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Towards the synthesis of a tetraketide and an α-diketone; two biosynthetic precursors of the fungal metabolite oudenone

Fei Zhou

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in

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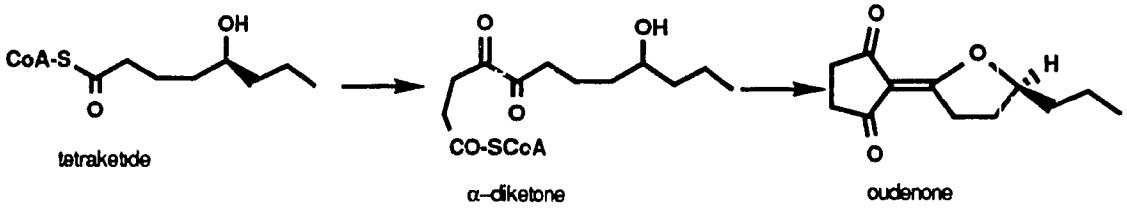
ABSTRACT

Synthesis of (S)-(-)-5-hydroxylthiolester derivative and the Biosynthesis of Oudenone, a Hypotensive Agent from *Oudemansiella radicata*

Fei Zhou

Oudenone **9** is a potent inhibitor of catecolamine biosynthesis, and as such, it exhibits strong hypotensive activity in test animals. As a fungal metabolite, it has an unusual structure composed of a 1,3-cyclopentanedione and a tetrahydrofuran ring moiety. Previous biosynthetic studies have suggested that the carbon skeleton of oudenone is derived from four acetate units and one succinate. The tetraketide **11** and α-diketone **10** are believed to be two advanced precursors of oudenone. The tetraketide **11** has been successfully synthesized as the NAC thioester derivative with a deuterium label at a specific position and the incorporation of **11** into the oudenone has been also achieved. A biomimetic approach leading to the synthesis of **10** is still under investigation.



11 **10** **9**

To My Lovely Wife Xiao-Jin ,
for her emotional support and understanding

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I would like to thank my supervisor Dr. Youla Tsantrizos for her encouragement, support, help and guidance during this project.

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Chapter 1. Introduction

1.1 General introduction

The chemistry of secondary metabolites has been of much interest to scientists and studied for over 3,000 years. The varied biological effects of natural products have been widely applied to the pharmaceutical, food and cosmetics industries. The determination of their chemical structure, as well as their biogenesis have been a challenging task. However structural assignments have become much simpler in recent years by modern spectroscopy.

Unlike the primary metabolites (e.g. amino acids, acetyl-CoA, sugars, nucleotides), which have a broad distribution in all living things and are involved in essential life processes, secondary metabolites do not appear to be essential to life.[1] Tens of thousands of secondary metabolites are known today, but little is known about their biosynthesis. Until the end of 1940's, *"Biosythetic"* studies were often limited to model experiments in chemical systems because of the great difficulty in following the metabolic fate of a compound in biological systems. This situation changed dramatically in the 1950's when the method of isotopic labelling was introduced.[2] In recent years, with the development of modern high resolution FT-NMR, the biosynthesis of natural products can be studied in great detail. Not only the primary precursors from which a metabolite is derived can be identified, but the advanced intermediates which lead to the formation of a metabolite can be followed.[3,4] Biosynthetic studies basically involve three major steps: a) administration (*feeding*) of isotopically labelled primary or advanced precursors, b) isolation of the labelled natural product (assuming that incorporation of the precursors has been achieved) and c) analysis of the labelling pattern in the structure of the metabolite by NMR.

The first step allows the identification of primary metabolites from which a metabolite is derived. The structure of a secondary metabolite often allows chemists to speculate their possible biological origin and perhaps the possible pathway by which they are constructed. A proposed biosynthetic scheme can subsequently be proven by feeding labelled primary precursors and intermediates.

For example, Oikawa and his coworkers recently reported the biosynthetic origins of solanapyrone A (**1**), a phytotoxin produced by the fungus *Alternaria solani*, using such labelling technique (Fig. 1).[5] Furthermore, they achieved the intact incorporation of the ^{2}H labelled diene-dienophile precursors [17-$^{2}H_2$, 18-$^{2}H_3$] (**4**) and [2-^{2}H, 3-^{2}H, 17-^{2}H, 18-$^{2}H_3$] (**3**), thus providing the first direct evidence of an enzyme catalyzed Diels-Alder reaction (Fig. 1).[6]

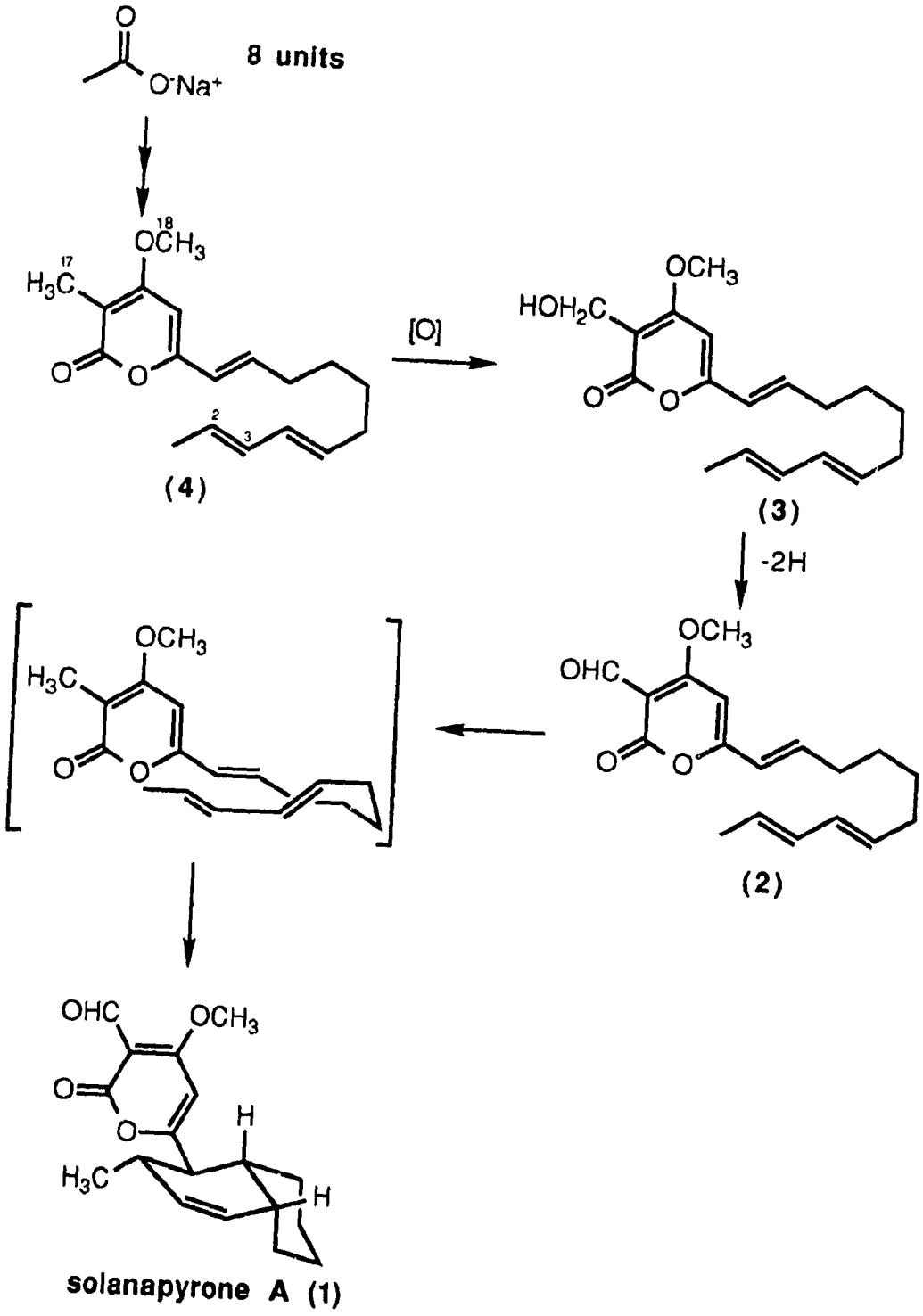


Figure 1. Biosynthesis of solanapyrone A (1).

1.2 Polyketide biosynthesis

Microorganisms and plants produce a collection of metabolites called polyketides that represent perhaps the largest group of secondary natural products[7]. Their diverse structures contain repeating acetate units and they are believed to be biosythesized by a mechanism analogous to the chain elongation steps of fatty acids biosynthesis.

In the biosynthesis of fatty acids the primary metabolites acetyl CoA and malonyl CoA are involved in the initiation and chain elongation processes, respectively. The biochemical reactions are catalyzed by a multi-enzyme complex, involved in the sequential addition, reduction of a carbonyl, dehydration and reduction of a double bond. These steps are repeated until the required length of the chain is formed (Fig. 2).

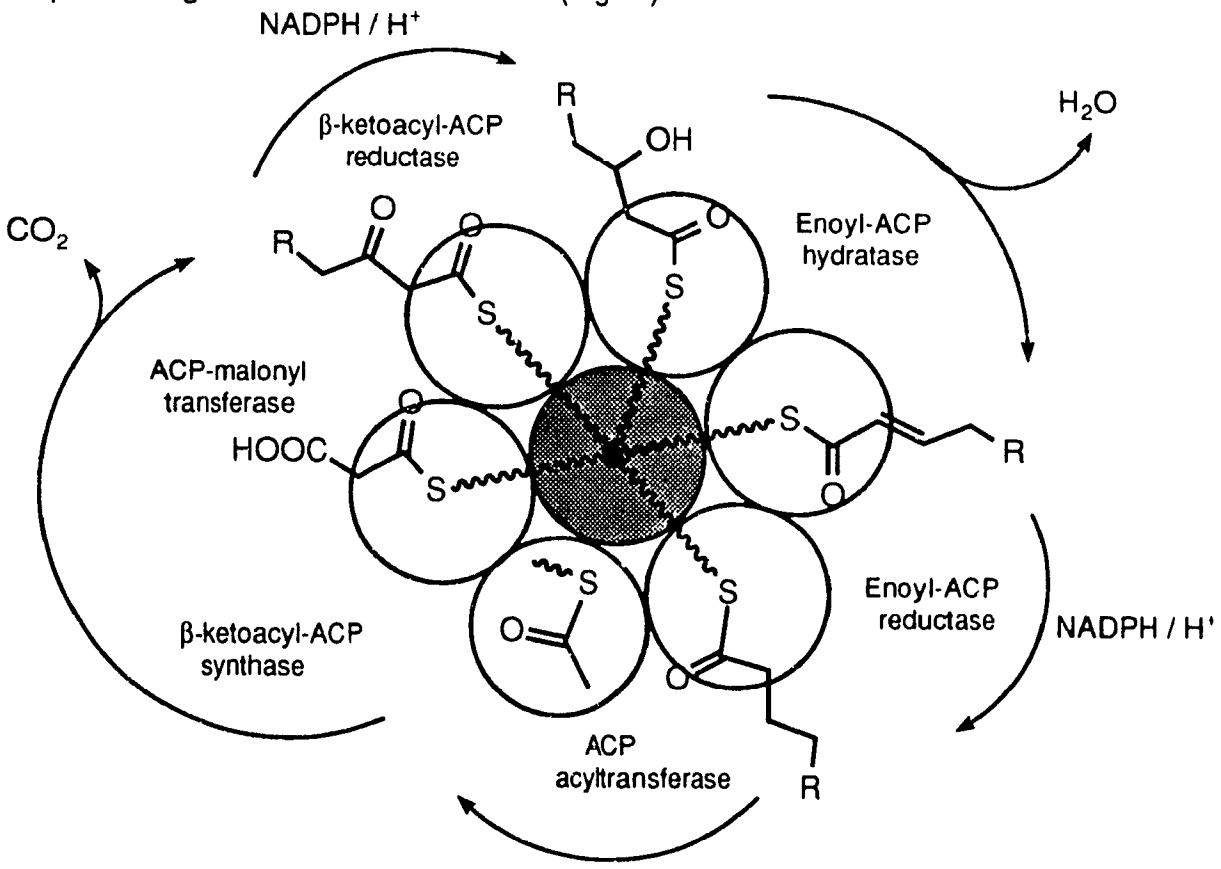


Figure 2. Fatty acid synthetase complex

In recent years, biosynthetic investigations have suggested that in polyketides the chain elongation process can bypass certain steps and that the oxidation level and stereochemistry at each carbon can be adjusted after each condensation.[3,4] This leads to the formation of different functional groups in the polyketide chain.

In 1990, Vederas and his coworkers[8,9] demonstrated the first example of intact incorporation of stable isotope labeled acetate derived *di-* and tetraketides, two intermediates believed to be involved in the biosynthesis of dehydrocurvularin, a macrolide phytotoxin from *Alternaria cineraiae*, provided another strong support for this hypothesis. In spite of these advances, the choice of substrates, the control of the reaction sequence, stereochemistry and termination of chain elongation in the polyketide biosynthesis are mostly unknown.[10]

Recently, Shen and Hutchinson[11] have reported the formation of the tetracenomycin(Tcm) F2 (8) from 1 acetyl CoA and 9 malonyl CoA units by an in *vitro* polyketide synthetase(PKS) preparation, which was generated by selective expression of Tcm PKS [TcmJKLMN] genes (Fig 3). Their results have provided a method for the investigation of substrate specificity, the detection of enzyme-bond biosynthetic intermediates and the generation of novel biosynthetic products through the manipulation of gene structure and organization.

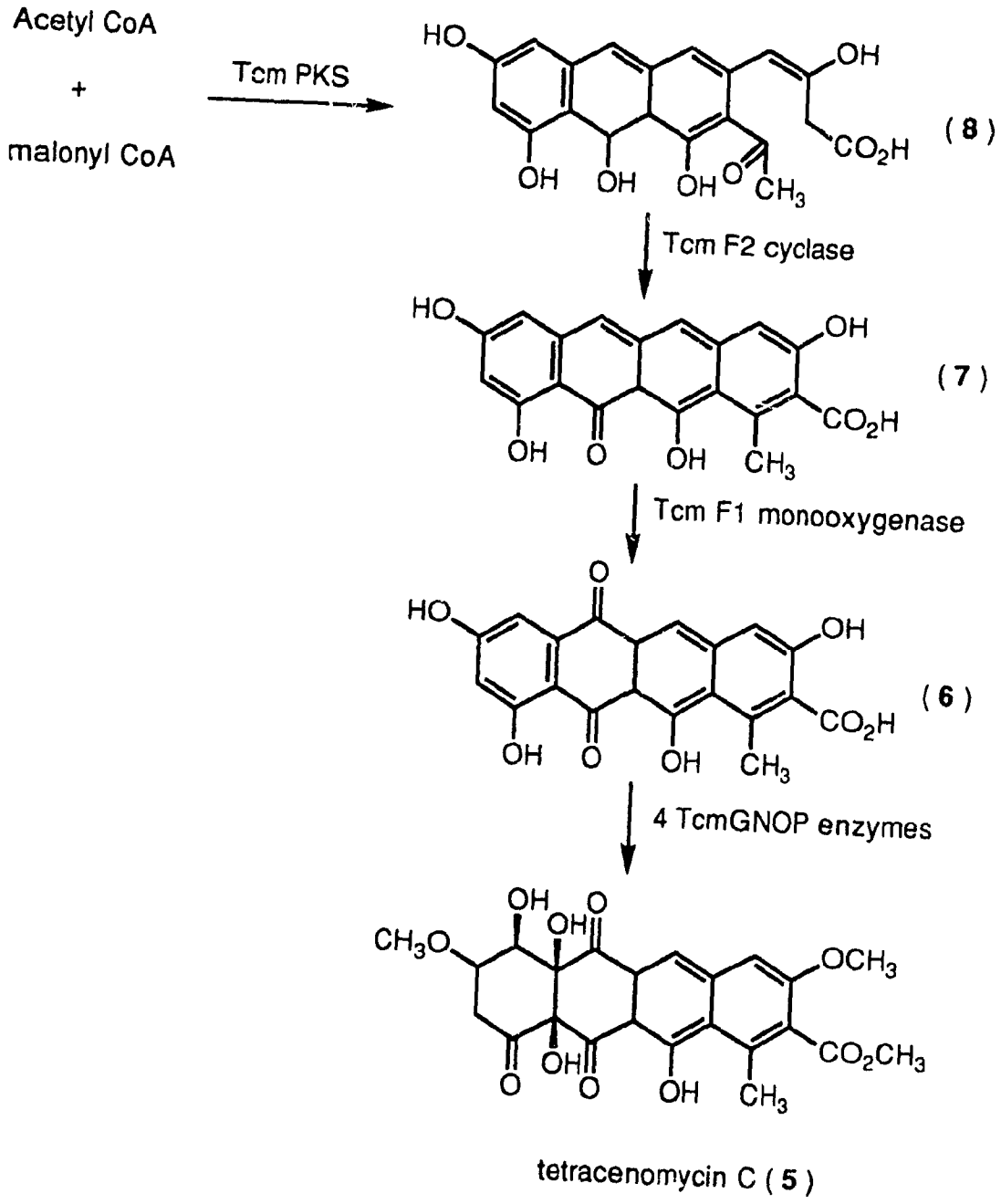
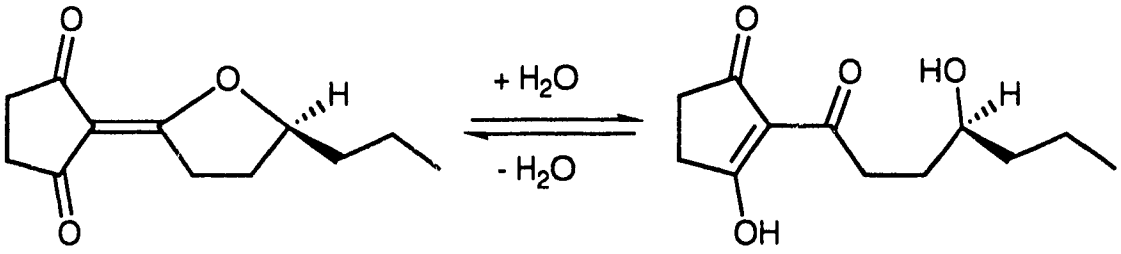


Figure 3. Biosynthetic pathway of the aromatic polyketide tetracenomycin C 1, an antitumor antibiotic from *Streptomyces glaucescens*.

