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Chemical predisposition in synthesis: application to the preparation of the pyrrolidine natural products, plakoridines A and B

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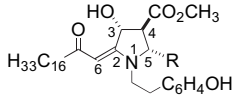
Abstract—The pyrrolidine natural products, plakoridines A and B, as well as an array of unnatural analogues, have been prepared using a five-step synthetic sequence modelled on a biogenetic theory. The key transformation involves a 'Mannich/Michael/internal-redox' cascade, which proceeds in yields of 31–63%.

1. Introduction

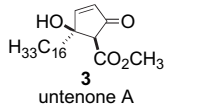
Marine sponges of the genus *Plakortis* are a rich source of oxidised fatty acid derivatives (oxylipins), many of which display quite potent bioactivities.[1] Plakoridines A and B (**1** and **2**) are two novel heterocyclic natural products belonging to this class of compounds, which were first isolated during the last decade from Japanese sponges collected in Okinawan waters (Fig. 1).[2,3] These unusual secondary metabolites have unprecedented structures containing a tyramine unit as well as a fully substituted pyrrolidine ring: initial biological studies have shown that **1** is cytotoxic towards murine lymphoma L1210 cells. In 2000, Ma and Sun described an elegant 14-step asymmetric synthesis of (−)-plakoridine A, which indicated that the natural products were essentially racemic (natural **1**: $[\alpha]_D^{20}$ −0.4 (*c* 0.5, $CHCl_3$); (−)-**1**: $[\alpha]_D^{22}$ −43.0 (*c* 0.5, $CHCl_3$)).[4]



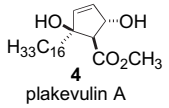
R = C_3H_7 : plakoridine A **1**
R = $C_{15}H_{31}$: plakoridine B **2**

Figure 1.

The plakoridines are members of a biogenetically related group of natural products, many of which have been isolated by the research group of Professor J. Kobayashi and which include untenone A (**3**),[5] plakevulin A (**4**),[6,7] manzamenones (e.g., **5**–**11**)[2,8,9] and plakorsins (e.g., plakorsin A (**12**)) (Fig. 2).[10] The members of this family of compounds are



3
untenone A



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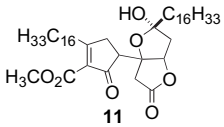
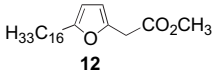
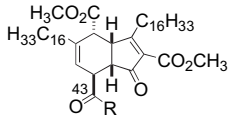
plakevulin A



11
manzamenone K



12
plakorsin A



manzamenone A **5** R = OH
manzamenone C **6** R = OEt
manzamenone D **7** R = NH_2
manzamenone E **8**

manzamenone F **9** R = OC_4H_9
manzamenone H **10** R = $HN(CH_2)_2C_6H_4OH$

Figure 2.

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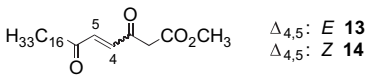
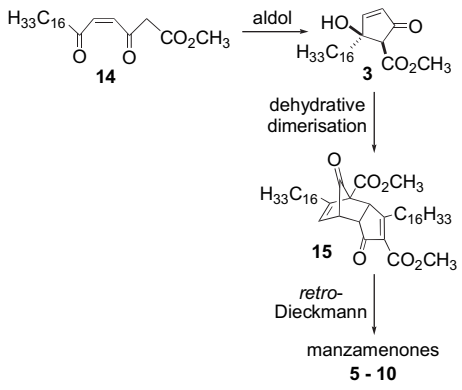


Figure 3.

characterised by the common structural features of at least one β-oxygenated carboxyl group and at least one fully saturated unbranched C_{16} alkyl chain.

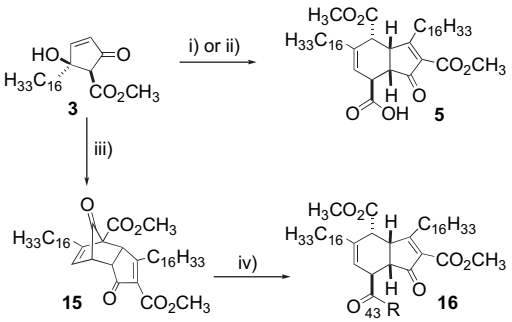
Kobayashi noted that many members of this family of oxylipins possess structures, which could be derived biosynthetically from (*E*)/(*Z*)-methyl-3,6-dioxo-4-docosenoate (**13**, **14**) (Fig. 3).

Prompted by this observation, we have suggested a plausible biosynthetic pathway, which interrelates (*Z*)-methyl-3,6-dioxo-4-docosenoate (**14**), untenone A (**3**) and many of the manzamenones (Scheme 1).[11–14] According to this proposal, aldol cyclisation of **14** leads to untenone A (**3**), which then undergoes dehydrative dimerisation to give the tricyclic adduct **15**. Subsequent attack at the reactive bridging carbonyl of **15** by different nucleophiles RH, followed by *retro*-Dieckmann ring-opening, gives a conjugated enol(ate), which undergoes kinetic protonation at the α-position and on the convex surface to give the bicyclic structure common to the majority of the manzamenones. An attractive feature of this scheme is that the inherent reactivity of the tricyclic intermediate **15** is ultimately manifested in differential functionalisation at C43 of the manzamenone skeleton, which is the natural locus of diversification.



Scheme 1.

Successful syntheses of several natural as well as unnatural manzamenones using approaches modelled on the biogenetic theory have provided support for the plausibility of the proposal (Scheme 2). Thus, simply stirring a mixture of **3** in water, at ambient temperature and in the presence of either a Brønsted acid surfactant combined catalyst (dodecyl-benzenesulfonic acid: 0.1 equiv) or a Lewis acid surfactant combined catalyst (scandium trisdodecylsulfate: 0.1 equiv) provided manzamenone A (**5**) in reproducible yields of 65–80%.[14] Alternatively, dehydrative dimerisation of untenone A to give adduct **15** has been achieved using trifluoroacetic anhydride: subsequent exposure of **15** to a range of O and N centred nucleophiles has provided a variety of manzamenone analogues differing with respect to the acyl substituent at C43.[15]



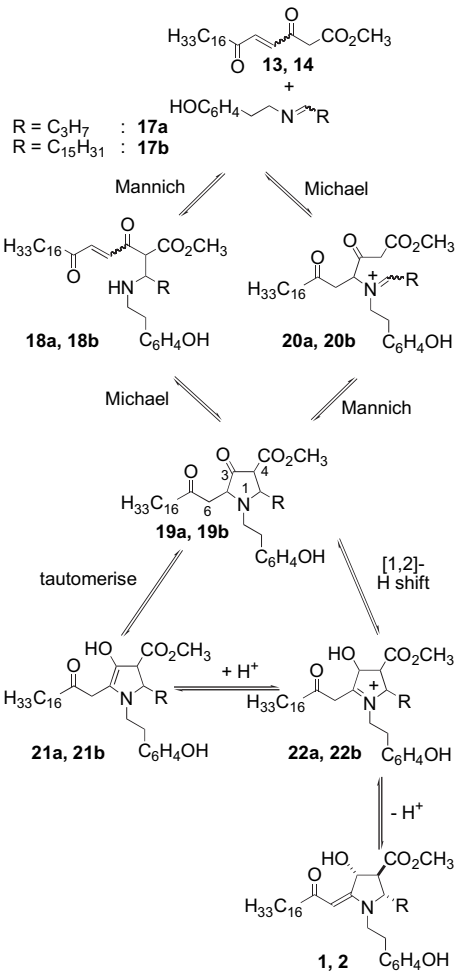
Scheme 2. Reagents and conditions: (i) scandium trisdodecylsulphate (0.1 equiv), H_2O, 25 °C, 1–24 h, 70–80%; (ii) dodecyl-benzenesulfonic acid (0.1 equiv), H_2O, 25 °C, 7–24 h, 65–70%; (iii) TFAA, $CDCl_3$, rt, 24 h; (iv) for ester derivatives: RH, rt, 24 h, 15–63% from **3**; for amide derivatives: RH, CH_2Cl_2, rt, 24 h, 10–15% from **3**.

Chemical predisposition refers to the kinetic reaction preferences bestowed on the functional groups in a molecule by their specific molecular context.[16] In the 'arena' of biochemical evolution, chemically predisposed reactions may be viewed as the starting points from which nature, the 'quintessential process development chemist',[17] evolves efficient enzyme-catalysed processes. The transformation of untenone A (**3**) into the manzamenones bears the hallmarks of a predisposed biochemical process and this is supported experimentally by the ease with which the transformations can be carried out in the laboratory.

It occurred to us that the plakoridines may also be products of a predisposed biochemical pathway, which commences with either (*E*)- or (*Z*)-methyl-3,6-dioxo-4-docosenoate (**13**, **14**) (Scheme 3).[18]

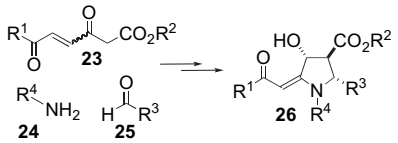
According to our proposal, reaction of **13** or **14** with the aldimines derived from tyramine and either butyraldehyde or hexadecanal would give pyrrolidinones **19a**, **19b**: this transformation might proceed by initial Mannich reaction followed by a '5-*exo*' Michael-type cyclisation, or alternatively by initial Michael reaction to give iminium species **20a**, **20b** followed by a '5-*endo*' Mannich cyclisation. The conversion of pyrrolidinones **19a**, **19b** to plakoridines A and B (**1**, **2**) involves, formally, an internal redox reaction whereby the ketone moiety of **19a**, **19b** is reduced to an alcohol and the exocyclic C–C single bond at C2 is oxidised to an alkene: the thermodynamic driving force for the transformation being formation of the vinylogous amide moiety present in the plakoridines. Mechanistically, this process might occur via a series of prototropic shifts somewhat akin to those which occur during the Amadori rearrangement: thus tautomerism of **19a**, **19b** could lead to enaminols **21a**, **21b**, which upon protonation would give iminium species **22a**, **22b**. Deprotonation of the *exo*-methylene group at C2 of **22a**, **22b** would then furnish the plakoridines. Iminium species **22a**, **22b** could alternatively be generated directly from pyrrolidinones **19a**, **19b** via a concerted [1,2]-hydrogen shift. The reversibility of the individual transformations of this sequence means that the relative stereochemistry of the plakoridines would be expected to be a consequence of thermodynamic control.

The challenging and quite unprecedented cascade sequence of reactions leading to the plakoridines inspired us to initiate



Scheme 3.

an investigation into the synthesis of these unusual natural products using an approach modelled on the biogenetic theory. Reports in the literature that plakoridine A (**1**),[19] as well as other members of this family of oxylipins,[20] displays inhibitory properties towards DNA polymerases α and β have provided additional stimulus for us to exploit the multi-component nature of the biosynthetic sequence in the preparation of novel analogues of the plakoridines. In this paper, we provide details of our investigations into the synthesis of an array of analogues of the plakoridines, which possess general structure **26** (Scheme 4). The successful outcome of these investigations will ultimately facilitate the generation of important SAR-data regarding the structural features, which are important for optimal bioactivity of this class of compounds towards DNA polymerases.

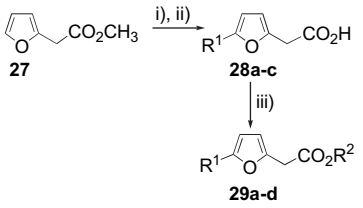


R^1= linear alkyl; R^2 = linear or branched alkyl;
R^3 = linear alkyl or aryl; R^4 = terminally substituted alkyl.

Scheme 4.

2. Results and discussion

Analogues of the furan fatty acid derivative, plakorsin A (e.g., **29a–d**), were envisaged to be the key intermediates for our synthetic investigations. Previously, we have reported full details of the synthesis of plakorsin A (**12**) starting from 2-furan acetonitrile;[13] our preferred starting material for the synthesis of analogues **29a–d** in the studies reported here was the methyl ester of 2-furanacetic acid (**27**) (Scheme 5). Acylation of **27** with the appropriate acid chloride followed by ketone reduction using the Huang-Minlon modification of the Wolff–Kishner conditions[21a,b] gave 5-alkyl-furan-2-yl acetic acids **28a–c** in acceptable yields. Subsequent esterification either under acid-catalysed conditions or in the presence of DCCI gave the desired ester derivatives **29a–d**. Representative yields for the individual transformations of this sequence are provided in Table 1.



Scheme 5. Reagents and conditions: (i) RCOCl, $SnCl_4$, CH_2Cl_2, −5 °C, 1 h; (ii) H_2NNH_2, NaOH, $HOCH_2CH_2OH$, Δ, 72 h; (iii) R^2OH, Amberlite® IR-120 (H), Δ, 72 h or R^2OH, DCCI, CH_2Cl_2, 0 °C, 1 h.

