Chemistry of the Mycalamides, Antiviral and Antitumour Compounds from a Marine Sponge. Part 3.[1,2] Acyl, Alkyl and Silyl Derivatives

Andrew M. Thompson,[a] John W. Blunt,*[,a] Murray H. G. Munro,*[,a] Nigel B. Perry[a] and Lewis K. Pannell[b]
[a] *Department of Chemistry, University of Canterbury, Christchurch, New Zealand*
[b] *NIDDKD, NIH, Bethesda, MD 20892, USA*

Acyl, alkyl and silyl derivatives of the hydroxy and *N*-amido functionalities of the antitumour and antiviral marine sponge metabolites mycalamides A and B have been prepared, their relative reactivities established and *in vitro* P388 leukemia bioassays performed.

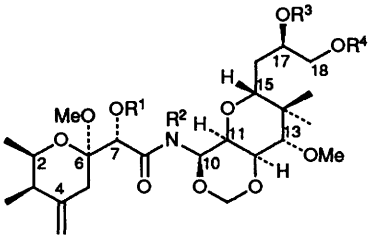
We have recently described the isolation and structures of two potent antiviral and antitumour compounds, mycalamides A[1] **1** and B[2] **2**, from the New Zealand marine sponge *Mycale sp.* The structure of the related compound onnamide A **37** from the Japanese sponge *Theonella sp.* has also been described.[3] These three compounds are related to pederin[4,5] **38** which has been isolated from the terrestrial blister beetle, *Paederus fuscipes.* In view of the biological potency of the mycalamides,[6,7] we have undertaken an extensive study of their chemistry in order to: (i) prepare analogues with increased potency or therapeutically more useful compounds with reduced toxicity; (ii) establish those features of the structure essential for the biological activity; and (iii) establish the conformational features of the active compounds. The potential to incorporate isotopic labels such as deuterium was also a consideration for possible future pharmacokinetic studies. The absolute stereochemistry of the mycalamides has recently been reported.[8] Here we describe the preparation of a range of acyl, alkyl and silyl derivatives of mycalamide, the biological properties of which have been studied.

Results and Discussion

Because of the limited supply of the mycalamides, all reactions have been conducted 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[9] experiments have aided in the assignment of ^{13}C NMR data. Selected NMR data are presented in Tables 1 and 2.

As the mycalamide structure features a number of hydroxy groups in different environments, it was desirable to determine the relative reactivities of these groups to a variety of different reagents.

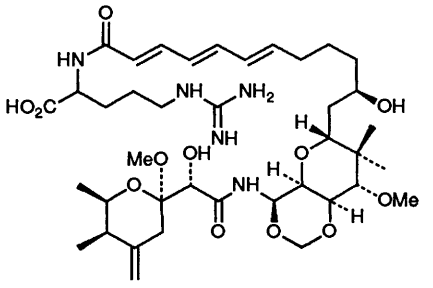
Silylation.—Silylation of mycalamide A **1** and mycalamide B **2** with *N,O*-bistrimethylsilylacetamide yielded the 7,17,18-trisTMS ether **3** and the 7,18-bisTMS ether **4** respectively (HREIMS, ^{1}H NMR) (addition of three and two TMS ether groups respectively, located on the available hydroxy groups, since the NH signal was still present; no rearrangements). Reaction of the trisTMS ether **3** with Bu_4NF gave a quantitative recovery of mycalamide A. This demonstrated the viability of using the TMS ethers as protection for the OH groups, since they could be uncovered under conditions which did not affect



1–36

	R^1	R^2	R^3	R^4
1	H	H	H	H
2	H	H	Me	H
3	$SiMe_3$	H	$SiMe_3$	$SiMe_3$
4	$SiMe_3$	H	Me	$SiMe_3$
5	H	H	Me	$SiMe_3$
6	H	H	$SiMe_3$	$SiMe_3$
7	H	H	H	$SiMe_2CMe_3$
8	H	H	$SiMe_2CMe_3$	$SiMe_2CMe_3$
9	H	H	Me	$SiMeCMe_3$
10	$SiMe_2CMe_3$	H	$SiMe_2CMe_3$	$SiMe_2CMe_3$
11	$SiMe_2CMe_3$	H	Me	$SiMe_2CMe_3$
12	COC_6H_4Br	H	H	H
13	H	H	H	COC_6H_4Br
14	COC_6H_4Br	H	H	COC_6H_4Br
15	H	H	COC_6H_4Br	COC_6H_4Br
16	COC_6H_4Br	H	COC_6H_4Br	COC_6H_4Br
17	COC_6H_4Br	H	Me	H
18	H	H	Me	COC_6H_4Br
19	COC_6H_4Br	H	Me	COC_6H_4Br
20	COMe	H	COMe	COMe
21	COMe	H	Me	COMe
22	H	H	Me	COMe
23	COMe	H	Me	H
24	COEt	H	COEt	COEt
25	Me	Me	H	H
26	Me	Me	H	Me
27	Me	Me	Me	Me
28	Me	Me	Me	H
29	Me	H	Me	Me
30	Me	H	H	Me
31	Me	H	Me	H
32	Me	H	H	H
33	H	H	H	Me
34	COMe	H	Me	Me
35	Me	H	Me	COMe
36	H	H	Me	Me

† Mass spectrometry acronyms: DCEIMS, direct insertion electron impact; DCIMS, direct insertion chemical ionisation; HREIMS, high resolution electron impact; HRFABMS, high resolution fast atom bombardment.



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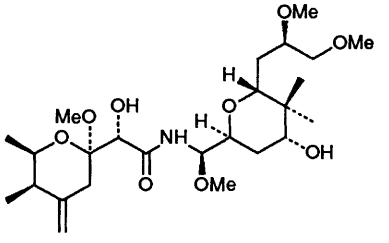

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the rest of the structure. However, it was found that these ethers (and other derivatives noted later) were extremely acid sensitive, *e.g.* when attempts were made to determine their NMR spectra in $CDCl_3$ solutions, decomposition of the compounds occurred, presumably arising from the effect of residual acidity in this solvent.[10] Initially, this problem was avoided by using CD_2Cl_2 as solvent for the NMR studies, but subsequently $CDCl_3$ was utilised routinely with a trace of pyridine included with the solvent.

As all hydroxy groups were protected under these conditions, attempts to obtain selectivity in the reactions with *N,O*-bistrimethylsilylacetamide were made by lowering the temperature of reactions to 0 °C. In this manner, the partially silylated derivatives 18-monoTMS ether **5** and 17,18-bisTMS ether **6** were obtained from mycalamides B and A respectively (HREIMS, ^{1}H NMR) (7-H broadened from coupling with the underivatised 7-OH; D_2O exch.). Some degree of selectivity in the silylation reactions had been achieved, with a preference for reaction at the less hindered 17- and 18-OH functions being displayed. However, the instability of these TMS ethers on storage and on exposure to very mild acid or base made them generally unsuitable for use as protecting groups.

Since *tert*-butyldimethylsilyl (TBDMS) ethers are known to be more stable to acids and bases than the TMS ethers,[11] the reactions of the mycalamides with *tert*-butyldimethylsilyl chloride were explored. The reaction of mycalamide A with this reagent in pyridine with dimethylaminopyridine as catalyst at 20 °C for 3 h gave a mixture of the 18-monoTBDMS **7** and 17,18-bisTBDMS **8** ethers, which were readily separable by chromatography. Similarly, reaction of mycalamide B under these conditions afforded the 18-monoTBDMS ether **9** (HREIMS, HRFABMS, ^{1}H NMR). These results again illustrate the preference for the silylation to occur at the 17- and 18-positions, rather than at the 7-OH group. Silylation with TBDMS chloride at the latter position could be achieved however by reacting each of mycalamides A and B as above, but at 60 °C for 2 days to give the fully silylated derivatives **10** and **11** respectively.

