DISSERTATION

STUDIES ON THE TOTAL SYNTHESIS OF PUTATIVE INTERMEDIATES IN THE BIOSYNTHESIS OF TAXOL

Submitted by

Steven Robert Lenger

Department of Chemistry

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY STEVEN ROBERT LENGER ENTITLED STUDIES ON THE TOTAL SYNTHESIS OF PUTATIVE INTERMEDIATES IN THE BIOSYNTHESIS OF TAXOL BE ACCEPTED AS FULFILLING IN PART THE REQUIREMENTS OF THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Advisor

Department Head

ABSTRACT OF DISSERTATION

STUDIES ON THE TOTAL SYNTHESIS OF PUTATIVE INTERMEDIATES IN THE BIOSYNTHESIS OF TAXOL

The exploration of several strategies for the synthesis of taxoids useful for studying the biosynthesis of taxol is presented. The approaches described include attempts to access the taxane carbon skeleton through closure of the B-ring across the C-2 – C-3 bond or the C-10 – C-11 bond, as well as attempts to form the A- and B-rings using an intramolecular Diels-Alder reaction.

Attempts to close the C-2 – C-3 bond employed an allyl silane to attack either a ketone, aldehyde, or acetal to close the B-ring in an intramolecular Sakurai reaction. The preparation of four substrates to suited to undergo this type of ring closure is described.

Attempts to close the B-ring across the C-10 – C-11 bond focused on converting a vinyl halide to the corresponding vinyl chromium or vinyl palladium and closing the B-ring through either a Nozaki-Hiyama-Kishi reaction or a Heck reaction. The preparation of six substrates suited to undergo ring closure through this means is described.

Attempts to form the A- and B-rings using an intramolecular Diels-Alder reaction were made. The preparation of three substrates suited to undergo ring closure through this means is described.

Steven Robert Lenger

Department of Chemistry

Colorado State University

Fort Collins, CO 80523

Spring 2003

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Chapter 1

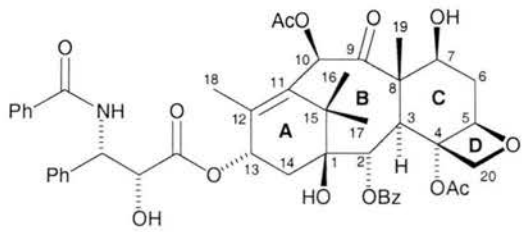
Taxol: Use, Production, Biosynthesis, and Synthesis

1.1 Objective

The work described herein has been undertaken with the goal of synthesizing compounds that could potentially be intermediates in the pathway by which taxol is biosynthesized in the Pacific Yew and other organisms using related enzymatic machinery.[1] Possessing methodology that would allow for the synthesis of such intermediates enriched in either stable or unstable isotopes would be invaluable in the effort to elucidate this biosynthetic pathway.

1.2 Taxol use and production

Taxol (**1**, Scheme 1.1) is a compound that generated much excitement in both the chemical and the biological communities after its isolation in 1970 by Wall and Wani[2]. In early studies, taxol showed excellent activity against a range of cancers, and it has matured into an important agent for the treatment of a number of solid tumors.[3] For the four-year period from 1998 through 2001, Bristol-Myers Squibb reported taxol sales of greater than $5.47 billion, an indication of how widely used taxol has become.



Taxol, 1

Scheme 1.1 Taxol numbering and ring designations

While taxol's unique structure and potentially useful activity made it an attractive target for chemists, its unusual mode of action made it a subject of much biological interest. Taxol has been shown to stabilize microtubules, structures that are intimately involved in determining cell shape and organelle position and movement[4]. When microtubules are stabilized, the cellular reorganization necessary for cell division cannot occur, effectively arresting the cell cycle at the transition between the G2 stage of interphase and the prophase stage of mitosis. This activity has both facilitated study of the cell cycle and lies at the heart of taxol's efficacy as an anti-neoplasitic agent.[5]

1.2.1 Clinical application and cost of treatment

Either by itself or in combination with cisplatin, taxol is used in the treatment of patients who suffer from breast cancer, ovarian cancer, non-small-cell lung cancer, and Kaposi's sarcoma, a type of cancer that affects the skin and mucous membranes.[6] Due to its poor solubility in water, taxol is administered intravenously as a solution in 1:1 polyoxyethylated castor oil and ethanol,[7] with an infusion time of three hours for a typical dose of 300 mg.

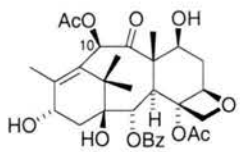
A typical treatment regimen for a patient suffering from breast cancer involves the repeated infusion of a 300 mg dose every three weeks for 3 months.[8] The cost of the 1.20 g of taxol required for this course of treatment was approximately $11,000 in November of 2002.[9] Even by modern pharmaceutical standards, this is an astronomical amount.

1.2.2 Taxol production

To a first approximation, the high cost of taxol is a function of its scarcity. Taxol occurs naturally in the bark of the Pacific Yew, *Taxus brevifolia*, from which it was

initially isolated. A full-grown yew tree yields about 10 kg of bark, and the isolation of taxol from yew bark proceeds in 0.01-0.02% yield, so each mature tree can provide 1-2 g of taxol.[5] Unfortunately, removal of a tree's bark is lethal, so this is not a truly renewable resource. Taxol isolated in this manner provided the material used for initial clinical trials and for early chemotherapeutic application.

Current production through semisynthesis, as carried out by Bristol-Myers Squibb, is significantly more ecologically friendly. Baccatin III (**2**) and 10-deacetylbaccatin III (**3**) can be isolated from judiciously removed twigs and needles of the European Yew, *Taxus baccata* (Scheme 1.2). The net isolation yield of baccatin III from this process is 0.1-0.2%, a significant improvement on direct taxol isolation, and most importantly, starts from a resource that is theoretically renewable. Starting with baccatin III, the semisynthesis consists of protection of the C-7 hydroxyl group, coupling to a side-chain equivalent, and deprotection of the C-7 hydroxyl group, a process that proceeds in 85-90% yield[10].



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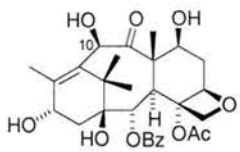


3

Scheme 1.2 Baccatin III (**2**) and 10-deacetylbaccatin III (**3**)

As will be discussed in Section 1.6, a great deal of excellent synthetic work has been done in the pursuit of taxol and related taxanes, including seven total syntheses of taxol. While these syntheses constitute impressive and often elegant pieces of work, they unmistakably establish that total synthesis is not capable of generating commercially useful amounts of taxol. The most concise and high-yielding of these syntheses, that

reported by Wender's group, requires 36 linear steps, proceeding with an approximate overall yield of 0.5% from (*R*)-pinene. This underscores the fact that future production of taxol and related drugs will rely on some kind of biological system to provide the advanced taxoids from which clinically effective agents can be assembled.

Much work has been done to identify the ideal biological system for the large-scale production of taxol and its relatives. The leading candidates in this search are cell-free biosynthesis, plant cell fermentation, and the fermentation of fungi found within various *Taxus* species.[11] The latter two techniques have shown particular promise. Fermentation of *Alternaria alternate*, for instance, yields up to 116 mg of taxol per liter, although sustaining this yield has been a problem.[12] Cultured plant tissue has proven to provide the most productive and reliable biological system for production of taxol. Methyl jasmonate elicitation of a *Taxus canadensis* cell line has been reported to generate a maximum taxol concentration of 117 mg/L, accumulating at up to 23.4 mg/L per day.[13] Taxol production in this cell line has also been shown to be enhanced by addition of acetyl-CoA.[14] In collaboration with Bristol-Myers Squibb, Phyton Inc. has commercialized a process of this nature, carrying it out on up to 75,000 liter scale, achieving taxol titers of up to 902 mg/L fermentation.[15] In order to take full advantage of this sort of system, detailed knowledge of the biosynthesis of taxol would be invaluable.

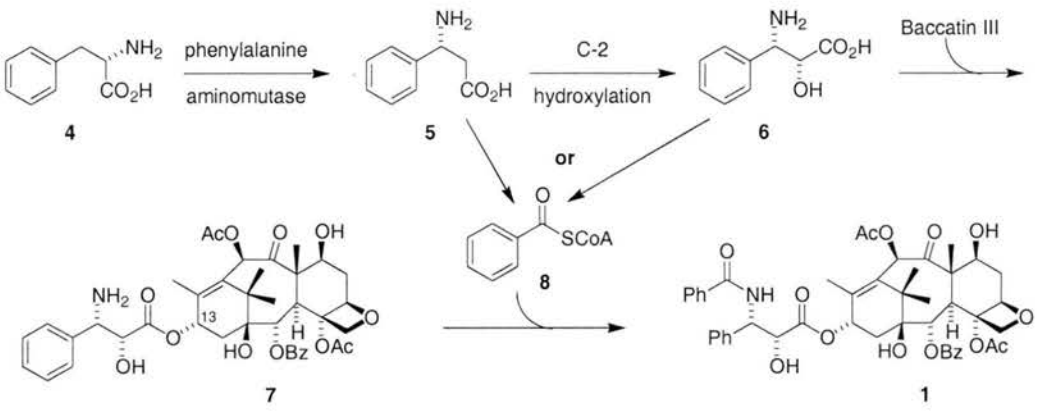
1.3 Taxol biosynthesis

The body of work directed toward the elucidation of the pathway by which taxol is biosynthesized by *Taxus* species can be roughly divided into three areas of focus, each investigated in its own period of time. Early work was aimed at learning about the last steps of the pathway, after which efforts to clarify the initial committed steps came to the

fore, and recent and ongoing work is directed toward the intervening steps, most of which must involve oxygenations and the manipulation of hydroxyl groups.

1.3.1 The end of the biosynthetic pathway: attaching the side chain

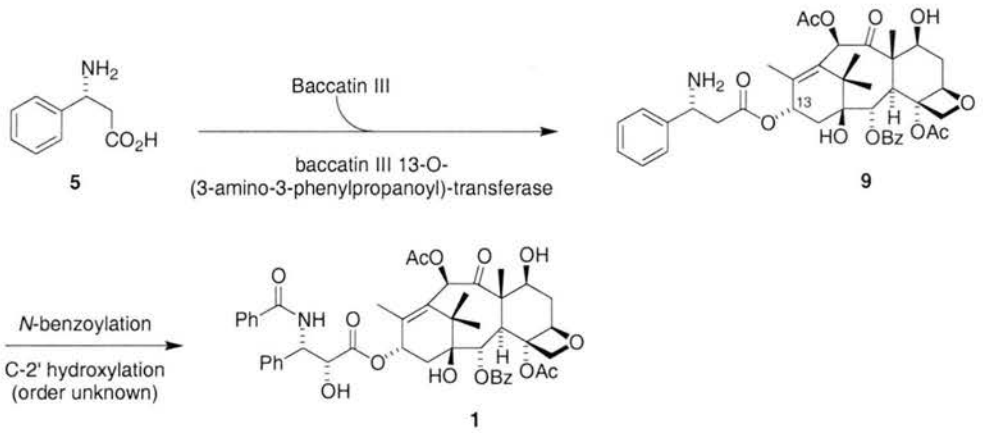
In 1993, Floss reported that the N-benzoylphenylisoserine side chain of taxol is derived entirely from phenylalanine (Scheme 1.3).[16] At that time, it was speculated that the phenylisoserine portion (**6**) was assembled in a two-step sequence consisting of the transformation of (*S*)-phenylalanine (**4**) to (*R*)-β-phenylalanine (**5**) followed by a C-2 hydroxylation, giving the required α-hydroxy-β-amino acid. The first of these steps was shown to be catalyzed by a novel phenylalanine aminomutase, which carries out an intramolecular transfer of nitrogen from the α position to the β position.[17] It was speculated that phenylisoserine was then coupled to the C-13 hydroxyl of baccatin III, giving debenzoyltaxol (**7**).[18] To this would be attached the necessary N-benzoyl group, supplied in the form of benzoyl CoA. Interestingly, this benzoyl group was shown to also be derived from phenylalanine via either β-phenylalanine (**5**) or phenylisoserine (**6**), a previously unknown means of attaining benzoyl CoA.



Scheme 1.3 Initially postulated side chain biosynthesis and attachment

In October of 2002, however, Croteau reported that the species with which

baccatin III is initially acylated is in fact (*R*)-β-phenylalanine, with the C-2' hydroxylation occurring after acylation of the C-13 hydroxyl.[19] This transformation is catalyzed by baccatin III 13-*O*-(3-amino-3-phenylpropanoyl)-transferase, an enzyme whose cDNA has been isolated and sequenced, and which, in recombinant form, shows appropriate activity. The order in which the product of this acylation, *N*-debenzoyl-2'-deoxytaxol (**9**, Scheme 1.4), undergoes N-benzoylation and C-2' hydroxylation has not yet been determined.

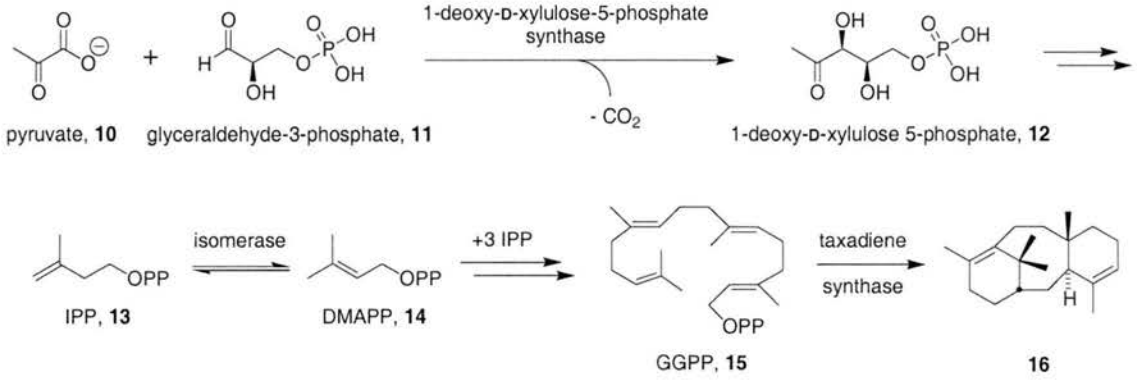


Scheme 1.4 Revised side chain biosynthesis and attachment, October 2002

1.3.2 The beginning of the biosynthetic pathway

As was the case with most terpenes and terpenoids, it was originally thought that the five-carbon isopentenyl pyrophosphate (IPP, **13**) and dimethylallyl pyrophosphate (DMAPP, **14**) units fused together to form the carbon skeleton of taxol came from acetyl CoA via the mevalonate pathway.[20] In the mid-1990s, however, Bacher demonstrated that in the case of taxoids these carbons were a product of the non-mevalonate or 1-deoxy-**D**-xylulose-5-phosphate/2-*C*-methyl-**D**-erythritol-4-phosphate pathway (Scheme 1.5).[21] Rather than starting from acetyl CoA, this pathway begins with the joining of

pyruvate (**10**) and glyceraldehyde-3-phosphate (**11**) (both produced during glycolysis) through the agency of 1-deoxy-D-xylulose-5-phosphate synthase.[22] Over the course of several steps this product is converted to IPP, after which point the mevalonate and non-mevalonate pathways are functionally indistinguishable. An isomerase ensures that the interconversion of IPP and DMAPP is facile enough to provide appropriate amounts of each building block. The head-to-tail addition of three IPP units to one molecule of DMAPP gives the next key intermediate in taxoid biosynthesis, geranylgeranyl pyrophosphate (GGPP, **15**). The last of these additions is catalyzed by GGPP synthase.[23]



Scheme 1.4 Non-mevalonate origins of taxa-4(5),11(12)-diene

1.3.3 Taxadiene synthase

In the first committed step of taxoid biosynthesis, GGPP undergoes an olefin-cation cascade catalyzed by taxadiene synthase to give taxa-4(5),11(12)-diene (**16**). The elucidation of the mechanism of this transformation has been the subject of a largely collaborative investigation carried out at the University of Washington (Floss), Washington State University (Croteau), the University of Illinois (Coates), and Colorado State University (Williams and Rithner).

An account of the first part of this investigation, conducted by the Floss and Croteau labs, was reported in 1996.[24] In this work, GGPP was synthesized in various deuterated forms and then subjected to the action of taxadiene synthase isolated from Pacific Yew stems. Analysis by mass spectrometry of the taxa-4(5),11(12)-diene isolated from these transformations provided insight about how this enzyme-mediated olefin-cation cascade might proceed (Table 1.1).

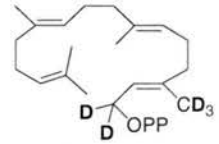
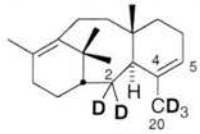
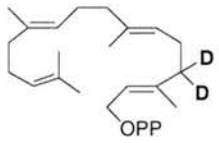
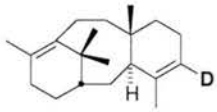
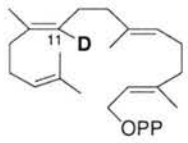
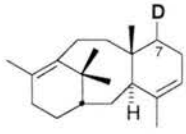
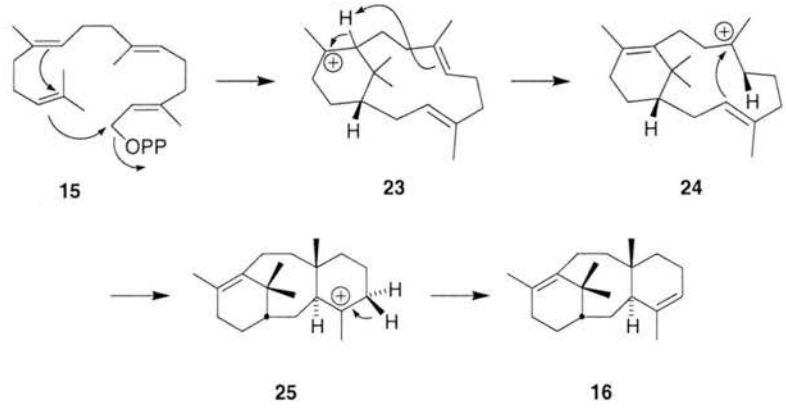
Substrate	Deuterium Atoms Lost	Suggested product
 **17**	0	 **18**
 **19**	1	 **20**
 **21**	0	 **22**

Table 1.1 Results of mass spectrometry study of taxadiene synthase products

When acting upon pentadeuterated GGPP **17**, taxadiene synthase produced taxa-4(5),11(12)-diene in which the parent ion detected by mass spectrometry was detected at *m/z* 277 rather than the *m/z* 272 observed with unlabeled GGPP. This observation demonstrated that the olefin-cation cascade likely proceeded directly to taxadiene **18** without the intervention of a 4(20) exocyclic olefin. This reaction mode was also suggested by the observation that dideuterated GGPP **19** was converted into taxa-4(5),11(12)-diene with a parent ion at *m/z* 273, indicating that one of the two deuterium atoms at what would become C-5 of the taxadiene was lost during the olefin-cation

cascade, giving rise, most likely, to taxadiene **20**. Furthermore, GGPP **21**, monodeuterated at what would eventually become the C-11 position of taxadiene gave rise to a taxa-4(5),11(12)-diene with a parent ion at *m/z* 273, indicating that the deuterium atom, which could not remain at C-11, had migrated to another position during the course of the olefin-cation cascade. Additionally, mass spectrometry showed that the fragment corresponding to the C-ring shifted from *m/z* 122 to *m/z* 123, suggesting that the product in this case was taxadiene **22**. This data indicated that the olefin-cation cascade likely proceeded by the pathway depicted in Scheme 1.6.

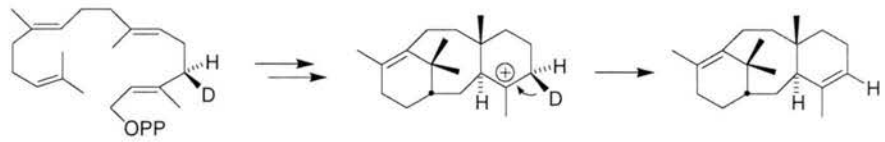


Scheme 1.6 Suggested pathway of olefin-cation cascade catalyzed by taxadiene synthase

While this part of the study shed much light on the probable sequence of events in this transformation, the evidence was somewhat circumstantial, owing to the inherent ambiguity of mass spectrometry. Once a cDNA encoding the taxadiene synthase from *Taxus brevifolia* was obtained,[25] however, overexpression of a truncated, functional version of this synthase allowed for production of sufficient amounts of taxa-4(5),11(20)-diene for more detailed study.[26] This subsequent, closer investigation included the use of asymmetrically labeled GGPP to study the facial selectivity of the C-5 proton abstraction, detailed NMR analysis to establish that the C-11 proton migrates to the C-7α position,

and molecular modeling that indicated a high probability that this migration would not require assistance.[27]

First, in a logical extension of the earlier mass spectrometry study, (4*R*)-[4-$^2H_1$]-GGPP **26** was synthesized and incubated with recombinant taxadiene synthase (Scheme 1.7). The taxa-4,(5),11(12)-diene isolated from this transformation gave a parent ion with *m/z* 272, indicating that the C-5 proton abstracted at the end of the cascade was that with a β disposition. As deduced from the rate of the transformation, a significant primary kinetic isotope effect was also observed when labeled GGPP **26** was employed as the enzyme substrate. This also led to the observation of appreciable (~10%) amounts of both the 4(20) and the 4(3) olefins.



26 **25** **16**

Scheme 1.7 Demonstration of which proton is the last to be abstracted

Second, by comparison of taxa-4(5),11(12)-diene generated by incubation of [10-$^2H_1$]-GGPP (**21**) (Table 1.1) with unlabeled taxadiene, Rithner at Colorado State University was able to confirm that the deuterium-transfer step proceeds from C-11 to C-7 (taxoid numbering). First, he assigned every proton on the C-ring using 1D DPFGSE-TOCSY and 2D NOESY-NMR. He then demonstrated that in the deuterium-bearing taxadiene the C-7α signal disappears, confirming this step of the proposed olefin-cation cascade and giving the exact location of the transferred proton in the final product.

In the third part of the work done to get a clearer picture of how the transformation of GGPP to taxadiene proceeds, work was done in the Williams' labs at Colorado State University to model some of the suspected cationic intermediates along

the cascade pathway. Using Spartan and MacSpartan (AM1 basis set, overall molecular charge of +1, trivalent carbon at C-12), it was shown that the C-11 proton is within ~2.2 Å of the C-7 carbon and seemingly perfectly poised for the transannular migration to the *re*-face at C-7 (Figure 1.1). Both the short distance this proton would need to travel and the highly congested environment in which it exists indicate that this proton transfer probably is not mediated by an amino acid on the enzyme.

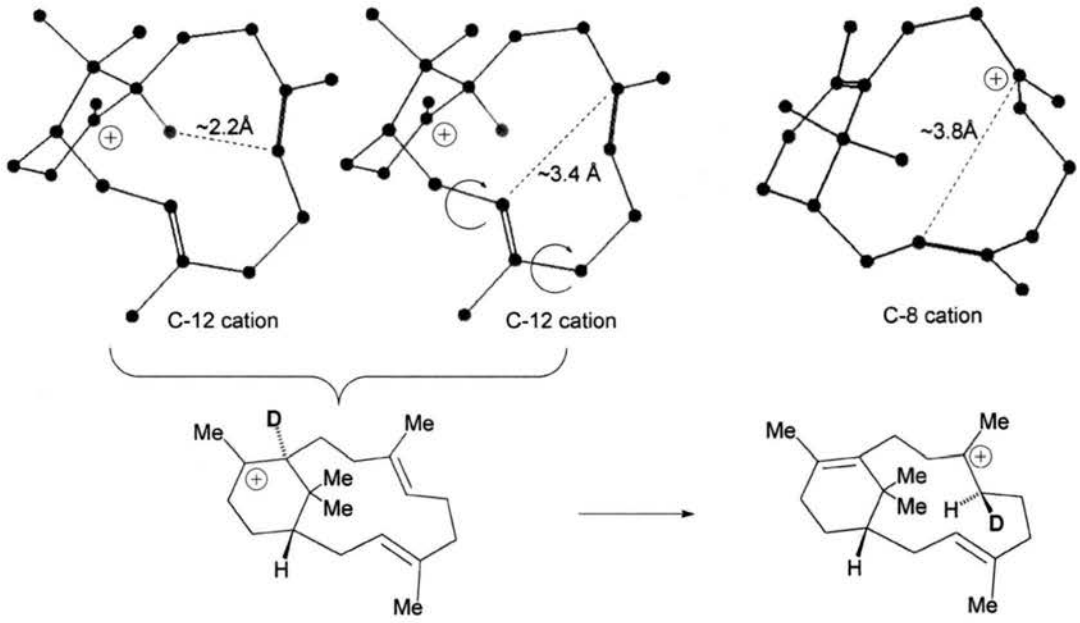


Figure 1.1 Molecular modeling of cationic intermediates in the olefin-cation cascade

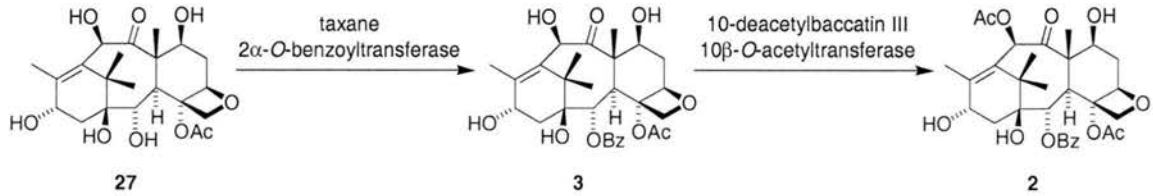
Taxadiene synthase is a remarkable terpene cyclase that appears to function by binding and ionizing its substrate GGPP to mediate an enantio- and face-selective polyolefin cation cascade that forms three carbon-carbon bonds, sets three stereogenic centers, and results in the loss of hydrogen. The seemingly unassisted intramolecular proton transfer mechanism of taxadiene synthase is unusual in this regard, suggesting that this enzyme type is capable of mediating complex olefin-cation cyclizations, with absolute stereochemical fidelity, by conformational control alone.

1.3.4 The intervening steps: attachment and manipulation of oxygen

Between the point at which taxa-4(5),11(12)-diene first appears and the point at which the process of adding the side chain to baccatin III begins, no fewer than eight oxygen atoms must be affixed to the taxane scaffold. Four of these oxygens become acylated, one undergoes oxidation to the corresponding ketone, two remain as free alcohols, and one becomes incorporated into the D-ring oxetane. The task of unraveling the secrets behind this series of manipulations is formidable. Some progress in this endeavor has been made, though, as the first three and last two steps in this sequence have been identified.

1.3.5 The last two steps leading up to baccatin III

In 2000, Croteau reported the isolation of a full-length cDNA clones for both taxane 2α-*O*-benzoyltransferase and 10-deacetylbaccatin III-10β-*O*-acetyltransferase.[28,29] The substrate for the first of these enzymes appears to be 2-debenzoyl-7,13-deacetylbaccatin III (**27**), whose benzoylation would provide 10-deacetylbaccatin III (**3**, Scheme 1.8). The identity of the substrate in this transformation was not rigorously established, though, because no naturally-occurring 2-deacetyltaxoid metabolites were available to use in assessing the activity of the recombinant enzyme. In place of the probable substrate Croteau used 2-debenzoyl-7,13-diacetylbaccatin III, which was selectively benzoylated on the C-2 hydroxyl. It was also demonstrated that this enzyme will not acylate simpler systems, as benzoylation of taxa-4(20),11(12)-2α,5α-diol[30] was unsuccessful.

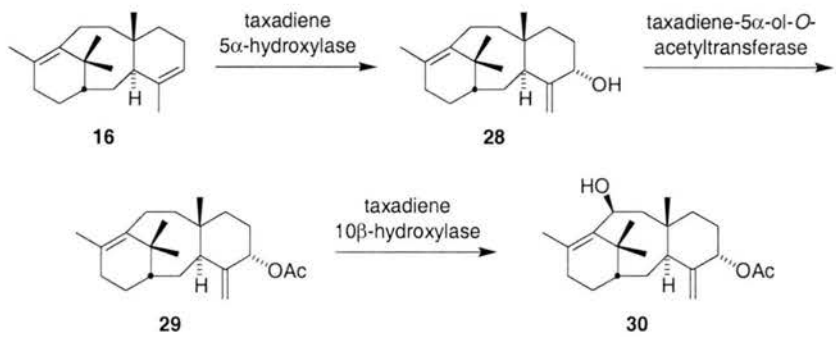


Scheme 1.8 The final acylations leading to baccatin III

After benzoylation, 10-deacetylbaccatin III 10β-*O*-acetyltransferase has been shown to selectively acetylate the C-10 hydroxyl of 10-deacetylbaccatin III. This gives baccatin III, whose involvement in taxol biosynthesis was described in Section 1.3.1 and depicted in Scheme 1.4.

1.3.6 Initial elaboration of taxa-4(5),11(12)-diene

The first oxygenation step after the formation of taxa-4(5),11(12)-diene has been shown to be catalyzed by taxadiene-5α-hydroxylase, transforming taxa-4(5),11(12)-diene into taxa-4(20),11(12)-dien-5α-ol (**28**, Scheme 1.9).[31] This alcohol, generated in tritiated form, has been confirmed as a biosynthetic intermediate leading to baccatin III and ultimately to taxol. The hydroxylase responsible for this transformation has been determined to be a membrane-bound, NADPH-dependent *Taxus* microsomal cytochrome P450 mixed-function monooxygenase, but a cDNA encoding the enzyme's sequence of amino acids has not yet been isolated.[32] It appears that this enzyme is not only



Scheme 1.9 The first three steps in the elaboration of taxa-4(5),11(12)-diene

responsible for the insertion of oxygen at C-5, but also mediates a concomitant migration of the 4(5) double bond to the 4(20) position.

In the second step in the process of elaborating taxa-4(5),11(12)-diene, taxa-4(20),11(12)-dien-5α-ol is acetylated to give taxa-4(20),11(12)-dien-5α-yl acetate (**29**). This transformation is catalyzed by taxadiene-5α-ol-*O*-acetyltransferase, an enzyme of 439 amino acid residues whose cDNA has been isolated and sequenced.[33] The recombinant enzyme has been shown to readily acetylate the hydroxyl group of taxa-4(20),11(12)-dien-5α-ol, but it will not acetylate any of the four free hydroxyls in 10-deacetylbaccatin III, an indication of its high substrate specificity.[34]

It has been demonstrated that the next step in the process of taxadiene elaboration involves the introduction of a β-disposed hydroxyl group at C-10 to give alcohol **30**. The cDNA encoding the enzyme responsible for this transformation, taxadiene-10β-hydroxylase, has been isolated and expressed in yeast (*Saccharomyces cerevisiae*).[35] Assessment of the substrate specificity has not been published to date.

1.4 Prior work toward synthesis of putative intermediates in the Williams group

When studying each transformation in a biosynthesis, it is invaluable to have access to the compound acting as substrate. Finding the enzyme responsible for each step is a process of dividing a cell's enzymes (and/or the genes encoding these enzymes) into subgroups, determining which group possesses ability to effect the desired reaction, and then further subdividing that group until the activity of interest can be assigned to a particular enzyme or combination of enzymes. This narrowing-down is simplified by having ample substrate to employ as a probe for enzyme activity in a given subgroup.

An even greater degree of certainty in this process is gained by using a substrate that contains an isotopic label. If the newly introduced isotope is stable (^{2}H or ^{13}C, for instance), the newly formed product can be correlated with the substrate employed using mass spectrometry. If the newly introduced isotope is unstable (^{3}H or ^{14}C, for instance), even minute amounts of substrate can be successfully used because product isolation can be guided by radioactivity. Both types of isotopic labeling are valuable, and so the ability to produce labeled substrates can be of central importance to the successful study of a biosynthetic pathway.

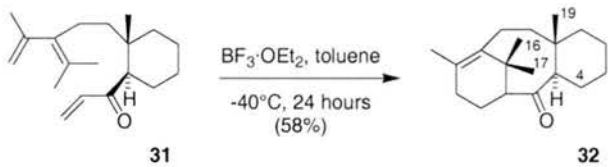
Since the early 1990s, the Williams group has been actively involved in the synthesis of compounds that either could be or have been shown to be intermediates in the biosynthesis of taxol. Both total synthesis and semi-synthesis have been employed in this pursuit, and success has been achieved with both approaches.

1.4.1 The first taxadiene synthesis and its extension to taxa-4(20),11(12)-dien-5α-ol

The first work toward the synthesis of taxol biosynthetic intermediates in the Williams group was conducted by graduate student Steven M. Rubenstein. The goal of his research was to synthesize the initial taxadiene produced in the first committed step of taxol biosynthesis. At the outset it was uncertain if this first taxane would be the 4(20),11(12) diene or the 4(5),11(12) diene, so a synthetic strategy was devised that could allow access to either.

The foundation for this strategy was provided by Jenkins, who in 1987 reported the Lewis acid-mediated conversion of triene **31** to taxane **32** in 58% yield (Scheme 1.10).[36] This was the first report of the use of an intramolecular Diels-Alder reaction to form the A and B rings of a taxoid system with both the geminal methyl groups (C-16

and C-17) and the angular methyl group (C-19) present. To be useful in the synthesis of the target taxadienes, however, a significant modification would be needed to introduce some sort of C-4 functionality from which the C-ring could be elaborated to include C-20, either as an exocyclic methylene or as an allylic methyl group.

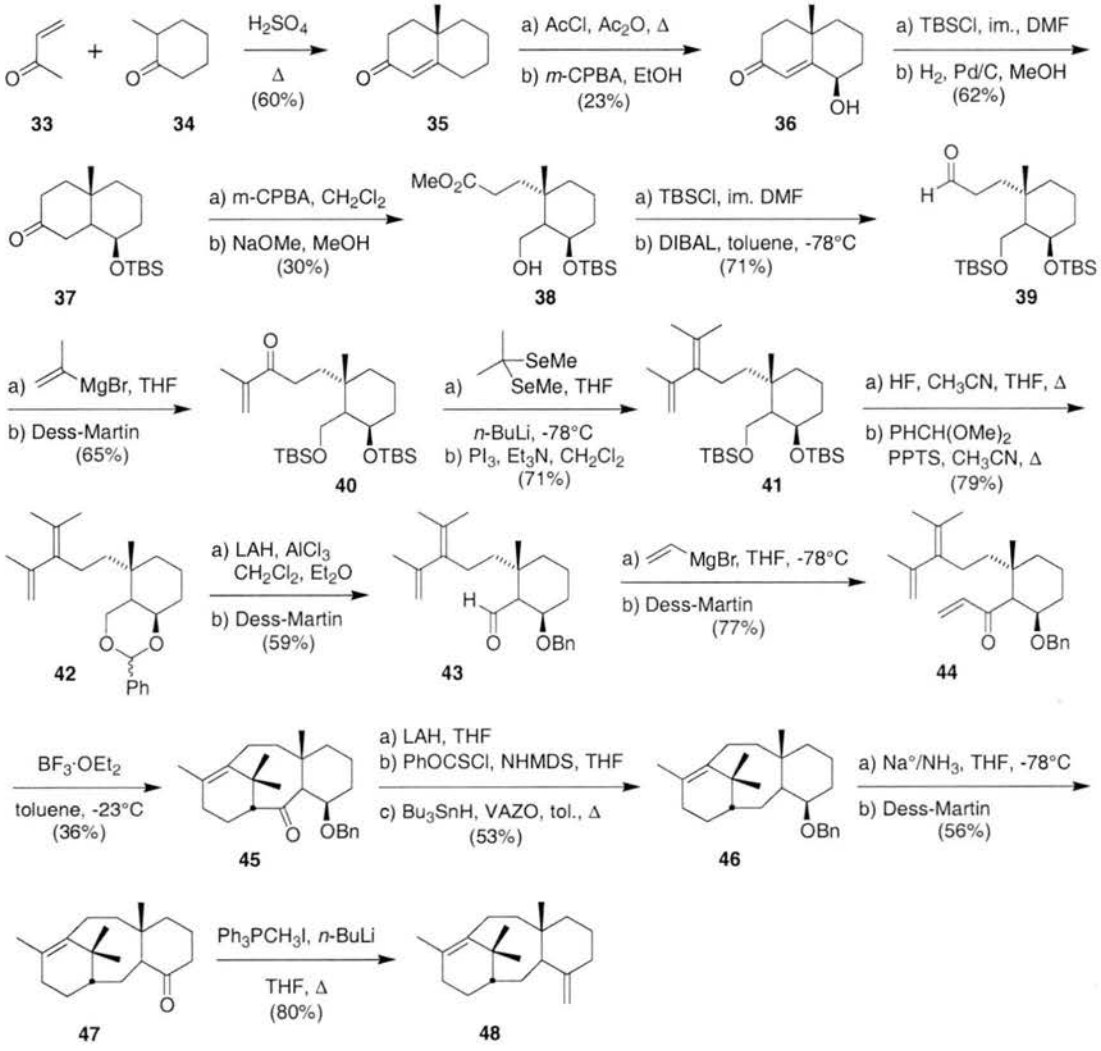


31 32

Scheme 1.10 Jenkins' successful taxoid-forming cycloaddition

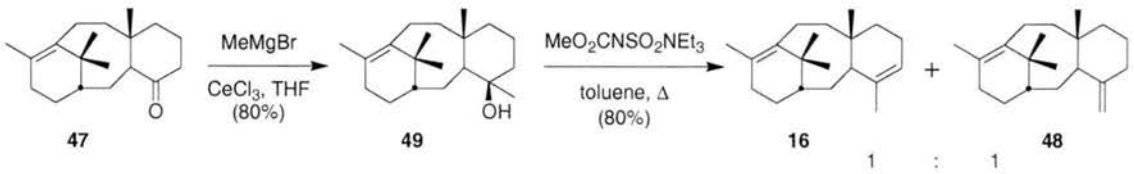
Williams and Rubenstein succeeded in functionalizing the future C-4 position by making the dienol acetate of Robinson annulation product **35** and then treating this diene with *m*-CPBA to give alcohol **36** (Scheme 1.11).[37] Protection of this alcohol as the silyl ether was followed by catalytic hydrogenation to give ketone **37**, which underwent Baeyer-Villiger oxidation to give a mixture of lactones. Hydrolysis of these lactones provided a mixture of hydroxy esters from which the required ester (**38**) could be isolated chromatographically, albeit in low yield since the major Baeyer-Villiger product (2:1 ratio) gave rise to the undesired ester. The primary hydroxyl group of ester **38** was protected as the silyl ether, after which the ester moiety was reduced to the corresponding aldehyde (**39**). To this aldehyde was added isopropenyl magnesium bromide, and the alcohol resulting from this addition was oxidized to give enone **40**. The nucleophilic α-selenoalkyllithium generated by treatment of 2,2-bis(methylseleno)propane with *n*-butyllithium attacked the carbonyl of enone **40** to give a mixture of tertiary alcohols, and PI_3-mediated elimination of the resulting hydroxyl group gave diene **41**. Next, the silyl protecting groups were removed and the resulting diol was converted to benzylidine acetal **42**. Reduction of this acetal provided a mixture of separable alcohols from which

the desired isomer could be isolated and oxidized to give aldehyde **43**. Cycloaddition substrate **44** was completed by a vinyl magnesium bromide addition followed by oxidation to the requisite enone, and the desired cycloaddition could be effected in yields as high as 36% to give ketone **45**. Reduction of this ketone was followed by Barton deoxygenation to give **46**, from which the benzyl protecting group could be removed using dissolving metal conditions. Subsequent oxidation of the resulting alcohol gave ketone **47**, and the synthesis of taxa-4(20),11(12)-diene (**48**) was completed with a Wittig olefination.



Scheme 1.11 Williams/Rubenstein taxa-4(20),11(12)-diene synthesis

Alternatively, ketone **47** could be treated with methyl magnesium bromide in the presence of cerium (III) chloride to give alcohol **49**, which upon treatment with Burgess' reagent underwent dehydration to give a chromatographically separable mixture of taxa-4(5),11(12)-diene (**16**) and taxa-4(20),11(12)-diene (Scheme 1.12).



Scheme 1.12 Modification used to generate taxa-4(5),11(12)-diene

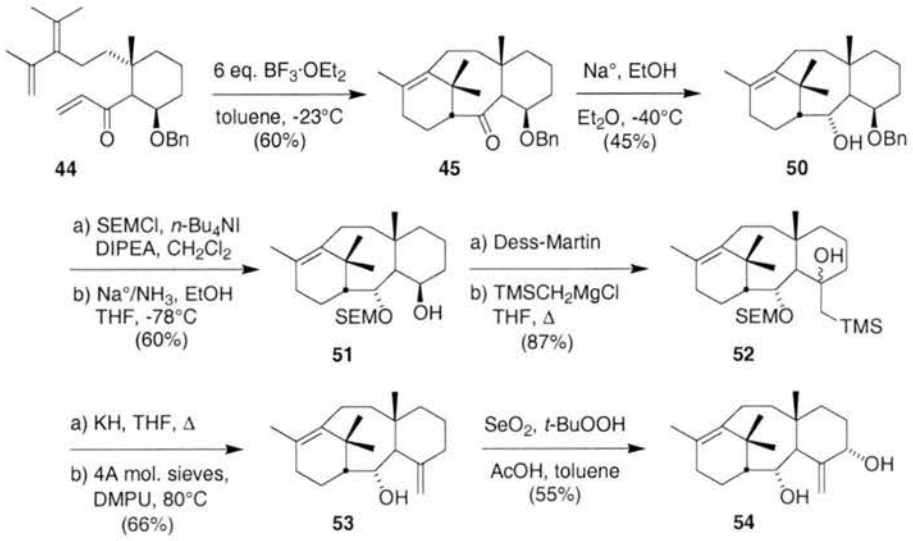
As mentioned above, taxa-4(5),11(12)-diene was shown to be the product of taxadiene synthase, and further work demonstrated that taxa-4(20),11(12)-diene was not even a transient participant in its production. While the 4(5),11(12) diene made by this synthesis was essential for the study of the subsequent biosynthetic steps, the 4(20),11(12) diene was also of value. Treatment of this diene with selenium dioxide provided a means by which taxa-4(20),11(12)-dien-5α-ol could be synthesized.[31] Since this was shown to be the product of taxadiene-5α-hydroxylase and the substrate for taxadiene-5α-ol-*O*-acetyltransferase, its synthesis was of great importance in studying these more advanced biosynthetic steps. This alcohol could also be easily converted to the corresponding acetate, the substrate upon which taxadiene-10β-hydroxylase has since been shown to act.

In addition to providing a synthesis from which several early biosynthetic intermediates could be produced, this route offered the advantage of allowing for late-stage incorporation of an isotopic label in a straight-forward manner.[38] Introducing a label into either taxa-4(20),11(12)-diene, taxa-4(20),11(12)-dien-5α-ol, or taxa-4(20),11(12)-dien-5α-yl acetate could be accomplished by carrying out the Wittig olefination of ketone **47** with an isotopically labeled phosphorus ylide. This ylide could

be made by mixing either [$^3H_3$] methyl iodide or [$^2H_3$] methyl iodide with triphenylphosphine and subsequent treatment with a strong base. The labeled taxadiene olefination product of this reaction could then be elaborated to the required alcohol or acetate. Alternatively, labeled taxa-4(5),11(12)-diene could be made using isotopically enriched methyl magnesium bromide (or iodide) to make alcohol **49**. Dehydration of the Grignard addition product would then deliver the desired taxadiene in labeled form. The same techniques have also been used to incorporate ^{13}C into the C-20 position of these biosynthetic intermediates.

1.4.2 Taxa-4(20),11(12)-dien-2α,5α-diol

The next lightly-oxygenated taxane made in the Williams group came from the efforts of Dr. Alfredo Vazquez as part of his work as a post-doctoral fellow. One of the objectives of his work was to synthesize taxa-4(20),11(12)-dien-2α,5α-diol, which was suspected as being one of the taxadiene-diols produced during the microsomal bioconversions carried out on taxa-4(20),11(12)-dien-5α-ol in *Taxus* species.



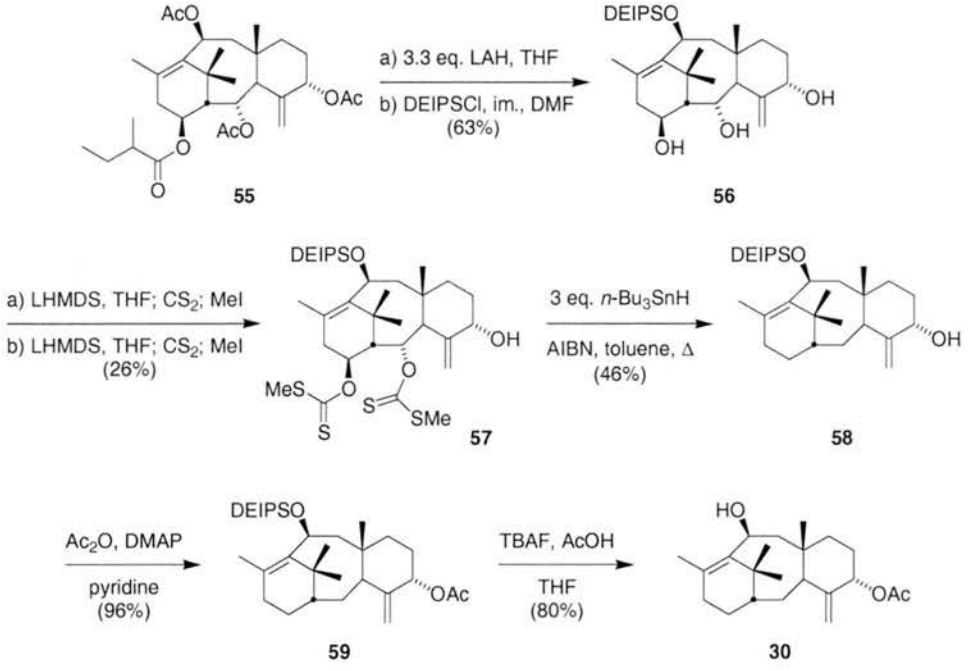
Scheme 1.13 Williams/Vazquez synthesis of taxa-4(20),11(12)-dien-2α,5α-diol

The synthesis of taxa-4(20),11(12)-dien-2α,5α-diol departed from the synthesis of the earlier taxadienes at the point of cycloaddition product **45** (Scheme 1.13).[30] Rather than reducing the carbonyl to the corresponding alcohol and carrying out a deoxygenation, ketone **45** was subjected to dissolving metal reduction using metallic sodium, giving a mixture of alcohols at the C-2 position. The alcohol with the required α-disposed hydroxyl group (**50**) could be chromatographically separated from its counterpart and then protected as the SEM ether. Removal of the benzyl protecting group gave alcohol **51**, which was oxidized to the corresponding ketone and then converted to tertiary alcohol **52** using trimethylsilylmethyl magnesium chloride. After the elimination stage of the Peterson olefination was carried out using potassium hydride in refluxing THF the SEM group was removed using warm DMPU to give free alcohol **53**. Allylic oxidation as before delivered the desired 2,5 diol (**54**) in moderate yield. This diol proved to be identical (GC-MS) to the suspected 2,5 diol isolated from the biological system.

1.4.3 Taxa-4(20),11(12)-dien-5α-acetoxy-10β-ol and taxadien-5α-acetoxy-2α,10β-diol

In addition to their success with total synthesis, the Williams group has recently enjoyed success in their semi-synthetic efforts to make more advanced putative intermediates in the biosynthesis of taxol, work carried out by Dr. Tohru Horiguchi during his time working in the group as a post-doctoral fellow. His initial target was taxa-4(20),11(12)-dien-5α-acetoxy-10β-ol, after which it was hoped taxa-4(20),11(12)-dien-5α-acetoxy-2α,10β-diol could be made using a similar strategy of selective deoxygenation.

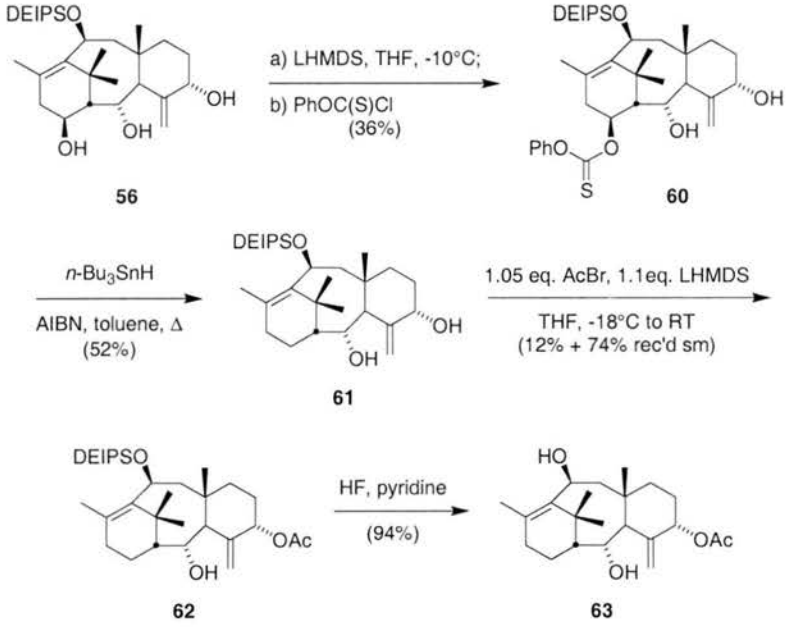
The starting material in this work was taxa-4(20),11(12)-dien-2α,5α,10β-triacetoxy-14β-(2-methyl)butyrate (**55**), a component of Japanese Yew heart wood (Scheme 1.14).[39] After removal of all of the acyl groups from **55** using lithium aluminum hydride, selective silyl protection of the C-10 hydroxyl furnished triol **56**. The C-2 and C-14 hydroxyls were converted to xanthates in two separate operations to give **57**, and the desired deoxygenations were effected under classical conditions to give alcohol **58**. After reacetylation of the C-5 hydroxyl, the silyl ether was cleaved to liberate the target taxa-4(20),11(12)-dien-5α-acetoxy-10β-ol (**30**) in 80% yield.



Scheme 1.14 Williams/Horiguchi synthesis of taxa-4(20),11(12)-dien-5α-acetoxy-10β-ol

Since the formation of dixanthate **57** occurred in two separate steps, with the initial xanthate forming at C-2, it was hoped that the difference in reactivity between the C-2 hydroxyl and the C-14 hydroxyl could be exploited to allow for the selective deoxygenation of C-14. Eventually it was found that treatment of triol **56** with lithium hexamethyldisilamide followed by trapping with phenyl thiochloroformate provided a

preparatively useful amount of a thiocarbonate (**60**) well-disposed for the desired deoxygenation (Scheme 1.15).[40] The balance of the material from formation of this thiocarbonate was useful material, consisting largely of recovered starting material and the dithiocarbonte analog of dixanthate **57**. Deoxygenation of **60** provided diol **61** in moderate yield, at which point it was necessary to selectively acetylate the C-5 hydroxyl in the presence of the free C-2 hydroxyl. After several unsuccessful efforts, conditions were found that allowed for this transformation to occur, delivering acetate **62** in a modest 12% yield, but with 74% recovery of diol **61**. Subsequent cleavage of the silyl ether from **62** delivered taxa-4(20),11(12)-dien-5α-acetoxy-2α,10β-diol (**63**) in high yield. This compound has not yet been shown to occur during taxol biosynthesis, but consideration of naturally occurring taxanes makes it or a close relative a likely candidate for inclusion.

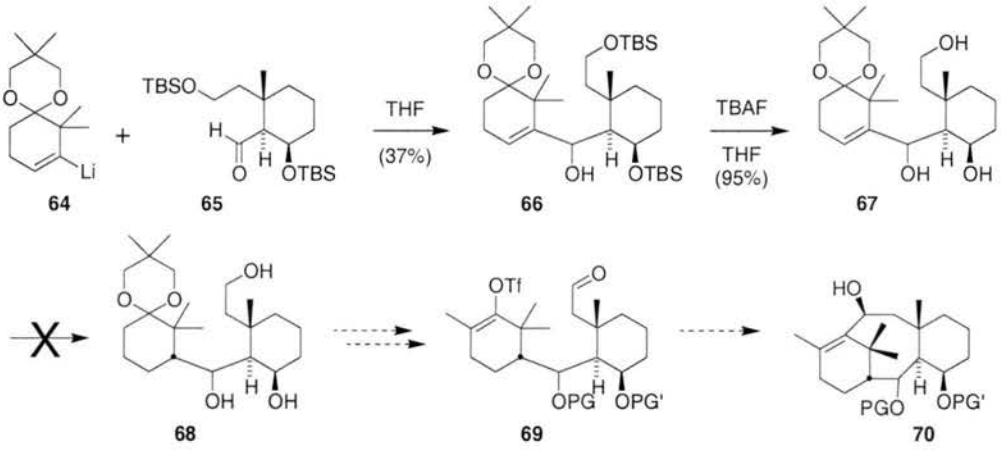


Scheme 1.15 Synthesis of taxa-4(20),11(12)-dien-5α-acetoxy-2α,10β-diol

1.4.4 Efforts aimed at the total synthesis of a C-10-hydroxy taxane

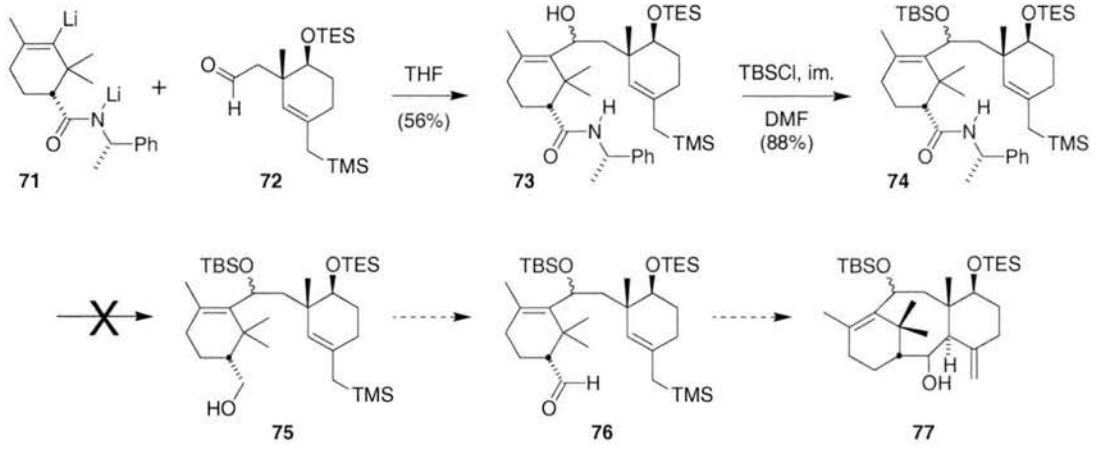
Between the successful synthesis of taxa-4(20),11(12)-dien-5α-acetate (1996) and the successful production of taxa-4(20),11(12)-dien-5α-acetate-10β-ol via semi-synthesis (2002), a great deal of effort was directed toward the production of a taxadiene that would possess functionality at C-10 amenable to the introduction of a β-disposed hydroxyl. Once it was demonstrated that this could not come from taxa-4(20),11(12)-dien-5α-ol through further allylic oxidation, the responsibility for this synthetic work belonged to Dr. Claude Quesnelle during his time working in the Williams group as a post-doctoral fellow. His efforts, though ultimately unsuccessful, provided excellent insight into the assembly of systems poised to undergo B-ring-forming cyclization and highlighted the abundant difficulties that exist in this undertaking.

These efforts can be classified into three separate avenues, two synthetic and one semi-synthetic. The first synthetic approach involved trying to achieve taxoid formation through closure of the B-ring at the C-10 – C-11 bond. Kishi had reported successful taxoid formation by a similar strategy, employing an intramolecular Nozaki-Hiyama-Kishi (NHK) reaction in the key cyclization step.[41,42] The compounds with which Kishi had reported this early success were not functionalized at C-4, so a somewhat different approach was going to be required to assemble a cyclization substrate arranged in a manner suitable for assembling the skeleton of a naturally occurring taxane. Toward this end, alcohol **66** was made by trapping organolithium species **64**, generated using a Shapiro reaction, with aldehyde **65** (Scheme 1.16). After removal of the TBS groups it was necessary to reduce the C-14 – C-1 double bond. Unfortunately, this reduction failed under a wide variety of conditions, so synthetic efforts were focused on other routes.



Scheme 1.16 Williams/Quesnelle work toward C-10 – C-11 B-ring closure

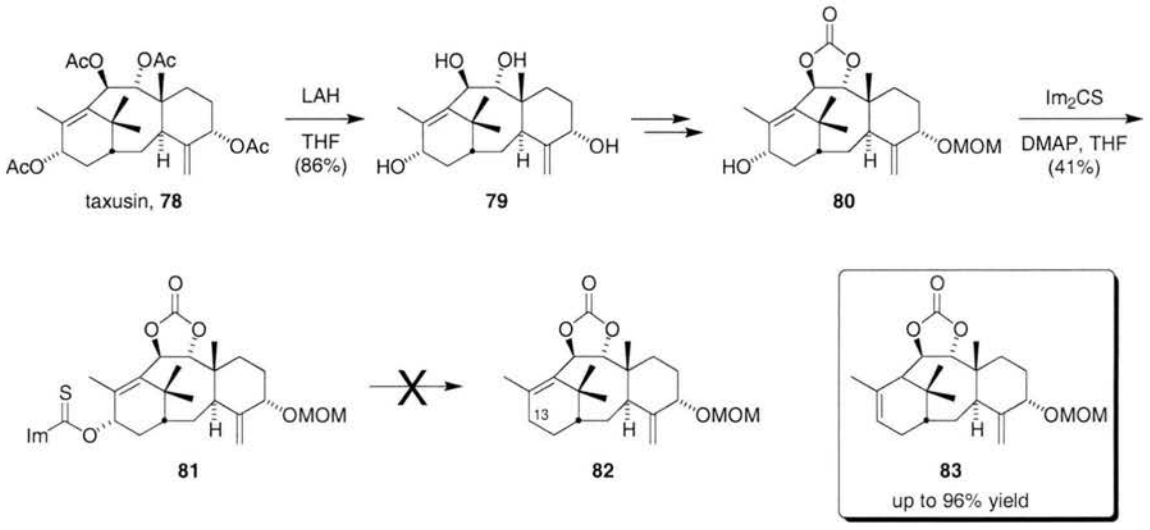
The second synthetic strategy involved trying to achieve taxoid formation through closure of the B-ring at the C-2 – C-3 bond. The requisite carbon atoms for this approach were brought together by using aldehyde **72** to trap organolithium species **71**, a species previously reported by Magnus (Scheme 1.17).[43] This coupling gave alcohol **73** as a mixture of diastereomers, a mixture which could be converted to silyl ethers **74** under standard conditions. Unfortunately, the required hydrolysis of the amide functionality in this molecule could not be effected, halting work toward cyclization substrate **76**.



Scheme 1.17 Williams/Quesnelle work toward C-2 – C-3 B-ring closure

The semi-synthetic approach was designed around the selective deoxygenation of taxusin (**78**, Scheme 1.18). After reductive removal of all of the acetate groups to give

tetraol **79**, standard hydroxyl protecting strategies allowed for the synthesis of alcohol **80**. This was converted to thioester **81** in the hope of being able to effect a radical deoxygenation at the C-13 position. Unfortunately, the desired deoxygenation could not be achieved. A rapid allylic transposition was found to occur during the course of the reaction to give C-12 – C-13 olefin **83** in yields as high as 96%. This side reaction was not entirely unexpected given the thermodynamic gain realized by migration of the double bond away from the bridgehead position. Similar difficulties were encountered when the hydroxyls at C-9 and C-10 were protected either together as the acetonide or separately as the diacetate.

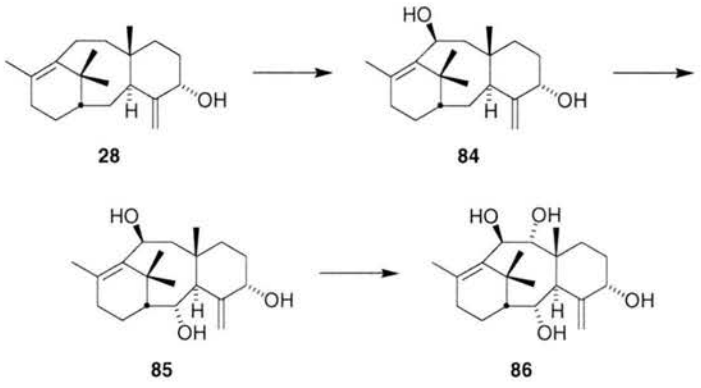


Scheme 1.18 A Williams/Quesnelle semi-synthetic approach starting with taxusin

1.5 The task set forth at the outset of this work

When the work described in this dissertation began in the spring of 1998, the most advanced of the early intermediates identified in taxol biosynthesis was taxa-4(20),11(12)-dien-5α-ol. It was expected that the next few major intermediates would be produced through a series of enzymatic hydroxylations. Consideration of naturally

occurring taxanes led to the hypothesis that the next three oxygens to be introduced would be at C-10, C-2, and C-9, quite possibly in that order (Scheme 1.19). Acylations and oxidations could occur during this process of elaboration, but a well-designed synthesis could handle these variations.



Scheme 1.19 Projected sequence of hydroxylations

The immediate goal of this work was the total synthesis of taxa-4(20),11(12)-dien-5α,10β-diol (**84**). The route chosen would hopefully allow for the introduction of oxygen at other positions from a common advanced intermediate, thereby providing access to compounds whose framework resembled triol **85** or tetraol **86**. Additionally, the synthesis should include a means by which an isotopic label could be introduced at a late stage.

1.6 B-ring formation in various taxane syntheses

Due to the intense interest taxol aroused in the organic chemistry community during the 1980s and 1990s, there was a wealth of applicable precedents to consider when designing the synthetic strategies presented in this document. While groups approached the taxane skeleton from a wide range of directions, the defining step in each synthesis could be considered to be that in which the eight-membered B-ring was formed.

Some of the more noteworthy strategies are depicted in Figure 1.2. Six examples cited in this figure are total syntheses of taxol, while the remainder are syntheses of either simpler taxoids or work done with model systems. The primary reason for the inclusion of these examples is the fact that each of these authors described a means for getting to a taxoid skeleton in which the C-16, C17, and C-19 methyl groups were all present and properly oriented.

The substantial strain arising from abutment of these methyl groups presents the most significant obstacle to the formation of the B-ring. A number of model systems have been reported in which an aromatic C-ring is employed, but such methodology almost always has failed when applied to systems possessing the angular methyl group, C-19.[44] Some research groups managed to sidestep this abutment at the time of B-ring closure (Mukaiyama,[53] Kuwajima,[50] Wender,[54] Swindell,[52] and Stork[51]), but each of these authors described the assembly of a final product possessing all of the necessary methyl groups. It is also worth noting that Holton's elegant work in the synthesis of taxol and taxusin is not included in Figure 1.2 because his choice of starting material circumvented the need to make any of the carbon-carbon bonds in the B-ring.[59]

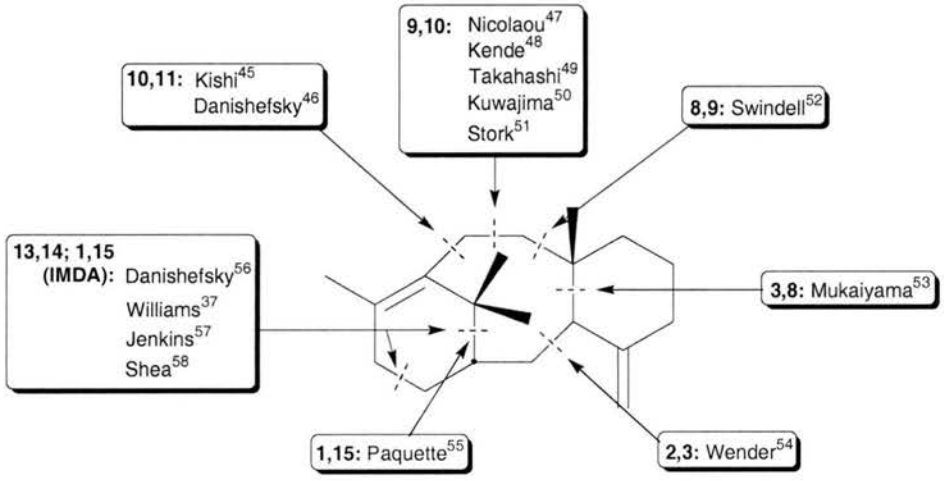


Figure 1.2 Means of B-ring closure in several noteworthy taxane syntheses

Of the ring-closing steps presented in Figure 1.2, Swindell's and Wender's stand out because they did not construct an eight-membered ring directly. Swindell used a photo [2+2] cycloaddition to form a bicyclo [4.2.0] ring system which later underwent fragmentation to make his B-ring. Wender used the attack of an organocopper species on a ketone to form a similar but more complex ring system, and this also was transformed to an eight-membered ring through fragmentation (Table 1.1).

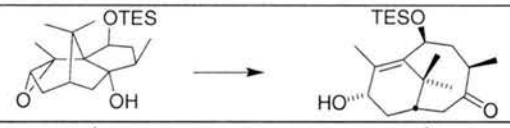
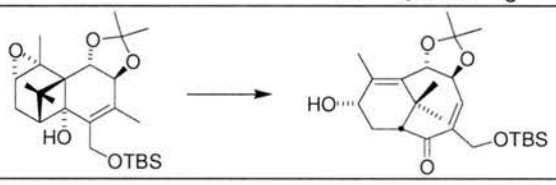
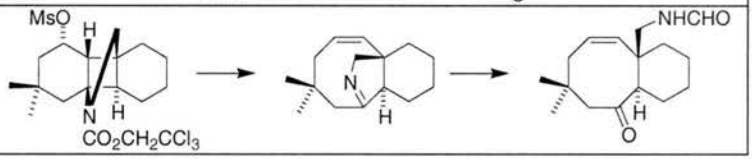
Holton	
Wender	
Swindell	

Table 1.1 Fragmentation approaches

Mukaiyama's total synthesis of taxol is the only example of getting to a taxane ring system using a strategy that requires the annulation of both the A and C rings onto a pre-assembled B-ring. While this led to a synthesis with a large number of linear steps, the greater freedom enjoyed by the B-ring cyclization substrate made possible an unusually high-yielding ring closure (70%). Equally unusual, Paquette has built very creatively on the ideological foundation set out by Martin,[60] employing an anionic oxy-Cope rearrangement to bring together the skeleton of pre-taxoid systems with 9-membered B-rings and 5-membered A-rings. He has then employed pinacol-type rearrangements to join the C-1 and C-15 carbons and establish the necessary eight-membered B-ring while expanding the A-ring to 6-carbons (Table 1.2).

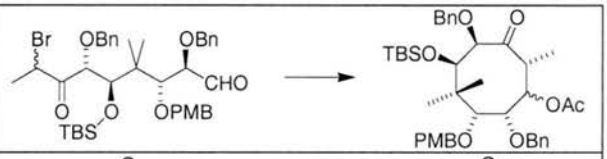
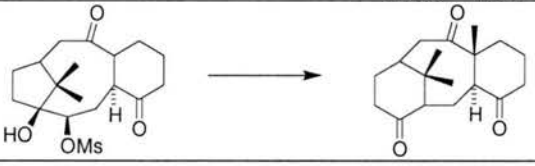
Mukaiyama	
Paquette	

Table 1.2 Approaches of Mukaiyama and Paquette

The C-9 – C-10 bond has been the most common point for closing the B-ring. Takahashi and Stork both used the displacement of a leaving group by a cyanohydrin enolate to form this bond. Stork did this with a system lacking a C-ring by using an enolate made from a protected C-9 cyanohydrin to attack a C-10 chloride. Takahashi closed this bond with a system containing both A- and C-rings by using an enolate made from a protected C-10 cyanohydrin to displace a C-9 tosylate. Kende and Nicolaou both used McMurray reactions to close the C-9 – C-10 bond, with Kende allowing the reaction to go all the way to the C-9 – C-10 olefin in poor yield and Nicolaou stopping the reaction at the C-9 – C-10 diol in comparably poor yield. Kuwajima's approach utilized the acetal variant of a Mukaiyama aldol reaction to form the C-9 – C-10 bond, with the creativity of this step lying in the fact that he employed a vinylogous silyl enol ether rather than the more common silyl enol ether as the attacking species (Table 1.3).

Closure of the B-ring at the C-10 – C-11 bond has led to the synthesis of a number of taxoids and to two total syntheses of taxol, one by Danishefsky and the other by Kishi. Kishi showed that this bond could be formed by using an intramolecular NHK reaction to unite either a C-11 triflate or a C-11 iodide with a C-10 aldehyde. After unsuccessfully trying to employ Kishi's NHK protocol, Danishefsky used an intramolecular Heck reaction to join a C-11 iodide with a C-10 terminal olefin.

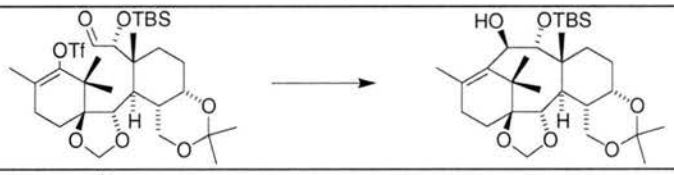
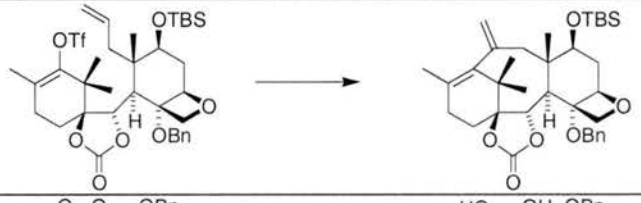
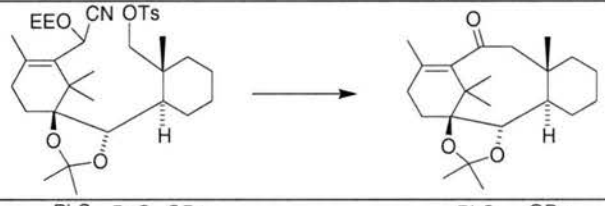
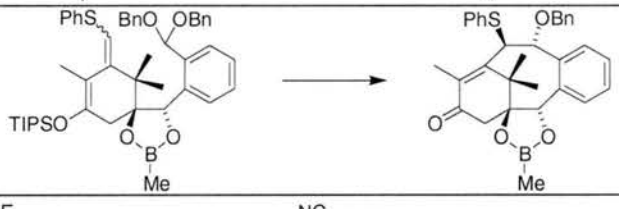
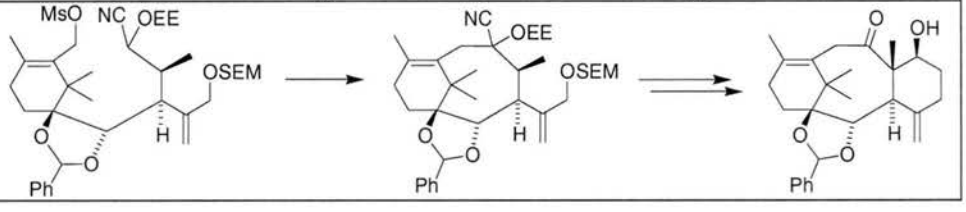
Kishi	
Danishefsky	
Nicolaou	
Kende	
Takahashi	
Kuwajima	
Stork	

Table 1.3 Closing the top of the B-ring

Jenkins, Shea, Danishefsky, and Williams have all reported the use of intramolecular Diels-Alder reactions to simultaneously form both the A- and B-rings of taxoid systems (Table 1.4). The significant elements of this work will be discussed at the beginning of Chapter 4. The unique characteristic separating the Williams synthesis (Scheme 1.11) from the others is that it allows for functionality to be introduced at C-4.

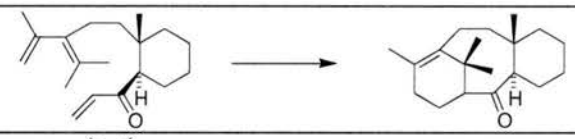
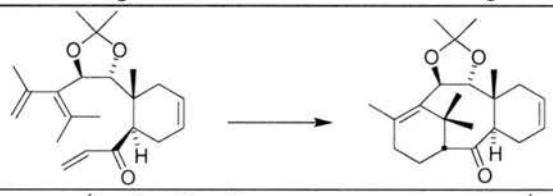
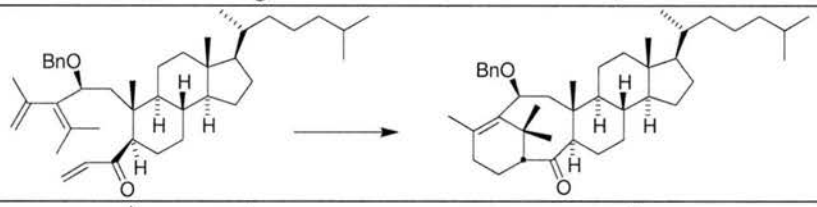
Jenkins	
Shea	
Danishefsky	
Williams	

Table 1.4 Intramolecular Diels-Alder approaches

Drawing from these examples and several others, synthetic strategies were laid out and executed in the hope of assembling some of the lightly oxygenated taxoids that were projected to be intermediates in the biosynthesis of taxol. The results of these efforts will be presented in the following chapters.

Chapter 2

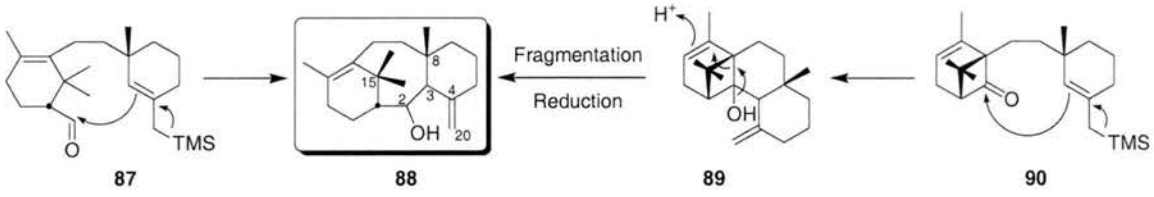
Approaches designed around initial formation of the C-10 – C-11 bond: Attempting to form the B-ring through a C-2 – C-3 ring closure

It is well-established that the formation of eight-membered rings is a significant challenge for the synthetic chemist.[61] This difficulty is exacerbated in the construction of the eight-membered B-ring of a taxane skeleton by the high level of strain arising from the transannular abutment of the geminal methyl groups on C-15 (C-16 and C-17) with the angular methyl group on C-8 (C-19). Research groups working in this area have developed several ways to approach this problem.

Some of the more fruitful approaches to this challenge have led to total syntheses of Taxol. The Nicolaou,[47] Danishefsky,[46] and Kuwajima[50] groups chose to close the top of the B-ring after the A- and C- rings had already been put in place. These strategies were all aided by the presence of a five-membered ring protecting connecting the C-1 and C-2 positions, imparting a conformational bias that made cyclization possible. The Mukaiyama group[53] chose to form the B-ring before either the A- or C-rings, allowing for a greater range of conformational freedom and therefore a reduction in the transannular strain. The Holton[59] and Wender[54] groups were able to use fragmentations to form the B-ring, after which they constructed the C- and D-rings to complete their syntheses of taxol.

As mentioned in the previous chapter, the Williams group conducted some preliminary investigations into a unique way to construct the B-ring of a taxane system. This involved coupling an A-ring synthon to a C-ring synthon across the top of the B-ring, and then closing the bottom of the B-ring by having a nucleophilic C-3 attack an

electrophilic C-2. An ideal nucleophilic species for this sort of transformation would be an allyl silane, which could be induced to undergo an intramolecular Sakurai reaction,[62] attacking either an aldehyde or a ketone (Scheme 2.1). The product of either approach would have an exocyclic methylene extending from the C-ring at C-4, thereby introducing the required double bond between C-4 and C-20 in the cyclization process.



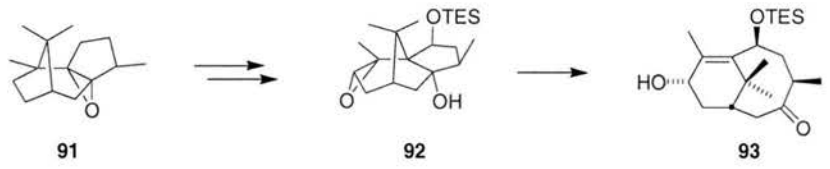
Scheme 2.1 Two means for exploiting a Sakurai reaction in taxane construction

The more obvious mode of cyclization is represented by the conversion of aldehyde **87** into taxane **88**. In this case the A-ring would possess a one-carbon electrophilic arm, such as the aldehyde in **87**, which could be attacked by the allyl silane to give a taxane skeleton directly. The less obvious mode of cyclization is represented by the conversion of ketone **90** into taxane **88** through the intermediacy of tetracyclic alcohol **89**. In this case, analogous to work from the Wender group,[54] the intermediate alcohol could hopefully be induced to undergo fragmentation, either via epoxidation of the trisubstituted olefin or via a cationic fragmentation upon subjection to acidic conditions. Each of the two modes of cyclization has been investigated, and this chapter will describe work done in both areas as well as some closely related approaches.

2.1 Verbenone-based A-rings

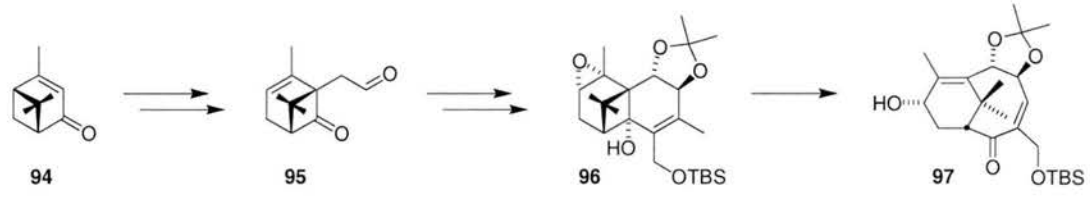
As mentioned above, both Holton and Wender used fragmentation approaches to circumvent the need to form the B-ring through a highly disfavored cyclization. Holton

began with β-patchoulene oxide (**91**, Scheme 2.2), elaborated it over the course of several steps into epoxide **92**, which upon fragmentation gave ketone **93**. This is a very clever sequence, but it does not lend itself especially well to adaptations that might allow for it to become the foundation of a more convergent synthesis.



Scheme 2.2 Holton's fragmentation approach

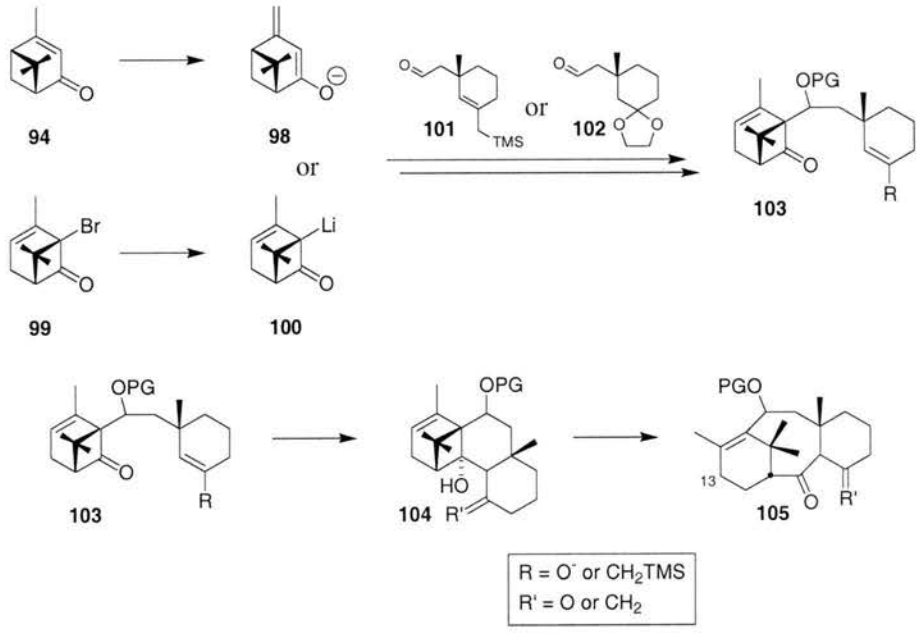
Wender's fragmentation approach began with verbenone (**94**, Scheme 2.3), which after alkylation, ozonolysis, and photo rearrangement gave aldehyde **95**. This was elaborated over seven steps to give epoxide **96**, which underwent base-mediated fragmentation to give ketone **97**. This approach could be adapted into a convergent synthesis if the electrophile in the initial alkylation step (prenyl bromide, in Wender's case) could be replaced with a more sophisticated C-ring synthon.



Scheme 2.3 Wender's fragmentation approach

Our initial synthetic strategy was based on generating a nucleophilic species from either verbenone (**97**) or a closely related compound (such as bromochrysanthenone, **99**), and having this attack the aldehyde portion of a suitable C-ring synthon (Scheme 2.4). Hopefully an intermediate capable of undergoing cyclization via either an intramolecular Sakurai reaction (**103**, R=CH_2TMS) or an intramolecular aldol condensation (**103**, R=O^-) could then be easily assembled within a few steps. Cyclization could be followed by

either of two protocols. Epoxidation and base-mediated ring opening following Wender's precedent could give a taxane identical to **105** except that it would possess a superfluous hydroxyl in the C-13 position. Alternatively, treatment with acid could potentially effect the fragmentation without the necessity of oxidation and subsequent deoxygenation.



Scheme 2.4 Initial synthetic strategy

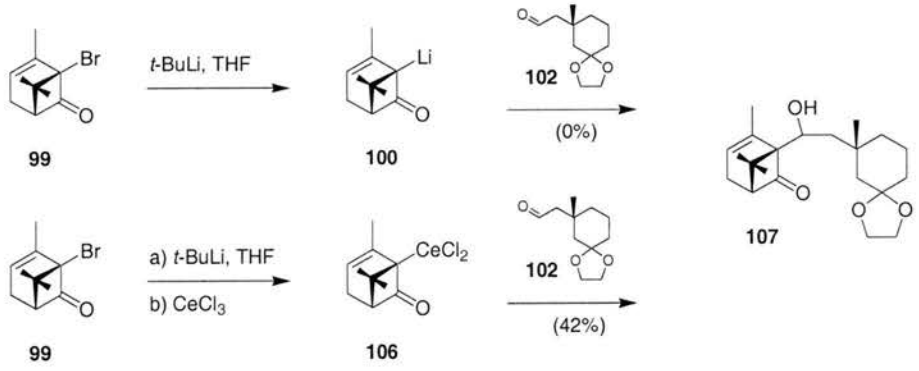
2.1.1 Attempted ring closure through aldol condensation

Wender's group has reported both the conversion of verbenone (**94**) to its potassium dienolate (**98**) and the conversion of bromochrysanthenone (**99**) to organolithium species **100**.[63] Both nucleophilic species were shown to be capable of attacking simple electrophiles – allylic halides for the potassium dienolate of verbenone and aromatic aldehydes for the chrysanthenone anion.

