Medicinally Active Dihalocyclopropanes

By

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Summary

Although mounting evidence suggests that Tamoxifen exerts cardioprotective effects against atherosclerosis, the findings that associate Tamoxifen and atherosclerosis at a molecular level are inconsistent and unclear.

Tamoxifen is a potent autophagy inducer and we believe that the cardioprotective effect of Tamoxifen may be arisen from this autophagy inducing ability. This is because recent studies have shown that enhanced autophagy may be beneficial at both early and advanced stages of atherosclerosis.

However, the estrogenic activity of Tamoxifen in the endometrium cells increases the risk of endometrial cancer in the patients treated with Tamoxifen. We, therefore, would like to minimize this risk by developing derivatives of Tamoxifen which induce autophagy, but with no apparent estrogenic activities.

We started our project based on the syntheses of dichlorocyclopropyl analogues of Tamoxifen as Magarian *et al.* reported that the introduction of a dichlorocyclopropyl moiety in place of the olefinic link in Tamoxifen can significantly reduce the estrogen agonist activity. We found that, however, those dichlorocyclopropanes were not particularly stable and degraded easily through dichlorocyclopropyl ring openings. Thus, we moved to synthesize the difluorocyclopropyl and other classes of derivatives of Tamoxifen.

A wide range of structurally different analogous of Tamoxifen have been synthesized and biologically tested for induction of autophagy, of which some display the desired autophagic activity in a dose-dependent manner but lower than that of Tamoxifen. Tamoxifen was observed to be cytotoxic to cells at high doses

(>5 μM), but this toxicity is not observed with our drug molecules. We ascertained that the presence of an amioethoxy basic side chain in conjunction with a diaryl backbone were essential pharmacophores for the induction of autophagy. Additionally, neither the antiestrogenic nor estrogenic activity is responsible for the autophagic effect of our drug candidates as they did not bind to estrogen receptor. Based on the results of ours and others in the literature, we propose that our novel aminoethoxy difluorocyclopropanes may be AEBS inhibitors. Selected compounds are in the process of being patented.

Declaration

This thesis is submitted to the University of Warwick in support of my application for the degree of Doctor of Philosophy. I certify that this thesis has not been submitted for a degree or any other qualification at this university or any other institution. All of the work presented in this thesis was carried out by myself at the University of Warwick between January 2013 and December 2014 except the biological data which was obtained from our collaborators at E3Bio by Kate Willetts and Dr. Jill Reckless.

Abbreviations

$(CH_3)_3SiI$	Iodotrimethylsilane
µM	Micromolar
5,6 ECs	Cholesterol-5,6-epoxides
5,6*α*-CE	Cholestan-5*α*,6*α*-epoxy-3*β*-ol
5,6*β*-CE	Cholestan-5*β*,6*β*-epoxy-3*β*-ol
ACAT	Acyl CoenzymeA: Cholesterol Acyl Transferase
Acyl-CoA	Cholesterol Acyltransferase
AEBS	Antiestrogen Binding Site
Ar	Aromatic
ATRA	All Trans-Retinoic Acid
BBr_3	Boron Tribromide
br.	IR: broad (peak) NMR: broad (peak)
BTMAB	Benzyltrimethylammonium Bromide
BuOK	Potassium butoxide
C5DS	3*β*-hydroxysterol-C^5-desaturase
CaM	Calmodulin Dependent Enzymes
CBr_2F_2	Dibromodifluoromethane
$CBrClF_2$	Bromochlorodifluoromethane
CCl_2	Dichlorocarbene
$Cd(CF_3)_2$	Bis(trifluoromethyl)cadmium
CDO-3*β*,5*α*-6*β*-*S*-GST	3*β*-5*α*-dihydroxycholestan-6*β*-yl-*S*-glutathione
CF_2	Difluorocarbene

ChEH	Cholesterol Epoxide Hydrolase
COSY	Correlation Spectroscopy
cPLA2	Cytosolic Phospholipase A2
CT	Cholestane-3β,5α,6β-triol
de	Diastereomeric excess
D8D7I	3β-hydroxysterol-Δ(8)-Δ(7)-isomerase
DDA	5α-Hydroxy-6β-[2-(1H-imidazol-4-yl)ethylamino]cholestan-3β-ol
DDB	5α-Hydroxy-6β-[3-(4-aminobutylamimo)propylamino]cholest-7-en-3β-ol
DFT	Density Functional Theory
DHA	Docosahexaenoic Acid
DHCR24	3β-Hydroxysterol-Δ^{24}-reductase
DHCR-7	3β-Hydroxysterol-Δ(7)-reductase
DHETs	Dihydroxyeicosatrienoic Acids
DMBA	Hormone-dependent-7,12-dimethylbenz[a]anthracene
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DPPE	*N*,*N*-Diethyl-2-[4-(phenylmethyl)-phenoxy]-ethanamine
ee	Enantiomeric Excess
E1	Estrone
E2	17b-estradiol
E3	Estriol
EETs	Epoxyeicosatrienoic Acids

EETs	Epoxyeicosatrienoic Acids
equiv.	Equivalent
ER	Estrogen Receptor
ER	Estrogen Receptor
ESI	Electrospray Ionization
EtOAc	Ethyl Acetate
FBS	Fetal Bovin Serum
GSTs	S-glutathione Transferases
h	Hour(s)
HCl	Hydrochloride H^+
HER2	Human Epidermal Growth Receptor
HMBC	Heteronuclear Multiple-Bond Correlation Spectroscopy
HMQC	Heteronuclear Multiple-Quantum Correlation Spectroscopy
HRMS	High Resolution Mass Spectrometry
HXEH	Hepoxilin Epoxide Hydrolase
IC50s	Half Maximal Inhibitory Concentration
K_m	Michaelis Constant
LDL	Low Density Lipoprotein
$LiClO_4$	Lithium Perchlorate
Lit.	Literature
LTA_4	Leukotriene A4
LTA_4H	Leukotriene A4 hydrolase
LTB_4	Leukotriene B4
LXRα	Liver X Receptor Alpha

LXRβ	Liver X Receptor Beta
MAD	Microwave-assisted Difluorocyclopropanation
MBPE	*N*-morpholino-2-[4-(benzyl)-phenoxy]-ethanamine
MCD	Monodansylcadaverine
Me_3SiCF_2Br	[Bromo(difluoro)methyl](trimethyl)silane
Me_3SiCF_2Cl	[Chloro(difluoro)methyl](trimethyl)silane
Me_3SiF	Fluoro(trimethyl)silane
Me_3SnCF_3	Trimethyl(trifluoromethyl)stannane
mEH	Microsomal Epoxide Hydrolase
mp	Melting Point
m/*z*	Mass-to-charge Ratio
nM	Nanomolar
NMR	Nuclear Magnetic Resonance
PBPE	*N*-pyrrolidino-(phenylmethyphenoxy)-ethanamine
PBr_3	Phosphorus Tribromide
$Pd(PH_3)_4$	Tetrakis-triphenyl Phosphine Palladium
Ph	Phenyl
pH	The Logarithmic Measure of H^+ Concentration
$PhHgCF_3$	Phenyl(trifluoromethyl)mercury
PhMgBr	Phenylmagnesium Bromide
pKa	Acid Dissociation Constant
PKC	Protein Kinase C
PTC	Phase Transfer Catalysis
ROS	Reactive Oxygen Species
RT	Room Temperature

RTD	Room Temperature Difluorocyclopropanation
sEH	Soluble Epoxide Hydrolase
SERMs	Selective Estrogen Receptor Modulators
siRNA	Small Interfering RNA
TBAHS	Tetrabutylammonium Hydrogensulphate
TBAT	Tetrabutylammonium triphenyldifluorosilicate
tert	Tertiary
TFDA	Trimethylsilyl Fluorosulfonyldifluoroacetate
TGF-β	Transforming Growth Factor Beta
THF	Tetrahydrofuran
THP	3,4-Dihydro-2H-pyran
TLC	Thin-layer Chromatography
TMS	Tetramethylsilane
TRAMP	Transgenic Adenocarcinoma of Mouse Prostate
UV	Ultra Violet
V_{max}	Maximal Velocity

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Chapter 1 Tamoxifen and Atherosclerosis

1.1 Tamoxifen

1.1.1 Breast cancer

Breast cancer is the most frequently diagnosed cancer in women.[1, 2] More than half million women died of breast cancer throughout the world in 2012, making breast cancer one of the most common cancer deaths among females.[2] In the UK, approximately 46,000 new cases of breast cancer were reported in 2010 which is one person every 10 minutes and an estimated 12,000 deaths as a result of the disease every year.[3]

Breast cancer can be broadly classified into four classes by receptors types:[4]

- Human epidermal growth receptor (HER2) positive
- Endocrine receptor (estrogen or progesterone receptors) positive
- Triple negative
- Triple positive

Breast cancer cells may or may not have receptors for chemical messengers, such as hormones, to bind and activate.[5] HER2-positive occurs when a patient tests positive for a protein called human epidermal growth factor receptor 2 (HER2).[1] The cancer cells produce surplus levels of HER2 as a result of gene mutation which in turn promotes the growth of cancer cells.[4] Similarly, endocrine receptor positive means that breast cancer cells grow in response to hormones estrogen and progesterone.[4] Cells that do not have any of these three receptor types are called triple-negative or conversely, triple positive if all these three receptors are present.[1, 6]

About 80% of all breast cancer cells are estrogen receptor (ER) and/or progesterone receptor (PR) positive.[7] These cells require estrogens to bind and activate the estrogen receptors in order to grow. The inhibition of estrogen receptors provides effective control of uterine growth and the growth of estrogen-dependent mammary tumours.[8] Tamoxifen (Tam) was one of the first antiestrogens discovered and approved for the treatment of breast cancer (Figure 1).[9]

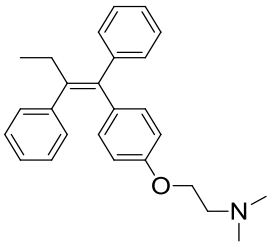


Figure 1: The structure of Tamoxifen.

Discovery of Nonsteroidal Antiestrogens and Tam

In the 1950s/1960s, coronary heart disease was a major target for drug development and Triparanol (Figure 2) was a potent lipid-lowering compound developed by Merrell.[10]

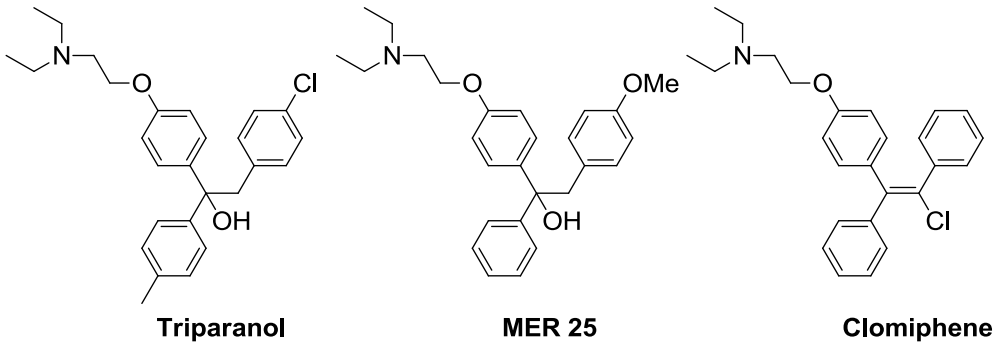


Figure 2: The structures of Triparanol and its analogues.

However, Triparanol was withdrawn from the clinical trials due to its toxicity. It was believed to inhibit the biosynthesis of cholesterol, leading to the accumulation of desmosterol and caused acute cataract formation in patients treated with the drug (Figure 3).[11, 12]

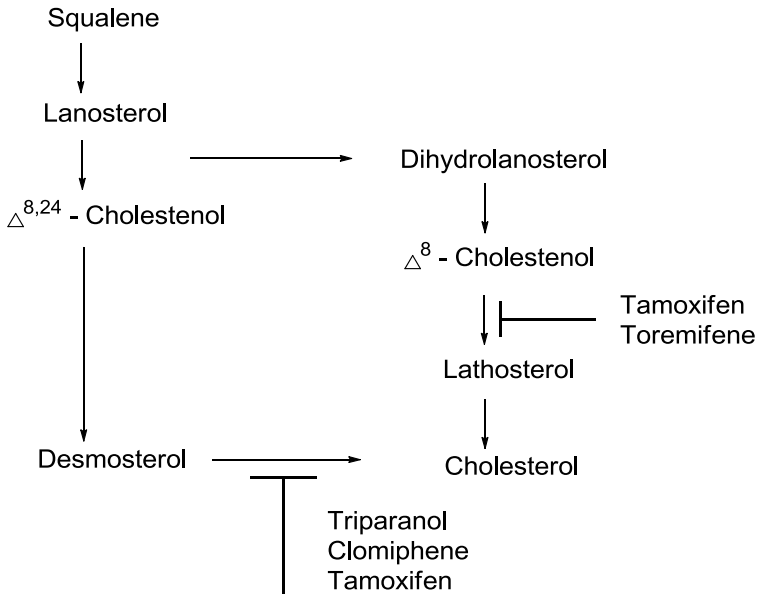


Figure 3: The inhibition of cholesterol biosynthesis pathway by Triparanol and its analogues.[13]

Triparanol and its analogues, MER 25 and clomiphene (Figure 2) had also been tested as a treatment for breast cancers since a lot of triphenylethylenes were known to be estrogenic and a correlation between estrogen and breast cancer had long been established.[14,15,13] Although MER 25 and clomiphene were found to be effective, the drugs were considered to be too toxic for long-term use.

Tamoxifen was first discovered by Dr. Arthur L. Walpole and his research team in the early 1960s at Imperial Chemical Industries (ICI) Pharmaceuticals.[13] He was leading a project conducting research into alternative compounds to Triparanol and its analogues as contraceptive pills owing to their apparent antiestrogen properties,

but with potentially less side effects. They found that while Tamoxifen was indeed an effective postcoital contraceptive in rats,[16] it was not useful in human contraception due to the distinct difference in pharmacology and physiology of ovulation between women and rats. In their clinical studies, Tamoxifen was found to promote ovulation rather than acting as an antifertility agent.[17]

It was not until 197l that Tamoxifen was redeveloped from a failed contraceptive agent and entered preliminary clinical studies as a treatment for breast cancers at the Christie Hospital in Manchester.[18] The studies showed a promising effect in advanced breast cancer in postmenopausal women. A further clinical trial was conducted by Harold W.C. Ward at the Queen Elizabeth Hospital, Birmingham in 1972. Ward showed that at a dose, 10 mg twice daily, of Tamoxifen gave a response rate of 77% with minor side effects such as hot flushes, gastro-intestinal intolerance and tumour pain.[18] By 1973, Tamoxifen was approved for the treatment of breast cancer by the Committee on the Safety of Medicines in the United Kingdom and sold under the trade name Nolvadex.[13]

Estrogen and Estrogen Receptors: How do they signal

Tamoxifen is a synthetic, non-steroidal, potent antagonist of ER in breast tissues. The natural ligands of ERs are steroidal estrogens. The sex hormone estrogens are a group of compounds which act as signaling molecules and are important for numerous physiologic processes. They play a key role in growth, differentiation, and function of various tissues,[19] which include the testes, epididymis and prostate in men, and the glands, uterus, vagina, and ovaries in women.[19] Estrogens are

present in both men and women, but women produce these hormones at much higher levels.

The three major naturally occurring estrogens in women are estrone (E1), 17b-estradiol (E2) and estriol (E3), and are steroids derived from cholesterol (Figure 4). The most potent and dominant estrogen found in women of reproductive ages is E2. E1 is most commonly found in postmenopausal women, while E3 is abundant primarily during pregnancy. Although E3 and E1 also have high affinities for ERs, E2 is a much stronger agonist.[20] Their levels in the body fluctuate with life stage, suggesting that they may have unique functions in biological and disease progression.[21]

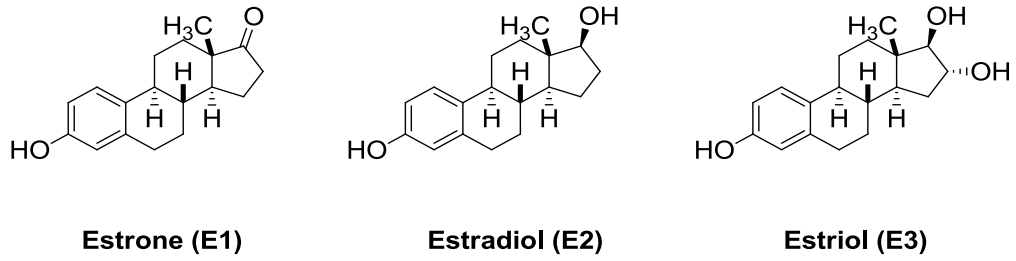


Figure 4: The structures of the estrogens.

Many studies suggest that estrogens inhibit bone resorption and help to protect against osteoporosis,[22] reduce the incidence of cardiovascular disease through regulation on blood lipids,[23] and it may also help to protect against cognitive decline and delay the onset of Alzheimer's disease.[24] However, prolonged estrogen exposure also contributes to increased risk of breast cancer, invasive carcinoma and uterine sarcoma.[19, 25]

The biological actions of estrogen are facilitated through their binding to ERs which are transcription factors that modulate the activity of different genes.[26] When an

estrogen molecule enters the cell, it binds to the estrogen receptor and causes a conformational change. Once activated, the estrogen receptor will dimerize to form an estrogen-receptor-ligand complex which then interacts with the estrogen response elements (EREs) in DNA.[27] After it becomes attached to the EREs, the dimer complex binds to various coregulators. These coregulators act as linker molecules between DNA binding proteins and protein transforming enzymes.[28] It has now been established that estrogen facilitates the interaction of the ER with coactivators which leads to activation of DNA transcription (Figure 5).[27]

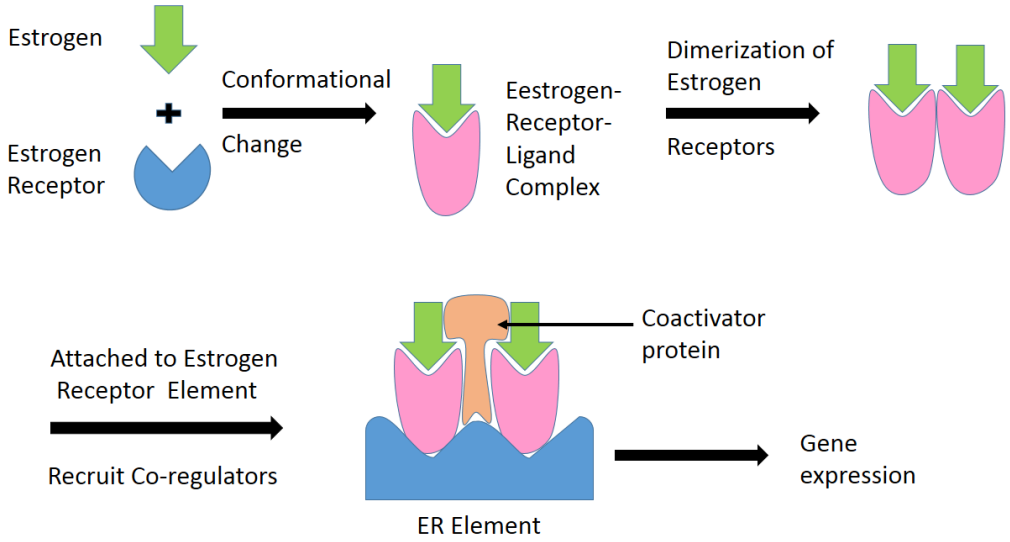


Figure 5: The binding mechanism of an estrogen to an estrogen receptor.

Emerging evidence suggests that the estrogen receptor's signaling pathway is much more complicated than the one just described. The estrogen receptors may regulate the physiological process through several distinct pathways. They could either activate the gene expression or repression through both genomic and non-genomic pathways, and with or without estrogen.[20]

The Antiestrogenic Effect of Tamoxifen in Breast Tissue

Tamoxifen is an antiestrogen of ERs in breast tissues. Instead of activating the ERs like estrogen does, it competitively binds to the ERs and blocks the estrogen-stimulated tumour growth. After binding and triggering the ER to form an antagonist-activated-dimer-complex, it selectively interacts with corepressors and exerts antiestrogenic effects, e.g. suppressing the DNA transcription (Figure 6).[27]

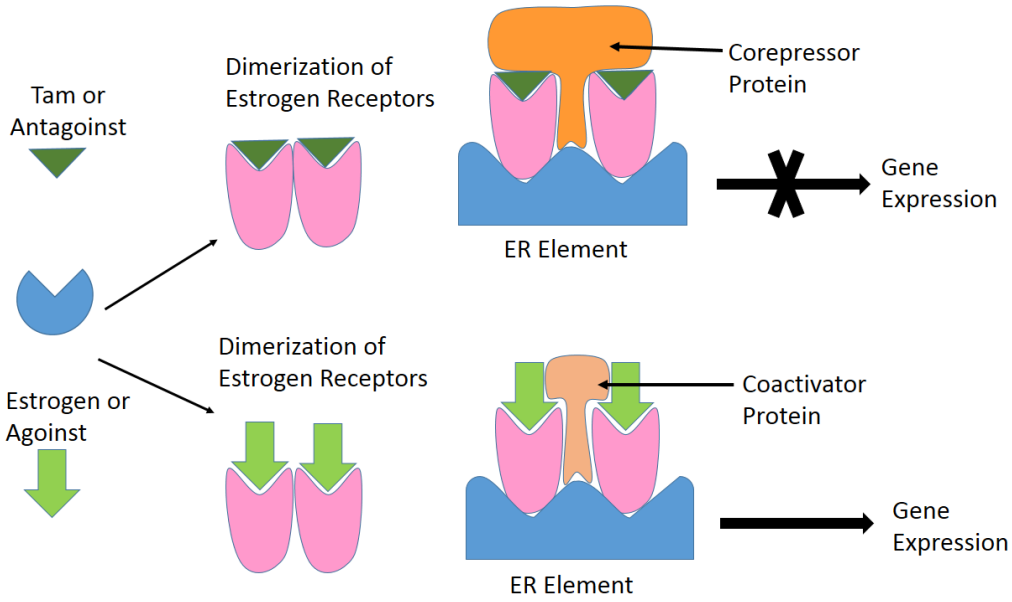


Figure 6: The binding of an antiestrogen to an estrogen receptor as compared to an estrogen.

Tamoxifen is widely used for the treatment of hormone-dependent breast tumours in pre- and post-menopausal women[29] and as a preventative therapy in woman at high risks of developing the diseases.[30] It is also the common treatment for male breast cancers.[31] In addition, Tamoxifen has been shown to be effective in treating other conditions such as infertility in women resulted from anovulatory disorders[32] and gynaecomastia related to high estrogenic level.[33]

Tamoxifen is a Selective Estrogen Receptor Modulator

Tamoxifen is a ligand for the ER with an affinity in the nanomolar range.[34] Tamoxifen belongs to a class of molecules called Selective Estrogen Receptor Modulators (SERMs). The characteristic of SERM compounds is that their mechanisms of action on estrogen receptors are different depending on the targeting tissues and genes. For example, Tamoxifen is a potent antagonist of estrogen receptor in breast tissue, however, in other tissues such as the endometrium, it behaves as an agonist.[19] The mixed agonist and antagonist activities of Tamoxifen are tissue- and cell-specific and may vary from one species to another.[19] In addition to its well-known anti-breast cancer properties, previous studies by others also reported that treatment with Tamoxifen may help prevention of osteoporosis[22, 35] and protection against atherosclerosis.[23] However, the estrogenic activity of Tamoxifen in the endometrium cells is also linked to increased risk of deep vein thrombosis[36] and uterus cancers[19] (Figure 7), and better SERMs are needed as a result.

In theory, it is possible to synthesize multiple-targeting SERMs displaying pure estrogenic agonist activity on one type of tissue and complete estrogenic antagonist activity on the other for the treatment of multiple diseases in women. Nevertheless, the exact molecular targets associated with the beneficial effects of Tamoxifen or other SERMs in many tissues remains unclear. The cardioprotective effect of Tamoxifen against atherosclerosis, in particular, has attracted much interest in the past decades. With atherosclerosis being the primary cause of heart disease and strokes, accounting for approximately 50% of deaths in the Western world,[37] perhaps it is not surprising that tackling heart disease remains one of the top priorities in drug research and developments.

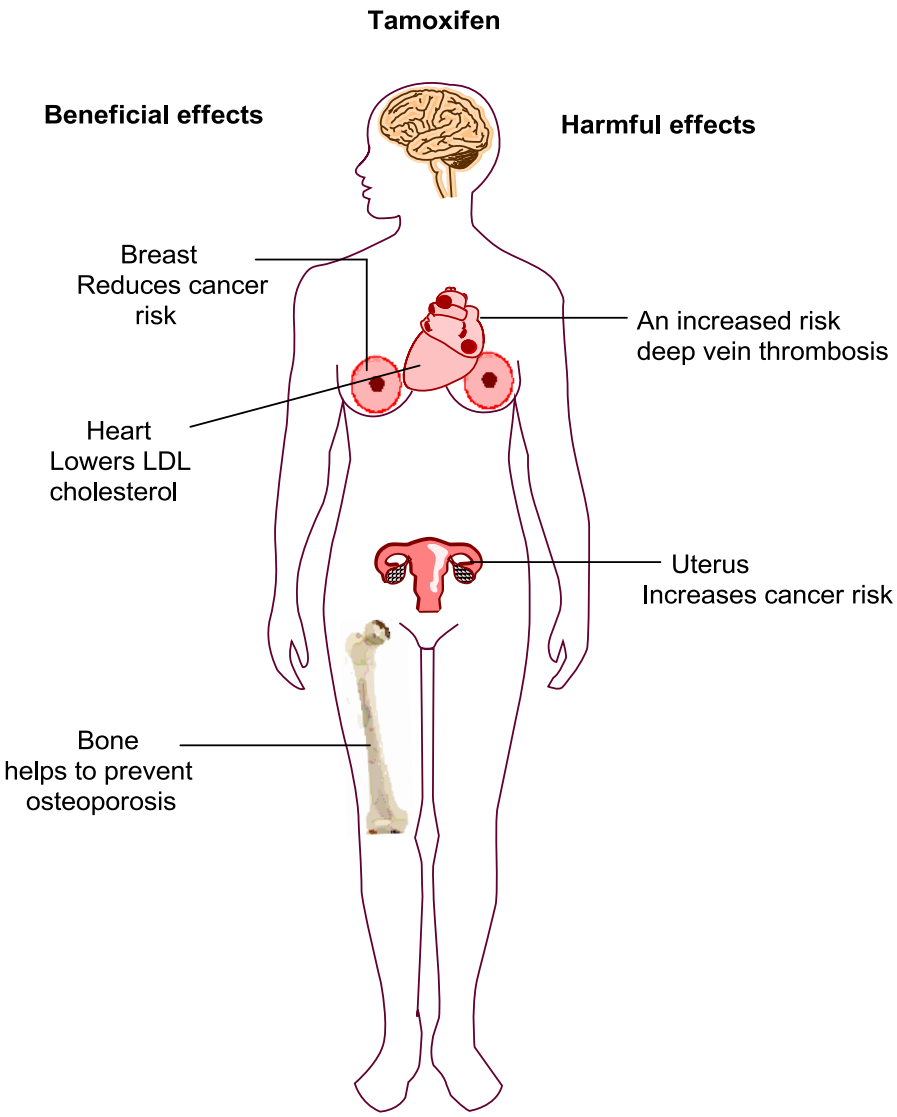


Figure 7: The potential beneficial and harmful effects of Tam.

1.1 Tamoxifen and Atherosclerosis

1.2.1 The Pathological Progression of Atherosclerosis

Atherosclerosis is a progressive disease resulting from the accumulation of lipids and fibrous elements in large arteries. Atherosclerotic lesions are broadly categorized into two types: stable and unstable plaques. The stable atherosclerotic plaques are generally rich in extracellular matrices and smooth muscle cells. They tend to be asymptomatic, while the unstable plaques are rich in macrophages and foam cells.[38]

The development of unstable atherosclerotic plaques is thought to be initiated by the accumulation of oxidatively modified low density lipoprotein (LDL) in the intima (the innermost layer of an artery or vien). The modified LDL stimulates the endothelial cells to produce a number of pro-inflammatory molecules, leading to the adhesion of monocytes to the vessel wall.[37] The monocytes then transmigrate into the intima where they proliferate, differentiate into macrophages and absorb the lipoproteins to form the foam cells.[37] After these foam cells die, they leave behind lipid-rich debris that can accumulate at the site of the developing lesion (Figure 8).

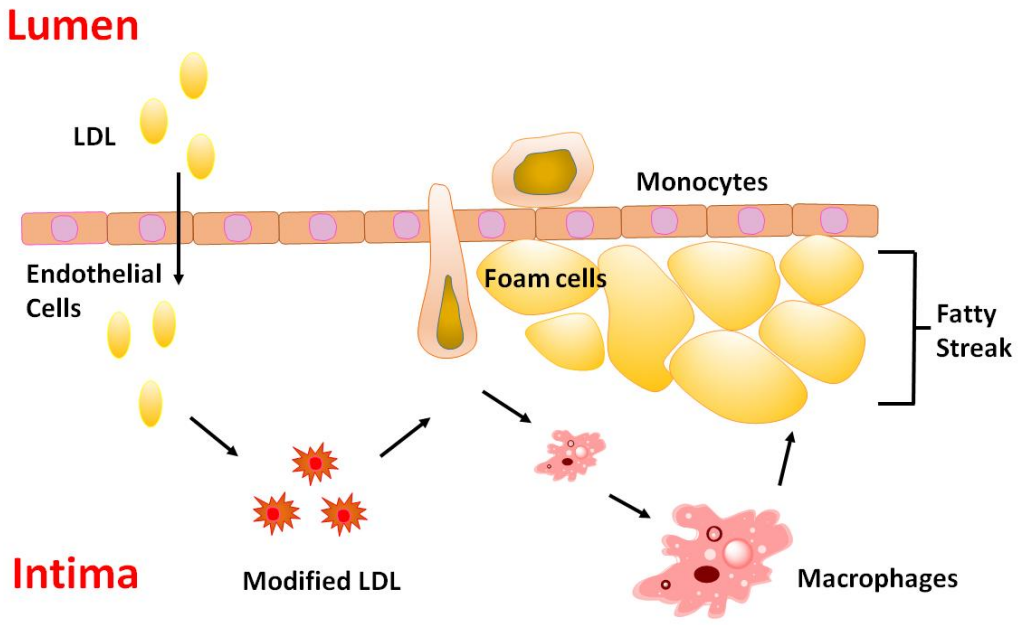


Figure 8: The development of fatty lesion and unstable plaques.

Over time the unstable plaques gradually increase in size and encroach on the lumen. These unstable plaques are weak and prone to rupture, leading to thrombus formation that can prevent or restrict blood flow in the arteries, causing myocardial infarction or stroke. The pathological process in which a stable, matrix-rich plaque phenotype transforms into an unstable pro-inflammatory phenotype is highly complicated, and the balance between inflammation and fibrosis is believed to be

mediated by transforming growth factor beta (TGF-β),[39] although the exact mechanism is not yet fully understood.

1.2.2 TGF-β and its possible anti-inflammatory role in atherosclerosis

TGF-β is a multifunctional cytokine that belongs to the transforming growth factor beta superfamily. It has been known to have roles in cell proliferation,[40] differentiation,[41] and other functions in many cell types. There are three structurally closely related isoforms of TGF-β in mammals, termed TGF-β1, TGF-β2, and TGF-β3 (Figure 9); all of which are known to bind to the same receptors and appear to exert similar functions.[42] Increasing evidence suggests that misregulation of the TGF-β concentration in plasma leads to the development of cancer,[43, 44] fibrotic disease,[45, 46] atherosclerosis,[39, 47-50] and many other diseases.[51]

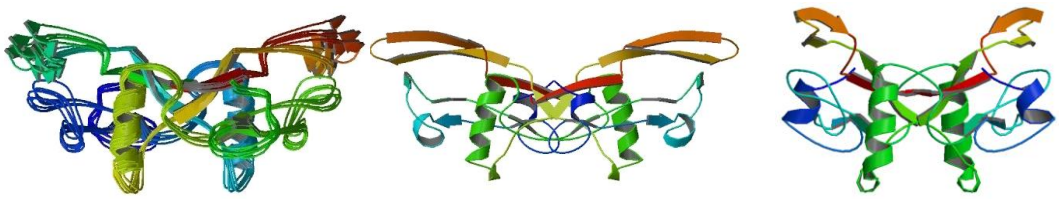


Figure 9: The structure 3 isoforms of TGF-β.

Image from www.rcsb.org PDB ID: 1KLA, 2TGI, 1TGK

Among the three TGF-β isoforms, TGF-β1 has been studied most extensively, particularly with its link to atherosclerosis, although the studies do not specify which ligand is accounted for the reported effects.[42]

TGF-β1 was first identified as an anti-inflammatory cytokine[52] and due to its anti-inflammatory properties. It has been proposed that TGF-β1 may function as an inhibitor of atherosclerosis.[47] In agreement of this hypothesis, it has been reported that expression of apolipoprotein A 1 in mice inhibited the activation of TGF-β, thus promoting smooth-muscle cell proliferation and the subsequent development of fatty lesions.[50] Consistent with this result are two independent but similar studies by Mallat *et al*.[47] and Lutgens *et al*.[39] In their studies, they employed neutralisation approaches to show that inhibition of TGF-β signalling in apolipoprotein E-deficient mice favours the formation of inflammatory cell-rich and collagen-poor lesions.

It has been demonstrated that Tamoxifen stimulates TGF-β production both in cancer cells and vascular smooth-muscle cells *in vitro*.[53] Grainger *et al*. postulated that Tamoxifen would inhibit atherosclerosis in mice[54] and their hypothesis has been shown to be correct. In various mice models, Grainger *et al*. were able to demonstrate that treatment with Tamoxifen triggered an increase in serum TGF-β1 levels and suppressed the diet-induced formation of lipid lesions in the aorta.[48, 55]

1.2.3 TGF-β and autophagy in atherosclerosis

Recent studies have, however, also shown that TGF-β is able to induce autophagy in certain human cancer cells.[56] Autophagy is a highly conserved homeostatic mechanism that involves lysosomal degradation of damaged and unwanted cellular components.[56] It is believed to play an important role in atherosclerosis and plaque progression, although the exact mechanism is still poorly understood.[57] The most convincing evidence for the beneficial effects of autophagy in atherosclerosis so far is probably an *in vivo* study conducted by Verheye and his co-workers. They

demonstrated that treatment with cholesterol-fed rabbits with everolimus, a derivative of rapamycin, led to a significant reduction (90%) of marcophage content via autophagic cell deaths without affecting the smooth muscle cells.[58] This finding is very significant since it is a common believe that presence of macrophages causes destabilization of atherosclerotic plaques.[58] Autophagy may exert its beneficial effect in atherosclerosis by degrading damaged intracellular organelles, thereby preventing oxidative injuries and cellular distresses.[59]

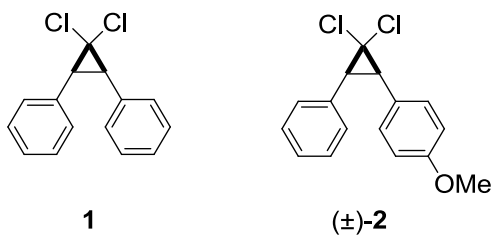
1.2.4 Other molecular targets of Tamoxifen

There is ample evidence of cardioprotective effects of Tam,[55, 60, 61] however, the findings that associate Tamoxifen and atherosclerosis at a molecular level are inconsistent and unclear. Some believe that Tamoxifen may mediate its cardio-protective effects by up-regulating serum TGF-β level which is, in turn, believed to stem from its antiestrogen ability.[62] The up-regulation of TGF-β may activate autophagy and/or have anti-inflammatory effects against atherosclerosis. On the other hand, others propose that it is the estrogenic activities that helps to reduce the incidence of coronary heart disease through down regulating the blood lipids.[23] It is also possible that the cardioprotective effects of Tamoxifen are through other mechanisms which are independent to its binding to the ERs. This is because Tamoxifen has also been demonstrated to inhibit cholesterol biosynthesis,[63-65] to act on P-glycoprotein and possibly cause interruption to the intracellular transport of cholesterol,[66, 67] to inhibit cholesterol acyltransferase (acyl-CoA) and the esterification of cholesterol,[68] and to prevent the apoprotein B of low-density lipoprotein (LDL) from being oxidative damaged.[69, 70] All these actions of

Tamoxifen could account for its cardioprotective effects and the exact underlying mechanisms are highly complex and have yet to be further confirmed or identified.

