Syntheses of Chorismate Analogs for Investigation of Structural Requirements for Chorismate Mutase

by

Chang-Chung Cheng

B.S., Department of Chemistry
National Chung-Hsing University
1986

Submitted to the Department of Chemistry
in Partial Fulfillment
of the Requirements for the Degree of

Doctor of Philosophy in
Organic Chemistry

at the

Massachusetts Institute of Technology

May, 1994

Signature of Author.....
Department of Chemistry
May 18, 1994

Certified by.....
Glenn A. Berchtold
Thesis Supervisor

Accepted by.....
Glenn A. Berchtold
Chairman, Departmental Committee
on Graduate Studies

This doctoral thesis has been examined by a Committee of the Department of Chemistry as Follows:

Professor Frederick D. Greene, II.....
Chairman

Professor Glenn A. Berchtold.....
Thesis Supervisor

Professor Julius Rebek, Jr.....

Foreword

Chang-Chung (Cliff) Cheng's Ph. D. thesis is a special thesis for me because of our association with the individual who supervised his undergraduate research and since Cliff will be the last student to receive the Ph. D. from me during my career at M.I.T..

Cliff received the B.S. degree in Chemistry from National Chung-Hsing University, Taichung, Taiwan in June 1986, and he enrolled in our doctoral program in September 1989. During his undergraduate career Cliff was engaged in research under the supervision of Professor Teng-Kuei Yang. Professor Yang also received his undergraduate training in chemistry at National Chung-Hsing University before coming to the United States to complete the Ph. D. in organic chemistry with Professor Hart at Ohio State University. After completion of his doctoral work, Professor Yang came to my laboratory as a Postdoctoral Research Associate in September 1983. After a year in my laboratory, Professor Yang returned to National Chung-Hsing University as a member of the faculty.

While he was in my research group, Professor Yang was involved in various studies associated with my interest in the chorismate pathway - an interest that began during the time when I was on sabbatical leave at the ETH in Zürich. It is befitting that Cliff's doctoral research completes the final investigations of the chorismate pathway from my laboratory.

Cliff and his undergraduate mentor, Professor Yang, are good friends; and it is a pleasure to have been associated with them as members of my research group and on social occasions. Both have introduced me to some magnificent Oriental cuisine in the Boston area.

May 10, 1994

Glenn A. Berchtold
Cambridge, Massachusetts

Syntheses of Chorismate Analogs for Investigation of Structural Requirements for Chorismate Mutase

by

Chang-Chung Cheng

Submitted to the Department of Chemistry on May 18, 1994 in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Organic Chemistry

Abstract

The syntheses of 3-[[1-(carboxy)ethenyl]oxy]-1-cyclopentene-1-carboxylic acid (**51**) and *trans*-3-[[1-(carboxy)ethenyl]oxy]-4-hydroxyl-cyclopentene-1-carboxylic acid (**52**) are reported. The thermal rearrangement of **51** and **52** was studied (**51**: $k_{30\ °C} = 2.4\times10^{-6}$ sec^{-1}; $t_{1/2} = 79$ h. **52**: $k_{30\ °C} = 7.1\times10^{-8}$ sec^{-1}; $t_{1/2} = 2710$ h). Compound **51** was a moderate substrate for chorismate mutase from *Bacillus subtilis*; the rearrangement is accelerated by a factor of 1.8×10^{3} ($K_m = 158$ μM; $k_{cat} = 4.2\times10^{-3}$ sec^{-1}). Compound **52** was an excellent substrate for chorismate mutase from *Bacillus subtilis*; the rearrangement is accelerated by a factor of 6.5×10^{6} ($K_m = 275$ μM; $k_{cat} = 0.46$ sec^{-1}). Analogs **51** and **52** were competitive inhibitors of chorismate mutase (**51**: $K_i = 27.5$ μM with K_m(chorismate) = 85.4 μM; **52**: $K_i = 71$ μM with K_m(chorismate) = 103 μM).

Detailed investigations of synthetic procedures to prepare the 6-aza-5,6-dihydro analog of chorismate (**80**) and the 7-aza analog of chorismate (**82**) were undertaken in order to acquire additional information on the structural effects on catalysis of the Claisen rearrangement by chorismate mutase. During these studies a new class of reagents, methyl N-substituted-2-aminoacrylates (**124**) in which the substituent was trifluoroacetyl, tosyl, or mesyl, was developed; and their utility in the Mitsunobu reaction to replace the hydroxyl group by the amido functionality of the reagent was demonstrated.

Attempted syntheses of (±)-5-carboxychorismate, **162**, with the ultimate goal of preparation of one or both of the pure enantiomers for investigations with isochorismate synthase are described.

Thesis Supervisor: Glenn A. Berchtold

Title: Professor of Chemistry

Acknowledgements

I would like to express my deepest appreciation to Professor Glenn Berchtold. His patience, guidance, and support throughout my years at M.I.T. have made the attainment of this goal possible. I will fondly remember Glenn's sense of humor and warm friendship, both in the lab and on the infamous group lunches and dinners, which helped to make my time at M.I.T. very enjoyable. I also enjoyed our coffee time of today at which we talked about not only science but world economy and politics as well. I am also grateful to his patience towards correcting this non-English manuscript and I feel very lucky to have worked with him.

I warmly acknowledge the help and friendship of all my colleagues: Dr. Tim Frigo for his help during my early days at M.I.T., my first baymate, Dr. Nina Quinn, and Dr. Gary Breton for their longtime friendship. They are the ones that represent the true meaning cooperation. To Professor Fred Greene, for his marvelous course on the mechanisms of chemical reactions.

The many hours I spent with other members of M.I.T. community, playing softball for Toxic Waste and volleyball with Danheiser's group, were also a special source of pleasure which I will always treasure. Thank to members of ROCSA/MIT for having made me feel closer to home.

I also have to thank the members of Spec Lab staff, especially Jim Simms and Jeanne Owens, for their friendship and expert assistance.

A final note of thanks must be given to my parents and my wife, whose pride in my accomplishments and support throughout the years has made all the work worthwhile. This thesis is dedicated to all of you. Thank you.

Table of Contents

Forword....2

Abstract....3

Acknowledgments....4

Table of Contents....5

Chapter 1: The Shikimate Pathway and the Biosynthesis of Enterobactin....13

Introduction....14

References and Notes....29

Chapter 2: Investigations of the Enzyme Chorismate Mutase (Part I): [3,3] Rearrangement of Chorismate to Prephenate....34

Introduction....35

Results and Discussion....46

Synthesis of 3-[[1-(Carboxy)ethenyl]oxy]-1-cyclopentene-1-carboxylic Acid (**51**)....46

Synthesis of *trans*-3-[[1-(Carboxy)ethenyl]oxy]-4-hydroxy-cyclopentene-1-carboxylic Acid (**52**)....48

Thermal Rearrangement of Dimethyl Esters **60** and **68**....51

NOE Experiments with Dimethyl Esters **60** and **68**....58

Thermal Rearrangements of **51** and **52**....60

Enzymatic Studies of **51** and **52** with Chorismate Mutase....62

Experimental....66

Methyl 2-Hydroxycyclopentane-1-carboxylate (**53**)....67

Methyl 2-Cyclopentene-1-carboxylate (**54**)....67

Methyl 6-Oxabicyclo[3.1.0]hexane-2-carboxylate (**56**)....68

Methyl (1α,5α)-6-Oxabicyclo[3.1.0]hexane-1-carboxylate (**58**)....69

Methyl 3-Hydroxy-1-cyclopentene-1-carboxylate (**57**).....69

Methyl 3-[1-(Methoxycarbonyl)-1-(dimethylphosphono)methoxy]-1-cyclopentene-1-carboxylate (**59**).....70

Methyl 3-[[1-(Methoxycarbonyl)ethenyl]oxy]-1-cyclopentene-1-carboxylate (**60**).....71

3-[[1-(Carboxy)ethenyl]oxy]-1-cyclopentene-1-carboxylic Acid (**51**).....72

Methyl 1-[2-(Methoxycarbonyl)-2-oxoethyl]-2-cyclopentene-1-carboxylate (**69**).....73

1-[2-(Carboxyl)-2-oxoethyl]-2-cyclopentene-1-carboxylic Acid (**77**).....74

Methyl 6-Oxabicyclo[3.1.0]-2-hexene-3-carboxylate (**62**)...74

Methyl *trans*-3-Acetoxy-4-hydroxy-1-cyclopentene-1-carboxylate (**63**).....76

Methyl *trans*-3-Acetoxy-4-trimethylsilyloxy-1-cyclopenten-1-carboxylate (**64**).....76

Methyl *trans*-3-Hydroxy-4-trimethylsilyloxy-1-cyclopentene-1-carboxylate (**65**).....77

Methyl *trans*-3-[1-(Methoxycarbonyl)-1-(dimethylphosphono)methoxy]-4-triethylsilyloxy-1-cyclopentene-1-carboxylate (**66**).....78

Methyl *trans*-3-[[1-(methoxycarbonyl)ethenyl]oxy]-4-triethylsilyloxy-1-cyclopentene-1-carboxylate (**67**).....79

Methyl *trans*-3-[[1-(methoxycarbonyl)ethenyl]-4-hydroxy]-1-cyclopentene-1-carboxylate (**68**).....80

NOE Experiments with **60** and **68**.....81

trans-3-[[1-(Carboxy)ethenyl]oxy]-4-hydroxy-cyclopentene-1-carboxylic Acid (**52**).....82

Methyl 4β-Hydroxy-1α-[2-(Methoxycarbonyl)-2-oxoethyl]-2-cyclopentene-1β-carboxylate (**70**).....82

4β-Hydroxy-1α-[2-(carboxyl)-2-oxoethyl]-2-cyclopentene-1β-carboxylic Acid (**76**).....83

Kinetics of Thermal [3,3] Rearrangement of **51** and **52**.....84

General Assay Procedure for Chorismate Mutase.....84

References and Notes.....86

Chapter 3: Investigations of the Enzyme Chorismate Mutase (Part II): [3,3] Rearrangement of Chorismate to Prephenate.....89

Introduction.....90

Results and Discussion.....92

Attempted Synthesis of Tetrahydropyridine Analog **80**.....92

Attempted Synthesis of α,β-Unsaturated Amino Acid Chorismate Analog **82**.....97

Experimental.....105

2,4-Pentadienol (**90**).....105

N-Hydroxymethyl-2,4-pentadienyl Carbamate (**92**).....105

N-Acetoxymethyl-2,4-pentadienyl.Carbamate.(**93**).....106

1-Aza-9-oxo-8-oxabicyclo[4.3.0]non-4-ene (**87**).....107

1-Methyl-2-hydroxymethyl-1,2,3,6-tetrahydropyridine (**96**).....108

1-Benzoyl-2-hydroxymethyl-1,2,3,6-tetrahydropyridine (**97**).....109

N-4-Butenylbenzylamine (**102**).....109

Methyl cis-4-Hydroxy-2-piperidinecarboxylate (**104**).....110

Methyl 1-Benzyl-*cis*-4-hydroxy-2-piperidinecarboxylate (**106**).....110

Methyl 1-Benzyl-*cis*-[1-(Methoxycarbonyl)-1-(dimethylphosphono)methoxy]-2-piperidinecarboxylate (**107**).....111

Methyl 1-Benzyl-*cis*-4-[[1-(Methoxycarbonyl)ethenyl]oxy]-2-piperidinecarboxylate (**108**).....112

Methyl (1β,2β,6β)-2-[N-*t*-(Butoxycarbonyl)-N-(*p*-toluenesulfonyl)amino]-7-oxabicyclo[4.1.0]hept-3-ene-4-carboxylate (**115**).....113

Methyl (1β,2β,6β)-2-*p*-Toluenesulphonylamido-7-oxabicyclo[4.1.0]hept-3-ene-4-carboxylate (**116**).....114

Methyl (1α,5α,6α)-5-Hydroxy-9-(*p*-toluenesulfonyl)-8-oxo-9-aza-7-oxabicyclo[4.3.0]non-2-ene-3-carboxylate (**117**).....114

Methyl 3-*p*-Toluenesulphonylamido-1,4-cyclohexadiene-1-carboxylate (**118**).....115

Methyl 3-[N-*t*-([*p*-butoxycarbonyl)-N-(p-toluenesulfonyl)amino]-1,4-cyclohexadiene-1-carboxylate (**119**).....116

Methyl (1α,5α,6α)-5-(Methanesulfonyloxy)-9-(*p*-toluenesulfonyl)-8-oxo-9-aza-7-oxabicyclo[4.3.0]non-2-ene-3-carboxylate (**120**).....117

Methyl (1α,5α,6α)-5-(*p*-Toluenesulfonyloxy)-9-(*p*-toluenesulfonyl)-8-oxo-9-aza-7-oxabicyclo[4.3.0]non-2-ene-3-carboxylate (**121**).....117

Trimethyl 2-(Toluenesulfonylamido)phosphonoacetate (**126**, R_2 = Ts).....118

Trimethyl 2-(Methanesulfonylamido)phosphonoacetate (**126**, R_2 = Ms).....119

Trimethyl 2-(Trifluoroacetamido)phosphonoacetate (**126**, R_2 = CF_3CO).....120

N-[1-(Methoxycarbonyl)ethenyl]-*p*-toluenesulfonylamide (**124**, R_2 = Ts).....120

N-[1-(Methoxycarbonyl)ethenyl]methanesulfonamide (**124**, R_2 = Ms).....122

N-[1-(Methoxycarbonyl)ethenyl]trifluoroacetamide (**124**, R_2 = CF_3CO).....124

Methyl (1β,2β,6β)-2-[N-(*p*-Toluenesulphonyl)[1-(methoxycarbonyl)ethenyl]amino]-7-oxabicyclo[4.1.0]hept-3-ene-4-carboxylate (**128**).....126

Methyl (1β,2β,6β)-2-[N-Trifluoroacetyl[1-(methoxycarbonyl)ethenyl]amino]-7-oxabicyclo[4.1.0]hept-3-ene-4-carboxylate (**129**).....127

Methyl (3β,4α,5β)-3-[N-(*p*-Toluenesulphonyl)[1-(methoxycarbonyl)ethenyl]amino]-4-hydroxy-5-bromocyclohex-1-ene-1-carboxylate (**133**).....128

Methyl (3β,4α,5β)-3-[N-(*p*-Toluenesulphonyl)[1-(methoxycarbonyl)ethenyl]amino]-4-hydroxy-5-iodocyclohex-1-ene-1-carboxylate (**134**).....128

Methyl 3α,4α-Dihydroxy-5β-phenylselenyl-1-cyclohexene-1-carboxylate (**138**).....129

Methyl *cis*-4-Hydroxy-3-triethylsilyloxy-1,5-cyclohexadiene-1- carboxylate (**140**).....130

Methyl (1β,4α,5α,6β)-4-Triethylsilyloxy-5-hydroxy-7-Oxabicyclo[4.1.0]hep-2-ene-2-carboxylate (**141**).....131

References and Notes.....133

Chapter 4: Investigations of the Enzyme Isochorismate Synthase: Conversion of Chorismate to Isochorismate.....135

Introduction.....136

Results and Discussion.....146

Attempted Synthesis of (±)-5-Carboxylatechorismate **162**.....146

Experimental.....152

Methyl 5-[1-(Methoxycarbonyl)-1-(dimethylphosphono)methoxy]-1,3-cyclohexadiene-1-carboxylate (**166**).....152

Methyl (1β,5β,6β)-5-[1-(Methoxycarbonyl)-1-(dimethylphosphono)methoxy]-7-oxabicyclo[4.1.0]hept-2-ene-3-carboxylate (**167**).....152

Methyl (1β,5β,6β)-5-[[1-(Methoxycarbonyl)ethenyl]oxy]-7-oxabicyclo[4.1.0]hept-2-ene-3-carboxylate (**168**).....153

Methyl (3β,4α,5β)-3-Cyano-4-hydroxy-5-[[1-(methoxycarbonyl)ethenyl]oxy]-1-cyclohexene-1-carboxylate (**169**).....154

Methyl (3β,4α,5β)-3-[[1-(Methoxycarbonyl)ethenyl]oxy]-4-hydroxy-5-cyano-1-cyclohexene-1-carboxylate (**176**)....155

(1α,6β,10α)-10-Cyano-8-methoxycarbonyl-4-methylene-2,5-dioxabicyclo[4.4.0]-dec-7-en-3-one (**177**).....156

Methyl 2-Oxo-3-bromo-2*H*-pyran-5-carboxylate (**179**).....157

Methyl (1α,2β,5β,6α)-5-Bromo-7,9,10-trioxa-11-oxotricyclo[4.3.0.$2^{2,5}$]undec-3-ene-3-carboxylate (**181**)....157

References and Notes.....159

Appendix: Selected NMR Spectra.....161

Figure 1: ^{1}H NMR of Compound **60**.....162

Figure 2: Enlargement at 4.5 - 5.5 ppm of ^{1}H NMR of Compound **60**.....163

Figure 3: ^{1}H NMR Decoupling of H_c of Compound **60**....164

Figure 4: Enlargement at 4.7 - 6.8 ppm of ^{1}H NMR of Decoupling H_c Compound **60**.....165

Figure 5: ^{1}H NMR of Compound **68**.....166

Figure 6: Enlargement at 4.4 - 5.6 ppm of ^{1}H NMR of Compound **68**.....167

Figure 7: ^{1}H NMR Decoupling of H_c of Compound **68**....168

Figure 8: Enlargement at 4.5 - 6.7 ppm of ^{1}H NMR of Decoupling H_c Compound **68**.....169

Figure 9: ^{1}H NMR of Compound **69**.....170

Figure 10: ^{1}H NMR of Compound **70**.....171

Figure 11: ^{1}H NMR of Compound **87**.....172

Figure 12: HETCOR of Compound **87**.....173

Figure 13: ^{1}H NMR of Compound **104**.....174

Figure 14: ^{1}H NMR of Compound **108**.....175

Figure 15: ^{1}H NMR of Compound **128**.....176

Figure 16: ^{1}H NMR of Compound **129**.....177

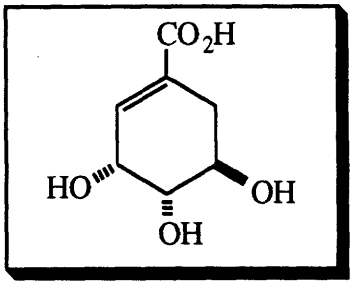
Figure 17: ^{1}H NMR of Compound **169**.....178

Figure 11: ^{1}H NMR of Compound **176**.....179

To My Parents
for Their Endless Love and Moral Support
and To My Lovely Wife
Cheng-Ping

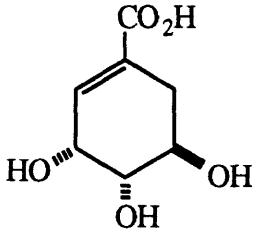
Chapter 1

The Shikimate Pathway and the Biosynthesis of Enterobactin



Introduction

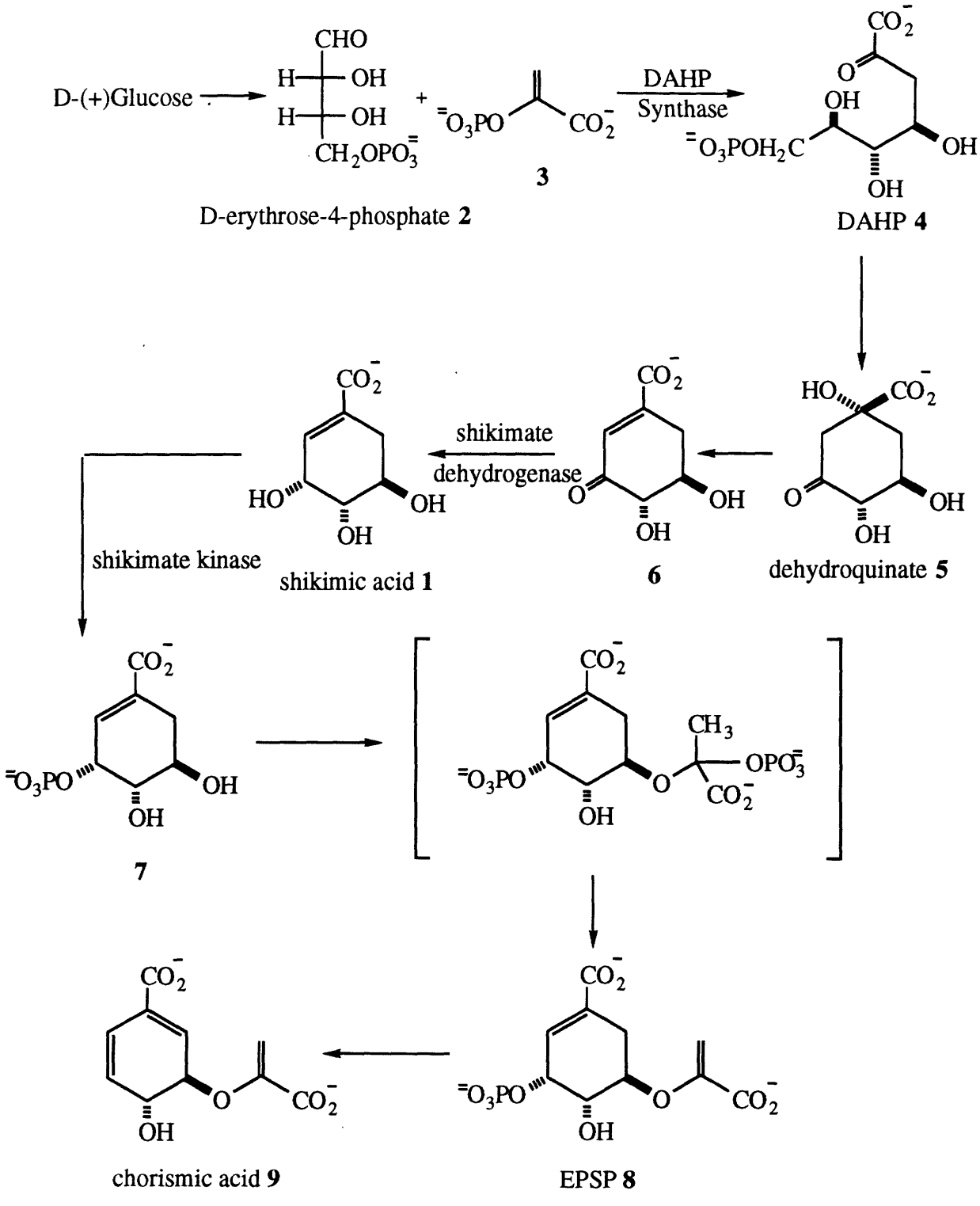
The shikimate pathway is one of the primary biosynthetic pathways for the synthesis of aromatic compounds from acyclic precursors in bacteria, plants, and fungi.[1] Shikimic acid (**1**) was first isolated in 1885 by Eijkman from the oriental plant Illicium religiosum.[2] The complete structure and absolute stereochemistry of **1** was determined in 1934.[3] Racemic **1** was resolved by Smissman in 1959.[4] Syntheses of (±)-**1** have been reported by a number groups.[5] Synthesis of (-)-**1** from (-) quinic acid,[6] D-mannose,[7] D-arabinose,[8] D-lyxose,[9] and by differentiation of the hydroxyl functions of (-)-**1**,[10] as well as synthesis of (-)-**1** by Berchtold and Pawlak from a kinetically resolved precursor[11] have been reported. In addition, all major intermediates in the biosynthesis of **1** have been isolated and characterized, and all of the enzymes involved in the numerous transformations have been identified and studied.[1,2]



shikimic acid **1**

The biosynthesis of **1** and its transformation to chorismic acid (**9**) is shown in Scheme 1. Phosphoenolpyruvate (**3**) is biosynthesized from D-glucose in one segment and is combined with D-erythrose-4-phosphate (**2**) to give 3-deoxy-D-arabinoheptulosonic acid 7-phosphate (DAHP, **4**). This reaction is an aldol type condensation, and is catalyzed by DAHP synthase. Transformation of **4** into 3-dehydroquinate (**5**) is catalyzed by a single enzyme 3-dehydroquinate synthase. Dehydration of **5** gives **6**. Reduction of **6** by shikimate dehydrogenase and NADPH results in the formation of shikimic acid.

Scheme 1

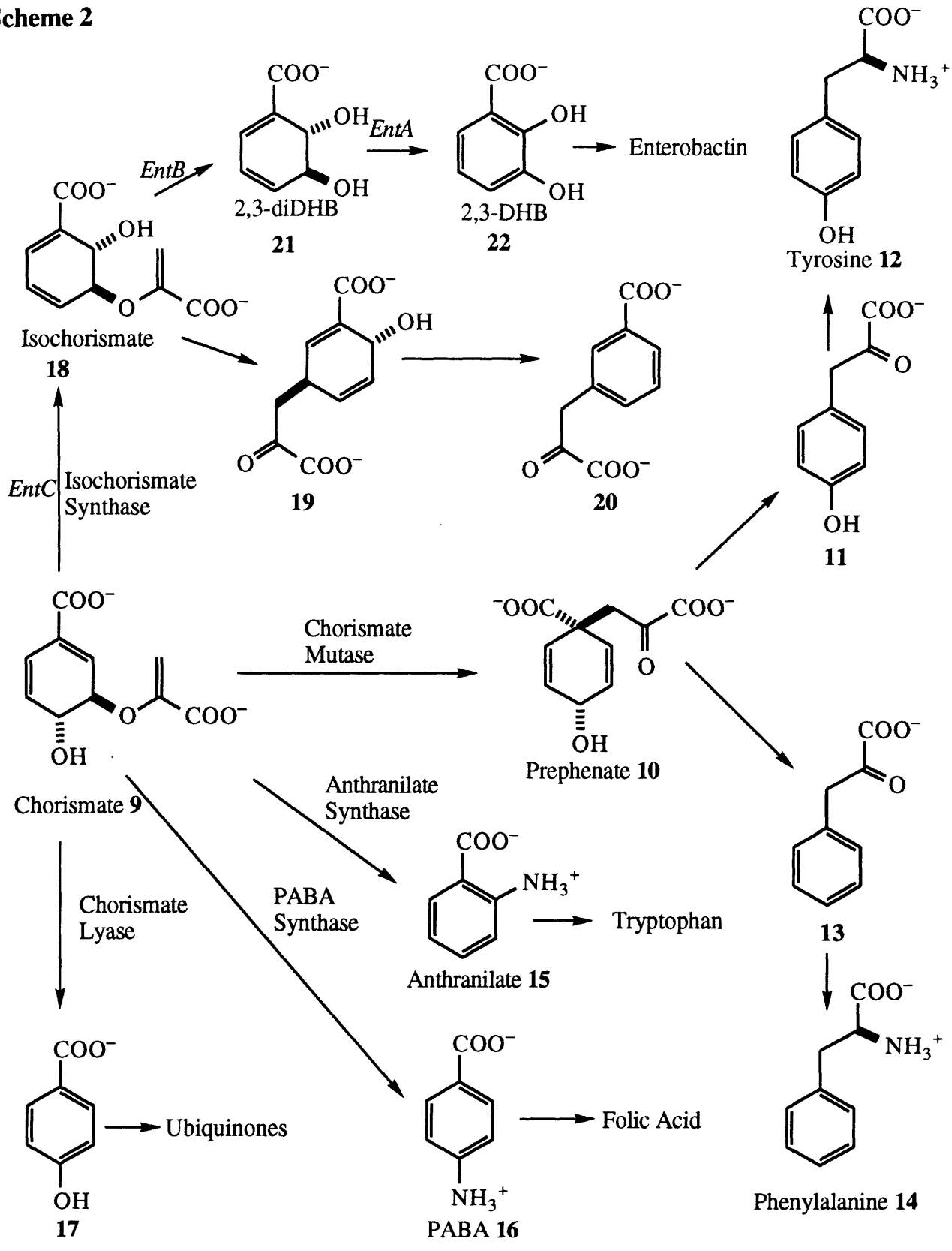

D-(+)Glucose
CHO
H—OH
H—OH
CH2OPO3=
D-erythrose-4-phosphate 2
=O3PO
CO2-
3
DAHP
Synthase
=O3POH2C
DAHP 4
dehydroquinate 5
shikimate
dehydrogenase
6
shikimic acid 1
shikimate kinase
7
CH3
OPO3=
chorismic acid 9
EPSP 8

Chorismic acid (**9**) is derived from shikimic acid in three steps. Conversion of **1** to **7** is catalyzed by shikimate kinase. Reaction of phosphoenolpyruvate (**3**) with **7** forms an intermediate[12] which subsequently gives enolpyruvyl shikimate phosphate (**8**) (EPSP).[13] Inorganic phosphate is eliminated from **8** by chorismate synthase in a net trans 1,4-elimination to give chorismic acid (**9**).[14]

Chorismic acid was first isolated in 1962 by Gibson from a mutant strain of *Klebiella pneumoniae* 62-1 (formally *Aerobacter aerogenes* 62-1).[15] The structure and stereochemistry were determined,[16] and the crucial role of chorismic acid in the biosynthesis of aromatic compounds, such as tyrosine and enterobactin, has been demonstrated through numerous investigations.[1] The first total synthesis of racemic chorismic acid was published in 1982 by McGowan and Berchtold,[17] and later that year, a synthesis was also accomplished by Ganem and co-workers.[18] An improved synthesis was reported the following year by Berchtold and co-workers.[19] The synthesis of optically pure (-)-**9** was accomplished by Pawlak and Berchtold.[10] A stereospecific synthesis of (±)-**9** has been addressed by Posner and Nelson.[20] Ganem et al have reported a synthesis of optically pure (-)-**9** from (-)-**1**.[21]

Some of the metabolites of chorismate are shown in Scheme 2. The diverse nature and structure of compounds that are biosynthesized from chorismic acid have made this part of the pathway one of the most extensively studied.[1,2] Until recently, there was not a great deal known about the mechanistic features, structure-function relationships, or active-site requirements of the enzymatic transformations of **9**. Molecular biologists have made many efforts to characterize the enzymes and their mechanisms.[22] Despite this, there remain many areas to be explored.

Scheme 2


EntB
EntA
2,3-diDHB
21
2,3-DHB
22
Enterobactin
Tyrosine 12
Isochorismate
18
19
20
11
EntC Isochorismate Synthase
Chorismate Mutase
Prephenate 10
Chorismate 9
Anthranilate Synthase
Anthranilate 15
Tryptophan
13
Chorismate Lyase
PABA Synthase
17
Ubiquinones
PABA 16
Folic Acid
Phenylalanine 14

One of the best characterized and most unique enzymes of the group is chorismate mutase. This enzyme catalyzes the [3,3] sigmatropic rearrangement (Claisen rearrangement) of **9** to prephenate (**10**). This rearrangement is the only known example of an enzyme catalyzed pericyclic reaction in primary metabolism.[16b]

Both phenylalanine and tyrosine are derived from chorismate through prephenate as shown in Scheme 2.[1] Under the action of prephenate dehydrogenase (PD), prephenate is decarboxylated with subsequent loss of hydride to NAD^+ to give *p*-hydroxyphenylpyruvate. Transamination of *p*-hydroxyphenylpyruvate gives tyrosine (**12**). Formation of phenylalanine (**14**) is the result of prephenate dehydratase-catalyzed decarboxylation and dehydration, followed by transamination.

The mechanisms of both the enzyme- and acid-catalyzed decarboxylation of prephenate were studied by Morrison, O'Leary and Cleland.[23] Hydride transfer to NAD^+ and loss of CO_2 were shown to be concerted for the reaction catalyzed by the bifunctional enzyme chorismate mutase-prephenate dehydrogenase (CM-PD) from *Escherichia Coli*. However, the acid-catalyzed reaction was established to occur by loss of water to give a carbocation intermediate that aromatized subsequently by decarboxylation.

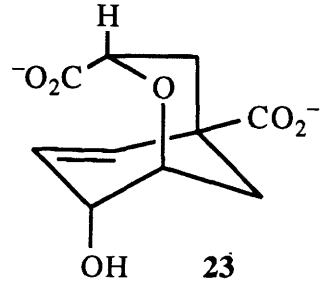
Many versions of bacterial chorismate mutase have been detected as part of a bifunctional polypeptide chain with fusion to either prephenate dehydrogenase or prephenate dehydratase activity.[24] Numerous structure / function studies of the mutase portion of the mutase / dehydrogenase or mutase / dehydratase enzymes have focused on their bifunctional character. Ganem and coworkers have utilized recombinant DNA techniques to obtain a monofunctional 109-amino acid

chorismate mutase from chorismate mutase-prephenate dehydratase from the N-terminal one-third of the protein from *E. coli.*[25] One of the small (14.5 kDa) monofunctional chorismate mutase enzymes has recently been purified to homogeneity.[26] The size and single activity make it an ideal choice for further structural and functional characterization.

Knowles et al employed molecular cloning of the gene from *Bacillus subtilis* to predict the structure of chorismate mutase as a homodimer.[26a] Jaffe et al determined the structure of the same chorismate mutase as a homotrimer from ^{13}C NMR studies of the enzyme-bound prephenate complex of *Bacillus subtilis* chorismate mutase.[27] Recently the crystal structure has been solved by Lipscomb and co-workers.[28]

Lipscomb deduced that *B. subtilis* chorismate mutase is a homotrimer, with β-sheets from each monomer packing to form the core of a pseudo-αβ-barrel with helices on the outside of the trimer. The subunits of the trimer are related by pseudo-3-fold symmetry. The active site of chorismate mutase has been determined by using data from a complex of the enzyme with an *endo*-oxabicyclic transition state analog (Bartlett's inhibitor K_i = 3 μM, **23,** Scheme 3).[29]

Scheme 3

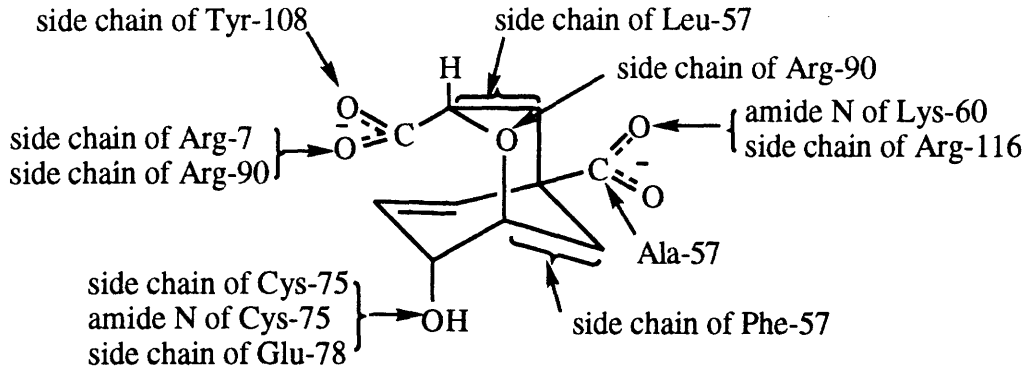


23

This inhibitor (**23**) is bound in the cleft formed from the interface between two adjacent subunits with three equivalent active sites for the trimer. The

structure of this complex has been refined to 2.2 Å, and it shows numerous interactions between enzyme and inhibitor (**23**). These interactions include hydrophobic contacts, ionic contacts, and polar contacts as shown in Scheme 4.

Scheme 4

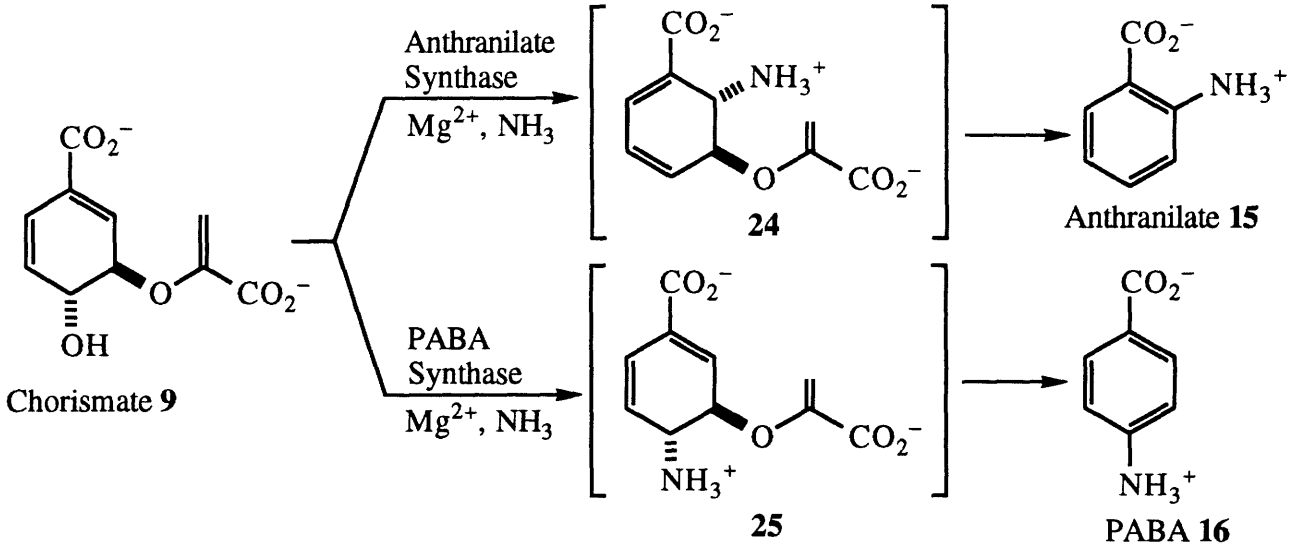


It remains unclear whether the mutase-catalyzed reaction is a concerted [3.3] process or whether it is a two-step reaction. This question is addressed further in Chapter 2.

Two other enzymes that have received detailed attention over the past few years are anthranilate synthase (AS) and *p*-aminobenzoate synthase (PABA). Anthranilate synthase catalyzes the conversion of **9** to anthranilate (**15**), which is the precursor to tryptophan in Scheme 5. This enzyme is composed of two subunits, AS I and AS II, which are encoded on the genes trpE and trpG.[30] AS I converts chorismate and ammonia to anthranilate in the presence of Mg^{2+}. AS II is responsible for the hydrolysis of glutamine to release ammonia for the amination of chorismate. The dihydro compound, *trans*-2-amino-2-deshydroxyisochorismate (**24**) is the established intermediate for the enzymatic reaction. Intermediate **24** was synthesized by the Berchtold group and the Ganem

group,[31,32] and was shown to be chemically and kinetically competent as an intermediate to anthranilate with pure enzyme.[31,32] Attempts to demonstrate accumulation of aminocyclohexadiene **24** in catalytical turnover of chorismate were not successful, perhaps reflecting that aromatization might not be rate-limiting.

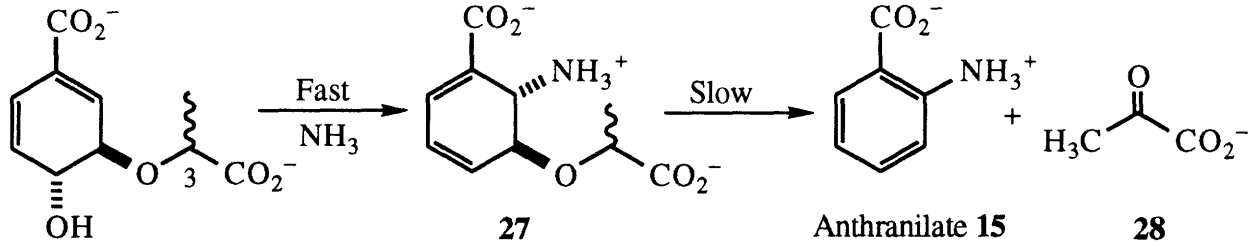
Scheme 5



To probe further for accumulation of aminocyclohexadiene intermediates, alternative substrates **26** for the enzyme were studied (Scheme 6).[33] Substitution of the C3 enolpyruvyl ether by a C3 (R)- or (S)-lactyl ether led to 10-20 fold decrease in k_{cat} values compared to the chorismate.[33] Anthranilate synthase displayed 12:1 of S:R lactyl ether preference by k_{cat} / K_m criteria such that the (S)-lactyl analogue of **27** was processed at 1 / 18 the catalytic efficiency of chorismate. UV-visible monitoring of the incubation solutions of these lactyl analogues indicated transient accumulation of the aminocyclohexadiene species (**27**) before further conversion to anthranilate. It is likely that the aromatization

step has been selectively slowed by the use of a poorer leaving in the elimination step (lactyl vs. enolpyruvyl ether).

Scheme 6



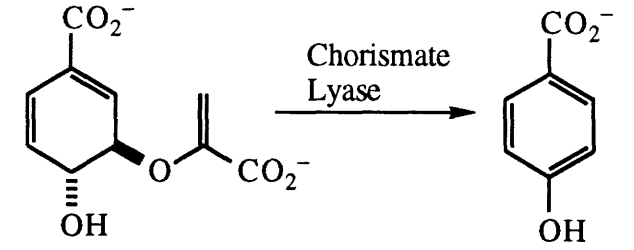
26 (R or S)

The conversion of chorismate to *p*-aminobenzoic acid (PABA) requires three separate proteins: PabA, PabB, and PabC. Together, PabA and PabB convert glutamine and chorismate to glutamate and 4-amino-4-*deshydroxy*-chorismate (**25**).[34] This aminochorismate analog is subsequently transformed to PABA by PabC as shown in Scheme 5.[35] Compound **25** was prepared as an intermediate by Teng and Ganem and proved to be chemically and kinetically competent.[36]

Another enzyme, chorismate lyase, is the least studied of the group of enzymes involved in the chorismate pathway. It catalyzes the loss of side chain and aromatization of chorismate to *p*-hydroxybenzoate **17** as shown in Scheme 7.[37] Compound **17** is then converted to the ubiquinones in subsequent steps.[1] It is known that the ubiC gene, encoding chorismate lyase, functions at the start of coenzyme Q biosynthesis, and a mutant strain of *E. coli* defective in ubiC gene has been isolated.[37] It seems likely that the aromatization step of **24** and **25** might be similar to that involved in the formation of **17**. A two-step mechanism involving an initial abstraction of the C4-H of chorismate by chorismate lyase should be followed by loss the C3-enolpyruvyl group to generate *p*-hydroxybenzoate directly

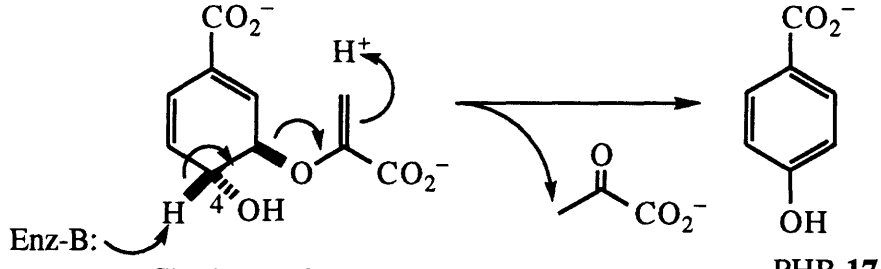
as shown in Scheme 8. A concerted cyclic mechanism is possible, but it was shown not to be the case in the formation of anthranilate.[38] The concerted mechanism is not likely for the enzyme-catalyzed reaction, but it is a reasonable mechanism for the thermal conversion of chorismate to **17**.[39]

Scheme 7



Chorismate **9** PHB **17**

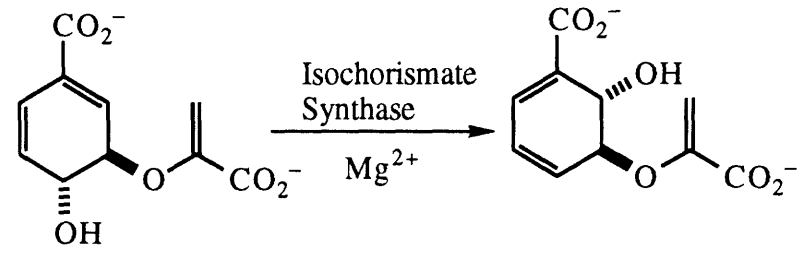
Scheme 8



Chorismate **9** PHB **17**

The chorismate metabolizing enzyme, isochorismate synthase, has been studied in detail only recently.[40] This enzyme catalyzes the transformation of chorismate (**9**) to isochorismate (**18**) as shown in Scheme 9. The overall reaction is believed to be similar to that catalyzed by anthranilate synthase. Isochorismate (**18**) was isolated by Gibson in 1967.[41] The structure and absolute stereochemistry were determined shortly thereafter,[42] and a synthesis or racemic **18** was reported by Busch and Berchtold.[43]

Scheme 9



Chorismate **9** Isochorismate **18**

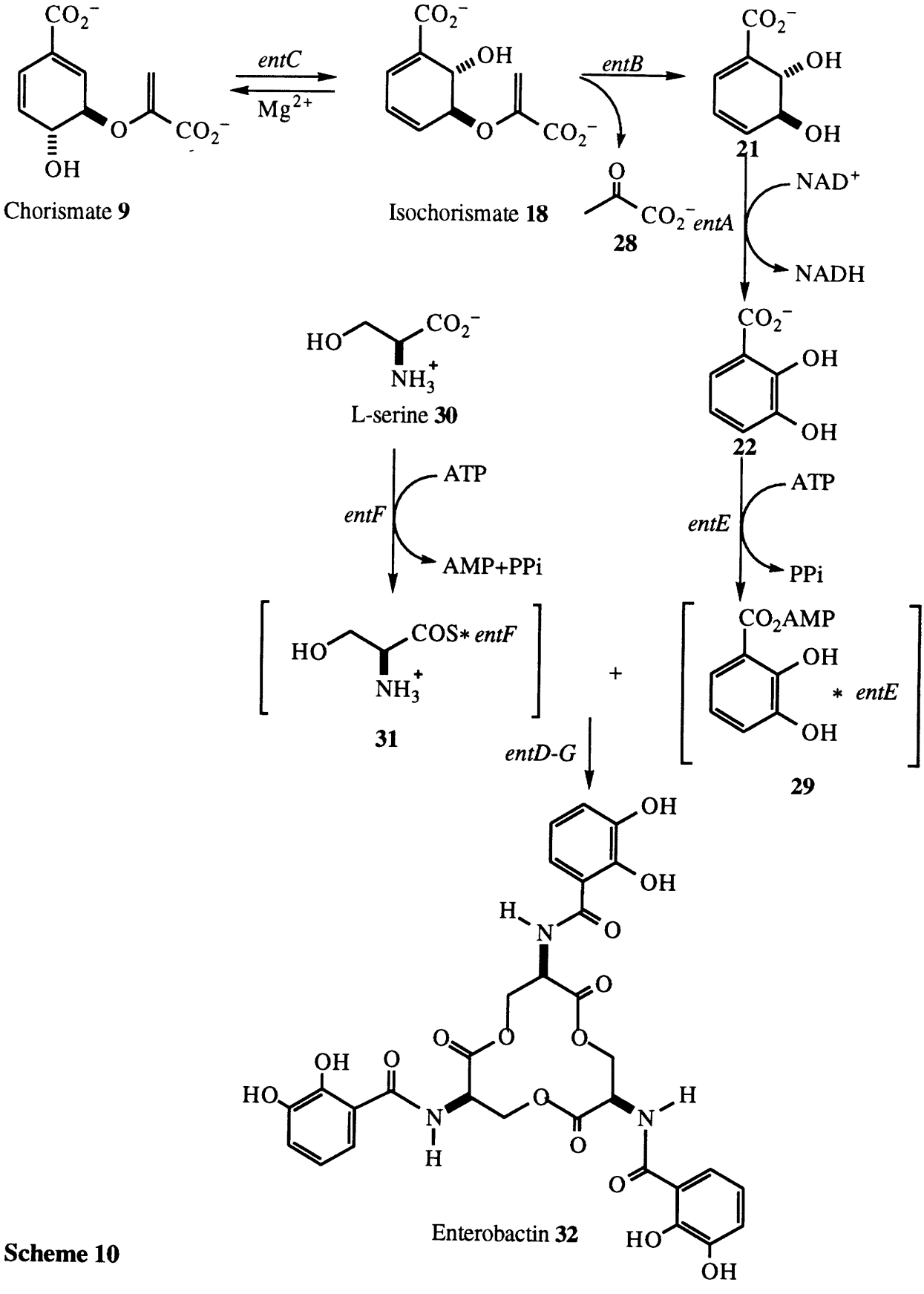
Under an iron-deficient environment many organisms, such as *E. coli*, synthesize potent iron-chelating compounds termed siderophores that permeate through the cell to search available iron (as ferric ion) for uptake back into the organism where it is provided for essential metabolic processes. Enterobactin is one of those siderophores.

Enterobactin (**32**), a cyclic, trimeric lactone, was discovered in 1970, and its structure was determined independently by Pollack and Nielands[44] and by O'Brien and Gibson.[45] It is the only known tricatecholamide siderophore which possesses a cyclic backbone composed of three units of N-2,3-dihydroxybenzoyl-L-serine. Two syntheses of **32** have been reported in the literature,[46] the first in 1977, was reported by Corey and Bhattacharyya.[47] The total synthesis of enterobactin (**32**) was also achieved by Rastetter et al.[48] Recently the enzymes, as well as several intermediates, have been purified or fully characterized utilizing recent advances in molecular biology (recombinant DNA techniques).[40]

The biosynthesis of enterobactin is accomplished when enterobacteria, such as *E. coli*, are in an iron-deficient environment. Seven gene products, *ent*A-G, are responsible for the biotransformations of enterobactin from chorismate as shown in Scheme 10. Isochorismate synthase (*ent*C) catalyzes the transformation of chorismate to isochorismate, which is subsequently transformed to *trans*-2,3-

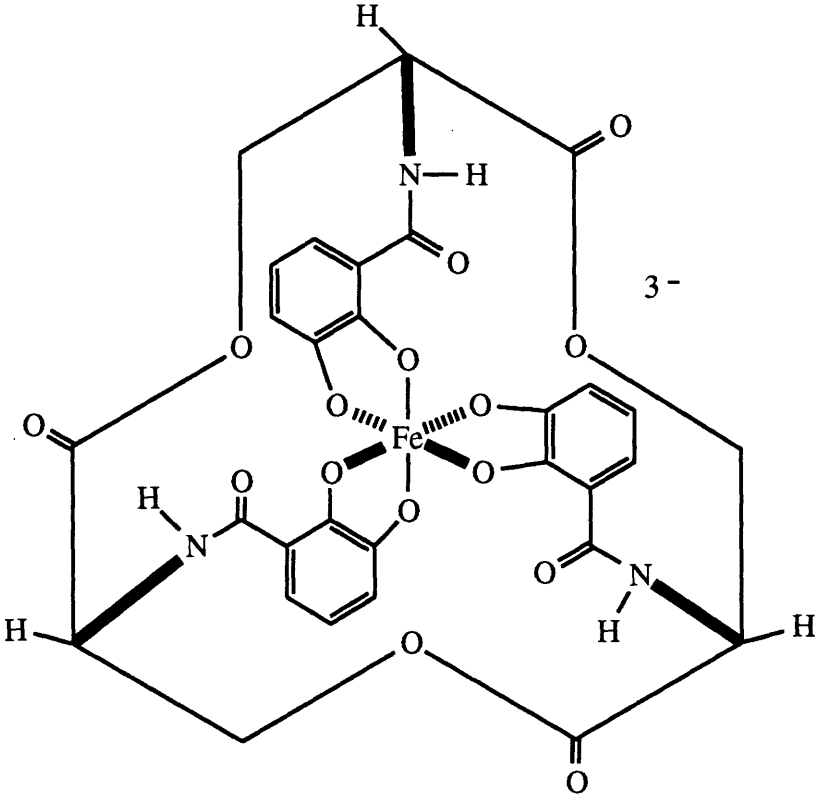
dihydro-2,3 dihydroxybenzoate (**21**) by isochorismatase (*ent*B). Aromatization of *trans*-2,3-dihydro-2,3-dihydroxybenzoate by 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase (*ent*A) leads to the formation of 2,3 dihydroxybenzoate (**22**). This is the best known segment of the pathway even though the enzymes have been purified only recently.[49]

The remaining four gene products *ent*D-G were observed to effect the final assembly process. Early studies have shown that *ent*E and *ent*F proteins catalyze the ATP-[^{32}P]pyrophosphate exchange reaction dependent upon 2,3-dihydroxybenzoate and L-serine, respectively.[50] Thus, carboxyl activation of 2,3-dihydroxybenzoate and L-serine monomers proceeds through the representative acyl adenylates. The final transformations are carried out by *ent*D and *ent*G proteins. Recently *ent*D has been sequenced,[51] and expression analysis indicated that *ent*D is membrane-bound, probably in the *E. coli* inner membrane. This suggests that enterobactin may be synthesized completely in a vectorial fashion with the final product exported outside the cell at the time of the final cyclization step, and *ent*D may serve such a function.


Chorismate 9
entC
Mg2+
Isochorismate 18
entB
21
28
entA
NAD+
NADH
L-serine 30
22
ATP
entF
AMP+PPi
ATP
entE
PPi
31
29
entD-G
Enterobactin 32

Scheme 10

The enterobactin-ferric ion complex has an estimated formation constant of 10^{52} M^{-1}. This is the strongest iron-chelating siderophore ever reported.[52] It can bind one atom of ferric ion to form a hexacoordinate complex via its three bidentate catechol units. Once formed, the ferric enterobactin complex **33** is transported from the environment through the cell envelope into the bacterial cell cytoplasm. The iron is made available to the cell, and enterobactin is hydrolyzed and transported back out of the cell.

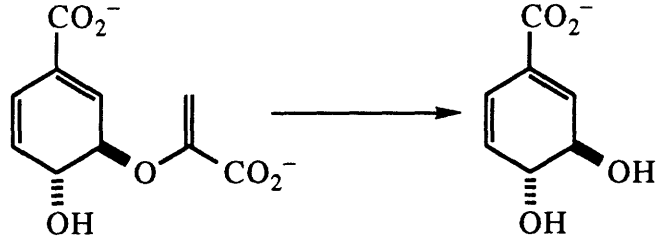


Enterobactin complex **33**

One of the transformations of the chorismate which differs from those discussed in Scheme 2 is the cleavage of the enolpyruvyl group giving *trans*-3,4-dihydro-3,4-dihydroxybenzoate (**34**) in Scheme 11. The metabolic function of **34**, if any, is not clearly understood. It may play a part, directly or indirectly, in metal

metabolism.[53,54] Attempts to demonstrate that **34** is a precursor of 3,4-dihydroxybenzoate in *Aerobacteria aerogenes* have not been successful. Since it has been demonstrated that isochorismatase, which catalyzes the hydrolysis of the enolpyruvyl of isochorismate, also catalyzes, albeit with poor turnover, the hydrolysis of the enolpyruvyl group of chorismate, the formation of **34** may be an artifact.[49a]

Scheme 11



Chorismate **9** **34**

This thesis will focus on the two major parts of the shikimate pathway. The conversion of chorismate to prephenate will be discussed, and the synthesis and enzymatic results of potential substrates will be described in detail in Chapter 2. The synthetic effort to prepare a chorismate analog with an α,β-unsaturated amino acid on the side chain will be discussed in Chapter 3. An approach to the synthesis of dihydropyridine analogs, potential suicide inhibitors, will be described in Chapter 3. Finally, an approach to the synthesis of a 5-carboxychorismate analog for the enzymatic study of conversion of chorismate to isochorismate by isochorismate synthase (*ent*C) will be addressed in Chapter 4.

References and Notes

1. (a) Weiss, U.; Edwards, J. M. *The Biosynthesis of Aromatic Compounds*; Wiley: New York, 1980. (b) Haslem, E. *The Shikimate Pathway*; Halstead Press, Wiley: New York, 1974.

2. Eijikman, J. F. *Recl. Trav. Chim. Pay-Bas* **1885**, *4*, 32.

3. Reference 1(a), p 104.

4. (a) Smissman, E. E.; Suh, J. T.; Oxman, M.; Daniel, R. *J. Am. Chem. Soc.* **1959,** *81,* 2909. (b) Smissman, E. E.; Suh, J. T.; Oxman, M.; Daniel, R. *J. Am. Chem. Soc.* **1962,** *84,* 1040.

5. (a) McCrindle, R.; Overton, K. H.; Raphael, R. A. *J. Am. Chem. Soc.* **1960,** *82,* 1560. (b) Grewe, R.; Hinrichs, I. *Chem. Ber.* **1964,** *97,* 443. (c) Grewe, R.; Kersten, S. *Chem. Ber.* **1967,** *100,* 2546. (d) Doshi, M. M. *Diss. Abstr.* **1964,** *24,* 3998. (e) Coblens, K. E.; Muralidharan, V. B.; Ganem, B. *J. Org. Chem.* **1982,** *47,* 5041. (f) Koreeda, M.; Ciufolini, M. A. *J. Am. Chem. Soc.* **1982,** *104,* 2308. (g) Bartlett, P. A.; McQuaid, L. A. *J. Am. Chem. Soc.* **1984,** *106,* 7854. (h) Rajapaksa, D.; Keay, B. A.; Rodrigo, R. *Can. J. Chem.* **1984,** *62,* 826. (i) Campbell, M. M.; Kaye, A. D.; Sainbury, M.; Yavarzadeh, R. *Tetrahedron Lett.* **1984,** *25,* 1629. (j) Campbell, M. M.; Kaye, A. D.; Sainbury, M.; Yavarzadeh, R. *Tetrahedron* **1984,** *40,* 2461. (k) Campbell, M. M.; Sainbury, M.; Yavarzadeh, R. *Tetrahedron* **1984,** *40,* 5063. (l) Roush, W. R.; Adam, M. A. personal communication. (m) Koreeda, M.; Teng, K.; Murata, T. *Tetrahedron Lett.* **1990,** *31,* 5997.

6. (a) Dangschat, G.; Fischer, H. O. L. *Biochim. Biophys. Acta* **1950,** *4,* 199. (b) Dangschat, G.; Fischer, H. O. L. *Naturwissenshafter* **1938,** *26,* 562. (c) Cleophax, J.; Mercier, D.; Gero, S. D. *Angew. Chem, Int. Ed. Engl.* **1971,** *10,* 652. (d) Cleophax, J.; Leboul, J.; Mercier, D.; Gaudemer, A.; Gero, S. D. *Bull. Soc. Chim. Fr.* **1973,** *95,* 7821.

7. (a) Yoshikawa, M.; Ikeda, Y.; Kayakiri, H.; Kitagawa, I. *Heterocycles* **1982,** *17,* 209. (b) Fleet, G. W. J.; Shingo, T. K. M. *J. Chem. Soc., Perkin Trans. 1* **1984,** 905. (c) Fleet, G. W. J.; Shingo, T. K. M. *J. Chem. Soc. Chem. Commun.* **1983,** 849.

8. Bestmann, H. J.; Heid, H. A. *Angew. Chem, Int. Ed. Engl.* **1971,** *10,* 336.

9. (a) Tadano, K.; Ueno, Y.; Iimura, Y.; Suami, T. *Carbohy. Chem.* **1987,** *6,* 245. (b) Mirza, S.; Harvey, J. *Tetrahedron Lett.* **1991,** *32,* 4111.

10. Chahoua, L.; Baltas, M.; Gorrichon, L.; Tisnes, P.; Zedde, C. *J. Org. Chem.* **1992,** *57,* 5798.

11. Pawlak, J.; Berchtold, G. A. *J. Org. Chem.* **1987,** *52,* 1765.

12. Anderson, K. S.; Johnson, K. A. *Chem. Rew.* **1990,** *90,* 1131.

13. (a) Grimshaw, C. E, Sogo, S. G.; Copley, S. D.; Knowles, J. R. *J. Am. Chem. Soc.* **1984**, *106*, 2699. (b) Asano, Y.; Lee, J. J.; Shieh, T. L.; Spreafico, F.; Kowal, C.; Floss, H. G. *J. Am. Chem. Soc.* **1985**, *107,* 4314.

