Department of Chemistry

Structure activity relationship studies of new ethylene antagonists

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of
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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgement has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any other university.

Signature: 

Date: …… 14/03/2016 ……

Dedication

To souls of

My parents

Miloud A. Musa

and

Torkiea Ibrahem Musa

I dedicate this humble work, to who were the reasons for my existence in this world

and

I ask Allah to mercy them

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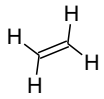
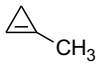
Abstract

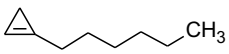
Ethylene is an essential hormone for plant development and fruit ripening, however it can lead to accelerated spoilage of horticulture produce postharvest. Ethylene can be controlled by inhibition of its biosynthesis or inhibition of the ethylene receptor in the plant. Since ethylene can act at low concentrations and can be produced from an external source, the inhibition of the ethylene receptor in the fruits and flowers is more effective than inhibition of ethylene biosynthesis. 1-Methylcyclopropene (1-MCP) is used commercially to inhibit ethylene and delay fruit ripening. 1-MCP is a volatile gas at room temperature and is unstable. In the search for new more practical ethylene antagonist, 1-hexylcyclopropene (1-HCP), benzocyclopropene and 1*H*-naphtho[*b*]cyclopropene were tested against of some climacteric fruit and variety of wax-flowers. These compounds were excellent inhibitors of ethylene action, e.g. flower drop and fruit ripening. Stable and easy to handle.



Ethylene
1

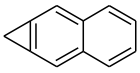


1-MCP
16



1-HCP
122

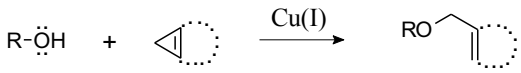
Benzocyclopropene
47



1*H*-Naphtho[*b*]cyclopropene
48

1-MCP and 1-HCP were synthesized and applied on some fruits and wax flowers and they showed high potency as ethylene antagonist at low concentration. They have suppressed the ethylene production and respiration rate, delayed the fruit ripening and wax flower abscission.

The mode of action of 1-MCP has been postulated to occur through a copper cofactor promoted inactivation the ethylene receptor. The high ring strain of cyclopropenes leads to ring opening to form a copper carbenoid intermediate. This intermediate then covalently bonds with neighboring amino acids thereby blocking the ethylene action. To mimic this response, copper complexes have been prepared and used to catalyse reaction of cyclopropenes and cycloproparenes with alcohols. The active ethylene antagonist reacted easily in presence of copper complex while those that were not antagonist did not react.



Water is the safest solvent that can be used to dissolve the ethylene antagonist compounds and applied on the edible fruits and vegetables. However as the cycloproparenes were insoluble or sparingly soluble in water several attempts were conducted to make water soluble analogues. 1,2-Dimethanolbenzocyclopropene was synthesized starting from dimethyl acetylenedicarboxylate and butadiene sulfone in three steps but unfortunately it decomposed immediately after preparation.

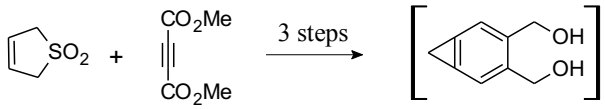


Table of contents

Declaration i

Acknowledgement iii

Abstract iv

Table of Contents vi

List of Figures xi

List of Schemes xiv

List of tables xviii

List of symbols and abbreviations xix

Chapter 1 1-46

1.1 Postharvest losses of fruits and vegetable 6

1.2 Ethylene biosynthesis and its inhibitors 7

1.3 Ethylene receptor 11

1.4 Synthetic complexes as models of ethylene receptor 13

1.5 Modulation of the ethylene response 15

1.5.1 Ethylene receptor agonists 15

1.5.2 Ethylene receptor antagonists 19

1.5.3 Role of hydrophobic chain 19

1.5.4 Natural occurring ethylene antagonists 20

1.5.5 Synthesized ethylene antagonists 23

1.6 1-Methylcyclopropene (1-MCP) 25

1.7 1-Substituted cyclopropenes 27

1.8 Differently substituted Cyclopropenes 28

1.9 Dialkylamine derivatives of 1-methylcyclopropenes 30

1.10 Proposed Action of 1-MCP and derivatives on the ethylene receptor 32

1.11 Synthesis of cyclopropenes 33

1.11.1 Synthesis of 1-methylcyclopropene 1-MCP 34

1.11.2 Synthesis of 3,3-Disubstituted 37

1.12 Cycloproparenes 37

1.12.1 Synthesis of cycloproparenes 38

1.12.2 Reaction of cycloproparenes 43

1.13 Aim of project 47

Chapter 2 **48-57**

2.1 Natural alkenes ethylene antagonist 48

2.2 Simple alkenes as ethylene antagonists 49

2.2.1 3,4-Epoxy-3-methyl-1-butene 52

2.2.2 Synthesis of isoprene oxide **106** 53

2.2.3 Effect of isoprene oxide **106** on abscission of flowers/buds in WX17 wax flowers 54

2.2.4 Effect of isoprene oxide **106** on climacteric ethylene production and respiration in some climacteric fruits 54

2.2.5 Reaction of isoprene oxide **106** with copper complex 56

Chapter 3 **58-76**

3.1 Mode of action of cyclopropene ethylene antagonists 58

3.2 Investigating the activity of compounds as ethylene agonist or antagonist in laboratory 60

3.2.1 Synthesis of copper complexes 61

3.3 Synthesis of cyclopropenes and their reactions with copper complexes 65

3.3.1 Effect of 1-HCP on ‘Kommeet’ tomato ((Solanum lycopersicum L)) 71

3.3.2 Effect of 1-HCP on ‘Black Amber’ Japanese plum fruit 72

3.3.3 Effect of 1-HCP on ‘Fuji’ apple 73

3.4 1,2,3,3-Tetrachlorocyclopropene **127** 75

Chapter 4 **77-95**

4.1 Synthesis of benzocyclopropene and 1*H*-naphtho[*b*]cyclopropene 79

4.2 Ring opening of 1*H*-naphtho[*b*]cyclopropene with copper complexes 84

4.3 Effect of benzocyclopropene and 1*H*-naphtho[*b*]cyclopropene on some climacteric fruits and wax flowers 86

4.4 Effect of cycloproparenes on abscission of flower (%) and vase life in wax flower. 87

4.5 'WX17' and 'WX73' wax flowers 88

4.6 Effect of Benzocyclopropene and 1*H*-naphtho[*b*]cyclopropene on some climacteric fruits 89

4.6.1 Effect of benzocyclopropene **47** on climacteric ethylene production and respiration rate in 'Tegan Blue' Japanese plum and 'Arctic Pride' nectarine 90

4.6.2 Effect of benzocyclopropene **47** on climacteric ethylene production in 'Kensington Pride' mango 91

4.6.3 Effect of benzocyclopropene **47** on climacteric ethylene production and respiration rate in 'Cripps Pink' apple 92

4.6.4 Effect of 1*H*-naphtho[*b*]cyclopropene **48** on 'fortune' plum 93

Chapter 5 **96-114**

5.1.1 Synthesis of a water soluble 1*H*-cyclopropa[*b*]naphthalene derivative 99

Chapter 6 **115-116**

6.1 General Conclusions 115

Chapter 7 **117-149**

7.1 Experimental 117

7.2 3,4-Epoxy-3-methylbutene (isoprene oxide) 118

7.3 1,2-Epoxyoctane 119

7.4 Allyl n-butyl ether 119

7.5 1,2-Dibromooctane 120

7.6 2-Bromooctene 121

7.7 1,1,2-Tribromo-2-hexylcyclopropane 122

7.8 1-Hexylcyclopropene (1-HCP) 122

7.9 Tris(triphenylphosphine)copper(I) iodide 123

7.10 Acetonitrile (1,10-phenanthroline)copper(I) iodide 124

7.11 3,5-Dimethyl-1*H*-pyrazole 124

7.12 tris(3,5-Dimethyl-1-pyrazolyl)methane. 125

7.13 Tetrakis(acetonitrile)copper(I) hexaflurophosphate 126

7.14 Copper(I)acetonitrile[tris(3,5-dimethyl-1-pyrazolyl)methane] hexaflurophosphate 126

7.15 Reaction of 1,2-epoxy-2-methyl-3-butene with ethanol in presence of Copper(I) complex **137** 127

7.16 Reaction of 1,2-epoxyoctane with copper complex **137** 128

7.17 Synthesis of 1-methylcyclopropene and reaction with butanol in presence of copper complex **137** 128

7.18 Synthesis of 1-methylcyclopropene and reaction with imidazole in presence of copper complex **137** 129

7.19 Reaction of 1,2,3,3-tetrachloro-1-cyclopropene with ethanol in presence of copper-complex **137** 130

7.20 Reaction of 1-HCP with methanol in the presence of AgNO3 130

7.21 Reaction of 1-HCP with methanol catalyzed by copper complex **137** 131

7.22 Trans-1,2-Dibromocyclohexane 131

7.23 1,3-Cyclohexadiene 132

7.24 7,7-Dichlorobicyclo[4.1.0]hept-2-ene 133

7.25 Benzocyclopropene 133

7.26 1,4-Dihydronaphthalene 134

7.27 1,1-Dichloro-1a,2,7,7a-tetrahydro-1*H*-Cyclopropa[*b*]naphthalene 135

7.28 1*H*-Naphtho[*b*]cyclopropene 135

7.29 Reaction of 1*H*-naphtho[*b*]cyclopropene with ethanol in presence of {[HC(3,5-$Me_2Pz)_3$]Cu(NCMe)}PF_6 complex (**137**) 136

7.30 Iodination of **67** 137

7.31 Attempted to etherification of adduct **196** 137

7.32 [2-(2-Methoxyethoxy) ethoxy]-Benzene. 138

7.33 2-[2-(2-Methoxyethoxy)ethoxy]ethyl (4-methylbenzenesulfonate) 139

7.34 Synthesis of 2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]-naphthalene. 139

7.35 Reduction of 2-naphthanol 140

7.36 Reduction of 2-naphthanol by using sodium hydride then sodium metal and t-BuOH 141

7.37 5,8-Dihydro-2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]naphthalene 142

7.38 Dichlorocarbene addition to 5,8-dihydro-2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy] naphthalene. 142

7.39 Bromination of **67** using formaldehyde and hydrogen bromide 143

7.40 Etherification of **218**. 144

7.41 Synthesis of 2-(2-(2-methoxyethoxy) ethoxy)ethanethiol $CH_3(OCH_2CH_2)_3SH$ 145

7.42 Reaction of **218** with 2-(2-(2-methoxyethoxy)ethoxy)ethane thiol using K_2CO_3 146

7.43 Dimethyl-1,4-cyclohexadiene-1,2-dicarboxylate **226** 146

7.44 Dichlorocarbene addition to 1,4-Cyclohexadiene-dicarboxylate **226** 147

7.45 7,7-Dichloro-bicyclo[4,1,0]hept-3-ene-1.2-dimethanol 148

7.46 Dehydrochlorination of **228** 148

References **150**

List of figures

Figure 1.1 Ethylene and the sycamore fig tree.[6] 1

Figure 1.2 The triple response of ethylene on the growth of etiolated pea plants.[15] ... 2

Figure 1.3 Schematic illustration of ethylene production in climacteric fruits as a function of time after harvest.[22] 4

Figure 1.4 Ethylene production after harvest of '*Golden delicious*' apple and avocado fruit.[23,25] 5

Figure 1.5 Ethylene biosynthesis in higher plant as shown in Yang Cycle.[26] 8

Figure 1.6 Inactivation of ACC oxidase by ACC analogs.[35] 11

Figure1.7 Receptor signal state mode l. The ethylene binding domain of ETR1 is depicted as a homodimer with each monomer containing three transmembrane helices. The region marked with pink shows the copper binding pocket and region marked in green are areas allowing the receptor to turn off. Adapted from Binder, et al., 2008.[42] 12

Figure 1.8 Complexes mimic ethylene receptor 14

Figure 1.9 Air stable ethylene copper(I) complex with a tridentate ligand $Cu(HB(3,5\text{-}Me_2Pz)_3(C_2H_4)$.[50] 15

Figure 1.10 Active ethylene antagonist compounds 26

Figure 1.11 The difference in the angle of benzocyclopropene and other cyclopropenes diagrams. 38

Figure 2.1 Natural terpenes 48

Figure 2.2 Analogues of 1-octene ……..…50

Figure 2.3 The ethylene production and respiration in climacteric "*Tegan Blue*" plum fruit at day 10 after treatment with different concentrations of isoprene oxide **115**. 55

Figure 2.4 The ethylene production and respiration in climacteric "*Fuji*" apple fruit at day 22 after treatment with different concentrations of isoprene oxide **115.** 56

Figure 3.1 Proposed mechanism of the mode of action of 1-MCP and its derivatives 58

Figure 3.2 Cyclopropenes to be tested 60

Figure 3.3 Models of copper(I) complexes adapted from Yunfan Zou 60

Figure 3.4 Cu(I)(phen)(CH_3CN) and [(bpy) CH_3CN]PF_6 complexes. 61

Figure 3.5 Copper complexes adapted from Hsu *et al* 61

Figure 3.6 The similarity between ethylene receptor domain and copper complex **137** 62

Figure 3.7 The comparison between histidine and 1*H*-imidazole and serine and methanol. 65

Figure 3.8 Setup of synthesis of 1-MCP and reaction with Cu-complex 66

Figure 3.9 Ethylene production and respiration climacteric at day 4 and day 10 of '*Kommeet*' tomato fruit treated with different concentrations of 1-HCP 72

Figure 3.10 Ethylene production and respiration climacteric at day 10 and day 14 of '*Black Amber*' plum treated with different concentrations of 1-HCP 73

Figure 3.11 Ethylene production and respiration climacteric at day 24 of '*Fuji*' apple treated with different concentration of 1-HCP 73

Figure 3.12 Effect of Tetrachlorocyclopropene **127** 50% wax flowers abscission . 755

Figure 4.1 Benzocyclopropene and 1*H*-naphtho[*b*]cyclopropene 79

Figure 4.2 Effect of benzocyclopropene on abscission of flower (%) and vase life in wax flower. 87

Figure 4.3 Effect of different concentration of benzocyclopropene **47** on the 50% abscission of wax flowers (WX17 and WX73) 88

Figure 4 . 4 Percent of flower/bud abscission after four days of treatment with 1*H*-naphtho[*b*]cyclopropene (100 nLL^{-1}) on flower/bud abscission (%) in ''WX73' and 'WX107' wax flowers. 89

Figure 4.5 Concentration of ethylene and CO_2 on the day of climacteric peak, vertical bars represent S.E. of means and are invisible when the values are smaller than the symbol. n = 5 replications. 91

Figure 4.6 Ethylene production at day 4 in '*Kensington Pride*' mango treated with (50 nLL^{-1}) and ethylene (10 μLL^{-1}) alone or their compensation. 92

Figure 4.7 Change of endogenous levels of ethylene and respiration rate in '*Cripps Pink*' apple on the 14^{th} day after treatment. 93

Figure 4.8 Concentration of ethylene (A) and CO_2 (B) on the day of climacteric peak in '*Fortune*' plum fruit treated with different concentration of 1*H*-naphtho[*b*]cyclopropene. 94

Figure 5.1 Benzocyclopropene **47** and 1*H*-naphtho[*b*]cyclopropene **48** ethylene antagonists..... 96

Figure 5.2 The resonance form of cyclopropenone 97

Figure 5.3 Effect of NH_3^+ group on 2-(aminomethyl)-2-cyclopropene-1-carboxylic acid **174** 97

Figure 5.4 Ethylene antagonist activity of water-soluble carboxylic cyclopropene .. 98

Figure 5.5 Introduce an ethylene glycol chain onto cycloproparene 98

List of Schemes

Scheme 1.1 Binding of ethylene to the copper in the ethylene receptor..... 13

Scheme 1.2 An stable copper(I) ethylene complex 14

Scheme1.3 Proposed model for action of ethylene and 1-methylcyclopropene adapted from the model proposed by Sisler and Serek (1997).[34]..... 32

Scheme 1.4 The mechanism of binding of 1-MCP to the ethylene receptor and the ring opining reaction.[63] 33

Scheme 1.5 Different ways to synthesis of cyclopropene 34

Scheme 1.6 Synthesis of 1-MCP 35

Scheme 1.7 Synthesis of 1-MCP 35

Scheme 1.8 Synthesis of 1-alkylcyclopropene 36

Scheme 1.9 Synthesis of cyclopropene by fluoride-induced elimination..... 36

Scheme 1.10 Synthesis of 3,3-dimethylcyclopropene 37

Scheme 1.11 Irradiation of 3*H*-indazoles to synthesis of cyclopropabenzene derivative..... 39

Scheme 1.12 Synthesis of 2-chloro-1,1-dimethyl-1*H*-cycloprop[*e*]azulene by irradiation of the azulenopyrazole..... 40

Scheme 1.13 Alder-Rickert synthesis of benzocyclopropene..... 40

Scheme 1.14 Aromatization of dihalocarbene adducts to synthesis of benzocyclopropene..... 41

Scheme 1.15 Synthesis of 1*H*-cyclopropa[*b*]naphthalene 41

Scheme 1.16 Attempt to synthesis of 1*H*-cyclopropene[*b*]anthracene..... 42

Scheme 1.17 Synthesis of 1-bromo-2-chlorocyclopropene..... 42

Scheme 1.18 Synthesis of 1-*H*-cycloprop[*b*]anthracene..... 43

Scheme 1.19 Reaction of benzocyclopropene with acids..... 43

Scheme 1.20 Dimerization of benzocyclopropene by Ag ion 44

Scheme 1.21 Reaction of 3-chloro-1*H*-cyclopropabenzene with methanol in presence of $AgNO_3$ 45

Scheme 1.22 Reaction of benzocyclopropene with metal carbene 45

Scheme 1.23 Reaction of 1*H*-cyclopropa[*b*]naphthalene with metal carbene 46

Scheme 2. 1 Synthesis of ether 51

Scheme 2. 2 Synthesis of 1,2-epoxyoctane 51

Scheme 2. 3 Synthesis of 1,2-epoxyoctane 51

Scheme 2. 4 Ring opening reaction of isoprene oxide 53

Scheme 2. 5 Synthesis of isoprene oxide **106** 54

Scheme 2.6 Reaction of 1,2-epoxy-2-methyl-3-butene with copper complex. 57

Scheme 2.7 Reaction of 1,2-epoxyoctane with copper complex 57

Scheme 3.1 Reaction of 1-HCP with $Cu(phen)(CH_3CN)PF_6$.[63] 59

Scheme 3.2 Ring opening reaction of 1-subsituted cyclopropenes in the presence of a nucleophile 59

Scheme 3.3 Synthesis of $Cu(PPh_3)_3I$ and $Cu(phen)(CH_3CN)PF_6$ 62

Scheme 3.4 Synthesis of tris(3,5-dimethylpyrazol)methane. 63

Scheme 3. 5 Synthesis of $[Cu(I)(NCCH_3)_4(CH)]I$. 64

Scheme 3.6 Synthesis of $[(CH)Cu(I)(3,5\text{-}Me_2Pz)_3(NCMe)]PF_6$. 65

Scheme 3.7 Synthesis of 1-MCP and reaction with Cu-complex and in butanol 67

Scheme 3.8 Synthesis of 1-MCP and reaction with Cu-complex and imidazole in CH_2Cl_2 68

Scheme 3.9 Synthesis 1-substitutied cyclopropene 68

Scheme 3.10 Proposed synthesis of 1-HCP 69

Scheme 3.11 Synthesis of 2-bromo-1-octene 69

Scheme 3.12 Synthesis of 1,1,2-tribromo-2-hexylcyclopropane 70

Scheme 3.13 Synthesis of 1-hexylcyclopropene 71

Scheme 3.14 Reaction of **127** with MeOH in presence of copper complex **137** 76

Scheme 4.1 The reaction of 3-chlorobenzocyclopropene with silver nitrate in methanol 77

Scheme 4.2 Reactions of benzocyclopropene with transition metal salts 78

Scheme 4.3 Proposed mechanism of the mode of action of benzocyclopropene and its derivatives 79

Scheme 4.4 Preparation of benzocyclopropene by Billups 80

Scheme 4.5 Alternative preparation of benzocyclopropene 80

Scheme 4.6 Preparation of 1,3-cyclohexadiene. 81

Scheme 4.7 Modified synthesis of benzocyclopropene from 1,3-cyclohexadiene. ... 82

Scheme 4.8 Preparation of 1*H*-naphtho[*b*]cyclopropene by Billups 82

Scheme 4.9 Modified synthesis of 1*H*-naphtha[*b*]cyclopropene from naphthalene .. 83

Scheme 4.10 Synthesis of 1*H*-cycloprop[*b*]anthracene 84

Scheme 4.11 Ring opening of reaction of 1*H*-naphtha[*b*]cyclopropene with silver salts 84

Scheme 4.12 Reaction of 1*H*-naphtha[*b*]cyclopropene with silver nitrate in ethanol 85

Scheme 4.13 Proposed reaction of benzocyclopropene with the ethylene receptor ETR1 95

Scheme 5.1 Proposed synthesis of a water soluble naphthalene derivative 99

Scheme 5.2 Iodination of **181** 100

Scheme 5.3 Attempted etherification of **196** 100

Scheme 5.4 Model reaction of Hosseinzadeh conditions 101

Scheme 5.5 Etherification of **196** with CuI, 1,10-phenanthroline and $CsCO_3$ 101

Scheme 5.6 Revised synthetic pathway 102

Scheme 5.7 Reaction of **208** with **209** 102

Scheme 5.8 Synthesis of 2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]-naphthalene. 103

Scheme 5.9 Reduction of 2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]-naphthalene 103

Scheme 5.10 Reduction of 2-naphthanol 104

Scheme 5.11 Reduction of sodium 2-naphthanoxide 104

Scheme 5.12 Synthesis of 5,8-dihydro-2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy] naphthalene. 105

Scheme 5.13 Addition of dichlorocarbene to **205** 105

Scheme 5.14 Bromomethylation of tetralin **216** 106

Scheme 5.15 Synthesis of an ether adduct via bromomethylation of **181** 106

Scheme 5.16 Bromomethylation of **181** using paraformaldehyde, acetic acid and hydrogen bromide 106

Scheme 5.17 Reaction of **181** by using formaldehyde and hydrogen bromide 107

Scheme 5.18 Reaction of **181** by using formaldehyde and hydrobromic acid 108

Scheme 5.19 Etherification of **218** 108

Scheme 5.20 Synthesis of 2-(2-(2-methoxyethoxy)ethoxy)ethanethiol. 109

Scheme 5.21 Reaction of brominated **218** with glycol thiol **223** 109

Scheme 5.22 Reaction of **218** with **223** using K_2CO_3 instead of NaH 110

Scheme 5.23 Synthesis of 3,4-dimethanolcyclopropa[*b*]benzene **229** 110

Scheme 5.24 Synthesis of dimethyl-1,4-cyclohexadiene-1,2-dicarboxylate. 111

Scheme 5.25 Synthesis of 7,7-dichloro-1,2-hexene-1,2-carboxylate 111

Scheme 5.26 Synthesis of 7,7-dichloro-1,2-hexene-1,2-carboxylate using NaO_2C-CCl_3. 112

Scheme 5.27 Synthesis of 7,7-dichloro-1,2-hexene-1,2-carboxylate 112

Scheme 5.28 Synthesis of 7,7-dichloro-bicyclo[4,1,0]hept-3-ene-1.2-dimethanol. 113

Scheme 5.29 Dehydrochlorination of 7,7-dichloro-bicyclo[4,1,0]hept-3-ene-1.2-dimethanol. 113

List of tables

Table 1.1 Activity of ACC synthase inhibitors..... 10

Table 1.2 Biological activity of ethylene and other alkenes and alkene-related compounds as determined by the Pea straight growth test.[16,28] 16

Table 1.3 Biological activity of ethylene and other alkenes and alkene-related compounds as determined by the pea straight growth test by (Burg and Burg, 1965, 1967). 17

Table 1.4 Effects of isocyanides and related compounds on pea seedling elongation.[53] 18

Table 1.5 Effect of ethylene and 1-alkenes on etiolated pea growth 20

Table 1.6 Naturally ethylene antagonist.[54,58] The numbers that under the molecules refer to the activity K_i ($\mu L\ L^{-1}$) 22

Table 1.7 Inhibition of ethylene action in plants by synthetic ethylene antagonists.[8,54] 24

Table 1.8 Inhibition of ethylene action in plants by synthetic six-membered cyclic compounds ethylene antagonists..... 25

Table 1.9 The minimum concentration of difference cyclopropenes and time of protection of banana fruits against ethylene.[68] 28

Table 1.10 Minimum concentration and time of insensitivity for different substituted cyclopropenes on banana fruit after 24 hrs of treatment.[60]... 30

Table 1.11 Protection of bananas by derivatives of 1-cyclopropenylmethylamine compounds applied as a gas.[70] 31

Table 2.1 Effect of the selected natural terpenes on abscission of verity of wax flowers, the concentration of the chemicals was 1 μM..... 49

Table 2.2 Effect of the synthesized simple alkenes analogues on abscission of varity of wax flowers 52

Table 3.1 Reaction of 1-HCP with ethanol in presence of catalyst 74

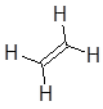
Table 4.1 Reaction of 48 with ethanol in presence of catalyst ……..... 86

List of symbols and abbreviations

ATP	Adenosine triphosphate
CTAB	Cetyltrimethyl ammonium bromide
DDQ	Dichloro-5,6- dicyanobenzoquinone
DMSO	Dimethyl sulfoxide
1-HCP	1-Hexylcyclopropene
1-MCP	1-Methylcyclopropene
PTC	Phase-transfer catalyst
TBAF	Tetrabutylammonium fluoride
TBAI	Tetrabutylammonium iodide
TEBA	Triethyl benzyl ammonium chloride

Chapter 1

Ethylene is a colourless gas with a faint sweetish smell and is the simplest unsaturated hydrocarbon. It has fascinated chemist and biologists for over a century because it affects a myriad of plant developmental processes including fruit ripening.[1] One of the earliest records of human influence in promoting fruit to ripen is found in the Old Testament (800 BC) where the prophet Amos described his profession as a scratcher of sycamore figs. The Greek philosopher Theophrastus in his thesis "Enquiry into Plants, IV" recognized that sycamore figs did not ripen unless they were scratched with an iron claw. The ancient Chinese also found that burning incense ripened pears.[2,3] In the 1860s, Russian farmers used wood smoke to fumigate young cucumber plants. The smoke increased the formation of fruitful female flowers and produced an earlier harvest.[4] At the beginning of the 19th century, when streets were illuminated by coal gas, it was observed that trees surrounding street lamps lost their leaves prematurely compared to other trees.[3,5] All of these historical observations can be traced back to the production and action of ethylene on plants.



Ethylene

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Figure 1.1 Ethylene and the sycamore fig tree.[6]

The biological role of ethylene was first identified by Dimitry Neljubow in 1901. He identified that ethylene is the active constituent in illuminating gas that caused the horizontal growth of etiolated pea seedlings. Early observations on the effect of ethylene on the growth of etiolated pea plants showed an inhibition of elongation, a horizontal growth and an increase in diameter. These three responses are known as the "triple response".[5,7,8,9,10] In 1935, with the advent of gas chromatography, scientists showed the importance of ethylene production as a plant growth regulator hormone.[11] It was found that it was produced in most plant tissues and cell types. Since it is a gas, it can diffuse from sites of synthesis through the interior or the exterior of the plant to influence many aspects of plant growth and development.[12,13,14]

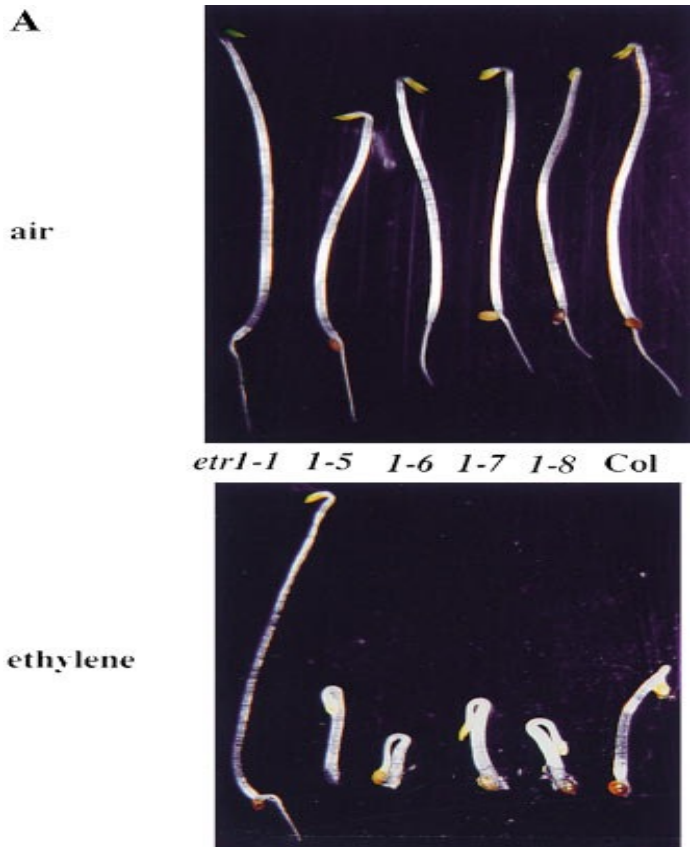


Figure 1.2 The triple response of ethylene on the growth of etiolated pea plants.[15]

Ethylene is a very important compound in agriculture and horticulture and its effects on plants have been studied for more than 100 years.[4,16] Endogenous ethylene production is induced for several reasons: rapid plant growth, such as the growing tips of roots, flowers; various stresses such as wounded or damaged tissue, disease, pathogens, insect predation, insufficient light, drought, flood or stimulus by some hormones (such as auxin, cytokinin and ethylene itself).[17] Ethylene production is regulated by a variety of developmental and environmental factors during the life of the plant. Ethylene has multiple effects on plant growth, development, and physiology at all stages from seed germination to tissue death. Other notable processes that are regulated by ethylene include seed germination, sex determination, flower opening, leaf and flower senescence and abscission, root hair development, cell-fate determination in the root outer skin, programmed cell death and response to stress factors such as drought, flooding, physical wounding, chilling injury, pathogen infection, and chemical inducers.[18,19]

Ethylene was suggested as a fruit ripening hormone when Denny in 1924 found that the smoke from combustion of kerosene was used to de-green citrus fruits contained ethylene as the active component.[3] Ethylene produced by ripe apples was proved chemically by Gane in 1934, when he analyzed the gases released by 60 lbs of ripening apples.[5] Since that time it has been clearly shown that ethylene is produced from most parts of higher plants including seedlings, roots, tubers, stems, leaves, flowers, and fruits.[4,8] The best known effect of ethylene is the promotion of ripening of climacteric fruit. Fruits can be classified as climacteric or non-climacteric depending on the patterns of ethylene production during maturation.[20] Non-climacteric fruits are those that do not depend on exogenous ethylene during maturation and do not ripen significantly after harvest, some examples are strawberry, cherry and pineapple. Climacteric fruits are fruits that ripen after harvest, such as tomato, apple, melon, pear, peach, banana, avocado and kiwifruit. Once separated from the plant, climacteric fruits pass through different sequential stages: the unripe stage, which is characterized by a low rate of ethylene production, followed by a rapid increase in production of ethylene during of the ripening process, and finally the ethylene production rapidly drops to low values when the fruits are ripe and ready to eat.[4,8,21,22]

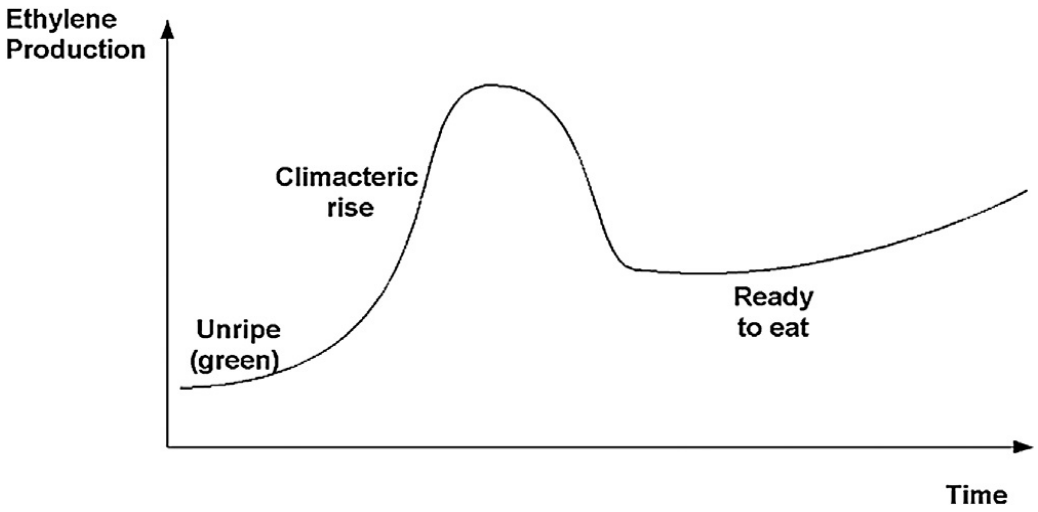


Figure 1.3 Schematic illustration of ethylene production in climacteric fruits as a function of time after harvest.[22]

An example of ethylene production in fruit (apples and avocados) is illustrated in Figure 1.4. Cin and his co-workers measured the respiration and ethylene production for '*Golden Delicious*' apples and they found that ethylene production increased steadily from the beginning of the experiment and peaked at 12 days after harvest of the fruit.[23] In the same trend avocado fruit showed a lower level of ethylene production occurs throughout the precimacteric period and the first increase in rate was noted at the beginning of the 6th day, then peaked at 9.5 days after harvest and reached the best soft and edible stage.[24] It is clear from Figure 1.4 that the production of ethylene in climacteric fruit increases steadily to reach the peak of the production of ethylene, and then declines sharply until the fruit becomes ripe and ready to eat.[23]

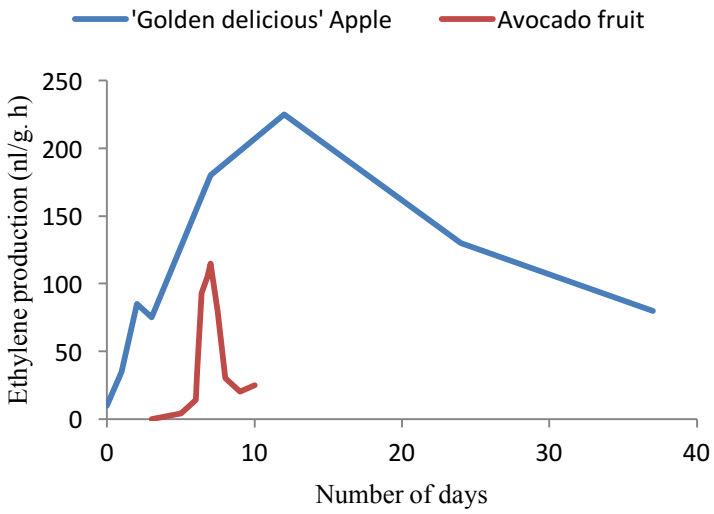


Figure 1.4 Ethylene production after harvest of '*Golden delicious*' apple and avocado fruit.[23,25]

Ethylene is recognized as ripening hormone of climacteric fruits during maturation since the 1920s.[16] It plays a crucial role in climacteric fruits by a range of ripening-associated actions such as colour changes, flavour development, and softening.[26,27] Ethylene initiates the catabolism in which the starch is converted into sugar in the fleshy part of the fruit.[28] It promotes degreening and color development in fruits as well. It is active in trace amounts and can act in extremely low concentration at part-per-million (µL per L) to part-per-billion (nL per L) of air. However, ethylene is not always beneficial. It can have detrimental effects as it accelerates senescence, enhances excessive softening of fruits, over ripens fruits, stimulates chlorophyll loss (e.g., yellowing of green vegetables), stimulates sprouting of potatoes, promotes discoloration (e.g., browning), develops russet spotting in lettuce and promotes abscission of leaves and flowers.[13,21] These effects are commercially important, particularly in the loss of fruit quality due to fruit softening.[13,27,28] The action of ethylene enhances the texture, colour and aroma of a ripe fruit making it more attractive for humans to eat. The unwanted action of ethylene accelerates the deterioration of these characteristics and makes fruit inedible and lead to commercial losses.

1.1 Postharvest losses of fruits and vegetable

According to a recent survey conducted by Jobling (Sydney Postharvest Laboratory) on several fruit and vegetables markets and retail stores around Sydney, the concentration of ethylene was between 0.017 - 0.06 ppm in the storage rooms.[21] This concentration is sufficient to cause a physiological effect on fruit.[29] The main source of ethylene is from the exhaust gases of vehicles and forklift trucks. The exposure of fruits and vegetables to ethylene during transportation and marketing storage rooms leads to loss of about 10 to 30% of fresh commodities. Postharvest losses in developing countries is a little bit higher than in developed countries, estimated to be between 2 and 23% in developed countries whereas in developing countries it ranges from 5 to 50% when estimated from the point of production to the consumer.[29,30] The main reason for the loss of shelf life is that ethylene exposure increases the rate at which the product deteriorates.

Even though the negative effect of ethylene causes a loss of both agricultural and horticultural products, it could be prevented. Endogenous metabolism is not the only source of ethylene that needs to be considered. It can come from either other plant materials held in the same storage chamber or from exhaust gases emanating from combustion devices operated in the vicinity.[29] A simple way to reduce the damaging effects of ethylene is to minimize ethylene buildup around the horticultural produce, for example electric forklifts rather than fuel powered ones. For ethylene sensitive products, it is important to avoid storing them with products that produce high levels of ethylene such as over ripe or rotting fruit. Ventilation of the storage area can help to reduce the level of ethylene around fresh produce. The effect of ethylene can also be lowered by using a number of chemical processes such as ethylene receptor antagonists and ethylene biosynthesis inhibitors.

1.2 Ethylene biosynthesis and its inhibitors

Endogenous production of ethylene occurs when methionine is converted to ethylene via a series of biochemical transformations known as the Yang cycle (figure1. 5). Firstly, methionine is converted to S-adenosyl-L-methionine (SAM) by addition of adenosine triphosphate (ATP) by action of S-adenosyl-L-methionine synthase. Then intramolecular substitution and cyclization, in presence of 1-aminocyclopropane-1-carboxylic acid synthase (ACC synthase), affords 1-aminocyclopropane-1-carboxylic acid. Finally, oxidation of 1-aminocyclopropane-1-carboxylic acid with oxygen and 1-aminocyclopropane-1-carboxylic acid oxidase (ACC oxidase) forms ethylene. Carbon dioxide and hydrogen cyanide are released as by products.[26,31] The key regulatory steps are the last two steps of ethylene biosynthesis, namely the conversion of S-adenosyl-L-methionine (SAM) to ethylene via 1-amino cyclopropane-1-carboxylic acid which is catalyzed by 1-amino cyclopropane-1-carboxylic acid (ACC) synthase, followed by ACC oxidase.

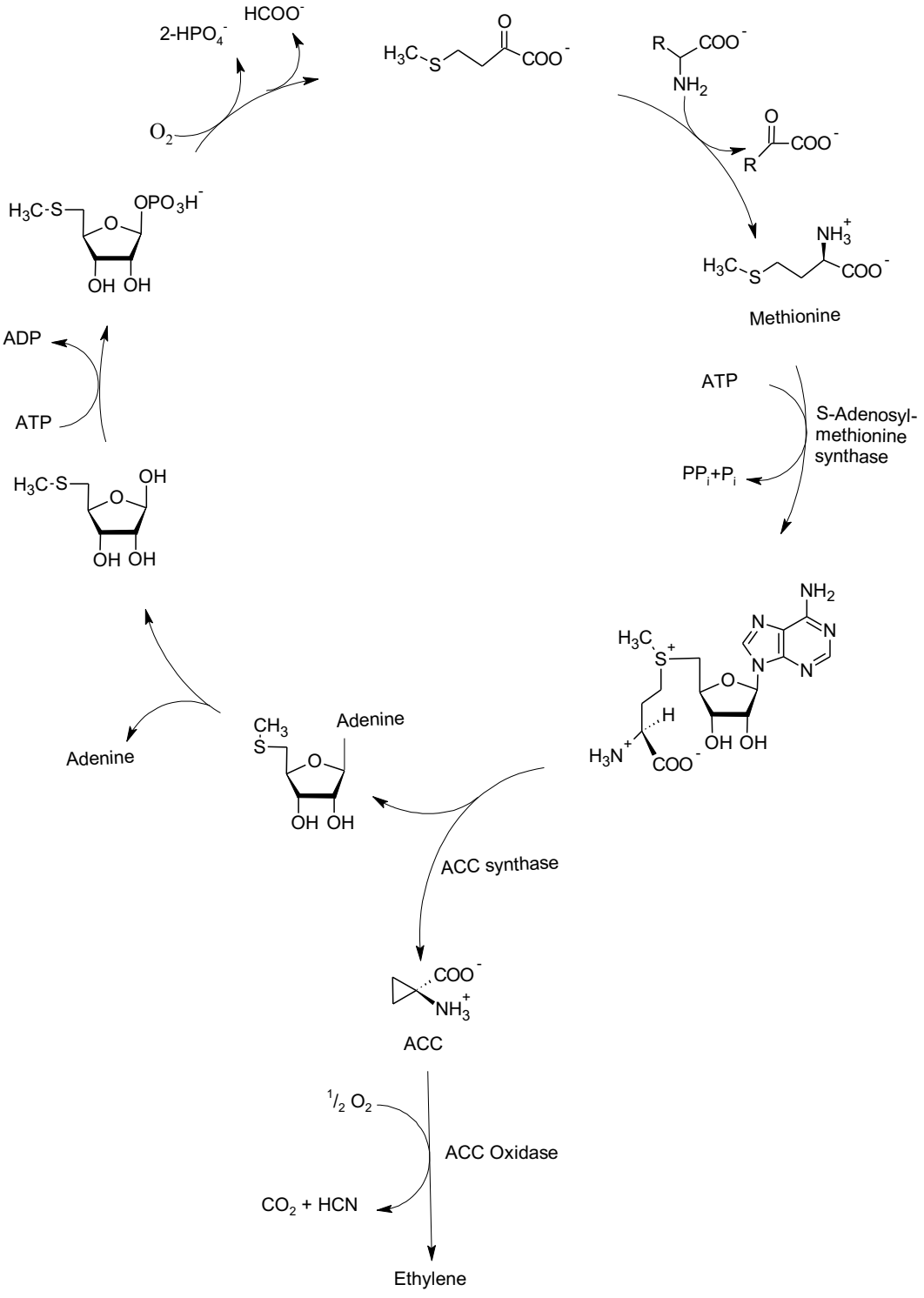
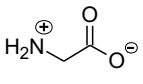
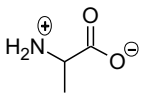
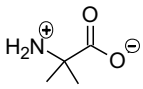
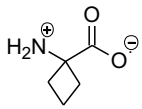
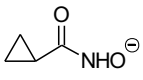
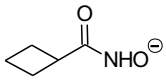
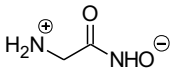
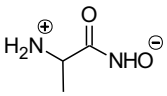
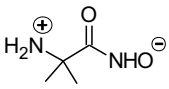
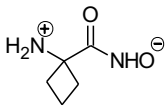


Figure 1.5 Ethylene biosynthesis in higher plant as shown in Yang Cycle.[26]

Environmental conditions can also lower the production of ethylene. Carbon dioxide acts by suppressing ethylene biosynthesis. CO_2 exerts its effect by promoting the conversion of ACC to ethylene, a decrease in CO_2 concentration leads to the inhibition of ethylene synthesis.[22,26] The level of oxygen has an effect on the rate ethylene production rate. ACC-Oxidase requires O_2 as co-substance, so low concentrations of O_2 (1-3%) inhibits the ethylene biosynthesis.[32,33] Some compounds can also affect the last two steps of ethylene biosynthesis such as (S)-*trans*-2-amino-4-(2-aminoethoxy)-3-butenoic acid **5** (AVG, Figure 1.6), *o*-(carboxylmethyl) hydroxyamine, cobalt ion and free radical scavengers.[26,34] Aminoethoxyvinylglycine (AVG), commercially marketed under name ReTain®, is an inhibitor for ACC synthase.[33]

A number of ethylene biosynthesis inhibitors have been developed which act on either ACC synthase or ACC oxidase. Pirrung and his colleagues reported that a series of 1-aminocyclopropane carboxylic acid analogs are inhibitors of ethylene production. They found these analogs are competitive with the (ACC) substrate for ACC synthase.[35] The table below shows inhibition activity of the ACC-hydroxamate analogs. The lower molar concentration means high inhibition activity.