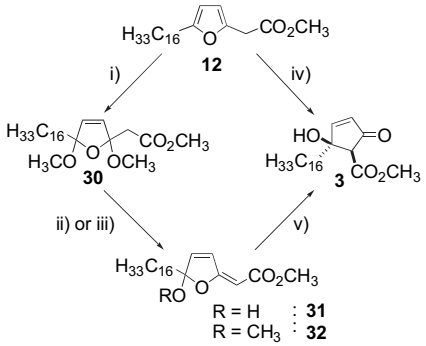
Table 1. Yields of individual transformations for the conversion of methyl ester **27** to plakorsin analogues **29a–d**

	R^1	R^2	Yield (%)		
			(i)	(ii)	(iii)
a	C_2H_5	CH_3	96	96	91
b	$C_{12}H_{25}$	CH_3	89	47	76
c	$C_{16}H_{33}$	C_2H_5	99	59	98
d	$C_{16}H_{33}$	$CH(CH_3)_2$	—	—	99

It was envisaged that under appropriately controlled conditions, oxidative cleavage of the furan ring in the plakorsin analogues would provide (*E*)- or (*Z*)-enediones analogous to **13** and **14**. Therefore, using plakorsin A (**12**) as a test substrate, we carried out investigations into the outcome of exposure of the 2,5-disubstituted furan ring-system to a variety of oxidation conditions.

As we have described previously, treatment of plakorsin A (**12**) with bromine in methanol gave the bis-acetal **30** as a mixture of diastereoisomers in good yield.[12,13,22] This compound is a masked form of (*Z*)-methyl-3,6-dioxo-4-docosenoate (**14**) and, accordingly, exposure of **30** to mildly acidic hydrolytic conditions[23] furnished **31**, a cyclic hemiketal tautomer of **14**. The structural identity of **31** was confirmed by comparison of its spectroscopic data with those of methyl ketal **32**, which was prepared in unambiguous fashion, by base-mediated elimination of methanol from **30** (Scheme 6).

Low temperature oxidation of **12** using a peracid (*m*-CPBA)[24] followed by a mildly basic aqueous work-up



Scheme 6. Reagents and conditions: (i) Br_2, CH_3OH, Na_2CO_3, rt, 2 h, 84%; (ii) 0.005 M H_2SO_4, H_2O, dioxane, rt, 1.5 h; (iii) KHMDS, THF, −78 °C to rt, 79%; (iv) *m*-CPBA, CH_2Cl_2, −10 °C to rt, 2 h then work-up with $NaHCO_{3(aq)}$, 63%; (v) $NaHCO_3$, H_2O, dioxane, rt, 1 h, 82% from **30**.

furnished untenone A (**3**) as the major product and as a single diastereoisomer. This transformation almost certainly proceeds via the intermediacy of (*Z*)-enedione **14**, or a tautomer thereof, and accordingly, exposure of cyclic hemiketal **31** to mildly basic conditions also furnished untenone A (**3**) in good yield.

These studies indicated that (*Z*)-enedione **14** has a propensity to undergo aldol cyclisation to give untenone A. This observation prompted us to conclude that the diastereoisomeric (*E*)-enedione **13** would be a more appropriate substrate for our synthetic studies towards the plakoridines. A useful procedure for the preparation of (*E*)-enediones from furan derivatives, which utilises pyridinium chlorochromate (PCC) as the oxidant, has been described by Piancatelli and co-workers.[25] Disappointingly, exposure of plakorsin A (**12**) to the conditions described by these authors gave (*E*)-enedione **13**, as its enol tautomer **33**, in variable and quite unsatisfactory yields. Fortunately, however, a satisfactory procedure for the preparation of **33** was developed, based on a report by Jurczak and Pikul:[26] thus, after much experimentation, it was found that treatment of a solution of **12** in a 5:1 mixture of acetone and water (pre-cooled to −20 °C) with 1 equiv of bromine, provided **33** in a yield of 63% after purification by flash chromatography (82% yield based on recovered **12**) (Scheme 7). If the reaction was carried out at temperatures higher than −10 °C, or if excess bromine was added, competitive bromination of the enolic product resulted in an erosion of the isolated yield of **33**. The generality of this procedure has been demonstrated by the preparation, in acceptable yields, of other analogues of **33** (e.g., **34**–**36**).

The C4/C5 double bond geometry of the products of these reactions was indicated as (*E*) by the magnitude of the vicinal coupling constants between C(4)*H* and C(5)*H* (15–16 Hz). Further confirmation of the structure of the products was provided by X-ray crystallographic analysis of a sample of the short-chain analogue **34** (Fig. 4).

With compounds **33**–**36** in-hand, we were in a position to investigate the reaction cascade leading to the core skeleton of the plakoridines. The ultimate aim of our investigations (vide supra) was to develop a procedure suitable for the preparation of a range of analogues of **1**, **2**. In the first instance therefore, we decided to investigate the reaction of the putative biosynthetic precursor **33** with an imine, which differed slightly from the one implicated in the biosynthesis of the natural products. Using the excellent procedure of Tashiro and co-workers, imine **37** was prepared under aqueous conditions from phenethylamine and propionaldehyde.[27] We were then pleased to discover that prolonged incubation at rt of a solution of this imine and enol **33** in $CDCl_3$ resulted in the generation of two isomeric plakoridine-type structures: clean samples of both compounds were obtained following careful purification by flash chromatography (Scheme 8).[18] The close similarity of the ^1H NMR spectral data of the major isomer **38** with those of plakoridine A,[2] and in particular, the similar magnitude of the vicinal coupling constants between the ring hydrogens of the respective pyrrolidine cores ($J_{3,4} \approx J_{4,5} \approx 6.0$ Hz for **38**; $J_{3,4}=J_{4,5}=5.5$ Hz for **1**) were in accord with the structural assignment shown for **38**. Furthermore, a significant NOE observed from C(3)*H* to C(5)*H* was consistent with a *syn* relative stereochemistry at these two centres in **38**. Tentative structural assignment of the minor isomer **39** as the C3 epimer of **38** was based on two pieces of evidence: an increased vicinal coupling constant between C(3)*H* and C(4)*H* ($J_{3,4}=8.5$ Hz) and the absence of an observable NOE from C(3)*H* to C(5)*H*.

The potential for interconversion of the two isomers **38** and **39** was confirmed by the finding that prolonged storage of a sample of the minor isomer **39** in $CDCl_3$ at rt, resulted in slow isomerisation to give a mixture of **38** and **39** enriched

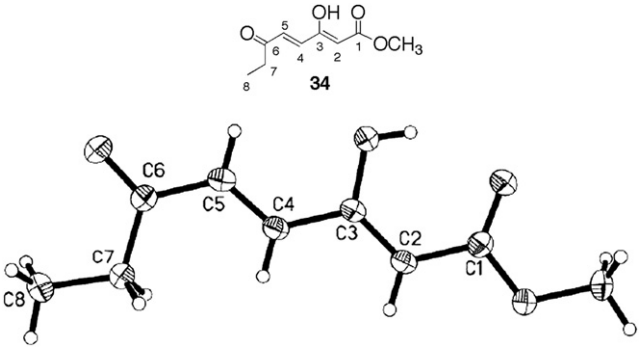
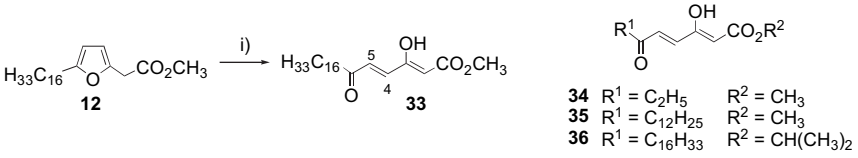
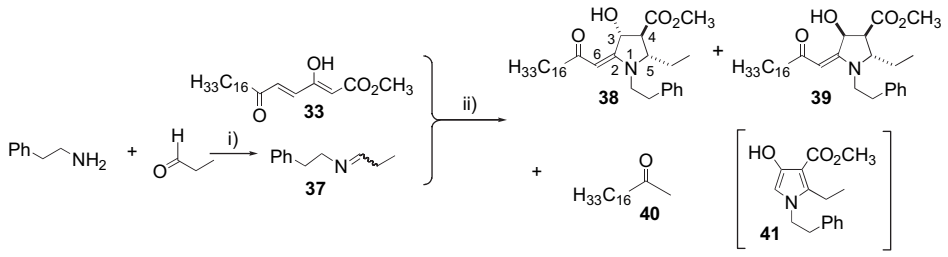


Figure 4. Crystal structure of **34** with ellipsoids at 50% probability.



Scheme 7. Reagents and conditions: (i) Br_2, acetone/H_2O (5:1), −20 to −10 °C, 6 h, 63% for **33**, 52% for **34**, 61% for **35**, 43% for **36**.



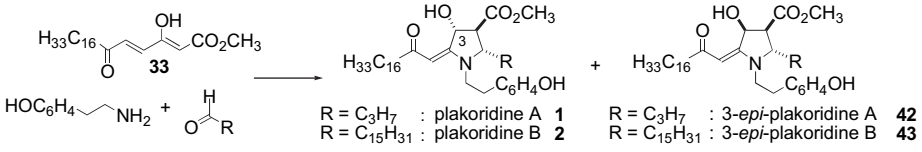
Scheme 8. Reagents and conditions: (i) H_2O, rt, 3 h, 97%; (ii) $CDCl_3$, rt, 12 days, 38% for **38**, 4% for **39**, 15% for **40**.

in the former (ratio of **38**:**39** 3:2 after 55 days). This observation is in accord with our initial suggestion that the relative stereochemistry of the natural plakoridines may be under thermodynamic control. Intriguingly, a third nonpolar compound was also isolated from the initial incubation reaction, which was identified as octadecan-2-one (**40**). Although a number of plausible mechanisms may result in the formation of this ketone, it seems likely that **40** is derived from a *retro*-Mannich reaction of an initial cyclised pyrrolidinone intermediate of type **19** (Scheme 3). A transformation of this kind would benefit from the generation of a hydroxylated pyrrole **41** (or tautomer thereof), but unfortunately, isolation of such an entity has not been possible.

The successful outcome of this initial study prompted us to investigate the preparation of the natural products themselves (Scheme 9).

It transpired that the imines necessary for the preparation of plakoridines A and B (**1** and **2**), derived from condensation of tyramine with either butyraldehyde or hexadecanal were unstable in a concentrated form. An alternative procedure was developed therefore, whereby the prerequisite imines were prepared in $CDCl_3$ in the presence of $MgSO_4$. The drying agent was then removed and a solution of enol **33** in $CDCl_3$ was added. The reactions were monitored by ^{1}H NMR spectroscopy and after a period of several days, substantial conversion to the natural products had occurred. A minor isomer was again generated in each case, believed to be the C3 epimer of the natural products (crude ratio of major isomer:minor isomer; ~3:1). Following purification by flash chromatography, plakoridines A and B (**1** and **2**) were isolated in 43 and 36% yields, respectively. The structural identity of our synthetic sample of **1** was confirmed by comparison of its ^{1}H NMR spectrum with that of (−)-plakoridine A[4] (Fig. 5).

The generality of the three-component coupling procedure outlined above for the synthesis of plakoridine-type structures has allowed the preparation of an array of analogues



Scheme 9.

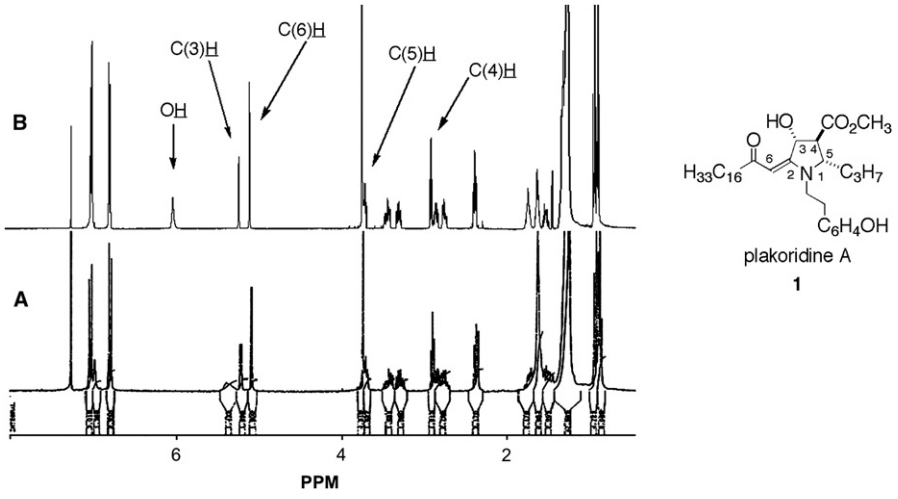


Figure 5. ^{1}H NMR spectra for (−)-plakoridine A[4] and (+/−)-plakoridine A, prepared according to Scheme 9. (A) ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of (−)-plakoridine A (reprinted with the kind permission from Professor Dawei Ma); (B) ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of (+/−)-plakoridine A.

of the natural products, using compounds **33**–**36** as substrates. Two alternative procedures were employed for these reactions, which differed with respect to the method of imine generation: method A involved preparation of the imines in an aqueous medium and method B, generally used for imines derived from long-chain aldehydes and/or from tryptamine, utilised dichloromethane as the reaction solvent. The crude product isomer ratios, as well as the isolated yields of the major products, are listed in Table 2: we feel that the yields of the reactions, which range between 31 and 63%, are acceptable given the complexity of the cascade sequence. Although it was feasible to isolate pure samples of the major 'natural' isomers from many of these reactions, it proved impossible, in most cases, to isolate pure samples of the minor isomeric components.

