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Mupirocin F: structure elucidation, synthesis and rearrangements

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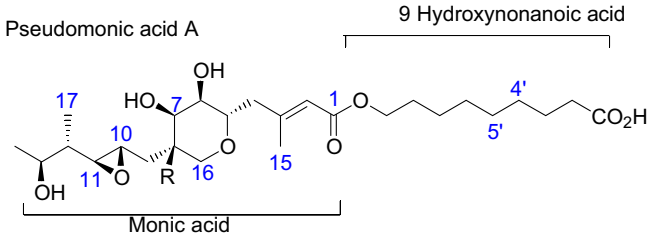
ABSTRACT

The structures of two novel metabolites, mupirocins F and F2, from extracts of the *mupF* mutant of *Pseudomonas fluorescens* were elucidated by spectroscopic methods. Methyl mupirocin F was synthesised from the triol methyl pseudomonate A by selective oxidation of the 7-hydroxyl group thus firmly establishing the structure of the natural product. Rearrangement of the densely functionalised skeleton led to unusual bicyclic and tricyclic products.

1. Introduction

The clinically important antibiotic mupirocin (major component being pseudomonic acid A, Fig. 1) is produced by the bacterium *Pseudomonas fluorescens*. It is used clinically as a topical antibiotic and has potent activity against *Staphylococcus aureus*, including MRSA.[1]

The pseudomonic acids are polyketide derived natural products and comprise a C_{17} unsaturated tetrahydropyran moiety (monic acid) linked to a C_9 saturated fatty acid (9-hydroxynonanoic acid). The presence of the 10,11-epoxide renders pseudomonic acid A unstable outside the range pH 4–9 leading to the formation of the rearrangement products **1** and **2** via intramolecular attack of the 7-hydroxyl onto the epoxide (Fig. 2).[2]



Pseudomonic acid A	R = H
Pseudomonic acid B	R = OH
Pseudomonic acid C	R = H, olefin C-10,11
Pseudomonic acid D	R = H, olefin C-4', 5'

Fig. 1. The pseudomonic acids.

The mupirocin gene cluster was one of the first *trans*-AT polyketide synthase containing clusters to be sequenced.[3] Extensive mutagenesis experiments have led to the isolation of a number of novel compounds and a better, although still incomplete, understanding of pseudomonic acid biosynthesis.[4] *MupF* encodes a putative ketoreductase acting late in the biosynthetic pathway. Interestingly a novel compound was isolated from cultures of *P. fluorescens* in which the *mupF* gene had been deleted and was named mupirocin F **3**.[5] Herein we report the structure elucidation of mupirocin F using spectroscopic methods and synthesis to firmly establish the structure. During the manipulation of these densely functionalised molecules rearrangements were observed leading to novel bicyclic and tricyclic compounds.

2. Results and discussion

2.1. Isolation and structure elucidation of mupirocins F and F2

Cultures of the *mupF* mutant of *P. fluorescens* were harvested after 50 h. HPLC analysis of the crude extract showed that no pseudomonic acid A was produced but a number of metabolites were apparent, some in only trace amounts. High resolution ESIMS

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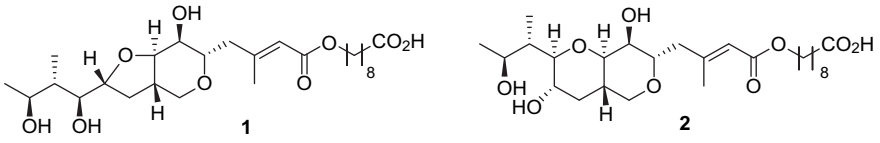


Fig. 2. Rearrangement products of pseudomonic acid A.

of one of the metabolites (4.0 mg from 1 L culture) gave a molecular formula of $C_{26}H_{24}O_9$, 2 amu less than pseudomonic acid A. Examination of the 1D and 2D NMR data revealed that it was closely related to pseudomonic acid A but contained a ketonic group (δ_C 209.3); the HMBC data indicated that this ketone was located at C-7. Hence the novel metabolite was assigned the structure **3**, and named mupirocin F (Fig. 3). The fermentation was repeated and on this occasion the crude extract was dissolved in methanol and treated with TMS diazomethane prior to purification, giving methyl mupirocin F **4** (5.0 mg from 1 L culture).

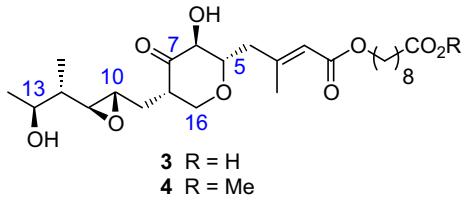


Fig. 3. Mupirocin F **3** and methyl mupirocin F **4**.

It was found that both mupirocin F **3** and methyl ester **4** were unstable if left for several weeks at room temperature giving a new product in each case. High resolution ESIMS established the same molecular formula for the new compounds as the parents ($C_{26}H_{42}O_9$ for mupirocin F **3** and $C_{27}H_{44}O_9$ for methyl mupirocin F **4**). From the chemical shifts of the signals assigned to 10-H and 11-H (δ 2.81, m and δ 2.65, dd, J 8, 2 cf. methyl mupirocin F δ 2.73, m and δ 2.70 dd, J 8, 2) it was concluded that the epoxide was still intact in the isomer. However there were significant differences in the vicinal coupling constants of 16-H_2 to 8-H (δ 4.33, dd, J 11, 7 and δ 3.38, app. t, J 11) compared with the parent **4** (δ 4.11, dd, J 12, 1 and δ 3.72, dd, J 12, 3) in accord with epimerisation having taken place at C-8 to give **5**. The structure was confirmed by 1D ROESY NMR experiments, which showed a key correlation between 6-H and 8-H indicating that both are on the same side of the THP ring and no ROE correlation between 5-H and 6-H in accord with their *anti* relationship. The formation of **5** can be rationalised via enolisation of the 7-ketone to give the side-chain at C-8 in a more favourable pseudoequatorial position rather than the pseudoaxial position in mupirocin F (Scheme 1).

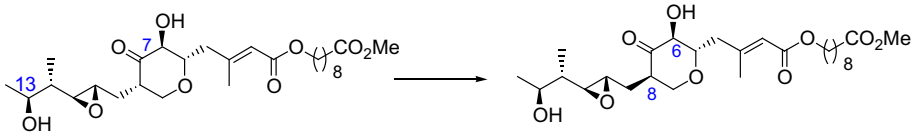
Further analysis of the extract of the *mupF* mutant of *P. fluorescens* led to isolation of a further new metabolite (22 mg/L). High resolution ESIMS established a molecular formula of $C_{26}H_{42}O_9$ for this more polar metabolite indicating an isomer of **3**. On examination of 1 and 2D NMR data the most striking differences compared with **3** were the lack of ketone and epoxide resonances. A carbon signal at 92.2 indicated the presence of an acetal or hemiacetal. The COSY data and the multiplicity of 5-H (doublet of doublets) showed that it was the end of a spin system with a quarternary carbon at C-6, suggesting that the hemiacetal is located at C-6. An HMBC correlation to this carbon was observed at 2.32 ppm, which corresponds to either 4-H or 8-H (the resonances overlap). The absence of epoxide resonances and the presence of two further R_2CHO resonances compared with mupirocin F (δ_H 3.89 and 4.25, δ_C 77.80 and 77.84) combined with all the 1D and 2D data were in accord with the tricyclic structure **7**, which was named mupirocin F2.

A plausible mechanism for the formation of the unusual tricyclic structure **7** from hydroxyketone **6** involves backside attack of the 7α-hydroxyl onto C-10 of the epoxide and attack of the oxirane oxygen to the 6-ketone forming the hemiacetal (Scheme 2). However, the origin of **6** is not proven but could be envisaged as being formed from isomerisation of mupirocin F **3**. No metabolite with a ketone at C-6 was observed in the crude extract of *P. fluorescens* Δ*mupF* mutant but this may be due to its rapid conversion to **7**. Mupirocin F **3** appears to be reasonably stable in vitro (except for epimerisation at C-8, vide infra) and there was no evidence of isomerisation to **6**. Hence we were intrigued by the possibility that **7** was a genuine metabolite of the organism, formed under enzymatic control. To gain evidence for the origin of **7**, mupirocin F **3** was fed to the wild type *P. fluorescens* (which produces neither **3** nor **7**) under standard fermentation conditions. A control experiment using identical fermentation conditions, but without inoculating the media was carried out. Standard extraction procedures were followed and the crude extracts analysed for the presence of **3** and **7**. In both cases residual **3** was observed, but **7** was not detected in either the inoculated or control experiment. Hence we have no evidence that **7** is formed directly from **3** either under enzymatic control, or as an artefact of the extraction process.

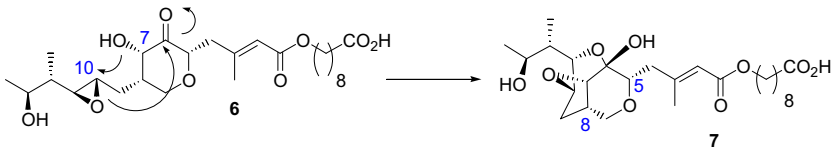
2.2. First proposed synthetic strategy to methyl mupirocin F

To firmly establish the structure of methyl mupirocin F **4** we embarked on its synthesis from pseudomonic acid A. The challenge was to effect a selective oxidation of the 7-hydroxyl in the presence of two further secondary alcohols (at C-6 and C-13) and a labile epoxide functionality. From the ^{1}H NMR spectra of pseudomonic acids combined with an X-ray crystal structure of ethyl pseudomonate C, it is evident that a chair conformation of the tetrahydropyran ring is preferred with the 7-hydroxyl group in an axial position (Fig. 4).[6] It has been reported that axial alcohols may be oxidised more rapidly than their equatorial counterparts[7] and hence we were optimistic that the required α-hydroxyketone **4** would be accessible from the parent triol, methyl pseudomonate A.