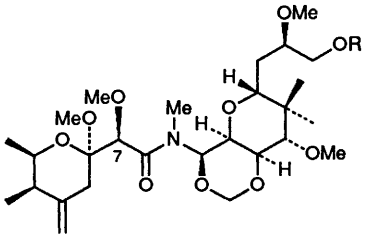
Acylation.—Acylation of the mycalamides with *p*-bromobenzoyl chloride was attempted in order to prepare crystalline compounds suitable for an X-ray crystallographic determination of the absolute stereochemistry of the mycalamides. This approach had earlier been successful for pederin.[12,13] Mycalamide A reacted very slowly with *p*-bromobenzoyl chloride in CH_2Cl_2 with triethylamine as catalyst at 20 °C to give an inseparable (by RP HPLC) mixture of 7-mono-*p*-bromobenzoyl-**12** and 18-mono-*p*-bromobenzoyl mycalamide A **13** (HRFABMS, ^{1}H NMR) [ester at C-18 for **13** deducted from $\Delta\delta_H$ 16-H, 17-H, 18-H; C-7 ester **12** $\Delta\delta_H$ + 1.4 (7-H)]. Acylation of mycalamide A with *p*-bromobenzoyl chloride in pyridine with dimethylaminopyridine as catalyst at 60 °C, gave a separable (by RP HPLC) mixture of 7,18-di-*p*-bromobenzoyl-**14**, 17,18-di-*p*-bromobenzoyl-**15** and 7,17,18-tri-*p*-bromobenzoyl-mycalamide A **16** (HRFABMS, ^{1}H NMR) [7,18-diester **14**, $\Delta\delta_H$ > + 0.6 (7-H, 18-H); 17,18-diester **15**, coupled 7-H–7-OH; 7,17,18-triester, $\Delta\delta_H$ large positive (7-H, 17-H, 18-H)]. Similarly, mycalamide B reacted with *p*-bromobenzoyl chloride in pyridine with dimethylaminopyridine as catalyst at 60 °C for 1 week yielding a separable (RP HPLC) mixture of 7-mono-*p*-bromobenzoyl-**17**, 18-mono-*p*-bromobenzoyl ester-**18** and 7,18-di-*p*-bromobenzoyl ester-**19** (HRFABMS, ^{1}H NMR). Although most of these esters were white solids, none of them could be induced to crystallise in a form suitable for X-ray crystallographic analysis.

Mycalamide A and B *p*-bromobenzoates were not ideal protected intermediates, since not only was their formation slow but, generally, mixtures of several products were obtained which required chromatographic separation; also their cleavage required strong base. Since acetyl derivatisation was used extensively in early studies of pederin,[14] being fast and high yielding, and at the same time giving products that had mild acid stability but were easily removed by bases, mycalamide A was treated with pyridine and acetic anhydride at 20 °C. A single product (TLC) 7,17,18-triacetate **20** (HREIMS, ^{1}H NMR) was readily obtained [δ_H 2.0–2.2 (3 × 3H); 7-H, 17-H, 18-H characteristic acetylation shift *ca.* + 1 ppm]. Similarly, mycalamide B afforded a single product, the 7,18-diacetate **21** (HREIMS, ^{1}H NMR).

The utility of these acetate esters as protecting groups was established by showing that they could be cleanly hydrolysed back to the parent compounds upon treatment with dilute aqueous methanolic K_2CO_3 at 20 °C for 2 h. While monitoring the progress of this reaction on mycalamide B 7,18-diacetate **21** (TLC), it was noticed that a compound of intermediate polarity appeared within minutes, but disappeared as the conversion into mycalamide B became complete. Reaction of the diester **21** was therefore halted after 1 h, and the intermediate product mycalamide B 18-monoacetate **22** (DCIMS, HREIMS, ^{1}H NMR) isolated (TLC) [δ_H 2.08 (3 H); broadened 7-H (7-H–7-OH)]. This selectivity in the removal of the C-7 ester group probably reflects the lower reactivity of primary acetate esters compared to secondary ones, due to steric factors.

In order to extend the range of derivatives for the study of structure/biological activity relationships, the isomeric 7-monoacetate ester of mycalamide B **23** was prepared by treatment of the previously described 18-mono-TMS ether of mycalamide B **5** with Ac_2O in pyridine for 3 h at 20 °C. This afforded a mixture which could be separated to give the 7,18-diacetate **21** and the 7-monoacetate **23** (HRFABMS, ^{1}H NMR). Thus, the TMS ether group was clearly useful in giving a good yield of the desired derivative, although it was not completely stable to the conditions as shown by the presence of up to 25% of the diacetate **21** in the product mixture.

To further extend the range of esters with varying sizes and polarities for the structure/biological activity study, the 7,17,18-tripropanoate ester **24** (HRFABMS, ^{1}H NMR) of mycalamide A was prepared in near-quantitative yield by treatment of mycalamide A with propionic anhydride in pyridine (most δ_H as for 7,17,18-triacetate **20**, except replacement of the three acetyl methyl resonances with complex multiplets in the alkyl region arising from the ethyl groups of the esters).



39 R = Me
40 R = H

Methylation.—Mycalamides A and B have structures similar to that of pederin, and it has been shown that these compounds all have very similar solution conformations.[2,15] The only differences between these structures occur in the substituents at C-10, C-12, C-13, C-17 and C-18. In particular, the interchange of hydroxy and methoxy groups at C-13, C-17 and C-18 in these structures is of interest in view of their similar potent biological activities. In order to further explore the importance of the location of the methyl ethers, a methylation study on mycalamides A and B was undertaken. Initially the method of Johnstone was used because of the mild reaction conditions, rapid reactions and high yields of permethylated products reported for reactions of alcohols and amides at room temperature.[16]

The reaction of mycalamide A with powdered KOH and MeI in dry dimethyl sulfoxide (DMSO) for 4 h at 20 °C gave a mixture of two major products and a third minor product (TLC) but only two resolved peaks were apparent by reverse phase HPLC. Preparative HPLC in fact yielded only one pure fraction, representing the major, most polar product, and a second fraction, which was a mixture of two components in a 4:1 ratio (^{1}H NMR). DCIMS and DEIMS showed that the first fraction consisted of a dimethyl mycalamide A derivative, while the second contained trimethylated compounds. The major product was identified as 7-*O*-methyl-*N*-methylmycalamide A **25** (^{1}H, ^{13}C NMR) [δ_H 3.45 (MeO), 3.19 (MeNCO); no NH; 10-H (d); irradiation of 7-H (difference NOE) gave enhancement the new Me resonances and 6-OMe; δ_C 28.8 (NMe), 58.9 (7-OMe); $\Delta\delta_C$ +9.8 (C-7), +3.6 (C-10), −4.1 (C-11)]. Repetition of the methylation reaction for a longer period yielded (TLC) pure samples of two compounds identified as 7,18-di-*O*-methyl-*N*-methylmycalamide A **26** [δ_C 59.12 (MeO); $\Delta\delta_C$ +1.4 (C-16), −1.9 (C-17), +10.0 (C-18)] and 7,17,18-tri-*O*-methyl-*N*-methylmycalamide A **27** (HREIMS, ^{13}C NMR) [δ_C 56.36 (MeO); $\Delta\delta_C$ +7.7 (C-17), −4.0 (C-18); assignments secured from a HMQC experiment].

Methylation of mycalamide B under similar conditions gave two compounds which were separated by reverse phase HPLC and identified as 7,17-di-*O*-methyl-*N*-methylmycalamide A **28** (HREIMS, NMR) and 7,17,18-tri-*O*-methyl-*N*-methylmycalamide A **27** ($\Delta\delta_C$ C-16, C-17 and C-18 as expected relative to **25**).

The importance of using dry DMSO for the above reactions was shown when a reaction of mycalamide A was carried out as above, but with 'wet' DMSO as solvent. Not only was the reaction slower, but a new product was observed in the product mixture which was separated and found (DCIMS and HREIMS) to be isomeric with the tetramethyl derivative **27**. The differences in the ^{1}H and ^{13}C NMR spectra were associated only with resonances for the C(5)–C(7) region of the structure. This suggested that an enolisation reaction at C(7)–C(8), involving the amide carbonyl group, had occurred, giving rise to the epimeric (7*R*)-7,17,18-tri-*O*-methyl-*N*-methylmycalamide A **39**. This epimerisation could also be effected through treatment of the 7,17-di-*O*-methyl-*N*-methyl derivative **28** with a mixture of MeONa in MeOH in a sealed tube at 90 °C for 3 d to give some (7*R*)-7,17-di-*O*-methyl-*N*-methylmycalamide A **40** after purification.