14. Reference 1(a), p 130.

15. (a) Gibson, M. I.; Gibson, F. *Biochim. Biophys Acta* **1962**, *65,* 160. (b) Gibson, F.; Jackman, L. M. *Nature (London)* **1963**, *198,* 388.

16. (a) Edwards, J. M.; Jackman, L. M. *Aust. J. Chem.* **1965**, *18,* 1227. (b) Retey, J.; Robinson, J. A. "Stereospecificity in Organic Chemistry and Enzymology"; Verlag Chemie, 1982, p148.

17. (a) McGowan, D. A.; Berchtold, G. A. *J. Am. Chem. Soc.* **1982**, *104,* 1153. (b) McGowan, D. A.; Berchtold, G. A. *J. Am. Chem. Soc.* **1982**, *104,* 7036.

18. Ganem, B.; Ikota, N.; Muralidharan, V. B.; Wade, W. S.; Young, S. D.; Yukimoto, Y. *J. Am. Chem. Soc.* **1982**, *104,* 6787.

19. Hoare, J. H.; Policastro, P. P.; Berchtold, G. A. *J. Am. Chem. Soc.* **1983**, *105,* 6264.

20. Posner, G.; Nelson, T. *Tetrahedron* **1990,** *46,* 4573.

21. Wood, H. B.; Ganem, B. *J. Am. Chem. Soc.* **1990**, *112,* 8907.

22. Liu, J. Ph.D thesis, Masschusettes Institute of Technology, **1990** and references cited therein.

23. Hermes, J. D.; Tipton, P. A.; Fisher, M. A.; O'Leary, M. H.; Morrison, J. F.; Cleland, W. W. *Biochemistry* **1984**, *23,* 6263.

24. Jenson, R.; Fisher, R. *Method Enzymol.* **1987**, 142.

25. Stewart, J; Wilson, D. W.; Ganem, B. *J. Am. Chem. Soc.* **1990**, *112,* 4582.

26. (a) Gray, J. V.; Golinelli-Pimpaneau, B.; Knowles, J. R. *Biochemistry* **1990**, *29,* 376. (b) Gray, J. V.; Eren, D.; Knowles, J. R. *Biochemistry* **1990**, *29,* 8872.

27. Rajagopalan, J. S.; Taylor, K. M.; Jaffe, E. K. *Biochemistry* **1993**, *32,* 3965.

28. Chook, Y. M.; Ke, H.; Lipscomb, W. N. *Proc. Natl. Acad. Sci. U.S.A.* **1993,** *90,* 8600.

29. Bartlett, P. A.; Johnson, C. R. *J. Am. Chem. Soc.* **1985**, *107,* 7792.

30. Zalkin, H. *Adv. in Enzymology* 1973, *38,* 1-39.

31. Teng, C.-Y. P.; Ganem, B. *J. Am. Chem. Soc.* **1984**, *106,* 2463.

32. Policastro, P. P.; Au, K. G.; Walsh, C. T.; Berchtold, G. A. *J. Am. Chem. Soc.* **1984**, *106,* 2443.

33. Delany, J. J. Ph.D thesis, Masschusettes Institute of Technology, **1988.**

34. (a) Ye, Q.-Z.; Liu,, J.; Walsh, C. T. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87,* 9391. (b) Anderson, K. S.; Kati, W. M.; Ye, Q.-Z.; Liu, J.; Walsh, C. T.; Benesi, A. J.; Johnson, K. A. *J. Am. Chem. Soc.* **1991**, *113,* 3198. (c) Roux, B.; Walsh, C. T *Biochemistry* **1992**, 6904.

35. (a) Nichols, B. P.; Seibold, A. M.; Doktor, S. Z. *J. Biol. Chem.* **1989**, *264,* 8597. (b) Green, J. M.; Nichols, B. P. *J. Biol. Chem.* **1991**, *266,* 12971.

36. Teng, C.-Y. P.; Ganem, B.; Doktor, S. Z.; Nichols, B. P.; Bhatnager, R. K.; Vining, L. C. *J. Am. Chem. Soc.* **1985**, *107,* 5008.

37. Lawrence, J.; Cox, G. B.; Gibson, F. *J. Bacteriol.* **1974**, *118,* 41.

38. (a) Onderka, D. K.; Floss, H. G. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1969,** *28,* 668. (b) Onderka, D. K.; Floss, H. G. *Biochim. Biophys. Acta* **1970**, *206*, 449.

39. Tamir, H.; Srinivasan, P. R. *Proc. Natl. Acad. Sci. U.S.A.* **1970,** *66,* 547.

40. (a) Liu, J.; Duncan, K.; Walsh, C. T. *J. Bacteriol.* **1989**, *171,* 789. (b) Liu, J.; Quinn, N. R.; Berchtold, G. A.; Walsh, C. T. *Biochemistry* **1990**, *29,* 1417.

41. Young, I. G.; Batterham, T. J.; Gibson, F. *Biochim. Biophys. Acta* **1968**, *165,* 567.

42. Young, I. G.; Batterham, T. J.; Gibson, F. *Biochim. Biophys. Acta* **1969**, *177,* 389.

43. Busch, R. F.; Berchtold, G. A. *J. Am. Chem. Soc.* **1983**, *105,* 3346.

44. (a) Pallak, J. R.; Nieland, J. B. *Biochim. Biophys. Res. Commun.* **1970**, *38,* 989. (b) Pallak, J. R.; Ames, B. N.; Nieland, J. B. *J. Bacteriol.* **1970**, *104,* 635.

45. (a) O'Brien, I. G.; Gibson, F. *Biochim. Biophys. Acta* **1970**, *215,* 393. (b) O'Brien, I. G.; Cox, G. B.; Gibson, F. *Biochim. Biophys. Acta* **1971**, *237,* 537.

46. Miller, M. J. *Chem. Rev.* **1989**, *89,* 1563,

47. Bhattacharyya, S.; Corey, E. J. *Tetrahedron Lettt.* **1977**, *45,* 3919.

48. Rastetter, W. H.; Erickson, T. J.; Venuti, M. C. *J. Org. Chem.* **1980**, *45,* 5012.

49. (a) Rusnak, F.; Liu, J.; Quinn, N.; Berchtold, G. A.; Walsh, C. T. *Biochemistry* **1990**, *29,* 1425. (b) Ozenberger, B. A.; Brickman, T. J.; McIntosh, M. A. *J. Bacteriol.* **1989**, *171*, 775. (c) Nahlik, M. S.; Ozenberger, B. A.; Brickman, T. J.; McIntosh, M. A. *J. Bacteriol.* **1989**, *171*, 784. (d) Harris, W.R.; Carrano, C. J.; Cooper, S. R.; Sofen, S. R.;

Avdeef, A. E.; Mcardle, J. V.; Raymond, K. N. *J. Am. Chem. Soc.* **1979**, *101*, 6097.

50. (a) Bryce, G. F.; Brot, N. *Biochemistry* **1972**, *11*, 1708. (b) Woodrow, G. C.; Young, I. G.; Gibson, F. *Biochim. Biophys. Acta* **1979**, *582,* 145. (c) Greenwood, K. T.; Luke, R. K. *Biochim. Biophys. Acta* **1976**, *454,* 285. (d) Greenwood, K. T.; Luke, R. K. *Biochim. Biophys. Acta* **1980**, *614,* 185.

51. (a) Armstrong, S. K.; Pettis, L. J.; McIntosh, M. A. *Mol. Microbil.* **1989**, *3,* 757. (b) Codere, P. E.; Earhart, C. F. *J. Gen. Microbil.* **1989**, *135,* 3043.

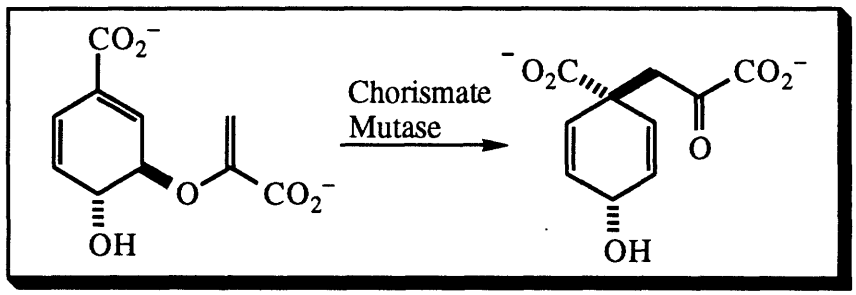
52. Reference 49(d).

53. Young, I. G.; Gibson, F. *Biochim. Biophys. Acta* **1969**, *177,* 182.

54. Young, I. G.; Gibson, F. ; McDonald, C. G. *Biochim. Biophys. Acta* **1969**, *5192,* 62.

Chapter 2

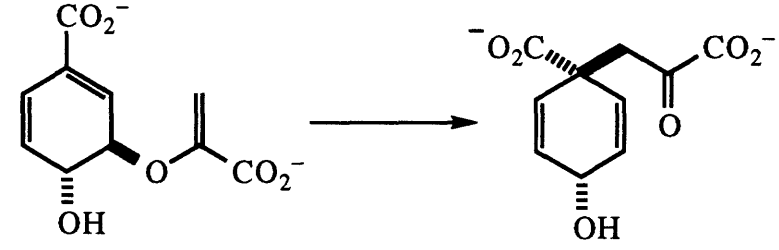
Investigations of the Enzyme Chorismate Mutase (Part I): [3,3] Rearrangement of Chorismate to Prephenate



Introduction

Among the different transformations in the shikimate pathway, perhaps the most interesting transformation is the enzyme-catalyzed [3,3] sigmatropic rearrangement of chorismate to prephenate (Scheme 1). This rearrangement, catalyzed by chorismate mutase (CM), is the only known example of an enzyme-catalyzed pericyclic reaction in primary metabolism.[1] The isolation and structural assignment of prephenate was reported by Weiss and Davis in 1954.[2] The stereochemistry of the hydroxyl group has been assigned by Plieninger.[3] The first total synthesis of disodium prephenate was achieved by Danishefsky.[4] Other syntheses have been reported by Plieninger,[3] Ramage,[5] and Berchtold.[6]

Scheme 1



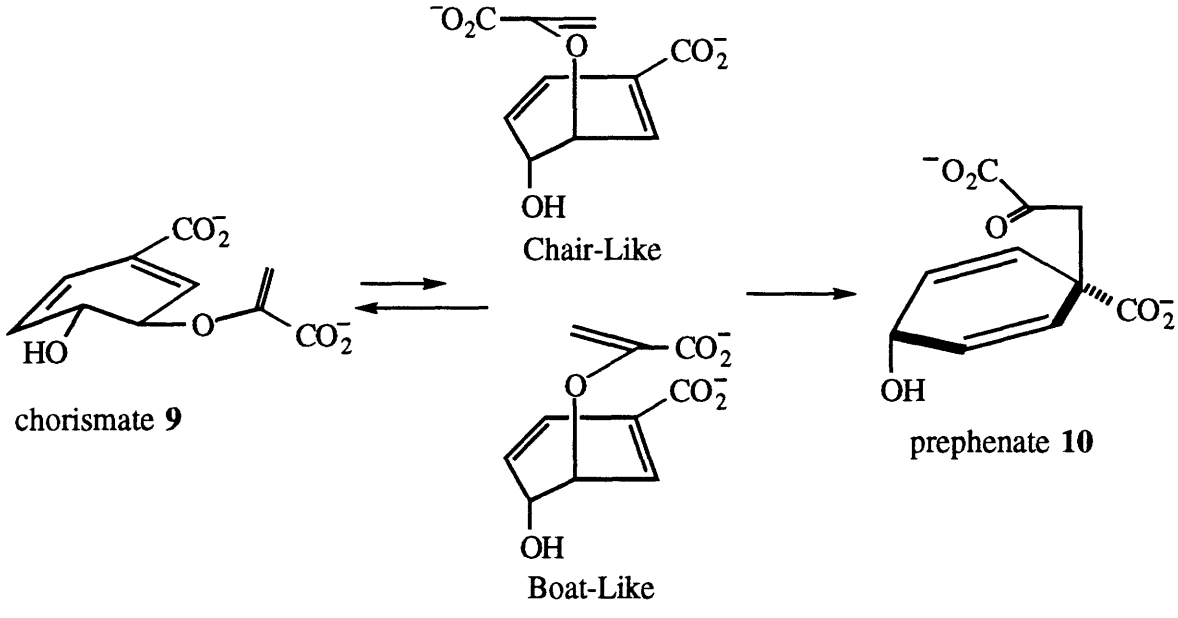
Chorismate **9** Prephenate **10**

The Claisen rearrangement of chorismate to prephenate has been the focus of many recent studies. Kinetic results showed that at pH 7.5 and 30 °C, chorismate mutase accelerates the rate of [3,3] rearrangement by a factor of 10^6 relative to the thermal rearrangement (without an enzyme or a catalyst) which has a half life of 15.7 h under the same conditions.[7a]

Molecular orbital calculations suggested that rearrangement through a chair-like transition state rather than a boat-like transition state should be favored (Scheme 2),[7] and the suggestion was supported with studies of chair-like geometry transition state analogs.[8] Experiments using specifically labeled

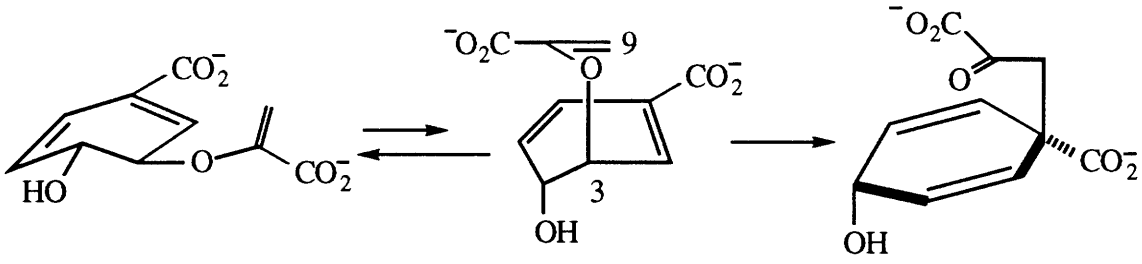
chorismate established that both the enzymatic[9] and thermal[10] reactions proceeded through the chair-like transition state. In addition, experiments using whole cell extracts from *E. coli* showed that both chorismate-mutase prephenate dehydrogenase and chorismate-mutase prephenate dehydratase catalyzed the rearrangement with chair-like geometry.[11] Experiments on substituent and solvent effects of the thermal rearrangement were carried out both by the Cornell group and the Indiana group.[12] The Cornell group suggested a dipolar transition state for the thermal rearrangement of chorismate analogs, while the Indiana group gave stronger emphasis to radical stabilizing properties of substituents.[12]

Scheme 2



Knowles and coworkers have conducted experiments on nonenzymatic and the enzyme-catalyzed rearrangement of chorismate to prephenate to determine the secondary tritium isotope effects at the bond-making position (C9) and bond-breaking position (C3) (Scheme 3).[13]

Scheme 3



chorismate **9** prephenate **10**

In the nonenzymatic (thermal) reaction (pH = 7.5, 30 °C), k_H / k_T is 1.149 for bond breaking (C3), and 0.092 for bond making (C9). This indicates an asymmetric transition state where the new bond is hardly formed, while the C3-O bond is substantially broken. In the enzymatic reaction (pH = 7.5, 30 °C), the value of k_H / k_T in both positions are unity within experimental error. It is likely that the isotope effects are suppressed in the enzymatic process, and the rate-limiting transition state occurs before the rearrangement. Knowles et al argued that the kinetically significant transition state presumably involves either the binding step of the small equilibrium proportion of the diaxial conformer of the substrate or an isomerization of enzyme-bound chorismate from the more stable conformer in which the carboxyvinyloxy group is equatorial to that in which the group is axial. Rearrangement would then proceed relatively rapidly from the higher energy axial conformer. Several possible mechanisms of chorismate mutase catalyzed rearrangement will be mentioned and discussed later in this chapter.

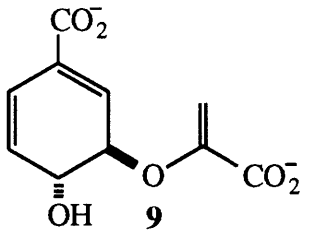
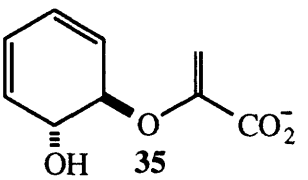
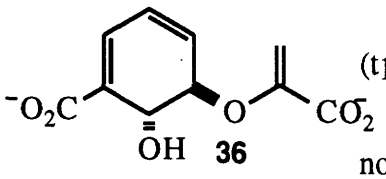
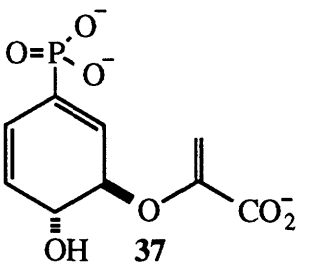
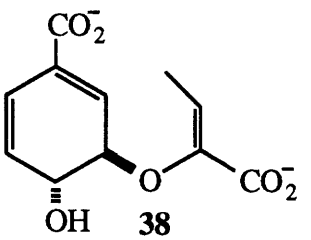
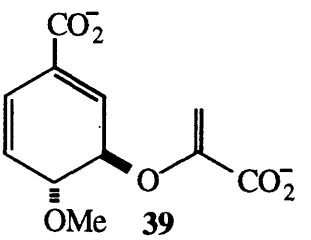
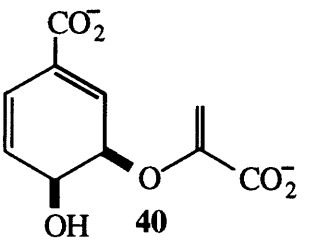
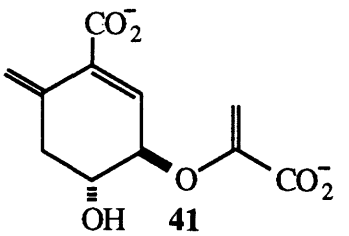
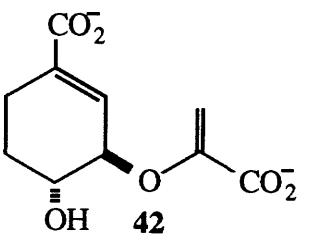
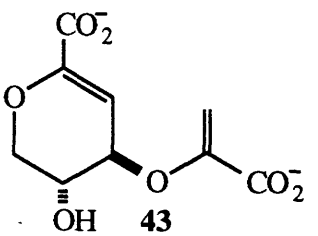
Substituent effects are very important for the rate of [3,3] rearrangement of chorismate and its derivatives. Berchtold and coworkers have synthesized several analogs of chorismate to explore the structural effects on both the uncatalyzed (thermal) and chorismate mutase catalyzed Claisen rearrangement at pH = 7.5, 30 °C (Table 1). Descarboxy analog **35** rearranges 10 times faster than **9**,[14] while

racemic isochorismate (**36**) rearranges 2.6 times faster due to the absence of an electron-withdrawing substituent at C1.[15] Replacing the C1 carboxylate group with a bulky stronger electron-withdrawing phosphate substituent (**37**) reduces the rate,[16] while attaching an electron-donating methyl group on the side chain (**38**) increases the rate.[17] Substitution of the C4 hydroxyl group with the methoxy group (**39**) gave a ~3-fold rate enhancement,[14] and 4-epichorismate (**40**) rearranges about 23 times faster than **9**.[6] Compound **41** was very stable under the reaction conditions. The 5,6-dihydro analog (**42**) rearranges at a rate about 3000 times slower than the rearrangement of chorismate, while the rate of rearrangement of dihydropyran analog **43** is approximately 70 times slower than **9**.[18]

All of the above observations can be explained by considering the dipolar nature of the rearrangement in addition to the effects of structural changes on the conformation of the analogs.[12] The ring carboxyl group of **9** and **36** and the phosphonate group of **37** could stablize the ground states of these molecules relative to **35** due to increased conjugation, with a resulting decrease of rate relative to **35**. Intramolecular hydrogen bonding of **9**, where the oxygen substituents are quasiequatorial, presumably stabilizes the ground state of **9**. In analog **39**, no hydrogen bonding is present, and in analog **40,** hydrogen bonding could be maintained in development of the transition state for rearrangement of **40**.[6] The fact that analogs **42** and **43** rearrange at a much slower rate than **9** suggests that there must be a significant electronic effect of the *endo* double bond at C5–C6 in **9** that increases the rate.

Table 1

Uncatalyzed and Catalyzed Claisen Rearrangement (30 °C, pH=7.5)

Compound	Relative rate[a]	Compound	Relative rate[a]
 **9**	1 ($t_{1/2}$ = 15.6 h) K_m = 0.14 mM	 **35**	10 ($t_{1/2}$ = 1.6 h) not a substrate
 **36**	2.6 ($t_{1/2}$ = 6 h) not a substrate	 **37**	0.4 ($t_{1/2}$ = 35 h) not a substrate
 **38**	2.6 ($t_{1/2}$ = 6 h) K_m = 4.0 mM	 **39**	2.8 ($t_{1/2}$ = 6.9 h) K_m = 1.9 mM
 **40**	23 ($t_{1/2}$ = 40 min) K_m = N / A	 **41**	0.00003 ($t_{1/2} = 5 \times 10^5$ h) not a substrate[b]
 **42**	0.0003 ($t_{1/2}$ = 49000 h) K_m = 0.55 mM	 **43**	0.014 ($t_{1/2}$ = 1200 h) K_m = 0.28 mM

a. Only for uncatalyzed (thermal) rate.

b. The rearrangement of **41** could be accelerated by the enzyme, but even with the increased rate the rearrangement would be too slow to measure under normal condition. It would require special conditions to see the rate enhancement such as the conditions used for compound **42**.

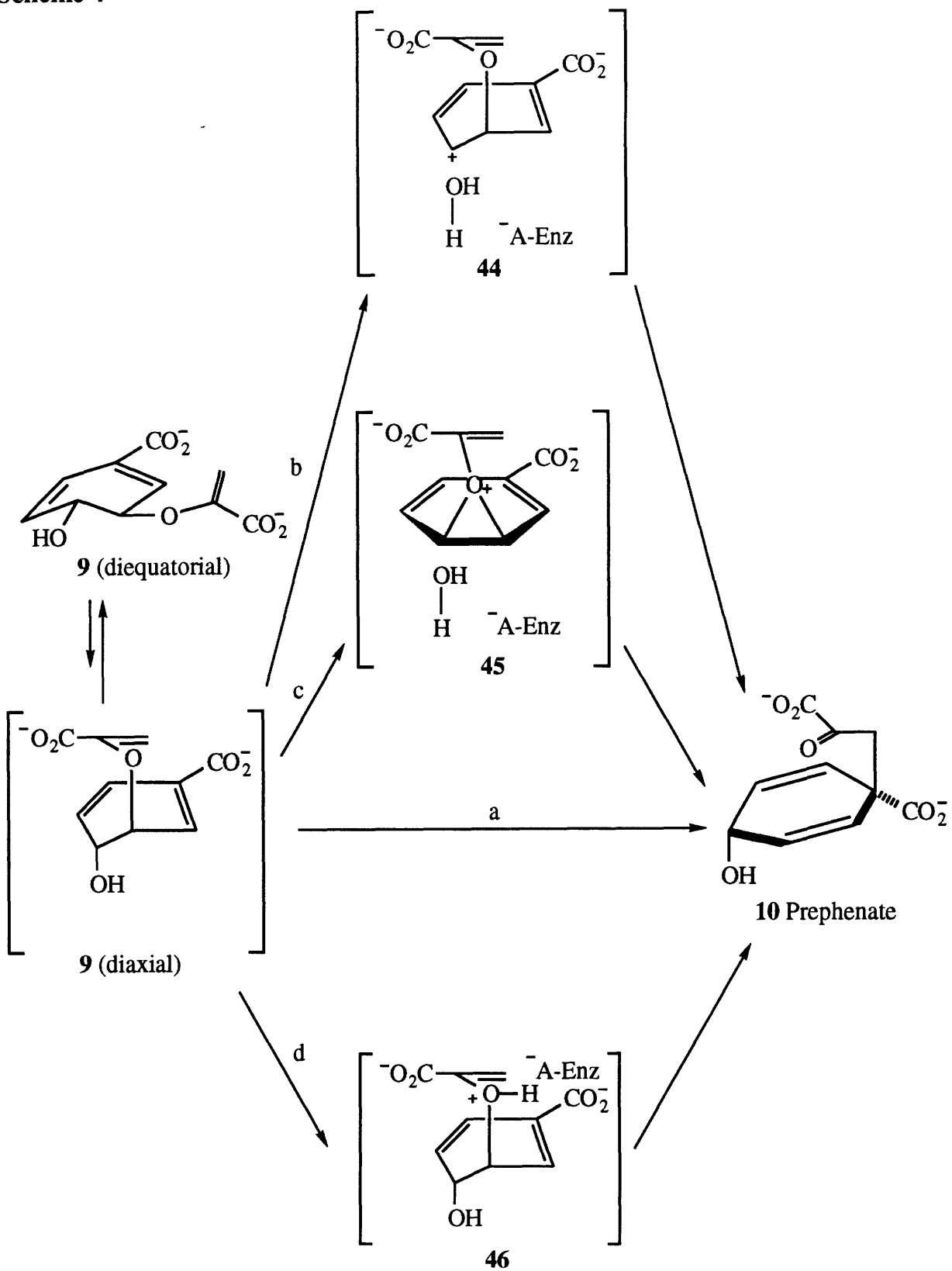
A fundamental question to be asked is, how does the enzyme accelerate the rate of Claisen rearrangement by a factor of about 10^6 ?

The activation parameters for both the thermal and enzymatic rearrangement of chorismate to prephenate have been determined.[7, 19] It was suggested that the enzymatic rate acceleration of more than 10^6 could be the result of a decrease in the entropy of activation to near zero and a reduction in the enthalpy of activation by about 5 kcal / mol.[19] To account for the rate enhancement and the lack of isotope effects observed experimentally, several possible enzymatic mechanisms were proposed (Scheme 4).[20]

First, since the rearrangement occurs from the pseudo-diaxial conformer of chorismate, the enzyme could procure the appropriate form of the substrate either by selective binding of **9** (diaxial conformer), or by first binding the predominant conformer of **9** (diequatorial conformer), and then performing a rate-limiting conformational isomerization to **9** (diaxial conformer), which then rearranges rapidly to enzyme-bound prephenate. Proton NMR studies indicated that as much as 10 - 20% of chorismate is present as the diaxial conformer **9** at equilibrium in aqueous solution.[21] The conformational equilibrium is rapid on the NMR time scale. These facts severely weaken any argument based upon the need for the enzyme to catalyze a conformational change, since the enzyme could select the appropriate diaxial form from the dynamic equilibrium mixture.

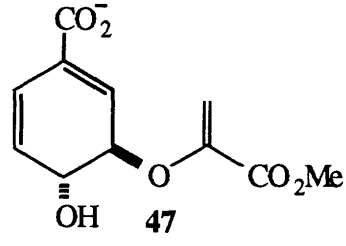
The second feasible mechanism outlined, path b in Scheme 4, suggests that the rate-limiting transition state might involve the protonation of the hydroxyl group at C4 and loss of H_2O to produce allylic cation **44**.[20] The third possible mechanistic suggestion, path c in Scheme 4, involves the transient formation of the oxiranium ion **45**.[22]

Scheme 4



The fourth mechanism, path d in Scheme 4, proposed by Knowles et al is the one involving heterolytic cleavage of chorismate at the C3–O bond, after protonation to form intermediate **46**, and subsequent addition of pyruvate to C1 to form prephenate (**10**).[20] A heterolytic pathway for the enzymatic reaction is supported by suggestions of Gajewski et al[12] and of Coates et al[23] that the transition state of a variety of non-enzymatic (thermal) Claisen rearrangements has considerable dipolar character.

In order to study the mechanism of [3,3] rearrangement in further detail, efforts have been made to establish the structural features that are essential for enzyme catalysis. Haslem and coworkers have reported investigations with **42** (Table 1) and **47**.[24] Monoester **47** was neither a substrate for chorismate mutase nor an inhibitor for the processing of **9**.[24] This observation established that the side-chain carboxylate group is essential for enzyme activity.

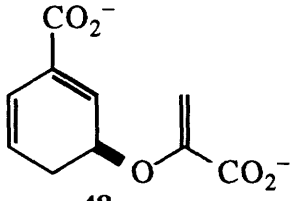


47

Haslem et al also reported that dihydrochorismate **42** did not display any tendency to rearrange with chorismate mutase, but it was a modest inhibitor. Berchtold and Delany reported that dihydro analog (**42**) is, in fact, an excellent substrate for chorismate mutase ($k_{cat} / k_{uncat} = 10^6$).[18] Observation of enzymatic catalysis for **42** requires special experimental conditions since the uncatalyzed (thermal) reaction is so slow compared to the uncatalyzed (thermal) rearrangement of chorismate. The 6-oxa-5,6-dihydrochorismate analog **43** reported by Berchtold and Delany is also a good substrate for chorismate mutase

(k_{cat} / k_{uncat} = 4 × 10^5).[18] Therefore, the sp^2 configuration at C5 and C6 is not necessary for mutase catalysis.

The importance of the C1 carboxylate group and the C4 hydroxyl group was determined by enzymatic studies on the following analogs in Table 1.[14] The chorismate analog lacking the C1 carboxylate group (**35**) and in which the carboxylate group was replaced with phosphonate group (**37**) were not substrates for chorismate mutase. Methyl ether **39** was a good substrate for chorismate mutase (k_{cat} / k_{uncat} = 2 × 10^4).[14] In the presence of large amount of enzyme, it was demonstrated that the Claisen rearrangement of enantiomerically pure **48** was accelerated at least 100-fold by chorismate mutase.[14]



48

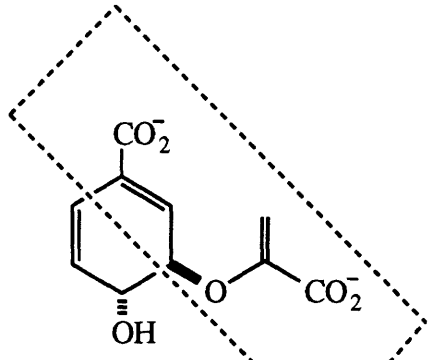
$t_{1/2}$ = 3.5 min
K_m = 0.01 mM

Attaching a methyl group at C9 on the side chain of the enolpyruvate moiety, compound **38**, increases the rate of rearrangement in the uncatalyzed (thermal) reaction (Table 1). This effect of a Z-methyl group has been noted previously for thermal Claisen-type rearrangements.[17a] Compound **38** is a fairly good substrate for the chorismate mutase catalyzed reaction (k_{cat} / k_{uncat} = 4.2 × 10^4).[17b]

From the results described above Berchtold and coworkers concluded that the hydroxyl group is neither essential for binding of the substrate to the active site of the enzyme nor for catalysis of the Claisen rearrangement. Therefore, the structural requirements for catalysis of the Claisen rearrangement by chorismate mutase are indicated in the dotted box as shown in Chart 1.[14] In addition to the

allyl vinyl ether moiety, the enzyme requires only the two carboxylate groups for the active site binding and catalysis. The hydroxyl group, although not required, may increases catalytic efficiency. Path b and path c proposed in Scheme 4 could thus be eliminated based on the results that the C4-hydroxyl group is not required for catalysis.

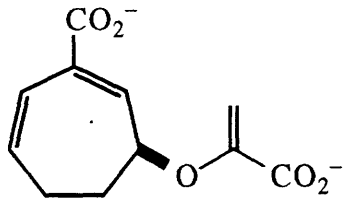
Chart 1



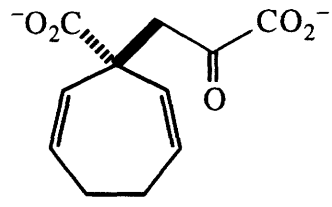
Chorismate **9**

Interestingly, the cycloheptadiene analog **49** (Chart 2), whose structure meets the structural requirements for the chorismate mutase catalysis, was reported by Pawlak and Berchtold.[25] The half-life for the uncatalyzed (thermal) rearrangement rate of compound **49** was 16 h at 80 °C in DMSO, and it was not a substrate for chorismate mutase-prephenate dehydrogenase (CM-PD), anthranilate synthase (AS), or *p*-aminobenzoate synthase (PABS);[25] however, it was a good inhibitor of the reaction catalyzed by these enzymes. The prephenate analog (**50**) in Chart 2 did not inhibit the dehydrogenase reactivity of CM-PD. Cycloheptadiene analog **49** could be a substrate for chorismate mutase; but due to the slow rate of rearrangement, catalysis is not observed under normal conditions as described earlier for **41** and **42** in Table 1.

Chart 2



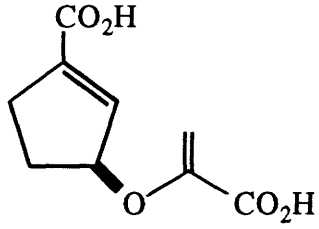
49



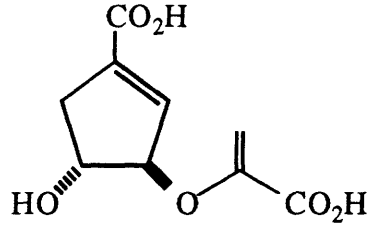
50

Since dihydrochorismate (**42**) and 6-oxa-5,6-dihydrochorismate (**43**) were extremely good substrates for chorismate mutase, and cycloheptadiene analog **49** could be a substrate, potential substrates **51** and **52** in Chart 3 were designed in order to further investigate the mechanism of the mutase-catalyzed rearrangement.

Chart 3



51



52

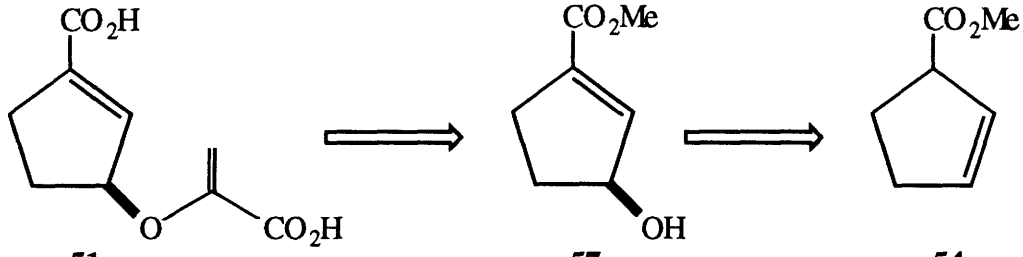
Of possible significance with compounds **51** and **52** is the fact that the dihedral angle C1–C5–C4 is less than that in six- or seven-membered analogs,[26] where the nature of the carbon center (C5) is crucial for the uncatalyzed (thermal) rate of rearrangement. Secondly, it would be interesting to examine the spatial tolerance of the active site of chorismate mutase; and steric effects might be explored with the five-membered analogs by comparison with the six and seven-membered ring compounds.

Results and Discusion

Synthesis of 3-[[1-(Carboxy)ethenyl]oxy]-1-cyclopentene-1-carboxylic Acid (51).

Retrosynthetic analysis indicated that **51** could be designed from β,γ-olefin **54** via allylic alcohol **57** in Scheme 5. Thus, epoxidation of **54,** followed by base-catalyzed rearrangement ought to give allylic alcohol **57.** The side-chain could be appended as described by Berchtold and coworkers for the total synthesis of optically pure chorismic acid,[6] and saponification would lead to compound **51.**

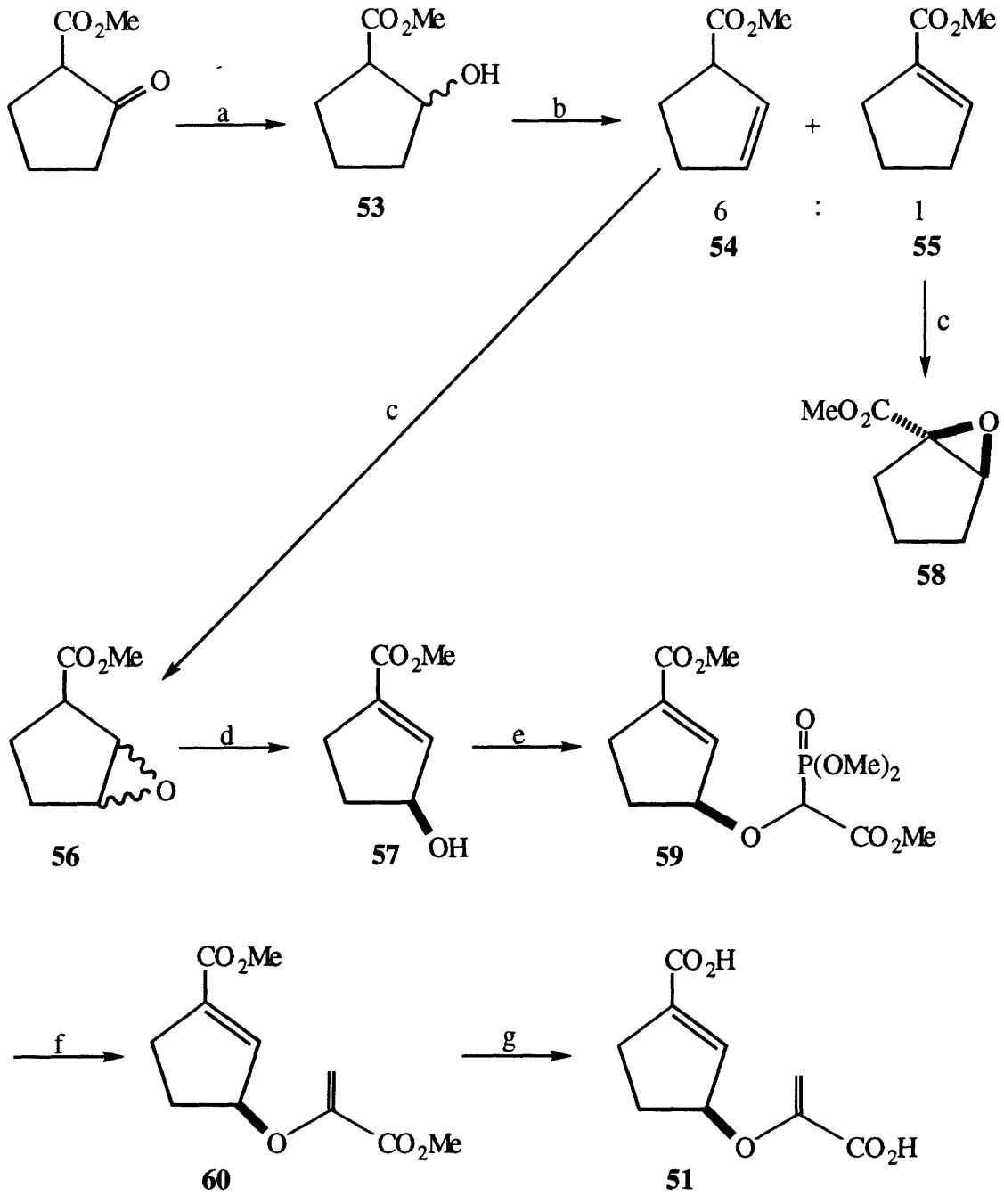
Scheme 5



51 **57** **54**

The route used for the synthesis of the cyclopentenyl analog of chorismate is depicted in Scheme 6. Methyl 2-oxocyclopentanecarboxylate was converted to a mixture of epimeric alcohols **53** in excellent yield by reaction with $NaBH_4$ in absolute methanol. Dehydration under the action of P_2O_5 in warm benzene gave regioisomers (α,β-olefin (**55**) / β,γ-olefin (**54**) in a 1:6 ratio (by NMR) in quantitative yield.[27] The ratio of regioisomers can also be determined either by oxidizing the mixture with MCPBA to provide epoxides **56** and **58** which are more easily separated or by analysis by MPLC.

Scheme 6[a]



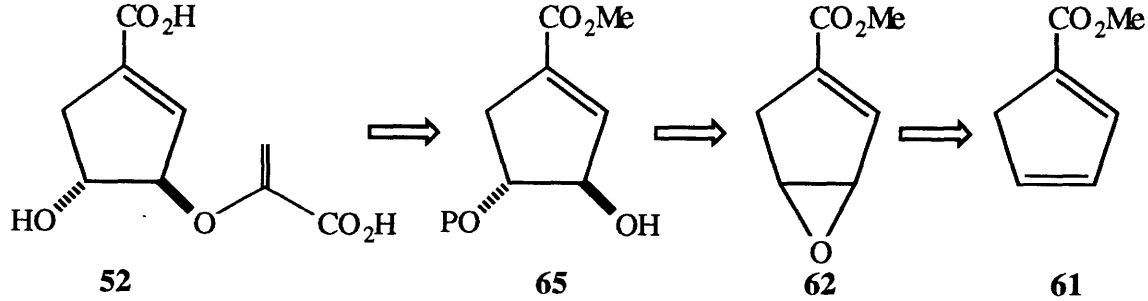
[a] Reagents: (a) $NaBH_4$, MeOH; (b) P_2O_5, benzene, Δ; (c) MCPBA, CH_2Cl_2-NaH_2PO_4, pH = 8; (d) NaOMe, MeOH; (e) $MeO_2CC(N_2)PO(OMe)_2$, Rh_2(Octanoate)$_4$, benzene, 60 °C; (f) $LiN(TMS)_2$, H_2CO, THF, -78 °C; (g) LiOH, THF-H_2O.

Epoxidation of **54** with MCPBA in CH_2Cl_2-$NaH_2PO_{4(aq)}$ proceeded smoothly to give epoxide **56**, which was rearranged with NaOMe in dry MeOH in an ice bath to give allylic alcohol **57** in an quantitative yield. Coupling of **57** and trimethyl diazophosphonoacetate with rhodium(II) octanoate catalysis in dry benzene at 60 °C gave phosphonate **59** in 85% yield after flash column chromatography. Ester **59** was converted to **60** by the Wittig type reaction with strong base, $LiN(TMS)_2$, and quenching with gaseous formaldehyde at -78 °C to give **60** in an excellent yield. Saponification of **60** gave diacid **51** in 75% yield. The overall yield to diacid **51** from methyl 2-oxocyclopentanecarboxylate was 33%.

Synthesis of *trans*-3-[[1-(Carboxy)ethenyl]oxy]-4-hydroxy-cyclopentene-1-carboxylic Acid (52)

The retrosynthesis of hydroxyl five-membered analog **52** is shown in Scheme 7. The key intermediate in this synthetic plan was the allylic epoxide **62** which was derived from cyclopentadiene derivative **61**. Thus, epoxide **62** opening, followed by protection and deprotection procedures would give allylic alcohol **65**. The side-chain could be constructed as described above in the synthesis of compound **51**, and saponification would lead to compound **52**.

Scheme 7



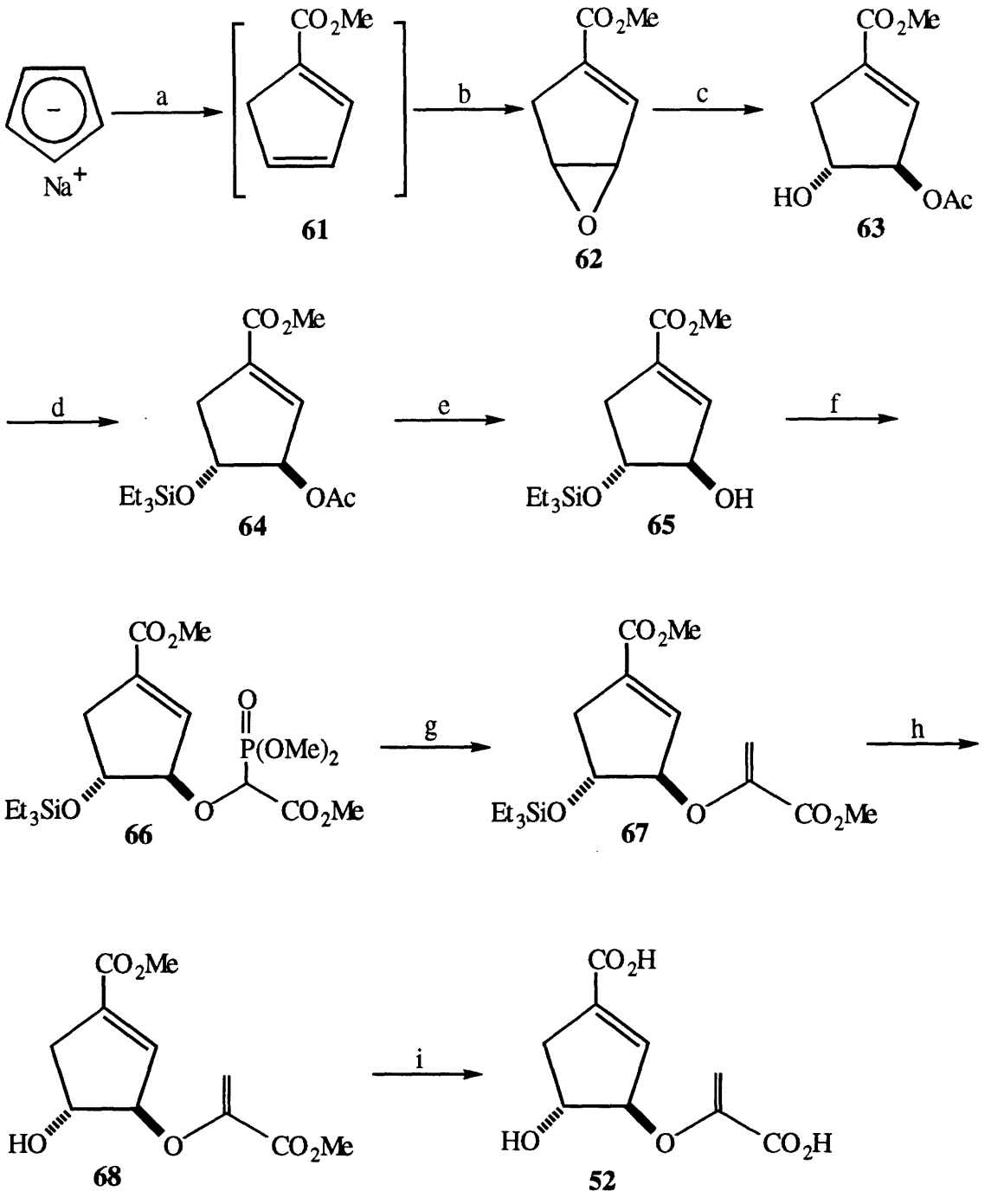
52 **65** **62** **61**

P = Protecting group

The synthesis of this novel compound **52** is shown in Scheme 8. The very unstable cyclopentadiene derivative **61** was prepared in situ by a modification of the procedure reported by Peters.[28] Without further purification, compound **61** was oxidized with MCPBA in CH_2Cl_2-$NaH_2PO_{4(aq)}$ to give key epoxide intermediate **62** in moderate yield after two steps. Epoxide **62** reacted with glacial acetic acid[29] at a temperature not higher than 40 °C and a reaction time not longer than 3 h to give alcohol **63** in good yield with high regioselectively. Unknown side products would appear at higher temperature or longer reaction time and lower the yield of desired product.

The free hydroxyl group of **63** was protected with triethylsilyl bromide in the presence of DBN in benzene to generate compound **64** in quantitative yield. Reaction of ester **64** with sodium methoxide in absolute methanol gave allylic alcohol **65** in good yield. Coupling of **65** and trimethyl diazophosphonoacetate with rhodium(II) octanoate catalysis in dry benzene at 60 °C gave phosphonate **66** in 70% yield after flash column chromatography. Ester **66** was transformed to **67** by the Wittig type reaction with strong base, $LiN(TMS)_2$, followed by quenching with gaseous formaldehyde at -78 °C to give **67** in quantitative yield. Desilylation of **67** with *tetra* n-butylammonium fluoride provided the homoallylic alcohol **68** in good yield. Saponification of **68** gave diacid **52** in 75% yield. The overall yield to diacid **52** from sodium cyclopentadienylide was 5%.

Scheme 8[a]

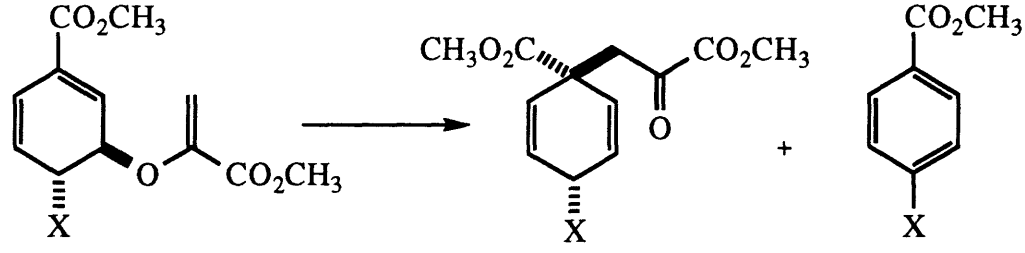


[a] Reagents: (a) reverse addition of chloroformate, THF; (b) MCPBA, CH_2Cl_2-NaH_2PO_4, pH = 8; (c) glacial acetic acid, 60 °C; (d) triethylsilylbromide, DBN, benzene; (e) NaOMe, MeOH; (f) $MeO_2CC(N_2)PO(OMe)_2$, $Rh_2(Octanoate)_4$, benzene, 60 °C; (g) $LiN(TMS)_2$, H_2CO, THF, -78 °C; (g) $(n\text{-}Bu)_4N^-F^+$, THF; (i) LiOH, THF-H_2O, 0 °C.

Thermal Rearrangement of Dimethyl Esters 60 and 68

Dimethyl esters **60** and **68** were quite stable and showed no tendency to rearrange in $CDCl_3$.. These were in stark contrast to the behavior of dimethyl 4-*deshydroxy*-chorismate (Scheme 9), which had a half-life for disappearence of starting material of 3.6 h at 30 °C in $CDCl_3$.[14] Dimethyl chorismate was more stable under these conditions; however, complete rearrangement of dimethyl chorismate was observed after heating for 36 h at 55 °C in DMSO-*d6*.[6]

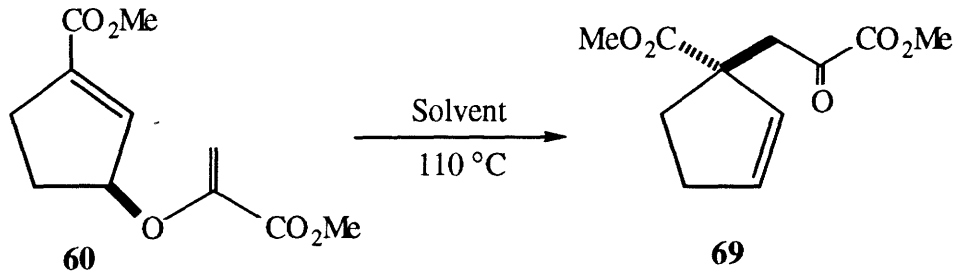
Scheme 9



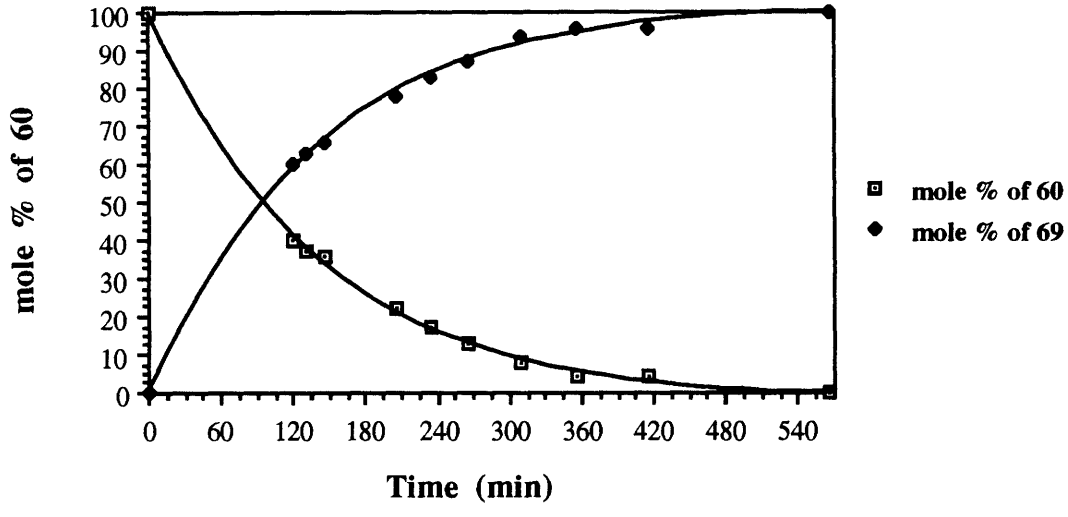
Dimethyl 4-*deshydroxy*-Chorismate, X = H
Dimethyl Chorismate, X = OH

In comparison, the half-lives for disappearance of **60** and **68** in DMSO-*d6* at 110 °C were 1.5 h and 6 h; and in *o*-dichlorobenzene-*d4* they were 1 h and 4.25 h, respectively. The data points to establish the half-lifes were collected by high field proton NMR and are in Scheme 10 and Scheme 11. The hydrolyzed trans diol product, **71**, was found during the heating of hydroxy dimethyl ester **68** only in DMSO-*d6*. It is undoubtedly due to a trace of water in DMSO-*d6*. The half-life for disappearance of **68** measured in DMSO-*d6* has been corrected for the formation of hydrolysis product.[30] No hydrolyzed product could be detected (^1H NMR) after heating dimethyl ester **60**. Complete transformation of **60** to **69** was

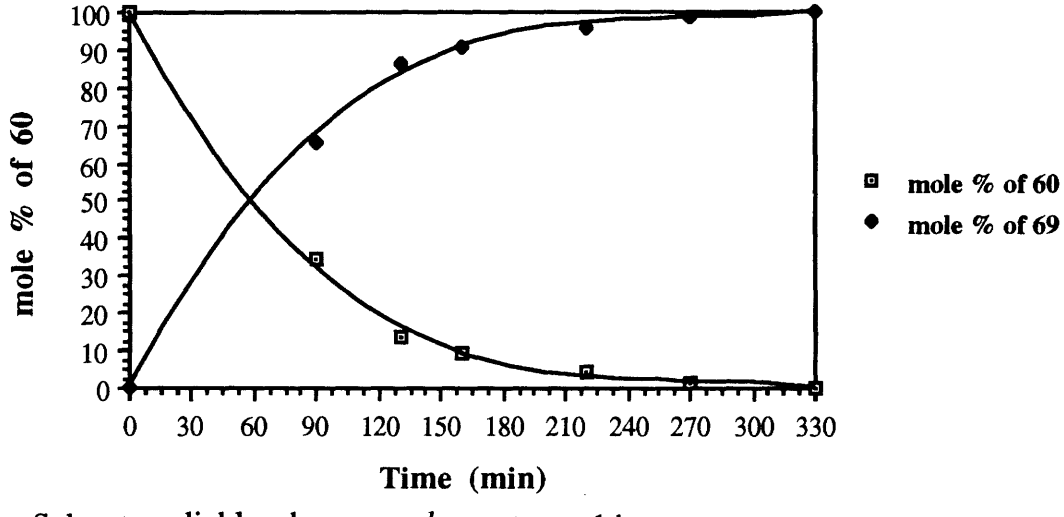
Scheme 10



60 **69**

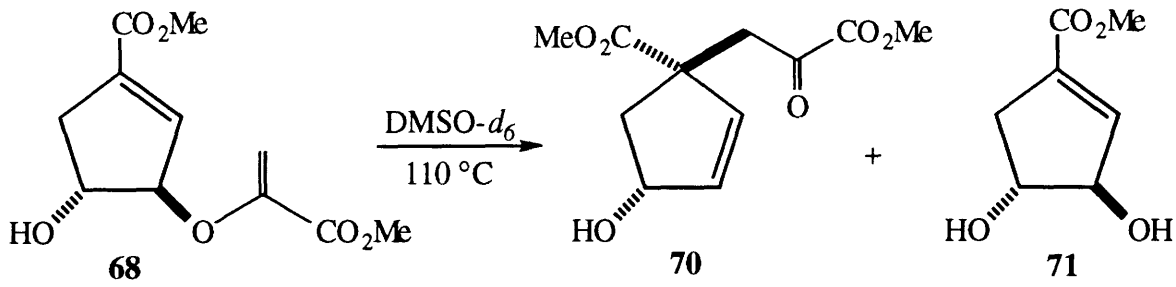


Solvent: DMSO-*d6* $t_{1/2}$ = 1.5 h

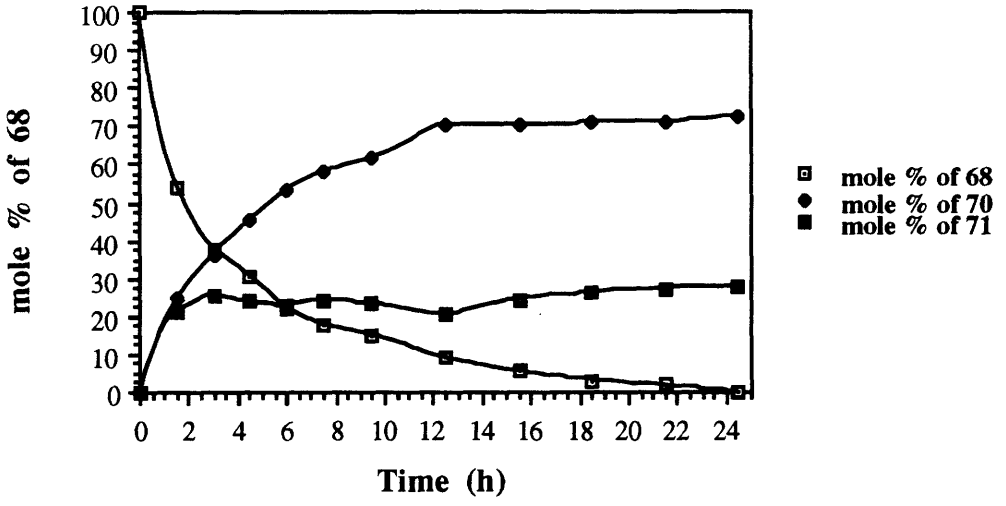


Solvent: *o*-dichlorobenzene-*d4* $t_{1/2}$ = 1 h

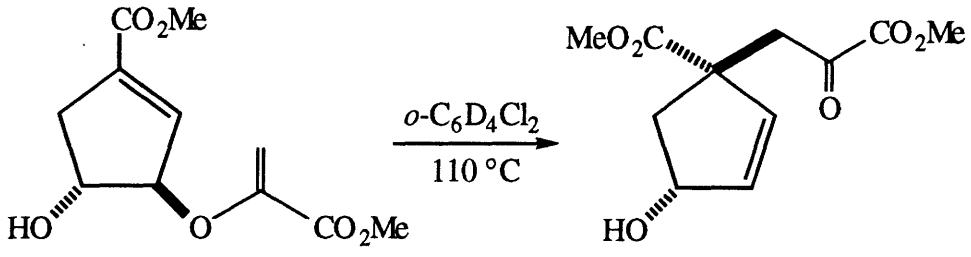
Scheme 11

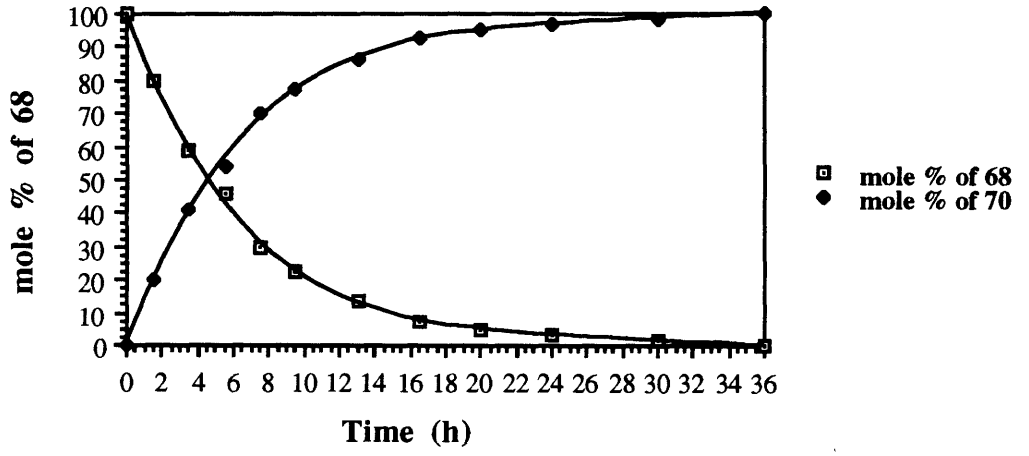


68 **70** **71**



Solvent: DMSO-*d6* $t_{1/2} = 6$ h



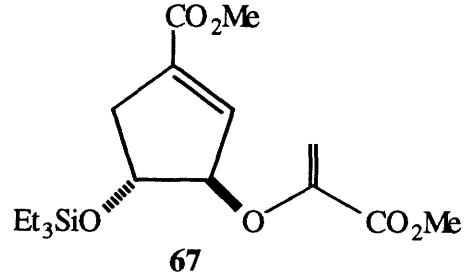


Solvent: *o*-dichlorobenzene-*d4* $t_{1/2} = 4.25$ h

observed after 9 h in DMSO and after 6 h in *o*-dichlorobenzene. Complete transformation of **68** to **70** was observed after 36 h in *o*-dichlorobenzene.