The initial synthetic strategy involved a selective protection of the 13-hydroxyl group, oxidation of the 7-OH followed by deprotection. Model studies were conducted on monic acid A **8**, which lacks the 9-hydroxynonanoic acid side-chain characteristic of pseudomonic acid A (Scheme 3).[8] Monic acid A **8** was methylated



Scheme 1. Epimerisation of methyl mupirocin F **4** leading to **5**.



Scheme 2. Proposed rearrangement of epoxy-ketone **6** to mupirocin F2 **7**.

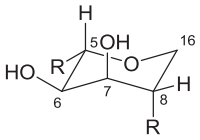
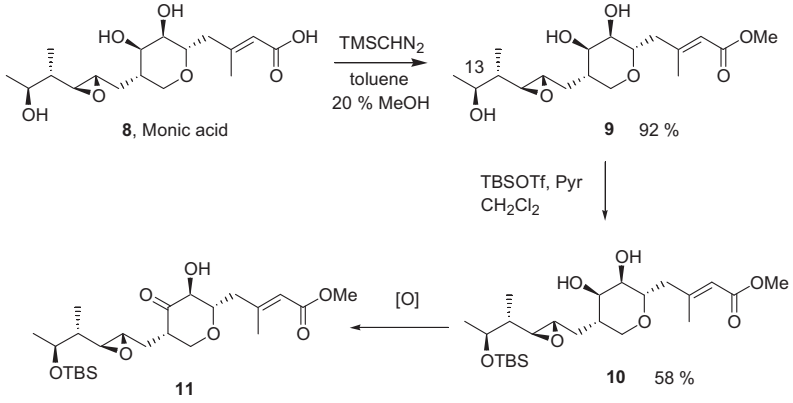


Fig. 4. Conformation of the tetrahydropyran ring of the PAs.

with TMS diazomethane giving methyl monate A **9**. A number of methods were investigated to selectively protect the 13-OH and the most satisfactory involved treatment of **9** with TBSOTf to give the monosilyl ether **10** in 58% yield.

mixture of products was formed. In contrast, in a model study with protected *syn* diol **12** the silyl group was removed using TBAF at room temperature giving epoxy alcohol **13** in 51% yield (Scheme 4).

Next mildly acidic conditions were investigated for the deprotection of silyl ether **11**. Treatment of **11** with 1% HCl in ethanol gave a new product with formula $C_{18}H_{29}O_7Cl$ (from ESIMS), which was assigned the bicyclic structure **14** (Scheme 5). The ^{13}C NMR spectrum clearly showed the loss of the carbonyl group (δ 209.1) and the appearance of a new signal at δ 98.1 characteristic of a hemiacetal carbon. HMBC unambiguously confirmed that a six-membered ring, as opposed to a five-membered ring, had been formed by



Scheme 3. Synthesis of hydroxyketone **11**.

Selective oxidation of the axial 7-OH of diol **10** to α-hydroxyketone **11** was investigated under various conditions including with Dess–Martin periodinane,[9] TPAP[10] and a Swern oxidation[11] as summarised in Table 1. The optimum conditions proved to be TEMPO/KBr/NaOCl[12] at room temperature giving ketone **11** in 50% yield along with recovered starting material **10** (42%) (entry 5, Table 1). The structure of **11** was confirmed by analysis of the ^1H NMR data, which lacked a signal for 7-H and showed a doublet for 6-H at δ 4.03 ($J_{6,5}$ 10) resonating downfield of the doublet of doublets at δ 3.26 ($J_{6,5}$ 9, 3) in **10**. Interestingly 7-ketone **11** was the only product isolated from the oxidation of diol **10**, no isomeric structure with a C-6 ketone was detected.

Table 1
Oxidation of diol **10** to ketone **11**

Entry	Oxidant	Equiv	Time (h)	Yield (%) of 10	Yield (%) of SM 11
1	Dess–Martin Periodinane	1.8	2	22	61
2	Pyridinium Dichromate	3	36	20	53
3	NMO/TPAP	0.05	48	15	46
4	DMSO/oxalyl chloride/NEt_3	2.4/1.2/5.5	4	Complex mixture	0
5	TEMPO/KBr/NaOCl	0.1	2.25	50	42

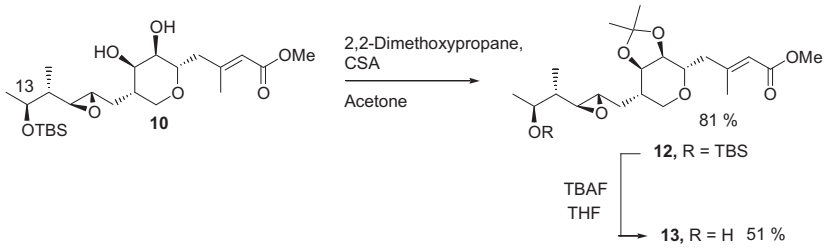
The final stage of the model synthesis of methyl mupirocin F **4** was removal of the silyl ether, which proved problematic due to the proximity of the labile epoxide functionality to the 7-ketone. On treatment of silyl ether **11** with either TBAF or HF/pyridine a complex

the coupling between C-7 (δ 98.1) and 11-H (δ 3.94). Molecular modelling revealed a preferred boat-chair conformation, which was in accord with the observed NOEs between 8-H and 11-H as well as between 9α-H and 10-H (Scheme 5). The formation of chloride **14** can be rationalised by attack of the oxirane oxygen onto the protonated 7-ketone with backside attack of the chloride at C-10.

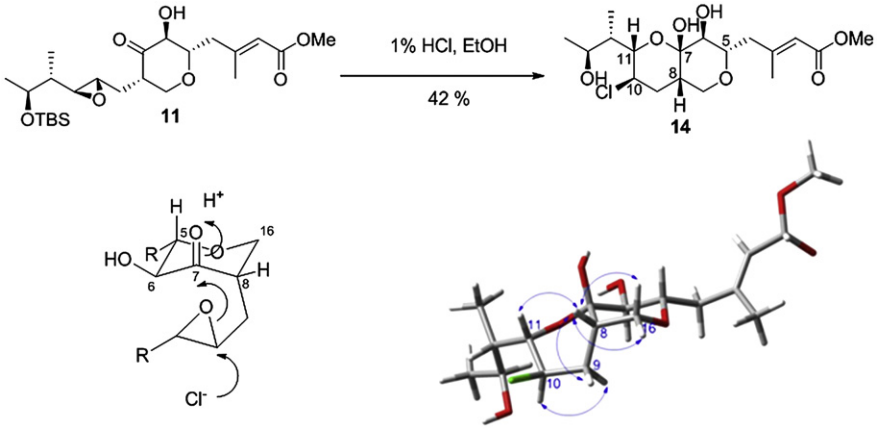
A further mild method to deprotect silyl ethers involves the use of potassium bisulfate[13] but when applied to silyl ether **11** a rearrangement took place leading to a novel tricyclic product **15** with molecular formula $C_{18}H_{30}O_7$ (from ESIMS). This unusual structure was assigned following extensive NMR investigations and confirmed by conversion to diacetate **16** with downfield shifts of the signals assigned to 6-H (from δ 3.50 in diol **15** to δ 5.09, d J 10 in **16**) and 10-H (from δ 3.86 in **15** to δ 4.4, dt, J 4, 2 in **16**). It was apparent that epimerisation at C-8 had occurred (as evidenced by a strong NOE between 6-H and 8-H) prior to formation of the new rings. Acetate **16** adopts a chair–chair–boat conformation and further NOEs are highlighted on the model shown in Scheme 6.

2.3. Synthesis of methyl mupirocin F

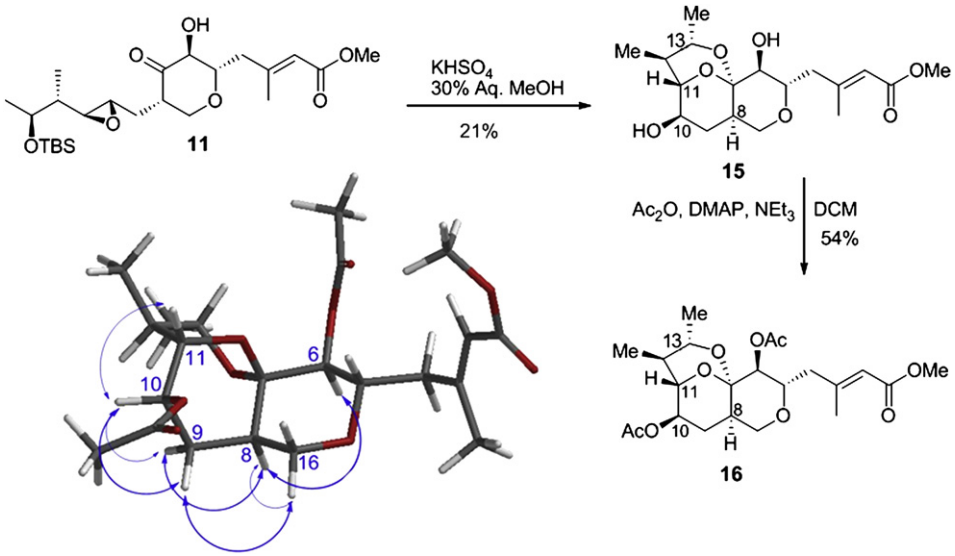
Whilst reaction of silyl ether **11** under acid conditions had led to fascinating rearrangement products **14** and **15**, the approach was not promising for the synthesis of our target methyl mupirocin F **4**. Hence the second strategy was to avoid the use of protecting groups. The optimised TEMPO mediated reaction conditions were used to oxidise methyl monate A **9** directly. A mixture of products



Scheme 4. Deprotection of acetonide **12**.