Tamoxifen is a potent inducer of autophagy.[71, 72] We and our collaborator, Grainger *et al.* believe that the cardioprotective effect of Tamoxifen may be arisen from this autophagy inducing ability. This is because studies by others recently have shown that enhanced autophagy may be beneficial at both early and advanced stages of atherosclerosis.[59, 73, 74] However, an increased incidence of endometrial cancer has also been reported in breast cancer patients treated with Tamoxifen and we would like to minimize this risk by developing derivatives of Tamoxifen with no apparent estrogenic activities, or at least, no intrinsic estrogenic activity in the endometrium cells.[19]

cis-1,1-Dichloro-2,3-diphenylcyclopropane **1** and its 4-methoxylated analogue **2** (Figure 10) were first synthesized and tested as pure antiestrogens by Magarian *et al.*[75] This class of derivatives of Tamoxifen may be of medicinal interest as the antiestrogenic activity of Tamoxifen was reported to up-regulate serum TGF-β level; and the up-regulation of TGF-β level has been shown to induce autophagy[56] which has been associated with the cardioprotective effects of Tamoxifen.[62]



1 (±)-**2**

Figure 10: Magarian's dichlorocyclopropyl analogues of Tamoxifen

Additionally, the replacement of a dichlorocyclopropyl moiety in place of the olefinic link in these derivatives of Tamoxifen can lead to a significant reduction or elimination of their estrogenic activity,[76-83] thus minimizing the risk of developing endometrial cancer. Therefore, we started our project based on Magarian's finding on dichlorocyclopropanes and aim to take a step-wise approach by first identifying the class or classes of derivatives of Tamoxifen with essential pharmacophores that could lead to induction of autophagy.

Chapter 2 Diaryldichlorocyclopropanes

2.1 Structure activity relationship studies and Magarian compounds

In the last few decades, Tamoxifen and its analogues have been extensively studied and the structure-activity relationship studies of these compounds reveal that the *Z*-arrangement of the α and β rings are crucial for the antiestrogenic activity (Figure 11).[78] In addition, it has been shown that the introduction of a dichlorocyclopropyl moiety in place of the olefinic link in these compounds can significantly reduce or eliminate their estrogen agonist activity.[76-83]

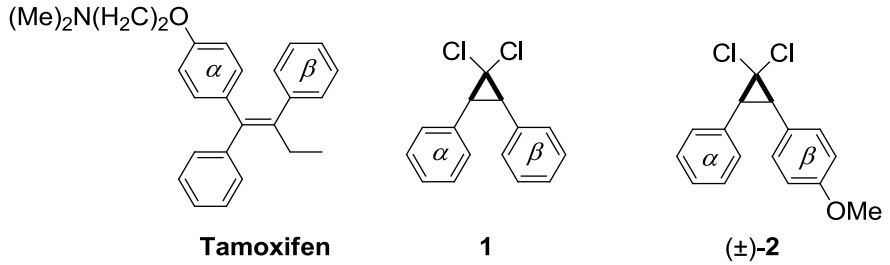


Figure 11: The structures of Tamoxifen and its dichlorocyclopropyl analogues

cis-1,1-Dichloro-2,3-diphenylcyclopropane (**1)** was first synthesized and tested for its biological activity as an antiestrogenic agent by Magarian *et al*. in 1975 in an attempt to reduce the undesirable estrogenic effects.[80] The compound displays antiestrogenic activity comparable to that of Tamoxifen against the hormone-dependent 7,12-dimethylbenz[a]anthracene (DMBA) induced rat mammary tumour model,[81] and is also found to be active against the hormone-independent DMBA-4 transplantable metastatic rat mammary tumour model,[82] although it produces no intrinsic uterotrophic effects. Magarian and his co-workers went on to synthesize some more derivatives of **1** and tested them for their anti-tubulin ability. Methoxylated analogue **2** was shown to have a five-fold increase in potency than **1**,

having an IC_{50} less than 1 μM for the MCF-7 cell line and less than 5 μM for the MDA-MB231 cell line.[77] In addition to the anti-proliferative ability against both the hormone-dependent and independent breast cancer cells demonstrated by Magarian *et al*.[77, 81, 82] and others,[84] **1** and **2** have also been shown to inhibit the growth of human prostate cancer cells in three transgenic adenocarcinoma of mouse prostate (TRAMP) models.[85] Again the 4-methoxylated analogue gives a higher potency with an IC_{50} less than 5 μM in all three cell lines.

Magarian's compounds **1** and **2** demonstrated that it is possible to synthesize dichlorocyclopropyl analogues of Tamoxifen which may have some desirable therapeutical effects, but without the estrogenic activities. Dichlorocyclopropyl stilbene derivatives have been tested for their antiestrogenic activity and anti-tumours properties, however, to the best of our knowledge, this class of compounds has not been tested for their ability to induce autophagy or in any atherosclerosis model. Thus, the focus of this project is to synthesize some dihalocyclopropyl Tamoxifen analogues and test their biological activities for autophagy *in vitro*, since it is believed that the cardioprotective effect of Tamoxifen may be arisen from its autophagy inducing ability.

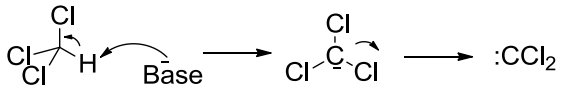
2.2 Synthetic routes for the preparation of dichlorocyclopropanes

2.2.1 Common methods for the synthesis of dichlorocyclopropanes

While there are numerous routes for the synthesis of cyclopropanes, methods for *gem*-dichlorocyclopropanes are very limited, with the great majority of the reactions reported involving the [2+1] cycloaddition of dichlorocarbenes to the olefins.[86] The

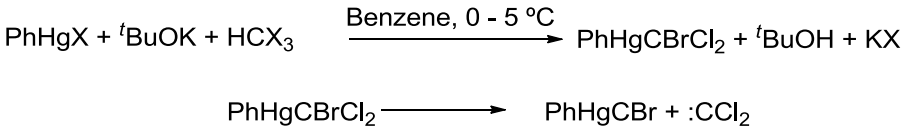
most important and widespread used methods for generating the dichlorocarbenes to date are the following:

1. α-elimination of hydrogen chloride from chloroform under concentrated aqueous solution of a base, often NaOH or *tert*-BuOK, in the presence of a lipophilic tetraalkyammonium salt as the phase transfer catalyst (Scheme 1).[86]



Scheme 1: α-elimination of hydrogen chloride from chloroform.

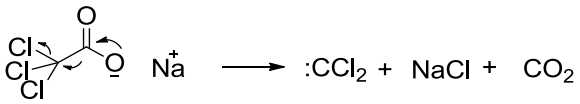
2. Thermal decomposition of a metal complex carbene precursor: trichloro- or bromodichloromethyl (phenyl) mercury (Scheme 2).[87] This method is very effective and allows transformation of alkenes with sensitive functional groups or alkenes of low reactivity to the desired *gem*-dichlorocyclopropane in good yields.[87] Unfortunately, in spite of their excellent reactivity, their high toxicity and subsequent lack of commercial availability have severely limited their use as a dichlorocarbene source. Their preparation is also both difficult and hazardous.



Scheme 2: Thermal decomposition of phenyl-(trichloro) or (bromodichloromethyl) mercury.

3. Decarboxylation of an alkali metal carbene precursor: e.g. sodium trichloroacetate (Scheme 3).[86] With unreactive alkenes (either due to electronic or steric reasons), a

large excess of the carbene precursor is often required, meaning the process inevitably becomes more expensive.



Scheme 3: Decarboxylation of sodium trichloroacetate.

There are certainly numerous ways of generating the dichlorocarbene, such as photolysis of dichlorocarbene complexes using visible or UV light,[88] lithiation of diethyl trichloromethyl phosphonate[89] and reaction of carbon tetrachloride with magnesium[90] or butyllithium[90] in the presence of alkenes, etc. They do not, however, offer any advantages over the methods presented earlier.[86]

Among all the methods that have been mentioned, phase transfer catalytic (PTC) dichlorocyclopropanation is probably the simplest and most favoured way of preparing the *gem*-dichlorocyclopropanes as it is easy to perform, has a low energy requirement and uses inexpensive, relatively non-toxic and commercially available reagents and solvent. Thus, for this particular project, PTC has been chosen as the primary method for the dichlorocyclopropanation step.

2.2.2 Mechanism of formation of dichlorocyclopropanes in PTC:

Although the term phase-transfer catalysis was introduced by Starks in 1971,[91] when he first demonstrated that reactions between two substances located in two immiscible phases could be accelerated by a phase transfer agent, the first interfacial mechanism for the generation of dichlorocarbene in a biphasic system was proposed by Maükosza[92] in 1975. According to his proposal (Figure 12), in the biphasic

system composed of an organic phase containing chloroform with alkene and an inorganic aqueous base, deprotonation of chloroform happens at the interface. Trichloromethyl anions generated form salts with sodium cations undergo ion exchange with quaternary ammonium salts to give organic soluble ion-pairs. These ion-pairs are extracted to the organic phase, where they dissociate reversibly to form dichlorocarbenes that are trapped by alkenes to afford the gem-dichlorocyclopropanes and the quaternary ammonium salts are re-generated.

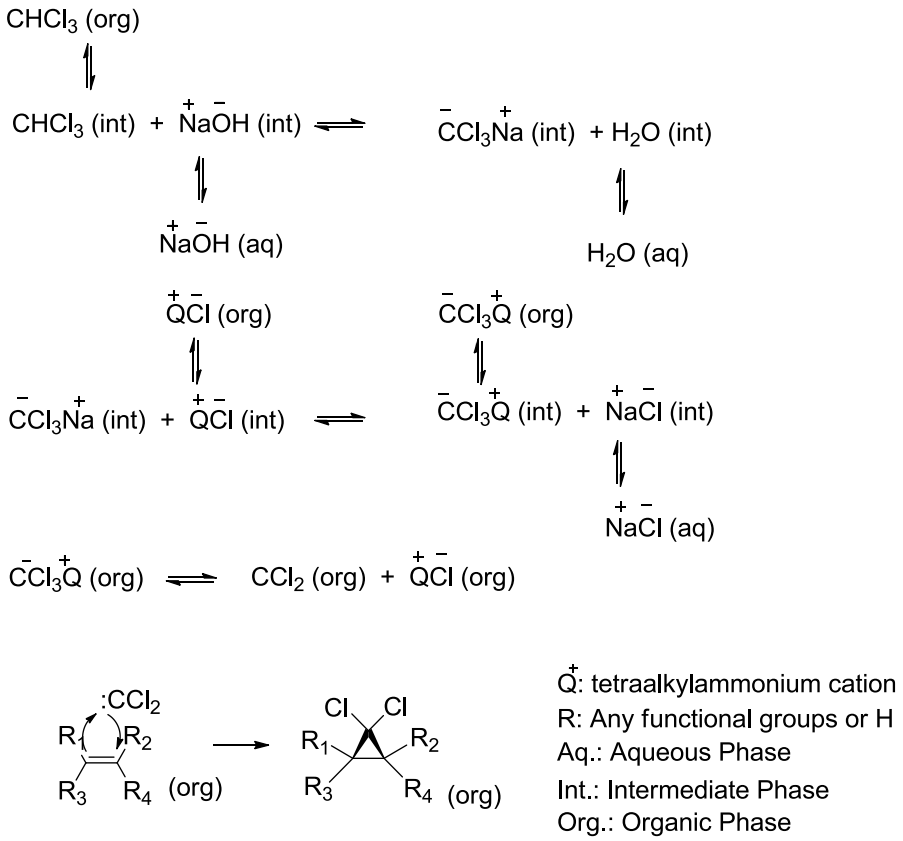


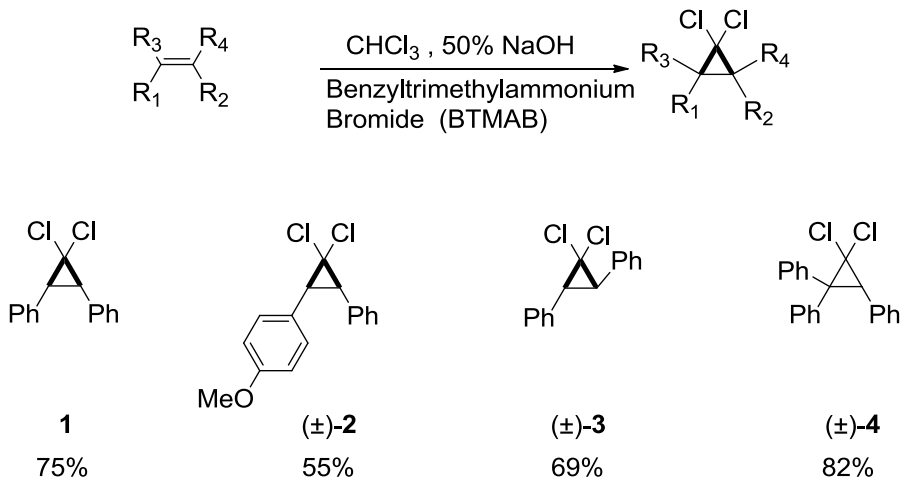
Figure 12: The interfacial mechanism proposed for the generation of dichlorocarbene in PTC.[33]

2.3 Research and development

2.3.1 Non asymmetric synthesis of dichlorocyclopropyl stilbenes

All the dichlorocyclopropyl compounds Magarian *et al.* synthesized and biologically tested were racemic, yet those compounds still displayed excellent therapeutic properties. Thus, this project began with the syntheses of some of those known, racemic dichlorocyclopropyl analogues of Tamoxifen.

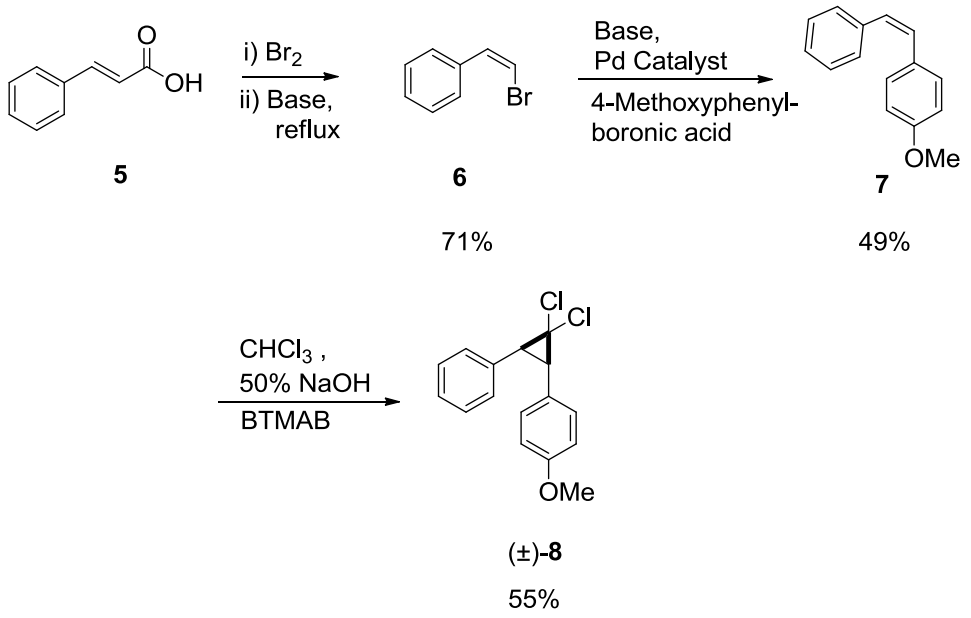
The synthesis of **1**, **3** and **4** are straightforward and can be achieved in one step with moderate to good yields by the PTC dichlorocyclopropanation method since the starting material triphenylethylene, *cis*- and trans-stilbenes are all commercially available (Scheme 4).



Scheme 4: The dichlorocyclopropyl analogues of Tamoxifen synthesized using PTC method

For a functionalized *rac*-1,1-dichloro-2,3-diarylcyclopropane such as **2**, an improved synthetic route from Magarian was adopted which involved the preparation of *cis*-*β*-bromosytrene that was then coupled with 4-methoxybenzeneboronic acid via Suzuki

coupling to give mainly the *cis*-isomer of 4-methoxy stilbene, followed by the PTC dichlorocyclopropanation (Scheme 5).



Scheme 5: An improved synthesis of *cis*-1,1-dichloro-2-(4-methoxyphenyl)-3-phenylcyclopropane (**2)**

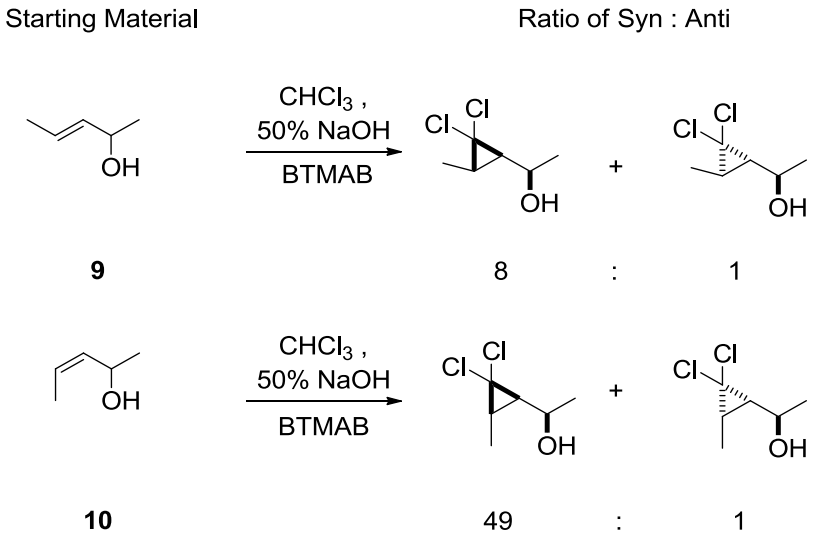
However, the synthesis of enantiomerically pure or enriched dichlorocyclopropanes is a topic of great importance if they are ever to become medicinally useful. Therefore, we intended to synthesize this class of compounds asymmetrically.

2.3.2 Asymmetric synthesis of dichlorocyclopropanes

2.3.2.1 Previous studies on asymmetric synthesis of dichlorocyclopropanes

Still *et al.*[93] has demonstrated that dichlorocarbene reacts stereoselectively with secondary allylic alcohols to give the *syn* - dichlorocyclopropanes (the dichlorocyclopropyl and the hydroxyl groups being on the same face) preferentially. Additionally, the *cis*-isomer is more diastereoselective than the corresponding *trans*-

isomer in giving the *syn*- products. Some of his findings are summarized in Scheme 6.



Scheme 6: Still's asymmetric synthesis of dichlorocyclopropanes

The diastereoselectivity of the dichlorocyclopropanation for *trans*- and *cis*-pent-3-en-2-ols (**9** and **10** respectively) may be explained by the alkene conformations of the starting materials using Houk's model shown in Figure 13. In both cases, the lowest energy conformations are the ones on the left hand side, in which the hydrogen eclipses the double bonds, reducing the 1,3-allaylic strain by having the alcohol group being away from the *cis* substituents (H and Me).

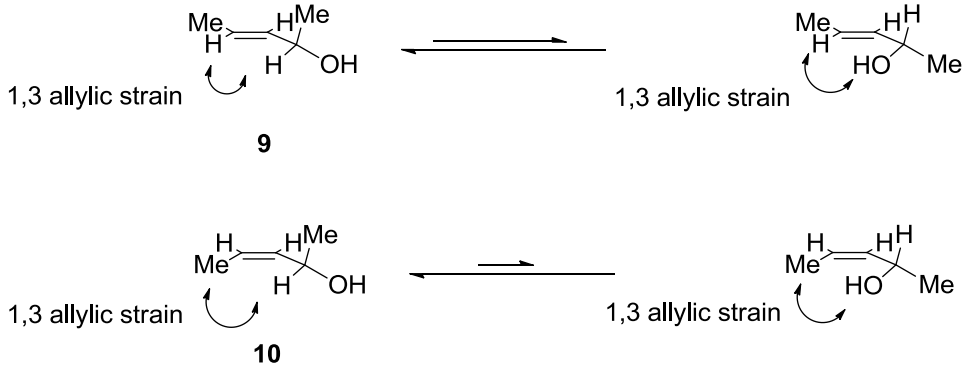


Figure 13: Houk's model

The dichlorocarbene then is more likely to attack the conformations with lowest energy from the less hindered face, giving the diastereomers as observed (Figure 14). The *cis*-alkene gives higher selectivity than its corresponding *trans*-alkene is because the 1,3-allylic strain in the higher energy conformation is greater for **10** (H and OH group) than **9** (Me and OH group) and therefore the reaction is more selective for the former than the latter.

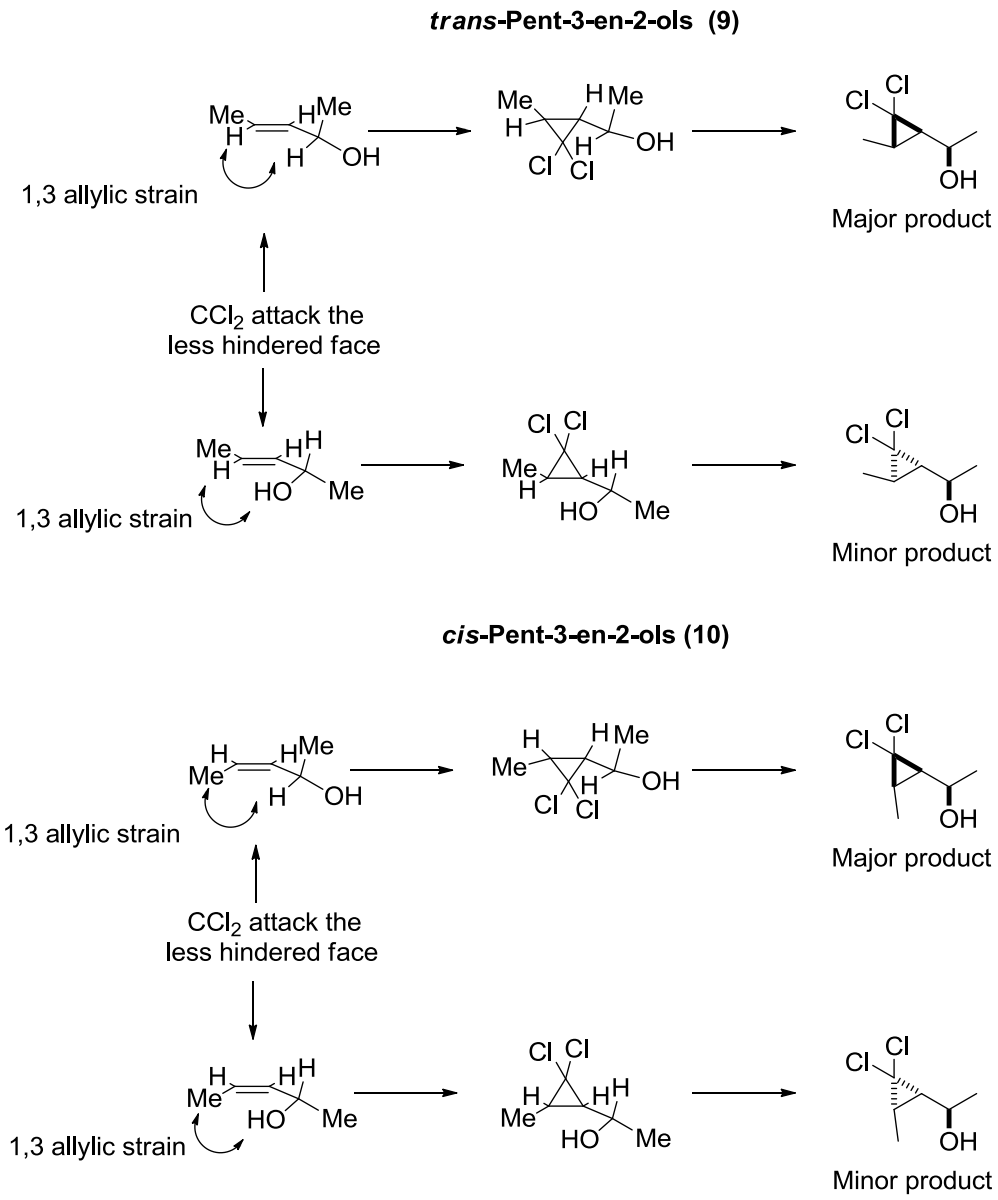


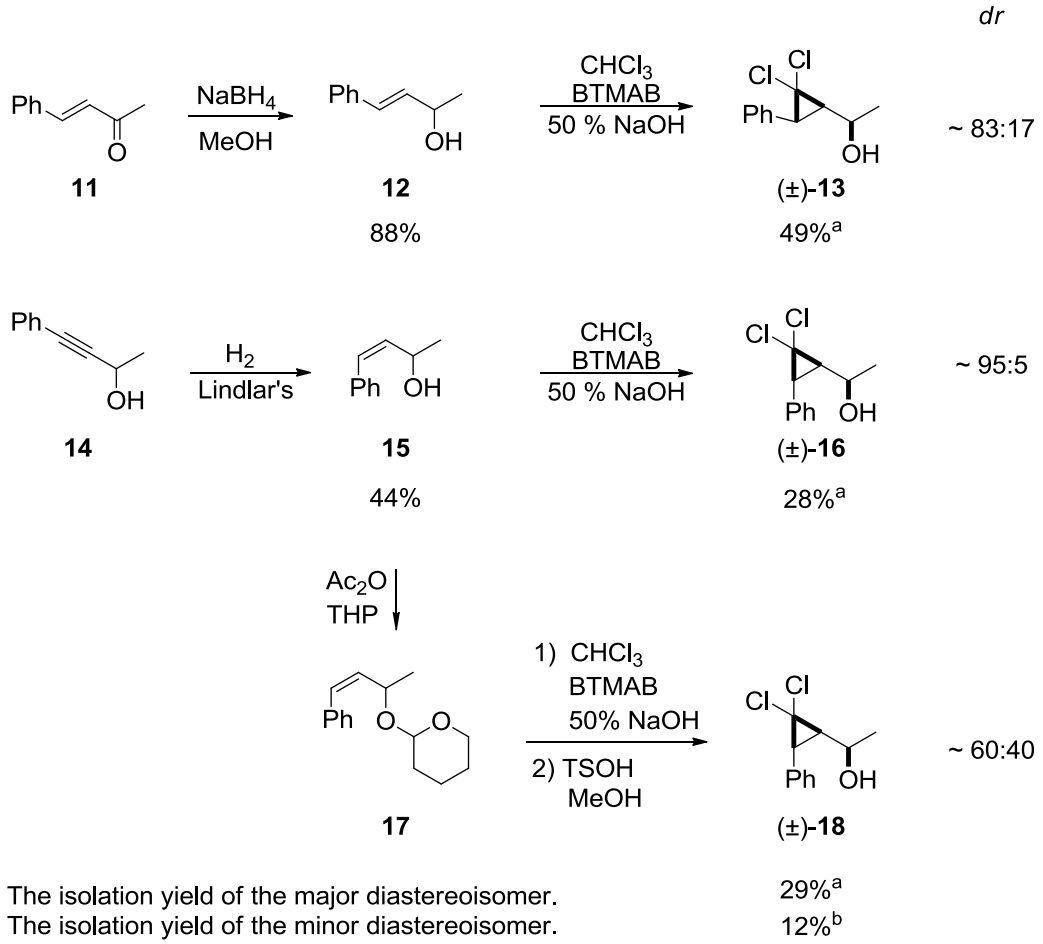
Figure 14: The stereoselective reactions of *cis*- and *trans*-pent-3-en-2-ols with dichlorocarbenes.

Based on the findings, we believed that if the stereochemistry of the directing groups, in this particular example, the hydroxyl group in the olefins can be controlled, then an asymmetric synthesis of dichlorocyclopropanes may be possible.

2.3.2.2. Diastereoselective synthesis of dichlorocyclopropanes using OH as a directing group and the challenges encountered

Still's finding has been tested on a few carefully selected aryl containing substrates and showed very positive results. For example, a mixture of diastereoisomers with a high diastereomeric ratio of about 83:17 was obtained when *trans*-ketone **11** was reduced with $NaBH_4$ to give the *trans*-alcohol **12** before being treated with dichlorocarbene derived from $CHCl_3$/NaOH to offer the dichlorocyclopropyl alcohol **13** (Scheme 7). A marked increase in the diastereomeric ratio was observed under the same reaction condition when a geometric isomer *cis*-**15** (synthesized via reduction of alkyne **14** using Lindlar's catalyst) was used as the substrate instead of the *trans*-**11**. It seems that only one diastereoisomer can be seen in the spectrum of the crude reaction product, suggesting the diastereomeric excess (*de*) is $\geq$ 95%.

Similarly, the diastereoselectivity of the dichlorocyclopropanation for **11** and **12** could be explained by using Houk's model shown in Figure 15. Again, the carbene is more likely to attack the lowest conformations which are the ones on the top left hand side for both substrates. The cycloaddition of carbenes to the alkenes are achieved from the less hindered faces, giving *syn*-dichlorocyclopropanes preferentially.



Scheme 7: The asymmetric synthesis of dichlorocyclopropanes from alcohol.

To confirm only one of two diastereoisomers was preferentially formed, we had synthesized both diastereoisomers by a slightly different route with the same starting alcohol **15**. The OH group of **15** was protected with 3,4-dihydro-2*H*-pyran (THP) and subsequent dichlorocyclopropanation of **17** gave a mixture of the THP-protected diastereoisomeric alcohols. The protected-dichlorocyclopropyl alcohols were not isolated and were directly used for the next deprotection step by simply stirring the reaction mixture in methanol with a few gains of (±)-camphorsulfonic acid to offer **18**. The two diastereoisomers of **18** were subsequently separated by standard silica chromatography and the ^{1}H NMR spectra of both diastereoisomers were then

compared with the ^{1}H NMR spectrum of the crude product of **16** which, indeed, showed that only one diastereoisomer was present.

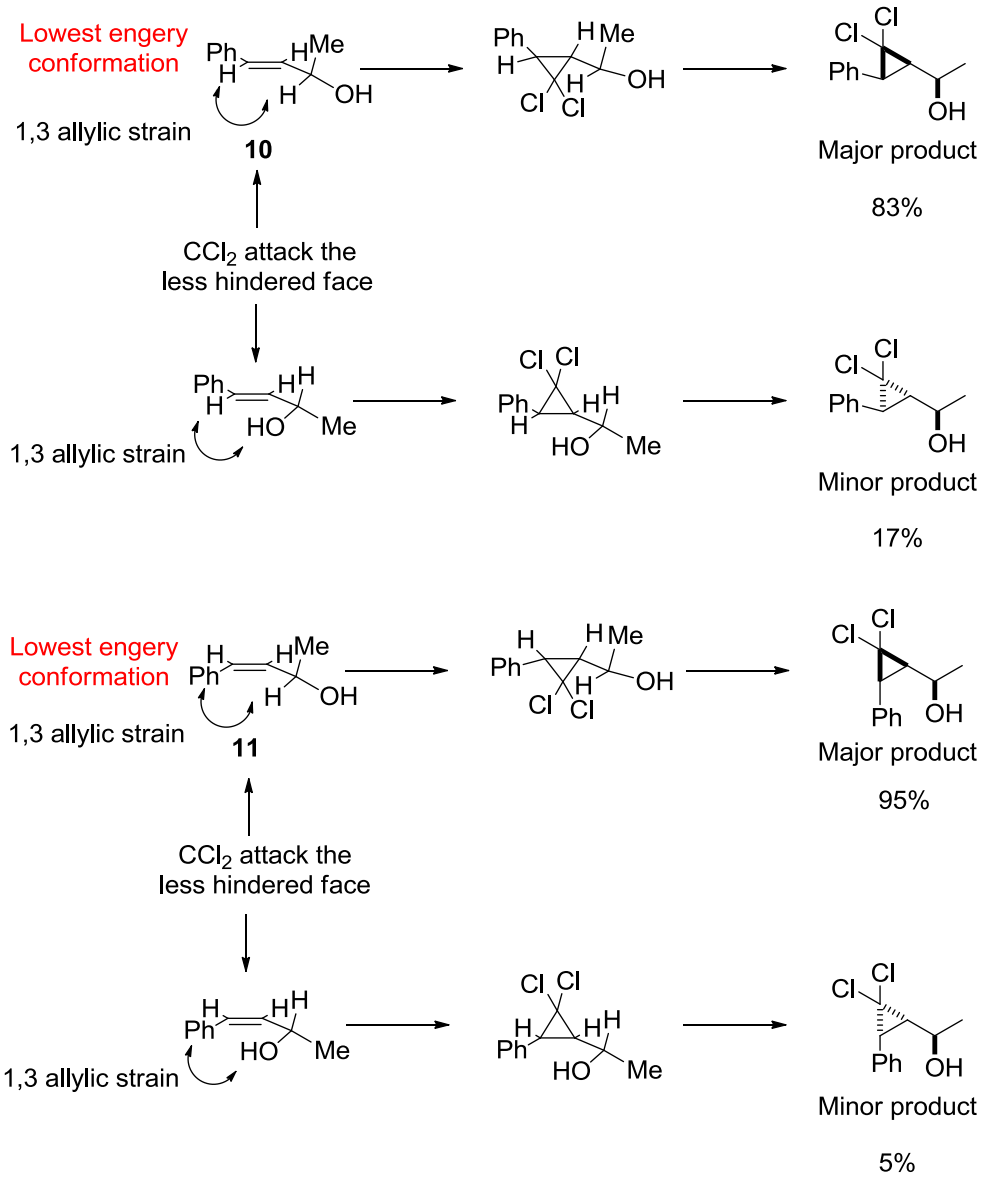


Figure 15: Diastereoselective dichlorocyclopropanation on allylic alcohols.

It is worthy to mention that for all the substrates tested the dichlorocyclopropyl products and the alkenes tend to be very similar in polarity. Indeed, they often come as a single spot in TLC plates under a wide range of solvent systems, making monitoring the progress of reactions by TLC very difficult or sometimes impossible.

Isolation of the products also proved to be problematic as all of the *gem*-dichlorocyclopropanes synthesized are essentially oils; the easiest and cheapest way of purifying those products in milligrams scale is probably by standard silica chromatography, which again relies greatly on the TLC analysis. Although it is not ideal and can be quite time-consuming and resource-wasting, a solution to this problem would be to collect different fractions and analyse each fraction by ^{1}H NMR spectroscopy.

Furthermore, for substrates like stilbenes and triphenylethylene, in which there are no other functional groups or activated C-H bonds apart from the alkenes that can react with the dichlorocarbene (for example, the synthesis of **1-4**), the reactions normally proceed quite cleanly with high conversion. The main side reactions are the carbene oligomerisation or hydrolysis of the dichlorocarbene whose by-products can be easily separated from the products during work up or by column chromatography. On the other hand, for substrates bearing an alcohol group and a C-H bond that has been activated by the presence of an adjacent hetroatom, oxygen and nitrogen for example, then insertion of carbene into both the O-H[94] and the C-H[95] bonds can occur. **20** was a by-product that was successfully identified from the reaction of **19** with $CHCl_3$/NaOH in the presence of benzyltrimethyl ammonium bromide as the phase transfer catalyst. From **20**, it is discovered that the dichlorocarbene insertion by-products can react further in the presence of other appropriate species, leading to a lot of side products being formed. The formation of **20** is possible via the mechanism shown in Figure 16.

