In compliance with the
Canadian Privacy Legislation
some supporting forms
may have been removed from
this dissertation.

While these forms may be included
in the document page count,
their removal does not represent
any loss of content from the dissertation.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

University of Alberta

The Biosynthesis of Lovastatin: Examining the Assembly and Elaboration Steps

by

John Sorensen 

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Department of Chemistry

Edmonton, Alberta

Fall 2003

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

National Library of Canada
Bibliothèque nationale du Canada

Acquisitions and Bibliographic Services
Acquisisitons et services bibliographiques

395 Wellington Street
Ottawa ON K1A 0N4
Canada

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence
ISBN: 0-612-88051-6
Our file Notre référence
ISBN: 0-612-88051-6

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

L'auteur conserve la propriété du droit d'auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou aturement reproduits sans son autorisation.

Canada

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

University of Alberta

Library Release Form

Name of Author: John Sorensen

Title of Thesis: The Biosynthesis of Lovastatin: Examining the Assembly and Elaboration Steps

Degree: Doctor of Philosophy

Year this Degree Granted: 2003

Permission is herby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as herein before provided, neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the authors' prior written permission.

Dated: SEPT 25/03

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled The Biosynthesis of Lovastatin: Examining the Assembly and Elaboration Steps submitted by John Sorensen in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Dr. John C. Vederas (Supervisor)

Dr. David R. Bundle

Dr. Derrick L. J. Clive

Dr. Monica M. Palcic

Dr. Michael A. Pickard

Dr. Craig A. Townsend (External)

Dated: Sept 23/03

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

In memory of my father

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

ABSTRACT

Lovastatin (**1**) is a widely prescribed inhibitor of HMG-CoA reductase, the critical enzyme involved in cholesterol biosynthesis in humans. Lovastatin and derivatives such as simvastatin (**4**) and atorvastatin (**6**) account for 5.4% ($11 billion USD) of all drug sales in the United States in 2002. Biosynthesis of **1** occurs via the polyketide pathway in the filamentous fungus *Aspergillus terreus*. It has been been established that an initial assembly of nine acetate units gives dihydromonacolin L (**11**), which is elaborated in a series of post-PKS reactions to give **1**. Heterologous expression of *lovB* and *lovC* genes in non-lovastatin producer *A. nidulans* led to the discovery that the main lovastatin nonaketide synthase (LNKS = LovB) along with an accessory enoyl reductase protein (LovC) is required for assembly of **11**. In addition, it was found that LovF is the lovastatin diketide synthase (LDKS) that catalyzes the assembly of the diketide side chain. Other enzymes, (*lovA*, *ORF17* and *lovD*) are responsible for the post-PKS oxidation and acylation of **11**.

Fermentation conditions for *A. nidulans lovB + lovC* were optimized to increase the yield of **11**. Adding either sodium [1-^{13}C] acetate or sodium [1-^{14}C] acetate leads to the production of **11** containing the corresponding isotope. Chemical degradation of the **11** thus produced was used to synthesize (3*R*)-5-((1*S*,2*S*,4a*R*,6*R*,8a*R*)-1,2,4a,5,6,7,8,8a-octahydro-2,6-dimethylnaphthalen-1-yl)-3-hydroxy-pentanethioic acid *S*-(2-acetylaminoethyl) ester (**24**), 3-((1*S*,2*S*,4a*R*,6*R*,8a*R*)-1,2,4a,5,6,7,8,8a-octahydro-2,6-dimethyl-naphthalen-1-yl)-propanethioic acid *S*-(2-acetylaminoethyl) ester (**25**) and ((1*S*,2*S*,4a*R*,6*R*,8a*S*)-1,2,4a,5,6,7,8,8a-octahydro-2,6-dimethyl-naphthalen-1-yl)-methanol (**29**) putative polyketide intermediates that may be involved in the assembly of **11**. In

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

preliminary experiments incorporation of intact intermediates, such as **24**, into **11** could not be detected.

During isolation of **11** a new compound, monacolin N **(33)**, was characterized which differs from 11 by the presence of an extra double bond in the linker arm between the lactone ring and decalin rings. Formation of **33** presumably results from a failure of the enoyl reductase activity of LovC during the assembly process.

In addition, a synthetic route to *N*-1'*S*-(1-phenyl-ethyl)- (*R*)-3-[(*tert*-butyldimethylsilyl)oxy]-6-(dimethoxyphosphinyl)-5-oxohexanamide **(42)**, a phosphonate precursor of the lactone ring of lovastatin was established.

A series of biotransformation experiments with *A. terreus* knockout mutants (blocked in the production of **1**) were conducted. It was found that *A. terreus lovA* (blocked in the transformation of **11** to **1**) degrades **11** to 3-((1*S*,2*S*,4a*R*,6*S*,8a*R*)-1,2,4a,5,6,7,8,8a-octahydro-2,6-dimethyl-6-hydroxy-naphthalen-1-yl)-propanoic acid by β-oxidation but retains the ability to transform the advanced intermediate monacolin J **(12)** to **1**. Additional experiments with two diastereomeric epoxide derivatives (**54a** and **54b**) of dihydromonacolin L **(11)** failed to yield detectable biotransformation products. Another mutant *A. terreus lovC* (blocked in the ability to produce **11**) was shown capable of converting exogenously added **11** to **1**, however transformation of analogues of **11**, such as monacolin N **(33)**, could not be detected.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Acknowledgements

I would like to acknowledge the outstanding support and encouragement of my research supervisor, Professor John C. Vederas. The time spent in his research group has been extremely rewarding and has prepared me for the future. I would also like to thank the members of the Vederas research group, both past and present, that have made working in this lab such an enjoyable experience. I would especially like to thank Mr. Doug Burr and Drs. Belen Mayo-Martin and Hanna Pettersson for their assistance in proofreading this manuscript. I would also like to mention the dedicated support staff in the Chemistry Department, especially those spectral services that have provided valuable assistance with this project.

I would like to thank Dr. Soledade Pedras for continuing advice and encouragement and Dr. Lois Browne for her friendship and advice.

I would like to thank the people that I have met both in and out of the Chemistry Department that have made my time in Edmonton memorable. The members of my family have also been especially encouraging during this time.

Financial support from the University of Alberta is also gratefully acknowledged.

Finally, and not least, a special "thank you" to my wife Lori. She has been especially patient especially during our time apart, and her support has helped make this project possible.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Table of Contents

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

List of Figures

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

List of Schemes

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

1. Introduction

1.1 Lovastatin and the statin drugs

Lovastatin (**1**) (Mevacor™) is the parent of a superfamily of prescription drugs known as the statins that are used for lowering cholesterol in humans[1,2]. Produced via the polyketide pathway in the filamentous fungus *Aspergillus terreus,* **1** was first reported in 1976[3,4] shortly after the isolation of the related fungal metabolite compactin (**2**)[5,6] from the fungus *Penicillium aurantiogrisem* (Fig. 1). Compound **2** is less potent than **1** and therefore is not as widely prescribed. However, **2** is still produced in large quantities for microbial transformation into the commonly prescribed pravastatin (**3**)[7,8] (Pravacol™). In addition to **1**, certain semi-synthetic analogues such as simvastatin (**4**)[9,10] (Zocor™) and fully synthetic analogues such as fluvastatin (**5**)[11] (Lescol™), atorvastatin (**6**)[12] (Lipitor™) and rosuvastatin (**7**)[13] (Crestor™) all compete for market share. In a report of the top 200 pharmaceutical products in the United States in 2002,[14] atorvastatin (**6**) and simvastatin (**4**) were the top-selling drugs with sales of $US 6.08 billion and $US 4.17 billion, respectively. This represents a total market share of 5.4% of all pharmaceutical drugs sold in the United States in 2002. Pravastatin (**3**) with a 0.9% market share sold only $US 1.76 billion and placed sixteenth on the list, while fluvastatin (**5**) was listed as the 189th top selling drug and lovastatin (**1**) slipped out of the top 200. By contrast sildenafil citrate (Viagra™) placed 39th on the list of top two hundred drugs. Clearly the control of cholesterol is a major concern in the US and around the world.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

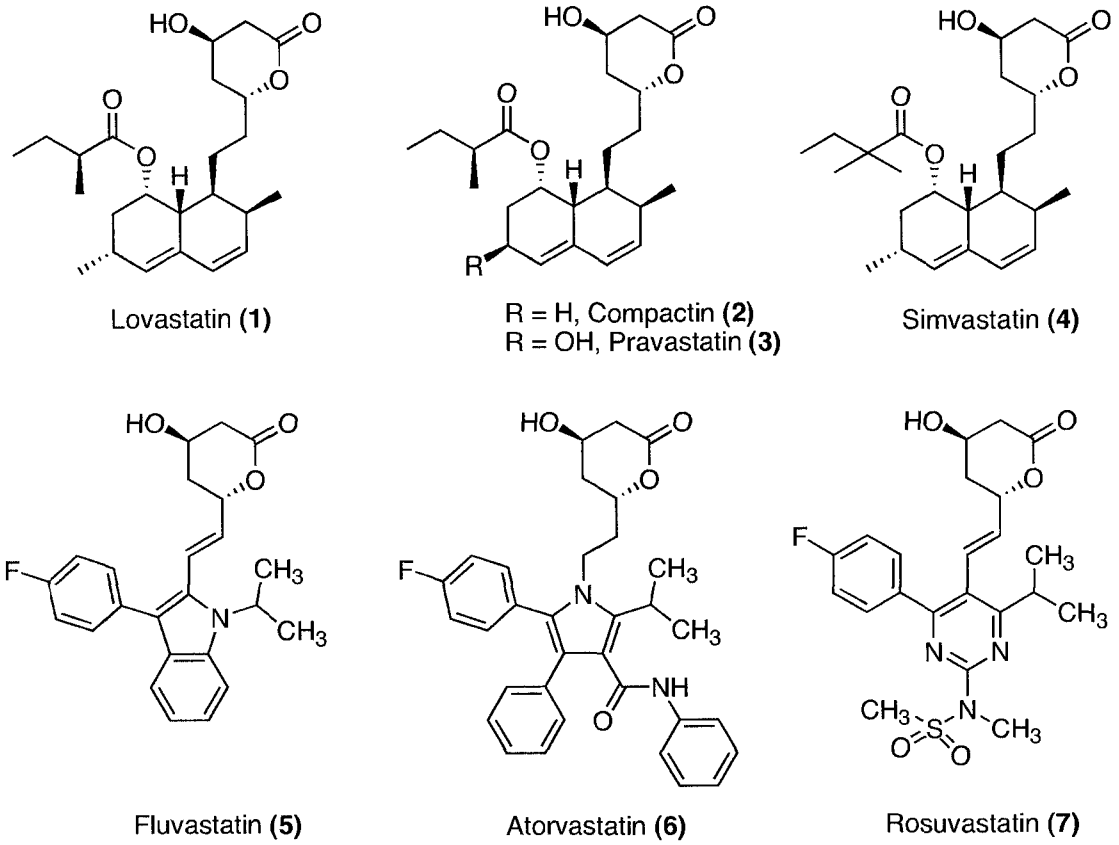


Figure 1. Lovastatin (**1**) and some of the statin family of drugs.

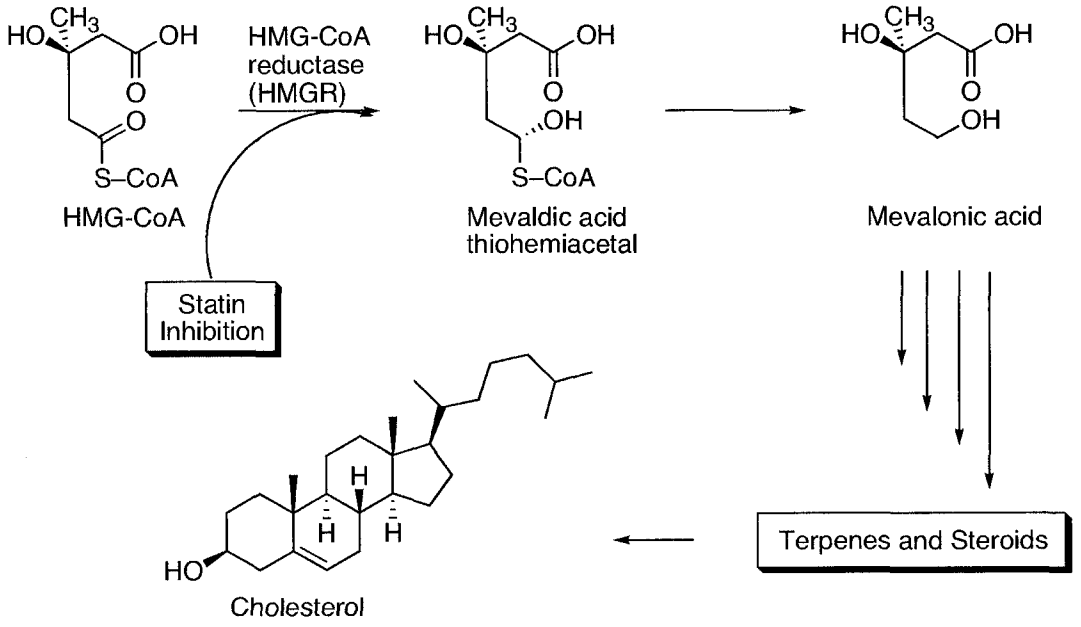
Interest in statins has been boosted by recent reports that this class of drug may have uses in treating conditions other than high cholesterol. It has been reported that statin drugs may have a lipid independent role in preventing heart disease[15] and may also be useful in reducing risk of cardiovascular disease[16] especially in diabetics.[17] In addition there is some indication that statins may even help prevent and potentially reverse the formation of the β-amloyid plaques associated with Alzheimers disease.[18,19] Early results also indicate that statins might be potential therapeutic agents for treating neuroinflammatory diseases such as multiple sclerosis.[20] Generating the most interest, however, are recent studies that show that treatment with statins may induce tumor-specific apoptosis and therefore represent a promising new cancer therapy.[21] These new

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

and apparently broad indications for therapy have led to statins being referred to as "the new aspirin."[22]

1.1.1 Lovastatin Mode of Action

Lovastatin **(1)** reduces cholesterol in humans by inhibiting the enzyme, hydroxymethylglutaryl-CoA reductase (HMGR), that catalyzes the reduction of the thioester of β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) to mevalonic acid (MVA) (Scheme 1).[3] This two-step reaction involves a reduction by NADPH of the thioester bond in HMG-CoA to a hemithioacetal followed by reduction to the primary alcohol (Scheme 1). Since mevalonic acid acts as a precursor for terpenes and steroids, including cholesterol, inhibition of HMGR shuts down this pathway and serves to lower production of cholesterol.



Scheme 1. Inhibition of HMG-CoA reductase (HMGR) by statin drugs.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Only recently has the mode of inhibition of HMGR by statin drugs been conclusively established through a series of X-ray crystal structures of human HMGR with various statins bound in the active site (Fig. 2).[23]

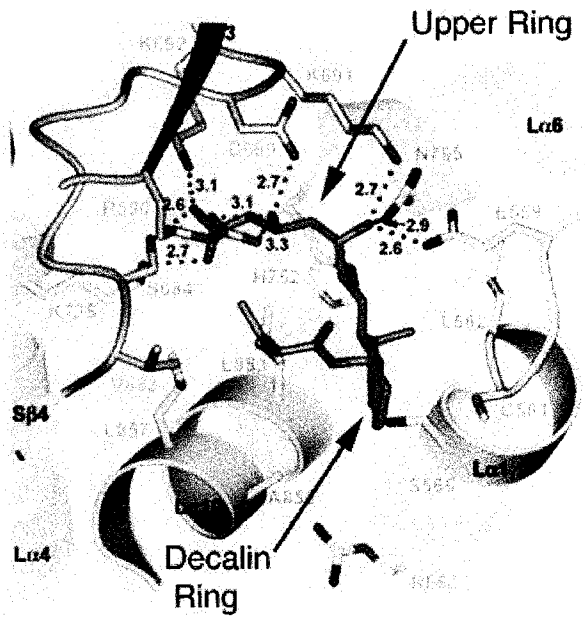


Figure 2. X-Ray structure of compactin (**2**) bound in the active site of HMGR. Oxygen atoms appear in red and numbers indicate distances in Å. Taken from: Istavan, E. S.; Deisenhofer, J. *Science* **2001**, *292*, 1160–1164.

The binding site of HMGR is composed of an HMG-CoA binding pocket next to an elongated NADPH binding site. The HMG-CoA binding site has a polar region that binds the HMG portion and a narrow pocket that accommodates the pantothenic acid region of the CoA portion of the substrate. In examining the X-ray structure it was found that, as expected, the HMG-like moiety common to all statins is held by polar residues in the HMG binding pocket. This X-ray study revealed that the binding of the upper ring portion has a high degree of similarity in all the statins studied as observed by measuring the atomic distances between substrate and active site residues. What had not been

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

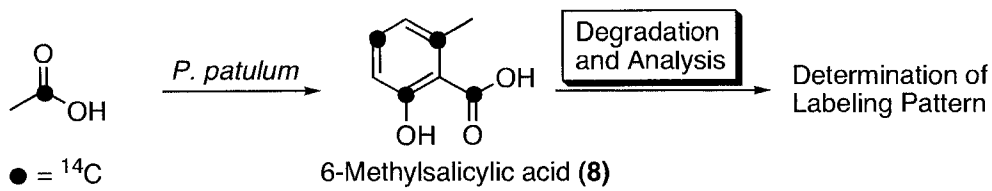
predicted is a change in the conformation of HMGR in the pantothenic acid binding region when the statin drugs are bound in the active site. This change in conformation maximizes the hydrophobic van der Waals interactions between the lower portion of the statin rings and the pantothenate binding pocket. These hydrophobic interactions are believed to be responsible for the tight binding of statins to HMGR as it was shown that a similar mode of binding occurs in the six different statins studied. Indeed, it is in this lower hydrophobic region where the various statin drugs differ in their structure. Figure 2 shows compactin (**2**) bound in the active site of HMGR with the lower decalin ring sitting in the pantothenoate binding pocket. The upper HMG-like ring, in the open-chain hydroxy acid form, sits above. The numbers indicate distances (in Å) to the polar residues in the HMG-CoA binding pocket.

Despite the interest and ongoing research in statin drugs, there still remain fundamental and interesting questions as to how these molecules, in particular **1**, are biosynthesized in Nature. As will be described below, **1** and its congeners are polyketides. This thesis will focus on elucidation of the biosynthesis of **1** in *A. terreus*.

1.2 Polyketide biosynthesis

Polyketide derived natural products comprise one of the most structurally diverse classes of compounds.[24,25] Polyketides were first shown to be produced from two carbon sodium acetate units by classic labeling and degradation studies done by Birch on 6-methylsalicylic acid (**8**) from *Penicillium patulum* (Scheme 2).[26] Sodium acetate labeled with ^{14}C at the C-1 (carbonyl) position led to the labeling pattern shown in Scheme 2.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

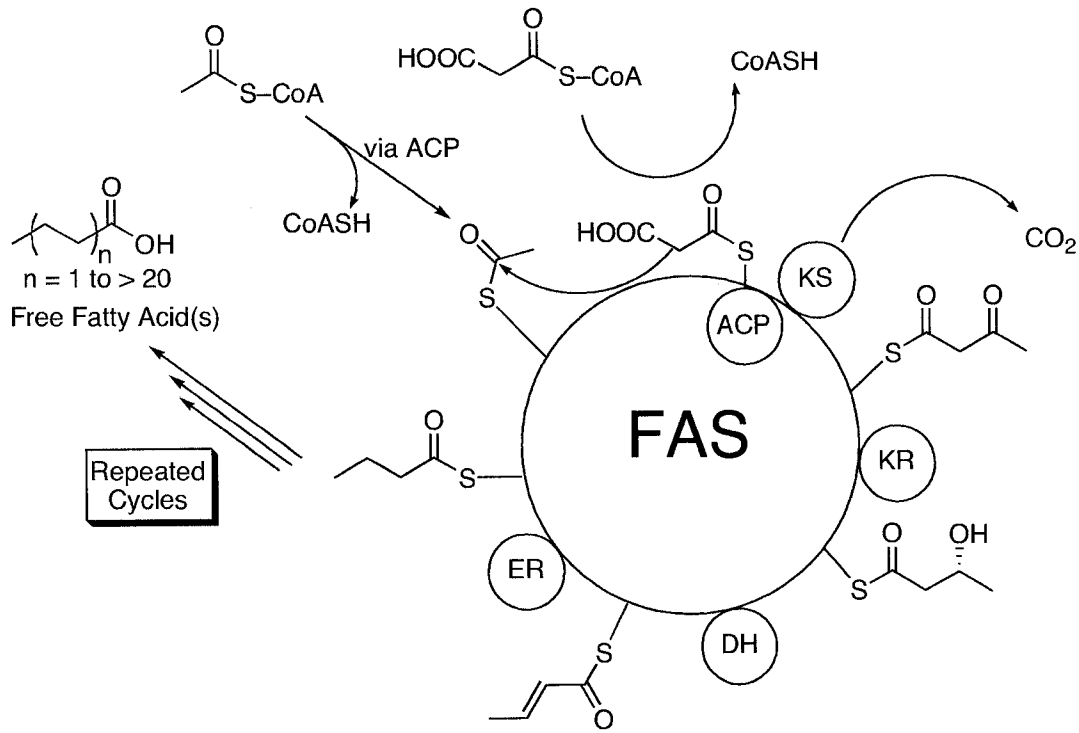


Scheme 2. 6-Methylsalicylic acid **(8)** and labeling pattern by sodium [1-^{14}C] acetate.

Isotopic incorporation studies such as these (eventually using stable isotopes) established that entire classes of compounds were derived from acetate. A very active area of research has developed aimed at elucidating the biosynthetic pathways.[27] Eventually it was found that acetyl-CoA acts as the two-carbon starter unit that primes the polyketide synthase (PKS) enzyme. The growing polyketide chain is then extended by two carbon atoms at each stage of the assembly process by malonyl-CoA (which acts as an activated form of acetyl-CoA) for the Claisen-type condensation used in chain extension.

The individual steps involved in this process are reminiscent of fatty acid biosynthesis as shown in Scheme 3.[28] Initial loading of acetyl-CoA onto the free thiol of the acyl carrier protein (ACP) domain of the fatty acid synthase (FAS) is followed by transfer of the acetyl group to an active site cysteine. Subsequent loading of malonyl-CoA onto the ACP then positions the acetate and malonate for a Claisen condensation (with concomitant decarboxylation of malonate) in a ketosynthase step (KS) that leads to chain extension. The carbonyl is then reduced, (ketoreductase = KR), eliminated (dehydratase = DH), and the resulting double bond hydrogenated (enoylreductase = ER) to leave a saturated carbon-carbon bond in the final product. A continuous repeat of this cycle leads to the assembly of long chain fatty acids.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



Scheme 3. Steps accomplished by fatty acid synthase (FAS).

A combination of some or all of these enzyme activities may be used during polyketide biosynthesis to produce metabolites with a range of functional groups. For example, if the chain is extended after the ketoreductase (KR) step a hydroxyl group will be carried through to the final polyketide product. It is also possible to have starter and extender units other than acetyl- and malonyl-CoA, but the fundamental repertoire of possible steps in the assembly of the polyketide remains the same.

More recently molecular biology and genetic approaches have helped develop an understanding of the processes involved in the assembly of these polyketide metabolites.[29] Probably the most striking example of this is the biosynthesis of 6-deoxyerythronolide B (6-dEB) (**9**), the macrolide core of the antibiotic erythromycin A (**10**).[30] The entire biosynthetic gene cluster responsible for 6-dEB (**9**) biosynthesis in the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

bacterium *Saccharopolyspora erythraea* has been identified. It consists of three genes that code for the production of three massive (~355 kDa) multi enzyme polypeptides that act as the 6-dEB (**9**) synthase (i.e. DEBS) (Fig. 3). Within smaller regions in these large polypeptides are the individual catalytic domains (i.e. KS, KR, DH, and ER) employed by DEBS.

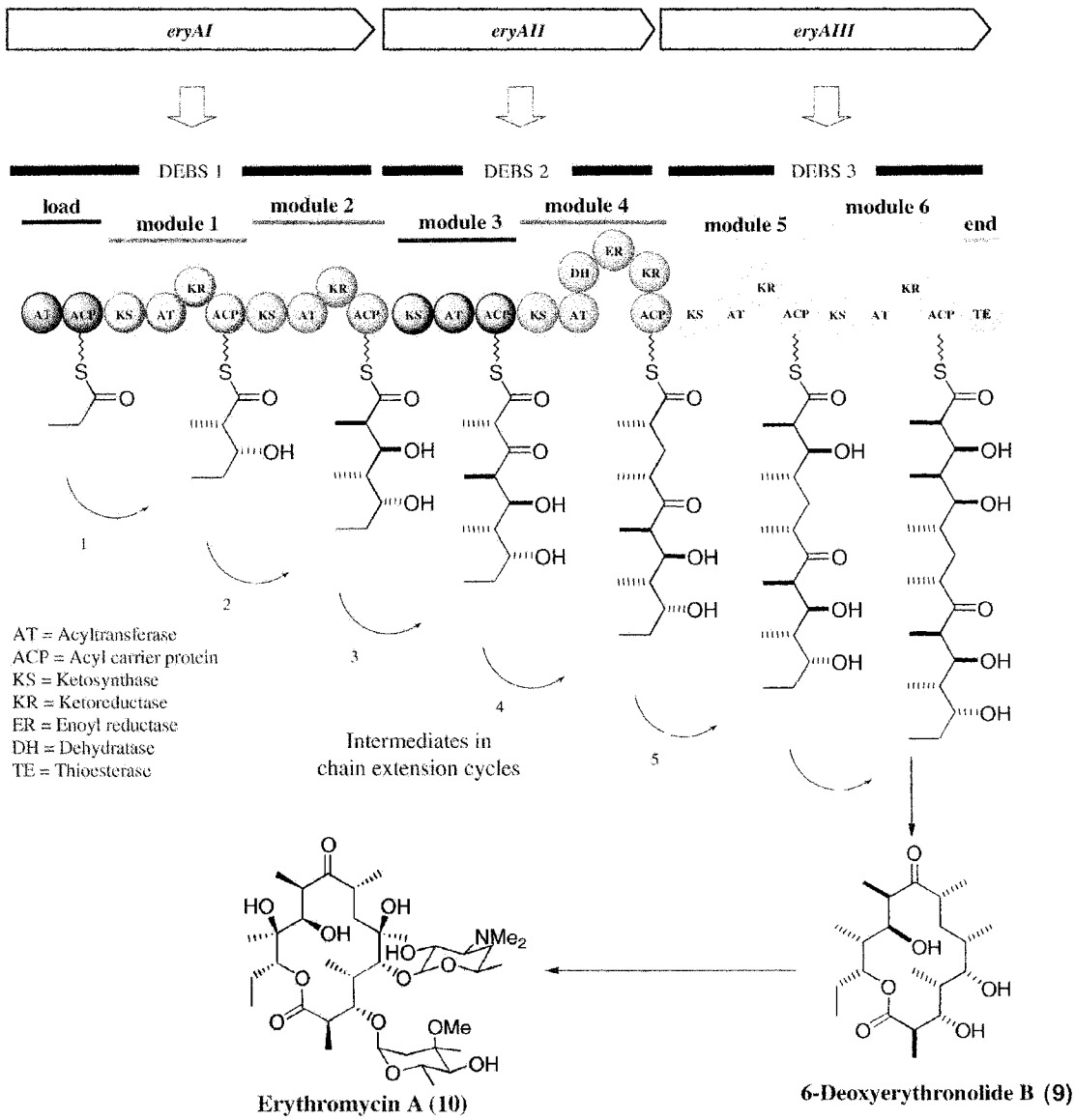


Figure 3. Biosynthesis of 6-deoxyerythronolide B (**9**). Taken from: Staunton, J.; Weissman, K. J. *Nat. Prod. Rep.* **2001**, *18*, 389.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Each one of these catalytic domains is used once and only once as the polyketide chain is extended from starter unit, in this case propinoyl Co-A, to completed polyketide (Fig. 3). This process can be thought of as analogous to an assembly line where each member of the team responsible for making the finished product has a very specific job to do. For example, DEBS has five separate and distinct ketoreductase (KR) domains, and each gets used once and only once in the assembly of 6-dEB (**9**). DEBS is classified as a Type I polyketide synthase and produces a functionalized and reduced (i.e. non-aromatic) polyketide characteristic of this group. Other iterative polyketide synthase systems are classified as Type II, and produce mainly aromatic polyketides, usually in bacteria. This class of PKS uses a multienzyme complex in an iterative fashion where each catalytic domain is used repetitively in the formation of the polyketide. Plants and some bacteria are known to employ an iterative Type III polyketide synthases that also build products in an iterative fashion similar to Type II synthases. Type III synthases are distinguished from Type I and Type II synthases by the lack of acyl carrier protein (ACP). Type III PKS enzymes are recognized for their ability to directly condense CoA thioesters onto the growing polyketide chain.[31]

The DEBS system has proven extremely amenable to alteration at the genetic level. It is possible to shut off the activity of individual domains through gene manipulation and thereby cause production of new products. For example, inactivation of the enoyl reductase (ER) activity in module 4, generates a new compound with an additional double bond incorporated into the 6-DEB (**9**) structure (Fig. 4).[32] This type of manipulation of secondary metabolite profiles at the gene expression level has come to be

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

known as "combinatorial biosynthesis" and is able to rapidly provide molecules in large quantity that would be difficult to access by chemical synthesis.[33,34]

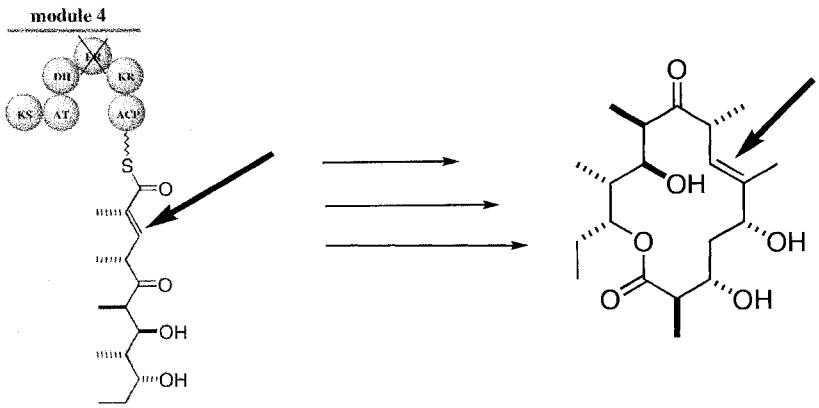


Figure 4. Deletion in module 4 in DEBS leads to new natural products. Adapted from Staunton, J.; Weissman, K. J. *Nat. Prod. Rep.* **2001**, *18*, 389.

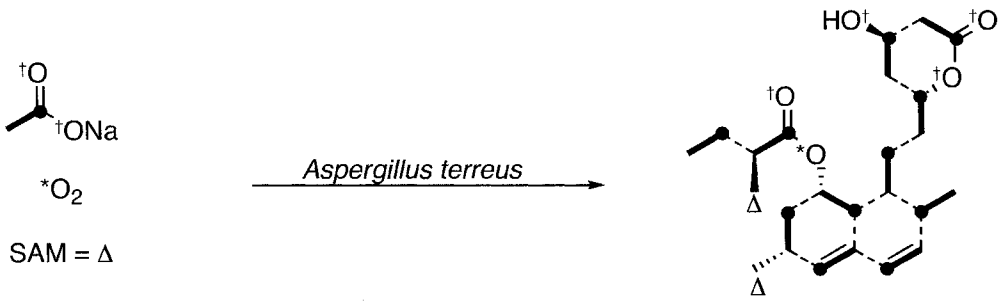
The manipulation of the genes involved in secondary metabolite production has also aided understanding of how a complex multienzyme system such as DEBS assembles highly functionalized natural products. Successful application of this approach to the biosynthesis of lovastatin (**1**) may lead to the production of new and more potent analogues. However, in order to generate new analogues of **1**, the individual steps involved in the biosynthesis of lovastatin must be more completely understood.

1.3 The Biosynthesis of Lovastatin

The biogenic origin of all the atoms in **1** has been deduced through tracer analysis using stable isotope incorporation and high field NMR (Scheme 4).[35,36] The pattern of isotopic labeling indicated a central nonaketide core that is assembled from nine intact acetate units with an additional methyl group introduced from *S*-adenosylmethionine

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(SAM). An intact diketide side chain, also with a SAM derived methyl group, is linked by an ester bond to an alcohol derived from molecular oxygen.



Scheme 4. Isotopic labeling of lovastatin (**1**) by *A. terreus*

Based upon the position of the isotopic labels in the natural product it was proposed that the biosynthesis of **1** occurs in two main stages. First is an assembly of two-carbon acetate and malonate units via a polyketide synthase (i.e. PKS) to form dihydromonacolin L (**11**), the nonaketide core of the lovastatin (**1**) structure. A series of post–PKS transformations then converts **11** into **1** (Fig. 5) through oxidation and acylation reactions. An additional PKS system is responsible for the synthesis of the diketide (*S*)-2-methylbutyryl side chain that is present in the final structure.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

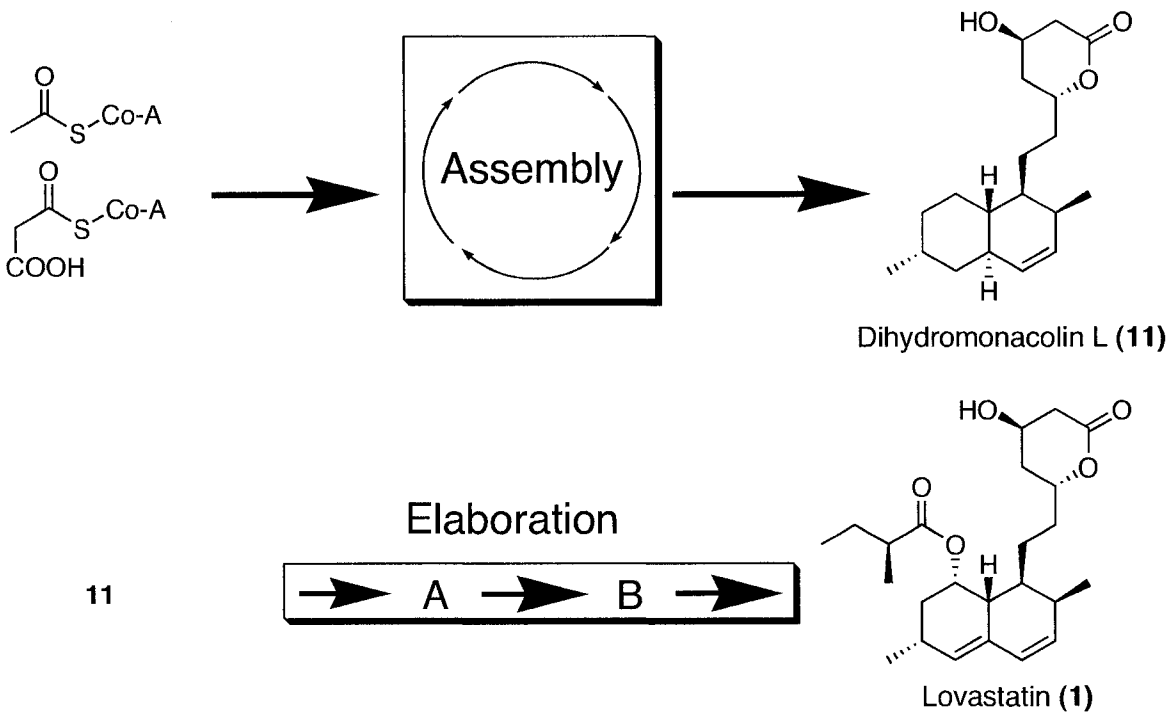


Figure 5. Assembly and elaboration during the biosynthesis of lovastatin (**1**).

Final confirmation of the proposed biosynthetic origin of **1** came from the identification and manipulation of the lovastatin (**1**) gene cluster in *A. terreus* in a collaborative effort with Hutchinson and co-workers (Fig. 6).[37] Following the pioneering studies of Reeves, McAda and others at MDS Panlabs,[38] some key genes (*lovB*, *lovF* *lovC*) involved in the biosynthesis of **1** were identified that appeared to code for the production of polyketide synthase enzymes based on sequence similarity to other known PKS genes. Other genes, such as *lovA* and *ORF17*, were believed responsible for the oxidative steps involved in the elaboration of **11** to the intermediate monacolin J (**12**). L*ovD* was shown to control the final esterification with the butyl side chain. Other genes in the cluster appear to be involved in regulation, transport and self-resistance.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

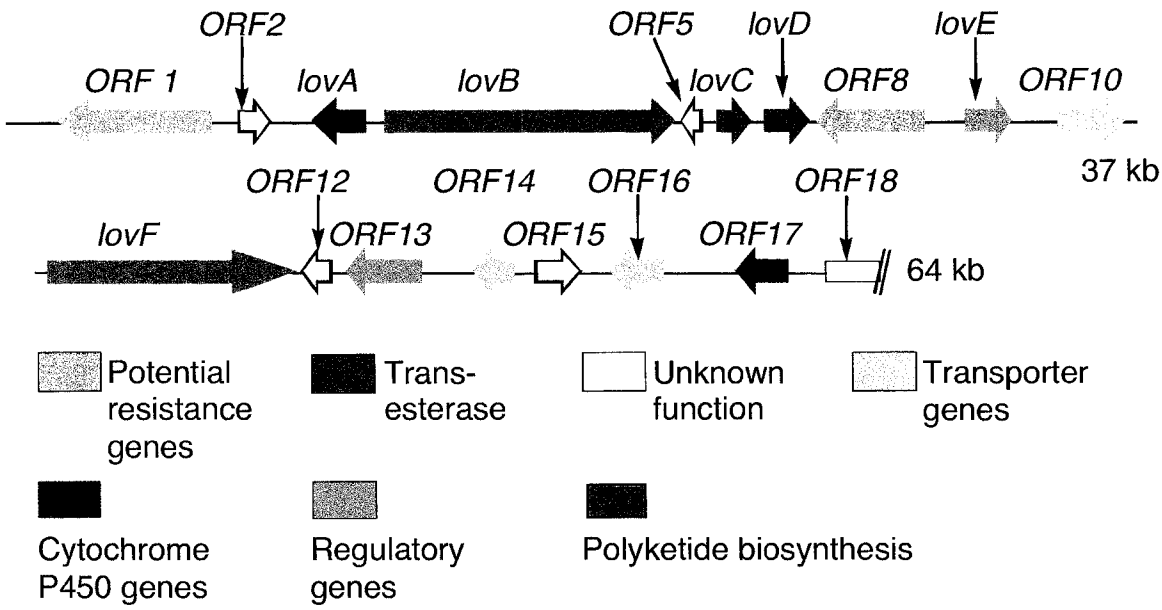


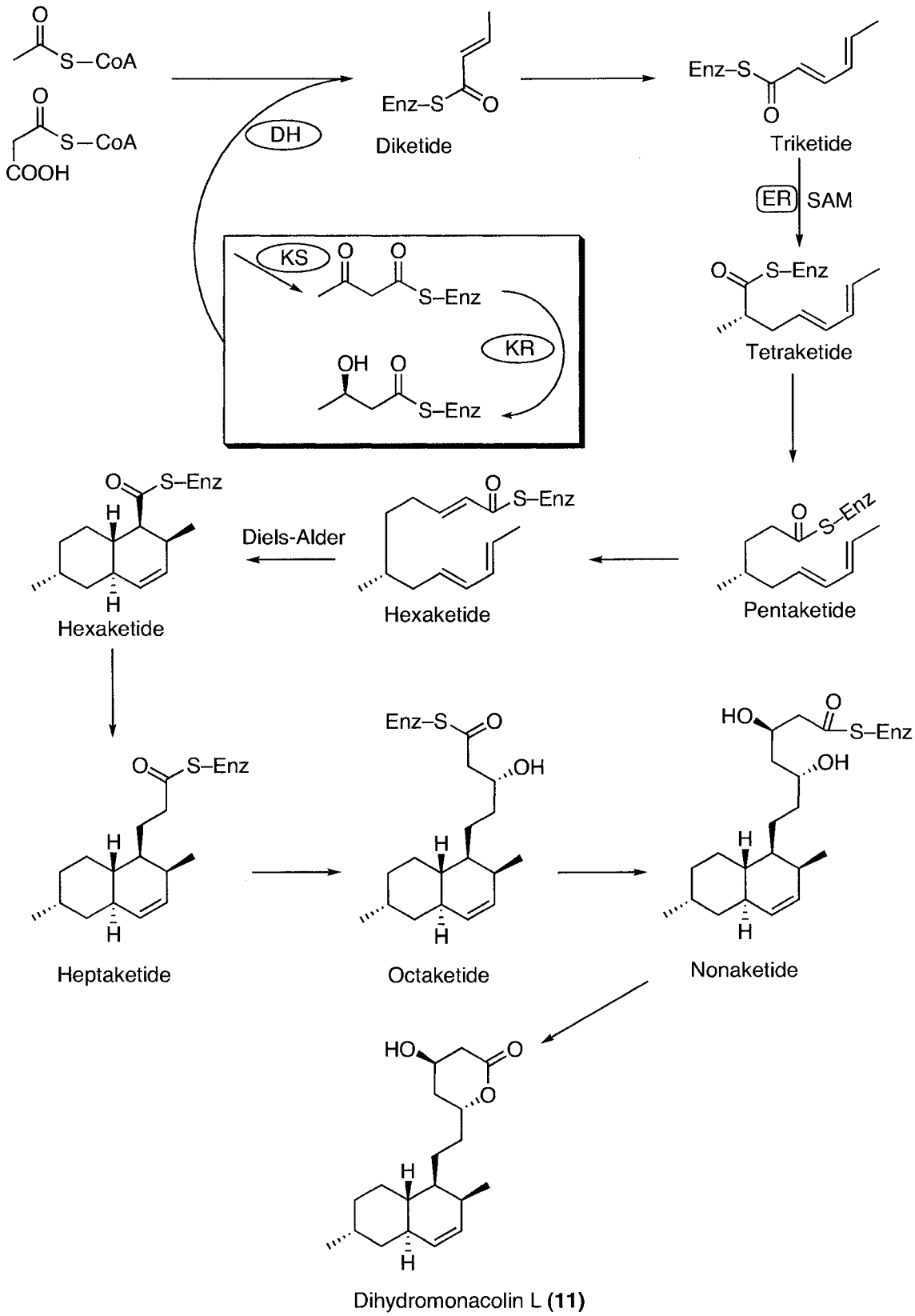
Figure 6. Lovastatin (**1**) biosynthesis gene cluster in *A. terreus*.

