Chemistry of the mycalamides, antiviral and antitumour compounds from a marine sponge. Part 5.[1–4] Acid-catalysed hydrolysis and acetal exchange, double bond additions and oxidation reactions

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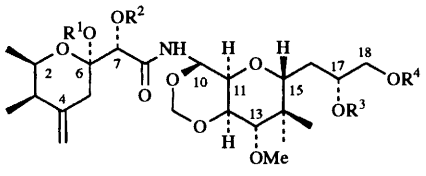
The acid-catalysed degradations of the potent antitumour and antiviral sponge metabolite mycalamide A and a triacetyl derivative have been examined. Acetal exchange reactions, catalytic hydrogenation, epoxidation and oxidation reactions have also been performed on mycalamides A and B. The major products derived from these reactions were characterised and tested for *in vitro* P388 anti-leukaemia activity and structure–activity relationships were deduced from these results.

Mycalamides A[1] **1** and B[2] **2** are potent antiviral and antitumour compounds isolated from a marine sponge, *Mycale* sp. We have recently described the reactions of mycalamide A and alkyl derivatives[3] with various basic nucleophiles.[4] This represented a continuation of extensive microscale structural modification studies of mycalamides A and B in which a major aim has been to develop structure–activity relationships for these compounds. We established[4] that the centrally located α-hydroxyamidoacetal functionality (specifically the C-7 OH and C-10 azaacetal units) is critical for the biological potency of the mycalamides, which reinforced the conclusions of a previous study.[3] The major aim of this present study was to determine the importance of some remaining key functional groups, including the exocyclic double bond and the C-6 acetal unit, and to examine further modifications to the C(16)–C(18) side chain.

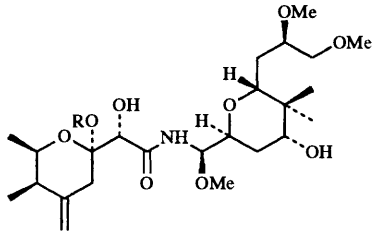
Previous work[3] had shown that mycalamide samples in $CDCl_3$ solution decomposed readily due to the trace acidity associated with this solvent.[5] However, the addition of 0.1% pyridine to the $CDCl_3$ was sufficient to prevent this decomposition, as previously described for the structurally related insect toxin pederin **9**.[6] Those workers involved in the total synthesis of pederin attributed this instability to the acid lability of the homoallylic C-6 acetal and C-10 azaacetal units, which necessitated delaying their introduction until the latest possible stage in the synthesis.[7]

Early chemical studies of pederin **9** established the identity of some major products derived from acid-catalysed hydrolysis and catalytic hydrogenations.[8] However, certain stereochemical features of these remained undefined due to limited spectroscopic characterisations, and, furthermore, the biological activity of some compounds was either not ascertained or was not well quantified. Importantly, dihydropederin **11** reportedly showed lower cytotoxicity than pederin while retaining potent antimitotic activity,[9] a result which was of considerable interest to us in our search for therapeutically more useful mycalamide derivatives.

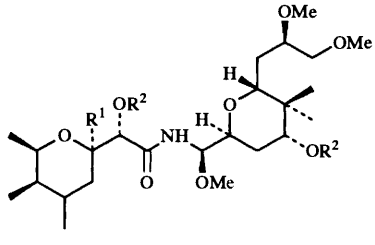
This report describes the results of detailed analyses of the acid-catalysed hydrolysis and catalytic hydrogenation of mycalamides A and B and some derivatives (to complement the earlier studies on pederin), together with similar studies of acetal exchange, elimination, epoxidation and oxidation reactions of these compounds. The structures (including stereochemistries) of the major products from these reactions were determined and possible mechanisms of formation are discussed (where relevant). Results from the *in vitro* P388 anti-leukaemia assay are also presented and further structure–activity relationships for the mycalamides are deduced.



	R^1	R^2	R^3	R^4
1	Me	H	H	H
2	Me	H	Me	H
3	H	H	H	H
4	H	H	Me	H
5	CD_3	H	H	H
6	Et	H	H	H
7	Me	Ac	Ac	Ac
8	H	Ac	Ac	Ac



9 R = Me
10 R = H



	R^1	R^2
11	OMe	H
12	OMe	Ac
13	H	H

Table 1 ^{1}H NMR spectroscopic data for mycalamide A derivatives[a,b]

	$\delta_H(J)$						
Proton	**3**	**15**	**22**	**23**	**26**	**35**	**36**
2-H	4.20 (2.6, 6.7)	4.18 (3.1, 6.5)	4.11 (2.7, 6.7)	3.97 (2.3, 6.6)	3.64 (2.2, 6.4)	4.02 (2.8, 6.6)	4.03 (2.8, 6.5)
2-Me	1.08 (6.6)	1.21 (6.5)	1.17 (6.6)	1.18 (6.6)	1.11 (6.4)	1.18 (6.6)	1.20 (6.6)
3-H	2.22 (2.6, 7.0)	2.45 (2.6, 7.0)	1.33 (2.7, 7.1)	1.47 (2.5, 3.9, 6.9)	1.41 (m)	2.23 (2.8, 7.1)	2.26 (2.8, 7.0)
3-Me	0.99 (7.0)	1.01 (7.0)	0.93 (7.1)	0.73 (7.1)	0.74 (7.0)	0.93 (7.1)	1.00 (7.1)
4-H			1.70 (m)	2.14 (3.7, 7.0, 12.8)	1.84 (m)		
4=CH(Z)	4.89 (2.0)	4.67 (m)				4.85 (1.9)	4.86 (1.8)
4=CH(E)	4.77 (1.9)	4.79 (m)				4.77 (1.9)	4.76 (1.8)
4-Me			1.16 (7.1)	0.88 (7.0)	0.93 (6.8)		
5a-H	2.81 (1.9, 13.8)		1.76 (5.6, 13.8)	1.23 (13.0)	1.40 (m)	2.06 (1.9, 14.2)	2.22 (2.0, 14.2)
5e-H	2.17 (13.8)	5.64	1.53 (2.9, 14.0)	1.62 (3.9, 13.9)	1.40 (m)	2.36 (14.1)	2.41 (14.0)
6-OMe			3.28	3.30		3.30	3.31
6-H					3.74 (4.0, 5.1, 8.8)		
7-H	4.08	4.57	4.21	4.23	4.00 (4.0)	4.27	4.31
7-OH							3.71
9-NH	7.59 (9.8)	7.23 (9.8)	7.45 (9.7)	7.46 (9.6)	7.41 (9.9)	7.47 (9.5)	7.51 (9.6)
10-H	5.82 (9.9)	5.86 (10.0)	5.85 (9.7)	5.84 (9.6)	5.83 (10.0)	5.88 (9.5)	5.83 (9.6)
10-OCH(R)	5.15 (7.0)	5.15 (6.8)	5.13 (7.0)	5.13 (6.9)	5.15 (7.0)	5.14 (7.0)	5.14 (6.9)
10-OCH(S)	4.89 (6.9)	4.88 (6.9)	4.87 (7.0)	4.87 (6.9)	4.88 (7.0)	4.88 (7.0)	4.88 (6.9)
11-H	3.93 (6.6, 10.0)	3.85 (6.9, 10.1)	3.86 (6.7, 9.7)	3.84 (6.7, 9.7)	3.88 (6.7, 10.1)	3.77 (6.6, 9.5)	3.77 (7.1, 9.7)
12-H	4.24 (6.8, 10.5)	4.24 (6.7, 10.6)	4.22 (6.7, 10.1)	4.22 (6.8, 10.1)	4.24 (6.8, 10.4)	4.21 (6.6, 10.1)	4.24 (7.1, 10.5)
13-H	3.47 (10.4)	3.48 (10.6)	3.46 (10.3)	3.45 (10.2)	3.48 (10.4)	3.50 (10.1)	3.47 (10.4)
13-OMe	3.56	3.56	3.56	3.56	3.56	3.57	3.57
14-Me(R)	0.97	0.97	0.98	0.98	0.98	0.93	1.00
14-Me(S)	0.87	0.86	0.88	0.88	0.87	0.87	0.85
15-H	3.58 (m)	3.56 (m)	3.63 (6.3)	3.63 (6.6)	3.61 (6.4, 8.5)	4.09 (5.2, 7.3)	3.64 (1.3, 10.5)
16-H	1.56 (m)	1.53 (m)	1.56 (m)	1.57 (m)	1.55 (m)	2.36 (2.6, 7.3)	1.67 (1.4, 5.7, 8.9, 14.5)
16-H	1.56 (m)	1.53 (m)	1.56 (m)	1.57 (m)	1.55 (m)	2.36 (2.0, 5.2)	1.37 (3.5, 3.5, 10.6, 14.4)
17-H	3.75 (m)	3.73 (m)	3.74 (m)	3.74 (m)	3.73 (m)	9.55 (2.0, 2.6)	3.50 (m, 17-H$_2$)
17-OH							2.96 (6.5)
18-H	3.56 (m)	3.56 (3.5, 11.3)	3.57 (3.5, 11.2)	3.58 (3.5, 11.4)	3.54 (3.5, 11.3)		
18-H	3.41 (5.6, 11.4)	3.37 (6.1, 11.4)	3.38 (5.9, 11.3)	3.37 (6.1, 11.2)	3.39 (5.9, 11.3)		