Bromochrysanthenone is not commercially available, but can be made in low (<25%) yield in two steps from verbenone. Therefore, the dienolate approach was the

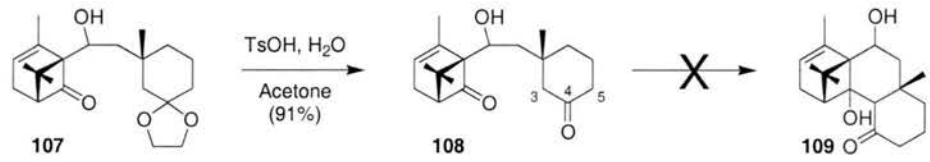
first to be tried. Unfortunately, when either verbenone's potassium dienolate was treated with either dioxolane-containing aldehyde **102** or allyl silane aldehyde **103**, the only coupled products were those resulting from the desired alkylation followed quickly by elimination of water from the product.

As Wender had reported, bromochrysanthenone readily underwent metal-halogen exchange to give organolithium species **100**. Their work had not specified, however, whether this anion was better suited to act as a nucleophile or as base, since none of the electrophiles they used had abstractable protons in the position α to the carbonyl undergoing attack. Our initial work made it clear that this anion was a strong base and a weak nucleophile, as it failed to give any of the desired coupling product when treated with aldehyde **102** (Scheme 2.5). Fortunately, this organolithium species could be smoothly converted[64] to the less basic, more nucleophilic organocerium variant (**106**), and with this species a decent coupling yield could be achieved.



Scheme 2.5 Improved coupling through use of organocerium intermediate

Deprotection of ketal **107** unmasked one carbonyl of the parent ketone (**108**, Scheme 2.6), but neither treatment with base (KO*t*Bu/THF or NaOMe/MeOH) nor with acid (TsOH/THF or TsOH/acetone) could induce this compound to undergo the intramolecular aldol reaction needed to assemble a tetracyclic fragmentation substrate.



Scheme 2.6 First attempted ring closure: intramolecular aldol condensation

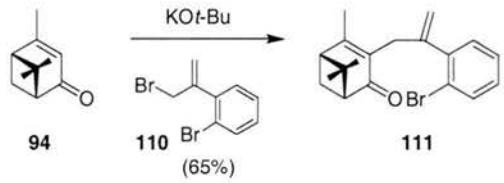
The aldol substrate (**108**) could form either of two enolates – one, with the double bond between C-4 and C-3, the other with the double bond between C-4 and C-5. It was believed that an enolate formed on the C-3 side could go on to react in the desired manner, forming a six-membered ring, while an enolate formed on the C-5 side would be much slower to react since cyclization from this position would require the formation of a very congested eight-membered ring. Attempted aldol reactions run under basic conditions employed alkoxides as bases, so that an equilibrium could be established between the two enolates and selectivity achieved on the basis of which would react more rapidly.

There is a very good possibility, however, that reversibility was at the root of the difficulties encountered with this transformation. In forming tetracyclic aldol product **109** a high entropic price must be paid, since a high degree of order would need to be imposed on a system which previously possessed a large number of degrees of freedom. Perhaps this price was high enough that, under equilibrating conditions, any aldol product being formed was rapidly undergoing a retro-aldol reaction.

This issue was addressed in two ways. On one hand, a substrate was assembled that could be capable of trapping some percentage of any transiently formed aldol product and in so doing pull the equilibrium in the desired direction. On the other hand, a substrate was assembled in which an allyl silane was the attacking species, effectively removing the possibility of having cyclization products revert back to starting material.

2.1.2 Alternative coupling approach and attempted trapping with A-ring epoxide

In their early studies into construction of taxane skeletons[65], Wender's group performed the alkylation of verbenone (**94**) with allyl bromide **110** in 65% yield (Scheme 2.7). They used the methylene of **111** as a hidden carbonyl group, unveiling it later through a hydroboration-oxidation-deformylation protocol. It was hoped that their C-ring synthon could be replaced by a more functionalized allyl bromide for our work.



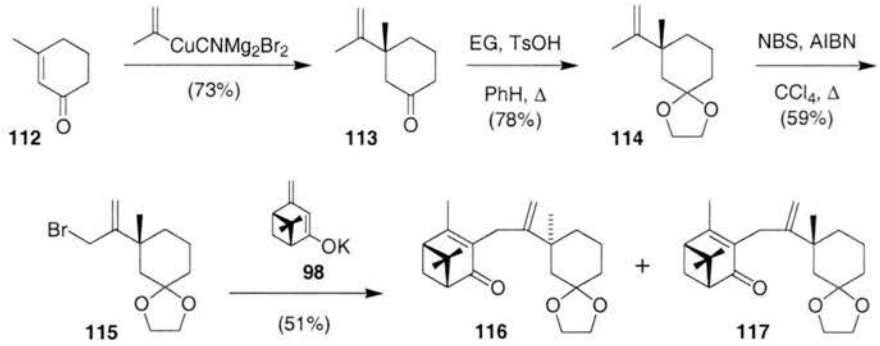
94 **110** (65%) **111**

Scheme 2.7 Alkylation from early work out of the Wender group

While using an allyl bromide as the electrophilic coupling partner would make the eventual introduction of oxygen at C-10 potentially difficult, this approach offered some advantages. First, the coupling could be easily done on large scale at 0°C, an improvement over having to employ metal-halogen exchange at very low temperatures in the previous approach. Second, the products arising when an aldehyde was the electrophile were a mixture of four diastereomers, since both the hydroxyl and the angular methyl group could occur with either an α or ß disposition. Using an allyl bromide as the electrophile would mean that the product would occur as a mixture of only two diastereomers, greatly simplifying purification and characterization. Third, the nucleophilic partner would be generated simply by treating verbenone with potassium *t*-butoxide, bypassing the low-yielding steps required to construct bromochrysanthenone.

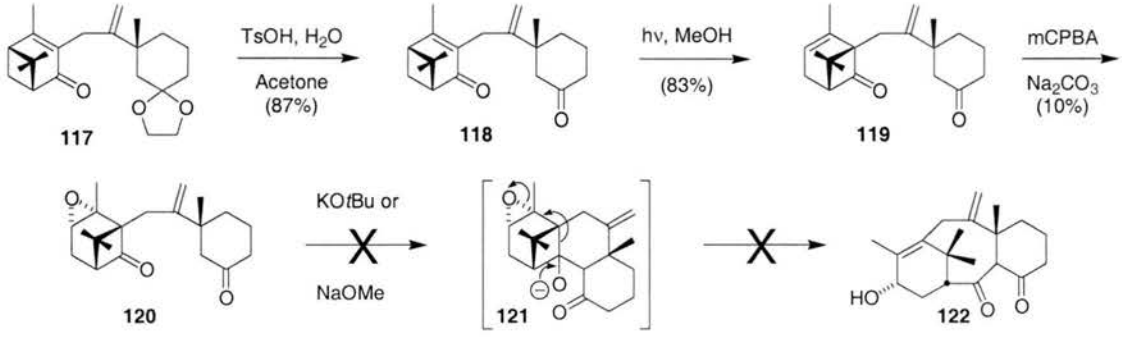
Assembly of a suitable allyl bromide that could serve as a C-ring synthon began with the conjugate addition of an isopropenyl cuprate to 3-methyl-2-cyclohexen-1-one

(**112**, Scheme 2.8). This went smoothly to give ketone **113**, and the carbonyl of the product was protected as the dioxolane (**114**) in good yield. Radical bromination selectively converted the isopropenyl group to the corresponding allyl bromide, providing C-ring synthon **115**. Treating this with the potassium dienolate of verbenone (**98**) gave a 51% yield of alkylation products, which could be chromatographically separated into equal amounts of each diastereomer (**116** and **117**).



Scheme 2.8 Assembly and coupling of an allyl bromide-based C-ring synthon

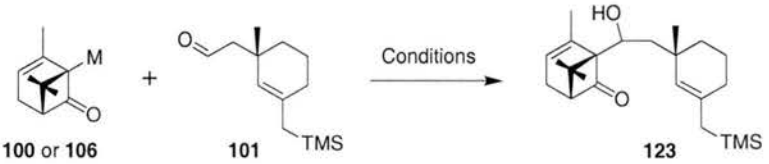
The C-ring carbonyl was unmasked under acidic conditions to give diketone **118**, after which photo rearrangement delivered chrysanthenone derivative **119** (Scheme 2.9). The trisubstituted double bond could be selectively epoxidized to give epoxide **120**, albeit in very low yield. Treatment of this compound with either potassium *t*-butoxide or sodium methoxide in methanol, however, failed to give either the desired taxane (**122**) or the envisioned intermediate (**121**), as the starting material was recovered unchanged.



Scheme 2.9 Epoxide assembly and failed cyclization

2.1.3 Allyl silane C-ring

Methodology had previously been developed in the Williams group for the assembly of an aldehyde similar to allyl silane aldehyde **101** (**72**, Scheme 1.17). This work was adapted to the construction of the required aldehyde, and proved to be robust and reliable on larger scale. On the other hand, the organocerium methodology employed in coupling bromochrysanthenone to aldehyde **102** was largely ineffective when allyl silane aldehyde **101** was used as the electrophilic partner (Table 2.1).



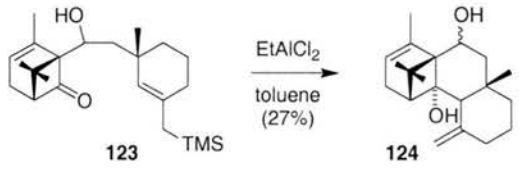
100 or **106** **101** **123**

Conditions	Notes	Scale, mmol	Yield
t-BuLi, $CeCl_3$	-105 °C	1.0	2.6%
t-BuLi, $CeCl_3$	Solid $CeCl_3$ addition	1.0	8.0%
t-BuLi, $CeCl_3$	Solid $CeCl_3$ addition	4.1	10.2%
t-BuLi	-105 °C	1.0	Decomposition
t-BuLi, $CeCl_3$	-105 °C	1.8	11.2%
t-BuLi, 5 mol % CuBr	-105 °C	0.5	Decomposition
t-BuLi, $CeCl_3$	-105 °C	0.5	5.1%
t-BuLi, $CeCl_3$	Phosphate quench	1.0	15.3%
t-BuLi, $CeCl_3$, $EtAlCl_2$	Attempted Cyclization	1.0	Decomposition
t-BuLi, $CeCl_3$, Benzyl Br.	Phosphate quench	0.5	13.4%

Table 2.1 Attempted optimization of chrysanthenone-based coupling to aldehyde **101**

A small amount of coupled product **123** could be produced, however, and some work was done to investigate the desired cyclization reaction. After trying tetrabutylammonium fluoride and a few different Lewis acids as agents for effecting the cyclization, some interesting reactivity was observed when the coupled product was treated with ethyl aluminum dichloride. One major product was isolated from this

reaction, albeit in 27% yield, and from all indications this was diol **124** (Scheme 2.10). The reaction was repeated, and the same product was observed. Two subsequent repetitions of this reaction, however, gave a very clean product that was in every way identical to that which had been identified as the desired diol except for its IR spectrum. The product of the first two reactions showed that the carbonyl stretch at ~1740 cm^{-1} in the starting material had disappeared in the product, and the dominant feature of the spectra was the broad band in the 3100 – 3600 cm^{-1} range, a characteristic region in which to detect hydroxyl O-H stretching. The product of the second two reactions, on the other hand, had as its dominant spectral feature a large band at 1740 cm^{-1}, and the broad band in the hydroxyl region was much less intense. This would indicate that the latter reactions gave only products resulting from clean protodesilation with allylic inversion of the allyl silane functionality.

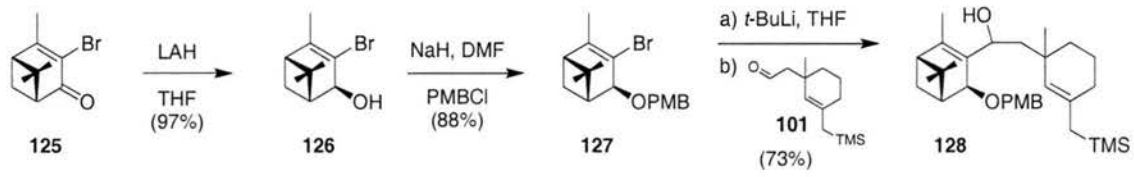


Scheme 2.10 Enigmatic intramolecular Sakurai reaction

In any case, success here was being defined as a capricious reaction going in 27% yield on the heels of a reaction that was going in 15% yield under the best of circumstances. It was impractical to accept so severe a bottleneck at this point in the synthesis, so an alternative approach was pursued toward the assembly of such a skeleton, an approach that would not rely on using a tertiary carbanion as a nucleophile.

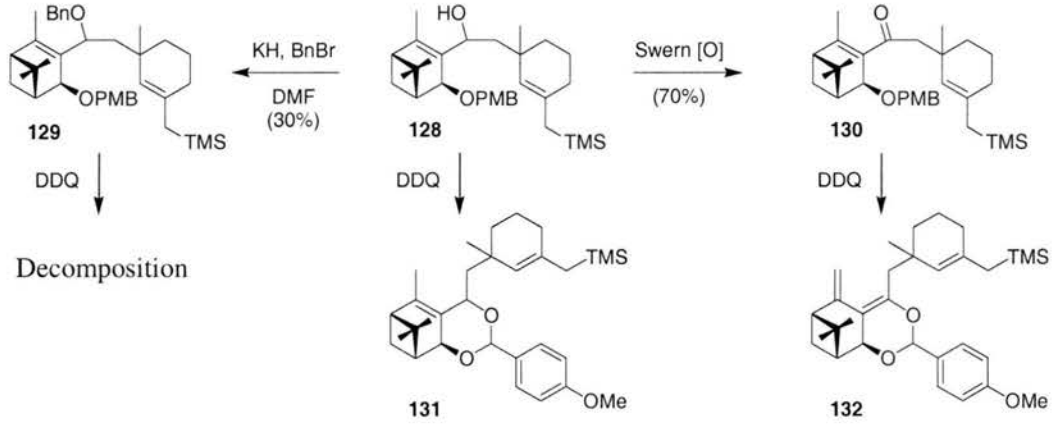
Toward this end bromoverbenone (**125**), an intermediate in the synthesis of bromochrysanthenone, was reduced to bromoverbenol (**126**). This alcohol could then be protected as *p*-methoxybenzyl ether **127**, which underwent metal-halogen exchange upon

treatment with *t*-butyllithium. The resulting vinyl carbanion proved to be a much better nucleophile, coupling with allyl silane aldehyde **101** in 73% yield (Scheme 2.11).



Scheme 2.11 Improved coupling using a vinylic anion

At this point, it was hoped that removal of the *p*-methoxybenzyl group, oxidation of the diol product to the corresponding diketone, and photo rearrangement would deliver a suitable substrate for the envisioned intramolecular Sakurai reaction that would be used to assemble the key tetracyclic intermediate (analogous to **124**, Scheme 2.10). Unfortunately, no effective means of removing the *p*-methoxybenzyl group could be found (Scheme 2.12). Removal of the *p*-methoxybenzyl protecting group from alcohol **128** led only to dibenzylidine acetal **131**. Oxidation of the secondary alcohol under Swern conditions gave ketone **130**, but attempts to remove the *p*-methoxybenzyl group led to the formation of exceptionally stable dibenzylidine acetal **132**. Protecting the troublesome hydroxyl as the benzyl ether could be carried out in low yield to give **129**, but attempts to remove the *p*-methoxybenzyl group resulted only in decomposition.

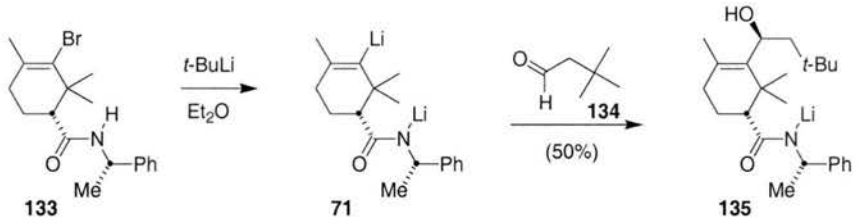


Scheme 2.12 Efforts to remove *p*-methoxybenzyl protecting group

Given the difficulty of removing the PMB group, efforts were shifted toward the assembly of an intramolecular Sakurai substrate in which the electrophilic component of the cyclization reaction would be an aldehyde, and whose product would be a taxane rather than one that would require the development of fragmentation methodology.

2.2 Attempted 8-membered ring formation using Sakurai conditions

In 1996, Magnus reported the construction of enantiopure amide **133** (Scheme 2.13) for use as a potential A-ring synthon in the construction of taxanes.[43] His group reported treatment of this amide with excess *t*-butyllithium gave dianion **71**, which they showed could attack a range of aldehydes, coupling with 30% to 80% yields. A yield of 50% was even reported for coupling to an aldehyde (**134**) with abstractable α protons. As described in the first chapter (Scheme 1.17), some prior work with this species had been done in the Williams group.

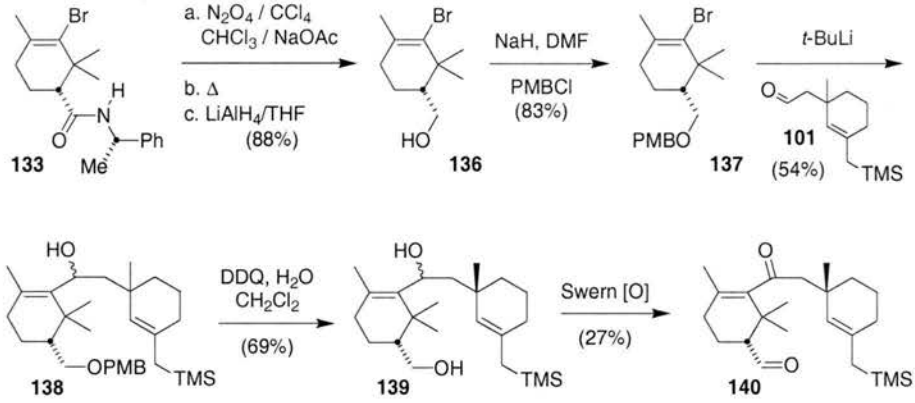


Scheme 2.13 A-ring synthon reported by the Magnus group

2.2.1 Sakurai substrate containing a C-2 aldehyde

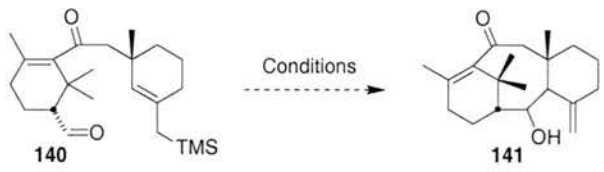
During this earlier work, a reductive adaptation of Magnus' unusual amide hydrolysis conditions was developed. This was used to give alcohol **136**, which was then protected as novel *p*-methoxybenzyl ether **137**. Metal-halogen exchange on this vinyl bromide gave the corresponding vinyllithium species, which could be trapped by

aldehyde **101** to give alcohol **138** in moderate yield (Scheme 2.14). Oxidative removal of the *p*-methoxybenzyl protecting group went in moderately good yield to give diol **139**. This diol was then oxidized under Swern conditions to dicarbonyl compound **140**, albeit in low (27%) yield. The low yield in this step was due to the tendency of a mono-oxidized intermediate to form a very stable intramolecular hemiacetal.



Scheme 2.14 Vinyl bromide coupling and elaboration to Sakurai substrate

Aldehyde **140** was seen as an ideal substrate for the desired cyclization reaction. By introducing a center of sp^2 rather than sp^3 hybridization at the C-10 position, a significant degree of stability would be imparted to the eight-membered ring in the desired product. It was hoped that the electrophilicity of the ketone carbonyl would not present a difficulty because of a) the greater reactivity of the aldehyde moiety, and b) the attenuation of the ketone's electrophilicity by virtue of its conjugation with the C-11 – C-12 double bond. Unfortunately, treatment of this substrate with any of a number of Lewis acids failed to bring about any sort of cyclization (Table 2.2).



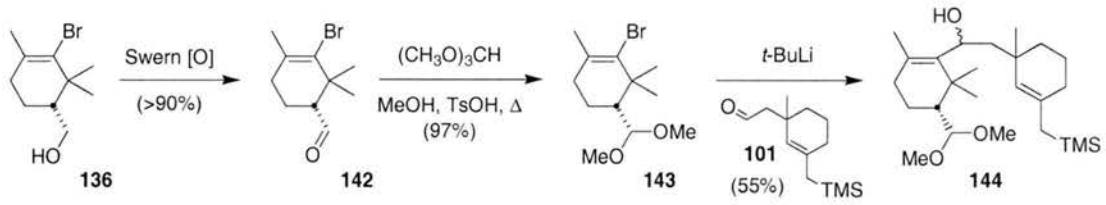
Conditions	Temp.	Result
TMSOTf	-78 °C	Decomposition
BF_3•Et_2O, Me_2AlCl, Toluene	-78 °C → 0 °C → RT	No Reaction
$EtAlCl_2$	0 °C → RT	No Reaction
$TiCl_4$	0 °C	Decomposition
BF_3•Et_2O, CH_2Cl_2	-78 °C → 0 °C → RT, 6 hrs	Decomposition
$TiCl_4$, CH_2Cl_2	-78 °C, 30 min	Exocyclic Methylene
$TiCl_4$, CH_2Cl_2	-78 °C, 45 min	Decomposition

Table 2.2 Attempted intramolecular Sakurai cyclizations with aldehyde **140**

2.2.2 Sakurai substrate containing a C-2 dimethyl acetal

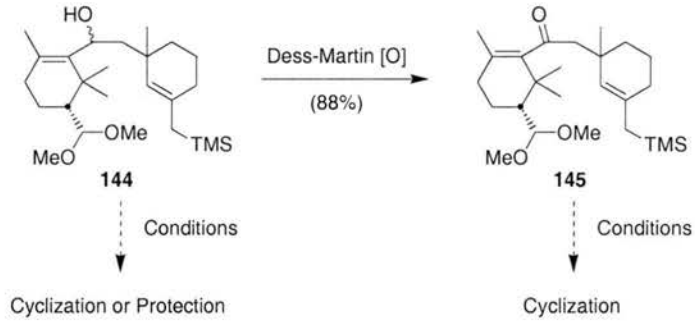
At this point it was postulated that perhaps the Sakurai reaction would have a better chance of succeeding if the aldehyde moiety could be replaced with a more electrophilic functional group. It is well-established that acetals can serve as more easily-activated versions of their parent aldehydes in Lewis acid-promoted versions of the Mukaiyama aldol and Sakurai reactions.[66] This ease of activation is due to the enhanced coordination to the Lewis acid by the two oxygen atoms of an acetal instead of the single oxygen of an aldehyde.

Work in the direction of an acetal cyclization substrate began with alcohol **136**, an early intermediate in the construction of the previous intramolecular Sakurai substrate. Oxidation using either Swern conditions (>90% yield), Dess-Martin conditions (75%), or pyridinium dichromate (75%) provided aldehyde **142**, which was converted to dimethyl acetal **143** in high yield (Scheme 2.15). Metal-halogen exchange proceeded smoothly, and this organolithium species could be trapped with aldehyde **101** in moderate yield.



Scheme 2.15 Coupling with a dimethyl acetal A-ring

Attempts were made to take alcohol **144** in two general directions. First it was subjected to Dess-Martin oxidation conditions, and a good yield of ketone **145** was realized (Scheme 2.16). This provided the acetal analog of the aldehyde substrate used as the previous Sakurai substrate, by a route that was actually more direct because it did not require protection and deprotection steps. Second, attempts were made to protect the hydroxyl of alcohol **58** in order to have a substrate with only one possible electrophilic site.



Scheme 2.16 Substrate manipulations and attempted cyclizations

As had been encountered with previous substrates, protection of secondary alcohol **144** was problematic due to its sterically congested environment, a condition probably exacerbated with this particular substrate by the likelihood of hydrogen bond formation between the hydroxyl and the acetal (Table 2.3). Furthermore, the sensitivity of the allyl silane functionality to both acid conditions and hydride bases limit the range of conditions that could be employed to carry out such a protection.

Substrate	Conditions	Result
144	KH, BnBr, DMF, DME	No Reaction
144	TBSCl, Imidazole, DMF, 80°C	No Reaction
144	$TiCl_4$, CH_2Cl_2, -78°C	Decomposition
145	$TiCl_4$, CH_2Cl_2, -78°C	Acetal Gone, Allyl Silane Remains
145	TMSOTf, CH_2Cl_2, -78°C	Acetal to Aldehyde, Allyl Silane Gone
145	$TiCl_4$, CH_2Cl_2, -78° → 0°C	Acetal to Aldehyde, Allyl Silane Gone

Table 2.3 Protection and cyclization conditions applied to alcohol **144** and ketone **145**

2.2.3 Cyclization efforts and conclusion

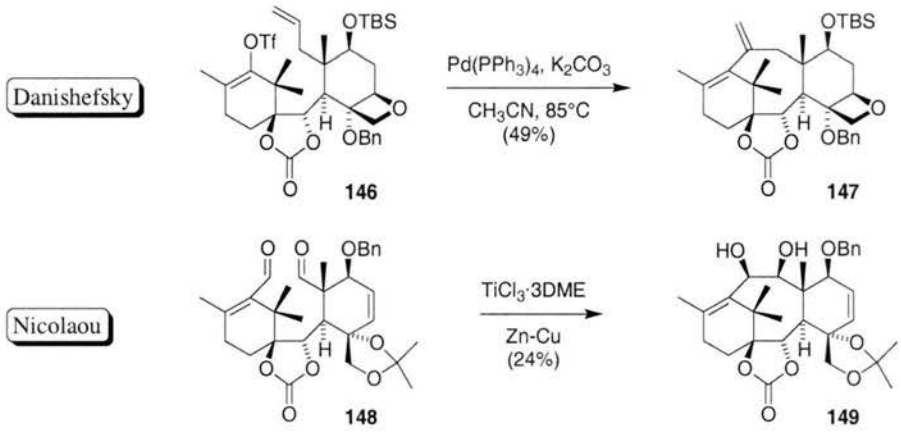
Attempts to induce cyclization using free alcohol **144** resulted only in decomposition, while attempts to cyclize ketone **145** gave only various aldehyde products, indicating that the acetal had been activated by the Lewis acid but was still not electrophilic enough to attract the π-electrons of the allyl silane.

Many difficulties were encountered in the attempts to form a taxane skeleton via a C-2 – C-3 ring closure. Among these were coupling difficulties – the A-rings best suited for cyclization were the worst suited for coupling; protection difficulties – the secondary alcohol of any coupling product was very difficult to mask effectively; and, most importantly, cyclization difficulties – under no conditions could a useful intramolecular aldol or intramolecular Sakurai cyclization be effected. Given these circumstances, the focus of our efforts was shifted to cyclization of the B-ring through formation of a C-10 – C-11 bond, as will be discussed in Chapter 3.

Chapter 3

Attempting to form the B-ring through a C-10 – C-11 ring closure

The key step in two of the first syntheses of Taxol, those of the Danishefsky[46] and Nicolaou[47] groups, consisted of a closure across the top of the B-ring in substrates containing functionalized A- and C-rings (Scheme 3.1). Given the enormous steric impediments faced in these reactions, they can be considered successes in spite of their rather low yields. It is especially noteworthy that both of these substrates benefited from a high degree of preorganization. In both cases, a cyclic carbonate bridged the hydroxyl groups at C-1 and C-2, preventing the substrates from adopting conformations in which the groups interacting in the cyclization step would lie far from one another.

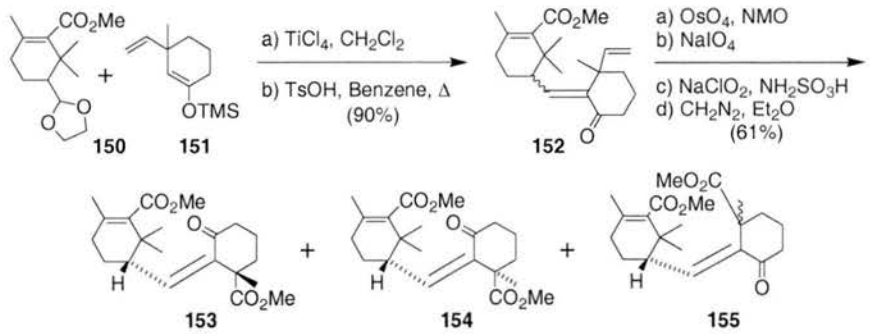


Scheme 3.1 Ring closures performed by Danishefsky and Nicolaou

Unfortunately, the relative simplicity of our targets, especially our initial target, the 5,10-diol, made any approach relying on such preorganization rather impractical. Work from both the Kishi and Kende labs, however, provided examples of closing the top of the B-ring in substrates with considerably less functionality.

3.1.1 Kende's taxatriene synthesis

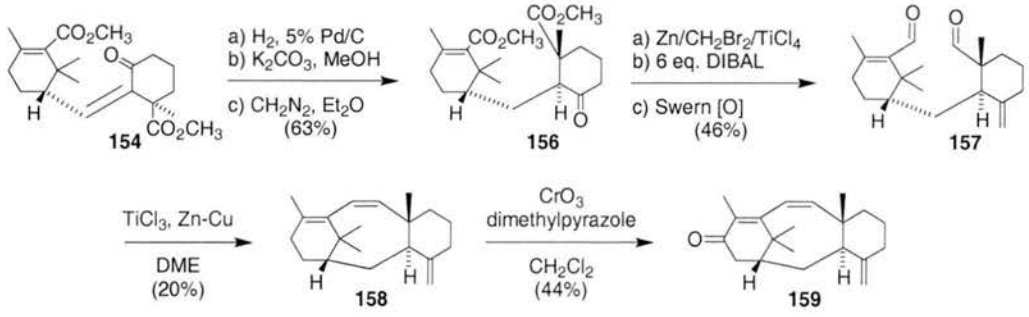
In 1986 Kende reported a racemic synthesis of a taxane triene possessing the "full and stereochemically correct carbon framework" of taxusin.[48] This triene (**159**, Scheme 3.3) was the first reported total synthesis of a taxane skeleton. The first key step in this synthesis was an acetal variant of a Mukaiyama aldol reaction between dioxolane **150** and silyl enol ether **151** (Scheme 3.2). This brought together all of the necessary carbon atoms for the tricyclic framework, leaving only the exocyclic methylene at C-20 to be added on at a later time. Following oxidative cleavage of the terminal olefin to the aldehyde and subsequent elaboration of the aldehyde to the methyl ester, the products could be separated into three fractions, the first a *Z* enone possessing the wrong disposition of the angular methyl group (**153**), the second a *Z* enone possessing the correctly disposed angular methyl group (**154**), and the third a mixture of the two E enones (**155**).



Scheme 3.2 First Steps of Kende's taxatriene synthesis

Reduction of the trisubstituted olefin, epimerization of the newly formed methine hydrogen, and restoration of the methyl esters lost during epimerization gave diester **156** (Scheme 3.3). Ketone olefination using Lombardo's conditions was followed by reduction of the esters to give the corresponding diol, which was oxidized under Swern

conditions to give dialdehyde **157**. This was the substrate for the second key step of this synthesis, the McMurray reaction which they envisioned employing to close the B-ring. This turned out to be a qualified success, providing triene **158** in 20% yield. The C-13 carbon was then oxidized using CrO_3, a transformation that would be valuable if this methodology were to be applied toward the synthesis of taxusin or Taxol.



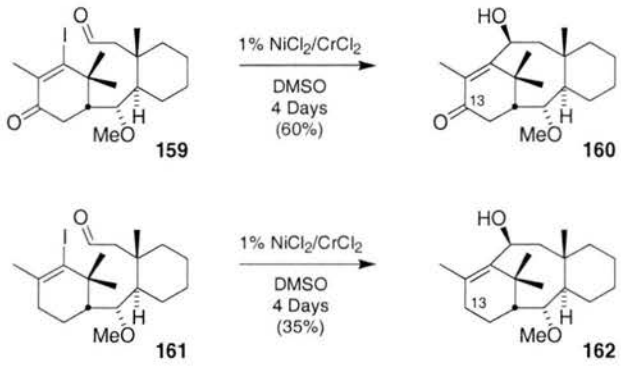
Scheme 3.3 Completion of Kende's taxatriene synthesis

From our perspective, the value in this work lay in the way by which the carbon framework of a cyclization substrate could be put together in a concise and controlled manner. Controlled functionalization of a triene such as **158** would most likely be very difficult to achieve, however, so other avenues were pursued in search of a means of ring closure that would be amenable to our purposes.

3.1.2 Kishi's work toward an effective B-ring closure

In 1993 Kishi reported two examples of the application of the Nozaki-Hiyama-Kishi[41,42] (NHK) reaction toward the formation of a taxane skeleton via closure of the C-10 – C-11 bond.[67,68] In the first example, vinyl iodide **159** was converted into taxane **160** in moderate yields (Scheme 3.4). The second important example from this work, the conversion of vinyl iodide **161** into taxane **162**, suffered from a much lower yield, but

when considered relative to the B-ring closures reported by Danishefsky and Nicolaou was still potentially useful methodology.



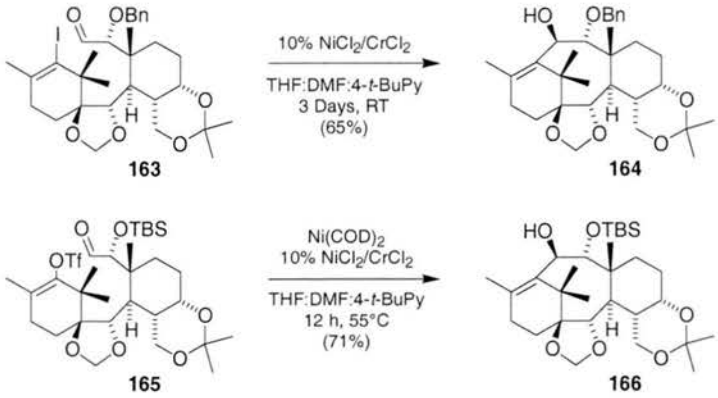
159 **160** **161** **162**

Scheme 3.4 Kishi's early application of the NHK reaction to B-ring closure

While intriguing, Kishi's results at this point hinted that this methodology may not function very well as a means for assembling the sort of taxane skeleton we needed. The rate-limiting step in these reactions is the activation of the carbon-iodine bond by nickel(0), which is necessary in the formation of the active organochromium species that closes on the aldehyde. As these results demonstrate, this activation occurs more readily with electron-poor vinyl iodides, such as enone **159**, than with electron-rich vinyl iodides, such as **161**. This would present us with a potential source of difficulty, since our ideal cyclization substrate would more closely resemble **161** rather than enone **159** in order to avoid having to tackle the problematic process of removing oxygen from the C-13 position of such compounds. Additionally, cyclization reactions of this nature had a reputation for being very difficult to reproduce (failing, for example, in the hands of the Danishefsky group with an aldehyde nearly identical to their intramolecular Heck substrate[1]) so other avenues (Chapter 2) were initially pursued.

Applying this methodology to a broader range of substrates was a problem not only for researchers at other institutions, but also for the Kishi group as they tried to

move forward with work in this area. Activation of the carbon-halogen bond continued to be a problem, and increasing the amount of nickel in the reaction, while aiding activation, had led to a significant degree of homo-coupling, leading to substrate dimers. Their first improvements in this area came in 1997, when they reported that the addition of 4-*t*-butyl pyridine to the reaction mixture brought the chromium(II) chloride into solution.[69] Prior to this point, reactions of this sort were heterogeneous, with neither the nickel(II) nor chromium(II) species solvated significantly. Bringing the chromium(II) into solution increased its availability, enabling nickel(II) loading (previously limited to 1% of the chromium content) to be increased to 33% of the chromium content without any sign of homo-coupling. This allowed for a substantial increase in the rate of activation, enabling aldehyde **163**, which was completely unreactive under the previous conditions, to cyclize with a 65% yield (Scheme 3.5). Since this reaction still required three days to reach completion, though, there was still room for improvement.



Scheme 3.5 Improved NHK B-ring closures from the Kishi group

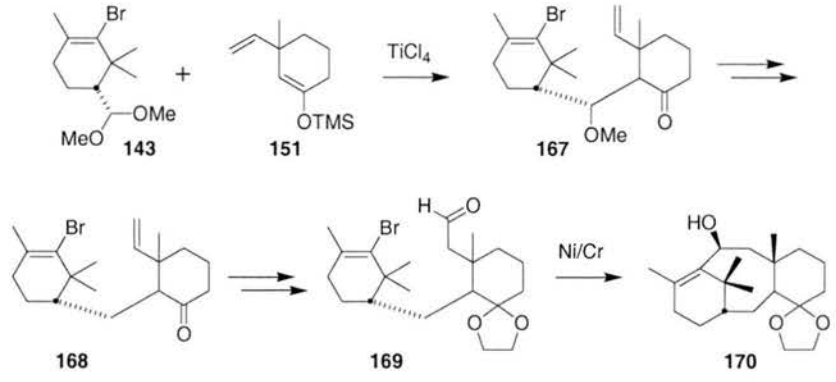
The next improvement from the Kishi group can be found in the Ph. D. thesis of X. C. Sheng,[70] which was submitted in 1998. In it are described modified conditions in which bis(cyclooctadiene) nickel(0) was added at the beginning of the reaction to provide a soluble source of nickel(0), the key species in the catalytic cycle whereby the carbon-

halogen bond is activated. With this abundance of soluble nickel(0), the transformation of triflate **165** to taxane **166** proceeded in 71% yield and was complete in only 12 hours.

These more recent improvements made cyclization via an NHK reaction a much more attractive approach than had initially been the case. It is worth noting, however, that in both of the substrates shown in Scheme 3.5 a dioxolane links the C-1 and C-2 oxygens, keeping the necessary reactive centers in close proximity to one another.

3.2 First generation cyclization substrate: the vinyl bromide approach

Our synthetic plan was to use Kende's methodology to assemble the skeleton of a substrate capable of undergoing cyclization via Kishi's NHK conditions. A logical starting point would be acetal **143**, which was available in enantiomerically pure form using methodology described in the last chapter (Scheme 3.6). This would hopefully couple with C-ring synthon **151** via an acetal variant of the Mukaiyama aldol reaction to give β-methoxy ketone **167**. Elimination of methanol followed by selective reduction of the conjugated double bond would hopefully deliver ketone **168**. The carbonyl of this ketone could be protected as the dioxolane, and subsequent hydroboration/oxidation could convert the terminal olefin to the primary alcohol, which could be oxidized to give

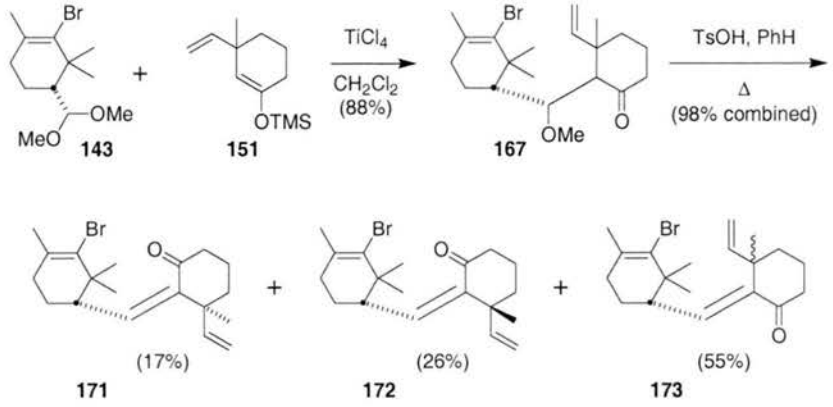


Scheme 3.6 Initial plan for assembling substrate for NHK reaction

aldehyde **169**. This would hopefully serve as a suitable substrate for closure of the B-ring via Kishi's application of the NHK reaction.

3.2.1 Early steps in work with vinyl bromide

Work in this direction began by preparing silyl enol ether **151** in one step through treatment of 3-methyl-2-cyclohexen-1-one with a mixed vinyl cuprate followed by trapping of the resulting enolate with chlorotrimethylsilane. The unpurified product of this reaction was then mixed with acetal **143**, dissolved in methylene chloride, and treated with titanium tetrachloride according to Kende's protocol to provide β-methoxy ketone **167** in good yield (Scheme 3.7). Elimination of methanol under acidic conditions, then, gave a product mixture which could be chromatographically separated into three discreet entities, which by NMR could be identified as one *Z* isomer (**171**), the other *Z* isomer (**172**), and the mixed *E* isomers, which were chromatographically inseparable (**173**).



Scheme 3.7 Mukaiyama aldol reaction and subsequent elimination

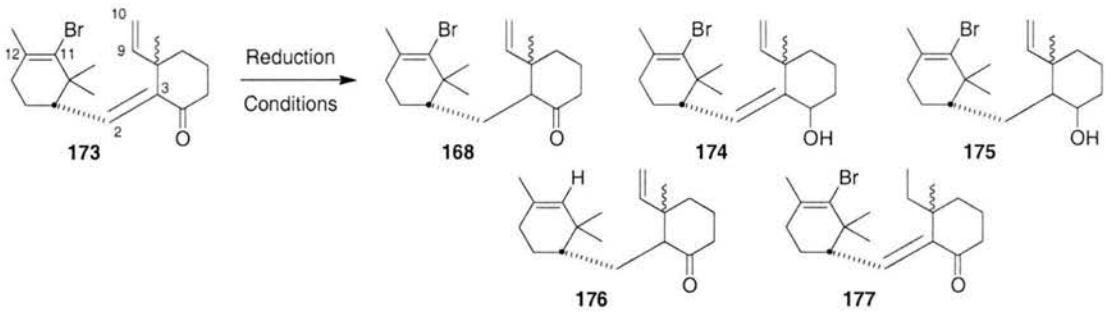
The first of these products, *Z* enone **171**, was crystalline, and X-ray analysis showed that it was the *Z* enone possessing the desired stereochemistry at the angular methyl group. The fact that the *Z* enone possessing the undesired stereochemistry could be isolated in higher yield than the *Z* enone possessing the desired stereochemistry,

coupled with the fact that the overall mass recovery from this reaction was very good, indicated that the mixture of *E* enones must be enriched with the desired diastereomer.

This was one of three reasons that subsequent work focused on the *E* enones. The second reason was that the E enones accounted for the majority of the material isolated from this reaction. The third reason for working with the mixture of *E* enones was that the next step in the synthesis was envisioned to be a hydride-type conjugate reduction of the enone double bond, and such reductions have been show to proceed more readily with *E* enones than with *Z* enones.[71]

3.2.2 Conjugate reduction

The component diastereomers of enone mixture **173** each contained three double bonds. Moving forward in the desired manner would require selective reduction of one of these, the double bond between C-2 and C-3. Achieving selectivity over the C-11 – C-12 double bond did not present much of a challenge because of its being tetrasubstituted and because of the encumbrance of the geminal methyl groups on C-15. In fact, in his taxatriene synthesis (Scheme 3.3), Kende was able to selectively reduce a C-2 – C-3 double bond in the presence of a C-11 – C-12 double bond by simply carrying out a catalytic hydrogenation at atmospheric pressure.[48] A greater challenge would be posed by the C-9 – C-10 double bond, which, by virtue of its being monosubstituted would be much more sterically accessible than the C-2 – C-3 double bond, and therefore likely to be reduced under conditions of heterogeneous catalysis much more rapidly.



	Reduction Conditions	% 168	% 174	% 175	% 176	% 177
1	Li^0, NH_3, Et_2O, EtOH, -78° C	0	0	0	50	0
2	Li^0, NH_3, Et_2O, EtOH, -33° C	0	0	0	18	0
3	Et_3SiH, $(Ph_3P)_3RhCl$, THF, reflux[72]	0	0	0	0	32
4	Et_3SiH, $(Ph_3P)_3RhCl$, THF, 45° C	0	0	0	0	25
5	Et_3SiH, $(Ph_3P)_3RhCl$, PhH, 70° C	0	0	0	0	80
6	$(Ph_3P)_3RhCl$, EtOH, DABCO, reflux	0	0	0	0	0
7	a) LAH, THF b) $RuCl_3$, NaOH, tol.[73]	0	100	0	0	0
8	Bu_3SnH, AIBN, toluene, reflux[74]	0	0	0	0	0
9	LAH, 4 CuI, THF[9]	0	45	0	0	0
10	LAH, THF	0	100	0	0	0
11	9-BBN, THF[75]	0	80	0	0	0
12	DIBAL, THF	15	75	0	0	0
13	DIBAL, THF, HMPA[76]	0	0	0	0	0
14	DIBAL, hexanes	15	60	15	0	0
15	4 Eq. $NaBH_4$, pyridine[77]	40	50	10	0	0
16	4 Eq. $NaBH_4$, pyridine (scaled up)	23	60	15	0	0
17	4 Eq. $NaBH_4$, pyridine, toluene	5	80	5	0	0
18	a) 20 Eq. $NaBH_4$, py. b) Dess-Martin	45	0	0	0	0

Table 3.1 Optimization of the conjugate reduction of enone **173**

Five different strategies were employed in hopes of realizing the desired selectivity, and these efforts are summarized in Table 3.1. Dissolving metal reductions are known to selectively effect 1,4 reductions on α,β-unsaturated ketones,[78] but the rate of this process is slower than the rate at which carbon-halogen bonds are reduced under these conditions (entries 1 and 2). Wilkinson's catalyst, while active, uncharacteristically gave exclusively reduction of the terminal olefin (entries 3-5). 1,2 Reduction could be achieved cleanly with lithium aluminum hydride, but attempts to convert this allylic

alcohol to the corresponding ketone via isomerization of the double bond through the intermediacy of ruthenium(III) chloride failed (entry 7). Using tributyltin hydride as a hydrogen atom source for reduction via successive single-electron transfer returned only unreacted starting enone (entry 8).

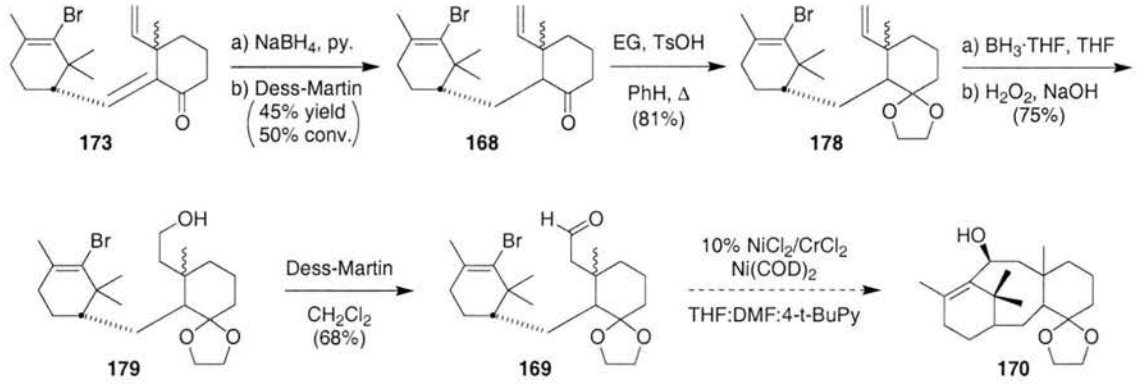
The fifth and most broadly explored means surveyed for carrying out the desired conjugate reduction involved the use of various reagents capable of effecting reduction through delivery of hydride. Since any such reagents will naturally have a great affinity for oxygen, finding a reagent combination that would preferentially deliver hydride to the β position of the enone rather than the carbonyl was expected to be a challenge. As mentioned, lithium aluminum hydride gave only reduction of the carbonyl (entry 10), as did 9-borabicyclononane (11) and *in situ* generated copper hydride species (9). As solvents of lower polarity were employed, diisobutylaluminum hydride began to give reasonably promising results, but even in the best case more than 60% of the product was that arising from reduction of the carbonyl (12-14).

Eventually a system consisting of excess sodium borohydride premixed with pyridine was settled upon as the best means for carrying out the conjugate reduction (entries 15-18). Addition of a 1.5 M solution of sodium borohydride in pyridine to enone **173** gave a product mixture consisting of three compounds: the product of 1,4 reduction, **168**; the product of 1,2 reduction, **174**; and the product of 1,4 reduction followed by 1,2 reduction, **175**. This product mixture could then be oxidized, either with Dess-Martin periodinane or with pyridinium dichromate to give a 45% yield of the conjugate reduction product and a 50% recovery of starting material. While not especially elegant, this allowed for as much as 700 mg of reduced ketone to be made at a time. Application

of these conditions to the conjugate reduction of pure *Z* enone, however, was entirely unsuccessful, giving only very slow 1,2 reduction.

3.2.3 Completion of initial cyclization substrate

With the methodology in place for generating useful amounts of ketone **168**, work could move forward, beginning with the protection of the carbonyl as dioxolane **178**, which could be also be accomplished using PPTS as a catalyst in place of TsOH, but at a much slower rate. Initial efforts to effect selective hydroboration of the terminal olefin of diene **178** using 9-borabicyclononane were unsuccessful, but using the much smaller borane-THF complex allowed for this transformation to proceed in good yield, giving alcohol **179**. Oxidation using Dess-Martin periodinane afforded the target cyclization substrate, aldehyde **169**, in 68% yield.



Scheme 3.8 Assembly of vinyl bromide cyclization substrate

When aldehyde **169** was subjected to Kishi's improved cyclization conditions, however, absolutely no reaction could be detected. Apparently, the nickel(0) in the reaction was unable to insert into the carbon-bromine bond at a detectable rate, and therefore the necessary organochromium species could not be formed.

While unfortunate, this was not a complete surprise. Kishi's cyclization substrates had all been either vinyl iodides or vinyl triflates, functionalities that have been shown to be far more readily activated than the corresponding bromides. Vinyl bromides have, however, worked with simpler systems[79] (adding 2-bromopropene to benzaldehyde, for example), but even in this case the reaction proceeded in reduced yield (87% for the bromide rather than 100% for the corresponding iodide) and took six times as long to go to completion. The easy accessibility of acetal **143** (Scheme 3.7) made a vinyl bromide-based approach worth trying, but the difficulty encountered in activation was not an unexpected result. Nonetheless, the synthetic methodology developed along this route was smoothly applied to future work, so at least the vinyl bromide approach served as an excellent model system.

3.3 Second generation cyclization substrate: the vinyl iodide approach

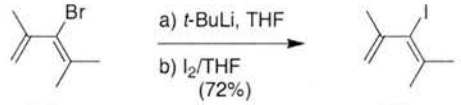
At this point, the synthesis of a cyclization substrate possessing either a vinyl triflate or a vinyl iodide at C-10 was clearly necessary. The best results reported to date with NHK reactions have employed vinyl iodides, and the conversion of vinyl bromides directly to vinyl iodides is a known process,[80] so the iodide analogous to vinyl bromide **169** was chosen as the target for a second generation cyclization substrate.

3.3.1 Developing useful bromine-iodine exchange methodology

Initially it was hoped that the bromine-iodine exchange could be carried out at a relatively late stage in the synthesis. This would allow us to be able to use advanced material currently in hand and keep to a minimum the number of steps though which a potentially reactive vinyl iodide would have to be carried. With that in mind, the ideal

substrate on which to carry out this exchange would be dioxolane **178**, which, thanks to the carbonyl being protected, lacked any especially sensitive functional groups. Since this was a reasonably advanced intermediate, a simpler, more easily accessible model system was desired for working out the exchange conditions.

Vinyl bromide **180** is an early intermediate in the synthesis of acetal **143**, and could be made on greater than 50 gram scale in two steps.[81] This abundance proved important because monitoring the exchange of bromine for iodine can be done conveniently with ^{13}C NMR, but cannot be done by ^{1}H NMR. Additionally, the steric environment around the carbon-bromine bond of this diene is similar to but not quite as demanding as that in the immediate vicinity of the carbon-bromine bond in advanced dioxolane **178**, so it was hoped this would serve well as a model. Nickel-mediated conditions reported to effect bromine-iodine exchange were unsuccessful,[80] but metal-halogen exchange could be smoothly executed with this substrate using *t*-butyllithium. Trapping this organolithium species with a solution of iodine in THF gave vinyl iodide **181** in good yield (Scheme 3.9).

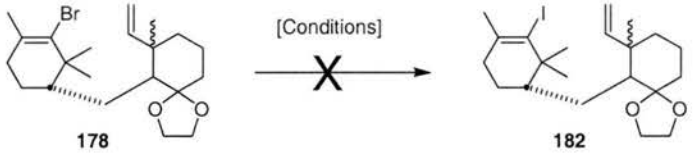


180 **181**

Scheme 3.9 Model system used for optimization of bromine-iodine exchange

Applying this metal-halogen exchange approach to dioxolane **178**, however, was almost completely unsuccessful (Table 3.2). Mass spectrometry indicated that a small amount of the desired vinyl iodide (**182**) was being formed, but it was too scant an amount to be detectable by ^{13}C NMR. Adding dimethoxyethane gave a small improvement in the metal-halogen exchange process, but warming this system gave only

starting material (entries 2 and 3). The next shift was toward a less hindered reagent, *n*-butyllithium, to effect metal-halogen exchange, but useful levels of anion formation could only be achieved at very high concentration and at temperatures too high to avoid side reactions (4-7). Attempts to trap this anion with iodine were not successful. Attempts to form the desired anion using lithium(0) in diethyl ether failed (8), but the desired organolithium or the corresponding organosodium could be cleanly formed using either of two dissolving-metal protocols (9-10). The organolithium species could also be formed using 4,4'-di-*t*-butyldibenzyl as a single-electron transfer reagent (11). Unfortunately, trapping any of these species with iodine failed. Transforming the vinyl



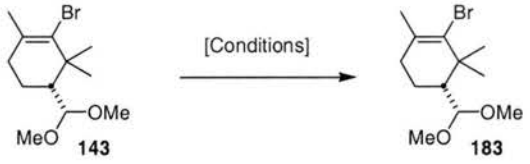
178 182

	Conditions	Result
1	a) 1.6 M *t*-BuLi/THF, -78° C b) 0.2 M I_2 in THF	Trace (<1%) product by HRMS
2	a) 1.6 M *t*-BuLi/THF-DME, -78° C b) 0.2 M I_2 in THF	Mostly sm, but some reduced
3	a) 1.6 M *t*-BuLi/THF-DME, -78° C → 0° C b) I_2 in THF	Only sm by APT
4	a) 2.6 M *n*-BuLi/THF, -78° C b) 0.2 M I_2 in THF	Only sm by APT
5	a) 2.5 M *n*-BuLi (45 eq)/THF-DME, -78° C → 0° C b) I_2 in THF	Mostly reduced sm
6	a) 2.0 M *n*-BuLi (4 eq)/Et_2O-DME, -78° C → 0° C	Only sm
7	a) 2.0 M *n*-BuLi (4 eq)/Et_2O, -78° C → 20° C	Only sm
8	a) Na-rich Li powder/Et_2O b) Solid I_2	Only sm by APT
9	a) $Li^0/NH_3/Et_2O$ b) I_2/Et_2O	Clean reduction
10	a) Na^0/HMPA/THF[82] b) Solid I_2	Clean reduction
11	a) Li^0/4,4'-di-*t*-butyldibenzyl/THF[83] b) Solid I_2	Clean reduction
12	a) $(Bu_3Sn)Cu(n\text{-}Bu)CNLi_2$/THF[84] b) Solid I_2	Very little reaction

Table 3.2 Attempts to effect bromine-iodine exchange with dioxolane **35**

bromide to the corresponding vinyl stannane through the agency of Lipshutz's mixed alkyl-stanyl cuprate also proved problematic (12). It appeared that the difficulties being encountered were largely a result of the steric congestion about the carbon-bromine bond of dioxolane **178**. This made metal-halogen exchange difficult and made the anion, when formed, too poor a nucleophile to successfully attack iodine.

Previous work had shown that not only could the vinyl bromide moiety of acetal **143** be easily converted to the corresponding organolithium, but this anion could also be trapped with an aldehyde electrophile (Scheme 2.15, Chapter 2). Early efforts showed that this anion could also be trapped with iodine (Table 3.3), a process which seemed to benefit from scaling up. The same outcome could be achieved by converting the vinyl bromide to the vinyl stannane using the Lipshutz methodology employed unsuccessfully earlier. This approach, however, introduced transition metals into the waste stream, a situation that would not be desirable given the scale that would have to be used if the bromine-iodine exchange were done this early in the synthesis. Fortunately, the process



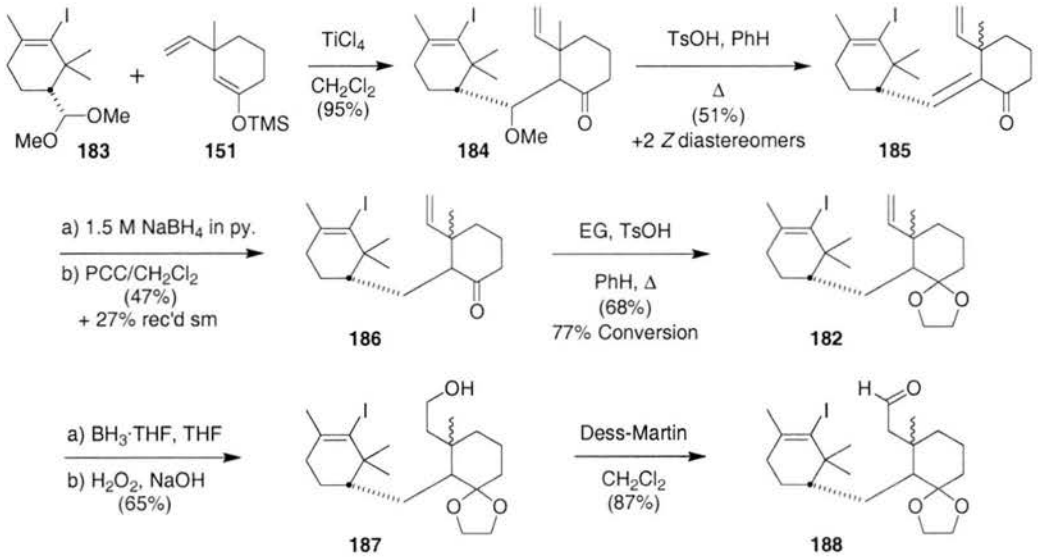
Conditions	Amount	Result
a) 1.6 M *t*-BuLi/THF b) I_2 in THF	157 mg	1:1 vinyl iodide:reduced vinyl
a) 1.6 M *t*-BuLi/THF b) I_2 in THF	454 mg	60% yield of vinyl iodide
a) $(Bu_3Sn)Cu(n\text{-}Bu)CNLi_2$/THF b) Solid I_2	1.73 g	58 % yield of vinyl iodide
a) 1.7 M *t*-BuLi/THF b) vacuum c) I_2 in THF	5.23 g	72% yield of vinyl iodide

Table 3.3 Optimization of bromine-iodine exchange with acetal **143**

of forming the vinyl iodide by way of the vinyl lithium could be improved by placing the reaction under vacuum after metal-halogen exchange. Doing so removed a good deal of the isobutylene (an artifact of metal-halogen exchange) that was interfering with iodine addition. This allowed for good yields of vinyl iodide **183** on a synthetically useful scale.

3.3.2 Synthesis of the first vinyl iodide cyclization substrate

Coupling vinyl iodide **183** to silyl enol ether **151** under Mukaiyama aldol conditions proceeded in slightly better yield than with the corresponding vinyl bromide, and subsequent elimination gave a comparable mixture of diastereomers, from which the mixed *E* enones (**185**) could be isolated in 51% yield (Scheme 3.10). Conjugate reduction proceeded in approximately the same fashion as with the vinyl bromide series, giving ketone **186** in 47% yield. Protection of the carbonyl gave dioxolane **182**, which underwent hydroboration/oxidation to give alcohol **187**. Dess-Martin oxidation provided the target cyclization substrate, aldehyde **188**, in 87% yield.



Scheme 3.10 Assembly of first vinyl iodide cyclization substrate

Unfortunately, when this aldehyde was subjected to Kishi's cyclization conditions no desired reaction occurred. While the starting iodide was never fully consumed in any of the attempts to effect this transformation, a significant amount of reduced material (in which the iodine was replaced by hydrogen) was produced in each of these reactions. This indicated that assembling a cyclization substrate possessing a vinyl iodide accomplished its objective, making possible the nickel insertion process and therefore allowing the crucial organochromium species to form. The failure of this intermediate to undergo the desired cyclization, then, could most likely be ascribed to conformational characteristics of the substrate that made extremely unlikely the possibility of the aldehyde carbonyl and the activated vinyl carbon encountering one another.

3.3.3 Changes aimed at adjusting substrate conformation and reactivity

Some analogues of aldehyde **188** were constructed in hopes of either addressing this potential conformational impediment or providing a substrate that could undergo cyclization by an alternate means. This was done in the hope that changing the conformation of the C-ring might allow the aldehyde carbonyl and the C-11 vinyl carbon to more readily occupy the same area of space. The C-ring can be expected to exist in either one of two chair-like conformations (Figure 3.1). In one, both the arm by which the A-ring is attached and the arm by which the aldehyde carbonyl is attached would occupy equatorial positions on the C-ring, and this would allow them to assume positions close to each other. In the other, both arms would occupy axial positions on opposite faces of the C-ring, a conformation in which cyclization is not possible. Proper choice of the substituent at C-4 could have a significant effect on which of the chairs is favored and how readily they could interconvert. This is because a substituent at this position would

be oriented in a manner that would allow it to either contribute to or alleviate 1,3-diaxial interactions with the groups on the quaternary center at C-8.

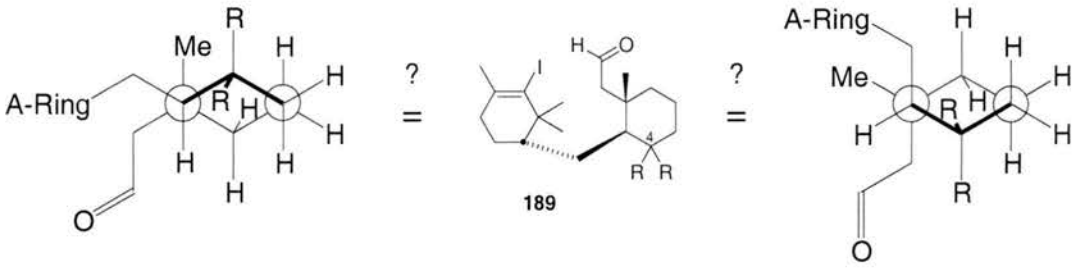
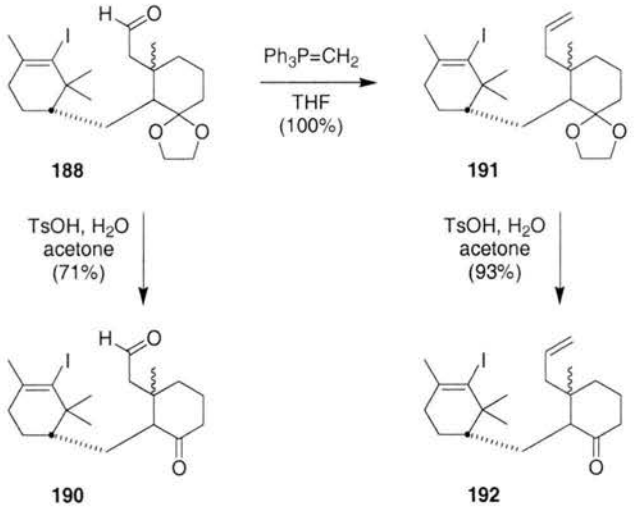


Figure 3.1 Possible C-ring conformations in cyclization substrates

To investigate what role, if any, substitution of this sort might play, the dioxolane at C-4 was removed from **188** in 71% yield, unmasking ketone **190** (Scheme 3.11). Subjection of this ketone to NHK cyclization conditions, however, led to the formation of no cyclized product in spite of a good degree of activation of the carbon-iodine bond, as determined by the amount of reduced iodide recovered from the reaction.



Scheme 3.11 Alternate cyclization substrates

In the course of his group's Taxol synthesis, Danishefsky reported unsuccessfully attempting an NHK cyclization on an aldehyde analog of their eventual cyclization substrate, terminal olefin **146** (Scheme 3.1). Their successful cyclization was

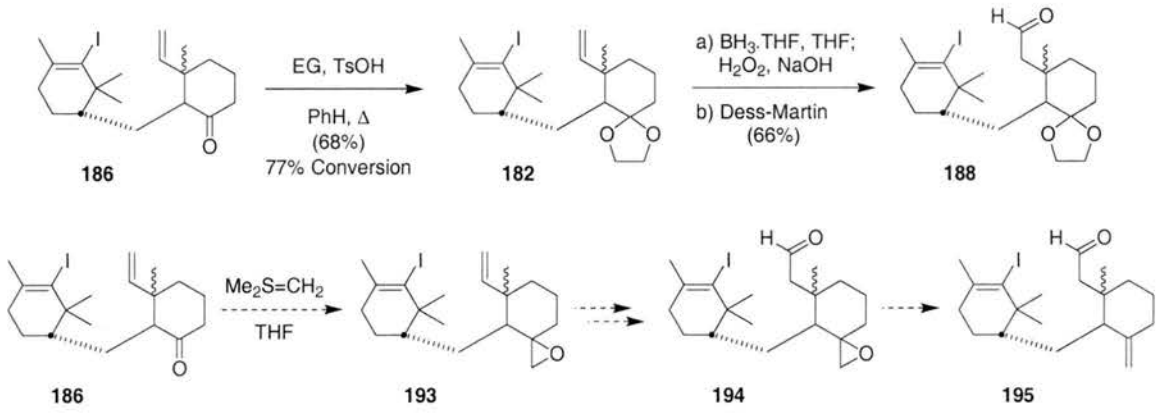
accomplished using an intramolecular Heck reaction, giving cyclized diene **147**, indicating that perhaps palladium-mediated cyclizations will work more reliably with this type of system than will NHK reactions. Wishing to investigate this, aldehyde **188** was subjected to Wittig conditions, giving terminal olefin **191** in quantitative yield. Some of this product was also deprotected to give ketone **192**, and both substrates were subjected to Danishefsky's cyclization conditions. In both instances, all of the starting material was slowly converted to a compound of lower chromatographic mobility, which was found to correspond to the product of palladium(0) into the carbon-iodine bond. Unfortunately, this intermediate could not be induced to cyclize.

Various other palladium sources were surveyed in hopes of effecting a cyclization. Some were chosen with an eye toward reducing the bulk around the activated carbon-iodine bond (such as the "ligandless" $Pd_2(dba)_3$).[85] Others were chosen in the hope that lowered phosphine levels would make the oxidative addition step more facile and allow for lower catalyst loading while also contributing to making the metal more accessible to the olefin it would need to coordinate (such as $(Ph_3P)_2PdCl_2$).[86] Still others were chosen with the hope of generating an intermediate in which the palladium would be effectively cationic, thereby accelerating the process of coordination to the electron-rich olefin ($Pd_2(dba)_3$ with Ag_3PO_4).[23] In all cases the starting vinyl iodide was consumed, but none of the conditions tried led to cyclization.

3.4 C-4-Centered spiroepoxide – a direct path to an ideal cyclization substrate

In the course of bringing along material to investigate these ring closures, a modification was envisioned that would hopefully allow the overall synthesis to be shortened by a step and keep to a minimum the number of acid-mediated steps that would

be necessary after cyclization. The modification would be made just after the conjugate reduction, when ketone **186** was in hand. Instead of protecting the carbonyl as the dioxolane followed by elaboration to aldehyde **188**, it could be treated with a sulfur ylide, hopefully giving spiroepoxide **193** (Scheme 3.12). This spiroepoxide could be elaborated in a similar fashion to give aldehyde **194**.



Scheme 3.12 Alternate directions to proceed from ketone **186**

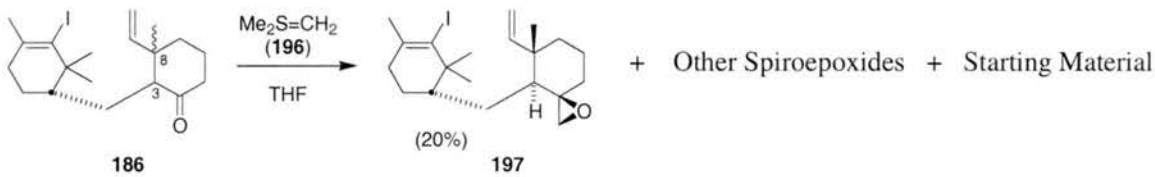
During his group's Taxol synthesis[46], Danishefsky needed to protect the C-11 – C-12 double bond. This was done by using *m*-chloroperoxybenzoic acid to convert the olefin to the corresponding epoxide. Several steps later, treatment with samarium(II) iodide converted the epoxide back to the olefin in 92% yield. It was hoped that such methodology could be applied to the conversion of epoxide **194** to olefin **195**.

This aldehyde would be an ideal cyclization substrate for several reasons. For one, the only oxygen atom would be that in the carbonyl, so potentially deleterious chelation of the Lewis-acidic intermediates of the NHK cyclization conditions by the oxygen atoms of a ketal or ketone would not be a problem. Additionally, only one reaction would be necessary after cyclization, a well-precedented allylic oxidation with selenium(IV) oxide to introduce an α-disposed alcohol at C-5. Furthermore, since a number of taxanes

possessing an exocyclic methylene between C-4 and C-20 have been isolated from natural sources, there was reason to believe that the product arising from cyclization of aldehyde **195** would not suffer from any particular instability and that the exocyclic methylene would not create an impediment to cyclization.

3.4.1 Spiroepoxide formation

The first step in assessing the viability of this alternative route was the treatment of ketone **186** with a sulfur ylide in hopes of generating the desired spiroepoxide. It should be noted at this point that this ketone was known to be a mixture containing both dispositions of the angular methyl group at C-8, and that the stereochemistry at C-3 was unknown and most likely a mixture of diastereomers in that regard as well. It was no surprise, then, that treatment of ketone **186** with sulfur ylide **196** gave a handful of products of varying mobility on silica gel (Scheme 3.13). The most polar of these products had the same mobility as the starting ketone, two more products were slightly less polar, and a fourth major spot was much less polar and could be cleanly separated from the others by chromatography. ^{1}H NMR indicated that this product was most likely a single diastereomer, and that the spiroepoxide had been successfully installed. The 20% yield was low, but if, to a first approximation, it can be assumed that the starting ketone was a mixture consisting of equal amounts of four diastereomers, the maximum yield would have been 25%.



186 197

Scheme 3.13 Spiroepoxide products arising from ketone **186**

As it turns out, the least polar product, spiroepoxide **197**, was crystalline, and X-ray analysis showed that it possessed the desired stereochemistry at all three chiral centers (C-1, C-3, and C-8, Figure 3.2). Therefore, installation of the spiroepoxide and resolution of the diastereomeric mixture could be performed in a single step.

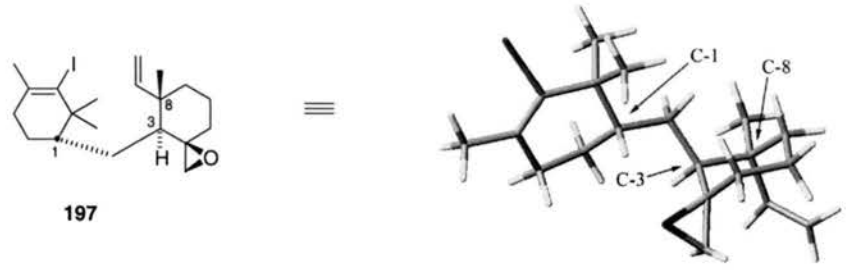
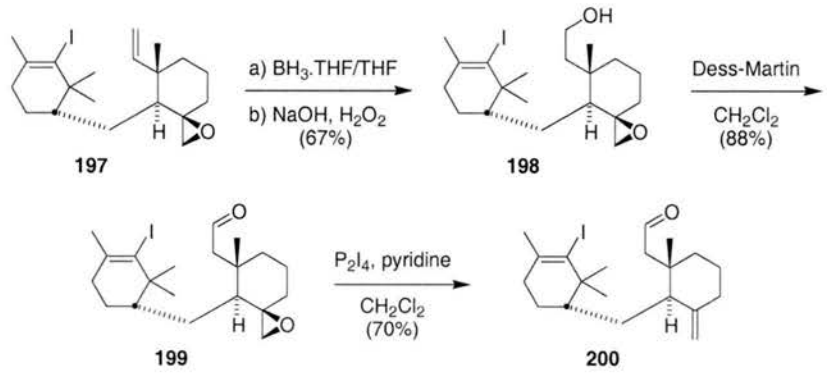


Figure 3.2 Structure of spiroepoxide **54** as determined by X-ray analysis

3.4.2 Assembly of cyclization substrate

Next it was necessary to elaborate terminal olefin of spiroepoxide **197** into the desired aldehyde. The first step in this process was the one with the greatest inherent risk, since the Lewis acidity of borane-THF complex could threaten the epoxide. As it turned out, this was not a major problem, and hydroboration followed by the usual basic peroxide workup furnished alcohol **198** in good yield (Scheme 3.14). This alcohol could then be smoothly oxidized under Dess-Martin conditions to provide aldehyde **199**.

At this point it was hoped the spiroepoxide could be converted to the exocyclic olefin using Danishefsky's samarium(II) iodide conditions. Treatment the epoxide with samarium(II) iodide, however, gave no reaction whatsoever. Fortunately, the desired transformation could be very cleanly effected using diphosphorous tetraiodide with pyridine in methylene chloride, giving diene **200** in good yield.



Scheme 3.14 Assembly of second generation vinyl iodide NHK cyclization substrate

3.4.3 Cyclization efforts and conclusions

The ideal cyclization substrate was now in hand. Should the key ring closure work, Kishi's work indicated that the product would most likely be that with the β-disposed alcohol at C-10, just as was desired. Allylic oxidation, then, would deliver the target molecule, the 5α, 10β taxadiene diol.

Reactions of this nature require impeccable reagents and meticulous technique, lessons learned in attempting cyclizations with previous substrates. When subjected to the conditions of the NHK reaction vinyl iodide **200** could be converted to the requisite vinyl chromium species with greater than 90% efficiency, as determined by the isolation of reduced vinyl iodide. Unfortunately, no taxane product of any kind was detected; only the product arising from protic quench of the activated vinylic species was recovered.

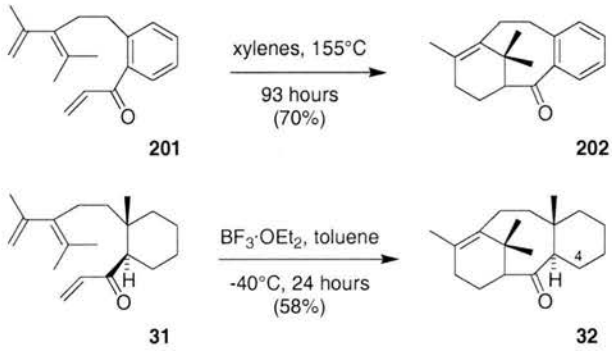
Throughout the process of refining the techniques and strategies employed in trying to effect an NHK-mediated cyclization, a good deal of communication took place with Jongwon Lim, who completed an unpublished total synthesis of Taxol while working in the Kishi labs as a graduate student. He provided a number of good insights and some excellent suggestions. Toward the later stages of this effort, he also provided

perspective: he said that the Kishi group has made more than 25 NHK cyclization substrates, and no more than 5 have cyclized successfully. Every possible step has to be taken to ensure cyclization, and even then there is no assurance of success.

Chapter 4

Attempting to form the A- and B-rings through an Intramolecular Diels-Alder reaction

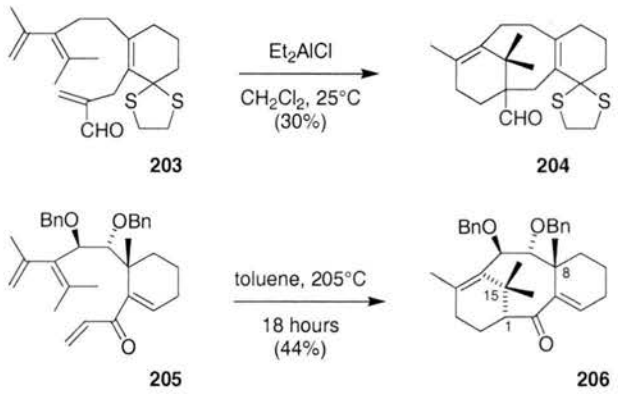
As early as 1983 Shea's group was investigating the feasibility of forming the A- and B-rings of a taxane skeleton through a type-II intramolecular Diels-Alder reaction (IMDA).[87] At that time they reported the assembly of triene **201**, which could be thermally induced to undergo cycloaddition, giving taxane analog **202** in 70% yield (Scheme 4.1). While promising, there was no guarantee that this approach would work on a substrate whose C-ring was saturated and possessed the necessary C-8 angular methyl group. In 1987, however, Jenkins reported the Lewis acid mediated conversion of triene **31** to taxoid **32** in 58% yield.[36]



Scheme 4.1 Cycloadditions from Shea and Jenkins giving taxane-type frameworks

With this groundwork in place, efforts turned toward functionalizing the taxane skeleton in a manner that could allow access to naturally occurring taxanes. Perhaps the most significant necessary modification would be the introduction of a one-carbon unit at C-4 to attach the C-20 carbon present in all natural taxanes. Toward that end, Shea reported in 1988 the transformation of tetraene **203** into potential taxane precursor **204**

(Scheme 4.2).[81] This was a capricious reaction, failing with several other Lewis acids, giving a 12% yield under thermal conditions, and failing under Lewis acidic or thermal conditions if the aldehyde were replaced with the corresponding methyl ester. He did not report any further elaboration of this product.



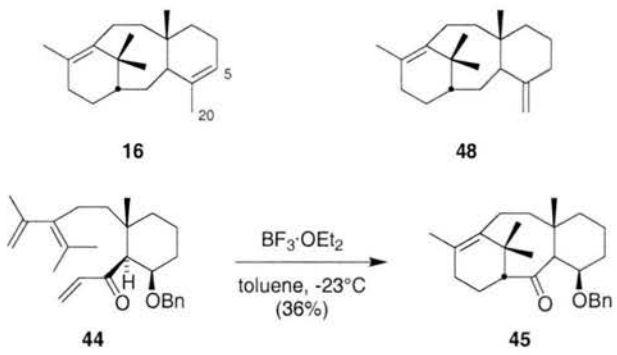
Scheme 4.2 Shea's first steps toward more functionalized taxanes

In 1994 their group reported the thermally induced intramolecular cycloaddition of tetraene **205** into potential taxane precursor **206**.[88] This approach not only allowed for the possibility of elaboration at C-4 through conjugate addition, but also introduced oxygenation at C-9 and C-10, necessary to access most natural taxanes. Unfortunately, the only tricyclic product of this cyclization was that with the C-15 geminal methyl groups on the opposite face of the B-ring as the angular methyl group at C-8, indicating that the stereochemistry of C-1 was incorrect. Reduction of the carbonyl of enone **206** allowed for determination of the relative stereochemistry by X-ray analysis, but no other elaboration of this compound was reported.

4.1.1 The Williams/Rubenstein extension of this work: a taxadiene total synthesis

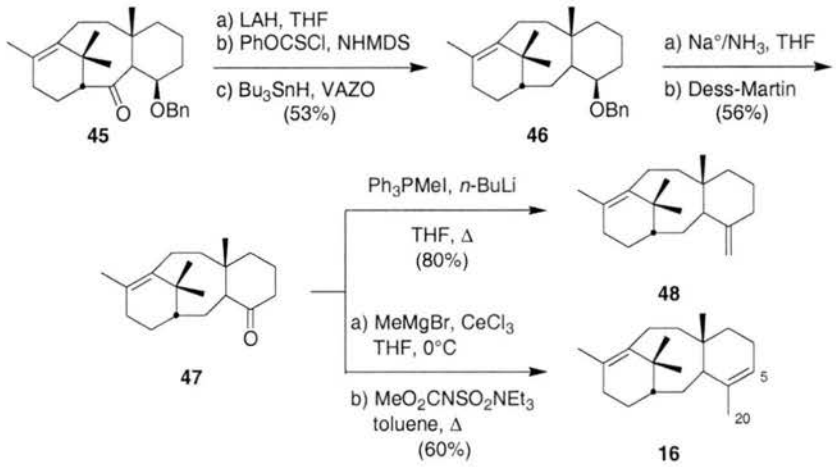
As discussed in the first chapter, the key requirement in the early work of the Williams group on taxol biosynthesis was a total synthesis that would allow access to

either taxa-4(5),11(12)-diene (**16**, Scheme 4.3) or taxa-4(20),11(12)-diene (**48**). They synthesized triene **44** in 19 steps, which upon treatment with $BF_3{\cdot}Et_2O$ in toluene underwent cycloaddition in the desired manner to afford taxane precursor **45**, albeit in 36% yield.[37]



Scheme 4.3 Targets and key cycloaddition of Williams' taxadiene synthesis

In Williams' case, further manipulation of the cycloaddition product was possible. Lithium aluminum hydride reduction of ketone **45** gave the corresponding alcohol, which was converted to its xanthate ester and subsequently deoxygenated to give taxoid **46** (Scheme 4.4). Reductive benzyl ether cleavage followed by oxidation gave ketone **47**, and Wittig olefination provided taxa-4(20),11(12)-diene (**48**). Alternatively, nucleophilic addition to ketone **47** followed by elimination provided taxa-4(5),11(12)-diene (**16**).

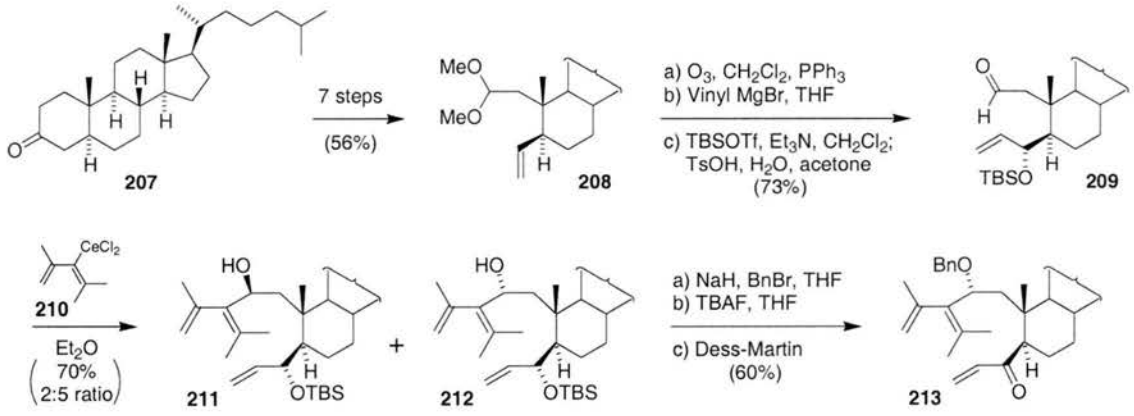


Scheme 4.4 Completion of Williams's taxadiene synthesis

4.1.2 Investigating functionality at C-10: Danishefsky's IMDA approach

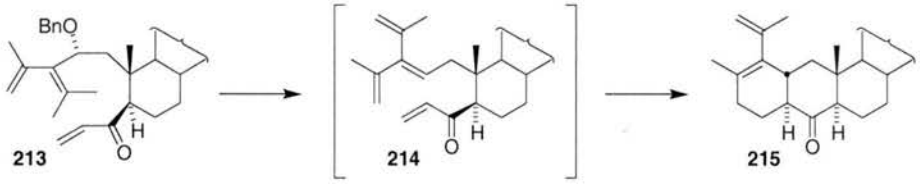
In the account of their ultimately successful efforts toward the total synthesis of taxol, the Danishefsky group described trying to assemble the requisite taxane skeleton through closure of the B-ring at both the C-1 – C-2 bond and the C-10 – C-11 bond.[46] At the same time, they were also looking into assembling a functionalized taxane skeleton through an intramolecular Diels-Alder reaction.[89,56] As was successful with their other work, the Danishefsky group took advantage of the ready availability of analogs built on steroid scaffolds to allow easy access to synthetically relevant substrates for studying the crucial steps of this pathway.