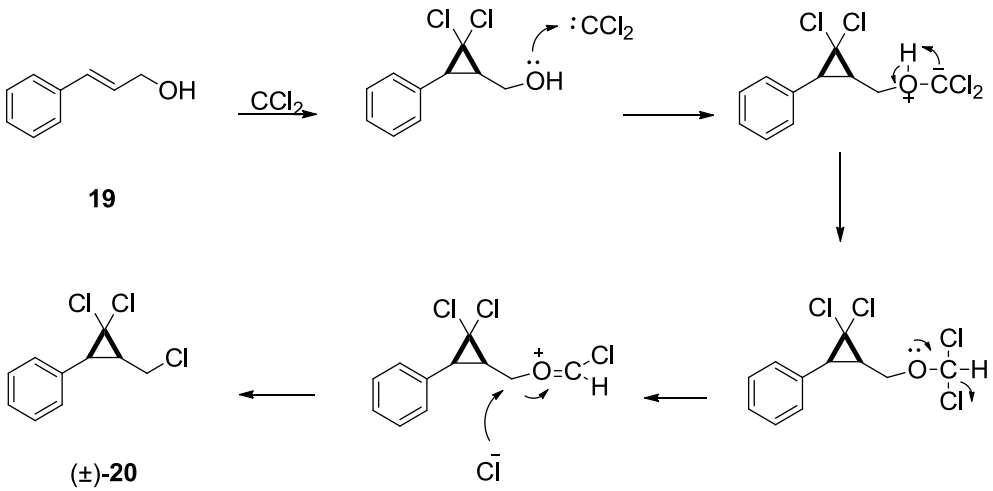
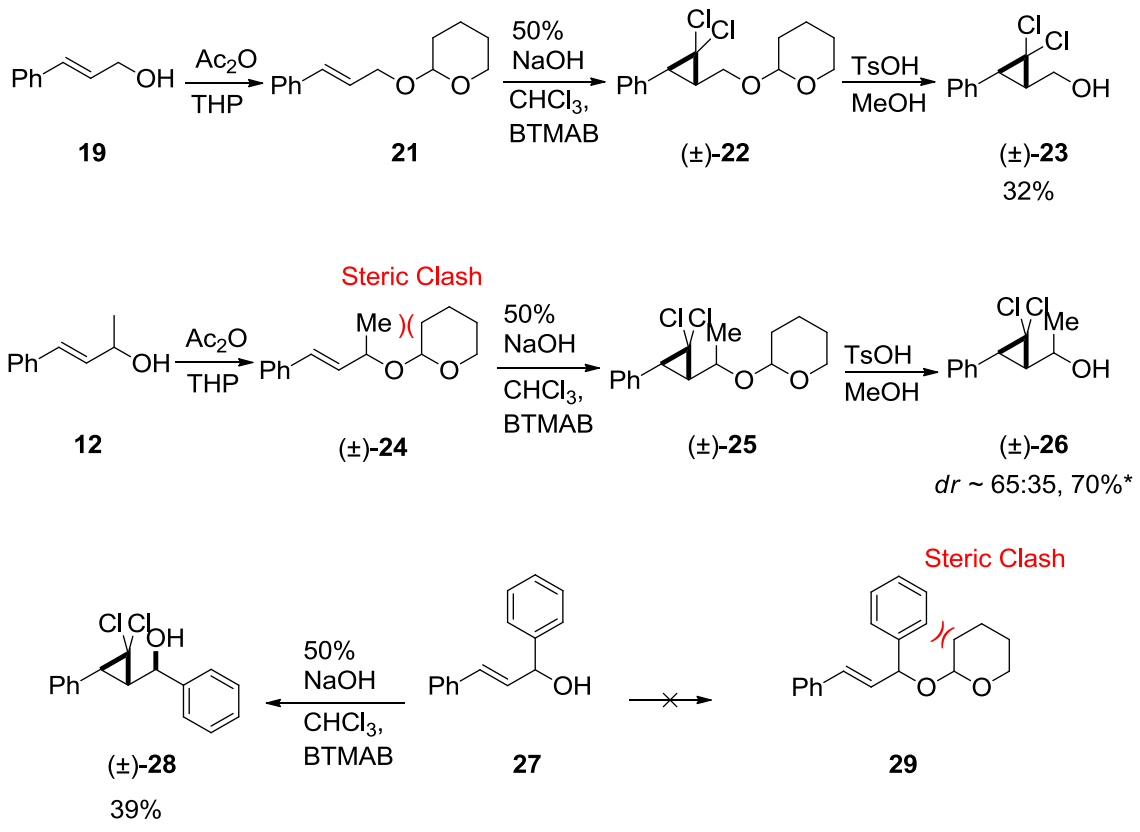


Figure 16: The possible mechanism for the formation of compound 18.

To minimise the side reactions, we had tried to protect the alcohols **12**, **19** and **27** with THP before the dichlorocyclopropanation step (Scheme 8)**.** Although the protection and deprotection using THP did limit the amount of undesired molecules being formed in the preparation of **23** and **26**, an extra stereocentre was introduced into the molecules which may potentially affect the diastereoselectivity. In fact, when **12** was protected with THP, the diastereomeric ratio of the cyclopropane product **26** reduced from ~83:17 to ~65:35. The reason for the decrease in selectivity is not certain, but the protection of an alcohol group with a THP resulted in creation of a new stereogenic centre which may have hindered or even altered which face the dichlorocarbene was attacking the alkenes.

Unsurprisingly, as the alpha position to the hydroxyl group becomes more sterically hindered, it becomes harder to protect the hydroxyl group. Attempts to protect the O-H group in **27** failed to give a reasonable amount of the protected alcohol **29**, but unexpectedly direct dichlorocyclopropanation of **27** proceeded smoothly to give **28** without too many side reactions.



Scheme 8: The asymmetric synthesis of dichlorocyclopropanes from alcohol.

In comparison to the direct synthesis of **12** and **27**, the yields of the dichlorocyclopropanes did not seem to benefit greatly from the protection of OH since it involves two more steps in the synthesis. Not only did the protection and deprotection steps rarely go to completion, but also some of the desired product may have been lost in the additional work up procedure. As a result, the use of THP as a protecting group was eliminated for this range of substrates.

2.3.2.3. Product formation vs reaction time studies using ^{1}H NMR spectroscopy

The difficulties in monitoring the progress of the dichlorocyclopropanations using TLC had prompted us to use ^{1}H NMR spectroscopy as an alternative method to

briefly examine the reaction rates. In our time study using substrate **12**, a small amount of the reaction mixture was taken out from the reaction flasks in 30 min intervals and submitted for ^{1}H NMR analysis.

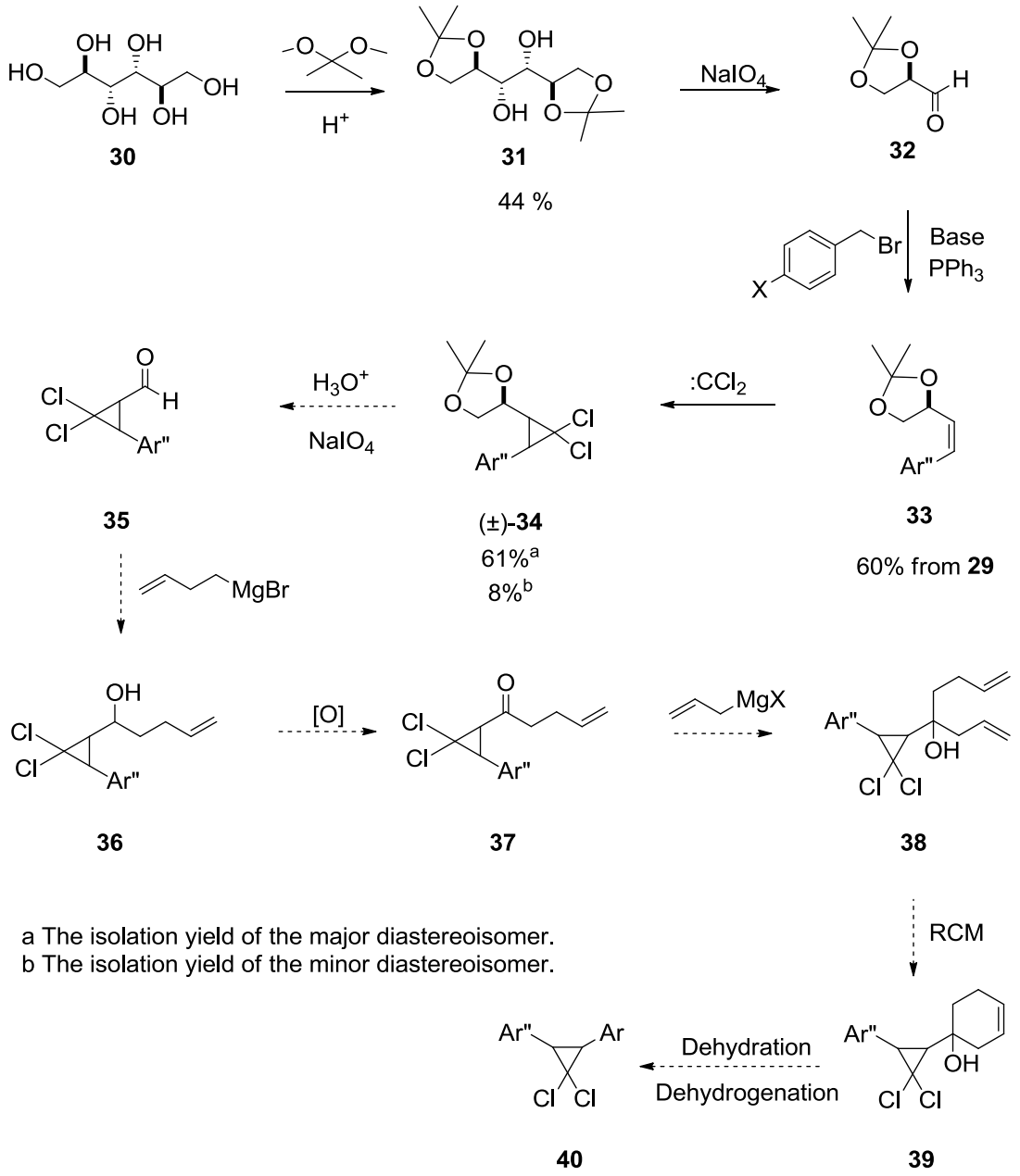
When monitoring the reaction, we found that alkenes bearing a free O-H group reacted much faster with dichlorocarbene than alkenes with a protected hydroxyl group (2 days), diaryl (6 hours) or triarylethylene (3 days). Within two hours, the majority of the starting material had converted to the desired dichlorocyclopropanes along with a small amount of side products that presumably resulted from carbene insertions into OH. Based on this observation, it is believed that dichlorocyclopropanation of the olefins is in competition with carbene insertion side reactions. The relative rate of dichlorocyclopropanation and carbene insertions depends on many factors, but mainly nucleophilicity and steric hindrance of the alkenes involved, electrophilicity of the dichlorocarbene generated in that particular environment and the types of bonds that are present to allow carbene insertion to take place.

Alkenes with an OH group are particularly susceptible to carbene insertions, although the dichloropropanation of such an alkene appears to happen at a faster rate than carbene insertion. Hence, at the beginning of the reaction when the concentration of the alkene is high, the dichlorocarbene reacts primarily with the alkene to offer the dichlorocyclopropane and the carbene insertion side reactions are less prominent. As the reaction proceeds towards to completion, the alkene still competes for the dichlorocarbene, but insertion of carbene into both the O-H and C-H bonds in the product becomes dominant since the concentration of

dichlorocyclopropyl product is now much higher than the alkene. This finding is very useful as it tells us that the reaction time is very crucial in determining the yields and the dichlorocyclopropanation has to be stopped as soon as it is completed, otherwise the products generated will start to degrade rapidly.

2.3.2.3 Synthetic routes proposed for asymmetric synthesis of 1, 1-dichloro-2, 3-diaryl cyclopropanes

Encouraged by Still's finding and the results of our own, we started to design a synthetic route for asymmetric synthesis of the dichlorocyclopropyl analogues of Tamoxifen (Scheme 9)**.** Starting with inexpensive *D*-mannitol **(30)**, 1,2-5,6-terminal diols were selectively protected with 2,2-dimethoxypropane using catalytic amount of *p*-toluene sulfonic acid. Subsequent oxidative cleavage of **31** with $NaIO_4$ gave aldehyde **32** which was not isolated and was used directly for the Wittig reaction in the next step. Reaction of **32** with benzyltriphenylphosphonium bromide and *n*-butyllithium provided the alkene **33** with *cis* and *trans* ratio of ~70:30. The two geometric isomers of **33** were subsequently separated by standard silica chromatography. Treatment of **33** with CCl_2 derived from $CHCl_3$/NaOH offered the diastereoisomeric dichlorocyclopropanes **34** with a ratio of ~88:12 that were also separable using column chromatography. Deprotection and oxidative cleavage of **34** should afford the target molecules **35** which would then be treated with butenyl magnesium bromide to give the secondary alcohol **36.** Oxidation of **36** would generate the ketone **37** which could be treated again with an appropriate Grignard reagent to give **38**. The tertiary alcohol **38** may undergo ring-closing metathesis to give **39**, and finally dehydration and dehydrogenation of **39** should give the enantiomerically pure/enriched dichlorocyclopropyl compound **40**.



Scheme 9: The proposed asymmetric syntheses of diaryldichlorocyclopropanes

However, only up to the synthesis **34** had been accomplished as we then realized that compound **2** and **4** were not particularly stable. Upon being stored in sealed vials at room temperature for a week, compound **2** decomposed, and so did compound **1** after a few weeks.

The decomposition of diaryl- or triaryldichlorocyclopropanes is not unknown. In fact, Magarian and his colleagues had reported that the attempts to purify *trans*-

diphenyldichlorocylclopropane **3** by sublimation[96] and **41** by standard silica chromatography[97] both resulted in the thermal loss of HCl with rearrangement to give the 2-chloro-1-phenylindene **42** and the 2-chlorodiarylindene **43** respectively (Figure 17).

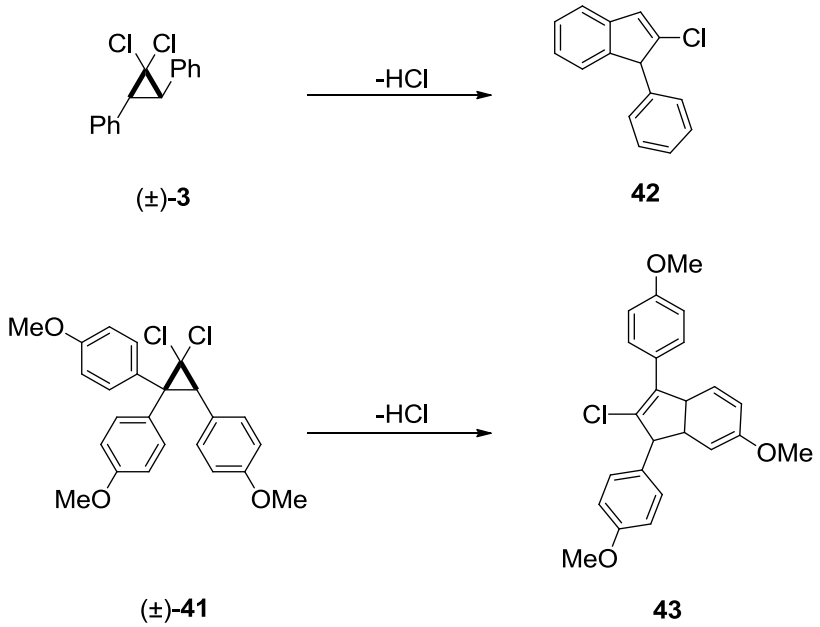


Figure 17: **40** and **41** were the degradation products from **3** and **39** identified by Magarian *et al.*

The thermal loss of HCl and rearrangement of **41** is believed to proceed via a concerted cleavage of the C^2-C^3 cyclopropyl bond with one of the chlorides as a leaving group to form an allyl cation.[97] The π electrons from one of the aromatic rings then attacks the allyl cation giving an indenyl cation, which subsequently loses a proton to complete the aromatization and afford **43**,[97] Figure 18.

The proposed mechanism of cyclopropyl ring opening postulated by Magarian *et al*. seems to be consistent with our finding. While the stability of unfunctionalized diphenyldichlorocyclopropanes **1** and **3** was found to be satisfactory and no

degradation was detected, compound **2** was quite unstable and decomposed at room temperature over a few weeks under the same handling conditions. This may be due to the fact that the electron donating methoxy group helps to stabilize the allyl cation formed upon ring opening, thus accelerating the degradation of **2**.

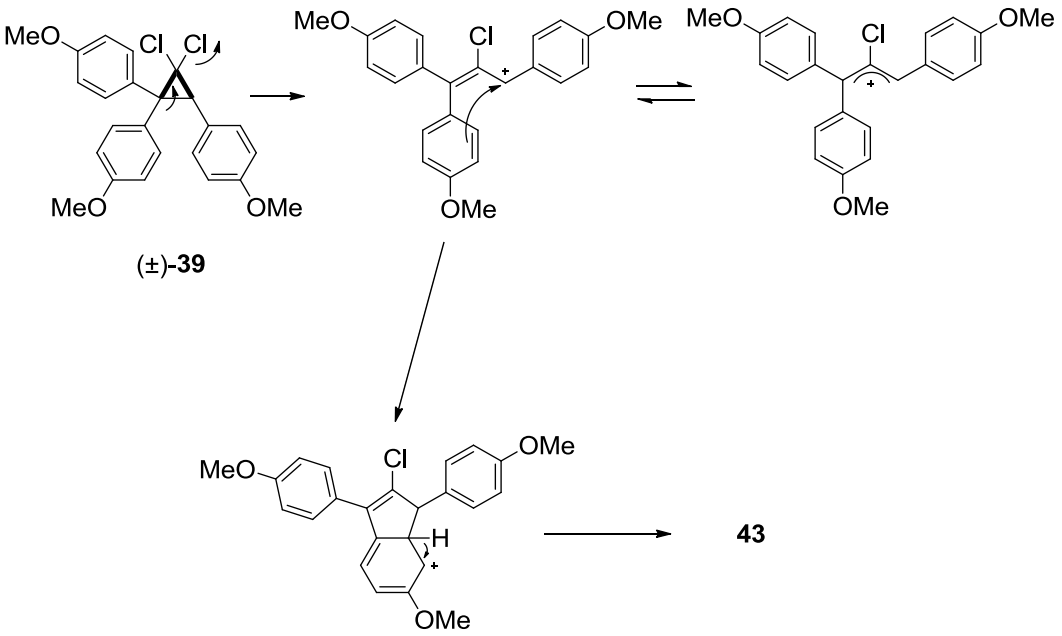


Figure 18: The proposed mechanism for the thermal loss of HCl and rearrangement of **41** to **43**.

The unstable nature of these dichlorocyclopropanes has not only led us to abandon the plan for their asymmetric synthesis, but has also prompted us to expand our range of desired compounds to include its difluorocyclopropyl analogues. The difluorocyclopropanes may offer higher stability due to the relatively stronger C-F bond.

Chapter 3 The Syntheses of Difluorocyclopropanes

3.1 Difluorocyclopropanes

3.1.1 The nature of fluorine as a substituent

Introduction of fluorine substituents into organic molecules often has a profound change in their physical, chemical and biological activities.[98] Fluorine atoms are very electronegative and fairly small in size which means that the C-F bond is very polar and has a relatively high ionic character. The 2*s* and 2*p* orbitals of fluorine also match more closely in size with those of carbon than any other of the halogens since they are elements of the same period and hence the orbital overlap of carbon with fluorine is much larger than with any other halides. All these reasons have contributed to the fact that C-F is one of the strongest single bonds in chemistry.

There are a number structural and thermochemical effects present in fluorinated organic compounds that result from the unique properties of fluorine. For example, there is a strong energetic preference for multiple fluorine substitutions at a carbon atom due to the increasing Coulombic interaction between the negatively charged fluorine atoms and the positively charged carbon.[99] These increased Coulombic interactions result in the strengthening of all carbon-carbon bonds neighbouring the fluorine substituents and lead to the inherent thermal stability of fluorinated organic compounds. In addition, fluorine has a strong tendency to be attached to a carbon bearing other carbons rather than hydrogens.[100] This is because tertiary carbons are more able to bear the positive charge that occurs when bonded to a highly electronegative atom.

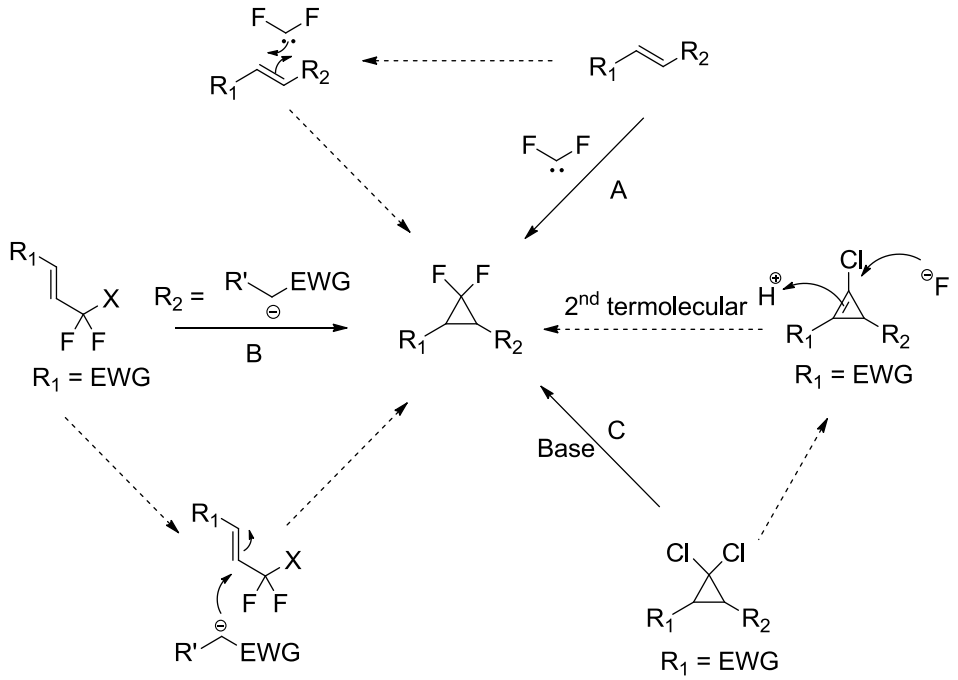
3.1.2 Fluorinated cyclopropane

In the special case of geminally difluorinated cyclopropanes, the effect is one of destabilization. The presence of two fluorine atoms on a cyclopropyl cabon is known to facilitate the cleavage of the C^2-C^3 bond. Fluorine is very electronegative and its strong σ-acceptor nature means that it withdraws significant electron density from the molecular orbital of the three-membered ring. However, as Durmaz *et al.* suggested that neither the π donation of the lone pair nor the σ-acceptor character is responsible for the shortening of vicinal (C^1-C^3 and C^1-C^2) bonds and the lengthening and thus, weakening of the distal (C^2-C^3) bond.[101] Of importance is the effect of fluorine substituents on the change of hybridization of the carbon molecular orbitals and steric interaction.[101] Due to fluorine's high electronegativity, it very much prefers to bond to carbon orbitals with high *p*-characters. As a considerable excess of *p*-character has already been utilized for the formation of the strained C-C bonds in cyclopropane, when one or more of the hydrogens are replaced by fluorine, the fluorine will "steal" *p*-character from the C-C bonds.[98] This re-distribution of *s*- and *p*-character on fluorine-bearing carbons results in the weakening of the C-C bonds, especially the C^2-C^3 bond and leads to a bond differentiation in the substituted cyclopropanes. The C^2-C^1-C^3 bond angle is also increased which intensifies the "strain" in the system.[98]

3.1.3 Synthesis of difluorocyclopropanes

While the syntheses of fluorinated cyclopropanes are numerous, methods for gem-difluorocyclopropane synthesis are very limited and the majority can be categorized into 3 types (Figure 19). The most common used is a [2+1] cycloaddition of a difluorocarbene or metal carbenoid to an alkene (**A**). Alternative routes for

synthesizing gem-difluorocyclopropanes that do not involve carbene include: a Michael addition of an anion to a double bond that is stabilized by an electron-withdrawing group followed by Michael initiated ring closure (**B**) and finally, consecutive elimination of hydrogen chloride and subsequent addition of fluoride ions and protonation to replace the chloride atoms in the dichlorocyclopropane (**C**).

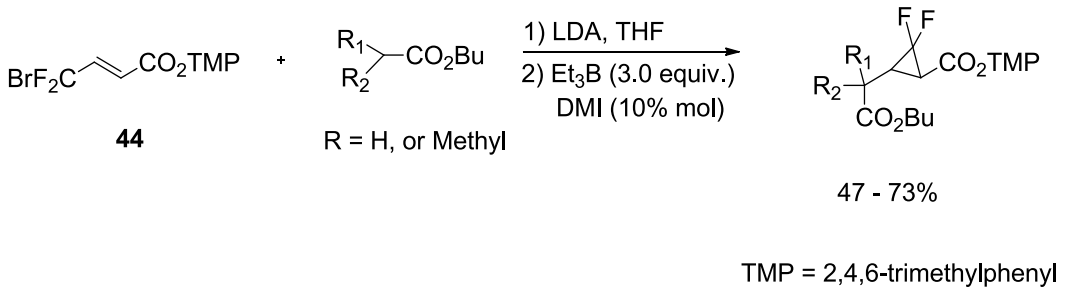


Where X is a leaving group, EWG is an electron withdrawing group

Figure 19: Common synthetic routes to difluorocyclopropanes

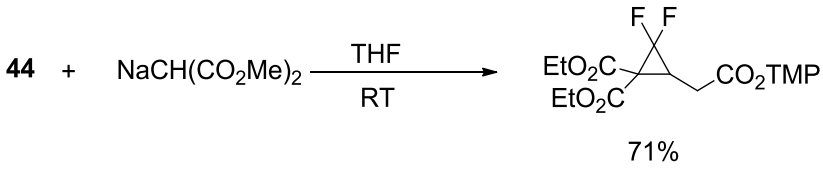
While examples for method **A** are various, the others are less well-known and utilized. In fact, not many successful examples have been reported using methods **B** and **C**. Perhaps it is worthwhile to give at least an example of each of those methods, but the main focus of this introduction will be on the carbene route since it is the most extensively studied and probably the most effective way of making the difluorocyclopropanes.

Method B: Taguchi reported an remarkable Michael addition followed by subsequent cyclisation process to synthesize functionalized difluorocyclopropanes through the reaction of 2,4,6-trimethylphenyl (TMP) ester of 4-bromo-4,4-difluorocarotonate **(44)** with the lithium enolate of an ester or amide (uiiScheme 10).[102] It was found that addition of triethylborane is necessary to initiate the cleavage of CF_2-Br bond after the Michael addition step, which then underwent an intramolecular substitution reaction.



uiiScheme 10: Michael addition of a lithium enolate followed by triethylborane promoted cyclisation

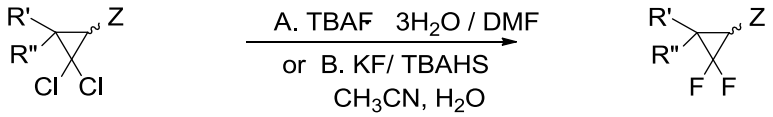
Interestingly, when **44** reacts with carbanions derived from active methylene compounds, such as malonate anion, only direct S_N2 displacement is observed at the malonate carbon and no triethylborane is needed (Scheme 11).[102]



Scheme 11: Michael addition of a maloate anion followed by cyclisation.

Method C: *gem*-difluorocyclopropanes could also be conveniently synthesized from the corresponding *gem*-dichlorocyclopropanes via a series of elimination-addition

processes (Scheme 12).[103] Although this reaction is very easy to perform, it can only be applied to substrates bearing a strong electron-withdrawing group and an α hydrogen atom must also be present. Yields of the products are usually quite low. Most importantly, the stereochemical outcome is very likely to be under thermodynamic control, meaning that *trans*-products will always be preferentially formed even when a *cis*-substrate is used.



(12 - 47%)

Z = $CO_2{}^tBu$, CO_2Pri, SO_2Ph, COPh; R = H, Me

Scheme 12: Displacement of chloride by fluoride atoms on the *gem*-dichlorocyclopropyl ring

3.2 Difluorocarbene

3.2.1 Structure of carbene in general

Carbenes are a family of neutral, two-coordinate carbon compounds with an oxidation state of II whose valence shells contain only six electrons, of those two are non-bonding electrons.[104, 105] The structure of carbene can either be bent or linear, depending on their degree of hybridization. Most singlet carbenes adopt a bent geometry with a sp^2 hybridization, because the two non-bonding electrons share the same p_x orbital (also known as σ orbital) while leaving the p_y orbital (or p_π) almost unchanged (Figure 20).[105]

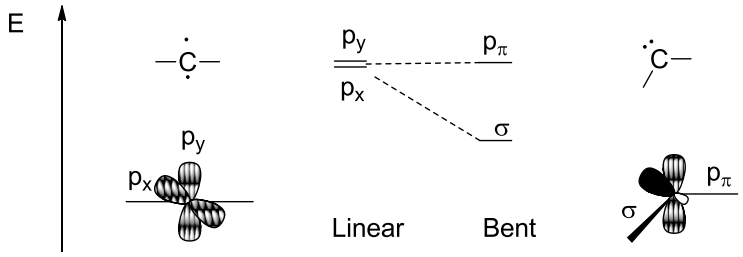


Figure 20: The general structure of a singlet carbene and triplet carbene.

This figure is reproduced from Bourissou *et al.*[105]

On the other hand, triplet carbenes have a linear *sp* hybridized structure with each of the two non-bonding electrons occupying one of the two degenerate orbitals (p_x and p_y) respectively.[105]

There are four possible electron configurations for carbenes as illustrated in Figure 21. The two nonbonding electrons can either be in the same σ or p_π orbitals, giving rise to two different $^1A_1$ states, though σ^2 is generally more stable than p_π^2.[105] When the two nonbonding electrons are placed in two different orbitals with parallel spin, the configuration is depicted as $\sigma^1 p_\pi^1$ with a $^3B_1$ state. Finally, an excited singlet state with a $\sigma^1 p_\pi^1$ configuration has a $^1B_1$ state.

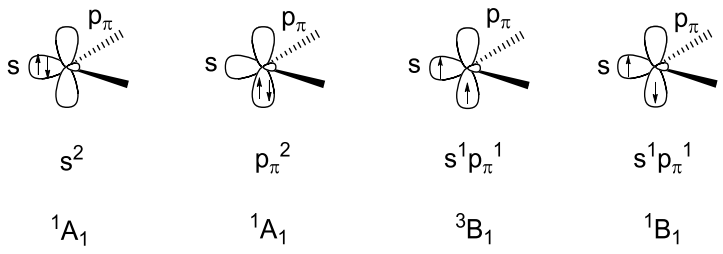


Figure 21: The four electronic configuration of carbenes

This figure is reproduced from Bourissou *et al.*[105]

As the two nonbonding electrons in the singlet carbene are spin-paired, it has a total spin of zero while that of triplet is one since there are two singly occupied orbitals and can be considered as diradicals. The group-state spin multiplicity of carbene is an important aspect, since it determines their reactivities.[105] If the energy gap between $\sigma - p_\pi$ orbital is large, typically above 2 eV, the singlet ground state is favoured, whereas a separation of less than 1.5 eV will most likely favour the triplet ground state.[106] The energy separation of $\sigma - p_\pi$ is, in turn, greatly influenced by the electronic and steric effects of substituents on the carbene.[105]

The electronic effects of substituents on a carbene can be divided into two parts: inductive effects and resonance effects. The inductive effects arise due to the electronegativity difference between the substituents and the carbon atom forming a σ bond. It has been demonstrated that electronegative substituents (e.g. F) favour a singlet ground state while electropositive substituents (e.g. Li) favour the triplet state (Figure 22),[107] although resonance effects also play an important factor on their ground state multiplicity.

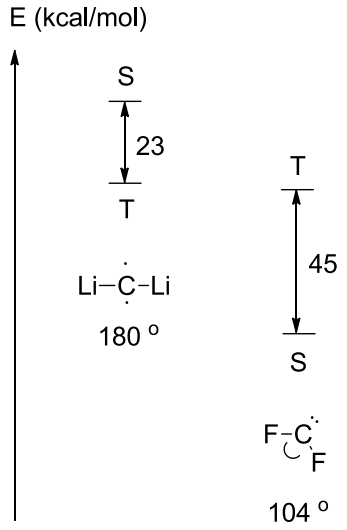


Figure 22: The effect of substituents' electronegativity on the ground state carbene spin multiplicity

This figure is reproduced from Bourissou *et al.*[105]

Figure 23 illustrates the effect of a) electronegative and b) electropositive substituents on the perturbation orbital diagram of carbenes. As shown, electronegative substituents lower the energy of the σ nonbonding orbital by inductively withdrawing electron density while leaving the p_π orbital unchanged. This results in an increase of $\sigma - p_\pi$ separation and thus favours the singlet ground state. Conversely, electropositive substituents donate their electron density to the carbon atom, pushing the energy of the non-bonding σ orbital closer to the empty p_π orbital. The $\sigma - p_\pi$ energy gap is reduced and therefore a triplet state is more favourable.

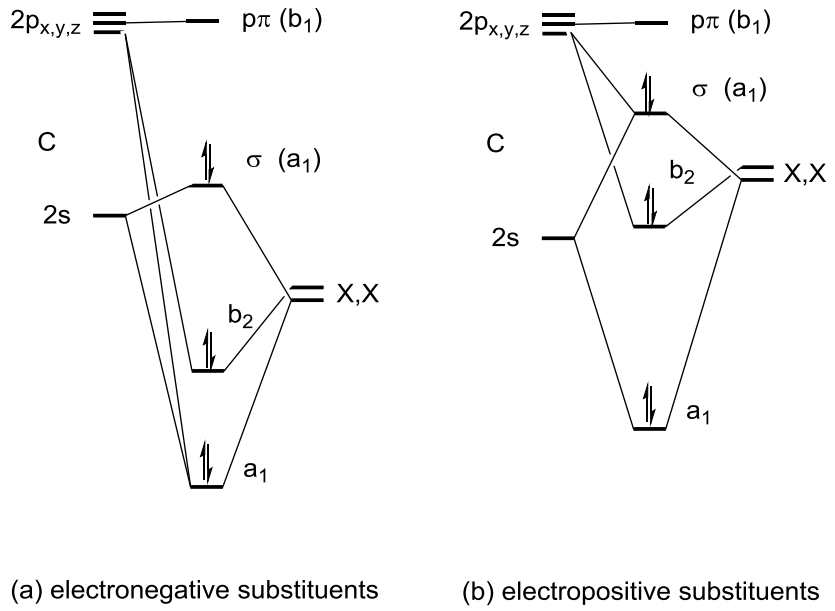


Figure 23: Pertubation orbital diagrams showing the influence of the inductive effects.

This figure is reproduced from Bourissou *et al.*[105]

Compared to inductive effects, however, resonance effects are in general of more importance.[105] Resonance effects involve the interaction of the carbon orbitals (p_x, p_y) with suitable *p* or π orbitals of the two subsitutents on carbene.[105] There are two classes of substituents that can induce these interactions, π electron-donating groups

Y (e.g. -F, -Cl, -Br, -I, $-NR_2$, $-PR_2$, -OR, -SR, $-SR_3$...) and π electron-withdrawing groups Z (e.g. -COR, -CN, CF_3, $-BR_2$, $-SiR_3$, $-PR_3^+$……). Thus, carbenes can have 3 different arrangements of substituents: the highly bent Y-C-Y carbene, the linear Z-C-Z carbene or quasi-linear Y-C-Z carbene.[105] Again, the effects of Y or Z groups on the $\sigma - p_\pi$ energy separation can be easily illustrated using a perturbation orbital diagram (Figure 24).

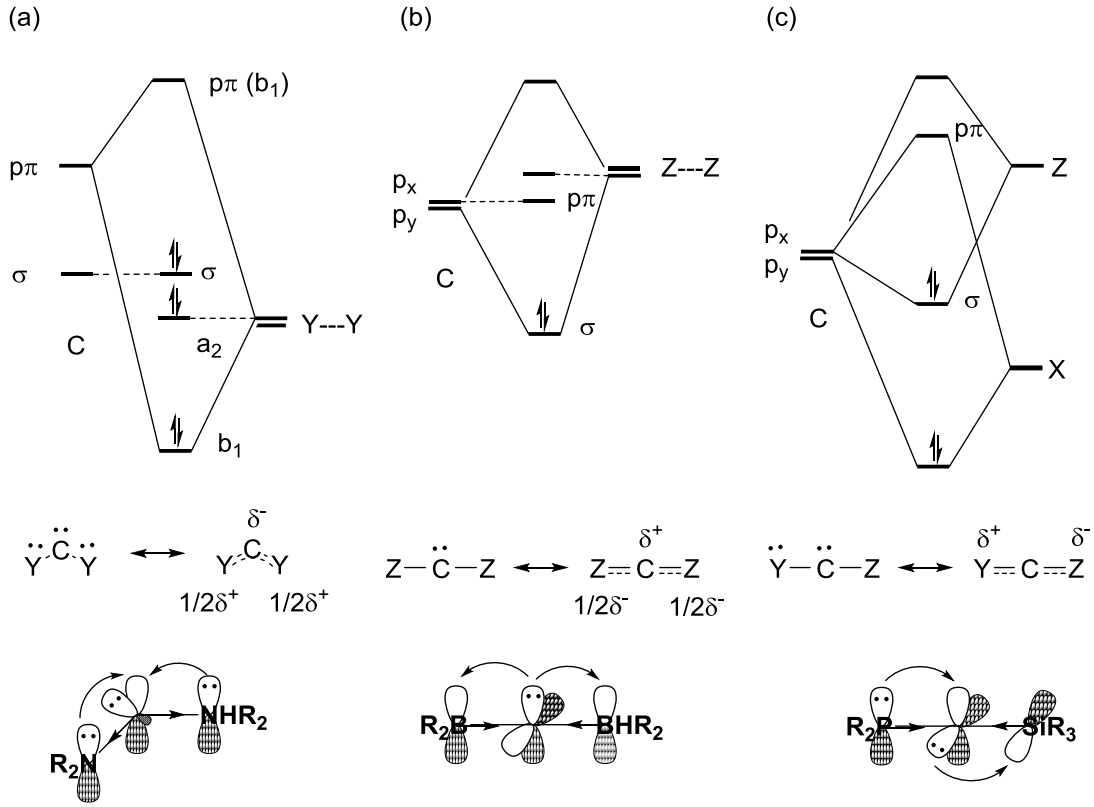


Figure 24: Pertubation orbital diagrams showing the influence of the resonance effects.

