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TETRAHEDRON

Synthesis and Evaluation of a Putative Acyl Tetramic Acid Intermediate in Tenellin Biosynthesis in *Beauveria bassiana*. A New Role for Tyrosine

M. Caragh Moore [1], Russell J Cox[2], Gordon R Duffin[3] and David O'Hagan[1]*

[1]Department of Chemistry, Science Laboratories, South Road, Durham, DH1 3LE.

[2]Current Address, School of Chemistry, University of Bristol, Cantock's Close, Bristol, BS8 1TS.

[3]Isotope Chemistry Laboratories, Sanofi Research Division, Alnwick Research Centre, Willowburn Avenue, Alnwick, Northumberland, NE66 2JH.

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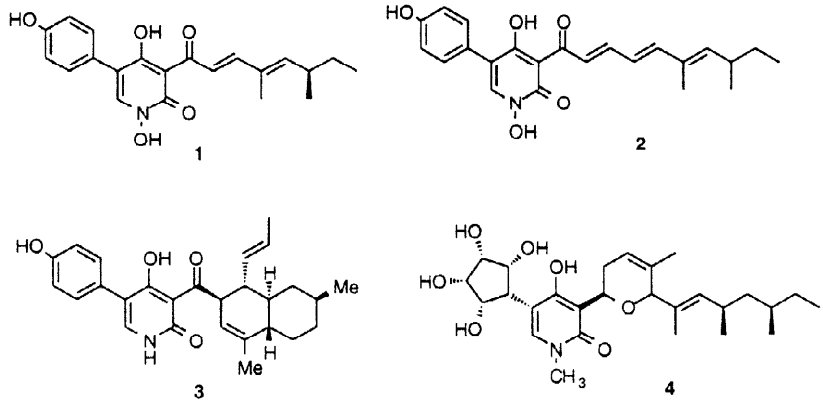
Abstract: The acyltetramic acid **6** previously proposed as a putative intermediate in tenellin biosynthesis in *Beauveria bassiana* has been synthesised in two isotopically labelled forms. This compound was not incorporated into tenellin and was not identifiable in extracts of *B. bassiana* and is unlikely to be involved in tenellin biosynthesis. On the other hand a re-evaluation of the role of tyrosine reveals that it is a good precursor to tenellin and is probably generated *in vivo* by the action of a phenylalanine hydroxylase directly from L-phenylalanine. Thus the long held contention that acyltetramic acid **6** undergoes oxidative ring expansion to a pyridone no longer appears a valid hypothesis for tenellin biosynthesis.

INTRODUCTION

Tenellin **1** is a secondary metabolite of *Beauveria bassiana* (Bals.) Vuill[1] and is one of a class of fungal metabolites which posess a 5-substituted 2-pyridone ring system with an acylated moiety at C-3. Other members of the class are bassianin **2**,[2] ilicicolin-H **3**[3] and funiculosin **4**.[4]



These compounds have been the subject of biosynthesis interest for many years[5,6,7,8,9] and it is established for tenellin **1**[2] and ilicicolin-H **3**[7] that they are of mixed biosynthetic origin being derived from a polyketide chain and an aromatic amino acid. In their early work on tenellin biosynthesis, Wright *et al.*[6] reported an efficient incorporation of L-[1-^{14}C]-phenylalanine into the metabolite but a relatively poor incorporation of L-[U-^{14}C]-tyrosine and drew the conclusion that L-phenylalanine and not L-tyrosine was involved in contributing the aryl ring and C-4 to C-6 of the pyridone ring system. In a subsequent experiment[8] DL-[1,3-$^{13}C_2$]-phenylalanine was

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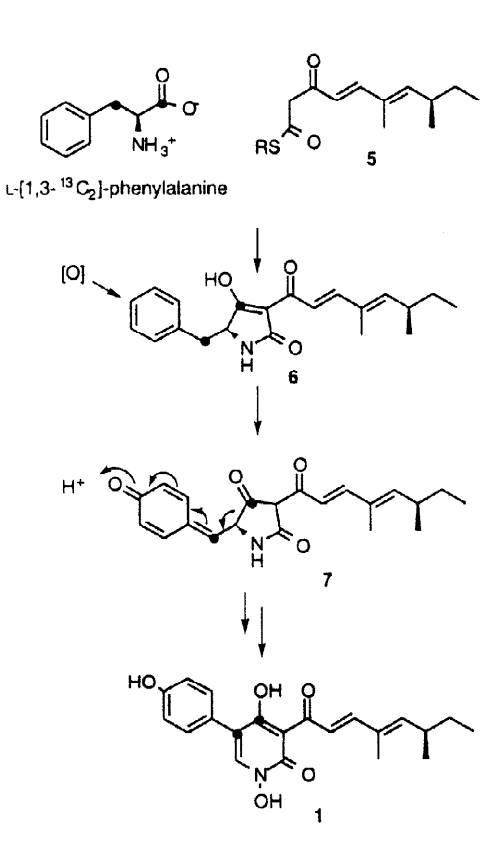
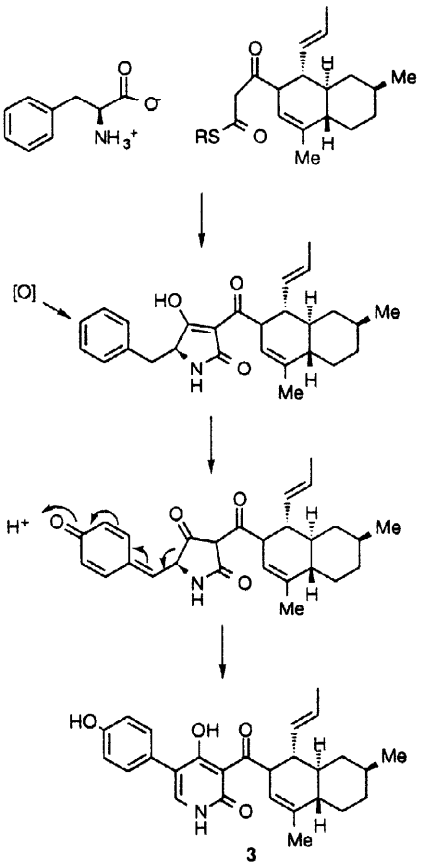
introduced into *B. bassiana* and the isotopic labels became contiguous in the resultant tenellin **1**, indicative of an intramolecular carbon skeletal rearrangement.



Scheme 1



Scheme 2

Hypothesis developed by Wright *et al.*[6] for the origin of tenellin **1** and ilicicolin H **3** involving the oxidation of acyltetramic acids to quininoid intermediates and then ring expansion to the respective pyridones.

