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# Biosynthetic Incorporation of Advanced Precursors into Dehydrocurvularin, a Polyketide Phytotoxin from *Alternaria cinerariae*

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*Dedicated to Professor A. Ian Scott on the occasion of his birthday*

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***Abstract:*** Biosynthesis of the polyketide, dehydrocurvularin **1**, by *Alternaria cinerariae* was examined by incorporation experiments with *N*-acetylcysteamine (NAC) thiolesters of potential labeled di-, tri-, tetra-, and pentaketide assembly intermediates, **5-10**. The results show that diketide and α,β-unsaturated tetraketide precursors can be utilized intact, whereas a saturated tetraketide can not, thereby suggesting that **1** is the initial PKS product. Curvularin **2** and **1** could not be interconverted by *A. cinerariae*, but 8-hydroxycurvularin **3** and **1** are transformed into each other by component(s) in the fermentation media.



## INTRODUCTION

Dehydrocurvularin **1** and its structural relatives, curvularin **2** and 8-hydroxycurvularin **3**, are produced by a number of phytopathogenic fungal species, such as *Curvularia*,[1] *Penicillium*,[2] *Cochliobolus*,[3] and *Alternaria* (Figure 1).[4] These macrolides possess interesting biological properties, including antifungal, phytotoxic and cytotoxic activities, and dehydrocurvularin **1** inhibits microtubule assembly.[1-4] Biogenetically-related octaketide and nonaketide analogs that have both an aromatic and a macrocyclic ring but with an altered mode of cyclization include bioactive compounds such as lasiodiplodins,[5] resorcylide, zearalenones, and monocillins.[6] Very recently, the *Curvularia* metabolite Ro 09-2210 **4** was shown to stop T-cell proliferation by inhibiting production of interleukin 2 ($IC_{50}$ 50 nM) through blocking the protein kinase MEK 1.[7] The interesting biochemical effects of curvularin derivatives have stimulated syntheses of **2**[8] and x-ray crystallographic examination of the conformations of **1** and **2**.[9] The presence of a variety of oxidation states along the backbone of the carbon skeleton of **1** prompted us to examine its biosynthesis and demonstrate that its assembly by *Alternaria cinerariae* (ATCC 11784) proceeds via a polyketide pathway.[10-12]

The elucidation of the genetic machinery involved in biosynthesis of a number of microbial polyketide metabolites has provided fundamental insight into the sequence of steps leading to construction of a host of natural products from simple short chain fatty acid precursors.[13,14] The central theme of polyketide biosynthesis is that iterative decarboxylative Claisen condensations of malonyl (or 2-alkyl substituted malonyl) thiolesters result in a growing carbon chain whose oxidation state at the previously attached unit can be reductively adjusted

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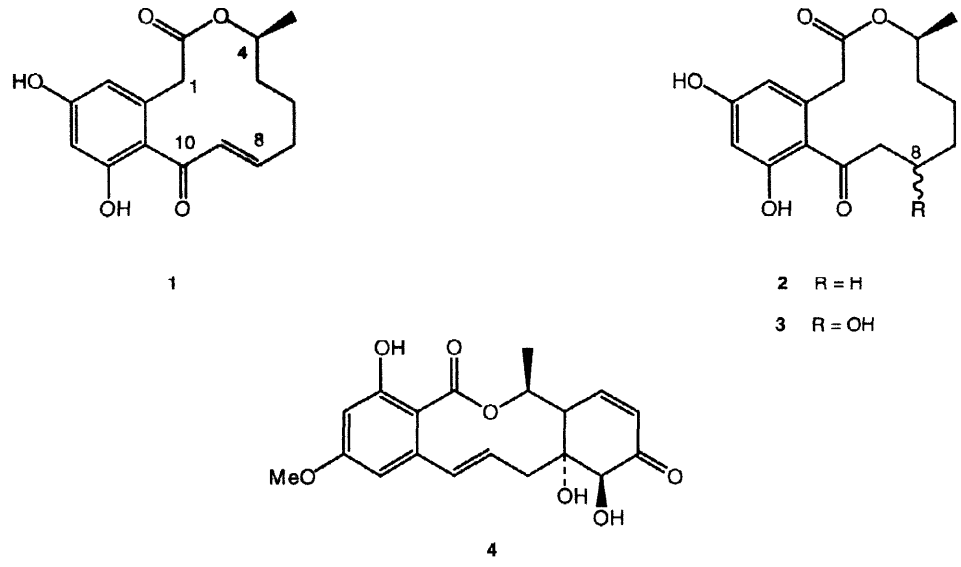



Fig. 1 Structures of dehydrocurvularin **1** and related metabolites

at each cycle.[13,14] In most cases, these investigations have focused on prokaryotic systems, primarily Actinomycetes, but interesting studies have been reported on fungal metabolites[15] such as aflatoxin,[16] cyclosporin A (the 4-(2-butenyl)-4-methyl-L-threonine (Bmt) portion),[17] 6-methylsalicylic acid,[18] and T-toxin.[19] Determining exactly what the partly-assembled PKS-bound intermediates are requires experimental verification, even with modular PKS enzymes where the genetic sequence indicates protein function, because certain enzyme sequences may remain "silent" and may not perform the expected transformation (e.g. rapamycin PKS).[13a] A very helpful tool in determining the partly assembled structures is loading of isotopically labeled putative intermediates as their *N*-acetylcysteamine thiolesters into the polyketide synthase (PKS) machinery using intact cells,[13,20,21] or more recently, cell-free systems.[17,22] Recent work shows that the PKS enzymes will readily accept and further elaborate correctly functionalized thiolesters and can also tolerate certain variations from the normally utilized structure if the natural intermediate is made unavailable.[22] However, even with purified PKS enzymes, at some phases of the assembly process the utilization of any exogenous precursors may be difficult or impossible.[17] Since the biosynthetic arrangement of bonds in dehydrocurvularin **1** derived intact from acetate had been previously determined,[10] in the current work we report chemical syntheses of multiply-labeled putative di-, tri-, tetra-, and pentaketide precursors of **1** and biosynthetic incorporation studies to assess oxidation states of PKS-bound intermediates (Figure 2). Studies on potential post-PKS transformations are also described.

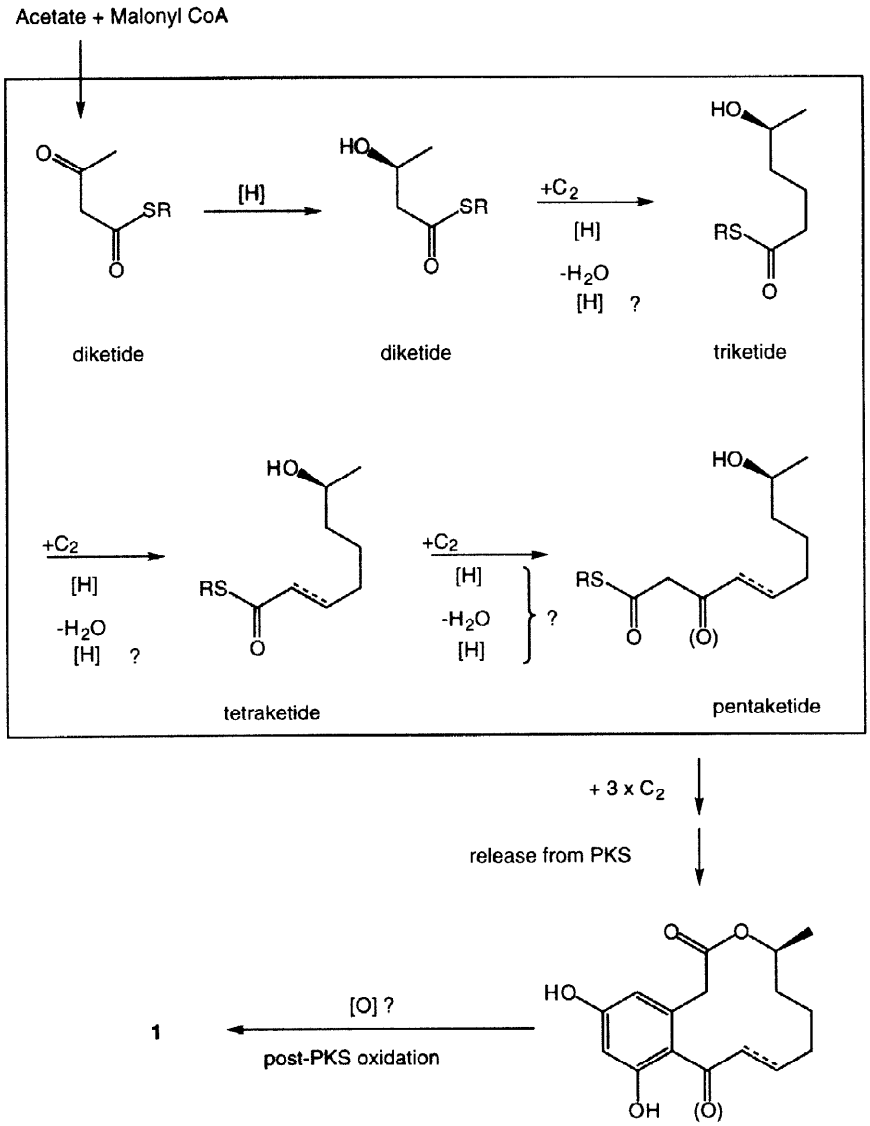


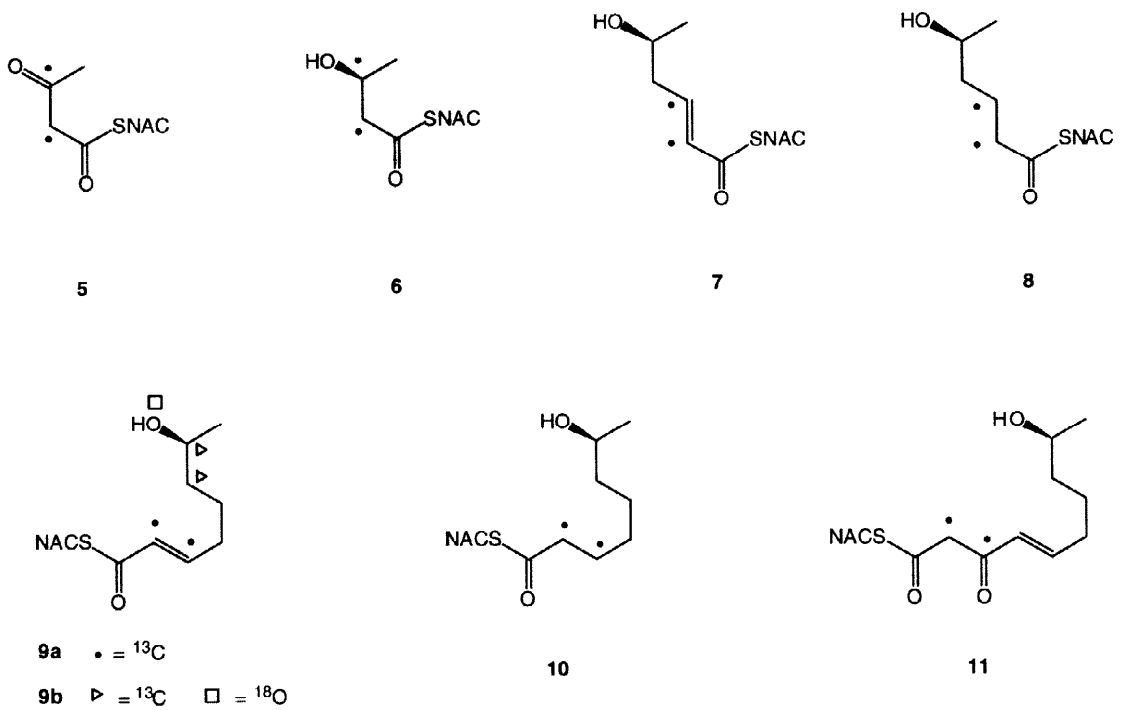

Fig. 2 Possible PKS assembly of dehydrocurvularin **1**

## RESULTS AND DISCUSSION

*Synthesis of Putative Advanced PKS Intermediates as Labeled NAC Esters.*

Based on the proposed assembly mechanism (Figure 2), a number of putative advanced precursors were investigated. Diketide intermediates can have two oxidation states, i.e. the β-ketobutanoate ester **5** and β-hydroxy ester **6**, with the stereochemistry of the latter presumably corresponding to that found in **1**. At the tri- and tetraketide stages the oxidation states of intermediates can not be easily predicted by extrapolating those found in **1** because of the co-occurrence of curvularin **2** and 8-hydroxycurvularin **3**. Therefore, both unsaturated and saturated potential precursors were targeted (**7**, **8**, **9a**, **10**, and **11**). The $^{13}$C-labels were placed at α,β-positions of all precursors since the competitive degradation of these compounds by highly effective β-oxidation

enzymes would generate singly labeled acetates.[23] These would be unlikely to recouple to a doubly labeled fragment due to dilution by unlabeled acetate from the fermentation medium. In order to eliminate the possibility of partial breakdown of the aliphatic chain at the "starter end" and to confirm that the hydroxyl oxygen is incorporated into **1**, a triply labeled tetraketide intermediate **9b** was also synthesized. The intact incorporation of labeled precursors can readily monitored by analyzing the final products with $^{13}C$ NMR spectrometry.[20]