This figure is reproduced from Bourissou *et al.*[105]

When both of the substituents are Y groups, the lone pairs of the Y substituents interact with the empty p_π orbital of carbene, creating a polarized four-electron three-centre π system (Figure 24a). The energy of p_π orbital is increased as a result of this interaction, but the energy of the σ orbital remains unchanged.[105] Therefore, the σ –

p_π energy gap increases, favouring the singlet ground state. (Y,Y)-carbenes usually have a bent geometry which can be depicted as a superposition of two zwitterionic structures with C-Y bonds acquiring some double bond character and the carbene centre bearing the negative charge.[105] The most common carbenes of this type are the dimethoxy and diaminocarbenes as well as the main focus of this introduction, dihalocarbenes.

According to Bourissou *et al.*[105], most (Z,Z)-carbenes would be singlet with linear structures. An example of this type is the diborylcarbenes (Figure 24b). As it is illustrated, the p_y orbital of carbon interacts with the vacant orbital of the Z substituents to form a polarized two-electron three-centre π system, whilst leaving the p_π orbital of the carbon unaffected,[105] thus, the (p_x, and p_y) orbitals are no longer degenerate. The C-Z bonds also have some double bond characters and are best represented by the superposition of two zwitterionic atom with the carbene centre now bearing a positive charge.[105]

The (Y,Z)-carbenes possess both types of electronic interactions discussed above (Figure 24c). The lone pair of the Y substituents interacts with the p_π orbital while the empty orbital of the Z substituent interacts with the p_x orbital of the carbon atom.[105] Consequently, both of the substituents are stabilized and both favour a singlet state.[105] The C-Y and C-Z bonds in this type of carbene also have double bond character and are described as examples of a " polarized allene-type system" by Bourissou *et al.* [105]

In conclusion, most CX_2 carbenes are predicted to be singlet with a highly bent geometry if X are elements or groups more electronegative than the central carbon atom[108] (e,g N to F) , and are triplet with either a linear or slightly bent geometry if X are elements or groups more electropostivie than the central carbon atom [108] (e,g Li or H). (Z,Z)- and (Y, Z) types of carbene are the exception in which the singlet carbenes also adopt a linear or quasi-linear structure.

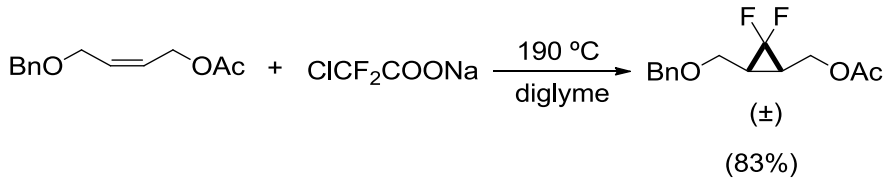
3.2.2 Properties of difluorocarbene

As has been mentioned previously, difluorocarbene is bent with a singlet electronic state. This is consistent with the stereospecific outcome of its addition reaction. Relative to other dihalocarbenes,:CF_2 is much more stable and less reactive. This is because among all halo substituents, fluorine atom provides the strongest electron donation via resonance to the empty *p* orbital of carbene and the strongest inductive stabilization of the two carbene's non-bonding electrons.[109]

The relatively unreactive nature of difluorocarbene means that only electron-rich olefins may react readily with difluorocarbene under mild condition to give the difluorocyclopropanes. For less nucleophilic alkenes, the reaction is much more difficult and challenging. This often requires :CF_2 to be generated at high temperature (>80 °C) to overcome the substantial energy barrier for addition.[98, 110] In most cases, the reactive intermediate is believed to involve a "free" difluorocarbene. This naked difluorocarbene is very electrophilic and can react with any substrates that can serve as stronger nucleophiles than the alkenes, e.g. water, in the reaction mixture. Thus, it is not surprising to know that a rigorously dry reaction conditions is often crucial for the successful addition of difluorocarbene to alkenes.

3.2.3 Methods for generating difluorocarbene

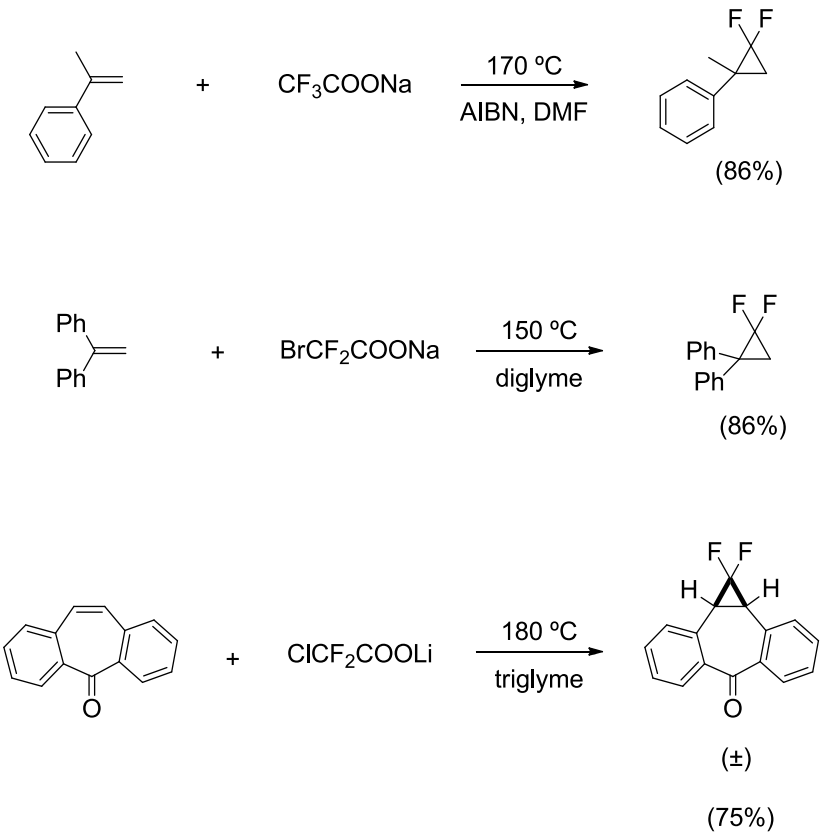
Over the last few decades, many methods have been developed for the generation of difluorocarbene. All except a few involve thermal fragmentation of a suitable difluorocarbene precursor with or without a catalyst. The first published and probably still the most frequently employed method is the pyrolysis of sodium chlorodifluoroacetate in refluxing diglyme at 190 °C (Scheme 13).[111]



Scheme 13: $ClCF_2COO^-Na^+$ is a thermal precursor of $:CF_2$

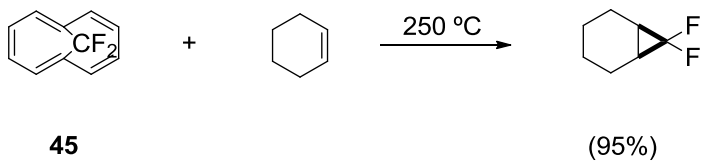
Since then, a few closely related reagents such as sodium trifluoroacetate[112] and bromodifluoroacetate[113] and other metal salts of chlorodifluoroacetate (Scheme 14)[114] have also been reported.

For this class of reactions, a large excess of reagents, in some cases more than tenfold equivalents, are usually required to drive the reaction to favour the difluorocyclopropanation. Useful to good yields can be obtained even for relatively unreactive alkenes and the reactions are performed under neutral reaction condition which means any acid or base sensitive functional groups are tolerated. Unfortunately, these methods are only limited to substrates with high thermal stability due to the rather harsh reaction conditions.



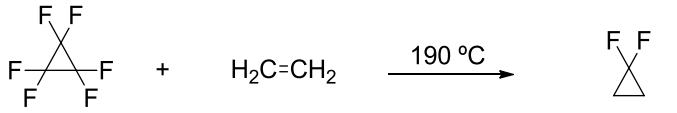
Scheme 14: Metal salts of halodifluoroacetate as thermal precursors of :CF_2

Another effective thermal source of :CF_2 is 11, 11- difluoro-l,6-methano-[l0]annulene **(45)** (Scheme 15)[115] which undergoes thermolysis upon being heated at 250 ºC; In the presence of an excess of cyclohexane, 7,7-difluoronorcarane was obtained in excellent 95% yield. Although effective, the very high temperature required has precluded its widespread application.



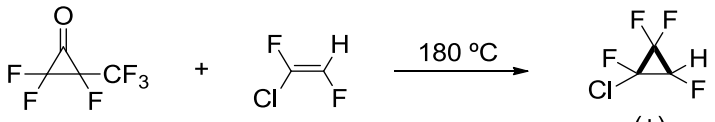
Scheme 15: Thermolysis of 11, 11- difluoro-l,6-methano-[l0]annulene to give :CF_2.

Some highly strained and polyfluorinated three membered rings, for example, perfluorocyclopropane **(46)** (Scheme 16)[116] and hexafluoropropylene oxide **(47)** (Scheme 17)[117] may also be decomposed thermally to extrude difluorocarbene at slightly lower temperature (>180 ºC). Yields are generally modest for reactive alkenes, but poor with unreactive ones.



46 (52%)

Scheme 16: Pyrolysis of perfluorocyclopropane to give :CF_2



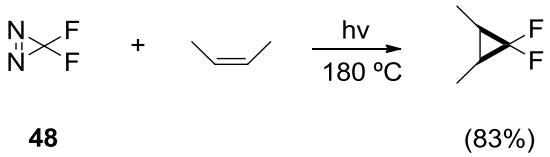
47 (57%)

Scheme 17: Photochemical precursor of :CF_2

Difluorodiazirine **(48)** (Scheme 18)[118] is another early difluorocarbene precursor developed. When photochemically heated at 180 ºC or above, it decomposes to give :CF_2 and N_2 gas. The reaction is very clean and the addition reactions with nucleophilic alkenes generally give good yields. The main drawback for this reagent is that it is potentially explosive and both its preparation and usage are hazardous.

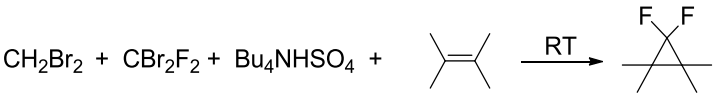
Attempts to generate the difluorocarbene at low temperature have also been explored. Balcerzak *et al*. (Scheme 19)[119] reported the first practical example of difluorocyclopropanation in a PTC system at room temperature by reaction of

CH_2Br_2 and CBr_2F_2 in the presence of alkenes and tetrabutylammonium hydrogensulphate (TBAHS) as a PTC catalyst.



48 (83%)

Scheme 18: Thermal decomposition of difluorodiazirine to give $:CF_2$



(70%)

Scheme 19: Difluorocyclopropanation in a phase-transfer catalysed (PTC) system

The reaction works by deprotonation of CH_2Br_2 with 60% aqueous solution of KOH and subsequent ion exchange with the TBAHS offers the lipophilic ion pair $CHBr_2^-Q^+$ (Figure 25). In the organic phase, it is possible that $^-CHBr_2Q^+$ reacts with CBr_2F_2 to give $HCBr_3$ and the reactive intermediate $CBrF_2Q^+$ which further dissociates into $:CF_2$ and tetrabutylammonium bromide. The $:CF_2$ generated under this environment can give difluorocyclopropanes with modest to good yields from electron rich alkenes, but fails to react with electron poor olefins. The conventional PTC method, e.g. dehydrohalogenation of a difluorohalomethane, does not work for difluoropropanation due to rapid base hydrolysis of difluorocarbene formed at the interfacial region by a concerted loss of proton and halide ion.[7, 25]

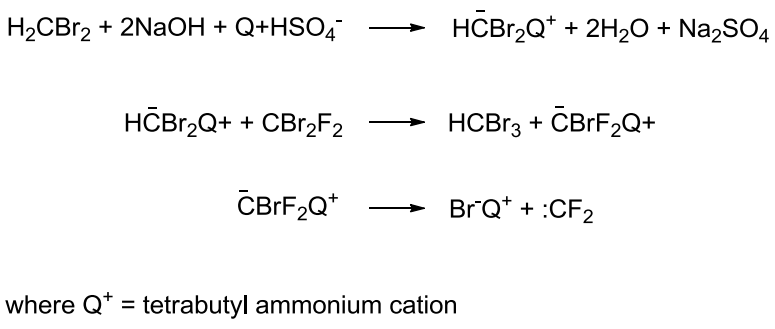
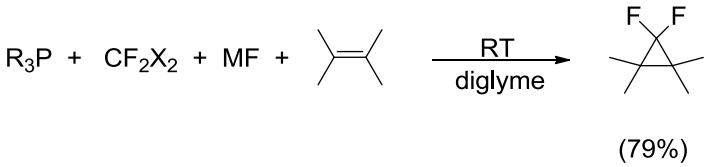


Figure 25: Possible reaction mechanism of CH_2Br_2 and CBr_2F_2 in the presence of KOH and TBAHS

Another noteworthy room temperature difluorocyclopropanation method is the use of an appropriate phosphine with difluorodihalomethane reported by Burton and Naae (Scheme 20).[120] They discovered that the treatment of bromodifluoromethylphosphonium bromide salt, formed from PPh_3 and CBr_2F_2, with either KF or CsF would generate $:CF_2$ which is able to covert nucleophilic alkenes to difluorocyclopropanes in good yield. Mechanistic study suggest that a CF_2Br^- anion is directly generated from bromodifluoromethylphosphonium bromide, followed by decomposition of this anion to $:CF_2$.



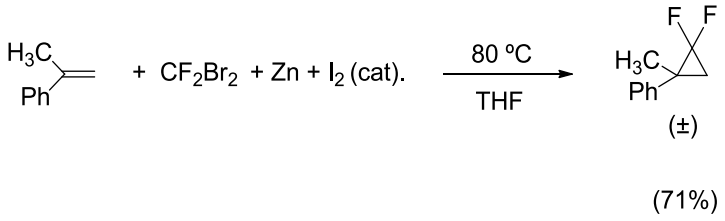
R = Ph or Me_2N; X = Br, Cl; M = K, Cs

Yield indicated when R = Ph, X = Br, M = Cs were used.

Scheme 20: Generation of $:CF_2$ via the reaction of PPh_3 and CBr_2F_2.

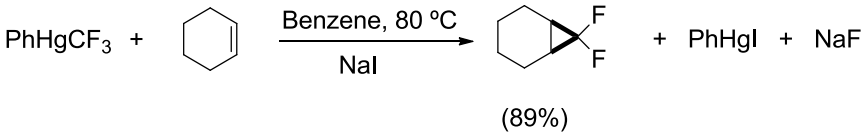
CBr_2F_2 is also used with zinc by Dolbier *et al*. (Scheme 21)[121] in a reaction similar to the Simmons-Smith reaction at ambient temperature. With a catalytic amount of I_2, the reactive species, thought to be a "free" carbene rather than a zinc

difluorocarbenoid, can transform alkenes to their corresponding difluorocyclopropanes in up to 96% yield.[121] Although this method only works well with electron-rich unsaturated systems, it is very mild and easy to perform and needs no special anhydrous conditions, thus it may be considered as a method of choice for reactive olefins.



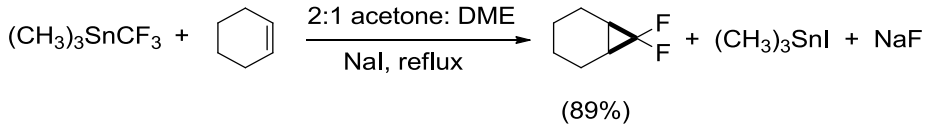
Scheme 21: Simmons-Smith like difluorocyclopropanation using CBr_2F_2, zinc and I_2.

The use of organometallic reagents as $:CF_2$ sources has also been widely reported. This class includes organomercury, organotin, organocadmium and organosilicon compounds.[102] One of the earliest organometallic $:CF_2$ precusors developed was $PhHgCF_3$ which was prepared by Seyferth *et al.*[122]. When $PhHgCF_3$ is treated with stoichiometric amount of sodium iodide, the nucleophilic iodide ion attacks on Hg and displaces the $^-CF_3$ anion. Subsequent loss of a fluoride ion from the trifluoromethyl anion gives difluorocarbene (Scheme 22).[122] Under these neutral reaction conditions, electron rich olefins give excellent yield of difluorocyclopropanes and even for relatively unreactive alkenes, difluorocyclopropanes can be obtained in modest yields.



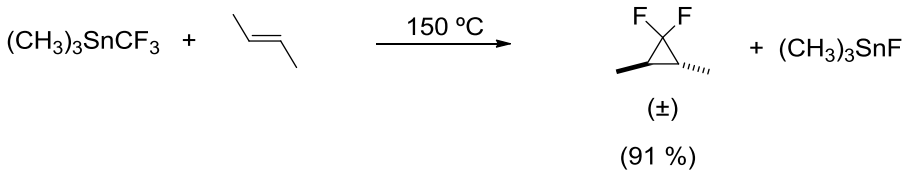
Scheme 22: Seyferth's reagent, $PhHgCF_3$, generates relatively reactive: CF_2 species

Me_3SnCF_3 is another example of an organometallic $:CF_2$ transferring agent. This reagent can give difluorocyclopropanes from their corresponding alkenes in good yield with or without the use of sodium iodide. In the presence of sodium iodide, a trifluoromethyl anion is thought to serve as an intermediate in the difluorocarbene formation and the mechanism of reaction may be similar to that of $PhHgCF_3$ (Scheme 23).[123]



Scheme 23: NaI catalyzed thermal decomposition of Me_3SnCF_3 to give $:CF_2$

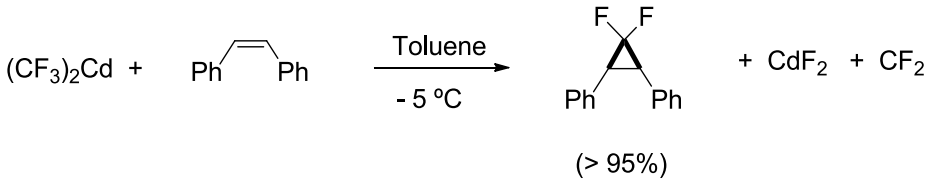
In the absence of sodium iodide, Me_3SnCF_3 undergoes thermolysis at a temperature of 150 ºC or above to liberate the difluorocarbene (Scheme 24).[124]



Scheme 24: Direct thermolysis of Me_3SnCF_3 to give $:CF_2$

Another milder, purely thermal source of $:CF_2$ of this class is $Cd(CF_3)_2$ (Scheme 25)[125] which is highly unstable in low coordinating solvents and decomposes easily even at -5 ºC to evolve $:CF_2$. The reactivity of the carbene species produced in this environment is probably the most reactive one to date. This is because the carbenes generated can be trapped by highly unreactive olefins at low temperature, e.g. *cis*- and *trans*-stilbene, to give difluorocyclopropanes in quantitative yields >95%. With all the reported examples given so far, the *cis*-diphenyldifluorocyclopropane is

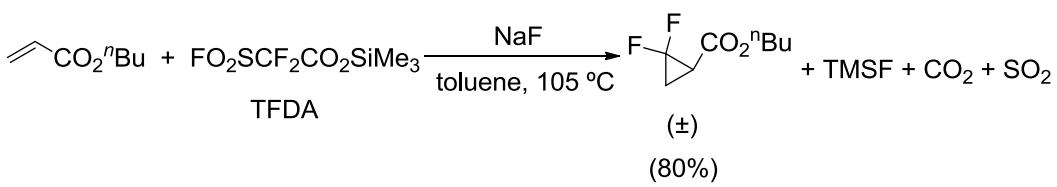
particularly challenging to prepare due to possible isomerisation of both the substrate and the product (at sufficiently high temperatures) and the very unreactive nature of a highly conjugated, non electron rich system toward a difluorocarbene.



Scheme 25: Thermal decomposition of $(CF_3)_2Cd$ to give $:CF_2$

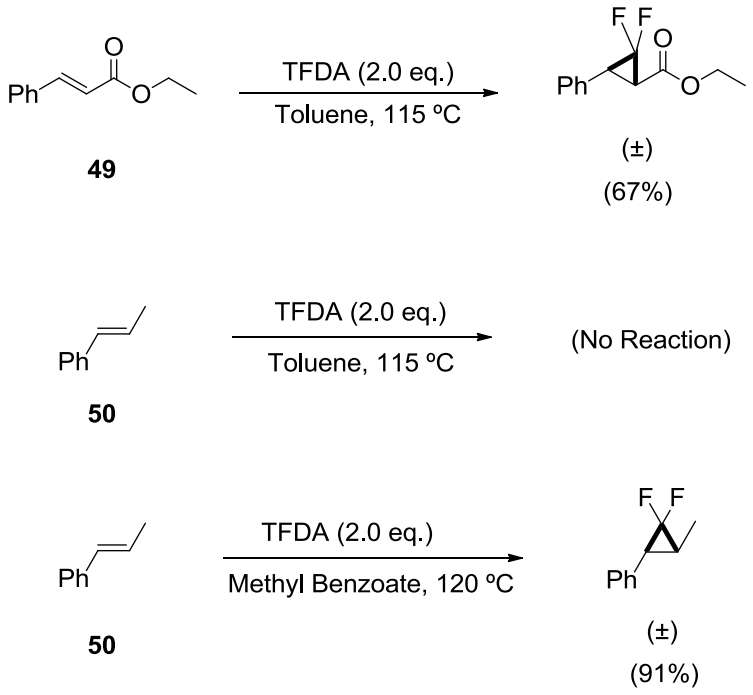
Despite the excellent reactivity of these organometallic $:CF_2$ transfering reagents, their toxicity and subsequent lack of commercial availability has limited their use in modern synthetic chemistry.[126] Their preparation is also both difficult and hazardous.[110, 126]

In comparison to other organometallic $:CF_2$ sources, organosilicon reagents are relatively non-toxic and easy to prepare. An effective reagent of this category is the recently reported trimethylsilyl fluorosulfonyldifluoroacetate (TFDA), which undergoes thermolysis in the presence of catalytic fluoride ion to extrude $:CF_2$ (Scheme 26Scheme 26).[126, 127] The difluorocarbene generated under these conditions can even add efficiently to relatively unreactive alkenes, such as acrylate esters, to give the corresponding difluorocyclopropanes in modest to good yields.



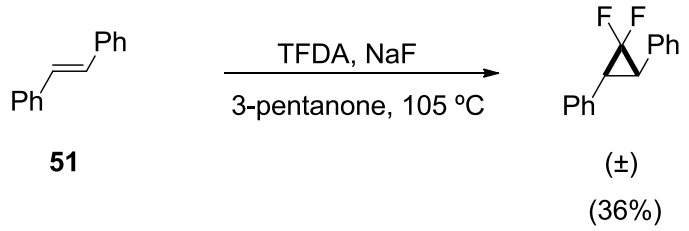
Scheme 26: NaF catalyzed thermal decomposition of TFDA to give $:CF_2$

The main drawback of TFDA is that there is no single "recipe" that can offer optimal results for all alkene substrates and this highly complicates the screening process for suitable reagents. In the first instance, 2.0 equiv. of TFDA in toluene at 115 ºC gave the desired difluorocyclopropane from ethyl cinnamate **(49)** in 67% yield, whilst *trans*-1-phenylpropene (**50)** did not react under the same reaction conditions (Scheme 27). The best reaction conditions found for **50** was 2.0 equiv. of TFDA in methyl benzoate at 120 ºC and an excellent yield of 91% was achieved (Scheme 27).



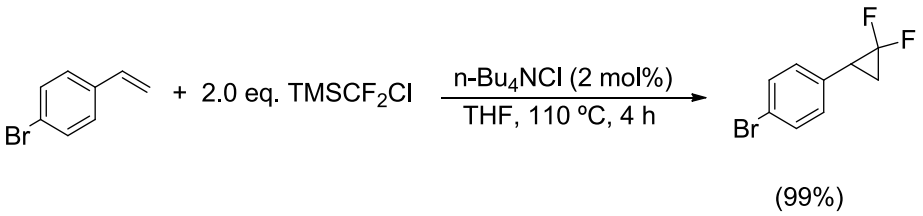
Scheme 27: Different substrates require different reaction conditions when using TFDA

In respect to the reactivity of carbenes formed under these conditions, they may still be quite reactive, but are not as superior as that of $(CF_3)_2Cd$, since a rather unsatisfactory yield of 36% was obtained compared to a quantitative yield of >95% when *trans*-stilbene (**51)** was used as the substrate (Scheme 28).

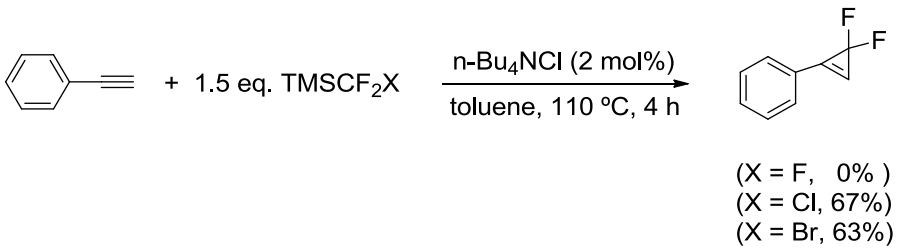


Scheme 28: TFDA only gave low yield of difluorocyclopropane from *trans*-stilbene.

Recently, Hu and his co-workers reported the use of Me_3SiCF_2Cl as a difluoromethylenation reagent which starts to decompose at temperatures above 80 ºC under the influence of catalytic amount of chloride ions to emit $:CF_2$. This method is very useful and not only allows the effective formation of difluorocyclopropane from alkenes (Scheme 29), but is also able to turn a range of alkynes to their difluorocyclopropene analogues in good yields (Scheme 30).



Scheme 29: Ddifluorocyclopropanation using Me_3SiCF_2Cl

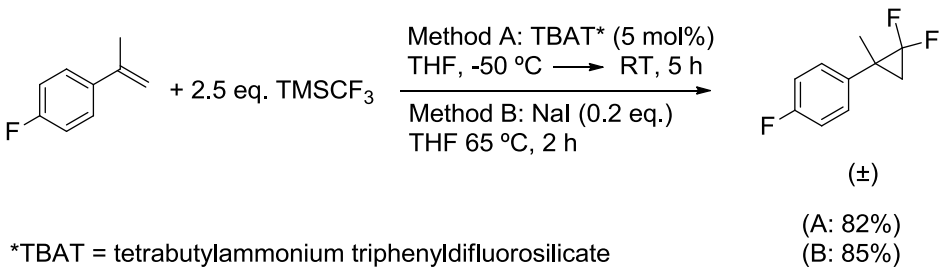


Scheme 30: Difluorocyclopropenation using Me_3SiCF_2X

Interestingly, the authors found that while Me_3SiCF_2Br may also serve as a difluorocarbene precursor with similar yields, Me_3SiCF_3 was inert under the same

reaction conditions (Scheme 30).[128] However, both Me_3SiCF_2Br and Me_3SiCF_2Cl are not commercially available and its preparation involves the use of ozone-depleting CBr_2F_2 and $CBrClF_2$[129] which are currently banned in many countries, thus limiting its practical synthetic potential.

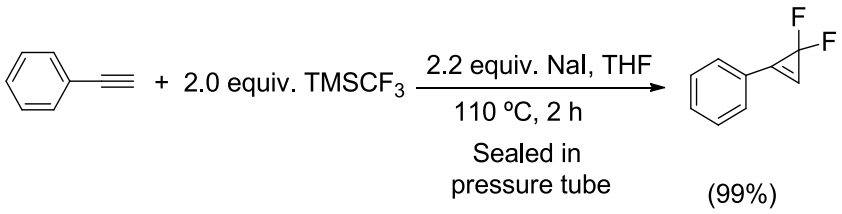
Me_3SiCF_3, also commonly referred to as the Ruppert–Prakash reagent, has been employed extensively as a trifluoromethylating agent, yet its use as a $:CF_2$ source has not been explored until very recently.[129] With an appropriate halide initiator, the Ruppert–Prakash reagent can give trifluoromethyl anion at low temperatures, yet this anion is susceptible to decomposition to $:CF_2$ and F^- anion and was recognized as a serious side reaction in trifluoromethylation.[130] Hu *et al*. exploited this side reaction and went on to demonstrate that the difluorocarbene generated can be trapped cleanly and conveniently by various alkenes and alkynes at low or elevated temperature to give their corresponding difluorocyclopropanes (Scheme 31) and difluorocyclopropenes in good to excellent yields (Scheme 32).[129]



Scheme 31: Difluorocyclopropanation of an alkene using Me_3SiCF_3

Of the three halodifluorotrimethylsilane reagents, Me_3SiCF_3 is probably the most useful since it is commercially available and inexpensive. This method also has many advantages over the others, either because the others are not commercially

available or their preparations are difficult or their thermolysis required harsh reaction conditions, and/or gave low product yields.

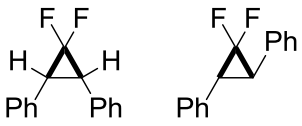


Scheme 32: Difluorocyclopropenation of an alkyne using Me_3SiCF_3

3.3 Research and development

3.3.1 Synthesis of 1,2-diaryl-difluorocyclopropanes and its challenges.

Numerous methods were investigated for the synthesis of difluorocyclopropyl stilbene analogues **52** and **53** (Figure 26). These include reaction of CH_2Br_2 and CBr_2F_2 with a strong aqueous base in the presence of tetrabutylammonium hydrogensulphate as a PTC catalyst,[119] thermal decomposition of sodium trifluoroacetate, chlorodifluoroacetate and bromodifluoroacetate,[112, 18, 19] and NaF induced thermal decomposition of TFDA.[131] All attempts failed to affect the difluorocyclopropanation on the relatively electron-deficient stilbenes.



52 (±)-**53**

Figure 26: Structure of *cis*- and *trans*-1,1-difluoro-2,3-diphenylcyclopropanes

Initially, it was believed that the energy barrier for addition of difluorocarbene to the relatively unreactive stilbenes may be too high for the low temperature PTC method and the thermal fragmenatation of sodium halodifluoroacetates to take effect. Thus, the temperatures of the pyrolysis reactions were gradually increased from 150 ºC up to 190 ºC; Still, no difluorocyclopropyl stilbenes were detected. Dolbier *et al*.[131] reported to have made the *trans*-difluorocyclopropyl stilbenes using TFDA in low yield (36%), yet when their procedure was followed exactly, their result could not be reproduced in our laboratory. The reaction temperature for NaF induced thermal decomposition of TFDA was also raised from 105 ºC to 190 ºC progressively, though it too failed to give any difluorocyclopropane.

It is known that slow rate of addition of reagents would increase the immediate ratio of difluorocarbene to available alkenes and hence favours product formation.[114] Nevertheless, attempts to improve the reaction conditions by adding the reagents over longer periods and with a greater excess of reagents than reported were all in vain and did not seem to afford the difluorocyclopropanes at all.

It was discovered from the literature that *cis*- and *trans*-difluorocyclopropyl stilbenes could interconvert via the cleavage of C^2-C^3 bond at temperature >150 °C, with irreversible formation of 2,2-difluoro-1-phenylindane **(54)** which further undergoes HF elimination to give a mixture of 1- and 3-phenyl-2-fluoroindene **(55** and **56)**, Figure 27.[132] Based on this finding, we concluded that the very high temperature needed for pyrolysis reactions will not work on our substrates.

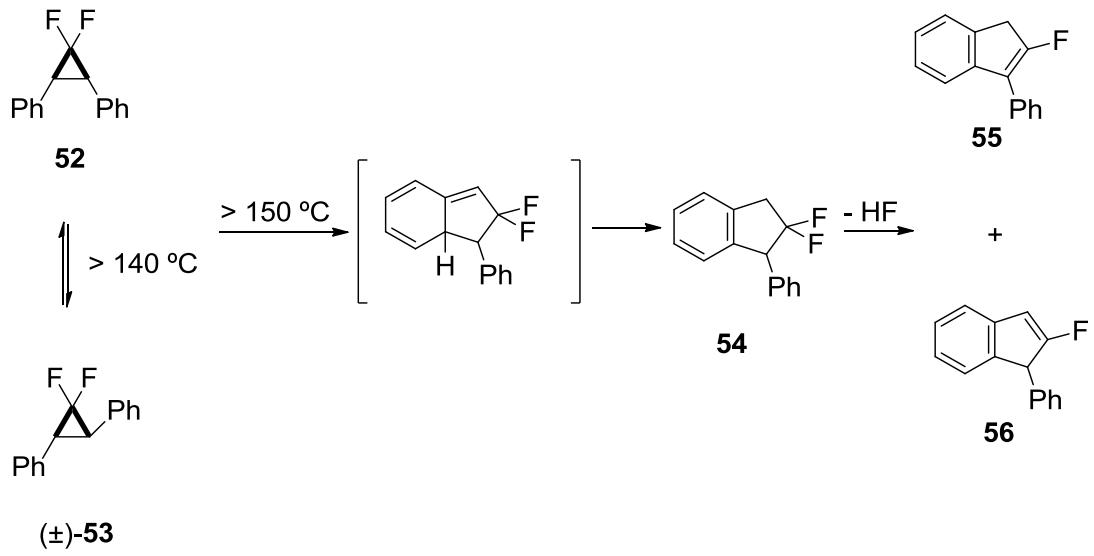


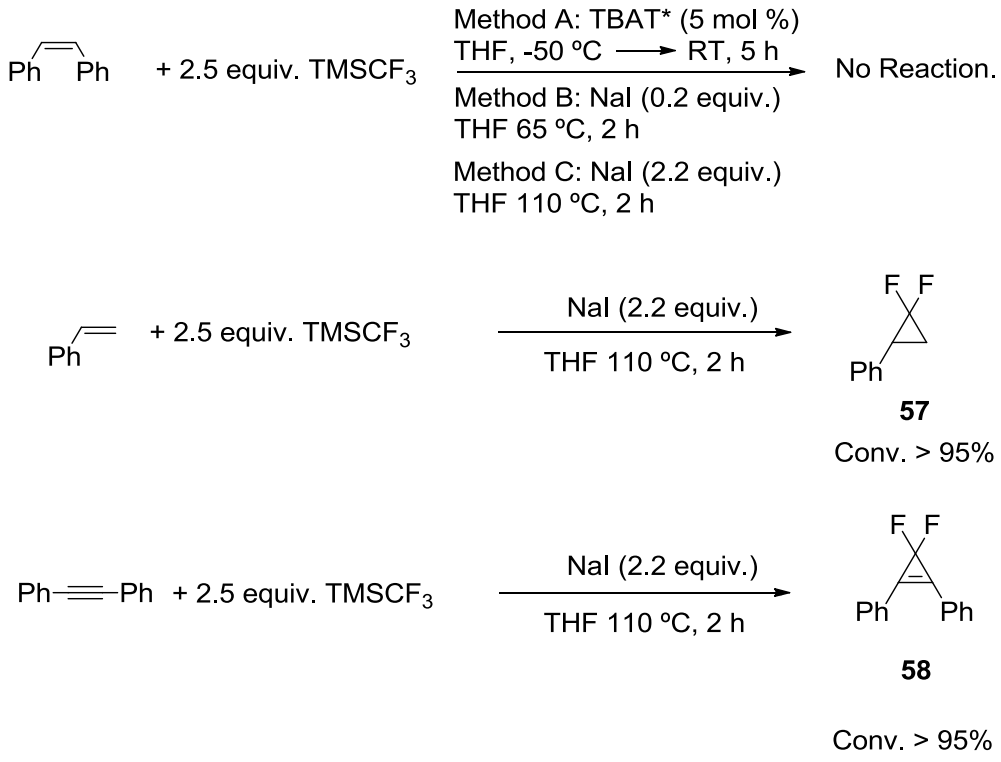
Figure 27: Thermal decomposition of diphenyldifluorocyclopropanes.[132]

Apart from TFDA, synthesis of *cis*-difluorocyclopropyl stilbene has only been reported using a few other methods, which include F_3CHgPh[133] and $(CF_3)_2Cd$.[125] Due to their high toxicities, these methods were not considered and other methods for effective difluorocyclopropanation of stilbenes were sought.