Table 1.1 Activity of ACC synthase inhibitors

			
33 mM	16 mM	1.9 mM	114 μM
			
71 μM		93 μM	
			
97 μM	49 μM	17 μM	0.6 μM

Yusuke Kosugi *et al*., managed to inhibit ACC oxidase activity by using substrate analogs. The analogs tested were 2-aminoisobutyrate (AIB) and its derivatives (Me-Ser and Me-Asp) and cycloalkane-amino acids (ACBC, ACPC and ACHC). The graph in Figure 1.7 shows the suppression of ACC oxidase activity by substrate analogs.[36]

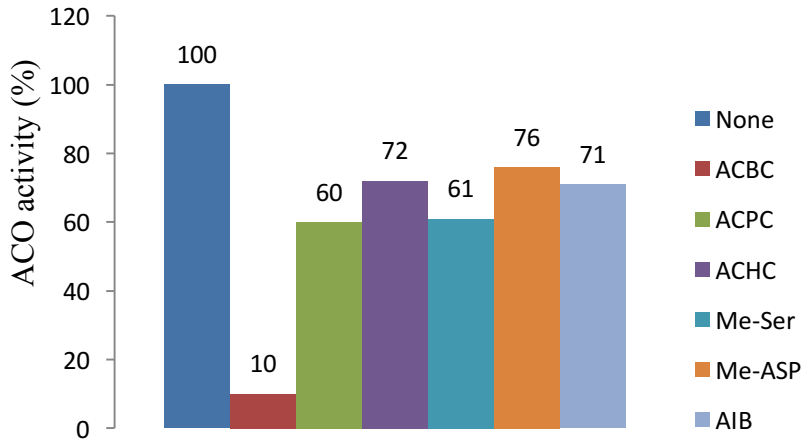


Figure 1.6 Inactivation of ACC oxidase by ACC analogs.[35]

1.3 Ethylene receptor

The ethylene receptor is a membrane bound receptor located in the endoplasmic reticulum of the plant cell. Studies on the ethylene receptor from Arabidopsis and Tomato showed that the ethylene binding domains are present within the *N*-terminal transmembrane domains of the receptor and have an electron-rich, hydrophobic pocket. In 1967, Burg and Burg suggested that a transition metal cofactor could provide the necessary chemistry for high-affinity interaction based on the known ability of olefins to form stable complexes with transition metals.[5] Based on a correlation between the relative ethylene-like activity of a number of compounds and their known order of binding to silver ion, Burg and Burg assumed the presence of a metal in the ethylene receptor.[37] Several transition metal cofactors were initially suggested including cobalt, nickel, copper, iron and zinc, however using ethylene receptor 1 (ETR1) exogenously expressed in yeast, it was shown that copper(I) acts as the co-factor for ethylene binding.[38] Thus the coordination of copper(I) to ethylene in the transmembrane portion of the receptor is critical.

The ethylene receptor consists of a family of membrane embedded proteins that bind ethylene. In Arabidopsis and some other plants, there are five receptors called ethylene receptor 1 and 2 (ETR1) and (ETR2), ethylene response sensor 1 and 2 (ERS1) and (ERS2), and ethylene insensitive 4 (EIN4). They are related to two-

component histidine kinase sensors and bind to ethylene through their *N*-terminal transmembrane domain of the receptors.[39,40] Characterization of the ethylene binding site in ETR1 has shown that it occurs in a hydrophobic pocket located at the *N*-terminus of the receptor and requires a transition metal, copper, as a co-factor.[41] The receptor is negatively regulated by the ethylene. When ethylene is absent, the receptor activates the protein kinase in the ethylene receptor complex (CTR1). CTR1 is composed of an amino-terminal domain and carboxy-terminal kinase domain, the amino-terminal domain of CTR1 can interact with the histidine kinase domains of ETR1. The CTR1 is a part of signaling cascade that regulates a variety of cellular process in case of absence of ethylene. When CTR1 is active, it represses further signaling. When ethylene binds to ETR1 the CTR1 inactivation allows signaling cascade to occur.[1,42,43]

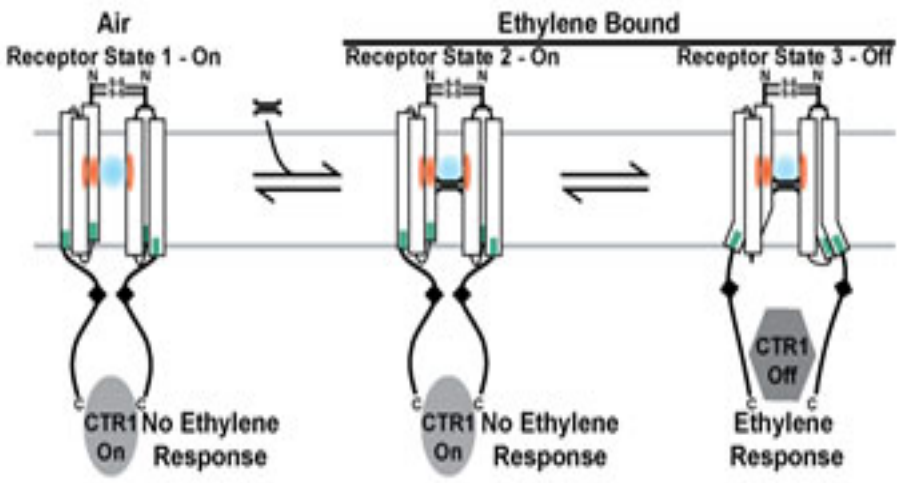
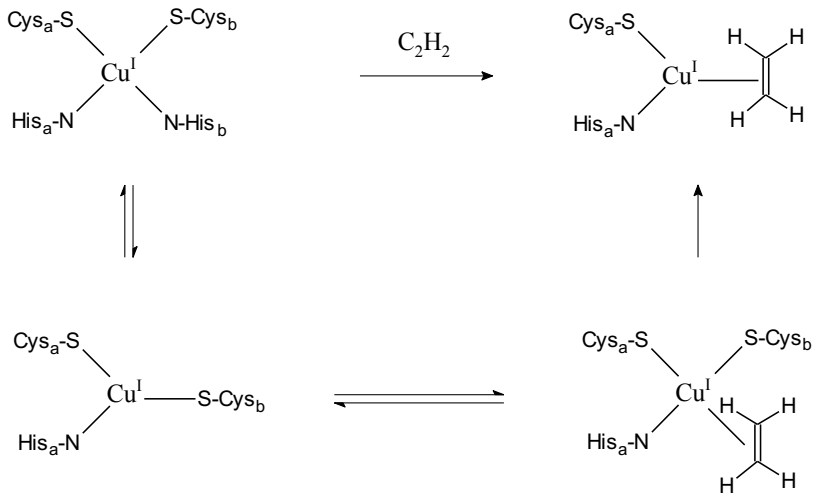


Figure1.7 Receptor signal state mode l. The ethylene binding domain of ETR1 is depicted as a homodimer with each monomer containing three transmembrane helices. The region marked with pink shows the copper binding pocket and region marked in green are areas allowing the receptor to turn off. Adapted from Binder, et al., 2008.[42]

The ethylene binding site is located within an ETR1 homodimer. The ETR1 homodimer is maintained through disulfide-linked cysteine residues (Cys^4 and Cys^6)

located at the *N*-terminal end of the protein and which form a covalent disulfide bond. The disulfide bonds are not required for ethylene signaling.[44] The functional unit of ETR1 is a dimer; each homodimer of the receptor contains three transmembrane segments. Cys[65] and His[69] are thought to coordinate the copper ion within the receptor domains.[45] There is one copper ion so one molecule of ethylene can bind per receptor dimer. The Cu(I) cofactor is required for high affinity ethylene binding in ETR1 receptors.[28] Binding ethylene to the ethylene receptor causes a changes in the coordination chemistry of the Cu(I) cofactor which results in conformational change in the binding site that is transmitted through the receptor dimer to downstream signaling elements.[46,28]

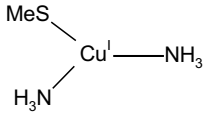


Scheme 1.1 Binding of ethylene to the copper in the ethylene receptor

1.4 Synthetic complexes as models of ethylene receptor

The idea that ethylene binds to a metal ion-based receptor has encouraged many studies by organometallic chemists to mimic the binding site. Ethylene and alkenes can use their π electrons to form metal-olefin bonds, σ donation or π back-donation is dominant in the copper(I) olefin complexes.[47] The coordination chemistry of copper ethylene complexes at the ethylene receptor site has inspired the synthesis of copper

complexes with ligand that can mimic the ethylene receptor. Pirrung and co-workers prepared simple copper complexes as models for the ligand-binding site of the ethylene receptor. These models were used to explain the properties of a variety agonists and antagonists of ethylene action. The thiolate of cysteine was replaced with methane thiolate and ammonia was used in the place of the nitrogen of histidine.



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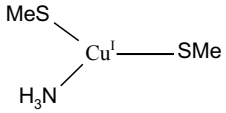

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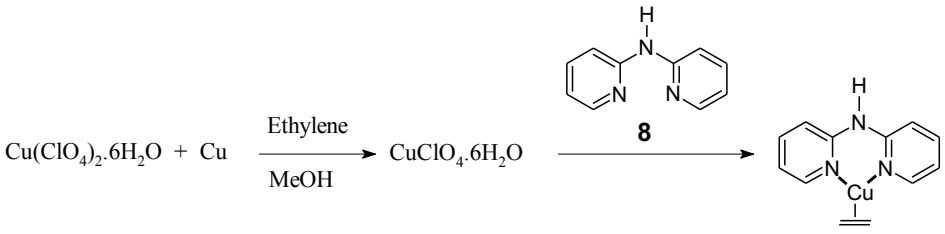
Figure 1.8 Complexes mimic ethylene receptor

A number of simple Cu(I) complexes can bind of ethylene, however most are air sensitive Thompson *et al*., in 1983, prepared and characterized copper-ethylene complex using a bidenate pyridyl ligand.[48,49] Copper(II) perchloride **9** was reduced to copper(I) **10** and reacted with 2,2'-bipyridylamine **8** in the presence of ethylene to give an stable copper complex and can be representative of the ethylene binding site.



9 **10** **11**

Scheme 1.2 An stable copper(I) ethylene complex

A number of copper(I) complexes with neutral tridentate pyrazole ligands have been prepared. In 1992, Perez and his co-workers synthesised a stable ethylene copper complex tris(3,5-dimethylpyrazol-1-yl)(hydro)borato-N_2,N_2`N_2``)copper **12**.[50] Also

copper(I) complexes of the all carbon analogue containing tris(pyrazol-1-yl)methane and tris(3,5-dimethylpyrazol-1-yl)methane ligand reported by Hsu et al. and have the same properties.[51]

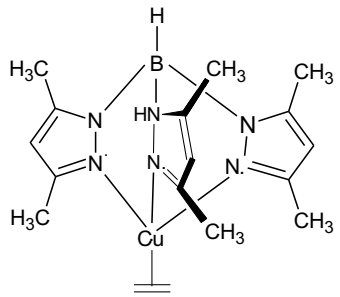


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Figure 1.9 Air stable ethylene copper(I) complex with a tridentate ligand Cu(HB(3,5-$Me_2Pz)_3(C_2H_4)$.[50]

1.5 Modulation of the ethylene response

Many organic compounds other than ethylene act on the ethylene receptor to produce a response.[52] Compounds that bind to the ethylene receptor and mimic the ethylene action are called ethylene agonists. Compounds that competitively binding on the ethylene receptor and prevent the action of ethylene are called ethylene antagonists. Currently direct binding assay on the ethylene receptor cannot be performed, so the testing of compounds other than ethylene is conducted using the dose response effect on the ethylene triple response, fruit ripening or flower senescence.

1.5.1 Ethylene receptor agonists

Compounds other than ethylene can cause a triple response in plants. A landmark study by Burg and Burg showed the agonistic effect of a range of simple organic molecules on the ethylene response of pea seedlings. Table 1.2 shows the ethylene-like action of some compounds based on the triple response criteria.[16] The essential structural requirement is the presence of a C-C π-bond because ethylene and

acetylene were active but ethane was not. Double bond is favored over triple bond. The response is selective as common small compounds do not cause the triple response for example H_2S, HCN, H_2O, CO_2 and KN_3. Activity is inversely related to molecular size; smaller molecules tend to be more active than the larger ones. The activity is also related to the electron withdrawing ability of the substituent. For example propylene and vinyl chloride are roughly the same size but vinyl chloride is about 20 times less active than propylene. Vinyl fluoride which is smaller than vinyl chloride is again less active, possibly due to the greater inductive withdrawing effect of the fluoride substituent. Acetylene showed less activity than ethylene even though the size of these two compounds is nearly same (Table 1.2).

Table 1.2 Biological activity of ethylene and other alkenes and alkene-related compounds as determined by the Pea straight growth test.[16,28]

Compound	K'_A relative to ethylene	ppm in gas phase for half-maximum activity
Ethylene	1	0.1
Propylene	130	10
Vinyl chloride	2,370	140
Vinyl fluoride	7,100	430
Vinyl bromide	220,000	1600
1-Butene	140,000	27,000

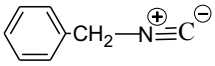
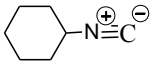
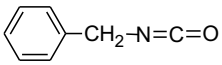
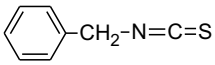
Table 1.3 Biological activity of ethylene and other alkenes and alkene-related compounds as determined by the pea straight growth test by (Burg and Burg, 1965, 1967).

Compound	K'_A relative to ethylene	ppm in gas phase for half-maximum activity
Ethylene	1	0.1
Acetylene	12,500	280
Allene	14,000	2900
Methylacetylene	45,000	800
Vinyl acetylene	765,000	11,000
Vinyl methyl ether	1,175,000	10,000
Butadiene	1,200,000	500,000
1,1-Difluoroethylene	2,060,000	350,000
Vinyl ethyl ether	5,440,000	30,000

In 1977, Sisler added isocyanides to the list of ethylene agonists. Some aliphatic and aromatic isocyanides mimic ethylene action and elicited an ethylene-like response. Pea seedling elongation was effected by isocyanides, isocyanates and isothiocyanates under different concentrations which is exhibit the triple response of shortening, bending and thickening of their epicotyls when exposed to isocyanides. Twenty three day old pea seedlings, having an average length of epicotyls prior to incubation 31±7 mm, were incubated for 3 days and the epicotyl length was measured and compared with ethylene affection on the same seedling.[53] Ethylene caused a half-maximal inhibition at a concentration about 0.3 µL/L, whereas the half-maximal inhibition of growth concentration of benzyl isocyanide and cyclohexyl isocyanide was little bit more than the concentration of ethylene and was estimated to be approximately 10 to 15 µL/L. The isocyanates and isothiocyanates also inhibited elongation of the pea seedling at certain concentration but did not show the other triple response such as bending or thickening of epicotyls. The isocyanates and isothiocyanates at high concentrations were toxic to the seedling as observed by discoloration and wilted appearance of the seedling and finally death. Table 1.4 shows the effects of isocyanides and related compounds on pea seedling elongation. The discoloration and wilted appearance of the pea seedlings when treated with higher concentrations

of isocyanates and isothiocyanates indicate these compounds have toxicity and the inhibition of elongation caused by these compounds is likely due to their toxicity.[53]

Table 1.4 Effects of isocyanides and related compounds on pea seedling elongation.[53]

Compound	Concentration μL/L	Increase in Epicotyl Length mm
Ethylene $H_2C=CH_2$	0 0.3 1.0 3.0	76 ± 4 36 ± 2 20 ± 1 8 ± 1
Benzyl isocyanide 	0 5 10 25 40 100	77 ± 4 48 ± 2 36 ± 3 31 ± 2 15 ± 1 8 ± 1
Cyclohexyl isocyanide 	0 10 100	74 ± 5 38 ± 1 4 ± 1
Benzyl isocyanate 	0 100 250 500	76 ± 4 57 ± 3 22 ± 2 Dead
Benzylisothiocyanate 	0 10 20 30	76 ± 2 29 ± 3 17 ± 2 Dead
Methyl isocyanate $H_3C-N=C=O$	0 100 400 600	81 ± 1 29 ± 3 17 ± 2 Dead
Methylisothiocyanate $H_3C-N=C=S$	0 10 20 40	85 ± 4 54 ± 3 60 ± 4 Dead

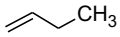
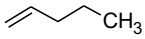
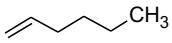
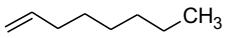
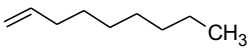
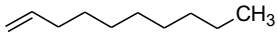
1.5.2 Ethylene receptor antagonists

Ethylene responses in plants can be prevented to some extent by a number of compounds which are inhibitors of ethylene action and can counteract ethylene action for several days.[31,54] Ethylene antagonists serve as competitive inhibitors of ethylene by occupying the binding sites without eliciting ethylene activity.[8] These antagonists compete with ethylene for binding to the ethylene receptor and much of the ethylene diffuses rapidly from the receptor, whereas inhibitory compounds bind for long periods. While they are bound, ethylene cannot bind.[37]

1.5.3 Role of hydrophobic chain

The presence of a double bond in the compound to provide π electrons to the metal in the ethylene receptor site on the plant is the most important factor to have response with the ethylene receptor.[55] Ethylene is the smallest alkene and has high potency to bind to the ethylene receptor. Not only ethylene but many other olefinic compounds interact with the binding site of the ethylene receptor and compete with ethylene on the ethylene receptor. 1-Alkenes change from being active ethylene agonists to ethylene antagonists as the chain length is extended.[56] Propylene and 1-butene have the same agonistic response as ethylene, but at higher concentrations in the ratio ethylene: propene: butene 1: 130: 140, 000.[28] 1-Butene being a pivotal compound active as both an agonist and as an antagonist, 1-butene and longer alkenes are competitive with ethylene for the receptor but are antagonists, inhibition increase as the length of alkenes increase. As the chain length is extended, the extra hydrophobic interaction make the 1-alkenes more potent.[56,57] This suggests the presence of an antagonistic hydrophobic binding site near the copper center.[58]

Table 1.5 Effect of ethylene and 1-alkenes on etiolated pea growth

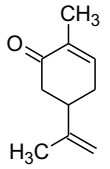
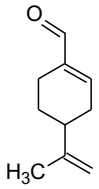
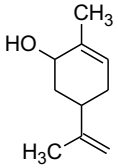
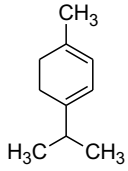
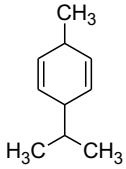
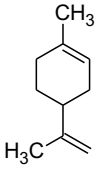
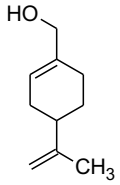
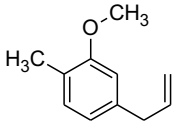
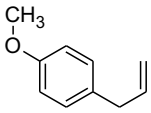
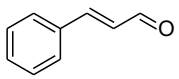
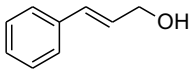
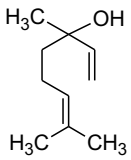
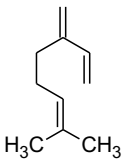
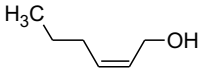
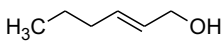
Compound	Structure	Activity K_m(µL L^{-1})	Inhibation K_i(µL L^{-1})
Ethylene	$H_2C{=}CH_2$	0.1 ± 0.02	=
Propene		10^3 ± 0.5	=
1-Butene		27000 ± 500	3300 ± 100
1-Pentene		Inactive	2300 ± 80
1-Hexene		Inactive	1100 ± 50
1-Octene		Inactive	400 ± 35
1-Decene		Inactive	300 ± 5
1-Dodcene		Inactive	246 ± 10

1.5.4 Natural occurring ethylene antagonists

There are many ethylene antagonists naturally produced by the plant itself such as terpenes which compete with ethylene for the ethylene receptor, even though these compounds are relatively large molecules they are easily accessible to the ethylene receptor-binding domain.[59] The presence of oxygen atoms in these compounds showed increasing activity, in particular when an oxygen atom was present in the allylic position. The monoterpene “linalool” which contains an oxygen atom showed significantly more activity than myrcene. This observation could be due to the oxygen atom acting as a hydrogen bond acceptor causing the molecule to bind tightly to the copper center or could be due to extra hydrogen bonding interactions within the binding site. Many natural compounds exhibit allelopathic inhibition of seed germination and growth of competing plants.[54,60] All terpenes tested that exhibit ethylene antagonist required continuous exposure.[54,58] Compounds that contain aldehyde, ketone, or methoxy group showed higher activity than these that have

hydroxyl groups. This is clear when comparing (+)carvone and carveol that have the same structure by differ in the oxidation states of the oxygen substituents, also *trans*-cinnamaldehyde and cinnamylalcohol have the same situation of the structure and activity. The first group of compounds in Table 1.5 are cyclic monoterpenes. The number and position of the double bond have little impact on the activity. The last two compounds (*cis* and *trans*-2-hexene-1-ol) exhibit a small difference activity perhaps due to the steric hindrance, the *cis* isomer being more accessible than the *trans* isomer.

Table 1.6 Naturally ethylene antagonist.[54,58] The numbers that under the molecules refer to the activity K_i (μL L^{-1})

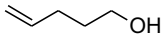
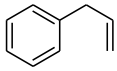
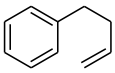
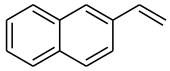
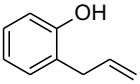
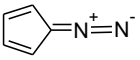
(+)Carvone  103	Perillaldehde  167	Carveol  337	α-Terpinene  400
γ-Terpinene  400	Limonene  588	Perillalcohol  693	
Engenol  101	Estragole  103	*trans*-Cinnamaldehyde  165	Cinnamyl alcohol  821
Monoterpenes (Linalool)  103	Myrcene  1333		
cis-2-Hexene-1-ol  175	*trans*-2-Hexene-1-ol  195		

1.5.5 Synthesized ethylene antagonists

Many synthesized ethylene antagonists have exhibited significant potency when applied and some of them have been successfully used by the agronomy industry to block ethylene responses.[42] Table 1.7 shows some of the synthesized antagonist known to date. Presence of the double bond at the end of the carbon chain of the first group of compounds plays the main role of the activity of the molecules as ethylene antagonist.[8] The activity is affected by other sections of the molecule possibly by steric hindrance.

Diazocyclopentadiene (DACP) also showed high activity as an ethylene antagonist whereas cyclopentadiene and cyclopentene do not. The activity is due to generation of carbene, diazo-compounds have a high specific reactive photo-affinity. According to the authors when diazocyclopentadiene is exposed to fluorescent light, nitrogen gas is extracted from the molecule generating a carbene. The carbene reacts immediately with the copper cofactor in the ethylene receptor to form a more stable complex and subsequently leads to block the ethylene responses.[61] Treatment a number of plants with DACP in presence of fluorescent light extended the period of inhibition of ethylene response.[56] The result in this table showed that *trans*-cyclooctene has higher activity than *cis*-cyclooctene that is because *trans*-isomer is a highly strained compound compared to the *cis*-isomer.[62] The activation energy of *trans*-cyclooctene is 74 KJ/mol, which is about 38 KJ/mol lower than the *cis*-cyclooctene. The strain energy increases the metal-binding potential of *trans*-cyclooctene as an ethylene antagonist.[63]

Table 1.7 Inhibition of ethylene action in plants by synthetic ethylene antagonists.[8,54]

Compound name	Compond Structure	Plant	K_i(μL L^{-1})
4-Pentene-1-ol		Banana	110
Allylbenzene		Banana	189
4-Phenyl-1-butene		Banana	206
2-Vinylnaphthalene		Banana	490
2-Allylphenol		Banana	995
Diazocyclopentadiene		Carnation	0.12
Cyclopentadiene		Banana	140
Cyclopentene		Pea	1,100
trans-Cyclooctene		Banana	0.78
cis-Cycloocene		Banana	512

A variety of six-membered carbocyclic compounds have activity as ethylene antagonists. The location and strain of the double bond is important. 2,5-Norbornadiene and norbornene have strained alkenes and this makes them more active. 2,5-Norbornadiene is more strained and is homoantiaromatic and its activity is twice that of norbornene.[8] Cyclohexane which has no double bonds showed no activity at all. Benzene is aromatic and this also leads to inactivity as ethylene antagonist (Table 1.8).

Table 1.8 Inhibition of ethylene action in plants by synthetic six-membered cyclic compounds ethylene antagonists

Compound name	Compound structure	Plant	K_i(μLL^{-1})
2,5-Norbornadiene		Pea & Banana	170 & 55
Norborene		Pea	360
1,3-Cyclohexadiene		Pea	488
1,4-Cyclohexadiene		Pea	4,650
Cyclohexene		Pea	6,060
Cyclohexane		Pea	Inactive
Benzene		Pea	Inactive

1.6 1-Methylcyclopropene (1-MCP)

There are four biologically active compounds that have been used extensively in scientific investigations: 2,5-norbornadiene, *trans*-cyclooctene, diazocyclopentadiene and 1-methylcyclopropene.[62] The most stable of these compounds was 2,5-norbornadiene. It is a liquid with a low boiling point that vaporizes easily at room temperature; this makes it easy to treat plant material in airtight chambers. The efficiency of 2,5-norbornadiene in preventing ethylene action was tested on carnation

flowers and some other different plant materials.[42] Both 2,5-norbomadiene and *trans*-cyclooctadiene, require continuous exposure, a high concentration, and have a very pungent and obnoxious odor and are toxic, therefore there have been no attempts to use them commercially.[37,61,64] Diazocyclopentadiene is a very effective antagonist but it has not been used because of its instability and its explosive nature at high concentrations.[58]

13 2,5-Norbornadiene

14 *trans*-Cyclooctene

15 Diazocyclopentadiene

16 1-Methylcyclopropene

Figure 1.10 Active ethylene antagonist compounds

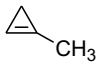
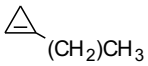
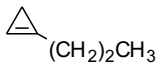
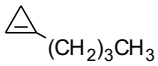
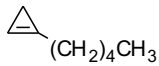
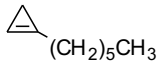
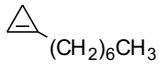
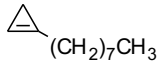
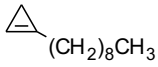
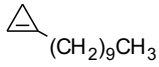
1-Methylcyclopropene (1-MCP) is the only ethylene receptor antagonist used commercially. It is a powerful antagonist of ethylene receptor and has been recently proposed to prolong shelf-life and delay ripening in the postharvest phase.[25,37,65] The action of 1-MCP is thought to inhibit ethylene action by interacting with the receptor and out competing with ethylene for binding sites.[66] Compared with ethylene, 1-MCP is active at much lower concentrations. The potency of 1-MCP for the ethylene receptor is about 10 times greater than that of ethylene.[67] 1-MCP is non-toxic and it leaves no detectable residues on the fruit and vegetables. It protects carnation and banana by a 24-h exposure at 0.5 nLL^{-1}.[37] 1-MCP is commercially available under the names EthylBloc® and SmartFresh™. The gaseous compound was developed as a powder complex 1-MCP with γ-cyclodextrine, so that 1-MCP is easily released when the powder is dissolved in water. 1-MCP is safe for humans, animals and the environment and in 1999 was approved by the Environmental Protection Agency (EPA) for use on ornamentals under the trade name of EthylBloc®. Subsequently it was developed and used for edible horticultural products like apples, avocados, persimmons, tomatoes, papaya, plums and several other crops and marketed as SmartFresh™. The apple was the first fruit harvest that received registration for 1-

MCP use, and 1-MCP use has been incorporated rapidly by food and agriculture industries around the world for use on various fruit and vegetables.[61,66,67]

1.7 1-Substituted cyclopropenes

A series of 1-alkyl substituted cyclopropenes has been tested as ethylene antagonists on banana fruit. The next table shows the minimum concentration of cyclopropenes and time of protection of banana fruits. Cyclopropene is very active but unstable. 1-Methylcyclopropene has the same activity as cyclopropene but is slightly more stable than cyclopropene. The minimum concentration of cyclopropene and 1-methylcyclopropene required for protecting bananas from ethylene and delay chlorophyll degradation in banana fruit peel for 12 days is 0.7 nLL^{-1} after a single 24 h exposure. As the aliphatic chain is extended with two and three carbons 1-ethyl- and 1-propylcyclopropene, the minimum concentration required to protect banana for same time is higher. Starting from 1-butylcyclopropene the minimum concentration required declines and the protection days increased to reach 0.3±0.01 nLL^{-1} and 36 days when 1-decyclopropene was used.[68] This concentration is even lower than 1-methylcyclopropene and the protection was three fold.

Table 1.9 The minimum concentration of difference cyclopropenes and time of protection of banana fruits against ethylene.[68]

Compound	Abreviation	Structure	Concentration (nLL^{-1})	Time (days)
Cyclopropene	CP		0.7 ± 0.05	12
1-Methylcyclopropene	1-MCP		0.7 ± 0.05	12
1-Ethylcycloprpene	1-ECP		4.0 ± 0.4	12
1-Propylcyclopropene	1-PCP		6.0 ± 0.3	12
1-Butylcyclopropene	1-BCP		3.0 ± 0.1	12
1-Pentylcyclopropene	1-PentCP		0.5 ± 0.01	14
1-Hexylcyclopropene	1-HCP		0.4 ± 0.01	20
1-Heptylcyclopropene	1-HeptCP		0.4 ± 0.01	21
1-Octylcyclopropene	1-OCP		0.3 ± 0.01	25
1-Nonylcyclopropene	1-NCP		0.4 ± 0.02	35
1-Decylcyclopropene	1-DCP		0.3 ± 0.01	36

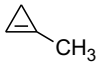
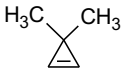
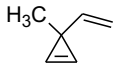
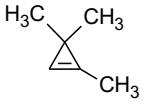
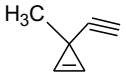
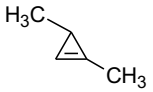
1.8 Differently substituted Cyclopropenes

Cyclopropene and 1-alkyl substituted cyclopropenes are more potent than the other substituted cyclopropenes. As the number of methyl group on the cyclopropene increases, the concentration required for inactivating the ethylene receptor increases, this is probably due to the steric effect on the binding of cyclopropene to ethylene receptor.[60] An exposure of 1-methylcyclopropene at (0.7 nLL^{-1}) for 24 h inactivates the ethylene receptor in banana whereas 1,3,3-trimethylcyclopropene needs 20,000

nLL^{-1}. The data in Table 1.10 shows the effect of the structure of the molecules on the concentration and the time insensitivity on banana fruit.

1-Methylcyclopropene has the same activity as cyclopropene for the protection carnation flowers, banana fruits, and tomato fruits, whereas its isomer 3-methylcyclopropene was shown to be less effective. 3-MCP required two to three times higher concentration than 1-MCP to have the same effect.[69] It is maybe due to the presence of the methyl group in the position 3 or the lack of a methyl group in the position 1 or both. Nevertheless, it does show the biological activity can be dramatically effected by small changes in the structure of molecules.[58] The methyl group adjacent to the double bond leads to increase the activity. As the number of methyl groups on the cyclopropene increases, the concentration required to prevent the ethylene response increases. This is probably due to the steric effects on the binding of cyclopropene to ethylene receptor. For instance 1,3,3-trimethylcyclopropene needs high concentration to inhibit the ethylene action, about 20,000 nLL^{-1}.[60,69] As the number of methyl groups increase on the three position the activity decreased. The minimum concentration of 3,3-dimethylcyclopropene 500 nLL^{-1} to protect banana fruit for only 7 days which is about 1000 times lower than 3-MCP. 1,2-Dimethylcyclopropene needs 3000 nLL^{-1} to protect banana fruit for 3 days only again probably due to the steric hindrance.[60,69]

Table 1.10 Minimum concentration and time of insensitivity for different substituted cyclopropenes on banana fruit.[60]

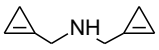
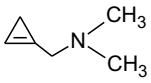
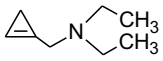
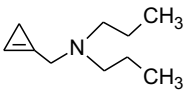
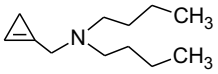
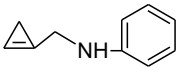
Compound	Structure	Concentration (nLL^{-1})	Time (days)
Cyclopropene		0.7	12
1-Methylcycloprpene		0.7	12
3-Methylcyclopropene		2.0	12
3,3-Dimethylcyclopropene		500	7
3-methyl-3-vinylcyclopropene		120	5
1,3,3-Trimethylcyclopropene		20000	12
3-Methyl-3-ethynulcyclopropene		240	5
1,3-Dimethylcyclopropene		250	12
1,2-Dimethylcyclopropene		3000	3

1.9 Dialkylamine derivatives of 1-methylcyclopropenes

The commercially available ethylene antagonist 1-MCP is volatile compound. It needs air tight rooms to apply and is likely to be ineffective outdoors. Also the time of protection against ethylene action is extended longer when other 1-substituted cyclopropene compounds were used. For instance, 1-decylcyclopropene protected bananas for 36 days at a low concentration but was still volatile. So the efforts had been made to develop ethylene inhibitor compounds that could be applied in open spaces. A salt of a cyclopropene compound could be applied outdoors and persist on the plant for an adequate time to block the ethylene receptor. Some dialkylamine derivatives of cyclopropenes were synthesized and applied on banana with a single

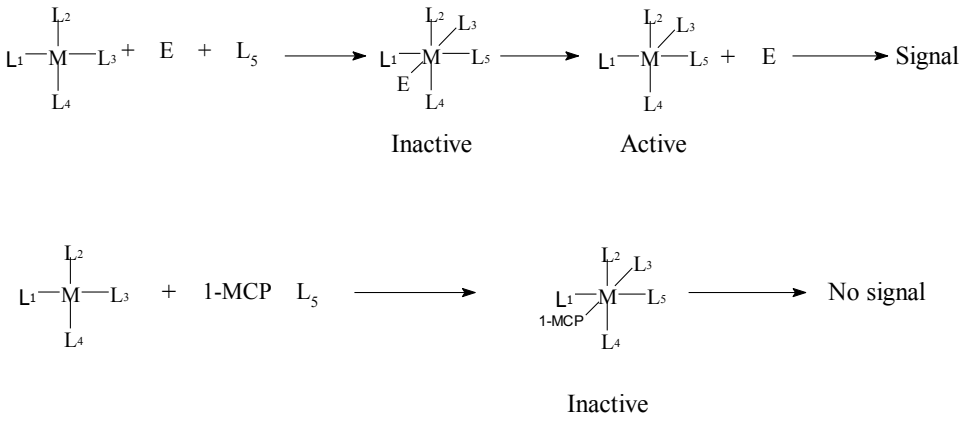
exposure for 24 h and found that they protected banana against ethylene. All bananas remained green for 26-28 days and hard for 32-34 days.[70] Table 1.11 below shows the minimum concentration of *N,N*-dialkyl-(1-cyclopropenylmethyl)amine when applied on banana as a gas and the number of days that protect banana from color degradation and softening.