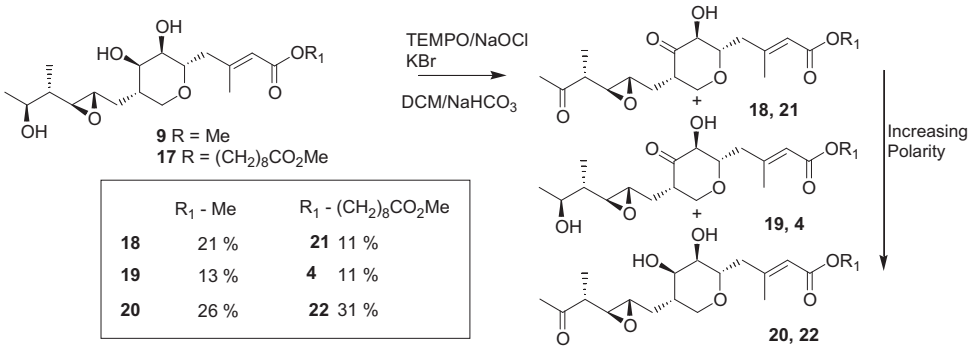
Scheme 5. Synthesis and molecular model showing key NOEs of bicyclic chloride **14**.



Scheme 6. Preparation of acetate **16** and molecular model showing NOEs.

was obtained, which were separated readily by silica gel column chromatography (Scheme 7). The least polar product was 7,13-diketone **18**, which was contaminated by an unknown impurity. The next compound eluted from the column was the required product **19** (13% yield) arising from selective oxidation of the 7-hydroxyl group. Finally the 13-ketone **20** was isolated as the major product (26% yield). Encouraged by these results in the model system we then turned to the oxidation of methyl pseudomonate A **17**. Using TEMPO, NaOCl and KBr **17** was converted to the 3 expected products 7,13-diketone **21** (11% yield), the desired α-hydroxyketone **4** (11% yield) arising from selective oxidation of 7-hydroxyl group and 13-ketone **22** (31% yield).

There was an excellent correlation between the ^{1}H NMR and ^{13}C NMR data obtained for the synthetic material **4** and that of the methylated natural product **4** thus confirming the structure of mupirocin F.



	R_1 - Me	R_1 - $(CH_2)_8CO_2Me$
18	21 %	**21** 11 %
19	13 %	**4** 11 %
20	26 %	**22** 31 %

Scheme 7. Oxidation of methyl monate A **9** and methyl pseudomonate A **17**.

3. Conclusion

In conclusion the structures of two novel metabolites, mupirocins F **3** and F2 **7** isolated from extracts of the *mupF* mutant of *P. fluorescens* have been elucidated by spectroscopic methods. Methyl mupirocin F **4** was synthesised from the triol methyl pseudomonate A **17** by selective oxidation of the 7-hydroxyl group thus firmly establishing the structure of the natural product. Methyl mupirocin F **4** was found to epimerise to 8-*epi*-mupirocin F **5** even when kept dry. Rearrangements of the epoxy-ketone **11** were shown to occur under acidic conditions due to the proximity of the labile epoxide functionality to the ketone leading to novel products **14** and **15**.

4. Experimental

4.1. General experimental details

All moisture or air sensitive reactions were carried out in oven-dried glassware under a positive pressure of nitrogen using standard syringe/septa techniques. Anhydrous solvents were obtained by passing through a modified Grubbs system of alumina columns, manufactured by Anhydrous Engineering. Routine monitoring of reactions was performed using precoated Merck-Keiselgel 60 F_{254} aluminium backed TLC plates. The spots were visualised by UV_{254} light, or potassium permanganate. Flash column chromatography[1] was performed using silica gel (obtained from Fluorochem Ltd.) as the adsorbent.

Melting points were determined on an electrothermal apparatus and are uncorrected. Optical rotations were recorded using with the sodium D line (λ=589 nm) on a Bellingham and Stanley ADP220 polarimeter and the $[\alpha]_D$ values are quoted in units 10^{-1} deg cm^2 g^{-1}. Infrared spectra were recorded on a Perkin–Elmer Spectrum One FT-IR spectrometer in the solid or liquid state. ^{1}H and ^{13}C NMR spectra were recorded using either a Jeol Delta/GX 400 MHz, a Jeol Eclipse 400 MHz or Varian VNMRS500 500 MHz spectrometer. The chemical shifts (δ) are reported in parts per million (ppm) and the coupling constants (J) are in Hertz (Hz). Tetramethylsilane was used as the internal reference for proton and carbon chemical shifts. DEPT 135, COSY, HBQC and HMBC NMR spectra were routinely used to definitively assign the signals of ^{1}H and ^{13}C NMR spectra. Electrospray (ESI) mass spectra were recorded on a Bruker Daltonics Apex 4e 7.0T FT-MS mass spectrometer.

4.2. Isolation of mupirocins F and F2

P. fluorescens Δ*mupF* was streaked on an L-agar plate and incubated at 25 °C overnight. A single colony was selected and used to inoculate 50 mL of L-medium in a 250 mL baffled flask. This seed culture was incubated at 25 °C overnight. The fermentation media (1 L in 10×500 mL baffled flasks; soya flour 25 g/L, spray-dried corn liquor 2.5 g/L, NH_4SO_4 5 g/L, $MgSO_4{\cdot}H_2O$ 0.5 g/L, Na_2HPO_4 1 g/L, KH_2PO_4 1.5 g/L, KCl 1 g/L, $CaCO_3$ 6.25 g/L, adjusted to pH 7.5 using $NaOH_{(aq)}$, then addition of glucose 4% w/v) was inoculated with 5% seed culture and incubated for 50 h at 22 °C. Centrifugation of the cells (7500 rpm for 30 min) yielded the aqueous extract, which was acidified to pH 4.5 using $HCl_{(aq)}$ and extracted with ethyl acetate (3×0.5 w/v). The organic extract was dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to give ~300 mg residue. This was subjected to Sephadex column (25×1.5 cm) and the colourful fractions were collected. A fraction of 90 mg was selected for further purification by gradient flash chromatography on normal-phase silica gel and eluted by MeOH in $CHCl_3$ from (0–15%) to give 21.2 mg mupirocin F2 **7** and 4.0 mg mupirocin F **3**.

Mupirocin F **3** was isolated as colourless viscous oil. $[\alpha]_D^{23}$ −27.4 (*c* 1.5, $CHCl_3$); ν_{max}/cm^{-1} 3422, 2930, 1708, 1650, 1223, 1149, 909, 728; δ_H (400 MHz, $CDCl_3$) 0.90 (3H, d, *J* 7.1, 17-H_3), 1.22 (3H, d, *J* 6.5, 14-H_3), 1.31 (1H, m, 12-H), 1.30–1.39 (8H, m, 4′-H_2, 5′-H_2, 6′-H_2, 7′-H_2), 1.65 (2H, m, 3′-H_2), 1.66 (2H, m, 8′-H_2), 2.02 (1H, ddd, *J* 14.4, 6.6, 6.1, 9-H*H*), 2.13 (1H, ddd, *J* 14.4, 9.3, 4.9 9-*H*H), 2.16 (3H, d, *J* 1.0, 15-H_3), 2.36 (2H, t, *J* 7.2, 2′-H_2), 2.46 (1H, dd, *J* 14.6, 9.6, 4-H*H*), 2.72 (1H, dd, *J* 7.8, 2.4, 11-H), 2.74 (1H, ddd, *J* 6.1, 4.9, 2.4, 10-H), 2.77 (1H, dd, *J* 14.6, 2.4, 4-*H*H), 2.82 (1H, ddd, *J* 9.3, 6.6, 3.2, 8-H), 3.42 (1H, ddd, *J* 9.6, 9.3, 2.4, 5-H), 3.73 (1H, dd, *J* 12.0, 3.2, 16-H*H*), 3.80 (1H, qd, *J* 6.5, 3.6, 13-H), 4.08 (1H, d, *J* 9.3, 6-H), 4.09 (2H, t, *J* 6.8, 9′-H_2), 4.13 (1H, d, *J* 12.0, 16-*H*H), 5.79 (1H, q, *J* 1.0, 2-H); δ_H (500 MHz, CD_3OD) 5.77 (1H, br s, 2-H), 4.12–4.05 (4H, m, 6-H, 16-*H*H, 9′-H), 3.75 (2H, m, 16-H*H*, 13-H), 3.49 (1H, td, 9.5, 2.5, 5-H), 2.74 (4H, m, 8-H, 10-H, 4-*H*H, 11-H), 2.46 (1H, dd, 14.7, 9.2, 4-H), 2.27 (2H, t, 7.5, 2′-H_2), 2.20 (3H, d, 1.2, 15-H_3), 2.09 (1H, ddd, 14.3, 9.5, 4.9, 9-*H*H), 1.94 (1H, dt, 14.3, 6.3, 9-H*H*), 1.65 (2H, m), 1.60 (2H, m,), 1.41–1.33 (8H, m, 4′-H_2, 5′-H_2, 6′-H_2, 7′-H_2), 1.19 (3H, d, 6.4, 14-H_3), 0.89 (3H, d, 7.0, 17-H_3); δ_C (100 MHz, $CDCl_3$) 12.4 (C-17), 19.2 (C-15), 20.8 (C-14), 24.6 (C-3′), 25.9 (C-7′), 28.6 (C-8′), 28.94 (C-4′), 28.85 and 29.0 (C-5′ and C-6′), 33.1 (C-9), 33.7 (C-2′), 42.5 (C-4), 48.9 (C-8), 42.5 (C-12), 54.6 (C-10), 60.8 (C-11), 63.8 (C-9′), 71.2 (C-13), 71.4 (C-16), 83.0 (C-5), 74.9 (C-6),118.4 (C-2), 155.1 (C-3), 166.6 (C-1), 178.5 (C-1′), 209.3 (C-7); HRESIMS 521.2710 [M]Na$^+$2.1 ppm (521.2721 expected for $C_{26}H_{42}O_9Na$).