In an attempt to obtain *O*-methylated derivatives without *N*-methylation, mycalamide A was treated with Ag_2O and MeI in refluxing benzene for 3 d to give a mixture from which the 7,17,18-tri-*O*-methyl **29** and 7,18-di-*O*-methyl **30** mycalamides A were isolated. Also present was a small amount of the 7,17-di-*O*-methyl derivative **35**, although this was not characterised as such until its preparation by a different method described below. A second, shorter (1.5 h) methylation reaction of mycalamide A gave a mixture from which the 7-mono-*O*-methyl **32** and 18-mono-O-methyl **33** mycalamide A derivatives were obtained (HREIMS, NMR) [δ_H (t, 10-H) (Table 1); $\Delta\delta_C$ +6–10 (*C*OMe) (Table 2)].

In order to complete the preparation of all the *O*-methylated derivatives, a reaction of mycalamide B with MeI and Ag_2O in refluxing benzene was carried out.[17] The products from this reaction could not be completely separated, but after acetylation of the mixture with acetic anhydride–pyridine, three products were isolated. These were the previously characterised mycalamide B 7,18-diacetate **21**, together with 17,18-di-*O*-methylmycalamide A 7-acetate **34** and 7,17-di-*O*-methylmycalamide A 18-acetate **35** (HREIMS, NMR). The data for the 7-acetate derivative **34** could be compared with that for the 17-*O*-methyl-7-acetate compound **23** described earlier, to show the addition of an extra methyl group on the 18-OH through the characteristic methylation shift of the C-18 resonance. Hydrolysis of the acetates **34** and **35** with K_2CO_3 in aq. MeOH afforded the remaining methylated derivatives, 17,18-di-*O*-methylmycalamide A **36** and 7,17-di-*O*-methylmycalamide A **31** respectively.

Biological Activity.—With the availability of the wide range of derivatives described above, it was possible to determine some of the features of the mycalamide structure which are essential for determining its biological activity. In Table 3 the activities of all of the derivatives are recorded as IC_{50} values (ng cm^{-3}) against the murine leukemia P388 cell line. For comparison, data are also included for onnamide and pederin. The most noticeable feature of the data is that methylation of the amide nitrogen together with the 7-OH group causes at least a 10^3 fold reduction in the activity. The trimethylsilyl ethers are all as active as the parent compounds, but this is probably because the TMS ethers are unstable and hydrolyse back to the parent compounds in the assay medium. The more stable *tert*-butyldimethylsilyl ethers all show marked reduction in activity relative to the comparably substituted *O*-methyl ethers, an effect which may be attributed to the presence of large, non-polar substituents. Derivatisation of the 7-OH group causes a 10–100 fold reduction in activity, whereas methylation of both the 17- and 18-OH groups (as found in pederin) makes the mycalamides as active as pederin. From these observations it was concluded that the centrally located α-hydroxyamidoacetal functionality is vitally important for the biological activity of the mycalamides.

Experimental

General Procedure.—IR spectra were recorded for chloroform solutions using a Pye Unicam SP3-300 spectrometer. UV spectra were recorded as methanol solutions on a Varian DMS 100 UV/VIS spectrophotometer. Mass spectra were recorded on Finnegan 4500 (DCI/DEI), JEOL SX102 (high resolution) mass spectrometers (NIH), or a Kratos MS80RFA mass spectrometer (UoC). NMR spectra were recorded on a Varian XL300 spectrometer, operating at 300 MHz for ^{1}H and at 75 MHz for ^{13}C nuclei. All samples for examination in

Table 1 ^{1}H NMR data for mycalamide A and its *O*-methyl ethers[a]

H	1	2	29	30	31	32	33	36
2-H	3.98 (2.7, 6.6)	4.02 (2.8, 6.6)	3.91 (2.7, 6.5)	3.89 (2.7, 6.6)	3.94 (2.7, 6.6)	3.90 (2.6, 6.7)	4.00 (2.7, 6.5)	4.05 (2.9, 6.6)
2-Me	1.19 (6.6)	1.19 (6.6)	1.16 (6.6)	1.16 (6.6)	1.17 (6.6)	1.17 (6.6)	1.19 (6.6)	1.21 (6.6)
3-H	2.23 (2.7, 7.0)	2.25 (2.8, 7.0)	2.19 (2.6, 7.2)	2.18 (2.6, 7.2)	2.21 (2.5, 7.0)	2.19 (2.6, 7.0)	2.24 (2.6, 7.1)	2.27 (2.5, 7.2)
3-Me	0.99 (7.0)	1.01 (7.1)	0.97 (7.0)	0.96 (7.2)	0.98 (7.0)	0.96 (7.0)	1.01 (7.1)	1.03 (7.1)
4=CH(*Z*)	4.84 (m)	4.85 (1.9)	4.82 (1.9)	4.81 (1.8)	4.83 (1.9)	4.82 (1.9)	4.84 (1.6)	4.86 (1.8)
4=CH(*E*)	4.72 (m)	4.71 (1.8)	4.71 (1.9)	4.70 (1.9)	4.71 (2.0)	4.71 (1.8)	4.74 (1.5)	4.74 (1.8)
5a-H	2.36 (m)	2.23 (1.9, 14.0)	2.43 (2.0, 14.2)	2.44 (1.9, 14.3)	2.41 (1.9, 14.3)	2.43 (2.0, 14.3)	2.31 (1.7, 14.0)	2.20 (1.9, 14.1)
5e-H	2.36 (m)	2.36 (14.0)	2.30 (14.2)	2.31 (14.5)	2.32 (14.4)	2.32 (14.1)	2.38 (13.9)	2.37 (14.3)
6-OMe	3.29	3.29	3.28	3.28	3.27	3.28	3.30	3.32
7-H/7-OH	4.31	4.28	3.87	3.88	3.89	3.88	4.29	4.28/3.92 (2.5)
7-OMe			3.55	3.54	3.57	3.56		
9-NH	7.51 (9.7)	7.53 (9.6)	7.13 (9.6)	7.13 (9.9)	7.25 (9.7)	7.18 (10.0)	7.49 (9.8)	7.55 (9.5)
10-H	5.87 (9.7)	5.78 (9.6)	5.81 (9.8)	5.90 (10.0)	5.82 (9.7)	5.91 (10.0)	5.85 (9.6)	5.81 (9.6)
10-OCH(*R*)	5.13 (6.9)	5.11 (7.0)	5.13 (7.0)	5.14 (7.0)	5.13 (6.9)	5.14 (6.9)	5.13 (6.9)	5.13 (6.9)
10-OCH(*S*)	4.87 (6.9)	4.84 (6.9)	4.82 (7.0)	4.83 (7.0)	4.85 (7.0)	4.85 (6.9)	4.87 (6.9)	4.86 (6.8)
11-H	3.86 (6.8, 9.7)	3.78 (6.8, 9.7)	3.84 (6.6, 9.9)	3.88 (6.7, 9.8)	3.80 (6.8, 10.0)	3.87 (6.9, 10.0)	3.84 (6.6, 9.6)	3.79 (6.6, 9.6)
12-H	4.22 (6.8, 10.3)	4.20 (6.8, 10.4)	4.21 (6.6, 10.4)	4.23 (6.8, 10.5)	4.23 (6.8, 10.5)	4.24 (6.9, 10.6)	4.21 (6.6, 10.2)	4.20 (6.6, 10.2)
13-H	3.46 (10.3)	3.43 (10.3)	3.44 (10.2)	3.47 (10.7)	3.45 (10.5)	3.48 (10.6)	3.44 (10.1)	3.44 (10.3)
13-OMe	3.55	3.54	3.55	3.55	3.56	3.56	3.55	3.56
14-Me(*R*)	0.87	0.85	0.87	0.88	0.86	0.88	0.87	0.87
14-Me(*S*)	0.97	0.97	0.97	0.97	0.97	0.97	0.99	0.99
15-H	3.62 (5.1, 7.5)	3.41 (3.3, 8.7)	3.32 (m)	3.58 (m)	3.43 (1.7, 10.0)	3.64 (5.1, 6.5)	3.54 (m)	3.37 (m)
16-H	1.54 (m)	1.54 (m)	1.62 (m)	1.60 (m)	1.57 (m)	1.56 (m)	1.60 (m)	1.62 (m)
16-H	1.54 (m)	1.54 (m)	1.62 (m)	1.52 (m)	1.46 (m)	1.56 (m)	1.54 (m)	1.57 (m)
17-H	3.74 (m)	3.20 (m)	3.29 (m)	3.82 (m)	3.22 (m)	3.75 (m)	3.79 (m)	3.24 (m)
17-OMe		3.24	3.29		3.30			3.25
18-H	3.57 (3.5, 11.3)	3.65 (3.3, 11.9)	3.45 (m)	3.30 (4.6, 9.6)	3.69 (3.0, 12.0)	3.60 (3.4, 11.0)	3.32 (4.2, 9.5)	3.46 (2.2, 10.3)
18-H	3.38 (6.2, 11.3)	3.46 (5.7, 11.9)	3.31 (m)	3.26 (5.7, 9.7)	3.45 (m)	3.38 (6.0, 11.0)	3.23 (6.2, 9.6)	3.31 (5.2, 10.3)
18-OMe			3.37	3.35			3.35	3.37

[a] Recorded in $CDCl_3$ with chemical shifts relative to δ_H ($CHCl_3$) 7.25 (coupling constants in Hz).