Table 1.11 Protection of bananas by derivatives of 1-cyclopropenylmethylamine compounds applied as a gas.[70]

Structure and name of compound	Activity K_m($\mu L\ L^{-1}$)	Protection time (days)
 *N,N*-Dicyclopropenylmethylamine	5.7 ± 0.3	33 ± 1
 *N,N*-Dimethyl_1-cyclopropenylmethyl)amine	73 ± 3	34 ± 1
 *N,N*-Diethyl(1-cyclopropenylmethyl)amine	59 ± 2	32 ± 1.5
 *N,N*-Dipropyl(1-cyclopropenylmethyl)amine	30 ± 2	33 ± 2
 *N,N*-Dibutyl(1-cyclopropenylmethyl)amine	184 ± 7	33 ± 1
 *N*-(1-methylcyclopropene)aniline	248 ± 10	33 ± 2

1.10 Proposed Action of 1-MCP and derivatives on the ethylene receptor

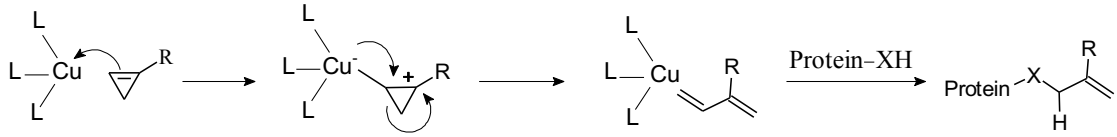
There are two proposed modes of action of cyclopropenes as ethylene antagonists. Sisler and Serek (1997) have proposed a model of mode of action for ethylene and 1-MCP where they have shown the changes occur in the receptor configuration through the withdrawal of electrons and substitution. Scheme 1.4 below, shows the mechanism of binding of 1-MCP to the ethylene receptor. Ligands (L_1-L_5) of unknown structure surround the metal centre present on the receptor where the ethylene or 1-MCP binds to it. In case of ethylene, ligand substitution occurs and ethylene is expelled causing some conformational change leading to an active receptor complex. Whereas with 1-MCP, it is too tightly bound to be expelled and subsequently an active receptor complex is not formed. This model can account for why ethylene is active and why 1-MCP blocks ethylene responses.[37]



Scheme1.3 Proposed model for action of ethylene and 1-methylcyclopropene adapted from the model proposed by Sisler and Serek (1997).[34]

Pirrung and co-workers (2008) have challenged the previous model. They proposed that ring-opening of the cyclopropene ring is responsible for the potency. According to their proposal, the copper cofactor in the ethylene receptor catalyses a reaction. 1-MCP binds to the copper metal through the double bond. Back bonding from the copper metal promotes a ring opening reaction and forms a copper carbenoid

intermediate. This intermediate then reacts irreversibly to make covalent bonds with adjacent amino acids of protein domain within the receptor site and this would inactivate and damage the ethylene receptor and thereby block the ethylene action.[28,63,71]



17 **18** **19**

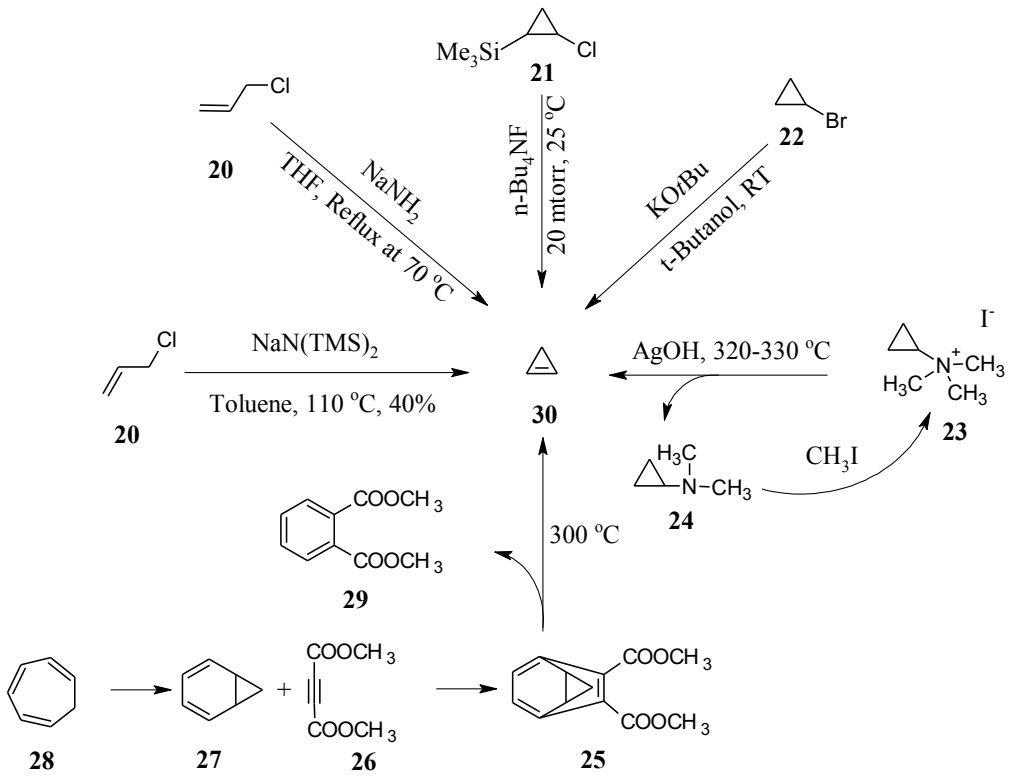
Scheme 1.4 The mechanism of binding of 1-MCP to the ethylene receptor and the ring opining reaction.[63]

1.11 Synthesis of cyclopropenes

Cyclopropenes have been shown as excellent ethylene receptor antagonists. They are a unique class of highly strained carbocyclic compounds. The strain leads to challenging synthesis and unusual reactivity. The synthesis and chemistry of cyclopropenes has been reviewed extensively and only key reactions will be highlighted.[72,73]

Several ways have been created to synthesise cyclopropene using either pyrolysis or strong bases to promote the elimination reaction. The first report of the synthesis of cyclopropene was by Demyanov and Dobrenko, who obtained the cyclopropene by the pyrolysis of trimethylcyclopropylammonium hydroxide on platinized clay at approximately 300 ^{o}C. To prevent oxidation and polymerization of the synthesized cyclopropene, the reaction was carried out under carbon dioxide atmosphere and away from direct light.[74] Cyclopropene can be obtained in 10% yield when allyl chloride is added dropwise to a suspension of sodium amide[75] or to a solution of sodium bis(trimethylsilyl)amide.[37,76] Cyclopropene can be synthesized from bromocyclopropane by elimination of HBr when treated with potassium *t*-butoxide.[77] Cyclopropene was obtained in low yield 1% as byproduct during reaction of cycloheptatriene with dimethyl acetylenedicarboxylate.[74] Cyclopropene was cleaved

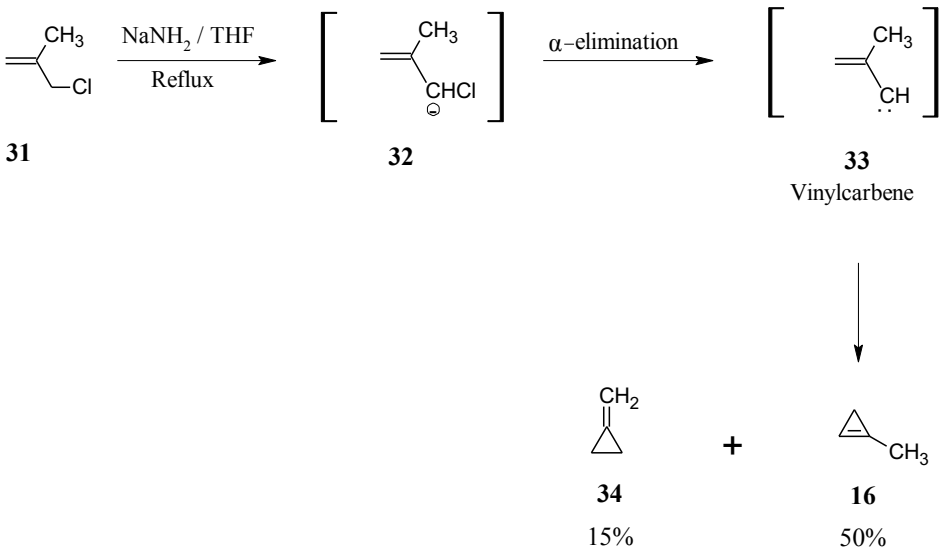
from the initial adducts by a retro Diels-Alder reaction. Cyclopropene has also been synthesized by elimination of β-halocyclopropylsilane using tetrabutylammonium fluoride in (65% to 75%) yield.[78]



Scheme 1.5 Different ways to synthesis of cyclopropene

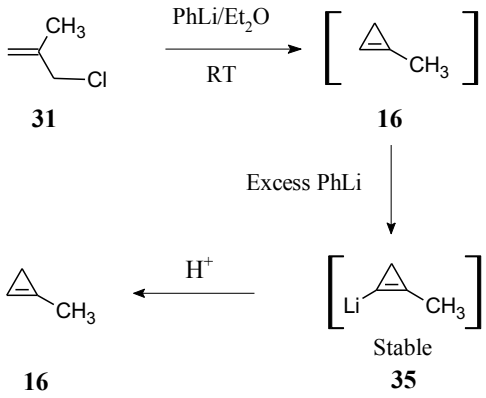
1.11.1 Synthesis of 1-methylcyclopropene 1-MCP

1-Methylcyclopropene (1-MCP) was prepared from β-methylallyl chloride and a strong base (sodium amide or phenyllithium) in one step. The proposed mechanism for this reaction is the strong base induces α-elimination of the allylic chloride to give a vinylcarbene intermediate which then cyclizes to the cyclopropene.[79]



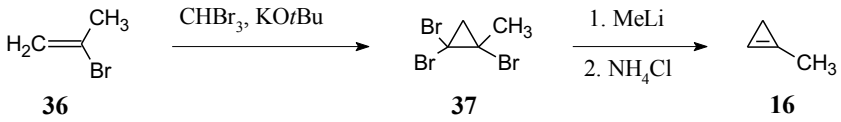
Scheme 1.6 Synthesis of 1-MCP

Due to the instability of 1-MCP and difficulty of storage it is necessary to perform the reaction each time a fresh sample is needed. Magid and co-workers in 1971 reported a new method using excess PhLi as strong base to get lithiocyclopropene which is stable in solution at -20 ^{o}C for at least three months. Subsequently large quantities can be prepared and stored, aliquots of which can then be neutralized to obtain a fresh 1-MCP desired quantity.[80]



Scheme 1.7 Synthesis of 1-MCP

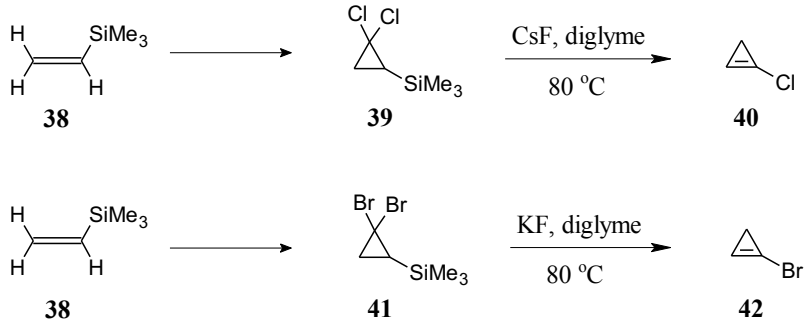
A general strategy applied to access 1-alkylcyclopropenes was based on treatment of alkenes with dibromocarbene to give the 1-substituted cyclopropene.[63] Treating 2-bromopropene **36** with dibromocarbene made from bromoform and potassium *t*-butoxide gave 1,1,2-tribromo-2-cyclopropanes **37**. Debromination and lithium-halogen exchange with an organolithium the lithiocyclopropene by metal-halogen exchange. Then quenching of this organometallic species with ammonium chloride solution gave the 1-methylcyclopropene **16**.



36 **37** **16**

Scheme 1.8 Synthesis of 1-alkylcyclopropene

Chan and Massuda, reported that 1-halocyclopropenes can be prepared readily by the fluoride-induced elimination of β-dihalocyclopropylsilanes.[81] *β*-substituted silicon compound do not eliminate easily. Chan and Massuda found that the elimination can be promoted by fluoride ion under mild conditions. When they heated a mixture of **39** and cesium fluoride in diglyme at 80 ^{o}C, the volatile product **40** was collected in a cold trap (-78 ^{o}C). A similar reaction of **41** with potassium fluoride in diglyme showed the formation of 1-bromocyclopropene **42**.



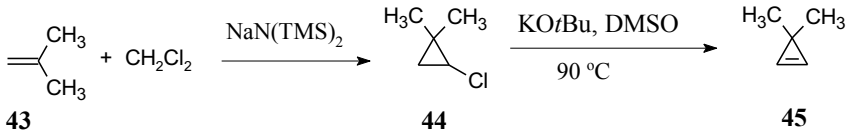
38 **39** **40**

38 **41** **42**

Scheme 1.9 Synthesis of cyclopropene by fluoride-induced elimination

1.11.2 Synthesis of 3,3-Disubstituted cyclopropene

3,3-Dimethylcyclopropene **45** can simply be obtained in a two steps synthesis with high yield (80%) by treating of 1,1-dimethylethylene **43** with dichloromethane in presence of sodium bis(trimethylsilyl)amide to obtained 1,1-dimethyl-2-chlorocyclopropane **44.** The elimination of hydrogen chloride was achieved with potassium *t*-butoxide in dimethyl sulfoxide at 90 ^{o}C.[76] The product mixture was collected in the trap at -78 ^{o}C, and then distillation of the product collected in the trap afforded the pure product.

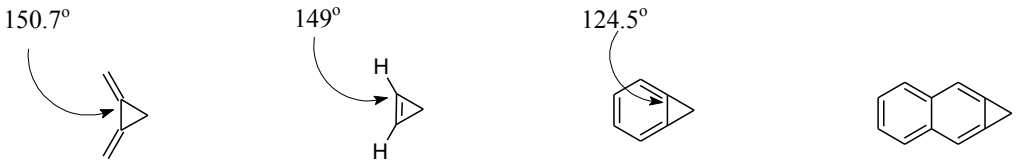


43 **44** **45**

Scheme 1.10 Synthesis of 3,3-dimethylcyclopropene

1.12 Cyclopropparenes

The cyclopropparenes are related to cyclopropenes. They are exemplified by the parent compound benzocyclopropene **47** where a benzene ring is fused onto a cyclopropene. The fusion of a benzene ring to a cyclopropene leads to greater geometric strain than either cyclopropene **30** or 1,2-dimethylenecyclopropane **46**. The strain energy of benzocyclopropene has been determined experimentally from silver-ion catalyzed methanolysis and found to be approximately 68 kcal mol^{-1}. This value is higher than the strain energy of cyclopropene (52.6 kcal mol^{-1}). A similar method has been applied to compute the strain energy for 1*H*-cyclopropa[*b*]naphthalene **48** and was found to be 65-67 kcal mol^{-1}.[76] However the strain is offset by the stability of the aromatic ring leading to some interesting reactivity. This family of compounds has been studied by chemists for many years and the chemistry has been reviewed extensively,[82,83,84] but has been forgotten over the past 5 years with no research papers being published.



46 **30** **47** **48**

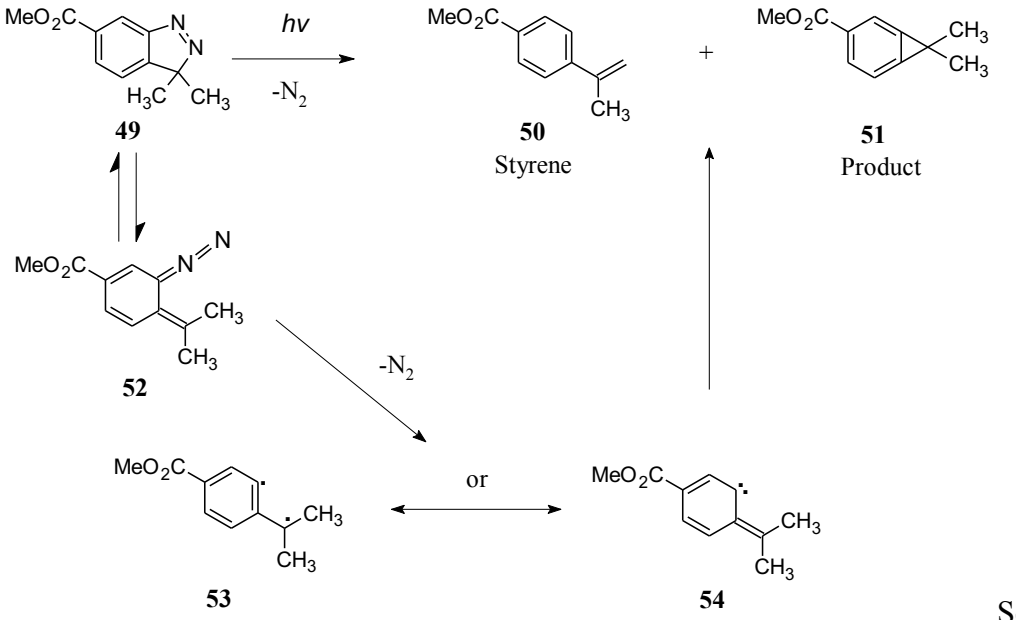
Figure 1.11 The difference in the angle of benzocyclopropene and other cyclopropenes diagrams.

1.12.1 Synthesis of cycloproparenes

Methods to synthesize cycloproparenes are limited due to the highly reactive nature of the cyclopropene ring fusion. The high strain energy of the ring system makes them unstable at modest temperatures and in the presence of electrophiles, transition metals or acids. However, cycloproparenes are stable to strong base and most synthetic procedures to prepare cycloproparenes are conducted under neutral or alkaline conditions. Nevertheless, four main synthetic methods have been developed, formation of the ring skeleton prior to aromatization is most common route.[85]

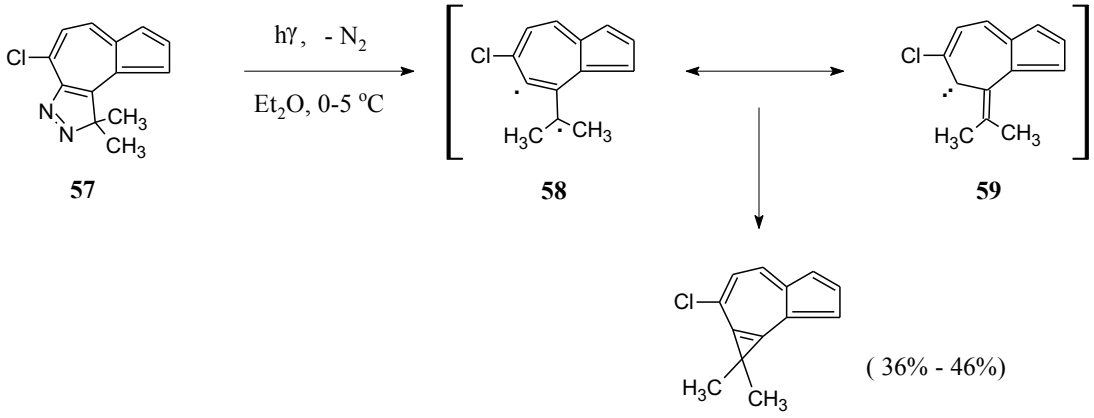
1.12.1.1 Irradiation of 3-*H*-indazoles

The first cyclopropabenzene derivative was described and synthesized by Anet and Anet in 1964.[82] The photochemical decomposition of a 3*H*-pyrazole to eliminate molecular nitrogen, generated a 1,1-disubstituted benzocyclopropene. The 3*H*-Indazole releases N_2 upon irradiation to form the intermediate biradical which then cyclizes to a cyclopropene. This method gave a low yield of benzocyclopropene **51** and the styrene derivative **50** was the major product. Another problem is only *gem*-disubstituted cycloproparenes can be made by this way as mono-substituted indazoles exclusively resides in the 1*H*-tautomer instead of the 3*H*-tautomer essential for this reaction.



Scheme 1.11 Irradiation of 3*H*-indazoles to synthesis of cyclopropabenzene derivative

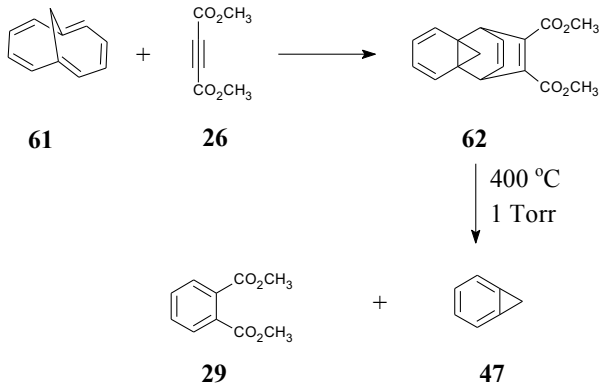
This method was successfully exploited in the synthesis of 2-chloro-1,1-dimethyl-1*H*-cycloprop[*e*]azulene **60**. Irradiation of the azulenopyrazole **57** in ether under nitrogen gave the cyclopropazulene in good yield (36% - 46%) because of the reduced strain of seven membered ring compared to the benzene ring (Scheme 1.12).[86]



Scheme 1.12 Synthesis of 2-chloro-1,1-dimethyl-1*H*-cycloprop[*e*]azulene by irradiation of the azulenopyrazole

1.12.1.2 Alder-Rickert method

The first synthesis of the parent compound benzocyclopropene was reported by Billups *et al*. A Diels-Alder reaction between dimethyl acetylenedicarboxylate and 1,6-methano[10]annulene,[10] gave the adduct **62**. This compound was subjected to flash vacuum pyrolysis to afford benzocyclopropene **47** and dimethyl phthalate **29**. This clever route was the first synthesis of the parent compound.[82,87]



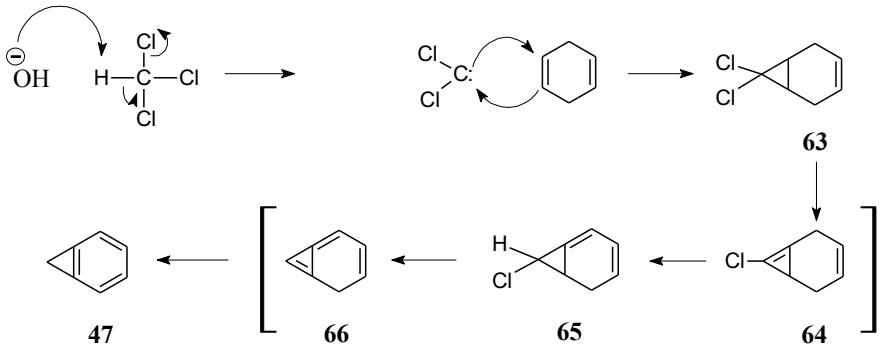
61 **26** **62** **29** **47**

Scheme 1.13 Alder-Rickert synthesis of benzocyclopropene

1.12.1.3 Aromatization of dihalocarbene adducts

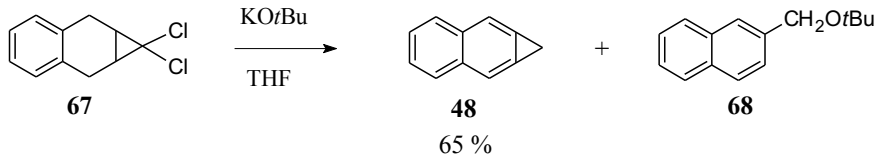
The most convenient method to synthesis benzocyclopropene was described by Billups and co-workers.[88] The reaction sequence starts with the formation of a 7,7-dichlorobicyclo[4.1.0]heptene **63** from addition of dichlorocarbene to an appropriate diene. Then treatment of **63** with potassium *tert*-butoxide afforded benzocyclopropene on large scale in 40% yield. The mechanism of dehydrochlorination proceeds through a sequence of base induced elimination and isomerization reactions,[88] with preservation of the original carbon skeleton.[89] The

dichlorocarbene adduct **63** is made by generating dichlorocarbene (made by the action of sodium hydroxide on chloroform) in the presence of 1,4-cyclohexadiene.

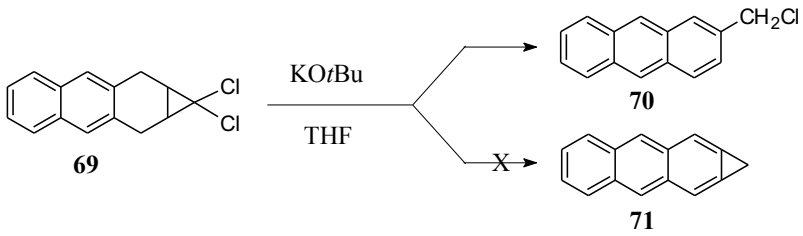


Scheme 1.14 Aromatization of dihalocarbene adducts to synthesis of benzocyclopropene.

1*H*-Cyclopropa[*b*]naphthalene **48** can be prepared by the same route in good yield 65%.[82] A byproduct in this reaction is 2-[(*t*-butoxy)methyl]naphthalene **68**. Disappointingly, the synthesis of 1-*H*-cycloprop[*b*]anthracene **71** by this method was unsuccessful. Treatment of 1,1-dichloro-1a,2,9,9a-tetrahydro-1*H*-cycloprop[*b*] anthracene with potassium *tert*-butoxide did not afford the desire product. The resulting product was mainly 2-chloromethylanthracene. The failure of this route to synthesise cyclopropa[*b*]anthracene has been ascribed to a greater degree of π-bond localization.[82]



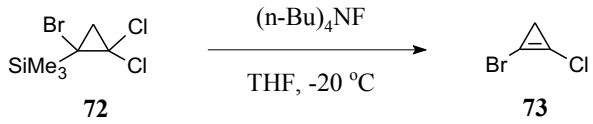
Scheme 1.15 Synthesis of 1*H*-cyclopropa[*b*]naphthalene



Scheme 1.16 Attempt to synthesis of 1*H*-cyclopropene[*b*]anthracene

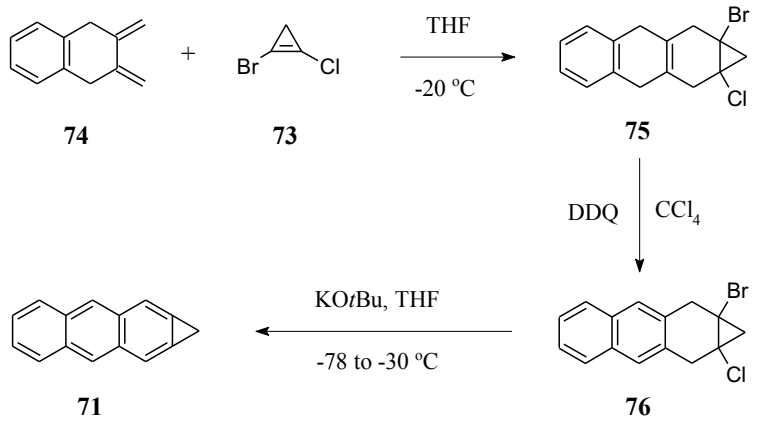
1.12.1.4 Dehydrohalogenation of dihalocyclopropene

A milder procedure to synthesise benzocyclopropene is the use of 1,2-dihalocyclopropene as a synthon. This method provided access to a number cycloproparenes that could not be gained by the previous method. The introduction of 1-bromo-2-chlorocyclopropene **73** represents a major advance in the synthesis of cycloproparenes. It is made by a fluoride induced elimination of cyclopropane **72** at -20 °C to give the dihalocyclopropene **73**.[85,90]



Scheme 1.17 Synthesis of 1-bromo-2-chlorocyclopropene

1*H*-Cyclopropa[*b*]anthracene was synthesized as shown in Scheme 1.18.[86] Cycloaddition of 1-bromo-2-chlorocyclopropene **73** with 1,2,3,4-tetrahydro-2,3-bis(methylene)-naphthalene **74** at -20 °C gave the adduct in good yield (76%). The adduct was then dehydrogenated to the naphthalene derivative **76** using DDQ in CCl_4. The resulting compound **76** was dehydrohalogenated using an excess of potassium *tert*-butoxide in THF at -78 °C to afford final product of 1*H*-cyclopro[*b*]anthracene.[85,90,92]



74 73 75 76 71

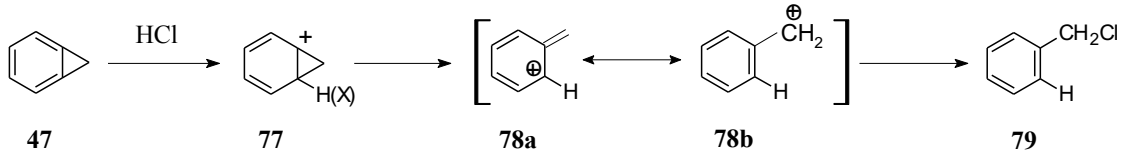
Scheme 1.18 Synthesis of 1-*H*-cycloprop[*b*]anthracene

1.12.2 Reaction of cyclopropparenes

The fusion of cyclopropene to a benzene ring results in geometrical distortions and strain which has consequences on the properties of the resulting cyclopropparene. The cleavage of the three-membered ring plays the main roles of benzocyclopropene chemistry due to the high strain energy of the cyclopropene.

1.12.2.1 Acid sensitivity

The benzocyclopropenes, like the cyclopropenes, are acid sensitive. The reaction with acids leads to ring open products. Reaction of benzocyclopropene with mineral acid is believed to proceed through H^+ addition on the central π-bond, leading to a cyclopropyl cation, which undergoes ring opening to the benzylic cation.[93]

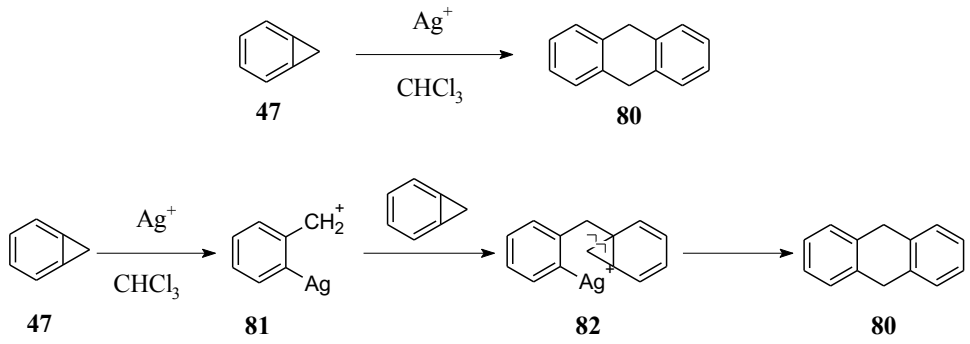


47 77 78a 78b 79

Scheme 1.19 Reaction of benzocyclopropene with acids

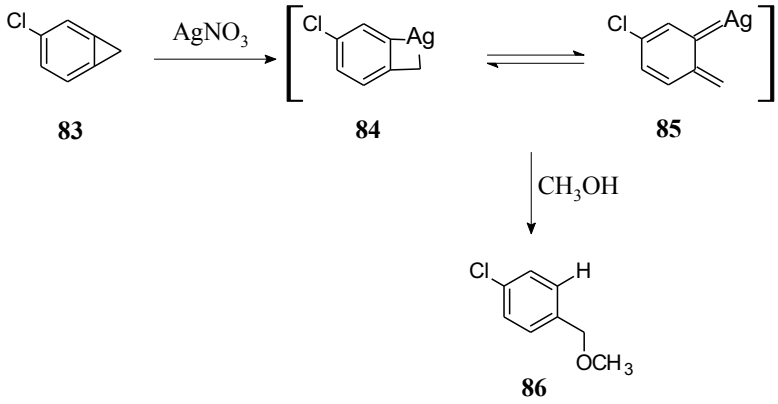
1.12.2.2 Reaction of cyclopropenes with silver salts

9,10-Dihydroanthracene is formed when benzocyclopropene is treated with silver ions in chloroform. Cleavage of the three-membered ring of benzocyclopropene by silver ion can be attributed to the strain energy associated with the cyclopropane ring system.[94]



Scheme 1.20 Dimerization of benzocyclopropene by Ag ion

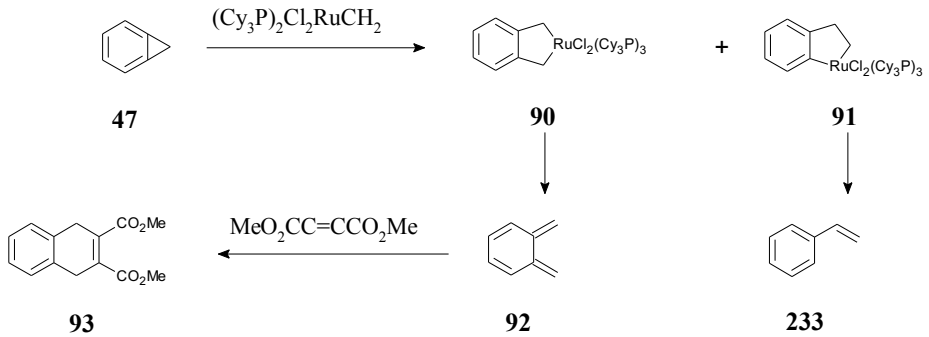
Silver salts promote reactions with cycloproparenes by inducing a ring opening reaction to form an organometallic intermediate **84** or **85**. The silver species intermediate is an excellent benzylating agent which reacts with alcohols, amines, and thiols to give benzylated derivatives. Billups et al. showed that 3-chloro-1*H*-cyclopropabenzene reacts with methanol in presence of $AgNO_3$ producing a ring opened product.[95]



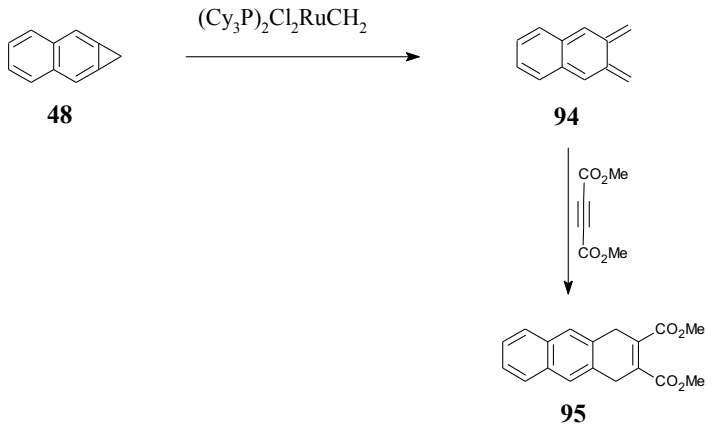
Scheme 1.21 Reaction of 3-chloro-1*H*-cyclopropabenzene with methanol in presence of $AgNO_3$

1.12.2.3 Reactions of cycloproparenes with metal carbenes

The cycloproparenes have an extraordinary reactivity toward organometallic reagents due to the high strain energy. Benzocyclopropene and cyclopropa[*b*]naphthalene react with Wilkinson's catalyst [chlorotris(triphenylphosphine)rhodium(I)] ($RhCl(PPh_3)_3$ to form metallacyclic compounds. Reaction of benzocyclopropene with the ruthenium complex ($RhCl(PPh_3)_3$ forms unstable intermediates 1- and 2-ruthenaindanes (**91** and **90**). Reductive elimination of these intermediates releases the hydrocarbons, o-xylylene and styrene, respectively.[96]



Scheme 1.22 Reaction of benzocyclopropene with metal carbene



Scheme 1.23 Reaction of 1*H*-cyclopropa[*b*]naphthalene with metal carbene

1.13 Aim of project

The main objective of this research is to gain insights into the mode of action of ethylene antagonists and to prepare a new series of compounds that can act as antagonists to the ethylene receptor to delay fruit from ripening and extend the shelf life of vase flowers. These new compounds will be designed to reduce post-harvest losses and be user friendly. The key properties of these antagonists should be:

- Potent antagonist of ethylene action.
- More stable than current antagonists of ethylene action, 1-MCP.
- Safe for people and environment.
- Easy to handle and apply.
- Cheap to prepare.

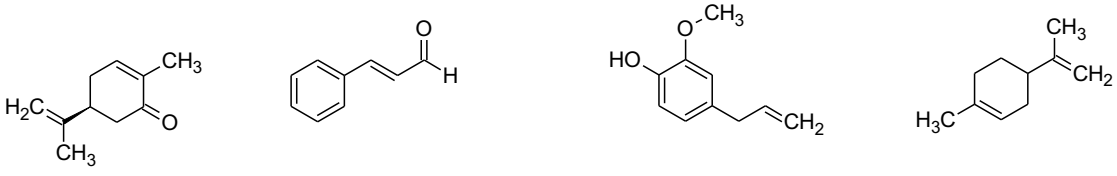
Copper complexes that can be mimicking the ethylene receptor on plant will be synthesized (see Section 1.4). They will be used to rationalize the effect of new inhibitors of ethylene action on fruit and flowers.

Chapter 2

Simple alkenes can be antagonist with good to modest potency (Chapter 1). Some of the alkenes bind to the ethylene receptor binding site and can act as either agonist or antagonist this activity depends on substituents on the alkene.[16] In this study a collection of alkenic compounds have been tested for their antagonist effect on ethylene action. All assays were conducted at the Department of Environment and Agriculture, Curtin University by Prof. Zora Singh and his research group.

2.1 Natural alkenes ethylene antagonist

Many naturally occurring terpenes are antagonists that compete with ethylene for the ethylene receptor. The phenomena of inhibition of growth of neighboring species is known as the allelopathy and may involve antagonist of ethylene action.[59] The presence of the double bonds in some natural compounds are important for their activity as ethylene antagonists.[28] Four readily available natural terpenes such as (carvone **96**, cinnamaldehyde **97**, eugenol **98** and limonene **99**) were selected to check there activity as ethylene antagonists. These compounds are particularly interesting as they can be obtain in near pure form in essential oils, which is important for crops to keep their "organic" credentials. The effect of these compounds on abscission of flowers/buds in different cultivars of wax flowers was conducted in by Sabah M. Abdalghani and Prof. Zora Singh from Department of Environment and Agriculture.



(+)Carvone **96** | Cinnamaldehyde **97** | Eugenol **98** | (-)Limonene **99**

Figure 2. 1 **Natural terpenes**

The 4 compounds were screened against different cultivars of wax flower (based on their availability). The cultivars were fumigated with each chemical 1 μM for 18 hours followed by exposure to ethylene (10 ppm) for 24 hours. The number of flowers or buds that had fallen was counted at day 4 after treatment. The reduction of flowers/buds abscission was obtained by normalising against the control experiment the control flowers was abscessed and the reduction percentage was calculated.

(+) or (-)Carvone and eugenol showed no reduction in abscission compared to the control. This is in contrast to the result of *trans*-cinnamaldehyde and (-)limonene which showed good reductions in abscission, 89% and 78% respectively.

Table 2.1 Effect of the selected natural terpenes on abscission of verity of wax flowers, the concentration of the chemicals was 1 μM

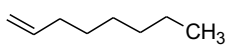
	Chemical	Cultivar of wax flower	% reduction of flowers/buds abscission over ethylene treated flower/buds *
1	(+)-Carvone	WX116	0
2	*trans*-Cinnamaldehyde (CA)	WX73	88.9
3	Eugenol	Muchae mauve	0
4	(-)-Limonene	WXFU	77.6

* Wax flowers/buds fumigated with one of chemicals tested followed by fumigation of ethylene (10 ppm)

2.2 Simple alkenes as ethylene antagonists

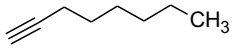
1-Alkenes can be competitive with ethylene on the ethylene receptor and act as either agonists or antagonists.[55] Based on the work of Sisler,[41] 1-octene was chosen as a model antagonist. Four other compounds containing same length chain were selected to test for their antagonistic activity: 1-Octyne, allyl butyl ether, butyl acrylate and 1,2-epoxyoctane. 1-Octyne differs from 1-octene in the number of π bonds. Allyl butyl ether and butyl acrylate have a double bond like 1-octene but have oxygen

atoms present in their chains. 1,2-Epoxyoctane has an epoxide ring instead of the double bond.



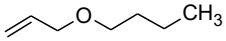
100

1-Octene



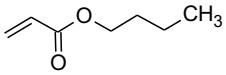
101

1-Octyne



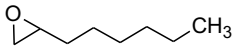
102

Butyl allyl ether



103

Butyl acrylate

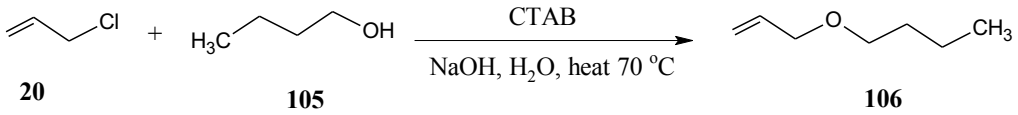


104

1,2-Epoxyoctane

Figure 2. 2 Analogues of 1-octene

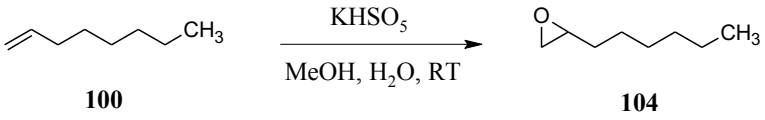
n-Butyl allyl ether was prepared by a Williamson etherification.[97] A mixture of allyl chloride **20**, 1-butanol **105**, water, sodium hydroxide, and cetyltrimethylammonium bromide was vigorously stirred at 70 ^{o}C overnight. Distillation of the crude product afforded the ether **106** in 97% yield. The ^{1}H NMR spectrum confirmed the product, showing a triplet at 0.91 ppm assigned to CH_3 group, two multiplets at 1.38 and 1.57 ppm belonging to 4 hydrogens of the butyl chain, a triplet at 3.42 assigned to 2 hydrogens on ether methylene of butyl chain, doublets of doublets at 3.95 ppm for the hydrogens between the vinyl group and the oxygen atom, two doublets at 5.15 and 5.26 ppm belonging to two hydrogens on the vinyl, and a multiplet at 5.91 ppm for the hydrogen on the vinyl group. The ^{1}H NMR data identical to that reported by Nalet'ko *et al*,.[98]



20 **105** **106**

Scheme 2. 1 Synthesis of ether

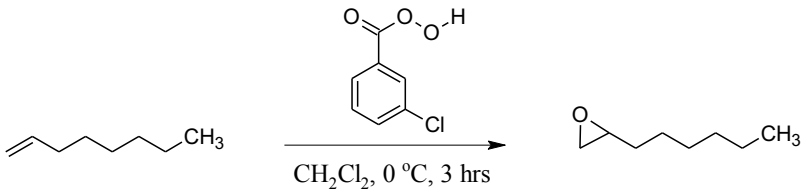
1,2-Epoxyoctane was made from 1-octene. A solution of potassium peroxymonosulfate ($KHSO_5$) in water and a solution of 1-octene in methanol was stirred overnight at room temperature. An oil was isolated in 25% yield which contained the epoxy **104** as well as other impurities so another procedure was used.



100 **104**

Scheme 2. 2 Synthesis of 1,2-epoxyoctane

1,2-Epoxyoctane was prepared by the procedure reported by May Abbott.[99,100] A solution of 1-octene and *m*-chloroperoxybenzoic acid in dichloromethane was stirred at 0°C for 3 hrs to give 1,2-epoxyoctane in 75% yield, without the need for further purification. The ^1H NMR spectrum was consistent with the structure. It showed triplet at 0.91 ppm for CH_3 end of the aliphatic chain, multiplets between 1.26 and 1.62 ppm belonging to 10 hydrogens of the chain, two doublets at 2.48 and 2.77 ppm and a multiplet at 2.92 ppm for the three hydrogens on the epoxide ring. The spectra was identical to that those reported by Arakelyan, et al,1989.[101]



100 **104**

Scheme 2. 3 Synthesis of 1,2-epoxyoctane.

This set of 5 compounds were applied on wax flowers. They were fumigated with one of chemicals (1 μM) then followed by fumigation of ethylene (10 ppm). Then the number of flowers fallen were counted and normalized with the control. All the synthesized simple alkenes in the table below showed did not reduce flower/bud abscission compared to the control except for allyl butyl ether which showed a small reduction. This was disappointing as 1-octene is known to act as an antagonist in pea shoots.[55]

Table 2.2 Effect of the synthesized simple alkenes analogues on abscission of varity of wax flowers

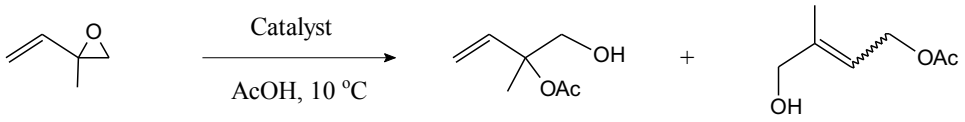
	Chemical	Cultivar of wax flower	Percent reduction flowers/buds abscission over ethylene treated flower/buds *
1	1-Octene	WX110	0%
2	1-Octyne	White spring	0%
3	Allyl butyl ether	WX110	9.6%
4	Butyl acrylate	WX17	0%
5	1,2-Epoxyoctane	WX17	0%

* Wax flowers/buds fumigated with one of chemicals tested followed by fumigation of ethylene (10 ppm).

2.2.1 3,4-epoxy-3-methyl-1-butene

3,4-Epoxy-3-methyl-1-butene or isoprene oxide **106** has an epoxide group adjacent to an alkene which may be a good ethylene antagonist. Epoxides relieve their ring strain when they undergo nucleophilic substitution. The ring opening reaction of isoprene oxide **106** with acetic acid leads to two products: 3-methyl-3-acetoxy-4-hydroxybut-1-ene **107** and *cis*- and *trans*-2-methyl-1-hydroxy-4-acetoxybut-2-ene

108. In the presence of copper(I) acetate the formation of **107** increases from 52% to 75%. Coordination between the double bond and the copper ion may promote this reaction. If applied to the ethylene receptor, parallels can be drawn between the copper cofactor and the copper salt used in this reaction.

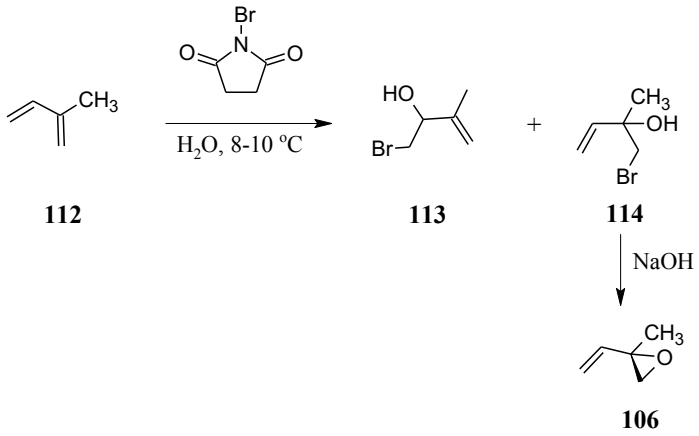


106		**107**	**108**
	none	52	15
	Cu(I)OAc	75	8

Scheme 2. 4 Ring opening reaction of isoprene oxide

2.2.2 Synthesis of isoprene oxide 106

Isoprene oxide **106**, was prepared by a two steps procedure described by Overman.[102] A suspension of isoprene in water was treated with *N*-bromosuccinimide and cooled at 8-10 °C for 2 hrs. The resulting crude bromohydrin (a mixture containing 1-bromo-2-methyl-3-buten-2-ol **114** as the major product and, 1-bromo-3-methyl-3-buten-2-ol **113** as the minor) was used directly in the next stage. The mixture of bromohydrins was treated with a solution of sodium hydroxide in the similar way that described by Arakelyan[101] to form the epoxide. The crude product was distilled to give the pure epoxide in 36% yield.[101]



Scheme 2. 5 Synthesis of isoprene oxide **106**

2.2.3 Effect of isoprene oxide 106 on abscission of flowers/buds in WX17 wax flowers

The effect of isoprene oxide **106** on abscission of flowers/buds in WX17 wax flowers was tested. The flowers were fumigated for 24 hours then followed by exposure to 10 ppm ethylene. There was a 44.1% reduction of flower and buds abscission. The reduction of abscission of flowers and buds shows that isoprene oxide competes with ethylene for the ethylene receptor and prevents the ethylene action, which delays the dropping of flowers and buds.