The first intramolecular Diels-Alder substrate of this nature they reported was triene **211** (Scheme 4.5). 5α-Cholestan-3-one (**207**) was converted in seven steps to acetal **208**,[90] and ozonolysis converted its terminal olefin into the corresponding aldehyde. Following vinyl addition and TBS protection, treatment with aqueous acid removed the acetal and liberated aldehyde **209**. Butadienyl species **210** was added into this aldehyde, giving alcohols **211** and **212** in a 2 : 5 ratio. The major product, α alcohol **212**, was carried on, and a protection-deprotection-oxidation sequence afforded triene **213**.



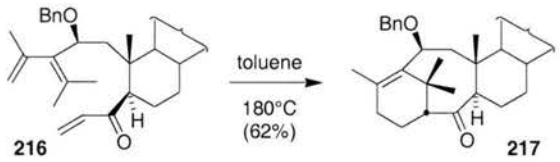
Scheme 4.5 Assembly of Danishefsky's first steroid-based IMDA substrate

They found, however, that triene **213** would not undergo the desired cycloaddition. When subjected to either thermal conditions or 5M lithium perchlorate, triene **213** eliminated benzyl alcohol, giving rise to tetraene **214**, which rapidly underwent a type I intramolecular Diels-Alder reaction to give diene **215** as the major product (Scheme 4.6). Treating **213** with either Lewis or Brønsted acids gave rise to rearrangement products.



Scheme 4.6 Deleterious elimination pathway observed by Danishefsky

They next carried β alcohol **211** through the same steps, giving triene **216** (Scheme 4.7). Initial treatment with $BF_3{\cdot}OEt_2$ gave the same rearrangement products observed with triene **213**. When heated to 197° in toluene, however, 14% of the desired steroid-taxane hybrid **217** was isolated. Lowering the temperature to 180° allowed them to obtain **217** in 62% yield.

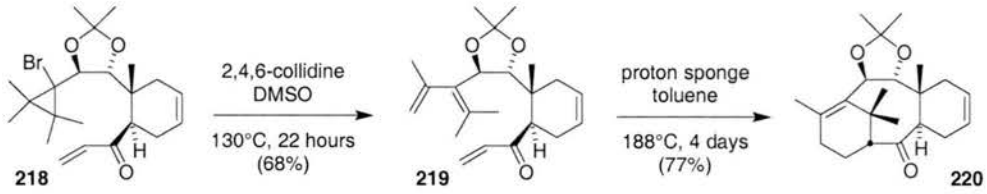


Scheme 4.7 Danishefsky's successful IMDA with β-disposed benzyloxy group

Though the Danishefsky group reports successfully translating the methodology developed with steroid-taxane hybrids to taxane construction in their Taxol total synthesis, they have not reported any attempts to transfer their intramolecular Diels-Alder methodology to substrates free of the steroid scaffold. It cannot be known for certain, then, if the steroid scaffold is necessary to constrain the C-ring in a conformational that allows the desired cycloaddition to occur.

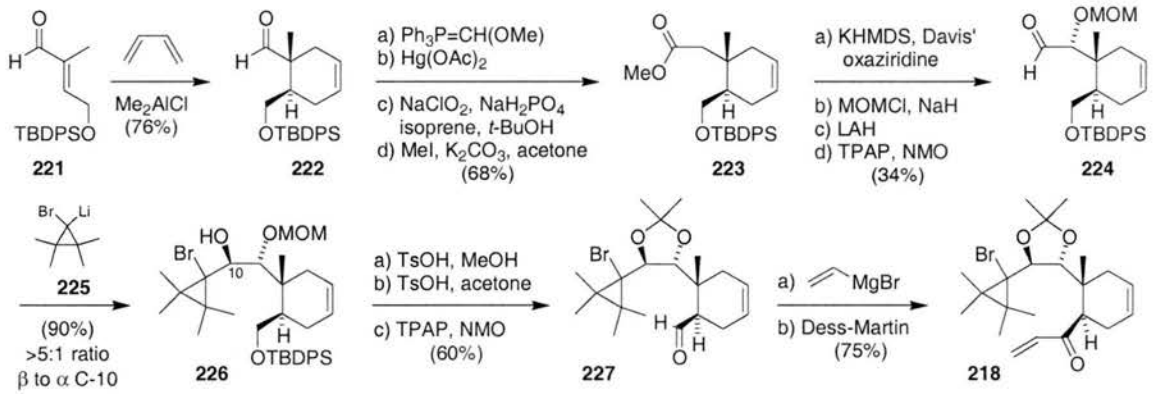
4.1.3 Shea's successful IMDA with functionality at C-9 and C-10

In 2001, Shea published a review of the type II intramolecular Diels-Alder reaction in which he included some recent work out of his labs that has thus far been otherwise unpublished.[58] The key steps of this newer work are the thermal opening of bromocyclopropane **218** to unmask the reactive diene of cycloaddition substrate **219** and the subsequent thermal intramolecular Diels-Alder reaction to give a 77% yield of taxane precursor **220** (Scheme 4.8).



Scheme 4.8 Key steps of Shea's recent IMDA work

Professor Shea was kind enough to provide us with a manuscript describing the synthesis of bromocyclopropane **218**. The key steps are the assembly of the C-ring through a Diels-Alder reaction between enone **221** and butadiene, the introduction of oxygen in a stereocontrolled manner at C-9 using Davis' oxaziridine, and the chelation-controlled addition of bromocyclopropyl anion **225** to give alcohol **226** (Scheme 4.9).

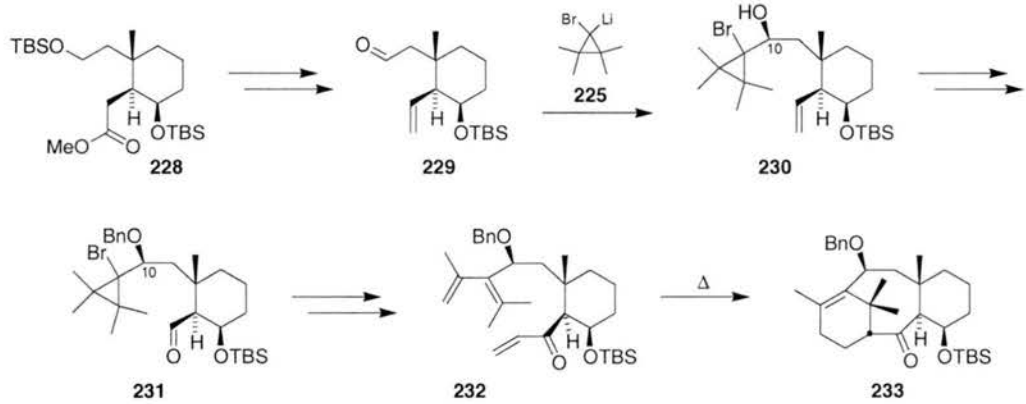


Scheme 4.9 Assembly of Shea's recent cycloaddition substrate

Shea has described taxane precursor **220** as a taxusin synthetic intermediate, but has not reported any work toward functionalizing C-5 in a manner that would allow for the introduction of the necessary C-20 carbon. With five allylic carbons, though, achieving such functionalization with any measure of selectivity would pose a significant challenge.

4.2 First synthesis of an IMDA substrate

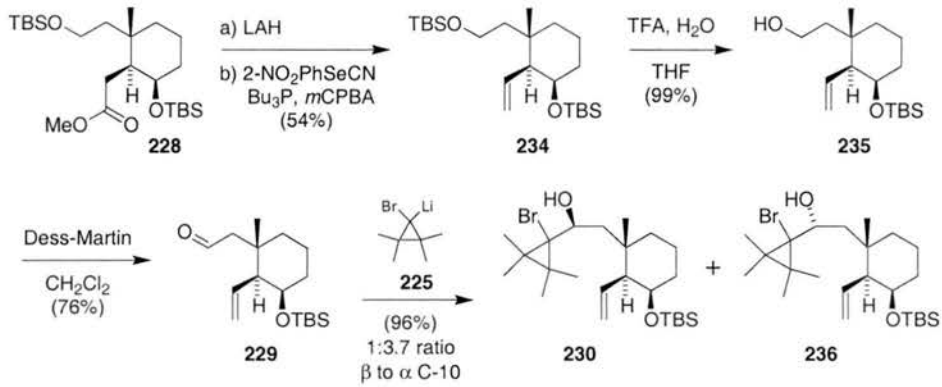
Our initial synthetic plan for assembling a substrate capable of undergoing an intramolecular Diels-Alder reaction began with ester **228**, a byproduct from the Williams taxadiene synthesis (Scheme 4.10).[37] Aldehyde **229** could be made through a sequence of reduction, elimination, selective deprotection, and oxidation. This aldehyde could be coupled with Seebach's bromocyclopropyl anion (**225**)[91] to give alcohol **230**, after which protection and ozonolysis could deliver aldehyde **231**. To this aldehyde could be added vinylmagnesium bromide, the resulting allylic alcohol could be oxidized to the corresponding enone, and then thermal opening of the cyclopropane could deliver the desired cycloaddition substrate, triene **232**. This would hopefully behave similarly to Danishefsky's substrate (**216**) when heated to give taxane precursor **233**.



Scheme 4.10 Initial plan for assembling IMDA substrate

4.2.1 Initial approach: C-4 hydroxyl protected as the TBS ether

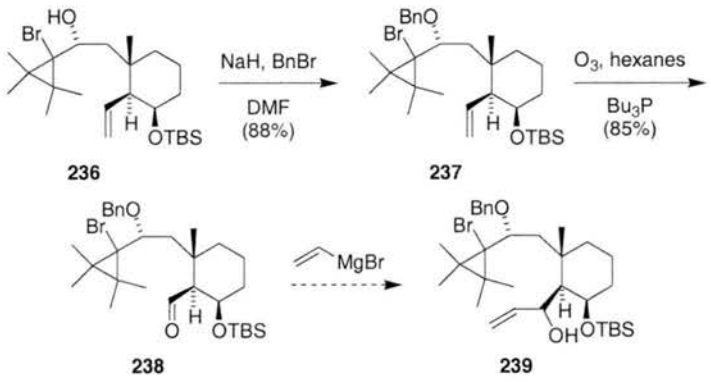
Work in this direction began with the reduction of ester **228** to the corresponding primary alcohol. This alcohol underwent elimination through the intermediacy of an aryl selenide to give terminal olefin **234** (Scheme 4.11). Treatment with aqueous acid allowed for selective removal of the primary TBS protecting group to give alcohol **235**, which was oxidized to aldehyde **229** under Dess-Martin conditions. A solution of this aldehyde in THF was then added to a cold (-105°C) solution of bromocyclopropyl anion **225**, giving in 96% yield a mixture of alcohols **236** and **230** in a 3.7 : 1 ratio. These diastereomers could be separated chromatographically, but it was not possible to determine conclusively which was the major product. Danishefsky's precedent[89] and a Spartan conformation search followed by semiempirical AM1 geometry optimization both indicated that alcohol **236**, with the α-disposed hydroxyl group should be the predominant product from this reaction. Since this could not be proven, however, initial work was done with the major diastereomer, later shown to be α alcohol **236**.



Scheme 4.11 Early steps in initial synthesis of IMDA substrate

Alcohol **236** was protected using benzyl bromide and sodium hydride, and ozonolysis on the crude benzyl ether gave aldehyde **238** (Scheme 4.12). At this point it

was hoped that vinylmagnesium bromide could add into this aldehyde to give allylic alcohol **239**. This addition, however, was unsuccessful when carried out in THF at -78°C (standard conditions), 0°C, and room temperature. Performing the reaction in DMF was equally ineffective.

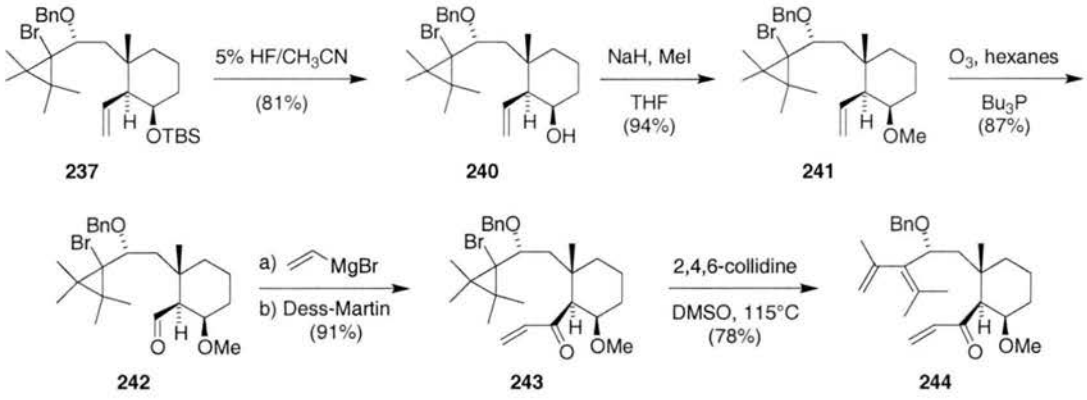


236 **237** **238** **239**

Scheme 4.12 Difficulty with vinyl addition to aldehyde

4.2.2 C-4 methyl ether as a small protecting group

Modeling indicated that the difficulties encountered with vinyl Grignard addition were perhaps a result of the significant steric impediment presented by the TBS protecting group on the C-4 hydroxyl. To determine if this were indeed the case, the TBS ether was replaced by a methyl ether. This was accomplished by using HF/CH_3CN to remove the silyl ether, giving alcohol **240**, and this alcohol was then converted to methyl ether **241** under standard conditions (Scheme 4.13). Ozonolysis gave aldehyde **242**, and treatment of this with vinylmagnesium bromide cleanly gave the desired allylic alcohol, which was oxidized to enone **243** using Dess-Martin periodinane. Heating enone **243** to 130°C in DMSO (Shea's ring-opening conditions) gave a poor yield of the desired triene, but lowering the reaction temperature to 115°C allowed for the unmasking of the diene in a very satisfactory 78% yield, giving triene **244**.



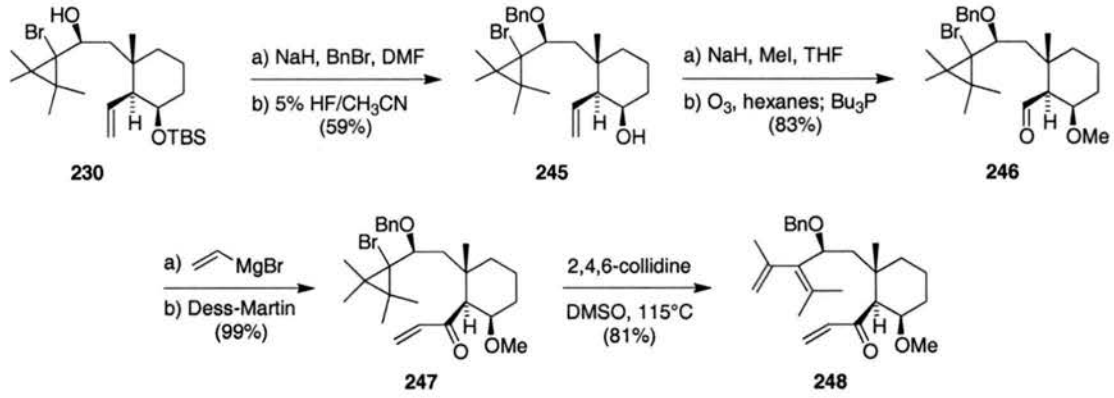
Scheme 4.13 Completion of the construction of the first IMDA substrate

Triene **244** did not react to any significant degree when heated to 180°C with proton sponge in toluene in a sealed tube. This lent credence to the theory that, in analogy to Danishefsky's report,[89] the species in hand was indeed that with the α-disposed benzyloxy group. Accepting the likelihood of this, work was begun on assembling an intramolecular Diels-Alder substrate starting with the minor coupling product, alcohol **230**.

4.3 Second synthesis of an IMDA substrate: β-disposed benzyloxy group

The experience gained synthesizing triene **244** allowed the construction of the triene with the epimeric benzyloxy group to go very smoothly. Benzyl protection of alcohol **230** went cleanly under standard conditions, and treating the crude reaction product with 5% HF/CH_3CN effected the desired deprotection, giving a 59% yield of alcohol **245** for the two-step sequence (Scheme 4.14). This alcohol was then cleanly converted to the corresponding methyl ether, and ozonolysis of the crude reaction product in hexanes delivered aldehyde **246** in 83% yield. Treatment of this aldehyde with vinyl Grignard gave the desired allylic alcohol, and this crude reaction product was oxidized using Dess-Martin periodinane to give enone **247** in nearly quantitative yield. This enone

was then subjected to the gentler ring opening conditions worked out with the other epimer, giving triene **248** in good yield.



Scheme 4.14 Completion of the construction of the second IMDA substrate

In the course of constructing triene **248**, it was found that enone **247** was a crystalline compound. High-quality crystals could be grown from a solution of the enone in pentane, and X-ray analysis showed that C-10 possessed an *S* configuration, meaning the benzyloxy group was indeed β-disposed (Figure 4.1).

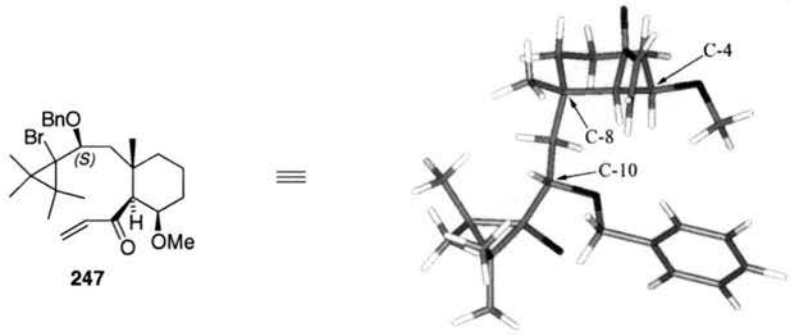
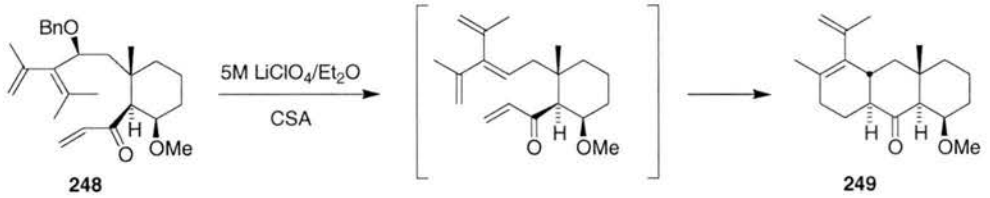


Figure 4.1 Structure of bromocyclopropane **247** as determined by X-ray analysis

Unfortunately, exposure of triene **248** to temperatures around 180°C in benzene, toluene, or xylenes, either in the presence or absence of proton sponge failed to deliver the desired taxane. Additionally, exposure of this triene to either $TiCl_4$ or $BF_3{\cdot}OEt_2$ in a series of different solvents resulted in the loss of both the benzyloxy and methoxy groups, while treatment with Me_2AlCl gave largely unreacted starting material. No reaction took

place when the triene was subjected to 5M lithium perchlorate in ether.[92] Addition of camphorsulfonic acid, on the other hand, which Grieco has introduced to effect more difficult cycloadditions[93], gave a clean, quick reaction in which the only product was tricyclic diene **249**. This arose from elimination of benzyl alcohol to give a tetraene, followed by a rapid type I intramolecular Diels-Alder cycloaddition (Scheme 4.15). Comparing the clean spectra of diene **249** to the spectra from earlier cycloaddition attempts showed that in most cases, a major component of the recovered material arose via this elimination-type I IMDA pathway.



Scheme 4.15 Elimination pathway observed with $LiClO_4/Et_2O$ or extended heating

4.4 Third synthesis of an IMDA substrate: α-disposed C-4 methoxy group

Perhaps the most significant difference between triene **248** and the intramolecular Diels-Alder substrates used successfully by Shea and Danishefsky is the presence of the methoxy group at C-4. This does not initially appear as though it would cause a problem. It is possible, though, that a substituent in this position could influence the conformation of the chair in which the C-ring prefers to reside.

As was the case with the NHK cyclization substrates made in Chapter 3, the desired taxane-forming reaction can only take place with the C-ring in the chair conformation that places both of the reactive arms in equatorial positions. In the case of intramolecular Diels-Alder substrates, the reactive arms are the enone connected to the C-

3 carbon and the diene moiety, which is tethered to the C-8 position. For these arms to be in equatorial positions on the C-ring, the angular methyl group at C-8 must occupy an axial position (Figure 3.2).

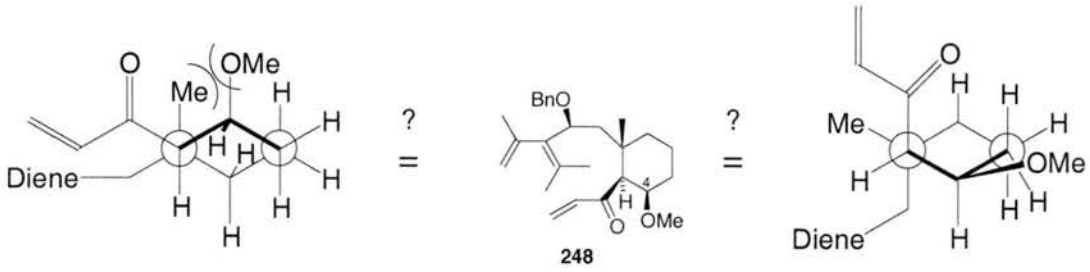


Figure 4.2 Possible C-ring conformations of IMDA substrates

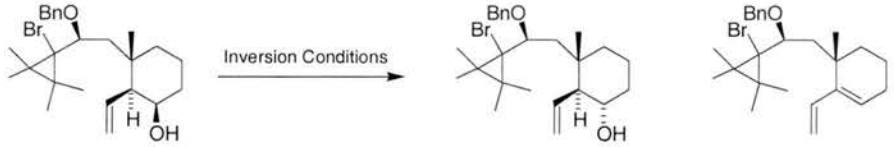
When the C-4 methoxy group is β-disposed, as is the case with triene **248**, the C-ring chair from which the desired cycloaddition can occur places both this methoxy and the angular methyl group on C-8 in axial positions on the same face of the ring. This placement gives rise to a significant and highly disfavored 1,3-diaxial interaction between the methoxy and methyl groups. An example of the effect this strain has on the conformation of the C-ring can be seen in the X-ray crystal structure of bromocyclopropane **247** shown in Figure 4.1. In order to avoid this interaction, the C-ring has assumed the chair conformation in which both the methyl and methoxy groups occupy equatorial positions, leaving the enone and latent diene arms in axial positions.

While such an impediment would not preclude the possibility of a non-trivial amount of the cycloaddition substrate assuming the conformation necessary for reaction, this would significantly decrease the amount of material poised to react in the desired fashion. This would allow side reactions that were too slow to plague the work of Shea and Danishefsky to become predominant. Such a condition would not be conducive a successful intramolecular Diels-Alder reaction. This problem could be alleviated,

however, by altering the disposition of the C-4 methoxy group so that it would possess an α disposition. Therefore, work was begun to construct an intramolecular Diels-Alder substrate possessing an α-disposed C-4 methoxy group.

4.4.1 Initial approach: hydroxyl inversion after bromocyclopropane addition

Hoping to keep to a minimum the amount of new chemistry that would have to be developed during the construction of an intramolecular Diels-Alder substrate possessing an α-disposed C-4 methoxy group, initial work focused on inverting the secondary hydroxyl group of alcohol **245**, encountered previously in Scheme 4.14. Mitsunobu conditions were naturally the first to be explored. Implementation of Martin's modified Mitsunobu conditions,[94] in which *p*-nitrobenzoic acid is employed as the nucleophilic partner, however, led to only a very quick, very clean elimination, giving diene **251** quantitatively (entries 1 and 2, Table 4.1).



245 **250** **251**

	Reaction Conditions	Scale	% 250	% 251
1	2 Eq. Ph_3P, 2 Eq. *p*-NO_2PhCO_2H, 2 Eq. DIAD, PhH	12 mg	0	62
2	5 Eq. Ph_3P, 5 Eq. *p*-NO_2PhCO_2H, 5 Eq. DIAD, PhH	11 mg	0	100
3	1) MsCl, Et_3N 2) KO_2, 18-crown-6, DME, DMSO	8 mg	29	60
4	1) MsCl, Et_3N 2) KO_2, 18-crown-6, 2 DME: 1 DMSO	6 mg	10	75
5	1) MsCl, Et_3N 2) KO_2, 18-crown-6, DME, DMSO	18 mg	12	75
6	1) MsCl, Et_3N 2) KO_2, 18-crown-6, DME, DMSO	39 mg	0	65

Table 4.1 Early attempts to invert the C-4 hydroxyl

A two-step protocol consisting of initial conversion of the β-hydroxyl to the corresponding mesylate and subsequent treatment of this mesylate with potassium

superoxide appeared to provide a promising starting point for developing useful inversion methodology.[95] On an 8 mg scale, 29% of the inverted alcohol could be isolated, although it was accompanied by a 60% yield of the diene resulting from elimination (entry 3). This reaction was very fast, so it was hoped that perhaps lowering solvent polarity would allow for suppression of the elimination pathway without curtailing the desired pathway of nucleophilic inversion. Potassium superoxide was entirely insoluble in a DME/THF solvent system, so the relatively moderate shift of solvent composition from 1 DME: 1 DMSO to 2 DME: 1 DMSO was investigated. This shift, however, did not have the desired outcome, actually leading to an increase the amount of diene (entry 4). Shifting to a more polar solvent system (1 DME: 2 DMSO) also failed to improve the ratio of inversion to elimination. Attempting to scale up the one modestly successful inversion reaction to 18 mg was accompanied by a significant drop in yield and selectivity (entry 5), and scaling up to 39 mg gave no inversion product at all.

It is worth noting that the only elimination product arising from these inversion attempts was diene **251**, with the internal double bond falling between C-3 and C-4. None of the other possible elimination product, which would possess a double bond between C-4 and C-5, was detected. This selectivity can attributed to two factors. First, the buildup of charge at C-3 would enjoy a measure of stability owing to its being allylic. Secondly, relative product energy would be expected play a significant role because a C-3 – C-4 double bond would be both trisubstituted and in conjugation with the terminal olefin, while a C-4 – C-5 double bond would enjoy neither of these advantages.

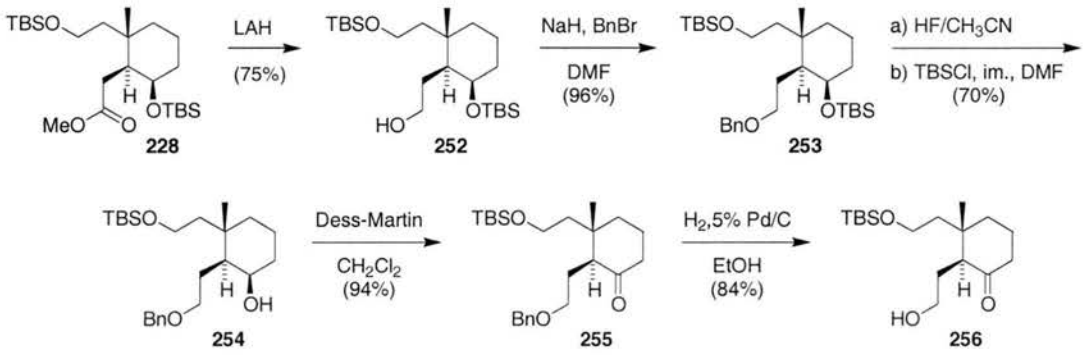
Two approaches were undertaken to try to achieve inversion while addressing the limitations inherent to this system. The first sought to avoid elimination-prone

intermediates by oxidizing the hydroxyl to the ketone, then carrying out a selective hydride reduction. The second approach was based on carrying out an inversion on a system in which the C-4 hydroxyl was no longer homoallylic.

4.4.2 Attempted hydroxyl inversion through oxidation then directed reduction

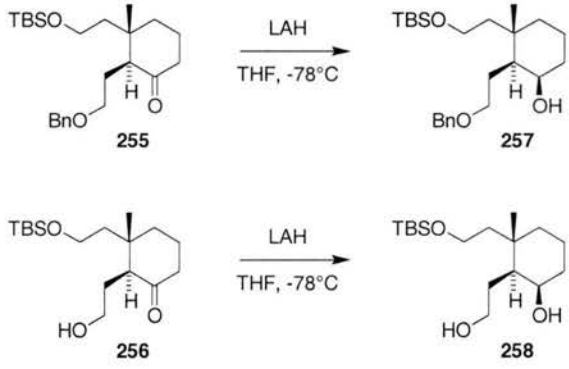
One method for inverting the stereochemistry of an alcohol is to oxidize the hydroxyl to the corresponding ketone and then carrying out a hydride reduction directed to the appropriate face of the ketone. Implementing such a strategy with alcohol **245** would have been dangerous because oxidation of the alcohol would have put C-3 in a situation in which it was both allylic and positioned α to a carbonyl. The enhanced acidity of the C-3 methine proton would make the position more susceptible to epimerization and increase the likelihood of the methine proton quenching a basic hydride source. In addition to this difficulty, alcohol **245** possessed no functionality that would lend itself particularly well to assisting in a chelation-controlled delivery of a hydride source to the top face of a C-4 carbonyl, as would be required.

Assembling an inversion substrate that both addressed the issue of C-3 acidity and incorporated functionality that would be suitable for a chelation-directed hydride delivery required manipulating C-ring starting point **228** in a new direction (Scheme 4.16). After reduction of the ester moiety in the manner employed earlier, giving alcohol **252**, the resulting hydroxyl was protected as the benzyl ether (**253**). Removal of both silyl protecting groups gave the corresponding diol, and the primary hydroxyl of this compound was selectively protected in good yield, giving secondary alcohol **254**. Oxidation under Dess-Martin conditions gave ketone **255** in high yield, and at this point the benzyl protecting group could be removed to give alcohol **256**.



Scheme 4.16 Assembly of substrates in which inversion could occur through reduction

The arm of alcohol **256** terminating in the free hydroxyl extends from the β face of the six-membered C-ring. It was hoped that this would enable the hydroxyl to chelate a hydride source and deliver it to the β face of the ketone. In case C-ring dynamics dictated that this hydroxyl deliver the hydride to the α face, some of benzyl ether **255** was saved to act as a complementary system.



Scheme 4.17 Undesired results of blocked and directed hydride reduction

Reduction of cyclohexanones has been shown to give primarily equatorial products when using small hydride sources, with lithium aluminum hydride showing the greatest equatorial selectivity.[96] When treated with lithium aluminum hydride at -78° C, however, both the free alcohol (**255**) and the benzyl ether (**256**) were converted into only β alcohols **257** and **258** respectively, with no α alcohol detected in either case (Scheme 4.17). Since all indications pointed to these conditions being the best available for the

desired transformation, and since no α alcohol could be detected in either case, it was decided to approach C-4 inversion from another direction.

4.4.3 Earlier hydroxyl inversion through non-homoallylic mesylate

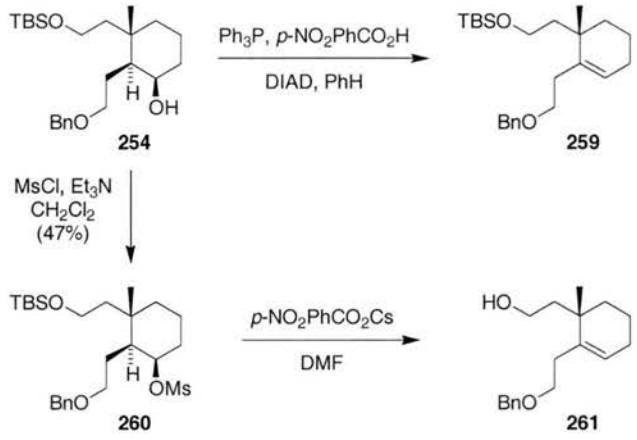
Altering C-3 in a way that would attenuate its ability to stabilize an accumulation of charge could only be approached in a manner that would address one of the responsible factors. Removing the two-carbon linker altogether was not an option because the stereochemistry at C-3, with the methine hydrogen α-disposed, needed to be maintained. The allylic nature of C-3, however, could be effectively masked.

The assembly of the first C-ring synthon employed in work toward intramolecular Diels-Alder substrates, aldehyde **229**, had been initiated by reducing ester **228** to the corresponding alcohol and then carrying out an aryl selenide-mediated elimination to deliver terminal olefin **234**. This olefin could be masked by delaying the elimination step until later in the synthesis. The primary hydroxyl group would require protection until such time as the elimination could be safely carried out.

An eye was kept toward the objective of synthesizing a C-ring with a masked olefin while work was being done on the assembly of a C-ring possessing a C-4 ketone suited for selective reduction (Scheme 4.16). Alcohol **254**, in fact, was an ideal candidate for assessing whether inversion could proceed more smoothly if C-3 were no longer an allylic position. Mitsunobu conditions offered the possibility of providing the most direct path to an inversion product, so these were the first to be tried. Unfortunately, a quick, clean elimination to olefin **259** was the only reaction observed (Scheme 4.18).

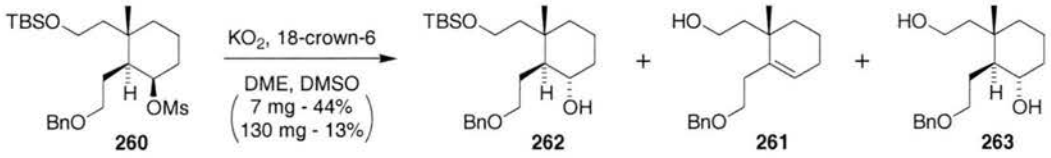
In order to try other inversion conditions, alcohol **254** was converted into mesylate **260**. Cesium carboxylates have been shown to be excellent nucleophiles when

used in DMF.[97] When a solution of mesylate **260** in DMF was treated with cesium *p*-nitrobenzoate, however, the product not only underwent complete elimination, it also lost its TBS protecting group, giving alcohol **261**.



Scheme 4.18 Initial inversion attempts with non-homoallylic alcohol

While Mitsunobu and cesium carboxylate conditions were entirely unsuccessful, initial work with potassium superoxide looked promising. The first time these conditions were employed with mesylate **260** (7 mg scale), a 44% yield of the desired inverted alcohol was realized (Scheme 4.19). The major product of this reaction was that arising from elimination with loss of the TBS group (**261**). Some product arising from inversion followed by loss of the TBS group was also recovered (**263**).

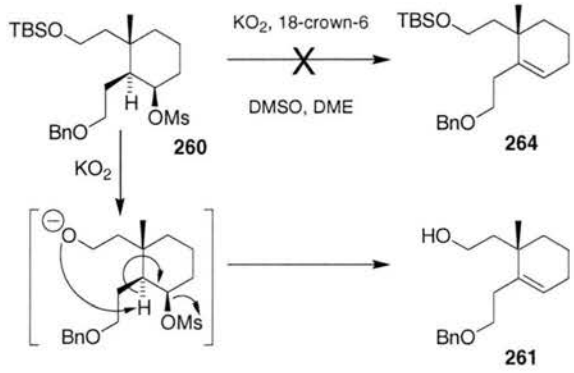


Scheme 4.19 Potassium superoxide inversion results with TBS protecting group

Scaling up (130 mg scale), however, tilted the product ratio dramatically in favor of the elimination product, giving only a 13% yield of the desired inversion product. Several attempts to improve the yield of this reaction were largely unsuccessful, but it was found that exceedingly dry solvents were necessary for reproducibility of results.

The presence of DMSO that was not thoroughly dried prevented the reaction from proceeding at all, presumably by accelerating the reduction of superoxide.

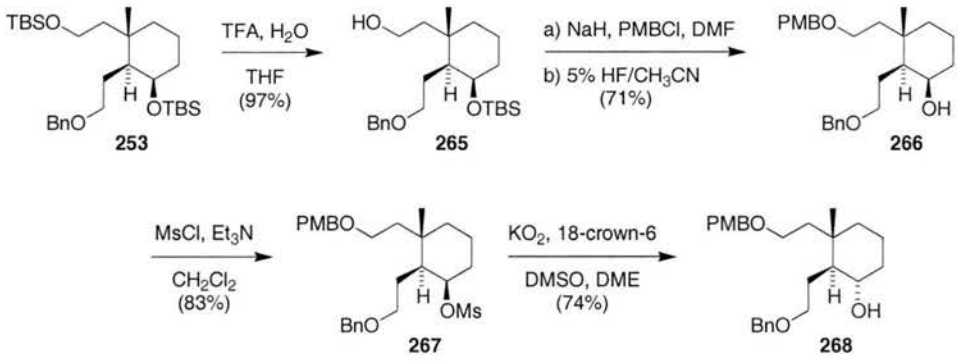
Interestingly, no elimination product was observed with the TBS group still in place. This prompted speculation that the major pathway of elimination may involve nucleophilic removal of the TBS group by superoxide followed by intramolecular abstraction of the methine proton, leading to elimination (Scheme 4.20). Using a protecting group less prone to nucleophilic removal was seen as a way to perhaps suppress this pathway.



Scheme 4.20 Mechanistic explanation for absence of **264** from inversion product mixture

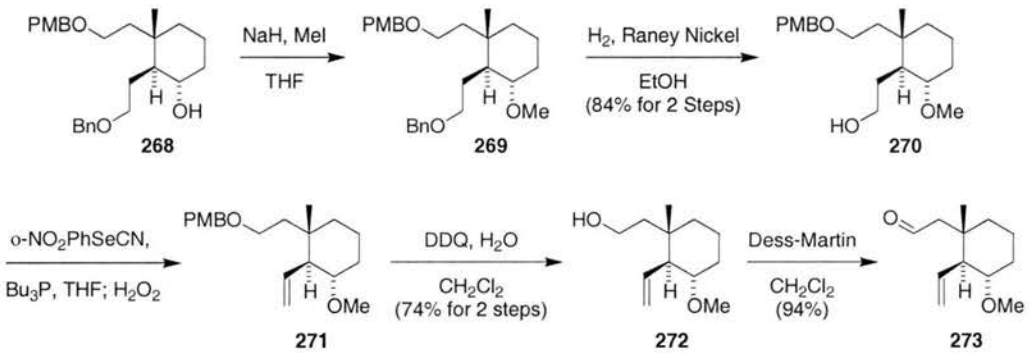
The *p*-methoxybenzyl group was seen as a protecting group that would not be susceptible to nucleophilic or basic removal but would be orthogonal to the benzyl group chosen to protect the other primary hydroxyl in this system. To introduce this group, the primary TBS group was selectively removed from benzyl ether **253**, and the resulting alcohol (**265**) was converted to the corresponding *p*-methoxybenzyl ether under standard conditions (Scheme 4.21). The TBS group was then removed from the secondary hydroxyl, giving alcohol **266**, and this was converted to mesylate **267**, which was found to be stable to column chromatography. When 18 mg of this mesylate were treated with potassium superoxide, a 62% yield of successfully inverted alcohol (**268**) was realized.

Better still, scaling up (87 mg and then 228 mg of mesylate) gave 74% yields of the desired α-disposed secondary alcohol.



Scheme 4.21 Inversion substrate with *p*-methoxybenzyl protected primary alcohol

Alcohol **268** was smoothly converted to methyl ether **269**, at which point it was necessary to selectively remove the benzyl protecting group without disturbing the *p*-methoxy benzyl group (Scheme 4.22). Reductive removal of the benzyl protecting group via hydrogenation catalyzed by 5% palladium on carbon gave only a 42% yield of the requisite alcohol, but when catalyzed by Raney nickel the benzyl removal went cleanly, giving alcohol **270** in good yield.[98] Selenium-mediated elimination "unmasked" the terminal olefin (**271**), from which the *p*-methoxybenzyl group was removed under oxidative conditions. Oxidation of alcohol **272** with Dess-Martin periodinane delivered aldehyde **273** in good yield.



Scheme 4.22 Elaboration of inversion product to aldehyde suitable for coupling

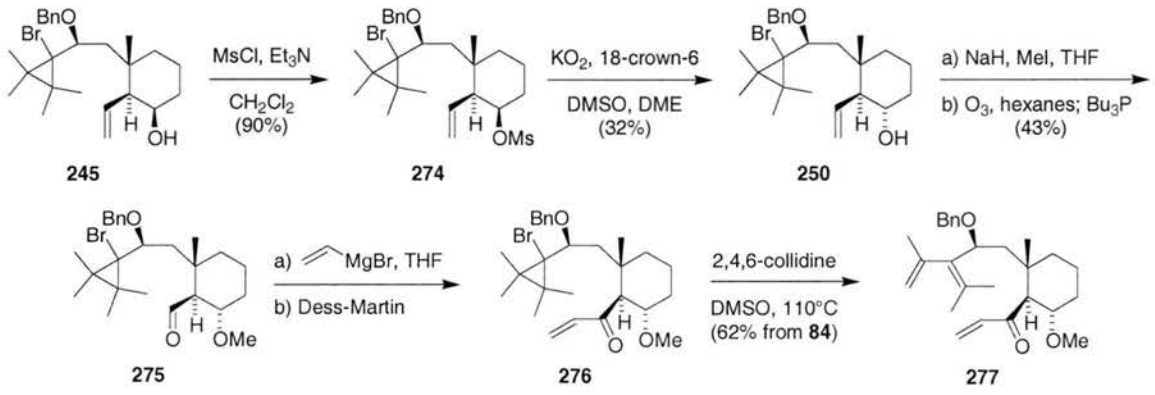
Aldehyde **273** was then treated with the same bromocyclopropyl anion that gave rise to the cyclopropane portion of **230**. The alcohol product of this reaction was carried a few more steps, but did not behave appropriately when it came time to open the cyclopropane. Characterization of intermediates after the coupling reaction was very challenging because, contrary to the case with earlier systems of this sort, the diastereomeric products of cyclopropyl addition could not be separated. Subsequent elaboration in the form of benzyl protection, ozonolysis, vinyl Grignard addition, and oxidation failed to provide diastereomers that could be separated.

Contributing to the problems of characterization, purification, and reactivity was the very real issue of throughput. Aldehyde **229**, used as the C-ring synthon for earlier intramolecular Diels-Alder substrates, was quickly and easily made, coming from ester **228** in four steps and 41% yield. Aldehyde **273**, on the other hand, was made in less than 17% yield over the course of twelve steps, seven of which required chromatography. The mounting difficulties of this approach prompted a return to investigations of inversion on the later-stage homoallylic system, in the hope that lessons learned in the course of work simpler mesylates would allow a greater degree of success in the context of more advanced intermediates.

4.4.4 Returning to a late, homoallylic mesylate as an inversion substrate

The first step taken to improve the process for inverting the C-4 hydroxyl in more advanced intermediates was to devise a means for generating the C-4 mesylate in very pure form. Earlier work indicated that mesylate **274** could be expected to behave unpredictably on silica gel, frequently undergoing a large amount of elimination during the course of chromatography. Further work, however, revealed that this mesylate could

be purified by rapid flash chromatography using a mobile phase in which the mesylate had very high mobility. This allowed for a minimal loss of material via elimination, allowing for high yields of the desired mesylate as a white solid (Scheme 4.23).



Scheme 4.23 A return to inversion using advanced intermediates

Applying the methodological advances made while studying the simpler systems, the inversion of mesylate **274** using potassium superoxide to give alcohol **250** was improved slightly and, more importantly, the reaction became more predictable. The key to successfully carrying out this transformation was the use of extremely dry solvents. Experimentally, this was accomplished by multiple treatment of the solvents with activated 4Å molecular sieves. Inversion product **250**, could then be converted to the corresponding methyl ether, which underwent ozonolysis to give aldehyde **275** in 43% yield. Attack on the aldehyde by vinylmagnesium bromide followed by Dess-Martin oxidation of the resulting alcohol cleanly provided enone **276**. Opening the bromocyclopropane under thermal conditions unmasked triene **277** in 62% yield from aldehyde **275**. Having demonstrated the lability of the benzyloxy and methoxy substituents in the presence of Lewis acids in work with earlier, closely related systems, cycloaddition efforts were limited to thermal conditions. Unfortunately, triene **277** failed to undergo the desired intramolecular Diels-Alder reaction under such conditions.

For four reasons it was becoming obvious at this point that a significant change of direction was necessary. First, the failure of the latest cycloaddition attempt did not bode well for any other closely related substrates. Second, the synthetic route to the cycloaddition substrate relied on two very low-yielding steps. The bromocyclopropane addition, which has given as high as a 96% yield, was doomed to be a point of major material loss by the fact that the addition product was a mixture of two diastereomers, with the undesired one predominating in a 3.7 : 1 ratio. Since the undesired diastereomer would not successfully a) undergo successful Mitsunobu inversion, b) be transformed into the mesylate or tosylate in preparation for inversion, or c) be oxidized to the corresponding ketone, setting up a subsequent reduction, this meant that 80% of the material coming into this step was going to be lost. The other low-yielding step, as discussed above, was the late-stage mesylate inversion. The third factor necessitating a change of direction was that the C-ring of all intramolecular Diels-Alder substrates to date was derived from a byproduct arising from a rather unselective Baeyer-Villager reaction along Williams' route to taxadiene.[37] Only a few grams of this material remained, so it was going to be necessary to make more or come up with a suitable alternate route better suited to our synthetic goals. The fourth reason for changing direction was that the semisynthetic work of Williams and Horiguchi had led to syntheses of both taxa-4(20),11(12)-dien-5α-acetoxy-10β-ol and a taxa-4(20),11(12)-dien-5α-acetoxy-2α,10β-ol.[39,40]

With methodology in hand for synthesizing these taxadienes, taxa-4(20),11(12)-dien-2α,5α,9α,10β-tetraol **86** became the next putative intermediate whose synthesis has not yet been accomplished, and would therefore be a potentially valuable target.

Furthermore, the substrate for an intramolecular Diels-Alder reaction leading to such a tetraol would more closely resemble that used by Shea to effect the successful (77% yield) cycloaddition described earlier (**219** to **220**, Scheme 4.8).[58] In their work they make no mention of elimination problems of the sort that have plagued our efforts to date.

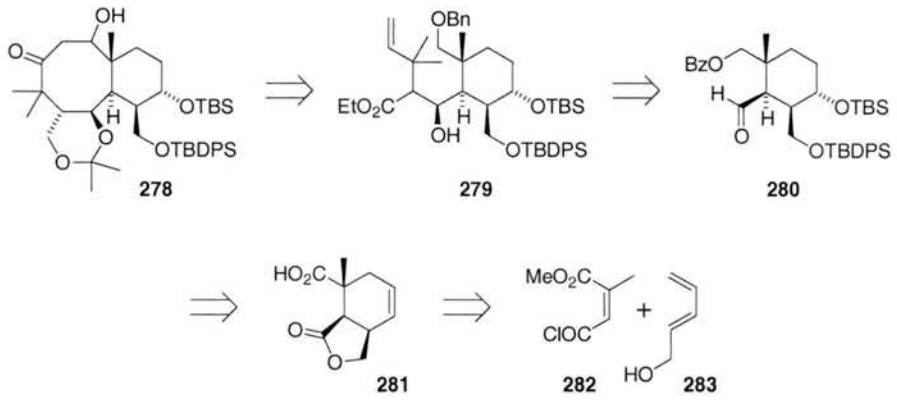
4.5 Efforts toward a taxadiene tetraol: a novel approach to an IMDA substrate

Given the pitfalls encountered during the previous route, a premium in planning out a new direction was put on the development of a practical synthesis. The guiding principles in this endeavor were caution and ease of implementation. This meant employing well-precedented reactions and sequences and keeping scalability in mind, making every effort to use inexpensive reagents and avoid chromatography if at all possible. This required eschewing flashy sequences that could potentially save steps in favor of more predictable methodologies. An eye was also kept on laying out a route on which a C-ring synthon could be generated that would be of general use for work in the area of taxane synthesis.

4.5.1 Ding's C-ring synthon

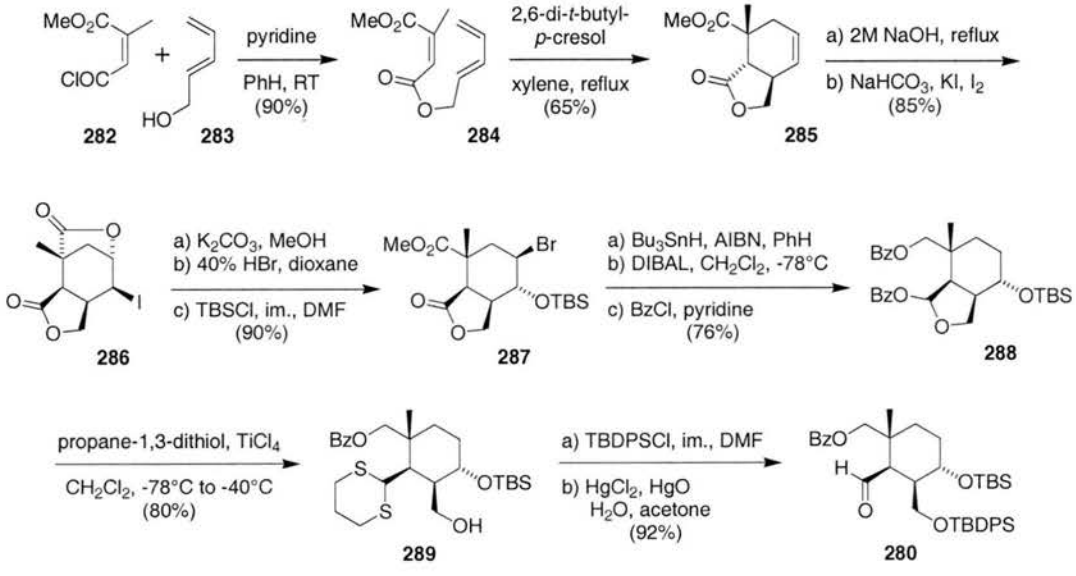
It was hoped that the later steps of the synthesis would be analogous to Shea's work toward taxusin, described in section 4.1.3. There existed, unfortunately, no high-probability means for slightly tweaking the early steps of Shea's synthesis to allow for functionalization at C-4, so a different approach to a C-ring synthon was necessary. The ground work for this was provided by Ding, whose synthesis of taxane CB ring system **278** built on the foundation of C-ring synthon **280** (Scheme 4.24).[99] This synthon was

assembled in 13 steps, starting from acid chloride **282** (prepared in three steps from citraconic anhydride) and pentadieneol **283** (prepared in two steps from acrolein).



Scheme 4.24 Ding's retrosynthesis

Ding's assembly of C-ring synthon **280** is depicted in Scheme 4.25. First among its noteworthy features is the intramolecular Diels-Alder reaction used to form bicyclic **285** from triene **284**, a transformation first reported by White in 1981.[100] After epimerization and concomitant saponification of the methyl ester to give *cis* γ-lactone **281**, iodolactonization delivered iodide **286**. Opening of the more strained upper lactone led to epoxide formation through alkoxide displacement of iodine, and this epoxide was

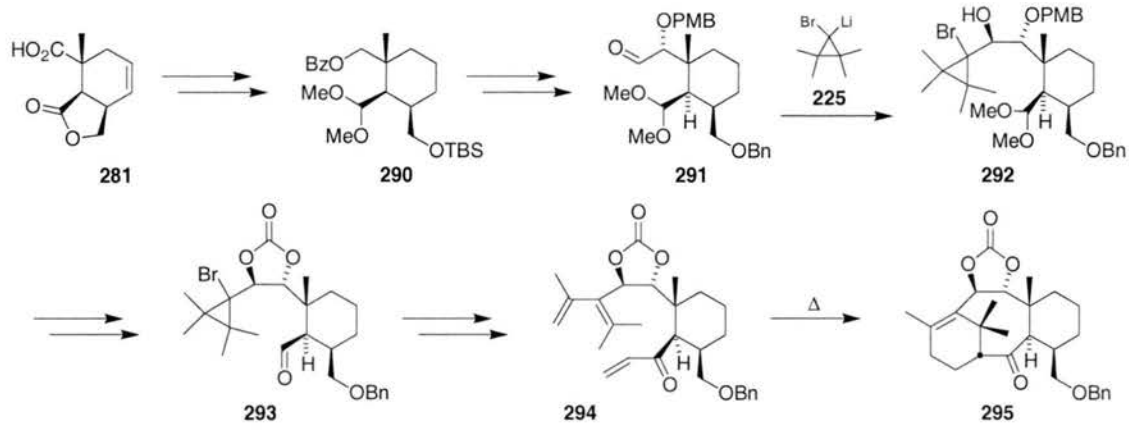


Scheme 4.25 Assembly of Ding's C-ring

opened with HBr, giving the desired α-disposed hydroxyl group at C-5. This hydroxyl was then protected as the *t*-butyldimethylsilyl ether (**287**). The superfluous bromine atom was removed using tributyltin hydride and AIBN, after which DIBAL reduction and treatment with benzoyl chloride gave dibenzoate **288**. This cyclic acetal was opened using propane-1,3-dithiol in the presence of $TiCl_4$ to give dithiane **289**. Hydroxyl protection and subsequent dithiane removal gave the C-ring synthon **280**.

4.5.2 Strategy for accessing taxadiene tetraol

Our synthetic plan for assembling a substrate capable of undergoing an intramolecular Diels-Alder reaction began with acid **281**, the product of the third step of Ding's C-ring synthesis (Scheme 4.26). Acetal **290** could be made through a sequence whose key steps closely resembled those employed by Ding in his assembly of aldehyde **280**. This acetal could be manipulated into an aldehyde such as **291**, whose most important feature would be the α-disposed *p*-methoxybenzyloxy group at what would become C-9. Its primary alcohol would also be protected as the benzyl ether rather than a silyl ether in order to avoid complications in the eventual removal of the dimethyl acetal. Coupling of aldehyde **291** with Seebach's bromocyclopropyl anion (**225**) would give alcohol **292**, and subsequent removal of the *p*-methoxybenzyl group, protection of the resulting diol as the cyclic carbonate, and acetal removal would deliver aldehyde **293**. To this aldehyde could be added vinylmagnesium bromide, the resulting allylic alcohol could be oxidized to the corresponding enone, and then thermal opening of the cyclopropane could deliver the desired cycloaddition substrate, triene **294**. This would hopefully behave similarly to Shea's cycloaddition substrate (**219**) when heated to give taxane precursor **295**.

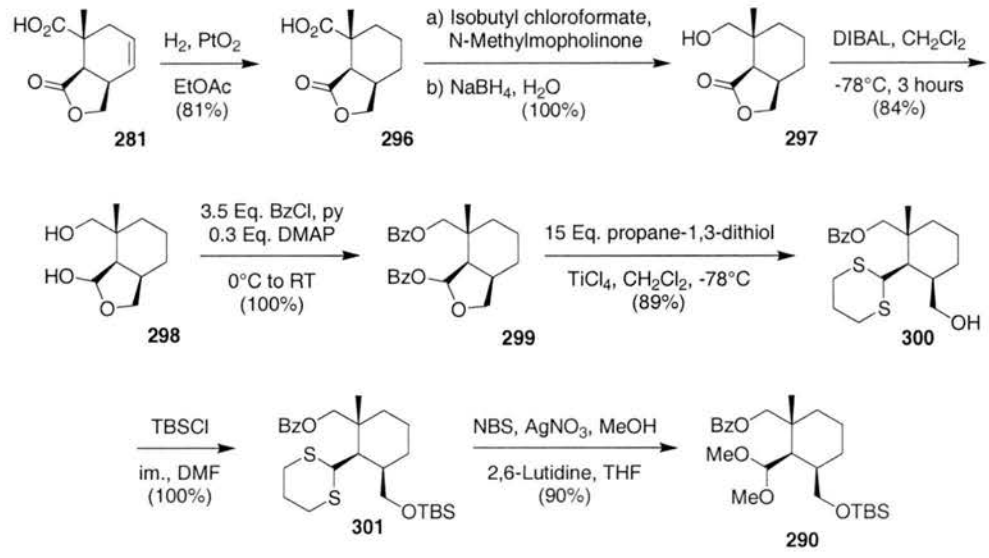


Scheme 4.26 Plan for accessing taxoid tetraol via IMDA reaction

4.5.3 A versatile C-ring for taxadiene synthesis

Adaptation of Ding's work to our needs began with carboxylic acid **89**, which was prepared on a twelve-gram scale in six steps using slightly modified versions of the reported procedures.[100,101] White reported using Adams' catalyst to reduce the double bond of an acid differing from **281** by only the addition of a methyl group in the upper allylic position, albeit with a 9.3% catalyst loading.[100] Fortunately, reduction of **281** went smoothly with only 1% catalyst loading to give **296** in good yield (Scheme 4.26). Initially, acid **296** was converted to the corresponding methyl ester in hopes of carrying out a DIBAL reduction of both the ester and the lactone in analogy to the transformation reported by Ding (between **287** and **288**, Scheme 4.25). Such a reduction, however, failed completely with this system, reducing only the lactone to the lactol under the gentle conditions employed by Ding. The lactone was reduced to the tetrahydrofuran under conditions forcing enough to reduce the ester. Reduction of **296** via the mixed anhydride, however, proceeded smoothly to give alcohol **297**, which could then be treated with DIBAL to give diol **298**. Formation of dibenzoate **299** also required more

strenuous conditions than those Ding reported, but after some modifications (use of DMAP, warming to room temperature, employing excess BzCl) the necessary dibenzoate could be formed in high yield.



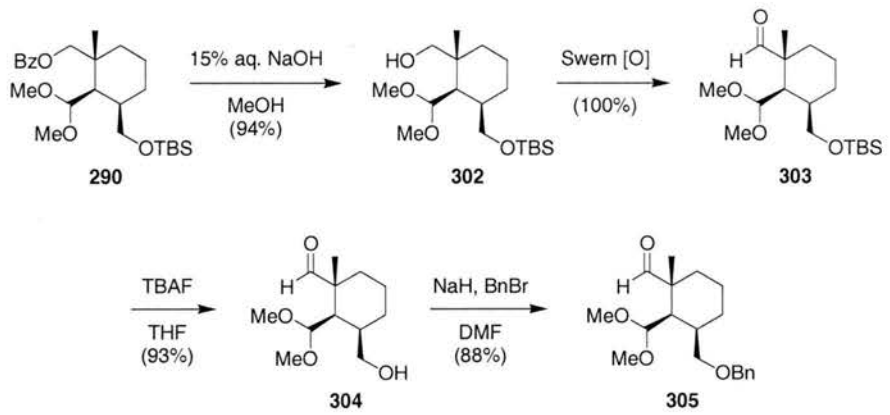
Scheme 4.27 Assembly of versatile C-ring synthon **290**

Ding reported opening the protected lactol of dibenzoate **288** in 80% yield using $TiCl_4$ and three equivalents of propane-1,3-dithiol, but such conditions only gave very poor yields when applied to **299**. Increasing the dithiol concentration and lowering the post-addition stir temperature from -40°C to -78°C, however, allowed for this dibenzoate to be converted into dithiane **300** in very good yield. It was hoped that the free hydroxyl of this dithiane could be protected as the benzyl ether, but the nucleophilicity of the sulfur atoms prevented this transformation from proceeding successfully under a variety of conditions. Formation of the *t*-butyldimethylsilyl ether, however, proceeded quantitatively, giving silyl ether **301**. Finding conditions for removing the dithiane of **301** that would be compatible with the presence of a primary silyl ether was not trivial, but eventually a hybridization of conditions used by Jones [102] with those used by Danishefsky[103] allowed for clean conversion of the dithiane to dimethyl acetal **290**.

Acetal **290** was the sort of versatile C-ring that had been envisioned, and its synthesis was in keeping with the goals laid out at its inception. The reactions were predictable and went in high yields, yields which improved as scale increased. Best of all, chromatography was only required after two steps, the formation of the dithiane and then its removal two steps later. Additionally, it was shown that either the benzoate or the silyl ether could be selectively removed in high yield, demonstrating that this synthon could be taken multiple directions.

4.5.4 Assembling a diene arm with appropriate functionality at C-9 and C-10

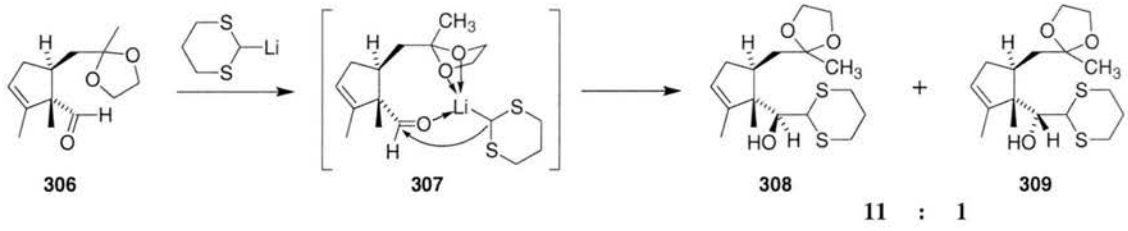
It was next required to begin the process of elaborating C-ring synthon **290** into aldehyde **291**. This α-hydroxy aldehyde was seen as coming from the attack of either a vinylic nucleophile or a deprotonated 1,3 dithiane on a C-9 aldehyde, followed by either ozonolysis or dithiane cleavage, respectively. The immediate goal, therefore, was to assemble an appropriate C-9 aldehyde.



Scheme 4.28 Assembling properly functionalized aldehyde **305**

The first step in this process was the removal of the benzoate group protecting the C-9 hydroxyl group, which proceeded in excellent yield (Scheme 4.28). Primary alcohol **302** was then oxidized cleanly to aldehyde **303** under Swern conditions. Attempts to

effect this oxidation using Dess-Martin conditions were only successful when pyridine was introduced as a co-solvent. In the absence of pyridine, trace acetic acid resulting from the Dess-Martin periodinane's coming into contact with moisture led to rapid displacement of one of the acetal methoxy groups by the C-9 hydroxyl, giving the corresponding tetrahydrofuranyl acetal. No such difficulties were encountered when Swern conditions were employed. Removal of the *t*-butyldimethylsilyl protecting group from aldehyde **303** was smoothly achieved using TBAF, giving alcohol **304**, and this alcohol was protected as the benzyl ether (**305**) under standard conditions.

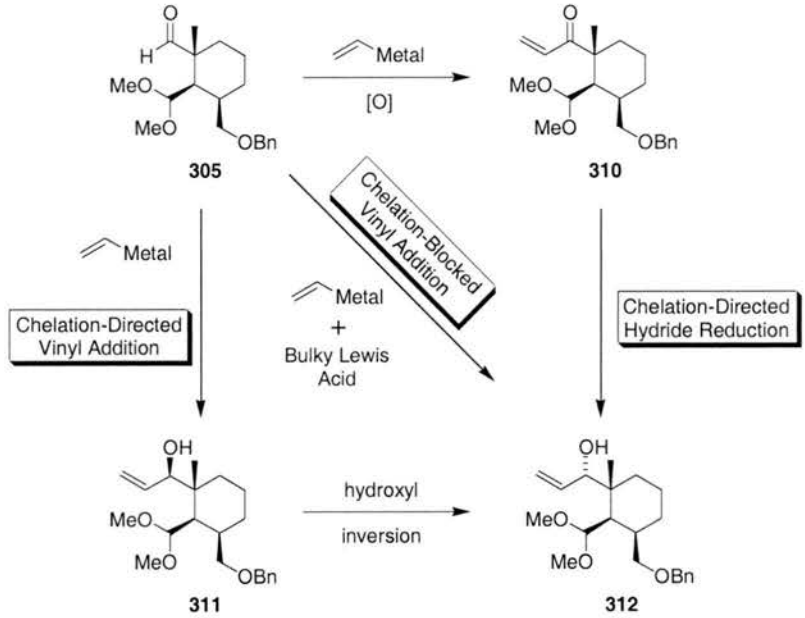


Scheme 4.29 Lansbury's use of an acetal to guide addition into an aldehyde

The use of a remote acetal to guide a carbanion to a particular face of an aldehyde was nicely demonstrated by Lansbury in 1985 during his work toward baldiulin.[104] When aldehyde **306** was treated with 2-lithio-1,3-dithiane at -65°C, both alcohols **308** and **309** were observed, with the β-disposed alcohol (**308**) predominating by an 11:1 ratio (Scheme 4.29). This selectivity was rationalized by transition state **307**, which depicts the trajectory of dithiane addition directed by the coordination of the two acetal oxygen atoms to lithium, while the rotation of aldehyde is restricted by the coordination of the aldehyde oxygen atom to lithium. This hypothesis was tested by the addition of five equivalents of HMPA to the reaction system. The HMPA was expected to coordinate to the lithium more vigorously than the aldehyde and acetal, removing any control over the

rotation of the aldehyde or trajectory of dithiane addition. As predicted, the product alcohols were generated in a 1:1 ratio.

In our work, we desired the kind of high selectivity demonstrated by Lansbury, but we required the selectivity to be in the opposite sense of what his group observed. There are three readily apparent ways for this to be accomplished (Scheme 4.30). The approach that initially looked the most appealing was addition to the aldehyde, oxidation of the resulting alcohol, and chelation-controlled delivering of a hydride reducing agent to the carbonyl. The next most obvious choice was addition to the aldehyde followed by inversion of the product alcohol, either via the mesylate or under Mitsunobu conditions. The third possible way to use this acetal to guide the addition in the desired sense would be to add a bulky Lewis acid to pre-coordinate to the available oxygen atoms, thereby blocking the trajectory of addition favored by acetal coordination.



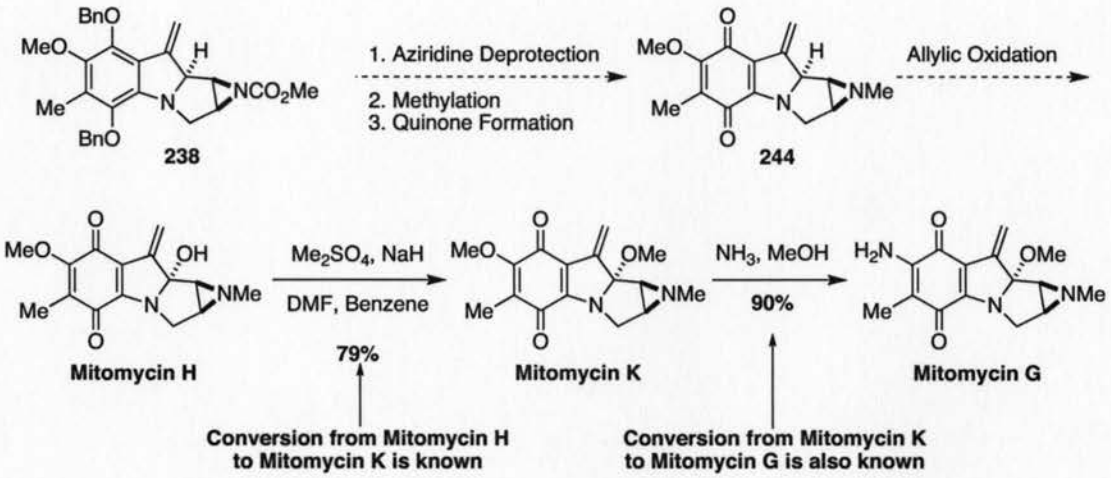
Scheme 4.30 Possible ways to introduce α-disposed hydroxyl in alcohol **312**

The first step in each of these processes is an addition into the aldehyde. Since selective removal of a dithiane in the presence of our dimethyl acetal would likely have

been problematic, it was determined that the carbonyl of required α-benzyloxy aldehyde **291** should come from addition of a formally anionic vinyl group. After protection of the resulting alcohol, oxidative cleavage of the double bond could liberate the desired aldehyde. Using a vinylic anion, however, did introduce the distinct disadvantage that one of the approaches discussed for achieving the desired stereochemistry would no longer be viable. Attempting to achieve the desired stereochemistry through a chelation-directed addition followed by hydroxyl inversion would be greatly complicated by the nature of the product alcohol. There would be a very good chance that the nucleophilic partner in an inversion reaction would be more likely to displace the activated hydroxyl via an S_N2' pathway rather than the required S_N2 mechanism required for successful inversion due to the hydroxyl being neopentyl and the olefin being monosubstituted.

Before investigating the other available means for generating α-disposed allylic alcohol **312**, it was important to determine the degree and sense of selectivity imparted during normal vinyl addition using vinylmagnesium bromide. The addition required excess Grignard reagent to go to completion, but the product allylic alcohols could be isolated in good yield (Scheme 4.31). The product was a chromatographically inseparable mixture of diastereomers, with the major one predominating by a ratio of 3:1 as determined by ^{1}H NMR. A solution of the product alcohols in methylene chloride was then treated with either *p*-toluenesulfonic acid or pyridinium *p*-toluenesulfonate (PPTS), and a rapid cyclization ensued to give tetrahydrofuranyl acetals **313** and **314**. These were different enough for a clean sample of the major diastereomer to be separated chromatographically from a mixture containing equal amounts of each diastereomer. Proton decoupling experiments allowed for the elucidation of which was the critical

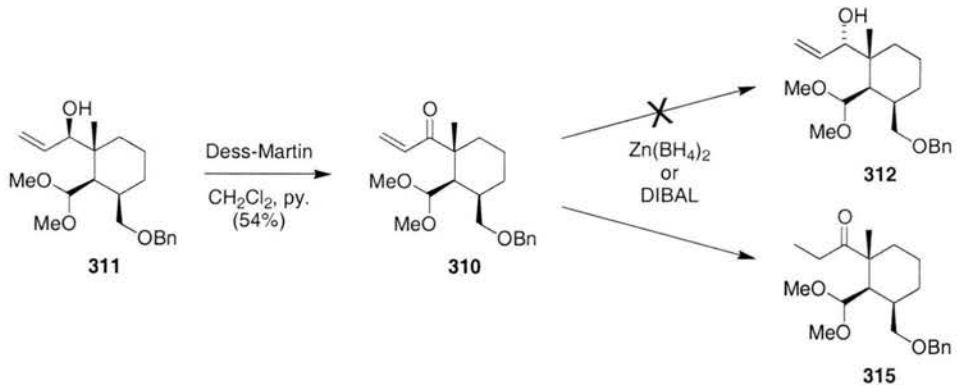
oxidation would give mitomycin H and subsequently mitomycins K and G by reported procedures.[93] This route, if successful, would be a unique and novel approach to several of the mitomycins.



Scheme 68: Planned Synthesis of *N*-Methyl Aziridine **244** and Proposed Route

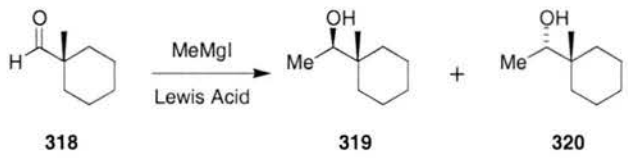
The first step in the sequence shown in Scheme 68 involved conversion of the methyl carbamate aziridine into the free aziridine and subsequent methylation to form the *N*-methyl aziridine moiety found in the natural products. It was anticipated that this sequence of steps would be relatively easy and would get us two steps closer to the natural product. As shown in Table 15, aziridine deprotection did not proceed smoothly at all. Under the conditions that were used, product **245** was formed in all instances but in very low yield. These reactions looked good when monitored by TLC but low yields were obtained upon workup and isolation. Complexation of the free aziridine to the metal used in the reduction seemed to be the major problem. Various workup conditions (including the use of Rochelle's salt) failed to provide the free aziridine **245** in higher yields than before. The use of Rochelle's salt actually gave a different compound by HNMR after workup.

both of these reagents gave strictly 1,4 reduction when used to treat enone **310**. In all likelihood, this is largely due to the carbonyl being neopentyl in nature.



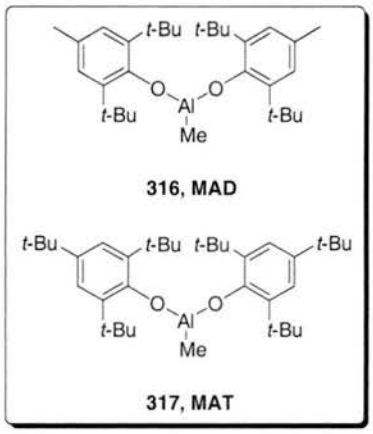
Scheme 4.32 Attempts at an acetal-directed 1,2 reduction of enone **310**

Having observed the inefficacy of this oxidation-reduction strategy, and having ruled out as impractical a strategy based on following vinyl addition with inversion, attention was turned toward the use of bulky Lewis acids to hopefully reverse the sense of the selectivity observed with chelation-controlled addition. Yamamoto has reported the use of methylaluminum bis(2,6-di-*t*-butyl-4-methylphenoxide) (MAD, **316**) and methylaluminum bis(2,4,6-tri-*t*-butylphenoxide) (MAT, **317**) as bulky Lewis acids capable of influencing the conformations of ketones and aldehydes in ways that dramatically alter the selectivity observed in alkylations and reductions of these electrophilic species (Scheme 4.33).[107] For instance, he was able to use both MAD and MAT to reverse tendency toward Cram selectivity for the addition of methylmagnesium iodide into aldehyde **318**. With no additive, the reaction goes in 81% yield, with the mixture of product alcohols containing greater than 80% of the Cram product, β-disposed alcohol **319**. When either MAD or MAT are added to a solution of the aldehyde prior to Grignard addition, comparably high anti-Cram selectivity is observed, with the reaction yield remaining around 80%.



318 319 320

Lewis Acid	% 123	% 124
none	82	18
MAD	22	78
MAT	23	77



316, MAD

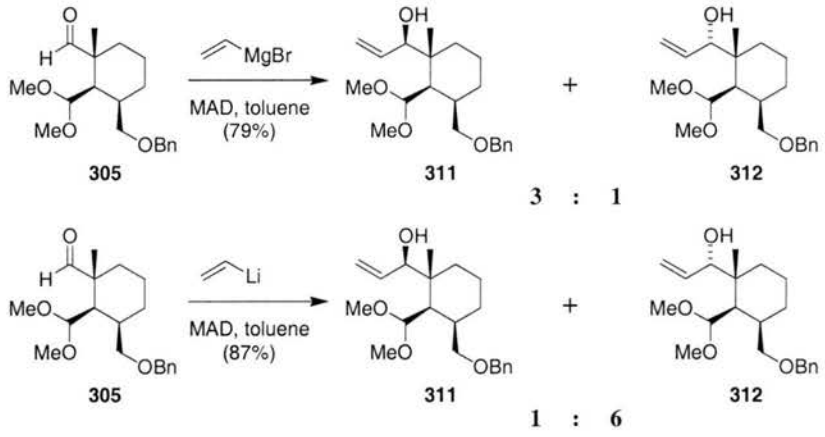
317, MAT

Scheme 4.33 Yamamoto's use of MAD and MAT to influence aldehyde alkylation

It was hoped that in our case, the presence of the dimethyl acetal in aldehyde **305** would enhance the effect Yamamoto observed with aldehyde **318**. Such an effect would be the result of a greater rigidity in the conformation of the aldehyde due to the propensity of the aluminum center to coordinate to the acetal oxygens as well as the carbonyl oxygen. When vinylmagnesium bromide was added to a solution of aldehyde **305** and three equivalents of MAD in toluene, however, the 3:1 product ratio still favored the undesired β-disposed alcohol, **311** (Scheme 4.34). Employing vinyllithium (prepared from tetravinyltin and *n*-butyllithium)[108] instead of vinylmagnesium bromide, on the other hand, gave an 87% yield of the allylic alcohols, with the desired α-disposed hydroxyl predominating in a 6:1 ratio. Scaling this reaction up from 5 mg to 48 mg scale gave a slight loss in selectivity (5.3 α:1 β), but an increase in yield to 95%. Carrying out the addition using vinyllithium without a Lewis acid additive gave the allylic alcohols in the same ratio (3:1 favoring β) as vinylmagnesium bromide.

The high level of selectivity achieved with the vinyllithium/MAD reagent combination proved difficult to realize on a consistent basis. Rather than getting a the 5 or 6:1 ratio favoring the properly disposed alcohol that had originally been obtained, all

subsequent reactions gave a product ratio of about 2.2:1 in the desired direction. Therefore, efforts were made to gain a greater understanding of the forces at work in this reaction (Table 4.2). The first aspect of this reaction studied was temperature (entries 4-6). First it was found that when the reaction was quenched after a few hours of stirring at -78° C, no product was formed. This suggested that a higher temperature was necessary for the reaction to take place. When the reaction was stirred for several hours at -60° C and then allowed to slowly warm to room temperature, however, the selectivity dropped a good deal, and became even worse at -40° C. This indicated that this reaction probably proceeds with the best selectivity when it is begun at -78° C and allowed to slowly warm.



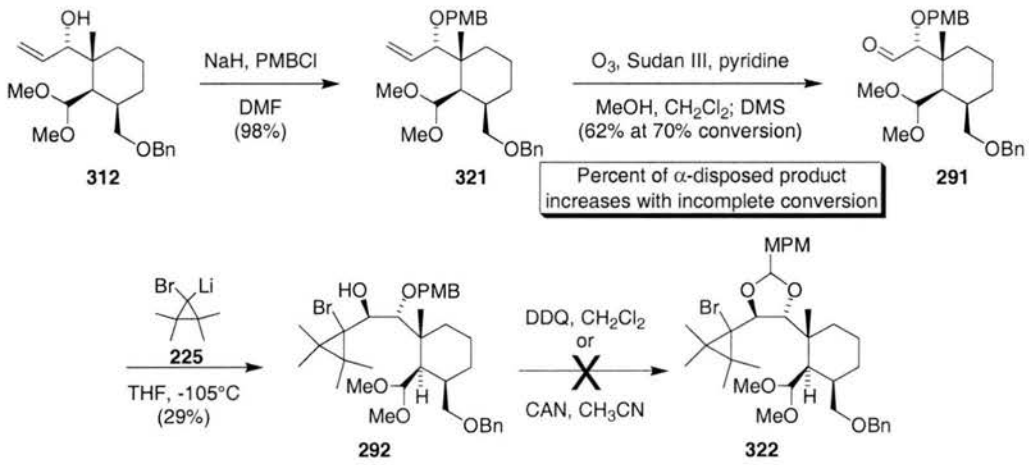
Scheme 4.34 Attempts to use MAD to reverse chelation-controlled vinyl addition

	Solvent	Temp.	312	311	conversion	MAD age
1	toluene	-78° → RT	**6**	**1**	100%	66 hours
2	toluene	-78° → RT	**5.3**	**1**	100%	114 hours
3	toluene	-78° → RT	**2.2**	**1**	100%	75 min.
4	toluene	-78°	No reaction		/	180 min.
5	toluene	-60° → RT	**1.7**	**1**	100%	155 min.
6	toluene	-40° → RT	**1.1**	**1**	100%	155 min.
7	CH_2Cl_2	-78° → RT	No reaction		/	110 min.
8	1 toluene / 1 Et_2O	-78° → RT	**1**	**5.5**	100%	130 min.
9	1 toluene / 1 hexanes	-78° → RT	**2.9**	**1**	100%	155 min.
10	1 toluene / 1 hexanes	-78° → RT	**3.8**	**1**	~50%	8 hours
11	1 toluene / 2 hexanes	-78° → RT	**4.5**	**1**	~50%	8 hours

Table 4.2 Behavior of chelation-blocked addition under a range of conditions

The next aspect of the addition reaction to be explored was the role of solvent. Using methylene chloride as solvent (entry 7) in the hope of getting better results with a more polar solvent (allowing reaction at lower temperature) that was still not going to coordinate to aluminum was unsuccessful, giving no detectable product. Increasing solvent polarity by adding ether to toluene (entry 8) surprisingly reversed the selectivity, giving the undesired β alcohol as the major product with a 5.5:1 ratio. Decreasing solvent polarity (entry 9), on the other hand, led to better selectivity, presumably by strengthening the coordination of aluminum to the acetal and aldehyde. The third factor considered was the age of the MAD reagent. Trends observed over the course of these reactions indicated that perhaps this reagent is more effective when given time to age, and entries 10 and 11 indicate that this may be the case. The best recent selectivity, then has been achieved using aged MAD in a very non-polar (2 hexanes: 1 toluene) solvent system.

With the methodology in place to generate α-disposed allylic alcohol **312** in high yield with good selectivity, attention turned toward the manipulation needed to get to aldehyde **291**. It was necessary to protect the hydroxyl group prior to oxidative cleavage



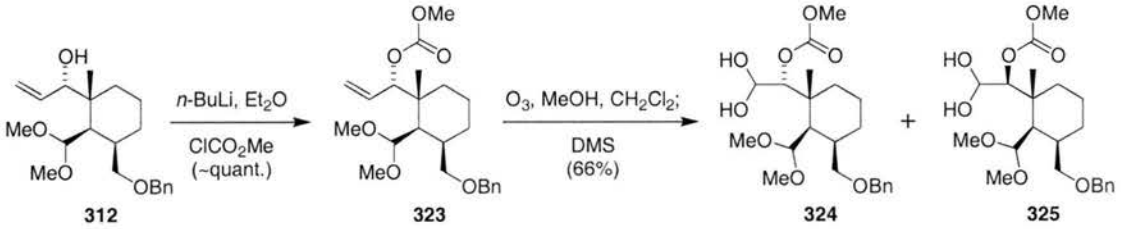
Scheme 4.35 Initial alcohol protection scheme and subsequent elaboration

of the double bond, and an ideal protecting group would be one capable of being easily converted from a hydroxyl protecting group to a diol protecting group. The *p*-methoxybenzyl group would theoretically provide such versatility, so alcohol **312** was protected as the *p*-methoxybenzyl ether, **321** (Scheme 4.35).

Because a *p*-methoxybenzyl protecting group can be oxidatively removed, cleaving the double bond to the corresponding aldehyde became a delicate operation. Initial attempts to carry out an ozonolysis in hexanes gave the desired aldehyde in fair yield, but the product was contaminated with a number of byproducts resulting from loss of the PMB group and subsequent side reactions. It was necessary to attenuate the activity of ozone in a way that would allow it to carry out the desired oxidative cleavage without affecting the PMB group. When faced with a similar situation, Schreiber successfully used pyridine as a co-solvent, speculating that pyridine was being oxidized to the corresponding N-oxide faster than his PMB group was being removed.[109] He also employed the ozonizable dye Sudan III as an internal standard to indicate when sufficient ozone had been delivered to effect the necessary cleavage.[110] When the ozonolysis of olefin **321** was carried out under Schreiber's conditions, halting ozone delivery when the dye lost its color, the desired aldehyde (**291**) could be isolated in 62% yield along with 30% recovered starting material. This was initially carried out on olefin possessing a 1:1 ratio of hydroxyl dispositions, and interestingly, the product was enriched in the desired α-disposed protected hydroxyl, while the recovered starting material was enriched in the undesired β-disposed protected hydroxyl. This pleasant surprise indicated that the selectivity achieved in the controlled vinyl addition could be enhanced two steps later if the ozonolysis step was carefully monitored.

