STRUCTURE ELUCIDATION OF NATURAL PRODUCTS FROM ENDOPHYTIC FUNGI AND HIGHER PLANTS
AND TOTAL SYNTHESIS OF MICROCARPALIDE

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAI'I IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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By

Anokha Sayani Ratnayake

Thesis Committee:

Thomas K. Hemscheidt, Chairperson
Marcus A. Tius
Richard E. Moore
Katja Michael
Michael Cooney
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Abstract

Part-1

Microcarpalide (1.1), a new alkyl-substituted nonenolide was isolated from fermentation broths of an unidentified fungus. Microcarpalide is weakly cytotoxic to mammalian cells and acts as a microfilament disrupting agent. The gross structure of 1.1 was elucidated by the application of spectroscopic methods, particularly high-resolution NMR spectroscopy. The relative stereochemistry of the C-4/C-5 diol function was determined as syn (threo) based on spectroscopic correlations. The absolute configuration at C-4/C-5 (determined by the exciton chirality method) was related by means of conformational analysis to the C-9 stereochemistry in order to elucidate the absolute configuration at C-9/C-10. The relative stereochemistry of the latter two centers was established as threo by the application of the J-based method of Murata. This is the first successful application of Murata’s method for assigning the relative configuration of carbon atoms that are part of a medium size ring (8≤11).

On the basis of a cytotoxicity assay, the crude organic extract of the Hawaiian endemic plant Tetraplasandra hawaiiensis was selected as a candidate for bioassay-guided fractionation. The major compound responsible for the in vitro cytotoxicity was isolated and identified by 1H and 13C NMR spectroscopy as the diacetylenic compound (+)-falcarindiol (1.12). The absolute configuration of (+)-falcarindiol (1.12) from T. hawaiiensis was unambiguously determined as 3(R),8(S) by chemical means (olefin cross-metathesis and advanced Mosher method) and by its independent synthesis.
Part-2

The proposed structure and stereochemical assignment of microcarpalide (1.1) was verified by total synthesis. A convergent approach was used to assemble the 10-membered lactone carbon skeleton of 1.1, employing ring-closing metathesis (RCM) as the key step for the formation of the medium-sized ring. The stereochemical outcome of the RCM reaction was studied by varying the choice of ruthenium alkylidene catalyst and diene substrate. The ability of the same metathesis catalysts to promote double-bond isomerization via ring opening metathesis (ROM) of the cyclized product was also examined.
List of Tables

1.1 Number and the ratio of conformers in 1.1 as a function of the solvent used ............... 6
1.2 $^1$H and $^{13}$C NMR data of 1.1 in CD$_3$CN at 400 MHz ($^1$H) and 100 MHz ($^{13}$C) .......... 7
1.3 NMR data of 1.7 in CDCl$_3$ at 500 MHz ($^1$H) and 125 MHz ($^{13}$C) ......................... 10
1.4 Characteristic $^{2,3}J$ values of 1.7 for the C-9/C-10 diol function (in CDCl$_3$) .............. 13
1.5 Characteristic $^{2,3}J$ values of 1.1 for the C-9/C-10 diol function (in MeOH-$d_4$) .......... 15
1.6 Distribution and biological activity of falcarindiol (1.12) ........................................ 26
1.7 Optical rotations and assigned configurations of falcarindiol (1.12) ............................ 32
1.8 Stereochemical analysis of falcarindiol (1.12) with (R)- and (S)-MPA and MTPA derivatives .............................................................................................................................................. 43
2.1 Preparation of p-methoxybenzaldehyde dimethyl acetal reagent ............................... 74
2.2 Attempted (Z)$\rightarrow$(E) isomerizations in 2.3b ................................................................ 89
2.3 Stereoselectivities observed during RCM of esters 2.3 and 4.19 .................................. 91
List of Figures

1.1 Formation of the isopropylidene acetal (1.7) ......................................................... 8
1.2 The H/H anti/gauche pair of rotamers in the C-8/C-11 unit of 1.7. ...................... 13
1.3 Formation of the acetyl derivative (1.7a) ................................................................. 13
1.4 Ring expansion in 1.7 ................................................................................................. 14
1.5 Selected ROESY correlations and J-values (in Hz) of 1.7 .................................. 16
1.6 The J-values of the major conformer of 1.7 in CDCl₃ ........................................... 20
1.7 Conformations arising from the changes in the double bond orientation in 1.1 .... 21
1.8 Allylic rearrangements in C17 oxylipins. ................................................................. 25
1.9 C18 acetylenes from Dendropanax ........................................................................ 25
1.10 Cytotoxins from T. hawaiiensis ............................................................................. 27
1.11 HMBC correlations within 1.12 ............................................................................ 28
1.12 Catalytic hydrogenation of the diacetylenes from P. oreoselinum ...................... 29
1.13 Symmetrical products expected from degradation of 1.12 ............................... 32
1.14 Oxidative degradation of 1.12 ................................................................................ 33
1.15 Grubbs' metathesis on the bis-TBS protected falcarindiol (1.12) ...................... 34
1.16 Intermolecular enyne metathesis .......................................................................... 34
1.17 Degradation of 1.12 by metathesis ........................................................................ 35
1.18 Δδ values (Δδ = δ_S - δ_R, 300 MHz, CDCl₃) .......................................................... 37
1.19 Approaches to the formation of the symmetrical diol (1.26) ............................. 39
1.20 Re-exposure of 1.26 to metathesis conditions ...................................................... 40
2.1 Energy diagrams for the progress of the RCM reaction between 2.3 and the catalyst
List of Schemes

1.1 The preparation of the *bis*-benzoate derivative from 1.7 ........................................ 17
1.2 Formation of the racemic allyl alcohol 1.28 ................................................................. 36
1.3 Synthetic route to the chiral diol 1.26 ................................................................. 37
1.4 Epimerization of allyl alcohols via the hydride shift mechanism .............................. 38
2.1 Retrosynthetic pathway to microcarpalide (1.1) ..................................................... 69
2.2 Cyclization of the diene to the lactone 1.1 ............................................................... 70
**Table of Contents**

Acknowledgments ............................................................................................................................ iii

List of Tables ...................................................................................................................................... vi

List of Figures ...................................................................................................................................... vii

List of Schemes ................................................................................................................................. vii

List of Abbreviations .......................................................................................................................... xii

Part 1: Structure Elucidation of Natural Products from Endophytic Fungi and Higher Plants ......................................................................................................................... 1

1.1 Introduction ...................................................................................................................................... 1

1.1.1 Fungal Secondary Metabolites .................................................................................................. 1

1.1.2 Endophytes as a Source of Pharmaceuticals ............................................................................ 2

1.1.3 Cytotoxic Plant Metabolites ..................................................................................................... 3

1.2 Cytotoxic Fungal Metabolites ......................................................................................................... 5

1.2.1 Microcarpalide .......................................................................................................................... 5

1.2.1.1 Isolation and Structure Determination of Microcarpalide .................................................... 6

1.2.1.2 Stereochemistry and Conformation of Microcarpalide......................................................... 11

1.2.1.3 Origin of Conformers in Microcarpalide .............................................................................. 20

1.3 Cytotoxic Plant Metabolites ........................................................................................................... 24

1.3.1 Falcarindiol Family of Compounds ......................................................................................... 24

1.3.1.1 History and Biology of Falcarindiol ..................................................................................... 25

1.3.1.1 Isolation and Structure Determination of Cytotoxic Diacetylenes from Tetraplasandra hawaiensis ......................................................................................................................... 26

1.3.1.2 Comparative Evaluation of the C-3 Stereochemistry of (+)-Falcarindiol ................. 29
1.3.2 Research Objective and Strategy .................................................. 32
  1.3.2.1 Symmetrical Analogues of Falcarindiol via Degradation and Synthesis..... 33
1.3.3 Evaluation of Results ......................................................................... 38
1.4 Conclusion ............................................................................................. 45
1.5 Experimental Section ............................................................................ 47
  1.5.1 General ............................................................................................ 47
    1.5.1.1 Spectral Analysis ...................................................................... 47
    1.5.1.2 General Operations .................................................................. 48
  1.5.2 Biological Material .......................................................................... 49
    1.5.2.1 Isolation of Fungal Strains .................................................... 49
    1.5.2.2 Cultivation of Fungal Strains ................................................ 50
  1.5.3 Extraction and Isolation ................................................................... 50
    1.5.3.1 Isolation of Microcarpalide (1.1) ........................................... 50
    1.5.3.2 Isolation of Falcarindiol (1.12) .............................................. 51
  1.5.4 Bioassay .......................................................................................... 52
  1.5.5 Physical Data ................................................................................... 53
  1.5.6 Synthetic Transformations .............................................................. 55
Part 2: Total Synthesis of Microcarpalide .................................................. 66
  2.1 Introduction ........................................................................................ 67
  2.2 Synthetic strategy .............................................................................. 68
    2.2.1 Synthesis of the Right-Hand (RH) Segment ................................ 70
    2.2.2 Synthesis of the Left-Hand (LH) Segment ................................... 74
    2.2.3 Macrocyclic Ring Closing Metathesis (RCM) .............................. 83
List of Abbreviations

<table>
<thead>
<tr>
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<tr>
<td>EC₅₀</td>
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<tr>
<td>EI</td>
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<td>EIMS</td>
<td>electron-impact mass spectrometry</td>
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<td>electro-spray ionization</td>
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FTIR        Fourier transformed infrared
FAB         fast atom bombardment
g           gram
$^1{\text{H}}$ proton isotope
HF          hydrogen fluoride
HREIMS       high resolution electron-impact mass spectrometry
HRMS        high resolution mass spectrometry
HMBC        heteronuclear multiple bond correlation
HMQC        heteronuclear multiple quantum coherence
HETLOC      heteronuclear half-filtered TOCSY
HSQC        heteronuclear single quantum coherence
HSQMBC      heteronuclear single quantum multiple bond correlation
Hz           hertz
h            hour
H$_2$O       water
HPLC        high performance liquid chromatography
IC$_{50}$ inhibitory concentration 50%
INEPT       insensitive nuclei enhancement by polarization transfer
$i$-Pr       isopropyl
IR          infrared
INADEQUATE  incredible nuclear abundance double quantum transfer experiment
$^nJ$        coupling constant via $n$ bonds
KB          human nasopharyngeal carcinoma cell line KB
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<td>M</td>
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</tr>
<tr>
<td>TsOH</td>
<td>toluenesulfonyl chloride</td>
</tr>
<tr>
<td>TMS-Cl</td>
<td>trimethylsilyl chloride</td>
</tr>
<tr>
<td>t-BuOH</td>
<td>tert-butyl alcohol</td>
</tr>
<tr>
<td>TOCSY</td>
<td>total correlated spectroscopy</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>v/v</td>
<td>volume per volume</td>
</tr>
<tr>
<td>(\lambda)</td>
<td>wavelength</td>
</tr>
<tr>
<td>(%)</td>
<td>percent</td>
</tr>
</tbody>
</table>
Part 1: Structure Elucidation of Natural Products from Endophytic Fungi and Higher Plants
1.1 Introduction

1.1.1 Fungal Secondary Metabolites

The total number of fungal species including undescribed ones, is estimated at around 250,000. This genetic diversity is reflected not only in morphological characteristics, but also in biochemical differences. These biochemical differences among the fungi can lead to the production of a wide range of secondary metabolites. Secondary metabolites are widely regarded as not being required for fungal growth but as conferring selective advantages to the producing organism.

A structurally diverse range of secondary metabolites has been observed in fungi. A subset thereof may serve as lead compounds for the development of drugs for pharmacological uses. For example, the penicillin, and the cephalosporin families of antibiotics are well known examples of drugs derived from fungi. Until recently, terrestrial micro-organisms have been the most common source of bioactive metabolites. However, in recent years the frequency of discovery of structurally novel compounds from these classical sources have declined.

One source of new bioactive metabolites left to be explored are microbes that have symbiotic relationships with plants. Only a few studies have been carried out on endophytes associated with tropical trees. This is a broad field of investigation that is almost entirely open to new findings. It could be expected that increased focus on these tropical microorganisms may lead to new pharmaceutical drugs.
1.1.2 Endophytes as a Source of Pharmaceuticals

Endophytic microorganisms are defined as fungi colonizing healthy plant tissue without causing overt symptoms or apparent injury to the host. These microbes have a symbiotic relationship with the host plant, receiving nutrients from it in exchange for protection against disease-carrying bacteria or fungi. Many, if not all woody plant stems harbor endophytes but because they are symptomless these fungi are difficult to detect and can only be successfully surveyed by plating out carefully prepared surface sterilized tissues. Endophytes are generally not considered organ-specific, and it is likely that many of the species isolated from stems also occur in leaves.

Most of the research with endophytes has been carried out using plants from temperate regions. The data from tropical regions is scarce, but preliminary results have shown that tropical plant-hosts contain a great diversity of endophytic microorganisms, many of them not yet classified and thus may belong to new genera or species. Potentially these organisms could provide new secondary metabolites that are of pharmaceutical value. As a part of an ongoing research program aimed at the discovery of naturally occurring biologically active natural products, we have been collecting and cultivating endophytic fungi from plants growing in Hawaii. Isolation and structure elucidation of one such new bioactive natural product from an endophytic fungus is described in part-1 of this dissertation. A short total synthesis of this compound is described in the second half of this dissertation.
1.1.3 Cytotoxic Plant Metabolites

Plants are capable of synthesizing an overwhelming variety of low-molecular weight organic compounds called secondary metabolites, many with unique and complex structures. Most of these compounds are differently distributed among limited taxonomic groups within the plant kingdom and, conversely, each plant species has a distinct profile of secondary metabolites. The biological function of many of the secondary products has been debated for a long time. However, considering their non-motile nature and lack of a sophisticated immune system, it is believed that plants may have developed their own chemical defense system against pathogens and predators. Indeed, many plant secondary metabolites are repellent or even poisonous to pests and herbivores. Thus, plant secondary metabolites are of tremendous importance, both for the plant itself (for plant-environment interactions) and for humans, since biological active metabolites can have high therapeutic value.

Secondary plant products have for thousands of years played an essential role in medicine. In traditional medicine, they have been used indirectly as food and herbs. In western medicine, a large number of human diseases are treated with natural product drugs, that have diverse chemical structures and biological activities. In fact over 50% of the top 25 selling prescription products are derivatives of natural product leads and approximately 25% of prescriptions handled in the USA contain a plant-derived natural product. For example, taxol, vincristine and camptothecin represent powerful anticancer compounds that are well-established plant derived molecules.

Because of the geographical isolation from the rest of the world, Hawaii has developed unique flora. Thus the possibility of finding unique biologically active natural
products from its flora is apparent. While the marine habitat surrounding Hawaii has been extensively explored for biologically active chemical constituents, the terrestrial higher plants have received little attention. Our group has been involved in the screening of several endemic Hawaiian plant extracts for antitumor activity. One of the bioactive plant metabolites that these efforts have yielded is discussed in part-1 of this dissertation.
1.2 Cytotoxic Fungal Metabolites

1.2.1 Microcarpalide

Microcarpalide (1.1) was isolated from fermentation broths of an unidentified endophytic fungus, obtained from the bark of *Ficus microcarpa* L. In A-10 cells, pure samples of 1.1 displayed an IC₅₀ value of 0.5-1.0 µg/mL. At concentrations above 20 µg/mL a cytotoxic effect was observed in the A-10 cell line (rat smooth muscle cells) as evidenced by cell loss. The cytotoxicity of 1.1 against cancer cells is similarly weak with IC₅₀ values in the KB (human nasopharyngeal carcinoma) and LoVo (human colon adenocarcinoma) cell lines of 50 and 90 µg/mL, respectively. This novel alkyl-substituted nonenolide is one of the members of a series of related poly-hydroxy macrocyclic lactone phytotoxins such as pinolidoxin (1.2), lethaloxin (1.3), the herbarums (1.4), and putaminoxin (1.5), all of which bear a propyl substituent at C-9. Achaetolide (1.6), as the closest analogue of microcarpalide (1.1), shares the same molecular formula, although obvious differences exist with respect to the hydroxylation pattern and double bond position.
1.2.1.1 Isolation and Structure Determination of Microcarpalide

Microcarpalide (1.1) was isolated by bioassay-guided fractionation of the ethyl acetate extract (0.25 g) of the fermentation broth (*Ficus microcarpa* L.) after repeated normal-phase chromatography as an optically active, colorless oil (18 mg, 7.2%) \([\alpha]_D^{22} -22 (c \ 0.67, \text{MeOH})\). The IR spectrum of 1.1 displayed significant bands at 3380 (br) and 1715 cm\(^{-1}\) indicating the presence of alcohol and ester functional groups, respectively. The UV/vis spectrum recorded in MeOH showed end absorption only.

Examination of the \(^1\)H NMR spectrum of 1.1 recorded in CD\(_3\)CN indicated the presence of a minor component (ratio of 3.5:1) in the chromatographically apparently homogeneous material. This ratio was a function of the solvent used for NMR, (Table 1.1) which suggested the presence of conformers.

**Table 1.1. Number and the ratio of conformers of 1.1 as a function of the solvent used**

<table>
<thead>
<tr>
<th>(^1)H NMR Solvent(^a)</th>
<th>Number of Conformers</th>
<th>Major: Minor Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDCl(_3)</td>
<td>2</td>
<td>1 : 1</td>
</tr>
<tr>
<td>Acetone-(d_6)</td>
<td>2</td>
<td>3 : 1</td>
</tr>
<tr>
<td>MeOH-(d_4)</td>
<td>3</td>
<td>7 : 2 : 1</td>
</tr>
<tr>
<td>CD(_3)CN</td>
<td>2</td>
<td>3.5 : 1</td>
</tr>
</tbody>
</table>

\(^a\) Owing to the limited solubility of 1.1 in other common NMR solvents, acetone-\(d_6\), MeOH-\(d_4\), CD\(_3\)CN and CDCl\(_3\) were the only solvents tried, with inferior results.

This interpretation was confirmed through the observation of exchange correlations in the NOESY spectrum of 1.1 (in CD\(_3\)CN), between several signals for the minor and the major conformer respectively. The \(^13\)C NMR spectrum of 1.1, which displayed many doubled and several extensively broadened resonances, further corroborated this interpretation. In fact the line broadening was so severe that even a simple carbon count for 1.1 could not be obtained with confidence. Further difficulties arose when 1.1 did not yield a clear-cut molecular ion under a variety of ionization
conditions (EI, FAB, CI, ES). Eventually a partial gross structure was established on the
basis of the resonances for the major conformer by analysis of COSY, gHSQC, and
gHMBC spectra recorded in CD$_3$CN. This analysis suggested that 1.1 contains a double
bond of trans geometry ($^2$J$_{HH}$=15.8 Hz) and a vicinal diol function.

Table 1.2. $^1$H and $^{13}$C NMR data of 1.1 in CD$_3$CN at 400 MHz ($^1$H) and 100 MHz ($^{13}$C)

<table>
<thead>
<tr>
<th>Major conformer</th>
<th>Minor conformer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C no</strong></td>
<td><strong>$\delta$ $^{13}$C (J in Hz)</strong></td>
</tr>
<tr>
<td>1</td>
<td>176.4</td>
</tr>
<tr>
<td>2</td>
<td>29.1</td>
</tr>
<tr>
<td>3</td>
<td>26.5</td>
</tr>
<tr>
<td></td>
<td>1.77, br dddd</td>
</tr>
<tr>
<td>4</td>
<td>73.4</td>
</tr>
<tr>
<td>5</td>
<td>72.4</td>
</tr>
<tr>
<td>6</td>
<td>134.6</td>
</tr>
<tr>
<td>7</td>
<td>126.7</td>
</tr>
<tr>
<td>8</td>
<td>36.7</td>
</tr>
<tr>
<td></td>
<td>2.14, br m</td>
</tr>
<tr>
<td>9</td>
<td>79.7</td>
</tr>
<tr>
<td>10</td>
<td>72.8</td>
</tr>
<tr>
<td>11</td>
<td>34.2</td>
</tr>
<tr>
<td>12</td>
<td>26.1</td>
</tr>
<tr>
<td>13</td>
<td>30.0</td>
</tr>
<tr>
<td>14</td>
<td>32.5</td>
</tr>
<tr>
<td>15</td>
<td>23.3</td>
</tr>
<tr>
<td>16</td>
<td>14.4</td>
</tr>
<tr>
<td>4$^c$</td>
<td>-</td>
</tr>
<tr>
<td>5$^c$</td>
<td>-</td>
</tr>
<tr>
<td>10$^c$</td>
<td>-</td>
</tr>
</tbody>
</table>

ND$^a$ = Not detected
$^b$ = Assignment is tentative because of signal overlap with H-2 and H-3
$^c$ = Exchangeable proton (OH)
On the basis of this information, we prepared the ketal derivative 1.7 \textsuperscript{12} (Figure 1.1) which displayed a much-improved ratio of minor to major conformer (1:7 in CDCl\textsubscript{3}). Gratifyingly, 1.7 proved to be amenable to mass spectrometric analysis. The low-resolution EIMS of 1.7 indicated a molecular mass of 340 and the HREIMS (found 340.2279, Δ -2.9 mmu) suggested a molecular formula of C\textsubscript{19}H\textsubscript{32}O\textsubscript{5} for 1.7 and hence one of C\textsubscript{16}H\textsubscript{28}O\textsubscript{5} (m/z 300) for 1.1.

![Diagram](https://example.com/diagram)

**Figure 1.1.** Formation of the isopropylidene acetal (1.7).

The combined analysis of the COSY, gHSQC, and gHMBC spectra of both 1.1 (in CD\textsubscript{3}CN) and 1.7 (in CDCl\textsubscript{3}) confirmed the gross structure of the ketal derivative 1.7. The proton connectivity within the nonenolide ring was deduced from COSY data and is in accord with the gHMBC spectra. The chemical shifts of the H-2 protons (δ\textsubscript{H-2} 2.54 and 2.32) suggested that the corresponding carbon atom C-2 (δ\textsubscript{C-2} 33.9) was adjacent to a carbonyl (δ\textsubscript{C-1} 170.4). The lactone structure followed from chemical shift considerations for the C-9 hydroxymethine proton (δ\textsubscript{H-9} 4.70) and from a gHMBC correlation of H-9 to C-1, which could be observed in the data set of 1.1 (major) but not in that of 1.7. The methine protons, H-6 and H-7, were attached to carbon atoms resonating at 129.3 and 130.2 ppm respectively, suggesting the presence of an internal double bond. The geometry of this double bond was established as *trans* based on the large coupling
constant observed between the vinylic protons ($^{3}J_{\text{H-6/H-7}}$ 15.6 Hz). The C-4/C-5 diol function was placed next to the double bond based on COSY correlations between the H-6 resonance at 5.33 ppm and the allylic signal (H-5) at 3.92 ppm. The diol was shown to be derivatized as the ketal known to be present within 1.7, on the basis of long-range correlations between the acetal carbon atom (C-17) at 108.8 ppm and the two hydroxymethine protons H-4 and H-5, resonating at 3.63 and 3.92 ppm respectively.

The presence of an exocyclic secondary free hydroxyl group at C-10 was readily inferred from the gHMBC data of 1.1. The structural features present within the ring including C-10 accounted for ten carbon atoms, all of the five oxygen atoms, and fifteen hydrogen atoms of the molecular formula as well as the three degrees of unsaturation of 1.1. This in turn suggested the presence of a C$_{6}$H$_{13}$ substituent, which had to be placed on C-10. A broad methylene envelope at 1.28-1.40 ppm and a broad three-proton triplet at 0.87 ppm in the $^{1}$H NMR spectra of 1.1 and of 1.7 suggested the presence of an $n$-hexyl unit.
## Table 1.3. NMR data of 1.7 in CDCl₃ at 500 MHz (¹H) and 125 MHz (¹³C)

<table>
<thead>
<tr>
<th>C no</th>
<th>²⁷C</th>
<th>δ¹H (J in Hz)</th>
<th>¹H COSY</th>
<th>HMBC (H→C)</th>
<th>NOE (H→H)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>170.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>33.9</td>
<td>2.54, ddd (13.4, 5.6, 3.6)</td>
<td>3</td>
<td>1, 3, 4</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.32, ddd (13.4, 12.0, 4.4)</td>
<td>3</td>
<td>1, 3, 4</td>
<td>4, 6, 9</td>
</tr>
<tr>
<td>3</td>
<td>25.5</td>
<td>1.97, dddd (15.3, 12.0, 8.1, 3.6)</td>
<td>2, 4</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.09, br m</td>
<td>2, 4</td>
<td>2, 4, 5</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>80.0</td>
<td>3.63, br ddd</td>
<td>3, 5</td>
<td>2, 6</td>
<td>2, 3, 6, 19</td>
</tr>
<tr>
<td>5</td>
<td>83.9</td>
<td>3.92, dd (9.3, 8.8)</td>
<td>4, 6</td>
<td>3, 4, 7</td>
<td>3, 7, 18</td>
</tr>
<tr>
<td>6</td>
<td>129.3</td>
<td>5.33, dd (15.6, 9.3)</td>
<td>5, 7</td>
<td>4, 8</td>
<td>2, 4, 8</td>
</tr>
<tr>
<td>7</td>
<td>130.7</td>
<td>5.78, ddd (15.6, 11.2, 4.7)</td>
<td>6, 8</td>
<td>5, 8</td>
<td>5, 8</td>
</tr>
<tr>
<td>8</td>
<td>32.2</td>
<td>2.42, br m</td>
<td>7, 9</td>
<td>6, 7, 10</td>
<td>7, 10, 11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.67, dddd (12.3, 11.2, 8.7)</td>
<td>7, 9</td>
<td>6, 7, 9, 10</td>
<td>6, 9</td>
</tr>
<tr>
<td>9</td>
<td>77.0</td>
<td>4.70, ddd (8.7, 4.7, 2.3)</td>
<td>8, 10</td>
<td>-</td>
<td>2, 8, 10, 11</td>
</tr>
<tr>
<td>10</td>
<td>73.8</td>
<td>3.65, br m</td>
<td>9, 11</td>
<td>8</td>
<td>8, 9, 11</td>
</tr>
<tr>
<td>11</td>
<td>33.6</td>
<td>1.48, br m</td>
<td>10</td>
<td>12</td>
<td>8, 9, 10</td>
</tr>
<tr>
<td>12</td>
<td>25.6</td>
<td>1.30-1.24</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>29.2</td>
<td>1.30-1.24</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>31.7</td>
<td>1.30-1.24</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>22.6</td>
<td>1.30-1.24</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>14.1</td>
<td>0.88, t (6.8)</td>
<td>CH₂-envelope</td>
<td>14, 15</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>108.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>27.1</td>
<td>1.41, s</td>
<td>-</td>
<td>17, 19</td>
<td>5</td>
</tr>
<tr>
<td>19</td>
<td>26.9</td>
<td>1.41, s</td>
<td>-</td>
<td>17, 18</td>
<td>4</td>
</tr>
</tbody>
</table>
1.2.1.2 Stereochemistry and Conformation of Microcarpalide

The Relative Stereochemistry. With the gross structure in hand, the relative stereochemistry of the C-4/C-5 diol function of 1.7 was examined. The large coupling constant of 8.8 Hz observed between H-4 and H-5 in 1.7 and the lack of any NOE correlation between these protons was indicative of a trans ring fusion and hence of syn stereochemistry at C-4/C-5.

\[
\begin{align*}
\text{syn (threo) stereochemistry} & \\
\text{3} \ J_{H-4/H-5} = 8.8 \text{ Hz} & \\
\text{(no NOE correlation)}
\end{align*}
\]

The relative stereochemistry of the vicinal diol moiety at C-9/C-10 was assigned by application of the J-based configuration analysis method of Murata. The J-based analysis is an empirical method, which allows the assignment of the diastereomeric relationship between vicinal methine systems. The combined use of \(2,3 \ J_{C/H}\) and \(3 \ J_{H/H}\) values\(^{13}\) and NOE/ROE data enables the identification of the predominant rotamer(s) from among the six possible staggered conformers arising from threo and erythro configurations.