In order to determine which genes control what part of the pathway, certain lovastatin biosynthesis genes from *A. terreus* were expressed in a heterologous host *Aspergillus nidulans*, which had none of the machinery to make **1**. It was found that when two putative polyketide synthase genes, *lovB* and *lovC*, were expressed together in *A. nidulans*, dihydromonacolin L (**11**) could be isolated from the fermentation cultures, thereby demonstrating that the LovB and LovC enzymes were the main polyketide synthase components. The assembly of **11** is accomplished by the large (335 kDa) lovastatin nonaketide synthase (LovB = LNKS) in conjunction with the smaller (39 kDa) accessory protein LovC. At least 27 individual chemical steps, reminiscent of those used in fatty acid biosynthesis, are required to assemble one acetyl-CoA and eight malonyl-CoA units into the intact polyketide **11** (Scheme 5). After initial loading of the starter acetate unit derived from acetyl-CoA, loading of malonate (from malonyl-CoA) onto the acyl carrier protein (ACP) of the PKS is followed by a Claisen-type ketosynthase (KS)

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

condensation to give a β-keto thioester intermediate. The ketone carbonyl is then reduced in a ketoreductase (KR) step, and the β-hydroxyl eliminated in a dehydratase (DH) step to generate the unsaturated diketide intermediate. This cycle of condensation, reduction and elimination is repeated to lengthen the chain to the triketide. In further assembly of the growing chain from the triketide to the tetraketide stage, an additional enzyme functionality, an enoyl reductase (ER), is employed to fully reduce the carbon-carbon double bond. An S-adenosylmethionine (SAM) derived methyl group becomes attached probably to the tetraketide β-keto thioester intermediate. The growing polyketide chain is then further assembled to the linear hexaketide triene. It has been shown that at this stage in the assembly of **11** there is an enzyme-catalyzed Diels-Alder reaction.[39] Purified LovB enzyme was shown to cyclize a linear triene precursor to give a product matching the *trans*-fused *endo* stereochemistry identical to that observed in **11**. Control experiments indicate that this stereochemistry is inaccessible via a thermal Diels-Alder reaction. LovB is the first purified Diels-Alderase enzyme and is one of a growing number of examples of the Diels-Alder reaction detected in Nature.[40-42] The cyclic hexaketide is then further assembled to the nonaketide stage (i.e. **11**) using a combination of the KS, KR, DH and ER enzyme activities. It is interesting that only two proteins, the LovB enzyme in conjunction with the accessory LovC protein, are responsible for all the chemical transformations necessary to assemble **11**.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

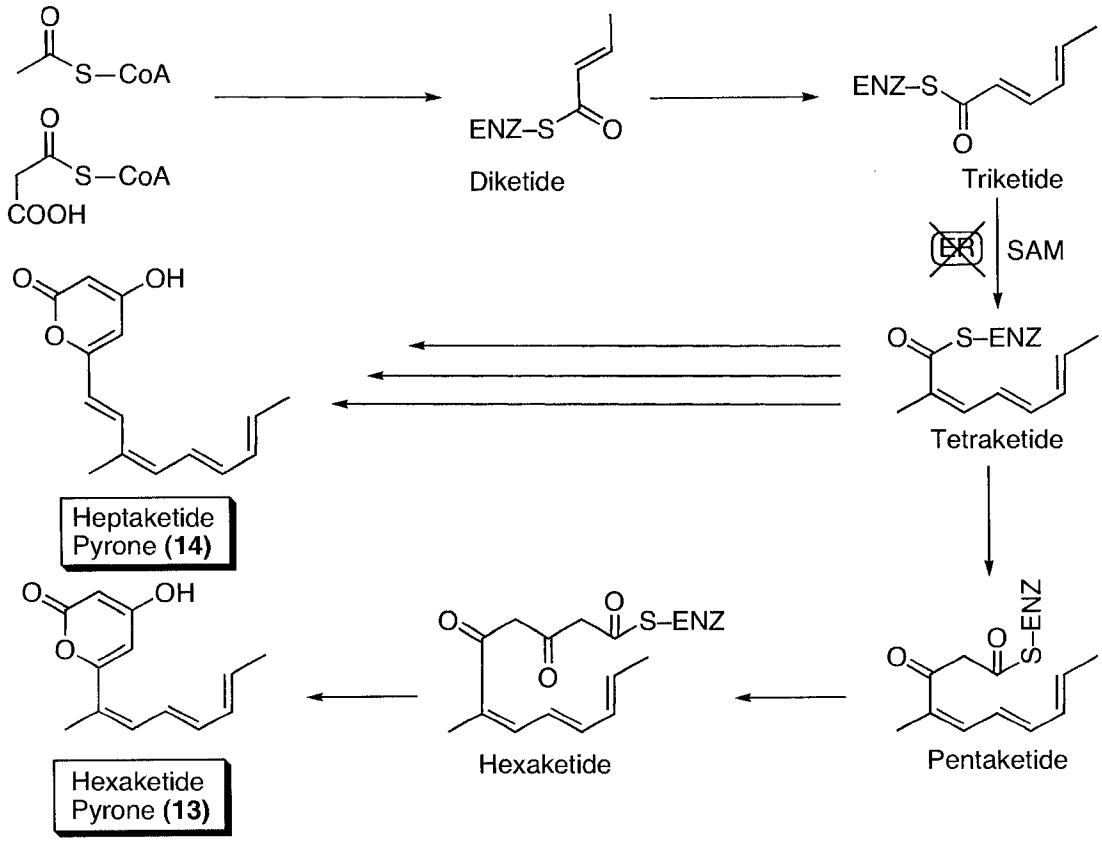


Scheme 5. Proposed polyketide synthase steps in the assembly of **(11)**

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Recently the entire biosynthetic gene cluster for compactin (**2**) in *Penicillium citrinum* has been reported.[43] The individual genes possess a high degree of similarity to those reported for lovastatin (**1**) biosynthesis, in particular the genes (*mlcA* and *mlcG*) correspond to the *lovB* and *lovC* genes in *A. terreus*. Although the *P. citrinum* nonaketide synthase (*mlcA*) contains a methyltransferase domain, it appears to be nonfunctional in this enzyme.

The role of LovC protein in the assembly of **11** was deduced when *lovB* gene was expressed alone in *A. nidulans*. It was found that the production of **11** was completely shunted to the production of the polyketide derived pyrones **13** and **14**, (Scheme 6).[37]



Scheme 6. Biosynthesis of pyrone shunt metabolites **13** and **14**.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

The examination of the structure of the shunt pyrones combined with the proposed assembly steps involved in the biosynthesis of **11** led to the conclusion that LovC acts in conjunction with LovB as an enoyl reductase (ER) during the assembly of **11**. This conclusion is supported by the genetic sequence of LovB and LovC. In the absence of LovC, it appears that LovB does not possess the ability to reduce the double bond of the tetraketide and that this failure leads to the production of **13** and **14** (Scheme 6).

Presumably LovC is also involved in the extension of the tetraketide to the pentaketide as well as of the cyclic hexaketide to heptaketide, as these steps require a fully saturated carbon-carbon bond to generate **11**. However, it appears that LovB possesses all the other catalytic domains normally associated with polyketide assembly, and that it can catalyse *different reactions* at each chain elongation step, with the structure of the enzyme bound product controlling the outcome. In contrast to the assembly line approach employed in the biosynthesis of 6-dEB by DEBS, the LNKS system uses more of a "team approach." Each member of the team (enzymatic domain) is utilized repeatedly to assemble the product. LovC acts as the "helper" that ensures correct functionalization at the tetraketide, pentaketide and heptaketide stages. Although LovB generates a reduced polyketide typical of Type I PKS systems, this repeated use of the same enzymatic domains classifies it as an iterative Type I PKS.[44] Such PKS systems are likely to occur in many fungi.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

1.4 Post-PKS transformations of polyketides

It is quite common for the products of polyketide biosynthesis to undergo additional transformation after release from the PKS enzyme complex.[45] Some of these post-PKS elaboration steps may be relatively simple, such as the hydroxylation and glycosylation steps involved in the transformation of 6-dEB (**9**) into erythromycin (**10**) (Fig. 7).

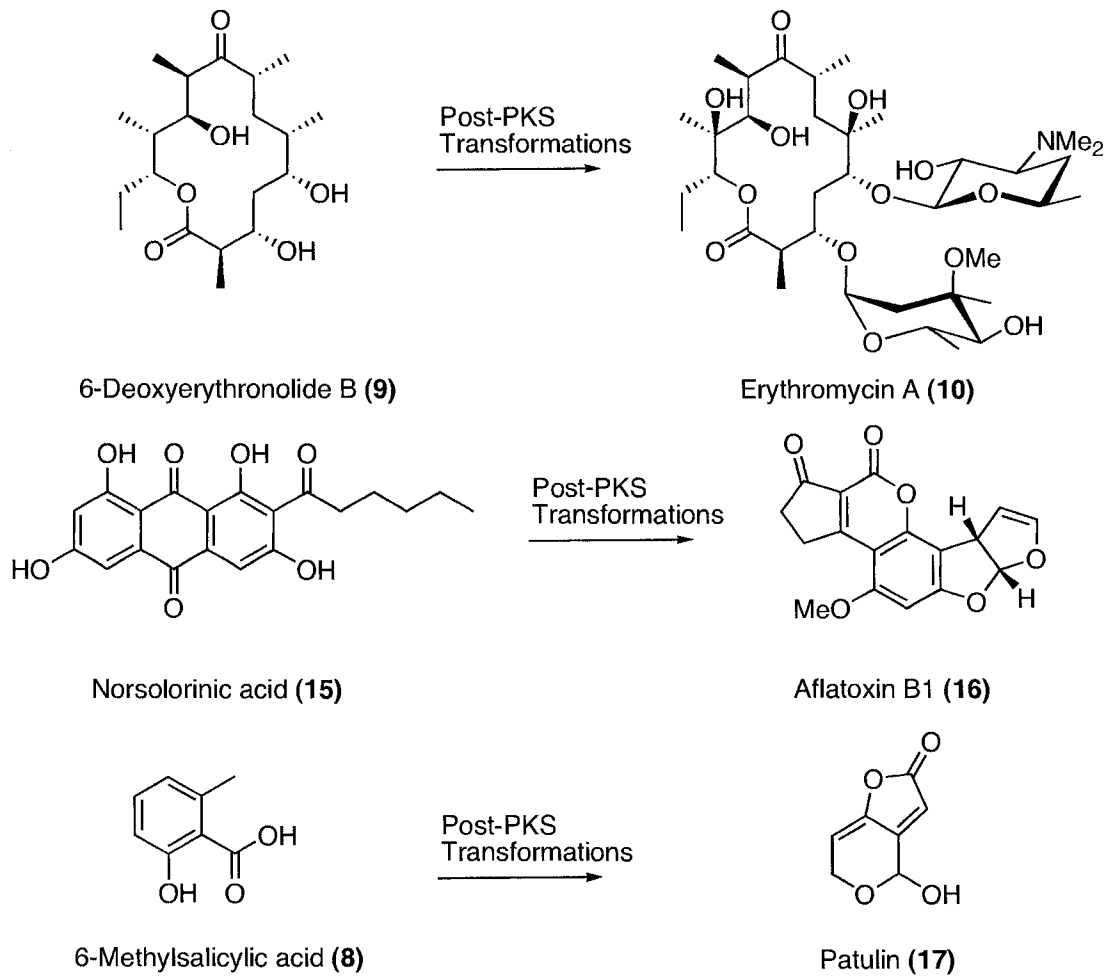


Figure 7. Selected examples of post-PKS transformations.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

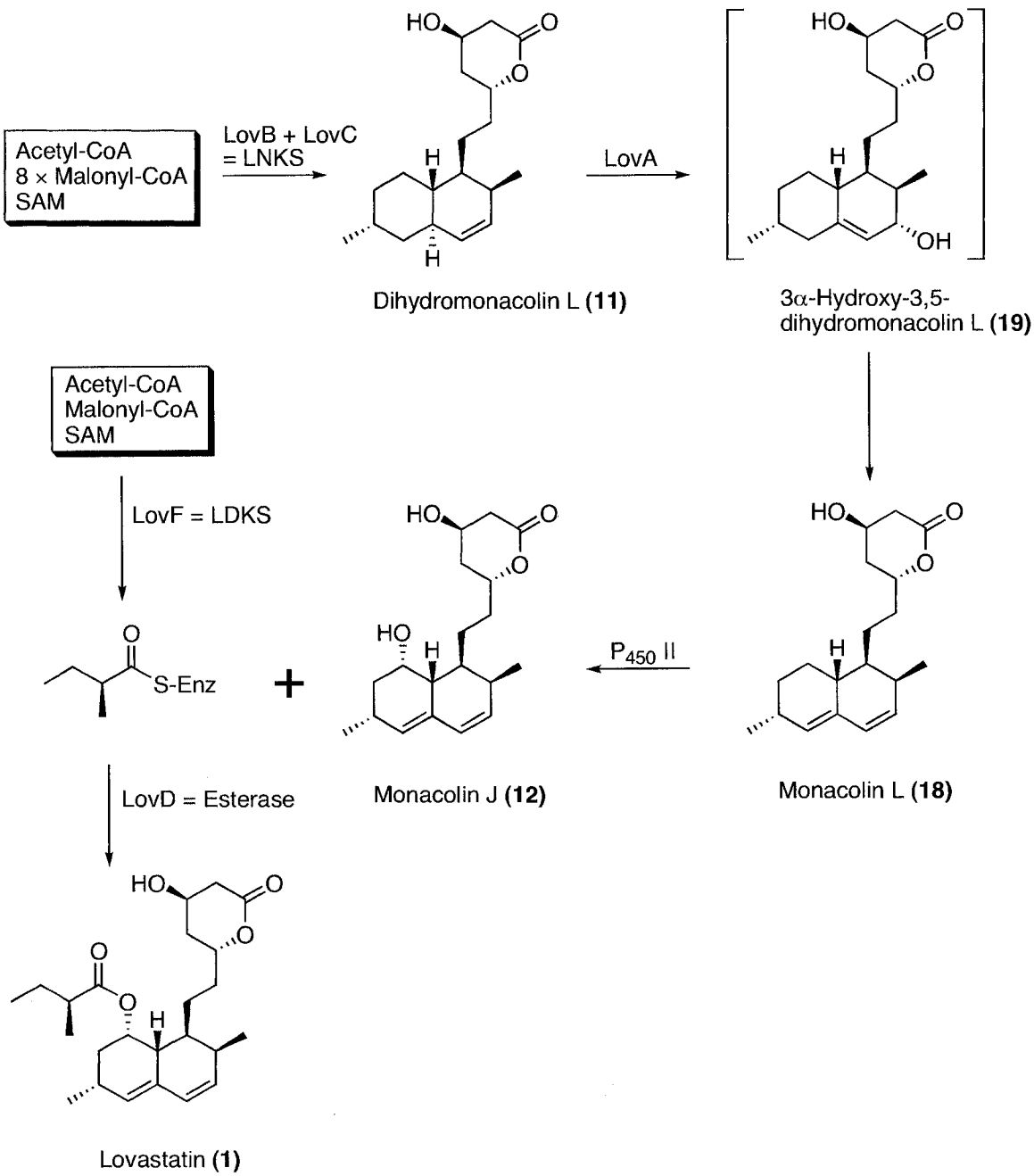
Other transformations may be more elaborate, such as those encountered in the conversion of the polyketide norsolorinic acid **(15)** into the potent hepatotoxin and peanut contaminant aflatoxin B1 **(16)**.[46-48] Oxidative post-PKS transformations may transform even simple compounds such as 6-methylsalicylic acid **(8)** to rearranged skeletons such as the carcinogen patulin **(17)**.[49] Recent work examining the post-PKS transformations involved in polyketide biosynthesis has focused on the individual steps and enzymes in these pathways. For example, cell free conversion of norsolorinic acid **(15)** to aflatoxin B1 **(16)** has been demonstrated in *Aspergillus parasiticus,*[47] and recent work has focused on the individual enzymes in this pathway, especially the P_{450} oxidation involved in the final step.[50] In another example, all of the individual steps involved in the post-PKS conversion of 6-dEB **(9)** into **(10)** have been identified through the use of mutants of *S. erythraea* blocked at critical genes.[51] The post-PKS enzyme system of *S. erythraea* can accept certain alternative analogues of 6-dEB **(9)**, resulting in the production of novel antibiotics.[52] The combination of the manipulation of the PKS assembly process coupled with controlled elaboration after assembly is a powerful approach to access new molecules.[53]

1.4.1 Post-PKS transformations leading to lovastatin (1).

During lovastatin **(1)** biosynthesis the PKS product, dihydromonacolin L **(11)**, is elaborated in a series of post-PKS transformations to the final product.[54,55] Two oxidative steps introduce an additional double bond at C-4a and C-5 and a hydroxyl at C-8. These steps are followed by the introduction of the butyryl side chain, which is assembled by a separate PKS (Scheme 7). As described in Section 1.2, *lovF* codes for the diketide synthase (LDKS) that produces the methylated side chain portion of **1**. Interestingly, this

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

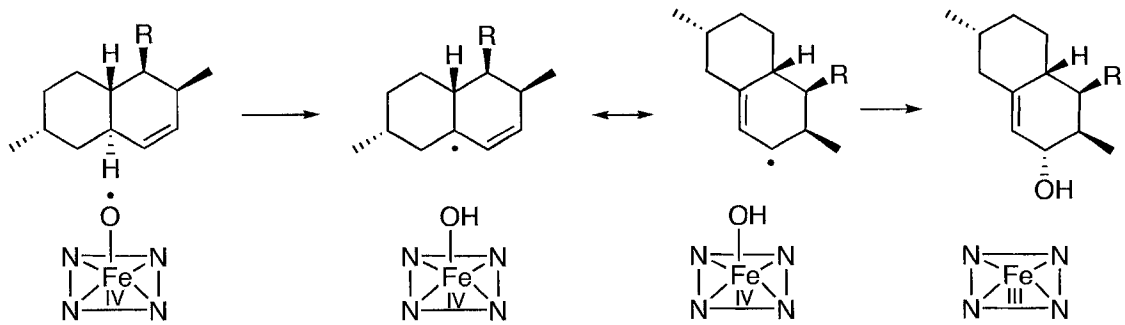
PKS produces a fully reduced polyketide, possesses enoyl reductase activity and has no requirement for LovC during assembly. In addition, it was shown that the LovF enzyme does not release the assembled polyketide product since in the absence of the *lovD* gene required for esterification, no free (*S*)-2-methylbutyric acid can be detected.[44]



Scheme 7. Proposed post-PKS transformations in lovastatin (**1**) biosynthesis.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

The P_{450} oxidation that installs the C-8 hydroxyl is likely to use a mechanism that is typical for this type of enzyme involving an initial hydrogen atom abstraction followed by an immediate 'rebound' of a hydroxy radical.[56] The oxidation involved in the transformation of **11** to **18** that introduces the second double bond appears unusual. This transformation has been proposed to occur via direct oxidation to an allylic alcohol intermediate **(19)** followed by a spontaneous dehydration to the diene system in **18**.[57] The unusual aspect of this proposal is that the oxidation process introduces the OH group with allylic rearrangement, as shown in Scheme 8.



Scheme 8. Proposed conversion of **11** to **19** by P_{450} enzyme.[57]

This rearrangement presumably would involve an initial hydrogen atom abstraction by the iron hydroxy radical of the P_{450} enzyme at C-4a to produce an allylic radical. This allylic radical then would need to shift in the enzyme active site to place C-3 directly above the iron-hydroxy intermediate in order for the hydroxylation to occur on this carbon. This type of shift of substrate is not common for hydroxylation by P_{450} enzymes.[58] "Molecular clock" experiments, using the well known rate of ring opening of the cyclopropylcarbinyl radical as a timing mechanism, have measured the time between hydrogen atom abstraction and hydroxyl radical rebound in the range of 80 – 200 fs; on the order of vibrational rate constants.[59] In order for the allylic radical to be hydroxylated

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

as proposed,[57] there would have to be a shift of the substrate in the enzyme active site in under 200 fs. Perhaps the delocalization energy of 14.0-14.5 kcal mol^{-1} that stabilizes the allyl radical[60] may extend its lifetime and allow such movement in the active site. However this does represent a departure from the typical P_{450} mechanism. The true role of **19** in the formation of **18**, and even the intermediacy of **18** in the pathway to **1** remain in doubt.[61]

1.4 Goals of this thesis

The aim of this thesis is twofold. First is to probe the steps involved in the PKS assembly of dihydromonacolin L (**11**). This will be done by synthesizing appropriate polyketide fragments and using enzyme transformation experiments to determine if they are incorporated intact into the structure of **11**. The second aim is to probe the post-PKS transformations involved in the elaboration of **11** to lovastatin (**1**). This will be examined by a series of biotransformation experiments using lovastatin (**1**) deficient mutants of *Aspergillus terreus*.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

2. Results and Discussion

2.1 *Aspergillus* Molecular Biology

The key development that facilitated this project was the identification of the lovastatin (**1**) biosynthetic gene cluster in *Aspergillus terreus* in collaboration with Prof. C. R. Hutchinson at the University of Wisconsin-Madision (now at Kosan Biosciences) (Fig. 8). As described in Section 1.4, the putative function of each gene was based on sequence similarity with other clusters of polyketide synthases.[37]

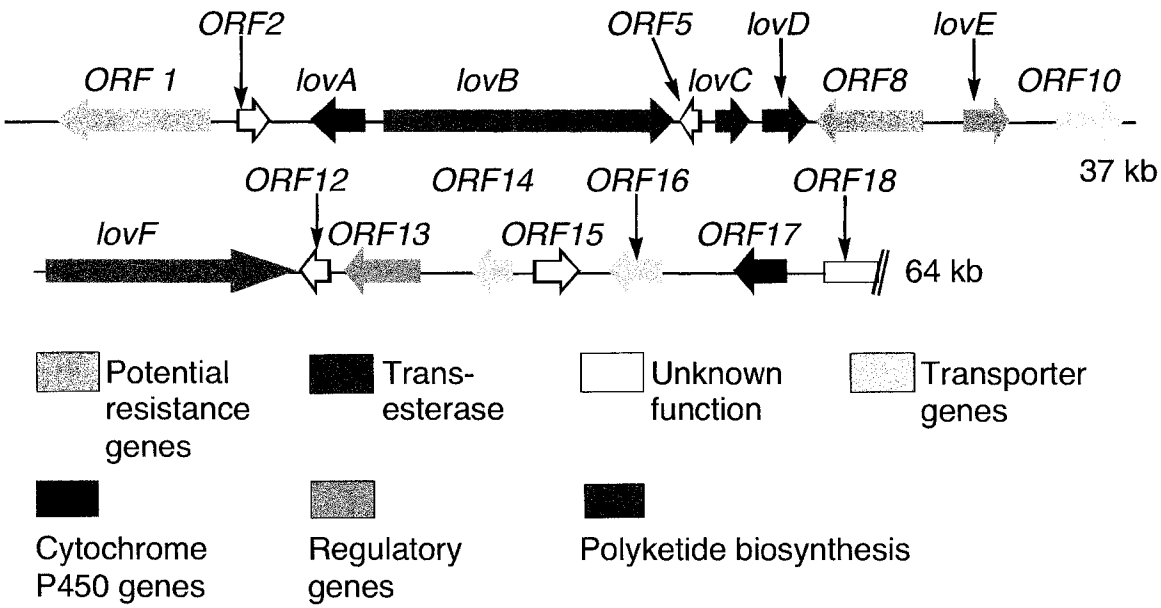


Figure 8. Lovastatin biosynthesis gene cluster in *A. terreus*

The *lovB*, *lovC* and *lovF* genes have a high degree of sequence similarity to other known polyketide synthase genes. The *lovA* and *ORF17* are believed to be involved in the two oxidative steps required for the elaboration of dihydromonacolin L (**11**) to lovastatin (**1**). Other key genes identified include *lovD*, which is an esterase necessary for the attachment of the 2-methylbutyryl side chain onto monacolin J (**12**). Other genes

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

are believed to be involved in regulation, transport, and self-resistance. The functions of the genes involved in lovastatin (**1**) biosynthesis were elucidated by the use of common molecular biology techniques. A series of lovastatin (**1**) deficient mutants of *A. terreus* were created by knocking out individual genes believed to be critical for the biosynthesis of **1**. Such mutants are not able to produce **1**, but would be expected to accumulate intermediates in the pathway to **1**, thereby assisting elucidation of the possible role of the missing gene. In addition, heterologous expression of key *A. terreus* genes in the non-lovastatin producer *Aspergillus nidulans* can allow the examination of the function of each gene or a combination of genes.

Two lovastatin-deficient mutants described in this project, *A. terreus lovC* and *A. terreus lovA,* were examined for their ability to produce intermediates related to **1**.[54] The first mutant *A. terreus lovC* has an insertional mutation in the *lovC* gene. This gene imports enoyl reductase activity and has been demonstrated to be required for the formation of dihydromonacolin L (**11**). The main acetate derived products of this mutant are the yellow pyrones **13** and **14** as described in Section 1.3 (See Scheme 6). However *A. terreus lovC* should contain all of the post-PKS machinery necessary for the elaboration of **11** to **1** and potentially be capable of biotransformation of advanced precursors into elaborated products.

The *lovA* gene disabled in *A. terreus lovA* by insertional inactivation is believed to be responsible for the oxidative introduction of the second double bond in the diene system of **1**. This gene appears to code for the production of a cytochrome P_{450} type of enzyme that catalyses the conversion of dihydromonacolin L (**11**) to monacolin L (**18**). Although lacking the ability to produce **1**, *A. terreus lovA* would be expected to produce

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

11 in fermentation cultures and contain the machinery necessary for the very late stage transformations, such as the ability to convert monacolin J (**12**) into **1**.

The *A. nidulans* transformants have proven useful in the elucidation of the functions of two of the lovastatin biosynthesis genes. For example, *A. nidulans lovB* (heterologous expression of *lovB* only) serves as a source of LovB protein which can be used in enzyme experiments. This transformant also produces the yellow pyrones **13** and **14** in fermentation cultures. In contrast, the *A nidulans* transformant expressing both *lovB* and *lovC* produces large amounts of dihydromonacolin L (**11**) in fermentation cultures.

2.2 Synthesis of Putative Polyketide Intermediates

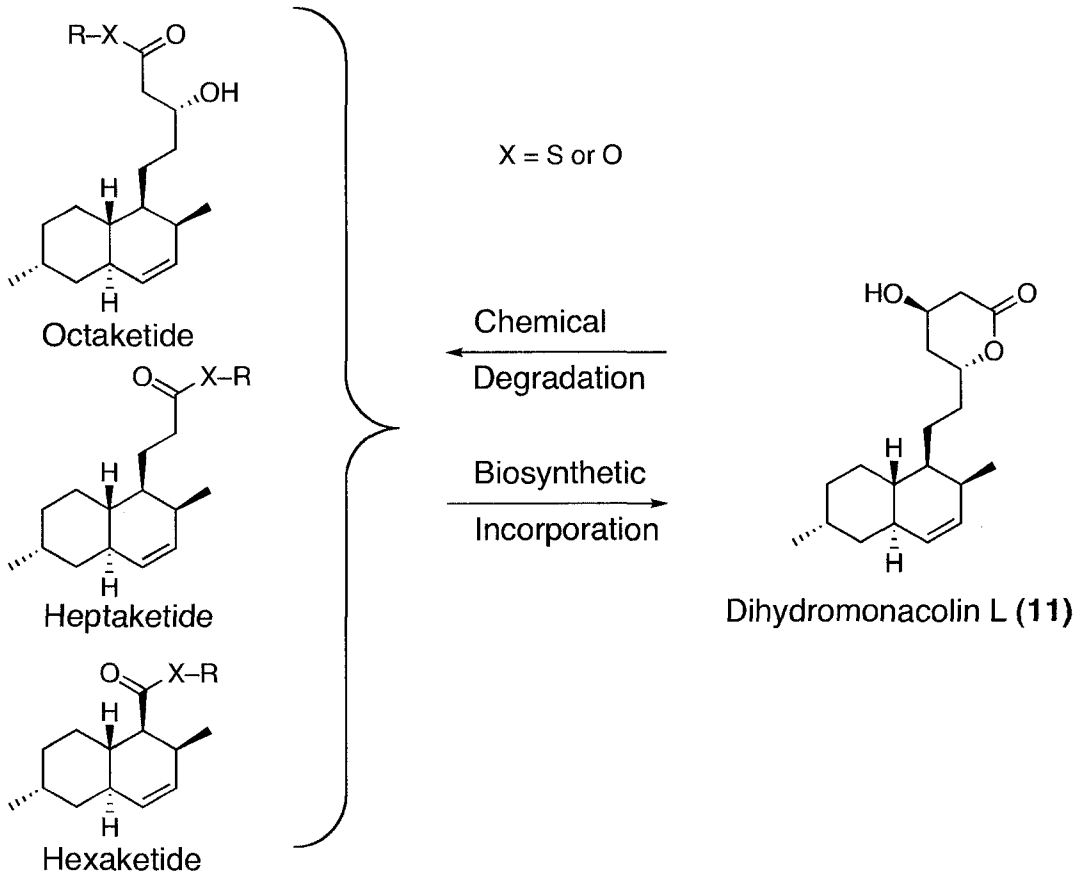
2.2.1 Dihydromonacolin L (11) isolation

In order to examine the individual steps involved in the assembly of dihydromonacolin L (**11**), putative polyketide intermediates enroute to **11** could be synthesized to determine if they could be incorporated intact into **11**. Previous attempts at such intact incorporation of labeled di- and triketides with whole cell systems were unsuccessful, and no intact labeling patterns could be detected in the skeleton of **11**.[62] Instead, the label became scrambled, presumably due to degradation of the labeled precursors by β-oxidation and reincorporation as monolabeled [^{13}C] acetates into the final polyketide product. Based on these results, it seemed that any attempt at intact incorporation would require cell free systems or pure enzymes.

One objective of this project was preparation of late stage polyketide precursors, such as the proposed octa-, hepta- and hexaketides enroute to **11** by the chemical

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

degradation of **11** itself (Scheme 9). These could then be used to determine if they can be accepted intact by the lovastatin nonaketide synthase (LovB) system to generate dihydromonacolin L (**11**) (Scheme 9).



Scheme 9. Proposed degradation of dihydromonacolin L (**11**).

Hence, a supply of dihydromonacolin L (**11**) is needed. Fortunately, the *Aspergillus nidulans lovB* + *lovC* transformant can provide **11** through fermentation. Initial yields of **11** were somewhat low (< 10 mg per liter) but optimization of the fermentation conditions of *A. nidulans lovB+lovC* gave increased production. Initially, spores of *A. nidulans lovB+lovC* are fermented in a glucose-based medium to establish

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

the culture. After 48 hrs, the mycelial mat is harvested by filtration and rinsed to remove any traces of the glucose medium. Glucose can act as a repressor of heterologous gene expression. The rinsed mycelia are then used to inoculate a lactose-based medium that contains a trace (i.e. 1 mL per liter) of an inducer of gene expression, cyclopentanone. After seven days the culture is harvested and **11** is extracted and purified by chromatography. Key changes that increase yields are: initial glucose-based fermentation culture grown for a longer period (4 days); and supplementation of the lactose-based medium with 1 g per liter of sodium acetate per day for the last five days of growth. Earlier studies showed a remarkable level of sodium [^{13}C] acetate incorporation into dihydromonacolin L (**11**) (ca. 70%) by cultures of *A. nidulans lovB+lovC*.[62] Hence, it seemed that the addition of sodium acetate might provide more precursor (i.e. acetyl-CoA) for the polyketide synthase system. However there is no direct evidence for this, and the increased yield may be due to a buffering of the pH or some other unidentified effect. The changes to the fermentation protocol resulted in an increase of the production per liter of **11** to 50 mg, which allowed the isolation of amounts sufficient for synthetic transformations.

2.2.2 Synthesis of the octaketide precursor

Ultimately the late stage precursors need to be made as their *N*-acetyl cysteamine (NAC) thioesters (Fig. 9). It has been established in other systems that NAC thioesters are acceptable substitutes for coenzyme A as substrates for polyketide synthases.[63,64] The strategy behind this approach is that N-acetyl cysteamine acts as a mimic for the pantothenate arm of coenzyme A and can allow loading of an acyl group onto the polyketide synthase. For example, as shown in Figure 9, a diketide may be loaded onto a

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

thiol in the enzyme active site in a fashion similar to the attachment of acetyl Co-A. This methodology allows advanced precursors, such as the diketide, to be utilized intact by the PKS systems and incorporated into the final products in a number of bacterial (cell-free or whole cell) and fungal (whole cell) systems.

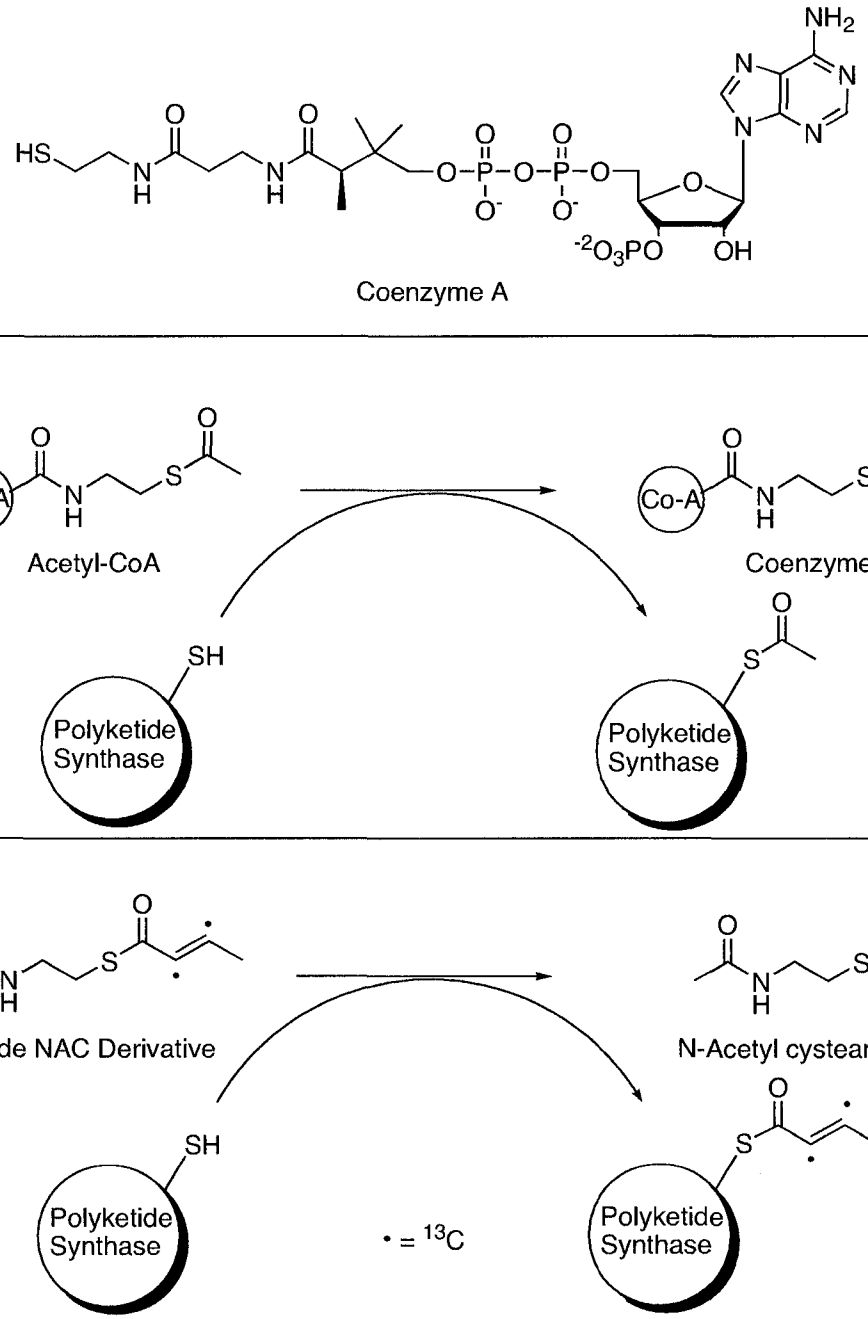


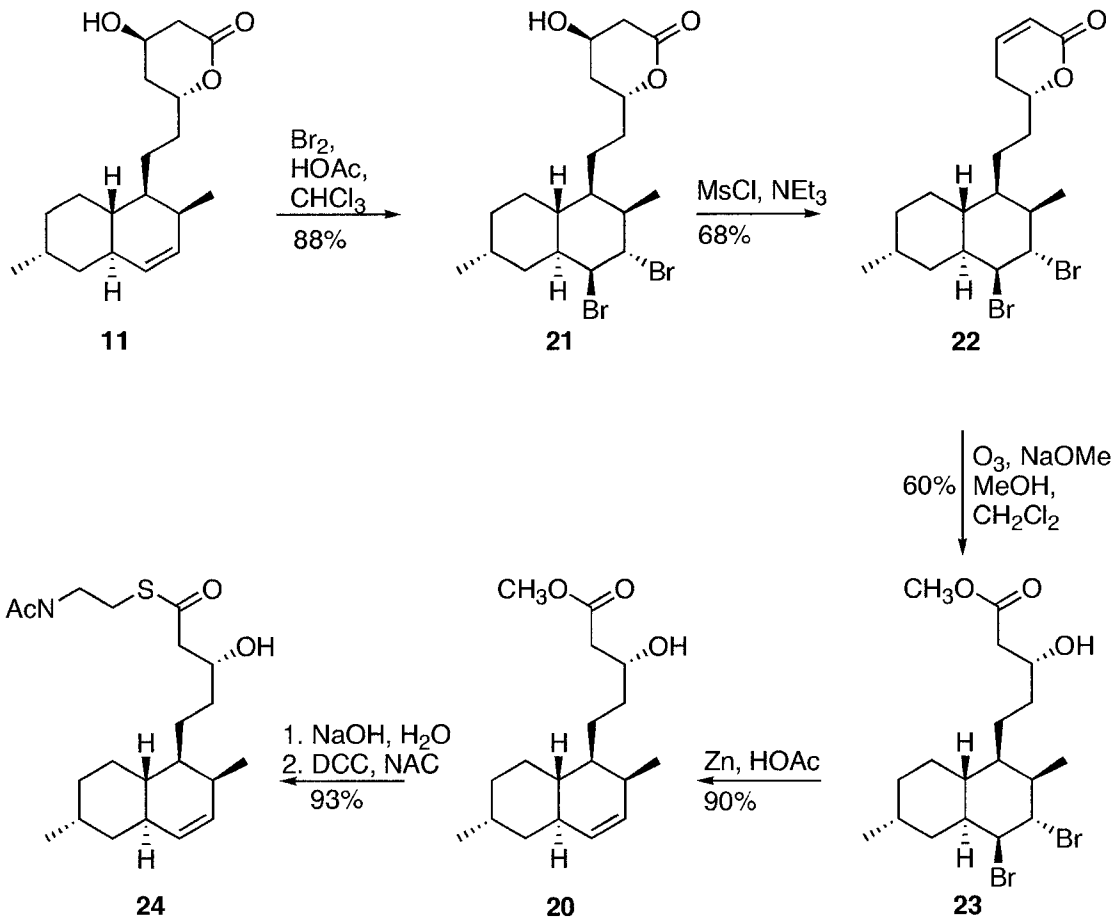
Figure 9. Use of N-acetyl cysteamine derivatives as coenzyme A mimics

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Synthesis of advanced precursors offers the opportunity to include multiple isotopic labels such as ^{13}C, ^{18}O and ^{2}H. Analysis by NMR spectroscopy of the final assembled product then allows determination of whether the fragment was incorporated intact as a unit into the final polyketide product.[65] Thus an initial goal was the preparation of an octaketide precursor that would also permit multiple labeling with stable isotopes.

A simple route from **11** to octaketide **20** could involve removal of two carbons from the upper ring by first forming a double bond through elimination and then cleaving by ozonolysis (Scheme 10). However, it is first necessary to protect the double bond already present in the decalin ring system with a group resistant to ozonolysis, as this more electron rich double bond would react preferentially over the double bond present in the upper ring. A suitable protecting group would be the dibromide derivative since bromination and debromination are usually easy to accomplish in high yield. Reaction of **11** with bromine in acetic acid forms **21**. Elimination of the alcohol in the upper ring proceeds readily via intermediate formation of the methanesulfonate using methanesulfonyl chloride and excess triethylamine. The α,β-unsaturated dibromide **22** can be cleaved by ozonolysis in the presence of methanol under basic conditions to give the methyl ester **23** directly.[66,67] The octaketide methyl ester **23**, is deprotected with Zn in acetic acid to regenerate the double bond in the decalin ring to afford **20**. Hydrolyis to the free acid and thioesterification by treatment with DCC followed by *N*-acetyl cysteamine yields NAC thioester **24** in 30% overall yield from **11**.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



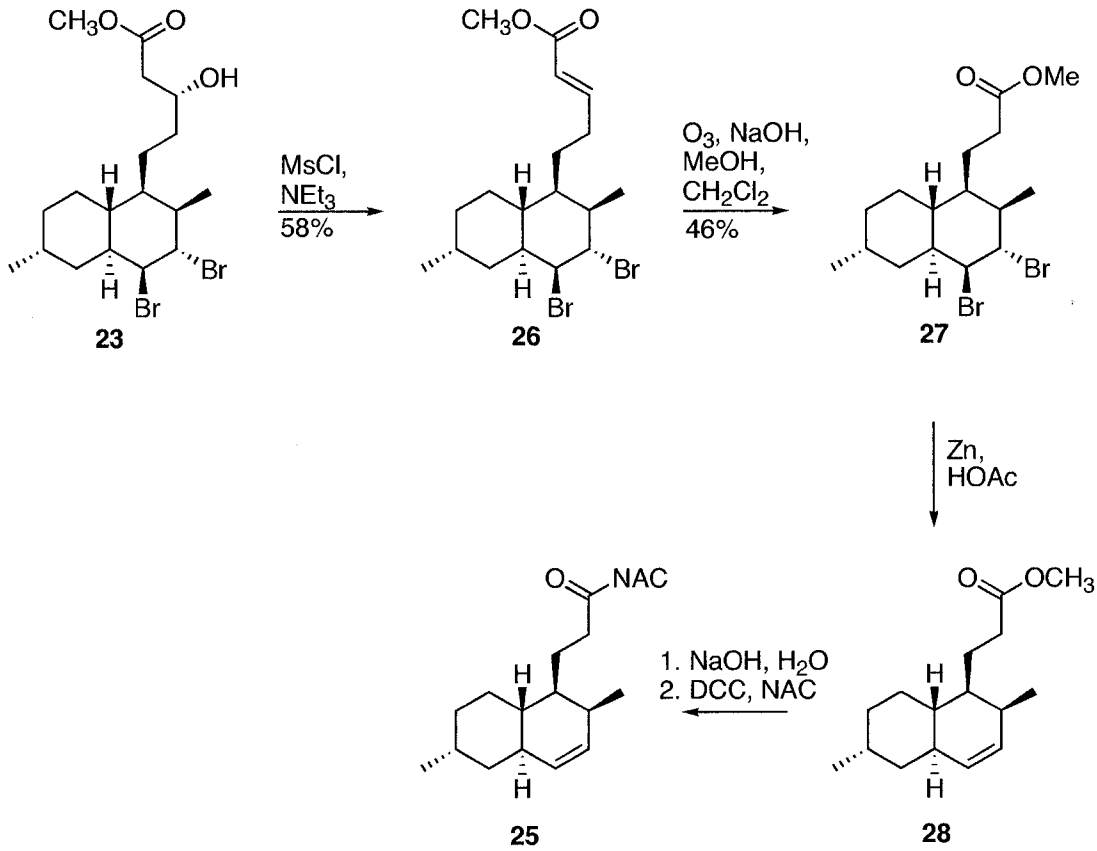
Scheme 10. Synthesis of octaketide NAC thioester **24**

2.2.3 Synthesis of the heptaketide precursor

With octaketide **20** available, a route to the heptaketide NAC precursor **25** could also be established (Scheme 11). The dibromide **23** can be used as a starting point for the synthesis. Treatment of **23** as before with methanesulfonyl chloride and excess triethylamine gives **26**. In this case, the reaction is quite slow, taking more than three days to get a reasonable amount of conversion of **23** to **26**. Changing the reaction conditions (by heating), the acylating group (i.e. TsCl vs MsCl), and using a different solvent did not lead to appreciably improved yields. Unlike the axial alcohol in the upper ring which is eliminated during the synthesis of **20**, this alcohol is part of a flexible chain

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

that may be partially hindered by the decalin ring, thereby resulting in a considerably slower rate of reaction.



