27. Synthesis of Putative Intermediates on the Monensin Biosynthetic Pathway and Incorporation Experiments with the Monensin-Producing Organism

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As a direct test of the *Cane-Westley* hypothesis concerning the mode of assembly of ether rings in the polyether class of ionophore antibiotics, we describe experiments culminating in the synthesis of three putative intermediates on the monensin biosynthetic pathway and incorporation experiments with these materials and the monensin-producing organism *Streptomyces cinnamonensis*. The putative intermediates synthesised include the trienes [21-^{3}H]-**7** and [13-^{3}H]-**10**, and the diene [9-^{3}H]-**11**. The results of the incorporation experiments conducted with whole cell cultures suggest that [13-^{3}H]-**10** and [21-^{3}H]-**7** are unable to cross the intact cell membrane of *S. cinnamonensis*, whereas diene [9-^{3}H]-**11** can gain entry to the cellular interior, but is then degraded efficiently, most likely by a pathway closely related to β-oxidation, without being specifically incorporated into the antibiotic.

Introduction. – Early biosynthetic studies [1] on monensin A, a commercially important polyether produced by fermentations of *Streptomyces cinnamonensis* [2], indicated that the backbone of this ionophore is constructed by the sequential coupling of precursors derived *in vivo* from one butyrate, seven propionate, and five acetate building blocks, with the MeO C-atom being derived additionally from methionine. Later, the natural-abundance ^{13}C-NMR spectrum of monensin A was assigned unambiguously [3–5] and through stable isotope labelling experiments using ^{13}C- and ^{18}O-doubly labelled acetate and propionate, as well as fermentations conducted under $^{18}O_2$, it was possible to identify the location of the building blocks in the C-backbone and to establish that four of the O-atoms in monensin A were derived from molecular O_2, with the remainder originating intact from the precursors [3] [6] [7]. Based upon these data, *Cane* and coworkers [3] [6] suggested that the heterocyclic rings in the ionophore might arise by the elegant pathway shown in *Scheme 1*, possibly involving a cascade of ring closures upon a triepoxide intermediate **1**, itself derived from a precursor **2** containing three double bonds each with (*E*)-configuration. This hypothesis was consistent with the earlier proposal by *Westley* [8], that lasalocid A and isolasalocid A might arise *via* alternative cyclisations of a common diepoxide intermediate, an idea also later supported [9] by results from ^{13}C- and ^{18}O-labelling experiments. Thereafter, related investigations upon narasin [10], lenoremycin [11], ICI139603 [12], and maduramicin [13] served to strengthen the view that the *Cane-Westley* polyene-polyepoxide concept [14] might be applied generally to account

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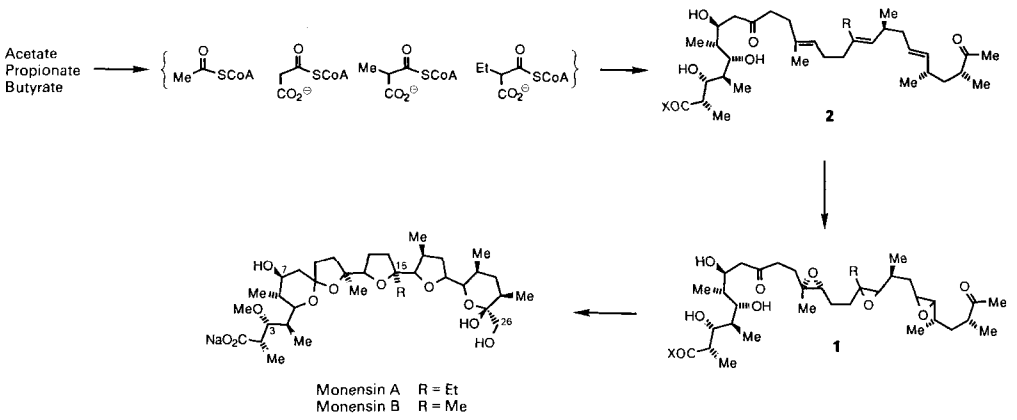
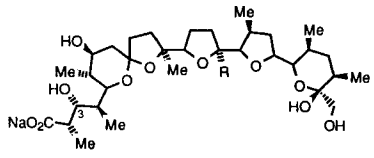
Scheme 1. *Hypothetical Biosynthetic Pathway to Monensin (X = OH or SR (carrier protein))* in vivo



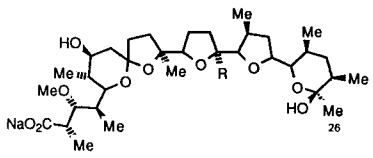
for the formation of cyclic ether rings within the entire family of polyether antibiotics, as well as in other classes of natural products [15].

More recent researches have sought direct evidence in support of the triene-triepoxide pathway to monensin. Despite substantial efforts in our laboratory, neither the triene **2**, nor closely related molecules have yet been isolated from the monensin-producing strain. The 3-*O*-demethylmonensins A and B (**3** and **4**) have been recovered [16] as minor components from *S. cinnamonensis* fermentation broths, and 26-deoxymonensins A and B (**5** and **6**) have been isolated from a mutant blocked in monensin production [17]. These metabolites, however, in labelled forms were not efficiently [17] incorporated into monensin A when fed to cultures of *S. cinnamonensis*. This could indicate that the hydroxylation and *O*-methylation events occur at an early stage in the elaboration of the polyether, and **3** and **5** are abortive shunt products. Or more likely, the metabolites failed to cross the cell membrane, possibly as a result of a natural mechanism for exporting and excluding polyethers from the cell interior.

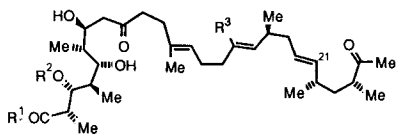
As a direct test for the *Cane-Westley* hypothesis, we embarked upon syntheses of putative intermediates in the monensin pathway, both with a view to feeding such materials to whole cell cultures and for attempted cell-free assays of enzymes operating late in the pathway. The triene **7**, a derivative of **2**, was an important objective although at the outset there existed no proof that **2** was indeed a *bona fida* intermediate. It seemed likely that no oxidative processes would occur before the entire C-backbone of the polyether had been assembled, *via* the so-called processive strategy [18] [19] catalysed by the monensin polyketide synthase complex. The uncertain timing of hydroxylation at C(26) and methylation at HO–C(3) were a major cause for concern, although the isolation of **3** and **5** implies that these events are not a prerequisite for the formation of the ether rings. In contrast, the presence of MeO–C(3) in the triene could block later steps if the enzymes acting late in the pathway strictly require a free OH at C(3). Also, the mutant producing **5** was available for the feeding and cell-free experiments, if required. The other major uncertainty appeared to be that the oxidative processes might require double bonds that were not of (*E*)-configuration, a factor that could at least be taken into account in planning the synthesis. In addition to the triene **7**, syntheses of **10** and **11**,



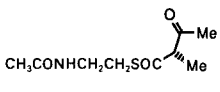
3 R = Et 3-*O*-demethylmonensin A
4 R = Me 3-*O*-demethylmonensin B



5 R = Et 26-deoxymonensin A
6 R = Me 26-deoxymonensin B



7 $R^1 = C_7H_{15}CONHCH_2CH_2S$, R^2 = H, R^3 = Me
8 R^1 = OH, R^2 = Me, R^3 = Et
9 R^1 = OH, R^2 = H, R^3 = Et



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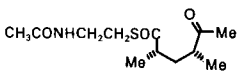

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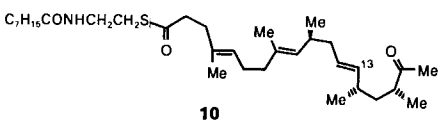

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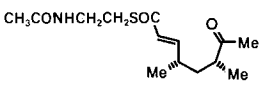

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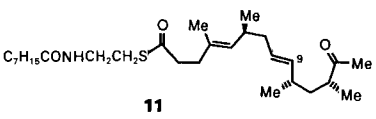

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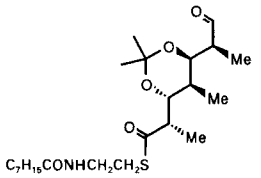
representing derivatives of putative chain-elongation intermediates, have also been completed. In analogy to fatty-acid biosynthesis, intermediates in the chain elongation *in vivo* almost certainly occur as enzyme-bound thioester species and should ideally be synthesised in an activated form, as thioesters, to facilitate their uptake and recognition by the presumptive polyketide synthase complex [19]. Exactly at which stage in the pathway the secondary metabolite is liberated as a free carboxylic acid is as yet unknown. At the outset, therefore, the primary synthetic targets were **7**, **10**, and **11**.

During the course of our work [20] [21], syntheses of the trienes **8** and **9** were described [22–24], although no results of feeding experiments with these materials have been reported. Also, recently, the syntheses of multiply labelled forms of **12**–**14**, again derivatives of putative chain-elongation intermediates, were described [25]. In this paper, we present full details of our syntheses of **7**, **10**, and **11**, in radiolabelled forms, and the results of feeding experiments with *S. cinnamonensis*.

Results and Discussion. – The synthesis of triene **7**, in labelled form, and in the quantities needed for in depth biochemical experiments, presents still a testing target for current synthetic methodology. Our earlier [20] retrosynthetic analysis identified the fragments **15**, **16**, and **17** as key synthons whose union could be achieved sequentially through a modified *Julia-Lythgoe* coupling [26–29], and a directed aldol reaction. A



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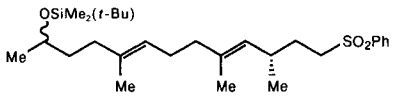

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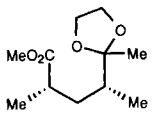

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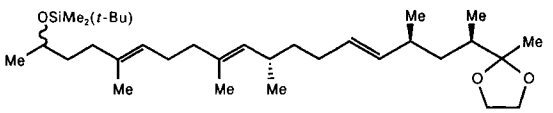

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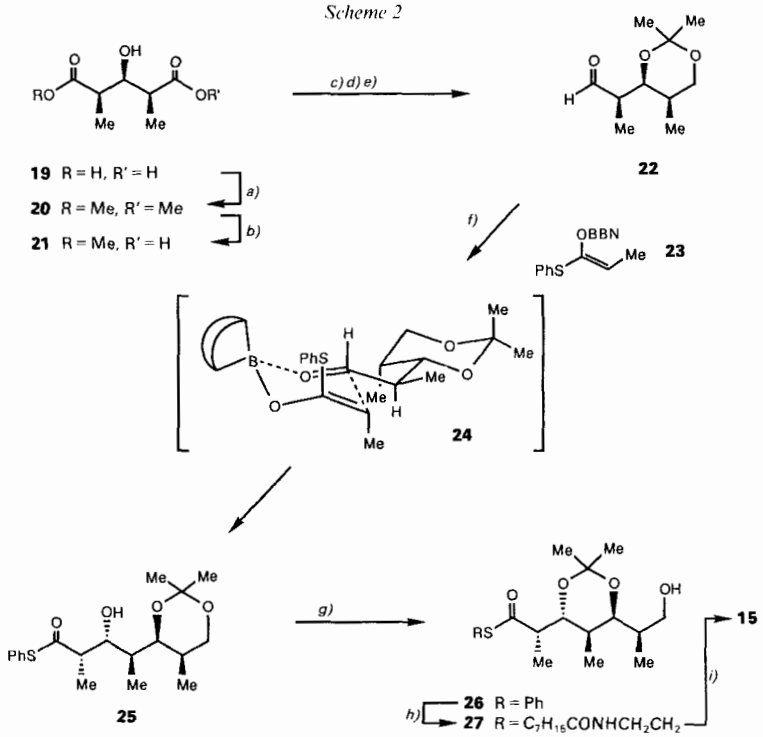
similar disconnection strategy was followed in the earlier syntheses of *Sih* and coworkers [22] [23] and *Evans* and *DiMare* [24]. Earlier model studies [20] to optimise the efficiency of the *Julia-Lythgo* coupling indicated that condensation of the sulfone **16** onto the ester **17** should proceed efficiently to afford, after reductive elimination, the triene fragment **18** having (all-*E*)-configuration. A synthesis of optically pure ester **17** was described earlier [20].

Left-Hand Fragment **15**. The synthesis of fragment **15** is shown in *Scheme 2*. The *'xylo'*-diacid **19** was prepared according to established methods [30] [31] and esterified by reaction with diazomethane. The *meso*-diester **20** was then treated with pig-liver esterase (PLE) which specifically hydrolyses the (*pro-R*)-ester group in accordance with previous literature precedence [32]. The best selectivity with this substrate was obtained [33] using 10% MeOH in 100 mM phosphate buffer at pH 6.8 and 0° which gave monoacid **21** in 99% yield (> 0.9 e.e.). Reduction of the monoacid with $BH_3 \cdot SMe_2$ afforded a diol, which was found to lactonise and eliminate H_2O upon storage for more than a few h. When protected immediately and then reduced with DIBAL (diisobutylaluminium hydride), aldehyde **22** was obtained in excellent yield. In the next step, a directed aldol reaction between **22** and the known [34] boron enolate **23** (BBN = 9-borabicyclo-[3.3.1]non-9-yl), at −90° in Et_2O afforded, after workup with H_2O_2, two aldol adducts in 5:1 ratio, and the major isomer **25** was obtained homogeneous in 54% yield. Interestingly [22] [23], when the reaction was performed at 0°, the same two aldol adducts were obtained in 1:2.4 ratio. The steric course of this aldol reaction can be analysed using the usual *Zimmerman*-type transition state model [35] [36] (as indicated in **24**), but the configuration of the major isomer obtained at −90° was proven unambiguously in a later step as **25**. The next goal was to unmask the primary OH group in **25**, and it was pleasing to discover that, upon treatment with acid, the isopropylidene group migrated to afford the desired product **26** as a crystalline material, in an optimised yield of 79%. The structure of **26** was confirmed by X-ray crystallography, and it is likely that the staggered conformation of the Me groups along the C-backbone, shown in the ORTEP diagram *(Fig. 1)*, contributes to the stability of the structure. A thioester exchange with *N*-octanoylcysteamine gave the thioester **27** as an oil, and *Swern* oxidation [37] of the primary alcohol then afforded the unstable completed left-hand fragment **15** which was used

Scheme 2



a) CH_2N_2, Et_2O. *b)* PLE, pH 6.8, 10% MeOH, 0°. *c)* $BH_3 \cdot SMe_2$, THF, −78°. *d)* $(CH_3)_2C(OMe)_2$, *Amberlyst 15*, MeCN. *e)* DIBAL, toluene, −90°. *f)* **23**, −90°, Et_2O. *g)* $(CH_3)_2C(OMe)_2$, TsOH, MeCN. *h)* $C_7H_{15}CONH(CH_2)_2SH$, $NaHCO_3$, MeOH. *i)* *Swern* oxidation.

immediately in the aldol coupling in order to avoid epimerisation. This relatively short sequence makes available the left-hand fragment **15**, in optically pure form, with the required thioester activation in 16% overall yield from diester **20**.