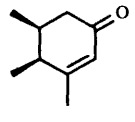
[a] Recorded in $CDCl_3$ with chemical shifts relative to $\delta_H(CHCl_3)$ 7.25 (coupling constants in Hz). [b] Full details of the spectroscopic results for compounds **4**, **6**, **14**, **16**, **17**, **18**, **20**, **21**, **24**, **25**, **27**, **28**, **29**, **30**, **31**, **32**, **33** and **34** available.

Results and discussion

Because of the limited supply of the mycalamides, all reactions were on a microscale, with typically only 0.5–2 mg of each product being obtained. Derivatives have been characterised by HRMS and NMR techniques. Wherever possible ^{1}H NMR spectral assignments have been verified by COSY and difference NOE methods, while in a few instances HMQC and HMBC experiments have aided in the assignment of ^{13}C NMR data. Selected NMR data are presented in Tables 1 and 2.

Acid-catalysed hydrolysis of mycalamides A and B

Pederin **9** and pseudopederin **10** underwent facile interconversion simply by heating in water or MeOH.[8,10] Prolonged hydrolysis of pseudopederin gave decomposition into a large number of non-isolable products. In contrast, mycalamide A **1** was not affected by hot water. Treatment of **1** with dilute HCl in MeOH gave a major, more polar product with time (HPLC), which was later identified as pseudomycalamide A **3**. However, the ^{1}H NMR spectrum of this product in $CDCl_3$, containing no added pyridine, showed the presence of unchanged mycalamide A, decomposed material (broad humps) and pederolactone[8] **14**, a known fragment from pederin. Flash silica gel chromatography gave purified material with similar ^{1}H NMR



14

and IR spectral data to the literature values.[11] Pederolactone **14** was probably derived from pseudomycalamide A **3** by an acid-catalysed transposition of the exocyclic double bond to the C(4)–C(5) position, followed by an oxidative cleavage of the (allylic) C-6 hemiacetal. (Several other mycalamide derivatives also gave **14** after prolonged contact with $CDCl_3$ in the absence of base.)

Optimal conditions for the synthesis of pseudomycalamide A **3** (HREIMS, ^{1}H NMR) consisted of a two-phase hydrolysis system, involving vigorous stirring of mycalamide A **1** in CH_2Cl_2 with aqueous toluene-*p*-sulfonic acid at 20 °C. HREIMS showed a facile loss of H_2O from the parent ion, which was confirmed by DCIMS using ammonia as the reagent gas. The ^{1}H and ^{13}C NMR spectra (in 0.1% pyridine/$CDCl_3$) of **3** showed the replacement of the 6-methoxy group and consequent significant chemical shift changes [$\Delta\delta_H$ +0.2 (2-H), +0.4 (5a-H), −0.2 (5e-H), −0.2 (7-H); $\Delta\delta_C$ +2.0 (C-5), −2.3 (C-6), +2.0 (C-7), +2.3 (C-8)]. Pseudomycalamides A **3** and B **4** (made by analogous reaction of mycalamide B **2**) were transformed back to mycalamides A and B in quantitative yield (TLC, HPLC) by treatment with toluene-*p*-sulfonic acid in MeOH at 20 °C. Other hydrolysis methods gave either complete decomposition (mycalamide A with trifluoroacetic acid in $CDCl_3$) or significant by-products and/or unchanged mycalamide A (as above).

The observation of a strong NOE interaction between 5e-H and 7-H, together with the significant chemical shift changes noted, were consistent with a different major C(6)–C(7) solution conformation for **3** to those previously described for mycalamide A.[2] This was supported by the ^{1}H NMR spectrum of pseudomycalamide B **4** in CD_2Cl_2, which showed the presence of long-range W coupling $J_{5a\text{-}H,6\text{-}OH}$ (2 Hz) [δ_{OH} 4.95 (d), removed on addition of D_2O]. Similar couplings have been reported for pseudopederin[11] **10** and related structures,[12,13]

Table 2 ^{13}C NMR spectroscopic data for mycalamide A derivatives[a]

Carbon	δ_C 3	15	22	23	26	35	36
C-2	68.95	76.59	64.92	70.88	77.6	69.61	69.79
2-Me	17.87	15.88	18.28	18.46	18.7	17.99	17.97
C-3	41.43	37.72	37.45	37.09	37.4	41.28	41.31
3-Me	11.75	13.17	13.04	4.36	4.8	12.17	12.04
C-4	146.49	142.90	32.11	28.94	34.0	145.07	145.13
4=CH_2	110.41	108.03				111.01	111.04
4-Me			20.81	18.85	19.2		
C-5	35.72	103.63	29.51	31.10	29.6	33.23	33.41
C-6	97.54	149.80	100.05	99.30	77.2	100.05	99.90
6-OMe			48.11	48.47		48.54	48.76
C-7	74.89	72.57	73.32	72.93	74.6	71.31	71.76
C-8	174.08	171.91	171.82	Not obs.	173.3	171.91	172.35
C-10	73.26	73.59	73.80	73.77	73.5	74.16	74.35
10-OCH_2	87.03	87.04	86.81	86.80	87.0	86.60	86.59
C-11	71.22	71.51	71.23	71.12	71.4	70.78	71.66
C-12	74.50	74.62	74.40	74.37	74.6	74.25	74.78
C-13	78.90	78.96	79.15	79.20	79.1	79.20	79.40
13-OMe	61.93	61.91	61.82	61.81	61.8	61.79	61.87
C-14	41.91	41.84	41.63	41.59	41.7	40.97	41.37
14-Me(*R*)	22.93	22.92	23.15	23.16	22.9	23.38	23.14
14-Me(*S*)	13.22	13.17	13.56	13.59	13.2	14.04	13.38
C-15	79.45	78.64	79.09	78.80	79.1	74.06	75.41
C-16	31.55	31.79	32.05	32.01	31.7	43.35	31.86
C-17	72.21	71.72	71.48	71.21	71.8	200.52	58.50
C-18	66.47	66.42	66.56	66.37	66.5		

[a] Recorded in $CDCl_3$ with chemical shifts relative to $\delta_C(CDCl_3)$ 77.01.

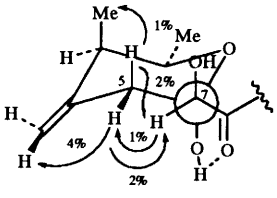


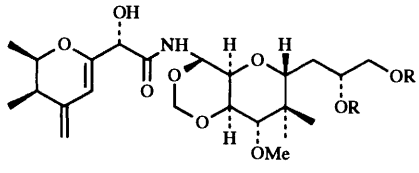
Fig. 1 Newman projection along C(6)–C(7) and observed NOE interactions (%) for the proposed solution conformation of pseudomycalamides A and B **3**, **4**

and implies a 'locked' 6-OH conformation, probably involving hydrogen bonding to the 8-carbonyl group (Fig. 1).