The inter-proton vicinal spin-coupling constants \(\tilde{J}_{H/H}\) are measured by the 1D TOCSY experiment\(^{14}\) or the homonuclear decoupling difference spectra while carbon-proton spin coupling constants \(2,3 \ J_{C/H}\) are measured by 2D NMR methods such as HSQMBBC\(^{15}\) and HETLOC (heteronuclear half-filtered TOCSY).\(^{16}\) This method has proven useful in elucidating the relative configuration of acyclic sub-units of natural products.\(^{17}\) However, there is little precedence for the application of this methodology in
cyclic systems, since in such systems non-staggered conformations may cause significant deviations in the dihedral angles from the anti and gauche orientation. Thus an unambiguous assignment of stereochemistry using this method may not be possible.

For the successful application of the $J$-based methodology to the vicinal centers C-9/C-10 of 1.1 or 1.7, five crucial NMR coupling constant parameters ($J_{H-9/H-10}$, $J_{H-9/C-11}$, $J_{H-10/C-8}$, $J_{H-10/C-9}$ and $J_{H-9/C-10}$) have to be unambiguously determined. Due to a more favorable conformational ratio (1:7 in CDCl$_3$), the initial configuration analysis was attempted on the ketal derivative 1.7. However, excessive broadening of the resonance for H-10 ($\delta_H$ 3.65), defeated all attempts to extract the $J_{C-8/H-10}$ value by a HETLOC experiment.

While four of the five couplings required for the analysis were determined successfully (Table 1.4), lack of a quantitative data point for $J_{C-8/H-10}$ reduced the certainty of the C-9/C-10 threo stereochemical assignment in 1.7. Since the application of the $J$-based methodology has little precedence in cyclic systems, especially to medium size rings, all five data points were regarded as crucial for an unambiguous stereochemical assignment.
Table 1.4. Characteristic $^{2,3}J$ values of 1.7 for the C-9/C-10 diol function (in CDCl$_3$)

<table>
<thead>
<tr>
<th>$J$ (Hz) (classification)</th>
<th>Classification for the threo diol</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^3 J$ (H-9, H-10)</td>
<td>+ 4.7 (medium)</td>
</tr>
<tr>
<td>$^3 J$ (H-9, C-11)</td>
<td>+1.4 (small)</td>
</tr>
<tr>
<td>$^3 J$ (H-10, C-8)</td>
<td>ND$^c$</td>
</tr>
<tr>
<td>$^2 J$ (H-10, C-9)</td>
<td>-1.5 (medium)</td>
</tr>
<tr>
<td>$^2 J$ (H-9, C-10)</td>
<td>-1.7 (medium)</td>
</tr>
</tbody>
</table>

$^a$ Values between large and small are regarded as medium. $^b$ Large, small and medium refer to the magnitude of the coupling constants. $^c$ Not detected.

![Table 1.4](image)

Figure 1.2. The H/H anti/gauche pair of rotamers in the C-8/C-11 unit of 1.7. The alternating A-1/A-3 pair of threo rotamers was identified using the $^3 J_{HH}$ and $^{2,3} J_{CH}$ values.

With the hope of removing the signal overlap between H-4 and H-10 and the signal broadening due to the exchangeable proton at C-10, which had previously obstructed the configuration analysis of 1.7, the corresponding acetylated ketal derivative 1.7a was prepared.$^{20}$

![Figure 1.3](image)

Figure 1.3. Formation of the acetyl derivative.
While the proton signals of H-10 and H-4 were now resolved, the signal for H-10 in 1.7a was still too broad to extract the coupling constant information, hence another derivative was required. A previous attempt to form the ketal derivative 1.7 from 1.1 with p-TsOH as the catalyst (acetone/DMP, RT) instead of PPTS, had resulted in isolation of the ring-expanded rearranged product 1.8 featuring an 11-membered lactone.

![Figure 1.4. Ring expansion of 1.7.](image)

The NMR spectra of 1.8 (500 MHz, CDCl₃) did not show any evidence for the existence of conformers, and all of the signals of interest were well separated. Thus we decided to carry out the J-based analysis using this 11-membered lactone (1.8). Unfortunately, due to a weak $^{3}J_{H,H}$ coupling interaction between H-9 and H-10 of 1.8 ($^{3}J_{H-9/H-10} = 0.4$ Hz), the HETLOC pulse sequence could not be applied successfully. In order to increase the magnitude of the $^{3}J_{H-9/H-10}$ value, the C-10 acetate derivative 1.8a was prepared (acetic anhydride, pyridine, DMAP, 0 °C, overnight) from 1.8. Even though the acetate 1.8a (500 MHz, CDCl₃) displayed an improved coupling ($^{3}J_{H-9/H-10} = 2.8$ Hz) between these two protons, the weak long range C-H correlations in both HETLOC and HSQMBC spectra interfered with the interpretation of the data.

Having failed to obtain the information needed to establish the relative configuration of C-9/C-10 using the ketal and the ketal ester derivatives of 1.1, the most viable alternative was to attempt the J-based analysis on the unprotected diol 1.1.
Although $^1$H NMR data of 1.1 in MeOH-$d_4$ indicated the presence of three conformers (7:2:1 ratio), the major one accounted for ~70% of the population. Moreover, the signals of the major were well separated from those of the minor conformers allowing the $J$-based analysis to be safely carried out on the major one.

Using MeOH-$d_4$ as the NMR solvent had the advantage of replacing the exchangeable proton at C-10 with deuterium, thus reducing the multiplicity of the resonance for H-10. Moreover, sufficient chemical shift dispersion of all proton resonances along with a $^3J_{H-9/H-10} = 4.4$ Hz was observed in this solvent. Therefore the diol 1.1 in MeOH-$d_4$ was subjected to configuration analysis by Murata’s method using the HETLOC pulse sequence. Gratifyingly, the characteristic coupling constants could be extracted from the HETLOC spectra of this sample (Table 1.5). This analysis strongly suggested threo configuration ($9S^*, 10S^*$) of the C-9/C-10 diol function of 1.3.

**Table 1.5.** Characteristic $^2_3J$ values of 1.1 for the C-9/C-10 diol function (in MeOH-$d_4$)

<table>
<thead>
<tr>
<th>$^2J$ (H-9, H-10)</th>
<th>$^3J$ (H-9, C-11)</th>
<th>$^3J$ (H-10, C-8)</th>
<th>$^2J$ (H-10, C-9)</th>
<th>$^2J$ (H-9, C-10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+4.4 (medium)</td>
<td>+1.8 (small)</td>
<td>+1.5 (small)</td>
<td>-0.9 (medium)</td>
<td>-0.3 (medium)</td>
</tr>
</tbody>
</table>

Classification for the *threo* diol

<table>
<thead>
<tr>
<th>$^2J$ (H-9, H-10)</th>
<th>$^3J$ (H-9, C-11)</th>
<th>$^3J$ (H-10, C-8)</th>
<th>$^2J$ (H-10, C-9)</th>
<th>$^2J$ (H-9, C-10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>medium</td>
<td>small</td>
<td>small</td>
<td>medium</td>
<td>medium</td>
</tr>
</tbody>
</table>

* Values between *large* and *small* are regarded as *medium*. * Large, small and medium refer to the magnitude of the coupling constants.
Conformation of the Ketal Derivative (1.7) Next the preferred conformation of 1.7 was established with the aid of a ROESY experiment. The important ROESY correlations and $J$-values are shown below.

![Diagram](image)

**Figure 1.5.** Selected ROESY correlations and $J$-values (in Hz) of 1.7.

Using the H-4/H-5 protons of known *anti* orientation (based on the lack of NOE correlations and the large $3J_{H-4/H-5} = 8.8$ Hz value) as a starting point, the ROESY analysis suggests that H-5 and H-7 are placed on the same face of the ring. Because of the *trans* configuration of the double bond ($3J_{H-6/H-7} = 15.6$ Hz), this in turn placed the vinyl proton H-6 on the opposite face of the ring from H-5. A consecutive network of ROESY correlations from H-4, H-6, H-8$^1$ and H-9 showed that these protons reside on one face of the ring, and are thus on the opposite face to H-5 and H-7.$^{22}$

This NOE-based result was corroborated by coupling constant analysis for 1.7 which was of particular importance with respect to the stereochemistry at C-9. Proton H-7 showed a large coupling to H-8$^1$ ($3J_{H-7/H-8} = 11.2$ Hz), which is indicative of a *trans* diaxial orientation. In turn, H-8$^1$ coupled to H-9 with $3J_{H-8^1/H-9} = 8.7$ Hz. The large $H-8^1/H-9$ coupling constant was of some concern as it is suggestive of an *anti* orientation and hence a *trans* relationship between these two protons rather than a *cis* one, as had been
concluded from the ROESY data. If H-8\(^1\) and H-9 were to reside on the same face of the ring at all, the magnitude of the coupling constant would suggest that they cannot be present in a staggered (gauche) conformation in 1.7 and hence a synperiplanar arrangement would have to be postulated instead. Coupling constant calculations\(^{23}\) based on dihedral angles of 0° and 180° predicted a value for \(J_{\text{H-8/H-9}}\) of 9.4 and 11.4 Hz, respectively. The former value was in excellent agreement with the experimental value of 8.7 Hz and suggested a small dihedral angle between H-8\(^1\) and H-9. This confirmed our earlier assignment, based on the ROESY data, of a cis relationship between these two protons. Since the orientation of H-4/H-5 protons was anti and the configuration of the C-9/C-10 diol function was threo (9\(^S\)*, 10\(^S\)*), the network of ROESY correlations from H-4, H-6, H-8\(^1\) and H-9 established the relative stereochemistry of the asymmetric centers in 1.1 as 4\(^R\)*, 5\(^R\)*, 9\(^S\)*, 10\(^S\)*.

**The Absolute Stereochemistry.** The absolute configuration of the C-4/C-5 threo diol function was established by the application of the exciton chirality method\(^{24}\) using the bis-p-methoxybenzoate derivative 1.9 (Scheme 1.1).

![Scheme 1.1](image)

**Scheme 1.1.** The preparation of the bis-benzoate derivative from 1.7.

It was expected that the chiral exciton coupling (\(\pi\rightarrow\pi^*\) transition) between the two strongly absorbing p-methoxybenzoate chromophores would generate a strong split CD
Cotton effect. However, since the benzoate at C-5 is flanked by a benzoate (C-4) and a double bond (C-6/C-7), exciton coupling between either chromophore (:two Cotton effects) could be anticipated. Therefore, in order to suppress the contribution from the undesired allylic-benzoate coupling to the exciton chirality split, the p-methoxybenzoate derivative (π→π* transition ~257 nm) was chosen, rather than an unsubstituted benzoate (π→π* transition ~230 nm). This would maximize the wavelength separation (Δλ_{max}) between the benzoates and the double bond π→π* (~195 nm), thus decreasing the Δε value (i.e. CD signal amplitude) of the allylic-benzoate Cotton effect.

The C-10 hydroxyl function was blocked in the form of an acetate to prevent the formation of the tris-benzoate and the possible complications in the CD spectrum due to long range interactions of the C-10 benzoyl residue with those on C-4 and C-5. Not surprisingly, conformational analysis of the triester 1.9 by 1H NMR in most of the commonly used NMR solvents was plagued by the presence of multiple conformers. However, 1H NMR data acquired in CD$_3$CN at 50 °C suggested the presence of one major conformer (≥ 90%). The coupling constant between H-4 and H-5 in 1.9 was determined to be 5.8 Hz. This value is considerably smaller than that observed in 1.7 for
these two protons ($^3J_{\text{H-4/H-5}} = 8.8 \text{ Hz}$) and suggested a difference in conformation between 1.7 and 1.9. However, after inspection of Dreiding molecular models this proved to be of no serious concern. The flexibility of the 1-oxa-cyclodecene system present within 1.9 is not sufficient to allow for inversion of the sense of helicity of the C-4/C-5 dibenzoate function during conformational twisting of the ring at ordinary temperature.

The CD spectrum of 1.9 in acetonitrile solution showed a first negative and a second positive Cotton effect ($\lambda_{\text{max}} 264 \text{ nm } \Delta\varepsilon = -7.6, \lambda_{\text{max}} 246 \text{ nm } \Delta\varepsilon = +6.5, \sum \Delta\varepsilon = -14.1$). This is indicative of negative exciton chirality and hence of $4R,5R$ absolute configuration. Since H-9 resides on the same face of the molecule as H-4, as shown by the ROESY correlations depicted in Figure 1.5, it follows that the absolute configuration at the C-9/C-10 threo diol of 1.1 is $9S,10S$. 
1.2.1.3 Origin of Conformers in Microcarpalide

Figure 1.6. The $J$-values of the major conformer of (1.7) in CDCl$_3$.

Inspection of the $^1$H NMR spectrum of the acetonide derivative (1.7) in CDCl$_3$ indicated a conformational ratio of 7:1. On the other hand, the $^1$H NMR spectrum of the parent triol (1.1) in CD$_3$CN, displayed two co-existing conformers in a 3.5:1 ratio. A detailed inspection of the $^1$H NMR spectrum of 1.7 suggested that the coupling constant data for the predominant conformer of 1.7 (in CDCl$_3$) are similar to those of the minor conformer of 1.1 (in CD$_3$CN). Thus, $^3J_{H-5/H-6}$ is large (9.4 Hz) in 1.1 (minor) and in 1.7, whereas in 1.1 (major) $^3J_{H-5/H-6}$ is small (2.0 Hz). Moreover, H-6 resonates upfield of H-7 in 1.1 (minor) ($\Delta\delta= -0.61$ ppm) and in 1.7 ($\Delta\delta= -0.45$ ppm), but downfield of H-7 in 1.1 (major) ($\Delta\delta= +0.2$ ppm). These chemical shift differences ($\Delta\delta$) are largely due to the shifting of the resonance of H-6 rather than that of H-7. This is perhaps a result of the reorientation of the diamagnetic shielding cone associated with the lactone carbonyl across the ring.
Prompted by the observation of drastic chemical shift ($\Delta\delta$) and coupling constant differences around the olefinic region of the major and the minor conformers in 1.1, we decided to investigate further differences in these conformations. It seemed likely that the difference in the alignment of the double bond (C-6/C-7) of the ten-membered lactone (1.1) would be responsible for such changes in the conformation. To verify this, it was necessary to compare the coupling constants and the NOE correlations of the major and the minor conformers.

![Diagram of conformations](image)

**Figure 1.7.** Conformations arising from the changes in the double bond orientation in 1.1.

A clear NOE was observed between the resonances for H-5 and H-6 of the (1.1) major, but not in the (1.1) minor, whereas H-5 and H-7 in 1.1 (minor) showed NOE to one another. These NOE data were in agreement with the coupling constant values obtained for the two conformers ($^3J_{H-5/H-6}$ is large (9.4 Hz) in 1.1 (minor), $^3J_{H-5/H-6}$ is small (2.0 Hz) in 1.1 (major)).

An attempt to extract coupling constants for the hydroxy methine protons H-4 and H-5 in both conformers of 1.1 failed due to excessive broadening of resonances, even after replacing the exchangeable protons at C-4 and C-5 with deuterium. A subsequent decoupling experiment also failed to yield the desired coupling constants. These
observations suggested that the broadening of the resonances for H-4 and H-5 in 1.1 is not due to the $^2J$-coupling with the exchangeable protons, but a result of conformational changes occurring on the NMR time scale.

In the left hand portion (C-8/C-9/C-10) of either the major or the minor conformers of lactone 1.1, the vinylic proton H-7 showed NOE correlations to both methylene protons at C-8. Thus no reliable information could be gathered based on the NOE correlations, concerning the configuration of this portion of the molecule. However, the magnitude of the $^3J_{H-8/H-9}$ values observed for the high and low field H-8 protons ($H^h$ and $H^l$, respectively) in 1.1 was reversed for the two conformers, while the coupling constant between both H-8 protons and the vinylic H-7 proton ($^3J_{H-7/H-8}$) remained unaffected. This showed that, in fact, the change in conformation in 1.1 arises due to the differences in the alignment of the olefinic bond (C-5/C-6), and that the rest of the ring (on either side of the double bond) undergoes rotational changes to accommodate this movement.

A subsequent variable temperature NMR experiment on microcarpalide 1.1 (in CD$_3$CN) was carried out using the 500 MHz NMR spectrometer to investigate the dynamics of interconversion of the major and the minor conformers. Even though there was evidence of moving together of the resonances of equivalent protons within the two conformers as a result of a gradual increase in temperature (15 °C → 45 °C → 75 °C), the frequency difference for the two peaks chosen for this analysis in the 500 MHz magnetic field, was too large for coalescence to occur. The coalescence temperature, at which the peaks merge into one broad peak, did not seem to lie within the range of room temperature to 75 °C.
In order to avoid potential problems due to ring-expansion, heating up the sample to higher temperatures was not attempted. Moreover, the choice of solvents was limited by the low solubility of the sample in common NMR solvents. Higher boiling NMR-solvents such as DMSO-$d_6$, and DMF-$d_7$ were not employed since the NMR instrument that was used for the analysis had a safe temperature limit of only up to ~100 °C.

It is worth mentioning that during a re-examination of a sample of the acetonide derivative (1.7) that had been stored away in a freezer for several months, the unexpected appearance of peaks due to the eleven-membered lactone (1.8) was observed. Approximately 70% of the ten-membered lactone (1.7) had rearranged to the eleven-membered lactone (1.8) on standing. A careful re-examination of the original spectra of the parent triol 1.1 (immediately after isolation), also showed the presence of the eleven-membered lactone (~10%). This was interesting, since previously during the formation of the ketal derivative (1.7) from 1.1 under acid catalyzed conditions (cat. $p$-TsOH), this phenomenon of ring-expansion had been encountered.

A subsequent computational study on the energy minimized structures (MM3) of the two compounds 1.7 and 1.8, showed that, in fact, the eleven membered lactone (1.8) is about 2 kcal/mol more stable than the ten-membered lactone (1.7). The above observations suggest that the ten-membered acetonide (1.7) prefers to rearrange to the thermodynamically more stable eleven-membered lactone (1.8) and that this rearrangement is facilitated by the presence of an acid catalyst.
1.3.1 Falcarindiol Family of Compounds

\[
\begin{align*}
1 & \quad 3 & \quad 8 & \quad Z & \quad 16 & \quad 17 \\
\text{HO} & & & & & \\
\end{align*}
\]

(1.10) \( R = \text{OH}; \ C_{16}\text{-}C_{17} = \text{dehydro} \\
(1.11) \( R = \text{H}; \ C_{16}\text{-}C_{17} = \text{dehydro} \\
(1.12) \( R = \text{OH} \\
(1.13) \( R = \text{H} \\

The distribution of polyacetylenes in the plant kingdom has shown regularity over seven families,\textsuperscript{25} namely Araliaceae, Campanulaceae, Compositae, Pittosporaceae, Oleaceae, Santalaceae and Umbelliferae. All these oxylipins display shared structural features, most notable being the \( C_{17} \) or \( C_{18} \) carbon skeleton and the terminal 3-hydroxy(or 3-oxo)hept-1-ene-4,6-diyne moiety. In addition the \( C_{17} \) compounds bear distinctive saturated aliphatic \(-C_{7}H_{15}\) termini, although \( C_{16}/C_{17} \) dehydro analogues\textsuperscript{26} such as dehydrofalcarindiol (1.10) and dehydrofalcarinol (1.11) are known. Those polyacetylenes that bear \( C_{9}/C_{10} \) unsaturation commonly possess \( Z \)-configuration across this olefinic bond, e.g. falcarindiol\textsuperscript{27} (1.12) and falcarinol\textsuperscript{28} (1.13). Oxidative modifications across the \( C_{9}/C_{10} \) double bond have given rise to a number of less common metabolites such as the dihydroxy, e.g. falcarintrioe\textsuperscript{9} (1.14), and \textit{cis}-epoxy polyacetylenes, e.g. panaxydol\textsuperscript{30} (1.15), and ginsenoyne I\textsuperscript{31} (1.16).
Occasionally, products of allylic rearrangements around the C-9/C-10 double bond are observed in C_{17} oxylipins (1.12 and 1.17) featuring C-8/C-9 (1.18) or C-10/C-11 (1.19) unsaturation.

![Chemical structures](image)

**Figure 1.8.** Allylic rearrangements in C_{17} oxylipins.

Other modifications such as reductions, oxidations and O-methylations in the C_{17} category of polyacetylenes have produced 1,2-dihydro, 4,5-ene, 8-oxo, and 10-methoxy variants, respectively. The C_{18} metabolites on the other hand are either C-17 carboxy or hydroxymethyl derivatives of 1.13. These C_{18} homologues of 1.13 seem less prevalent in nature and have so far only been isolated from leaves of the plant *Dendropanax trifidus* (Araliaceae).

![Chemical structure](image)

**Figure 1.9.** C_{18} acetylenes from *Dendropanax*.

### 1.3.1.1 History and Biology of Falcarindiol

Numerous examples of cytotoxic polyacetylenic natural products have been identified, most frequently from plants belonging to the family Umbelliferae, and to a lesser extent from the family Araliaceae. Falcarindiol (1.12), a cytotoxic diacetylene, is
one of the more commonly encountered members of this series, and has been repeatedly discovered along with related naturally occurring polyacetylenes, from various plant sources (Table 1.6). The first report of 1.12 was in 1966\(^{36}\) (from Umbelliferae), the gross structure of which was determined to be heptadeca-1,9(Z)-diene-4,6-diyne-3,8-diol in 1969.\(^{35}\) Falcarindiol (1.12) has displayed potent and varied biological activity (antifungal and anticancer) in *in vitro* assays (Table 1.6).

**Table 1.6. Distribution and Biological Activity of Falcarindiol**

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Plant Family</th>
<th>Plant Source</th>
<th>Active solvent</th>
<th>Activity/Cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Dendropanax arboreus</em></td>
<td>Araliaceae</td>
<td>Leaves</td>
<td>95% EtOH</td>
<td>Human and rat tumor cell lines</td>
</tr>
<tr>
<td><em>Artemisia borealis</em></td>
<td>Asteraceae</td>
<td>Whole plant</td>
<td>CH(_2)Cl(_2)</td>
<td>Antifungal</td>
</tr>
<tr>
<td><em>Peucedanum oreoselinum</em></td>
<td>Umbelliferae</td>
<td>Roots</td>
<td>Diethyl ether</td>
<td>Antifungal</td>
</tr>
<tr>
<td><em>Daucus carota L.</em></td>
<td>Umbelliferae</td>
<td>Roots</td>
<td>Diethyl ether</td>
<td>Antifungal</td>
</tr>
<tr>
<td><em>Schefflera digitata</em></td>
<td>Araliaceae</td>
<td>Leaves</td>
<td>Diethyl ether</td>
<td>Antimycotic</td>
</tr>
<tr>
<td><em>Glehnia littoralis</em></td>
<td>Apiaceae</td>
<td>Roots</td>
<td>MeOH/CHCl(_3)(1:1, v/v)</td>
<td>Antimicrobial(^c)</td>
</tr>
</tbody>
</table>

**1.3.1.1 Isolation and Structure Determination of Cytotoxic Diacetylenes from **

*Tetraplasandra hawaiiensis*

The Hawaiian endemic plant *Tetraplasandra hawaiiensis*, the genus of which belongs to the family Araliaceae, was examined for cytotoxic components.\(^{37}\) The lipophilic extract of the crushed, freeze-dried leaves of *T. hawaiiensis* was partitioned between a series of organic and aqueous solvents in a modified Kupchan scheme. Activity-directed fractionation (against the KB cancer cell line) of the organic extract (toluene layer, 4.4 g) followed by repeated flash column chromatography on silica gel led
to the isolation of a group of oxylipins of which falcarindiol (1.12) was the major constituent (37 mg, 0.84 %). Two other known metabolites, epoxy-falcarindiol \(^{31}\) (1.20) (3.5 mg, 0.08 %) and crithmumdiol \(^{38}\) (1.21) (1.0 mg, 0.02 %) were isolated only in trace amounts. All three compounds 1.12, 1.20 and 1.21 were recovered as colorless oils. Falcarindiol (1.12) was determined to be a C\(_{17}\)-acetylene (C\(_{17}\)H\(_{24}\)O\(_{2}\)-H\(_{2}\)O) by means of HREIMS (found 342.1671, \(\Delta 0\) mmu). The high C–H ratio observed in the molecular formula suggested multiple unsaturation (UN = 6).

The gross structures of these (1.12, 1.20 and 1.21) were established by the analysis of 1D and 2D NMR spectra in combination with FTIR data. Even though the IR spectra of all three compounds showed characteristic absorptions corresponding to hydroxy (\(br \sim 3400\) cm\(^{-1}\)) and alkene (\(sh \sim 1645\) cm\(^{-1}\)) functions, the absorption band(s) typical of an acetylenic function (weak, 2230, 2150 cm\(^{-1}\)) were apparent only in the spectrum of falcarindiol (1.12). The proton framework on either side of the diyne fragment was determined by means of \(^1\)H–\(^1\)H-COSY experiments, in the form of partial structures. The configuration of the internal olefinic bonds was evident from coupling
constant values ($^3J_{H-9/H-10} = 10.8 \text{ Hz} \ (\text{cis})$ for 1.12, $^3J_{H-9/H-10} = 10.4 \text{ Hz} \ (\text{cis})$ and $^3J_{H-4/H-5} = 16.5 \text{ Hz} \ (\text{trans})$ for 1.21) extracted from $^1\text{H} \ \text{NMR}$ spectra recorded in CDCl$_3$. The $^1\text{H} \ \text{NMR}$ spectra of the three acetylenes bore a close resemblance, with a downfield set of vinylic and hydroxymethine protons resonating between 4.5-6.2 ppm and a second set of signals belonging to the saturated -C$_7$H$_{15}$ moiety resonating at 1.3-1.5 ppm.

The existence of a terminal double bond in each of these compounds was evident from the appearance of a characteristic proton splitting pattern that was consistent with $^3J_{HH}$ coupling between cis ($^3J_{H-H-2} \approx 10-11 \text{ Hz}$) trans ($^3J_{H-H-2} \approx 16-17 \text{ Hz}$) and geminal ($^2J_{H-H-1} \approx 0.9 \text{ Hz}$) sets of vinylic-protons. The $^{13}\text{C}$-chemical shift values, typical of a conjugated network of four sp-carbons (79.8, 70.3, 68.7, 78.2 and 78.5, 70.2, 70.1, 78.0) appeared in the spectra of 1.12 and 1.21, respectively. These acetylenic units were connected to the rest of the framework by means of HMBC correlations.

![Figure 1.11. HMBC correlations within 1.12.](image)

Obvious differences were seen in the proton spectrum of crithmumdiol (1.21) with regard to its epoxy function. The oxirane ring protons H-9 and H-10 resonated at 3.14 ppm and 3.05 ppm, respectively, with a $^3J_{HH}$ value ($^3J_{H-9/H-10} = 4.0 \text{ Hz}$) indicative of a cis relationship between these protons. The relative stereochemistry between the H-8 and H-9 protons ($^3J_{H-8/H-9} = 7.3 \text{ Hz}$) in 1.21 was assigned as erythro based on the empirical rule$^{39}$ proposed for predicting the stereochemical outcome of OsO$_4$ oxidations of allylic alcohols.$^{40}$ The spectroscopic data of these three known diacetylenes (1.12, 1.20}
and 1.21) from Tetraplasandra hawaiiensis were in excellent agreement with those of the literature\textsuperscript{31,38} (The HREIMS data for 1.20 and 1.21 were not recorded due to limited sample availability).