Central Fragment **16**. The construction of fragment **16** followed straightforwardly, using the *Claisen* rearrangement [38] to generate stereoselectively each trisubstituted

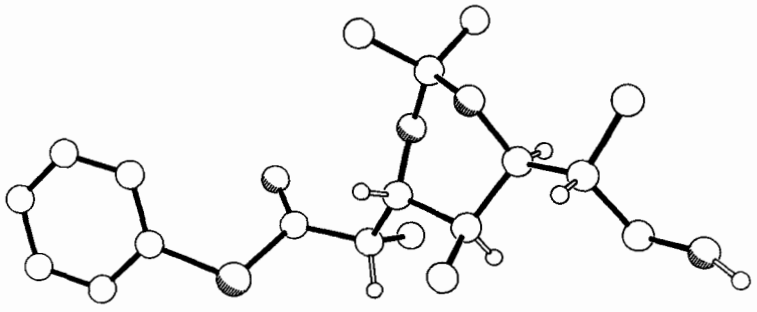
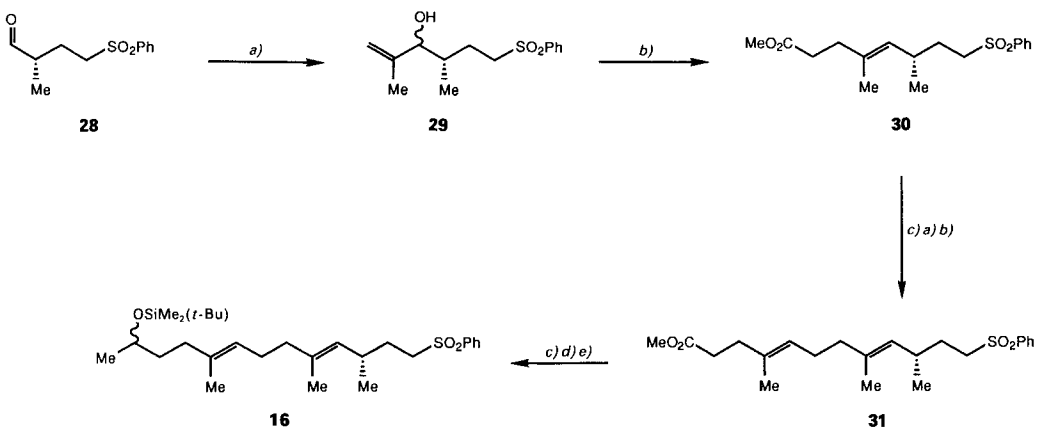


Fig. 1. *ORTEP Diagram from the X-ray structure determination of thioester* **26**

Scheme 3

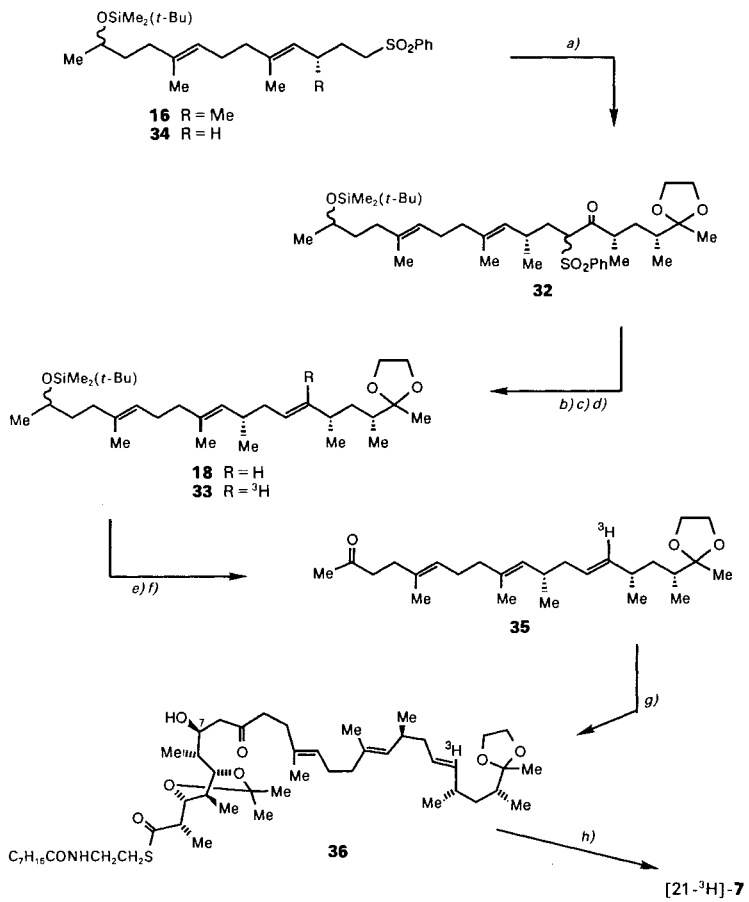


a) CH_2CBrCH_3, Mg, THF. *b)* $CH_3C(OMe)_3$, trimethylbenzoic acid, toluene, 110°. *c)* DIBAL, toluene, −90°. *d)* MeLi, Et_2O. *e)* *t*-$BuMe_2SiCl$, imidazole, DMF.

(*E*)-double bond *(Scheme 3)*. For the first *Claisen* rearrangement, allylic alcohol **29** was prepared as a mixture of diastereoisomers following addition of a *Grignard* reagent prepared from 2-bromopropene to aldehyde **28**, derived in seven steps (*i*) 3,4-dihydro-2*H*-pyran, TsOH; *ii*) $LiAlH_4$; *iii*) TsCl, pyridine; *iv*) NaI; *v*) $CH_2(CO_2Me)SO_2Ph$, NaH, DMF; *vi*) LiCl, DMSO, 145°; *vii*) *Swern* oxidation) from commercially available methyl (*R*)-3-hydroxy-2-methylpropanoate. The reaction of **29** with trimethyl orthoacetate and trimethylbenzoic acid at 110° afforded **30** in 76% yield. Careful control of the reaction temperature was necessary to ensure a high stereoselectivity, and the proportion of (*Z*)-isomer formed was < 3% (by ^{13}C-NMR). After reduction with DIBAL (−90°), another round of *Grignard* addition, and rearrangement, the (*E*,*E*)-diene **31** was obtained in > 97% isomeric purity and in 35% overall yield from the chiral sulfone **28**. From **31**, the required central fragment **16** could then be generated efficiently in three further steps *(Scheme 3)*.

Fragment Coupling Reactions. The first fragment coupling was based on earlier model studies [20] and involved addition of the lithium anion derived from **16** to the ester **17** to afford a α-ketosulfone **32** *(Scheme 4)*. It was convenient at this point to introduce a radiolabel, by reduction of the oxo group with [^{3}H]$NaBH_4$. Subsequent benzoylation and reductive elimination with Na-Hg amalgam at −30° afforded the tritiated (all-*E*)-triene **33** with a specific activity of 1.9×10^{11} dpm/mmol. The stereoselectivity of the reductive elimination was determined by ^{13}C-NMR analysis to be 10:1 (*E*,*E*,*E*)/(*Z*,*E*,*E*). This (*E*/*Z*) ratio was larger than that seen (4:1) in our model studies [20] using sulfone **34**, most likely due both to the lower temperature used here for the reductive elimination and to the presence now of a Me substituent in β-position to the sulfone group of **16**. Chain branching α to the sulfone moiety has been reported previously [29] to increase the predominance of the (*E*)-isomer in the *Julia-Lythgoe* reaction. The silyl protecting group of **33** was then removed (Bu_4NF) and the resulting alcohol oxidised to afford the tritiated ketone **35**.

Scheme 4



a) BuLi, THF, then **17**, −78 to 0°. *b)* $NaBH_4$ or [^{3}H]$NaBH_4$, 60 h, EtOH. *c)* BuLi, PhCOCl. *d)* Na-Hg (6%), THF/MeOH/AcOEt, −30°. *e)* Bu_4NF, THF. *f)* $(COCl)_2$, DMSO, Et_3N, −60°. *g)* LDA, THF, −80°, then **15**. *h)* aq. HCl, MeCN.

The final coupling reaction was achieved by a directed aldol addition of the lithium enolate, derived under kinetic control from **35**, onto the freshly prepared aldehyde **15**. A 5:1 ratio of aldol adducts, diastereoisomeric at C(7), were isolated from which the predominant isomer, obtained in an unoptimised yield of 32%, was expected to have the required (7*S*)-configuration. In accordance with the *Felkin-Anh* model [39–41], the enolate derived from **35** under kinetic control would be expected to attack the aldehyde from its sterically least hindered face leading predominantly to the *Cram* addition product, diastereoisomer **36**. This prediction is in agreement with the outcome of several closely related aldol reactions described elsewhere [22–24] [42–45], although confirmation must await future investigations. The high-resolution CI mass spectrum of **36** was consistent with the formula $C_{49}H_{86}NO_8S$ [$(M + H)^+$]. From the 360-MHz 2D-COSY and ^{1}H-NMR

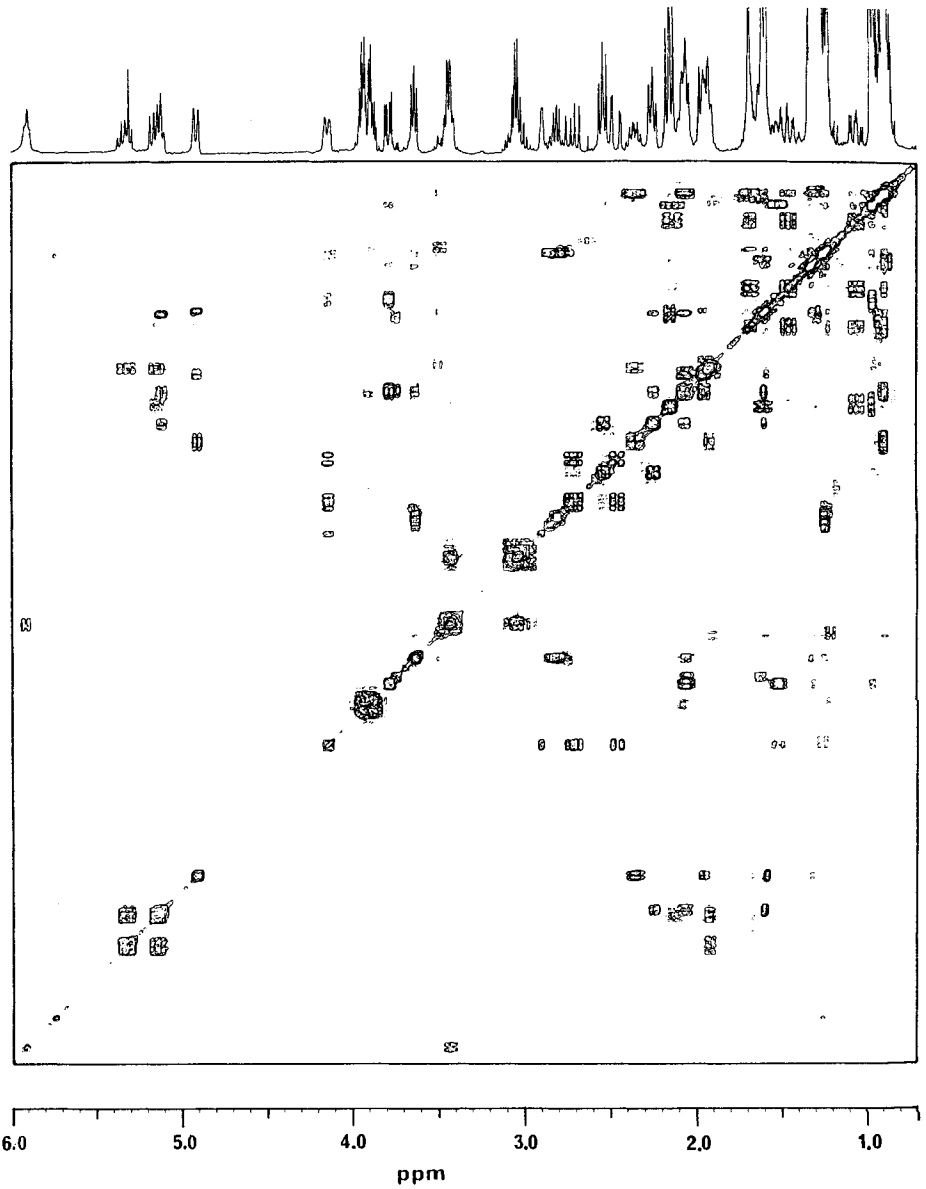
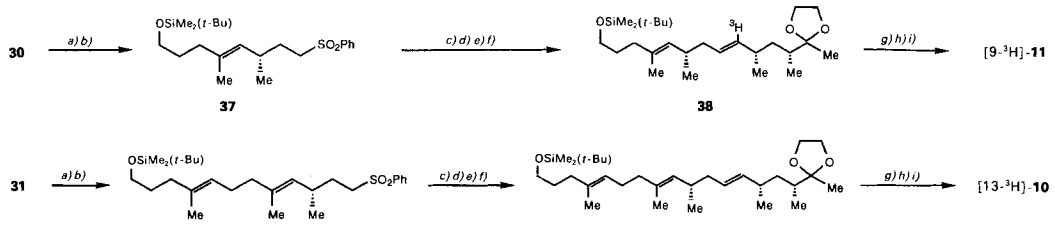


Fig. 2. *2D-COSY 360-MHz ^{1}H-NMR Spectrum of* **36**. Accumulated with $SI2 = 2$ K and $SI1 = 1$ K, transformed with an unshifted sine-bell squared weighting, and shown after symmetrisation of the 2D transform.

Scheme 5



a) DIBAL, 0°. *b)* (*t*-Bu)Me_2SiCl, imidazole, DMF. *c)* BuLi, THF, then **17**, −78 to 0°. *d)* [^{3}H]$NaBH_4$, 60 h, EtOH. *e)* BuLi, PhCOCl. *f)* Na-Hg (6%), THF/MeOH/AcOEt, −30°. *g)* Bu_4NF. *h)* CrO_3, H^+. *i)* $C_7H_{15}CONH(CH_2)_2SH$, DCC, Me_2NPy.

spectra *(Fig. 2)*, assignments could be made that confirmed the expected constitution of **36**. These data together with those from $^{13}C\{^1H\}$-NMR spectra were fully consistent with the proposed structure. In the final step, cautious treatment of **36** with HCl in MeCN led to smooth deprotection and the isolation of tritiated product [21-^{3}H]-**7** in 61% yield, after purification by prep. TLC. The 360-MHz ^{1}H 2D-COSY and 90.5-MHz ^{13}C 1D and DEPT-NMR spectra and the CI-MS were fully consistent with the assigned structure (see *Exper. Part*).