Acetal exchange and elimination of mycalamide A

Mycalamide A was converted into a trideuterio derivative **5** (HRFABMS, ^1H NMR) by the use of a catalytic amount of trifluoroacetic acid (TFA) in CD_3OD solution [$\Delta\delta_H$ −2 Hz (7-H), loss of 6-methoxy], a reaction which was reversible. A similar reaction of mycalamide A with either TFA or pyridinium-*p*-toluene sulfonate (PPTS) in dry EtOH was very slow at 20 °C. After 4 days, the latter gave *ca.* 60% conversion into the less polar 6-ethoxymycalamide A **6** (HRFABMS, ^1H NMR, ^{13}C NMR) and a minor component (< 10%), which were separated by C_{18} reversed-phase HPLC.

No reaction was observed for a solution of mycalamide A and PPTS in isopropyl alcohol after 1 day at 20 °C (TLC), but heating at 55 °C for 1 h gave a mixture of unchanged mycalamide A, pseudomycalamide A **3**, pederolactone **14** and a new component, the same as the minor component observed in the EtOH–PPTS reaction above. This new compound was purified by HPLC (34% yield) and identified as the elimination product, Δ^5-mycalamide A **15** (HRFABMS, ^1H NMR, ^{13}C NMR). The ^1H and ^{13}C NMR spectra of **15** showed no 6-OMe resonance and the presence of a double bond at C(5)–C(6) [δ_H 5.64 (s, 5-H); δ_C 103.6 (C-5), 149.8 (C-6), assigned by HMQC and HMBC experiments], together with related chemical shift changes for neighbouring groups. It was subsequently found



15 R = H
16 R = Ac

that **15** could also be obtained in about 90% yield by simply heating a solution of mycalamide A and pyridine (or triethylamine) in dimethyl sulfoxide at 150 °C for 3 h (no reaction occurred in the absence of base) and this enabled the complete characterisations described above.

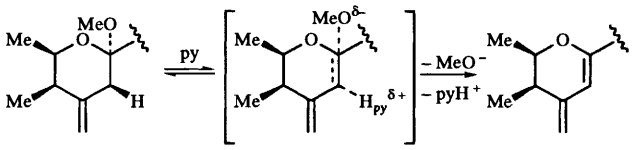
The acid-catalysed hydrolysis and acetal exchange mimic those of glycosides, occurring *via* a cyclic carboxonium ion intermediate (S_N1). The latter being homoallylic, it is not surprising that elimination (E1) competes with substitution, particularly for poorer nucleophiles and higher temperatures. The base-catalysed mechanism probably lies between E2 and E1cb, since, while 5a-H and 6-OMe are antiperiplanar, methoxide is a poor leaving group and 5a-H is slightly acidic (being allylic), so the transition state may have some carbanion character (Scheme 1).[14]

Having characterised reactions occurring at the homoallylic C-6 acetal of mycalamide A, we then examined the two-phase hydrolysis of mycalamide A triacetate **7**. The rationale for this was that the use of the moderately acid-stable acetate ester as a protecting group for the three hydroxyls of mycalamide A would improve the likelihood of isolating stable fragments, by increasing their mass and reducing polarity.

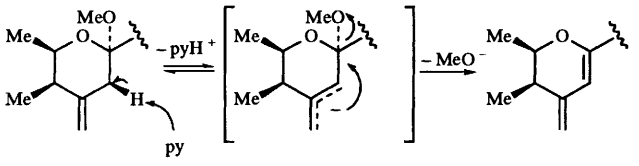
Acid-catalysed hydrolysis of mycalamide A triacetate

The two-phase hydrolysis of mycalamide A triacetate **7** in CH_2Cl_2 with 0.1 mol dm^{-3} aqueous toluene-*p*-sulfonic acid at 80 °C for 1 day gave a mixture of products, which were separated by preparative silica gel TLC and then reversed-phase HPLC. The major component was identified as the elimination product, (*E*)-neomycalamide A triacetate **17** (HRFABMS, ^1H

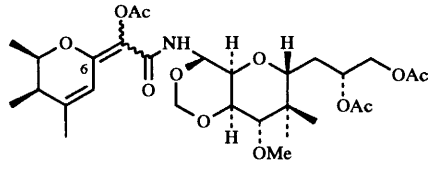
Base-catalysed elimination (E2)



Base-catalysed elimination (E1cb)



Scheme 1 Base-catalysed elimination mechanisms for the formation of Δ^5-mycalamide A **15**

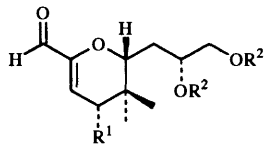


17 6*E*
18 6*Z*

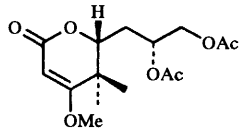
NMR, ^{13}C NMR), isolated in 40% yield. The ^1H NMR spectrum contained new coupled resonances at δ_H 6.01 (q) and δ_H 1.92 (d), $J_{5\text{-H},4\text{-Me}}$ (1.5), similar to those observed for pederolactone **14**, and no resonances for 4=CH_2, 5-H_2, 6-OCH_3 and 7-H. Related chemical shift changes and the ^{13}C NMR data were consistent with a conjugated planar dienone structure having a vinyl acetate group at C-7, [ν_{max}/cm^{-1} 1765 (C=O)], while NOE interactions between 5-H and 7-$OCOCH_3$ and between NH and both 2-H and 2-CH_3 established the *E* stereochemistry at C(6)=C(7). The structure had earlier been observed for other C-7 ethers and esters of mycalamides prepared in $CDCl_3$ solutions containing no added base.

The second major product (16% yield) was identified as an isomer of **17**, *Z*-neomycalamide A triacetate **18** (HRFABMS, ^1H NMR), but was evidently less stable than **17** since some rearrangement to **17** had occurred during purification. In the ^1H NMR spectrum of **18**, the chemical shift of 5-H was 1.3 ppm further downfield than for **17**, consistent with deshielding by the 8-carbonyl group. Attempted deacetylation of **17** or **18** (K_2CO_3, MeOH–water) gave decomposition.

Several minor components were also purified which could not be fully characterised because of lack of material. These included pseudomycalamide A triacetate **8** (unstable), Δ^5-mycalamide A 17,18-diacetate **16** (HRFABMS, ^1H NMR), in which the allylic C-7 acetate had been hydrolysed [δ_H 4.56 (d, 7-H), 4.29 (7-OH), the latter removed on addition of D_2O, $J_{7\text{-H},7\text{-OH}}$ (4.4)] and two fragments from the right-hand portion of the triacetate **7**. The first, by analogy to pederenal[11] **19** (derived from the hydrolysis of pederin in hexane–sulfuric acid), was proposed to be the unsaturated aldehyde **20** (DCIMS, IR, ^1H NMR), formed by hydrolysis of the acetal units at C-10 and (10-)OCH_2O-C-12 and elimination of the resulting β-hydroxy aldehyde. This structure followed from the ^1H NMR data [δ_H 9.16 (s, 10-H), 5.84 (d, 12-H), 3.61 (13-H), $J_{12\text{-H},13\text{-H}}$ (2.6)], including observed NOE interactions (*e.g.* between 10-H and 12-H), and the FTIR data [ν_{max}/cm^{-1} 1741, 1700 (C=O) and 1637 (C=C)], which were similar to those reported for **19**.[11] The second was probably the unsaturated lactone **21** (HRFABMS, ^1H NMR) [δ_H 5.05 (12-H), NOE between 12-H and 13-OCH_3]. The structure **21** was also consistent with chemical shift



	R^1	R^2
19	H	Me
20	OMe	Ac



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changes (compared to **7**) [$\Delta\delta_H$ +0.2 (13-OCH_3), +0.2 (14-CH_3S), +0.7 (15-H)], and evidence from the FTIR spectrum [ν_{max}/cm^{-1} 1740, 1720 (C=O), 1665 and 1614 (C=C)], and could arise from an oxidative cleavage of the enol form of **20**, by analogy to the formation of pederolactone **14**.

The above acid-catalysed reactions of the mycalamides have led to the characterisation of useful derivatives to test the biological importance of the C-6 acetal as well as revealing some interesting chemistry which has partially explained the acid-catalysed decomposition pathway for the mycalamides. We next considered reactions of the exocyclic C-4 double bond.