2.2.4 Effect of isoprene oxide 106 on climacteric ethylene production and respiration in some climacteric fruits

Two fruits "Tegan blue" plums and "Fuji" apples were fumigated with different concentrations (0, 500, 100, 2000 nLL^{-1}) for 18 hours. These experiments were conducted by Prof. Zora Singh and his research group.

After fumigation of "*Tegan blue*" plums with 500 and 1000 nLL^{-1} of isoprene oxide **106** the climacteric peak of ethylene were observed 10 and 12 days post treatment,

with an ethylene concentration of 1.91 and 3.88 µmolkg^{-1}h^{-1} respectively. The fruit treated with isoprene oxide **106** (250 nLL^{-1}) produced more ethylene than the control. The control fruit showed 76-fold less ethylene production than fruit treated with 1000 nLL^{-1} of isoprene oxide **106**. The respiration peak in the fruit treated with the same concentration of isoprene oxide **106** was observed on day 10 after treatment. The concentration of CO_2 was 0.17 mmolkg^{-1}h^{-1}. The concentration of CO_2 in fruit treated with 1000 nLL^{-1} was 1.15-fold more than control fruit. In conclusion, isoprene oxide **106** is acting as an agonist rather than an antagonist in "Tegan Blue" plums.

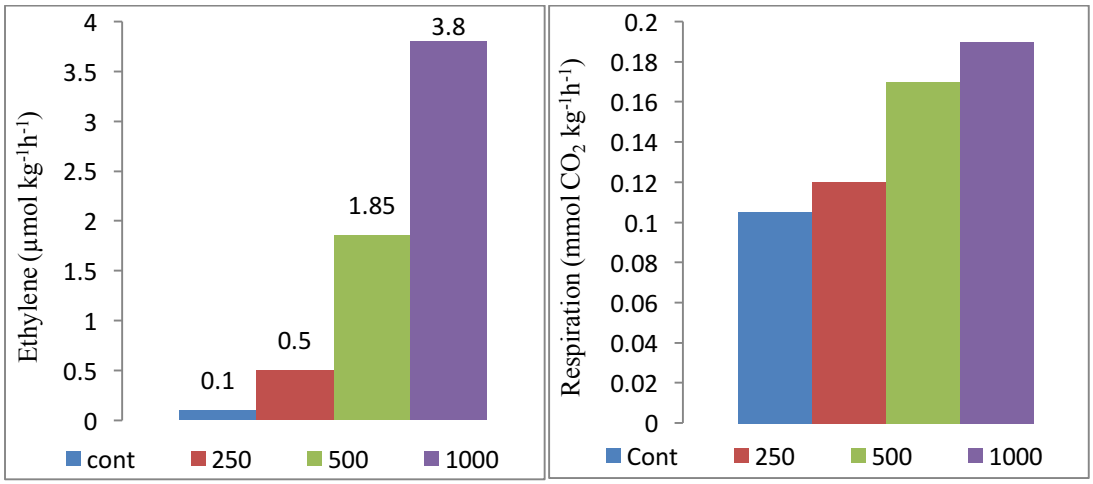


Figure 2. 3 The ethylene production and respiration in climacteric "*Tegan Blue*" plum fruit at day 10 after treatment with different concentrations of isoprene oxide **115**.

The climacteric ethylene peak was observed on day 22 after treatment of the "*Fuji*" apple with all concentrations of isoprene oxide **106.** The fruits treated with 250 nLL^{-1} and 500 nLL^{-1} showed no difference in ethylene production compared to the control. The fruits treated with 125 and 1000 nLL^{-1} exhibited a high level of ethylene production (1.28 and 1.35-fold) respectively, higher than control ethylene production. The respiration climacteric at day 22 after treatment in "*Fuji*" apple exhibited some fluctuations when treated with different concentrations of isoprene oxide **106**, the lowest level of CO_2 concentration was in the control and 500 nLL^{-1} of

isoprene oxide **106** treated fruit. The concentration of CO_2 in fruits treated with 1000 nLL^{-1} was 13-fold higher than the control. Whereas isoprene oxide **106** may work as ethylene agonist at 1000 nLL^{-1} concentration.

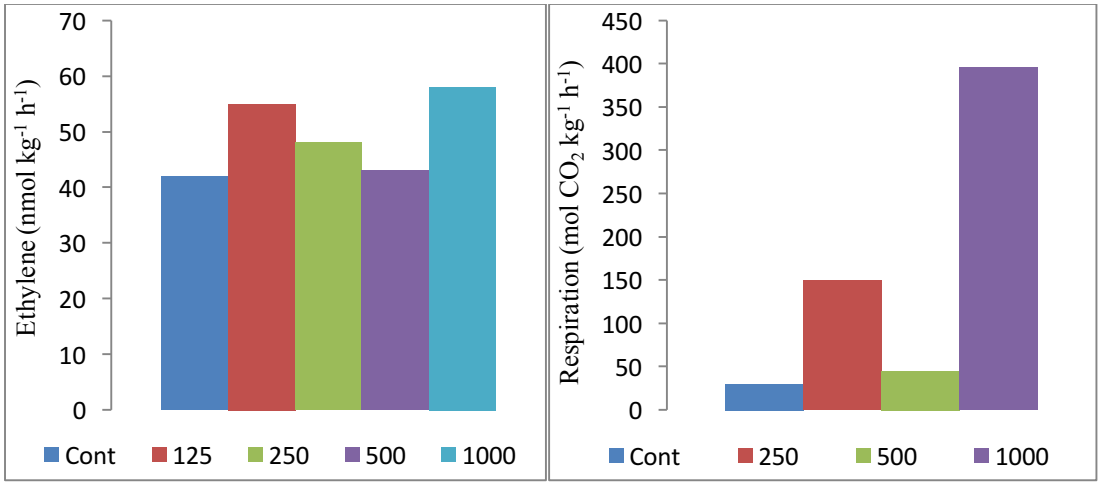
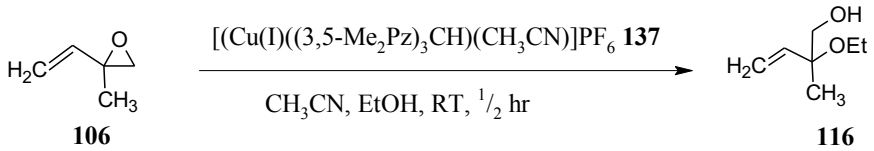


Figure 2. 4 The ethylene production and respiration in climacteric "*Fuji*" apple fruit at day 22 after treatment with different concentrations of isoprene oxide **115.**

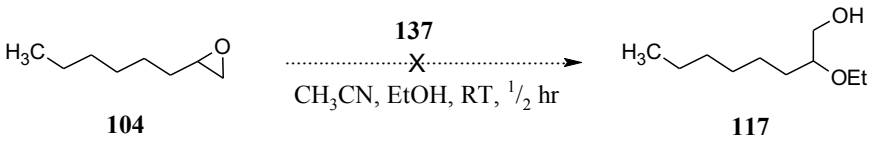
2.2.5 Reaction of isoprene oxide 106 with copper complex

A copper complex, $[(Cu(I)((3,5\text{-}Me_2Pz)_3CH)(CH_3CN)]PF_6$ **137**, was synthesized and was used as a proxy for the ethylene receptor (see Chapter 3). The chemistry of isoprene oxide **106** was compare to 1,2-epoxyoctane **104** as one showed good activity as an ethylene agonist/antagonist and the other was inactive when applied on plants. Both compounds are characterized by the presence of an epoxide ring but differ by the presence of an alkene. The ring opening reaction will be tested in the presence of a copper salt as a proxy for the ethylene receptor.



Scheme 2.6 Reaction of 1,2-epoxy-2-methyl-3-butene with copper complex.

A solution of isoprene oxide **106** in acetonitrile was added to a stirred solution of complex **137** in dry ethanol (30 mL). After 30 minutes, the ^{1}H NMR spectrum of crude product showed consumption of starting material. Flash chromatography of the crude product gave **116** in 12% yield. The compound **116** was identified by its ^{1}H NMR spectrum. The vinyl hydrogens are shifted little bit downfield from 5.52 to 5.90 ppm compare to isoprene oxide, to two doublets at 5.35 ppm and 5.21 ppm. The two signals for the hydrogens on the oxirane ring shifted from 2.65 ppm to 3.50 ppm after the ring was opened and two new signals for ethoxy group, one quartet at 3.75 ppm for CH_2 and a triplet for CH_3 at 1.28 ppm were observed.



Scheme 2.7 Reaction of 1,2-epoxyoctane with copper complex

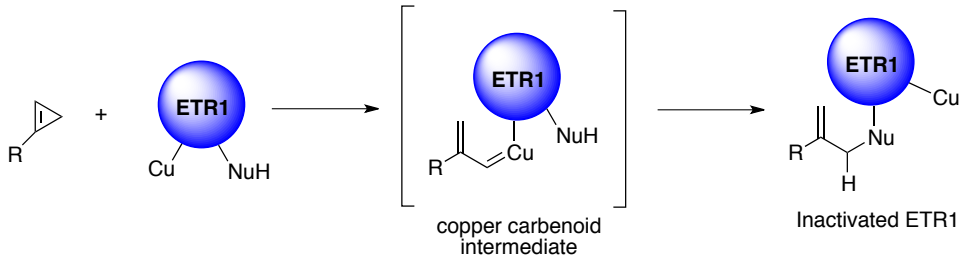
The reaction were as in Scheme 4.6 were same condition applied to 1,2-epoxyoctane **104** but only starting material was recovered.

Comparing the divergent result of both the copper catalyzed reactions of isoprene oxide **106** and 1,2-epoxyoctane **104** with their effect on flowers and fruits, shows that copper catalyzed reaction may be a proxy for the ethylene receptor. In this limited case, compounds that were active ethylene agonists/antagonists participated in copper catalyzed reaction whereas inactive compounds did not. New agonist and antagonist compound could be checked their activity before being applied on plants by using these simple reactions and will be exploited in the following chapters.

Chapter 3

3.1 Mode of action of cyclopropene ethylene antagonists

1-Alkylcyclopropenes have been shown to be effective inhibitors for the ethylene receptor in plants.[68,72,73] The most plausible mode of action of 1-MCP and its derivatives was postulated by Pirrung.[63] It involves the copper(I) cofactor located within the ETR1 receptor. 1-MCP and its derivatives are thought to react with the copper cofactor within the ethylene receptor and cleave the cyclopropene ring. This newly formed copper carbenoid intermediate then reacts with a nucleophile adjacent to the binding site to form a covalent bond. This causes the antagonist effect by blocking further molecules of ethylene from binding to the receptor.

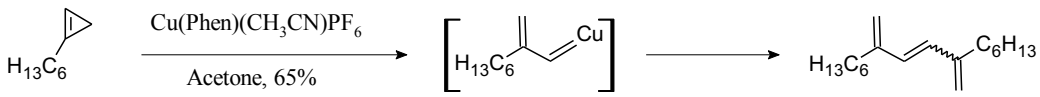


118 **119** **120** **121**

Figure 3.1 Proposed mechanism of the mode of action of 1-MCP and its derivatives

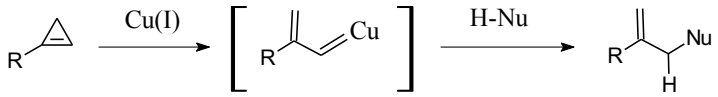
This proposed mode of action is based on the model reaction below (Scheme 3.2). When 1-HCP in acetone was mixed with a copper(I) source, $Cu(phen)(CH_3CN)PF_6$, a dimer was formed as a mixture of stereoisomers (E:Z 2:1). The most likely mechanism for the formation of this product is the copper carbenoid intermediate **123** which dimerizes, a common reaction in copper carbene chemistry. A closer representation of the mode of action of 1-MCP is if the copper carbenoid intermediate reacts with a nucleophile to give an insertion product. To date no such

reaction has been reported for cyclopropenes. In this, chapter we will compare the reactivity of some cyclopropene derivatives in ring opening reactions and their ability to antagonize the action of ethylene.



112 **123** **124**

Scheme 3.1 Reaction of 1-HCP with $Cu(phen)(CH_3CN)PF_6$.[63]

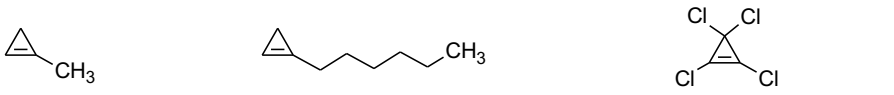


118 **125** **126**

Scheme 3.2 Ring opening reaction of 1-subsituted cyclopropenes in the presence of a nucleophile

Three cyclopropenes were applied on horticulture produce and reacted with copper complexes to test the above hypothesis. 1-Methylcyclopropene (1-MCP), 1-hexylcyclopropene (1-HCP) **112** and 1,2,3,3-tetrachlorocyclopropene **127** were chosen as they are cyclopropenes with different substitution patterns. The efficacy of 1-MCP is well known as an ethylene antagonist and is used commercially to delay fruits over ripening and abscission of flowers and leaves.[67] 1-MCP and 1-HCP are similar, and differ by the length of the alkyl chain. This increase of the length by the chain slightly increases the potency of 1-HCP. The longer chain length declined the minimum required concentration from 0.7 nLL^{-1} to 0.4 nLL^{-1} and time of protection of banana fruits by 1-HCP against ethylene was increased from 12 days to 20 days.[68] Also 1-HCP is a liquid and is easier to handle than 1-MCP. 1,2,3,3-

Tetrachlorocyclopropene **127** is a commercially available cyclopropene and has yet to be tested as an ethylene antagonist.



1-Methylcyclopropene **16** | 1-Hexylcyclopropene **112** | 1,2,3,3-tetrachlorocyclopropene **127**

Figure 3.2 Cyclopropenes to be tested

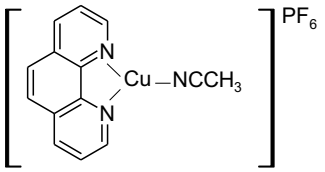
3.2 Investigating the activity of compounds as ethylene agonist or antagonist in laboratory

A copper(I) cofactor associated with the ethylene-binding domain of ETR1 is required for high-affinity ethylene-binding activity.[28,45] Rodriguez and co-workers hypothesized that cysteine and histidine amino acid residues may serve as ligands for a Cu(I) ion in the ETR1 binding site for ethylene. Based on this hypothesis Cu(I) complexes with amine and thiol ligands were designed to mimic a binding site of the ethylene receptor. Yunfan Zou has used many models of Cu(I) complexes to study the binding of the ethylene in the ethylene receptor such as $[Cu(I)(SMe)_2NH_3)_2]^{-1}$, $Cu(I)(SMe)(NH_3)(H_2O)$, $Cu(I)(SMe)_2(NH_3)$ and $Cu(I)(SMe)(NH_3)_2$.[48]

128 | **129** | **130** | **131**

Figure 3.3 Models of copper(I) complexes adapted from Yunfan Zou

Pirrung and co-workers used Cu(phen)(CH_3CN)PF_6 and Cu(bpy)(CH_3CN)PF_6 as copper complexes to mimic the ethylene receptor and show the mechanism of reaction of these complexes with strained alkenes.[48] Hsu *et al*., have reported stable Cu(I) ethylene complexes. These copper complexes contain either tri(pyrazole-1-yl)methane or tri(3,5-dimethyl pyrazol-1-yl)methane ligands.[51] Hanna *et al*., had studied the copper(I) complexes [$Cu(PPh_3)_3X$] and [$Cu(PPh_3)_3(CH_3CN)$]X where X is Cl, Br or I. For the purpose of this study, some of the copper complexes needed to be prepared.



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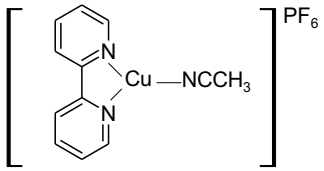

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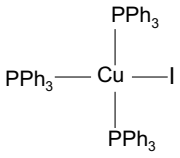
Figure 3.4 Cu(I)(phen)(CH_3CN) and [(bpy) CH_3CN]PF_6 complexes.



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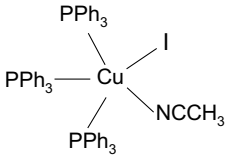


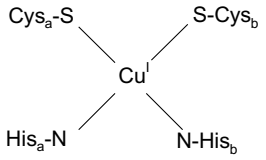
135

Figure 3.5 Copper complexes adapted from Hsu *et al*.

3.2.1 Synthesis of copper complexes

Four air stable copper(I) complexes were chosen to be tested with the cyclopropenes. These compounds were chosen on the basis of ease of synthesis and reasonable solubility in organic solvents. The counter ion used for the complexes was iodide

then later PF_6 to avoid counter anion acting as a nucleophile in the reaction. Along with simple complexes, tris(3,5-dimethylpyrazol)methane was chosen as a ligand for copper(I) as it resembles histidine binding to copper cofactor in ETR1.



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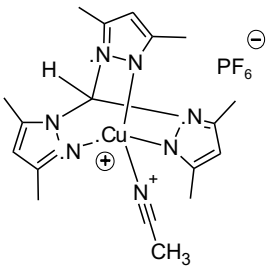
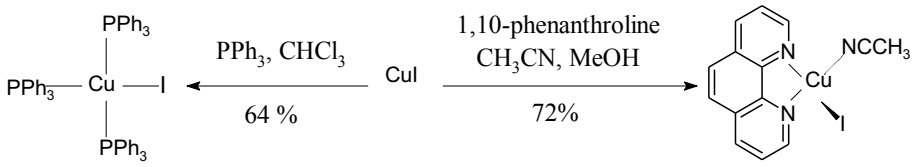


137

Figure 3.6 The similarity between ethylene receptor domain and copper complex **137**

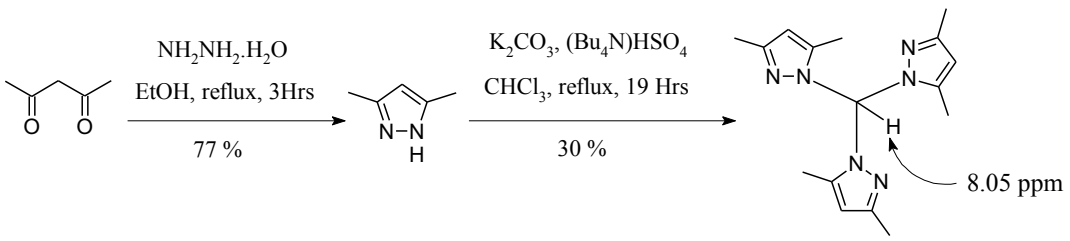
Simple air stable copper(I) complexes were synthesized in a one-step reaction. Tris(triphenylphosphine)copper iodide was prepared by precipitation of copper(I) iodide and triphenylphosphine in 64% yield.[103] Reaction of CuI with 1,10-phenanthroline in degassed acetonitrile at 50 ^oC afforded dark red crystals of copper(I)-1,10-phenanthroline acetonitrile iodide in 72% yield.[104] The ^1H NMR spectrum showed three multiplets at 8.46, 7.96 and 7.26 ppm assigned to 1,10-phenanthroline and singlet at 1.36 ppm due to methyl group of the coordinated acetonitrile.



134 **138** **139**

Scheme 3.3 Synthesis of $Cu(PPh_3)_3I$ and $Cu(phen)(CH_3CN)PF_6$.

Tris(3,5-dimethylpyrazol)methane **142** was made in two steps. 1*H*-3,5-dimethylpyrazole **141** was made by heating a solution of acetylacetone and hydrazine hydrate in ethanol under reflux for three hours. The condensation product was isolated in good yield (77%) as colourless crystals.[105] The ^{1}H NMR spectrum contained 2 singlets. The first peak at 5.83 ppm integrated for 1 hydrogen and was ascribe to the three hydrogens the other signal at 2.26 ppm integrated for 6 hydrogens and were assigned to methyl groups. The ^{1}H NMR spectrum was identical to that reported by Sigma-Aldrich. The next reaction in the sequence was a 3-fold substitution reaction of chloroform with 3,5-dimethylpyrazole **141**. A mixture of 3,5-dimethylpyrazole, anhydrous K_2CO_3, and phase transfer agent $(Bu_4N)HSO_4$ in chloroform was heated under refluxed overnight. Tris(3,5-dimethyl-1-pyrazolyl)methane **142** was obtained after chromatography in a 30% yield. ^{1}H NMR spectrum had characteristic signals for tridentate ligand. A signal at 8.05 ppm integrating for 1 hydrogen was assigned to the hydrogen on the newly formed trisubstituted carbon. Singlets at 5.92 ppm integrating for 3 hydrogens and two singlets at 2.18 and 2.01 ppm integrating for 9 hydrogens each indicated three pyrazole ring were present. The ^{1}H NMR was identical to that reported by Reger *et al*. and Neves *et al.*[106,107]

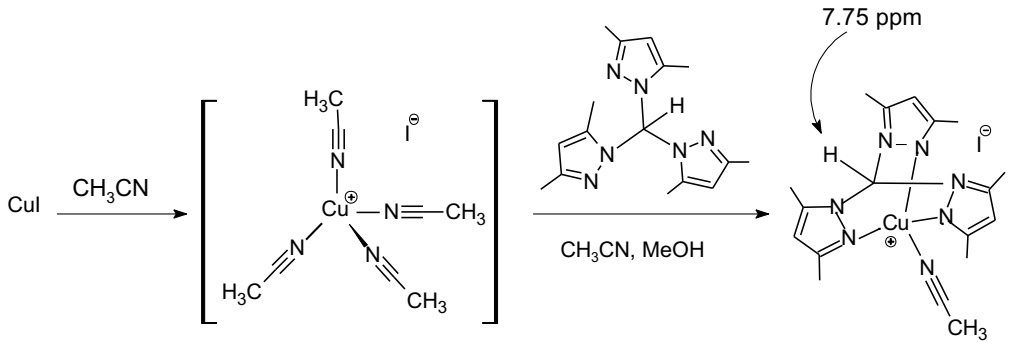


140 **141** **142**

Scheme 3.4 Synthesis of tris(3,5-dimethylpyrazol)methane.

Complexation of the above tridentate ligand with copper iodide was performed in good yields. Copper(I) iodide in acetonitrile was initially heated under reflux until the solution became colorless. The $[Cu(I)(NCCH_3)_4]I$ was not isolated, but used immediately in the next step. A degassed solution of the tridentate ligand in

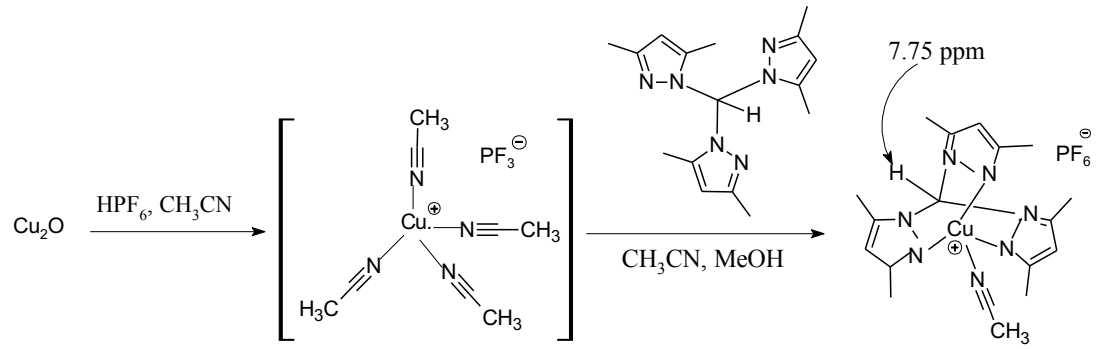
acetonitrile was then added and the resulting mixture heated under reflux for a further 2 days. The resulting solid was collected by vacuum filtration to give the complex as colourless crystals. The ^{1}H NMR spectrum indicated the complexation had occurred by the upfield shift of the aliphatic CH on the ligand from 8.05 to 7.75 ppm. The presence of a new singlet at 2.28 ppm was attributed to the bound acetonitrile.



138 **143** **144**

Scheme 3. 5 Synthesis of $[Cu(I)(NCCH_3)_4(CH)]I$.

As iodide can act as a nucleophile in the model ring opening reactions, it was replaced with hexaflurophosphate counter anion. This complex was made in a similar way to the iodide. Copper(I) oxide was reacted with hexafluorophosphoric acid in acetonitrile. A white solid was isolate in 83% yield which was identified as $Cu(CH_3CN)_4PF_6$. The melting point, ^{1}H NMR and IR were identical to that reported.[108] This copper salt and tris(3,5-dimethylpyrazol)methane were dissolved in acetonitrile and the solution was stirred for 10 minutes. When methanol was added a white precipitate was deposited. The precipitate was recrystallized from dichloromethane and methanol (1 : 1) to give colourless crystals.[109,110] ^{1}H NMR spectrum showed the same peaks and the upfield shift that happen in the copper acetonitrile iodide with small different in the shift where aliphatic CH on the ligand shifted from 8.05 to 7.80 ppm. The new singlet at 2.37 ppm was attributed to the bound acetonitrile. The IR spectrum confirmed the structure of the complex. ^{1}H NMR and IR spectrum were identical to that reported.[109,110]



145 **146** **137**

Scheme 3.6 Synthesis of [(CH)Cu(I)(3,5-Me_2Pz)$_3$(NCMe)]PF_6.

3.3 Synthesis of cyclopropenes and their reactions with copper complexes

Alcohols and 1*H*-imidazole were used in reactions of cyclopropenes with the newly prepared copper complexes because they have similar functional groups to serine and histidine side chains which may be present near the copper cofactor in the ETR1 ethylene binding site. For experimental ease butanol and methanol were used as nucleophiles. In later experiments imidazole was used to mimic a histidine residue. Thiols were not use to mimic cysteine due to their smell.

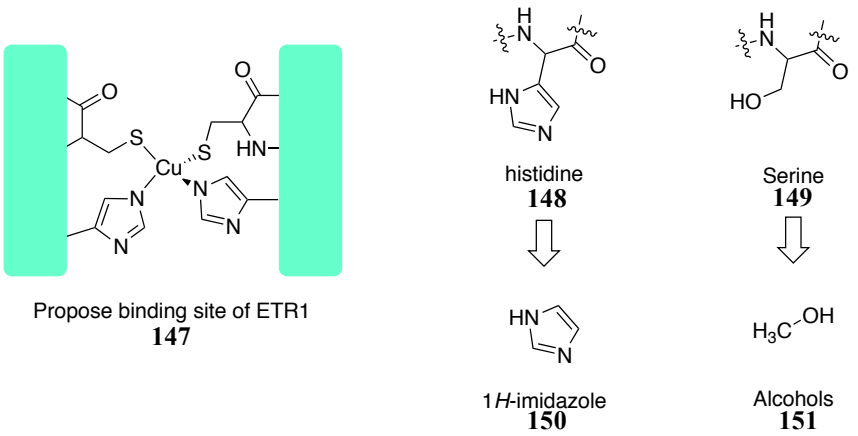
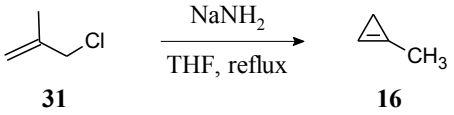


Figure 3.7 The comparison between histidine and 1*H*-imidazole and serine and methanol.

1-MCP was prepared by the known procedure that was reported by Fisher and Applequist.[79] 1-MCP was made by γ-elimination of hydrogen chloride from methallyl chloride. When this compound was treated with sodium amide in gently refluxing anhydrous tetrahydrofuran, the produced 1-MCP gas was collected in a trap at -86 ^{o}C by passing a slow stream of nitrogen over the reaction.[79] The photo in Figure 3.8 shows the experimental setup. The presence of the reflux condenser removes the methallyl chloride from the flow of nitrogen. Collecting and handling 1-MCP is difficult, so the stream of 1-MCP in nitrogen was bubble directly through a solution of butanol and the copper complexes.



31 **16**

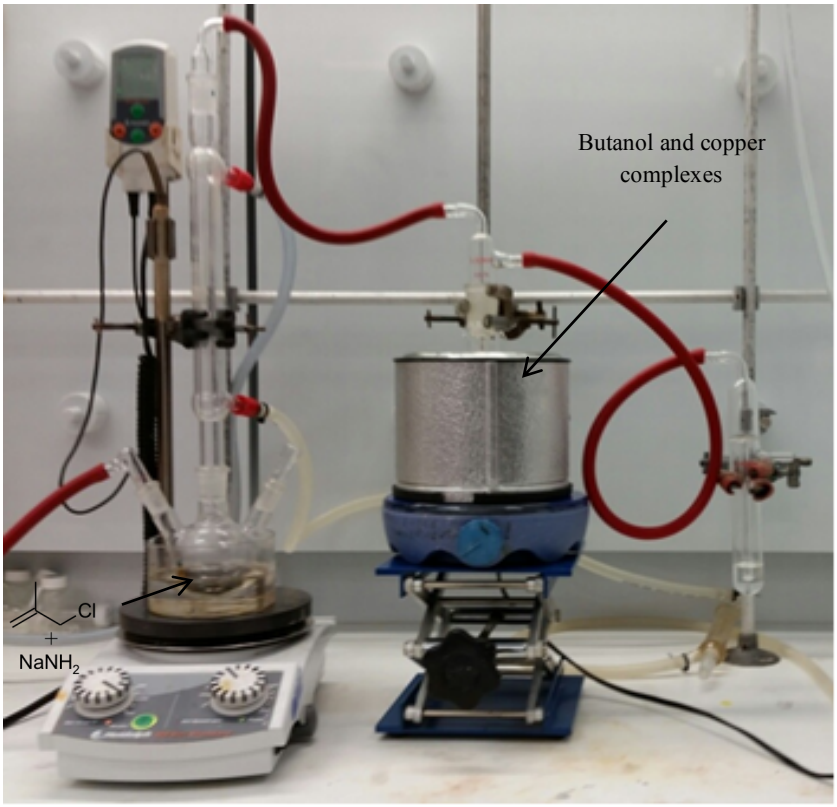
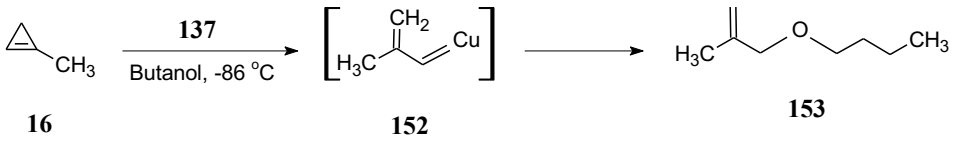


Figure 3.8 Setup of synthesis of 1-MCP and reaction with Cu-complex

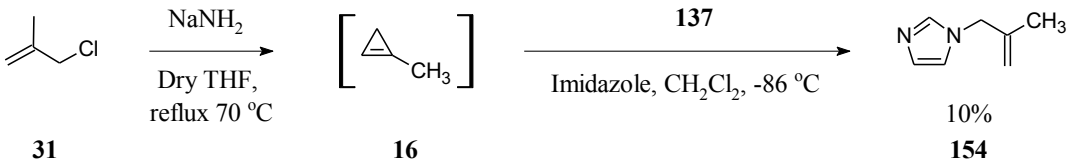
The reaction of 1-MCP with butanol catalysed by the copper complex **137** gave a complex mixture of products. However methallyl butyl ether was isolated in very small amounts (2%) after successive chromatography. The ^{1}H NMR spectrum showed two singlets at 4.97 and 4.87 ppm for the alkene. It also showed a singlet at 3.89 ppm for the 2 hydrogens between the oxygen atom and the vinylic group, and another singlet at 1.79 ppm integrating to 3 hydrogens of the allylic methyl group. The proposed formation of methallyl butyl ether is as follows. 1-MCP reacts with the copper(I) to form a carbenoid species which then insert into the O-H bond of butanol to give the observed product **153**.



16 **152** **153**

Scheme 3.7 Synthesis of 1-MCP and reaction with Cu-complex and in butanol

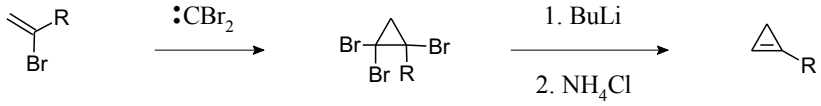
The same reaction was performed using a solution of imidazole in dichloromethane. A product was isolated in about 10% yield which was identified as 1-(2-methyl-2-propen-1-yl)-1*H*-imidazole **154** by NMR. The ^{1}H NMR spectrum showed three multiplet signals between 6.81 and 7.53 ppm assigned to the imidazole ring, two singlets at 4.80 and 4.95 ppm for the germinal vinylic hydrogens, a singlet at 4.43 ppm attributed to the allylic methylene and a singlet at 1.65 ppm for the allylic methyl group. This spectral data was identical with that reported by Kamijo *et al.*[111] However, this reaction was not a good representation of ETR1 as the dichloromethane solution containing imidazole turn blue upon addition of the copper(I) complex indicating oxidation of copper(I) to copper(II).



31 **16** **154**

Scheme 3.8 Synthesis of 1-MCP and reaction with Cu-complex and imidazole in CH_2Cl_2

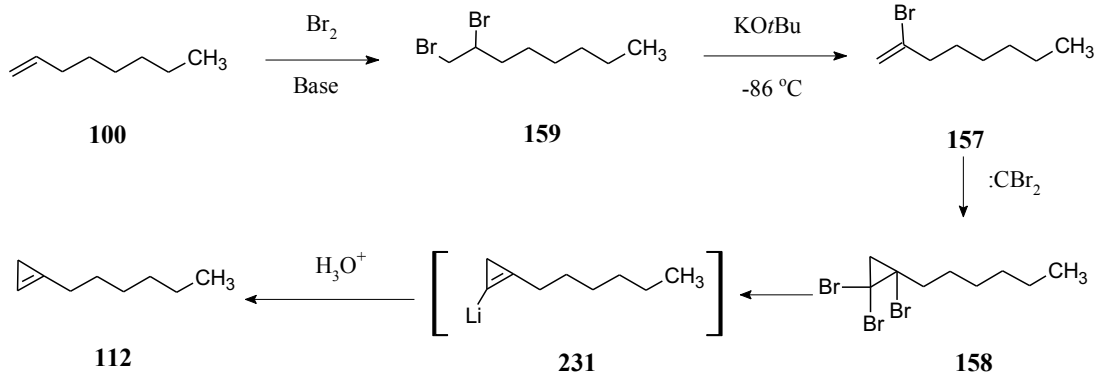
1-Hexylcyclopropene was the next target of study as a liquid and it is easier to handle than 1-MCP. Pirrung and co-workers reported the steps to synthesis 1-substituted cyclopropene based on treatment of the vinyl bromide **155** with dibromocarbene generated *in situ* to give the tribromocyclopropane **156**. Exhaustive debromination with BuLi gave the 1-substituted cyclopropene **16**.



155 **156** **112**

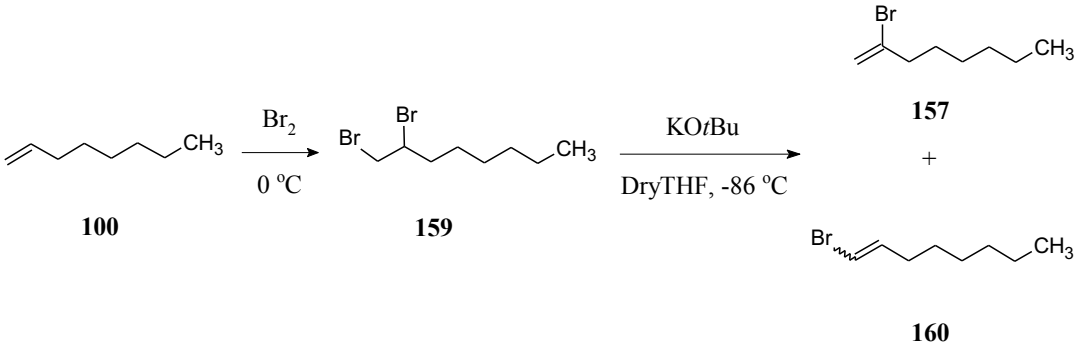
Scheme 3.9 Synthesis 1-substitutied cyclopropene

1-HCP was made in a 4 steps sequence starting from 1-octene. This procedure is a modified procedure to that reported. 2-Bromo-1-octene would be made from 1-octene by addition of bromine followed by elimination. A dibromocarbene addition to the alkene would then afford the cyclopropane **158**. Treatment of **158** with BuLi would give the lithiated species **231**, which would give 1-HCP upon hydrolysis



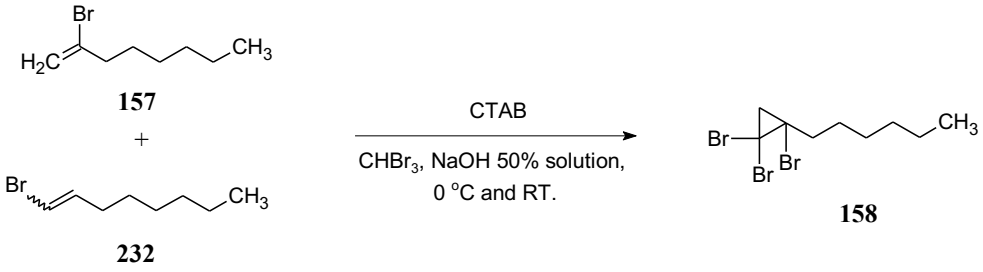
Scheme 3.10 Proposed synthesis of 1-HCP

1,2-Dibromooctane **159** was synthesized using the procedure used to convert cyclohexene to 1,2-dibromocyclohexene**159**.[112] Bromine was added dropwise to a solution of 1-octene **100** in dichloromethane at 0 °C. The reaction turned colourless at the end of the reaction. The solvent was removed to afford the pure dibromide **159** in 63% yield. The ^{1}H NMR spectrum matched that reported by Kabalka.[113] 1,2-Dibromooctane **159** was then treated with potassium *tert*-butoxide at -86 °C to give an isomeric mixture of alkenes.[114] The resulting oil (84%) was a mixture of (E/Z)-1-bromooctene **160** and 2-bromooctene **157** in a 1 : 2 ratio based on the integration of ^{1}H MNR peaks of each isomer. This mixture was used directly in the next step as the isomers were inseparable by distillation or chromatography. 2-Bromo-1-octene had two characteristic singlets at 5.50 and 5.30 ppm in the ^{1}H NMR spectrum.



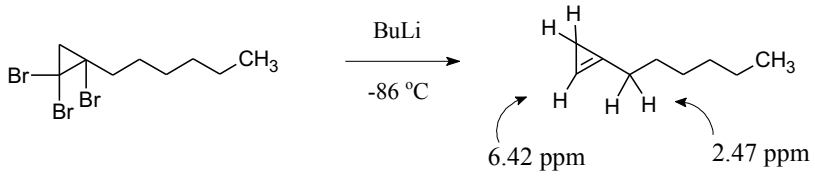
Scheme 3.11 Synthesis of 2-bromo-1-octene

The foregoing mixture containing 2-bromo-1-octene **157** was used in the cyclopropanation reaction. Standard dichlorocyclopropanation conditions were used. The reaction used by Sydnee for in the synthesis of 1,1,2-tribromo-1-hexylcyclopropane **158** was followed however triethylbenzylammonium chloride (TEBA) was replaced by cetyltrimethylammonium bromide (CTAB) as a phase transfer catalyst. Stirring a mixture of bromoform, sodium hydroxide and CTAB gave 1,2,2-tribromo-1-hexylcyclopropane **158** in 50% yield after chromatography.



Scheme 3.12 Synthesis of 1,1,2-tribromo-2-hexylcyclopropane

1-Alkylcyclopropenes have been prepared by treating of 1,2,2-tribromo-1-alkyl-cyclopropane **158** with methyllithium in ether, however a modified procedure was used. 1,2,2-tribromo-1-hexyl-cyclopropane **158** was treated with butyllithium at -86 ^{o}C for 10 minutes and a further 10 minutes at 0 ^{o}C. Hydrolysis of the lithiated intermediate gave 1-hexylcyclopropene as a brown oil in 76% yield. The structure of the compound was confirmed by the ^{1}H NMR spectrum which showed a triplet at 6.42 ppm assigned to the vinylic hydrogen and a triplet of doublets at 2.47 ppm assigned to the a cyclic allylic methylene. The spectra was identical to at reported by Yoo, and Chung.[115] The compound is stable for 3 weeks at -20 ^{o}C.



158 **112**

Scheme 3.13 Synthesis of 1-hexylcyclopropene

1-Methylcyclopropene is commercially used as an ethylene antagonist. It is applied on several of vegetables, fruits and ornamental plants and its activity is well known. 1-hexylcyclopropene has longer alkyl chain which made it more effective than 1-methylcyclopropene and is slightly more stable.[68] Having made 1-HCP, it was tested by Dr. Shamim Ahmed Khan and Prof. Zora Sigh at Department of Environment and Agriculture Curtin University against some flowers and climacteric fruits as an ethylene antagonist.

3.3.1 Effect of 1-HCP on '*Kommeet*' tomato ((*Solanum lycopersicum L*))

1-Hexylcyclopropene at different concentrations (500, 1000, 2000 nLL^{-1}) was applied by fumigation on '*Kommeet*' tomato fruit in a 60 L tight container for 18 hours. Day 4 after treatment showed the climacteric peak for the control fruits whereas the fruits treated with all concentrations of 1-HCP delayed climacteric ethylene production for 6 days which suppressed the production of ethylene till day 10 after treatment. Low concentrations (500, 1000 nLL^{-1}) of 1-HCP were more effective than 2000 nLL^{-1} concentration. The graph below illustrates the ethylene production and CO_2 respiration at days 4 and 10 after treatment. At day 4, both ethylene production and respiration are clearly suppressed compared to the control. At day 10 after treatment ethylene production increased compared to the control but respiration remained subdued.