Table 2 ^{13}C NMR data for mycalamide A and its *O*-methyl ethers[a]

C	1	2	29	30	31	32	33	36
C-2	69.81	69.63	69.39	69.32	69.52	69.40	69.48	69.58
2-Me	17.88	19.94	17.82	17.80	17.85	17.81	17.93	18.01
C-3	41.37	41.27	41.42	41.45	41.36	41.40	41.35	41.30
3-Me	12.02	12.15	11.80	11.73	11.88	11.77	12.07	12.28
C-4	145.65	145.10	146.20	146.45	145.77	146.27	145.50	145.00
4=CH_2	110.55	111.03	110.02	109.79	110.37	109.95	110.71	111.23
C-5	33.75	33.66	34.16	34.20	34.19	34.14	33.72	33.61
C-6	99.83	99.92	99.91	99.97	100.00	100.02	99.85	100.01
6-OMe	48.94	48.59	49.01	49.14	48.98	49.18	48.86	48.56
C-7	72.92	71.75	82.98	83.16	82.39	82.99	72.52	71.45
7-OMe			60.20	60.45	60.39	60.50		
C-8	171.82	171.79	170.06	170.43	170.33	170.56	171.73	171.78
C-10	73.74	73.93	73.11	73.07	73.51	73.22	73.83	73.94
10-OCH_2	86.84	86.49	86.49	86.74	86.61	86.83	86.72	86.47
C-11	71.26	70.94	70.59	71.26	71.41	71.60	70.87	70.62
C-12	74.42	74.44	74.41	74.47	74.64	74.55	74.31	74.33
C-13	79.15	79.30	79.48	79.38	79.27	79.22	79.34	79.43
13-OMe	61.81	61.77	61.80	61.86	61.86	61.89	61.77	61.76
C-14	41.66	41.46	41.57	41.81	41.59	41.81	41.54	41.47
13-Me(*R*)	13.49	13.35	13.45	13.35	13.18	13.24	13.72	13.66
14-Me(*S*)	23.11	23.17	23.18	23.03	23.12	22.98	23.25	23.34
C-15	78.99	75.51	75.81	79.08	75.49	78.99	78.56	75.99
C-16	31.99	29.68	29.83	32.48	29.94	32.03	32.49	29.95
C-17	71.64	78.80	77.66	69.96	79.72	71.65	69.71	—
17-OMe		56.65	56.81		56.87			56.87
C-18	66.49	66.68	72.87	76.19	64.69	63.45	76.05	72.63
18-OMe			59.19	59.10			59.05	59.20

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

deuteriochloroform were normally prepared and stored in the presence of 0.1% pyridine to prevent acid-catalysed decomposition. Chemical shifts are expressed as ppm on the δ scale, relative to the following solvent reference peaks; ^{1}H, $CHCl_3$ δ 7.25; ^{13}C, $CHCl_3$ δ 77.01. Silica gel column chromatography was performed using Davisil, 35-70 μm, 60 Å, typically 200–300 mg in a Pasteur pipette, using solvent gradients starting with light petroleum–ethyl acetate and progressing to mixtures of ethyl acetate–ethanol. Analytical and preparative silica gel TLC was performed on Merck DG P.K.60 F_{254} plates, of 0.2 mm thickness. Visualisation of TLC plates containing mycalamide derivatives was achieved using an anisaldehyde dip reagent

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

Mycalamide	IC_{50}/ng cm^{-3}
1	0.5
2	0.1
37	0.4
38	0.07
Silyl derivatives	
3	1.3
4	0.2
5	0.1
6	1.2
7	28
8	1 200
9	23
10	>12 500
11	600
Benzoate derivatives	
12	23
13	1
14	140
15	57
16	115
17	24
18	20
19	350
Acyl derivatives	
20	45
21	20
22	0.9
23	2
24	85
Alkyl derivatives	
25	1 000
26	1 500
27	2 000
28	2 500
29	85
30	105
31	95
32	80
33	0.1
34	1
35	500
36	0.07
39	5 000

[0.1:1:100 (by volume) anisaldehyde–conc. sulfuric acid–glacial acetic acid) then heating at 120 °C for 3–5 min. The yellow, orange or brown colour obtained for most derivatives could be accentuated by iodine. Reverse phase (C18) analytical and preparative HPLC was performed on a Shimadzu LC-4A instrument, equipped with a LKB Bromma 2142 Differential Refractometer (a refractive index recorder for semi-quantitative detection), using an Alltech Econosphere C18 column, of dimensions 250 mm × 4.6 mm and 5 μm particle size, with the stated mixtures of methanol (AnalaR grade, distilled and filtered) and water (deionised). Solvents were distilled and filtered (or further purified by standard procedures). Microscale reactions were performed in either a 1 or 5 cm^3 reactivial and stirred magnetically. All technical grade solvents having b.p. <80 °C were distilled prior to use, as were pyridine and acetic anhydride. Benzene and DMSO were AnalaR grade, and the latter was usually predried over molecular sieves (type 4A). All other reagents were CP or AnalaR grades and used without further purification, unless stated otherwise. Mycalamides A **1** and B **2** were obtained from a *Mycale sp.* sponge as described previously.[1,2]

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

Trimethylsilylation Reactions of Mycalamides A and B.—(*a*) Mycalamide A **1** (2.5 mg) was stirred with *N,O*-bis-trimethylsilylacetamide (0.1 cm^3) in pyridine (0.1 cm^3) at room temp. overnight. Water (0.3 cm^3) was added and the mixture extracted with CH_2Cl_2 (3 × 0.2 cm^3). Chromatography of the product on silica (200 mg) eluted with light petroleum-ethyl acetate (4:1) gave a fraction of *mycalamide A tris-trimethylsilyl ether* **3** as an oil (3 mg); ^{1}H and ^{13}C NMR data in Supplementary Tables 1 and 2* (HREIMS. M^+ − MeOH, 687.3651. *M* − MeOH, 687.3654).

(*b*) Treatment of mycalamide B **2** (2.6 mg) under identical conditions gave *mycalamide B bis-trimethylsilyl ether* **4** as an oil (3.1 mg); ^{1}H and ^{13}C NMR data in Supplementary Tables 1 and 2 (HREIMS. M^+ − MeOH, 629.3418. *M* − MeOH, 629.3415).

(*c*) Mycalamide B **2** (2.0 mg) was treated with *N,O*-bis-trimethylsilylacetamide (0.1 cm^3) in pyridine (0.1 cm^3) at 0 °C for 5 min. Water (0.5 cm^3) was added and the mixture extracted with CH_2Cl_2 (3 × 0.2 cm^3). Chromatography of the product on silica (200 mg) eluted with light petroleum–ethyl acetate (2:1) gave a fraction of *mycalamide B* 18-*mono-trimethylsilyl ether* **5** as an oil (2.1 mg); ^{1}H and ^{13}C NMR data in Supplementary Tables 1 and 2 (HREIMS. M^+ − MeOH, 557.3022. *M*-MeOH, 557.3020).

(*d*) Identical treatment of mycalamide A **1** (1 mg) gave *mycalamide A* 17,18-*bis-trimethylsilyl ether* **6** as an oil (1.2 mg); ^{1}H NMR data in Supplementary Table 1 (HREIMS. M^+ − MeOH, 615.3263. *M* − MeOH, 615.3259).

Cleavage of Trimethylsilyl Ethers.—Mycalamide A tris-trimethylsilyl ether **3** (0.5 mg) was stirred with Bu_4NF (1 mol dm^{-3}; 1 mm^3) in THF (0.1 cm^3) at room temp. for 20 min. Water (0.5 cm^3) was added and the mixture extracted with CH_2Cl_2 (3 × 0.2 cm^3). The solvent was removed to give the crude organic product, which was almost pure mycalamide A (TLC, ^{1}H NMR).