Previous work has shown that the rate of Claisen rearrangement of chorismate and chorismate analogs shows a solvent dependence.[12, 21] The increase in rate with increasing solvent polarity has been used to argue that the rearrangement involves a dipolar transition state. Similar conclusions have been drawn with other related Claisen rearrangements in various solvents.[23] Esters **60** and **68** do not show a significant change in rate of rearrangement as a function of solvent polarity (*o*-dichlorobenzene-d_4 vs DMSO-d_6) and, in fact, the rate was slightly faster in the less polar solvent for both compounds.

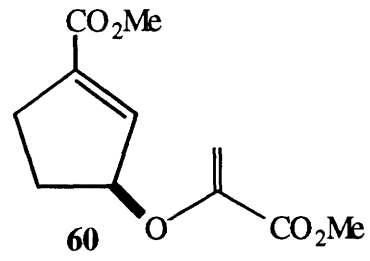
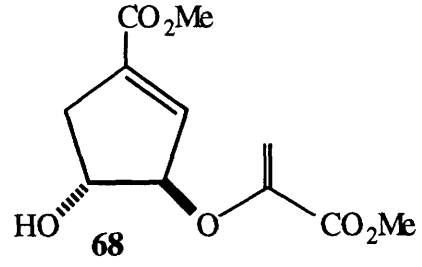
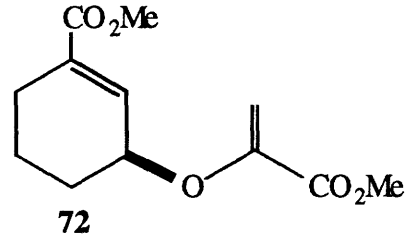
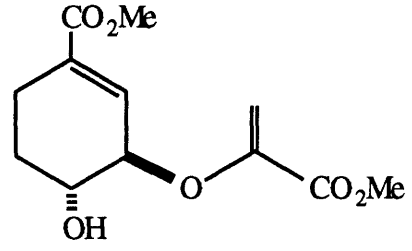
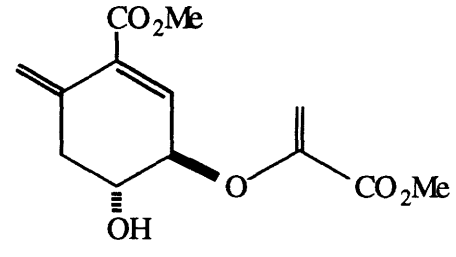
The half-lives for the thermal rearrangement of **60** and **68** are compared with the half-lives for rearrangement of the six-membered chorismate analogs[31] **72** - **74** in Table 2. It is interesting to note that the ratio for the half-life for rearrangement of **60**:**68** is equal to the corresponding ratio for **72**:**73**. Intramolecular hydrogen-bonding to retard **68** and **73** from adapting the chair conformation for Claisen rearrangement is not expected to be an important factor for the four-fold decrease in rate for the C4 hydroxy derivatives at 110 °C. In fact, silyl derivative **67** rearranged with the same half-life, 6 h, as **68** in DMSO-d_6 under the same conditions.[33] Hence the rate-retarding effect of the C4 hydroxyl substituent appears to be due to an inductive effect.



67

Table 2

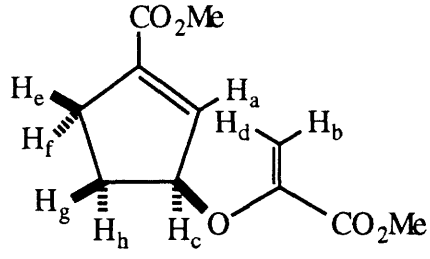
Uncatalyzed Thermal Claisen Rearrangement

Compound	Half life
 **60**	$t_{1/2}$ = 1.5 h (DMSO-*d6*, 110 °C) $t_{1/2}$ = 1 h (*o*-Dichlorobenzene-*d4*, 110 °C)
 **68**	$t_{1/2}$ = 6 h (DMSO-*d6*, 110 °C) $t_{1/2}$ = 4.25 h (*o*-Dichlorobenzene-*d4*, 110 °C)
 **72**	$t_{1/2}$ = 25.3 h (DMSO-*d6*, 110 °C)
 **73**	$t_{1/2}$ = 100 h (DMSO-*d6*, 110 °C)
 **74**	$t_{1/2}$ = 29.3 h (DMSO-*d6*, 110 °C)

On the other hand it appears that hydrogen-bonding does have some influence on the rate of Claisen rearrangement of dimethyl chorismate which showed no tendency to rearrange in $CDCl_3$ at 30 °C while the 4-epi analog, which can adapt the chair conformation for rearrangement without disrupting the hydrogen bonding, rearranged under same conditions with a half-life of 2.3 h.[6] This is a rather special case since presence of the C5–C6 double bond in these cases has a significant effect on increasing the rate of rearrangement, and a stereoelectronic effect due to the lone pairs on the C4 hydroxyl oxygen atom or the alignment of the C4–O sigma bond with the olefinic π system is likely to be important.[32] The observation that the 4-methoxy analog (dimethyl ester of **39**, Table 1) rearranges with a half-life of 185 h under the same conditions[14] suggests that all of these factors may be important.

It is difficult to predict whether conformational effects or the difference in bond angles in the carbocyclic systems are significant factors responsible for the faster rate of arrangement of the five-membered carbocyclic derivatives. The C1–C5–C4 bond angle for the five-membered analogs **60**, **67**, and **68** are estimated to be 102°, 103.5°, and 103.5°, respectively, while the C1–C6–C5 bond angle for the six-membered analogs **72** and **73** is 113°.[26] Diesters **60** and **68** were analyzed by ^1H NMR spectroscopy, and an unusual long-range coupling of 0.5 Hz was observed between the *Z*-enolpyruvyl hydrogen and the C3 hydrogen for both compounds. This long-range coupling has not been observed previously in the six-membered analogs studied in these laboratories. Irradiation of the C3 hydrogen of **60** (H_c in Table 3) at 5.20 - 5.14 ppm changed the absorption of the *Z*-9 hydrogen (H_d in Table 3) at 4.66 ppm from a doublet of doublets to a doublet. Similar results were observed on irradiation of the C3 hydrogen of **68**. A detailed analysis of the ^1H NMR spectrum of **60** and **68** is provided in Tables 3 and 4.

Table 3. ^{1}H NMR Data for Compound 60

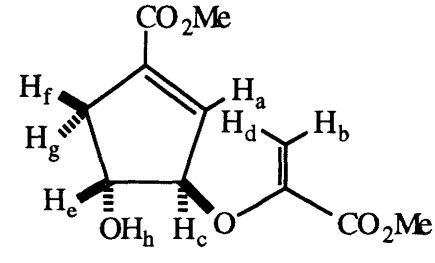


proton (d)[a]	coupling partner (J)[b,c]	mult
a (6.80)	c (2.8), e (2.8), f (2.8)	app. q (ddd)
b (5.44)	d (2.8)	d
c (5.20–5.14)	a (2.8), d (0.5), e, f, g, h,	ddm
d (4.66)	b (2.8), c (0.5)	dd
e (2.87–2.75)	a (2.8), c, f, g, h	dm
f (2.63–2.50)	a (2.8), c, e, g, h	dm
g (2.49–2.37)	c, e, f, h	m
h (2.12–2.00)	c, e, f, g	m

a. Chemical shifts are in ppm downfield from tetramethylsilane.

b. Coupling constants are in Hz. c. This includes only coupled protons, for which the assignment is unambiguous.

Table 4. ^{1}H NMR Data for Compound 68



proton (d)[a]	coupling partner (J)[b]	mult
a (6.70)	c (3.9), e (1.3), f (2.0), g (1.8)	dddd
b (5.52)	d (2.8)	d
c (4.94)	a (3.9), d (0.5), e (4.7), f (1.4), g (1.8)	ddddd
d (4.89)	b (2.8), c (0.5)	dd
e (4.61)	a (1.3), c (4.7), f (7.5), g (4.0), h (4.7)	ddddd
f (3.13)	a (1.8), c (1.8), e (7.5), g (17.1)	dddd
g (2.52)	a (1.4), c (2.1), e (4.0), f (17.2)	dddd
h (2.17)	e (4.7)	d

a. Chemical shifts are in ppm downfield from tetramethylsilane.

b. Coupling constants are in Hz.

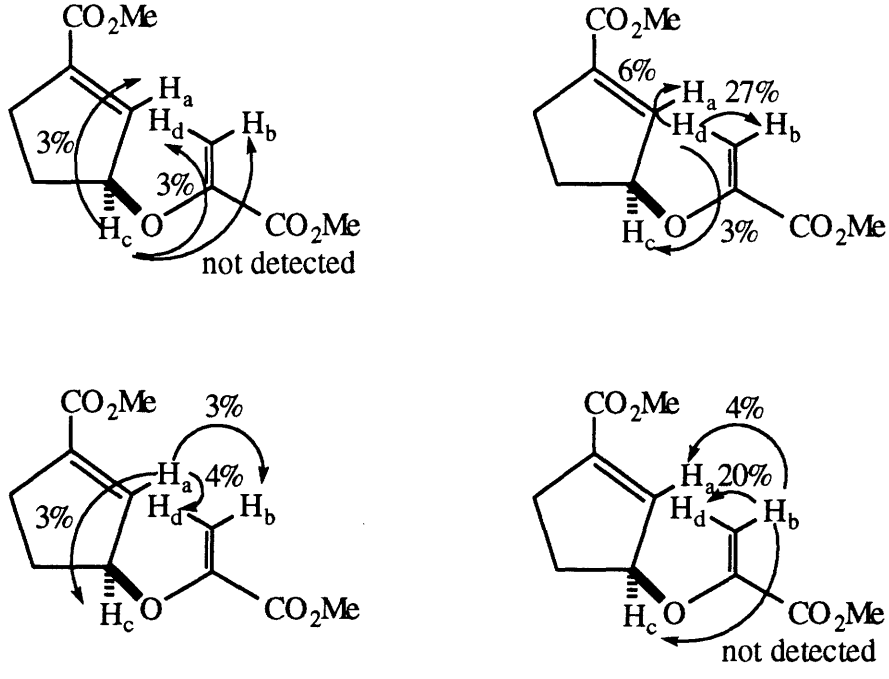
NOE Experiments with Dimethyl Esters 60 and 68

Hilvert and coworkers have reported some interesting nuclear Overhauser effects to support the conformation of chorismate in aqueous solution and the conformation of antibody-bound chorismate with the antibody that catalyzed the Claisen rearrangement of chorismate.[34] For chorismate in aqueous solution, irradiation of the *Z*-9 enolpyruvyl hydrogen gave a small positive NOE to the C2 hydrogen as expected for the pseudodiequatorial conformer (Scheme 3) which has been established as the predominant conformer in aqueous solution.[34a] On the other hand in the presence of the catalytic antibody, NOE's were observed not only between the *Z*-9 enolpyruvyl hydrogen and the C2 hydrogen but also between the *E*-9 enolpyruvyl hydrogen and the C6 hydrogen. These observations provide evidence that the antibody binds the higher energy pseudodiaxial conformer (Scheme 3).

NOE measurements with **60** and **68** were undertaken in $CDCl_3$ to see whether the data would provide any useful information about their solution

conformation. The NOE results with **60** and **68** are listed in Scheme 12 and 13, respectively. Presaturation of proton H_c of **60** gives a 3% NOE to H_d, and no enhancement was detected for H_b.

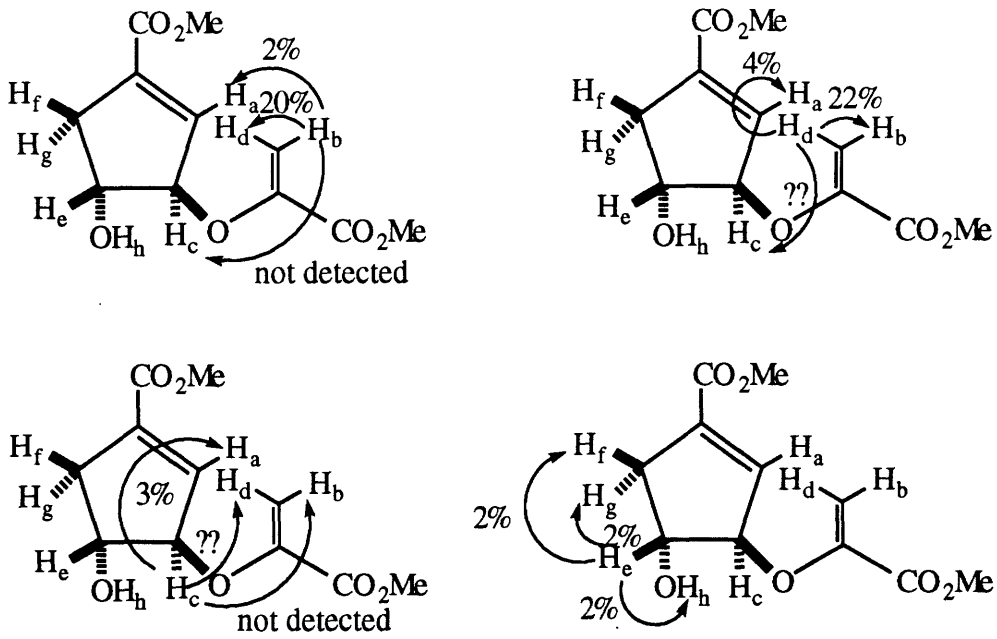
Scheme 12



Scheme 13 shows the NOE results of compound **68** in $CDCl_3$ with tetramethylsilane as internal standard. For compound **68** irradiation of H_d results in a small positive NOE to H_a and a moderate NOE to H_b as expected for an orientation of the enolpyruvyl side chain similar to that for the predominant confomer of chorismate in aqueous solution. The small negative NOE apparent to H_c when H_d is preirradiated is probably an off-resonance effect of irradiation at the frequency of H_d, which produces some spillover saturation to H_c. This effect was also observed by Hilvert and coworkers.[34a] Irradiation of H_b does not give a detectable NOE at H_f, and this observation also suggests that the enolpyruvate side chain is not positioned over the cyclopentenyl ring in solution. The small NOE

that is detected for H_a when H_d is preirradiated likely arises from spin diffusion through H_b.

Scheme 13



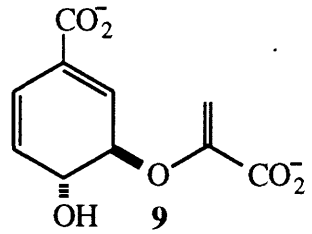
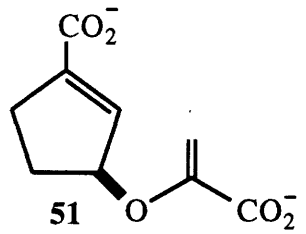
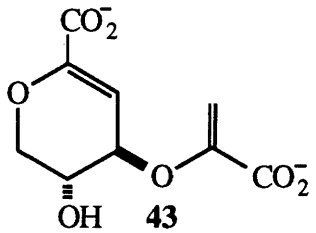
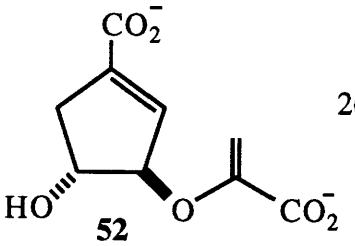
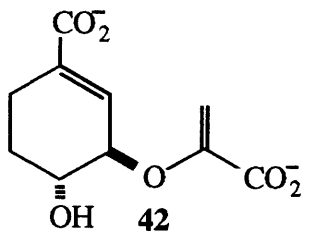
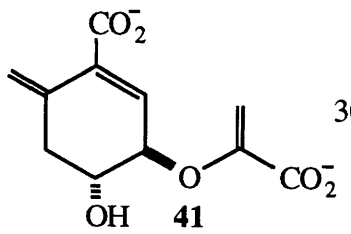
As discussed above, the rearrangement of these two analogs **60** and **68** to **69** and **70** requires a large conformational change in which diequatorial conformers that predominate in solution are convered to the diaxial conformers on the way to the geometrically constrained transition state.

Thermal Rearrangements of 51, 52

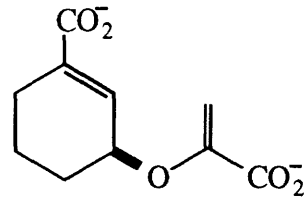
Analogs **51** and **52** rearranged thermally at 30 °C at a rate 5 times and 170 times, respectively, more slowly than chorismate (**9**), but **52** rearranged 18 times faster than **42** (Table 5). Although diester **72** was not hydrolyzed to the diacid **75** by previous investigators for measurement of the rate of rearrangement, it appears reasonable that **51** rearranges ~18 times faster than the diacid **75** derived from **72**

Table 5

Thermodynamic Data for Uncatalyzed Claisen Rearrangement (30 °C, pH = 7.5)

Compound	$\Delta G^{\ddagger}$(Kcal/mol)	$\Delta H^{\ddagger}$(Kcal/mol)	$\Delta S^{\ddagger}$(cal/mol °K)	$k_{30\,°C}(sec^{-1})$
 **9**	24.7	20.7	-12.8	1.22×10^{-5} ($t_{1/2}$ = 15.6 h)
 **51**	25.5	22.1	-11.4	2.4×10^{-6} ($t_{1/2}$ = 79 h)
 **43**	27.2	26.9	-1.0	1.6×10^{-7} ($t_{1/2}$ = 1200 h)
 **52**	26.3	24.7	-5.3	7.1×10^{-8} ($t_{1/2}$ = 2710 h)
 **42**	29.4	25.9	-11.6	3.9×10^{-9} ($t_{1/2}$ = 49000 h)
 **41**	30.8	29.4	-4.7	3.7×10^{-10} ($t_{1/2} = 5\times10^{5}$ h)

since diesters **60** and **68** both rearranged 17 times faster than **72** and **73** respectively, in DMSO-d_6 at 110 °C (Table 2).



75

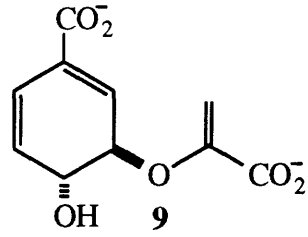
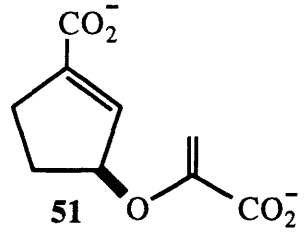
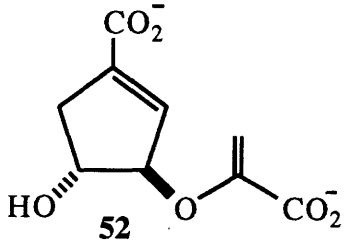
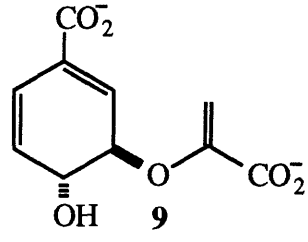
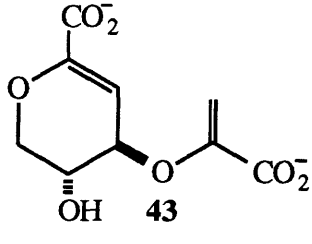
Arrhenius plots[7a] were obtained for the rearrangements of **51** and **52**. From the data, activation parameters for the rearrangement at 30 °C were obtained (Table 5). Chorismate (**9**), 5,6-dihydrochorismate (**42**) and analog **51** show similar $\Delta S^{\ddagger}$ values for reaarrangement. On the other, pyran **43,** hydroxy analog **52,** and *exo*-methylene analog **41** have less negative $\Delta S^{\ddagger}$ values. This is perhaps due to the rotational freedom of the different ring systems.

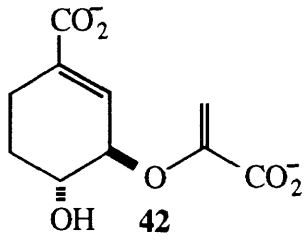
Enzymatic Studies of 51 and 52 with Chorismate Mutase

Cyclopentenyl analogs **51** and **52** were tested as substrates and as inhibitors for chorismate mutase. The data are presented in Table 7. The first entry for **9** and the entries for **51** and **52** are results with the monofunctional chorismate mutase from *Bacillus subtilis*.[35] The second entry for **9** and the entries for **42** - **43** are results with the mutase activity of the bifunctional enzyme chorismate mutase-prephenate dehydrogenase from *Escherichia coli*.[36] Unfortunately our sample of the bifunctional enzyme lost all activity on storage, and our source of the enzyme was no longer available. Nonetheless, the comparisons with the two different enzymes is meaningful. The kinetic parameters are given in Table 7.

Table 7

Enzymatic Data for Catalyzed Claisen Rearrangement by Chorismate Mutase (30 °C, pH = 7.5)[a]

Compound	$k_{uncat}(sec^{-1})$[b]	$k_{cat}(sec^{-1})$	Acceleration	$K_m(\mu M)$	$K_i(\mu M)$
 **9**	1.22×10^{-5} ($t_{1/2}$ = 15.6 h)	50[c, 34]	4×10^{6}	100[34]	
 **51**	2.4×10^{-6} ($t_{1/2}$ = 79 h)	4.2×10^{-3}[c]	1760	158	27.5
 **52**	7.1×10^{-8} ($t_{1/2}$ = 2710 h)	0.46[c]	6.5×10^{6}	275	71
 **9**	1.22×10^{-5} ($t_{1/2}$ = 15.6 h)	25[d]	2×10^{6}	140	
 **43**	1.6×10^{-7} ($t_{1/2}$ = 1200 h)	0.07[d]	4×10^{5}	280	



42	3.9×10^{-9} ($t_{1/2}$ = 49000 h)	0.012[d]	3×10^{6}	55

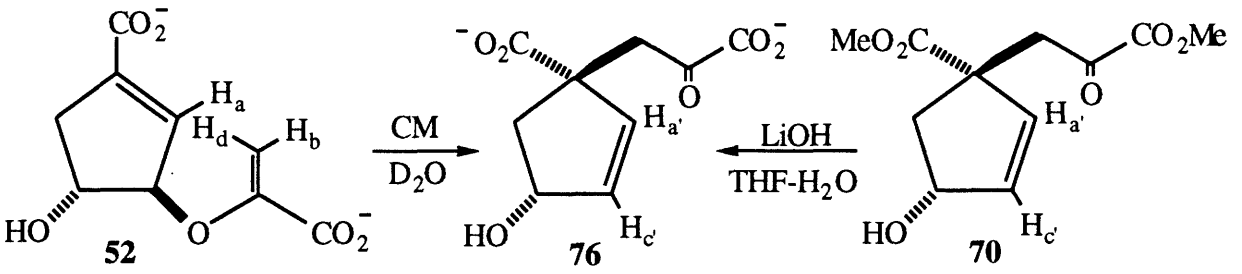
a. See experimental section for the details of the enzymatic measurements. b. The uncatalyzed (thermal) rate were measured at 30 °C, pH = 7.5. c. The catalyzed rate was measured by the monofunctional enzyme, chorismate mutase. d. The catalyzed rate was measured by the bifunctional enzyme, CM-PD.

Remarkably, the mutase accelerates the rearrangement of **52** by a factor of 6.5×10^{6} over the rate of the thermal reaction, whereas the rate acceleration observed for *des*hydroxy analog **51** by the enzyme was only a factor of 1.8×10^{3}. The difference in rate enhancement between **51** and **52** is about 3.7×10^{3}, which was similar to that estimated as a maximum difference in rate enhancement between chorismate (**9**) and *des*hydroxychorismate (**48**) from earlier work in this laboratory (~10^{4}).[14] It must be pointed out that results with **51** and **48** utilized a large excess of enzyme and, consequently, whether results are from steady state conditions is open to question. In the present study the concentration of chorismate mutase used to observe enzyme-catalyzed turnover of **51** was 10^{5} times the concentration used to follow the metabolism of chorismate under normal conditions. And for **52** it was 2×10^{3} times the concentration used to follow the metabolism of chorismate under normal conditions. These results support the contention that the C4 hydroxyl group of chorismate is not necessary for enzymatic activity, but the presence of the hydroxyl group provides for more effective binding and increased turnover by the enzyme.

That the enzyme-catalzyed product was the product of Claisen rearrangement (**76**) was established by the fact that it was identical to the product

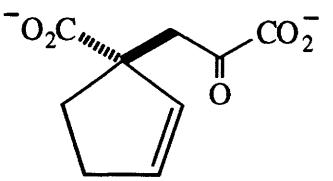
from base-catalyzed hydrolysis of **70** (Scheme 14). In addition the metabolism of **52** by chorismate mutase in D_2O could be monitored by ^1H NMR. It was possible to follow the disappearance of protons H_a, H_b, and H_d of **52** (Scheme 14) and the appearance of $H_{a'}$ and $H_{c'}$ as the reaction progressed.

Scheme 14



Since the turnover of **51** and **52** is slow compared to chorismate, it was possible to measure their activity as competitive inhibitors of the processing of chorismate by chorismate mutase. Both were good competitive inhibitors. The *des*hydroxy analog **51** had a K_i = 27.5 μM ([**51**] = 102 μM) with the K_m for chorismate (**9**) equal to 85.4 μM. The hydroxy analog **52** had a K_i = 71 μM ([**52**] = 107 μM) with the K_m for chorismate (**9**) equal to 103 μM.

Unfortunately, the inhibition of compounds **76** (Scheme 14) and **77** to CM-PD could not be studied since the enzyme activity was lost on storage. Presumably, both compounds **76** and **77** might competive inhibitors for CM-PD based on the inhibition studies of compounds **51** and **52** to chorismate mutase.

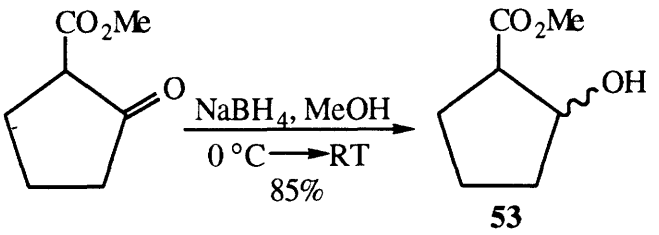


77

Experimental

^{1}H and ^{13}C NMR spectra were obtained in $CDCl_3$ unless otherwise noted on Bruker 250 MHz or Varian 300 MHz instruments. Chemical shift values are reported in parts per million downfield from tetramethylsilane. IR spectra were recorded using salt plates (oils) or KBr pellets (solids). Melting points were uncorrected. Mass spectra were recorded on a Finnegan MAT 2800 using electron ionization or fast atom bombardment techniques. All reactions were carried out under a positive pressure of nitrogen unless otherwise noted. Reaction product solutions were concentrated using a Büchi evaporator at aspirator pressure. High vacuum pressure is $< 10^{-3}$ mm Hg. Reaction mixtures were stirred magnetically unless otherwise noted. Tetrahydrofuran was distilled from sodium and benzophenone. All other reagents that required distillation were purified as described in Perrin and Perrin.[37]

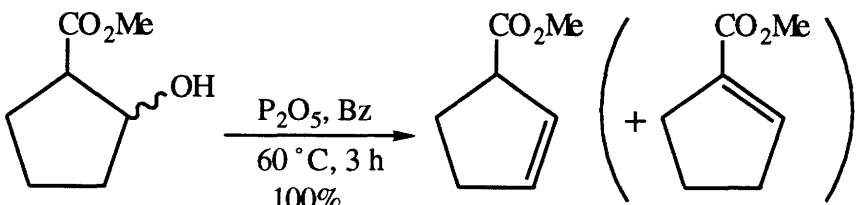
Methyl 2-Hydroxycyclopentane-1-carboxylate (53).



53

To a solution of methyl 2-oxocyclopentanecarboxylate (5 g, 4.5 mL, 351 mmol) in dry MeOH (50 mL) was added $NaBH_4$ (2 g, 527 mmol, 1.5 equiv) slowly at 0 °C. After addition was complete, the reaction mixture was stirred at room temperature for 12 h, and monitored by TLC which indicated starting material had disappeared. The reaction solution was quenched with H_2O and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated to give a white solid. The solid was triturated with CH_2Cl_2, passed through a pad of celite, and concentrated to give a pale yellow oil. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:2). Compound **53** (4.3 g, $R_f = 0.34$) was obtained as a colorless oil (85% yield). IR (neat) 3429, 2951, 2875, 1725, 1434, 1200, 1138 cm^{-1}; ^1H NMR (300 MHz) δ 4.3 - 4.5 (1 H, m), 3.8 - 3.7 (3 H, two singlets), 2.8 - 2.6 (1 H, m), 2.1 - 2.5 (6 H, m); MS m/z (relative intensity) 144 (M^+, 22.9), 129 (16.2), 114 (25), 101 (36), 87 (100), 67 (33); HRMS calc for $C_7H_{12}O_3$: 144.0786; Found 144.0785.

Methyl 2-Cyclopentene-1-carboxylate (54).

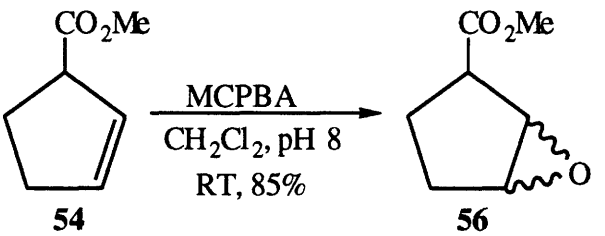


53 **54** : **55**

6 : 1

To compound **53** (1.5 g, 10.4 mmol) in benzene (25 mL) was added P_2O_5 (1.5 g, 10.4 mmol, 1 equiv). The mixture was heated to reflux for 3 h and then cooled to room temperature. The mixture was poured into cold H_2O, extracted with ether, and washed with brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo to give regioisomers (α, β-olefin/β, γ-olefin 1:6 by NMR). The mixture was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 6:1) to give **54** (1.12 g, R_f = 0.45) as a colorless oil (86% yield). IR (neat) 2948, 2918, 2857, 1734, 1627, 1435, 1196, 1171 cm^{-1}; ^1H NMR (300 MHz) δ 6.0 - 5.8 (1 H, m), 5.8 - 5.7 (1 H, m), 3.69 (3 H, s), 3.6 - 3.5 (1 H, m), 2.6 - 2.3 (4 H, m); ^{13}C NMR (75 MHz) δ 174.1, 143.4, 133.4, 120.6, 51.5, 50.3, 32.0, 26.3; MS m/z (relative intensity) 126 (M^+, 8.6), 111 (12.3) 95 (18), 87 (100), 67 (42); HRMS calc for $C_7H_{10}O_2$: 126.0681; Found 126.0680.

Methyl 6-Oxabicyclo[3.1.0]hexane-2-carboxylate (56).

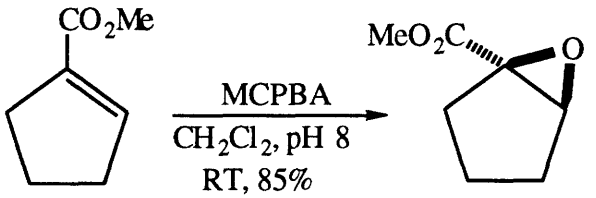


54 **56**

To compound **54** (2.5 g, 19.8 mmol) in CH_2Cl_2-NaH_2PO_4(aq) (40 mL, v/v = 1:1) was added *m*-CPBA (7.6 g, 50-60% purity, 1.2 equiv) at 0 °C. The reaction was stirred at room temperature for 12 h and monitored by TLC which indicated starting material had disappeared. The mixture was poured into saturated $Na_2S_2O_3$(aq) to destroy excess *m*-CPBA and was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:4) to give **56** (2.40 g, R_f = 0.35) as a pale yellow oil (85% yield). IR (neat) 3032, 2955, 2857, 1739,

1437, 1319, 1270, 1204 cm^{-1}; ^1H NMR (300 MHz) δ 6.0 - 5.8 (1 H, m), 3.75 (3 H, s), 3.70 - 3.67 (1 H, m), 2.89 (1 H, td, J = 2.4, 1.5 Hz), 2.21 - 2.07 (1 H, m), 1.90 - 1.62 (3 H, m); ^{13}C NMR (75 MHz) δ 172.7, 57.2, 56.2, 51.7, 45.1, 26.8, 21.2; MS (FAB) m/z (relative intensity) 127 ([M - CH_3]$^+$, 41.2), 111 ([M - OCH_3]$^+$, 57.7).

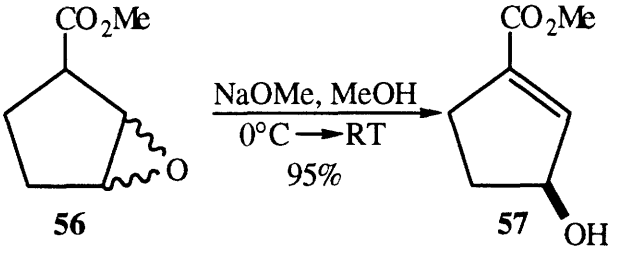
Methyl (1α,5α)-6-Oxabicyclo[3.1.0]hexane-1-carboxylate (58).



55 **58**

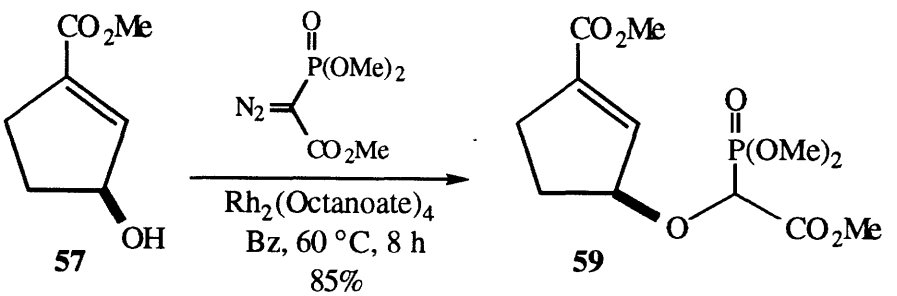
To compound **55** (1.25 g, 9.9 mmol) in CH_2Cl_2-NaH_2PO_4(aq) (20 mL, v/v = 1:1) was added *m*-CPBA (3.8 g, 50-60% purity, 1.2 equiv) at 0 °C. The reaction was stirred at room temperature for 12 h and monitored by TLC which indicated starting material disappeared. The mixture was poured into saturated $Na_2S_2O_3$(aq) to destroy excess *m*-CPBA and was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, filtered, concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:4) to give **58** (1.20 g, R_f = 0.50) as a pale yellow oil (85% yield). IR (neat) 2956, 2851, 1735, 1439, 1495, 1282, 1204 cm^{-1}; ^1H NMR (300 MHz) δ 3.77 (3 H, s), 3.72 - 3.67 (1 H, ABX, m) 2.22 - 2.0 (3 H, m), 1.78 - 1.62 (3 H, m); ^{13}C NMR (75 MHz) δ 169.9, 63.7, 62.9, 52.2, 27.1, 26.6, 19.0; MS (FAB) m/z (relative intensity) 127 ([M - CH_3]$^+$, 41.2), 111 ([M - OCH_3]$^+$, 57.7).

Methyl 3-Hydroxy-1-cyclopentene-1-carboxylate (57).



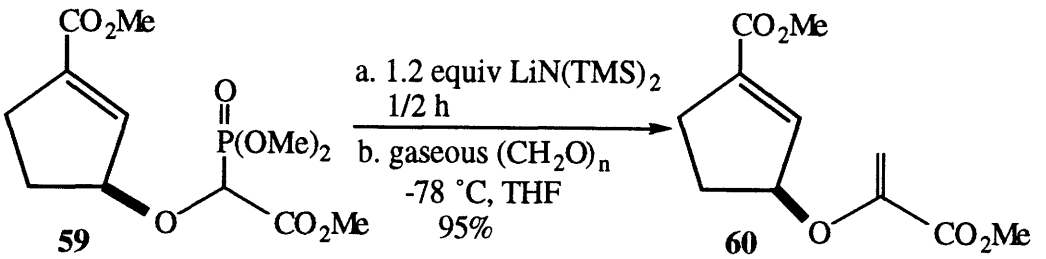
To compound **56** (1.5 g, 10.6 mmol) in dry MeOH (60 mL) in an ice bath NaOMe (687 mg, 12.72 mmol, 1.2 equiv) was added slowly. After addition was complete the reaction mixture was stirred at room temperature for 2 h and monitored by TLC which indicated starting material disappeared. The reaction solution was quenched with H_2O and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated to give a pale yellow oil. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 2:1) to give **57** (1.43 g, $R_f = 0.28$) as a colorless oil (95% yield). IR (neat) 3416, 2951, 1719, 1632, 1438 cm^{-1}; ^1H NMR (300 MHz) δ 6.72 - 6.69 (1 H, m), 5.02 - 4.94 (1 H, m), 3.76 (3H, s), 2.80 - 2.66 (1 H, m), 2.56 - 2.34 (2H, m), 1.86 - 1.74 (2 H, m), 1.74 - 1.64 (1 H, br); ^{13}C NMR (75 MHz) δ 166.7, 143.2, 138.4, 51.7, 33.4, 29.9; MS m/z (relative intensity) 142 (M^+, 2.8), 127 (47.9) 111 (19.1), 83 (100); HRMS calc for $C_7H_{10}O_3$: 142.0630; Found 142.0631.

Methyl 3-[1-(Methoxycarbonyl)-1-(dimethylphosphono)methoxy]-1-cyclopentene-1-carboxylate (59).



To starting material **57** (52 mg, 0.37 mmol) in dry benzene was added rhodium(II) octanoate (15 mg, 18.5 μmol, 0.05 equiv) and trimethyl diazophosphonoacetate (115 mg, 0.56 mmol, 1.5 equiv). The reaction mixture was brought to 60 °C for 12 h. Solvent was removed, and the crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 5:1) to give **59** (102 mg, R_f = 0.2) as a pale yellow oil (85% yield). IR (neat) 3005, 2944, 2844, 1750, 1720, 1712, 1635, 1434 cm^{-1}; ^1H NMR (300 MHz) δ 6.75 - 6.70 (1 H, m), 4.90 - 4.78 (1 H, m), 4.50 - 4.40 (1 H, two doublets), 3.88 - 3.80 (9 H, m), 3.77 - 3.75 (3 H, two singlets), 2.80 - 2.68 (1 H, m), 2.55 - 2.26 (2 H, m), 2.10 - 1.90 (1 H, m); MS m / z (relative intensity) 322 (M^+, 0.11), 291 (3.0) 199 (13), 182 (100), 125 (92.4), 93 (92.7); HRMS calc for $C_7H_{10}O_2$: 322.0818; Found 322.0821.

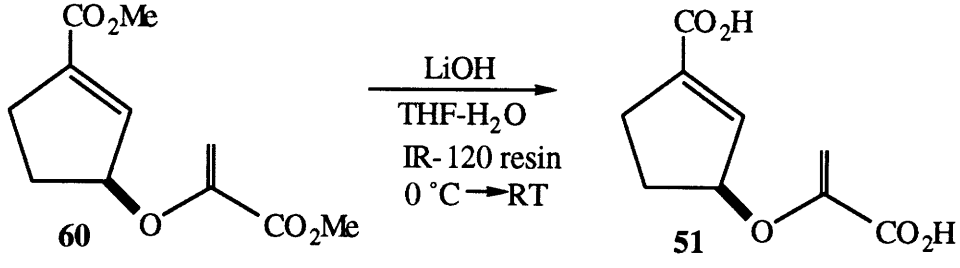
Methyl 3-[[1-(Methoxycarbonyl)ethenyl]oxy]-1-cyclopentene-1-carboxylate (60).



To phosphonate **59** (200 mg, 0.62 mmol) in dry THF (12 mL) was added a 1 M solution of LiN(TMS)2 in THF (1.0 mL, 1.0 mmol, 1.5 equiv) at -78 °C (acetone-Dry Ice bath) via syringe. After the mixture was stirred for additional 20 min, gaseous formaldehyde (generated from paraformaldehyde, 168 mg) was bubbled into the solution under a N_2 stream at -78 °C (acetone-Dry Ice bath) over 10 min, and the reaction was continued for 2 h and monitored by TLC which indicated starting material disappeared. The reaction was quenched at -78 °C by the addition of saturated $NH_4Cl_{(aq)}$, followed by extraction with ethyl acetate.

The organic layer was washed with brine, dried over $MgSO_4$, filtered, concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:5) to give **60** (133 mg, R_f = 0.35) as a colorless oil (95% yield). IR (neat) 3006, 2950, 1728, 1621, 1439, 1355, 1309, 1244, 1209, 1179, 1108, 1036 cm^{-1}; ^1H NMR (300 MHz) δ 6.8 (1 H, q, J = 2.8 Hz), 5.44 (1 H, d, J = 2.8 Hz), 5.20 - 5.14 (1 H, m), 4.66 (1 H, dd, J = 2.8, 0.5 Hz), 3.80 (3 H, s), 3.77 (3 H, s), 2.87 - 2.75 (1 H, m), 2.63 - 2.50 (1 H, m), 2.49 - 2.37 (1 H, m), 2.12 - 2.00 (1 H, m); ^{13}C NMR (75 MHz) δ 165.0, 164.5, 150.1, 141.1, 138.0, 95.6, 82.2, 52.3, 51.7, 30.1, 29.7; MS m / z (relative intensity) 226 (M^+, 0.14), 195 (3.14), 194 (4.61), 125 (100), 93 (74.47), 59 (49.21); HRMS calc for $C_{11}H_{14}O_5$: 226.0841; Found 226.0841.

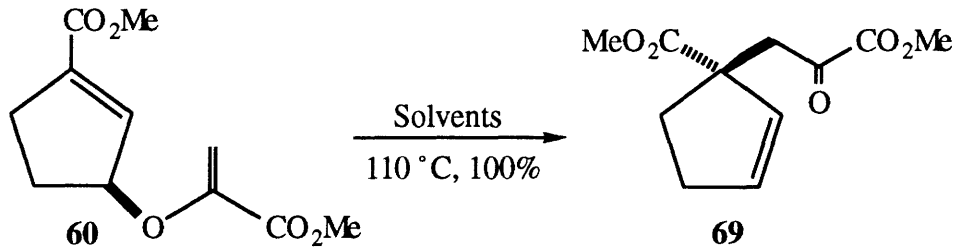
3-[[1-(Carboxy)ethenyl]oxy]-1-cyclopentene-1-carboxylic Acid (51).



To a solution of dimethyl ester **60** (50 mg, 0.22 mmol) in THF-H_2O (1:1, 3 mL) at 0 °C (ice-H_2O) was added lithium hydroxide monohydrate (26 mg, 0.46 mmol, 2 equiv) in 0.7 mL of H_2O via syringe. The mixture was warmed to room temperature, stirred for 3 h, and monitored by TLC which indicated reaction was complete. Amberlite® IR-120 (plus) ion-exchange resin was added, and the pH was adjusted to 3. The resin was removed by filtration, and the filtrate was concentrated to give **51** as an off-white solid (40 mg, mp.: 119 - 121 °C, 92% yield). IR (neat) 3402, 3067, 2944, 2863, 1716, 1703, 1621, 1433, 1295, 1204, 1102, 1005, 934 cm^{-1}; ^1H NMR (300 MHz in CD_3OD) δ 6.78 - 6.71 (1 H, m), 5.42 (1 H, d, J = 3 Hz), 5.28 - 5.20 (1 H, m), 4.70 (1 H, d, J = 3 Hz), 2.78 - 2.68 (1

H, m), 2.60 - 2.40 (2 H, m), 2.08 - 1.95 (1 H, m); ^{13}C NMR (75 MHz in CD_3OD) δ 168.0, 166.4, 151.5, 142.7, 139.4, 96.1, 84.1, 52.3, 31.0, 30.8; UV (H_2O, pH = 7.5) λ_{max} = 243 nm, ε = 1882; MS m / z (relative intensity) 198 (M^+, 0.24), 181 (0.64), 125 (100), 111 (100), 93 (18.46); HRMS calc for $C_9H_{10}O_5$: 198.0528; Found 198.0527.

Methyl 1-[2-(Methoxycarbonyl)-2-oxoethyl]-2-cyclopentene-1-carboxylate (69).



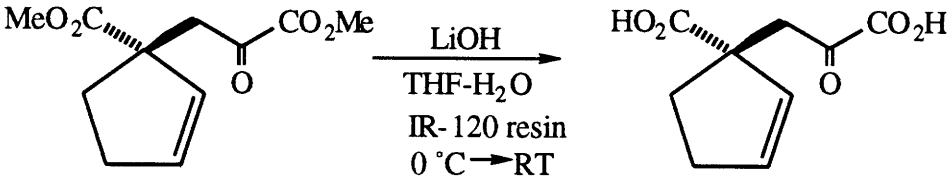
60 69

Method A: Compound **60** (50 mg, 0.22 mmol) in DMSO (3 mL) was heated at 110 °C for 9 h. The reaction was monitored by TLC, which indicated starting material had disappeared. The solvent was removed under high vacuum to give a brown oil. The crude product was purified by MPLC on silica gel (ethyl acetate/petroleum ether 1:2) to give **69** (48 mg, R_f = 0.35) as a colorless oil (96% yield). IR (neat) 3070, 3023, 2858, 1740, 1730, 1433, 1388, 1276, 1194, 1104, 1056 cm^{-1}; ^1H NMR (300 MHz) δ 6.0 - 5.8 (1 H, dm, J = 5.8 Hz), 5.8 - 5.7 (1 H, dm, J = 5.8 Hz), 3.88 (3 H, s), 3.67 (3 H, s), 3.33 (1 H, d, J = 18.3 Hz), 3.19 (1 H, d, J = 18.3 Hz), 2.60 - 2.34 (3 H, m) 1.90 - 1.76 (1 H, m); ^{13}C NMR (75 MHz) δ 191.9, 175.4, 161.1, 134.3, 132.3, 57.0, 52.9, 52.3, 46.8, 34.1, 31.8; MS m/z (relative intensity) 226 (M^+, 1.4), 194 (6.0), 167 (32.8), 139 (81.6), 59 (47.9); HRMS calc for $C_{11}H_{14}O_5$: 226.0841; Found 226.0845.

Method B: Compound **60** (50 mg, 0.22 mmol) in *m*-dichlorobenzene (3 mL) was heated at 110 °C for 7 h. The reaction was monitored by TLC which

indicated starting material had disappeared. The solvent was removed under high vacuum to give a brown oil. The crude product was purified by MPLC on silica gel (ethyl acetate/petroleum ether 1:2) to give **69** (46 mg, R_f = 0.35) as a colorless oil (92% yield). IR (neat) 3070, 3023, 2858, 1740, 1730, 1433, 1388, 1276, 1194, 1104, 1056 cm^{-1}; ^1H NMR (300 MHz) δ 6.0 - 5.8 (1 H, dm, J = 5.8 Hz), 5.8 - 5.7 (1 H, dm, J = 5.8 Hz), 3.88 (3 H, s), 3.67 (3H, s), 3.33 (1 H, d, J = 18.3 Hz), 3.19 (1 H, d, J = 18.3 Hz), 2.60 - 2.34 (3 H, m) 1.90 - 1.76 (1H, m); ^{13}C NMR (75 MHz) δ 191.9, 175.4, 161.1, 134.3, 132.3, 57.0, 52.9, 52.3, 46.8, 34.1, 31.8.

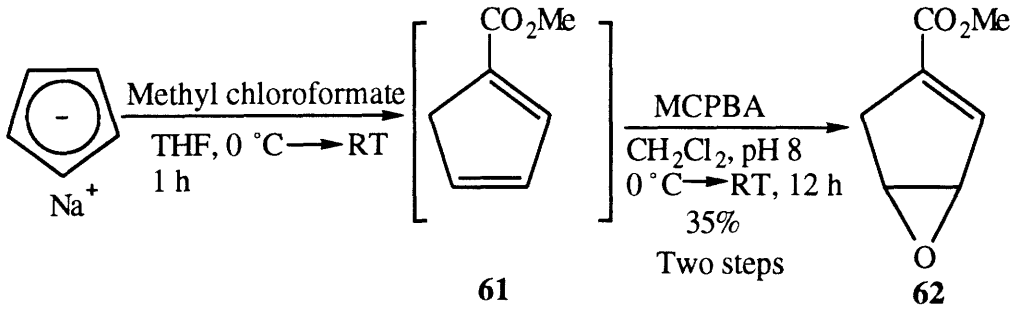
1-[2-(Carboxyl)-2-oxoethyl]-2-cyclopentene-1-carboxylic Acid (77).



69 **77**

To a solution of dimethyl ester **69** (15 mg, 66 μmole) in THF-H_2O (1:1, 1 mL) at 0 °C (ice-H_2O) was added lithium hydroxide monohydrate (7.66 mg, 0.132 mmol, 2 equiv) in 0.5 mL of H_2O via syringe. The mixture was warmed to room temperature, stirred for 3 h, and monitored by TLC which indicated reaction was complete. Amberlite® IR-120 (plus) ion-exchange resin was added, and the pH was adjusted to 3. The resin was removed by filtration, and the filtrate was concentrated to give **77** as a colorless oil (12 mg, 92% yield). ^1H NMR (500 MHz in CD_3OD) δ 6.03 - 5.91 (1 H, m), 5.77 - 5.70 (1 H, m), 2.85 (1 H, dd, J = 16.6, 8.3 Hz), 2.66 (1 H, dd, J = 16.6, 5.9 Hz), 2.60 - 2.44 (4 H, m).

Methyl 6-Oxabicyclo[3.1.0]-2-hexene-3-carboxylate (62).

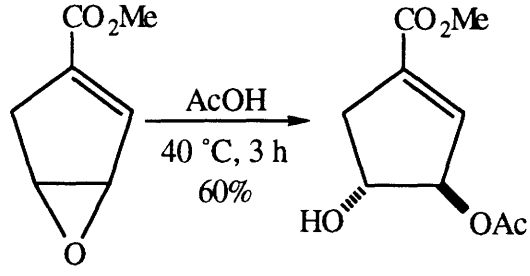


61 62

Intermediate methyl cyclopentadiene-1-carboxylate was made by a modification of the procedure reported by Peters.[28] To a stirred solution of methyl chloroformate (6.62 g, 5.4 mL, 70 mmol) in dry THF (70 mL) was added dropwise a 2 M solution of cyclopentadienylsodium in THF (35 mL, 70 mmol) under N_2 at 0 °C. After the addition was complete, the resulting red solution was stirred for an additional 30 min at room temperature. The reaction solution was quenched with H_2O and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated to give dark red oil. The crude product was used in the next step without further purification. To the crude compound in CH_2Cl_2-$NaH_2PO_{4(aq)}$ (150 mL, v/v = 1:1) was added *m*-CPBA (24 g, 50-60% purity, 1.0 equiv) at 0 °C. The reaction was stirred at room temperature for 12 h, and monitored by TLC which indicated starting material had disappeared. During the reaction the color of the reaction solution changed from pink to yellow then white. The mixture was poured into saturated $Na_2S_2O_{3(aq)}$ to destroy excess *m*-CPBA and was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by MPLC on silica gel (ethyl acetate/petroleum ether 1:4) to give **62** (3.40 g, R_f = 0.45) as a colorless oil (35% yield the two steps). IR (neat) 3060, 3016, 2870, 1719, 1614, 1439, 1360, 1290, 1195 cm^{-1}; ^1H NMR (300 MHz) δ 7.02 (1 H, dd, J = 3.3, 2.0 Hz), 3.97 (1 H, dd, J = 2.77, 3.3 Hz), 3.88 - 3.86 (1 H, m), 3.75 (3 H, s), 2.92 (1 H, dt, J = 19.2, 2.1 Hz), 2.61 (1 H, ddd, J = 18.6,

3.5, 2.0 Hz); ^{13}C NMR (75 MHz) δ 164.6, 141.5, 140.9, 58.0, 56.8, 51.6, 34.8; MS m/z (relative intensity) 139 ([M - H]$^+$, 12.2), 125 (53.8), 99 (22.8), 81 (61.3), 53 (100); HRMS([M - H]$^+$) calc for $C_7H_8O_3$: 139.0395; Found 139.0395.

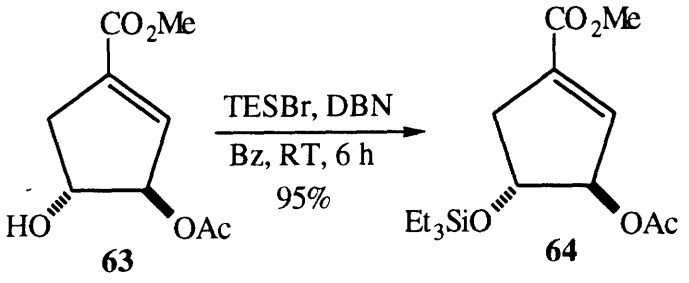
Methyl *trans*-3-Acetoxy-4-hydroxy-1-cyclopentene-1-carboxylate (63).



62 **63**

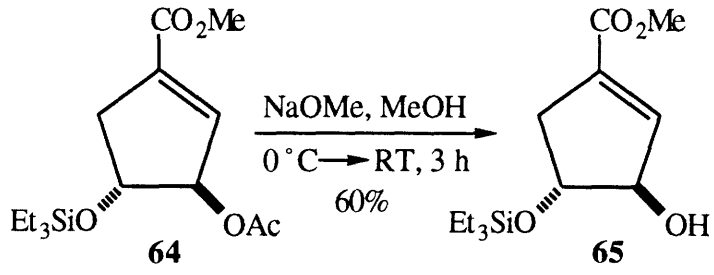
Compound **62** (170 mg, 1.2 mmol) in acetic acid (10 mL) was heated to 40 °C for 3 h. The reaction was monitored by TLC which indicated starting material had disappeared. The solvent was removed under high vacuum to give a brown oil. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:2) to give **63** (144 mg, R_f = 0.25) as a colorless oil (60% yield). IR (neat) 3060, 3016, 2870, 1719, 1614, 1439, 1360, 1290, 1195 cm^{-1}; ^1H NMR (300 MHz) δ 6.53 (1 H, ddd, J = 2.1, 2.1, 1.9 Hz), 5.49 (1 H, dtd J = 3.1, 2.1, 2.1 Hz), 4.45 (1 H, m), 3.77 (3 H, s), 3.16 (1 H, br), 3.09 (1 H, dddd, J = 17.1, 8.0, 1.6, 2.1 Hz), 2.57 (1 H, dddd, J = 17.1, 5.5, 2.0, 2.1 Hz), 2.12 (3 H, s); ^{13}C NMR (75 MHz) δ 172.1, 164.4, 138, 136.4, 87.8, 78.1, 51.9, 38.2, 29.6, 20.8; MS m/z (relative intensity) 200 (1.78), 172 (2.6), 140 (18.2), 126 (61), 81 (61.3), 43 (100); HRMS calc for $C_9H_{12}O_5$: 200.0685; Found 200.0686.

Methyl *trans*-3-Acetoxy-4-trimethylsilyloxy-1-cyclopenten-1-carboxylate (64).



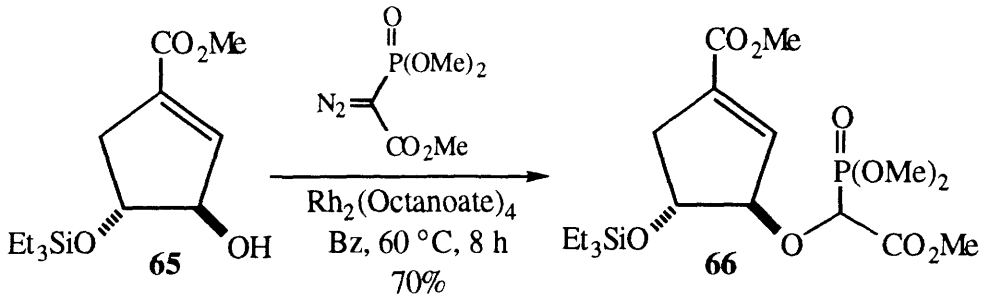
To a stirred solution of compound **63** (120 mg, 0.6 mmol) in dry benzene (10 mL) was added triethylsilyl bromide (200 μL, 0.9 mmol, 1.5 equiv). DBN (124 μL, 1.0 mmol, 1.6 equiv) was added, and the mixture was stirred at room temperature for 6 h. The reaction was monitored by TLC which indicated starting material had disappeared. The mixture was poured into 10% $NaH_2PO_{4(aq)}$ and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:8) to give **64** (175 mg, R_f = 0.45) as a pale yellow oil (95% yield). IR (neat) 2955, 2927, 2877,1746, 1728, 1633, 1433, 1350, 1229, 1113 cm^{-1}; ^1H NMR (300 MHz) δ 6.59 (1 H, dddd, J = 3.7, 2.1, 1.8, 1.6 Hz), 5.56 (1 H, dddd J = 7.3, 5.3, 4.5, 1.6 Hz), 4.44 (1 H, dddd, 7.3, 5.3, 4.5, 1.6 Hz), 3.75 (3 H, s), 2.95 (1 H, dddd, J = 16.6, 7.3, 1.6, 1.6 Hz), 2.47 (1 H, dddd, J = 16.5, 5.2, 2.1, 1.6 Hz), 2.08 (3 H, s), 0.95 (9 H, t, J = 7.7 Hz), 0.61 (6 H, q, J = 7.8 Hz); ^{13}C NMR (75 MHz) δ 170.3, 164.6, 137.6, 137.2, 85.6, 76.8, 51.7, 38.9, 29.7, 20.8, 6.5, 4.5; MS m/z (relative intensity) 314 (1.53), 255 (0.4), 240 (4.26), 145 (100); HRMS calc for $C_{15}H_{26}O_5Si$: 314.1545; Found 314.1548.

Methyl *trans*-3-Hydroxy-4-trimethylsilyloxy-1-cyclopentene-1-carboxylate (65).