1.3 Oudenone

Oudenone (**9**) is a fungal metabolite isolated from *Oudemansiella radicata*.[12] It is an inhibitor of tyrosine hydroxylase and phenylalanine hydroxylase, two key enzymes involved in the biosynthesis of catecholamines. As such, oudenone exhibits a strong hypotensive effect in test animals. Its structure consists of a 1,3-cyclopentanedione and a tetrahydrafurane ring moiety, but it can undergo a dynamic structural change to form an open chain trione (**9a**) by the addition of water. Oudenone has an apparent pKa = 4.1, thus it exists as **9** in acidic solutions and as **9a** in neutral or basic solutions(Fig. 4).



(**9**) (**9a**)

uv_{max} = 284nm, pH < 3 uv_{max} = 246nm, pH > 7

Figure 4. Structure of oudenone.

Based on what is already known about natural products, a scheme involving a mixed polyketide origin was proposed for the biosynthesis of oudenone (Fig. 5).

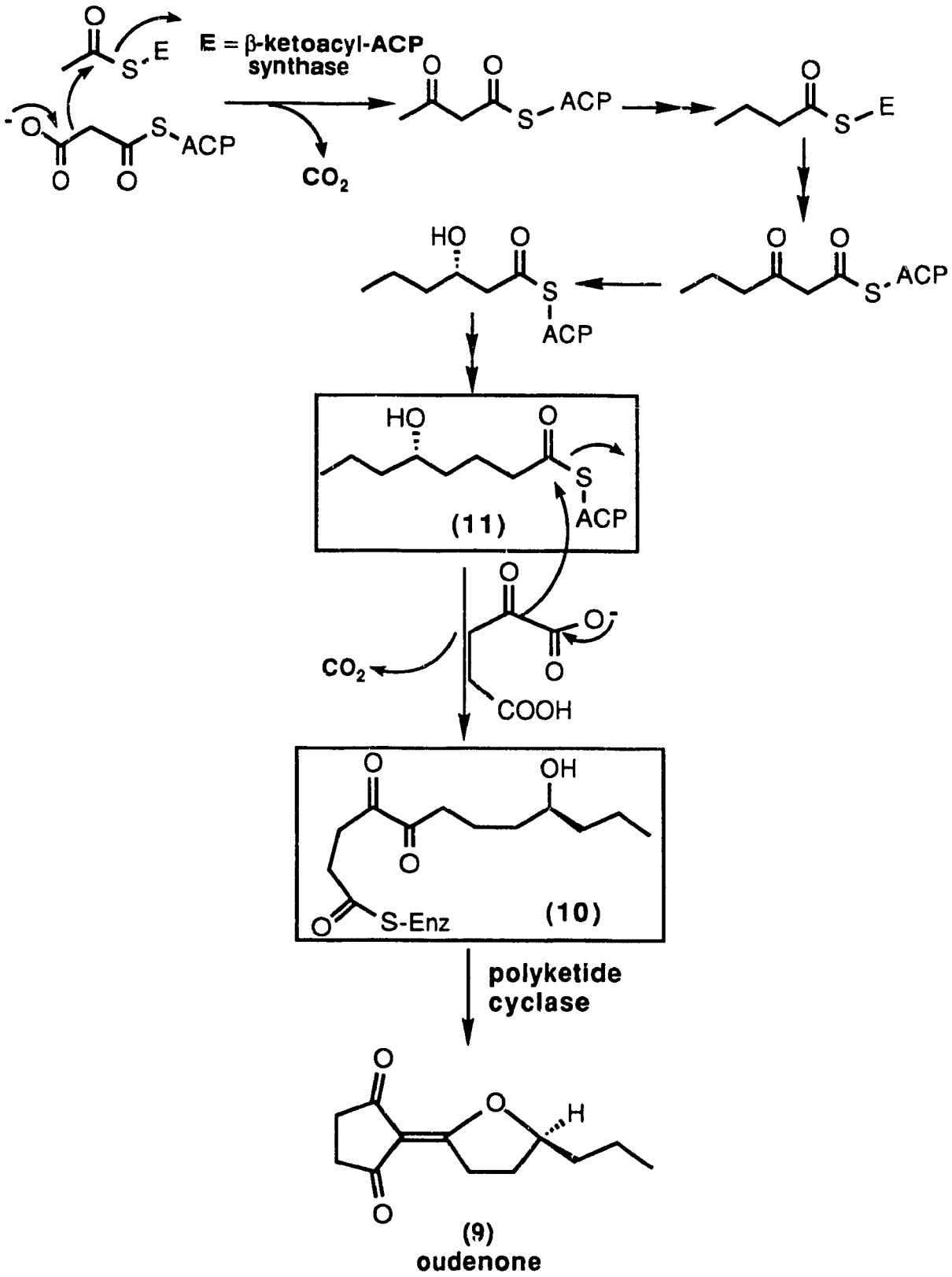


Figure 5. Proposed biosynthesis of oudenone

A *"head to head"* condensation of a succinic unit and a tetraketide chain is very unusual in polyketide biosynthesis; as previously described a typical polyketide biosynthesis involves a *"head to tail"* condensation (acetate / malonate addition). In addition, the cyclization of the open diketone **10** to oudenone **9** constitutes another interesting aspect of this research project.

Due to the inherent chemical instability of open chain polyketides, the mechanistic steps and relevant enzymes involved in the cyclizations of polyketides are mostly unknown. Recently, Shen and Hutchinson[13] have reported the purification and characterization of the first discrete enzyme which catalyzes the intramolecular aldol condensation-dehydration (Fig. 3, from compound **8** to **7**) in the biosynthesis of Tetracenomycin C in *Streptomyces glaucescens* . Their work has provided support for the existence of a " polyketide cyclase " enzyme which may be responsible for the final structural construction of natural products, such as oudenone.

Data from previous incorporations of ^{13}C labelled primary precursors into oudenone[14] have strongly supported the biosynthetic scheme outlined in Figure 5. Previous results are summarized in Figure 6. Incorporation of [1-^{13}C] sodium acetate and [1,2-$^{13}C_2$] sodium acetate into oudenone suggested that the tetrahydrofuran moiety and the attached *n*-propyl side chain of oudenone are derived from 4 units of acetate, random incorporation on the cyclopentanedione part is assumed due to the conversion of acetyl CoA into α-ketoglutarate or succinate *via* the Krebs cycle, but incorporation of [1,4-$^{13}C_2$] succinate unit into the oudenone was not observed, suggested that α-ketoglutarate might be the precursor responsible for the formation of the cyclopentanedione ring.

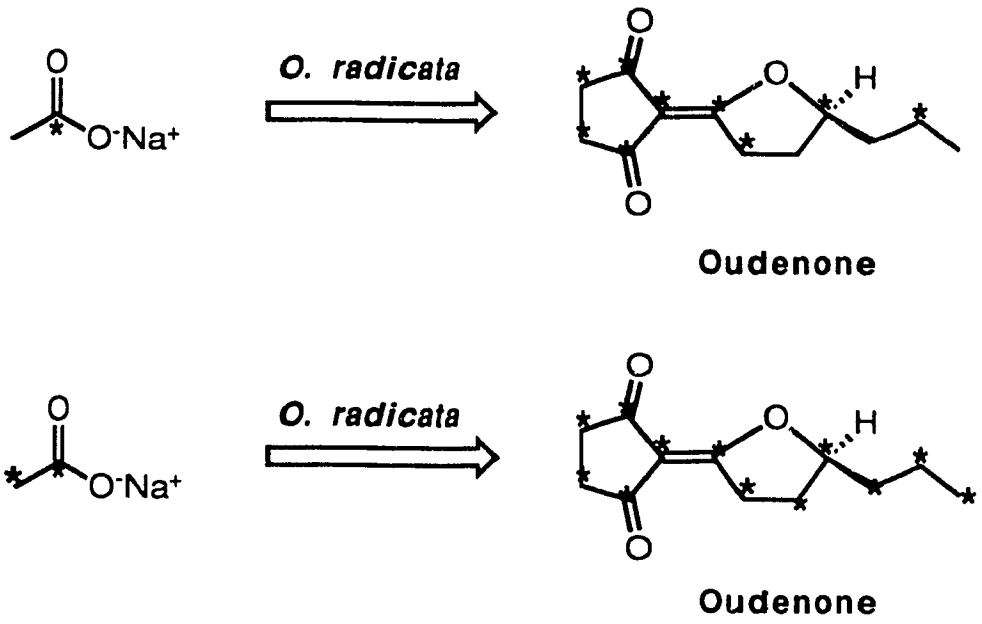


Figure 6. Incorporation of ^{13}C labeled primary precursors into oudenone ("*" indicates ^{13}C label).

Further insight into the proposed biosynthetic scheme can be obtained by the incorporation of isotopically labelled precursors **11** and **10** (Fig. 5) into the metabolite **9**. Attempts to incorporate the ^{13}C labelled butyrate were not successful, most likely due to the efficient degradation of short fatty acids by β-oxidation. However, it is expected that the synthetic precursors **11** and **10** will have a much greater chance of incorporation into oudenone. In order to optimize the incorporation of these compounds, their NAC-thioester analogues, along with β-oxidase inhibitors will have to be fed to the *O. radica* cultures. Thus, the isotopically labelled (S)-5-hydroxyoctanoic NAC-thioester derivative and the α-diketone derivative must be synthesized.

In the following chapters, there are details of studies of the synthesis of two target compounds, the (S)-5-hydroxyoctanoate NAC-thioester derivative **11** and the α-diketone **10**, which are believed to be the two advanced precursors in

the biosynthesis of oudenone (**9**), also, the results from intact incorporation of labelled compound **11** will be discussed.

Chapter 2: Results and discussion

2.1 Synthesis of the deuterium labeled tetraketide 11, as its N-acetylcysteamine thioester.

In order to gain support for the proposed biosynthesis of oudenone (Fig. 5), we decided to explore the intact incorporation of the tetraketide **11** into the structure of oudenone by cultures of *O. radicata*. We chose to use the corresponding N-acetylcysteamine (NAC) thioester (**11**) for incorporation experiment, since NAC derivatives of polyketide intermediates have been shown to give better incorporation results than their parent carboxylic aids.[8,9] It is believed that the synthetic NAC derivatives of polyketide intermediate mimic the natural thioester intermediates of coenzyme A and the acyl carrier protein which are involved in the biosynthesis of polyketides. Compound **11** was synthesized following the general scheme outlined in Figure 7.

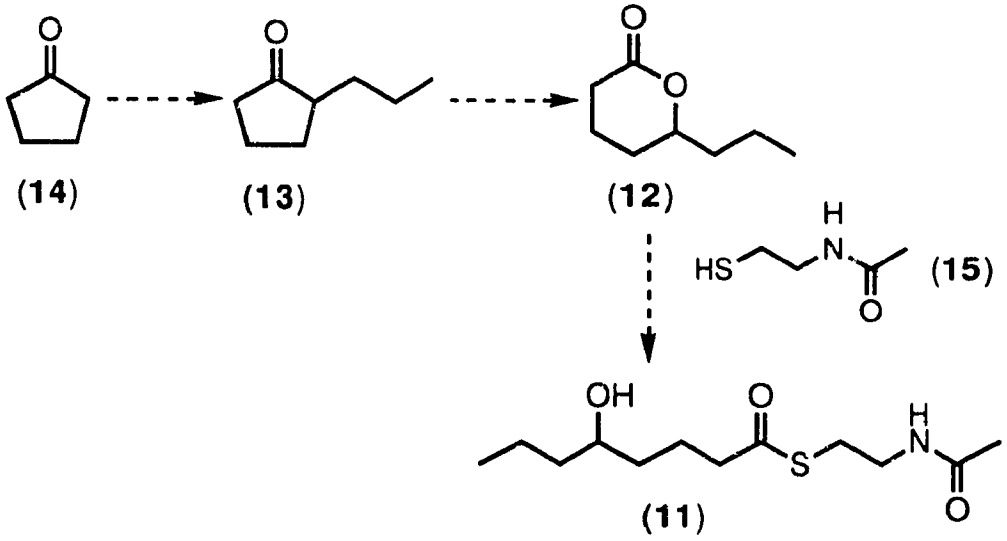


Figure 7. General outline of the synthesis of tetraketide 11.

Initially, direct α-alkylation of cyclopentanone **14** was attempted using propyl bromide in basic conditions (Fig. 8). However, these reactions lead primarily to

the formation of the dimer **6**, formed via aldol condensation, and only a small amount of the desired product was formed.

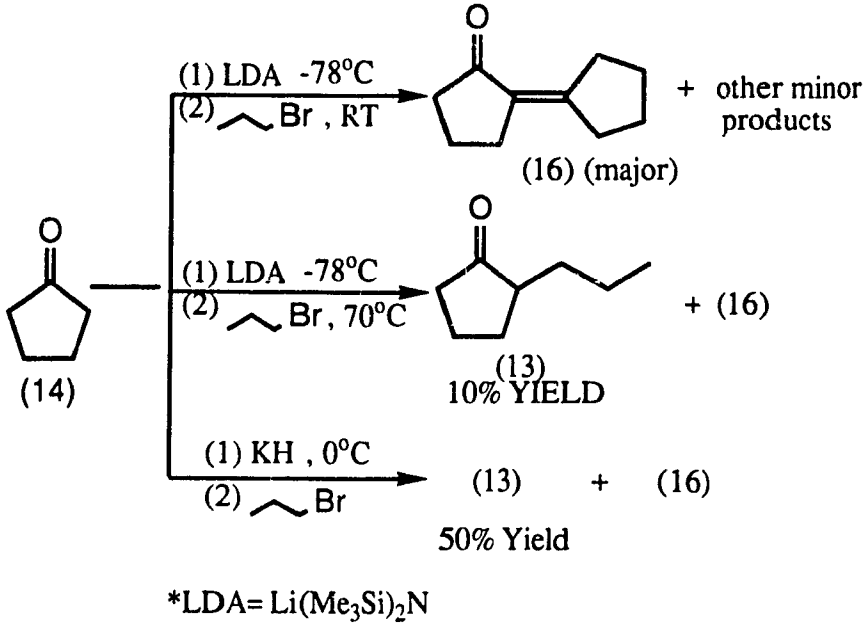


Figure 8. α-Alkylation of cyclopentanone.

Similar results from reaction of ketones with sodium hydride had been reported. It was suggested that ketones, especially unhindered cyclic or methyl ketones, suffer substantial aldol condensation in competition with metalation by lighter hydrides.[15] However the best yields were achieved when KH was used as the base (50% yield). This could be due to the high reactivity of KH which would react with the ketone form removing it from the aldol equilibrium (Fig. 9). Furthermore, aldo formation appears to be favored by tightly associating cations (Li+>K+).[16]

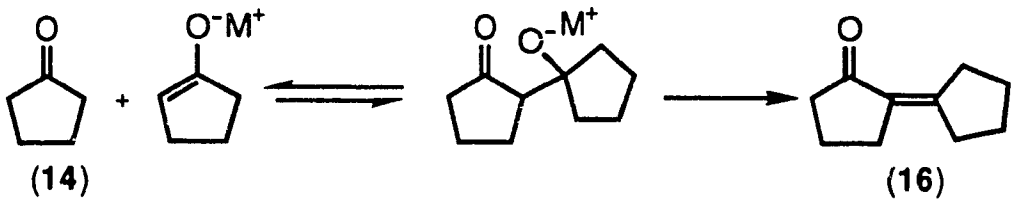


Figure 9. The aldol condensation of cyclopentanone

An alternate route leading to the synthesis of compound **13** is outlined in Fig. 10. Ethyl 2-oxocyclopentanecarboxylate (**17**) was easily deprotonated by NaH to form a stable intermediate which was then alkylated with propyl bromide to give compound **18**. After hydrolysis of the ethyl ester and decarboxylation, compound **13** was obtained in 78-83% yield. Baeyer-Villager oxidation of **13** yielded the δ-valerolactone **12** in 85% yield.