Mupirocin F2 **7** was isolated as colourless viscous oil. $[\alpha]_D^{23}$ −12.5 (*c* 3.2, MeOH); ν_{max}/cm^{-1} 3425, 2929, 2856, 2120, 1710, 1649, 1457, 1396, 1223, 1149; δ_H (400 MHz, methanol-d_4) 0.82 (3H, d, *J* 6.9, 17-H_3), 1.10 (3H, d, *J* 6.3, 14-H_3), 1.30–1.40 (8H, m, 4′-H_2, 5′-H_2, 6′-H_2, 7′-H_2), 1.59 (2H, m, 3′-H_2), 1.65 (2H, m, 8′-H_2), 1.71 (1H, dqd, *J* 10.3, 6.9, 4.4 12-H), 2.02 (2H, m, 9-H_2), 2.16 (3H, d, *J* 1.2, 15-H_3), 2.26 (2H, t, *J* 7.3, 2′-H_2), 2.32 (1H, dd, *J* 14.6, 10.5, 4-H*H*), 2.32 (1H, m, 8-H), 2.59 (1H, dd, *J* 14.6, 2.4, 4-*H*H), 3.26 (1H, dd, *J* 10.5, 2.4, 5-H), 3.74 (1H, dd, *J* 12.3, 3.2, 16-H*H*), 3.89 (1H, dd, *J* 10.3, 1.5, 11-H), 3.91 (1H, d, *J* 6.1, 7-H), 4.01 (1H, d, *J* 12.3, 16-*H*H), 4.06 (2H, t, *J* 6.6, 9′-H), 4.10 (1H, qd, *J* 6.3, 4.4, 13-H), 4.25 (1H, ddd, *J* 6.1, 3.2, 1.5, 10-H), 5.70

(1H, q, *J* 1.2, 2-H); δ_C (100 MHz, methanol-d_4) 9.7 (C-17), 17.3 (C-14), 19.1 (C-15), 26.3 (C-3′), 26.3 (C-9), 27.2 (C-7′), 29.9 (C-8′), 30.2 (C-4′), 30.3 and 30.4 (C-5′ and C-6′), 35.2 (C-2′), 38.7 (C-4), 40.6 (C-8), 41.6 (C-12), 64.9 (C-9′), 67.9 (C-16), 68.4 (C-13), 77.80 (C-10), 77.84 (C-11), 79.2 (C-7), 80.2 (C-5), 92.2 (C-6),118.4 (C-2), 159.2 (C-3), 168.5 (C-1), 178.2 (C-1′); HRESIMS 521.2711 [M]Na^+1.9 ppm (521.2721 expected for $C_{26}H_{42}O_9Na$).

The sample of mupirocin F **3** was left at room temperature for 2 weeks after which time a ~1:1 mixture of 3 and 8-*epi*-mupirocin was present. The isomers were separated by HPLC using a 55–59% gradient of methanol in H_2O+0.0005% formic acid over 28 min at 40 °C (mupirocin F3 **1**: <1 mg, 22.0 min, 8-*epi*-mupirocin F: <1 mg, 23.0 min). The 8-epimer was obtained as a colourless oil; δ_H ($CDCl_3$, 500 MHz) 5.77 (1H, br s, 2-H), 4.29 (1H, dd, 11.3, 6.9, 16-H), 4.07 (2H, t, 6.7, 9′-H_2), 4.07 (1H, obscured m, 13-H), 3.94 (1H, dd, 9.6, 1.2, 6-H), 3.76 (1H, m, 13-H), 3.49 (1H, td, 9.6, 2.5, 5-H), 3.37 (1H, t, 11.3, 16-H), 2.91 (1H, m, 8-H), 2.84 (1H, td, 5.6, 2.2, 10-H), 2.77 (1H, br d, *J* 14.6, 4-H), 2.70 (1H, dd, 7.6, 2.2, 11-H), 2.44 (1H, dd, *J* 14.6, 9.3, 4-H), 2.19 (3H, s, 15-H_3), 2.19 (2H, obscured t, 2′-H_2), 2.02 (1H, dt, 14.4, 5.8, 9-H), 1.64 (2H, m), 1.60 (2H, m), 1.41 (1H, obscured m, 12-H), 1.39–1.33 (8H, m, 4′-H_2, 5′-H_2, 6′-H_2, 7′-H_2), 1.19 (3H, t, *J* 6.4, 14-H_3), 0.92 (3H, d, *J* 7.1, 17-H_3); δ_C ($CDCl_3$, 125 MHz, observed via 2D NMR experiments) 169.6 (C-1′), 159.2 (C-3), 119.8 (C-2), 83.7 (C-5), 78.1 (C-6), 72.6 (C-16), 71.1 (C-13), 64.8 (C-9′), 61.6 (C-11), 55.3 (C-10), 47.9 (C-8), 44.3 (C-4), 42.8 (C-13), 38.0 (C-2′), 30.1 (CH_2), 29.8 (CH_2), 27.1 (CH_2), 27.0 (C-9), 26.9 (C-3′), 20.0 (C-14), 19.0 (C-15), 11.2 (C-17), C1, C7 not observed; HRESIMS 521.2712 [M]Na^+2.9 ppm (521.2727 expected for $C_{26}H_{42}O_9Na$).

4.3. Isolation of methyl mupirocins F and F2

Mutant mupF was streaked on an L-agar plate and incubated at 25 °C overnight. A single colony was selected and used to inoculate 50 mL of L-medium in a 250 mL baffled flask. This seed culture was incubated at 25 °C overnight. The fermentation media (1 L in 10×500 mL baffled flasks; soya flour 25 g/L, spray-dried corn liquor 2.5 g/L, NH_4SO_4 5 g/L, $MgSO_4 \cdot H_2O$ 0.5 g/L, Na_2HPO_4 1 g/L, KH_2PO_4 1.5 g/L, KCl, 1 g/L, $CaCO_3$ 6.25 g/L, adjusted to pH 7.5 using $NaOH_{(aq)}$, then addition of glucose 4% w/v) was inoculated with 5% seed culture and incubated for 50 h at 22 °C. Centrifugation of the cells (7500 rpm for 30 min) yielded the aqueous extract, which was acidified to pH 4.5 using $HCl_{(aq)}$ and extracted with ethyl acetate (3×0.5 w/v). The organic extract was dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to give 165 mg crude extract. This extract was dissolved in methanol (2 mL), and TMS diazomethane (2 M solution in diethyl ether, 1 mL, 2 mmol) was added. The reaction was stirred at room temperature for 1 h, then all solvents were removed using a flow of nitrogen. The crude material was partially purified on deacidified silica (using triethylamine) using 40% ethyl acetate in petroleum ether. The mixture was further purified by reversed-phase HPLC using a Phenomenex Luna C_{18} column at 25 °C with a gradient of 60%–62% MeOH in H_2O+0.005% formic acid over 42 min. This yielded two fractions methyl mupirocin F **4** (5.0 mg, t_R 34.8 min) and methyl 8-*epi*-mupriocin **5** (2.9 mg, t_R 36.9 min).

Methyl mupirocin F **4** was obtained as a colourless oil. $[\alpha]_D^{20}$ +26 (*c* 0.23, MeOH); ν_{max}/cm^{-1} 2931, 2857, 1713, 1221, 1148, 1111; δ_H ($CDCl_3$, 500 MHz) 5.78 (1H, br d, *J* 1.0, 2-H), 4.11 (1H, dd, 11.9, 0.6, 16-H), 4.09–4.05 (3H, m, 6-H, 9′-H_2), 3.78 (1H, m, 13-H), 3.72 (1H, dd, *J* 11.9, 3.1, 16-H), 3.67 (3H, s, OCH_3), 3.60 (1H, d, *J* 4.0, OH), 3.40 (1H, dt, *J* 9.5, 2.5, 5-H), 2.81 (1H, m, 8-H), 2.76 (1H, m, 4-H), 2.73 (1H, m, 10-H), 2.70 (1H, dd, *J* 7.6, 2.1, 11-H), 2.44 (1H, dd, *J* 14.7, 9.2, 4-H), 2.30 (2H, t, *J* 7.4, 2′-H_2), 2.21 (3H, d, *J* 1.0, 15-H_3), 2.12 (1H, ddd, *J* 14.5, 9.3, 5.1, 9-H), 2.01 (1H, dt, *J* 14.5, 6.1, 9-H), 1.65–1.57 (4H, m), 1.35–1.29 (9H, m, 12-H, 4′-H_2, 5′-H_2, 6′-H_2, 7′-H_2), 1.21 (3H, d, *J* 6.4, 14-H_3), 0.89 (3H, d, *J* 6.5 17-H_3); δ_C ($CDCl_3$, 125 MHz) 209.2 (C-7), 174.3 (C-1′), 166.6 (C-1), 155.1 (C-3), 118.35 (C-2), 83.0 (C-5), 75.0 (C-6), 71.4 and 71.2 (C-16 and C-13), 63.9 (C-9′), 60.9 (C-11), 54.5 (C-10), 51.4 (CH_3, OCH_3), 48.8 (C-8), 43.9 (C-4), 42.6 (C-13), 34.1, 33.1, 29.11, 29.06, 29.03, 28.7, 25.9, 24.9 (each CH_2), 20.8 (C-14), 19.2 (C-15), 12.4 (C-17); HRESIMS 535.2873 [M]Na^+1.9 ppm (535.2883 expected for $C_{27}H_{44}O_9Na$).