The synthesis of putative diketide precursors starts with readily available $^{13}C$-labeled sodium acetate (99% isotopic purity), from which labeled ethyl acetate and acetyl chloride are made. Ethyl [2,3-$^{13}C$]acetoacetate **12** is then prepared from ethyl [2-$^{13}C$]acetate and [1-$^{13}C$]acetyl chloride as shown in Figure 3. Careful

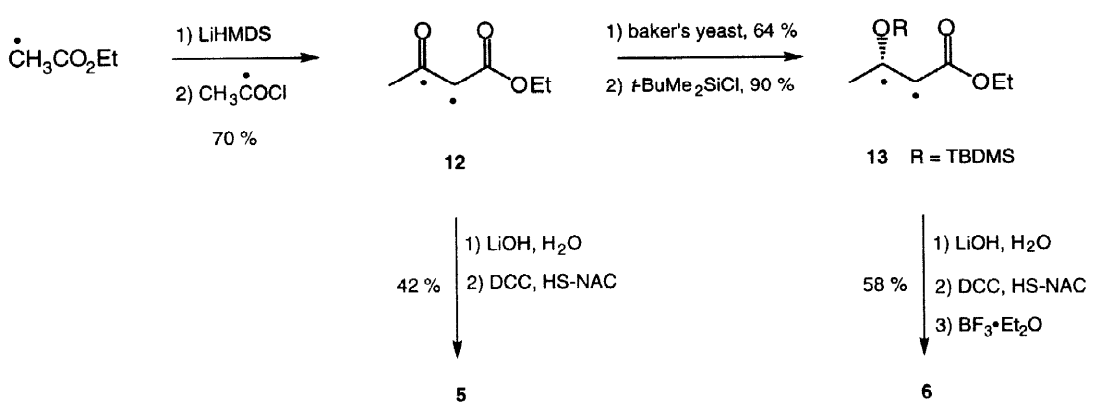



Fig. 3 Synthesis of $^{13}C$-labeled diketide NAC esters

hydrolysis of **12** gives the corresponding acid, which upon coupling with *N*-acetylcysteamine (NAC) provides NAC thiolester **5** in 42% yield after two steps. The *S*- alcohol (>90% ee) is obtained in 64% yield from Baker's yeast reduction of **12**. After protection of the hydroxyl group with TBDMS to yield **13**, the ester functionality is hydrolyzed and coupled with NAC. Removal of the silyl group with boron trifluoride etherate completes the synthesis of diketide precursor **6** in 58% yield from **13**.

For construction of a possible triketide precursor, mono-$^{13}$C-labeled ethyl ester **13a**, prepared in similar manner to **13**, is reduced to the alcohol, and then converted to the corresponding aldehyde by Swern oxidation (Figure 4). Wittig reaction[24] extends the chain by two carbons to give the doubly $^{13}$C-labeled α,β-unsaturated ester **14** as the *E*-isomer, with no detectable amount of *Z*-isomer by $^{1}$H NMR. Saponification (95%) of **14** with lithium hydroxide followed by esterification (93%) of the acid with *N*-acetylcysteamine leads to the NAC thiol ester. Attempts to remove the silyl group with boron trifluoride, tetrabutylammonium fluoride, or a hydrogen fluoride-pyridine complex failed. However, treatment with Dowex50 ($H^+$) resin in methanol at 40 °C by Corey's

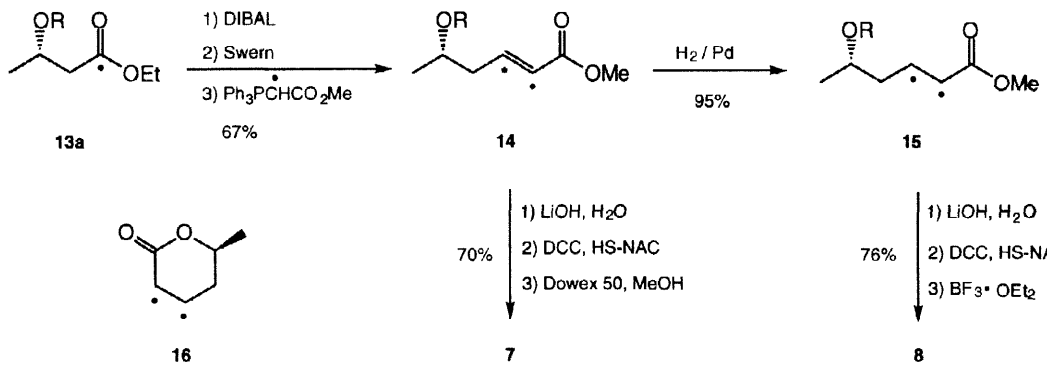



Fig. 4 Synthesis of $^{13}$C-labeled triketide NAC esters

procedure[25] cleaves the silyl group to produce the [2,3-$^{13}$C]-labeled precursor **7** in 78% yield. Hydrogenation and subsequent saponification of **15**, followed by DCC coupling with N-acetylcysteamine provides the NAC ester. Reaction with boron trifluoride etherate in cold dichloromethane affords the precursor **8** in 98% yield. As expected, **8** cyclizes readily on silica gel to form a known lactone **16**.[26] Therefore **8** was directly used for feeding experiments without further purification.

The tetraketide precursors are synthesized as shown in Figure 5, adopting the chain extension strategy described for the synthesis of triketides. Mono-labeled compound **15a** is available in a similar manner to the doubly-labeled compound **15** (Figure 4). This is then extended by two carbons to give **17** as mixture of two isomers, *E*:*Z* = 64:36. After separation by silica gel chromatography, the *E*-isomer is hydrolyzed and coupled with NAC. Removal of the silyl group with boron trifluoride etherate gives precursor **9a**. Hydrogenation of the

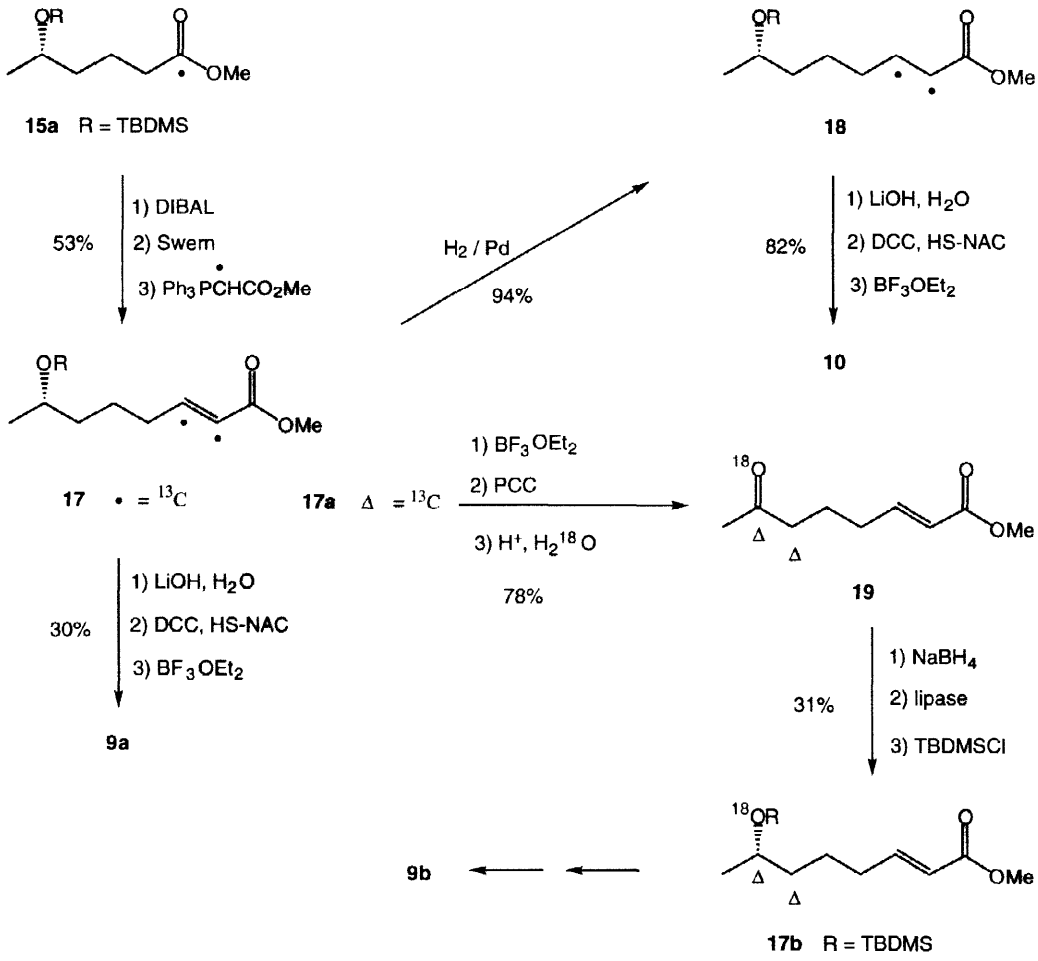



Fig. 5 Synthesis of $^{13}C$-labeled tetraketide NAC esters

isomeric *E*:*Z* mixture **17** generates the saturated ester **18** as a pure *S*-isomer, which is converted in the same fashion as the corresponding unsaturated compound to provide the saturated precursor **10** (82% overall yield from **18**).

Introducing an oxygen-18 atom into the molecule is the key step for the synthesis of the tetraketide NAC ester **9b**. Since ketones can undergo oxygen exchange reasonably rapidly in water,[27] preparation of the keto compound **19** was targeted (Figure 5). The silyl ether **17a**, obtained using methods similar to those for the preparation of **17**, reacts with boron trifluoride etherate to give the hydroxy compound in 87% yield (with 12% recovery of **17a**). Oxidation with pyridinium chlorochromate (PCC) gives the $^{16}O$-keto compound **19** in 90% yield. The ketone oxygen of **19** exchanges with $^{18}O$-water under acidic conditions[27] to give the corresponding $^{18}O$ labeled keto compound **19a**. This is then immediately reduced without purification to the corresponding hydroxy product with sodium borohydride in methanol (89%). The oxygen-18 labeled racemic mixture can be resolved by lipase catalyzed transesterification[28] to provide the *S*-alcohol (49%). This reacts with TBDMS chloride in the presence of imidazole to produce the silyl ether **17b** (72%). The desired thiolester **9b** (isotopic purity 99% $^{13}C_2$, 66% $^{18}O$) is obtained from **17b** in an overall 24% yield using the same strategy developed for the synthesis of its analogue **9a**.

Several strategies were investigated to construct the pentaketide **11**, and an approach which generates the fully functionalized skeleton in protected form is shown in Figure 6. Condensation of the aldehyde **20** and reagent **21**[29] affords **22**, which has the stereochemically correct carbon framework. However, attempted removal of the ester functionality of **22** leads to complex mixtures. Protection of the carbonyl group as an *O*-

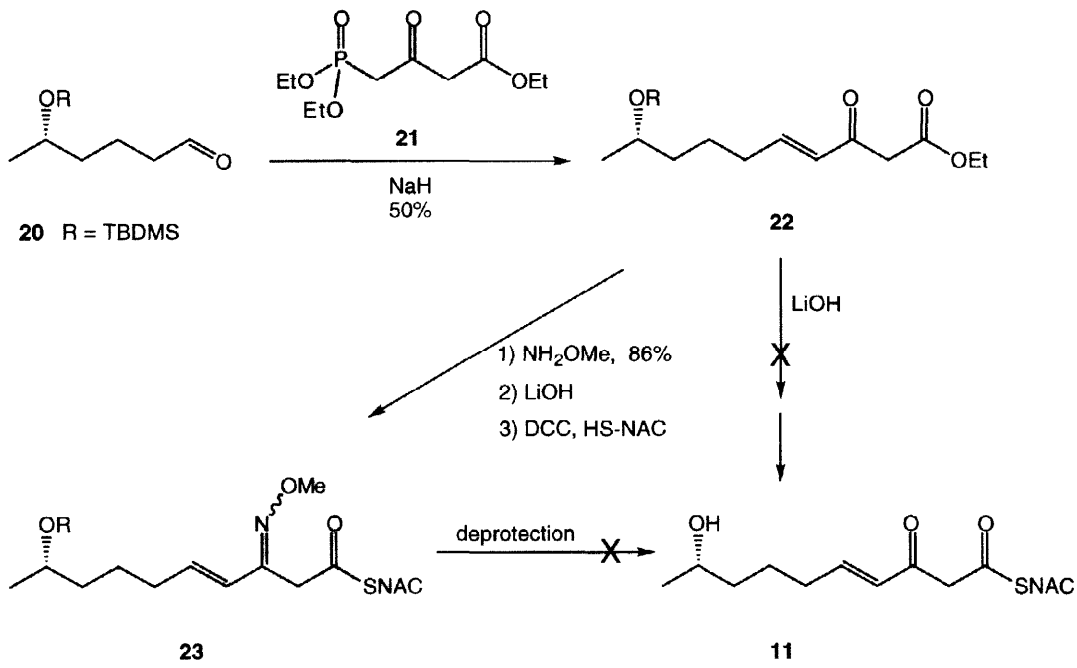


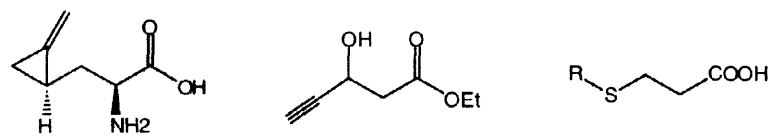

Fig. 6 An approach to synthesis of pentaketide NAC ester **11**

methyl oxime overcomes this problem, and also avoids the risk of decarboxylation of the free β-keto acid. With this modification, the NAC thiolester **23** could be readily made. Unfortunately, the *O*-methyl oxime is resistant to removal. Treatment with Amberlyst-15 as described by Sakamoto *et al.*[30] results in loss of the silyl group, leaving the oxime untouched. With Corey's titanium reagent[31] compound **23** undergoes slow decomposition to form a complex mixture, and none of the desired product could be detected. The relative instability of the functionalized intermediates and possibly the target pentaketide **11**, as well as the necessity to produce these compounds with double isotopic labeling, caused us to abandon this approach.