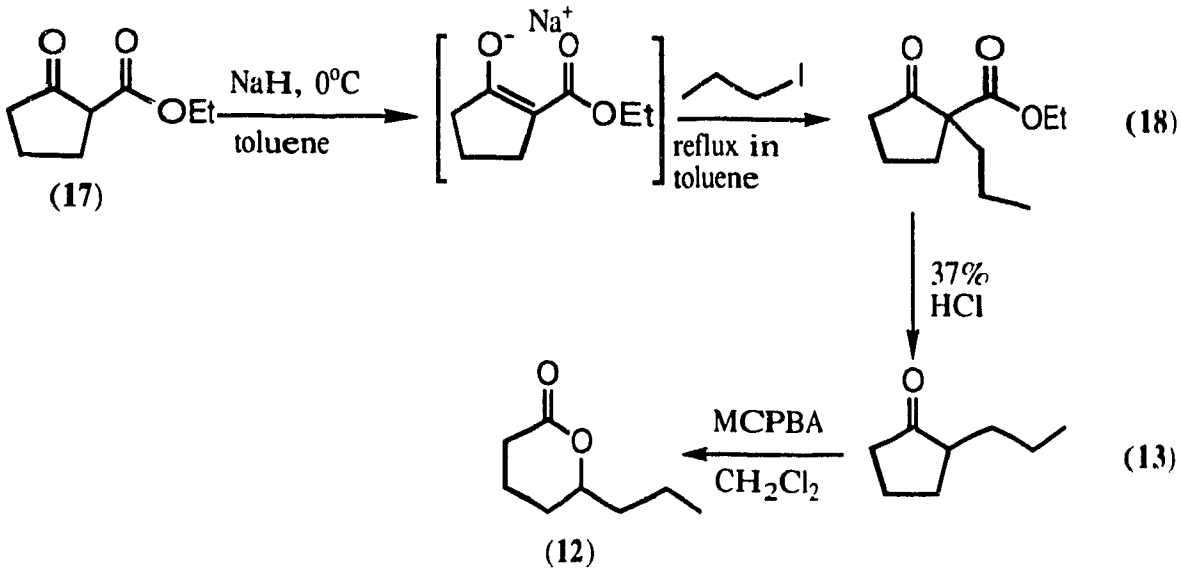


Figure 10. Synthesis of δ-valerolactone 12.

A number of synthetic methodologies exist for the conversion of a carboxylic acid to a thioester. For example, the carboxylic acid can be easily converted to an acid chloride which can subsequently be converted to the thioester with a thiol in the presence of base (Fig. 11, reaction 1). In addition, esters and lactones can be converted to thioesters directly by the addition of an activated sulfide anion, formed in situ by the reaction of a mercaptan with $Al(CH_3)_3$ (Fig. 11, reactions 2, 3).[17]

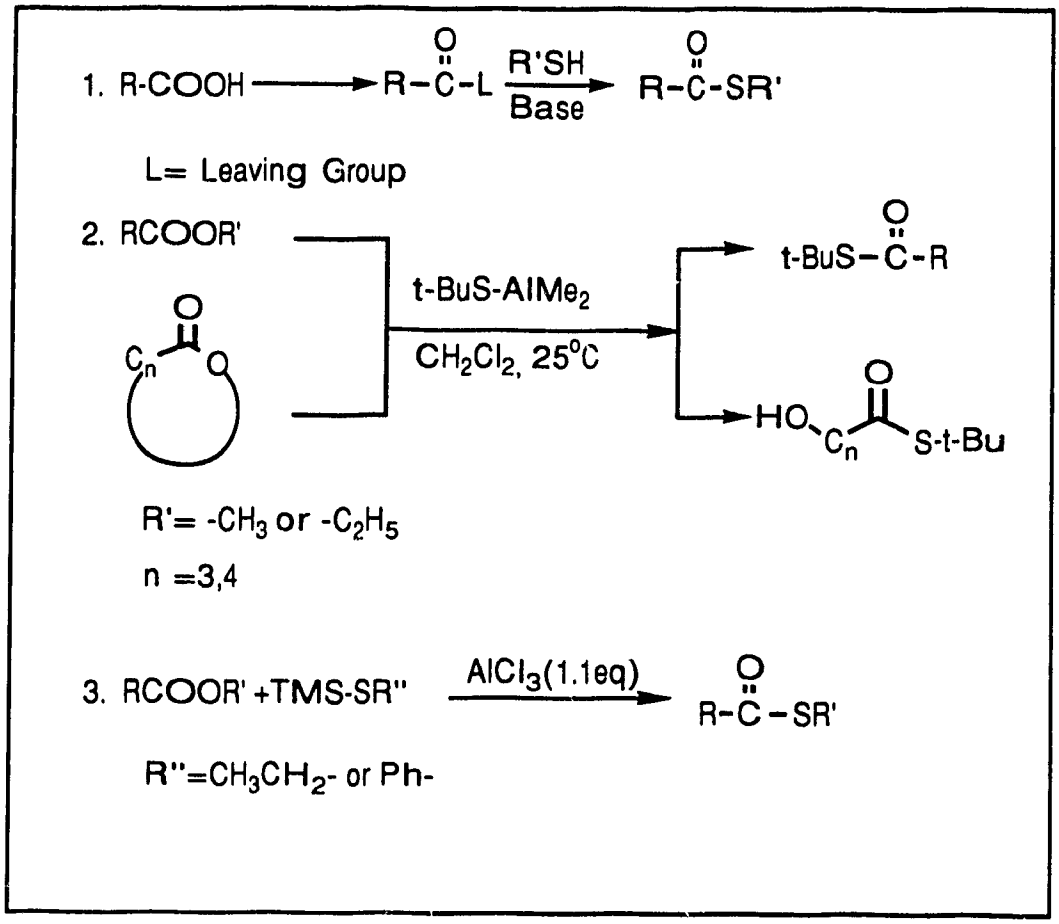


Figure 11. Literature methods for preparation of thioesters.

Initially, we tried to form compound **11** by reacting the lactone **12** with a thallium salt of NAC (**19**) (Fig. 12), generated in situ from TlOEt and NAC.[18] This approach was attempted several times, in different solvents and at different temperatures, but the desired product was not obtained. Similar results were obtained whe formation of the thioester **11** was attempted using NAC derivative **20** or **21** (Fig. 12). In all of these cases, there was no detectable product formed and the unreacted starting materials was recovered. A reasonable explanation for these results may be that the lactone is much more stable than the thioester, thus the equilibrium for this reaction lies far to the left.

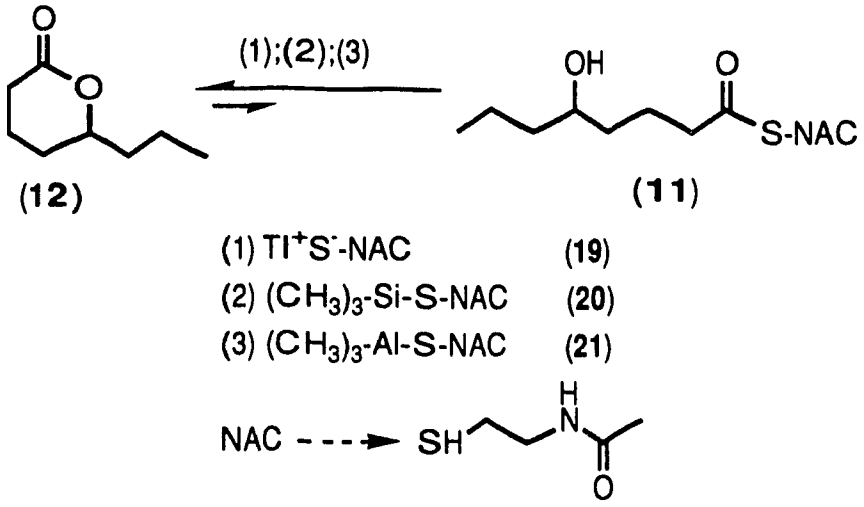


Figure 12. Attempts for preparation of thiolester derivative

Since the lactone (12) could not be converted directly to the thioester, another scheme was designed and followed (Fig. 13). The compound **12** was first converted to the 5-hydroxyl methyl octanate (22) and the hydroxyl group was protected as the *t*-butyldimethylsilyl (TBDMS) ether **23**.[19] It is worth noting that the yield of the latter reaction was found to be highly dependent on the ratio of TBDMS-Cl to base used. When the ratio of TBDMS-Cl : Imidazole was 1:2, the yield was only about 50%, with the remaining 50% of the material being mostly the lactone **12**. Whereas, when the ratio of TBDMSCl : Imidazole was increased to 1:3, a yield of 83% was obtained and no lactone was recovered. This could be due to the high sensitivity of the starting material to acid; trace amounts of moisture, which would react with TBDMS-Cl to produce HCl, could catalyze the formation of **12** from the methyl ester **22**.

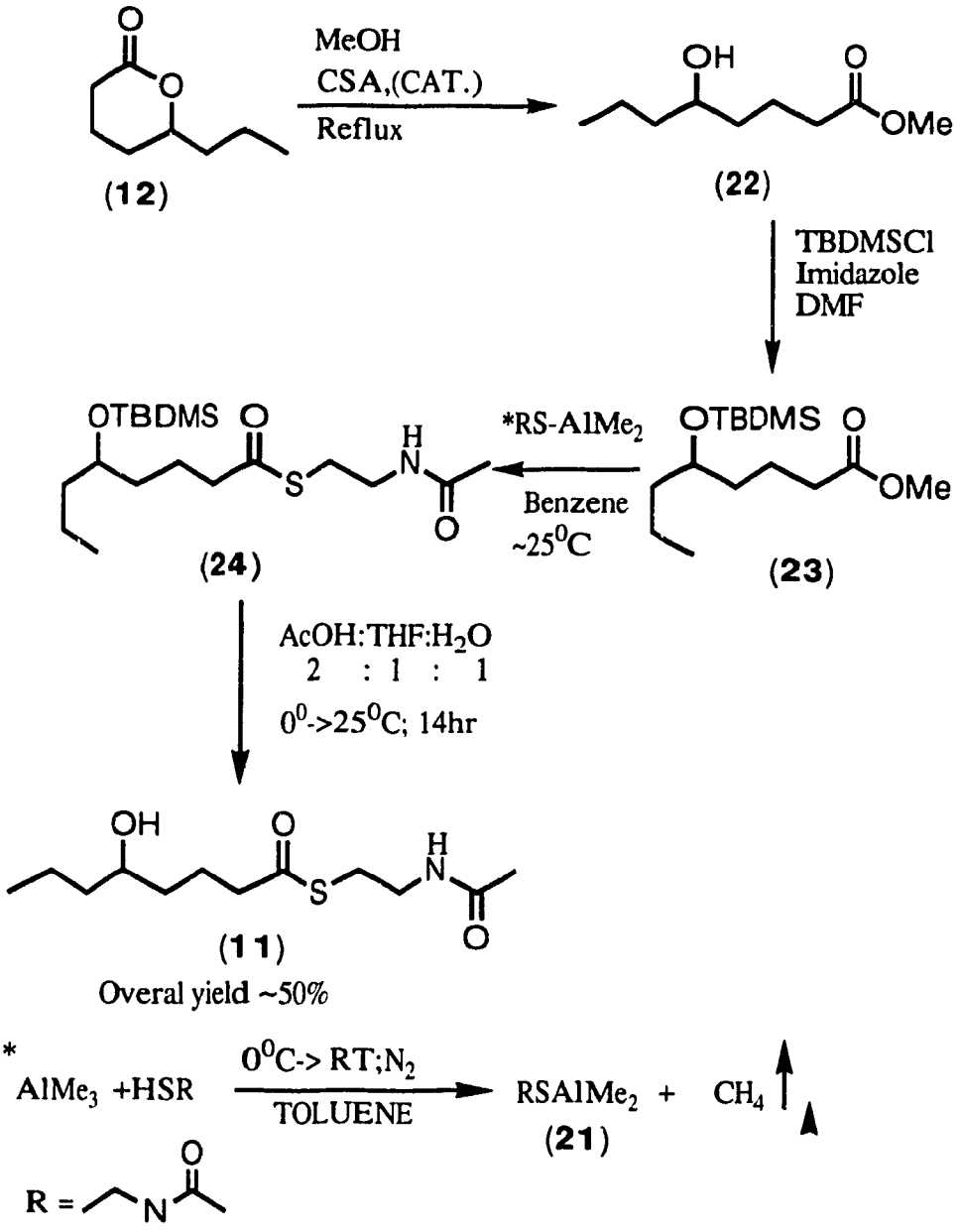


Figure 13. Synthesis of Compound 11

Once the alcohol is protected, the methyl ester **23** reacts readily with $(CH_3)_2$Al-NAC (21) (generated in situ from $AlMe_3$ and N-acetylcysteamine) to form the thioester derivative **24** with ~60% yield. The reaction was first carried out in toluene at 60°C-80°C for 14 hours, but better results were obtained when

benzene was used as the solvent and the reaction was carried out at room temperature.

The mechanism involved in this reaction is believed to follow the steps outlined in Figure 14. The carbonyl carbon is first activated through the coordination of the oxygen with the aluminum, followed by nucleophilic attack by the sulfur and rearrangement leading to the formation of the thiol ester **24**.

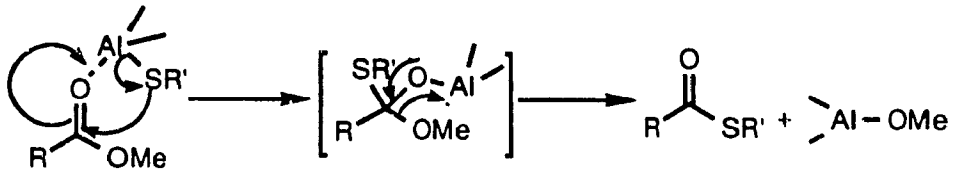
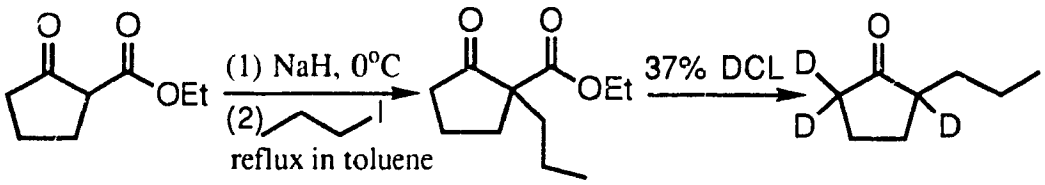


Figure 14. Mechanism for the Formation of Compound 24.

In the last step of our synthesis, the TBDMS protecting group was removed with acetic acid to give the desired thioester derivative of our tetraketide **11**. Compound **11** was found to be unstable at room temperature over a period of many days, slowly decomposing to the corresponding lactone **12** and NAC. However, we assumed that it would be stable for 24~48 hours in the liquid cultures of *O. radicata* (26-27°C, pH 4~5), in order to have a chance of being incorporated into the structure of oudenone. [Since the conditions of the TBDMS deprotection reaction involved a 24-hour exposure to 50% acetic acid, we believe that this assumption is not unreasonable.]

2.2 Stereoselective synthesis of deuterium labeled compound 11

In order to follow the possible incorporation of the advanced biosynthetic precursor **11** into the structure of oudenone by *O. radicata* cultures, the deuterium labeled compound **11** was synthesized. Replacement of HCl for DCl during the hydrolysis and subsequent decarboxylation of compound **18** to **13'**, allowed us to obtain the deuterium labeled 2-propyl cyclopentanone (Fig. 15). ^{1}H and ^{2}H NMR and mass spectral data were consistent with the incorporation of three deuterium atoms at the positions shown in figure 15 for approximately 90% of the sample.



(17) **(18)** **(13')**

Figure 15. Synthesis of deuterium labeled 2-propyl cyclopentanone.