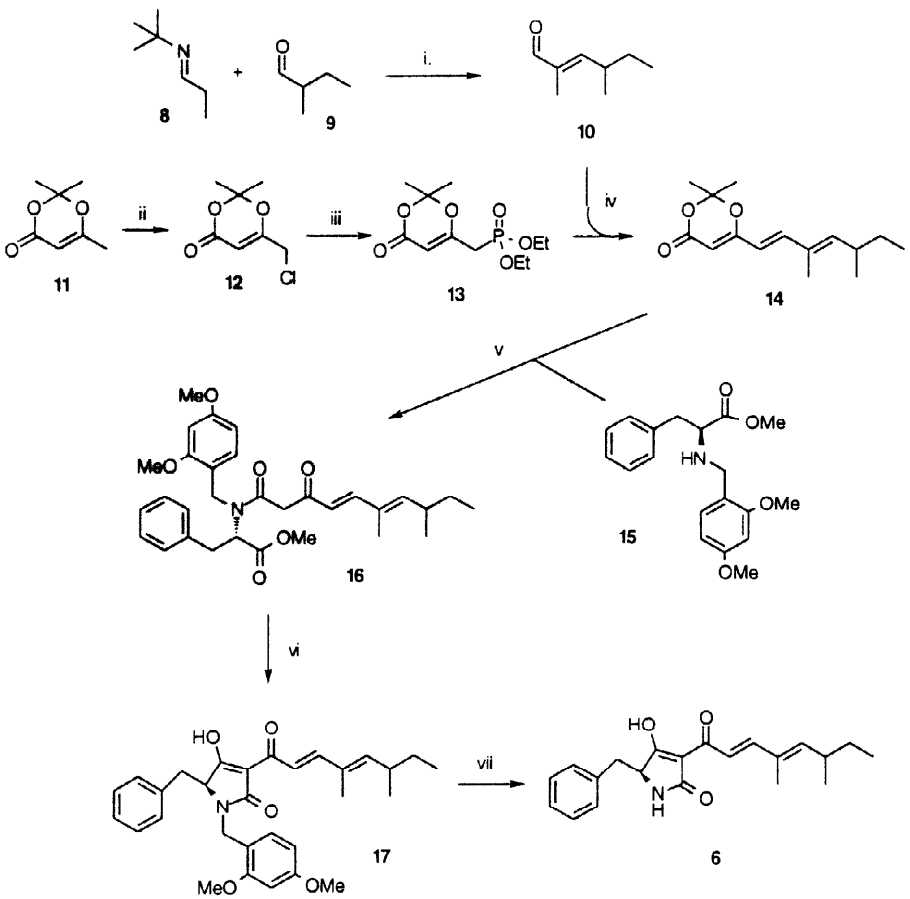
These authors proposed[6] the process shown in Scheme 1 where L-phenylalanine combines with a polyketide moiety to generate the acyltetramic acid intermediate **6** by analogy to the biosynthesis of many known acyl tetramic acids in fungi. Oxidation at the *para* position of the aryl ring, could generate a quinoid intermediate to prime a ring expansion to the pyridone, and satisfy the labelling pattern which emerged from the DL-[1,3-$^{13}C_2$]-phenylalanine experiment. This hypothesis links aryl oxidation with the ring expansion. A biosynthetic study[7] on ilicicolin-H **3** has also demonstrated the incorporation (0.5%) of L-[U-^{14}C]-phenylalanine and DL-[^{15}N]-phenylalanine into **3** and these authors interpreted the results by extending the hypothesis for tenellin **1** to ilicicolin H **3**, as illustrated in Scheme 2.

In order to test this hypothesis, which has emerged as a consensus for the biosynthesis of these metabolites, a synthesis for acyltetramic acid **6** has been developed and the compound prepared, isotopically labelled in separate preparations, with ^{13}C and ^{2}H isotopes. The results of the subsequent feeding experiments with **6** do not support its role as an intermediate in tenellin biosynthesis.

RESULTS AND DISCUSSION

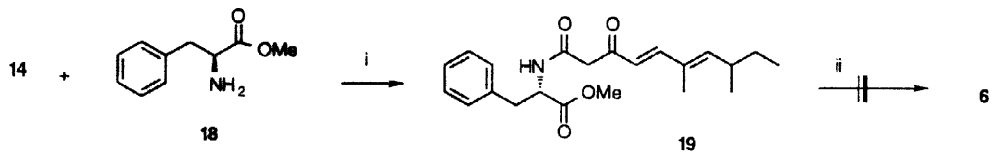
Synthesis

The approach to the construction of the putative acyltetramic acid **6** was based on a biomimetic strategy involving the condensation of an elaborated polyketide moiety **14** with an appropriately protected phenylalanine **15**. The required acetonide **14** was prepared by a Wadsworth-Emmons olefination between *E*-2,4-dimethylhex-2-enal **10** and phosphonate **13**. Aldehyde **10** was prepared as a racemate as previously described[10] by lithiation of imine **8** with BuLi and then reaction with 2-methylbutyraldehyde **9** as shown below in Scheme 3.



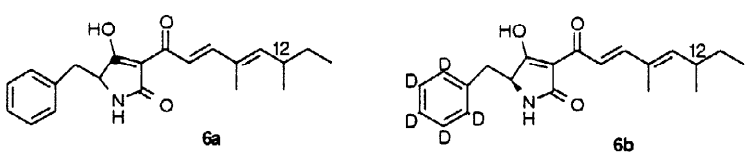
Scheme 3 i. BuLi, THF, -78°C, 95%; ii. LDA, C_2Cl_6, THF, C_2Cl_6,-50°C, 55%, iii. $P(O)OEt_2$, K^tBuO, DMF, 84%; iv. LiHMDS, 0°C, 43%; v. PPTS, toluene, 110°C, 3h, 62%; vi. K^tBuO, ^{t}BuOH, 15 min, 25°C, 90-100%; vii. TFA, 5 min, 95%.

Appropriate work up furnished **10**, a compound which was unstable at room temperature but could be stored at -20°C. The phosphonate **13** was first prepared by Boeckman *et al.*[11] for the synthesis of tetramic acids, permitting Wadsworth-Emmons olefination under mild conditions. A modification of this synthesis was employed which involved lithiation of freshly distilled acetone diketene adduct **11** in THF at -70°C followed by quenching the resultant yellow precipitate with hexachloroethane. In order to maximise the yield of this reaction it proved necessary to transfer the precipitate *via* Teflon tubing and add it dropwise into the hexachloroethane in THF at -50°C. The resultant chlorinated acetone diketene adduct **12** was treated with potassium diethylphosphite in DMF to generate **13**. Following methodology of Jones and Tankard[12], treatment of **13** with lithium hexamethyldisilazide and then reaction with aldehyde **10** gave acetonide **14** which could be purified over silica gel and stored at 0°C until required.



Scheme 4 i. PPTS, toluene, 110°C, 1h, 54%; ii. K^{t}BuO, ^{t}BuOH, 15min.

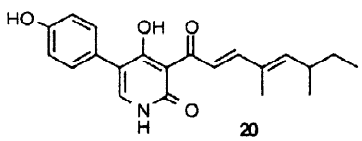
The direct condensation of acetonide **14** and L-phenylalanine methyl ester **18** proved to be straightforward[12] generating the β-ketoamide **19** in moderate yield. However this compound was resistant to Dieckmann cyclisation for generation of **6** under mild conditions affording only the corresponding carboxylic acid as a result of ester hydrolysis. More forcing conditions resulted only in decomposition. In the total synthesis of the acyltetramic acids tirandamycin[13] and ikarugamycin,[14] N-protection was employed for successful cyclisation. Schessinger first described[15] the use of the dimethoxybenzyl group in the preparation of tetramic acids and this appeared an appropriate protecting group to use. Thus N-(2,4-dimethoxybenzyl)phenylalanine methyl ester **15** was prepared by treatment of L-phenylalanine methyl ester with 2,4-dimethoxybenzaldehyde, followed by *in situ* reduction with $NaBH_3CN$. Condensation of **14** and **15** was accomplished under acidic conditions to generate **16** and then Dieckmann cyclisation proceeded smoothly to give the protected acyltetramic acid **17**. In the event, deprotection of **17** was straightforward giving the desired compound **6**, which was fully characterised. Both ^{1}H-NMR and ^{13}C-NMR revealed that this compound was a mixture of two tautomers (85:15) consistent with previous NMR analysis of acyltetramic acids.[16] The synthesis was repeated to deliver the two isotopically labelled forms of the acyltetramic acid **6a** and **6b**. These compounds were prepared from DL-[1-^{13}C]-phenylalanine and L-[*phenyl*-$^2H_5$]-phenylalanine respectively. As a consequence of the racemic labelled amino acid and the epimeric C-12 stereogenic centre, compound **6a** is a mixture of four stereoisomers whereas compound **6b** prepared from the L-amino acid is a mixture of two diastereoisomers.