*Biosynthetic Incorporation Experiments with Labeled NAC Esters.*

A major problem encountered with attempted loading of advanced precursors onto the PKS enzymes is competitive degradation of these compounds by highly effective β-oxidation enzymes.[23] Initial experiments in which **6** was added to wild type *A. cinerariae* (ATCC 11787) under various conditions gave dehydrocurvularin **1** whose $^{13}C$ NMR spectra indicated complete degradation of the putative precursor to acetate prior to incorporation (i.e., enhanced singlets at every carbon). It was only with the combined use of high glucose replacement media, a UV-generated mutant of *A. cinerariae* deficient in the ability to grow on fatty acids, and the addition of 4-pentynoic acid as a potential β-oxidation inhibitor, that intact utilization of a significant portion

(12%) of **6** could be achieved. Under these conditions, carbon-coupled signals could be seen at C-4 and C-5 of **1**, thereby indicating that the four carbon chain had been incorporated without cleavage of its C-2 to C-3 bond. Nevertheless, even with these special conditions, the majority of the precursor **6** was degraded by β-oxidation prior to incorporation. In order to avoid the use of special mutants for such experiments, several other potential β-oxidation inhibitors were tested for their ability to enhance intact incorporation of diketide **6** and tetraketide **9a** into **1** with wild type *A. cinerariae*. Compounds such as hypoglycin **24**,[32] ethyl 3-hydroxypentynoate **25**,[33] 3-(tetradecylthio)propanoic acid **26**,[34] and 3-(octylthio)propanoic acid **27**[34] (Figure 7) permit use of the wild type fungus for intact incorporation of these precursors, with **25** and **26** being the most effective (Table 1).




**24** **25** **26** R = $Me(CH_2)_{13}$

**27** R = $Me(CH_2)_7$

Fig 7. β-Oxidation inhibitors used to assist incorporation of NAC esters

Table 1. Effect of β-Oxidation Inhibitors on Intact Incorporation of **9a** into Dehydrocurvularin **1**

| inhibitor | amount added (mg/125 mL) | intact incorporation (%)[a] |
|---|---|---|
| none | | 7 |
| hypoglycin **24** | 36 | 7[b] |
| ethyl 3-hydroxypentynoate **25** | 36 | 16 |
| 3-(tetradecylthio)propanoic acid **26** | 15 | 70 |
| 3-(octylthio)propanoic acid **27** | 15 | 7 |

[a]Minimum value determined by integration of $^{13}C$ NMR spectra of coupled resonances (intact incorporation) and singlets (natural abundance + incorporation of degraded precursor) for C-8 and C-9 of **1**. Absolute (total) incorporation rate of precursor was 1-3%. [b]Negligible effect for this precursor, but **24** enhances incorporation of other precursors such as **6**.

Solutions of **5** and **6** (30-40 mg each) with ethyl 3-hydroxypentynoate (**25**) (35 mg) as well as compounds **7**-**10** (30-40 mg each) with 3-(tetradecylthio)propanoic acid (**26**) (35 mg) were individually administered in 98% ethanol (2.5 mL) in six portions to the high glucose fermentation culture of wild type *A. cinerariae* (125 mL). The dehydrocurvularin from each of these experiments was then purified and analyzed by $^{13}C$ NMR spectrometry. Intact incorporation is calculated based on integration ratio of the coupled signals and the singlet corresponding to the natural abundance signal and any labeling from degraded precursor (Table 2).

For example, the $^{1}H$-decoupled $^{13}C$ NMR spectra of **1** derived from **5** or **6** show two sets of coupled signals for C-4 and C-5 at 72.82 and 34.73 ppm and indicate a minimum of 22% and 14% intact incorporation, respectively. Careful separation of the fermentation extracts also affords 8-hydroxycurvularin **3**, which shows identical labeling patterns and exists as a mixture of two diastereomers at C-8 in the ratio 1 to 2.6.[35] This compound had been previously reported in *Alternaria tomato*[4c] and *Penicillium* sp. 511.[2c]

Table 2. Intact Incorporation of NAC Esters into **1**

| Precursor | diketide | | triketide | | tetraketide | | |
|---|---|---|---|---|---|---|---|
| | **5** | **6** | **7** | **8** | **9a** | **9b** | **10** |
| Incorporation[a] | 22% | 14% | none[b] | none[b] | 70% | 70% | none[b] |

[a]Integration ratio of coupled signals to singlet composed of natural abundance signal and labeling by degraded precursor. [b]Detection limits are ca 2-3% intact incorporation.

Neither of the triketides **7** or **8** show any detectable amount of intact incorporation. This suggests that the PKS machinery may permit loading of external precursors only at certain chain lengths or oxidation states which allow effective competition with the endogenously generated intermediates that are already covalently attached to the PKS. Such a situation has been observed for loading of NAC esters in a cell-free fungal PKS system that makes the Bmt portion of cyclosporin A.[17] Other possible reasons for the lack of successful intact incorporation may be an inability of the substrate to cross the cell membrane or rapid breakdown of the exogenously added precursor by cellular degradation pathways such as thiolester hydrolysis or β-oxidation. In the case of the saturated triketide **8**, it is also possible that intramolecular cyclization to the lactone **16** occurs more rapidly in the fermentation medium than uptake by the PKS.

Both forms of the unsaturated tetraketide **9a** and **9b** showed substantial intact incorporation into **1** (Table 2). The $^{1}H$-decoupled $^{13}C$ NMR spectrum of **1** derived from **9a** shows two sets of coupled signals for C-8 and C-9 at 149.6 and 132.5 ppm, and that of **1** derived from **9b** shows two sets of coupled signals for C-4 and C-5 with $^{18}O$-induced isotope shift observed at C-4.[20] This confirms that the entire chain with the hydroxyl group can be utilized without breakdown or dehydration. Interestingly, the corresponding saturated NAC ester **10** could not be incorporated under identical conditions. Since the physical properties of the unsaturated compound **9** and its saturated derivative **10** are reasonably similar, these results suggest that the double bond is essential for incorporation at the tetraketide stage and that the next unit is added to form an unsaturated pentaketide chain such as that in **11**. Unfortunately, as mentioned above, NAC ester **11** could not be synthesized in unprotected form to test this hypothesis.

*Studies on Post-PKS Transformations Leading to 1.*

After PKS enzymes assemble a basic skeleton in a predominantly reductive fashion,[13] the initially-released product may be transformed further by other enzymatic processes, such as oxidation, acylation, alkylation or glycosylation to give final metabolites. We observed earlier[10] that the origin of the oxygen atom at the C-10

ketone is unclear because $^{18}O$ label could not be detected after incorporation of either [$^{18}O$]acetate or oxygen-18 gas. This is probably caused by a facile exchange with unlabeled water from the media during formation of **1**, but could possibly also be due to an unexpectedly small isotope shift that defies observation by $^{13}C$ NMR analysis.[20,27,36] Hence, despite the failure to incorporate the saturated tetraketide derivative **10** into **1** and the facile utilization of the corresponding unsaturated analog **9**, the exact biosynthetic relationship of dehydrocurvularin **1**, curvularin **2**, and 8-hydroxycurvularin **3** is uncertain. It could be that the polyketide machinery may produce a less oxidized precursor, for example, curvularin **2**, which is released and subsequently transformed by oxidative enzymes to **1**. Alternatively, **1** could be reduced to **2** in a post-PKS process, or both metabolites may be produced independently by one or more PKS enzymes. Two approaches were investigated in an effort to elucidate the relationship of **1-3**, namely blocking of possible oxidative $P_{450}$ enzymes during fermentation and direct attempts to interconvert these metabolites with *A. cinerariae*.

Cytochrome $P_{450}$ inhibitors ancymidol (**28**) and metyrapone (**29**) (Figure 8) have been used very effectively in biosynthetic studies of betaenone B by *Phoma betae* ,[37] chaetoglobosin by *Chaetomium subaffine*[38]


**28** **29**

Fig. 8 $P_{450}$ oxidation inhibitors

as well as of other metabolites.[39] New less oxidized compounds were isolated and identified as plausible precursors in the biosynthetic pathways. However, administration of ancymidol to *A. cinerariae* in moderate concentration (2.0 mM) killed the fungus. At lower concentrations, the culture growth was suppressed to some extent, but sufficient quantities of metabolites were produced for HPLC analysis. Comparison with a control in which no inhibitor was employed shows no new compounds except for the presence of a peak due to ancymidol. Similar experiments with metyrapone (final concentrations 0.5-5.0 mM) demonstrate that this oxidation inhibitor also does not significantly affect the metabolite types and amounts, and that added metyrapone is reduced by *A. cinerariae* to the corresponding alcohol in all cases.[40] The results suggest that oxidative $P_{450}$ enzymes may not be involved in formation of **1-3**.

In order to examine whether more highly reduced derivatives of dehydrocurvularin **1** can be transformed to this product by *A. cinerariae*, labeled **1a** was generated by incorporation of doubly $^{13}C$-labeled sodium acetate[10] and chemically transformed to $^{13}C$-labeled curvularin **2a**, alcohol **30** and the 8,9-dihydro-10-deoxy derivative **31** (Figure 9). Thus hydrogenation of **1a** in the presence of $PtO_2$ converts it to labeled curvularin **2a** in 95%

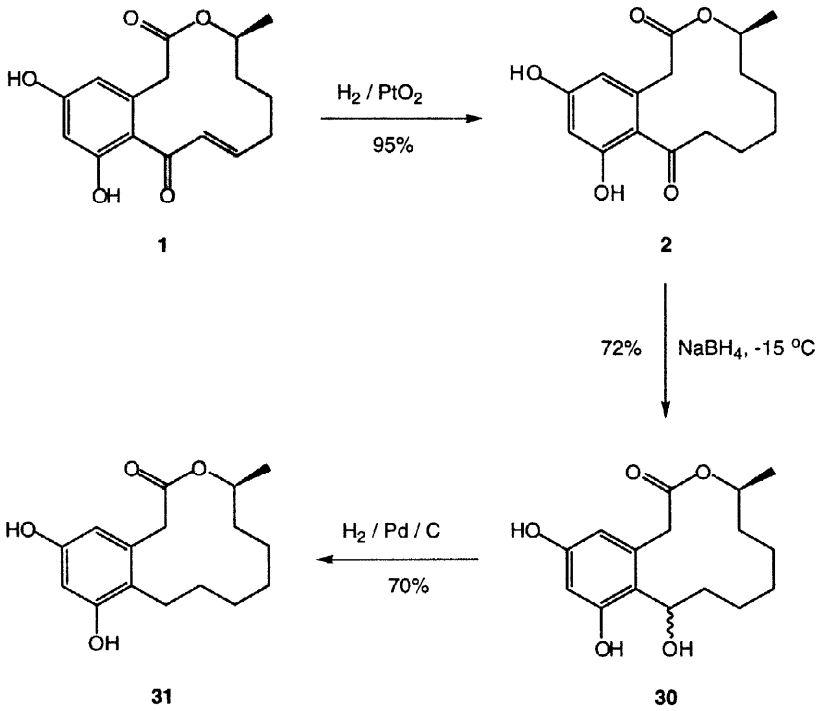



Figure 9 Preparation of curvularin analogs

yield.[10] Reduction with sodium borohydride at -15 °C provides $^{13}C$-labeled **30** as a mixture of two diastereomers (ca. 1:1 ratio). Removal of the benzylic hydroxyl of **30** by hydrogenolysis affords $^{13}C$-labeled 10-deoxycurvularin **31**. Attempts to transform **2a**, **30** or **31** with cultures of *A. cinerariae* in the presence or absence of β-oxidation inhibitor **26** did not result in any detectable transformation of these precursors into **1**. However, during these experiments it became apparent that dehydrocurvularin **1** and 8-hydroxycurvularin **3** are in equilibrium in the fungal culture. Addition of labeled dehydrocurvularin **1a** to *A. cinerariae* generates 8-hydroxycurvularin **3a** which shows the same labeling pattern and enrichment of carbon-13. Similarly, addition of labeled **3a** to the cultures gives labeled **1a**. To determine whether the interconversion is enzyme-mediated, a series of experiments were performed. The fungal culture is slightly acidic (ca. pH 5.5-6.0) throughout the fermentation. However, incubation of dehydrocurvularin **1** in pH 5 or pH 6 phosphate buffers at 28 °C shows no detectable formation of 8-hydroxycurvularin **3**, thereby excluding simple acid-catalyzed hydration or dehydration. As a control, the fermentation culture of *A. cinerariae* was autoclaved at 121 °C for 20 min to kill the fungus, and acetate-labeled dehydrocurvularin **1a** was added. Surprisingly, the autoclaved mixture can still perform the transformation. Finally, acetate-labeled dehydrocurvularin **1a** was added to sterile potato dextrose media without fungus, and incubated under the same conditions. It was found that the media alone also accomplishes this transformation. This inter-conversion can therefore be interpreted as a Michael addition and elimination of water catalyzed by some component(s) in the potato dextrose broth. This indicates that the co-occurence of **3** as a mixture of diastereomers with **1** is due primarily to non-fungal transformation.