Preparation of tert-*Butyldimethylsilyl Ethers.*—(*a*) Mycalamide A **1** (1 mg), *tert*-butyldimethylchlorosilane (8 mg), dimethylaminopyridine (1 mg) and triethylamine (5 mm^3) were stirred in pyridine (0.2 cm^3) at room temp. for 3 h. Water (0.5 cm^3) was added and the mixture extracted with CH_2Cl_2 (3 × 0.3 cm^3). Chromatography of the product on silica (200 mg) eluting with light petroleum–ethyl acetate (4:1) gave, as a minor fraction, *mycalamide A* 17,18-*bis*-tert-*butyldimethylsilyl ether* **8** as an oil (0.4 mg); ^{1}H and ^{13}C NMR data in Supplementary Tables 3 and 4 (HRFABMS. MK^+, 770.4145. *M*K, 770.4097). Further elution with light petroleum–ethyl acetate (2:1) gave the major fraction, *mycalamide A* 18-*mono*-tert-*butyldimethylsilyl ether* **7** as an oil (0.8 mg); ^{1}H and ^{13}C NMR data in Supplementary Tables 3 and 4 (HRFABMS. MK^+, 656.3214. *M*K, 656.3232).

(*b*) Mycalamide A **1** (2.6 mg), *tert*-butyldimethylchlorosilane (~20 mg), dimethylaminopyridine (2 mg) and triethylamine (30 mm^3) were stirred in pyridine (0.3 cm^3) at 60 °C for 2 d. The solution was concentrated under nitrogen, water (0.5 cm^3) added and the mixture extracted with CH_2Cl_2 (3 × 0.3 cm^3). Chromatography of the product on silica (200 mg) eluting with light petroleum–ethyl acetate (9:1) gave, as a minor fraction,

* ^{1}H and ^{13}C NMR data (nine tables) are available as a Supplementary publication [Supp. pub. no. 56878 (10 pp.)]. For details of the Supplementary publications scheme see 'Instructions for Authors', *J. Chem. Soc., Perkin Trans. 1*, 1992, Issue 1.

mycalamide A tris-tert-*butyldimethylsilyl ether* **10** as an oil (1.5 mg); ^{1}H and ^{13}C NMR data in Supplementary Tables 3 and 4 (HRFABMS. MH^+ – MeOH, 814.5145. *M*H – MeOH, 814.5141). The major fraction, eluted with light petroleum–ethyl acetate (3:1) was mycalamide A 17,18-bis-*tert*-butyldimethylsilyl ether **8** (2.5 mg).

(*c*) Mycalamide B **2** (3 mg), *tert*-butyldimethylchlorosilane (12 mg), dimethylaminopyridine (1 mg) and triethylamine (20 mm^3) were stirred in pyridine (0.2 cm^3) at room temp. for 20 h. Water (0.5 cm^3) was added and the mixture extracted with CH_2Cl_2 (3 × 0.3 cm^3). Chromatography of the product on silica (200 mg) eluting with light petroleum–ethyl acetate (3:2) gave a fraction of *mycalamide B* 18-*mono*-tert-*butyldimethylsilyl ether* **9** as an oil (1.5 mg): ^{1}H and ^{13}C NMR data in Supplementary Tables 1 and 4 (HRFABMS. MH^+ – MeOH, 600.3535. *M*H$^+$ – MeOH, 600.3568). A second fraction, eluted with ethanol–ethyl acetate (1:19) was mycalamide B **2** (1.7 mg).

(*d*) Mycalamide B **2** (2.8 mg), *tert*-butyldimethylchlorosilane (25 mg), dimethylaminopyridine (6 mg) and triethylamine (40 mm^3) were stirred in pyridine (0.3 cm^3) at 80 °C for 2 d. The solution was concentrated under nitrogen, water (0.5 cm^3) added, and the mixture extracted with CH_2Cl_2 (3 × 0.3 cm^3). The product was chromatographed on silica (200 mg). The methanol-soluble portion of the major fraction, which eluted with light petroleum–ethyl acetate (7:1) was *mycalamide B bis*-tert-*butyldimethylsilyl ether* **11** as an oil (2.5 mg); ^{1}H and ^{13}C NMR data in Supplementary Tables 1 and 4 (HRFABMS. MH^+ – MeOH, 714.4383. *M*H$^+$ – MeOH, 714.4432).

Preparation of p-*Bromobenzoyl Esters.*—(*a*) Mycalamide A **1** (2.7 mg), *p*-bromobenzoyl chloride (5 mg) and triethylamine (4 mm^3) were stirred in CH_2Cl_2 (0.3 cm^3) at room temp. for 24 h. The solvent was removed and the crude product chromatographed on silica (200 mg). Two fractions, eluted with light petroleum–ethyl acetate (1:1) and ethyl acetate, were a mixture of two products and unresolved reagent (TLC). These were combined (4.2 mg) and subjected to preparative reverse phase HPLC (20% water in methanol), giving two fractions. One of these was *mycalamide A* 18-*mono*-p-*bromobenzoate* **13** as an oil or white solid (1.8 mg); λ_{max}(MeOH)/nm 244 (ε/dm^3 mol^{-1} cm^{-1} ~5700); ^{1}H NMR data in Supplementary Table 5 (HRFABMS. MK^+, 724.1709. *M*K, 724.1735). The other fraction was *mycalamide A* 7-*mono*-p-*bromobenzoate* **12** as an oil or white solid (0.7 mg); λ_{max}(MeOH)/nm 245 (ε/dm^3 mol^{-1} cm^{-1} ~6500); ^{1}H NMR data in Supplementary Table 5 (HRFABMS. MH^+ – MeOH, 654.1914. *M*H – MeOH, 654.1914).

(*b*) Mycalamide A **1** (5.0 mg), *p*-bromobenzoyl chloride (11 mg), dimethylaminopyridine (1 mg) and triethylamine (10 mm^3) were stirred in pyridine (0.4 cm^3) at 75 °C overnight. The solution was concentrated under nitrogen, water (0.4 cm^3) was added and the mixture extracted with CH_2Cl_2 (3 × 0.4 cm^3). The solvent was removed to give the crude product (17 mg), which was a mixture of *p*-bromobenzoate esters and reagent (TLC). Preparative reverse phase HPLC (10% water in methanol) gave four fractions. (i) Mycalamide A 18-mono-*p*-bromobenzoate **13** (2 mg). (ii) *Mycalamide A* 7,18-*di*-p-*bromobenzoate* **14** an oil or white solid (1 mg); λ_{max}(MeOH)/nm 245 (ε/dm^3 mol^{-1} cm^{-1} ~21000); ^{1}H NMR data in Supplementary Table 5, ^{13}C NMR data in Supplementary Table 2 except the *p*-bromobenzoate resonances δ_C ($CDCl_3$) 165.72 and 164.77 (7- and 18-OCO), 131.82, 131.74, 131.50, 131.28, 131.14, 129.04, 128.73 and 128.17 (7- and 18-$OCOC_6H_4Br$) (HRFABMS. MK^+, 908.1109. *M*K, 908.1083). (iii) Mycalamide A 17,18-di-p-*bromobenzoate* **15** an oil or white solid (1.2 mg); ^{1}H NMR data in Supplementary Table 5. (iv) *Mycalamide A tri*-p-*bromobenzoate* **16** as an oil or white solid (1.1 mg); ^{1}H NMR data in Supplementary Table 5 (HRFABMS. MH^+ – MeOH – $C_6H_4{}^{79}BrCO_2H$, 820.1175, *M*H – MeOH – $C_6H_4{}^{79}BrCO_2H$, 820.1157); FABMS *m*/*z* 1076 (MNa^+, 5%), 1074 (MNa^+, 5), 1022 (MNa^+ – MeOH, 14), 1021 (8), 1020 (MNa^+ – MeOH, 16), 823 (27), 822 (51), 821 (37), 820 (MH^+ – CH_3OH – $C_6H_4{}^{79}BrCO_2H$, 100), 819 (22) and 818 (50).