The use of Me_3SiCF_3 as a difluorocarbene source in the presence of an appropriate initiator both at low and elevated temperature published by Hu[36] was also attempted. Again, it failed to react with the electron poor stilbenes while reported examples, such as the preparation of **57** and **58** were easily repeated (Scheme 33).

It was concluded at this point that the stilbenes are extremely unreactive toward the difluorocarbene, probably because of the presence of an extended π-conjugated system rather than being electron poor. This may as well explain why in the literature, examples of difluorocyclopropanation on 1,1-diarylethenes are plentiful,

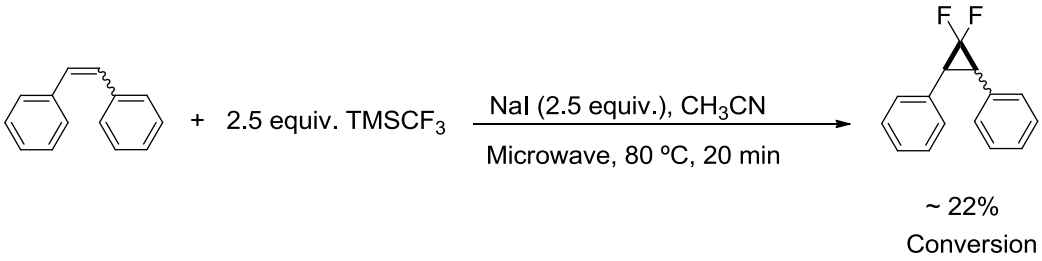
while only a few involve 1,2-diarylethenes in spite of them being structurally very similar.



Scheme 33: No difluorocyclopropanation of stilbene was observed using Hu's methods.

3.3.2 Microwave assisted difluorocyclopropanation (MAD)

We then tried to facilitate the difluorocyclopropanation of stilbenes with Me_3SiCF_3 using a microwave reactor since microwave irradiation can be more effective than conventional heating.[134] To our delight, some of the stilbenes did transform into difluorocyclopropanes after heating the reaction mixture in a sealed microwave tube for just 20 minutes at 80 ºC (Scheme 34), although the conversion was only ~22%.

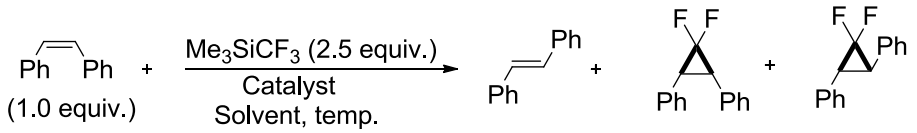


Scheme 34: MAD of stilbenes using Me_3SiCF_3

Despite much of our effort to optimize the reactions, however, the conversion never exceeded 30%. Replacement of NaI with a different salt: NaF, NaCl, NaBr, KF, KCl, KBr, KI and CsF resulted in no or only trace amounts of product being formed. When THF, toluene, diglyme, DMF and DMSO were used as solvents instead of CH_3CN, only a trace amount of difluorocyclopropanes was detected. If the reaction temperature was below 80 ºC, only small quantities of difluorocyclopropane were formed in the 20 minutes timescale, indicating that a sustainable high temperature (>80 ºC) is required to overcome the energy barrier for the addition of $:CF_2$ to the olefins. Conversion of *trans*-stilbene to the desired *trans*-difluorocyclopropane increased with increasing reaction temperature, but only up to ~30% at 130 ºC; At 140 ºC, isomerisation of *trans*-difluorocyclopropane to *cis*-difluorocyclopropane was observed in a small percentage (~2%) which was in strong agreement with Roth's finding.[132]

With *cis*-stilbene, the situation is a little bit more complicated. Table 1 shows a short summary of selected reactions when *cis*-stilbene was used as the model substrate. At 80 ºC, about 22% of *cis*-stilbene was converted to difluorocyclopropane, while a hefty amount of starting material (~41%) was isomerized into the *trans*-stilbene which was also accompanied by the formation of *trans*-difluorocyclopropane (~1%, **Entry 5**). Interestingly, at temperature ≥ 120 ºC, *trans*-difluorocyclopropane (16%)

was detected as the major cyclopropane product (**Entry 7).** The *cis*- and *trans*-isomerisation was increased from 41% to 57%, whereas the overall formation of difluorocyclopropane was reduced from 23% to a mere 17%.



59 **51** **52** **(±)-53**

Entry	Solvent	Catalyst (equiv.)	°C	Min	% of crude reaction mixture			
					59	**51**	**52**	**53**
1	THF	NaI (2.5)	80	20	~99	Trace	0	0
2	Toluene	NaI (2.5)	80	20	~99	Trace	0	0
3	Diglyme	NaI (2.5)	80	20	~99	Trace	0	0
4	CH_3CN	NaI (2.5)	60	20	~99	Trace	0	0
5	***CH_3CN***	***NaI (2.5)***	***80***	***20***	***~36***	***~41***	***~22***	***~1***
6	CH_3CN	NaI (2.5)	100	20	~34	~50	~15	~1
7	***CH_3CN***	***NaI (2.5)***	***120***	***20***	***~26***	***~57***	***~1***	***~16***
8	*CH_3CN*	*NaI (2.5)*	*80*	*10*	*~63*	*~20*	*~17*	*0*
9	CH_3CN	KF (2.5)	80	20	~99	Trace	0	0
10	CH_3CN	CsF (0.2)	60	20	~99	Trace	Trace	0
11	THF	CsF (0.2)	60	20	~99	Trace	Trace	0
12	Toluene	CsF (0.2)	60	20	~95	Trace	~5	0
13	Toluene	CsF (0.5)	60	20	~98	Trace	~2	0

Table 1: Difluorocyclopropanation using *cis*-stilbene as a model substrate; the conversions were measured by ^{1}H NMR spectroscopy.

There are two possible pathways by which *trans*-difluorocyclopropane may be formed from *cis*-stilbene, Figure 28:

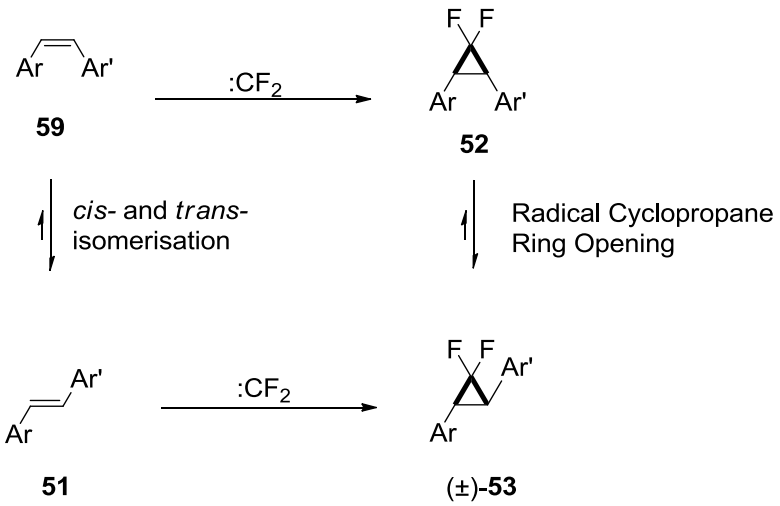
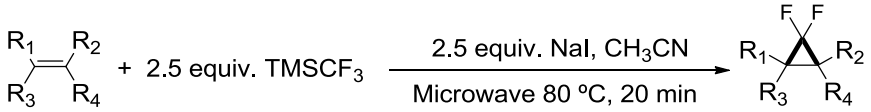


Figure 28: Possible pathways for the formation of *trans*-diphenyldifluorocyclopropane from *cis*-stilbene

From the isomerization and conversion of *cis*-stilbene to *trans*-stilbene and difluorocyclopropanes (41% vs. 23% at 80 °C), we can conclude that the energy barrier for isomerization is much lower than difluorocyclopropanation, implying selective preparation of *cis*-difluorocyclopropanes is very difficult to achieve.

To the best of our knowledge, difluorocyclopropanation using microwave irradiation had not been explored before and this method provides a fast and convenient way to the syntheses of difluorocycloproapanes in just 20 minutes. The conversion shown in **Entry 5** may seem trivial in the first instance, however, when the method is applied for more reactive alkenes, the conversion is remarkable, Scheme 35 and Table 2:



Scheme 35: Typical reaction condition for MAD method.

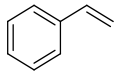
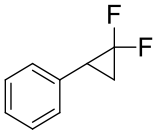
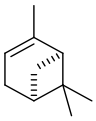
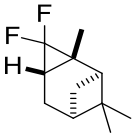
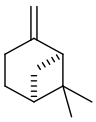
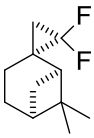
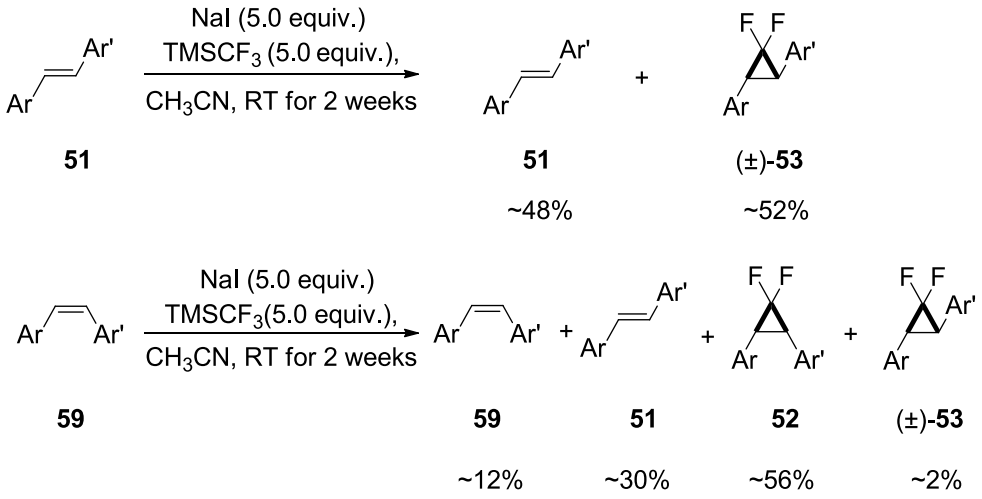
Substrate	Product	% of Conversion	*de*
		>98	-
		>98	>99
		>98	>93

Table 2 shows conversions of more reactive olefins using MAD method

The very high conversion obtained with other alkenes further provides evidence that stilbenes are, indeed, very unreactive towards difluorocarbene.

3.3.3 Room temperature difluorocyclopropanation (RTD)

Apart from the MAD, we also serendipitously discovered that the $:CF_2$ generated from $TMSCF_3$ using the same catalyst and the same solvent can react with the stilbenes very slowly at room temperature. Upon being sealed in a pressure tube and stirred at room temperature for two weeks, both *cis*- and *trans*-difluorocyclopropanes can be obtained in a more stereoselective manner from their corresponding stilbenes (Scheme 36).



Scheme 36: Conversion of *cis*- and *trans*-stilbenes to difluorocyclopropanes using RTD method.

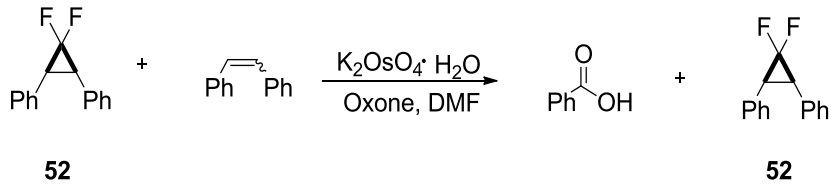
It is worth noting that the formation of both *trans*-stilbene and *trans*-difluorocyclopropane were still observed even when the difluorocyclopropanation of *cis*-stilbene was conducted at room temperature. However, unlike MAD method where the *trans*-stilbene was the main product, the RTD method actually gave the desired *cis*-difluorocyclopropane from its corresponding *cis*-stilbene as the major product. This further illustrates that the synthesis of *cis*-1,2-diaryl-2,3-difluorocyclopropanes is extremely difficult to accomplish and this may be the reason why successful synthesis of the named compound has only ever reported with the use of highly toxic but also extremely effective $Cd(CF_3)_2$ as the carbene precursor.

The only drawback of the RTD is the very long reaction time required for good conversion; with 5 equivalent of $TMSCF_3$ and NaI, stilbenes required at least 2 weeks to get ~50% conversion. For reactive olefins such as styrene, only 2.5 equivalent of reagents were needed to achieve a conversion of >98% in 4 days. The reaction time may be reduced by using more equivalents of reagents, although by

doing that this will decrease the cost efficiency. This method is, however, more selective with fewer limitations than the MAD and is more suited to sensitive substrates.

3.3.4 Isolation of difluorocyclopropanes

It was impossible to isolate the diaryldifluorocyclopropanes from the mixture by standard silica chromatography since the stilbenes and the difluorocyclopropanes have very similar polarities on silica in many different solvent systems. To isolate the products, the reaction mixture was treated with osmium tetroxide and oxone and the stilbenes were oxidatively cleaved, whereas the difluorocyclopropanes were left unchanged which could then be easily separated from the carboxylic acids (Scheme 37).



Scheme 37: Typical reaction conditions for oxidative cleavage of unreacted stilbenes

3.3.5 Mechanism of NaI with $TMSCF_3$ in CH_3CN

Hu *et al*. recently examined the reaction between Cl^- anion produced from *n*-Bu_4NCl and $TMSCF_2Cl$ using the density functional theory (DFT) model to evaluate the possible reaction pathways of difluorocarbene with alkenes.[128] They proposed that a pentacovalent silicate transition state **60** was formed after Cl^- attacked on the silicon centre, a process well established by others.[135, 136] A chlorodifluoro-methyl anion

was then released from **60** which readily underwent α-elimination of a chloride ion to give difluorocarbene (Figure 29).

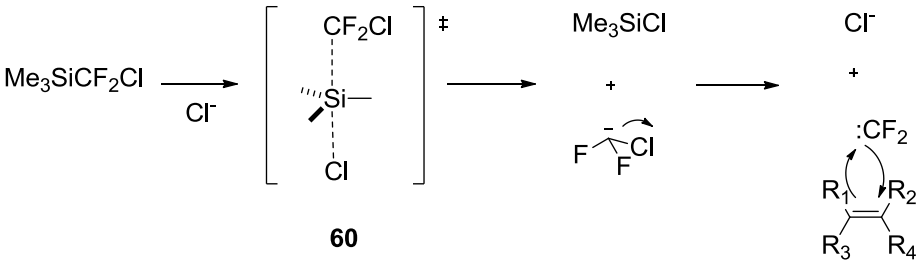


Figure 29: Hu's proposed mechanism for the reaction of $TMSCF_2Cl$ and *n*-Bu_4NCl

Mechanistically, it is not unreasonable to assume that NaI and $TMSCF_3$ in CH_3CN may react in a similar fashion to *n*-Bu_4NCl and $TMSCF_2Cl$, in which a five-coordinated silicate complex **61** may also be involved. Upon decomposition of the ate complex, Me_3SiI (iodide may undergo rapid halide exchange with the fluoride ion in the solution media to give Me_3SiF and re-generate NaI) and $^-CF_3$ are formed which subsequently loses a fluoride ion to generate $:CF_2$ (Figure 30).

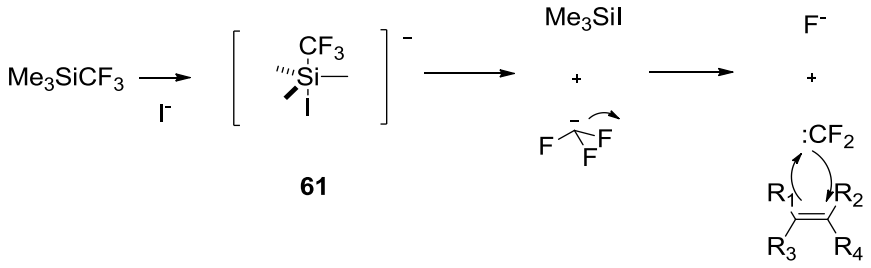


Figure 30: The possible mechanism of reaction between NaI and $TMSCF_3$

The mechanism above, however, may be a little over-simplified as it cannot give a plausible explanation for a phenomenon that we had observed in our preliminary experiments. We found that the reaction tubes had to be securely sealed, otherwise

little or no difluorocyclopropanes would be formed even if the reactions were carried out at room temperature and it is the reason why pressure tubes were used in our experiments instead of normal reaction flasks. In addition, we observed that a large amount of gas was produced during the reactions.

Although we did not try to identify the gaseous product, the formation of HCF_3, which is a gas, from the reaction of $^{-}CF_3$ with residual water was well documented by Prakash, who introduced $TMSCF_3$ to a vocabulary of organic chemistry over the last few decades.[129] By assuming $^{-}CF_3$ must have abstracted a proton from water residue only, the reactions were subsequently carried out under vigorously dried conditions with the use of anhydrous CH_3CN as well as ultra dried NaI. Nevertheless, it did not seem to reduce the amount of gas being produced.

CH_3CN is frequently used as a solvent in fluorine chemistry,[137] but we realized that it is not chemically inert. In fact, the strongly basic $^{-}CF_3$ (pKa of HCF_3 = 31)[135] released from $TMSCF_3$ upon addition of a ^{-}F anion from tetramethylammonium fluoride is known to deprotonate a neighbouring CH_3CN (pKa = 25)[138] molecule to give gaseous CF_3H and a $^{-}CH_2CN$ anion.[135] According to Prakash, Me_3SiF would be formed as the end product, presumably along with $Me_4N^+CH_2CN^-$ (Figure 31).

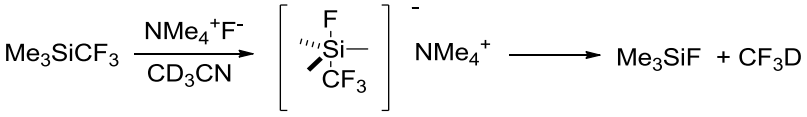
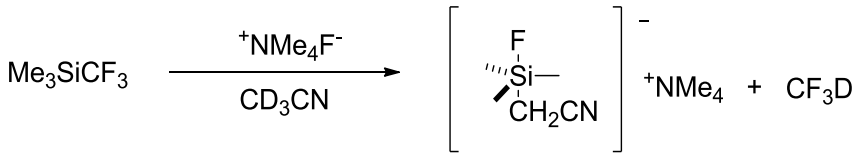


Figure 31: Prakash *et al.*'s proposed formation of Me_3SiF and $Me_4N^+CH_2CN^-$

Adams *et al*., on the other hand, suggested that the $^{-}CH_2CN$ anion then re-attacks the silicon centre to form a pentacoordinate silicate complex **62**, which is stable in acetonitrile for at least a day in the absence of fluoride or moisture (Figure 32).[135]



62

Figure 32: Adams *et al.* suggested formation of a pentacoordinate silicate complex

When the five-coordinated complex collapses back to a four coordinated silicate, it could either be an active source of fluoride or $^{-}CH_2CN$, depending on the reaction conditions.[135] Undoubtedly, their mechanistic models may not be directly applicable to our reactions, since NaI is used as the initiator rather than tetramethylammonium fluoride. What can be drawn from their findings is that the whole reaction mechanism is probably under equilibrium, and all the reactions may be reversible, except the difluorocyclopropanation where the energy difference between alkenes and difluorocyclopropanes is probably too high (Figure 33).

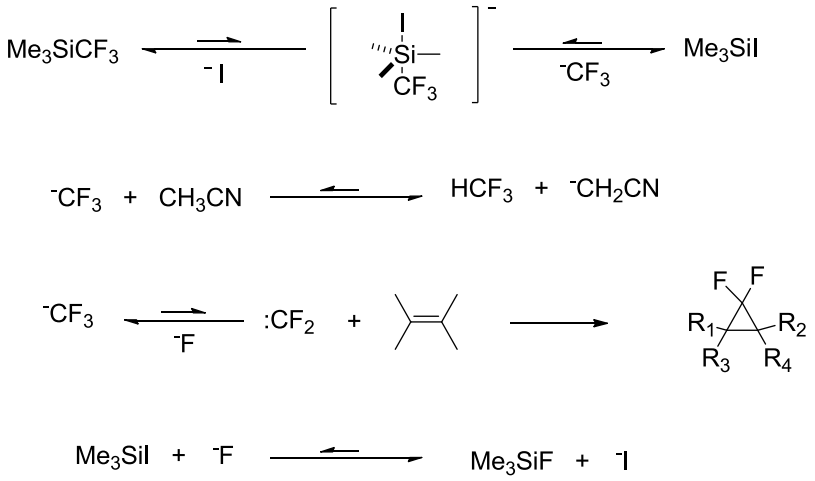


Figure 33: Our proposed mechanism between $TMSCF_3$ and NaI in CH_3CN

As we can see from Figure 33 above, $^{-}CF_3$ can be lost by proton abstraction from CH_3CN to give HCF_3 as a gas, so if the pressure tubes are tightly sealed, then the loss of gaseous HCF_3 from the reaction is diminished. This, in turns, favours the decomposition of $^{-}CF_3$ to $:CF_2$, thus favouring the formation of difluorocyclopropanes.

3.3.6 Synthesis of functionalized cis- and trans-stilbenes

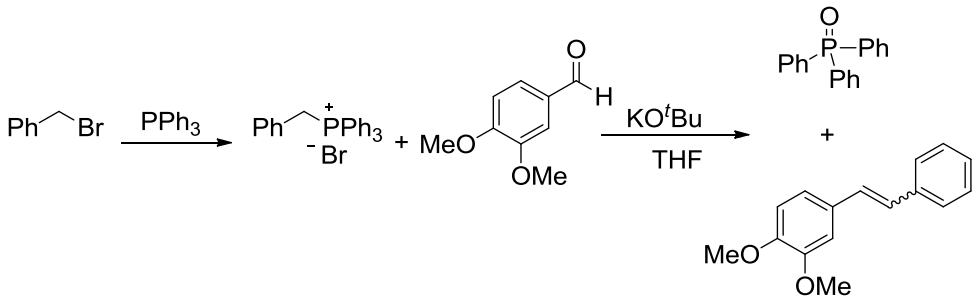
As none of the non-difluorocarbene methods investigated had worked, we decided to focus on the [2+1] cycloaddition of difluorocarbene to the diarylethene in the meantime. To synthesize the diaryldifluorocyclopropanes, we first had to prepare the functionalized *cis*- and *trans*-stilbenes since they are not usually commercially available.

Different methoxylated analogues of stilbene were investigated for this project mainly due to two reasons. First and foremost, **2** was one of the most active antiestrogenic and anti-tumour compounds Magarian *et al.* discovered and was shown to have a five-fold increase in potency than the non-functionalised diphenyldichlorocyclopropanes **1**.[77] This indicates the methoxy group on the phenyl ring is an important pharmacophore for the significant increase of antiestrogenic and anti-tumour activities of these analogues. Therefore, we would like to determine whether the position of the substituents has any pronounced effect on its biological activities.

Secondly, the methylated analogues of stilbene resembles Resveratrol structurally, a natural polyphenol found in grapes, berries, peanuts and other plants. Resveratrol

was found to be effective against a wide range of age-associated diseases including cancer, diabetes and cardiovascular diseases[139] and has been demonstrated to induce autophagy by others. [140-143]

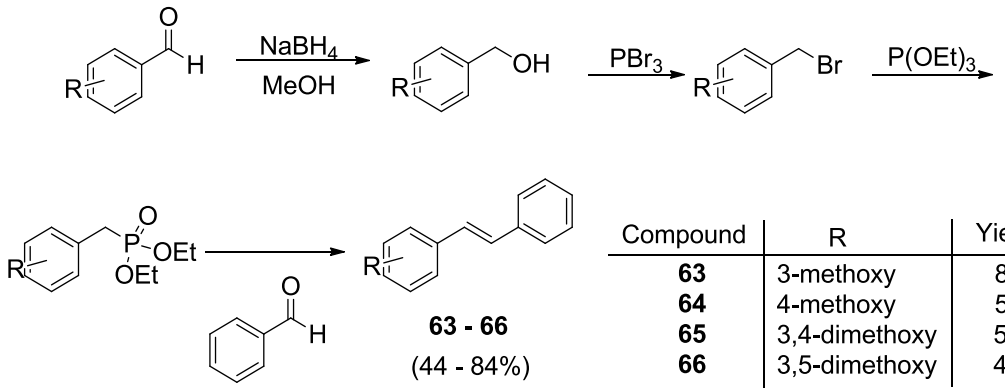
Many routes can lead to functionalized stilbenes, yet only few provide the alkenes in a selective manner. Not only Wittig reactions typically give a mixture of both *cis*- and *trans*-isomers, but the formation of a large amount of triphenylphosphine oxide as a by-product also make the purification of products very challenging and time-consuming (Scheme 38).



Scheme 38: Wittig reaction to give alkenes and triphenylphosphine oxide

As a result, the utilisation of Wittig reaction for alkene synthesis was abandoned and the stereoselective syntheses of *trans*-stilbenes **(63 – 66)** were achieved via Horner-Wadsworth-Emmons (a modified Wittig reaction using phosphonate-stabilized carbanions) reactions instead. Briefly, mono- or di-methoxy substituted benzaldehydes were reduced to give primary alcohols which were followed by conversion to the benzyl bromide using PBr_3 (Scheme 39). Treatment of the benzyl bromide with triethyl phosphite afforded benzylphosphonic acid diethyl esters and subsequent reaction with a benzaldehyde offered predominately the *trans*-stilbenes as solids, which were easily purified by re-crystallisation. Although quite a few steps

were involved, the overall yields of the products were good and no chromatographic purification was needed at any stage.

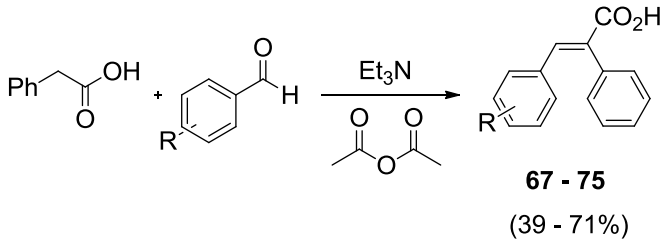


Compound	R	Yield %
63	3-methoxy	84
64	4-methoxy	56
65	3,4-dimethoxy	57
66	3,5-dimethoxy	44

Scheme 39: Typical reaction conditions for Horner-Wadsworth-Emmons reactions

Preparations of substituted *cis*-stilbenes, on the other hand, are much harder to perform in a selective manner since they are thermodynamically less table than their *trans*-counterparts and hence their formations are not favoured. Their isolation from a mixture of both *cis*- and *trans*-isomers is also very difficult, but generally achievable with slow and careful standard silica chromatographic techniques.

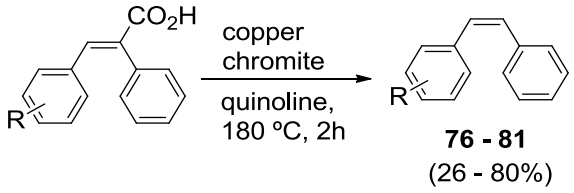
Perkin condensation of phenylacetic acid and aryl aldehyde to give aryl cinnamic acid has been extensively studied both for its mechanism and synthetic applications. Synthetically, it is very useful as it allows a fast and convenient access to a range of *α*,*β*-unsaturated aromatic acids, which could subsequently be transformed to their corresponding stilbenes. Mechanistically, it is interesting because *cis*-diarylethenes are preferentially formed as the major products after the decarboxylation of the adducts.



Compound	R	Yield %
67	4-F	56
68	4-Cl	68
69	3-OMe	39
70	4-OMe	43
71	2,3-OMe	71
72	3,4-OMe	38
73	3,5-OMe	60
74	2,4,5-OMe	48
75	3,4,5-OMe	63

Scheme 40: Typical reaction conditions for Perkin condensations.

A wide series of aryl cinnamic acids have been successfully prepared using the method shown in Scheme 40, with yields ranging from 39 – 71%. Although the yields in general were not that impressive, the syntheses were really easy to perform and the starting materials were readily accessible and inexpensive. After decarboxylation of the aryl cinnamic acids with copper chromite and quinoline at 180 ºC (Scheme 41), a mixture of *cis*- and *trans*-stilbenes were obtained in good to excellent yields, with a combined yields of *cis*- and *trans*-isomers ranging between 68 - 98%.

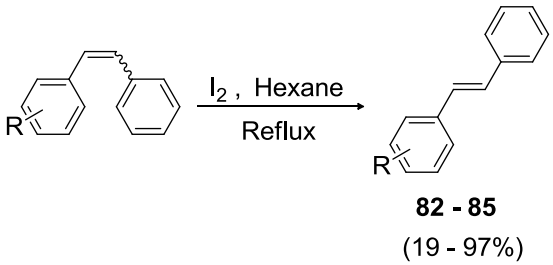


Compound	R	Yield %
76	3-OMe	73
77	4-OMe	78
78	3,4-OMe	80
79	3,5-OMe	56
80	2,3-OMe	26
81	3,4,5-OMe	64

Scheme 41: Typical reaction conditions for copper chromite assisted decarboxylation

Careful separation of the two stereoisomers on silica chromatography permitted, at least, a fraction of the total *cis*-stilbenes to be isolated cleanly in most cases except 4-chloro and 4-fluoro stilbenes. These stilbene analogues were the only cases in which separation of the two geometric isomers on standard silica chromatography were

found impossible to achieve as they were very non-polar in nature and both isomers of these two compounds were eluted out quickly at the same time. The remaining isomeric stilbene mixtures were conveniently turned to predominately *trans*-stilbenes using a catalytic amount of iodide in refluxing hexane for two days (Scheme 42). The crude reaction mixtures were then purified by recrystallization to offer the *trans*-diarylethenes. This iodine catalyzed isomerisation of *cis*-stilbene allows a rapid expansion of the *trans*-stilbenes library.

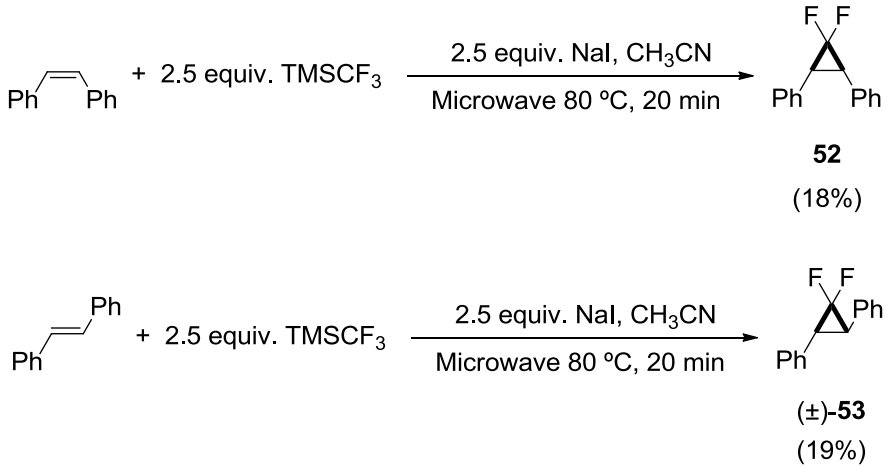


Compound	R	Yield %
82	4-F	93
83	4-Cl	97
84	2,3-OMe	38
66	3,5-OMe	19
85	3,4,5-OMe	29

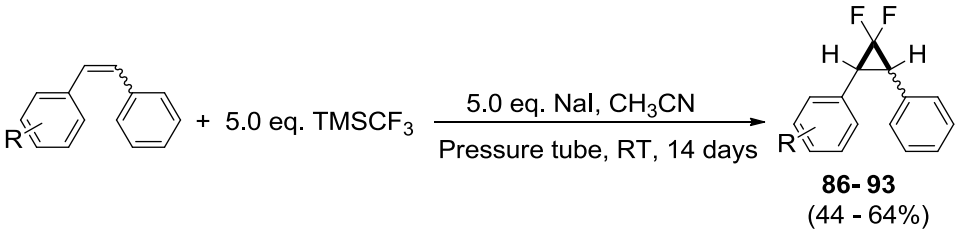
Scheme 42: Typical reaction conditions for *cis*- and *trans*- stilbene isomerisation

3.3.7 Synthesis of difluorocyclopropanes

After the required stilbene substrates were prepared, we used both MAD and RTD developed in our laboratory for the syntheses of diarylfluorocyclopropanes. The MAD is very fast and convenient and was used for the syntheses of diphenyldifluorocyclopropanes **52** and **53**, Scheme 43. However, it is not as stereoselective as the RTD method. Thus, all other functionalized diaryldifluorocyclopropanes (**86 - 93**)were achieved using the RTD method, Scheme 43.



Scheme 43: Microwave-assisted difluorocyclopropanation (MAD) of *cis*- and *trans*-stilbene



Compound	*cis*- or *trans*-	R	Yield %
86	*cis*-	3-methoxy	44%
87	*cis*-	4-methoxy	60%
88	*cis*-	2,3-dimethoxy	57%
89	*cis*-	3,4-dimethoxy	60%
90	*cis*-	3,5-dimethoxy	37%
91	*cis*-	3,4,5-trimethoxy	36%
92	*trans*-	3-methoxy	64%
93	*trans*-	3,4-dimethoxy	43%

Scheme 44: Typical reaction conditions for RTD of stilbene and its analogues

Although slow, the RTD method is very easy to perform and is very suitable for stereoselective syntheses of difluorocyclopropanes derived from alkenes, such as *cis*-stilbene, in which the energy barrier for isomerisation is lower than that for difluorocyclopropanation.

Chapter 4 Structure and Activity Relationship Studies of Tamoxifen and Its Analogues for Autophagy.

4.1 Biological testing of functionalized *cis*- and *trans*-stilbenes and their dihalocyclopropyl derivatives

As discussed earlier, Tamoxifen is a well-known multi-target drug. In addition to its high affinity to the ERs, Tamoxifen binds with relatively high affinity to an intercellular site called the antiestrogen binding site (AEBS). Additionally, it has been shown to have affinities to a number of other molecular targets which include protein kinase C (PKC), calmodulin (CaM)-dependent enzymes and Acyl CoenzymeA: Cholesterol Acyl Transferase (ACAT).[34] Any one or more of these targets could account for its cardioprotective effects and therefore it is very difficult to study the mechanism of action at a molecular level.

It is known that, however, Tamoxifen is a potent autophagy inducer and there is a strong correlation between enhancing autophagy and cardioprotection[34] and common age-related diseases.[144] Consequently, in our project we focused on finding novel molecules which could induce autophagy and were structurally similar to Tamoxifen. This chapter reports data obtained from our collaborators at E3Bio by Kate Willetts and Dr. Jill Reckless in various biological assays. Tamoxifen had been used as a positive control in all of our assays, whereas for the negative control dimethyl sulfoxide (DMSO) vehicles were used. Monodansylcadaverine (MCD) is an autofluorescent dye developed as a specific *in vivo* marker for lysosomal/autophagic vacuoles.[145] MCD accumulates in autophagic vacuoles possibly due to a combination of ion trapping in acidic compartments and specific

interactions of this molecule with autophagic vacuole membrane lipids.[146] However, it is known to produce a high background and weak fluorescent signal. AB139484 is an optimized autophagy detection assay which uses a novel 488 nm-excitable green fluorescent dye that selectively labels autophagic vacuoles while minimizing the staining of lysosomes. The assay allows for a convenient, specific and quantitative measure of the autophagic vacuoles and monitoring autophagic flux by the fluorescence intensity using fluorescence microscopy, flow cytometry and fluorescence microplate assay.

In our first structural activity relationship study, we tested compounds which predominantly only contain *cis*- (part A) and *trans*- (part B) stilbene backbone moieties as well as the dichlorocyclopropyl and acrylic acid derivatives of Tamoxifen (Figure 34) without the amino side chains.