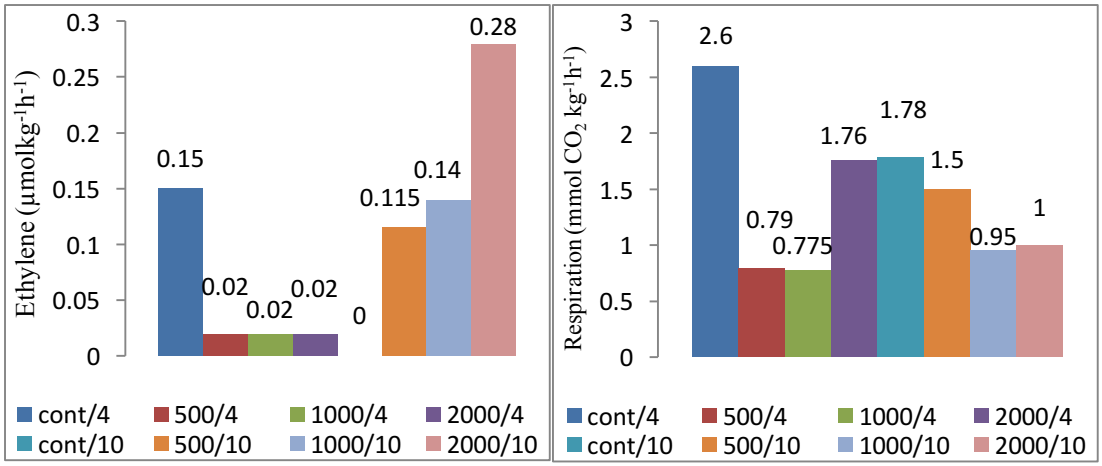


Figure 3.9 Ethylene production and respiration climacteric at day 4 and day 10 of '*Kommeet*' tomato fruit treated with different concentrations of 1-HCP

3.3.2 Effect of 1-HCP on '*Black Amber*' Japanese plum fruit

The '*Black Amber*' plum fruit exhibited delayed of climacteric peak of production of ethylene by 4 days when treated with 500 and 1000 nLL^{-1} of 1-HCP compared with untreated fruit, and was delayed 2 days when treated with 250 nLL^{-1}. The control showed the ethylene climacteric peak at day 10 whereas the treated fruits ethylene climacteric peak at day 14 after treatment. The graphs below show the ethylene production and respiration at day 10 and 14 after treatment. All treated fruits showed suppression of ethylene production till day 14 after treatment whereas the control fruits peaked at day 10.

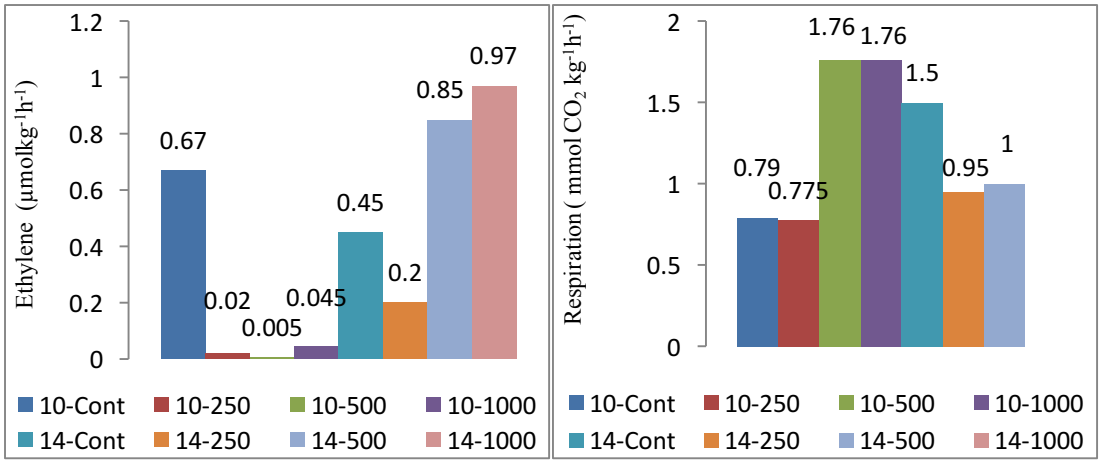


Figure 3.10 Ethylene production and respiration climacteric at day 10 and day 14 of '*Black Amber*' plum treated with different concentrations of 1-HCP

3.3.3 Effect of 1-HCP on '*Fuji*' apple

When '*Fuji*' Apple fruit were treated with different concentrations (125, 250, 500 and 1000 nLL^{-1}) of 1-HCP the climacteric ethylene production was observed at day 24 after treating. Treatment of the fruit with 250 nLL^{-1} of 1-HCP exhibited a significantly lower effect in ethylene production. The climacteric ethylene peak was (196.65 nmol $Kg^{-1}h^{-1}$) whereas it was 6.68-fold lower when treated with 125 nLL^{-1} of 1-HCP.

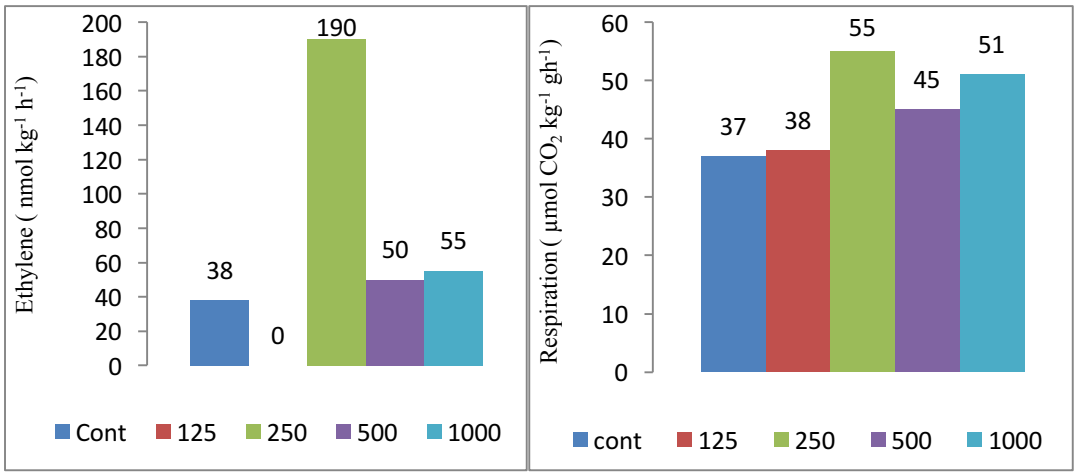
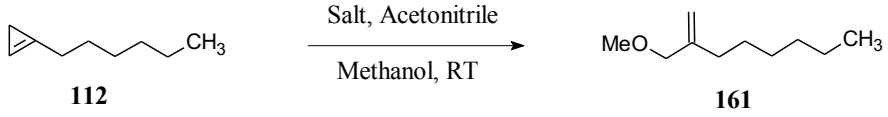


Figure 3.11 Ethylene production and respiration climacteric at day 24 of '*Fuji*' apple treated with different concentration of 1-HCP

The above post-harvest assays show 1-HCP is as effective as 1-MCP in the suppression of ethylene production and respiration and subsequently delay the fruits over ripening. The reaction of 1-HCP with methanol in presence of a copper complex was trailed to reinforce the hypothesis that the copper(I) within the ethylene receptor is reacting with the cyclopropenes. Silver nitrate was also tested as Ag(I) can replace Cu(I) as a cofactor in ETR1.[116] The compounds that have the ethylene antagonist activity should react with methanol catalysed by copper or silver to give ring opened products. 1-HCP was reacted with methanol in presence of $AgNO_3$ and copper salt **137** under the same reaction conditions and both salts gave the same product with some difference in yield. A solution of 1-HCP in acetonitrile was added to a stirred solution of silver nitrate or copper complex **137** (0.016 mol) in dry methanol under nitrogen atmosphere. The reaction mixture was stirred for 30 minutes to afford 2-(methoxmethyl)oct-1-ene **161** after chromatography. The ^{1}H NMR spectrum showed the key characteristic signals. It had two singlets at 4.98 and 4.89 ppm integrating for 1 hydrogen each for the alkene, a singlet at 3.85 ppm integrating for the allylic ether hydrogens and a singlet at 3.31 ppm for the methoxy group. The ^{1}H NMR spectrum was identical to that the one reported by Miyano *et al*.[117]

Table 3.1 Reaction of 1-HCP with ethanol in presence of catalyst



112 **161**

Substrate	Salt	mol%	time (min)	product	yield %
1-HCP	$AgNO_3$	60	30	114	23
1-HCP	Copper(I) complex **137**	60	30	114	56

3.4 1,2,3,3-Tetrachlorocyclopropene 127

Tetrachlorocyclopropene **127** was purchased from Merck and tested against wax flowers. Although it has the strained three membered ring same as 1-MCP, was not effective as an ethylene antagonist. Tetrachlorocyclopropene **127** was applied on WX73 and WX17 wax flowers. The tests were conducted by Shamim Ahmed Khan and Prof Zora Singh at Department of Environment and Agriculture Curtin University. Tetrachlorocyclopropene gave no protection in reducing the rate of flower abscission. At day 8 and 11.5, 50% of the control flowers of WX17 and WX73 flowers respectively were dropped. Whereas the 50% abscission of WX17 flowers was observed at day 2 after treatment with (0, 10, 50, 100 nLL^{-1}) of **127** followed with ethylene same as that flowers treated with ethylene only and negligible enhancement with WX73 flowers which delay the 50% abscission of the flowers only one day after the ethylene treatment.

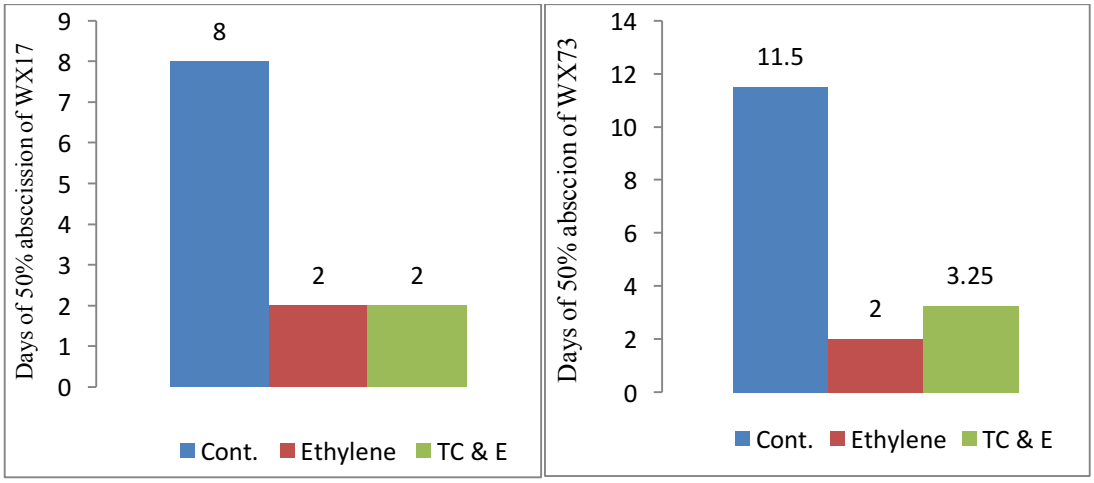
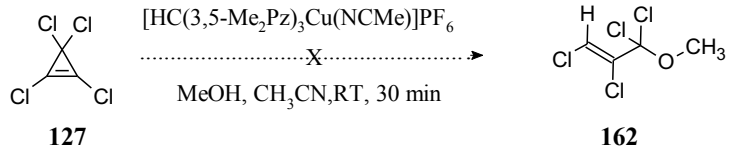


Figure 3.12 Effect of Tetrachlorocyclopropene **127** 50% wax flowers abscission

Tetrachlorocyclopropene **127** did not delay the abscission flowers when applied on WX17 and WX73 wax flowers. This similar observation translated to the chemistry as well. When **127** was reacted with methanol in presence of copper complex **137** under the same reaction conditions described in Scheme 3.8, no reaction occurred. Starting material **127** was recovered and **162** or its degradation products were not

detected. This result reinforces the cyclopropene ring-opening hypothesis catalyzed by copper(I) cofactor and shows that compounds that have similar reaction may be good antagonist of ethylene.



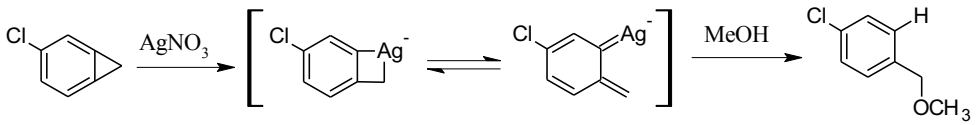
127 **162**

Scheme 3.14 Reaction of **127** with MeOH in presence of copper complex **137**

Chapter 4

The experiments in Chapter 3 add to the evidence that 1-MCP and its derivatives inhibit the ethylene receptor by copper cofactor promoted irreversible binding. Although 1-MCP is very potent, it suffers in several aspects that prevent its wide spread use in agriculture. 1-MCP is a volatile gas at room temperature with a boiling point at about -12 °C. It is unstable and decomposes readily even when kept at -20 °C. It also decomposes in solution (CCl_4) within 2 days at ambient temperature. Another restriction of 1-MCP is its application by fumigation. It requires enclosed rooms making it quite complicated to apply. Nevertheless, the concentration of 1-MCP gas in a sealed container with plant material declines with time, only about a third of the initial amount of 1-MCP remains in the container after 24 hrs.[37,118]

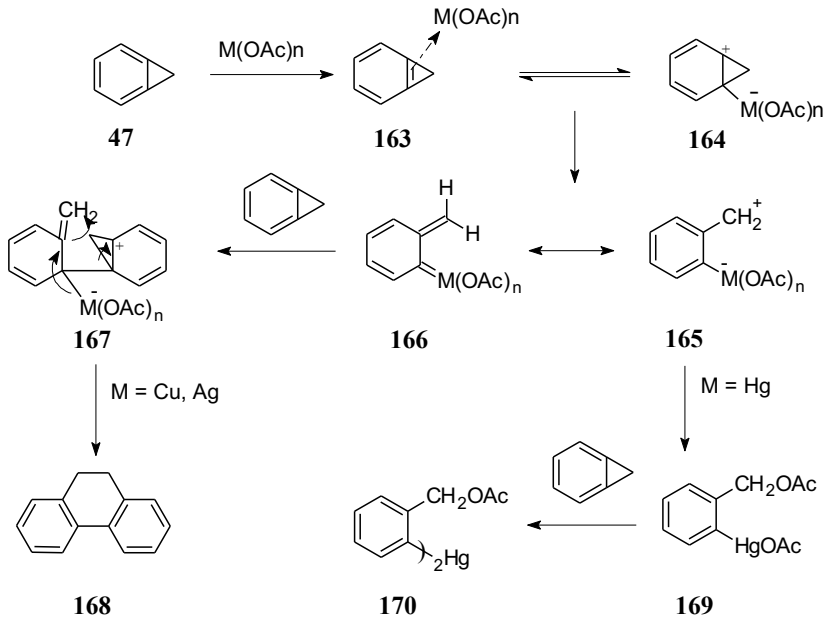
The search for new potent and more user-friendly ethylene receptor antagonist was undertaken. In searching the literature, a potential lead compound was found. Billups reported a reaction that was reminiscent of the proposed ring-opening mechanism of inhibition of 1-MCP (Scheme 4.1). The reaction described the ring opening of benzocyclopropene in the presence of silver(I) salts. This seemed particularly relevant as silver(I) can replace copper(I) as a cofactor in the ethylene receptor.[119] Cycloproparenes have a fused three-membered ring that can be readily cleaved by silver salts. The reaction intermediates are excellent benzylating species.[95] The Ag(I) catalyzed reaction of benzocyclopropene with alcohols proceeds easily at 0 °C in aprotic solvents giving the corresponding benzylated derivatives in high yield.



83 **84** **85** **86**

Scheme 4.1 The reaction of 3-chlorobenzocyclopropene with silver nitrate in methanol

In a further poorly cited report by Shirafuji and Nozaki, ring opening reactions of benzocyclopropene did occur with other transition metals, namely copper and mercury.[120] Insertion of the transition metal into the benzocyclopropene forms a metalocycle **165** which can lead to two different outcomes. The reaction of benzocyclopropene with $Hg(OAc)_2$ at room temperature gave the benzyl acetate **169** which reacted immediately with a second molecule of benzocyclopropene to afford bis(o-acetoxymethylphenyl)mercury **170**. The reaction benzocyclopropene with $Cu(OAc)_2$ or $Ag(OAc)_2$ gave 9,10-dihydrophenanthrene. With these transition metals the intermediate **165** reacts with a second molecule of benzocyclopropene to give the phenanthrene derivative **168**.



Scheme 4.2 Reactions of benzocyclopropene with transition metal salts

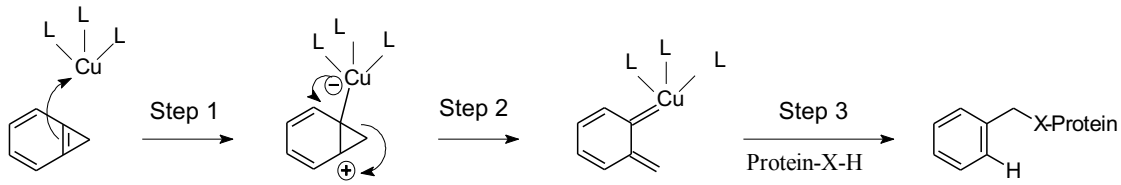
Based on these observations a similar mode of inhibition of the ethylene receptor could be proposed for benzocyclopropene and its derivatives. To investigate the possibility of these compounds acting as ethylene receptor antagonist, benzocyclopropene (1*H*-cyclopropabenzene) and naphtho[*b*]cyclopropene (1*H*-cyclopropa[*b*]naphthalene) were investigated. They were chosen for their simplicity

and apparent ease of synthesis. Benzocyclopropene is a liquid at room temperature (b.p. 150.9 °C)[85] and 1*H*-naphtho[*b*]cyclopropene is a solid (m.p. 86-87 °C).[121] The properties of benzocyclopropenes would make them easier to handle than 1-MCP which is a gas.

47 **48**

Figure 4.1 Benzocyclopropene and naphtho[*b*]cyclopropene

Benzocyclopropene **47** and 1*H*-naphtho[*b*]cyclopropene **48** are more strained than cyclopropene however the ring fusion aromatic ring increases its stability. The hypothesis is than these compounds would behave in the same way as 1-methylcyclopropene. The cycloproparenes **47** and **48** could react with the copper(I) cofactor in ETR1 leading to a reaction intermediate which benzylates the binding site of the ETR1 to inactivate it. (Scheme 4.3).



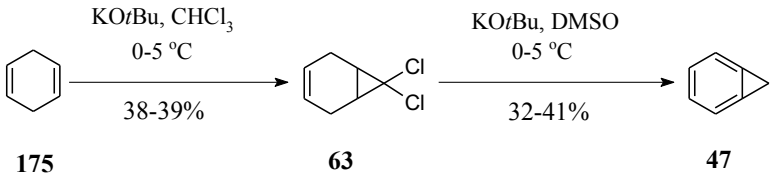
171 **172** **173** **174**

Scheme 4.3 Proposed mechanism of the mode of action of benzocyclopropene and its derivatives

4.1 Synthesis of benzocyclopropene and 1*H*-naphtho[*b*]cyclopropene

Benzocyclopropene has been prepared in several different ways. The most common method is the one described in *Organic Synthesis* by Billups and Chow, starting from 1,4-cyclohexadiene. Addition of dichlorocarbene to 1,4-cyclohexadiene is achieved

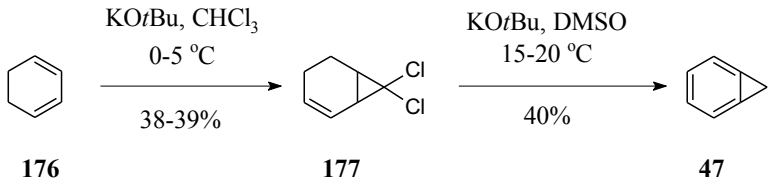
by the action of NaOH on chloroform to give the adduct **63** in 38% yield. Treatment of this adduct with potassium *t*-butoxide in DMSO followed by distillation gave benzocyclopropene in 32-41% yield. Although this synthesis is the method of choice to prepare benzocyclopropene, 1,4-cyclohexadiene is very expensive (Aldrich $225/25g) and an alternate method was sort.



175 **63** **47**

Scheme 4.4 Preparation of benzocyclopropene by Billups

Both Banwell and coworkers and Davalian and coworkers reported the synthesis of benzocyclopropene using 7,7-dichlorobicyclo[4.1.0]hept-2-ene **177**. This dichlorocarbene adduct was prepared from 1,3-cyclohexadiene **176** which is easier to access.[145,148] The yields are equivalent to the *Organic Synthesis* procedure and therefore this synthetic route was adopted.

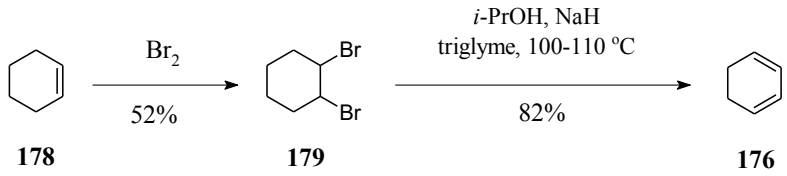


176 **177** **47**

Scheme 4.5 Alternative preparation of benzocyclopropene

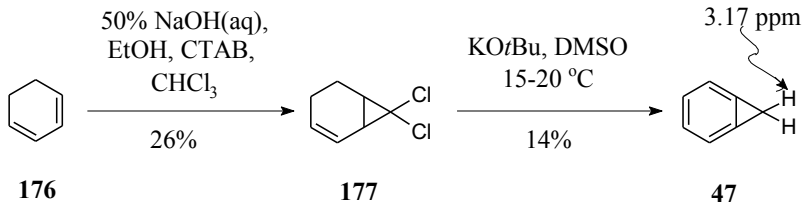
1,3-Cyclohexadiene **176** was made by a two-step process from cyclohexene. Bromine was added to cyclohexene at 0 °C to afforded 1,2-dibromocyclohexane **179** as a pale yellow liquid, (64% yield). The ^{1}H NMR spectrum was identical to that reported by Kabalka *et al*.[122] Debromination of 1,2-dibromocyclohexane **179** was

performed using sodium isopropoxide generated *in situ* by reacting isopropanol with sodium hydride. Addition of the dibromide to this solution of sodium isopropoxide under slight vacuum gave 1,3-cyclohexadiene **176**, which was isolated by distillation to give the diene in 88% yield. The ^{1}H NMR spectrum characteristically had 2 signals at 5.88 and 5.77 ppm integrating for 2 hydrogens due to the new vinylic hydrogens.



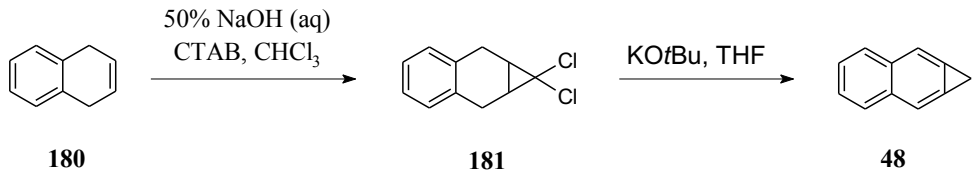
Scheme 4.6 Preparation of 1,3-cyclohexadiene.

The cyclopropanation method by employed Billups and co-workers was modified slightly. Ethanol and then chloroform were added to a mixture of 1,3-cyclohexadiene, 50% sodium hydroxide solution and cetyltrimethylammonium bromide at room temperature and then stirred vigorously for one hour at 0 ^{o}C. The crude product was purified by column chromatography to afford the adduct in 26% yield. Dehydrochlorogenation of the adduct was performed with potassium *tert*-butoxide in dimethyl sulfoxide. After some experimentation, benzocyclopropene was isolated from the reaction mixture by pouring it in to water and extracting with petroleum spirits. Benzocyclopropene is very sensitive to acids and transition metal ions so all glassware and solvents were vigorously cleaned to be free of acids and metal ions. An example is the use deuterochloroform directly from the bottle for NMR analysis which led to immediate decomposition of the benzocyclopropene to benzyl chloride. Only $CDCl_3$ stored over anhydrous K_2CO_3 was use. Benzocyclopropene is temperature sensitive so all the workup operations were performed at about 0 ^{o}C. The yield of the product using this method was 14%. The key signal in the ^{1}H NMR spectrum was the cyclopropane methylene at 3.17 ppm which was identical to that reported by Billups.[123]



Scheme 4.7 Modified synthesis of benzocyclopropene from 1,3-cyclohexadiene.

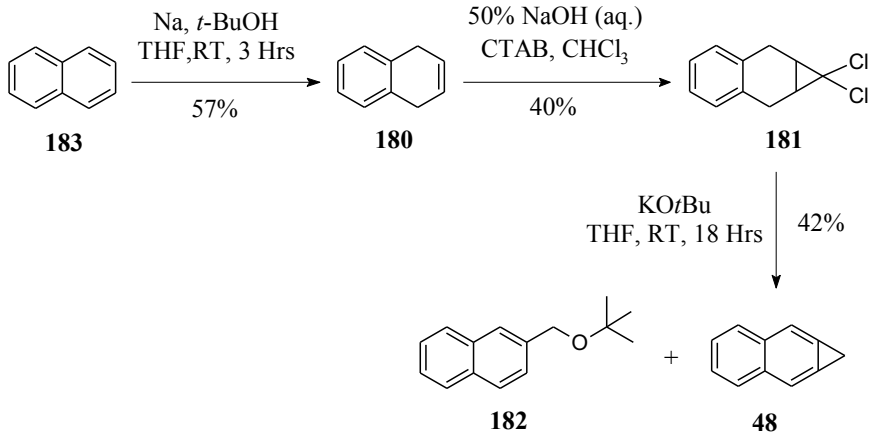
Unfortunately benzocyclopropene has a strong stench, so a less volatile cycloproparene, 1*H*-naphtho[*b*]cyclopropene **181** was also prepared. Synthesis of 1*H*-cyclopropa[*b*]naphthalene **181** is analogous to the synthesis of benzocyclopropene. It has been prepared by the method describe by Billups.[124] The preparation of 1*H*-cyclopropa[*b*]naphthalene was made by the action of potassium *tert*-butoxide on the dichlorocarbene adduct of 1,4-dihydronaphthalene **180**. The starting material of this synthesis is naphthalene.[85]



Scheme 4.8 Preparation of 1*H*-naphtho[*b*]cyclopropene by Billups

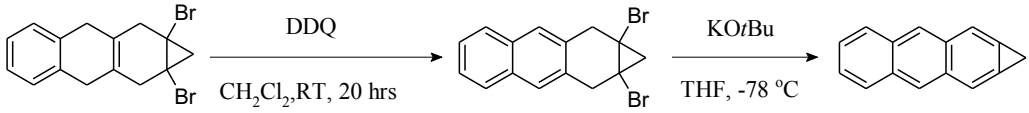
Reduction of naphthalene with sodium and *tert*-butanol in THF was performed following the procedure reported by Abdullah et al.[125] After adding the sodium metal pieces to the solution the reaction mixture change to blue, then *tert*-butanol was added. After 3 hrs when the reaction mixture was pale yellow the residual sodium metal was removed by the gravity filtration to afford the product **180** in 57% yield. The conversion of 1,4-dihydronaphthene **180** to 1*H*-naphtho[*b*]cyclopropene **48** follows the procedure outlined by Billups. Addition of dichlorocarbene to 1,4-dihydronaphthalene **180** to chloroform, sodium hydroxide and

cetyltrimethylammonium bromide (CTAB) was performed in 40% yield.[84] Unlike benzocyclopropene the elimination, isomerization reaction using potassium *tert*-butoxide was performed in THF. The reaction produced two compounds **48** and **182**. The ^{1}H NMR spectrum of **48** showed two apparent doublets at 7.56 and 7.13 ppm and singlet at 6.93 ppm belonging to the aromatic rings and singlet at 3.20 ppm belonging to hydrogens on cyclopropene. The small amount of the *tert*-butyl ether **182** was removed by a chromatography.



Scheme 4.9 Modified synthesis of 1*H*-naphtha[*b*]cyclopropene from naphthalene

1*H*-Cycloprop[*b*]anthracene was also prepared but not used in the biological assay. It was made from the Diels-Alder adduct **184** which was available in the lab, by a method analogous to the synthesis of cyclopropa[*b*]anthracene described by Billups.[82] The adduct **184** was added to a solution of DDQ in dichloromethane and stirred at room temperature for 20 hrs to afford a yellow crystals of **185** in 80% yield. The naphthalene derivative **185** was treated with potassium *tert*-butoxide in dry THF at -78 ^{o}C for 2 hrs. To give 1*H*-cycloprop[*b*]anthracene **71** in 42% yield. The ^{1}H NMR spectrum showed a singlet at 3.56 ppm for the cyclopropene CH_2 and multiplets for aromatic rings between 7.67 and 8.41 ppm. The ^{1}H NMR spectrum was identical to that reported by Billups.[90]

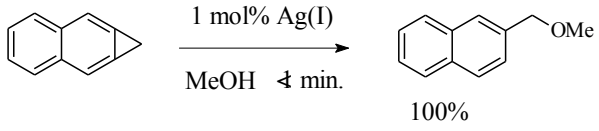


184 **185** **71**

Scheme 4.10 Synthesis of 1*H*-cycloprop[*b*]anthracene

4.2 Ring opening of 1*H*-naphtho[*b*]cyclopropene with copper complexes

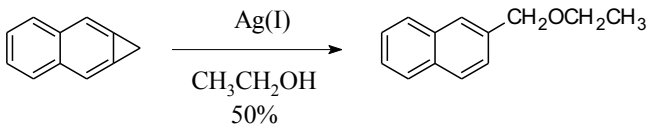
As benzocyclopropene proved to be extremely smelly, the ring opening studies were conducted exclusively on 1*H*-naphtho[*b*]cyclopropene. To date, no ring opening reaction have been conducted using Cu(I) complexes, however it has been reacted with silver salts to give ring opened products. Billups reacted 1*H*-naphtho[*b*]cyclopropene with Ag(I) and report a quantitative yield of 2-(methoxymethyl)-naphthalene **89** in less than one minute. For this study the same conditions as in Chapter 3 were used to confirm the Ag(I) catalyzed reaction occurred in our hands.



48 **89**

Scheme 4.11 Ring opening of reaction of naphtha[*b*]cyclopropene with silver salts

1*H*-Naphtho[*b*]cyclopropene was initially reacted with silver nitrate (50 mol %) in ethanol to confirm the reported observation. When a solution of 1*H*-naphtho[*b*]cyclo propene in dichloromethane was added to a solution of silver nitrate in ethanol, 1*H*-naphtho[*b*]cyclopropene was consumed in less than 30 minutes. Isolation of the major product in the reaction showed three distinctive signals in the ^{1}H NMR spectrum a triplet at 1.25 ppm and a quartet at 3.55 ppm for the ethyl group, a singlet at 4.65 ppm for the benzylic methylene and 7 aromatic signals. The ^{1}H NMR data was the same as reported by Saraçoğlu *et al*.[93]

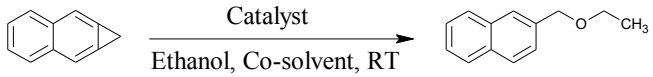


48 **186**

Scheme 4.12 Reaction of 1*H*-naphtha[*b*]cyclopropene with silver nitrate in ethanol

Having shown that silver nitrate reacts with 1*H*-naphtho[*b*]cyclopropene a series of copper(I) salts were trialed. The three membered ring was easily opened when copper complexes were used. Copper triphenylphosphine iodide complex gave lower yield (23%) compared to $AgNO_3$, whereas the multidenate nitrogen ligands, complexes **139** and **137,** gave good yields 66 and 60% respectively. Surprisingly, $Au(PPh_3)Cl$ did not catalyse the ring opening reaction and only starting material was recovered. To confirm that the copper ion plays a primary role in the ring opening reactions, a control experiment was run. When compound **48** was stirred with ethanol alone for 2 hours at room temperature 1*H*-naphtho[*b*]cyclopropene was recover in 100% yield.

Table 4. 1 Reaction of **48** with ethanol in presence of catalyst



48 **186**

	Complex	Co-solvent	Time	Yield %
1	Control	- -	2 hrs	N.R
2	$AgNO_3$	CH_2Cl_2	30 min	50
3	$Cu(PPh_3)I$	CH_2Cl_2	30 min	23
4	Cu(I) (1,10-phen-$NCCH_3$)I (**139**)	CH_2Cl_2	30 min	66
5	[(Cu(I)((3,5-$Me_2Pz)_3CH)(CH_3CN)PF_6$ (**137**)	CH_3CN	30 min	60
6	$[Au(PPh_3)]Cl$	- -	30 min	N.R

4.3 Effect of benzo[*b*]cyclopropene and 1*H*-naphtho[*b*]cyclopropene on some climacteric fruits and wax flowers

Having shown that a mechanism similar to the proposed mode of action of 1-MCP could be envisaged, both compounds were tested with fruits and flowers. The compounds benzocyclopropene and 1*H*-naphtho[*b*]cyclopropene were tested by Dr. Shamim A.K.U Khan and Prof. Zora Singh at the Department of Environment & Agriculture. The compounds were tested on seasonal flowers and fruits. Various mature climacteric fruits were used in this study '*Tegan Blue*' Japanese plum, '*Arctic Pride*' nectarine, '*Kensington Pride'* mango, '*Cripps Pink*' and '*Jazz*' apple and wax flower ('WX17' and 'WX73'). Fruits were gathered freshly in early morning free from defects, blemishes and diseases, flowering stems of flower (*Chamelaucium Desf*.) were collected from mature bushes grown at Department of Agriculture and Food Western Australia (DAFWA).

4.4 Effect of cycloproparenes on abscission of flower (%) and vase life in wax flower.

Gratifyingly benzocyclopropene effectively inhibited the action of ethylene on wax flowers. The qualitative effect can be seen in the photos in Figure 4.2. In the control experiment Geraldton wax flowers were monitor with and without exposure to ethylene (10 μLL^{-1}). The effect is stark. Exposure to ethylene leads to complete flower loss (abscission) whereas the unexposed flowers remain reasonable intact. When fumigated with benzocyclopropene, both sets of flowers, either exposed or not exposed, to ethylene retained their flowers. This indicates that benzocyclopropene prevents the action of ethylene does not deteriorate the plant.

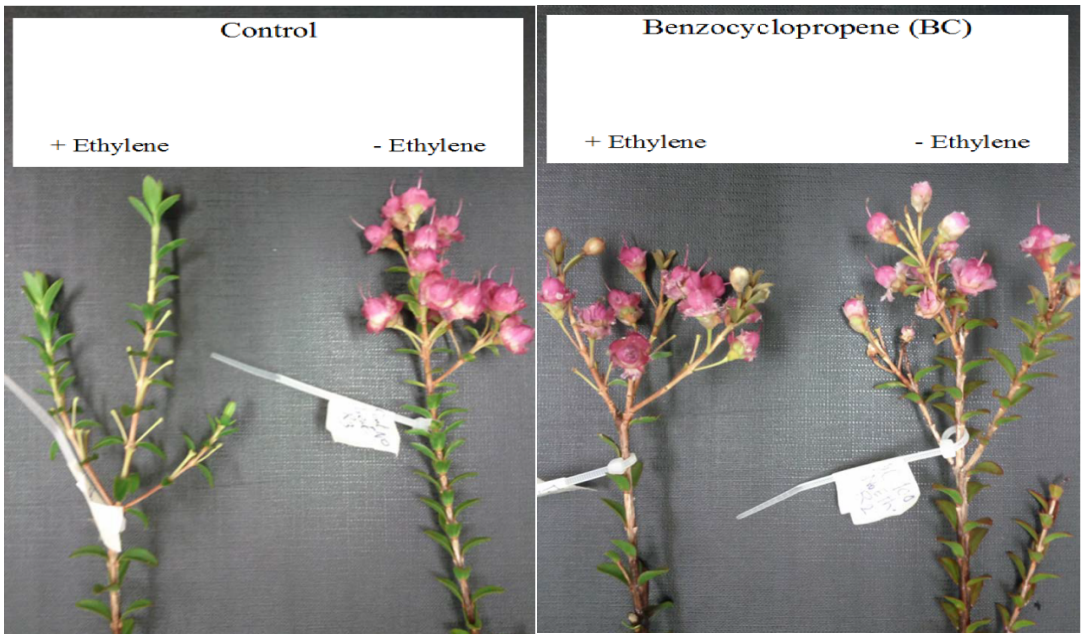


Figure 4.2 Effect of benzocyclopropene on abscission of flower (%) and vase life in wax flower.

4.5 'WX17' and 'WX73' wax flowers

To obtain quantitative data, the number of abscised flowers was counted and the percentage flower fall calculated. Fumigation with benzocyclopropene **47** (100 nLL^{-1}) followed by exposure to ethylene (10 μLL^{-1}) significantly reduced the abscission of flower in wax flower (WX17). Abscission of 50% flowers happened more than four days later than the flowers treated with ethylene alone. The flowers that treated with concentrations of benzocyclopropene **47** (10 $nLL^{-1}$and 50 nLL^{-1}) did show significant difference with flowers that treated with ethylene (10 μLL^{-1}) alone where the same percentage of flower fallen within 2 to 3 days of treatment. Fumigation of wax flowers (WX73) with benzocyclopropene **47** (50 nLL^{-1}) followed by treatment with ethylene (10 μLL^{-1}) exhibited significantly reduced loss of floral organs to about 6 days than that treated with ethylene alone. Flowers treated with benzocyclopropene **47** (100 nL L^{-1}) followed by (10 μLL^{-1}) treatment of ethylene where less potent and 50% flowers abscessed by 2 days after treatment.

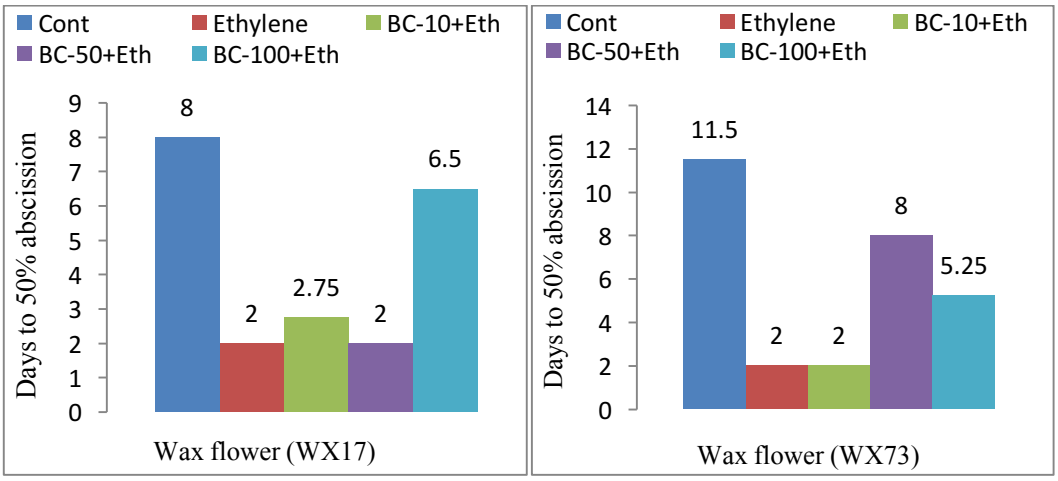


Figure 4.3 Effect of different concentration of benzocyclopropene **47** on the 50% abscission of wax flowers (WX17 and WX73)

1*H*-Naphtho[*b*]cyclopropene like benzocyclopropene showed good activity as ethylene antagonist. It successfully delayed flower abscission in wax flowers. The treatment of 1*H*-naphtho[*b*]cyclopropene **48** (100 nLL^{-1}) also significantly ($P \leq 0.05$) suppressed the rate of flower/bud abscission in WX73 (0%) and WX107 (22.82%) wax flowers in comparison to the ethylene treated flowers. Suppressed flower/bud abscission was also observed in WX73 and WX107 wax flowers (38.11% and 25.51% respectively), even when the 1*H*-naphtho[*b*]cyclopropene **48** treatment was followed by ethylene treatment (10 μLL^{-1}) (Figure 4.9). The highest level of flower/bud abscission in all genotypes was noted from the ethylene treated flowers.

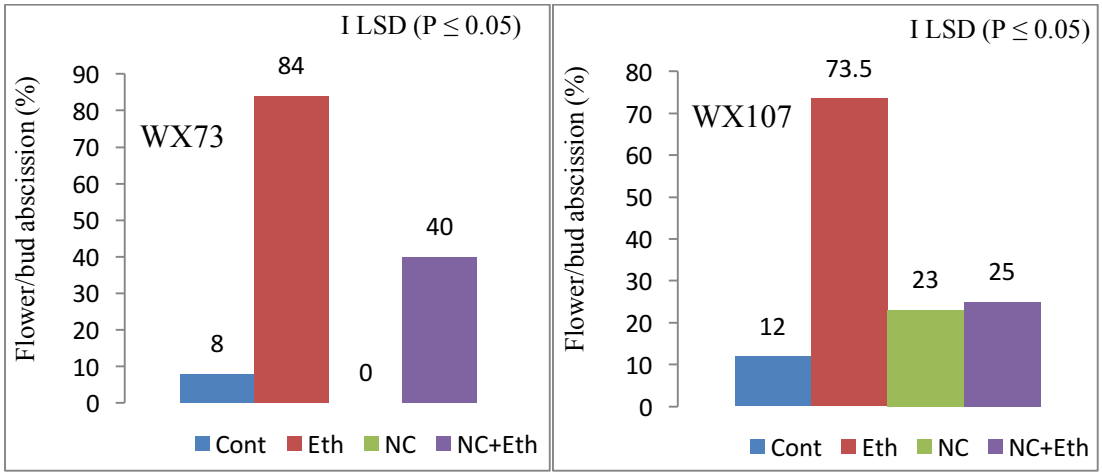


Figure 4. 4 Percent of flower/bud abscission after four days of treatment with 1*H*-naphtho[*b*]cyclopropene (100 nLL^{-1}) on flower/bud abscission (%) in ‘‘WX73’ and ‘WX107’ wax flowers.

4.6 Effect of Benzo[*b*]cyclopropene and 1*H*-naphtho[*b*]cyclopropene on some climacteric fruits

Fruits, despite having been detached from the tree are living organism and continue to respire during the postharvest. Respiration continues through utilization of stored carbohydrates and affects the quality of fruits such as flavor, sweetness, water content and weight. So reducing the rate of respiration is important to extend the postharvest life of fruits.[126] There is a correlation between the rate of respiration and

the ethylene production during fruit ripening stages. For example, a sharp rise in ethylene production of "*Mcintosh*" apples is followed by the rise in CO_2 production.[127] In this study some fruits were treated with different concentrations of benzocyclopropene and 1*H*-naphtho[*b*]cyclopropene (0 and 100 nLL^{-1}) by using 60 L sealed plastic containers then ethylene production and respiration were determined during the ripening stage.