Biosynthesis studies

After supplementation of *B. bassiana* flasks with [4-^{13}C]-**6a** at a final concentration of 2.0mM and in a minimum volume of ethanol, tenellin production was similar to that in control flasks. There was no apparent problem with secondary metabolite production associated with adding **6a** to the fungal culture. Isolation and HPLC purification of the resultant tenellin gave an analytically pure sample, however there was no evidence for any isotope enrichment at C-4 after ^{13}C-NMR analysis. In order to increase the sensitivity of the analysis, an experiment was repeated with [*phenyl*-$^2H_5$]- **6b**. The natural abundance of deuterium (0.013%) is significantly lower than that of ^{13}C (1.1%) and a lower incorporation of the deuterium labelled precursor should be detectable above natural abundance after ^{2}H-NMR of the isolated tenellin. In the event, the recovered tenellin gave a ^{2}H-NMR spectrum with no evidence of any isotope enrichment in the aryl hydrogens. Negative incorporations always raise the possibility that the putative precursor could not penetrate the cells, and this cannot be discounted. However in an effort to identify **6** in the fungal extract, HPLC analysis of the crude acetone extract

of several of the broth supernatants and various stages of growth and of a mature broth of *B. bassiana* was carried out using synthetic **6** as a reference compound. There was no observable component in the broth or mycelium extracts which co-eluted by HPLC with compound **6**. A single minor metabolite was identifiable by HPLC in this study and was analysed by LC-MS searching for candidate molecular ions by selective ion monitoring. This compound had an ion of 354 (M+1) and a base peak of 230 and corresponded to tenellin minus an oxygen atom. It was clear from the ^{1}H-NMR of a small HPLC purified sample (~4.5mg) of the metabolite that it contained a *para*-substituted aromatic moiety and it is tentatively assigned structure **20**. Compound **20** is either the penultimate precursor to tenellin prior to N-oxidation to a hydroxamic acid, or it is a reduced product generated by metabolism of tenellin **1**.



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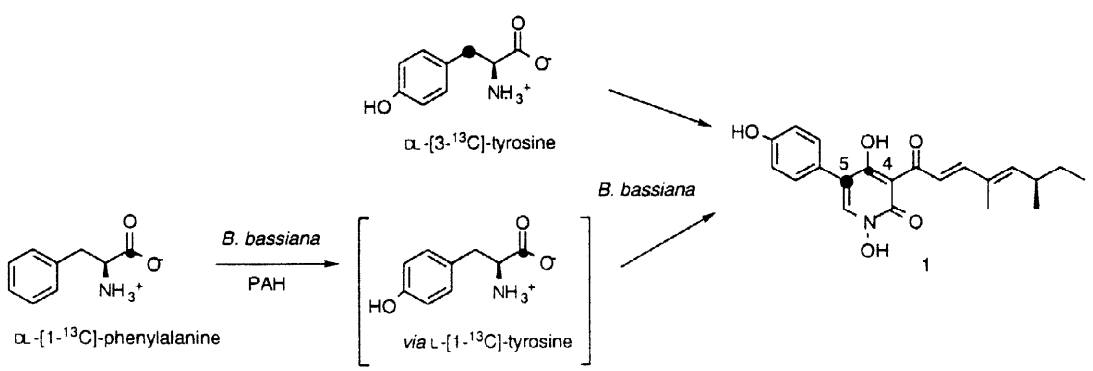
In conclusion there was no evidence for the presence of **6** as a minor metabolite in the *B. bassiana* culture and at the end of this study we are forced to conclude that **6** is not a *bone fide* intermediate in tenellin biosynthesis. This is reinforced by the studies described below.

Tyrosine revisited

The above outcome led us to reconsider a role for L-tyrosine in tenellin biosynthesis, particularly as the early experiments[6] had been carried out only with radiolabelled L-[U-^{14}C]-tyrosine, and it seemed appropriate to repeat this experiment with a [^{13}C]-labelled amino acid. Accordingly the *B. bassiana* medium was supplemented with DL-[3-^{13}C]-tyrosine to a final concentration of 3.5 mM. The resultant tenellin, which was isolated after the experiment, was purified by HPLC. Analyses by ^{13}C-NMR revealed a carbon-13 enrichment (5.1%) of the signal (δ = 110.9 ppm) corresponding to C-5 of tenellin. Thus DL-[3-^{13}C]-tyrosine was efficiently incorporated into tenellin in a regiospecific manner.



Scheme 5 Tyrosine and phenylalanine are both efficiently incorporated into tenellin **1** in *B. bassiana*.

An experiment was then conducted, repeating the incorporation of DL-[1-^{13}C]-phenylalanine at 3.5mM. This led to an enrichment (7.4%) in the resultant ^{13}C-NMR spectrum of the signal (δ = 173.1 ppm) corresponding to C-4 of tenellin **1**. Tyrosine is incorporated approximately 20% less efficiently than phenylalanine (5.1% versus

7.4%), and this observation has been confirmed in three additional comparative feeding experiments.[17] This may be due to differential transport of tyrosine and phenylalanine across membranes and location at the site of biosynthesis in the fungal cells or perhaps there are other less easily defined factors influencing this bias. Nonethe-less, both tyrosine and phenylalanine are efficiently incorporated into tenellin. It is unlikely that tyrosine is converted to phenylalanine and more reasonable that there is a phenylalanine hydroxylase (PAH) activity operating in *B. bassiana* which converts phenylalanine to tyrosine as illustrated in Scheme 5. The observation is clearly consistent with the inability of **6** to become incorporated into tenellin **1**, as the phenolic hydroxyl is introduced from tyrosine and not as a later stage modification of the aryl ring.

These results open up new possibilities for tenellin **1** biosynthesis. For example a *para*-hydroxylated acyltetramic acid, and not **6**, may now emerge as a late intermediate on the biosynthetic pathway. Alternatively tyrosine or a derivative, may be acted upon by a mutase enzyme to generate an isomerised intermediate with the correct connectivity to link up with a polyketide chain and generate a pyridone ring system directly. The delineation of these possibilities is currently being explored in our laboratory.

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EXPERIMENTAL

General Methods

IR Spectra were recorded on a Perkin-Elmer 257 Spectrometer and mass spectra were recorded on a VG-7070E instrument. NMR spectra were obtained on Varian VXR-400(S), Bruker AC-250, Varian XL200 instruments in $CDCl_3$ unless otherwise stated. Chemical shifts are quoted relative to TMS for ^{1}H- and ^{13}C-NMR spectra. All solvents were dried and distilled prior to use, reactions requiring anhydrous conditions were carried out under nitrogen and column chromatography was carried out over silica gel (Merck, Kieselgel 60, 230-400 mesh). A tenellin producing culture of *Beauveria bassiana* (Bals.) Vuill. (No 110.25), was obtained from the CBS culture collection, Oosterstraat, Delft, Netherlands and tenellin was cultured and isolated as previously described.[6,9] HPLC was carried out on a Varian 9012 pump with UV detector and a Hypersil 5 C18 (250 x 5mm, 5μcron) column. A Finnigan Matt MS TSQ700 triple stage spectrometer was used for LCMS analysis.

(1,1-Dimethyl-N-propylidene) ethylamine (8)

Propionaldehyde (29g, 0.5mol) was added dropwise over the course of two hours to *tert*-butylamine (36.5g, 0.5mol), stirred at 0°C. When addition was complete stirring was stopped and KOH pellets (20g) were added. The solution was left to separate at 0°C overnight before the yellow upper layer was carefully decanted. This was distilled three times over KOH at atmospheric pressure and the title compound collected under dry N_2 as a clear colourless oil (25.4g, 224mmol, 44.9%, b.p. 103-105°C, 1 atm), which was stored over molecular sieves, at 0°C. δ_H, 0.96 (3H, t, J 7.6, CH_3), 1.10 (9H, s, 3x CH_3), 2.17 (2H, qd, J 7.6 & 4.9, CH_2), 7.52 (1H, t, J 4.9, CH); δ_c, 11.18 (CH_3), 30.16 (3x CH_3), 55.90 (CH_2), 102.11 (C), 160.62 (CH); ν_{max} (neat)/cm^{-1} 2964, 2933, 2872, 1671, 1461, 1361, 1227, 1215; m/z (EI) 114 (M+1, 18.82%), 113 (M+, 30.20%), 98 (100%).