Once the desired aldehyde was in hand, treatment with bromocyclopropyl anion **225** provided alcohol **292**, although in rather poor yield. This yield was not optimized because the product alcohol could not be converted to benzylidine acetal **322** using the oxidative conditions expected to effect this transformation. Furthermore, attempting to remove the *p*-methoxybenzyl group altogether under reductive conditions resulted in the loss of bromine and under oxidative conditions gave several products. Therefore, it was necessary to return to alcohol **312** and try protecting the hydroxyl with other groups that would still be compatible with the functionality present.

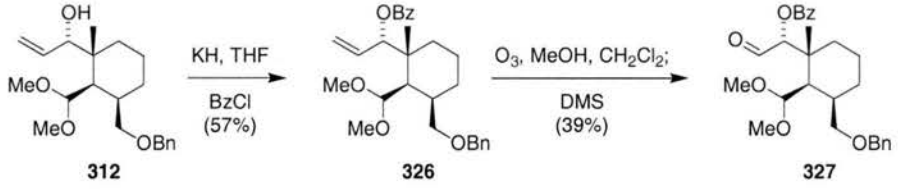
Because of both the failure to convert the *p*-methoxybenzyl group to the corresponding acetal and because of the sensitivity of the dimethyl acetal to acidic conditions, it was determined at this point that an acetal would no longer be possible for the protection of the C-9 and C-10 hydroxyls. In its place a cyclic carbonate could perhaps be used to protect these hydroxyls. Hoping to make this in the most direct manner possible, the first group chosen to protect alcohol **312** was the methyl carbonate. It was hoped that the alkoxide resulting from cyclopropyl addition could then attack a carbonate such as **323** and go directly to the desired cyclic carbonate. This required methyl carbonate was made in quantitative yield by using *n*-butyllithium to convert alcohol **312** to the corresponding lithium alkoxide and then trapping with methyl chloroformate (Scheme 4.36). Ozonolysis of carbonate **323**, however, did not give the



Scheme 4.36 Methyl carbonate alcohol protection scheme

desired aldehyde, but rather two easily separable hydrates, one arising from each diastereomer. Attempts to convert these hydrates to the desired aldehyde through dehydration were unsuccessful.

Conversion to the corresponding benzoate (**326**) was the third means used to protect alcohol **312** (Scheme 4.37). This could be done in moderate yield by converting the alcohol to the potassium alkoxide using potassium hydride and then trapping with benzoyl chloride. Ozonolysis of benzoate **326** provided a modest yield of aldehyde **327**, an aldehyde that is also prone to hydrate formation but does so at a much slower rate than the corresponding carbonate.

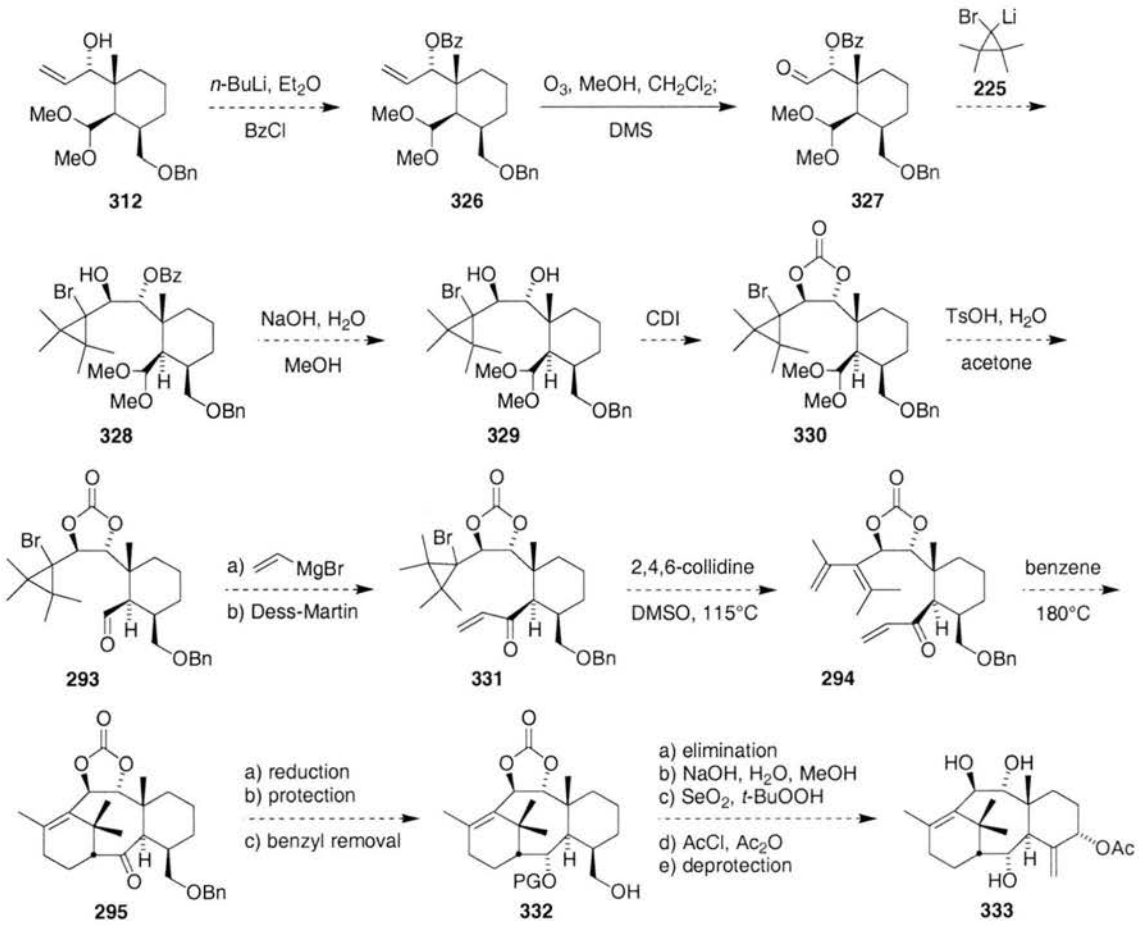


Scheme 4.37 Benzoyl alcohol protection scheme

4.6 Future Direction

The precise means by which a synthesis of this nature could be completed is not entirely clear, but the general nature of the synthetic task is rather predictable (Scheme 4.38). Deviation from this route may be necessary in the form of an alternate protection strategy, but the nature and order of carbon-carbon bond formation will most likely need to occur in the manner described. Shea has demonstrated (Scheme 4.9) that the group protecting the C-9 hydroxyl in compounds such as benzoate **327** imparts a profound influence on the facial selectivity of organolithium addition into the C-10 aldehyde. He rationalized his excellent selectivity in the transformation of aldehyde **224** to alcohol **226** by suggesting that both oxygens of his MOM protecting group chelate the lithium and

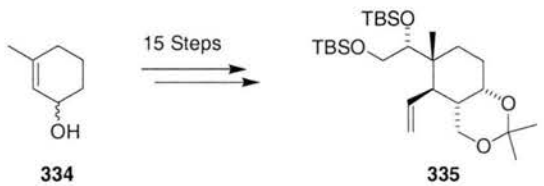
guide his cyclopropyl nucleophile to the desired face of the aldehyde. This suggests that a protecting group that can also act as a Lewis base might be best suited to facilitating a highly selective addition of bromocyclopropyl anion **225**. Alternatively, the C-9 hydroxyl could be protected as the benzyl ether, relying in this case on coordination to the dimethyl acetal to provide the direction needed for a selective addition. Both the C-9 and C-20 benzyl ethers could be removed in the next step and then the adjacent hydroxyls at C-9 and C-10 could be protected as the carbonate.[111] Should addition of the bromocyclopropyl anion into aldehyde **327** and subsequent benzoate removal to give diol **329** be successful, protection of the diol using a dialkyl silyl dichloride could provide a compound that is more stable under the conditions that would be used to introduce the



Scheme 4.38 Strategy for completing tetraol synthesis using current direction

vinyl group on the way to enone **331**. This may also widen the range of possibilities available in the last few steps of the synthesis. Once an aldehyde such as **293** is in hand, the remaining steps through the Diels-Alder reaction are reasonably well-precedented based on work described herein. Reduction of the ketone carbonyl in **295** could be challenging in the presence of the cyclic carbonate, which may necessitate silyl protection of the C-9, C-10 diol. Of the remaining steps, only acetylation of the C-5 hydroxyl could be potentially problematic, but this hydroxyl should be far less sterically encumbered than those at C-9 and C-10.

Looking at this synthesis more broadly, problems could arise from the fact that C-4 of cycloaddition substrate **294** possesses *R* stereochemistry, which could exert a negative effect on the conformation of the C-ring in response to the 1,3-diaxial interaction that could arise between the angular methyl group and C-20. This could be addressed in either of two ways. First, C-20 could be deprotected, oxidized to the aldehyde oxidation state, and epimerization could be attempted in the hope that the relief of the diaxial interaction would provide the thermodynamic driving force necessary for this to be possible. The second way to address this and possibly other issues would be to employ the C-ring synthon reported by Kishi in 1993 (Scheme 4.39).[112] This C-ring was made in enantiomerically pure form by using an early-stage Noyori asymmetric hydrogenation to enable subsequent resolution. This synthesis also manages to set the C-9 hydroxyl with the desired α disposition using a [2,3] Wittig rearrangement.



334 **335**

Scheme 4.39 Kishi's C-ring synthon

4.7 Conclusions

As with the rest of the work describe here, the intramolecular Diels-Alder approaches tried came painfully close to success without actually realizing it. Three different substrates were subjected to cycloaddition conditions, with each new substrate more promising than the one before. None gave any of the desired products, but they were shown to take part in the side reactions common to this milieu. Work toward a fourth intramolecular Diels-Alder substrate has progressed to within a handful of steps of another cycloaddition, along the way developing a new C-ring synthesis that is particularly well-suited to bringing up large amounts of material.

A great range of approaches have been explored in the hope of using total synthesis as a tool to further the study of taxol biosynthesis. Subtleties of conformation, substitution, and electronics have exacerbated the inherent difficulties faced when trying to form the congested B-ring of a taxoid system. While the goal laid out at the outset of this work has not yet been realized, hopefully these efforts will provide the foundation from which more successful attempts can be launched. Perhaps the greatest contribution of this work, though, will be to underscore the apparent reality that semi-synthesis is the most practical means by which lightly oxygenated taxanes can be obtained.

References

[1] Paclitaxel is the generic name for Taxol, a registered trademark of Bristol-Myers Squibb. Because of its greater familiarity, `taxol' is used throughout.

[2] *Plant Antitumor Agents. VI. The Isolation and Structure of Taxol, a Novel Antileukemic and Antitumor Agen from* Taxus brevifolia, Wani, M.C. ; Taylor, H.L.; Wall, M. E.; Coggon, P.; McPhail, A.T. *J. Am. Chem. Soc.*, 1971, **93**, 2325-2327.

[3] *Clinical overview of the taxanes*, Goldspiel, B. R. *Pharmacotherapy*, 1997, **17**(5, Pt. 2), 110S-125S.

[4] *Promotion of microtubule assembly in vitro by taxol*, Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature*, 1979, **277**, 665-667.

[5] *Chemistry and Biology of Taxol*, Nicolaou, K. C.; Dai, W. M.; Guy, R. K., *Angew. Chem. Int.Ed. Engl.* 1994, **33**, 15.

[6] Food and Drug Administration New Drug Applications approved: a) breast cancer – NDA 20-262/S-033; b) ovarian cancer (used with cisplatin) – NDA 20-262/S-031; c) non-small cell lung cancer (used with cisplatin) – NDA 20-262/S-024; d) Kaposi's sarcoma – NDA 20-262/S-032.

[7] Full prescribing information for taxol courtesy Bristol-Myers Squibb Company.

[8] Dosage for a patient weighing 165 lbs.

[9] Cost and dosage information courtesy of Hematology Oncology Associates, Fort Collins, CO. Precise cost for 1.20 g taxol was $10,960 in November of 2002.

[10] *Highly efficient, practical approach to natural taxol*, Denis, J. N.; Greene, A. E.; Guenard, D.; Gueritte-Voegelein, F.; Mangatal, L.; Potier, P. *J. Am.Chem. Soc.* 1988, **110**, 5917-5919.

[11] *Biosynthesis of Taxol*, Rohr, J. *Angew. Chem. Int. Ed. Engl.* 1997, **36**, 2190-2195.

[12] *Fermentation for taxol production*, Dahiya, J. S. (Novopharm Ltd.), *PCT Int. Appl.* WO 9632490 A1, 1996, 30 pp.

[13] *The Kinetics of Taxoid Accumulation in Cell Suspension Cultures of* Taxus *Following Elicitation With Methyl Jasmonate*, Ketchum, R. E. B.; Gibson, D. M.; Croteau, R. B.; Shuler, M. L. *Biotechnol. Bioeng.*, 1999, **62**, 97-105.

[14] *Kinetic Studies of Paclitaxel Production by* Taxus canadensis *Cultures in Batch and Semicontinuous with Total Cell Recycle*, Phisalaphong, M.; Linden, J. C. *Biotechnol. Prog.* 1999, **15**, 1072-1077.

[15] *Enhanced production of taxanes by cell cultures of Taxus species*, Bringi, V.; Kakrade, P. G.; Prince, C. L.; Roach, B. L. *PCT Int. Appl.* WO 9744476 A1, 1997, 106 pp.

[16] *Biosynthesis of Taxoids. Mode of Formation of the Taxol Side Chain*, Fleming, P. E.; Mocek, U.; Floss, H. G. *J. Am. Chem. Soc.*, 1993, **115**, 805-807.

[17] *Detection of a Phenylalanine Aminomutase in Cell-Free Extracts of* Taxus brevifolia *and Preliminary Characterization of Its Reaction*, Walker, K. D.; Floss, H. G. *J. Am. Chem. Soc.*, 1998, **120**, 5333-5334.

[18] *Biosynthesis of Taxoids. Mode of Attachment of the Taxol Side Chain*, Fleming, P. E.; Knaggs, A. R.; He, X.-G.; Mocek, U.; Floss, H. G. *J. Am. Chem. Soc.*, 1994, **116**, 4137-4138.

[19] *Molecular cloning and heterologous expression of the C-13 phenylpropanoid side chain-CoA acyltransferase that functions in Taxol biosynthesis*, Walker, K.; Fujisaki, S.; Long, R.; Croteau, R. *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 12715-12720.

[20] *Biosynthetic building blocks of* Taxus canadensis *taxanes*, Zamir, L. O.; Nedea, M. E.; Garneau, F. X. *Tetrahedron Lett.*, 1992, **33**, 5235-5236.

[21] *Studies on the biosynthesis of taxol: The taxane carbon skeleton is not of mevalonoid origin*, Eisenreich, W.; Menhard, B.; Hylands, P. J.; Zenk, M. H.; Bacher, A. *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 6431-6436.

[22] *Non-mevalonate isoprenoid biosynthesis: enzymes, genes and inhibitors*, Lichtenthaler, H. K. *Biochem. Soc. Trans.*, 2000, **28**, 785-789.

[23] *Cloning and Functional Expression of a cDNA Encoding Geranylgeranyl Diphosphate Synthase from* Taxus canadensis *and Assessment of the Role of this Prenyltransferase in Cells Induced for Taxol Production*, Hefner, J.; Ketchum, R. E. B.; Croteau, R. *Arch. Biochem. Biophys.*, 1998, **360**, 62-74.

[24] *Mechanism of Taxadiene Synthase, a Diterpene Cyclase That Catalyzes the First Step in Taxol Biosynthesis in Pacific Yew*, Lin, X.; Hezari, M.; Koepp, A. E.; Floss, H. G.; Croteau, R. *Biochemistry*, 1996, **35**, 2968-2977.

[25] *A cDNA clone for taxadiene synthase, the diterpene cyclase that catalyzes the committed step of Taxol biosynthesis*, Wildung, M.R.; Jin, Q.; Dalal, D.; Oliver, J.S.; Coates, R.M.; Croteau, R. *J. Biol. Chem.*, 1996, **271**, 9201-9204.

[26] *Heterologous expression and characterization of a 'pseudomature' form of taxadiene synthase involved in paclitaxel (Taxol) biosynthesis, and evaluation of a potential intermediate and inhibitors of the multistep diterpene cyclization reaction*, Williams, D.C.; Wildung, M.R.; Croteau, R. *Arch. Biochem. Biophys.*, 2000, **379**, 137-146.

[27] *Intramolecular proton transfer in the cyclization of geranylgeranyl diphosphate to the taxadiene precursor of taxol catalyzed by recombinant taxadiene synthase*, Williams, D. C.; Carroll, B. J.; Jin, Q.; Rithner, C. D.; Lenger, S. R.; Floss, H. G.; Coates, R. M.; Williams, R. M.; Croteau, R. *Chem. Biol.*, 2000, **7**, 969-977.

[28] *Taxol biosynthesis: molecular cloning of a benzoyl-CoA:taxane 2α-O-benzoyltransferase cDNA from* Taxus *and functional expression in* Escherichia coli, Walker, K.; Croteau, R. *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 13591-13596.

[29] *Molecular cloning of a 10-deacetylbaccatin III-10-*O*-acetyl transferase cDNA from* Taxus *and functional expression in* Escherichia coli, Walker, K.; Croteau, R. *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 583-587.

[30] *Studies on the Biosynthesis of Taxol. Synthesis of Taxa-4(20),11(12)-dien-2α,5α-diol*, Vazquez, A.; Williams, R.M., *J. Org. Chem.* 2000, **65**, 7865-7869.

[31] *Cytochrome P450-catalyzed hydroxylation of taxa-4(5),11(12)-diene to taxa-4(20),11(12)-diene-5α-ol: the first oxygenation step in taxol biosynthesis*, Hefner, J.; Rubenstein, S. M.; Ketchum, R. E. B.; Gibson, D. M.; Williams, R. M.; Croteau, R. *Chem. Biol.*, 1996, **3**, 479-489.

[32] *Taxol biosynthetic genes*, Walker, K.; Croteau, R. *Phytochemistry*, 2001, **58**, 1-7.

[33] *Molecular Cloning of a Taxa-4(20),11(12)-dien-5α-ol-*O*-Acetyl Transferase cDNA from* Taxus *and Functional Expression in* Escherichia coli, Walker, K.; Schoendorf, A.; Croteau, R. *Arch. Biochem. Biophys.*, 2000, **374**, 371-380.

[34] *Partial Purification and Characterization of Acetyl Coenzyme A: Taxa-4(20),11(12)-dien-5α-ol O-Acetyl Transferase That Catalyzes the First Acylation Step of Taxol Biosynthesis*, Walker, K.; Ketchum, R. E. B.; Hezari, M.; Gatfield, D.; Golenowski, M.; Barthol, A.; Croteau, R. *Arch. Biochem. Biophys.*, 1999, **364**, 273-279.

[35] *Molecular cloning of a cytochrome P450 taxane 10β-hydroxylase cDNA from* Taxus *and functional expression in yeast*, Schoendorf, A.; Rithner, C. D.; Williams, R. M.; Croteau, R. B. *Proc. Natl. Acad. Sci. USA*, 2001, **98**, 1501-1506.

[36] *A new synthesis of substituted dienes and its application to an alkylated taxane model system*, Bonnert, R. V.; Jenkins, P. R. *J. Chem. Soc., Chem. Commun.*, 1987, **20**, 1540-1541.

[37] *Studies on the Biosynthesis of Taxol: Total Synthesis of Taxa-4(20),11(12)-diene and Taxa-4(5),11(12)-diene. The First Committed Biosynthetic Intermediate*, Rubenstein, S. M.; Williams, R. M. *J. Org. Chem.* 1995, **60**, 7215-7223.

[38] *Synthesis of Stable and Radioisotopomers of Taxa-4(5),11(12)-diene, Taxa-4(20),11(12)-diene and Taxa-4(20),11(12)-dien-5-a -ol. Early Intermediates in Taxol*

Biosynthesis, Rubenstein, S. M.; Vazquez, A.; Williams, R.M. *J. Labeled Cmpd & Radiopharm.* 2000, **43**, 481-491.

[39] *Studies on Taxol Biosynthesis. Preparation of 10b-hydroxy taxadien-5a-yl acetate by Deoxygenation of a Taxadiene Tetra-acetate Obtained from Japanese Yew*, Horiguchi, T.; Rithner, C. D.; Croteau, R.; Williams, R.M. *J. Org. Chem.* 2002, **67**, 4901-4903.

[40] *Studies on Taxol Biosynthesis. Preparation of Taxa-4(20),11(12)-dien-5α-acetoxy-2α,10β-diol derivatives by Deoxygenation of a Taxadiene Tetra-acetate Obtained from Japanese Yew*, Horiguchi, T.; Rithner, C.D.; Croteau, R.; Williams, R.M. *Tetrahedron* 2002, **58**, (In Press).

[41] *Catalytic Effect of Nickel(II) Chloride and Palladium(II) Acetate on Chromium(II)-Mediated Coupling Reactions of Iodo Olefins with Aldehydes*, Jin, H.; Uenishi, J.; Christ, W. J.; Kishi, Y. *J. Am. Chem. Soc.*, 1986, **108**, 5644-5646.

[42] *Reactions of Alkenylchromium Reagents Prepared from Alkenyl Trifluoromethanesulfonates (Triflates) with Chromium(II) Chloride under Nickel Catalysis*, Takai, K.; Tagashira, M.; Kuroda, T.; Oshima, K.; Utimoto, K.; Nozaki, H. *J. Am. Chem. Soc.*, 1986, **108**, 6048-6050.

[43] *Concise Synthesis of Taxol A-Ring Components: Remote Diastereoselective Additions of Alkenyl Lithiums to Aldehydes*, Frost, C.; Linnane, P.; Magnus, P.; Spyvee, M. *Tetrahedron Lett.*, 1996, **37**, 9139-9142.

[44] *Taxane Diterpene Synthesis Strategies. A Review*, Swindell, C. S. *Org. Prep. Proced. Int.*, 1991, **23**, 465-543.

[45] *Total synthesis of taxol*, Lim, J. *Ph. D Dissertation, Harvard University*, 2000, 141pp.

[46] *Total Synthesis of Baccitin III and Taxol*, Danishefsky, S. J.; Masters, J.J.; Young, W. B.; Link, J.T.; Snyder, L. B.; Magee, T. V.; Jung, D. K.; Isaacs, R. C. A.; Bornmann, W. G.;Alaimo, C.A.; Coburn, C.A.; DiGrandi, M. J. *J. Am. Chem. Soc.* 1996, **118**, 2843.

[47] a) *Total Synthesis of Taxol. 1. Retrosynthesis, Degradation, and Reconstitution.* Nicolaou, K. C.; Nantermet, P.G.; Ueno, H.; Guy, R. K. Coulandouros, E.A.; Sorensen, E.J. *J. Am. Chem. Soc.* 1995, **117**, 624. b) *Total Synthesis of Taxol. 2. Construction of A and C ring intermediates and initial attempts to construct the ABC ring system.* Nicolaou, K. C.; Liu, J.J.; Yang, H.; Claiborne, C.F.; Hwang, C.K.; Nakada, M.; Nantermet, P.G.; Ueno, H.; Guy, R. K.; Sorensen, E.J. *J. Am. Chem. Soc.* 1995, **117**, 634. c) *Total Synthesis of Taxol. 3. Formation of Taxol's ABC Ring Skeleton.* Nicolaou, K. C.; Liu, J.J.; Yang, H.; Claiborne, C.F.;Renaud, J.; Nantermet, P.G.; Guy, R. K.; Shibayama, K. *J. Am. Chem. Soc.* 1995, **117**, 645. d) *Total Synthesis of Taxol. 4. The Final Stages and Completion of the Synthesis.* Nicolaou, K. C.; Ueno, H.; Liu, J.J.; Yang, H.; Renaud, J.; Nantermet, P.G; Paulvannan, K.; Chadha, R. *J. Am. Chem. Soc.* 1995, **117**, 653.

[48] *Synthesis of a Taxane Triene,* Kende, A. S.; Johnson, S.; Sanfilippo, P.; Hodges, J. C.; Jungheim, L. N. *J. Am. Chem. Soc.*, 1986, **108**, 3513-3515.

[49] *Synthesis of Taxoid Ring Systems: AC → ABC Approach by Way of Intramolecular Alkylation*, Takahashi, T.; Iwamoto, H., Nagashima, K.; Okabe, T.; Doi, T., *Angew. Chem. Int. Ed.*, 1997, **36**, 1319-1321.

[50] *Enatioselective Total Synthesis of (-)-Taxol*, Kusama, H.; Hara, R.; Kawahara, S.; Nishimori, T.; Kashima, H.; Nakamura, N.; Morihara, K.; Kuwajima, I. *J. Am. Chem. Soc.*, 2000, **122**, 3811-3820.

[51] *An Enantioselective Construction of the ABC System of Taxol*, Stork, G.; Manabe, K.; Liu, L. *J. Am. Chem. Soc.*, 1998, **120**, 1337-1338.

[52] *Stereoselective Construction of the Taxinine AB System through a Novel Tandem Aldol-Payne Rearrangement Annulation*, Swindell, C. S.; Patel, B. P. *J. Org. Chem.*, 1990, **55**, 3-5

[53] *Asymmetric total synthesis of taxol*, Mukaiyama, T.; Shiina, I.; Iwadare, H.; Saitoh, M.; Nishimura, T.; Ohkawa, N.; Sakoh, H.; Nishimura, K.; Tani, Y.; Hasegawa, M.; Yamada, K.; Saitoh, K., *Chem.--Eur.* J. 1999, **5**, 121-161.

[54] a) *The Pinene Path to Taxanes. 5. Stereocontrolled Synthesis of a Versatile Taxane Precursor*, Wender, P. A.; Badham, N. F.; Conway, S. P.; Floreancig, P. E.; Glass, T. E.; Granicher, C.; Houze, J. B.; Janichen, J.; Lee, D.; Marquess, D. G.; McGrane, P. L.; Meng, W.; Mucciaro, T. P.; Muhlebach, M.; Natchus, M. G.; Paulsen, H.; Rawlins, D. B.; Satkofsky, J.; Shuker, A. J.; Sutton, J. C.; Taylor, R. E.; Tomooka, K. I., *J. Am. Chem. Soc.* 1997, **119**, 2755. b) *The Pinene Path to Taxanes. 5. A Concise Stereocontrolled Synthesis of Taxol*, Wender, P. A.; Badham, N. F.; Conway, S. P.; Floreancig, P. E.; Glass, T. E.; C.; Houze, J. B.; Krauss, N. E.; Lee, D.; Marquess, D. G.; McGrane, P. L.; Meng, W.; Natchus, M. G.; Shuker, A. J.; Sutton, J. C.; Taylor, R. E., *J. Am. Chem. Soc.* 1997, **119**, 2757.

[55] *Enantiospecific Total Synthesis of Natural (+)-Taxusin. 1. Retrosynthesis, Advancement to Diastereomeric* trans-$\Delta^{9,10}$*-Tricyclic Olefinic Intermediates, and the Stereocontrol Attainable Because of Intrinsic Rotational Barriers Therein*, Paquette, L. A.; Zhao, M. *J. Am. Chem. Soc.*, 1998, **120**, 5203-5212.

[56] *The First Successful Intramolecular Diels-Alder Reaction Leading to a Baccatin III Construct Bearing Oxygen Functionality at C10*, Park, T. K.; Kim, I. J.; Danishefsky, S. J.; de Gala, S. *Tetrahedron Lett.*, 1995, **36**, 1019-1022.

[57] *A synthesis of an alkylated taxane model system*, Bonnert, R. V.; Jenkins, P. R. *J. Chem. Soc. Perkin Trans. I*, 1989, **20**, 413-418.

[58] *The Type 2 Intramolecular Diels-Alder Reaction: Synthesis and Chemistry of Bridgehead Alkenes*, Bear, B. R.; Sparks, S. M.; Shea, K. M. *Angew. Chem. Int. Ed.*, 2001, **40**, 820-849.

[59] a) *First Total Synthesis of Taxol. 1. Functionalization of the B Ring* Holton, R. A.; Somozoa, C.; Kim, H. B.; Liang, F.; Biediger, R. J.; Boatman, D.; Shindo, M.; Smith, C. C.; Kim, S.; Nadizadeh, H.; Suzuki, Y.; Tao, C.; Vu, P.; Tang, S.; Zhang, P.; Murthi, K.K.; Gentile, L. S.; Liu, J.H. *J. Am. Chem. Soc.* 1994, **116**, 1597. b) *First Total Synthesis of Taxol. 2. Completion of the C and D Rings*. Holton, R. A.; Somozoa, C.; Kim, H. B.; Liang, F.; Biediger, R. J.; Boatman, D.; Shindo, M.; Smith, C. C.; Kim, S.; Nadizadeh, H.; Suzuki, Y.; Tao, C.; Vu, P.; Tang, S.; Zhang, P.; Murthi, K.K.; Gentile, L. S.; Liu, J.H. *J. Am. Chem. Soc.* 1994, **116**, 1599.

[60] *Alkoxide-accelerated sigmatropic rearrangements. A novel entry to the bicyclo[5.3.1]undec-7-ene system of the taxane diterpenes*, Martin, S. F.; White, J. B.; Wagner, R. *J. Org. Chem.*, 1982, **47**, 3190-3192.

[61] *Ring closure reactions of bifunctional chain molecules.* Illuminati, G.; Mandolini, L., *Acc. Chem. Res.*, 1981, **14**, 95-102.

[62] *Chemistry of organosilicon compounds. 89. Syntheses of g,d-unsaturated alcohols from allylsilanes and carbonyl compounds in the presence of titanium tetrachloride*, Hosomi, A.; Sakurai, H. *Tetrahedron Lett.*, 1976, **16**, 1295-1298.

[63] *The Pinene Path to Taxol: Readily Accessible A-Ring Building Blocks Based on Novel Alkyl- and Alkenyllithium Reagents with Internal Carbonyl Groups*, Wender, Paul A.; Wessjohann, Ludger A.; Peschke, Bernd; Rawlins, David B., *Tetrahedron Lett.* (1995) **36**, 7181-7184.

[64] *Comparative Analysis of Molecular-Recognition Levels Attained during Capture of Chiral Cyclopentenyl Organometallics by Conformationally Immobilized Ketonic Systems*, Paquette, Leo A.;He, Wei; Rogers, Robin D. *J. Org. Chem.* (1989) **54**, 2291-2300.

[65] *A New and Practical Approach to the Synthesis of Taxol and Taxol Analogues: The Pinene Path*, Wender, Paul A.; Mucciaro, Thomas P. *J. Am. Chem. Soc.* **1992**, *114*, 5878-5879.

[66] *Cross-Coupling Reactions Based on Acetals*, Mukaiyama, T.; Murakami, M., *Synthesis*, **1987**, 1043-1054.

[67] *Synthetic Studies Toward the Taxane Class of Natural Products*, Kress, M. H.; Ruel, R.; Miller, W. H.; Kishi, Y. *Tetrahedron Lett.*, 1993, **34**, 5999-6002.

[68] *Investigations of the Intramolecular Ni(II)/Cr(II)-Mediated Coupling Reaction: Application to the Taxane System*, Kress, M. H.; Ruel, R.; Miller, W. H.; Kishi, Y. *Tetrahedron Lett.*, 1993, **34**, 6003-6006.

[69] *Ni(II)/Cr(II)-Mediated Coupling Reaction: Beneficial Effects of 4-*tert*-Butylpyridine as an Additive and Development of New and Improved Workup Procedures*, Stamos, D. P.; Sheng, X. C.; Chen, S. S.; Kishi, Y. *Tetrahedron Lett.*, 1997, **38**, 6355-6358.

[70] *Total Synthesis of (+)-O-Cinnamoyltaxicin-I Triacetate*, Sheng, X. C., Ph. D. Thesis, 1998.

[71] *New and Effective Reagents for 1,4 Reduction of α,β-Unsaturated Ketones, $LiAlH_4$-CuI and Its Reactive Species H_2AlI*, Ashby, E. C.; Lin, J. J.; Kovar, R. *J. Org. Chem.*, 1976, **41**, 1939-1942.

[72] *Selective Reduction of α,β-unsaturated Terpene Carbonyl Compounds using Hydrosilane-Rhodium(I) complex combinations*, Ojima, I.; Kogure, T.; Nagai, Y. *Tetrahedron Lett.*, 1972, **13**, 5035-5038.

[73] *$RuCl_3$-NaOH as a Reagent for Chirality Transfer. Synthesis of Chiral β-Deuteriated Ketones by Asymmetric Induction*, Smadja, W.; Ville, G.; Georgoulis, C. *J. C. S. Chem. Comm.* 1980, 594-595.

[74] *Organotin Hydride-Catalyzed Conjugate Reduction of α,β-Unsaturated Ketones*, Hays, D. S.; Scholl, M.; Fu, G. C. *J. Org. Chem.*, 1996, **61**, 6751-6752.

[75] *Conjugate Reduction of α,β-Unsaturated Carbonyl Compounds by Catecholborane*, Evans, D. A.; Fu, G. C. *J. Org. Chem.* 1990, **55**, 5678-5680.

[76] *Methylcopper(I)-Catalyzed Selective Conjugate Reduction of α,β-Unsaturated Carbonyl Compounds by Diisobutylaluminum Hydride in the Presence of Hexamethylphosphoric Triamide*, Tsuda, T.; Hayashi, T.; Satomi, H., Kawamoto, T.; Saegusa, T. *J. Org. Chem.*, 1986, **51**, 537-540.

[77] *Addition Reaction of Conjugated Double Bonds. Part II. The Occurrence of 1,4- or 1,2-Addition in the Reduction of Some Aryl α,β-Unsaturated Ketones with Metal Hydrides*, Iqbal, K.; Jackson, W. R. *J. Chem. Soc. C.*, 1968, 616-620.

[78] *Partial synthesis of 9,10-syn-diterpenes via tosylhydrazone reduction: (-)-(9β)-pimara-7,15-diene and (-)-(9β)-isopimaradiene*. Chu, M.; Coates, R. M. *J. Org. Chem.* 1992, **57**, 4590-4597.

[79] *Carbon-Carbon Bond Formations Involving Organochromium(III) Reagents*, Fürstner, A. *Chem. Rev.* 1999, **99**, 991-1045.

[80] *Synthesis of Vinyl Iodides From Vinyl Bromides and Potassium Iodide by Means of Nickel Catalyst*, Takagi, K.; Hayama, N.; Inokawa, S. *Chem. Lett.*, 1978, 1435-1436.

[81] *Synthetic efforts directed towards the taxol skeleton. The saturated C-ring approach*, Shea, K. J.; Haffner, C. D. *Tetrahedron Lett.*, 1988, **29**, 1367-1370.

[82] *Reactions Involving Electron Transfer. I. Reductions of 2,2,6,6-Tetramethyl-4-hepten-3-one*, Bowers, K. W.; Giese, R. W.; Grinshaw, J.; House, H. O.; Kolodny, N. H.; Kronberger, K.; Roe, D. K. *J. Am. Chem. Soc.*, 1970, **92**, 2783-2799.

[83] *DTBB-Catalysed Lithiation of 4-Functionalised 1-Chloro-Butenes*, Huerta, F. F.; Gómez, C.; Yus, M. *Tetrahedron*, 1996, **52**, 13243-13254.

[84] *Transmetalation Reaction of Higher Order Cyanocuprates: Direct Formation of Trialkyltin Cuprates From Tin Hydrides Which Bypasses Organolithium Intermediates*, Lipshutz, B. H.; Ellsworth, E. L.; Dimock, S. H.;, Reuter, D. C. *Tetrahedron Lett.*, 1989, **30**, 2065-2068.

[85] *Controlling Stereoselection in Intramolecular Heck Reactions by Tailoring the Palladium Catalyst*, Madin, A.; Overman, L. E. *Tetrahedron Lett.*, 1992, **33**, 4859-4862.

[86] *Synthesis of the Benzophenone Fragment of Balanol via an Intramolecular Cyclization Event*, Denieul, M.; Laursen, B.; Hazell, R.; Skrydstrup, T. *J. Org. Chem.*, 2000, **65**, 6052-6060.

[87] *The tricyclo[9.3.1.03,8]pentadecane ring system. A short synthesis of the C-aromatic taxane skeleton*, Shea, K. J.; Davis, P. D. *Angew. Chem., Int. Ed. Engl.*, 1983, **95**, 419-420.

[88] *Synthesis of a C-1 epi taxinine intermediate using the type 2 intramolecular Diels-Alder approach*, Jackson, R. W.; Shea, K. J. *Tetrahedron Lett.*, 1994, *35*, 1317-1320.

[89] *On the Importance of the Stereochemistry at C10 in Governing the Feasibility of the Intramolecular Diels-Alder Route to Baccatin III Constructs: Surprising Results in the 10R-9-Deoxy Series*, Kim, I. J.; Park, T. K.; Danishefsky, S. J. *Tetrahedron Lett.*, 1995, **36**, 1015-1018.

[90] *A novel intramolecular Heck reaction: synthesis of a cholesterol-baccatin III hybrid*, Masters, J. J.; Jung, D. K.; Danishefsky, S. J.; Snyder, L. B.; Park, T. K.; Isaacs, R. C. A.; Alaimo, C. A.; Young, W. B. *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 452-455.

[91] *Reactions of 1-bromo-1-lithiocyclopropanes with ketones and aldehydes. New method of converting olefins into oxaspiropentanes, cyclobutanones, cyclopropyl ketones, and lactones* Braun, M.; Dammann, R.; Seebach, D. *Chem. Ber.* 1975, **108**, 2368-2390.

[92] *Dramatic Rate Accelerations of Diels-Alder Reactions in 5 M Lithium Perchlorate-Diethyl Ether: The Cantharidin Problem Reexamined.* Grieco, P. A.; Nunes, J. J.; Gaul, M. D. *J. Am. Chem. Soc.*, 1990, **112**, 4595-4596.

[93] *Acid Catalyzed Intramolecular Diels-Alder Reactions in Lithium Perchlorate-Diethyl Ether: Enhanced Reaction Rates and Diastereoselectivity*, Grieco, P. A.; Handy, S. T.; Beck, J. P. *Tetrahedron Lett.*, 1994, **35**, 2663-2666.

[94] *Efficacious Modification of the Mitsunobu Reaction for Inversion of Sterically Hindered Secondary Alcohols*, Martin, S. F.; Dodge, J. A. *Tetrahedron Lett.*, 1991, **32**, 3017-3020.

[95] *Superoxide Ion as a Synthetically Useful Oxygen Nucleophile*, Corey, E. J.; Nicolaou, K. C.; Shibasaki, M.; Machida, Y.; Shiner, C. S. *Tetrahedron Lett.*, 1975, **16**, 3183-3186.

[96] *Lithium perhydro-9b-boraphenalylhydride. Active reducing agent of unusually high stereoselectivity for the reduction of cyclic and bicyclic ketones*, Brown, H. C.; Dickason, W. C. *J. Am. Chem. Soc.*, 1970, **92**, 709-710.

[97] *Cesium Carboxylates in Dimethylformamide. Reagents for Introduction of Hydroxyl Groups by Nucleophilic Substitution and for Inversion of Configuration of Secondary Alcohols*, Kruizinga, W. H.; Strijtveen, B.; Kellogg, R. M. *J. Org. Chem.*, 1981, **46**, 4321-4323.

[98] *On the Selectivity of Deprotection of Benzyl, MPM (4-Methoxybenzyl) and DMPM (3,4-Dimethoxybenzyl) Protecting Groups for Hydroxyl Functions*, Horita, K.; Yoshioka, T.; Tanaka, T.; Oikawa, Y.; Yohemitsu, O. *Tetrahedron*, 1986, **42**, 3021-3028.

[99] *New ring construction strategy in taxane synthesis: stereocontrolled synthesis of taxane CB-ring system*, Wei, C. Q.; Zhao, G.; Jiang, X. R. ; Ding, Y. *J. Chem. Soc., Perkin Trans. 1*, 1999, 3531–3536.

[100] *Intramolecular Diels-Alder Reactions of Sorbyl Citraconate and Mesaconate Esters*, White, J. D.; Sheldon, B. G. *J. Org. Chem.*, 1981, **46**, 2273-2280.

[101] *A novel and convenient synthesis of 3-Methylfuran-2(5H)-one*. Nefkens, G. H. L.; Thuring, J. W. J. F.; Zwanenburg, B. *Synthesis*, 1997, 290-292.

[102] *Chemistry of Tricarbonyl Hemiketals and Application of Evans' Technology to the Total Synthesis of the Immunosuppressant (-)-FK506*, Jones, T. K.; Reamer, R. A.; Desmond, R.; Mills, S. *J. Am. Chem. Soc.*, 1990, **112**, 2998-3017.

[103] *Development of a Fully Synthetic Stereoselective Route to 6-Deoxyerythronolide B by Reiterative Applications of the Lewis Acid Catalyzed Diene Aldehyde Cyclocondensation Reaction: A Remarkable Instance of Diastereofacial Selectivity*, Danishefsky, S. J.; Myles, D. C. *J. Org. Chem.*, 1990, **55**, 1636-1648.

[104] *Total Synthesis of Pseudoguaianolides IV: A Stereoselective Approach to Balduilin,* Lansbury, P. T.; Mazur, D. J.; Springer, J. P. *J. Org. Chem.*, 1985, **50**, 1632-1636.

[105] *Synthetic and Mechanistic Studies of the Retro-Claisen Rearrangement. 3. A Route to Enantiomerically Pure Vinyl Cyclobutane Diesters via a Highly Diastereoselective Syn SN2' Reaction and Their Rearrangement to Enantiomerically Pure Dihydrooxacenes*, Boeckman, R. K., Jr.; Reeder, M. R. *J. Org. Chem.*, 1997, **62**, 6456-6457.

[106] *Reaction of Diisobutylaluminum Hydride with Selected Organic Compounds Containing Representative Functional Groups*, Yoon, N. M.; Gyoung, Y. S. *J. Org. Chem.*, 1985, **50**, 2443-2450.

[107] *Amphiphilic Reactions by Means of Exceptionally Bulky Organoaluminum Reagents. Rational Approach for Obtaining Unusual Equatorial, Anti-Cram, and 1,4 Selectivity in Carbonyl Alkylation*, Maruoka, K.; Itoh, T.; Sakurai, M.; Nonoshita, K.; Yamamoto, H. *J. Am. Chem. Soc.*, 1988, **110**, 3588-3597.

[108] *Actinide Diene (2-Butene-1,4-diyl) Complexes. Synthesis Including a Methyl-Induced β-Hydrogen Elimination Reaction, Structures, Structural Dynamics, Thermochemistry, and Reactivity*, Smith, G. M.; Suzuki, H.; Sonnenberger, D. C.; Day, V. W.; Marks, T. J. *Organometallics*, 1986, **5**, 549-561.

[109] *Total Synthesis of FK506 and an FKBP Probe Reagent, (C_8, C_9-$^{13}C_2$)-FK506*, Nakatsuka, M.; Ragan, J. A.; Sammakia, T.; Smith, D. B.; Uehling, D. E.; Schreiber, S. L. *J. Am. Chem. Soc.*, 1990, **112**, 5583-5601.

[110] *A Convenient Method for the Control of Selective Ozonizations of Olefins*, Veysoglu, T.; Mitscher, L. A.; Swayze, J. K. *Synthesis*, 1980, 807-810.

[111] *A synthesis of forskolin. Hydroxylation of 9-deoxyforskolin*, Hrib, N. J. *Tetrahedron Lett.*, 1987, **28**, 19-22.

[112] *A Concise Synthesis of Enantiomerically Pure Taxane C-Ring* via *the [2,3] Wittig Rearrangement*, Kress, M. H.; Kaller, B. F.; Kishi, Y. *Tetrahedron Lett.*, 1993, **34**, 8047-8050.

Experimental Section

5.1 General Procedures

Unless otherwise noted, materials were obtained from commercially available sources and used without purification. Toluene was freshly distilled from CaH_2. Diethyl ether and THF were freshly distilled from sodium benzophenone ketyl. 4Å molecular sieves were activated by heating three times for one minute at the highest setting in a microwave followed by cooling under argon.

All reactions requiring anhydrous conditions were performed under a positive pressure of argon using oven-dried glassware (120°C) that was cooled in a dessicator, unless stated otherwise.

Column chromatography was performed on Merck silica gel Kiesel 60 (230-400 mesh).

Mass spectra were obtained on Fisons VG Autospec.

^{1}H NMR, ^{13}C NMR, HSQC and nOe experiments were recorded on a Varian 300 or 400 MHz spectrometer. Spectra were recorded in $CDCl_3$ and chemical shifts (δ) were given in ppm using $CHCl_3$ as reference peak (7.27 ppm). Proton ^{1}H NMR were tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet), coupling constant in hertz, and number of protons. When appropiate, the multiplicity of a signal is denoted as "br" to indicate the signal was broad.

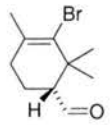
IR spectra were recorded on a Nicolet Avatar 320 series FT-IR spectrometer.

5.1 Experimental Procedures

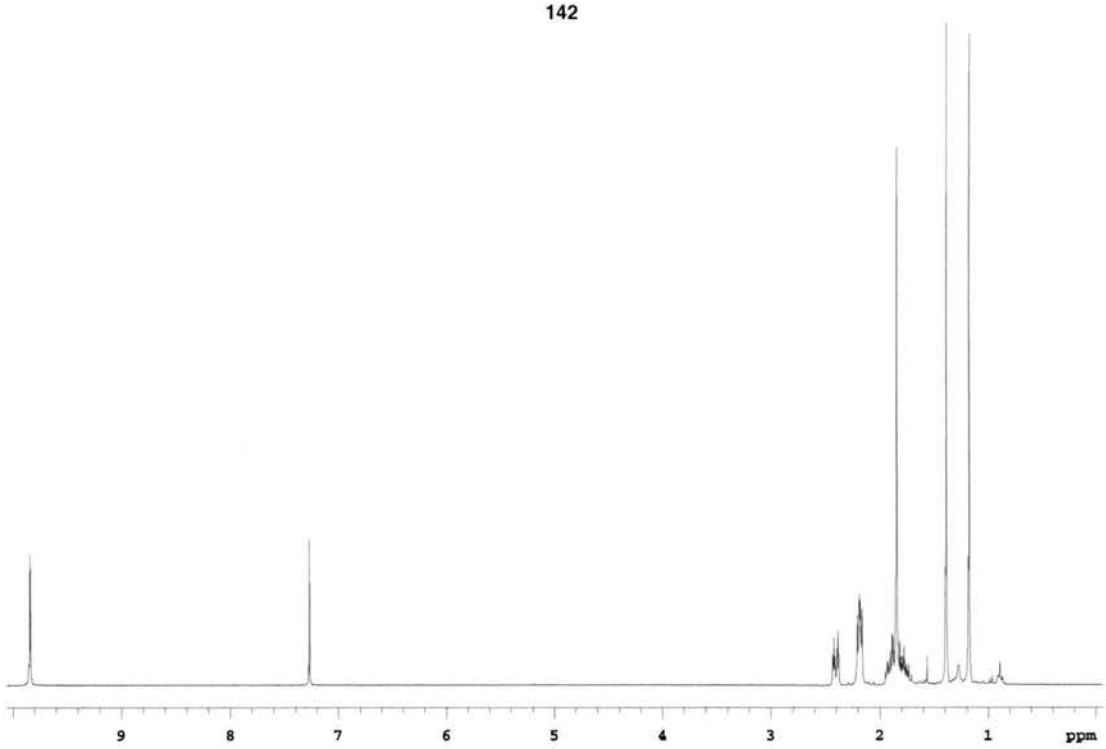
136 142

1-(*R*)-3-Bromo-2,2,4-trimethyl-cyclohex-3-enecarbaldehyde 142.

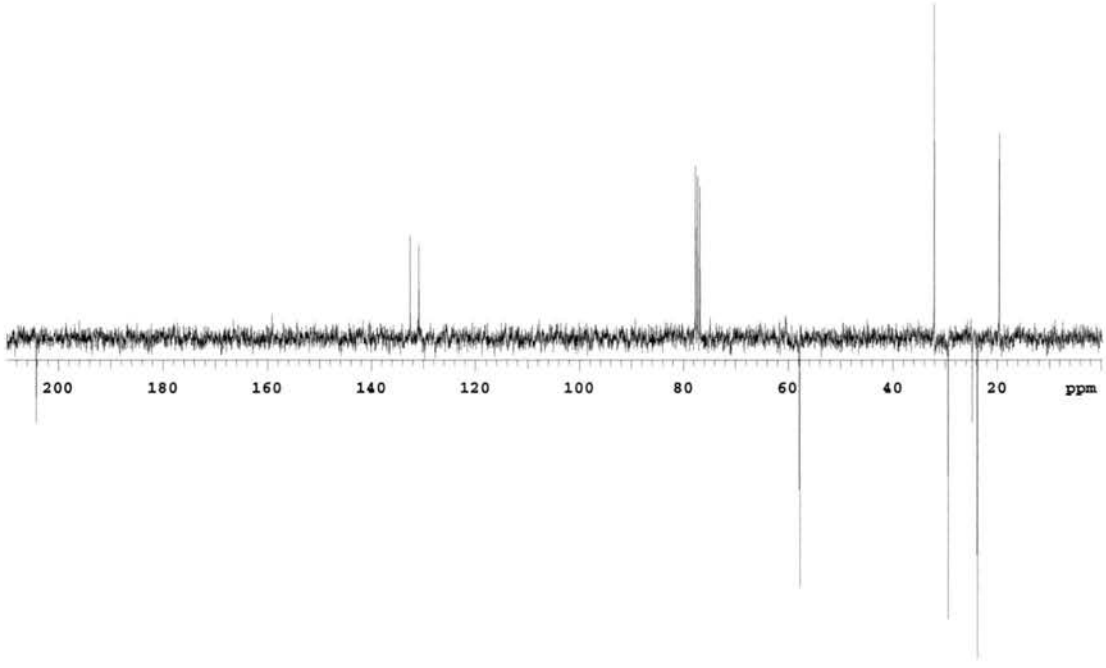
Oxalyl chloride (409 μL, 4.58 mmol) dissolved in 30 mL CH_2Cl_2 and cooled to -78°C. DMSO (875 μL, 12.33 mmol) added via syringe. Stirred 15 min, then solution of alcohol **136** (814.4 mg, 3.52 mmol) in 5 mL CH_2Cl_2 added via cannula with 5 mL rinse. Stirred 15 min, then Et_3N (2.95 mL, 21.14 mmol) added via syringe. Stirred at -78°C for 15 min, then bath removed and reaction allowed to warm to room temp. Stirred 15 min after removal of bath, then quenched with 15 mL water, separated, and aqueous extracted three times with 10 mL CH_2Cl_2. Combined organics washed once with 10 mL water, twice with 10 mL brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purified by flash chromatography (7H: 1Et_2O, 27 g silica) to yield 652.9 mg pale oil (81% yield). ^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 1.17 (3H, s), 1.38 (3H, s), 1.83 (3H, s), 1.84 (2H, br. m), 2.17 (2H, dd, J=7.51Hz, J=4.95Hz), 2.39 (1H, ddd, J=10.98Hz, J=2.93Hz, J=2.93Hz), 9.83 (1H, d, J=2.57Hz). ^{13}C NMR (75MHz) ($CDCl_3$) δ: 19.57, 23.79, 24.75, 29.43, 32.08, 57.75, 60.40, 130.89, 132.54, 204.22.



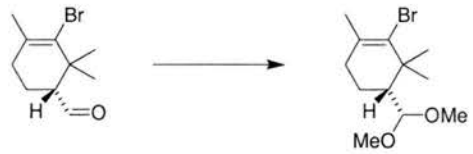
142



^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx454f11-12



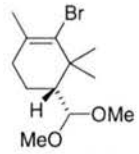
^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx435apt



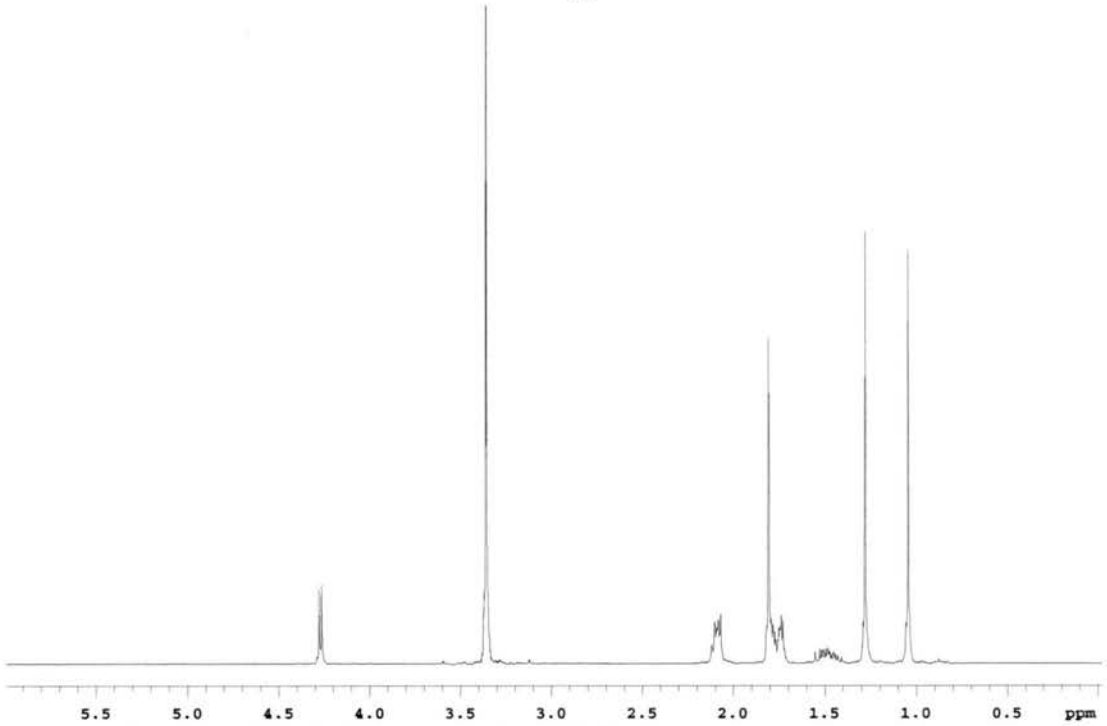
142 143

2-Bromo-4-(*R*)-dimethoxymethyl-1,3,3-trimethyl-cyclohexene 143.

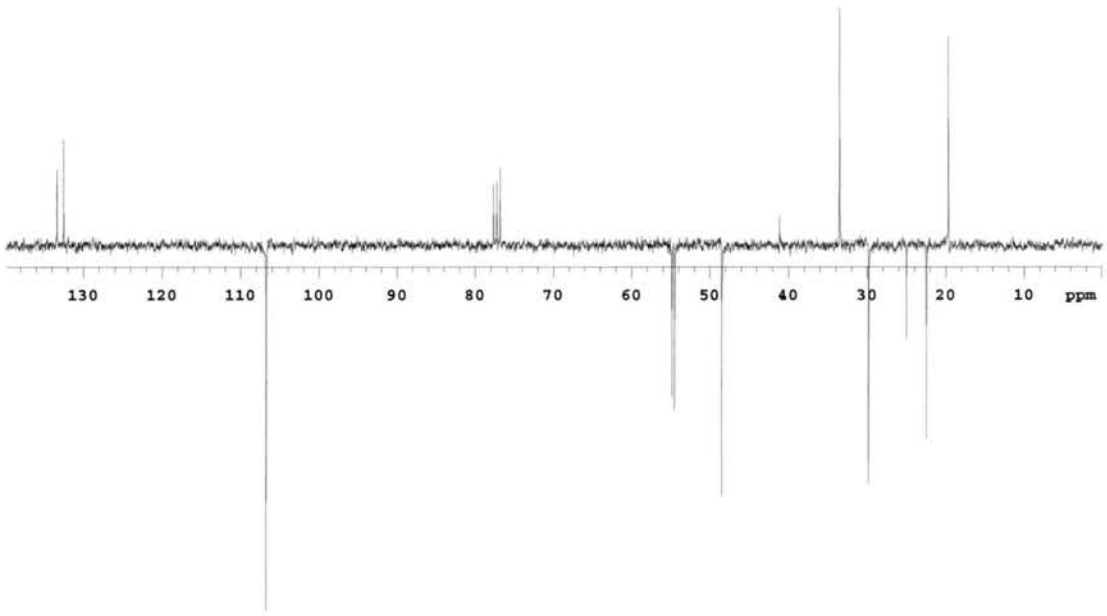
Aldehyde **142** (947 mg, 4.10 mmol) and *p*-toluenesulfonic acid monohydrate (65 mg) dissolved in 10 mL methanol and 10 mL trimethyl orthoformate and heated to reflux. Refluxed 14 hours, then diluted with 50 mL Et_2O, washed once with 5 mL 1M NaOH: 5 mL brine, twice with 5 mL water, twice with 5 mL brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purified by flash chromatography (5H: 1 Et_2O, 30 g silica) to yield 1.08 g colorless oil (95% yield). ^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 1.05 (3H, s), 1.28 (3H, s), 1.49 (1H, br. m), 1.74 (2H, m), 1.81 (3H, s), 2.09 (2H, m), 3.35 (3H, s), 3.36 (3H, s), 4.27 (1H, d, J=4.02Hz). ^{13}C NMR (75MHz) ($CDCl_3$) δ: 19.65, 22.48, 24.99, 29.81, 33.48, 41.09, 48.44, 54.46, 54.81, 106.73, 132.51, 133.35.



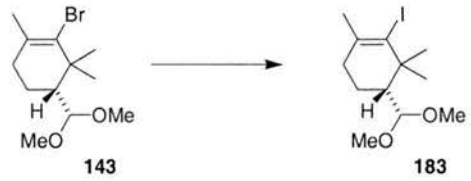
143



^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx438f4-6



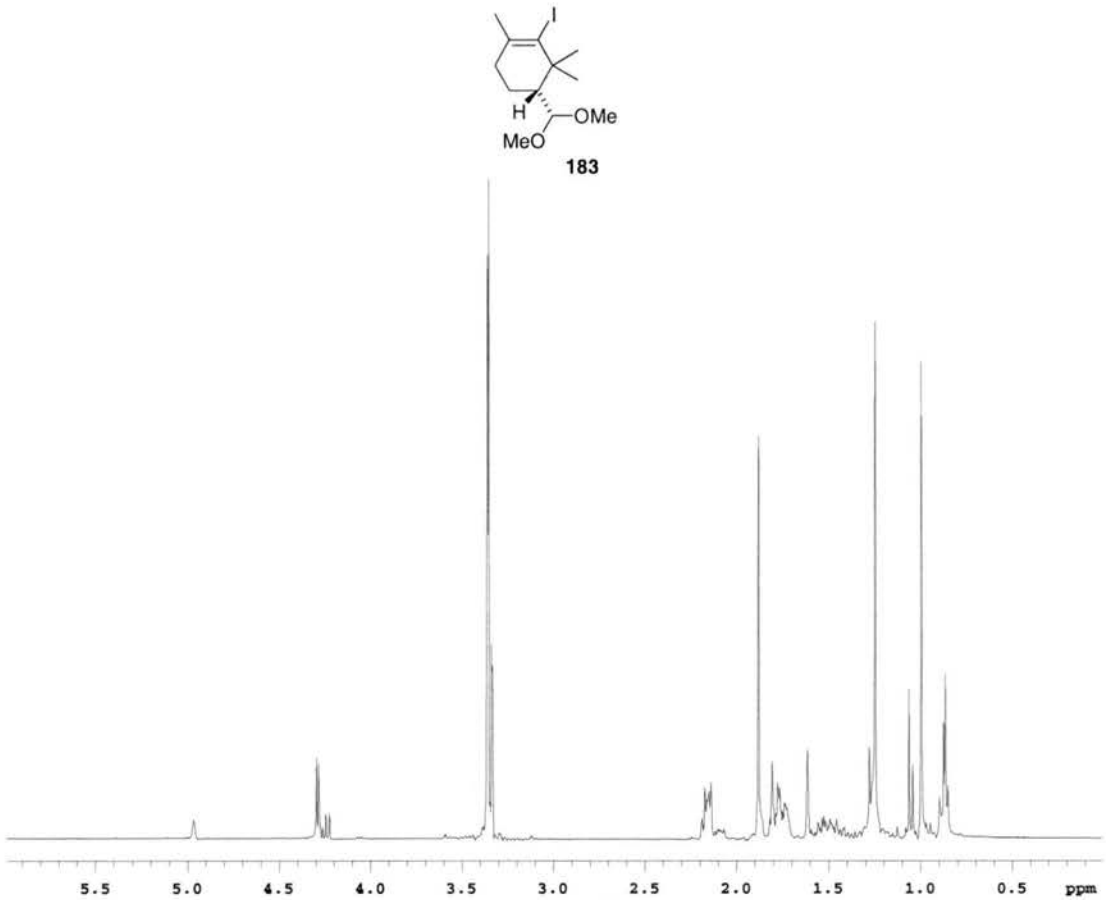
^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx438apt



143 183

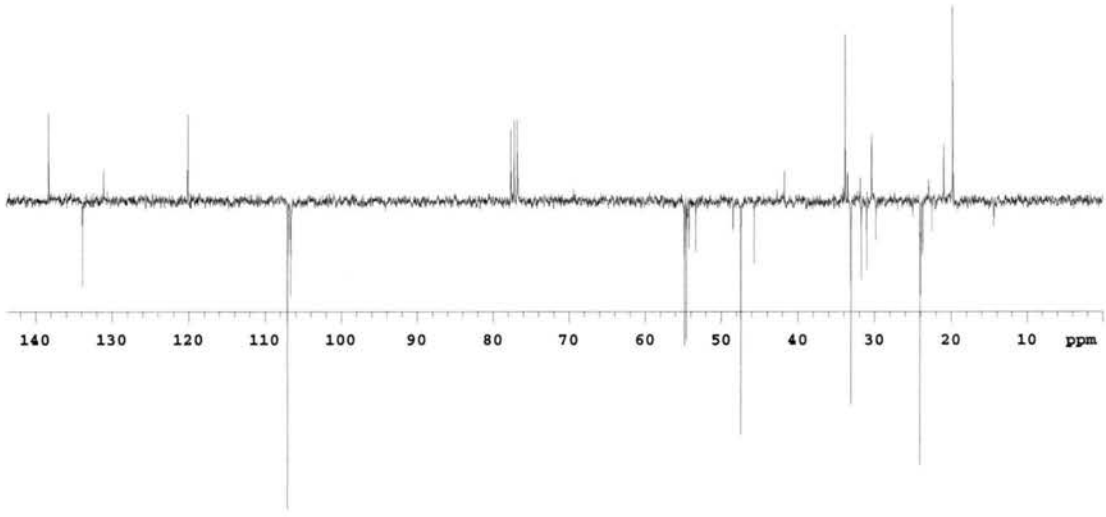
4-(*R*)-Dimethoxymethyl-2-iodo-1,3,3-trimethyl-cyclohexene 183.

Vinyl bromide **143** (5.23g, 18.87 mmol) dissolved in 60 mL THF and cooled to -78° C. 22.2 mL of 1.7 M *t*-Butyllithium (37.73 mmol, 2.0 eq) added via syringe over 5 minutes. Stirred 5 min., vacuum (~5 mmHg) applied for 10 min. (until bubbling stops), stirring continued 10 more min., then a solution of I_2 (7.18g, 1.5 eq) in 50 mL THF added via cannula. Reaction allowed to warm slowly to room temp. over 3 hours, then quenched with 25 mL sat. $Na_2S_2O_3$ sol. Diluted with 200 mL Et_2O and 20 mL water, separated, and aqueous extracted twice with 25 mL Et_2O. Combined organics washed with 20 mL brine, dried with $MgSO_4$, and concentrated *in vacuo*. Resulting oil purified by flash chromatography (10 H: 1 Et_2O, 300g silica) to yield 4.62g vinyl iodide as colorless oil (72% yield). ^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 1.00 (3H, s), 1.25 (3H, s), 1.49 (1H, br. m), 1.75 (2H, m), 1.88 (3H, s), 2.16 (2H, m), 3.35 (3H, s), 3.36 (3H, s), 4.29 (1H, d, J=3.66Hz). ^{13}C NMR (75MHz) ($CDCl_3$) δ TMS: 19.81, 24.07, 31.75, 33.07, 33.83, 41.74, 47.41, 54.57, 54.83, 107.05, 120.12, 138.28.

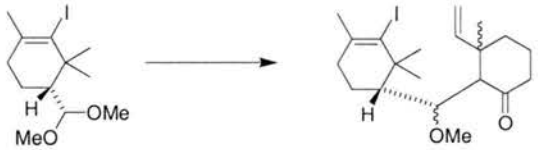


183

^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx671f8-15

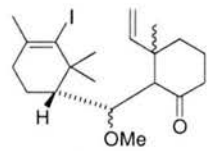


^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx671apt

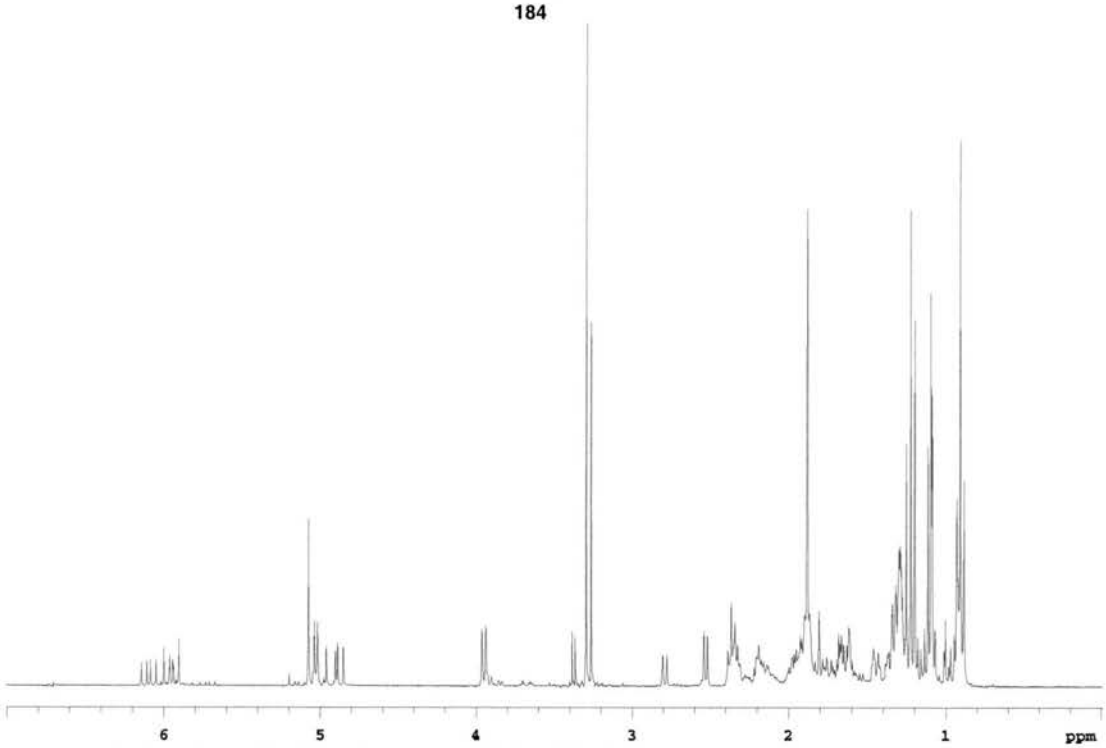


2-(*R,S*)-[(3-Iodo-2,2,4-trimethyl-1-(*S*)-cyclohex-3-enyl)-(*R,S*)-methoxy-methyl]-3-(*R,S*)-methyl-3-vinyl-cyclohexanone 184.

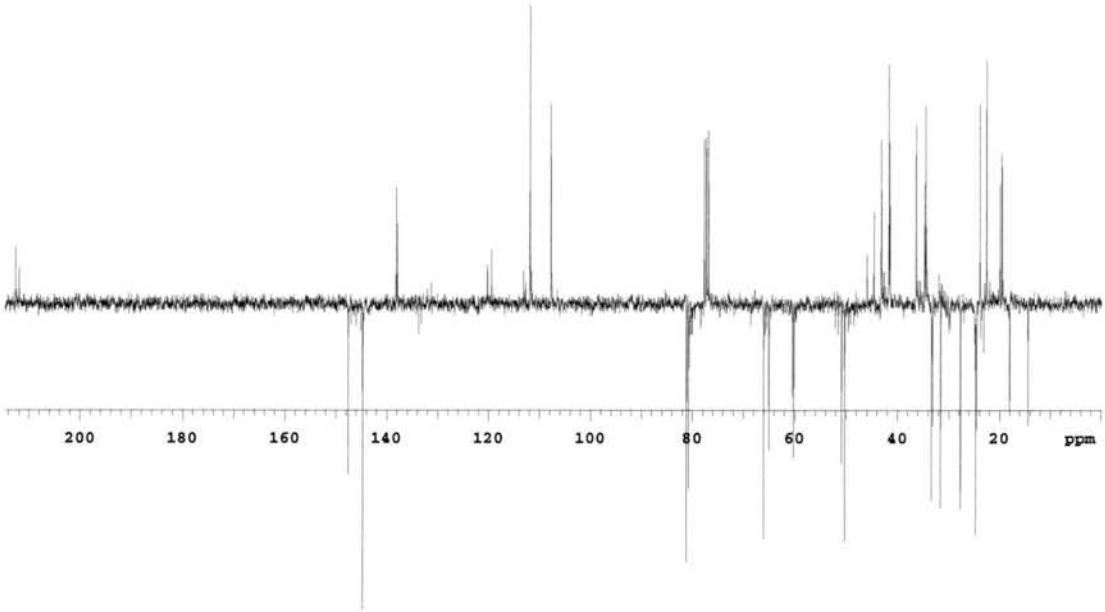
Dimethyl acetal **183** (1.629 g, 5.025 mmol) and silyl enol ether **151** (2.166 g, 10.30 mmol) dissolved in CH_2Cl_2 (60 mL) and cooled to –78° C. $TiCl_4$ (829 μL, 7.54 mmol) added via syringe. Stirred 1 h, then quenched at –78° C with 10 mL sat. Na_2CO_3 sol., diluted with 100 mL ether and 10 mL water, separated, and aqueous extracted twice with 15 mL ether. Combined organics washed twice with 5 mL water, twice with 5 mL brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purified by flash chromatography (10 H: 1 Et_2O, 110 g silica) to yield 2.059 g of aldol product as a pale oil (95% yield). ^1H NMR (300MHz) ($CDCl_3$) δ TMS: 1.05 (½ 3H,s), 1.06 (½ 3H, s), 1.08 (½ 3H, s), 1.16 (½ 3H, s), 1.19 (½ 3H, s), 1.22 (2H, m), 1.27 (3H, m), 1.41 (1H, d, J=9.16Hz), 1.61 (2H, m), 1.85 (3H, m), 1.90 (2H, m), 2.14 (1H, m), 2.31 (2H, m), 2.49 (½ 1H, d, J=6.96Hz), 2.76 (½ 1H, d, J=8.05Hz), 3.23 (½ 3H, s), 3.26 (½ 3H, s), 3.92 (1H, d, J=7.69Hz), 4.86 (1H, dd, J=18.13Hz, J=14.10Hz), 5.00 (1H, dd, J=6.78Hz, J=0.92Hz), 5.92 (½ 1H, dd, J=17.03Hz, J=11.54Hz), 6.06 (½ 1H, dd, J=17.58Hz, J=10.62Hz). ^{13}C NMR (75MHz) ($CDCl_3$) δ: (14.36, 17.99), (19.52, 19.91), (22.57, 23.85), (24.50, 24.65), (27.68, 31.55), (33.13, 33.22), (34.51, 34.66), 36.33, (41.46, 41.65), 43.16, (44.57, 45.91), (50.24, 50.86), (60.12, 60.42), (64.97, 66.03), (80.67, 81.10), (107.70, 111.83), (119.43, 120.23), (137.85, 138.14), (144.69, 147.59), (211.93, 212.60).



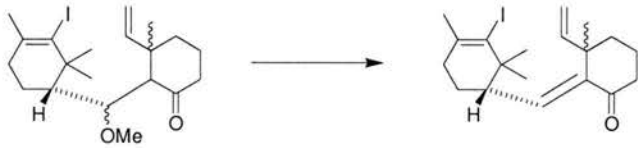
184



^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx672f8-11



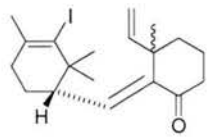
^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx672f8-11apt



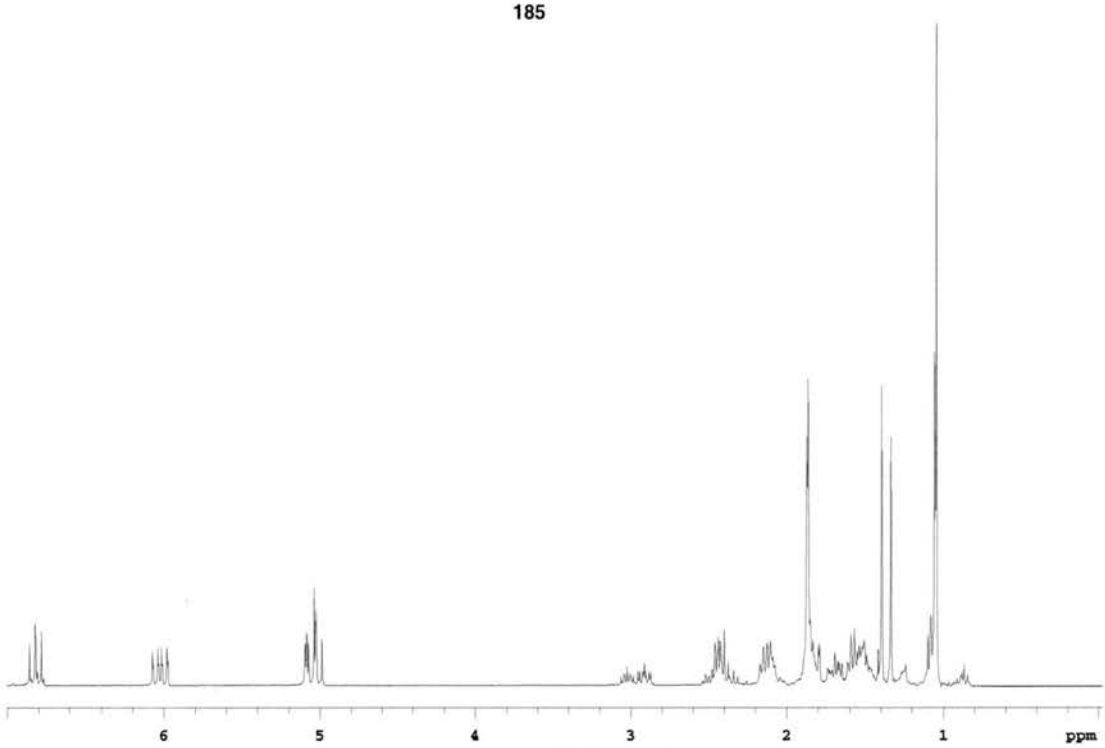
184 185

2-(3-Iodo-2,2,4-trimethyl-1-(*S*)-cyclohex-3-enylmethylene)-3-(*R,S*)-methyl-3-vinyl-cyclohexanone 185.

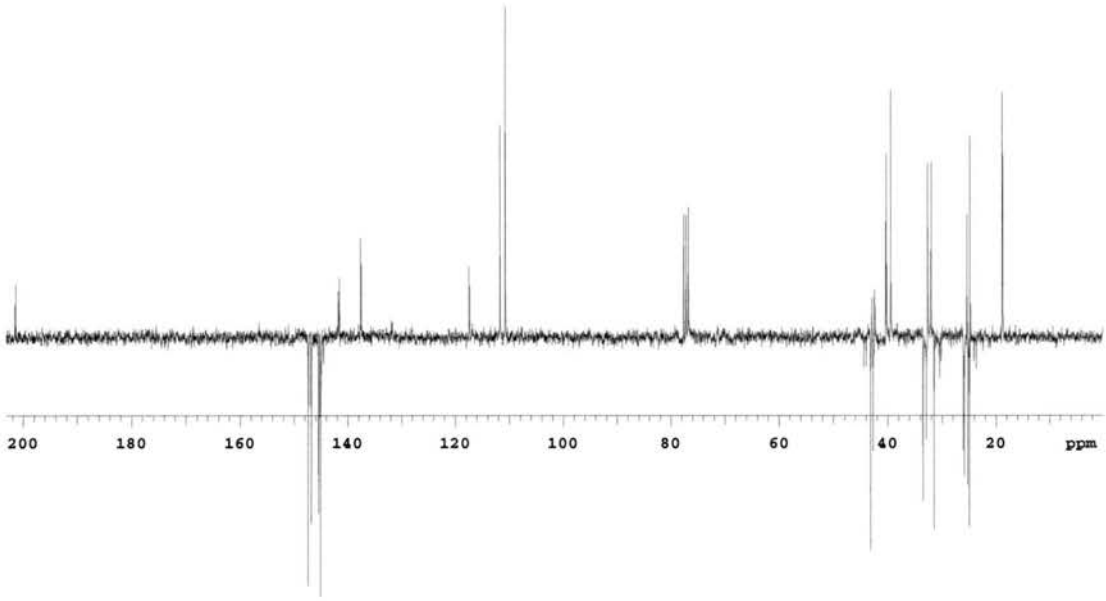
Mukaiyama aldol product **184** (2.059g, 4.784 mmol) and *p*-toluenesulfonic acid (75 mg) were dissolved in 40 mL of benzene and refluxed for 2h. Reaction cooled to room temp, diluted with 80 mL ether, washed twice with 5 mL sat. Na_2CO_3 sol., once with 5 mL brine, dried with $MgSO_4$ and concentrated *in vacuo* to a yellow oil. Purified by flash chromatography (8 H: 1 Et_2O, 145 g silica) to give 285.1 mg of the first Z isomer, 504.6 mg of the second Z isomer, and 962.9 mg of mixed *E* isomers, 1.7526 g in all (92% yield). **Mixed *E* isomers:** ^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 1.05 (3H,s), 1.06 (3H, s), 1.34 (½ 3H, s), 1.39 (½ 3H, s), 1.51 (2H, m), 1.58 (1H, dd, J=12.45Hz, J=6.23Hz), 1.69 (1H, m), 1.85 (2H, m), 1.86 (½ 3H, s), 1.87 (½ 3H, s) 2.13 (2H, m), 2.43 (2H, m), 2.91 (½ 1H, ddd, J=12.09Hz, J=9.16Hz, J=3.30Hz), 3.02 (½ 1H, ddd, J=11.99Hz, J=6.68Hz, J=5.13Hz), 5.03 (1H, dd, J=14.65Hz, J=10.99Hz), 6.03 (1H, dd, J=17.58Hz, J=10.63Hz), 6.82 (1H, dd, J=11.36Hz, J=11.36Hz). ^{13}C NMR (75MHz) ($CDCl_3$) δ TMS: (18.73, 18.83), (24.85, 25.39), (24.91, 25.19), (25.88, 26.03), 31.48, 32.03, 32.67, (32.91, 33.48), 39.54, (40.27, 40.40), (42.39, 42.91), (42.71, 43.17), (110.78, 111.75), (117.37, 117.50), (137.49, 137.70), (141.58, 141.79), (145.06, 145.43), (146.81, 147.33), (201.40, 201.57).



185



^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx676f16-28



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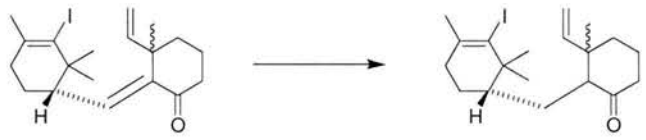
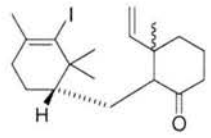


185 186

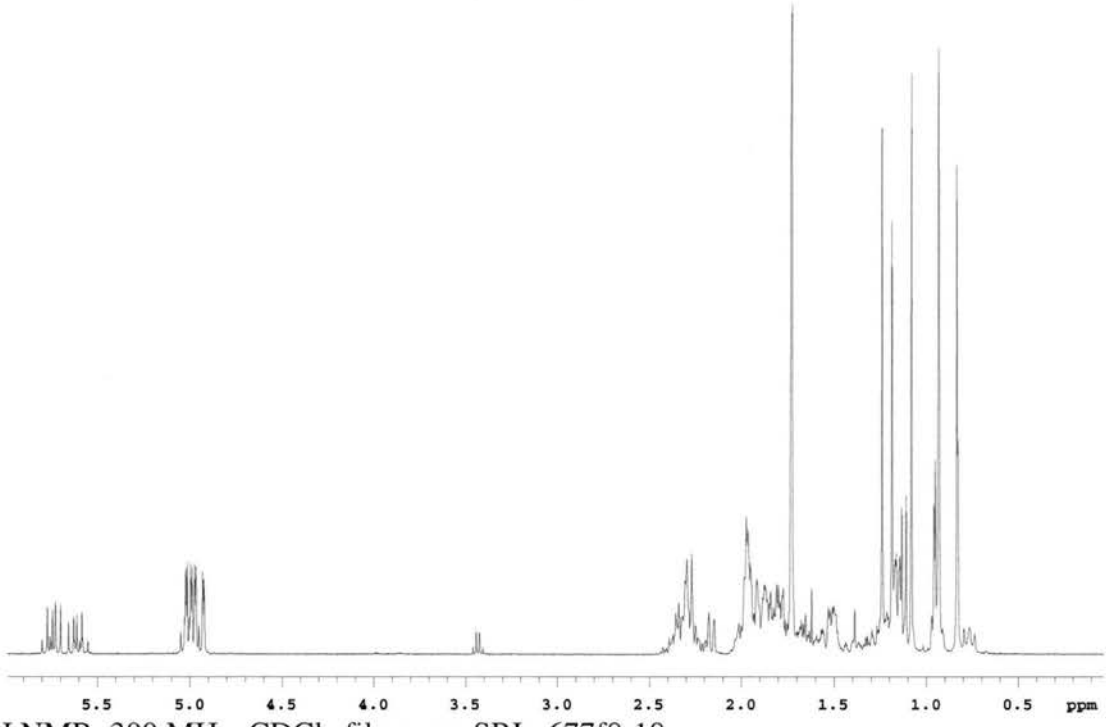
2-(*S*)-(1-(*S*)-3-Iodo-2,2,4-trimethyl-cyclohex-3-enylmethyl)-3-(*R,S*)-methyl-3-vinyl-cyclohexanone 186.