1.3.1.2 Comparative Evaluation of the C-3 Stereochemistry of (+)-Falcarindiol

There are conflicting reports in the literature concerning the absolute sterochemistry at C-3 of the diacetylene oxylipin (+)-falcarindiol (1.12). The absolute configuration of (+)-1.12 from Peucedanum oreoselinum (Umbelliferae) \{[\alpha]_D +284 (c 1.0, ether)\} had first been assigned as (3R,8S) by Lemmich in 1981 on the basis of chemical correlation studies.\textsuperscript{41}

\[
\text{Figure 1.12. Catalytic hydrogenation of the diacetylenes form } P. \text{ oreoselinum.}
\]

Accordingly, (+)-1.12 under catalytic hydrogenation conditions (Pd/BaSO\textsubscript{4}, 0°C, 8h) produced four reaction products, heptadecane, (−)-heptadecan-3-ol (1.22), (+)-heptadecan-8-ol, and (−)-heptadecan-3,8-diol. The absolute configuration of (−)-(1.22) derived form (+)-1.12 was assigned as 3(R) by comparison to a previous study by the same author, where the absolute configuration of a sample of (−)-(1.22) derived from (−)-falcarinol (1.13) under similar catalytic hydrogenation conditions was shown to possess
3(R)-configuration. However, in 1996, the Boyd group from the NCI proposed a (3S,8S)-configuration for a similarly strongly dextrorotatory sample of (+)-1.12 \([\alpha]_D +300\) isolated from *Dendropanax arboreus* (Araliaceae). The assignment of the stereochemistry of (+)-1.12 in *D. arboreus* was based in part on biogenetic considerations since a sample of (+)-1.13 isolated from the same plant had been shown to have 3(S)-stereochemistry. It was deemed to be unlikely that two biogenetically related compounds, 1.12 and 1.13, isolated from the same source would have the opposite configuration at C-3. This conjecture was corroborated by an application of the advanced Mosher method to the same samples of (+)-1.13 and (+)-1.12.

It should be noted, however, that the analysis of chemical shift changes in bis-MTPA esters of 1,2-diols, may not always allow a safe assignment of the stereochemistry, as demonstrated by Riguera. The main problem with stereochemical assignments in such a system arises because of the common practice of considering the MTPA directly bound to the \(-\text{OH}\) as the sole contributor to the \(\Delta\delta^{SR}\) value and disregarding the combined shielding/deshielding effects produced by all of the MTPAs present in the poly-ester. In addition, in the case of (+)-1.12, one has to rely on the analysis of \(\Delta\delta\)-values from resonances for protons on only one side of the MTPA-plane, a practice that the developers of the method and also Riguera have cautioned against.

The assignment of the 3(S)-stereochemistry to (+)-1.13 from *D. arboreus* was confirmed by an application of the exciton chirality method using the *p*-bromobenzoate derivative \((\lambda_{\text{max}} 248 \text{ nm} \Delta\epsilon = +8.7, \lambda_{\text{max}} 239 \Delta\epsilon = +7.6)\) by Boyd, based on allylic-benzoate coupling. Following an independent study by Shim et al. using CD spectra of a sample of the *p*-bromobenzoate derivative of 1.13 \([\alpha]_D\) was not recorded) from Korean
ginseng roots (Araliaceae), the absolute stereochemistry was assigned as 3(S) with a negative exciton chirality ($\lambda_{\text{max}}$ 313 $\Delta\varepsilon = -4.5$, $\lambda_{\text{max}}$ 244 $\Delta\varepsilon = -4.8$). The sign of the exciton chirality of the allylic benzoate system of 1.13 reported by Shim et al. conflicted with that of the NCI group. This suggests that when a chiral secondary alcohol is flanked by two chromophores, an isolated double bond and a conjugated diyne, it is not readily apparent which chromophore would couple preferentially to the benzoate chromophore.\textsuperscript{48} Thus the reliance on CD studies for an unambiguous assignment of the absolute stereochemistry at C-3 appeared unsatisfactory.

Without reference to the report from the NCI, in 1999 Cai et al.\textsuperscript{49} published a chemical synthesis of (+)-(3R,8S)-1.12. The stereocenters were introduced by transformations of L-tartaric acid and D-xylose. The former gave rise to the C-8 stereochemistry, while the latter was used to generate the absolute configuration at C-3. The synthetic product had an optical rotation $\{[\alpha]_D +211.7 (c 0.65, \text{CHCl}_3)\}$ and other spectroscopic data reportedly matching those of (+)-(3R,8S)-1.12 isolated from Glehnia littoralis (Apiaceae) $\{[\alpha]_D +219.4 (c 4.6, \text{CHCl}_3)\}$\textsuperscript{50}

Unfortunately, the optical rotation value in ether solution was not reported for either the natural material from G. littoralis or the synthetic sample. Hence no comparison can be made to the samples of (+)-1.12 used by either the NCI group or by Lemmich for the determination of the absolute configuration of (+)-1.12 as (3S,8S) and (3R,8S), respectively.
1.3.2 Research Objective and Strategy

The sample of (+)-1.12 isolated from *T. hawaiiensis*\(^{37}\) showed optical rotations matching (within experimental error) all of the literature values reported for either of the diastereomers of 1.12 for which an absolute configuration had been proposed (Table 1.7).

**Table 1.7. Optical rotations and assigned configurations of falcarindiol (1.12)**

<table>
<thead>
<tr>
<th>Entry #</th>
<th>([\alpha]_D) reported</th>
<th>Conditions</th>
<th>Assigned Configuration of 1.12</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>+284</td>
<td>c 1.0, ether</td>
<td>3R, 8S</td>
</tr>
<tr>
<td>2</td>
<td>+300</td>
<td>c 0.14, ether</td>
<td>3S, 8S</td>
</tr>
<tr>
<td>3</td>
<td>+219.4</td>
<td>c 4.6, CHCl(_3)</td>
<td>3R, 8S</td>
</tr>
<tr>
<td>4</td>
<td>+302</td>
<td>c 1.0, ether</td>
<td>This work</td>
</tr>
<tr>
<td>5</td>
<td>+276</td>
<td>c 0.14, ether</td>
<td>This work</td>
</tr>
<tr>
<td>6</td>
<td>+250</td>
<td>c 0.46, CHCl(_3)</td>
<td>This work</td>
</tr>
</tbody>
</table>

Hence an unambiguous assignment of the absolute configuration of the sample from *T. hawaiiensis* could not be made on the basis of the chiroptical data available in the literature. Therefore the need to pursue an unambiguous determination by an independent method was apparent. It was foreseen that a confirmation of either of the proposed absolute configurations might be obtained if it were possible to modify the C-1/C-2 and C-9/C-10 double bonds present in 1.12 in such a way that a symmetrical compound is formed. The degradation product would either be meso or chiral if (+)-1.12 possesses a (3R,8S)- or (3S,8S)-configuration, respectively (Figure 1.13).

![Figure 1.13. Symmetrical products expected from degradation of 1.12.](image-url)
Consequently, an oxidative degradation route and an alternative Grubbs metathesis\textsuperscript{51} route were examined as potential approaches to accomplish the favored degradation of \textbf{1.12} under mild conditions.

1.3.2.1 Symmetrical Analogues of Falcarindiol via Degradation and Synthesis

**Oxidative Degradation (Route A).** The \textit{bis}-TBS derivative of falcarindiol (\textbf{1.12a}) was prepared (TBS-trflate, 2,6-lutidine, CH\textsubscript{2}Cl\textsubscript{2}, 0-25 °C, 5h) and used to explore the oxidative degradation pathway. Direct cleavage of the terminal (C\textsubscript{1}=C\textsubscript{2}) and the internal (C\textsubscript{9}=C\textsubscript{10}) olefinic bonds in \textbf{1.12a} was expected to yield the symmetrical \textit{bis}-carboxylic acid product \textbf{1.23} under osmium tetroxide-promoted catalytic oxidative conditions.\textsuperscript{52,53}

![Figure 1.14. Oxidative degradation of 1.12.](image)

However, all attempts to reduce this idea to practice using oxidative methods on \textbf{1.12a} failed, as no characterizable products could be isolated. The oxidative approach to olefin double bond cleavage was thus abandoned.

**Metathesis on the \textit{bis}-TBS protected falcarindiol (Route B).** At this point, the olefin cross-metathesis\textsuperscript{54} of \textbf{1.12a} was considered as an alternative to oxidative degradation. In the simplest implementation of this approach, ethylene gas or allyltrimethylsilane would serve as the second olefin in a reaction catalyzed by one of Grubbs’ ruthenium carbenes.
Figure 1.15. Grubbs' metathesis on the bis-TBS protected falcarindiol (1.12).

A review of the available literature did not provide reassurance that removal of the C$_7$H$_{15}$ chain, the desired reaction, would prevail over potential competing ones such as intermolecular enyne metathesis$^{55}$ (Figure 1.16) in the multifunctional environment of 1.12a. However, side reactions proved to be much less of a problem than we had anticipated.

Figure 1.16. Intermolecular enyne metathesis.
In fact, exposure of bis-TBS-protected falcarindiol (1.12a), to the first or the second generation Grubbs’ catalysts (10 mol %, 1.24 and 1.25), using either ethylene gas or excess allyltrimethylsilane (5 equiv), did not yield any of the expected product (Figure 1.15), even under forcing conditions (refluxing in CH₂Cl₂ for prolonged periods of time). Recognizing that the bulky TBS protecting group might be responsible for this lack of reactivity, the unprotected diol (1.12) was chosen as the substrate for the next set of metathesis reactions.

Metathesis on the unprotected falcarindiol (Route C). In the presence of the first-generation Grubbs’ catalyst (10-20 mol %, 1.24) under varied reaction conditions (CH₂Cl₂, RT→reflux, 16-40 h), 1.12 failed to undergo the anticipated degradation. However, using the second-generation Grubbs catalyst (10 mol %, CH₂Cl₂, 16 h, room temperature, ethylene-filled double balloon, 1 mM (+)-1.12),⁵⁶ quantitative conversion of 1.12 to a single, slightly more polar compound (1.26) was observed by TLC.

\[ \text{HO} \quad \text{Mes-N\textsubscript{2}N-Mes-C\textsubscript{7}H\textsubscript{15}} \]

\[ \text{HO} \quad \text{Mes-N\textsubscript{2}N-Mes-C\textsubscript{7}H\textsubscript{15}} \]

\[ \text{1.12} \quad \text{1.26} \]

\[ \text{CH₂Cl₂, reflux, 16 h} \]

Figure 1.17. Degradation of 1.12 by metathesis

The product was isolated in 81% yield by careful chromatography and crystallization. The gross structure was shown to be 1.26 (¹H NMR, 500 MHz, CDCl₃). We deemed chromatography to be necessary due to the large specific rotation of 1.12 \([\alpha]_D^{23} +302 (c 1.0, \text{Et}_2\text{O})\). Potential contamination of 1.26 by small amounts of 1.12,
not detectable by $^1$H NMR or TLC, might induce a measurable optical rotation in samples of 1.26 and hence might lead to an erroneous conclusion as to its configuration.

In the event, the crystalline sample of 1.26 isolated from the cross-metathesis reaction possessed marginal optically activity $\{[\alpha]_D^{23} +5 \ (c \ 3.8, \ CHCl_3)\}$, which was suspiciously low when compared to that of (+)-1.12 $\{[\alpha]_D^{23} +250 \ (c \ 0.46, \ CHCl_3)\}$. Further experiments using the second-generation ruthenium catalyst (1.25) proved that the use of ethylene as the donor olefin is preferable over the more nucleophilic allyltrimethylsilane because the latter yielded an inseparable mixture of olefin geometrical isomers. 57

**Synthesis of the Symmetrical Analogues.** For comparison purposes, a synthetic sample of (3S,8S)-1.26 was prepared. The lithium acetylide, generated in situ by reacting (trimethylsilyl)acetylene (1.27) with n-BuLi,58 was converted to 5-trimethylsilylpent-1-en-4-yne-3-ol (±)-1.28 in quantitative yield, by reaction with acrolein.

![Scheme 1.2. Formation of the racemic allyl alcohol 1.28.](image)

The racemic 5-trimethylsilylpent-1-en-4-yne-3-ol (±)-1.28 was resolved using lipase from *Pseudomonas*. 59,60 The remaining nonacylated (S)-1.28a $\{[\alpha]_D^{23} +38 \ (c \ 2.0, \ CHCl_3), \ 95\% \ ee\}$ was desilylated and converted to the bromide (S)-1.30 using Isobe's convenient one-pot procedure. 61 Dimerization of (S)-1.30 under Cadiot-Chodkiewicz conditions62 yielded (S,S)-1.26 $\{[\alpha]_D +102 \ (c \ 3.8, \ CHCl_3)\}$. 
Scheme 1.3. Synthetic route to the chiral diol 1.26.

The assignment of (S)-configuration to the nonacylated (+)-enantiomer of 1.28a, and hence of (S,S)-configuration to (+)-1.26, was based on the results of an application of the advanced Mosher method to (+)-1.28a. Moreover, in enzymatic resolutions of similarly substituted propargylic alcohols the remaining starting material was shown to have an (S)-configuration. Compound (S)-1.28a was chosen for the analysis because it bears the TMS group, which yielded one data point on the right side of the MTPA plane.

Figure 1.18. \( \Delta \delta \) values (\( \Delta \delta = \delta_S - \delta_R \), 300 MHz, CDCl$_3$).
1.3.3 Evaluation of Results

When compared to the optical rotation of the synthetic sample of \((3S,8S)-1.26\) \([\alpha]_D +102 (c 3.8, \text{CHCl}_3)\), the optical rotation of the sample of 1.26 from the degradation was noticeably low \([\alpha]_D +5 (c 3.8, \text{CHCl}_3)\). This observation could be indicative of meso stereochemistry in the latter. However, it has been reported that ring-closing metathesis of certain allylic alcohols using the first generation catalyst \((\text{PCy}_3)_2\text{Cl}_2\text{Ru}=\text{CHPh}\) may lead to the formation of methylketones (1.31) in a side-reaction.\(^63\) The mechanism the authors proposed for this transformation could, in principle, also explain the isolation of an optically inactive, epimerized sample of 1.26 from the degradation of \((+)-1.12\) (Scheme 1.4).

Accordingly, a stereorandom sample of low or null optical activity might also result from epimerization of 1.12 or of 1.26 during the cross-metathesis by a variant of the hydride shift mechanism first proposed by Hoye.\(^64\) This loss of stereochemical information may occur during cross-metathesis of 1.12 with ethylene if metallocyclobutane formation is slower than rearrangement. Alternatively, even if the former is competitive with the putative isomerization, erosion of optical purity may result over larger time scales during a degenerate cross-metathesis between ethylene and the

\[
\begin{align*}
\text{R} & \quad \text{OH} \quad \text{RuLn} \\
\longrightarrow & \quad \text{epimerization} \\
\text{R} & \quad \text{H} \quad \text{RuLn} \\
\downarrow & \\
\text{R} & \quad \text{OH} \quad \text{RuLn} \\
\text{R} & \quad \text{H} \quad \text{RuLn} \\
\longrightarrow & \quad \text{R} \quad \text{OH} \quad \text{RuLn} \\
\end{align*}
\]

\[(1.31)\]

Scheme 1.4. Epimerization of allyl alcohols via the hydride shift mechanism.
degradation product, after 1.26 has been generated form 1.12. Under either of these two scenarios, one would expect to observe the formation of a mixture of chiral and meso diastereomers during a net epimerization process. Similarly, a synthetic sample of (±)-1.26 obtained by dimerization of (±)-1.30 should generate a mixture of the racemate and the meso isomers. In principle, the components of such mixtures are distinguishable by chromatography or NMR. However, both 1H as well as 13C NMR spectra of 1.26, obtained either by degradation of (+)-1.12 or by dimerization of (±)-1.30 showed only five resonances. Although this outcome suggested the presence of only one diastereomer, the same result would be observed if coincidentally the meso isomer 1.26 can not be distinguished from the chiral one by TLC and/or NMR.

\[
\begin{align*}
\text{HO} & \quad \text{Br} \\
\text{(±)-1.30} & \quad \text{dimerization} \\
& \quad \text{synthesis procedure} \\
& \quad \text{HO} \\
& \quad \text{(±)-1.26}
\end{align*}
\]

\[
\begin{align*}
\text{HO} & \quad \text{C}_7\text{H}_{15} \\
(3R, 8S)-(1.12) & \quad \text{degradation} \\
& \quad \text{metathesis} \\
& \quad \text{meso-(1.26)} \\
& + \quad \text{C}_7\text{H}_{15}
\end{align*}
\]

**Figure 1.19.** Approaches to the formation of the symmetrical diol (1.26).

Analytical HPLC on a chiral stationary phase was employed to clarify this interpretation of results. Upon chromatography on a Chiralcel OD column, a sample of the symmetrical (±)-dial 1.26 derived from (±)-1.30 yielded three well-resolved peaks in a 1:2:1 ratio. This result, albeit not necessarily in that elution order, is to be expected in a successful separation and resolution of a statistical mixture of meso and chiral
diastereomers. Synthetic (+)-(3S,8S)-1.26 \([\alpha]_D +102 (c \ 3.8, \ \text{CHCl}_3)\) from dimerization of (+)-1.30 showed only one peak, which co-eluted with the last peak from the stereorandom sample of 1.26. Last, 1.26 \([\alpha]_D +5 (c \ 3.8, \ \text{CHCl}_3)\) obtained from degradation of (+)-1.12 from \emph{T. hawaiensis} gave rise to only one peak, which eluted at the same retention time as the large peak due to the meso diastereomer of 1.26 in the synthetic sample. This result suggests that epimerization does not accompany cross-metathesis when (+)-1.12 is being degraded to 1.26 because the product is the pure meso isomer. It is important to note that the same meso isomer is also found exclusively if the crude degradation reaction mixture is analyzed directly by chiral HPLC without prior chromatographic purification and crystallization of 1.26.

\begin{figure}[ht]
\centering
\includegraphics[width=\textwidth]{figure120.png}
\caption{Re-exposure of 1.26 to metathesis conditions}
\end{figure}

(+)-(3S, 8S)-1.26 \([\alpha]_D +75 (c \ 0.67, \ \text{CHCl}_3)\)  \[\alpha]_D +77 (c \ 0.67, \ \text{CHCl}_3)\)

In an additional control experiment, synthetic (+)-1.26 was subjected to the cross-metathesis conditions used for degradation of (+)-1.12 (Figure 1.20). This did not result in any noticeable change in the optical purity of (+)-1.26, as shown by chiral HPLC analysis of the crude reaction mixture and by polarimetry. These results proved unambiguously that (+)-1.26 from \emph{T. hawaiensis} has the same (3R,8S)-stereochemistry.
as the material obtained by Cai et al. through total synthesis. Upon cross-metathesis with ethylene, a sample of this configuration is expected to yield the meso isomer of 1.26, as is observed experimentally.

Having established the absolute stereochemistry of (+)-1.26 from T. hawaiiensis unambiguously, the validity of the application of the advanced Mosher method (a NMR based technique) to such diyne-diol systems was examined. This method requires derivatization of the alcohol functions at C-3 and C-8 of 1.12 with (R)- and (S)-methoxytrifluoromethylphenylacetic acid (MTPA, Mosher's reagent) or its analogue methoxyphenylacetic acid (MPA), followed by NMR analysis of the resulting esters. The chemical shift differences (Δδ) between these diastereomers would indicate whether the secondary asymmetric centers (C-3 and C-8) in 1.12 are (R) or (S), based on an established empirical model.

The difference in chemical shift (Δδ) observed for the protons in the substrate are due to shielding/deshielding effects on the protons in the alcohol backbones induced by the aromatic ring in the MPA and MTPA derivatives. For a given secondary alcohol, the MPA ester may exist in two conformations whereas the MTPA ester would give rise to
three low energy conformations. While either derivative can be used, $\Delta \delta$ values observed in MTPA derivatives are generally smaller than those obtained using MPA because of the greater complexity of the conformational composition in the former. The resulting smaller shifts of resonances occasionally makes it difficult to interpret the NMR shifts ($\Delta \delta$), consequently making the data less reliable for configuration assignment. Therefore we chose to carry out our initial investigations using the (R)- and (S)-MPA ester derivatives of (+)-falcariindiol (1.12).

Examination of the $^1$H NMR spectra of the latter derivatives (15 °C, 500 MHz, CDCl$_3$) showed two sets of doublets for the allylic proton H-8, in a ratio of 2:1 for the bis-(S)-MPA and 2:1.5 for the bis-(R)-MPA derivatives, respectively. This doubling of the H-8 signal could be due to either existence of conformers or epimerization at C-8 during the derivatization. Since the allylic proton at C-3 in both (R)- and (S)-MPA derivatives remained a clean doublet, it was deemed unlikely that epimerization should occur only at C-8. Thus the doubling of resonances for H-8 is likely due to restricted rotation in that portion of the molecule. Observation of TOCSY transfer between these two sets of doublets for the H-8 proton, when either one is excited, subsequently confirmed this interpretation. TOCSY transfer between corresponding resonances in epimeric compounds is not possible.

Next, for the purpose of comparison with the literature data, the bis-MTPA ester derivatives of 1.12 were also prepared (Table 1.8). Even though the same doubling of the resonance for the H-8 proton (ratio 1:1) was observed for the bis-(S)-MTPA ester derivative of 1.12 (15 °C, 500 MHz, CDCl$_3$), the bis-(R)-MTPA ester was conformationally homogeneous. The difference in conformer distribution observed
between the (S)-MTPA and (R)-MTPA ester derivatives can be explained on the basis of their diastereomeric relationship.

**Table 1.8.** Stereochemical analysis of falcarindiol with (R)- and (S)-MPA and MTPA derivatives

<table>
<thead>
<tr>
<th>bis-esters of Falcarindiol</th>
<th>H-1E</th>
<th>H-1Z</th>
<th>H-2</th>
<th>H-3</th>
<th>H-9</th>
<th>H-10</th>
<th>H-11</th>
<th>H-12</th>
<th>C-3 center</th>
<th>C-8 center</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T. hawaiensis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bis-(MPA)</td>
<td>+105</td>
<td>+70</td>
<td>+95</td>
<td>b</td>
<td>-85</td>
<td>-45</td>
<td>-50</td>
<td>-</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>bis-(MTPA)</td>
<td>+40</td>
<td>+30</td>
<td>+50</td>
<td>b</td>
<td>-20</td>
<td>-45</td>
<td>-10</td>
<td>-</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td><strong>D. arboreus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bis-(MTPA)</td>
<td>-27</td>
<td>-37</td>
<td>-44</td>
<td>b</td>
<td>-43</td>
<td>-18</td>
<td>-11</td>
<td>-6</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

* CDCl₃/500MHz. * Resonances of protons directly attached to esterified centers are not subject to analysis. * (Δδᵣₛ = δᵣ - δₛ). * (Δδₛᵣ = δₛ - δᵣ).

- R = MTPA/MPA

In the bis-MPA and bis-MTPA ester derivatives of (+)-1.12 from *T. hawaiensis*, the resonances for protons H-9, H-10, and H-11 all showed negative Δδ values, while those for the resonances for H-1E, H-1Z, and H-2 were all positive, indicating a (3R,8S)-configuration. Conversely, the sample of (+)-1.12 from *D. arboreus* (Table 1.8) had shown all negative values, which had been interpreted as indicating a (3S,8S)-configuration. Our results, being clearly different (Table 1.8), indirectly support this assignment. Thus the results of analyses of the Δδ values of the bis-MPA and bis-MTPA esters according to the established model were in accordance with (3R,8S)-stereochemistry of the *T. hawaiensis* sample of (+)-1.12.

Unfortunately, we could not obtain a sample of (+)-1.12 from *D. arboreus* for degradation by our method for a rigorous confirmation. However, it appears that Nature
does indeed elaborate two diastereomeric forms of (+)-1.12, which cannot be distinguished by polarimetry. Hence, all assignments of stereochemistry to samples of 1.12 and analogous compounds (falconinol,66 panaxytriol,67 panaxydol67) using this latter method must be regarded as suspect.
1.4 Conclusion

The isolation and structure determination of microcarpalide (1.1), a novel fungal metabolite with microfilament disrupting properties, was described in detail. The interpretation of spectroscopic data of microcarpalide (1.1) was hampered by the existence of conformers in solution. However, by incorporating a conformational constraint, it was possible to restrict free rotation in that part of the molecule, forcing the molecule to adopt a strongly preferred conformation. The vicinal diol function (C-4/C-5) was used successfully to introduce such a conformational control element, the isopropylidene acetal.

Even though the application of Murata’s methodology to the configuration assignment of acyclic subunits of natural products has been well documented, there is little precedence for the application of this methodology in cyclic systems, especially in medium size (8≤11) rings. Despite the limited number of applications of this methodology to cyclic systems, the relative stereochemistry of C-9/C-10 asymmetric centers in microcarpalide (1.1) was established as threo based on Murata analysis. The validity of the application of Murata’s method for assigning the relative configuration of carbon atoms that are part of a medium size ring (8≤11) was thus demonstrated using microcarpalide (1.1) as the target molecule. Since such 1-oxadecenolide natural products are common in Nature, the prospect of extending this methodology to other members of this series of herbicidal agents now seems promising. Finally, the absolute configuration of C-4/C-5 threo diol was established based on exciton splitting observed for the bis-p-methoxybenzoate derivative of microcarpalide (1.1).
Falcarindiol (1.12), a known C_{17} diacetylene, was identified as the major component responsible for in vitro cytotoxicity observed in the endemic Hawaiian plant *Tetraplasandra hawaiiensis*.

When chiroptical data on either diastereomers of (+)-falcarindiol (1.12), isolated from several different plant families, were compared with those of (+)-1.12 from *T. hawaiiensis*, it was evident that based on optical rotation data alone, an unambiguous assignment of the absolute configuration of the sample form *T. hawaiiensis* cannot be made.

In order to resolve conflicting stereochemical reports regarding the absolute stereochemistry of 1.12, we employed olefin cross-metathesis using Grubbs' second generation catalyst and ethylene gas to degrade falcarindiol (1.12) to the symmetrical 1,9-decadiene-4,6-diyn-3,8-diol. The reaction was completely selective for net removal of the aliphatic side chain. Degradation of (+)-falcarindiol (1.12) from *Tetraplasandra hawaiiensis* yielded a meso product as shown by chiral HPLC. Hence, (+)-falcarindiol from this source has a (3R,8S)-configuration.