E-2,4-Dimethyl-2-hexenal (10)

Butyllithium (1.6M in hexanes, 34.5ml, 55mmol) was added dropwise over 10 minutes to a solution of *freshly distilled* **8** (5.65g, 50 mmol) in tetrahydrofuran (30ml) at -78°C *via* dry N_2 flushed syringe. The solution was warmed to 0°C, stirred for 20 min and then cooled to 78°C. 2-Methylbutyraldehyde (3.88g, 45mmol, distilled b.p.92°C, 1atm) was added dropwise *via* a dry N_2 flushed syringe and the solution allowed to warm to 0°C and stirred for 4h at this temperature. An aqueous slurry of oxalic acid (30g in 110ml H_2O) was added and the biphasic mixture was vigorously stirred for 72h at room temperature. The mixture was filtered and the filtrate extracted into dichloromethane (3x75ml). The combined organic layers were dried ($MgSO_4$) and evaporated *in vacuo* to yield the crude aldehyde as a pale yellow aromatic oil (5.42g, 42.9mmol, 95.4%) and the product was purified *immediately* by chromatography (CH_2Cl_2). The oil was stable for a few days at 20°C under dry N_2, but needed to be repurified prior to use. δ_H, 0.84 (3H, t, J 7.3, CH_3), 1.02 (3H, d, J 6.6, CH_3), 1.40 (2H, m, CH_2),

1.71 (3H, d, J 1.4, CH_3), 2.58 (1H, m, CH), 6.22 (1H, dq, J 9.9 & 1.4, CH), 9.36 (1H, s, CHO); δ_c, 11.3 (CH_3), 13.8 (CH_3), 21.4 (CH_3), 31.4 (CH_2), 37.1 (CH), 140.1 (C), 162.2 (CH), 197.4 (CO); ν_{max} (neat)/cm^{-1} 2962, 2929, 2874, 2817, 2707, 1689, 1640, 1458, 734.35; m/z (EI) 126 (M+, 10.88%), 41 (100%); Found 126.1040, $C_8H_{14}O$ (M+) requires 126.1045.

2,2-Dimethyl-6-chloromethyl-1,3-dioxin-4-one (12)

Freshly distilled **11** (7.1g, 50 mmol, b.p. 36°C, 0.03 mbar) was added dropwise to a solution of LDA (50mmol) stirred at -78°C. A bright yellow suspension formed which was stirred for 15 minutes. It was then carefully transferred dropwise, over a period of 30 minutes, *via* dry Teflon tubing to a stirred solution of hexachloroethane (17.7g, 75 mmol), in tetrahydrofuran (30 ml), which was cooled to -50°C under dry N_2. The red solution thus formed was allowed to warm slowly to -20°C over 1 hour, after which the reaction mixture was quenched by addition of ice cold dilute aqueous HCl (150 ml). The acidic solution was shaken to dispel colour and extracted into diethyl ether (3x 100ml). The combined ether layers were washed with a saturated aqueous solution of $NaHCO_3$ (2x 50ml), dried ($MgSO_4$), and reduced *in vacuo* to afford a yellow oil containing crystalline hexachloroethane. This was removed by trituration with hexane (2x 50 ml). The product could be purified by chromatography (CH_2Cl_2) (4.9g, 27.7mmol, 55.5%), but was routinely used without further purification. δ_H, 1.66 (6H, s, 2x CH_3), 3.99 (2H, s, CH_2), 5.50 (1H, s, CH); δ_c, 26.7 (2x CH_3), 43.0 (CH_2Cl), 97.5 (CH), 109.4 (C), 162.3 (C), 166.6 (CO); ν_{max} (neat)/cm^{-1} 3102, 3000, 2945, 1729, 1642, 1391, 1377, 1274, 1203, 1015.

2,2-Dimethyl-6-(diethylphosphonomethyl)-1,3-dioxin-4-one (13)

Diethylphosphite (20.7g, 150 mmol, distilled b.p. 27°C, 0.001mbar) was added dropwise to a solution of potassium *tert*-butoxide (16.8g, 150 mmol) in DMF (100 ml), cooled to 0°C. After 30 minutes, 2,2-dimethyl-6-chloromethyl-1,3-dioxin-4-one **12** (5.50g, 31.14mmol) in DMF (25ml) was added dropwise over 20 minutes, and the deep purple solution generated was stirred at 0°C for a further 30 minutes. Dropwise addition of concentrated aqueous HCl (6ml) discharged the colour, and the pale brown mixture was filtered through celite, and the celite pad washed with diethyl ether (100ml). The combined organic washings were evaporated *in vacuo* keeping the bath temperature below 40°C. Excess dimethylformamide and diethyl phosphite were removed by vacuum distillation (0.01 mbar, < 45°C) affording the crude product as a thick brown oil, which was diluted with ethyl acetate and left at 0°C for 12h. The solution was decanted from the crystals thus formed, and reduced *in vacuo*. Purification by chromatography (ethyl acetate, R_f 0.19) yielded the title compound as a pale yellow oil (7.27g, 26.13mmol, 83.9%) which was stored under N_2 at 0°C. δ_H, 1.28 (6H, t, J 7.0, 2x CH_3), 1.65 (6H, s, 2x CH_3), 2.75 (2H, d, J 22.5, CH_2), 4.07 (4H, m, 2x CH_2) 5.33 (1H, d, J 3.7, CH); δ_c, 16.2 (d, J_{13CP} 6.57, 2x CH_3), 24.5 (2x CH_3), 31.0 (d, J_{13CP} 132.00, CH_2), 61.90 (d, J_{13CP} 6.58, 2x CH_2), 95.5 (d, J_{13CP} 8.81, CH), 106.6 (C), 159.9 (C), 164.25 (CO); ν_{max} (neat)/cm^{-1} 3400 (br), 2984, 2933, 2910, 1729, 1633, 1443, 1375, 1258, 1024.

2,2-Dimethyl-6-(*E,E*-3,5-dimethylhepta-1,3-dienyl)-1,3-dioxin-4-one (14)

To a solution of LHMDS (15 mmol) in tetrahydrofuran (100ml), cooled to 0°C, and stirred under dry N_2 was added a solution of 2,2-dimethyl-6-(diethylphosphonomethyl)-1,3-dioxin-4-one (4.17g, 15mmol), in THF (30ml) *via* a dry N_2 flushed syringe. The red solution was stirred for 20 minutes before cooling to -78°C. Freshly purified **10** (1.89g, 15mmol) was added and the solution allowed to warm to room temperature over a period of 4 hours, followed by stirring for a further 8 hours at this temperature. Solvent was removed by evaporation *in vacuo* (bath temperature below 40°C), and the resulting red solid dissolved in dichloromethane (30ml). Colloidal inorganics were removed by vacuum filtration through glass wool, and the filtrate reduced *in vacuo*. The title compound was purified by chromatography (dichloromethane, R_f0.21) to give a pale yellow oil (1.59g, 6.37mmol, 42.5%). δ_H, 0.80 (3H, t, J 7.0, CH_3), 0.94 (3H, d, J6.25, CH_3), 1.32 (2H, m, CH_2) 1.66 (6H, s, 2x CH_3), 1.75 (3H, s, CH_3), 2.41 (1H, m, CH), 5.25 (1H, s, CH), 5.60 (1H, d, J 9.0, CH), 5.84 (1H, d, J 14.5, CH), 6.92 (1H, d, J 14.5, CH); δ_c, 11.9 (CH_3), 12.3 (CH_3), 20.2 (CH_3), 25.0 (CH_3), 25.0 (CH_3), 30.0 (CH_2), 34.9 (CH), 93.5 (CH), 106.1 (C), 117.0 (CH), 131.7 (C), 143.3 (CH), 148.0 (CH), 162.1 (C), 164.1 (CO); ν_{max} (neat)/cm^{-1} 2960, 2926, 2871, 1720, 1623, 1387, 1374, 1271, 1203, 1017; m/z (CI) 251 (44.2%), 167 (100%); Found 251.1647, $C_{15}H_{23}O_3$ (MH+) requires 251.1647.

L-N-(2,4-Dimethoxybenzyl) phenylalanine methyl ester (15)

Methanolic HCl was added to a stirred solution of L-phenylalanine methyl ester (4.33g, 24.16mmol) in methanol (100ml) to adjust to pH 6. 2,4-Dimethoxybenzaldehyde (4.82g, 29.00mmol) was then added, the solution stirred at 20°C for 30 min and then $NaBH_3CN$ (2.20g, 35.01mmol) was added and the reaction stirred for a further 16h.