*Conclusion*

In summary, a number of advanced precursors as di-, tri- and tetraketides were synthesized in multiply labeled form and tested for incorporation into dehydrocurvularin **1** by *A. cinerariae*. The results show that both diketides **5** and **6** are precursors in the biosynthetic pathway, and that the double bond of the tetraketide **9** is essential for incorporation. This strongly supports the hypothesis that the unsaturated tetraketide represents the final oxidation state achieved on the PKS enzyme prior to addition of the next $C_2$ unit. Since the penta-, hexa-, hepta-, and octaketide PKS intermediates require no reduction (i.e. they retain the β-carbonyl oxygen of the PKS-bound β-ketothiolester), this suggests that dehydrocurvularin **1** is the initial PKS product. Studies on the post-PKS transformations involving blocking of $P_{450}$ oxidation and attempted conversions of fully-assembled reduced derivatives **2a**, **30** or **31** also support this hypothesis. Interestingly, this work reveals that dehydrocurvularin **1** and 8-hydroxycurvularin **3** are interconvertible in the fermentation culture, and that the hydration-dehydration reaction is catalyzed by some component(s) from the potato dextrose broth.

## ACKNOWLEDGEMENTS

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## EXPERIMENTAL[41]

**Fermentation of *A. cinerariae* and Isolation of Dehydrocurvularin 1 and 8-Hydroxycurvularin 3**. The cultures of *A. cinerariae* (ATCC 11784) were maintained on slants made from potato dextrose agar (Difco, 39 g/L). Spore suspensions from 2 slants were used to inoculate ten 500 mL Erlenmeyer flasks containing 125 mL of potato dextrose broth (Difco, 24 g/L). The flasks were then placed on a rotary fermentor and incubated at 26 °C and 165 rpm for 7 days in the dark. The cultures were filtered and washed with EtOAc (500 mL), the filtrate was extracted with EtOAc (3 x 800 mL). Concentration after drying ($Na_2SO_4$) provided a yellow gum (343 mg). Flash chromatography on $SiO_2$ gave dehydrocurvularin **1** (217 mg) and 8-hydroxycurvularin (**3**) (79 mg). Melting point and spectroscopic data for **1** were previously reported.[10] Compound **3** is a mixture of two isomers (1:2.6 ratio, determined by $^1H$ NMR): mp 115-117 °C (lit.[2b] 150-152 °C for α-isomer, 138-140 °C for β-isomer); IR (KBr) 3414 (br s), 2931 (m), 1703 (s), 1611 (s), 1267 (s), 1160 (m), 842 (m) $cm^{-1}$; $^1H$ NMR (300 MHz, acetone-$d_6$) δ 9.05 (br s, 1 H, Ar-OH), 8.75 (br s, 1 H, Ar-OH), 6.40 (d, 1 H, $J$ = 2.3 Hz, Ar-H), 6.34 (d, 1 H, $J$ = 2.3 Hz, Ar-H), 4.82 (m, 1 H, H-4), 4.10 (m, 1 H, H-8), 3.85-2.75 (m, 4 H, 2 x H-1, 2 x H-9), 1.80-1.20 (m, 6 H, 2 x H-5, 2 x H-6, 2 x H-7), 1.10 (d, 3 H, $J$ = 6.2 Hz, $CH_3$); $^{13}C$ NMR (50 MHz, acetone-$d_6$) for the major isomer δ 204.47 (C-10), 170.50 (C-2), 160.02 (C-11), 158.06 (C-13), 136.55 (C-15), 120.74 (C-16), 111.72 (C-14), 102.32 (C-12), 73.03 (C-4), 66.82 (C-8),

53.96 (C-9), 39.09 (C-1), 35.16 (C-5), 32.13 (C-7), 22.12 (C-6), 21.02 ($\underline{C}H_3$); for the minor isomer δ 204.06 (C-10), 170.32 (C-2), 160.16 (C-11), 158.74 (C-13), 137.07 (C-15), 120.22 (C-16), 112.10 (C-14), 102.32 (C-12), 71.21 (C-4), 67.57 (C-8), 53.27 (C-9), 39.55 (C-1), 35.74 (C-5), 31.61 (C-7), 22.12 (C-6), 21.02 ($\underline{C}H_3$); HRMS (EI) calcd for $C_{16}H_{20}O_6$ 308.1260, found 308.1261 ($M^+$, 5); MS(FAB, Cleland) *m/z* (relative intensity) 309 ($MH^+$, 85), 291 (39), 195 (100).

**Incorporation of Advanced Precursors.** The same conditions as described above were used except that after 96 h, the mycelia from two flasks were filtered and washed with replacement media consisting of: glucose (100 g); $Na_2HPO_4$ (1 g); $MgSO_4.7H_2O$ (0.5 g); KCl (0.5 g); $FeSO_4.7H_2O$ (0.01 g) per litre. The washed mycelia were further incubated in a flask containing 125 mL of replacement media. Labeled precursor in 98% EtOH (1 mL) and β-oxidation inhibitor (typically 35 mg) in 98% EtOH (1 mL) were then added in six equal portions at 12 h intervals. The culture was extracted 12 h after the last feeding.

**Curvularin 2 and ($^{13}$C)-Curvularin 2a.** The procedure for isotopic labeling of dehydrocurvularin and its reduction to **2** (or **2a**) have previously reported with full spectroscopic data.[10]

**NAC [2,3-$^{13}C_2$]Acetoacetate 5.** Ethyl [2,3-$^{13}C_2$]acetoacetate **12** was hydrolyzed following Hall's procedure[42] to give the lithium acetoacetate in 51% yield. The lithium salt (216 mg, 2.00 mmol) was dissolved in a minimum amount of $H_2O$. The solution was then cooled in an ice bath, and acidified with cold dilute $H_2SO_4$ to pH 1. After saturation with NaCl the solution was extracted with ether (4 x 10 mL), and dried ($Na_2SO_4$). After concentration *in vacuo* the colorless oil (194 mg) was dissolved in $CH_2Cl_2$ (20 mL) at 0 °C. Molecular sieves (5 g) were added and stirred for 15 min. Solutions of DCC (454 mg, 2.20 mmol) and DMAP (10 mg) in $CH_2Cl_2$ (5 mL), and *N*-acetylcysteamine (262 mg, 2.20 mmol) in $CH_2Cl_2$ (5 mL) were added simultaneously *via* two syringes. The mixture was then stirred at room temperature overnight. After filtration and evaporation of solvent the residue was purified by $SiO_2$ column chromatography (5% $CH_3OH$ in $CHCl_3$) to afford the product (281 mg) as a mixture of **5** and *N*-acetylcysteamine (ca. 1 : 1 ratio, determined by $^1$H NMR). The product was further purified using MPLC (Merck, RP-8, 40-63 μm), eluting with 95:5 $H_2O$-$CH_3CN$, to give pure **5** (42% yield from **12**): IR ($CHCl_3$ cast) 3287 (br), 1667 (s), 1547 (s) cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$), for keto form δ 6.21 (br s, 1 H, N$\underline{H}$), 3.69 (dd, 2 H, *J* = 131.0, 6.2 Hz, $^{13}CO^{13}C\underline{H}_2$), 3.43 (q, 2 H, *J* = 6.1 Hz), 3.08 (t, 2 H, *J* = 6.4 Hz), 2.25 (dd, 3 H, *J* = 6.2, 1.3 Hz, $C\underline{H}_3{}^{13}CO$), 1.96 (s, 3 H); for enol form δ 12.55 (s, 1 H), 6.21 (br s, 1 H), 5.45 (dd, 1 H, *J* = 167.3, 4.4 Hz, $^{13}C=^{13}C\underline{H}$), 3.43 (q, 2 H, *J* = 6.1 Hz), 3.04 (t, 2 H, *J* = 6.3 Hz), 1.96 (s, 3 H), 1.93 (dd, 3 H, *J* = 6.6, 4.2 Hz, $C\underline{H}_3{}^{13}C$); $^{13}$C NMR (100 MHz, $CDCl_3$), for keto form δ 199.83 (d, *J* = 36.3 Hz, enriched, $^{13}\underline{C}O$), 192.12, 170.51, 57.93 (d, *J* = 36.3 Hz, enriched, $^{13}\underline{C}H_2COO$), 39.05, 30.54 ($\underline{C}H_3{}^{13}CO$), 29.10, 23.02; for enol form δ 194.11, 173.91 (d, *J* = 69.9 Hz, enriched, $^{13}\underline{C}=^{13}CH$), 170.38, 99.78 (d, *J* = 69.9 Hz, enriched, $^{13}C=^{13}\underline{C}H$), 39.76, 27.66, 23.08, 20.90 ($\underline{C}H_3{}^{13}C$); HRMS (EI) calcd for $^{13}C_2C_6H_{13}NO_3S$ 205.0683, found 205.0680; MS (CI, $NH_3$) *m/z* (relative intensity) 223 ($MNH_4^+$, 71), 206 ($MH^+$, 21).

**NAC *(S)*-[2,3-$^{13}C_2$]-3-Hydroxybutyrate 6.** The procedure for the conversion of **12** to **5** was used in the first step to convert ethyl ester **13** to the corresponding NAC ester. To remove the silyl protecting group, boron trifluoride diethyl etherate (1.50 mL, 12 mmol) was added to a solution of silyl ether (162 mg, 0.51 mmol) in

$CH_2Cl_2$ (50 mL) at 0 °C. After stirring at 0 °C for 2 h the solution was neutralized with 5% $Na_2CO_3$, extracted with $CHCl_3$ (4 x 50 mL), and the combined extracts were dried ($Na_2SO_4$). Purification by column chromatography with EtOAc afforded the labeled β-hydroxy thiolester **6** (98.8 mg, 95% yield in desilylation, 58% yield from **13**). IR ($CHCl_3$ cast) 1653 (s), 1550 (m), 1290 (m), 1037 (m) $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 6.22 (br s, 1 H), 4.45 and 4.06 (dm, 1 H, $J$ = 145 Hz, $^{13}C\underline{H}(OH)$), 3.43 (m, 2 H), 3.05 (dt, 2 H, $J$ = 6.4, 4.2 Hz), 2.88 and 2.57 (dm, 2 H, $J$ = 129 Hz, $^{13}C\underline{H}_2$), 2.84 (br s, 1 H), 2.00 (s, 3 H), 1.24 (dt, 3 H, $J$ = 6.2, 4.7 Hz, $C\underline{H}_3{}^{13}CH(OH)$); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 199.31(d, $J$ = 46.0 Hz), 170.48), 64.45 (d, $J$ = 36.0 Hz, enriched, $CH_3{}^{13}\underline{C}H(OH)$), 52.46 (d, $J$ = 36.0 Hz, enriched, $^{13}\underline{C}H_2$), 39.22, 28.78, 23.16, 22.70 (t, $J$ = 19.6 Hz, $\underline{C}H_3{}^{13}CH(OH)$); MS (CI, $NH_3$) 208 ($MH^+$, 100), 225 ($MNH_4^+$, 96).