4.6.1 Effect of benzocyclopropene 47 on climacteric ethylene production and respiration rate in 'Tegan Blue' Japanese plum and '*Arctic Pride'* nectarine

Mature fruit of the Japanese plum '*Tegan Blue*' (*Prunus salicina Lindl*.) were treated with benzocyclopropene (0 and 100 nLL^{-1}). The result showed suppressed ethylene production (0.85 fold) and delayed climacteric peak by 2 days in comparison to the control fruits. The respiration climacteric peak occured 8 days after treatment of '*Tegan Blue*' plum fruit with benzocyclopropene (100 nLL^{-1}) alone and exhibited a suppressed climacteric respiration peak of 0.28 mmol CO_2 $Kg^{-1}h^{-1}$. These results are also comparable to 1-MCP at (100 nLL^{-1})

The onset of climacteric peak of ethylene was not affected by the treatment compared to the control ones and the climacteric ethylene peaked on the 9^{th} day after treatment. The fruit treated with benzocyclopropene **47** (50 nLL^{-1}) produced a significantly suppressed climacteric peak of ethylene (0.76-fold) compared to control fruit. Whereas the climacteric respiration peak of the control '*Arctic Pride'* nectarine peaked on the 10^{th} day after the treatment, the respiration climacteric peak was suppressed when the fruit treated with benzocyclopropene **47** (50 nLL^{-1}), compared to the control.

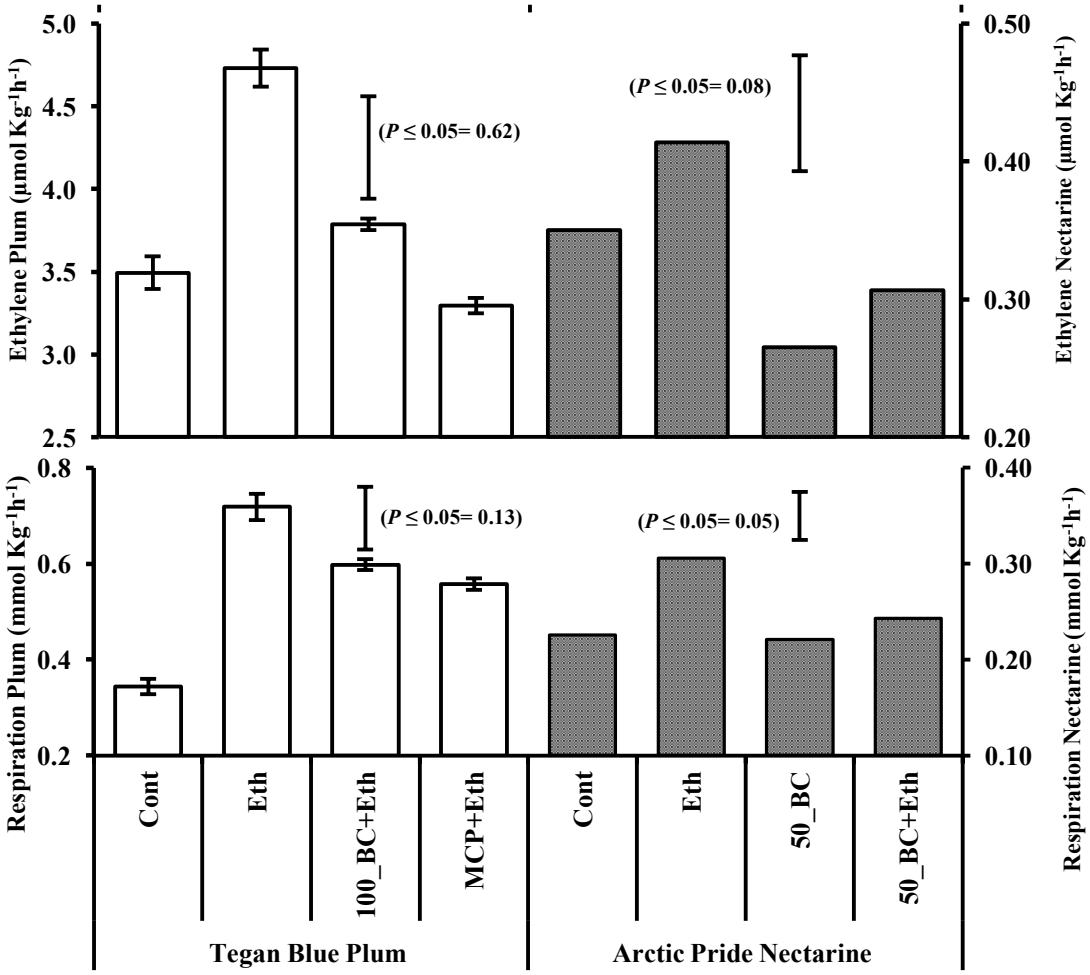


Figure 4.5 Concentration of ethylene and CO_2 on the day of climacteric peak, vertical bars represent S.E. of means and are invisible when the values are smaller than the symbol. n = 5 replications.

4.6.2 Effect of benzocyclopropene 47 on climacteric ethylene production in 'Kensington Pride' mango

The climacteric ethylene peak in the control '*Kensington Pride*' mango fruit was observed on the 4th day after treatment whilst, the benzocyclopropene **47** (50 nLL^{-1}) treated fruit exhibited significantly suppressed ethylene peak (0.92-fold) compared to control fruits.

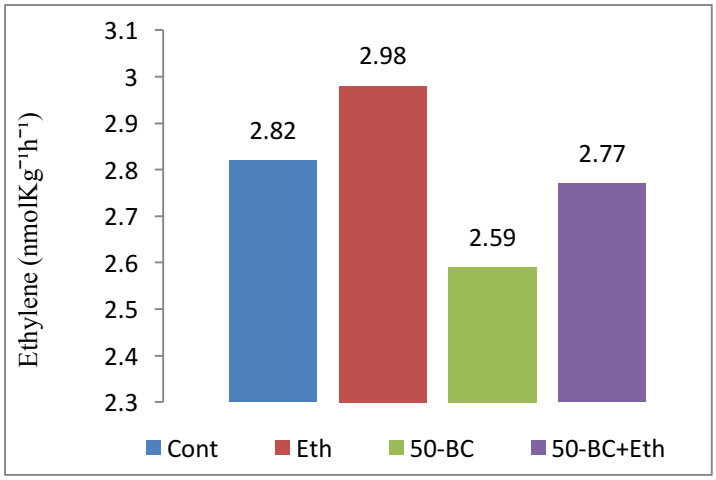


Figure 4.6 Ethylene production at day 4 in '*Kensington Pride*' mango treated with (50 nLL^{-1}) and ethylene (10 μLL^{-1}) alone or their compensation.

4.6.3 Effect of benzocyclopropene 47 on climacteric ethylene production and respiration rate in 'Cripps Pink' apple

Treating '*Cripps Pink*' apple fruit with benzocyclopropene **47** (1, 10, 100 nLL^{-1}) delayed the climacteric ethylene peak more than week than the control fruit, the climacteric peak was observed on 14th day after treatment with benzocyclopropene (1000 nLL^{-1}). Suppression of the climacteric peak of ethylene was significant when treated with benzocyclopropene **47** (1, 10, 100 and 1000 nLL^{-1}). Similar to climacteric ethylene production peak, the climacteric respiration peak in '*Cripps Pink*' apple fruit was observed on the 14th day after treatment and the treatment exhibited suppression climacteric of CO_2 (0.96, 0.92 and 0.95-fold) when treated with benzocyclopropene **47** (10, 100 and 1000 nLL^{-1}) respectively compared to the control fruit.

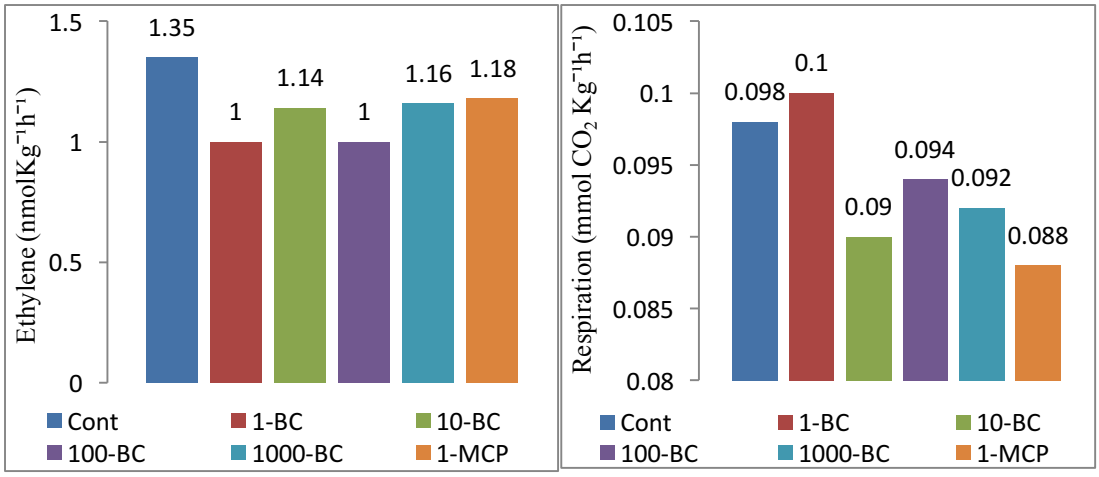


Figure 4.7 Change of endogenous levels of ethylene and respiration rate in '*Cripps Pink*' apple on the 14th day after treatment.

4.6.4 Effect of 1*H*-naphtho[*b*]cyclopropene 48 on 'fortune' plum

'*Fortune*' plum fruit was treated with different concentrations of 1*H*-cyclopropa[*b*]naphthalene **48** (1, 10, 100, 1000 nLL^{-1}). The results exhibited some changes in ethylene production at climacteric peak in '*Fortune*' plum as described in the graph in Figure 4.8. Treatment of plum fruit with 1*H*-cyclopropa[*b*]naphthalene **48** showed delay of the climacteric ethylene production by 4 to 6 days compared to the control fruits. The production of ethylene at the climacteric peak of fruit treated with ethylene alone was higher than the control one, whereas the production of ethylene in fruits that treated with 1*H*-naphtho[*b*]cyclopropene **48** followed with ethylene was suppressed and showed less production of ethylene than the control fruits. The rate of respiration increased steadily till day 10 after treatment then showed a sharp decline on day 12 after treatment in all concentrations of 1*H*-cyclopropa[*b*]naphthalene **48** treated fruits. The respiration rate was significantly suppressed in the fruit treated with 1*H*-cyclopropa[*b*]naphthalene **48** (1 and 10 nLL^{-1}).

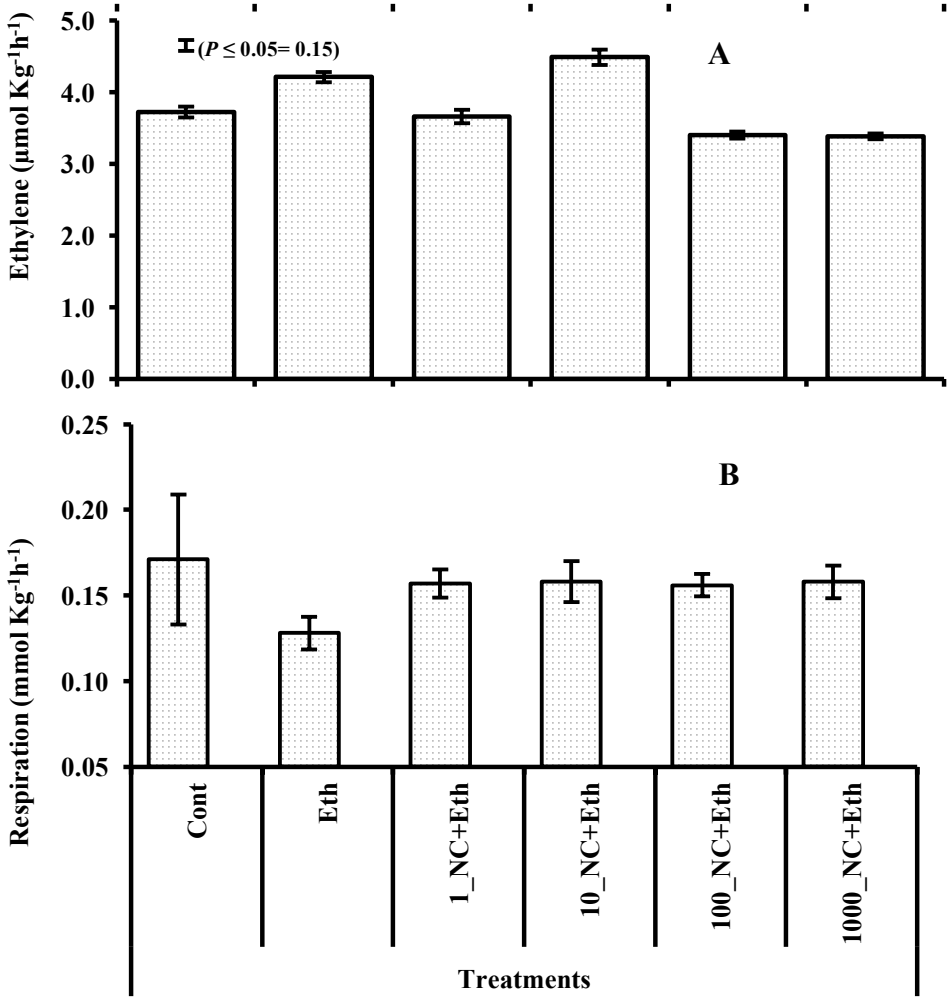
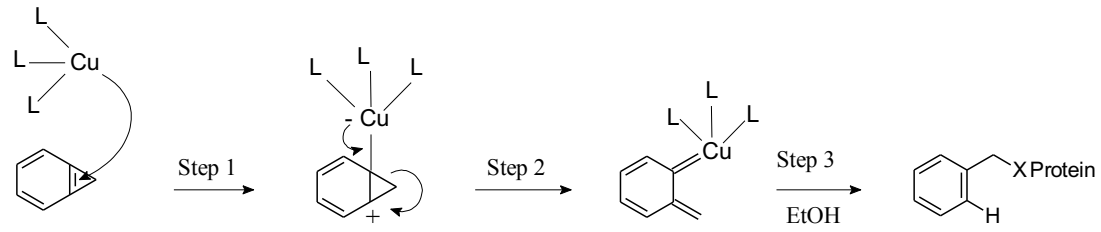


Figure 4.8 Concentration of ethylene (A) and CO_2 (B) on the day of climacteric peak in '*Fortune*' plum fruit treated with different concentration of 1*H*-naphtho[*b*]cyclopropene.

In conclusion, both benzocyclopropene and 1*H*-naphtho[*b*]cyclopropene are potent antagonists of ethylene action. They also react similarly to the proposed mode of action of 1-MCP in the presence of copper(I) salts. They did not show any toxicity on fruit or cultivars of wax flowers. Thus, the cycloproparenes are a new class of compounds that can act as antagonist of ethylene action, which are easier to handle than 1-MCP. The proposed mode of action of the cycloproparene is shown in Scheme 4.13. The copper ion coordinates the medial double bond and promotes the reaction of benzocyclopropene to the carbenoid intermediate **173.** This intermediate reacts with neighbouring protein residue to form a covalent bond and inactivate the ethylene receptor.



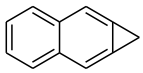
171 **172** **173** **174**

Scheme 4.13 Proposed reaction of benzocyclopropene with the ethylene receptor ETR1

Chapter 5

Benzocyclopropene and 1*H*-naphtho[*b*]cyclopropene are effective inhibitors of fruit ripening and flower senescence. However these molecules are hydrocarbons and poorly water soluble. Water solubility is important for the practical application of these compounds in agriculture and horticulture. For example horticultural produce could be sprayed with an aqueous solution of cyclopropenes to retard ripening or senescence. The cycloproparene could be dissolve in water using an organic solvent (such as ethanol) or a surfactant (such as Tween 20), however this may affect the quality of the horticultural produce. No water soluble or partially water soluble cycloproparenes have been made to date and is due to their fairly restricted methods of synthesis, so this would be a challenge.

47



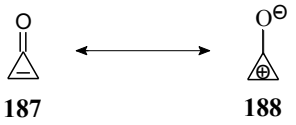
48

Figure 5.1 Benzocyclopropene **47** and 1*H*-naphtho[*b*]cyclopropene **48** ethylene antagonists

One of the targets in this work was to find water soluble compounds that can be active as ethylene antagonist. To make these compounds water soluble a polar functional group needs to be attached to the cycloproparenes, such as carboxylate, hydroxyl, ammonium group or a polyethylene glycol chain. Some attempts have been reported by Grichko to make water soluble cyclopropenes.[128]

The potency of cyclopropene derivatives as ethylene antagonists varies substantially depending on their molecular structure. A collection of cyclopropene derivatives were prepared and tested for their solubility in water and their ethylene antagonist activity. Cyclopropenes with alcohols, carboxylic acids, amines and amino acids were readily soluble in water however their potency as ethylene antagonists varied

greatly. For example, cyclopropenone was water soluble however it was inactive as an antagonist. The lack of antagonism can be related to aromatic cycloproplium cation, which would impede the coordination for the cyclopropene with the copper(I) cofactor in ETR1.



187 **188**

Highly water soluble and inactive

Figure 5.2 The resonance form of cyclopropenone

In light of the above example, substitution of the cyclopropene was then investigated. A cyclopropene with a carboxylic acids derivative at the 3 position **189** greatly reduces the potency of the ethylene antagonist.[126] Presence of NH_3^+ group on 2-(aminomethyl)-2-cyclopropene-1-carboxylic acid **190** appears to decreased the activity compared to its analogue, 2-cyclopropene-1-carboxylic acid **189.** The extra steric requirement of the aminomethyl group is likely to impede the binding of the compound to the binging site of the ethylene receptor.

189 **190**

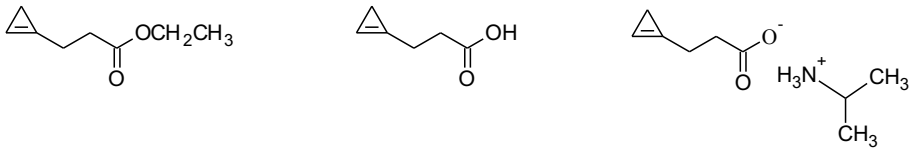
Highly soluble
740 ± 79 nL/L

Highly soluble
1200 ± 400 nL/L

Figure 5.3 Effect of NH_3^+ group on 2-(aminomethyl)-2-cyclopropene-1-carboxylic acid **174**

Having a cyclopropene with a carboxylic acid derivative on an extended carbon chain appears to provide a balance between potency and water solubility. In this way, the electron withdrawing carboxylic acid derivative does not affect the chemistry of

the cyclopropene, yet it can solubilize the molecule in water. The three cyclopropene derivatives in Figure 5.4 all have moderate antagonistic properties and are all water soluble.



191

Soluble
160 ± 66 nL/L

192

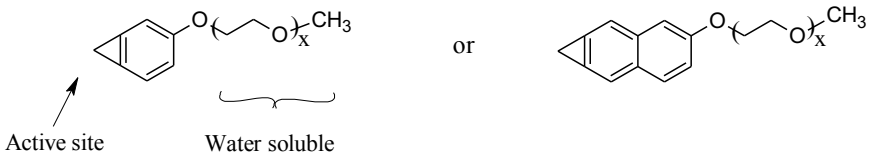
Soluble
410 ± 76 nL/L

193

Soluble
214 ± 79 nL/L

Figure 5.4 Ethylene antagonist activity of water-soluble carboxylic cyclopropene

Based on the above observations, the synthetic target for water-soluble cycloproparenes is drawn in Figure 5.5. The water solubilizing group, a polyethylene glycol, will be attached to the cycloproparene in a position that is furthest away from the cyclopropene ring fusion. The polyethylene glycol chain would allow a Billups-like protocol to be used in their synthesis as it is one of the few functional groups that would survive the strongly basic conditions used.

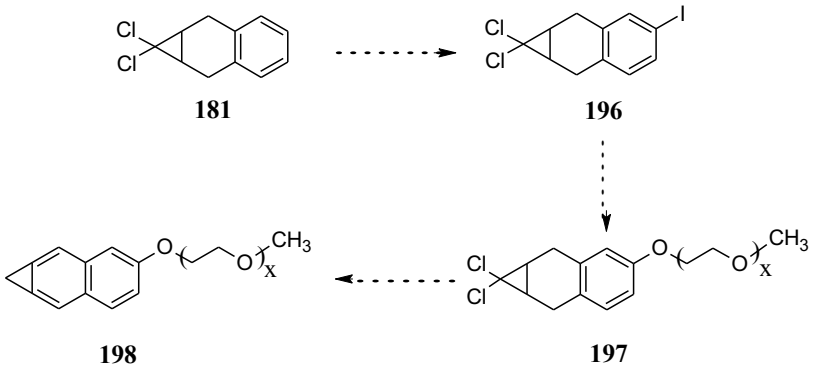


194 **195**

Figure 5.5 Introduce an ethylene glycol chain onto cycloproparene

5.1.1 Synthesis of a water soluble 1*H*-cyclopropa[*b*]naphthalene derivative

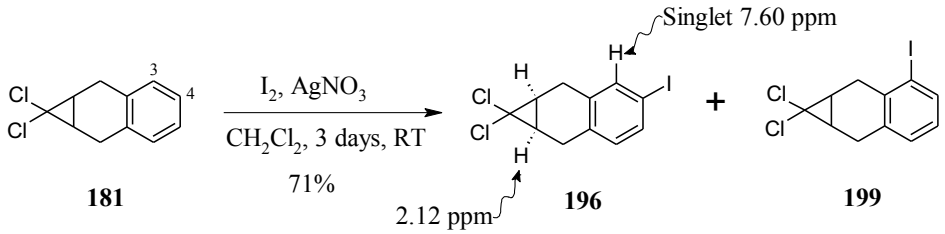
One of the most straightforward synthetic pathways to a water soluble cycloproparene is the one described in Scheme 4.9. It does not deviate significantly from the synthesis of the parent compound described in Chapter 4. The dichlorocarbene adduct **181** can be made on a reasonable scale. Electrophilic aromatic iodination could give the iodinated derivative either in the 3 or 4 positions, however there is a risk that the cyclopropane ring of **196** may ring open under the harsh reaction conditions. Hosseinzadeh *et al*. converted aryl iodides to ethers in the presence of KF/Al_2O_3 and CuI. Using these conditions the iodide **196** could be converted to aryl ether **197**. The treatment of this compound under the standard conditions described by Billups should afford the cycloproparene.



Scheme 5.1 Proposed synthesis of a water soluble naphthalene derivative

The iodination of **181** can be performed by reacting $AgNO_3$ with I_2 to form of I^+.[129] Iodination of **181** could give two possible products giving iodides at position 3 (**196**) or at position 4 (**199**). When the dichlorocarbene adduct **181** was reacted with iodine and silver nitrate at room temperature for 3 days, two new products was isolated. Thankfully, the cyclopropane ring remained intact and products were identified as the isomeric iodide **196** and **199** in a 6 : 1 ratio. The iodide **196** was the predominant product and isolated in 71% yield. The ^{1}H NMR spectrum of **196** showed a triplet at 2.12 ppm due to the bridgehead hydrogens which indicated the cyclopropane ring was intact, two multiplets at the range of 2.62 to 3.30 ppm belonging to 4 hydrogens

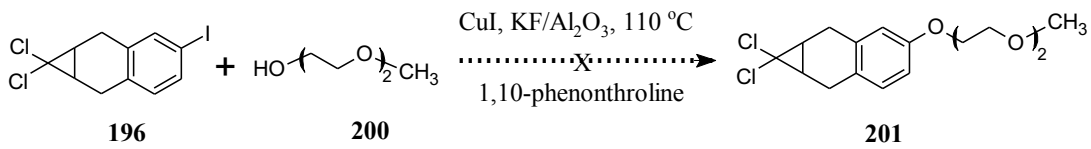
that were similar to the hydrogens on the cyclohexane ring of the starting material. Two doublets at 6.84 and 7.45 ppm for the two adjacent hydrogens on the aromatic ring and singlet at 7.60 for the other hydrogen on the aromatic ring indicated the substitution pattern shown. Unfortunately the isomers were inseparable.



181 **196** **199**

Scheme 5.2 Iodination of **181**

Etherification of the iodides **196** and **199** was attempted by using the same reaction conditions that was reported by Hosseinzadeh. It was shown that KF/Al_2O_3, CuI and 1,10-phenanthroline promoted the C-O coupling of aryl iodide with polyethylene glycols. Using the reported conditions a mixture of the iodides **196** and **199** (1 equiv.) and **200** (30 equiv.) were added CuI (0.1 equiv.) and 1,10-phenanthroline (0.2 equiv.) and then followed by KF/Al_2O_3 (5 equiv.) was heated under reflux at 110 ^oC for 18 hrs. Unfortunately only starting material was recovered and longer reaction times lead to degradation of the starting material.

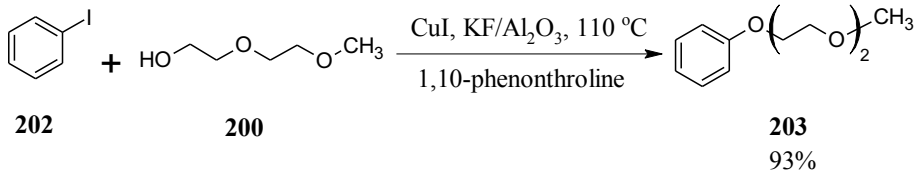


196 **200** **201**

Scheme 5.3 Attempted etherification of **196**

To ensure that the etherification reaction was been performed correctly, one of the reactions described by Hosseinzadeh was attempted. Iodobenzene **202** and 2-(2-

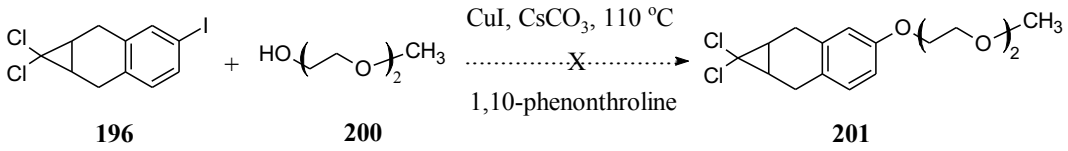
methoxyethoxy)-ethanol **200** where reacted using the same conditions as above. It afforded the aryl ether in excellent yield (93%), which suggests the substrate **196** is the cause for the lack of reactivity.



202 **200** **203**
93%

Scheme 5.4 Model reaction of Hosseinzadeh conditions

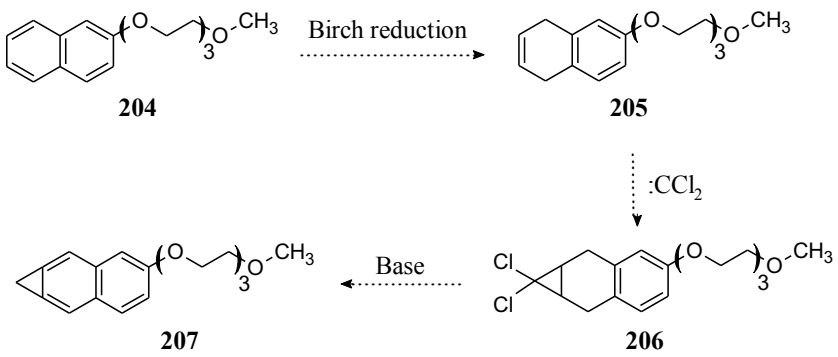
In a similar paper, KF/Al_2O_3 was substituted with cesium carbonate to give aryl ether **201** in good yield (70% - 94%).[130] When a mixture of cesium carbonate (2 equiv.), 1,10-phenanthroline (0.2 equiv.) and CuI (0.1 equiv.) compounds **196** and **199** (1 equiv.) and **200** (30 equiv.) in toluene was stirred at 110 °C for 24 hrs, no aryl ether was observe. The ^{1}H NMR spectrum of the crude reaction mixture showed a complex mixture of products.



196 **200** **201**

Scheme 5.5 Etherification of **196** with CuI, 1,10-phenanthroline and $CsCO_3$

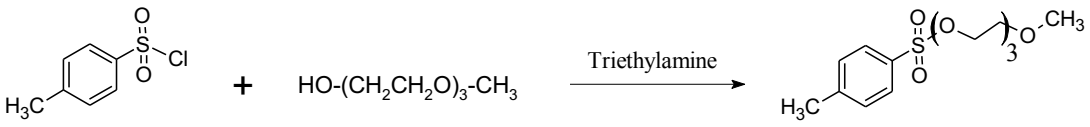
Since the iodination of the dichlorocarbene adduct follow by etherification was unsuccessful an alternative pathway to **206** was envisaged. This method introduces the ether at the earliest stage of the synthesis. A Birch reduction of the naphthyl ether **204** could give **205**. Dichlorocarbene can then be added to the alkene in **205** under the same reaction conditions that were used to prepare the dichlorocarbene adduct **181.** Treatment of **206** with a strong base should afford 1*H*-naphtho[*b*]cyclopropene derivative following the Billups protocol.



204 **205** **207** **206**

Scheme 5.6 Revised synthetic pathway

The naphthyl ether **204** was made in a two-step process. The required tosylate **210** was obtained by tosylation of triethylene glycol monomethyl ether as described by Fu.[131] Triethylene glycol monomethyl ether **209** was tosylated with excess of 4-toluenesulfonyl chloride **208** in presence of trimethylamine to afford the pure tosylate **210** as a viscous liquid (71% yield). The ^{1}H NMR spectrum shows two singlets at 2.44 and 3.37 ppm assigned $PhCH_3$ and OCH_3 respectively, two apparent doublets at 7.80 and 7.34 ppm belonging to four protons of the aromatic ring and multiple signals from 3.53 to 4.21 ppm for the 12 hydrogen of the chain. The ^{1}H NMR spectrum identical to those reported by Szabo *et al*.[132]

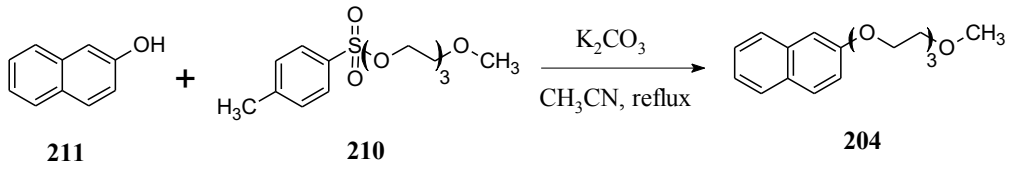


208 **209** **210**

Scheme 5.7 Reaction of **208** with **209**

The substitution reaction was undertaken using slightly modified conditions reported by Kimura and co-workers for the synthesis of [2-[2-(2-methoxyethoxy)ethoxy] ethoxy]benzene.[132] Thus treating a mixture of 2-naphthol **211** and **210** with potassium carbonate in acetonitrile under reflux for 36 hrs gave the desire product ether in 35% yield. The ^{1}H NMR spectrum shows multiplets between 7.80 to 7.11

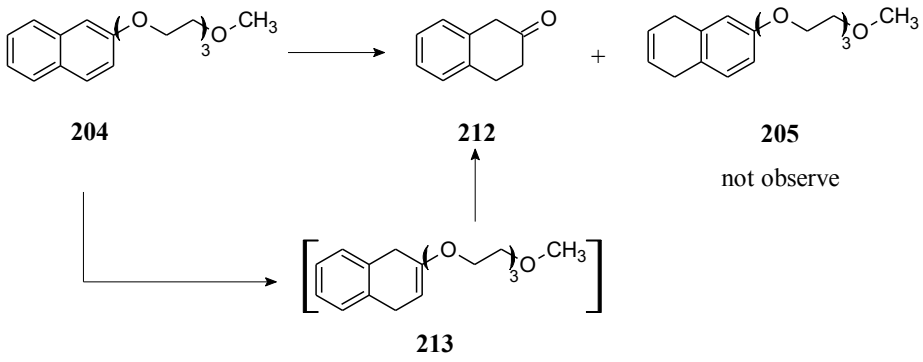
ppm assigned to 7 hydrogens of the aromatic rings, other multiplets from 3.60 to 4.30 ppm belonging to 12 hydrogens of the chain and singlet at 3.38 ppm for methyl group.



211 **210** **204**

Scheme 5.8 Synthesis of 2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]-naphthalene.

Unfortunately the reduction of the naphthyl ether **204** did not give the desired product. When the ether **204** was reduced under the same conditions as the reduction of naphthalene to 1,4-dihydronaphthalene (Scheme 10, Chapter 4), a new product was observed. The ^{1}H NMR spectrum had 4 aromatic signals (7.25-7.10 ppm), singlet at 3.59 ppm for one hydrogen, and two triplets at 3.07 and 2.56 ppm belonging to 4 hydrogens. The compound was identified as 2-tetralone. The formation of this product is as follows. The Birch reduction occurs at the more electron rich benzene ring to give the vinyl ether **205,** and then upon acidic workup the vinyl ether hydrolyses to the ketone to give 2-tetralone.



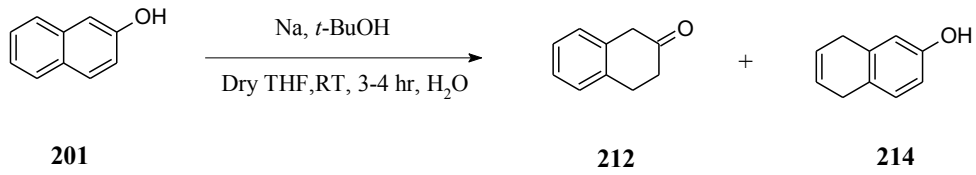
204 **212** **205**

not observe

213

Scheme 5.9 Reduction of 2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]-naphthalene

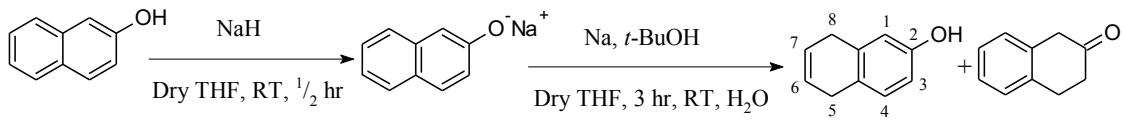
In light of this result, investigation into the reduction of 2-naphthol **211** was conducted. Small pieces of sodium metal were added to a solution of **211** in THF, stirred for 1 hour then *tert*-butanol was added and stirred for 3 hrs. The majority of the product of the reduction of 2-naphthanol **211** with sodium metal was 2-tetralone **212** with some traces of 5,8-dihydro-2-naphthalenol **214**.



201 **212** **214**

Scheme 5.10 Reduction of 2-naphthanol

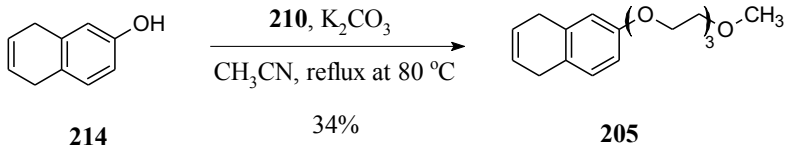
Conversion of 2-naphthol **211** to the naphthoxide salt **215** may favor the reduction of the other aromatic ring. 2-Naphthol **211** was treated with sodium hydride to get sodium 2-naphtholate **215.** This salt was then treated with sodium metal by the same manner described above and acidified to afford 5,8-dihydro-2-naphthol **214** in (24% yield) and 2-tetralone **212** (53% yield). ^{1}H NMR spectrum showed singlet at 7.74 ppm for the hydrogen at position 1, two doublets at 7.67 and 6.98 ppm for hydrogens at positions 3 and 4 respectively, triplet at 5.89 ppm assigned to the hydrogens on the double bond and multiplets at 3.33 ppm for C-5 and C-8 hydrogens. The ^{1}H NMR spectrum was identical to the data that reported by Marshall and Deghenghi.[132]



211 **215** **214** **212**

Scheme 5.11 Reduction of sodium 2-naphthanoxide

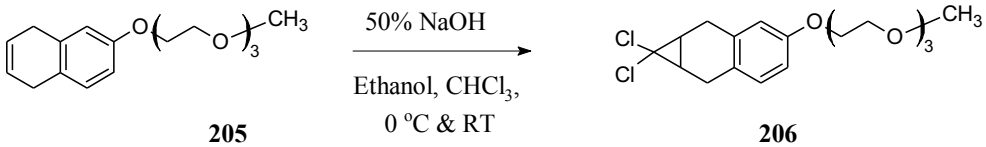
Etherification of 5,8-dihydro-2-naphthol **214** was performed using the same method described earlier for preparation of **204**. Under these conditions the ether **205** was obtained in 34% yield. The ^{1}H NMR data showed the same signals as the ^{1}H NMR spectrum of starting material **214** multiple signals at 7.07-7.82 ppm, a doublet at 5.90 ppm plus the new signals of the chain in the range of 3.50 to 4.20 ppm.



214 **205**

Scheme 5.12 Synthesis of 5,8-dihydro-2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy] naphthalene.

Having small amounts of **205** in hand, the addition of dichlorocarbene to the alkene was undertaken. The same reaction conditions were used as in the preparation of **181** (Chapter 4). Unfortunately only trace amounts of the adduct was observe. It had distinctive signals at 3.26 and 2.79 ppm indicative of the cyclopropane ring. The low yield may be due to the increased solubility of the product in water leading to low recovery. Since the 3 steps to this point were low yielding this approach was abandoned.



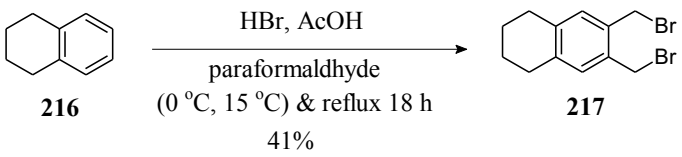
205 **206**

Traces

Scheme 5.13 Addition of dichlorocarbene to **205**

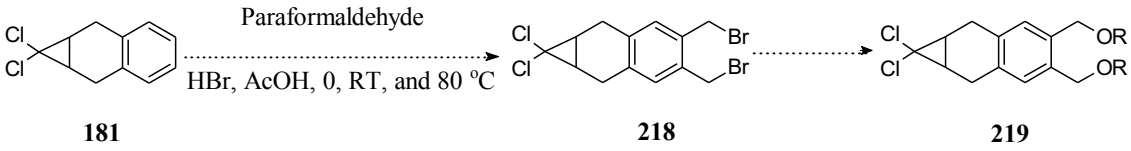
As direct etherification onto the aromatic ring proved to be difficult a simpler way was envisaged. Kotha reported that tetralin **216** could be bromomethylated with paraformaldehyde and HBr in acetic acid to give the bis-bromomethylated product **217**. A similar reaction could be envisaged for the preparation of the ether **221**.

Bromomethylation of **181** could give the dibromide **218.** The benzylic bromide should then undergo a substitution reaction with an alcohol to give the required ethers **218.**



216 **217**

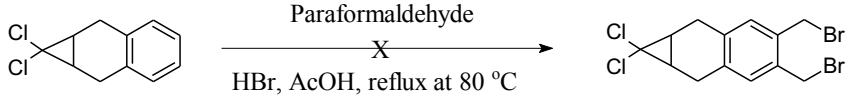
Scheme 5.14 Bromomethylation of tetralin **216**



181 **218** **219**

Scheme 5.15 Synthesis of an ether adduct via bromomethylation of **181**

A mixture of **181** and paraformaldehyde were added rapidly to HBr in glacial AcOH at 0 °C then heated to 80 °C for 18 hrs. The ^{1}H NMR spectrum of the crude reaction mixture was messy and shown no characteristic methylene signals for the product.

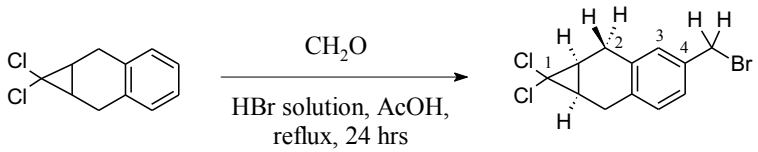


181 **218**

Scheme 5.16 Bromomethylation of **181** using paraformaldehyde, acetic acid and hydrogen bromide

The replacement of paraformaldehyde with aqueous formaldehyde solution (25% $^{W}/_{V}$) gave excellent results. When a solution of formaldehyde, hydrobromic acid and

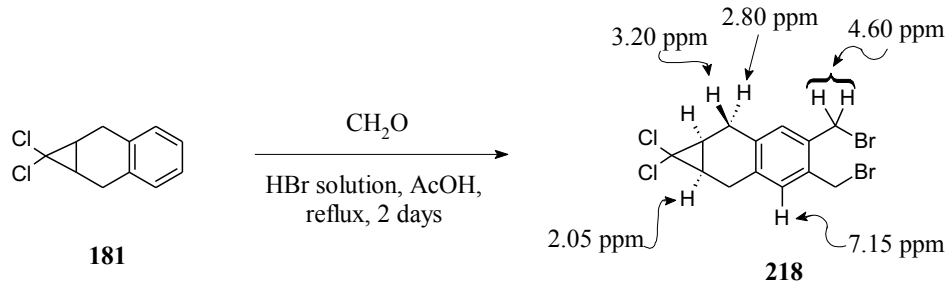
181 were heated under reflux for 24 hours, white crystals of 4-bromomethyl substituted product **220** was obtained in 65% yield. ^{1}H NMR spectrum showed two doublets at 7.15 ppm and 7.07 ppm integrating for 2 adjacent hydrogen atoms and singlet at 7.13 ppm for the other hydrogen on the aromatic ring, a singlet at 4.45 ppm integrating to 2 hydrogens of the CH_2Br, two doublets at 3.21 and 2.79 ppm assigned to 4 hydrogens of cyclohexane ring and triplet at 2.05 ppm assigned to 2 hydrogen on the bridge between cyclopropane and cyclohexane rings. ^{13}C NMR spectrum showed 12 signals showing an extra carbon atom in the molecule.



