The squalestatins: cleavage of the bicyclic core *via* the novel 6,8-dioxabicyclo[3.2.1]octane ring system

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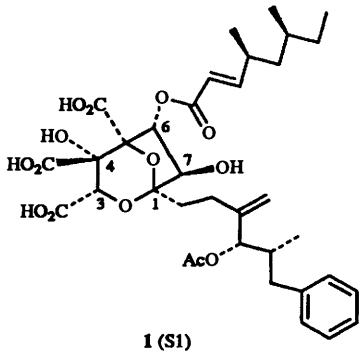
Squalestatin S1 **1** has been converted into its 4,7-bis(2-methoxyethoxymethyl) ether 4,5-dimethyl ester **11** and thence to its 3-(*tert*-butoxycarbonyl)amino derivative **12** *via* a Schmidt degradation. Acid-catalysed hydrolysis of **12** brought about a molecular rearrangement of the 2,8-dioxa- to the novel 6,8-dioxa-bicyclo[3.2.1]octane ring system **3**. Oxidation of **3** followed by methanolysis gave the novel spiroketal **17**. Treatment of **3** with trimethyl phosphonoacetate gave the acyclic derivative **18**.

We have recently described the isolation[1] and structure elucidation[2] of the squalestatins, a novel group of fungal metabolites isolated from a previously unknown *Phoma* species (*coelomycetes*). Squalestatin S1 **1**† is a potent and selective

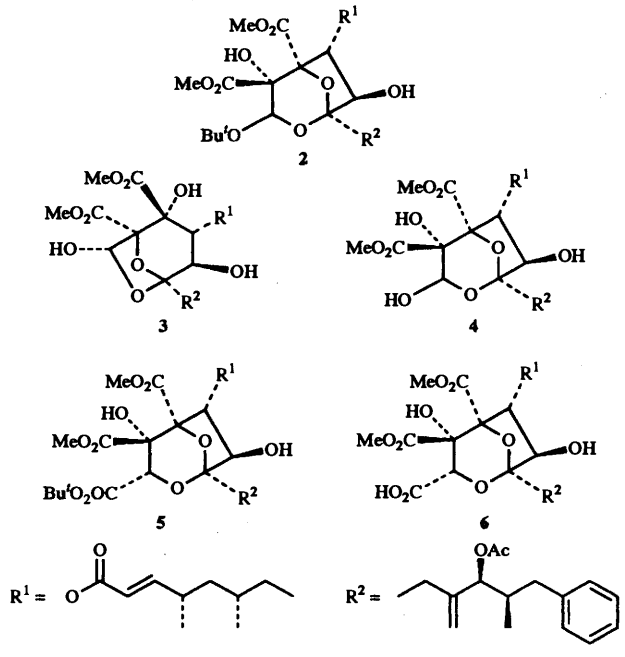


1 (S1)

inhibitor of both rat and *Candida* squalene synthase (SQS); 50% inhibition of rat liver microsomal SQS activity is observed *in vitro* at a concentration of 12 nmol dm^{-3}. Furthermore, when S1 is administered orally to marmosets a 50% reduction in serum cholesterol levels is observed at a dose of 10 mg kg^{-1} day^{-1} for 7 days.[3] In this species S1 has a profound and extended effect on lipids [50–60% decrease in serum cholesterol levels during 7 days after a single intravenous (iv) dose of 1 mg kg^{-1}].[4] Squalestatin **1** incorporates the highly substituted 2,8-dioxabicyclo[3.2.1]octane system possessing carboxylic acid groups at C-3, C-4 and C-5, hydroxy groups at C-4 and C-7, a lipophilic side-chain at C-1, and an α,β-unsaturated ester chain at C-6. Subsequent to our publications, the group at Merck has published the isolation of zaragozic acids, the structure of zaragozic acid A[5,6] being identical with that of squalestatin **1**. More recently the group at Tokyo Noko University–Mitsubishi[7] have also isolated squalestatin **1** from *Setosphaeria khartoumensis*.

As a part of our chemical programme aimed at the modification of the complex squalestatin structure and the identification of the key structural features responsible for the biological activity, we have reported on the C-1 chain-length requirements,[8] on the role of the tricarboxylic acid moiety,[9] on C-6 and C-7 modifications,[10] on the 6,7-dideoxy,[11] 3-decarboxy,[12] monocyclic,[13] acyclic[14] and 3-hydroxymethyl[15] analogues, and on modifications at the allylic centre.[16] In this paper, we report an improved procedure for the rearrangement of the squalestatin core to the 6,8-dioxabicyclo[3.2.1]octane ring system, and our attempts to cleave the ketal moiety of the squalestatin nucleus leading to a novel spiroketal or an acyclic derivative.

In one of our earlier communications[1,2] we have reported on the acid catalysed cleavage and subsequent rearrangement of the 3-*tert*-butoxy derivative **2** to the novel lactol **3** (56%).

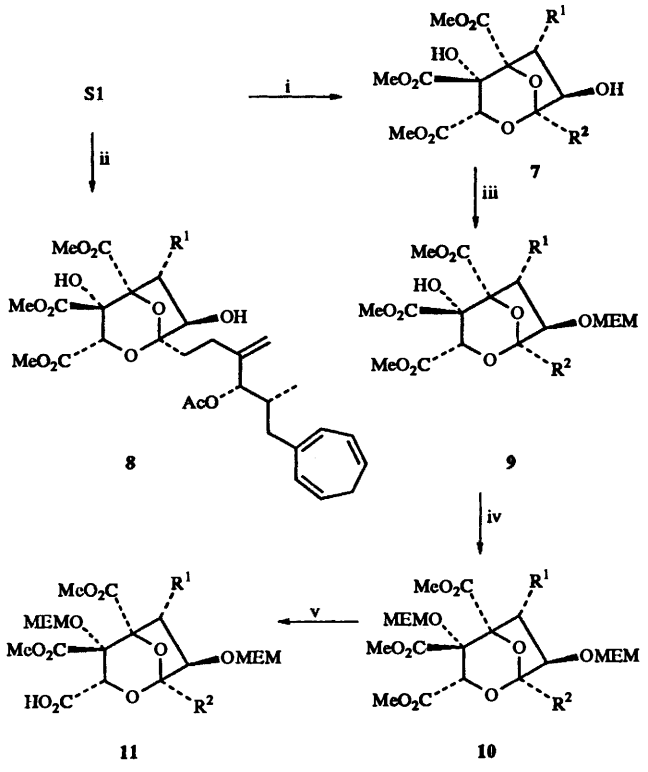


Mechanistically, this transformation is thought to proceed *via* the intermediate lactol **4** to the thermodynamically favoured six-membered lactol **3** by transketalisation. The *tert*-butoxy derivative **2** was obtained as a by-product (15%) of the photolysis of the peroxy ester **5**. Alternatively, lactol **3** was obtained as an unexpected product (10%) during an attempted Barton decarboxylation[17] of the 3-carboxylic acid **6**.