(*c*) Mycalamide B **2** (11 mg), *p*-bromobenzoyl chloride (26 mg), dimethylaminopyridine (2 mg) and triethylamine (20 mm^3) were stirred in pyridine (0.6 cm^3) at 55 °C for 1 week. The solution was concentrated under nitrogen (to 0.1 cm^3) then water (0.3 cm^3) was added and the mixture extracted with CH_2Cl_2 (4 × 0.4 cm^3). The solvent was removed to give the crude product (50 mg), which was a mixture of *p*-bromobenzoate esters and reagent (TLC). Preparative reverse phase HPLC (10% water in methanol, then 18% water in methanol) gave four fractions. (i) Mycalamide B **2** (1.2 mg). (ii) *Mycalamide B* 7-*mono*-p-*bromobenzoate* **17** as an oil or white solid (0.8 mg); ^{1}H NMR data in Supplementary Table 3 (HRFABMS. MK^+, 738.1874. *M*K, 738.1892). (iii) *Mycalamide B* 18-*mono*-p-*bromobenzoate* **18** as an oil or white solid (5.2 mg); ^{1}H NMR data in Supplementary Table 3, ^{13}C NMR data in Supplementary Table 2 except the *p*-bromobenzoate resonances δ_C ($CDCl_3$) 164.56 (18-OCO), 131.78, 131.14, 129.03 and 128.18 (18-$OCOC_6H_4Br$) (HRFABMS. MNa^+, 722.2155. *M*Na, 722.2152). (iv) *Mycalamide B di*-p-*bromobenzoate* **19** as an oil or white solid (2.4 mg); ^{1}H NMR data in Supplementary Table 3, ^{13}C NMR data in Supplementary Table 2 except the *p*-bromobenzoate resonances δ_C ($CDCl_3$) 165.51, 164.71 (7- and 18-OCO), 131.91, 131.69, 131.41, 131.14, 129.16, 128.71, 128.15, 128.01 (7- and 18-$OCOC_6H_4Br$) (HRFABMS. MH^+ – MeOH, 852.1414. *M*H – MeOH, 852.1419); DCIMS (NH_3) *m*/*z* 872 (15%), 871 (55), 870 (32), 869 (MNH_4^+ – MeOH, 100), 868 (16), 867 (51), 854 (17), 853 (8), 852 (MH^+ – MeOH, 52) and 850 (18).

Preparation of Acetate Esters.—(*a*) A solution of mycalamide A **1** (6.5 mg) in pyridine (0.1 cm^3) and acetic anhydride (0.1 cm^3) was stirred at room temp. overnight. Water (2 cm^3) was added and the mixture extracted with CH_2Cl_2 (3 × 2 cm^3). The solvent was removed to give *mycalamide A triacetate* **20** as an oil (8 mg); ν_{max}/cm^{-1} ($CHCl_3$) 3380, 2940, 2870, 1740, 1705, 1570, 1375, 1110, 1100 and 1030; ν_{max}/cm^{-1} (film) 3370, 3070, 2954, 2925, 2855, 1741, 1678, 1588, 1522, 1463, 1368, 1230, 1145 and 1034; ^{1}H and ^{13}C NMR data in Supplementary Tables 5 and 2 (HREIMS. M^+ – MeOH, 597.27536. *M* – MeOH, 597.2785); DCIMS (NH_3) *m*/*z* 647 (MNH_4^+, 13%), 617 (29), 616 (37), 615 (MNH_4^+ – MeOH, 100), 542 (35), 318 (42), 317 (44), 299 (30), 286 (58), 285 (41), 270 (28), 269 (60) and 257 (30); DCIMS (CH_4) *m*/*z* 598 (MH^+ – MeOH, 71%), 538 (MH^+ – MeOH – $MeCO_2H$, 100), 299 (53), 269 (56), 240 (41) and 208 (55).

(*b*) A solution of mycalamide B **2** (2.0 mg) in pyridine (0.1 cm^3) and acetic anhydride (0.1 cm^3) was stirred at room temp. for 5 h. Water (0.5 cm^3) was added and the mixture extracted with $CHCl_3$ (3 × 0.3 cm^3). The solvent was removed to give *mycalamide B diacetate* **21** as an oil (2.5 mg); ν_{max}($CHCl_3$)/cm^{-1} 3390, 2940, 2890, 1740, 1710, 1380, 1125, 1100, 1030 and 910; ^{1}H and ^{13}C NMR data in Supplementary Tables 6 and 7 (HREIMS. M^+ – MeOH, 569.2848. *M* – MeOH, 569.2836) (HRFABMS. MNa^+, 624.2973. *M*Na, 624.2996); DCIMS (NH_3) *m*/*z* 619 (MNH_4^+, 22%), 589 (16), 588 (32), 587 (MNH_4^+ – MeOH, 100), 570 (MH^+ – MeOH, 25), 318 (19), 290 (25), 258 (62), 257 (26) and 241 (45).

(*c*) Mycalamide B 18-mono-trimethylsilyl ether **5** (1.2 mg) was dissolved in pyridine (0.1 cm^3) and acetic anhydride (0.1 cm^3) and stirred for 3 h at room temp. Water (0.4 cm^3) was added and the mixture extracted with CH_2Cl_2 (3 × 0.3 cm^3). Removal of the solvent gave a 3:1 mixture (1.2 mg) of

mycalamide B 7-monoacetate **23** and mycalamide B diacetate **21** (TLC, ^{1}H NMR). Chromatography of this mixture on silica (200 mg), and elution with ethyl acetate gave, as the major fraction, *mycalamide B 7-monoacetate* **23** as an oil (0.8 mg); ^{1}H and ^{13}C NMR data in Supplementary Tables 6 and 7 (HRFABMS. MH^+ – MeOH, 528.2836. *M*H – MeOH, 528.2808); DCIMS (NH_3) *m/z* 577 (MNH_4^+, 15%), 547 (28), 546 (30), 545 (MNH_4^+ – MeOH, 100), 530 (10), 529 (11) and 528 (MH^+ – MeOH, 34).

Hydrolysis of Acetates **20** *and* **21**.—(*a*) Mycalamide A triacetate **20** (0.5 mg) was stirred with K_2CO_3 (0.2 mg) in methanol–water (9:1) (0.2 cm^3) at room temp. for 2 h. Silica gel TLC indicated complete hydrolysis to mycalamide A.

(*b*) Mycalamide B diacetate **21** (2.2 mg) was stirred with K_2CO_3 (0.3 mg) in methanol–water (9:1) (0.3 cm^3) at room temp. for 12 h. The solution was concentrated under nitrogen (to 0.1 cm^3), water (0.5 cm^3) was added and the mixture extracted with CH_2Cl_2 (3 × 0.4 cm^3). The solvent was removed to give 2 mg of *ca.* 90% mycalamide B **2** by TLC and ^{1}H NMR spectroscopy, containing a small amount of a mycalamide B monoacetate.

(*c*) Mycalamide B diacetate **21** (2.5 mg) was stirred with K_2CO_3 (0.5 mg) in methanol–water (9:1) (0.3 cm^3) at room temp. for 1 h. Water (2.5 cm^3) was added and the mixture extracted with CH_2Cl_2 (3 × 2 cm^3). The solvent was removed and the crude organic products subjected to preparative silica gel TLC (ethyl acetate). Two bands of silica were recovered (R_f 0.2 and 0.7) and eluted with ethanol–ethyl acetate (1:3) to give two fractions, pure mycalamide B (0.5 mg) and *mycalamide B 18-monoacetate* **22** as an oil (1.7 mg); ^{1}H and ^{13}C NMR data in Supplementary Tables 6 and 7 (HREIMS. M^+ – MeOH, 527.2749. *M* – MeOH, 527.2730); DCIMS (NH_3) *m/z* 577 (MNH_4^+, 1%), 560 (MH^+, 1), 547 (15), 546 (35), 545 (MNH_4^+ – MeOH, 100), 530 (16), 529 (27) and 528 (MH^+ – MeOH, 88).