The progress of each of the reactions was monitored by ^{1}H NMR spectroscopy and in many cases, this provided evidence for the intermediacy of a pyrrolidinone intermediate, which was present as a mixture of two diastereoisomers. For example, in the case of the synthesis of compound **52**, the appearance and relatively slow disappearance of two ABX coupled systems were consistent with the formation of diastereoisomeric intermediates having general structure **55** (Fig. 6).

We reasoned that the use of an aromatic amine in the cascade sequence might lessen the thermodynamic drive for the formation of the vinylogous amide moiety of the plakoridine structures and allow the isolation of a pyrrolidinone intermediate. Accordingly, we exposed the short-chain enol **34** to Schiff's base **56** derived from aniline and benzaldehyde. After stirring for a period of 24 h, the enol **34** had been completely consumed to be replaced by three new principal compounds (ratio ~7:3:1). Continued monitoring by ^{1}H NMR over a period of 3 days indicated no further changes and, in particular, no evidence for the formation of plakoridine-type structures. The major product from this reaction was isolated by crystallisation from ethyl acetate, however, it proved impossible to grow crystals suitable for X-ray analysis. The ^{1}H NMR spectrum of this material was consistent with that expected for a pyrrolidinone intermediate: the observation of NOEs between C(2)*H* and C(4)*H*, as well as between both C(2)*H* and C(4)*H* and the *ortho* hydrogens of the phenyl substituent at C(5) are in accord with the relative stereostructure **57** depicted in Scheme 10.

Storage of a sample of **57** in either $CDCl_3$ or C_6D_6 resulted in quite rapid equilibration with two other species, which were the same as those generated in the original incubation reaction. We believe the major of these to be enol tautomer **58**

Table 2. Isomer ratios and isolated yields for preparation of plakoridine analogues **44**–**54**

Compound number	R^1	R^2	R^3	R^4	Synthetic method[a]	Isomer ratio[b] (major:minor)	Yield of major isomer (%)
44	$C_{16}H_{33}$	CH_3	C_6H_5	3-Indolyl-$(CH_2)_2$	B	28:1	38
45	$C_{16}H_{33}$	CH_3	C_6H_5	$C_6H_5(CH_2)_2$	A	19:1	39[c]
46	$C_{16}H_{33}$	CH_3	$C_{15}H_{31}$	$C_6H_5(CH_2)_2$	B	4:1	60
47	$C_{16}H_{33}$	CH_3	$C_{15}H_{31}$	3-Indolyl-$(CH_2)_2$	B	6:1	63
48	$C_{16}H_{33}$	$CH(CH_3)_2$	C_2H_5	$C_6H_5(CH_2)_2$	A	3:1	63
49	$C_{12}H_{25}$	CH_3	C_6H_5	$C_6H_5(CH_2)_2$	A	19:1	60
50	$C_{12}H_{25}$	CH_3	C_6H_5	3-Indolyl-$(CH_2)_2$	B	28:1	38
51	$C_{12}H_{25}$	CH_3	C_2H_5	$C_6H_5(CH_2)_2$	A	3:1	40
52	$C_{12}H_{25}$	CH_3	C_6H_5	$C_6H_5CH_2$	A	7:1	31[d]
53	C_2H_5	CH_3	C_6H_5	$C_6H_5CH_2$	A	8:1	57
54	C_2H_5	CH_3	$C_{15}H_{31}$	$C_6H_5(CH_2)_2$	B	3:1	55

[a] Method A: imine was prepared by stirring a mixture of the appropriate aldehyde and amine in water for 3 h at rt; method B: imine was prepared by stirring a mixture of the appropriate aldehyde and amine in dichloromethane for 3 h at rt.
[b] Isomer ratios were estimated by comparison of the integrals for C(6)*H* in the ^{1}H NMR spectra of the crude product mixtures.
[c] Sample was contaminated with <5% of the minor isomer.
[d] Sample was contaminated with <10% of the minor isomer.

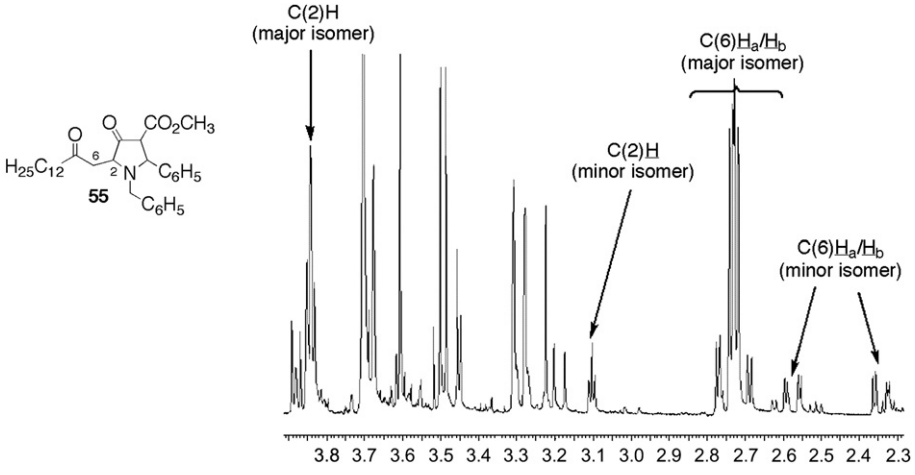
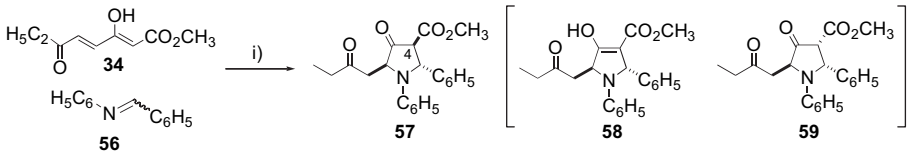


Figure 6. ^{1}H NMR spectrum of the intermediate product mixture from reaction of enol **35** with benzylidene benzylamine.



Scheme 10. Reagents and conditions: (i) $CDCl_3$, rt, 24h, 55%.

and we have assigned the minor component **59** to be the C4 epimer of **57**: the gradual disappearance of the ^1H NMR signal for C(4)*H* of **57** in the presence of D_2O is in accord with these conclusions.

3. Conclusions

In conclusion, we have prepared the plakoridines A (**1**) and B (**2**) as well as an array of analogues of the natural products, in five linear steps from the methyl ester of 2-furanacetic acid (**27**). The synthetic approach was modelled on a plausible and apparently unprecedented biosynthetic pathway involving a three-component Mannich/Michael reaction sequence followed by an 'internal redox' process. We consider that the yield of the key biomimetic transformation (31–63%) is reasonable given the complexity of the cascade sequence. Spectroscopic evidence for the intermediacy of a pyrrolidinone intermediate has been provided and a sample of such a species has been obtained by altering the amine and aldehyde partners in the cascade process. Further studies have indicated that the relative stereochemistry of the natural products may be under thermodynamic control. The successful preparation of an array of natural and unnatural plakoridines will allow further assessment of the inhibitory properties of this unusual structural type towards DNA polymerases α and β.[15] The full results of these SAR studies will be reported in due course.

4. Experimental

4.1. General

Solvents were dried and distilled before use. Chromatography was performed over Merck silica gel 60 (40–63 μm). IR spectra were recorded on a Perkin–Elmer 881 spectrometer, an AT1-Mattson Genesis Series FTIR spectrometer or a JASCO FT/IR-4100 spectrometer. ^1H and ^{13}C spectra were recorded on a Varian Inova 400 MHz spectrometer, a Varian Inova 300 MHz spectrometer or a Bruker AMX 500 MHz spectrometer. Chemical shifts are referenced to the residual solvent peak. Mass spectra were recorded on a Micromas Trio 2000 quadrupole spectrometer (EI/CI, low resolution), a Thermo Finigan MAT 95 XP spectrometer (EI/CI, high resolution) and a Micromass Platform spectrometer (electrospray). Melting points were recorded using a Sanyo Gallenkamp MPD350 heater and are uncorrected.

For the purpose of consistency and clarity, the numbering scheme employed for the presentation of spectroscopic data for the plakoridine analogues is depicted in Figure 7 for compound **38**.

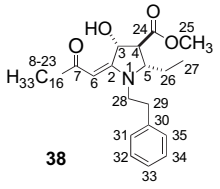


Figure 7.

4.2. Representative procedure for Friedel–Crafts acylation of 2-furanacetic acid methyl ester

4.2.1. (5-Dodecanoylfuran-2-yl)acetic acid methyl ester. A 1 M solution of tin tetrachloride in dichloromethane (43 mL, 43 mmol) was added dropwise via cannula to a solution of dodecanoyl chloride (8.2 mL, 35.5 mmol) in dichloromethane (10 mL) at −5 °C. The reaction mixture was stirred at this temperature for 45 min. A solution of 2-furanacetic acid methyl ester (**27**) (5.0 g, 35.5 mmol) in dichloromethane (10 mL) was then added dropwise over a period of 10 min and the reaction mixture was stirred at −5 °C for a further 45 min. The mixture was poured onto ice, stirred for 30 min and the resulting two-phase mixture was separated. The organic layer was washed with water, dried ($MgSO_4$) and then concentrated in vacuo. The residue was dissolved in diethyl ether and filtered through a pad of Celite® to remove tin residues. The filtrate was concentrated in vacuo to give the title compound as an orange solid (10.2 g, 89%); R_f 0.31 (petroleum ether:ethyl acetate, 6:1); mp 35.5–37.8 °C; ν_{max} (film)/cm^{-1} 2925s (C–H), 1746m (C═O, ester), 1679m (C═O, ketone), 1517m, 1464w, 1438w, 1264w, 1214m; δ_H (300 MHz, $CDCl_3$) 0.85 (3H, t, *J* 6.7, C(18)H_3), 1.24–1.29 (16H, m, C(10)H_2–C(17)H_2), 1.63–1.72 (2H, m, C(9)H_2), 2.74 (2H, t, *J* 7.5, C(8)H_2), 3.72 (3H, s, CO_2CH_3), 3.76 (2H, s, C(2)H_2), 6.39 (1H, d, *J* 3.0, C(4)*H*), 7.11 (1H, d, *J* 3.0, C(5)*H*); δ_C (75 MHz, $CDCl_3$) 14.3 (*C*(18)H_3), 22.9, 24.7, 29.57, 29.65, 29.7, 29.8, 32.1 (*C*(9)H_2 to *C*(17)H_2, some overlapping), 34.3 (*C*(2)H_2), 38.6 (*C*(8)H_2), 52.7 (CO_2CH_3), 111.1 (*C*(4)H), 118.5 (*C*(5)H), 152.5 (*C*(3) and *C*(6)), 169.0 (*C*(1)O), 189.6 (*C*(7)O); *m*/*z* (CI/NH_3) 340 ($[M+NH_4]^+$, 100%), 323 ($[M+H]^+$, 15), 199 (10); found 340.2484, $C_{19}H_{34}NO_4$ ($[M+NH_4]^+$) requires 340.2482.

4.2.2. Data for (5-acetylfuran-2-yl)acetic acid methyl ester. Pale yellow oil; R_f 0.21 (petroleum ether:diethyl ether, 1:1); ν_{max} (film)/cm^{-1} 3123w and 2955w (C–H), 1742s (C═O, ester), 1674s (C═O, ketone), 1517s, 1437m, 1296s, 1218s, 1019m; δ_H (300 MHz, $CDCl_3$) 2.37 (3H, s, C(8)H_3), 3.70 (3H, s, CO_2CH_3), 3.72 (2H, s, C(2)H_2), 6.37 (1H, d, *J* 3.6, C(4)*H*), 7.08 (1H, d, *J* 3.6, C(5)*H*); δ_C (75 MHz, $CDCl_3$) 26.0 (*C*(8)H_3), 34.2 (*C*(2)H_2), 52.6 (CO_2CH_3), 111.2 (*C*(4)H), 119.0 (*C*(5)H), 152.4, 152.8 (*C*(3) and *C*(6)), 168.9 (*C*(1)O), 186.5 (*C*(7)O); *m*/*z* (CI/NH_3) 200 ($[M+NH_4]^+$, 100%), 183($[M+H]^+$, 15).