We envisaged that Schmidt degradation of the carboxylic acid **6** would provide an aminal at C-3 which on hydrolysis should give the lactol **3** *via* **4** in a more direct and efficient manner. The trimethyl ester **7** was originally obtained by diazomethane treatment of S1 in methanol.[2] Although this esterification method generally worked very well, on one occasion, when excess diazomethane was used over a prolonged

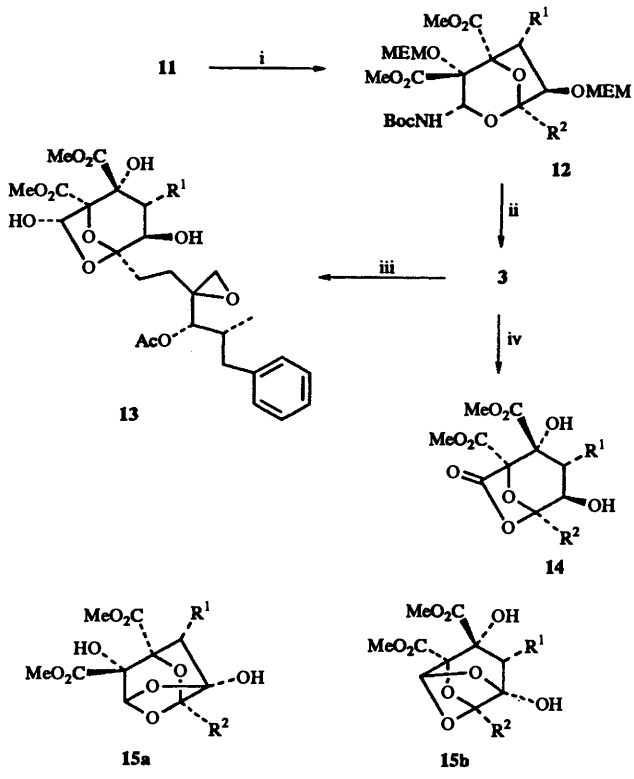
† Systematic name: (1*S*,3*S*,4*S*,5*R*,6*R*,7*R*)-1-{3-[(1*S*,2*R*)-1-acetoxy-2-benzylpropyl]but-3-enyl}-4,7-dihydroxy-6-{[(2*E*,4*S*,6*S*)-4,6-dimethyloct-2-enoyl]oxy}-2,8-dioxabicyclo[3.2.1]octane-3,4,5-tricarboxylic acid.

reaction time, a complex mixture was obtained from which the cycloheptatriene **8** was isolated (3%) presumably *via* carbene insertion[18] (Scheme 1). This complication, coupled with the



Scheme 1 *Reagents:* i, $NaHCO_3$, MeI, DMF; ii, CH_2N_2, MeOH; iii, MEM-Cl, Pr^i_2NEt, $ClCH_2CH_2Cl$; iv, NaH, MEM-Cl, DMF; v, NaOH, THF, H_2O

toxicity and explosive nature of diazomethane, led us to the development of an alternative procedure. Thus, treatment of S1 with sodium hydrogen carbonate and iodomethane in *N,N*-dimethylformamide (DMF) provided the trimethyl ester **7** routinely in high yield (90%) and on large scales (> 50 g). Before the Schmidt degradation was attempted the free hydroxy groups were protected in order to avoid possible reaction of the 4-hydroxy group with the derived acyl azide or isocyanate group at C-3. Thus, treatment of diol **7** with an excess of 2-methoxyethoxymethyl chloride (MEM-Cl) in the presence of diisopropylethylamine in 1,2-dichloroethane provided selectively the 7-MEM derivative **9** (75%); the only other minor product formed was the 4,7-bis(MEM) derivative **10**. The mono-MEM derivative **9** was smoothly converted into **10** (90%) using excess MEM-Cl and sodium hydride in DMF. Selective saponification of the trimethyl ester **10** to the dimethyl ester **11** was achieved using aqueous dilute sodium hydroxide in tetrahydrofuran.[9] The carboxylic acid **11** was found to be unstable to storage over a period of a few days in the cold (3 °C). Schmidt degradation of **11** with diphenylphosphoryl azide[19] and triethylamine in the presence of *tert*-butyl alcohol gave the expected *tert*-butoxycarbonylamino derivative **12** (48%) which on hydrolysis with aqueous formic acid provided the lactol **3** (57%) as a single anomer (Scheme 2). The structure of **3** was deduced from NMR studies. The unusually large coupling constant (*J* 9 Hz) between 3-H and 4-H (Fig. 1) suggested their *trans*-diaxial relationship in a chair conformation of a six-membered ring as opposed to the usual coupling constant of 2 Hz between 6-H and 7-H in the normal squalestatin core. A strong NOE between 3-H and 7-H showed their close proximity and confirmed the anomeric configuration shown. The alternative ring system in **3** was established by an



Scheme 2 *Reagents:* i, $(PhO)_2PON_3$, Et_3N, Bu^tOH; ii, HCO_2H, H_2O; iii, NIS, Bu_4NI, CH_2Cl_2; iv, Jones reagent

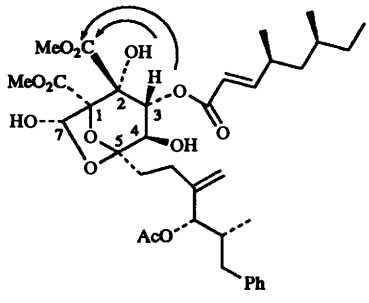


Fig. 1 Key long range ^1H–^{13}C correlations in **3**

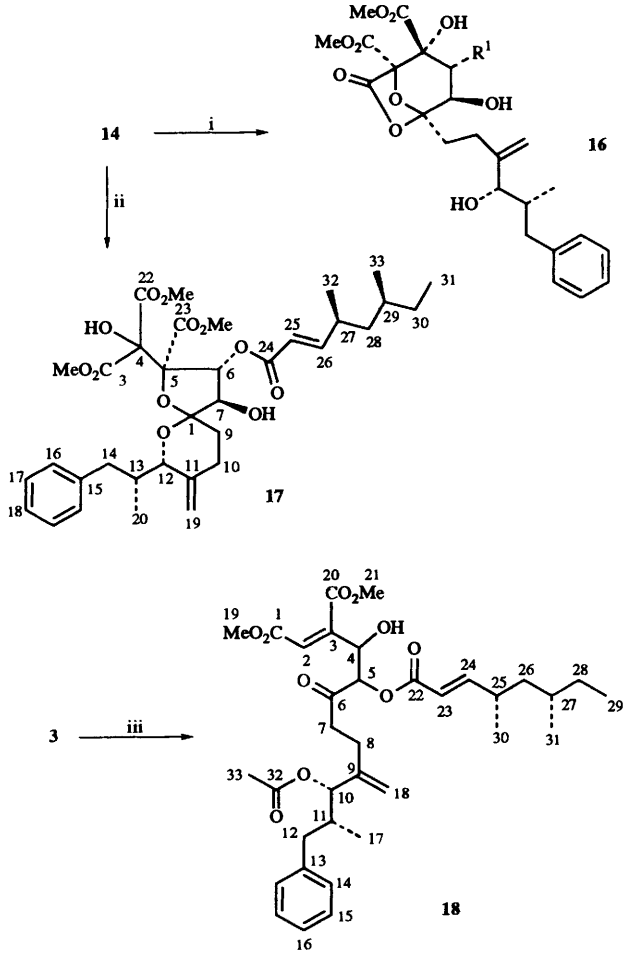
inverse detected heteronuclear multiple bond correlation (HMBC) experiment optimised for long range couplings of 6 Hz. The key long range ^1H–^{13}C correlations are summarised in Fig. 1. Additionally lactol **3** was totally unreactive towards oxidation by sodium periodate or lead tetraacetate confirming thus the absence of the 1,2-diol moiety found in the alternative structure **4**.