181 **220**

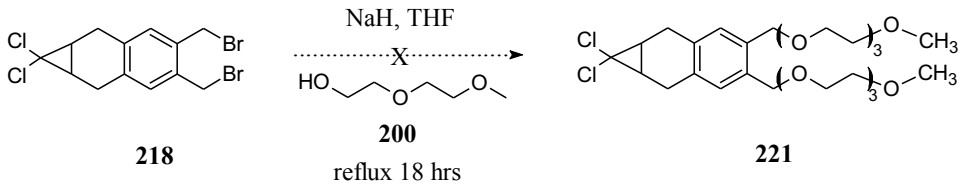
Scheme 5.17 Reaction of **181** by using formaldehyde and hydrogen bromide

To obtain dibromide **218,** the amount of hydrogen bromide was doubled and the reaction was heated for 2 days. White crystals dibromide **218** were isolated in 74% yield. The ^{1}H NMR spectra could be easily assigned. ^{1}H NMR showed two singlets at 7.15 ppm and 4.60 ppm integrating for 2 hydrogen on the aromatic ring and 4 hydrogens on CH_2Br respectively, two doublets at 3.20 and 2.80 ppm assigned to 4 hydrogens of cyclohexane ring and triplet at 2.05 ppm assigned to 2 hydrogen on the bridge between cyclopropane and cyclohexane rings. ^{13}C NMR spectrum showed 7 peaks due to the symmetry of the compound. It showed two signals at 135.4 and 134.4 ppm belonging to 2 carbons on the aromatic ring and 131.3 ppm assigned to CH of the aromatic ring. The cyclopropane was intact due to the signal at 65.9 ppm for CCl_2 carbon. The new bromomethyl groups were observed by a signal at 24.5 ppm.



Scheme 5.18 Reaction of **181** by using formaldehyde and hydrobromic acid

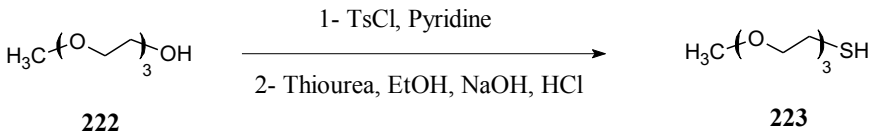
Etherification of adduct **218** could be conducted by using the alkoxide of **162** and performing a substitution reaction. A report by Teh-Chang[133] was followed to substitute bromine with 2-(2-methoxyethoxy)-ethanol **200** and formation of the ether compound **221**. Sodium hydride (1.00 mmol) was added to a solution of **200** (1.00 mmol) in THF followed by the addition of a solution of dibromide **208**. Unfortunately only starting material was recovered.



Scheme 5.19 Etherification of **218**

In light of the above reaction, a better nucleophile was prepared by the procedure of Foos and co-workers. Thiolates are good nucleophiles, ethane thiol-2-[2-(2-methoxy ethoxy)ethoxy] **223** was made.[134] The hydroxyl group of **222** was first converted to a good leaving group using *p*-toluenesulfonyl chloride in pyridine. The resulting oily tosylate was treated with a solution of thiourea in aqueous sodium hydroxide solution. The isolated yellow oily product was purified by vacuum distillation (60 ^{o}C, 1 torr) to obtain a yellow liquid of **223** in 54% yield. The ^{1}H NMR spectrum showed multiplets at 3.50 to 3.80 ppm for 10 hydrogens, singlet at 3.38 ppm for 3 hydrogens

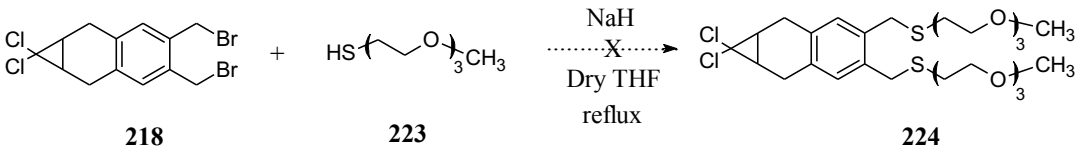
of methoxy group, doublet triplet at 2.70 ppm belonging to 2 hydrogen of SCH_2 and triplet at 1.58 ppm assigned to SH hydrogen. The ^1H NMR data was identical to that reported by Foos.[134]



222 **223**

Scheme 5.20 Synthesis of 2-(2-(2-methoxyethoxy)ethoxy)ethanethiol.

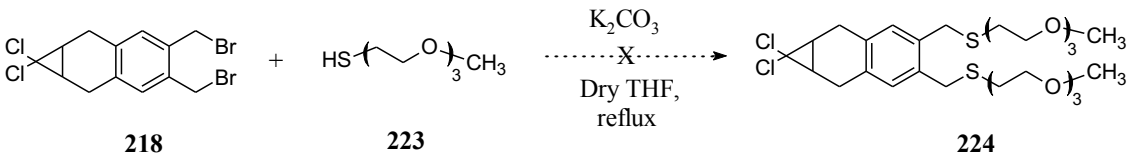
The substitution reaction of the dibromide **218** with the thiol **223** was performed using a method reported by Kantekin.[135] Sodium hydride was added to a solution of thiol **223** in dry THF and was heated under reflux for 24 hrs. Then a solution of the bromide **218** was added at room temperature. Unfortunately the desired product was not formed. ^1H NMR of the recovered material only showed degradation.



218 **223** **224**

Scheme 5.21 Reaction of brominated 218 with glycol thiol **223**

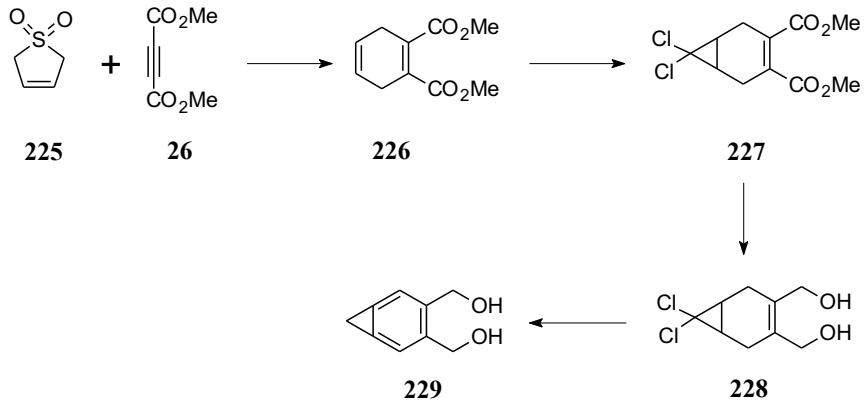
A weaker base was then used to avoid decomposing the starting bromide. Sodium hydride was replaced with potassium carbonate. The bromide **218**, thiol **223** and K_2CO_3 were heated under reflux for 12 hrs. Unfortunately this method also gave a complex mixture by ^1H NMR.



218 **223** **224**

Scheme 5.22 Reaction of **218** with **223** using K_2CO_3 instead of NaH

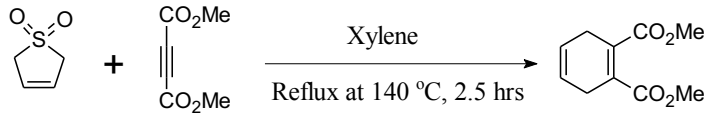
Because of the all previous attempts were ended in failure, the diol **229** became the new target for a water-soluble cycloproparene. The presence of a diol should make the molecule water soluble. Thus, the new synthesis would start from butadiene sulfone **225** and dimethylacetylenedicarboxylate (DMAD). A Diels-Alder reaction between butadiene, generated *in situ* by the cheletropic elimination of sulfur dioxide, and DMAD would afford the cyclohexadiene **226**. Addition of dichlorocarbene would add to the most electron rich alkene to give **227**. Then reduction of the diester **227** to the diol **228** followed by dehydrochlorination should give the water soluble benzocyclopropene **229**. (Scheme 5. 21).



225 **26** **226** **227** **228** **229**

Scheme 5.23 Synthesis of 3,4-dimethanolcyclopropa[*b*]benzene **229**

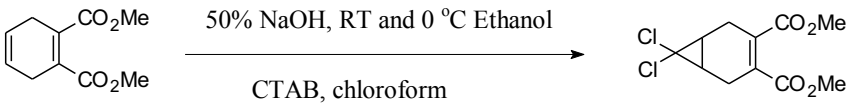
Dimethyl-1,4-cyclohexadiene-1,2-dicarboxylate **226** has been synthesized by reaction butadiene with dimethyl acetylene dicarboxylate **26**. DiFrancesco and Pinhas conduct the cycloaddition reaction between butadiene sulfone and dimethyl acetylene dicarboxylate in refluxing xylene.[136] At this temperature butadiene sulfone **225** releases 1,3-butadiene which then reacts with dimethyl acetylenedicarboxylate **226**. Used the same method, dimethyl 1,4-cyclohexadiene-1,2-dicarboxylate **226** was obtained in (47% yield). ^{1}H NMR spectrum was identical to that reported by DiFrancesco and Pinhas, a singlet at 5.70 ppm was assigned to the 2 vinylic hydrogens, a singlet at 2.99 ppm to the 4 allylic hydrogens and the singlets at 3.77 ppm were assigned to the ester methyl groups.



225 **26** 47% **226**

Scheme 5.24 Synthesis of dimethyl-1,4-cyclohexadiene-1,2-dicarboxylate.

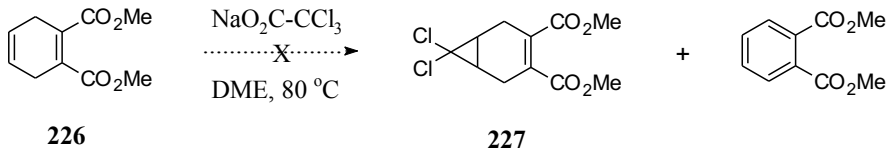
The same reaction conditions were use as described in Chapter 4, Scheme 10 for the addition of dichlorocarbene. This method gave only some trace amounts of the desired product. The low yield may be due to hydrolysis of the esters or Michael addition to the unsaturated ester.



226 **227**
Traces

Scheme 5.25 Synthesis of 7,7-dichloro-1,2-hexene-1,2-carboxylate

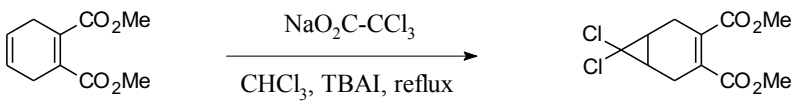
To avoid the strongly basic conditions used above reaction sodium trichloroacetate was used to generate dichlorocarbene.[137] A mixture of 1,4-cyclohexadiene-dicarboxylate **226** and sodium trichloroacetate in dimethoxyethane was heated at 80 ^{o}C but the final colorless liquid was not the desired product. 1,4-Cyclohexadiene-dicarboxylate **226** seems to have oxidized to dimethyl phthalate. The ^{1}H NMR spectrum showed two doublets at 7.73 and 7.54 ppm assigned to the aromatic ring and singlet at 3.66 ppm for the CH_3 on the ester group.



226 **227**

Scheme 5.26 Synthesis of 7,7-dichloro-1,2-hexene-1,2-carboxylate using NaO_2C-CCl_3.

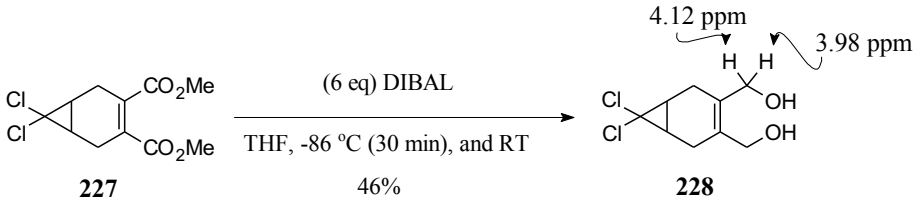
An alternative method using tetrabutylammonium iodide (TBAI) as a phase transfer catalyst to promote the generation of dichlorocarbene was used. Heating a mixture of **167**, sodium trichloroacetate, TBAI (0.01 mol%) in chloroform under reflux afforded the adduct in 86% yield. ^{1}H NMR and IR spectra confirmed the structure of the compound. ^{1}H NMR spectrum showed a singlet at 3.75 ppm assigned to the methyl group, multiplets at 2.80 ppm for 2 hydrogens on the bridgehead carbons, and two doublets at 2.55 and 1.95 ppm belonging to the 4 hydrogens on cyclohexene ring. The ^{13}C NMR spectrum had characteristic signals at 168.1, 131.7, and 64.1 for the ester, alkene and cyclopropane



226 86% **227**

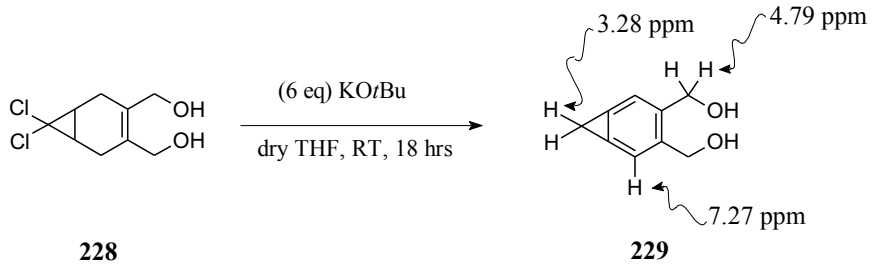
Scheme 5.27 Synthesis of 7,7-dichloro-1,2-hexene-1,2-carboxylate

The dichlorocarbene adduct **227** was reduced using diisobutylaluminumhydride (DIBAL) at -86 ^{o}C to afford the alcohol in 46% yield. The ^{1}H NMR spectrum showed two doublets at 4.12 and 3.98 ppm integrating for the 4 hydrogen of methanolic group. The bridgehead hydrogen appeared at 2.60 ppm. The signals of the other 4 hydrogens were still at the same positions 2.35 and 1.90 ppm.



Scheme 5.28 Synthesis of 7,7-dichloro-bicyclo[4,1,0]hept-3-ene-1.2-dimethanol.

Same procedure of dehydrochlorination that was described in Chapter 4 was applied to the diol **227**. The alcohol **228** was treated with potassium *tert*-butoxide in dry dimethyl sulfoxide at room temperature for 18 hrs. The ^{1}H NMR spectrum of the crude product showed that the compound was formed. It has a singlet at 7.27 ppm belonging to the aromatic ring, singlet at 4.79 ppm assigned to the 2 hydrogens on the methanol group and singlet at 3.28 ppm for the 2 hydrogens on the cyclopropane ring. Unfortunately the compound polymerized within several hours and further purification was not possible.



Scheme 5.29 Dehydrochlorination of 7,7-dichloro-bicyclo[4,1,0]hept-3-ene-1.2-dimethanol.

Although the synthesis of water soluble ethylene antagonist was not achieved, a number of new compounds were synthesized during the attempts of synthesis water soluble compounds. The water soluble benzocyclopropene-2,3-dimethanol was made but, it polymerized within, several hours and further purification was not possible.

Chapter 6

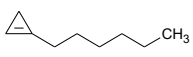
6.1 General Conclusions

The aim of the project was to find the potent ethylene antagonist compounds that can be used to control the ethylene action on fruits and flowers and therefore delay the fruits overripening and flower abscission. This would lead to a reduction in post-harvest losses of horticultural produce. 1-MCP is commercially used nowadays as an ethylene antagonist. It is a gas and subsequently it needs special requirements to apply. The target of this study was to find new ethylene antagonists that are stable, easy to handle and safe for people and the environment.

1-MCP has been shown to be effective inhibitor for the ethylene receptor in plants. Similar compound containing the strained three membered rings with different substitutions (1-hexylcyclopropene and 1,2,3,3-tetrachloropropene) were tested and their activity compared with 1-MCP. 1-HCP **112** and 1-MCP **16** showed high potency as ethylene antagonists, whereas TC **127** did not show any activity either as an ethylene agonist or antagonist. Cycloproparenes are cyclopropene analogs contain the same active site with more stability. Benzocyclopropene **47** and 1*H*-naphto[*b*]cyclopropene **48** were synthesized and their activities were tested. Both were showed good activity as ethylene antagonist on fruits and flowers with suppressed of ethylene production and respiration, thereby delaying fruit overripening and flower abscission.

1-Methylcyclopropene

16



1-Hexylcyclopropene

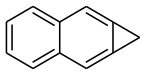
112

1,2,3,3-tetrachlorocyclopropene

127

47

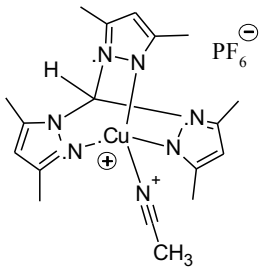
Benzocyclopropene



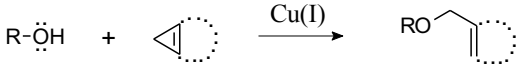
48

1*H*-Naphtho[*b*]cyclopropene

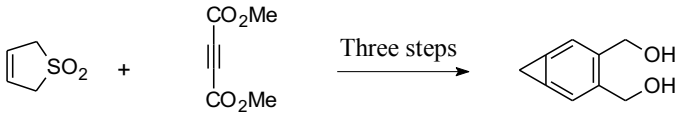
The copper complex $[(CH)Cu(I)(3,5\text{-}Me_2Pz)_3(CNMe)]PF_6$ **137** was synthesized to mimic the ethylene receptor in plants. The cyclopropenes and cycloproparenes that were applied on fruits and flowers were reacted with the copper complex **137** and ethanol. The active antagonists **115**, **16**, **112**, **47** and **48** reacted easily with alcohol in presence of the copper complex while those that were not (**104** and **127**) did not react. The strained three membered ring binds to the copper ion leading to ring opening and forms a copper carbenoid intermediate. This intermediate could then make covalent bonds with alcohol and form stable ether product.



137



Water soluble ethylene antagonists were one of the targets in this study and many attempts were conducted to synthesis these. Only benzocyclopropene-2,3-dimethanol was made, but it was unstable and it polymerized with in several hours.

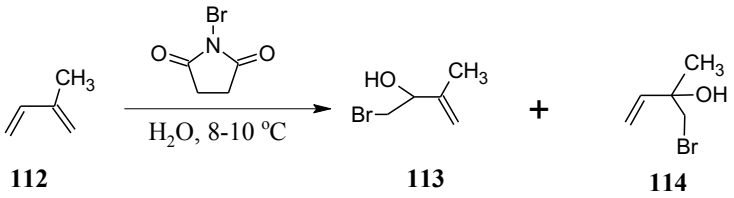


Chapter 7

7.1 Experimental

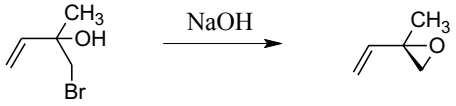
All reactions involving moisture or air-sensitive reagents were performed under a positive pressure of nitrogen. Glassware was dried in an oven at 120ºC and allowed to cool under nitrogen. All materials were obtained from commercial sources and used without further purification. NMR experiments were performed on a Bruker Ultra Shield Avance III 400 spectrometer (^{1}H, 400.1 MHz; ^{13}C, 100.6 MHz). Chemical shifts (δ) are expressed in ppm with reference to the solvent resonances of $CDCl_3$: (^{1}H, 7.26 ppm; ^{13}C, 77.16 ppm). Infrared spectra were recorded on a Perkin Elmer Fourier Transform-IR spectrometer 100 equipped with a ZnSe-diamond crystal ATR accessory; spectra were acquired between 4000-650 cm^{-1}. Melting points were determined on a Crown Scientific Barnstead Electrothermal 9100 apparatus. Flash chromatography was achieved using SiliaFlash® P60 silica gel (230-400 mesh, SiliaCycle, Canada) with the solvents stated. TLC was completed on Merck aluminum backed silica gel 60 F254 sheets and visualised by using short-wave UV light (λ = 254 nm) for aromatics and potassium permanganate solutions. A -86^{o}C bath was obtained by a solid/liquid slurry of ethyl acetate using liquid nitrogen.

7.2 3,4-Epoxy-3-methylbutene (isoprene oxide)



112 **113** **114**

A suspension of 2-methyl-1,3-butadiene **112** (20 g, 0.29 mol) and water (74.20 mL) was stirred vigorously at 8-10 ^{o}C. Then *N*-bromosuccinimide (52.33 g, 0.29 mol) was added in portions. The reaction mixture was stirred for a further 2 hrs at 10 – 22 ^{o}C. The resulting reaction mixture was extracted with diethyl ether. The organic phase was washed with water (100 mL) and with saturated sodium chloride solution (1 x 100 mL) and dried over anhydrous sodium sulfate. Diethyl ether was removed by vacuum distillation to get crude bromohydrin (32 g, 67%). The crude product mainly contained bromohydrin **114.** ^{1}H NMR: δ 5.90 (dd, *J* = 17.25, 10.7 Hz, 1H); 5.36 (dd, *J* = 17.25, 1.0 Hz, 1H); 5.19 (dd, *J* = 10.7, 1.0 Hz, 1H); 3.47 (s, 2H); 1.42 (s, 3H). The ^{1}H NMR data matches reported values by Arakelyan *et al.*[101] This crude product was used directly in the next stage.

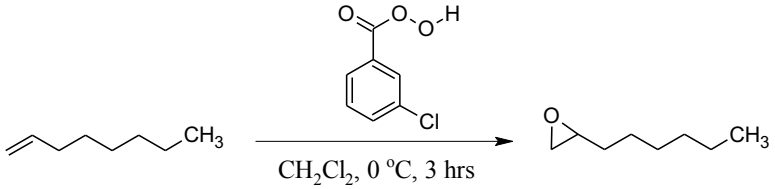


114 **115**

The forgoing crude bromohydrin (20.80 g, 0.126 mol) was added dropwise to vigorously stirred solution of aqueous sodium hydroxide (30% w/v, 33.6 mL) at 10 – 15 ^{o}C. The reaction mixture was stirred for 2 hrs. The organic layer was separated and dried over a minimum quantity of anhydrous sodium sulfate. The oil was distilled at 45 ^{o}C at atmospheric pressure to get pure isoprene oxide **115** as a colourless oil (7.10 g, 67%). ^{1}H NMR: δ 5.55 (ddd, *J* = 17.4, 10.7, 1.1 Hz, 1 H); 5.26 (dt, *J* = 17.4, 1.3 Hz, 1 H); 5.12 (dd, *J* = 10.7, 1.3 Hz, 1 H); 2.71 (dd, *J* = 5.3, 1.3 Hz,

1 H); 2.66 (dd, *J* = 5.3, 1.0 Hz, 1H); 1.36 (d, *J* = 1.4 Hz, 3H). The ^{1}H NMR data matches that reported by Arakelyan *et al*.[101]

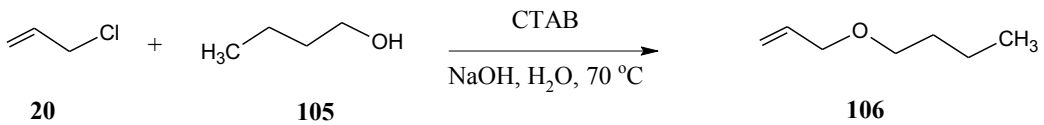
7.3 1,2-epoxyoctane



100 **104**

A solution of *m*-chloroperoxybenzoic acid (5.95 g, 0.035 mol) in dichloromethane (30 mL) was added to solution of 1-octene **100** (3.13 mL, 0.02 mol) in dichloromethane (20 mL). The reaction was stirred at 0^{o}C for 3 hrs then washed with saturated potassium carbonate solution (1 x 100 mL). The organic layer was dried over anhydrous sodium sulfate. Evaporation of the solvent under reduced pressure gave an oil (3.61 g, 75%) of 1,2-epoxyoctane **104**. ^{1}H NMR: δ 2.92 (ddt, *J* = 6.6, 3.8 Hz, 1 H); 2.77 (dd, *J* = 2.8, 3.8 Hz, 1H); 2.48 (dd, *J* = 5.1, 2.8 Hz, 1 H); 1.64-1.51 (m, 1 H); 1.50-1.42 (m, 1 H); 1.41-1.26 (m, 8 H); 0.91 (t, *J* = 6.6 Hz, 3 H). The spectra was identical to that reported by Corrêa *et al*.[152]

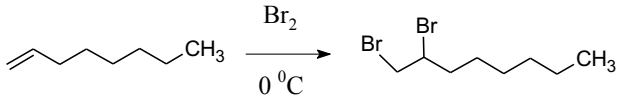
7.4 Allyl n-butyl ether



20 **105** **106**

A mixture of allyl chloride **20** (8.15 mL, 0.10 mol), 1-butanol **105** (7.42 mL, 0.10 mol), water (5 mL), cetyltrimethylammonium bromide (1.82 g, 0.005 mol) and sodium hydroxide (8.00 g, 0.20 mol) was heated to 70 ^{o}C with vigorous stirring overnight. The reaction was then allowed to cool and the solid residue was separated by filtration. The residue was washed several times with diethyl ether. The filtrate was dried over anhydrous sodium sulfate and concentrated under reduced pressure to give an oily residue. The oil was purified by distillation to afford the ether as a colourless oil (9.70 g, 97%). ^{1}H NMR: δ 5.91 (ddt, *J* = 17.2, 10.3, 5.6 Hz, 1H); 5.26 (dd, *J* = 17.2, 1.6 Hz, 1H); 5.15 (dd, *J* = 10.3, 1.6 Hz, 1H); 3.95 (d, *J* = 5.6 Hz, 1H); 3.74 (d, *J* = 2.4 Hz, 1H): 2.08-1.20 (m, 6H); 0.91 (t, CH_3). The ^{1}H NMR data identical to that reported by Nalet'ko *et al*.[98]

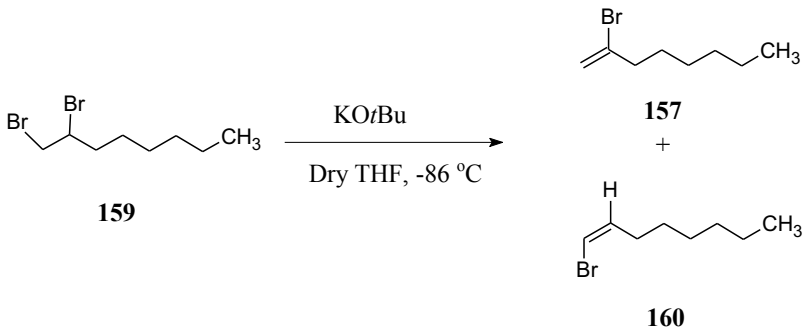
7.5 1,2-Dibromooctane



100 **159**

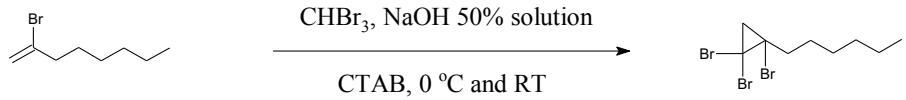
Bromine (9.30 g, 0.08 mol) was added dropwise to a stirred solution of 1-octene (30 mL, 0.27 mol) and dichloromethane (200 mL). The reaction was stirred at 0 ^{o}C until the solution was turned colorless. The mixture was concentrated under reduced pressure to give 1,2-dibromooctane **159** as a pale yellow liquid (47 g, 63%). ^{1}H NMR: δ 4.17 (dd, *J* = 9.4, 4.5, 3.3 Hz, 1 H); 3.85 (dd, *J* = 10.2, 4.5 Hz 1H); 3.70-3.57 (m, 1 H); 2.14 (m, *J* = 14.7, 10.2, 3.3 Hz, 1 H); 1.88-1.24 (m, 1H); 1.53 (s, 1H); 1.38-1.24 (m, 6 H); 0.96-0.85 (m, 3 H). The ^{1}H NMR spectra were matched to those reported by Kabalka *et al*.[109]

7.6 2-Bromooctene



A solution of 1,2-dibromooctane **159** (20.70 g, 0.0761 mol) in dry tetrahydrofuran (100 mL) under nitrogen was cooled to -86 °C. Potassium *tert*-butoxide (9.56 g, 0.085 mol) was added in portions to the reaction mixture. The mixture was stirred for 30 minutes at -86°C and then allowed to warm to room temperature. The resulting mixture was diluted with dichloromethane (100 mL) and poured into water (100 mL). Two crystals of 2,6-di-*tert*-butyl-4-methylphenol were added to the organic layer. The mixture was extracted with petroleum spirits (3 x 50 mL). The combined organic extracts were washed with saturated sodium chloride solution, dried over anhydrous magnesium sulfate and concentrated under reduced pressure to afford mixture of (*E/Z*)-1-bromooctene and 2-bromooctene as a yellow oil (12.00 g, 84%). ^{1}H NMR: δ 5.50 (d, *J* = 1.5 Hz, 1H); 5.38 (d, *J* = 1.5 Hz, 1 H); 2.42 (td, *J* = 7.4, 1.2 Hz, 2 H); 2.28-2.14 (m, 1H); 1.54 (d, *J* = 7.4 Hz, 2 H); 1.46-1.21 (m, 7 H); 0.99-0.85 (m, 3 H). The ^{1}H NMR spectrum matched that reported by Miranda *et al*.[138] The mixture was used directly in the next step.

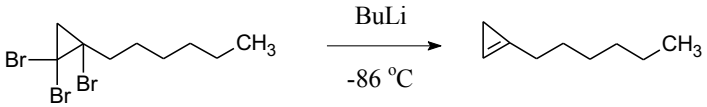
7.7 1,1,2-Tribromo-2-hexylcyclopropane



157 **158**

A solution of sodium hydroxide (4.03 g, 0.01 mol) and water (4 mL) was added in one portion to a vigorously stirred at 0 °C solution of the mixture isomers of 2-bromooctene (4.90 g, 0.025 mol) and cetyltrimethylammonium bromide (0.09 g, 0.0003 mol) in bromoform (2.20 mL, 0.025 mol). The reaction was allowed to warm to room temperature and vigorously stirred for a further 20 hrs. The resulting mixture was diluted with dichloromethane (50 mL) and water (50 mL), and then filtered through celite. The organic phase was separated and concentrated to afford a black oil. The oil was purified by flash chromatography (petroleum spirits) to obtain a 1,2,2-tribromo-1-hexylcyclopropane **158** as a yellow oil (4.70 g, 50%). ^{1}H NMR: δ 3.74 (d, *J* = 9.3 Hz, 1H); 2.04 (d, *J* = 5.7 Hz, 1 H); 1.94 (d, *J* = 5.7 Hz, 1H); 1.82 (d, *J* = 9.3 Hz, 1 H); 1.53 (m, 1 H); 0.93-0.89 (m, 3 H). The ^{1}H NMR spectra were matched to those reported by Sydnes *et al*.[139]

7.8 1-Hexylcyclopropene (1-HCP)

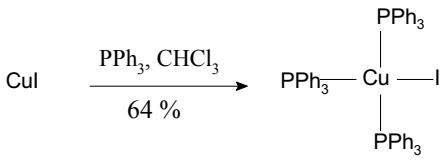


158 **122**

Butyllithium in hexanes 1.6 M (5.00 mL, 0.008 mol) was added dropwise to a cooled (-86 °C) solution of 1,2,2-tribromo-1-hexylcyclopropane **158** (1.00 g, 0.0028 mol) in dry tetrahydrofuran (5 mL). After 10 minutes the solution was allowed to warm to 0

^{o}C and stirred for further 10 minutes. Saturated ammonium chloride solution (5 mL) was added to the solution and the organic phase was separated. The aqueous phase was extracted with diethyl ether (13 mL) and the organic phase was collected. The combined organic phase was washed with water (13 mL) and then saturated sodium chloride solution (13 mL) and dried over anhydrous sodium sulfate. The product solution was concentrated to afford brown oil of 1-hexylcyclopropene **122** (0.26 g, 76%). ^{1}H NMR: δ 6.42 (t, *J* = 1.59 Hz, 1 H); 2.47 (td, *J* = 7.2, 1.3 Hz, 1H); 1.59 (d, *J* = 0.9 Hz, 2 H); 1.3 (d, *J* = 7.2 Hz, 2H); 2.16 – 1.04 (m, 8H); 0.86 (s, 3H). The ^{1}H NMR spectrum is identical to that reported by S. K. Yoo, and J. W. Chung.[111]

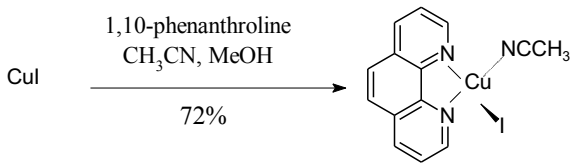
7.9 Tris(triphenylphosphine)copper(I) iodide



138 **134**

The complex was synthesized by the procedure that reported by Hanna *et al.* Triphenylphosphine (5.24 g, 0.02 mol) was dissolved in chloroform (40 mL), then copper(I) iodide (3.80 g, 0.02 mol) was added and stirred for one hour. The resulting precipitate was filtered and the residue dried to afforded tris(triphenylphosphine) copper(I) iodide **134** (1.65 g, 64 %).[103]

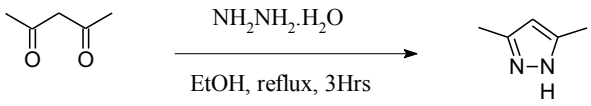
7.10 Acetonitrile (1,10-phenanthroline)copper(I) iodide



138 **139**

Copper(I) iodide (1.32 g, 0.01 mol) in degassed acetonitrile (10 mL) was heated under reflux until the solution became colourless. Then a solution of 1,10-phenanthroline (1.39 g, 0.01 mol) in degassed methanol (30 mL) was added. The colour of the solution turned to dark red and the reaction was stirred for a further five minutes. The precipitate was collected by vacuum filtration to obtain acetonitrile(1,10-phenanthroline)copper(I) iodide complex **139** as a red solid powder (2.32 g, 72%). ^{1}H NMR: δ 8.46 (d, *J* = 8.3 Hz, 1H); 7.96 (s, 1H); 7.26 (s, 1H); 1.36 (s, 3H). IR: 3046, 2164, 1505, 1416, 1139, 843, and 725. Calculated: C = 40.84%, H = 2.69, N = 10.21%,; Found C = 36.87%, H = 1.57, N = 6.99%.

7.11 3,5-Dimethyl-1*H*-pyrazole

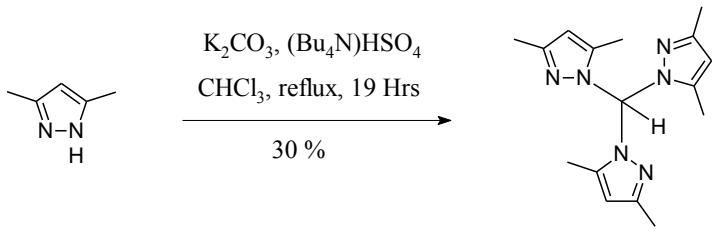


140 **141**

A mixture of acetylacetone **140** (7 g, 012 mol) and hydrazine hydrate (7 g, 85%) in ethanol (20 mL) was heated under reflux for 3 hrs. The solvent was removed under reduced pressure and the slightly yellow crystals were dried at reduced pressure to

afford a 3,5-dimethylpyrazole **141** (5.2 g, 77%). ^{1}H NMR: δ 5.96-5.72 (m, 1H); 2.26 (d, *J* = 0.6 Hz, 6H). The ^{1}H NMR spectrum matched that reported by K. Anandarajagopal *et al*.[153]

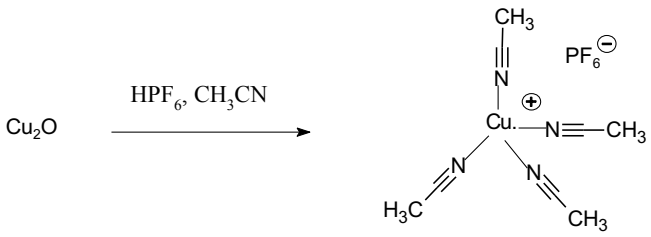
7.12 *tris*(3,5-Dimethyl-1-pyrazolyl)methane.



141 **142**

A mixture of 3,5-dimethyl-1*H*-pyrazole **141** (2 g, 0.02 mol), anhydrous K_2CO_3 (14.37 g, 0.1 mol), $(Bu_4N)HSO_4$ (0.35 g, 0.001 mol) and chloroform (25 mL) was heated under reflux for two days. The mixture was filtered and the residue washed with hot CH_2Cl_2. The combined filtrates were washed with water (3 x 20 mL), dried over anhydrous calcium chloride and concentrated under reduced pressure. The residue was subjected to column chromatography (10% MeOH/DCM) to give colourless crystals of **142** (0.70 g, 30%). ^{1}H NMR: δ 8.07 (s, 1H); 6.08-5.69 (m, 3H); 2.18 (s, 9H); 2.01 (d, *J* = 0.8 Hz, 9H). The ^{1}H NMR spectrum matched that reported by Reger *et al*.; Neves *et al*.[106,107]

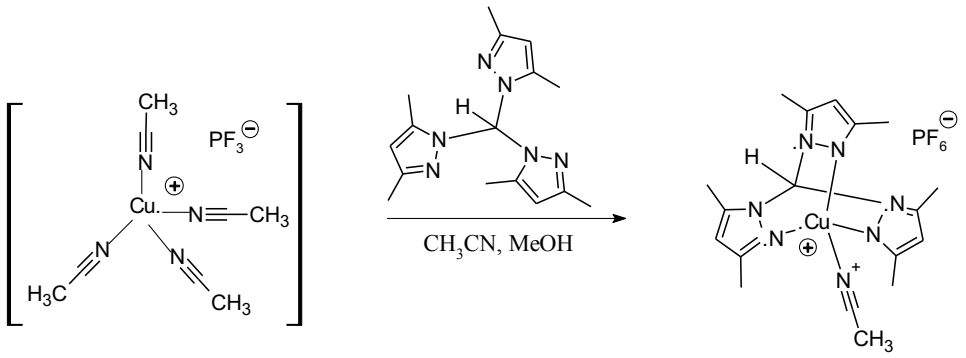
7.13 Tetrakis(acetonitrile)copper(I) hexaflurophosphate



145 **146**

60% HPF_6 (2.5 mL) was added in 0.5 mL portions to a stirred suspension of Cu_2O (1 g, 0.007 mol) in MeCN (20 mL). The reaction was exothermic, the hot solution was stirred for 3 minutes and allowed to cool, and the precipitate was collected by vacuum filtration and washed with a small amount of acetonitrile. The filtrate was cooled to -20^{o}C for 3 hrs and the resulting precipitates collected by vacuum filtration. The residue was washed with ether. The combined precipitate was then redissolved in acetonitrile (25 mL) and filtered. Ether (25 mL) added to the filtrate and cooled to -20 ^{o}C overnight. The white precipitate was filtered by vacuum filtration and the residue washed with ether. The collected solid was dried in vacuo to afford copper complex **146** as a colourless solid (2.50 g, 83%). ^{1}H NMR: δ 2.02 (s). The ^{1}H NMR spectrum was identical to that was reported by Jarvis *et al*.[104]

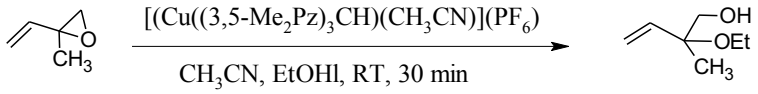
7.14 Copper(I)acetonitrile[tris(3,5-dimethyl-1-pyrazolyl)methane] hexaflurophosphate



146 **137**

$[Cu^{I}(CH_3CN)_4](PF_6)$ (0.375 g, 0.001 mol) was dissolved in acetonitrile (40 mL) under nitrogen. *Tris*(3,5-Me_2Pz)methane (0.298 g, 0.001 mol) was added to the solution in one portion and the solution stirred for 10 minutes. Methanol (35 mL) was added to the reaction mixture and a white precipitate formed. The precipitate was collected by vacuum filtration and was recrystallized from CH_2Cl_2 : MeOH (1 : 1) to afford colourless crystals of **137** (0.427 g, 78%). ^{1}H NMR: δ 7.72 (s, 1H); 6.02 (s, 3H); 2.72-2.06 (m, 18H) 1.97-1.61 (m, 3H). The ^{1}H NMR spectrum matched that reported by Reger and Collins; Cvetkovic *et al*.[109,110]

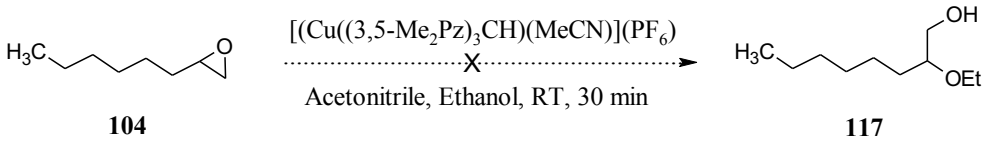
7.15 Reaction of 1,2-epoxy-2-methyl-3-butene with ethanol in presence of Copper(I) complex 109



115 **116**

A solution of 1,2-epoxy-2-methyl-3-butene **115** (0.42 g, 0.005 mol) in acetonitrile was added to a stirred solution of (1 equivalent) copper complex **137** (2.73 g, 0.005 mol) in dry ethanol (30 mL) the mixture was stirred for 30 minutes. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in diethyl ether and washed with water (2 x 10 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was subjected to column chromatography (1% ethyl acetate in petroleum spirit) to give the alcohol **116** as an oil (0.072 g, 12%). ^{1}H NMR: δ 5.90 (dd, J = 17.9, 10.8 Hz, 1H); 5.51-5.09 (m, 2H); 3.49 (m, 2H); 3.56-3.26 (m, 2H); 1.55 (s, 3H); 1.29 (s, 3H).

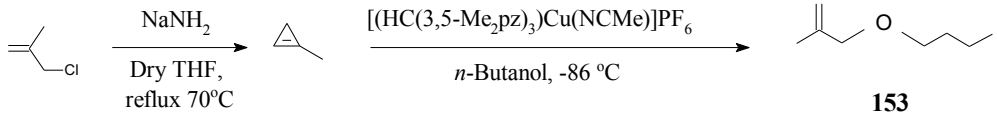
7.16 Reaction of 1,2-epoxyoctane with copper complex 137



104 **117**

A solution of 1,2-epoxyoctane **104** (0.64 g, 0.005 mol) in acetonitrile (5 mL) was added to a stirred solution of (1 equivalent) copper complex **137** (2.73 g, 0.005 mol) in dry ethanol (30 mL) the mixture was stirred for 30 minutes. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in diethyl ether and washed with water (2 x 10 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The NMR spectrum showed only starting material.