It is important to note the choice of stable isotope, as well as the position of labeling in the precursor. It was decided to use ^{2}H instead of ^{13}C for several reasons: a) the source of ^{2}H label is fairly inexpensive, b) the natural abundance of deuterium is so low that it would not interfere with the incorporation studies, even if the incorporation of labelled **11** into oudenone was very low, and c) the random incorporation of label due to the inevitable β -oxidation of compound **11** is not expected to be a problem in this study. Random incorporation of label is often observed with ^{13}C labeled precursors. However, it was expected that any degradation of **11'** by β-oxidation will lead to total loss of the deuterium label at the carbon derived from C-5 of **11'** (Fig. 16). Thus, the presence of any deuterium at the C-9 of oudenone would strongly

suggest that tetraketide **11'** was incorporated intact and that it is an advanced biosynthetic precursor of oudenone (Figure 16).

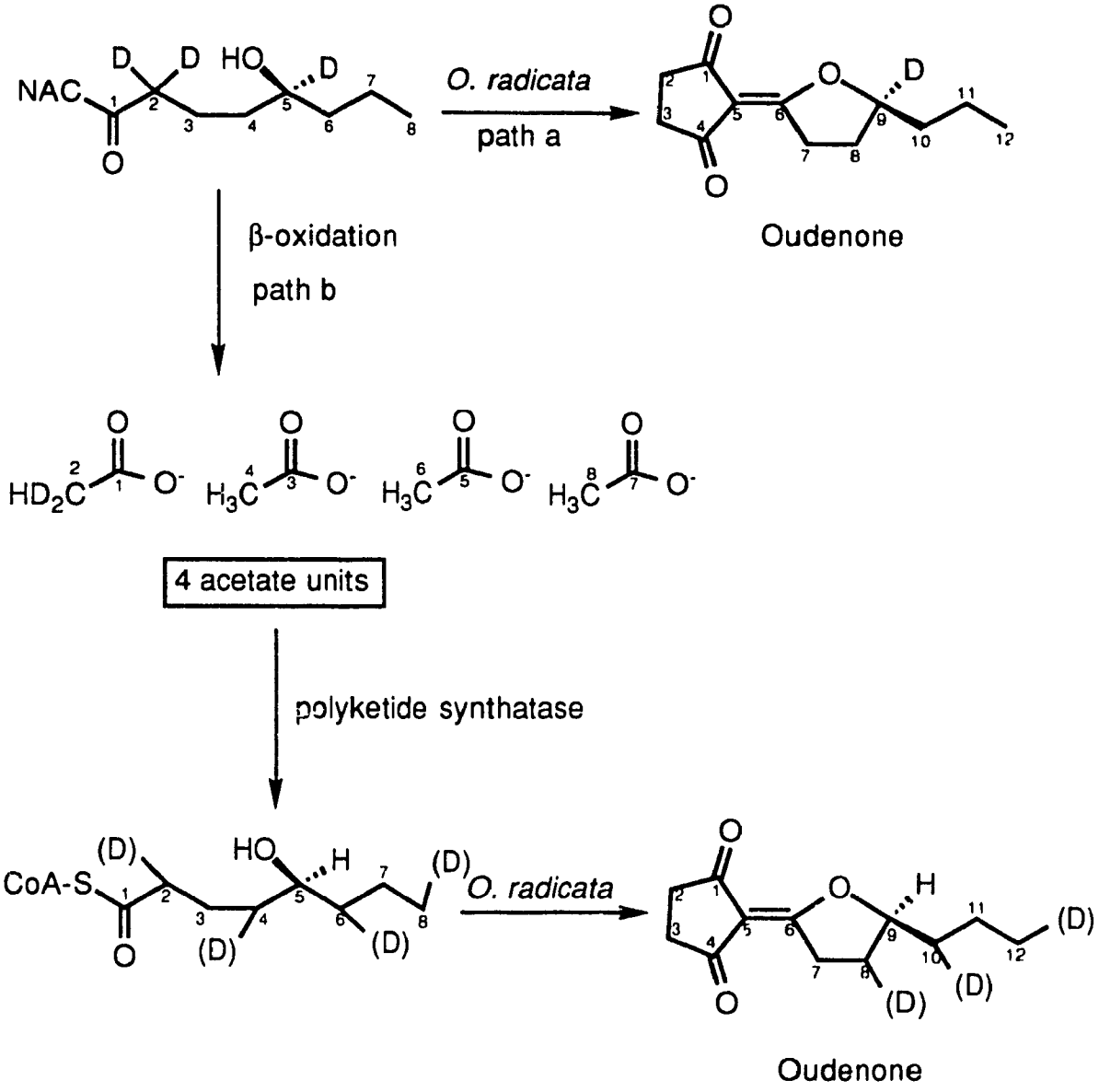


Figure 16. Possible incorporation of deuterium label into oudenone

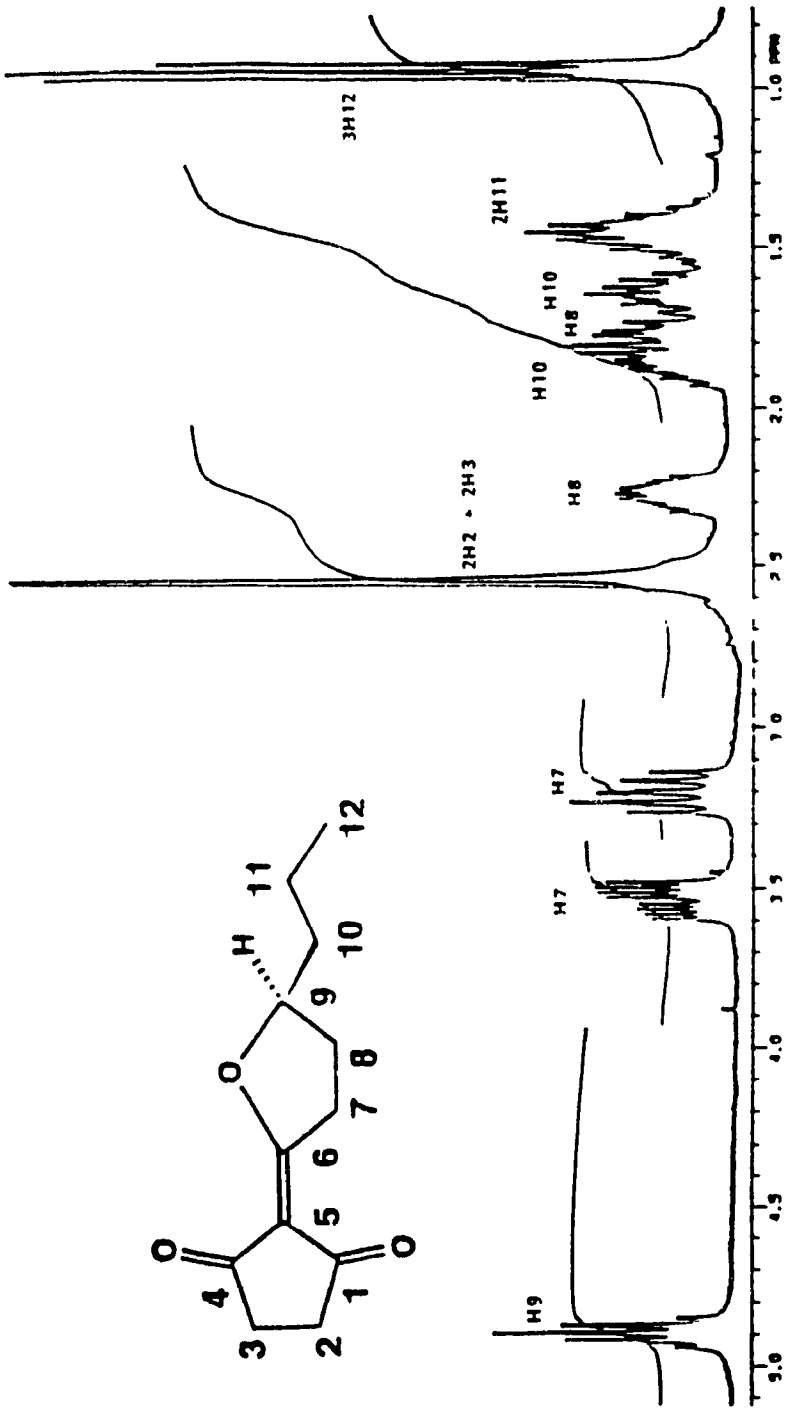


Figure 17. ^{1}H NMR spectrum of oudenone.

Concerning the position of the label,, the chemical shift of H9 is clearly separated from all other signals in the ^1H NMR spectrum of oudenone.(Fig. 17) Therefore, the level of precursor incorporation can be easily and accurately measured from the relative integration of H9 in the ^1H NMR of the labeled oudenone.

In most cases, it is expected that an enzyme will only accept an enantiomerically pure compound as its substrate. In some cases the opposite enantiomer may still be able to bind to the active site and behave as a competitive inhibitor of the enzyme. In order to avoid any such possible problems, the (S)-enantiomer of **11'** was synthesized, based on our proposed biosynthetic scheme (Fig. 5) which assumes that the absolute stereochemistry at C-9 of oudenone is the same as that of the C-5 of the tetraketide **11**.

Many methods are available which permit the separation of the two enantiomers from a racemic mixture. The most commonly used method involves the conversion of the two enantiomers to diastereomers by derivatization with a chiral resolving agent. This process allows the separation of the two compounds (diastereomers) by column chromatography and recovery of each pure enantiomer after removal of the chiral resolving agent.

Based on this idea, two chiral resolving reagents were used in an effort to resolve the racemic mixture of 2-propylcyclopentanone (**13**) or the lactone **12** as showing in Figures 18 and 19, respectively. Acetalization of 2-propylcyclopentanone with chiral reagent **25** gave a pair of diastereomers. However, the two diastereomers were not separable by TLC and could only be observed by GC-MS and NMR. Similar results were obtained from the reaction of the lactone and (R)-methylphenylamine **26**.

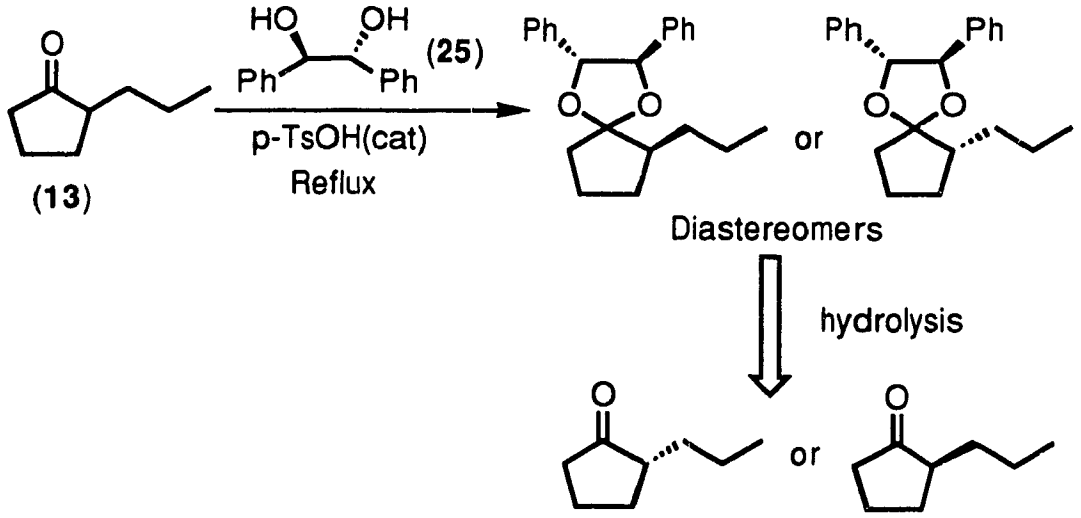


Figure 18. Approach to resolve the racemic 13 using chiral reagent 25.

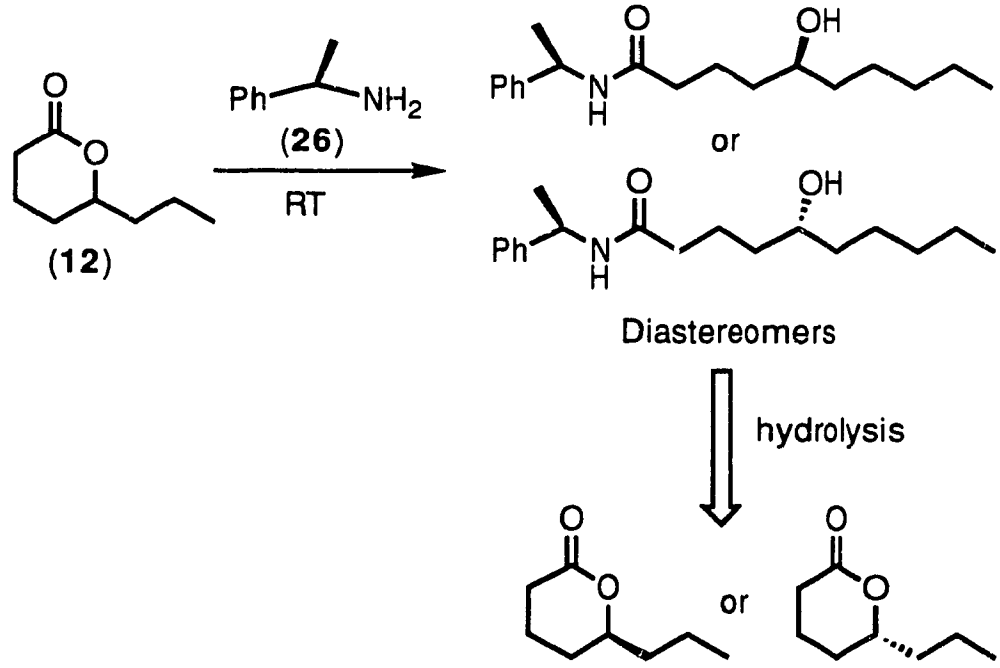


Figure 19. Approach to resolve the racemic 12 using chiral reagent 26.

Recently, chiral α-monosubstituted cyclopentanones were isolated *via* asymmetric acetalization of their racemic mixture using the chiral hydroxy thiol **29**.[20] The chiral hydroxyl thiol **29** was synthesized using Eliel's original procedures,[21] as outlined in Figure 20.

Commercially available (+)-pulegone (**27**) was reacted with benzyl mercaptan in the presence of 10% aqueous NaOH to give **28**. Compound **28** was then reduced by Na/NH_3 to give the isomeric mixture of hydroxyl thiols **29a-d**. Although the literature method involved the separation of diastereomers by preparative HPLC, we were able to isolate the isomers **29a** and **29b** by flash column chromatography in approximately 68% and 20% yields respectively. However, the separation of these hydroxylthiols from the minor components is the most difficult and time-consuming step in this synthetic scheme. Pure chiral oxathiane can also be prepared by condensation of the mixed hydroxylthiols with paraformaldehyde followed by crystallization of the main component **30a** (Fig. 21). Oxidative hydrolysis of compound **30a** with $AgNO_3$ and N-chlorosuccininide, followed by $LiAlH_4$ reduction can regenerate the enantiomerically pure hydroxylthiol **29b**.

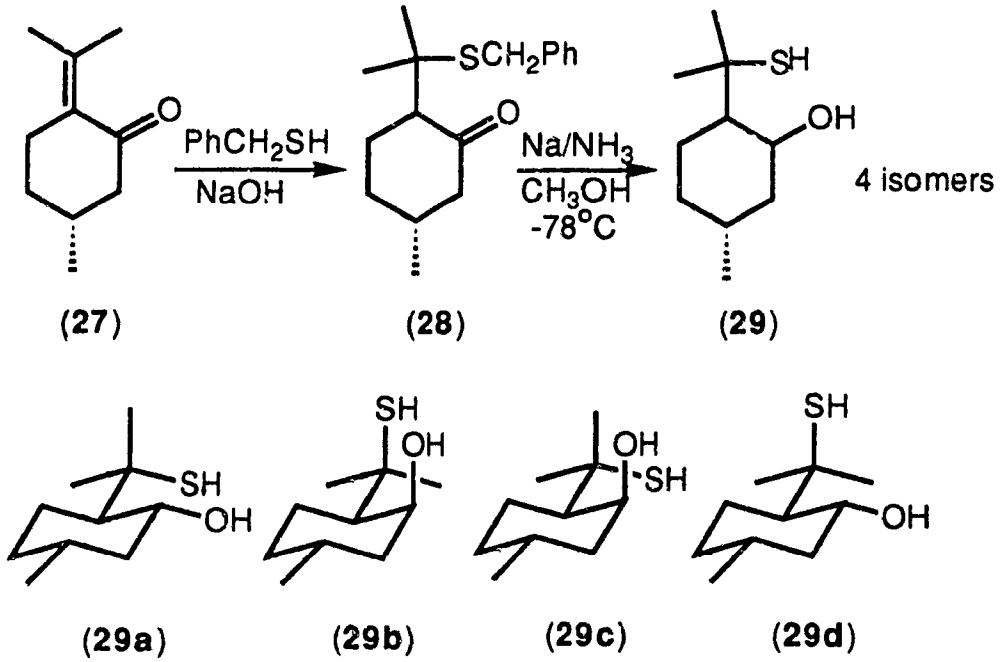


Figure 20. Synthesis of chiral hydroxythiol 29.