4.2.3. Data for (5-hexadecanoylfuran-2-yl)acetic acid methyl ester. Orange solid; mp 65.4–66.7 °C; ν_{max} (solid state)/cm^{-1} 2918s and 2848s (C–H), 1732s (C═O, ester), 1664s (C═O, ketone), 1520m, 1217s; δ_H (300 MHz, $CDCl_3$) 0.86 (3H, t, *J* 6.7, C(22)H_3), 1.15–1.35 (24H, m, C(10)H_2 to C(21)H_2), 1.62–1.73 (2H, m, C(9)H_2), 2.75 (2H, t, *J* 7.5, C(8)H_2), 3.73 (3H, s, CO_2CH_3), 3.77 (2H, s, C(2)H_2), 6.40 (1H, d, *J* 3.3, C(4)*H*), 7.12 (1H, d, *J* 3.3, C(5)*H*); δ_C (75 MHz, $CDCl_3$) 14.3 (*C*(22)H_3), 22.9, 24.7, 29.5, 29.56, 29.59, 29.65, 29.7, 29.8, 29.89, 29.92, 32.1 (*C*(9)H_2 to *C*(21)H_2, some overlapping), 34.3 (*C*(2)H_2), 38.6 (*C*(8)H_2), 52.6 (CO_2CH_3), 111.1 (*C*(4)H), 118.5 (*C*(5)H), 152.4 and 152.5 (*C*(3) and *C*(6)), 169.0 (*C*(1)O), 189.7 (*C*(7)O); *m/z* (CI/NH_3) 396 ([M+NH_4]$^+$, 100%), 379 ([M+H]$^+$, 15) (+ve ion electrospray); found 378.2766, $C_{23}H_{38}O_4$ (M$^+$) requires 378.2765.

4.3. Representative procedure for the preparation of (5-alkylfuran-2-yl)acetic acids

4.3.1. (5-Dodecylfuran-2-yl)acetic acid (28b). A mixture of (5-dodecanoylfuran-2-yl)acetic acid methyl ester (10.2 g, 31.5 mmol) and hydrazine monohydrate (18.3 mL, 378 mmol) in ethylene glycol (85 mL) was heated under reflux for 1 h. Potassium hydroxide pellets (10.2 g, 181 mmol) were added cautiously and the reaction mixture was heated under reflux for a further 72 h and then allowed to cool. Water (40 mL) was added and the reaction mixture was heated to ~60 °C and stirred for 30 min. The reaction mixture was cooled to room temperature and acidified to pH 4 with 2 M aqueous hydrochloric acid solution. The product was extracted into diethyl ether (3×30 mL), the combined organic extracts were dried ($MgSO_4$) and then concentrated in vacuo. Purification by flash column chromatography (petroleum ether:ethyl acetate, 6:1) afforded the title compound as a colourless solid (4.38 g, 47%); R_f 0.24 (SiO_2; petroleum ether: ethyl acetate, 6:1); mp 56.3–57.5 °C; ν_{max} (film)/cm^{-1} 2918m (C–H), 1712m (C═O, carboxylic acid), 1467w, 1444w, 1254w, 1179w; δ_H (300 MHz, $CDCl_3$) 0.90 (3H, t, *J* 6.6, C(18)H_3), 1.21–1.31 (18H, m, C(9)H_2 to C(17)H_2), 1.57–1.67 (2H, m, C(8)H_2), 2.59 (2H, t, *J* 7.6, C(7)H_2), 3.69 (2H, s, C(2)H_2), 5.93 (1H, d, *J* 3.0, C(5)*H*), 6.13 (1H, d, *J* 3.0, C(4)*H*); δ_C (75 MHz, $CDCl_3$) 14.4 (*C*(18)H_3), 23.0, 28.2, 28.3, 29.4, 29.6, 29.8, 29.92, 29.94, 32.2 (*C*(7)H_2 to *C*(17)H_2, some overlapping), 34.2 (*C*(2)H_2), 105.8 (*C*(5)H), 109.2 (*C*(4)H), 145.0 and 156.8 (*C*(3) and *C*(6)), 176.3 (*C*(1)O); *m/z* (CI/NH_3) 312 ([M+NH_4]$^+$, 100%), 295 ([M+H]$^+$, 8), 250 (10), 95 (12); found 312.2532, $C_{18}H_{34}NO_3$ ([M+NH_4]$^+$) requires 312.2533.

4.3.2. (5-Ethylfuran-2-yl)acetic acid (28a) and (5-hexadecylfuran-2-yl)acetic acid (28c). Data for (5-ethylfuran-2-yl)acetic acid (**28a**) and (5-hexadecylfuran-2-yl)acetic acid (**28c**) were as described previously.[13]

4.4. Representative procedures for esterification of (5-alkylfuran-2-yl)acetic acids

4.4.1. Method 1: preparation of methyl ester derivatives.

4.4.1.1. (5-Dodecylfuran-2-yl)acetic acid methyl ester (29b). Amberlite® IR-120 (H) (4.20 g) was added to a solution of (5-dodecylfuran-2-yl)acetic acid (**28b**) (4.20 g, 14.2 mmol) in methanol (40 mL) and the reaction mixture was heated under reflux for 72 h. The resin was then removed by hot filtration and the filtrate was concentrated in vacuo. Purification of the residue by flash column chromatography (SiO_2; petroleum ether:ethyl acetate, 25:1) furnished the title compound as a colourless oil (3.33 g, 76%); R_f 0.46 (petroleum ether:ethyl acetate, 25:1); ν_{max} (film)/cm^{-1} 2925s (C–H), 1745s (C═O, ester), 1463w, 1436w, 1268w, 1223w, 1141w; δ_H (300 MHz, $CDCl_3$) 0.89 (3H, t, *J* 6.7, C(18)H_3), 1.22–1.31 (18H, m, C(9)H_2 to C(17)H_2), 1.57–1.64 (2H, m, C(8)H_2), 2.58 (2H, t, *J* 7.6, C(7)H_2), 3.65 (2H, s, C(2)H_2), 3.73 (3H, s, CO_2CH_3), 5.91 (1H, d, *J* 3.0, C(5)*H*), 6.10 (1H, d, *J* 3.0, C(4)*H*); δ_C (75 MHz, $CDCl_3$) 14.4 (*C*(18)H_3), 23.0, 28.27, 28.30, 29.5, 29.62, 29.64, 29.8, 29.91, 29.94, 32.2 (*C*(7)H_2 to *C*(17)H_2, many overlapping), 34.3 (*C*(2)H_2), 52.5 (CO_2CH_3), 105.7 (*C*(5)H), 108.7 (*C*(4)H), 145.7 and 156.6 (*C*(3) and *C*(6)), 170.4 (*C*(1)O); *m/z* (CI/NH_3) 326 ([M+NH_4]$^+$, 100%), 309 ([M+H]$^+$, 13), 249 (3); found 326.2689, $C_{19}H_{36}NO_3$ ([M+NH_4]$^+$) requires 326.2690.

4.4.1.2. (5-Ethylfuran-2-yl)acetic acid methyl ester (29a) and plakorsin A (12). Data for (5-ethylfuran-2-yl)-acetic acid methyl ester (**29a**) and plakorsin A (**12**) were as described previously.[13]

4.4.2. Method 2: preparation of ethyl and isopropyl ester derivatives.

4.4.2.1. (5-Hexadecylfuran-2-yl)acetic acid ethyl ester (29c). A solution of (5-hexadecylfuran-2-yl)acetic acid (**28c**) (270 mg, 0.77 mmol), ethanol (54 mL, 0.93 mmol) and 4-dimethylaminopyridine (9 mg, 0.077 mmol) in dichloromethane (5.7 mL) was cooled to 0 °C. A solution of *N*,*N*′-dicyclohexylcarbodiimide (191 mg, 0.93 mmol) in dichloromethane (2 mL) was added and the reaction mixture was stirred at 0 °C for 1 h. The precipitated dicyclohexylurea was removed by filtration, washed with ice-cold dichloromethane and the filtrate was concentrated in vacuo. Residual dicyclohexylurea was removed by trituration with a minimum volume of ice-cold dichloromethane followed by filtration. Purification by flash column chromatography (SiO_2; petroleum ether:diethyl ether, 18:1) yielded the title compound as a colourless oil, which solidified on standing (286 mg, 98%); R_f 0.57 (petroleum ether:diethyl ether, 7:3); mp 33.4–34.1 °C; ν_{max} (film)/cm^{-1} 2921s and 2851s (C–H), 1742s (C═O, ester), 1567w, 1467m, 1218m, 1174m, 1138m; δ_H (300 MHz, $CDCl_3$) 0.92 (3H, t, *J* 6.7, C(22)H_3), 1.23–1.40 (26H, m, C(9)H_2 to C(21)H_2), 1.31 (3H, t, *J* 7.2, OCH_2CH_3), 1.60–1.70 (2H, m, C(8)H_2), 2.61 (2H, t, *J* 7.5, C(7)H_2), 3.66 (2H, s, C(2)H_2), 4.22 (2H, q, *J* 7.2, $CO_2CH_2CH_3$), 5.94 (1H, d, *J* 3.0, C(5)*H*), 6.13 (1H, d, *J* 3.0, C(4)*H*); δ_C (75 MHz, $CDCl_3$) 14.4 (*C*(22)H_3), 23.0, 28.3, 29.5, 29.7, 29.8, 29.96, 29.99, 32.2 (*C*(7)H_2 to *C*(21)H_2 and OCH_2CH_3, many overlapping), 34.5 (*C*(2)H_2), 61.3 ($CO_2CH_2CH_3$), 105.7 (*C*(5)H), 108.6 (*C*(4)H), 145.9 and 156.4 (*C*(3) and *C*(6)), 170.0 (*C*(1)O); *m/z* (CI/NH_3) 396 ([M+NH_4]$^+$, 100%), 379 ([M+H]$^+$, 70), 167 (40), 88 (45), 74 (80); found 396.3478, $C_{24}H_{46}O_3N$ ([M+NH_4]$^+$) requires 396.3472.

4.4.2.2. Data for (5-hexadecylfuran-2-yl)acetic acid isopropyl ester (29d). Colourless solid; R_f 0.69 (petroleum ether:diethyl ether, 7:3); mp 28.3–29.0 °C; ν_{max} (film)/cm^{-1} 2924s and 2853s (C–H), 1740s (C═O, ester), 1566w,

1466m, 1374w, 1266m, 1223m, 1179m, 1108s; δ_H (300 MHz, $CDCl_3$) 0.92 (3H, t, *J* 6.6, C(22)H_3), 1.23–1.37 (32H, m, C(9)H_2 to C(21)H_2 and OCH(CH_3)$_2$), 1.60–1.69 (2H, m, C(8)H_2), 2.61 (2H, t, *J* 7.6, C(7)H_2), 3.61 (2H, s, C(2)H_2), 5.08 (1H, septet, *J* 6.3, $CO_2CH(CH_3)_2$), 5.94 (1H, d, *J* 3.0, C(5)*H*), 6.12 (1H, d, *J* 3.0, C(4)*H*); δ_C (75 MHz, $CDCl_3$) 14.4 (*C*(22)H_3), 22.0 (OCH(*C*H_3)$_2$), 23.0, 28.3, 29.5, 29.7, 29.9, 30.0, 32.2 (*C*(7)H_2 to *C*(21)H_2, many overlapping), 34.9 (*C*(2)H_2), 68.7 (CO_2CH(CH$_3$)$_2$), 105.7 (*C*(5)H), 108.5 (*C*(4)H), 146.1, 156.3 (*C*(3) and *C*(6)), 169.5 (*C*(1)O); *m/z* (CI/NH_3) 410 ([M+NH_4]$^+$, 100%), 393 ([M+H]$^+$, 50), 305 (30), 96 (30); found 392.3279, $C_{25}H_{44}O_3$ (M^+) requires 392.3285.