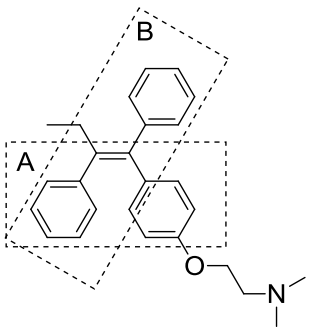


Figure 34: Part A and B moieties of Tamoxifen

The compounds tested could be categorized into three different classes of compounds which include 1,2-diarylethenes, diaryl-2-propenoic acids and di- and tri-aryldichlorocyclopropanes. Our decision to test 1,2-diarylethenes is based on the

fact that resveratrol and other stilbene analogues have been reported to induce autophagy.[140-143]

Resveratrol (Figure 35), in particular, has attracted a lot of interest in the past decade. It is a natural polyphenol which is found in grapes, berries, peanuts and other plants. Many preclinical and clinical studies have demonstrated that resveratrol is effective against a wide range of age-associated diseases including cancer, diabetes, and neurodegenerative, cardiovascular and pulmonary diseases through modulation of multiple cell signalling pathways.[139] In addition to Resveratrol, all other 1,2-diarylethenes and their derivatives synthesized and biologically tested are shown in Figure 35.

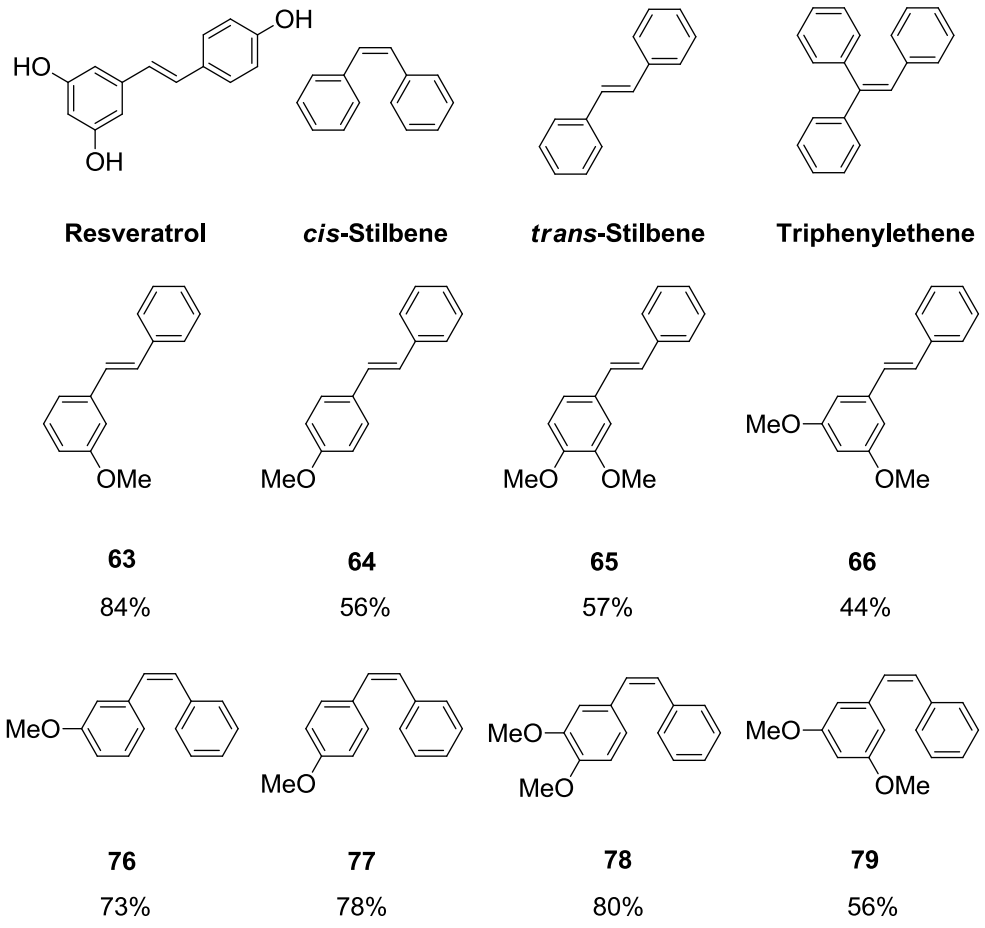
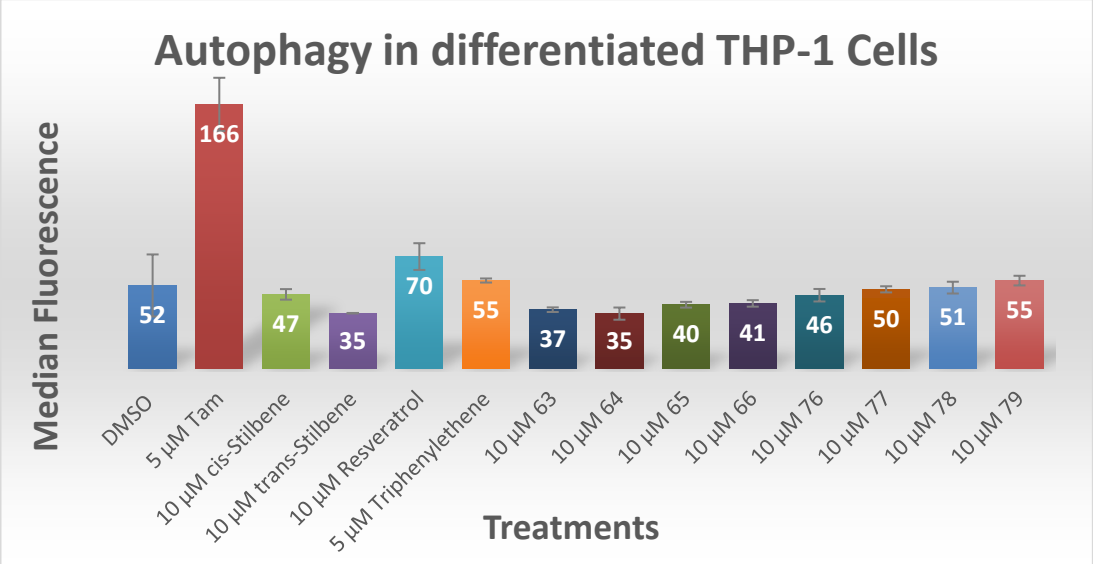


Figure 35: The structures of functionalized stilbenes synthesized and biologically tested.

As can be seen clearly seen from **Graph 1**, none of the compounds of these classes shown in Figure 35 induced a significant difference in the autophagic assays compared to the controls. In fact, the 1,2-diarylethenyl or triphenylethylene derivates of Tamoxifen did not seem to stimulate autophagy at all except resveratrol.



Graph 1: Measure induction of autophagy of stilbene and its analogues shown in Figure 35 using Tamoxifen (Tam) and DMSO as positive and negative controls respectively.

Resveratrol was reported to be a potent autophagy inducer by others,[147] but it had only had a marginal effect in our biological assays. Interestingly, the *cis*-isomers (*cis*-stilbene and compound **76** to **79**) appeared to give consistently and slightly better results than their *trans*-counterparts (*trans*-stilbene and compound **63** to **66**), although the DMSO control also appeared to have higher autophagic flux on the fluorescence microscopy than the *trans*-diarylethenes. This could be a result of experimental errors as the standard error bar of the control was much higher than the other compounds tested. In order to draw a less ambiguous conclusion about whether *cis*-stilbene and its derivatives are any better than their *trans*-isomers, further

investigation is needed. However, even if there was an experimental error in the control and the *cis*-isomers were in fact better than the *trans*-isomers, the statistical difference between those compounds would not be insignificant.

The stilbene precursors, 2,3-diaryl-2-propenoic acids (Figure 36), were also tested because some of these compounds have been found to be biologically active by other researchers.[148]

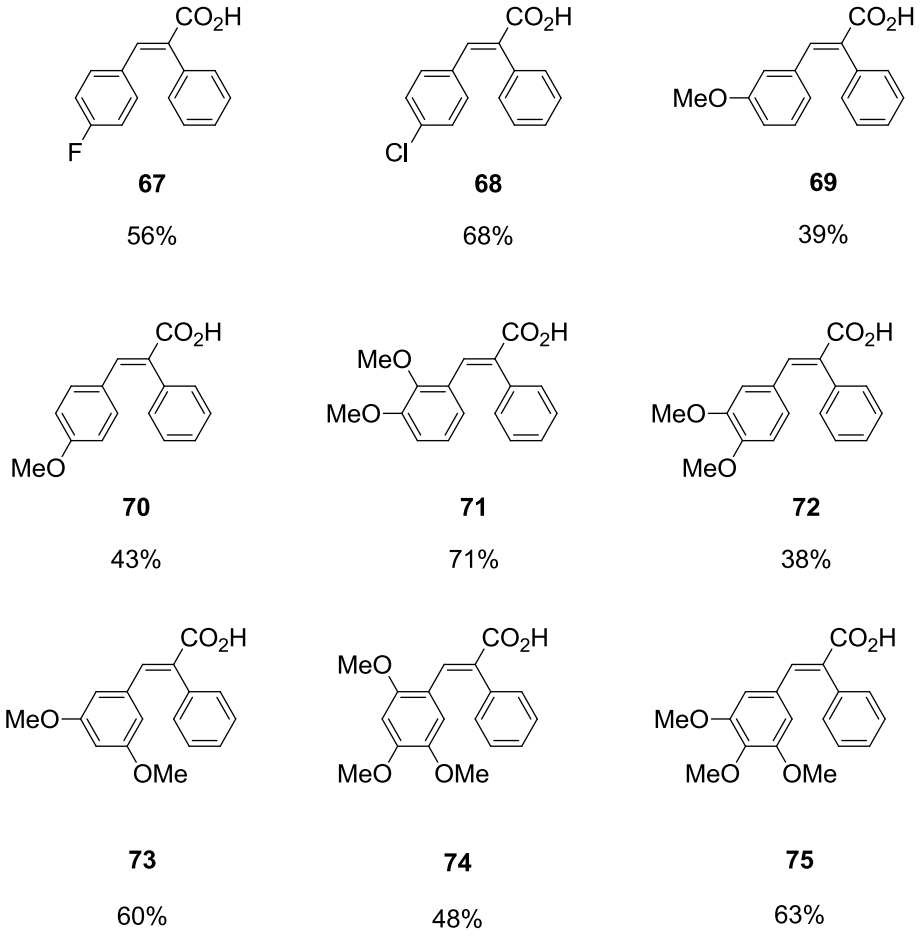
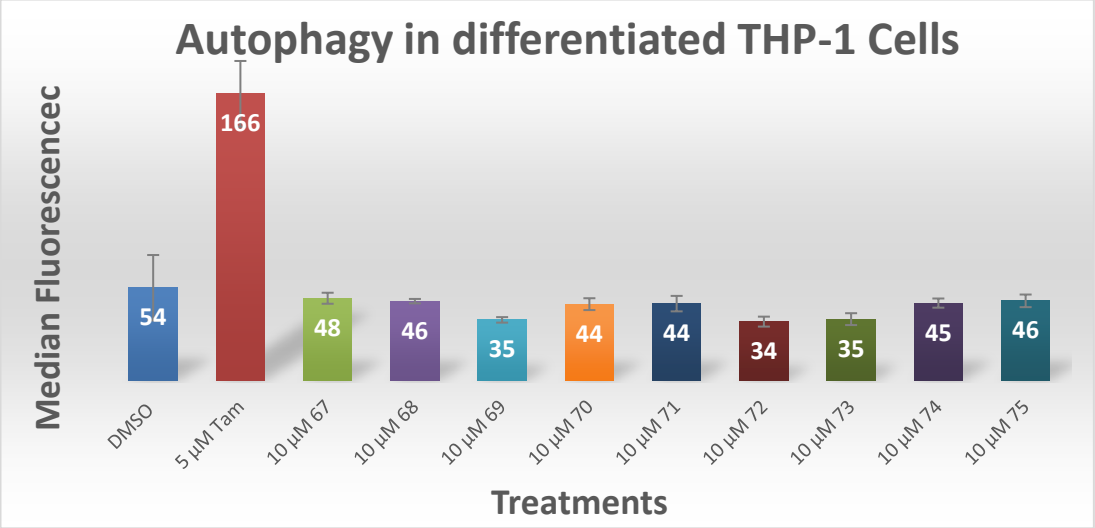


Figure 36: The structures of 2,3-diaryl-2-propenoic acids synthesized and biologically tested.

Graph 2 illustrates the results of the autophagic assays for the diarylpropenoic derivative of Tamoxifen shown in Figure 36. Treatment of THP-1 cells with these

compounds did not result in induction of autophagy. On the other hand, autophagy was potently induced by Tamoxifen at a concentration half that of the tested compounds.



Graph 2: Measure induction of autophagy of 2,3-diaryl-2-propenoic acids shown in Figure 36 using Tamoxifen (Tam) and DMSO as positive and negative controls respectively.

The di- and tri-arydichlorocyclopropanes are derivatives of stilbene and Tamoxifen, in which the olefinic link is replaced by a dichlorocyclopropyl moiety. The presence of a cyclopropyl ring is thought to reduce or eliminate their estrogenic activity. This class of compounds and its derivatives (Figure 37) may be of medicinal interest as Magarian and others have demonstrated that they have antiestrogenic and anti-tumour activities.[76-83, 149]

Graph 3 illustrates the test results for the dichlorocyclopropyl analogous of Tamoxifen shown in Figure 37. Again, none of the compounds of this class induced autophagy in the biological assays.

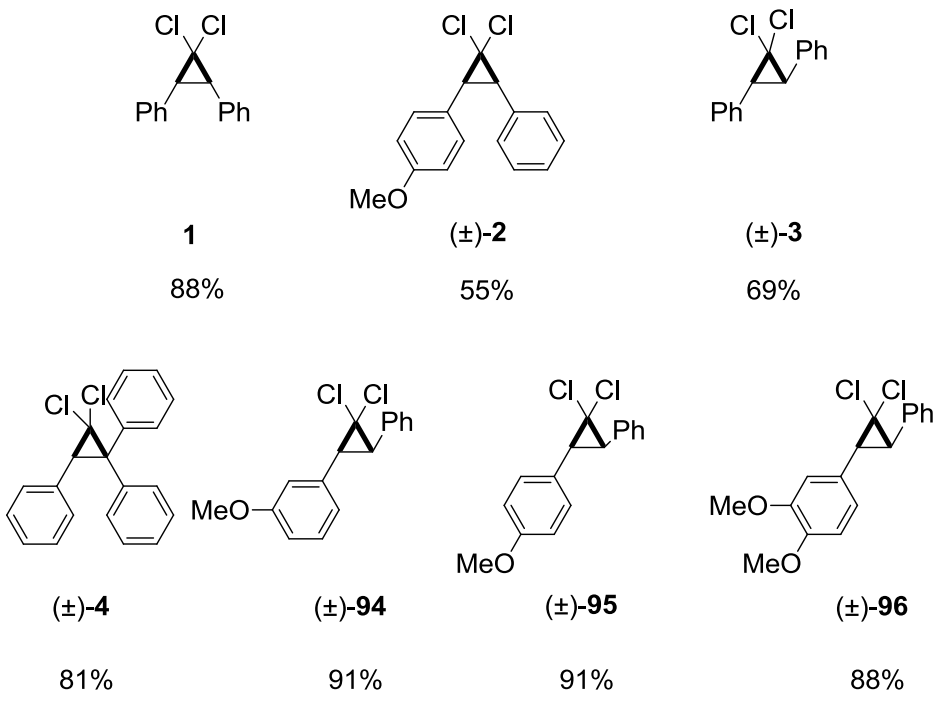
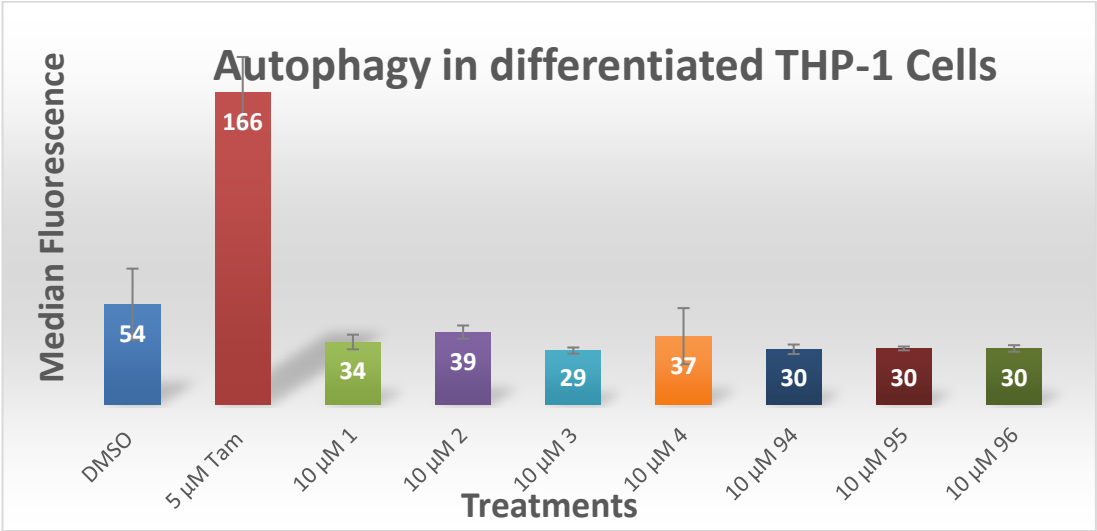


Figure 37: The structures of di- and triaryl-dichlorocyclopropanes synthesized and biologically tested.



Graph 3: Measure induction of autophagy of dichlorocyclopropanes shown in Figure 37 using Tamoxifen (Tam) and DMSO as positive and negative controls respectively.

The first biological testing results were certainly very disappointing. However, from our earlier experience we knew that *di*- and *tri*-aryldichlorocyclopropanes were not particularly stable and degraded easily at room temperature, thus it may be possible that the inactivity of these compounds in our biological assays were attributed to

their instability. Consequently, we aimed to synthesize some more dihalocyclopropyl analogues of Tamoxifen which were more stable for our second biological testing.

4.2 Biological testing result for the difluorocyclopropyl stilbenes and aminoethoxy derivative of Tamoxifen

4.2.1 Difluorocyclopropyl analogues of Tamoxifen

In comparison to dichlorocyclopropanes, the syntheses of difluorocyclopropyl compounds were much more difficult to achieve due to the highly unreactive nature of difluorocarbene toward electron poor olefins. A lot of effort had been made to try to find good practical methods for the synthesis of *gem*-difluorocyclopropanes. However, none of the methods we tried giving the desired diaryldifluorocyclopropanes. The use of highly toxic Cadmium complex $Cd(CF_3)_2$ is the only example ever reported for the successful difluococyclopropanation of *cis*-stilbene. However, its use had not been considered in our laboratory due to its high toxicity. Although the RTD we developed generally requires long reaction times for unreactive alkenes, it is particularly suitable for unstable substrates such as *cis*-stilbene, in which the energy barrier for isomerization is much lower than the cycloaddition of difluorocarbene, making the selective synthesis of their products very challenging. Consequently, the difluorocyclopropyl compounds illustrated in Figure 38, all except **52**, **53** and **58** were synthesized using the RTD method.

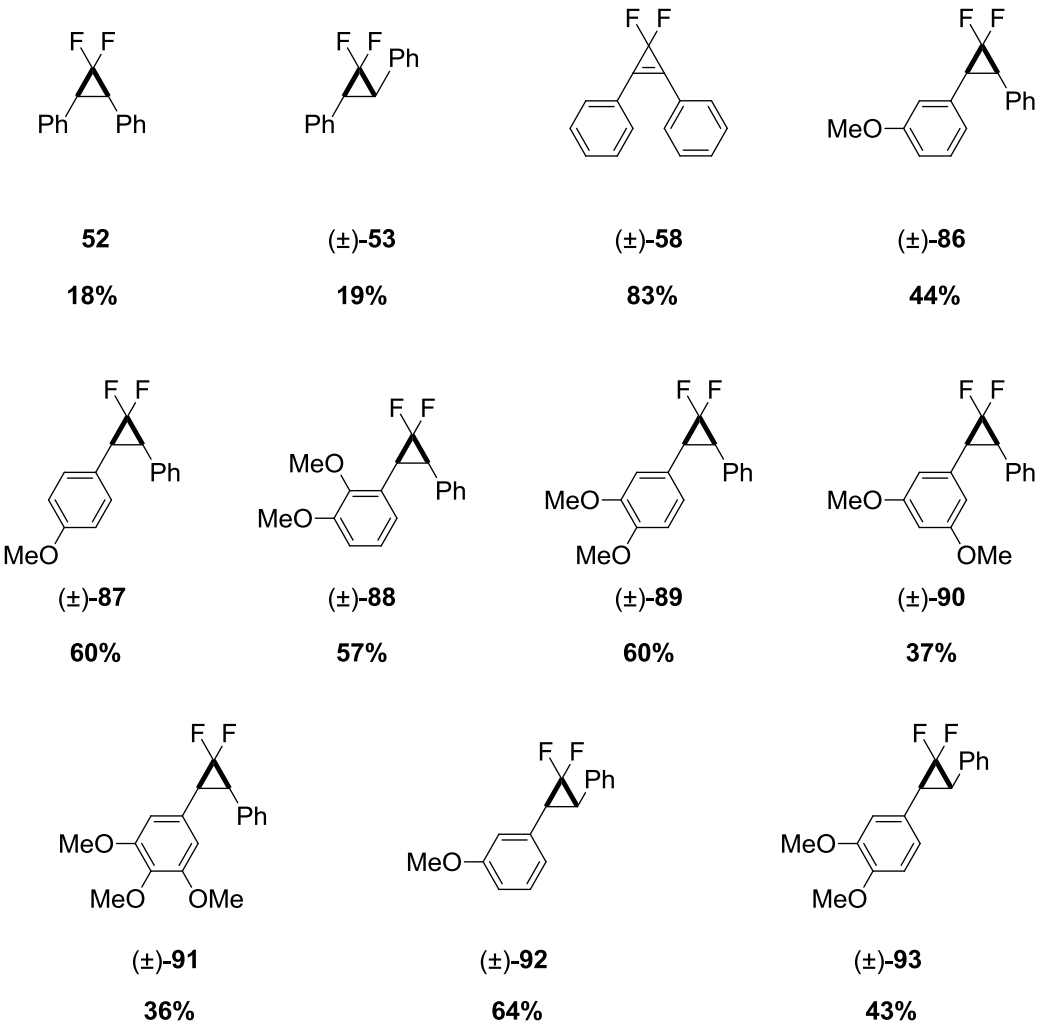
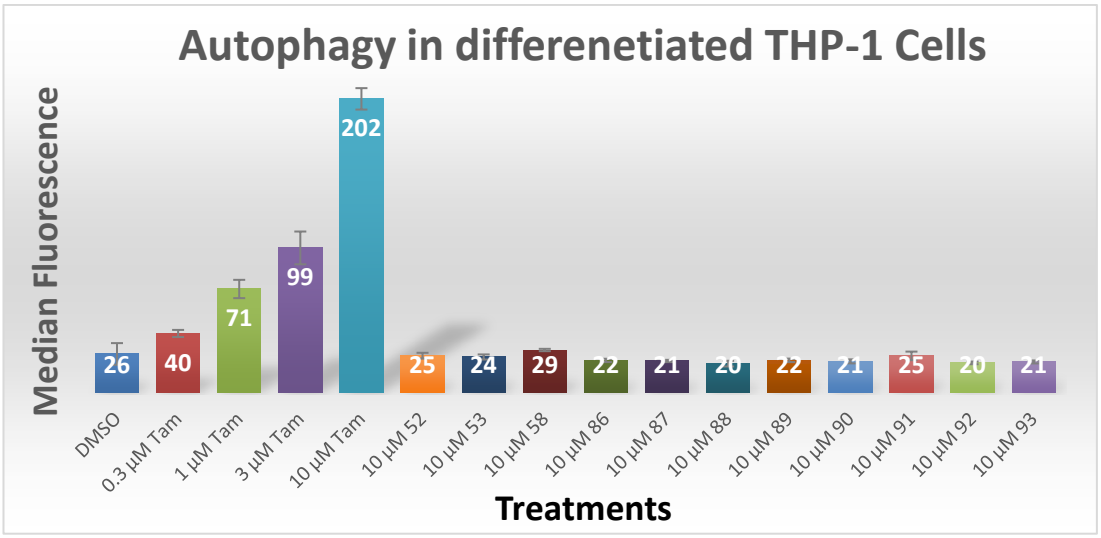


Figure 38: The structures of diaryldifluorocyclo- propanes and propene synthesized and tested.

As can be seen from **Graph 4**, when differentiated THP-1 cells were treated with 0.3, 1.0, 3.0, and 10.0 µM of Tamoxifen as positive controls, Tamoxifen potently induced autophagy in a dose-dependent manner from a concentration as low as 0.3 µM. In contrast, treatment with those 1,2-diaryldifluorocyclopropanes gave similar results as their corresponding dichlorocyclopropyl analogues and no sign of any autophagy was observed at a concentration as high as 10 µM (**Graph 4**), despite those difluorocyclopropyl derivatives of Tamoxifen being more stable than its dichlorocyclopropyl counterparts and did not degrade under normal handling

condition. This result, although disappointing, helped to eliminate the possibility that the inactivity of diaryldichlorocyclopropanes was due to their instability.



Graph 4: Measure induction of autophagy of 1,2-diaryldifluorocyclopropanes shown in Figure 38 using Tamoxifen (Tam) and DMSO as positive and negative control respectively.

As all the compounds tested in the first biological assays were without the amino side chain, we also tried to synthesize some difluorocyclopropyl analogues with part C and part D moieties to test for activity (Figure 39).

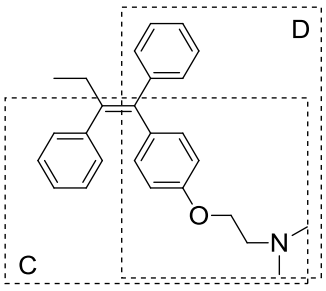
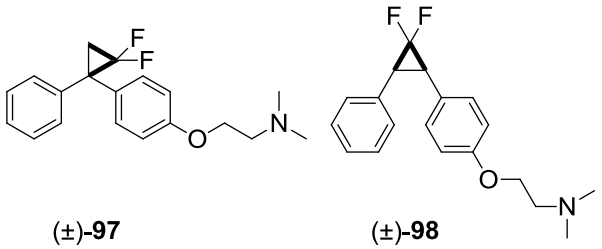


Figure 39: Part C and D moieties of Tamoxifen

4.2.2 The syntheses of aminoethoxy derivatives of Tamoxifen

Introducing an amino side chain to the 1,1-diaryl and 1,2-diaryl analogues was found to be quite challenging and a number of methods were attempted for the synthesis of compounds **97** and **98**.

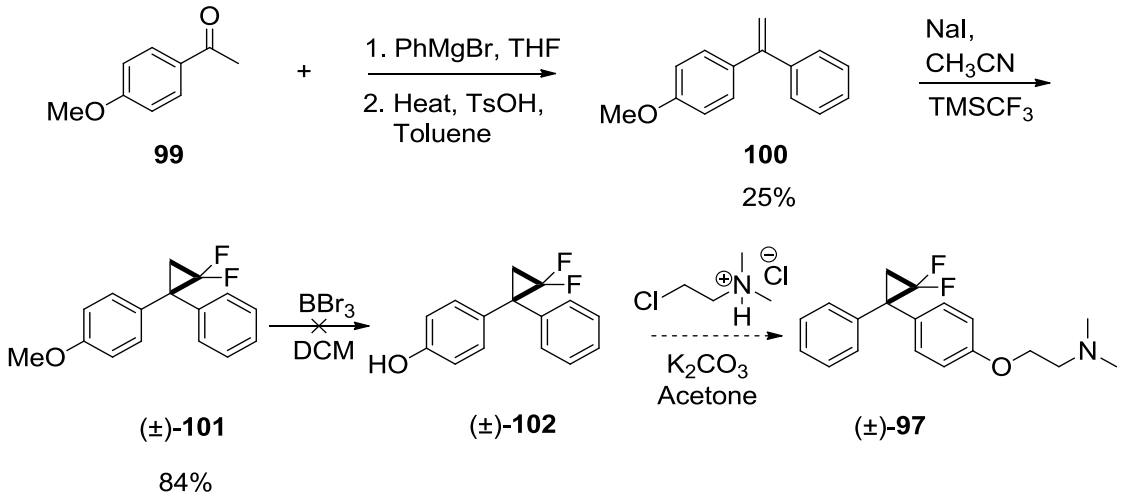


(±)-**97** (±)-**98**

Figure 40: The structures of difluorocyclopropyl analogues of Tamoxifen

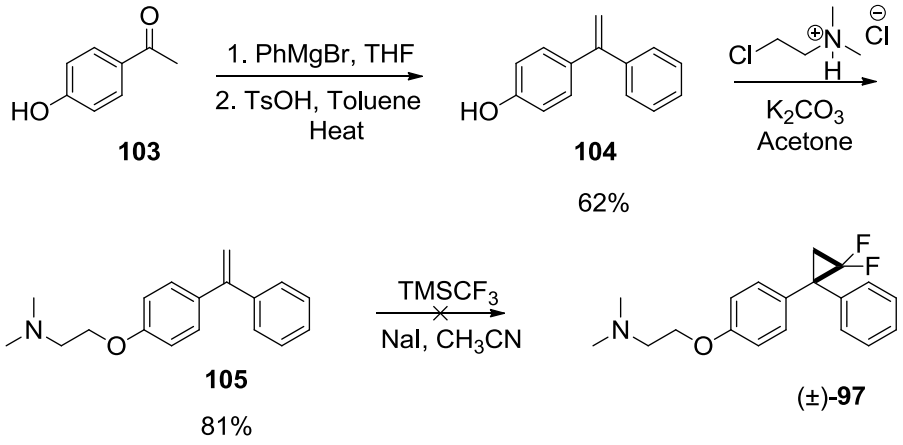
From our experience, $^{-}CF_3$ is able to attack an electrophilic center or abstract a proton from other reagents and solvent before decomposing into $:CF_2$. In addition to reacting with nucleophiles such as alkenes in the cycloaddition, the difluorocarbene has also been reported to insert into C-H,[150] C-Br,[150] Si-H,[151] O-H[152] and also other bonds.[153] All these possible side reactions led us to decide to protect the OH group with a methyl ether and attempt to synthesize **97** via Scheme 45.

As it is illustrated in Scheme 45, the reaction of 4-methoxyacetophenone (**99**) with Grignard reagent PhMgBr would provide a tertiary alcohol, which was not isolated but was directly used for the next dehydration step. The dehydration of alcohol was achieved by adding a few gains of *p*-toluenesulfonic acid in refluxing toluene to give the intermediate **100**. Reaction of **100** with $TMSCF_3$ in the presence of NaI offered **101** smoothly. However, **100** was found to be degraded when the demethylating agent BBr_3 was used to cleave the methyl ether in the deprotection step.



Scheme 45: Proposed route for synthesis of **97**

Subsequently, we tried to introduce the aminoethoxy functional group before the difluorocyclopropanation step and without protection of the alcohol group. **104** was synthesized by the coupling of 4-hydroxyacetophenone **103** with the Grignard reagent PhMgBr followed by dehydration as previously described. The alkylation of **104** with 2-chloro-*N*,*N*-dimethyl ethylamine in the presence of a weak base potassium carbonate in refluxing acetone gave **105** as expected, Scheme 46. However, the difluorocyclopropanation step failed to afford the desired product **97.** Instead, a complex mixture was formed which could not be purified using standard silica chromatography.



Scheme 46: Proposed route for synthesis of **97**

It was considered that the difluorocarbene may have attacked the nucleophilic nitrogen atom of the tertiary amine. Although nothing in the literature specifically documents the reaction between difluorocarbene and a tertiary amine, the electrophilic attack of dichlorocarbene[154] or other type of carbenes[155] by amines giving rise to *N*-ylides are well recognized. The *N*-ylides are not stable which may undergo further rearrangement (Figure 41:)[154] to give a complex mixture containing many side products.

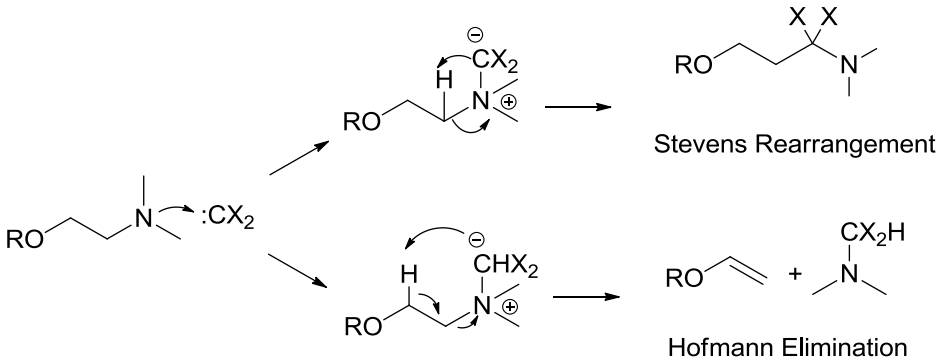
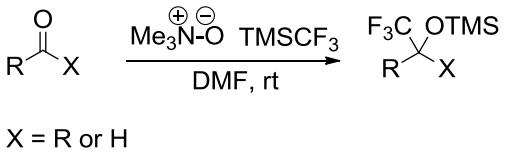


Figure 41: Rearrangements of *N*-ylides

Upon knowing that TMS-protected trifluoromethylated alcohols could be prepared from both aldehyde and ketone using $TMSCF_3$ in one step (Scheme 47),[156] it was realized that the phenol may not need to be protected at all.



Scheme 47: One pot syntheses of TMS-protected trifluoromethylated alcohols using $TMSCF_3$

It was postulated that the $(CH_3)_3SiCF_3$ or $(CH_3)_3SiI$ generated in situ may be able to form a trimethylsilyl-protected phenol with the phenolic ion (Figure 42).

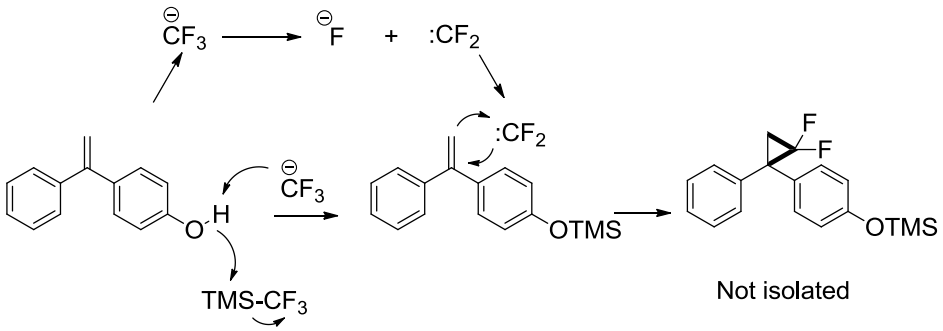
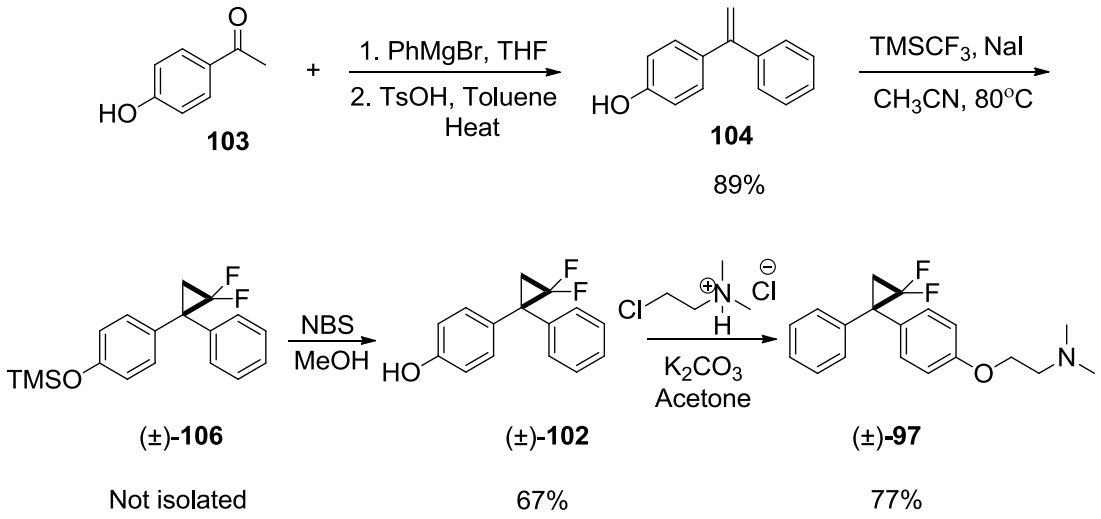


Figure 42: One pot synthesis of TMS-protected difluorocyclopropanel from 4-(1-phenylvinyl)phenol.