*Putative Chain-Elongation Intermediates [13-^{3}H]-***10** *and [9-^{3}H]-***11**. The methodology used for the syntheses of [13-^{3}H]-**10** and [9-^{3}H]-**11** was similar to that described above for the synthesis of [21-^{3}H]-**7**. A modified *Julia-Lythgoe* coupling reaction between sulfone **37** and ester **17** afforded an α-ketosulfone *(Scheme 5)*, that upon sequential reduction with [^{3}H]$NaBH_4$, benzoylation, and reductive elimination gave the tritiated diene **38**. Deprotection, oxidation, and coupling to *N*-octanoylcysteamine afforded the required diene [9-^{3}H]-**11**. Triene [13-^{3}H]-**10** was prepared in a similar fashion from ester **31**, as shown in *Scheme 5*.

Feeding Experiments. Fermentations of *Streptomyces cinnamonensis* strain A3823.5 typically result in the production of both monensin A and monensin B in *ca.* 10:1 ratio [46]. When grown in shake flask cultures in a complex medium containing substantial amounts of soybean meal, soybean oil, lard oil, and methyl oleate, cells start to produce monensin after *ca.* 24 h and continue to do so for a further 4–5 days. Under optimal conditions, the titre of antibiotic can reach 3–4 mg/ml although, typically, 1–2 mg/ml of pure monensin A can be recovered from the fermentation broth. It is likely that any hydrophobic materials fed to the producing cultures will dissolve in the oils present in the fermentation broth. These oils stimulate antibiotic production [47] and most likely, after entry into the cell and processing through primary metabolism, provide the bulk of the C-atom source needed for the biosynthesis of the antibiotic.

The materials synthesised in this work should act only as precursors of monensin B. In a straightforward test for a specific incorporation, therefore, the only tritiated product will be monensin B. On the other hand, if the precursor is degraded into primary metabolites prior to incorporation of the label, enrichments should occur both in monensin A and B. In the first feeding experiment, the triene [21-^{3}H]-**7** (4 mg, $5.8 \cdot 10^8$ dpm) was added portionwise as a EtOH solution to cultures of *S. cinnamonensis*. After 6

days, the fermentation was halted and the whole broth extracted with AcOEt. A radio-TLC analysis of the extract (containing $4.6 \cdot 10^8$ dpm ^3H) indicated the presence of a single major radioactive component migrating with the starting triene [21-^{3}H]-**7**. This extract was fractionated by elution from an *LH-20 Sephadex* column with CH_2Cl_2, and the radioactive material, after further purification by prep. TLC, showed a ^{1}H-NMR spectrum that was identical to that of triene [21-^{3}H]-**7**, indicating that the bulk of the material fed had been recovered intact (60% recovery). The monensins A and B were also isolated, and both were found to contain only very low levels of ^3H; upon repeated recrystallisation, the specific activity of both continued to decrease and was 78 dpm/mg in monensin A after 9 recrystallisations and 422 dpm/mg in monensin B after 8 recrystallisations. Hence, a negligible level of incorporation of the ^3H label had occurred.

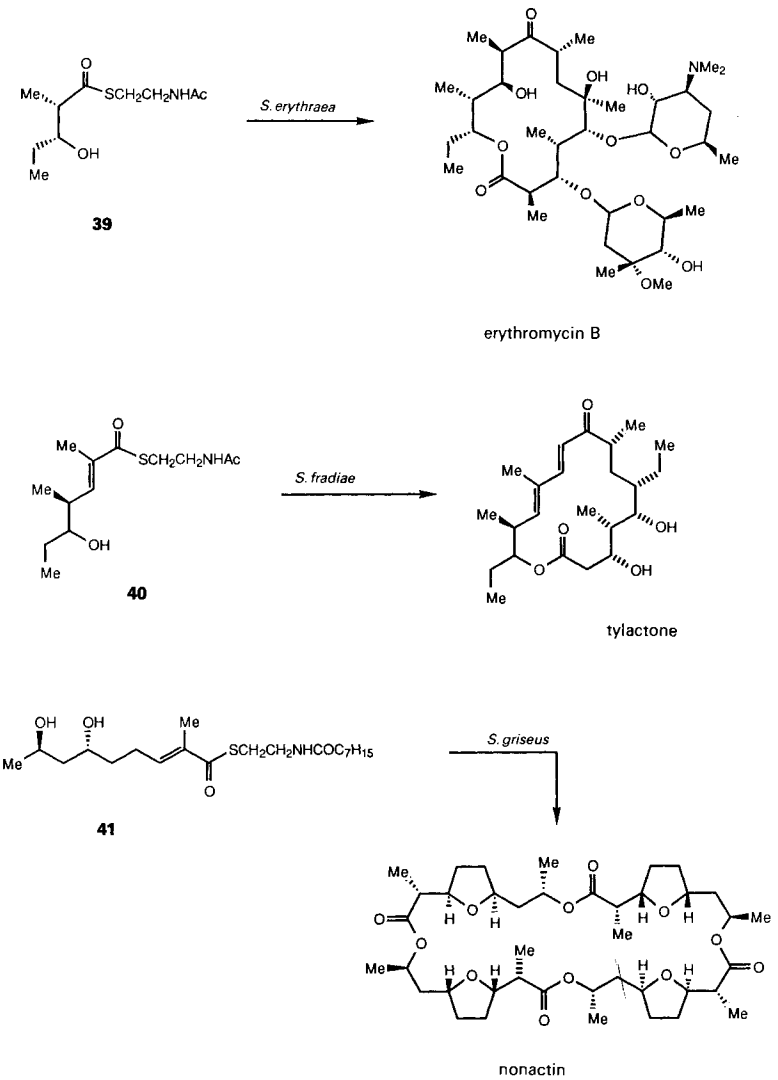
In order to avoid any possible complications arising from the lack of an OH group at C(26) in our labelled triene, a second feeding experiment was carried out with the 26-deoxymonensin-producing mutant [17] *S. cinnamonensis* DMA300. The same feeding regime was followed, except that the shake culture was left a total of 10 days before extraction. Most of the radioactivity was extracted (90% recovery) into AcOEt and was again associated with unchanged triene **7**, whereas negligible incorporations into deoxymonensins A and B were observed. Interestingly, a radio-TLC analysis of the crude extract revealed that a second minor radio-labelled component was present ($< 1\%$ of total radioactivity), although its characterisation proved impossible.

Similar results were obtained upon feeding the truncated triene [13-^{3}H]-**10** to either strain A3823.5 or DMA300. None of the monensins or 26-deoxymonensins produced contained significant amounts of ^3H label, and in each experiment, 90% of the labelled triene administered to the shake culture were recovered intact. In the case of diene [9-^{3}H]-**11** (specific activity 1.96×10^{11} dpm/mmol), however, after feeding to cultures of strain A3823.5 and extraction of the fermentation broth with AcOEt, 68% of the ^3H label remained in the aqueous layer as volatile material, most likely either tritiated H_2O or AcOH. None of diene [9-^{3}H]-**11** was recovered, whereas, after purification, the monensins A and B that had been produced possessed specific activities of 3.53×10^7 and 3.18×10^7 dpm/mmol, respectively.

Conclusions. – An efficient, convergent synthetic route to the triene **7** has been developed that allows its preparation on a scale large enough for initial biochemical experiments with the monensin-producing organism. Two of the key synthons needed for the assembly of **7** can be prepared in optically pure form by relatively short synthetic routes, and for one of these, **26**, the relative configuration was established unambiguously by X-ray methods. Because of its linear format, the route to the central fragment **16** containing two trisubstituted double bonds is less amenable to the large-scale production of this synthon and is not easily adapted to the synthesis of isomers containing trisubstituted (*Z*)-double bonds. A similar problem exists in earlier syntheses of related trienes by the groups of *Sih* [22] [23] and *Evans* [24]. It is notable that new organometallic chemistry developed by *Kocienski* and coworkers [48] [49] may offer a viable alternative approach that overcomes these limitations of the *Claisen*-type chemistry used to date.

The incorporation experiments described here help to define the scope and limitations of the whole-cell approach to studies of monensin biosynthesis. Upon feeding either labelled **7** or **10** to relevant strains of *S. cinnamonensis*, no significant incorporations into

Scheme 6



monensin B or deoxymonensin B were observed, most plausibly because these molecules are unable to cross the cell membrane and access the biosynthetic enzymes. It is noteworthy that upon feeding labelled 3-*O*-demethylmonensin A (**3**) and 26-deoxymonensin A (**5**) to intact cells of *S. cinnamonensis*, a similar problem [17] was encountered. The roles of the trienes **7** and **10** in monensin biosynthesis, therefore, remain unsettled. In contrast, upon feeding the labelled diene **11**, > 90% of the ^3H label appeared as volatile material in the fermentation broth. This demonstates that **11** can cross the cell membrane, but is then efficiently degraded, presumably by β-oxidation. The reduced size of **11**, as compared to

the trienes, is most likely the key factor in its ability to permeate the cell membrane. Its degradation by β-oxidation would lead to the transfer of ^3H label firstly to $NAD(P)^+$, and thereafter to the aqueous medium, as well as to intermediates in monensin biosynthesis, hence accounting for the observed equal but low levels of ^3H incorporation into both monensins A and B. Given that permeability to the cell membrane is no longer a problem, it is surprising that **11** does not function more effectively as a substrate for the enzymes active in the monensin pathway. There are now several reported examples where the intact incorporation of chain-elongation intermediates in both macrolide and ionophore antibiotic biosynthesis in *Streptomycetes* does occur and competes effectively with β-oxidation, when the precursors are added to shake cultures of the relevant producing strain. This includes the incorporation of **39** into erythromycin B in *S. erythraea* [50], the incorporation of **40** into tylactone [51], a precursor of tylosin in *S. fradiae*, and the incorporation of both enantiomers of **41** into nonactin in *S. griseus* [52]. The likely explanations for the failure of **11** to incorporate selectively into monensin B are *i*) that the β-oxidation pathway is more active in *S. cinnamonensis* under the growth conditions used, *ii*) that compartmentalisation within the cells prevents the precursor from accessing the biosynthetic enzymes, and *iii*) that **11** is not a suitable substrate for the monensin biosynthetic enzymes.

These considerations suggest new experiments that might more successfully illuminate aspects of this fascinating, but so far intractable problem. The results of other studies (see following paper) in particular provide the impetus to attempt additional incorporation experiments, possibly using cells made more permeable to exogenous materials or using cell-free extracts.

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Experimental Part

General. All reactions under anh. conditions were performed under O_2-free N_2 except where otherwise stated. Pig-liver esterase (PLE) was carboxylic hydrolase E.C. 3.1.1.1. Type 1 from porcine liver in 3.2M $(NH_4)_2SO_4$ from *Sigma Chemicals Ltd.* HPLC: *DuPont-8800* instrument and UV detector or *Waters-600* instrument with a model *481* UV detector and a *Waters ODS* semiprep. column. Flash column chromatography (FC) [53]: *Macherey-Nagel* silica gel *60* (230–400 mesh). *Florisil* CC: *Aldrich Florisil* (100–200 mesh). Prep. TLC: *Merck* silica gel *60F* UV_{254} plates. Anal. TLC: 0.25-mm precoated silica gel plates (*Merck 60F* UV_{254}); visualisation by UV fluorescence and vanillin spray. ^3H-Radioactivity measurements: *Packard Minaxi Tri-Carb 4000* series scintillation counter, using external standardisation. Autoradiography: *Amersham Hyperfilm* ^3H, *RPN12* plates. M.p.: electrothermal apparatus; not corrected. Optical rotations: optical activity *AA 100* polarimeter. IR spectra: *Perkin-Elmer-298* spectrophotometer; thin films in nujol between NaCl discs or solns. in 0.1-mm NaCl cells. ^1H-NMR spectra: at 90, 270, or 360 MHz using *Jeol FX90Q*, *Jeol GX270*, or *Bruker AM360* spectrometers; tetramethylsilane (TMS) as internal standard (= 0 ppm), except where otherwise stated; *J* in Hz. ^{13}C-NMR spectra: at 67.9 MHz (*Joel GX270*) and 90.5 MHz (*Bruker AM360*); δ in ppm rel. to TMS (= 0 ppm). MS and HR-MS: *V. G. 70-250-S.E.* double focusing mass spectrometer with a *Hewlett Packard 5790A* series GLC system; some samples were recorded at the *SERC* Mass Spectrometry Centre, University College of Swansea, on a *ZAB-2E* double-focusing reverse-geometry mass spectrometer.

(2R,3S,4S)-1-Methyl Hydrogen 3-Hydroxy-2,4-dimethylpentanedioate (**21**). To a soln. of **20** [30] (2.0 g, 9.8 mmol) in 0.1M phosphate buffer (pH 6.8, 48 ml) and MeOH (6 ml), bovine-serum albumin (50 mg) and pig-liver esterase (PLE; 2 ml, 3200 units) were added, and the pH of the stirred soln. was maintained at pH 6.8. After 24 h, the rate of reaction had decreased dramatically, and so more PLE (0.5 ml, 800 units) was added. After consumption

of 1 equiv. of base (usually after 2 days), the pH of the soln. was increased to pH 8 and the soln. extracted with Et_2O (4 × 20 ml). The aq. phase was acidified to pH 2.5, saturated with NaCl, and then extracted continuously with Et_2O overnight. The Et_2O phase was dried ($MgSO_4$) and evaporated: pale yellow solid **21** which was not purified further (1.85 g, 99%). $[\alpha]_D^{20} = -8.4$ ($c = 6$, EtOH). IR (nujol): 3380*m*, 2800*m*, 1735*s*, 1705*s*. ^{1}H-NMR (60 MHz, $CDCl_3$): 1.1 (*d*, $J = 7$, Me–C(2), Me–C(4)); 2.65 (*m*, H–C(2), H–C(4)); 3.73 (*s*, MeO); 4.17 (*m*, H–C(3)); 4.95 (br. *s*, OH). CI-MS (NH_3): 208 (100, $M + NH_4$), 191 (54, $M + H$), 173 (9).