Scheme 11. Synthesis of the heptaketide NAC thioester **25**

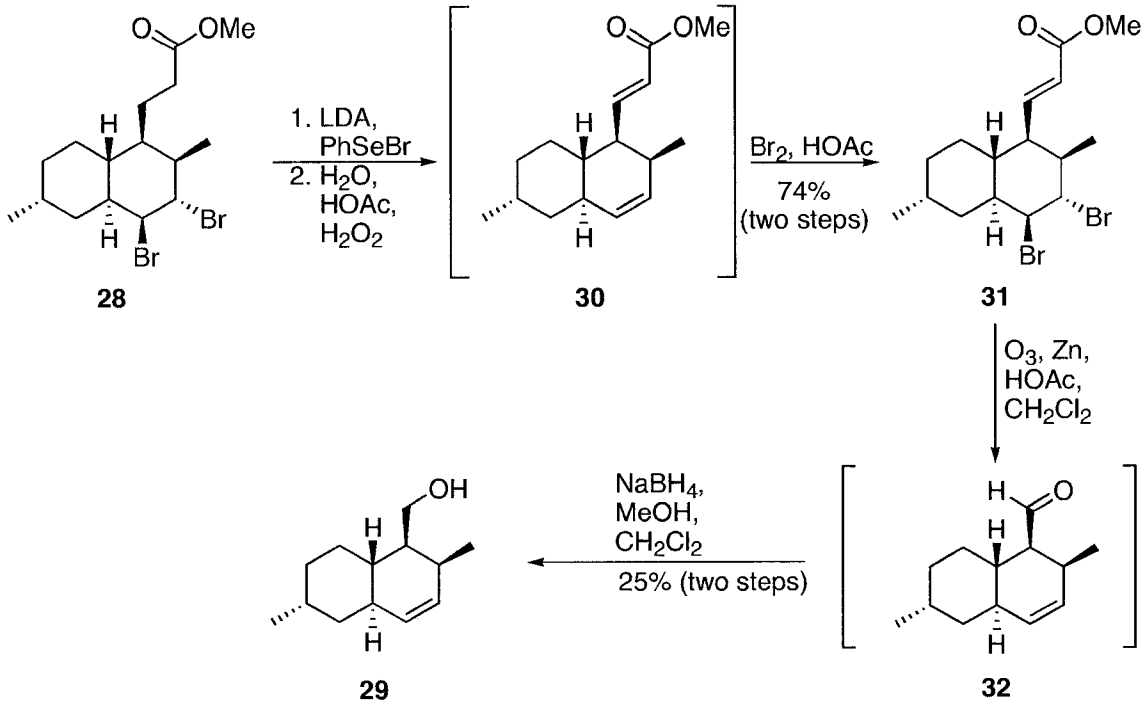
After the double bond is introduced, cleavage with ozone under basic conditions in the presence of methanol leads to the formation of heptaketide methyl ester **27**. The dibromide protecting group can be removed using Zn in acetic acid to regenerate the double bond and give **28**. Hydrolysis to the free acid and thioesterification with DCC and *N*-acetylcysteamine leads to the formation of **25** (Scheme 11). Conversion of **28** to **25** was done by Dr. Hiroyuki Morita, a post-doctoral fellow in our research group.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

2.2.4. Synthesis of the hexaketide precursor

The dibromide-protected intermediate **28** can be used as a precursor of the hexaketide unit **29** (Scheme 12). However, an alcohol functionality that could be used to introduce an additional double bond is absent in this case. Formation of the α-phenylselenyl derivative by treatment of the enolate anion of **28** with phenylselenyl bromide followed by *in situ* oxidation to the selenoxide with hydrogen peroxide, and spontaneous fragmentation[68,69] gives α,β-unsaturated heptaketide **30** having two double bonds. Interestingly the reaction of the dibromide **28** with LDA regenerates the double bond in the decalin ring. It appears that the LDA is reacting as a nucleophile with one of the bromine atoms leading to elimination of the other to reform the olefin. Hence it was necessary to re-introduce the dibromide protecting group by treating **30** with Br_2 in HOAc to give **31**. The isolated double bond in the decalin ring reacts with bromine preferentially over the more electron deficient α,β-unsaturated system in the side chain.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



Scheme 12. Synthesis of hexaketide alcohol **29**

In this synthesis, it is no longer necessary to produce an intermediate with a protected double bond after ozonolysis, and therefore a workup with Zn and acetic acid can be used.[70,71] This not only deprotects the double bond, but also reductively cleaves the ozonide to the aldehyde **32**, accomplishing two transformations in one step.

The hexaketide aldehyde **32** is quite volatile, and in order to make isolation and characterization more convenient, it was reduced *in situ* after removal of the Zn and acetic acid by treatment with sodium borohydride and methanol. This produces the hexaketide alcohol **29** in 4 steps and 18 % yield from **28**. The hexaketide alcohol **29**, is less volatile and can be stored for later oxidization to the acid and conversion to the hexaketide NAC ester. Due to small quantities available and initial difficulties with

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

incorporations of advanced precursors as NAC thioesters (see below), the final conversion of **29** to the NAC thioester was not attempted.

2.2.5 Intact incorporation experiments

Preliminary experiments aimed at determining if the polyketide fragments could be incorporated intact into the dihydromonacolin L (**11**) skeleton are being conducted using LovB enzyme in conjunction with a fellow graduate student, Mr. Doug Burr. The heptaketide NAC thioester **25**, is typically incubated overnight with LovB enzyme, ^{14}C-labelled malonyl-CoA and the appropriate cofactors, such as NADPH and FAD. The enzyme buffer is extracted with organic solvent, and the extract is examined for the presence of ^{14}C-labelled **11**. Since LovB enzyme is incapable of synthesizing **11** on its own from malonyl-CoA, any **11** detected in these experiments should be the result of incorporation of the intact polyketide fragment and extension with two additional malonate units. This extension occurs past the stage where there is an obivous requirement for LovC enzyme to properly assemble **11**. The ^{14}C radiolabel assists detection of **11** by isotope dilution analysis since the amount expected to be present is extremely small (i.e. < 6 μmole assuming 100% conversion). If radiolabeled **11** is produced it can be isolated from the crude enzyme extract by adding unlabeled **11** and purifying to a constant specific activity. If upon repurification of **11** from the enzyme extract gives radioactive material of constant specific activity, this would indicate that there was production of **11** by the enzyme.

To date no radiolabeled **11** has been detected in any of these preliminary experiments. One explanation is that perhaps, as has been the case in other systems, the NAC thioester is not being accepted as a substrate by the LovB enzyme. A possible

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

solution involves synthesis of the coenzyme A derivative of the heptaketide (and other polyketide precursors) in hopes that it may be a more suitable substrate for the enzyme, as has been successfully demonstrated in other polyketide synthase systems.[72,73] In addition, these preliminary experiments have been done using crude cell free extracts from *A. nidulans lovB*. It may be that other enzymes or cell constituents present in this mixture are interfering with the incorporation of the intact polyketide precursor. It may be that pure LovB enzyme is necessary for the successful incorporation of the heptaketide or other advanced fragments into dihydromonacolin L (**11**). The synthesis and enzyme testing of heptaketide-CoA derivatives with pure LovB enzyme is also under investigation by Mr. Doug Burr.

An additional possibility is that the LovB enzyme alone lacks the ability to elaborate the heptaketide without the assistance of LovC. The extension of heptaketide to octaketide and nonaketide does not require any enoyl reductase activity. However, the influence of the LovC protein may be more subtle than merely providing the required enoyl reductase activity. LovC protein may control the conformation of LovB and its ability to accept substrates other than acetyl and malonyl-CoA. Cell free extracts of *A. nidulans lovB+lovC* have been shown capable of synthesizing **11** when acetyl and malonyl-CoA (and appropriate cofactors) are provided. This adds an additional level of complexity to the experiment, since adding ^{14}C-malonyl-CoA to the cell free extract will produce ^{14}C labeled **11**, with or without the presence of the intact heptaketide. To confirm intact incorporation of a putative polyketide precursor, the precursor itself must contain an isotopic label, either ^{13}C or ideally ^{14}C. Although ^{14}C increases the sensitivity of detection in the final product **11**, this would require a multi-step radioactive synthetic

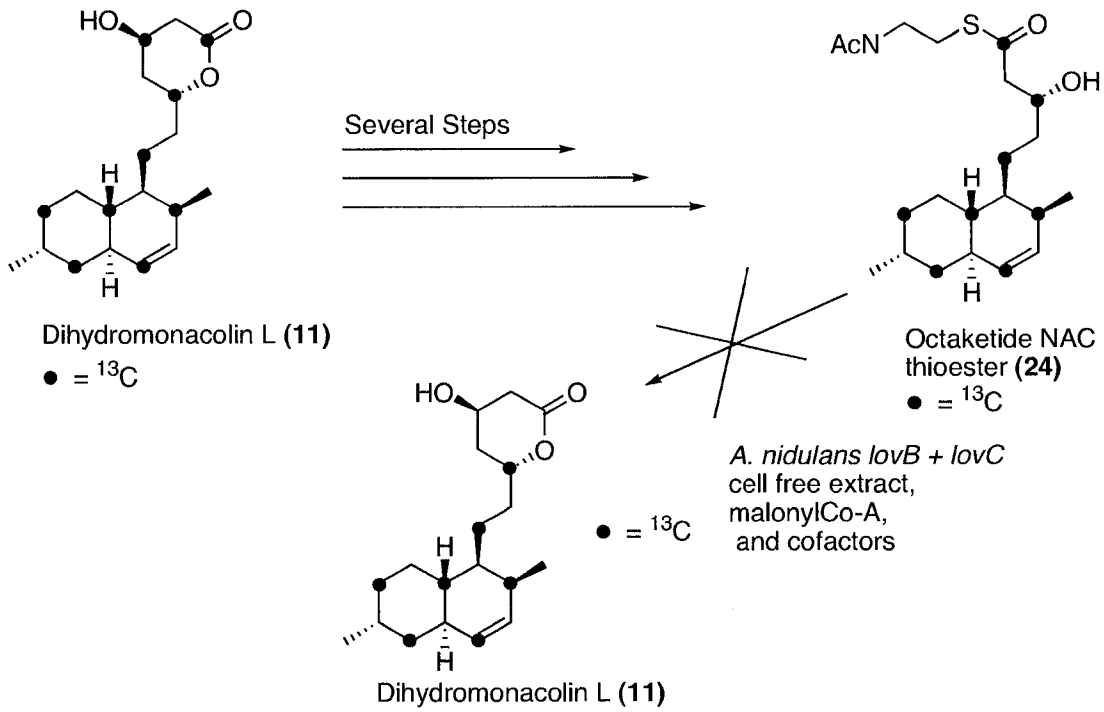
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

sequence starting with radiolabeled **11**. Although not as sensitive as ^{14}C detection by liquid scintillation counting, modern NMR techniques such as HMQC can offer a high degree of sensitivity for ^{13}C analysis.

A preliminary experiment of this type was attempted. The ^{13}C labeled octaketide NAC ester **24** (ca. 20% ^{13}C incorporation) was synthesized from ^{13}C labeled **11** (obtained from sodium [1-^{13}C] acetate) by following the synthetic scheme outlined in Scheme 10. Labeled compound **24** was incubated with the cell free extract of *A. nidulans lovB* + *lovC*, malonyl CoA and cofactors in enzyme buffer overnight. However, upon extraction of the enzyme buffer with CH_2Cl_2 and examination of the organic extract by NMR and mass spectrometry no trace of **11** could be detected (Scheme 13).

Although preliminary experiments to incorporate intact polyketide fragments have failed, there are still chances for success. Coenzyme A derivatives of the polyketide fragments may have a better chance of being accepted as substrates for the enzyme system. In addition, experiments with a cell free or pure LovB and LovC enzymes and ^{13}C labeled precursors may result in the production of [^{13}C]-**11**.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



Scheme 13. Attempted intact incorporation of ^{13}C Octaketide **24**

2.3 Isolation of Monacolin N (33)

During isolation of large amounts of dihydromonacolin L (**11**) for the synthetic work described in Section 2.2, a new product was purified from *A. nidulans lovB+lovC* fermentation cultures.[74] Produced at a level ca. 1% of that of **11** (i.e < 0.5 mg per liter), it can be isolated from the less polar fractions of the crude *A. nidulans lovB+lovC* culture extract. It was given the name monacolin N (**33**), and its spectral data appears very similar to **11**. However, both the ^{1}H NMR and high-resolution mass spectrometry indicate an extra double bond in the structure. ^{13}C NMR spectroscopy also confirms the presence of an additional pair of sp^2 carbons. The 2D-gCOSY (^{1}H-^{1}H) and gHMQC (^{13}C-^{1}H) NMR correlation experiments show that this additional double bond is present in the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

linker arm connecting the decalin ring to the upper lactone moiety. Final confirmation of the structure of monacolin N (**33**) is provided by X-ray crystallography (Figure 10).

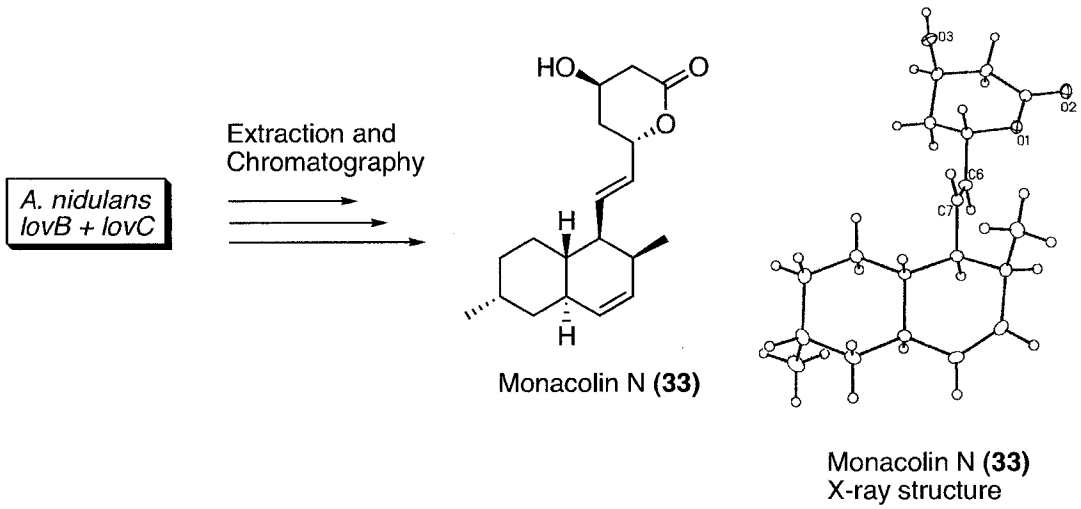
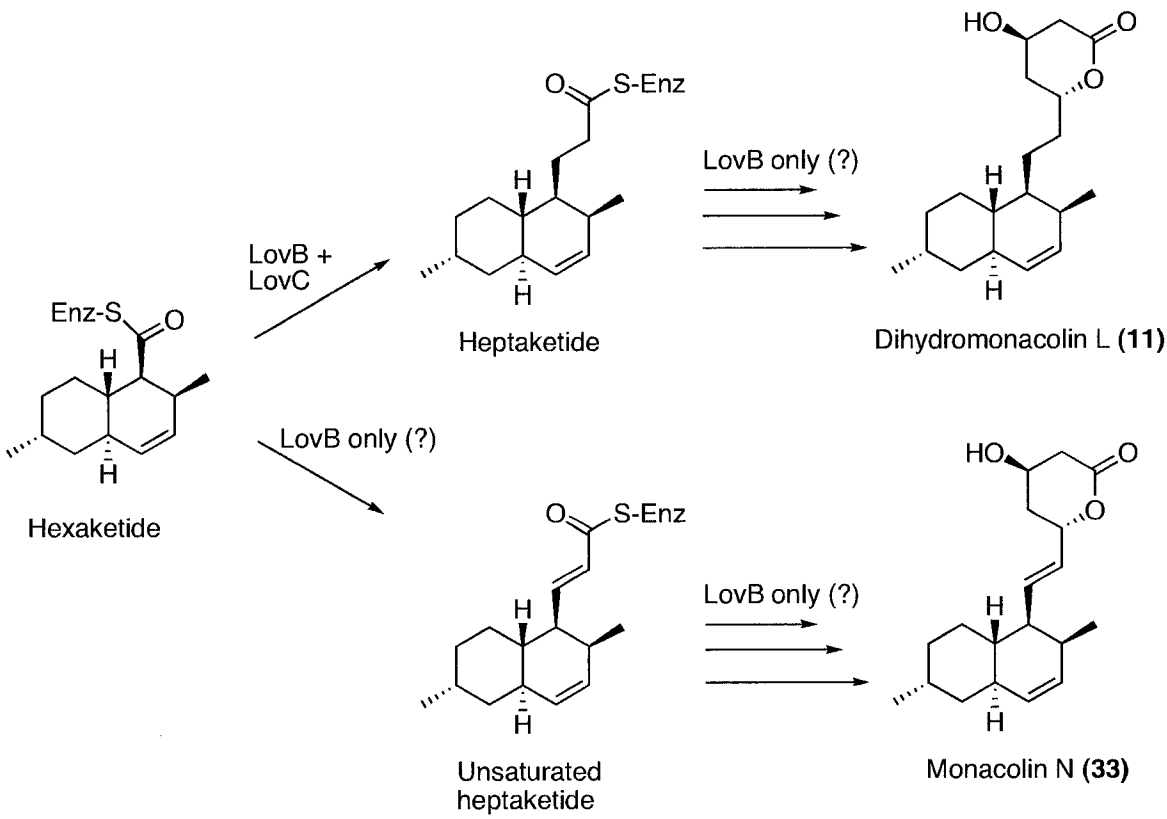


Figure 10. Structure of monacolin N (**33**) with X-ray structure confirmation.

The extra double bond in monacolin N (**33**) occurs in a position where LovC enzyme would normally be expected to accomplish an enoyl reductase step to form the fully saturated moiety present in dihydromonacolin L (**11**). It appears that LovC fails to reduce the double bond as the growing polyketide chain is elaborated from hexaketide to heptaketide. However, unlike failure of LovC at the tetraketide stage which leads to formation of truncated pyrone products **13** and **14** (Scheme 6), in this case LovB enzyme can continue to add the remaining two malonate units in the proper fashion to give monacolin N (**33**) (Scheme 14).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



Scheme 14. Biosynthesis of monacolin N (**33**)

This result seems somewhat surprising in light of the proposed mechanism for how LovB enzyme operates. LovB has all the necessary machinery for polyketide synthesis (apart form an enoyl reductase ability) and, together with LovC, is all that is necessary for the production of **11**. Therefore, what guides the next round of chain extension is presumably the shape and size of the growing polyketide chain. For example, the methyl transferase domain is used once and only once at the tetraketide stage, presumably activated by the size and shape of the tetraketide. This most likely occurs before LovC uses the enoyl reductase activity since the methyl group is present in both **11** and the shunt pyrones. However, the change in structure of the tetraketide containing an extra double bond leads to improper functioning of LovB in the next cycle

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

of chain extension. This results in the formation of pyrones **13** and **14**. However, in the case of monacolin N (**33**), the change in structure caused by the introduction of an extra double bond is apparently tolerated by the LovB enzyme, and the remaining two malonate units are added properly. It is not at all clear *a priori* that this structural modification should be tolerated by LovB in light of pyrone formation at the tetraketide stage.

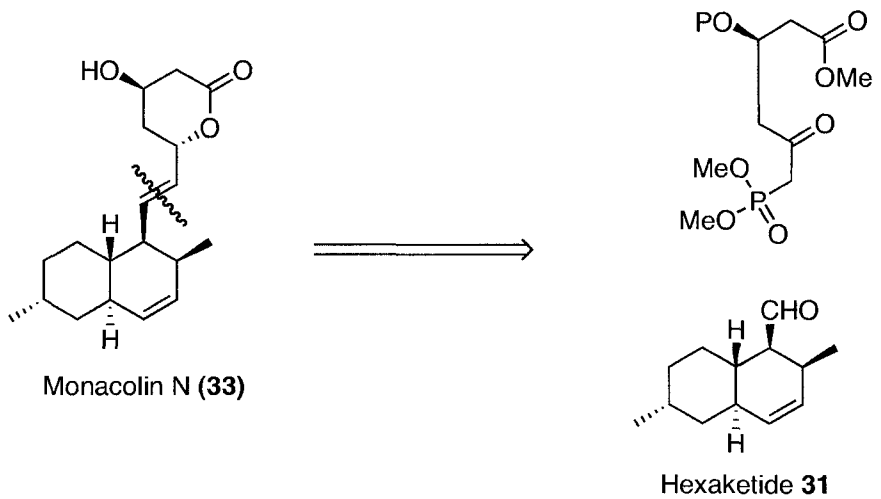
There is no clear explanation for why LovC fails to accomplish the expected enoyl reductase (ER) activity during the formation of monacolin N (**33**). One possibility is that uneven levels of expression of the *lovB* and *lovC* genes in the *A. nidulans lovB+lovC* transformant result in a deficiency of LovC enzyme with respect to LovB. It may also be that even with similar levels of expression there is an incomplete association between the two enzymes in *A. nidulans lovB+lovC*. There is some experimental evidence to support this, since fermentation cultures of *A. nidulans lovB+lovC* also produce the same yellow pyrone shunt metabolites seen when only *lovB* gene is present. It is possible that incomplete association between LovB and LovC enzyme is caused by the lack of other *A. terreus* enzymes in the *A. nidulans* transformant. The genes for enzymes responsible for secondary metabolism in bacteria and fungi are known to cluster together, and the corresponding enzymes may associate inside the cell. Perhaps enzymes which are missing in the *A. nidulans lovB+lovC* transformant provide an extra degree of association between LovB and LovC, and it is this that prevents the formation of shunt metabolites such as pyrones and **33** in the wild type *A. terreus*. There is also the possibility of other auxiliary factors that may affect the heterologous expression in *A. nidulans*.[75] Nonetheless, monacolin N (**33**) provides an interesting tool to examine the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

association between LovB and LovC enzymes. It may be possible to control the generation of other analogues of **11** through blocking of function at intermediate stages, and perhaps even make new analogues of lovastatin (**1**) enzymatically.

2.4 Synthesis of Statin Upper Ring Fragment

In order to produce monacolin N (**33**) and analogues in amounts sufficient for further experimentation a synthetic route to this molecule was investigated. A common approach to lovastatin (**1**) and other statin molecules involves coupling between the upper lactone with the lower decalin ring (or a decalin ring analogue).[76] There are numerous syntheses of precursors of the statin lactone ring.[77-80] Hence, a disconnection at the double bond in the linker arm between the upper and lower ring systems seemed a reasonable approach to **33** (Scheme 15). This idea was used previously to synthesize lovastatin (**1**), compactin (**2**) and their analogues,[81,82] and involves a Horner-Emmons-Wadsworth type coupling. This is especially attractive in making monacolin N (**33**) since the phosphonate coupling step would leave a double bond in the appropriate place.



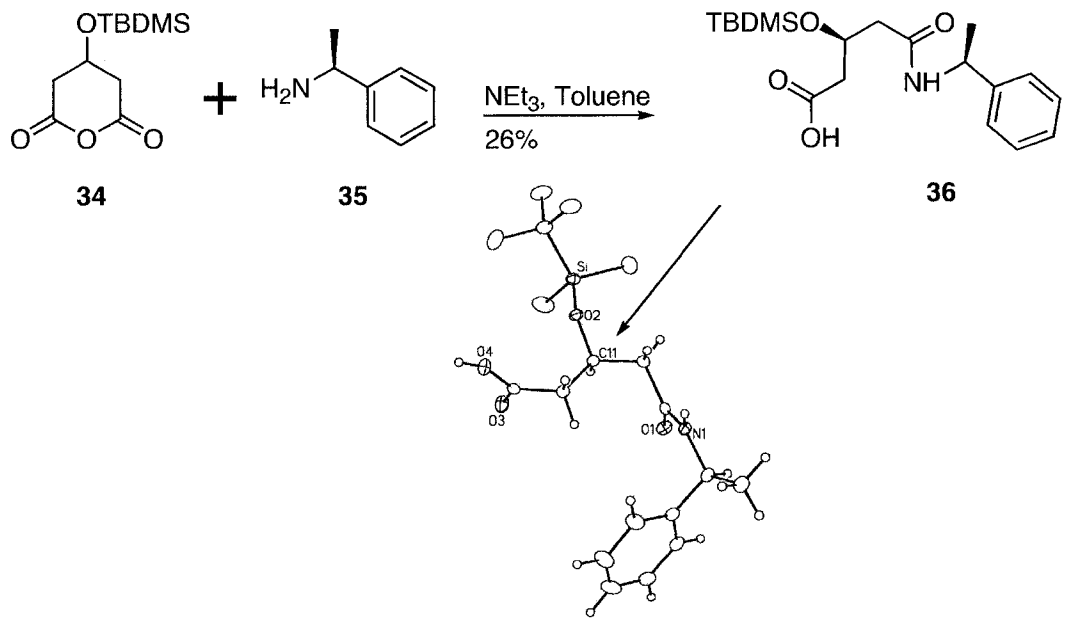
Monacolin N (**33**) Hexaketide **31**

Scheme 15. Disconnection of monacolin N (**33**)

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Following a modification of the Heathcock approach[83,84] developed by Karanewsky et al.[85] at Bristol-Myers Squibb, TBDMS protected 3-hydroxyglutaric anhydride (**34**) reacts with (*S*)-α-methylbenzylamine (**35**) to form amide acid **36** (Scheme 16). The desired *syn* diastereomer can be separated from the unwanted *anti* by rinsing the crude reaction mixture with cold diethyl ether and isolating the desired stereoisomer from the ether soluble portion. The stereochemistry of the acid **36** isolated following this procedure was confirmed by X-ray crystallography (Scheme 16).

Although it is possible to separate the diasteromers, this is a tedious process and takes many successive ether washes to obtain stereochemically pure material. An alternative is to simply react the crude residue after the ring opening reaction with methyl iodide to generate the amide ester (**37**). At this stage, the separation of diasteromers is easily accomplished by flash column chromatography.

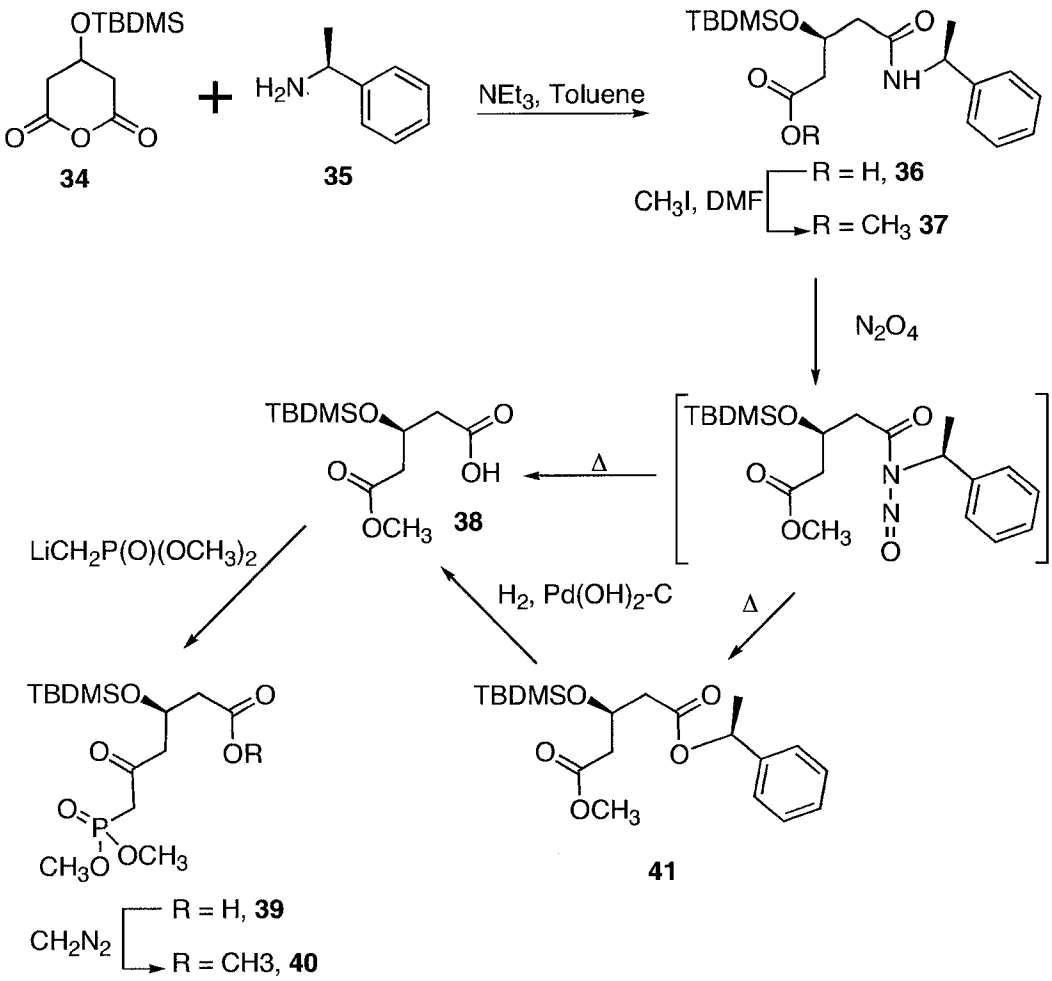


Scheme 16. Synthesis of amide acid **36** and X-ray structure. Key stereogenic center is indicated.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

The Karanewsky modification of the Heathcock approach (Scheme 17) requires the hydrolysis of amide **37** to free acid **38**. The ester **38** reacts with two equivalents of the lithium anion of dimethyl methylphosphonate to give β-keto phosphonate **39**. The acid **39** is esterified by treatment with diazomethane (CH_2N_2) to give phosphonate **40**, which is ready for the Horner-Emmons-Wadsworth coupling reaction. The strategy of this approach was to prevent β-elimination of the protected alcohol that may occur in the presence of the strong lithium phosphonate base. The acid was utilized because the first equivalent of phosphonate base would deprotonate to generate the carboxylate anion. This would raise the pKa of the C-H bonds alpha to the carboxylate carbonyl and thereby hinder the competing elimination reaction. The difficulty with this approach is that it requires the selective hydrolysis of an amide in the presence of a more reactive ester. In order to accomplish this transformation amide **37** was reacted with gaseous (and toxic) N_2O_4 to generate the *N*-nitroso amide which, upon heating, gives a mixture of the half ester **38**, and the benzyl ester **41**. Although the benzyl ester **41** can be cleaved by hydrogenolysis to give half ester **38** this step adds another level of difficulty to the synthesis. Free acid **38** then reacts with an excess (3.5 eq) of lithium phosphonate to generate the acylated product **39**. Finally the free acid is protected as the methyl ester by treatment with diazomethane to give **40**.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



Scheme 17. The Karanewsky approach to phosphonate **40**.

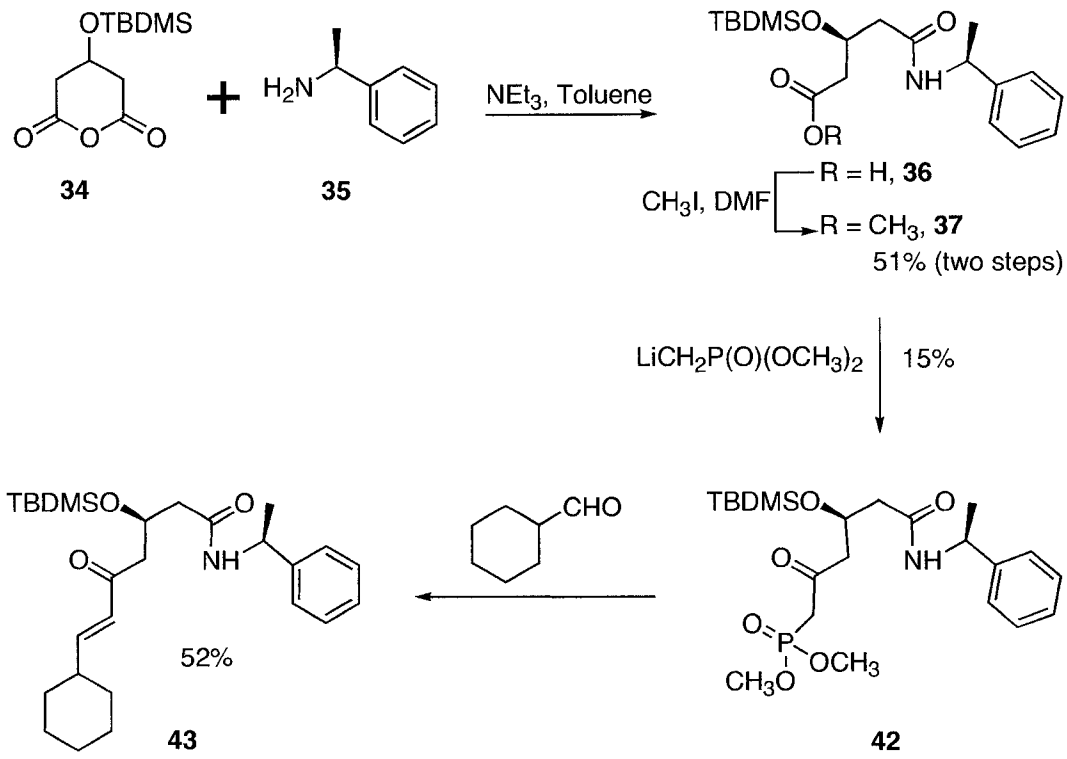
An improvement to this rather complicated approach seemed possible. It appeared that perhaps the proper protecting group to prevent elimination was already present in the form of the acidic N-H bond in amide **37**. The first equivalent of the lithium phosphonate would deprotonate the amide to generate an anion that would serve the same purpose as the carboxylate anion in the Karanewsky approach and raise the pKa of the C-H bond alpha to the amide. The second equivalent of phosphonate would then react with the ester **37** to generate the β-ketophosphonate **42** having the distal end as an amide (Scheme 18). This would obviate the need for the final diazomethane step to

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

protect the carboxylate as an ester, since an amide would not be expected to interfere with the Horner-Emmons-Wadsworth reaction. Most attractive was the elimination of the need for the selective hydrolysis of an amide in the presence of an ester and the use of toxic N_2O_4. This approach was successful in producing the desired product **42**. Although initial yields of the acylation reaction were somewhat disappointing (i.e. < 15%), this methodology has been improved by another graduate student in the Vederas group, Mr. Jamie Côté, who has achieved 60% yield with no detectable amount of β-elimination product.

A test reaction of the phosphonate **42** was conducted to ensure that the presence of the acidic N-H bond would not pose a problem for the Horner-Emmons-Wadsworth reaction. The anion of **42** was reacted for 2 days with cyclohexanecarboxaldehyde to give a 50% yield of the expected Horner-Emmons-Wadsworth adduct **43**. Optimization of such condensations are also being investigated by Mr. Côté.

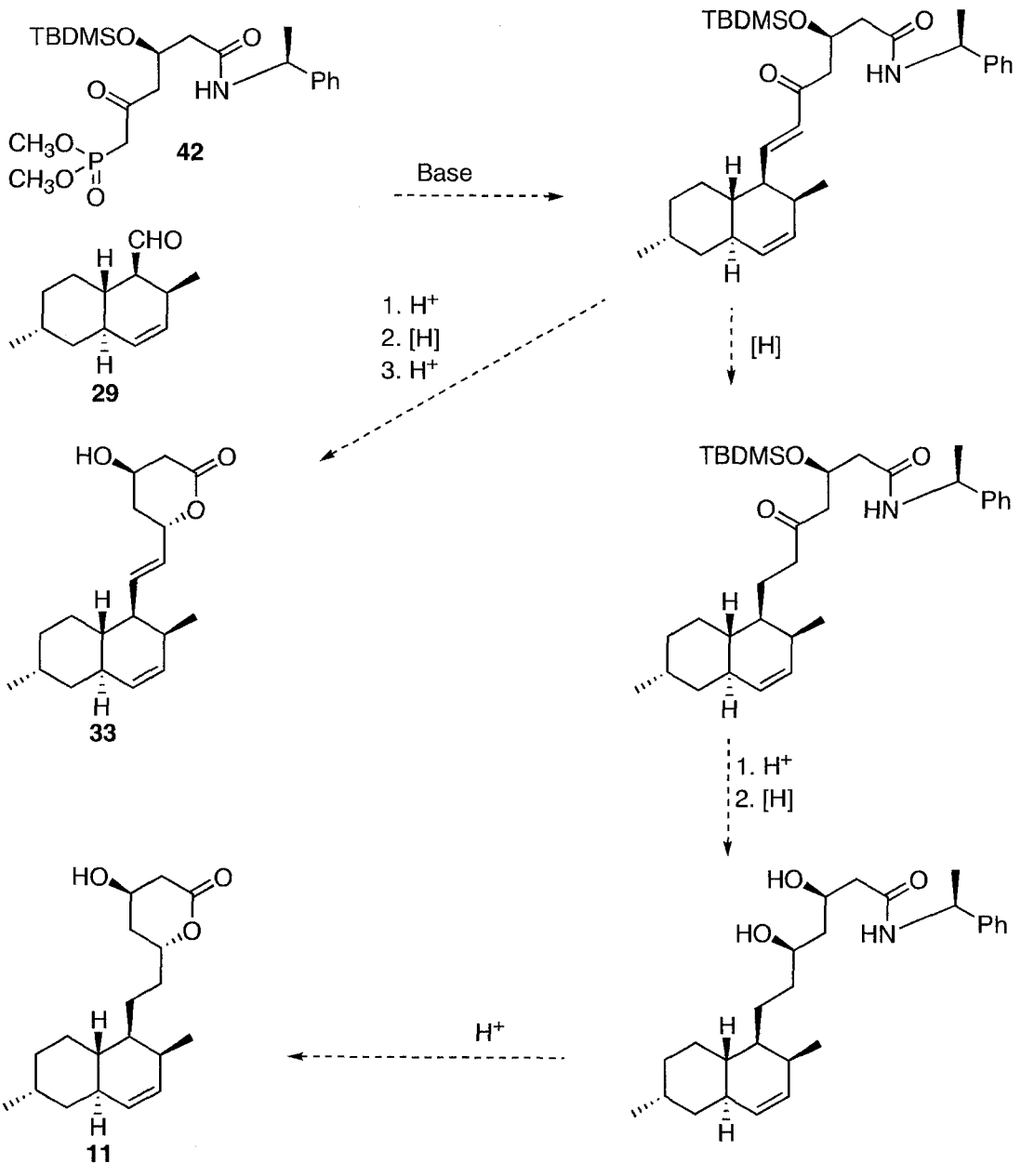
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



Scheme 18. Synthesis of **42** and test of Horner-Emmons-Wadsworth reaction.

The amide phosphonate (**42**) should react with the hexaketide aldehyde **32** (Section 2.2.3) to give the desired Horner-Emmons-Wadsworth product. However, current lack of sufficient amounts of **32** has delayed this attempt. It is possible to envision a chemical synthesis of both dihydromonacolin L (**11**) and monacolin N (**33**) using these two precursors (Scheme 19). The synthesis of **11** would involve coupling as already discussed, followed by a reduction of the double bond, and another reduction of the carbonyl to the alcohol. The silyl protecting group may have to be removed first as the free alcohol is required for chelation control during the reduction of the carbonyl.[77] The ultimate step would be the cyclization of the lactone ring under acidic conditions with concomitant removal of the amide to yield **11**. A similar approach, leaving the double bond intact is expected to yield **33**.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



Scheme 19. Proposed synthesis of **11** and **33**.

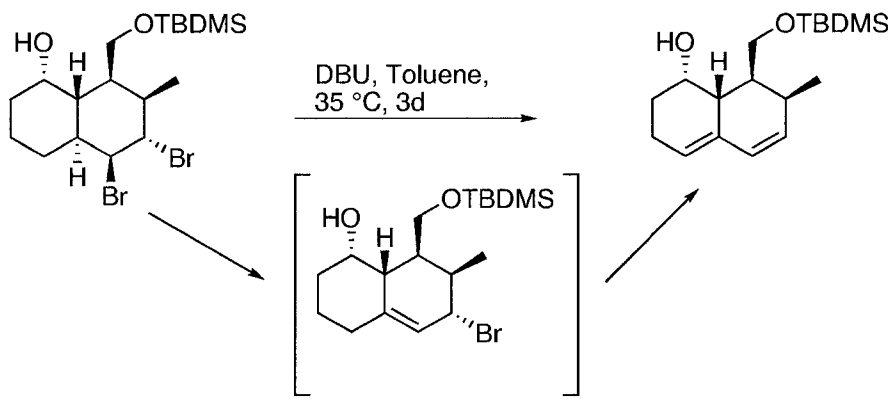
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

2.5 Attempted synthesis of monacolin L (18)

A controversial question in the biosynthesis of lovastatin (**1**) is whether monacolin L (**18**) is an intermediate in the transformation of **11** to **1**. In order to examine this it is necessary to obtain **18** in reasonable quantities. Currently no fermentation process produces an appreciable amount of monacolin L (**18**), and therefore a synthetic (or semi-synthetic) route was sought. There are two possible approaches from dihydromonacolin L (**11**) and from monacolin J (**12**) that were examined.