Methyl 8-epi-mupirocin F **5** was obtained as a colourless oil. ν_{max}/cm^{-1} 2933, 2856, 1714, 1223, 1148, 1108; δ_H ($CDCl_3$, 500 MHz) 5.78 (1H, br d, *J* 1.0, 2-H), 4.33 (1H, dd, *J* 11.3, 7.0, 16-H), 4.08 (2H, t, *J* 6.7, 9′-H), 3.92 (1H, br d, 9.5, 6-H), 3.81 (1H, quint, 6.4, 13-H), 3.67 (3H, s, OCH_3), 3.59 (1H, d, *J* 3.7, OH), 3.42 (1H, td, *J* 9.5, 2.5, 5-H), 3.38 (1H, t, *J* 11.3, 16-H), 2.90 (1H, m, 8-H), 2.81 (1H, m, 10-H), 2.77 (1H, br d, *J* 14.6, 4-H), 2.65 (1H, dd, *J* 8.2, 2.5, 11-H), 2.45 (1H, dd, *J* 14.6, 9.5, 4-H), 2.30 (2H, t, *J* 7.6, 2′-H_2), 2.22 (3H, d, *J* 1.0, 15-H), 1.99 (1H, m, 9-H), 1.67–1.53 (4H, m), 1.36–1.29 (9H, 12-H, 4′-H_2, 5′-H_2, 6′-H_2, 7′-H_2), 1.21 (3H, d, *J* 6.4, 14-H), 0.94 (3H, d, *J* 7.3, 17-H_3); δ_C ($CDCl_3$, 125 MHz, observed via 2D NMR experiments) 208.2 (C-7), 174.3 (C-1′), 166.6 (C-1), 155.7 (C-3), 118.4 (C-2), 83.1 (C-5), 76.9 (C-6), 71.8 (C-16), 71.5 (C-13), 64.0 (C-9′), 61.5 (C-11), 54.0 (C-10), 51.6 (OCH_3), 47.0 (C-8), 44.0 (C-4), 42.9 (C-13), 34.2 (C-2′), 29.0, 26.0, 25.0 (each CH_2), 26.8 (C-9), 20.8 (C-14), 19.4 (C-15), 12.8 (C-17); HRESIMS 535.2867 [M]Na^+3.0 ppm (535.2883 expected for $C_{27}H_{44}O_9Na$).

4.4. Methyl monate A 9

Trimethylsilyldiazomethane (2 M in Et_2O, 5.68 ml, 11 mmol) was added dropwise to a solution of monic acid **8** (3 g, 9 mmol) in dry toluene (32 ml) and HPLC grade MeOH (8 ml). The reaction was monitored by TLC and had reacted to completion after 1 h, the reaction mixture was diluted with EtOAc (30 ml) and AcOH (10% aqueous, 20 ml) added. The aqueous phase was extracted with EtOAc, (3×30 ml) the organic layers combined, washed with brine (50 ml), dried with $MgSO_4$, filtered and the solvent removed in vacuo. The resulting crude material was a colourless oil and trituration with petrol yielded methyl ester **9** as a white crystalline solid (2.95 g, 92%). Mp 121–123 °C, [lit.[14] mp 124–125 °C][1]; $[\alpha]_D^{23}$ −10 (*c* 1, $CHCl_3$) [lit.[14] $[\alpha]_D^{20}$ −11.1 (*c* 1.5, $CHCl_3$)][1]; ν_{max} (neat)/cm^{-1} 3467 and 3405 (OH), 2918 and 2877 (CH), 1713 (C=O) and 1647 (C=C); δ_H (400 MHz, MeOD) 0.94 (3H, d, *J* 7, 17-H_3), 1.19 (3H, d, *J* 7, 14-H_3), 1.40 (1H, m, 12-H), 1.68 (2H, m, 9-H), 1.94 (1H, m, 8-H), 2.17 (3H, s, 15-H_3), 2.21 (1H, m, 4-H*H*), 2.63 (1H, dd, *J* 16, 2, 4-*H*H), 2.71 (1H, dd, *J* 8, 2, 11-H), 2.80 (1H, app td, *J* 6, 2, 10-H), 3.35 (1H, dd, *J* 10, 3, 6-H), 3.55 (1H, dd, *J* 12, 2, 16-*H*H), 3.63 (3H, s, OCH_3), 3.73 (1H, m, 5-H), 3.78 (1H, m, 13-H), 3.84 (1H, m, 16-H*H*), 3.87 (1H, m, 7-H), 5.72 (1H, m, 2-H).

4.5. Methyl monate A 13-*tert*-butyldimethylsilyl ether 10

TBDMSOTf (1.92 ml, 8 mmol) was added dropwise to a solution of methyl ester **9** (2 g, 5.5 mmol) and pyridine (0.9 ml, 11 mmol) in dry DCM (50 ml). Methyl ester **9** was not initially soluble in DCM but as the reaction progressed it became soluble. The reaction was left to stir for 1 h and quenched with saturated $NaHCO_3$ solution (50 ml). The aqueous phase was extracted with DCM (3×30 ml), the organic layers combined, washed with brine (50 ml), dried with $MgSO_4$, filtered and the solvent removed in vacuo. The resulting crude product was purified by flash column chromatography using a gradient eluent system of 30%–50% EtOAc/petrol to give the *silyl ether* **10** as a white crystalline solid (1.67 g, 3.5 mmol, 58%). $[\alpha]_D^{23}$ −12 (*c* 1, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3467 and 3404 (OH), 2933 and 2910 (CH), 1719 (C=O) and 1650 (C=C); δ_H (400 MHz, MeOD) 0.01 and 0.02 (2× 3H, 2× s, 2× SiMe), 0.83 (9H, s, Si^{t}Bu), 0.87 (3H, d, *J* 7, 17-H_3), 1.11 (3H, d, *J* 7, 14-H_3), 1.29 (1H, m, 12-H), 1.55–1.63 (2H, m, 9-H_2), 1.87 (1H, m, 8-H), 2.10 (3H, d, *J* 1, 15-H_3), 2.15 (1H, m, 4-H), 2.56 (1H, br d, *J* 14, 4-H), 2.62 (1H, dd, *J* 8, 2, 11-H), 2.70 (1H, *J* 6, 2, 10-H), 3.26 (1H, dd, *J* 9, 3, 6-H), 3.48 (1H, dd, *J* 12, 1, 16-H*H*), 3.57

(3H, s, OCH_3), 3.65 (1H, td, *J* 9, 3, 5-H), 3.76 (1H, m, 16-*H*H), 3.78 (1H, m, 7-H), 3.85 (1H, m, 13-H), 5.67 (1H, m, 2-H); δ_C (100 MHz, MeOD) −4.7 (Si—CH_3), −4.1 (Si—CH_3), 12.7 (C-17), 18.9 (Si—$C(CH_3)_3$), 19.2 (C-15), 21.1 (C-14), 25.4 (3× ^{t}Bu—CH_3), 32.9 (C-9), 41.8 (C-8), 43.9 (C-4), 44.4 (C-12), 51.2 (OCH_3), 57.0 (C-10), 60.8 (C-11), 66.3 (C-16), 70.0 (C-6), 71.6 (C-13), 76.1 (C-7), 83.0 (C-5), 117.9 (C-2), 159.3 (C-3), 168.7 (C-1). Found (ESI): 495.2729 $[MNa]^+$, ($C_{24}H_{44}O_7SiNa$ requires 495.2748); *m*/*z* (ESI) 495 ([MNa^+], 100%).

4.6. Methyl 7-ketomonate A 13-*tert*-butyldimethylsilyl ether 11

Silyl ether **10** (0.6 g, 1.27 mmol) was dissolved in DCM (100 ml) and saturated $NaHCO_3$ solution (50 ml), KBr (30.2 mg, 0.25 mmol) and TEMPO (20 mg, 0.13 mmol) were added and the solution left to stir for 0.25 h. NaOCl (1.8 ml, 1.4 mmol) was added and the reaction left to stir for 2 h in which time the colour changed from an orange to a colourless solution. The two phases were separated and the aqueous layer extracted with DCM (3×30 ml) the organic layers combined, washed with brine (50 ml), dried with $MgSO_4$, filtered and the solvent removed in vacuo. The resulting crude product was purified by flash column chromatography using an eluent system of 20% EtOAc/petrol to give the *α-hydroxyketone* **11** as a colourless oil (297 mg, 0.63 mmol, 50%). $[\alpha]_D^{23}$ +16 (*c* 0.25, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3484 (OH), 2930 and 2857 (CH), 1714 (C=O) and 1651 (C=C); δ_H (400 MHz, $CDCl_3$) 0.001 and 0.023 (2× 3H, 2× s, 2× SiMe), 0.83 (9H, s, Si^{t}Bu), 0.84 (3H, d, *J* 6, 17-H_3), 1.14 (3H, d, *J* 6, 14-H_3), 1.31 (1H, m, 12-H), 1.89 (1H, app dt, *J* 15, 7, 9-*H*H), 2.12 (1H, m, 9-H*H*), 2.19 (3H, d, *J* 1, 15-H_3), 2.42 (1H, dd, *J* 15, 9, 4-*H*H), 2.63 (1H, m, 10-H), 2.69 (1H, dd, *J* 8, 2, 11-H), 2.77 (2H, m, 4-H*H*, 8-H), 3.35 (1H, td, *J* 9, 2, 5-H), 3.66 (1H, dd, *J* 12, 3, 16-*H*H), 3.67 (3H, s, OCH_3), 3.82 (1H, qd, *J* 6, 3, 13-H), 4.03 (1H, d, *J* 10, 6-H), 4.09 (1H, dd, *J* 12, 2, 16-H*H*); δ_C (100 MHz, $CDCl_3$) −5.0 (Si—CH_3), −4.3 (Si—CH_3), 12.3 (C-17), 18.0 (Si—$C(CH_3)_3$), 19.2 (C-15), 20.8 (C-14), 25.8 (3× ^{t}Bu—CH_3), 33.2 (C-9), 42.7 (C-12), 43.8 (C-4), 48.9 (C-8), 50.9 (OCH_3), 54.3 (C-10), 59.0 (C-11), 70.2 (C-13), 71.4 (C-16), 74.8 (C-6), 83.0 (C-5), 117.8 (C-2), 155.6 (C-3), 166.9 (C-1), 209.1 (C-7); Found (ESI): 493.2605 $[MNa]^+$, ($C_{24}H_{42}O_7SiNa$ requires 493.2592); *m*/*z* (ESI) 493 ([MNa^+], 100%). Starting material **10** (0.24 g, 42%) was recovered.