In conclusion, it was demonstrated that olefin cross-metathesis using ethylene can be a viable alternative to the classical oxidative degradation procedures for natural products containing double bonds. Additionally, cross-metathesis can be performed under mild conditions with easy-to-handle Ru-based catalysts. The commercial availability of such well-defined single-component homogeneous catalysts (1.24 and 1.25) has made the olefin metathesis reaction practical for organic synthesis.
1.5 Experimental Section

1.5.1 General

1.5.1.1 Spectral Analysis

**Routine NMR and Mass Analysis.** NMR spectra were determined on Varian Unity Inova 400/500 spectrometers, operating at 400/500 MHz and 100/125 MHz, respectively, or on an 11.75 T instrument operating at 500 MHz for $^1$H and at 125 MHz for $^{13}$C. Chemical shifts are reported in $\delta$ (ppm) units and are referenced to residual solvent signals as internal references. {i.e., CHCl$_3$ (7.26, in CDCl$_3$), MeOH (3.30 in MeOH-$d_4$), Acetone (4.04 in Acetone-$d_6$) or CH$_3$CN (1.93 in CD$_3$CN)}; $^{13}$C chemical shifts are referenced to the solvent CDCl$_3$ (77.0), MeOH-$d_4$ (49.0) Acetone-$d_6$ (29.8) or CD$_3$CN (1.3). Multiplicities are indicated as: br (broad), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Coupling constants ($J$) are reported in Hertz (Hz). One-bond $^1$H—$^{13}$C connectivities were determined by gHMQC; Two- and three-bond connectivities were determined by gHMBC; All chemical shift assignments are based on detailed analysis of COSY, TOCSY, gHMQC and gHMBC analysis. Mass spectra were obtained using a VG-70SE mass spectrometer operating in the EI mode.

**IR, UV, CD, Optical Rotations.** Infrared spectra were recorded on a Perkin-Elmer IR 1600 spectrometer. UV spectra were obtained on a Hewlett Packard 8453 spectrometer. CD measurements were taken on a JASCO J-600 spectropolarimeter. Optical rotations were measured on a JASCO DIP-370 polarimeter. UV-spectra were acquired in spectroscopy grade methanol, chloroform, or diethyl ether and CD-spectra were obtained in spectroscopy grade methanol at 20 °C.
1.5.1.2 General Operations

All plant materials were freeze-dried using a FTS-systems, INC, Flexi-Dry™ μp freeze-dryer and stored in the cold, away from light. Normal-phase column chromatography was performed on silica gel (200-425 mesh/type 60 or 70-230 mesh ASTM, Kieselgel 60) from Fisher Scientific or on Extract-clean silica cartridges (500 mg-2 g, Alltech). Reversed-phase flash column chromatography was performed on small-scale high capacity C8 coated silica gel columns (Extract-clean cartridge, 500 mg) from Alltech. Merck KgaA RP-8 F254S pre-coated silica plates (0.25 mm) served for thin-layer chromatography (TLC). All solvents were filtered through a 0.22 μm Nylon filter prior to use as HPLC solvents. Isocratic HPLC was performed using Gilson 802B Manometric Module coupled to a Linear UVIS 200 Absorbance Detector. The detection occurred at 254 nm unless mentioned otherwise. The following HPLC columns were used: Econosil C18 (10 μm, 250×10 mm, Alltech), Econosil Silica (10 μm, 250×10 mm, Alltech) and ODS (2) Luna (5 μm, 250×4.6 mm, Phenomenex). Exact chromatographic conditions are specified in the corresponding experimental sections. For chiral HPLC analysis, a Chiralcel OD (250×4.6 mm) column was utilized. Ethyl acetate, hexanes, methylene chloride, and methanol used for chromatography purposes were distilled before use. Other chromatography solvents (chloroform, acetone, methyl tert-butyl ether, and toluene) were used as received.

Tetrahydrofuran was distilled from sodium metal and benzophenone. Pyridine and 2,6-lutidine were dried over potassium hydroxide (pellets) and freshly distilled from barium oxide prior to use. Acetic anhydride, toluene, hexanes and methylene chloride were distilled from calcium hydride. Methanol was distilled from Mg(OMe)₂. N-
bromosuccinimide was recrystallized from deionized water. Acetone was purchased in reagent grade and used as received. Anisic anhydride was synthesized from p-anisic acid according to the literature procedure. All moisture sensitive reactions were performed under static nitrogen or argon atmosphere in oven-dried or flame-dried glassware. Solvents and other chemicals were obtained from standard commercial sources. Purity and homogeneity of all materials was determined from TLC, $^1$H NMR, $^{13}$C NMR and HPLC.

1.5.2 Biological Material

1.5.2.1 Isolation of Fungal Strains

The area of bark *Ficus microcarpa* L. to be sampled was surface sterilized by squirting 70% aqueous ethanol on the bark and letting it evaporate. A piece of bark was removed by means of a flame-sterilized pocket knife and transferred to the laboratory in a sterile polyethylene bag. Within 2h, the sample was surface-sterilized by repeated treatment (3× for 1 min each) with 70% (v/v) aqueous ethanol and rinsed with sterile water. The sample was divided into several pieces with a dissecting knife or sterile forceps, and individual pieces were incubated at room temperature on Petri dishes containing water-agar. Fungal colonies growing from the bark were removed after 2-3 days and individually subcultured on potato-dextrose agar supplemented with novobiocin (10 µg/mL). Individual colonies were subcultured repeatedly on potato-dextrose agar at 27 °C in the dark until judged to be axenic. Liquid cultures were inoculated by adding agar plugs (∅ ≈ 5 mm) from actively growing cultures. The taxonomic identification of the fungal stains obtained form *Ficus microcarpa* L. has so far been unsuccessful. The
strain 112/13 (for 1.1) has been deposited in the University of Hawaii chemistry department's culture collection under the access number 112/13.

1.5.2.2 Cultivation of Fungal Strains

Preparative-scale fermentation (4 L) was carried out in 2 L Erlenmeyer flasks for 3 weeks as standing cultures at room temperature in the dark. Each flask contained 0.5 L of potato-dextrose extract (Difco) in deionized water. After autoclaving for 15 min at 121 °C and cooling, cultures were inoculated from a 1-week-old broth culture in potato-dextrose medium by transfer of approximately 5 mL of mycelial suspension.

1.5.3 Extraction and Isolation

1.5.3.1 Isolation of Microcarpalide (1.1)

The mycelium was filtered off and washed with water. The filtrate was extracted with ethyl acetate (4×1 L), and the combined organic extracts were washed with water (0.5 L) and brine (0.5 L), dried over MgSO₄, and evaporated to dryness in vacuo to yield 250 mg of an oil. The crude oil was dissolved in acetone/ethyl acetate (1:2 mL) and adsorbed on to 0.5 g of silica. The silica-coated oil was next applied to a silica gel column (20 g, 15 mm×20 cm) pre-equilibrated with CH₂Cl₂:cyclohexane (1:1). The column was washed with a step gradient of CH₂Cl₂:EtOAc (1:0, 2:1, 1:2, 0:1). The active fractions containing 1.1 were successively eluted with CH₂Cl₂:EtOAc 1:2 (200 mL) and EtOAc (neat, 100 mL), combined and concentrated to dryness in vacuo to give a pale yellow residue (26 mg) which was further purified by flash chromatography (silica gel, 3 g, Alltech) with an isocratic solvent system of isopropanol:CH₂Cl₂ (1:7). Following a
final purification involving preparative TLC {(20x20 cm, Kieselgel 60 F$_{254}$, 1 mm, hexane:EtOAc (2:5)}, 1.1 was isolated as a pure solid (18 mg; 7.2% of extract mass).

1.5.3.2 Isolation of Falcarindiol (1.12)

The freeze-dried leaves (115 g) of Tetraplasandra hawaiiensis (family Araliaceae) were crushed to a coarse powder and extracted with isopropanol:CH$_2$Cl$_2$ (2:1). The crude extract (12 g) was partitioned between a series of organic and aqueous solvents {aqueous MeOH (90%)/hexanes; aqueous MeOH (80%)/toluene}, respectively. The organic residue from the toluene layer (4.4 g) was subjected to flash chromatography on a silica gel column (150 g, 40 mm x 45 cm), pre-equilibrated with CH$_2$Cl$_2$:cyclohexane (2:1). The column was eluted with a step-gradient of CH$_2$Cl$_2$ in ethyl acetate (1:0, 330 mL; 2:1, 330 mL; 0:1, 330 mL). The active fraction (942 mg) was further purified on silica gel (20 g, 15 mm x 20 cm) with a second step-gradient of ethyl acetate in hexanes (1:5, 1:3, 1:2; each 100 mL). The active fractions eluted at the mid-section of the step-gradient were combined and evaporated to dryness in vacuo. The final purification of the dry residue (45 mg) following two successive columns {silica gel (15 g, 10 mm x 16 cm)} with ethyl acetate:hexanes (1:4) and CHCl$_3$:acetone (7:1) respectively, yielded falcarindiol 1.12 as a colorless oil (37 mg, 0.84 % of extract mass). Two other known metabolites, epoxy-falcarindiol 1.20 (3.5 mg, 0.08 %) and crithmumdiol 1.21 (1.0 mg, 0.02 %) were isolated from the latter-section of the former step-gradient of ethyl acetate in hexanes, following further purification (18 mg) engaging preparative TLC {(20x20 cm, Kieselgel 60 F$_{254}$, 1 mm, CHCl$_3$:acetone (7:1)}. 
1.5.4 Bioassay

The microfilament immunofluorescence assay was performed as described. Briefly, A10 cells, rat smooth muscle cells, were grown on cover slips in Basal medium Eagle containing 10% fetal calf serum. The cells were treated for 24 h with various dilutions of fractions or with pure samples dissolved in DMF (final concentration ≤2.5%). Phalloidin and vehicle served as positive and negative controls, respectively. Cells were fixed with 3% paraformaldehyde for 20 min, permeabilized with 0.2% Triton X-100 for 2 min and then reduced with 1% NaBH₄ in PBS (phosphate-buffered saline) three times for 5 min each. The microfilaments were stained with a TRITC-phalloidin conjugate in PBS for 45 min. After repeated washing of the slips, the chromatin was stained with 4,6-diamidino-2-phenylindole. The cover slips were mounted on microscope slides and examined by means of a Zeiss Axioplan fluorescence microscope. Activity was scored semiquantitatively on a + to ++++ scale reflecting the percentage of cells displaying changes in the microfilament network. Cytotoxicity was determined by the sulforhodamine B assay. Cytotoxicity against cancer cell lines LoVo (human colon adenocarcinoma) and KB (human nasopharyngeal carcinoma) were determined by IC₅₀ values based on the sulforhodamine B assay.
1.5.5 Physical Data

**Microcarpalide (1.1):** colorless oil; $[\alpha]_D^{23} -22$ (c 0.67, MeOH); FTIR (liquid film) $\nu_{max}$ 3380, 2925, 2855, 1715, 1435, 1365, 1230, 1160, 1065, 980 cm$^{-1}$; $^1$H NMR (400 MHz, CD$_3$CN) and $^{13}$C NMR (100 MHz, CD$_3$CN) see Table 1.2.

**Falcarindiol (1.12):** colorless oil; $[\alpha]_D^{23} +302$ (c 1.0, Et$_2$O); FTIR (liquid film) 3320, 2230, 2150, 1645, 1460, 1300, 1120, 1020, 930, 880 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 5.97 (ddd, J= 17.1, 10.2, 5.4 Hz, 2H), 5.61 (ddd, J= 10.8, 7.5, 0.9 Hz, 1H), 5.59 (ddd, J= 10.8, 8.1, 1.2 Hz, 1H), 5.49 (ddd, J= 17.1, 0.9, 0.9 Hz, 2H), 5.26 (ddd, J= 10.2, 0.9, 0.9 Hz, 2H), 5.20 (dd, J= 8.1, 5.1 Hz, 1H), 4.94 (dd, J= 6.6, 5.4 Hz, 1H), 2.10 (m, 2H), 1.92 (d, J= 6.6 Hz, 1H), 1.84 (d, J= 5.1 Hz, 1H), 1.46-1.36 (m, 2H), 1.27-1.23 (m, 8H), 0.88 (t, J= 6.9 Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 135.8, 134.7, 127.6, 117.3, 79.8, 78.2, 70.3, 68.7, 63.5, 58.6, 31.8, 29.2, 29.1, 29.0, 27.7, 22.6, 14.1; EIMS 242 (M-H$_2$O)$^+$, 171, 157, 129, 115, 91, 77; HREIMS calcd for (C$_{17}$H$_{24}$O$_2$-H$_2$O)$^+$ 242.1776, found 242.1671.

**Epoxy-falcarindiol (1.20):** colorless oil; $[\alpha]_D^{23} +87$ (c 0.64, MeOH); FTIR (liquid film) 3320, 2360, 1655, 1465, 1375, 1260, 1020 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 5.94 (ddd, J= 17.1, 10.2, 5.4 Hz, 1H), 5.50 (br m, 1H), 5.32 (br m, 1H), 4.37 (d, J= 7.3 Hz, 1H), 3.14 (dd, J= 7.3, 4.0 Hz, 1H), 0.15 (d, J= 4.0 Hz, 1H), 2.11 (m, 1H), 1.67-1.58 (m, 1H), 1.40-1.32 (m, 2H), 1.27-1.23 (m, 8H), 0.88 (t, J= 6.9 Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 135.7, 117.5, 78.5, 78.0, 70.2, 70.1, 63.5, 60.8, 58.0, 57.9, 31.7, 29.4, 29.3, 29.1, 27.5, 22.5, 14.1.
Crithmumdiol (1.21): colorless oil; [α]_D^{23} +36 (c 0.1, MeOH); FTIR (liquid film) 3415 (br), 17010, 1645, 1555, 1460, 1410, 1260, 1160, 1025 cm⁻¹; \(^1\)H NMR (500 MHz, CDCl₃) δ 6.15 (dd, J= 16.0, 5.8 Hz, 1H), 5.85 (ddd, J= 16.5, 10.4, 5.9 Hz, 1H), 5.78 (ddd, J= 16.0, 1.6, 1.6 Hz, 1H), 5.59 (m, 1H), 5.55 (m, 1H), 5.29 (ddd, J= 10.4, 1.1, 1.1 Hz, 1H), 5.27 (m, 1H), 5.19 (ddd, J= 10.4, 1.2, 1.1 Hz, 1H), 4.68 (ddd, J= 5.9, 5.9, 1.2 Hz, 1H), 2.14-2.09 (m, 2H), 1.35 (m, 2H), 1.31-1.26 (m, 8H), 0.87 (t, J= 6.9 Hz, 3H); \(^{13}\)C NMR (75 MHz, CDCl₃) δ 143.8, 138.1, 133.8, 128.7, 116.2, 109.8, 90.3, 82.6, 73.1, 58.6, 31.8, 29.3, 29.2, 29.1, 27.6, 22.6, 14.1.
1.5.6 Synthetic Transformations

A solution of diol 1.1 (1.0 mg, 0.003 mmol) in dry acetone (0.016 mL) was treated with DMP (0.003 mL, 0.024 mmol) in the presence of a catalytic amount of TsOH at room temperature. After complete conversion of the starting material to the acetonide derivative 1.8, as indicated by TLC, the reaction mixture was diluted with EtOAc and washed with 5% NaHCO₃. The organic layer was concentrated to dryness in vacuo to give approximately 1.0 mg (88% yield) of 1.8 as a colorless liquid: [α]D²³⁻⁸³ (c 0.07, CHCl₃); FTIR (liquid film) νmax 3460, 2925, 2860, 1735, 1440, 1370, 1235, 1160, 1055 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 5.84 (ddd, J = 15.9, 7.9, 7.9 Hz, 1H), 5.53 (dd, J = 15.9, 8.4 Hz, 1H), 4.92 (ddd, J = 9.0, 5.3, 0.4 Hz, 1H), 3.97 (dd, J = 8.4, 8.4 Hz, 1H), 3.87 (ddddd, J = 9.7, 5.0, 3.3, 0.4 Hz, 1H), 3.62 (m, 1H), 2.52-2.34 (m, 4H), 2.22-2.11 (m, 2H), 1.71 (br m, 1H), 1.57 (m, 1H), 1.40 (s, 3H), 1.39 (s, 3H), 1.32-1.23 (br m, 8H), 0.86 (t, J = 6.9 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 172.6, 132.1, 127.8, 108.0, 82.7, 81.4, 74.1, 69.7, 38.0, 32.5, 31.9, 31.6, 29.0, 27.5, 27.0, 26.9, 25.1, 22.5, 14.1.
The acetonide derivative 1.8 (1.0 mg, 0.003 mmol) was dissolved in anhydrous pyridine (0.05 mL) and cooled to 0 °C under N₂ with stirring. Acetic anhydride (0.05 mL, 0.53 mmol) was added dropwise followed by catalytic amount of DMAP, and allowed to stir overnight at 0 °C. The reaction mixture was concentrated to dryness in vacuo and the resulting crude residue was purified on silica gel (hexane:EtOAc 5:1.3) to afford approximately 1.10 mg (94% yield) of monoacetyl derivative 1.8a as colorless liquid: ^1H NMR (500 MHz, CDCl₃) δ 5.81 (ddd, J = 15.6, 8.3, 6.8 Hz, 1H), 5.51 (dd, J = 15.6, 8.4 Hz, 1H), 5.15 (ddd, J = 5.5, 5.5, 2.1 Hz, 1H), 5.01 (ddd, J = 9.0, 5.0, 2.1 Hz, 1H) 3.93 (dd, J = 8.4, 8.4 Hz, 1H), 3.65 (ddd, J = 8.4, 8.4, 5.1 Hz, 1H), 2.56 (br m, 1H), 2.47 (m, 1H), 2.40 (dddd, J = 14.8, 8.3, 5.1, 1.1 Hz, 1H) 2.21 (m, 1H), 2.14 (s, 3H), 2.11 (br m, 2H), 1.54 (m, 1H), 1.48 (m, 1H), 1.40 (s, 3H), 1.39 (s, 3H), 1.27-1.23 (br m, 8H), 0.86 (t, J = 6.8 Hz, 3H); ^13C NMR (125 MHz, CDCl₃) δ 173.5, 170.4, 132.3, 127.6, 108.1, 81.6, 81.5, 73.1, 71.0, 35.5, 31.6, 31.5, 31.4, 29.0, 27.1, 27.0, 26.6, 25.1, 22.5, 22.1, 14.0.
Following the general procedure described above for the protection of 1,2 diols, diol 1.1 (1.0 mg, 0.003 mmol) was subjected to the above described reaction conditions using PPTS as the acid catalyst instead of TsOH, to afford 0.95 mg (84% yield) of the corresponding acetonide derivative 1.7 as a colorless oil: $^1$H NMR (500 MHz, CDCl$_3$) δ 5.78 (ddd, $J = 15.6, 11.2, 4.7$ Hz, 1H), 5.33 (dd, $J = 15.6, 9.3$ Hz, 1H), 4.70 (ddd, $J = 8.7, 4.7, 2.3$ Hz, 1H), 3.92 (dd, $J = 9.3, 8.8$ Hz, 1H), 3.65 (br m, 1H), 3.63 (ddd, $J = 8.8, 8.1, 0.7$ Hz, 1H), 2.67 (ddd, $J = 12.3, 11.2, 8.7$ Hz, 1H), 2.54 (ddd, $J = 13.4, 5.6, 3.6$ Hz, 1H) 2.42 (ddd, $J = 12.3, 4.7, 2.3$ Hz, 1H), 2.32 (ddd, $J = 13.4, 12.0, 4.4$ Hz, 1H), 2.09 (ddddd, $J = 15.3, 5.6, 4.4, 0.7$ Hz, 1H), 1.97 (ddddd, $J = 15.3, 12.0, 8.1, 3.6$ Hz, 1H), 1.48 (br m, 2H), 1.41 (s, 6H), 1.30-1.24 (br m, 8H), 0.88 (t, $J = 6.8$ Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 170.4, 130.7, 129.3, 108.8, 83.9, 80.0, 77.0, 73.8, 33.9, 33.6, 32.2, 31.7, 29.2, 27.1, 26.9, 25.6, 25.5, 22.6, 14.1.
Following the above described procedure for acetylation of alcohols, 1.7 (0.95 mg, 0.003 mmol) afforded 1.0 mg (88% yield) of the acylated derivative 1.7a as a colorless liquid: $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 5.67 (ddd, $J$ = 15.5, 11.4, 4.5 Hz, 1H), 5.33 (dd, $J$ = 15.5, 9.2 Hz, 1H), 5.05 (br m, 1H), 4.87 (ddd, $J$ = 8.8, 4.4, 2.5 Hz, 1H), 3.91 (dd, $J$ = 9.2, 8.8 Hz, 1H), 3.63 (br ddd, 1H), 2.68 (br m, 1H), 2.54 (ddd, $J$ = 13.1, 5.0, 4.0 Hz, 1H) 2.30 (ddd, $J$ = 13.1, 12.8, 4.8 Hz, 1H), 2.23 (br m, 1H), 2.09 (m, 1H), 2.00 (m, 1H), 1.62 (br m, 2H), 1.41 (s, 3H), 1.40 (s, 3H), 1.33-1.25 (br m, 8H), 0.86 (t, $J$ = 6.8 Hz, 3H); (compound 1.17a was treated as an intermediate and was not fully characterized by NMR).

To a stirred solution of 1.7a (1.0 mg, .003 mmol) in THF (0.10 mL) was added 1N HCl (0.10 mL) in one portion at room temperature. After 4.5 h, TLC analysis indicated completion of deprotection of diol 1.7b. The reaction mixture was partitioned between satd NaHCO$_3$ and EtOAc and the organic layer was evaporated to dryness in
*vacuo* to yield approximately 0.80 mg (89% yield) of the desired product 1.7b as a colorless liquid: (compound 1.7b was treated as an intermediate and was not characterized by NMR).

To a solution of 1.7b (0.80 mg, 0.002 mmol) and DMAP (catalytic amount) in dry pyridine (0.06 mL) at 0 °C was added anisic anhydride (6.0 mg, 0.021 mmol) in one portion. The resulting mixture was allowed to stir overnight at 0 °C. After complete conversion of the starting diol 1.7b to the di-benzoyl derivative 1.9, as indicated by TLC, 3-(Dimethylamino)propylamine (0.006 mL, 0.044 mmol) was added dropwise at room temperature and allowed to stir for 0.5 h. The reaction mixture was diluted with diethyl ether:pentane 1:1 (v/v, 0.5 mL) and washed successively with ice cold 1N HCl (3×0.5 mL), 5% NaHCO₃ (2×0.5 mL) and brine. The organic extract was concentrated to dryness in *vacuo* and the residue was subjected to column purification (silica gel, hexane:EtOAc 6:0.5, hexane:EtOAc 6:1.5) to give approximately 0.75 mg (52% yield) of pure bis-anisoyl derivative 1.9 as a colorless oil: $[\alpha]_D^{23} -48$ (c 0.03, CH₃CN); UV (CH₃CN) $\lambda_{max}$ nm (log ε) 195 (4.2), 258 (4.0); FTIR (liquid film) $\nu_{max}$ 2925, 2850, 1735, 1605, 1580, 1500, 1255, 1165, 1100, 1025 cm⁻¹; CD (CH₃CN) $\lambda_{ext}$ nm ($\Delta$ε) 264 (-4), 246 (+3); $^1$H NMR (500 MHz, CH₃CN, 50 °C) δ 8.7 (d, $J = 8.7$ Hz, 4H), 7.02 (d, $J = 8.6$ Hz, 4H), 5.87 (dd, $J = 15.8$, 2.5 Hz, 1H), 5.68 ($br$ m, 1H), 5.59 ($br$ m, 1H), 5.32 (ddd, $J = 5.8$, 4H).
5.8, 1.3 Hz, 1H) 5.12 (ddd, J = 11.5, 4.6, 3.1 Hz, 1H), 5.05 (ddd, J = 6.6, 6.6, 1.3 Hz, 1H), 3.87 (s, 6H), 2.57 (m, 2H), 2.46-2.25 (br m, 2H), 2.20 (br m, 2H) 2.10 (s, 3H), 1.60 (m, 1H), 1.30 (br m, 9H), 0.89 (t, J = 6.9 Hz, 3H).

To a solution of (trimethylsilyl)acetylene 1.27 (1.04 g, 1.5 mL, 10.6 mmol) in dry THF (42 mL) at -78 °C was added dropwise a solution of n-butyllithium (2.4 M in hexanes, 4.42 mL, 10.6 mmol) under a N₂ atmosphere. After the addition was complete, the solution was allowed to warm to -60 °C over a period of 15-30 min, at which point an ice-cold solution of acrolein (0.71 g, 0.85 mL, 12.7 mmol) in THF (25 mL) was introduced slowly via a cannula. The resulting mixture was allowed to gradually warm to 0 °C over a period of 1.5 h. After stirring for an additional 1 h at room temperature, the mixture was quenched with cold satd NH₄Cl. The aqueous phase was extracted with Et₂O (×3), dried (MgSO₄) and concentrated to dryness in vacuo. The resulting crude oil was purified by Kugelrohr distillation (bp 85-90 °C/20 mm Hg) to give 1.63 g (95% yield) of pure racemic allyl alcohol (±)-1.28 as a colorless liquid. FTIR (film) 3355 (br), 2175, 1640, 1405, 1250, 1115, 1030, 985, 845, 760 cm⁻¹; ^1H NMR (300 MHz, CDCl₃) δ 5.94 (ddd, J = 17.1, 10.2, 5.4 Hz, 1H), 5.44 (dd, J = 17.1, 0.9 Hz, 1H), 5.19 (dd, J = 10.2, 0.9 Hz, 1H), 4.85 (brd, J = 5.4 Hz, 1H), 2.60 (brs, 1H), 0.16 (s, 9H); ^13C NMR (75 MHz, CDCl₃) δ 136.9, 116.7, 104.3, 91.2, 63.6, -0.03 (3C).
To a well-stirred suspension of lipase (*Pseudomonas fluorescens*, 0.03 g, 0.15 mass equiv), ground activated 4Å molecular sieves (0.22 g, 1.0 mass equiv) and racemic allyl alcohol (±)-1.28 (0.22 g, 1.45 mmol) in dry hexane (12 mL) was added vinyl acetate (0.53 mL, 5.8 mmol) in one portion. The suspension was allowed to stir under N₂ at room temperature for 21 h and the course of the reaction was monitored by ¹H NMR analysis. At the end of this period, the mixture was filtered through a pad of Celite, and evaporated to dryness under reduced pressure. The crude product mixture, following flash chromatography, gave acetate (R)-1.29 (eluent: 3% Et₂O:hexane) 0.20 g (35% yield, ee > 98%) and alcohol (S)-1.28a ((eluent: 10% Et₂O:hexane) 0.16 g (35% yield, ee > 98%) as colorless oils: (S)-1.28a ≥95 % ee; [α]D²³ +38 (c 2.0, CHCl₃); for spectroscopic data see (±)-1.28.

A solution of alcohol (S)-1.28a (0.05 g, 0.32 mmol) in acetone (2 mL) was treated with N-bromosuccinimide (0.07 g, 0.39 mmol) and a catalytic amount of powdered AgNO₃ (0.004 g, 0.02 mmol). The reaction mixture was wrapped with aluminum foil to exclude light and stirred at room temperature for 3 h. The mixture was diluted with cold water and extracted with Et₂O (x3). The combined organic layers were dried (MgSO₄),
concentrated to dryness in vacuo and subjected to flash column chromatography (silica gel, 10% Et$_2$O:hexane) to afford 0.03 g (55% yield) of bromo acetylene ($S$)-1.30 as a clear liquid: [$\alpha$]$_D^{25} +38$ (c 1.9, CHCl$_3$); FTIR (film) 3380 (br), 2215, 1640, 1405, 1265, 1120, 1015, 985, 935, 885, 800, 725 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 5.95 (ddd, J= 17.1, 9.9, 5.4 Hz, 1H), 5.46 (ddd, J= 17.1, 0.9, 0.9 Hz, 1H), 5.25 (ddd, J= 9.9, 0.9, 0.9 Hz, 1H), 4.90 (ddd, J= 6.3, 5.4, 0.9 Hz, 1H), 2.15 (d, J= 6.3 Hz, 1H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 136.4, 117.2, 79.0, 64.2, 47.2.