(−)-(2R,3S,4R)-Methyl 3,5-(Isopropylidenedioxy)-2,4-dimethylpentanoate. $BH_3 \cdot Me_2S$ (1.7 ml, 25 mmol) was added dropwise within 10 min to a soln. of **21** (0.5 g, 2.63 mmol) in THF (1 ml) at 0°, under N_2. After stirring at r.t. for 24 h, the soln. was cooled to 0° and treated with brine. The white precipitate was extracted with Et_2O (6 × 5 ml) and the combined org. extract washed with brine and dried ($MgSO_4$). After evaporation, the residue was purified by FC (AcOEt/petroleum ether 2:1): unstable diol (0.32 g, 69.0%). ^{1}H-NMR (360 MHz, D_2O): 0.92 (*d*, $J = 7.0$, Me–C(4)); 1.20 (*d*, $J = 7$, Me–C(2)); 1.70 (*m*, H–C(4)); 2.78 (*m*, H–C(2)); 3.46 (*dd*, H–C(5)); 3.58 (*dd*, H–C(5)); 3.72 (*s*, CO_2Me); 3.85 (*dd*, H–C(3)).

The diol (0.32 g, 1.82 mmol), dry MeCN (0.8 ml), 2,2-dimethoxypropane (2.8 ml), and *Amberlyst 15* (6 mg) were stirred overnight at r.t. The mixture was filtered and the solvent evaporated. The residue was purified by FC (AcOEt/petroleum ether 1:19): title compound (0.27 g, 69.1%). $[\alpha]_D^{20} = -0.7$ ($c = 2.0$, CCl_4). IR: 1745*s*, 1100*s*. ^{1}H-NMR (360 MHz, $CDCl_3$): 1.08 (*d*, Me–C(4)); 1.21 (*d*, Me–C(2)); 1.38 (*s*, 3 H, Me_2C); 1.45 (*s*, 3 H, Me_2C); 1.52 (*m*, H–C(4)); 2.60 (*m*, H–C(2)); 3.55 (*dd*, 1 H–C(5)); 3.70 (*s*, MeO); 3.95 (*dd*, H–C(3)); 4.10 (*dd*, 1 H–C(5)). ^{13}C-NMR (90.5 MHz, $CDCl_3$): 12.9, 15.8, 19.8, 30.6, 31.2 (5 *q*); 43.0, 53.0 (2 *d*); 67.0 (*t*); 74.2 (*d*); 99.4, 176.2 (2 *s*). EI-MS: 201 (1.9, $M - CH_3$), 141 (1.8), 127 (3.4), 85 (3.8). Anal. calc. for $C_{11}H_{20}O_4$: C 60.9, H 9.1; found: C 61.1, H 9.3.

(2R,3S,4R)-3,5-(Isopropylidenedioxy)-2,4-dimethylpentanal (**22**). The foregoing ester (175 mg, 0.81 mmol) in toluene (1.2 ml) at −78° was treated dropwise over 2 h with a soln. of DIBAL (0.70 ml, 1.5M in hexane) and toluene (0.34 ml). After 50 min at −78°, the mixture was quenched with H_2O-sat. AcOEt (4 ml) at −78° and then allowed to warm to r.t., whereupon Et_2O (10 ml) was added. When the soln. was shaken with brine (15 ml), a white precipitate formed which gradually dissolved in the aq. layer. The aq. layer was extracted with Et_2O (3 × 10 ml) and the combined org. phase dried ($MgSO_4$) and evaporated. The resulting residue was purified by FC (AcOEt/petroleum ether 1:4); **22** (131 mg, 85%). ^{1}H-NMR (360 MHz, $CDCl_3$): 1.09 (*d*, Me); 1.13 (*d*, Me); 1.39 (*s*, 3 H, Me_2C); 1.45 (*s*, 3 H, Me_2C); 1.64 (*m*, H–C(4)); 2.61 (*m*, H–C(2)); 3.58 (*dd*, 1 H–C(5)); 4.08 (*dd* 1 H–C(5)); 4.12 (*m*, H–C(3)); 9.71 (*d*, CHO).

(+)-(2S,3R,4S,5S,6R)-S-Phenyl 3-Hydroxy-5,7-(isopropylidenedioxy)-2,4,6-trimethylheptanethioate (**25**). A soln. of 9-borabicyclo[3.3.1]non-9-yl trifluoromethanesulfonate (BBN-OTf; 3 ml, 0.5M in hexane, 1.5 mmol) was injected into a flask and the hexane removed before Et_2O (3 ml) was added and the soln. cooled to 0°. *S*-Phenyl propanethioate (248.9 mg, 1.5 mmol) and $(i\text{-}Pr)_2EtN$ (262.5 µl, 1.5 mmol) were dissolved in Et_2O (3 ml), cooled to 0°, and added over 5 min to the BBN-OTf soln. The resulting soln. was allowed to warm to r.t., stirred for 10 min, before cooling to −90°. Then, **22** (186 mg, 1.0 mmol) in Et_2O (1.0 ml) was added and the mixture allowed to warm to −2° over 1.5 h. The mixture was quenched with MeOH (12 ml), 0.5M phosphate buffer (pH 7.5, 10.5 ml), and H_2O_2 (1.8 ml, 30%), allowed to warm to r.t., and stirred for 5 min before evaporating most of the solvent. The concentrate was then extracted with AcOEt/petroleum ether 1:2 (5 × 20 ml). The combined org. phase was washed with sat. aq. sodium thiosulfite, dried (Na_2SO_4), and evaporated and the product purified firstly by *Florisil* CC (Et_2O/petroleum ether 1:4) and then by prep. TLC (silica-gel plate preeluted with 1% Et_3N and dried; AcOEt/petroleum ether 1:3): 175 mg (53%) of **25**. $[\alpha]_D^{24} = +35.8$ ($c = 1.7$, $CHCl_3$). UV ($CHCl_3$): 251 (7260), 271 (infl., 1760). IR ($CHCl_3$): 3500*m* (br.), 1690*s*, 1485*m*, 1460*m*. ^{1}H-NMR (360 MHz, $CDCl_3$): 0.95 (*d*, Me); 1.18 (*d*, Me); 1.32 (*d*, Me); 1.41 (*s*, Me); 1.47 (*s*, Me); 1.70 (*m*, H–C(6)); 1.80 (*m*, H–C(4)); 2.95 (*m*, H–C(2)); 3.00 (*d*, OH); 3.57 (*dd*, 1 H–C(7)); 3.93 (*m*, H–C(3)); 4.12 (*dd*, 1 H–C(7)); 4.13 (*m*, H–C(5)); 7.42 (*s*, Ph). ^{13}C-NMR (90.5 MHz, $CDCl_3$): 11.6, 12.0, 12.5, 19.3, 29.7 (5 *q*); 33.0, 38.7, 50.7 (3 *d*); 68.0 (*t*); 72.8, 74.5 (2 *d*); 99.0, 127.4 (2 *s*); 129.3, 129.6, 134.5 (3 *d*); 202.2 (*s*). FAB-MS: 353 (5.6, $M + H$), 337 (13.5), 295 (11), 227 (21), 259 (5.6), 243 (8.1), 239 (8.2), 221 (8.0), 185 (35.0), 167 (51.4), 129 (100). Anal. calc. for $C_{19}H_{28}O_4S$: C 64.75, H 8.0, S 9.1; found: C 64.9, H 7.8, S 9.0.

(+)-(2S,3R,4R,5S,6R)-S-Phenyl 7-Hydroxy-3,5-(isopropylidenedioxy)-2,4,6-trimethylheptanethioate (**26**). A soln. of **25** (15.0 mg, 0.04 mmol) in dry MeCN (0.5 ml) and 2,2-dimethoxypropane (0.5 ml) was treated with pyridinium *p*-toluenesulfonic acid (1 mg) and stirred overnight under Ar. The reaction was stopped by addition of Na_2SO_4 (5 mg) and $NaHCO_3$ (5 mg). The mixture was filtered, the solvent evaporated, and the product purified by prep. TLC (silica-gel plate pre-eluted with 1% Et_3N; AcOEt/petroleum ether 1:4): 9.3 mg (62%) of **26**. M.p. 94.5–95° (pentane). $[\alpha]_D^{22} = +62.3$ ($c = 0.9$, $CHCl_3$). UV: 251 (7250), 269 (infl. 1730). IR: 3500 (br.), 1705*s*, 1485*m*, 1465*m*, 1445*m*. ^{1}H-NMR (360 MHz, C_6D_6, ref. C_6H_6 7.3 ppm): 0.6 (br. *s*, OH); 1.04 (*d*, Me); 1.22 (*d*, Me); 1.43 (*d*,

Me); 1.46 (*s*, Me); 1.51 (*s*, Me); 1.82 (*m*, H–C(6)); 2.12 (*m*, H–C(4)); 2.90 (*m*, H–C(2)); 3.26 (*dd*, 1 H–C(7)); 3.38 (*dd*, 1 H–C(7)); 3.80 (*dd*, H–C(5)); 3.97 (*dd*, H–C(3)); 7.2, 7.55 (*m*, Ph). ^{13}C-NMR (90.5 MHz, $CDCl_3$): 12.6, 13.0, 14.0, 24.0, 25.0 (5 *q*); 35.7, 36.0, 52.2 (3 *d*); 64.8 (*t*); 70.8, 76.2 (2 *d*); 101.2, 127.5 (2 *s*); 129.3, 129.4, 134.6 (3 *d*); 199.7 (*s*). FAB-MS: 353 (37, *M* + H), 295 (12), 277 (25), 207 (34), 167 (36), 155 (33.5), 139 (52), 129 (100). Anal. calc. for $C_{19}H_{28}O_4S$: C 64.75, H 8.0, S 9.1; found: C 64.8, H 8.0, S 9.4.

(+)-(2S,3R,4R,5S,6R)-S-[2'-(Octanamido)ethyl] 7-Hydroxy-3,5-(isopropylidenedioxy)-2,4,6-trimethylheptanethioate (**27**). Compound **26** (13.7 mg, 37.8 μmol) and *N*-octanoylcysteamine (= *N*-(2-mercaptoethyl)-octanamide; 9.2 mg, 45.4 μmol) in MeOH (1 ml) were treated with $NaHCO_3$ (2 mg) and stirred for 48 h at r.t. H_2O (1 ml) was added and the mixture extracted with Et_2O. The combined org. phase was washed with H_2O and brine, dried ($MgSO_4$), and evaporated. The product was purified by prep. TLC (AcOEt/petroleum ether 1:1, then CH_2Cl_2/MeOH 95:5): 15.5 mg (94.8%) of **27**. $[\alpha]_D^{23} = +19.5$ (*c* = 0.10, $CHCl_3$). UV (CH_2Cl_2): 237.5 ($3.4 \cdot 10^3$). IR ($CHCl_3$): 3500*m*, 3300*m*, 1675*s*, 1520*s*. ^{1}H-NMR (270 MHz, $CDCl_3$): 0.87 (*t*, Me); 0.93 (*d*, Me); 1.04 (*d*, Me); 1.24 (*d*, Me); 1.28 (*m*, 4 CH_2); 1.31 (*s*, 3 H, Me_2C); 1.33 (*s*, 3 H, Me_2C); 1.62 (*m*, CH_2CH_2CON); 1.77 (*m*, H–C(6)); 1.95 (*m*, H–C(4)); 2.05 (br. *s*, OH); 2.16 (*m*, CH_2CON); 2.80 (*m*, H–C(2)); 3.02 (*m*, CH_2S); 3.43 (*m*, CH_2N); 3.50 (*m*, H–C(5)); 3.60 (*m*, H–C(3), CH_2(7)); 5.90 (br., NH). ^{13}C-NMR (67.9 MHz, $CDCl_3$): 12.7, 13.1, 14.1, 14.2 (4 *q*); 22.7 (*t*); 24.2, 25.0, 25.7 (3 *q*); 25.9 (*t*); 28.7; 29.3, 31.8 (2 *t*); 35.6 (*d*); 35.6 (*t*); 36.8 (*d*); 39.6 (*t*); 52.3 (*d*); 64.6 (*t*); 70.8, 76.4 (2 *d*); 100.9, 173.6, 202.3 (3 *s*). FAB-MS: 446 (24.4, *M* + H), 405 (19), 204 (58.3), 170 (41.8), 75 (76.8), 57 (100). HR-MS: 446.29480 (*M* + H, $C_{23}H_{43}NO_5S$ + H, calc. 446.29395).

(+)-(2S,3R,4R,5S,6S)-S-[2'-(Octanamido)ethyl] 6-Formyl-3,5-(isopropylidenedioxy)-2,4-dimethylheptanethioate (**15**). DMSO (36.3 μl, 0.53 mmol) was added slowly to oxalyl chloride (23.3 μl, 0.267 mmol) in CH_2Cl_2 (0.5 ml) cooled to −60°, and the soln. was stirred for 2 min. Then, **27** (75.3 mg, 0.165 mmol) was added in CH_2Cl_2 (0.5 ml) and the resulting soln. stirred for 45 min at −60° before warming to −10°. Et_3N (125 μl) was added and the resulting soln. warmed to r.t. H_2O was added and the mixture extracted with CH_2Cl_2. The combined org. phase was washed with H_2O and brine, dried ($MgSO_4$), and evaporated. The crude **15** was used immediately for the aldol coupling. ^{1}H-NMR (270 MHz, $CDCl_3$): 0.87 (*t*, $CH_3(CH_2)_6CON$); 1.14 (*d*, Me); 1.24 (*d*, Me); 1.25 (*d*, Me); 1.28 (*m*, 4 CH_2); 1.31 (*s*, 3 H, Me_2C); 1.33 (*s*, 3 H, Me_2C); 1.62 (*m*, CH_2CH_2CON); 2.02 (*m*, H–C(4)); 2.12 (*t*, CH_2CON); 2.58 (*m*, H–C(6)); 2.70 (*m*, H–C(2)); 2.97 (*m*, CH_2S); 3.35 (*m*, CH_2N); 3.58 (*m*, H–C(3)); 3.85 (*dd*, H–C(5)); 6.12 (br. *t*, NH); 9.65 (*d*, H–C(7)).