Having established the identity of lactol **3** and an improved procedure for its preparation, we examined its potential as an intermediate towards the synthesis of monocyclic squalestatin analogues. We envisaged that selective oxidation of the anomeric hydroxy group would provide a γ-lactone which on transesterification with methanol would give a monocyclic squalestatin derivative. Reaction between lactol **3** and *N*-iodosuccinimide in the presence of tetrabutylammonium iodide,[20] a reagent which we have used previously quite successfully for the selective oxidation of lactols to lactones[21] in the presence of other hydroxy groups, gave the epoxide **13** as a 2:1 mixture of diastereoisomers. Oxidation of lactol **3** with Jones reagent provided the γ-lactone **14** (40%), characterised by its high IR carbonyl absorption at 1815 cm^{-1}, and an unstable byproduct (10%) which was tentatively assigned as having the tricyclic core shown in compounds **15a** and **15b**. The molecular

formula of **15**, $C_{36}H_{48}O_{13}$, was determined from its high resolution mass spectrum and suggested an oxidation product. The ^{13}C NMR spectrum in $CDCl_3$ indicated an additional ketal type tertiary carbon atom at δ 108.1 and the loss of a secondary alcohol carbon. The two side chains were intact, and two tertiary hydroxy groups were observed at δ 3.82 and 4.99 in the ^1H NMR spectrum. An HMBC experiment revealed a correlation between the acetal proton at δ 5.40 and the ketal carbon at 108.1 which suggests that these atoms must be within a three bond vicinity. Hence this product must be a tricyclic derivative and both structures **15a** and **15b** are compatible with the data. Mechanistically this product is formed by oxidation of the secondary hydroxy group, followed by ketalisation with the anomeric hydroxy group.

Oxidation of **3** using tetrapropylammonium perruthenate–*N*-methylmorpholine-*N*-oxide in acetonitrile in the presence of 4 Å sieves[22] was less clean and gave a lower yield of the γ-lactone **14** than the Jones oxidation.

Attempts to open the lactone **14** using dry methanol and sodium hydrogen carbonate,[23] gaseous ammonia in tetrahydrofuran, or pyrrolidine in dichloromethane all gave more polar complex mixtures from which no discrete products could be isolated. Treatment of lactone **14** with thionyl chloride in methanol provided selectively the allylic alcohol **16** (Scheme 3).



Scheme 3 *Reagents:* i, $SOCl_2$, MeOH; ii, camphorsulfonic acid, MeOH; iii, NaH, $(MeO)_2POCH_2CO_2Me$, THF

Similar transesterification of the allylic acetate with methanol and hydrochloric acid was observed previously.[2,15] Reaction of lactone **14** with methanol in the presence of (±)-camphorsulfonic acid gave the same allylic alcohol **16** which on heating to reflux for 5 days gave a new product identified by a series of NMR experiments as the spiroketal **17**. The high resolution mass spectrum indicated a molecular formula of $C_{35}H_{48}O_{12}$, which corresponds to a gain of a methyl group. Examination of the ^1H and ^{13}C NMR spectra (Table 1) showed the presence of three methyl esters and the retention of a ketal carbon. These data are in accord with methanolysis of the lactone **16** to give a trimethyl ester and the participation of the allylic alcohol in the transketalisation at C-1. The absence of coupling between 6-H and 7-H and the ^{13}C shift of C-5 (assigned by a long range correlation from 7-H) suggested that transketalisation back to a five-membered ring had occurred. In order to obtain confirmation, NMR spectra were obtained in $[^2H_6]$dimethyl sulfoxide ($[^2H_6]$DMSO). Secondary isotope multiplet NMR of partially labelled entities (SIMPLE)[24,25] was then applied. The observation of three-bond secondary deuterium isotope shifts (0.050 and 0.036 ppm) for the ^{13}C signals of two of the methyl ester carbonyls served to establish the ring contraction.

Wadsworth–Emmons reaction of the lactol **3** was envisaged to provide first an olefin at the hemiacetal centre, unmasking thus the C-1 ketone moiety which could then react further to give a product possessing most of the squalestatin functionality except its bicyclic core. Thus, treatment of lactol **3** with trimethyl phosphonoacetate and sodium hydride in tetrahydrofuran provided a mixture from which the major component **18** (30% by HPLC) was isolated in 11% as a single diastereoisomer. The molecular formula of this product, $C_{35}H_{48}O_{10}$, was determined from its high resolution mass spectrum. Examination of the 1D ^1H and ^{13}C NMR data (Table 2) showed that the side chains were intact, two methyl esters were present, and that the core to which these were all linked contained a trisubstituted double

Table 1 ^1H and ^{13}C NMR data in $CDCl_3$ for compound **17**[a]

Position[b]	δ_C	δ_H
1	110.8	—
3	169.1	—
4	81.2	—
5	91.9	—
6	79.3	6.38 (s)
7	81.1	4.01 (s)
9	30.6	1.97 (ddd, *J* 5, 11.5, 13.5), 1.82 (dt, *J* 13.5, 5)
10	29.2	2.44 (m), 2.30 (m)
11	145.1	—
12	75.1	4.76 (d, *J* 3.5)
13	36.9	2.19 (m)
14	40.6	2.48 (dd, *J* 10.5, 13.5), 2.97 (dd, *J* 3.5, 13.5)
15	141.7	—
16	129.0	7.20 (m)
17	128.0	7.28 (t, *J* 8)
18	125.5	7.20 (m)
19	107.6	4.81 (s), 4.92 (s)
20	12.8	0.80 (d, *J* 7)
22	166.4	—
23	167.5[c]	—
24	164.4	—
25	118.2	5.70 (d, *J* 16)
26	156.5	6.87 (d, *J* 8, 16)
27	36.9	2.36 (m)
28	43.0	1.07 (m), 1.33 (m)
29	31.7	1.29 (m)
30	29.5	1.08 (m), 1.27 (m)
31	10.9	0.82 (t, *J* 7)
32	20.1	0.95 (d, *J* 7)
33	18.8	0.81 (d, *J* 7)
3-OMe	54.3	3.92 (s)
22-OMe	53.1	3.82 (s)
23-OMe	52.1	3.69 (s)

[a] Non-standard numbering has been used in the assignment of the NMR data. See structure **17** for numbering scheme used. [b] Assignments are based on DEPT, HMQC and HMBC spectra in addition to the normal 1D data. [c] HMBC spectrum shows correlation to 6-H.

Table 2 ^1H and ^{13}C NMR data in $CDCl_3$ for compound **18**[a]

Position[b]	δ_C	δ_H
1	165.2	—
2	124.1	6.36 (d, *J* 2)
3	144.2	—
4	70.5	5.01 (m)
5	77.7	5.27 (d, *J* 3)
6	205.2	—
7	37.4	2.83 (dt, *J* 19, 7), 2.61 (ddd, *J* 19, 7, 6)
8	25.2	2.39 (m), 2.27 (dt, *J* 16, 7)
9	144.7	—
10	78.7	5.01 (d, *J* 5)
11	36.8	2.07 (m)
12	39.8	2.69 (dd, *J* 13, 5), 2.37 (dd, *J* 13, 9)
13	140.1	—
14	128.9	7.13 (d, *J* 7)
15	128.2	7.29 (t, *J* 7)
16	125.9	7.19 (t, *J* 7)
17	13.6	0.84 (d, *J* 7)
18	111.9	4.97 (s), 4.94 (s)
19	51.9	3.75 (s)
20	166.1	—
21	52.4	3.80 (s)
22	165.5	—
23	117.6	5.86 (dd, *J* 16, 1)
24	157.5	6.95 (dd, *J* 16, 8)
25	34.3	2.45 (m)
26	43.1	1.44–1.22 (m), 1.18–1.08 (m)
27	31.7	1.44–1.22 (m)
28	29.7	1.44–1.22 (m), 1.18–1.08 (m)
29	10.9	0.85 (t, *J* 7)
30	19.8	1.05 (d, *J* 7)
31	18.8	0.85 (d, *J* 7)
32	170.4	—
33	20.9	2.10 (s)
4-OH	—	3.21 (d, *J* 7)

[a] Non-standard numbering has been used in the assignment of the NMR data. See structure **18** for numbering system used. [b] Assignments are based on DEPT, HETCOR and HMBC spectra in addition to the normal 1D data.