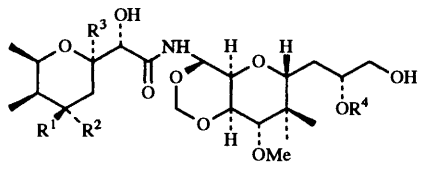
Hydrogenation of mycalamides A and B over Adams catalyst

Hydrogenation of mycalamide A over platinum oxide (Adams catalyst) gave a 3:2 ratio of two isomers of dihydromycalamide A (HRFABMS, ^1H NMR). These isomers were incompletely resolved by analytical C_{18} reversed-phase HPLC and by silica gel TLC. However, by collecting cuts either side of the observed double peak (HPLC) and recycling the central mixture, it was possible to obtain two samples each consisting of 9:1 mixtures of the two isomers. The ^1H NMR spectra of both isomers of dihydromycalamide A were then assigned by COSY and NOE experiments, and the stereochemistries deduced.

For the major isomer, **22**, NOE interactions were observed between 2-H, 6-OCH_3 and 4-CH_3 and between 3-CH_3 and 5a-H which required that these groups exist in diaxial relationships. Thus, the O(1)–C(6) ring was in the expected chair conformation, in agreement with the observed vicinal proton-proton coupling constants (data in Table 1), having the C-4 stereochemistry shown (designated 4β for H_2 addition from the *re* face of the double bond). Similarly, for the minor isomer, **23**, observed NOE interactions between 2-H, 4-H and 6-OCH_3 and between 3-CH_3 and 5a-H required the same chair conformation for the O(1)–C(6) ring, but with a 4α-H stereochemistry at C-4.

Hydrogenation of mycalamide B under the same conditions gave the two isomers of dihydromycalamide B **24** and **25** (HRFABMS, ^1H NMR, ^{13}C NMR), which were separated as above. HMQC experiments were recorded on both isomers and an HMBC experiment recorded on the major isomer, which together enabled complete assignments of their ^{13}C NMR spectra and those of the mycalamide A derivatives by comparison.

In contrast to these results, the hydrogenation of pederin **9** over Adams catalyst reportedly yielded a single isomer **11** (after crystallisation), but the stereochemistry at C-4 was not defined.[8] Examination of the ^1H NMR data reported for



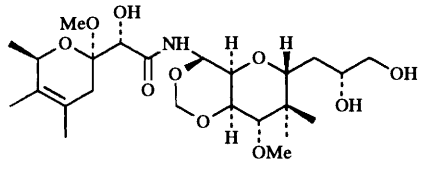
	R^1	R^2	R^3	R^4
22	H	Me	OMe	H
23	Me	H	OMe	H
24	H	Me	OMe	Me
25	Me	H	OMe	Me
26	Me	H	H	H

dihydropederin diacetate[11] **12** suggested strongly that this was the 4α-H isomer since both the 3-CH_3 and 4-CH_3 resonances were relatively shielded (δ_H 0.88 and 0.69), as found for **23**, due to a gauche interaction. The reason for this apparent difference in stereochemical outcome for similar reactions of pederin and the mycalamides remains unclear.

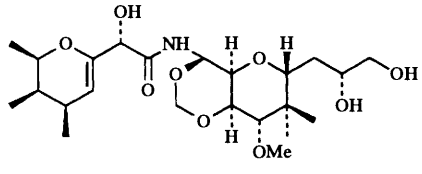
Both isomers of dihydromycalamides A and B underwent very facile hydrolysis at C-6 to give the hemiacetal derivatives (almost complete within 2 min in 'pure' $CDCl_3$). This instability towards acid, despite removal of the exocyclic double bond, is consistent with the known reactivities of glycosides and related cyclic acetals.

Hydrogenation of mycalamide A over palladium-on-charcoal

The reactivity of the homoallylic 6-acetal functionality present in the mycalamides and pederin was further illustrated by the reported isolation of both dihydropederin **11** and the hydrogenolysis product, dihydrodeoxy pseudopederin **13** (undesignated stereochemistry), from the hydrogenation of pederin over 10% palladium-on-charcoal catalyst.[8] A similar reaction on mycalamide A gave mostly dihydromycalamide A (**22** and **23**), together with two new products, which were separated by reversed-phase HPLC and identified as **27** and **26**.



27



28

The first product, **27**, isolated in 18% yield, was the Δ^3 isomer of mycalamide A (HRFABMS, ^{1}H NMR). The ^{1}H NMR spectrum showed loss of the 4=CH_2 and 3-H resonances and contained a new methyl singlet [δ_H 1.63 (4-CH_3)], together with significant chemical shift changes [$\Delta\delta_H$ +0.2 (2-H), +0.6 (3-CH_3), −0.3 (5e-H), assigned by NOE experiments]. Long-range allylic and homoallylic couplings were also observed between 2-H, 3-CH_3, 4-CH_3 and 5-H_2. For example, irradiation of 3-CH_3/4-CH_3 enabled resolution of the coupling between 2-H and 5-H_2 [δ_H 2.24 (dd, 5a-H), 2.08 (dd, 5e-H), $J_{5a\text{-}H,5e\text{-}H}$ (17.3), $J_{5a\text{-}H,2\text{-}H}$ (2.7), $J_{5e\text{-}H,2\text{-}H}$ (2.2)], where the larger coupling is known to be associated with a *transoid* relationship of the protons.[15] The transposition of the exocyclic double bond of mycalamide A into the ring is not unexpected for hydrogenation over a palladium catalyst and the Δ^3-mycalamide A isomer is less susceptible towards hydrogenation (having a tetrasubstituted double bond).

The second product, **26**, also isolated in 18% yield, was the expected C-6 hydrogenolysis product (HRFABMS, ^{1}H NMR, ^{13}C NMR), having the 6-methoxyl replaced by a proton [δ_H 3.74 (ddd, 6-H), 4.00 (d, 7-H), $J_{6\text{-}H,7\text{-}H}$ (4.0), $J_{5e\text{-}H,6\text{-}H}$ (5.1), $J_{5a\text{-}H,6\text{-}H}$ (8.8)], in addition to the expected hydrogenation at C-4. Observed NOE interactions between 2-H, 4-H and 6-H required these protons to be in diaxial relationships, thus establishing both the stereochemistries at C-4 and C-6 and the usual chair conformation of the O(1)–C(6) ring. Note that proton chemical shift changes between **26** and **23** [$\Delta\delta_H$ −0.3 (2-H, 4-H), −0.2 (5e-H, 7-H)] were entirely consistent with loss of the deshielding 6-OMe group.[16] Furthermore, the use of HMQC and HMBC experiments (at 500 MHz) enabled the indirect acquisition of ^{13}C NMR data (Table 2) which would otherwise have been unavailable due to the small sample size, and these data were also in agreement with the structure shown.

The similarity of the ^{1}H NMR spectral data reported[11] for dihydrodeoxy pseudopederin **13** to data for **26** suggested that these compounds have the same stereochemistries at C-4 and C-6. These arise firstly by transposition of the exocyclic double bond of mycalamide A into the C(4)–C(5) ring position and a stereoselective catalytic replacement of the 6-OMe group by a hydrogen atom from the same side. Secondly, hydrogenation of the C(4)=C(5) double bond occurs from the now unhindered lower (*si,si*) face in preference to the more-hindered upper (*re,re*) face, having an adjacent axial methyl group at C-3, to give the observed products.

Hydrogenation of Δ^5-mycalamide A over Adams catalyst

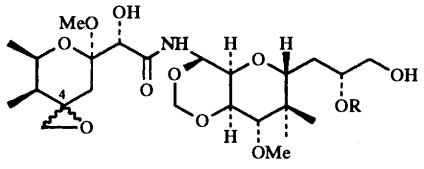
In order to obtain sufficient quantities of **26** to perform all the characterisations described above, it was necessary to carry out a second synthesis. The availability of Δ^5-mycalamide A **15** in near-quantitative yield by the mild base-catalysed elimination described above provided an alternative hydrogenation route to **26**, in which the 4α-H isomer would be favoured by the same steric factors described above. In practice, however, the hydrogenation of Δ^5-mycalamide A over Adams catalyst in MeOH gave a mixture of four major components, which were separated by reversed-phase HPLC.