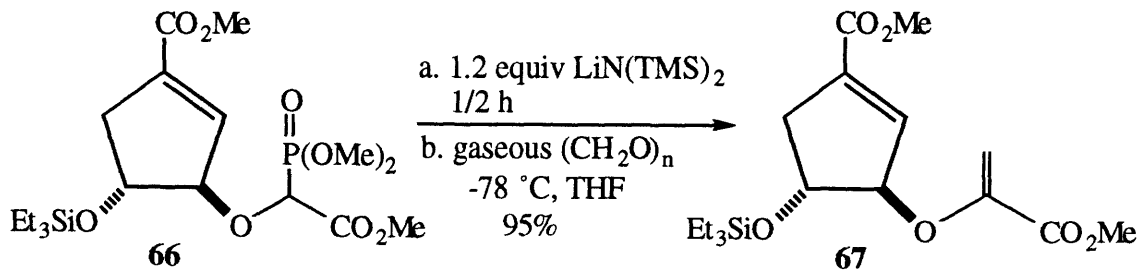
To a solution of compound **64** (188.4 mg, 0.6 mmol) in dry MeOH (12 mL) NaOMe (64.8 mg, 1.2 mmol, 2 equiv) was added slowly at 0 °C. After addition was complete the reaction mixture was stirred at room temperature for 3 h and was monitored by TLC which indicated starting material had disappeared. The reaction solution was quenched with H_2O and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated to give a pale yellow oil. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:6) Compound **65** (98 mg, $R_f = 0.28$) was obtained as a colorless oil (60% yield). IR (neat) 3441, 2955, 2914, 2877, 1727, 1633, 1439, 1355, 1103 cm^{-1}; ^1H NMR (300 MHz) δ 6.60 (1 H, dddd, J = 3.6, 2.1, 1.6, 0.2 Hz), 4.73 (1 H, ddddd J = 4.9, 3.6, 2.0, 1.6, 1.0 Hz), 4.24 (1 H, dddd, 7.3, 5.9, 4.9, 0.2 Hz), 3.75 (3 H, s), 2.93 (1 H, dddd, J = 16.3, 7.3, 1.6, 1.5 Hz), 2.42 (1 H, dddd, J = 16.4, 5.9, 2.1, 2.0 Hz), 1.83 (1 H, d, J = 0.9 Hz), 0.98 (9 H, t, J = 8.2 Hz), 0.64 (6 H, q, J = 8.0 Hz); ^{13}C NMR (75 MHz) δ 165.0, 141.5, 136.4, 83.9, 81.1, 51.6, 38.7, 6.7, 4.7; MS m/z (relative intensity) 272 (39), 240 (30), 241 (41.2), 103 (93), 75 (100); HRMS calc for $C_{13}H_{24}O_4Si$: 272.1444; Found 272.1443.

Methyl *trans*-3-[1-(Methoxycarbonyl)-1-(dimethylphosphono)methoxy]-4-triethylsilyloxy-1-cyclopentene-1-carboxylate (66).



To starting material **65** (91 mg, 0.335 mmol) in dry benzene rhodium(II) octanoate (15 mg, 18.5 μmol, 0.05 equiv) and trimethyl diazophosphonoacetate (140 mg, 0.67 mmol, 2 equiv) were added. The reaction mixture was brought to 60 °C for 12 h. Solvent was removed, and the crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 5:1) to give **66** (106.1 mg, $R_f = 0.33$) as a pale yellow oil (70% yield). IR (neat) 3008, 2958, 2877, 2124, 1754, 1715, 1633, 1437, 1289, 1109, 1031 cm^{-1}; ^1H NMR (300 MHz) δ 6.7 - 6.6 (1 H, m), 4.8 - 4.7 (1 H, d J = 20 Hz), 4.7 - 4.5 (1 H, m), 3.75 (3 H, s), 4.65 - 4.5 (1 H, d, J = 20 Hz), 4.4 - 4.5 (1 H, m), 3.7 - 3.9 (12 H, m), 2.9 - 3.1 (2 H, m), 2.3 - 2.5 (2 H, m), 1.0 - 0.9 (9 H, two triplets), 0.5 - 0.7 (6 H, two quartets); MS m/z (relative intensity) 452 (4.7), 420 (42), 253 (45), 185 (64), 182 (100), 124 (75); HRMS calc for $C_{18}H_{33}O_9PSi$: 452.1632; Found 452.1628.

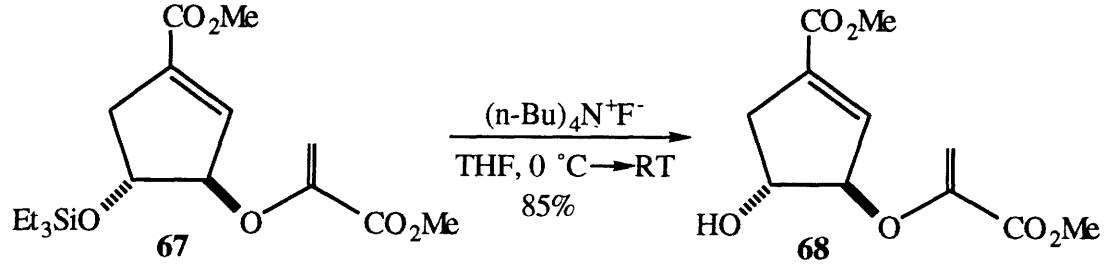
Methyl *trans*-3-[[1-(methoxycarbonyl)ethenyl]oxy]-4-triethylsilyloxy-1-cyclopentene-1-carboxylate (67).



To phosphonate **66** (151 mg, 0.335 mmol) in dry THF (9 mL) a 1 M solution of $LiN(TMS)_2$ in THF (0.44 mL, 0.44 mmol, 1.3 equiv) was added at -78

°C(acetone-Dry Ice bath) via syringe. After the mixture was stirred for an additional 20 min, gaseous formaldehyde (generated from paraformaldehyde, 100 mg) was bubbled into the solution under a N_2 stream at -78 °C (acetone-Dry Ice bath) over 10 min. The reaction was continued for 3 h and was monitored by TLC which indicated starting material had disappeared. The reaction was quenched at -78 °C by the addition of saturated $NH_4Cl_{(aq)}$ followed by extraction with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, filtered, concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:8) to give **67** (113 mg, R_f = 0.45) as a colorless oil (95% yield). IR (neat) 2953, 2912, 2890, 1741, 1729, 1627, 1461, 1438, 1354, 1313, 1231, 1196, 1160, 1109 cm^{-1}; ^1H NMR (300 MHz) δ 6.67 (1 H, dddd, J = 2.3, 2.0, 1.6, 0.2 Hz), 5.47 (1 H, d, J = 5.5 Hz), 4.98 (1 H, ddddd, J = 4.3, 2.3, 1.6, 1.6, 0.5 Hz), 4.8 (1 H, dd, J = 2.8, 0.5 Hz), 4.56 (1H, dddd, J = 7.5, 5.2, 4.4, 0.2 Hz), 3.80 (3 H, s), 3.76 (3 H, s), 3.02 (1 H, dddd, J = 16.6, 7.5, 1.6, 1.6 Hz), 2.48 (1 H, dddd, J = 16.7, 5.3, 2.2, 1.6 Hz), 0.95 (9 H, t, J = 8.2 Hz), 0.62 (6 H, q, J = 8.2 Hz); ^{13}C NMR (75 MHz) δ 164.6, 163.3, 150.3, 137.5, 136.4, 96.1, 89.6, 77.7, 52.3, 51.8, 39.2, 6.6, 4.6; MS m/z (relative intensity) 356 (10.4), 324 (10), 255 (19.4), 187 (100), 115 (61), 87 (74); HRMS calc for $C_{17}H_{28}O_6Si$: 356.1655; Found 356.1658.

Methyl *trans*-3-[[1-(Methoxycarbonyl)ethenyl]-4-hydroxy]-1-cyclopentene-1-carboxylate (68).

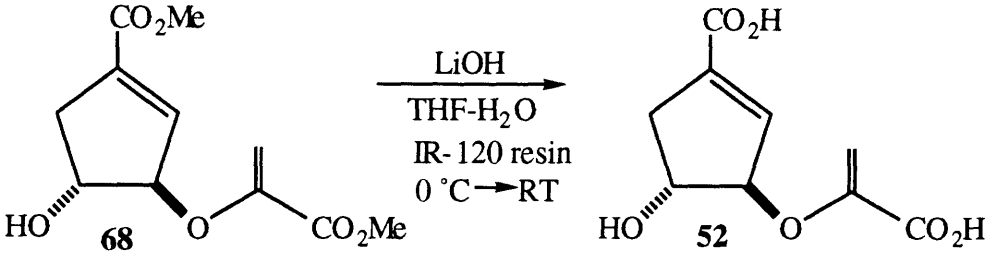


To solution of compound **67** (197 mg, 0.553 mmol) in dry THF (10 mL) a 1 M solution of $(n\text{-}Bu)_4N^+F^-$ in THF (0.72 mL, 0.72 mmol, 1.3 equiv) was added at 0 °C. The solution was stirred at room temperature for 3 h and monitored by TLC which indicated starting material had disappeared. The reaction was quenched by the addition of saturated $NH_4Cl_{(aq)}$, followed by extraction with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:3) to give **68** (114 mg, R_f = 0.35) as a off-white solid (mp.: 47 - 48 °C, 85% yield). IR (neat) 3479, 3005, 2955, 2853, 1732, 1713, 1622, 1440, 1353, 1308, 1206, 1172 cm^{-1}; ^1H NMR (300 MHz) δ 6.70 (1 H, dddd, J = 3.9, 2.0, 1.8, 1.3 Hz), 5.52 (1 H, d, J = 2.8 Hz), 4.94 (1 H, ddddd, J = 4.7, 3.9, 1.8, 1.4, 0.5 Hz), 4.89 (1 H, dd, J = 2.8, 0.5 Hz), 4.61 (1 H, ddddd, J = 7.5, 4.7, 4.7, 4.0, 1.3 Hz), 3.81 (3 H, s), 3.77 (3 H, s), 3.13 (1 H, dddd, J = 17.1, 7.5, 1.8, 1.8 Hz), 2.52 (1 H, dddd, J = 17.2, 4.0, 2.1, 1.4 Hz), 2.17 (1 H, d, J = 4.7 Hz); ^{13}C NMR (75 MHz) δ 164.6, 163.6, 150.0, 138.1, 136.3, 97.2, 89.3, 76.5, 52.5, 51.9, 38.5; MS m/z (relative intensity) 242 (2.8), 210 (9.5), 141 (100), 81 (81.2), 53(75.3); HRMS calc for $C_{11}H_{24}O_6$: 242.0790; Found 242.0790.

NOE Experiments with 60 and 68.

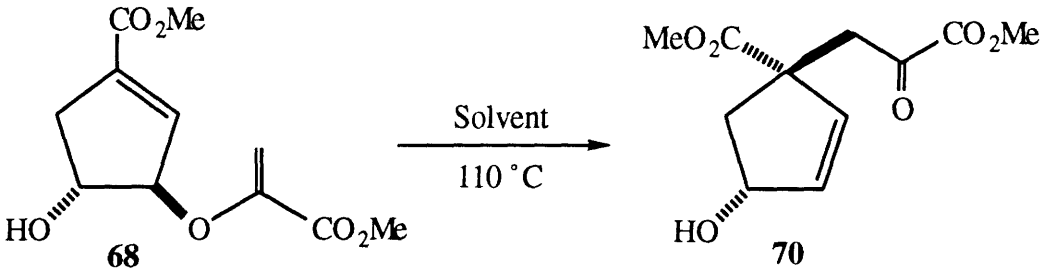
NOE measurements with **60** (5 mg) and **68** (5 mg) in anhydrous $CDCl_3$ (1 mL) respectively with tetramethylsilane as internal standard were measured after three freeze-thaw procedures, high vacuum pump (< 10^{-3} mm Hg). The NOE experiment was measured with the Varian Unity 300 MHz spectrometer system. The results are provided in Scheme 12 and in Scheme 13.

***trans*-3-[[1-(Carboxy)ethenyl]oxy]-4-hydroxy-cyclopentene-1-carboxylic Acid (52).**



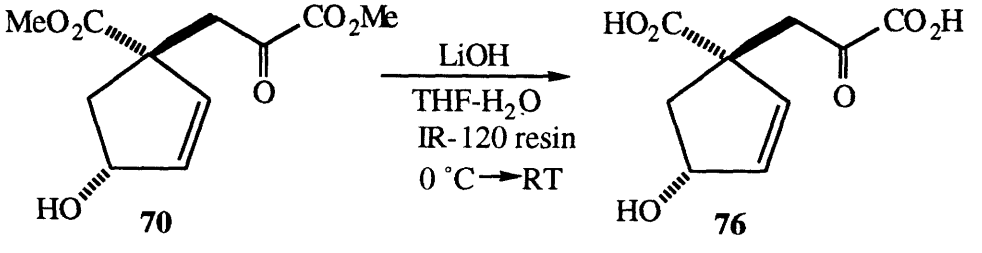
To a solution of dimethyl ester **68** (60 mg, 0.25 mmol) in THF-H_2O (1:1, 2 mL) at 0 °C (ice-H_2O) was added lithium hydroxide monohydrate (29 mg, 0.5 mmol, 2 equiv) in 0.7 mL of H_2O via syringe. The mixture was warmed to room temperature, stirred for 3 h, and monitored by TLC which indicated reaction was complete. Amberlite® IR-120 (plus) ion-exchange resin was added, and the pH was adjusted to 3. The resin was removed by filtration, and the filtrate was concentrated to give **52** as an off-white solid (45 mg, mp.: 130 - 131 °C, 84% yield). ^1H NMR (500 MHz in CD_3OD) δ 6.66 (1 H, d, J = 2 Hz), 5.44 (1 H, d, J = 3.0 Hz), 4.95 - 4.90 (2H, m, overlaping with H_2O), 4.44 (1H, ddd, J = 4.4, 4.0, 3.4 Hz), 3.0 (1 H, ddd, J = 17.0, 7.2, 2.0 Hz), 2.41 (1 H, ddd, J = 17.0, 4.5, 2.0 Hz); ^{13}C NMR (75 MHz) δ 167.4, 166.2, 151.8, 140.2, 137.2, 96.6, 90.4, 76.9, 39.8; UV (H_2O, pH = 7.5) λ_{max} = 240 nm, ε = 1700; MS m/z (relative intensity) 196 ([M - H_2O]$^+$, 2.47), 169 (51.67), 126 (80.48), 98 (66.51), 69 (62.75); HRMS ([M - H_2O]$^+$) calc for $C_9H_{10}O_6$: 196.0372; Found 196.0371.

Methyl 4β-Hydroxy-1α- [2-(methoxycarbonyl)-2-oxoethyl]-2-cyclopentene-1β-carboxylate (70).



Compound **68** (50 mg, 0.21 mmol) in *m*-dichlorobenzene (3 mL) was heated at 110 °C for 36 h. The reaction was monitored by TLC which indicated starting material had disappeared. The solvent was removed under high vacuum to give a brown oil. The crude product was purified by MPLC on silica gel (ethyl acetate/petroleum ether 1:2) to give **70** (46 mg, R_f = 0.35) as a colorless oil (92% yield). IR (neat) 3382, 2951, 2912, 1753, 1730, 1713, 1436, 1392, 1352, 1284, 1205, 1158 cm^{-1}; ^1H NMR (300 MHz) δ 6.11 (1 H, dd, J = 5.5, 2.4 Hz), 5.86 (1 H, d, J = 5.5 Hz), 4.79 - 4.74 (1 H, ddbr, J = 2.0, 1.5 Hz), 3.86 (3 H, s), 3.70 (3 H, s), 3.46 (1 H, d, J = 19.7 Hz), 3.12 (1 H, d, J = 19.7 Hz), 2.78 - 2.66 (1 H, br), 2.50 (1 H, dd, J = 14.5, 1.5 Hz), 1.97 (1 H, dd, J = 14.5, 2.0 Hz); ^{13}C NMR (75 MHz) δ 201.6, 192.2, 191.4, 137.4, 135.5, 76.2, 56.0, 53.1, 46.5, 44.2, 29.7; MS m/z (relative intensity) 242 (0.11), 225 (0.77), 210 (3.34), 183 (45.1), 165(26.23), 155 (93.66), 123 (76.65), 95 (100); HRMS calc for $C_{11}H_{24}O_6$: 242.0790; Found 242.0792.

4β-Hydroxy-1α-[2-(carboxyl)-2-oxoethyl]-2-cyclopentene-1β-carboxylic Acid (76).



To a solution of dimethyl ester **70** (12 mg, 50 μmole) in THF-H_2O (1:1, 1 mL) at 0 °C (ice-H_2O) was added lithium hydroxide monohydrate (6 mg, 0.1 mmol, 2 equiv) in 0.5 mL of H_2O via syringe. The mixture was warmed to room temperature, stirred for 6 h, and monitored by TLC which indicated reaction was complete. Amberlite® IR-120 (plus) ion-exchange resin was added, and the pH was adjusted to 3. The resin was removed by filtration, and the filtrate was concentrated to give **76** as an off-white solid (9 mg, mp.: 135 - 137 °C, 84% yield). ^1H NMR (250 MHz in CD_3OD) δ 5.9 - 5.7 (2 H, m), 4.90 - 4.80 (1H, m, overlaping with H_2O), 2.77 - 2.60 (2 H, m), 2.28 - 2.18 (2 H, m).

Kinetics of Thermal [3,3] Rearrangement of 51 and 52.

The Claisen rearrangement of **51** and **52** was monitored as follows: Samples of substrate (3.0 mg) were dissolved in 0.7 mL of 0.1 M deuterated phosphate buffer (pH = 7.0) in an NMR tube. The sample was heated in a constant temperature bath at the designated temperature. ^1H NMR spectra were recorded periodically. The rate constants, k_{rearr}, were determined from the slope of the graph of ln {[starting material]/[[starting material]+[product]]} versus time. The concentrations of starting material and product were determined by the integral values of the vinyl protons of each species. In all cases, the disappearance of starting material obeyed first-order kinetics to at least 90% completion. The Arrhenius equation was used to solve for $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$. For compound **51**: $k_{50\ °C} = 2.36 \times 10^{-5}\ s^{-1}$; $k_{30\ °C} = 2.44 \times 10^{-6}\ s^{-1}$. For compound **52**: $k_{50\ °C} = 9.01 \times 10^{-6}\ s^{-1}$; $k_{30\ °C} = 7.12 \times 10^{-8}\ s^{-1}$.

General Assay Procedure for Chorismate Mutase.

For studies with chorismate mutase the reaction was monitored by disappearance of substrate (chorismate (**9**) λ = 273 nm, ε = 2630. Compound **51** λ

= 243 nm, ε = 1882. Compound **52** λ = 240 nm, ε = 1700). All assay were performed at 30 °C in a buffer of 100 mM Tris·HCl (pH = 7.5), 1 mM EDTA, 1 mM DTT, and 0.1 mg/mL bovine serum albumin. The concentrations of chorismate mutase in the cell were diluted from 10 mg/mL[38] to 1 ng/mL for the test of chorismate (**9**), 100 μg/mL for the test of compound **51**, and 2 μg/mL for the test of compound **52**. K_m and V_{max} values were obtained by monitoring the reaction rate at varied substrate concentrations. Inhibition constants were calculated from the apparent K_m of chorismate in the presence of 0.5 - 1.0 mM of inhibitor.

References and Notes

1. Retey, J.; Robinson, J. A. *Stereospecificity in Organic Chemistry and Enzymology*; Verlag Chemie: 1982, p 148.

2. Weiss, U.; Gilvarg, C; Mingiol, E. S.; Davis, B. D. *Science,* **1954,** *119,* 774.

3. Pleiniger, H.; Keilich, G. *Chem. Ber.* **1959,** *92,* 2897.

4. (a) Danishefsky, S.; Hirama, M.; Fritsch, N.; Clardy, J. *J. Am. Chem. Soc.* **1979**, *101,* 7013. (b) Danishefsky, S.; Hirama, M. *J. Am. Chem. Soc.* **1977**, *99,* 7740.

5. Ramage, R.; Macleod, A. M. *J. Chem. Soc., Chem. Commun.* **1984,** 1008.

6. Hoare, J. H.; Policastro, P. P.; Berchtold, G. A. *J. Am. Chem. Soc.* **1983**, *105,* 6264.

7. (a) Andrews, P. R.; Smith, G. D.; Young, I. G. *Biochemistry* **1973,** *12,* 3492. (b) Andrews, P. R.; Haddon, R. C. *Aust. J. Chem.* **1979,** *32,* 1921.

8. Andrews, P. R.; Cain, E. N.; Rizzardo, E.; Smith, G. D. *Biochemistry* **1977,** *16,* 4848.

9. Sogo, S. G.; Widlanski, T. S.; Hoare, J. H.; Grimshaw, C. E.; Berchtold, G. A.; Knowles, J. R. *J. Am. Chem. Soc.* **1984**, *106,* 2701.

10. Copley, S. D.; Knowles, J. R. *J. Am. Chem. Soc.* **1985**, *107,* 5306.

11. Asano, Y.; Lee, J. J.; Shieh, T. L.; Spreafico, F.; Kowal, C.; Floss, H. G. *J. Am. Chem. Soc.* **1985**, *107,* 4314.

12. Gajewski, J. J.; Jurayi, J. Kimbrough, D. R.; Gande, M. E.; Ganem, B.; Carpenter, B. K. *J. Am. Chem. Soc.* **1987,** *109,* 1170.

13. Guilford, W. J.; Copley, S. D.; Knowles, J. R. *Biochemistry* **1983**, *22,* 4494.

14. Pawlak, J. L.; Padykula, R.E.; Kronis, J. D.; Aleksejczyk, R. A.; Berchtold, G. A. *J. Am. Chem. Soc.* **1987**, *111,* 3374.

15. Busch, R. F.; Berchtold, G. A. *J. Am. Chem. Soc.* **1983**, *105,* 3346.

16. Ganey, M. V. Ph. D. Thesis, Massachusetts Institute of Technology, **1986.**

17. (a). Wilcox, C. S.; Babston, R. E. *J. Am. Chem. Soc.* **1986**, *108*, 6636. (b). Lesuisse, D.; Berchtold, G. A. *J. Org. Chem.* **1988**, *53,* 4992.

18. Delany, J. J.; Padykula, R.E.; Berchtold, G. A. *J. Am. Chem. Soc.* **1992**, *114,* 1394.

19. Gorisch, H. *Biochemistry* **1978,** 17, 3700.

20. Guilford, W. J.; Copley, S. D.; Knowles, J. R. *J. Am. Chem. Soc.* **1987**, *109,* 5013.

21. Copley, S. D.; Knowles, J. R. *J. Am. Chem. Soc.* **1987**, *109,* 5008.

22. Eschenmoser, A. personal communication to Professor Glenn A. Berchtold.

23. Coates, R. M.; Rogers, B. D.; Hobbs, S. J.; Peck, D. R.; Curran, D. P. *J. Am. Chem. Soc.* **1987**, *109,* 1160.

24. Ife, R. J.; Ball, L. F.; Lowe, P.; Haslem, E. *J. Chem. Soc., Perkin Trans. 1* **1976,** 1776.

25. Pawlak, J. L.; Berchtold, G. A. *J. Org. Chem.* **1988**, *53,* 4063.

26. The dihedral angles were determined with Chem 3D Plus Version 3.1a from Cambridge Scientific Computing, Inc. after minimizing structural energies.

27. Scharf, H.-D.; Korte, F.; *Chem. Ber.* **1964,** 2425.

28. Peters, D. *J. Chem. Soc.* **1959,** 1757.

29. Pawlak, J. L.; Berchtold, G. A. *J. Org. Chem.* **1987**, *52,* 1765.

30. Frost, A. A.; Pearson, R. G. *Kinetics and Mechanism*; Wiley: New York, 1961.

31. Kronis, J. D.; Berchtold, G. A. Unpublished observations.

32. Barton, D. H. R.; Hartwig, W.; Motherwell, W. B. *J. Chem. Soc., Chem. Commun* **1982**, 447

33. This reaction was followed through two half-lives due to problems associated with the competitive reaction involving hydrolysis of the enolpyruvyl group.

34. Campbell, A. P.; Tarasow, T. M.; Massefski, W.; Write, P. E.; Hilvert, D. *Proc. Natl. Acad. Sci. USA* **1993,** *90,* 8663.

35. Gray, J. V.; Golinelli-Pimpaneau, B.; Knowles, J. R. *Biochemistry* **1990,** *29,* 376.

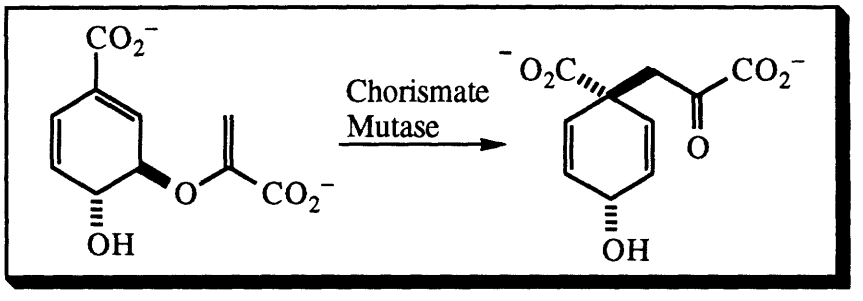
36. Hermes, J. D.; Tipton, P. A.; Fisher, M. A.; O'Leary, M. H.; Morrison, J. F.; Cleland, W. W. *Biochemistry* **1984**, *23,* 6263.

37. Perrin, D. D.; Armarege, W. L. F.; Perrin, D. R. *Purification of Laboratory Chemicals;* Pergmon: 1980, 2nd ed..

38. It was a gift from Professor Knowles at Harvard University.

Chapter 3

Investigations of the Enzyme Chorismate Mutase (Part II): [3,3] Rearrangement of Chorismate to Prephenate



Introduction

In order to acquire more information on the nature of catalysis by chorismate mutase, it was desirable to examine the electronic character of chorismate at C6. Only three chorismate analogs had been reported with structural change at C6: 5,6-Dihydrochorismate (**42**), *exo*-methylene analog **41**, and 6-oxa-5,6-dihydrochorismate analog **43**.[1]

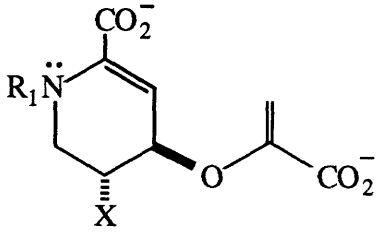
There are problems with analogs such as **41**. The steric limitations of the active site are such that **41** does not bind as a competitive inhibitor. 5,6-Dihydrochorismate (**42**), which sterically differs from chorismate (**9**) only by an sp^3 hybridized CH_2 group rather than an sp^2 hybridized CH group, binds only one-fourth as well as chorismate (**9**). Clearly, the enzyme is sensitive to the steric bulk present at C5–C6. Second, the electronic effect of an electron-rich substituent at C6 is very important for both thermal and enzymatic rearrangement. However, the *exo*-methylene group does not provide any rate acceleration, even though a double bond is in direct conjugation with the C1 position. The reason for this is thought to be twofold: first, an s-trans diene shows less electron delocalization than a cyclic s-cis diene and second, the *exo*-methylene perturbs the cyclohexane ring geometry so as to stabilize the diequatorial ground state of the analog. The energy barrier for rearrangement is increased substantially by this stabilization.

The rate of rearrangement of dihydropyran analog **43** is approximately 70 times slower than **9**.[1] The fact that analogs **42** and **43** rearrange at a much slower rate than **9** suggests that there must be a significant electronic effect of the *endo* double bond at C5–C6 in **9** that increases the rate. The 6-oxa-5,6-dihydrochorismate analog **43** reported by Berchtold and Delany is also a good

substrate for chorismate mutase ($k_{cat} / k_{uncat} = 4 \times 10^5$).[1] Therefore, the sp^2 configuration at C5 and C6 is not necessary for mutase catalysis, and the structural requirements for chorismate mutase catalyzed rearrangement have been discussed in Chapter 2.

A useful substrate analog must have a small electron-donating group at either C6 of the ring or C3 of the side chain. Tetrahydropyridine analogs in Chart 1 appeared to be reasonable candidates. If either an S_N1 or S_N2 process were occurring for the enzymatic rearrangement, tetrahydropyridine analogs would be expected to have a higher V_{max} than 6-oxa-5,6-dihydrochorismate analog **43**, but it would probably not be higher than that for chorismate (**9**). Since the tetrahydropyridine analogs suggested are enamines, they should be very reactive under S_N1 or S_N2 conditions. This structural factor could manifest itself as an increase in V_{max} compared to analog **43**. Since the effect is only seen after binding, K_m should not be greatly affected. The steric concerns mentioned above should be little or less important for pyran **43**. We expect tetrahydropyridine analogs to have an association constant between those of chorismate (**9**), 5,6-dihydrochorismate (**42**), and pyran **43**, except when R_1 = Me (**80** and **81**), steric effects are likely to be significant.

Chart 1

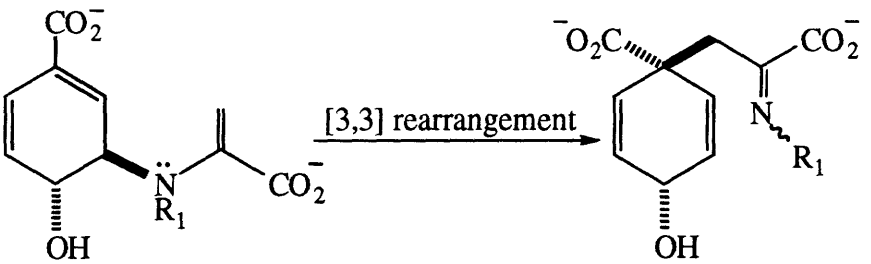


78. R_1 = H, X = H
79. R_1 = H, X = OH
80. R_1 = Me. X = H
81. R_1 = Me, X = OH

Another substrate type of interest is that with an α,β-unsaturated amino acid at the side chain (Scheme 1) instead of the enolpyruvate moiety. This type of

analog might have a faster non-catalyzed (thermal) rate of [3,3] rearrangement due to the stronger electron-donating group at C3 where nitrogen replaces oxygen in the side chain . However, compared to the enolpyruvate counterpart it is thermodynamically less favorable for the rearrangement of **82** and **83** due to the weak imine bond of rearranged products **84** and **85** in Scheme 1 compared to the carbonyl group from rearrangement of the enolpyruvate derivatives.

Scheme 1



82. R_1 = H
83. R_1 = Me

84. R_1 = H
85. R_1 = Me

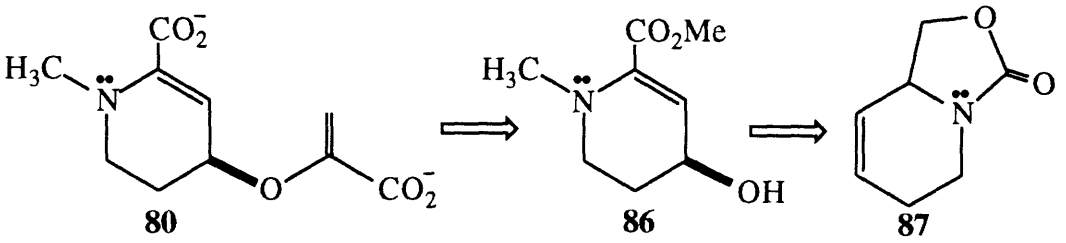
Therefore, it is difficult to predict the uncatalyzed (thermal) rate of rearrangement and V_{max} of compounds **82** and **83** relative to chorismate (**9**) and pyran **43**.

Results and Discussion

Attempted Synthesis of Tetrahydropyridine Analog 80.

Retrosynthetic analysis indicated that **80** could be designed from bicyclic intermediate **87** via allylic alcohol **86** in Scheme 2. Thus, epoxidation of **87**, followed by base-catalyzed rearrangement after cleavage of the 5-membered ring with ester formation would give allylic alcohol **86**. The side-chain could be appended as described by Berchtold and co-workers for the total synthesis of optically pure chorismic acid,[2] and saponification would lead to compound **80**.

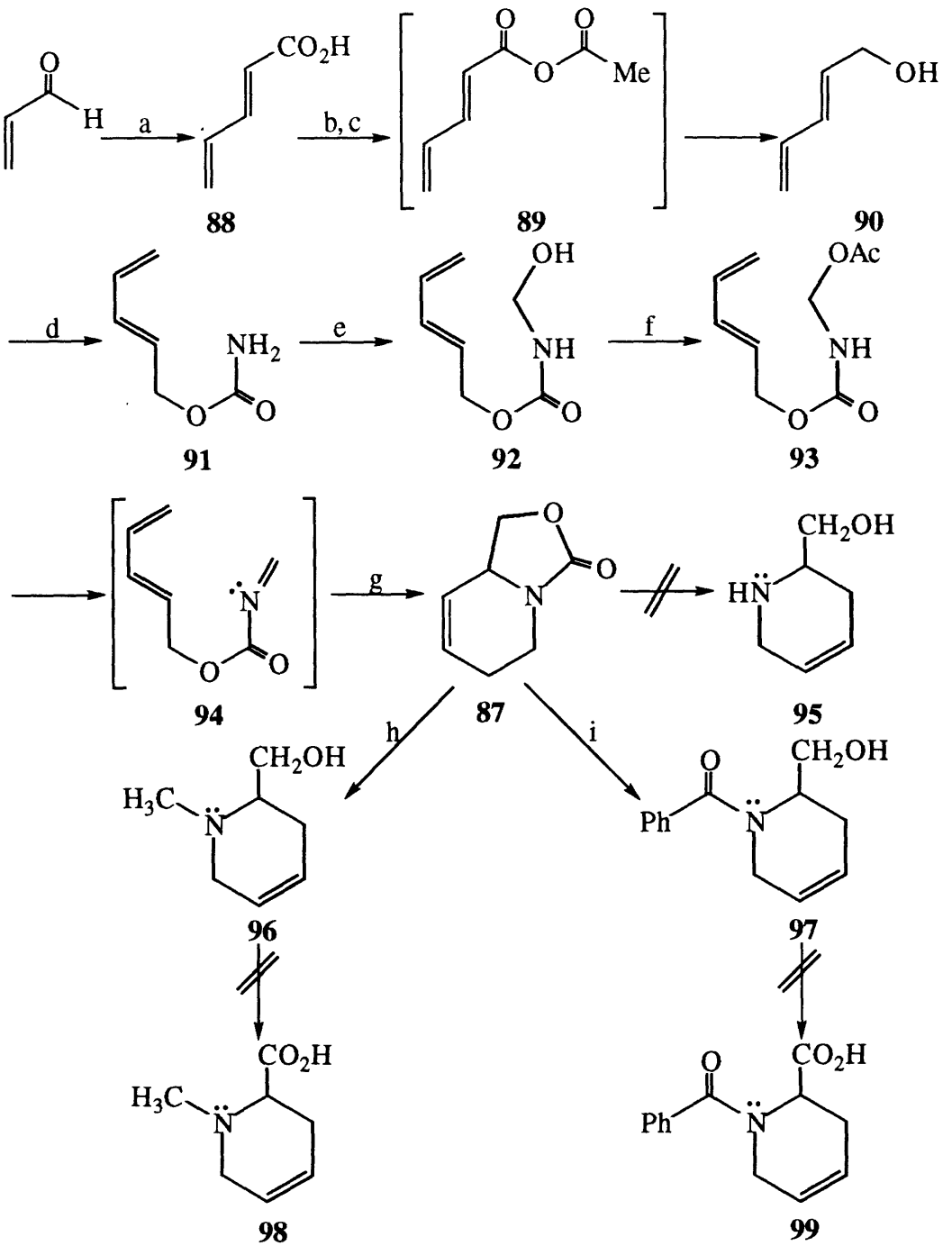
Scheme 2



We sought to synthesize **87** via an intramolecular Diels-Alder reaction of imine **94**. Intermediate **94** was formed through thermolysis of compound **93**, which was synthesized as outlined in Scheme 3. Acrolein was treated with malonic acid in anhydrous pyridine to give diene acid **88** in quantitative yield.[3] Diene acid **88** was reduced by $NaBH_4$ after treatment with methyl chloroformate to afford 2,4-pentadienol (**90**) in 85% yield.[4] Alcohol **90** was transformed in 77% yield to carbamate **91** on treatment with NaOCN and TFA in Et_2O.[5] Carbamate **91** was converted into the corresponding methylol **92** in good yield with formaldehyde and Cs_2CO_3 in THF. Methylol **92** was then transformed to Diels-Alder precursor **93** with acetic anhydride-pyridine at room temperature in 85% yield. Pyrolysis of methylol acetate **93** in *o*-dichlorobenzene at 210 °C produced a single cycloadduct **87** in 43% yield. This is the first successful case using a carbamate derivative without an activated dienophile.[5]

Compound **87** did not undergo basic hydrolysis on treatment with NaOH or $Ba(OH)_2$ under reflux conditions to form hydroxyl amine **95**. However, compound **87** was reduced by excess LAH under drastic conditions to form the hydroxy *N*-methyl amine **96** in quantitative yield.[6] Compound **87** also was converted into the corresponding hydroxyl phenyl amide **97** in good yield with 3 equiv of phenyl magnesium bromide under standard conditions.

Scheme 3[a]

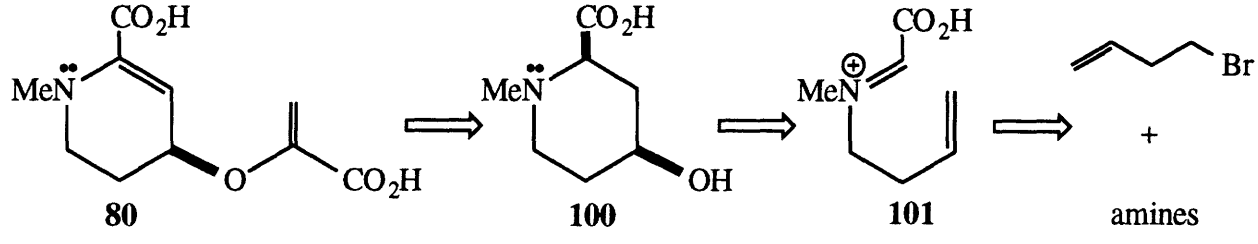


[a] Reagents: (a) ref. 3; (b) methyl chloroformate, NEt_3, THF; (c) $NaBH_4$, THF-H2O; (d) NaOCN, trifluoroacetic acid, Et_2O; (e) paraformaldehyde, Cs_2CO_3, THF; (f) acetic anhydride, pyridine, CH_2Cl_2; (g) 210 °C, 1,2-dichlorobenzene; (h) 3 equiv LAH, THF, reflux; (i) 3 equiv PhMgBr, ether.

Compounds **96** and **97** were oxidized to amino acids **98** and **99**, respectively, on treatment of CrO_3 in methylene chloride, but neither product could be purified. Many attempts to isolate pure amino acids **98** and **99** or the methyl esters of **98** and **99** failed. This route had to be abandoned since the amino acids and derivatives could not be purified.

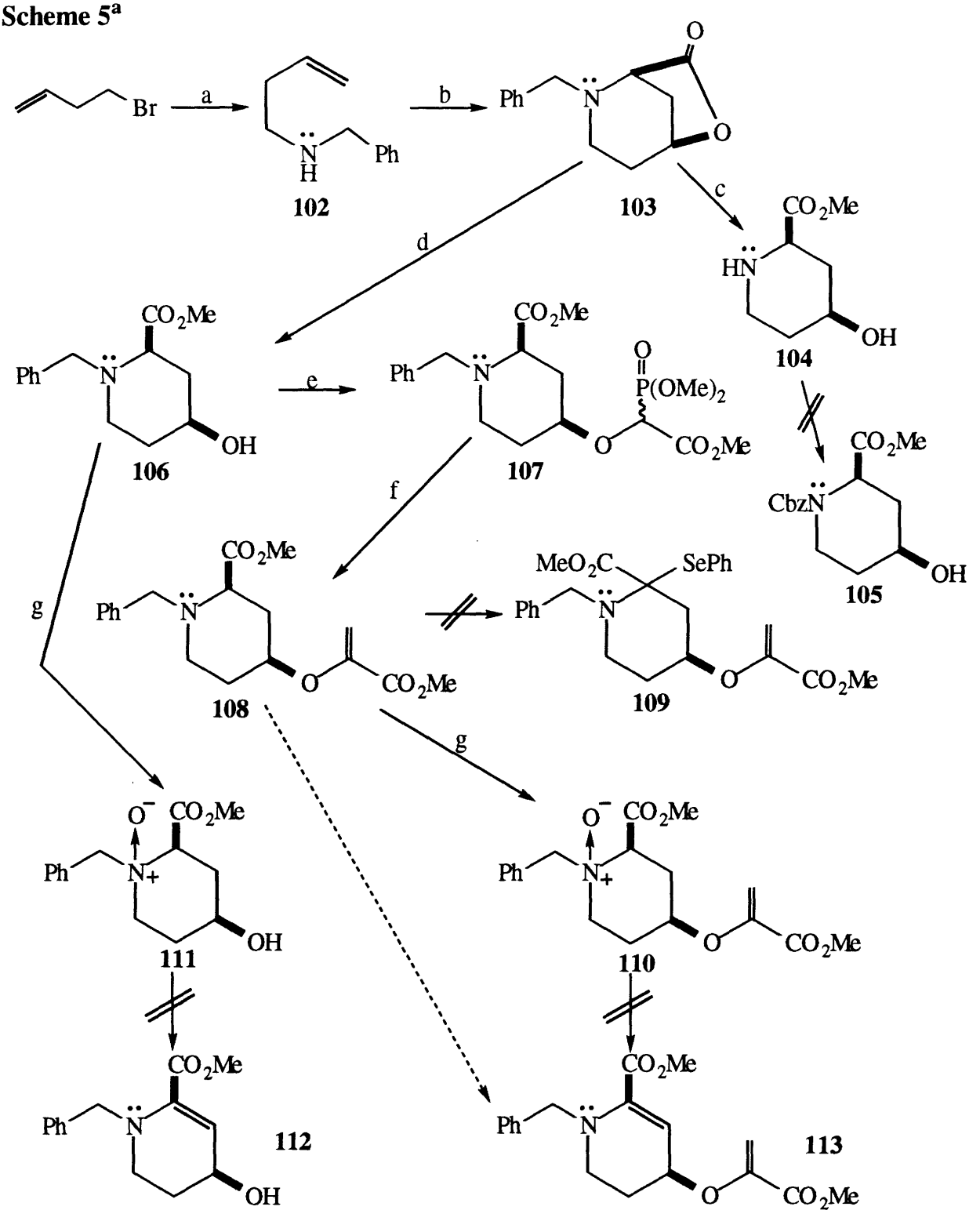
Hays et al reported the synthesis of a derivative of 2-piperidinecarboxylic acid having a skeleton similar to **80** (Chart 1).[7] Retrosynthetic analysis was modified to use the reported procedure[7] for synthesis of **80** from hydroxy 2-piperidinecarboxylic acid **100** via iminium ion **101** with cyclization under aqueous conditions (Scheme 4).

Scheme 4



The route used for the synthesis is depicted in Scheme 5. A modified procedure was used to prepare homoallylamine **102** as the starting material, which was formed from the reaction of 4-bromo-1-butene and benzyl amine. When compound **102** was treated with glyoxylic acid in aqueous acetonitrile, lactone **103** was isolated as the sole reaction product.[7] Subsequent debenzylation and lactone cleavage occurs in one step when **103** is hydrogenated in 10% formic acid in methanol in the presence of 5% Pd black to produce a quantitative yield of methyl ester **104**, for which the NMR data at δ 5.31 (m, 4 H, $CHOCH_3$) was incorrectly assigned in the literature.[7] Unfortunately amino methyl ester **104** could not be protected as carbobenzoxy ester **105** by the reported procedure.[7] Therefore, the

Scheme 5[a]



[a] Reagents: (a) $BnNH_2$, DMAP, $CHCl_3$, Δ ; (b) glyoxylic acid, CH_3CN-H_2O; (c) 5% Pd black, 10% formic acid, MeOH; (d) 1 equiv $ZnCl_2$, formic acid (cat.), MeOH; (e) $MeO_2CC(N_2)PO(OMe)_2$, Rh_2(Octanoate)$_4$, benzene, 60 °C; (f) $LiN(TMS)_2$, H_2CO, THF, -78 °C; (g) 30% H_2O_2, CH_2Cl_2.

benzyl group was used as a protecting group, and only the lactone was cleaved on treatment with 1 equiv $ZnCl_2$ and a catalytical amount of formic acid in methanol to produce *N*-benzyl amino methyl ester **106** in quantitative yield. Coupling of **106** and trimethyl diazophosphonoacetate with rhodium(II) octanoate catalysis in dry benzene at 60 °C gave phosphonate **107** in 85% yield after flash column chromatography. Ester **107** was converted to **108** by the Wittig-type reaction with strong base, $LiN(TMS)_2$, and quenching with gaseous formaldehyde at -78 °C to give **108** in an excellent yield.

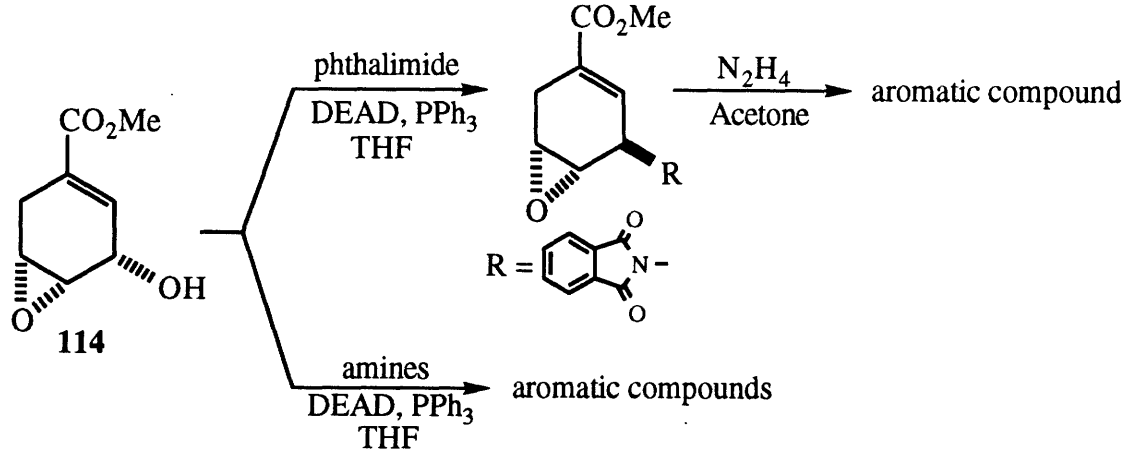
Exposure of **108** to strong base, LDA, in THF along with PhSeBr at -78 °C to room temperature gave no reaction; starting material **108** was recovered (85%). According to the literature,[8] enamines can be formed via imines or iminium salts upon treatment with 30% H_2O_2 to produce the *N*-oxide followed by the elimination of the trifluoroacetate formed in the presence of trifluoroacetic anhydride. This procedure was not successful with compound **110** and alcohol **111**. Hydrolysis of the enolpyruvate moiety of **110** was found under the acidic conditions. No desired product **113** could be detected. Synthesis of these analogs was terminated at this stage.

Attempted Synthesis of α,β-Unsaturated Amino Acid Chorismate Analog 82.

Since the synthesis of *cis*-epoxy alcohol **114** (Scheme 6) was developed by Berchtold and coworkers more than a decade ago, it has been used on the total synthesis of shikimate and chorismate-type structures.[2] The Mitsunobu reaction is an exceptionally useful and general method in organic synthesis whereby one can replace a hydroxyl group by a wide range of nucleophiles with inversion of configuration.[9] Both inter– and intramolecular versions of the Mitsunobu reaction are well documented. A variety of nitrogen nucleophiles have been utilized in this

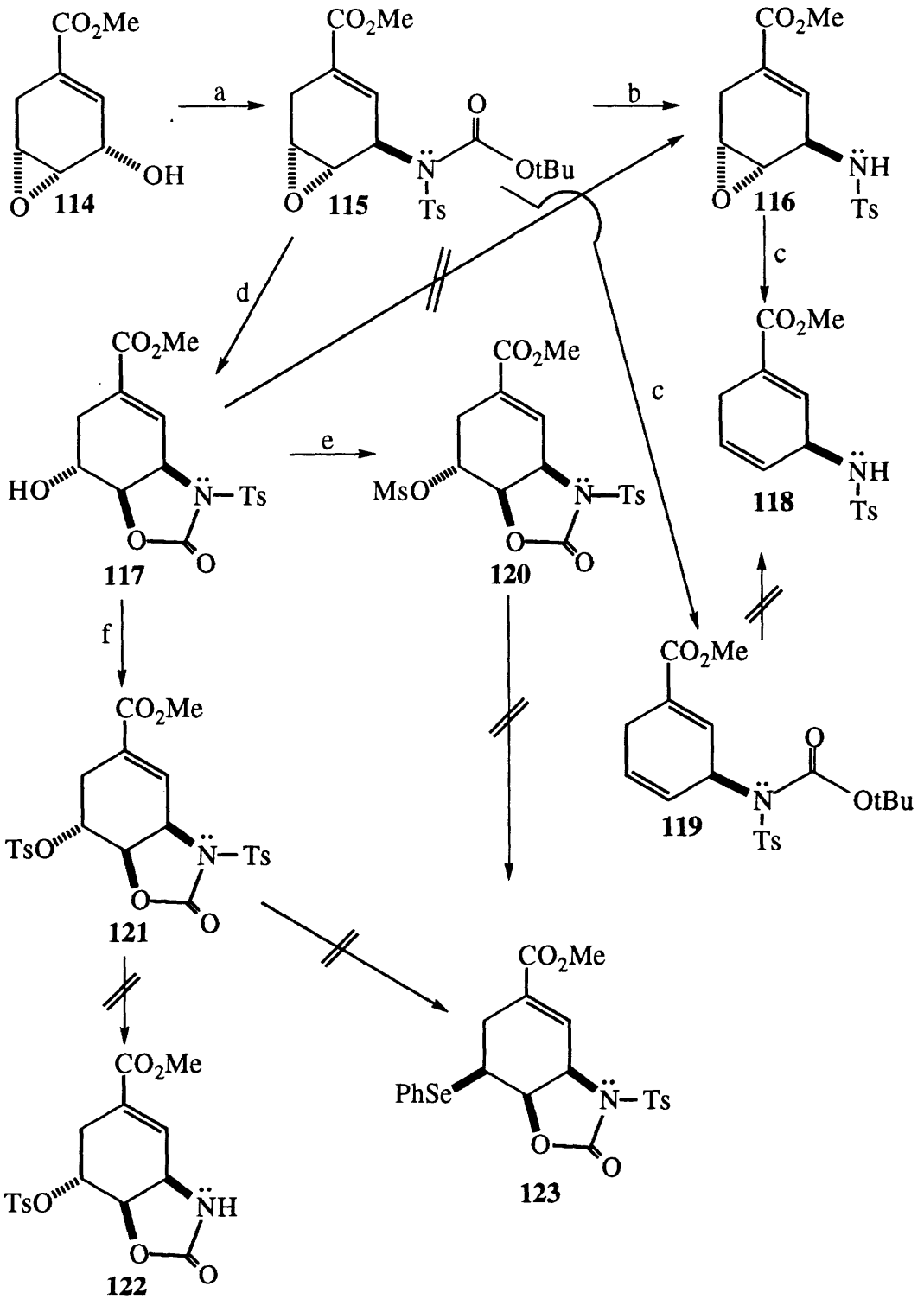
procedure to afford amines and amine derivatives.[9, 10] *cis*-Epoxy alcohol **114** tends to aromatize under either acidic or basic conditions. Attempts to replace the hydroxyl group to form an amine via Mitsunobu reaction (Scheme 6)[11] or by direct S_N2 displacement by an amine with a suitable derivative of the hydroxyl group resulted in formation of aromatic products.

Scheme 6



It is very important to develop good amino nucleophiles with a lower pK_a for the reaction with *cis*-epoxy alcohol **114** to prevent aromatization. *N*-BOC *p*-toluenesulfonamide developed by Weinreb et al[10e] recently can be coupled with *cis*-epoxy alcohol **114** under Mitsunobu conditions to afford a sulfonamide-protected amine **115** in good yield (Scheme 7). Surprisingly, the BOC group had to be removed in DMSO under drastic conditions to form compound **116** since ring closure occurred to produce **117** in quantitative yield under standard acidic conditions. Attempted coupling of **116** and excess trimethyl diazophosphonoacetate with rhodium(II) octanoate catalysis in dry benzene at 60 °C only led to deoxygenated product **118** in 60% yield after flash column chromatography. No insertion product was found. Compound **115** deoxygenated

Scheme 7[a]



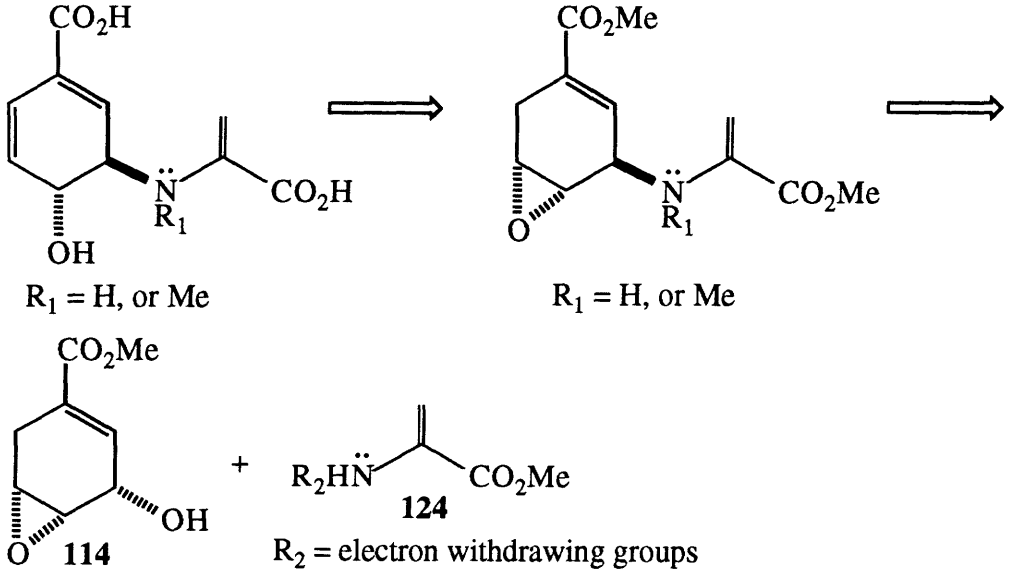
[a] Reagents: (a) *N*-BOC *p*-toluenesulfonamide, DEAD, PPh_3, THF ; (b) DMSO, Δ; (c) $MeO_2CC(N_2)PO(OMe)_2$, $Rh_2(Octonate)_4$, benzene, 60 °C; (d) trifluoroacetic acid, CH_2Cl_2 ; (e) MsCl, DMAP, benzene; (f) TsCl, DMAP, benzene.

to form **119** in moderate yield under similar conditions, and **119** aromatized under the acidic reaction conditions.

Alcohol **117** was not transformed to **116** on treatment with base under mild or reflux conditions; at least 90% of the starting material was recovered. Alcohol **117** could be converted into mesylate **120** and tosylate **121** in quantitative yield, respectively. However, no elimination or displacement occurred on exposure of **120** and **121** to strong base or PhSeLi in THF. Reductive cleavage of the tosyl group led to aromatic material. This route was abandoned at this stage.

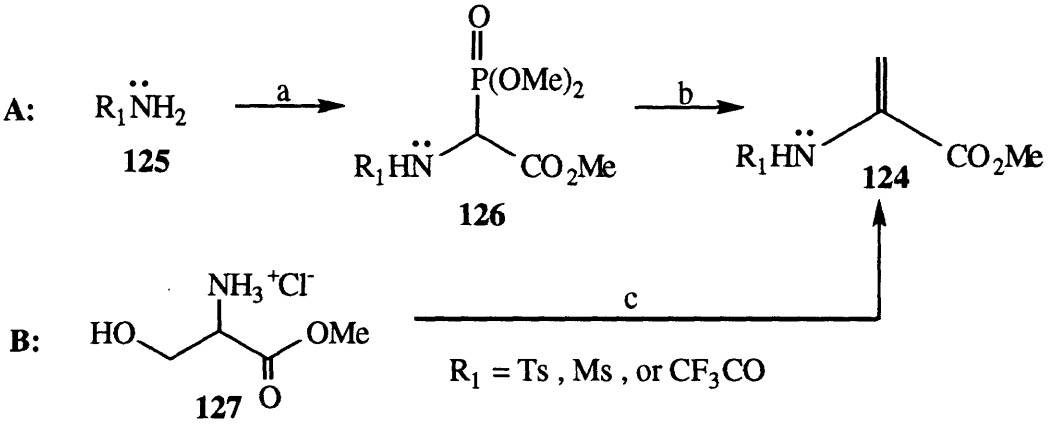
Since the only difference between chorismate (**9**) and compounds **82** or **83** is the side chain, another attractive and short route simply uses an α,β-unsaturated amino acid methyl ester as a nucleophile under Mitsunobu conditions. Apparently enamines have not been described as components of Mitsunobu reactions.[12, 13] As we have discussed above, good amino nucleophiles for the reaction with *cis*-epoxy alcohol **114** ought to have a lower pK_a to prevent aromatization. The enamine **124** in Scheme 8 is a good candidate as a nucleophile for the Mitsunobu reaction.

Scheme 8



The *N*-acyl α,β-unsaturated amino acid methyl esters **124** were prepared in a good yield (40 – 55%) by coupling of **125** and trimethyl diazophosphonoacetate with rhodium(II) octanoate catalysis in dry benzene at 60 °C to afford phosphonate **126** in an excellent yield after flash column chromatography. Esters **126** were then converted to **124** by the Wittig-type reaction with strong base, $LiN(TMS)_2$, etheral $ZnCl_2$, and quenching with gaseous formaldehyde at -78 °C to give **124** in reasonable yield (40 – 50%, Method A in Scheme 9). It is worth mention that the etheral $ZnCl_2$ is a key reagent to stabilize the enolate of **126** and to chelate both gaseous formaldehyde and the enolate of **126**.