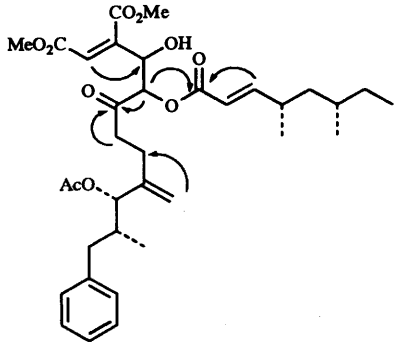
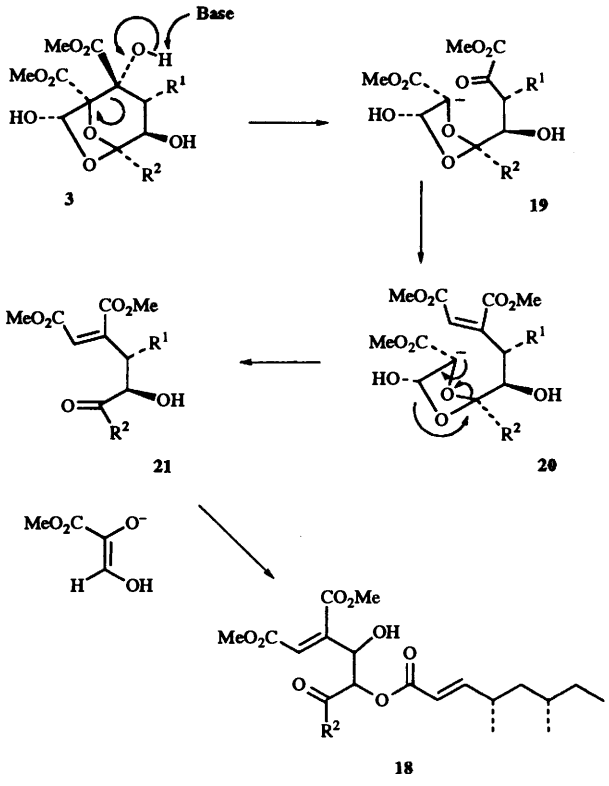


Fig. 2 Key long range ^1H–^{13}C correlations in **18**

bond. In addition two CH–O groups and a ketone carbonyl were also present. Proton homonuclear decoupling established that the allylic coupling (2 Hz) from 2-H was to the CH of the secondary alcohol group. This in turn was coupled to the other CH–O group. Analysis of the ^1H–^{13}C correlation data (both one bond and long range) enabled the full structure to be put together (Fig 2). The geometry of the double bond follows from the chemical shift of 2-H and was confirmed by the observation of an NOE at 4-H when 2-H was irradiated. A plausible mechanism for the formation of **18** is outlined in Scheme 4. The first step is envisaged to be a retro-Claisen condensation to form ketone **19**, reaction of **19** with the Wadsworth–Emmons reagent to produce olefin **20**, which then breaks down to ketone **21**, followed by a 1,2-migration of the octenoate side chain to provide **18**.

Finally, we attempted to deprotect the dimethyl esters of



Scheme 4

γ-lactone **14** and spiroketal **17** using lithium iodide in 2,4,6-collidine (2,4,6-trimethylpyridine) but under these conditions no discrete products were isolated. In conclusion methodology has been developed for the cleavage of the bicyclic core of the squalestatins *via* the intermediacy of lactol **3** under both acidic conditions to provide the spiroketal **17** and under basic conditions to provide the acyclic derivative **18**.

Experimental

Organic solutions were dried over $MgSO_4$, and column chromatography was performed on silica gel 60 (Merck, Art no. 9385). Ether refers to diethyl ether. Analytical and preparative HPLC were performed on Spherisorb 5 ODS-2 columns. IR spectra were recorded on a Nicolet 5SXC or a Bio-Rad FTS-7 FTIR spectrometer. NMR spectra were recorded on Bruker AM 500, AM 250 or Varian VXR 400 spectrometers using standard pulse sequences. Chemical shifts are expressed as parts per million downfield from internal tetramethylsilane. All *J* values are in Hz. Samples for SIMPLE NMR were made up as previously described.[2] Electron-impact mass spectrometry (EI 70 eV) was performed on a Finningan MAT 8400, positive ammonia chemical-ionisation (CI), desorption chemical-ionisation (DCI) and fast-atom-bombardment (FAB) on a VG Autospec spectrometer. High resolution mass spectrometry was conducted on a Kratos Concept or a VG Autospec spectrometer.

Trimethyl ester of 1

A suspension of the tricarboxylic acid **1** (50 g, 72.4 mmol) and sodium hydrogen carbonate (54.72 g, 652 mmol) in DMF (500 cm^3) was treated with methyl iodide (40.6 cm^3, 652 mmol) and the mixture was stirred at 20 °C for 2 days. The reaction mixture was filtered and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in ethyl acetate and washed with water, dilute aq. hydrochloric acid and brine, dried and then chromatographed on silica gel eluting with ethyl acetate–cyclohexane (1 : 2) to give the trimethyl ester **7** (47.75 g,

90%) as a white foam; $\nu_{max}(CHBr_3)/cm^{-1}$ 3547, 1770, 1735 and 1648; δ_H(250 MHz; $CDCl_3$) 0.8–0.9 (9 H, m, CH_3), 1.04 (3 H, d, *J* 7, =CHCHCH_3), 1.08–1.4 (5 H, m), 2.10 (3 H, s, AcO), 2.06–2.5 (7 H, m), 2.70 (1 H, dd, *J* 14 and 5, CH_2Ph), 3.3 (1 H, br, 7-OH), 3.76, 3.81 and 3.93 (3 H each, s, CO_2CH_3), 3.84 (1 H, s, 4-OH), 4.05 (1 H, d, *J* 2, 7-H), 4.97 and 5.00 (1 H each, s, =CH_2), 5.10 (1 H, d, *J* 5, C*H*OAc), 5.26 (1 H, s, 3-H), 5.75 (1 H, d, *J* 16, CH=C*H*CO_2), 5.81 (1 H, d, *J* 2, 6-H), 6.84 (1 H, dd, *J* 16 and 9, C*H*=CHCO$_2$) and 7.1–7.3 (5 H, m, Ph).