**NAC *(S)*-[2,3-$^{13}C_2$]-5-Hydroxyhex-2-enoate 7.** The procedure for the conversion of **12** to **5** was used to construct the NAC thiolester. To remove the silyl protecting group, the silyl ether (808 mg, 2.33 mmol) and AG 50W-X8 ion exchange resin (Bio-Rad, 1.7 meq/mL, 8.10 mL, 13.8 mmol) were added to a flask containing MeOH (60 mL). The mixture was heated at 40 °C for 30 min and filtered. The filtrate was concentrated to give a liquid residue, which after purification by flash chromatography (silica, 2 x 6 cm, EtOAc) gave **7** (425 mg, 78% yield, 70% yield from **14**): $[\alpha]_D$ +8.0° (*c* 0.48, $CHCl_3$); IR ($CHCl_3$ cast) 3280 (br), 1656 (s), 1579 (m), 1550 (m) $cm^{-1}$; $^1H$ NMR (360 MHz, $CDCl_3$) δ 7.16 and 6.73 (ddt, 1 H, $J$ = 136, 15.0, 7.7 Hz, $^{13}C\underline{H}={}^{13}CHCOS$), 6.45 and 6.00 (ddt, 1 H, $J$ = 154, 15.0, 1.9 Hz, $^{13}CH={}^{13}C\underline{H}COS$), 5.91 (br s, 1 H), 4.13 (m, 1 H), 3.47 (dt, 2 H, $J$ = 6.4, 5.5 Hz), 3.11 (t, 2 H, $J$ = 6.4 Hz), 2.38 (m, 2 H, $C\underline{H}_2{}^{13}CH$), 1.97 (s, 3 H), 1.25 (d, 3 H, $J$ = 6.3 Hz); $^{13}C$ NMR (90 MHz, $CDCl_3$) δ 190.04 (d, $J$ = 62.0 Hz), 170.57), 142.53 (d, $J$ = 69.6 Hz, enriched, $^{13}\underline{C}H={}^{13}CHCOS$), 130.25 (d, $J$ = 69.6 Hz, enriched, $^{13}CH={}^{13}\underline{C}HCOS$), 66.40, 41.65 (d, $J$ = 42.6 Hz, $\underline{C}H_2{}^{13}CH$), 39.53, 28.19, 23.27 (d, $J$ = 3.7 Hz), 23.04; MS (CI, $NH_3$) 234 ($MH^+$, 100), 251 ($MH_4^+$, 83).

**NAC (*S*)-[2,3-$^{13}C_2$]-5-Hydroxyhexanoate 8**. The procedure for the conversion of **13** to **6** was used to transform **15** to **8** in 76% yield. Analyses were done directly after the deprotection without further purification: IR ($CHCl_3$ cast) 3293 (br s), 2929 (s), 1686 (m), 1686 (s), 1656 (s), 1554 (m), 1021 (m) $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 6.25 (br s, 1 H), 3.77 (m, 1 H), 3.40 (q, 2 H, $J$ = 6.2 Hz), 3.00 (t, 2 H, $J$ = 6.4 Hz), 2.58 (dm, 2 H, $J$ = 128.7 Hz, $^{13}C\underline{H}_2CO$), 2.39 (br s, 1 H, O$\underline{H}$), 1.95 (m, 3 H), 1.72 (dm, 2 H, $J$ = 132.6 Hz, $^{13}C\underline{H}_2{}^{13}CH_2CO$), 1.45 (m, 2 H), 1.17 (d, 3 H, $J$ = 6.2 Hz); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 199.82 (d, $J$ = 47.2 Hz), 170.32, 67.25, 43.78 (d, $J$ = 33.9 Hz, enriched, $^{13}\underline{C}H_2COS$), 39.47, 38.47 (d, $J$ = 35.1 Hz, 28.43, 23.44, 23.06, 21.74 (enriched, d, $J$ = 33.9 Hz, $^{13}\underline{C}H_2{}^{13}CH_2CO$); MS (CI, $NH_3$) *m/z* (relative intensity) 253 ($MNH_4^+$, 28), 236 ($MH^+$, 47); HRMS (EI) *m/z* (relative intensity) 119.0396 (60), 117.0825 (18).

**NAC (*S*)-[2,3-$^{13}C_2$]-7-Hydroxyoctanoate 9a**. The method for the conversion of **13** to **6** was adapted to convert **17** to **9a** in 30% yield: $[\alpha]_D$ +3.90° (*c* 1.43, $CHCl_3$); IR ($CHCl_3$ cast) 3286 (br m), 2930 (s), 2857 (m), 1693 (s), 1646 (s), 1556 (m) $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 6.00 (br s, 1 H), 3.79 (m, 1 H), 3.42 (q, 2 H, $J$ = 6.3 Hz), 3.02 (t, 2 H, $J$ = 6.4 Hz), 2.57 (dm, 2 H, $J$ = 130.1 Hz, $^{13}C\underline{H}_2CO$), 1.96 (s, 3 H), 1.75 (br s, 1 H), 1.66 (dm, 2 H, $J$ = 134.6 Hz, $^{13}C\underline{H}_2{}^{13}CH_2CO$), 1.50-1.30 (m, 6 H), 1.18 (d, 3 H, $J$ = 6.2 Hz); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 199.97 (d, $J$ = 45.3 Hz), 170.38, 67.72, 43.88 (d, $J$ = 33.4 Hz, enriched, $^{13}\underline{C}H_2CO$), 39.55, 38.89, 28.77 (d, $J$ = 34.6 Hz, $\underline{C}H_2{}^{13}CH_2$), 28.38, 25.46 (d, $J$ = 33.4 Hz, enriched,

$^{13}\underline{C}H_2{}^{13}CH_2CO$), 25.37, 23.48, 23.05; **MS** (CI, $NH_3$) *m/z* (relative intensity) 395 ($MNH_4^+$, 7), 378 ($MH^+$, 100); HRMS (EI) *m/z* (relative intensity) 362.2120 (4), 320.1626 (100).

**NAC *(S)*-[6,7-$^{13}C_2$,7-*hydroxy*-$^{18}O$]-7-Hydroxyoct-2-enoate 9b.** The method for the conversion of **13** to **6** was used to transform **17b** to **9b** (isotopic purity 99% $^{13}C_2$, 66% $^{18}O$): IR ($CH_2Cl_2$ cast) 3378 (br), 2930 (m), 1658 (s), 1634 (m), 1558 (m) $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 6.93 (dt, 1 H, *J* = 15.6, 7.0 Hz), 6.15 (dt, 1 H, *J* = 15.6, 1.5 Hz), 5.87 (br s, 1 H), 3.98 and 3.63 (dm, 1 H, *J* = 141 Hz, $^{13}C\underline{H}(^{18/16}OH)$), 3.46 (q, 2 H, *J* = 6.2 Hz), 3.09 (t, 2 H, *J* = 6.3 Hz), 2.23 (m, 2 H), 1.97 (s, 3 H), 1.7-1.4 (m, 4 H, $^{13}C\underline{H}_2C\underline{H}_2$), 1.21 (dt, 3 H, *J* = 6.0, 4.4 Hz, $C\underline{H}_3{}^{13}CH(^{18/16}OH)$); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 190.40, 170.25, 146.18 (d, *J* = 2.6 Hz), 128.55, 67.76 (dd, *J* = 38.3, 2.0 Hz, enriched, $^{13}\underline{C}H(^{18/16}OH)$), 39.82 ($CO\underline{C}H_3$), 38.52 (d, *J* = 38.4 Hz, enriched, $^{13}\underline{C}H_2$), 32.20 (d, *J* = 4.2 Hz), 28.29, 24.12 (t, *J* = 17.3 Hz, $^{13}CH_2\underline{C}H_2$), 23.67 (t, *J* = 19.2 Hz, $\underline{C}H_3{}^{13}CH(^{18/16}OH)$), 23.23; **MS** (CI, $NH_3$) 262 ($MH^+(^{16}O)$, 41.4), 264 ($MH^+$ ($^{18}O$), 77.8).

**NAC *(S)*-[2,3-$^{13}C_2$]-7-Hydroxyoctanoate 10**. The method for the conversion of **13** to **6** was adapted to transform **18** to **10** in 82% yield: $[\alpha]_D$ +3.90° (*c* 1.43, $CHCl_3$); IR ($CHCl_3$ cast) 3286 (br m), 2930 (s), 2857 (m), 1693 (s), 1646 (s), 1556 (m) $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 6.00 (br s, 1 H), 3.79 (m, 1 H), 3.42 (q, 2 H, *J* = 6.3 Hz), 3.02 (t, 2 H, *J* = 6.4 Hz), 2.57 (dm, 2 H, *J* = 130.1 Hz, $^{13}C\underline{H}_2CO$), 1.96 (s, 3 H), 1.75 (br s, 1 H), 1.66 (dm, 2 H, *J* = 134.6 Hz, $^{13}C\underline{H}_2{}^{13}CH_2CO$), 1.50-1.30 (m, 6 H), 1.18 (d, 3 H, *J* = 6.2 Hz); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 199.97 (d, *J* = 45.3 Hz), 170.38, 67.72, 43.88 (d, *J* = 33.4 Hz, enriched, $^{13}\underline{C}H_2CO$), 39.55, 38.89, 28.77 (d, *J* = 34.6 Hz, $\underline{C}H_2{}^{13}CH_2$), 28.38, 25.46 (d, *J* = 33.4 Hz, enriched, $^{13}\underline{C}H_2{}^{13}CH_2CO$), 25.37, 23.48, 23.05; MS (CI, $NH_3$) *m/z* (relative intensity) 395 ($MNH_4^+$, 7), 378 ($MH^+$, 100); HRMS (EI) *m/z* (relative intensity) 362.2120 (4), 320.1626 (100).

**Ethyl [2,3-$^{13}C_2$]Acetoacetate 12**. A modification of the procedure of Cane and Block[43] was used. LiHMDS was prepared by the addition of *n*-BuLi (2.5 M in hexane, 20.5 mL, 51 mmol) to hexamethyldisilazane (HMDS) (10.8 mL, 51.2 mmol) in THF (20 mL) at -78 °C. After the mixture had been warmed to room temperature, the solvent and unreacted HMDS were removed *in vacuo*, and further dried under high vacuum. The white solid lithium salt was redissolved in THF (30 mL) at -78 °C. Ethyl [2-$^{13}$C] acetate (2.05 g, 23.0 mmol) was added and the solution stirred for 15 min at -78 °C. [1-$^{13}$C]Acetyl chloride (1.83g, 23.0 mmol) was introduced dropwise, and the mixture was stirred for 1 h at -78 °C. After warming to 20 °C, the reaction mixture was quenched by addition of 2N HCl (100 mL), and the solution was extracted with ether (3 x 100 mL). The combined organic layers were washed with brine, and dried ($Na_2SO_4$). After concentration *in vacuo* the resulting yellow residue was distilled to afford **12** (2.13 g, 70%): bp 75-78 °C (12 mm Hg); $^1H$ NMR (300 MHz, $CDCl_3$) δ 4.20 (q, 2 H), 3.45 (dd, 2 H, *J* = 130.2, 6.2 Hz, $^{13}CO^{13}C\underline{H}_2$), 2.27 (dd, 3 H, *J* = 7.4, 1.4 Hz, $C\underline{H}_3{}^{13}CO$), 1.29 (t, 3 H, *J* = 7.2 Hz); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 200.54 (d, *J* = 38.5 Hz, enriched, $CH_3{}^{13}\underline{C}O$), 167.07, 61.36, 50.12 (d, *J* = 37.7 Hz, enriched, $^{13}\underline{C}H_2CO$), 26.79 ($\underline{C}H_3{}^{13}CO$), 14.06; HRMS (EI) *m/z* (relative intensity) calcd for $^{13}C_2C_4H_{10}O_3$ 132.0697, found 132.0712 ($M^+$, 13), 87.0354 (24).

**Ethyl *(S)*-[2,3-$^{13}C_2$]-3-(*tert*-Butyldimethylsiloxy)butyrate 13.** A modification of the procedure of Mori was employed.[44] Thus ethyl [2,3-$^{13}$C2]acetoacetate **12** (1.60 g, 12.1 mmol) in 98% EtOH (10 mL) was added to a vigorously stirred solution of glucose (22.0 g) and baker's yeast (Fleischmann's, 20.0 g) in 0.1M

potassium phosphate buffer (320 mL, pH 7) at 30 °C. The solution was stirred at 30 °C for ca. 4 h until the extract showed a negative test on $FeCl_3/CHCl_3$. Celite 545 (20 g) and ether (100 mL) were added to the solution, and the mixture was filtered through a pad of Celite 545. The filtrate was then extracted with ether (6 x 200 mL). The combined organic phases were washed with brine (200 mL), dried ($Na_2SO_4$), and concentrated. The resulting residue was distilled under reduced pressure to yield the alcohol (1.03 g, 64%). Bp 70-72 °C (10 mm Hg). The above alcohol (1.17 g, 8.79 mmol), *tert*-butyldimethylsilyl chloride (1.60 g, 10.6 mmol) and imidazole (900 mg, 13.2 mmol) were dissolved in DMF (20 mL), and the solution was stirred at room temperature overnight. The mixture was diluted with *n*-hexane (100 mL) and then washed with water (3 x 100 mL). The dried ($Na_2SO_4$) organic layer was concentrated and distilled *in vacuo* to give **13** (2.15 g, 99%): bp 95-100 °C (8 mm Hg); IR ($CH_2Cl_2$ cast) 2957 (m), 2930 (m), 2858 (m), 1740 (s), 1255 (m) $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 4.46 and 4.11 (dm, 1 H, $J$ = 140 Hz, $^{13}C\underline{H}(OSi)$), 4.12 (dq, 2 H, $J$ = 7.1, 4.0 Hz), 2.63 and 2.31 (dm, 1H, $J$ = 130 Hz, $^{13}C\underline{H}H$ ), 2.52 and 2.21 (dm, 1 H, $J$ = 127 Hz, $^{13}CH\underline{H}$), 1.27 (t, 3 H, $J$ = 7.1 Hz,), 1.20 (dt, 3 H, $J$ = 6.0, 4.4 Hz, $C\underline{H}_3{}^{13}CH(OSi)$), 0.89 (s, 9 H, $(C\underline{H}_3)_3C$), 0.08 (s, 6 H); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 171.68 (t, $J$ = 28.5 Hz), 65.84 (d, $J$ = 38.8 Hz, enriched, $^{13}\underline{C}H(OSi)$), 60.23, 44.95 (d, $J$ = 38.9 Hz, $^{13}\underline{C}H_2$), 25.70, 23.70 (t, $J$ = 19.5 Hz, $\underline{C}H_3{}^{13}CH(OSi)$), 17.92, 14.16, -4.54 and -5.07; MS (CI, $NH_3$) 249 ($MH^+$, 48).