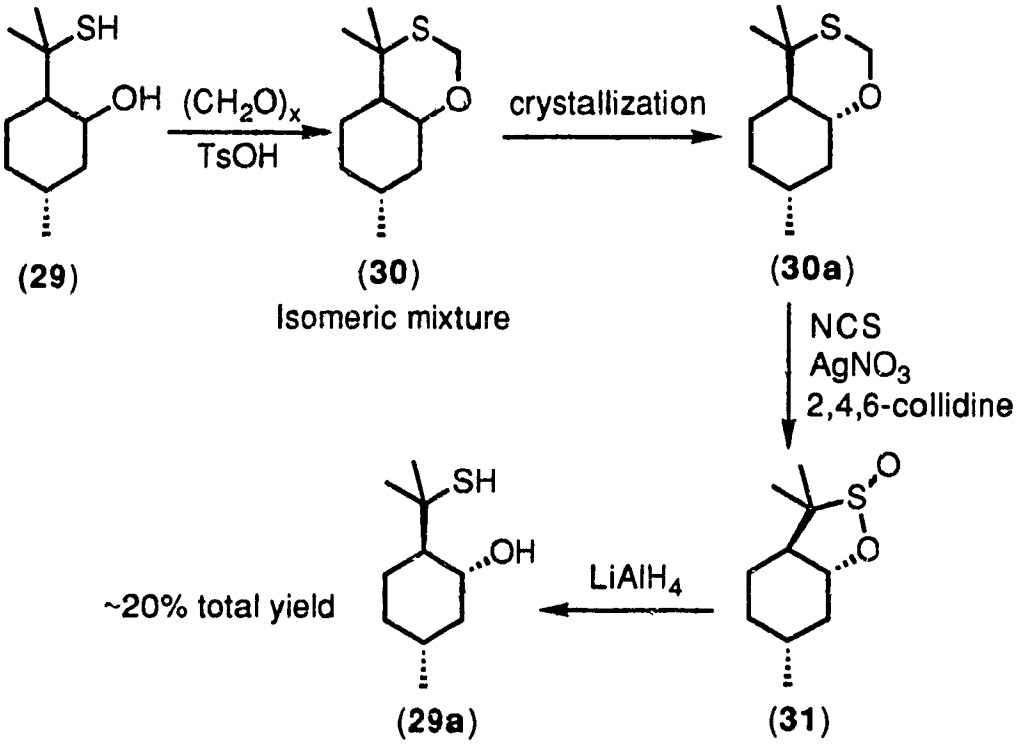


Figure 21. Synthesis of diastereomeric pure hydroxythiol 29a.

Hydroxylthiol **29b** was purified from the mixture of **29** by repetitive column chromatography and it was used to synthesize two acetals **32a** and **32b** in a 2:1 ratio and a 90% overall yield.(Fig.22.) The diastereoselectivity observed is due to the steric hindrance between the α-alkyl group and the methyl group next to the sulfur. However, the NMR spectrum showed that almost 50% of deuterium label was lost during the acetalization reaction, most likely due to the keto-enol equilibrium of cyclopentanone which would exist under the acidic condition of this reaction. In order to solve this problem, all exchangeable protons were first exchanged with deuterium using D_2O (e.g. *p*-toluene sulfuric acid, as well as the hydroxyl and thiol protons were exchanged for deuterium). In addition, a small amount of D_2O was added to the anhydrous toluene solvent and any source of $^1H^+$ was carefully avoided. Consequently, the deuterium label in the final sample (compound **11'**) was shown to be approximately 98% based on the integration ratios in the ^1H NMR spectra of diastereomer **32d**. (2S)-(+)-2-propylcyclopentanone was obtained in ~50% yield after hydrolysis of the major diastereomer **32d** using NCS and $AgNO_3$ in the presence a large excess of 2,4,6-collidine in 80% aqueous acetonitrile. Excess of base was used in order to prevent the possible racemization due to the rapid rise of acidity during the reaction.

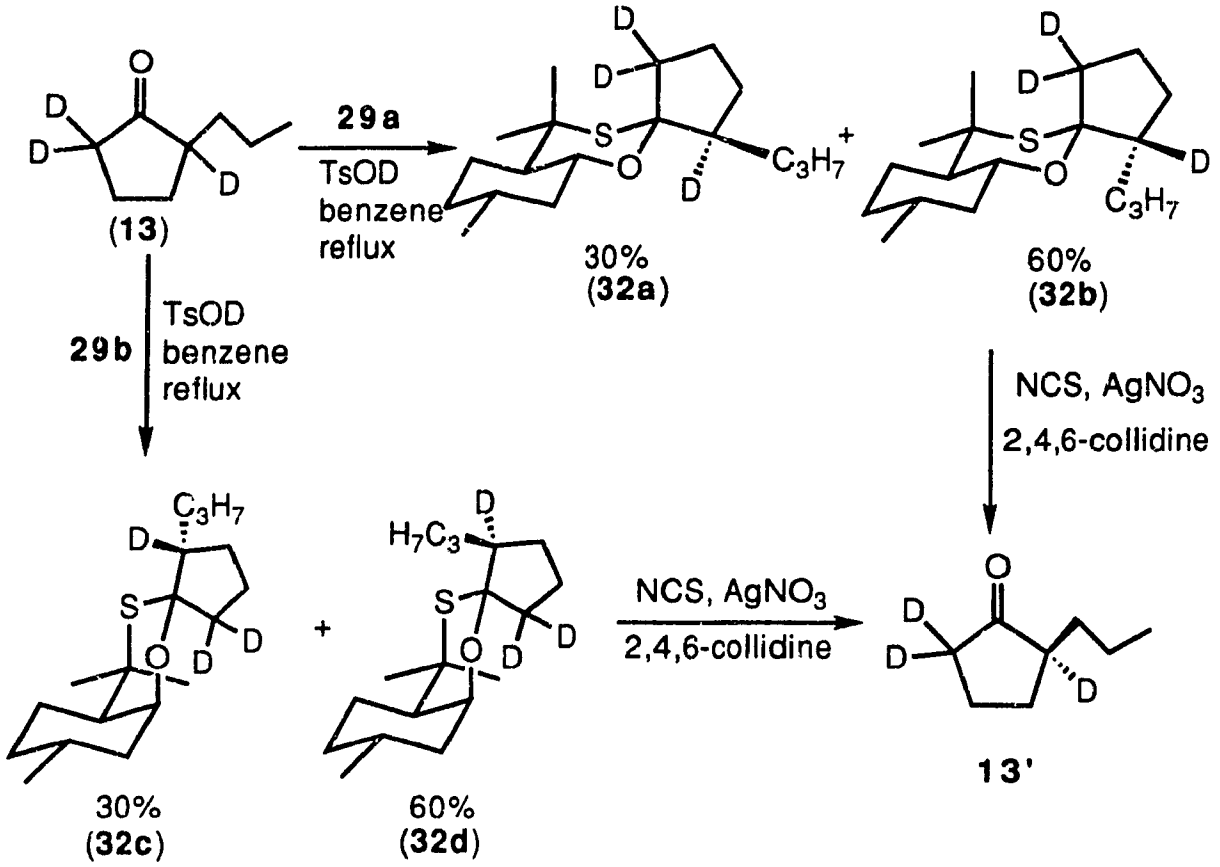


Figure 22. Preparation of (S)-(+)-2-propylcyclopentanone 13'.

The enantiomerically pure (2S)-(+)-[2-^{2}H, 5-$^2H_2$]-2-Propylcyclopentanone was subsequently converted to the final product (5S)-[2-$^2H_2$, 5-^{2}H]-5-hydroxyloctanoyl NAC thioester **11'** following the synthetic scheme described previously (Fig. 14).

Although the absolute configuration of the (+)-2-propyl cyclopentanone **13'** was confirmed from the X-ray crystallographic data of the corresponding oxathiane diastereomer **32d**, its enantiomeric purity still remains unknown. Several attempts were made to determine the %*ee* (enantiomeric excess) of (S)-(-)-lactone **12'** and the TBDMS thioester derivative **24'** using NMR in the presence of chiral shift reagents and chiral HPLC, respectively. Unfortunately, none of these experiments could provide a reliable measurment of the %*ee* for

these compounds. However, since there is an insignificant loss of deuterium label at the chiral carbon (calculated from corresponding mass spectrum data), it is assumed that the extent of racemization during conversion of thioacetal **32d** to the final product **11'** should be also insignificant.

2.2. Intact incorporation of the advanced precursor (5S)-[2-$^2H_2$, 5-^2H]-5-hydroxyloctanoyl NAC thioester 11' into oudenone.

Deuterium labelled compound **11'** was dissolved in a small amount of 100% EtOH and fed to growing cultures of *Oudemansiella radicata* , along with the β-oxidase inhibitor 3-(tetradecylthio)propanoic acid. Examination of the oudenone isolated from the cultures by ^2H NMR showed a deuterium enrichment at the expected chemical shift, corresponding to H9 (Fig. 23). As previously discussed, this result strongly supports our hypothesis that the tetraketide is the true precursor of oudenone; in addition, the chiral center of the oudenone structure is derived from the (S)-tetraketide, as predicted.

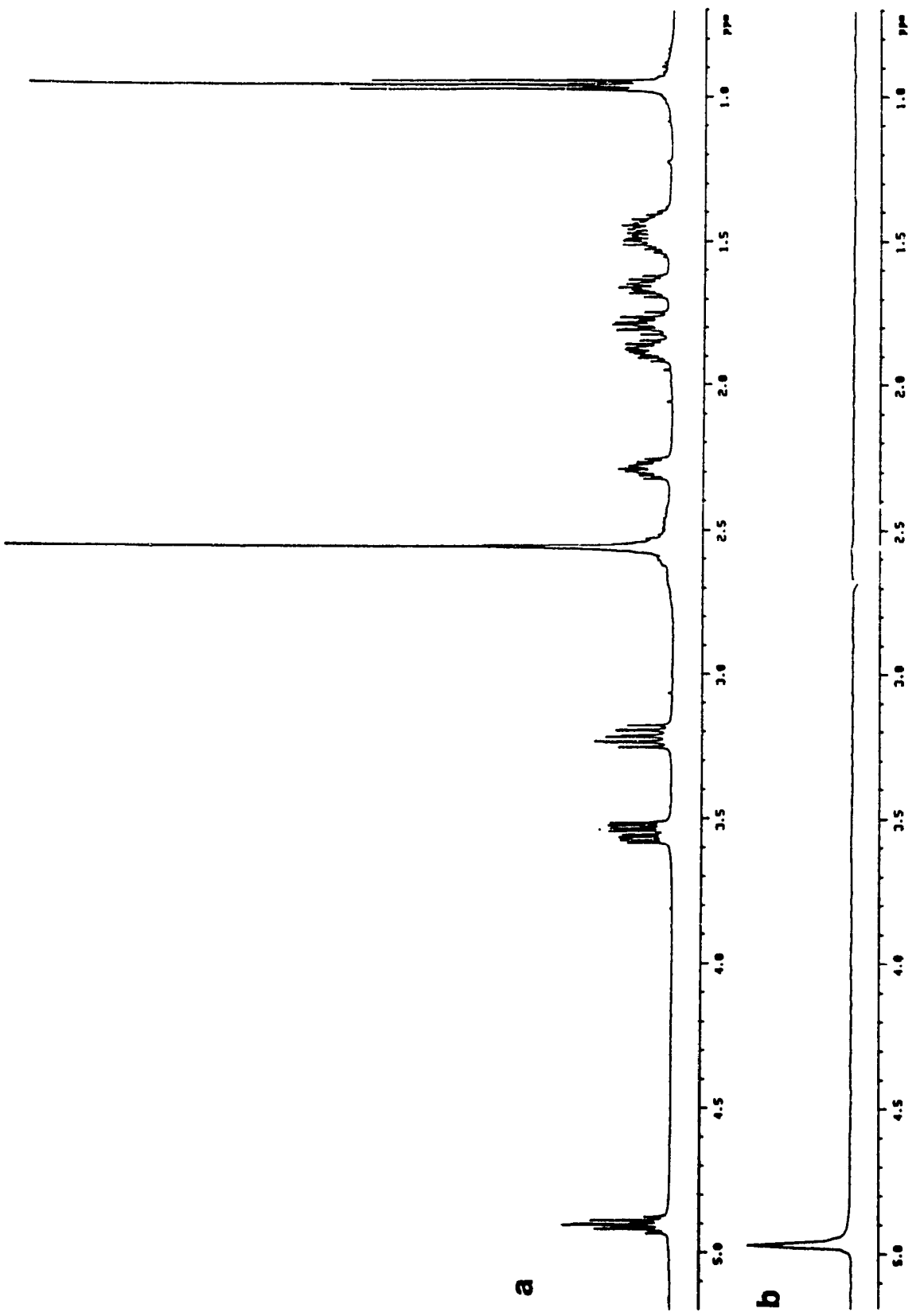


Figure 23. a)^{1}H-NMR spectrum of standard oudenone. b) ^{2}H-NMR spectrum of oudenone isolated from the fermentation inwhich deuterium labelled compound 11' had been fed.

2.3. Approach towards the synthesis of an α-diketone derivative 10.

Previous incorporation experiments (2.1 & 2.2) suggested that oudenone is biosynthetically derived from one unit of succinate and four units of acetate. It has been proved that the acetate units are incorporated into the structure of oudenone as the advanced precursor **11'**. Therefore, we proposed that the α-diketone derivative **10** could be the direct precursor of oudenone. A "head to head" condensation between a succinate unit and a tetraketide is quite unlikely, but the decarboxylation of an α-keto acid, such as α-ketoglutarate, could produce a nucleophile which could undergo C-C bond formation. Thiazolium dependent enzymes are known to catalyze such biochemical reactions and a reasonable mechanism for the formation of the α-diketone **10** is shown in Figure 24.

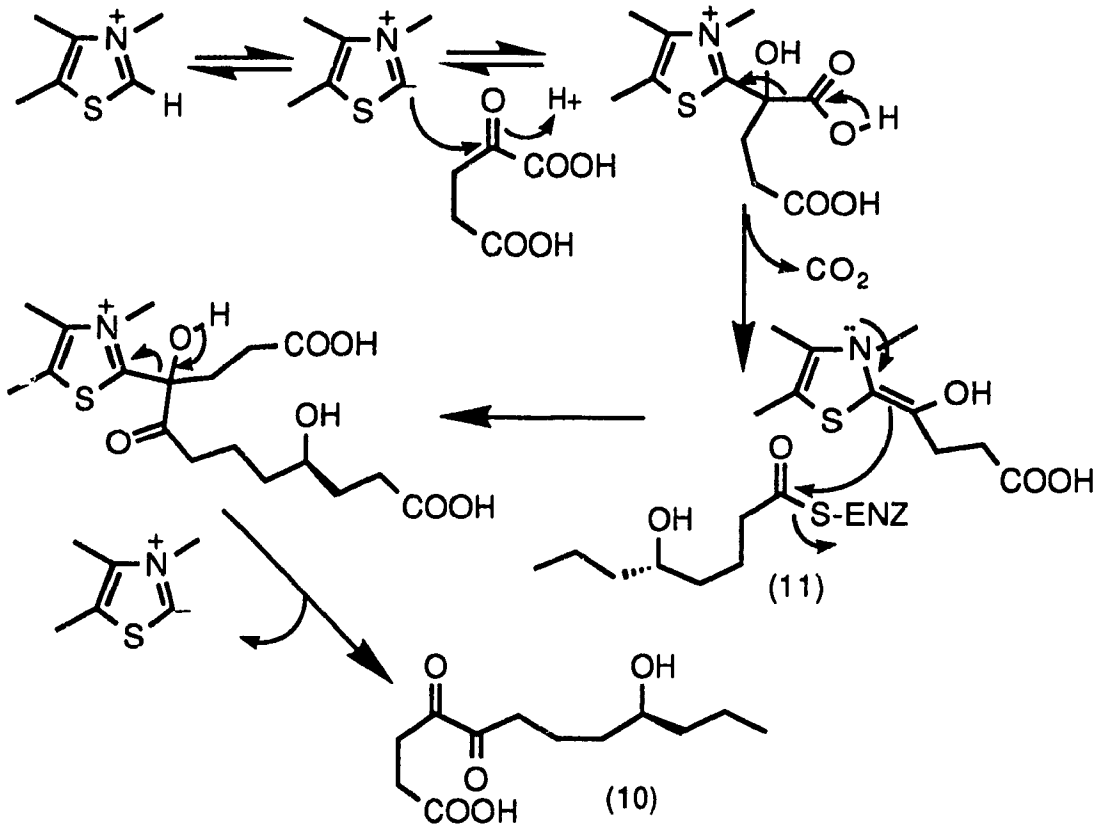


Figure 24. Proposed mechanism for the formation of α-diketone intermediate.