Treatment of 1 with diazomethane

A solution of **1** (151 mg, 0.22 mmol) in methanol (4 cm^3) was treated with an excess of diazomethane in ether (0.4 mol dm^{-3}; 20 cm^3) and the solution was allowed to stand at 20 °C for 15 h whilst the solvents were allowed to evaporate. The residue was chromatographed on silica gel eluting with methanol–dichloromethane (1:49) and rechromatographed on silica eluting with ethyl acetate–cyclohexane (1:1) to give the trimethyl ester **7** (97 mg, 60%) and a mixture (30 mg). This mixture was purified further by HPLC on a Spherisorb 5 ODS-2 column (15 × 0.46 cm) eluting with 80% MeCN–H_2O) at a flow rate of 0.25 cm^3 min^{-1}, detecting at 210 nm and collecting the component with a t_r of 11.5 min. The appropriate fractions were combined and the MeCN was removed under reduced pressure. The aqueous residue was extracted with ethyl acetate and the organic phase was washed with brine, dried and then evaporated to give cycloheptatriene **8** as a gum (5 mg, 3%); δ_H(500 MHz; $CDCl_3$) 0.80–0.89 (9 H, m, CH_3), 1.04 (3 H, d, *J* 7, =CHCHCH_3), 1.08–1.19 (2 H, m), 1.24–1.4 (3 H, m), 1.96 (1 H, m), 2.08 (3 H, s, AcO), 2.08–2.49 (9 H, m), 3.27 (1 H, br, 7-OH), 3.76, 3.81 and 3.93 (3 H each, s, CO_2CH_3), 3.82 (1 H, s, 4-OH), 4.08 (1 H, d, *J* 2, 7-H), 4.96 (2 H, s, =CH_2), 5.05 (1 H, d, *J* 5, C*H*OAc), 5.26 (1 H, s, 3-H), 5.33 and 5.39 (1 H each, dt, *J* 10 and 6), 5.74 (1 H, dd, *J* 16 and 1, CH=C*H*CO_2), 5.82 (1 H, d, *J* 2, 6-H), 6.01 (1 H, d, *J* 10), 6.10 (1 H, dd, *J* 10 and 5), 6.34 (1 H, d, *J* 5) and 6.83 (1 H, dd, *J* 16 and 9, C*H*=CHCO$_2$); *m/z* (EI) 746 ($M^{+\cdot}$).

Preparation of the 7-(2-methoxyethoxymethyl) ether 9

Diol **7** (18.9 g, 25.79 mmol) was dissolved in dichloroethane (190 cm^3), diisopropylethylamine (22.8 cm^3, 130 mmol) and 2-methoxyethoxymethyl chloride (14.7 cm^3, 129 mmol) and the mixture was heated under reflux for 20 h. The mixture was diluted with aqueous citric acid (1 mol dm^{-3}; 160 cm^3) and brine (40 cm^3) and extracted with ether (400 cm^3). The organic phase was washed with aqueous citric acid, water, aqueous sodium hydrogen carbonate and brine, dried, evaporated to a gum (32.2 g) and then chromatographed on silica gel eluting with toluene–tetrahydrofuran–methanol (20:1:1) to give 7-(2-methoxyethoxymethyl) ether **9** (15.86 g, 75%) [Found: (DCI/NH_3) $(M + NH_4)^+$, 838.4259. $C_{42}H_{60}O_{16}$ requires ($M + NH_4$), 838.4225] (Found: C, 60.4; H, 7.4. $C_{42}H_{64}NO_{16}$·0.75H_2O requires C, 60.5; H, 7.4%); ν_{max}(KBr)/cm^{-1} 3474, 1770, 1732 and 1657; δ_H(250 MHz; $CDCl_3$), 0.8–0.9 (9 H, m, CH_3), 1.03 (3 H, d, *J* 7.5, =CHCHCH_3), 1.03–1.4 (5 H, m), 2.10 (3 H, s, AcO), 2.02–2.50 (7 H, m), 2.72 (1 H, dd, *J* 14 and 5.5, CH_2Ph), 3.34 (3 H, s, CH_3OCH$_2$), 3.5 (2 H, m), 3.70, 3.76, 3.95 (3 H each, s, CO_2CH_3), 3.6–3.8 (2 H, m), 3.84 (1 H, s, 4-OH), 4.14 (1 H, d, *J* 2, 7-H), 4.81 and 4.92 (1 H each, d, *J* 7.5, OCH_2O), 4.99 and 5.02 (1 H each, s, =CH_2), 5.13 (1 H, d, *J* 5, C*H*OAc), 5.23 (1 H, s, 3-H), 5.72 (1 H, d, *J* 16, CH=C*H*CO_2), 6.40 (1 H, d, *J* 2, 6-H), 6.83 (1 H, dd, *J* 16 and 8.5, C*H*=CHCO$_2$) and 7.1–7.3 (5 H, m, Ph); *m/z* (DCI/NH_3) 838 $(M + NH_4)^+$.

Preparation of 4,7-bis(2-methoxyethoxymethyl) ether 10

The 7-MEM ether **9** (21.17 g, 25.8 mmol) in DMF (250 cm^3) was treated with sodium hydride (60% oil dispersion; 1.12 g, 28 mmol) under nitrogen at 0 °C, followed after 30 min by the addition of 2-methoxyethoxymethyl chloride (8 cm^3, 70 mmol). The temperature was allowed to rise to 20 °C and the mixture was stirred for 4 days. The mixture was diluted with aqueous citric acid and extracted with ether (3 × 350 cm^3). The organic phase was washed with water and brine, dried, evaporated and then chromatographed on silica gel eluting with toluene–tetrahydrofuran–methanol (20:1:1) to give 4,7-bis(2-methoxyethoxymethyl) ether **10** as a gum (21.1 g, 90%) (Found: C, 58.5; H, 7.4. $C_{46}H_{68}O_{18}$·2H_2O requires C, 58.5; H, 7.7%); ν_{max}(KBr)/cm^{-1} 1767, 1738, 1731 and 1650; δ_H(250 MHz; $CDCl_3$) 0.8–0.9 (9 H, m, CH_3), 1.02 (3 H, d, *J* 7.5, =CHCHCH_3), 1.05–1.42 (5 H, m), 2.0–2.5 (7 H, m), 2.11 (3 H, s, AcO), 2.71 (1 H, dd, *J* 14 and 5, CH_2Ph), 3.30 and 3.34 (3 H each, s, CH_3OCH$_2$), 3.5 (4 H, m), 3.70 (3 H, s, CO_2CH_3), 3.7–3.85 (4 H, m), 3.76 (3 H, s, CO_2CH_3), 3.88 (3 H, s, CO_2CH_3), 4.10 (1 H, d, *J* 2, 7-H), 4.8 and 4.92 (1 H each, d, *J* 7.5, OCH_2O), 4.98 and 5.02 (1 H each, s, =CH_2), 5.05–5.13 (3 H, m), 5.30 (1 H, s, 3-H), 5.70 (1 H, d, *J* 16, CH=C*H*CO_2), 6.55 (1 H, d, *J*, 2, 6-H), 6.82 (1 H, dd, *J* 16 and 8, C*H*=CHCO$_2$) and 7.1–7.3 (5 H, m, Ph); *m/z* (DCI/NH_3) 926 $(M + NH_4)^+$.