To an ice-cold solution of 70% aqueous EtNH$_2$ (0.060 mL) and catalytic CuCl (0.002 g, 0.02 mmol) in MeOH (0.074 mL, degassed with Ar) was added NH$_2$OH·HCl (0.007 g, 0.11 mmol) dissolved in water (0.026 mL). To the above mixture at 0 °C was added a solution of bromo acetylene ($S$)-1.30 (0.052 g, 0.32 mmol) in MeOH (0.65 mL). After the addition was complete, the reaction mixture was removed from the ice-bath and stirred at room temperature overnight. The solution was diluted with cold water and extracted with Et$_2$O. The organic layers were dried (MgSO$_4$), concentrated under reduced pressure and the resulting crude residue was purified on silica gel (20%-30% EtOAc-hexane) to yield 0.011 g (80% yield) of symmetrical dimer ($S,S$)-1.26 as a white crystalline solid: [$\alpha$]$_D^{24} +102$ (c 0.38, CHCl$_3$); mp 77-78 °C; FTIR (film) 3320 (br), 2355, 2145, 1635, 1405, 1260, 1115, 1015, 985, 935, 865 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 5.95 (ddd, J= 17.1, 10.2, 5.4 Hz, 2H), 5.48 (ddd, J= 17.1, 0.9, 0.9 Hz, 2H), 5.27 (ddd, J=
10.2, 0.9, 0.9 Hz, 2H), 4.95 (br m, 2H), 2.03 (d, J= 4.8 Hz, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 135.9 (2C), 117.7 (2C), 78.6 (2C), 70.3 (2C), 63.7 (2C).

A solution of second-generation Grubbs' catalyst (0.011 g, 0.013 mmol, 10 mol%) in degassed anhydrous CH$_2$Cl$_2$ (45.5 mL) at room temperature was stirred vigorously under an atmosphere of ethylene gas. After bubbling a steady stream of ethylene gas for 15 min, a solution of (+)-1.12 (0.035 g, 0.13 mmol) in degassed CH$_2$Cl$_2$ (70 mL) under ethylene gas was added slowly via a cannula to the above reaction flask at room temperature. Progress of the reaction was monitored by TLC analysis. After stirring overnight (16 h), the solvent was removed in vacuo and the residue was purified by flash column chromatography (silica gel, 20-30% EtOAc:hexane) to afford the symmetrical dimer 1.26-(meso) as a slightly brown solid. The crude solid was recrystallized in Et$_2$O:hexane to give 0.018 g (81% yield) of pure product as white crystalline material: $[\alpha]_D^{23}$ +5 (c 0.7, CHCl$_3$); mp 49-51 °C; FTIR (film) 3300 (br), 2360, 2145, 1640, 1400, 1325, 1260, 1115, 1015, 985, 930, 865 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$) δ 5.94 (ddd, J= 17.0, 10.1, 5.4 Hz, 2H), 5.48 (d, J= 17.0 Hz, 2H), 5.27 (d, J= 10.1 Hz, 2H), 4.95 (d, J= 5.4 Hz, 2H), 2.36 (br s, 2H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 135.6 (2C), 117.5 (2C), 78.3 (2C), 70.1 (2C), 63.4 (2C).
General procedure for the preparation of MTPA-esters:

A 0.01 M solution of alcohol product in anhydrous CH₂Cl₂ at 0 °C was treated successively with 2,6-lutidine (8 mol equiv), MTPA-Cl (4 mol equiv per OH) and DMAP (1 mol equiv per OH) under N₂. The mixture was allowed to stir at room temperature for 15 h and treated with 3(N,N-dimethylamino)propylamine (0.3 mol equiv over acid chloride). After stirring for an additional 10 min, the solvent was removed in vacuo. The residue was dissolved in a minimum amount of CH₂Cl₂ and applied to a silica gel column (5% Et₂O-hexane) to afford pure ester in 85-90% yield:

(+)-(3R,8S)-1.12-bis-(S)-MTPA-ester: ^1^H NMR (500 MHz, CDCl₃) 7.52-7.50 (m, 5H), 7.41-7.38 (m, 5H), 6.40 (d, J= 9.0 Hz, 1H), 6.09 (d, J= 5.4 Hz, 1H), 5.92 (m, 1H), 5.73 (br m, 1H), 5.60 (d, J= 16.5 Hz, 1H), 5.46 (m, 1H), 5.43 (d, J= 10.5 Hz, 1H), 3.63 (s, 3H), 3.58 (s, 3H), 2.15 (m, 2H), 1.45-1.17 (m, 10H), 0.88 (t, J= 6.9 Hz, 1H).

(+)-(3R,8S)-1.12-bis-(R)-MTPA-ester: ^1^H NMR (500 MHz, CDCl₃) 7.52-7.49 (m, 5H), 7.41-7.39 (m, 5H), 6.35 (d, J= 8.6 Hz, 1H), 6.11 (d, J= 5.8 Hz, 1H), 5.82 (ddd, J= 16.9, 10.2, 5.8 Hz, 1H), 5.77 (m, 1H), 5.55 (m, 1H), 5.52 (d, J= 16.9 Hz, 1H), 5.37 (d, J= 10.2 Hz, 1H), 3.60 (s, 3H), 3.57 (s, 3H), 2.17 (m, 2H), 1.40-1.24 (m, 10H), 0.87 (t, J= 7.1 Hz, 1H).
Procedure for MPA-ester formation:

Falcarindiol (0.001 g, 0.004 mmol) in anhydrous CH₂Cl₂ (1 mL) was cooled to 0 °C under N₂ with stirring. EDCI (0.007 g, 0.023 mmol) and O-methylmandelic acid (0.001 g, 0.012 mmol) were added followed by DMAP (0.003 g, 0.023 mmol). The mixture was allowed to stir at room temperature for 17 h, poured into cold satd NH₄Cl and extracted with Et₂O (×3). The combined organic extracts were washed with brine, dried (MgSO₄) and concentrated to dryness in vacuo. The crude residue was purified on silica gel (35% CH₂Cl₂-hexane, CH₂Cl₂) to provide 0.0017 g (81% yield) of the desired bis-MPA-ester as a colorless liquid:

(+)-(3R,8S)-1.12-(S)-bis-MPA-ester: ¹H NMR (500 MHz, CDCl₃) δ 7.52-7.30 (m, 10H), 6.18 (d, J= 9.0 Hz, 1H), 5.94 (d, J= 5.5 Hz, 1H), 5.66 (m, 2H), 5.47 (br m, 1H), 5.30 (d, J= 17.0 Hz, 1H), 5.20 (d, J= 10.0 Hz, 1H), 2.10 (m, 2H), 1.45-1.17 (m, 10H), 0.88 (t, J= 6.9 Hz, 1H); (+)-(3R,8S)-1.12-bis-(R)-MPA-ester: ¹H NMR (500 MHz, CDCl₃) δ 7.52-7.30 (m, 10H), 6.15 (d, J= 9.0 Hz, 1H), 5.94 (d, J= 6.0 Hz, 1H), 5.85 (m, 1H), 5.57 (m, 1H), 5.51 (d, J= 17.5 Hz, 1H), 5.34 (d, J= 10.5 Hz, 1H), 5.30 (dd, J= 10.5, 0.9 Hz, 1H), 2.00 (m, 2H), 1.45-1.17 (m, 10H), 0.88 (t, J= 6.9 Hz, 1H).
Part 2: Total Synthesis of Microcarpalide
2.1 Introduction

During the structure elucidation, the relative stereochemistry of the C-9/C-10 asymmetric centers of microcarpalide (1.1) was established as *threo* by the application of the *J*-based method of Murata.\(^6\) However, there is little precedence for the use of this *J*-based methodology in cyclic systems, especially in medium size (8≤11) rings. As pointed out by Murata,\(^7\) because of the importance of strain for the overall energy of the molecule, in such systems the local conformer population distribution may be skewed from the predominant staggered one observed in an acyclic system of the same relative configuration. Such changes in conformer distribution will affect the magnitude of \(2J\) and \(3J\) coupling constants, which are used to establish the relative configuration around two stereogenic atoms. This may lead to an erroneous assignment since the *J*-based method is essentially an empirical method. An additional complication for the application of Murata’s method to 1.1 arises from the presence of essentially three large substituents at C-9 of 1.1. Therefore a brief synthesis of microcarpalide (1.1) was pursued as a means of validating the application of the *J*-based methodology to the stereochemical assignment of medium size rings and to confirm the proposed structure of 1.1.
2.2 Synthetic strategy

The total synthesis of 1.1\(^{70}\) was pursued as a means of confirming the lactone structure and validating the \(J\)-based stereochemical assignment at C-9/C-10 asymmetric centers. For this reason, it was essential to develop a flexible yet stereochemically unambiguous approach. Because the target molecule (1.1) contains a double bond, the most straightforward entry into its 10-membered heterocycle was envisioned to be via intramolecular ring-closing metathesis (RCM) of suitable diolefin precursors. This approach was chosen over the alternative macrolactonization since, in addition to its precedence for successful application to a number of ring sizes,\(^{71}\) RCM also offered the benefit of a more convergent synthesis.

![Diagram of macrolactonization](image)

The question of C-6/C-7 double bond stereochemistry was ignored for the time being since no firm basis appears to exist as of yet that allows one to predict the stereochemistry of the newly formed double bond in the RCM reaction of olefins. It was also important that the alcohol functions at C-4, C-5, and C-10 be blocked by suitable protecting group(s) which should be selectively removable under mild conditions in steps prior to and following completion of ring closure. If macrolactonization was employed as the ring closing method, it was expected that the C-10 protecting group would inevitably depress the rate of formation of the desired 10-membered ring. On the other hand, no such complications were foreseen for RCM and formation of oxadecenes by this process.
was preceded.\textsuperscript{72} The retrosynthetic approach to the formation of the carbon skeleton of microcarpalide (1.1) is summarized in (Scheme 2.1). RCM allows the nonenolide to be deconvoluted into two rather simple diolefin fragments, 2.1 and 2.2. Accordingly, disconnection of the ester linkage in the diolefinic acyclic substrate (2.3) would generate a secondary alcohol segment (2.2) and a carboxylic acid segment (2.1). The 2.1 and 2.2 segments in turn could be derived from readily available starting materials.

\begin{center}
\textbf{Scheme 2.1. Retrosynthetic pathway to microcarpalide}
\end{center}

The desired stereogenic centers in 2.1 and 2.2 (C-4/C-5 and C-9/C-10 respectively) could easily be generated by asymmetric dihydroxylation\textsuperscript{73} of the corresponding (\textit{E})-double bonds. The resulting vicinal diol function in 2.1, if protected as an isopropylidene derivative, could selectively be removed under mild acidic conditions\textsuperscript{74} without affecting the C-1 ester linkage even at later stages of the reaction pathway. The
carboxyl function at C-1 in 2.1 could be generated from the corresponding alcohol precursor by a single-step oxidation\(^{75}\) process. The C\(_6\)H\(_{13}\) aliphatic side chain in 2.2 can be a part of the starter unit and be carried through the synthesis. A final Swern-Wittig olefination\(^{76}\) sequence would generate the terminal double bonds in 2.1 and 2.2, thus completing the synthesis of the two fragments.

An esterification\(^{77}\) step between the C-9 secondary alcohol function in 2.2 and the C-1 carboxyl function of 2.1 was anticipated to form the desired linkage between C-1/C-9 in 2.3. The resulting diene precursor (2.3) in the presence of a suitable ruthenium alkylidene catalyst\(^{78}\) would set the stage for a final cyclization via an intramolecular ring-closing metathesis to yield the ten-membered lactone 1.1.

![Scheme 2.2. Cyclization of the diene to the lactone 1.1.](image)

**2.2.1 Synthesis of the Right-Hand (RH) Segment**

1,4-Butanediol (2.4) was chosen as the starting material for the synthesis of the RH segment of microcarpalide (1.1). Selective monoprotection\(^{79}\) of the symmetrical diol (2.4) with 1 equiv of tert-butyldimethylsilyl chloride, followed by catalytic free radical oxidation\(^{80}\) led to the corresponding aldehyde (2.5) in near quantitative yield. Conversion of aldehyde (2.5) to the trans-\(\alpha,\beta\)-unsaturated ethyl ester (2.6) proceeded smoothly under
Masamune's reaction conditions in 93% (crude) yield. TLC analysis (hexanes:EtOAc 4:1) of the crude product (2.6) gave evidence for the formation of both cis and trans double bond isomers ($E/Z$ ratio $\geq$40:1). This observation was further confirmed by the $^1$H NMR spectrum of the sample mixture of 2.6. Following chromatographic purification on silica gel, the desired (E)-isomer of 2.6 was recovered in 91% yield.

![Scheme 2.3](image)

Key: a) NaH, THF, TBDMSCl, RT, N$_2$, overnight, 100%; b) TEMPO free radical, CH$_2$Cl$_2$, 5% NaOCl, KBr, 0°C, 30 min-1 h, 95%; c) DBU, LiCl, CH$_3$CN, triethylphosphonoacetate, RT, N$_2$, 91%;

Scheme 2.3

Enantioselective asymmetric dihydroxylation (AD) of the trans olefin (2.6) according to Sharpless' procedure$^{73}$ yielded the desired diol ($4R,5S$) 2.7 as a colorless oil. Exposure of the 1,2-diol (2.7) to DMP and $p$-TsOH (cat.) for 5 h at RT and subsequent TLC analysis of the crude mixture (hexanes:EtOAc 3:1) revealed the unexpected formation of three different products (2.8, 2.8a, and 2.8b).

![Scheme 2.4](image)

Key: d) ADmix-β, H$_2$O/t-BuOH, 0°C, 12 h, 89%; e) DMP, acetone, $p$-TsOH, RT, 5 h, 87% (combined);

Scheme 2.4
The two less polar products ($R_f = 0.69$ and $0.54$ in hexanes:EtOAc 3:1) were identified as the desired silyl ether-protected acetal 2.8 and the undesired methoxydimethylether-protected acetal 2.8a, respectively. The most polar product ($R_f = 0.19$ in hexanes:EtOAc 3:1) was identified by spectroscopic means as the desilylated ketal 2.8b. The three products amounted to 87% combined recovery. The observed desilylation and hence the formation of the undesired products (2.8a and 2.8b) was likely to be a result of the acidity of the $p$-TsOH catalyst\textsuperscript{82} used.

Accordingly, the reaction conditions were adjusted by replacing the latter with a mild acid catalyst, (PPTS). The conversion of 2.7 to 2.8 when repeated under these modified conditions (DMP, acetone, PPTS, overnight, RT) proceeded smoothly in 81% yield. Direct reduction of the ester group with LiAlH\textsubscript{4}/THF furnished compound 2.9 in 98% from compound 2.8. Compound 2.9 following Swern oxidation\textsuperscript{83} and Wittig olefination\textsuperscript{84} under standard conditions gave the desired terminal alkene 3.1 in 68% overall yield for the two steps.

![Scheme 2.5](image)

Key: f) LiAlH\textsubscript{4}, THF, 0 °C-RT, N\textsubscript{2}, 2.5 h, 98%; g) oxalyl chloride, DMSO, CH\textsubscript{2}Cl\textsubscript{2}, Et\textsubscript{3}N, N\textsubscript{2}, -78 → -60 0 °C; h) CH\textsubscript{3}PPh\textsubscript{3}Br, n-BuLi, THF, -78 °C to RT, overnight, 68%;

**Scheme 2.5**

The silyl ether protecting group in (3.1) was cleaved selectively under mild conditions with Et\textsubscript{3}N·3HF to expose the primary alcohol function. Direct oxidation of the
alcohol (3.2) was readily carried out with PDC-DMF to the corresponding carboxylic acid (2.1) in 92% yield.

Key: i) Et₃N·3HF, CH₂Cl₂, 12 h, RT, 89%; j) pyridinium dichromate, DMF, 24 h, RT, 92%.

**Scheme 2.6**

The 10-step sequence (a-j) described above completed the transformation of 1,4-butane dinol (2.4) into the RH segment (2.1) with an overall yield of 34%.
2.2.2 Synthesis of the Left-Hand (LH) Segment

Method A. The elongation of the starter unit, \(n\)-heptanal (3.3) under Horner-Wadsworth-Emmons\(^8^5\) reaction conditions gave the \(\alpha,\beta\)-unsaturated ester (3.4) in 88% yield. The ester (3.4) so formed, following asymmetric dihydroxylation,\(^7^3\) produced the anticipated 1,2-diol (3.5) in good yield.

![Chemical structures](image)

Key: a) DBU, LiCl, CH\(_3\)CN, triethylphosphonoacetate, RT, \(N_2\), 88%; b) ADmix-\(\alpha\), H\(_2\)O/t-BuOH, 0\(^\circ\) C, 12 h, 90%;

Scheme 2.7

The conversion of diol (3.5) to \(p\)-methoxybenzylidene acetal (3.6) was initially designed to be carried out with anisaldehyde dimethyl acetal under standard conditions (anisaldehyde dimethyl acetal, Amberlyst, 4 Å MS, toluene, 65 \(^\circ\)C).\(^8^6\) For this purpose, a fresh sample of anisaldehyde dimethyl acetal reagent was required. The optimum conditions for the synthesis of the latter reagent was experimentally determined (Table 2.1, Exp. No. 3) after examining a few standard literature procedures.\(^8^6,8^7,8^8\)

Table 2.1. Preparation of \(p\)-methoxybenzaldehyde dimethyl acetal reagent

<table>
<thead>
<tr>
<th>Exp. No</th>
<th>Reagents/Conditions</th>
<th>Catalyst</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anisaldehyde, trimethyl orthoformate, 0 (^\circ)C→RT, (N_2), overnight</td>
<td>Amberlyst-15</td>
<td>39%</td>
</tr>
<tr>
<td>2</td>
<td>Anisaldehyde, MeOH, Et(_3)N, 0 (^\circ)C→RT, (N_2), 45 min</td>
<td>TiCl(_4) (CH(_2)Cl(_2)), 1 mol%</td>
<td>50%</td>
</tr>
<tr>
<td>3</td>
<td>Anisaldehyde, trimethyl orthoformate, MeOH, reflux, 15 h</td>
<td>TsOH, 1.3 mol%</td>
<td>88%</td>
</tr>
</tbody>
</table>
Based on two parallel experiments carried out under otherwise comparable experimental conditions (Amberlyst, toluene, 4 Å MS, 65 °C), the conversion of (3.5) to (3.6) was found to yield inferior results in the presence of anisaldehyde dimethyl acetal (67%) when compared to the corresponding aldehyde (85%). Subsequent exposure of 3.6 to LiAlH₄ resulted in the formation of the alcohol (3.7) in excellent yield (Scheme 2.8).

![Chemical structure](image)

**Scheme 2.8**

The substrate 3.7 in particular seemed to hold an added advantage, since the terminal hydroxyl function in 3.7 could also assist in achieving regioselectivity during the subsequent ketal ring opening. Acetals derived from 1,2-diols bearing such neighboring hydroxyl groups have been selectively cleaved at the acetal C-O bond proximate to the hydroxyl group using a combination of boron reagents.

![Chemical structure](image)

**Scheme 2.9**
Accordingly, initial treatment of 3.7 with BH$_3$·SMe$_2$ for 1h (0 °C→RT) followed by BF$_3$·Et$_2$O for 5 min at 0 °C should exclusively yield the product (3.8). The mechanism that the author proposed$^{90,91}$ (Scheme 2.10) suggested the formation of an oxyborane intermediate (3.9) upon addition of one-mole equivalent of BH$_3$·SMe$_2$. This coordination is accompanied by the evolution of one-mole equivalent of hydrogen gas. Subsequent addition of one-mole equivalent of BF$_3$·Et$_2$O would trigger a 1,3-hydride shift$^{92}$ via transition-state 4.0, bringing the reaction to completion within 5 minutes with the exclusive formation of 4.1.

\[
\begin{align*}
\text{(4.0)} & \quad \text{(4.1)}
\end{align*}
\]

Scheme 2.10

Unfortunately, substrate 3.7 failed to undergo the anticipated ring opening under the above-described conditions. Even though oxyborane-directed reductive acetal cleavage has been carried out on five-membered isopropyl and five- and six-membered benzylidene acetals$^{90}$ with good selectivity and yield, it has not been demonstrated on $p$-methoxybenzylidene acetals. It was reasoned that the $p$-methoxy function on the benzene ring may be interfering with the driving force necessary for the 1,3-hydride shift mechanism by stabilizing the partial positive charge on the ketal carbon (4.0, Scheme
A control experiment with isopropylidene ketal (4.2), carried out under similar reaction conditions proceeded smoothly to yield the expected product (4.3) in quantitative yield. The outcome of this control experiment further supported the earlier argument that a hydride shift malfunction may have been the reason for the failure of the desired conversion (3.7 → 3.8).

\[
\text{Scheme 2.11}
\]

It appears that the stabilizing effect of the 4-methoxy function improves the lifetime of the benzylic cation intermediate thereby attenuating the driving force for the 1,3-H¹ shift mechanism. Consequently, the reaction was allowed to run for a longer period of time in the presence of BF₃·OEt₂ at 0 °C. In spite of the latter modification, no improvement in product yield was observed. The use of BF₃·THF⁹³ instead of BH₃·SMe₂-BF₃·EtO₂ also failed to effect the desired transformation. Therefore, the need for a more efficient route to the LH segment (2.2) was apparent.

**Method B.** Even though this new pathway required a few additional steps, it offered some added advantages with regard to purity and simplicity of the chemistry employed in the transformations. A freshly distilled sample of octanal (4.4) was used as the starting material for the synthesis. Octylaldehyde (4.4) in the presence of excess malonic acid under modified Knoevenagel-condensation⁹⁴ conditions, produced the corresponding trans-β,γ-unsaturated acid (4.5) in good yield with high stereochemical purity (¹H NMR, CDCl₃). The conversion of carboxylic acid (4.5) to the ethyl ester (4.6)
was achieved via a TMS-Cl catalyzed esterification\textsuperscript{95} step. Following a standard metal-hydride reduction, the ester (4.6) was converted to the desired homoallylic alcohol (4.7) in excellent yield.

Key: a) malonic acid, xylene, piperidine, reflux, 24 h, 75%; b) EtOH, TMS-Cl, 24 h, RT, N\textsubscript{2}, 100%; c) LiAlH\textsubscript{4}, THF, 0 °C-RT, 5 h, N\textsubscript{2}, 100%; d) Ph\textsubscript{3}P, p-methoxyphenol, DIAD, THF, reflux, 4 h, 95%;

\textbf{Scheme 2.12}

The homoallylic 4-methoxyphenyl ether derivative (4.8)\textsuperscript{96} was prepared from the corresponding (E)-3-decen-1-ol (4.7). This ether derivative (4.8) was identified as an ideal substrate for the subsequent asymmetric dihydroxylation (AD) step when compared to the parent alcohol (4.7) itself. This notion was extracted from the work of Corey \textit{et al.},\textsuperscript{97} where a homoallylic 4-methoxyphenyl group was shown to be superior to other alcohol protecting groups in AD-reactions with respect to both yield and enantiomeric purity of the resulting product. Accordingly, the homoallylic anisole ethers have the ability to participate in hydrophobic and aryl-aryl interactions with the U-shaped binding pocket of the AD-catalyst, thereby enhancing enantioselectivity.

Consequently, asymmetric dihydroxylation of 4.8 via Corey’s procedure gave the desired diol (4.9) in high yield and excellent enantioselectivity (≥ 96% ee, chiral HPLC), as anticipated.
Acylation of 4.9 and successive deprotection of the 4-methoxyphenyl\(^{98}\) and the acetyl groups proceeded smoothly to yield the desired triol (4.12) in near quantitative yields. In contrast, direct removal of the \(p\)-methoxyphenyl group in 4.9 allowed only 60% recovery of the pure product (4.12). The low product recovery was mainly due to difficulties encountered during extraction of triol (4.12) from the CAN/CH\(_3\)CN/H\(_2\)O mixture into the EtOAc layer followed by an equally laborious chromatographic purification step.

Key: e) ADmix-\(\alpha\), H\(_2\)O/t-BuOH, 0 °C, 24 h, 97%;

**Scheme 2.13**

Key: f) Ac\(_2\)O, pyridine, DMAP, 0 °C, 11 h, N\(_2\), 97%; g) CH\(_3\)CN/H\(_2\)O (4:1), CAN, 0 °C, 45 min; h) NH\(_2\)OH, MeOH, overnight, RT, 82%;

**Scheme 2.4**
Differentiation of the three hydroxyl groups in 4.12 was expected to be achieved via the formation of the corresponding 6-membered p-methoxybenzylidene ketal derivative (4.14). Thus, reaction of p-methoxybenzaldehyde dimethyl acetal with 4.12 gave an equilibrium mixture of 5-membered (4.13) and 6-membered (4.14) ketals in a 1:2 ratio. The two products were easily separated by flash column chromatography (20-30% EtOAc in hexanes + 1% triethylamine). Additional 4.14 was obtained by isomerization of the dioxolane (4.13) in the presence of catalytic TsOH in DMF at room temperature. The remaining unprotected secondary hydroxyl function in 4.14 was subsequently blocked by exposure to excess of TBS-Cl (4.4 molar equiv).

Key: i) p-methoxybenzaldehyde dimethyl acetal, p-TsOH, DMF, reflux, 2.5 h, N₂; j) p-TsOH, toluene, 20 h, RT, N₂, 65%; k) TBS-Cl, Et₃N, DMAP, CH₂Cl₂, 24 h, 0°C-RT, N₂, 93%.

Scheme 2.15

The next step of the synthesis was critical in the sense that it required the 6-membered benzylidene ketal in 4.15 to be selectively cleaved in such a fashion that only the C-3 alcohol function would be exposed, thereby setting the stage for a final Swern-
Wittig sequence. This crucial regioselective ring opening step had to be attempted with several different reducing agents (NaBH₃CN, BH₃·THF/M(OTf)n, DIBAL-H) before optimum conditions were realized.

The reductive-cleavage of benzylidene acetals of carbohydrates has been extensively studied using the sodium cyanoborohydride-trimethylsilyl chloride system and was shown to favor the secondary 4-methoxybenzyl ether regioisomer. Attempted cleavage of the 1,3-dioxane in 4.15 under these conditions (NaBH₃CN, TMS-Cl, CH₃CN, 0 °C→RT, 5 h) led to extensive hydrolysis of the substrate yielding the triol (4.12) as the major product. The undesired primary 4-methoxybenzyl ether derivative (4.16a) was also formed as a side-product. To avoid such hydrolytic degradations, the reaction was repeated at ice-bath temperature in the presence of activated 3Å molecular sieves. Despite these modifications, problems due to bulk decomposition could not be minimized to an extent that this methodology became of any practical significance.

\[
\begin{align*}
\text{OTBS} & \quad n\text{C}_6\text{H}_{13} \quad 0 \quad H \quad \text{MP} \\
\text{H} & \quad \text{O} \\
\text{ reduction} & \\
\text{major (4.12)} & \\
\text{(4.15)} & \\
\text{NaBH}_3\text{CN} & \\
\end{align*}
\]

\[
\begin{align*}
\text{OH} & \quad \text{OH} \\
\text{OR}_1 & \quad \text{OR}_2 \\
\text{OTBS} & \quad n\text{C}_6\text{H}_{13} \\
\text{(4.16)} & \quad \text{R}_1 = \text{PMB}, \text{ R}_2 = \text{H} \\
\text{(4.16a)} & \quad \text{R}_1 = \text{H}, \text{ R}_2 = \text{PMB} \\
\end{align*}
\]

\[\text{Scheme 2.16}\]

Therefore, the regioselectivity of the reductive ring opening using DIBAL-H was examined as an alternative to the NaBH₃CN approach.
The addition of DIBAL-H, when carried out at $-78 \, ^\circ\text{C}$ in dry toluene with careful temperature control as the reaction progressed, gave the best yield compared to either hexane or CH$_2$Cl$_2$ as solvents. However, at higher temperatures ($-50 \, ^\circ\text{C}$), product loss due to reductive cleavage$^{105}$ of the TBS protecting group became a major side reaction.