2.5.1 Dehydrogenation of dihydromonacolin L (11)

With large amounts of dihydromonacolin L (**11**) available from the fermentation of *A. nidulans lovB+lovC*, it seemed that dehydrogenation of this molecule could give **18**. Work done by Funk and Zeller in their synthesis of compactin (**2**) (Scheme 20) suggested application of the same sequence to a derivative of **11**.[86]



Scheme 20. Precedent for double elimination

This approach involved formation of a dibromide, and then treatment with base to cause a double elimination, thereby formally resulting in dehydrogenation of the parent mono-

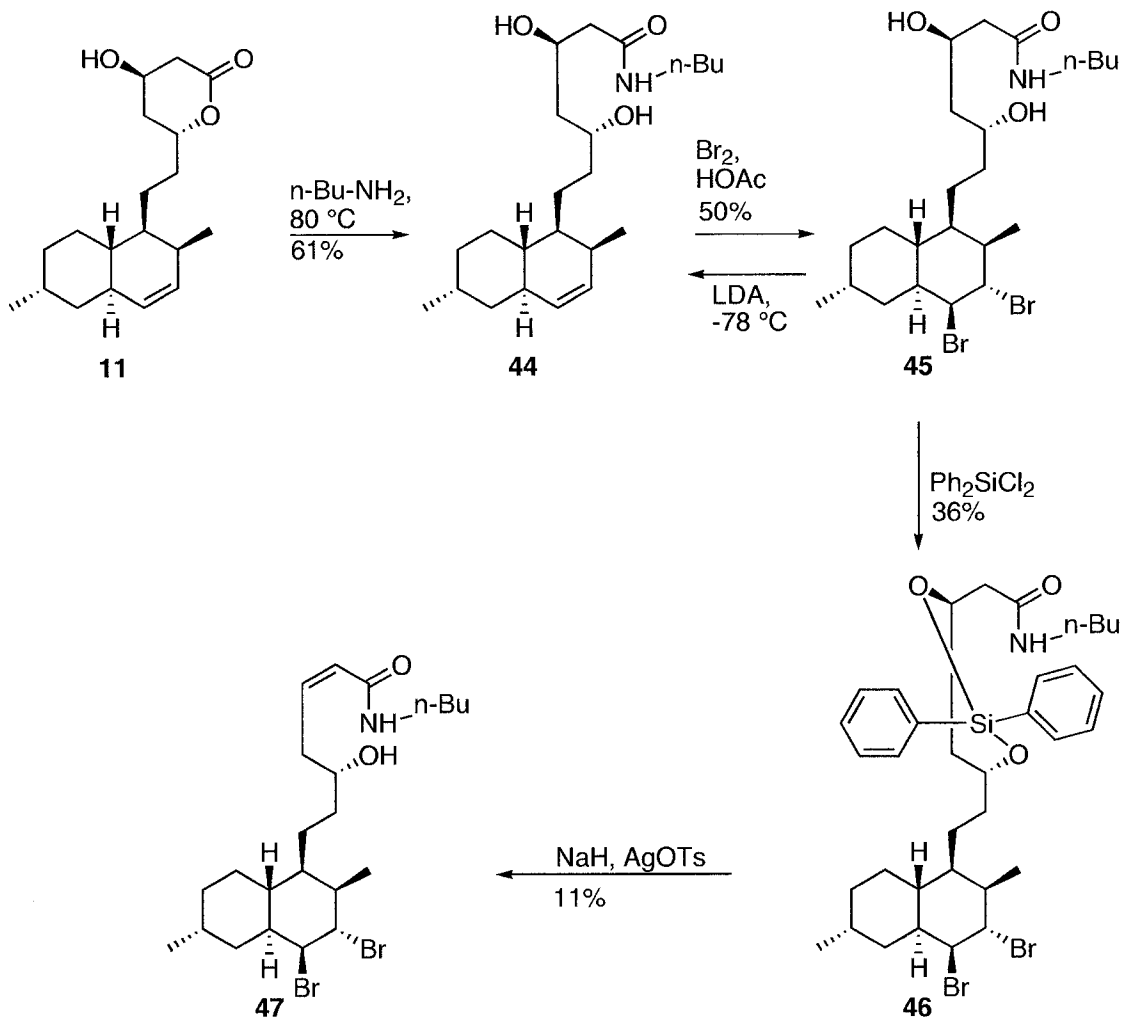
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

olefin. The authors propose a mechanism where the first equivalent of base removes the tertiary proton to form an allylic bromide. The second equivalent then removes a proton from the methylene group to eliminate the second bromine atom and form the diene system.

A concern about treatment of dihydromonacolin L (**11**) with strong base is the possible elimination of alcohol to yield an undesired α,β-unsaturated lactone. This is a problem encountered during the synthesis of simvastatin (**4**) in the attempted methylation of the lithium enolate of the side chain ester in lovastatin (**1**). To prevent this, the *n*-butyl amide derivative is made by reacting lovastatin (**1**) with neat n-butyl amine.[87] The acidic N-H bond in the amide derivative is deprotonated first, thereby raising the pKa of the alpha C-H bonds and preventing the formation of the elimination product. The *n*-butyl amide derivative seemed to be a reasonable protecting group for the same process in dihydromonacolin L (**11**).

The *n*-butyl amide derivative **44** can be formed by heating a solution of **11** in neat n-butyl amine to reflux. Amide **44** reacts with elemental bromine to give the dibromide derivative **45** in 50% yield. Numerous attempts to eliminate dibromide **45** to generate the diene system were unsuccessful. No elimination product could be detected using a variety of bases and only unchanged **45** could be recovered (Scheme 21). In addition, treatment with strong base such as LDA results in the regeneration of the decalin ring double bond. As in the formation of **30** (Scheme 12) the base appears to act as a nucleophile on bromine, thereby leading to dehalogenation.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



Scheme 21. Attempted synthesis of monacolin L (**18**)

It seemed that perhaps Ag^+ ion would assist the elimination by complexing to the bromide ion. Various silver salts were tested, such as AgOTf. However such reactions resulted in the recovery of unchanged starting material **45**. The two free hydroxyl groups were next protected as silyl ethers in order to prevent possible interaction with the Ag^+ ion. Treatment of dibromide amide **45** with dichlorodiphenylsilane gave the protected derivative **46**. The only identifiable transformation of **46** upon treatment with NaH and AgOTs, was formation of the unwanted elimination product **47**.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

A possible explanation for the failure of the elimination reaction to form the desired diene system may be the blocking effect of the methyl group at the C-6 position in dihydromonacolin L (**11**) (Fig. 11). In the literature precedent[86] (Scheme 20) there is no methyl group at the C-6 position, and perhaps this allows the base to access the tertiary proton from below. In the case of the dihydromonacolin L (**11**) derivatives, (**45** or **46**) the axial methyl group at C-6 position may cause steric interference that prevents the base from getting to the tertiary proton.

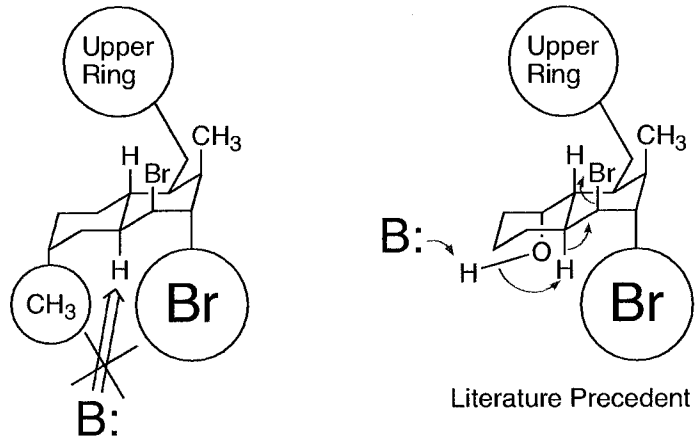


Figure 11. Cartoon representation of steric hindrance in decalin ring of **11**, and possible assistance by the C-8 alcohol in the literature precedent.

The derivatives **45** and **46** also have a large side chain with an amide functionality that is not present in the literature precedent. This side chain may also play a role in hindering the removal of the proton at C-4a perhaps because the amide proton is more acidic. In addition, the literature precedent has an axial hydroxyl group at the C-8 position. This may assist by acting as an internal base (after deprotonation) to remove the C-4a hydrogen. The stereochemistry of the dibromide derivative **22** of dihydromonacolin L was confirmed by crystallography and the orientation of the bromine atom on C-3 and

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

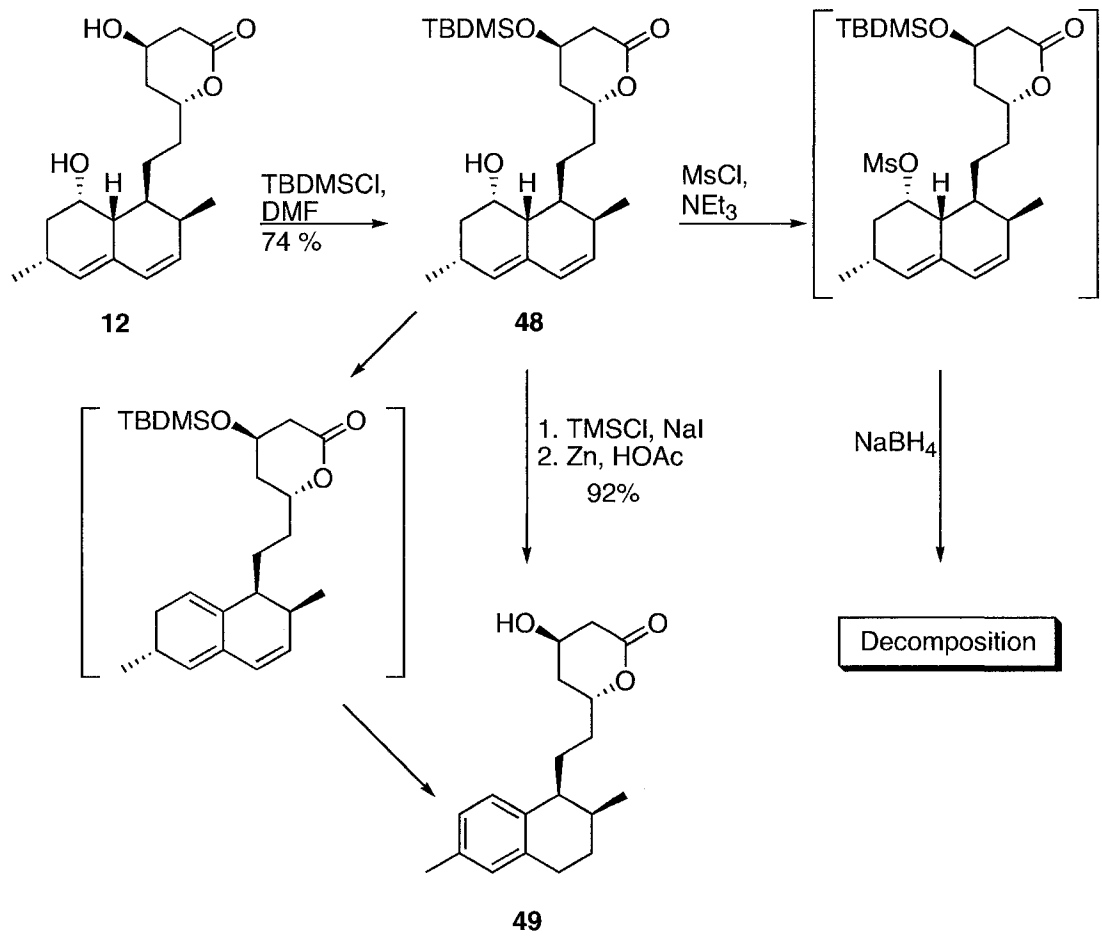
C-4 in **45** and **46** is assumed to be the same based on NMR chemical shifts and coupling constants.

2.5.2 Deoxygenation of monacolin J (12)

A second route to monacolin L (**18**) was also considered wherein the C-8 alcohol of monacolin J (**12**) (which already possess the desired diene system), could be removed by reduction. The initial step involves selective protection of the less hindered upper ring alcohol as the silyl ether by treatment of **12** with TBDMSCl in DMF to give **48** in good yield (Scheme 22).[88] The next step is to react the remaining alcohol in the decalin ring with an activating agent of some kind, and then substitute the C-O bond with a C-H bond. Literature precedent shows that a series of secondary alcohols was successfully deoxygentated by reacting the methanesulfonate derivative of the alcohol with $NaBH_4$.[89,90] Monacolin J derivative **48** does react with methanesulfonyl chloride to give the corresponding mesylate but subsequent reaction with $NaBH_4$ yields only a complex mixture. The mesylate is quite unstable and cannot be easily isolated from the crude reaction mixture. It decomposes at room temperature to a complex mixture of products. A milder alternative is to react the protected monacolin J derivative **48** with TMSCl followed by NaI.[91] In principle, this forms a secondary iodide, which can be dehalogenated with Zn in acetic acid. However, the product of this reaction was aromatized compound **49**. This substance presumably arises from the elimination of the activated alcohol or iodide to give a triene system. Acid catalyzed rearrangement of the external double bond leads to the formation of the stable aromatic system. It appears that

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

the axial C-8 alcohol very readily undergoes elimination, as opposed to deoxygenation, and that perhaps products similar to **49** may occur in the mesylation reaction. This approach to monacolin L (**18**) was abandoned.

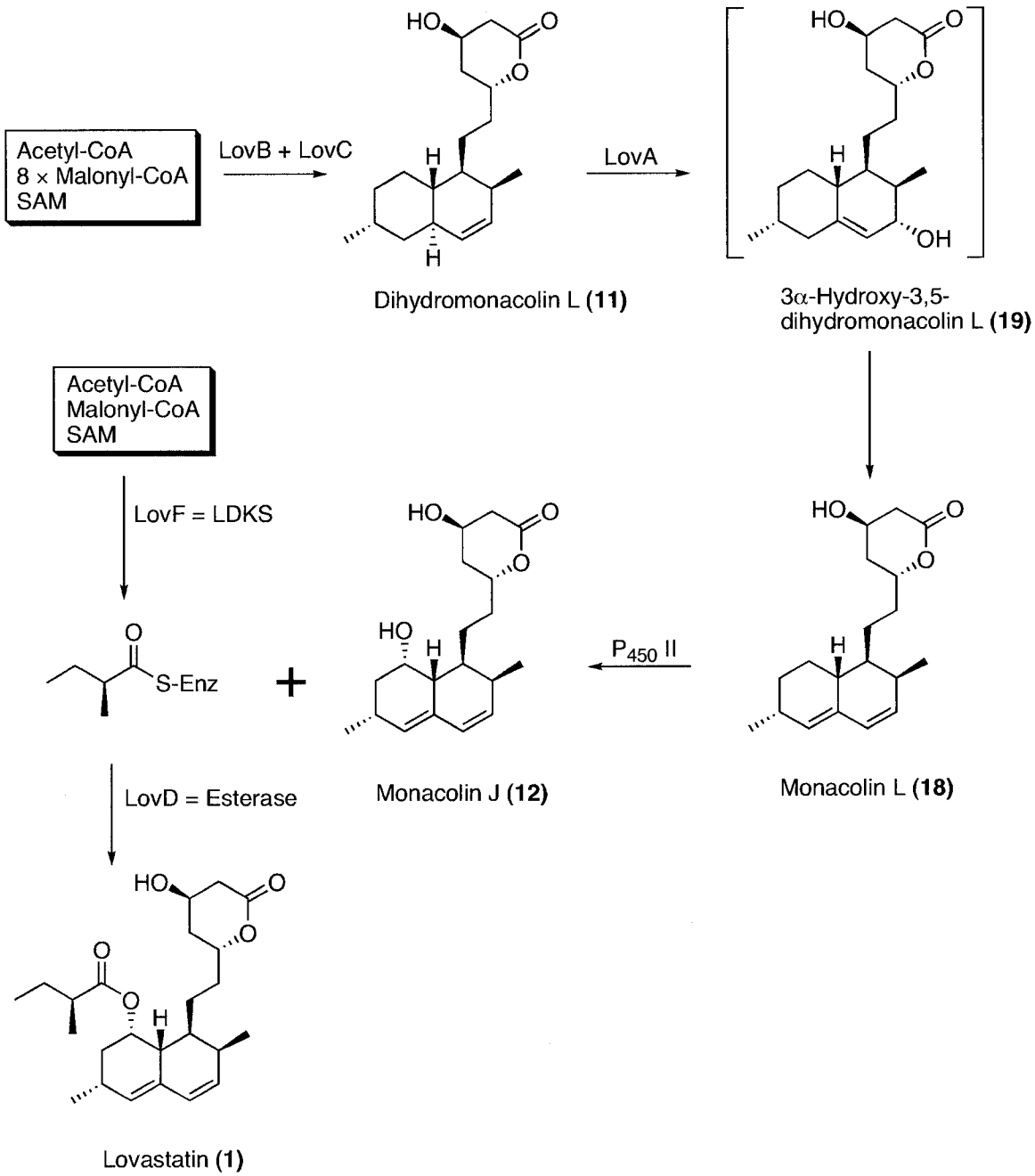


Scheme 22. Attempted deoxygenation of monacolin J (**12**)

2.6 Post-PKS Transformations

An additional objective of this thesis is to investigate the enzymatic transformations after the PKS assembly of dihydromonacolin L (**11**) that lead to the formation of lovastatin (**1**) (Scheme 23).[54]

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



Scheme 23. Proposed post-PKS transformations in lovastatin (**1**) biosynthesis

The first proposed enzymatic step in the post-PKS elaboration, involves the transformation of **11** to **18** by the introduction of a second double bond,[57] and is believed to be under the control of the *lovA* gene (Scheme 23). A second P_{450} type enzyme, under

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

the control of *orf17* may be responsible for the oxidative step that introduces the C-8 hydroxyl. As described previously, the diketide side chain is synthesized by the lovastatin diketide synthase (i.e. LDKS = LovF) and the esterification reaction is under the control of the *lovD* gene. The key to this part of the project is the use of lovastatin (**1**) deficient mutants of *A. terreus* that are missing one or more of the required genes. Mutants of this type would be expected to accumulate intermediates associated with lovastatin (**1**) biosynthesis from steps before the blocked gene. In addition, it would be expected that the biotransformation machinery after the block should be intact and, if fed appropriate precursors, should be capable of transforming them into lovastatin (**1**) or related molecules. Two of these lovastatin deficient *A. terreus* mutants were examined, one lacking the *lovA* gene for the first post-PKS oxidation and the other lacking the *lovC* gene required for biosynthesis of **11**.

2.6.1 *A. terreus lovA* biotransformations

Lovastatin deficient mutant *A. terreus lovA* is missing the *lovA* gene believed to be responsible for the introduction of the second double bond of the diene system. This mutant would be expected to possess intact the assembly machinery (i.e. LovB and LovC enzymes) necessary for the production of dihydromonacolin L (**11**), but should lack the ability to transform **11** into **1**. As expected, preliminary HPLC examination of fermentation cultures of *A. terreus lovA* showed that no lovastatin (**1**) could be detected. However, somewhat surprisingly, no dihydromonacolin L (**11**) could be detected either. The first objective was to account for the absence of dihydromonacolin L (**11**) in the fermentation culture.

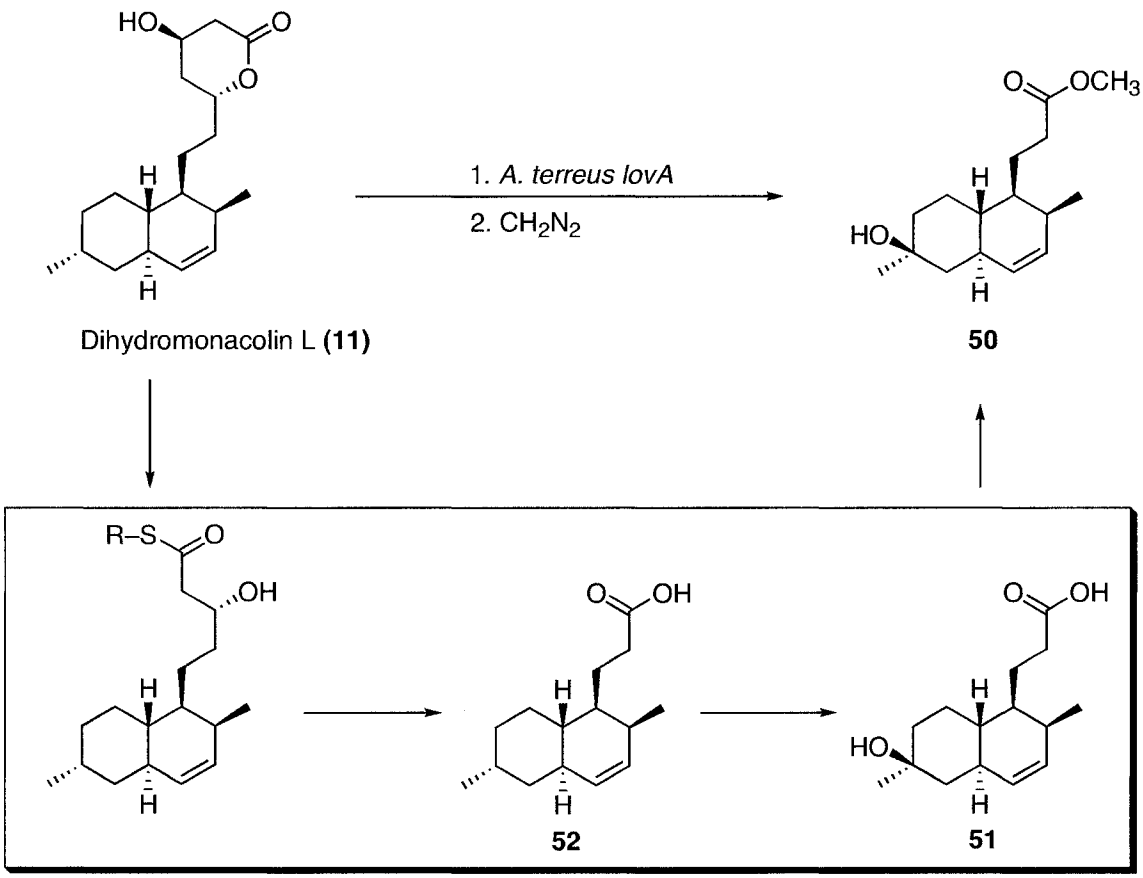
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Radioactive [^{14}C]-dihydromonacolin L (**11**) was produced by feeding sodium 1-[^{14}C] acetate to cultures of *A. nidulans lovB+lovC*. After extraction and isolation, the ^{14}C labeled dihydromonacolin L (**11**) has a specific activity of 0.185 μCi per mg. A portion of this sample of [^{14}C]-**11** was fed (as an EtOH solution) to a growing culture of *A. terreus lovA* mutant over a period of three days. At the end of this time, the *A. terreus lovA* culture was extracted and the major band of radioactivity was purified to homogeneity by preparative TLC. During isolation the culture extract was treated with diazomethane (CH_2N_2) to aid in the purification by transforming any free acid groups present to their methyl esters. The major radioactive compound isolated from these cultures appeared to possess the decalin ring but its mass spectrum indicated a molecular formula with four carbons and one oxygen atom less than that of **11**. In addition the ^{1}H NMR spectrum did not display the protons attached directly to carbons bearing hydroxyl groups, and one of the methyl groups was now a singlet. This information along with correlations in the 2D NMR experiments, especially gCOSY and gHMQC, led to assignment of the structure as **50** (Scheme 24).

The free acid **51**, appears to arise from β-oxidation of dihydromonacolin L (**11**) to a heptaketide **52**. This is then further oxidized, presumably by a P_{450} enzyme, to introduce the hydroxyl group at C-6 position. β-Oxidation is a ubiquitous process and is a common catabolism for fatty acids and natural products,[92,93] including statins in mouse liver preparations.[94] Hydroxylation at C-6 is the first step of metabolism of lovastatin (**1**) in the human liver,[95] and similar processes occur in fungi, for example the microbial conversion of compactin (**2**) to pravastatin (**3**).[96] Compound **50** can also be isolated after diazomethane treatment of the extracts from cultures of *A. nidulans lovB+lovC*

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

suggesting that similar enzymatic degradative processes occur in both *A. terreus* and *A. nidulans*.



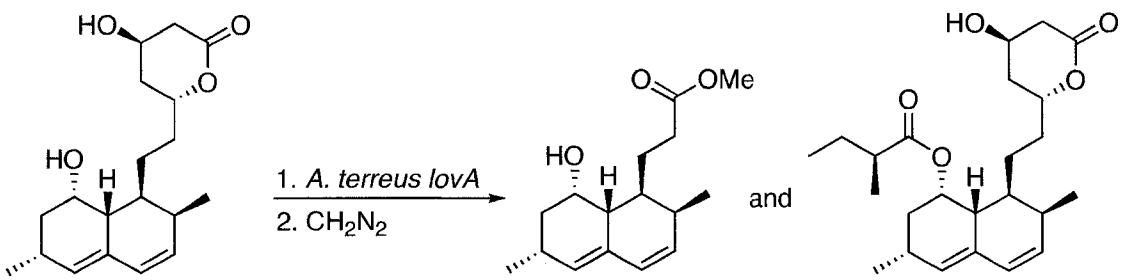
Scheme 24. Compound **50** and proposed biotransformation of **11**.

A small amount of heptaketide free acid **52** can also be isolated from *A. nidulans lovB+lovC* fermentation culture supernatant (ca. 2 – 3 mg per liter). Presumably this is the result of β-oxidation of **11** and export out of the cells before hydroxylation by a P_{450} enzyme. Presumably the heptaketide acid **52** is also released into the supernatant in *A. terreus lovA*, but the expected amounts are quite small and difficult to detect. Careful examination of the **11** fed to the *A. terreus lovA* cultures revealed that it was not contaminated with any **51** or with **52**.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

These results suggest that any **11** produced in *A. terreus lovA* is completely degraded by β-oxidation. The *lovA* gene may have a role in regulating the enzymes of the β-oxidation pathway, and its absence may result in this pathway being over-expressed. It is somewhat unexpected that inactivation of one of the genes in the biosynthetic pathway would completely shunt the metabolism.

The *A. terreus lovA* mutant would be expected to possess the ability to convert monacolin J (**12**) to lovastatin (**1**) since the enzymes responsible for this transformation are after the *lovA* block. To test this, a biotransformation experiment was conducted where an EtOH solution of **12** was administered to a culture of *A. terreus lovA*. Analysis of the extract of the fermentation broth of this culture after methylation with diazomethane revealed the presence of lovastatin (**1**) along with a small amount of **53** (Scheme 25). Compound **53** is presumably the result of degradation of **12** via a β-oxidation pathway, in this case without the hydroxylation by a P_{450} type enzyme.



Monacolin J (**12**) **53** Lovastatin (**1**)

Scheme 25. Biotransformation of monacolin J (**12**) by *A. terreus lovA*

Additional experiments were conducted to determine if there may be other possible oxidized intermediates on the pathway from **11** to **1**. As described in Section 1.3.1, there is some question as to the intermediates involved in the introduction of the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

second double bond of the diene system. It is known that epoxides may also be the product of P_{450} type enzymes,[97] and therefore may potentially be intermediates on the pathway to **1**. It is also believed that there is no discrete radical intermediate formed in alkene epoxidation.[98] Epoxidation of the double bond in **11** could allow for ring opening to an allylic alcohol as seen in **19**, which could then go on to produce **18** after dehydration. To test this hypothesis, two diastereomeric epoxides **54a** and **54b** were synthesized from dihydromonacolin L (**11**) by treatment with MCPBA and separation by HPLC. The stereochemistry of the epoxides was confirmed by X-ray crystallography of **54a** (Fig. 12).

In a separate experiment, ^{14}C-labeled **11** was used in the synthesis to generate the epoxides **54a** and **54b** with radioactive labels. Each diasteromer of the radiolabeled epoxide was added separately to cultures of *A. terreus lovA*, and culture extracts were examined for the presence of ^{14}C-labeled **1**. However, in neither case could any **1** be detected by TLC radioactive analysis nor could any of the original epoxide be recovered. Control experiments show that the epoxides are quite sensitive to the culture medium, which contains peptides and amino acids. Treatment of **54a** with the methyl ester of glycine leads to nucleophilic ring opening of the epoxide. Therefore it is likely that the epoxides suffer from nucleophilic attack either in the medium or on the surface of the cell before being exposed to the post-PKS enzymes. Experiments with purified enzymes will be required to determine the possible intermediacy of epoxides **54a** or **54b**.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

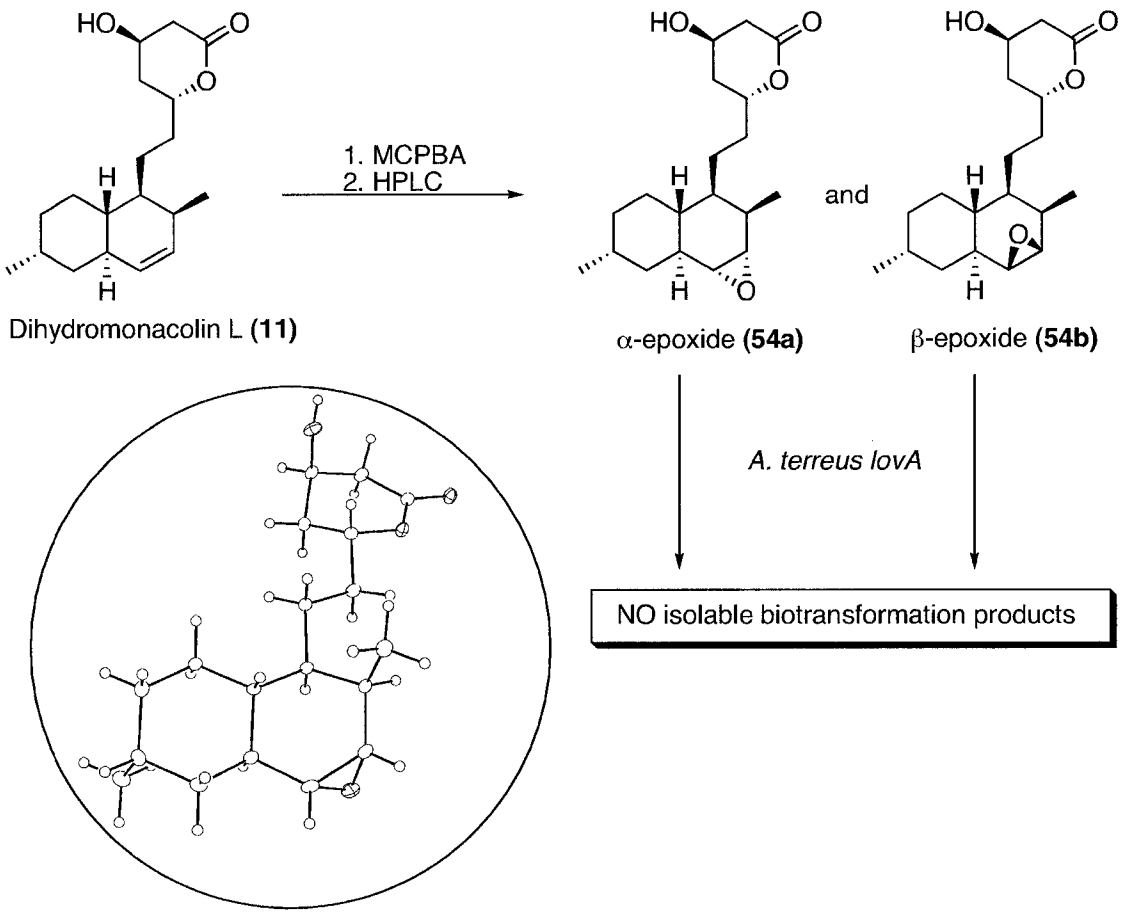


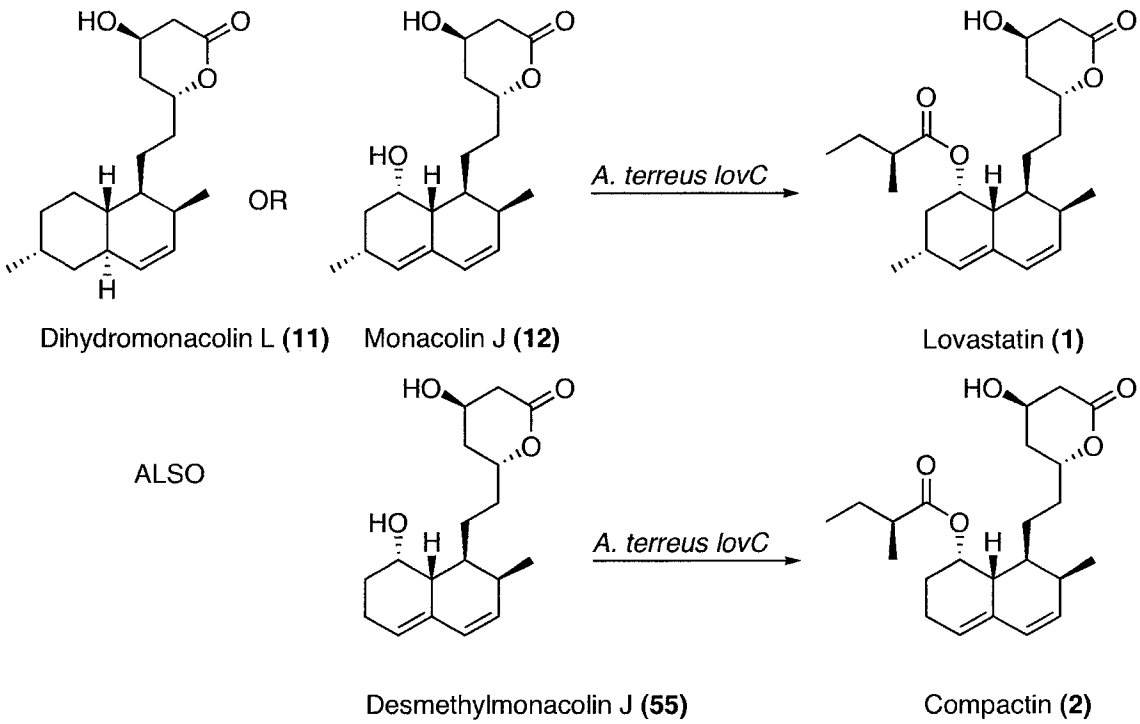
Figure 12. Synthesis and separation of epoxides **54a** and **54b**. X-ray structure of α-isomer **54a**.

In summary, the *A. terreus lovA* mutant does not possess the ability to produce lovastatin (**1**), and does not accumulate **11** as expected, but rather efficiently converts it into **51**. *A. terreus lovA* does possess a limited ability to transform monacolin J (**12**) into **1**, with some of the **12** being degraded by β-oxidation. The intermediacy of epoxides **54a** or **54b** remains uncertain.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

2.6.2 *A. terreus lovC* biotransformations

A. terreus lovC mutant has an insertional mutation in the key *lovC* gene required for the production of dihydromonacolin L (**11**), and as a result lacks the ability to make lovastatin (**1**). However, it would be expected that this mutant possesses the entire elaboration machinery necessary to convert **11** to **1**. If **11** is added (as an EtOH solution) to fermentation cultures of *A. terreus lovC*, lovastatin (**1**) is produced. In addition, monacolin J (**12**) is also efficiently converted by this organism into **1**. Since *A. terreus lovC* can accomplish all the post-PKS elaboration steps it seemed possible to employ this capability to make analogues of lovastatin (**1**). A successful example is the conversion of desmethylmonacolin J (**55**), which has no C-6 methyl group, into compactin (**2**) by *A. terreus lovC*.[55]



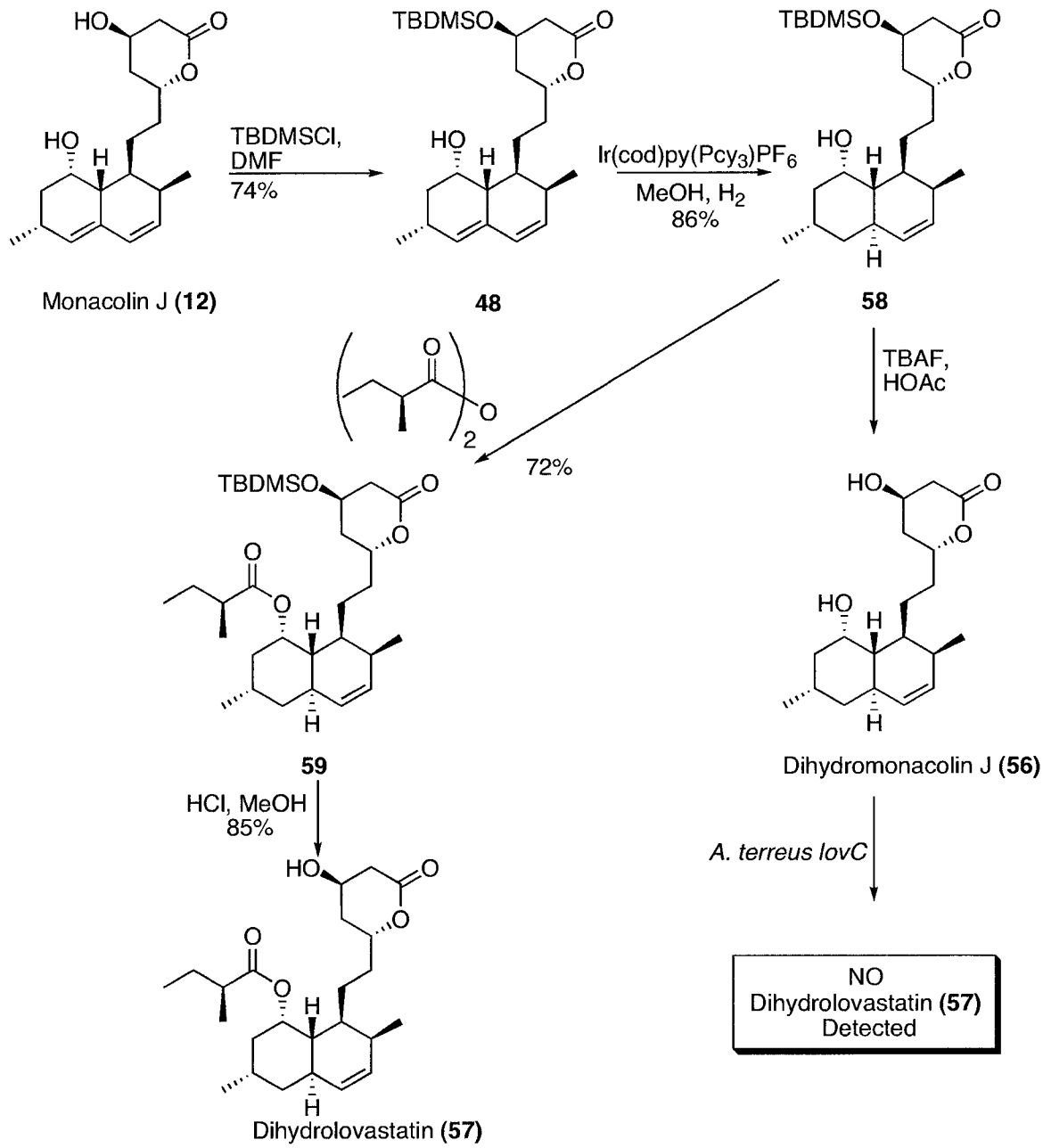
Scheme 26. Biotransformations by *A. terreus lovC*

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

To examine the scope of such biotransformations, dihydromonacolin J **(56)** was added as an EtOH solution to a growing culture of *A. terreus lovC* (Scheme 27). However, HPLC analysis showed no trace of dihydrolovastatin **(57)**, and no obvious products of biotransformation of **56** could be isolated. A standard sample of **57** was synthesized (Scheme 27) for use as an HPLC standard.

Protection of monacolin J **(12)** generates **48** which was selectively reduced using an iridium catalyst[99,100] to **58** by Dr. Joanna Harris, a postdoctoral fellow in our research group. This can be acylated with (*S*)-2-methylbutyric anhydride[101] to give **59**. The silyl protecting group was then removed under acidic conditions to provide a standard sample of **57**, whose spectral data matched those described in the literature.[102]

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



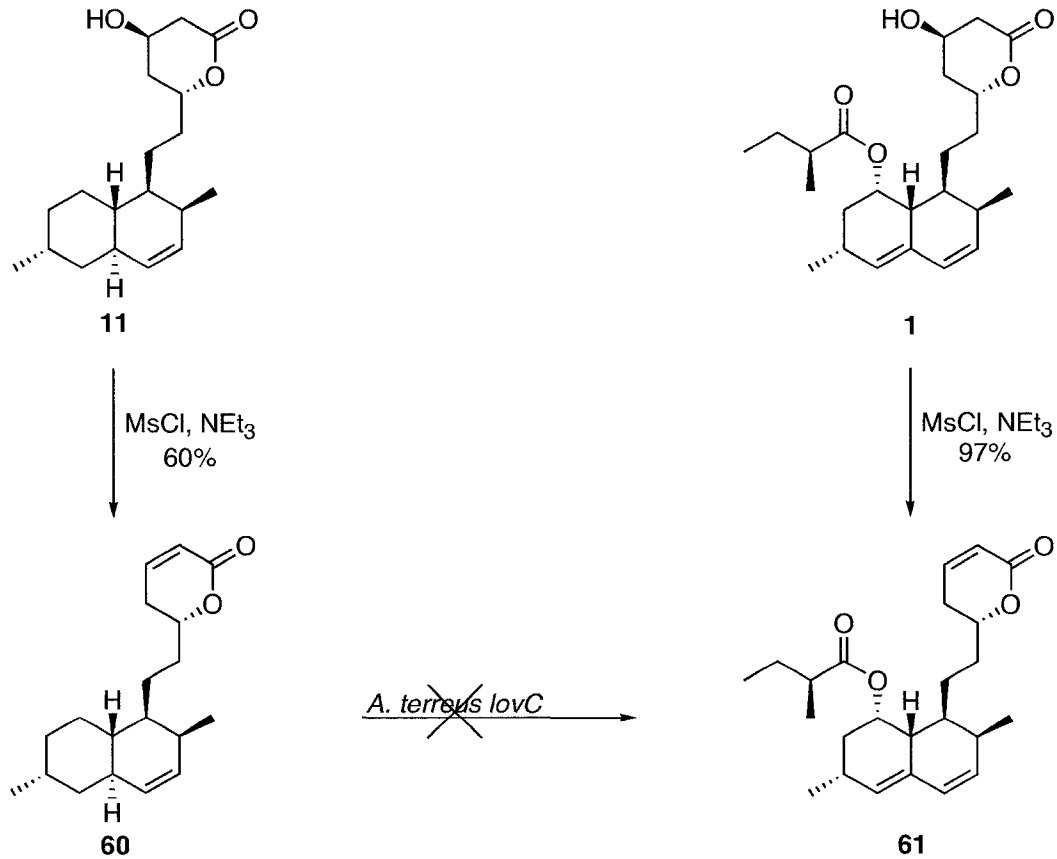
Scheme 27. Synthesis of dihydromonacolin J **(56)** and dihydrolovastatin **(57)**.