Preparation of carboxylic acid 11

The trimethyl ester **10** (23 g, 25 mmol) in tetrahydrofuran (230 cm^3) was treated with aqueous sodium hydroxide (0.1 mol dm^{-3}; 250 cm^3) and the mixture was stirred at 20 °C for 16 h. The mixture was then poured into water (300 cm^3), acidified with aqueous citric acid and extracted with ether (3 × 200 cm^3). The organic phase was washed with water (300 cm^3), dried, evaporated and then chromatographed on silica gel eluting with chloroform–methanol (20:1, 10:1) to give carboxylic acid **11** as a gum (11.4 g, 51%) (Found: C, 57.9; H, 7.35. $C_{45}H_{66}O_{18}$·2H_2O requires C, 58.05; H, 7.6%); $\nu_{max}(CHBr_3)/cm^{-1}$ 3400–2500 and 1733; δ_H(250 MHz; $CDCl_3$), 0.8–0.9 (9 H, m, CH_3), 1.01 (3 H, d, *J* 7, =CHCHCH_3), 1.05–1.4 (5 H, m), 2.10 (3 H, s, AcO), 2.0–2.5 (7 H, m), 2.70 (1 H, dd, *J* 14 and 5, CH_2Ph), 2.8–3.1 (1 H, br, OH), 3.32 (6 H, s, CH_3OCH$_2$), 3.3–3.4 (2 H, m), 3.4–3.58 (2 H, m), 3.65 (3 H, s, CO_2CH_3), 3.65–4.0 (4 H, m), 3.84 (3 H, s, CO_2CH_3), 4.09 (1 H, br s, 7-H), 4.75 and 4.87 (1 H each, d, *J* 7, OCH_2O), 4.96 and 4.99 (1 H each, s, =CH_2), 5.0–5.15 (3 H, m), 5.25 (1 H, br s, 3-H), 5.70 (1 H, d, *J* 15, CH=C*H*CO_2), 6.50 (1 H, br s, 6-H), 6.80 (1 H, dd, *J* 15 and 7, C*H*=CHCO$_2$) and 7.1–7.3 (5 H, m, Ph).

Schmidt degradation of carboxylic acid 11

A suspension of the carboxylic acid **11** (1.1 g, 1.23 mmol) in *tert*-butyl alcohol (14 cm^3) and triethylamine (0.21 cm^3, 1.51 mmol) was treated with diphenylphosphoryl azide (0.32 cm^3, 1.5 mmol) and the mixture was heated to reflux under nitrogen for 15 h. The suspension was concentrated under reduced pressure and the residue was chromatographed on silica gel eluting with ethyl acetate–cyclohexane (1:3) to give 3-(*tert*-butoxycarbonyl)amino derivative **12** (576 mg, 48%) as a gum [Found: (CI) $(M + H)^+$, 966.5062. $C_{49}H_{76}NO_{18}$ requires ($M + H$), 966.5057]; $\nu_{max}(CHBr_3)/cm^{-1}$ 1727; δ_H(400 MHz; $CDCl_3$), 0.8–0.9 (9 H, m, CH_3), 1.02 (3 H, d, *J* 7, =CHCHCH_3), 0.9–1.4 (5 H, m), 1.44 (9 H, s, Bu^{t}OCO), 2.0–2.45 (7 H, m), 2.10 (3 H, s, AcO), 2.70 (1 H, dd, *J* 14 and 6, CH_2Ph), 3.34 (6 H, s, CH_3OCH$_2$), 3.34–3.6 (4 H, m), 3.70 and 3.83 (3 H each, s, CO_2CH_3), 3.7–4.0 (4 H, m), 4.09 (1 H, br s, 7-H), 4.80–5.03 (5 H, m), 5.06 (1 H, d, *J* 5, C*H*OAc), 5.33 (1 H, d, *J* 5.5), 5.71 (1 H, d, *J* 16, CH=C*H*CO_2), 6.03 (1 H, d, *J* 10, 3-H), 6.45 (1 H, d, *J* 10, NH), 6.61 (1 H, br s, 6-H), 6.82 (1 H, dd, *J* 16 and 7.5, C*H*=CHCO$_2$) and 7.1–7.3 (5 H, m, Ph); *m/z* (DCI/NH_3) 983 $(M + NH_4)^+$.

Formic acid hydrolysis of 3-(*tert*-butoxycarbonyl)amino derivative 12

The Boc protected amine **12** (560 mg, 0.58 mmol) was dissolved in formic acid (20.2 cm^3) and water (6.7 cm^3) and the mixture was heated at 65–70 °C for 2 h. The reaction mixture was

concentrated under reduced pressure and the residue was dissolved in toluene (75 cm^3) and evaporated to dryness. This process was repeated three times until all the water had been removed. The resulting yellow gum (400 mg) was chromatographed on silica gel eluting with ethyl acetate–cyclohexane (1:3) to give lactol **3** (227 mg, 57%) as a gum [Found: (FAB +ve) $(M + H)^+$, 691.3329. $C_{36}H_{51}O_{13}$ requires (M + H), 691.3306]; $\nu_{max}(CHBr_3)/cm^{-1}$ 3477, 1736 and 1647; δ_H(500 MHz; [$^2H_6$]DMSO) 0.80 (3 H, d, J 7, $CHCH_3$), 0.84 (3 H, t, J 7, CH_2CH_3), 0.86 (3 H, d, J 7, $CHCH_3$), 1.01 (3 H, d, J 7, =CHCHCH_3), 1.1–1.4 (5 H, m), 1.87–2.04 (2 H, m), 2.11 (3 H, s, AcO), 2.10–2.21 (3 H, m), 2.4–2.5 (2 H, m), 2.64 (1 H, dd, J 14 and 6, CH_2Ph), 3.55 (1 H, dd, J 9 and 7, C*H*OH), 3.63 and 3.66 (3 H each, s, CO_2CH_3), 4.94 and 4.95 (1 H each, s, =CH_2), 5.01 (1 H, d, J 5, C*H*OAc), 5.22 (1 H, d, J 9, C*H*OCOCH=CH), 5.54 (1 H, d, J 7, OH), 5.83 (1 H, d, J 16, CH=C*H*CO_2), 5.92 (1 H, br s, OH), 6.06 (1 H, d, J 5, OC*H*OH), 6.80 (1 H, dd, J 16 and 9, C*H*=$CHCO_2$), 7.15 (1 H, d, J 5, OCHO*H*), 7.19 (2 H, d, J 8, *o*-Ph), 7.20 (1 H, t, J 8, *p*-Ph) and 7.30 (2 H, t, J 8, *m*-Ph); δ_C(125 MHz; [$^2H_6$]DMSO) 170.4 (s), 169.6 (s), 165.5 (s), 164.7 (s), 155.3 (d), 146.3 (s), 140.0 (s), 128.9 (d), 128.3 (d), 125.9 (d), 119.0 (d), 110.6 (t), 109.7 (s), 94.2 (d), 91.4 (s), 77.9 (d), 77.4 (s), 74.6 (d), 70.0 (d), 52.6 (q), 52.1 (q), 42.6 (t), 39.3 (t), 35.9 (d), 33.6 (d), 31.4 (d), 30.1 (t), 29.2 (t), 24.6 (t), 20.8 (q), 20.0 (q), 18.7 (q), 13.6 (q) and 11.0 (q); m/z (DCI/NH_3) 708 $(M + NH_4)^+$.