Preparation of Propanoate Ester **24**.—A solution of mycalamide A (2.8 mg) in pyridine (0.1 cm^3) and propionic anhydride (0.1 cm^3) was stirred at room temp. overnight. Water (0.4 cm^3) was added and the mixture extracted with CH_2Cl_2 (3 × 0.4 cm^3). The solvent was removed to give *mycalamide A tripropanoate* **24** as an oil (3.7 mg); ^{1}H NMR data in Supplementary Table 6, together with propanoate resonances $\delta_H(CDCl_3)$ 2.50, 2.48 (2 × qd, *J* 7.5, 16.5, 7-$OCOCH_2$), 2.32 and 2.25 (2 × q, *J* 7.5, 17- and 18-$OCOCH_2$), 1.19, 1.12 and 1.07 (7- and 18-$OCOCH_2CH_3$); ^{13}C NMR data in Supplementary Table 2, except propanoate resonances δ_C ($CDCl_3$) 173.95, 173.21 and 173.12 (7-, 17- and 18-OCO), 27.55, 27.46 and 27.34 (7-, 17- and 18-$OCOCH_2$), 9.11, 9.02 and 8.90 (7-, 17- and 18-$OCOCH_2CH_3$) (HRFABMS. MNa^+, 694.3356. *M*Na, 694.3414); DCIMS (NH_3) *m/z* 689 (MNH_4^+, 20%), 657 (MNH_4^+ – MeOH, 24), 404 (23), 346 (52), 331 (44), 314 (28) and 244 (85).

Preparation of N,O-*Methyl Derivatives*.—(*a*) A solution of mycalamide A **1** (3 mg), powdered KOH (3 mg) and MeI (3 mm^3) in DMSO (0.2 cm^3) was stirred at room temp. for 4 h. Water (2 cm^3) was added and the mixture extracted with CH_2Cl_2 (3 × 2 cm^3). Preparative reverse phase HPLC (30% water–methanol) of the crude product (3.5 mg) gave two fractions. (i) 7-O-*Methyl*-N-*methylmycalamide A* **25** as an oil (1.9 mg); ^{1}H and ^{13}C NMR data in Supplementary Tables 8 and 9 (HREIMS. M^+ – MeOH, 499.2798. *M* – MeOH, 499.2781); DCIMS (NH_3) *m/z* 549 (MNH_4^+, 3%), 519 (6), 518 (20), 517 (MNH_4^+ – MeOH, 66), 502 (10), 501 (29) and 500 (MH^+ – MeOH, 100. (ii) Mixture (4:1) (1 mg) of two compounds obtained in larger quantities as described below, and subsequently characterised as 7,18-di-*O*-methyl-*N*-methylmycalamide A **26** and 7,17-di-*O*-methyl-*N*-methylmycalamide A **28**.

(*b*) A solution of mycalamide A **1** (5.5 mg), powdered KOH (14 mg) and MeI (10 mm^3) in DMSO (0.3 cm^3) was stirred at room temp. for 3.5 h. Water was added (0.5 cm^3) and the mixture transferred onto a reverse phase column (200 mg C18, equilibrated to water), which was then flushed with water (6 cm^3) and eluted with MeOH (6 cm^3). The methanol fraction was evaporated to dryness (5.5 mg), subjected to preparative silica gel TLC (ethyl acetate). Two bands of silica were recovered and eluted with ethanol–ethyl acetate (1:9) (4 cm^3) to give two fractions. (i) 7,18-*Di*-O-*methyl*-N-*methylmycalamide A* **26** as an oil (1 mg); ^{1}H and ^{13}C NMR data in Supplementary Tables 8 and 9 (HREIMS. M^+ – MeOH, 513.2928. *M* – MeOH, 513.2938); DCIMS (NH_3) *m/z* 563 (MNH_4^+, 10%), 533 (19), 532 (37), 531 (MNH_4^+ – MeOH, 100), 516 (17), 515 (32) and 514 (MH^+ – MeOH, 95). (ii) 7,17,18-*Tri*-O-*methyl*-N-*methylmycalamide A* **27** as an oil (3.5 mg); ^{1}H and ^{13}C NMR data in Supplementary Tables 8 and 9 (HREIMS. M^+ – MeOH, 527.3068. *M* – MeOH, 527.3094); DCIMS (NH_3) *m/z* 577 (MNH_4^+, 1%), 547 (4), 546 (6), 545 (MNH_4^+ – MeOH, 19), 531 (5), 530 (19), 529 (30) and 528 (MH^+ – MeOH, 100).

(*c*) A solution of mycalamide B **2** (2 mg), powdered KOH (2 mg) and MeI (2 mm^3) in DMSO (0.3 cm^3) was stirred at room temp. for 24 h. Water was added (0.5 cm^3) and the mixture extracted with CH_2Cl_2 (3 × 0.3 cm^3) to give a mixture of two compounds (TLC) (3 mg). Preparative reverse phase HPLC (30% water–methanol) gave two fractions which were 7,17,18-tri-*O*-methyl-*N*-methylmycalamide A **27** (0.6 mg) and 7,17-*di*-O-*methyl*-N-*methylmycalamide A* **28** as an oil (1.2 mg); ^{1}H and ^{13}C NMR data in Supplementary Tables 8 and 9 (HREIMS. M^+ – MeOH, 513.2945. *M* – MeOH, 513.2938); DCIMS (NH_3) *m/z* 563 (MNH_4^+, 2%), 533 (9), 531 (MNH_4^+ – MeOH, 34), 516 (25) and 514 (MH^+ – MeOH, 100).

(*d*) A solution of mycalamide A **1** (4 mg), powdered KOH (7.2 mg) and MeI (5 mm^3) in non-dried DMSO (0.3 cm^3) was stirred at room temp. for 20 h, then at 40 °C for 4 h. Water (0.5 cm^3) was added, and the mixture transferred onto a reverse phase column (200 mg C18, equilibrated to water), which was then flushed with water (6 cm^3) and eluted with methanol (6 cm^3). The methanol fraction (4.3 mg) was separated by preparative silica gel TLC (ethyl acetate) into three fractions which were 7,18-di-*O*-methyl-*N*-methylmycalamide A **26** (0.5 mg), 7,17,18-tri-*O*-methyl-*N*-methylmycalamide A **27** (1.5 mg) and (7R)-7,17,18-*tri*-O-*methyl*-N-*methylmycalamide A* **39** as an oil (1 mg); ^{1}H and ^{13}C NMR data in Supplementary Tables 8 and 9 (HREIMS. M^+ – MeOH, 527.3078. *M* – MeOH, 527.3094); DCIMS (NH_3) *m/z* 577 (MNH_4^+, 8%), 547 (15), 546 (30), 545 (MNH_4^+ – MeOH, 88), 530 (19), 529 (37) and 528 (MH^+ – MeOH, 100).

(*e*) A solution of 7,17-di-*O*-methyl-*N*-methylmycalamide A **28** (1.2 mg) in sodium methoxide–methanol (1 mol dm^{-3}; 0.3 cm^3) was stirred at 90 °C for 3 d. The solvent was removed and the residue partitioned in water–chloroform (1:1) (4 cm^3) followed by extraction with $CHCl_3$ (3 × 1 cm^3). Chromatography of the extract on silica (200 mg) yielded, as the major fraction, eluted with ethanol–ethyl acetate, (1:19) a 1:3 mixture (0.9 mg) of starting material **28** and its epimer at C-7 by ^{1}H NMR spectroscopy. Further mixtures of the two epimers were combined (2 mg), reacted as above, then subjected to preparative silica gel TLC (2 × ethyl acetate). Two fractions obtained were 7,17-di-*O*-methyl-*N*-methylmycalamide A **28** (0.6 mg) and (7R)-7,17-*di*-O-*methyl*-N-*methylmycalamide A* **40** as an oil (1.2 mg); ^{1}H and ^{13}C NMR data in Supplementary Tables 8 and 9 (HREIMS. M^+ – MeOH, 513.2960. *M* – MeOH, 513.2938); DCIMS (NH_3) *m/z* 563 (MNH_4^+, 3%), 533 (5), 532 (13), 531 (MNH_4^+ – MeOH, 45), 516 (14), 515 (28) and 514 (MH^+ – MeOH, 100).

Preparation of O-*Methyl Derivatives*.—(*a*) A solution of mycalamide A **1** (4.2 mg), Ag_2O (25 mg) and MeI (8 mm^3) in PhH (0.3 cm^3) was stirred at 80 °C for 3 d. Preparative TLC of the product (4.6 mg) (silica gel developed in ethyl acetate) gave three fractions each of which was individually rechromatographed by preparative silica gel TLC to give the pure compounds. Fraction 1 was 7,17,18-*tri*-O-*methylmycalamide A* **29** as an oil (2.0 mg); ^1H and ^{13}C NMR data in Tables 1 and 2 (HREIMS. M^+ − MeOH, 513.2916. *M* − MeOH, 513.2938); DCIMS (NH_3) *m/z* 563 (MNH_4^+, 10%), 533 (18), 532 (37), 531 (MNH_4^+ − MeOH, 100), 516 (11), 515 (22) and 514 (MH^+ − MeOH, 74). Fraction 2 was 7,18-*di*-O-*methylmycalamide A* **30** as an oil (1.2 mg); ^1H and ^{13}C NMR data in Tables 1 and 2 (HREIMS. M^+ − MeOH, 499.2779. *M* − MeOH, 499.2781); DCIMS (NH_3) *m/z* 549 (MNH_4^+, 7), 519 (22), 518 (30), 517 (MNH_4^+ − MeOH, 100), 502 (11), 501 (16) and 500 (MH^+ − MeOH, 53). Fraction 3 was 7,17-di-*O*-methylmycalamide A **31** (0.5 mg) which was subsequently characterised after its alternative preparation described below.