**Methyl (*S*)-[2,3-$^{13}C_2$]-5-(*tert*-Butyldimethylsilyloxy)hex-2-enoate 14**. To a cold (-78 °C) solution of ester **13a** (prepared in the same fashion as **13**) (5.42 g, 21.9 mmol) in $CH_2Cl_2$ (100 mL) was added DIBAL (20.0 mL, 109 mmol) in $CH_2Cl_2$ (30 mL). The solution was stirred at -78 °C for 1.5 h, warmed to -30 °C and then quenched by adding methanol. The mixture was diluted with ether (200 mL), and washed with saturated aqueous potassium sodium tartrate (4 x 100 mL), brine (2 x 100 mL). The dried ($Na_2SO_4$) ether layer was concentrated *in vacuo* to give the alcohol (3.89 g, 87%), which was subsequently oxidized by Swern procedure. To a cold (-78 °C) solution of oxalyl chloride (2.36 mL, 26.7 mmol) in $CH_2Cl_2$ (50 mL) was added DMSO (3.80 mL, 53.4 mmol) in $CH_2Cl_2$ (15 mL). After 5 min the above alcohol (3.22 g, 15.7 mmol) in $CH_2Cl_2$ (20 mL) was added. The mixture was stirred at -78 °C for 45 min before triethylamine (22 mL, 160 mmol) was added, and then warmed to room temperature. The mixture was diluted with ether (200 mL), washed with 1N HCl (3 x 150 mL), brine (150 mL). The dried ($Na_2SO_4$) ether layer was concentrated and purified by $SiO_2$ chromatography (5:1 hexane-$Et_2O$) to afford the aldehyde (1.84 g, 79%). The aldehyde (1.62 g, 7.98 mmol) and [2-$^{13}$C](carbomethoxymethylene)triphenylphosphorane (2.95 g, 8.78 mmol) were dissolved in benzene (60 mL). The mixture was heated under reflux overnight (ca. 16h). The precipitate formed was removed by filtration, and washed with hexane. The filtrate was then concentrated and purified by $SiO_2$ chromatography (5:1 hexane-$Et_2O$) to afford **14** (2.02 g, 97%), exclusively as the *E*-isomer: IR ($CH_2Cl_2$ cast) 2955 (s), 2930 (s), 2858 (m), 1727 (s), 1663 (m), 837 (s) $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 6.96 (ddtd, 1 H, $J$ = 155.6, 15.7, 7.5, 1.8 Hz, $^{13}C\underline{H}=^{13}CHCO$), 5.84 (dd, 1 H, $J$ = 162.2, 15.7 Hz, $^{13}CH=^{13}C\underline{H}CO$), 3.93 (m, 1 H), 3.73 (s, 3 H), 2.38-2.26 (m, 2 H), 1.16 (d, 3 H, $J$ = 6.1 Hz), 0.88 (s, 9 H), 0.05 (s, 3 H, 0.04 (s, 3 H; $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 166.78 (d, $J$ = 56.6 Hz), 146.27 (d, $J$ = 70.5 Hz, enriched, $^{13}\underline{C}H=^{13}CHCO$), 122.76 (d, $J$ = 70.5 Hz, enriched, $^{13}CH=^{13}\underline{C}HCO$), 67.58, 51.35, 42.43 (d, $J$ = 43.0 Hz), 25.78, 23.71, 18.05, -4.57 and -4.85; MS (CI, $NH_3$) *m/z* (relative intensity) 278 ($MNH_4^+$, 100), 259 ($MH^+$, 48); HRMS (EI) *m/z* (relative intensity) 245.1478 (5), 203.1012 (90), 159.1202 (85), 133.0686 (100).

**Methyl (*S*)-[2,3-$^{13}C_2$]-5-(*tert*-Butyldimethylsilyloxy)hexanoate 15**. Under an atmosphere of $H_2$, the unsaturated ester **14** (1.02 g, 3.95 mmol) in EtOAc (25 mL) was stirred in the presence of palladium catalyst (10% Pd/C, 100 mg) for 2 h. The mixture was then filtered, and washed with EtOAc. After the concentration *in vacuo*, the crude product was purified with $SiO_2$ column chromatography (5% $Et_2O$ in hexane) to give **15** as an oil (0.97 g, 95%): IR ($CHCl_3$ cast) 2955 (s), 2930 (s), 2858 (s), 1744 (s) $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 3.80 (m, 1 H), 3.67 (s, 3 H), 2.30 (dm, 2 H, $J$ = 127.8 Hz, $^{13}C\underline{H}_2CO$), 1.81 (m, 1 H, $^{13}C\underline{H}H^{13}CH_2CO$), 1.60-1.35 (m, 3 H, $^{13}CH\underline{H}^{13}CH_2CO$), 1.12 (d, 3 H, $J$ = 6.1 Hz), 0.88 (s, 9 H), 0.05 (s, 6 H); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 174.21 (d, $J$ = 57.3 Hz), 68.14, 51.40, 38.97 (d, $J$ = 35.0 Hz), 34.09 (d, $J$ = 34.1 Hz, enriched, $^{13}CH_2{}^{13}\underline{C}H_2CO$), 25.87, 23.69, 21.19 (d, $J$ = 34.1 Hz, enriched, $^{13}\underline{C}H_2{}^{13}CH_2CO$), 18.09, -4.42 and -4.77; MS (CI, $NH_3$) *m/z* (relative intensity) 280 ($MNH_4^+$, 1), 263 ($MH^+$, 61); HRMS (EI) *m/z* (relative intensity) 231.1695 (11), 205.1168 (29).

**Methyl (*E* and *Z*)-(*S*)-[2,3-$^{13}C_2$]-7-(*tert*-Butyldimethylsilyloxy)oct-2-enoate 17**. The procedure employed for the conversion of **13a** to **14** was followed to transform **15a** to **17** in 53% yield. A mixture of *E*- and *Z*-isomers (*E*:*Z* = 64:36) was obtained, which was separated with $SiO_2$ chromatography. For *E*-isomer: $[\alpha]_D$ +11.0° (*c* 2.08, $CHCl_3$); IR (neat) 2952 (s), 2930(s), 2857 (m), 1727 (s), 1256 (m), 836 (s) $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$) δ 6.97 (ddt, 1 H, $J$ = 154.1, 15.5, 7.1 Hz, $^{13}C\underline{H}=^{13}CHCO$), 5.82 (ddt, 1 H, $J$ = 160.1, 15.5, 1.5 Hz, $^{13}CH=^{13}C\underline{H}CO$), 3.80 (m, 1 H), 3.71 (s, 3 H), 2.21 (m, 2 H, $C\underline{H}_2{}^{13}CH$), 1.75-1.32 (m, 4 H), 1.12 (d, 3 H, $J$ = 6.1 Hz), 0.89 (s, 9 H), 0.05 (s, 6 H); $^{13}C$ NMR (50 MHz, $CDCl_3$) δ 166.95 (d, $J$ = 75.5 Hz), 149.19 (d, $J$ = 70.5 Hz, enriched, $^{13}\underline{C}H=^{13}CHCO$), 120.59 (d, $J$ = 70.5 Hz, enriched, $^{13}CH=^{13}\underline{C}HCO$), 67.98, 51.03, 38.86, 32.02 (d, $J$ = 41.3 Hz, $\underline{C}H_2{}^{13}CH$), 25.68, 24.00, 23.66, 17.88, -4.53 and -4.89; MS (CI, $NH_3$) 306 ($MNH_4^+$, 85), 289 ($MH^+$, 53); MS (EI) 273.1798 (3), 231.1322 (100).

For *Z*-isomer: $[\alpha]_D$ +8.64° (*c* 1.77, $CHCl_3$); IR ($CH_2Cl_2$ cast) 2953 (s), 2929(s), 2857 (m), 1726 (s), 1171 (s), 836 (s), $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$) δ 6.15 (ddt, 1 H, $J$ = 152.1, 11.5, 7.5 Hz, $^{13}C\underline{H}=^{13}CHCO$), 5.71 (dd, 1 H, $J$ = 164.1, 11.5 Hz, $^{13}CH=^{13}C\underline{H}CO$), 3.74 (m, 1 H), 3.63 (s, 3 H), 2.60 (m, 2 H, $C\underline{H}_2{}^{13}CH$), 1.65-1.25 (m, 4 H), 1.06 (d, 3 H, $J$ = 6.2 Hz), 0.82 (s, 9 H), -0.02 (s, 6 H); $^{13}C$ NMR (50 MHz, $CDCl_3$) δ 166.80 (d, $J$ = 75.5 Hz), 150.48 (d, $J$ = 69.4 Hz, enriched, $^{13}\underline{C}H=^{13}CHCO$), 119.43 (d, $J$ = 69.4 Hz, enriched, $^{13}CH=^{13}\underline{C}HCO$), 68.19), 50.83, 39.11, 28.80 (d, $J$ = 40.6 Hz, $\underline{C}H_2{}^{13}CH$), 25.82, 24.40, 23.73, 18.02, -4.42 and -4.84; MS (CI, $NH_3$) *m/z* (relative intensity) 306 ($MNH_4^+$, 85), 289 ($MH^+$, 53); HRMS (EI) *m/z* (relative intensity) 273.1798 (3), 231.1322 (100).

**Methyl (*S*)-[6,7-$^{13}C_2$,7-*siloxy*-$^{18}O$]-7-(*tert*-Butyldimethylsiloxy)oct-2-enoate 17b.** The ketoester **19** (333 mg, 1.96 mmol) was dissolved in dry EtOH (5 mL), and to this solution $NaBH_4$ (152 mg, 4 mmol) in EtOH (2 mL) was added at 0 °C. The mixture was stirred for 1.5 h at 0 °C and 3.5 h at room temperature. Aqueous 0.5N HCl (20 mL) was added to the reaction mixture. The aqueous phase was extracted with ether (5 x 20 mL), the combined organic phases were dried over $Na_2SO_4$ and concentrated to afford the racemic alcohol (309 mg, 89%). A modification of the procedure of Klibanov and coworkers was used to resolve the *S*-form.[28] A mixture of racemic alcohol (288 mg, 1.70 mmol), trichloroethyl butyrate (0.44 g, 2 mmol), and porcine pancreatic lipase (1.7 g, predried 3 days at high vacuum before use) in dry ether (10 mL) was stirred at room temperature for 20 h. The mixture was filtered, and the filtrate was concentrated chromatographed on silica to afford the *S*-alcohol (140.6 mg, 49%). The protection procedure for the conversion

of **12** to **13** was then applied to convert the *S*-alcohol (137 mg, 0.777 mmol) to **17b** (163 mg, 72%): IR ($CH_2Cl_2$ cast) 2952 (m), 2929 (m), 2857 (m), 1728 (s), 1568 (m) $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 6.97 (dt, 1 H, *J* = 15.6, 7.0 Hz), 5.82 (dt, 1 H, *J* = 15.6, 1.3 Hz), 3.91 and 3.61 (dm, 1 H, *J* = 140 Hz, $^{13}C\underline{H}(^{18/16}OSi)$), 2.23 (m, 2 H), 1.6-1.3 (m, 4 H, $^{13}C\underline{H_2}C\underline{H_2}$), 1.12 (dt, 3 H, *J* = 6.0, 4.4 Hz, $C\underline{H_3}^{13}CH(^{18/16}OSi)$), 0.87 (s, 9 H), 0.07 (s, 6 H); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 167.18, 149.58 (d, *J* = 3.0 Hz), 120.93, 68.20 (dd, *J* = 39.2, 3.0 Hz, enriched, $^{13}\underline{C}H(^{18/16}OSi)$), 51.38 ($O\underline{C}H_3$), 39.11 (d, *J* = 39.4 Hz, enriched, $^{13}\underline{C}H_2$), 32.24 (d, *J* = 4.3 Hz), 25.88, 24.19 (t, *J* = 17.5 Hz, $^{13}CH_2\underline{C}H_2$), 24.01 (t, *J* = 19.3 Hz, $\underline{C}H_3{}^{13}CH(^{18/16}OSi)$), 18.11, -4.37 and -4.73; MS (CI, $NH_3$) 289 ($MH^+(^{16}O)$, 5.9), 291 ($MH^+(^{18}O)$, 9.3).