The solvent was removed *in vacuo,* water (50ml) added and the solution extracted into diethyl ether (3x 100ml). The organic extracts were combined, washed with an aqueous solution of $FeSO_4$, dried ($MgSO_4$), filtered and reduced *in vacuo,* to afford a crude product as a pale yellow oil. Impurities of 2,4-dimethoxybenzylalcohol were removed by distillation under reduced presure (furnace temparature 110°C, 0.1mmHg). The product could be further purified by chromatography (hexane: ethylacetate, 50 : 50). (3.71g, 11.26mmol, 46.6%) $[\alpha]_D^{21}$ = -65.22 (c = 0.01, EtOH). $\delta_H(CDCl_3)$ 2.14 (1H, bs, NH), 2.89 (1H, dd, J_{gem} 13.8, J_{vic} 7.8, CH_2), 2.97 (1H, dd, J_{gem} 13.8, J_{vic} 6.5, CH_2), 3.49 (1H, dd, J_{vic} 7.8, J_{vic} 6.5, CH), 3.59 (3H, s, $COOCH_3$), 3.61 (3H, s, OCH_3), 3.76 (3H, s, OCH_3), 3.79 (2H, d, J 8.1, CH_2), 6.39 (2H, m, Ph), 7.16 (6H, m, Ph); $\delta_c(CDCl_3)$ 39.5, (CH_2), 47.1 (NCH_2), 51.5 ($COO\underline{C}H_3$), 55.0 (OCH_3), 55.2 (OCH_3), 61.8 (CH), 98.2 (Ph CH), 103.4 (Ph CH), 119.8 (Ph CH), 126.5 (Ph CH), 128.3 (Ph CH), 129.0 (Ph CH), 130.3 (Ph C), 137.3 (Ph C), 158.5 (Ph CO), 160.1 (Ph CO), 174.7 (CO); Found 329.1627, $C_{19}H_{23}NO_4$ (M^+) requires 329.1627.

DL-[1-^{13}C]-N-(2,4-Dimethoxybenzyl) phenylalanine methyl ester (15a); was prepared as **15** from DL-[1-^{13}C]-phenylalanine methyl ester (0.598g, 3.32mmol) to give **15a** (0.877g, 2,65mmol, 80.4%). $\delta_H(CDCl_3)$ 2.20 (1H, bs, NH), 2.95 (2H, m, CH_2), 3.50 (1H, m, CH), 3.59 (3H, d, J_{H13C} 3.7, $COOCH_3$), 3.61 (3H, s, OCH_3), 3.74 (3H, s, OCH_3), 3.70 (2H, d, J 9.0, CH_2), 6.35 (2H, m, Ph), 7.17 (6H, m, Ph); $\delta_c(CDCl_3)$ 41.61, (CH_2), 49.26 (NCH_2), 53.58 (d, J 2.87, $COO\underline{C}H_3$), 57.06 (OCH_3), 57.28 (OCH_3), 63.81 (d, J 59.2, CH), 100.34 (Ph CH), 105.56 (Ph CH), 121.99 (Ph CH), 128.59 (Ph CH), 130.37 (Ph CH), 131.16 (Ph CH), 132.38 (Ph C), 139.46 (Ph C), 160.59 (Ph CO), 162.17 (Ph CO), 176.85 (CO, enriched); ν_{max} (neat)/cm^{-1} 3334 (br), 3026, 3000, 2948, 2835, 2250 (w), 2064 (w), 1693, 1613, 1588, 1506, 1463, 1289, 1261, 1208, 1156, 1036, 733, 700; m/z (EI)

L-[C_6D_5]-N-(2,4-Dimethoxybenzyl) phenylalanine methyl ester (15b); was prepared as **15** from L-[C_6D_5]-phenylalanine methyl ester (0.919g, 4.99mmol) to give **15b**; (1.459g, 4.36mmol, 87.5%) $[\alpha]_D^{21}$ =-69.23 (c = 0.01, EtOH). $\delta_H(CDCl_3)$ 2.12 (1H, bs, NH), 2.89 (1H, dd, J_{gem} 13.5, J_{vic} 7.6, CH_2), 2.97 (1H, dd, J_{gem} 13.5, J_{vic} 6.2, CH_2), 3.49 (1H, dd, J_{vic} 7.6, J_{vic} 6.26, CH), 3.59 (3H, s, $COOCH_3$), 3.61 (3H, s, OCH_3), 3.74 (3H, s, OCH_3), 3.80 (2H, d, J 8.1 CH_2), 6.37 (2H, m, Ph), 7.00 (1H, d, J 8.1, Ph); $\delta_c(CDCl_3)$ 39.4, (CH_2), 47.2 (NCH_2), 51.5 ($COO\underline{C}H_3$), 55.0 (OCH_3), 55.24 (OCH_3), 61.9 (CH), 98.3 (Ph CH), 103.4 (Ph CH), 119.8 (Ph CH), 127.4-129.0 (m, Ph CD), 130.3 (Ph C), 137.1 (Ph C), 158.5 (Ph CO), 160.1 (Ph CO), 174.7 (CO); ν_{max} (neat)/cm^{-1} 3335 (br), 2998, 2949, 2836, 2362 (w), 2273 (w), 1733, 1612, 1588, 1505, 1460, 1206, 1155, 1035; m/z (EI) 333 (M-1, 1.98%).

L-N-(2,4-dimethoxybenzyl)-N-(*E,E*-3-oxo-6,8-dimethyldeca-4,6-dienoyl) phenylalanine methyl ester (16)
To a solution of **14** (0.261g, 1.04mmol) and L-N-(2,4-dimethoxybenzyl)-phenylalanine methyl ester (0.412g, 1.25mmol) in toluene (6ml) was added pyridinium para-toluene sulfonate (0.261g, 1.04mmol), and the mixture heated to reflux temperature for 3 hours with stirring. After cooling the solvent was removed *in vacuo* and the resultant red oil purified by silica gel flash chromatography (petroleum ether : ethyl acetate, 6 : 4, R_f 0.51) yielding the title compound as a yellow oil (0.339g, 0.650mmol, 62.5%). NMR spectra were complicated by the presence of both the enol and keto forms. δ_H, 0.83 (3H, 2x t, J 7.4, CH_3), 0.96 (3H, 2x d, J 6.4, CH_3), 1.30 (2H, m, CH_2), 1.72, 1.79 (3H, 2x s, CH_3), 2.42 (1H, m, CH), 3.11, 3.37 (2H, m, CH_2), 3.56, 3.59 (3H, 2x s, $COO\underline{C}H_3$), 3.72, 3.73 (3H, s, OCH_3), 3.76, 3.77 (3H, s, OCH_3), 3.94, 4.34 (2H, m, CH_2), 4.09, 4.50 (1H, m, CH), 5.15 (1H, s, $CO\underline{C}H_2CO$), 5.54, 5.77 (1H, 2x d, J 9.6, CH), 5.72, 6.21 (1H, 2x d J 15.6, CH), 6.35 (2H, m, Ph), 7.16 (7H, m, Ph & CH), 14.2 (0.5H, bs, OH) ; δ_c, 11.9, 12.4 (CH_3), 20.1, 20.3 (CH_3), 30.0, 30.12 (CH_2), 34.8, 35.1 (CH), 35.2, 35.5 (CH_2), 46.5, 47.8, 48.7 (CH_2), 52.0, 52.1 ($COO\underline{C}H_3$), 55.1 (OCH_3), 55.3 (OCH_3), 60.2, 60.5 (CH), 89.4 ($CO\underline{C}H_2CO$), 98.2, 98.5 (Ph CH), 103.7 (Ph CH), 116.0, 116.6 (Ph C), 120.4, 123.6 (CH), 126.4 (Ph CH), 128.3, 128.5 (Ph CH), 129.3, 129.4 (Ph CH), 130.5, 129.1 (Ph CH), (Ph CH), 131.8, 132.0 (Ph C), 138.0, 138.3 (C), 141.2, 150.0 (CH), 145.6, 150.6 (CH), 157.8 (Ph $\underline{C}OCH_3$), 158.7 (Ph $\underline{C}OCH_3$), 160.3, 160.9 (CON), 170.2, 171.3 ($\underline{C}OOCH_3$), 172.9 (C=$\underline{C}$OH), 193.8 (CO). ν_{max} (neat)/cm^{-1} 2957, 2927, 2871, 2360, 1697, 1629, 1579, 1465, 1456, 1208, 529; m/z (CI) 522 (M+1, 3.51%), 330 (100%); Found 522.2855, $C_{31}H_{40}NO_6$ (MH+) requires 522.2856.