(3RS,4S)-2,4-Dimethyl-6-(phenylsulfonyl)hex-1-en-3-ol (**29**). Oxalyl chloride (168 mg, 1.32 mmol) was dissolved in CH_2Cl_2 (3 ml) and cooled to −60°. DMSO (207 mg, 2.65 mmol) was added and the mixture stirred for 2 min. (2*S*)-2-Methyl-4-(phenylsulfonyl)butan-1-ol (190 mg, 0.83 mmol) in CH_2Cl_2 (0.5 ml) was added and the mixture stirred for a further 30 min at −60° before warming to −10°. Et_3N (0.65 ml) was added and the mixture warmed to 20°. H_2O (10 ml) was then added and the mixture extracted with CH_2Cl_2 (3 × 10 ml). The combined org. phase was washed with H_2O and brine, dried (Na_2SO_4), and evaporated: *(2S)-2-methyl-4-(phenylsulfonyl)butanal* (**28**) as an oil which was not purified before use. ^{1}H-NMR (270 MHz, $CDCl_3$): 1.05 (*d*, Me); 1.7 (*m*, CH_2(3)); 2.0 (*m*, H–C(2)); 3.05 (*m*, CH_2(4)); 7.5–7.9 (*m*, Ph); 9.5 (*s* CHO).

Mg (21.9 mg, 0.90 mmol) and I_2 (cat.) were warmed with 2-bromopropene (99.6 mg, 0.82 mmol) in THF (4 ml) at r.t. until a clear soln. had formed. Then, **28** was added in THF (0.5 ml) and the soln. stirred for 16 h. The reaction was quenched with sat. aq. NH_4Cl and 10% HCl soln. Most of the org. solvent was evaporated before extracting with Et_2O. The combined org. phase was washed with 5% aq. HCl soln. and brine, dried (Na_2SO_4), and evaporated. The product was purified by FC (Et_2O): 154.3 mg (68.5%) of **29**. $[\alpha]_D^{22} = -1.6$ (*c* = 1.6, $CHCl_3$). UV (MeOH): 254 (660), 266 (910), 272 (810), 249 (infl.), 253 (sh). IR ($CHCl_3$): 3100*m*, 1592*w*, 1450*s*, 1385*m*, 1160*vs*. ^{1}H-NMR (270 MHz, $CDCl_3$): 0.75 (2*d*, *J* = 7, Me); 1.55 (2*s*, Me–C(2)); 1.5–2.0 (*m*, CH_2(5), H–C(4), OH); 3.10 (*m*, CH_2(6)); 3.71 (*m*, H–C(3)); 4.80 (*m*, CH_2(1)); 7.5–7.9 (*m*, Ph). ^{13}C-NMR (67.9 MHz, $CDCl_3$): 13.5, 18.6, 26.6, 34.5, 54.5, 78.2, 111.9, 128.2, 129.2, 133.8, 139.1, 146.05. CI-MS (NH_3): 269 (2.4, *M* + H), 257 (7.5), 143 (20), 125 (16), 110 (11.1), 109 (100). HR-MS: 286.14821 (*M* + NH_4, $C_{14}H_{20}O_3S$ + NH_4, calc. 286.1477).

(6S,4E)-Methyl 4,6-Dimethyl-8-(phenylsulfonyl)oct-4-enoate (**30**). A mixture of **29** (0.77 g, 2.87 mmol), toluene (20 ml), trimethyl orthoacetate (1.89 g, 15.9 mmol), and trimethylbenzoic acid (60 mg, cat.) was stirred at 110° for 4 h and then cooled. After evaporation of the toluene, the residue was dissolved in CH_2Cl_2, the org. phase washed with H_2O and brine, dried (Na_2SO_4), and evaporated, and the product purified by FC (Et_2O/petroleum ether 1:1): 0.64 g (69.0%) of **30**. $[\alpha]_D^{23} = -2.09$ (*c* = 2.50, $CHCl_3$). UV (MeOH): 258 (660), 266 (910), 272 (810), 249 (infl., 253 (sh)). IR ($CHCl_3$): 1732*s*, 1592*w*, 1455*m*, 1310*s*, 1160*vs*. ^{1}H-NMR (270 MHz, $CDCl_3$): 0.85 (*d*, Me); 1.53 (*s*, Me); 1.70 (*m*, CH_2(7)); 2.20 (*m*, CH_2(3)); 2.35 (*m*, CH_2(2), H–C(6)); 2.95 (*m*, CH_2(8)); 3.57 (*s*, CO_2Me); 4.75 (*d*, H–C(5)); 7.5–7.9 (*m*, Ph). ^{13}C-NMR (67.9 MHz, $CDCl_3$): 16.2, 21.1, 29.9, 31.4, 32.8, 34.6, 51.5, 54.6, 129.7, 134.2, 128.0, 129.2, 133.6, 139.1, 173.6. FAB-MS: 325 (61.8, *M* + H), 309 (12.8), 293 (100), 182 (14.6), 151 (83.5), 109 (34.6). Anal. calc. for $C_{17}H_{24}O_4S$: C 62.9, H 7.4, S 9.9; found: C 62.9, H 7.5, S 9.9.

(3RS,8S,6E)-2,6,8-Trimethyl-10-(phenylsulfonyl)deca-1,6-dien-3-ol. DIBAL (2.06 ml, 1.5M in hexane, 3.0 mmol) in toluene (0.8 ml) was added slowly over 2 h to **30** (0.77 g, 2.37 mmol) in toluene (8 ml) at −90°. After stirring for a further h, AcOEt sat. with H_2O (4 ml) was added. The mixture was then warmed to r.t. and sat. aq. NaCl soln. added before extracting with Et_2O. The combined org. phase was washed with brine, dried (Na_2SO_4), and evaporated: aldehyde as an oil which was not purified further. ^{1}H-NMR (90 MHz, $CDCl_3$): 0.91 (*d*, Me); 1.66 (*s*, Me); 1.80 (*m*, CH_2(7)); 2.1 (*m*, CH_2(3)); 2.25 (*m*, CH_2(2)); 2.35 (*m*, H–C(6)); 2.95 (*m*, CH_2(8)); 4.7 (*m*, H–C(5); 7.5–7.9 (*m*, Ph); 9.75 (*t*, *J* = 3, CHO). Then, 2-bromopropene (291.5 mg, 2.42 mmol) was added to Mg (64.0 mg, 2.61 mmol) and I_2 (cat.) in THF (10 ml). The mixture was stirred at r.t., until a clear soln. had formed. The foregoing aldehyde was added in THF (2 ml) and the soln. stirred for 16 h. The reaction was quenched with sat. aq. NH_4Cl and 10% HCl soln. Most of the org. solvent was evaporated before extracting the concentrate with Et_2O. The combined org. phase was washed with 5% HCl soln. and brine, dried (Na_2SO_4), and evaporated. The resulting residue was purified by FC (Et_2O/petroleum ether 1:1): 0.55 g (68.9%) of title compound. $[\alpha]_D^{22} = +1.32$ (*c* = 1.82, $CHCl_3$). UV (MeOH): 258 (660), 266 (910), 272 (810), 249 (infl.), 253 (sh). IR ($CHCl_3$): 3610*m* (br.), 1160*s*, 1095*m*. ^{1}H-NMR (270 MHz, $CDCl_3$): 0.90 (*d*, Me); 1.61 (*s*, Me); 1.73 (*s*, Me); 1.6–1.8 (*m*, CH_2(9), CH_2(4)); 2.0 (*m*, CH_2(5)); 2.4 (*m*, H–C(8), OH); 3.11 (*m*, CH_2(10)); 4.0 (*t*, H–C(3)); 4.8–4.95 (*m*, CH_2(1), H–C(7)); 7.5–7.9 (*m*, Ph). ^{13}C-NMR (67.9, $CDCl_3$): 16.4, 16.45, 17.7, 21.2, 21.3, 30.1, 31.5, 33.2, 33.3, 35.7, 54.8, 75.5, 111.1, 111.2, 129.0, 129.1, 129.3, 128.1, 135.4, 135.8, 147.5, 147.6. FAB-MS: 337 (9.5, *M* + H), 319 (34), 293 (11), 143 (37), 121 (43), 109 (100), 95 (56). Anal. calc. for $C_{19}H_{28}O_3S$: C 67.8, H 8.4, S 9.5; found: C 67.6, H 8.14, S 9.4.

(10S,4E,8E)-Methyl 4,8,10-Trimethyl-12-(phenylsulfonyl)dodeca-4,8-dienoate (**31**). Trimethyl orthoacetate (190 mg, 1.6 mmol) and trimethylbenzoic acid (10 mg, cat.) were added to the foregoing alcohol (98.0 mg, 0.29 mmol) in toluene (8 ml), and the mixture was stirred at 110° for 3 h before cooling and evaporating the toluene. The residue was dissolved in CH_2Cl_2, the soln. washed with H_2O and brine, dried (Na_2SO_4), and evaporated, and the residue purified by FC (Et_2O/petroleum ether 1:1): 93.1 mg (81.3%) of **31**. $[\alpha]_D^{23} = +1.92$ (*c* = 0.84, $CHCl_3$). UV (MeOH): 258 (660), 266 (910), 272 (810), 249 (infl.), 253 (sh). IR ($CHCl_3$): 1745*s*, 1492*w*, 1455*m*, 1160v*s*. ^{1}H-NMR (270 MHz, $CDCl_3$): 0.89 (*d*, Me); 1.52 (*s*, Me); 1.60 (*s*, Me); 1.71 (*m*, CH_2(11)); 1.95 (*m*, CH_2(6), CH_2(7)); 2.35 (*m*, CH_2(3), CH_2(2), H–C(10)); 3.05 (*m*, CH_2(12)); 3.65 (*s*, CO_2Me); 4.75 (*d*, *J* = 10, H–C(9)); 5.11 (*t*, *J* = 8, H–C(5)); 7.6–7.9 (*m*, Ph). ^{13}C-NMR (67.9 MHz, $CDCl_3$): 16.0, 16.4, 21.3 (3 *q*); 26.5 (*d*); 30.1 (*t*); 31.5 (*d*); 33.0, 34.6, 39.5, 54.8 (4 *t*); 124.8, 128.1, 128.9 (3 *d*); 129.3 (*s*); 133.5, 133.6 (2 *d*); 135.7, 136.7, 173.9 (3 *s*). FAB-MS: 393 (18.0, *M* + H), 361 (43.5), 219 (21.6), 201 (13.0), 189 (13.0), 175 (14.6), 165 (19.3), 153 (13.1), 152 (13.9), 141 (51.8), 109 (71), 81 (100). Anal. calc. for $C_{22}H_{32}O_4S$: C 67.3, H 8.2, S 8.2; found: C 67.4, H 8.1, S 7.9.

(2RS,11S,5E,9E)-5,9,11-Trimethyl-13(phenylsulfonyl)trideca-5,9-dien-2-ol. DIBAL (316 µl, 1.5M in hexane, 0.46 mmol) mixed with toluene (100 µl) was added slowly over 2 h to **31** (142 mg, 0.36 mmol) in toluene (1 ml) at −90°. After stirring for 1 h, AcOEt sat. with H_2O (2 ml) was added and the mixture warmed to r.t. Sat. aq. NaCl soln. was added and the mixture extracted with Et_2O. The combined org. phase was washed with brine, dried (Na_2SO_4), and evaporated: aldehyde as an oil which was not purified further. ^{1}H-NMR (90 MHz, $CDCl_3$): 0.90 (*d*, Me); 1.45 (*s*, Me); 1.55 (*s*, Me); 2.0 (*m*, CH_2(6), CH_2(7)); 2.35 (*m*, H–C(10), CH_2(3), CH_2(2)); 3.0 (*t*, CH_2(12)); 4.70 (*d*, H–C(9)); 5.1 (*d*, H–C(5)); 7.6–7.9 (*m*, Ph); 9.7 (*t*, H–C(1)).

MeLi (0.8M, 1.5 ml, 1.1 mmol) was added slowly to the foregoing aldehyde dissolved in Et_2O (2 ml) at −78°. This was stirred for 30 min at −78° before warming to −40° for 15 min. The mixture was then cooled to −78° and sat. aq. NH_4Cl soln. added before extracting with Et_2O. The combined org. phase was washed with H_2O and brine, dried (Na_2SO_4), and evaporated. The residue was purified by prep. TLC (Et_2O) title compound (93.9 mg, 85%; with 9.4% recovery of starting material). $[\alpha]_D^{22} = +5.71$ (*c* = 3.15, $CHCl_3$). UV (MeOH): 258 (660), 265 (910), 272 (810), 249 (infl.), 253 (sh). IR ($CHCl_3$): 3500*w*, 1450*s*, 1150*s*, 1095*m*. ^{1}H-NMR (270 MHz, $CDCl_3$): 0.92 (*d*, Me); 1.20 (*d*, Me); 1.58 (*s*, Me); 1.60 (*s*, Me); 1.61 (*m*, CH_2(3)); 1.71 (*m*, CH_2(12)); 2.02 (7 H, CH_2(4), CH_2(8), CH_2(7), OH); 2.35 (*m*, H–C(11)); 3.02 (*m*, CH_2(13)); 3.80 (*m*, H–C(2)); 4.78 (*d*, H–C(10)); 5.1 (*t*, H–C(6)); 7.5–7.9 (*m*, Ph). ^{13}C-NMR (67.9 MHz, $CDCl_3$): 16.2, 16.4, 21.4, 23.5, 26.3, 30.3, 31.6, 36.2, 37.5, 39.6, 54.9, 68.2, 124.2, 128.2, 129.1, 129.4, 133.8, 135.8, 139.2. EI-MS: 378 (3.4, M^+), 360 (8.5), 318 (5.7), 149 (22.7), 109 (100). HR-MS: 378.2225 (M^+, $C_{22}H_{34}O_3S$, calc. 378.2228).

(3S,12RS,4E,8E)-12-[(tert-Butyl)dimethylsilyloxy]-3,5,9-trimethyl-1-(phenylsulfonyl)trideca-4,8-diene (**16**). The foregoing alcohol (63 mg, 0.17 mmol), imidazole (34.1 mg, 0.5 mmol), and (*t*-Bu)Me_2SiCl (37.7 mg, 0.25 mmol) in DMF (2 ml) were stirred for 20 h at r.t. Then, sat. aq. NH_4Cl soln. was added and the mixture extracted with Et_2O. The combined org. layers were washed with H_2O and brine, dried (Na_2SO_4), and evaporated. The product was purified by prep. TLC (Et_2O): 73 mg (71.3%) as an oil. $[\alpha]_D^{20} = -2.3$ (*c* = 1.6, $CHCl_3$). UV (MeOH): 258 (660), 266 (900), 272 (810), 249 (infl.), 253 (sh). IR ($CHCl_3$): 1450, 1370*m*, 1140*s*. ^{1}H-NMR (270 MHz, $CDCl_3$): 0.04 (*s*, Me_2Si); 0.90 (*s*, *t*-BuSi); 0.92 (*d*, Me); 1.18 (*d*, Me); 1.58 (*s*, Me); 1.60 (*s*, Me); 1.50 (*m*, CH_2(11)); 1.71 (*m*, CH_2(2)); 2.0 (*m*, CH_2(10), CH_2(6), CH_2(7)); 2.35 (*m*, H–C(3)); 3.0 (*m*, CH_2(1)); 3.8 (*m*, H–C(12)); 4.78 (*d*,

H–C(4)); 5.11 (*t*, H–C(8)); 7.5–7.9 (*m*, Ph). ^{13}C-NMR (67.9 MHz, $CDCl_3$): –4.5, –4.2, 16.3, 16.5, 18.4, 21.4, 23.9, 26.1, 26.8, 29.9, 30.3, 31.6, 36.1, 38.4, 39.8, 55.0, 68.6, 123.8, 128.9, 135.6, 136.1, 128.2, 129.4, 133.8, 139.4. CI-MS (NH_3): 494 (72, M + 2 H), 435 (100), 361 (98), 219 (10), 163 (8), 109 (28). HR-MS: 491.30242 (M – H, $C_{28}H_{48}O_3SSi$ – H, calc. 491.30151).