4.5. One-pot procedure for the conversion of plakorsin A (12) to (+/−)-untenone A (3)

meta-Chloroperbenzoic acid (46 mg, 0.27 mmol) was added to a stirred solution of plakorsin A (**12**) (75 mg, 0.21 mmol) in dichloromethane (3 mL) at −10 °C. The reaction mixture was allowed to warm to room temperature and stirred for 2.5 h when it was washed thoroughly with a saturated aqueous solution of sodium hydrogen carbonate (3×10 mL) and brine (10 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by flash column chromatography (SiO_2; petroleum ether:ethyl acetate, 9:1) furnished the title compound as a colourless solid (44 mg, 56%); analytical data were as described previously.[13]

4.6. Representative procedure for the oxidation of (5-alkylfuran-2-yl)acetates to give (*E*)-enediones

4.6.1. (2*Z*,4*E*)-3-Hydroxy-6-oxo-docosa-2,4-dienoic acid methyl ester (33). A solution of bromine (42 μL, 0.82 mmol) in a mixture of acetone and water (5:1, 1 mL) was added to a solution of plakorsin A (**12**) (300 mg, 0.82 mmol) in a mixture of acetone and water (5:1, 6.5 mL) at −20 °C. The reaction mixture was stirred at −20 °C for 3 h and then warmed to −10 °C. After a further 3 h, the reaction mixture was poured into diethyl ether (15 mL) and the resulting two-phase mixture was separated. The organic layer was washed with brine (3×10 mL), dried ($MgSO_4$) and concentrated in vacuo. Purification by flash column chromatography (SiO_2; petroleum ether:ethyl acetate, 25:1) yielded the title compound as a colourless solid (196 mg, 63% [82% based on recovered starting material]); R_f 0.19 (petroleum ether:ethyl acetate, 25:1); mp 82.5–83.5 °C; ν_{max} (film)/cm^{-1} 3019m, 2927m and 2855m (C–H), 1659m (C=O, ketone), 1588m, 1216s; δ_H (300 MHz, $CDCl_3$) 0.88 (3H, t, *J* 6.7, C(22)H_3), 1.21–1.36 (26H, m, C(9)H_2 to C(21)H_2), 1.59–1.68 (2H, m, C(8)H_2), 2.61 (2H, t, *J* 7.3, C(7)H_2), 3.79 (3H, s, CO_2CH_3), 5.34 (1H, s, C(2)*H*), 6.77 (1H, d, *J* 15.3, C(4)*H*), 6.93 (1H, d, *J* 15.3, C(5)*H*), 11.64 (1H, br s, O*H*); δ_C (75 MHz, $CDCl_3$) 14.4 (*C*(22)H_3), 23.0, 24.1, 29.5, 29.64, 29.67, 29.73, 29.9, 30.0, 32.2 (*C*(8)H_2 to *C*(21)H_2, many overlapping), 42.5 (*C*(7)H_2), 51.9 (CO_2CH_3), 96.9 (*C*(2)H), 132.0 (*C*(5)H), 134.5 (*C*(4)H), 166.7 (*C*(3)O), 171.6 (*C*(1)O), 200.0 (*C*(6)O); *m/z* (APCI) 379 ([M−H]$^-$, 70%), 348 ([M−CH_3OH]$^-$, 100); found 379.2841, $C_{23}H_{39}O_4$ [M−H]$^-$ requires 379.2854.

4.6.2. Data for (2*Z*,4*E*)-3-hydroxy-6-oxo-octa-2,4-dienoic acid methyl ester (34). Colourless crystals; mp 70.2–71.9 °C; R_f 0.45 (petroleum ether:ethyl acetate, 3:1); ν_{max} (solid state)/cm^{-1} 3070br w (O–H), 2976w and 2937w (C–H), 1657s (C=O, ketone), 1583s, 1446s, 1336s, 1188s; δ_H (300 MHz, $CDCl_3$) 1.12 (3H, t, *J* 7.3, C(8)H_3), 2.65 (2H, q, *J* 7.3, C(7)H_2), 3.78 (3H, s, CO_2CH_3), 5.34 (1H, s, C(2)*H*), 6.78 (1H, dd, *J* 15.7, 1.5, C(4)*H*), 6.92 (1H, d, *J* 15.7, C(5)*H*), 11.60 (1H, d, *J* 1.5, O*H*); δ_C (75 MHz, $CDCl_3$) 8.0 (*C*(8)H_3), 35.6 (*C*(7)H_2), 51.9 (CO_2CH_3), 96.9 (*C*(2)H), 131.8 (*C*(5)H), 134.5 (*C*(4)H), 166.7 (*C*(3)O), 172.5 (*C*(1)O), 200.4 (*C*(6)O); *m/z* (CI/NH_3) 202 ([M+NH_4]$^+$, 100%), 185 ([M+H]$^+$, 22); found 184.0729, $C_9H_{12}O_4$ (M^+) requires 184.0730.

4.6.3. Data for (2*Z*,4*E*)-3-hydroxy-6-oxo-octadeca-2,4-dienoic acid methyl ester (35). Colourless solid; R_f 0.16 (petroleum ether:ethyl acetate, 25:1); mp 74.5–75.0 °C; ν_{max} (film)/cm^{-1} 3453br w (O–H), 3019s and 2927s (C–H), 1710m (C=O, ester), 1658m (C=O, ketone), 1588m, 1447m, 1216s; δ_H (300 MHz, $CDCl_3$) 0.88 (3H, t, *J* 6.6, C(18)H_3), 1.19–1.30 (18H, m, C(9)H_2 to C(17)H_2), 1.58–1.66 (2H, m, C(8)H_2), 2.60 (2H, t, *J* 7.3, C(7)H_2), 3.79 (3H, s, CO_2CH_3), 5.34 (1H, s, C(2)*H*), 6.80 (1H, dd, *J* 15.6, 1.6, C(4)*H*), 6.93 (1H, d, *J* 15.6, C(5)*H*), 11.61 (1H, d, *J* 1.6, O*H*); δ_C (75 MHz, $CDCl_3$) 14.4 (*C*(18)H_3), 23.0, 24.2, 29.5, 29.6, 29.66, 29.73, 29.90, 29.92, 32.2, 42.5 (*C*(8)H_2, to *C*(17)H_2), 47.9 (*C*(7)H_2), 52.0 (CO_2CH_3), 97.0 (*C*(2)H), 132.0 (*C*(5)H), 134.6 (*C*(4)H), 166.7 (*C*(3)O), 172.6 (*C*(1)O), 200.0 (*C*(6)O); *m/z* (CI/NH_3) 342 ([M+NH_4]$^+$, 75%), 325 ([M+H]$^+$, 35); found 324.2294, $C_{19}H_{32}O_4$ (M^+) requires 324.2295.

4.6.4. Data for (2*Z*,4*E*)-3-hydroxy-6-oxo-docosa-2,4-dienoic acid isopropyl ester (36). Colourless solid; R_f 0.23 (petroleum ether:ethyl acetate, 25:1); mp 85.1–86.9 °C; ν_{max}(KBr disc)/cm^{-1} 2914s and 2849s (C–H), 1695m (C=O, ester), 1640s (C=O, ketone), 1590s, 1473s, 1375m, 1239s, 1110m; δ_H (300 MHz, $CDCl_3$) 0.91 (3H, t, *J* 6.6, C(22)H_3), 1.22–1.40 (26H, m, C(9)H_2 to C(21)H_2), 1.32 (6H, d, *J* 6.3, OCH(CH_3)$_2$), 1.62–1.71 (2H, m, C(8)H_2), 2.63 (2H, t, *J* 7.4, C(7)H_2), 5.15 (1H, septet, *J* 6.3, $CO_2CH(CH_3)_2$), 5.32 (1H, s, C(2)*H*), 6.80 (1H, dd, *J* 15.6, 1.5, C(4)*H*), 6.95 (1H, d, *J* 15.6, C(5)*H*), 11.80 (1H, d, *J* 1.5, O*H*); δ_C (75 MHz, $CDCl_3$) 14.4 (*C*(22)H_3), 22.1 (OCH(*C*H_3)$_2$), 23.0, 24.2, 29.5, 29.63, 29.67, 29.7, 29.8, 29.93, 29.96, 32.2 (*C*(8)H_2 to *C*(21)H_2, some overlapping), 42.5 (*C*(7)H_2), 68.7 (CO_2CH(CH$_3$)$_2$), 97.8 (*C*(2)H), 131.7 (*C*(5)H), 134.7 (*C*(4)H), 166.5 (*C*(3)O), 171.9 (C(1)O), 200.2 (*C*(6)O); *m/z* (−ve ion electrospray) 407 ([M−H]$^-$, 100%), 273 (80) (+ve ion electrospray); found 431.3132, $C_{25}H_{44}O_4Na$ [M+Na]$^+$ requires 431.3132.

4.7. Representative procedures for the preparation of plakoridine analogues using isolated imines

Imines were prepared and isolated using one of the two alternative procedures.

4.7.1. Method A. The amine (1 equiv) was added to a rapidly stirred mixture of the appropriate aldehyde (1 equiv) and water (*c*=0.85 M). The resulting suspension was stirred at room temperature for 3 h and the reaction mixture was

then extracted three times with dichloromethane. The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo to yield the required imine, which was used, when required, without further purification.

4.7.2. Method B. The amine (1 equiv) was added to a 0.03 M solution of the appropriate aldehyde (1 equiv) in dichloromethane. The reaction mixture was stirred at room temperature for 3 h when $MgSO_4$ was added and the reaction mixture was stirred for a further 30 min. The magnesium sulfate was removed by filtration and the filtrate was concentrated in vacuo to yield the required imine, which was used, when required, without further purification.

4.7.3. (3*R,4*S**,5*S**)-1-(2-(1*H*-Indol-3-yl)-ethyl)-2-(2′-oxo-octadec-*E*-ylidene)-3-hydroxy-5-phenyl-pyrrolidine-4-carboxylic acid methyl ester (44).** A solution of benzylidine-2-(1*H*-indol-3-yl)ethylamine (76 mg, 0.31 mmol) in deuterochloroform (1.0 mL) was added to (2*Z*,4*E*)-3-hydroxy-6-oxo-docosa-2,4-dienoic acid methyl ester (**33**) (117 mg, 0.31 mmol). The reaction mixture was stirred at room temperature for 10 days and then concentrated in vacuo. Purification by flash column chromatography (SiO_2; petroleum ether:ethyl acetate, 4:1) yielded the title compound as a pale yellow oil (74 mg, 38%); R_f 0.13 (petroleum ether:ethyl acetate, 3:1); ν_{max} (film)/cm^{-1} 3300br (O–H), 2923s and 2852s (C–H), 1739s (C=O, ester), 1618m (C=O, vinylogous amide), 1526s, 1458s, 1250m, 1173m; δ_H (300 MHz, $CDCl_3$) 0.93 (3H, t, *J* 6.8, C(23)H_3), 1.24–1.42 (26H, m, C(10)H_2 to C(22)H_2), 1.61–1.71 (2H, m, C(9)H_2), 2.38 (2H, ~t, *J* 7.8, C(8)H_2), 2.81–2.91 (1H, m, one of C(33)H_2), 3.04–3.22 (2H, m, one of C(32)H_2 and one of C(33)H_2), 3.23 (1H, ~t, *J* 7.1, C(4)*H*), 3.44–3.54 (1H, m, one of C(32)H_2), 3.71 (3H, s, CO_2CH_3), 4.63 (1H, d, *J* 7.5, C(5)*H*), 5.25 (1H, s, C(6)*H*), 5.34 (1H, d, *J* 6.6, C(3)*H*), 6.96 (1H, d, *J* 2.4, C(35)*H*), 7.08–7.42 (9H, m, C(27)*H* to C(31)*H* and C(38)*H* to C(41)*H*), 7.46 (1H, br s, O*H*), 8.34 (1H, br s, N*H*); δ_C (75 MHz, $CDCl_3$) 14.4 (*C*(23)H_3), 21.6 (*C*(33)H_2), 23.0, 26.6, 29.7, 29.87, 29.96, 30.0, 32.2 (*C*(9)H_2 to *C*(22)H_2, many overlapping), 44.8 (*C*(8)H_2), 45.5 (*C*(32)H_2), 52.8 (CO_2CH_3), 57.0 (*C*(4)H), 69.5 (*C*(5)H), 76.1 (*C*(3)H), 90.9 (*C*(6)H), 111.7, 112.1, 118.5, 119.8, 122.52, 122.55, 127.2, 128.2, 129.3, 129.4 (*C*(27)H and *C*(31)H, *C*(28)H and *C*(30)H, *C*(29)H, *C*(35)H, *C*(38)H to *C*(41)H and 2× quaternary), 136.6, 138.4 (2×quaternary), 166.2 (*C*(2)), 172.2 (*C*(24)O), 200.6 (*C*(7)O); *m/z* (+ve ion electrospray) 651 ([M+Na]$^+$, 73%), 629 ([M+H]$^+$, 55), 196 (55); found 629.4323, $C_{40}H_{57}N_2O_4$ ([M+H]$^+$), requires 629.4313.