Preparation of epoxide 13

The lactol **3** (30 mg, 43 μmol) in dichloromethane (0.25 cm^3) was treated with a solution of *N*-iodosuccinimide (48.4 mg, 0.21 mmol) and tetrabutylammonium iodide (15.9 mg, 43 μmol) in dichloromethane (0.75 cm^3) and the resulting brown mixture was stirred for 70 h at 20 °C. The reaction mixture was diluted with dichloromethane (19 cm^3) and washed with saturated aqueous sodium thiosulfate (7 cm^3), water (3 × 7 cm^3) and brine (7 cm^3). The organic phase was dried and chromatographed on silica gel eluting with ethyl acetate–cyclohexane (1:3, 1:1) to give the epoxide **13** as a 2:1 mixture of diastereoisomers (14.7 mg, 69%) [Found: (CI) $(M + H)^+$, 707.3254. $C_{36}H_{51}O_{14}$ requires (M + H), 707.3279]; δ_H(400 MHz; $CDCl_3$) major diastereoisomer: 0.8–0.9 (9 H, m, CH_3), 1.02 (3 H, d, J 7, =CHCHCH_3), 1.1–1.47 (5 H, m), 2.07 (3 H, s, AcO), 1.84–2.88 (8 H, m), 2.68 and 2.82 (2 H, 2 d, J 5, epoxide-CH_2), 3.68 (1 H, t, J 8, C*H*OH), 3.77 and 3.88 (3 H each, s, CO_2CH_3), 4.05 (1 H, br, OCHO*H*), 4.97 (1 H, d, J 4, C*H*OAc), 5.28 (1 H, d, J 8, C*H*OCOCH=CH), 5.78 (1 H, d, J 16, CH=C*H*CO_2), 6.19 (1 H, d, J 6, OC*H*OH), 6.90 (1 H, dd, J 16 and 8, C*H*=$CHCO_2$) and 7.1–7.3 (5 H, m, Ph); δ_C(100 MHz; $CDCl_3$) major diastereoisomer: 170.2 (s), 169.6 (s), 166.1 (s), 165.0 (s), 157.6 (d), 139.5 (s), 128.8 (d), 127.9 (d), 125.7 (d), 117.4 (d), 110.0 (s), 94.8 (d), 90.9 (s), 76.4 (s), 74.3 (d), 74.1 (d), 72.0 (d), 57.7 (s), 53.7 (q), 52.7 (q), 48.9 (t), 42.8 (t), 39.5 (t), 35.7 (d), 34.0 (d), 31.5 (d), 29.3 (t), 25.7 (t), 23.6 (t), 20.3 (q), 19.5 (q), 18.6 (q), 13.7 (q) and 10.6 (q); m/z (DCI/NH_3) 724 $(M + NH_4)^+$.

Jones oxidation of lactol 3

Jones reagent (2.67 mol dm^{-3}; 0.049 cm^3) was added dropwise to a solution of lactol **3** (95 mg, 0.14 mmol) in acetone (2.5 cm^3) and the orange solution was stirred at 20 °C for 0.5 h. A further portion of Jones reagent was added (0.018 cm^3) and the mixture was stirred for a further 1 h. The solution was then partitioned between ethyl acetate (15 cm^3) and saturated aqueous sodium hydrogen carbonate (5 cm^3). The aqueous phase was extracted with ethyl acetate (15 cm^3), and the combined organic phases were washed with water (10 cm^3) and brine (10 cm^3), dried and then chromatographed on silica gel eluting with ethyl acetate–cyclohexane (1:3, 1:2) to give the lactone **14** (38.3 mg, 40%) as a colourless gum [Found: (FAB +ve) $(M + H)^+$, 689.3153. $C_{36}H_{49}O_{13}$ requires (M + H), 689.3173]; analytical HPLC t_r 8.82 min 95.4% on a Spherisorb 5 ODS-2 column (15 cm × 0.46 cm) using 65% MeCN–H_2O as eluent at a flow rate of 2 cm^3 min^{-1} and detecting at 210 nm; $\nu_{max}(CHBr_3)/cm^{-1}$ 1815, 1753, 1731 and 1633; δ_H(400 MHz; $CDCl_3$), 0.83–0.9 (9 H, m, CH_3), 1.06 (3 H, d, J 7, =CHCHCH_3), 1.08–1.47 (5 H, m), 2.12 (3 H, s, AcO), 2.03–2.52 (7 H, m), 2.71 (1 H, dd, J 13 and 5, CH_2Ph), 2.96 (1 H, d, J 6, CHO*H*), 3.85 and 3.89 (3 H each, s, CO_2CH_3), 3.97 (1 H, dd, J 8.5 and 6, C*H*OH), 4.03 (1 H, s, OH), 5.03 and 5.06 (1 H each, s, =CH_2), 5.09 (1 H, s, J 5, C*H*OAc), 5.38 (1 H, d, J 8.5, C*H*OCOCH=CH), 5.81 (1 H, d, J 16, CH=C*H*CO_2), 6.94 (1 H, dd, J 16 and 8, C*H*=$CHCO_2$) and 7.1–7.3 (5 H, m, Ph); δ_C(100 MHz; $CDCl_3$) 170.2, 169.5, 166.6, 163.6, 162.6, 158.7, 144.9, 140.3, 129.1, 128.3, 126.1, 117.5, 112.6, 110.8, 83.8, 79.0, 76.2, 74.5, 70.9, 54.2, 53.8, 43.2, 39.9, 37.1, 34.6, 31.9, 30.2, 29.7, 24.0, 21.0, 20.0, 19.1, 14.0 and 11.1; m/z (DCI/NH_3 +ve) 706 $(M + NH_4)^+$.

Further elution of the column gave a mixture (26.9 mg) which was purified by preparative reversed-phase HPLC on Spherisorb 5 ODS-2 column (25 × 2.2 cm) using 70% MeCN–H_2O as eluent at a flow rate of 15 cm^3 min^{-1} and detecting at 210 nm. The appropriate fractions were combined and the acetonitrile was removed under reduced pressure. The aqueous phase was extracted with ethyl acetate and the organic solution was dried and then evaporated under reduced pressure. The first compound to be eluted off the column (t_r 11 min) was obtained as a colourless gum (6.9 mg, 7%) identified as the lactol **3**; δ_H(250 MHz; $CDCl_3$) 0.8–0.9 (9 H, m, CH_3), 1.03 (3 H, d, J 7.5, =CHCHCH_3), 0.9–1.42 (5 H, m), 2.10 (3 H, s, AcO), 2.1–2.5 (7 H, m), 2.65 (1 H, br, OH), 2.70 (1 H, dd, J 14 and 6, CH_2Ph), 3.58 (1 H, br d, J 7.5, OH), 3.74 (1 H, br t, J 7, C*H*OH), 3.80 (1 H, s, OH), 3.81 and 3.91 (3 H each, s, CO_2CH_3), 5.02 (2 H, s, =CH_2), 5.11 (1 H, d, J 5, C*H*OAc), 5.28 (1 H, d, J 9, C*H*OCOCH=), 5.80 (1 H, d, J 16, CH=C*H*CO_2), 6.18 (1 H, d, J 7, OC*H*OH), 6.90 (1 H, dd, J 16 and 8, C*H*=$CHCO_2$) and 7.1–7.3 (5 H, m, Ph). The second compound to be eluted off the column (t_r 16 min) was obtained as a colourless gum (10.1 mg, 10%) identified as the unstable tricycle **15** [Found: (CI) $(M + H)^+$, 689.3163. $C_{36}H_{49}O_{13}$ requires (M + H), 689.3173]; analytical HPLC t_r 6.94 min 82.7% on a Spherisorb 5 ODS-2 column (15 cm × 0.46 cm) using 65% MeCN–H_2O as eluent, at a flow rate of 2 cm^3 min^{-1} and detecting at 210 nm; δ_H(400 MHz; $CDCl_3$) 0.8–0.9 (9 H, m, CH_3), 1.00 (3 H, d, J 7, =CHCHCH_3), 0.94–1.50 (5 H, m), 2.09 (3 H, s, AcO), 2.00–2.47 (7 H, m), 2.70 (1 H, dd, J 13 and 5, CH_2Ph), 3.8 and 3.92 (3 H each, s, CO_2CH_3), 3.82 (1 H, s, OH), 4.99 (2 H, s, =CH_2, OH), 5.01 (1 H, s, =CH_2), 5.10 (1 H, d, J 5, C*H*OAc), 5.40 (1 H, s, OCHO), 5.79 (1 H, d, J 15.5, CH=C*H*CO_2), 5.99 (1 H, s, C*H*OCOCH=CH), 6.95 (1 H, dd, J 15.5 and 8, C*H*=$CHCO_2$) and 7.08–7.32 (5 H, m, Ph); δ_C(100 MHz; $CDCl_3$) 170.1 (s), 168.5 (s), 168.4 (s), 164.4 (s), 159.4 (d), 145.6 (s), 140.3 (s), 129.1 (d), 128.3 (d), 126.0 (d), 117.5 (d), 111.9 (t), 109.1 (s), 108.1 (s), 100.5 (d), 88.6 (s), 79.3 (d), 76.8 (d), 74.8 (s), 53.8 (q), 53.1 (q), 43.1 (t), 40.0 (t), 36.9 (d), 34.5 (d), 32.0 (d), 29.7 (t), 29.6 (t), 24.9 (t), 21.1 (q), 19.9 (q), 19.1 (q), 14.0 (q) and 11.1 (q); m/z (DCI/NH_3) 689 $(M + H)^+$.