(*b*) A solution of mycalamide A **1** (7 mg), Ag_2O (55 mg) and MeI (9 mm^3) in PhH (0.4 cm^3) was stirred in a sealed vial at 95 °C for 1.5 h. Preparative TLC of the product (7.3 mg) [silica gel, 2 × light petroleum–ethyl acetate (1 : 7)] gave four fractions. Fraction 1 was mycalamide A **1** (0.8 mg). Fraction 2 was subjected to further preparative silica gel TLC [3 × light petroleum–ethyl acetate (1:5)] to give 7-O-*methylmycalamide A* **32** as an oil (1.2 mg); ^1H and ^{13}C NMR data in Tables 1 and 2 (HREIMS. M^+ − MeOH, 485.2618. *M* − MeOH, 485.2625); DCIMS (NH_3) *m/z* 535 (MNH_4^+, 10%), 505 (28), 504 (28), 503 (MNH_4^+ + MeOH, 100), 488 (12), 487 (9) and 486 (MH^+ − MeOH, 30). Fraction 3 was a 3:2 mixture (2.0 mg) of 17-*O*-methylmycalamide A **2** and 18-*O*-methylmycalamide A **33**, while fraction 4 was pure 18-O-*methylmycalamide A* **33** as an oil (2.8 mg); ^1H and ^{13}C NMR data in Tables 1 and 2 (HREIMS, M^+ − MeOH, 485.2617. *M*-MeOH, 485.2625); DCIMS (NH_3) *m/z* 535 (MNH_4^+, 16%), 505 (24), 504 (33), 503 (MNH_4^+ − MeOH, 100), 487 (19) and 486 (MH^+ − MeOH, 66).

(*c*) A solution of mycalamide B **2** (6 mg), Ag_2O (40 mg) and MeI (12 mm^3) in PhH (0.4 cm^3) was stirred in a sealed vial at 90 °C for 2 h. The product was dissolved in pyridine (0.1 cm^3) and acetic anhydride (0.1 cm^3) and the mixture stirred at room temp. for 16 h. Water (2.5 cm^3) was added and the mixture extracted with CH_2Cl_2 (3 × 2 cm^3). Preparative TLC the product [silica gel developed in light petroleum–ethyl acetate (1:3)] gave three fractions. Fraction 1 was 7,17-*di*-O-*methylmycalamide A* 18-*monoacetate* **35** as an oil (1.7 mg); ^1H and ^{13}C NMR data in Supplementary Tables 6 and 7 (HREIMS. M^+ − MeOH, 541.2881. *M* − MeOH, 541.2887); DCIMS (NH_3) *m/z* 591 (MNH_4^+, 2%), 562 (9), 561 (34), 560 (32), 559 (MNH_4^+ − MeOH, 100), 545 (11), 544 (36), 543 (30) and 542 (MH^+ − MeOH, 99). Fraction 2 was 17,18-*di*-O-*methylmycalamide A* 7-*monoacetate* **34** as an oil; ^1H and ^{13}C NMR data in Supplementary Tables 6 and 7 (HREIMS. M^+ − MeOH, 541.2866. *M* − MeOH, 541.2887); DCIMS (NH_3) *m/z* 592 (10%), 591 (MNH_4^+, 20), 561 (26), 560 (33), 559 (MNH_4^+ − MeOH, 100), 544 (10), 543 (14) and 542 (MH^+ − MeOH, 43). Fraction 3 was mycalamide B diacetate **21** (2.4 mg). 7,17-Di-*O*-methylmycalamide A 18-monoacetate **35** (1.8 mg) was stirred with K_2CO_3 (1.2 mg) in methanol–water (9:1) (0.4 cm^3) at room temp. for 5 h. The solution was concentrated under nitrogen (to 0.1 cm^3), water (2.5 cm^3) was added and the mixture extracted with CH_2Cl_2 (3 × 2 cm^3). Preparative TLC of the product [silica gel 2 × light petroleum–ethyl acetate (1:5)] gave 7,17-*di*-O-*methylmycalamide A* **31** as an oil (1.2 mg); ^1H and ^{13}C NMR data in Tables 1 and 2 (HREIMS. (M^+ − MeOH, 499.2794. *M* − MeOH, 499.2781), DCIMS (NH_3) *m/z* 549 (MNH_4^+, 6%), 520 (4), 519 (20), 518 (40), 517 (MNH_4^+ − MeOH, 100), 502 (13), 501 (29) and 500 (MH^+ − MeOH, 79). 17,18-Di-O-methylmycalamide A 7-monoacetate **34** (2 mg) was stirred with K_2CO_3 (0.4 mg) in methanol–water (9:1) (0.4 cm^3) at room temp. for 1.5 h. The solution was concentrated under nitrogen (to 0.1 cm^3), water (2.5 cm^3) was added and the mixture extracted with CH_2Cl_2 (3 × 2 cm^3) to give pure 17,18-*di*-O-*methylmycalamide A* **36** as an oil (1.8 mg); ^1H and ^{13}C NMR data in Tables 1 and 2 (HREIMS. (M^+ − MeOH, 499.2773. *M* − MeOH, 499.2781); DCIMS (NH_3) *m/z* 549 (MNH_4^+, 7%), 520 (6), 519 (23), 518 (29), 517 (MNH_4^+ − MeOH, 99), 503 (5), 502 (19), 501 (29) and 500 (MH^+ − MeOH, 100).

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References

1 Part 1, N. B. Perry, J. W. Blunt, M. H. G. Munro and L. K. Pannell, *J. Am. Chem. Soc.*, 1988, **110**, 4850.
2 Part 2, N. B. Perry, J. W. Blunt, M. H. G. Munro and A. M. Thompson, *J. Org. Chem.*, 1990, **55**, 223.
3 S. Sakemi, T. Ichiba, S. Kohmoto, G. Saucy and T. Higa, *J. Am. Chem. Soc.*, 1988, **110**, 4851.
4 C. Cardani, D. Ghiringhelli, R. Mondelli and A. Quilico, *Tetrahedron Lett.*, 1965, 2537.
5 C. Cardani, D. Ghiringhelli, A. Quilico and A. Selva, *Tetrahedron Lett.*, 1967, 4023.
6 N. S. Burres and J. J. Clement, *Cancer Res.*, 1989, **49**, 2935.
7 A. Brega, A. Falaschi, L. De Carli and M. Pavan, *J. Cell Biol.*, 1968, **36**, 485.
8 C. Y. Hong and Y. Kishi, *J. Org. Chem.*, 1990, **55**, 4242.
9 A. Bax and M. F. Summers, *J. Am. Chem. Soc.*, 1986, **108**, 2093.
10 A. T. Chapman, *J. Am. Chem. Soc.*, 1935, **57**, 419.
11 E. J. Corey and A. Venkateswarlu, *J. Am. Chem. Soc.*, 1972, **94**, 6190.
12 A. Furusaki, T. Watanabe, T. Matsumoto and M. Yanagiya, *Tetrahedron Lett.*, 1968, 6301.
13 A. Bonamartini Corradi, A. Mangia, M. Nardelli and G. Pelizzi, *Gazz. Chim. Ital.*, 1971, **101**, 591.
14 C. Cardani, D. Ghiringhelli, R. Mondelli and A. Quilico, *Gazz. Chim. Ital.*, 1966, **96**, 3.
15 N. B. Perry, J. W. Blunt, M. H. G. Munro, A. M. Thompson, unpublished results.
16 R. A. W. Johnstone and M. E. Rose, *Tetrahedron*, 1979, **35**, 2169.
17 G. C. Hopkins, J. P. Jonak, H. J. Minnemeyer and H. Tieckelmann *J. Org. Chem.*, 1987, **32**, 4040.

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