Scheme 9[a]

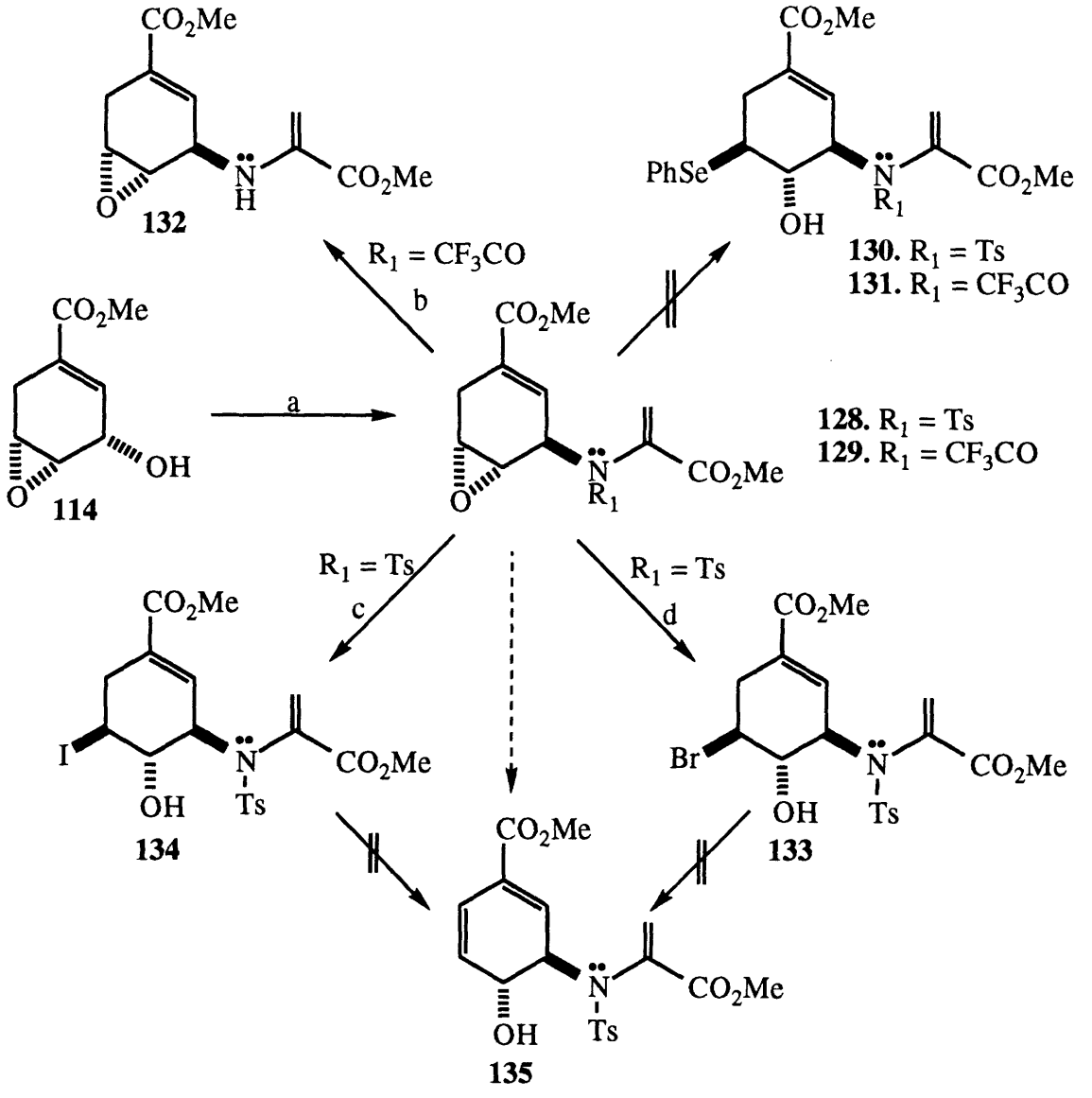


[a] Reagents: (a) $MeO_2CC(N_2)PO(OMe)_2$, $Rh_2(Octanoate)_4$, benzene, 60 °C ; (b) $LiN(TMS)_2$, etheral $ZnCl_2$, H_2CO, THF, -78 °C; (c) 2.5 equiv R_2Cl or trifluoroacetic anhydride, 5 equiv NEt_3-DMAP, THF.

The second strategy involving protection and elimination occurred in one step. The methyl ester of serine·HCl (**127**) and R_1Cl or trifluoroacetic anhydride in THF were added in one portion to excess base to give **124** in reasonable yield (55%, Method B in Scheme 9).

Mitsunobu reaction of **124** with *cis*-epoxy alcohol **114** provided the corresponding displacement products **128** and **129** in good yield (Scheme 10), but the mesyl derivative did not undergo reaction, and starting material was recovered.

Scheme 10[a]



[a] Reagents: (a) **124**, DEAD, PPh_3, THF; (b) $NaHPO_4$, THF; (c) HI, acetone; (d) TESBr, PPh_3, acetonitrile.

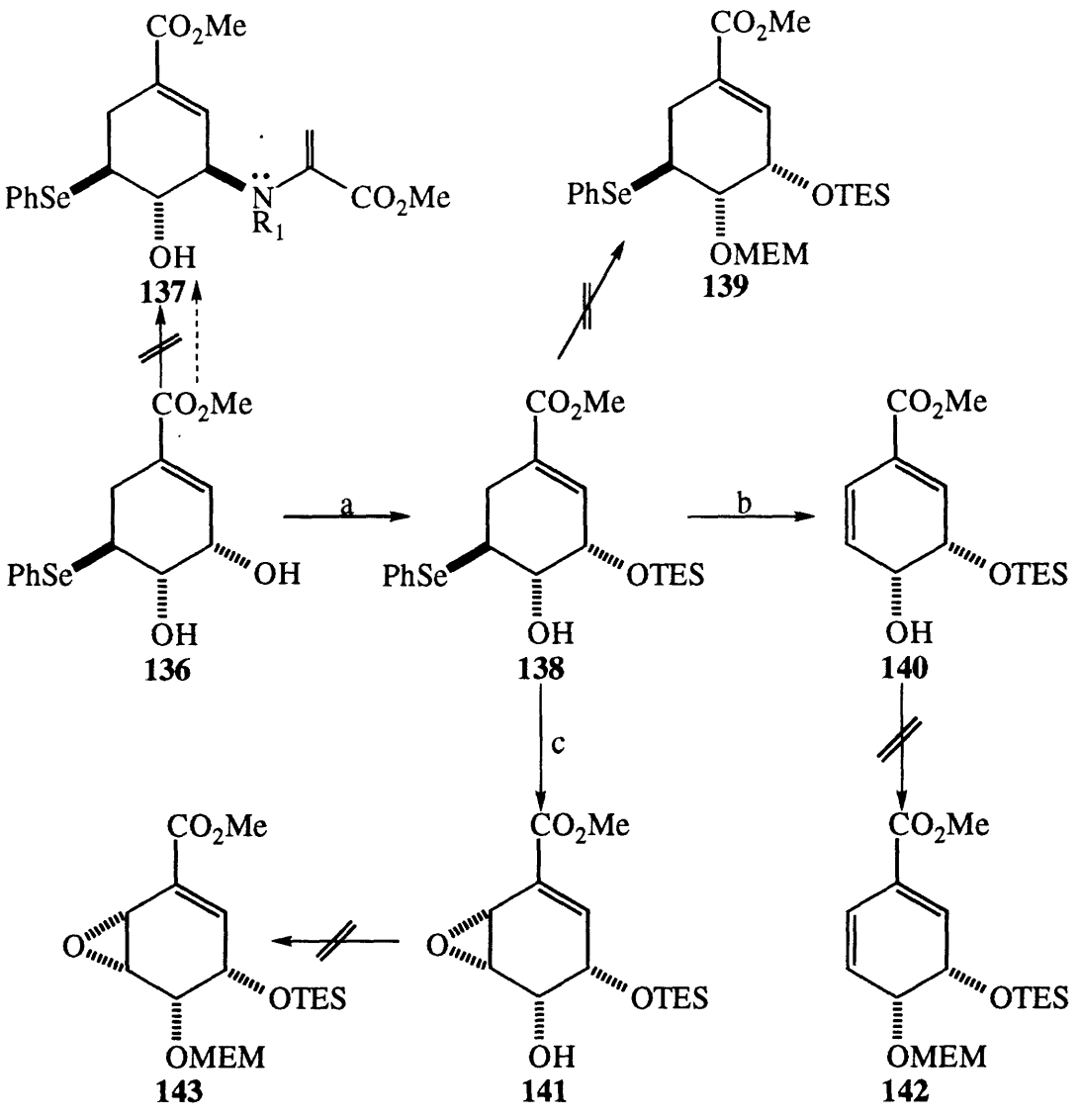
Reaction of compounds **128** and **129** with PhSeLi in THF or $(PhSe)_2$ with $NaBH_4$ in THF afforded the 1,4-addition adduct at the side-chain, and no desired

products, **130** and **131**, were found. The trifluroacetyl group in compound **129** can be hydrolyzed under mild conditions to furnish enamine **132** in low yield, but **132** decomposed on standing. Bartlett et al reported that similar epoxide derivatives were transformed to allylic alcohols on treatment with TMSBr and PPh_3 in acetonitrile and basic work-up.[14] However, only bromohydrin **133** was formed under these same conditions. Halo alcohols **133** and **134** failed to react with DBN in benzene.

The Mitsunobu reaction of **124** with *cis*-epoxy alcohol **114** can in fact be successfully used in good yield, but 1,4-addition reactions at the side-chain are a crucial problem for further steps to generate the allylic alcohol in the ring. Therefore another possible route for the synthesis of **82** was developed (Scheme 11).

Mitsunobu reaction of **124** with *cis*-diol **136**[11] did not provide the corresponding displacement product **137**, and the starting material was recovered (Scheme 10). The allylic hydroxyl group of **136** was protected as the silyl ether to afford **138** in excellent yield on the treatment with TESBr and triethyl amine in benzene. But the homoallyl alcohol group of **138** was not converted into the corresponding MEM ether **139** in CH_3Cl under reflux conditions due to the steric hindrance of the neighboring triethylsilyl group. Homoallyl alcohol **139** was transformed to cyclohexadienyl carboxylate **140** and *cis*-epoxy alcohol **141** in good yields with 1 equiv and 2 equiv MCPBA in CH_2Cl_2, respectively. Both compounds **140** and **141** aromatized during attempts to prepare the MEM ether derivative. The efforts to synthesize **82** were abandoned at this stage.

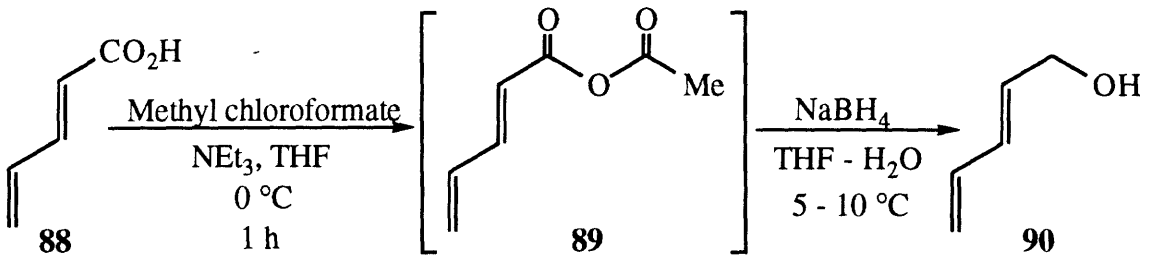
Scheme 11[a]



[a] Reagents: (a) TESBr, NEt_3, benzene; (b) 1 equiv MCPBA, CH_2Cl_2; (c) 2 equiv MCPBA, CH_2Cl_2.

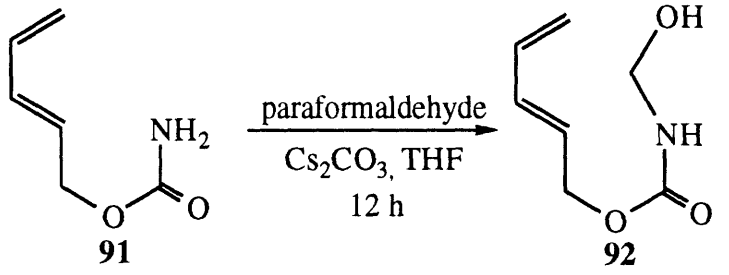
Experimental

2,4-Pentadienol (90).



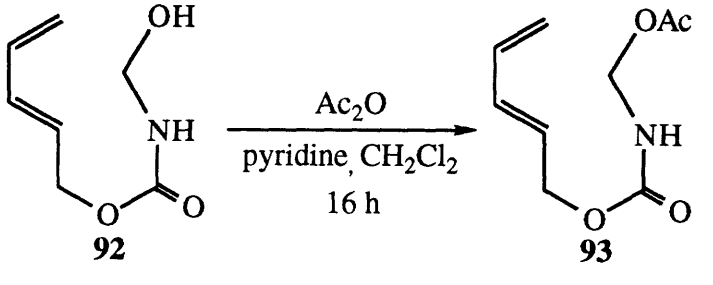
A solution of methyl chloroformate (7.2 g, 6 mL, 77 mmole, 1.5 equiv) in dry THF (10 mL) was added dropwise at -5 °C during the course of 30 min to a solution of diene acid[3] (5 g, 51 mmole) and triethylamine (5.2 g, 7.2 mL, 51 mmole, 1 equiv) in dry THF (100 mL). The resulting solution was stirred for another 30 min at the same temperature. The white precipate (triethylammonium chloride) was filtered off, washed with 20 mL of dry THF, and the combined filtrate and washings were added during 30 min to a solution of sodium borohydride (3 g, 77 mmole, 1.5 equiv) in water (20 mL) at 5 - 10 °C with external cooling. Violent evolution of gas was observed. After the addition was complete, the reaction mixture was stirred at room temperature for 3 h, then acidified with 6 N $HCl_{(aq)}$ and extracted. The combined etheral solution was washed with saturated $Na_2CO_{3(aq)}$, brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel (diethyl ether 100%) to give **90** (3.95 g, R_f = 0.45) as a pale yellow oil (85% yield). IR (neat) 3337, 2920, 2865, 1604, 1538, 1412, 1244, 1186, 1131 cm^{-1}; ^1H NMR (300 MHz) δ 6.42- 6.19 (2 H, m), 5.88 - 5.79 (1 H, m), 5.25 - 5.08 (2 H, m), 4.17 (2 H, d, J = 5.1 Hz), 2.42 (1 H, br); ^{13}C NMR (75 MHz) δ 136.3, 132.5, 131.7, 117.4, 76.6, 62.9.

N-Hydroxymethyl-2,4-pentadienyl Carbamate (92).



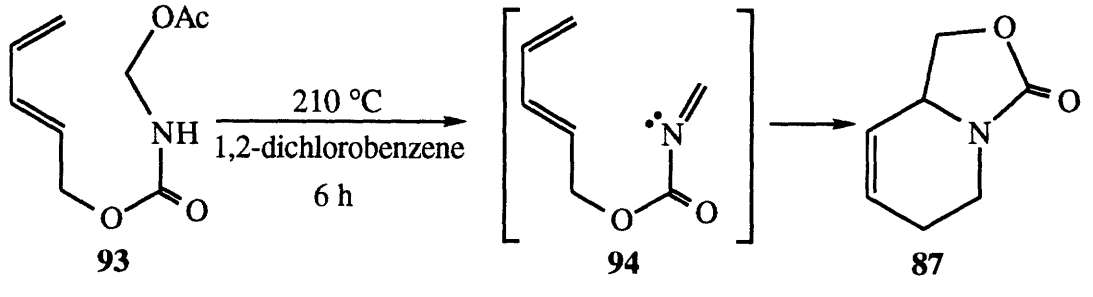
A mixture of carbamate **91**[5] (1 g, 8 mmole), paraformaldehyde (1.2 g, 40 mmole, 5 equiv), and cesium carbonate (2.6 g, 8 mmole, 1 equiv) in dry THF (25 mL) was stirred at room temperature under nitrogen for 12 h. The solvent was removed in vacuo. The residue was taken up in ether (100 mL) and washed with four 25-mL portions of water. The organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo to an oily residue that solidified on standing to a slightly yellowish solid. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:4) to give **92** (755 mg, $R_f = 0.35$) as a off-white solid (60% yield, mp. 77 - 78 °C). IR (KBr) 3324, 3250, 3047, 3004, 2901, 1697, 1604, 1486, 1457, 1414, 1146, 1103, 1086 cm^{-1}; ^1H NMR (300 MHz) δ 6 .42- 6.23 (2 H, m), 5.85 - 5.26 (1 H, m), 5.24 - 5.14 (1 H, br), 5.33 - 5.15 (2 H, m), 4.86 - 4.14 (4 H, m), 3.07 - 2.95 (1 H, br); ^{13}C NMR (75 MHz) δ 156.6, 135.8, 134.6, 128.0, 127.2, 118.7, 72.4, 66.1, 65.3; MS m/z (relative intensity) 157 (M^+, 0.8), 139 (10.7), 83 (100), 67 (55.6), 55 (57.6); LRMS for $C_7H_{11}NO_3$: 157.

N-Acetoxymethyl-2,4-pentadienyl Carbamate (93).



A stirred solution of methylol **92** (500 mg, 3.2 mmole) and anhydrous pyridine (253 mg, 260 μL, 3.2 mmole, 1 equiv) in CH_2Cl_2 (20 mL) under nitrogen was treated dropwise with acetic anhydride (520 mg, 480 μL, 4.8 mmole, 1.5 equiv) over 30 min. The resulting solution was stirred at room temperature for another 16 h. The mixture was poured into ice-water (20 mL), and the organic phase was separated and washed with 1 N $HCl_{(aq)}$, water, saturated $NaHCO_3$, water, and brine. The solution was dried over anhydrous $MgSO_4$ and concentrated in vacuo to give the desired product as a pale yellow oil. The crude product was then purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:6) to give **93** (541 mg, R_f = 0.33) as a colorless oil (85% yield). IR (NaCl) 3351, 3088, 3043, 2981, 2947, 2894, 1736, 1660, 1605, 1527, 1445, 1409, 1369, 1261, 1097, 1048 cm^{-1}; ^1H NMR (300 MHz) δ 6.40 - 6.21 (2 H, m), 6.18 - 6.17 (1 H, br), 5.82 - 5.72 (1 H, m), 5.30 - 5.13 (4 H, m), 4.64 (2 H, d, J = 4.64), 2.06 (3H, s); ^{13}C NMR (75 MHz) δ 171.5, 155.6, 135.7, 134.5, 126.9, 118.6, 72.4, 66.5, 65.3, 20.8; MS m/z (relative intensity) 199 (M^+, 0.3), 139 (0.4), 111 (2.4), 83 (8.2), 67 (42.1), 55 (27.2), 43 (100); LRMS for $C_9H_{13}NO_4$: 199.

1-Aza-9-oxo-8-oxabicyclo[4.3.0]non-4-ene (87).

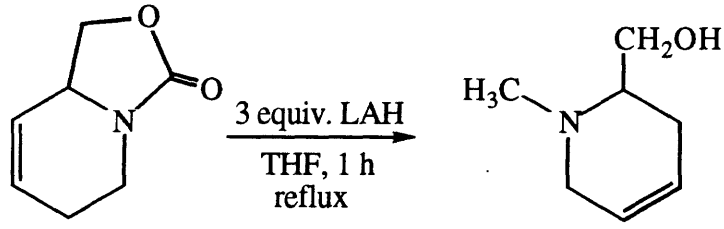


93 **94** **87**

A solution of compound **93** (250 mg, 1.26 mmole) in a large volume of 1,2-dichlorobenzene (300 mL) [molar ratio (solvent/solute) > 250:1] was heated to 210 °C for 6 h. The solution was cooled, and the solvent was removed under high vacuum. The residue was chromatographed by MPLC on silica gel (ethyl

acetate/petroleum ether 1:1) to give **87** (76 mg, R_f = 0.33) as a pale yellow oil (43% yield). IR (NaCl) 3034, 2980, 2917, 2842, 1745, 1648, 1480, 1452, 1420, 1383, 1290, 1235, 1092, 1062, 1023, 1004 cm^{-1}; ^1H NMR (300 MHz) δ 5.98 - 5.92 (1 H, dm, J = 9.8 Hz), 5.66 (1H, dm, J = 10 Hz), 4.48 (1H, dd, J = 8.1, 8.7 Hz), 4.43 - 4.35 (1 H, m), 3.95 (1H, dd, J = 5.7, 7.5), 3.93 - 3.80 (1H, m), 3.08 (1 H, ddd, J = 4.8, 11.5, 13.7 Hz), 2.47 - 2.32 (1 H, m), 2.10 - 1.97 (1 H, m); ^{13}C NMR (75 MHz) δ 157.2, 126.8, 125.4, 67.2, 51.9, 37.4, 22.7; MS m/z (relative intensity) 139 (M^+, 100), 81 (72.1), 67 (54.9), 55 (84.5), 41 (80.2); LRMS for $C_7H_9NO_2$: 139.

1-Methyl-2-hydroxymethyl-1,2,3,6-tetrahydropyridine (96).

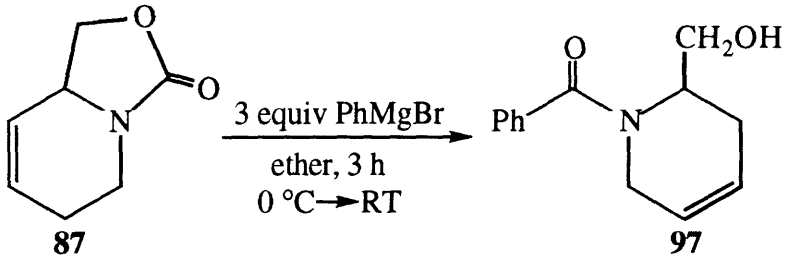


87 **96**

To a solution of lithium aluminum hydride (44 mg, 1.17 mmole, 3 equiv) in dry THF (5 mL) compound **87** (50 mg, 0.39 mmole) dissolved in 1 mL of dry THF was added slowly. The resulting solution was heated to reflux for 1 h and monitored by TLC which indicated starting material disappeared. The reaction was quenched with cold 1 N $NaOH_{(aq)}$ (1.5 mL). The white precipate was filtered off and washed with ether. The combined filtrate and the washings were washed with water, brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel ethyl acetate/petroleum ether 2:1) to give **96** (37.5 mg, R_f = 0.35) as a pale yellow oil (75% yield). ^1H NMR (300 MHz) δ 6.06 - 5.96 (1H, m), 5.67 - 5.77 (1H, m), 5.15

- 5.06 (1H, mbr), 3.82 - 3.64 (3 H, m), 3.32 - ,3.14 (1 H, m), 2.39 - 2.25 (1H, m), 2.20 (3 H, s), 2.18 - 1.95 (1H, m).

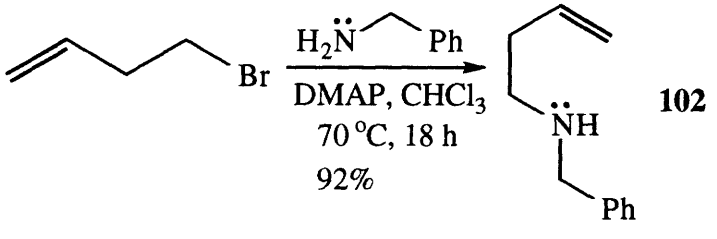
1-Benzoyl-2-hydroxymethyl-1,2,3,6-tetrahydropyridine (97).



87 **97**

To a solution of compound **87** (50 mg, 0.39 mmole) in dry ether (5 mL) was added a 1 M solution of PhMgBr in THF (1.2 mL, 1.2 mmol, 3 equiv) at 0 °C via syringe. The resulting solution was stirred at room temperature for 3 h and monitored by TLC which indicated starting material disappeared. The reaction was quenched with cold 1 N $HCl_{(aq)}$. The white emulsion was poured into water and extracted with ether. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel ethyl acetate/petroleum ether 1:3) to give **97** (55 mg, R_f = 0.35) as a pale yellow oil (65% yield). ^1H NMR (250 MHz) δ 7.46 (5 H, s), 6.06 - 5.96 (1H, m), 5.67 - 5.77 (1H, m), 5.18 - 5.08 (1H, mbr), 3.88 - 3.68 (3 H, m), 3.37 - 3.20 (1 H, m), 2.39 - 2.25 (1H, m), 2.18 - 1.95 (1H, m).

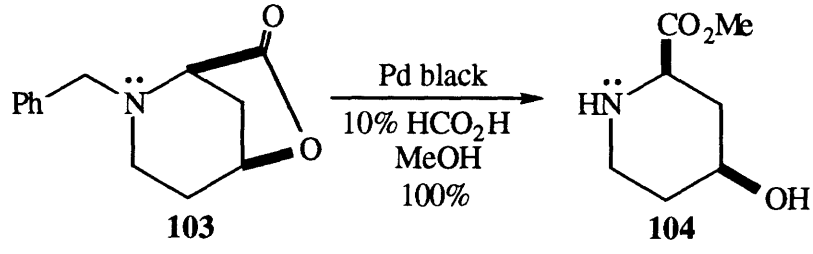
N-4-Butenylbenzylamine (102).



102

A mixture of 4-dimethylaminopyridine (DMAP, 1.7 g, 14 mmol, 0.5 equiv) and 4-bromo-1-butene (5.7 g, 4.3 mL, 42 mmole, 1.5 equiv) were added in one portion to a solution of benzyl amine (3 g, 3.1 mL, 28 mmole) in $CHCl_3$ (60 mL). The mixture was heated to 70 °C for 18 h. Solvent was removed under vacuum; and the residue was dissolved in ethyl acetate, passed through a pad of silica gel to remove base and amino salts, and concentrated in vacuo to give a dark red oil. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:2) to give **102** (4.15 g, $R_f = 0.4$) as a colorless oil (92% yield). ^1H NMR and IR data are the same as reported.[15]

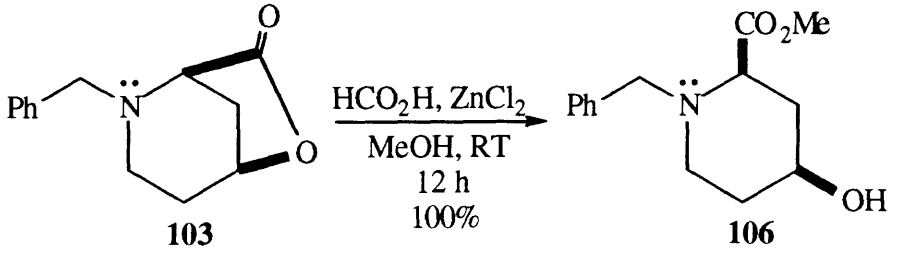
Methyl cis-4-Hydroxy-2-piperidinecarboxylate (104).



103 **104**

Lactone **103**[7] (500 mg, 2.3 mmole) and Pd black (5 mg, 0.05 mmole, 0.02 equiv) in 10 mL of 10% HCO_2H–methanol (v/v) was stirred at room temperature for 30 min, and the reaction was monitored by TLC which indicated starting material disappeared. The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated to give **104** as a pale yellow oil (320 mg, 100% yield). IR (NaCl) 3417, 3028, 2950, 1743, 1589, 1446, 1357, 1269, 1077 cm^{-1}; ^1H NMR (300 MHz) δ 3.85 - 3.70 (1 H, m), 3.75 (3 H, s), 3.40 - 3.38 (1H, m), 3.26 - 3.19 (1H, m), 2.70 - 2.60 (1H, m), 2.35 - 2.24 (1H, m), 1.97 - 1.87 (2H, m), 1.45 - 1.32 (1H, m); ^{13}C NMR (75 MHz) δ 172.9, 68.1, 56.7, 52.2, 42.7, 37.8, 34.8.

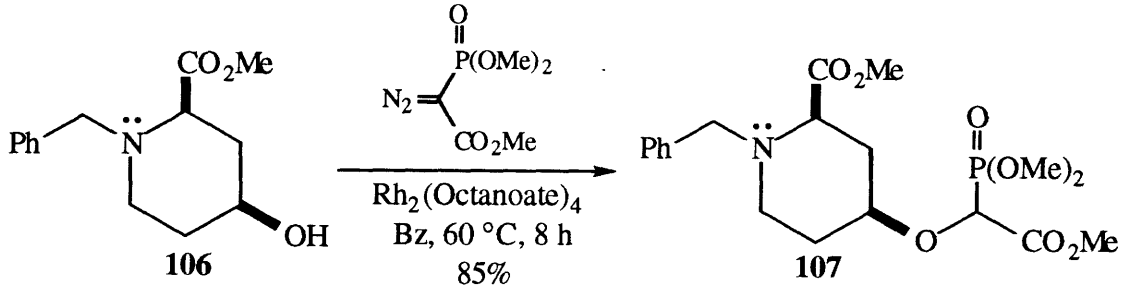
Methyl 1-Benzyl-*cis*-4-hydroxy-2-piperidinecarboxylate (106).



103 **106**

A solution of lactone **103**[7] (500 mg, 2.3 mmole), two drops of formic acid, and ZnCl2 (308 mg, 2.3mmole, 1 equiv) in 10 mL of MeOH were stirred at room temperature for 12 h. The reaction mixture was concentrated to give a white solid. The residue was dissolved in water and extracted with ethyl acetate. The organic layer was washed with saturated NaHCO3(aq), brine, dried over Na2SO4, filtered, and concentrated in vacuo to give a yellowish oil. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:1) to give **106** (570 mg, R_f = 0.33) as an off-white solid (100% yield, mp. 58 - 59 °C). IR (KBr) 3417, 3028, 2950, 1746, 1495, 1453, 1439, 1367, 1276 cm^{-1}; ^1H NMR (300 MHz) δ 7.33 - 7.27 (5 H, m), 3.80 (1H, d, J = 13.2 Hz), 3.76 (3 H, s), 3.41 (1 H, d, J = 13.5 Hz), 3.21 (1H, dd, J = 3.7, 8.6 Hz), 3.06 - 2.97 (1H, m), 2.22 - 2.08 (2H, m), 1.93 - 1.82 (2 H, m), 1.67 - 1.54 (2 H, m); ^{13}C NMR (75 MHz) δ 173.9, 137.6, 129.0, 128.0, 127.0, 66.6, 62.8, 59.7, 51.7, 47.2, 37.1, 33.5; MS m/z (relative intensity) 249 (M^+, 2.2), 191 (25.6), 172 (16.0), 146 (10.2), 91 (100), 65 (19.1); HRMS calc for C14H19NO3: 249.1365; Found 249.1365.

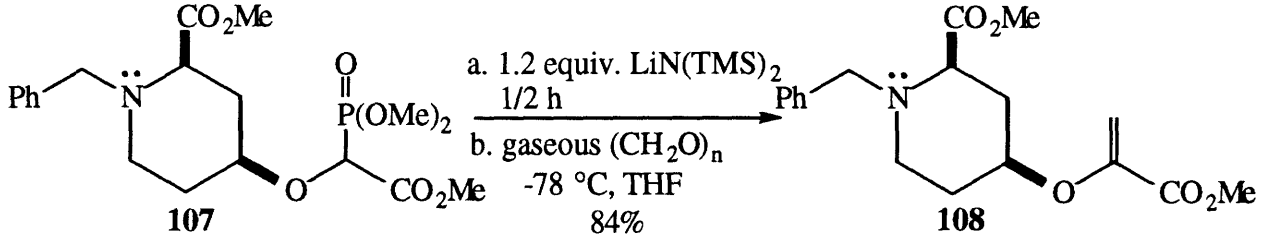
Methyl 1-Benzyl-*cis*-[1-(Methoxycarbonyl)-1-(dimethylphosphono)methoxy]-2-piperidinecarboxylate (107).



106 **107**

To starting material **106** (300 mg, 1.2 mmol) in dry benzene was added rhodium(II) octanoate (15 mg, 18.5 μmol, 0.02 equiv) and trimethyl diazophosphonoacetate (370 mg, 1.8 mmol, 1.5 equiv). The reaction mixture was brought to 60 °C for 8 h. Solvent was removed, and the crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 5:1) to give **107** (438 mg, R_f = 0.2) as a pale yellow oil (85% yield). ^1H NMR (300 MHz) δ 7.33 - 7.26 (5 H, m), 4.43 - 4.49 (1 H, two singlets), 3.85 - 3.76 (12 H, m), 3.37 - 3.27 (1 H, m), 3.14 - 2.96 (1 H, m), 2.26 - 1.65 (6 H, m).

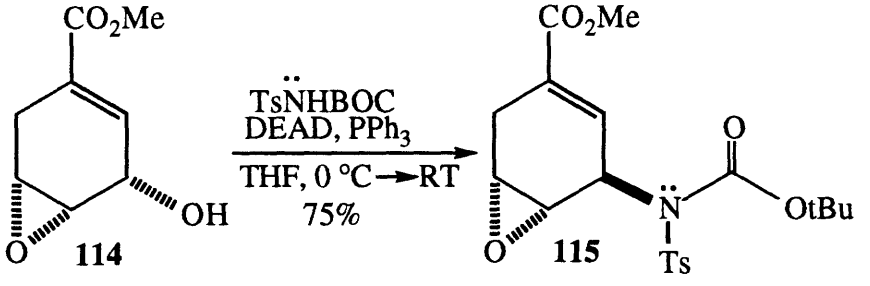
Methyl 1-Benzyl-*cis*-4-[[1-(Methoxycarbonyl)ethenyl]oxy]-2-piperidinecarboxylate (108).



To phosphonate **107** (250 mg, 0.58 mmol) in dry THF (12 mL) was added a 1 M solution of LiN(TMS)2 in THF (873 μL, 0.87 mmole, 1.5 equiv) at -78 °C (acetone-Dry Ice bath) via syringe. After the mixture was stirred for an additional 20 min, gaseous formaldehyde (generated from paraformaldehyde, 168 mg) was bubbled into the solution under a N_2 stream at -78 °C (acetone-Dry Ice bath) over 10 min. The reaction was continued for 2 h and monitored by TLC which indicated starting material disappeared. The reaction was quenched at -78 °C by the addition of saturated $NH_4Cl_{(aq)}$, followed by extraction with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:5) to give **108** (165 mg, R_f = 0.35) as a colorless oil (85% yield). IR (neat) 3019, 2952, 2845, 1741,

1727, 1622, 1495, 1452, 1437 cm^{-1}; ^1H NMR (300 MHz) δ 7.33 - 7.26 (5 H, m), 5.43 (1H, d, J = 2.61 Hz), 4.63 (1H, d, J = 2.45 Hz), 4.11 - 4.04 (1H, m), 3.90 (1H, d, J = 13.37 Hz), 3.78 (3H, s), 3.74 (3H, s), 3.49 (1H ,d, J = 13.53 Hz), 3.26 - 3.20 (1H, m), 3.14 - 3.06 (1H, m), 2.25 - 2.09 (3H, m), 1.96 - 1.77 (2H, m); ^{13}C NMR (75 MHz) δ 172.7, 163.5, 149.1, 137.8, 128.8, 128.0, 127.0, 96.0, 72.4, 61.5, 59.4, 52.0, 51.5, 46.3, 33.1, 29.4; MS m/z (relative intensity) 333 (M^+, 1.1), 274 (100), 172 (100), 91 (100), 65 (13.4); HRMS calc for $C_{14}H_{19}NO_3$: 333.1576; Found 333.1575.

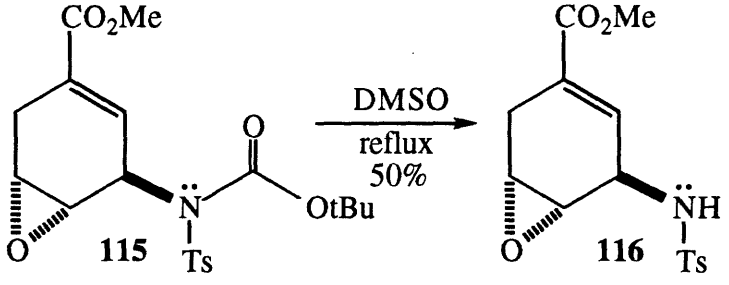
Methyl (1β,2β,6β)-2-[N-*t*-(Butoxycarbonyl)-N-(*p*-toluenesulfonyl)amino]-7-oxabicyclo[4.1.0]hept-3-ene-4-carboxylate (115).



N-BOC *p*-toluenesulfonamide (600 mg, 2.21 mmol, 1.5 equiv) was dissolved in dry THF (10 mL) and triphenylphosphine (771 mg, 2.94 mmol, 2 equiv) was added. The solution was stirred under nitrogen and *cis*-epoxy alcohol **114** (250 mg, 1.47 mmol) was added, followed by addition of 95% diethyl azodicarboxylate (DEAD, 365 μL, 3.2 mmol, 2.2 equiv) at 0 °C. The mixture was stirred at room temperature for 12 h and monitored by TLC which indicated starting material disappeared. The solvent was removed in vacuo and the crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:5) to give **115** (470 mg, R_f = 0.35) as a colorless oil (75% yield). IR (neat) 2984, 2938, 1729, 1710, 1667, 1598, 1454, 1438, 1371, 1359, 1278, 1253, 1169, 1150, 1088, 1050 cm^{-1}; ^1H NMR (300 MHz) δ 7.83 -

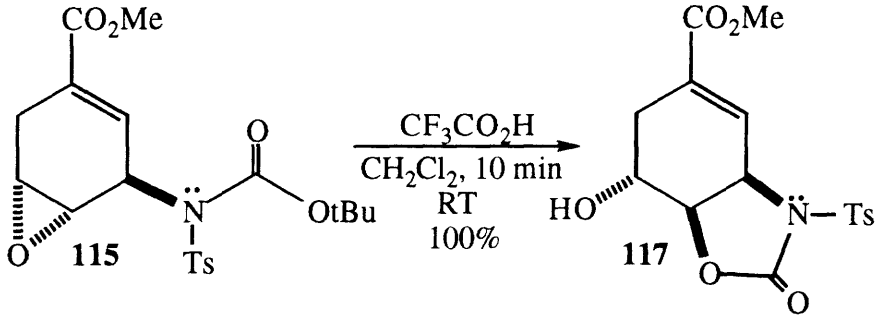
7.78 (2 H, m), 7.36 - 7.31 (2 H, m), 6.66 - 6.61 (1 H, m), 5.50 - 5.45 (1 H, m), 3.77 (3 H, s), 3.54 - 3.52 (1 H, m), 3.39 -3.36 (1 H, m), 2.98 - 2.88 (1 H, m), 2.81 - 2.70 (1 H, m), 2.46 (3 H, s), 1.36 (9 H, s); ^{13}C NMR (75 MHz) δ 166.6, 150.3, 144.5, 137.1, 131.0, 129.4, 128.0, 127.9, 85.2, 52.9, 52.4, 52.0, 51.9, 27.8, 24.3, 21.5; MS m/z (relative intensity) 367 ([M - C_4H_8]$^+$, 17.7), 323 (0.2), 305 (6.9), 212 (60.4), 155 (32.0) 136 (34.6), 91 (89.4), 57 (100); HRMS calc for $C_{20}H_{25}NO_7S$ ([M - C_4H_8]$^+$): 367.0726; Found 367.0723.

Methyl (1β,2β,6β)-2-*p*-Toluenesulphonylamido-7-oxabicyclo[4.1.0]hept-3-ene-4-carboxylate (116).



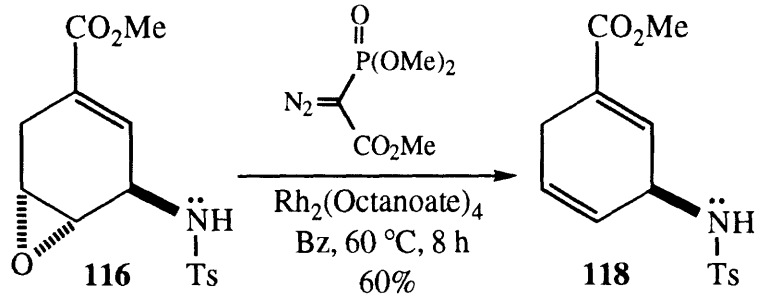
Starting material **115** (420 mg, 1 mmol) in dimethyl sulfoxide (DMSO, 15 mL) was heated under reflux for 30 min. The solvent was removed under high vacuum to give a brown oil. The crude product was purified by MPLC on silica gel (ethyl acetate/petroleum ether 1:3) to give **116** (154 mg, R_f = 0.35) as a colorless oil (50% yield). ^1H NMR (250 MHz) δ 7.83 - 7.77 (2 H, m), 7.38 - 7.34 (2 H, m), 6.41 - 6.34 (1 H, m), 4.53 (1 H, d J = 7.7 Hz), 4.38 - 4.28 (1 H, m), 3.73 (3 H, s), 3.38 - 3.35 (1 H, m), 3.27 - 3.22 (1 H, m), 3.10 - 2.88 (1 H, m), 2.65 - 2.52 (1 H, m), 2.45 (3 H, s).

Methyl (1α, 5α, 6α)-5-Hydroxy-9-(*p*-toluenesulfonyl)-8-oxo-9-aza-7-oxabicyclo[4.3.0]non-2-ene-3-carboxylate (117).



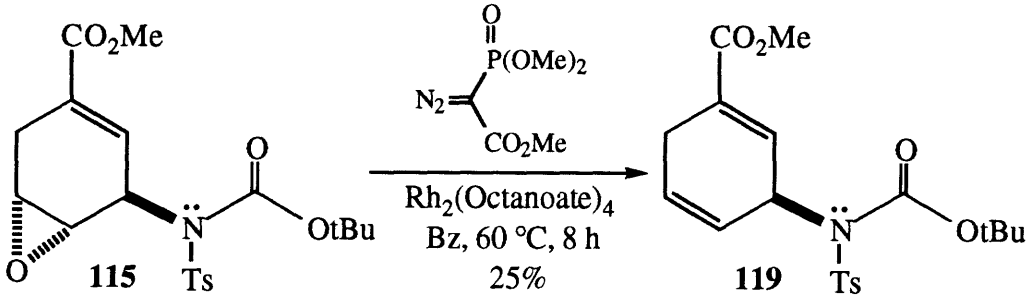
Trifluoroacetic acid (27 mg, 17.5 μL, 236 μmol, 1 equiv) was added to compound **115** (100 mg, 236 μmol) in CH_2Cl_2 (10 mL) at room temperature. The reaction was stirred at room temperature for 10 min and monitored by TLC which indicated starting material disappeared. The mixture was poured into saturated $NaHCO_3$ (aq) and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:2) to give **117** (87 mg, R_f = 0.33) as an off-white solid (100% yield, mp. 57 - 59 °C). IR (KBr) 3443, 2947, 2926, 2852, 1781, 1718, 1655, 1607, 1438, 1438, 1364, 1253, 1174, 1121, 1090, 1016, 915, 810, 662 cm^{-1}; ^1H NMR (300 MHz) δ 7.98 - 7.93 (2 H, m), 7.39 - 7.33 (2 H, m), 7.22 - 7.19 (1 H, m), 5.10 - 5.05 (1 H, m), 4.64 - 4.58 (1 H, m), 4.31 - 4.23 (1 H, m), 3.81 (3 H, s), 2.71 - 2.48 (2 H, m), 2.45 (3 H, s), 1.95 (1 H, m, J = 4.07 Hz); ^{13}C NMR (75 MHz) δ 166.0, 151.0, 145.8, 135.0, 131.1, 131.0, 129.8, 128.6, 75.0, 65.6, 54.6,52.2, 27.8, 21.6; MS m/z (relative intensity) 367 ($[M - C_4H_8]^+$, 17.7), 323 (0.2), 305 (6.9), 212 (60.4), 155 (32.0) 136 (34.6), 91 (89.4), 57 (100); HRMS calc for $C_{16}H_{17}NO_7S$: 367.0726; Found 367.0706.

Methyl 3-*p*-Toluenesulphonylamido-1,4-cyclohexadiene-1-carboxylate (118).



Rhodium(II) octanoate (15 mg, 18.6 μmol, 0.07 equiv) and trimethyl diazophosphonoacetate (87.4 mg, 420 μmol, 1.5 equiv) were added to **116** (90 mg, 280 μmol) in dry benzene (15 mL). The reaction mixture was heated at 60 °C for 8 h. Solvent was removed, and the crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:4) to give **118** (52 mg, R_f = 0.4) as a pale yellow oil (60% yield). ^{1}H NMR (300 MHz) δ 7.82 - 7.78 (2 H, m), 7.37 - 7.31 (2 H, m), 6.68 - 6.63 (1 H, m), 5.92 - 5.83 (1 H, m), 5.51 - 5.43 (1 H, m), 4.57 - 4.43 (2 H, brm), 3.73 (3 H, s), 2.97 - 2.71 (2 H, m), 2.42 (3 H, s).

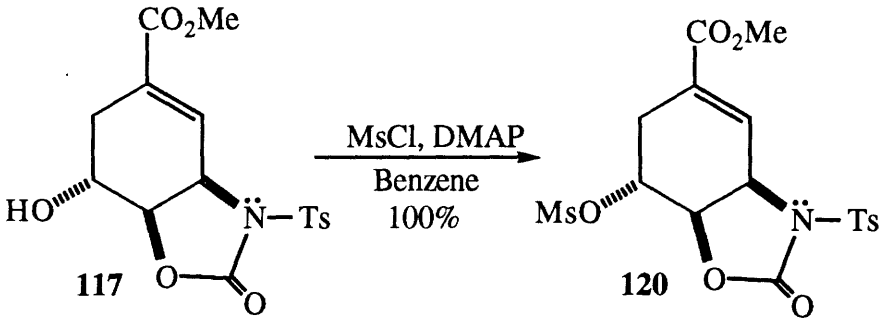
Methyl 3-[N-*t*-([*p*-butoxycarbonyl)-N-(p-toluenesulfonyl)amino]-1,4-cyclohexadiene-1-carboxylate (119).



Rhodium(II) octanoate (5 mg, 6.2 μmol, 0.07 equiv) and trimethyl diazophosphonoacetate (25 mg, 120 μmol, 1.4 equiv) were added to **115** (35 mg, 83 μmol) in dry benzene (2 mL). The reaction mixture was heated at 60 °C for 8 h. Solvent was removed, and the crude product was purified by flash column

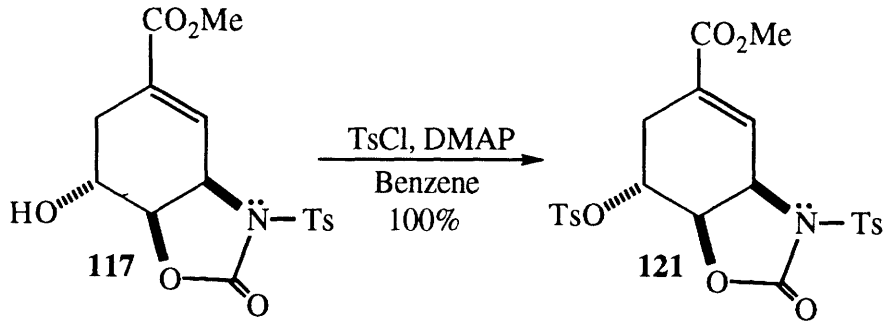
chromatography on silica gel (ethyl acetate/petroleum ether 1:8) to give **119** (8.5 mg, R_f = 0.33) as a pale yellow oil (25% yield). ^{1}H NMR (300 MHz) δ 7.85 - 7.81 (2 H, m), 7.35 - 7.31 (2 H, m), 6.91 - 6.86 (1 H, m), 6.0 - 5.92 (1 H, m), 5.78 - 5.65 (2 H, m), 3.77 (3 H, s), 3.08 - 2.77 (2 H, s), 2.44 (3 H, s).

Methyl (1α, 5α, 6α)-5-(Methanesulfonyloxy)-9-(*p*-toluenesulfonyl)-8-oxo-9-aza-7-oxabicyclo[4.3.0]non-2-ene-3-carboxylate (120).



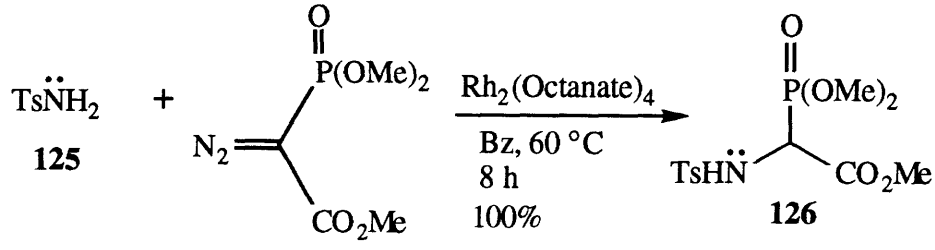
Methanesulfonyl chloride (116 μL, 1.5 mmol, 1.5 equiv) and 4-dimethylaminopyridine (DMAP, 240 mg, 1.5 mmol, 1.5 equiv) were added to **117** (360 mg, 1 mmol) in benzene (30 mL) at room temperature. The reaction was stirred at room temperature for 3 h and monitored by TLC which indicated starting material disappeared. Solvent was removed to give a yellow oil. The residue was dissolved in ethyl acetate, passed through a pad of silica gel, and concentrated to give **120** as a pale yellow oil, which was pure enough to use without further purfication. (440 mg, 100% yield). ^{1}H NMR (300 MHz) δ 7.95 - 7.92 (2 H, m), 7.39 - 7.36 (2 H, m), 7.25 - 7.22 (1 H, m), 5.17 - 5.08 (2 H, m), 4.87 - 4.81 (1 H, m), 3.82 (3 H, s), 3.07 (3 H, s), 2.88 - 2.73 (2 H, m), 2.46 (3 H, s).

Methyl (1α, 5α, 6α)-5-(*p*-Toluenesulfonyloxy)-9-(*p*-toluenesulfonyl)-8-oxo-9-aza-7-oxabicyclo[4.3.0]non-2-ene-3-carboxylate (121).



p-Toluenesulfonyl chloride (55 mg, 288 μmol, 1.5 equiv) and 4-dimethylaminopyridine (DMAP, 30 mg, 163 μmol, 0.85 equiv) were added to compound **117** (70 mg, 191 μmol) in benzene (5 mL) at room temperature. The reaction was stirred at room temperature for 3 h and monitored by TLC which indicated starting material disappeared. Solvent was removed to give a yellow oil. The residue was disolved in ethyl acetate, passed through a pad of silica gel, and concentrated to give **121** as a pale yellow oil which was pure enough to use without further purfication (98 mg, 100% yield). ^{1}H NMR (500 MHz) δ 7.95 - 7.92 (2 H, m), 7.76 - 7.72 (2 H, m), 7.38 - 7.32 (4 H, m), 7.15 - 7.12 (1 H, m), 5.10 - 5.06 (1 H, m), 4.95 - 4.91 (1 H, m),4.72 - 4.68 (1 H, m), 3.78 (3 H, s), 2.68 - 2.62 (1 H, m), 2.52 - 2.48 (1 H, m), 2.47 (3 H, s), 2.43 (3 H, s).

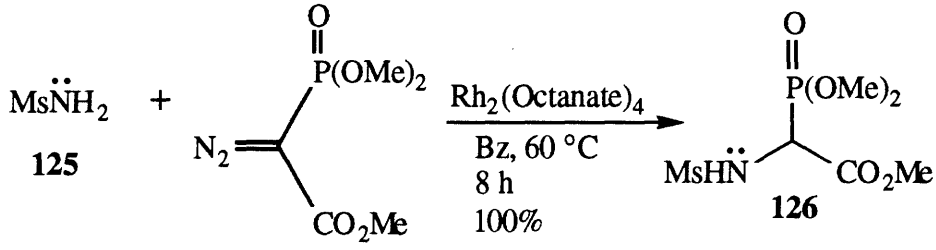
Trimethyl 2-(Toluenesulfonylamido)phosphonoacetate (126, R2 = Ts).



Rhodium(II) octanoate (15 mg, 18.5 μmol, 0.03 equiv) and trimethyl diazophosphonoacetate (182 mg, 876 μmol, 1.5 equiv) were added to toluenesulfonamide (100 mg, 584 μmol) in dry benzene (10 mL). The reaction mixture was heated at 60 °C for 8 h. Solvent was removed, and the crude product

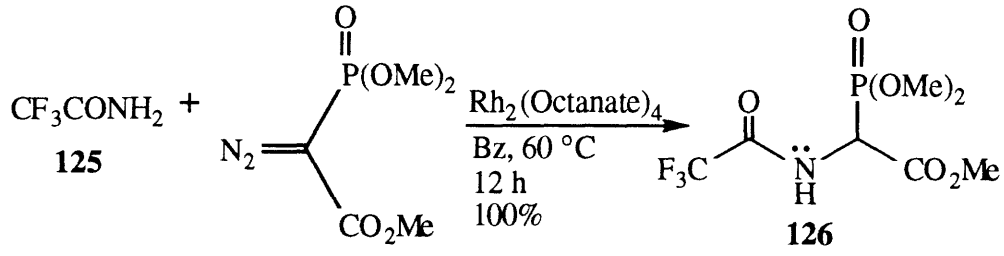
was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 4:1) to give **126** (R_2 = Ts) (205 mg, R_f = 0.30) as a white solid (mp 127 - 128 °C, 100% yield). IR (KBr) 3441, 3241, 3146, 2946, 1741, 1657, 1632, 1599, 1330, 1251, 1162, 1035, 935 cm^{-1}; ^1H NMR (300 MHz) δ 7.74 - 7.71 ((2 H, m), 5.43 - 5.39 (1 H, two broad doublets), 4.51 - 4.39 (1 H, two doublets), 3.85 - 3.79 (6 H, two doublets), 3.53 - 3.52 (3 H, two singlets), 2.4 (3 H, s); MS m/z (relative intensity) 351 (M^+, 2.5), 292 (38.5) 242 (38.6), 196 (92.2), 155 (100) 91 (35.5); HRMS calc for $C_{12}H_{18}NO_7PS$: 351.0542; Found 351.0550.

Trimethyl 2-(Methanesulfonylamido)phosphonoacetate (126, R_2 = Ms).



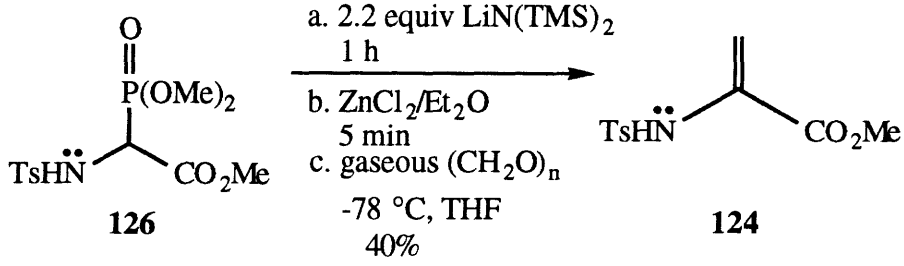
Rhodium(II) octanoate (25 mg, 30.8 μmol, 0.08 equiv) and trimethyl diazophosphonoacetate (1.25 g, 6 mmol, 1.5 equiv) were added to methanesulfonamide (400 mg, 4 mmol) in dry benzene (50 mL). The reaction mixture was heated at 60 °C for 8 h. Solvent was removed, and the crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 5:1) to give **126** (R_2 = Ms) (1 g, R_f = 0.34) as a pale yellow solid (mp 80 - 81 °C, 100% yield). IR (KBr) 3118, 3026, 2964, 2933, 1753, 1476, 1440, 1322, 1286, 1245, 1215, 1153, 1122, 1055, 1035 cm^{-1}; ^1H NMR (300 MHz) δ 5.42 - 5.37 (1 H, two broad doublets), 4.71 - 4.59 (1 H, two doublets), 3.91 - 3.84 (6 H, m), 3.05 (3 H, s); MS m/z (relative intensity) 275 (M^+, 0.7), 216 (88.1) 196 (73.6), 136 (14.0), 127 (13.9), 110 (100); HRMS calc for $C_6H_{14}NO_7PS$: 275.0229; Found 275.0235.

Trimethyl 2-(Trifluoroacetamido)phosphonoacetate (126, R_2 = CF_3CO).

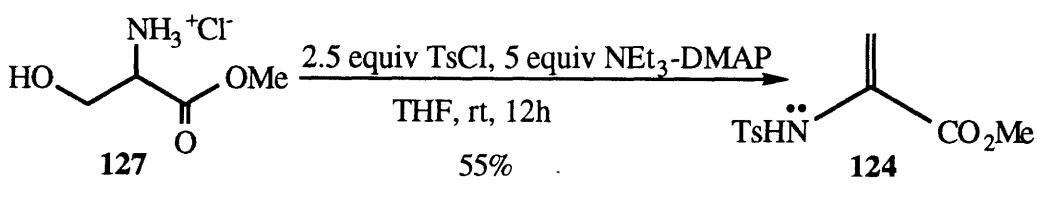


Rhodium(II) octanoate (25 mg, 30.8 μmol, 0.04 equiv) and trimethyl diazophosphonoacetate (1.85 g, 8.89 mol, 1 equiv) were added to trifluoroacetamide (1 g, 8.85 mmol) in dry benzene (30 mL). The reaction mixture was heated at 60 °C for 12 h. Solvent was removed, and the crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 4:1) to give **126** (R_2 = CF_3CO) (1.2 g, R_f = 0.30) as a yellow solid (mp 62 - 63 °C, 46% yield). IR (KBr) 3307, 3016, 2963, 2919, 2855, 1739, 1449, 1417, 1298, 1250, 1223, 1196, 1099, 1051, 1029, 916, 836 cm^{-1}; ^1H NMR (300 MHz) δ 5.42 - 5.37 (1 H, two broad doublets), 4.65 - 4.55 (1H, doublet), 3.93 - 3.85 (9 H, m), 3.45 - 3.35 (1 H, br); MS m/z (relative intensity) 275 (M^+, 0.7), 216 (88.1) 196 (73.6), 136 (14.0), 127 (13.9), 110 (100); HRMS calc for $C_7F_3H_{11}NO_6P$: 275.0229; Found 275.0235.

N-[1-(Methoxycarbonyl)ethenyl]-*p*-toluenesulfonylamide (124, R_2 = Ts).



Method A: A 1 M solution of $LiN(TMS)_2$ in THF (1.1 μL, 1.0 mmol, 2 equiv) at -78 °C (acetone-Dry Ice bath) was added via syringe to phosphonate **126** (170 mg, 0.48 mmol) in dry THF (5 mL). After the mixture was stirred for 20 min, a 1M etheral solution of $ZnCl_2$ (0.53 mL, 0.53 mmol, 1.1 equiv) was added at -78 °C (acetone-Dry Ice bath) via syringe, and the mixture was stirred for another 10 min. Gaseous formaldehyde (generated from paraformaldehyde, 145 mg) was bubbled into the solution under a N_2 stream at -78 °C (acetone-Dry Ice bath) over 10 min, and the reaction was continued for 2 h and monitored by TLC which indicated starting material disappeared. The reaction was quenched at -78 °C by the addition of saturated $NH_4Cl_{(aq)}$, followed by extraction with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:2) to give **124** (R_2 = Ts) (50 mg, R_f = 0.4) as an off-white solid (mp 96 - 97 °C, 40% yield). IR (KBr) 3278, 3234, 3029, 2954, 2911, 2858, 1730, 1634, 1596, 1424, 1338, 1295, 1166, 1091, 1005 cm^{-1}; ^1H NMR (300 MHz) δ 7.75 (2 H, ddd, J = 8.4, 1.8, 1.5 Hz), 7.30 (2 H, ddd, J = 8.4, 1.8, 1.4 Hz), 7.10 (1 H, d, J = 1.5 Hz), 5.70 (1H, d, J = 1.2 Hz), 5.60 (1 H, dd, J = 1.5, 1.2 Hz), 3.75 (3 H, s), 2.42 (3H, s); ^{13}C NMR (75 MHz) δ 163.6, 144.2, 135.6, 130.9, 129.6, 127.5, 106.9, 53.1, 21.5; MS m/z (relative intensity) 255 (M^+, 3.8), 191 (44.4), 155 (37.8), 132 (30.1), 91 (100); HRMS calc for $C_{11}H_{13}NO_4S$: 255.0567; Found 255.0567.