4.7.4. Data for (3*R,4*S**,5*S**)-1-phenethyl-2-(2′-oxo-octadec-*E*-ylidene)-3-hydroxy-5-phenylpyrrolidine-4-carboxylic acid methyl ester (45).** A pale yellow oil; R_f 0.47 (petroleum ether:ethyl acetate, 3:1); ν_{max} (film)/cm^{-1} 3426m (O–H), 2924s and 2853s (C–H), 1741m (C=O, ester), 1626w (C=O, vinylogous amide), 1528m, 1458m; δ_H (500 MHz, $CDCl_3$) 0.81 (3H, t, *J* 7.0, C(23)H_3), 1.18–1.27 (26H, m, C(10)H_2 to C(22)H_2), 1.54–1.60 (2H, m, C(9)H_2), 2.34 (2H, ~td, *J* 7.0, 2.5, C(8)H_2), 2.56 (1H, ddd, *J* 13.8, 8.5, 5.0, one of C(33)H_2), 2.76–2.82 (1H, m, one of C(33)H_2), 2.94–3.00 (1H, m, one of C(32)H_2), 3.12 (1H, ~t, *J* 7.0, C(4)*H*), 3.30 (1H, ddd, *J* 13.8, 9.0, 5.0, one of C(32)H_2), 3.63 (3H, s, CO_2CH_3), 4.47 (1H, d, *J* 7.2, C(5)*H*), 5.13 (1H, s, C(6)*H*), 5.22 (1H, d, *J* 6.6, C(3)*H*), 6.94–6.96 (2H, m, Ar-C*H*), 7.14–7.33 (8H, m, Ar-C*H*); δ_C (100 MHz, $CDCl_3$) 14.3 (*C*(23)H_3), 22.8, 25.4, 29.5, 29.7, 29.8, 31.5 (*C*(9)H_2 to *C*(22)H_2, many overlapping), 32.0 (*C*(33)H_2), 43.7 (*C*(8)H_2), 46.3 (*C*(32)H_2), 52.6 (*C*(4)H), 56.7 (CO_2CH_3), 69.3 (*C*(5)H), 75.7 (*C*(3)H), 90.8 (*C*(6)H), 126.8 (Ar-*C*H), 127.8 (Ar-*C*H), 128.6 (Ar-*C*H), 128.7 (Ar-*C*H), 129.0 (Ar-*C*H), 129.1 (Ar-*C*H), 137.9, 138.1 (*C*(26) and *C*(34)), 165.6 (*C*(2)), 171.7 (*C*(24)O), 200.1 (*C*(7)O); *m/z* (+ve ion electrospray) 590 ([M+H]$^+$, 100%); found 590.4203, $C_{34}H_{56}NO_4$ ([M+H]$^+$), requires 590.4204.

4.7.5. Data for (3*R,4*S**,5*S**)-1-phenethyl-2-(2′-oxo-octadec-*E*-ylidene)-3-hydroxy-5-pentadecylpyrrolidine-4-carboxylic acid methyl ester (46).** A pale yellow oil; R_f 0.21 (petroleum ether:ethyl acetate, 9:1); ν_{max} (film)/cm^{-1} 3225br (O–H), 2925s, 2853s, 1741s (C=O, ester), 1626m (C=O, vinylogous amide), 1538s, 1466m, 1172w; δ_H (500 MHz, $CDCl_3$) 0.81 (6H, ~t, *J* 6.8, C(23)H_3 and C(40)H_3), 1.10–1.30 (52H, m, C(10)H_2 to C(22)H_2 and C(27)H_2 to C(39)H_2), 1.40–1.47 (1H, m, one of C(26)H_2), 1.51–1.56 (2H, m, C(9)H_2), 1.65–1.70 (1H, m, one of C(26)H_2), 2.29 (2H, td, *J* 7.8, 2.8, C(8)H_2), 2.75 (1H, ddd, *J* 14.4, 8.9, 5.8, one of C(42)H_2), 2.81–2.87 (1H, m, one of C(42)H_2), 2.84 (1H, ~t, *J* 5.7, C(4)*H*), 3.26 (1H, ddd, *J* 14.4, 8.9, 5.8, one of C(41)H_2), 3.39 (1H, ddd, *J* 14.4, 8.9, 5.8, one of C(41)H_2), 3.63–3.76 (1H, m, C(5)*H*), 3.67 (3H, s, CO_2CH_3), 5.03 (1H, br s, C(6)*H*), 5.13 (1H, d, *J* 5.7, C(3)*H*), 6.89 (1H, br s, O*H*), 7.11 (2H, d, *J* 7.4, C(44)*H* and C(48)*H*), 7.18 (1H, t, *J* 7.4, C(46)*H*), 7.25 (2H, t, *J* 7.4, C(45)*H* and C(47)*H*); δ_C (75 MHz, $CDCl_3$) 14.4 (*C*(23)H_3 and *C*(40)H_3, coincident), 23.0, 24.5, 26.5, 29.6, 29.7, 29.79, 29.84, 29.88, 29.9, 30.0, 32.2, 32.4, 33.3 (*C*(9)H_2 to *C*(22)H_2, *C*(26)H_2 to *C*(39)H_2 and *C*(42)H_2, many overlapping), 43.8 (*C*(8)H_2), 46.2 (*C*(41)H_2), 52.5 (*C*(4)H), 52.8 (CO_2CH_3), 65.6 (*C*(5)H), 76.1 (*C*(3)H), 90.0 (*C*(6)H), 127.2 (*C*(46)H), 128.9 (*C*(44)H and *C*(48)H), 129.1 (*C*(45)H and *C*(47)H), 138.3 (*C*(43)), 165.9 (*C*(2)), 173.0 (*C*(24)O), 200.0 (*C*(7)O); *m/z* (+ve ion electrospray) 746 ([M+Na]$^+$, 100%), 724 ([M+H]$^+$, 93); found 724.6229, $C_{47}H_{82}NO_4$ ([M+H]$^+$) requires 724.6238.

4.7.6. Data for (3*R,4*S**,5*S**)-1-(2-(1*H*-indol-3-yl)-ethyl)-2-(2′-oxo-octadec-*E*-ylidene)-3-hydroxy-5-pentadecylpyrrolidine-4-carboxylic acid methyl ester (47).** A pale yellow oil; R_f 0.25 (petroleum ether:ethyl acetate, 3:1); ν_{max} (film)/cm^{-1} 3300br (O–H), 2923s and 2852s (C–H), 1739m (C=O, ester), 1618w (C=O, vinylogous amide), 1526s, 1465m; δ_H (500 MHz, $CDCl_3$) 0.81 (6H, ~t, *J* 6.9, C(23)H_3 and C(40)H_3), 1.10–1.26 (52H, m, C(10)H_2 to C(22)H_2 and C(27)H_2 to C(39)H_2), 1.40–1.51 (3H, m, C(9)H_2 and one of C(26)H_2), 1.64–1.70 (1H, m, one of C(26)H_2), 2.18 (2H, t, *J* 7.6, C(8)H_2), 2.83 (1H, ~t, *J* 6.1, C(4)*H*), 2.89–2.94 (1H, m, one of C(42)H_2), 3.00–3.06 (1H, m, one of C(42)H_2), 3.33–3.39 (1H, m, one of C(41)H_2), 3.46–3.52 (1H, m, one of C(41)H_2), 3.66 (3H, s, CO_2CH_3), 3.69 (1H, ddd, *J* 8.9, 6.1, 2.8, C(5)*H*), 5.00 (1H, s, C(6)*H*), 5.12 (1H, d, *J* 6.1, C(3)*H*), 6.94 (1H, d, *J* 2.4, C(44)*H*), 7.00 (1H, br s, O*H*), 7.09 (1H, t, *J* 7.6, C(48)*H* or C(49)*H*), 7.15 (1H, t, *J* 7.6, C(48)*H* or C(49)*H*), 7.31 (1H, d, *J* 7.6, C(47)*H* or C(50)*H*), 7.52 (1H, d, *J* 7.6, C(47)*H* or C(50)*H*), 8.06 (1H, br s, N*H*); δ_C (75 MHz, $CDCl_3$) 14.4 (*C*(23)H_3 and *C*(40)H_3, coincident), 22.2, 23.0, 24.5, 26.6, 29.6, 29.7, 29.81, 29.83, 29.9, 29.95, 29.99, 32.2, 33.3

($C(9)H_2$ to $C(22)H_2$, $C(26)H_2$ to $C(39)H_2$ and $C(42)H_2$, many overlapping), 43.7 ($C(8)H_2$), 45.0 ($C(41)H_2$), 52.6 ($C(4)$H), 52.8 (CO_2CH_3), 65.5 ($C(5)$H), 76.3 ($C(3)$H), 90.4 ($C(6)$H), 111.7, 112.3, 118.5, 120.0, 122.5, 122.7, 127.3, 136.6 ($C(43)$, C(44)H, $C(46)$, $C(47)$H to $C(50)$H and $C(51)$), 166.2 ($C(2)$), 173.2 ($C(24)$O), 200.0 ($C(7)$O); *m/z* (+ve ion electrospray) 785 ($[M+Na]^+$, 100%); found 785.6165, $C_{49}H_{82}N_2O_4Na$ ($[M+Na]^+$) requires 785.6167.

4.7.7. Data for (3*R,4*S**,5*S**)-1-phenethyl-2-(2′-oxo-octadec-*E*-ylidene)-3-hydroxy-5-ethylpyrrolidine-4-carboxylic acid isopropyl ester (48).** A pale yellow oil; R_f 0.16 (petroleum ether:ethyl acetate, 9:1); ν_{max} (film)/cm^{-1} 3222br (O–H), 2924s and 2853s (C–H), 1732s (C=O, ester), 1625m (C=O, vinylogous amide), 1535s, 1466s, 1249m, 1180m, 1107s; δ_H (300 MHz, $CDCl_3$) 0.79–0.84 (6H, m, C(23)H_3 and C(26)H_3), 1.10–1.28 (32H, m, C(10)H_2 to C(22)H_2 and $OCH(CH_3)_2$), 1.48–1.57 (3H, m, C(9)H_2 and one of C(25)H_2), 1.72–1.80 (1H, m, one of C(25)H_2), 2.29 (2H, td, *J* 7.5, 3.2, C(8)H_2), 2.72–2.78 (1H, m, one of C(28)H_2), 2.77 (1H, ~t, *J* 6.0, C(4)*H*), 2.84 (1H, ddd, *J* 14.4, 9.0, 4.5, one of C(28)H_2), 3.25 (1H, ddd, *J* 14.4, 9.0, 4.5, one of C(27)H_2), 3.40 (1H, ddd, *J* 14.4, 9.0, 4.5, one of C(27)H_2), 3.58–3.62 (1H, m, C(5)*H*), 4.98 (1H, septet, *J* 6.2, $CO_2CH(CH_3)_2$), 5.03 (1H, br s, C(6)*H*), 5.13 (1H, d, *J* 6.0, C(3)*H*), 6.88 (1H, br s, O*H*), 7.12 (2H, d, *J* 7.0, C(30)*H* and C(34)*H*), 7.17–7.20 (1H, m, C(32)*H*), 7.26 (2H, d, *J* 7.0, C(31)*H* and C(33)*H*); δ_C (75 MHz, $CDCl_3$) 8.7, 14.4 ($C(23)H_3$ and $C(26)H_3$), 21.9, 22.0, 23.0, 25.9, 26.5, 29.4, 29.82, 29.85, 29.9, 30.0, 32.2, 32.3 ($C(9)H_2$ to $C(22)H_2$, $OCH(CH_3)_2$, $C(25)H_2$ and $C(28)H_2$, many overlapping), 43.7 ($C(8)H_2$), 46.1 ($C(27)H_2$), 52.4 ($C(4)$H), 66.7 ($C(5)$H), 69.3 ($CO_2CH(CH_3)_2$), 76.0 ($C(3)$H), 90.5 ($C(6)$H), 127.2 ($C(32)$H), 128.9 ($C(30)$H and $C(34)$H), 129.1 ($C(31)$H and $C(33)$H), 138.3 ($C(29)$), 166.3 ($C(2)$), 172.1 ($C(24)$O), 200.0 ($C(7)$O); *m/z* (+ve ion electrospray) 592 ($[M+Na]^+$, 13%), 570 ($[M+H]^+$, 100); found 570.4509, $C_{36}H_{60}NO_4$ ($[M+H]^+$) requires 570.4517.

4.7.8. Data for (3*R,4*S**,5*S**)-1-phenethyl-2-(2′-oxo-tetradec-*E*-ylidene)-3-hydroxy-5-phenylpyrrolidine-4-carboxylic acid methyl ester (49).** A pale yellow oil; R_f 0.19 (petroleum ether:ethyl acetate, 5:1); ν_{max} (film)/cm^{-1} 3255br (O–H), 2924s and 2852s (C–H), 1739s (C=O, ester), 1625m (C=O, vinylogous amide), 1533s, 1457m, 1251m; δ_H (300 MHz, $CDCl_3$) 0.92 (3H, t, *J* 6.6, C(19)H_3), 1.24–1.41 (18H, m, C(10)H_2 to C(18)H_2), 1.64–1.73 (2H, m, C(9)H_2), 2.43–2.48 (2H, m, C(8)H_2), 2.67 (1H, ddd, *J* 13.8, 8.7, 5.0, one of C(29)H_2), 2.86–2.96 (1H, m, one of C(29)H_2), 3.00–3.13 (1H, m, one of C(28)H_2), 3.23 (1H, ~t, *J* 6.9, C(4)*H*), 3.41 (1H, ddd, *J* 13.8, 8.7, 5.0, one of C(28)H_2), 3.74 (3H, s, CO_2CH_3), 4.59 (1H, d, *J* 6.9, C(5)*H*), 5.25 (1H, s, C(6)*H*), 5.33 (1H, d, *J* 6.9, C(3)*H*), 7.05–7.08 (2H, m, 2×aromatic C*H*), 7.25–7.43 (8H, m, 8×aromatic C*H*); δ_C (75 MHz, $CDCl_3$) 14.4 ($C(19)H_3$), 23.0, 26.6, 29.6, 29.8, 29.9, 30.0, 31.7, 32.2 ($C(9)H_2$ to $C(18)H_2$ and $C(29)H_2$, some overlapping), 43.9 ($C(8)H_2$), 46.5 ($C(28)H_2$), 52.8 (CO_2CH_3), 56.9 ($C(4)$H), 69.6 ($C(5)$H), 76.0 ($C(3)$H), 91.0 ($C(6)$H), 127.1, 128.1, 128.9, 129.1, 129.4 ($C(23)$H and $C(27)$H, $C(24)$H and $C(26)$H, $C(25)$H, $C(31)$H and $C(35)$H, $C(32)$H and $C(34)$H and $C(33)$H), 138.2, 138.5 ($C(22)$ and $C(30)$), 165.9 ($C(2)$), 172.1 ($C(20)$O), 200.5 ($C(7)$O); *m/z* (+ve ion electrospray) 556 ($[M+Na]^+$, 100%); found 556.3405, $C_{34}H_{47}NO_4Na$ ($[M+Na]^+$) requires 556.3397.