Mixed *E* enone isomers **185** (962.9 mg, 2.417 mmol) dissolved in 1.5 M $NaBH_4$ in pyridine (28 mL, 17 eq) at room temp. Stirred 38 hours, then quenched with 10 mL sat. NH_4Cl sol. over 35 min., diluted with 200 mL Et_2O, 200 mL hexanes, and 10 mL water, and separated. Combined organics washed 5 times with 10 mL water, 7 times with 10 mL sat. $CuSO_4$ sol., twice with 5 mL water, once with 10 mL brine, dried with $MgSO_4$ and concentrated *in vacuo* to a yellow oil, which was pushed through a 30g silica gel column eluted with 1 H: 1 Et_2O. Fractions containing product and intermediates concentrated *in vacuo*. Residue dissolved in 30 mL CH_2Cl_2 and PCC (782mg, 3.262 mmol) added. Stirred 12 hours, then diluted with 150 mL Et_2O, filtered through a pad of $MgSO_4$ and concentrated *in vacuo*. Residue purified by flash chromatography (10 H: 1 Et_2O, 50 g silica) to give 454.6 mg conjugate reduction product (47% yield) and 263.8 mg recovered starting enone (27% recovery). ^1H NMR (300MHz) ($CDCl_3$) δ TMS: 0.88 (½ 3H, s), 0.98 (3H, s), 1.12 (½ 3H, s), 1.16 (1H, d, J=9.36Hz), 1.20 (1H, d, J=7.40Hz), 1.23 (½ 3H, s), 1.28 (½ 3H, s), 1.55 (1H, dd, J=8.58Hz, J=3.12Hz), 1.68 (2H, m), 1.77 (3H, s), 1.90 (4H, m), 2.01 (2H, dd, J=8.32Hz, J=4.42Hz), 2.30 (3H, m), 4.99 (1H, dd, J=17.54Hz, J=3.12Hz), 5.05 (1H, dd, J=10.73Hz, J=10.92Hz), 5.66 (½ 1H, dd, J=17.55Hz, J=10.92Hz), 5.78 (½ 1H, dd, J=17.35Hz, J=11.12Hz). ^{13}C NMR (125.89MHz) ($CDCl_3$) δ: 17.84, (21.90, 21.93), (22.74, 23.28), (23.47, 23.58), (24.76, 26.46), (25.79, 27.30), 28.51, (33.35, 33.39), 35.68, (38.60, 40.45), 42.06, (43.85, 44.23),

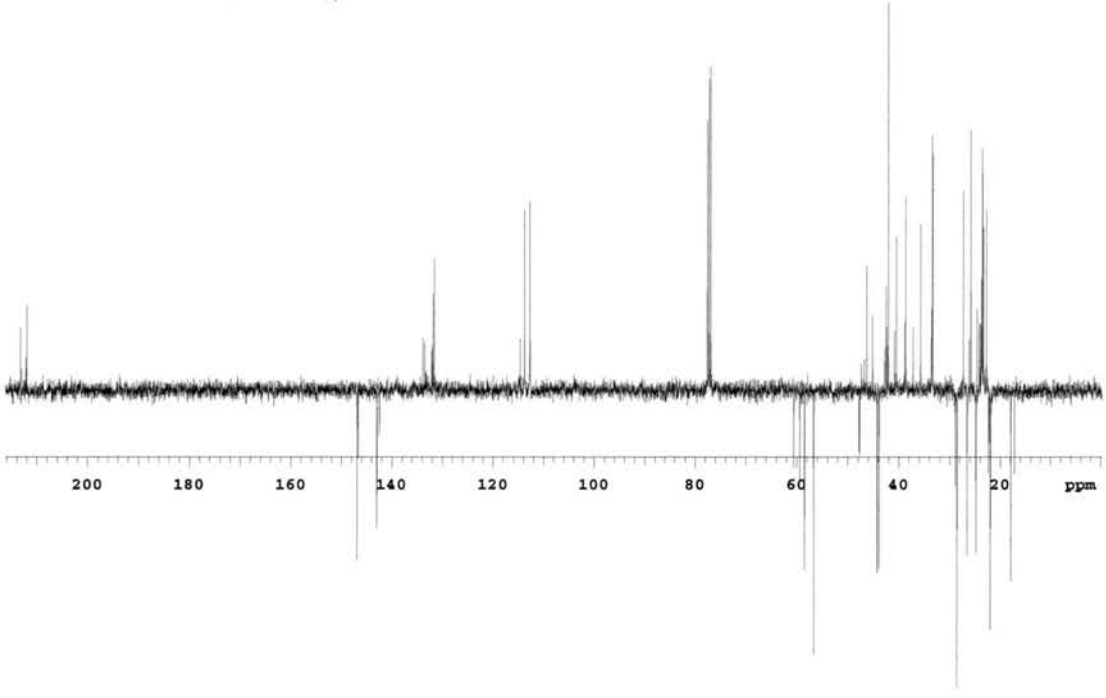
(45.12, 46.23), (56.69, 58.47), (131.55, 131.81), (133.47, 133.84), (142.91, 146.89), (212.22, 213.19).



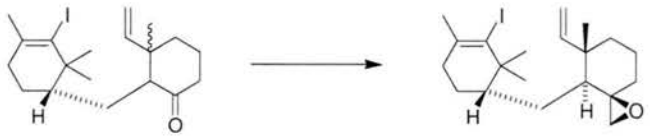
186



^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx677f8-18



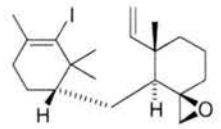
^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx677apt



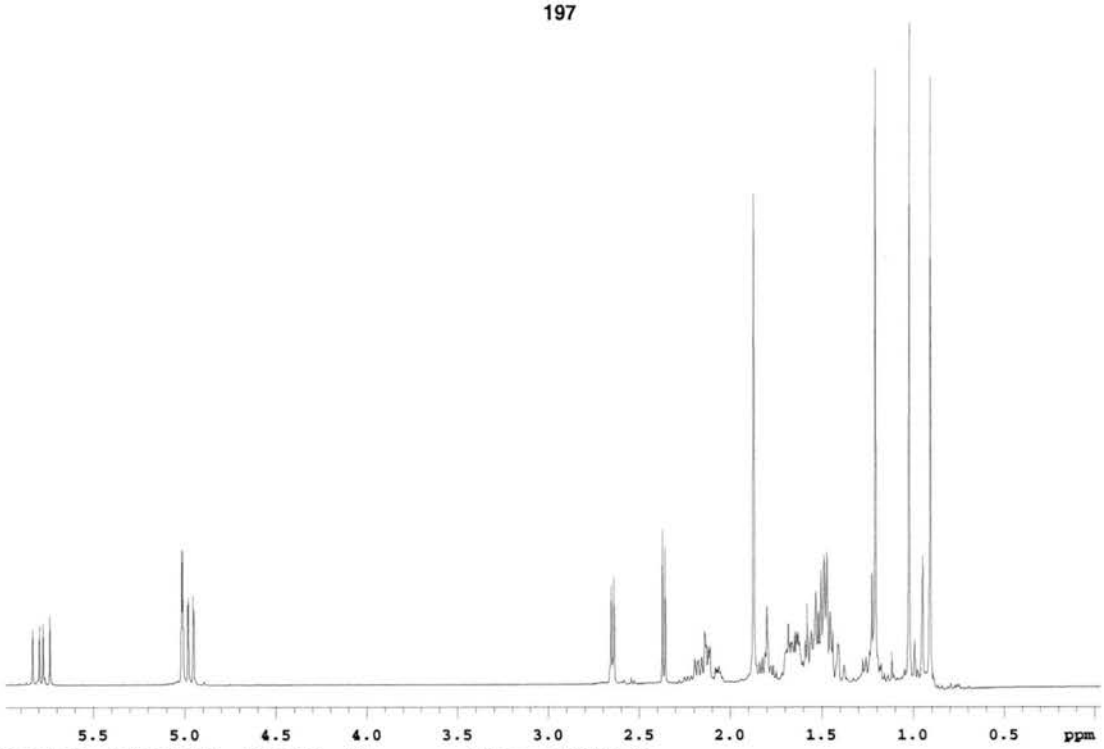
186 197

4-(*S*)-(1-(*S*)-3-Iodo-2,2,4-trimethyl-cyclohex-3-enylmethyl)-5-(*S*)-methyl-5-vinyl-1-oxa-spiro-(*R*)-[2.5]octane 197.

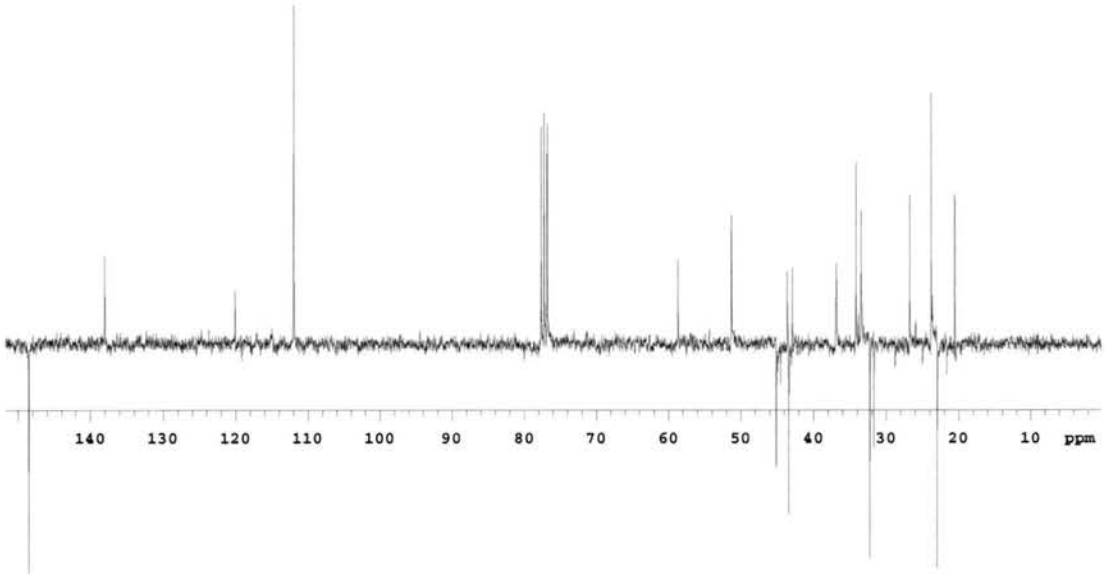
n-BuLi (2.0M in cyclohexane, 191.5 μL, 0.383 mmol) added to a suspension of trimethylsulfonium iodide (90.3 mg, 0.442 mmol) in 2 mL THF at 0°C. Stirred 55 min., then a sol. of ketone # in 2 mL THF added via cannula with two 1 mL rinses. Reaction stirred at 0°C 90 min, then quenched with 5 mL water, diluted with 20 mL Et_2O, separated, and aqueous extracted twice with 15 mL Et_2O. Combined organics washed twice with 2 mL water, once with 3 mL brine, dried with $MgSO_4$, and concentrated *in vacuo*. Purified by flash chromatography (20H:1Et_2O, 8 g silica) to yield spiroepoxide **6** as 22.2 mg white solid (18% yield, 73% of possible) as the first compound off the column. ^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 0.91 (3H, s), 0.95 (1H, s), 1.02 (3H, s), 1.21 (3H, s), 1.23 (1H, s), 1.50 (7H, m), 1.66 (2H, m), 1.80 (1H, s), 1.87 (3H, s), 2.14 (2H, m), 2.36 (1H, d, J=4.39Hz), 2.65 (1H, d, J=4.76Hz), 4.97 (1H, dd, J=8.95Hz, J=0.89Hz), 5.01 (1H, d, J=1.47Hz), 5.79 (1H, dd, J=17.22Hz, J=10.99Hz). ^{13}C NMR (75MHz) ($CDCl_3$) δ TMS: 20.56, 22.99 (2C), 23.81, 26.77, 31.70, 32.24, 33.45, 34.17, 36.87, 42.91, 43.38, 43.60, 45.09, 51.30, 58.69, 111.97, 120.07, 138.01, 148.49.



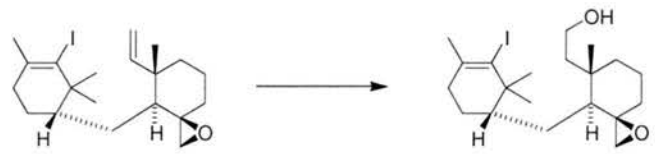
197



^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx722f5-6



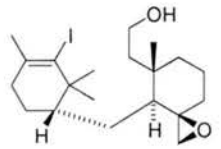
^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx739apt



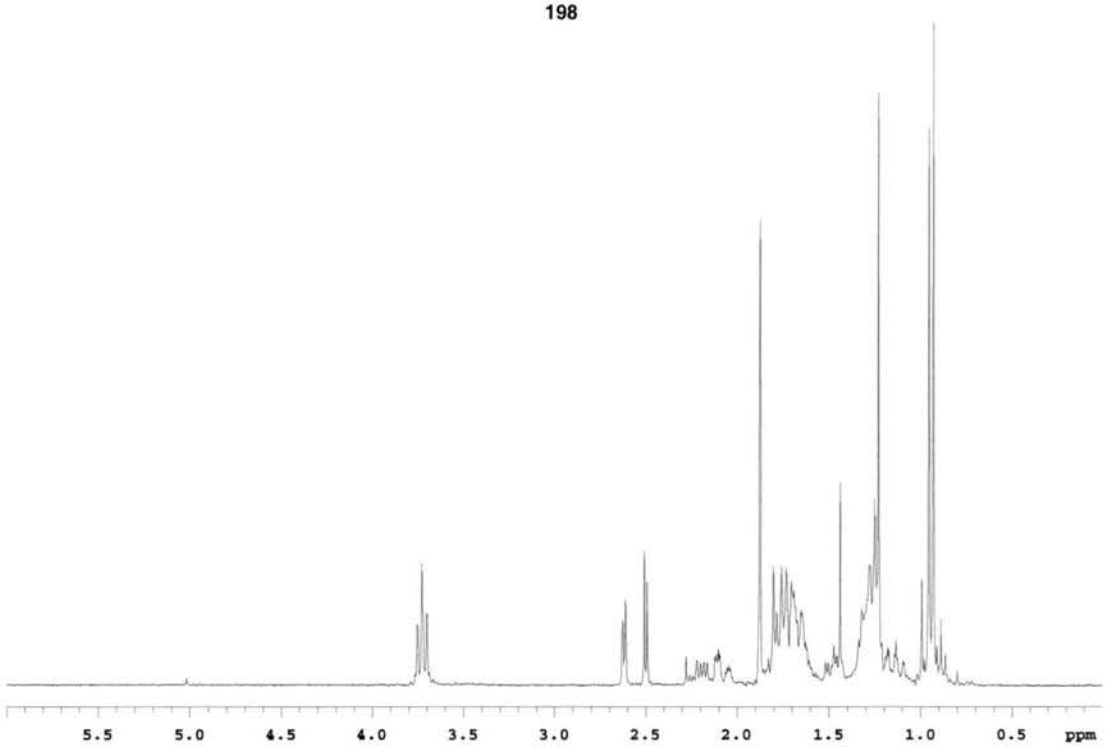
197 198

2-[4-(*S*)-(1-(*S*)-3-Iodo-2,2,4-trimethyl-cyclohex-3-enylmethyl)-5-(*S*)-methyl-1-oxa-spiro-(*R*)-[2.5]oct-5-yl]-ethanol 198.

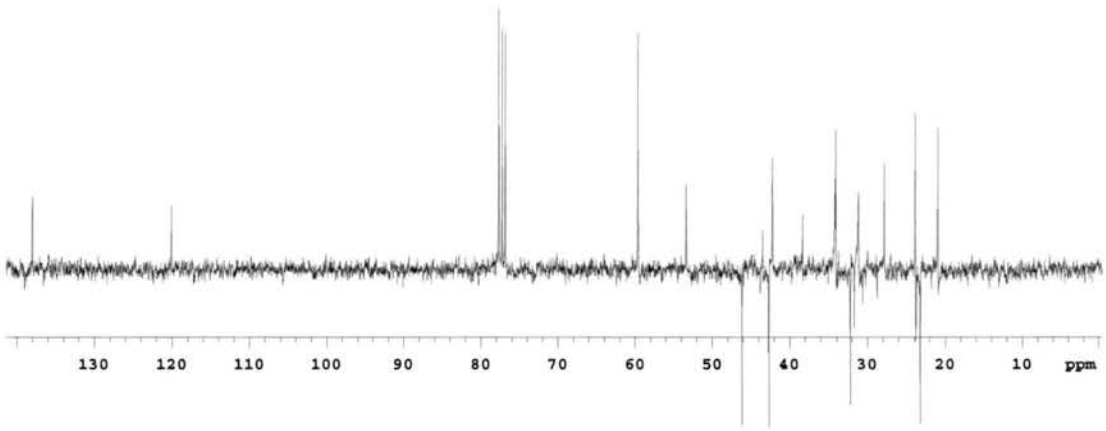
Spiroepoxide **198** (32.4 mg, 0.078 mmol) dissolved in 1.5 mL THF at room temp. BH_3•THF (1.0 M in THF, 117 μL, 0.117 mmol) added via syringe, reaction stirred 40 min, then quenched at 0° C with 500 μL 1 M NaOH sol. and 500 μL 30% H_2O_2 sol. and diluted with 3 mL THF. Stirred overnight, then diluted with 15 mL ether, separated, and aqueous extracted twice with 3 mL ether. Combined organics washed twice with 1 mL sat. $NaHCO_3$, dried with $MgSO_4$ and concentrated *in vacuo*. Purified by flash chromatography (1H:1Et_2O, 1.5 g silica) to yield 22.6 mg white foam (67% yield). ^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 0.93 (3H, s), 0.95 (3H, s), 1.23 (3H, s), 1.28 (6H, m), 1.44 (1H, s), 1.72 (8H, m), 1.87 (3H, s), 2.08 (1H, m), 2.21 (1H, m), 2.50 (1H, d, J=4.76Hz), 2.62 (1H, d, J=4.76Hz), 3.73 (2H, t, J=7.60Hz). ^{13}C NMR (75MHz) ($CDCl_3$) δ TMS: 20.95, 23.18, 23.74, 23.85, 31.16, 32.16, 34.08, 34.17, 38.32, 42.23, 42.67, 43.45, 46.11, 53.37, 59.62 (2C), 120.08, 138.00.



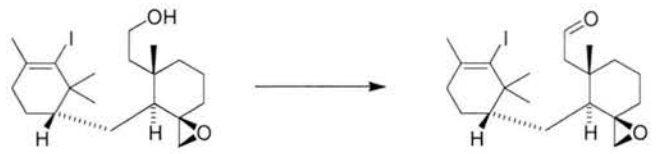
198



^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx740



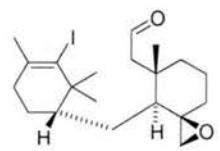
^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx740apt



198 199

[4-(*S*)-(1-(*S*)-3-Iodo-2,2,4-trimethyl-cyclohex-3-enylmethyl)-5-(*S*)-methyl-1-oxa-spiro-(R)-[2.5]oct-5-yl]-acetaldehyde 199.

Dess-Martin reagent (44.3 mg, 0.105 mmol) added to a room temp. sol. of alcohol **198** (22.6 mg, 0.052 mmol) in 2.5 mL CH_2Cl_2. Stirred 45 min, then quenched with 1 mL sat. $Na_2S_2O_3$ and 1 mL sat. Na_2CO_3, diluted with 3 mL CH_2Cl_2, separated, and aqueous extracted three times with 1.5 mL CH_2Cl_2. Combined organics dried with $MgSO_4$ and concentrated *in vacuo*. Purified by flash chromatography (2H:1Et_2O, 1.5 g silica) to yield 19.2 mg white foam (85% yield). ^{1}H NMR (400MHz) ($CDCl_3$) δ TMS: 0.96 (3H, s), 1.15 (3H, s), 1.23 (3H, s), 1.25 (1H, s), 1.29 (2H, m), 1.39 (1H, d, J=10.02Hz), 1.48 (1H, m), 1.62 (3H, m), 1.77 (4H, m), 1.87 (3H, s), 2.08 (1H, ddd, J=17.48Hz, J=5.44Hz, J=2.45Hz), 2.21 (1H, ddd, J=17.26Hz, J=10.87Hz, J=5.33Hz), 2.42 (1H, dd, J=14.82Hz, J=2.67Hz), 2.50 (1H, d, J=4.91Hz), 2.59 (1H, dd, J=14.82Hz, J=3.20Hz), 2.60 (1H, d, J=5.11Hz), 9.88 (1H, t, J=2.88Hz). ^{13}C NMR (100MHz) ($CDCl_3$) δ TMS: 20.81, 23.21, 23.77, 24.29, 27.81, 30.84, 31.65, 32.13, 33.61, 33.95, 34.70, 39.57, 42.36, 43.37, 45.74, 53.00, 59.08, 119.77, 138.06, 203.15.



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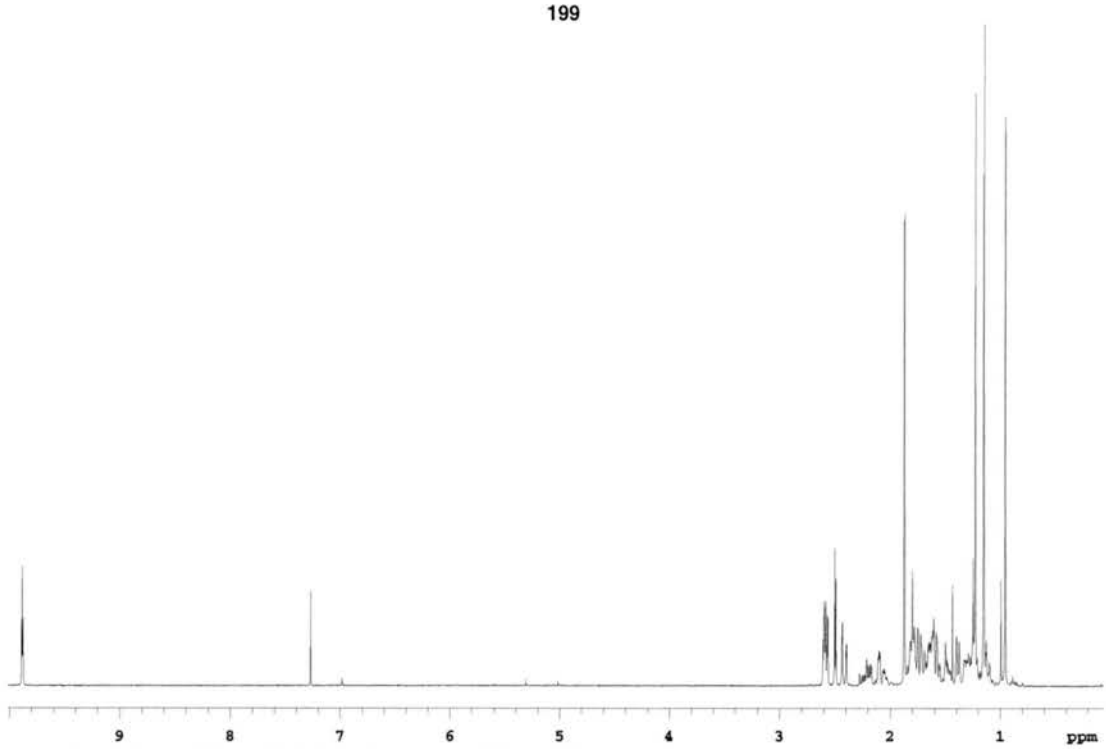
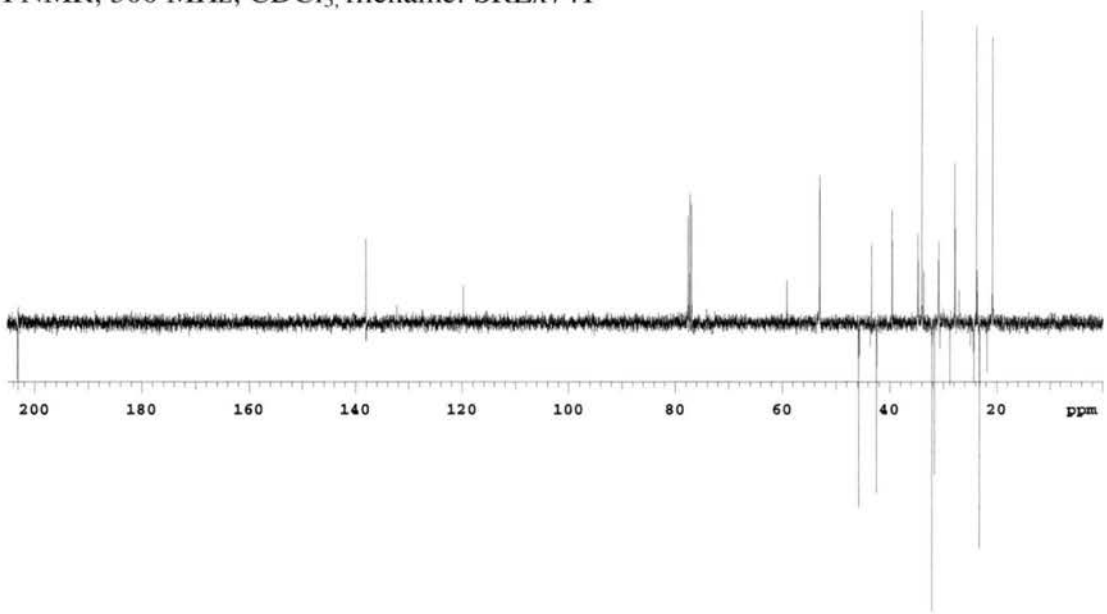
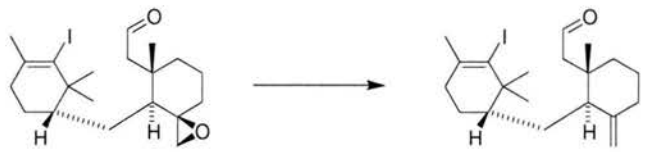


^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx741



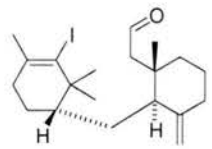
^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx741apt



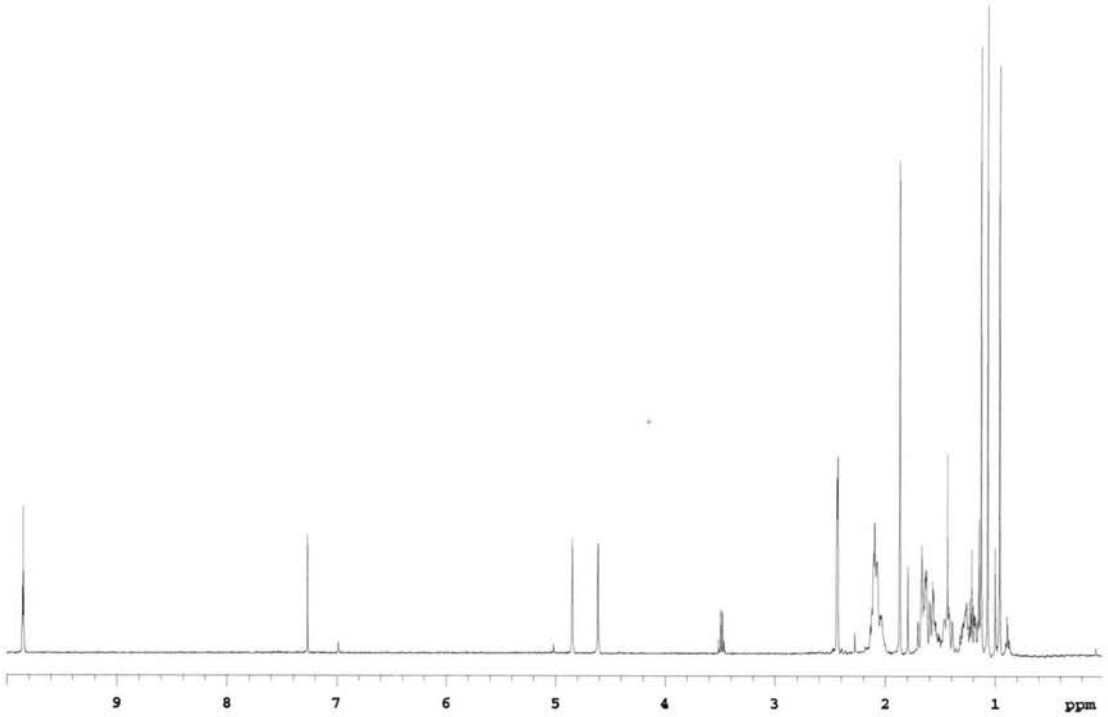
199 200

[2-(*R*)-(1-(*S*)-3-Iodo-2,2,4-trimethyl-cyclohex-3-enylmethyl)-1-(*S*)-methyl-3-methylene-cyclohexyl]-acetaldehyde 200.

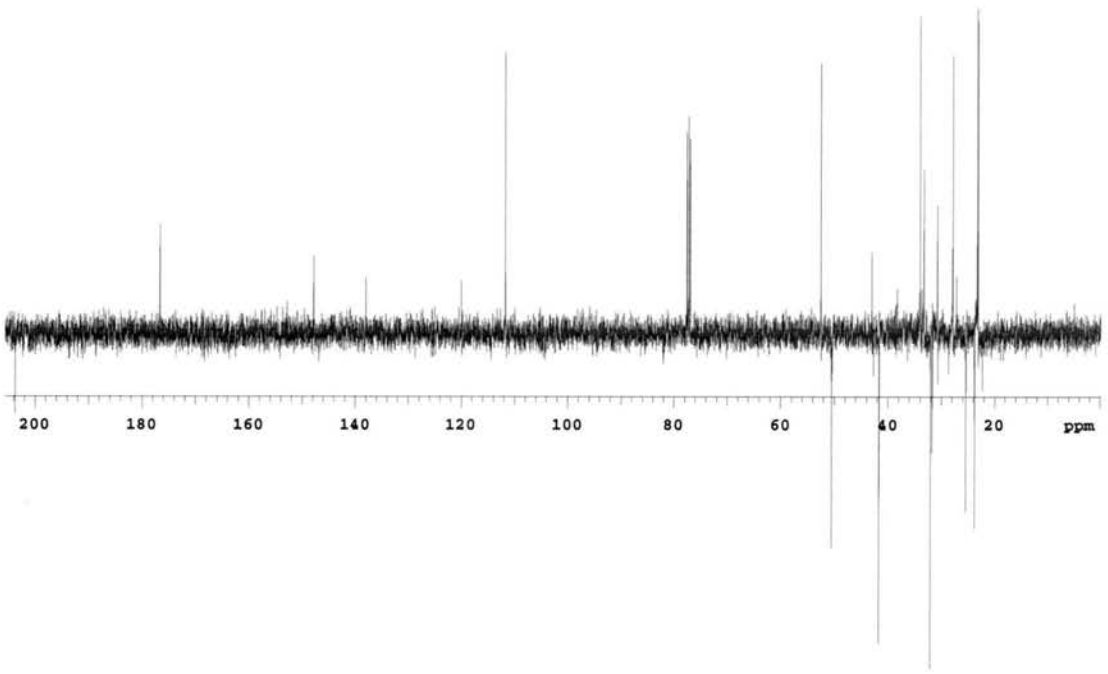
P_2I_4 (80.2 mg, 0.134 mmol) added to a room temp. sol. of spiroepoxide **199** (19.2 mg, 0.045 mmol) and pyridine (350 μL) in 3.5 mL CH_2Cl_2 and heated to reflux for 14 hours. Quenched with 4 mL water, stirred 45 min., diluted with 10 mL Et_2O, separated, and aqueous extracted three times with 3 mL Et_2O. Combined organics dried with $MgSO_4$ and concentrated *in vacuo*. Purified by flash chromatography (5H:1Et_2O, 3 g silica) to yield 12.9 mg white solid (70% yield) and 2.5 mg recovered starting material. ^{1}H NMR (400MHz) ($CDCl_3$) δ TMS: 0.96 (3H, s), 1.07 (3H, s), 1.13 (3H, s), 1.15 (1H, s), 1.20 (1H, m), 1.28 (1H, m), 1.44 (1H, m), 1.56 (2H, m), 1.65 (3H, m), 1.87 (3H, s), 2.07 (5H, m), 2.44 (2H, d, J=2.77Hz), 4.61 (1H, s), 4.85 (1H, s), 9.86 (1H, t, J=2.98Hz). ^{13}C NMR (100MHz) ($CDCl_3$) δ TMS: 23.08, 23.23, 23.72, 25.36, 27.09, 27.83, 30.70, 31.68, 31.99, 33.21, 33.95, 41.60, 42.97, 50.52, 52.45, 111.77, 119.99, 138.04, 147.83, 203.72.



200



^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx742

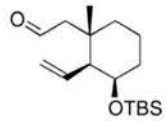


^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx742apt

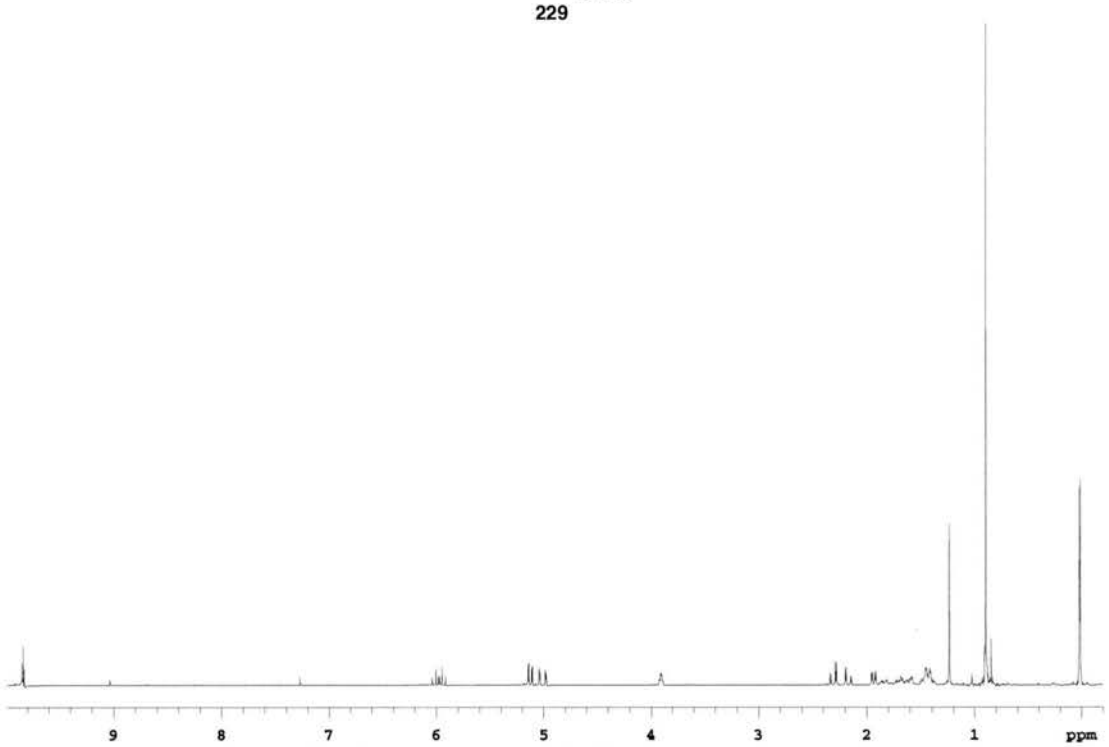
235 229

[3-(*R*)-(tert-Butyl-dimethyl-silanyloxy)-1-(*S*)-methyl-2-(*S*)-vinyl-cyclohexyl]-acetaldehyde 229.

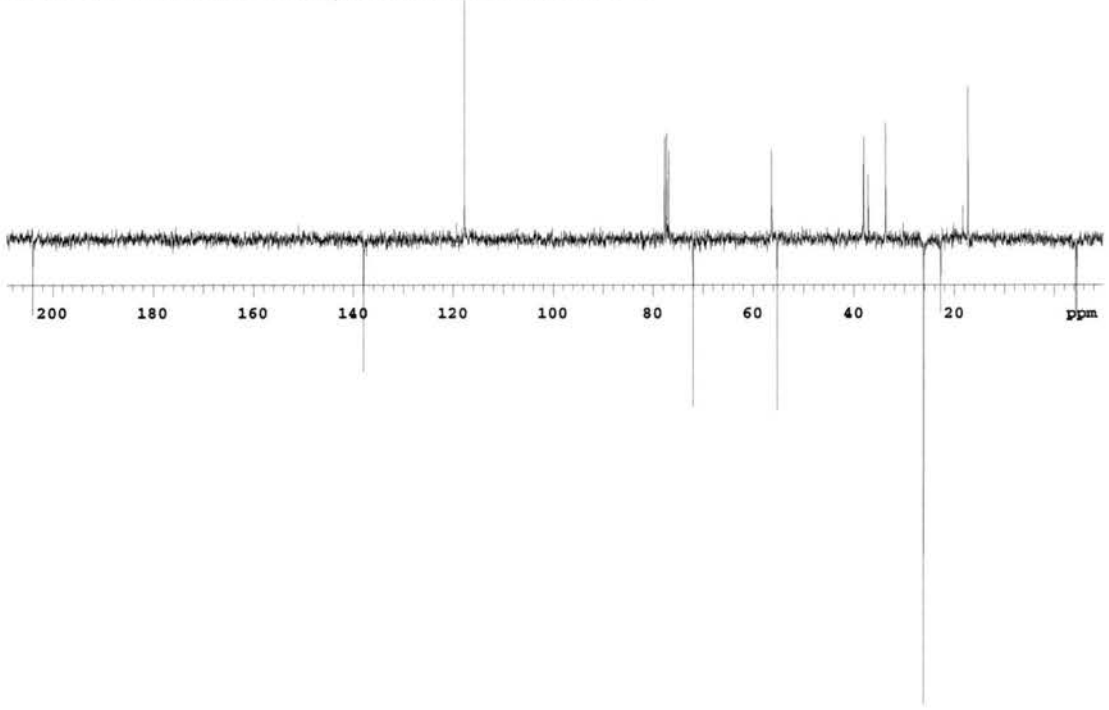
Dess-Martin periodinane (667 mg, 1.57 mmol) added to a room temp. sol. of alcohol **235** (391.1 mg, 1.31 mmol) in 20 mL CH_2Cl_2. Stirred 30 min., then quenched with 3 mL sat. $Na_2S_2O_3$ sol. and 3 mL sat. $NaHCO_3$ sol., stirred overnight, separated, and aqueous extracted twice with 10 mL CH_2Cl_2. Combined organics dried with $MgSO_4$ and concentrated *in vacuo*. Purified by flash chromatography (8 Hex:1 Et_2O, 13 g silica) to yield 294 mg colorless oil (76% yield). ^1H NMR (300MHz) ($CDCl_3$) δ TMS: 0.01 (3H, s), 0.02 (3H, s), 0.90 (9H, s), 1.24 (3H, s), 1.43 (3H, m), 1.66 (2H, m), 1.83 (1H, m), 1.94 (1H, dd, J=9.85Hz, J=3.15Hz), 2.17 (1H, dd, ½ ABq, J=14.70Hz, J=3.15Hz), 2.31 (1H, dd, ½ ABq, J=14.60Hz, J=3.20Hz), 3.90 (1H, dd, J=7.30Hz, J=3.10Hz), 5.01 (1H, ddd, J=17.18Hz, J=2.23Hz, J=0.63Hz), 5.12 (1H, dd, J=10.10Hz, J=2.30Hz), 5.97 (1H, ddd, J=17.20Hz, J=10.05Hz, J=10.05Hz), 9.84 (1H, t, J=3.10Hz). ^{13}C NMR (75MHz) ($CDCl_3$) δ TMS: -4.64, -4.33, 17.28, 18.34, 22.72, 26.12 (3C), 33.69, 37.11, 38.06, 55.21, 56.32, 71.99, 117.76, 137.98, 203.93.



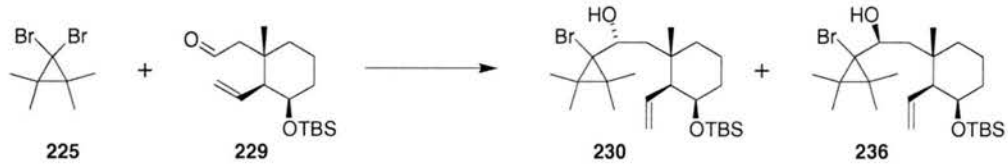
229



^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx839f5-8



^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx839f5-8apt



225 229 230 236

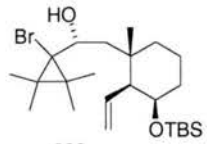
t-BuLi (1.7M in pentane, 2.33 mL, 3.96 mmol) added via syringe to a -105°C solution of 1,1-dibromo-2,2,3,3-tetramethylcyclopropane (531.7 mg, 2.08 mmol) in 10 mL THF. Stirred 10 min., then solution of aldehyde **229** in 7 mL THF added dropwise via addition funnel over 10 min. with 1 mL THF rinse. Allowed to warm slowly to room temp. overnight, then quenched with 3 mL sat. NH_4Cl sol., diluted with 25 mL Et_2O and 1 mL water, separated, and aqueous extracted twice with 6 mL Et_2O. Combined organics washed with 2 mL brine, dried with $MgSO_4$, and concentrated *in vacuo*. Flash chromatography (10 Hex:1 EtOAc, 45 g silica) yields 94.7 mg first diastereomer and 354.0 mg second diastereomer as colorless oils (96% yield).

1-(*R*)-(1-Bromo-2,2,3,3-tetramethyl-cyclopropyl)-2-[3-(*R*)-(tert-butyl-dimethyl-silanyloxy)-1-(*S*)-methyl-2-(*S*)-vinyl-cyclohexyl]-ethanol 230 (MAJOR).

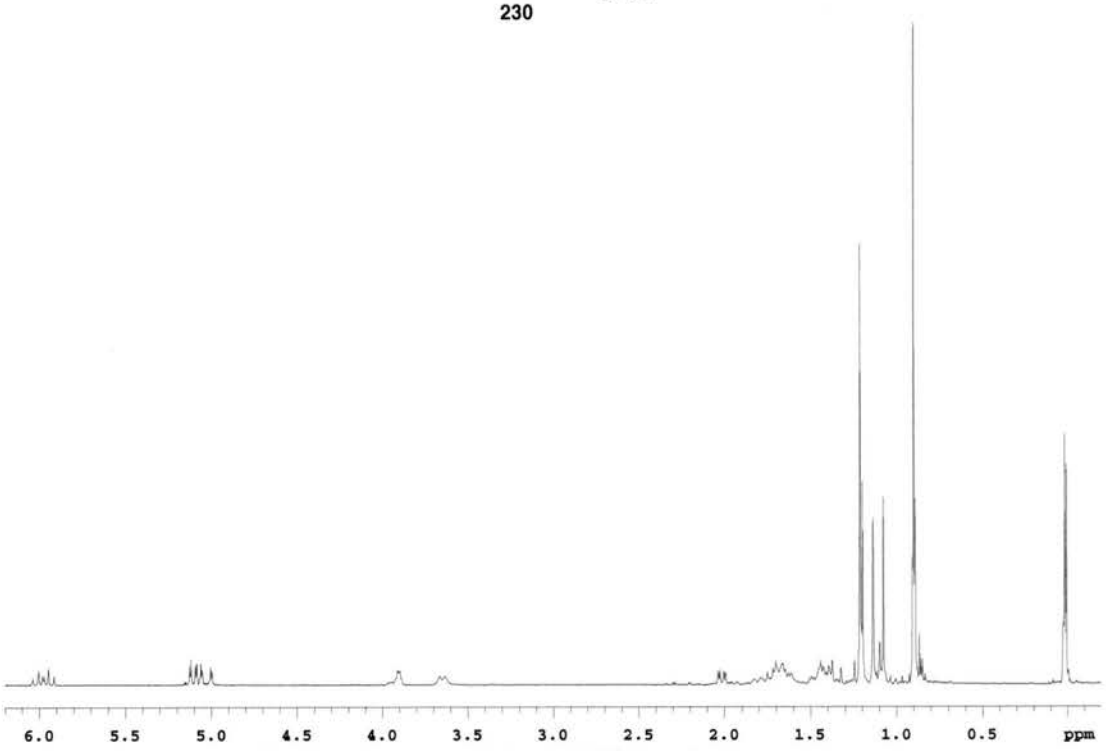
^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 0.01 (3H, s), 0.02 (3H, s), 0.90 (9H, s), 1.08 (3H, s), 1.14 (3H, s), 1.20 (3H, s), 1.21 (1H, m), 1.21 (6H, s), 1.41 (3H, m), 1.71 (4H, m), 2.02 (1H, dd, J=9.90Hz, J=3.30Hz), 3.65 (1H, d, J=9.70Hz), 3.90 (1H, dd, J=7.00Hz, J=3.10Hz), 5.03 (1H, ddd, J=17.18Hz, J=2.43Hz, J=0.58Hz), 5.11 (1H, dd, J=10.30Hz, J=2.50Hz), 5.98 (1H, ddd, J=17.00Hz, J=10.10Hz, J=10.10Hz). ^{13}C NMR (75MHz) ($CDCl_3$) δ TMS: -4.57, -4.25, 17.62, 17.88, 18.53, 22.86, 23.31, 23.40, 26.17 (3C), 26.71, 34.02, 36.35, 38.23, 43.36, 50.26, 55.25, 63.43, 70.37, 71.73, 72.92, 116.83, 139.82.

1-(*S*)-(1-Bromo-2,2,3,3-tetramethyl-cyclopropyl)-2-[3-(*R*)-(tert-butyl-dimethyl-silanyloxy)-1-(*S*)-methyl-2-(*S*)-vinyl-cyclohexyl]-ethanol 236 (MINOR).

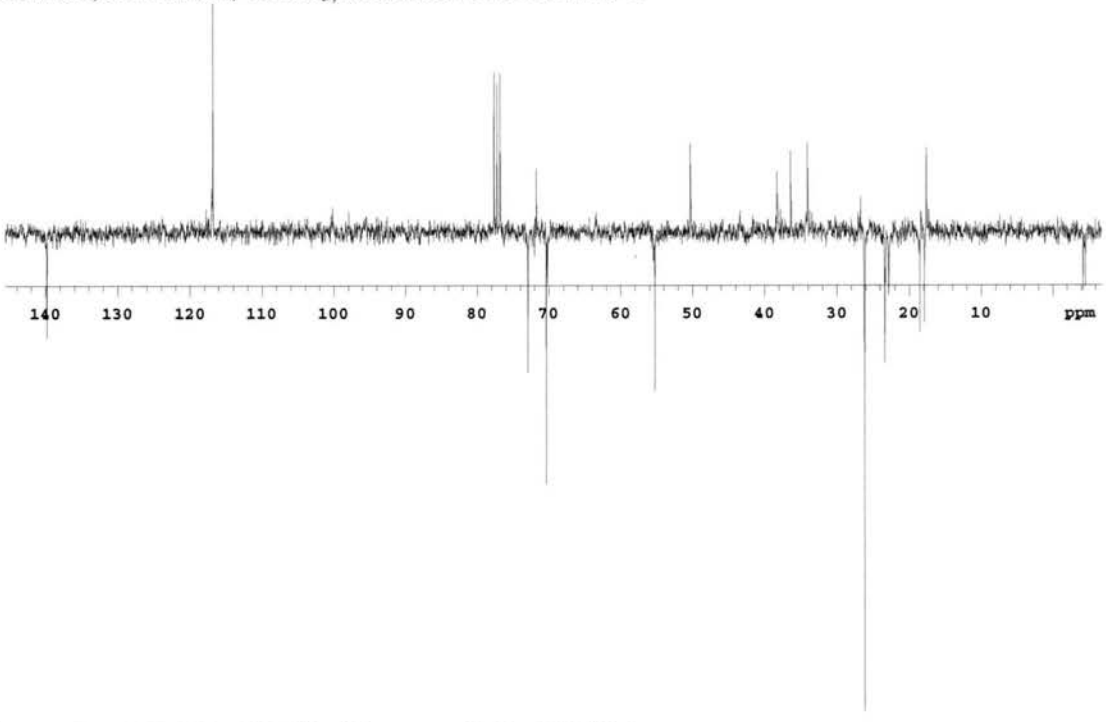
^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 0.02 (3H, s), 0.03 (3H, s), 0.90 (9H, s), 1.10 (3H, s), 1.14 (3H, s), 1.21 (3H, s), 1.21 (1H, m), 1.22 (6H, s), 1.41 (3H, m), 1.65 (4H, m), 2.02 (1H, dd, J=15.20Hz, J=9.85Hz), 3.65 (1H, d, J=10.20Hz), 3.95 (1H, ddd, J=6.60Hz, J=3.13Hz, J=3.13Hz), 5.04 (1H, ddd, J=17.10Hz, J=2.45Hz, J=0.65Hz), 5.11 (1H, dd, J=10.20Hz, J=2.60Hz), 5.98 (1H, ddd, J=17.00Hz, J=10.08Hz, J=10.08Hz).



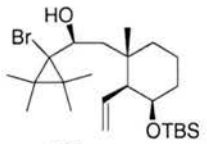
230



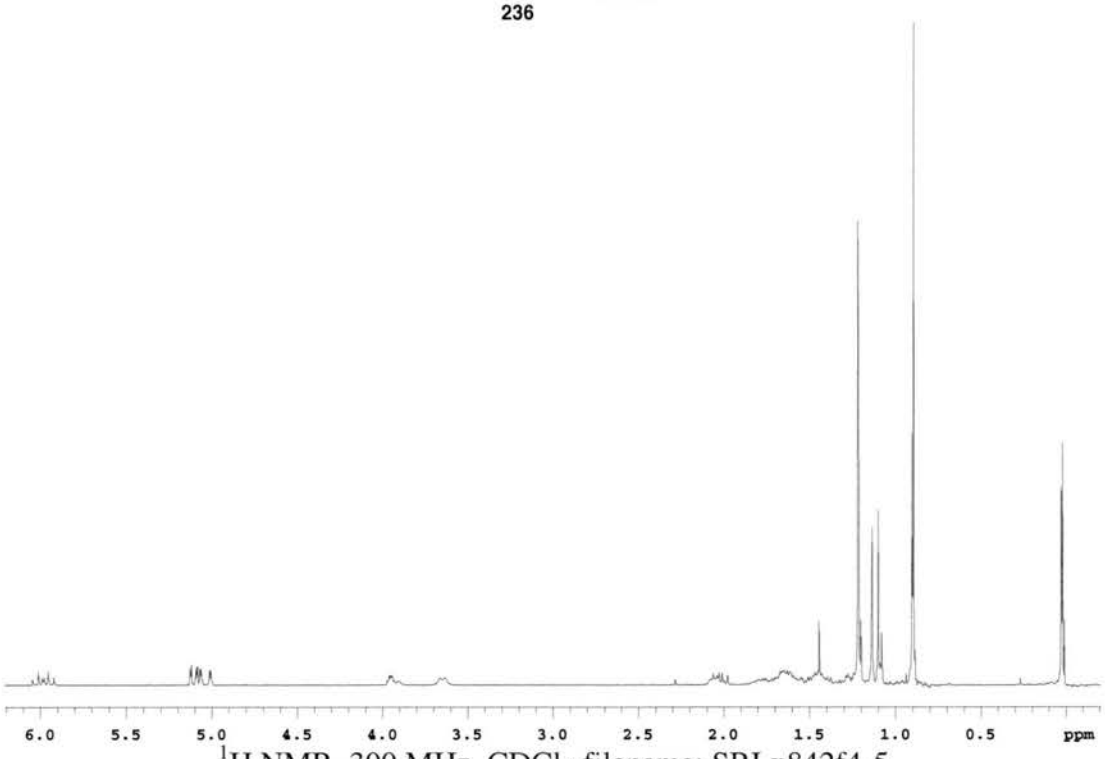
^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx842f6-8



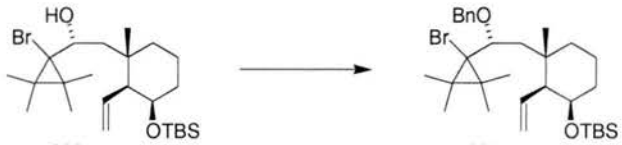
^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx842f6-8apt



236



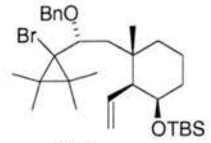
^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx842f4-5



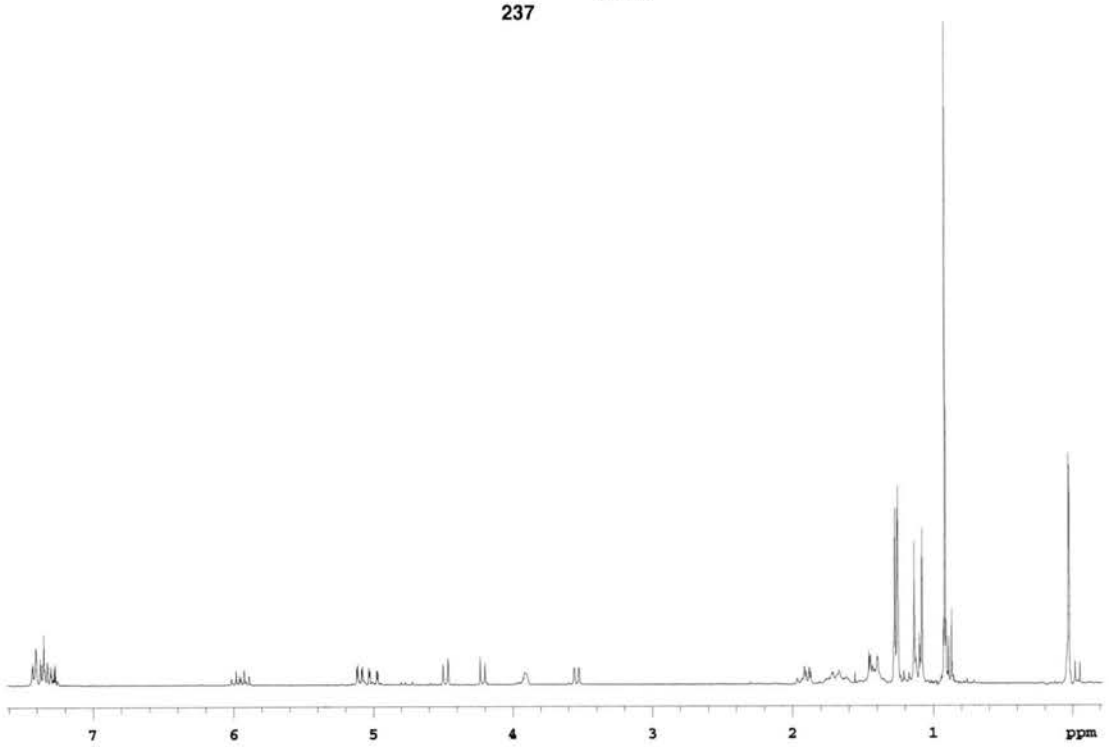
236 237

{1-(*R*)-3-[2-(*R*)-Benzyloxy-2-(1-bromo-2,2,3,3-tetramethyl-cyclopropyl)-ethyl]-3-(*S*)-methyl-2-(*S*)-vinyl-cyclohexyloxy}-tert-butyl-dimethyl-silane #.

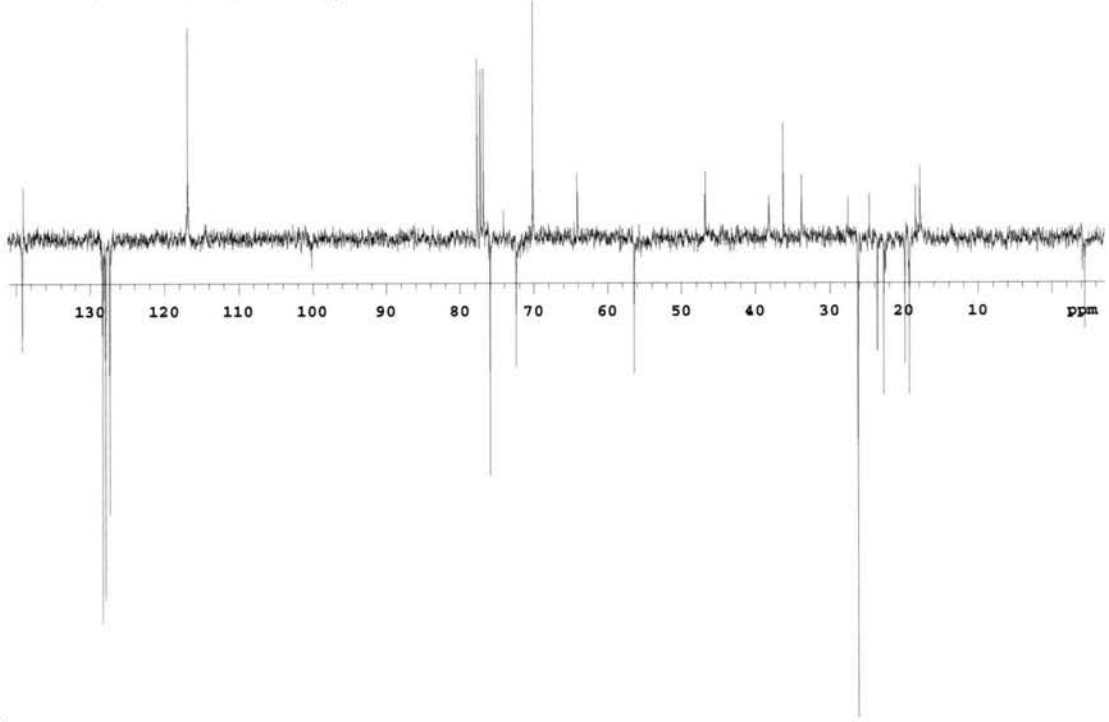
NaH (74 mg of 60% suspension, 1.84 mmol) added to a 0°C sol. of alcohol **236** (290.9 mg, 0.614 mmol) in 5 mL DMF. Bath removed, stirred at room temp. 30 min., then benzyl bromide (292 μL, 2.46 mmol) added via syringe. Stirred overnight, diluted with 20 mL Et_2O, quenched with 3 mL sat. NH_4Cl sol., separated, and aqueous extracted twice with 6 mL Et_2O. Combined organics washed twice with 2 mL water, once with 2 mL brine, dried with $MgSO_4$, and concentrated *in vacuo*. Purified by flash chromatography (100% Hex → 40 Hex:1 Et_2O, 40 g silica) to yield 303.0 mg colorless oil (88% yield). ^1H NMR (300MHz) ($CDCl_3$) δ TMS: 0.02 (3H, s), 0.03 (3H, s), 0.92 (9H, s), 1.08 (3H, s), 1.14 (3H, s), 1.25 (3H, s), 1.26 (3H, s), 1.28 (3H, s), 1.43 (5H, m), 1.69 (3H, m), 1.89 (1H, dd, J=9.90Hz, J=3.40Hz), 3.54 (1H, dd, J=9.90Hz, J=0.90Hz), 3.91 (1H, m), 4.22 (1H, ½ ABq, J=10.20Hz), 4.48 (1H, ½ ABq, J=10.50Hz), 5.00 (1H, dd, J=15.60Hz, J=2.55Hz), 5.10 (1H, dd, J=10.25Hz, J=2.55Hz), 5.95 (1H, ddd, J=17.20Hz, J=10.15Hz, J=10.15Hz), 7.35 (5H, m). ^{13}C NMR (75MHz) ($CDCl_3$) δ TMS: -4.55, -4.24, 17.84, 18.42, 19.29, 19.87, 22.49, 22.73, 23.57, 24.67, 26.19 (3C), 27.53, 33.76, 36.22, 38.15, 46.76, 56.42, 64.08, 70.13, 72.39, 74.09, 75.93, 116.90, 127.37, 127.93, 128.32, 139.04, 139.17.



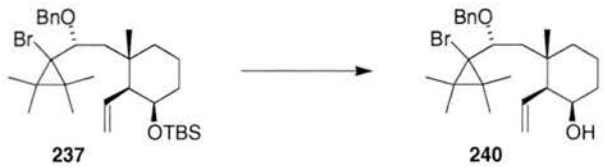
237



^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx854f31

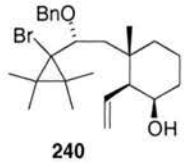


^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx854apt

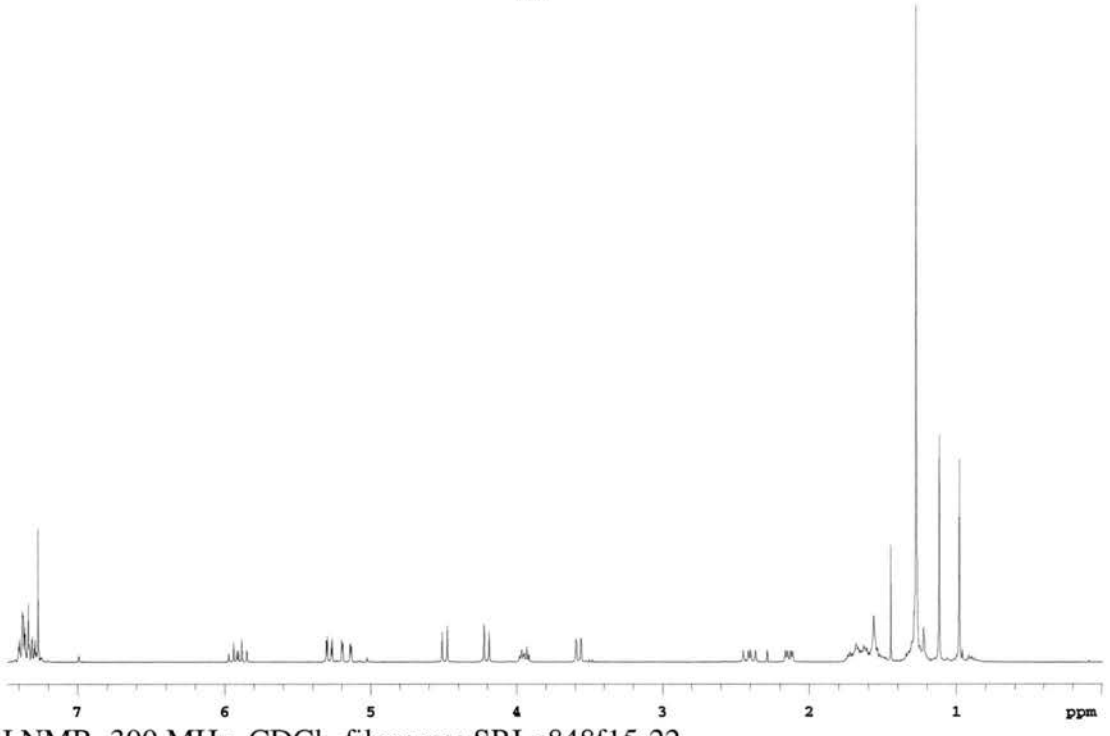


3-[2-(*R*)-Benzyloxy-2-(1-bromo-2,2,3,3-tetramethyl-cyclopropyl)-ethyl]-3-(*S*)-methyl-2-(*S*)-vinyl-1-(*R*)-cyclohexanol 240.

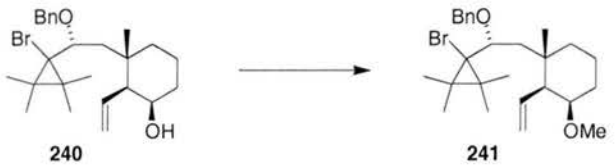
5% HF in CH_3CN (1 mL) added to TBS ether **237** (83.3 mg, 0.148 mmol) at room temp. Stirred 30 min., then diluted with 10 mL Et_2O, quenched slowly with 2.5 mL $NaHCO_3$ sol., stirred 30 min., separated, and aqueous extracted twice with 5 mL Et_2O. Combined organics washed with 1 mL brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purified by flash chromatography (3 Hex:1 Et_2O, 6 g silica) to yield 54.0 mg colorless oil (81% yield). ^1H NMR (300MHz) ($CDCl_3$) δ TMS: 0.98 (3H, s), 1.12 (3H, s), 1.28 (9H, s), 1.29 (2H, m), 1.45 (1H, s), 1.62 (5H, m), 2.14 (1H, dd, J=10.25Hz, J=4.55Hz), 2.41 (1H, dd, J=15.00Hz, J=10.10Hz), 3.57 (1H, dd, J=9.90Hz, J=1.10Hz), 3.95 (1H, ddd, J=10.90Hz, J=4.40Hz, J=4.40Hz), 4.20 (1H, ½ ABq, J=10.40Hz), 4.49 (1H, ½ ABq, J=10.40Hz), 5.17 (1H, ddd, J=17.03Hz, J=2.38Hz, J=0.58Hz), 5.28 (1H, dd, J=10.30Hz, J=2.40Hz), 5.91 (1H, ddd, J=16.80Hz, J=10.15Hz, J=10.15Hz), 7.34 (5H, m).



240



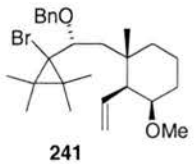
^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx848f15-22



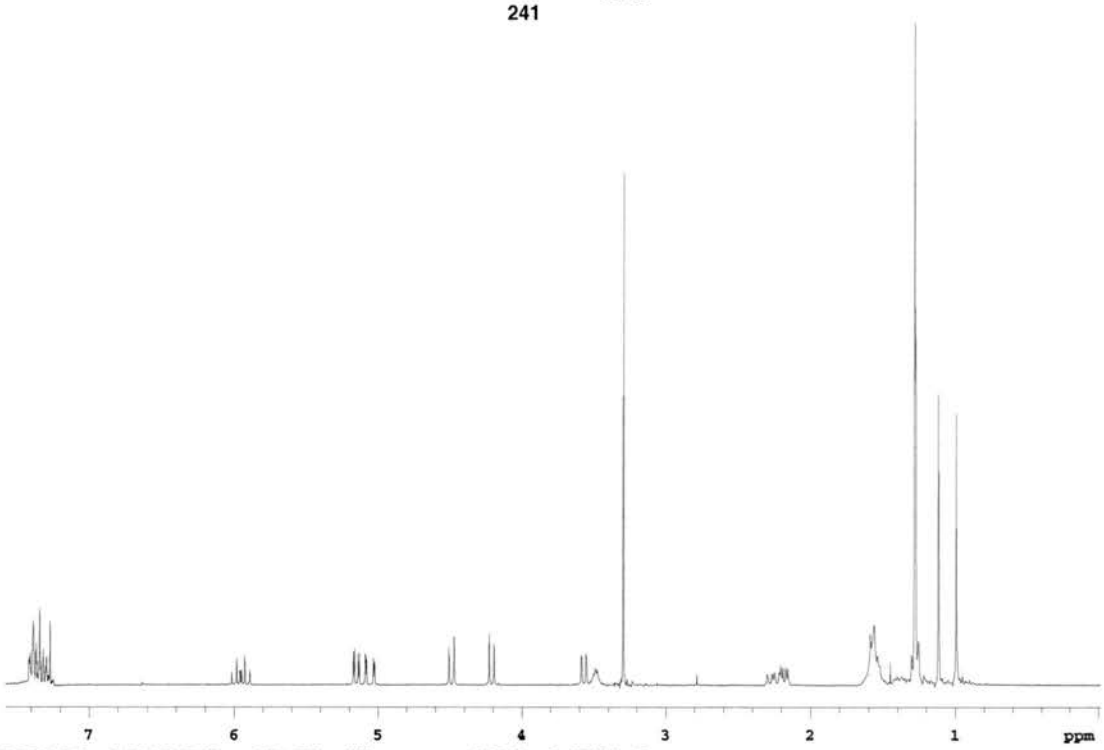
240 241

[1-(*R*)-(1-Bromo-2,2,3,3-tetramethyl-cyclopropyl)-2-(3-(*R*)-methoxy-1-(*S*)-methyl-2-(*S*)-vinyl-cyclohexyl)-ethoxymethyl]-benzene 241.

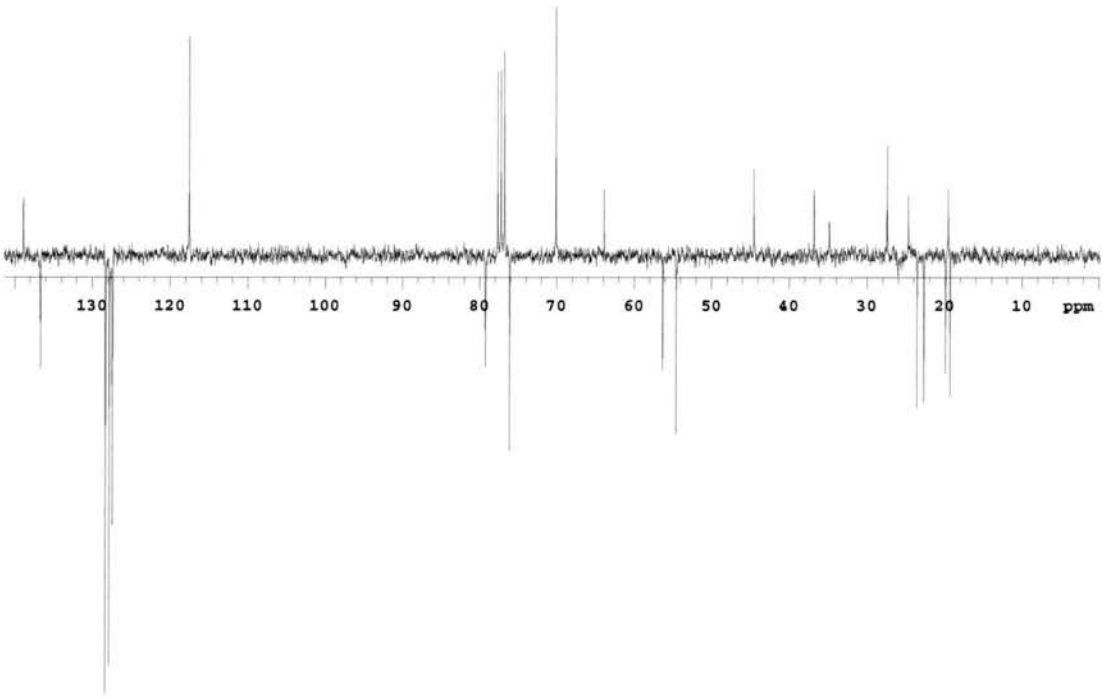
NaH (11.3 mg of 60% suspension, 0.282 mmol) added to a 0°C sol. of alcohol **240** (31.7 mg, 0.071 mmol) in 2 mL THF. Bath removed, stirred at room temp. 15 min., then iodomethane (44 μL, 0.706 mmol) added via syringe. Stirred overnight, diluted with 10 mL Et_2O, quenched with 2 mL water, separated, and aqueous extracted twice with 3 mL Et_2O. Combined organics dried with $MgSO_4$, and concentrated *in vacuo*. Purified by flash chromatography (5 Hex:1 Et_2O, 3 g silica) to yield 30.9 mg colorless oil (94% yield). ^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 0.99 (3H, s), 1.12 (3H, s), 1.27 (2H, m), 1.28 (9H, s), 1.56 (5H, m), 2.18 (1H, dd, J=10.20Hz, J=4.30Hz), 2.25 (1H, ddd, J=14.95Hz, J=9.80Hz, J=0.35Hz), 3.30 (3H, s), 3.49 (1H, m), 3.57 (1H, dd, J=9.70Hz, J=0.90Hz), 4.21 (1H, ½ ABq, J=10.20Hz), 4.49 (1H, ½ ABq, J=10.20Hz), 5.05 (1H, ddd, J=17.03Hz, J=2.38Hz, J=0.58Hz), 5.15 (1H, dd, J=10.25Hz, J=2.35Hz), 5.95 (1H, ddd, J=16.80Hz, J=10.15Hz, J=10.15Hz), 7.34 (5H, m). ^{13}C NMR (75MHz) ($CDCl_3$) δ TMS: 19.31, 19.59, 19.90, 22.69, 23.57 (2C), 24.70, 27.42, 34.82, 36.81, 44.51, 54.54, 56.30, 63.89, 70.11, 76.10, 79.24, 117.54, 127.40, 127.81, 128.31, 136.66, 138.87.



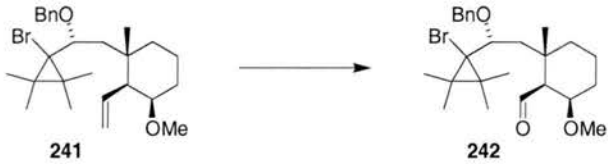
241



^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx865f4-6

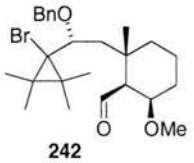


^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx865apt

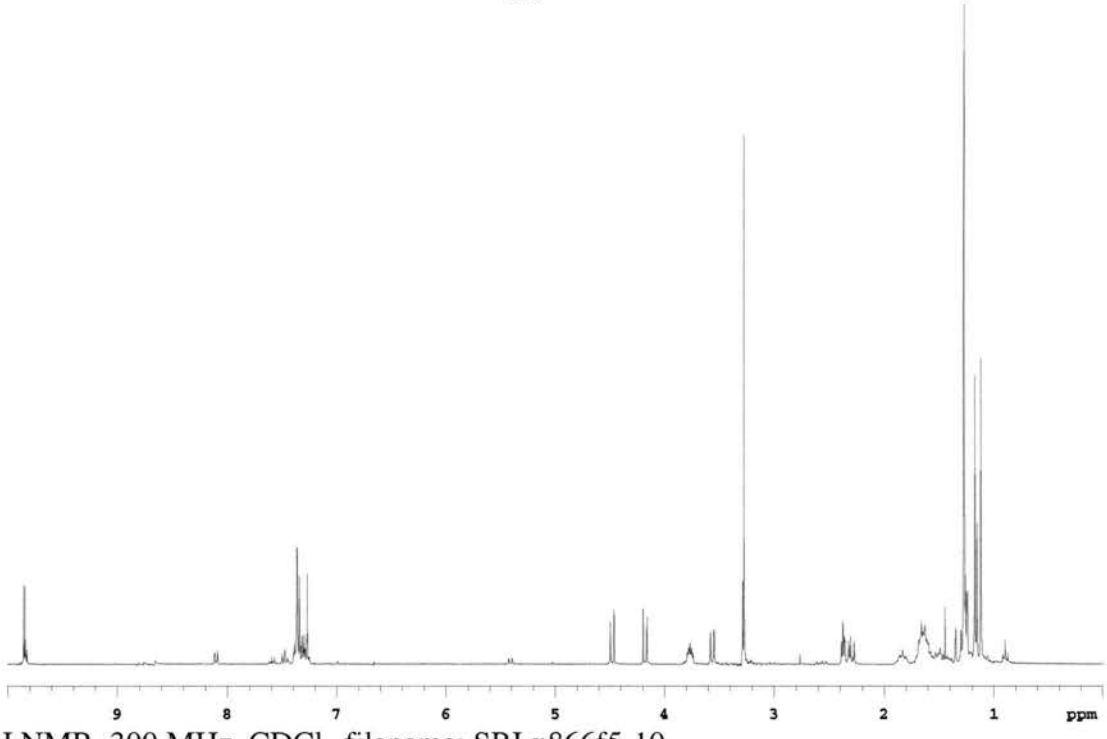


2-[2-(*R*)-Benzyloxy-2-(1-bromo-2,2,3,3-tetramethyl-cyclopropyl)-ethyl]-6-(*R*)-methoxy-2-(*S*)-methyl-1-(*S*)-cyclohexanecarbaldehyde 242.

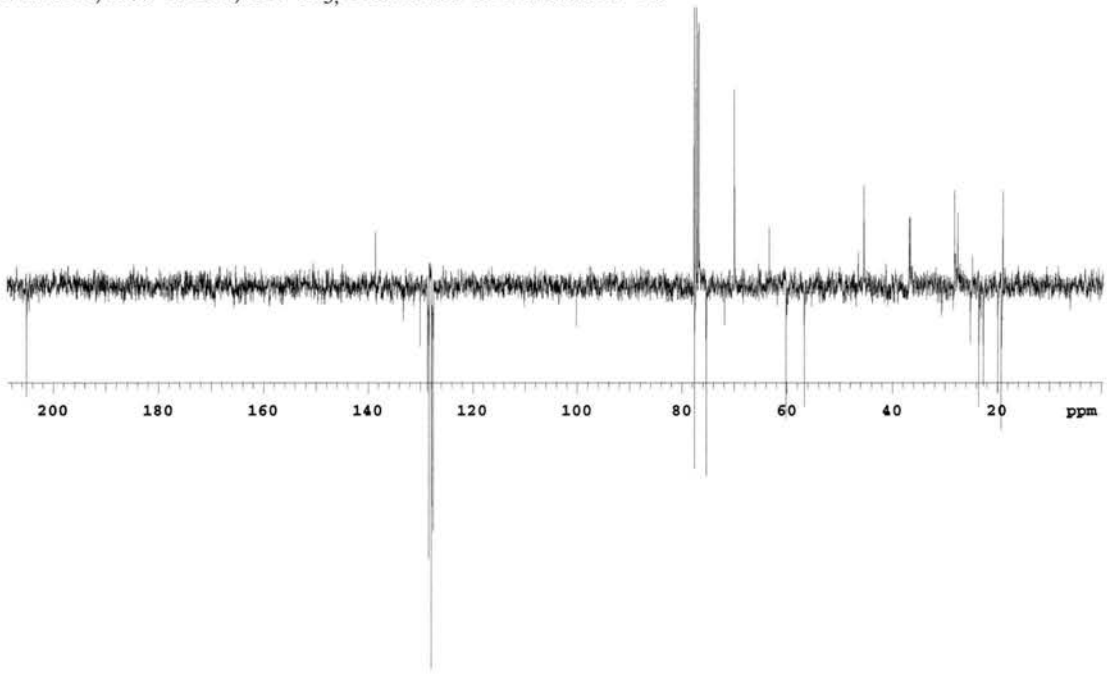
Ozone bubbled for 2 min. through a -78°C solution of alkene **241** (25.8 mg, 0.056 mmol) in 6 mL hexanes. Resulting light blue sol. stirred 20 min., then tributylphophine (41.6 μL, 0.167 mmol) added via syringe. Reaction allowed to warm slowly to room temp overnight, then concentrated *in vacuo*. Purified by flash chromatography (5 Hex:1 Et_2O, 3 g silica) to yield 22.5 mg colorless oil (87% yield). ^1H NMR (300MHz) ($CDCl_3$) δ TMS: 1.12 (3H, s), 1.18 (3H, s), 1.27 (2H, m), 1.28 (9H, s), 1.64 (4H, m), 1.83 (1H, m), 2.31 (1H, dd, J=15.00Hz, J=9.80Hz), 2.37 (1H, dd, J=4.20Hz, J=4.20Hz), 3.27 (3H, s), 3.56 (1H, dd, J=9.70Hz, J=0.90Hz), 3.77 (1H, m), 4.18 (1H, ½ ABq, J=10.20Hz), 4.48 (1H, ½ ABq, J=10.30Hz), 7.35 (5H, m), 9.85 (1H, d, J=3.90Hz). ^{13}C NMR (75MHz) ($CDCl_3$) δ TMS: 18.88, 19.20, 19.89, 22.64, 23.52, 25.11, 27.55, 28.15, 36.55, 36.74, 45.39, 56.64, 60.08, 63.32, 69.94, 75.31, 77.55, 127.52, 127.86, 128.38, 138.57, 205.07.



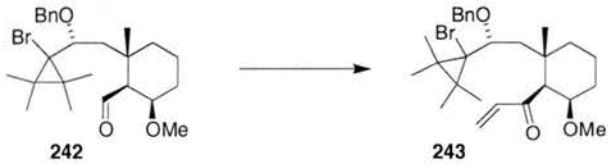
242



^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx866f5-10

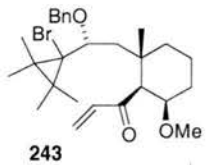


^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx866apt

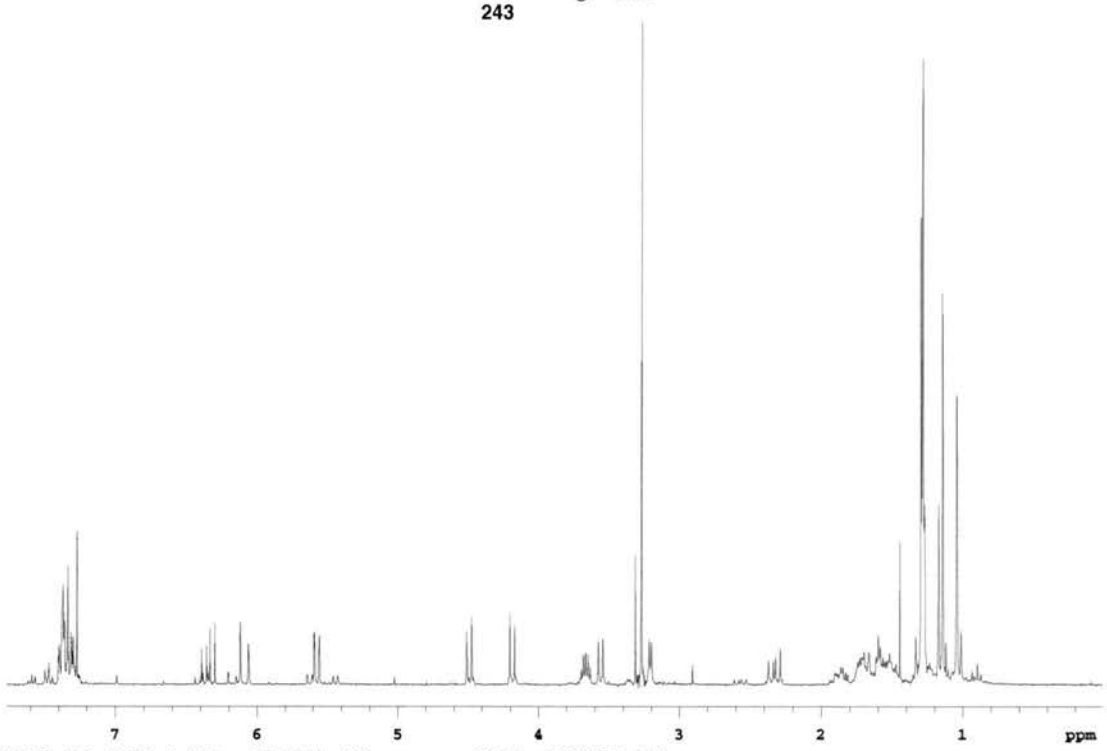


1-{2-[2-(*R*)-Benzyloxy-2-(1-bromo-2,2,3,3-tetramethyl-cyclopropyl)-ethyl]-6-(*R*)-methoxy-2-(*S*)-methyl-1-(*S*)-cyclohexyl}-2-propenone 243.