The major product, isolated in 18% yield, was the partial hydrogenation product, **28** (HRFABMS, ^{1}H NMR). This compound displayed several long-range couplings, as shown by selective proton decoupling experiments, for example, between 4-H and 7-H [$J_{4\text{-}H,7\text{-}H}$ (1.5)] and between 5-H and both 3-H (W coupling) and 7-H. The C-4 stereochemistry was verified by NOE experiments, and was the expected 4α-H isomer, as discussed above.

The second major product, isolated in 16% yield, was the desired product, **26**, obtained above (^{1}H NMR). The remaining two products were (unexpectedly) 4α- and 4β-dihydromycalamide A, **23** and **22**, in a combined yield of 21%. These appear to arise from the re-addition of MeOH at the very reactive C-6 position, and could possibly have been avoided by using a less nucleophilic solvent. The low overall yields were suggestive of partial decomposition, consistent with the presence of the unstable allylic hydroxy group (C-7) in **15**.

Epoxidation of mycalamides A and B

The reactions of mycalamides A and B with *m*-chloroperbenzoic acid in CH_2Cl_2 gave 3:2 mixtures of their respective epimeric epoxides, **29** and **30**, and **31** and **32** (HRFABMS, ^{1}H NMR), which were separated by preparative silica gel TLC. Their ^{1}H NMR spectra showed the presence of long-range couplings



4-O R
29 α H
30 β H
31 α Me
32 β Me

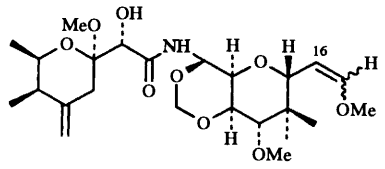
between 3-H and 5e-H for both isomers (W coupling) and between 5a-H and the more distant 4*Z*-CH (assigned by NOE experiments) for the minor isomer only. The latter coupling has been observed for other exocyclic epoxides having a pseudo-axial (epoxide) methylene proton and an adjacent axial methylene proton and has been ascribed to favourable orbital overlap between a delocalised electron density system above the C–C bond of the strained epoxide ring and the adjacent σ orbital of the axial methylene proton.[17]

This evidence suggested that the minor isomer had a 4β stereochemistry at C-4 (compounds **30** and **32**) and this was further supported by the observation of an NOE interaction between the axial 3-CH_3 and the 4*Z*-CH proton for the major isomer only. The ^{13}C NMR data, assigned by an HMQC experiment on the major isomer, were also consistent with these assignments. Note also that the observed major isomer corresponds to a preferential attack of the peracid from the lower (*si*, axial) face of the exocyclic double bond, which is known to be favoured in epoxidations.[18]

Selective oxidation of mycalamides A and B

Previous studies have shown that the biological potency of the mycalamides can be fine tuned by modifications to the C(16)–C(18) side chain.[3] This is also demonstrated by the reported activities of the structurally related natural products pederin **9**, onnamide A[19] and theopederins A–E.[20] Selective oxidation of the primary hydroxy groups of mycalamides A and B would enable further structural modifications to this important region. In addition, the reduction of a carbonyl functionality would present an opportunity for the introduction of a stable isotopic label for potential use in mode of action or pharmacokinetic studies. Tris(triphenylphosphine)-ruthenium(II) chloride is one of the few reagents which reportedly performs this selective transformation and would be compatible with other functionalities present in the mycalamides.[21]

The reaction of mycalamide B with tris(triphenylphosphine)ruthenium(II) chloride and solid potassium carbonate in benzene at 60 °C gave a mixture of starting material and several products, which were separated by preparative silica gel TLC, followed by reversed-phase HPLC. Two major products were obtained which were isomers of molecular formula of $C_{24}H_{39}NO_9$ (HRFABMS), corresponding to a loss of CH_3OH from mycalamide B. The ^1H NMR spectra of both isomers established that the structure of mycalamide B up to C-15 has been preserved. However, there was no aldehyde resonance, but rather resonances indicative of an unsaturated vinyl ether substructure [*e.g.* δ_H 6.39 (d, 17-H), 4.57 (dd, 16-H), $J_{16\text{-H},17\text{-H}}$ (12.6); 15-H from dd to d; $\Delta\delta_H$ +0.4 (15-H), +0.2 (17-OCH_3)]. These were, therefore, *E* and *Z* Δ^{16}-normycalamide B, **33** and **34**, in which the stereochemistry of the new C(16)=C(17) double bond was deduced from the size of $J_{16\text{-H},17\text{-H}}$ (12.6 and 6.6, respectively) and confirmed by NOE experiments. Partial ^{13}C NMR data for the *E* isomer were also obtained indirectly from an HMQC experiment, and these data



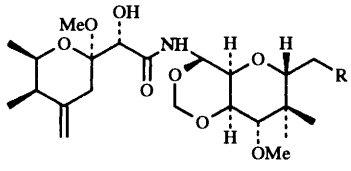
33 16*E*
34 16*Z*

[δ_C 147.0 (C-17), 55.9 (17-OCH_3)] were also consistent with the proposed structure.

The mechanism of oxidation of alcohols by ruthenium complexes reportedly involves the initial formation of a ruthenium alkoxide, which then undergoes hydride transfer from the α-carbon to the metal and (usually) cleavage of the metal–oxygen bond to give the carbonyl derivative.[22] The observed product may arise from an alternative base-promoted eliminative decarbonylation of the intermediate ruthenium–aldehyde complex involving the initial oxidation of mycalamide B at C-18. Such hydridocarbonylations are known to occur for tertiary phosphine complexes of group VIII metals such as ruthenium and rhodium, being favoured by the enhanced stability of the resulting metal–carbonyl complexes.[23]

The reaction of mycalamide A with tris(triphenylphosphine)ruthenium(II) chloride (only) in benzene gave about 50% conversion to a single product after 2 days at 20 °C. However, if the reaction mixture was heated to 50 °C with added solid potassium carbonate, then only decomposition was observed. The above product was purified by chromatography on silica gel and identified as normycalamide A aldehyde, **35** (HRFABMS, ^1H NMR, ^{13}C NMR). Whereas the aldehyde functionality was observable in both the FTIR spectrum [ν_{max}(film)/cm^{-1} 1725 (C=O)] and the ^1H and ^{13}C NMR spectra recorded in $CDCl_3$ [δ_H 9.55 (t, 17-H), $J_{16\text{-H},17\text{-H}}$ (2.0, 2.6), δ_C 200.5 ppm (C-17)], when the latter were recorded in CD_3OD, only a 1:1 mixture of the isomeric C-17 hemiacetals was observed. The loss of C-18 was deduced from the MS and NMR data, and probably involves a similar mechanism to that described above for mycalamide B.

Subsequent experiments showed that **35** could be obtained in quantitative yield by the oxidation of mycalamide A with lead tetraacetate in benzene–pyridine at 20 °C (TLC, NMR). In contrast, mycalamide B did not react with this reagent, even on mild heating. The aldehyde **35** was easily reduced by sodium



35 R = CHO
36 R = CH_2OH
37 R = OH

borohydride in MeOH at 20 °C to give normycalamide A **36** (HRFABMS, ^1H NMR) in quantitative yield [δ_H 3.50 (m, 17-H_2), 2.96 (t, 17-OH, removed on addition of D_2O), $J_{17\text{-H},17\text{-OH}}$ (6.6)]. Here the use of $NaBD_4$ would enable the incorporation of deuterium at C-17 as a stable isotopic label. It is also interesting to note that **36** is the one-carbon homologue of theopederin E **37**, a cytotoxic compound isolated from a *Theonella* sponge.[20]

Biological activity

The structural diversity of the products described above has enabled an assessment of the biological importance of many features of the mycalamide structure. The cytotoxicities of these

Table 3 IC_{50} Values of mycalamide derivatives against P388 cells

Compound	IC_{50} (ng cm^{-3})
1	0.5
2	0.1
3	9
4	4
6	1
15	120
16	270
17	1300
18	220
20	500
21	125
22	0.2
23	0.8
24	0.1
25	0.8
26	250
27	58
28	650
29	115
30	16
31	90
32	4
33	34
34	10
35	0.2
36	0.2

products against the murine leukaemia P388 cell line have been measured as IC_{50} values (in ng cm^{-3}) and recorded in Table 3.