4.7. Protected methyl monate 12

2,2-Dimethoxypropane (0.13 ml, 1.06 mmol) was added to a solution of TBDMS ether **10** (0.1 g, 0.21 mmol) with a catalytic amount of camphorsulfonic acid (4.8 mg, 0.02 mmol) in HPLC grade acetone (15 ml). The reaction was left to stir for 2 h and was quenched with saturated $NaHCO_3$ (15 ml) and the aqueous phase extracted with EtOAc (3×15 ml). The organic layers were combined, washed with brine (30 ml), dried with $MgSO_4$, filtered and the solvent removed in vacuo. The resulting crude product was purified by flash column chromatography using a gradient eluent system of 10%–30% EtOAc/petrol to give the *acetonide* **12** as a colourless oil (87.6 mg, 81%). $[\alpha]_D^{23}$ −36 (*c* 0.25, $CHCl_3$); ν_{max} (neat)/cm^{-1} 2930 and 2858 (CH), 1716 (C=O) and 1650 (C=C); δ_H (400 MHz, $CDCl_3$) 0.001 and 0.021 (2× 3H, 2× s, 2× SiMe), 0.85 (9H, s, Si^{t}Bu), 0.89 (3H, d, *J* 7, 17-H_3), 1.15 (3H, d, *J* 6, 14-H_3), 1.33 (3H, s, $OCCH_3$), 1.35 (1H, m, 12-H), 1.47 (3H, s, $OCCH_3$), 1.61–1.76 (2H, m, 9-H_2), 2.15 (1H, m, 8-H), 2.16 (3H, d, *J* 1, 15-H_3), 2.20 (1H, ddd, *J* 14, 9, 1, 4-*H*H), 2.46 (1H, br d, *J* 14, 1, 4-H*H*), 2.67 (1H, dd, *J* 8, 2, 11-H), 2.73 (1H, m, 10-H), 3.41 (1H, td, *J* 9, 3, 5-H), 3.64 (3H, s, OCH_3), 3.66–3.74 (3H, m, 7-H, 16-H_2), 3.84 (1H, qd, *J* 8, 6, 13-H), 4.13 (1H, dd, *J* 5, 3, 6-H), 5.72 (1H, m, 2-H); δ_C (100 MHz, $CDCl_3$) −5.0 (Si—CH_3), −4.3 (Si—CH_3), 12.5 (C-17), 18.0 (Si—$C(CH_3)_3$), 19.0 (C-15), 20.7 (C-14), 25.8 (3× ^{t}Bu—CH_3), 26.3 ($OCCH_3$), 28.3 ($OCCH_3$), 33.8 (C-9), 35.0 (C-12), 42.9 (C-8), 43.9 (C-4), 50.8 (C-1′), 55.0 (C-10), 59.4 (C-11), 67.1 (C-16), 70.2 (C-13), 74.2 (C-7), 75.6 (C-6), 76.3 (C-5), 108.8 ($OCCH_3$), 117.2 (C-2), 156.6 (C-3), 166.7 (C-1); Found (ESI): 535.3072 $[MNa]^+$, ($C_{27}H_{48}O_7SiNa$ requires 535.3061); *m*/*z* (ESI) 535 ([MNa^+], 100%).

4.8. Methyl monate acetonide 13

The acetonide TBDMS ether **12** (56 mg, 0.11 mmol) was dissolved in THF (10 ml) and TBAF was added (0.87 ml, 0.87 mmol) over 6 h and the reaction left to stir for a further 1 h. The reaction was quenched with water (15 ml) and diluted with EtOAc (10 ml) and the aqueous phase was extracted with EtOAc (3×20 ml). The organic layers were combined, washed with brine (30 ml), dried with $MgSO_4$, filtered and the solvent removed in vacuo. The resulting crude product was purified by flash column chromatography using a gradient eluent system of 10%–50% EtOAc/petrol to give *acetonide* **13** as a colourless oil (22 mg, 51%). $[\alpha]_D^{23}$ −39 (*c* 1, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3498 (OH), 2933 (CH), 1713 (C=O) and 1649 (C=C); δ_H (400 MHz, $CDCl_3$) 0.89 (3H, d, *J* 7, 17-H_3), 1.15 (3H, d, *J* 6, 14-H_3), 1.25 (1H, m, 12-H), 1.30 (3H, s, $OC(CH_3)$), 1.47 (3H, s, $OC(CH_3)$), 1.61–1.74 (2H, m, 9-H), 2.12 (1H, m, 8-H), 2.13 (3H, d, *J* 1, 15-H_3), 2.17 (1H, ddd, *J* 14, 9, 1, 4-*H*H), 2.43 (1H, br d, *J* 14, 1, 4-H*H*), 2.60 (1H, dd, *J* 8, 2, 11-H), 2.78 (1H, ddd, *J* 7, 5, 2, 10-H), 3.39 (1H, td, *J* 9, 3, 5-H), 3.61 (3H, s, 1′-Me), 3.60–3.71 (3H, m, 7-H, 16-H_2), 3.76 (1H, app quint, *J* 6, 13-H), 4.09 (1H, m, 6-H), 5.69 (1H, m, 2-H); δ_C (100 MHz, $CDCl_3$) 12.8 (C-17), 19.0 (C-15), 20.6 (C-14), 26.3 ($OCCH_3$), 28.3 ($OCCH_3$), 33.8 (C-9), 35.0 (C-12), 42.9 (C-8), 43.9 (C-4), 50.8 (C-1′), 55.5 (C-10), 61.5 (C-11), 66.9 (C-16), 71.3 (C-13), 74.1 (C-7), 75.6 (C-6), 76.3 (C-5), 108.8 ($OCCH_3$), 117.2 (C-2), 156.5 (C-3), 166.7 (C-1); Found (ESI): 421.2199 $[MNa]^+$, ($C_{21}H_{34}O_7Na$ requires 421.2197); *m*/*z* (ESI) 421 ([MNa^+], 100%).

4.9. Rearrangement product 14

α-Hydroxyketone **11** (113 mg, 0.24 mmol) was stirred vigorously in EtOH (4 ml) to which 12 M HCl_{aq} (2 equiv, 0.48 mmol, 40 μL) was added. The reaction was left to stir for 1 h at room temperature before more EtOH (4 ml) mixed with 12 M HCl_{aq} (2 equiv, 0.48 mmol, 40 μL) was added. The mixture was stirred at room temperature for a further 1.5 h before saturated aqueous $NaHCO_3$ (40 ml) solution and EtOAc (40 ml) were added. The layers were separated and the aqueous phase was extracted with EtOAc (4×20 ml). The combined organic extracts were washed with brine (40 ml), dried with $MgSO_4$, filtered and concentrated in vacuo. The resulting crude colourless oil was purified by flash column chromatography on silica gel, eluting with 40% EtOAc/petrol to give *alcohol* **14** (41 mg, 0.1 mmol, 42%). $[\alpha]_D^{23}$ +3 (*c* 1, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3431 (OH), 2930 (CH), 1715 (C=O), 1648 (C=C); δ_H (400 MHz, C_6D_6) 0.86 (3H, d, *J* 7, 17-H_3), 1.11 (3H, d, *J* 6, 14-H_3), 1.53–1.65 (2H, m, 9-*H*H and 12-H), 2.01 (1H, app dt, *J* 14, 6, 9-H*H*), 2.20–2.26 (1H, m, 8-H), 2.27–2.36 (1H, m, 4-*H*H), 2.40 (3H, d, *J* 1, 15-H_3), 2.72 (1H, br d, *J* 14, 4-H*H*), 3.41 (3H, s, OMe), 3.42 (1H, dd, *J* 12, 1, 16-*H*H), 3.48–3.51 (2H, m, 6-H and 5-H), 3.65 (1H, dd, *J* 12, 4, 16-H*H*), 3.81 (1H, app quint, *J* 6, 13-H), 3.86 (1H, m, 10-H), 3.94 (1H, app t, *J* 8, 11-H); δ_C (100 MHz, C_6D_6) 13.3 (C-17), 19.8 (C-15), 21.0 (C-14), 35.1 (C-9), 36.4 (C-8), 43.7 (C-4), 44.6 (C-12), 50.9 (OCH_3), 59.8 (C-10), 68.9 (C-16), 72.2 (C-13), 73.8 (C-6 or C-5), 77.4 (C-5 or C-6), 79.3 (C-11), 98.1 (C-7), 118.6 (C-2), 157.4 (C-3), 167.2 (C-1). Found (ESI): 415.1505 $[MNa]^+$, ($C_{18}H_{29}O_7ClNa$ requires 415.1494); *m*/*z* (ESI) 415 ([MNa^+], 100%), 416 (20), 417 (30), 325 (24), 287 (18), 138 (10).

4.10. Rearrangement product 15

α-Hydroxyketone **11** (64 mg, 0.14 mmol) was stirred in 30% aqueous methanol (6 ml) and $KHSO_4$ was added (7.4 mg, 0.05 mmol). The reaction was left to stir for 2.5 h and concentrated in vacuo to give a colourless oil. The resulting crude material was dissolved in methanol and dry loaded onto a silica column and

eluted with 50% EtOAc/petrol to yield *tricyclic ether* **15** (10 mg, 21%) as a colourless oil; $[\alpha]_D^{25}$ −12 (*c* 0.5, $CHCl_3$); δ_H (400 MHz, C_6D_6) 0.57 (3H, d, *J* 7, 17-H_3), 0.86 (1H, ddd, *J* 15, 4, 1.5, 9-*H*H), 0.92 (1H, m, 12-H), 1.09 (3H, d, *J* 6, 14-H_3) 1.73 (1H, app dt, *J* 12, 5, 8-H), 2.03 (1H, ddd, *J* 15, 6, 4, 9-*H*H), 2.19 (1H, ddd, *J* 14, 9, 1, 4-*H*H), 2.37 (3H, d, *J* 1, 15-H_3), 2.71 (1H, br d, *J* 14, 4-H*H*), 2.92 (1H, m, 10-H), 3.32 (1H, d, *J* 10, 6-H), 3.36 (1H, m, 11-H), 3.39 (3H, s, OCH_3), 3.40 (1H, m, 16-*H*H), 3.51 (1H, app dt, *J* 10, 3, 5-H), 3.56 (1H, dd, *J* 12, 5, 16-H*H*), 3.78 (1H, dq, *J* 10, 6, 13-H), 4.08 (1H, app t, *J* 12, 16-H*H*), 6.04 (1H, m, 2-H); δ_C (125 MHz, C_6D_6) 18.0 (C-17), 19.6 (C-15), 19.8 (C-14), 24.0 (C-9), 39.1 (C-8), 41.3 (C-12), 44.1 (C-4), 50.7 (OCH_3), 67.8 (C-10), 69.7 (C-16), 71.4 (C-13), 78.1 (C-6), 78.7 (C-5), 81.6 (C-11), 96.7 (C-7), 118.2 (C-2), 158.0 (C-3), 167.2 (C-1). Found (ESI): 379.1746 $[MNa]^+$, ($C_{18}H_{28}O_7Na$ requires 379.1727); *m*/*z* (ESI) 379 ([MNa^+], 100%).