The succinate portion of α-ketogluterate could condense with the activated tetraketide **11**, *via* thiamine-dependent decarboxylation, to form the α-diketone **10**. Recently, Grue-Sorensen and Spenser[22] demonstrated a similar mechanism in the biosynthesis of the *Ephedra* alkaloids (Fig. 25). Formation of the α-diketone **33**, via decarboxlation of pyruvate and condensation with benzoic acid (or its CoA thioester), was proposed as the advanced precursor to the alkaloids **34a-34b**.

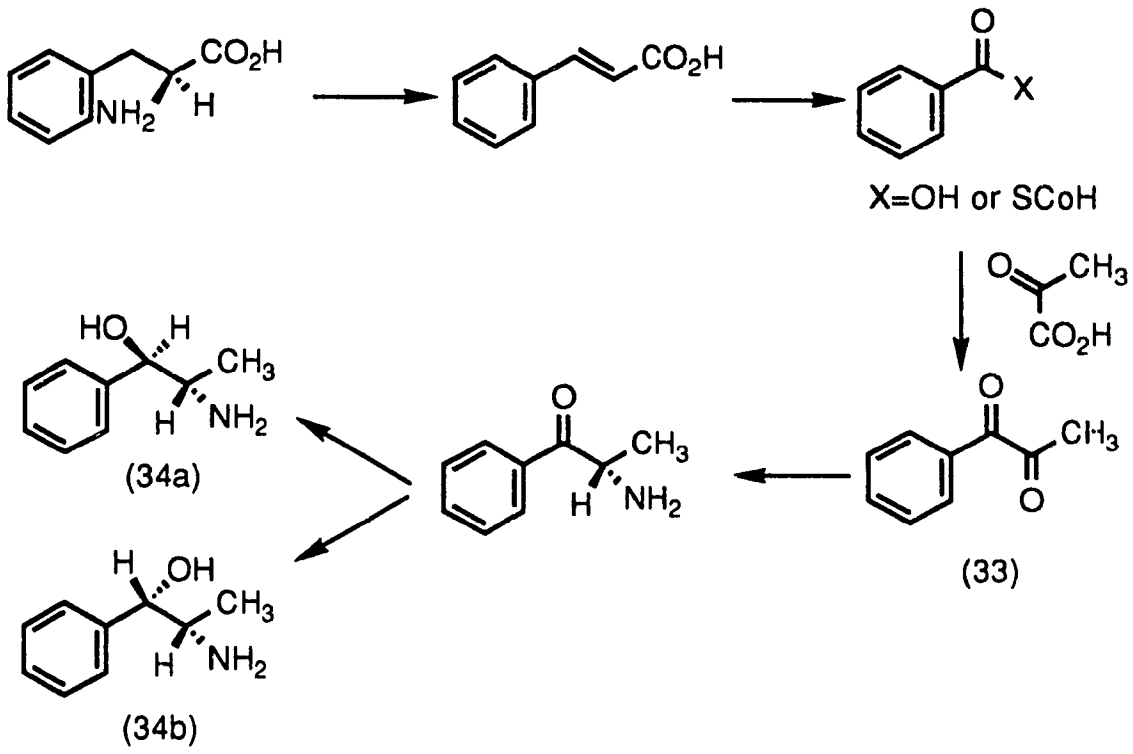


Figure 25. Biosynthetic pathway to the Ephedra alkaloids 34a and 34b.

Based on our hypothesis, a biomimatic approach towards the synthesis of the α-diketone **10** was attempted. In the past, thiazolium derivatives have been used to catalyze similar condensation reactions. For example, the condensation of formaldehyde to form 1,3-dihydroxyacetone **36** was catalyzed by the benzothiazolium salt **35** under basic conditions[23](Fig.26). Similarly, the condensation of aldehyde **37** with ethyl acrylate led to the formation of the γ-keto-ester **38** catalyzed by the catalyst **39**.[24] (Fig. 27)

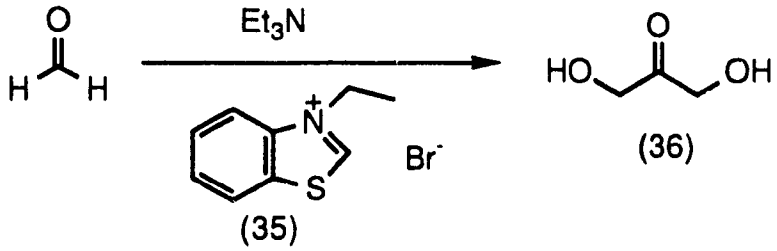


Figure 26. Synthesis of compound 36.

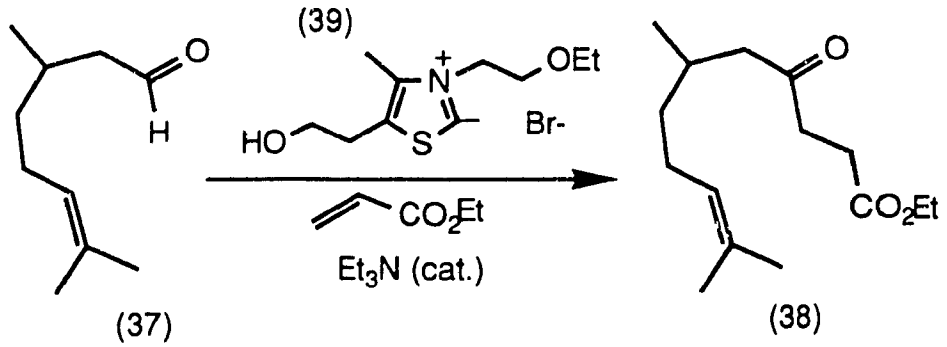


Figure 27. Synthesis of compound 38.

The benzothiazolium salt **35** was prepared by refluxing benzothiazole in the presence of ethyl bromide. This compound was initially used as a catalyst in two different model reactions (Fig. 28). However, the desired products (**40** or **43**) were not obtained under a variety of different conditions. In all cases the products formed in these reactions were the spiral thiazolium dimer **46** and small amounts of compound **47** and **48** of which the latter was found to be fairly unstable. The structure elucidation of compounds **46,47** and **48** was achieved primarily from their NMR and MS data and that of the major product **46** was confirmed by X-ray crystallography. The synthesis of this compound was first reported in the late 1960, however, its structural assignment was based only on elemental analysis and the observation that CO_2 gas was produced during course of the reaction.[25] The mechanism proposed for its formation is outlined in Figure 29.

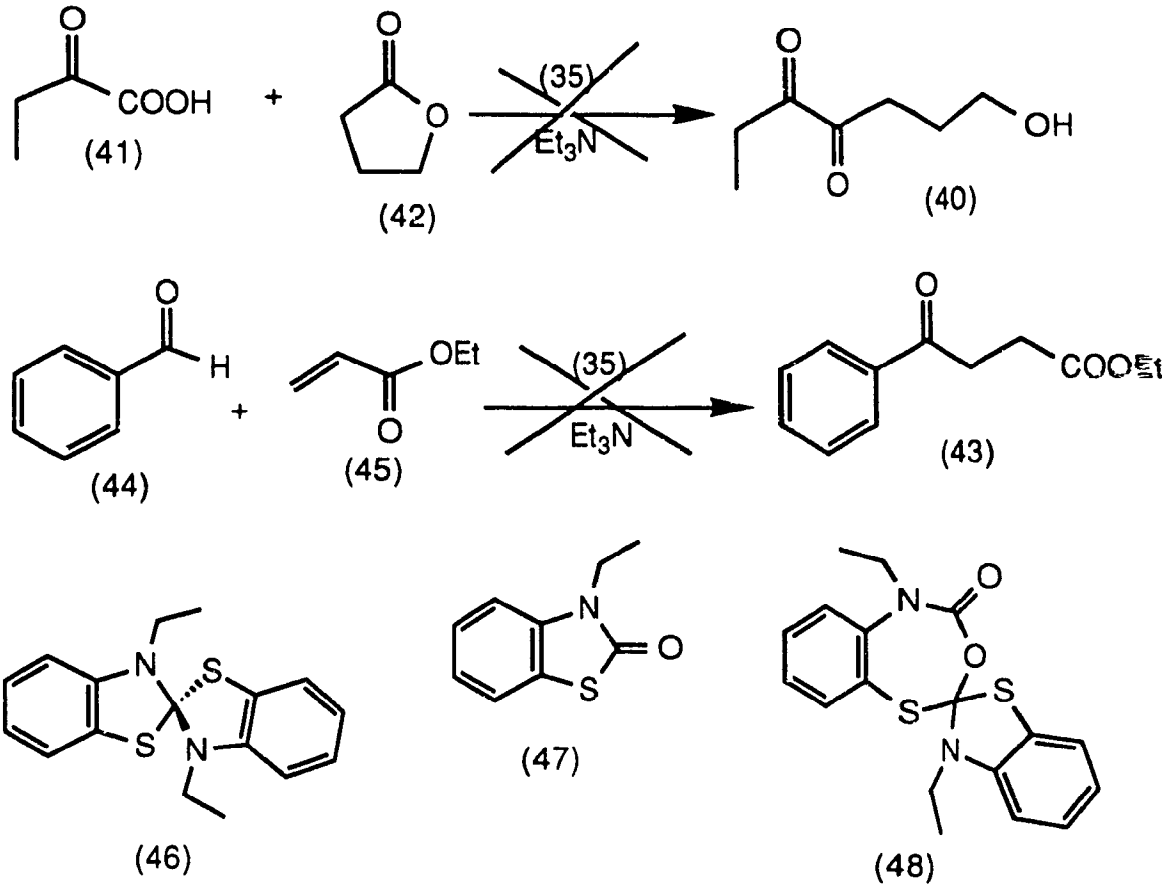


Figure 28. Reactions catalyzed by benzothiazolium salt 35.

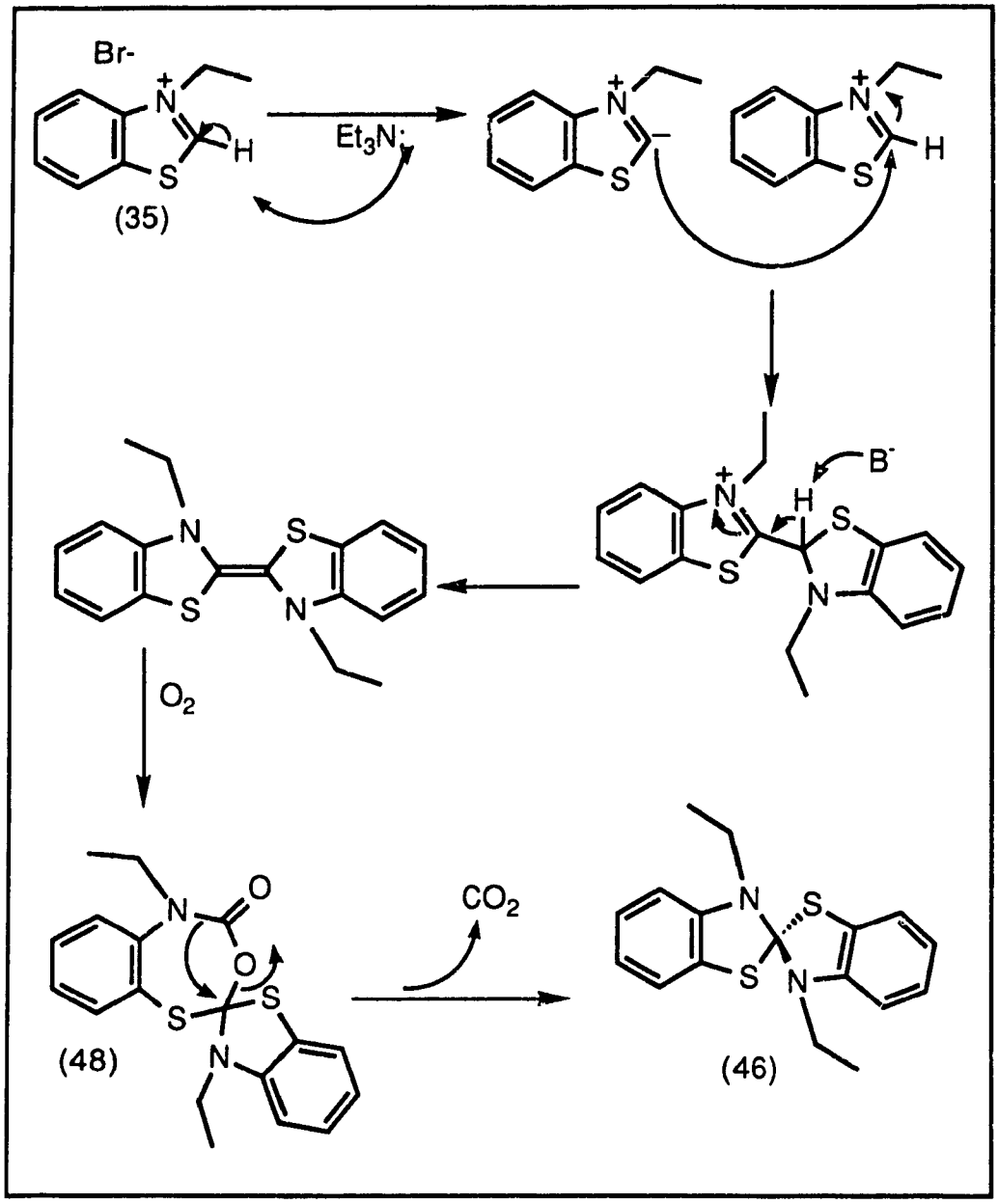


Figure 29. Proposed mechanism for the formation of compound 46.

It was then decided to use the bromide of 3-(2-ethoxyethyl)-5-(2-hydroxyethyl)-4-methyl-1,3-thiazolium (39) as catalyst for the same type of condensation reaction. Compound **39** was originally used as a catalyst in the condensation of citronellal **37** with ethyl acrylate.(Fig. 27) Preparation of this catalyst was carried out following a modified literature procedure,[24] using an

excess of 2-ethoxyethyl bromide as the solvent. The yield was greatly increased up to 98% compared to the literature yield of 76%.

Catalyst **39** was tested in the condensation of benzaldhyde with γ-butyrolactone and with succinic anhydride.(Fig. 30&31) Although in both cases the desired α-diketones **49** or **53** were not formed, the benzoin **50** and its derivative **52** were obtained in low yields. Therefore, C-C bond formation was achieved in the presence of catalyst **39**. Further investigation is needed in order to find the optimum substrates and reaction conditions which would lead to the formation of the desired α-diketone **10**.

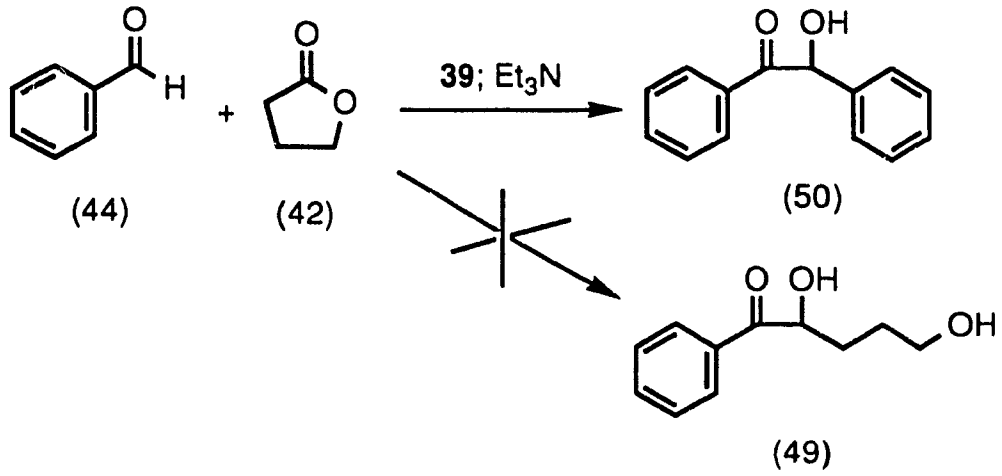


Figure 30. Formation of compound 50.