It may be that **56** is not accepted as a substrate by the post-PKS elaboration machinery in *A. terreus lovC*. However dihydrolovastatin **(57)** is a natural product that has been isolated from cultures of *A. terreus*, and it may be that the amounts of **57** produced are below our detection limits. This question could potentially be answered by

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

repeating the experiments with larger amounts of **56** or using radiolabeled **56** in conjunction with isotope dilution analysis.

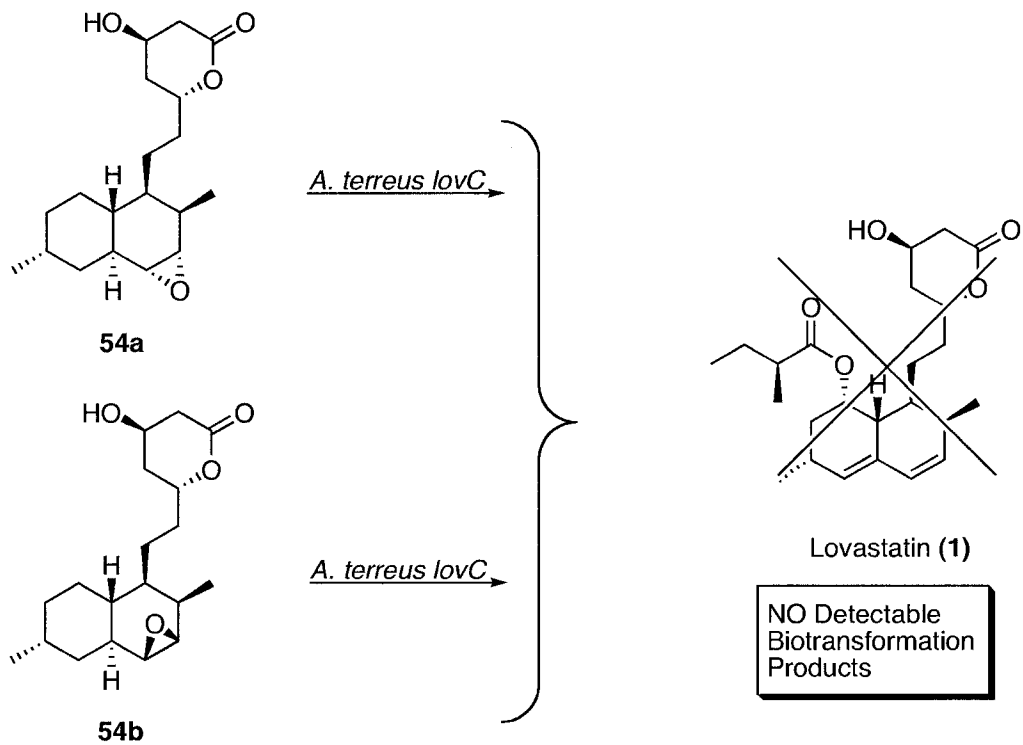
To further probe the substrate specificity of the post-PKS enzyme complex in *A. terreus lovC*, a biotransformation experiment was attempted with α,β-unsaturated dihydromonacolin L (**60**). This compound could potentially form α,β-unsaturated lovastatin **61**. Both **60** and a standard sample of **61** can be readily synthesized from the respective natural products via the methanesulfonates (Scheme 28). However, no trace of **61** could be detected after addition of **60** to *A. terreus lovC*. It may be that the change in conformation of the upper ring due to two sp^2 carbons makes **60** unacceptable to the post-PKS elaboration system.



Scheme 28. Attempted biotransformation of **60**.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

The diastereomeric epoxides **54a** and **54b**, described in Section 2.6.1 and examined with *A. terreus lovA*, were also added separately to the *lovC* mutant (Scheme 29). As in the earlier biotransformation experiment, no conversion of either radiolabeled epoxide diastereomer into lovastatin (**1**) could be detected and no epoxide could be recovered from the fermentation broth. As described previously, the most likely explanation is that the epoxides are too chemically unstable and do not survive the medium.



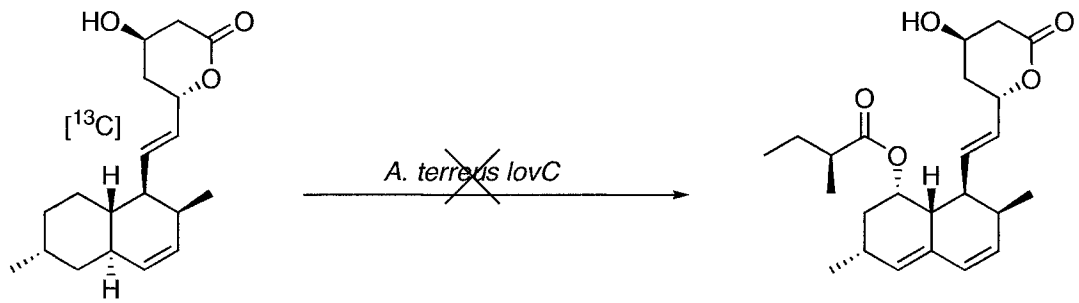
Scheme 29. Epoxides **54a** and **54b** attempted biotransformation with *A. terreus lovC*

One final experiment conducted with the *A. terreus lovC* mutant was the attempted biotransformation of monacolin N (**33**). If it were to be transformed by the post-PKS enzyme complex, it would be expected to generate an unsaturated lovastatin

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

analogue such as **62** (Scheme 30). The additional double bond in **62** is located similarily as in currently marketed drugs such as rosuvastatin (**7**) (Scheme 30 and Fig. 1). Since **7** shows higher inhibition of HMG-CoA reductase (HMGR) than **1**,[13] **62** could display a greater HMGR inhibition than lovastatin (**1**).

Although a synthetic path to monacolin N (**33**) has been established as described in Section 2.4, to detect the products of a biotransformation experiment incorporation of isotopic labels in **33** is advantageous. The most convenient way of incorporating this label is addition of sodium [1-^{13}C] acetate to fermentation cultures of *A. nidulans lovB+lovC*, followed by purification of ^{13}C-labeled **33**. The labeled monacolin N (**33**) was then fed to growing cultures of *A. terreus lovC* and the culture extracts were examined for the presence of **62** or related products. Unfortunately, lovastatin analogue (**62**) could be detected in the extracts of the fermentation cultures, and ca. 25% of monacolin N (**33**) was recovered unchanged. No other metabolite from the biotransformation of **33** could be readily isolated. Control experiments conducted in parallel wherein dihydromonacolin L (**11**) was added to *A. terreus lovC* cultures (inoculated from the same batch of spores) resulted in the isolation of lovastatin (**1**).



Monacolin N (**33**) Lovastatin analouge (**62**)

Scheme 30. Attempted biotransformation of monacolin N (**33**)

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Apparently the extra double bond present in the linker arm of **33** changes the molecule's shape sufficiently that it is no longer an acceptable substrate for the post-PKS elaboration enzymes. It is possible that the biotransformation is occurring, but is producing **62** at a level below our detection limits. In order to determine this, the experiment would need to be repeated with radiolabeled **33** followed by isotope dilution analysis.

2.7 Summary and future work

This thesis presents work done to examine both the assembly (PKS) and elaboration (post-PKS) steps involved in the biosynthesis of lovastatin (**1**). A synthetic strategy that provides putative late stage polyketide intermediates such as octaketide **24**, heptaketide **25** and hexaketide **29** has been developed. Starting from the natural product, dihydromonacolin L (**11**), a series of nine synthetic steps gives hexaketide **29** with a 1% overall yield. The somewhat unusual feature of this synthetic strategy is the use of a dibromide to protect a double bond from cleavage by ozonolysis. Compounds **24** and **25** will prove valuable in investigating the individual steps and the intermediates involved in the assembly of **11** from acetate and malonate units. Studies to determine intact incorporation are currently underway.

In addition a new compound monacolin N (**33**) has been isolated from fermentation cultures of *A. nidulans* expressing the lovastatin biosynthesis genes *lovB* and *lovC*. Monacolin N (**33**) has an additional double bond in the linker arm between the upper lactone and lower decalin ring system that is not present in the related

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

dihydromonacolin L (**11**). The presence of this double bond seems to indicate a failure of the enoyl reductase activity of the LovC enzyme, which is tolerated by LovB as the chain is fully (and properly) extended to the nonaketide. The implications of this with respect to lovastatin biosynthesis and the role of both LovB and LovC enzyme have been discussed.

Also presented are some recent efforts towards the development of a synthetic precursor for the upper lactone ring of the statins. The synthesis of a phosphonate precursor **42** that can be used in a coupling with an appropriate aldehyde is described and improvements in the synthesis have been reported. The elimination of several steps from the previously reported synthesis has streamlined the process.

Unsuccessful efforts towards the synthesis of monacolin L (**18**), a proposed intermediate in the biosynthesis of lovastatin (**1**), have been described. Attempts to dehydrogenate dihydromonacolin L (**11**) through the use of a dibromide intermediate did not result in the formation of the desired product. Attempts to deoxygenate monacolin J (**12**) resulted in the formation of the undesired aromatized product (**49**).

A series of biotransformation experiments aimed at probing the role of both the *lovA* and *lovC* gene have been done. Lovastatin deficient mutant, *A. terreus lovA*, can convert monacolin J (**12**) to lovastatin (**1**), but it does not accumulate dihydromonacolin L (**11**). It was found that *A. terreus lovA* converts **11** to the oxidation product **51**, and the proposed pathway responsible for this conversion has been presented. An additional lovastatin (**1**) deficient mutant, *A. terreus lovC*, can convert both **11** and **12** to **1**, and has so far demonstrated an ability to transform analogues such as desmethylmonacolin J (**55**)

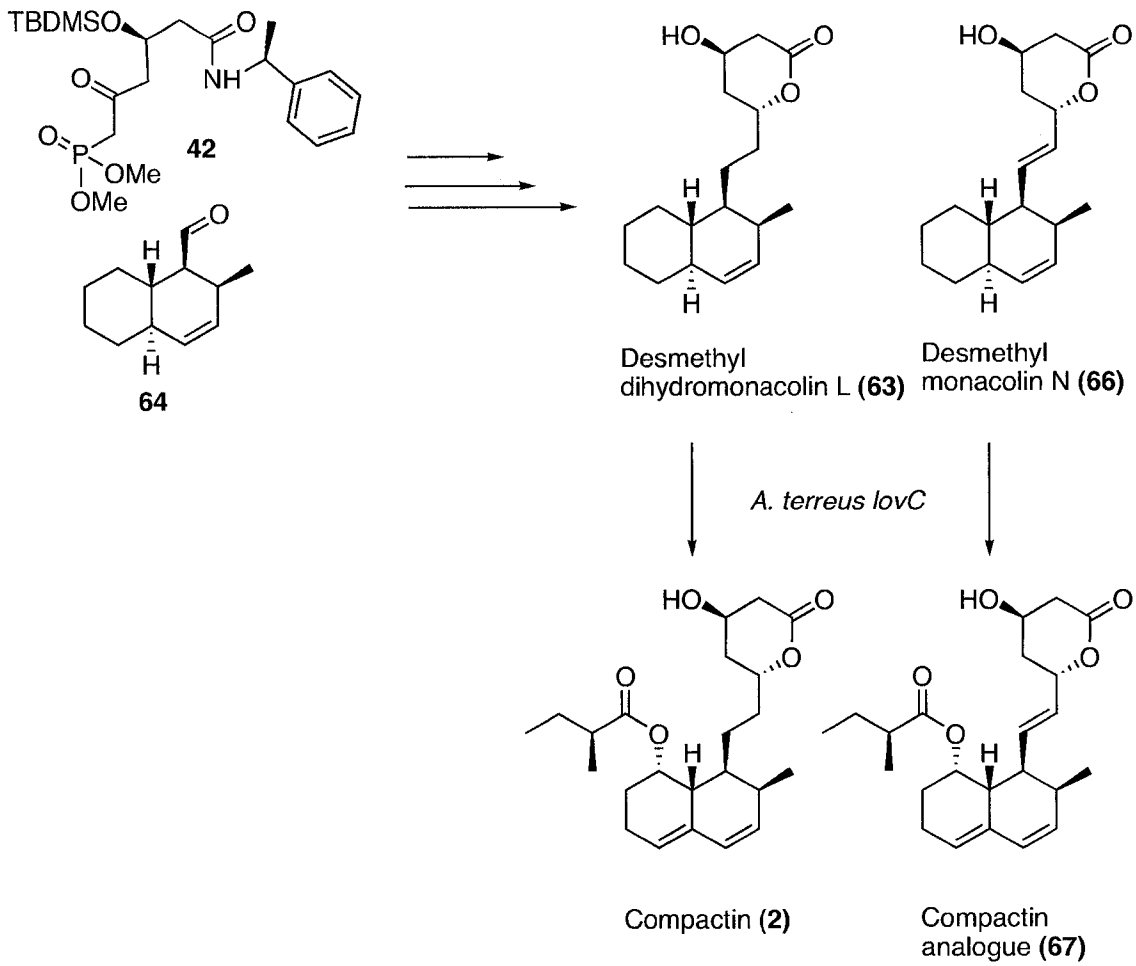
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

to compactin (**2**). Conversion of analogues such as monacolin N (**33**) to lovastatin derivatives such as **62** has not yet been detected.

The future of this project could proceed in several directions. It remains to be seen if any of the polyketide precursors synthesized in this project can be converted into dihydromonacolin L (**11**) by LovB and LovC. It may also be possible to observe monacolin N (**33**) production when the hexaketide fragment is incubated with LovB alone. This would demonstrate that only LovB is necessary for the final three assembly steps.

This project has also laid the framework for the combination of the synthesis of the statin upper ring precursor with biotransformation experiments involving *A. terreus lovC*. Although it has been shown that *A. terreus lovC* can transform desmethylmonacolin J (**55**) to compactin (**2**), it remains to be seen if the desmethyl analogue of dihydromonacolin L (**63**) can be transformed into compactin (**2**) (Scheme 31). Combining the statin upper ring precursor **63**, described in Section 2.4, and the synthesis of the desmethyl hexaketide analogue **64** recently completed by Mr. Jamie Côté (Scheme 31) should allow formation of **63** and **66**. These compounds may be transformed into compactin (**2**) or **67**. In addition, this route could provide acess to a synthesis of **11** and **33** by combining **42** with the hexaketide aldehyde **32**.

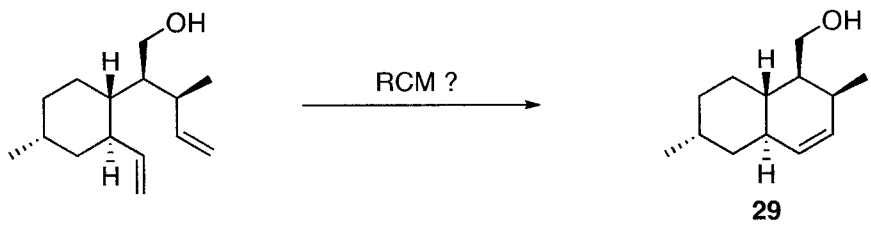
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



Scheme 31. Proposed synthesis and biotransformation of desmethyl analogues **63** and **64**.

A key synthetic challenge would be to develop a more efficient synthesis of dihydromonacolin L (**11**) and the hexaketide **29**. Although this thesis reports a synthesis of **11**, the starting material is available from fermentation cultures and takes considerable effort to isolate and purify. There has been one other reported synthesis of a hexaketide fragment like **29** which involves over 14 chemical steps with a ca. 1% total yield.[103] The aspect that presents a synthetic challenge is that both methyl groups are axial. Perhaps one approach may be to close the decalin ring in the last step by forming the double bond using olefin metathesis (Scheme 32).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



29

Scheme 32. Proposal for the synthesis of the hexaketide fragment **29**.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

3. Experimental Procedures

3.1 General Experimental Methods

3.1.1 Reagents, solvents and solutions

All chemicals were purchased from Aldrich Chemical Company, Inc., Madison WI, or Sigma Chemical Company, St. Louis, MO. Compounds containing stable isotopes (i.e ^{13}C) were purchased from Cambridge Isotope Laboratories Inc., Andover MA. All non-aqueous reactions involving air or moisture sensitive reactants were done under a positive pressure of dry argon in oven-dried glassware. Reagents and solvents were reagent grade and used as such. Tetrahydrofuran was dried by distillation from sodium and benzophenone under dry argon atmosphere. Toluene was dried by distillation from molten sodium under argon. Dichloromethane, pyridine, chloroform and triethylamine were distilled from calcium hydride under an atmosphere of dry argon. Diazomethane (CH_2N_2) was prepared as a diethyl ether solution from Diazald (*N*-methyl-*N*-nitroso-*p*-toluenesulfonamide) according to Aldrich technical bulletin AL-113. Evaporation refers to removal of volatile solvent by reduced pressure at 30 °C using a rotary evaporator. Unless otherwise specified, solutions of NH_4Cl, $NaHCO_3$, HCl, NaOH, and KOH refer to aqueous solutions. Brine refers to a saturated solution of NaCl.

3.1.2 Purification techniques

Analytical TLC was done on glass plates (1.5 cm × 5 cm) precoated (0.25 mm) with silica gel (Merck, Silica Gel 60 F_{254}). Compounds were visualized by exposure to UV light and by dipping the plates in a solution of 1% $Ce(SO_4)_2 \cdot 4\ H_2O$ and 2.5% $(NH_4)_2Mo_7O_{24} \cdot 4\ H_2O$ in 10% H_2SO_4 followed by heating on a hot plate. Flash column

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

chromatography was done according to Still[104] employing silica gel grade 60 (Rose Scientific, 230-400 mesh).

3.1.3 Instrumentation for compound characterization

Preparative HPLC was performed on a RANIN Dynamax high performance liquid chromatograph employing a manual injector, fraction collector and single wavelength detector. Reversed phase HPLC was conducted utilizing a Waters radial compression module with a Bondapak C_{18} 25 × 100 mm column (15-20 μm particle size) with a guard column of similar packing. Mobile phase for RP-HPLC typically consisted of 70% H_2O – 30% CH_3CN to 100% CH_3CN over 25 min, linear gradient. Normal phase HPLC was done on the same RANIN system using a Waters radial compression Porasil silica gel column (125 Å, 25 × 100 mm). NP-HPLC mobile phase employed was 30% EtOAc – 70% hexane to 70% EtOAc – 30% hexane over 27 min.

NMR spectra were recorded on a Varian Inova 600, Inova 500, Inova 300 or Unity 500 spectrometer. For ^1H (300, 500 or 600 MHz) δ values were referenced to $CHCl_3$ (7.24 ppm), and for ^{13}C (125.8 or 150.9 MHz) δ values were referenced to $CDCl_3$ (77.0 ppm). First-order behaviour was assumed in all analysis. Additional assignments were done using pulsed field gradient versions of shift correlation spectroscopy (gCOSY), heteronuclear multiple quantum coherence (gHMQC), and heteronuclear multiple bond correlation (gHMBC).

Melting points are uncorrected and were determined on a Kauffmann Block microscope or a Thomas-Hoover apparatus using open capillary tubes. Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a micro cell (100 mm path length 1 ml). IR spectra were recorded on a Nicolet Magna-IR 750 with Nic-Plan microscope FT-

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

IR spectrometer. Mass spectra were recorded on a Krator AEI MS–50 (HREIMS) and a ZabSpec Isomass VG (HRESMS).

Radioactivity was determined using standard liquid scintillation procedures in plastic 10 mL scintillation vials with Beckman Ready-gel scintillation cocktail. The scintillation counter used was a Beckman LS 5000TD with automatic quench control to directly determine decompositions per minute (dpm) in the labelled samples against a quench curve prepared from Beckman ^{14}C quenched standards. Radiolabelled compounds were also detected by a TLC assay, using a Berthold LB2760 2D-TLC scanner.

3.1.4 Biological media preparations

The water used for biological media preparations was purified on an ELGA Maxima model water purifier, and had a resistivity of 18 MΩ or greater.

Aspergillus nidulans growth medium was prepared by dissolving 20 g of glucose, 20 g of yeast extract, 1 g of peptone and 1 mL of a 1 mg/mL solution of para–aminobenzoic acid in 1 L of distilled deionized water. *A. nidulans* production medium was prepared by dissolving in 1 L of distilled deionized water 1 mL trace elements solution (1.0 g $FeSO_4 \cdot 7\ H_20$, 8.8 g $ZnSO_4 \cdot 7\ H_2O$, 0.4 g $CuSO_4 \cdot 5\ H_20$, 0.15 g $MnSO_4 \cdot 4\ H_2O$, 0.1 g $Na_2B_7O_7 \cdot 10\ H_2O$, 0.05 g $(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$, 0.5 mL conc. HCl dissolved in 1L H_2O), 100 mL of 10 × AMM salts (60 g $NaNO_3$, 5.2 g KCl, 15.2 g KH_2PO_4 dissolved in 1 L of H_2O and adjusted to pH 6.5), 1 ml of a 1 mg/mL para-aminobenzoic acid solution, and 0.9 mL of cyclopentanone and autoclaving. After cooling to room temperature 2.5 mL of a sterile solution of 20 % $MgSO_4 \cdot 7H_2O$ and 25 mL of a sterile 40 % lactose solution were added to the production media.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Spores of *A. nidulans lovB+lovC* were propagated on potato dextrose agar (containing 0.1 g L^{-1} streptomycin sulfate) by spotting an aqueous suspension of spores using sterile toothpicks. Plates were maintained for 14 d in the dark at 30 °C. Spores were harvested by suspending them in sterile water (10 mL per plate) and rubbing with a flame sterilized glass rod. Spores were collected by centrifugation at 3500 rpm and stored at –20 °C as a concentrated aqueous suspension.

Aspergillus terreus growth medium consists of 2 g Hunts' tomato paste, 0.5 g oat flour, 0.5 g glucose, 0.25 g corn steep liquor, and 0.5 mL trace elements all dissolved in 50 mL H_2O and autoclaved. The trace elements solution consisted of 19 mg $(NH_3)_6Mo_7O_{24} \cdot 4\ H_2O$, 25 mg $CuCl_2 \cdot 2\ H_2O$, 56 mg H_3BO_3, 76 mg $CaCl_2$, 200 mg $ZnSO_4 \cdot 7\ H_2O$, 670 mg $MnSO_4 \cdot H_2O$ and 1 g $FeSO_4$ dissolved in 1 L of water. *A. terreus* production media consists of 45 g glucose, 24 g peptonized milk, 2.5 g yeast extract and 2.5 g PEG 2000 dissolved in 1 L of water and autoclaved. *Penicillium aurantiogrisem* growth medium consists of 0.25 g corn steep solids, 1.0 g Pablum, 1.5 g glucose, 2.0 mg trace elements (as for *A. terreus*) and 4.2 g Hunts' tomato paste dissolved in 100 mL water and autoclaved. *P. aurantiogriseum* production medium consists of 24.5 g succinic acid, 2.0 g peptone, 10 g glucose, 60 g malt extract dissolved in 2 L of water and autoclaved. All biological media solutions and equipment were autoclaved at 121 °C and 15 psi of steam pressure for 15 min.

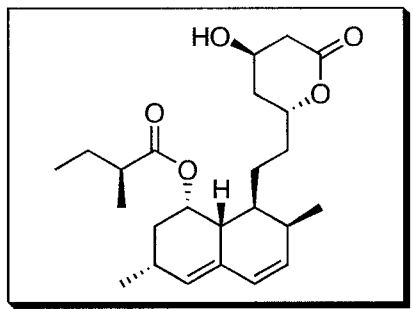
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

3.2 Synthesis and Characterization of Compounds

Lovastatin (1)

[1*S*-[1α(*R**),3α,7β,8β(2*S**,4*S**),8aβ]]-1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran2-yl)ethyl]-1-naphthalenyl-2-methylbutanoate]

CAS Registry Number [75330-75-5]



Spores of *Aspergillus terreus* were used to inoculate 50 mL of growth media in a 125 mL Erlenmeyer flask. After shaking at 200 rpm and 30 °C for 48 hr, the contents of the flask were transferred to 1 L of production media in a 2 L Erlenmeyer flask. After 7 days shaking at 200 rpm and 30 °C, the culture was harvested by vacuum filtration to remove the mycelia. The aqueous broth was acidified with conc HCl (pH < 2) and extracted with 1.5 L CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), filtered and evaporated to give 2.3 g crude extract. This residue was suspended in 50 mL toluene and held at reflux with a CaH_2 Soxhlet for 1 h to remove H_2O. The crude lactonized extract (2.4 g) was fractionated by flash column chromatography to give 18.0 mg pure lovastatin (**1**). The spectral data for this isolated sample matched in every way with a standard sample. The complete characterization data for lovastatin (**1**) is reproduced below, with permission, from the Ph.D. dissertation of Karine Auclair.[62]

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

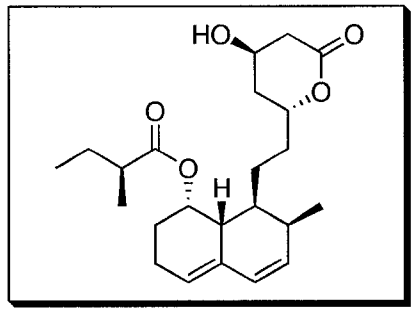
$[\alpha]_D^{25}$ = +314 (c 1.8, CH_3CN), lit. $[\alpha]_D^{25}$ = +323 (c 0.84, CH_3CN); UV (MeOH soln) λmax 238, 204 nm; FTIR ($CHCl_3$, cast) 3435 (br m), 2963 (m), 1725 (s), 1258 (m), 1187 (m) cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 5.94 (d, 1H, *J* = 9.7 Hz, H-4), 5.74 (dd, 1H, *J* = 9.7, 6.0 Hz, H-3), 5.47 (m, 1H, H-5), 5.34 (ddd, 1H, *J* = 3.1, 3.0, 3.1 Hz, H-8), 4.59 (m, 1H, H-11), 4.31 (m, 1H, H-13), 3.29 (br s, 1H, OH), 2.67 (dd, 1H, *J* = 17.7, 5.0 Hz, H_a-14), 2.59 (dd, 1H, *J* = 17.7, 2.4 Hz, Hb-14), 2.40 (m, 1H, H-6), 2.36–2.27 (m, 2H, H-2, H-2'), 2.23 (dd, 1H, *J* = 12.0, 3.1 Hz, H-8a), 1.96 – 1.77 (m, 4H, H-7, Ha-10, Ha-12), 1.68 – 1.57 (m, 3H, H-1, H_a-3', H_b-12), 1.49 – 130 (m, 3H, H-9, H_b-3'), 1.25 (m, 1H, H_b-10), 1.06 (d, 3H, *J* = 7.0 Hz, 2'-Me), 1.03 (d, 3H, *J* = 7.3 Hz, 6-Me), 0.85 (d, 3H, *J* = 7.0 Hz, 2-Me), 0.84 (t, 3H, *J* = 7.5 Hz, H-4'); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.8 (C-1'), 170.8 (C-15), 133.0 (C-3), 131.5 (C-4a), 129.5 (C-5), 128.2 (C-4), 76.5 (C-11), 67.9 (C-8), 62.4 (C-13), 41.4 (C-2'), 38.5 (C-14), 37.2 (C-8a), 36.5 (C-1), 36.0 (C-12), 32.9 (C-10) 32.6 (C-7), 30.6 (C-2), 27.4 (C-6), 26.7 (C-3'), 24.2 (C-9), 22.8 (6-Me), 16.2 (2'-Me), 13.8 (2-Me), 11.6 (C-4'); HRESMS $[M+H]^+$ = 405.2683 (405.2641 calcd for $C_{24}H_{36}O_5$ + H).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Compactin (2)

(1*S*-(1*R*,7*S*,8*S*(2*S*,4*S*),8a*S*))-(1,2,3,7,8,8a-hexahydro-7-methyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl) ethyl]-1-naphthyl-2-methylbutanoate

CAS Registry Number [73573-88-3]



A concentrated spore suspension (10 μL) of *Penicillium aurantiogriseum* was used to inoculate 100 mL of growth media in a 250 mL Erlenmeyer flask. After maintaining at 30 °C with shaking at 200 rpm for 48 h the contents of the flask was transferred to 1 L of production media in a 2 L Erlenmeyer flask. This culture was maintained at 30 °C with shaking at 200 rpm for 7 days at which point it was harvested by vacuum filtration to remove mycelia. The aqueous broth was acidified with 2N HCl (pH < 2) and extracted with 1.5 L of CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), filtered and evaporated to give 45 mg crude extract. This extract was suspended in 50 mL toluene and held at reflux with a CaH_2 Soxhlet for one hour. The crude lactonized extract (44.1 mg) was fractionated by flash column chromatography to give 10.2 mg compactin (**2**). The spectral data for this isolated compound matched in every way with a standard sample. The complete characterization data for compactin (**2**) is reproduced below, with permission, from the Ph.D. thesis of Karine Auclair.[62]

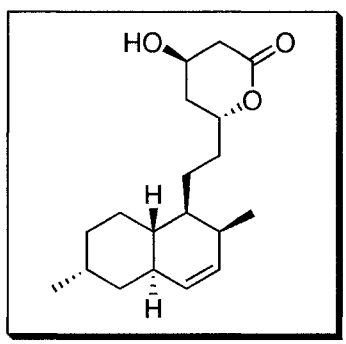
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

$[\alpha]_D^{25}$ = +158 (c 0.5, $CHCl_3$), lit. $[\alpha]_D^{25}$ = +283 (*c* 0.84, acetone), FTIR ($CHCl_3$, cast) 3407 (br w), 2925 (m), 1725 (s), 1251 (m), 1077 (m) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.96 (d, 1H, *J* = 9.6 Hz, H-4), 5.72 (dd, 1H, *J* = 9.6, 6.0 Hz, H-3), 5.54 (br d, 1H, H-5), 5.32 (br t, 1H, *J* = 4.3 Hz, H-8), 4.60 (m, 1H, H-11), 3.86 m, (1H, H-13), 2.72 (dd, 1H, *J* = 17.6, 5.2 Hz, Ha-14), 2.60 (ddd, 1H, *J* = 17.6, 4.0, 1.5 Hz, Hb-14), 2.36 (m, 3H, H-2, H-6), 2.31 (m, 1H, H-8a), 2.13 (m, 4H, H-1, H-7, OH), 1.95 (m, 2H, H-12), 1.45 – 1.24 (m, 6H, H-3', H-9, H-10), 1.11 (d, 3H, *J* = 7.0 Hz, 2-Me), 0.88 (m, 6H, 2-Me, 4'-Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.9 (C-1'), 170.5 (C-15), 133.7 (C-4a), 132.8 (C-3), 128.3 (C-4), 123.8 (C-5), 76.3 (C-11), 67.7 (C-8), 62.7 (C-13), 41.8 (C-6), 38.7 (C-2'), 37.6 (C-14), 36.9 (C-8a), 26.3 (C-12), 33.1 (C-10), 31.0 (C-2), 26.8 (C-3'), 26.3 (C-7), 24.1 (C-9), 21.0 (C-1), 17.0 (2'-Me), 13.9 (2-Me), 11.8 (4'-Me); HREIMS $[M]^+$ = 390.2409 (390.2406 calcd for $C_{23}H_{34}O_5$), 270.2 (8%), 210.1 (16%), 184.1 (60%), 145.1 (87%), 57.1 (100%).

Dihydromonacolin L (11)

4*R*-tetrahydro-4-hydroxy-6-[2-((1*S*,2*S*,4a*R*,6*R*,8a*R*)-1,2,4a,5,6,7,8,8a-octahydro-2,6-dimethyl-1-naphthalenyl)ethyl]-2H-pyran-2-one

CAS Registry Number [86827-77-2]



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Dihydromonacolin L (**11**) was isolated from a strain of *Aspergillus nidulans* expressing the lovastatin *lovB* and *lovC* biosynthesis genes.[37] The typical isolation procedure is described below. A concentrated spore suspension (10 μL) was used to inoculate 1 L of *A. nidulans* growth media and the culture was incubated at 30 °C and 200 rpm for 4 days. The mycelia were harvested by filtering through sterile miracloth (Calbiochem) and were thoroughly rinsed with 2 L of a 1 % lactose solution. The wet mycelia were divided into 4 equal portions and used to inoculate 4 × 1 L of *A. nidulans* production media. Cultures were incubated at 30 °C and 200 rpm for 2 days, after which point the growth was supplemented with 1 g L^{-1} sodium acetate added each day for an additional 5 days. The cultures were harvested by removing the mycelia by vacuum filtration, and the combined broth (4 L) was acidified with 2 N HCl (pH < 2) and extracted with CH_2Cl_2 (2 × 1.5 L). The combined organic layers were dried (Na_2SO_4), filtered and evaporated to provide ca. 500 mg of crude extract, which was fractionated by flash column chromatography to provide 300 mg of pure dihydromonacolin L (**11**). (Typical yields, 50 – 70 mg L^{-1} of production media).

[^{14}C]–Dihydromonacolin L (**11**) was produced from 1 L of *A. nidulans lovB* + *lovC* production culture fermented as described above, except that 250 μCi of sodium [1–^{14}C] acetate (fed as a 5 mL aqueous solution 4 times per day) was added in addition to the unlabelled sodium acetate. Culture was harvested and extracted as above and the crude extract (97 mg) was fractionated by preparative TLC to give 22.5 mg of pure [^{14}C]–dihydromonacolin L (**11**) with a specific activity of 0.185 μCi mg^{-1}. The ^{1}H NMR of the [^{14}C]–dihydromonacolin L (**11**) was identical to that of the unlabelled compound.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

For unlabelled **11**: mp 162-163°C, lit mp 163-164 °C[21]; $[\alpha]_D^{25}$ = + 115 (*c* 0.22, methanol), lit. $[\alpha]_D^{25}$ = + 123.9 (*c* 0.5, methanol); UV (MeCN solution) λmax 228 nm (br 210-234); R_f = 0.5 (EtOAc 100%); FTIR (microscope) 3391 (br m), 3018 (w), 2910 (s), 1713 (s), 1254 (s), 1062 (s), 1047 (s) cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 5.57 (ddd, 1H, *J* = 9.8, 4.9, 2.7 Hz, H-3), 5.28 (d, 1H, *J* = 9.8 Hz, H-4), 4.67 (m, 1H, H-11), 4.38 (m, 1H, H-13), 2.72 (dd, 1H, *J* = 17.6, 5.13 Hz, H-14), 2.60 (ddd, 1H, *J* = 17.6, 3.7, 1.6 Hz, H-14), 2.21 (m, 1H, H-2), 2.00 (m, 1H, H-6), 1.95 (m, 1H, H-12), 1.89 (m, 1H, H-4a), 1.81-1.73 (m, 2H, H-10 and H-12), 1.62 (m, 2H, H-9), 1.58-1.42 (m, 5H, H-5, H-7, H-8a and H-10), 1.07 (dq, 1H, *J* = 11.9, 3.7 Hz, H-8), 0.97 (dq, 1H, *J* = 11.9, 3.0 Hz, H-8), 0.96 (d, 3H, *J* = 7.3 Hz, 6-CH_3), 0.82 (d, 3H, *J* = 6.9 Hz, 2-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4 (C-15), 132.6 (C-3), 131.6 (C-4), 76.1 (C-11), 62.8 (C-13), 41.4 (C-8a), 40.0 (C-8), 38.9 (C-5), 38.7 (C-14), 37.3 (C-4a), 36.1 (C-12), 33.2 (C-10), 32.3 (C-7), 32.0 (C-2), 27.5 (C-6), 23.7 (C-1), 23.6 (C-9), 18.2 (6-CH_3), 14.9 (2-CH_3); HREIMS $[M]^+$ = 306.2190 (306.2195 calcd for $C_{19}H_{30}O_3$), (M^+, 27%), 288.2 (18%), 161.1 (100%), 105.1 (99%).

Crystal data for dihydromonacolin L (11)

This experiment was done by Dr. Robert MacDonald of the University of Alberta X-ray crystallography laboratory. Data were acquired on a Bruker P4/RA/SMART 1000 CCD diffractometer. All intensity measurements were performed using graphite monochromated Mo-Kα radiation (λ = 0.71073 Å). 4a,5–Dihydromonacolin L (**11**) ($C_{19}H_{30}O_3$) was obtained as white crystals, space group $P2_12_12_1$ (No. 19), *a* = 5.5947 (9), *b* = 9.6504 (17), *c* = 31.930 (6) Å, *V* = 1723.9 (5) $Å^3$, *Z* = 4, *T* = -80°C, ρ_{calcd} = 1.181 g

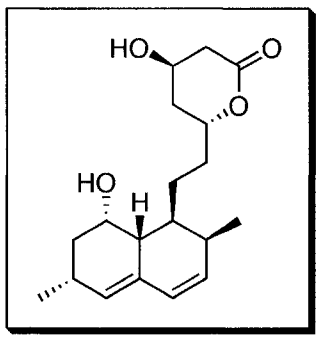
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

cm^{-3}, μ = 0.078 mm^{-1} 10870 reflections measured, 3287 unique which were used in all least squares calculations, $R_1(F)$ = 0.0620 (for 899 reflections with $F_o^2 \geq 2\sigma\ (F_o^2)$), $wR_2(F^2)$ = 0.2159 for all unique reflections). Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Center.

Monacolin J (12)

4*R*-tetrahydro-4-hydroxy-6-[2-((1*S*,2*S*,6*S*,8*S*,8a*R*)-1,2,6,7,8,8a-hexahydro-8-hydroxy-2,6-dimethyl-1-naphthalenyl)ethyl]-2H-pyran-2-one

CAS Registry Number [79952-42-4]



Monacolin J (**12**) was produced by the basic hydrolysis of lovastatin (**1**) as follows. Lovastatin (**1**) (200 mg) was suspended in 10 mL of aqueous 1 N LiOH and heated at reflux overnight. The reaction was cooled to room temperature, acidified with 2 N HCl (pH < 2) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were dried (Na_2SO_4) filtered and evaporated to give 243 mg of crude residue. This was dissolved in 25 mL toluene and heated at reflux with a CaH_2 Soxhlet for one hour. After cooling to room temperature, the solvent was evaporated and the residue (208 mg) was

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

fractionated by flash column chromatography (40 % EtOAc in hexanes) to give 140 mg (89 % yield) pure monacolin J (**12**).

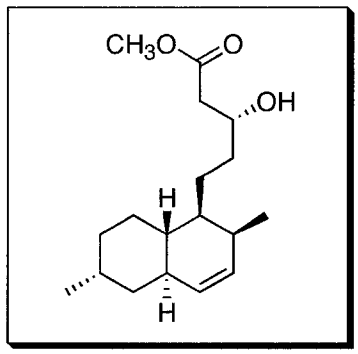
Monacolin J (**12**) was also isolated from *A. terreus* fermentation cultures grown as described for the isolation of lovastatin. The more polar fractions of the flash column separation (40% EtOAc in hexane) gave 10.0 mg pure **12**.

$[\alpha]_D^{25}$ = +17.3 (c 0.14, CH_3OH); R_f = 0.25 (EtOAc 100%) IR (microscope) 3236 (br s), 2927 (s), 2879 (s), 1707 (m), 1645 (m), 1451 (s), 1318 (s), 1093 (s), 1075 (s), 1060 (s), 1049 (s), 1026 (s), 973 (s), 858 (s) cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 5.93 (d, 1H, *J* = 10.0 Hz, H-4), 5.75 (dd, 1H, *J* = 10.0, 6.2 Hz, H-3), 5.45 (br s, 1H, H-5), 4.23 (br dd, 1H, *J* = 6.5, 2.8 Hz, H-8), 3.92 (m, 1H, H-11), 3.79 (m, 1H, H-13), 3.68 (br t, 2H, *J* = 6.5 Hz, H-14), 2.38 (m, 2H, H-2, H-6), 2.13 (br dd, 1H, H-12), 1.92–1.68 (m, 4H, H-7, H_a-9, H_a-12), 1.67–1.50 (m, 3H, H-1, H_a-10, H_b-12), 1.41 (m, 1H, H_b-10), 1.31 (m, 1H, H-9), 1.18 (d, 3H, *J* = 7.4 Hz, 6-Me), 0.89 (d, 3H, *J* = 6.9 Hz, 2-Me); ^{13}C NMR (125 MHz, CD_3OD) δ 190.0 (C-15), 134.1 (C-4), 133.2 (C-4_a), 130.6 (C-3) 130.0 (C-5), 71.7 (C-8), 69.2 (C-11), 65.9 (C-13), 60.1 (C-14), 45.4 (C-7), 41.0 (C-10), 39.8 (C-8_a), 37.6 (C-1), 37.1 (C-12), 35.6 (C-9), 32.1, 29.1 (C-2, C-6), 23.65 (6-Me), 14.3 (2-Me); HREIMS $[M]^+$ 320.1983 (320.1988 calcd. for $C_{19}H_{28}O_4$) 320.2 (2%), 302.2 (9%), 198.1 (58%), 157.1 (100%), 105.1 (41%).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Ocatketide Methyl Ester 20

Methyl 3*R*-5-((1*S*,2*S*,4a*R*,6*R*,8a*R*)-1,2,4a,5,6,7,8,8a-octahydro-2,6-dimethyl-naphthalen-1-yl)-3-hydroxypentanoate



A sample of 259 mg (0.63 mmol) octaketide dibromide **23** was dissolved in 25 mL CH_2Cl_2. To this solution was added 124 mg (1.89 mmol) Zn dust and 2.5 mL glacial acetic acid and the reaction was stirred overnight at rt. The reaction mixture was filtered and diluted with 50 mL CH_2Cl_2 and washed with satd $NaHCO_3$ (1 × 50 mL) and water (1 × 50 mL). The organic layer was dried (Na_2SO_4), evaporated and purified by flash column chromatography (5% ethyl acetate in hexane) to give **20** (142 mg, 90%) as a clear oil.