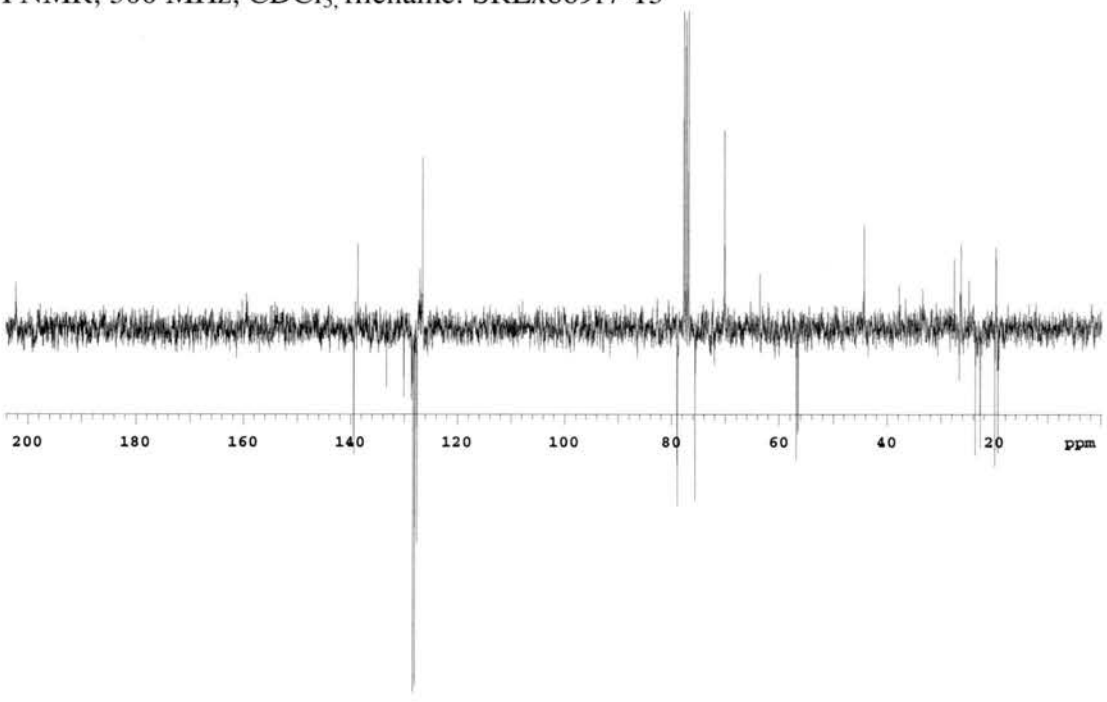
Vinyl magnesium bromide (1.0 M in THF, 145 μL, 0.145 mmol) added via syringe to a -78°C sol. of aldehyde **242** (22.5 mg, 0.048 mmol) in 4 mL THF. Stirred 90 min., then quenched with 2 mL ½ sat. NH_4Cl sol., diluted with 10 mL Et_2O, separated, and aqueous extracted twice with 3 mL Et_2O. Combined organics dried with $MgSO_4$ and concentrated *in vacuo* to yield 26.1 mg pale residue. This residue dissolved in 2 mL CH_2Cl_2 at room temp. and Dess-Martin periodinane (62 mg, 0.146 mmol) added. Stirred 30 min., then quenched with 1 mL sat. $Na_2S_2O_3$ sol. and 1 mL sat. $NaHCO_3$ sol., stirred overnight, separated, and aqueous extracted twice with 3 mL CH_2Cl_2. Combined organics dried with $MgSO_4$ and concentrated *in vacuo*. Purified by flash chromatography (5 Hex:1 Et_2O, 3 g silica) to yield 21.6 mg colorless oil (91% yield). ^1H NMR (300MHz) ($CDCl_3$) δ TMS: 1.04 (3H, s), 1.14 (3H, s), 1.27 (2H, m), 1.28 (3H, s), 1.29 (3H, s), 1.30 (3H, s), 1.55 (2H, m), 1.70 (2H, m), 1.86 (1H, m), 2.33 (1H, dd, J=14.95Hz, J=9.85Hz), 3.21 (1H, d, J=4.60Hz), 3.27 (3H, s), 3.56 (1H, dd, J=9.50Hz, J=0.70Hz), 3.67 (1H, ddd, J=10.00Hz, J=4.90Hz, J=4.90Hz), 4.19 (1H, ½ ABq, J=10.00Hz), 4.49 (1H, ½ ABq, J=10.30Hz), 5.58 (1H, dd, J=10.45Hz, J=1.25Hz), 6.09 (1H, dd, J=17.40Hz, J=1.50Hz), 6.34 (1H, dd, J=17.40Hz, J=10.40Hz), 7.35 (5H, m). ^{13}C NMR (75MHz) ($CDCl_3$) δ TMS: 19.39, 19.76, 19.93, 22.66, 23.57, 24.76, 26.30, 26.57, 27.55, 37.74, 44.25, 56.47, 56.77, 63.56, 70.13, 75.61, 78.90, 126.54, 127.52, 127.93, 128.34, 138.65, 139.37, 202.28.



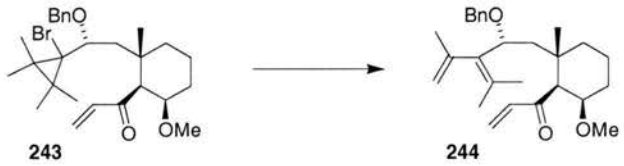
243



^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx869f7-15

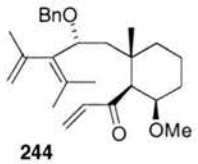


^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx869apt

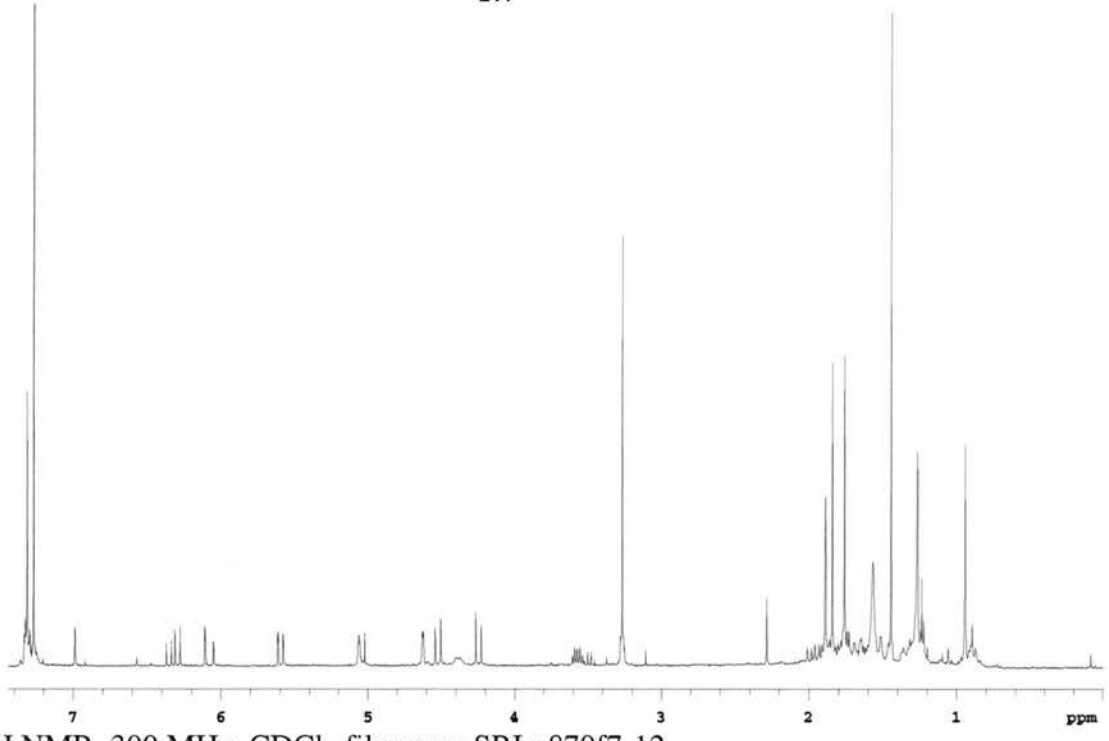


1-[2-(2-(*R*)-Benzyloxy-3-isopropenyl-4-methyl-pent-3-enyl)-6-(*R*)-methoxy-2-(*S*)-methyl-(*S*)-cyclohexyl]-2-propenone 244.

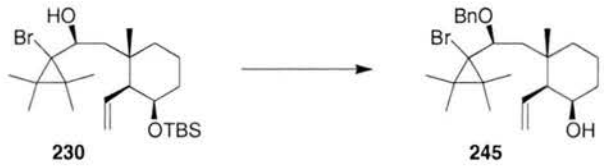
Bromocyclopropane **243** (6.6 mg, 0.013 mmol) and 2,4,6-collidine (35.5 μL, 0.27 mmol) dissolved in 2 mL DMSO and heated to 115°C in sand bath. Stirred 12 hours, cooled to room temp, diluted with 20 mL Et_2O, 2 mL brine, and 1 mL water, shaken vigorously, separated, and aqueous extracted twice with 10 mL 1 Hex:1 Et_2O. Combined organics washed with 1 mL brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purified by flash chromatography (7 Hex:1 Et_2O, 2.3 g silica) to yield 4.3 mg (78% yield). ^1H NMR (300MHz) ($CDCl_3$) δ TMS: 0.94 (3H, s), 1.26 (3H, m), 1.79 (5H, br. m), 1.76 (3H, s), 1.84 (3H, s), 1.89 (3H, dd, J=1.50Hz, J=0.90Hz), 2.28 (1H, s), 3.27 (3H, s), 3.28 (1H, m), 3.57 (1H, ddd, J=10.70Hz, J=5.08Hz, J=5.08Hz), 4.24 (1H, ½ ABq, J=11.40Hz), 4.38 (1H, m), 4.52 (1H, ½ ABq, J=11.30Hz), 4.63 (1H, dd, J=2.50Hz, J=0.90Hz), 5.06 (1H, dd, J=2.55Hz, J=1.45Hz), 5.59 (1H, dd, J=10.40Hz, J=1.30Hz), 6.08 (1H, dd, J=17.40Hz, J=1.30Hz), 6.32 (1H, dd, J=17.40Hz, J=10.40Hz), 7.31 (5H, m).



244



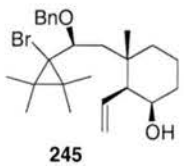
^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx870f7-12



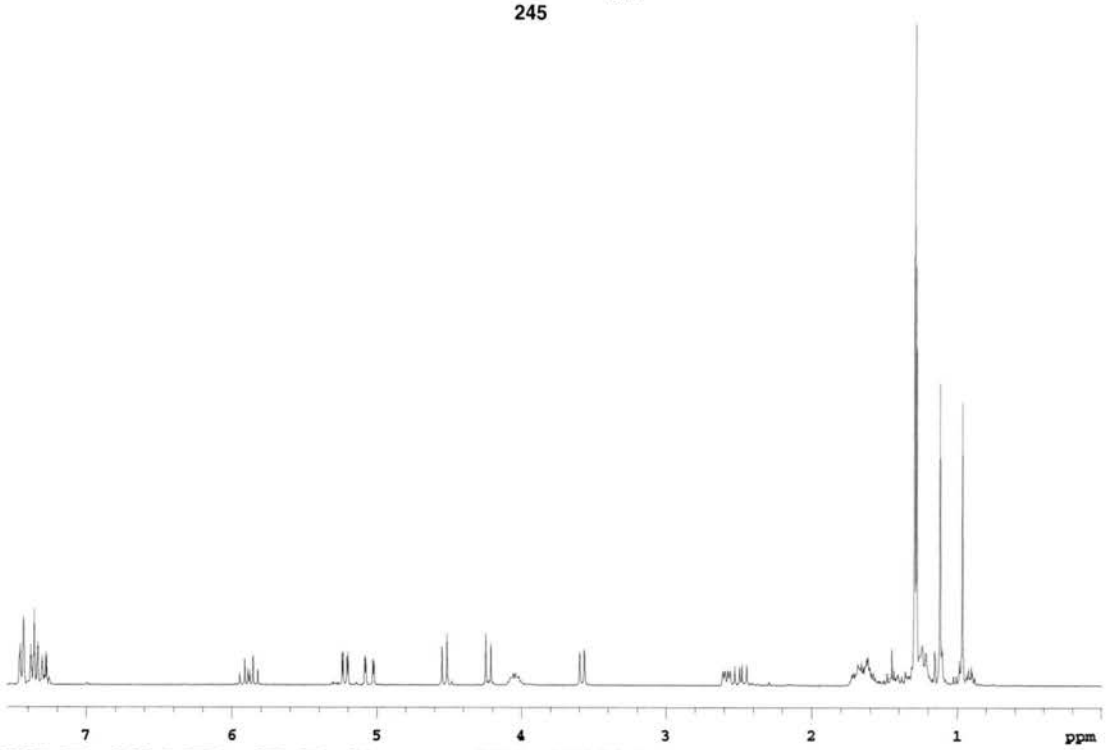
230 245

3-[2-(*S*)-Benzyloxy-2-(1-bromo-2,2,3,3-tetramethyl-cyclopropyl)-ethyl]-3-(*S*)-methyl-2-(*S*)-vinyl-1-(*R*)-cyclohexanol 245.

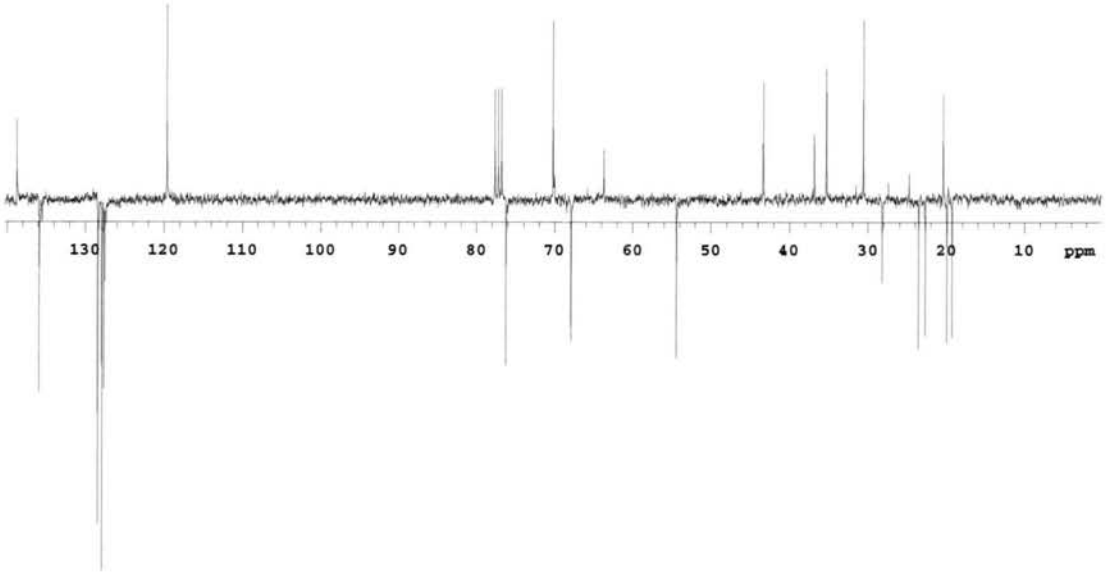
NaH (27.7 mg of 60% suspension, 0.693 mmol) added to a 0°C sol. of alcohol **230** (82.0 mg, 0.173 mmol) in 3 mL DMF. Bath removed, stirred at room temp. 30 min., then benzyl bromide (103 μL, 0.866 mmol) added via syringe. Stirred overnight, diluted with 20 mL Et_2O, quenched with 3 mL sat. NH_4Cl sol. and 1 mL water, separated, and aqueous extracted twice with 5 mL Et_2O. Combined organics washed twice with 2 mL water, once with 2 mL brine, dried with $MgSO_4$, and concentrated *in vacuo* to yield 177.9 mg pale oil. To this residue was added 5% HF in CH_3CN (1 mL) at room temp. Stirred 30 min., then diluted with 10 mL Et_2O, quenched slowly with 5 mL ½ sat. Na_2CO_3 sol., stirred 45 min., separated, and aqueous extracted twice with 5 mL Et_2O. Combined organics washed with 1 mL brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purified by flash chromatography (3 Hex:1 Et_2O, 6 g silica) to yield 46.1 mg colorless oil (59% yield). ^1H NMR (300MHz) ($CDCl_3$) δ TMS: 0.96 (3H, s), 1.12 (3H, s), 1.28 (3H, s), 1.28 (2H, m), 1.29 (6H, s), 1.45 (1H, s), 1.62 (5H, m), 2.48 (1H, dd, J=15.10Hz, J=9.80Hz), 2.58 (1H, dd, J=10.40Hz, J=4.60Hz), 3.58 (1H, dd, J=9.80Hz, J=1.00Hz), 4.05 (1H, m), 4.23 (1H, ½ ABq, J=10.20Hz), 4.53 (1H, ½ ABq, J=10.30Hz), 5.05 (1H, dd, J=17.05Hz, J=2.35Hz), 5.22 (1H, dd, J=10.05Hz, J=2.35Hz), 5.88 (1H, ddd, J=16.80Hz, J=10.28Hz, J=10.28Hz), 7.35 (5H, m). ^{13}C NMR (75MHz) ($CDCl_3$) δ TMS: 19.27, 19.94, 20.43, 22.68, 23.55, 24.76, 28.16, 30.56, 35.29, 36.80, 43.30, 54.35, 63.69, 67.87, 70.20, 76.23, 119.64, 127.56, 127.88, 128.40, 135.89, 138.76.



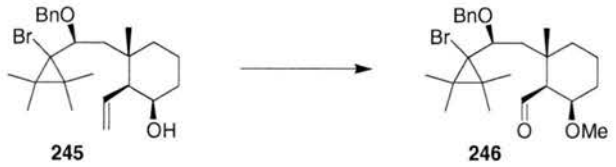
245



^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx878f6-11

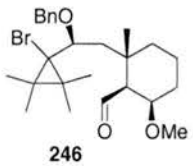


^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx878apt

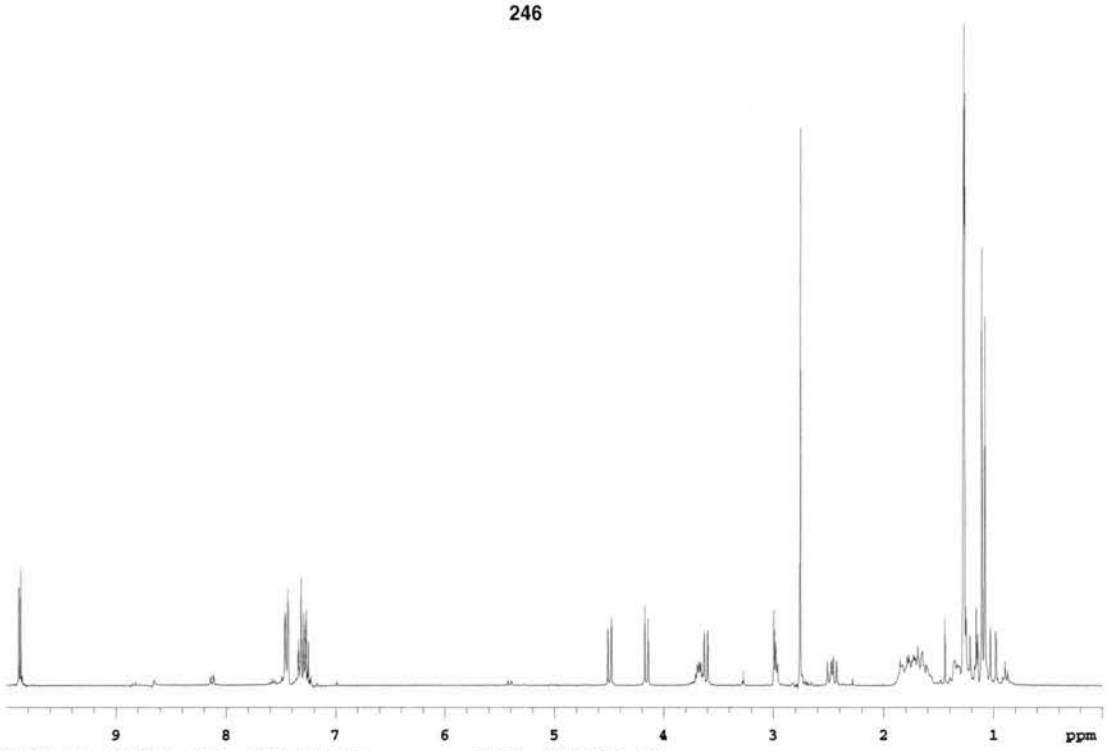


2-[2-(*S*)-Benzyloxy-2-(1-bromo-2,2,3,3-tetramethyl-cyclopropyl)-ethyl]-6-(*R*)-methoxy-2-(*S*)-methyl-1-(*S*)-cyclohexanecarbaldehyde 246.

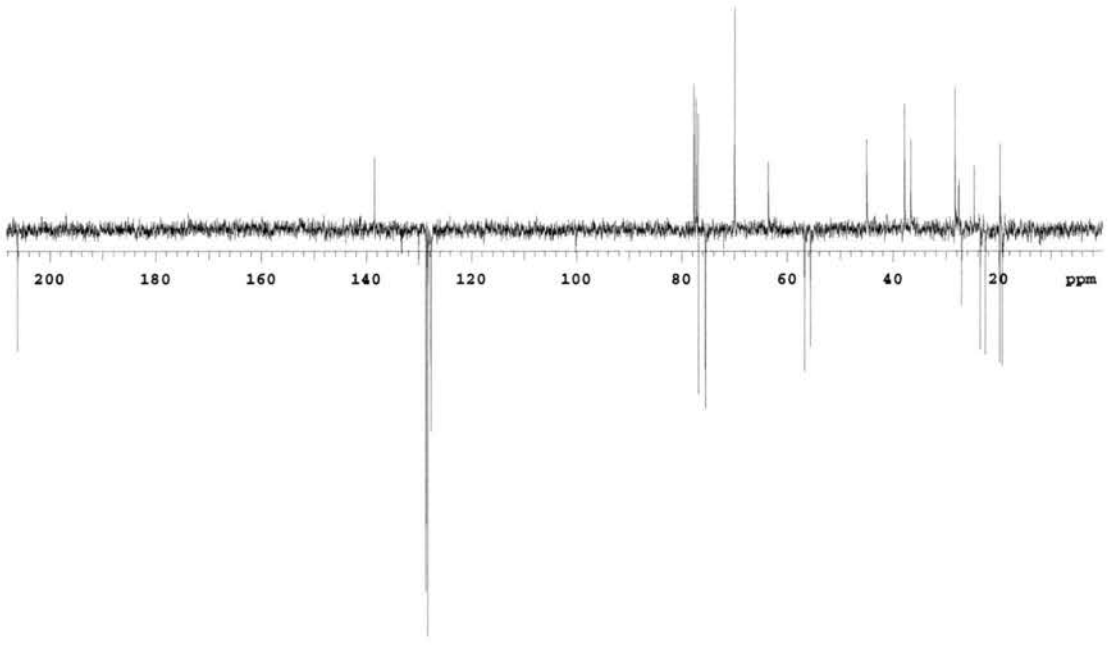
NaH (16.5 mg of 60% suspension, 0.410 mmol) added to a 0°C sol. of alcohol **245** (46.1 mg, 0.103 mmol) in 2 mL THF. Bath removed, stirred at room temp. 15 min., then iodomethane (64 μL, 1.03 mmol) added via syringe. Stirred overnight, diluted with 10 mL Et_2O, quenched with 2 mL water, separated, and aqueous extracted twice with 3 mL Et_2O. Combined organics dried with $MgSO_4$, and concentrated *in vacuo*. Residue dissolved in 8 mL hexanes, cooled to -78°C, and ozone bubbled through the solution for 3 min. Resulting light blue sol. stirred 25 min., then tributylphophine (77 μL, 0.309 mmol) added via syringe and bath removed. Reaction stirred 3.5 hours, then concentrated *in vacuo*. Purified by flash chromatography (5 Hex:1 Et_2O, 4.5 g silica) to yield 39.6 mg colorless oil (83% yield for 2 steps). ^1H NMR (300MHz) ($CDCl_3$) δ TMS: 1.08 (3H, s), 1.11 (3H, s), 1.27 (3H, s), 1.27 (2H, m), 1.28 (6H, s), 1.72 (5H, m), 2.47 (1H, dd, J=15.00Hz, J=9.50Hz), 2.76 (3H, s), 2.98 (1H, dd, J=4.90Hz, J=4.90Hz), 3.61 (1H, d, J=9.00Hz), 3.68 (1H, ddd, J=10.00Hz, J=5.00Hz, J=5.00Hz), 4.16 (1H, ½ ABq, J=9.30Hz), 4.49 (1H, ½ ABq, J=9.50Hz), 7.30 (3H, m), 7.45 (2H, m), 9.88 (1H, d, J=5.30Hz). ^{13}C NMR (75MHz) ($CDCl_3$) δ TMS: 19.31, 19.74, 19.81, 22.55, 23.53, 24.65, 27.05, 28.31, 36.68, 37.84, 44.98, 55.62, 56.73, 63.54, 69.94, 75.44, 76.78, 127.69, 128.30, 128.62, 138.46, 206.16.



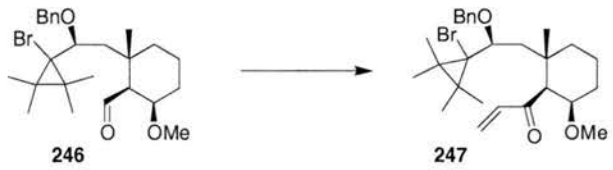
246



^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx880f5-12

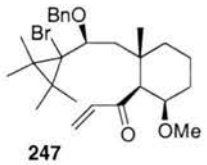


^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx880apt



1-{2-[2-(*S*)-Benzyloxy-2-(1-bromo-2,2,3,3-tetramethyl-cyclopropyl)-ethyl]-6-(*R*)-methoxy-2-(*S*)-methyl-1-(*S*)-cyclohexyl}-2-propenone 247.

Vinyl magnesium bromide (1.0 M in THF, 425 μL, 0.425 mmol) added via syringe to a -78°C sol. of aldehyde **246** (39.6 mg, 0.085 mmol) in 4 mL THF. Stirred 2.5 hours, then quenched with 3 mL ½ sat. NH_4Cl sol., diluted with 15 mL Et_2O, separated, and aqueous extracted twice with 3 mL Et_2O. Combined organics dried with $MgSO_4$ and concentrated *in vacuo*. . This residue dissolved in 6 mL CH_2Cl_2 at room temp. and Dess-Martin periodinane (144 mg, 0.340 mmol) added. Stirred 15 min., then quenched with 1.5 mL sat. $Na_2S_2O_3$ sol. and 1.5 mL sat. $NaHCO_3$ sol., stirred 5 min., diluted with 10 mL hexanes, separated, and aqueous extracted twice with 3 mL Et_2O. Combined organics washed with 1.5 mL brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purified by flash chromatography (5 Hex:1 Et_2O, 4.5 g silica) to yield 41.2 mg amorphous white solid (99% yield for 2 steps). ^1H NMR (300MHz) ($CDCl_3$) δ TMS: 0.93 (3H, s), 1.10 (3H, s), 1.27 (2H, m), 1.29 (6H, s), 1.30 (3H, s), 1.54 (1H, dddd, J=13.55Hz, J=13.55Hz, J=4.05Hz, J=4.05Hz), 1.74 (3H, m), 1.97 (1H, ddd, J=13.35Hz, J=13.35Hz, J=4.33Hz), 2.54 (1H, dd, J=15.10Hz, J=10.00Hz), 2.90 (3H, s), 3.21 (1H, d, J=4.60Hz), 3.64 (2H, m), 4.09 (1H, d, J=5.10Hz), 4.17 (1H, ½ ABq, J=9.50Hz), 4.61 (1H, ½ ABq, J=9.50Hz), 5.51 (1H, dd, J=10.50Hz, J=1.20Hz), 5.89 (1H, dd, J=17.45Hz, J=1.15Hz), 6.18 (1H, dd, J=17.50Hz, J=10.50Hz), 7.32 (3H, m), 7.49 (2H, m). ^{13}C NMR (75MHz) ($CDCl_3$) δ TMS: 19.18, 19.87, 20.01, 22.62, 23.49, 24.63, 27.53, 35.95, 37.16, 43.40, 50.67, 56.40, 63.47, 70.07, 76.04, 78.61, 126.56, 127.81, 128.36, 128.42, 138.63, 140.38, 203.98.



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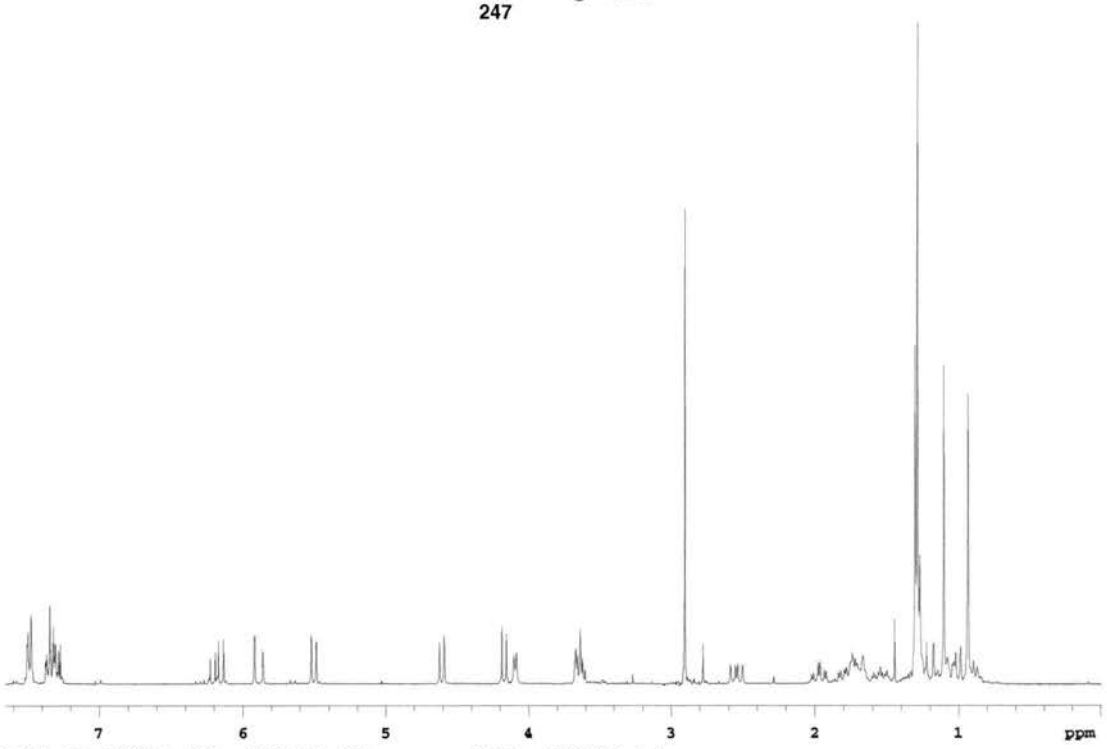
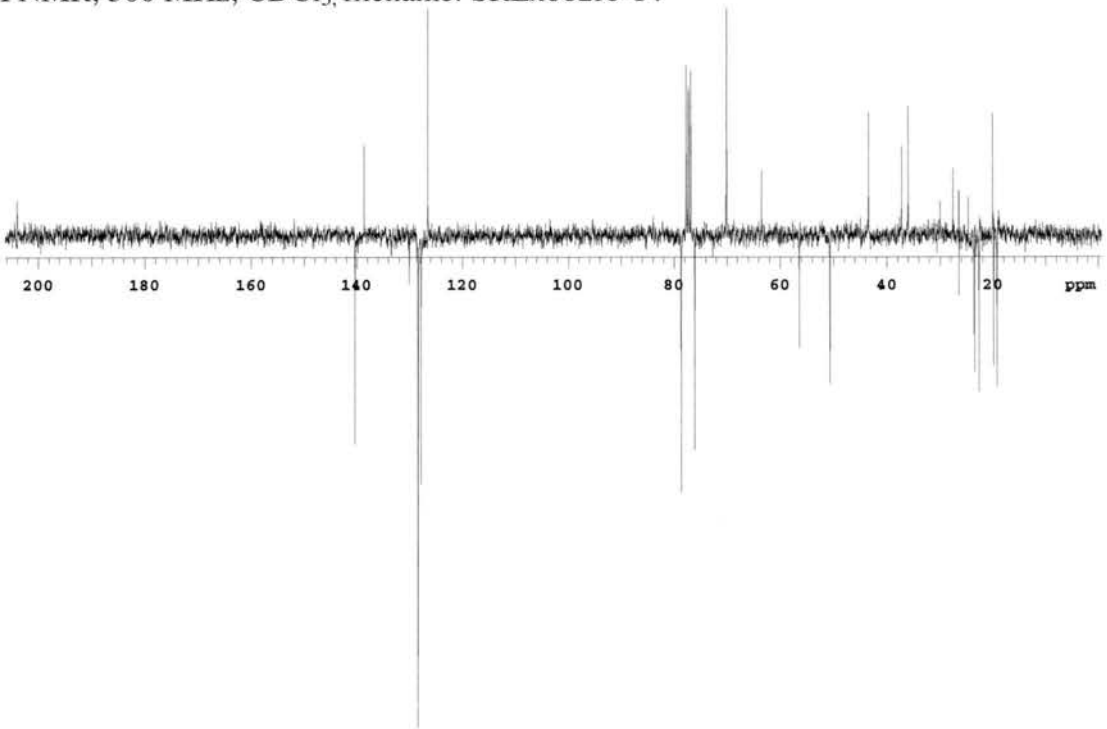
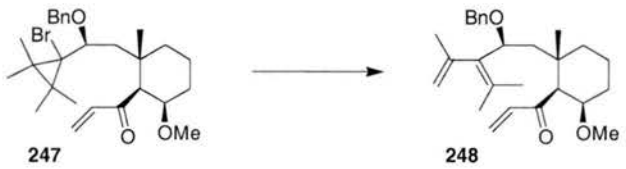


^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx882f6-14

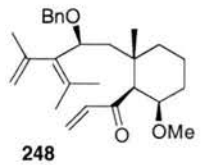


^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx882apt



1-[2-(2-(*S*)-Benzyloxy-3-isopropenyl-4-methyl-pent-3-enyl)-6-(*R*)-methoxy-2-(*S*)-methyl-(*S*)-cyclohexyl]-2-propenone 248.

Bromocyclopropane **247** (6.5 mg, 0.013 mmol) and 2,4,6-collidine (42 μL, 0.317 mmol) dissolved in 2 mL DMSO and heated to 115°C in sand bath. Stirred 12 hours, cooled to room temp, diluted with 20 mL Et_2O, 2 mL brine, and 1 mL water, shaken vigorously, separated, and aqueous extracted twice with 10 mL 1 Hex:1 Et_2O. Combined organics washed with 1 mL brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purified by flash chromatography (7 Hex:1 Et_2O, 4 g silica) to yield 4.4 mg (81% yield). ^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 0.90 (3H, s), 1.11 (2H, m), 1.57 (2H, s), 1.68 (1H, m), 1.76 (3H, s), 1.80 (2H, m), 1.85 (3H, s), 1.91 (3H, dd, J=1.40Hz, J=0.90Hz), 2.20 (1H, dd, J=15.20Hz, J=9.70Hz), 3.11 (3H, s), 3.67 (1H, ddd, J=10.80Hz, J=5.43Hz, J=5.43Hz), 3.84 (1H, d, J=5.10Hz), 4.26 (1H, ½ ABq, J=11.10Hz), 4.45 (1H, m), 4.60 (1H, ½ ABq, J=10.70Hz), 4.63 (1H, m), 5.07 (1H, dd, J=2.50Hz, J=1.40Hz), 5.50 (1H, dd, J=10.55Hz, J=1.15Hz), 5.99 (1H, dd, J=17.55Hz, J=1.25Hz), 6.25 (1H, dd, J=17.60Hz, J=10.40Hz), 7.35 (5H, m).



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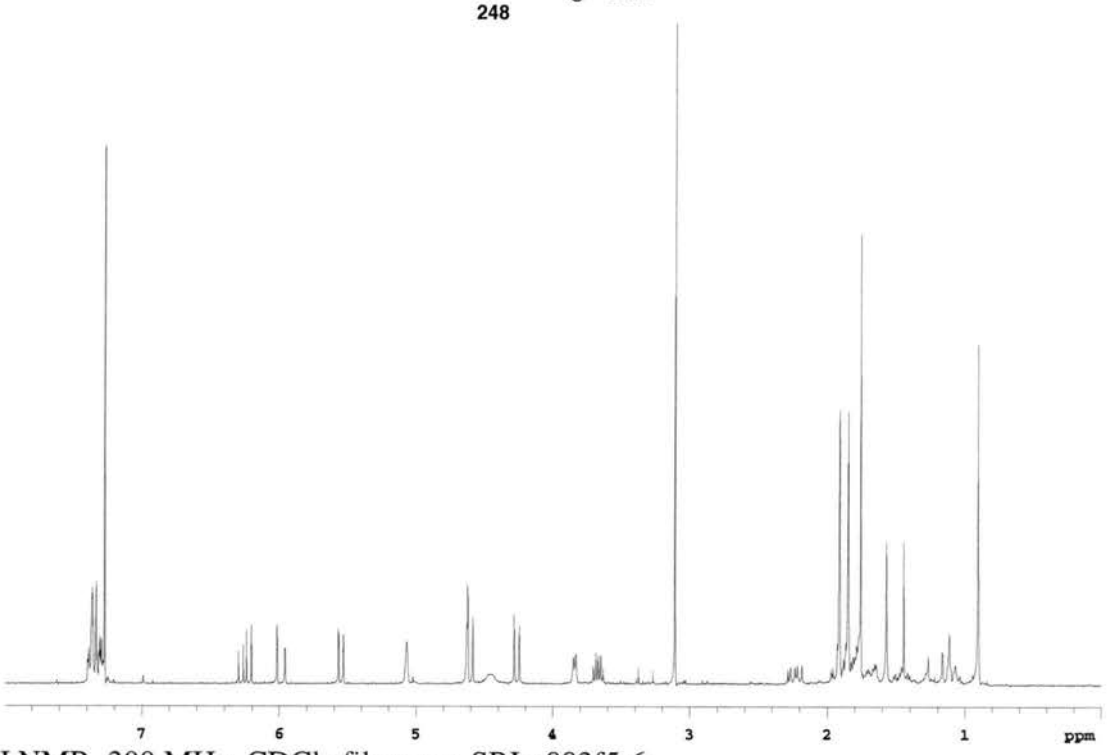
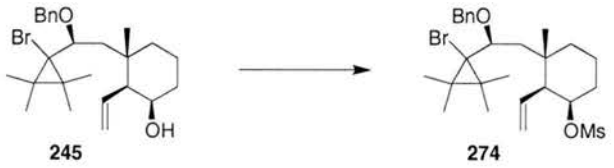
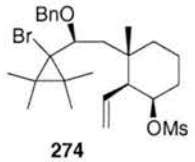


^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx883f5-6

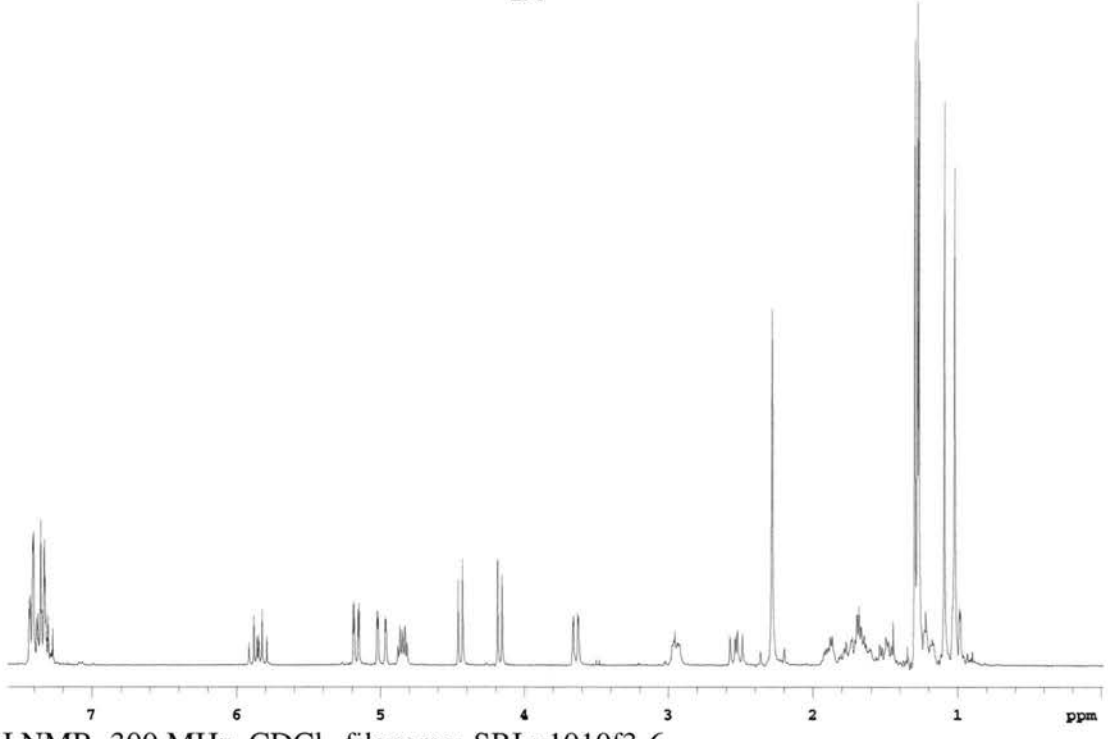


Methanesulfonic acid 3-[2-(*S*)-benzyloxy-2-(1-bromo-2,2,3,3-tetramethyl-cyclopropyl)-ethyl]-3-(*S*)-methyl-2-(*S*)-vinyl-(*R*)-cyclohexyl ester 274.

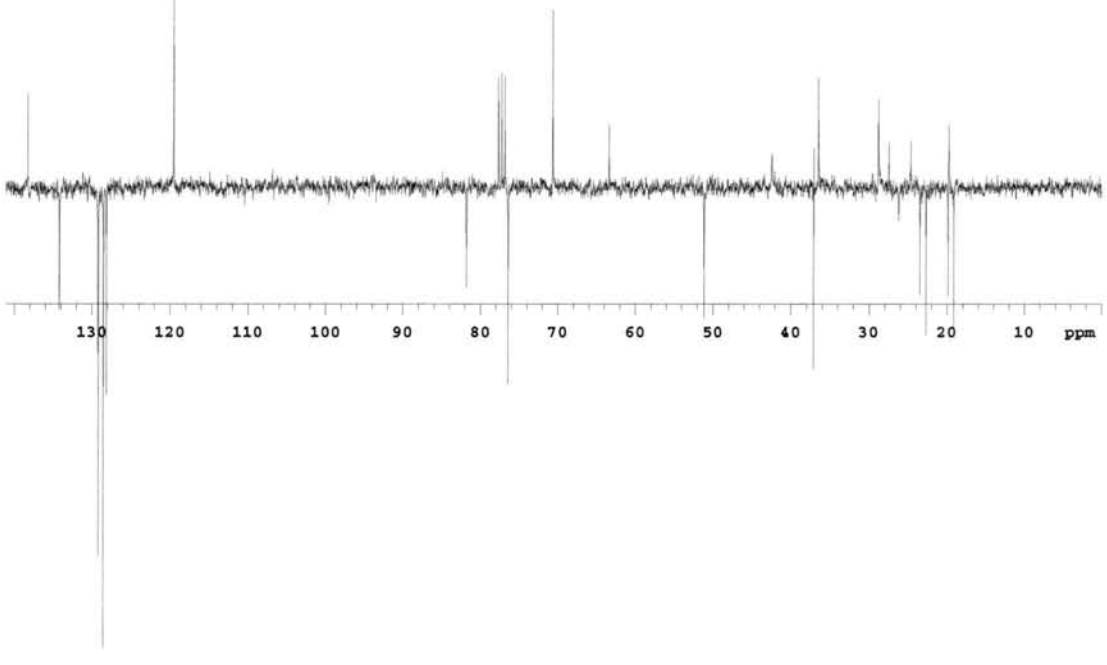
Methanesulfonyl chloride (31.1 μL, 0.402 mmol) added to a room temp. sol. of alcohol **245** (90.3 mg, 0.201 mmol) and triethylamine (78.4 μL, 0.563 mmol) in 3 mL CH_2Cl_2. Stirred 15 hours, then diluted with 7 mL Et_2O and 7 mL pentane. Washed with 3 mL ½ sat. Na_2CO_3 sol., then with 3 mL water, dried with $MgSO_4$, and concentrated *in vacuo*. Purified by flash chromatography (1 Hex:1 Et_2O, 3 g silica) to yield 94.9 mg white solid (90% yield). ^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 1.02 (3H, s), 1.10 (3H, s), 1.22 (2H, m), 1.27 (3H, s), 1.28 (3H, s), 1.30 (3H, s), 1.49 (1H, m), 1.70 (3H, m), 1.89 (1H, m), 2.28 (3H, s), 2.53 (1H, dd, J=15.20Hz, J=10.60Hz), 2.95 (1H, m), 3.64 (1H, dd, J=10.20Hz, J=1.85Hz), 4.17 (1H, ½ ABq, J=9.30Hz), 4.44 (1H, ½ ABq, J=9.40Hz), 4.85 (1H, ddd, J=10.10Hz, J=4.83Hz, J=4.83Hz), 4.99 (1H, dd, J=16.85Hz, J=1.83Hz), 5.17 (1H, dd, J=10.10Hz, J=2.15Hz), 5.85 (1H, ddd, J=16.80Hz, J=10.18Hz, J=10.18Hz), 7.35 (5H, m). ^{13}C NMR (75MHz) ($CDCl_3$) δ TMS: 19.14, 19.77, 19.83, 22.69, 23.46, 24.61, 26.19, 27.44, 28.77, 36.46, 37.07, 42.41, 51.13, 63.37, 70.66, 76.41, 81.79, 119.55, 128.12, 128.53, 129.18, 134.19, 138.22.



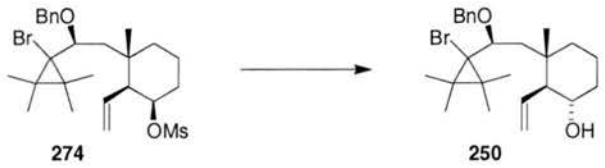
274



^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx1010f3-6

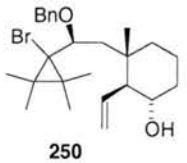


^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx1010apt



3-[2-(*S*)-Benzyloxy-2-(1-bromo-2,2,3,3-tetramethyl-cyclopropyl)-ethyl]-3-(*S*)-methyl-2-(*S*)-vinyl-1-(*S*)-cyclohexanol 250.

Potassium Superoxide (5.3 mg, 0.074 mmol) added to a room temp. sol. of mesylate **274** (~9.7 mg, 0.018 mmol) and 18-crown-6 (19.5 mg, 0.074 mmol) in 500 μL DMSO and 500 μL DME. Stirred 1 hour, then diluted with 10 mL Et_2O, 10 mL pentane, 2 mL brine, and 1 mL water, shaken vigourously, separated, and aqueous extracted twice with 4 mL 1:1 pentane: Et_2O. Combined organics washed twice with 1 mL brine, dried with $MgSO_4$, and concentrated *in vacuo*. Purified by flash chromatography (2 Hex:1 Et_2O, 3 g silica) to yield 2.4 mg clear film (29% yield for 2 steps). ^1H NMR (300MHz) ($CDCl_3$) δ TMS: 1.02 (3H, s), 1.10 (3H, s), 1.20 (1H, m), 1.25 (3H, s), 1.26 (3H, s), 1.27 (3H, s), 1.36 (1H, dd, J=13.30Hz, J=5.20Hz), 1.44 (1H, s), 1.59 (3H, m), 1.71 (1H, dd, J=9.75Hz, J=9.75Hz), 1.87 (1H, br. s), 2.08 (1H, m), 2.22 (1H, dd, J=24.20Hz, J=13.90Hz), 3.53 (1H, m), 3.59 (1H, dd, J=10.20Hz, J=1.05Hz), 4.21 (1H, ½ ABq, J=10.20Hz), 4.48 (1H, ½ ABq, J=10.10Hz), 5.23 (1H, dd, J=17.00Hz, J=2.35Hz), 5.32 (1H, dd, J=10.20Hz, J=2.20Hz), 5.74 (1H, ddd, J=17.00Hz, J=10.13Hz, J=10.13Hz), 7.35 (5H, m).



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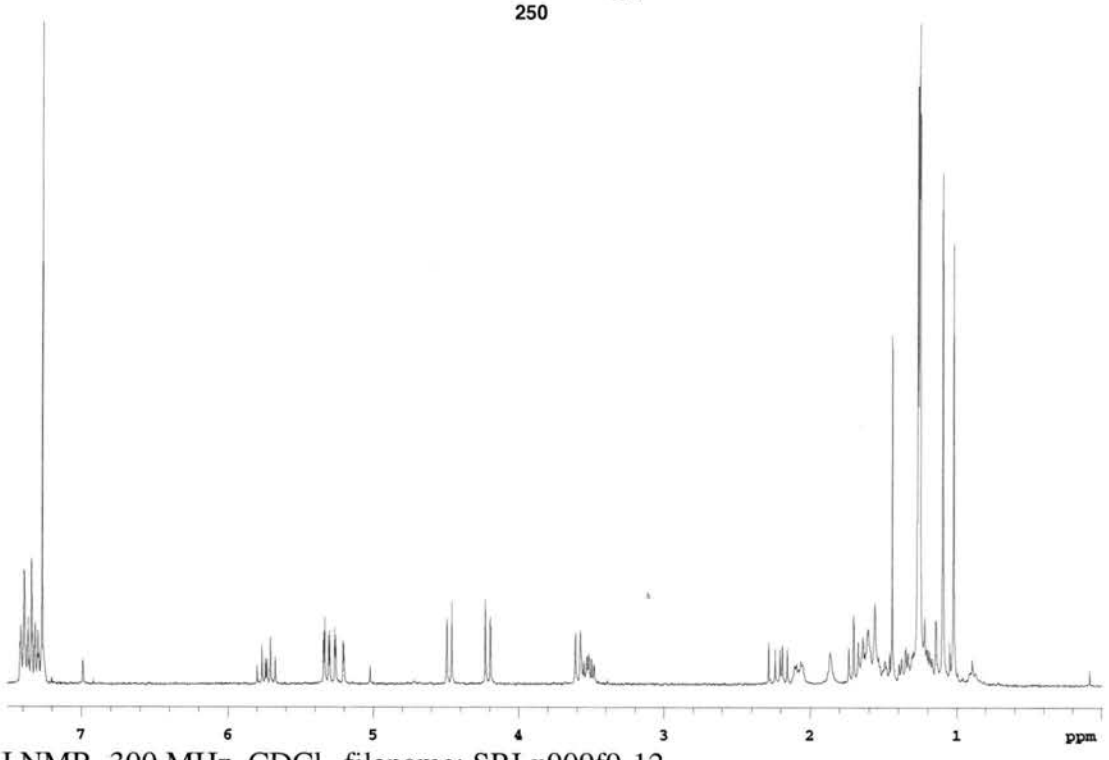
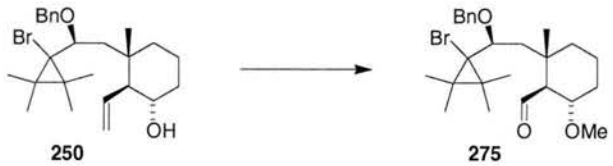


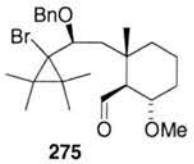
^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx909f9-12



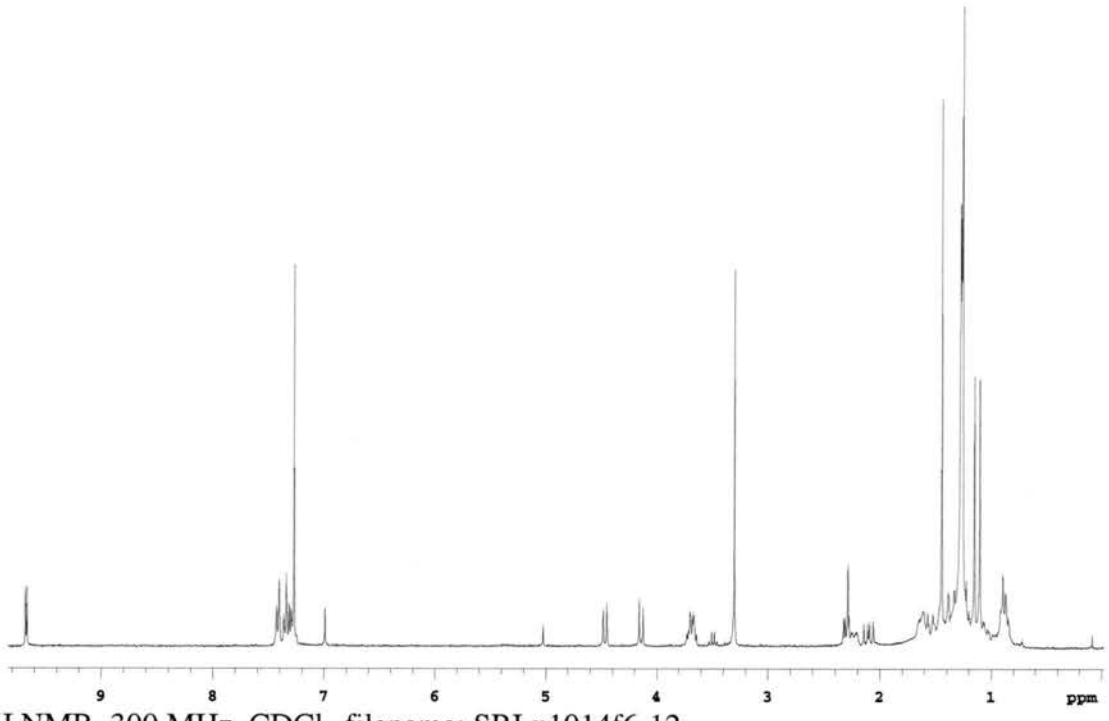
250 275

2-[2-(*S*)-Benzyloxy-2-(1-bromo-2,2,3,3-tetramethyl-cyclopropyl)-ethyl]-6-(*S*)-methoxy-2-(*S*)-methyl-1-(*S*)-cyclohexanecarbaldehyde 275.

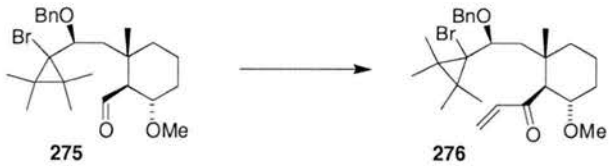
NaH (2.7 mg of 60% suspension, 0.068 mmol) added to a 0°C sol. of alcohol **250** (6.1 mg, 0.014 mmol) in 2 mL THF. Bath removed, stirred at room temp. 15 min., then iodomethane (8.4 μL, 0.136 mmol) added via syringe. Stirred overnight, diluted with 8 mL Et_2O, quenched with 1.5 mL water, separated, and aqueous extracted twice with 3 mL Et_2O. Combined organics dried with $MgSO_4$, and concentrated *in vacuo*. This residue was dissolved in 6 mL hexanes, cooled to -78°C, and ozone was bubbled through the solution for 3 min. The resulting light blue sol. was stirred 20 min., then tributylphophine (10.1 μL, 0.041 mmol) was added via syringe. The reaction was allowed to warm slowly to room temp overnight, then concentrated *in vacuo*. Purified by flash chromatography (5 Hex:1 Et_2O, 2.5 g silica) to yield 2.7 mg colorless oil (43% yield for 2 steps). ^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 1.10 (3H, s), 1.15 (3H, s), 1.27 (3H, s), 1.28 (3H, s), 1.32-1.39 (2H, m), 1.44 (3H, s), 1.50-1.57 (2H, m), 1.59-1.67 (2H, m), 2.10 (1H, dd, J=14.80Hz, J=10.30Hz), 2.17-2.25 (1H, m), 2.30 (1H, dd, J=10.60Hz, J=4.30Hz), 3.30 (3H, s), 3.64-3.73 (2H, m), 4.14 (1H, ½ ABq, J=10.30Hz), 4.46 (1H, ½ ABq, J=10.10Hz), 7.29-7.43 (5H, m), 9.67 (2H, d, J=4.20Hz).



275

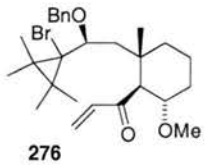


^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx1014f6-12

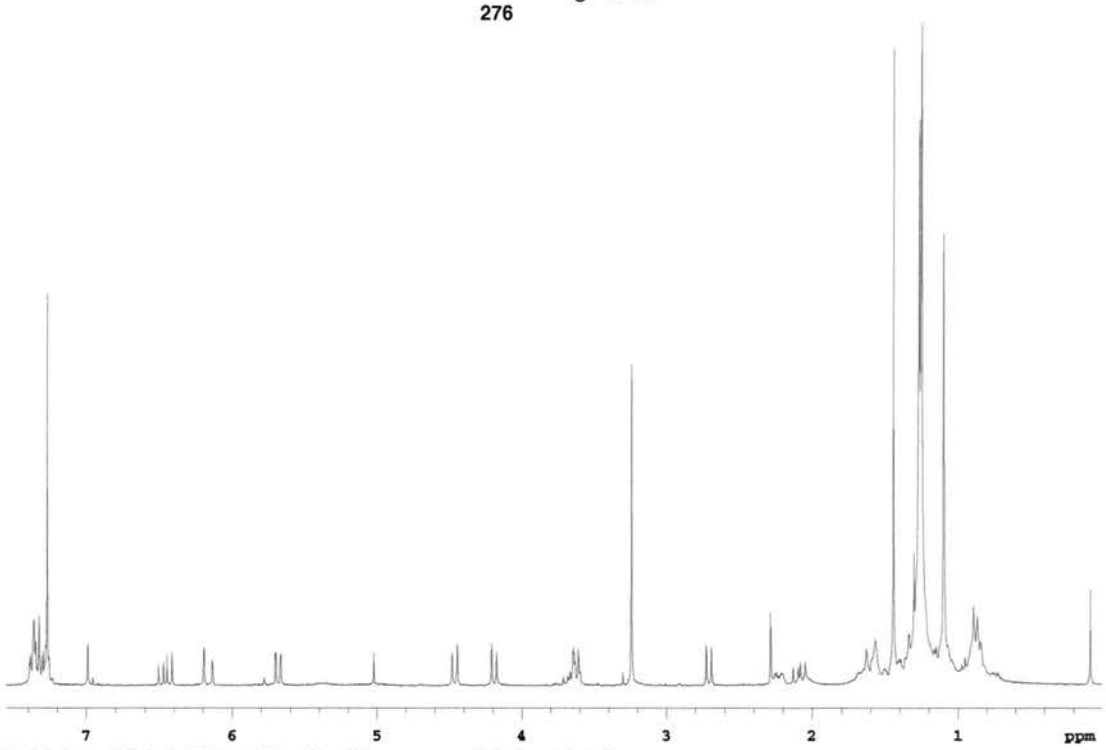


1-{2-[2-(*S*)-Benzyloxy-2-(1-bromo-2,2,3,3-tetramethyl-cyclopropyl)-ethyl]-6-(*S*)-methoxy-2-(*S*)-methyl-1-(*S*)-cyclohexyl}-2-propenone 276.

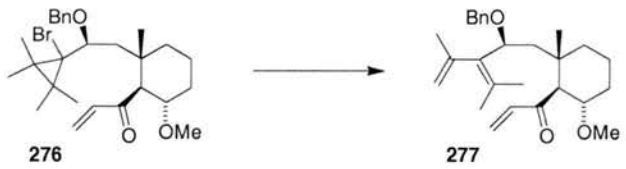
Vinyl magnesium bromide (1.0 M in THF, 41 μL, 0.041 mmol) added via syringe to a -78°C sol. of aldehyde **275** (2.7 mg, 0.0058 mmol) in 2 mL THF. Stirred 2 h., then quenched with 2 mL ½ sat. NH_4Cl sol., diluted with 7 mL Et_2O, separated, and aqueous extracted twice with 3 mL Et_2O. Combined organics dried with $MgSO_4$ and concentrated *in vacuo*. Dess-Martin periodinane (62 mg, 0.146 mmol) added to a room temp. sol. of this residue in 2 mL CH_2Cl_2. Stirred 90 min., then quenched with 700 μL sat. $Na_2S_2O_3$ sol. and 1.4 mL sat. Na_2CO_3 sol., stirred overnight, separated, and aqueous extracted twice with 3 mL CH_2Cl_2. Combined organics washed with 1 mL sat. Na_2CO_3 sol., dried with $MgSO_4$ and concentrated *in vacuo* to yield 3.6 mg colorless oil. ^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 1.10 (3H, s), 1.25 (3H, s), 1.25 (3H, s), 1.27 (3H, s), 1.23-1.29 (2H, m), 1.44 (3H, s), 1.54-1.64 (4H, m), 2.09 (1H, dd, J=14.65Hz, J=10.05Hz), 2.18-2.27 (1H, m), 2.71 (1H, d, J=10.40Hz), 3.24 (3H, s), 3.60-3.65 (2H, m), 4.19 (1H, ½ ABq, J=10.40Hz), 4.46 (1H, ½ ABq, J=10.40Hz), 5.68 (1H, dd, J=10.50Hz, J=1.30Hz), 6.16 (1H, dd, J=17.3Hz, J=1.40Hz), 6.46 (1H, dd, J=17.35Hz, J=10.45Hz), 7.27-7.39 (5H, m).



276

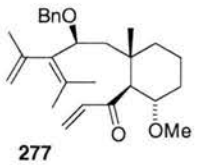


^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx1016

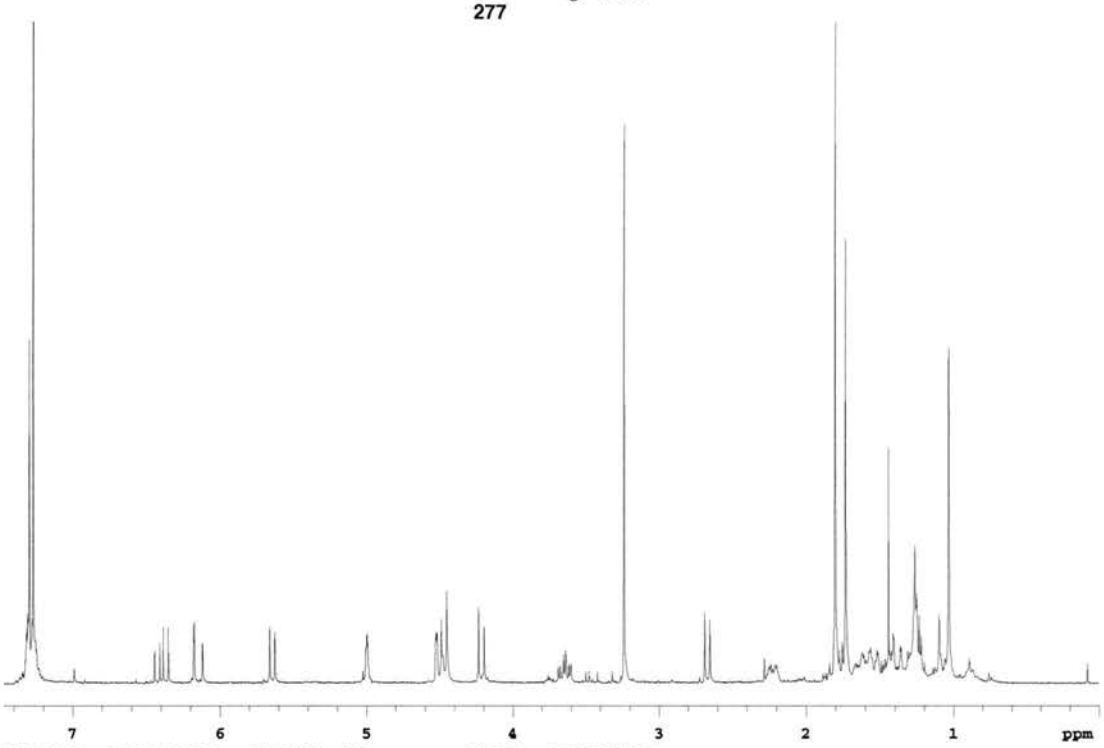


1-[2-(2-(*S*)-Benzyloxy-3-isopropenyl-4-methyl-pent-3-enyl)-6-(*S*)-methoxy-2-(*S*)-methyl-(*S*)-cyclohexyl]-2-propenone 277.

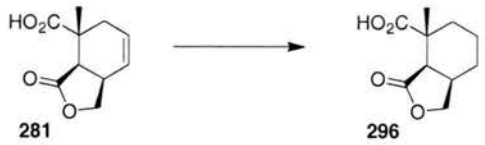
Bromocyclopropane **276** (~2.9 mg, 0.0058 mmol) and 2,4,6-collidine (19.2 μL, 0.145 mmol) dissolved in 1.5 mL DMSO and heated to 115°C in sand bath. Stirred 12 hours, cooled to room temp, diluted with 10 mL Et_2O, 1 mL brine, and 0.5 mL water, shaken vigorously, separated, and aqueous extracted twice with 3 mL 1 Hex:1 Et_2O. Combined organics washed with 1 mL brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purified by flash chromatography (2 Hex:1 Et_2O, 2 g silica) to yield 1.5 mg (62% yield for three steps). ^1H NMR (300MHz) ($CDCl_3$) δ TMS: 1.03 (3H, s), 1.23-1.29 (3H, m), 1.38 (1H, dd, J=14.90Hz, J=2.00Hz), 1.50-1.63 (3H, m), 1.74 (3H, s), 1.81 (6H, s), 2.19-2.26 (1H, m), 2.67 (1H, d, J=10.40Hz), 3.24 (3H, s), 3.65 (1H, ddd, J=10.75Hz, J=10.75Hz, J=4.53Hz), 4.21 (1H, ½ ABq, J=11.50Hz), 4.47 (1H, ½ ABq, J=11.30Hz), 4.48 (1H, m), 4.52 (1H, dd, J=2.60Hz, J=0.90Hz), 5.00 (1H, dd, J=2.70Hz, J=1.60Hz), 5.64 (1H, dd, J=10.50Hz, J=1.30Hz), 6.14 (1H, dd, J=17.4Hz, J=1.50Hz), 6.40 (1H, dd, J=17.40Hz, J=10.40Hz), 7.27-7.32 (5H, m).



277

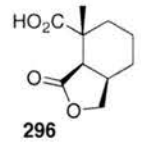


^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx1017f5-7

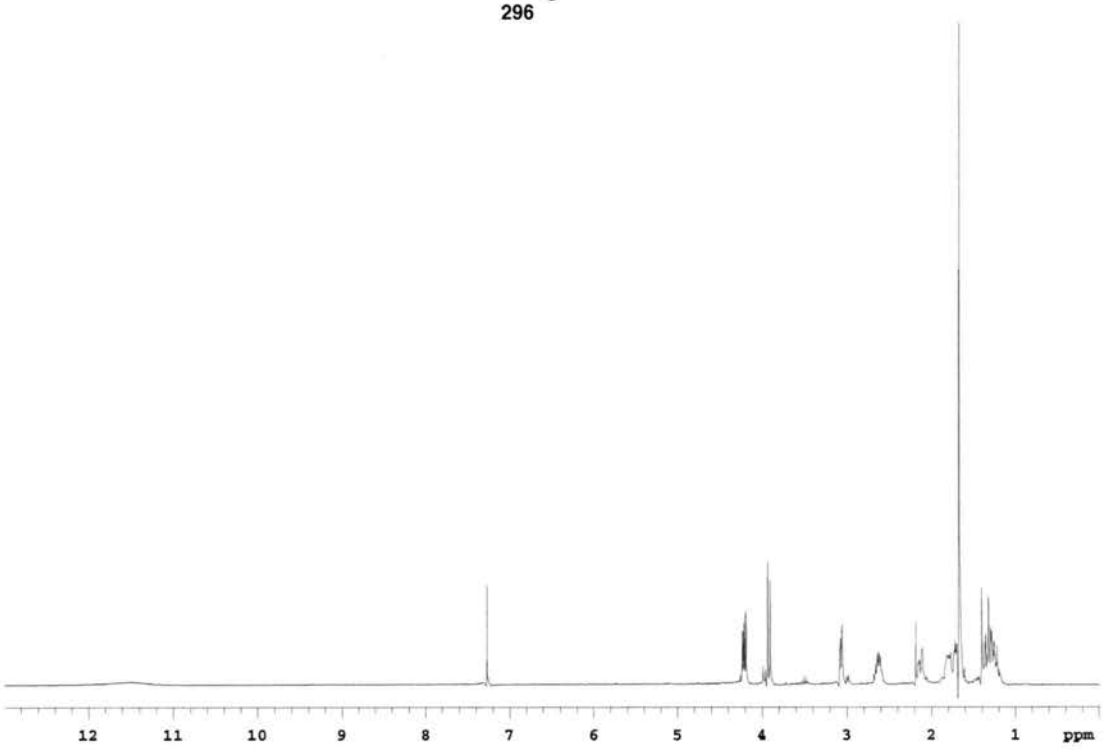


3a(*R*)-7a(*R*)-4-(*R*)-Methyl-3-oxo-octahydro-isobenzofuran-4-carboxylic acid 296.

Alkene **281** (12.85 g, 65.5 mmol) dissolved in 500 mL EtOAc in a 2 L round-bottom flask and stirred under argon for 30 min. To this solution was added PtO_2 (149 mg, 0.655 mmol), the flask was purged with 1 balloon of H_2, after which 2 balloons full of H_2 were attached. After the reaction was stirred at room temp for 36 hours, occasionally refilling the balloons and swirling the flask to get Pt^0 off the walls, a couple spatula scoops of activated carbon were added. The reaction was stirred 5 more min., then filtered through celite with EtOAc rinses and concentrated *in vacuo* to give 12.66 g white solids. These solids were taken up in 50 mL Et_2O, warmed to reflux, and then 25 mL hexanes added. Solids filtered off to give a first crop of about 4 g. The mother liquor was concentrated *in vacuo* and the resulting solids dissolved in 10 mL warm EtOAc, to which was added 30 mL hexanes. The resulting suspension was allowed to cool to room temp, then cooled to 0°C. Solids filtered off to give a second crop of about 6.5 g. Combined crops dried overnight in a vacuum dessicator to yield 10.45 g of acid **296** as a white powder (81% yield). ^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 1.21-1.41 (3H, m), 1.67 (3H, s), 1.69-1.74 (1H, m), 1.76-1.85 (1H, m), 2.13 (1H, dddd, J=10.90Hz, J=3.10Hz, J=1.65Hz, J=1.65Hz), 2.57-2.69 (1H, m), 3.07 (1H, d, J=6.10Hz), 3.92 (1H, d, J=8.80Hz), 4.21 (1H, dd, J=8.80Hz, J=4.60Hz), 11.10-12.10 (1H, br. s).



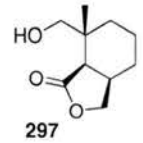
296



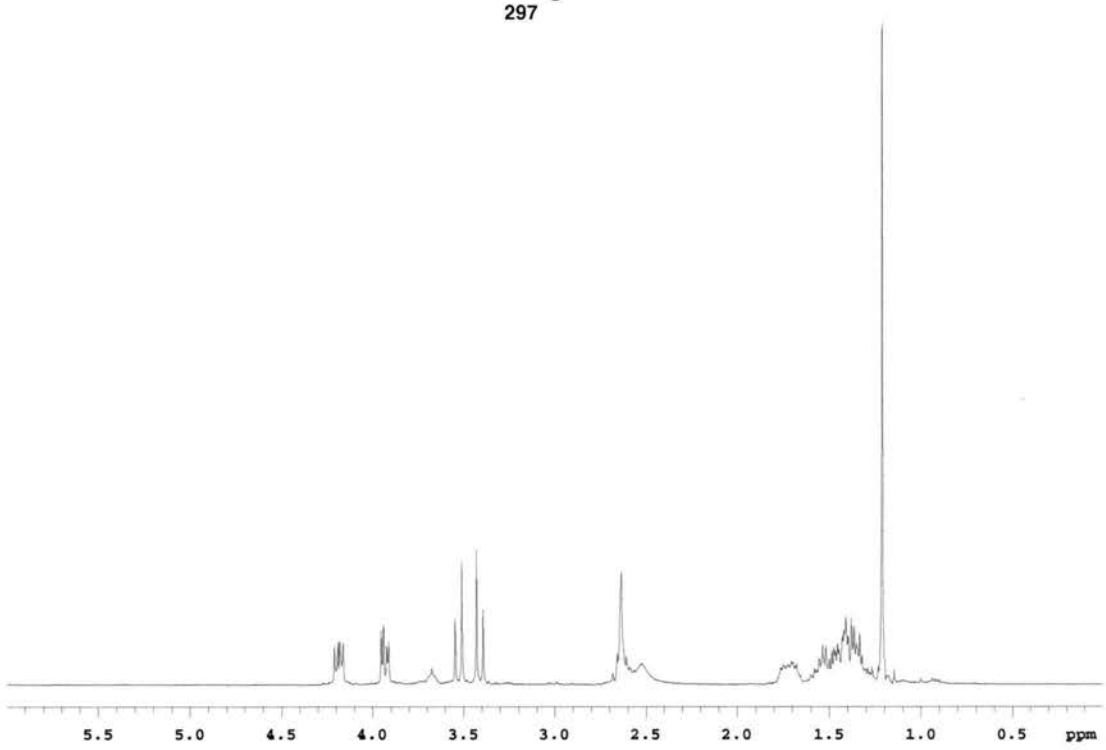
^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx1047cr

3a-(*R*)-7a-(*R*)-7-(*R*)-Hydroxymethyl-7-methyl-hexahydro-isobenzofuran-1-one 297.

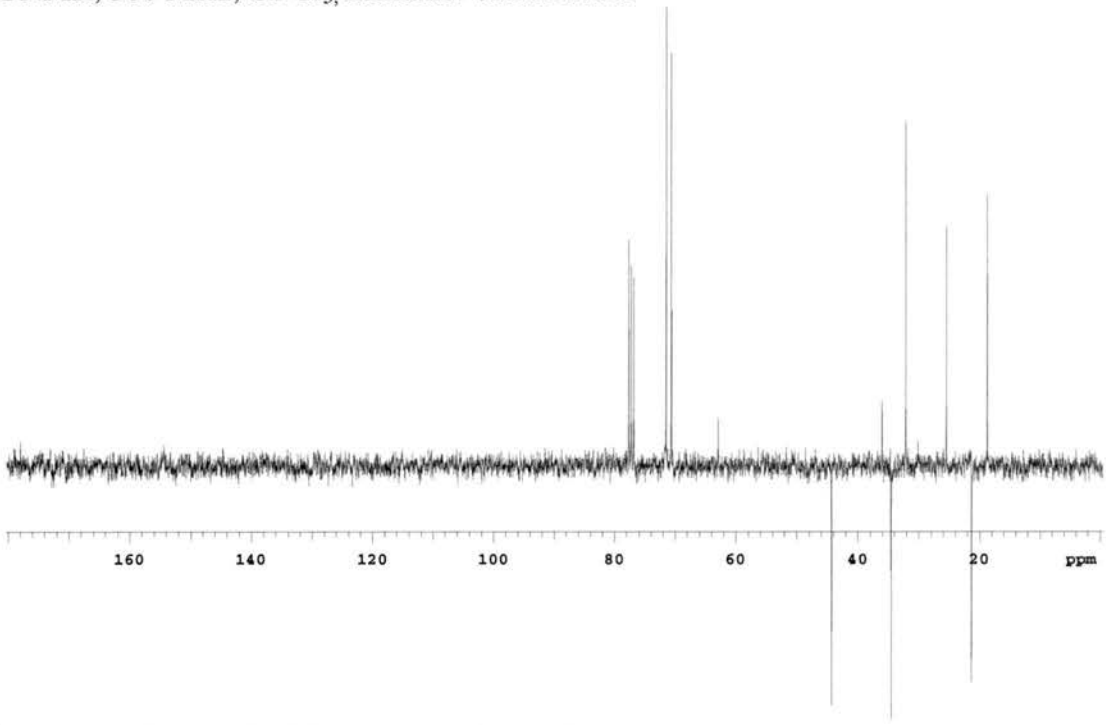
Acid **296** (448.5 mg, 2.26 mmol) and N-methylmorpholine (274 μL, 2.49 mmol) were dissolved in 5 mL THF and cooled to 0°C. To this solution was added isobutyl chloroformate (323 μL, 2.49 mmol) slowly via syringe. Stirred 45 min., then solids filtered off through a coarse frit with 5 mL of THF rinses. Filtrate cooled to 0°C, and a sol. of $NaBH_4$ (214 mg, 5.66 mmol) in 700 μL water was added via syringe with a 200 μL rinse. Stirred 15 h., then diluted with 15 mL Et_2O, filtered through a pad of $MgSO_4$ with 20 mL of Et_2O rinses, and concentrated *in vacuo* to give 418.7 mg of alcohol **297** as a pale oil (100% yield). ^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 1.21 (3H, s), 1.30-1.60 (5H, m), 1.65-1.78 (1H, m), 2.52 (1H, br. s), 2.64 (2H, s), 3.41 (1H, ½ ABq, J=11.00Hz), 3.53 (1H, ½ ABq, J=10.90Hz), 3.93 (1H, dd, J=8.70Hz, J=3.70Hz), 4.19 (1H, dd, J=8.80Hz, J=5.70Hz).). ^{13}C NMR (75MHz) ($CDCl_3$) δ TMS: 18.74, 21.32, 25.44, 32.13, 34.55, 36.03, 44.31, 70.61, 71.45, 177.97. IR: 3402, 2929, 2869, 1760 cm^{-1}.



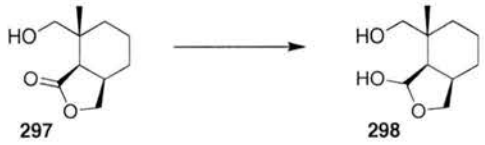
297



^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx1070cr

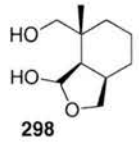


^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx1070crapt

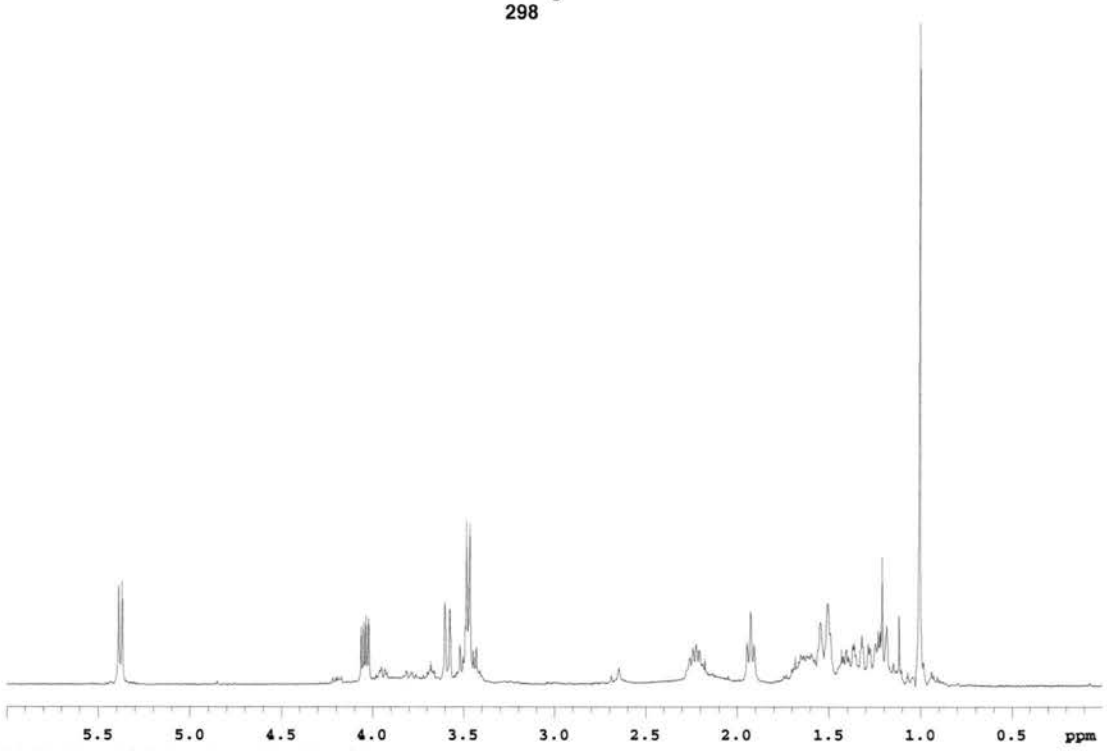


3a-(*R*)-7a-(*R*)-7-(*R*)-Hydroxymethyl-7-methyl-octahydro-isobenzofuran-1-ol 298.

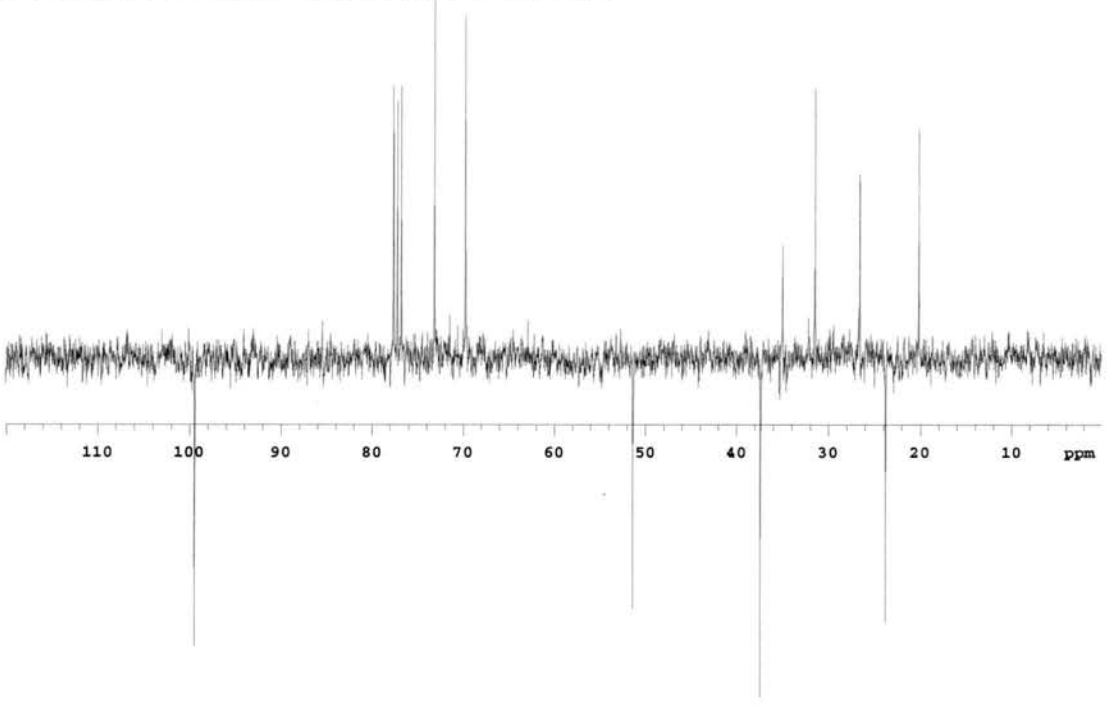
Lactone **297** (416.8 mg, 2.26 mmol) was dissolved in 15 mL CH_2Cl_2 and cooled to -78°C. To this sol. was added DIBAL (4.98 mL of a 1.0 M sol. in CH_2Cl_2, 4.98 mmol) via syringe over 3 min. Stirred 2.5 h., then bath removed and reaction quenched with 500 µL MeOH. Stirred 15 min., then 2.3 g $Na_2SO_4 \cdot 10\ H_2O$ and 15 mL CH_2Cl_2 added. Stirred overnight, then filtered through a coarse frit with 30 mL of EtOAc rinses and concentrated *in vacuo* to give 344.7 mg of diol **298** as an off-white amorphous solid (82% yield). ^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 1.01 (3H, s), 1.18-1.44 (4H, m), 1.53 (2H, d, J=12.40Hz), 1.57-1.71 (1H, m), 1.93 (1H, dd, J=5.75Hz, J=5.75Hz), 2.18-2.28 (1H, m), 3.46 (1H, ½ ABq, J=10.80Hz), 3.51 (1H, ½ ABq, J=10.80Hz), 3.59 (1H, dd, J=8.00Hz, J=1.00Hz), 4.04 (1H, dd, J=8.00Hz, J=4.30Hz), 5.38 (1H, d, J=5.80Hz). ^{13}C NMR (75MHz) ($CDCl_3$) δ TMS: 20.16, 23.78, 26.60, 31.47, 34.98, 37.40, 51.37, 69.75, 73.16, 99.47.