The importance of the C-6 acetal is shown by the retention of activity with a C-6 ethoxy substituent (compound **6**), a 20–40-fold drop in activity for a 6-hydroxy substituent as in compounds **3** and **4** (in agreement with limited data reported[9] for pseudopederin **10** and pederin **9**) and further losses ($> 10^2$-fold) with elimination or hydrogenolysis at C-6 (compounds **15–18**, **26**, **28**). These results complement those of previous studies,[3,4] which had established that derivatisation of 7-OH was highly detrimental (*ca.* 10^2-fold deactivation), that the C-10 acetal functionality was important and that cleavage fragments were essentially inactive.

Of most significance were results for compounds involving derivatisation or transposition of the exocyclic double bond. 4β-Dihydromycalamide A **22** was significantly more active than mycalamide A but 4β-dihydromycalamide B **24** had the same activity as mycalamide B. The 4α isomers were 4–8 times less active, which may explain the reportedly reduced cytotoxicity of dihydropederin **11** compared to pederin **9**.[9] Surprisingly, Δ^3-mycalamide A **27** was 10^2-fold less active than mycalamide A. The epoxide derivatives **29–32** were also much less active, although there was an even more pronounced isomer effect.

Modifications to the C-16 side chain were expected to be well tolerated, as noted above. Surprisingly, both Δ^{16}-normycalamide B isomers (**33** and **34**) were *ca.* 10^2-fold less active than mycalamide B. However, the C-17 aldehyde and alcohol derivatives (**35** and **36**) were significantly more active than both mycalamide A and theopederin E[20] **37** (reported[20] P388 IC_{50} of 9 ng cm^{-3}). Thus several derivatives of mycalamide A have been prepared which have enhanced activity but none of these are more active than mycalamide B.

The 'tight' structure–activity correlations observed for the mycalamides is consistent with the high potency displayed, suggesting that these compounds interact with a specific, as yet undefined, target binding site, where the structure of the natural products may be fairly optimal, despite the apparent flexibility. Opportunities for further analogue development would appear to lie principally with more side chain modifications, at least until the site of action is better defined. Undoubtedly, the high potency of these novel antiviral and antitumour natural products will continue to stimulate further interest and investigation.

Experimental

General procedure

The same general procedure was used as described previously.[3] In addition, FTIR spectra were recorded from sample films on KBr plates using a Perkin-Elmer 1600 series spectrometer or a Bio-Rad Digilab Division FTS-40 spectrometer. NMR spectra were recorded on a Varian XL300 spectrometer, operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C nuclei, except for some HMQC and HMBC data, which were recorded on a VXR500S spectrometer operating at 500 MHz for ^{1}H. Mycalamides A **1** and B **2** were obtained from a *Mycale* sp. sponge as described previously.[1,2]

WARNING: The mycalamides and several of their derivatives cause severe dermatitic reactions if allowed to come into contact with the skin.

Preparation of pseudomycalamides A and B (and pederolactone)

(a) A solution of mycalamide A **1** (2.7 mg) in CH_2Cl_2 (0.2 cm^3) was rapidly stirred with aqueous toluene-*p*-sulfonic acid (0.05 mol dm^{-3}; 0.2 cm^3) at 20 °C for 4 h. After addition of water (2 cm^3) to the mixture it was neutralised with dilute aqueous NaOH and extracted with CH_2Cl_2 (3 × 2 cm^3). The combined extracts were evaporated to give *pseudomycalamide* A **3** as an oil (2.3 mg); ^{1}H and ^{13}C NMR data in Tables 1 and 2 (HREIMS.† $M^+ - H_2O$, 471.2477. *M* − H_2O, 471.2468); DCIMS (NH_3) *m/z* 508 (27%), 507 (MNH_4^+, 100), 491 (34), 490 (26), 489 ($MNH_4^+ - H_2O$, 72), 474 (14), 473 (29) and 472 ($MH^+ - H_2O$, 90).

(b) A solution of mycalamide B **2** (5.5 mg) in CH_2Cl_2 (0.5 cm^3) was rapidly stirred with aqueous toluene-*p*-sulfonic acid (0.05 mol dm^{-3}; 0.2 cm^3) at 20 °C for 48 h after which it was extracted with CH_2Cl_2 (3 × 0.3 cm^3). The combined extracts were washed with water (3 × 0.3 cm^3) and evaporated to give pseudomycalamide B **4** (5.1 mg, *ca.* 90%) (TLC), containing a small amount of mycalamide B. Preparative reversed-phase HPLC (20% water in methanol) on a sub-sample (1.2 mg) gave pure *pseudomycalamide* B **4** as an oil (0.9 mg); ^{1}H and ^{13}C NMR data in Supplementary Tables 1 and 3‡ (HREIMS. $M^+ - H_2O$, 485.2614. *M* − H_2O, 485.2625); DCIMS (NH_3) *m/z* 522 (20%), 521 (MNH_4^+, 64), 504 (MH^+, 51), 503 ($MNH_4^+ - H_2O$, 72), 487 (42) and 486 ($MH^+ - H_2O$, 100).

(c) A solution of mycalamide A **1** (5 mg) in dilute hydrochloric acid (0.1 mol dm^{-3}; 0.45 cm^3) and methanol (0.05 cm^3) was stirred at 20 °C for 1 h after which it was neutralised with dilute aqueous sodium hydroxide and evaporated. Extraction of the residue with $CHCl_3$ (3 × 1 cm^3) gave, after filtration and evaporation, an oil, which was taken up in pyridine-free $CDCl_3$ and examined by ^{1}H NMR spectroscopy (see text). Chromatography of the oil on silica gel (200 mg), eluting with light petroleum–ethyl acetate (1 : 1), gave as the major product, crude pederolactone **14** as an oil (1 mg), which decomposed during subsequent purification attempts; ν_{max}(film)/cm^{-1} 2956, 2926, 2853, 1720, 1645, 1461, 1377, 1260, 1166. 1096, 1072 and 1031; ^{1}H NMR data in Supplementary Table 1.‡

† Throughout, the first value given is that found, and the second value that expected by calculation.

‡ Supplementary publication no. SUP NO. 57079 (4 pp.). For details of the supplementary publication scheme, see Instructions for Authors (1995), 1995, issue 1.

Methanolysis of pseudomycalamides A and B

(a) A solution of pseudomycalamide A **3** (0.1 mg) in methanol (0.1 cm^3) containing toluene-*p*-sulfonic acid (5×10^{-4} mol dm^{-3}) was stirred at 20 °C for 1 d to give pure mycalamide A (HPLC).

(b) Similar treatment of pseudomycalamide B **4** (0.5 mg) for 4 h gave mycalamide B (TLC, HPLC).

Preparation of 6-alkoxy derivatives

(a) A solution of mycalamide A **1** (1.1 mg) in CD_3OD (0.6 cm^3) containing trifluoroacetic acid (0.01 mol dm^{-3}) was kept at 20 °C overnight to give [6-methoxy-$^2H_3$]*mycalamide* A **5** as an oil (1.1 mg); ^1H NMR data as for mycalamide A **1** except for the loss of 6-OMe (HRFABMS. MNa^+, 529.2808. *M*Na, 529.2814); DCIMS (NH_3) *m/z* 524 (MNH_4^+, 16%), 491 (14), 490 (22), 489 ($MNH_4^+ - CD_3OH$, 81) and 472 ($MH^+ - CD_3OH$, 16).

(b) A solution of mycalamide A **1** (3.3 mg) in dry ethanol (0.3 cm^3) containing pyridinium toluene-*p*-sulfonate (1×10^{-3} mol dm^{-3}) was stirred at 20 °C for 4 d. The solution was concentrated under nitrogen and then treated with dilute aqueous sodium hydroxide (2 cm^3, pH 9) and extracted with CH_2Cl_2 (3×2 cm^3). The combined extracts were evaporated and the crude product (3.2 mg) subjected to preparative reversed-phase HPLC (40% water in methanol) to give two fractions, pure mycalamide A (1.0 mg) and *6-ethoxymycalamide* A **6** as an oil (2.0 mg); ^1H and ^{13}C NMR data in Supplementary Tables 1 and 3 (HRFABMS. MNa^+, 540.2785. *M*Na, 540.2785); DCIMS (NH_3) *m/z* 535 (MNH_4^+, 6%), 491 (4), 490 (6), 489 ($MH_4^+ - EtOH$, 100), 474 (5), 473 (5) and 472 ($MH^+ - EtOH$, 65).