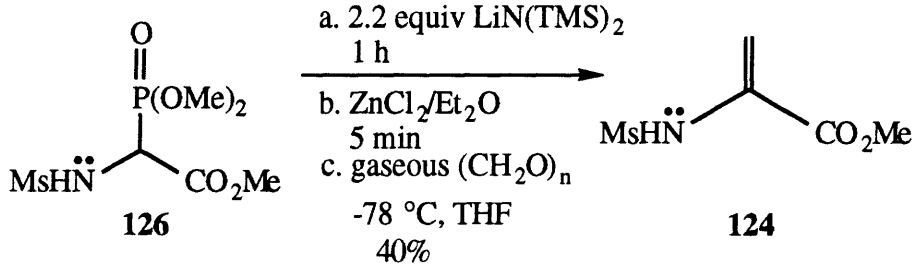


127 **124**

Method B: To a solution of the methyl ester of serine·HCl (1 g, 6.5 mmol) in dry THF (60 mL) trimethylamine (2.25 mL, 16.25 mmol, 2.5 equiv) and 4-

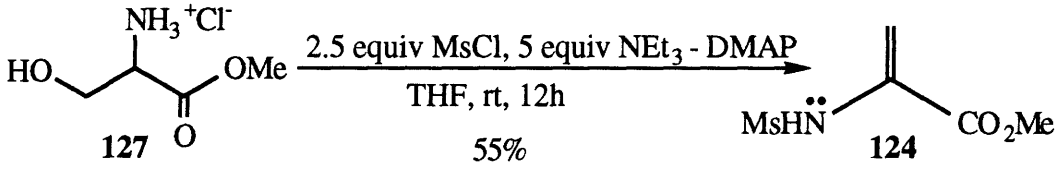
dimethylaminopyridine (DMAP, 3.1 g, 16.25 mmol, 2.5 equiv) were added in one portion. The mixture was stirred for 20 min. *p*-Toluenesulfonyl chloride (3.1g, 13 mmol, 2.5 equiv) was added, and the mixture was stirred for another 12 h. Solvent was removed; and the residue was dissolved in ethyl acetate, passed through a pad of silica gel to remove base and amino salts, and concentrated in vacuo to give dark red oil. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:2) to give **124** (R_2 = Ts) (912 mg, R_f = 0.4) as a off-white solid (mp.: 96 - 97 °C,.40% yield). IR (KBr) 3278, 3234, 3029, 2954, 2911, 2858, 1730, 1634, 1596, 1424, 1338, 1295, 1166, 1091, 1005 cm^{-1}; ^1H NMR (300 MHz) δ 7.75 (2 H, ddd, J = 8.4, 1.8, 1.5 Hz), 7.30 (2 H, ddd, J = 8.4, 1.8, 1.4 Hz), 7.10 (1 H, d, J = 1.5 Hz), 5.70 (1H, d, J = 1.2 Hz), 5.60 (1 H, dd, J = 1.5, 1.2 Hz), 3.75 (3 H, s), 2.42 (3H, s); ^{13}C NMR (75 MHz) δ 163.6, 144.2, 135.6, 130.9, 129.6, 127.5, 106.9, 53.1, 21.5; MS m/z (relative intensity) 255 (M^+, 3.8), 191 (44.4), 155 (37.8), 132 (30.1), 91 (100); HRMS calc for $C_{11}H_{13}NO_4S$: 255.0567; Found 255.0567.

N-[1-(Methoxycarbonyl)ethenyl]methanesulfonamide (124, R_2 = Ms).



Method A: To phosphonate **126** (R_2 = Ms) (1.05 g, 3.82 mmol) in dry THF (18 mL) was added via syringe a 1 M solution of $LiN(TMS)_2$ in THF (8.01 mL, 8.01 mmol, 2.1 equiv) at -78 °C (acetone-Dry Ice bath). After the mixture was stirred for 20 min, a 1 M etheral solution of $ZnCl_2$ (4.2 mL, 4.2 mmol, 1.1 equiv) was added via syringe at -78 °C (acetone-Dry Ice bath), and the mixture was

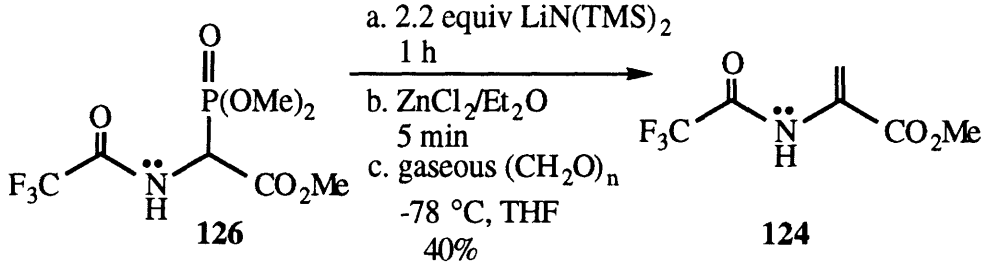
stirred for another 10 min. Gaseous formaldehyde (generated from paraformaldehyde, 1.146 g) was bubbled into the solution under a N_2 stream at -78 °C (acetone-Dry Ice bath) over 10 min, and the reaction was continued for 2 h and monitored by TLC which indicated starting material disappeared. The reaction was quenched at -78 °C by the addition of saturated $NH_4Cl_{(aq)}$, followed by extraction with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:1) to give **124** (R_2 = Ms) (252 mg, R_f = 0.25) as a pale yellow oil (40% yield). IR (neat) 3278, 3016, 2956, 2926, 1735, 1718, 1628, 1573, 1447, 1421, 1336, 1291, 1995, 1145, 1004, 974 cm^{-1}; ^1H NMR (300 MHz) δ 6.9 (1 H, br), 5.80 (1 H, d, J = 1.5 Hz), 5.70 (1 H, d, J = 1.5 Hz), 3.87 (3 H, s), 3.04 (3 H, s); ^{13}C NMR (75 MHz) δ 163.4, 131.3, 106.7, 53.3, 38.6; MS m/z (relative intensity) 180 ($[M+H]^+$, 1.7), 191 (44.4), 150 (6.6), 95 (56.1), 80 (100); HRMS calc for $C_5H_9NO_4S$ $[M+H]^+$: 180.0331; Found 180.0329.



Method B: To a solution of the methyl ester of serine·HCl (2 g, 12.9 mmol) in dry THF (50 mL), trimethylamine (4.5 mL, 32.25 mmol, 2.5 equiv) and 4-dimethylaminopyridine (DMAP, 4 g, 32.25 mmol, 2.5 equiv) were added in one portion. The mixture was stirred for 20 min. Methanesulfonyl chloride (2.5 mL, 32 mmole, 2.5 equiv) was added, and the mixture was stirred for another 12 h. Solvent was removed; and the residue was dissolved in ethyl acetate, passed through a pad of silica gel to remove base and amino salts, and concentrated in vacuo to give dark red oil. The crude product was purified by flash column

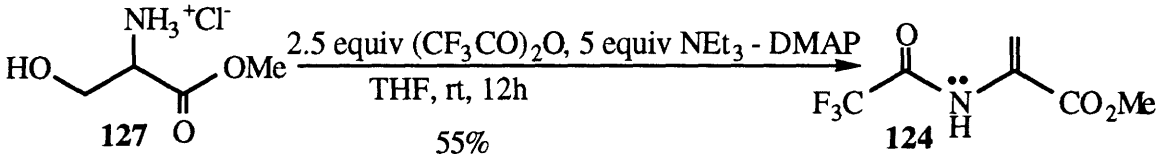
chromatography on silica gel (ethyl acetate/petroleum ether 1:1) to give **124** (R_2 = Ms) (1.2 g, R_f = 0.25) as a pale yellow oil (55% yield). IR (neat) 3278, 3016, 2956, 2926, 1735, 1718, 1628, 1573, 1447, 1421, 1336, 1291, 1995, 1145, 1004, 974 cm^{-1}; ^1H NMR (300 MHz) δ 6.9(1 H, br), 5.80 (1 H, d, J = 1.5 Hz), 5.70 (1 H, d, J = 1.5 Hz), 3.87 (3 H, s), 3.04 (3 H, s); ^{13}C NMR (75 MHz) δ 163.4, 131.3, 106.7, 53.3, 38.6; MS m/z (relative intensity) 180 ($[M+H]^+$, 1.7), 191 (44.4), 150 (6.6), 95 (56.1), 80 (100); HRMS calc for $C_5H_9NO_4S$ $[M+H]^+$: 180.0331; Found 180.0329.

N-[1-(Methoxycarbonyl)ethenyl]trifluoroacetamide (124, R_2 = CF_3CO).



Method A: To phosphonate **124** (R_2 = CF_3CO) (1.05 g, 3.82 mmol) in dry THF (18 mL) was added via syringe a 1 M solution of $LiN(TMS)_2$ in THF (8.01 mL, 8.01 mmol, 2.1 equiv) at -78 °C (acetone-Dry Ice bath). After the mixture was stirred for 20 min, a 1 M etheral solution of $ZnCl_2$ (4.2 mL, 4.2 mmol, 1.1 equiv) was added via syringe at -78 °C (acetone-Dry Ice bath), and the mixture was stirred for another 10 min. Gaseous formaldehyde (generated from paraformaldehyde, 1.146 g) was bubbled into the solution under a N_2 stream at -78 °C (acetone-Dry Ice bath) over 10 min, and the reaction was continued for 2 h and monitored by TLC which indicated starting material disappeared. The reaction was quenched at -78 °C by the addition of saturated $NH_4Cl_{(aq)}$, followed by extraction with ethyl acetate. The organic layer was washed with brine, dried

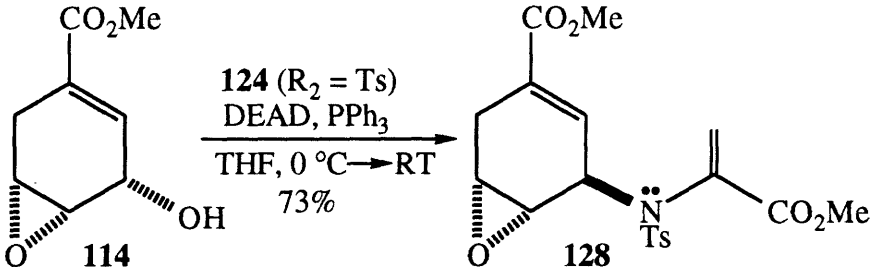
over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:4) to give **124** ($R_2 = CF_3CO$) (300 mg, $R_f = 0.33$) as a pale yellow oil (40% yield). IR (neat) 3381, 3152, 2953, 2923, 2854, 1740, 1715, 1641, 1536, 1441, 1387, 1357, 1297, 1213, 1158, 994, 964 cm^{-1}; ^1H NMR (500 MHz) δ 8.85 - 8.45 (1 H, br), 6.72 (1 H, d, J = 1.0 Hz), 6.14 (1 H, d, J = 1.0 Hz), 3.91 (3 H, s); ^{13}C NMR (75 MHz) δ 163.2, 154.9 (1 C, q, J = 37.8 Hz), 124.9, 115.1 (1 C, q, J = 285.9 Hz), 112.1, 53.3; MS m/z (relative intensity) 197 (M^+, 47.3), 165 (100), 138 (30.2), 69 (51.2); HRMS calc for $C_6F_3H_6NO_3$: 197.0300; Found 197.0298.



Method B: To a solution of the methyl ester of serine·HCl (1 g, 6.5 mmol) in dry THF (50 mL), trimethylamine (2.25 mL, 16.25 mmol, 2.5 equiv) and 4-dimethylaminopyridine (DMAP, 3.1 g, 16.25 mmol, 2.5 equiv) were added in one portion. The mixture was stirred for 20 min. Trifluoroacetic anhydride (1 mL, 32.25 mmol, 2.5 equiv) was added, and the mixture was stirred for another 12 h. Solvent was removed; and the residue was dissolved in ethyl acetate, passed through a pad of silica gel to remove base and amino salts, and concentrated in vacuo to give yellow oil. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:4) to give **124** ($R_2 = CF_3CO$) (705 mg, $R_f = 0.33$) as a pale yellow oil (55% yield). IR (neat) 3381, 3152, 2953, 2923, 2854, 1740, 1715, 1641, 1536, 1441, 1387, 1357, 1297, 1213, 1158, 994, 964 cm^{-1}; ^1H NMR (500 MHz) δ 8.85 - 8.45 (1 H, br), 6.72 (1 H, d, J = 1.0 Hz), 6.14 (1 H, d, J = 1.0 Hz), 3.91 (3 H, s); ^{13}C NMR (75 MHz) δ 163.2, 154.9 (1 C, q, J = 37.8 Hz), 124.9, 115.1 (1 C, q, J = 285.9 Hz), 112.1, 53.3; MS

m/z (relative intensity) 197 (M^+, 47.3), 165 (100), 138 (30.2), 69 (51.2); HRMS calc for $C_6F_3H_6NO_3$: 197.0300; Found 197.0298.

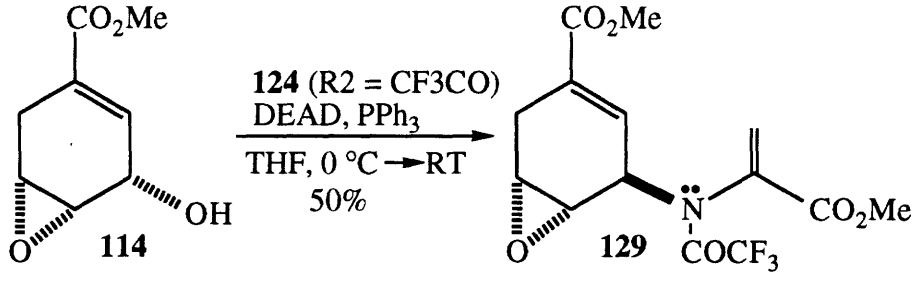
Methyl (1β,2β,6β)-2-[N-(*p*-Toluenesulphonyl)[1-(methoxycarbonyl)ethenyl]amino]-7-oxabicyclo[4.1.0]hept-3-ene-4-carboxylate (128).



Sulfonamide **124** (R_2 = Ts) (200 mg, 0.78 mmol, 1 equiv) was dissolved in dry THF (15 mL) and triphenylphosphine (307 mg, 1.17 mmol, 1.5 equiv) was added. The solution was stirred under nitrogen; and **114** (133.5 mg, 0.78 mmol) was added, followed by addition of diethyl azodicarboxylate (DEAD, 160 μL, 0.94 mmol, 1.2 equiv, purity: 95%) at 0 °C. The mixture was stirred at room temperature for 12 h and monitored by TLC which indicated starting material disappeared. The solvent was removed in vacuo and the crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:2) to give **128** (470 mg, R_f = 0.33) as a white solid (73% yield, mp. 107 - 108 °C). IR (neat) 2984, 2938, 1729, 1710, 1667, 1598, 1454, 1438, 1371, 1359, 1278, 1253, 1169, 1150, 1088, 1050 cm^{-1}; ^1H NMR (300 MHz) δ 7.83 - 7.78 (2 H, m), 7.36 - 7.31 (2 H, m), 6.66 - 6.61 (1H, m), 5.50 - 5.45 (1 H, m), 3.77 (3 H, s), 3.54 - 3.52 (1 H, m), 3.39 -.3.36 (1H, m), 2.98 - 2.88 (1H, m), 2.81 - 2.70 (1H, m), 2.46 (3H, s), 1.36 (9H, s); ^{13}C NMR (75 MHz) δ 166.6, 150.3, 144.5, 137.1, 131.0, 129.4, 128.0, 127.9, 85.2, 52.9, 52.4, 52.0, 51.9, 27.8, 24.3, 21.5; MS m/z

(relative intensity) 367 (m/z, 0.2), 325 (3.1), 283 (3.0), 253 (13.6), 220 (12.2) 192 (21.1), 91 (23.7); HRMS calc for $C_{19}H_{21}NO_7S$: 407.1039; Found 407.1036.

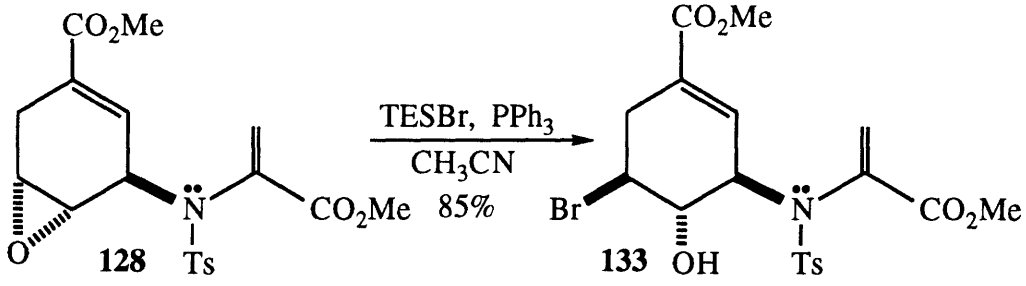
Methyl (1β,2β,6β)-2-[N-Trifluoroacetyl[1-(methoxycarbonyl)ethenyl]amino]-7-oxabicyclo[4.1.0]hept-3-ene-4-carboxylate (129).



Trifluoroacetamide **124** (R_2 = CF_3CO) (870 mg, 4.42 mmol, 1 equiv) was dissolved in dry THF (20 mL), and triphenylphosphine (1.55 g, 5.3 mmol, 1.2 equiv) was added. The solution was stirred under nitrogen and **114** (752 mg, 4.42 mmol) was added, followed by addition of diethyl azodicarboxylate (DEAD, 878 μL, 5.3 mmol, 1.2 equiv, purity: 95%) at 0 °C. The mixture was stirred at room temperature for 12 h and monitored by TLC which indicated starting material disappeared. The solvent was removed in vacuo and the crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:2) to give **129** (771 mg, R_f = 0.35) as a pale yellow oil (50% yield). IR (neat) 3002, 2958, 2922, 2850, 1725, 1712, 1664, 1619, 1439, 1348, 1299, 1258, 1204, 1145, 1101, 1078 cm^{-1}; ^1H NMR (300 MHz) δ 6.89 - 6.84 (1 H, m), 5.84 - 5.80 (1 H, m), 5.78 (1 H, s), 5.09 (1 H, s), 3.81 (3 H, s), 3.78 (3 H, s), 3.52 - 3.47 (1 H, m), 3.46 - 3.43 (1H, m), 3.07 - 2.97 (1 H, m), 2.79 - 2.69 (1 H, m); ^{13}C NMR (75 MHz) δ 166.3, 163.2, 146.1 (1 C, q, J = 35.7 Hz), 138.8, 130.3, 128.5, 115.8 (1 C, q, J = 285 Hz), 109.4, 68.3, 52.5, 52.1, 50.4, 49.9, 24.4; MS m/z

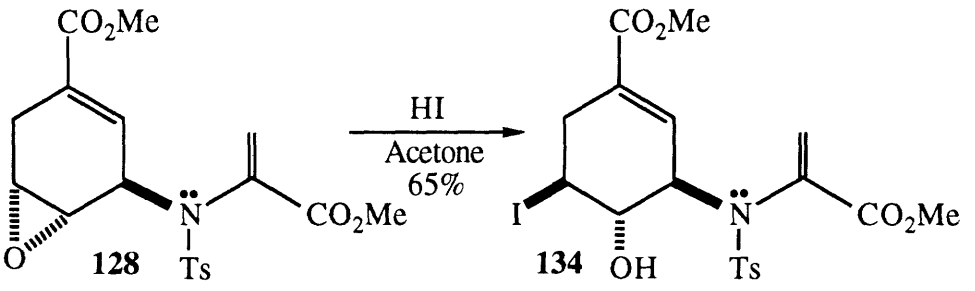
(relative intensity) 349 (M^+, 2.8), 318 (5.8), 153 (100), 121 (15.6), 93 (33.4) 59 (88.5); HRMS calc for $C_{14}F_3H_{14}NO_7$: 349.0773; Found 349.0778.

Methyl (3β,4α,5β)-3-[N-(*p*-Toluenesulphonyl)[1-(methoxycarbonyl)ethenyl]amino]-4-hydroxy-5-bromocyclohex-1-ene-1-carboxylate (133).



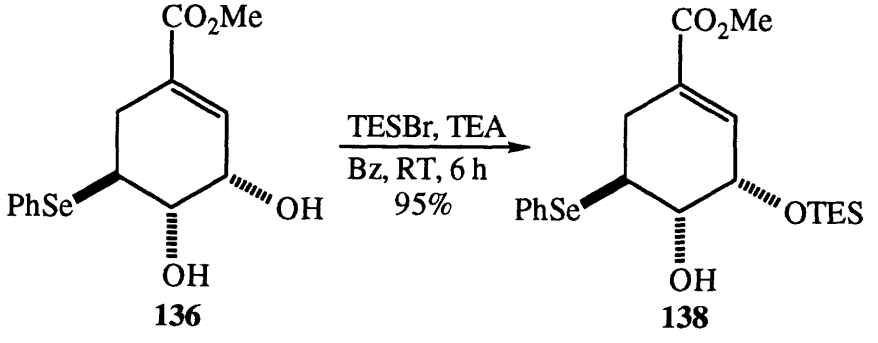
To compound **128** (50 mg, 0.12 mmol) and triphenylphosphine (4 mg, 15 μmol, 0.13 equiv) in acetonitrile (3 mL) was added triethylsilyl bromide (25 mg, 0.13 mmol, 1.1 equiv) at 0 °C. The reaction was stirred at room temperature for 9 h and monitored by TLC which indicated starting material disappeared. Solvent was removed to give yellow oil. The crude product was purified by MPLC on silica gel (ethyl acetate/petroleum ether 1:3) to give **133** (30 mg, R_f = 0.35) as a pale yellow oil (51% yield). ^{1}H NMR (500 MHz) δ 7.79 - 7.76 (2 H, m), 7.34 - 7.31 (2 H, m), 6.71 - 6.68 (1 H, m), 6.53 (1 H, s), 5.83 (1 H, s), 4.58 - 4.51 (1 H, m), 4.15 - 4.12 (1 H, br), 4.12 - 4.04 (1 H, m), 3.83 (3 H, s), 3.76 (3 H, s), 3.57 - 3.52 (1 H, m), 3.20 - 3.14 (1 H, m), 2.71 - 2.62 (1 H, m), 2.42 (3 H, s).

Methyl (3β,4α,5β)-3-[N-(*p*-Toluenesulphonyl)[1-(methoxycarbonyl)ethenyl]amino]-4-hydroxy-5-iodocyclohex-1-ene-1-carboxylate (134).



To compound **128** (21 mg, 52 μmol) in acetone (3 mL) was added two drops of a 47 - 51% aqueous solution of hydrogen iodide at room temperature. The reaction was stirred at room temperature for 2 h and monitored by TLC which indicated starting material disappeared. Solvent was removed to give a yellow oil. The residue was dissolved in ethyl acetate, passed through a pad of silica gel, and concentrated to give **134** as a pale yellow oil which was pure enough to use without further purfication. (25 mg, 85% yield). ^{1}H NMR (300 MHz) δ 7.78 - 7.73 (2 H, m), 7.35 - 7.31 (2 H, m), 6.71 - 6.63 (1 H, m), 6.53 (1 H, s), 5.82 (1 H, s), 4.57 - 4.49 (1 H, m), 4.26 - 4.23 (1 H, br), 4.19 - 4.13 (1 H, m), 3.83 (3 H, s), 3.72 (3 H, s), 3.57 - 3.43 (1 H, m), 3.24 - 3.18 (1 H, m), 2.92 - 2.77 (1H, m), 2.41 (3H, s).

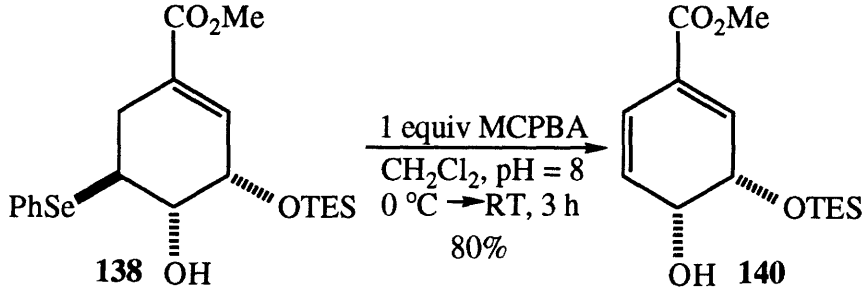
Methyl 3α,4α-Dihydroxy-5β-phenylselenyl-1-cyclohexene-1-carboxylate (138).



To a stirred solution of diol **136**[11] (327 mg, 1 mmol) in dry benzene (15 mL) was added triethylsilyl bromide (192.3 mg, 1 mmol, 1 equiv). Triethylamine

(0.2 mL, 1.2 mmol, 1.2 equiv) was added, and the mixture was stirred at room temperature for 6 h. The reaction was monitored by TLC which indicated starting material had disappeared. The mixture was poured into 10% $NaH_2PO_{4(aq)}$ and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:8) to give **138** (360 mg, R_f = 0.45) as a pale yellow oil (95% yield). IR (neat) 3520, 3054, 2954, 2899, 2809, 1721, 1655, 1579, 1461, 1438, 1385, 1253, 1103, 1045, 1013, 956, 906, 826 cm^{-1}; ^1H NMR (300 MHz) δ 7.61 - 7.57 (2 H, m), 7.32 - 7.24 (3 H, m), 6.67 - 6.65 (1 H, m), 4.63 - 4.41 (1 H, m), 3.77 - 3.75 (1 H, m), 3.74 (3 H, s), 3.02 - 2.93 (1 H, m), 2.82 (1 H, d, J = 3 Hz), 2.56 - 2.47 (1 H, m), 0.97 (9 H, t, J = 7.8 Hz), 0.66 (6 H, q, J = 8.1 Hz); ^{13}C NMR (75 MHz) δ 166.6, 136.8, 134.9, 130.3, 129.1, 128.0, 127.7, 69.7, 66.8, 51.9, 40.8, 28.1, 4.8; MS m/z (relative intensity) 442 (6.4), 413 (3.2), 267 (100), 153 (25.3); HRMS calc for $C_{20}H_{30}O_4SeSi$: 442.1079; Found 442.1079.

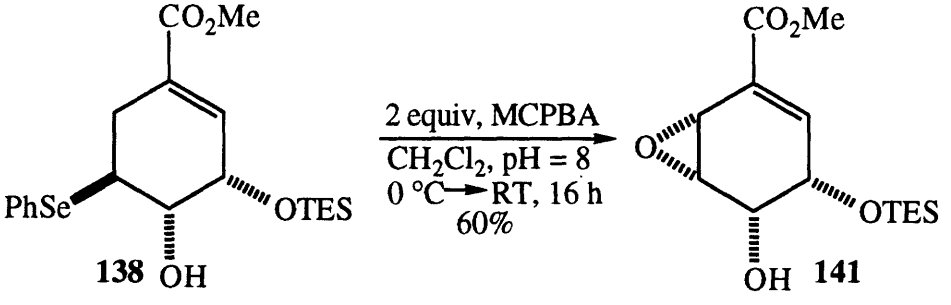
Methyl *cis*-4-Hydroxy-3-triethylsilyloxy-1,5-cyclohexadiene-1-carboxylate (140).



To compound **138** (51 mg, 115 μmol) in CH_2Cl_2-$NaH_2PO_{4(aq)}$ (3 mL, v/v = 1:1) was added m-CPBA (40 mg, 50-60% purity, 1.0 equiv) at 0 °C. The reaction was stirred at room temperature for 3 h and monitored by TLC which indicated starting material had disappeared. The mixture was poured into

saturated $Na_2S_2O_{3(aq)}$ to destroy excess m-CPBA and was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by MPLC on silica gel (ethyl acetate/petroleum ether 1:3) to give **140** (26 mg, R_f = 0.35) as a colorless oil (80% yield). ^1H NMR (300 MHz) δ 6.79 (1 H, d, J = 3.0 Hz), 6.50 (1 H, d, J = 9.6 Hz), 6.08 (1 H, dd J = 4.95, 9.75 Hz), 4.45 (1 H, dd, J = 3.15, 6.15 Hz), 4.09 (1 H, ddd, J = 4.5, 5.1, 6.2 Hz), 3.97 (1 H, dd, J = 2.77, 3.3 Hz), 3.79 (3 H, s), 2.56 (1 H, d, J = 4.5 Hz), 0.99 (9 H, t, J = 7.8 Hz), 0.68 (6 H, q, J = 7.9 Hz).

Methyl (1β,4α,5α,6β)-4-Triethylsilyloxy-5-hydroxy-7-Oxabicyclo[4.1.0]hep-2-ene-2-carboxylate (141).



To compound **138** (110 mg, 226 μmol) in CH_2Cl_2-$NaH_2PO_{4(aq)}$ (6 mL, v/v = 1:1) was added m-CPBA (200 mg, 50-60% purity, 2.5 equiv) at 0 °C. The reaction was stirred at room temperature for 16 h and was monitored by TLC which indicated starting material had disappeared. The mixture was poured into saturated $Na_2S_2O_{3(aq)}$ to destroy excess m-CPBA and was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by MPLC on silica gel (ethyl acetate/petroleum ether 1:3) to give **141** (41 mg, R_f = 0.45) as a colorless oil (60% yield). IR (neat) 3520, 2957, 2971, 2867, 1720, 1649, 1438, 1408, 1373, 1252, 1147, 1117, 1077, 1011, 966, 865 cm^{-1}; ^1H NMR (300 MHz) δ 6.96 (1 H, dd, J = 5.4, 2.1 Hz), 4.45 (1 H, ddd, J = 1.8, 5.4, 5.5 Hz), 4.01 (1 H, ddd, J = 2.1,

5.4, 10.2 Hz), 3.90 (1 H, dd, J = 2.1, 4.2Hz), 3.83 (3 H, s), 3.63 (1 H, ddd, J = 1.8, 2.1, 4.2 Hz), 2.93 (1 H, d, J = 10.2 Hz), 0.98 (9 H, t, J = 7.8 Hz), 0.65 (6 H, q, J = 7.7 Hz); ^{13}C NMR (75 MHz) δ 165.4, 141.3, 130.7, 87.2, 65.4, 56.6, 52.4, 47.8, 6.8, 5.0.

References and Notes

1. Delany, J. J.; Padykula, R.E.; Berchtold, G. A. *J. Am. Chem. Soc.* **1992**, *114,* 1394.

2. Hoare, J. H.; Policastro, P. P.; Berchtold, G. A. *J. Am. Chem. Soc.* **1983**, *105,* 6264.

3. Berchtold, G. A.; Ciabattoni, J.; Tunick, A. A. *J. Org. Chem.* **1965,** *30,* 3679.

4. Oids, S; Ohki, E. *Chem. Pharm. Bull.* **1969**, *17*, 1990.

5. Nader, B.; Bailey, T. R.; Franck, R. W.; Weinreb, S. M. *J. Am. Chem. Soc.* **1981**, *103*, 7573.

6. Maryanoff, B. E.; Almond, H. R., Jr. *J. Org. Chem.*, **1986,** *51,* 3295.

7. Hays, S. J.; Malone, T. C.; Johnson, G. *J. Org. Chem.*, **1991,** *56,* 4084.

8. Grierson, D. S.; Harris, M.; Husson, H.-P. *J. Am. Chem. Soc.* **1980**, *102*, 1064.

9. Mitsunobu, O. *Synthesis* **1981,** 1.

10. (a) Pfister, J. R. *Synthesis* **1984,** 696. (b) Miller, M. *J. Acc. Chem. Res.* **1986,** *19,* 49. (c) Slusarska, E.; Zwierzak, A. *Liebigs Ann. Chem.* **1986,** 402. (d) Sammes, P. G.; Thetford, D. *J. Chem. Soc., Perkin Trans 1,* **1989,** 655. (e) Henry, J. R.; Marcin, L. R.; McIntosh, M. C.; Scola, P. M.; Harris, G. D.; Weinreb, S. M. *Tetrahedron Lett.,* **1989,** *30,* 5709. (e) Campbell, J. A.; Hart, D. A. *J. Org. Chem.,* **1993,** *58,* 2900.

11. McManus, M. Ph.D thesis, Massachusetts Institute of Technology, **1986.**

12. Bittner, S.; Assaf, Y.; Krief, P.; Pomerantz, M.; Ziemnicka, B. T.; Smith, C. G. *J. Org. Chem.,* **1985,** 50, 1712.

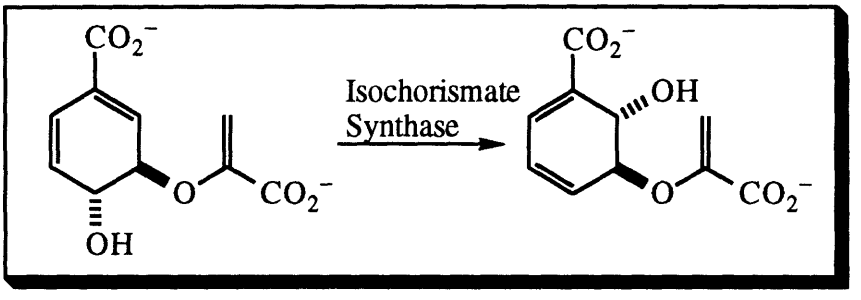
13. Cimarusti, C. M. *Gazz. Chim. Ital.* **1986,** *116,* 169.

14. Bartlett, P. A.; McQuaid, L. A. *J. Am. Chem. Soc.* **1984**, *106*, 7854.

15. Davies, K. M.; Dewar, M. J. S.; Rona, P. *J. Am. Chem. Soc.* **1967,** *89,* 6294.

Chapter 4

Investigations of the Enzyme Isochorismate Synthase: Conversion of Chorismate to Isochorismate



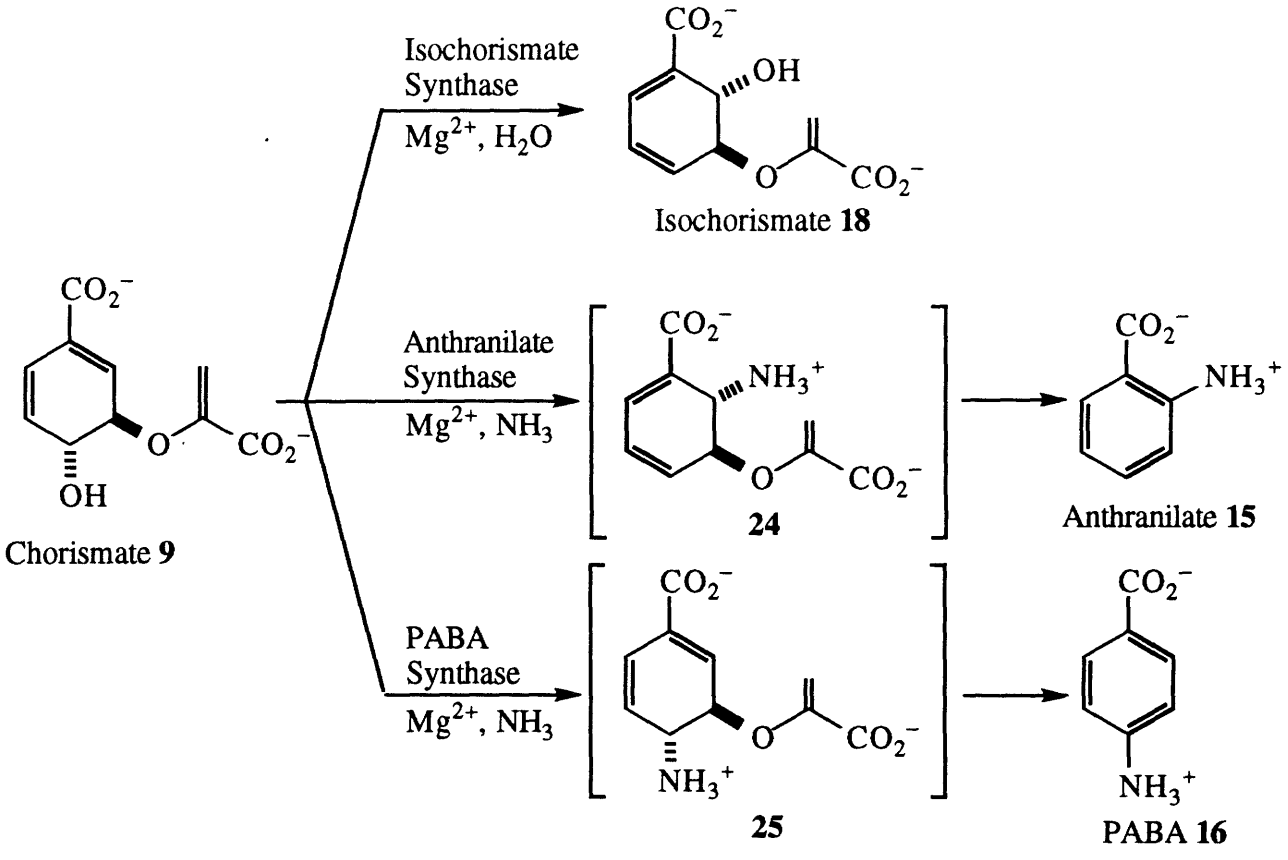
Introduction

The rational design of analogs provides a valuable way to gain information on how a biological transformation occurs. A properly designed compound could act as an inhibitor of the natural substrate, as an alternate substrate, or as a suicide substrate (mechanism-based inhibitor). Inhibitor studies often give valuable information on how the enzyme binds the substrate at the active site. Transition-state inhibitors help define the shape of the enzyme active site. Alternate substrates can provide useful information about substrate specificity, but probably of greater importance is the potential to form a covalently bound substrate-enzyme complex. Such a complex could give invaluable information as to the location and make-up of the enzyme active site.

While the mechanism of the transformation of chorismic acid to isochorismic acid remains unsolved, the rational design of analogs seemed a logical way to help clarify this important biological transformation which eventually leads to the formation of bacterial siderophore enterobactin.

Isochorismate synthase (*ent*C) and its role in the biosynthesis of 2,3-dihydroxybenzoate leading to the siderophore enterobactin were first discovered by Gibson and coworkers.[1] It catalyzes the equivalent of a 1,5 double-S_N2' displacement of the 4-hydroxyl group in chorismate (**9**) with H_2O to give the dihydroaromatic diene isochorismate (**18**).[2] Studies have shown that the DNA sequences of *E. Coli ent*C and two other chorismate utilizing enzymes anthranilate synthase and PABA synthase, have about 20% gene homologies.[2] These observations suggest that these enzymes may share a common mechanism for the 1,5 double-S_N2' displacement reaction (Scheme 1).

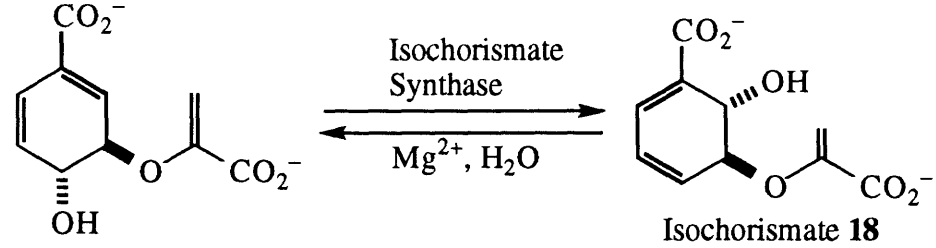
Scheme 1



Before studying the mechanism of the *ent*C catalyzed 1,5 double-S_N2' displacement in detail, an initial characterization of isochorismate synthase was necessary. A joint effort by Walsh[3] and Berchtold group established the K_{eq} of the enzyme-reaction (Scheme 2). The Walsh group determined the K_{eq} to be 0.56 by spectrophotometric assay.[3] With the availability of a large quantity of pure *ent*C, the two groups designed a ^1H NMR experiment to determine the equilibrium constant. Using the integration of two sets of unique olefinic protons for chorismate and isochorismate K_{eq} was calculated to be 0.66 in favor of chorismate.[3] This is in reasonable agreement with the K_{eq} determined by the Walsh group. The NMR experiment showed no detectable intermediates or by-

products, not even the known thermal degradation products of chorismate (**9**) or isochorismate (**18**).

Scheme 2



Chorismate **9** Isochorismate **18**

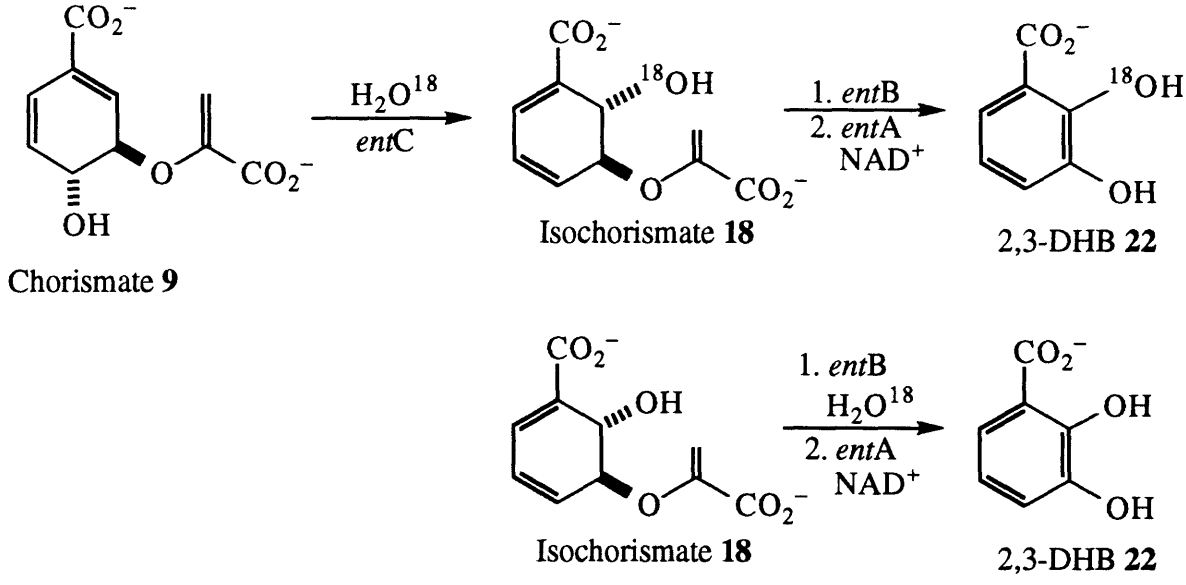
$$Keq = \frac{[\text{isochorismate}]}{[\text{chorismate}]} = \frac{V\text{max,f}}{V\text{max,r}} \times \frac{K\text{m,r}}{K\text{m,f}}$$

Another question in the transformation of chorismate to isochorismate was whether the C2 hydroxyl group of isochorismate is derived from water or from intramolecular transfer of the C4 hydroxyl group of chorismate. An ^{18}O experiment distinguished between the two alternatives.[3] When chorismate was metabolized by *ent*C in 1 : 1 H_2O^{18}–H_2O^{16} as solvent, incorporation of 50% O^{18} was noted in the 2,3-dihydroxybenzoate derived from isochorismate (analysis by mass spectrometry). Control reactions showed no O^{18} incorporation. These results proved that the nucleophile comes from the solvent rather than from intramolecular transfer of the C4 hydroxyl group in chorismate(Scheme 3).

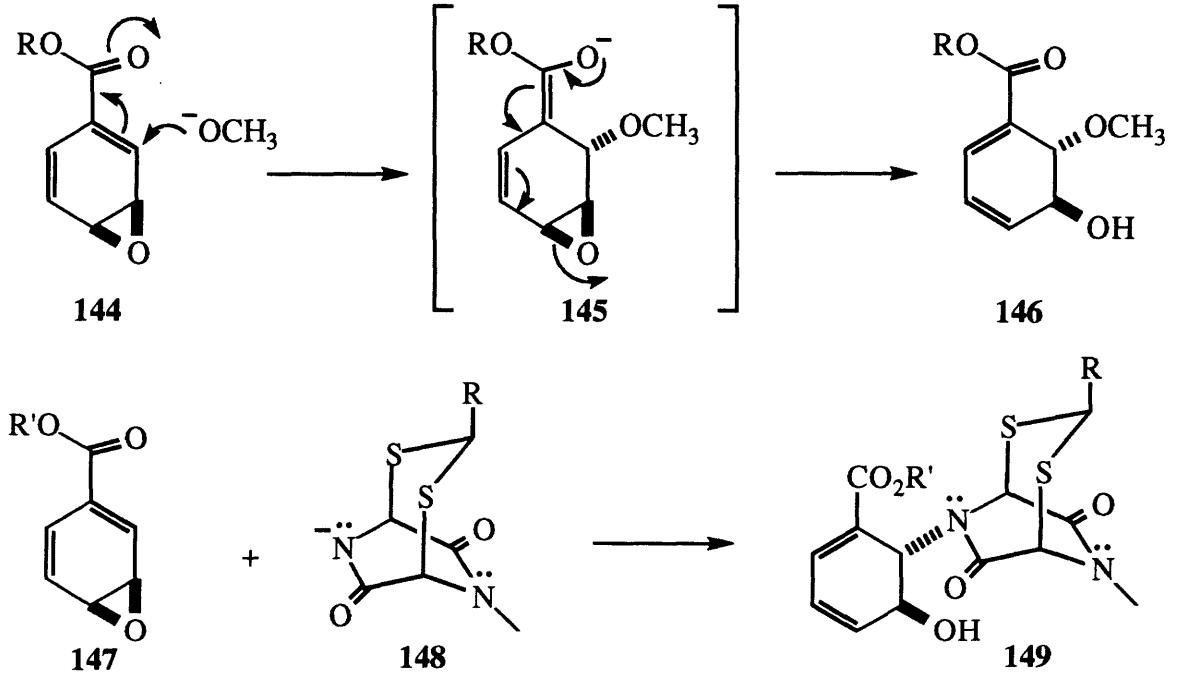
With the initial characterization of isochorismate synthase complete, several mechanisms were proposed for the conversion of chorismate to isochorismate. The double-S_N2' reaction has some chemical precedent. For example, Berchtold and coworkers reported the opening of benzene oxide **144** (R = Me or *t*-Bu) with methoxide ion or hydroxide ion (Scheme 4). The latter reaction was utilized in their synthesis of 2,3-dihydro-2,3-dihydroxybenzoate.[4]

Later, this same reaction was used with a nitrogen nucleophile by Fukuyama and Kishi in the total synthesis of gliotoxin (Scheme 4).[5] In both cases, the

Scheme 3

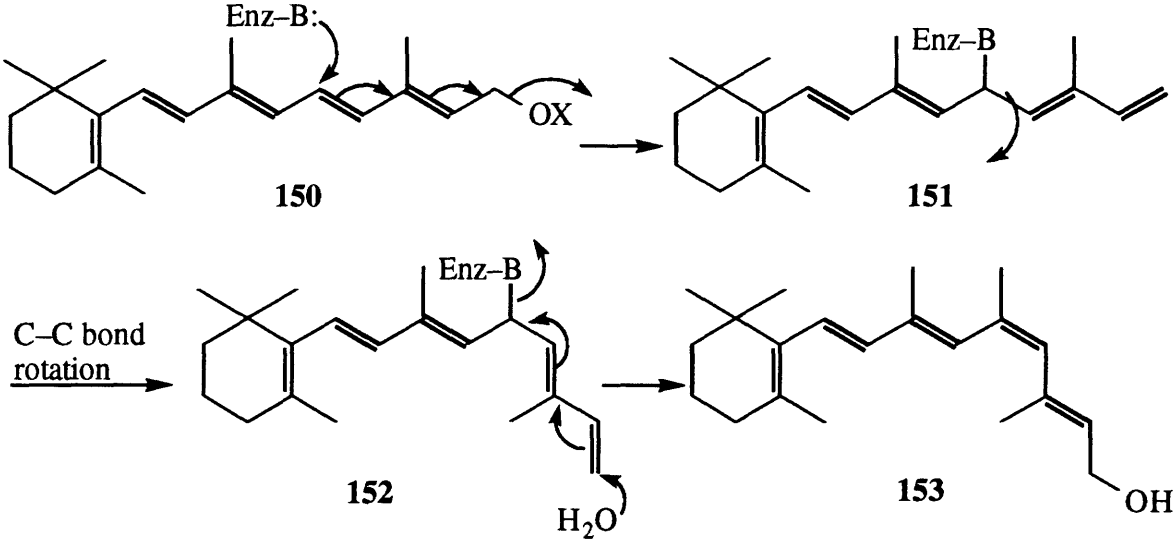


Scheme 4



authors proposed a Michael addition followed by the opening of the epoxide group as indicated in Scheme 4. In biological systems, the double-S_N2' displacement has also been proposed in the conversion of all-*trans*-retinol (**150**) to 11-*cis*-retinol (**153**) during the visual cycle (Scheme 5).[6]

Scheme 5



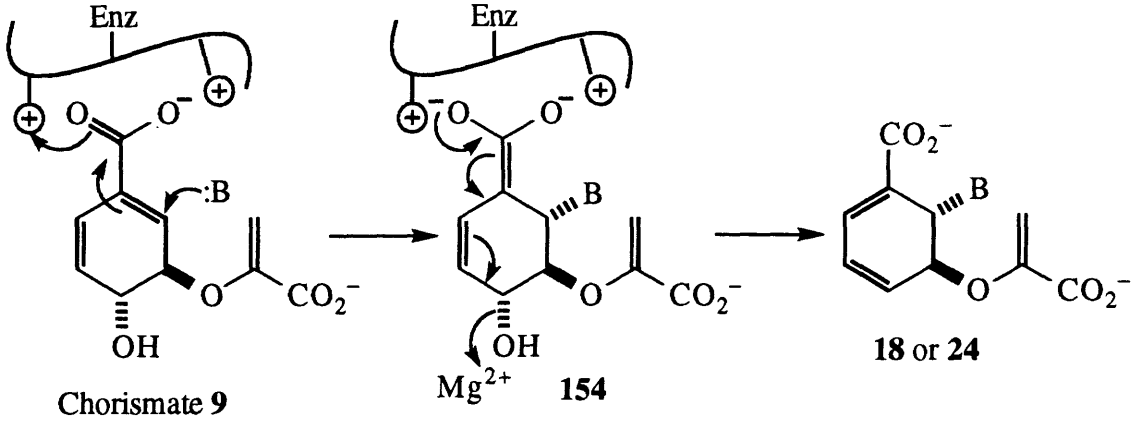
One unique feature of the enzyme-catalyzed double-S_N2' reaction is the requirement for Mg^{2+} ion, possibly to serve as a Lewis acid to make the C4-hydroxyl group of chorismate a better leaving group. It is also likely that Mg^{2+} may play an even more significant role in the catalytic process. Four mechanisms proposed for the conversion of chorismate to isochorismate are discussed below. Since isochorismate synthase shares significant homology with anthranilate synthase and PABA synthase, unifying mechanisms have been proposed for all three.

Mechanism A: Michael Addition and Elimination

When water or ammonia is added in a Michael fashion at C2, intermediate **154** is formed (Scheme 6). The dienolate intermediate **154** can be stabilized by

positively charged residues on the enzyme. Once formed, the C4 hydroxyl group can be displaced in a reverse Michael reaction. Mg^{2+} ion acts as a general acid to facilitate the elimination of the C4 hydroxyl group. This mechanism can account for the mechanism of *ent*C and AS, but can not be used to explain the PABA synthase-catalyzed reaction.

Scheme 6

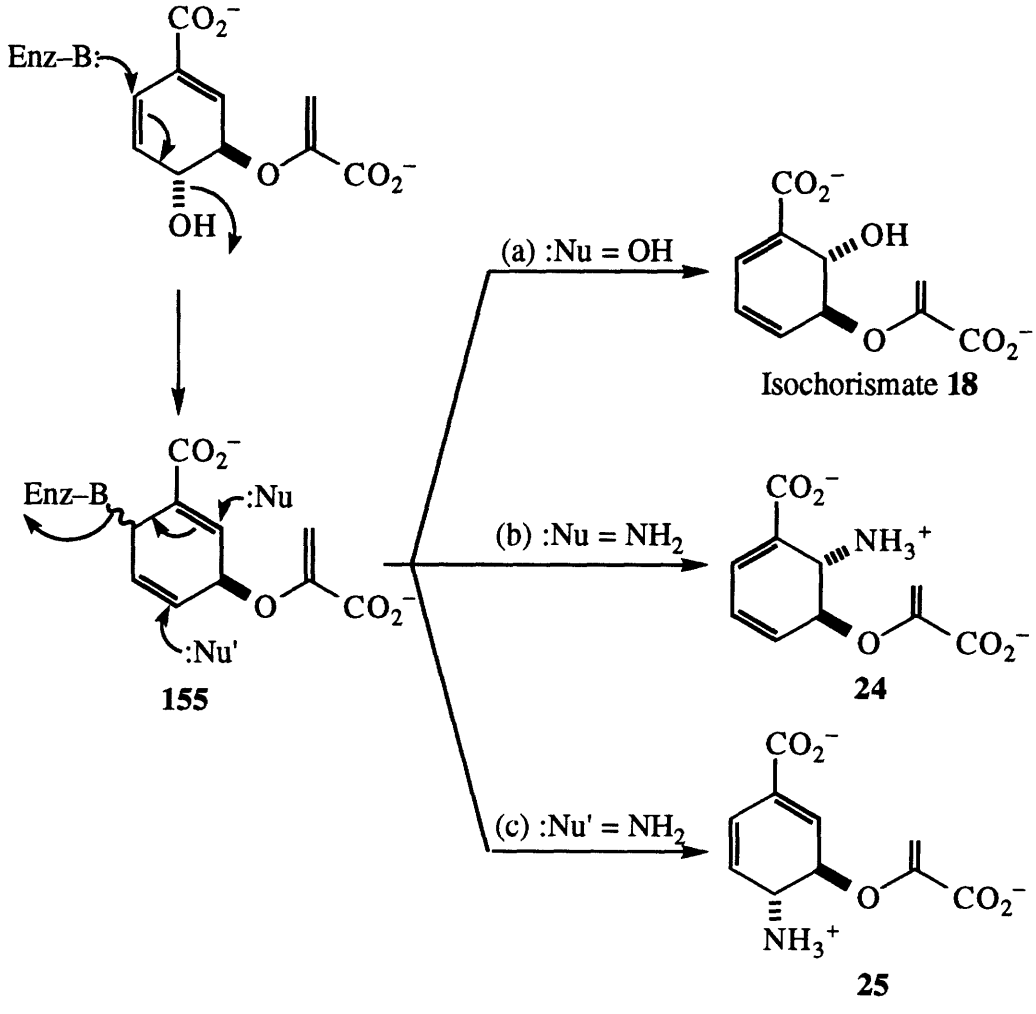


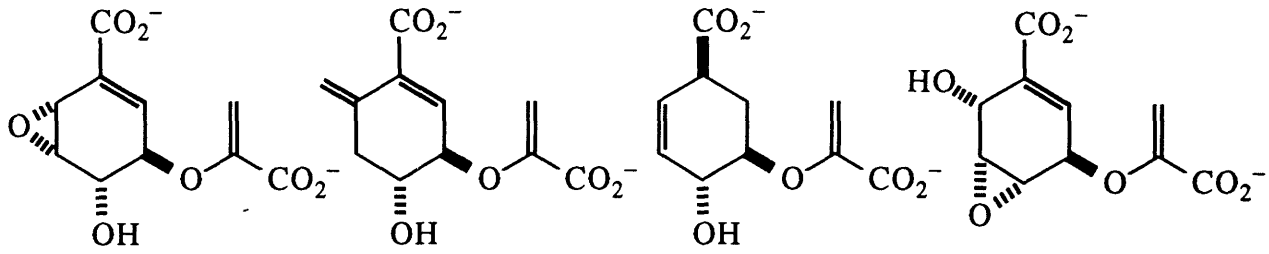
Mechanism B: Catalysis with a nucleophile from the enzyme active site

The mechanism shown in Scheme 7 is well precedented in other classes of enzymes such as the serine and cystine protease families.[7] In this mechanism, the double-S_N2' reaction involves two consecutive S_N2' reactions. An active site on the enzyme acts as a nucleophile for the first S_N2' reaction and as a leaving group for the second S_N2'. The major advantage of this mechanism is that the putative intermediate can lead to all three products involved in isochorismate, anthranilate and *p*-aminobenzoate formation. S_N2' addition of water at C2 would lead directly to isochorismate (path a). When ammonia is the nucleophile, addition at C2 position followed by elimination of pyruvate would give anthranilate (path b). The addition of ammonia at C4 can lead to 4-amino-4-deoxychorismate (path c), the intermediate for PABA synthase.[8] During the investigation of AS and PABA

synthase, compounds **156**[9], **41**[10], and **157**[11] were synthesized to serve as feasible mechanism-based inhibitors to capture the putative nucleophile from the enzyme active site. Unfortunately, none of them gave time-dependent inhibition of either AS or PABA.[12] Compound **158**, which is an analog of **155**, was synthesized by Quinn and Berchtold and was tested with *ent*C.[13] It showed no behavior as a substrate or as an inhibitor. It is unknown whether the epoxide group at the C4–C5 position blocks the compound from the binding active site of isochorismate synthase. Since the enzymatic results of epoxide **158** were inconclusive, no conclusions could be drawn.

Scheme 7



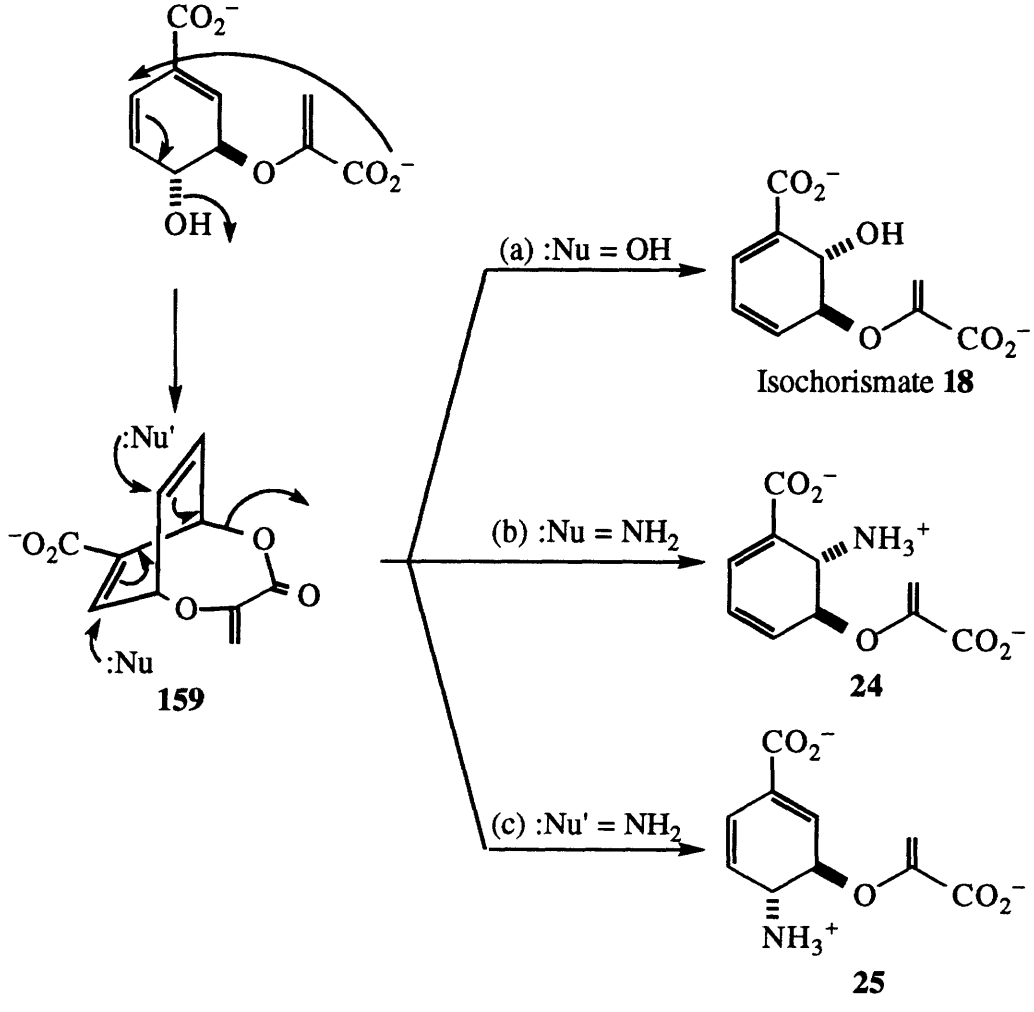


156 41 157 158

Mechanism C: Covalent catalysis with a nucleophile from the substrate itself

The intramolecular nucleophilic displacement of the C4 hydroxyl group by the enolpyruvyl carboxylate group is similar to mechanism B (Scheme 8).