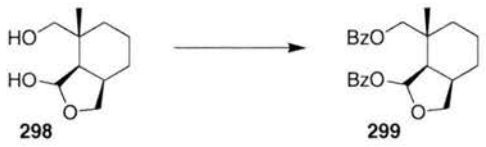
298



^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx1071cr

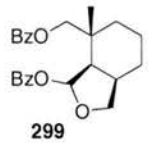


^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx1071crapt

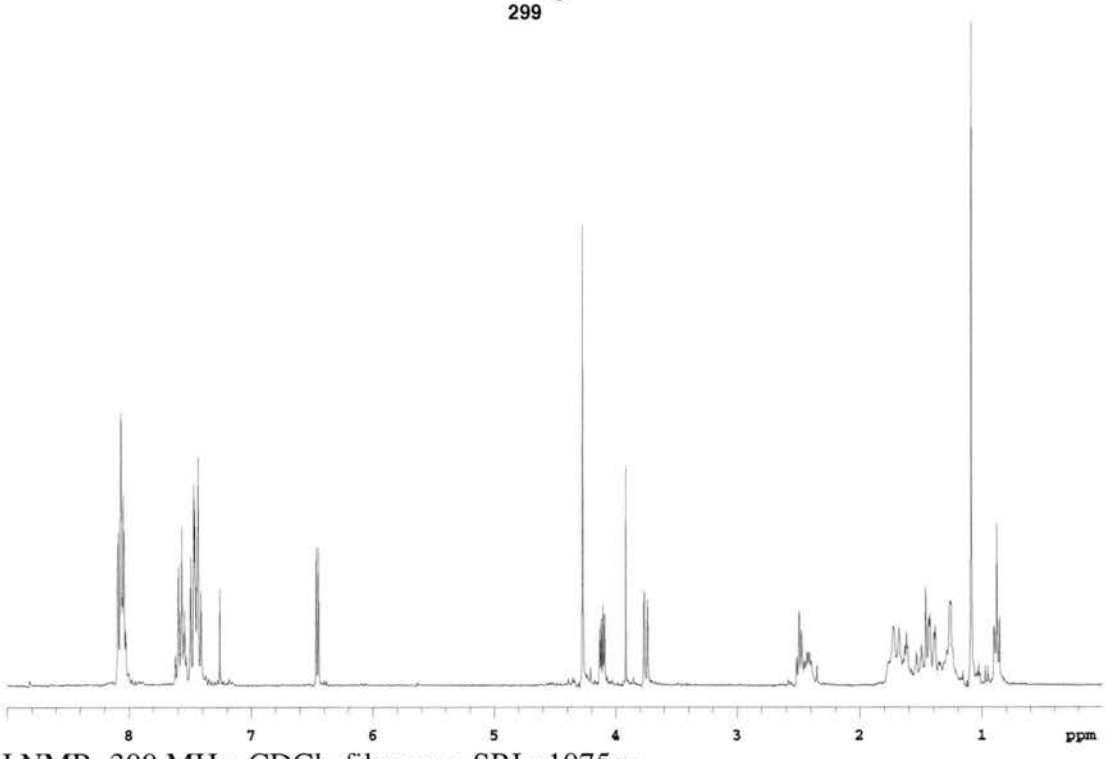


Benzoic acid 3a-(*R*)-7a-(*R*)-7-(*R*)-methylbenzoate-7-methyl-octahydro-isobenzofuran-1-yl ester 299.

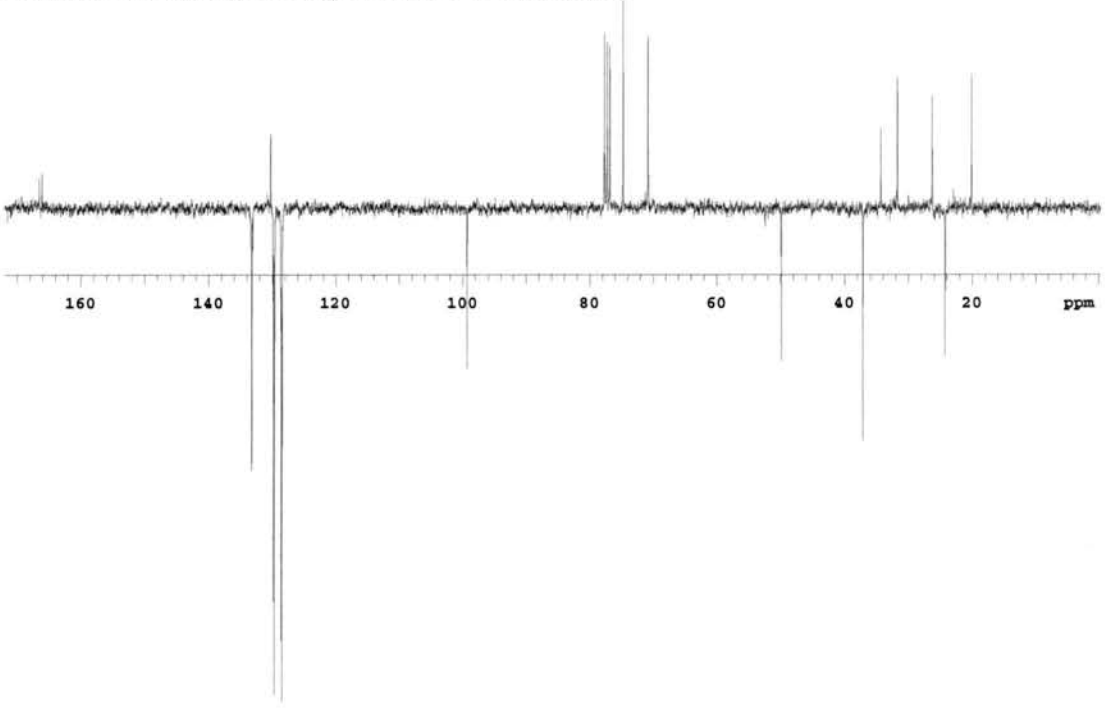
Diol **298** (344.7 mg, 1.85 mmol) and DMAP (22.6 mg, 0.185 mmol) dissolved in 7 mL pyridine and cooled to 0°C. To this solution was added benzoyl chloride (473 μL, 4.07 mmol) via syringe over 2 min. Stirred 1 h. at 0°C, then bath removed and reaction stirred at room temp. for 36 h. Reaction cooled back to 0°C and 279 μL BzCl and 50 mg DMAP added. Bath removed and stirred for 60 min., after which reaction is complete. Quenched with 3 mL MeOH, stirred 30 min., then diluted with 30 mL EtOAc and 20 mL 10% HCl sol., separated, and aqueous extracted twice with 20 mL EtOAc. Combined organics washed with 10 mL sat. $NaHCO_3$ sol., twice with 10 mL brine, dried with $MgSO_4$ with concurrent treatment with activated carbon, filtered through celite and concentrated *in vacuo* with four 75 mL toluene azeotropes to give 689.7 mg of dibenzoate **299** as a pale oil (94% yield). ^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 1.10 (3H, s), 1.23-1.32 (2H, m), 1.39-1.57 (2H, m), 1.62-1.79 (2H, m), 2.44 (1H, dd, J=10.45Hz, J=5.15Hz), 2.51 (1H, dd, J=5.95Hz, J=5.95Hz), 3.77 (1H, d, J=8.30Hz), 4.13 (1H, dd, J=8.20Hz, J=4.20Hz), 4.39 (2H, s), 6.47 (1H, d, J=5.70Hz), 7.42-7.52 (4H, m), 7.54-7.64 (2H, m), 8.04-8.12 (4H, m). ^{13}C NMR (75MHz) ($CDCl_3$) δ TMS: 20.09, 24.22, 26.26, 31.68, 34.31, 37.07, 49.84, 70.78, 74.71, 99.28, 128.46, 128.58, 129.67, 129.83, 130.23, 130.27, 133.22, 133.27, 166.05, 166.50.



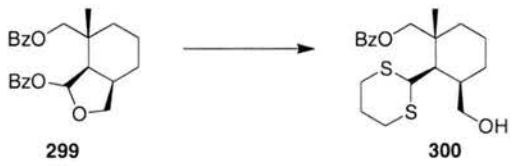
299



^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx1075cr

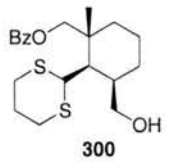


^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx1075apt



Benzoic acid 2-(*R*)-[1,3]dithian-2-yl-3-(*R*)-hydroxymethyl-1-(*R*)-methyl-cyclohexylmethyl ester 300.

Dibenzoate **299** (527.9 mg, 1.34 mmol) and 1,3-propanedithiol (2.02 mL, 20.1 mmol) dissolved in 12 mL CH_2Cl_2 and cooled to -78°C. To this sol. was added $TiCl_4$ (220.1 μL, 2.01 mmol) via syringe. Stirred 60 min., then quenched with 5 mL sat. $NaHCO_3$ sol. and 10 mL H_2O. Diluted with 20 mL CH_2Cl_2, separated, and aqueous extracted twice with 15 mL CH_2Cl_2. Combined organics washed with 8 mL brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purified by flash chromatography (3 Hex:2 EtOAc, 20 g silica) to yield 430.2 mg of dithiane **300** as a white foam (85% yield). ^1H NMR (300MHz) ($CDCl_3$) δ TMS: 1.30 (3H, s), 1.47-1.58 (3H, m), 1.59-1.69 (1H, m), 1.74-1.91 (3H, m), 2.03-2.14 (2H, m), 2.16-2.27 (2H, m), 2.82 (2H, ddd, J=14.20Hz, J=3.70Hz, J=3.70Hz), 2.86-3.03 (2H, m), 3.68-3.77 (1H, m), 4.00-4.08 (1H, m), 4.22 (1H, ½ ABq, J=11.20Hz), 4.27 (1H, ½ ABq, J=11.00Hz), 4.60 (1H, d, J=2.60Hz), 7.45 (2H, dd, J=7.50Hz, J=7.50Hz), 7.57 (1H, dddd, J=7.40Hz, J=7.40Hz, J=1.68Hz, J=1.68Hz), 8.03-8.08 (2H, m). ^{13}C NMR (75MHz) ($CDCl_3$) δ TMS: 20.40, 24.01, 26.24, 26.78, 33.38, 34.40, 38.91, 40.56, 47.32, 51.04, 65.23, 71.13, 128.51, 129.66, 130.32, 133.04, 166.54.



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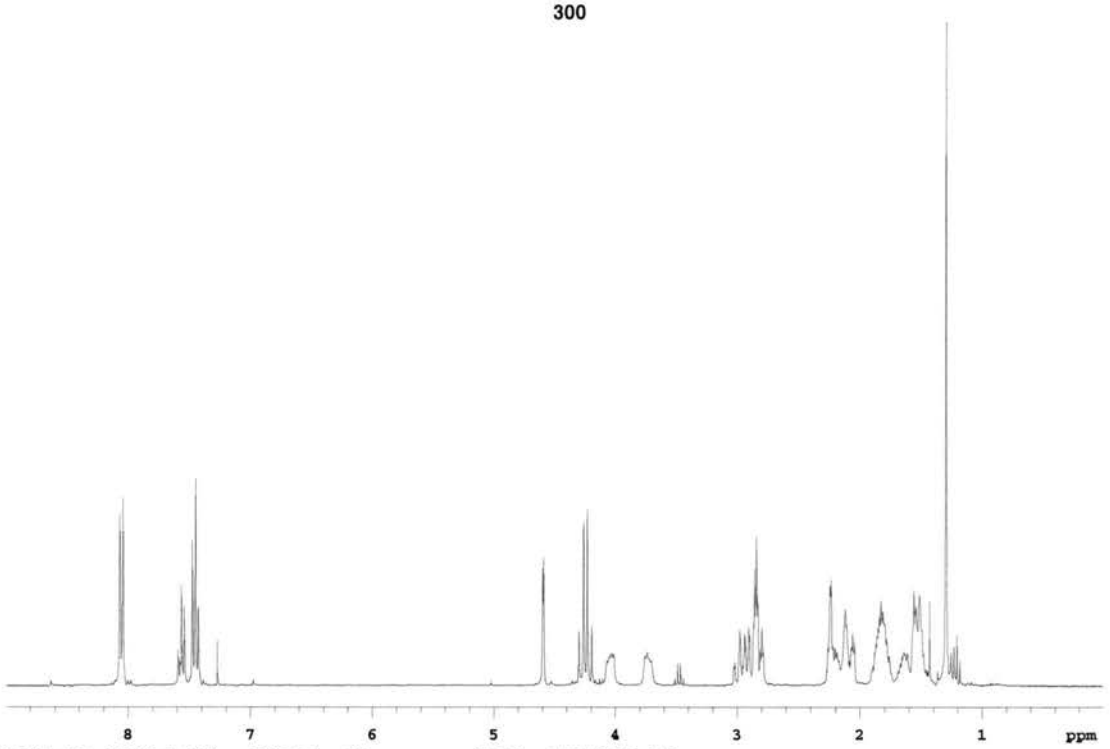
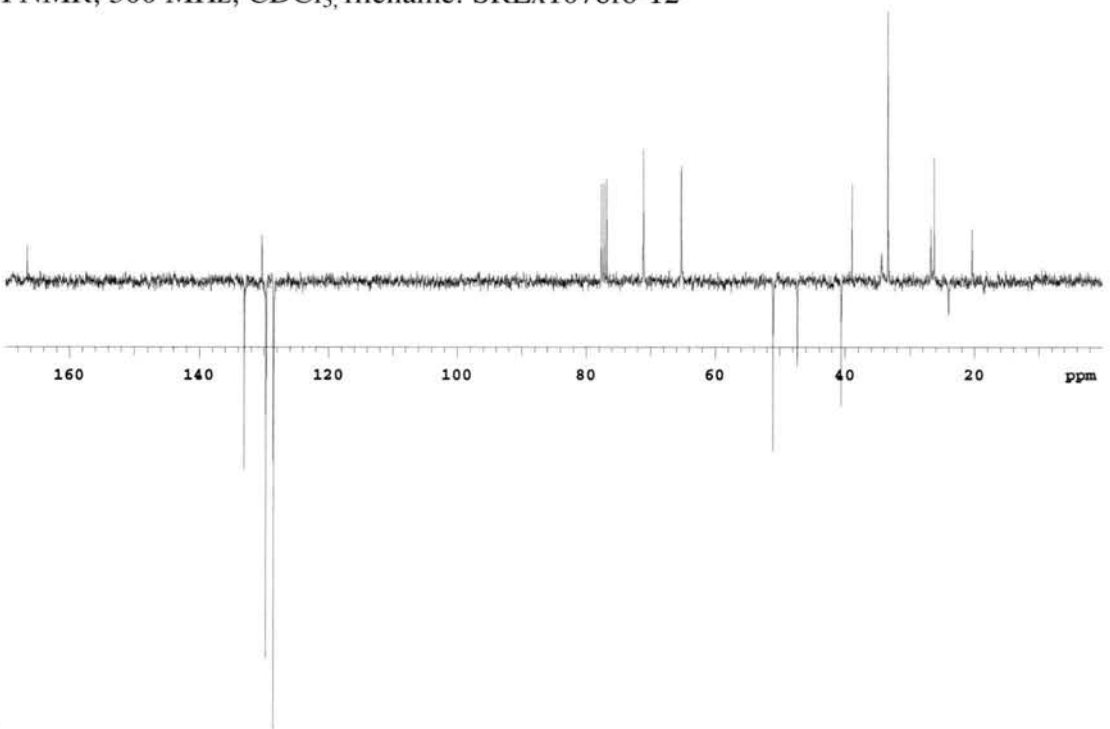


^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx1078f6-12

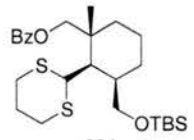


^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx1078apt

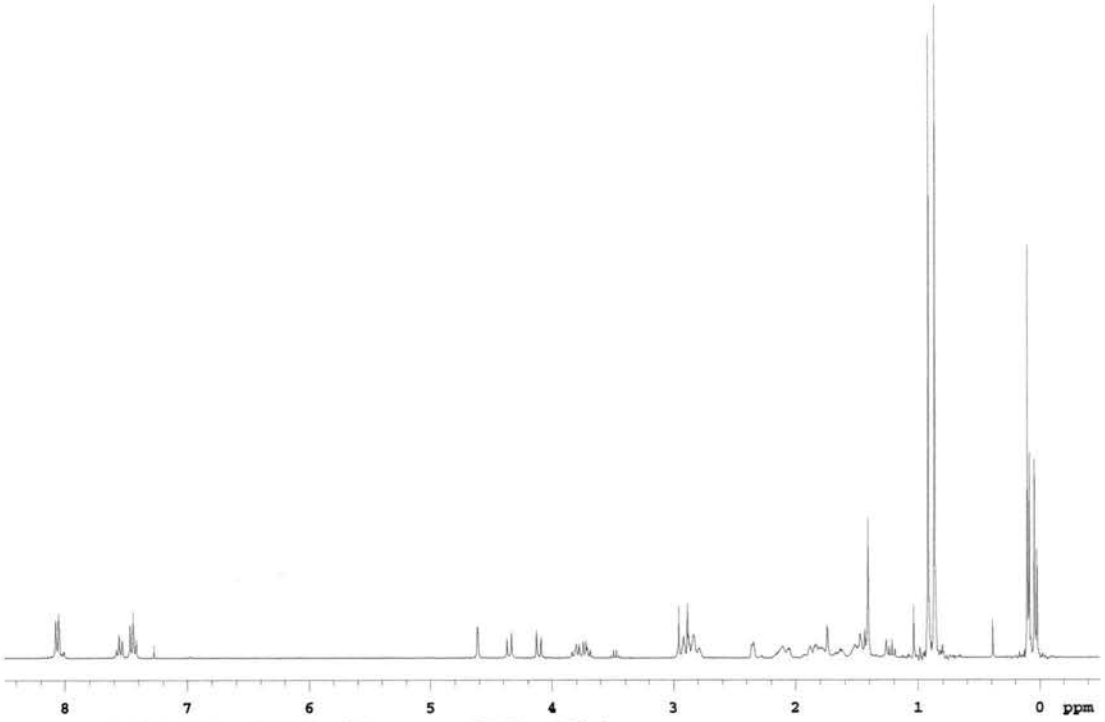
300 301

Benzoic acid 3-(*R*)-(tert-butyl-dimethyl-silanyloxymethyl)-2-(*R*)-[1,3]dithian-2-yl-1-(*R*)-methyl-cyclohexylmethyl ester 301.

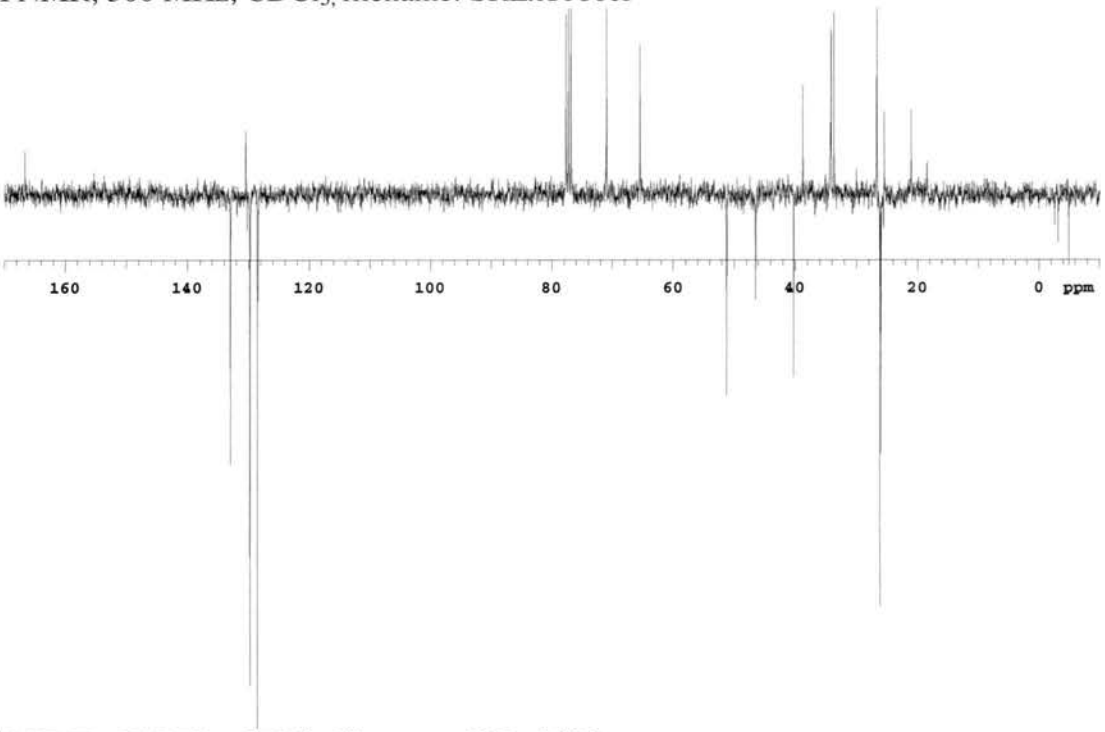
Alcohol **300** (117.9 mg, 0.310 mmol), *t*-butyldimethylsilyl chloride (93.4 mg, 0.620 mmol), and imidazole (63.3 mg, 0.929 mmol) dissolved in 750 μL DMF at room temp. Stirred 19 h., then diluted with 10 mL Et_2O and 10 mL hexanes, washed thrice with 2 mL water, once with 2 mL brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purified by flash chromatography (2 Hex:1 Et_2O, 4 g silica) to give 137.7 mg of silyl ether **301** as a pale oil (90% yield). ^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 0.04 (3H, s), 0.08 (3H, s), 0.87 (9H, s), 1.41 (3H, s), 1.43-1.55 (3H, m), 1.59-1.60 (1H, m), 1.74 (1H, s), 1.76-1.90 (2H, m), 2.03-2.18 (2H, m), 2.35 (1H, d, J=4.40Hz), 2.77-2.97 (4H, m), 3.71 (1H, dd, J=10.20Hz, J=7.00Hz), 3.81 (1H, dd, J=9.80Hz, J=7.60Hz), 4.10 (1H, ½ ABq, J=11.20Hz), 4.35 (1H, ½ ABq, J=11.10Hz), 4.61 (1H, d, J=2.10Hz), 7.44 (2H, dd, J=7.40Hz, J=7.40Hz), 7.56 (1H, dddd, J=7.40Hz, J=7.40Hz, J=1.53Hz, J=1.53Hz), 8.04-8.09 (2H, m). ^{13}C NMR (75MHz) ($CDCl_3$) δ TMS: -3.23, -2.60, 18.45, 21.00, 25.43, 26.18, 26.62, 33.62, 34.09, 34.20, 38.69, 40.20, 46.44, 51.18, 65.44, 70.95, 128.47, 129.72, 130.39, 132.95, 166.61.



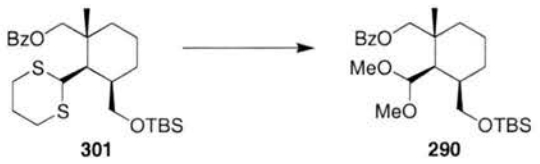
301



^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx1086cr



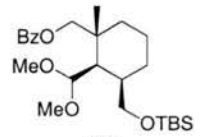
^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx1086crapt



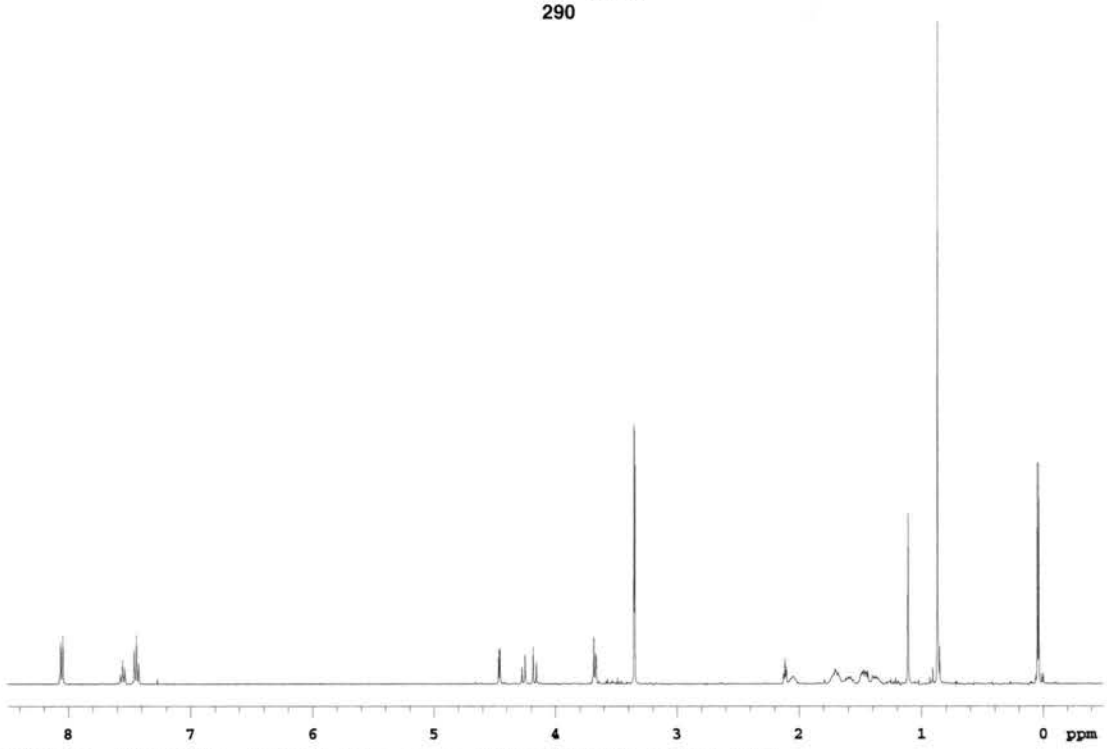
301 290

Benzoic acid 3-(*R*)-(tert-butyl-dimethyl-silanyloxymethyl)-2-(*R*)-dimethoxymethyl-1-(*R*)-methyl-cyclohexylmethyl ester 290.

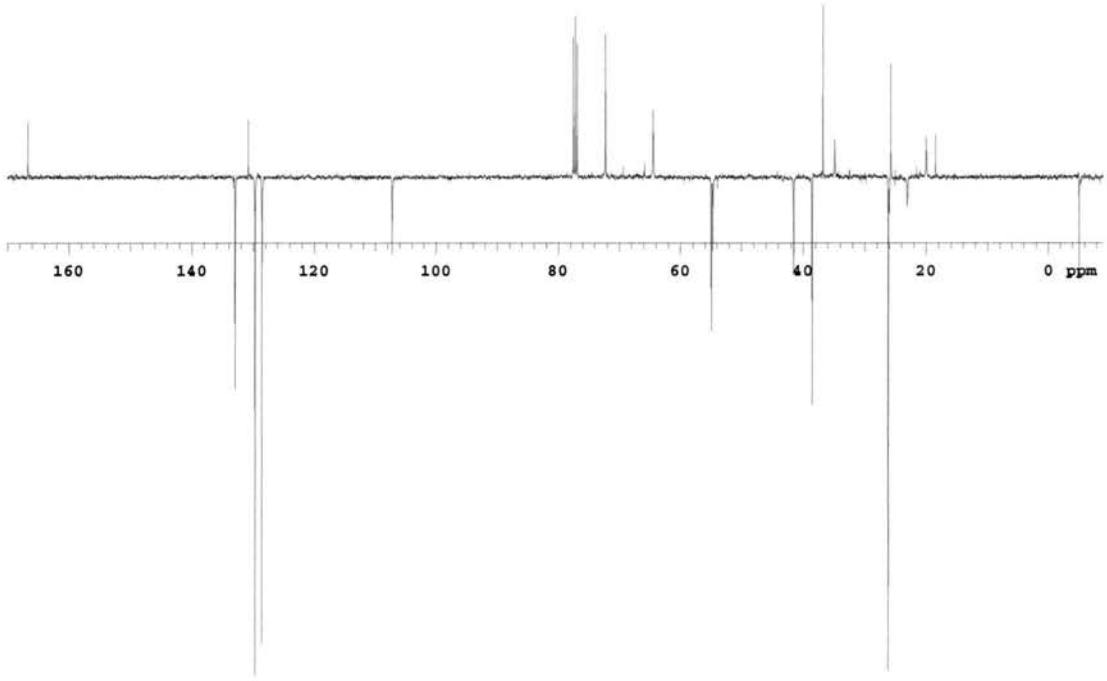
Silver nitrate (175.8 mg, 1.035 mmol) dissolved/suspended in 2 mL MeOH at room temp. N-bromosuccinimide (163.7 mg, 0.920 mmol) added, followed by 2,6-lutidine (267.9 μL, 2.30 mmol). The resulting white slurry was stirred 30 min. in the dark, after which a sol. of dithiane **301** (113.8 mg, 0.230 mmol) in 1 mL THF was added via pipet with two 500 μL THF rinses. Resulting light yellow suspension stirred 40 min., then quenched with 2.5 mL sat. $Na_2S_2O_3$ sol., and diluted with 3 mL water and 3 mL sat. $NaHCO_3$ sol. This was extracted thrice with 10 mL Et_2O, and the combined organics were washed with 3 mL brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purified by flash chromatography (2 Hex:1 Et_2O, 3 g silica) to give 82.8 mg of acetal **290** as a pale oil (80% yield). ^1H NMR (400MHz) ($CDCl_3$) δ TMS: 0.03 (3H, s), 0.04 (3H, s), 0.87 (9H, s), 1.11 (3H, s), 1.33-1.41 (1H, m), 1.43-1.50 (2H, m), 1.55-1.63 (1H, m), 1.66-1.75 (2H, m), 2.01-2.09 (1H, m), 2.11 (1H, dd, J=4.80Hz, J=4.80Hz), 3.34 (3H, s), 3.35 (3H, s), 3.68 (1H, dd, J=7.00Hz, J=2.20Hz), 4.17 (1H, ½ ABq, J=10.90Hz), 4.26 (1H, ½ ABq, J=10.90Hz), 4.46 (1H, d, J=4.50Hz), 7.44 (2H, dd, J=7.60Hz, J=7.60Hz), 7.56 (1H, dddd, J=7.50Hz, J=7.50Hz, J=1.33Hz, J=1.33Hz), 8.04-8.07 (2H, m). ^{13}C NMR (100MHz) ($CDCl_3$) δ TMS: -5.11, 18.47, 19.98, 25.74, 26.14, 34.85, 36.75, 38.52, 41.52, 54.65, 54.93, 64.46, 72.26, 107.21, 128.52, 129.66, 130.79, 132.95, 166.70.



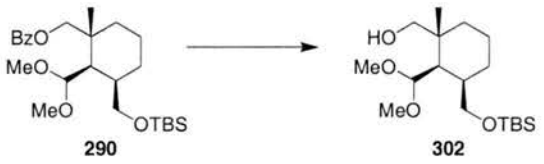
290



^{1}H NMR, 400 MHz, $CDCl_3$, filename: SRLx1100f3-5MHz400

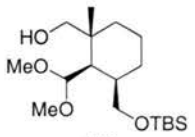


^{13}C NMR, 100 MHz, $CDCl_3$, filename: SRLx1100apt

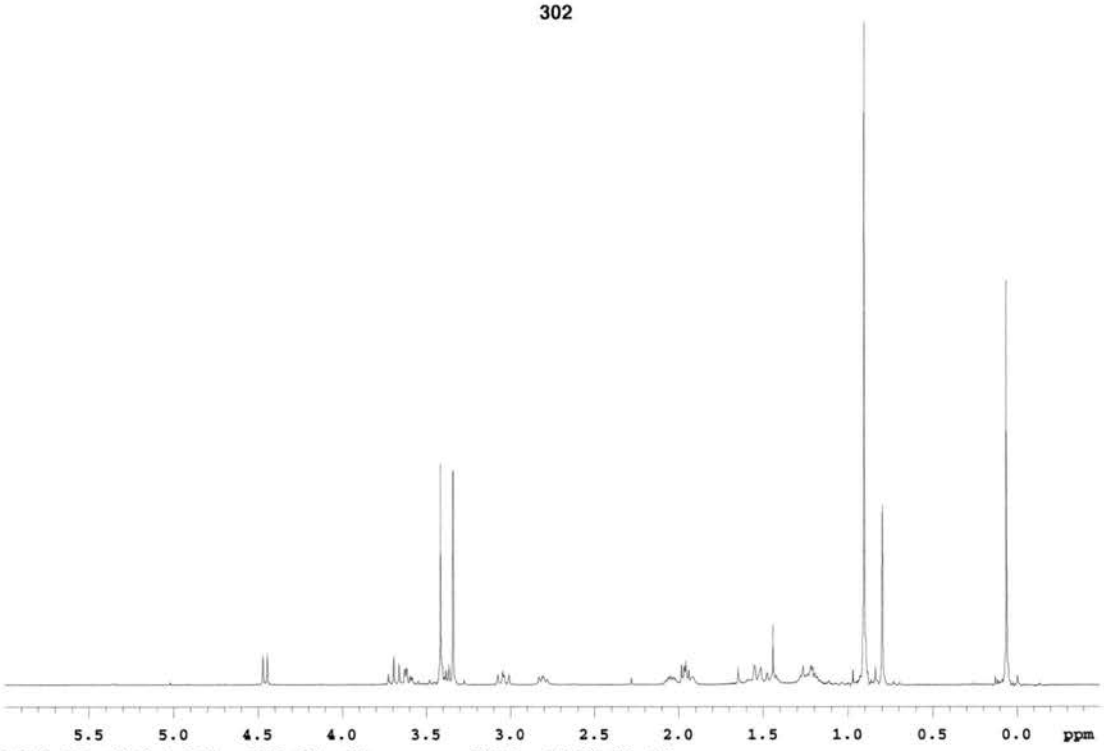


[3-(*R*)-(tert-Butyl-dimethyl-silanyloxymethyl)-2-(*R*)-dimethoxymethyl-1-(*R*)-methyl-cyclohexyl]-methanol 302.

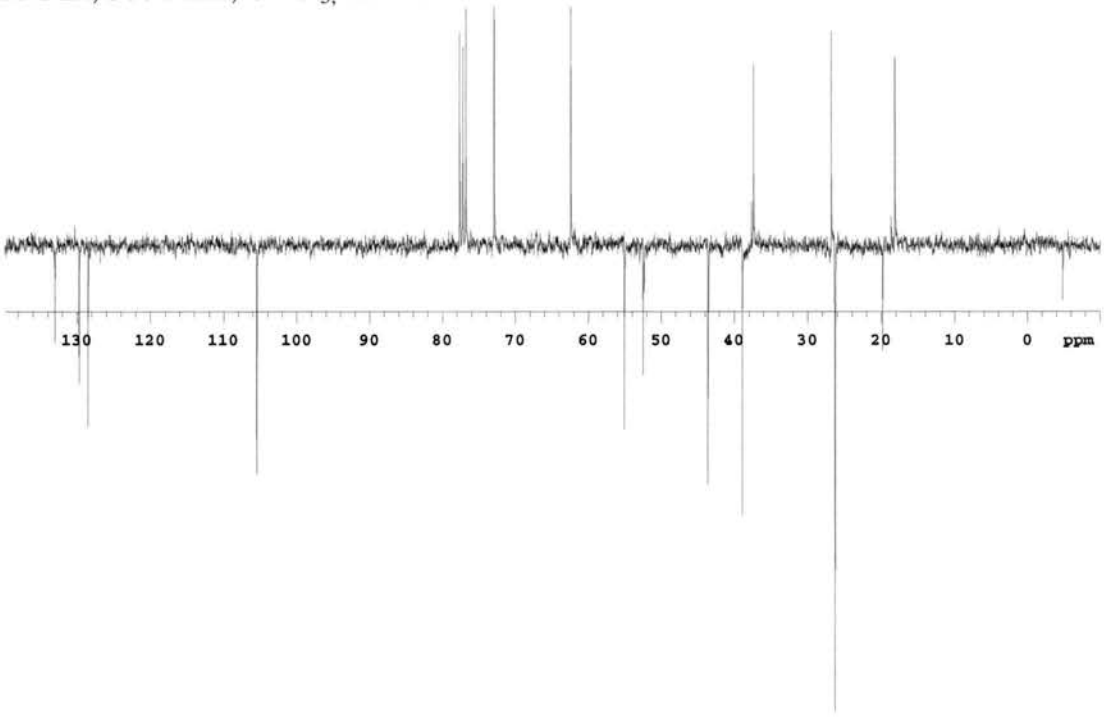
A 15% aqueous solution of sodium hydroxide (7 mL) was added to a solution of benzoate **290** (1.729 g, 3.836 mmol) in 50 mL methanol and heated to reflux. Stirred 1 hour, cooled to room temp, then ~30 mL methanol removed by rotary evacuation. Diluted with 30 mL hexanes and 30 mL Et_2O, separated, and aqueous extracted twice with 30 mL 1 Et_2O:1 hexanes. Combined organics washed with 15 mL sat. Na_2CO_3, twice with 15 mL brine, dried with Na_2CO_3 and $MgSO_4$, and concentrated *in vacuo* to yield 1.254 mg of alcohol **302** as a pale oil (94% yield). ^1H NMR (300MHz) ($CDCl_3$) δ TMS: 0.06 (6H, s), 0.80 (3H, s), 0.90 (9H, s), 1.15-1.29 (3H, m), 1.40-1.46 (1H, m), 1.47-1.56 (2H, m), 1.90-1.93 (1H, m), 1.96 (1H, dd, J=7.65Hz, J=5.15Hz), 2.00-2.09 (1H, m), 2.81 (1H, dd, J=8.60Hz, J=6.40Hz), 3.04 (1H, dd, J=11.35Hz, J=8.75Hz), 3.34 (3H, s), 3.41 (3H, s), 3.61 (1H, ddd, J=9.50Hz, J=3.60Hz, J=0.50Hz), 3.69 (1H, dd, J=9.60Hz, J=9.60Hz), 4.46 (1H, d, J=7.70Hz). ^{13}C NMR (75MHz) ($CDCl_3$) δ TMS: -4.87, 18.14, 18.66, 19.81, 26.30, 26.82, 37.40, 37.64, 38.87, 43.58, 55.04, 62.40, 72.92, 105.46.



302



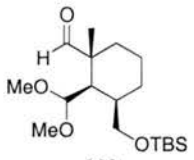
^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx1101f6-13



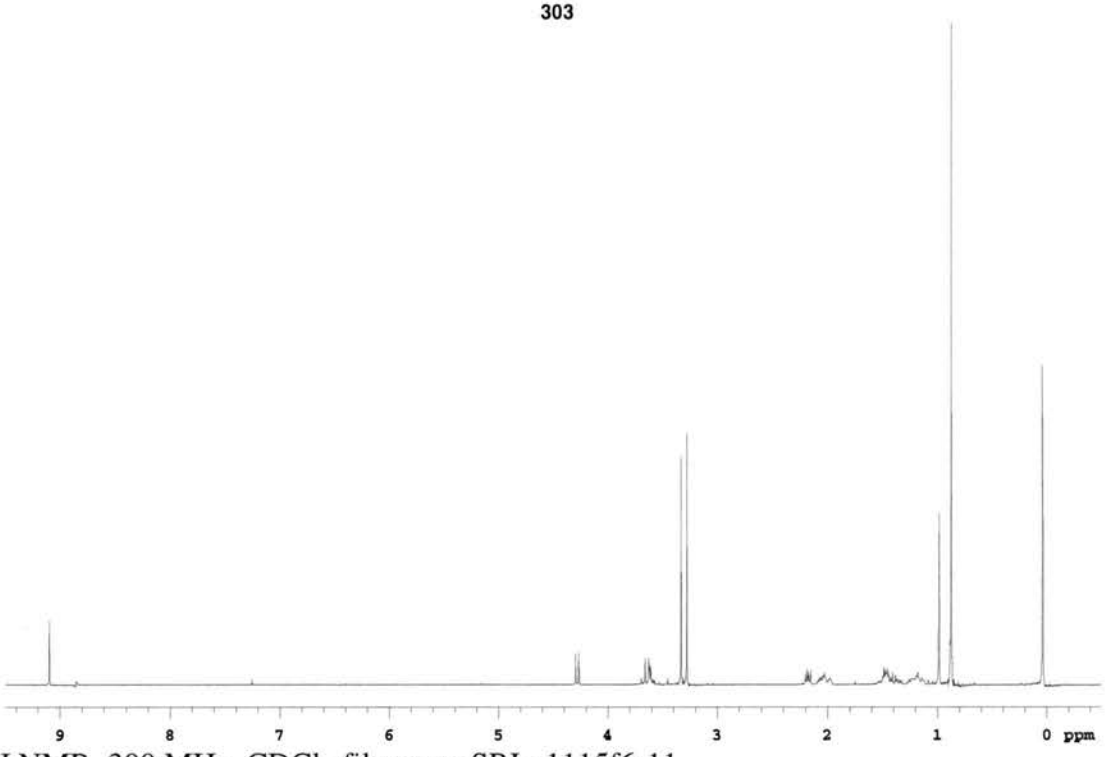
^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx1113apt

3-(*R*)-(tert-Butyl-dimethyl-silanyloxymethyl)-2-(*R*)-dimethoxymethyl-1-(*R*)-methyl-cyclohexanecarbaldehyde 303.

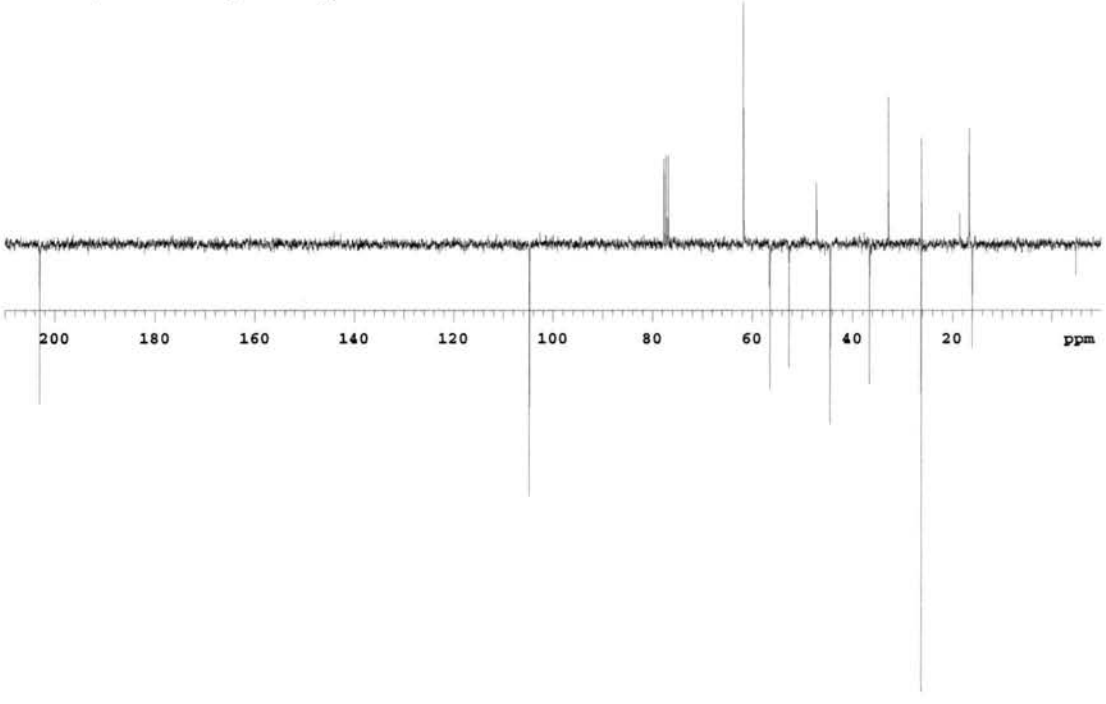
Oxalyl chloride (600 μL, 6.87 mmol) dissolved in 15 mL CH_2Cl_2 and cooled to -78°C. DMSO (854 μL, 12.03 mmol) added via syringe. Stirred 8 min, then solution of alcohol **302** (1.191 g, 3.44 mmol) in 7 mL CH_2Cl_2 added via cannula with 3 mL rinse. Stirred 15 min, then Et_3N (2.87 mL, 20.62 mmol) added via syringe. Stirred at -78°C for 15 min, then bath removed and reaction allowed to warm to room temp. Stirred 30 min after removal of bath, then quenched with 10 mL water, diluted with 25 mL hexanes, separated, and aqueous extracted twice with 10 mL hexanes. Combined organics washed once with 5 mL water, once with 5 mL brine, dried with $MgSO_4$ and concentrated *in vacuo* to yield 1.24 g aldehyde **303** as a pale oil, which was used crude in the next reaction. On smaller scale, purification by flash chromatography (7H: $1Et_2O$, 27 g silica) yielded 652.9 mg pale oil (81% yield). ^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 0.05 (6H, s), 0.89 (9H, s), 1.00 (3H, s), 1.13-1.29 (2H, m), 1.39 (1H, ddd, J=11.70Hz, J=11.70Hz, J=5.17Hz), 1.43-1.52 (2H, m), 1.97-2.06 (1H, m), 2.03-2.12 (2H, m), 2.19 (1H, dd, J=8.80Hz, J=5.30Hz), 3.29 (3H, s), 3.34 (3H, s), 3.58-3.71 (2m), 4.29 (1H, d, J=8.60Hz), 9.11 (1H, s). ^{13}C NMR (75MHz) ($CDCl_3$) δ TMS: -4.93, 16.03, 16.70, 18.60, 26.18, 26.23, 32.79, 36.57, 44.39, 47.14, 52.62, 56.44, 61.68, 104.74, 203.01.



303



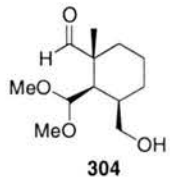
^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx1115f6-11



^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx1115apt

2-(*R*)-Dimethoxymethyl-3-(*R*)-hydroxymethyl-1-(*R*)-methyl-cyclohexanecarbaldehyde 304.

Tetrabutylammonium fluoride (1.0 M in THF, 2.53 mL, 2.53 mmol) was added via syringe to a 0°C solution of TBS ether **303** (670.9 mg, 1.947 mmol) in 10 mL THF. Stirred 6 min, then bath removed. Stirred 16 hours at room temperature, then diluted with 50 mL Et_2O, quenched with 5 mL sat. NH_4Cl sol. and 2 mL water, separated, and aqueous extracted twice with 10 mL Et_2O. Combined organics dried with Na_2CO_3 and $MgSO_4$ and concentrated *in vacuo*. Flash chromatography (3 EtOAc:1 hexanes, 20 g silica) yields 416.3 mg alcohol **304** as a pale oil (93% yield for two steps). ^1H NMR (300MHz) ($CDCl_3$) δ TMS: 1.02 (3H, s), 1.20-1.37 (2H, m), 1.40-1.53 (3H, m), 1.85-1.95 (2H, m), 1.96-2.06 (1H, m), 2.22 (1H, dd, J=7.90Hz, J=5.30Hz), 2.30 (1H, br. s), 3.32 (3H, s), 3.35 (3H, s), 3.62 (1H, dd, J=10.75Hz, J=7.75Hz), 3.72 (1H, dd, J=10.70Hz, J=5.60Hz), 4.40 (1H, d, J=7.90Hz), 9.14 (1H, s). ^{13}C NMR (75MHz) ($CDCl_3$) δ TMS: 16.68, 17.77, 26.81, 32.40, 37.46, 43.74, 47.41, 52.76, 56.59, 63.08, 105.26, 203.27.



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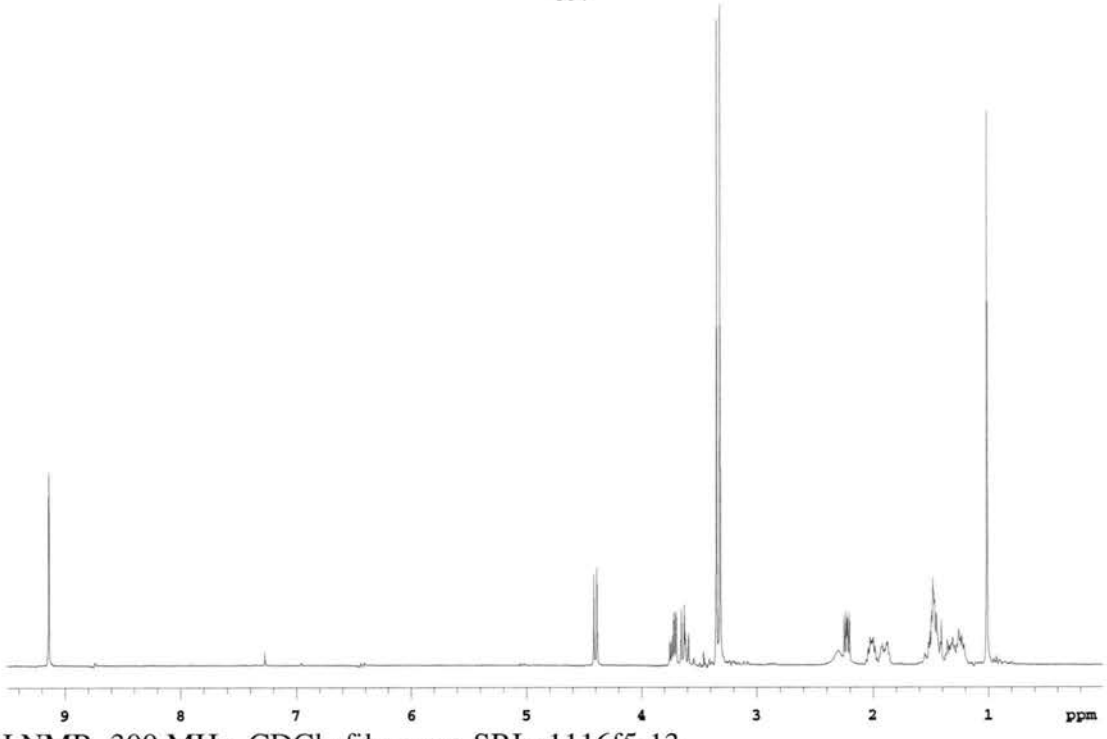
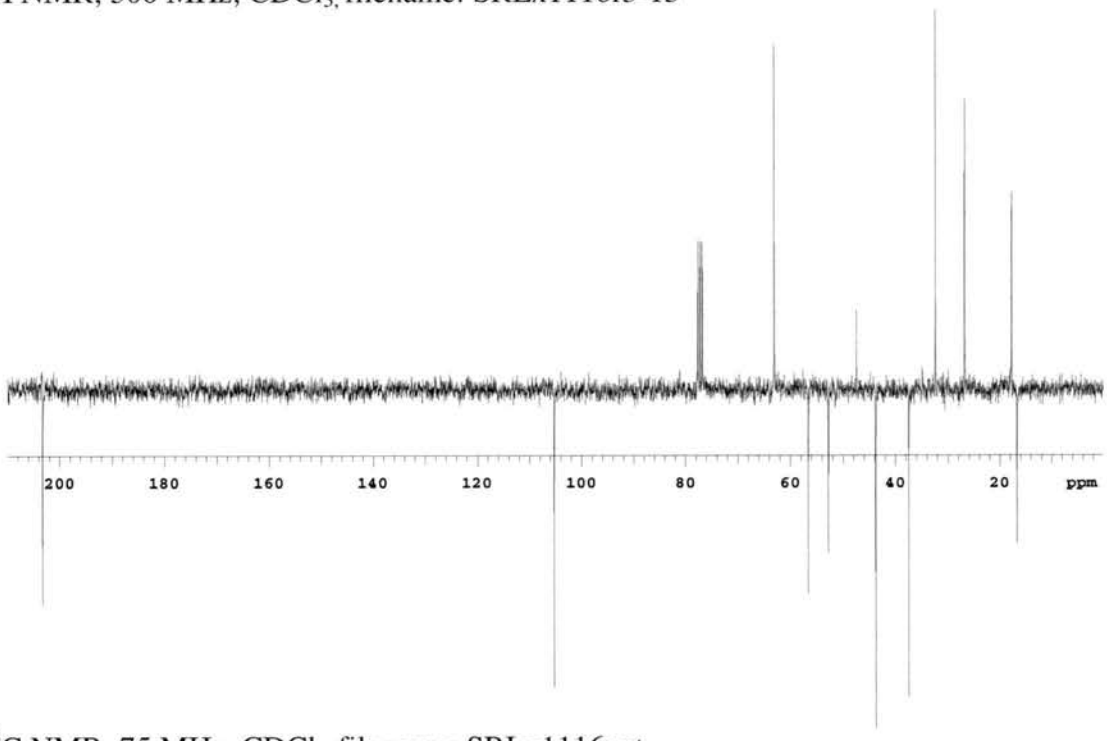
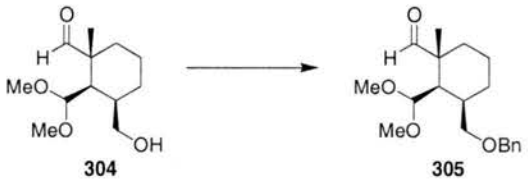


^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx1116f5-13



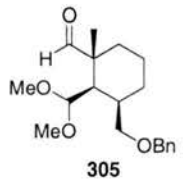
^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx1116apt



304 305

3-(*R*)-Benzyloxymethyl-2-(*R*)-dimethoxymethyl-1-(*R*)-methyl-cyclohexanecarbaldehyde 305.

NaH (32 mg of 60% suspension, 0.804 mmol) added to a 0°C sol. of alcohol **304** (46.3 mg, 0.201 mmol) in 2 mL DMF. Bath removed, stirred at room temp. 7 min., then benzyl bromide (31.1 μL, 0.261 mmol) added via syringe. Stirred 3 hours, diluted with 15 mL Et_2O, quenched with 1 mL sat. NH_4Cl sol. and 1 mL water, separated, and aqueous extracted twice with 3 mL Et_2O. Combined organics washed twice with 2 mL water, once with 2 mL brine, dried with $MgSO_4$, and concentrated *in vacuo*. Purified by flash chromatography (2 hexanes:1 Et_2O, 2.5 g silica) to yield 59.1 mg of benzyl ether **305** as a colorless oil (92% yield). ^1H NMR (300MHz) ($CDCl_3$) δ TMS: 0.99 (3H, s), 1.13-1.21 (1H, m), 1.27-1.55 (4H, m), 2.02 (1H, dd, J=13.40Hz, J=3.30Hz), 2.20 (1H, dd, J=8.80Hz, J=5.30Hz), 2.27-2.36 (1H, m), 3.29 (3H, s), 3.33 (3H, s), 3.51 (1H, dd, J=8.95Hz, J=4.05Hz), 3.57 (1H, dd, J=9.25Hz, J=9.25Hz), 4.30 (1H, d, J=8.80Hz), 4.50 (1H, ½ ABq, J=12.10Hz), 4.55 (1H, ½ ABq, J=11.90Hz), 7.26-7.40 (5H, m), 9.13 (1H, s). ^{13}C NMR (75MHz) ($CDCl_3$) δ TMS: 15.73, 16.74, 27.22, 32.63, 33.77, 44.59, 47.06, 53.04, 56.47, 69.40, 73.04, 104.92, 127.62, 127.67, 128.43, 138.55, 202.83.



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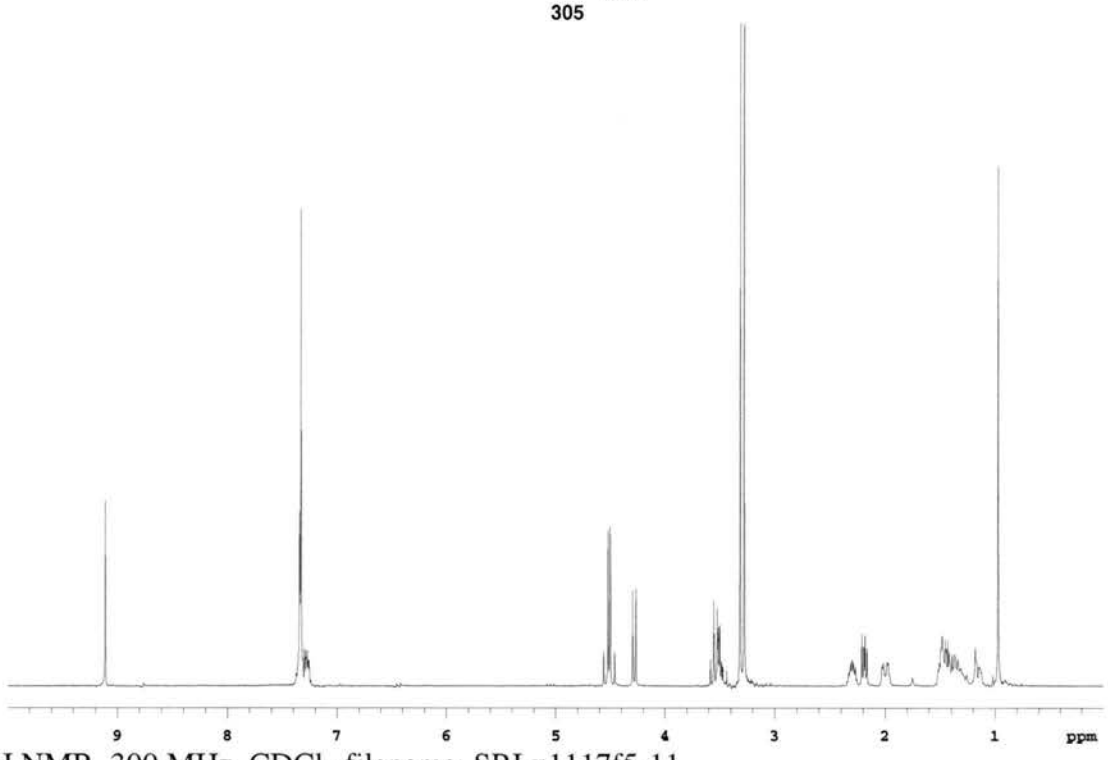
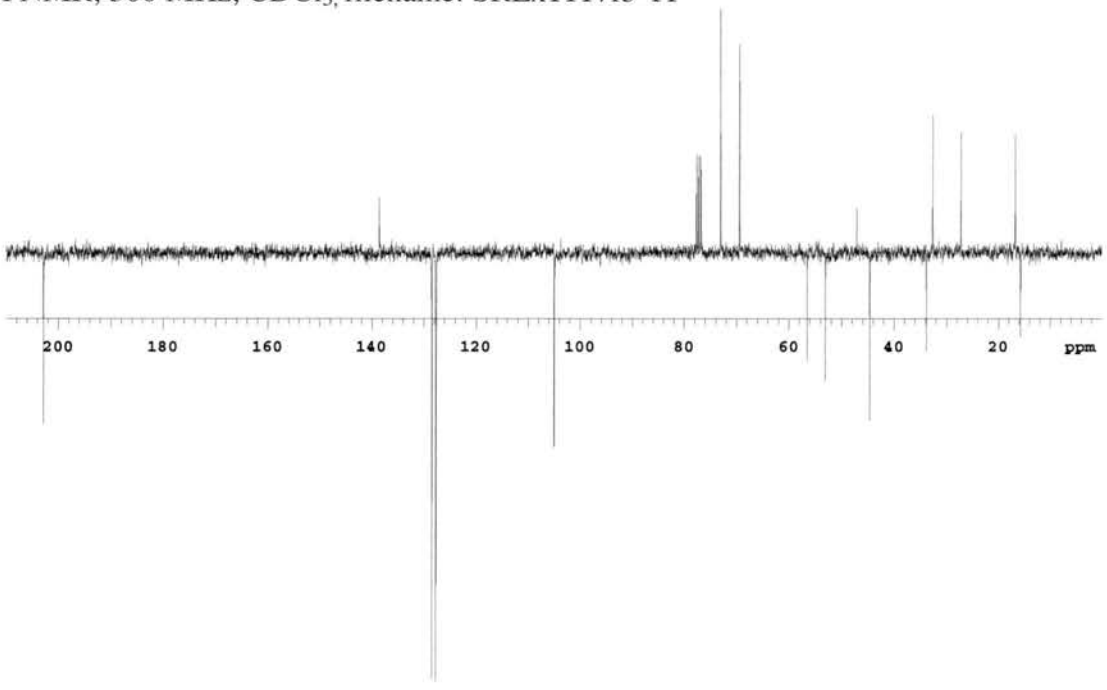


^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx1117f5-11

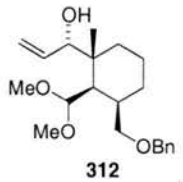


^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx1117apt

305 312

(*S*)-1-[3-(*R*)-Benzyloxymethyl-2-(*R*)-dimethoxymethyl-1-(*R*)-methyl-cyclohexyl]-prop-2-en-1-ol 312.

A 1.0 M solution of methylaluminum bis(2,6-di-*t*-butyl-4-methylphenoxide in toluene (448 μL, 0.448 mmol) was added via syringe to a -78°C solution of aldehyde **305** (47.8 mg, 0.149 mmol) in 3 mL toluene. Stirred 15 minutes, then vinyllithium (0.5 M in Et_2O, 1.19 mL, 0.597 mmol) added slowly via syringe. Stirred 2.5 hours with gradual warming to room temperature as dry ice dissipated, then quenched with 2 mL MeOH. Diluted with 5 mL Et_2O, then 2 mL ½ saturated NH_4Cl solution added, layers separated, and aqueous extracted twice with 4 mL Et_2O. Combined organics washed with 1.5 mL brine, dried with Na_2CO_3 and $MgSO_4$ and concentrated *in vacuo*. Purified by flash chromatography (3 hexanes:2 Et_2O, 3.5 g silica) to yield 49.5 mg of alcohol **312** as a colorless oil, with the α-displaced alcohol predominating by a ratio of 5.3:1 (92% yield). ^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 0.93 (3H, s), 1.21-1.29 (2H, m), 1.39-1.58 (3H, m), 1.64-1.76 (1H, m), 2.19-2.29 (2H, m), 2.38 (1H, br. s), 3.34 (3H, s), 3.35 (3H, s), 3.57 (1H, dd, J=8.60Hz, J=8.60Hz), 3.62 (1H, dd, J=9.25Hz, J=4.85Hz), 4.32-4.37 (1H, m), 4.42 (1H, d, J=3.80Hz), 4.53 (2H, dd, J=5.50Hz, J=4.20Hz), 5.19 (1H, ddd, J=10.47Hz, J=1.68Hz, J=0.98Hz), 5.25 (1H, ddd, J=17.23Hz, J=1.48Hz, J=1.48Hz), 5.96 (1H, ddd, J=17.07Hz, J=10.43Hz, J=6.63Hz), 7.26-7.37 (5H, m). ^{13}C NMR (75MHz) ($CDCl_3$) δ TMS: 19.02, 20.15, 26.64, 33.68, 36.38, 40.45, 43.46, 54.96, 55.53, 73.14, 73.56, 76.66, 108.64, 116.46, 127.46, 127.76, 128.37, 137.80, 138.97.



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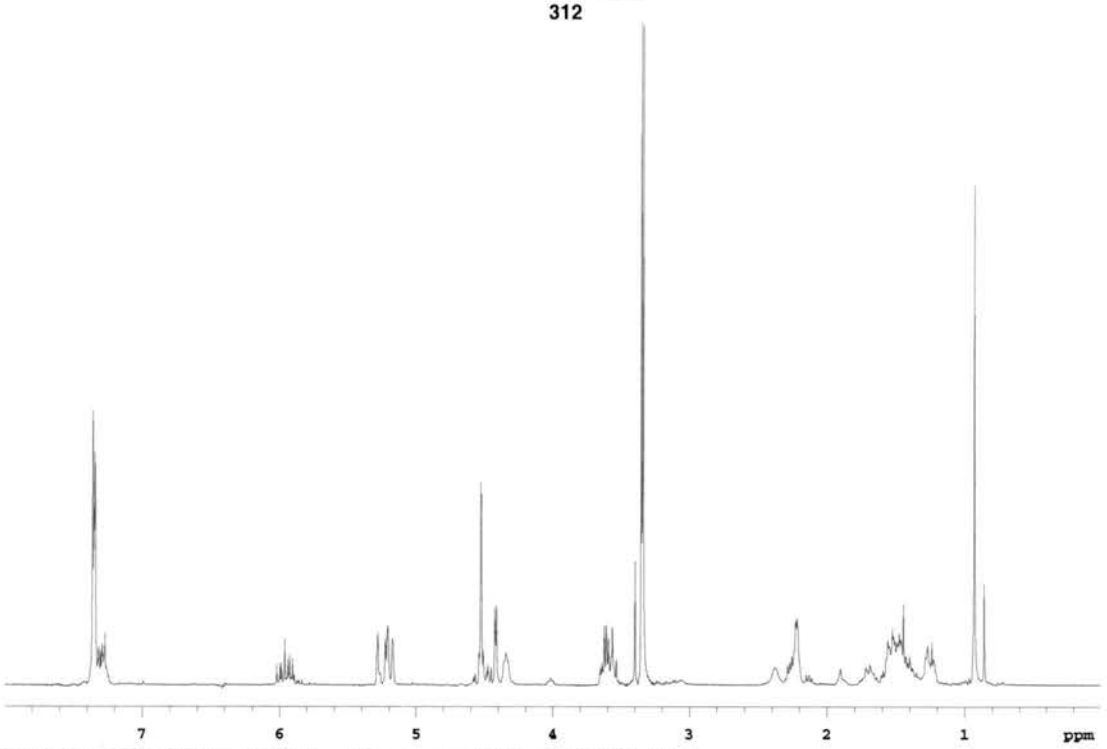
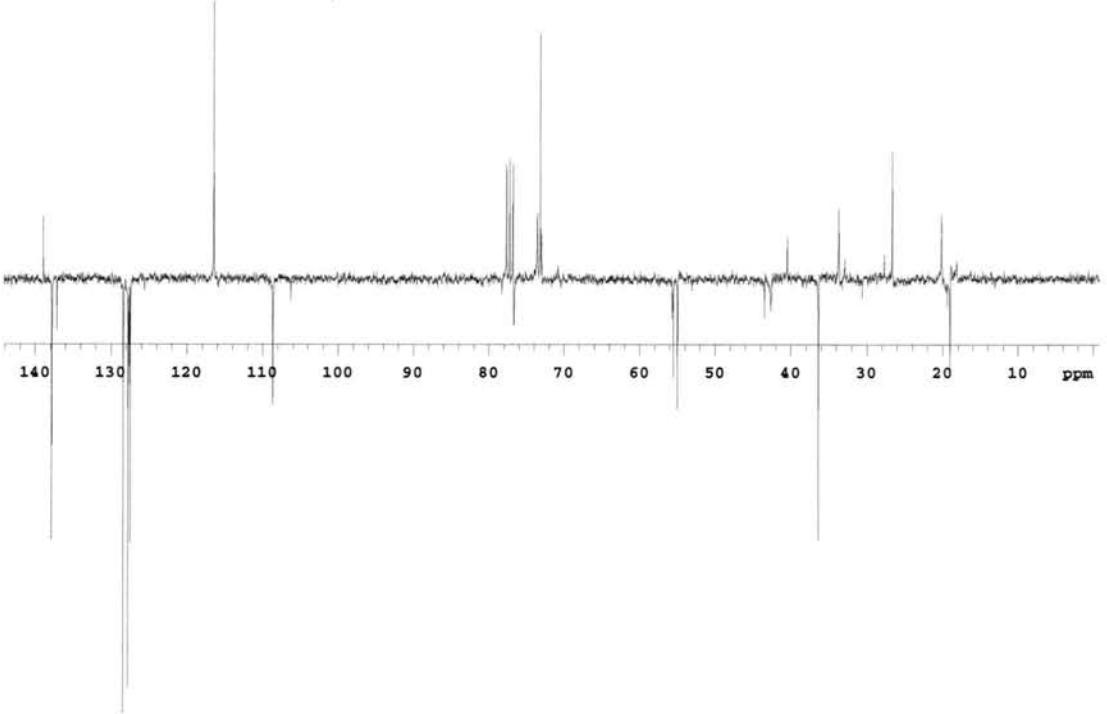


^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx1139f6-13

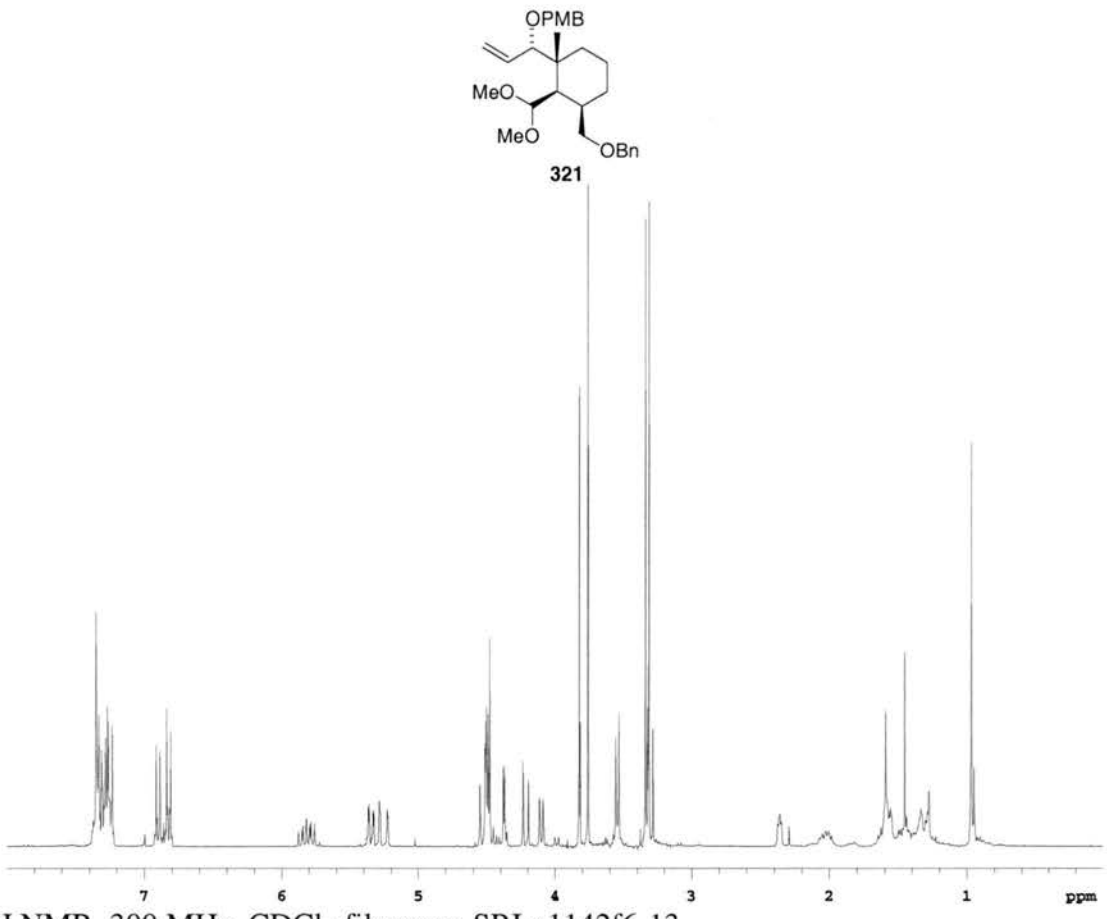


^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx1139apt

312 321

{2-(*R*)-Dimethoxymethyl-3-(*R*)-[1-(*S*)-(4-methoxy-benzyloxy)-allyl]-3-methyl-(*R*)-cyclohexyl}-methyl benzyl ether 321.

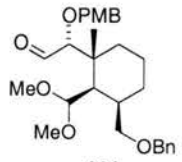
NaH (106 mg of 60% suspension, 2.64 mmol) added to a 0°C sol. of alcohol **312** (230.2 mg, 0.661 mmol) in 2.5 mL DMF. Bath removed, stirred at room temp. 7 min., then *p*-methoxybenzyl chloride (116.4 μL, 0.859 mmol) added via syringe. Stirred 18 hours, diluted with 10 mL hexanes and 10 mL Et_2O, quenched with 2.5 mL sat. NH_4Cl sol. and 2.5 mL water, separated, and aqueous extracted twice with 10 mL 1 hexanes: 1Et_2O. Combined organics washed with 4 mL water, with 4 mL brine, dried with Na_2CO_3 and $MgSO_4$, and concentrated *in vacuo*. Purified by flash chromatography (3 hexanes:1 Et_2O, 15 g silica) to yield 303.5 mg of benzyl ether **321** as a pale oil (98% yield). ^1H NMR (300MHz) ($CDCl_3$) δ TMS: 0.97 (3H, s), 1.27-1.37 (2H, m), 1.41-1.50 (1H, m), 1.54-1.65 (3H, m), 1.97-2.08 (1H, m), 2.36 (1H, dd, J=3.90Hz, J=3.20Hz), 3.32 (3H, s), 3.34 (3H, s), 3.54 (2H, d, J=6.70Hz), 3.76 (3H, s), 4.10 (1H, d, J=7.90Hz), 4.21 (1H, ½ ABq, J=11.40Hz), 4.38 (1H, d, J=2.90Hz), 4.47-4.51 (2H, m), 4.53 (1H, ½ ABq, J=11.40Hz), 5.25 (1H, ddd, J=17.22Hz, J=2.03Hz, J=0.78Hz), 5.35 (1H, dd, J=10.50Hz, J=2.20Hz), 5.82 (1H, ddd, J=17.70Hz, J=10.00Hz, J=7.25Hz), 6.82 (2H, ddd, J=9.08Hz, J=2.48Hz, J=2.48Hz), 7.25 (2H, d, J=8.80Hz), 7.29-7.36 (5H, m).



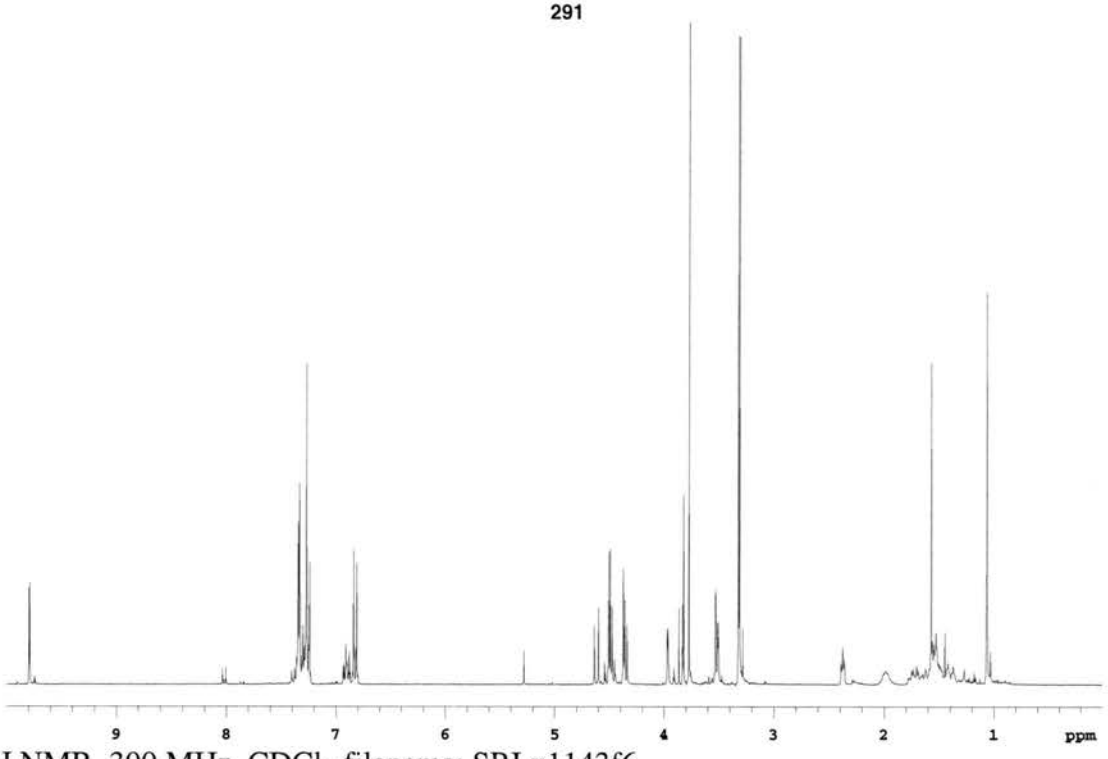
^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx1142f6-13

(3-(*R*)-Benzyloxymethyl-2-(*R*)-dimethoxymethyl-1-(*R*)-methyl-(*R*)-cyclohexyl)-(4-methoxy-benzyloxy)-acetaldehyde 291.

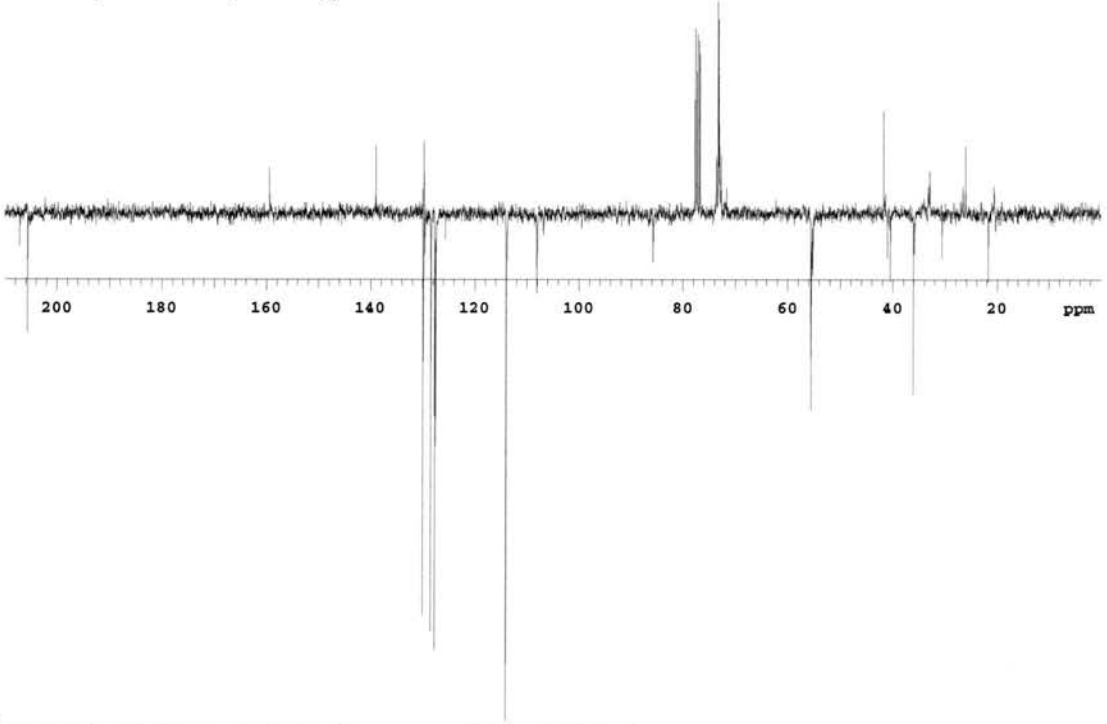
Alkene **321** (97.1 mg, 0.207 mmol, 2.2:1 ratio favoring α *p*-methoxybenzyloxy), pyridine (450 μL), and Sudan III dye solution (0.10% in MeOH, 200μL) dissolved in 5 mL MeOH and 5 mL CH_2Cl_2 and cooled to -78°C. Ozone bubbled through solution until red color had completely disappeared, then argon bubbled through reaction for 7 min. Dimethyl sulfide (761 μL, 10.36 mmol) added via syringe. Bath removed, and reaction stirred 16 hours at room temperature. (Rxn initially looks bad by TLC (2 Hex: 1 EtOAc), but gradually improves with stirring.) Diluted with 15 mL hexanes and 15 mL Et_2O, washed twice with 3 mL water, twice with 3 mL sat. $CuSO_4$ sol., dried with Na_2CO_3 and $MgSO_4$ and concentrated *in vacuo*. Purified by flash chromatography (2 Hex:1 Et_2O, 10 g silica) to yield 49.2 mg aldehyde **291** as a pale oil (50% yield, 3.5:1 ratio favoring α *p*-methoxybenzyloxy) and 44.1 mg recovered olefin (45% recovery). ^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 1.07 (3H, s), 1.34-1.44 (1H, m), 1.46-1.57 (3H, m), 1.61-1.79 (2H, m), 1.94-2.03 (1H, m), 2.38 (1H, dd, J=4.40Hz, J=4.40Hz), 3.31 (3H, s), 3.32 (3H, s), 3.51 (1H, d, J=2.40Hz), 3.53 (1H, s), 3.77 (3H, s), 3.96 (1H, d, J=2.60Hz), 4.36 (1H, ½ ABq, J=10.60Hz), 4.37 (1H, d, J=3.80Hz), 4.47-4.51 (2H, m), 4.62 (1H, ½ ABq, J=11.40Hz), 6.83 (2H, ddd, J=9.18Hz, J=2.58Hz, J=2.58Hz), 7.26 (2H, d, J=9.00Hz), 7.28-7.36 (5H, m), 9.79 (1H, d, J=2.60Hz). ^{13}C NMR (75MHz) ($CDCl_3$) δ TMS: 20.65, 21.70, 26.07, 30.60, 32.97, 36.05, 40.43, 41.04, 41.73, 55.44, 55.58, 73.12, 73.24, 73.60, 108.05, 113.90, 127.43, 127.62, 128.37, 129.70, 129.86, 139.01, 159.41, 205.64.