Preparation of Δ^5-mycalamide A

(a) A solution of mycalamide A **1** (2.2 mg) in dry isopropyl alcohol (0.3 cm^3) containing pyridinium toluene-*p*-sulfonate (1×10^{-3} mol dm^{-3}) was stirred at 20 °C for 16 h and then at 55 °C for 1 h. After this the solution was concentrated under nitrogen and then treated with dilute aqueous sodium hydroxide (2 cm^3, pH 10) and extracted with CH_2Cl_2 (3×2 cm^3). The combined extracts were evaporated and the crude product (2 mg) was subjected to preparative reversed-phase HPLC (45% water in methanol) to give two fractions, mycalamide A (0.7 mg) and Δ^5-*mycalamide* A **15** as an oil (0.5 mg); ν_{max}(film)/cm^{-1} 2960, 2926, 2855, 1679, 1600, 1529, 1454, 1385, 1266, 1194, 1109, 1074 and 1031; ^1H and ^{13}C NMR data in Tables 1 and 2 (HRFABMS. MK^+, 510.2132. *M*K, 510.2105).

(b) A solution of mycalamide A **1** (3.8 mg) and pyridine (10 mm^3) in DMSO (0.6 cm^3) was heated at 150 °C for 3 h and then diluted with water (1 cm^3) and transferred onto a reverse phase column (200 mg C_{18}, equilibrated to water). This was then flushed with water (5 cm^3) and eluted with methanol (5 cm^3). The methanol fraction was evaporated to dryness to give Δ^5-mycalamide A **15** (3.6 mg, *ca.* 90%) (^1H NMR spectroscopy) containing a small amount of mycalamide A.

Acid-catalysed hydrolysis of mycalamide A triacetate

A solution of mycalamide A triacetate **7** (5.5 mg) in CH_2Cl_2 (2 cm^3) was rapidly stirred with aqueous toluene-*p*-sulfonic acid (0.1 mol dm^{-3}; 1 cm^3) at 75 °C for 24 h and then at 85 °C for 6 h in a sealed vial. The resulting solution was neutralised with dilute aqueous sodium hydroxide and then extracted with CH_2Cl_2 (3×2 cm^3). The combined extracts were evaporated and the residue subjected to preparative silica gel TLC [light petroleum–ethyl acetate (1:1)]. Four bands of silica were recovered and eluted with ethyl acetate to give four fractions: (i) (E)-*neomycalamide A triacetate* **17** as an oil (3.0 mg); ν_{max}(film)/cm ^{1} 3380, 2960, 2925, 2853, 1765, 1740, 1670, 1644, 1525, 1370, 1225, 1175, 1110 and 1030; ^1H and ^{13}C NMR data in Supplementary Tables 1 and 3 (HRFABMS. MH^+, 598.2862. *M*H, 598.2864); DCIMS (NH_3) *m/z* 616 (7%), 615 (MNH_4^+, 34), 600 (13), 599 (31), 598 (MH^+, 100), 573 ($MNH_4^+ - CH_2CO$, 28), 571 (18), 557 (24) and 556 ($MH^+ - CH_2CO$, 66). (ii) (Z)-*Neomycalamide A triacetate* **18** as an oil (1.2 mg); ν_{max}(film)/cm^{-1} 3375, 2956, 2925, 2854, 1774, 1740, 1664, 1640, 1590, 1514, 1460, 1370, 1228, 1108 and 1028; ^1H and ^{13}C NMR data in Supplementary Tables 1 and 3 (HRFABMS. MH^+, 598.2857. *M*H, 598.2864). (iii) Mixture (1.5 mg) which was further purified by preparative reversed-phase HPLC (40% water in methanol) to give mycalamide A triacetate **7** (0.4 mg), crude pseudomycalamide A triacetate **8** (unstable, incompletely characterised) as an oil (0.4 mg) and Δ^5-*mycalamide A 17,18-diacetate* **16** as an oil (0.5 mg); ^1H NMR data in Supplementary Table 1 (HRFABMS. MH^+, 556.2765. *M*H, 556.2758). (iv) Mixture (0.8 mg) which was further purified by preparative reversed-phase HPLC (47% water in methanol) to give two fractions.

(a) Aldehyde fragment **20** as an oil (0.3 mg); ν_{max}(film)/cm^{-1} 2963, 2926, 2854, 1741, 1700, 1637, 1450, 1372, 1230, 1093, 1044 and 1027; ^1H NMR data in Supplementary Table 1; DCIMS (NH_3) *m/z* 348 (6%), 347 (13), 346 (MNH_4^+, 100), 315 (11) and 314 ($MNH_4^+ - MeOH$, 27).

(b) *Lactone fragment* **21** as an oil (0.2 mg); ν_{max}(film)/cm^{-1} 2960, 2925, 2853, 1740, 1720, 1665, 1614, 1455, 1371, 1229, 1095 and 1031; ^1H NMR data in Supplementary Table 1 (HRFABMS. MK^+, 353.0987. *M*K, 353.1003).

Preparation of dihydromycalamides A and B

(a) Hydrogen was bubbled through a stirred solution of mycalamide A **1** (5.0 mg) in methanol (0.8 cm^3) containing platinum oxide (2 mg) at 20 °C for 1 h. The resulting solution was filtered through Celite and then evaporated to give the crude product (4.8 mg) which was a 2:1 mixture of dihydromycalamide A epimers. Preparative reversed-phase HPLC (35% water in methanol), recycling central mixtures, gave overall three fractions: (i) *ca.* 90% 4α-*dihydromycalamide* A **23** as an oil (1.4 mg); ^1H and ^{13}C NMR data in Tables 1 and 2 (HRFABMS. MNa^+, 528.2751. *M*Na, 528.2785); (ii) mixture (0.5 mg) of 4α- and 4β-dihydromycalamide A (**23** and **22**); (iii) *ca.* 90% *4β-dihydromycalamide A* **22** as an oil (2.5 mg); ^1H and ^{13}C NMR data in Tables 1 and 2 (HRFABMS. MNa^+, 528.2813. *M*Na, 528.2785).

(b) Similar treatment of mycalamide B **2** (5.5 mg) gave a 3:2 mixture of dihydromycalamide B epimers (5.5 mg). Preparative reversed-phase HPLC as above gave overall three fractions: (i) *ca.* 90% *4α-dihydromycalamide B* **25** as an oil (1.8 mg); ^1H and ^{13}C NMR data in Supplementary Tables 2 and 3 (HRFABMS. $MNa^+ - MeOH$, 510.2725. *M*Na – MeOH, 510.2679); (ii) mixture (0.5 mg) of 4α- and 4β-dihydromycalamide B (**25** and **24**); (iii) *ca.* 90% *4β-dihydromycalamide B* **24** as an oil (2.7 mg); ^1H and ^{13}C NMR data in Supplementary Tables 2 and 3 (HREIMS. $MH^+ - MeOH$, 487.2765. *M*H – MeOH, 487.2781; DCIMS (NH_3) *m/z* 537 (MNH_4^+, 4%), 507 (7), 506 (34), 505 ($MNH_4^+ - MeOH$, 100), 490 (6), 489 (27) and 488 ($MH^+ - MeOH$, 90).

Preparation of Δ^3-mycalamide A and 4α-dihydro-6-deoxy-pseudomycalamide A

Hydrogen was bubbled through a stirred solution of mycalamide A **1** (4.5 mg) in methanol (0.8 cm^3) containing 10% palladium-on-charcoal (4.5 mg) at 20 °C for 5 h. The resulting solution was filtered through Celite and evaporated. Preparative reversed-phase HPLC (40% water in methanol) of the crude product gave six fractions: (i) Δ^3 *mycalamide* A **27** as an oil (0.7 mg); ^1H NMR data in Supplementary Table 2 (HRFABMS. $MH^+ - MeOH$, 472.2548. *M*H – MeOH, 472.2547); (ii)

mycalamide A **1** (0.5 mg); (iii) *4α-dihydro-6-deoxypseudomycalamide* A **26** as an oil (0.7 mg); ^{1}H and ^{13}C NMR data in Tables 1 and 2 (HRFABMS. MH^+, 476.2854. *M*H, 476.2860); (iv) *ca.* 90% 4α-dihydromycalamide A **23** (0.8 mg); (v) mixture (0.4 mg) of 4α- and 4β-dihydromycalamide A (**23** and **22**); (vi) *ca.* 90% 4β-dihydromycalamide A **22** (0.9 mg).