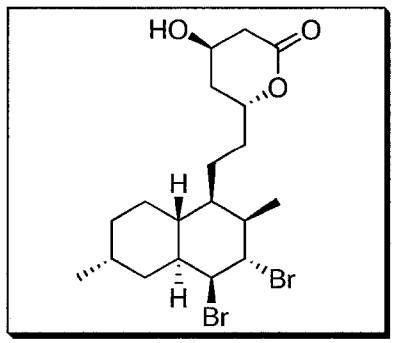
$[\alpha]^{20}_D$ = +73.8 (*c* 0.10, $CHCl_3$); R_f = 0.60 (50% EtOAc in hexane); FTIR ($CHCl_3$ cast) 3413, 3007, 2955, 2916, 1740, 1437, 1378, 1288, 1247, 1195, 1170, 984, 865, 777, 723 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 5.56 (ddd, 1H, *J* = 9.6, 4.8, 3.0 Hz H-3), 5.27 (d, 1H, *J* = 9.6 Hz, H-4), 3.64 (s, 3H, OCH_3), 2.37 (ddd, 1H, *J* = 15.6, 10.2, 5.4 Hz, Ha-2'), 2.20 (m, 1H, H-2), 2.18 (m, 1H, Hb-2'), 1.99 (m, 1H, H-6), 1.95-1.86 (m, 2H, H-4a and Ha-1'), 1.60-1.46 (m, 5H, H-1, 7 and Ha-5, 8), 1.34 (m, 1H, Hb-1'), 1.24 (dt, 1H, *J* =

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

13.2, 4.8 Hz, Hb-5), 1.09 (dq, 1H, J = 12.6, 4.2 Hz, Hb-8), 1.00-0.95 (m, 1H, H-8a), 0.96 (d, 3H, J = 7.2 Hz, 6-CH_3), 0.82 (d, 3H, J = 6.6 Hz, 2-CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.3 (C-3'), 132.4 (C-3), 131.5 (C-4), 68.2 (OCH_3), 51.5 (C-11), 41.2 (C-1), 40.0 (C-8a), 39.0 (C-5), 37.3 (C-4a), 34.1 (C-12), 32.3 (C-7), 32.1 (C-10), 31.9 (C-2), 27.5 (C-6), 24.0 (C-9), 23.7 (C-8), 18.3 (6-CH_3), 15.0 (2-CH_3); HREIMS $[M]^+$ = 294.2202 (294.2195 calcd for $C_{18}H_{30}O_3$) 294.2 (25%), 276.2 (20%), 202.2 (41%), 176.1 (100%), 163.1 (89%), 161.1 (50%).

Dihydromonacolin L dibromide 21

[4*R*-tetrahydro-4-hydroxy-6-[2-(1*R*,2*R*,3*S*,4*S*,4a*S*,6*R*,8a*R*-decahydro-3,4-dibromo-2,6-dimethyl-1-naphthalenyl)ethyl]-2H-pyran-2one]



A sample of 50 mg (0.163 mmol) of dihydromonacolin L (**11**) was dissolved in 2 mL of $CHCl_3$. To this solution was added 0.25 ml glacial acetic acid and 12.4 μL (39.2 mg, 0.245 mmol, 1.5 eq) elemental bromine. The reaction was stirred at room temperature for 1 h at which point 10 mL of a 0.25 M soln. of $Na_2S_2O_4$ was added, and the mixture was stirred vigorously for 10 min. It was then diluted with 30 mL of CH_2Cl_2 and placed in a separatory funnel. The organic layer was separated and washed with satd

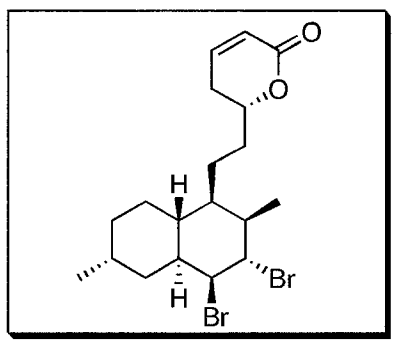
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

$NaHCO_3$ (1 × 10 mL), H_2O (1 × 10 mL) and brine (1 × 10 mL). The organic phase was then dried (Na_2SO_4), filtered and evaporated to give ca. 75 mg of crude product. This was purified by flash column chromatography (40% EtOAc in hexane) to give 70 mg (88%) pure dihydromonacolin L dibromide (**21**).

$[\alpha]_D^{25}$ = + 40.2 (c = 0.43, $CHCl_3$); Rf = 0.50 (EtOAc 100%); FTIR ($CHCl_3$ cast) 3431 (br s), 2924 (s), 1713 (s), 1382 (s), 1175 (m), 1070 (m), 755 (m), 616 (w) cm^{-1} ; ^1H NMR (500 MHZ, $CDCl_3$) δ 4.94 (m, 1H), 4.64 (ddd, 1H, *J* = 11.1, 7.5, 3.7 Hz), 4.44 (m, 1H), 4.37 (m, 1H), 2.72 (dd, 1H, *J* = 17.5, 5.0 Hz), 2.60 (ddd, 1H, *J* = 17.5, 3.5, 1.5 Hz), 2.36 – 2.29 (m, 1H), 2.16 – 2.09 (m, 1H), 2.06 – 2.00 (m, 2H), 1.96 – 1.85 (m, 2H), 1.78 – 1.62 (m, 4H), 1.59 – 1.48 (m, 2H), 1.38 – 1.47 (m, 2H), 1.30 – 1.10 (m, 4H), 1.26 (d, 3H, *J* = 7.8 Hz), 1.00 (d, 3H, *J* = 7.2 Hz); ^{13}C NMR (125 MHZ, $CDCl_3$) δ 170.2, 75.7, 62.8, 60.23, 60.0, 39.5, 38.8, 38.6, 38.3, 36.2, 34.9, 34.7, 32.7, 31.1, 26.9, 23.6, 23.5, 17.9, 17.6; HRESMS $[M+Na]^+$ = 487.04569 (487.04594 cacld for $C_{19}H_{30}O_3Br_2$ + Na).

α–β-Unsaturated dihydromonacolin L dibromide 22

6-[2-((1*R*,2*R*,3*S*,4*S*,4a*S*,6*R*,8a*R*)-decahydro-3,4-dibromo-2,6-dimethyl-1-naphthalenyl)ethyl]-5,6-dihydro-pyran-2-one



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

A sample of 152 mg (0.327 mmol) dihydromonacolin L dibromide (**21**) was dissolved in 5 ml of CH_2Cl_2 and cooled to 0 °C. To this stirred solution was added 114 μL (82.8 mg, 0.819 mmol, 2.5 eq) triethylamine followed by 28.4 μL (41.2 mg, 0.360 mmol, 1.1 eq) methanesulfonyl chloride. The mixture was allowed to warm to rt and stirred for 1 h, at which point the reaction was quenched with 10 mL of 1N HCl and stirred vigorously for 10 min. The organic layer was diluted with 20 mL CH_2Cl_2, placed in a separatory funnel and the layers separated. The organic layer was washed with H_2O (1 × 10 mL), satd $NaHCO_3$ (1 × 10 mL), H_2O (1 × 10 mL), brine (1 × 10 mL). It was then dried (Na_2SO_4), filtered and evaporated to give 148 mg of crude product. The residue was purified by flash column chromatography (20% EtOAc in hexane) to give 98.6 mg (68%) of pure **22**.

$[\alpha]_D^{25}$ = + 5.5 (*c* 1.58, $CHCl_3$); R_f = 0.75 (EtOAc 100%); FTIR 2924 (s), 2851 (s), 1719 (s), 1383 (m), 1244 (s), 1024 (m), 817 (m), 615 (m) cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 6.85 (ddd, 1H, *J* = 9.9, 4.4, 4.2 Hz), 4.94 (brs, 1H), 4.44 (brs, 1H), 4.41 – 4.37 (m, 1H) 2.35 – 2.31 (m, 3H) 2.12 (dd, 1H, *J* = 11.0, 3.3 Hz), 2.06–2.02 (m, 1H), 1.92 (tt, 1H, *J* = 11.0, 3.9 Hz) 1.78 (m, 4H), 1.53 (m, 3H), 1.40 (dd, 1H, *J* = 11.0, 4.0 Hz) 1.34 – 1.30 (m, 1H), 1.27 (d, 3H, *J* = 7.9 Hz), 1.23 – 1.20 (m, 1H), 1.18 – 1.11 (m, 1H), 1.00 (d, 3H, *J* = 7.14 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.3, 144.8, 121.4, 77.9, 60.2, 60.0, 39.5, 38.8, 38.4, 34.9, 34.7, 32.1, 31.2, 29.7, 27.0, 23.7, 23.5, 18.1, 17.7; HRESMS $[M + Na]^+$ 469.0351 (469.0354 calcd for $C_{19}H_{28}O_2Br_2$ + Na).

Crystal data for α–β-unsaturated dihydromonacolin L dibromide 22

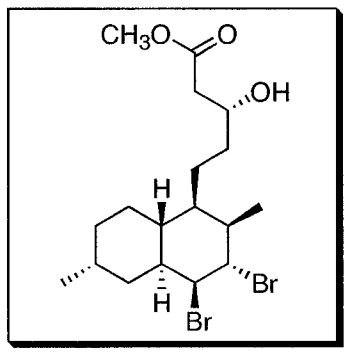
This experiment was performed by Dr. Robert MacDonald of the University of Alberta X-ray crystallography laboratory. $C_{19}H_{28}Br_2O_3$: *M* = 448.23, 0.50 × 0.30 × 0.29

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

mm, orthorhombic, a = 12.1661(8), b = 12.2760(8), c = 13.0349(9) Å, V = 1946.8(2) Å^3, T = 193 K, space group $P2_12_12_1$ (No. 19), Z = 4, μ(Mo-Kα) = 4.171 mm^{-1}, 9750 reflections measured, 3966 unique (R_{int} = 0.0256) which were used in all least squares calculations, $R_1(F)$ = 0.0235 (for 3497 reflections with $F_o^2 \geq 2\sigma(F_o^2)$), $wR_2(F^2)$ = 0.0497 (for all unique reflections).

Octaketide dibromide 23

Methyl 3*R*-5-((1*R*,2*R*,3*S*,4*S*,4a*S*,6*R*,8a*R*)-decahydro-3,4-dibromo-2,6-dimethylnaphthalen-1-yl)-3-hydroxypentanoate



A sample of 41.8 mg (0.089 mmol) α,β-unsaturated dihydromonacolin L dibromide **22** was dissolved in 10 mL CH_2Cl_2. To this solution was added 1 mL of a 2.5 M methanolic NaOH and the mixture was cooled to –78 °C. A rapid stream of O_3 (in O_2) was bubbled through the mixture. An immediate orange color developed and the O_3 stream was stopped when a faint blue color persisted (ca. 1 h) and the flask was purged with O_2 for 5 min. The reaction was warmed to rt, diluted with 30 mL of CH_2Cl_2 and the base was neutralized with 20 mL of 2 N HCl. The organic layer was separated and

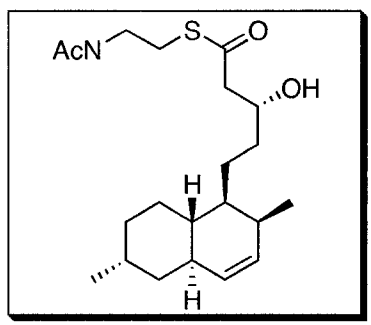
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

washed with H_2O (1 × 20 mL), satd $NaHCO_3$ (1 × 20 mL), H_2O (1 × 20 mL), brine (1 × 20 mL). It was then dried (Na_2SO_4), filtered and evaporated to give 34.7 mg of crude product. The crude residue was fractionated by preparative TLC (50% EtOAc in hexane, $R_f = 0.55$) to give 25.4 mg (60%) of pure **23**.

^1H NMR (500 MHz, $CDCl_3$) δ 4.94 (m, 1H, H-3), 4.45 (m, 1H, H-4), 3.99 – 3.96 (m, 1H, H-11), 3.70 (s, 3H, CH_3) 2.85 (br s, 1H, OH), 2.49 (dd, 1H, J = 16.5, 3.0 Hz, H-$12_{a/b}$), 2.41 (dd, 1H, J = 16.5, 9.0 Hz, H-$12_{a/b}$), 2.36 – 2.31 (m, 1H, H-2), 2.16 – 2.09 (m, 1H), 1.94 – 1.88 (m, 1H), 1.79 – 1.66 (m, 3H), 1.55 – 1.45 (m, 4H), 1.43 – 1.35 (m, 1H), 1.28 – 1.10 (m, 4H), 1.26 (d, 3H, J = 8 Hz), 1.00 (d, 3H, J = 7.5 Hz); HRESMS $[M+Na]^+$ = 475.0454 (475.0459 cacld for $C_{18}H_{30}O_3Br_2$ + Na).

Octaketide N-acetylcysteamine thioester 24

3*R*-5-((1*S*,2*S*,4a*R*,6*R*,8a*R*)-1,2,4a,5,6,7,8,8a-octahydro-2,6-dimethyl-naphthalen-1-yl)-3-hydroxy-pentanethioic acid *S*-(2-acetylaminoethyl) ester



A sample of 98.1 mg (0.42 mmol) octaketide free acid in 3 mL CH_2Cl_2 was treated with a solution of *N*-acetylcysteamine (61.5 mg, 0.62 mmol) in 3 mL CH_2Cl_2 and a solution of 128.2 mg (0.62 mmol) DCC and 1.5 mg 4-(dimethylamino)pyridine in 3 mL

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

CH_2Cl_2 over 5 minutes at –20°C. The resultant cloudy white solution was stirred overnight at room temperature. The mixture was filtered, and the filtrate was concentrated. The residue was purified by flash column chromatography eluting with 70% ethyl acetate in hexane. The fractions containing title compound were collected, and concentrated. The resultant cloudy white solution was filtered twice to remove 1,3-dicyclohexyl-urea, and solvent was removed *in vacuo* to **24** (139.0 mg, 99%) as a white solid.

A ^{13}C enriched sample of **24** was prepared by the above method starting with 10.0 mg of ^{13}C **11** (ca. 60% enrichment) and 25 mg unlabeled **11** to make 2 mg of **24** with ca 20% ^{13}C enrichment.

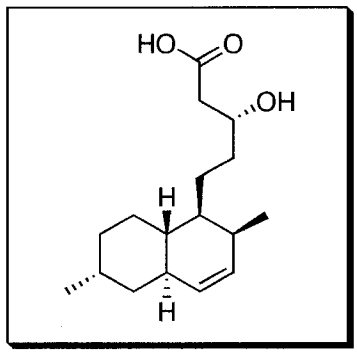
$[\alpha]_D^{25}$ = + 72.9 (*c* 0.10, $CHCl_3$); FTIR ($CHCl_3$ cast) 3280, 3075, 3006, 2957, 2913, 2869, 1690, 1652, 1552, 1440, 1371, 1287, 1195, 1088, 1042, 978, 936, 777, 724, 599 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.83 (br s 1H, N*H*CH_2CH_2S), 5.55 (ddd, 1H, *J* = 10.0, 5.0, 3.0 Hz H-3), 5.27 (d, 1H, *J* = 10.0 Hz, H-4), 3.40 (q, 2H, *J* = 6.5 Hz, NHC*H*$_2$$CH_2S$), 3.00 (t, 2H, *J* = 6.5 Hz, $NHCH_2CH_2S$), 2.61 (ddd, 1H, *J* = 15.0, 10.5, 5.0 Hz, Ha-2'), 2.45 (ddd, 1H, *J* = 15.0, 9.5, 6.5 Hz, Hb-2'), 2.19 (m, 1H, H-2), 1.99 (m, 1H, H-6), 1.95-1.84 (m, 2H, H-4a and Ha-1'), 1.93 (s, 3H, OC*H*$_3$), 1.56-1.44 (m, 5H, H-1, H-7 and Ha-5, H-8), 1.36 (m, 1H, Hb-1'), 1.24 (dt, 1H, *J* = 13.0, 5.0 Hz, Hb-5), 1.08 (dq, 1H, *J* = 12.0, 4.0 Hz, Hb-8), 1.00-0.93 (m, 1H, H-8a), 0.95 (d, 3H, *J* = 7.5 Hz, 6-C*H*$_3$), 0.82 (d, 3H, *J* = 7.0 Hz, 2-C*H*$_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 200.3 ($CH_3CONHCH_2CH_2SCO$), 170.0 ($CH_3CONHCH_2CH_2SCO$), 132.2 (C-3), 131.6 (C-4), 42.1 (C-2'), 41.0 (C-1), 39.9 (C-8a), 39.8 ($CH_3CONHCH_2CH_2SCO$), 38.9 (C-5), 37.3 (C-4a), 32.3 (C-7), 31.9 (C-2), 28.6 ($CH_3CONHCH_2CH_2SCO$), 27.5 (C-6), 24.8 (C-1'), 23.6

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(C-8), 23.3 ($CH_3CONHCH_2CH_2SCO$), 18.2 (6-CH_3), 15.0 (2-CH_3); HREIMS $[M\text{-}Ac]^+$ = 337.20754 (337.20755 calcd for $C_{19}H_{31}O_2NS$) 337.2 (1%), 218 (100%), 176.1 (40%), 159.1 (40%).

Synthesis and characterization of the free acid

3*R*-5-(1*S*,2*S*,4a*R*,6*R*,8a*R*-1,2,4a,5,6,7,8,8a-octahydro-2,6-dimethyl-naphthalen-1-yl)-3-hydroxypentanoic acid



A sample of 143 mg (0.56 mmol) octaketide methyl ester (**20**) was dissolved in 10 mL THF and treated with aqueous 2 mL 3M KOH. The mixture was stirred for 1 day at 50°C. After most of the THF was removed *in vacuo* and water (20 mL) was added, this solution was acidified with 2N HCl (pH < 2) and then extracted with CH_2Cl_2 (4 x 50 ml). The organic layer was dried over Na_2SO_4, and concentrated. Purification by flash column chromatography (30% ethyl acetate in hexane containing 0.5% acetic acid) gave the free acid (123 mg, 93%) as a white solid.

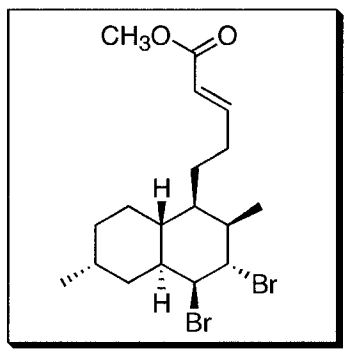
$[\alpha]_D^{25}$ = +120.3 (*c* 0.10, $CHCl_3$); R_f = 0.25 (EtOAc 100%) IR ($CHCl_3$ cast) 3020, 2952, 2915, 2860, 1696, 1445, 1411, 1377, 1310, 1297, 1267, 1238, 1211, 720 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.57 (ddd, 1H, *J* = 10.0, 5.0, 3.0 Hz, H-3), 5.28 (d, 1H, *J* =

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

10.0 Hz, H-4), 2.42 (ddd, 1H, J = 15.5, 10.0, 5.0 Hz, Ha-2'), 2.26-2.19 (m, 2H, H-2 and Hb-2'), 2.00 (m, 1H, H-6), 1.96-1.97 (m, 2H, H-4a and Ha-1'), 1.60-1.44 (m, 5H, H-1, 7 and Ha-5, 8), 1.36 (m, 1H, Hb-1'), 1.25 (dt, 1H, J = 13.0, 4.5 Hz, Hb-5), 1.10 (dq, 1H, J = 12.0, 4.0 Hz, Hb-8), 1.02-0.94 (m, 1H, H-8a), 0.96 (d, 3H, J = 7.5 Hz, 6-CH_3), 0.82 (d, 3H, J = 7.0 Hz, 2-CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 179.9 (C-3'), 132.3 (C-3), 131.6 (C-4), 41.06 (C-1), 40.0 (C-8a), 39.0 (C-5), 37.3 (C-4a), 32.3 (C-7), 32.0 (C-2'), 31.9 (C-2), 27.5 (C-6), 23.8 (C-1'), 23.7 (C-8), 18.3 (6-CH_3), 15.0 (2-CH_3); HREIMS $[M]^+$ = 280.2037 (280.2039 calcd for $C_{17}H_{28}O_3$) 280.2 (15%), 262.2 (9%), 176.1 (47%), 163 (100%).

α,β-Unsaturated octaketide dibromide 26

Methyl 5-((1*R*,2*R*,3*S*,4*S*,4a*S*,6*R*,8a*R*)-decahydro-3,4-dibromo-2,6-dimethyl-naphthalen-1-yl)-2-penteneoate



A sample of 100.0 mg (0.221 mmol) octaketide dibromide **23** was dissolved in 5 mL of CH_2Cl_2 and cooled to 0 °C. To this solution was added 92.4 μL (67.1 mg, 0.663 mmol, 3 eq) triethylamine followed by 26 μL (38.0 mg, 0.3315 mmol, 1.5 eq) methanesulfonyl chloride, and the reaction mixture was allowed to warm to room

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

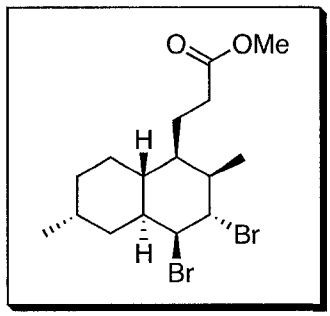
temperature. After three days the reaction was quenched by the addition of 10 mL of 1 N HCl with vigorous stirring for 1 min. CH_2Cl_2 (25 mL) was used to dilute the organic layer and the acidic aqueous layer was separated and discarded. The organic layer was then washed with H_2O (1 × 20 mL), satd. $NaHCO_3$ (1 × 20 mL), H_2O (1 × 20 mL), and brine (1 × 20 mL). After drying over Na_2SO_4, the organic layer was filtered and evaporated to give 120 mg of crude product. Unreacted starting material could be separated by flash column chromatography (20% EtOAc in hexane R_f = 0.58) to give 61.2 mg of α,β,-unsaturated octaketide dibromide **26** (58% yield).

$[\alpha]_D^{25}$ = + 29.5 (c 2.96, $CHCl_3$), FTIR ($CHCl_3$ cast) 2925 (s), 1725 (s), 1657 (m), 1435 (m), 1270 (m), 1041 (m), 616 (m) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.90 (ddd, 1H, *J* = 15.6, 7.4, 6.6 Hz), 5.79 (d, 1H, *J* = 15.6 Hz), 4.93 (m, 1H), 4.44 (m, 1H), 3.70 (s, 3H), 2.37 – 2.35 (m, 1H), 2.21 – 2.09 (m, 2H), 2.05 – 1.99 (m, 2H), 1.94 – 1.89 (m, 1H), 1.78 – 1.67 (m, 3H), 1.56 – 1.49 (m, 2H), 1.40 (dd, 1H), *J* = 11.0, 4.0 Hz), 1.36 (dd, 1H, *J* = 11.0, 4.0 Hz), 1.25 (d, 3H, J = 8.0 Hz), 1.23 – 1.15 (m, 1H), 1.12 (dd, 1H, *J* = 12.5, 5 Hz), 1.00 (d, 3H, *J* = 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.0, 148.9, 121.1, 60.1, 59.9, 51.4, 39.2, 38.5, 38.4, 34.8, 34.7, 31.1, 29.3, 26.9, 26.6, 23.6, 18.0, 17.6; HRESMS $[M + Na]^+$ = 457.03486 (457.03482 calcd for $C_{18}H_{28}O_2Br_2$ + Na).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Heptaketide dibromide 27

Methyl 3-((1*R*,2*R*,3*S*,4*S*,4a*S*,6*R*,8a*R*)-decahydro-3,4-dibromo-2,6-dimethyl-naphthalen-1-yl)-propanoate



α,β-Unsaturated octaketide dibromide **26** (102 mg, 0.236 mmol) was dissolved in 25 mL of CH_2Cl_2 and cooled to –78 °C. To this was added 10 mL of 2.5 M methanolic NaOH. A rapid stream of O_3 (in O_2) was bubbled through until a blue color persisted (ca. 60 min). Excess O_3 was purged with O_2 and the reaction mixture was allowed to warm to room temperature, at which point 10 mL of 2 N HCl and 25 mL CH_2Cl_2 were added and vigorously mixed. The organic layer was separated and washed with H_2O (1 × 20 mL) and brine (1 × 20 mL). It was then dried over Na_2SO_4, filtered and evaporated to give 84.1 mg of crude product. This residue was fractionated by flash column chromatography (5% EtOAc in hexane, R_f = 0.25) to give 45.0 mg (46%) of heptaketide dibromide **27**.

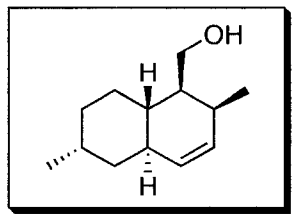
$[\alpha]_D^{25}$ = + 11.1 (c 0.051, $CHCl_3$); FTIR 2925 (s), 1739 (s), 1383 (m), 1282 (m), 1161 (m), 614 (m) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.92 (m, 1H), 4.45 (m, 1H), 3.66 (s, 3H), 2.37 – 2.26 (m, 2H), 2.18 – 2.10 (m, 2H), 2.06 – 1.99 (m, 2H), 1.94 – 1.88 (m, 1H), 1.78 – 1.72 (m, 2H), 1.56 – 1.49 (m, 1H), 1.41 – 1.21 (m, 5H), 1.26 (d, 3H, J = 8.0

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Hz), 1.00 (d, 3H, J = 7.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.0, 60.0, 59.9, 51.6, 39.2, 38.7, 38.4, 34.9, 34.7, 31.5, 31.1, 26.9, 23.8, 23.6, 18.0, 17.6; HRESMS $[M+Na]^+$ = 431.0191 (431.0191 calcd for $C_{16}H_{26}O_2Br_2$ + Na).

Hexaketide alcohol 29

((1*S*,2*S*,4a*R*,6*R*,8a*S*)-1,2,4a,5,6,7,8,8a-octahydro-2,6-dimethyl-naphthalen-1-yl)-methanol



A sample of 8.5 mg (0.021 mmol) **30** was dissolved in 5 mL CH_2Cl_2 and cooled to –78 °C. A rapid stream of O_3 (in O_2) was bubbled through until a blue color persisted. Excess O_3 was purged with a stream of O_2, and then 1.0 mL HOAc and 50 mg (0.76 mmol) Zn dust were added. The reaction mixture was warmed to room temperature and stirred 1 h. It was diluted with 10 mL CH_2Cl_2, filtered and washed with satd $NaHCO_3$ (1 × 10 mL) and H_2O (1 × 10 mL). It was then dried (Na_2SO_4) and filtered. To this CH_2Cl_2 solution was added 20 mg (0.53 mmol) $NaBH_4$ in 1 mL of MeOH, and the mixture was stirred 1 h at rt. The $NaBH_4$ was neutralized with 10 mL of 0.2 N HCl, and the organic layer was separated and washed with H_2O (1 × 10 mL). It was then dried (Na_2SO_4) and filtered to give 2.4 mg of crude product. Flash column chromatography (40% EtOAc in hexane) gave 1.0 mg (25%) pure hexaketide alcohol **29**.

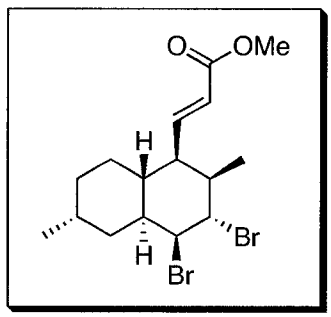
R_f = 0.18 (5% EtOAc in hexane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.58 (ddd, 1H, J = 9.8, 4.9, 2.5), 5.27 (d, 1H, J = 9.8 Hz), 3.64 (m, 2H), 2.35 – 2.32 (m, 1H), 2.27 – 2.23

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(m, 1H), 2.02 – 1.97 (m, 2H), 1.91 – 1.87 (m, 2H), 1.70 – 1.50 (m, 4H), 1.10 – 1.06 (m, 2H), 0.96 (d, 3H, J = 7.0 Hz), 0.82 (d, 3H, J = 7.0 Hz); ^{13}C NMR (125 MHz, CDCl3) δ 132.8, 131.6, 63.5, 37.8, 29.6, 27.6, 24.1, 18.4, 15.4;

α,β-Unsaturated heptaketide dibromide 31

Methyl 3-((1*R*,2*R*,3*S*,4*S*,4a*S*,6*R*,8a*R*)-decahydro-3,4-dibromo-2,6-dimethyl-naphthalen-1-yl)-propeneoate



A solution of 45.0 mg (0.109 mmol) heptaketide dibromide **28** in 2 mL of THF was cooled to –78 °C and to this solution was added 54.5 μL of a 2 M LDA (in pentane/THF/benzene) solution. The reaction mixture was stirred for 15 min. This was followed by the addition of a solution of 25.7 mg (0.109 mmol) phenylselenenyl bromide in 0.75 mL THF. This mixture was stirred at –78 °C for 10 min and then warmed to 0 °C and stirred for 30 min. At this point 0.5 mL H_2O, 0.25 mL HOAc, and 0.5 mL 30% H_2O_2 were added in succession, and this mixture stirred at 0 °C for 10 min and then allowed to warm to room temperature and stirred for 1 h. The mixture was diluted with 30 mL CH_2Cl_2 and extracted with 2 N HCl (1 × 10 mL). The organic layer was washed with satd $NaHCO_3$ (1 × 10 mL), H_2O (3 × 10 mL) and brine (1 × 10 mL), dried over Na_2SO_4,

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

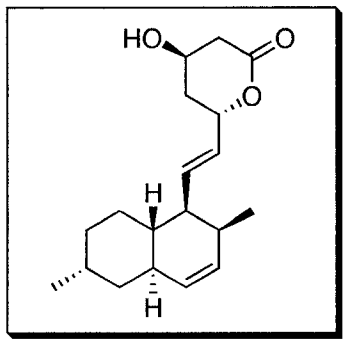
filtered and evaporated to give 64.0 mg of crude product. The crude product was a mixture of ca. 1.7 heptaketide dibromide : 1 debrominated heptaketide, based upon the integration of signals in the ^{1}H NMR spectrum. This crude product was then redissolved in 10 mL CH_2Cl_2 and 56.2 μL (175.3 mg, 1.09 mmol, 10 eq) elemental bromine were added, and the reaction mixture was stirred for 20 min at rt. This was diluted with 20 mL CH_2Cl_2 and 10 mL 0.25 M $Na_2S_2O_3$ were used to neutralize any remaining Br_2. The organic layer was separated and washed with H_2O (1 × 10 mL) satd $NaHCO_3$ (1 × 10 mL), H_2O (1 × 10 mL) and brine (1 × 10 mL), dried over Na_2SO_4, filtered and evaporated to give 76.8 mg of crude product. Analysis of the ^{1}H NMR spectrum of the crude product showed that it was now exclusively the α,β-unsaturated heptaketide dibromide **30**. Purification by flash column chromatography (5% EtOAC in hexane, R_f = 0.30) yielded 35.0 mg (74%) pure **30**.

$[\alpha]_D^{25}$ = + 6.0 (c 0.57, $CHCl_3$); FTIR ($CHCl_3$ cast) 2925 (s), 1725 (s), 1655 (w), 1435 (m), 1163 (m), 1132 (m), 620 (m) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.81 (dd, 1H, *J* = 15.6, 10.0 Hz), 5.85 (d, 1H, *J* = 15.6 Hz), 4.89 (m, 1H), 4.44 (m, 1H), 3.71 (s, 3H), 2.76 (ddd, 1H, *J* = 10.0, 10.0, 5.0 Hz), 2.38 – 2.26 (m, 2H), 2.18 – 2.12 (m, 2H), 2.10 – 2.00 (m, 2H), 1.80 – 1.72 (m, 2H), 1.56 – 1.44 (m, 2H), 1.35 (d, 3H, *J* = 8.0 Hz), 1.01 (d, 3H, *J* = 7.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.6, 150.7, 122.6, 59.0, 58.8, 51.5, 45.0, 42.7, 38.0, 34.0, 33.8, 30.9, 27.3, 25.3, 19.0, 18.0;

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Monacolin N (33)

(4*R*)-tetrahydro-4-hydroxy-6-[2-((1*S*,2*S*,4a*R*,6*R*,8a*S*)-1,2,4a,5,6,7,8,8a-octahydro-2,6-dimethyl-1-naphthalenyl)ethenyl]-2H-pyran-2-one



Monacolin N (**33**) was isolated from *A. nidulans lovB + lovC* fermentation cultures grown as described for the isolation of dihydromonacolin L (**11**). Monacolin N (**33**) was purified by flash column chromatography (40% EtOAc in hexane) from the crude residue of the fermentation cultures as follows. A crude culture extract (615 mg) was applied in $CHCl_3$ to a 16.5 cm × 3 cm column of silica gel packed in hexane. The column was flushed with 200 mL of hexane and then eluted with 40% EtOAc in hexane. Fractions (3 ×100 mL) were collected, then 20 mL fractions were collected, and combined according to TLC analysis. The fraction containing **33** (tube 24 – 26) was evaporated to give 5.6 mg **33** contaminated with a trace of **11**. Final purification was accomplished on a 6.3 cm × 1 cm column of silica gel eluted with 30% EtOAc in hexane. The above procedure yielded 2.8 mg of pure **33** from 8 L of *A. nidulans lovB + lovC* fermentation broth.

R_f = 0.5 (EtOAc, 100%); FTIR ($CHCl_3$ cast) 2929 (m), 1724 (s), 1419 (m), 1197 (m), 1039 (m), 640 (m) cm^{-1} ; ^1H NMR (600 MHz, $CDCl_3$) δ 5.71, (ddd, 1H, *J* = 15.2,

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

9.8, 0.8 Hz), 5.55 (ddd, 1H, J = 9.8, 4.4, 3.1 Hz), 5.49 (dd, 1H J = 15.4, 6.8 Hz), 5.30 (d, 1H, J = 10.0 Hz), 5.15 (ddd, 1H, J = 10.0, 6.8, 3.1 Hz) 4.38 (m, 1H), 2.75 (dd, 1H, J = 17.7, 5.1 Hz), 2.60 (ddd, 1H, J = 17.7, 4.0, 1.6 Hz), 2.25–2.18 (m, 2H), 2.05–1.97 (m, 2H), 1.92–1.85 (m, 2H), 1.52–1.44 (m, 4H), 1.33 (dd, 1H, J = 12.8, 3.1 Hz), 1.28 (dd, 1H, J = 13.2, 4.8 Hz), 1.26-1.22 (m, 2H), 0.96 (d, 3H, J = 7.2 Hz) 0.90 (d, 3H, J = 6.9 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.1, 136.8, 132.4, 131.2, 128.1, 76.1, 62.8, 47.0, 39.1, 38.9, 38.8, 36.9, 36.6, 35.8, 32.2, 27.7, 25.2, 18.4, 16.6; HREIMS $[M]^+$ 304.2043 (304.2039 calcd for $C_{19}H_{28}O_3$) 304.2 (14%), 286.2 (12%), 216.2 (33.0%), 161.1 (100%), 93.1 (94.1%) 81.1 (96%).

Crystal data for monacolin N (33)

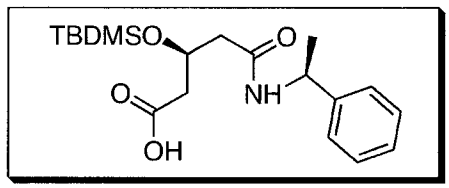
This experiment was conducted by Dr. Michael Ferguson of the University of Alberta X-ray crystallography laboratory. $C_{19}H_{28}O_3$: M = 304.41, 0.42 × 0.28 × 0.05 mm, orthorhombic, a = 5.5364(8), b = 9.9736(14), c = 31.173(4) Å, V = 1721.3(4) Å^3, T = 193 K, space group $P2_12_12_1$ (No. 19), Z = 4, μ(Mo-Kα) = 0.077 mm^{-1}, 8084 reflections measured, 3504 unique (R_{int} = 0.0413) which were used in all least squares calculations, $R_1(F)$ = 0.0444 (for 2789 reflections with $F_o^2 \geq 2\sigma(F_o^2)$), $wR_2(F^2)$ = 0.1111 (for all unique reflections). Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Center.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Glutaric amide acid 36

(3*S*,1'*S*)-3-[(*tert*-butyldimethylsilyl)oxy]-5-[(1-phenylethyl)amino]-5-oxopentanoic acid

CAS Registry Number [121331-22-4]



3-(*tert*-Butyldimethylsilyloxy)-glutaric anhydride **34** (244 mg, 1.0 mmol) was dissolved in 10 mL toluene and cooled to –78 °C. To this solution was added 280 μL (102 mg, 1.0 mmol) NEt_3 followed by 129 μL (121 mg, 1 mmol) of *S*-(-)-α-methylbenzylamine **(35)**. The reaction mixture was stirred at –78 °C for 4 h and then warmed to rt over 1 h. The reaction was quenched by the addition of 10 mL THF and 10 mL of 5% $KHSO_4$. This mixture was diluted with 25 mL EtOAc and the layers were separated. The organic layer was extracted with 5% $KHSO_4$ (1 × 10 mL), then washed with H_2O (3 × 10 mL) and brine (1 × 10 mL). The organic layer was dried (Na_2SO_4), filtered and evaporated to give 315 mg crude product. This residue was rinsed with ice cold Et_2O (20 mL) and the Et_2O soluble portion was kept. The solvent was evaporated, and the washing procedure was repeated, keeping the Et_2O soluble portion. Evaporation of the solvent left a residue of 97.0 mg (26%) of the desired diastereomer **36**.

$[\alpha]_D^{25}$ = - 15.2 (*c* = 1.5, $CHCl_3$); FTIR ($CHCl_3$ cast) 3319 (br s), 2927 (s), 2855 (m), 1696 (s), 1618 (s), 1559 (m), 1211 (m), 1097 (m), 778 (m); ^{1}H NMR δ 7.31 (m, 2H, Ar-H), 7.30 (m, 2H, Ar-H), 7.25 – 7.22 (m, 1H, Ar-H), 6.50 (d, 1H, *J* = 7.5 Hz, N-H),

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

5.12 (dq, 1H, J = 7.5, 7.5 Hz, C-1'), 4.48 – 4.43 (m, 1H, C-3), 2.55 – 2.53 (m, 2H), 2.50 – 2.48 (m, 2H), 1.47 (d, 3H, J = 7.5 Hz, CH_3), 0.83 (s, 9H, *t*-Bu), 0.09 (s, 3H, Si-CH_3), 0.07 (s, 3H, Si-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.2, 170.4, 142.8, 128.7 (×2), 127.4, 126.1 (×2), 66.7, 49.0, 43.2, 41.4, 25.7 (×3), 21.8, 17.8, -5.0 (×2); HREIMS $[M]^+$ = 365.2031 (365.2022 cacld for $C_{19}H_{31}NO_4Si$) 365.2 (2%), 308.1 (36%), 204.1 (9%), 120.0 (19%), 112.0 (44%), 105.1 (100%).

Crystal data for 36

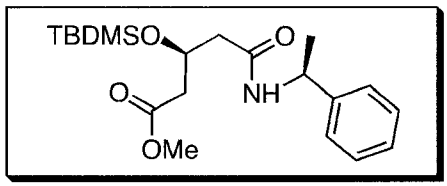
This experiment was conducted by Dr. Michael Ferguson of the University of Alberta X-ray crystallography laboratory. $C_{19}H_{31}NO_4Si$: M = 365.54, 0.71 × 0.12 × 0.08 mm, orthorhombic, a = 6.6059(8), b = 11.9309(15), c = 27.716(4) Å, V = 2184.4(5) Å^3, T = 193 K, space group $P2_12_12_1$ (No. 19), Z = 4, μ(Mo-Kα) = 0.128 mm^{-1}, 10020 reflections measured, 4453 unique (R_{int} = 0.0468) which were used in all least squares calculations, $R_1(F)$ = 0.0564 (for 3397 reflections with $F_o^2 \geq 2\sigma(F_o^2)$), $wR_2(F^2)$ = 0.1280 (for all unique reflections).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Glutaric amide ester 37

(3*S*,1'*S*)-3-[(*tert*-butyldimethylsilyl)oxy]-5-[(1-phenyl-ethyl)amino]-5-oxopentanoic acid methyl ester

CAS Registry Number [121331-23-5]



3-(*tert*-Butyldimethylsilyloxy)glutaric anhydride (**34**) (488 mg, 2.0 mmol) was dissolved in 10 mL toluene and cooled to –78 °C. To this solution was added 560 μL (203 mg, 2.0 mmol) NEt_3 followed by 258 μL (242 mg, 2.0 mmol) of *S*-(-)-α-methylbenzylamine (**35**). The reaction mixture was stirred at –78 °C for 4 h then warmed to rt over 1 h. The reaction was quenched by the addition of 10 mL THF and 10 mL of 5% $KHSO_4$. This mixture was diluted with 25 mL CH_2Cl_2 and the layers separated. The organic layer was washed with H_2O (3 × 10 mL) and brine (1 × 10 mL), dried (Na_2SO_4), filtered and evaporated to give 808 mg crude product. This residue was dissolved in 10 mL DMF and 580 mg (4 mmol) K_2CO_3 and 500 μL (8 mmol) CH_3I were added. This mixture was stirred overnight at rt. The reaction was quenched by dilution with 30 mL CH_2Cl_2 and the addition of 10 mL H_2O. After stirring 10 min, the layers were separated and the organic layer was washed with H_2O (3 × 10 mL) and brine (1 × 10 mL), then dried (Na_2SO_4), filtered and evaporated to give 895 mg crude product. This residue was

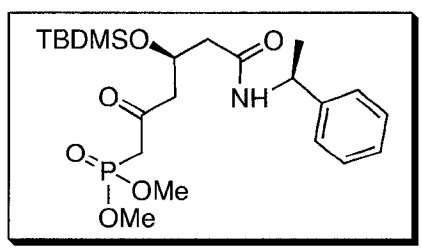
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

fractioned by flash column chromatography (40% EtOAc in hexane, R_f = 0.50) to give 387 mg (51%) of **37**.

$[\alpha]_D^{25}$ = -26.7 (c = 1.55, $CHCl_3$) FTIR ($CHCl_3$ cast) 3296 (br s), 2953 (s), 2929 (s), 1740 (s), 1642 (s), 1543 (s), 1255 (m), 1207 (m), 1155 (m), 1097 (m), 778 (m), 699 (m); ^{1}H NMR (500 MHz $CDCl_3$) δ 7.31 (m, 1H, Ar-H), 7.30 (m, 1H, Ar-H), 7.29 (m, 2H, Ar-H), 7.24 – 7.20 (m, 1H, Ar-H), 6.52 (d, 1H, *J* = 7.5 Hz, N-H), 5.10 (dq, 1H, *J* = 7.5, 7.5 Hz, C-1'), 4.48 (dddd, 1H, J = 6.0, 6.0, 6.0, 6.0 Hz, C-3), 3.62 (s, 3H, OCH_3), 2.50 (dd, 1H, *J* = 15.0, 6.0 Hz), 2.48 (m, 1H), 2.43 (dd, 1H, *J* = 8.0, 6.0 Hz), 2.36 (dd, 1H, *J* = 15.0, 6.0 Hz), 1.45 (d, 3H, *J* = 7.5 Hz, CH_3), 0.84 (s, 9H, *t*-Bu), 0.08 (s, 3H, Si-CH_3) 0.05 (s, 3H, Si-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.4, 169.1, 143.4, 128.6 (×2) 127.2, 126.1 (×2), 66.7, 51.5, 48.6, 43.8, 41.2, 25.6 (×3), 22.0, 17.8, -4.8, -5.1; HREIMS $[M]^+$ = 379.2171 (379.2179 cacld for $C_{20}H_{33}NO_4Si$) 379.2 (3.2%), 322.1 (100%), 218.1 (51%), 112.0 (43%), 105.0 (93%).