4.7.9. Data for (3*R,4*S**,5*S**)-1-(2-(1*H*-indol-3-yl)-ethyl)-2-(2′-oxo-tetradec-*E*-ylidene)-3-hydroxy-5-phenyl-pyrrolidine-4-carboxylic acid methyl ester (50).** A yellow oil; R_f 0.47 (petroleum ether:ethyl acetate, 3:1); ν_{max} (film)/cm^{-1} 3326br (O–H), 2924s and 2852m (C–H), 1738s (C=O, ester), 1618w (C=O, vinylogous amide), 1525s, 1457m, 1437w, 1250w; δ_H (300 MHz, $CDCl_3$) 0.94 (3H, t, *J* 6.6, C(19)H_3), 1.25–1.40 (18H, m, C(10)H_2 to C(18)H_2), 1.62–1.70 (2H, m, C(9)H_2), 2.38 (2H, t, *J* 7.6, C(8)H_2), 2.82–2.93 (1H, m, one of C(29)H_2), 3.05–3.24 (2H, m, one of C(28)H_2 and one of C(29)H_2), 3.24 (1H, ~t, *J* 7.2, C(4)*H*), 3.45–3.58 (1H, m, one of C(28)H_2), 3.72 (3H, s, CO_2CH_3), 4.63 (1H, d, *J* 7.2, C(5)*H*), 5.25 (1H, s, C(6)*H*), 5.35 (1H, d, *J* 6.3, C(3)*H*), 7.00 (1H, d, *J* 2.1, C(31)*H*), 7.10–7.43 (10H, m, C(23)*H* to C(27)*H*, C(34)*H* to C(37)*H* and O*H*), 8.35 (1H, br s, N*H*); δ_C (75 MHz, $CDCl_3$) 14.4 ($C(19)H_3$), 21.6 ($C(29)H_2$), 23.0, 26.6, 29.7, 29.9, 29.96, 29.99, 32.2 ($C(9)H_2$ to $C(18)H_2$, some overlapping), 43.8 ($C(8)H_2$), 45.5 ($C(28)H_2$), 52.8 (CO_2CH_3), 57.0 ($C(4)$H), 69.5 ($C(5)$H), 76.1 ($C(3)$H), 90.9 ($C(6)$H), 111.7, 112.1, 118.5, 119.8, 122.6, 127.3, 128.2, 129.3, 129.4 ($C(23)$H and $C(27)$H, $C(24)$H and $C(26)$H, $C(25)$H, $C(34)$H, $C(35)$H, $C(36)$H, $C(37)$H and 2×quaternary *C*), 136.6, 138.4 (2×quaternary *C*), 166.3 ($C(2)$), 172.3 ($C(20)$O), 200.6 ($C(7)$O); *m/z* (+ve ion electrospray) 595 ($[M+Na]^+$, 20%), 573 ($[M+H]^+$, 70), 295 (25); found 573.3689, $C_{36}H_{49}N_2O_4$ ($[M+H]^+$) requires 573.3687.

4.7.10. Data for (3*R,4*S**,5*S**)-1-phenethyl-2-(2′-oxo-tetradec-*E*-ylidene)-3-hydroxy-5-ethylpyrrolidine-4-carboxylic acid methyl ester (51).** A yellow oil; R_f 0.47 (petroleum ether:ethyl acetate, 3:1); ν_{max} (film)/cm^{-1} 2924s and 2853s (C–H), 1740s (C=O, ester), 1624m (C=O, vinylogous amide), 1535s, 1463m, 1248w; δ_H (300 MHz, $CDCl_3$) 0.93 (3H, t, *J* 6.9, C(19)H_3 or C(23)H_3), 0.94 (3H, t, *J* 7.5, C(19)H_3 or C(23)H_3), 1.26–1.37 (18H, m, C(10)H_2 to C(18)H_2), 1.61–1.71 (3H, m, C(9)H_2 and one of C(22)H_2), 1.82–1.93 (1H, m, one of C(22)H_2), 2.39–2.45 (2H, m, C(8)H_2), 2.87 (1H, ddd, *J* 14.8, 9.0, 5.8, one of C(25)H_2), 2.93–3.02 (1H, m, one of C(25)H_2), 2.96 (1H, ~t, *J* 6.0, C(4)*H*), 3.37 (1H, ddd, *J* 14.8, 9.0, 5.8, one of C(24)H_2), 3.53 (1H, ddd, *J* 14.8, 9.0, 5.8, one of C(24)H_2), 3.74–3.82 (1H, m, C(5)*H*), 3.80 (3H, s, CO_2CH_3), 5.16 (1H, s, C(6)*H*), 5.26 (1H, d, *J* 6.0, C(3)*H*), 7.23–7.42 (5H, m, C(27)*H* to C(31)*H*); δ_C (75 MHz, $CDCl_3$) 8.6, 14.4 ($C(19)H_3$ and $C(23)H_3$), 23.0, 25.7, 26.6, 29.4, 29.8, 29.9, 30.0, 32.2, 32.3 ($C(9)H_2$ to $C(18)H_2$, $C(22)H_2$ and $C(25)H_2$, some overlapping), 43.8 ($C(8)H_2$), 46.1 ($C(24)H_2$), 51.9 ($C(4)$H), 52.8 (CO_2CH_3), 66.3 ($C(5)$H), 76.0 ($C(3)$H), 90.6 ($C(6)$H), 127.2 ($C(29)$H), 128.9 (either $C(27)$H and $C(31)$H or $C(28)$H and $C(30)$H), 129.1 (either $C(27)$H and $C(31)$H or $C(28)$H and $C(30)$H), 138.2 (C(26)), 166.1 ($C(2)$), 173.0 ($C(20)$O), 200.1 ($C(7)$O); *m/z* (+ve ion electrospray) 508 ($[M+Na]^+$, 90%), 486 ($[M+H]^+$, 100), 417, (10), 212 (38); found 486.3574, $C_{30}H_{48}NO_4$ ($[M+H]^+$) requires 486.3578.

4.7.11. Data for (3*R,4*S**,5*S**)-1-benzyl-2-(2′-oxo-tetradec-*E*-ylidene)-3-hydroxy-5-phenylpyrrolidine-4-carboxylic acid methyl ester (52).** A pale yellow oil

contaminated with <10% of a minor diastereoisomer; R_f 0.50 (petroleum ether:ethyl acetate, 3:1); ν_{max} (film)/cm^{-1} 2925s and 2853m (C–H), 1739m (C=O, ester), 1628w (C=O, vinylogous amide), 1535s, 1457m, 1250w; δ_H (300 MHz, $CDCl_3$) 0.92 (3H, t, *J* 6.6, C(19)H_3), 1.24–1.37 (18H, m, C(10)H_2 to C(18)H_2), 1.47–1.65 (2H, m, C(9)H_2), 2.36–2.41 (2H, m, C(8)H_2), 3.21 (1H, ~t, *J* 6.4, C(4)*H*), 3.75 (3H, s, CO_2CH_3), 3.97 (1H, d, *J* 16.1, one of C(28)H_2), 4.48 (1H, d, *J* 16.1, one of C(28)H_2), 4.89 (1H, d, *J* 6.4, C(5)*H*), 5.34 (1H, s, C(6)*H*), 5.41 (1H, d, *J* 6.4, C(3)*H*), 7.07–7.43 (11H, m, C(23)*H* to C(27)*H* and C(30)*H* to C(34)*H* and O*H*); δ_C (75 MHz, $CDCl_3$) 14.4 (*C*(19)H_3), 23.0, 26.4, 29.6, 29.7, 29.8, 29.9, 32.2 (*C*(9)H_2 to *C*(18)H_2), 43.9 (*C*(8)H_2), 48.3 (*C*(28)H_2), 52.9 (CO_2CH_3), 56.7 (*C*(4)H), 69.2 (*C*(5)H), 76.1 (*C*(3)H), 91.6 (*C*(6)H), 127.4, 128.1, 128.2, 129.1, 129.3, 129.4 (*C*(23)H and *C*(27)H, *C*(24)H and *C*(26)H, *C*(25)H, *C*(30)H and *C*(34)H, *C*(31)H and *C*(33)H and *C*(32)H), 134.6, 138.3 (*C*(22) and *C*(29)), 166.6 (*C*(2)), 172.4 (*C*(20)O), 201.0 (*C*(7)O); *m/z* (+ve ion electrospray) 520 ([M+H]$^+$, 100%); found 520.3422, $C_{33}H_{46}NO_4$ [M+H]$^+$ requires 520.3421.

4.7.12. Data for (3*R,4*S**,5*S**)-1-benzyl-2-(2′-oxo-but-*E*-ylidene)-3-hydroxy-5-phenylpyrrolidine-4-carboxylic acid methyl ester (53).** A pale yellow oil; R_f 0.12 (petroleum ether:ethyl acetate, 5:1); ν_{max} (film)/cm^{-1} 3185br (O–H), 2972m (C–H), 1737s (C=O, ester), 1628m (C=O, vinylogous amide), 1535s, 1457s, 1251m; δ_H (300 MHz, $CDCl_3$) 1.13 (3H, t, *J* 7.5, C(9)H_3), 2.43 (2H, q, *J* 7.4, C(8)H_2), 3.32 (1H, ~t, *J* 6.0, C(4)*H*), 3.75 (3H, s, CO_2CH_3), 3.97 (1H, d, *J* 15.9, one of C(18)H_2), 4.48 (1H, d, *J* 15.9, one of C(18)H_2), 4.90 (1H, d, *J* 6.6, C(5)*H*), 5.34 (1H, s, C(6)*H*), 5.41 (1H, d, *J* 6.0, C(3)*H*), 7.07–7.10 (2H, m, 2×aromatic C*H*), 7.15 (1H, br s, O*H*), 7.29–7.43 (8H, m, 8×aromatic C*H*); δ_C (75 MHz, $CDCl_3$) 36.7 (*C*(8)H_2), 48.3 (*C*(18)H_2), 52.9 (CO_2CH_3), 56.7 (*C*(4)H), 69.2 (*C*(5)H), 76.1 (*C*(3)H), 91.1 (*C*(6)H), 127.4, 128.1, 129.1, 129.3, 129.4 (*C*(13)H and *C*(17)H, *C*(14)H and *C*(16)H, *C*(15)H, *C*(20)H and *C*(24)H, *C*(21)H and *C*(23)H and *C*(22)H), 134.6, 138.3 (*C*(12) and *C*(19)), 166.5 (*C*(2)), 172.4 (*C*(10)O), 201.3 (*C*(7)O); *m/z* (+ve ion electrospray) 402 ([M+Na]$^+$, 15%), 380 ([M+H]$^+$, 12); found 380.1863, $C_{23}H_{26}NO_4$ ([M+H]$^+$) requires 380.1856.