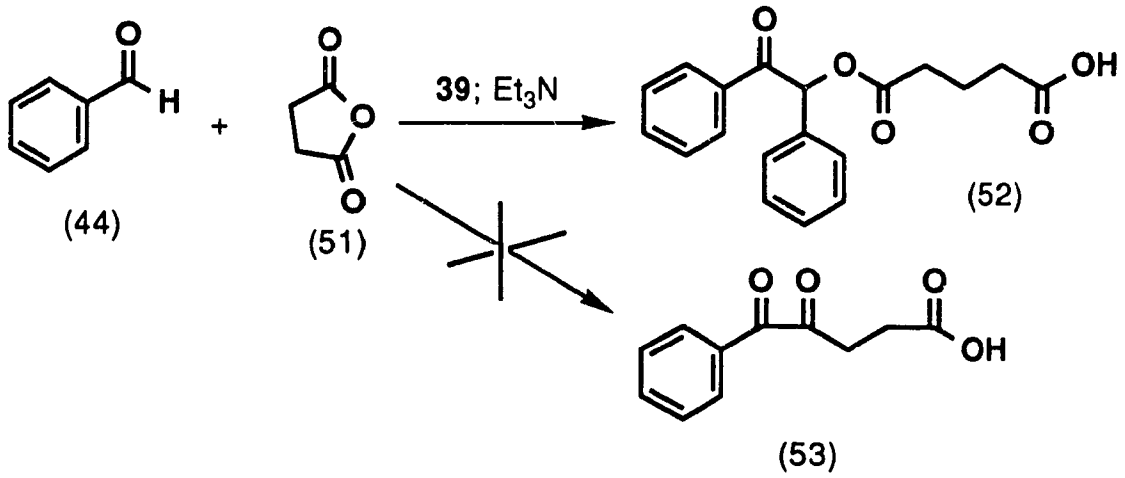


Figure 31. Formation of compound 52.

Chapter 3. Conclusions:

The proposed advanced precursor of oudenone, (S)-(-)-5-hydroxyl octanoic acid was successfully synthesized as its NAC-thiolester derivative **11'**, having better than 95% deuterium at the desired position (C-5). The 7-step reaction scheme, starting from the chiral 2-propylcyclopentanone, led to the formation of the final product in approximately 35% overall yield.

The N-acetylcysteamine thioester of the advanced precursor (5S)-[2-$^{2}H_{2}$,5-^{2}H]-5-hydroxyoctanoic acid **11'** was efficiently incorporated into oudenone. This result strongly supports our hypothesis that oudenone **9** is derived from one succinate unit and a tetraketide **11'**.

The approach towards the synthesis of the α-diketone derivative was not fully explored, but some useful information was obtained from our studies in this part of the project.

Chapter 4. Experimental:

4.1 General: Nuclear Magnetic Resonance spectra were obtained at 20-22°C using JEOL 270 MHz-CPF, Varian XL-300 MHz and Varian Unity 500 MHz instruments. ^{1}H, ^{2}H and ^{13}C-NMR chemical shifts are quoted in ppm and are referenced to the internal deuterated solvent downfield from tetramethylsilane (TMS).

Mass spectra were performed at the Biomedical Mass Spectrometry Unit, McGill University. High resolution $(NH_3)Cl$ MS spectra were obtained using a ZAB 2F HS instrument (resolution: 10^4).

Melting points were measured using a Gallenkamp capillary apparatus and they are uncorrected. Ultraviolet spectra were recorded on a Hewlett Packard 8452A DIODE ARRAY Spectrophotometer. Infrared spectra were recorded on a Bomem Michelson 102 FTIR spectrophotometer calibrated to the 1602 cm^{-1} line of polystyrene. Optical rotations were measured using a Jasco model DIP-140 Digital Polarimeter.

Chromatography: Flash column chromatography was carried out on Merck Kieselgel 60, 230-400 mesh, #9385 silica gel. Reverse phase flash column chromatography was carried out on silica gel (Merck Kieselgel 60, 230-400 mesh, #9385) reacted with *n*-octadecyltrichlorosilane. C18 reverse-phase silica gel was prepared following previously reported procedures.

Fermentation of *Oudemansiella radicata* ATCC 20295 for Precursor Feeding Experiments. Autoclaved seed medium (2 g glucose, 5 g Avicel microcrystalline

cellulose, 0.5 g yeast extract, 100 mL nanopure H_2O, in a 500 mL flask) was inoculated with 10-15 small scrapings of mycelia from an agar plate of *O. radicata*. The culture was grown in an incubator shaker at 26.5° C and 140 rpm for 17 days. Oudenone producing cultures were initiated by inoculation of the sterile production medium (2 g glucose, 0.5 g Bacto-peptone, 0.3 g yeast extract, 0.3 g KH_2PO_4, 0.1 g $MgSO_4 \cdot 7H_2O$, 100 mL nanopure H_2O, in a 500 mL flask) with 10 mL of seed culture and grown under the same conditions. Oudenone (3) production was usually observed after 6-7 days of growth and a maximum concentration was reached approximately 3-4 days later.

For feeding experiments involving the advanced precursor, tetraketide 11, the vegetative growth of the original production culture were transferred into an autoclaved 250 mL centrifuge tube and centrifuged at 10,000 rpm (Sorvall GSA rotor) for 15 min. The mycelia were then resuspended in replacement medium (10 g glucose, 0.3 g KH_2PO_4, 0.1 g $MgSO_4 \cdot 7H_2O$, 100 mL nanopure H_2O), transfered into a 500 mL flask and reincubated on a shaker at 26.5°C and 140 rpm. The ^2H labeled substrate **11'** (22 mg) was dissolved in absolute ethanol and administered in ~100 mL aliquots with β-oxidase inhibitor within 3 days.

UV Assays for Monitoring Oudenone Production. Fermentation broth aliquots (3 mL) were withdrawn from an actively growing culture every 12-24 h. The sample was filtered and diluted to 10 mL with distilled H_2O. A 1.0 mL sample was subsequently added to a 4.0 mL phosphate buffer (pH = 7.0) solution and to a 4.0 mL 0.1N HCl solution. The UV_{max} of oudenone is at 246 nm in phosphate buffer (pH = 7.0) , whereas it shifts to 284 nm in acid (0.1 N HCl). Thus, the amount of oudenone present in the fermentation broth was estimated

by measuring the difference in UV absorbance between the phosphate buffer and HCl solutions of the broth at 246 nm.

Isolation and Purification of Oudenone (9). The fermentation broth (100 mL) was filtered through cheese cloth and then extracted with *n*-butanol (3 x 100 mL). Evaporation of the butanol under high vacuum gave a light brown syrup which was redissolved in EtOAc (10 mL) and extracted with H_2O (pH = 7.0, 3 x 10 mL). The pH of the aqueous layer was adjusted to 3.0 with 1.0 N HCl and extracted with EtOAc (3 x 10 mL). The EtOAc layer was dried on anhydrous $MgSO_4$ and evaporated to dryness to give 10-20 mg of crude oudenone as a yellow oil. Pure oudenone was isolated after reversed flash column chromatography on C18 silica. The column was eluted with a linear gradient from 0.1% AcOH / 99.9% H_2O to 0.1% AcOH / 49.9% H_2O / 50% MeOH. The elution of oudenone from the column was monitored by UV.

4.2. Synthesis Part.

1. 3-(tetradecylthio)propanoic acid(β-oxidase inhibitor)

To a 100ml round bottom flask, 3-(tetradecylthio)propanic ethyl ester(0,5g) was dissolved in 80% THF in water (50ml), and was cooled down to 0°C in an ice-bath, 2 eq.(1.5ml) of 2M NaOH solution was added dropwise, the reaction mixture was then stirred for 24hr at room temprature. The pH of the reaction mixture was adjusted to 2-3 by adding 1N HCl, and then the reaction mixture was extracted with EtOAc (3x20ml), dried over MgSO4, and concentrated to give a white crystalline the product (0.44g, 95% yield) which was confirmed by ^1H NMR.

2. 2-Propylcyclopentanone(13)

Mathod 1: 2-Propylcyclopentanone (13). In a dry round bottom flask (500ml), sodium hydride (2.52 g,105.6 mmol) was suspended in toluene (300 ml) and cooled in an ice-bath. Ethyl-2-oxocyclopentanecarboxylate **17** (15 g, 96 mmol) dissolved in 50 ml of toluene was added dropwise into the flask *via* a syringe over a period of 20 min. The reaction mixture was allowed to warm up to R.T. and stir for 15 min. Propyl iodide (32.60g,192mmol) was added and the mixture was refluxed for 48 h. The reaction mixture was then cooled to R.T., quench with H_2O (~1 mL) and extracted with 200 mL of H_2O. The aqueous layer was further extracted with EtOAc (2 x 100 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated under reduced pressure to give compound **18** as a yellow oil (22 g, ~100%). Compound **18** was used in the synthesis of **13** without further purification.

TLC [silica, hexanes:EtOAc (10:1)]: R_f = 0.22

^1H NMR ($CDCl_3$): δ 0.90 (t, J=7.3 Hz, 3H, $-CH_3$), 1.25 (t, J=7.3 Hz, 3H, $-CH_3$), 1.08-1.42 (m, 2H), 1.48-1.63 (dt, 1H), 1.8-2.2 (m, 4H), 2.25-2.58 (m, 3H), 4.10-4.21 (q, J= 7.3 Hz, 2H).

^{13}C NMR ($CDCl_3$): δ 214.7, 170.8, 61.0, 60.3, 37.7, 35.8, 32.5, 19.4, 18.0, 14.2, 13.9.

Concentrated HCl (37%, 100 mL) was added to crude **18** (22 g) and the mixture was allowed reflux for 24 h. The reaction mixture was then extracted with Et_2O (2 x 100 mL), washed with saturated $NaHCO_3$ (2 x 100 mL) and brine (100 mL), dried over $MgSO_4$, and concentrated under reduced pressure to give **13** as a crude oil. After vacuum distillation, 9.9g of pure **13** was obtained, an 82% overall yield from 17.

Method 2: In a dry round bottom flask, potassium hydride suspension in mineral oil were transfered and washed with dry THF 3 times via syringe under N_2, and dried under high vacuum to give the pure KH powder (0.79g, 0.197mol), 8ml of THF was added, followed by the addition of cyclopentanone (1.482g, 0.176mol) dropwise at 0°C, then the reaction mixture was allowed to stir at room temperature for 15min, then propyl bromide (2.43g, 0.197mol) was added and the reaction mixture was stirred for another 15min. 10 ml of H_2O was added to quench the reaction. The reaction mixture was extracted with ether, the combined organic layer was dried over $MgSO_4$, concentrated to give the crude oil. This was further purified by flash column chromatography to give the product **13** (50% yield). (The main side product **16** was from the self-condensation of cyclopentanone according to the GC-Mass spectrum data and ^1H, ^{13}C NMR).

TLC [silica, hexanes:EtOAc (4:1)]: $R_f = 0.71$

^1H NMR ($CDCl_3$): δ 0.80 (t, J=7.3 Hz, 3H, $-CH_3$), 1.08-2.30 (5m, 11H).

^{13}C NMR ($CDCl_3$): δ 221.5, 48.7, 37.9, 31.7, 29.4, 20.55, 20.52, 13.8.

[2-^{2}H, 5-$^2H_2$]-2-Propylcyclopentanone (13')

The deuterium labeled **13'** was obtained *via* hydrolysis and decarboxylation of **18** in 37% DCl in D_2O.

^1H NMR ($CDCl_3$): δ 0.87 (t, J=7.3 Hz, 3H, $-CH_3$), 1.1-1.5 (m, 4H), ~1.6-1.8 (m, 2H), ~1.9-2.0 (m, 1H), 2.15-2.25 (m, 1H).

^{13}C NMR ($CDCl_3$): δ 221.9, 48.4 (t), 37.5 (m), 31.7, 29.4, 20.7, 20.5, 14.0.

^2H NMR ($CDCl_3$): δ 2.03-2.08 (2D) 2.26 (1D).

MS [$(NH_3)Cl$, direct inlet, 58°C], m/z (% relative intensity, assignment): 147 [73.6, $(M+NH_4)^+$ of **13'** with 3 deuterium atoms], 146 [24.2, $(M+NH_4)^+$ of **13'** with 2 deuterium atoms], 145 [2.2, $(M+NH_4)^+$ of **13'** with 1 deuterium atom].

Cis-and trans-7-benzylthiomenthone (28)

To a round bottom flask(1L), pulegone **27** (100g, 0.657mol) and benzylmercaptan (93.7g, 0.747mol) were dissolved in 250ml of THF, then 4ml of 10%NaOH aqueous solution was added, the reaction mixture was allowed to be refluxed for 6 hrs, cooled down to room temperature, transfered into a seperatory funnel and washed with two 200ml portions of saturated aqueous NaCl. The combined aqueous layer were extracted with ether (3x150ml). The combined organic layer was dried over anhydrous $MgSO_4$, and concentrated under vacuum. This residual oil was distilled in a good vaccum to give a fraction of b.p. 150°-160°C of **28** which weighed 150g (83%yield).

^{1}H NMR($CDCl_3$): δ (ppm) 7.3-7.5 (m, aromatic, 5H), 3.83 (s, 2H, $SC\underline{H}_2$), 1.7 (s, 3H, $C\underline{H}_3CSH$), 1.5 (s, 3H, $C\underline{H}_3CSH$) and other peaks.

^{13}C NMR($CDCl_3$): δ (ppm) 210.7, 138.5, 128.9, 128.4, 126.7, 57.6, 52.2, 48.0, 36.6, 34.4, 33.1, 29.6, 27.7, 23.8, 22.2 and peaks due to the minor isomer.

Isomeric mixtures of Hydroxythiol derivative (29)

An oven dried 3L 3 neck round bottom flask was equipped with a Hershberg stirrer and a 1L Dewar condenser filled with dry ice-acetone which was connected to the N_2 gas, the remaining neck of the flask was capped with a rubber septum through which approximately 2000ml of ammonia was condensed into the flask via a glass tube. 75g of clean sodium was added piece by piece to this liquid ammonia with slow stirring (the liquid ammonia turned dark blue), then 150g of 7-benzylthiomenthone **28** and 43.5ml of MeOH in 300ml of anhydrous ether were added dropwise via a pressure-equalized addition funnel over 2hr to the vigorously stirred solution. Stirring is continued an additional 30 min following which 90 ml of MeOH was added over 30 min. Then the solution is allowed to warm up slowly, the additional funnel and the

condenser were removed to allow the ammonia to evaporate for 12 hr. The flask was immersed in an ice-bath and 400 ml of distilled water was added continually to the residue left by evaporation of the ammonia. The solution was extracted with ether(2x200ml) which were discarded. The aqueous layer was poured into a mixture of 300 ml of HCl 37% and 600g of ice, then extracted with ether (4x200ml). The combined ether extracts were washed with 200 ml of water, 200 ml of saturated aqueous NaCl solution, dried over $MgSO_4$ and concentrated under vacuum. The residue liquid was placed under high vacuum for 2 hr to remove the remaining solvent to give an orange oil that is the isomeric mixture of **29**.(100g, 98%yield)

4 isomers(29a,29b,29c,29d) were obtained in pure form by flash column chromatography using 3.5% EtOAC in hexanes as eluent.(repeated several times). The ratio was determined by ^1H NMR (**29a** 68%, **29b,c** 21%, **29d** 11%)

29a(major): ^1H NMR($CDCl_3$): δ (ppm) 3.73 (dt, J=4Hz, 10Hz, 1H, C<u>H</u>OH), 3.27(s, 1H, O<u>H</u>), 2.15 (s, 1H, S<u>H</u>), 1.68-2.0 (m,3H), 1.56 (s, 3H,C<u>H</u>$_3$CSH), 1.45 (s, 3H, C<u>H</u>$_3$CSH), 1.16-1.48 (m, 2H), 0.9-1.15 (m, 3H) 0.95 (d, J=6Hz, 3H, C<u>H</u>$_3$CH)

^{13}C NMR($CDCl_3$): δ (ppm) 72.9, 54.6, 47.1, 45.2, 34.7, 34.5, 31.2, 28.5, 26.7, 21.8.

29b: ^1H NMR($CDCl_3$): δ (ppm) 4.35 (m, 1H, C<u>H</u>OH), 2.62(br, 1H, O<u>H</u>), 1.87 (s, 1H, S<u>H</u>), 1.5-2.0 (m,6H), 1.45 (s, 3H,C<u>H</u>$_3$CSH), 1.43 (s, 3H, C<u>H</u>$_3$CSH), 1.2-1.35(m, 2H), 1.17 (d, J=8Hz, 3H, C<u>H</u>$_3$CH)

^{13}C NMR($CDCl_3$): δ (ppm) 68.8, 52.0, 47.1, 39.3, 33.7, 32.3, 30.9, 26.2, 21.1, 17.0.

29C: ^{1}H NMR($CDCl_3$): δ (ppm) 4.30 (m, 1H, C<u>H</u>OH), 2.81 (br,1H, O<u>H</u>), 1.6-1.9 (m, 6H), 1.41 (s, 6H,C<u>H</u>$_3$CSH), 1.25 (m, 1H), 0.9-1.1 (m, 2H) 0.82 (d, J=6Hz, 3H, C<u>H</u>$_3$CH)

^{13}C NMR($CDCl_3$): δ (ppm) 67.9, 51.7, 46.9, 42.9, 35.1, 33.9, 31.0, 25.7, 22.1, 21.8.