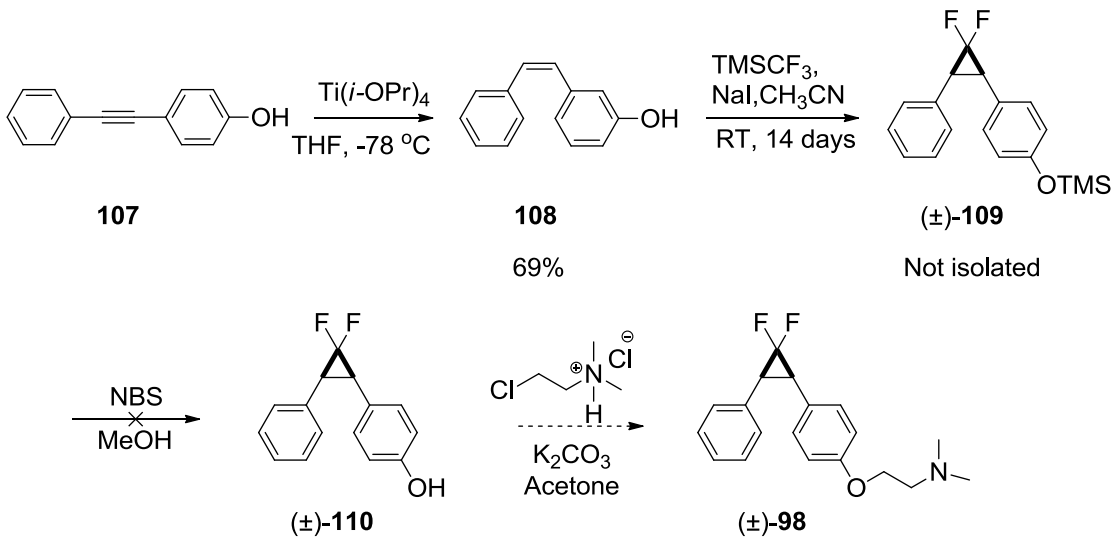
To test this hypothesis, **104**, prepared from coupling of 4-hydroxyacetophenone (**103**) with the Grignard reagent followed by dehydration as previously described, was subjected to difluorocyclopropanation directly without any phenol protection. It was found that compound **105** was, indeed, formed cleanly and smoothly as expected. The trimethylsilyl-protected phenol was not isolated and was subsequently deprotected cleanly and easily by stirring it directly with 0.2 equiv. of *N*-bromosuccinimide in MeOH. Alkylating **102** with 2-chloro-*N*,*N*-dimethyl ethylamine hydrochloride under mild basic conditions offered the target compound **97** in good yields (>75%), Scheme 48.



Scheme 48: The successful synthetic route for **97**

It was noteworthy that 1-(4-hydroxyphenyl)-1-phenylethene (**104**) was very unstable and was oxidized within hours if it was not stored under nitrogen and at low temperature, whilst its analogue 1-(4-methoxyphenyl)-1-phenylethene (**100**) was found to be chemically quite inert under ambient and normal handling conditions.

Following the successful synthesis of **97**, a similar method was utilized for the synthesis of its regioisomer, 1-[4-[2-(dimethylamino)ethoxy]phenyl]-2-phenyl-3,3-difluorocyclopropane **98** (Scheme 49). Although the trimethylsilyl-protected phenol **109** was observed in the crude reaction mixture, **110** was not obtainable as the cyclopropyl ring was destroyed during the TMS deprotection step.



Scheme 49: The proposed synthetic routes for the preparation of **97**

During the attempted syntheses of **97** and **98**, we came across 1,1-diarylmethanes as another class of derivatives of Tamoxifen reported in the literatures. This class of compounds was reported to bind selectively to AEBS which is the second highest affinity binding site for Tamoxifen after ERs.[34] Tamoxifen is known to induce two

different types of effects against tumour cells depending on the dosage. At low doses (< 0.1 μM), it is anti-proliferative to ER-positive cell lines and the anti-proliferation is reversible by adding estrogen E2 which acts as an agonist to ERs and stimulates the growth of cancer cells. At high doses (>1 μM), Tamoxifen is cytotoxic to the tumour cells and its cytotoxicity cannot be reversed by addition of estrogen E2, implying Tamoxifen may mediate this effect through other cellular targets which is independent of ERs.[34, 157] Interestingly, selective ligands of AEBS also display anti-tumour activities and antiviral properties.[68]

4.2.3 Tamoxifen and ligands of AEBS inhibit biosynthesis of cholesterol

4.2.3.1 Molecular identity of AEBS and its biological actions

Two decades ago, Sutherland *et al.* noticed that Tamoxifen bound to another binding site with high affinity in addition to the estrogen receptors.[158] The site was termed microsomal antiestrogen binding site (AEBS) since it is located within the microsomes of cells.[159] Previous studies reported that Tamoxifen and its derivatives resulted in the accumulation of zymosterol in the blood of patients.[63] This coupled with the fact that oxysterols such as 7-ketocholesterol, 6-ketocholestanol and 7-ketocholestanol (Figure 43), are another class of ligands that bind to AEBS with high affinities, led Poirot *et al.* to hypothesize that there may be a possible link between the AEBS and lipid and sterol metabolism.[160]

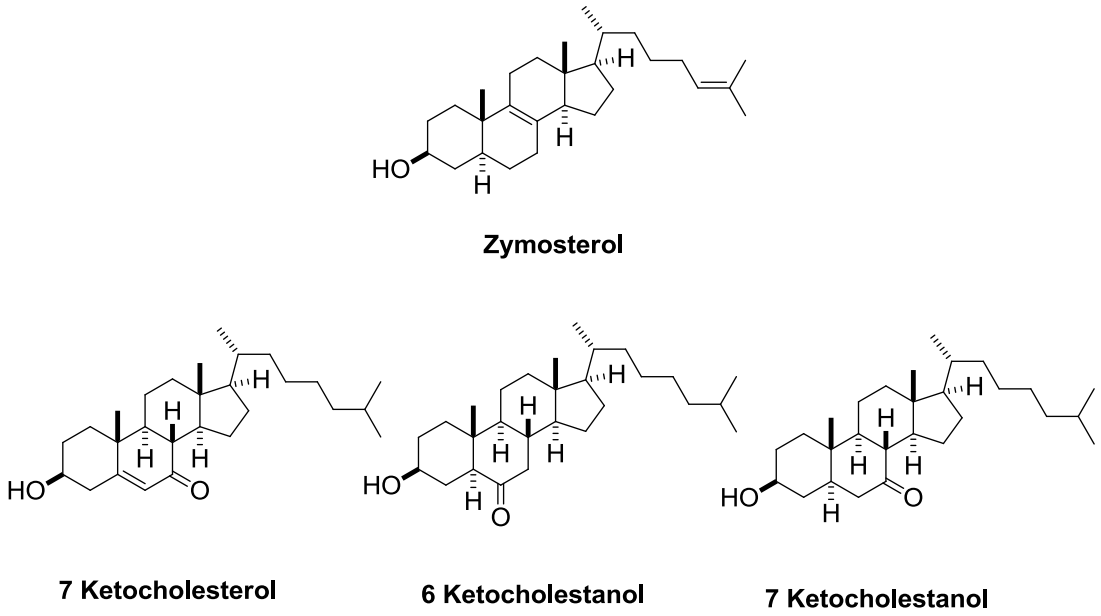


Figure 43: The structures of cholesterol precursors.

Poirot *et al.* went on to confirm the hypothesis by showing that AEBS is involved in the post-lanosterol intermediates of cholesterol biosynthesis and inhibition of AEBS leads to an accumulation of cholesterol precursors.

Poirot *et al.* also reported that the treatment of PBPE (Figure 44), a selective AEBS ligand, and Tamoxifen at low dosages (both 0.5 μm) to the human breast adenocarcinoma cell line MCF-7 led to a massive intracellular accumulation of sterols. For Tamoxifen treatments, 5α-cholest-8-en-3β-ol (zymostenol) was detected as the major metabolite.[159] When treated with PBPE, two major sterols were isolated and identified which were 5α-cholest-8-en-3β-ol, and cholesta-5,7-dien-3β-ol (7-dehydrocholesterol), suggesting the selective AEBS ligand, PBPE, affected at least two enzymatic steps.[159]

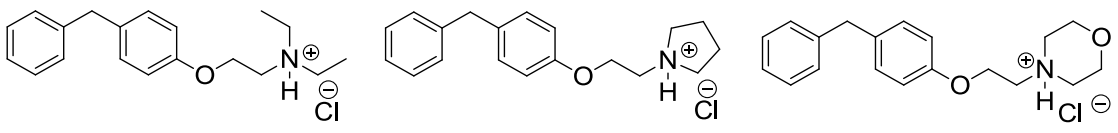


Figure 44: The structures of known AEBS ligands

Zymostenol and 7-dehydrocholesterol are substrates of 3*β*-hydroxysterol-Δ(8)-Δ(7)-isomerase (D8D7I) and 3*β*-hydroxysterol-Δ(7)-reductase (DHCR-7) respectively and accumulation was due to a noncompetitive inhibition of these two enzymes (Figure 45).[161] This finding led Poirot *et al.* to identify AEBS as a hetero-oligomeric enzyme complex which consists of D8D7I and DHCR-7 as subunits.[159] Additionally, Poirot *et al.* showed that the coexpression of D8D7I and DHCR-7 is essential and sufficient to reconstruct the AEBS in mammalian cells.[159]

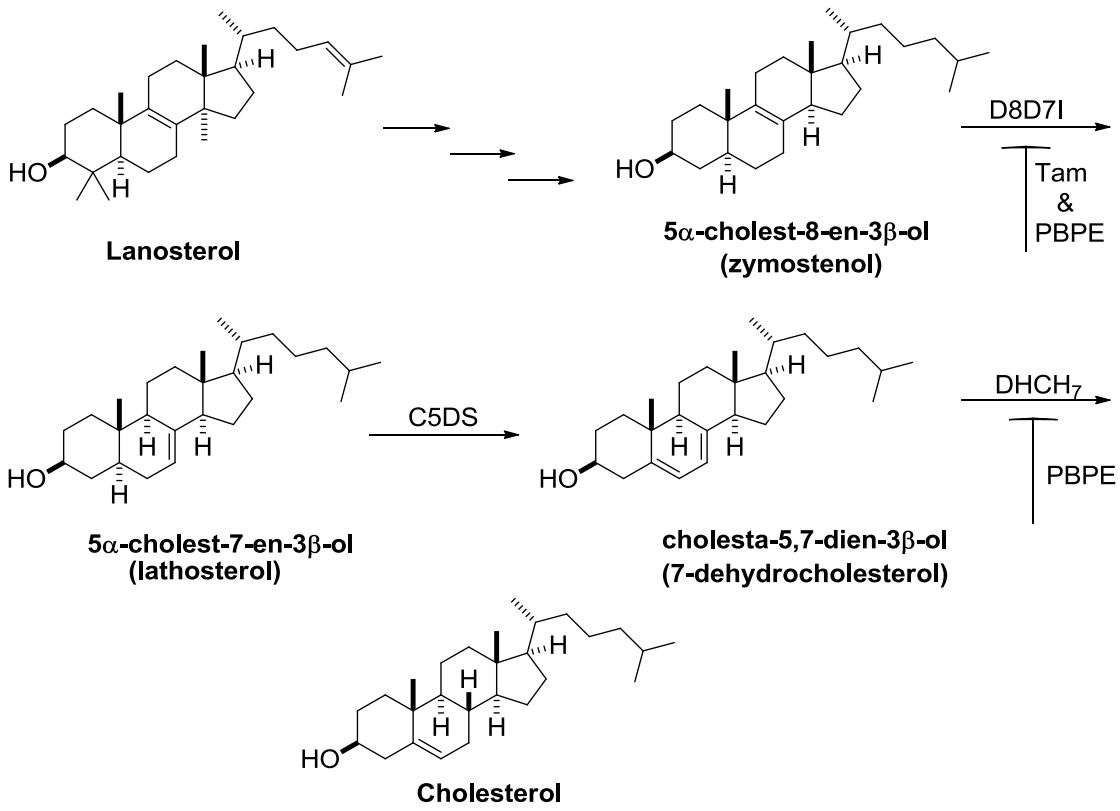


Figure 45: The proposed biosynthesis pathway of cholesterol

This figure is reproduced from Kedjouar *et al.*[159]

At high dosage (PBPE = 40 μM, Tamoxifen = 10 μM), Tamoxifen and PBPE were reported to trigger active cell death and induction of autophagy,[161] indicating that binding to AEBS could result in both apoptosis and autophagy depending on the concentration of ligands. Other diphenylmethyl compounds, such as DPPE, were also potent inhibitors of AEBS and produced similar sterol accumulation profiles, active cell death and autophagy in MCF-7 cells, but PBPE was found to have the highest affinity for AEBS in the series.[159, 161]

During the last decade, research has shown that apoptosis (self-killing) and autophagy (self-eating) are two very closely related, coordinately regulated biological processes. In general, autophagy is a cellular stress adaption which promotes cell survival and suppresses apoptosis. However, in some certain cases, autophagy may lead to induction of apoptosis or autophagic cell death resulting from excessive cytoplasm degradation.[162] The underlying mechanisms which control the decision of autophagy to act as a cell survival or cell death are still poorly understood.[162]

4.2.3.2 Different classes of AEBS ligands inhibit different enzymes or steps in the cholesterol biosynthesis

The binding of the microsomal antiestrogen binding site by Tamoxifen and other selective AEBS ligands such as PBPE and DPPE leads to a significant concentration- and time-dependent accumulation of cholesterol precursors.[161] Interestingly, different classes of AEBS ligands have been shown to inhibit different enzymes or steps in the cholesterol biosynthesis pathway, leading to different sterol accumulation profiles. Selective Estrogen Receptor Modulators bearing a hydroxyl

group on their aromatic backbone such as 4-OH-Tamoxifen and triparanol, inhibit the 3β-hydroxysterol-Δ^{24}-reductase (DHCR24) and give rise to the accumulation of cholest-5,24-diene-3β-ol (desmosterol). Ring B oxysterols such as 7-ketocholesterol, 6-ketocholestanol and 7-ketocholestanol are inhibitors of D8D7I, leading to accumulation of zymostenol, while Raloxifene is able to inhibit both cholesterogenic enzymes D8D7I and DHCR24, causing an accumulation of zymosterol (Figure 46).[163]

An interesting observation was made by Poirot *et al.* that the accumulation of zymostenol in MCF-7 cell reached a plateau 24 h after treatment with Tamoxifen and other selective AEBS ligands and decreased significantly in favour of oxysterols after 48 h, indicating a reactive oxygen species producing system may be present.[159] Indeed, Tamoxifen and 7-ketocholesterol have been shown to produce reactive oxygen species,[164-166] which lead to these cholesterol precursors being further oxidized into different oxysterol species that might have different pharmacological properties.[159]

It was established that the accumulation of cholesterol precursors and the stimulation of reactive oxygen species contribute to the autophagic effects, cell differentiation and apoptotic properties of Tamoxifen, other SERMs and selective AEBS ligands in breast cancer cells.[71, 160, 161] Interestingly, it was found that co-treating MCF-7 cells with high doses of vitamin E reversed the effect of diminishing zymostenols.[160] Moreover, treatment with vitamin E inhibited the cell differentiation and active cell death induced by PBPE, Tamoxifen, and 7-dehydrocholesterol, but not autophagy.[160, 161] Together, these data suggest that zymostenol was transformed by

oxidation and the accumulation of sterols alone is enough for induction of macroautophagy, whereas apoptosis requires both the sterols accumulation and production of reactive oxygen species.[71]

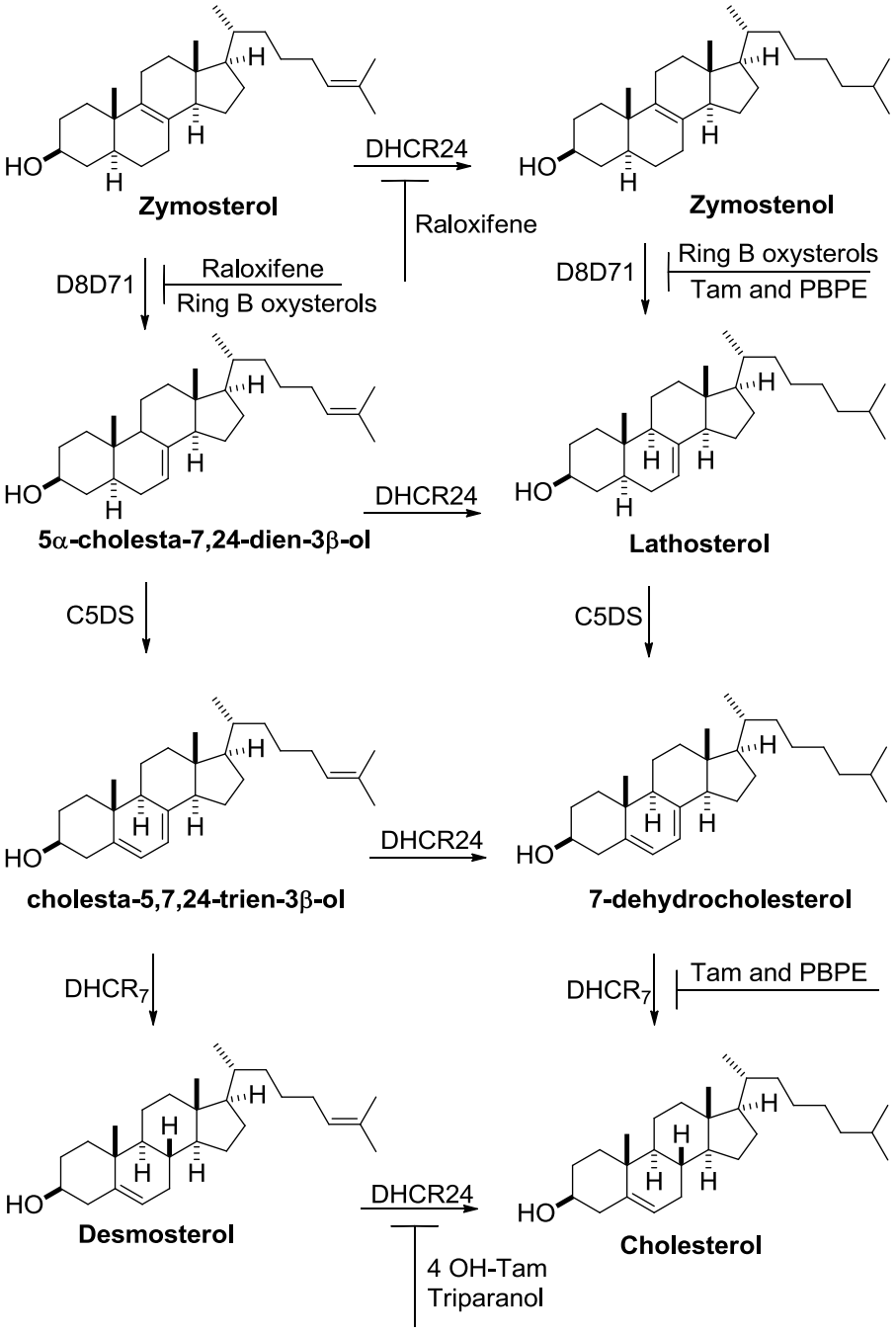


Figure 46: Inhibition of cholesterol biosynthesis by different classes of AEBS ligands

This figure is reproduced from Kedjouar *et al*.[159]

4.2.3.2 Different classes of AEBS ligands and their link to ChEH

Several structurally different classes of ligands of AEBS have been identified which include Selective Estrogen Receptor Modulators and diphenylmethanes that contain an aminoethoxy basic side chain, such as Tamoxifen, 4-OH Tamoxifen and raloxifene for the former and DPPE and PBPE for the latter. AEBS has no affinity for estrogens or other non-cationic SERMs. Additionally, AEBS has been reported to bind to poly-unsaturated fatty acids such as arachidonic acid and docosaheaxaenoic acid as well as ring B-oxysterols, for example, 7-ketocholestrol, 6- and 7-ketocholestanol.[167] The last oxysterol is an autoxidation product of zymosterol and the substrate of D8D7I.[168] Interestingly, 7-ketocholestrol and 6- and 7-ketocholestanol are also potent inhibitors of cholesterol epoxide hydrolase (ChEH),[169] a microsomal epoxide hydrolase which catalyzes the *trans*-hydration of cholesterol-5,6-epoxides (5,6-ECs) into cholestane-3β,5α,6β-triol (CT), Figure 47, indicating AEBS may be pharmacologically and structurally associated with ChEH. Indeed, according to Poirot *et al.* all AEBS ligands are inhibitors of ChEH and it has even been proposed that AEBS and ChEH could be a single entity[170] since experiments indicated that the enzymes which form the AEBS also participated in the catalytic activity of ChEH.[171] The knockdown of either D8D7I or DHCR7 using siRNA resulted in partial inhibition of the ChEH activity and the knockdown of both D8D7I and DHCR7 enzymes almost completely abolished the hydrolyzing actions of ChEH, whereas the co-expression of both D8D7I and DHCR7 fully reconstructed the ChEH activities.[171] Moreover, Poirot *et al.* demonstrated that the substrates of ChEH, 5,6-CEs and its hydration product (CT) are competitive ligands of Tamoxifen binding to the AEBS.[171] Conversely, every AEBS ligand tested inhibited ChEH and the higher affinity of these ligands for AEBS, the more potently they inhibit

ChEH.[171] These results clearly show that AEBS and ChEH are very closely related if not a single entity.

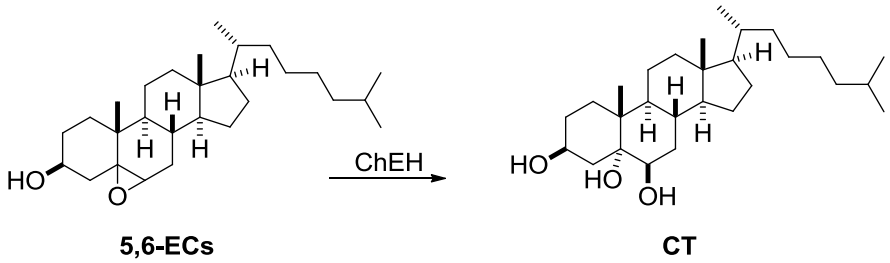


Figure 47: *trans*-Hydration of 5,6-ECs into CT

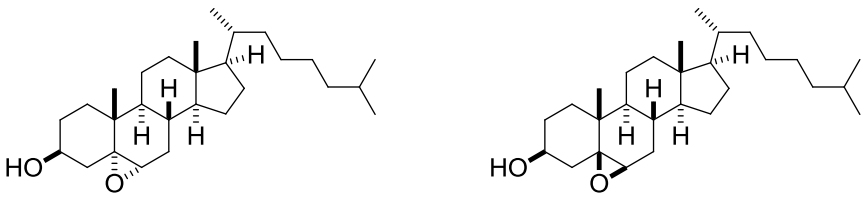
It was also demonstrated in the single knockdown experiments that D8D7I and DHCR7 had different influences on the kinetic parameters of ChEH activity. The V_{max} of the enzymatic activity of ChEH was observed to diminish considerably when only D8D7I was knocked down, whereas the single knockdown of DHCR7 led to an increase of the K_m value.[171] From these data, Poriot *et al.* suggests that D8D7I may have a catalytic role in the ChEH activity, whilst DHCR7 plays an important role in regulating and assisting substrate binding.[171]

4.2.3.3 The molecular action of AEBS/ChEH

ChEH is widely distributed in mammals. It belongs to an epoxide hydrolase family which catalyze the hydration of reactive epoxides with water to yield 1,2-diol products. Generally speaking, the hydration is energetically more favourable and leads to more stable products being formed, however exceptions do exist.[172] In vertebrates, a number of epoxide-hydrolase enzymes have been identified so far which includes leukotriene-A4 hydrolase (LTA_4H), hepoxilin-epoxide hydrolase (HXEH), microsomal epoxide hydrolase (mEH), soluble epoxide hydrolase (sEH)

and cholesterol 5,6-epoxide hydrolase. Both LTA_4H and sEH are bifunctional enzymes with great structural complexity which catalyze not only the epoxide hydrolase activities, but also aminopeptidase and phosphate phosphatase activities respectively.[173] If AEBS and ChEH were really a single entity, then it would be a tri-functional protein complex consisting of two closely related enzymes, D8D7I and DHCR-7, as subunits, but is involved in three enzymatic steps in the sterols and lipid metabolism. All epoxide hydrolases, except ChEH, have been cloned and molecularly characterized. The greater complexity of ChEH probably provides the reason why ChEH is the only member of the epoxide hydrolase family whose cloning and purification has never been reported before.[173]

The only known substrate of ChEH is cholesterol-5,6-epoxide (5,6-ECs) which can exist in two diastereoisomeric forms: cholestan-5*α*,6*α*-epoxy-3*β*-ol (5,6*α*-CE) and cholestan-5*β*,6*β*-epoxy-3*β*-ol (5,6*β*-CE), Figure 48. The binding of ChEH is highly specific for both 5,6-CEs, yet the enzyme in liver microsomes from several species shows an approximate 2 to 2.5 fold preference for 5,6*α*-EC over 5,6*β*-EC under physiological pH conditions.[174]



5,6*α*-EC **5,6*β*-EC**

Figure 48: Structure of 5,6*α*-CE and 5,6*β*-CE

4.2.3.4 The stability and toxicity of 5,6-ECs

Owing to their highly polarized C-O bond and three-membered strained ether ring, epoxides in general are quite unstable and react readily with electron rich nucleophiles. Some reactive epoxides have been reported to have mutagenic, toxic and carcinogenic effects.[175] An example of these is styrene oxide which is a potent alkylating agent that can form a covalent adduct with critical biological targets such as DNA and proteins, thus contributing to its mutagenicity and carcinogenicity. Most epoxides produced in organisms are, however, of intermediate reactivity and are relatively stable at physiological conditions, hence they do not present a serious danger to cells and organisms.[175]

Unlike zymostenol which is very sensitive to oxidants,[159] 5,6-ECs are relatively stable under physiological conditions[176] and exceptionally stable under non-catalytic chemical conditions.[177] It was reported that when treating 5,6α-CE and 5,6β-CE with 2-aminoethanol in refluxing ethanol for 2 days, only 2% of the expected aminoethanolic compound was formed from 5,6α-CE and no reaction was observed with 5,6β-CE under the same reaction conditions.[177] The desired product obtained from 5,6α-CE was subsequently increased from the extremely low yield of 2% to an unsatisfactory yield of 25% in the presence of a strong Lewis acid ($LiClO_4$) and even in these reaction conditions, 5,6β-CE still did not react.[177] In contrast to the lower reactivity of 5,6β-CE toward nucleophiles being observed, it was reported that at pH 2, the rate of hydrolysis of 5,6β-EC was approximately six times faster than that of 5,6α-EC.[177] These results highlight a difference in the stability of these two diastereoisomers in different pH environments.

The hydrolysis of both diastereoisomers of 5,6-CEs by ChEH yields CT as the exclusive product.[174] It was found that CT as well as its further oxidative metabolites are mutagenic and cytotoxic and are associated with increased lipid peroxidation. 5,6-ECs may be derived from the photo oxidation of cholesterol in response to UV radiation and fears had been raised over the formation of 5,6-ECs in biological systems as cholesterol epoxides were suspected to associate with an increased skin cancer risk.[178, 179] Although the mutagenicity of 5,6-ECs had been examined in numerous studies, conflicting results were reported. *In vivo*, the tumourigenicity of 5,6-CEs was evaluated in the rat mammary gland and both disastereosiomers of the cholesterol epoxide were demonstrated to be non-tumour promoting agents.[180] *In vitro*, Smith *et al.* studied air-aged samples of cholesterol in several strains of *Salmonella typhimurium* and found that 5,6-ECs were not mutagenic, although some unidentified autoxidation products of cholesterol were.[181] This finding was subsequently confirmed by Cheng *et al.* and his group found that CT was instead mutagenic and suggested that reactive oxygen species (ROS) might play an important role in this effect.[182] On the contrary, Sevanian *et al.* reported that 5,6-CEs were weak mutagenic agents at high concentration in Chinese hamster lung fibrolasts,[183-185] with the β isomer being a more potent mutagen than the α isomer. The cytotoxicity of 5,6-CEs had also been investigated and compared; it was established that the β-cholesterol epoxide was more toxic than its α-cholesterol epoxide counterpart.[183, 184]

5,6-ECs are the major autoxidation products of cholesterol.[186] Interestingly, the inhibition of AEBS also leads to accumulation of 5,6-ECs resulting from the secondary oxidative metabolites of cholesterol precursors inhibiting ChEH

activity.[171] This finding is in a strong agreement to Poirot *et al.*'s suggestion that AEBS and ChEH is an single entity.[170] In their studies, they demonstrated that the enzymes constituting AEBS are also involved in the catalytic activity of ChEH.[171] In addition, Poirot *et al.* suggests that it is highly unlikely that 5,6-ECs are directly mutagenic and carcinogenic substances themselves as some well-known drugs such as Tamoxifen and raloxifene are potent inhibitors of AEBS and lead to accumulation of 5,6-ECs.[173, 187] In fact, several lines of evidence demonstrated that inhibition of AEBS and accumulation of 5,6-ECs contribute to the anticancer and chemopreventive activities of these compounds.[173] Tamoxifen inhibits AEBS activity in a nanomolar concentration range and is widely used as a treatment or prevention of breast cancer, while omega-3 docosahexaenoic acid, also a potent inhibitor of ChEH, is a common dietary supplement which was demonstrated to increase survival of metastatic breast cancer patients undergoing chemotherapy.[173] Consistent with these results was the beneficial effect of DPPE, another potent AEBS inhibitor, reported in a phase III clinical trial in which a substantial improvement on the overall survival rate was observed in patients co-treated with doxorubicin.[173]

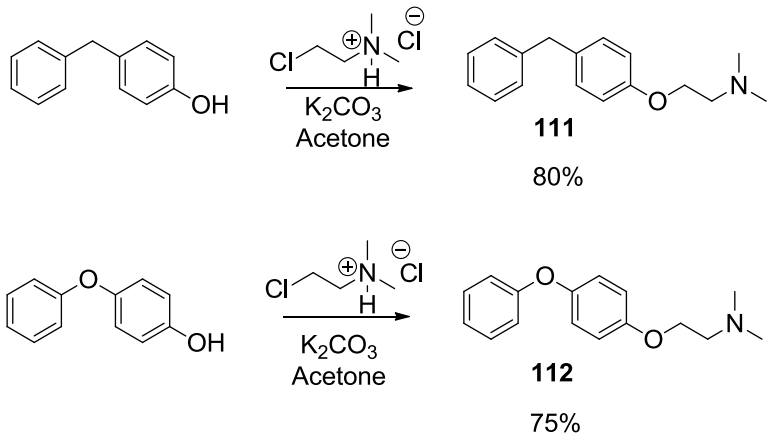
The role of ChEH was initially proposed to be a detoxifying enzyme similar to that of microsomal epoxide hydrolase,[169] though this possibility may be ruled out.[171] As opposed to detoxification, the cytotoxicity[183, 185] and mutagenicity[183, 185] of CT, resulting from the hydrolytic action of ChEH, and its further oxidative products[182] were measured to be greater than both the substrates of ChEH in various studies. These data indicate that the inhibition of ChEH and CT production as well as its

mutagenic metabolites exerts a protective rather than harmful effect on biological systems.

4.2.4 Aminoethoxy-1,1-diarylmethanes – another class of selective AEBS ligands

Diphenylmethane derivatives of Tamoxifen are selective ligands of AEBS that do not bind ERs.[34, 157, 188] DPPE, in particular, has been extensively studied and was reported to have a 22-fold weaker affinity than Tamoxifen for AEBS.[189] In *in vitro* studies, DPPE has been shown to induce apoptosis and inhibit proliferation of tumour cells through its binding to AEBS.[160] DPPE had been developed as far as phase III clinical trial testing for the treatment of metastatic/recurrent breast cancer before a clear understanding of the mechanism of action was established.[161] Although DPPE failed to show any advantage in response rate, response duration and progression-free survival, it significantly improved the overall survival rate in patients co-treated with doxorubicin.[190]

111, which is the diphenylmethane homologue of Tamoxifen, has also been tested by Poirot *et al.* for its affinity to AEBS. **111** was found to have a slightly higher affinity than DPPE, but was still 17 times weaker than Tamoxifen.[189] In their studies, they had also tested a series of other phenoxyphenol derivatives that, too, were also potent AEBS ligands.[191] This class of compounds is certainly worth further investigation and consequently **111** and **112** were synthesized. Their syntheses were easily achieved by alkylating the commercially available 4-benzylphenol or 4-phenoxyphenol with 2-chloro-*N*,*N*-dimethyl- hydrochloride ethanamine as shown in Scheme 50.



Scheme 50: The synthesis of analogues of DPPE

Therefore, in addition to the 1,1-diaryldifluorocyclopropyl derivative of Tamoxifen and its precursors, the compounds listed in Figure 49 were also sent for biological testings.

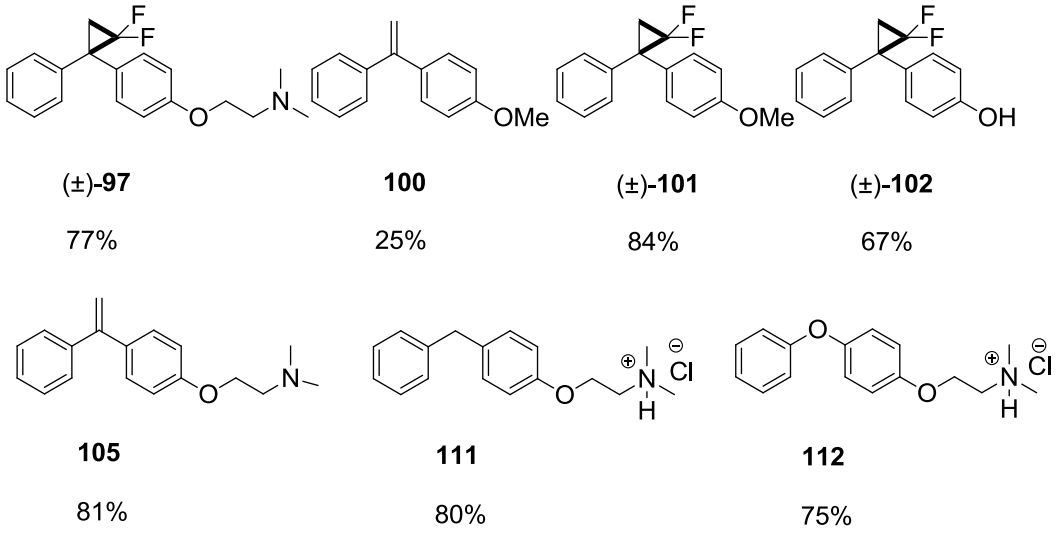
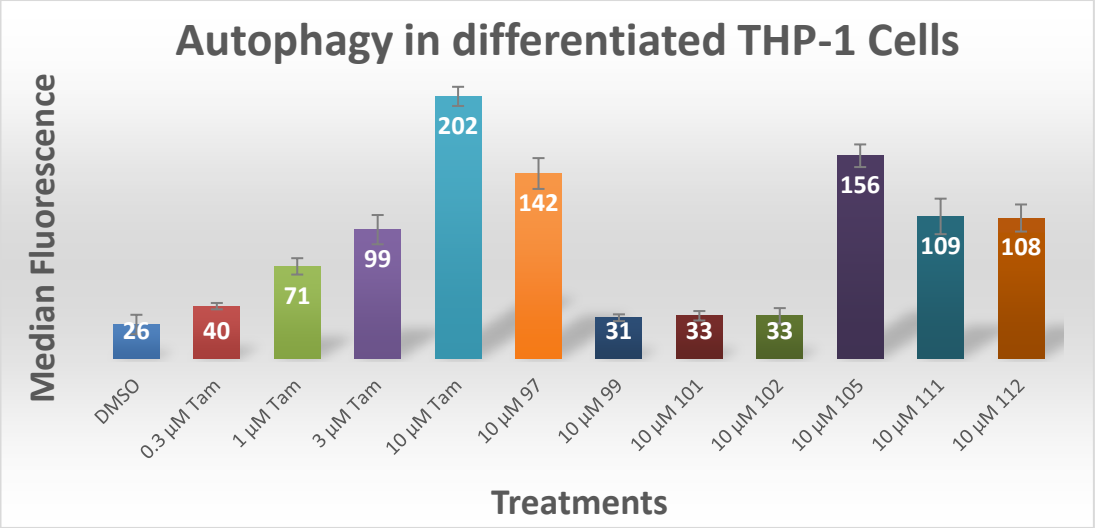


Figure 49: Structures of the compounds for second round of biological testing.