Amide phosphonate 42

N-1'*S*-(1-phenyl-ethyl)- (*R*)-3-[(*tert*-butyldimethylsilyl)oxy]-6-(dimethoxyphosphinyl)-5-oxohexanamide



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Dimethyl methylphosphonate (61.0 μL, 70.0 mg, 0.566 mmol, 2.5 eq) was dissolved in 2 mL THF and cooled to –78 °C. To this solution was added 180 μL of a 2.5 M soln of n-butyl lithium in hexane (0.452 mmol, 2 eq) and the mixture was stirred at –78 °C for 30 min. A solution of 84.7 mg (0.226 mmol) of **36** in 1 mL of THF was added and the mixture was stirred for 2.5 h while maintaining the temperature at –78 °C. The reaction was quenched by the addition of 1 mL of satd NH_4Cl, followed by warming to rt. This mixture was diluted with 25 mL CH_2Cl_2 and washed with H_2O (2 × 10 mL) and brine (1 × 10 mL), then dried (Na_2SO_4), filtered and evaporated to give 75 mg crude product. The crude residue was fractioned over silica gel by flash column chromatography (100% EtOAc, $R_f = 0.15$) to give 15.6 mg (15%) of pure **42**.

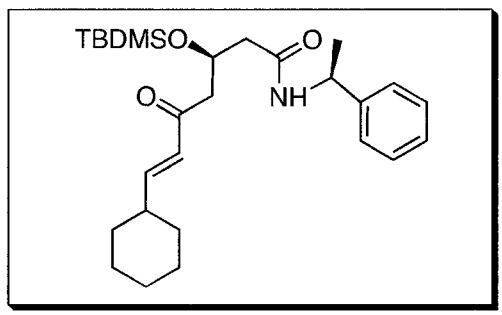
FTIR ($CHCl_3$ cast) 3291 (br s), 2956 (s), 2928 (s), 1713 (s), 1646 (s), 1542 (s), 1252 (s), 1032 (s), 701 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 – 7.29 (m, 3H, Ar-H), 7.24 – 7.22 (m, 2H, Ar-H), 6.56 (br d, 1H, *J* = 7.5 Hz, N-*H*), 5.09 (dq, 1H, *J* = 7.5, 7 Hz, H-1'), 4.47 (m, 1H, H-3), 3.69 (d, 3H, $^3J_{PH}$ = 9.1 Hz, OCH_3), 3.67 (d, 3H, $^3J_{PH}$ = 9.1 Hz, OCH_3), 3.02 (dd, J_{HH} = 13.9 Hz, $^2J_{PH}$ = 22.6 Hz, H-$6_{a/b}$), 2.96 (dd, J_{HH} = 13.9 Hz, $^2J_{PH}$ = 22.3 Hz, H-$6_{a/b}$), 2.76 (dd, 1H, *J* = 16.5, 6.0 Hz, H-$4_{a/b}$), 2.67 (dd, 1H, *J* = 16.5, 5.5 Hz, H-$4_{a/b}$), 2.47 (dd, 1H, *J* = 14.5, 5.0 Hz, H-$2_{a/b}$), 2.31 (dd, 1H, *J* = 14.5, 4.5 Hz, H-$2_{a/b}$), 1.44 (d, 3H, *J* = 7.0 Hz, CH_3'), 0.84 (s, 9H, *t*-Bu), 0.09 (s, 3H, Si-CH_3), 0.05 (s, 3H, Si-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 200.0 (d, $^3J_{PC}$ = 6.9 Hz, C-5), 169.2 (C-1), 143.5 (Ar-C), 128.6 (×2, Ar-C), 127.3 (Ar-C), 126.2 (×2, Ar-C), 65.9 (C-3), 52.9 (d, $^2J_{PC}$ = 7.0 Hz, OCH_3), 52.8 (d, $^2J_{PC}$ = 6.4 Hz, OCH_3), 49.6 (d, $^3J_{PC}$ = 1.6 Hz, C-4), 48.9 (C-1'), 43.5 (C-2), 42.3 (d, $^1J_{PC}$ = 127.7 Hz, C-6), 25.7 (×3, t-Bu-CH_3), 22.0 (CH_3'), 17.8 (t-Bu-C), -4.96 (Si-CH_3), -5.01 (Si-CH_3); HREIMS $[M]^+$ = 471.2194 (471.2206 calcd for $C_{22}H_{38}O_6NSiP$),

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

471.2 (3%), 456.2 (4%), 414.1 (100%), 310.1 (18%), 267.1 (13%), 120.1 (43%), 105.1 (84%).

Cyclohexyl adduct 43

N-1'*S*-(1-phenyl-ethyl)-(*R*)-3-[(*tert*-butyldimethylsilyl)oxy]-7-cyclohexyl-5-oxo-6-hepteneamide



A sample of **42** (14.3 mg, 0.030 mmol) was dissolved in 1 mL THF in a 1 mL Reacti-Vial (Wheaton Glass). To this solution was added 30 mg (0.71 mmol) LiOH • H_2O and the mixture was stirred at rt for 30 min, at which point 50 μL (0.413 mmol) of cyclohexanecarboxaldehyde was added. The reaction mixture was stirred at rt for 2 days. The mixture was diluted with 30 mL CH_2Cl_2 and washed with H_2O (2 × 10 mL) and brine (1 × 10 mL). It was then dried (Na_2SO_4), filtered and evaporated to give 33.0 mg crude product. The final product was purified by preparative TLC (30% EtOAc in hexane, R_f = 0.39) to give 7.2 mg (52%) of **43**.

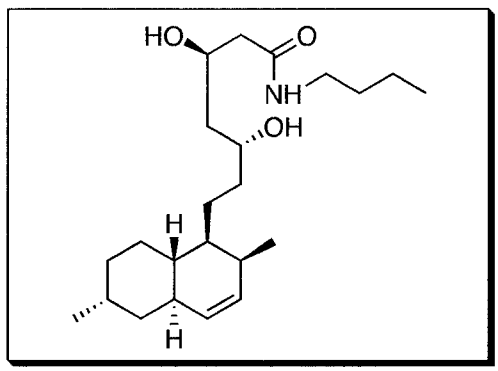
$[\alpha]_D^{25}$ = - 2.4 (c 0.72, $CHCl_3$); FTIR 3307 (br m), 2928 (s), 2854 (m), 1645 (s), 1539 (m), 1255 (w), 1091 (m), 836 (m), 778 (m) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.35 – 7.30 (m, 3H), 7.25 – 7.22 (m, 2H), 6.79 (br d, 1H, J = 8.1 Hz), 6.52 (dd, 1H, J =

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

16.0, 6.7 Hz), 5.89 (dd, 1H, *J* = 16.0, 1.4 Hz), 5.12 (dq, 1H, *J* = 8.1, 7.0 Hz), 4.52 – 4.48 (m, 1H), 2.63 (dd, 1H, *J* = 15.5, 6.5 Hz), 2.59 (dd, 1H, *J* = 15.5, 5.6 Hz), 2.52 (dd, 1H, *J* = 14.8, 5.0 Hz), 2.30 (dd, 1H, *J* = 14.8, 4.1 Hz), 2.06 – 2.02 (m, 1H), 1.75 – 1.64 (m, 5H), 1.44 (m, 1H), 1.45 (d, 1H, *J* = 7.0 Hz), 1.31 – 1.23 (m, 3H), 1.09 – 1.03 (m, 3H), 0.84 (s, 9H), 0.09 (s, 3H), 0.02 (s, 3H); ^{13}C NMR δ 198.7, 169.5, 153.3, 143.7, 128.7, 128.6 (×2), 127.2, 126.2 (×2), 66.7, 48.5, 45.4, 43.8, 40.6, 31.7, 31.6, 25.9, 25.8 (×3), 25.7 (×2), 22.0, 17.8, -4.9, -5.0; HREIMS $[M]^+$ = 457.2990 (457.3012 calcd for $C_{27}H_{43}NO_3Si$) 457.3 (1%), 398.2 (31%), 334 (6%), 294.1 (10%), 237.1 (23%), 105.0 (100%).

Dihydromonacolin L n-butyl amide 44

N-butyl 3*R*,5*R*-7-(1*S*,2*S*,4a*R*,6*R*,8a*R*-1,2,4a,5,6,7,8,8a-octahydro-2,6-dimethyl-naphthalen-1-yl)-3-5-dihydroxyheptanamide



A solution of dihydromonacolin L (**11**) (98.9 mg, 0.323 mmol) in 2 mL n-butyl amine was held at reflux (80 °C) for 1 h and then cooled to rt. The mixture was diluted with 30 mL CH_2Cl_2 and extracted with 2 N HCl (2 × 10 mL). The organic layer was

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

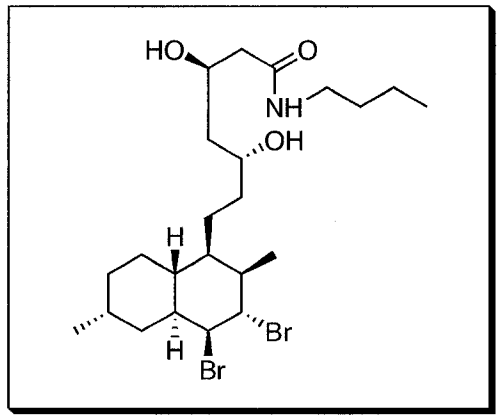
washed with H_2O (3 × 10 mL), dried (Na_2SO_4), filtered and evaporated to give 75.5 mg (61%) **44**.

$[\alpha]_D^{25}$ = + 44.9 (*c* = 1.0, $CHCl_3$); R_f = 0.20 (EtOAc, 100%) FTIR (CH_2Cl_2 cast) 3307 (br), 2931 (s), 1645 (s), 1555 (s), 1440 (s), 1089 (m), 720 (m); ^{1}H NMR (600 MHz, $CDCl_3$), δ 5.65 (ddd, 1H, *J* = 9.8, 4.8, 2.8 Hz, H-4), 5.26 (d, 1H, *J* = 9.8 Hz, H-3), 4.21 (m, 1H, H-13), 3.86 (m, 1H, H-11), 3.22 (q, 2H, *J* = 6.6 Hz), 2.31 (m, 2H, H-14), 2.22 (m, 1H, H-2), 1.98 (m, 1H, H-6), 1.87 (m, 1H, H-4a) 1.60 – 1.50 (m, 6H), 1.49 – 1.40 (m, 6H), 1.35 – 1.30 (m, 2H, H-3'), 1.29 – 1.21 (m, 2H), 1.16 – 1.12 (m, 2H), 1.07 (d, 1H, *J* = 3.6 Hz), 1.05 (d, 1H, *J* = 4.2 Hz), 0.95 (d, 3H, *J* = 7.3 Hz, CH_3-6), 0.90 (t, 3H, *J* = 7.5 Hz, CH_3-4'), 0.81 (d, 3H, *J* = 7.0 Hz, CH_3-2); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.8 (C-15), 132.8 (C-4), 131.5 (C-3), 72.7 (C-11), 69.6 (C-13), 42.9 (C-14), 42.3 (C-12), 41.5 (CH), 40.0 (CH), 39.1 (CH_2), 38.9 (CH_2), 37.3 (C-4a), 35.4 (C-10), 32.3 (CH_2), 31.9 (C-2), 31.5 (CH_2), 27.4 (C-6), 23.8 (CH_2), 23.6 (CH_2), 20.0 (C-3'), 18.2 (CH_3-6), 14.9 (CH_3-2), 13.7 (CH_3-4'); HREIMS $[M]^+$ = 379.3088 (379.3087 cacld for $C_{23}H_{41}O_3N$) 379.3 (17%), 361.3 (5%), 328.3 (14%), 214.1 (18%), 186.1 (39%), 176.1 (82%), 144.1 (100%).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Dihydromonacolin L n-butylamide dibromide 45

N-butyl 3*R*,5*R*-7-((1*R*,2*R*,3*S*,4*S*,4a*S*,6*R*,8a*R*)-decahydro-3,4-dibromo-2,6-dimethyl-naphthalen-1-yl)-3-5-dihydroxyheptanamide



A sample of **44** (14.2 mg, 0.038 mmol) was dissolved in 3 mL $CHCl_3$. To this was added 200 μL (5 eq) of a 5% Br_2 in HOAc soln. The reaction mixture was stirred at rt for 2 h, then diluted with 30 mL CH_2Cl_2 and quenched with 10 mL of 0.25 M $Na_2S_2O_3$. After stirring vigorously for 10 min the organic layer was separated and washed with satd $NaHCO_3$ (1 × 10 mL) and H_2O (3 × 10 mL). It was then dried (Na_2SO_4), filtered and evaporated to give 15.3 mg of crude product. Dihydromonacolin L n-butylamide dibromide was purified from the residue by flash column chromatography to give 10.1 mg (50%) pure **45**.

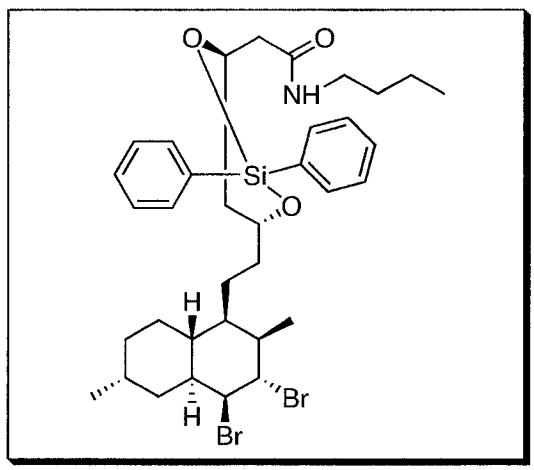
$[\alpha]_D^{25}$ = + 2.5 (*c* = 1.16, $CHCl_3$); R_f = 0.20 (EtOAc, 100%) FTIR ($CHCl_3$ cast) 3306 (br s), 2927 (s), 1644 (s), 1553 (s), 1454 (s), 1381 (s), 1090 (s), 756 (s), 616 (m); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.85 (br s, 1H), 4.94 (m, 1H), 4.45 (m, 1H), 4.23 – 4.20 (m, 1H), 3.88 – 3.84 (m, 1H), 3.24 (q, 2H, *J* = 7.2 Hz), 2.36 – 2.30 (m, 2H), 2.14 – 2.09

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(m, 1H), 2.07 – 2.00 (m, 1H), 1.93 – 1.87 (m, 1H), 1.78 – 1.72 (m, 2H), 1.70 – 1.64 (m, 1H), 1.60 – 1.41 (m, 7H), 1.40 – 1.30 (m, 4H), 1.25 (d, 3H, J = 7.8 Hz), 1.21 – 1.10 (m, 3H), 1.00 (d, 3H, J = 7.2 Hz), 0.91 (t, 3H, J = 7.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.0, 72.3, 69.7, 60.5, 60.3, 42.7, 42.6, 39.6, 39.2, 38.8, 38.5, 35.0, 34.9, 34.8, 31.6, 31.2, 27.0, 23.7, 23.6, 20.1, 18.0, 17.6, 13.7; HRESMS $[M+H]^+$ = 538.1527 (538.1526 calcd for $C_{23}H_{41}NO_3Br_2$ + H).

Diphenylsilyloxy-dihydromonacolin L n-butylamide dibromide 46

N-butyl 2-{4*R*,6*R*-6-[2-((1*R*,2*R*,3*S*,4*S*,4a*S*,6*R*,8a*R*)-decahydro-3,4-dibromo-2,6-dimethylnaphthalen-1-yl)-ethyl]-2,2-diphenyl-[1,3,2]dioxasilinan-4-yl}-acetamide



A sample of dihydromonacolin L n-butylamide dibromide **45** (176 mg, 0.327 mmol) was dissolved in 4 mL CH_2Cl_2. To this solution was added 114 μL (82.7 mg, 0.82 mmol, 2.5 eq) NEt_3 and 75.6 μL (91.0 mg, 0.360 mmol, 1.1 eq) dichlorodiphenylsilane. The reaction mixture was stirred under Ar for 2 h. The reaction mixture was diluted with 25 mL CH_2Cl_2 and washed quickly once with 0.1 N HCl. The organic layer was washed

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

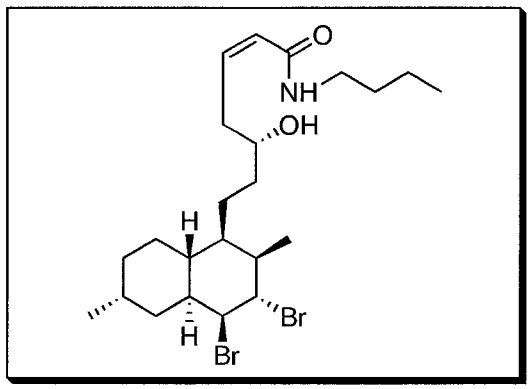
with H_2O (3 × 10 mL), dried (Na_2SO_4), filtered and evaporated to give 232 mg of crude product. This residue was fractioned by flash column chromatography (20% EtOAC in hexane) to give 84.5 mg (36%) pure **46**

$[\alpha]_D^{25}$ = + 30.1 (*c* 1.47, $CHCl_3$); R_f = 0.39 (50% EtOAc in hexane) FTIR ($CHCl_3$ cast) 3302 (br m), 2956 (s), 2927 (s), 1644 (s), 1429 (s), 1125 (s), 1116 (s), 699 (s), 517 (s); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.66 (m, 2H, Ar-H), 7.56 (m, 2H, Ar-H), 7.46 – 7.40 (m, 4H, Ar-H), 7.36 – 7.34 (m, 2H, Ar-H), 6.27 (br s, 1H, N-H), 4.91 (s, 1H, H-3), 4.51 (m, 1H, H-13), 4.44 (s, 1H, H-4), 4.12 (m, 1H, H-11), 3.27 (ddd, 1H, *J* = 13.0, 7.1, 6.1 Hz, H-16_a), 3.15 (ddd, 1H, *J* = 13.0, 7.1, 5.5 Hz, H-16_b), 2.34 (m, 1H, H-2) 2.44 (d, 2H, *J* = 5.4 Hz, H-14) 2.04 – 2.00 (m, 1H, H-6), 1.95 – 1.90 (m, 1H, H-1), 1.84 – 1.79 (m, 2H), 1.76 – 1.71 (m, 2H, H-12), 1.61 – 1.48 (m, 4H), 1.41 – 1.31 (m, 4H, H-17), 1.29 – 1.21 (m, 2H, H-18), 1.25 (d, 3H, *J* = 7.9 Hz, CH_3-2), 1.02 (d, 3H, *J* = 7.15 Hz, CH_3-6), 0.84 (t, 3H, *J* = 7.3 Hz, H-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3 (C-17), 134.5 (2 × Ar-C), 134.4 (2 × Ar-C), 132.8 (Ar-C), 132.3 (Ar-C), 130.8 (Ar-C), 130.6 (Ar-C), 128.1 (2 × Ar-C), 127.8 (2 × Ar-C), 73.0 (C-11), 71.2 (C-13), 60.3 (C-4), 60.2 (C-3), 45.2 (C-14), 42.1 (C-12), 39.1 (C-16), 38.9 (C-2), 38.6 (C-1), 38.4 (CH_2), 35.0 (CH), 34.9 (CH_2), 34.8 (CH), 31.7 (CH_2), 31.2 (CH_2), 27.0 (H-6), 23.7 (CH_2), 22.9 (CH_2), 20.1 (C-18), 18.1 (CH_3-6), 17.7 (CH_3-2), 13.8 (C-19); HRESMS $[M+Na]^+$ = 740.1753 (740.1746 calcd for $C_{35}H_{49}NO_3SiBr_2$+Na).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

α,β-Unsaturateddihydrodromonacolin L n-butyl amide dibromide 47

N-butyl 5*R*-7-(1*R*,2*R*,3*S*,4*S*,4a*S*,6*R*,8a*R*-decahydro-3,4-dibromo-2,6-dimethyl-naphthalen-1-yl)-5-hydroxy-2-hepteneamide



To a solution of **46** (22.3 mg, 0.031 mmol) in 2 mL THF was added 18 mg (0.75 mmol) of NaH (freshly rinsed in hexane) and 30 mg (0.108 mmol) AgOTs. The reaction mixture was heated at reflux for 1 h. The reaction was quenched with satd NaCl soln added dropwise until the evolution of H_2 ceased. The mixture was then diluted with 20 mL H_2O, acidified with 2 N HCl (pH < 2), and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with H_2O (3 × 10 mL), dried (Na_2SO_4), filtered and evaporated to give 16.5 mg of crude product. This residue was purified by preparative TLC (EtOAc–hexane 1:1, two elutions, $R_f = 0.12$) to give 1.7 mg (11%) **47**.

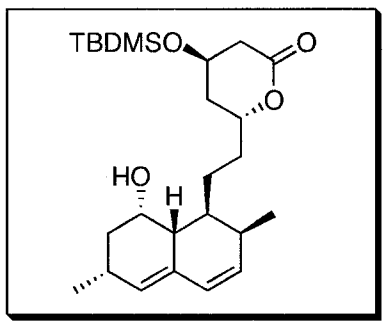
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.80 (ddd, 1H, *J* = 15.0, 7.8, 7.2 Hz), 5.82 (d, 1H, *J* = 15.0 Hz) 5.39 (m, 1H), 4.94 (m, 1H), 4.45 (m, 1H), 3.72 (m, 1H), 3.30 (q, 2H, *J* = 6.9 Hz), 2.40 – 2.24 (m, 6H), 2.18 – 2.08 (m, 1H), 2.06 – 2.00 (m, 1H), 1.96 – 1.87 (m, 1H), 1.80 – 1.65 (m, 4H), 1.60 – 1.28 (m, 9H), 1.25 (d, 3H, *J* = 7.2 Hz), 1.00 (d, 3H, *J* = 7.2

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Hz), 0.91 (t, 3H, J = 7.2); HRESMS $[M+Na]^+$ = 542.1240 (542.1245 calcd for $C_{23}H_{39}Br_2NO_2$ + Na).

tert-*Butyldimethylsilyloxymonacolin J (48)*

4*R*-tetrahydro-4-(*tert*-butyldimethylsilyl)oxy-6-[2-(1*S*,2*S*,6*S*,8*S*,8a*R*-1,2,6,7,8,8a-hexahydro-8-hydroxy-2,6-dimethyl-1-naphthalenyl)ethyl]-2H-pyran-2-one



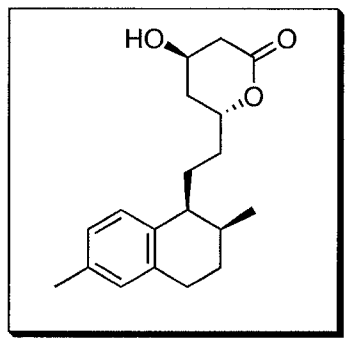
tert-Butyldimethylsilyloxy-monacolin J (**48**) was prepared according to the procedure of Willard and Smith.[101] Monacolin J (**12**) (100 mg, 0.312 mmol) and 107 mg (1.56 mmol, 5 eq) imidazole were dissolved in 4 mL dimethylformamide (DMF). To this was added 117 mg (0.78 mmole, 2.5 eq) *tert*-butyldimethylsilyl chloride (TBDMSCl) as a solution in 1mL DMF. The mixture was stirred at room temperature for 18 h, then diluted with 30 mL CH_2Cl_2 and extracted with 0.2 N HCl (1 × 10 mL). The organic layer was washed with H_2O (3 × 10 mL), dried (Na_2SO_4), filtered and evaporated to give 198 mg crude residue. The product was purified by flash column chromatography (20% EtOAc in hexanes) to give 100 mg (74% yield) *tert*-butyldimethylsilyloxymonacolin J (**48**).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

$[\alpha]_D^{25}$ = -10.1 (c 1.35, CH_2Cl_2); R_f = 0.25 (50% EtOAc in hexane); FTIR ($CHCl_3$ cast) 3446 (b), 2954 (s), 1734 (s), 1254 (s), 1082 (s), 735(s) $^{cm-1}$; ^1H NMR (600 MHz, $CDCl_3$) δ 5.95 (d, 1H, *J* = 9.5 Hz), 5.77 (dd, 1H, *J* = 6, 9.5 Hz), 5.52 (s, 1H), 4.65 (m, 1H), 4.27 (q, 1H, *J* = 3.5), 4.21 (s, 1H), 2.58 (dd, 1H, *J* = 17.5, 4 Hz), 2.53 (ddd, 1H, *J* = 17.5, 3, 1.5 Hz), 2.42 (m, 1H), 2.35 (s, 1H, *J* = 6.5), 2.14 (dd, 1H, *J* = 12, 2.5 Hz), 1.91–1.72 (m, 6H), 1.69 (ddd, 1H, *J* = 15.0, 3.0 Hz), 1.45 (m, 2H), 1.17 (d, 3H, *J* = 7.5 Hz), 0.88 (d, 3H, *J* = 7), 0.86 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.5, 133.7, 131.3, 130.0, 128.4, 76.5, 65.3, 63.5, 39.3, 38.8, 36.9, 36.5, 35.8, 33.0, 30.8, 27.4, 25.7, 24.3, 23.8, 18.0, 14.0, -4.8, -4.9; HREIMS $[M]^+$ 434.28466 (434.28525 calcd for $C_{25}H_{42}O_4Si$), 434.3 (7%), 359.2 (17%), 284.2 (24%), 159.1 (100%), 101.0 (59%).

Aromatic elimination product 49

(4*R*)-tetrahydro-4-hydroxy-6-[2-((1*S*,2*S*),-1,2,3,4-tetrahydro-2,6-dimethyl-1-naphthalenyl)ethyl]-2H-pyran-2-one



A sample of 11.0 mg (0.025 mmol) of **48** and 5.4 mg (0.063 mmol, 2.5 eq) NaI were dissolved in 1 mL CH_3CN. To this mixture was added 6.4 μL (5.5 mg, 0.050 mmol,

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

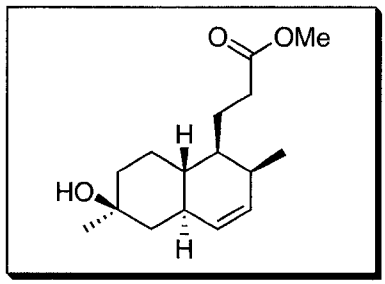
2.0 eq) chlorotrimethylsilane (TMSCl) and the mixture stirred at rt for 1 h. At this point 8.2 mg (0.126 mmol, 5 eq) Zn powder and 0.5 mL HOAc were added and the reaction mixture was stirred an additional 1 h. The mixture was filtered through a cotton plug, diluted with 30 mL CH_2Cl_2 and extracted with satd $NaHCO_3$ (1 × 10 mL). The organic layer was washed with H_2O (3 × 10 mL), dried (Na_2SO_4), filtered and evaporated to give 7.0 mg (92%) of **49**.

$[\alpha]_D^{25}$ = + 34.1(c = 1.0, $CHCl_3$); R_f = 0.62 (50% EtOAc in hexane); FTIR ($CHCl_3$ cast) 3434 (br s), 2948 (s), 2922 (s), 1711 (s), 1254 (s), 1069 (m) 1040 (m); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.91 (ap d, 1H, *J* = 7.5 Hz,), 6.83 (ap d, 1H, J = 7.5 Hz), 4.80 – 4.74 (m, 1H), 4.24 – 4.39 (m, 1H), 2.90 (dd, 1H, *J* = 13.0, 4.5 Hz), 2.87 – 2.82 (m, 2H), 2.76 (dd, 1H, *J* = 17.7, 5.3 Hz), 2.69 – 2.62 (m, 3H), 2.36 (dd, 1H, *J* = 16, 10.5 Hz), 2.28 (s, 3H), 2.01 – 1.97 (m, 1H), 1.94 – 1.89 (m, 1H), 1.84 – 1.68 (m, 5H), 1.36 (dd, 1H, *J* = 13.0, 6.0 Hz), 1.34 (dd, 1H, *J* = 13.0, 6.0 Hz), 1.32 (dd, 1H, *J* = 13.0, 6.0 Hz), 1.02 (d, 3H, *J* = 6.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.0, 137.4, 135.0, 134.4, 133.1, 127.6, 127.1, 75.7, 62.9, 38.8 (× 2), 36.0, 34.6, 31.9, 28.7, 26.5, 24.6, 21.8, 19.6; HREIMS $[M]^+$ = 302.1874 (302.1882 calcd for $C_{19}H_{26}O_3$) 302.2 (19%), 284.2 (6%), 199.1 (21%), 198 (100%), 172.1 (46%), 157.1 (29%).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

β-Oxidation product 50

Methyl 3-((1*S*,2*S*,4a*R*,6*S*,8a*R*)-1,2,4a,5,6,7,8,8a-octahydro-2,6-dimethyl-6-hydroxy-naphthalen-1-yl)-propanoate



The title compound, β-oxidation product **50**, was isolated from a biotransformation experiment as follows. *A. nidulans* growth media (1 L) was inoculated with 10 mL of a concentrated suspension of *Aspergillus terreus lovA* spores and incubated at 30 °C and 200 rpm for 2 days. To the growing culture was added a solution of unlabelled 4a,5–dihydromonacolin L (**11**) (30 mg) and 52.5 nCi (ca. 0.5 mg) of ^{14}C–4a,5–dihydromoancolin L (**11**) in 6 mL EtOH. This dihydromonacolin L solution (0.5 mL) was added to the cultures three times a day until completely used. After seven days total growth, the mycelia were removed by vacuum filtration, the broth was acidified with 2 N HCl (pH < 2) and extracted with CH_2Cl_2 (1 × 1 L, 1 × 0.5 L). The combined organic layers were dried (Na_2SO_4), filtered and evaporated to give 208 mg crude extract. The crude extract was fractionated by preparative TLC (EtOAc R_f = 0.34) to give a fraction (26.3 mg) containing the majority of the radioactivity. A portion of this radioactive fraction was treated with CH_2N_2 (Et_2O soln.), and fractionated by preparative

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

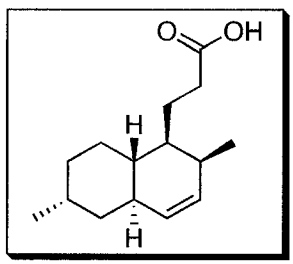
TLC (EtOAc R_f = 0.43) to isolate β-oxidation product **50** as the major radioactive component (1.7 mg, 3.4 nCi).

This same product could be isolated from the CH_2Cl_2 extract of *A. nidulans lovB + lovC* production cultures grown as described above. A portion (23.2 mg) of the crude CH_2Cl_2 extract was treated with CH_2N_2 (Et_2O soln) to give 26.1 mg crude methylated product which was fractionated by HPLC to give 2.2 mg pure β-oxidation product **50**.

FTIR (cast) 3297 (b), 3008 (s), 1737 (s), 1437 (m), 1223 (s), 1170 (s) cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 5.58 (ddd, 1H, *J* = 9.5, 4.5, 2.5 Hz, H-3), 5.30 (d, 1H, *J* = 9.5 Hz, H-4), 3.65, (s, 3H, OCH_3), 2.37 (ddd, 1H, *J* = 15, 10, 5 Hz, H-$10_{a/b}$), 2.24 (m, 1H, H-2), 2.18 (ddd, 1H, *J* = 15, 9.5, 6.5 Hz, H-$10_{a/b}$), 1.89 (m, 2H, H-9), 1.80, 1.78 (d, 1H, *J* = 2.5, H-4_a), 1.69, 1.67, 1.47, 1.23 (s, 3H, 6-CH_3), 1.02, 0.83 (d, 3H, *J* = 7 Hz, 2-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.3, 132.9, 130.3, 71.2, 51.6, 47.1, 41.1, 41.0, 40.8, 39.2, 32.0, 31.9, 27.0, 26.1, 24.3, 14.8; HREIMS $[M-H_2O]^+$ = 248.1773 (248.1763 calcd. for $C_{16}H_{24}O_2$) , 217.1 (18%), 192.1 (14%), 161.1 (100%), 119.1 (52%), 105.1 (78%).

Heptaketide free acid 52

3-((1*S*,2*S*,4a*R*,6*R*,8a*R*)-1,2,4a,5,6,7,8,8a-octahydro-2,6-dimethyl-naphthalen-1-yl)-propanoic acid



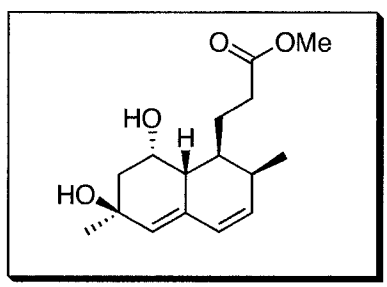
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Heptaketide **52** was isolated from cultures of *A. nidulans lovb+lovC* as follows. *A. nidulans lovB+lovC* production cultures (4 L) were fermented as described for the isolation of **11**. These were extracted with CH_2Cl_2 (2 × 1.5 L) *without* acidification of the culture broth. The organic layer was dried (Na_2SO_4), filtered and evaporated to give ca. 50 mg of crude residue. Purification by flash column chromatography (EtOAc 100%) gave 6.5 mg of pure **52**.

$[\alpha]^{20}_D$+120.3° (*c* 0.10, $CHCl_3$); FTIR (cast) 3020, 2952, 2915, 2860, 1696, 1445, 1411, 1377, 1310, 1297, 1267, 1238, 1211, 720 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.57 (ddd, 1H, *J* = 10.0, 5.0, 3.0 Hz), 5.28 (d, 1H, *J* = 10.0 Hz), 2.42 (ddd, 1H, *J* = 15.5, 10.0, 5.0 Hz), 2.26-2.19 (m, 2H), 2.00 (m, 1H), 1.96-1.97 (m, 2H,), 1.60-1.44 (m, 5H), 1.36 (m, 1H), 1.25 (dt, 1H, *J* = 13.0, 4.5 Hz), 1.10 (dq, 1H, *J* = 12.0, 4.0 Hz), 1.02-0.94 (m, 1H), 0.96 (d, 3H, *J* = 7.5 Hz), 0.82 (d, 3H, *J* = 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.9, 132.3, 131.5, 41.0, 39.9, 38.9, 37.3, 32.3, 32.0, 31.9, 27.5, 23.8, 23.6, 18.2, 15.0; HREIMS $[M]^+$ 236.1777 (236.1776 calcd. for $C_{15}H_{24}O_2$) 236.2 (12%), 221.1 (4%), 181.1 (25%), 176.1 (65%), 163.1 (100%).

β-Oxidation product methyl ester 53

Methyl 3-((1*S*,2*S*,6*S*,8*S*,8a*R*)-1,2,6,7,8,8a-hexahydro-8-hydroxy-2,6-dimethyl-6-hydroxy-naphthalen-1-yl)-propanoate



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

β-Oxidation metabolite **53** was isolated as follows. *A. terreus lovA* spores were used to inoculate flasks (2 × 100 mL) of *A. nidulans* growth media. After 4 days the mycelia were harvested by filtration and transferred to *A. nidulans* production media (2 × 100 mL). After 48 hours, a solution of monacolin J (**12**) in EtOH (30 mg in 3.0 ml EtOH) was administered in intervals (0.5 mL of above solution every 12 h until completely used). After seven days total growth in the production media, the culture broth was harvested by vacuum filtration. The combined broth was acidified and extracted with EtOAc (2 × 100 ml). The combined organic extracts were dried, filtered and evaporated to give 42.0 mg crude extract. The crude extract was fractionated by preparative TLC (EtOAc – Hexane 4:1 $R_f = 0.25$) to give a crude sample of **53**. This was further purified (after treatment with CH_2N_2 (Et_2O soln.) to give 0.1 mg of the methyl ester of **(53)**.

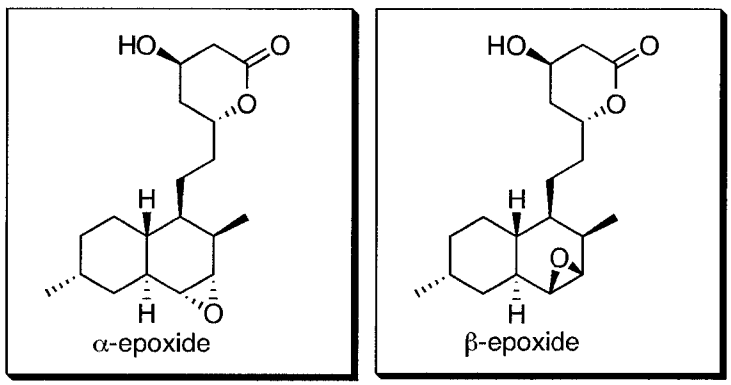
^{1}H NMR (600 MHz $CDCl_3$) δ 5.95 (d, 1H, J = 10.0 Hz), 5.75 (dd, 1H, J = 9.1, 6.2 Hz), 5.55 (m, 1H), 4.28 (m, 1H), 3.68 (s, 3H), 2.50–2.20 (m, 6H), 1.80–1.20 (m, 10H), 1.16 (d, 3H, J = 7.4 Hz), 0.88 (d, 3H, J = 7.0 Hz).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Dihydromonacolin L epoxide (54a) and (54b)

α-epoxide **54a** = (4*R*)-tetrahydro-4-hydroxy-6-[2-((1*S*,2*R*,3*R*,3a*R*,6*R*,7a*S*,8*R*)-decahydro-2,6-Dimethyl-1-oxa-cyclopropa[*a*]naphthalen-3-yl)-ethyl]-2H-pyran-2-one

β-epoxide **54b** = (4*R*)-tetrahydro-4-hydroxy-6-[2-((1*R*,2*R*,3*R*,3a*R*,6*R*,7a*S*,8*S*)-decahydro-2,6-Dimethyl-1-oxa-cyclopropa[*a*]naphthalen-3-yl)-ethyl]-2H-pyran-2-one



A sample of 50.0 mg (0.163 mmole) dihydromonacolin L (**11**) was dissolved in 4 mL of $CHCl_3$ under Ar. To this solution was added dropwise 50.1 mg (0.244 mmol, 1.5 eq) of *meta*-chloroperbenzoic acid (85% purity) dissolved in 1 mL of $CHCl_3$. The mixture was stirred under Ar at room temperature for 90 min. The mixture was diluted with 25 mL of CH_2Cl_2 and extracted with 5% $NaHSO_3$ (1 × 10 mL), satd. $NaHCO_3$ (1 × 10 mL), and H_2O (3 × 10 mL). The organic layer was dried (Na_2SO_4), filtered and evaporated to give 54.1 mg crude product, which was fractionated by flash column chromatography (40% EtOAc in hexanes, R_f = 0.27 EtOAc) to yield 11.3 mg of **54a** and 11.2 mg of **54b**. (44% total yield of pure **54a** and **54b**).

For dihydromonacolin L α-epoxide (**54a**): $[\alpha]_D^{25}$ = +90 (c 0.95, $CHCl_3$); FTIR (cast) 3392 (b), 2915 (s), 2876 (s), 1709 (s), 1257 (s), 1044 (s), 842 (s); ^{1}H NMR (500

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

MHz, $CDCl_3$) δ 4.63 (dddd, 1H, *J* = 11.0, 11.0, 7.6, 3.8), 4.36 (m, 1H), 2.98 (d, 1H, *J* = 2.4 Hz), 2.72 (d, 1H, J = 3.7 Hz), 2.69 (d, 1H, *J* = 5.0 Hz), 2.61 (dd, 1H, *J* = 3.6, 1.7 Hz), 2.56 (dd, 1H, *J* = 3.7, 1.7 Hz), 2.30 m, 1H), 2.05 (m, 2H), 1.93 (m, 2H), 1.8–1.6 (m, 2H), 1.6–1.3 (m, 10H), 0.95 (d, 3H, *J* = 7.1 Hz), 0.89 (d, 3H, *J* = 7.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4, 76.0, 62.8, 58.8, 57.5, 38.6, 38.8, 37.4, 36.3, 36.2, 36.1, 33.0, 32.0, 30.3, 27.6, 23.9, 23.7, 18.1, 10.2; HREIMS $[M]^+$ = 322.2145 (322.2144 calcd. for $C_{19}H_{30}O_4$) 322 (5%), 307 (5%), 191 (48%), 179 (100%), 95 (65%).

For dihydromonacolin L β-epoxide **(54b)**: ^1H MNR (600 MHz, $CDCl_3$) δ 4.65 (dddd, 1H, *J* = 11.5, 11.5, 7.3, 4.0 Hz), 4.37 (m, 1H), 3.17, (dd, 1H, *J* = 3.9, 5.5 Hz), 2.91 (d, 1H, *J* = 3.9 Hz), 2.71 (dd, 1H, *J* = 17.6, 5.0 Hz), 2.60 (ddd, 1H, *J* = 17.6, 3.7, 1.7 Hz), 2.15 (m, 1H), 2.05 (m, 1H), 1.90 (m, 2H), 1.75 (m, 2H), 1.65–1.40 (m, 8H), 1.20 (m, 2H), 0.97 (d, 3H, *J* = 7.2 Hz), 0.92 (d, 3H, *J* = 7.1 Hz); ^{13}C NMR (125 MHZ, $CDCl_3$) δ 170.2, 75.8, 62.8, 58.2, 57.0, 41.5, 38.6, 37.6, 35.9, 35.6, 34.6, 33.2, 31.7, 28.9, 26.9, 23.2, 22.7, 18.0, 9.5.

Crystal data for dihydromonacolin L α-epoxide (54a)

This experiment was performed by Dr. Robert MacDonald of the University of Alberta X-ray crystallography laboratory. Data were acquired on a Bruker P4/RA/SMART 1000 CCD diffractometer. All intensity measurements were performed using graphite monochromated Mo-Kα radiation (λ = 0.71073 Å). Dihydromonacolin L α-epoxide **(53a)** ($C_{19}H_{30}O_4$) was obtained as white crystals, space group $P2_12_12_1$ (No. 19), *a* = 5.5318 (11), *b* = 9.7123 (19), *c* = 32.163 (7) Å, *V* = 1728.0 (6) $Å^3$, *Z* = 4, *T* = -80°C, ρ_{calcd} = 1.239 g cm^{-3}, μ = 0.085 mm^{-1}. 9765 reflections measured, 3482 unique which were used in all least squares calculations, $R_1(F)$ = 0.0538 (for 2478 reflections with $F_o^2 \geq 2\sigma$

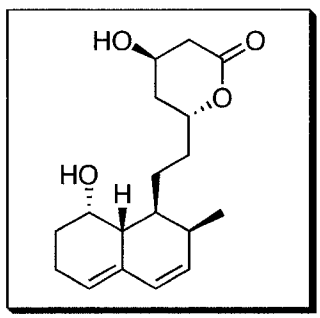
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(F_o^2)), $wR_2(F^2)$ = 0.1044 for all unique reflections). The absolute stereochemistry of **54a** cannot be directly determined from the data, but it is correct as shown based on conversion of **11** to **1** and to **54a**. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Center.