Treatment of lactone 14 with thionyl chloride in methanol

A stirred solution of lactone **14** (10 mg, 0.015 mmol) in methanol (0.5 cm^3) at −10 °C was treated cautiously with thionyl choride (0.0011 cm^3, 0.015 mmol), the mixture was allowed to warm to 20 °C and then stirred for 43 h. The mixture was concentrated under reduced pressure and the residue (11.6 mg) was purified by PLC (one plate 20 × 5 cm eluting with ethyl acetate–cyclohexane, 1:2) to give the allylic alcohol **16** (5.2 mg, 54%) as a colourless gum: analytical HPLC t_r 5.03 min 86% pure on a Spherisorb 5 ODS-2 column (15 × 0.46 cm) using 65% MeCN–H_2O as eluent at a flow rate of 2 cm^3 min^{-1}

and detecting at 210 nm; δ_H(250 MHz; $CDCl_3$) 0.8–0.9 (9 H, m, CH_3), 1.05 (3 H, s, *J* 7, =CHCHCH_3), 0.78–1.45 (5 H, m), 1.93–2.5 (7 H, m), 2.76 (1 H, dd, *J* 14 and 6, CH_2Ph), 3.85 and 3.89 (3 H each, s, CO_2CH_3), 3.94–4.07 (3 H, m), 5.01 and 5.14 (1 H each, s, $=CH_2$), 5.38 (1 H, d, *J* 9, C*H*OCOCH=CH), 5.81 (1 H, d, *J* 16, CH=C*H*CO_2), 6.94 (1 H, dd, *J* 16 and 8, C*H*=CHCO_2) and 7.15–7.3 (5 H, m, Ph); *m/z* (DCI/NH_3) 664 $[(M + NH_4)^+$, 100%] and 646 $\{[(M + NH_4) - H_2O]^+, 60\}$.

Treatment of lactone 14 with methanol in the presence of (±)-10-camphorsulfonic acid

A solution of the lactone **14** (92 mg, 0.13 mmol) and (±)-10-camphorsulfonic acid (CSA) (31 mg, 0.13 mmol) in anhydrous methanol (2.3 cm^3) was heated to reflux for 16 h. At this stage TLC indicated a 1:1 mixture of lactone **14** and allylic alcohol **16**. A further quantity of CSA (15.6 mg, 0.07 mmol) was added and the mixture was heated to reflux for a total of 5 days and then evaporated to dryness under reduced pressure. The residue was dissolved in ethyl acetate (50 cm^3), washed with aqueous sodium hydrogen carbonate (2 × 15 cm^3) and brine (15 cm^3), dried and evaporated to a gum (69.4 mg), which was purified by preparative reversed-phase HPLC on a Spherisorb 5 ODS-2 column (25 × 2.2 cm) using 70% MeCN–H_2O as eluent at a flow rate of 15 cm^3 min^{-1} acid detecting at 210 nm. The fractions eluting with a t_r of 38 min were combined, concentrated under reduced pressure and then extracted with ethyl acetate. The organic phase was washed with brine, dried and evaporated to dryness to give spiroketal **17** as a colourless gum (17.8 mg, 21%) [Found: (LSIMS +ve) $(M + H)^+$, 661.3240. $C_{35}H_{49}O_{12}$ requires (*M* + H), 661.3224]; analytical HPLC t_r 6.3 min 89% pure on Spherisorb ODS-2 column (15 × 0.46 cm) using 80% MeCN–H_2O as eluent at a flow rate of 2 cm^3 min^{-1} and detecting at 210 nm; for NMR data see Table 1; *m/z* (DCI/NH_3) 661 $(M + H)^+$.

Treatment of lactol 3 with trimethyl phosphonoacetate

A suspension of sodium hydride (60% oil dispersion; 5.5 mg, 0.14 mmol) in tetrahydrofuran (0.2 cm^3) was treated with a solution of trimethyl phosphonoacetate (0.023 cm^3, 0.14 mmol) in tetrahydrofuran (1 cm^3) and the mixture was stirred at 20 °C under nitrogen until the hydrogen gas evolution ceased. A solution of lactol **3** (38 mg, 0.055 mmol) in tetrahydrofuran (2 cm^3) was added dropwise to the mixture and the solution was stirred under nitrogen for 0.5 h. The reaction mixture was then treated with dilute aqueous hydrochloric acid (1 cm^3) and extraced with ethyl acetate (2 × 3 cm^3). The combined organic extracts were washed with dilute hydrochloric acid (2 cm^3), water (2 cm^3), saturated aqueous sodium hydrogen carbonate (2 × 3 cm^3) and brine (2 cm^3), dried, evaporated to a clear gum (37.1 mg) and then purified by HPLC on a Spherisorb 5 ODS-2 column (25 × 2.2 cm) eluting with 70% MeCN–H_2O at a flow rate of 15 cm^3 min^{-1}, detecting at 210 nm and collecting the component with a t_r of 28 min. The appropriate fractions were concentrated under reduced pressure and then extracted with ethyl acetate. The organic phase was washed with brine, dried and then evaporated to give the acyclic derivative **18** (3.8 mg, 11%) as a colourless gum [Found: (FAB +ve) $(M + H)^+$, 629.3310. $C_{35}H_{49}O_{10}$ requires (*M* + H), 629.3325]; analytical HPLC t_r 15.42 min, 93.2% pure on a Spherisorb 5 ODS-2 column (25 × 0.46 cm) eluting with 70% MeCN–H_2O at a flow rate of 1.5 cm^3 min^{-1}, detecting at 210 nm; *m/z* (FAB +ve) 651 $(M + Na)^+$ and 629 $(M + H)^+$; *m/z* (FAB −ve) 627 $(M - H)^-$; for NMR data see Table 2.

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