DL-[1-^{13}C]-N-(2,4-dimethoxybenzyl)-N-(*E,E*-3-oxo-6,8-dimethyldeca-4,6-dienoyl) phenylalanine methyl ester (16a)
To a solution of **14** (0.409g, 1.63mmol) and DL-[1-^{13}C]-N-(2,4-dimethoxybenzyl)-phenylalanine methyl ester (0.628g, 1.90mmol) in toluene (15ml) was added pyridinium para-toluene sulfonate (0.415g, 1.65mmol), and the mixture heated to reflux temperature for 3 hours with stirring. After cooling the solvent was removed *in vacuo* and the resultant red oil purified by silica gel flash chromatography (petroleum ether : ethyl acetate, 6 : 4, R_f 0.51) yielding the title compound as a yellow oil (0.323g, 0.618mmol, 37.9%). The NMR spectra were

complicated by the presence of both the the enol and keto forms. δ_H, 0.85 (3H, 2x t, J 7.4, CH_3), 0.98 (3H, 2x d, J 6.7, CH_3), 1.30 (2H, m, CH_2), 1.74 1.82 (3H, 2x s, CH_3), 2.45 (1H, m, CH), 3.14, 3.39 (2H, m, CH_2), 3.56, 3.59 (3H, 2x d, J_{H13C} 3.9, $COO\underline{CH}_3$), 3.74, 3.75 (3H, 2x s, OCH_3), 3.78, 3.79 (3H, 2x s, OCH_3), 4.09, 4.40 (1H, m, CH), 5.14 (1H, s, $CO\underline{CH}_2CO$), 5.56, 5.79 (1H, 2x d, J 9.6, CH), 5.74, 6.24 (1H, 2x d J 15.6, CH), 6.39 (2H, m, Ph), 7.20 (7H, m, Ph & CH), 14.0 (0.5H, bs, OH); δ_c, 11.8 12.19 (CH_3), 20.0, 20.2 (CH_3), 29.8, 30.0 (CH_2), 34.6, 35.0 (CH), 35.0, 35.4 (CH_2), 46.5, 47.6, 48.7 (CH_2), 51.8, 52.0 ($\underline{C}OOCH_3$), 54.9, 55.0 (OCH_3), 55.2, 55.2 (OCH_3), 60.2, 61.4 (2x d, J 67.0, CH) 89.4 ($CO\underline{CH}_2CO$), 98.04, 98.4 (Ph CH), 103.5, 103.6 (Ph CH), 115.8, 116.4 (Ph C), 120.3, 123.5 (CH), 126.3, 126.2 (Ph CH), 128.2, 128.4 (Ph CH), 129.1, 129.3 (Ph CH), 130.4, 129.1 (Ph CH), 131.7, 131.9 (Ph C), 137.9, 138.2 (C), 141.1, 149.8 (CH), 145.4, 150.5 (CH), 157.7 (Ph $\underline{C}OCH_3$), 158.6 (Ph $\underline{C}OCH_3$), 160.2, 160.8 (CON), 170.1, 170.6 ($\underline{C}OOCH_3$, enriched), 176.6 (C=$\underline{C}$OH), 193.7 (CO); ν_{max} (neat)/cm^{-1} 2957, 2927, 2871, 2360, 1697, 1629, 1579, 1465, 1456, 1208, 529; m/z (CI) 523 (M+1, 17.34%), 331 (100%).

L-[C_6D_5]-N-(2,4-dimethoxybenzyl)-N-(*E,E*-3-oxo-6,8-dimethyldeca-4,6-dienoyl) phenylalanine methyl ester (16b)

To a solution of **14** (0.851g, 3.40mmol) and L-[C_6D_5]-N-(2,4-dimethoxybenzyl)-phenylalanine methyl ester (1.34g, 4.00mmol) in toluene (15ml) was added pyridinium para-toluene sulfonate (0.851g, 3.40mmol), and the mixture heated to reflux temperature for 3 hours with stirring. After cooling the solvent was removed *in vacuo* and the resultant red oil purified by silica gel flash chromatography (petroleum ether : ethyl acetate, 6 : 4 R_f 0.51) yielding the title compound as a yellow oil (0.947g, 1.80mmol, 52.9%). The NMR spectra were complicated by the presence of both the enol and keto forms. δ_H 0.83 (3H, 2x t, J 7.4, CH_3), 0.96 (3H, 2x d, J 6.4, CH_3), 1.31 (2H, m, CH_2), 1.72 1.80 (3H, 2x s, CH_3), 2.42 (1H, m, CH), 3.11, 3.37 (2H, m, CH_2), 3.56, 3.59 (3H, 2x s, $COO\underline{CH}_3$), 3.72, 3.73 (3H, s, OCH_3), 3.76, 3.77 (3H, s, OCH_3), 3.95, 4.32 (2H, m, CH_2), 4.10, 4.51 (1H, m, CH), 5.14 (1H, s, $CO\underline{CH}_2CO$), 5.54, 5.77 (1H, 2x d, J 9.6, CH), 5.73, 6.21 (1H, 2x d J 15.6, CH), 6.35 (2H, m, Ph), 6.91, 6.97 (1H, 2x d, J 8.6, Ph), 7.06, 7.29 (1H, 2x d, J 15.6, CH), 14.0 (0.5H, bs, OH); δ_c, 11.9, 12.4 (CH_3), 20.0, 20.3 (CH_3), 29.9, 30.1 (CH_2), 34.7, 35.1 (CH), 35.1, 35.4 (CH_2), 46.5, 47.7, 48.7 (CH_2), 52.0, 52.1 ($COO\underline{C}H_3$), 55.1, 55.1 (OCH_3), 55.2, 55.3 (OCH_3), 60.2, 60.5 (CH), 89.4 ($CO\underline{CH}_2CO$), 98.2 (Ph CH), 98.5 (Ph CH), 103.6 (Ph CH), 116.0, 116.6 (Ph C), 120.4, 123.5 (CH), 129.2, 130.4 (Ph CH), 131.8, 132.0 (Ph C), 137.8, 138. (C), 141.2, 149.9 (CH), 145.6, 150.6 (CH), 157.8 (Ph $\underline{C}OCH_3$), 158.7 (Ph $\underline{C}OCH_3$), 160.3, 160.9 (CON), 170.2, 171.3 ($\underline{C}OOCH_3$), 172.9 (C=$\underline{C}$OH), 193.8 (CO); ν_{max} (neat)/cm^{-1} 2961, 2933, 2871, 2362, 1738, 1613, 1583, 1506, 1454, 1496, 1206, 1156, 1118, 1031; m/z (CI) 527 (M+1, 6.85%), 335 (100%).