6–Desmethylmonacolin J (55)

(4*R*)-tetrahydro-4-hydroxy-6-[2-((1*S*,2*S*,8*S*,8a*R*)-1,2,6,7,8,8a-hexahydro-8-hydroxy-2-methyl-1-naphthalenyl)ethyl]-2H-pyran-2-one

CAS Registry Number [58889-19-3]



A solution of compactin (**2**) (55 mg, 0.13 mmol) and LiOH (60 mg, 1.3 mmol) in H_2O (15 mL) was heated at reflux for one day. The reaction mixture was cooled in an ice bath, acidified with 2 N HCl (pH < 1) and extracted with EtOAc (5 × 30 mL) and $CHCl_3$ (4 × 20 mL). The combined organic extracts were dried (Na_2SO_4), filtered and evaporated. The resulting residue was taken up in toluene (60 mL) and heated at reflux in a Sohxlet containing CaH_2 for 1 h. After cooling the toluene was evaporated and the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

residue fractionated by flash column chromatography (EtOAc 100%, R_f = 0.11) to yield 30 mg of **55** as a colorless oil (75%).

Desmethylmonacolin J (**55**) could also be isolated from fermentation cultures of *P. aurauantigrisum* grown as described for the production of compactin (**2**).

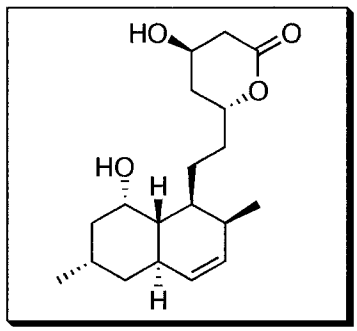
$[\alpha]_D^{25}$ = +78 (c 0.15, $CHCl_3$); FTIR (cast) 3407 (br s), 2928 (s), 1710 (s), 1256 (s), 1075 (s), 754 (s); ^{1}H NMR (500 MHz $CDCl_3$) δ 5.92 (d, 1H, *J* = 9.6 Hz), 5.71 (dd, 1H, *J* = 9.6, 6.0 Hz), 5.52 (br d, 1H, *J* = 2.1 Hz), 4.69 (m, 1H), 4.52 (m, 1H), 4.21 (br s, 1H), 2.67 (dd, 1H, *J* = 17.7, 5.0 Hz), 2.59 (ddd, 1H, *J* = 17.7, 3.7, 1.7 Hz), 2.31 (m, 2H), 2.14 (m, 2H), 1.96 (m, 2H0, 1.82–1.62 (m, 5H), 1.53–1.39 (m, 2H), 0.88 (d, 3H, *J* = 7.0 Hz); ^{13}C NMR (75 MHZ, $CDCl_3$) δ 170.8, 133.3, 133.0, 128.3, 123.6, 76.2, 64.4, 62.6, 38.8, 38.5, 36.4, 36.1, 32.6, 30.8, 29.1, 23.8, 20.3, 13.9; HREIMS $[M]^+$ 306.1824 (306.1831 calcd for $C_{18}H_{26}O_4$), (306.1, 1%), 288.1 (5.5%), 272.3 (6%), 185.1 (37%), 171.1 (9%), 169.1 (10%), 145.1 (100%).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Dihydromonacolin J (56)

(4*R*)-tetrahydro-4-hydroxy-6-[2-((1*S*,2*S*,4a*R*,6*S*,8*S*,8a*R*)-1,2,4a,5,6,7,8,8a-octahydro-8-hydroxy-2,6-dimethyl-1-naphthalenyl)ethyl]-2H-pyran-2-one

CAS Registry Number [79902-68-4]



A sample of 30.0 mg (0.071 mmole) 4a,5–(*tert*-butyldimethylsiloxy)dihydromonacolin J **(58)** was dissolved in 4 mL THF and cooled to 0 °C. Glacial acetic acid (20 μL, 0.355 mmole, 5 eq) was added, followed 10 min later by 100 μL of a 1 M soln of tetrabutylammonium fluoride (TBAF) in THF (0.10 mmole, 1.5 eq). The mixture was stirred 2 h at 0 °C, then warmed to rt and stirred an additional 2 h. Analytical TLC (EtOAc R_f = 0.23) displayed mostly starting material so an additional 100 μL of 1M TBAF in THF was added, and the mixture was stirred at rt overnight. The solvent was evaporated, and the residue was dissolved in 20 mL CH_2Cl_2 and extracted with 0.4 M $CaCl_2$ (1 × 10 mL). This was washed with H_2O (3 × 10 mL), dried (Na_2SO_4), filtered and evaporated to give 20.0 mg crude product. Dihydromonacolin J **(56)** was purified by flash column chromatography over silica gel (1% – 5% *i*-PrOH in CH_2Cl_2) to yield 13.6 mg (60% yield) pure product.

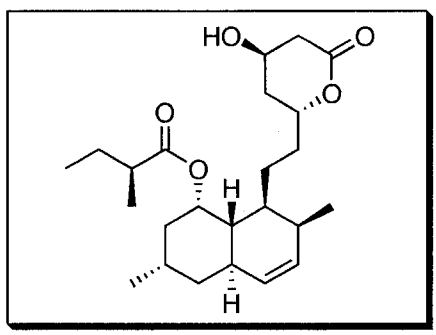
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

$[\alpha]_D^{25}$ = +137.4 (c 0.38, MeOH); R_f = 0.12 (5% i-PrOH in CH_2Cl_2) FTIR (cast), 3361 (b), 2906 (s), 1703 (s), 1259 (s), 1039 (s) cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 5.60 (ddd, 1H, *J* = 9.7, 4.8, 2.6 Hz), 5.35 (d, 1H, *J* = 10.1 Hz), 4.68 (m, 1H), 4.36 (p, 1H, *J* = 3.7 Hz), 4.14 (d, 1H, *J* = 2.8 Hz), 2.71 (dd, 1H, *J* = 18.0, 5.1 Hz), 2.60 (ddd, 1H, *J* = 18.0, 3.8, 1.8 Hz), 2.01 – 1.90 (m, 3H), 1.82 – 1.20 (m, 16 H), 1.18 (d, 3H, *J* = 7.5 Hz), 0.83 (d, 3H, *J* = 7.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.4, 132.3, 131.5, 76.1, 67.1, 62.8, 42.7, 39.3, 39.0, 38.6, 37.0, 36.3, 32.8, 31.4, 29.7, 27.0, 23.0, 21.6, 15.0; HREIMS $[M]^+$ 322.2139 (322.2144 calcd. for $C_{19}H_{30}O_4$) 322.2 (1%), 286.2 (10%), 200.1 (18%), 159.1 (79%), 105.1 (100%).

4a,5–Dihydrolovastatin (57)

((1*S*)-((1*R*, 3*S*, 4a*R*, 7*S*, 8*S*(2*S*, 4*S*), 8a*R*)-1,2,3,4,4a,7,8,8a-octahydro-3,7-dimethyl-8- (2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl) ethyl)-1-naphthyl) 2-methylbutanoate

CAS Registry Number [77517-29-4]



Compound **59** (7.7 mg, 0.0148 mmole) was dissolved in 1 mL of 10% HCl in MeOH and stirred at rt for 1 h. The mixture was diluted with 30 mL CH_2Cl_2 and extracted

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

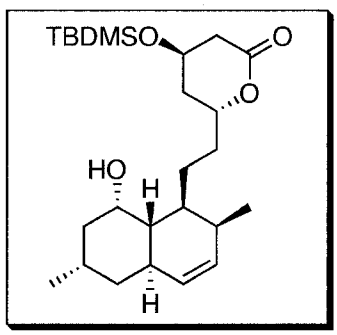
with $NaHCO_3$ (3 × 10 mL). The organic layer was washed with H_2O (3 × 10 mL), dried and evaporated to give 5.0 mg of **57**.

R_f = 0.45 (EtOAc, 100%); ^1H MNR (300 MHz $CDCl_3$) δ 5.62 (m, 1H), 5.35 (d, 1H, *J* = 9.6 Hz), 5.17 (m, 1H), 4.55 (m, 1H), 4.35 (m, 1H), 2.74 (d, 1H, *J* = 5.0 Hz), 2.68 (d, 1H, *J* = 5.0 Hz), 2.61 (dd, 1H, *J* = 3.8, 1.5 Hz), 2.52 (dd, 1H, *J* = 4.2, 1.5 Hz), 2.43 (d, 1H, *J* = 6.0 Hz), 2.4–2.2 (m, 2H), 2.05–1.75 (m, 6H), 1.70–1.40 (m, 10H), 1.15 (d, 3H, 7.0 Hz), 1.08 (d, 3H, *J* = 7.4 Hz), 0.88 (t, 3H, *J* = 8 Hz), 0.82 (d, 3H, *J* = 7.0 Hz).

4a,5 – Dihydro(*tert*-butyldimethylsilyloxy)monacolin J (58)

(4*R*)-tetrahydro-4-(*tert*-butyldimethylsilyl)oxy-6-[2-(1*S*,2*S*,4a*R*,6*S*,8*S*,8a*R*-1,2,4a,5,6,7,8,8a-octahydro-8-hydroxy-2,6-dimethyl-1-naphthalenyl)ethyl]-2H-pyran-2-one

CAS Registry Number [79902-69-5]



The following experiment was done by Dr. Joanna Harris of the University of Alberta. The iridium complex $Ir(cod)py(Pcy_3)PF_6$ was synthesized according to the procedure of Stork and Kahne.[99] The selective reduction of *tert*-butyldimethylsilyloxymonacolin J **(48)** was achieved by modification of the procedure of

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

DeCamp et al.[100] To a solution of *tert*-butyldimethylsilyloxymonacolin J **(48)** (0.103 g, 0.24 mmol) in CH_2Cl_2 (1.7 mL) was added MeOH (0.1 mL) and Ir-complex (2 mg, 2.5 μmol). The mixture was hydrogenated for 10 min. Analytical TLC (20% EtOAc in hexane R_f = 0.37) displayed presence of starting material, therefore more Ir-complex (2 mg, 2.5 μmol) was added and the mixture was hydrogenated for an additional 10 min. This process was repeated until no starting material remained by TLC (total 8 mg Ir-complex added). The mixture was passed through a pad of Florisil to remove the catalyst and the solvent was evaporated under reduced pressure to give 4a,5-(*tert*-butyldimethylsilyloxy)dihydromonacolin J **(58)** (105 mg, quant.) as a mixture (86 : 14) of the desired *olefin* and over-reduced tetra-hydro material. The tetrahydro product was removed by flash column chromatography (50% EtOAc in hexanes) to give pure 4a,5-(*tert*-butyldimethylsilyloxy)dihydromonacolin J **(58)**.

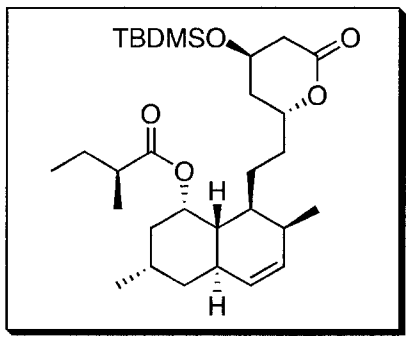
$[\alpha]_D^{25}$ = +76 (c 1.20, CH_2Cl_2); R_f = 0.30 (25% EtOAc in hexane); FTIR (cast) 3463 (b), 1733 (s), 1253 (s) cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ 5.61 (ddd, 1H, *J* = 9.7, 4.9, 2.6 Hz), 5.36 (d, 1H, *J* = 9.8 Hz), 4.66 (m, 1H,), 4.27 (m, 1H), 4.16 (m,1H), 2.59 (dd, 1H, *J* = 17.5, 4.3 Hz), 2.56 (ddd, 1H, *J* = 17.5, 3.5, 1.4 Hz), 2.52 - 1.01 (m, 20H), 1.19 (d, 1H, *J* = 7.4), 0.87 (m, 9H), 0.81 (d, 3H, *J* = 7.1), 0.06 (3 H, s), 0.05 (3 H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.0, 170.3, 132.6, 131.0, 76.3, 69.8, 63.6, 41.8, 41.7, 39.3, 38.6, 37.6, 36.8, 35.6, 33.2, 31.3, 30.9, 26.7, 26.7, 25.7, 23.1, 21.0, 16.5, 14.9, 11.7 –4.8, -4.9; HREIMS $[M]^+$ 436.3005 (436.3009 calcd. for $C_{25}H_{44}O_4Si$) 436 (1%), 418 (6%), 361 (28%), 343 (8%) 75 (100%).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(tert-Butyldimethylsilyloxy)dihydrolovastatin (59)

(1*S*-(1*R*, 3*S*, 4a*R*, 7*S*, 8*S*(2*S*, 4*S*), 8a*R*))-(1,2,3,4,4a,7,8,8a-octahydro-3, 7-dimethyl-8- (2-(tetrahydro-4-(tert-butyldimethylsilyl)oxy-6-oxo-2H-pyran-2-yl) ethyl)-1-naphthyl) 2-methylbutanoate

CAS Registry Number [85614-05-7]



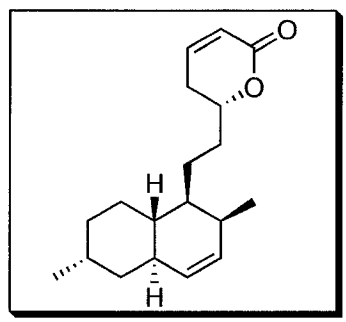
A sample of 14.0 mg (0.032 mmole) 4a,5–dihydro-*tert*-butyldimethylsilylmonaolcin J **(58)** and 5 mg (0.041 mmole) 4-dimethylaminopyridine were placed in a 1 mL React-vial (Wheaton Glass) and dissolved in 0.5 mL pyridine. To this mixture was added 100 μL (93.4 mg, 0.50 mmole) (*S*)-2-methylbutyric anhydride, and the mixture was stirred at rt for 72 h. The mixture was diluted with 30 mL CH_2Cl_2 and washed with 1 N HCl (1 × 10 mL). The organic layer was washed with H_2O, (3 × 10 mL), dried (Na_2SO_4), filtered and evaporated to give ca. 50 mg of crude residue, which was purified by flash column chromatography (10% EtOAc in hexane, R_f = 0.30 in 25% EtOAc in hexane) to give 11.9 mg (72% yield) of pure 4a,5–dihydro-(*tert*-butyldimethylsilyloxy)lovastatin **(59)**.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

$[\alpha]_D^{25}$ = +73 (c 0.20, $CHCl_3$); FTIR (cast) 2295 (s), 1727 (s), 1253 (s), 1079 (s) cm-1; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.62 (ddd, 1H, *J* = 9.5, 4.5, 2.5 Hz), 5.36 (d, 1H, *J* = 9.5 Hz), 5.15 (d, 1H, *J* = 2.5 Hz), 4.55 (m, 1H), 4.25 (p, 1H, *J* = 3.5 Hz), 2.57 (dd, 1H, *J* = 17, 4.5 Hz), 2.52 (dd, 1H, *J* = 17, 1 Hz), 2.45 (m, 1H), 2.41 (m, 1H), 2.32 (q, 1H, *J* = 7 Hz), 2.27 (m, 1H), 2.02 (m, 1H), 1.89 (d, 1H, *J* = 15 Hz), 1.83–1.80 (m, 2H), 1.72–1.44 (m, 5H), 1.32–1.22 (m, 2H), 1.17 (d, 2H, *J* = 7 Hz), 1.10 (d, 3H, *J* = 7), 1.07 (d, 3H, *J* = 7.5), 0.93 (t, 3H, *J* = 7.5 Hz), 0.86 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.0, 170.1, 132.6, 131.0, 76.3, 69.8, 63.6, 41.8, 41.7, 39.3, 38.6, 37.6, 36.8, 35.6, 33.2, 31.3, 30.9, 26.8, 26.7, 26.6, 25.6,.23.1, 21.0,.17.9, 16.5, 16.4, 14.9, 11.7, -4.8, -4.9, HREIMS $[M]^+$ = 520.3577 (520.3584 calcd for $C_{30}H_{52}O_5Si$) (520.3, 1.4%), 463.3 (1.3%), 361.2 (74%), 269.2 (17%), 227.2 (31%), 174.1 (67%), 75.0 (81%), 57.0 (100%).

α,β-Dehydro-4a,5-dihydromonacolin L (60)

6-[2-((1*S*,2*S*,4a*R*,6*R*,8a*R*)-1,2,4a,5,6,7,8,8a-octahydro-2,6-dimethyl-1-naphthalenyl)ethyl]-5,6-dihydro-pyran-2-one



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

A sample of 18.4 mg (0.060 mmole) dihydromonacolin L (**11**) was dissolved in 0.5 mL CH_2Cl_2. To this solution was added 7.1 μL (10.3 mg, 0.90 mmole, 1.5eq), methanesulfonyl chloride and 2.5 μL (18.2 mg, 0.18 mmole, 3 eq) triethylamine, and the reaction mixture was stirred at rt 1 h. The mixture was diluted with 30 mL CH_2Cl_2 and extracted with 0.2 N HCl (1 × 10 mL) and satd $NaHCO_3$ (1 × 10 mL). The organic layer was washed with H_2O (3 × 10 mL), dried (Na_2SO_4), filtered and evaporated to give 21.0 mg crude product. Product was fractionated by preparative TLC (hexane – EtOAc, 1:1, R_f = 0.54) to give 10 mg (60%) pure α,β-dehydro-dihydromonacolin L (**60**).

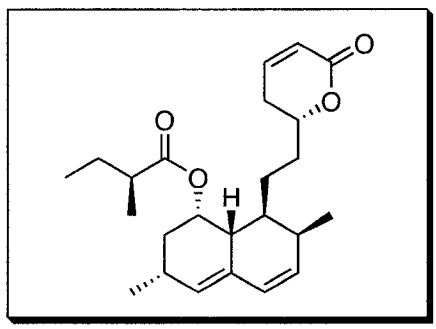
$[\alpha]_D^{25}$ = +8.6 (c 1.00, CH_2Cl_2); FTIR (cast) 2915 (s), 1717 (s), 1389 (s), 1248 (s), 818 (s) cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 6.85 (ddd, 1H, *J* = 9.5, 9.5, 4.4 Hz), 6.00 (d, 1H, *J* = 9.8 Hz), 5.57 (ddd, 1H, *J* = 9.7, 4.7, 2.8 Hz), 5.28 (d, 1H, *J* = 9.8 Hz), 4.41 (dddd, 1H, *J* = 12.3, 12.3, 7.6, 4.7 Hz), 2.33 (m, 2H), 2.21 (m, 1H), 2.11 (m, 1H), 1.91–1.87 (m, 2H), 1.62–1.41 (m, 8H), 1.34–1.22 (m, 2H), 1.08 (m, 1H), 0.99 (dd, 1H, *J* = 10.3, 2.6 Hz), 0.96 (d, 3H, *J* = 7.3 Hz), 0.83 (d, 3H, *J* = 7.0); ^{13}C NMR (150 MHz, $CDCl_3$) δ 164.3, 144.8, 132.5, 131.6, 121.3, 78.0, 41.1, 39.6, 38.3, 38.2, 37.0, 32.0, 31.8, 31.5, 29.0, 27.1, 23.2, 17.7, 14.6; HREIMS $[M]^+$ 288.20866 (288.20892 calcd. for $C_{19}H_{28}O_2$) (19%), 273.2 (17%), 228.2 (22%), 176.1 (85%), 161.1 (71%), 105.1 (100%).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

α,β-Dehydrolovastatin (61)

(1*S*)-[(1*R*(*R**),3*R*,7*R*,8*R*(2*S**,4*S**),8a*R*)]-[1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-(6-oxo-2,3-dihydro-pyran2-yl)ethyl]-1-naphthalenyl] 2-methyl-butanoate

CAS Registry Number [109273-98-5]



Lovastatin (**1**) (33.0 mg, 0.082 mmol) was dissolved in 3 mL CH_2Cl_2. To this solution was added 34.7 μL (25.2 mg, 0.249 mmol, 3 eq), triethylamine, followed by 9.6 μL (14 mg, 0.123 mmol, 1.5 eq) methanesulfonyl chloride and the reaction mixture was stirred at rt for 1 h. The mixture was diluted with CH_2Cl_2 and extracted with 0.1 N HCl (1 × 10 mL), satd $NaHCO_3$ (1 × 10mL), and H_2O (3 × 10 mL). This was then dried (Na_2SO_4) and filtered to give 40.4 mg of crude residue which was fractionated by flash column chromatography (20% EtOAc in hexane, R_f = 0.50 30% EtOAc in hexane) to give 30.7 mg pure α,β-dehydrolovastatin (**61**).

$[\alpha]_D^{25}$ = +136.4 (c 3.30, CH_2Cl_2); FTIR (cast) 2963 (s), 1723 (s), 1383 (s), 1247 (s), 818 (s) cm^{-1};^{1}H NMR (600 MHz, $CDCl_3$) δ 6.83 (ddd, 1H, *J* = 9.8, 6.1, 2.4 Hz), 5.98 (ddd, 1H, *J* = 9.7, 2.6, 0.9 Hz), 5.97 (d, 1H, *J* = 10.5 Hz), 5.76 (dd, 1H, *J* = 9.5, 6.1 Hz), 5.50 (m, 1H), 5.36 (g, 1H, *J* = 3.2 Hz), 4.31 (dddd, 1H, *J* = 11.5, 11.5, 5.3, 3.5 Hz), 2.42

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(m, 1H), 2.37–2.30 (m, 3H), 2.25–2.19 (m, 2H), 1.96–1.87 (m, 3H), 1.69–1.59 (m, 3H), 1.51–1.28 (m, 4H), 1.08 (d, 3H, J = 7.0 Hz), 1.05 (d, 3H, J = 7.4 Hz), 0.87 (d, 3H, J = 7.0 Hz), 0.85 (t, 3H, J = 7.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.6, 164.2, 144.7, 133.0, 131.6, 129.7, 128.3, 121.5, 78.5, 67.7, 41.4, 37.3, 36.6, 32.7, 32.4, 30.7, 29.5, 27.4, 26.8, 24.2, 22.8, 16.2, 13.8, 11.7; HREIMS $[M]^+$ 386.2453 (386.2457 calcd. for $C_{24}H_{34}O_4$) 386.2 (2%), 284.2 (17%), 198.1 (64%), 159.1 (100%).

3.3 Biotransformation Experiments

3.3.1 Production of ^{13}C labeled monacolin N (33)

A. nidulans production media (3 × 1L each in a 2 L Erlenmeyer) were inoculated with freshly rinsed *A. nidulans lovB* + *lovC* mycelia following the procedure described for dihydromonacolin L **(11)** production. The cultures were maintained at 30 °C and 200 rpm for 48 h, at which point their growth was supplemented with 0.5 g sodium [1-^{13}C] acetate (isotopic purity 99%) and 0.5 g NaOAc per day for the next 5 days. The cultures were harvested and extracted as described for dihydromonacolin L **(11)** production. This procedure yielded 313 mg of crude residue. Monacolin N **(33)** was purified from this residue by a series of chromatographic separations (40% EtOAc in hexane) over silica gel to give 2.0 mg of ^{13}C enriched monacolin N **(33)** (^{13}C enrichment ca. 50%).

3.3.2 Biotransformation of [^{13}C] monacolin N (33)

A 2 L Erlenmeyer flask containing 1 L of *A. nidulans* growth media was inoculated with spores of *A. terreus lovC*. After growth at 200 rpm and 30 °C for 52 h the mycelia were filtered through miracloth and thoroughly rinsed with 2 L of sterile 1% lactose solution, then transferred to 1 L of *A. nidulans* production media and shaken at

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

200 rpm and 30 °C for 48 h. At this point a solution of 9.0 mg monacolin N (**33**) (2.0 mg ^{13}C enriched and 7.0 mg natural abundance) in 6 mL EtOH was administered in 1 mL portions at 4 h intervals until completely used (i.e. 24 h). The cultures were incubated an additional 12 h and then harvested by vacuum filtration. The broth was acidified with conc HCl (pH < 2) and extracted with CH_2Cl_2 (1 × 1 L, 1 × 0.5 L). The organic layer was dried (Na_2SO_4), filtered and evaporated to give 28.1 mg crude culture extract. This extract (24.1 mg) was applied in $CHCl_3$ to a 22.8 cm × 1.27 cm column of silica gel packed with 40% EtOAc in hexane. The column was eluted with 40% EtOAc in hexane and 20 mL fractions were collected and combined according to TLC. The tubes were combined in to five fractions and each fraction examined by ^1H NMR. In addition the column was flushed with 500 mL 10% MeOH in CH_2Cl_2 and the residue from this wash examined by ^1H NMR. In no instance could any amount of transformed **33** be detected by NMR. However from this purification ca. 2 mg of **33** was recovered unchanged.

3.3.3 Control experiment (biotransformation of dihydromonacolin L (11))

A 1 L culture of *A. terreus lovC* mycelia in *A. nidulans* production media was prepared as described above for monacolin N (**33**). After 48 h growth at 200 rpm and 30 °C a solution of 13.6 mg [^{13}C] dihydromonacolin L (**11**) (ca. 50% enrichment) in 6 mL EtOH was added in 1 mL portions every 4 h until completely used (i.e. 24 h). The ^{13}C enriched dihydromonacolin L (**11**) was purified from the sodium [1-^{13}C] acetate supplemented *A. nidulans lovB* + *lovC* culture extract described in the above section. The *A. terreus lovC* culture was harvested 12 h after the final feeding and extracted in a manner identical to that described above to give 40.2 mg crude extract. This extract was

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

fractioned by flash column chromatography to give 1.7 mg ^{13}C enriched lovastatin (**1**) and 2.2 mg recovered **11** as identified by ^{1}H NMR spectroscopy.

3.3.4 Attempted biotransformation of 24

A cell free extract was prepared from *A. nidulans lovB + lovC* mycelia as described previously.[37] A solution of ^{13}C labeled **24** was added to 50 mL of cell free extract and incubated overnight with 20 mg malonyl-CoA, 10.0 mg FAD, 32.6 mg NADPH, at which point the enzyme buffer was extracted with CH_2Cl_2 (3 × 10 mL) and the organic layer was dried (Na_2SO_4), filtered and evaporated to give 10 mg of crude residue. This residue was examined by high field NMR, however no trace of **11** could be detected.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

4. References

(1) Downton, C.; Clark, I. *Nat. Rev. Drug. Discov.* **2003**, *2*, 343-343.

(2) Endo, A. H., K. *Nat. Prod. Rep.* **1993**, *10*, 541 - 550.

(3) Alberts, A. W.; Chen, J.; Kuron, G.; Hunt, V.; Huff, J.; Hoffman, C.; Rothrock, J.; Lopez, M.; Joshua, H.; Harris, E.; Patchett, A.; Monaghan, R.; Currie, S.; Stapley, E.; Albersschonberg, G.; Hensens, O.; Hirshfield, J.; Hoogsteen, K.; Liesch, J.; Springer, J. *Proc. Natl. Acad. Sci. USA* **1980**, *77*, 3957-3961.

(4) Endo, A. *J. Antibiot.* **1979**, *32*, 852-854.

(5) Endo, A.; Kuroda, M.; Tsujita, Y. *J. Antibiot.* **1976**, *29*, 1346-1348.

(6) Brown, A. G.; Smale, T. C.; King, T. J.; Hasenkamp, R.; Thompson, R. H. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1165-1173.

(7) Serizawa, N.; Nakagawa, K.; Tsujita, Y.; Terahara, A.; Kuwano, H. *J. Antibiot.* **1983**, *36*, 608-610.

(8) Serizawa, N.; Nakagawa, K.; Hamano, K.; Tsujita, Y.; Terahara, A.; Kuwano, H. *J. Antibiot.* **1983**, *36*, 604-607.

(9) Hoffman, W. F.; Alberts, A. W.; Anderson, P. S.; Chen, J. S.; Smith, R. L.; Willard, A. K. *J. Med. Chem.* **1986**, *29*, 849-852.

(10) Mauro, V. F.; Macdonald, J. L. *DICP Ann. Pharmacother.* **1991**, *25*, 257-264.

(11) Repic, O.; Prasad, K.; Lee, G. T. *Org. Process Res. Dev.* **2001**, *5*, 519-527.

(12) Roth, B. D.; Blankley, C. J.; Chucholowski, A. W.; Ferguson, E.; Hoefle, M. L.; Ortwine, D. F.; Newton, R. S.; Sekerke, C. S.; Sliskovic, D. R.; Stratton, C. D.; Wilson, M. W. *J. Med. Chem.* **1991**, *34*, 357-366.

(13) Carswell, C. I.; Plosker, G. L.; Jarvis, B. *Drugs* **2002**, *62*, 2075-2085.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(14) Vaczek, D. *Pharmacy Times* **2003**, 20-21.

(15) Liao, J. K. *Int. J. Cardiol.* **2002**, *86*, 5-18.

(16) Chapman, J.; Shepherd, J. *Atherosclerosis Supp.* **2003**, *4*, 1-2.

(17) Collins, R.; Armitage, J.; Parish, S.; Sleight, P.; Peto, R. *Lancet* **2003**, *361*, 2005-2016.

(18) Michikawa, M. *J. Neurosci. Res.* **2003**, *72*, 141-146.

(19) Crisby, M.; Carlson, L. A.; Winblad, B. *Alz. Dis. Assoc. Dis.* **2002**, *16*, 131-136.

(20) Stuve, O.; Youssef, S.; Steinman, L.; Zamvil, S. S. *Curr. Opin. Neurol.* **2003**, *16*, 393-401.

(21) Wong, W. W. L.; Dimitroulakos, J.; Minden, M. D.; Penn, L. Z. *Leukemia* **2002**, *16*, 508-519.

(22) Kmietowicz, Z. *Brit. Med. J.* **2001**, *323*, 1145-1145.

(23) Istvan, E. S.; Deisenhofer, J. *Science* **2001**, *292*, 1160-1164.

(24) Staunton, J.; Weissman, K. J. *Nat. Prod. Rep.* **2001**, *18*, 380-416.

(25) Rawlings, B. J. *Nat. Prod. Rep.* **1999**, *16*, 425-484.

(26) Birch, A. J.; Massy, R. A.; Moye, C. J. *Aus. J. Chem.* **1955**, *8*, 539-544.

(27) Simpson, T. J. *Top. Curr. Chem.* **1998**, *195*, 1-48.

(28) Kawaguchi, A.; Iwamoto-Kihara, A.; Sato, N. In *Comprehensive Natural Products Chemistry*; Sankawa, U., Ed.; Elsevier Science Ltd.: Oxford, UK, 1999; Vol. 1, pp 1-23.

(29) Bentley, R.; Bennett, J. W. *Annu. Rev. Microbiol.* **1999**, *53*, 411-446.

(30) Rawlings, B. J. *Nat. Prod. Rep.* **2001**, *18*, 190–227.

(31) Shen, B. *Curr. Opin. Chem. Biol.* **2003**, *7*, 285-295.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(32) Donadio, S.; Mcalpine, J. B.; Sheldon, P. J.; Jackson, M.; Katz, L. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 7119-7123.

(33) Rodriguez, E.; McDaniel, R. *Curr. Opin. Microbiol.* **2001**, *4*, 526-534.

(34) Fu, H.; Khosla, C. *Mol. Divers.* **1996**, *1*, 121-124.

(35) Wagschal, K.; Yoshizawa, Y.; Witter, D. J.; Liu, Y. Q.; Vederas, J. C. *J. Chem. Soc., Perkin Trans. 1* **1996**, 2357-2363.

(36) Moore, R. N.; Bigam, G.; Chan, J. K.; Hogg, A. M.; Nakashima, T. T.; Vederas, J. C. *J. Am. Chem. Soc.* **1985**, *107*, 3694-3701.

(37) Kennedy, J.; Auclair, K.; Kendrew, S. G.; Park, C.; Vederas, J. C.; Hutchinson, C. R. *Science* **1999**, *284*, 1368-1372.

(38) Hendrickson, L.; Davis, C. R.; Roach, C.; Nguyen, D. K.; Aldrich, T.; McAda, P. C.; Reeves, C. D. *Chem. Biol.* **1999**, *6*, 429-439.

(39) Auclair, K.; Sutherland, A.; Kennedy, J.; Witter, D. J.; Van den Heever, J. P.; Hutchinson, C. R.; Vederas, J. C. *J. Am. Chem. Soc.* **2000**, *122*, 11519-11520.

(40) Laschat, S. *Angew. Chem. Int. Edit.* **1996**, *35*, 289-291.

(41) Ichihara, A.; Oikawa, H. In *Comprehensive Natural Products Chemistry*; Sankawa, U., Ed.; Elsevier Science Ltd.: Oxford, UK, 1999; Vol. 1, p 367–409.

(42) Stocking, E. M.; Williams, R. M. *Angew. Chem. Int. Edit.* **2003**, *42*, 3087–3115.

(43) Abe, Y.; Suzuki, T.; Ono, C.; Iwamoto, K.; Hosobuchi, M.; Yoshikawa, H. *Mol. Genet. Genomics* **2002**, *267*, 636-646.

(44) Hutchinson, C. R.; Kennedy, J.; Park, C.; Kendrew, S.; Auclair, K.; Vederas, J. *Anton. Leeuw. Int. J. G.* **2000**, *78*, 287-295.

(45) Staunton, J.; Wilkinson, B. *Top. Curr. Chem.* **1998**, *195*, 49-92.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(46) Townsend, C. A.; Minto, R. E. In *Comprehensive Natural Products Chemistry*; Sankawa, U., Ed.; Elsevier Science Ltd.: Oxford, UK, 1999; Vol. 1, p 443–473.

(47) Watanabe, C. M. H.; Townsend, C. A. *J. Am. Chem. Soc.* **1998**, *120*, 6231-6239.

(48) Hsieh, D. P. H.; Lin, M. T.; Yao, R. C.; Singh, R. *J. Agric. Food Chem.* **1976**, *24*, 1170-1174.

(49) Shoolingin-Jordan, P. M.; Campuzano, I. D. G. In *Comprehensive Natural Products Chemistry*; Sankawa, U., Ed.; Elsevier Science Ltd.: Oxford, UK, 1999; Vol. 1, p 345–367.

(50) Udwary, D. W.; Casillas, L. K.; Townsend, C. A. *J. Am. Chem. Soc.* **2002**, *124*, 5294-5303.

(51) Summers, R. G.; Donadio, S.; Staver, M. J.; WendtPienkowski, E.; Hutchinson, C. R.; Katz, L. *Microbiology* **1997**, *143*, 3251-3262.

(52) Carreras, C.; Frykman, S.; Ou, S.; Cadapan, L.; Zavala, S.; Woo, E.; Leaf, T.; Carney, J.; Burlingame, M.; Patel, S.; Ashley, G.; Licari, P. *J. Biotechnol.* **2002**, *92*, 217-228.

(53) Rix, U.; Fischer, C.; Remsing, L. L.; Rohr, J. *Nat. Prod. Rep.* **2002**, *19*, 542-580.

(54) Sorensen, J. L.; Auclair, K.; Kennedy, J.; Hutchinson, C. R.; Vederas, J. C. *Org. Biomol. Chem.* **2003**, *1*, 50-59.

(55) Auclair, K.; Kennedy, J.; Hutchinson, C. R.; Vederas, J. C. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1527-1531.

(56) Groves, J. T.; Mcclusky, G. A. *J. Am. Chem. Soc.* **1976**, *98*, 859-861.

(57) Nakamura, T.; Komagata, D.; Murakawa, S.; Sakai, K.; Endo, A. *J. Antibiot.* **1990**, *43*, 1597-1600.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(58) Silverman, R. B.; *The Organic Chemistry of Enzyme Catalyzed Reactions*; Revised Edition.; Academic Press: San Diego, 2002, p 193–205.

(59) Newcomb, M.; Toy, P. H. *Acc. Chem. Res.* **2000**, *33*, 449-455.

(60) Korth, H. G.; Trill, H.; Sustmann, R. *J. Am. Chem. Soc.* **1981**, *103*, 4483-4489.

(61) Treiber, L. R.; Reamer, R. A.; Rooney, C. S.; Ramjit, H. G. *J. Antibiot.* **1989**, *42*, 30-36.

(62) Auclair, K. Ph.D. Thesis, *Biosynthetic Studies on the Polyketide Lovastatin : Enzyme-catalyzed Diels-Alder Reactions*; University of Alberta: Edmonton, Alberta, Canada, 1999.

(63) Yue, S.; Duncan, J. S.; Yamamoto, Y.; Hutchinson, C. R. *J. Am. Chem. Soc.* **1987**, *109*, 1253-1255.

(64) Cane, D. E.; Yang, C. C. *J. Am. Chem. Soc.* **1987**, *109*, 1255-1257.

(65) Liu, Y. Q.; Li, Z.; Vederas, J. C. *Tetrahedron* **1998**, *54*, 15937-15958.

(66) Marshall, J. A.; Garofalo, A. W. *J. Org. Chem.* **1993**, *58*, 3675-3680.

(67) Sundararaman, P.; Walker, E. C.; Djerassi, C. *Tetrahedron Lett.* **1978**, 1627-1628.

(68) Reich, H. J.; Reich, I. L.; Renga, J. M. *J. Am. Chem. Soc.* **1973**, *95*, 5813–5815.

(69) Clive, D. L. J. *Tetrahedron* **1978**, *34*, 1049-1132.

(70) Patel, D. V.; Vanmiddlesworth, F.; Donaubauer, J.; Gannett, P.; Sih, C. J. *J. Am. Chem. Soc.* **1986**, *108*, 4603-4614.

(71) Mayr, H.; Baran, J.; Will, E.; Yamakoshi, H.; Teshima, K.; Nojima, M. *J. Org. Chem.* **1994**, *59*, 5055-5058.

(72) Abe, I.; Morita, H.; Nomura, A.; Noguchi, H. *J. Am. Chem. Soc.* **2000**, *122*, 11242-11243.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(73) Abe, I.; Takahashi, Y.; Lou, W. W.; Noguchi, H. *Org. Lett.* **2003**, *5*, 1277-1280.

(74) Sorensen, J. L.; Vederas, J. C. *Chem. Commun.* **2003**, 1492-1493.

(75) Pfeifer, B. A.; Khosla, C. *Microbiol. Mol. Biol. Rev.* **2001**, *65*, 106-+.

(76) Blackwell, C. M.; Davidson, A. H.; Launchbury, S. B.; Lewis, C. N.; Morrice, E. M.; Reeve, M. M.; Roffey, J. A. R.; Tipping, A. S.; Todd, R. S. *J. Org. Chem.* **1992**, *57*, 1935-1937.

(77) Ghosh, A. K.; Lei, H. *J. Org. Chem.* **2002**, *67*, 8783-8788.

(78) Fernandes, R. A.; Kumar, P. *Eur. J. Org. Chem.* **2002**, 2921-2923.

(79) Reddy, M. V. R.; Brown, H. C.; Ramachandran, P. V. *J. Organomet. Chem.* **2001**, *624*, 239-243.

(80) Reddy, P. P.; Yen, K. F.; Uang, B. J. *J. Org. Chem.* **2002**, *67*, 1034-1035.

(81) Blackwell, C. M.; Davidson, A. H.; Launchbury, S. B.; Lewis, C. N.; Morrice, E. M.; Reeve, M. M.; Roffey, J. A. R.; Tipping, A. S.; Todd, R. S. *J. Org. Chem.* **1992**, *57*, 5596-5606.

(82) Araki, Y.; Konoike, T. *J. Org. Chem.* **1997**, *62*, 5299-5309.

(83) Rosen, T.; Heathcock, C. H. *J. Am. Chem. Soc.* **1985**, *107*, 3731-3733.

(84) Theisen, P. D.; Heathcock, C. H. *J. Org. Chem.* **1988**, *53*, 2374-2378.

(85) Karanewsky, D. S.; Malley, M. F.; Gougoutas, J. Z. *J. Org. Chem.* **1991**, *56*, 3744-3747.

(86) Funk, R. L.; Zeller, W. E. *J. Org. Chem.* **1982**, *47*, 180-182.

(87) Askin, D.; Verhoeven, T. R.; Liu, T. M. H.; Shinkai, I. *J. Org. Chem.* **1991**, *56*, 4929-4932.

(88) Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190–6191.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(89) Hutchins, R. O.; Kandasamy, D.; Maryanoff, C. A.; Masilamani, D.; Maryanoff, B. E. *J. Org. Chem.* **1977**, *42*, 82-91.

(90) Hutchins, R. O.; Kandasamy, D.; Dux, F.; Maryanoff, C. A.; Rotstein, D.; Goldsmith, B.; Burgoyne, W.; Cistone, F.; Dalessandro, J.; Puglis, J. *J. Org. Chem.* **1978**, *43*, 2259-2268.

(91) Morita, T.; Okamoto, Y.; Sakurai, H. *Synthesis* **1981**, 32-33.

(92) Baltazar, M. F.; Dickinson, F. M.; Ratledge, C. *Microbiology* **1999**, *145*, 271-278.

(93) Valenciano, S.; DeLucas, J. R.; Pedregosa, A.; Monistrol, I. F.; Laborda, F. *Arch. Microbiol.* **1996**, *166*, 336-341.

(94) Prueksaritanont, T.; Ma, B.; Fang, X. J.; Subramanian, R.; Yu, J.; Lin, J. H. *Drug. Metab. Dispos.* **2001**, *29*, 1251-1255.

(95) Jacobsen, W.; Kirchner, G.; Hallensleben, K.; Mancinelli, L.; Deters, M.; Hackbarth, I.; Benet, L. Z.; Sewing, K. F.; Christians, U. *Drug. Metab. Dispos.* **1999**, *27*, 173-179.

(96) Tsujita, Y.; Kuroda, M.; Shimada, Y.; Tanzawa, K.; Arai, M.; Kaneko, I.; Tanaka, M.; Masuda, H.; Tarumi, C.; Watanabe, Y.; Fujii, S. *Biochim. Biophys. Acta* **1986**, *877*, 50-60.

(97) de Montellano, P. R. O.; De Voss, J. J. *Nat. Prod. Rep.* **2002**, *19*, 477-493.

(98) Miller, V. P.; Fruetel, J. A.; Demontellano, P. R. O. *Arch. Biochem. Biophys.* **1992**, *298*, 697-702.

(99) Stork, G.; Kahne, D. E. *J. Am. Chem. Soc.* **1983**, *105*, 1072-1073.

(100) Decamp, A. E.; Verhoeven, T. R.; Shinkai, I. *J. Org. Chem.* **1989**, *54*, 3207-3208.

(101) Willard, A. K.; Smith, R. L. *J. Labelled. Compd. Radiopharm.* **1982**, *19*, 337-344.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(102) Hanessian, S.; Roy, P. J.; Petrini, M.; Hodges, P. J.; Difabio, R.; Carganico, G. *J. Org. Chem.* **1990**, *55*, 5766-5777.

(103) Witter, D. J.; Vederas, J. C. *J. Org. Chem.* **1996**, *61*, 2613-2623.

(104) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923-2925.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.