The final Swern-Wittig olefination sequence$^{76}$ was carried out efficiently with an overall yield of 83% for the two steps (l and m). Oxidative removal of the PMB ether group with DDQ$^{106}$ gave access to the LH-segment (2.2) in a total of fifteen steps with a 13% yield for the entire transformation.

The alcohol function in 2.2 (left-hand segment) and the carboxylic acid function in 2.1 (right-hand segment) were coupled efficiently in the presence of EDCI/DMAP$^{77}$ to form the acyclic diene 2.3. Alcohol (2.2) and diene (2.3) displayed similar $R_f$ values ($R_f = 0.44$, hexane:EtOAc 8.5:1 (v/v)) in the solvent system chosen for chromatographic
purposes. Therefore, a slight excess of 2.1 over 2.2 was used in order to simplify chromatographic purification of the ester (2.3).

Scheme 2.19

2.2.3 Macrocyclic Ring Closing Metathesis (RCM)

Increasing attention has been directed toward the synthesis of medium and large rings using metal-catalysed ring-closing olefin metathesis (RCM). Ruthenium alkylidenes (Grubbs et al.)\textsuperscript{107} and molybdenum alkylidenes (Schrock et al.)\textsuperscript{108} are two of the most commonly used initiators for RCM.\textsuperscript{78} The commercial availability of such well-defined single-component homogeneous catalysts (1.24 and 1.25) has made the olefin metathesis reaction practical for organic synthesis.\textsuperscript{109} The classical Grubbs catalyst 1.24 is known to be highly sensitive toward the substitution pattern of the alkenes.\textsuperscript{110} On the other hand the second-generation ruthenium carbene catalyst 1.25,\textsuperscript{111} where one phosphine ligand (PCy\textsubscript{3}) of 1.24 has been replaced by a 1,3-dimesityl-4,5-dihydroimidazol-2-ylidene ligand, not only exhibits higher activity in RCM and cross metathesis relative to the
parent complex 1.24, but also provides excellent functional group tolerance.¹¹² Thus, catalyst 1.25 has been successfully applied to several difficult cases that were beyond the scope of parent complex 1.24.¹¹³,¹¹⁴ In fact, Ru-catalyzed RCM has proven to be highly efficient and is becoming recognized as one of the most straightforward and reliable methods for the synthesis of large (≥12) carbo- and heterocycles.¹¹⁵,¹¹⁶

The formation of medium ring systems (8≤11), such as the ten-membered lactone in microcarpalide (1.1), however, is largely unexplored and poses considerable challenges.¹¹⁷ Because of the inherent ring strain, eight- to eleven-membered cycloalkenes in particular are prone to the reverse process,¹¹⁸ i.e. to ring opening metathesis (ROM) or ring opening metathesis polymerization (ROMP) since the release of the ring strain provides a formidable driving force for the latter processes.¹¹⁹

\[
\text{Scheme 2.20}
\]

The assumption that only conformationally predisposed dienes can act as suitable starting materials has been exercised in many cases by incorporating appropriate conformational control elements designed to force the substrate to adopt a favorable conformation for ring closure.¹²⁰ Such modifications have been shown to facilitate the RCM and to stabilize the product formed against the competing ROMP pathway. For
example, during the synthesis of herbarum I (4.18), the isopropylidene acetal acts as a temporary constraint which adequately shapes the diene and simultaneously confers bias upon the stereochemistry of the newly formed double bond giving complete (E)-selectivity.

Subsequently, several other macrolides of the pinolidoxin series (8≤11) have been synthesized using RCM as the key step for the formation of the medium-sized ring. In general the number of successful applications of RCM to this series is still rather limited.

2.2.3.1 Stereoselectivity of Ring Closing Metathesis (RCM)

The vast majority of RCM based syntheses of medium-sized rings using catalyst 1.24 is known to provide (E/Z) mixtures with the (E)-isomer frequently being favored. The (E/Z) selectivity of the RCM product is often difficult to control or predict, since the selectivity changes with the ring size and the position of the olefin. As demonstrated in the recent RCM study of epothilone, even a functionality far from the metathesis reaction site is capable of affecting the (E/Z) ratio of the products. On the other hand, NHC-containing metathesis catalysts (1.25 and 1.25a) were recently shown to be particularly (E)-selective (ring ≥ 14), by enriching the product initially formed in the thermodynamically more favored alkene via subsequent isomerization.
Kinetic studies involving 1.24 with 14-membered lactones have failed to exhibit any significant evidence of secondary metathesis isomerizations in ring-closed products. Complete (Z)-selectivity of RCM reactions with 1.25 has also been reported. The choice of solvent (CH$_2$Cl$_2$, toluene or benzene) and the reaction temperature (reflux or RT) have also been shown to have a significant influence on the stereochemical outcome of the metathesis reactions.

2.2.3.2 Ring Closing Metathesis (RCM) of Microcarpalide

The RCM of 2.3 catalyzed by 1.24 (10 mol%) in CH$_2$Cl$_2$ at high dilution (0.00056M) proceeded extremely sluggishly to yield a stereoisomeric mixture of lactones (2.3a and 2.3b) after 147 h at reflux in 96% combined yield based on recovered starting material. The $E$/Z ratio was approximately 2:1 as determined by $^1$H NMR (CDCl$_3$). Inspection of NMR spectra of samples taken at various time points during the reaction...
suggests that this ratio does not change over the course of the reaction. Subsequently, the (E)-isomer (2.3a) \(^{3}J_{\text{H-H}8} = 15.5 \text{ Hz}\) was isolated by flash column chromatography (silica gel, hexanes-EtOAc). The 2.3a-(E) was sequentially treated with TREAT-HF and dil HCl in THF\(^{74}\) in order to remove the TBS and isopropylidene protecting groups respectively. The synthetic triol (1.1), so obtained was identical with the natural product (1.1) by \(^{1}H\) MNR (500 MHz, CD\(_{3}\)CN), \(^{13}C\) NMR (125 MHz, CD\(_{3}\)CN), TLC and \([\alpha]_{D}\) \{synthetic \([\alpha]_{D} = -23, c 0.12 (\text{MeOH}); \text{natural } [\alpha]_{D} = -22, c 0.67 (\text{MeOH})\}.

Exposure of 2.3 to the second-generation Ru-catalyst 1.25 bearing the imidazolylidene ligand\(^{131}\) under otherwise identical conditions \{cat.(10 mol\%) in CH\(_{2}\)Cl\(_{2}\) at high dilution (0.00056M)\} resulted in fast (15 min), almost quantitative conversion (91\%) to lactone 2.3b, which was shown to be exclusively of (Z)-stereochemistry \(^{3}J_{\text{H-H}8} = 10.6 \text{ Hz}\). Surprisingly, the cyclization product 2.3b of (Z)-configuration was conformationally homogeneous at room temperature as evident by \(^{1}H\) NMR. The (E)-isomer (2.3a) existed under the same conditions as two conformers in a ratio of 3.5:1 as had already been observed for the desilylated ketal during the structure elucidation of microcarpalide (1.1).
2.2.4 Evaluation of Results of Ring Closing Metathesis (RCM)

**Isomerization Studies.** Complete (Z)-selectivity of the second-generation catalyst 1.25 has been reported recently during the synthesis of the oxadecenolide natural product herbarumin and pinolidoxin.\(^\text{121}\) It has also been observed that in the case of the 14-membered lactone zearalenone that catalyst 1.25 can effect (Z) to (E)-isomerization of the cyclized product to give a more favorable \(E/Z\) ratio.\(^\text{115}\) In order to establish whether the observed double bond geometry distributions in the cyclized products 2.3a and 2.3b obtained using the two catalysts (1.24 and 1.25) are a result of equilibration rather than of a kinetic effect, similar isomerizations were attempted under several different experimental conditions (Table 2.2).

Treatment of pure lactone (Z)-2.3b with either of Grubbs’ catalysts (1.24 or 1.25, 10 mol%) for ~ 40h in refluxing CH\(_2\)Cl\(_2\) or benzene, respectively, did not result in any isomerization of the double bond as shown by \(^1\)H NMR. Moreover, lactone (Z)-2.3b also resisted photoisomerization\(^\text{132,133}\) to the (E)-isomer in toluene solution containing thiophenol during 24-40 h of exposure to sunlight or artificial UV radiation (UV-lamp).
Table 2.2. Attempted \((Z)\rightarrow (E)\) isomerizations in 2.3b

<table>
<thead>
<tr>
<th>Reaction conditions</th>
<th>Time (hours)</th>
<th>Catalyst (10 mol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhSH / toluene / hv</td>
<td>40</td>
<td>-</td>
</tr>
<tr>
<td>CH₂Cl₂ / reflux</td>
<td>38</td>
<td>1.24</td>
</tr>
<tr>
<td>CH₂Cl₂ / reflux</td>
<td>38</td>
<td>1.25</td>
</tr>
<tr>
<td>C₆H₆ / reflux</td>
<td>42</td>
<td>1.24</td>
</tr>
<tr>
<td>C₆H₆ / reflux</td>
<td>42</td>
<td>1.25</td>
</tr>
<tr>
<td>C₆H₆ / RT / I₂</td>
<td>22</td>
<td>-</td>
</tr>
</tbody>
</table>

Scheme 2.25

In view of the strong preference (91% yield) for the formation of \((Z)-2.3b\) from acyclic precursor 2.3 under the influence of 1.25, isomerization of \((E)-2.3a\) to \((Z)-2.3b\) by the second-generation catalyst 1.25 was also examined. Surprisingly, complete isomerization of the \((E)-2.3a\) to \((Z)-2.3b\) isomer was observed within 15 min in refluxing CH₂Cl₂ with no starting material remaining in the reaction mixture as evidenced by \(^1\)H NMR. At this point there was no direct evidence which suggested that the strong preference for the formation of \((Z)-2.3b\) isomer from acyclic precursor 2.3 catalyzed by 1.25 is a result of a secondary isomerization event. Monitoring of the reaction by \(^1\)H NMR did not indicate that a detectable amount of \((E)-2.3a\) isomer was present at any time (15 min \(\rightarrow\) 48 h / CH₂Cl₂, reflux) during the reaction. Thus a possible \((E)\) to \((Z)\)-isomerization reaction would have to occur at a rate that is either higher or at least comparable to that of cyclization.
Comparative Synthetic Model Studies. In order to gain some further insight into the factors determining the stereoselectivity of the RCM reaction during the formation of 1-oxacyclodecenolides related to 1.1, the diastereomeric cyclization substrate 4.19 ([α]D = +9, c 1.0 (CHCl3)) was prepared from 2.1 and 2.2a (Scheme 2.26). The LH segment (2.2a) was prepared in a similar manner to its enantiomer 2.2 (Scheme 2.26) via asymmetric dihydroxylation (AD) of the corresponding (E)-3-decen-1-ol (4.7) using ADmix-β. The cyclization precursors 2.3 and 4.19 were indistinguishable from each other by 1H NMR (500 MHz, CDCl3).

The acyclic ester 4.19 was cyclized using the classical Grubbs catalyst 1.24 (10 mol%) under high dilution conditions (0.00056 M, CH2Cl2, reflux, 1.5 h) to yield lactone 4.19a in 96% isolated yield with complete (E)-selectivity ([α]D = +24, c 0.3 (CHCl3)). The second-generation catalyst 1.25 under the same experimental conditions delivered a chromatographically inseparable mixture of two products in a ratio of 2:1 (Table 2.3).
The two compounds could clearly be distinguished in crude reaction mixtures by the low-field resonances in their 500 MHz $^1$H NMR (CDCl$_3$) spectra.

**Table 2.3. Stereoselectivities observed during RCM of esters 2.3 and 4.19**

<table>
<thead>
<tr>
<th>Entry No.</th>
<th>Ester</th>
<th>Catalyst$^a$</th>
<th>Product</th>
<th>Yield</th>
<th>$E/Z$ ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.3</td>
<td>1.24</td>
<td>2.3a &amp; 2.3b</td>
<td>96$^b$ (147 h)</td>
<td>2:1</td>
</tr>
<tr>
<td>2</td>
<td>2.3</td>
<td>1.25</td>
<td>2.3b</td>
<td>91 (15 min)</td>
<td>0:100</td>
</tr>
<tr>
<td>3</td>
<td>4.18</td>
<td>1.24</td>
<td>4.19a</td>
<td>96 (1.5 h)</td>
<td>100:0</td>
</tr>
<tr>
<td>4</td>
<td>4.18</td>
<td>1.25</td>
<td>4.19a &amp; 4.19b</td>
<td>96 (1.5 h)</td>
<td>2:1</td>
</tr>
</tbody>
</table>

$^a$ Catalyst loading of 10 mol% was used in all cases; $^b$ Based on recovered starting material, 60% conversion.

The major compound was identified as the (E)-isomer 4.19a while the minor one was the (Z)-4.19b isomer on the basis of 1D-TOCSY data. The double bond configuration was assigned on the basis of $^3J_{H-6/H-7}$ coupling constant values {$(E)$-4.19a = 15.5 Hz, $(Z)$-4.19b = 10.2 Hz}. It is noteworthy that only the (E)-isomer of 2.3a existed as two conformers (in CDCl$_3$), whereas the other three lactones (2.3b, 4.19a and 4.19b) were found to be conformationally homogeneous at room temperature in the same NMR solvent.

![Scheme 2.27](image)
2.3 Conclusion

- The structure of the anti-microfilament agent microcarpalide (1.1) was confirmed by an enantioselective synthesis. This result validates the application of the $J$-based method of configurational analysis to stereocenters that are part of medium sized rings ($8\leq11$).

- All attempts to isomerize the double bond of the pure lactone ($Z$)-2.3b to the ($E$)-isomer 2.3a with the first generation Grubbs catalyst 1.24 (10 mol%, reflux) failed. This observation was consistent with that in the recent study by Grubbs et al.$^{115}$ where the catalyst 1.24 was shown to be incapable of facilitating secondary metathesis isomerization ($E\leftrightarrow Z$) in 14-membered lactones.

- The stereoisomeric ratio ($E/Z$) of the products observed during the RCM of the acyclic diene (2.3) with catalyst 1.24 is a result of a kinetically controlled reaction and appears to be irreversible.

- A computational study by Fürstner et al.$^{121}$ supports these conclusions. Comparison of energy minimized structures (MM3) of the acetal-protected decenolide
herbarumin I (4.18) indicated that the (Z)-lactone is ca. 3.5 kcal/mol more stable than the isomeric (E)-lactone. The authors suggest that only under kinetic control might it be possible to obtain the targeted (E)-alkenes with reasonable selectivity. Hence the (Z)-isomer 2.3b being the thermodynamic product, the preferential formation of 2.3a would confirm that the product ratio is kinetically controlled.

The exposure of the acyclic diene (2.3) to the second-generation Ru-catalyst 1.25 (10 mol%, reflux) led to the rapid (15 min) and exclusive formation of the (Z)-isomer 2.3b. Not surprisingly, complete isomerization of (E)-2.3a to (Z)-2.3b was achieved by catalyst 1.25 within 15 min in refluxing CH₂Cl₂. Indeed, a kinetic study by Grubbs et al.¹¹⁵ was in agreement with the above observation, where the catalyst 1.25 was shown to be capable of changing the initial (£/Z) ratio of macro-lactone products to give more favorable (£/Z) ratios via secondary metathesis isomerization. This in turn suggests that the choice of the metathesis catalyst is important, because one could avoid those ruthenium carbene complexes that are known to favor the retro-reaction. Moreover, treatment of the pure lactone (Z)-2.3b with catalyst 1.25 (10 mol%) for ~40 h in refluxing CH₂Cl₂ or benzene did not result in any isomerization of the double bond.

Thus the RCM reaction between 2.3 and the catalyst 1.25 could proceed by either of the two following pathways (Figure 2.1).
Figure 2.1 Energy diagrams for the progress of the RCM reaction between 2.3 and the catalyst 1.25.

In order for plot B to be true, the rate of (E) to (Z)-isomerization reaction would have to occur at a rate that is either higher or at least comparable to that of cyclization. To investigate the above possibility, two parallel reactions, the (E)-2.3a to (Z)-2.3b isomerization of the lactones and cyclization of the acyclic diene 2.3 was carried out under identical conditions (at high dilution (0.00056M), CH$_2$Cl$_2$, RT} in the presence of the catalyst 1.25 (10 mol%). The progress of the reactions was monitored by TLC. After 15 h at room temperature, no evidence of (E) to (Z) isomerization was observed. However, complete conversion of the acyclic precursor 2.3 to the (Z)-isomer 2.3b was observed under identical conditions. Moreover, no detectable amount of the (E)-isomer 2.3a could be observed (TLC) at any stage of the latter conversion. Thus, the plot A seems to hold true for the RCM reaction with catalyst 1.25, yielding the kinetically and thermodynamically favored (Z)-product 2.3b.
2.4 Experimental Section

2.4.1 General

For details regarding instrumentation and techniques employed in routine NMR and mass analysis, refer to section 1.5. For chiral HPLC analysis, a Chiralcel OD (250×4.60 mm) column was utilized. Melting points of solids were measured by a Fluke 51 II digital thermometer coupled to a Mel-Temp II device. For molecular modeling (MM3) computations and graphics, Alchemy 2000 software (Tripos Inc.) was employed. Physical models were built using Dreiding molecular models. Lithium chloride was finely ground and flame-dried under high vacuum (1.0 mm Hg) immediately before use. Trimethylsilyl chloride was distilled from calcium hydride. Reagents were added inside a glove-bag framework, when highly hydroscopic reagents such as NaBH₃CN were employed. Reflux reactions that required the use of a water aspirator were connected to a calcium chloride tube, and performed under a nitrogen atmosphere. Oxalyl chloride was freshly distilled prior to use. DMSO was distilled under vacuum at 22 mm Hg with the aid of an oil pump. Methyltriphenylphosphonium bromide was dried overnight at 60 °C employing a Kugelrohr distillation apparatus under vacuum (1.0 mm Hg). Triethylamine and pyridine were distilled over barium oxide and stored over potassium hydroxide. Molecular sieves (4 Å) were activated prior to use, by heating overnight at 200 °C under vacuum (1.0 mmHg) in a Kugelrohr distillation apparatus.
2.4.1.1 Synthetic Transformations