4.7.13. Data for (3*R,4*S**,5*S**)-1-phenethyl-2-(2′-oxo-but-*E*-ylidene)-3-hydroxy-5-pentadecylpyrrolidine-4-carboxylic acid methyl ester (54).** A pale yellow oil; R_f 0.25 (petroleum ether:ethyl acetate, 5:1); ν_{max} (film)/cm^{-1} 3205br (O–H), 2925s and 2854m (C–H), 1740s (C=O, ester), 1627m (C=O, vinylogous amide), 1537s, 1466s, 1172m; δ_H (500 MHz, $CDCl_3$) 0.67 (3H, t, *J* 7.0, C(26)H_3), 0.92 (3H, t, *J* 7.5, C(9)H_3), 0.97–1.16 (26H, m, C(13)H_2 to C(25)H_2), 1.27–1.34 (1H, m, one of C(12)H_2), 1.52–1.58 (1H, m, one of C(12)H_2), 2.20 (2H, qd, *J* 7.5, 2.5, C(8)H_2), 2.61 (1H, ddd, *J* 14.7, 9.1, 6.0, one of C(28)H_2), 2.68–2.73 (1H, m, one of C(28)H_2), 2.70 (2H, ~t, *J* 5.8, C(4)*H*), 3.13 (1H, ddd, *J* 14.7, 9.1, 6.0, one of C(27)H_2), 3.26 (1H, ddd, *J* 14.7, 9.1, 6.0, one of C(27)H_2), 3.50–3.54 (1H, m, C(5)*H*), 3.53 (3H, s, CO_2CH_3), 4.90 (1H, br s, C(6)*H*), 5.01 (1H, d, *J* 5.5, C(3)*H*), 6.72 (1H, br s, O*H*), 6.97–7.13 (5H, m, C(30)*H* to C(34)*H*); δ_C (75 MHz, $CDCl_3$) 10.3 (*C*(9)H_3), 14.4 (*C*(26)H_3), 23.0, 24.5, 29.6, 29.7, 29.8, 29.87, 29.92, 29.95, 31.2, 32.4, 33.3 (*C*(12)H_2 to *C*(25)H_2 and *C*(28)H_2, some overlapping), 36.5 (*C*(8)H_2), 46.2 (*C*(27)H_2), 52.4 (*C*(4)H), 52.7 (CO_2CH_3), 65.6 (*C*(5)H), 76.1 (*C*(3)H), 89.9 (*C*(6)H), 128.9 (either *C*(30)H and *C*(34)H or *C*(31)H and *C*(33)H), 129.1 (either *C*(30)H and *C*(34)H or *C*(31)H and *C*(33)H), 138.3 (*C*(29)), 165.9 (*C*(2)), 173.0 (*C*(10)O), 200.4 (*C*(7)O); *m/z* (+ve ion electrospray) 550 ([M+Na]$^+$, 70%), 528 ([M+H]$^+$, 100); found 528.4048, $C_{33}H_{54}NO_4$ [M+H]$^+$ requires 528.4047.

4.7.14. (3*R,4*S**,5*S**)-1-Phenethyl-2-(2′-oxo-octadec-*E*-ylidene)-3-hydroxy-5-ethylpyrrolidine-4-carboxylic acid methyl ester (38) and (3*R**,4*R**,5*R**)-1-phenethyl-2-(2′-oxo-octadec-*E*-ylidene)-3-hydroxy-5-ethylpyrrolidine-4-carboxylic acid methyl ester (39).** Data for (3*R**,4*S**,5*S**)-1-phenethyl-2-(2′-oxo-octadec-*E*-ylidene)-3-hydroxy-5-ethylpyrrolidine-4-carboxylic acid methyl ester (**38**) and (3*R**,4*R**,5*R**)-1-phenethyl-2-(2′-oxo-octadec-*E*-ylidene)-3-hydroxy-5-ethylpyrrolidine-4-carboxylic acid methyl ester (39) were as reported previously.[18]

4.8. Representative procedure for the preparation of the natural plakoridines

4.8.1. (+/−)-Plakoridine B (2). Tyramine (28.8 mg, 0.21 mmol) was added to a solution of hexadecanal (50.4 mg, 0.21 mmol) in $CDCl_3$ (12 mL). The reaction mixture was stirred at room temperature for 3 h when $MgSO_4$ was added and the reaction mixture was stirred for a further 30 min. The $MgSO_4$ was removed by filtration and the filtrate was added to (2*Z*,4*E*)-3-hydroxy-6-oxo-docosa-2,4-dienoic acid methyl ester (**33**) (80 mg, 0.21 mmol). The reaction mixture was stirred at room temperature for 11 days and then concentrated in vacuo. Purification of the residue by flash column chromatography (SiO_2, petroleum ether:ethyl acetate, 5:1) yielded the title compound as a pale yellow oil (56 mg, 36%). R_f 0.30 (petroleum ether:-ethyl acetate, 3:1); ν_{max} (film)/cm^{-1} 3252br (O–H), 2923s and 2853s (C–H), 1741s (C=O, ester), 1613m (C=O, vinylogous amide), 1516s, 1466s, 1247m; δ_H (500 MHz, $CDCl_3$) 0.89 (6H, ~t, *J* 6.9, C(23)H_3 and C(40)H_3), 1.21–1.35 (54H, m, C(10)H_2 to C(22)H_2 and C(27)H_2 to C(39)H_2), 1.48–1.55 (1H, m, one of C(26)H_2), 1.58–1.65 (2H, m, C(9)H_2), 1.72–1.78 (1H, m, one of C(26)H_2), 2.35–2.39 (2H, m, C(8)H_2), 2.74 (1H, ddd, *J* 14.1, 8.8, 5.6, one of C(42)H_2), 2.82–2.88 (1H, m, one of C(42)H_2), 2.91 (1H, ~t, *J* 5.8, C(4)*H*), 3.26–3.32 (1H, m, one of C(41)H_2), 3.43 (1H, ddd, *J* 14.1, 8.8, 5.6, one of C(41)H_2), 3.71 (1H, ddd, *J* 8.8, 5.8, 2.9, C(5)*H*), 3.75 (3H, s, CO_2CH_3), 5.09 (1H, s, C(6)*H*), 5.22 (1H, d, *J* 5.8, C(3)*H*), 5.53 (1H, br s, O*H*), 6.80 (2H, d, *J* 8.6, C(45)*H* and C(47)*H*), 7.01 (1H, br s, O*H*), 7.04 (2H, d, *J* 8.6, C(44)*H* and C(48)*H*); δ_C (75 MHz, $CDCl_3$) 14.4 (*C*(23)H_3 and *C*(40)H_3), 23.0, 24.5, 26.7, 29.6, 29.7, 29.8, 29.89, 29.94, 31.5, 32.2, 33.3 (*C*(9)H_2 to *C*(22)H_2, *C*(26)H_2 to *C*(39)H_2 and *C*(42)H_2, many overlapping), 43.7 (*C*(8)H_2), 46.5 (*C*(41)H_2), 52.4 (*C*(4)H), 52.9 (CO_2CH_3), 65.8 (*C*(5)H), 76.2 (*C*(3)H), 90.5 (*C*(6)H), 116.0 (*C*(45)H and *C*(47)H), 129.7 (*C*(46)), 130.0 (*C*(44)H and *C*(48)H), 155.4 (*C*(43)), 166.3 (*C*(2)), 173.0 (*C*(24)O), 200.2 (*C*(7)O); *m/z* (+ve ion electrospray) 762 ([M+Na]$^+$, 100%); found 740.6188, $C_{47}H_{82}NO_5$ ([M+H]$^+$) requires 740.6188.

4.8.2. Data for (+/−)-Plakoridine A (1). A pale yellow oil; R_f 0.18 (petroleum ether:ethyl acetate, 3:1); ν_{max} (film)/cm^{-1} 3300br (O–H), 2924s and 2853s (C–H), 1740s (C=O, ester), 1613m (C=O, vinylogous amide), 1516s, 1466s, 1236m; δ_H (500 MHz, $CDCl_3$) 0.88 (3H, t, J 7.1, C(23)H_3 or C(28)H_3), 0.93 (3H, t, J 7.4, C(23)H_3 or C(28)H_3), 1.21–1.35 (28H, m, C(10)H_2 to C(22)H_2 and C(27)H_2), 1.47–1.55 (1H, m, one of C(26)H_2), 1.59–1.65 (2H, m, C(9)H_2), 1.70–1.77 (1H, m, one of C(26)H_2), 2.36–2.39 (2H, m, C(8)H_2), 2.74 (1H, ddd, J 14.2, 8.9, 5.5, one of C(30)H_2), 2.82–2.88 (1H, m, one of C(30)H_2), 2.91 (1H, ~t, J 5.6, C(4)H), 3.27–3.33 (1H, m, one of C(29)H_2), 3.44 (1H, ddd, J 14.2, 8.9, 5.5, one of C(29)H_2), 3.70–3.75 (1H, m, C(5)H), 3.74 (3H, s, CO_2CH_3), 5.10 (1H, br s, C(6)H), 5.24 (1H, d, J 5.6, C(3)H), 6.03 (1H, br s, OH), 6.81 (1H, d, J 8.6, C(33)H and C(35)H), 7.02–7.05 (3H, m, C(32)H, C(36)H and OH); δ_C (75 MHz, $CDCl_3$) 14.2, 14.4 (C(23)H_3 and C(28)H_3), 17.9, 23.0, 26.7, 29.6, 29.8, 30.0, 31.5, 32.2 (C(9)H_2 to C(22)H_2, C(27)H_2 and C(30)H_2, many overlapping), 35.5 (C(26)H_2), 43.7 (C(8)H_2), 46.5 (C(29)H_2), 52.4 (C(4)H), 52.9 (CO_2CH_3), 65.7 (C(5)H), 76.2 (C(3)H), 90.5 (C(6)H), 116.0 (C(33)H and C(35)H), 129.8 (C(34)), 130.0 (C(32)H and C(36)H), 155.3 (C(31)), 166.2 (C(2)), 173.0 (C(24)O), 200.2 (C(7)O); m/z (+ve ion electrospray) 594 ([M+Na]$^+$, 100%), 572 ([M+H]$^+$, 12); found 572.4313, $C_{35}H_{58}NO_5$ [M+H]$^+$ requires 572.4310.

4.9. Synthesis of (2*R**,4*R**,5*S**)-1-phenyl-2-(2′-oxo-butyl)-3-oxo-5-phenylpyrrolidine-4-carboxylic acid methyl ester (57)

A solution of benzylidine-aniline (135 mg, 0.74 mmol) in deuterochloroform (0.6 mL) was added to (2*Z*,4*E*)-3-hydroxy-6-oxo-octa-2,4-dienoic acid methyl ester (**34**) (125 mg, 0.74 mmol). The reaction mixture was stirred at room temperature for 24 h and then concentrated in vacuo and the residue was triturated with ether. The resulting solid was recrystallised from ethyl acetate to yield the title compound as a colourless microcrystalline solid (149 mg, 55%); mp 130.2–132.1 °C (decomp.); R_f 0.15 (petroleum ether:diethyl ether, 5:1); ν_{max} (film)/cm^{-1} 3002w, 2952w and 2900w (C–H), 1760m (C=O, five-membered ketone), 1731s (C=O, ester), 1704s (C=O, ketone), 1597m, 1501m, 1337s, 1258s, 1223s, 1110s; δ_H (500 MHz, $CDCl_3$) 0.87 (3H, t, J 7.3, C(9)H_3), 2.08 (1H, dq, J 17.5, 7.3, C(8)H_2), 2.19 (1H, dq, J 17.5, 7.3, one of C(8)H_2), 3.04 (1H, dd, J 18.0, 3.5, one of C(6)H_2), 3.41 (1H, dd, J 18.0, 4.8, one of C(6)H_2), 3.65 (1H, d, J 8.3, C(4)H), 3.88 (3H, s, CO_2CH_3), 4.81 (1H, ~t, J 4.0, C(2)H), 5.62 (1H, d, J 8.3, C(5)H), 6.64 (2H, d, J 7.6, C(13)H and C(17)H), 6.74 (1H, t, J 7.6, C(15)H), 7.12 (2H, t, J 7.6, C(14)H and C(16)H), 7.22 (1H, t, J 7.4, C(21)H), 7.29 (2H, t, J 7.4, C(20)H and C(22)H), 7.39 (2H, d, J 7.4, C(19)H and C(23)H); m/z (+ve ion electrospray) 388 ([M+Na]$^+$, 100%), 366 ([M+H]$^+$, 35%).

4.10. X-ray crystallographic analysis of (2*Z*,4*E*)-3-hydroxy-6-oxo-octa-2,4-dienoic acid methyl ester (34)

Crystal data for **34**: $C_9H_{12}O_4$, M=184.19, monoclinic, space group $P2_1/c$, Z=4, a=3.970(5), b=25.875(5), c=9.245(5) Å, β=101.507(5)°, U=930.6(13)Å^3, d_{calcd}=1.315 Mg/m^3. Intensity data were collected using a Mo Kα Bruker Apex CCD diffractometer;[28] 5280 reflections were collected, of which 1917 were unique, R_{int}=0.0777. Data processing was carried out using SAINT[29] and the structure was solved by direct methods using SHELXS97.[30] All nonhydrogen atoms were refined anisotropically, and hydrogens were included in calculated positions using the riding method. Refinement on F^2 was carried out using SHELXL97,[30] Final $R1$=0.0410, $wR2$=0.0844 for 1044 data with $I>2\sigma(I)$. All calculations were carried out using the SHELXTL package.[28] Crystallographic data (excluding structure factors) have been deposited in the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 612879. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44 1223 336033 or email: deposit@ccdc.cam.ac.uk].

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