291



^{1}H NMR, 300 MHz, $CDCl_3$, filename: SRLx1143f6



^{13}C NMR, 75 MHz, $CDCl_3$, filename: SRLx1175apt

Appendix 1

X-ray Crystal Structure Data for Compound 171

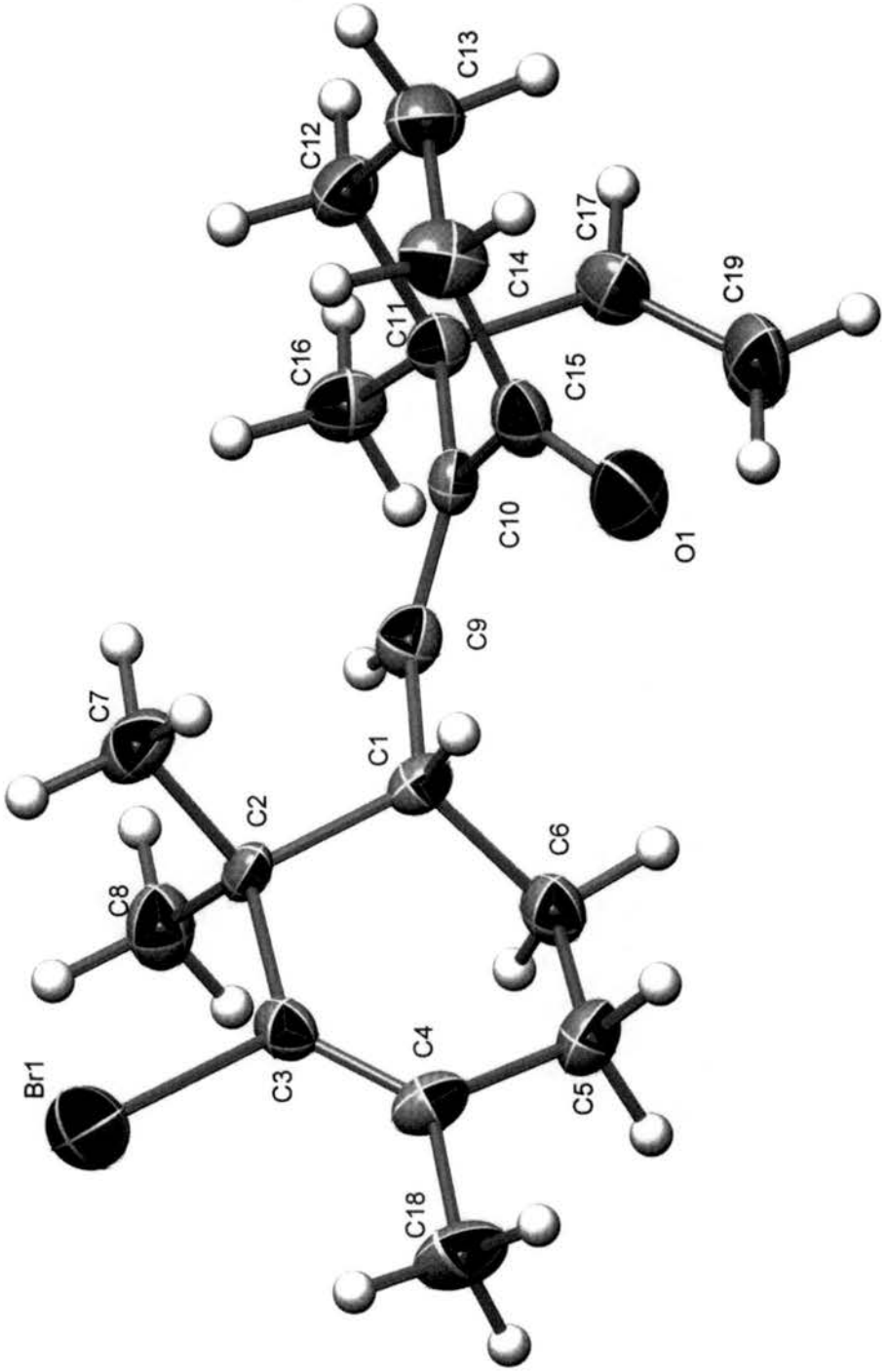

C13
C12
C17
C14
C19
C11
C16
C15
C10
O1
C9
C7
C1
C2
C6
C8
C4
Br1
C3
C5
C18

Table 1. Crystal data and structure refinement for **171**.

Identification code	rwccd19m	
Empirical formula	C_{19} H_{27} Br O	
Formula weight	351.32	
Temperature	171(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	$P2_1/n$	
Unit cell dimensions	a = 11.436(3) Å	α= 90°.
	b = 13.132(3) Å	β= 106.111(5)°.
	c = 12.311(3) Å	γ = 90°.
Volume, Z	1776.1(7) $Å^3$,4	
Density (calculated)	1.314 Mg/m^3	
Absorption coefficient	2.312 mm^{-1}	
F(000)	736	
Crystal size	0.20 x 0.25 x 0.50 mm	
θ range for data collection	2.15 to 28.34°.	
Limiting indices	$-15 \leq h \leq 14$, $-15 \leq k \leq 17$, $-16 \leq l \leq 14$	
Reflections collected	11832	
Independent reflections	4314 [R_{int} = 0.1377]	
Completeness to theta = 28.34°	97.2 %	
Absorption correction	None	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4314 / 0 / 191	
Goodness-of-fit on F^2	0.800	
Final R indices [I>2σ(I)]	R1 = 0.0551, wR2 = 0.0999	
R indices (all data)	R1 = 0.1582, wR2 = 0.1207	
Largest diff. peak and hole	0.505 and -0.442 $e.Å^{-3}$	

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for **171**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
Br(1)	4207(1)	8340 (1)	3200 (1)	52(1)
O(1)	5315 (3)	6605 (2)	-1505 (3)	48(1)
C(1)	3569 (4)	7295 (3)	-186 (3)	26(1)
C(2)	3533 (3)	8081 (3)	768 (3)	25(1)
C(3)	3833 (4)	7475 (3)	1874 (3)	28(1)
C(4)	3837 (3)	6490 (3)	2010 (3)	31(1)
C(5)	3492 (4)	5774 (3)	1010 (3)	34(1)
C(6)	2894 (4)	6321 (3)	-90 (3)	33(1)
C(7)	4472 (4)	8903 (3)	777 (4)	42(1)
C(8)	2269 (4)	8567 (3)	577 (4)	42(1)
C(9)	3107 (4)	7724 (3)	-1361 (3)	31(1)
C(10)	3677 (3)	7782 (3)	-2152 (3)	24(1)
C(11)	3111 (3)	8226 (3)	-3328 (3)	28(1)
C(12)	3991 (3)	9025 (3)	-3578 (4)	35(1)
C(13)	5250 (4)	8608 (3)	-3469 (4)	41(1)
C(14)	5814 (4)	8142 (4)	-2290 (4)	42(1)
C(15)	4965 (4)	7421 (3)	-1952 (3)	34(1)
C(16)	1878 (4)	8728 (3)	-3460 (3)	38(1)
C(17)	2926 (4)	7388 (4)	-4225 (4)	39(1)
C(18)	4188 (4)	5935 (4)	3136 (3)	47(1)
C(19)	2998 (4)	6406 (4)	4043 (4)	55(2)

Table 3. Bond lengths [Å] and angles [°] for **171**.

Br(1)-C(3)	1.936(4)
C(1)-C(9)	1.504(5)
C(1)-C(2)	1.573(5)
C(2)-C(3)	1.531(5)
C(3)-C(4)	1.303(5)
C(4)-C(18)	1.519(5)
C(9)-C(10)	1.316(5)
C(10)-C(11)	1.528(5)
C(11)-C(17)	1.531(6)
C(12)-C(13)	1.511(5)
C(14)-C(15)	1.495(6)
O(1)-C(15)	1.220(5)
C(1)-C(6)	1.516(5)
C(2)-C(7)	1.521(5)
C(2)-C(8)	1.537(5)
C(4)-C(5)	1.513(6)
C(5)-C(6)	1.517(5)
C(10)-C(15)	1.501(6)
C(11)-C(16)	1.524(5)
C(11)-C(12)	1.543(5)
C(13)-C(14)	1.543(6)
C(17)-C(19)	1.308(6)
C(9)-C(1)-C(6)	109.9(3)
C(6)-C(1)-C(2)	112.0(3)
C(7)-C(2)-C(8)	109.8(3)
C(7)-C(2)-C(1)	107.8(3)
C(8)-C(2)-C(1)	112.1(3)
C(4)-C(3)-Br(1)	118.8(3)
C(3)-C(4)-C(5)	121.4(4)
C(5)-C(4)-C(18)	112.8(4)
C(1)-C(6)-C(5)	110.9(3)
C(9)-C(10)-C(15)	121.7(4)
C(15)-C(10)-C(11)	114.4(3)

C(16)-C(11)-C(17)	107.2(3)
C(16)-C(11)-C(12)	108.6(3)
C(17)-C(11)-C(12)	108.2(3)
C(12)-C(13)-C(14)	111.5(3)
O(1)-C(15)-C(14)	121.6(4)
C(14)-C(15)-C(10)	116.0(4)
C(9)-C(1)-C(2)	113.4(3)
C(7)-C(2)-C(3)	112.3(3)
C(3)-C(2)-C(8)	108.7(3)
C(3)-C(2)-C(1)	106.1(3)
C(4)-C(3)-C(2)	128.4(4)
C(2)-C(3)-Br(1)	112.8(3)
C(3)-C(4)-C(18)	125.7(4)
C(4)-C(5)-C(6)	112.5(3)
C(10)-C(9)-C(1)	128.3(4)
C(9)-C(10)-C(11)	123.9(4)
C(16)-C(11)-C(10)	113.7(3)
C(10)-C(11)-C(17)	110.3(3)
C(10)-C(11)-C(12)	108.7(3)
C(13)-C(12)-C(11)	113.2(3)
C(15)-C(14)-C(13)	112.2(3)
O(1)-C(15)-C(10)	122.4(4)
C(19)-C(17)-C(11)	126.6(4)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for **171**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

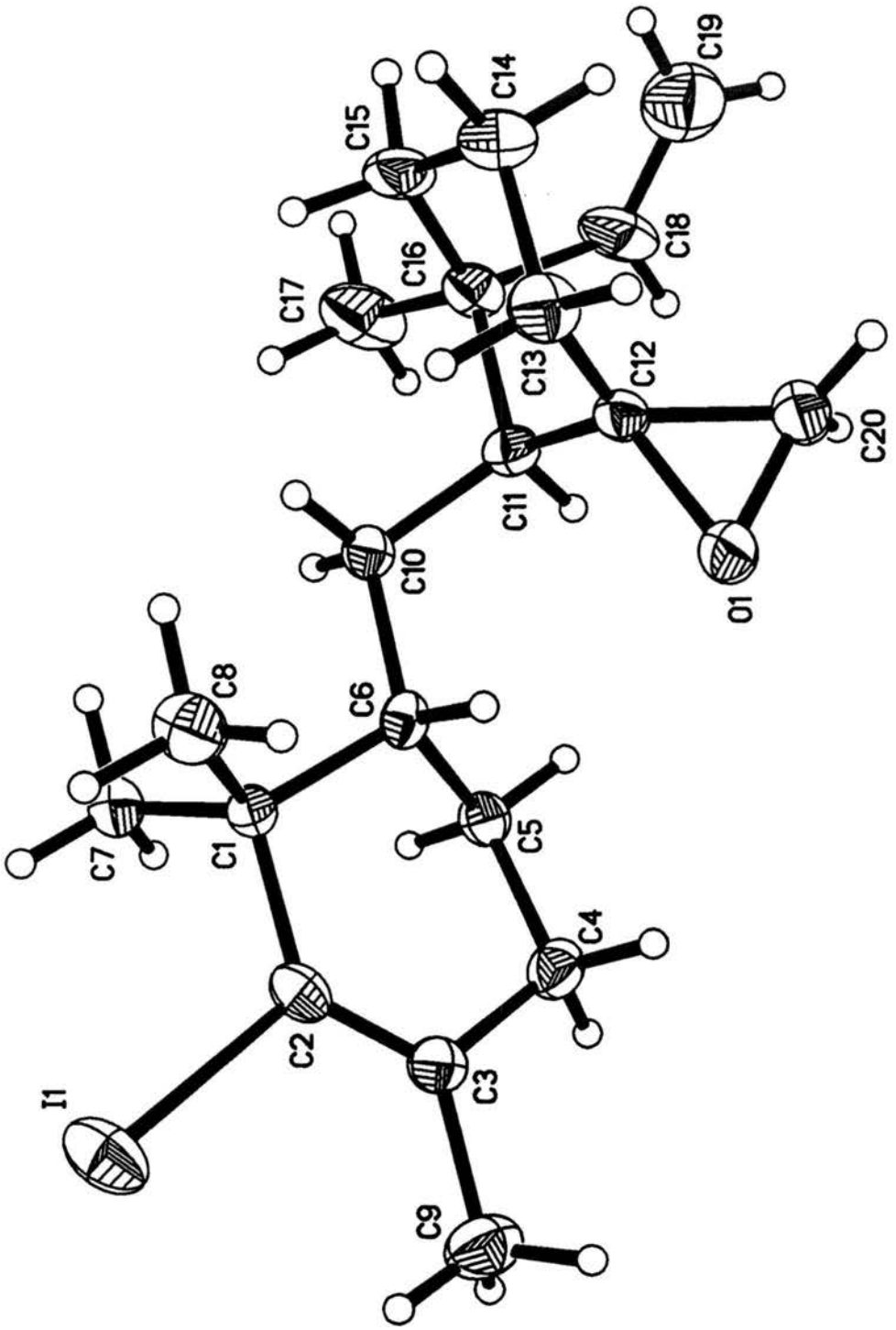
	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
Br(1)	70(1)	54(1)	27(1)	-11(1)	6(1)	-18(1)
O(1)	57(2)	40(2)	45(2)	8(2)	10(2)	20(2)
C(1)	27(2)	25(3)	25(2)	4(2)	3(2)	-4(2)
C(2)	36(3)	16(2)	22(2)	1(2)	6(2)	-4(2)
C(3)	29(3)	32(3)	21(2)	-4(2)	6(2)	-2(2)
C(4)	28(2)	33(3)	31(3)	10(2)	4(2)	-3(2)
C(5)	38(3)	24(3)	38(3)	4(2)	6(2)	0(2)
C(6)	39(3)	33(3)	24(2)	-5(2)	5(2)	-1(2)
C(7)	53(3)	32(3)	37(3)	1(2)	7(2)	-16(2)
C(8)	53(3)	33(3)	35(3)	1(2)	4(2)	10(2)
C(9)	33(3)	28(3)	29(3)	2(2)	2(2)	0(2)
C(10)	27(2)	17(2)	24(2)	-2(2)	4(2)	1(2)
C(11)	30(2)	27(3)	26(2)	1(2)	6(2)	2(2)
C(12)	40(3)	27(3)	36(3)	5(2)	9(2)	1(2)
C(13)	44(3)	41(3)	42(3)	5(2)	16(2)	4(2)
C(14)	31(3)	53(3)	41(3)	7(2)	8(2)	9(2)
C(15)	43(3)	30(3)	25(2)	-6(2)	4(2)	4(2)
C(16)	40(3)	41(3)	30(3)	9(2)	5(2)	4(2)
C(17)	51(3)	40(3)	21(2)	-2(2)	4(2)	3(2)
C(18)	56(3)	49(3)	33(3)	19(2)	6(2)	-2(3)
C(19)	87(4)	40(4)	36(3)	-16(2)	12(3)	-8(3)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for **171**.

	x	y	z	U(eq)
H(1A)	4442	7108	-77	32
H(5A)	2926	5251	1147	41
H(5B)	4232	5421	938	41
H(6A)	2887	5869	-735	39
H(6B)	2039	6484	-122	39
H(7A)	4463	9407	1362	62
H(7B)	4276	9238	37	62
H(7C)	5282	8594	938	62
H(8A)	2285	9048	1189	63
H(8B)	1667	8034	566	63
H(8C)	2050	8929	-148	63
H(9A)	2302	7987	-1556	38
H(12A)	3648	9288	-4355	41
H(12B)	4056	9604	-3049	41
H(13A)	5781	9163	-3600	49
H(13B)	5203	8081	-4054	49
H(14A)	6570	7775	-2291	50
H(14B)	6035	8696	-1724	50
H(16A)	1309	8229	-3303	57
H(16B)	1560	8982	-4235	57
H(16C)	1970	9297	-2928	57
H(17A)	2739	7604	-4990	47
H(18A)	4395	6433	3754	71
H(18B)	4893	5497	3177	71
H(18C)	3503	5517	3206	71
H(19A)	3182	6151	-3292	66
H(19B)	2867	5947	-4662	66

Appendix 2

X-ray Crystal Structure Data for Compound 197


I1
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C3
C9
C4
C5
C1
C7
C8
C6
C10
C11
C12
C13
C16
C17
C15
C14
C18
C19
O1
C20

Table 1. Crystal data and structure refinement for **197**.

Identification code	rwccd20 (Lenger/Williams)	
Empirical formula	$C_{20}H_{31}IO$	
Formula weight	414.35	
Temperature	293(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	$P2_12_12_1$	
Unit cell dimensions	a = 11.1632(17) Å	$\alpha = 90°$.
	b = 12.802(2) Å	$\beta = 90°$.
	c = 13.349(2) Å	$\gamma = 90°$.
Volume, Z	1907.7(5) $Å^3$,4	
Density (calculated)	1.443 Mg/m^3	
Absorption coefficient	1.680 mm^{-1}	
F(000)	848	
Crystal size	0.40 x 0.30 x 0.20 mm	
θ range for data collection	2.20 to 28.31°.	
Limiting indices	$-14 \leq h \leq 14$, $-15 \leq k \leq 16$, $-14 \leq l \leq 17$	
Reflections collected	12898	
Independent reflections	4548 [$R_{int} = 0.0347$]	
Completeness to theta = 28.34°	98.2 %	
Absorption correction	SADABS	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4548/ 0 / 199	
Goodness-of-fit on F^2	0.687	
Final R indices [$I>2\sigma(I)$]	R1 = 0.0276, wR2 = 0.0516	
R indices (all data)	R1 = 0.0519, wR2 = 0.0549	
Absolute structure parameter	0.000(17)	
Largest diff. peak and hole	0.444 and -0.482 $e.Å^{-3}$	

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for rwccd20. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
I(1)	4104(1)	416(1)	2111(1)	51(1)
O(1)	-135(2)	-3397(2)	1522(2)	42(1)
C(1)	3359(3)	-1889(2)	1729(2)	25(1)
C(2)	3072(3)	-772(2)	1400(2)	28(1)
C(3)	2316(3)	-464(3)	699(2)	29(1)
C(4)	1666(3)	-1263(3)	71(2)	33(1)
C(5)	2230(3)	-2335(2)	151(2)	29(1)
C(6)	2417(3)	-2620(2)	1250(2)	24(1)
C(7)	4631(3)	-2154(3)	1402(3)	37(1)
C(8)	3260(3)	-1975(3)	2876(2)	42(1)
C(9)	2057(3)	655(3)	389(3)	47(1)
C(10)	2710(3)	-3790(2)	1366(2)	26(1)
C(11)	1672(3)	-4529(3)	1146(2)	28(1)
C(12)	570(3)	-4321(2)	1776(2)	29(1)
C(13)	748(3)	-4510(2)	2873(2)	39(1)
C(14)	1058(3)	-5664(2)	3039(2)	42(1)
C(15)	2111(3)	-5991(3)	2370(2)	43(1)
C(16)	1972(3)	-5721(2)	1255(2)	32(1)
C(17)	3148(3)	-5985(3)	715(3)	60(1)
C(18)	998(4)	-6336(3)	719(3)	49(1)
C(19)	366(4)	-7102(3)	1044(3)	68(1)
C(20)	-631(3)	-4407(2)	1357(3)	44(1)

Table 3. Bond lengths [Å] and angles [°] for rwccd20.

I(1)-C(2)	2.130(3)
O(1)-C(20)	1.425(4)
O(1)-C(12)	1.461(3)
C(1)-C(7)	1.524(4)
C(1)-C(2)	1.530(4)
C(1)-C(8)	1.539(4)
C(1)-C(6)	1.547(4)
C(2)-C(3)	1.321(4)
C(3)-C(4)	1.508(4)
C(3)-C(9)	1.519(5)
C(4)-C(5)	1.514(4)
C(5)-C(6)	1.526(4)
C(6)-C(10)	1.541(4)
C(10)-C(11)	1.525(4)
C(11)-C(12)	1.513(4)
C(11)-C(16)	1.569(5)
C(12)-C(20)	1.457(4)
C(12)-C(13)	1.497(4)
C(13)-C(14)	1.534(4)
C(14)-C(15)	1.534(4)
C(15)-C(16)	1.536(5)
C(16)-C(18)	1.521(5)
C(16)-C(17)	1.535(4)
C(18)-C(19)	1.283(5)
C(20)-O(1)-C(12)	60.64(18)
C(7)-C(1)-C(2)	108.8(3)
C(7)-C(1)-C(8)	109.7(3)
C(2)-C(1)-C(8)	109.7(3)
C(7)-C(1)-C(6)	112.3(2)
C(2)-C(1)-C(6)	107.7(2)
C(8)-C(1)-C(6)	108.6(3)
C(3)-C(2)-C(1)	128.0(3)
C(3)-C(2)-I(1)	116.6(2)

C(1)-C(2)-I(1)	115.2(2)
C(2)-C(3)-C(4)	119.9(3)
C(2)-C(3)-C(9)	126.5(3)
C(4)-C(3)-C(9)	113.4(3)
C(5)-C(4)-C(3)	112.1(3)
C(4)-C(5)-C(6)	109.9(2)
C(5)-C(6)-C(10)	111.0(2)
C(5)-C(6)-C(1)	110.2(2)
C(10)-C(6)-C(1)	113.7(2)
C(11)-C(10)-C(6)	115.0(2)
C(12)-C(11)-C(10)	113.6(2)
C(12)-C(11)-C(16)	107.1(3)
C(10)-C(11)-C(16)	115.0(2)
O(1)-C(12)-C(20)	58.45(18)
O(1)-C(12)-C(13)	115.4(2)
C(20)-C(12)-C(13)	119.0(3)
O(1)-C(12)-C(11)	116.9(2)
C(20)-C(12)-C(11)	121.4(3)
C(13)-C(12)-C(11)	114.0(3)
C(12)-C(13)-C(14)	109.1(3)
C(15)-C(14)-C(13)	110.6(3)
C(14)-C(15)-C(16)	115.2(3)
C(18)-C(16)-C(15)	114.3(3)
C(18)-C(16)-C(17)	106.1(3)
C(15)-C(16)-C(17)	108.6(3)
C(18)-C(16)-C(11)	107.9(3)
C(15)-C(16)-C(11)	109.2(2)
C(17)-C(16)-C(11)	110.7(3)
C(19)-C(18)-C(16)	129.0(4)
O(1)-C(20)-C(12)	60.92(19)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for rwccd20. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
I(1)	60(1)	44(1)	48(1)	-12(1)	0(1)	-18(1)
O(1)	27(1)	30(1)	68(2)	4(1)	-2(1)	2(1)
C(1)	23(2)	26(2)	26(2)	3(1)	-2(1)	-2(1)
C(2)	29(2)	30(2)	26(2)	-3(1)	6(1)	-7(1)
C(3)	26(2)	28(2)	34(2)	0(2)	7(1)	2(2)
C(4)	29(2)	39(2)	32(2)	8(2)	0(2)	2(2)
C(5)	33(2)	27(2)	28(2)	-3(1)	-9(1)	-5(2)
C(6)	21(2)	31(2)	21(2)	-1(1)	2(1)	3(1)
C(7)	30(2)	34(2)	47(2)	3(2)	-5(2)	-2(2)
C(8)	54(2)	44(2)	27(2)	-1(2)	-6(2)	-11(2)
C(9)	47(2)	37(3)	56(2)	6(2)	7(2)	10(2)
C(10)	24(2)	27(2)	29(2)	0(1)	3(1)	1(2)
C(11)	33(2)	25(2)	24(2)	1(2)	-1(1)	0(2)
C(12)	26(2)	21(2)	40(2)	1(1)	1(1)	-1(1)
C(13)	36(2)	41(2)	38(2)	0(2)	11(2)	3(2)
C(14)	52(2)	38(2)	37(2)	7(2)	5(2)	-1(2)
C(15)	43(2)	21(2)	66(3)	10(2)	5(2)	3(2)
C(16)	34(2)	22(2)	40(2)	-6(1)	7(2)	1(1)
C(17)	61(3)	29(2)	89(3)	-9(2)	27(2)	2(2)
C(18)	59(3)	25(2)	63(2)	0(2)	11(2)	-10(2)
C(19)	73(3)	49(3)	82(3)	-3(2)	-7(2)	4(2)
C(20)	30(2)	32(2)	71(2)	4(2)	-3(2)	-1(2)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for rwccd20.

	x	y	z	U(eq)
H(4A)	837	-1303	287	40
H(4B)	1673	-1042	-624	40
H(5A)	2994	-2339	-195	35
H(5B)	1715	-2849	-165	35
H(6A)	1656	-2495	1596	29
H(7A)	5185	-1686	1719	56
H(7B)	4695	-2086	688	56
H(7C)	4815	-2860	1592	56
H(8A)	3844	-1527	3183	62
H(8B)	3402	-2685	3076	62
H(8C)	2472	-1767	3083	62
H(9A)	2505	1124	807	70
H(9B)	1217	795	463	70
H(9C)	2287	754	-297	70
H(10A)	3368	-3961	921	32
H(10B)	2981	-3912	2047	32
H(11A)	1441	-4415	446	33
H(13A)	22	-4334	3235	46
H(13B)	1391	-4072	3122	46
H(14A)	1267	-5775	3736	51
H(14B)	365	-6092	2886	51
H(15A)	2221	-6739	2432	52
H(15B)	2833	-5658	2619	52
H(17A)	3325	-6713	802	90
H(17B)	3787	-5574	993	90
H(17C)	3069	-5832	14	90
H(18A)	838	-6128	65	59
H(19A)	480	-7350	1692	82
H(19B)	-205	-7409	631	82
H(20A)	-704	-4645	669	53
H(20B)	-1268	-4636	1799	53

Appendix 3

X-ray Crystal Structure Data for Compound 247

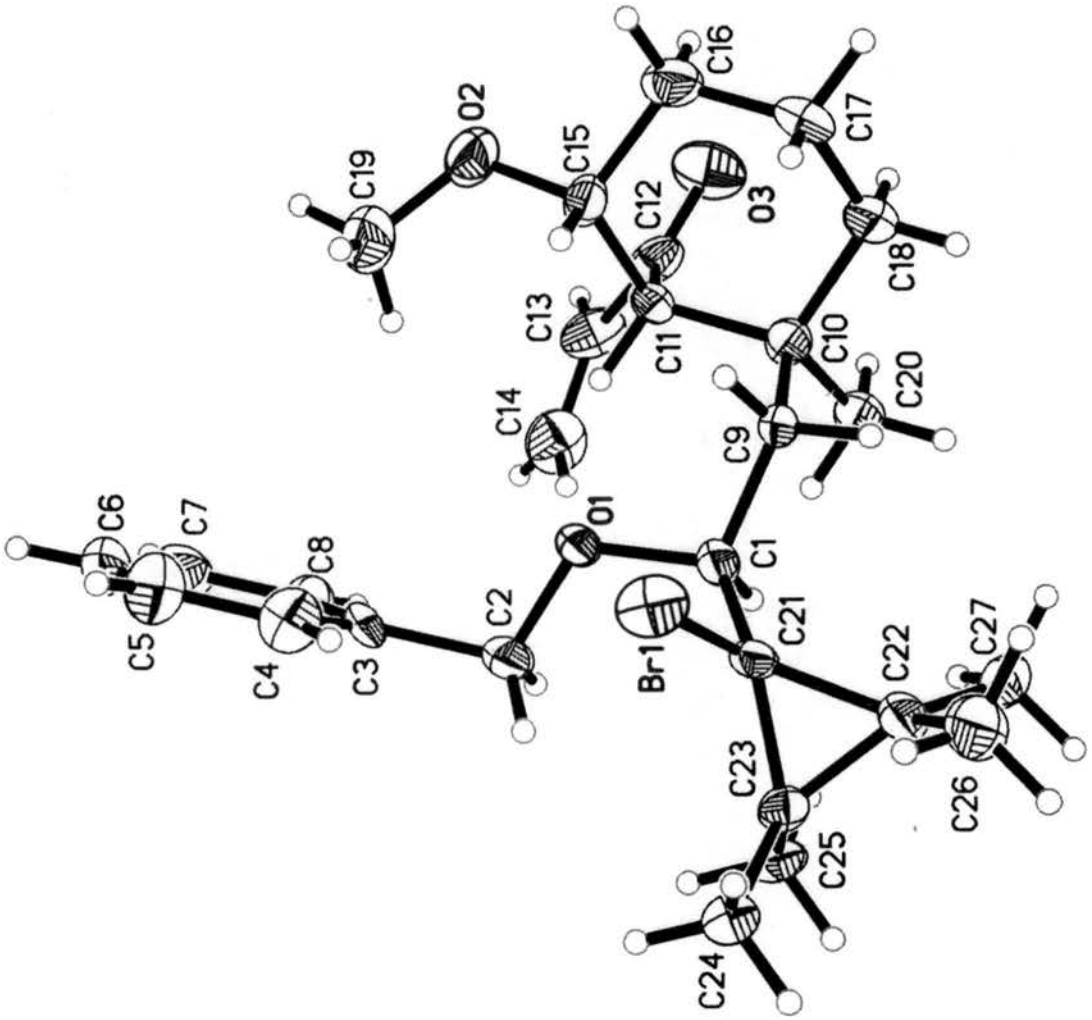
C6
C7
C8
C5
C4
C3
C2
O1
C1
Br1
C21
C22
C27
C23
C26
C25
C24
C9
C14
C13
C11
C10
C20
C12
O3
C18
C15
O2
C19
C16
C17

Table 1. Crystal data and structure refinement for **247**.

Identification code	rw23	
Empirical formula	$C_{27}\ H_{39}\ Br\ O_3$	
Formula weight	491.49	
Temperature	298(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P2(1)/c	
Unit cell dimensions	a = 7.0815(9) Å	α= 90°.
	b = 17.455(2) Å	β= 95.404(2)°.
	c = 20.684(3) Å	γ = 90°.
Volume	2545.4(6) $Å^3$	
Z	4	
Density (calculated)	1.283 Mg/m^3	
Absorption coefficient	1.639 mm^{-1}	
F(000)	1040	
Crystal size	0.08 x 0.25 x 0.28 mm^3	
Theta range for data collection	3.06 to 23.25°.	
Index ranges	-7<=h<=7, -19<=k<=17, -22<=l<=22	
Reflections collected	12883	
Independent reflections	3628 [R(int) = 0.1728]	
Completeness to theta = 23.25°	99.8 %	
Absorption correction	None	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3628 / 0 / 281	
Goodness-of-fit on F^2	0.751	
Final R indices [I>2sigma(I)]	R1 = 0.0509, wR2 = 0.0744	
R indices (all data)	R1 = 0.1724, wR2 = 0.0975	
Largest diff. peak and hole	0.342 and -0.308 e.$Å^{-3}$	

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for **247**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
Br(1)	4674(1)	314(1)	3059(1)	65(1)
O(1)	8053(5)	258(2)	2273(2)	46(1)
O(2)	7675(6)	1439(3)	395(2)	74(1)
O(3)	11215(6)	2510(3)	956(2)	86(2)
C(1)	8371(8)	764(3)	2816(3)	41(2)
C(2)	8711(9)	-504(3)	2376(3)	57(2)
C(3)	8087(12)	-972(3)	1781(3)	51(2)
C(4)	6230(12)	-1223(4)	1687(4)	75(2)
C(5)	5631(14)	-1650(5)	1140(5)	103(3)
C(6)	6920(20)	-1824(5)	707(5)	108(4)
C(7)	8752(16)	-1574(5)	792(4)	90(3)
C(8)	9324(11)	-1152(4)	1336(4)	66(2)
C(9)	7806(8)	1566(3)	2571(3)	42(2)
C(10)	9177(8)	2002(4)	2161(3)	42(2)
C(11)	9302(8)	1613(3)	1486(3)	40(2)
C(12)	11001(10)	1859(5)	1138(3)	54(2)
C(13)	12463(9)	1284(5)	1023(3)	65(2)
C(14)	12717(10)	623(5)	1301(3)	80(3)
C(15)	7452(8)	1721(4)	1036(3)	48(2)
C(16)	6847(9)	2556(4)	978(3)	62(2)
C(17)	6633(9)	2904(3)	1637(3)	66(2)
C(18)	8477(9)	2831(3)	2065(3)	60(2)
C(19)	7539(10)	642(4)	334(3)	83(2)
C(20)	11162(8)	2038(3)	2532(3)	58(2)
C(21)	7282(8)	512(3)	3380(3)	41(2)
C(22)	7670(9)	869(4)	4058(3)	48(2)
C(23)	8141(9)	35(4)	3954(3)	41(2)
C(24)	6948(8)	-588(3)	4231(3)	60(2)
C(25)	10195(8)	-213(3)	3996(3)	55(2)
C(26)	5988(9)	1079(3)	4432(3)	77(2)
C(27)	9265(9)	1441(3)	4184(3)	69(2)

Table 3. Bond lengths [Å] and angles [°] for **247**.

Br(1)-C(21)	1.934(6)
O(1)-C(2)	1.419(6)
O(1)-C(1)	1.430(6)
O(2)-C(19)	1.400(6)
O(2)-C(15)	1.437(6)
O(3)-C(12)	1.211(7)
C(1)-C(21)	1.522(7)
C(1)-C(9)	1.529(6)
C(2)-C(3)	1.507(7)
C(3)-C(8)	1.365(8)
C(3)-C(4)	1.382(8)
C(4)-C(5)	1.388(9)
C(5)-C(6)	1.373(11)
C(6)-C(7)	1.362(10)
C(7)-C(8)	1.375(9)
C(9)-C(10)	1.548(7)
C(10)-C(20)	1.538(7)
C(10)-C(18)	1.536(7)
C(10)-C(11)	1.562(7)
C(11)-C(12)	1.521(7)
C(11)-C(15)	1.546(7)
C(12)-C(13)	1.477(9)
C(13)-C(14)	1.295(7)
C(15)-C(16)	1.521(7)
C(16)-C(17)	1.512(7)
C(17)-C(18)	1.512(7)
C(21)-C(23)	1.528(8)
C(21)-C(22)	1.534(7)
C(22)-C(27)	1.512(7)
C(22)-C(23)	1.514(8)
C(22)-C(26)	1.526(7)
C(23)-C(25)	1.512(7)
C(23)-C(24)	1.522(7)

C(2)-O(1)-C(1)	115.8(4)
C(19)-O(2)-C(15)	114.2(5)
O(1)-C(1)-C(21)	111.7(4)
O(1)-C(1)-C(9)	106.7(5)
C(21)-C(1)-C(9)	112.4(5)
O(1)-C(2)-C(3)	108.6(5)
C(8)-C(3)-C(4)	119.6(7)
C(8)-C(3)-C(2)	121.1(7)
C(4)-C(3)-C(2)	119.3(8)
C(3)-C(4)-C(5)	119.9(8)
C(6)-C(5)-C(4)	118.6(9)
C(7)-C(6)-C(5)	122.0(10)
C(6)-C(7)-C(8)	118.7(9)
C(3)-C(8)-C(7)	121.1(8)
C(1)-C(9)-C(10)	118.3(5)
C(20)-C(10)-C(18)	107.1(5)
C(20)-C(10)-C(9)	109.8(5)
C(18)-C(10)-C(9)	108.9(5)
C(20)-C(10)-C(11)	109.7(5)
C(18)-C(10)-C(11)	109.7(5)
C(9)-C(10)-C(11)	111.5(5)
C(12)-C(11)-C(15)	110.1(5)
C(12)-C(11)-C(10)	114.5(5)
C(15)-C(11)-C(10)	111.7(5)
O(3)-C(12)-C(13)	118.5(7)
O(3)-C(12)-C(11)	122.7(7)
C(13)-C(12)-C(11)	118.8(6)
C(14)-C(13)-C(12)	127.2(7)
O(2)-C(15)-C(16)	108.0(5)
O(2)-C(15)-C(11)	110.7(5)
C(16)-C(15)-C(11)	112.4(5)
C(15)-C(16)-C(17)	111.5(5)
C(16)-C(17)-C(18)	109.8(5)
C(17)-C(18)-C(10)	114.0(5)
C(1)-C(21)-C(23)	123.9(5)
C(1)-C(21)-C(22)	121.5(5)

C(23)-C(21)-C(22)	59.2(4)
C(1)-C(21)-Br(1)	109.0(4)
C(23)-C(21)-Br(1)	117.8(4)
C(22)-C(21)-Br(1)	118.0(4)
C(27)-C(22)-C(23)	119.2(5)
C(27)-C(22)-C(26)	111.1(5)
C(23)-C(22)-C(26)	119.8(5)
C(27)-C(22)-C(21)	119.5(5)
C(23)-C(22)-C(21)	60.2(4)
C(26)-C(22)-C(21)	118.7(5)
C(25)-C(23)-C(22)	119.4(5)
C(25)-C(23)-C(24)	109.8(5)
C(22)-C(23)-C(24)	119.7(5)
C(25)-C(23)-C(21)	120.8(5)
C(22)-C(23)-C(21)	60.6(4)
C(24)-C(23)-C(21)	119.2(5)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for **247**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
Br(1)	53(1)	76(1)	65(1)	12(1)	6(1)	-1(1)
O(1)	74(3)	29(3)	35(3)	1(3)	9(2)	6(3)
O(2)	106(4)	71(4)	44(3)	2(3)	1(3)	-20(3)
O(3)	93(4)	70(4)	98(4)	31(3)	26(3)	-20(3)
C(1)	56(4)	32(4)	37(4)	-3(4)	9(4)	0(3)
C(2)	90(5)	34(5)	46(5)	5(4)	7(4)	14(4)
C(3)	88(6)	19(4)	45(5)	-5(4)	2(5)	4(4)
C(4)	85(6)	75(6)	65(6)	-3(5)	4(5)	0(5)
C(5)	130(9)	85(8)	89(8)	-4(7)	-28(7)	-23(6)
C(6)	215(14)	54(7)	49(7)	-2(5)	-16(8)	-31(8)
C(7)	179(11)	49(6)	46(7)	5(5)	30(7)	0(6)
C(8)	112(7)	47(5)	40(5)	-5(4)	11(5)	7(5)
C(9)	66(4)	32(4)	28(4)	-4(3)	3(3)	5(4)
C(10)	45(4)	42(5)	39(5)	1(4)	3(4)	-6(4)
C(11)	48(4)	28(4)	42(4)	5(3)	1(4)	-8(3)
C(12)	53(5)	74(6)	36(5)	14(4)	4(4)	-19(5)
C(13)	51(5)	96(7)	50(5)	18(5)	14(4)	2(5)
C(14)	83(5)	92(7)	70(6)	-7(5)	28(5)	4(5)
C(15)	58(5)	49(5)	36(5)	-4(4)	0(4)	-12(4)
C(16)	62(5)	52(6)	67(6)	17(4)	-11(4)	-11(4)
C(17)	93(6)	34(5)	70(6)	10(4)	0(5)	13(4)
C(18)	80(5)	39(5)	59(5)	8(4)	-5(4)	-3(4)
C(19)	113(6)	78(7)	59(5)	-14(5)	10(5)	-9(5)
C(20)	78(5)	42(4)	53(5)	6(4)	6(4)	-17(4)
C(21)	63(4)	28(4)	32(4)	7(3)	13(3)	4(3)
C(22)	69(5)	35(5)	42(5)	0(4)	17(4)	2(4)
C(23)	50(4)	45(5)	31(4)	4(3)	12(3)	-8(3)
C(24)	87(5)	52(5)	41(4)	5(4)	6(4)	-4(4)
C(25)	70(4)	58(5)	35(4)	15(4)	4(3)	3(4)
C(26)	117(6)	61(5)	57(5)	-4(4)	35(5)	17(5)
C(27)	112(6)	58(5)	37(5)	0(4)	4(4)	-17(5)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for **247**.

	x	y	z	U(eq)
H(1A)	9729	764	2961	50
H(2A)	8192	-721	2753	68
H(2B)	10083	-509	2452	68
H(4A)	5383	-1106	1990	90
H(5A)	4380	-1816	1067	124
H(6A)	6541	-2121	345	130
H(7A)	9598	-1687	488	108
H(8A)	10575	-986	1403	79
H(9A)	7609	1879	2946	50
H(9B)	6592	1524	2314	50
H(11A)	9441	1062	1567	47
H(13A)	13292	1411	717	78
H(14A)	11928	467	1611	97
H(14B)	13689	304	1191	97
H(15A)	6441	1428	1215	57
H(16A)	5648	2591	711	74
H(16B)	7784	2843	766	74
H(17A)	5629	2644	1838	79
H(17B)	6291	3440	1587	79
H(18A)	8313	3052	2486	72
H(18B)	9445	3126	1874	72
H(19A)	7731	496	-103	125
H(19B)	6304	478	432	125
H(19C)	8488	405	630	125
H(20A)	11654	1529	2597	86
H(20B)	11080	2279	2946	86
H(20C)	11992	2330	2286	86
H(24A)	5647	-427	4205	90
H(24B)	7398	-678	4677	90
H(24C)	7047	-1052	3988	90
H(25A)	10941	183	3822	82
H(25B)	10303	-675	3751	82
H(25C)	10645	-303	4442	82
H(26A)	4988	713	4339	115
H(26B)	5539	1581	4304	115
H(26C)	6377	1076	4889	115
H(27A)	10303	1296	3942	104
H(27B)	9683	1447	4639	104
H(27C)	8824	1941	4050	104

Appendix 4

Publications

Intramolecular proton transfer in the cyclization of geranylgeranyl diphosphate to the taxadiene precursor of taxol catalyzed by recombinant taxadiene synthase

David C Williams[1], Brian J Carroll[2], Qingwu Jin[3], Christopher D Rithner[4], Steven R Lenger[4], Heinz G Floss[2], Robert M Coates[3], Robert M Williams[4] and Rodney Croteau[1]

Background: The committed step in the biosynthesis of the anticancer drug taxol in yew (*Taxus*) species is the cyclization of geranylgeranyl diphosphate to taxa-4(5),11(12)-diene. The enzyme taxadiene synthase catalyzes this complex olefin cation cyclization cascade involving the formation of three rings and three stereogenic centers.

Results: Recombinant taxadiene synthase was incubated with specifically deuterated substrates, and the mechanism of cyclization was probed using MS and NMR analyses of the products to define the crucial hydrogen migration and terminating deprotonation steps. The electrophilic cyclization involves the ionization of the diphosphate with closure of the A-ring, followed by a unique intramolecular transfer of the C11 proton to the *re*-face of C7 to promote closure of the B/C-ring juncture, and cascade termination by proton elimination from the β-face of C5.

Conclusions: These findings provide insight into the molecular architecture of the first dedicated step of taxol biosynthesis that creates the taxane carbon skeleton, and they have broad implications for the general mechanistic capability of the large family of terpenoid cyclization enzymes.

[1]Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340, USA
[2]Department of Chemistry, University of Washington, Seattle, WA 98195-1700, USA
[3]Department of Chemistry, University of Illinois, Urbana, IL 61801, USA
[4]Department of Chemistry, Colorado State University, Fort Collins, CO 80523, USA

Correspondence: Robert M Williams
E-mail: rmw@chem.colostate.edu

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Introduction

The diterpenoid taxol [1] (paclitaxel, **1**, Figure 1) is now well-established as a potent chemotherapeutic agent, showing excellent activity against a range of cancers and is currently approved for treating refractory ovarian, metastatic breast, and non-small-cell lung cancers, as well as Kaposi's sarcoma [2]. (Paclitaxel is the generic name for Taxol, a registered trademark of Bristol-Myers Squibb. Because of its greater familiarity, 'taxol' is used throughout.) The limited supply of the drug from the original source, the bark of the Pacific yew (*Taxus brevifolia* Nutt.; Taxaceae), prompted intensive efforts to devise alternative means of production [3,4]. These efforts have yielded a commercially viable semisynthesis of taxol and its analogs from advanced taxane diterpenoid (taxoid) metabolites that are more readily available from yew [5,6]. However, with increasing applications in chemotherapy, both in treatment of additional cancer types and for earlier disease intervention, the supply and cost of these drugs will remain important issues [7]. Total syntheses of taxol have been achieved by several elegant routes [8–13] but the yields are too low to be practical, and it is clear that in the foreseeable future the supply of taxol and its synthetically useful progenitors must rely on biological methods of production [7]. The development of improved biological processes must be based upon a detailed understanding of the pathway for taxol biosynthesis, the enzymes which catalyze the sequence of reactions and their mechanisms of action, and the genes encoding these enzymes, especially those responsible for slow steps of the pathway.

Taxadiene synthase from *Taxus* species catalyzes the first committed step in the biosynthesis of taxol and related taxoids by the cyclization of the universal diterpenoid precursor (*E*,*E*,*E*)-geranylgeranyl diphosphate (GGPP, **2**) to the parent olefin (**6**), which undergoes an extended series

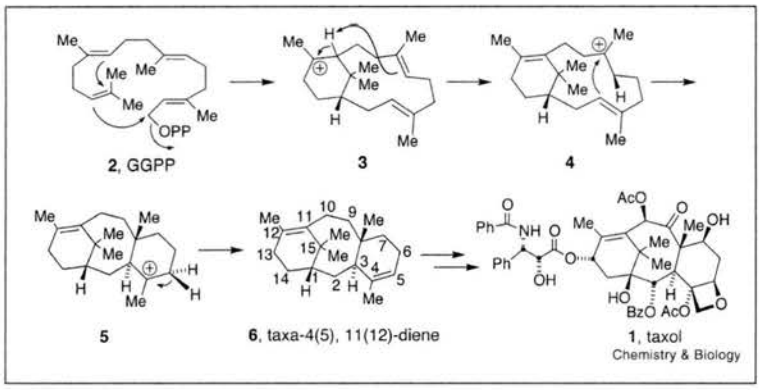

Figure 1. Cyclization of geranylgeranyl diphosphate to taxol.

of oxygenation and acylation reactions (Figure 1) [14,15]. This enzyme, which has been isolated from both yew saplings [16] and cell cultures [17], catalyzes a slow, but apparently not rate limiting, step in the taxol biosynthetic pathway [17]. Initial assessment of the mechanism of taxadiene synthase [18] indicates that the reaction involves the ionization and cyclization of GGPP to a transient verticillyl intermediate (**3**), proposed to have the 11R configuration to allow intramolecular transfer of the C11 proton to C7 (**4**) to initiate transannular B/C-ring closure to the taxenyl cation (**5**), followed by deprotonation at C5 to yield taxa-4(5),11(12)-diene (**6**). No other details of the stereochemical mechanism of this novel enzyme are known.

A cDNA encoding the taxadiene synthase from *T. brevifolia* has been obtained by a homology-based PCR cloning method [19]. A truncated version of the enzyme, in which the plastidial targeting peptide of the preprotein has been deleted, has been functionally overexpressed in *Escherichia coli* and shown to resemble the native enzyme in kinetic properties [20]. This advance has made available sufficient amounts of the enzyme, and thus the enzyme product, to permit a more detailed study of the electrophilic cyclization reaction cascade.

Results and discussion

Chain termination: C5-deprotonation

To examine the stereochemistry of the C5-deprotonation of the terminal taxenyl C4-carbocationic intermediate of the reaction sequence (**5**, Figure 1), the appropriate deuterium labeled substrate, (4*R*)-[4-$^2H_1$]GGPP (**7**, Figure 2) (84 mol% ^2H), was prepared and incubated at preparative scale with the purified recombinant taxadiene synthase. The olefinic products of the reaction were isolated and subjected to GC-MS analysis for comparison to the prod-

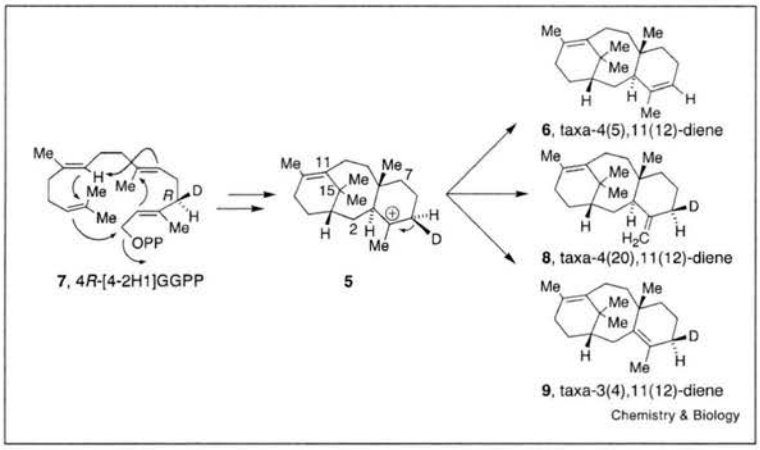

Figure 2. Cyclization of deuterium labeled GGPP.

ucts identically obtained from the recombinant enzyme using unlabeled substrate. The taxa-4(5),11(12)-diene (**6**) enzymatically synthesized from the deuterium labeled acyclic precursor bore no detectable deuterium compared to the product prepared from the unlabeled precursor, thereby indicating that the C4 β-hydrogen is selectively and entirely lost in the terminating deprotonation step (see Figure 2). Interestingly, the use of the alternate substrate also permitted observation of the phenomenon of isotopically sensitive branching [21,22], in which the deuterium-dependent slowing of the C5-deprotonation of the common taxenyl cation intermediate promoted alternative deprotonations for terminating the reaction cycle. With the unlabeled substrate, taxadiene synthase yields principally taxa-4(5),11(12)-diene (**6**, 94%), with lesser amounts (~5%) of the 4(20),11(12)-isomer (**8**) resulting from deprotonation from the C20 methyl group, and negligible proton loss from the bridgehead methine (C3) to give the 3(4),11(12)-isomer (**9**) [20]. With the deuterated substrate, the proportion of taxa-4(5),11(12)-diene (**6**) in the enzymatic cyclization products decreased to 77.6%, with a concomitant increase in the proportions of the 4(20),11(12)-isomer (**8**) to 11.1% and of the 3(4),11(12)-isomer (**9**) to 9.2%; the latter two isomers fully retained the deuterium at C5.

Although there was relatively little change in the absolute rate of formation of the 4(20)-isomer, partitioning of the taxenyl carbocation to the 3(4)-isomer was substantially enhanced in response to the primary deuterium kinetic isotope effect on the C5 methylene deprotonation. An overall rate suppression resulting from the deuterium substitution on total olefin formation of about 50% was determined from the total ion chromatogram (compared to control experiments run in parallel with unlabeled substrate), indicating a kinetic isotope effect for the C5-deprotonation of $k_H/k_D \sim 2.1$. This latter result is of note, since earlier studies based on apparent secondary kinetic isotope effects had suggested that the initial ionization of the diphosphate ester substrate was rate limiting [18] and potentially capable of masking downstream isotopically sensitive steps.

A-ring to C-ring cascade

To examine the stereochemistry of the intramolecular hydrogen migration from C11 of the verticillyl intermediate (**3**) to C7 in the second ring closure step, [10-$^2H_1$]GGPP (**10**, >99 mol% ^2H, Figure 3) was prepared as previously described [18]. Incubation at preparative scale with the purified recombinant enzyme at saturating levels of the deuterated substrate yielded ~100 μg of taxa-4(5),11(12)-diene (**6**) by calibrated GC-MS analysis of the reaction products (an overall rate suppression of ~50% was also observed due to C10 deuterium substitution, yielding $k_H/k_D = 2.0$ similar to that previously noted for the C5-deprotonation). Evaluation of the mass spectrum revealed that >99% of the deuterium originally present at C10 of GGPP (C11 of the intermediate) had migrated to C7 of the taxane skeleton as assessed by shift of the C-ring fragment ion at *m/z* 122 to *m/z* 123 (and of the parent ion from *m/z* 272 to *m/z* 273), thereby demonstrating the complete fidelity of the intramolecular proton transfer.

The olefin fraction of the enzymatic reaction products was purified by a combination of open column silica gel chromatography (with pentane) and reversed phase (C_{18}) HPLC (with acetonitrile) to afford ~10 μg of pure C7-deuterated taxa-4(5),11(12)-diene (**6**) following transfer to deuterobenzene and removal of residual pentane used to partition the product from the HPLC solvent. The complete assignment of the ^1H NMR spectrum of taxa-4(5),11(12)-diene in $CDCl_3$ has been described previously [14] and these assignments have been reconfirmed in C_6D_6 for the present work. Since earlier work had indicated that deuterium from [10-$^2H_1$]GGPP should reside at C7 of taxadiene following transfer from C11 of the verticillyl intermediate [18], the assignments most relevant to defining the regiochemistry of the product include those for the C-ring H5, H6 (α and β), H7 (α and β), and the H19 methyl protons.

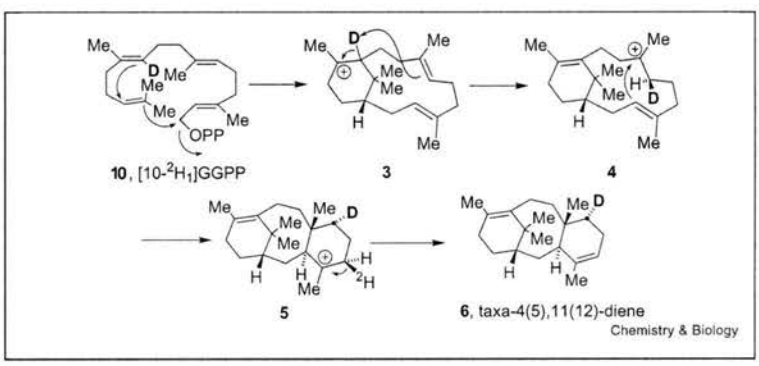

Figure 3. Stereochemistry of intramolecular hydrogen migration.

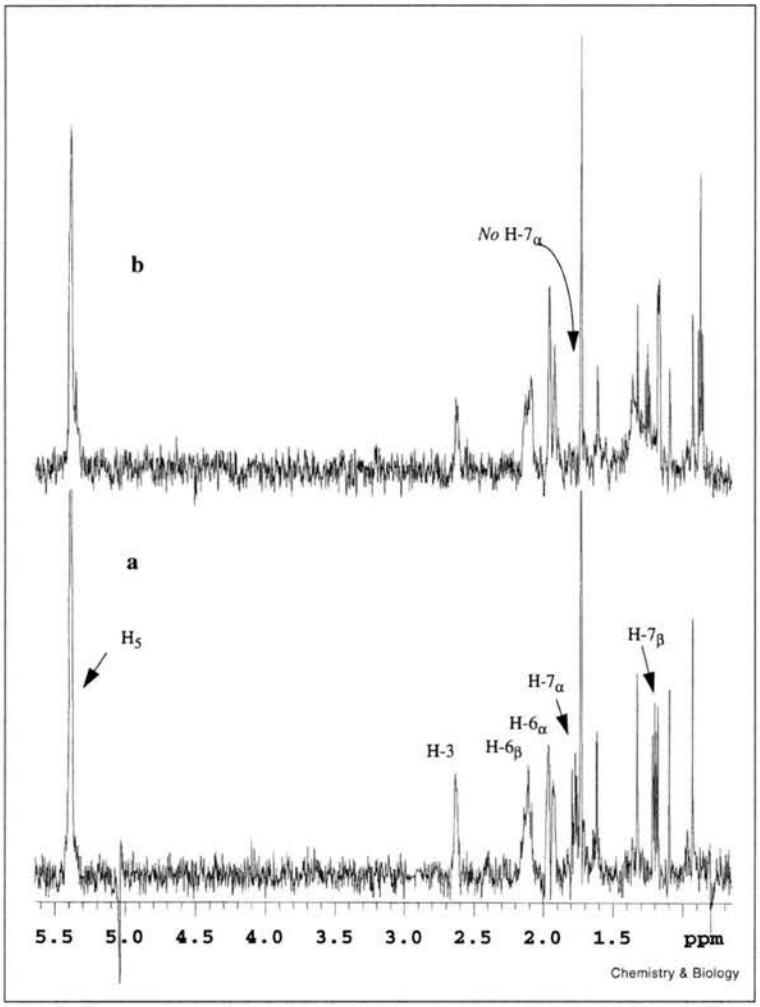

Figure 4. Double pulsed field gradient spin echo-selective TOCSY of **6** (C_6D_6) produced from **10(b)** and of unlabeled **6(a)**. The H5 signal at δ5.38 was selectively excited in each case followed by a 65 ms mixing period. The predominant signals following the mixing period are those of the C-ring. Note the presence of the 7_α proton signal in **a** at δ1.76 but its absence in **b** following deuterium atom transfer. Other features to note are the collapse of the 7_β, 6_α, and 6_β proton fine structure in **b** as compared with **a**.

The relatively small amount of biosynthetic [^{2}H]taxadiene available (~10 μg) precluded the use of two-dimensional NMR techniques. Consequently, 1D DPFGSE-TOCSY [23] experiments were done to explore ways of discriminating among the various spin systems in the olefin, with the hope that an unambiguous excitation of the H7 protons would result. The presence of H5 at δ5.38 ppm, alone in this region of the spectrum, provided an entry point to define the C-ring system. Figure 4 illustrates the results of the 1D DPFGSE-TOCSY experiment, following selective excitation of H5 and a 65 ms mixing period. The most notable features of this spectrum are the doublet of doublets at δ1.19 ppm (assigned to $H7_\beta$), the multiplet at δ1.76 ($H7_\alpha$), the singlet at δ1.73 (C20 methyl), and the broad triplet and doublet at δ2.11 and δ1.94 ($H6_\beta$ and $H6_\alpha$, respectively). Since the interpretation of the result of deuterium incorporation into taxadiene rests upon the correct stereochemical assignments for the C7 protons, it was imperative to confirm these assignments.

A two-dimensional NOESY-NMR experiment (with a 400 ms mixing time) was conducted for this purpose (Figure 5). Through-space correlations among protons on the β- (top) face of the molecule were examined. Cross-peaks were observed correlating the diagonal signal at 0.93 ppm (C19 methyl) with signals at δ1.19 (C17 methyl), at δ1.39 and δ1.81 ($H9_{\alpha,\beta}$), δ2.11 ($H6_\beta$), δ1.72 ($H2_\beta$), and δ1.19 ($H7_\beta$). No cross-peak was observed between the C19 signal and the signal at δ1.76 ppm, which is consistent with the latter assignment as the $H7_\alpha$ proton. Correlations with the diag-

onal signal for the $H7_\beta$ proton (δ1.19 ppm) were carefully examined. As expected, there was a strong cross-peak found at δ1.76 ppm assigned to the $H7_\alpha$ proton. There were also other cross-peaks at δ0.93 (C19 methyl), δ1.39 ($H9_\alpha$), δ1.94 and δ2.11 ($H6_\alpha$ and $H6_\beta$, respectively). In contrast, the signal assigned to the $H7_\alpha$ proton (δ1.76 ppm) had fewer correlations, including those for H3 (δ2.62) and the C18 methyl (δ1.60), which is consistent with the stereochemical assignment to the α- (bottom) face of the olefin.

With completion of the regio- and stereochemical NMR assignments, the enzymatically derived, deuterium labeled taxadiene sample was examined. Residual pentane (from the final solvent partitioning step) was removed from the sample by transfer to and repeated evaporation from C_6D_6; otherwise, pentane signals completely dominate the 1D ^1H NMR spectrum in the crucial δ0.5–δ1.5 ppm region (Figure 4). By this means, the pentane signal intensities were reduced sufficiently to permit repetition of the 1D DPFGSE-TOCSY experiment, this pulsed field gradient technique being especially efficient at de-phasing those signals not correlated with the selected proton resonance. The 1D DPFGSE-TOCSY spectrum of the deuterium labeled olefin, with selective excitation of the H5 proton at δ5.38 ppm, is clear of artifacts including residual pentane signals (Figure 4b) and appears remarkably similar to that of the unlabeled standard (Figure 4a) with a few important exceptions. The signal for the $H7_\alpha$ proton (1.76 ppm), clearly present in the standard, has disappeared in the labeled olefin. The signal at δ1.19 ppm, assigned to the $H7_\beta$ proton, has collapsed to an apparent doublet ($J = 6$ Hz) consistent with the loss of its geminal $H7_\alpha$ partner. Finally, the NMR patterns for both of the H6 protons have been altered. These results are entirely consistent with transfer of the deuterium atom from C10 of the acyclic

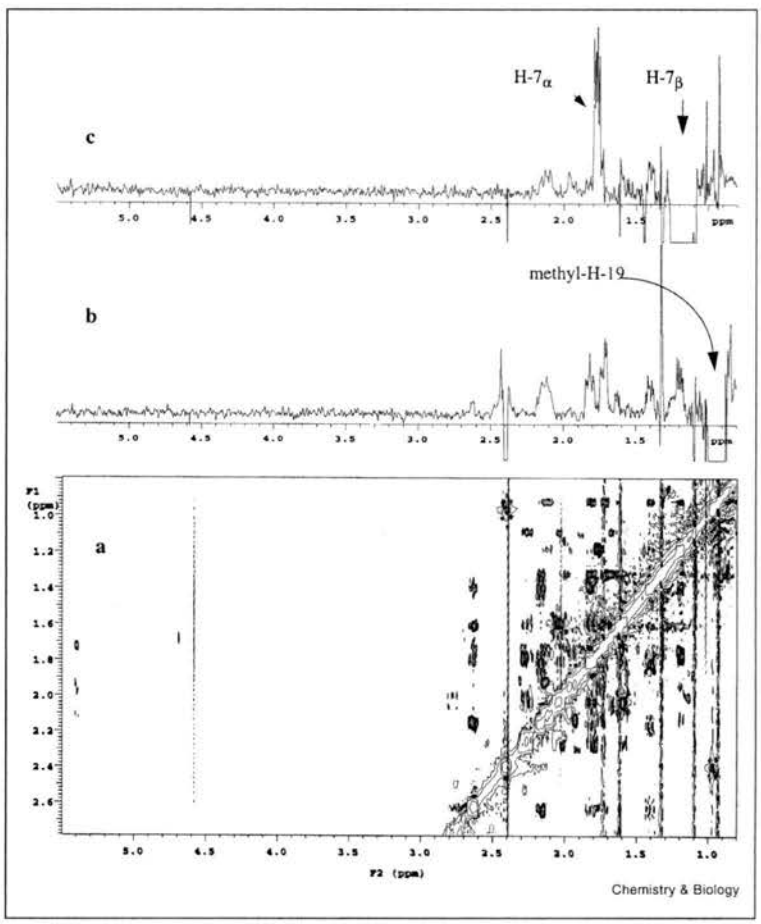

Figure 5. NOESY NMR spectrum of **6** (C_6D_6). Inset **a** is a 500 MHz two-dimensional NOESY spectrum. Insets **b** and **c** are slices of the 2D spectrum taken through the methyl H19 and $H7_\beta$ positions, respectively.

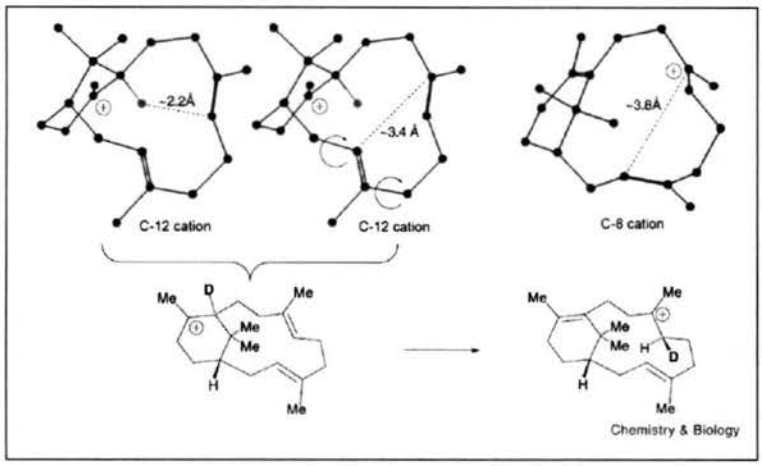

Figure 6. Molecular modeling of the C11 to C7 hydrogen migration converting the verticillen-12-yl carbocation to its 8-yl isomer prior to cyclization of the C-ring.

precursor exclusively to the $H7_{\alpha}$ position of the taxadiene product.

Transition state modeling

Modeling of the intermediate C12 carbocation resulting from the C11–C15 closure in the initially formed macrocycle (Figure 6), with minimization of this structure using MacSpartan (AM1 basis set, overall molecular charge of +1, trivalent carbon at C12), reveals that the C11 proton is within ~2.2 Å of the C7 carbon and seemingly perfectly poised for the transannular migration to the *re*-face at C7. Furthermore, inspection of the conformation of the C12 carbocation strongly suggests that it is unlikely that an amino acid at the enzyme active site could gain access to the C11 proton to mediate the C11–C7 transfer, since this proton is buried deep within the concave face of the 12-membered macrocycle. Based on both the experimental evidence and the conformational analysis, it appears that an unassisted intramolecular transfer is the most plausible mechanism for the initiation of the final ring closure step

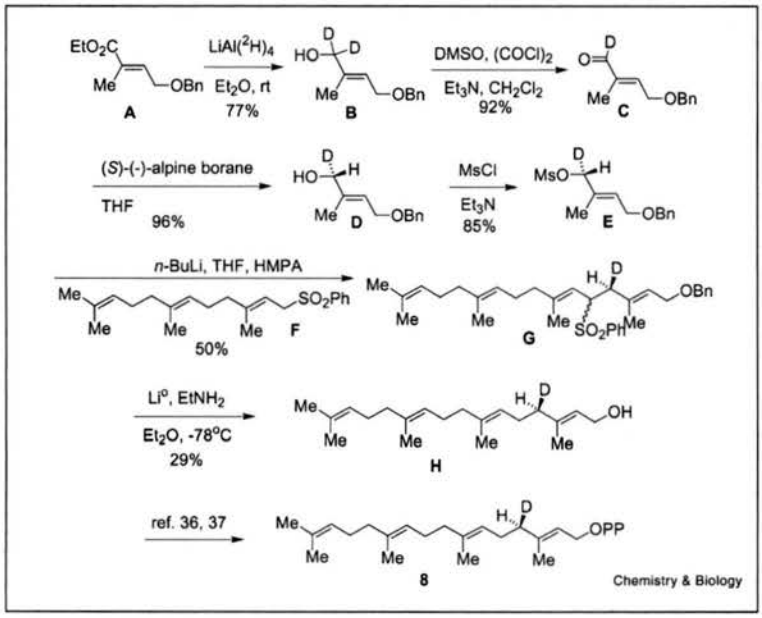

Figure 7. Synthesis of all *trans*-(4*R*)-[4-$^{2}H_1$]GGPP (**8**).

mediated by taxadiene synthase. Thus, the C7–C8 olefin serves as the Brønsted base that quenches the incipient carbocation at C12 and no active site enzyme base needs to be invoked for this process.

Of further interest in this enzymatic reaction is the facial bias of the final C8–C3 olefin cation cyclization. The established stereochemistry of the taxane B/C-ring junction dictates attack by the C3/C4 π-electrons on the C8 cation (formed immediately following the C11 to C7 proton migration) from *the same face* to which the newly installed proton at C7 has migrated (overall *syn* addition of H^+ and C3 to the 7,8 double bond). Modeling of this process (Figure 6), which formally involves a criss-cross of bond formation across the C7/C8 double bond, proved to be insightful. The molecular model of the C8 carbocation suggests that, upon pyramidalization at C7 and fashioning of the C11/C12 olefin, the conformation of the 12-membered ring twists, relative to the C12-cationic center, thereby rocking the C3/C4 π-electrons into the correct facial orientation for capture of the C8 cation and establishing the *trans*-fused B/C-ring juncture.

There is precedent in enzymatic terpenoid cyclization reactions for apparently similar deprotonation–reprotonation steps involving transient formation of olefinic intermediates, with reprotonation of a distal double bond to initiate subsequent rearrangements or ring closures. For example, in the diterpene series, an intramolecular proton transfer in a pimarenyl intermediate promotes a syn addition/methyl migration to yield the abietane skeleton catalyzed by recombinant abietadiene synthase [24]. Related internal proton transfers also occur in cyclization reactions catalyzed by sesquiterpene synthases [25,26]; however, in the best defined example (5-epi-aristolochene synthase), the proton transfer step is clearly mediated by an active site base [27].

A remarkable olefin cation cascade

The overall stereochemical mechanism for the conversion of GGPP to taxa-4(5),11(12)-diene (Figure 3) is shown to involve the following five processes: (1) Walden inversion at the diphosphate-bearing carbon (C1) coupled with antiperiplanar C1–C14 and C11–C15 bond formation initiated by the ionization of the diphosphate group and leading to the verticillen-12-yl carbocation (**3**) (the stereochemical outcome at C1 and C15 was elucidated by separate enzymatic cyclizations of (*R*)-[1-$^2H_1$]- and (*S*)-[1-$^2H_1$]-, and(*E*)-[16,16,16-$^2H_3$]GGPP and 600 MHz ^1H NMR analyses of the resulting [2-$^2H_1$]- and [16,16,16-$^2H_3$]taxadienes; unpublished results, D.C.W., Q.J., R.M.C., R.C.); (2) conformational inversion of the A-ring accompanied by rotations of the macrocyclic loop; (3) a unique intramolecular transfer of the C11 proton to the *re*-face of C7 to generate the isomeric verticillen-8-yl carbocation; (4) bond rotations and closure of the B/C-ring junction via capture of the C8 carbocation by the transannular C3/C4 π-electrons to form the penultimate taxen-4-yl intermediate; and (5) elimination of the 5-β proton from a twist boat conformer to form taxadiene.

Significance

Taxadiene synthase is a remarkable terpene cyclase that appears to function by binding and ionization its substrate GGPP to mediate an enantio- and face-selective polyolefin cation cascade that involves the formation of three carbon–carbon bonds, three stereogenic centers, and the loss of hydrogen in 'a single step'. The seemingly unassisted intramolecular proton transfer mechanism of taxadiene synthase is thus strikingly unusual in this regard, and suggests that this enzyme type is capable of mediating complex olefin cation cyclizations, *with absolute stereochemical fidelity, by conformational control alone.* These findings provide insight into the molecular architecture of the first dedicated step of taxol biosynthesis that creates the taxane carbon skeleton, and these observations have broad implications for the general mechanistic capability of the large family of terpenoid cyclization enzymes that catalyze similar electrophilic reaction cascades.

Materials and methods

Substrate preparation

All *trans*-GGPP (**2**, 98%) and [10-$^2H_1$]GGPP (**10**, >90%; >99 mol% ^2H) were prepared and purified as previously described [18]. All *trans*-(*R*)-[4-$^2H_1$]GGPP (**8**, 93%; 84 mol% ^2H) was prepared from previously described [28] ester **A** (25:1 *E*:*Z* ratio by ^1H NMR) and the known [29–31] sulfone **F** (Figure 7). Ester **A** was reduced with $LiAl^2H_4$ to dideuterated alcohol **B**, oxidized to monodeuterated aldehyde **C** by the Swern method, and reduced asymmetrically to **D** with (*S*)-alpine borane. Camphanate derivatization and ^1H NMR analysis showed a 95:5 enantiomeric ratio for **D** [32]. Following conversion to the mesylate **E** and coupling [33] to sulfone **F**, the product **G** was obtained in 50% yield after purification. The sulfone group and benzyl ether protecting group were removed by reduction with Li in $EtNH_2$ at −78°C to give (*R*)-[4-$^2H_1$]geranylgeraniol **H** in 29% yield following $AgNO_3$ silica gel argentation chromatography to remove a contaminating double bond isomer. To determine the enantiomeric purity of **H**, the asymmetric epoxidation [34] of the 2,3 double bond was carried out to differentiate the two protons adjacent to the epoxide ring by ^2H NMR analysis. Integration of the relevant peaks at 1.51 and 1.35 ppm showed a ratio of 1:8 which, by assuming the same enantioselectivity as in the epoxidation of geraniol [34], gave an estimated *R*:*S* enantiomer ratio of 91:9. Alcohol **H** was converted to the corresponding diphosphate ester (30% yield) by established procedures [35,36]. A single component was observed upon cellulose TLC and no impurities were observed in the ^1H and ^{31}P NMR spectra; the latter (D_2O, 162 MHz) displayed two doublets at −9.54 and −5.72 ppm (J = 20.8 Hz).

Enzymatic conversions

The truncated (M60) version of recombinant taxadiene synthase, in which the plastidial transit peptide has been deleted, was overexpressed in *E. coli* and purified (>96%) as previously described [20]. Incubations were carried out in 3 ml of the standard assay buffer [20] containing 1 mg of enzyme and a 2 mM concentration of the appropriate substrate. After immediate extraction with pentane to remove any organic soluble contaminants, the reaction mixture was maintained at 31°C until in excess of 100 μg of olefin product had been generated. Following incubation, the pentane-soluble reaction products were extracted for capillary GC-MS analysis [20] to establish overall conversion, the distri-

bution of taxadiene isomers, and the deuterium content of these olefinic products. In preparation for the NMR analysis of the product derived from [10-$^2H_1$]GGPP (**10**), the olefin mixture was purified by silica gel column chromatography with hexane, followed by reversed phase (C_{18}) HPLC with acetonitrile. The concentrated acetonitrile eluent was partitioned between pentane and water, and the pentane phase was transferred to deuterobenzene (99.96 mol%) and repeatedly concentrated under N_2 (without drying which results in decomposition [14]) to remove residual solvent, ultimately yielding ~10 μg of pure taxa-4(5),11(12)-diene (**6**) in a 500 μl sample volume.

MS and NMR spectrometry

Protocols for capillary GC-MS analysis of the taxadiene isomers have been described in detail elsewhere [14,18,20]. All NMR spectra were recorded on a Varian Unity-500 spectrometer at 25°C using a very sensitive ^1H indirect detection probe. 1D double pulsed field gradient spin echo TOCSY (DPFGSE-TOCSY) spectra were obtained with a mixing time of 65 ms. 2D NOESY spectra were acquired using mixing times of 400 ms. 2D spectra were collected as 256 (t_1)×2048 (t_2) complex points with a sweep width of 7 kHz in each dimension. Data were processed using Varian, Inc. VNMR software. The final data size, after linear prediction in t_1 and zero-filling in both dimensions, was 1024 (f_1)×4096 (f_2) complex points.

Acknowledgements

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References

1. Wall, M.E. and Wani, M.C. (1995). Paclitaxel: from discovery to clinic. in *Taxane Anticancer Agents: Basic Science and Current Status.* (Georg, G.I., Chen, T.T., Ojima, I. and Vyas, D.M., eds.), pp. 18–30, American Chemical Society.
2. Goldspiel, B.R. (1997). Clinical overview of the taxanes. *Pharmacotherapy* **17**, 1105–1255.
3. Kingston, D.G.I. (1991). The chemistry of taxol. *Pharmacol. Ther.* **52**, 1–34.
4. Cragg, G.M., Schepartz, S.A., Suffness, M. & Grever, M.R. (1993). The taxol supply crisis. New NCI policies for handling the large-scale production of novel natural product anticancer and anti-HIV agents. *J. Nat. Prod.* **56**, 1657–1668.
5. Holton, R.A., Biediger, R.J. and Boatman, P.D. (1995). Semisynthesis of Taxol and Taxotere. in *Taxol: Science and Applications.* (Suffness, M., ed.), pp. 97–121, CRC Press, Boca Raton.
6. Commerçon, A., Bourzat, J.D., Didier, E. and Lavelle, F. (1995). Practical semisynthesis and antimitotic activity of docetaxel and side-chain analogs. in *Taxane Anticancer Agents: Basic Science and Current Status.* (Georg, G.I., Chen, T.T., Ojima, I. and Vyas, D.M., eds.), pp. 223–246, American Chemical Society.
7. Suffness, M. (1995). Overview of paclitaxel research: progress on many fronts. in *Taxane Anticancer Agents: Basic Science and Current Status.* (Georg, G.I., Chen, T.T., Ojima, I. and Vyas, D.M., eds.), pp. 1–17, American Chemical Society.
8. Holton, R.A., Kim, H.-B., Samoza, C., Liang, F., Biediger, R.J., Boatman, P.D., Shindo,., Smith, C.C., Kim, S., Nadizadeh, H., Suzuki, Y., Tao, C., Vu, P., Tang, S., Zhang, P., Murthi, K.K., Gentile, L.N. & Liu, J.H. (1994). First total synthesis of Taxol. Completion of the C and D rings. *J. Am. Chem. Soc.* **116**, 1599–1600.
9. Nicolaou, K.C., Yang, Z., Liu, J.J., Nantermet, P.G., Guy, R.K., Claiborne, C.F., Renaud, J., Couladouros, E.A., Paulvannan, K. & Sorensen, E.J. (1994). Total synthesis of Taxol. *Nature* **367**, 630–634.
10. Masters, J.J., Link, J.T., Snyder, L.B., Young, W.B. & Danishefsky, S.J. (1995). A total synthesis of Taxol. *Angew. Chem. Int. Ed. Eng.* **34**, 1723–1726.
11. Wender, P.A., Badham, N.F., Conway, S.P., Floreancig, P.E., Glass, T.E., Houze, J.B., Krauss, N.E., Lee, D., Marquess, D.G., McGrane, P.L., Meng, W., Natchus, M.G., Shuker, A.J., Sutton, J.C. & Taylor, R.E. (1997). The pinene path to taxanes. 6. A concise stereocontrolled synthesis of Taxol. *J. Am. Chem. Soc.* **119**, 2757–2759.
12. Morihira, K., Hara, R., Kawahara, S., Nishimori, T., Nakamura, N., Kusama, H. & Kuwajima, I. (1998). Enantioselective total synthesis of Taxol. *J. Am. Chem. Soc.* **120**, 12980–12981.
13. Mukaiyama, T., Shiina, I., Iwadare, H., Saitoh, M., Nishimura, T., Ohkawa, N., Sakoh, H., Nishimura, K., Tani, Y., Hasegawa, M., Yamada, K. & Saitoh, K. (1999). Asymmetric total synthesis of Taxol. *Chem. Eur.* **5**, 121–161.
14. Koepp, A.E., Hezari, M., Zajicek, J., Vogel, B.S., LaFever, R.E., Lewis, N.G. & Croteau, R. (1995). Cyclization of geranylgeranyl diphosphate to taxa-4,11(12)-diene is the committed step of Taxol biosynthesis in Pacific yew. *J. Biol. Chem.* **270**, 8686–8690.
15. Walker, K. & Croteau, R. (1999). Taxol biosynthesis. *Rec. Adv. Phytochem.* **33**, 31–50.
16. Hezari, M., Lewis, N.G. & Croteau, R. (1995). Purification and characterization of taxa-4(5),11(12)-diene synthase from Pacific yew (*Taxus brevifolia*) that catalyzes the first committed step of Taxol biosynthesis. *Arch. Biochem. Biophys.* **322**, 437–444.
17. Hezari, M., Ketchum, R.E.B., Gibson, D.M. & Croteau, R. (1997). Taxol production and taxadiene synthase activity in *Taxus canadensis* cell suspension cultures. *Arch. Biochem. Biophys.* **337**, 185–190.
18. Lin, X., Hezari, M., Koepp, A.E., Floss, H.G. & Croteau, R. (1996). Mechanism of taxadiene synthase, a diterpene cyclase that catalyzes the first step of Taxol biosynthesis in Pacific yew. *Biochemistry* **35**, 2968–2977.
19. Wildung, M.R., Jin, Q., Dalal, D., Oliver, J.S., Coates, R.M. & Croteau, R. (1996). A cDNA clone for taxadiene synthase, the diterpene cyclase that catalyzes the committed step of Taxol biosynthesis. *J. Biol. Chem.* **271**, 9201–9204.
20. Williams, D.C., Wildung, M.R. & Croteau, R. (2000). Heterologous expression and characterization of a 'pseudomature' form of taxadiene synthase involved in paclitaxel (Taxol) biosynthesis, and evaluation of a potential intermediate and inhibitors of the multistep diterpene cyclization reaction. *Arch. Biochem. Biophys.* **379**, 137–146.
21. Jones, J.P., Korzekwa, K.R., Rettie, A.E. & Trager, W.F. (1986). Isotopically sensitive branching and its effect on the observed intramolecular isotope effects in cytochrome P-450 catalyzed reactions: A new method for the estimation of intrinsic isotope effects. *J. Am. Chem. Soc.* **108**, 7074–7078.
22. Harada, N., Miwa, G.T., Walsh, J.S. & Lu, A.Y.H. (1984). Kinetic isotope effects on cytochrome P450-catalyzed oxidation reactions (evidence for the irreversible formation of an activated oxygen intermediate of cytochrome P-448). *J. Biol. Chem.* **259**, 3005–3010.
23. Stott, K., Keeler, J., Van, Q.N. & Shaka, A.J. (1997). One-dimensional NOE experiments using pulsed field gradients. *J. Magn. Reson.* **125**, 302–324.
24. Ravn, M.M., Coates, R.M., Jetter, R. & Croteau, R. (1998). Stereospecific intramolecular proton transfer in the cyclization of geranylgeranyl diphosphate to (−)-abietadiene catalyzed by a recombinant cyclase from grand fir (*Abies grandis*). *J. Chem. Soc. Chem. Commun.* **1998**, 21–22.
25. Ravn, M.M., Coates, R.M., Flory, J.E., Peters, R.J. & Croteau, R. (2000). Stereochemistry of the cyclization-rearrangement of (+)-copalyl diphosphate to (−)-abietadiene catalyzed by recombinant abietadiene synthase from *Abies grandis*. *Org. Lett.* **2**, 573–576.
26. Cane, D.E. (1999). Sesquiterpene biosynthesis: cyclization mechanisms. in *Comprehensive Natural Products Chemistry, Vol. 2, Isoprenoids Including Carotenoids and Steroids.* (Cane, D.E., ed.), pp. 155–200, Elsevier Science, Amsterdam.
27. Rising, K.A., Starks, C.M., Noel, J.P. & Chappell, J. (2000). Demonstration of germacrene A as an intermediate in 5-epi-aristolochene synthase catalysis. *J. Am. Chem. Soc.* **122**, 1861–1866.
28. Marshall, J.A., Trometer, J.D., Blough, B.E. & Crute, T.D. (1988). Stereochemistry of S_N2' additions to acyclic vinyloxiranes. *J. Org. Chem.* **53**, 4274–4282.
29. Eren, D. & Keinan, E. (1988). Total synthesis of linear polyprenoids. 3.1 Syntheses of ubiquinones via palladium-catalyzed oligomerization of monoterpene monomers. *J. Am. Chem. Soc.* **110**, 4356–4362.
30. Guertin, K.R. & Kende, A.S. (1993). Chemoselective catalytic oxidation of sulfides to sulfones with tetrapropylammonium perruthenate (TPAP). *Tetrahedron Lett.* **34**, 5369–5372.
31. Torii, S., Uneyama, K. & Matsunami, S. (1980). Stereoselective synthesis of (±)-irones. *J. Org. Chem.* **45**, 16–20.
32. Lampe, D., Mills, S.J. & Potter, B.V.L. (1992). Total synthesis of the

second messenger analogue D-myo-inositol 1-phosphorothioate 4,5-bisphosphate: optical resolution of DL-1-*O*-allyl-2,3,6-tri-*O*-benzyl-myo-inositol and fluorescent labeling of myo-inositol 1,4,5-triphosphate. *J. Chem. Soc. Perkin* **1**, 2899–2906.

33. Yee, N.K.N. & Coates, R.M. (1992). Total synthesis of (+)-9, 10-syn- and (+)-9,10-anti-copalol via epoxy trienylsilane cyclizations. *J. Org. Chem.* **57**, 4598–4608.
34. Katsuki, T. & Sharpless, K.B. (1980). The first practical method for asymmetric epoxidation. *J. Am. Chem. Soc.* **102**, 5974–5976.
35. Collington, E.W. & Meyers, A.I. (1971). A facile and specific conversion of allylic alcohols to allylic chlorides without rearrangement. *J. Org. Chem.* **36**, 3044–3045.
36. Woodside, A.B., Huang, Z. & Poulter, C.D. (1993). Trisammonium geranyl diphosphate. *Org. Synth. Coll. Vol.* **VIII**, 616–620.