**Methyl (*S*)-[2,3-$^{13}C_2$]-7-(*tert*-Butyldimethylsilyloxy)octanoate 18**. The procedure employed for the conversion of **14** to **15** was used. Thus, unsaturated ester **17** as a mixture of *E* and *Z* isomers (517 mg, 1.79 mmol) was hydrogenated in the presence of 10% Pd/C (55 mg) to afford **18** (488 mg, 94%): $[\alpha]_D$ +8.93° (*c* 1.49, $CHCl_3$); IR ($CHCl_3$ cast) 2930 (s), 1743 (s), 1255 (m), 1051 (m), 836 (s), 774 (m) $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 3.76 (m, 1 H), 3.66 (s, 3 H), 2.30 (dm, 2 H, *J* = 127.6 Hz, $^{13}C\underline{H_2}CO$), 1.63 (dm, 2 H, *J* = 136.0 Hz, $^{13}C\underline{H_2}{}^{13}CH_2CO$), 1.50-1.20 (m, 6 H), 1.10 (d, 3 H, *J* = 6.0 Hz), 0.89 (s, 9 H), 0.04 (s, 6 H); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 174.03 (d, *J* = 56.6 Hz), 68.47), 51.37, 39.50, 34.03 (d, *J* = 33.9 Hz, enriched, $^{13}\underline{C}H_2CO$), 29.20 (d, *J* = 34.6 Hz, $\underline{C}H_2{}^{13}CH_2$), 25.87, 25.34, 24.93 (d, *J* = 33.9 Hz, enriched, $^{13}\underline{C}H_2{}^{13}CH_2CO$), 23.78, 18.11, -4.43 and -4.75; MS (CI, $NH_3$) *m/z* (relative intensity) 308 ($MNH_4^+$, 4), 291 ($MH^+$, 64); HRMS (EI) *m/z* (relative intensity) 275.1959 (4), 259.2002 (10) 233.1483 (78), 201.1220 (100).

**Methyl [6,7-$^{13}C_2$,7-oxo-$^{18}O$]-7-Oxo-2-octenoate 19**. The silyl ether **17a** (753 mg, 2.61 mmol) was deprotected in the same way as in conversion of **13** to **6**. Column chromatography (silica, EtOAc) afforded the free alcohol (394 mg, 87%) along with recovery of **17a** (93.0 mg, 12%). To a solution of the alcohol (387 mg, 2.22 mmol) in $CH_2Cl_2$ (10 mL) was added 4 Å molecular sieves (1 g) and PCC (0.65 g, 3.0 mmol). The mixture was stirred for 80 min at room temperature, then poured into ether (100 mL) and filtered. The filtrate was concentrated to give the keto compound (343 mg, 90%). A modification of the literature procedure[27] was used for isotope exchange. A mixture of the keto compound (338 mg, 1.96 mmol), $H_2{}^{18}O$ (97% $^{18}O$, 500 mg, 25.0 mmol, Cambridge Isotope Laboratories, Andover MA), THF (2 mL), and $CF_3CO_2H$ (10 μL) was heated at 70 °C for 9 h. The mixture was transferred to a separatory funnel and the organic phase was collected, dried ($Na_2SO_4$), and concentrated to give **19** (99% recovery), which was used immediately for the next reaction.

**Ethyl (*S*)-9-(*tert*-Butyldimethysilyloxy)-3-oxodec-4-enoate 22**. To a suspension of NaH (60% dispersion in mineral oil, 33 mg, 0.79 mmol) in THF (5 mL) was added a solution of **21** (105 mg, 0.395 mmol) prepared by literature method.[29] After 30 min stirring, the aldehyde **20** (91 mg, 0.40 mmol) in THF (5 mL) was added. The mixture was then stirred at room temperature overnight. The reaction mixture was acidified to pH 2 with 2N HCl, then extracted with $CH_2Cl_2$ (3 x 20 mL). The combined organic extracts were washed with brine, and dried ($Na_2SO_4$). Purification by $Si_2O$ column chromatography (5% $Et_2O$ in hexane) gave **22** (68 mg, 50%): IR ($CHCl_3$ cast) 2930 (m), 2857 (m), 1744 (m), 1666 (m), 1599 (m), 1236 (s), 836 (s) $cm^{-1}$; $^1H$ NMR (360 MHz, $CDCl_3$) keto:enol = 30:70; for keto form δ 6.88 (dt, 1 H, *J* = 15.9, 6.9 Hz, C<u>H</u>=CHCO), 6.16 (dt, 1 H, *J* = 15.9, 1.5 Hz), 4.20 (q, 2 H, *J* = 7.1 Hz), 3.79 (m, 1 H), 3.58 (s, 2 H), 2.22 (m, 2 H), 1.70-1.40 (m, 4 H),

1.27 (t, 3 H, $J$ = 7.1 Hz), 1.12 (d, 3 H, $J$ = 6.1 Hz), 0.89 (s, 9 H), 0.05 (s, 6 H); for enol form δ 11.91 (d, 1 H $J$ = 1.5 Hz), 6.65 (dt, 1 H, $J$ = 15.5, 7.1 Hz), 5.75 (dd, 1 H, $J$ = 15.5, 1.5 Hz), 4.98 (s, 1 H), 4.21 (q, 2 H, $J$ = 7.1 Hz), 3.79 (m, 1 H), 2.19 (m, 2 H), 1.70-1.40 (m, 4 H), 1.30 (t, 3 H, $J$ = 7.1 Hz), 1.12 (d, 3 H, $J$ = 6.1 Hz), 0.89 (s, 9 H), 0.05 (s, 6 H); $^{13}$C NMR (75 MHz, $CDCl_3$) for keto form δ 192.11, 167.40, 149.80, 129.64, 68.16, 61.29, 46.97, 39.05, 32.59, 25.87, 24.10, 23.80, 18.10, 14.08, -4.39 and -4.74; for enol form δ 173.01, 169.53, 140.91, 124.42, 90.00, 68.30, 60.00, 39.13, 32.59, 25.87, 24.65, 23.80, 18.10, 14.27, -4.39 and -4.74; MS (CI, $NH_3$) *m/z* (relative intensity) 360 ($MNH_4^+$, 10), 343 ($MH^+$, 3); HRMS (EI) *m/z* (relative intensity) calcd for $C_{18}H_{34}O_4Si$ 342.2226, found 342.2224 ($M^+$, 0.8), 327.1988 (3), 285.1526 (100).

**NAC (*S*)-9-(*tert*-Butyldimethylsilyloxy)-3-oxodec-4-enoate *O*-Methyloxime 23.** To a solution of **22** (19.4 mg, 0.0567 mmol) and O-methylhydroxylamine hydrochloride (8.0 mg, 0.091 mmol) in methanol (1 mL) was added pyridine (10 μl, 0.12 mmol). The mixture was then stirred at room temperature for 3 h. After the addition of 0.25 M HCl (10 mL), the solution was extracted with $CHCl_3$ (3 x 10 mL). The combined extracts were dried ($Na_2SO_4$) and concentrated to give the TBDMS-protected *O*-methyloxime ethyl ester (18.0 mg, 86%) as a colorless oil. The procedure for the conversion for **12** to **5** was used to transform the ethyl ester (18.0 mg, 0.048 mmol) to the NAC ester **23** (13 mg, 55% from **22**): IR ($CHCl_3$ cast) 3280 (br w), 2929 (s), 2855 (m), 1654 (s), 1554 (m), 1053 (s), $cm^{-1}$; $^1$H NMR (360 MHz, $CD_2Cl_2$) δ 6.18-5.99 (m, 2 H), 5.77 (br s, 1 H), 3.88 (s, 3 H), 3.80 (m, 1 H), 3.76 (s, 2 H), 3.37 (q, 2 H, $J$ = 6.3 Hz), 3.00 (t, 2 H), 2.17 (m, 2 H), 1.90 (s, 3 H), 1.80-1.23 (m, 4 H), 1.10 (d, 3 H, $J$ = 6.0 Hz), 0.88 (s, 9 H), 0.05 (s, 6 H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ 194.35, 170.17, 151.56, 137.75, 126.46, 68.68, 62.24, 40.06, 39.67, 39.47, 33.28, 29.14, 26.02, 25.31, 23.95, 23.27, 18.33, -4.34 and -4.67; MS (FAB, Cleland) *m/z* (relative intensity) 445 ($MH^+$, 53), 194 (100).

**10-Dihydrocurvularin 30.** To a solution of curvularin **2** (103 mg, 0.352 mmol) in 95% ethanol (20 mL) cooled to -15 °C was slowly added a solution of $NaBH_4$ (20 mg, 0.53 mmol) in 95% ethanol (5 mL). The mixture was stirred at 0 °C for 1 h before a saturated solution of $NH_4Cl$ was added. Ethanol was then removed *in vacuo*, and the residue was extracted four times with $CHCl_3$, then dried ($Na_2SO_4$). Flash chromatography on $SiO_2$ (2:1 hexane-EtOAc) gave two fractions, which were identified as the two isomers of **30**, the less polar isomer (35.5 mg, 35%) and the more polar isomer (38.8 mg, 38%). For the less polar isomer: IR (KBr) 3452 (s), 3393 (s), 3271 (br m), 2940 (m), 1700 (s), 1655 (m), 1460 (m), 1318 (s), 1135 (s) $cm^{-1}$; $^1$H NMR (300 MHz, acetone-$d_6$) δ 8.86 (br s, 1 H), 8.11 (br s, 1 H), 6.28 (d, 1 H, $J$ = 2.8 Hz), 6.22 (d, 1 H, $J$ = 2.8 Hz), 5.32 (br s, 1 H), 5.14-5.04 (m, 2 H), 3.65 (d, 2 H), 3.38 (br d, 1 H, $J$ = 17.0 Hz), 1.91-1.09 (m, 10 H), 1.19 (d, 3 H, $J$ = 7.0 Hz); $^{13}$C NMR (50 MHz, acetone-$d_6$) δ 173.53, 158.82, 158.16, 134.05, 121.21, 111.78, 104.13, 78.81, 69.56, 40.29, 34.88, 32.96, 27.57, 24.54, 23.53, 20.53; HRMS (EI) *m/z* (relative intensity) calcd for $C_{16}H_{22}O_5$ 294.1467, found 294.1486 ($M^+$, 13), 292 (45), 205 (3), 195 (100), 167 (94), 150 (65).

**10-Deoxycurvularin 31.** Under an atmosphere of $H_2$ a solution of **30** (10 mg, 0.034 mmol) and 10% palladium over carbon (8 mg) in ethyl acetate (2 mL) was stirred for 5 h. After filtration and evaporation of solvent the crude material was purified with column chromatography (3:2 $CHCl_3$-EtOAc) to afford **31** (7.0 mg, 70%). IR (KBr) 3405 (br s), 2929 (m), 1702 (s), 1629 (m), 1600 (m), 1318 (m), 1208 (m), 1134 (s) $cm^{-1}$; $^1$H NMR (400 MHz, acetone-$d_6$) δ 7.99 (s, 1 H), 7.89 (s, 1 H), 6.31 (d, 1 H, $J$ = 2.4 Hz), 6.28 (d, 1 H, $J$ = 2.4

Hz), 5.07 (m, 1 H), 3.69 (d, 1 H, $J$ = 15.5 Hz), 3.36 (d, 1 H, $J$ = 15.5 Hz), 2.54 (m, 2 H), 1.84-1.69 (m, 2 H), 1.66-1.49 (m, 2 H), 1.14-1.10 (m, 4 H), 1.18 (d, 3 H, $J$ = 6.3 Hz); $^{13}$C NMR (100 MHz, acetone-$d_6$) δ 171.99, 157.21, 156.39, 135.80, 120.03, 111.21, 102.45, 73.32, 40.29, 33.95, 27.39, 27.34, 26.91, 25.36, 25.12, 20.86; MS (EI) *m/z* (relative intensity) calcd for $C_{16}H_{22}O_4$ 278.1518, found 278.1514 ($M^+$, 71), 219.1383 (8), 207.0657 (20), 181.0500 (100).