Hydrogenation of Δ^5-mycalamide A over Adams catalyst

Hydrogen was bubbled through a stirred solution of Δ^5-mycalamide A **15** (3.8 mg) in methanol (0.8 cm^3) containing platinum oxide (4 mg) at 20 °C for 8 h. The resulting solution was filtered through Celite and evaporated. Preparative reversed-phase HPLC (43% water in methanol) of the crude product gave five fractions: (i) *Δ^5-4α-dihydromycalamide A* **28** as an oil (0.7 mg); ^{1}H NMR data in Supplementary Table 2 (HRFABMS. MH^+, 474.2721. *M*H, 474.2703); (ii) mixture (0.5 mg) of uncharacterised minor products; (iii) 4α-dihydro-6-deoxypseudomycalamide A **26** (0.6 mg); (iv) mostly 4α-dihydromycalamide A **23** (0.3 mg); (v) mostly 4β-dihydromycalamide A **22** (0.5 mg).

Preparation of epoxide derivatives

(a) A solution of mycalamide A **1** (4.5 mg) and *m*-chloroperbenzoic acid (4.5 mg) in CH_2Cl_2 (0.3 cm^3) was stirred at 20 °C for 1 d after which it was evaporated and the crude product subjected to preparative silica gel TLC [2 × ethanol–ethyl acetate (1 : 19)]. Three bands of silica were recovered and eluted with ethanol–ethyl acetate (1 : 4) to give three fractions: (i) *mycalamide* A *4α-epoxide* **29** as an oil; ^{1}H and ^{13}C NMR data in Supplementary Tables 2 and 3 (HRFABMS. MNa^+, 542.2594. *M*Na, 542.2578); DCIMS (NH_3) *m/z* 537 (MNH_4^+, 10%), 505 (MNH_4^+ − MeOH, 22) and 488 (MH^+ − MeOH, 17); (ii) mixture (0.6 mg) consisting of mostly mycalamide A 4α-epoxide **29**, but containing a trace of mycalamide A 4β epoxide **30**; (iii) *mycalamide* A *4β-epoxide* **30** as an oil; ^{1}H and ^{13}C NMR data in Supplementary Tables 2 and 3 (HRFABMS. MNa^+, 542.2599. *M*Na, 542.2578); DCIMS (NH_3) *m/z* 537 (MNH_4^+, 24%), 505 (MNH_4^+ − MeOH, 23) and 488 (MH^+ − MeOH, 15).

(b) A solution of mycalamide B **2** (4.0 mg) and *m*-chloroperbenzoic acid (6 mg) in CH_2Cl_2 (0.3 cm^3) was stirred at 20 °C for 20 h. The solvent was removed and the crude product subjected to preparative silica gel TLC [2 × ethanol–ethyl acetate (1 : 19)]. Two bands of silica were recovered and eluted with ethanol–ethyl acetate (1 : 4) to give two fractions: (i) *mycalamide* B *4α-epoxide* **31** as an oil; ^{1}H and ^{13}C NMR data in Supplementary Tables 2 and 3 (HRFABMS. MNa^+, 556.2714. *M*Na, 556.2734); DCIMS (NH_3) *m/z* 551 (MNH_4^+, 14%), 519 (MNH_4^+ − MeOH, 23) and 502 (MH^+ − MeOH, 36); (ii) *mycalamide* B *4β-epoxide* **32** as an oil; ^{1}H and ^{13}C NMR data in Supplementary Tables 2 and 3 (HRFABMS. MNa^+, 556.2756. *M*Na, 556.2734); DCIMS (NH_3) *m/z* 551 (MNH_4^+, 17%), 542 (16), 528 (50), 519 (MNH_4^+ − MeOH, 32), 503 (23) and 502 (MH^+ − MeOH, 75).

Preparation of (*E*)- and (*Z*)-Δ^{16}-normycalamide B

A solution of mycalamide B **2** (3.5 mg), potassium carbonate (6 mg) and tris(triphenylphosphine)ruthenium(II) chloride (12 mg) in dry benzene (0.4 cm^3) was stirred at 60 °C for 7 h and then evaporated. The crude product was subjected to preparative silica gel TLC [light petroleum–ethyl acetate (1 : 1)]; three bands of silica were recovered and eluted with ethyl acetate to give three fractions; (i) mixture (1 mg) of reagent-derived material and mycalamide B **2**; (ii) mixture (8 mg) of reagent-derived material and several very minor oxidation products (not characterised); (iii) mixture (2 mg) which was further purified by preparative reversed-phase HPLC (35% water in methanol) to give two major fractions. One of these was (*E*)-*Δ^{16}-normycalamide B* **33** as an oil (0.5 mg); ν_{max}(film)/cm^{-1} 3353, 3065, 2954, 2921, 2849, 1678, 1656, 1529, 1462, 1438, 1378, 1261, 1102, 1072, 1031 and 1012; ^{1}H and ^{13}C NMR data in Supplementary Tables 2 and 3 (HRFABMS. MH^+ − MeOH, 454.2410. *M*H − MeOH, 454.2441). The other fraction was (*Z*)-*Δ^{16}-normycalamide* B **34** as an oil (0.5 mg); ^{1}H NMR data in Supplementary Table 2 (HRFABMS. MH^+ − MeOH, 454.2401. *M*H − MeOH, 454.2441).

Preparation of normycalamide A aldehyde

(a) A solution of mycalamide A **1** (3.5 mg) and tris(triphenylphosphine)ruthenium(II) chloride (5 mg) in dry benzene (0.5 cm^3) was stirred at 20 °C for 2 d and then evaporated. The crude product was subjected to chromatography on silica gel (200 mg). The major fraction, which was eluted with ethyl acetate, was a mixture (6 mg) of reagent-derived material and normycalamide A aldehyde **35** (characterised after its alternative preparation described below). A minor fraction, which eluted with ethanol-ethyl acetate (1 : 19), was a mixture (1.5 mg) of reagent-derived material and mycalamide A **1**.

(b) A solution of mycalamide A **1** (4.0 mg) and lead tetraacetate (10 mg) in pyridine (0.2 cm^3) and benzene (0.2 cm^3) was stirred at 20 °C for 1.5 h after which it was concentrated under nitrogen (to 0.1 cm^3). It was then diluted with water (2 cm^3) and extracted with CH_2Cl_2 (3 × 2 cm^3). Filtration and evaporation of the combined extracts gave *normycalamide A aldehyde* **35** as an oil (3.8 mg); ν_{max}(film)/cm^{-1} 3350, 3065, 2968, 2924, 2852, 1725, 1678, 1527, 1454, 1380, 1260, 1100 and 1033; ^{1}H and ^{13}C NMR data in Tables 1 and 2 (HRFABMS. MH^+ − MeOH, 440.2305. *M*H − MeOH, 440.2284).

Preparation of normycalamide A

A solution of normycalamide A aldehyde **35** (1.5 mg) and sodium borohydride (1.2 mg) in methanol (0.3 cm^3) was stirred at 20 °C for 15 min and then concentrated under nitrogen (to 0.1 cm^3). Water (2 cm^3) was added to the mixture which was then extracted with CH_2Cl_2 (3 × 2 cm^3). Evaporation of the combined extracts gave *normycalamide A* **36** as an oil (1.5 mg); ν_{max}(film)/cm^{-1} 3348, 3072, 2969, 2928, 2882, 1676, 1529, 1453, 1382, 1263, 1107, 1074 and 1035; ^{1}H and ^{13}C NMR data in Tables 1 and 2 (HRFABMS. MH^+ − MeOH, 442.2426. *M*H − MeOH, 442.2441).

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