*(2R,4S,6RS,8S,17RS,9E,13E)-17-[(*tert*-Butyl)dimethylsilyloxy]-4,8,10,14-tetramethyl-2-(2'-methyl-1',3'-dioxolan-2'-yl)-6-(phenylsulfonyl)octadeca-9,13-dien-5-one* (**32**). BuLi (0.5M, 0.38 ml) was added to **16** (93.6 mg, 0.19 mmol) in THF (0.4 ml) at –78°. After stirring for 30 min, **17** (30.8 mg, 0.143 mmol) in THF (0.25 ml) was added slowly and the resulting soln. stirred at –78° for 75 min and at 0° for a further 15 min. Phosphate buffer (pH 8) was added at 0° and the mixture stirred for 15 min before extracting with Et_2O. The combined org. phase was washed with H_2O and brine, dried ($MgSO_4$), and evaporated. The product was purified by prep. TLC (AcOEt/CH_2Cl_2 1:4): 43.2 mg (67.4%) of **32** and 55.8 mg of **16**. UV (MeOH): 258 (660), 265 (910), 272 (810), 249 (infl.), 253 (sh). IR ($CHCl_3$): 1712*s*, 1450*m*, 1370*m*, 1150*s*. ^{1}H-NMR (270 MHz, $CDCl_3$): 0.04 (*s*, Me_2Si); 0.89 (*d*, Me); 0.95 (*s*, *t*-Bu); 0.97, 1.04 (2*d*, 3 H, Me); 1.05 (*d*, Me); 1.12, 1.14 (2*d*, 3 H, Me); 1.35 (*s*, Me); 0.9–1.5 (*m*, CH_2(3)); 1.48 (*s*, Me); 1.63 (*s*, Me); 1.75 (*m*, H–C(2)); 1.85–2.2 (*m*, CH_2(15), CH_2(11), CH_2(12), CH_2(7), H–C(8)); 3.25 (*m*, H–C(4)); 3.79 (*m*, H–C(17)); 3.97 (*m*, $(CH_2O)_2$); 4.30 (*d*, J = 10, H–C(6)); 4.78 (*d*, J = 10, H–C(9)); 5.08 (*t*, J = 7.8, H–C(13)); 7.5–7.8 (*m*, Ph). ^{13}C-NMR (67.9 MHz, $CDCl_3$): –4.50, –4.18, 1.21, 15.6, 16.4, 16.6, 16.9, 18.3, 20.1, 20.3, 22.1, 23.9, 26.1, 26.7, 26.75, 30.0, 30.4, 34.66, 35.1, 35.3, 36.1, 38.4, 38.68, 39.72, 39.8, 46.2, 46.7, 64.6, 64.7, 64.8, 68.6, 71.9, 72.15, 77.4, 112.4, 123.6, 123.8, 128.7, 129.0, 129.6, 129.8, 134.3, 135.7, 136.6, 137.7, 205.1. CI-MS (NH_3): 694 (12.6, M + NH_4), 554 (42.1), 537 (4.8), 274 (2.5), 230 (4.2), 175 (5.6), 160 (8.9), 87 (7.1), 52 (100). HR-MS: 694.4533 (M + NH_4, $C_{38}H_{64}O_6SSi$ + NH_4, calc. 694.4543).

*(2R,4S,5RS,6RS,8S,17RS,9E,13E)-17-[(*tert*-Butyl)dimethylsilyloxy]-4,8,10,14-tetramethyl-2-(2'-methyl-1',3'-dioxolan-2'-yl)-6-(phenylsulfonyl)octadeca-9,13-dien-5-yl Benzoate*. At r.t., **32** (119 mg, 0.176 mmol) and $NaBH_4$ (10 mg, 0.26 mmol) were stirred in EtOH (1 ml) for 5 h. The solvent was then evaporated, the residue redissolved in H_2O and extracted with Et_2O, the combined org. phase washed with H_2O and brine, dried ($MgSO_4$), and evaporated; and the crude product carried forward onto the next stage. The residue was dissolved in THF (2 ml) with 1,10-phenanthroline (2 mg) and cooled to –78°. BuLi (*ca.* 100 µl) was added dropwise, until there was a permanent colour change. Benzoyl chloride (100 µl, excess) was added and the soln. allowed to warm to 0° over 4 h. Et_3N (100 µl) was added and the resulting soln. warmed to r.t. and then added to an equal quantity of H_2O before extracting with Et_2O. The combined org. phase was washed with H_2O and brine, dried ($MgSO_4$), and evaporated, and the product purified by prep. TLC (petroleum ether/AcOEt 4:1): title compound (105 mg, 87.0%) as an oil. It was possible to separate two diastereoisomers.

Diastereoisomer with lower R_f: UV (MeOH): 258 (695), 270 (900), 272 (885), 280 (infl.). IR ($CHCl_3$): 1720*s*, 1455, 1360*m*, 1155*s*, 835*s*. ^{1}H-NMR (270 MHz, $CDCl_3$): 0.04 (2*s*, Me_2Si); 0.82 (*d*, Me); 0.92 (*s*, *t*-Bu); 0.96 (*d*, Me); 1.08 (*d*, Me); 1.13 (*d*, Me); 1.17 (*s*, Me); 0.9–1.2 (*m*, CH_2(3)); 1.4–1.8 (*m*, CH_2(15), CH_2(16), CH_2(7)); 1.50 (*s*, Me); 1.54 (*s*, Me); 1.8–2.0 (*m*, CH_2(11), CH_2(12)); 2.14 (*m*, H–C(8)); 2.42 (*m*, H–C(4), H–C(2)); 3.68 (*m*, H–C(17)); 3.80 (*m*, H–C(6), $(CH_2O)_2$); 4.59 (*d*, J = 10, H–C(9)); 4.96 (*t*, J = 8, H–C(13)); 5.38 (*m*, H–C(5)); 7.4–7.9 (*m*, 2 Ph). ^{13}C-NMR (67.9 MHz, $CDCl_3$): –4.48, –4.15, 15.85, 16.3, 16.5, 16.6, 18.35, 19.95, 21.5, 24.0, 26.1, 26.6, 31.7, 32.8, 33.9, 36.1, 37.0, 38.15, 38.4, 39.8, 63.5, 64.6, 68.9, 72.9, 112.4, 123.8, 128.3, 128.9, 129.2, 129.3, 133.2, 133.5, 135.4, 136.4, 140.4, 165.4. CI-MS (NH_3): 800 (24.1, M + NH_4), 783 (2.2), 694 (81.1), 660 (14.7), 475 (11.8), 405 (17.4), 230 (13.5), 175 (20.2), 160 (30.8). HR-MS: 800.4981 (M + NH_4, $C_{45}H_{70}O_7SSi$ + NH_4, calc. 800.4955).

Diastereoisomer with higher R_f: ^{1}H-NMR (270 MHz, $CDCl_3$): 0.04 (2*s*, Me_2Si); 0.83 (*d*, Me); 0.92 (*s*, *t*-Bu); 0.98 (*d*, Me); 1.08 (*d*, Me); 1.10 (*s*, Me); 1.16 (*d*, Me); 1.60 (*s*, Me); 1.62 (*s*, Me); 0.9–1.2 (*m*, CH_2(3)); 1.4–1.8 (*m*, CH_2(15), CH_2(16), CH_2(7)); 1.8–2.1 (*m*, H–C(8), CH_2(11), CH_2(12)); 2.64 (*m*, H–C(2)); 2.96 (*m*, H–C(4)); 3.4 (*m*, H–C(6)); 3.55–3.88 (*m*, H–C(17), $(CH_2O)_2$); 4.34 (*d*, J = 10, H–C(9)); 5.08 (*t*, J = 8, H–C(13)); 5.25 (*d*, J = 12, H–C(5)); 7.4–7.9 (*m*, 2 Ph). ^{13}C-NMR (67.9 MHz, $CDCl_3$): –4.48, –4.13, 16.3, 16.7, 16.8, 18.35, 18.9, 20.0, 21.9, 24.0, 26.1, 26.9, 29.6, 33.9, 34.6, 36.1, 37.6, 38.4, 39.85, 40.3, 63.9, 64.4, 64.6, 68.7, 77.0, 112.4, 123.7, 128.5, 128.8, 129.25, 129.3, 129.8, 130.3, 133.25, 133.5, 135.7, 136.9, 140.4, 165.4.

*(2R,4S,5RS,6RS,8S,17RS,9E,13E,13E)-17-[(*tert*-Butyl)dimethylsilyloxy]-4,8,10,14-tetramethyl-2-(2'-methyl-1',3'-dioxolan-2'-yl)-6-(phenylsulfonyl)[5-^{3}H]octadeca-9,13-dien-5-yl Benzoate*. $NaBH_4$ (0.2 mg, 5.3 µmol) was added to **32** (140 mg, 0.207 mmol) in EtOH (1 ml) and stirred for 30 min. [^{3}H]$NaBH_4$ (0.55 mg, 13.2 µmol, 100 mCi) was added and the soln. stirred for 42 h. Then, $NaBH_4$ (20 mg, excess) was added and the soln. left for a further 12 h. After evaporation, the residue was redissolved in H_2O and extracted with Et_2O and the combined Et_2O phase washed with H_2O and brine, dried ($MgSO_4$), and evaporated. The resulting alcohol was benzoylated by the procedure described above for non-labelled material. For spectral data, see above.

*(2R,4S,8S,17RS,5E,9E,13E)-17-[(*tert*-Butyl)dimethylsilyloxy]-4,8,10,14-tetramethyl-2-(2'-methyl-1',3'-dioxolan-2'-yl)octadeca-5,9,13-triene* (**18**). Na_2HPO_4 (0.32 g) was added to the foregoing unlabelled benzoate (93

mg, 0.119 mmol) in THF (6 ml), MeOH (1.2 ml), and AcOEt (0.6 ml) under Ar, and the suspension was cooled to −30°. Na-Hg (6%) amalgam (0.8 g) was added and the suspension stirred for 1 h. More Na-Hg amalgam (0.8 g), Na_2HPO_4 (0.32 g), and MeOH (0.6 ml) were then added at 1-h intervals for a further 4 h, and the mixture was stirred for a further 3 h at −30°. The mixture was then poured into petroleum ether (60 ml) and filtered through *Celite*. The filtrate was washed with brine, dried ($MgSO_4$), and evaporated and the product purified FC (Et_2O/CH_2Cl_2/petroleum ether 1:1:18): **18** (47.1 mg, 76.2%) as an oil. $[\alpha]_D^{22} = +15.5$ (*c* = 1.13, $CHCl_3$). IR ($CHCl_3$): 1380*s*, 1255*s*, 1050*m*, 970*s*. ^{1}H-NMR (270 MHz, $CDCl_3$): 0.04 (*s*, Me_2Si); 0.87 (*s* + 2*d*, *t*-Bu, 2 Me); 0.90 (*d*, Me); 1.12 (*d*, Me); 1.20 (*m*, H–C(3)); 1.25 (*s*, Me); 1.4–1.7 (*m*, CH_2(16), H–C(3)); 1.60 (*s*, 2 Me); 1.85–2.2 (H–C(4), CH_2(11), CH_2(12), CH_2(7), H–C(8), CH_2(15)); 2.35 (*m*, H–C(2)); 3.76 (*m*, H–C(17)); 3.91 (*m*, $(CH_2O)_2$); 4.93 (*d*, H–C(9)); 5.11 (*m*, H–C(13), H–C(5)); 5.32 (*m*, H–C(6)). ^{13}C-NMR: (67.9 MHz, $CDCl_3$): −4.5, −4.2, 14.4, 16.3, 16.4 (5 *q*); 18.3 (*s*); 20.4, 20.8, 22.0, 23.9, 26.1 (5 *q*); 33.0, 35.0 (2 *d*); 36.1, 38.4 (2 *t*); 38.8 (*d*); 39.1, 39.9, 40.8, 64.65, 64.6 (5 *t*); 68.7 (*d*); 112.7 (*s*); 124.15, 128.0, 131.1 (3 *d*); 133.4, 135.2 (2 *s*); 136.8 (*d*). CI-MS (NH_3): 538 (47.3, *M* + NH_4), 521 (42.5, *M* + H), 459 (100), 389 (70.5), 345 (24.0), 327 (93.2), 109 (34.6). HR-MS: 521.43950 (*M* + H, $C_{32}H_{60}O_3Si$ + H, calc. 521.43899).

*(2*R*,4*S*,8*S*,17*RS*,5*E*,9*E*,13*E*)-17-[(*tert*-Butyl)dimethylsilyloxy]-4,8,10,14-tetramethyl-2-(2′-methyl-1′,3′-dioxolan-2′-yl)[5-^{3}H]octadeca-5,9,13-triene* (**33**) was prepared from the tritiated precursor using the procedure described above.