## REFERENCES AND NOTES


1. (a) Musgrave, O. C. *J. Chem. Soc.* **1956**, 4301-4305. (b) Munro, H. D.; Musgrave, O. C.; Templeton, R. *J. Chem. Soc. (C)* **1967**, 947-948.
2. (a) Lai, S.; Shizuri, Y.; Yamamura, S.; Kawai, K.; Terada, Y.; Furukawa, H. *Chem. Lett.* **1990**, 589-592. (b) Lai, S.; Shizuri, Y.; Yamamura, S.; Kawai, K.; Terada, Y.; Furukawa, H. *Tetrahedron Lett.* **1989**, *30*, 2241-2144. (c) Kobayashi, A.; Hino, T.; Yata, S.; Itoh, T.; Sato, H.; Kawazu, K. *Agric. Biol. Chem.* **1988**, *52*, 3119-3123. (d) Raistrick, H.; Rice, F. A. H. *J. Chem. Soc. (C)* **1971**, 3069-3070.
3. Ghisalberti, E. L.; Rowland, C. Y. *J. Nat. Prod.* **1993**, *56*, 2175-2177.
4. (a) Robeson, D. J.; Strobel, G. A.; Strange R. N. *J. Nat. Prod.* **1985**, *48*, 139-141. (b) Robeson, D. J.; Strobel, G. A. *Z. Naturforsch.* **1981**, *36*, 1081-1083. (c) Hyeon, S.-B.; Ozaki, A.; Suzuki, A.; Tamura, S. *Agric. Biol. Chem.* **1976**, *40*, 1663-1664.
5. For leading references see: (a) Fürstner, A.; Kindler, N. *Tetrahedron Lett.* **1996**, *37*, 7005-7008. (b) Bracher, F. Schulte, B. *J. Chem. Soc., Perkin Trans. 1* **1996**, 2619-2622. (c) Jones, G. B.; Huber, R. S. *Synlett.* **1993**, 367-368.
6. Turner, W. B.; Aldridge, D. C. *Fungal Metabolites II*; Academic Press: New York, 1983, pp. 167-178.
7. Williams, D. H.; Wilkinson, S. E.; Purton, T.; Lamont, A.; Flotow, H.; Murray, E. J. *Biochemistry* **1998**, *37*, 9579-9585.
8. Curvularin synthesis: (a) Bracher, F.; Schulte, B. *Leibigs Ann.* **1997**, *9*, 1979-1982. (b) Bracher, F.; Schulte, B. *Nat. Prod. Lett.* **1995**, *7*, 65-68. (c) Gerlach, H. *Helv. Chim. Acta* **1977**, *60*, 3039-3044.
9. (a) Almassi, F.; Ghisalberti, E. L.; Skelton, B. W.; White, A. H. *Aust. J. Chem.* **1994**, *47*, 1193-1197. (b) Ghisalberti, E. L.; Hockless, D. C. R.; Rowland, C.; White, A. H. *Aust. J. Chem.* **1993**, *46*, 571-575.
10. Arai, K.; Rawlings, B. J.; Yoshizawa, Y.; Vederas, J. C. *J. Am. Chem. Soc.* **1989**, *111*, 3391-3399.
11. For preliminary communications see: (a) Li, Z.; Martin, F. M.; Vederas, J. C. *J. Am. Chem. Soc.* **1992**, *114*, 1531-1533. (b) Li, Z.; Martin, F. M.; Reese, P. B.; Yoshizawa, Y.; Vederas, J. C. In *Biosynthesis and Metabolism of Secondary Metabolite Natural Products*, Petroski, R. J. and McCormick, S. P. Eds., Plenum: New York, 1992, pp 27-39 (c) Yoshizawa, Y.; Li, Z.; Reese, P. B.; Vederas, J. C. *J. Am. Chem. Soc.* **1990**, *112*, 3212-3213.
12. For acetate incorporation experiments into curvularin type metabolites by a hybrid strain of *Penicillium citreoviride B* see: Lai, S.; Shizuri, Y.; Yamamura, S.; Kawai, K.; Niwa, M.; Furukawa, H. *Heterocycles*, **1991**, *32*, 307-310.
13. For recent reviews see: (a) Staunton, J.; Wilkinson, B. In *Biosynthesis: Polyketides and Vitamins, Topics in Current Chemistry*; Leeper, F. J., Vederas, J. C., Eds.; Springer-Verlag: Berlin, 1998, pp.49-92.

(b) Hopwood, D. A. *Chem. Rev.* **1997**, *97*, 2465-2497. (c) Rawlings, B. J. *Nat. Prod. Rep.* **1997**, *14*, 523-556. (d) Staunton, J. *Curr. Opinion Chem. Biol.* **1998**, *2*, 339-345.

14. For recent leading references see: (a) Hitchman, T. S.; Crosby, J.; Byrom, K. J.; Cox, R. J.; Simpson, T. J. *Chem. Biol.* **1998**, *5*, 35-47. (b) Weissman, K. J.; Smith, C. J.; Hanefeld, U.; Aggarwal, R.; Bycroft, M.; Staunton, J.; Leadlay, P. *Angew. Chem. Int. Ed.* **1998**, *37*, 1437-1440. (c) Bao, W.; Wendt-Pienkowski, E.; Hutchinson, C. R. *Biochemistry* **1998**, *37*, 8132-8138. (d) Gokhale, R. S.; Lau, J.; Cane, D. E.; Khosla, C. *Biochemistry* **1998**, *37*, 2524-2528. (e) McPherson, M.; Khosla, C.; Cane, D. E. *J. Am. Chem. Soc.* **1998**, *120*, 3267-3268. (f) Schroeder, J.; Raiber, S.; Berger, T.; Schmidt, A.; Schmidt, J.; Soares-Sello, A. M.; Bardshiri, E.; Strack, D.; Simpson, T. J.; Veit, M.; Schroeder, G. *Biochemistry* **1998**, *37*, 8417-8425.
15. Bennett, J. W. *Can. J. Bot.* **1995**, *73*, S917-S924.
16. (a) Watanabe, C. M. H.; Townsend, C. A. *J. Am. Chem. Soc.* **1998**, *120*, 6231-6239. (b) Feng, G. H.; Leonard, T. J. *Appl. Environ. Microbiol.* **1998**, *64*, 2275-2277. (c) Brown, D. W.; Yu, J. H.; Kelkar, H. S.; Fernandes, M.; Nesbitt, T. C.; Keller, N. P.; Adams, T. H.; Leonard, T. J. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 1418-1422. (d) Yu, J. J.; Chang, P. K.; Cary, J. W.; Wright, M.; Bhatnagar, D.; Cleveland, T. E.; Payne, G. A.; Linz, J. E. *Appl. Environ. Microbiol.* **1995**; *61*, 2365-2371.
17. Offenzeller, M.; Santer, G.; Totschnig, K.; Su, Z.; Moser, H.; Traber, R.; Schneider-Scherzer, E. *Biochemistry* **1996**, *35*, 8401-8412.
18. For leading references see: (a) Child, C. J.; Shoolingin-Jordan, P. M. *Biochem. J.* **1998**, *330*, 933-937. (b) Bedford, D. J.; Schweizer, E.; Hopwood, D. A.; Khosla, C. *J. Bacteriol.* **1995**, *177*, 4544-4548. (c) Fujii, I.; Ono, Y.; Tada, H.; Gomi, K.; Ebizuka, Y.; Sankawa, U. *Molec. Gen. Genet.* **1996**, *253*, 1-10.
19. Yang, G.; Rose, M. S.; Turgeon, B. G.; Yoder, O. C. *Plant Cell*, **1996**, *8*, 2139-2150.
20. For reviews of isotopic methods to study secondary metabolism see: (a) Simpson, T. J. In *Biosynthesis: Polyketides and Vitamins, Topics in Current Chemistry*; Leeper, F. J., Vederas, J. C., Eds.; Springer-Verlag: Berlin, 1998, pp. 1-48. (b) Vederas, J. C. *Nat. Prod. Rep.* **1987**, *4*, 277-337.
21. (a) Yue, S.; Duncan, J. S.; Yamamoto, Y.; Hutchinson, C. R. *J. Am. Chem. Soc.* **1987**, *109*, 1253-1255. (b) Cane, D. E.; Yang, C. C. *J. Am. Chem. Soc.* **1987**, *109*, 1255-1257.
22. Chuck, J.-A.; McPherson, M.; Huang, H.; Jacobsen, J. R.; Khosla, C.; Cane, D. E. *Chem. Biol.* **1997**, *4*, 757-766.
23. For leading references to β-oxidation in yeasts and fungi see: (a) Kunau, W. H.; Dommes, V.; Schulz, H. *Prog. Lipid Res.* **1995**, *34*, 267-342. (b) Henke, B,; Girzalsky, W.; Berteaux-Lecellier, V.; Erdmann, R. *J. Biol Chem.* **1998**; *273*, 3702-3711. (c) Mathieu, M.; Modis, Y.; Zeelen, J. P.; Engel, C. K.; Abagyan, R. A.; Ahlberg, A.; Rasmussen, B.; Lamzin, V. S.; Kunau, W. H.; Wierenga, R. K. *J. Molec. Biol.* **1997**; *273* : 714-728.
24. House, H. O.; Jones, V. K.; Frank, G. A. *J. Org. Chem.* **1964**, *29*, 3327-3333.
25. Corey, E. J.; Ponder, J. W.; Ulrich, P. *Tetrahedron Lett.* **1980**, *21*, 137-140.
26. (a) Mori, K.; Senda, S. *Tetrahedron* **1985**, *41*, 541-546. (b) Seebach, D.; Renaud, P. *Helv. Chim. Acta* **1985**, *68*, 2342-2349.
27. Diakur, J.; Nakashima, T. T.; Vederas, J. C. *Can. J. Chem.* **1980**, *58*, 1311-1315 and references therein.
28. Kirchner, G.; Scollar, M. P.; Klibanov, A. M. *J. Am. Chem. Soc.* **1985**, *107*, 7072-7076.

29. (a) Moorhoff, C. M.; Schneider, D. F. *Tetrahedron Lett.* **1987**, *28*, 559-562. (b) Ley, S. V.; Woodward, P. R. *Tetrahedron Lett.* **1987**, *28*, 345-346. (c) Pietrusiewicz, K. M.; Monkiewicz, J. *Tetrahedron Lett.* **1986**, *27*, 739-742. (d) Bodalski, R.; Pietrusiewicz, K. M.; Monkiewicz, J.; Koszuk, J. *Tetrahedron Lett.* **1980**, *21*, 2287-2290.
30. Sakamoto, T.; Kikugawa, Y. *Synthesis* **1993**, 563-564.
31. Corey, E. J.; Niimura, K.; Konishi, Y.; Hashimoto, S.; Hamada, Y. *Tetrahedron Lett.* **1986**, *27*, 2199-2202.
32. Li, D.; Zhou, H. Q.; Dakoji, S.; Shin, I. J.; Oh, E.; Liu, H. W. *J. Am. Chem. Soc.* **1998**, *120*, 2008-2017 and references therein.
33. Although **25** has not been directly demonstrated to inhibit β-oxidation enzymes, other alkynoic acids such as 4-pentynoic acid are effective at stopping precursor degradation by this route: see ref. 11b and Cane, D. E.; Prabhakaran, P. C.; Tan, W.; Ott, W. R. *Tetrahedron Lett.* **1991**, *32*, 5475-5460.
34. (a) Spydevold, O.; Bremer, J. *Biochim. Biophys. Acta* **1989**, *1003*, 72-79. (b) Skorve, J.; Ruyter, B.; Rustan, A. C.; Christiansen, E. N.; Drevon, C. A.; Berge, R. K. *Biochem. Pharmacol.* **1990**, *40*, 2005-2012. (c) Hovik, R.; Osmundsen, H.; Berge, R.; Aarsland, A.; Bergseth, S.; Bremer, J. *Biochem. J.* **1990**, *270*, 167-173. (d) Aarsland, A.; Berge, R. K. *Biochem. Pharmacol.* **1991**, *41*, 53-61.
35. Under typical fermentation conditions *A. cinerariae* can produce 200 mg of **1** and 50 mg of **3** per litre of culture broth.
36. For an example of unusually small $^{18}O$-induced isotope shifts in $^{13}C$ NMR spectra which required transformation to other functional groups for detection see: (a) Wagschal, K.; Yoshizawa, Y.; Witter, D. J.; Liu, Y.; Vederas, J. C. *J. Chem. Soc., Perkin Trans. 1* **1996**, 2357-2363. (b) Yoshizawa, Y.; Witter, D. J.; Liu, Y.; Vederas, J. C. *J. Am. Chem. Soc.* **1994**, *116*, 2693-2694.
37. (a) Oikawa, H.; Ichihara, A.; Sakamura, S. *J. Chem. Soc., Chem. Commun.* **1990**, 908-909. (b) Oikawa, H.; Ichihara, A.; Sakamura, S. *J. Chem. Soc., Chem. Commun.* **1988**, 600-602.
38. (a) Oikawa, H.; Murakami, Y.; Ichihara, A. *Tetrahedron Lett.* **1991**, *32*, 4533-4536. (b) Oikawa, H.; Murakami, Y.; Ichihara, A. *J. Chem. Soc., Perkin. Trans. I* **1992**, 2949-2953.
39. Peters, S.; Schmidt, W.; Beerhues, L. *Planta* **1998**, *204*, 64-69. (b) Kammerer, L.; De-Eknamkul, W.; Zenk, M. H. *Phytochemistry* **1994**, *36*, 1409-1416. (c) Engelhardt, D.; Weber, M. M. *J. Steroid Biochem. Mol. Biol.* **1994**, *49*, 261-267. (d) Pospisil, S.; Sedmera, P.; Havlicek, V.; Tax, J. *Appl. Environ. Microbiol.* **1994**, *60*, 1561-1564.
40. Reduction of metyrapone has been observed with S. cinnamonensis during studies on monensin biosynthesis, but in this case deoxymonensins A and B accumulated. See ref. 39d.
41. (a) General procedures and instrumentation have recently been described: see ref. 36a and Harris, J. M.; McDonald, R.; Vederas J. C. *J. Chem. Soc., Perkin Trans. 1*, **1996**, 2669-2674. (b) Hypoglycin **24** was a generous gift by Prof. E. A. Kean (University of the West Indies, Kingston, Jamaica). (c) β-Oxidation inhibitors **25-27** were synthesized by literature procedures.[33,34] (d) Ancymidol **28** and metyrapone **29** are now available from Sigma Chemical Co.
42. Hall, L. M. *Biochem. Prep.* **1963**, *10*, 1-3.
43. Block, M. H.; Cane, D. E. *J. Org. Chem.* **1988**, *53*, 4923-4928.
44. Mori, K. *Tetrahedron* **1981**, *37*, 1341-1342.