4.11. Acetylation of diol 15

Triethylamine (9 μl, 0.066 mmol), acetic anhydride (9 μl, 0.066 mmol) and a crystal of DMAP (cat.) were added to a solution of tricyclic ether **15** (8 mg, 0.022 mmol) in dry DCM (2 ml). The reaction was left to stir for 0.2 h before being quenched with saturated $NaHCO_3$ solution (2 ml). The aqueous phase was extracted with DCM (3×5 ml), the organic layers combined, dried with $MgSO_4$, filtered and the solvent removed in vacuo. The crude material was purified by flash column chromatography using an eluent system of 25% EtOAc/petrol to give *acetylated ether* **16** (5 mg, 0.012 mmol, 54%) as a colourless oil; $[\alpha]_D^{25}$ −10 (*c* 0.1, $CHCl_3$); δ_H (500 MHz, C_6D_6) 0.65 (3H, d, *J* 7, 17-H_3), 0.95–1.06 (2H, m, 12-H and 9-H*H*), 1.04 (3H, d, *J* 6.5, 14-H_3), 1.54 (3H, s, OAc), 1.74 (1H, m, 8-H), 1.79 (3H, s, OAc), 2.11 (1H, ddd, *J* 15.5, 6, 4.5, 9-*H*H), 2.26 (1H, m, 4-*H*H), 2.27 (3H, d, *J* 1, 15-H_3), 2.33 (1H, ddd, *J* 14, 8.5, 1, 4-H*H*), 3.36 (3H, s, OCH_3), 3.53 (1H, dd, *J* 11.5, 5.5, 16-*H*H), 3.73 (1H, dq, *J* 10, 6, 13-H), 3.78 (1H, m, 11-H), 4.05 (1H, ddd, *J* 10, 8.5, 4, 5-H), 4.08 (1H, app t, *J* 11.5, 16-H*H*), 4.41 (1H, app dt, *J* 4, 1.5, 10-H), 5.09 (1H, d, *J* 10, 6-H), 5.93 (1H, m, 2-H); δ_C (125 MHz, C_6D_6) 17.7 (C-17), 19.1 (C-15), 19.3 (C-14), 20.9 (C-9 or $OCOCH_3$), 21.2 (C-9 or $OCOCH_3$), 21.3 (C-9 or $OCOCH_3$), 38.8 (C-8), 41.6 (C-12), 44.4 (C-4), 50.7 (OCH_3), 69.2 (C-10), 69.5 (C-16), 71.2 (C-13), 75.3 (C-5), 77.3 (C-6), 78.7 (C-11), 96.7 (C-7), 118.5 (C-2), 156.5 (C-3), 169.7 (C-1), 169.91 (2× $OCOCH_3$). Found (ESI): 463.1942 $[MNa]^+$, ($C_{18}H_{28}O_7Na$ requires 463.1939); *m*/*z* (ESI) 463 ([MNa^+], 100%).

4.12. Oxidation of methyl monate 9

Methyl monate **9** (0.19 g, 0.53 mmol) was dissolved in DCM (100 ml) and saturated $NaHCO_3$ solution (50 ml), KBr (13 mg, 0.11 mmol) and TEMPO (8 mg, 0.05 mmol) was added and the solution left to stir for 0.25 h. NaOCl (0.9 ml, 0.58 mmol) was added and the reaction left to stir for 2 h in which time the colour changed from an orange to a colourless solution. The two phases were separated and the aqueous layer extracted with DCM (3×30 ml) the organic layers combined, washed with brine (50 ml), dried with $MgSO_4$, filtered and the solvent removed in vacuo. The resulting crude product was purified by flash column chromatography using an eluent system of 70% EtOAc/petrol to give *diketone* **18** as a colourless oil (40 mg, 0.11 mmol, 21%), which was not clean enough for full characterisation; *α-hydroxyketone* **19** as a colourless oil (25 mg, 0.07 mmol, 13%); $[\alpha]_D^{24}$ +16 (*c* 1, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3434 (OH), 2973 and 2856 (CH), 1712 (C=O) and 1649 (C=C); δ_H (400 MHz, $CDCl_3$) 0.89 (3H, d, *J* 7, 17-H_3), 1.21 (3H, d, *J* 6.5, 14-H_3), 1.31 (1H, m, 12-H), 2.00 (1H, app dt, *J* 14.5, 6.5, 9-*H*H), 2.12 (1H, ddd, *J* 14.5, 9, 5, 9-H*H*), 2.22 (3H, d, *J* 1, 15-H_3), 2.45 (1H, ddd, *J* 14.5, 9.5, 1, 4-*H*H), 2.70 (1H, dd, *J* 7.5, 2, 11-H), 2.74 (1H, m, 10-H), 2.78 (1H, m, 4-H*H*), 2.82 (1H, m, 8-H), 3.40 (1H, app dt, *J* 9.5, 2.5, 5-H), 3.69 (3H, s, OMe), 3.71 (1H, dd, *J* 11.5, 3, 16-*H*H), 3.78 (1H, m, 13-H), 4.07 (1H, d, *J* 9.5, 6-H), 4.11 (1H, dd, *J* 11.5, 1, 16-H*H*), 5.79 (1H, m, 2-H); δ_C (100 MHz, $CDCl_3$) 12.4 (C-17), 19.3 (C-15), 20.8 (C-14), 33.1 (C-9), 42.6 (C-12), 43.8 (C-4), 48.8 (C-8), 50.9 (OMe), 54.5 (C-10), 60.8 (C-11), 71.2 (C-16), 71.4 (C-13), 74.5 (C-6), 83.0 (C-5), 117.9 (C-2), 155.5 (C-3), 166.9 (C-1), 209.2 (C-7). Found (ESI): 379.18 $[MNa]^+$, ($C_{18}H_{28}O_7SiNa$ requires 379.1733); *m*/*z* (ESI) 379 ([MNa^+], 100%). *Ketone* **20** as a colourless oil (50 mg, 0.14 mmol, 26%); $[\alpha]_D^{24}$ −22 (*c* 1, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3442 (OH), 2972 and 2857 (CH), 1713 (C=O) and 1644 (C=C); δ_H (400 MHz, $CDCl_3$) 1.13 (3H, d, *J* 7, 17-H_3), 1.73 (2H, app dd, *J* 7.5, 6, 9-H_2), 2.00 (1H, m, 8-H), 2.20 (3H, d, *J* 1, 15-H_3), 2.23 (3H, s, 14-H_3), 2.27 (1H, dd, *J* 15, 9.5, 4-*H*H), 2.41 (1H, app quint, *J* 7, 12-H), 2.60 (1H, br d, *J* 15, 4-H*H*), 2.81 (1H, dd, *J* 7, 2, 11-H), 2.85 (1H, app dt, *J* 6, 2, 10-H), 3.45 (1H, dd, *J* 9, 3.5, 6-H), 3.53 (1H, dd, *J* 12, 2, 16-*H*H), 3.67 (3H, s, OMe), 3.74 (1H, app dt, *J* 9, 3, 5-H), 3.86 (1H, dd, *J* 12, 3, 16-H*H*), 3.91 (1H, app t, *J* 3.5, 7-H), 5.75 (1H, m, 2-H); δ_C (100 MHz, $CDCl_3$) 12.3 (C-17), 19.0 (C-15), 29.4 (C-14), 31.4 (C-9), 39.4 (C-8), 42.8 (C-4), 49.1 (C-12), 50.9 (OMe), 56.2 (C-10), 59.0 (C-11), 65.2 (C-16), 68.9 (C-6), 70.3 (C-7), 74.7 (C-5), 117.1 (C-2), 157.0 (C-3), 167.0 (C-1), 210.0 (C-13).