5-L-N-(2,4-Dimethoxybenzyl)-3-(*E,E*-4,6-dimethylocta-2,4-dienoyl)-5-benzyl-2,4-dioxopyrrolidine (17)

To a stirred solution of freshly sublimed potassium *tert*-butoxide (0.165ml of 1M solution in *tert*-butanol, 0.615mmol) in *tert*-butanol (7ml) under nitrogen, was added a solution of **16** (0.161g, 0.31mmol) in *tert*-butanol (5ml). After 15 mins stirring the red solution was quenched with dilute aqueous hydrochloric acid (5ml). The organics were extracted into diethyl ether (1x 30ml), washed with water (1x 5ml), dried ($MgSO_4$) and evaporated *in vacuo* to give afford the title compound as a yellow oil (0.152g, 0.31mmol, 100%). Two tautomers in the ratio 84 : 16 were observed in the NMR spectra. δ_H, 0.82, 0.83 (3H, 2x t, J 7.5, CH_3), 0.97 (3H, d, J 6.5, CH_3), 1.31 (2H, m, CH_2), 1.84 (16%), 1.88 (84%) (3H, d, J 1.01, CH_3), 2.30 (16%), 2.50 (84%) (1H, m, CH), 3.14 (2H, d, J 4.6, CH_2), 3.72 (3H, s, OCH_3), 3.76 (3H, s, OCH_3), 3.85 (84%), 3.99 (16%), (1H, t, J 4.3, CH), 4.14 (84%), 4.21(16%) (1H, d, J_{gem} 14.6, CH_2), 4.97 (84%), 4.98 (16%) (1H, d, J_{gem} 14.8, CH_2), 5.78 (1H, d, J 10.0, CH), 6.41 (2H, m, Ph), 7.13 (7H, m, Ph & CH), 7.42 (1H, d, J 15.9, CH); δ_c, 11.9 (CH_3), 12.4 (CH_3), 20.0 (CH_3), 29.9 (CH_2), 35.2 (CH), 35.2 (CH_2), 38.4 (CH_2), 55.3 (OCH_3), 55.4 (OCH_3), 62.6 (16%), 64.9 (84%), (CH), 98.4 (Ph CH), 100.3 (C), 104.3 (Ph CH), 116.0 (CH), 116.1 (Ph C), 126.8 (Ph CH), 128.3 (Ph CH), 129.5 (Ph CH), 131.3 (Ph CH), 133.0 (Ph C), 135.7 (C), 149.5 (CH), 151.5 (CH), 158.5 (Ph C), 160.8 (Ph C), 173.8 (CON & COH), 194.4 (84%), 204.6 (16%) (CO). ν_{max} (neat)/cm^{-1} 3028, 2960, 2926, 2871, 2836, 2360 (w), 2341 (w), 1694, 1614, 1570, 1507, 1454, 1293, 1261, 1208, 1036, 613; m/z (CI) 490 (M+1, 9.14%), 151 (100%); Found 490.2593, $C_{30}H_{36}NO_5$ (MH^+) requires 490.2593.

5-DL-[4-^{13}C]-N-(2,4-Dimethoxybenzyl)-3-(*E,E*-4,6-dimethylocta-2,4-dienoyl)-5-benzyl-2,4-dioxopyrrolidine (17a)

To a stirred solution of freshly sublimed potassium *tert*-butoxide (0.139g, 1.24mmol) in *tert*-butanol (20ml) under nitrogen, was added a solution of **16a** (0.323g, 0.618mmol) in *tert*-butanol (10ml). After 30 mins stirring the red solution was quenched with dilute aqueous hydrochloric acid (5ml). The organics were extracted into diethyl ether (1x 50ml), washed with water (1x 10ml), dried ($MgSO_4$) and evaporated *in vacuo* to give afford the title compound as a yellow oil (0.298g, 0.61mmol, 98.7%). Two tautomers in the ratio 84 : 16 were observed in

the NMR spectra. δ_H, 0.75 (3H, 2x t, J 7.3, CH_3), 0.90 (3H, d, J 6.6, CH_3), 1.30 (2H, m, CH_2), 1.76 (16%), 1.79 (84%) (3H, s, CH_3), 2.40 (1H, m, CH), 3.08 (2H, dd, J 4.7 J_{H13C} 4.7, CH_2), 3.68 (3H, s, OCH_3), 3.69 (3H, s, OCH_3), 3.78 (84%), 3.94 (16%) (1H, m, CH), 4.08 (84%) 4.16 (16%) (1H, d, J_{gem} 14.7, CH_2), 4.90 (84%), 4.96 (16%) (1H, d, J_{gem} 14.9, CH_2), 5.70 (1H, d, J 9.9, CH), 6.34 (2H, m, Ph), 7.12 (7H, m, Ph & CH), 7.36 (1H, d, J 15.6, CH); δ_c, 14.0 (CH_3), 14.5 (CH_3), 22.1 (CH_3), 32.0 (CH_2), 37.1 (CH), 37.3 (CH_2), 40.5 (CH_2), 57.4 (2x OCH_3), 64.6 (16%), 66.4 (84%) (d, J 40.5, CH), 100.4 (Ph CH), 102.4 (d, J 63.4, C), 106.4 (Ph CH), 118.0 (CH), 118.2 (Ph C), 128.8 (Ph CH), 130.4 (Ph CH), 131.6 (Ph CH), 133.3 (Ph CH), 135.0 (Ph C), 137.7 (C), 151.6 (CH), 153.5 (CH), 160.6 (Ph C), 162.8 (Ph C), 174.4 (dd, CON) 175.9 (d, COH), 196.1 (84%) 204.8, (16%) (CO, enriched), 209.2 (84%), 211.4 (16%) (CO); ν_{max} (neat)/cm^{-1} 2958, 2924, 2871, 1664, 1607, 1560, 1507, 1450, 1290, 1207, 1157, 1035, 612, 490.

L-5-[C_6D_5]-N-(2,4-Dimethoxybenzyl)-3-(*E,E*-4,6-dimethylocta-2,4-dienoyl)-5-benzyl-2,4-dioxopyrrolidine (17b)

To a stirred solution of freshly sublimed potassium *tert*-butoxide (2.91ml of 1M solution in *tert*-butanol, 2.92mmol) in *tert*-butanol (30ml) under nitrogen, was added a solution of **16b** (0.792g, 1.46mmol) in *tert*-butanol (20ml). After 15 min stirring the red solution was quenched with dilute aqueous hydrochloric acid (10ml). The organics were extracted into diethyl ether (1x 60ml), washed with water (1x 15ml), dried ($MgSO_4$) and evaporated *in vacuo* to give afford the title compound as a yellow oil (0.722g, 1.46mmol, 99.7%). Two tautomers in the ratio 84 : 16 were observed in the NMR. δ_H, 0.82, 0.83 (3H, 2x t, J 7.3, CH_3), 0.97 (3H, d, J 6.7, CH_3), 1.30 (2H, m, CH_2), 1.84 (16%), 1.86 (84%) (3H, d, J 0.81, CH_3), 2.44 (1H, m, CH), 3.14 (2H, d, J 4.9, CH_2), 3.75 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 3.85(84%), 3.99 (16%) (1H, t, J 4.7, CH), 4.14 (84%), 4.22 (16%) (1H, d, J_{gem} 14.6, CH_2), 4.96 (84%), 5.05 (16%) (1H, d, J_{gem} 14.9, CH_2), 5.77 (1H, d, J 9.7, CH), 6.41 (2H, m, Ph), 7.01 (1H, d, J 9.7, Ph), 7.04 (1H, d, J 15.4, CH), 7.42 (84%), 7.43 (16%) (1H, d, J 15.4, CH); δ_c, 11.9 (CH_3), 12.4 (CH_3), 20.0 (CH_3), 30.0 (CH_2), 35.1 (CH), 35.2 (CH_2), 38.4 (CH_2), 55.3 (OCH_3), 55.4 (OCH_3), 62.6 (16%), 64.9 (84%) (CH), 98.4 (Ph CH), 100.3 (C), 104.3 (Ph CH), 116.0 (CH), 116.1 (Ph C), 127.5-129.4 (m, 3x Ph CD) 131.3 (Ph CH), 133.0 (Ph C), 135.5 (C), 149.5 (CH), 151.5 (CH), 158.5 (Ph C), 160.8 (Ph C), 173.8 (CON) 174.7 (COH), 194.4 (84%) 202.8 (16%) (CO); ν_{max} (neat)/cm^{-1} 2959, 2928, 2873, 2269, 1608, 1564, 1506, 1449, 1289, 1261, 1206, 1155, 1099; m/z (CI) 495 (M+1, 8.34%), 151 (100%).