*(11*S*,15*S*,17*R*,5*E*,9*E*,13*E*)-5,9,11,15-Tetramethyl-17-(2′-methyl-1′,3′-dioxolan-2′-yl)[14-^{3}H]octadeca-5,9,13-trien-2-one* (**35**). Bu_4NF (1.0M, 250 µl) was added in portions to **33** (69 mg, 0.133 mmol) in THF (1 ml) and stirred at r.t. for 16 h. H_2O was added and the mixture extracted with Et_2O. The combined org. phase was washed with H_2O and brine, dried ($MgSO_4$), and evaporated. The crude product was used directly. ^{1}H-NMR (270 MHz, $CDCl_3$): 0.88 (2*d*, 2 Me); 0.95 (*d*, Me); 1.18 (*d*, Me); 0.9–1.2 (*m*, 1 H–C(16)); 1.21 (*s*, Me); 1.4–1.7 (*m*, 1 H–C(16), CH_2(3)); 1.56 (*s*, Me); 1.58 (*s*, Me); 1.8–2.15 (10 H, CH_2(4), H–C(17), CH_2(12), CH_2(7), CH_2(8), H–C(15)); 2.35 (*m*, H–C(11)); 2.55 (*m*, OH); 3.78 (*m*, H–C(2)); 3.90 (*m*, $(CH_2O)_2$); 4.91 (*d*, H–C(10)); 5.12 (*m*, H–C(6)); 5.15 (*m*, H–C(14)); 5.31 (*m*, H–C(13)). ^{13}C-NMR (67.9 MHz, $CDCl_3$): 14.4, 16.1, 16.4, 20.8, 22.9, 23.6, 26.7, 33.0, 34.9, 36.3, 37.5, 38.9, 39.1, 39.8, 40.8, 64.6, 64.7, 68.2, 112.7, 124.9, 128.0, 131.2, 133.3, 135.0, 136.9.

DMSO (33.0 mg, 0.42 mmol) was added slowly to oxalyl chloride (26.8 mg, 0.21 mmol) in CH_2Cl_2 (0.5 ml) cooled to −60° and stirred for 2 min. The foregoing alcohol in CH_2Cl_2 (0.5 ml) was added and the mixture stirred at −60° for 1 h before warming to −10°. Et_3N (10 µl) was added, the soln. warmed to r.t., an equal volume of H_2O added, and the mixture extracted with CH_2Cl_2. The combined org. phase was washed with brine, dried ($MgSO_4$), and evaporated and the product purified by FC (petroleum ether/Et_2O 70:30): **35** (40.1 mg, 74.1%) as an oil. $[\alpha]_D^{22} = +18.5$ (*c* = 0.875, $CHCl_3$). IR ($CHCl_3$): 1715*s*, 975*m*, 860*m*. ^{1}H-NMR (270 MHz, $CDCl_3$): 0.87 (2*d*, 2 Me); 0.96 (*d*, Me); 1.18 (*m*, 1 H–C(16)); 1.21 (*s*, Me); 1.45 (*m*, 1 H–C(16)); 1.56 (*s*, Me); 1.58 (*s*, Me); 1.68 (*m*, H–C(17)); 1.90, 2.05 (2*m*, CH_2(7), CH_2(8), CH_2(12), H–C(15)); 2.12 (*s*, Me); 2.25 (*t*, CH_2(4)); 2.35 (*m*, H–C(11)); 2.51 (*t*, CH_2(3)); 3.89 (*m*, $(CH_2O)_2$); 4.90 (*d*, H–C(10)); 5.10 (*m*, H–C(6)); 5.15 (*m*, H–C(14)); 5.31 (*m*, H–C(13)). ^{13}C-NMR (67.9 MHz, $CDCl_3$): 14.35, 16.2, 16.4, 20.4, 20.8, 22.86, 26.7, 30.05, 32.95, 33.7, 34.9, 38.9, 39.95, 40.8, 42.6, 64.6, 64.7, 112.7, 125.0, 127.9, 131.2, 133.2, 133.5, 136.9, 209.05. CI-MS (NH_3): 422 (63.9, *M* + NH_4), 405 (47.2, *M* + H), 378 (14.2), 343 (19.9), 312 (13.0), 295 (26.5), 271 (20.2), 230 (23.6), 181 (38.2), 132 (65.1), 125 (42.7), 87 (100). HR-MS: 405.33817 (*M* + H, $C_{26}H_{44}O_3$ + H, calc. 405.3369).

*(2*S*,3*R*,4*R*,5*S*,6*R*,7*S*,18*S*,22*S*,24*R*,12*E*,16*E*,20*E*)-S-[2′-(Octanamido)ethyl] 7-Hydroxy-3,5-(isopropylidenedioxy)-2,4,6,12,16,18,22-heptamethyl-24-(2′-methyl-1′,3′-dioxolan-2′-yl)-9-oxo[21-^{3}H]pentadodeca-12,16,20-trienethioate* (**36**). BuLi was added to (i-Pr)$_2$NH (150 µl, 1.075 mmol) in THF (9.4 ml) cooled to −20° and stirred for 20 min (→ LDA, 0.108 mmol ml^{-1}). This LDA (1 ml, 0.108 mmol) was added to **35** (39 mg, 96.5 µmol) in THF (1 ml) at −78° and stirred at −78° for 20 min. Then, **15** (75 mg) in THF (0.5 ml) was added and stirred for 1 h before sat. aq. NH_4Cl soln. (50 µl) was added. The soln. was warmed to r.t., filtered through a plug of Na_2SO_4, and evaporated. The product was purified by prep. TLC (petroleum ether/AcOEt:1, then CH_2Cl_2/AcOEt 85:15): **36** (26 mg, 32.1%) as an oil; 1.37×10^8 dpm mg^{-1}, 1.17×10^{11} dpm mmol^{-1}. $[\alpha]_D^{22} = +10.1$ (*c* = 1.0, CH_2Cl_2). UV (CH_2Cl_2): 237.5 ($3.3 \cdot 10^3$). ^{1}H-NMR (360 MHz, $CDCl_3$): 0.86 (*t*, Me); 0.88 (3*d*, 3 Me); 0.96 (*d*, Me); 0.97 (*d*, Me); 1.12 (*m*, 1 H–C(23)); 1.22 (*s*, Me); 1.25 (*d*, Me); 1.28 (*m*, $CH_3(CH_2)_4(CH_2)_2CON$); 1.32 (*s*, Me); 1.34 (*s*, Me); 1.49 (*m*, 1 H–C(23)); 1.54 (H–C(6)); 1.58 (*s*, Me); 1.62 (*s*, Me); 1.63 (*m*, $CH_3(CH_2)_4CH_2CH_2CON$); 1.65 (*m*, H–C(24)); 1.92 (*m*, CH_2(19)); 1.95 (*m*, CH_2(15)); 2.06 (*m*, H–C(4), CH_2(14)); 2.14 (*m*, H–C(22), CH_2CON); 2.25 (*m*, CH_2(11)); 2.37 (*m*, H–C(18)); 2.47 (*dd*, *J* = 16, 3, 1 H–C(8)); 2.54 (*m*, CH_2(10)); 2.71 (*dd*, *J* = 16, 8, 1 H–C(8)); 2.82 (*m*, H–C(2)); 2.90 (br. *s*, OH); 3.04 (*m*, NCH_2CH_2S); 3.43 (*m*, NCH_2CH_2S); 3.64 (*m*, H–C(3)); 3.78 (*m*, H–C(5)); 3.92 (*m*, $(CH_2O)_2$); 4.15 (*m*, H–C(7)); 4.82 (*d*, *J* = 9, H–C(17)); 5.12 (*t*, *J* = 8, H–C(13)); 5.16 (*m*, H–C(21)); 5.32 (*m*, H–C(20)); 5.92 (*t*, *J* = 6, NH). ^{13}C-NMR (67.9 MHz, $CDCl_3$): 9.24; 12.5, 13.3, 14.25, 14.35, 16.3, 16.4, 20.4, 20.8 (8 *q*); 22.3 (*t*); 22.9, 24.2, 25.2 (3 *q*); 25.9 (*t*); 26.8 (*d*); 28.8, 29.2, 29.4, 31.9 (4 *t*); 33.0 (*d*); 33.4

(*t*); 34.9, 35.0 (2 *d*); 36.9 (*t*); 37.4, 38.9 (2 *d*); 39.1, 39.8, 40.7, 42.5, 47.5 (5 *t*); 52.4 (*d*); 64.6, 64.7 (2 *t*); 66.0; 70.6, 76.4 (2 *d*); 101.0, 112.7 (2 *s*); 125.4, 127.9 (2 *d*); 131.2; 133.3, 133.3 (2 *s*); 136.9 (*d*); 173.6, 202.4, 212.2 (3 *s*). FAB-MS: 870 (3.6, *M* + Na), 846 (1.5, *M* – H), 790 (2.1), 772 (4.9), 358 (4.2), 300 (6.9), 204 (59.4), 170 (40.5), 109 (41.0), 87 (100). HR-MS: 848.60752 (*M* + H, $C_{49}H_{85}O_8NS$ + H, calc. 848.60741).

(2S,3R,4R,5S,6R,7S,18S,22S,24R,12E,16E,20E)-S-[2'-(Octanamido)ethyl] 3,5,7-Trihydroxy-2,4,6,12,16,18,22,24-octamethyl-9,25-dioxo[21-^{3}H]hexadodeca-12,16,20-trienethioate ([21-^{3}H]-**7**). A soln. (100 µl) of conc. HCl soln. in MeCN (20 µl of conc. HCl in 10 ml of MeCN) and H_2O (5 µl) were added to **36** (9.0 mg, 1.2 µmol) in MeCN (1 ml). (TLC monitoring: completion after 48 h.) The solvent was evaporated and the residue chromatographed by prep. TLC (petroleum ether/AcOEt 1:1): [21-^{3}H]-**7** (5 mg, 61.0%). $[\alpha]_D^{22} = -23.3$ (*c* = 0.25, CH_2Cl_2). UV (CH_2Cl_2): 237.5 ($3.2 \cdot 10^3$). ^{1}H-NMR (360 MHz, $CDCl_3$): 0.88 (*t*, Me); 0.91 (*d*, Me); 0.97 (2*d*, 2 Me); 1.00 (*d*, Me); 1.03 (*d*, Me); 1.12 (*d*, Me); 1.22 (*m*, 1 H–C(23)); 1.3 (*m*, $CH_3(CH_2)_4(CH_2)_2CON$); 1.35 (*m*, H–C(6)); 1.52 (*m*, H–C(4)); 1.60 (*s*, Me–C(12), Me–C(16)); 1.64 (*m*, 1 H–C(23)); 1.65 (*m*, $CH_3(CH_2)_4CH_2CH_2CON$); 1.96 (*m*, CH_2(15), CH_2(19), H–C(22)); 2.06 (*m*, CH_2(14)); 2.11 (*s*, Me(26)); 2.17 (*m*, CH_2(11)); 2.26 (*m*, CH_2CON); 2.37 (*m*, H–C(18)); 2.48 (*m*, 1 H–C(8)); 2.48 (*m*, CH_2(10)); 2.49 (*m*, H–C(24)); 2.59 (*dd*, *J* = 4, 16, 1 H–C(8)); 2.81 (*m*, H–C(2)); 3.15 (*m*, NCH_2CH_2S); 3.58 (*m*, H–C(5)); 3.60 (*m*, H–C(7)); 3.62 (*m*, H–C(3)); 3.67 (*m*, NCH_2CH_2S); 4.90 (*d*, H–C(17)); 5.10 (*t*, H–C(13)); 5.16 (*m*, H–C(21)); 5.34 (*m*, H–C(20)); 6.98 (*t*, NH). ^{13}C-NMR (67.9 MHz, $CDCl_3$): 8.4, 8.81, 12.8, 13.5, 14.3, 16.0, 16.25, 16.5, 20.9, 21.9, 22.85, 26.0, 26.9, 29.3, 29.5, 29.9, 31.9, 32.0, 32.9, 33.4, 35.3, 36.8, 38.9, 39.8, 40.1, 40.9, 40.8, 43.6, 43.9, 45.35, 46.0, 49.5, 77.4, 78.8, 81.4, 125.15, 128.5, 131.1, 133.5, 136.35, 174.1, 201.9, 213.4. FAB-MS: 768 (24.1, *M* – H_2O + Na), 746 (16.1, *M* – H_2O + H), 728 (3.4), 595 (10.5), 551 (17.3), 507 (20.4), 463 (13.6), 204 (37.4), 170 (24.7), 109 (100). HR-MS: 746.53750 (*M* – H_2O + H, $C_{44}H_{77}O_7NS$ – OH, calc. 746.53933).

*(3S,4E)-8-[(*tert*-Butyl)dimethylsilyloxy]-3,5-dimethyl-1-(phenylsulfonyl)oct-4-ene* (**37**). A soln. of **30** (1.3 g, 4.1 mmol) in THF (5 ml) was added to DIBAL (8.3 ml, 1.5 M in hexane, 12.5 mM) and stirred at 0° for 12 h. AcOEt sat. with H_2O was added, followed by sat. aq. NaCl soln. and the mixture extracted with CH_2Cl_2. The org. phase was washed with brine, dried (Na_2SO_4), and evaporated, and the resulting residue purified by FC (AcOEt/CH_2Cl_2): *(6S,4E)-4,6-dimethyl-8-(phenylsulfonyl)oct-4-en-1-ol* (1.1 g, 93%) as a colourless oil. UV (EtOH): 267 (857), 273 (774), 260 (655), 255 (sh, 476). IR (CCl_4): 3530 (br.), 3030*s*, 3010*s*, 2980*s*, 1600*w*, 1310*s*, 1270*s*, 1220*s*, 1150*s*, 1090*s*, 900*s*. ^{1}H-NMR (270 MHz, $CDCl_3$): 0.85 (*d*, Me); 1.40–1.70 (*m*, CH_2(2), CH_2(7)); 1.55 (*s*, Me–C(4)); 1.90 (*t*, CH_2(3)); 2.31 (*m*, H–C(6)); 2.60 (br. *s*, OH); 2.95 (*m*, CH_2(8)); 3.55 (*t*, CH_2(1)); 4.75 (*d*, *J* = 9, H–C(5)); 7.45–7.85 (*m*, Ar). ^{13}C-NMR (67.9 MHz, $CDCl_3$): 16.15, 21.1, 29.9, 30.6, 31.2, 35.6, 54.5, 62.05, 127.85, 128.8, 129.2, 133.6, 135.5, 138.8.

(*t*-Bu)Me_2SiCl (0.55 g, 3.6 mmol) and imidazole (2.07 g, 30 mmol) were added to the above alcohol (0.9 g, 3.0 mmol) in DMF (30 ml) and stirred for 15 h at r.t. The soln. was then poured into H_2O and extracted with CH_2Cl_2. The extract was washed with 0.1% aq. HCl soln., sat. aq. $NaHCO_3$ soln. and brine, dried (Na_2SO_4), and evaporated. The residue was chromatographed by FC (CH_2Cl_2): **37** (1.16 g, 95%) as a colourless oil. UV (EtOH): 267 (857), 273 (774), 260 (655), 255 (476). IR (CCl_4): 2980*s*, 2970*s*, 2860*s*, 1450*m*, 1330*s*, 1260*s*, 1160*s*. ^{1}H-NMR (270 MHz, $CDCl_3$): 0.05 (*s*, Me_2Si); 0.88 (*s*, *t*-BuSi); 0.90 (*d*, Me); 1.40–1.80 (*m*, CH_2(2), CH_2(7)); 1.53 (*s*, Me–C(5)); 1.95 (*t*, CH_2(6)); 2.35 (*m*, H–C(3)); 3.01 (*m*, CH_2(1)); 3.53 (*t*, CH_2(8)); 4.75 (*d*, *J* = 9, H–C(4)); 7.50–7.90 (*m*, Ar). ^{13}C-NMR (67.9 MHz, $CDCl_3$): –5.2, 16.4, 18.4, 21.3, 26.0, 30.1, 31.2, 31.5, 35.8, 54.8, 62.7, 128.1, 128.8, 129.3, 133.7, 135.8, 139.2.