7.17 Synthesis of 1-methylcyclopropene and reaction with butanol in presence of copper complex 137

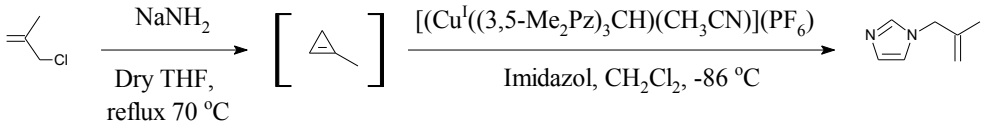


31 **16** **153**

A solution of dry methallyl chloride **31** (1.54 g, 0.017 mol) in THF (5 mL) was added dropwise to a slurry of sodamide (1.03 g, 0.026 mol) in anhydrous THF (10 mL). The reaction mixture heated under reflux for 3 hours after the addition of methallyl chloride. During this time a slow stream of nitrogen was passed over the reaction into a trap containing butanol (20 mL) and (Cu-complex **137**) (2.73 g, 0.005 mol) at -86 ^{o}C. The trap was allowed to warm to room temperature. Diethyl ether (25 mL) was added to the butanol solution and washed with water (1 x 25 mL) dried over

anhydrous Na_2SO_4 and concentrated under reduced pressure. The mixture was diluted in (40 : 60) hexane in petroleum spirit (25 mL) and washed with water (10 x 25 mL) then dried over anhydrous sodium sulfate and the solvent removed under reduced pressure to get about 2% yield of the ether **153**. ^{1}H NMR: δ 4.98 – 4.94 (m, 2H); 4.88-4.96 (m, 2H); 3.87 (m, 2H); 3.37 (t, 2H); 1.39 (s, 2H); 1.79 (s, 3H); 0.88 (s, 3H) The ^{1}H NMR spectrum was identical that reported by Donohoe *et al*.[141]

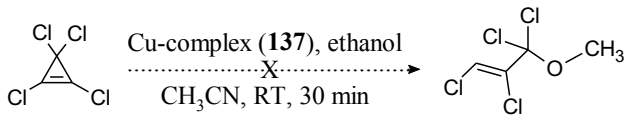
7.18 Synthesis of 1-methylcyclopropene and reaction with imidazole in presence of copper complex 137



31 **16** **154**

The same reaction was performed but replacing butanol with a solution of imidazole (10 g, 0.181 mol) in dichloromethane (20 mL). The product was isolated in 10% yield which was identified as 1-(2-methyl-2-propen-1-yl)-1*H*-imidazole **154**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48 (s, 1H); 7.08 (s, 1H); 6.89 (s, 1H); 4.96 (s, 1H); 4.80 (s, 1H); 4.45 (s, 2H); 1.68 (s, 3H). This data was identical that reported by Tetsuhide Kamijo *et al*.[107]

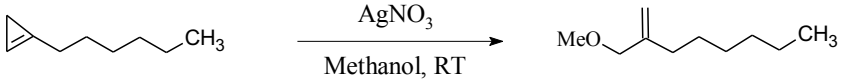
7.19 Reaction of 1,2,3,3-tetrachloro-1-cyclopropene with ethanol in presence of copper-complex 137



127 **162**

A solution of 1,2,3,3-tetrachlorocyclopropene **127** (15 mg, 0.084 mmol) and ethanol (5 mL) was added to Cu-complex **137** in a mixture of CH_3CN (3 mL) and ethanol (5 mL). The mixture was stirred for half an hour at room temperature, and the solvent was removed under reduced pressure. Ether (20 mL) was added to the residue and the ethereal layer washed with water (2 x 20 mL), and then dried under anhydrous sodium sulfate and concentrated to give an oil the ^{1}H NMR spectrum showed only starting material.

7.20 Reaction of 1-HCP with methanol in the presence of AgNO3

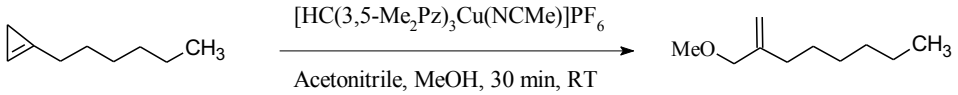


122 **161**

A solution of 1-hexylcyclopropene **122** (12.3 mg, 1 mmol) in acetonitrile (5 mL) was added to a stirred solution of silver nitrate (9 mg, 0.05 mmol) in dry methanol (20 mL). The mixture was stirred for 30 min and the solvent was removed under reduced pressure. The residue was diluted with ether (20 mL) and the ethereal layer washed with water (2 x 20 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by flash chromatography (10% ethyl acetate/petroleum spirits) to give **161** as an oil (36 mg, 23%). ^{1}H NMR: δ 4.98 (d, J =

4.5 Hz, 1 H); 4.89 (d, J = 4.5 Hz, 1H); 3.85 (s, 2H); 3.31 (s, 3H); 2.17 (s, 2H); 1.73 – 1.01 (m, 8H); 0.88 (t, 3H). The ^{1}H NMR spectrum was identical that reported by S. Miyano *et al*.[117]

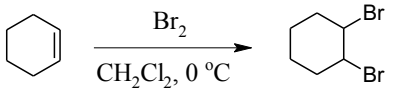
7.21 Reaction of 1-HCP with methanol catalyzed by copper complex 137



112 **161**

A solution of 1-HCP **112** (12.3 mg, 1 mmol) in acetonitrile (5 mL) was added to a stirred solution of [HC(3,5-$Me_2Pz)_3$Cu(NCMe)]PF_6 (**137**) (9 mg, 0.02 mmol) in dry methanol (20 mL) under nitrogen atmosphere. The reaction mixture was stirred for 30 minutes and then concentrated under reduced pressure. The residue was diluted in diethyl ether (20 mL) and washed with water (2 x 20 mL). The organic extract was dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford oily product. The oil was subjected to flash chromatography (petroleum spirit) to give 2-(methoxmethyl)oct-1-ene **161** as a colourless oil (0.088 g, 56%). ^{1}H NMR spectrum was the same as the one described above.

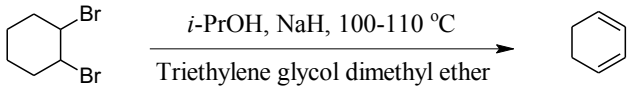
7.22 *trans*-1,2-Dibromocyclohexane



178 **179**

Bromine (29 g, 0.363 mol) was added dropwise to a stirred solution of cyclohexene **178** (30 mL, 0.296 mol) in dichloromethane (20 mL). The reaction mixture was stirred until the solution turned colourless. The mixture was concentrated under vacuum and gave the 1,2-dibromide **179** as a pale yellow liquid. (46 g, 64%). ^{1}H NMR: δ 4.44 (m, 2 H); 1.90-1.85 (m, 4H); 1.79 (d, *J* = 3.1 Hz, 2 H); 1.53-1.47 (m, 2H). The ^{1}H NMR spectrum were matched that reported by Karki and Magolan.[142]

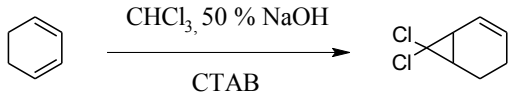
7.23 1,3-Cyclohexadiene



179 **176**

Sodium hydride 70% in mineral oil (8.917 g, 0.223 mol) was added in small portions to the stirred solution of triethylene glycol dimethyl ether (50 mL) and isopropyl alcohol (30 mL), in a 500 mL three-neck round-bottomed flask fitted with a magnetic stirrer and setup for a simple vacuum distillation. The temperature of the reaction flask was raised to 100-110 °C and the receiving flask was cooled to -86 °C as a rapid stream of nitrogen was passed through the system. After most of the isopropyl alcohol was removed by distillation, the receiver was changed. 1,2-Dibromo cyclohexane **179** (24.2 g, 0.1 mol) was added dropwise to the reaction mixture and the temperature of the reaction mixture was maintained at 100-110 °C. The addition required about 30 minutes. The distillate was washed with water (4 x 20 mL) and dried with anhydrous magnesium sulfate to give colourless oil (14.60 g). The diene was distilled at atmospheric pressure under nitrogen to give 1,3-cyclohexadiene **176** (7.02 g, 88%). ^{1}H NMR: δ 5.88 (d, *J* = 1.5 Hz, 2H); 5.83-5.77 (m, 2 H); 2.15 (dd, *J* = 2.6, 1.5 Hz, 4H). Spectral data for this compound matched that provided by Sharaby *et al*.[143]

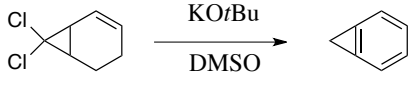
7.24 7,7-Dichlorobicyclo[4.1.0]hept-2-ene



176 **177**

A solution of 1,3-cyclohexadiene **176** (80.5 g, 1 mol), aqueous solution of sodium hydroxide (200 g, 50% w/v) and cetyltrimethylammonium bromide (2 g, 0.0055 mol) was stirred vigorously at 0 ^{o}C. Ethanol (5 mL) and then chloroform (80 mL) were added successively. The mixture was then stirred for 1 hour at 0 ^{o}C and then 1 hour at room temperature. Water (200 mL) was added to the reaction mixture and the organic phase separated. The organic phase was washed with water (2 x 100 mL), dried over anhydrous calcium chloride and concentrated under reduced pressure. The oil was purified by flash chromatography (10% ethyl acetate/petroleum spirits) to afford the adduct **177** as a colourless oil (42.0 g, 26%). ^{1}H NMR: δ 5.92-5.87 (m, 2 H); 2.36 (s, 1 H); 1.83 (d, J = 6.8 Hz, 1 H); 1.50 (s, 1 H); 1.29 (s, 1 H); 1.15 (dt, J = 2.8, 1.1 Hz, 2 H). The ^{1}H NMR spectrum matched that reported by Ketley *et al*.; Davalian *et al*.[144,145]

7.25 Benzocyclopropene

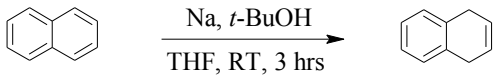


177 **47**

Potassium *tert*-butoxide (2.75 g, 0.025 mol) was added in small portions to a solution of bicyclo[4,1,0]hept-2-ene-7,7-dichloro **177** (1.00 g, 0.006 mol) in dry DMSO (30 mL) under nitrogen. The resulting dark brown reaction mixture was stirred for 30

minutes and the volatile material distilled at room temperature under low pressure (1 mmHg) into a -86 ^{o}C trap. The distillate was diluted in petroleum spirit (20 mL) and washed with saturated sodium chloride solution (4 x 30 mL) and water (2 x 30 mL) and dried under anhydrous sodium sulfate. The solvent was carefully removed on the rotary evaporator at 0 ^{o}C to give benzocyclopropene as a colourless oil (80 mg, 14%). ^{1}H NMR: δ 7.25-7.20 (m, aromatic H); 3.19 (s, 2 H). The ^{1}H NMR spectrum matched that reported by Billups *et al*.[123]

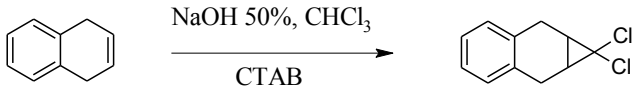
7.26 1,4-Dihydronaphthalene



183 **180**

Sodium metal (15 g, 0.65 mol) was added in small pieces to a solution of naphthalene (30 g, 0.23 mol) in dry tetrahydrofuran (100 mL) over a period of 10-15 minutes. The solution was stirred until a blue colour was observed. Then a solution of *tert*-butanol (48 g, 0.65 mol) and tetrahydrofuran (48 mL) was added dropwise over 10-20 minutes. The mixture was stirred at room temperature for 3 hours. The remaining sodium metal was removed by gravity filtration. The filtrate was washed with water (2 x 50 mL). The organic phase was concentrated under reduced pressure to give 1,4-dihydronaphthalene **180** as a colourless solid (17.5 g, 57%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.19-7.09 (m, 2 H); 5.95-5.90 (m, 2 H); 3.40 (d, J = 1.6 Hz, 4 H). The ^{1}H NMR data matched that reported by Pétrier and Suslick.[146]

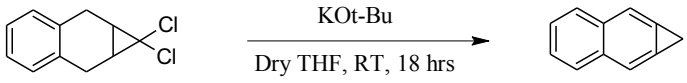
7.27 1,1-dichloro-1a,2,7,7a-tetrahydro-1*H*-Cyclopropa[*b*]naphthalene



180 **67**

To a solution of 1,4-dihydronaphthalene **180** (17 g, 0.131 mol), sodium hydroxide (50 g, 50% w/v) aqueous solution and cetyltrimethylammonium bromide (0.567 g, 0.0016 mol) was added ethanol (2 mL) and then chloroform (23 mL) were added successively. The temperature of the reaction mixture was strictly maintained at room temperature during the addition. The mixture was stirred for 1 hour at 0 ^{o}C and then a further 1 hour at room temperature. Water (100 mL) was added, the organic phase separated. The organic phase was washed with water (2 x 50 mL), dried over anhydrous calcium chloride and the solvent was removed under reduced pressure. The residue was purified by column chromatography (30% ethyl acetate /petroleum spirit) to afford a pale yellow oil **67** (7.56 g, 27%). ^{1}H NMR: δ 7.10 (s, 4 H); 3.28-3.19 (m, 2H); 2.81 (dd, *J* = 17.1, 1.4 Hz, 2H); 2.11-1.99 (m, 2H). The ^{1}H NMR data matched that reported by Műller and Rodriguez; Kelly *et al*.[147,148]

7.28 1*H*-naphtho[*b*]cyclopropene

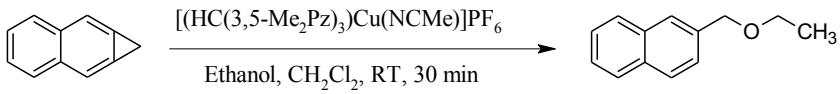


76 **48**

Potassium *tert*-butoxide (11 g, 0.098 mol) was added in small portions to a solution of **67** (4.00 g, 0.028 mol) in dry tetrahydrofuran (60 mL). The reaction mixture stirred for 18 hours under nitrogen atmosphere at room temperature. Petroleum spirits (20 mL) was added to the reaction mixture. The organic phase washed with saturated sodium chloride solution (4 x 20 mL) and water (2 x 10 mL) and dried over anhydrous sodium sulfate and the solvent removed under vacuum. The mixture was purified by flash chromatography (petroleum spirits) to afford pure 1*H*-

naphtho[*b*]cyclopropene **48** as a colourless solid (1.113 g, 42%). ^{1}H NMR: δ 7.88 (d, *J* = 9.5, 1 H); 7.58 (s, 2 H); 7.45 (dd, *J* = 6.2, 3.3 Hz, 1 H); 3.52 (d, 2 H). The ^{1}H NMR data matched that reported by Saraçoğlu *et al*.[93]

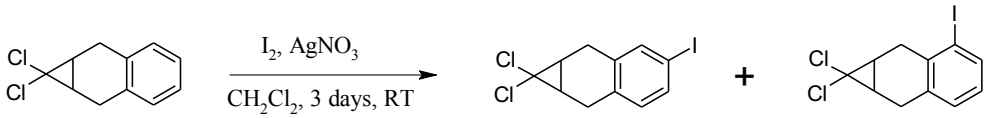
7.29 Reaction of naphtho[*b*]cyclopropene with ethanol in presence of {[HC(3,5-$Me_2Pz)_3$]Cu(NCMe)}PF_6 complex (137)



48 **186**

A solution of 1*H*-naphtho[*b*]cyclopropene **48** (15 mg, 0.107 mol) in CH_2Cl_2 (5 mL) was added to a stirred solution of complex **137** (9 mg, 0.05 mol) in ethanol (20 mL). The mixture was stirred for 30 minutes at room temperature and then the solvent was removed under reduced pressure. Ether (20 mL) was added to the residue and the ethereal layer washed with water (2 x 20 mL) and then dried under anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by flash chromatography (10 % ethyl acetate/petroleum spirits) to afford **186** as a colourless solid (11.9 mg, 60 %). ^{1}H NMR: δ 8.03-7.71 (m, 4H); 7.72-7.29 (m, 3H); 4.68 (s, 2H); 3.60 (q, *J* = 7.0 Hz, 2H); 1.28 (t, 3H). The ^{1}H NMR data matched that reported by Saraçoğlu *et al*.[93]

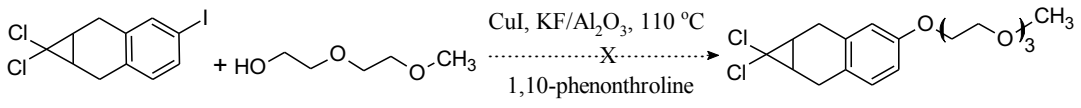
7.30 Iodination of 67



67 **196** **199**

1,1-Dichloro-1a,2,7,7a-tetrahydro-1*H*-cyclopropa[*b*]naphthalene **67** (0.21 g, 0.001 mol) was added to mixture of iodine (0.51 g, 0.004 mol) and silver nitrate (0.62 g, 0.004 mol) in dichloromethane (20 mL). The reaction mixture was stirred for three days at room temperature. The reaction mixture was filtered. The filtrate was washed with 10% aqueous sodium metabisulfate solution (1 x 10 mL) and water (3 x 10 mL), was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (5% of ethyl acetate in petroleum spirit) to afford 1,1-dichloro-9-iodo-1a,2,7,7a-tetrahydro-1*H*-cyclopropa[*b*]naphthalene **196** and its isomer **199** as a colourless oil (0.19 g, 71%). ^{1}H NMR: δ 7.68 (d, *J* = 7.5 Hz, 1 H); 7.57 (s, 1 H); 7.08 (d, *J* = 7.5 Hz, 1 H); 3.30-3.13 (m, 1 H); 2.93-2.62 (m, 2 H); 2.13-1.98 (m, 1 H); 1.55 (s, 2 H). The isomers were inseparable.

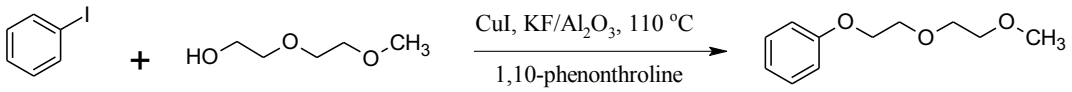
7.31 Attempted to etherification of adduct 196



196 **200** **201**

To a solution of diethylene glycol methyl ether **200** (4.13 mL, 0.03 mol) and iodides **196** and **199** (0.338 mg, 0.001 mol) under nitrogen atmosphere, were added successively CuI (19 mg, 0.0001 mol) and 1,10-phenonthroline (40 mg, 0.00022 mol) then KF/Al_2O_3 5 equivalent (780 mg) were added successively. The mixture was stirred at 110^{o}C for 18 hrs. The reaction mixture was filtered and the filtrate was concentrated. The ^{1}H NMR spectrum showed that the product may decomposed.

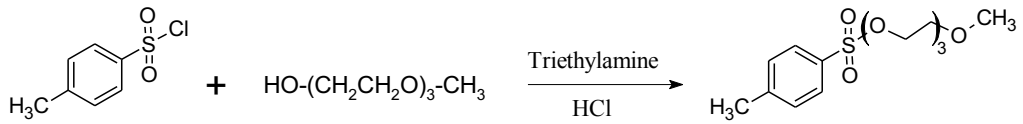
7.32 [2-(2-methoxyethoxy) ethoxy]-Benzene.



202 **200** **203**
93%

Copper(I) iodide (0.019 g, 0.1 mmol) and 1,10-Phenonthroline (0.04 g, 0.22 mmol) then KF/Al_2O_3 5 equivalents (0.78 g) were added to a solution of 2-(2-methoxyethoxy)-ethanol **200** (5 mL, 30 mmol) and iodobenzene **202** (0.204 g, 1 mmol) under nitrogen atmosphere. The mixture was stirred at 110 ^{o}C for 18 hrs the reaction mixture was filtered and the filtrate was concentrated under reduced pressure and the product was purified by column (hexane) which afforded **203** (0.133 g, 93%). ^{1}H NMR: δ 7.27 (d, *J* = 1.3 Hz, 1 H); 6.96-6.93 (m, 1 H); 6.91 (t, *J* = 1.3 Hz, 1 H); 4.20-4.07 (m, 1 H); 3.92-3.82 (m, 1 H); 3.79-3.71 (m, 1 H); 3.70-3.68 (m, 1 H); 3.67-3.64 (m, 1 H); 3.59-3.52 (m, 1H); 3.38 (s, 3 H). The ^{1}H NMR data matched that reported by Kimura *et al*.[149]

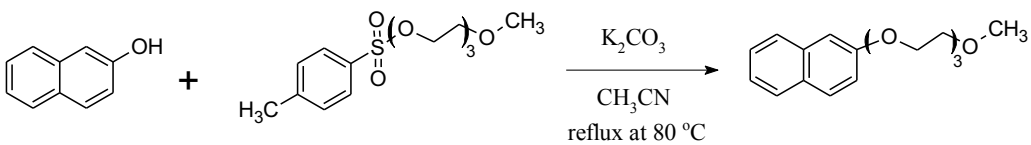
7.33 2-[2-(2-methoxyethoxy)ethoxy]ethyl(4-methylbenzenesulfonate)



208 **209** **210**

To a solution of trimethylamine (100 mL) and 2-[2-(2-methoxyethoxy)ethoxyethanol **209** (25 mL, 0.143 mol) in an ice bath 4-toluenesulfonyl chloride **208** (30 g, 0.157 mol) (10% excess) was added and the mixture was stirred for 6 hrs. Then concentrated HCl (150 mL) and ice (200 g) were added. The product was extracted with diethyl ether and washed with water (3 x 100 mL) and dried over anhydrous magnesium sulfate. Further filtration and solvent evaporation gave the product **210** (35.6 g, 71%) as a viscous liquid. ^{1}H NMR: δ 7.78 (s, 2H); 7.33 (d, *J* = 8.2 Hz, 2H); 4.14 (d, *J* = 14.1 Hz, 1H); 3.68 (d, *J* = 8.2 Hz, 3H); 3.57 (s, 7H)); 3.51 (s, 1H); 3.35 (s, 2H); 2.44 (s, 3 H). ^{13}C NMR: δ 144.49 (C), 133.01 (C), 129.88 (CH), 128.20 (CH), 71.87 (CH_2), 71.32 (CH_2), 70.70 (CH_2), 70.52 (CH_2), 69.01 (CH_2), 68.63 (CH_2), 58.97 (O-CH_3), 21.60 (CH_3). IR: 2876, 1598, 1452, 1353, 1189, 1175, 1096, 1017, 916, 815, 773, 662. Calculated: C = 52.81%, H = 6.96%; found: C = 52.53%, H = 7.14%. The ^{1}H NMR data matched that reported by Liu and Baker.[150]

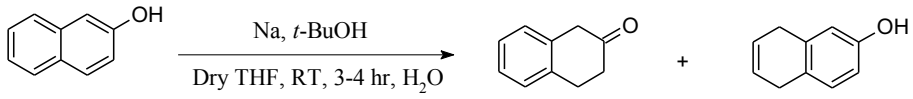
7.34 Synthesis of 2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]-naphthalene.



211 **210** **204**

2-Naphthol **211** (8.6 g, 0.06 mol) was added to solution of 2-[2-(methoxyethoxy)ethoxy)ethyl-4-methylbenzene sulfonate **210** (9.54 g, 0.03 mol) and potassium carbonate (8.2 g, 0.06 mol) in acetonitrile (50 mL). The mixture solution was heated at reflux for 36 hrs. Then the solvent was removed under reduced pressure. The product was purified by chromatography with (1:1) petroleum spirit and ethyl acetate to give **204** (6 g, 35%). ^{1}H NMR: δ 7.80-7.11 (m, 7 H); 3.58-3.73 (m, 12 H); 3.38 (s, 3 H). ^{13}C NMR: δ 156.77 (C), 134.51 (C), 129.36 (CH), 129.04 (C), 127.63 (CH), 126.76 (CH), 126.33 (CH), 123.64 (CH), 119.02 (CH), 106.78 (CH), 71.95 (CH_2), 70.89 (CH_2), 70.70 (CH_2), 70.60 (CH_2), 69.76 (CH_2), 67.45 (CH_2), 59.04 (CH_3). IR: 3058, 2873, 1628, 1469, 1257, 1216, 1104, 969. Calculated: C = 70.32%, H = 7.64%, Found: C = 69.85%, H = 7.93%. HRMS (EI) m/z $C_{17}H_{22}O_4$ [M – H] + requires 290.1518, found. 291.1578.

7.35 Reduction of 2-naphthanol

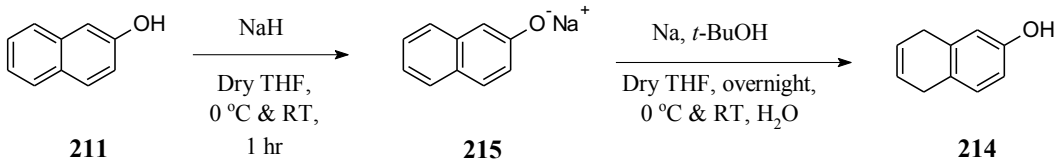


211 **212** **214**

Small pieces of sodium metal (1.62 g, 70 mmol) were added to a solution of 2-naphthol (2 g, 14 mmol) over a period of 10-15 minutes. The reaction mixture was stirred for 3 hrs. The solution of tert-butanol (3.2g) in dry THF (1 : 1) was added slowly over 10-20 minutes. The reaction mixture was stirred for another 3 hrs and the sodium metal was removed by gravity filtration. Water (25 mL) was added to the filtrate and then petroleum spirt (25 mL) was added the organic phase was extracted. The aqueous phase was acidified with 1 M HCl and extracted with petroleum spirit. The organic extractions were combined dried over anhydrous magnesium sulfate

then the solvent removed under reduced pressure gave a yellow oil containing **212** and only very small traces **214** by ^{1}H NMR spectroscopy.

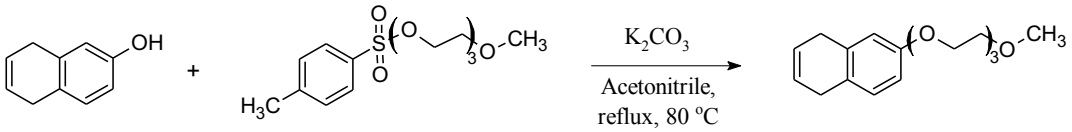
7.36 Reduction of 2-naphthanol by using sodium hydride then sodium metal and *t*-BuOH



211 **215** **214**

To a solution of naphth-2-ol **211** (1 g, 0.007 mol) in dry THF (20 mL), sodium hydride (0.277 g, 0.012 mol) was added slowly with stirred for one hour during this time the colour of the reaction mixture changed to yellowish green. Then sodium metal (0.81 g, 0.035 mol) was added. The mixture was stirred overnight and then solution of *tert*-BuOH (2 g, 0.027 mol) in THF (2 mL) was added and stirred overnight. Water was added to the reaction mixture. The resulting solution was transferred to separating funnel and extracted with petroleum spirits. The combined organic extracts were concentrated under reduced pressure and flash chromatography (10% ethyl acetate/petroleum spirits) gave a pure product **215** (0.24 g, 24%). ^{1}H NMR: δ 7.82-7.07 (m, 3 H); 5.90 (dt, J = 7.8, 1.7 Hz, 2 H); 3.33 (s, 4 H). The ^{1}H NMR data matched that reported by Marshall and Deghenghi.[151]

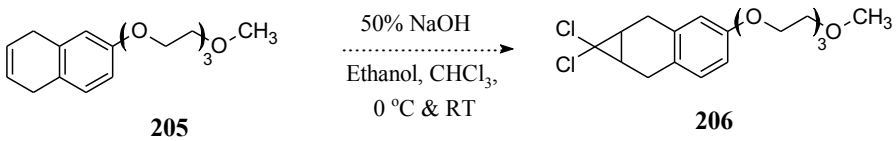
7.37 5,8-Dihydro-2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]naphthalene



214 **210** **205**

5,8-Dihydro-2-naphthol **214** (1.00 g, 6.8 mmol) was added to a solution of 2-[2-(2-methoxyethoxy)ethoxy]ethyl-4-methylbenzenesulfonate **210** (1.10 g, 3.6 mmol) and potassium carbonate (0.93 g, 6.7 mmol) in acetonitrile (20 mL). The reaction mixture was heated under reflux for 36 hours. The solvent was removed under reduced pressure and the residue was subjected to flash chromatography (50% EtOAc/P.S) to afford 5,8-dihydro-2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]naphthalene **205** as a dark brown oil (0.66 g, 34%). ^{1}H NMR: δ 7.91-6.56 (m, 3 H); 6.11-5.78 (m, 2H); 4.49-3.60 (m, 12 H); 3.39 (s, 3 H); 3.38 (d, *J* = 1.4 Hz, 2 H). ^{13}C NMR: δ 165.37 (C), 136.62 (C), 135.14 (C), 128.63 (CH), 127.03 (CH), 124.76 (CH), 126.33 (CH), 122.64 (CH), 118.15 (CH_2), 108.87 (CH_2), 74.93 (CH_2), 71.89 (CH_2), 70.34 (CH_2), 70.20 (CH_2), 69.56 (CH_2), 66.45 (CH_2), 64.34 (CH_3). IR: 3073, 2807, 1480, 1234, 1016, 963. Calculated: C = 69.84%, H = 8.27%, Found: C = 69.93%, H = 8.11%.

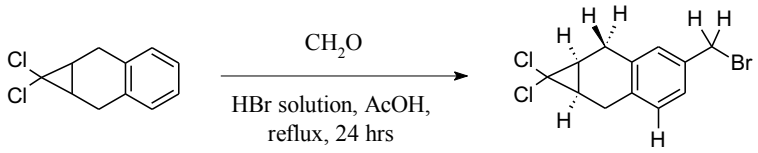
7.38 Dichlorocarbene addition to 5,8-dihydro-2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy] naphthalene.



205 **206**
Traces

Ethanol (0.015 mL) and then chloroform (3 mL) were added successively to a vigorously stirred solution of 5,8-dihydro-2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy] naphthalene **205** (3 g, 0.01 mol), aqueous solution of sodium hydroxide (2 g, 50% w/v) and cetyltrimethylammonium bromide (0.02 g, 55 micromol) at 0 ^{o}C. The mixture was then stirred for 1 hour at 0 ^{o}C and then 1 hour at room temperature. Water (5 mL) was added to the reaction mixture and the organic phase separated. The organic phase was washed with water (2 x 10 mL), dried over anhydrous calcium chloride and concentrated under reduced pressure. The ^{1}H NMR spectrum of the crude product showed some traces of the product.

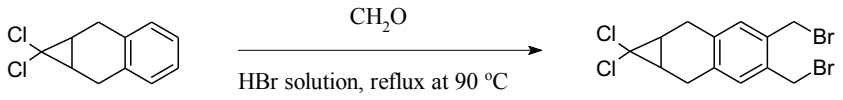
7.39 Bromination of 67 using formaldehyde and hydrogen bromide



67 **220**

Formaldehyde solution (25% $^{W}/_{V}$, 2 mL, 0.07 mol), hydrobromic acid (33%, 8.3 mL) and compound **67** (5 g, 0.0235 mol) were mixed and the reaction mixture was heated under reflux for 24 hours. The reaction was allowed to cool to room temperature and poured into cold water (50 mL). The resulting mixture neutralized using saturated $NaHCO_3$ solution and extracted with ethyl acetate (3 x 20 mL). The combined organic extracts were dried over anhydrous sodium sulfate, purified by column chromatography (1% ethyl acetate/petroleum spirits) to afford colourless crystals (4.41 g, 63%) of 1,1-dichloro-1a,2,7,7a-tetrahydro-6-bromomethylcyclopropa [*b*]naphthalene **220**. The ^{1}H NMR and ^{13}C spectrum confirmed that the compound was synthesized has only one bromomethyl group **220**, The ^{1}H NMR: δ 7.15 (d, J = 8.3 Hz, 1H); 7.13 (s, 1H); 7.07 (d, J = 8.3 Hz, 1H); 4.45 (s, 2H); 3.28 – 3.16 (m, 1H); 2.84 – 2.77 (m, 1H); 2.08 – 2.02 (m, 1H). ^{13}C NMR δ: 135.62 (CH); 134.28 (CH); 134.25 (CH); 129.06 (C), 128.96 (C), 126.83 (C); 65.98 (CCl); 33.52 (CH_2); 27.08 (CH_2); 27.01 (CH_2Br); 24.70 (CH); 24.62 (CH). IR: 3015, 2975, 2904, 1204, 827,

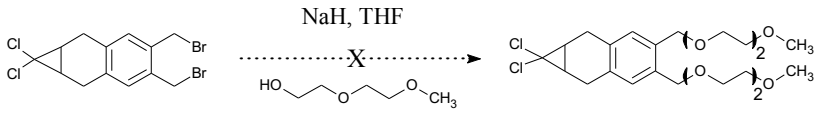
696. Calculated: C = 47.10%, H = 3.62%: found C = 44.93%, H = 3.20%. Molecular ion not found.



67 **218**

Under the same reaction conditions, a new experiment was stirred for five days and afforded compound **218** (6.8 g, 74%). ^{1}H NMR: δ 7.11 (s, 2 H); 4.62 (s, 4 H); 3.27-3.15 (m, 2 H); 2.80 (dt, *J* = 17.0, 1.5 Hz, 2 H); 2.11-1.99 (m, 2 H). ^{13}C NMR δ: 135.35 (C); 134.37 (C); 131.28 (CH); 65.88 (C); 30.19 (CH_2); 26.84 (CH); 24.49 (CH_2). Calculated: C = 39.14%, H = 3.03%: found C = 37.63%, H = 2.83%. Molecular ion not found.

7.40 Etherification of 218.

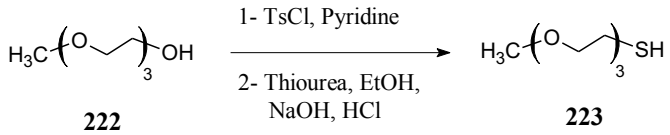


218 **221**

Sodium hydride (0.8 g, 0.0334 mol) was added in portion to a solution of **212** (6.28 g, 0.0368 mol) and diethylene glycol (4 g, 0.0334 mol) in dry THF (40 mL). After refluxing and stirring for 18 hrs, the excess NaH was filtered off and the filtrate was concentrated under reduced pressure. The crude product was purified by flash

chromatography 50% ethyl acetate in petroleum spirit, ^{1}H NMR data showed only starting material.

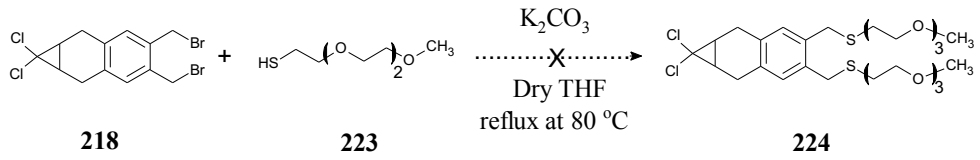
7.41 Synthesis of 2-(2-(2-methoxyethoxy)ethoxy)ethanethiol $CH_3(OCH_2CH_2)_3SH$



222 **223**

Triethylene glycol monomethyl ether **222** (10 g, 0.06 mol) was dissolved in pyridine (12 mL) and cooled to 0 ^{o}C. *p*-Toluenesulfonyl chloride (11.60 g 0.06 mol) dissolved in pyridine (12 mL) was added dropwise under nitrogen. The reaction was stirred for 2.5 hrs, at which point the flask was sealed and stored at 2 ^{o}C overnight. This mixture was then added to ice water (120 mL) and stirred for 1 hour. Extraction of this solution with CH_2Cl_2 permitted isolation of the tosylate as an oil. It was immediately dissolved in a minimum amount of ethanol and was added to solution of thiourea (4.64 g 0.06 mol) in water (15 mL). The reactants were refluxed for 2 hrs and then a solution of NaOH (2.68 g, 0.07 mol) in water (20 mL) was added and reflux was maintained for 1.5 hrs. The solution was cooled to room temperature, concentrated, and neutralized with HCl solution. The product was extracted from this aqueous solution with CH_2Cl_2 and isolated as yellow oil that was purified by vacuum distillation (60 ^{o}C, 1 torr). To give the required product **223** (5.9 g, 54%). ^{1}H NMR: δ 3.79-3.53 (m, 10 H); 3.38 (s, 3H); 2.70 (dt, *J* = 8.2, 6.5 Hz, 2 H); 1.58 (t, *J* = 8.2 Hz, 1 H). The ^{1}H NMR data were identical to that those reported by Foos and co-workers.[134]

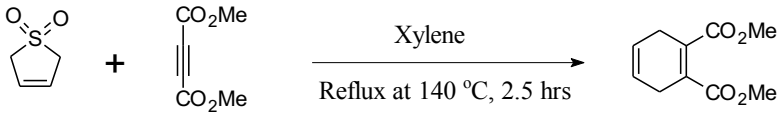
7.42 Reaction of 218 with 2-(2-(2-methoxyethoxy)ethoxy)ethane thiol using K_2CO_3



218 **223** **224**

Potassium carbonate (0.004 g, 0.029 mmol) and cyclopropa[*b*]naphtho-1,1-dichloro-4,5-bis(bromomethyl)-1a,2,7,7a-tetrahydro **218** (0.046 g, 0.09 mmol) solution was added to the solution of ethane-thiol-2-(2-(2-methoxyethoxy)ethoxy) **223** (0.04 g, 0.22 mmol) in a dry THF (2 mL), then the reaction mixture was heated at reflux for 12 hrs at 80 ^{o}C. The reaction mixture was filtered to remove the precipitate salt and concentrated under reduced pressure. The residue was diluted with dichloromethane and was washed with water (2 x 10 mL). The resultant dichloromethane solution was concentrated under vacuum followed by an additional washing of the product with 10 mL hexane to remove unreacted **218**. But unfortunately ^{1}H NMR spectrum did not show any product.

7.43 Dimethyl-1,4-cyclohexadiene-1,2-dicarboxylate 226

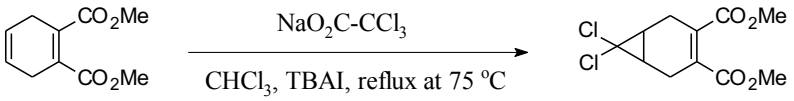


225 **26** **226**

A solution of dimethyl acetylenedicarboxylate **26** (1.00 mL, 0.008 mol), butadiene sulfone **225** (5.40 g, 0.045 mol) and xylene (8 mL) was heated under reflux under nitrogen atmosphere for 2.5 hrs. After 2.5 hrs the yellow reaction mixture was

allowed to cool to room temperature and concentrated under reduced pressure. The residue was purified by column chromatography (10% ethyl acetate/petroleum spirits) to give the adduct **226** as a yellow liquid (0.75 g, 47%). ^{1}H NMR: δ 5.93 (m, 2 H), 3.78 (s, 6 H), 3.00 (d, *J* = 1.2 Hz, 4 H). The ^{1}H NMR spectrum was identical that reported by DiFrancesco and Pinhas.[136]

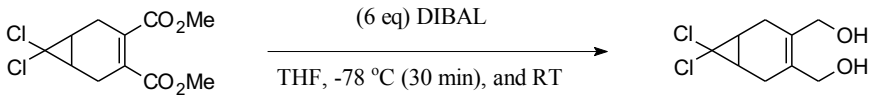
7.44 Dichlorocarbene addition to 1,4-Cyclohexadiene-dicarboxylate 226



226 **227**

1,4-Cyclohexadiene-dicarboxylate **226** (0.20 g, 0.1 mmol) was added to a solution of sodium trichloroacetate (3 g, 16 mmol) and tetrabutylammonium iodide (0.005 g, 0.014 mmol) in chloroform (20 mL) and the reaction mixture was stirred under reflux under nitrogen overnight. The reaction was allowed to cool to room temperature. Then the reaction mixture was diluted in chloroform (20 mL), washed with water (5 x 25 mL), dried under anhydrous calcium chloride and the solvent was removed under reduced pressure. The residue was purified by column chromatography (10% ethyl acetate in petroleum spirits) to afford **227** as a colourless solid (0.172 g, 87%). ^{1}H NMR: δ 3.76 (s, 6 H); 2.87-2.75 (m, 2 H); 2.55 (d, *J* = 7.6 Hz, 2 H); 1.98-1.94 (m, 2 H). ^{13}C NMR δ: 168.11 (C); 131.68 (C); 64.06 (C); 52.37 (CH); 23.84 (CH_2); 21.56 (CH_3). IR: 3058, 2873, 1736, 1628, 1600, 1469, 1390, 1352, 1258, 1216, 1104, 970, 838, 747. Calculated: C = 47.33%, H = 4.33%, Found: C = 47.32%, H = 4.11%. HRMS (EI) m/z $C_{11}H_{12}O_4Cl_2$ [M – H] + requires 278.0113, found 300.9988.

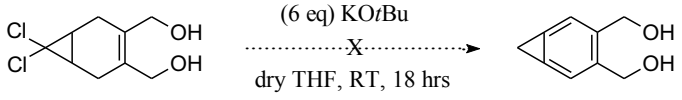
7.45 7,7-Dichloro-bicyclo[4,1,0]hept-3-ene-1.2-dimethanol



227 **228**

Diisobutylaluminium hydride (1.364 g, 0.01 mol) and toluene solution was added dropwise to a solution of the ester **227** (0.10 g, 0.0004 mol) in dry THF (10 mL) at -78 °C. The reaction was stirred for 30 minutes at -78 °C, and then it was allowed to warm to room temperature overnight. 1 M HCl solution (10 mL) was added to the reaction mixture and extracted with ethyl acetate (3 x 20 mL). The combined organic extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography (40% ethyl acetate in petroleum spirit) to give colourless solid **228** (0.038 g, 46%). ^{1}H NMR: δ 4.23 (m, 4 H); 2.74 - 2.58 (m, 2H); 2.34 (d, *J* = 10.9 Hz, 2 H); 1.89 (d, *J* = 10.9 Hz, 2 H). ^{13}C NMR δ: 131.04 (C), 65.31 (C), 62.55 (CH), 24.83 (CH), 23.53 (CH_2). IR: 3291, 2878, 2830, 1679, 1422, 1261, 1086, 995, 784. Calculated: C = 48.45%, H = 5.42%; Found: C = 48.32%, H = 5.33%. HRMS (EI) m/z $C_9H_{12}O_2Cl_2$ [M – H] + requires 222.0214, found 245.009.

7.46 Dehydrochlorination of 228



228 **229**

7,7-Dichloro-bicyclo[4,1,0]hept-3-ene-1.2-dimethanol **228** (0.0485 g, 0.2 mmol) was added to a solution of potassium *tert*-butoxide (0.154 g, 1.4 mmol) in dry THF (8 mL) the reaction mixture was stirred for 36 hrs. Petroleum spirits (10 mL) was added to the brown mixture and washed with brine water (3 x 10 mL) and water (1 x 10 mL) and then the organic phase was dried under anhydrous magnesium sulfate and the solvent was removed under reduced pressure to give 229 which polymerized rapidly. ^{1}H NMR: δ 7.27 (s, 2H); 4.79 (s, 4H); 3.28 (s, 4H).

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