Scheme 8

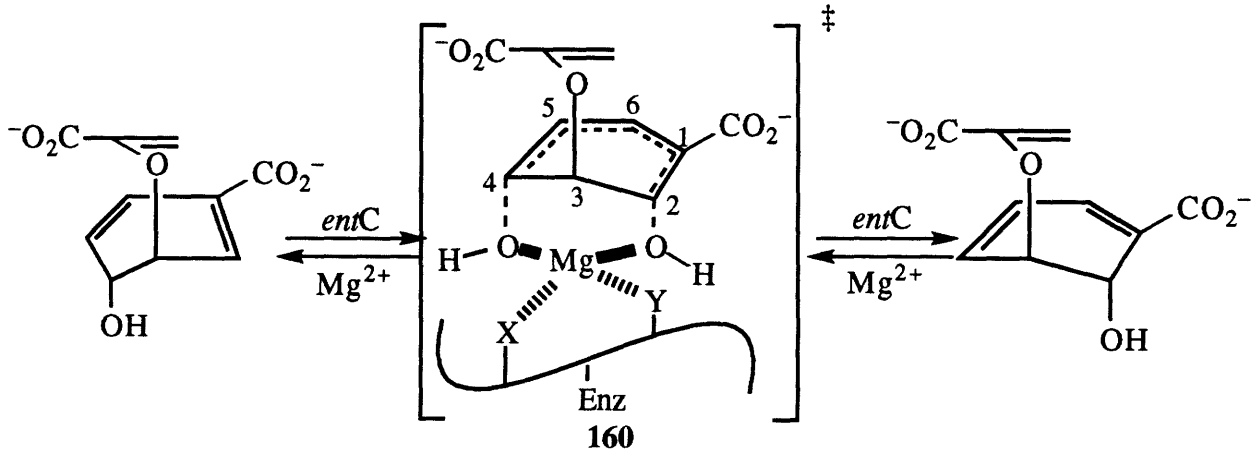


A second S_N2' reaction with water or ammonia at C2 position with subsequent opening of the lactone would yield **18** and **24** respectively. If ammonia were to attack at the C4 position, intermediate **25** would be formed. For stereochemical reasons this pathway was considered unlikely.

Mechanism D: Concerted mechanism involving a Mg-bound transition state

The fact that Mg^{2+} can form hexacoordinate complexes with oxygen ligands suggests that Mg^{2+} may play a more significant role in the catalysis. It may help deliver the incoming hydroxyl group (Scheme 9). In this mechanism, the magnesium ion is bound to the enzyme with at least one water molecule in its coordination sphere. In the transition state, the C4–O bond is partially broken while a C2–O bond is partially formed, with concomitant shifting of the diene π-bonds within the six-membered ring. The transition state has a boat-like geometry. When the Mg-bound water is replaced with ammonia, the intermediate for AS is formed.

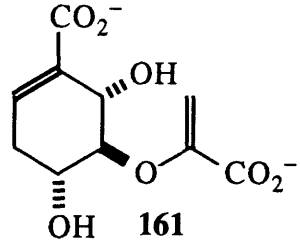
Scheme 9



160

Compound **161** was synthesized and tested by Bartlett and coworkers and found to be a good competitive inhibitor of the enzyme *ent*C (K_i = 0.36 μM, vs.

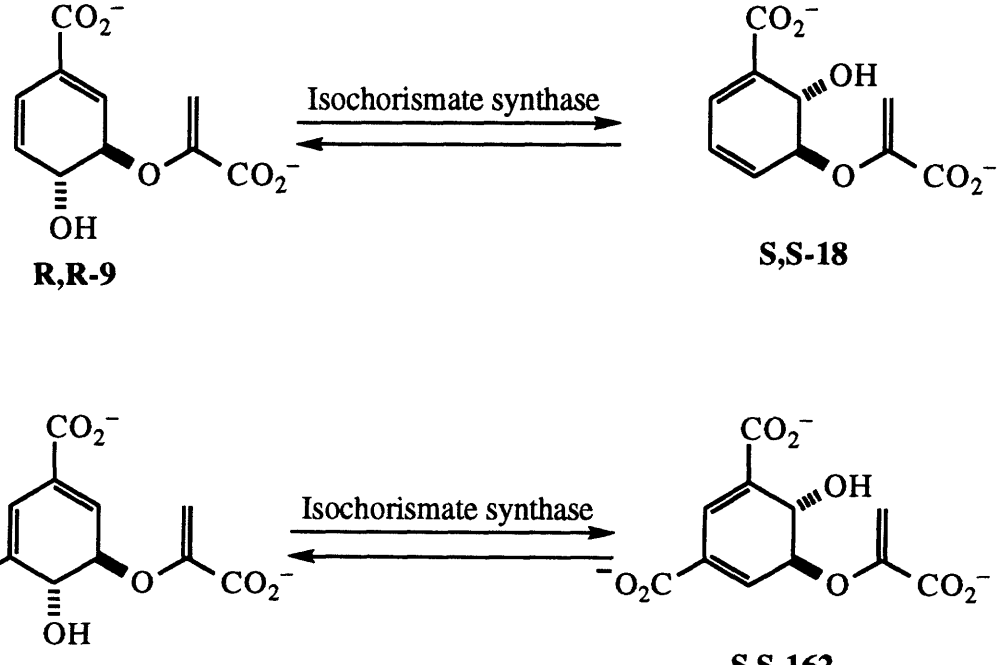
K_m = 7.0 μM for chorismate).[14] This result supports mechanism D. However, there is still insufficient evidence to eliminate conclusively any of the other three mechanistic possibilities. Only after some of the mechanism-based inhibitors, putative intermediates and transition state analogs are made can we gain more insight into the mechanism of these reactions.



161

In view of the equilibrium established between **R,R-9** and **S,S-18** in the isochorismate synthase-catalyzed reaction (K_{eq} ~ 0.56 - 0.66) and considering the structural relationship between chorismate and isochorismate, it was of

Scheme 10



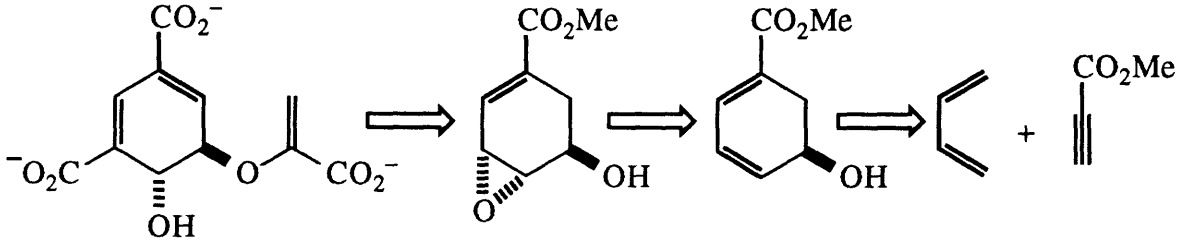
interest to prepare the 5-carboxy derivatives of **R,R-9** and **S,S-18** (Scheme 10) since the two are enantiomers, **R,R-162** and **S,S-162**, respectively. Assuming the enzyme can tolerate the addition of the carboxyl group, **R,R-162** would be a substrate for the forward reaction; and **S,S-162** would be a substrate for the reverse reaction. This is a unique situation in which racemization of either enantiomer should occur via what is an overall double S_N2' displacement reaction. Consequently, a synthetic approach to (±)-**162** was planned with the intention of resolving an intermediate to one or both of the pure enantiomers somewhere in the synthetic pathway.

Results and Discussion

Attempted Synthesis of (±)-5-Carboxychorismate (162).

Retrosynthetic analysis indicated that **162** could be designed from *trans*-epoxy alcohol **164** via cyclohexadiene derivative **165** in Scheme 11. The side-chain could be appended as described by Berchtold and coworkers for the total synthesis of optically pure chorismic acid, and saponification would lead to compound **162.**

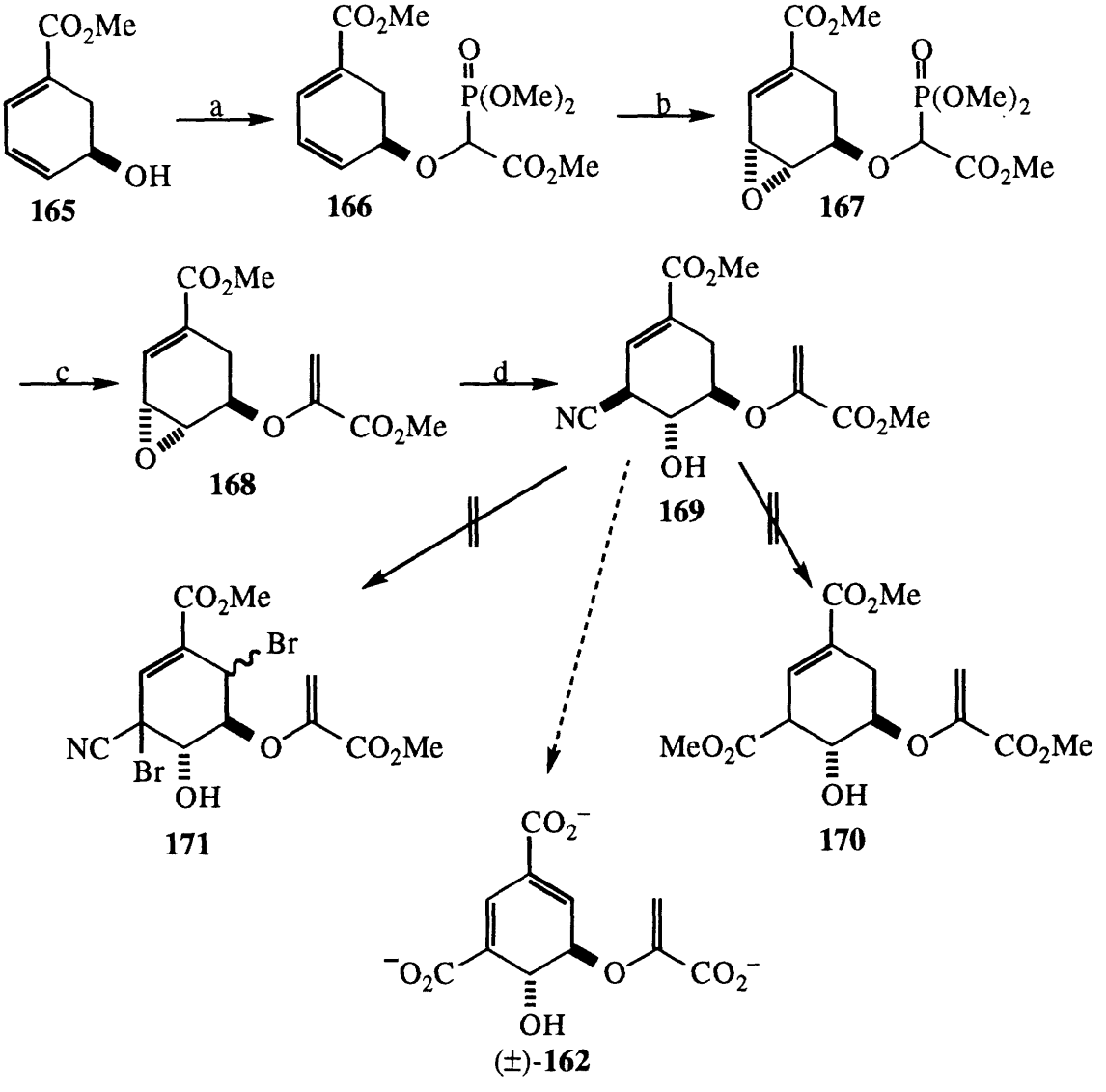
Scheme 11



(+)-**162** or (-)-**162** **164** **165**

The route used for the attempted synthesis of the (±)-**162** is depicted in Scheme 12. Cyclohexadienyl alcohol **165**[15] was converted into compound **166** by the coupling of trimethyl diazophosphonoacetate with rhodium(II) octanoate

Scheme 12[a]

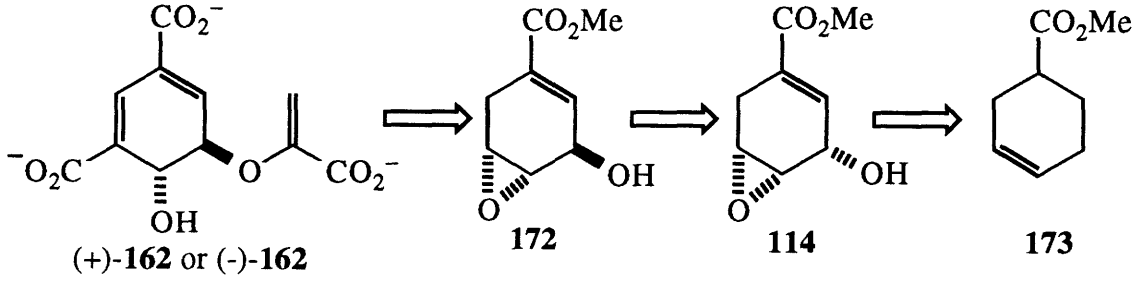


[a] Reagents: (a) $MeO_2CC(N_2)PO(OMe)_2$, $Rh_2(Octanoate)_4$, benzene, 60 °C ; (b) MCPBA, CH_2Cl_2, pH = 8; (c) $LiN(TMS)_2$, H_2CO, THF, -78 °C; (c) TMSCN, $ZnCl_2$, CH_2Cl_2, aqueous work-up.

catalysis in dry benzene at 60 °C gave phosphonate **166** in a high yield after flash column chromatography. Epoxidation of **166** with MCPBA in CH_2Cl_2-$NaH_2PO_{4(aq)}$ proceeded smoothly to give epoxide **167**. Wittig-type reaction of **167** by treatment with strong base, $LiN(TMS)_2$, and quenching with gaseous formaldehyde at -78 °C gave **168** in an excellent yield. Trimethylsilyl cyanide reacted with epoxide **168** under catalysis by $ZnCl_2$[16] to produce *β*-hydroxy nitrile **169** in a good yield. Acidic hydrolysis of *β*-hydroxy nitrile **169** in methanolic HCl led to aromatic material. Bis bromination of *β*-hydroxy nitrile **169** with NBS in CCl_4 was very sluggish. No desired product (**171**) was found after purification by preparative plate chromatography. This route was abandoned at this stage in view of the number of steps that would be required to complete the synthesis.

Another route has been widely used for the syntheses of chorismate analogs.[17] Retrosynthetic analysis indicated that **162** could be designed from *trans*-epoxy allylic alcohol **172**, derived from compound **114**, via cyclohexene derivative **173** in Scheme 13. The side-chain could be appended as described by Berchtold and coworkers for the total synthesis of optically pure chorismic acid, and saponification would lead to compound **162.**

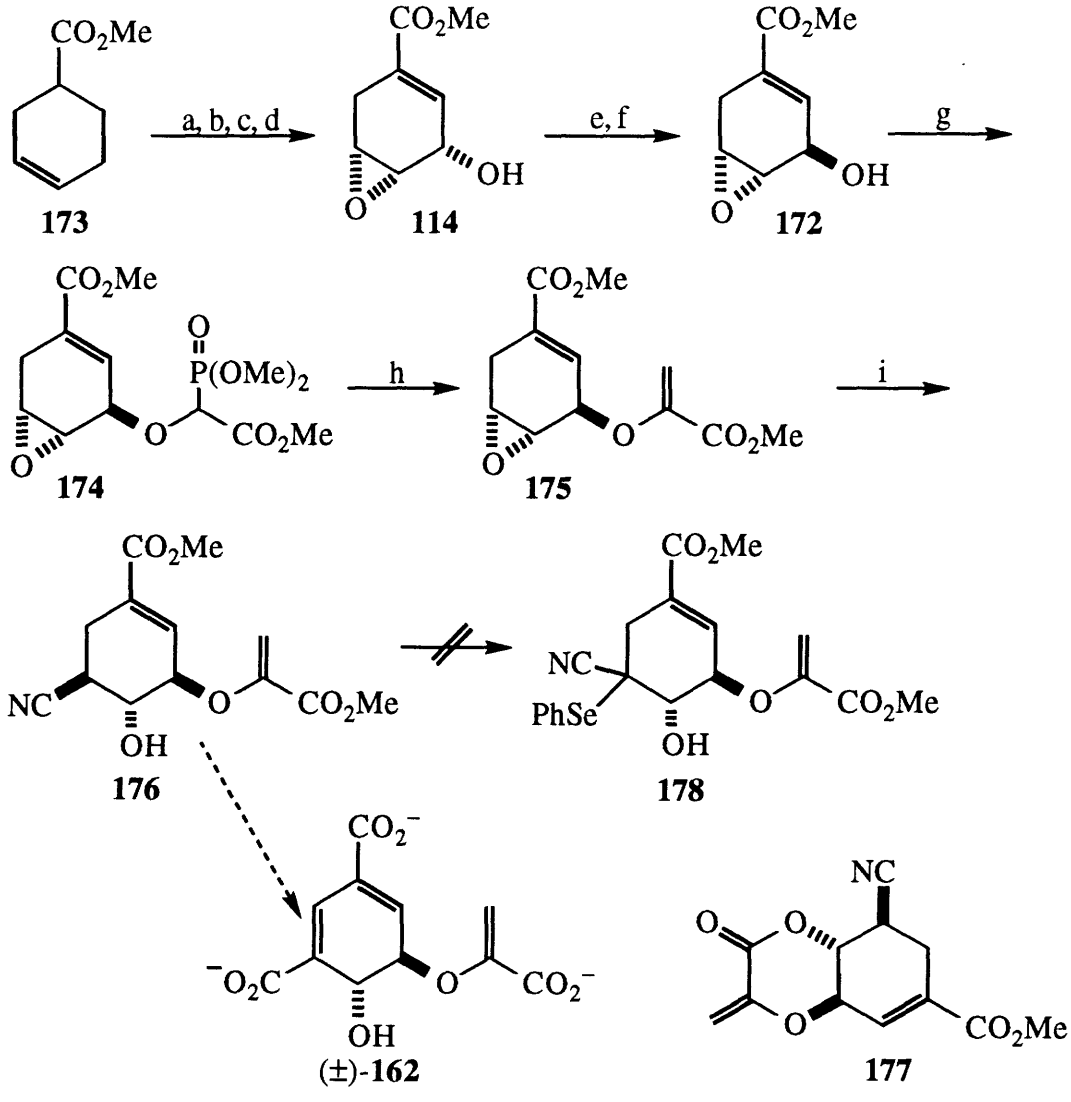
Scheme 13



The route used for the synthesis is depicted in Scheme 14. Coupling of *trans*-epoxy allylic alcohol **172** and trimethyl diazophosphonoacetate with

rhodium(II) octanoate catalysis in dry benzene at 60 °C gave phosphonate **174** in a quantitative yield after flash column chromatography. Ester **174** was transformed to **175** by the Wittig-type reaction with strong base, $LiN(TMS)_2$, followed by quenching with gaseous formaldehyde at -78 °C to give **175** in a

Scheme 14[a]

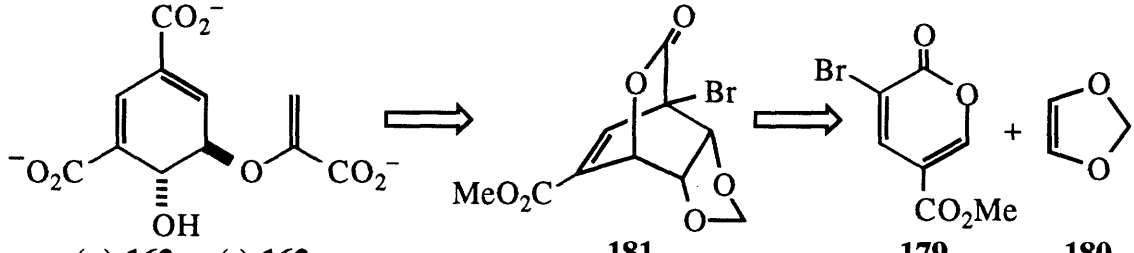


[a] Reagents: (a) NBS, CCl_4; (b) NaI, acetone; (c) $^1O_2$, Rose Bangel, acetone, hυ; (d) DBN, benzene; (e) acetic acid, DEAD, PPh_3, THF; (f) NaOMe, MeOH, H^+; (g) $MeO_2CC(N_2)PO(OMe)_2$, $Rh_2(Octanoate)_4$, benzene, 60 °C ; (h) $LiN(TMS)_2$, H_2CO, THF, -78 °C; (i) TMSCN, $ZnCl_2$, CH_2Cl_2, aqueous work-up.

good yield. Trimethylsilyl cyanide reacted with epoxide **175** under catalysis by $ZnCl_2$[16] to produce the β-hydroxy nitrile **176** in a good yield along with side product **177**. This cyclization was not found for the reaction of allylic epoxide **168** in Scheme 12. Exposure of **176** to strong base, LDA, in THF along with PhSeBr at -78 °C to room temperature gave an unknown product, and no desired product **178** was detected. This route was abandoned at this stage.

Posner et al reported the synthesis of highly functionalized cyclohexenes having a skeleton similar to **162**.[18] Retrosynthetic analysis was modified to use the reported procedure[18] for synthesis of **162** from the Diels-Alder adduct **181** via methyl bromocoumalate (**179**) with 1,3-dioxole (**180**)[18] in CH_2Cl_2 under drastic conditions (Scheme 15).

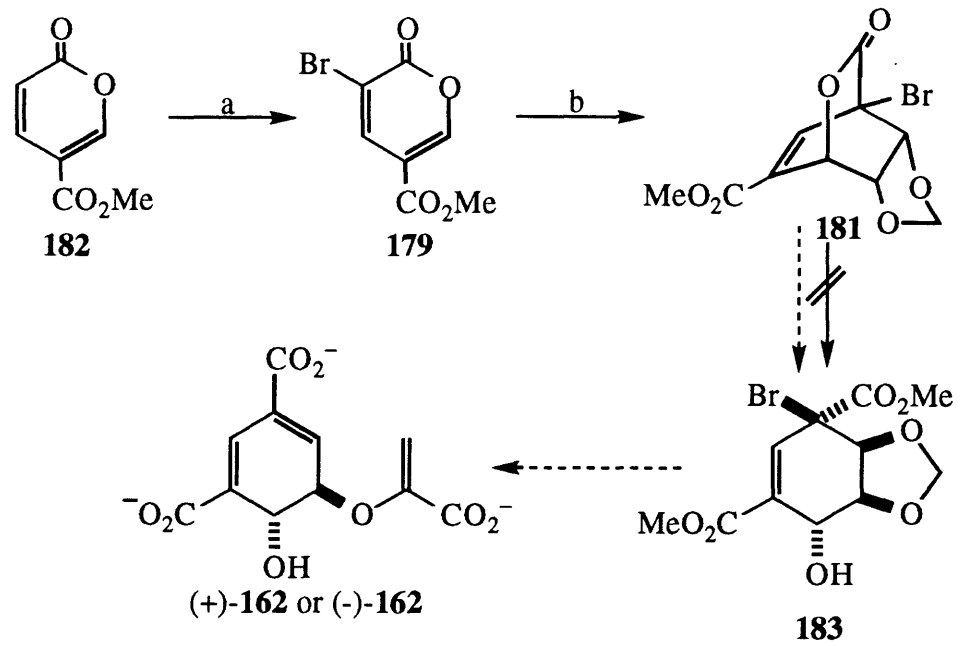
Scheme 15



The route used for the synthesis is outlined in Scheme 16. Bromination of methyl coumalate (**182**) was accomplished by a reported procedure in excellent yield.[19] Cycloaddition of **179** with 1,3-dioxole (**180**)[18] in CH_2Cl_2 under drastic conditions in the presence of base afforded predominately endo-adduct **181** in which the dioxole oxygen atoms are trans to the lactone bridge. Methanolysis[18] of the lactone of dioxole cycloadduct **181** did not provide cyclohexene **183** at room temperature or with heating.

At this stage we were notified by the Walsh group at Harvard Medical School that the isochorismate synthase available no longer had any activity, and there were no plans to obtain an additional supply of the enzyme. Consequently, synthetic efforts to prepare **162** were terminated.

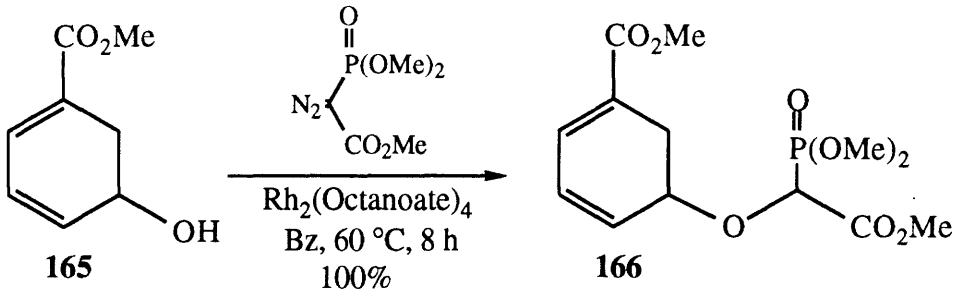
Scheme 16[a]



[a] Reagents: (a) Br_2, CCl_4, Δ; (b) **180**, Ba_2CO_3, CH_2Cl_2, Δ.

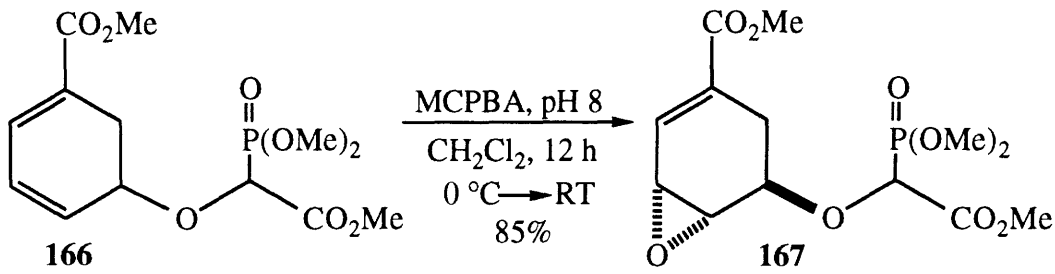
Experimental

Methyl 5-[1-(Methoxycarbonyl)-1-(dimethylphosphono)methoxy]-1,3-cyclohexadiene-1-carboxylate (166).



To starting material **165**[15] (500 mg, 4.1 mmol) in dry benzene was added rhodium(II) octanoate (30 mg, 37 μmol, 0.01 equiv) and trimethyl diazophosphonoacetate (1.26 g, 6.15 mmol, 1.5 equiv). The reaction mixture was brought to 60 °C for 8 h. Solvent was removed, and the crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 5:1) to give **166** (1.45 g, R_f = 0.2) as a pale yellow oil (100% yield). IR (neat) 3006, 2958, 2908, 2856, 1751, 1709, 1438, 1263, 1199, 1102, 1034 cm^{-1}; ^1H NMR (250 MHz) δ 7.1 - 7.0 (1H, m), 6.40- 6.19 (2 H, m), 4.52 - 4.30 (2 H, m), 3.90 - 3.75 (12 H, m), 3.13 - 2.96 (1 H, m), 2.68 -2.50 (1 H, m); MS m/z (relative intensity) 334 (M^+, 0.6), 182 (38.5), 109 (65.6), 93 (100); LRMS for $C_{13}H_{19}O_8P$: 334.

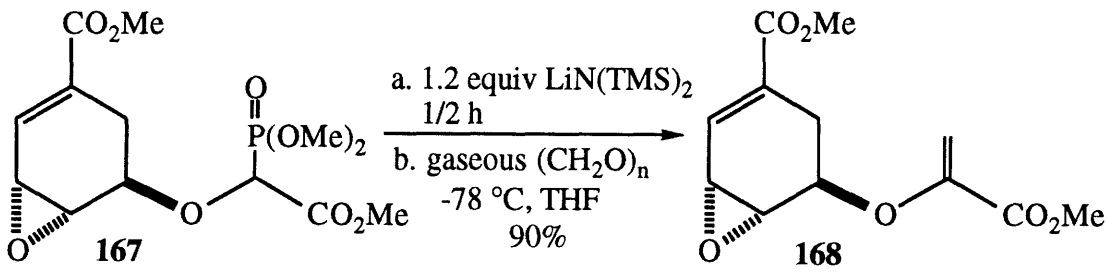
Methyl (1β,5β,6β)-5-[1-(Methoxycarbonyl)-1-(dimethylphosphono)methoxy]-7-oxabicyclo[4.1.0]hept-2-ene-3-carboxylate (167).



166 167

To compound **166** (1.0 g, 3.0 mmole) in CH_2Cl_2-$NaH_2PO_{4(aq)}$ (40 mL, v/v = 1:1) was added m-CPBA (1.25 g, 50-60% purity, 1.2 equiv) at 0 °C. The reaction was stirred at room temperature for 12 h and monitored by TLC which indicated starting material disappeared. The mixture was poured into saturated $Na_2S_2O_{3(aq)}$ to destroy excess m-CPBA and was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:4) to give **167** (890 mg, R_f = 0.35) as a pale yellow oil (85% yield). ^1H NMR (300 MHz) δ 7.16 - 7.10 (1H, m), 4.73 - 4.71 (1 H, m), 4.37 - 4.43 (1 H, m), 3.88 - 3.75 (13 H, m), 3.58 - 3.52 (1 H, m), 2.96 -2.85 (1 H, m), 2.26 - 2.16 (1H, m).

Methyl (1β,5β,6β)-5-[[1-(Methoxycarbonyl)ethenyl]oxy]-7-oxabicyclo[4.1.0]hept-2-ene-3-carboxylate (168).

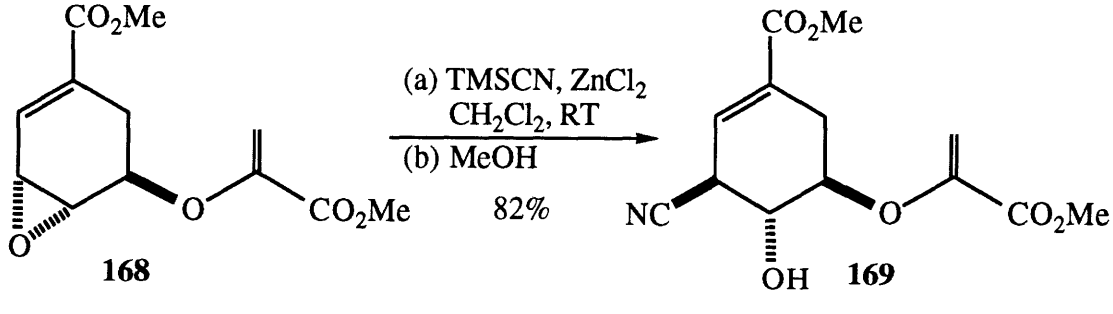


167 168

To phosphonate **167** (200 mg, 0.57 mmole) in dry THF (10 mL) was added via syringe a 1 M solution of $LiN(TMS)_2$ in THF (855 μL, 0.855 mmole, 1.5 equiv) at -78 °C (acetone-Dry Ice bath). After the mixture was stirred for an additional

20 min, gaseous formaldehyde (generated from paraformaldehyde, 171 mg) was bubbled into the solution under a N_2 stream at -78 °C (acetone-Dry Ice bath) over 10 min. The reaction was continued for 3 h and monitored by TLC which indicated starting material disappeared. The reaction was quenched at -78 °C by the addition of saturated $NH_4Cl_{(aq)}$, followed by extraction with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:2) to give **168** (130 mg, R_f = 0.35) as a colorless oil (90% yield). IR (neat) 3007, 2946, 2908, 2856, 1724, 1713, 1644, 1619, 1438, 1263, 1210, 1108, 1044 cm^{-1}; ^1H NMR (300 MHz) δ 7.16 (1H, dd, J = 3.1, 4.08 Hz), 5.55 (1 H, d, J = 2.77 Hz), 4.86 (1 H, dd, J = 0.5, 2.77 Hz),4.82 (1 H, dddd, J = 0.5, 2.0, 2.2, 5.4 Hz), 3.78 (3 H, s), 3.76 (3 H, s), 3.70 (1H, ddd, J = 2.0, 2.2, 4.1 Hz), 3.56 (1 H, t, J = 4.07 Hz), 3.00 (1 H, ddd, J = 2.0, 2.0, 17.9 Hz), 2.28 (1 H, ddd, J = 3.1, 5.4, 17.9 Hz); ^{13}C NMR (75 MHz) δ 165.8, 163.0, 149.5, 132.4, 130.4, 98.1, 69.9, 53.8, 52.1, 51.9, 46.6, 24.7; MS m/z (relative intensity) 254 (M^+, 0.4), 222 (7.1), 153 (100), 125 (58.5), 93 (37.7), 59 (85.6); LRMS for $C_{12}H_{14}O_6$: 254.

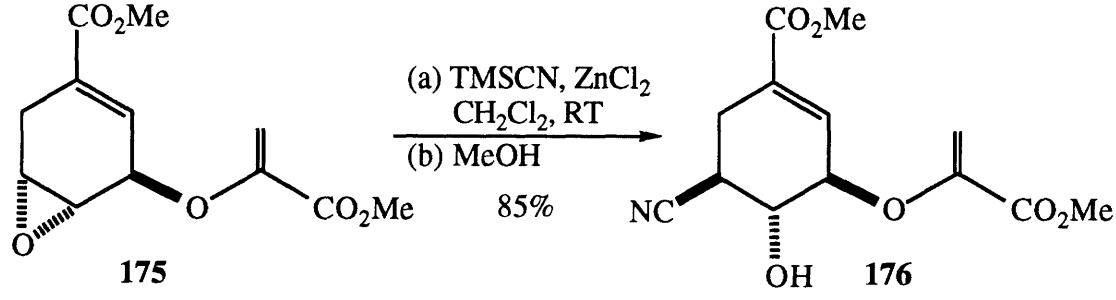
Methyl (3β,4α,5β)-3-Cyano-4-hydroxy-5-[[1-(methoxycarbonyl)ethenyl]oxy]-1-cyclohexene-1-carboxylate (169).



To epoxide **168** (50 mg, 0.2 mmole) in dry CH_2Cl_2 (5 mL) was added via syringe anhydrous zinc dichloride (27 mg, 0.2 mmole, 1 equiv), and trimethylsilyl

cyanide (30 mg, 40μL, 0.3 mmole, 1.5 equiv). The resulting mixture was stirred at room temperature for 12 h and monitored by TLC which indicated starting material disappeared. The reaction was quenched at room temperature by the addition of MeOH and stirred for another 30 min. The reaction mixture was concentrated, and the residue was dissolved in water and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous Na_2SO_4,, filtered, and concentrated in vacuo to a yellow oil. The crude product was purified by MPLC on silica gel (ethyl acetate/petroleum ether 1:1) to give **169** (46 mg, R_f = 0.33) as a pale yellow oil (82% yield). IR (neat) 3583, 3472, 3462, 3452, 3444, 3166, 3004, 2918, 2851, 1720, 1659, 1622, 1439 cm^{-1}; ^1H NMR (300 MHz) δ 6.78 (1H, t, J = 2.7 Hz), 5.61 (1 H, d, J = 2.7 Hz), 4.97 (1 H, d, J = 3 Hz), 4.62 - 4.56 (1 H, m), 4.11 - 3.96 (2 H, m), 3.83 (3H, s), 3.78 (3 H, s), 3.66 (1 H, br), 3.11 (1 H, ddd, J = 1.5, 5.2, 17.5 Hz), 2.39 (1 H, dddd, J = 3, 3.9, 5.4, 17.5 Hz); ^{13}C NMR (75 MHz) δ 165.4, 163.7, 149.4, 136.2, 128.2, 99.0, 75.3, 59.0, 52.3, 52.0, 28.4; MS m/z (relative intensity) 281 (M^+, 0.2), 255 (1.5), 189 (100), 125 (58.5), 93 (60.0), 59 (58.4); LRMS for $C_{13}H_{15}NO_6$: 281.

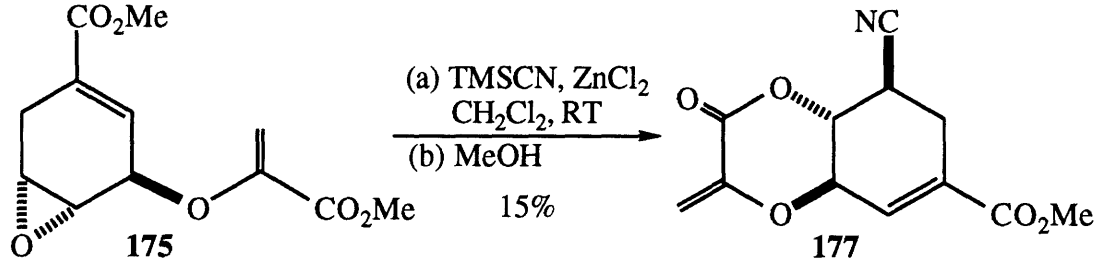
Methyl (3β,4α,5β)-3-[[1-(Methoxycarbonyl)ethenyl]oxy]-4-hydroxy-5-cyano-1-cyclohexen-1-carboxylate (176).



To epoxide **175**[17] (200 mg, 0.79 mmole) in dry CH_2Cl_2 (10 mL) was added via syringe anhydrous zinc dichloride (106 mg, 0.79 mmole, 1 equiv) and trimethylsilyl cyanide (118 mg, 158 μL, 1.185 mmole, 1.5 equiv). The resulting

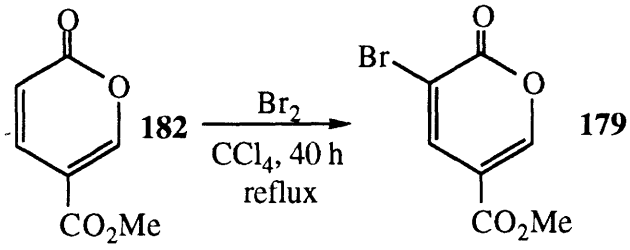
mixture was stirred at room temperature for 12 h and monitored by TLC which indicated starting material disappeared. The reaction was quenched at room temperature by the addition of MeOH and stirred for another 30 min. The reaction mixture was concentrated, and the residue was dissolved in water and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to a yellow oil. The crude product was purified by MPLC on silica gel (ethyl acetate/petroleum ether 1:1) to give **176** (189 mg, R_f = 0.3) as a pale yellow oil (85% yield). IR (neat) 3585, 3477, 3452, 3444, 3166, 3026, 3004, 2918, 2851, 1724, 1662, 1620, 1434 cm^{-1}; ^1H NMR (300 MHz) δ 6.82 - 6.77 (1H, m), 5.62 (1 H, d, J = 3.3 Hz), 4.94 (1 H, d, J = 3.3 Hz), 4.68 - 4.62 (1 H, m), 4.15 - 3.96 (2 H, m), 3.84 (3H, s), 3.77 (3 H, s), 3.21 - 3.10 (1 H, m), 2.73 - 2.60 (1 H, m); ^{13}C NMR (75 MHz) δ 165.4, 163.5, 149.6, 133.3, 130.4, 98.8, 78.5, 74.1, 58.0, 52.5, 52.2, 34.4.

(1α,6β,10α)-10-Cyano-8-methoxycarbonyl-4-methylene-2,5-dioxabicyclo[4.4.0] dec-7-en-3-one (177).



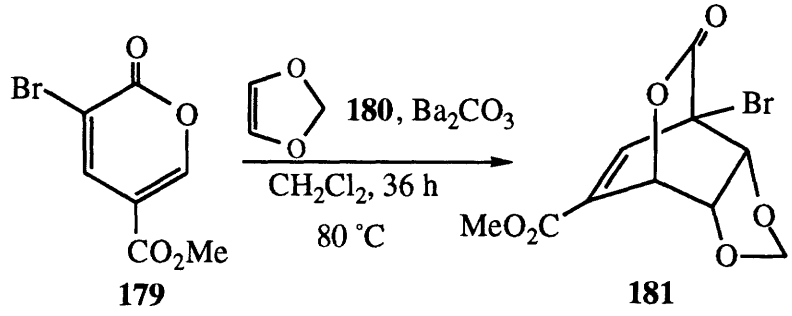
Lactone **177** is the side product of the reaction as shown above. This product was purified by MPLC on silica gel (ethyl acetate/petroleum ether 1:1) to give **177** (28 mg, R_f = 0.5) as a pale yellow oil (15% yield). ^1H NMR (300 MHz) δ 6.82 - 6.77 (1H, m), 5.72 (1 H, d, J = 3.3 Hz), 5.17 (1 H, d, J = 3.3 Hz), 4.92 - 4.90 (1H, m), 4.78 - 4.72 (1 H, m), 4.45 - 4.41 (1H, m), 3.78 (3H, s), 2.90 - 2.79 (1 H, m), 2.53 - 2.41 (1H, m).

Methyl 2-Oxo-3-bromo-2*H*-pyran-5-carboxylate (179).



To methyl coumalate (3.1 g, 20 mmole) in carbon tetrachloride (60 mL) bromine (4.8 g, 1.54 mL, 30 mmole, 1.5 equiv) was added dropwise, and the resulting mixture was heated at reflux for 40 h. The reaction mixture was concentrated, and the residue was washed with saturated $Na_2S_2O_{3(aq)}$ and extracted with ether. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo to a yellow solid. The crude product was purified by flash column chromatography on silica gel (diethyl ether/petroleum ether 2:1) to give **179** (3.9 g, $R_f = 0.55$) as a pale yellow solid (84% yield, mp. = 128 - 129 °C). ^1H NMR (300 MHz) δ 8.30 (1H, d, J = 3.3 Hz), 8.17 (1H, d, J = 3.3 Hz), 3.90 (3 H, s).

Methyl (1α,2β,5β,6α)-5-Bromo-7,9,10-trioxa-11-oxotricyclo[4.3.0.2^{2,5}]undec-3-ene-3-carboxylate (181).



A 10 mL hydrolysis tube was charged with 3-bromo-2-pyrone (**179**) (163 mg, 0.7 mmole), barium carbonate (138 mg, 0.7 mmole), 1,3-dioxole (**180**)[18] (50 mg, 0.7 mmole) and dry CH_2Cl_2 (2 mL). The tube was sealed under nitrogen and

warmed to 80 °C for 36 h. The reaction was cooled, diluted with methylene chloride, and filtered. Solvent was removed under vacuum. The crude product was purified by MPLC on silica gel (ethyl acetate/petroleum ether 1:2) to give **181** (150 mg, R_f = 0.35) as a colorless oil (70% yield). ^{1}H NMR (300 MHz) δ 7.25 - 7.21 (1 H, m), 5.82 (1 H, dd, J = 2.3, 4.9 Hz), 5.12 (1 H, s), 4.96 (1H, s), 4.81 (1H, dd, J= 4.9, 7.5 Hz), 4.58 (1 H, dd, J = 2.3, 7.5 Hz).

References and Notes

1. (a) Young, I. G.; Batterham, T. J.; Gibson, F. *Biochim. Biophys. Acta* **1968**, *165,* 567. (b) Young, I. G.; Batterham, T. J.; Gibson, F. *Biochim. Biophys. Acta* **1969**, *177,* 389.

2. (a) Ozenberger, B. A.; Brickman, T. J.; McIntosh, M. A. *J. Bacteriol.* **1989**, *171*, 775. (b) Nahlik, M. S.; Ozenberger, B. A.; Brickman, T. J.; McIntosh, M. A. *J. Bacteriol.* **1989**, *171*, 784.

3. Liu, J.; Quinn, N. R.; Berchtold, G. A.; Walsh, C. T. *Biochemistry* **1990**, *29,* 1417.

4. DeMarinis, R. M; Filer, G. N.; Waraszkiewicz, S. M.; Berchtold, G. A. *J. Am. Chem. Soc.* **1974**, *96,* 1193.

5. Fukuyama, T; Kishi, Y. *J. Am. Chem. Soc.* **1976,** *98,* 6723.

6. Law, W. C.; Kim, S.; Rando, R. R. *J. Am. Chem. Soc.* **1989,** *111,* 793.

7. Neurath, H. *Science* **1984,** *224,* 350.

8. Teng, C.-Y. P.; Ganem, B.; Doktor, S. Z.; Nichols, B. P.; Bhatnager, R. K.; Vining, L. C. *J. Am. Chem. Soc.* **1985**, *107,* 5008.

9. Ife, R. J.; Ball, L. F.; Lowe, P.; Halsam, E. *J. Chem. Soc., Perkin Trans. 1* **1976,** 1776.

10. Kronis, J. D.; Berchtold, G. A. Unpublished observations.

11. Padykula, R. E.; Berchtold, G. A. Unpublished observations.

12. Walsh, C. T.; Erios, M. D.; Walts, A. E.; Delany, J. J.; Berchtold, G. A. *Biochemistry* **1987**, *26,* 4734.

13. Quinn, N. R. Ph.D thesis, Masschusettes Institute of Technology, **1990**.

14. Kozlowski, M. C.; Bartlett, P. A. *J. Am. Chem. Soc.* **1991,** *113,* 5897.

15. McGowan, D. A.; Berchtold, G. A. *J. Am. Chem. Soc.* **1982**, *104,* 1153.

16. Gassman, P. G.; Guggenheim, T. L. *J. Am. Chem. Soc.* **1982**, *104,* 1504.

17. Hoare, J. H.; Policastro, P. P.; Berchtold, G. A. *J. Am. Chem. Soc.* **1983**, *105,* 6264.

18. Posner, G. H.; Nelson, T. D. *Tetrahedron* **1990,** *46,* 4573.

19. Posner, G. H; Harrison, W.; Wettlaufer, D. G. *J. Org. Chem.* **1985,** *50,* 5041.

Appendix

Selected NMR Spectra

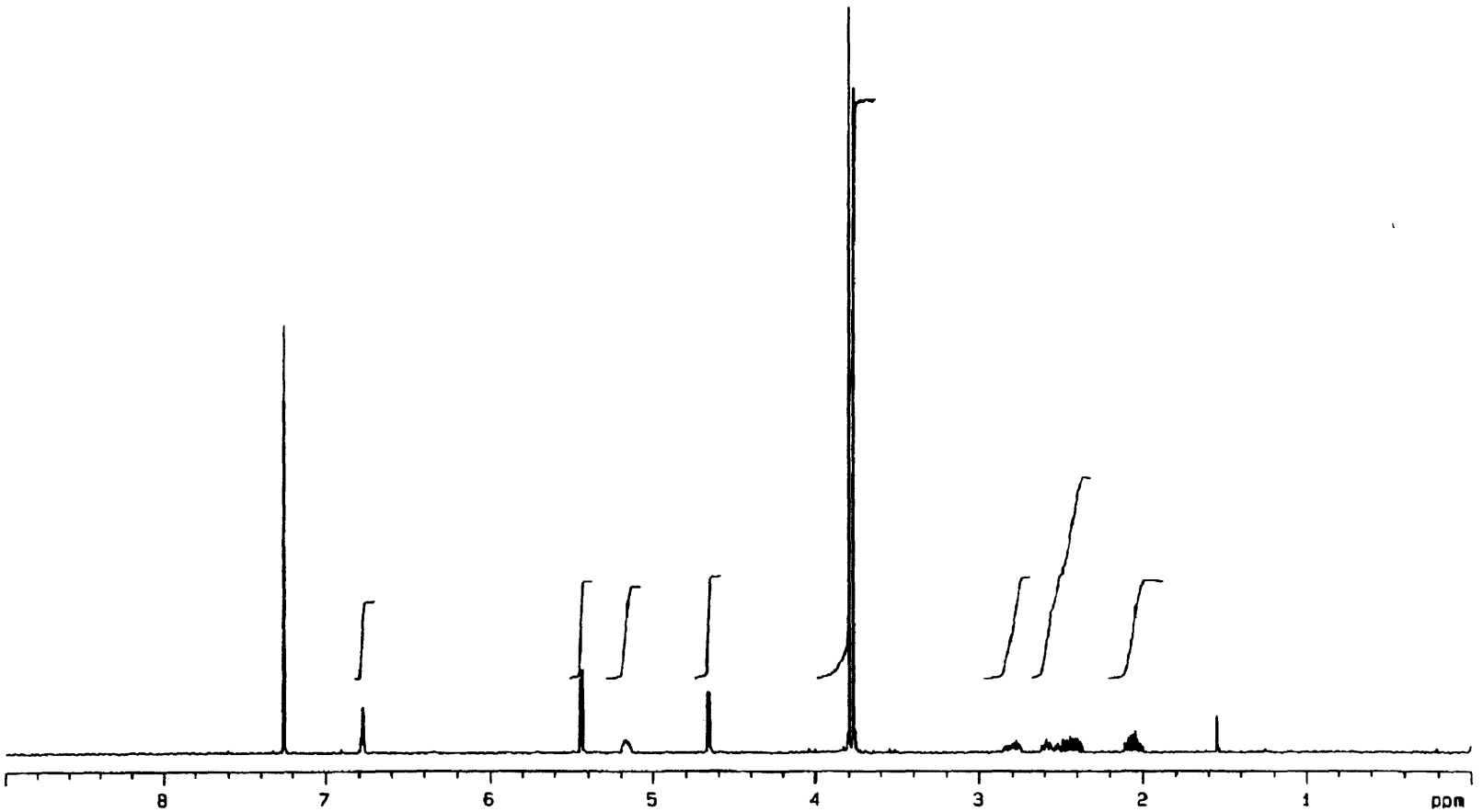
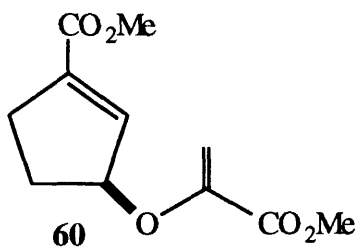


Figure 1: ^{1}H NMR of Compound **60.**



60

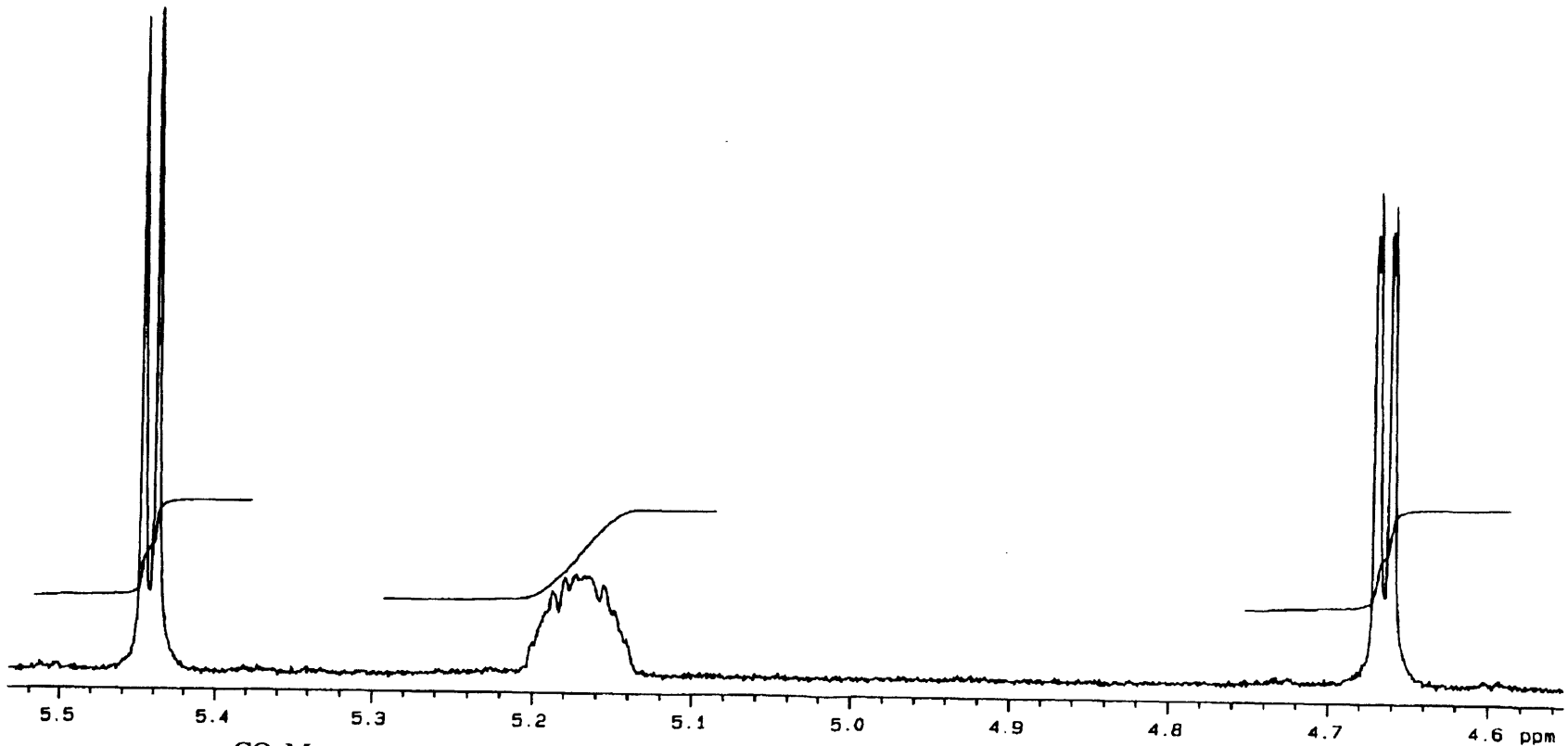
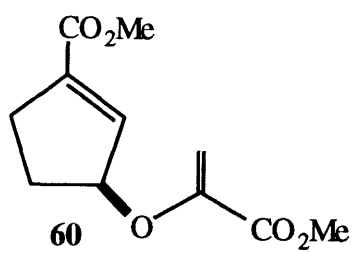
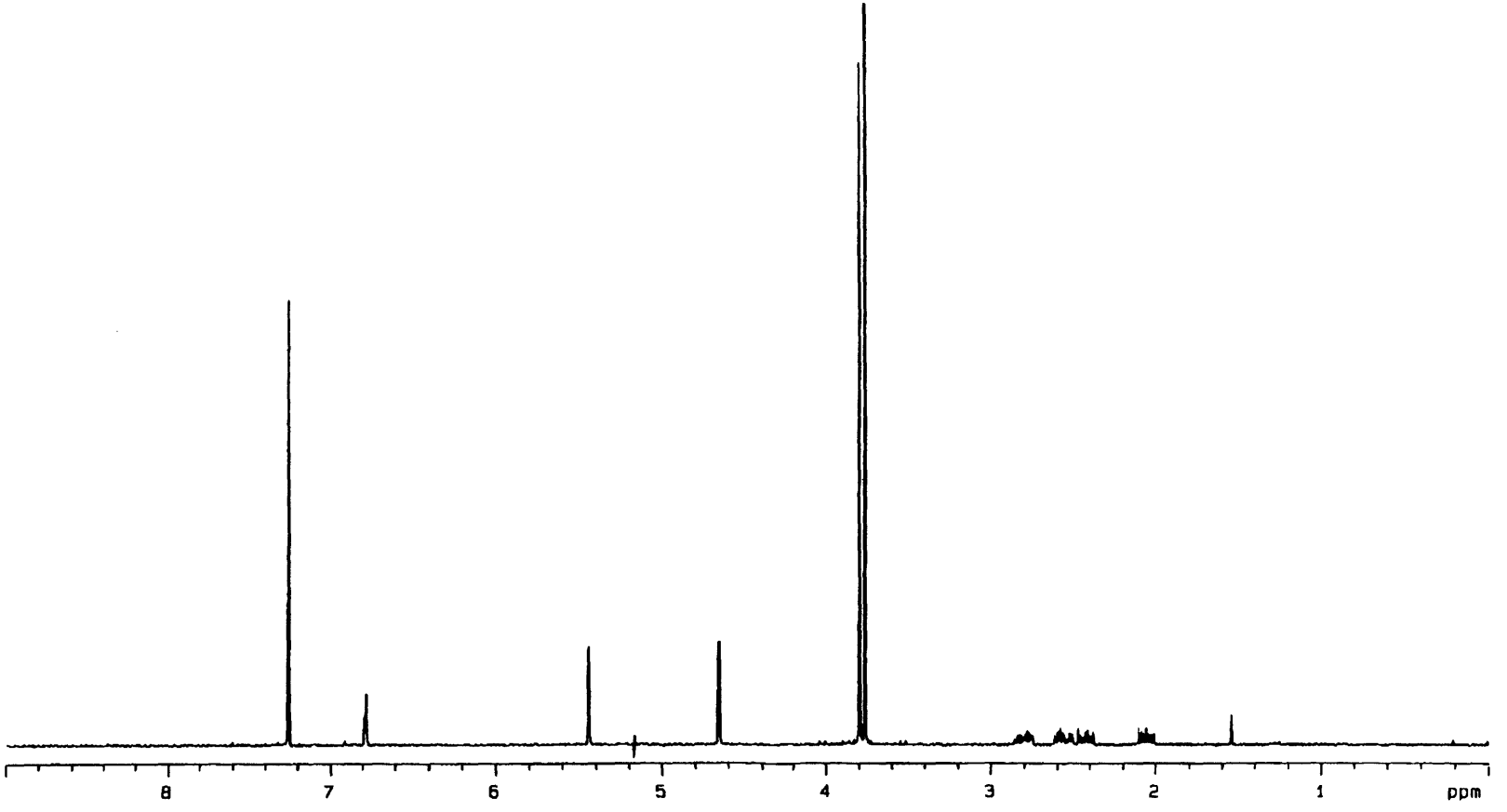


Figure 2: Enlargement at 4.5 - 5.5 ppm of ^{1}H NMR of Compound **60.**



60



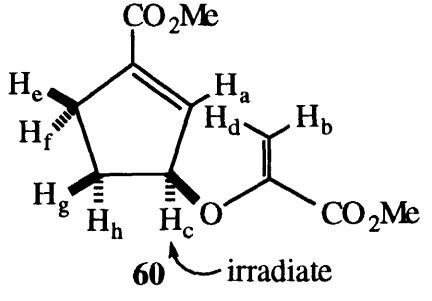
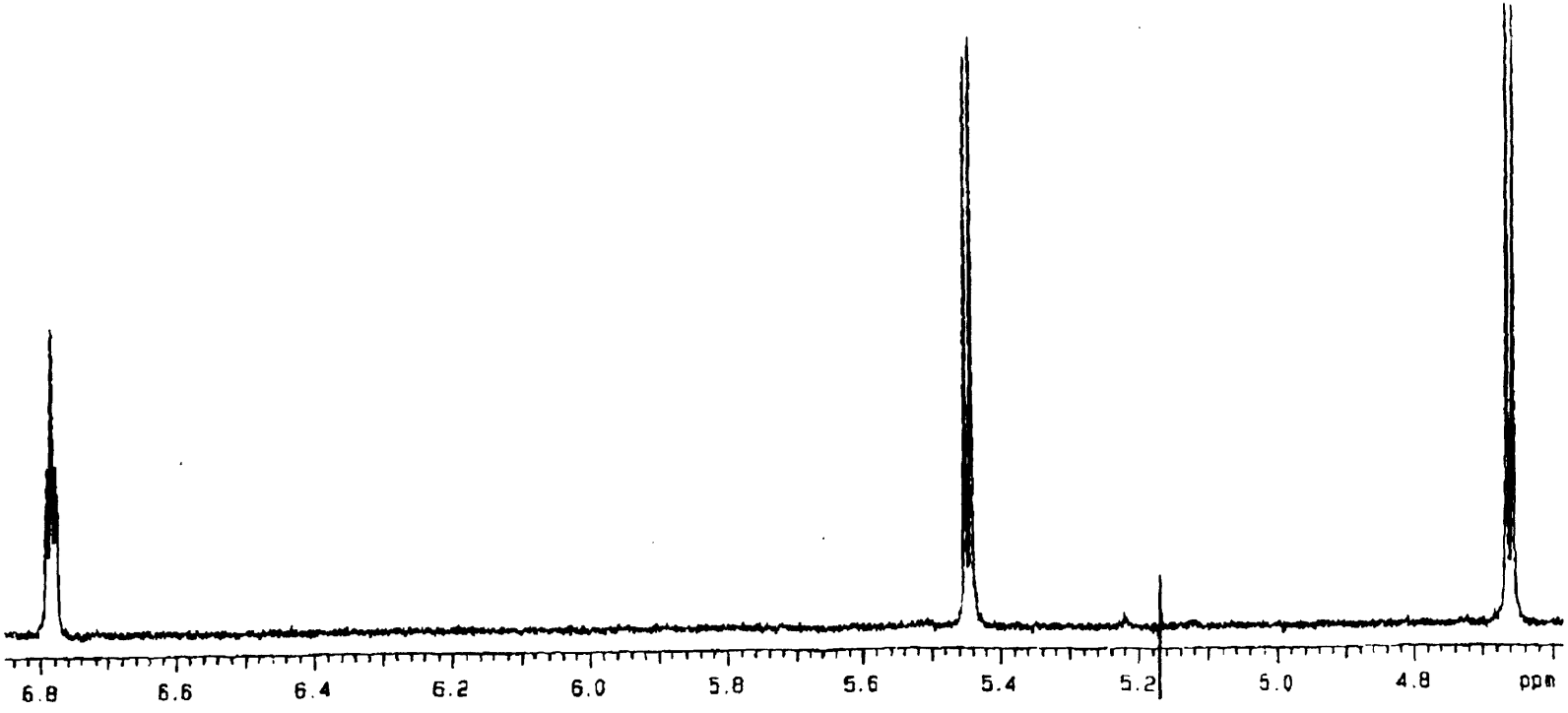