*(2R,4S,8S,5E,9E)-13-[(*tert*-Butyl)dimethylsilyloxy]-4,8,10-trimethyl-2-(2'-methyl-1',3'-dioxolan-2'-yl)-[5-^{3}H]trideca-5,9-diene* (**38**). Using the modified *Julia* olefination as described above for **33**, **38** was prepared from **37** and **17**. IR (CCl_4): 2960*s*, 2940*s*, 2880*s*, 1260*s*, 1100*s*, 980*m*, 845*s*. ^{1}H-NMR (270 MHz, $CDCl_3$): 0.05 (*s*, Me_2Si); 0.9 (2*d* + *s*, 2 Me, *t*-Bu); 0.95 (*d*, Me); 1.05 (*m*, 1 H–C(3)); 1.22 (*s*, Me–C(2')); 1.44 (*m*, 1 H–C(3)); 1.59 (*s*, Me); 1.60–1.80 (*m*, H–C(2), CH_2(12)); 1.90–2.05 (*m*, CH_2(7), CH_2(11)); 2.13 (*m*, H–C(4)); 2.35 (*m*, H–C(8)); 3.55 (*t*, CH_2(13)); 3.90 (*m*, $(CH_2O)_2$); 4.93 (*d*, *J* = 10, H–C(9)); 5.14 (*dd*, *J* = 14, 7 H–C(5)); 5.32 (*dt*, *J* = 14, 7, H–C(6)). ^{13}C-NMR (67.9 MHz, $CDCl_3$): 14.2, 16.4, 20.5, 20.9, 22.9, 30.9, 32.9, 34.9, 36.1, 39.0, 39.1, 40.8, 62.9, 64.6, 64.7, 112.7, 128.0, 131.3, 133.2, 136.9. HR-MS: 439.3619 (*M* + H, $C_{26}H_{50}O_3Si$ + H, calc. 439.3607).

(6S,10S,12R,4E,8E)-S-[2'-(Octanamido)ethyl] 4,6,10,12-Tetramethyl-13-oxo[9-^{3}H]tetradeca-4,8-diene-thioate ([9-^{3}H]-**11**). Diene **38** (80 mg) was added to Bu_4NF in THF (2 ml, 1.0M). After stirring for 16 h, H_2O was added and the product extracted with Et_2O. After washing with H_2O and brine, the soln. was dried (Na_2SO_4) and evaporated: alcohol (50 mg, 84%). *Jones* reagent [54] was added dropwise to a soln. of this alcohol (20 mg, 0.08 mmol) in acetone (1 ml), until the soln. remained orange. The reaction was quenched with sat. $NaHCO_3$ soln. After extraction with Et_2O (5 × 2 ml), the org. layer was dried (Na_2SO_4) and evaporated. The dry residue in CH_2Cl_2 (1 ml) was treated with *N,N*-dicyclohexylcarbodiimide (60 mg, 0.26 mmol), 4-(dimethylamino)pyridine (3.3 mg, 0.03 mmol), and *N*-octanoylcysteamine (57 mg, 0.26 mmol) and the mixture stirred for 15 h at r.t. This was filtered

through *Celite* with CH_2Cl_2, washed with brine, dried (Na_2SO_4), and evaporated to afford, after prep. TLC (1:1, Et_2O/petroleum ether 1:1), [9-^{3}H]-**11** (15 mg, 55%) as a semi-solid. $[\alpha]_D^{22} = +12.2$ ($c = 1.22$, $CHCl_3$). UV (EtOH): 243 (740), 265 (sh), 272 (sh). IR ($CHCl_3$): 3480 (br.), 3460 (br.), 2980*s*, 2850*m*, 1710*s*, 1690*s*, 1510*m*, 1450*m*, 965*m*. ^{1}H-NMR (270 MHz, $CDCl_3$): 0.87 (*t*, Me); 0.89 (*d*, Me); 0.97 (*d*, Me); 1.04 (*d*, Me); 1.10–1.40 (*m*, $CH_3(CH_2)_4(CH_2)_2CON$, 1 H–C(11)); 1.55–1.75 (*m*, $CH_3(CH_2)_4CH_2CH_2CON$, 1 H–C(11)); 1.60 (*s*, Me); 1.92 (*t*, CH_2(7)); 2.05 (*m*, H–C(10)); 2.12 (*s*, Me); 2.16 (*t*, CH_2CON); 2.30 (*m*, CH_2(3), H–C(6)); 2.48 (*m*, H–C(12)); 2.63 (*m*, CH_2(2)); 3.02 (*t*, NCH_2CH_2S); 3.44 (*m*, NCH_2CH_2S); 4.95 (*d*, $J = 9$, H–C(5)); 5.14 (*dd*, $J = 15,8$, H–C(9)); 5.29 (*dt*, $J = 15,7$, H–C(8)); 5.88 (br., NH). ^{13}C-NMR (67.9 MHz, $CDCl_3$): 14.2, 16.1, 16.4, 20.8, 21.8, 22.8, 25.9, 28.2, 28.8, 29.2, 29.4, 31.9, 33.0, 35.3, 35.4, 36.9, 39.7, 40.1, 40.6, 43.1, 45.4, 128.1, 131.4, 132.2, 136.6, 173.5, 199.7, 213.2. EI-MS: 480 (55.5, $M + H$), 277 (120), 204 (100). HR-MS: 480.35114 ($M + H$, $C_{28}H_{49}O_3NS + H$, calc. 480.35137).

(10S,14S,16R,4E,8E,12E)-S-[2'-Octanamidoethyl] 4,8,10,14,16-Pentamethyl-17-oxo[13-^{3}H]octadeca-4,8,12-trienethioate ([13-^{3}H]-**10**). From **31** using the procedures described for **30** → → [9-^{3}H]-**11**. [13-^{3}H]-**10**: UV (EtOH): 243 (740), 265 (sh), 272 (sh). IR ($CHCl_3$): 3480 (br.), 3460 (br.), 2960*s*, 2920*s*, 2840*m*, 1710*s*, 1685*s*, 1510*m*, 1450*m*, 945*m*. ^{1}H-NMR (360 MHz, $CDCl_3$): 0.89 (*t*, Me); 0.91 (*d*, Me); 0.98 (*d*, Me); 1.03 (*d*, Me); 1.17–1.35 (*m*, $CH_3(CH_2)_4(CH_2)_2CON$, 1 H–C(15)); 1.55–1.70 (*m*, $CH_3(CH_2)_4CH_2CH_2CON$, 1 H–C(15)); 1.59 (*s*, Me); 1.61 (*s*, Me); 1.90–2.00 (*m*, CH_2(7), CH_2(11)); 2.06 (*t*, CH_2(6)); 2.10 (*m*, H–C(14)); 2.11 (*s*, Me); 2.17 (*t*, CH_2CON); 2.30–2.40 (*m*, CH_2(3), H–C(10)); 2.50 (*m*, H–C(16)); 2.67 (*m*, CH_2(2)); 3.02 (*t*, NCH_2CH_2S); 3.43 (*m*, NCH_2CH_2S); 4.91 (*d*, $J = 9$, H–C(9)); 5.15 (*m*, H–C(5), H–C(13)); 5.35 (*m*, H–C(12)); 5.84 (br., NH). ^{13}C-NMR (67.9 MHz, $CDCl_3$): 14.3, 16.0, 16.2, 16.4, 20.9, 21.9, 22.8, 25.9, 26.8, 28.2, 28.7, 29.2, 29.4, 29.9, 31.9, 32.9, 35.3, 35.4, 36.9, 39.7, 40.1, 40.8, 43.1, 45.3, 125.8, 128.5, 131.2, 132.8, 133.3, 136.3, 173.5, 199.9, 213.3. CI-MS (NH_3): 548 (18.3, $M + H$), 345 (5.1), 204 (100). HR-MS: 548.4137 ($M + H$, $C_{33}H_{57}O_3NS + H$, calc. 548.4140).

*Attempted Incorporation of Triene [21-^{3}H]-***7** *into Monensin B with* S.cinnamonensis *Strain A3823.5*. The growth of *S.cinnamonensis* A3823.5 in liquid cultures has been described previously [5]. Triene [21-^{3}H]-**7** (4 mg, 5.8×10^8 dpm) in EtOH was added in equal proportions to a 60-ml shake flask culture containing complex production medium [5] on days 3, 4, and 5. After 6 days, the fermentation was stopped, the medium centrifuged (10000 rpm, 10 min *Beckman-JA20* rotor), and the supernatant extracted with AcOEt. The cells were washed with AcOEt and the combined extracts dried and evaporated: brown oil (0.5 g). Only 3.0×10^6 dpm (0.7% of total added) remained after extraction in the aq. broth, whilst most was extracted into the AcOEt (4.6×10^8 dpm, 83% recovery). The crude extract was first analysed by autoradiography and radio-TLC. This revealed only a single major radioactive component (R_f 0.75; AcOEt). The extract was fractionated by eluting from an *LH-20 Sephadex* column (75 × 2.5 cm) with dry MeOH (flow rate 0.2 ml min^{-1}, 3-ml fractions). *Fr. 43–46* contained most of the radioactive material and some monensin A and B, while *Fr. 47–51* contained most of the monensin components. *Fr. 43–46* were evaporated, and the residue was fractionated further by TLC (AcOEt/petroleum ether 4:1) to afford homogeneous radioactive material (1.8 mg, 45% recovery of radioactivity) whose ^{1}H-NMR was identical to that of triene [21-^{3}H]-**7**. Further purification of the extract by FC (AcOEt/CH_2Cl_2 75:25, then AcOEt (100%)) furnished monensin A (12.0 mg) and monensin B (1.21 mg) which were recrystallised to constant activity from MeOH/H_2O. Monensin A (m.p. 267–268° ([55]: 268°)) and B (m.p. 259.5–260.5° ([55]: 258–260°)) contained insignificant amounts of radioactivity (monensin A < 100 dpm mg^{-1}; monensin B 422 dpm mg^{-1}).

*Attempted Incorporation of Triene [21-^{3}H]-***7** *into 26-Deoxymonensin B with* S.cinnamonensis *Strain DMA 300*. The growth of this strain and the isolation and characterisation of 26-deoxymonensin A and B has been described previously [17]. Triene [21-^{3}H]-**7** (4 mg, 5.8×10^8 dpm) in EtOH was added in equal proportions to the medium on days 3, 4, and 5. After 10 days, the fermentation was stopped and the medium centrifuged (10000 rpm, 10 min *Beckman-JA20* rotor). The cells and supernatant were extracted with AcOEt and the combined org. extracts dried ($MgSO_4$) and evaporated: brown oil (0.5 g, radioactivity recovered in org. phases, $5.5 \cdot 10^8$ dpm, 90.8% of total added; radioactivity recovered in the aq. phase, $2.9 \cdot 10^6$ dpm, 0.5% of total added). The crude extract was initially analysed by autoradiography and scintillation counting. These revealed a single major radioactive component (R_f 0.75; AcOEt). The extract was fractionated by eluting from a *LH-20 Sephadex* column (75 × 2.5 cm) with CH_2Cl_2 (flow rate 0.2 ml min^{-1}, fraction size 3 ml). The fractions from the *LH-20 Sephadex* column were further fractionated by HPLC (*C18* reverse phase, MeCN/H_2O 95:5) to afford 26-deoxymonensins A and B in the ratio 5:1. The specific radioactivites of these two components were determined, and they were both shown to contain insignificant levels of ^{3}H.

*Attempted Incorporation of Diene [9-^{3}H]-***11** *into Monensin A with* S.cinnamonensis *Strain A3823.5*. The general procedure for the growth and extraction of the cultures was similar to that described above, except that diene [9-^{3}H]-**11** (10 mg, 4.1×10^9 dpm) was added in equal portions on days 2, 3, and 4 and the fermentation stopped after 9 days (radioactivity recovered in org. phases, $6.0 \cdot 10^8$ dpm, 14.6%; radioactivity recovered in the aq.

phase, $2.8 \cdot 10^9$ dpm, 68.3%). The monensins A and B were purified as before to give monensin A ($3.53 \cdot 10^7$ dpm $mmol^{-1}$) and monensin B ($3.18 \cdot 10^7$ dpm $mmol^{-1}$). Distillation of the aq. phase, after extraction, showed that 95% of the remaining radioactivity was volatile (b.p. < 120°).

*Attempted Incorporation of Diene [9-^{3}H]-***11** *into Deoxymonensin B with* S. cinnamonensis *Strain DMA 300.* The procedure for the growth and extraction of the cultures was similar to that used with [21-^{3}H]-**7**, except that diene [9-^{3}H]-**11** (10 mg, 4.1×10^9 dpm) was added in equal portions on days 2, 3, and 4 and the fermentation stopped after 9 days (radioactivity recovered in org. phases, $8.0 \cdot 10^8$ dpm, 19.5% of total added; radioactivity recovered in the aq. phase, $2.7 \cdot 10^9$ dpm, 65.9% of total added). Distillation of the aq. phase showed that 95% of the remaining radioactivity was volatile, whilst the deoxymonensins A and B, as in the previous experiment, contained comparable low levels of ^{3}H activity.

*Attempted Incorporation of Triene [13-^{3}H]-***10** *into Monensin B with* S. cinnamonensis *Strain A3823.5.* The procedure for the growth and extraction of the cultures was similar to that used for [21-^{3}H]-**7**, with [13-^{3}H]-**10** (5 mg, 2.1×10^9 dpm) added in equal portions on days 2, 3, and 4 and the fermentation stopped after 9 days (radioactivity recovered in org. phases, 2.08×10^9 dpm, 99.0% of total added; radioactivity recovered in the aq. phase, $2.07 \cdot 10^7$ dpm, 1.0% of total added). The crude extract was fractionated as before, resulting in 90% recovery of [13-^{3}H]-**10**. When the monensins A and B obtained from this fermentation were recrystallised to constant activity, they were found to contain insignificant amounts of radioactivity.

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