Sodium hydride (0.6 g, 20.0 mmol, as a 80% dispersion in mineral oil) was placed in a 250 mL round bottom flask equipped with a magnetic stirrer and was washed with anhydrous THF (4x10 mL) under a stream of N₂. At the end of this period, the flask was placed in a water bath at room temperature and the well-stirred suspension was treated with anhydrous THF (39 mL) followed by 1,4-butanediol (2.4) (1.77 mL, 20.0 mmol) dropwise (over ~10 min), during which time H₂ evolution ceased. After ~45 min to the resulting heterogeneous slurry, was added TBDMS-Cl (3.0 g, 20.0 mmol) in THF (5 mL) in one portion. After stirring for 45 min, the mixture was poured into a separatory funnel with ether and washed successively with 10% K₂CO₃ (2x50 mL), and brine, dried (MgSO₄) and concentrated to dryness in vacuo to give 4.08 g (100% yield) of pure product 2.4a as a colorless liquid: FTIR (liquid film) 3380 (br), 2360, 1640, 1470, 1390, 1255, 1100, 835, 775 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 3.65 (m, 4H), 2.61 (br t, J= 5.6 Hz, 1H), 1.68-1.61 (m, 4H), 0.90 (s, 9H), 0.07 (s, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 63.5, 62.8, 30.3, 30.0, 26.1 (3C), 18.5, -5.2 (2C); EI-MS 147 (M-tertBu)⁺, 105, 75; HREIMS calcd for (C₁₀H₂₄O₂- tertBu)⁺ 147.0841, found 147.0818.
To a vigorously stirring solution of monosilylated alcohol 2.4a (4.0 g, 19.6 mmol) in CH$_2$Cl$_2$ (24.5 mL) at 0 °C was added a 0.5 M aqueous solution of KBr (0.233 g, 1.96 mmol) followed by a CH$_2$Cl$_2$ solution of TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy) free radical (0.034 g, 0.196 mmol). To the resulting mixture was added NaOCl (5% active chlorine) portion wise (~50 mL) at pH 8.6 (the pH of NaOCl was adjusted to 8.6 prior to use by adding solid NaHCO$_3$). After the addition was complete, the solution (a deep red-orange color) was allowed to stir for an additional 4-5 min at 0 °C until the hypochlorite was consumed. The progress of the reaction was monitored by $^1$H NMR analysis of the crude organic phase. The two phase mixture was transferred to a separatory funnel and the organic layer was separated, dried (MgSO$_4$) and concentrated to dryness in vacuo to yield 3.96 g (100% yield) of the corresponding carbonyl derivative 2.5 as a colorless oil: FTIR (liquid film) 3380 (br), 2715, 1725, 1470, 1255, 1100, 1005, 835, 775 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.80 (t, J= 1.7 Hz, 1H), 3.67 (t, J= 6.1 Hz, 2H), 2.52 (td, J= 7.1, 1.7 Hz, 2H), 1.88 (br m, 2H), 0.90 (s, 9H), 0.06 (s, 6H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 208.5, 63.1, 40.9, 29.5, 26.1, 26.0, 25.8, 18.5, -5.2, -5.1; EIMS 145 (M-tertBu)$^+$, 105, 75; HREIMS caled for (C$_{10}$H$_{22}$O$_2$Si- tertBu)$^+$ 145.0685, found 145.0690.
A 500 mL round bottom flask was charged with anhydrous LiCl (0.997 g, 23.5 mmol) and dry CH₃CN (235 mL) under N₂ in a water bath at room temperature. To the resulting suspension was added dropwise, triethylphosphonoacetate (4.67 mL, 23.5 mmol) and DBU (2.93 mL, 19.6 mmol) followed by 4-siloxy aldehyde 2.5 (3.96 g, 19.6 mmol) with vigorous stirring. After 1.5 h, TLC analysis indicated complete consumption of the starting material. The reaction mixture was concentrated to dryness in vacuo and partitioned between 5% NaHCO₃ and diethyl ether. The aqueous phase was re-extracted twice more with diethyl ether. The combined organic extracts were washed successively with 5% NaHCO₃, 1N HCl and brine, dried (MgSO₄) and concentrated in vacuo and purified on a column of silica gel (hexane:EtOAc 10:1) to afford 3.57 g (91% yield) of desired (E)-isomer 2.6 as a colorless liquid: FTIR (liquid film) 1725, 1655, 1470, 1365, 1255, 1205, 1160, 1100, 1045, 835, 775 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 6.98 (ddd, J= 15.6, 7.1, 7.1 Hz, 1H), 5.82 (ddd, J= 15.6, 1.5, 1.5 Hz, 1H), 4.18 (q, J= 7.1 Hz, 2H), 3.62 (t, J= 6.1 Hz, 2H), 2.27 (m, 2H), 1.66 (m, 2H), 1.28 (t, J= 7.1 Hz, 3H), 0.88 (s, 9H), 0.04 (s, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 166.7, 149.0, 121.4, 62.1, 60.1, 31.1, 28.6, 25.9 (3C), 18.5, 14.3, -5.4 (2C); EIMS 257 (M-CH₃)⁺, 227, 215 (M- tertBu), 187, 169, 103, 95, 75; HREIMS calcd for (C₁₄H₂₉O₃Si-CH₃)⁺ 257.1573, found 257.1591.
To a well stirred two-phase mixture of tert-butyl alcohol (19 mL) and water (19 mL) was added AD-mix-β (5.35 g) at room temperature. The resulting suspension was treated with methanesulfonamide (0.363 g, 3.82 mmol), cooled to 0 °C and trans-α,β-unsaturated ester 2.6 (1.04 g, 3.82 mmol) was added at once. The heterogeneous slurry was stirred vigorously at 0 °C for 36 h and quenched by the addition of Na2SO3 (5.74 g, 45 mmol). The reaction mixture was allowed to warm to room temperature with stirring over a period of 45 min and extracted with CH2Cl2 (4×5 mL). The combined organic extracts were washed with 2N KOH, dried (MgSO4) and concentrated to dryness in vacuo. The crude product was purified on silica gel (EtOAc:hexane 2:3) to provide 1.04 g (89% yield) of diol 2.7 as a clear liquid: [α]D23 +9.3 (c 0.6, CHCl3); FTIR (film) 3450 (br), 1730, 1645, 1470, 1250, 1090, 835, 775 cm⁻¹; 1H NMR (300 MHz, CDCl3) δ 4.03 (br, 1H), 3.90 (br, 1H), 3.69-3.57 (m, 4H), 3.02 (br s, 1H), 2.88 (br s, 1H), 1.76-1.61 (m, 4H), 1.26 (t, J= 7.2 Hz, 3H), 0.86 (s, 9H), 0.03 (s, 6H); 13C NMR (75 MHz, CDCl3) δ 165.2, 73.7, 72.4, 63.3, 62.0, 31.1, 29.2, 26.1 (3C), 18.2, 14.3, -5.2 (2C); EIMS 249 (M-tertBu)+, 231, 215, 175, 145, 131, 104; HREIMS calcd for (C14H30O5Si-tertBu)+ 249.1158, found 249.1128.
A solution of diol 2.7 (1.04 g, 3.40 mmol) in dry acetone (17 mL) was treated with DMP (3.4 mL, 27.6 mmol) in the presence of a catalytic amount of PPTS (0.085 g, 0.340 mmol, 10 mol%). After allowing the reaction mixture to stir at room temperature for 4.5 h, solid powdered K₂CO₃ (0.582 g, 4.21 mmol) was added and the resulting suspension was stirred for an additional 10 min. The reaction mixture was filtered through Celite, and the filtrate was concentrated to dryness in vacuo. The crude residue was purified by flash column chromatography (silica gel, 15% EtOAc:hexane) to afford 0.95 g (81% yield) of isopropylidene derivative 2.8 as a clear liquid: [α]D²¹ +14 (c 0.6, CHCl₃); FTIR (film) 2735, 1760, 1470, 1380, 1255, 1210, 1100, 835, 775 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 4.24-4.13 (m, 2H), 4.08 (m, 2H), 3.61 (m, 2H), 1.71-1.54 (m, 4H), 1.41 (s, 3H), 1.39 (s, 3H), 1.24 (t, J= 7.3 Hz, 3H), 0.85 (s, 9H), 0.00 (s, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 166.5, 108.5, 79.5, 79.3, 62.9, 61.3, 30.2, 29.0, 27.4, 26.1 (3C), 26.0, 18.1, 14.3, -5.1 (2C); EIMS 331 (M-CH₃)⁺, 289 (M-tertBu), 231, 215, 185, 145, 89, 75; HREIMS calcd for (C₁₇H₃₄O₅Si-CH₃)⁺ 331.1941, found 331.1940.
To a well-stirred solution of 2.8 (0.95 g, 2.75 mmol) in dry THF (5 mL) at 0 °C was added dropwise an ethereal suspension of LiAlH₄ (0.21 g, 5.50 mmol) under N₂. After the addition was complete, stirring was continued for an additional 4 h at 0 °C. The reaction mixture was successively quenched with 0.105 mL of water, 0.105 mL of 15% NaOH and 0.315 mL of water, dried (MgSO₄) and filtered through Celite. The filtrate was concentrated to dryness in vacuo to give 0.763 g (91% yield) of isopropylidene alcohol 2.9 as a colorless liquid: FTIR (film) 3425 (br), 1470, 1375, 1255, 1070, 835 cm⁻¹; ^1H NMR (300 MHz, CDCl₃) δ 4.02-3.66 (m, 4H), 3.68-3.52 (m, 2H), 1.74-1.52 (m, 4H), 1.41 (s, 3H), 1.40 (s, 3H), 2.11 (br s, 1H), 0.89 (s, 9H), 0.04 (s, 6H); ^13C NMR (75 MHz, CDCl₃) δ 108.9, 81.7, 76.9, 63.1, 62.3, 29.6, 29.3, 27.6, 27.3, 26.2 (3C), 18.6, -5.1 (2C).
To a well stirred solution of oxalyl chloride (0.143 mL, 1.64 mmol) in CH₂Cl₂ (1.43 mL) at -78 °C under N₂ was added dropwise a solution of DMSO (0.188 mL, 2.63 mmol) in CH₂Cl₂ (0.87 mL) over a period of 5 min. After 15 min, a -60 °C solution of alcohol 2.9 (0.20 g, 0.66 mmol) in CH₂Cl₂ (0.70 mL) was added and the reaction mixture was allowed to slowly warm to -70 °C over a period of ~30 min. At the end of this period, triethylamine (0.458 mL, 3.29 mmol) was introduced dropwise over ~5 min and the resulting white slurry was allowed to stir for 1 h at -70 °C. The mixture was diluted with ether and filtered through Celite. The filtrate was washed successively with cold 5% KHSO₄, cold NaHCO₃ and brine, dried (MgSO₄) and evaporated to dryness in vacuo. The resulting crude aldehyde 3.0 (a yellow oil) was handled at 0 °C and used immediately, without further purification. To a stirred slurry of methyltriphenylphosphonium bromide (0.47 g, 1.32 mmol) in anhydrous THF (6.5 mL) at -78 °C under N₂ was added dropwise, n-BuLi (2.4 M solution in hexane, 0.494 mL, 1.18 mmol). The resulting bright yellow solution was allowed to stir at -78 °C for 1 h. At the end of the hour, a pre-cooled solution of aldehyde 3.0 (0.20 g, 0.66 mmol) in THF (1.06 mL) was added and the resulting pale yellow mixture was allowed to slowly return to ambient temperature overnight with stirring. The crude mixture was diluted with ether and filtered through Celite. The filtrate was washed with satd NH₄Cl, satd NaHCO₃, and brine, dried (MgSO₄) and concentrated to dryness in vacuo. The residual yellow oil was subjected to flash
chromatography (silica gel, hexanes:EtOAc 8:1) to give 0.132 g (67% yield) of the
desired terminal alkene 3.1 as a clear liquid: [α]D\textsuperscript{24} -2.2 (c 2.5, CHCl\textsubscript{3}); FTIR (liquid
film) 1470, 1380, 1255, 1170, 1095, 1055, 835, 775 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) \textsuperscript{δ}
5.76 (ddd, J= 17.4, 10.3, 7.5 Hz, 1H), 5.32 (br ddd, J= 17.4, 0.9, 0.9 Hz, 1H), 5.20 (br
ddd, J= 10.3, 0.7, 0.9 Hz, 1H), 3.95 (br dd, J= 8.1, 7.5 Hz, 1H), 3.65 (m, 1H), 3.59 (m,
2H), 1.66-1.55 (m, 4H), 1.37 (s, 3H), 1.36 (s, 3H), 0.84 (s, 9H), 0.007 (s, 6H); \textsuperscript{13}C NMR
(75 MHz, CDCl\textsubscript{3}) \textsuperscript{δ} 135.7, 118.6, 108.6, 82.9, 80.7, 63.0, 29.3, 28.4, 27.5, 27.1, 26.1(3C),
18.3, -5.1 (2C); EIMS 285 (M-CH\textsubscript{3})\textsuperscript{+}, 185, 131, 111, 98, 75; HREIMS calcd for
(C\textsubscript{16}H\textsubscript{32}O\textsubscript{3}Si-CH\textsubscript{3})\textsuperscript{+} 285.1886, found 285.1867.
A solution of **3.1** (0.36 g, 1.20 mmol) in anhydrous CH₂Cl₂ (8.74 mL) at room temperature was treated with triethylamine (18 drops) followed by TREAT·HF (0.524 mL, 3.21 mmol). The reaction mixture was allowed to stir under N₂ at room temperature for 12 h until complete conversion to the desired product **3.2**, as indicated by TLC (hexane:EtOAc 1:1). The mixture was diluted with CH₂Cl₂ and cold 5% NaHCO₃. The aqueous phase was extracted with CH₂Cl₂, washed with brine, dried (MgSO₄) and concentrated to dryness in vacuo. The crude residual oil was chromatographed on silica gel (hexane:EtOAc 4:1) to afford 0.220 g (99% yield) of pure alcohol **3.2** as a colorless liquid: [α]_D^20 = −39.6 (c 2.4, CHCl₃); FTIR (liquid film) 3405 (br), 2985, 1650, 1465, 1380, 1250, 1220, 1165, 1070, 900 cm⁻¹; ^1H NMR (300 MHz, CDCl₃) δ 5.77 (ddd, J= 17.4, 10.1, 7.3 Hz, 1H), 5.34 (br d, J= 17.4 Hz, 1H), 5.23 (br d, J= 10.1 Hz, 1H), 3.97 (dd, J= 8.1, 7.3 Hz, 1H), 3.65 (br m, 3H), 2.25-2.55 (br, 1H), 1.70-1.65 (m, 4H), 1.40 (s, 3H), 1.39 (s, 3H); ^13C NMR (75 MHz, CDCl₃) δ 135.0, 119.3, 108.7, 82.7, 80.5, 62.5, 29.3, 28.3, 27.2, 26.9; EIMS 171 (M-CH₃)⁺, 111, 98, 83; HREIMS calcd for (C₁₀H₁₈O₃·CH₃)⁺ 171.1021, found 171.1011.
A solution of primary alcohol 3.2 (0.22 g, 1.19 mmol) in anhydrous DMF (5.9 mL) under N₂, was treated with pyridinium dichromate (1.80 g, 4.77 mmol). The dark brown solution was allowed to stir at room temperature for 24 h, quenched with water and extracted with EtOAc (3 x). The combined organic layers were washed successively with water, 5% KHSO₄ and brine, dried (MgSO₄) and concentrated to dryness in vacuo to give 0.22 g (92% yield) of pure carboxylic acid 2.1 as a colorless oil: [α]D₂¹ + 9.0 (c 0.4, CHCl₃); FTIR (liquid film) 3070 (br), 1710, 1425, 1375, 1240, 1165, 1070, 935, 870, 810 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 5.73 (ddd, J = 17.3, 10.3, 7.3 Hz, 1H), 5.31 (br d, J = 17.3 Hz, 1H), 5.20 (br d, J = 10.3 Hz, 1H), 3.95 (dd, J = 7.9, 7.3 Hz, 1H), 3.64 (ddd, J = 8.2, 7.9, 3.5 Hz, 1H), 2.43 (m, 2H), 1.98-1.50 (m, 2H), 1.34 (s, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 179.0, 135.1, 119.7, 109.2, 82.7, 79.6, 30.6, 27.4, 27.1, 26.7; EIMS 185 (M-CH₃)+, 125, 98, 83; HREIMS calcd for (C₁₀H₁₆O₄-CH₃)+ 185.0814, found 185.0818.
To a mixture of malonic acid (46.83 g, 450 mmol) and piperidine (0.015 mL, 0.15 mmol) in xylene (120 mL) at reflux was added octyl aldehyde 4.4 (23.46 mL, 150 mmol) dropwise over a period of 1 h. After 2 h, additional malonic acid (150 mmol) was added and the mixture was allowed to reflux overnight, cooled to room temperature and extracted with EtOAc (3x). The combined organic layers were washed with 5% NaHSO₃, water and brine, dried (MgSO₄) and concentrated to dryness in vacuo. The resulting crude residual oil was purified by Kugelrohr distillation (bp 120-130 °C/4.0 mm Hg) to give 19.2 g (75% yield) of the desired carboxylic acid 4.5 as a clear liquid: FTIR (liquid film) 3035 (br), 1715, 1455, 1415, 1290, 1220, 965 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 11.00-12.02 (br s, 1H), 5.59 (dtt, J= 15.4, 6.5, 1.2 Hz, 1H), 5.51 (dtt, J= 15.4, 6.5, 1.2 Hz, 1H), 3.07 (ddt, J= 6.7, 1.2 Hz, 2H), 2.03 (tddt, J= 6.7, 6.5, 1.2, 1.2 Hz, 2H), 1.27 (m, 8H), 0.88 (t, J= 6.7 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 178.0, 135.6, 120.9, 37.9, 32.6, 31.9, 29.3, 29.0, 22.8, 14.2; ElMS 170 (M)+, 152, 134, 123, 110, 100, 84; HREIMS caleld for (C₁₀H₁₈O₂)⁺ 170.1307, found 170.1260.
A stirred solution of 4.5 (8.0 g, 47 mmol) in dry absolute EtOH (230 mL) under N$_2$ with stirring was treated with TMS-Cl (13.14 mL, 103 mmol). The reaction mixture was stirred at room temperature for 12 h and the solvent was removed under reduced pressure. The resulting crude residue was diluted with EtOAc and washed with cold 5% NaHCO$_3$, dried (MgSO$_4$) and concentrated to dryness in vacuo to give 9.32 g (100% yield) of $\beta,\gamma$-unsaturated ethyl ester 4.6 as a colorless oil: FTIR (liquid film) 1735, 1460, 1370, 1160, 1030, 965 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 5.53 (m, 2H), 4.13 (q, J= 7.1 Hz, 2H), 3.01 (ddd, J= 5.6, 1.0, 1.0 Hz, 2H), 2.01 (m, 2H), 1.34-1.23 (m, 11H), 0.87 (t, J= 6.8 Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 173.2, 134.9, 121.7, 60.6, 38.4, 32.7, 32.0, 29.3, 29.0, 22.8, 14.4, 14.2; EIMS 198 (M$^+$), 152, 123, 110, 96, 88; HREIMS calcd for (C$_{12}$H$_{22}$O$_2$)$^+$ 198.1620, found 198.1669.
To a well-stirred solution of ester 4.6 (9.3 g, 47 mmol) in dry THF (81 mL) at 0 °C was added dropwise a ethereal suspension of LiAlH₄ (3.57 g, 94 mmol) under N₂. After the addition was complete, stirring was continued for an additional 5 h at 0 °C. The reaction mixture was successively quenched with 1.76 mL of water, 1.76 mL of 15% NaOH and 5.30 mL of water, dried (MgSO₄) and filtered through Celite. The filtrate was concentrated to dryness in vacuo to give 7.33 g (100% yield) of the desired alcohol 4.7 as a colorless liquid: FTIR (liquid film) 3380 (br), 1650, 1460, 1050, 965 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 5.49 (dtt, J= 15.2, 6.6, 1.1 Hz, 1H), 5.30 (dtt, J= 15.2, 7.0, 1.3 Hz, 1H), 3.58 (br m, 2H), 2.19 (ddd, J= 13.2, 7.0, 1.1 Hz, 2H), 1.95 (ddd, J= 13.2, 6.6, 1.3 Hz, 2H), 1.37-1.14 (m, 8H), 0.82 (t, J= 6.8 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 134.7, 125.9, 62.2, 36.2, 32.9, 31.9, 29.7, 29.1, 22.8, 14.3; EIMS 156 (M)⁺, 138, 110, 95, 81, 68; HREIMS calcd for (C₁₀H₂O)⁺ 156.1514, found 156.1523.
To a solution of the homoallylic alcohol 4.7 (2.0 g, 12.8 mmol), 4-methoxyphenol (4.77 g, 38 mmol) and PPh₃ (4.36 g, 16.6 mmol) in dry THF (42 ml) was added dropwise diethyl azodicarboxylate (3.28 mL, 16.6 mmol) at 23 °C. The reaction mixture was heated at reflux for 4 h under N₂, cooled to room temperature and concentrated to dryness in vacuo. The crude residue was subjected to flash chromatography (2% EtOAc:hexane) to afford 3.2 g (95% yield) of pure p-anisyl ether 4.8 as a colorless oil: FTIR (liquid film) 1590, 1510, 1465, 1440, 1385, 1290, 1230, 1180, 1105, 1045, 970, 825 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 6.84 (m, 4H), 5.57 (dt, J= 15.4, 6.6 Hz, 1H), 5.48 (dt, J= 15.4, 6.0 Hz, 1H), 3.92 (t, J= 6.8 Hz, 2H), 3.77 (s, 3H), 2.46 (td, J= 6.8, 6.6 Hz, 2H), 2.02 (td, J= 6.6, 6.6 Hz, 2H), 1.39-1.28 (m, 10H), 0.89 (t, J= 6.6 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 154.1, 153.4, 133.3, 125.7, 115.9, 115.8, 114.9 (2C), 68.9, 55.9, 36.9, 32.8, 32.0, 29.6, 29.3, 22.8, 14.2; EIMS 262 (M)⁺, 236, 137, 124, 109, 97, 83; HREIMS calcd for (C₁₇H₂₀O₂)⁺ 262.1933, found 262.1939.
To a well-stirred two phase mixture of tert-butyl alcohol (30 mL) and water (30 mL) was added AD-mix-α (8.44 g) at room temperature. The resulting suspension was treated with methanesulfonamide (0.573 g, 6.02 mmol), cooled to 0 °C and homoallylic 4-methoxyphenyl ether 4.8 (1.58 g, 6.03 mmol) was added at once. The heterogeneous slurry was stirred vigorously at 0 °C for 24 h and quenched by the addition of Na₂SO₃ (9.0 g, 71 mmol). The reaction mixture was allowed to warm to room temperature with stirring over a period of 45 min, and extracted with CH₂Cl₂ (4×5 mL). The combined organic extracts were washed with 2N KOH, dried (MgSO₄) and concentrated to dryness in vacuo. The crude product was purified on silica gel (EtOAc:hexane 2:3) to provide 1.50 g (84% yield) of diol 4.9 as a white crystalline solid of >99% ee (chiral HPLC): mp 63-65 °C; [α]D²³ -11 (c 2.3, CHCl₃); FTIR (film) 3370 (br), 1645, 1510, 1465, 1380, 1290, 1230, 1110, 1040, 815 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 6.86-6.82 (m, 4H), 4.03 (dd, J= 7.2, 5.5 Hz, 2H), 3.70 (s, 3H), 3.54 (ddd, J= 9.2, 4.7, 3.4 Hz, 1H), 3.33 (ddd, J= 8.4, 4.7, 3.5 Hz, 1H), 2.89 (d, J= 5.7 Hz, 1H), 2.76 (d, J= 5.7 Hz, 1H), 1.88 (dddd, J= 14.3, 7.2, 7.2, 3.4 Hz, 1H), 1.75 (ddddd, J= 14.3, 9.2, 5.5, 5.5 Hz, 1H), 1.49-1.39 (m, 2H), 1.38-1.28 (m, 8H), 0.88 (t, J= 7.0 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 154.2, 152.9, 115.7 (2C), 114.9 (2C), 74.8, 72.5, 66.4, 55.9, 33.7, 33.4, 32.0, 29.6, 25.9, 22.9, 14.3; EIMS 296 (M)⁺, 278, 262, 180, 163, 124, 109; HREIMS calcd for (C₁₇H₂₈O₄)⁺ 296.1988,
found 296.2022; HPLC (chiral) Chiralcel OD at 23 °C, λ = 280 nm, 15% hexanes-2-propanol, retention times 7.95 (3R,4R), 10.03 min (3S,4S) at 1 mL/min flow rate.

Diol 4.9 (1.49 g, 5.03 mmol) was dissolved in anhydrous pyridine (3.05 mL) and cooled to 0 °C under N₂ with stirring. Acetic anhydride (3.09 mL, 33 mmol) was added portionwise followed by a catalytic amount of DMAP (0.061 g, 0.50 mmol, 10 mol%) and the mixture was allowed to stir for 11 h at 0 °C; the progress of the reaction was monitored by TLC, hexane:EtOAc 3:1. The reaction was quenched with ice, stirred for an additional 10 min and poured into water (25 mL). The aqueous phase was extracted with diethyl ether (3×25 mL). The combined extracts were washed successively with water (3×25 mL), cold 1N HCl and brine, dried (MgSO₄) and concentrated to dryness in vacuo to afford 1.85 g (97% yield) of 4.10 as a colorless oil: [α]₀₂⁵ +31 (c 0.9, CHCl₃); FTIR (liquid film) 3045, 1740, 1730, 1590, 1510, 1465, 1440, 1370, 1290, 1230, 1110, 1040, 950, 825 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 6.81 (m, 4H), 5.26 (ddd, J = 7.6, 5.2, 4.3 Hz, 1H), 5.06 (ddd, J = 6.6, 6.6, 4.3 Hz, 1H), 3.94 (ddd, J = 9.4, 6.1, 6.1 Hz, 1H), 3.76 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.02-1.98 (m, 2H), 1.58-1.53 (m, 2H), 1.26-1.24 (m, 8H), 0.87 (t, J = 6.7 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 170.5, 170.3, 154.0, 152.8, 115.7 (2C), 114.7 (2C), 73.9, 71.2, 64.9, 55.7, 51.7, 30.8, 30.6, 29.3, 25.2, 22.6, 20.9, 20.8, 14.0; EIMS 380 (M)⁺, 257, 197, 155, 124, 81; HREIMS calcd for (C₂₁H₃₂O₆)⁺ 380.2199, found 380.2179.
To an ice-cold solution of 4.10 (1.91 g, 5.03 mmol) in CH₃CN-H₂O (4:1, v/v; 62 mL) was added ceric ammonium nitrate (CAN) (6.89 g, 12.6 mmol) in one portion. The reaction mixture was stirred at 0 °C for 45 min and partitioned between diethyl ether and satd NaHCO₃. The aqueous phase was re-extracted twice more with diethyl ether and the combined organic layers were washed (5% NaHCO₃, brine), dried (MgSO₄), and concentrated to dryness in vacuo. The crude residue was chromatographed on silica gel (CH₂Cl₂, hexane:EtOAc 3:2) to give 1.37 g (100% yield) of alcohol 4.11 as a colorless liquid: [α]_D^{26} +34 (c 0.4, CHCl₃); FTIR (liquid film) 3450 (br), 1735, 1720, 1460, 1430, 1370, 1225, 1020, 955 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 5.14 (ddd, J= 10.1, 4.1, 3.5 Hz, 1H), 5.02 (ddd, J= 7.9, 5.6, 4.1 Hz, 1H), 3.69-3.65 (m, 1H), 3.54-3.47 (m, 1H), 2.28 (br s, 1H), 2.08 (s, 3H), 2.07 (s, 3H), 1.85-1.74 (m, 2H), 1.85-1.63 (m, 2H), 1.26 (br m, 8H), 0.87 (t, J= 6.7, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 171.6, 170.5, 74.1, 71.2, 58.2, 33.9, 31.7, 30.7, 29.3, 25.1, 22.6, 20.9, 20.8, 14.0.
To a stirred solution of **4.11** (1.37 g, 5.03 mmol) in CH$_3$OH (9.0 mL) at room temperature was added conc. NH$_4$OH (9.0 mL) in one portion. After the end of the second hour, additional conc. NH$_4$OH (9.0 mL) was added and stirring was continued overnight until complete conversion to the triol product, as indicated by TLC (neat EtOAc). The reaction mixture was concentrated to dryness in vacuo and purified by flash column chromatography (silica gel, hexane:EtOAc 1:1, EtOAc). The resulting crystalline residue was recrystallized (hexane) to give 0.78 g (82% yield) of pure triol **4.12** as a white crystalline solid: mp 58-59 °C; [α]$_D^{26}$ +37 (c 0.4, CH$_3$OH); FTIR (film) 3220 (br), 1460, 1340, 1180, 1140, 1070, 1025, 940 cm$^{-1}$; $^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 3.74-3.67 (m, 2H), 3.56 (ddd, J= 9.0, 4.4, 4.4 Hz, 1H), 3.36 (ddd, J= 8.3, 4.4, 4.4 Hz, 1H), 1.74-1.61 (m, 2H), 1.53-1.39 (m, 2H), 1.34-1.27 (m, 8H), 0.87 (t, J= 6.9 Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 74.6, 73.8, 60.6, 35.3, 32.0, 29.8, 29.4, 25.9, 22.8, 14.2; EIMS 115 (M-75)$^+$, 97, 87, 75, 69; HREIMS calcd for (C$_{10}$H$_{22}$O$_3$-75)$^+$ 115.1569, found 115.1113.
A solution of triol 4.12 (0.78 g, 4.12 mmol) in anhydrous DMF (8.25 mL) at room temperature under N₂ was treated with 4-methoxybenzaldehyde dimethyl acetal (1.06 mL, 6.19 mmol) in the presence of a catalytic amount of TsOH (0.036 g, 0.21 mmol, 5 mol%). The reaction mixture was allowed to stir for 2.5 h at reflux, cooled to ambient temperature and concentrated under reduced pressure. The resulting residue was dissolved in ether, washed with satd. cold NaHCO₃ and brine, dried (MgSO₄) and concentrated to dryness in vacuo. Analysis of the residual oil (TLC, hexane:EtOAc 3:2) showed formation of the desired six-membered acetal 4.14 along with the undesired five-membered acetal 4.13. The two products were separated by column chromatography to yield the six-membered acetal 4.14 (20% EtOAc:hexane + 1% triethylamine) and the five-membered acetal 4.13 (30% EtOAc:hexane + 1% triethylamine) in the ratio of 2:1 (6-mem/5-mem) yielding 0.85 g (67% yield) of the desired product 4.14 as a colorless liquid: 4.14 [α]D²³ -1.7 (c 1.8, CHCl₃); FTIR (liquid film) 3460 (br), 1615, 1515, 1465, 1395, 1305, 1250, 1175, 1105, 1035, 830, 800 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.41 (m, 2H), 6.89 (m, 2H), 5.48 (s, 1H), 4.28 (ddd, J= 11.4, 5.1, 0.9 Hz, 1H), 3.94 (ddd, J= 12.0, 11.4, 2.7 Hz, 1H), 3.80 (s, 3H), 3.72 (ddd, J= 11.4, 6.6, 2.7 Hz, 1H), 3.60-3.53 (m, 1H), 1.90 (ddddd, J= 12.5, 12.0, 11.4, 5.1 Hz, 1H), 1.51-1.45 (m, 3H), 1.34-1.25 (m, 8H), 0.88 (t, J= 6.6 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 160.1, 131.1, 127.6 (2C), 113.8
A stirred solution of the five-membered acetal 4.13 (1.02 g, 3.33 mmol) in dry toluene (16.3 mL) under N₂ was re-exposed to a catalytic amount of TsOH (0.006 g, 0.03 mmol, 1 mol%) for 20 h at room temperature. The crude mixture was neutralized with satd. cold NaHCO₃ and extracted with EtOAc (3x). The combined organic layers were washed with 5% cold NaHCO₃ and brine, dried (MgSO₄) and concentrated to dryness in vacuo and subjected to flash chromatography (silica gel, hexane:EtOAc 20%, 30% with 1% triethylamine) to afford 0.67 g (65% yield) of the desired six-membered acetal 4.14 as a clear liquid.
A well stirred solution of 4.14 (0.92 g, 2.98 mmol) in anhydrous CH₂Cl₂ (6 mL) at room temperature was treated with triethylamine (2.0 mL, 14 mmol) followed by DMAP (0.36 g, 2.98 mmol). The reaction mixture was cooled to 0 °C and TBDMS-Cl (1.98 g, 13 mmol) was added in one portion and allowed to stir under N₂ at 0 °C—room temperature for 24 h until complete conversion to the desired product 4.15, as indicated by TLC (hexane:EtOAc 4:1). The mixture was diluted with ether and washed with cold water, 5% cold KHSO₄, 5% cold NaHCO₃, and brine, dried (MgSO₄) and concentrated to dryness in vacuo. The crude residual oil was chromatographed on silica gel (hexane:EtOAc 6:1 + 1% triethylamine) to afford 1.17 g (93% yield) of the corresponding monosilylated derivative 4.15 as a clear liquid: [α]D24~ -10 (c 1.5, CHCl₃); FTIR (liquid film) 1615, 1515, 1465, 1395, 1300, 1250, 1170, 1115, 1035, 830, 775 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 7.40 (d, J= 8.8 Hz, 2H), 6.87 (d, J= 8.8 Hz, 2H), 5.43 (s, 1H), 4.26 (ddd, J= 11.2, 4.8, 0.9 Hz, 1H), 3.91 (ddd, J= 11.7, 11.2, 2.7 Hz, 1H), 3.80 (s, 3H), 3.77 (ddd, J= 11.3, 5.7, 2.3 Hz, 1H), 3.73 (ddd, J= 9.0, 5.7, 3.4 Hz, 1H), 1.84 (ddddd, J= 12.3, 11.7, 11.3, 4.8 Hz, 1H), 1.58-1.36 (m, 3H), 1.317-1.24 (m, 8H), 0.88 (t, J= 6.7 Hz, 3H), 0.86 (s, 9H), 0.05 (s, 3H), 0.03 (s, 3H), -0.02 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 160.1, 131.6, 127.8 (2C), 113.7 (2C), 101.8, 80.7, 74.3, 67.4, 55.5, 32.2, 32.1, 29.8, 26.2 (3C), 26.0, 25.8, 22.9, 18.4, 14.4, -4.0, -4.5; EIMS 422 (M)+, 381(M-tertBu),
365, 227, 199, 171, 121, 75; HREIMS calcd for (C₂₄H₄₂O₄Si)⁺ 422.2852, found 422.2809.