4.13. Oxidation of methyl pseudomonate 17

Methyl pseudomonate **17** (0.18 g, 0.35 mmol) was dissolved in DCM (100 ml) and saturated $NaHCO_3$ solution (50 ml), KBr (8 mg, 0.07 mmol) and TEMPO (6 mg, 0.04 mmol) was added and the solution left to stir for 0.25 h. NaOCl (0.5 ml, 0.39 mmol) was added and the reaction left to stir for 2 h in which time the colour of the solution changed from orange to a colourless. The two phases were separated and the aqueous layer extracted with DCM (3×30 ml) the organic layers combined, washed with brine (50 ml), dried with $MgSO_4$, filtered and the solvent removed in vacuo. The resulting crude product was purified by flash column chromatography using an eluent system of 60% EtOAc/petrol to give *diketone* **21** as a colourless oil (20 mg, 0.04 mmol, 11%); $[\alpha]_D^{24}$ −5.2 (*c* 2.3, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3416 (OH), 2928 and 2857 (CH), 1712 (C=O) and 1648 (C=C); δ_H (400 MHz, $CDCl_3$) 1.12 (3H, d, *J* 6.5, 17-H_3), 1.29–1.40 (8H, m, 4′-H_2, 5′-H_2, 6′-H_2, 7′-H_2), 1.60–1.69 (4H, m, 8′-H_2, 3′-H_2), 2.03 (1H, m, 9-*H*H), 2.18 (1H, ddd, *J* 14.5, 9.5, 5, 9-H*H*), 2.23 (3H, d, *J* 1, 15-H_3), 2.24 (3H, s, 14-H_3), 2.32 (2H, t, *J* 7.5, 2′-H_2), 2.35 (1H, m, 12-H), 2.46 (1H, dd, *J* 14.5, 9.5, 4-H*H*), 2.78 (1H, m, 10-H), 2.78 (1H, m, 4-*H*H), 2.83 (1H, dd, *J* 8, 2, 11-H), 2.89 (1H, m, 8-H), 3.43 (1H, app td, *J* 9.5, 2.5, 5-H), 3.69 (3H, s, OMe), 3.75 (1H, dd, *J* 12, 3, 16-*H*H), 4.07 (1H, d, *J* 9.5, 6-H), 4.09 (2H, t, *J* 7, 9′-H_2), 4.12 (1H, dd, *J* 12, 1, 16-H*H*), 5.79 (1H, m, 2-H); Found (ESI): 533.28 $[MNa]^+$, ($C_{27}H_{42}O_9Na$ requires 533.2727); *m*/*z* (ESI) 533 ([MNa^+], 100%). *α-hydroxyketone* **4** as a colourless oil (20 mg, 0.04 mmol, 11%); $[\alpha]_D^{24}$ +20 (*c* 0.2, MeOH); ν_{max} (neat)/cm^{-1} 3446 (OH), 2930 and 2856 (CH), 1713 (C=O) and 1648 (C=C); δ_H (400 MHz, $CDCl_3$) 0.89 (3H, d, *J* 6.5, 17-H_3), 1.21 (3H, d, *J* 6, 14-H_3), 1.28–1.37 (9H, m, 4′-H_2, 5′-H_2, 6′-H_2, 7′-H_2, 12-H), 1.59–1.66 (4H, m, 8′-H_2, 3′-H_2), 2.01 (1H, app dt, *J* 14.5, 6, 9-*H*H), 2.12 (1H, ddd, *J* 14.5, 9.5, 5, 9-H*H*), 2.21 (3H, d, *J* 1, 15-H_3), 2.30 (3H, t, *J* 7.5, 2′-H_2), 2.44 (1H, ddd, *J* 15, 9, 1, 4-*H*H), 2.70 (1H, dd, *J* 7.5, 2.5, 11-H), 2.73–2.76 (H, m, 4-HH and 10-H), 2.81 (1H, m, 8-H), 3.40 (1H, app dt, *J* 9, 2.5, 5-H), 3.67 (3H, s, OMe), 3.72 (1H, dd, *J* 12, 3, 16-*H*H), 3.78 (1H, app quint, *J* 6.5, 13-H), 4.07 (1H, d, *J* 9, 6-H), 4.08 (2H, t, *J* 7, 9′-H_2), 4.11 (1H, dd, *J* 12, 1, 16-H*H*), 5.78 (1H, m, 2-H); δ_C (100 MHz, $CDCl_3$) 12.4 (C-17), 19.2 (C-15), 20.8 (C-14), 24.9 (C-3′), 25.9 (C-7′), 28.6 (C-8′), 29.0 and 29.1 (C-4′, C-5′ and C-6′), 33.1 (C-9), 34.0 (C-2′), 42.6 (C-12), 43.9 (C-4), 48.8 (C-8), 51.5 (OMe), 54.5 (C-10), 60.8 (C-11), 63.9 (C-9′), 71.2 (C-13), 71.4 (C-16), 74.9 (C-6), 83.0 (C-5), 118.3 (C-2), 155.1 (C-3), 166.6 (C-1), 174.3 (C-1′), 209.2 (C-7); Found (ESI): 535.29 $[MNa]^+$, ($C_{27}H_{44}O_9Na$ requires 535.2883); *m*/*z* (ESI) 535 ([MNa^+], 100%). 13-*Ketone* **22** as a colourless oil (56 mg, 0.11 mmol, 31%); $[\alpha]_D^{24}$ −29 (*c* 2, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3460 (OH), 2930 and 2858 (CH), 1710 (C=O) and 1646 (C=C); δ_H (400 MHz, $CDCl_3$) 1.14 (3H, d, *J* 7.5, 17-H_3), 1.28–1.37 (8H, m, 4′-H_2, 5′-H_2, 6′-H_2, 7′-H_2),

1.57–1.67 (4H, m, 8′-H_2, 3′-H_2), 1.74 (2H, app t, *J* 6, 9-H_2), 1.98–2.05 (1H, m, 8-H), 2.21 (3H, d, *J* 1, 15-H_3), 2.24 (3H, s, 14-H_3), 2.30 (3H, t, *J* 7.5, 2′-H_2), 2.31 (1H, m, 4-*H*H), 2.42 (1H, app quint, *J* 7.5, 12-H), 2.60 (1H, br d, *J* 14.5, 4-H*H*), 2.81 (1H, dd, *J* 7.5, 2, 11-H), 2.84 (1H, dt, *J* 6, 2, 10-H), 3.47 (1H, dd, *J* 9, 3.5, 6-H), 3.55 (1H, dd, *J* 11.5, 2.5, 16-*H*H), 3.66 (3H, s, OMe), 3.75 (1H, dt, *J* 9, 3, 5-H), 3.88 (1H, dd, *J* 11.5, 3, 16-H*H*), 3.93 (1H, app t, *J* 3.5, 7-H), 4.06 (2H, t, *J* 7, 9′-H_2), 5.75 (1H, m, 2-H); δ_C (100 MHz, $CDCl_3$) 12.4 (C-17), 19.2 (C-15), 25.0 (C-3′), 26.1 (C-7′), 28.8 (C-8′), 29.1 and 29.2 (C-4′, C-5′ and C-6′), 29.5 (C-14), 31.5 (C-9), 34.2 (C-2′), 39.6 (C-8), 43.0 (C-4), 49.2 (C-12), 51.6 (OMe), 56.3 (C-10), 59.1 (C-11), 63.9 (C-9′), 65.4 (C-16), 69.2 (C-6), 70.5 (C-7), 74.9 (C-5), 117.8 (C-2), 156.7 (C-3), 166.9 (C-1), 174.5 (C-1′), 210.0 (C-13); Found (ESI): 535.29 $[MNa]^+$, ($C_{27}H_{44}O_9Na$ requires 535.2883); *m*/*z* (ESI) 535 ($[MNa^+]$, 100%).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2011.05.021.

References and notes

1. Sutherland, R.; Boon, R. J.; Griffith, K. E.; Masters, P. J.; Slocombe, B.; White, A. R. *Antimicrob. Agents Chemother.* **1985**, *27*, 495–498; For a recent review see: Thomas, C. M.; Hothersall, J.; Willis, C. L.; Simpson, T. J. *Nat. Rev. Microbiol.* **2010**, *8*, 281–289.
2. Clayton, J. P.; Oliver, R. S.; Rogers, N. H.; King, T. J. *J. Chem. Soc., Perkin Trans. 1* **1979**, 838–846.
3. El-Sayed, A. K.; Hothersall, J.; Cooper, S. M.; Stephens, E.; Simpson, T. J.; Thomas, C. M. *Chem. Biol.* **2003**, *10*, 419–430; For a recent review see: Piel, J. *Nat. Prod. Rep.* **2010**, *27*, 996–1047.
4. Cooper, S. M.; Cox, R. J.; Crosby, J.; Crump, M.; Hothersall, J.; Laosripaiboon, W.; Simpson, T. J.; Thomas, C. M. *Chem. Commun.* **2005**, 1179–1181; Wu, J.; Cooper, S. M.; Cox, R. J.; Crosby, J.; Hothersall, J.; Simpson, T. J.; Thomas, C. M.; Willis, C. L. *Chem. Commun.* **2007**, 2040–2042; Wu, J.; Hothersall, J.; Mazzetti, C.; O'Connell, Y.; Shields, J. A.; Cox, R. J.; Crosby, J.; Simpson, T. J.; Thomas, C. M.; Willis, C. L. *ChemBioChem* **2008**, *9*, 1500–1508.
5. Hothersall, J.; Wu, J.; Rahman, A. S.; Shields, J. A.; Haddock, J.; Johnson, N.; Cooper, S. M.; Stephens, E. R.; Cox, R. J.; Crosby, J.; Willis, C. L.; Simpson, T. J.; Thomas, C. M. *J. Biol. Chem.* **2007**, *282*, 15451–154614.
6. Clayton, J. P.; O'Hanlon, P. J.; Rogers, N. H.; King, T. J. *J. Chem. Soc., Perkin Trans. 1* **1982**, 2827–2833.
7. Schreiber, J.; Eschenmoser, A. *Helv. Chim. Acta* **1955**, 1529–1536.
8. Silyl protected methyl 7-ketomonate C (with a 10,11-double bond rather than the epoxide) was prepared as an intermediate in the total synthesis of methyl monate C van Innis, L.; Plancher, J. M.; Markó, I. E. *Org. Lett.* **2006**, *8*, 6111–6114.
9. Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155–4156.
10. Griffith, W. P.; Ley, S. V.; Whitcombe, G. P.; White, A. D. *J. Chem. Soc., Chem. Commun.* **1987**, 1625–1627.
11. Omura, K.; Swern, D. *Tetrahedron* **1978**, *34*, 1651–1660.
12. Anelli, P. L.; Biffi, C.; Montanari, F.; Quici, S. *J. Org. Chem.* **1987**, *52*, 2559–2562.
13. Arumugam, P.; Karthikeyan, G.; Perumal, P. T. *Chem. Lett.* **2004**, *33*, 1146–1147.
14. Clayton, J. P.; Luk, K.; Rogers, N. H. *J. Chem. Soc., Perkin Trans. 1* **1979**, 308–313.