Figure 3: ^{1}H NMR Decoupling of H_c of Compound **60.**



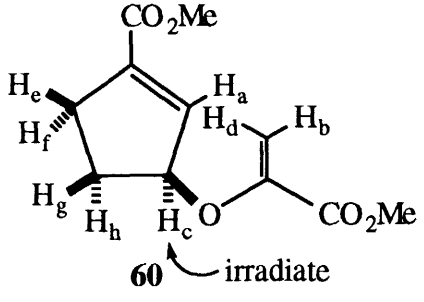


Figure 4: Enlargement at 4.7 - 6.8 ppm of ^{1}H NMR Decoupling H_c of Compound **60.**

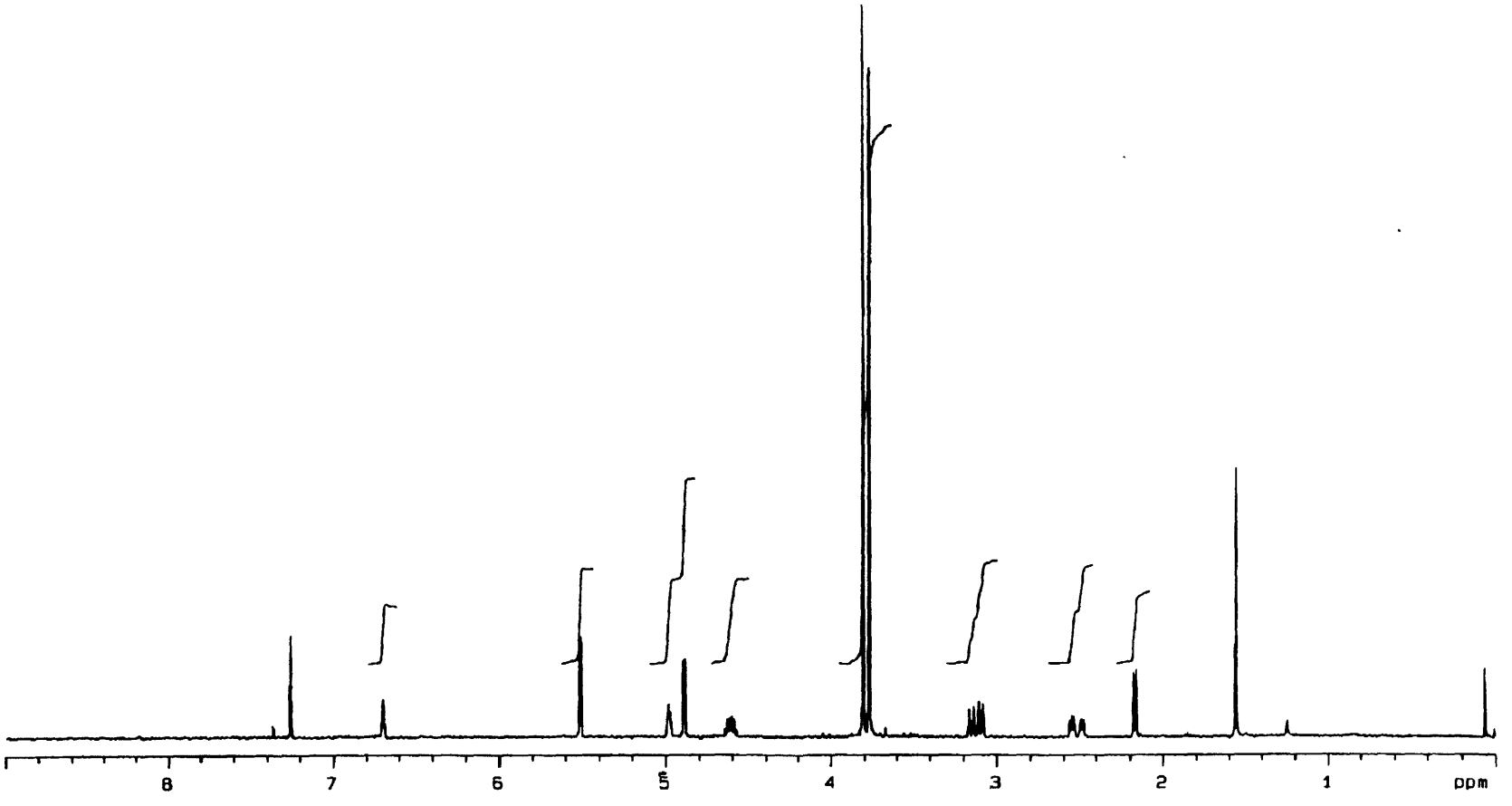
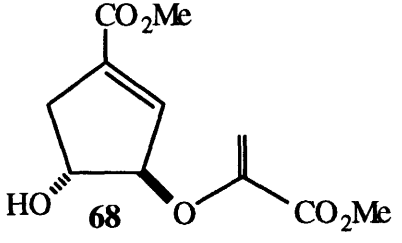
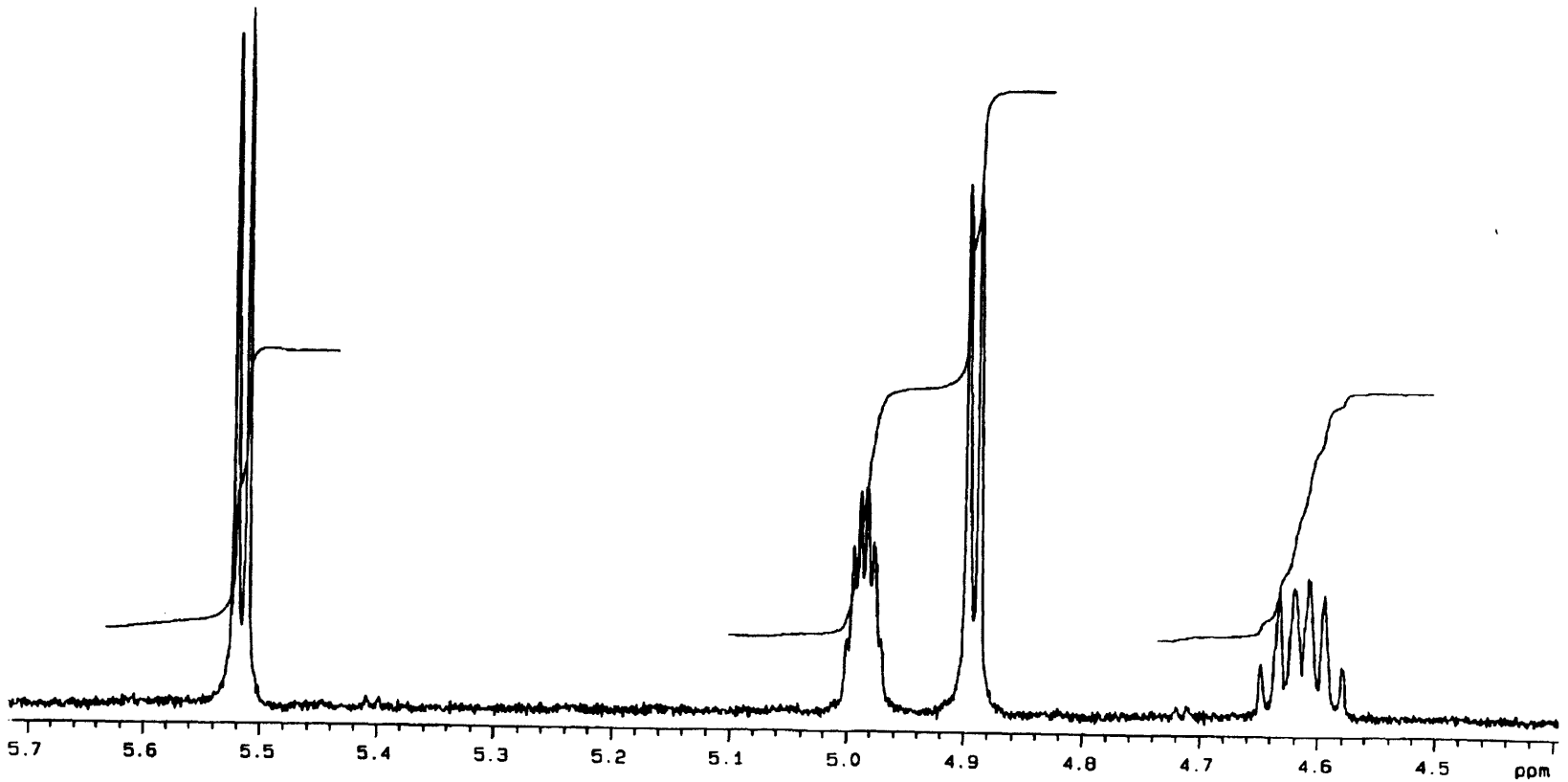
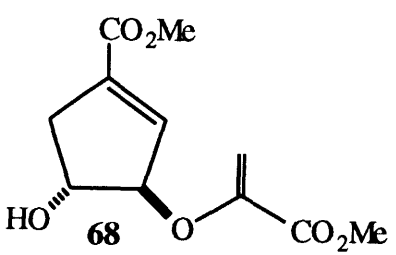


Figure 5: ^{1}H NMR of Compound **68.**



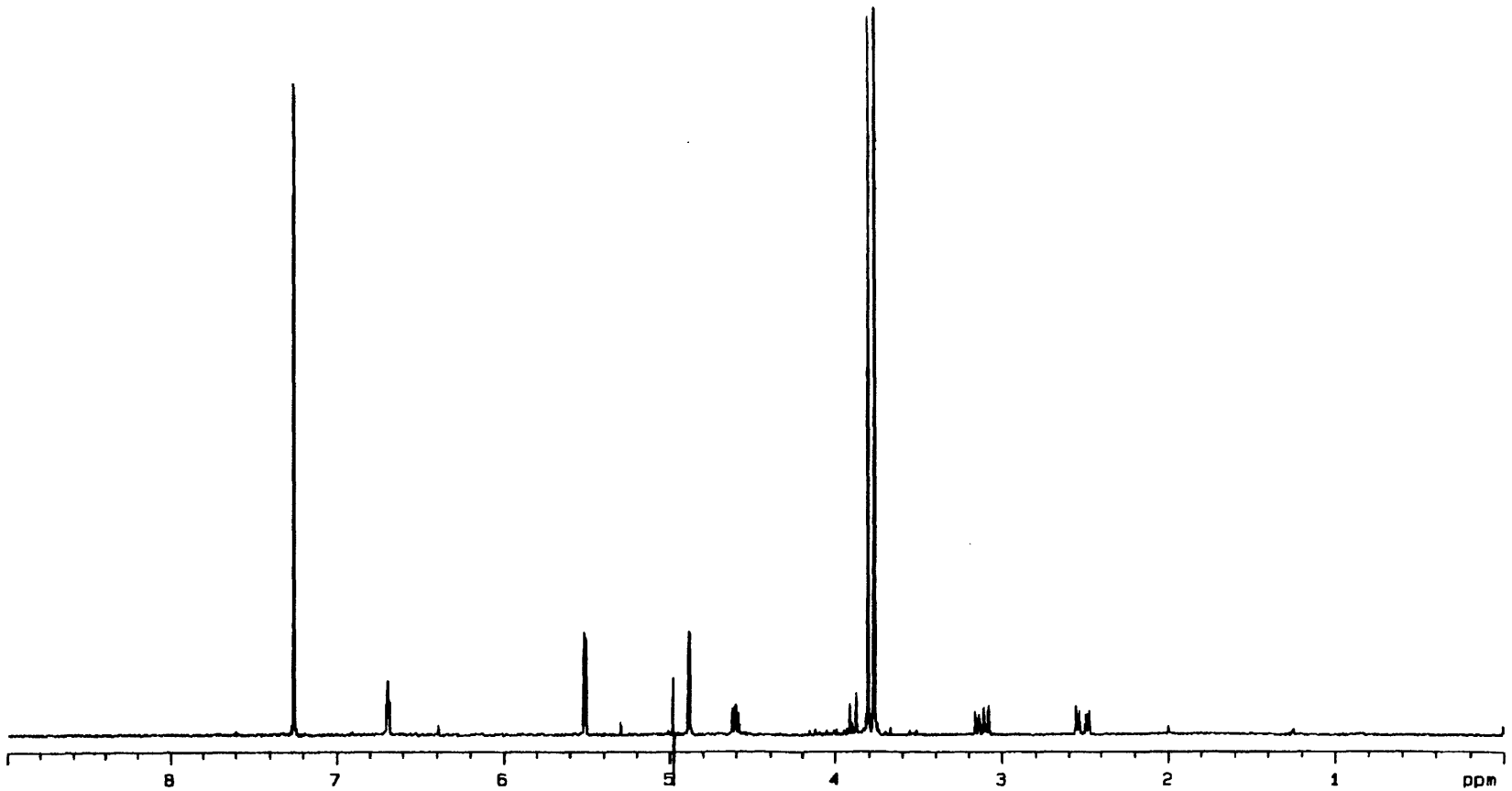
68





68

Figure 6: Enlargement at 4.4 - 5.6 ppm of ^{1}H NMR of Compound **68.**



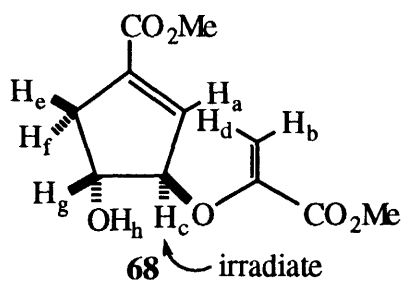
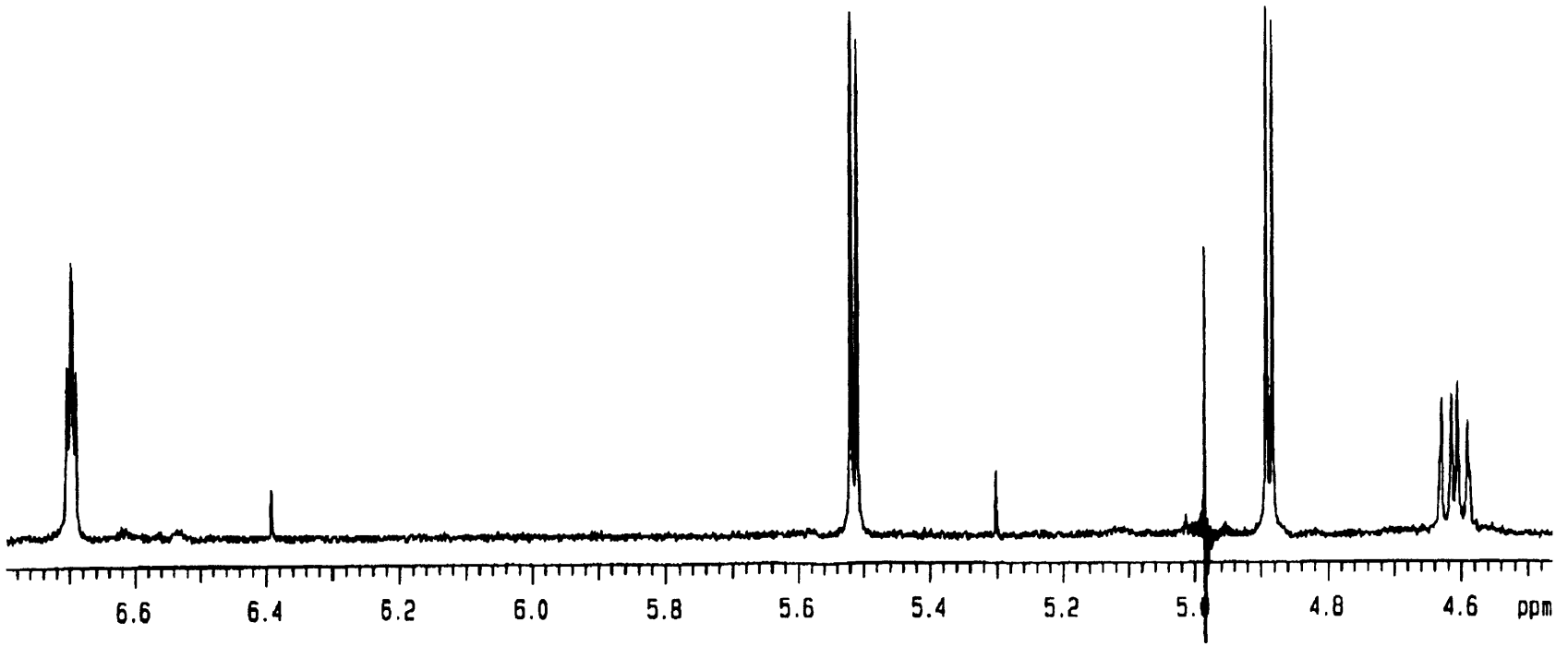


Figure 7: ^{1}H NMR Decoupling of H_c of Compound **68.**



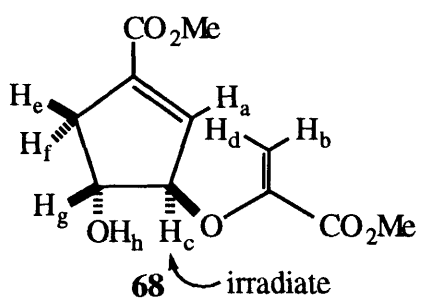
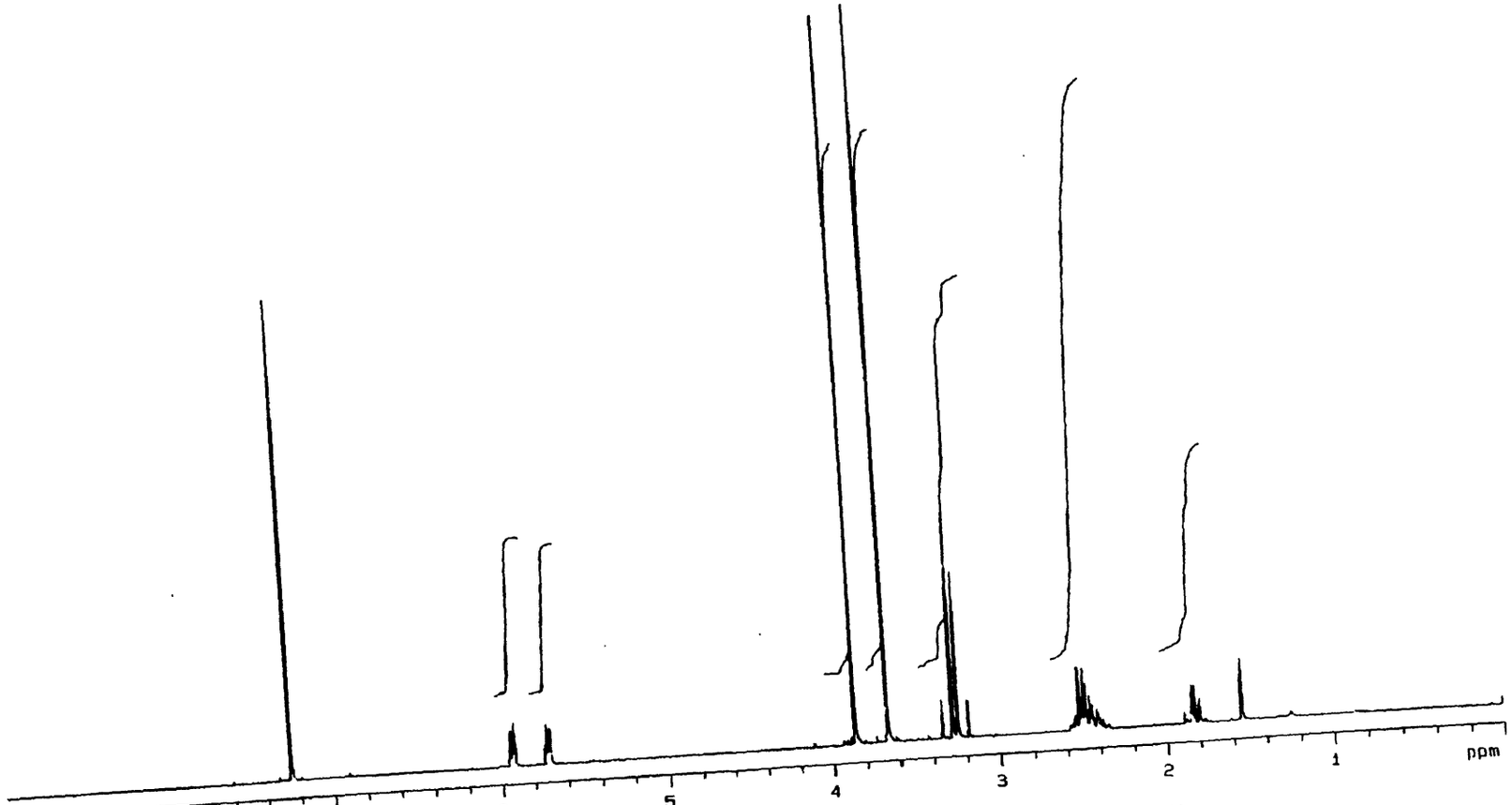
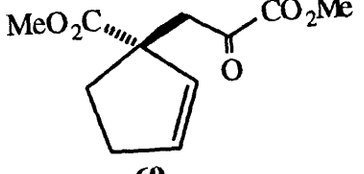


Figure 8: Enlargement at 4.5 - 6.7 ppm of ^{1}H NMR Decoupling H_c of Compound **68.**





69

Figure 9: ^{1}H NMR of Compound **69.**

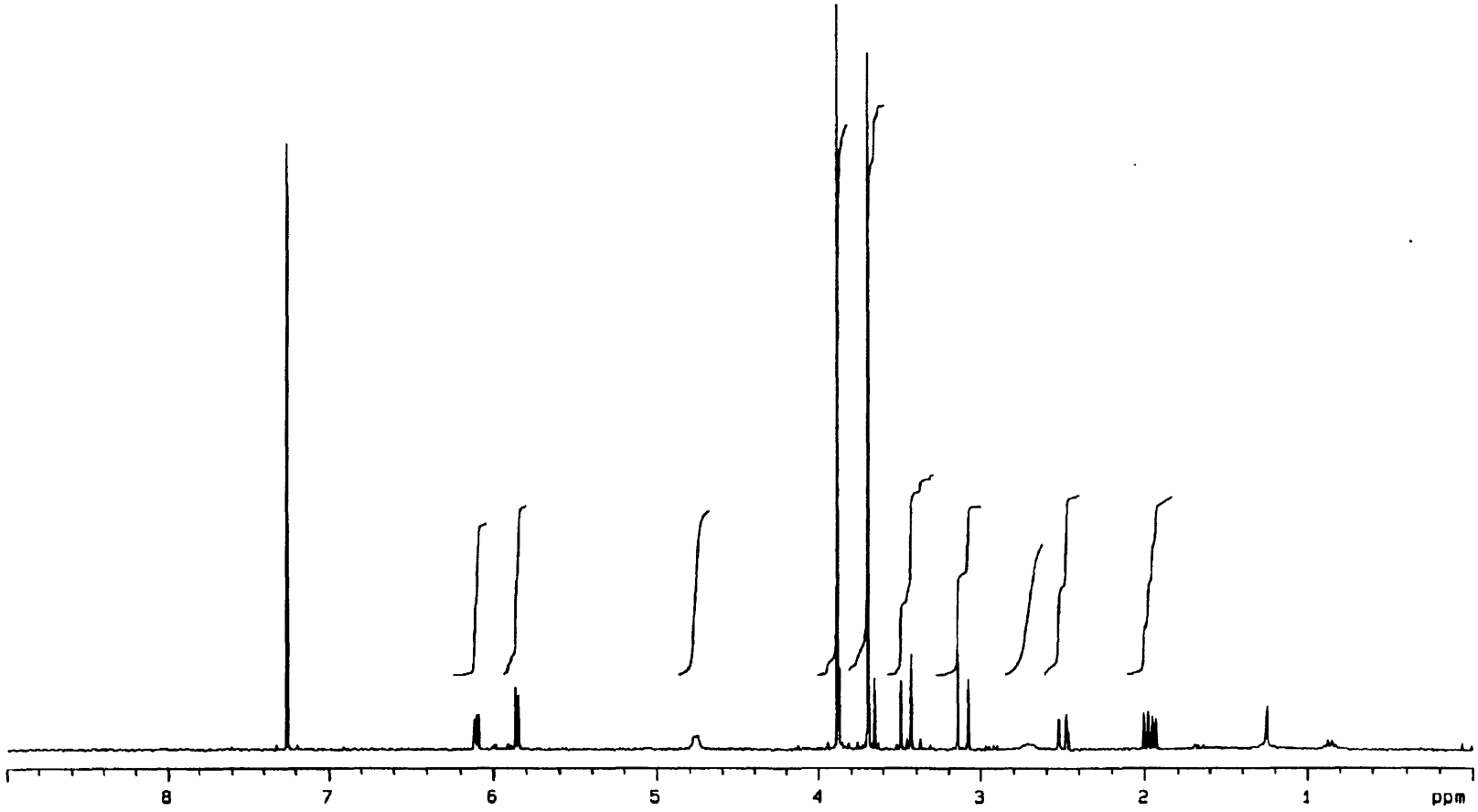
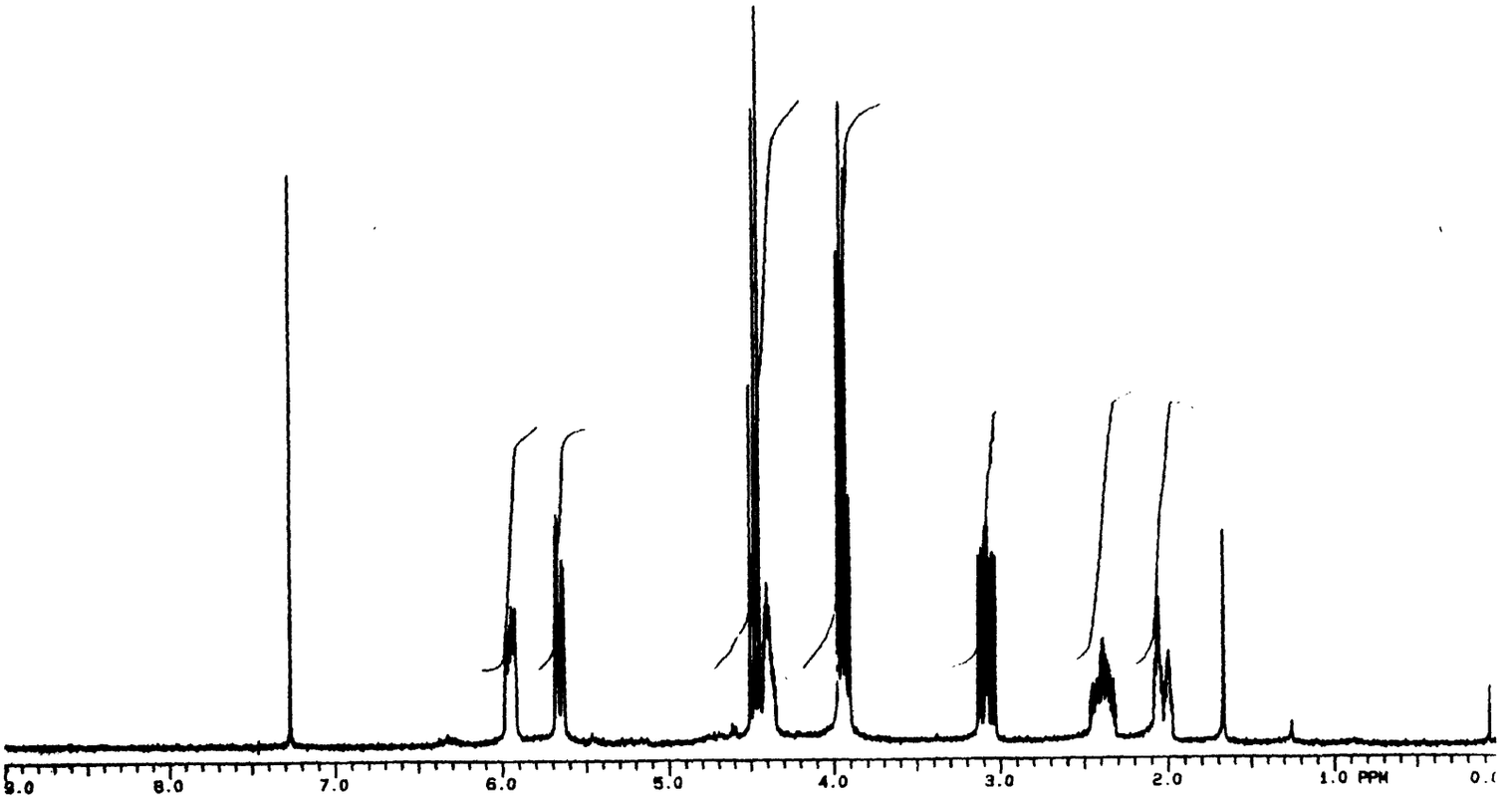
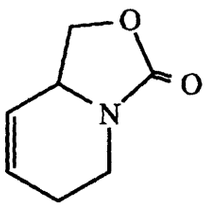


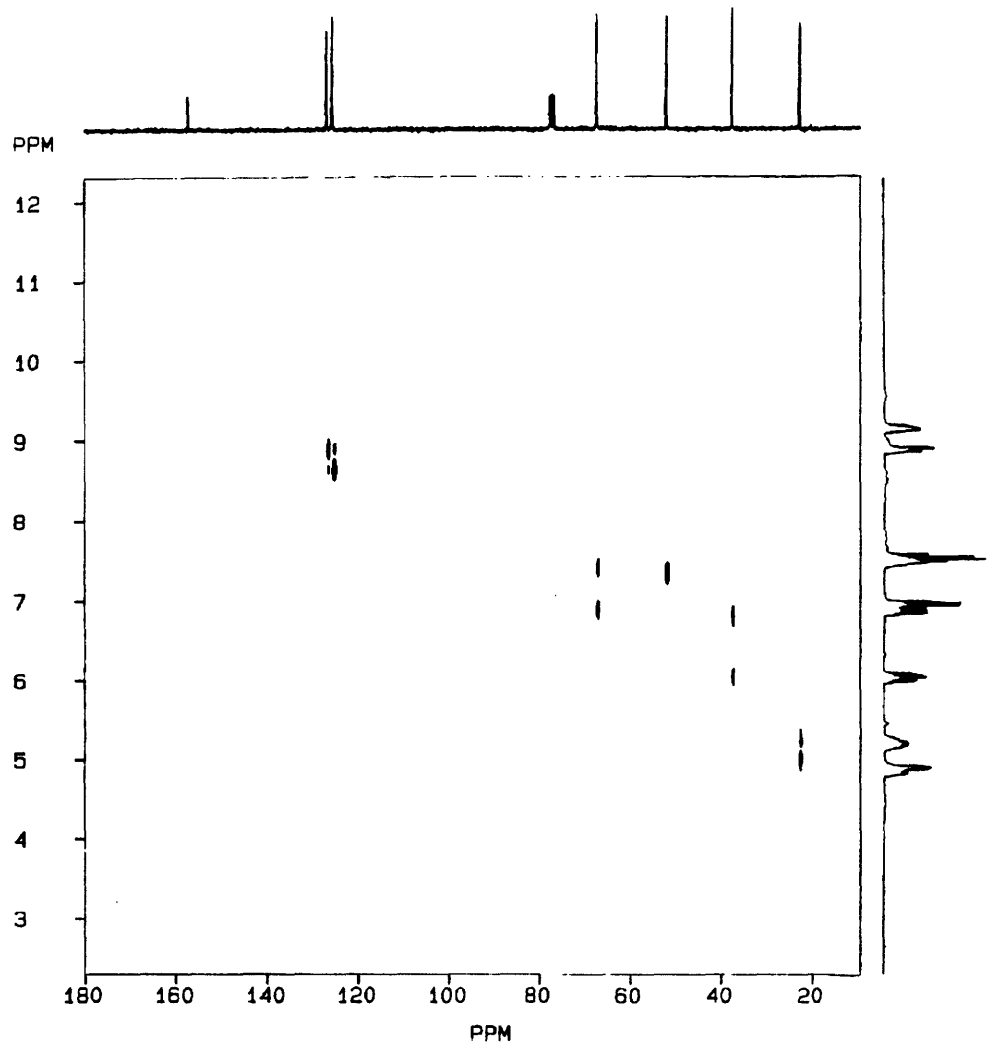
Figure 10: ^{1}H NMR of Compound **70.**

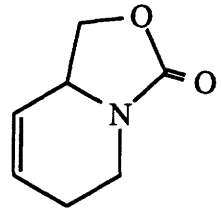




87

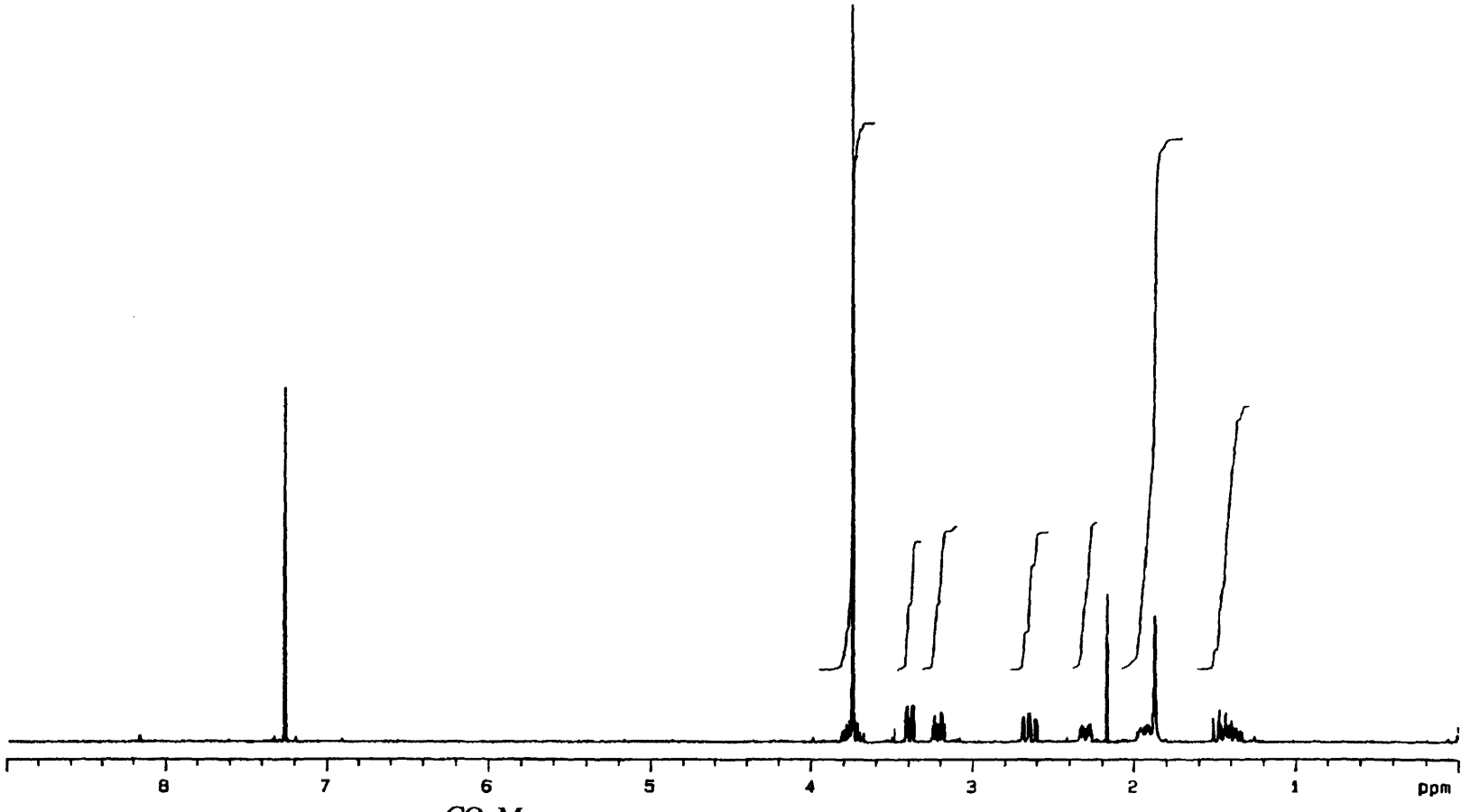
Figure 11: ^{1}H NMR of Compound **87.**

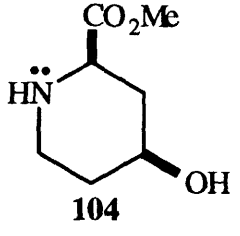




87

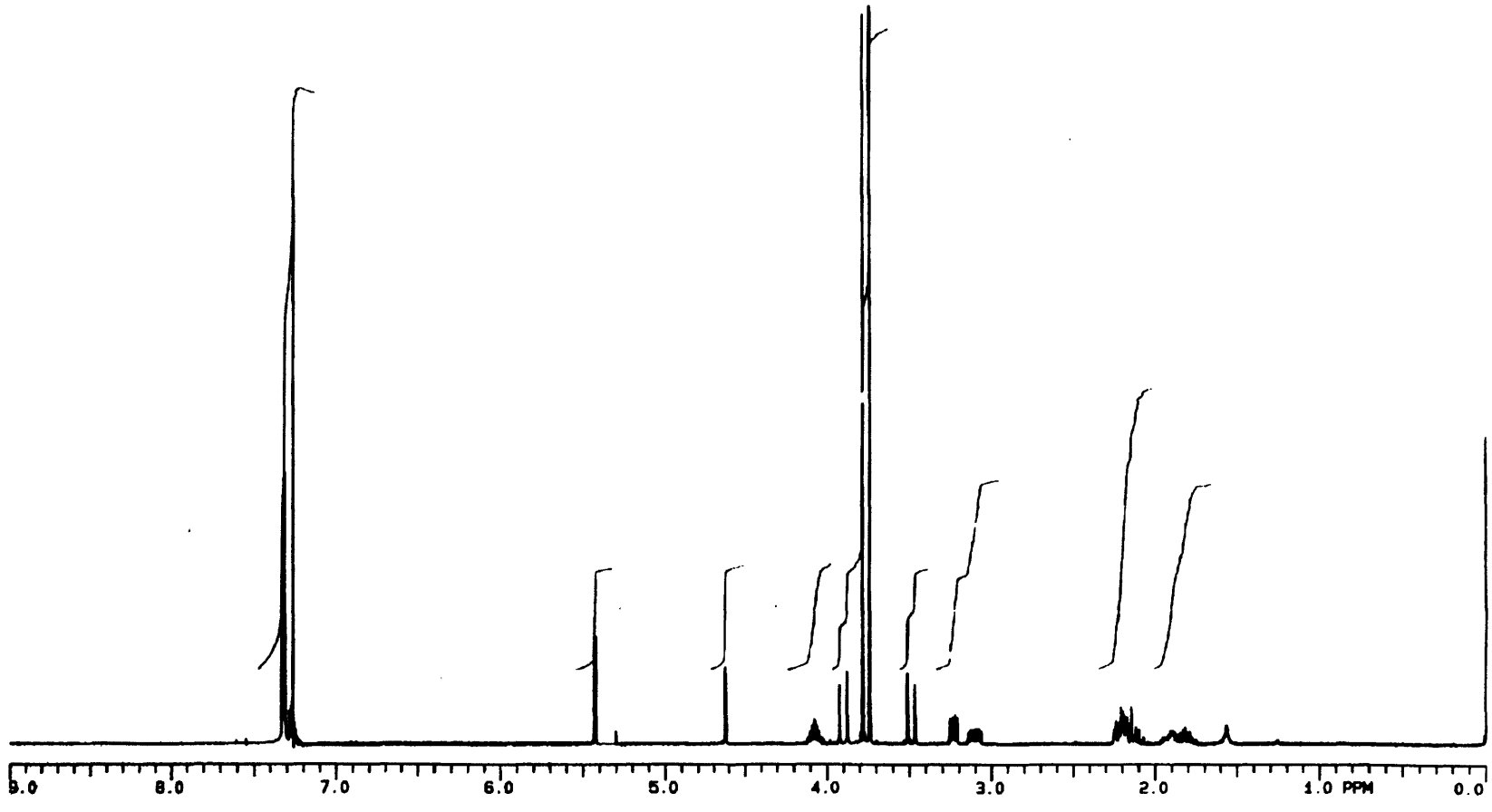
Figure 12: HETCOR of Compound **87.**

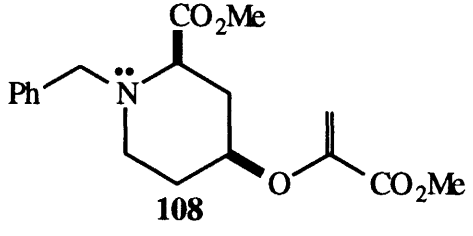




104

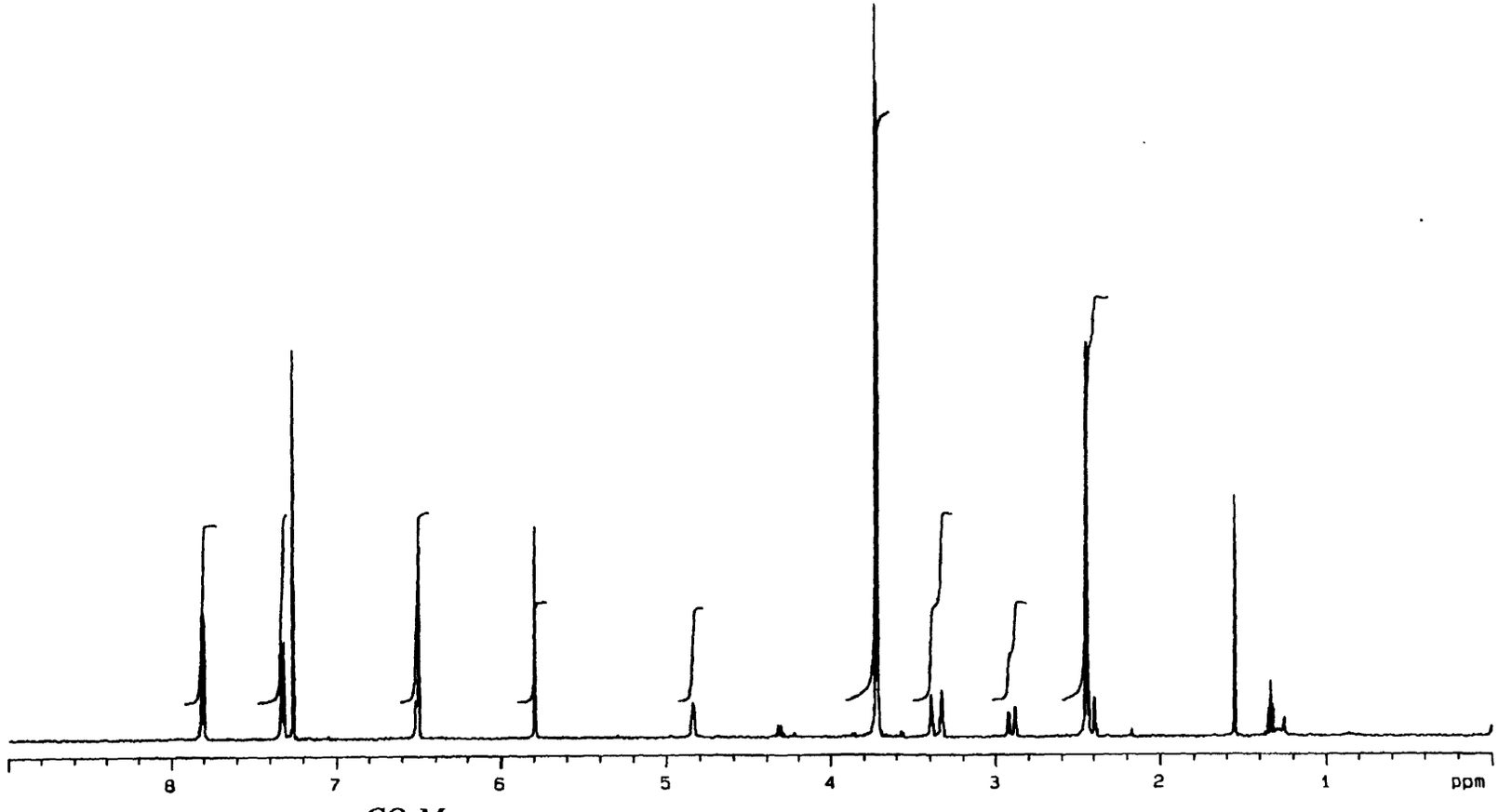
Figure 13: ^{1}H NMR of Compound **104.**

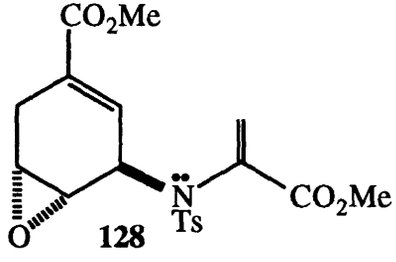




108

Figure 14: ^{1}H NMR of Compound **108.**





128

Figure 15: ^{1}H NMR of Compound **128.**

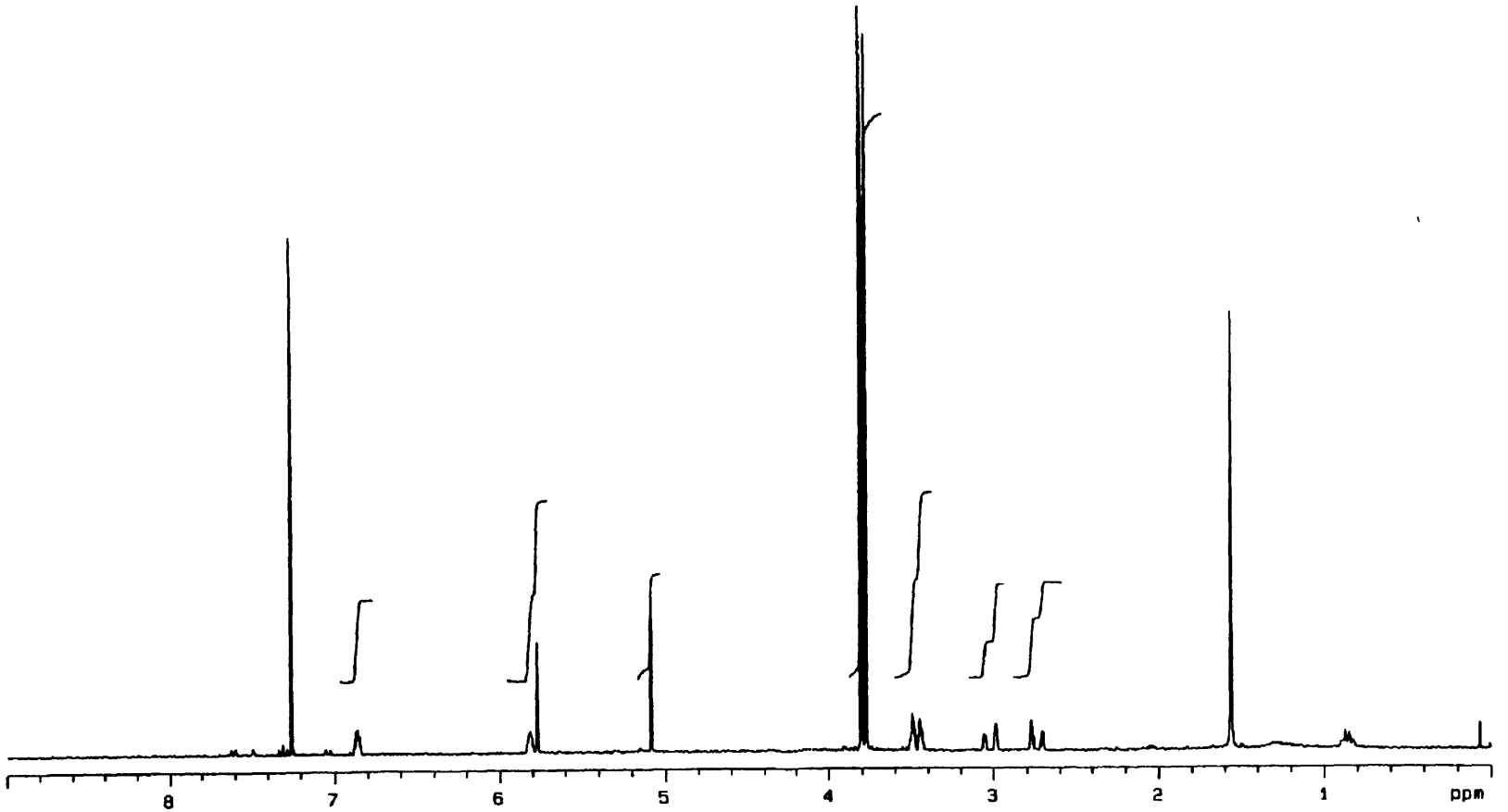
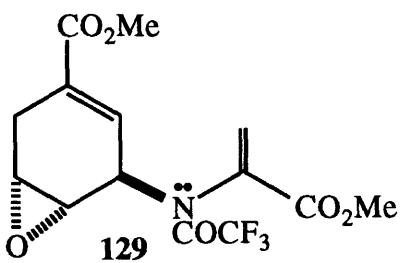


Figure 16: ^{1}H NMR of Compound **129.**



129

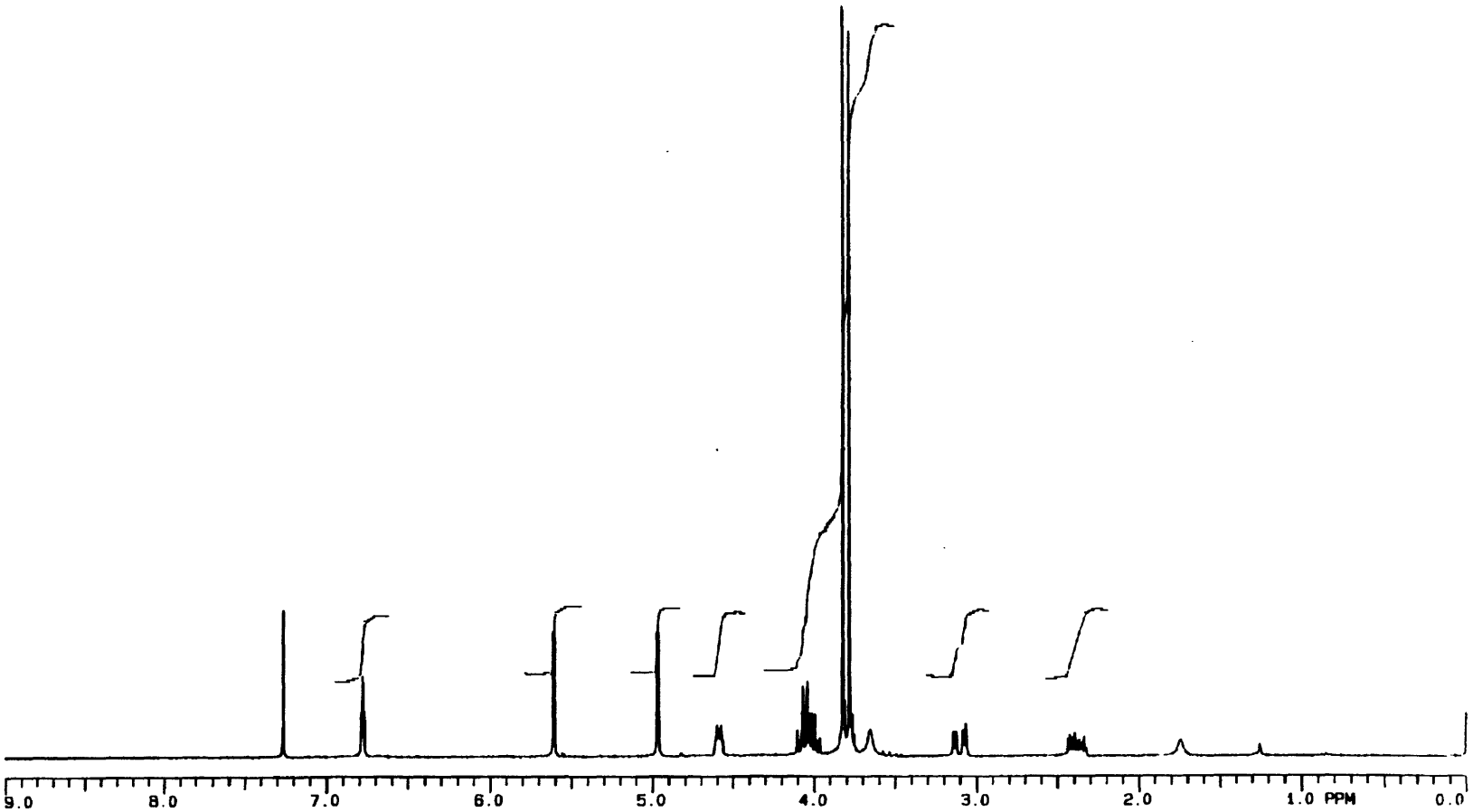
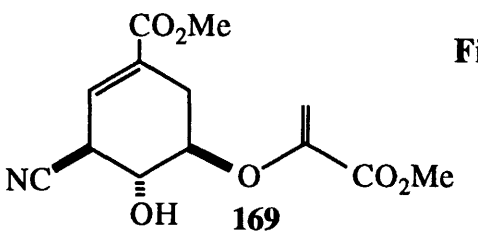


Figure 17: ^{1}H NMR of Compound **169.**



169

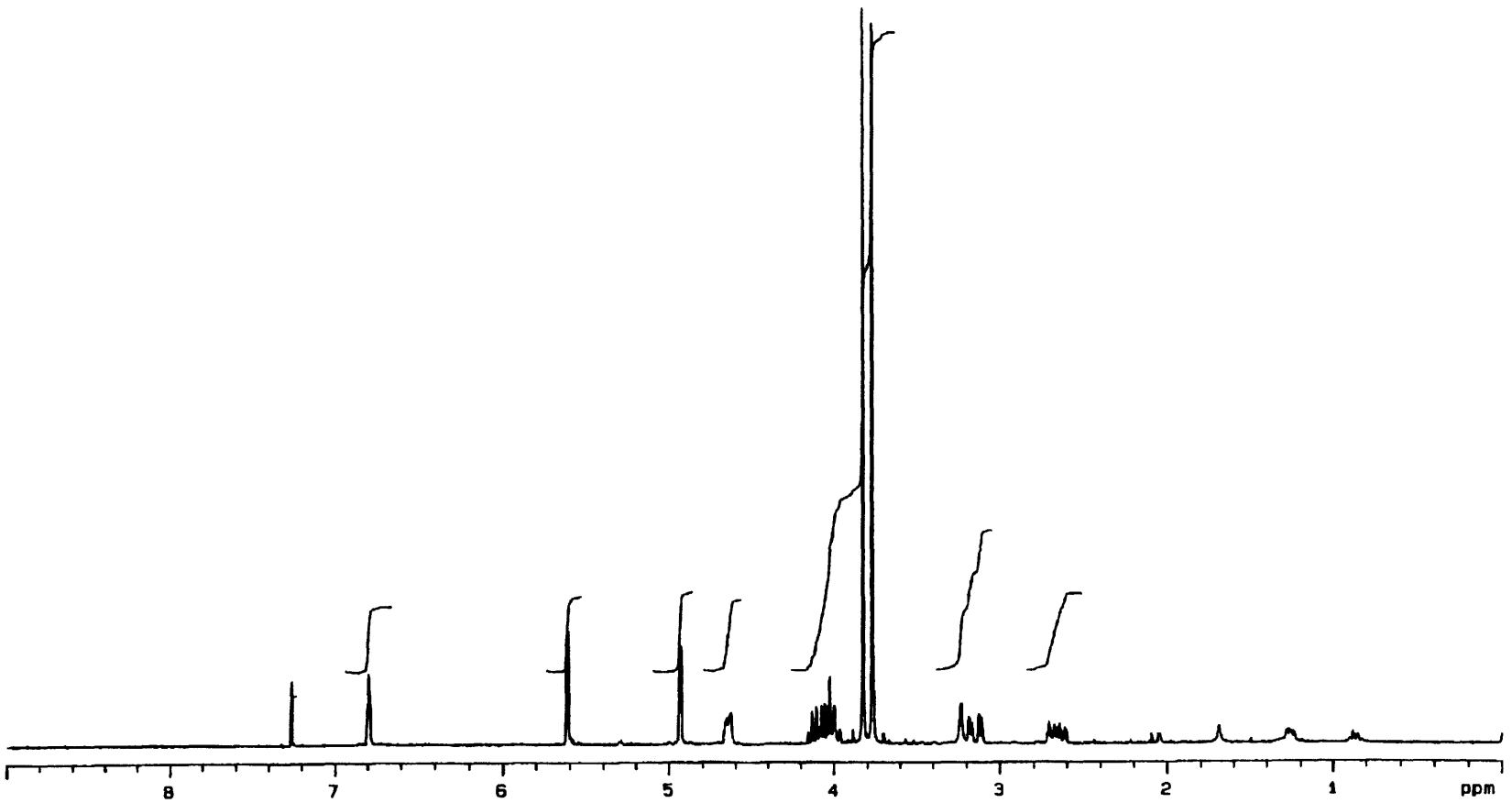
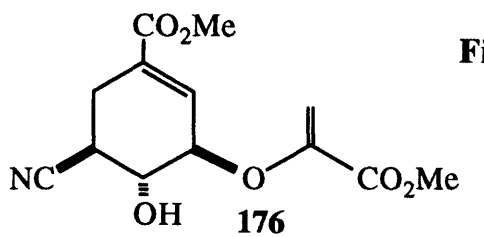


Figure 18: ^{1}H NMR of Compound **176.**



176