L-5-3-(*E,E*-4,6-Dimethylocta-2,4-dienoyl)-5-benzyl-2,4-dioxopyrrolidine (6)

Compound **17** (0.129g, 0.263mmol) was dissolved in trifluoroacetic acid (5ml) and the bright red solution stirred under nitrogen for 5 min. The reaction mixture was quenched with ice, resulting in the formation of a pale yellow precipitate. This was extracted into dichloromethane (2x 50ml), washed with saturated aqueous $NaHCO_3$ solution, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was redissolved in methanol (6ml), and filtered to remove white solid impurities. The filtrate was reduced *in vacuo* to give the title compound as a waxy yellow solid (84.5mg, 0.249mmol, 94.7%). Two tautomers in the ratio 85 : 15 were observed in the NMR spectra. δ_H, 0.84 (3H, t, J 7.3, CH_3), 1.00 (85%), 1.11(15%) (3H, d, J 6.2, CH_3), 1.31 (2H, m, CH_2), 1.86 (15%) 1.90 (85%) (3H, s, CH_3), 2.49 (1H, m, CH), 2.65 (1H, m, CH_2), 3.26 (1H, m, CH_2), 3.99 (85%), 4.10(15%) (1H, bm, CH), 5.66 (15%), 5.85 (85%) (1H, d, J 9.7, CH), 6.32 (1H, bs, NH), 7.12 (1H, J 15.6, CH), 7.22 (5H, bm, Ph), 7.52 (85%), 7.96 (15%) (1H, d, J 15.4, CH); δ_c, 11.9 (CH_3), 12.3 (CH_3), 20.0 (CH_3), 29.9 (CH_2), 35.3 (CH), 38.2 (CH_2), 61.0 (15%), 63.5 (85%) (CH), 99.4 (C), 115.7 (CH), 127.0 (Ph CH), 128.7 (Ph CH), 129.1 (Ph CH), 133.2 (Ph C), 136.7 (C), 150.5 (CH), 152.3 (CH), 175.4, 175.6 (CON & COH), 194.3 (CO). ν_{max} (neat)/cm^{-1} 3189 (br), 3062, 2958, 2923, 2871, 2852, 2345, 1701, 1654, 1612, 1570, 1431, 1288, 980, 694; m/z (EI) 339 (M+, 8.16%), 91 (100%), Found 339.1834, $C_{21}H_{25}NO_3$ (M+) requires 339.1834.

5-DL-[4-^{13}C]-3-(*E,E*-4,6-Dimethylocta-2,4-dienoyl)-5-benzyl-2,4-dioxopyrrolidine (6a)

Compound **17a** (0.296g, 0.603mmol) was dissolved in trifluoroacetic acid (10ml) and the bright red solution stirred under nitrogen for 5 min. The reaction mixture was quenched with ice, resulting in the formation of a pale yellow precipitate. This was extracted into dichloromethane (2x 50ml), washed with saturated aqueous $NaHCO_3$ solution, dried ($MgSO_4$), and concentrated *in vacuo* . The residue was redissolved in methanol (10ml), and filtered to remove white solid impurities. The filtrate was reduced *in vacuo* to give the title compound as a waxy yellow solid (0.161g, 0.472mmol, 78.3%). Two tautomers in the ratio 85 : 15 were observed in the NMR spectra. δ_H, (very broad) 0.78 (3H, t, J 7.2, CH_3), 0.93 (3H, d, J 6.4, CH_3), 1.27 (2H, m, CH_2), 1.83 (3H, s, CH_3), 2.43 (1H, m, CH), 2.57 (1H, m, CH_2), 3.20 (1H, m, CH_2), 3.93 (1H, m, CH), 5.76 (1H, d, J 8.4, CH), 6.32 (1H, bs, NH), 7.16 (6H, m, CH & Ph) 7.43 (1H, d, J 15.20); δ_c, 11.9 (CH_3), 12.4 (CH_3), 20.0 (CH_3), 29.9 (CH_2), 35.2 (CH), 38.3 (CH_2), 62.7 (d, J 40.0, CH), 99.4 (d J 59.0 C), 116.2 (br, CH), 126.8 (Ph CH), 128.6 (Ph CH), 129.1 (Ph CH), 133.0 (Ph C), 136.8 (C), 149.4 (br, CH), 151.6 (br, CH), 174.8, 175.9 (br, CON & COH), 194.1 (85%),

202.3 (15%) (CO, enriched); ν_{max} (neat)/cm^{-1} 3188 (br), 3063, 2958, 2925, 2870, 2345, 1677, 1645, 1609, 1566, 1431, 1288, 980, 695; m/z (EI) 340 (M^+, 33.03%).

5-L-[C_6D_5]-3-(*E,E*-4,6-Dimethylocta-2,4-dienoyl)-5-benzyl-2,4-dioxopyrrolidine (6b)
Compound **17b** (0.671g, 1.36mmol) was dissolved in trifluoroacetic acid (25ml) and the bright red solution stirred under nitrogen for 5 min. The reaction mixture was quenched with ice, resulting in the formation of a pale yellow precipitate. This was extracted into dichloromethane (2x 75ml), washed with saturated aqueous $NaHCO_3$ solution, dried ($MgSO_4$), and concentrated *in vacuo* . The residue was redissolved in methanol (20ml), and filtered to remove white solid impurities. The filtrate was reduced *in vacuo* to give the title compound as a waxy yellow solid (0.457g, 1.33mmol, 97.8%). Two tautomers in the ratio 85 : 15 were observed in the NMR. δ_H, 0.86 (3H, t, J 7.2, CH_3), 1.01(85%), 1.11(15%) (3H, d, J 6.8, CH_3), 1.34 (2H, m, CH_2), 1.86 (15%), 1.92 (85%) (3H, s, CH_3), 2.50 (1H, m, CH), 2.65 (1H, m, CH_2), 3.32 (1H, m, CH_2), 4.01 (85%), 4.18 (15%) (1H, m, CH), 5.70 (15%), 5.87 (85%) (1H, d, J 10.0, CH), 6.28 (1H, bs, NH), 7.14 (1H, J 15.6, CH), 7.55 (85%), 7.96 (15%) (1H, d, J 15.6, CH); δ_D 7.41 (bs, Ph); δ_c, 11.9 (CH_3), 12.4 (CH_3), 20.0 (CH_3), 29.9 (CH_2), 35.3 (CH), 38.3 (CH_2), 61.5 (15%), 63.4 (85%) (CH), 99.4 (C), 115.7 (CH), 128.5 (m, Ph CD), 133.1 (Ph C), 136.6 (C), 150.7 (CH), 152.5 (CH), 175.5, 175.6 (CON & COH), 194.1 (85%) 202.7 (15%) (CO); ν_{max} (neat)/cm^{-1} 3185 (br), 3063, 2967, 2954, 2922, 2870, 1658, 1620, 1568, 1427, 1292, 978, 721; m/z (EI) 344 (M^+, 18.25%).

Feeding Experiments to *B. bassiana*
Compound **6a** (136mg) was dissolved in ethanol (8ml) and pulse fed (0.5ml aliquots) to four *B. bassiana* cultures[6,9] on days 4, 5, 7 and 8 to a final concentration of 2.0 mM. Compound **6b** (103mg) was similarly administered to a final concentration of 1.5mM. DL-[1-^{13}C]-Phenylalanine and DL-[3-^{13}C]-tyrosine were each fed at a final concentration of 3.5mM in the medium. In all cases tenellin was isolated on day 10 by soxhlet extraction of the mycelium into acetone and then purification by HPLC (prep., C_{18}, reverse phase column eluting with MeOH : H_2O : TFA (85 : 15 : 0.1%)). Each tenellin sample was analysed by either ^{13}C-NMR [$^2H_6$]-DMSO or ^{2}H-NMR in DMSO.

Isolation of (20)
Compound **20** was the only perceptable minor metabolite identified after HPLC of the acetone extract of *B. bassiana* cells. This compound eluted before tenellin (6min *versus* 12 min at 1ml/min, see conditions above) This peak was subjected to LC-MS in selective ion monitoring (SIM) mode and contained ions for 354 (M+1) and 230 (100%). Repeated preparative HPLC generated a sample of **20** (4.5mg). δ_H, 0.80 (3H, t, CH_3), 0.99 (3H, d, CH_3), 1.38 (2H, m, CH_2), 1.80 (3H, s, CH_3), 5.96 (1H, d, CH), 6.59, (2H, d, 2 x CH), 6.99 (1H, d, CH), 7.00 (2H, d, 2 x CH), 7.2 (1H, s, CH), 9.22 (1H, bs, OH).

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