29d: ^{1}H NMR($CDCl_3$): δ (ppm) 3.95 (dt, J=4Hz, 10Hz, 1H, C<u>H</u>OH), 2.82(br,1H, O<u>H</u>), 2.02 (s, 1H, S<u>H</u>), 1.5 (s, 3H,C<u>H</u>$_3$CSH), 1.4 (s, 3H, C<u>H</u>$_3$CSH), 1.1-1.9 (m, 5H), 0.95 (d, J=7Hz, 3H, C<u>H</u>$_3$CH), 0.8-1.0 (m, 3H)

^{13}C NMR($CDCl_3$): δ (ppm) 68.9, 55.7, 47.3, 41.8, 34.7, 31.3, 28.8, 27.5, 21.7, 18.6.

Oxathianes 32c,32d: The deuterium labeled, racemic **13** (756 mg, 5.9 mmol) dissolved in dry benzene (20 mL) was added to a solution of hydroxythiol **29b** (1.10 g, 5.8 mmol) in dry benzene (60 mL). A catalytic amount of *p*-toluenesulfonic acid (~10 mg) and D_2O was added, the flask was fitted with a Dean-Stark trap and the reaction mixture was refluxed for 24 h. The benzene was then removed by evaporation, the crude product was dissolved in Et_2O (100 mL), washed with a saturated, aqueous $NaHCO_3$ (2 x100 mL) and brine (100 mL). The ether layer was dried over $MgSO_4$ and concentrated under reduced pressure to give the diastereometic mixture of oxathiane **32d** and **32c** in ~2:1 ratio (1.58 g, 91% total yield). Flash column chromatography (repeated twice) using a linear solvent gradient from pure hexanes to 0.25% EtOAc in hexanes permitted the isolation of the desired diastereomer **32d** in 62% yield (1.06 g). Compound **32d** was crystallized from pentane.

<u>Major product **32d**</u>

mp = 34.5 - 35.0°C.

TLC (silica gel, hexanes): R_f = 0.48.

^{1}H NMR ($CDCl_3$): δ 0.80 (d, J = 5.9 Hz, -CH_3), 0.88 (t, J = 7.3 Hz, -CH_3), ~0.9-1.0 (m, 2H), 1.09 (s, -CH_3), ~1.2-1.4 (m, 4H), ~1.5-1.7 (m, 1H), 1.50 (s, -CH_3), ~1.7-1.9 (m, 9H), 4.01 (br s, 1H).

^{13}C NMR ($CDCl_3$): δ 14.4, 21.6, 21.7, 22.3, 22.4, 25.9, 28.4, 29.5, 30.5, 32.2, 34.8, 40.5 (m), 41.8, 43.7, 44.1, 51.4 (t), 65.6, 89.8.

Minor product **32c**

TLC (silica gel, hexanes): R_f = 0.39.

^{1}H NMR ($CDCl_3$): δ 0.80 (d, J = 5.9 Hz, -CH_3), 0.88 (t, J = 7.3 Hz, -CH_3), 1.1 (s, -CH_3), 1.6 (s, -CH_3), ~0.8-2.0 (m, 16H), 4.01 (br s, 1H).

^{13}C NMR ($CDCl_3$): δ 14.5, 20.5, 21.6, 21.8, 22.3, 25.7, 27.4, 30.3, 32.2, 32.5, 34.8, 38.5 (m), 41.8, 43.9, 51.5 (t), 67.9, 93.1.

(2S)-(+)-[2-^{2}H, 5-$^2H_2$]-2-Propylcyclopentanone (13')

Compound **32d** (920 mg, 3.09 mmol) was dissolved in a mixture of CH_3CN : THF (2 mL: 0.5 mL) and then quickly added into a vigorously stirred, ice cold solution of 80% aqueous CH_3CN containing N-chlorosuccinimide (2.52 g, 18.8 mmol), $AgNO_3$ (3.36 g, 19.78 mmol) and 2,4,6-collidine (4.56 g, 37.7 mmol). The mixture was stirred in an ice-bath for 30 min, followed by the succesive addition of saturated Na_2SO_3 and brine (3 mL of each). A solvent mixture of hexanes : CH_2Cl_2 (30 mL of each) was added, the mixture was filtered and the filter cake was washed thoroughly with hexanes : CH_2Cl_2 (1:1). The organic layer was dried over anhydrous $MgSO_4$ and concentrated to give **(S)-(+)-13'** as a crude oil. Purification by flash coloum chromatography using Et_2O : petroleum ether (1:25) as the eluting solvent, followed by vacuum distillation gave the pure product with ~50% yield (198 mg).

$[\alpha]_D$ = ~ + 103 (c 0.312, THF).

(3S)-(-)-[3-^{2}H, 6-$^2H_2$]-3-Propyl-δ-valerolactone (12'):

m-Chloroperbenzoic acid (1,7 g, 9.85 mmol) was added to a solution of **(S)-13'** (1.2 g, 9.52 mmol) in CH_2Cl_2 (40 mL) and the reaction mixture was stirred at R.T. for 24 h. It was then filtered, washed with saturated $NaHCO_3$ (3 x 30 mL), dried over $MgSO_4$ and concentrated under reduced pressure to give a crude oil. After vacuum distillation, lactone **12'** was isolated as a colorless oil, in 85% yield (1.2 g).

TLC [silica, EtOAc: hexanes (1:4)]: R_f = 0.22.

$[a]_D$ = -63.4° (c 3.38, $CHCl_3$)

^{1}H NMR ($CDCl_3$): δ 0.9 (t, J= 7.3 Hz, 3H, CH_3), 1.3-1.9 (m, 8H, $4CH_2$).

^{13}C NMR ($CDCl_3$): δ 13.7, 18.0, 18.1, 27.5, 27.8 (m), 37.6, 79.7 (t), 171.9

^{2}H NMR ($CDCl_3$): δ 2.28 and 2.42 (2^2H6), 4.17 (s, 1^2H3).

Non-labeled 3-Propyl-δ-valerolactone (12)

^{1}H NMR ($CDCl_3$): δ 0.9 (t, J = 7.3 Hz, 3H, CH_3), 1.3-1.9 (m, 8H, $4CH_2$), 2.3-2.6 (2 m, 2H, 2H6), 4.3 (m,1H, H3).

^{13}C NMR ($CDCl_3$): δ 13.6, 17.9, 18.2, 27.5, 29.2, 37.7, 80.1, 171.7

(5S)-(-)-[2-$^2H_2$, 5-^{2}H]-5-Hydroxyloctanoyl methyl ester (22'):

Lactone **12'** (0.876g), fresh distilled methanol (15 mL) and catalytic amounts of (±)-10-camphorsulfonic acid (10 mg) were added to a dry round bottom flask (25 mL). The reaction mixture was refluxed for 12 h under N_2, cooled back to room temperature and partitioned between Et_2O (20 mL) and saturated $NaHCO_3$ aqueous solution (10 mL) . The aqueous layer was extracted further with Et_2O (3x10 mL), the organic layers were combined, dried over anhydrous

$MgSO_4$ and concentrated under reduced pressure to give crude **22'**. Flash column chromatography using 25% EtOAc in hexanes, gave pure **22'** as a colorless oil (1.036g, 96.5% yield).

TLC [silica, EtOAc: hexanes (2:3)]: R_f = 0.5.

$[\alpha]_D$ = -1.6° (c 2.94, THF)

^{1}H NMR($CDCl_3$): δ 0.87 (t, J=6.6, 3H, $-CH_3$), 1.2-1.8 (m, 8H, $4CH_2$), 3.61(s, 3H, $-CO_2CH_3$).

^{13}C NMR($CDCl_3$): δ 13.9, 18.6, 20.7, 33.4 (m), 36.4, 39.3, 51.3, 70.3 (t), 174.1

Non-labeled 5-hydroxyloctanoate methyl ester 22

^{1}H NMR($CDCl_3$): δ 0.89 (t, J=6.6, 3H, $-CH_3$), 1.2-1.9 (m, 8H, $4CH_2$), 2.38 (t, J=7.3 Hz, 2H, 2H2), 3.65 (m, 1H, H5), 3.61(s, 3H, $-CO_2CH_3$).

^{13}C NMR($CDCl_3$): δ 14.6, 19.3, 21.5, 34.2, 37.0, 39.9, 51.6, 70.8, 172.9

(5S)-[2-$^2H_2$, 5-^{2}H]-5-*t*-Butyldimethylsilyloxyloctanoate methyl ester (23'): *t*-Butyldimethylsilyl chloride (525 mg, 3.48 mmol) and imidazole (564 mg, 8.28 mmol) were dissolved in dry DMF (1 mL). Methyl ester **22'** (145 mg, 828 µmol) was added and the reaction mixture was stirred under N_2, at R.T. for 48 h. The reaction was subsequently quenched with the addition of Et_2O (10 mL) and brine (5 mL). The aqueous layer was further extracted with Et_2O (2x10 mL), the ether layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give the crude **23'**. Flash column chromatography using 5% EtOAc in hexanes as the eluting solvent, lead to the isolation of **23'** as a colourless oil (206 mg, 86% yield).

TLC [silica, EtOAc: hexanes (1:20)]: R_f = 0.46

$[\alpha]_D$ = -0.46° (c 3.9, $CHCl_3$)

^{1}H NMR ($CDCl_3$): δ 0.01 (s, 6H, Si-$2CH_3$), 0.86 (s, 9H, *t*-butyl-Si), 0.86 (t, J = 5.3, -CH_3), 1.2-1.7 (m, 8H, $4CH_2$), 3.63 (s, 3H, CO_2CH_3).

^{13}C NMR($CDCl_3$): δ -4.5, 14.3, 18.1, 18.5, 20.8, 26.0, 34.2 (m), 36.4, 39.3, 51.4, 71.6 (t), 174.1.

Non-labeled compound 23

^{1}H NMR ($CDCl_3$): δ 0.01 (s, 6H, Si-$2CH_3$), 0.86 (s, 9H, *t*-butyl-Si), 0.86 (t, J = 5.3, -CH_3), 1.2-1.7 (m, 8H, $4CH_2$), 2.28 (t, J=7.3, 2H, 2H2), 3.63 (m, 1H, H5), 3.63 (s, 3H, CO_2CH_3).

^{13}C NMR($CDCl_3$): δ -4.5, 14.3, 18.1, 18.5, 20.8, 26.0, 34.2, 36.4, 39.3, 51.4, 71.6, 174.1.

(5S)-[2-$^2H_2$, 5-^{2}H]-5-*t*-Butyldimethylsilyloxyloctanoyl NAC thioester 24':

Dry benzene (2.5 mL) and trimethylaluminum (1.7 mL of a 2 M solution in toluene, 3.4 mmol) were added in an oven-dried RB flask (10 mL) under N_2 and the mixture was cooled in ice-bath. A solution of N-acetylcysteamine (407 mg, 3.42 mmol) in dry benzene (1 mL) was added and the reaction mixture was allowed to warm up to RT while stirring for 15 min. A solution of compound **23'** (200 mg, 0.680 mmol) in dry benzene (1 mL) was added and the mixture was stirred at RT for 24 h. The reaction was quenched with the addition of Et_2O (20 mL) and KH_2PO_4 buffer (pH=7, 20 mL). The aqueous layer was further extracted with ether (3x20 mL), the combined organic layer was washed with brine (20 mL), dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a yellow oil. This crude product was purified by flash column chromatography (60% EtOAc in hexanes) to give pure **24'** in 63% yield (162 mg).

TLC [silica, EtOAc: MeOH (10:1)]: R_f = 0.59

$[\alpha]_D$ = -0.406° (c 2.96, $CHCl_3$)

^1H NMR($CDCl_3$): δ 0.02 (s, 6H, Si-$2CH_3$), 0.86 (s, 9H, *t*-Bu-Si), 1.2-1.5 (m, 6H, $3CH_2$), 1.6-1.8 (m, 2H, CH_2), 1.94 (s, 3H, $COCH_3$), 2.98 (t, J=6.3 Hz, 2H, S-CH_2), 3.41 (dt, J_1=J_2=6.3 Hz, 2H, NCH_2), 5.85 (br, 1H, NH).

^{13}C NMR($CDCl_3$): δ -4.5, 14.2, 18.0, 18.4, 21.4, 23.1, 25.8, 28.4, 36.0, 39.2, 39.7, 170.2, 199.9.

MS [(NH_3) CI, direct inlet, 240°C], m/z (% relative intensity, assignment): 321 [55.1, $(M+H^+ - NHAc)^+$ of **24'** with 3 deuterium atoms], 320 [15.5, $(M+H^+ - NHAc)^+$ of **24'** with 2 deuterium atoms], 319 [2.1, $(M+H^+ - NHAc)^+$ of **24'** with 1 deuterium atoms], 176 [71.1, $(M+H^+ - TBDMSiD - CH_2CH_2NHAc)^+$ of **24'** with 3 deuterium atoms], 86 [100.0, $(CH_2CH_2NHAc)^+$].

Non-labeled compound 24

^1H NMR($CDCl_3$): δ 0.02 (s, 6H, Si-$2CH_3$), 0.86 (s, 9H, *t*-Bu-Si), 1.2-1.5 (m, 6H, $3CH_2$), 1.6-1.8 (m, 2H, CH_2), 1.94 (s, 3H, $COCH_3$), 2.55 (t, J=7.5 Hz, 2H, -$COCH_2$) 2.98 (t, J=6.3 Hz, 2H, S-CH_2), 3.41 (dt, J_1=J_2=6.3 Hz, 2H, NCH_2), 3.63 (m, 1H, -CH-OSi), 5.85 (br, 1H, NH).

^{13}C NMR($CDCl_3$): δ -4.5, 14.2, 18.0, 18.4, 21.4, 23.1, 25.8, 28.4, 36.0, 39.2, 39.7, 44.2, 71.5, 170.2, 199.9.

(5S)-[2-$^2H_2$, 5-^2H]-5-hydroxyloctanoyl NAC thioester 11':

In a round bottom flask (10 mL), 3 mL of acetic acid and 1.5 mL of H_2O were mixed. The flask was dipped into an ice-bath and a THF solution (1.5 mL) of the silyl ether **24'** (140 mg) was added. The reaction was stirred at 0°C for 5 minutes, then R.T. for 14hr. After that, the reaction mixture was neutralized with

aqueous $NaHCO_3$ and then extracted with EtOAc (3x20 mL). The combined EtOAc layer were dried over anhydrous $MgSO_4$, concentrated under reduced pressure and further dried under high vacuum to yield the final product **11'** as an oil (90 mg, ~100%yield).

TLC [silica, EtOAc]: $R_f = 0.25$

$[\alpha]_D = -1.86^o$ (c 1.72, $CHCl_3$)

^1H NMR($CDCl_3$): δ 0.89 (m, 3H, CH_3), 1.22-2.95 (m, 8H, $4CH_2$), 1.96 (s, 3H, $-NCOCH_3$), 2.99 (t, J=6.3, 2H, $-CH_2-SCO$), 3.41 (dt, $J_1=J_2=6.3$ Hz, 2H, $-NCH_2-$), 5.86 (br, 1H, NH).

^{13}C NMR($CDCl_3$): δ 14.0, 18.8, 21.8, 23.2, 28.6, 36.4, 39.6, 39.7, 43.9 (m), 71.1 (t), 170.3, 200.1

Non-labeled compound 11

^1H NMR($CDCl_3$): δ 0.89 (m, 3H, CH_3), 1.22-2.95 (m, 8H, $4CH_2$), 1.96 (s, 3H, $-NCOCH_3$), 2.58 (t, J=7.3, 2H, $-SCO-CH_2-$), 2.99 (t, J=6.3, 2H, $-CH_2-SCO-$), 3.41 (dt, $J_1=J_2=6.3$ Hz, 2H, $-NCH_2-$), 3.58 (m, 1H, $-CHOH$), 5.86 (br, 1H, NH).

^{13}C NMR($CDCl_3$): δ 14.0, 18.8, 21.8, 23.2, 28.6, 36.4, 39.6, 39.7, 43.9, 71.1, 170.3, 200.1

Benzothiazolium salt 39:

In a dry round bottom flask(100mL), a mixture of benzothiazole(24.9g), and ethyl bromide (21.8g) was refluxed for 2 days under N_2. The mixture was cooled down to the room temp., the solidified product was collected by filteration(the filtrate contained starting materials which could be used again) and was purified by recrystallization from ethanol-ether to give the white

needle-like crystals(8.8g, 30% yield). The NMR spectrum of the product showed identical chemical shift to those in the literature.

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