To a well-stirred solution of 4.15 (1.17 g, 2.77 mmol) in dry toluene (55 mL) at −78 °C was added dropwise a 1.0 M solution of DIBAL-H (28 mL, 28 mmol) in hexane under N₂. After the addition was complete, the stirring was continued for an additional 12 h at −78 °C. The mixture was quenched with MeOH (48 mL) and successively treated with satd NH₄Cl (40 mL) and 1.0 M NaK tartrate (40 mL). The resulting gel was extracted with CH₂Cl₂, dried (MgSO₄) and concentrated to dryness in vacuo to give 0.60 g (51% yield) of the desired p-methoxyphenyl ether derivative 4.16 as a clear oil. [α]D° +34 (c 0.5, CHCl₃); FTIR (liquid film) 3425 (br), 2065, 1615, 1585, 1515, 1470, 1385, 1360, 1300, 1255, 1175, 1070, 835, 775, 665 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 7.25 (d, J= 8.7 Hz, 1H), 6.88 (d, J= 8.7 Hz, 1H), 4.55 (d, J= 11.26 Hz, 1H), 4.46 (d, J= 11.26 Hz, 1H), 3.81 (s, 3H), 3.82 (m, 2H), 3.70 (br m, 2H), 3.51 (ddd, J= 8.5, 4.7, 4.3 Hz, 1H), 2.56 (t, J= 5.9 Hz, 1H), 1.87 (ddddd, J= 14.6, 7.5, 4.7, 2.5 Hz, 1H), 1.70 (ddddd, J= 14.6, 8.5, 4.4, 2.3 Hz, 1H), 1.62-1.57 (m, 2H), 1.47-1.21 (m, 8H), 0.88 (m, 12H), 0.05 (s, 3H), 0.03 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 159.3, 130.3, 129.4 (2C), 113.8 (2C), 81.3, 72.1, 71.6, 61.3, 55.3, 31.8, 31.4, 29.4, 26.2, 25.8 (4C), 22.6, 18.0, 14.1, -4.4, -4.7; EIMS
To a well-stirred solution of oxalyl chloride (0.11 mL, 1.25 mmol) in CH$_2$Cl$_2$ (1.09 mL) at -78 °C under N$_2$ was added dropwise a solution of DMSO (0.14 mL, 2.0 mmol) in CH$_2$Cl$_2$ (0.65 mL) over a period of 5 min. After 15 min, a -60 °C solution of alcohol 4.16 (0.21 g, 0.5 mmol) in CH$_2$Cl$_2$ (0.55 mL) was added and the reaction mixture was allowed to slowly warm to -70 °C over a period of ~30 min. At the end of this period, triethylamine (0.35 mL, 2.5 mmol) was introduced dropwise over ~5 min and the resulting white slurry was allowed to stir for 1 h at -70 °C. The mixture was diluted with ether and filtered through Celite. The filtrate was washed successively with cold 5% KHSO$_4$, cold NaHCO$_3$, and brine, dried (MgSO$_4$) and evaporated to dryness in vacuo. The resulting crude aldehyde 4.17 (a yellow oil) was handled at 0 °C and used immediately without further purification. To a stirred slurry of methyltriphenylphosphonium bromide (0.357 g, 1.0 mmol) in anhydrous THF (5 mL) at -78 °C under N$_2$ was added dropwise n-BuLi (2.4 M solution in hexane, 0.36 mL, 0.9 mmol). The resulting bright yellow solution was allowed to stir at -78 °C for 1 h. At the end of the hour, a pre-cooled solution of aldehyde 4.17 (0.21 g, 0.5 mmol) in THF (0.831 mL) was added and the resulting pale yellow mixture was allowed to slowly return to
ambient temperature overnight with stirring. The crude mixture was diluted with ether and filtered through Celite. The filtrate was washed with satd NH₄Cl, satd NaHCO₃, and brine, dried (MgSO₄), and concentrated to dryness in vacuo. The residual yellow oil was subjected to flash chromatography (silica gel, hexane:EtOAc 8:1) to give 0.174 g (83% yield) of the desired terminal alkene 4.18 as a clear liquid. [α]D 20 —22 (c 0.7, CHCl₃); FTIR (liquid film) 3075, 1640, 1615, 1585, 1515, 1465, 1440, 1300, 1250, 1175, 1085, 1040, 910, 835, 775 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.26 (d, J= 6.8 Hz, 2H), 6.88 (d, J= 8.6 Hz, 2H), 5.89 (dddd, J= 17.1, 14.1, 7.0, 7.0 Hz, 1H), 5.09 (br m, 1H), 5.02 (br m, 1H), 4.50 (s, 2H), 3.82 (s, 3H), 3.72 (br ddd, J= 8.1, 4.4, 4.4 Hz, 1H), 3.36 (ddd, J= 8.8, 4.4, 3.1 Hz, 1H), 2.39 (br m, 2H), 2.18 (br m, 2H), 1.35-1.22 (m, 8H), 0.88 (br m, 12H), 0.03 (s, 3H), 0.007 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 137.0, 131.3, 129.6 (2C), 116.3, 113.9 (2C), 82.0, 72.7, 72.2, 55.5, 33.8, 32.1, 31.3, 29.7, 26.3, 26.1 (3C), 22.9, 18.5, 14.4, -4.5, -4.3; EIMS 363 (M-tertBu)+ 293, 277, 262, 243, 229, 121; HREIMS calcd for (C₂₃H₄₄O₃Si-tertBu)+ 363.2355, found 363.2337.
To an ice-cold solution of p-methoxybenzyl ether derivative 4.18 (0.174 g, 0.41 mmol) in CH₂Cl₂-H₂O (18:1, v/v; 5.15 mL) was added DDQ (0.11 g, 0.5 mmol) in one portion. The reaction mixture was stirred at 0 °C for 3 h and partitioned between CH₂Cl₂ and cold 5% NaHCO₃. The aqueous phase was re-extracted twice more with CH₂Cl₂ and the combined organic layers were washed (5% cold NaHCO₃, brine) dried (MgSO₄) and concentrated to dryness in vacuo. The crude residue was chromatographed on silica gel (MTBE:hexane 1:6.5) to give 0.11 g (92% yield) of alcohol 2.2 as a colorless liquid: [α]D²³ +3 (c 0.8, CHCl₃); FTIR (liquid film) 3470 (br), 3075, 1640, 1470, 1385, 1360, 1255, 1080, 835, 775 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ; 5.78 (dddd, J= 17.1, 14.1, 7.0, 7.0 Hz, 1H), 5.04 (m, 2H), 3.46 (m, 2H), 2.17-2.12 (m, 4H), 1.56-1.16 (m, 8H), 0.83 (s, 9H), 0.80 (t, J= 6.8 Hz, 3H), 0.01 (s, 3H), 0.007 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 135.6, 117.2, 74.5, 72.3, 38.8, 33.9, 32.0, 29.7, 26.1 (3H), 25.3, 22.8, 14.3, -3.9, -4.4; EIMS 285 (M-CH₃)⁺ 259, 243 (M-tertBu), 229, 189, 105, 95, 75; HREIMS calcd for (C₁₇H₃₆O₂Si-CH₃)⁺ 285.2250, found 285.2259.
To a well stirred solution of alcohol 2.2 (0.017 g, 0.087 mmol), and acid 2.1 (0.020 g, 0.067 mmol) in dry CH$_2$Cl$_2$ (0.45 mL) at 0 °C under N$_2$ was added DMAP (0.008 g, 0.067 mmol) followed by EDCI-HCl (0.04 g, 0.13 mmol) in one portion. The resulting mixture was allowed to return to ambient temperature over a period of 3-4 h, and continued to stir at this temperature for 3 days. The reaction mixture was diluted with ether, washed with cold satd NH$_4$Cl, cold 5% NaHCO$_3$ and brine, dried (MgSO$_4$) and concentrated to dryness in vacuo. The crude product was purified by flash column chromatography (silica gel, CH$_2$Cl$_2$:hexane 1:2, 1:1) to afford 0.022 g (69% yield) of pure ester 2.3 as a colorless oil: $[\alpha]_D^{24}$ -4 (c 1.0, CHCl$_3$); FTIR (liquid film) 3080, 1735, 1645, 1470, 1375, 1255, 1165, 1070, 985, 930, 835, 775 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 5.74 (ddd, $J$ = 17.1, 10.3, 7.5 Hz, 1H), 5.65 (m, 1H), 5.31 (ddd, $J$ = 17.1, 1.3, 0.9 Hz, 1H), 5.20 (ddd, $J$ = 10.3, 1.3, 0.7 Hz, 1H), 5.00 (m, 2H), 4.82 (ddd, $J$ = 9.5, 4.4, 3.5 Hz, 1H), 3.39 (dd, $J$ = 8.3, 7.5 Hz, 1H), 3.62 (m, 2H), 2.51-2.29 (m, 3H), 2.16 (m, 1H), 1.85 (m, 1H), 1.74 (m, 1H), 1.35 (s, 3H), 1.34 (s, 3H), 1.21-1.95 (m, 10H), 0.83 (s, 9H), 0.82 (br t, 3H), 0.01 (s, 3H), 0.007 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 172.8, 135.2, 134.8, 119.5, 117.4, 109.0, 82.7, 79.8, 75.5, 72.2, 33.6, 32.2, 32.0, 31.1, 29.7, 27.4, 27.2, 27.1, 26.0 (3C), 25.6, 22.8, 18.3, 14.3, -4.2 (2C); EIMS 482 (M)$^+$, 467 (M-CH$_3$)$^+$, 425
Following the above procedure for EDCI-HCl promoted condensation, alcohol 2.2a (0.020 g, 0.067 mmol) with acid 2.1 (0.017 g, 0.087 mmol) after 3 days afforded 0.026 g (83% yield) of pure ester 4.19 as a colorless oil: [α]_D^22 +9 (c 1.0, CHCl_3); FTIR (liquid film) 3080, 1735, 1645, 1470, 1375, 1255, 1165, 1070, 985, 930, 835, 775 cm⁻¹; 

¹H NMR (300 MHz, CDCl_3) δ 5.74 (ddd, J = 17.1, 10.3, 7.5 Hz, 1H), 5.65 (m, 1H), 5.31 (ddd, J = 17.1, 1.3, 0.9 Hz, 1H), 5.20 (ddd, J = 10.3, 1.3, 0.7 Hz, 1H), 5.00 (m, 2H), 4.82 (ddd, J = 9.5, 4.4, 3.5 Hz, 1H), 3.39 (dd, J = 8.3, 7.5 Hz, 1H), 3.62 (m, 2H), 2.51-2.29 (m, 3H), 2.16 (m, 1H), 1.94 (m, 1H), 1.88 (m, 1H), 1.35 (s, 3H), 1.34 (s, 3H), 1.21-1.95 (m, 10H), 0.83 (s, 9H), 0.82 (br t, 3H), 0.01 (s, 3H), 0.007 (s, 3H); 

¹³C NMR (75 MHz, CDCl_3) δ 172.9, 135.2, 134.9, 119.5, 117.4, 109.0, 82.8, 79.7, 75.5, 72.2, 33.6, 32.2, 32.0, 31.1, 29.7, 27.4, 27.1, 27.0, 26.0 (3C), 25.7, 22.8, 18.2, 14.3, -4.3 (2C); EIMS 482 (M)⁺, 467 (M-CH₃)⁺, 425 (M-tBu)⁺, 397, 367, 339, 317, 299, 199, 125, 98; HREIMS calcd for (C₂₇H₅₀O₅Si)⁺ 482.3427, found 482.3418.
Macrocyclization via ring closing Metathesis (RCM); representative procedure.

To a solution of Grubbs' catalyst (0.0017 g, 0.002 mmol, 10 mol%) in degassed anhydrous CH₂Cl₂ (25 mL) at reflux was added a solution of acyclic ester (0.010 g, 0.021 mmol) in degassed CH₂Cl₂ (12 mL) via a cannula over a period of ~45 min. The reaction mixture was allowed to reflux for 1.5-134 h (progress of the reaction was monitored by a combination of TLC and ¹H-NMR). The mixture was allowed to return to room temperature and the solvent was removed in vacuo. The residue was purified by flash column chromatography (silica gel, EtOAc:hexane) to afford the cyclized product(s) in 91-96% yield.

Following the general procedure for the preparation of macrocycles via ring closing metathesis, ruthenium carbene 1.24 with 2.3 ester (0.012 g, 0.025 mmol) in refluxing CH₂Cl₂, after 147 h afforded 0.01 g (96% yield) of the desired 10-membered lactone 2.3a/b as a mixture of (E) and (Z) isomers (E/Z = 2:1). Clear liquid; [α]ᵢ²⁵ \text{D} –38 (c 0.3, CHCl₃); FTIR (liquid film) 2360, 1735, 1470, 1365, 1255, 1165, 1065, 975, 835, 775 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 5.64 (ddd, J = 15.4, 11.2, 4.6 Hz, 1H), 5.25 (dd, J = 15.4, 9.2 Hz, 1H), 4.68 (ddd, J = 8.6, 5.1, 2.4 Hz, 1H), 3.84 (dd, J = 9.2, 8.8 Hz, 1H), 3.65 (ddd, J = 7.0, 5.1, 5.1 Hz, 1H), 3.57 (m, 1H), 2.52 (ddd, J = 12.5, 11.2, 8.6 Hz, 1H), 2.45
Following the general procedure for ring closing metathesis, Ru-catalyst 1.25 with 2.3 ester (0.0185 g, 0.038 mmol) in refluxing CH₂Cl₂, after 15 min led to the rapid exclusive cyclization to desired (Z)-isomer 2.3b yielding 0.016 g (91% yield). Clear liquid; [α]D^25 +21 (c 0.8, CHCl₃); FTIR (liquid film) 1735, 1660, 1470, 1365, 1240, 1180, 1055, 890, 835, 775 cm⁻¹; ^1H NMR (500 MHz, CDCl₃) δ 5.74 (ddd, J= 10.6, 10.6, 7.3 Hz, 1H), 5.49 (dd, J= 10.6, 9.0 Hz, 1H), 4.92 (ddd, J= 11.8, 4.4, 2.4 Hz, 1H), 4.49 (dd, J= 9.0, 9.0 Hz, 1H), 3.71 (m, 1H), 3.65 (m, 1H), 2.65 (ddd, J= 17.3, 6.0, 2.6 Hz, 1H), 2.60 (ddd, J= 12.8, 11.8, 10.6 Hz, 1H), 2.34 (ddd, J= 17.3, 13.0, 2.7 Hz, 1H), 2.18 (m, 1H), 2.11 (ddd, J= 12.8, 7.3, 2.4 Hz, 1H), 2.07 (m, 1H), 1.54-1.40 (m, 2H), 1.39 (s, 3H), 1.37 (s, 3H), 1.34-1.25 (m, 8H), 0.89 (s, 9H), 0.88 (t, J= 6.7 Hz, 3H), 0.06 (s, 3H), 0.05 (s, 3H); ^13C NMR (75 MHz, CDCl₃) δ 171.0, 131.0, 130.7, 107.9, 81.8, 77.2, 74.2, 73.2, 33.8, 32.4, 32.0, 29.7, 29.5, 28.3, 27.3, 27.2, 26.0 (3C), 25.1, 22.8, 18.2, 14.3, -4.0, -4.4;
Following the general procedure for macrocyclization via ring closing metathesis, Ru-carbene 1.24 with ester 4.19 (0.012 g, 0.025 mmol) in refluxing CH$_2$Cl$_2$, after 1.5 h afforded 0.011 g (96% yield) of the desired 10-membered macrolide 4.19a in a stereoisomerically pure (E)-form. Clear liquid: [α]$_D^{22}$ +24 (c 0.3, CHCl$_3$); FTIR (liquid film) 1735, 1670, 1470, 1365, 1255, 1180, 1150, 1165, 975, 835, 775 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) δ 5.70 (ddd, J= 15.4, 11.2, 4.4 Hz, 1H), 5.26 (dd, J= 15.4, 9.4 Hz, 1H), 4.90 (ddd, J= 11.2, 6.2, 3.7 Hz, 1H), 3.91 (dd, J= 9.4, 8.6 Hz, 1H), 3.68 (m, 1H), 3.51 (m, 1H), 2.46 (m, 1H), 2.33 (ddd, 12.1, 4.4, 3.7 Hz, 1H), 2.17-1.96 (m, 4H), 1.49-1.41 (m, 2H), 1.38 (s, 3H), 1.37 (s, 3H), 1.35-1.16 (m, 8H), 0.85 (brt, 3H), 0.84 (s, 9H), 0.03 (s, 3H), 0.01 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 174.4, 135.6, 129.5, 108.2, 82.7, 82.4, 76.4, 73.4, 35.8, 33.5, 32.5, 32.0, 29.7, 27.4, 27.3, 27.2, 26.0 (3C), 24.8, 22.8, 18.4, 14.3, -4.2 (2C); EIMS 454 (M)$^+$, 439, 397, 379, 339, 321, 295, 279, 247, 229, 201, 149, 110; HREIMS calcd for (C$_{25}$H$_{46}$O$_5$Si)$^+$ 454.3115, found 454.3120.
Following the general procedure for ring closing metathesis, Ru-catalyst 1.25 with 4.19 ester (0.0185 g, 0.038 mmol) in refluxing CH$_2$Cl$_2$, after 1.5 h led to an inseparable mixture of (E:Z)-isomers 4.19a and 4.19b in the ratio of 2:1 respectively. Clear liquid; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 5.71 (ddd, J= 10.5, 10.2, 7.0 Hz, 1H), 5.59 (dd, J= 10.2, 9.4 Hz, 1H), 4.85 (ddd, J= 8.8, 5.4, 1.5 Hz, 1H), 4.21 (dd, J= 9.4, 8.7 Hz, 1H), 3.89 (ddd, J= 8.7, 4.2, 2.8 Hz, 1H), 3.73 (ddd, J= 8.2, 5.4, 3.8 Hz, 1H), 2.75 (ddd, J= 15.0, 7.5, 4.2, 2.0 Hz, 1H), 2.67 (ddd, J= 16.8, 11.8, 2.0 Hz, 1H), 2.40 (ddd, J= 16.8, 7.5, 2.5 Hz, 1H), 2.22 (ddd, J= 15.0, 11.8, 2.8 Hz, 1H), 2.13 (m, 1H), 2.02 (m, 1H), 1.42 (s, 3H), 1.41 (s, 3H), 1.37-1.21 (m, 10H), 0.88 (brt, 3H), 0.86 (s, 9H), 0.05 (s, 6H).

Following the general macrocyclic RCM procedure, (E)-isomer of 10-membered lactone 2.3a (0.002 g, 0.004 mmol) when re-exposed to Ru-catalyst 1.25 (10 mol%) in refluxing CH$_2$Cl$_2$, after 15 min, following flash chromatography (silica gel, hexane:EtOAc 10:1) afforded 0.019 g (98% yield) of desired isomerized product, (Z)-isomer 2.3b.
A solution of 2.3a (0.008 g, 0.018 mmol) in anhydrous CH$_2$Cl$_2$ (0.615 mL) at room temperature was treated with triethylamine (4 drops) followed by TREAT-HF (0.038 mL, 0.23 mmol). The reaction mixture was allowed to stir under N$_2$ at room temperature for 3 days until complete conversion to the desired product, as indicated by TLC (hexane:EtOAc 1:1). The mixture was diluted with ether and washed with cold 5% NaHCO$_3$ and brine, dried (MgSO$_4$) and concentrated to dryness in vacuo. The crude residual oil was chromatographed on silica gel (hexane:EtOAc 4:1) to afford 0.006 g (99% yield) of pure alcohol as a white solid: [α]$_D$$^{22}$ -16 (c 0.86, CHCl$_3$); FTIR (liquid film) 3455 (br), 1735, 1460, 1370, 1240, 1055, 800 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 5.84 (ddd, J= 15.8, 8.0, 8.0 Hz, 1H), 5.53 (dd, J= 15.8, 8.4 Hz, 1H), 4.92 (m, 1H), 3.97 (dd, J= 8.4, 8.4 Hz, 1H), 3.87 (ddd, J= 8.5, 4.3, 4.3 Hz, 1H), 3.63 (br m, 1H), 2.53-2.11 (m, 6H), 1.72 (m, 2H), 1.51-1.45 (br s, 1H), 1.40 (s, 3H), 1.39 (s, 3H), 1.35-1.25 (m, 8H), 0.87 (t, J= 6.7 Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 172.6, 132.1, 127.7, 108.0, 82.7, 81.4, 74.1, 69.7, 38.0, 32.5, 31.9, 31.6, 29.7 (2C), 29.0, 26.9, 25.1, 22.5, 14.1.
A white solid; \([\alpha]_D^{22} +24\) (c 0.2, CHCl3); FTIR (film) 3485, 1735, 1440, 1365, 1240, 1180, 1155, 1060, 975, 860, 800, 755 cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl3) \(\delta\) 5.73 (ddd, \(J = 15.5, 11.1, 4.6\) Hz, 1H), 5.32 (dd, \(J = 15.5, 9.4\) Hz, 1H), 4.93 (ddd, \(J = 11.3, 8.1, 4.0\) Hz, 1H), 3.94 (dd, \(J = 9.4, 8.5\) Hz, 1H), 3.64 (br m, 1H), 3.53 (ddd, \(J = 9.9, 8.5, 2.8\) Hz, 1H), 2.52 (ddd, \(J = 14.1, 7.4, 2.0\) Hz, 1H), 2.37 (ddd, 12.1, 4.6, 4.0 Hz, 1H), 2.29 (ddd, \(J = 12.1, 11.3, 1.1\) Hz, 1H), 2.18 (m, 2H), 2.12 (m, 1H), 1.69 (d, \(J = 5.9\) Hz, 1H), 1.49-1.47 (m, 2H), 1.41 (s, 3H), 1.40 (s, 3H), 1.30-1.24 (m, 8H), 0.87 (t, \(J = 6.8\) Hz, 3H); \(^{13}\)C NMR (75 MHz, CDCl3) \(\delta\) 171.8, 135.3, 129.7, 108.3, 82.6, 82.4, 76.8, 72.8, 36.3, 34.0, 32.4, 31.9, 29.4, 27.4, 27.2 (2C), 25.5, 22.8, 14.3; EIMS 340 (M\(^+\)), 325 (M-CH\(_3\))\(^+\), 265, 238, 198, 180, 123, 110, 85; HREIMS calcd for (C\(_{19}\)H\(_{32}\)O\(_5\))\(^+\) 340.2249, found 340.2249.

A solution of the acetonide (0.006 g, 0.018 mmol) in 1N HCl-THF (1:1, v/v, 0.6 mL) was stirred at room temperature for 4.5 h. The progress of the reaction was monitored by TLC (hexane:EtOAc 2:1). At the end of this period, the reaction mixture
was neutralized with cold satd NaHCO₃ and extracted with EtOAc (3×2 mL). The combined organic layers were concentrated to dryness in vacuo to give 0.005 g (100% yield) of desired triol 1.1 as a white solid: [α]₀²²° = -23 (c 0.12, CH₂OH); FTIR (film) 3380 (br), 1710, 1435, 1365, 1260, 1225, 1155, 1070, 1020, 800 cm⁻¹; ¹H NMR (300 MHz, CD₃CN) δ same as natural product microcarpalide; ¹³C NMR (125 MHz, CDCl₃) δ 176.4, 134.4, 126.6, 79.5, 73.7, 72.8, 72.3, 36.6, 34.1, 32.5, 29.9, 29.1, 26.1, 23.3, 14.3.

Colorless liquid: FTIR (film) 3425 (br), 2360, 1720, 1655, 1550, 1410, 1260, 1090, 1020, 800 cm⁻¹; ¹H NMR (300 MHz, CD₃CN) δ 5.51 (ddd, J= 15.4, 10.3, 5.0 Hz, 1H), 5.24 (dd, J= 15.4, 9.6 Hz, 1H), 4.77 (ddd, J= 11.4, 4.7, 3.7 Hz, 1H), 3.56 (ddd, J= 10.2, 9.0, 3.3 Hz, 1H), 3.56-3.50 (m, 1H), 3.53 (dd, J= 9.6, 9.0 Hz, 1H), 2.43 (ddd, J= 12.0, 10.2, 2.1 Hz, 1H), 2.24-2.10 (m, 5H), 1.41-1.37 (m, 2H), 1.36-1.26 (m, 8H), 0.87 (t, J= 6.9 Hz, 3H).
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(1) Pearce, C. In Advances in Applied Microbiology, 1997, 44, 1-80.

(2) Stierle, A. A.; Stierle, D. B. Studies in Natural Products Chemistry 2000, 24 (Bioactive Natural Products (Part E)), 33-977.


(4) Taxonomic identification of strain 112/13 has so far been unsuccessful. The strain has been deposited in the UH Chemistry Department’s culture collection under the accession number 112/13.


(12) The use of PPTS for acid-catalyzed reactions has been quite successful and is particularly useful when TsOH is too strong an acid given the functionality in a particular substrate.


(19) Application of Murata analysis to diol 1.1 was thought to yield inconclusive results as a consequence of the existence of several conformers of 1.1 in common NMR solvents (Table 1.1).

(21) The HETLOC pulse sequence could not be applied successfully. (The HETLOC pulse sequence relies on a TOCSY magnetization transfer, which is more efficiently measured in more strongly coupled systems).

(22) H-8\textsuperscript{h} and H-8\textsuperscript{l} indicates the H-8 proton resonating at high and low field, respectively.


(39) During OsO₄ oxidation of allyl alcohols and their derivatives, the relative stereochemistry between the preexisting hydroxyl or alkoxy group and the adjacent newly introduced hydroxyl group of the major product in all cases is erythro.


(82) The use of PPTS for acid-catalyzed reactions has been quite successful and is particularly useful when \( p\)-TsOH is too strong an acid for the functionality in a given substrate.


(92) The Lewis acid (BF\(_3\)·OEt\(_2\)) should not be added before the completion of formation of (3.9). Otherwise, the added BF\(_3\) will promote intramolecular transacetalization to yield the rearranged product.


(116) Note that cycloalkenes of 12 ring atoms can be selectively formed by ring-closing alkyne metathesis followed by semi-reduction. Rings with 8-11 ring atoms, however, are hardly accessible by this method due to ring strain at the


(130) The $^1$H NMR resonances due to H-9 of the (E)-isomer and those for H-6 and H-5 of the (Z)-isomer are baseline separated at 300 MHz from all other resonances in crude reaction mixtures. (E/Z)-ratios were determined by repeated, careful integration.


Appendix: NMR Spectra

400 MHz $^1$H NMR spectrum of microcarpalside (1.1) in CD$_3$CN
100 MHz $^1$H NMR spectrum of microcarpalide (1.1) in CD$_3$CN
400 MHz $^1$H NMR spectrum of microcarpalide (1.1) in CD$_3$OD
FTIR spectrum (liquid film) of microcarpalide (1.1)
500 MHz $^1$H NMR spectrum of the ketal derivative (1.7) in CDCl$_3$
125 MHz $^{13}$C NMR spectrum of the ketal derivative (1.7) in CDCl$_3$
CD spectrum of the dibenzoate derivative (1.9) in CH$_3$CN
500 MHz $^1$H NMR spectrum of the eleven-membered lactone (1.8) in CDCl$_3$
Variable temperature NMR experiment on microcarpalide 1.1 (in CD$_3$CN, 500 MHz NMR)
(15 °C → 45 °C → 75 °C)
300 MHz $^1$H NMR spectrum of the acyclic diene (2,3) in CDCl$_3$
300 MHz $^1$H NMR spectrum of the (E)-isomer (2.3a) in CDCl$_3$
300 MHz $^1$H NMR spectrum of the (Z)-isomer (2.3b) in CDCl$_3$
300 MHz $^1$H NMR spectrum of the (E)-isomer (4.19a) in CDCl$_3$
500 MHz $^1$H NMR spectrum of the (E/Z)-isomers (4.19a) and (4.19b) in CDC$_6$ (E:Z = 2:1)
300 MHz $^1$H NMR spectrum of falcarniol (1.12) in CDCl$_3$
100 MHz $^{13}$C NMR spectrum of falcarindiol (1.12) in CDCl$_3$
500 MHz $^1$H NMR spectrum of crithmumdiol (1.21) in CDCl$_3$
500 MHz $^1$H NMR spectrum of the epoxy-derivative (1.20) in CDCl$_3$
500 MHz $^1$H NMR spectrum of the symmetrical diol (3S,8S)-1.26 in CDCl$_3$
125 MHz $^{13}$C NMR spectrum of the symmetrical diol (3S,8S)-1.26 in CDCl$_3$
Assignment of (S)-configuration to (+)-1.28 using (S)-MTPA (Mosher) derivative
Δδ values (Δδ = δ_S - δ_R, 500 MHz, CDCl₃)

(S)-MTPA (Mosher) derivative

(R)-MTPA (Mosher) derivative
Determination of the enantiomeric excess:
The (S)-MTPA (Mosher) derivative of 1.28 (300 MHz, CDCl₃)
Natural Product

(meso)-1.26
retention time (min) - 16.28 (R,S)

Synthetic (±)-1.26
retention times (min) - 13.90 (R,R)
16.27 (R,S)
19.33 (S,S)

Synthetic (+)-(S,S)-1.26
retention time (min) - 19.22 (S,S)

HPLC (chiral) Chiralcel OD (250×4.6 mm)
23 °C, λ = 254 nm, 15% iPrOH-hexane, 1 mL/min