Graph 5 shows the results for the autophagic assays of those 1,1-diarylmethenes and 1,1-diarylethenes shown in Figure 49. To our delight, some of the compounds did exhibit moderate autophagic activities. Interestingly, all compounds lacking a basic

side chain in their structures did not induce autophagy (compound **100** to **102**). On the other hand, compounds bearing an aminoethoxy side chain (**97, 105, 111** and **112**) were potent autophagy inducers, although Tamoxifen was determined to be more potent under the same concentrations. The most active compound among the aminoethoxy derivatives of Tamoxifen tested was **105**, followed by **97**; **111** and **112** were also active (**Graph 5**), but they were less effective as autophagy inducers and appeared to be as equally potent as one another.



Graph 5: Measure induction of autophagy of the 1,1-diaryl-methenes and ethenes shown in Figure 49 using Tamoxifen (Tam) and DMSO as positive and negative controls respectively.

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4.2.5 The importance of aminoethoxy basic side chains for inducing autophagy

In addition to ERs and AEBS, Tamoxifen is also reported to bind to other cellular targets such as PKC, CaM and ACAT.[34] However, no correlation between diphenylmethanes and other common molecular targets of Tamoxifen has been reported, except AEBS. Additionally, the diphenylmethane derivatives of Tamoxifen such as DPPE and PBPE have been demonstrated to bind to AEBS with high affinities and specificity and do not affect CaM[192, 193] and PKC dependent

pathways[194] and did not inhibit ACAT.[68] Therefore, it is highly likely that the autophagic effects of both **111** and **112** in THP-1 cells and their difluorocyclopropyl analogues are attributed to the binding of AEBS due to the high structural similarities between our drug candidates and those well-known AEBS ligands.

A closer inspection of the first and second biological testing data and the structures of compounds tested reveals that all aminoethoxy derivatives of Tamoxifen are potent autophagy inducers, whereas no autophagic activity was observed in the THP-1 cell lines with compounds which lack the amino side chains. This indicates that the aminoethoxy moiety is a crucial pharmacophore for the induction of autophagic activities of Tamoxifen and its derivatives.

Likewise, the presence of a protonatable amine side chain is found to be crucial for binding to AEBS, whereas for binding to the ERs it could be very influential to the binding affinity, but not essential.[34] For instance despite **113**, Metabolite X and Y of Tamoxifen all lacking a protonatable side chain, they still display high binding affinities for the ERs (Figure 50). While **113** and metabolite X are 10 times and 2 times weaker than Tamoxifen respectively, the affinity of Metabolite Y is comparable to Tamoxifen for binding to the ERs.[34] In contrast, metabolite X and Metabolite Y of Tamoxifen and the analogue **113** do not exhibit any binding affinity for the AEBS.[34] This finding is consistent to our belief that the autophagic effects of our compounds are a result of interaction with the AEBS and not the ERs.

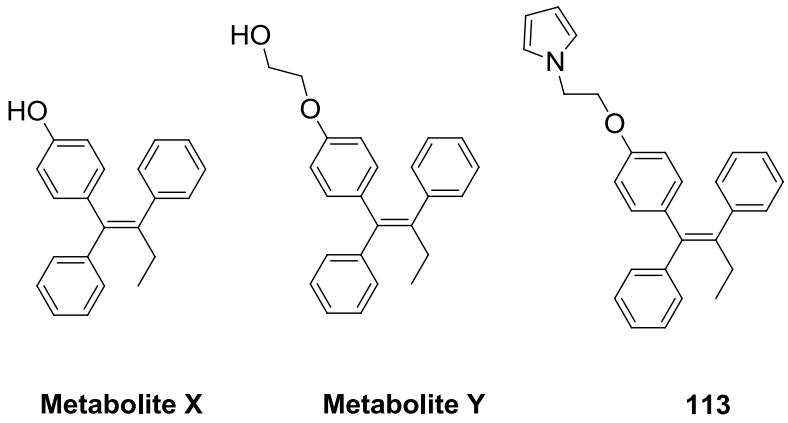


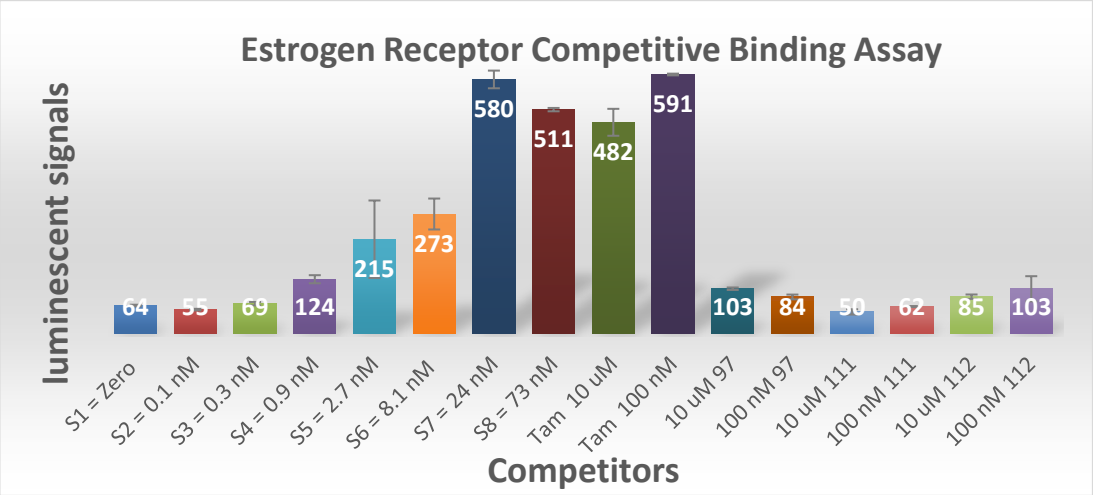
Figure 50: Metabolite of Tamoxifen and its analogue

4.2.6 Biological testing results for their affinity to ERs.

To confirm that that neither the antiestrogenic nor estrogenic activities through direct interaction with ERs is responsible for the autophagic effect of our drug molecules, an *in vitro* estrogen receptor competitive immunoassay was conducted on the compounds which induced autophagy. The assay works by using an ED-steroid hormone conjugate which could be recognized by the estrogen receptor. The ED hormone is able to compete with ligands for the binding site of estrogen receptor binding site. In the presence of ER ligands, the binding sites are occupied by the free ligands and the free ED-conjugate complements with Enzyme Acceptor to form an active enzyme that can hydrolyze substrates to produce luminescent signals. In the absence of ER ligands, the ED-conjugates are bound to the ERs and are unavailable for complementation, leading to low luminescent signals. The strength of these signals is proportional to how strongly a ligand can compete for binding to the estrogen receptors. The signals produced are then compared with the standards with known concentration of 17β-estradiol.

Graph 6 shows the relative binding affinity of Tamoxifen and our compounds **97**, **111** and **112** in comparison to 17*β*-estradiol. As illustrated, Tamoxifen displays a binding affinity in the same order of magnitude as 17*β*-estradiol (100 nm of Tamoxifen vs S8), whereas our compounds are several orders of magnitude lower than that of Tamoxifen and 17*β*-estradiol (10 µM vs S4).

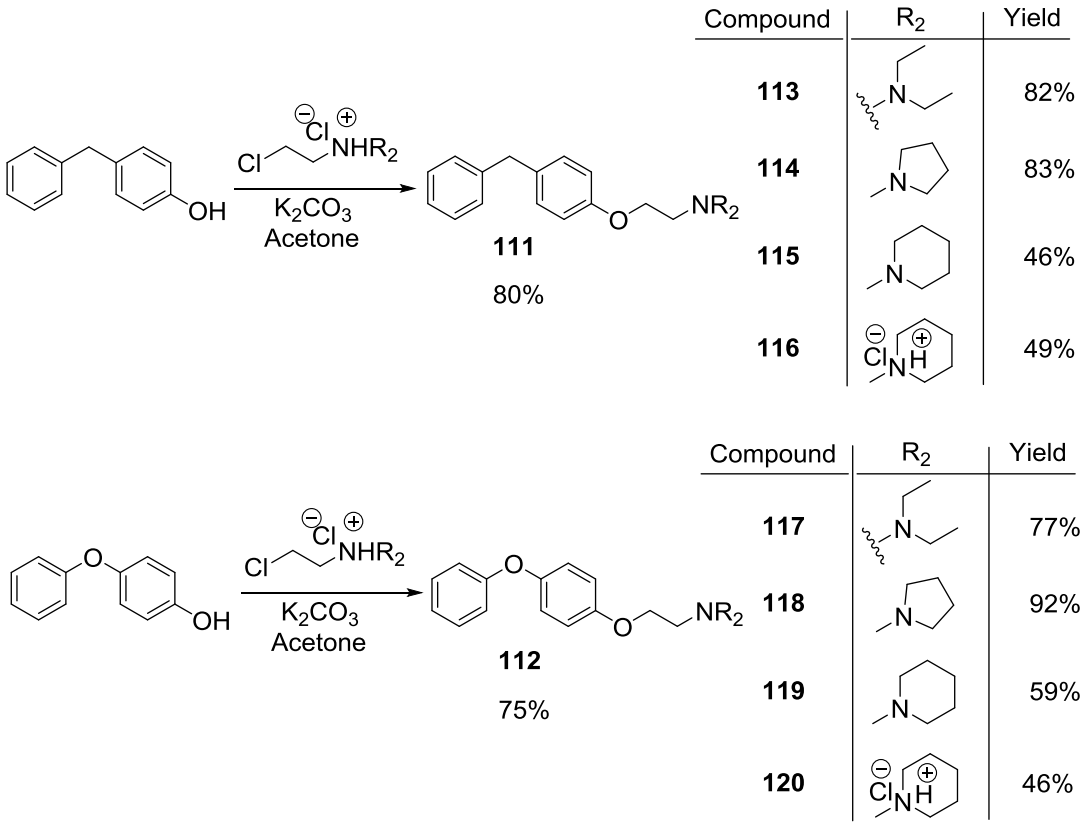
Despite 1,1-diarylethylene **105** being the most active compounds among the series, it would not be suitable as a drug candidate and is of little medicinal interest. The presence of a terminal alkene moiety is susceptible to electrophilic attack by chemical species or oxidative metabolism by the cytochrome P450 enzyme and they would be chemically and enzymatically very unstable and thus, it was not tested for its binding affinity to ER.



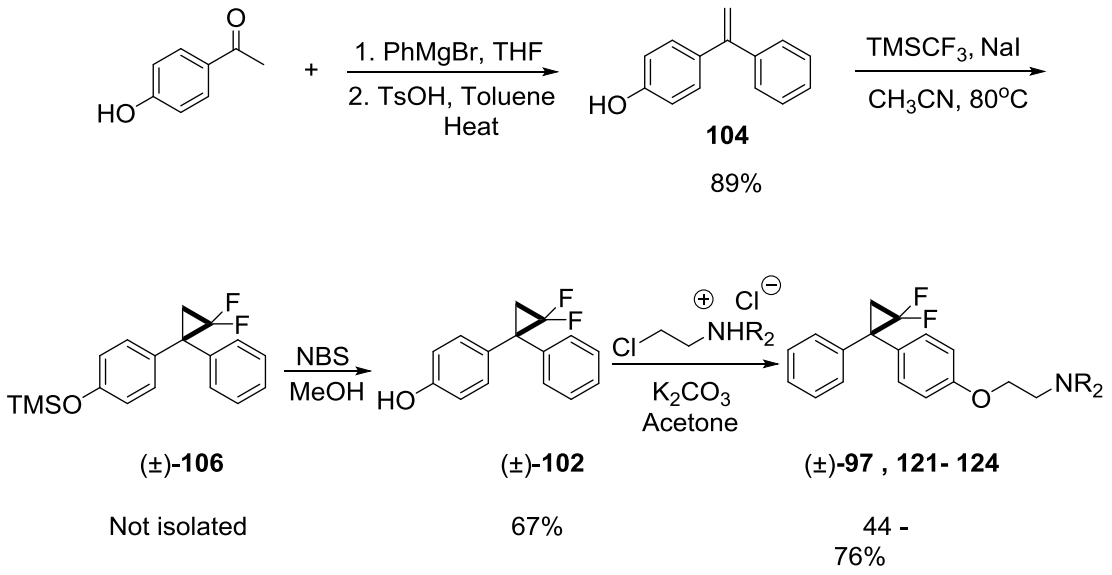
Graph 6: Estrogen receptor competitive binding assay of Tamoxifen (Tam) and compound **97**, **111** and **112** shown in Figure 49 using 17*β*-estradiol (S1 – S8) as positive dose dependent controls.

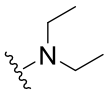
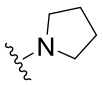
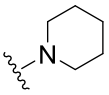
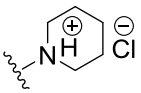
4.3 Biological testing result for other aminoethoxy diarylmethyl analogues of Tamoxifen synthesized for this project

Among all the different classes of compounds tested in our previous biological analyses, only the derivatives of Tamoxifen with an aminoethoxy side chain are potent inducers of autophagic effects. Thus, we explored the structure-activity relationship of a few more analogues based on our previous findings. Scheme 51: Other phenoxyphenyl- and phenoxyphenoxyethanamines synthesized and biologically tested. and Scheme 52: Other 1,1-diaryldifluorocyclopropylethanamines synthesized and biologically tested.show other diarylaminoethoxy compounds that have been synthesized for this project and their preparations are similar to their related analogues reported previously.



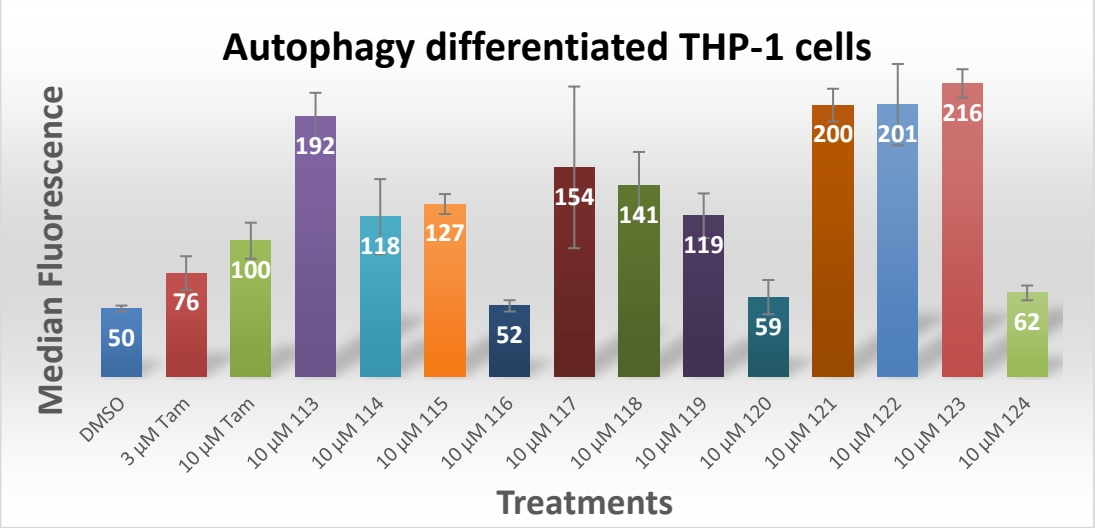
Scheme 51: Other phenoxyphenyl- and phenoxyphenoxyethanamines synthesized and biologically tested.



Compound	R_2	Yield
121		69%
122		76%
123		65%
124		44%

Scheme 52: Other 1,1-diaryldifluorocyclopropylethanamines synthesized and biologically tested.

As illustrated in **Graph 7**, many of the aminoethoxy derivatives of Tamoxifen shown in Scheme 51 and 52 induced autophagy to various extents. These data confirmed our previous hypothesis that the presence of a basic aminoethoxy side chain is an important pharmacophore for induction of autophagy.

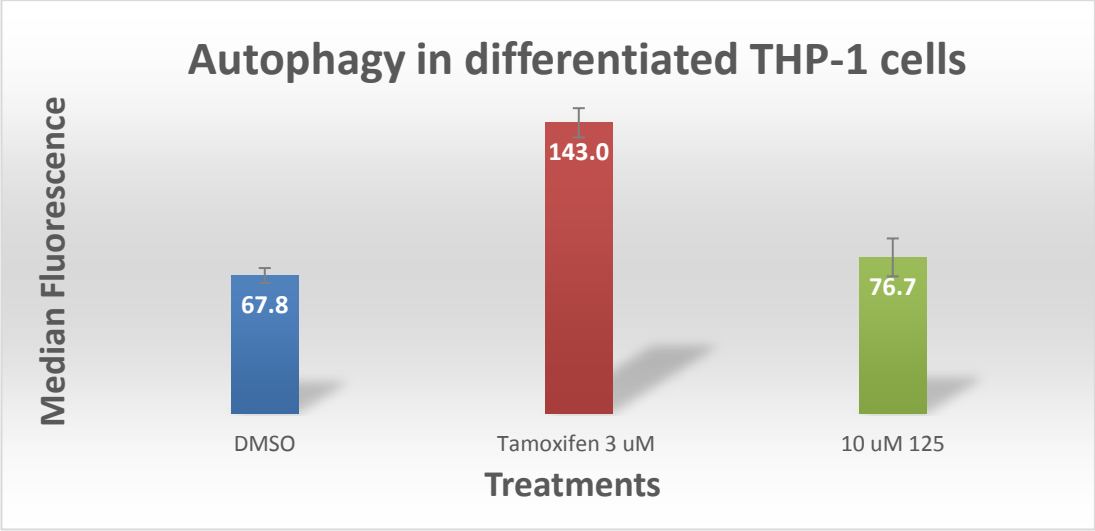


Graph 7: Measure induction of autophagy of our drug candidates shown in Scheme 51 and Scheme 52 using Tamoxifen (Tam) and DMSO as positive control and negative controls respectively.

The most active among the series were the difluorocyclopyl analogues **121** to **123** which were found to be consistently more potent than any other phenoxyphenolic **(113 – 116)** and phenoxybenzyl **(117 – 120)** derivatives of Tamoxifen. **113** and **114** are DPPE and PBPE respectively that have been extensively studied by Poirot *et al.* and others for their affinity binding to AEBS and induction of autophagic activities.[161] They were once again proven to be potent inducers of autophagy in our independent studies. Interestingly, PBPE was the most potent AEBS ligand in the 1,1-diphenylmethane series reported by Poirot *et al.* and was found to have at least a 3-5 times higher affinity for AEBS than DPPE.[171] However, their affinity for AEBS did not seem to translate directly into their induction of autophagy in our biological assays as DPPE was found to be a more potent inducer than PBPE. Moreover, analogues with a morpholine moiety attached to the aminoethoxy side chain, **116**, **120** and **124,** were found to be relatively inactive, implying that the presence of an additional hydrogen-acceptor is probably unfavourable. This finding is in contrast to

Poirot *et al.*'s result who found **116** and **120** to be more active than DPPE, although they studied for their affinity to AEBS and not for their induction of autophagy.[191]

If the molecular target of our compounds is AEBS, then it is perfectly sensible to expect a positive correlation between the binding affinity of our compounds for AEBS and the induced autophagic response, unless the compounds are cytotoxic to cells or autophagy is overdone which could lead to either apoptosis or autophagic cell deaths. We have tested the morpholine analogue **124** against Tamoxifen and DMSO vehicle again since the difluorocyclopropyl series is more potent than the derivatives of benzylphenol or phenoxy phenol. However, similar negative results were obtained for the morpholine analogues confirming that the results were not due to practical/experimental errors (**Graph 8**).

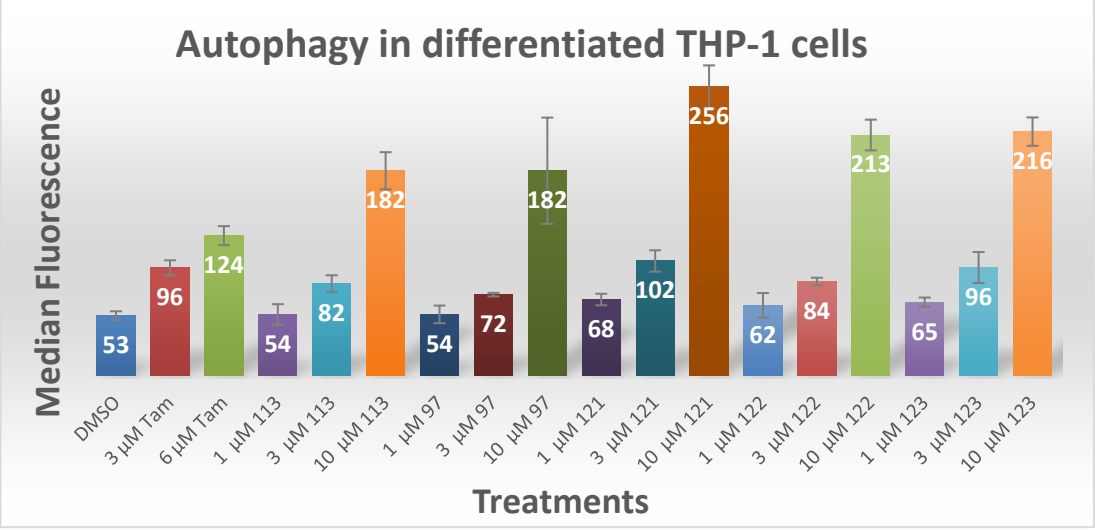


Graph 8: Measure induction of autophagy of **125** using DMSO and Tamoxifen as controls

Tamoxifen is at least equal if not more potent than any of the compounds we synthesized and tested. Nevertheless, its potency as an autophagic inducer is not reflected in our biological assay when cells were treated with high dose of

Tamoxifen. Tamoxifen was observed to be cytotoxic to cells at high doses (>5 µM), but DPPE, PBPE or our novel aminoethoxy difluorocyclopropanes were not cytotoxic. The relatively low autophagic activity detected at 10 µM treatment of Tamoxifen (**Graph 7**) was probably due the cytotoxicity of Tamoxifen on the differentiated THP-1 cells, making direct comparison of data rather difficult or unreliable as the concentrations for the treatments of Tamoxifen have to be within low and narrow ranges.

As the difluorocyclopropyl compounds simulate the highest level of autophagy responses, they were further evaluated against Tamoxifen and DPPE at different dosages. **Graph 9** demonstrates clearly that Tamoxifen, DPPE and the difluorocyclopropanes promoted induction of autophagy in a concentration-dependent manner.



Graph 9: Treatment of Tamoxifen (Tam) and its analogue induce autophagy in a concentration-dependent manner, DMSO was used as the negative control.

In conclusion, we have synthesized a wide range of structurally different analogous of Tamoxifen and have subsequently tested them for their autophagy inducing abilities, of which some display the desired autophagic activity comparable to Tamoxifen. We believe that the presence of an amioethoxy basic side chain in conjunction with a diaryl backbone were essential pharmacophores for the induction of autophagy as compounds lacking a basic side chain in their structures did not induce autophagy. Additionally, neither the antiestrogenic nor estrogenic activity is responsible for the autophagic effect of our drug candidates, as they did not bind to estrogen receptor. Tamoxifen was observed to be cytotoxic to cells at high doses (>5 μM) and this toxicity is not observed with our drug molecules. Based on the results of ours and others in the literature, we propose that our novel aminoethoxy difluorocyclopropanes may be AEBS inhibitors.

Chapter 5 Summary for Recent Development on AEBS/ChEH and Future Work

5.1 Recent development on AEBS/ChEH

5.1.1 Metabolites of 5,6 ECs

In addition to cholestane-3*β*,5*α*,6*β*-triol (CT), several other common metabolites of cholesterol-5,6-epoxides (5,6-ECs) have been identified and some display interesting pharmacological properties. 5,6-ECs have been reported to be sulfated into 5,6-EC-3*β*-sulfate by sulfatase SULTSB1b (Figure 51), with 5,6*α*-EC having higher activity than 5,6*β*-EC.[195] Interestingly, 5,6*α*-EC-3*β*-sulfate, but not 5,6*β*-EC-3*β*-sulfate, is an antagonist of alpha and beta liver receptors (LXR*α* and LXR*β*) and was reported to contribute to the induced cells differentiation and apoptosis in breast cancer cells by Tamoxifen.[196, 197]

Additionally, the 3*β*-hydroxyl group on ring A can be esterified by cholesterol acyltransferases to give 5,6-EC-3*β*-fatty acid ester in human sera (Figure 51). Besides CT and its metabolites, cholesteryl esters were also found to be associated with cancer development[196, 198] and these findings may have contributed to the impression that the 5,6-ECs were direct-acting mutagens.[185]

3*β*-5*α*-Dihydroxycholestan-6*β*-yl-*S*-glutathione (CDO-3*β*,5*α*-6*β*-*S*-GST) is another common metabolite of 5,6-ECs reported which is synthesized by the enzymes *S*-glutathione transferases (GSTs) in the rat liver (Figure 51).[199] It is not known whether the product of this addition reaction has any significant biological properties, but the primary role of GSTs is to detoxify electrophilic compounds by

catalyzing the conjugation of electrophilic substrates to glutathione,[200] thus the formation of CDO-3β,5α-6β-*S*-GST may offer a more water soluble product which could be more readily eliminated than 5,6-ECs with high lipid solubility.

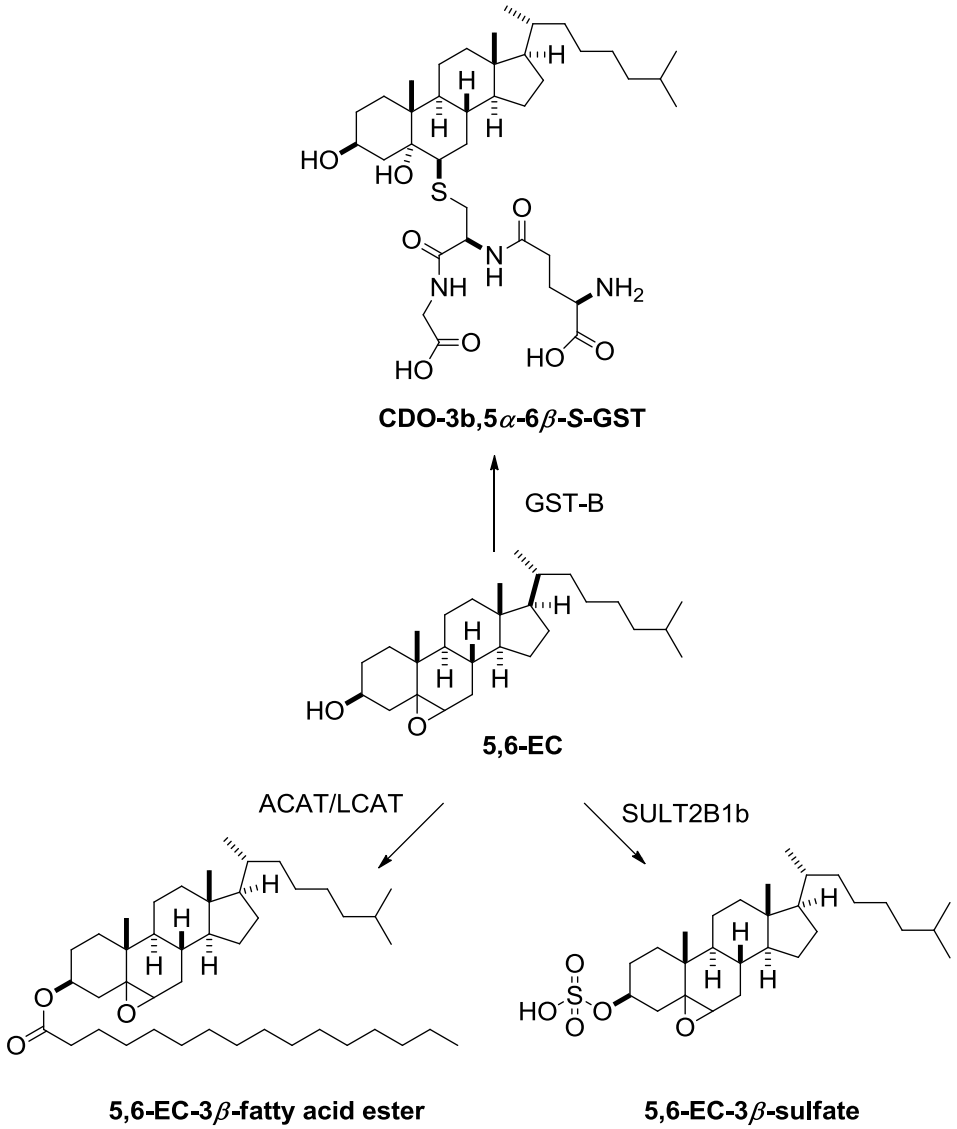


Figure 51: The known metabolite of 5,6-ECs

This figure is reproduced from Poirot *et al* [168]

5.1.2 The discovery of DDA and its pharmacological properties

Recently, Poirot *et al.* also reported the discovery of 5α-hydroxy-6β-[2-(1*H*-imidazol-4-yl)ethylamino]cholestan-3β-ol (DDA) in mammal tissues and normal

cells which is formed by enzyme catalysed conjugation of 5,6α-EC and histamine (His) (Figure 52).[170] DDA is a highly potent selective inhibitor of AEBS/ChEH, although the enzyme involved in the biosynthesis of DDA has yet to be identified.

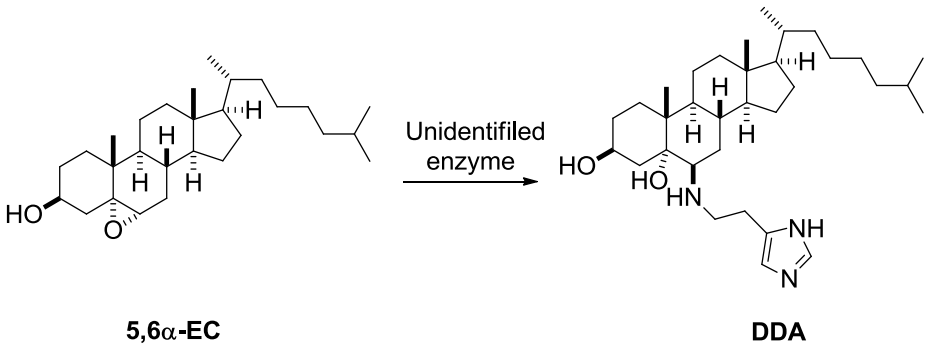


Figure 52: The structure of DDA

AEBS was earlier described by Brades *et al.* as having a histamine or histamine-like binding cavity. DPPE, a selective ligand of AEBS, was found to compete for the histamine binding site and its structure is very similar to the aminoethyl ether groups of antihistamines.[194, 201] Histamine binds to AEBS,[170] so do the antagonists of histamine receptors such as hydroxyzine and phenyltoloxamine (Figure 53), of which the former was demonstrated to have affinity for AEBS approximately equal to DPPE,[201] and the latter is effectively a regio-isomer of DPPE.

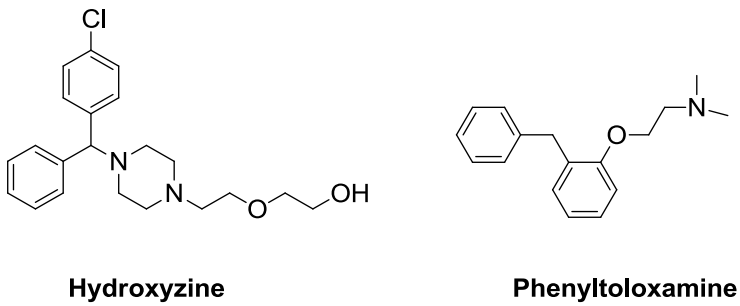
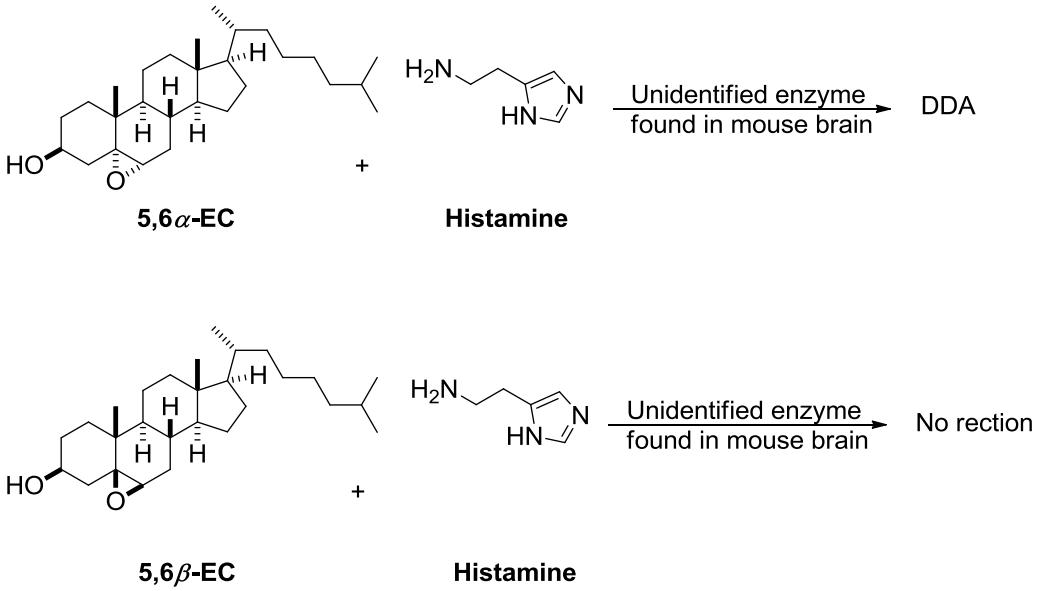


Figure 53: The structure of histamine antagonists: hydroxyzine and phenyltoloxamine.

Cholesterol epoxides are also competitive ligands of Tamoxifen binding to AEBS.[171] Therefore, DDA was first chemically synthesized by Poirot *et al.* based on the hypothesis that CEs and histamine may be concentrated at the AEBS binding site. He postulated that a ring-opening reaction of the electrophilic CEs by the nucleophilic histamine may be possible under proximity-induced catalysis due to the two substrates being spatially close to each other.[189, 202]

This hypothesis was found to be correct as DDA was detected in many mammal tissues at a concentration ranging from approximately 50 ng per gram of tissue for mouse brain to 300 ng per gram of tissues for mouse liver.[170] In addition, DDA was detected in human plasma and fetal bovin serum (FBS) in the several nM concentration ranges, implying that DDA was present in the circulation.[170] In comparison however, DDA was not detectable in all of the human or mouse tumour cells tested by Poirot *et al.* who suggested a possible down regulation of DDA in tumour cells may occur as opposed to normal cells.[170]

Poirot *et al.* then went on to demonstrate that DDA is synthesized through an unidentified enzyme which could be found in mouse brain, a tissue reported to be rich in AEBS.[203] They incubated 5,6α-EC and His with brain homogenate and observed the transformation of 5,6α-EC and His into DDA.[170] The presence of an active DDA synthase in the brain homogenate was crucial for the transformation and if the enzyme was denatured by boiling or treating the brain homogenate with pronase, no reaction would occur.[170] Furthermore, the DDA synthase seems to be highly specific for 5,6α-EC as 5,6β-EC was found to be inactive under similar treatment (Scheme 53).[170]



Scheme 53: Enzyme catalyzed formation of DDA

In *in vitro* studies, DDA was shown to induce tumour cell differentiation in P19, U937 and SK-Mel-28 cell lines at 1 μM, a concentration 5 to 20 fold lower than that of Tamoxifen, PBPE and all trans-retinoic acid (ATRA) respectively.[189] Additionally, DDA was demonstrated to have a high potency to kill tumour cells and was active against a range of different lines tested at the same micromolar concentration range.[189] In i*n vivo* studies, DDA also proved to be effective against cancers. Mice crafted with melanoma (B16F10) and mammary (TS/A) cells were treated with DDA and such treatments led to significant prohibition of tumour growth and improved animal survival rates of 40% (B16F10) and 60% (TS/A) at day 50 compared to none in the control groups.[170] The high anti-tumour activity of DDA demonstrated by *in vivo* studies suggests that DDA may have clinical importance in the treatment and prevention of cancers.

5.1.3 Other synthetic 5,6α-epoxysteroids reported

Along with DDA, Poirot *et al.* also synthesized a range of 5,6*α*-epoxysteroids from various natural amines and tested their biological activities. The most active compound among the series after DDA was DDB. Interestingly, DDA is a highly potent inhibitor of ChEH, but the regioisomer of DDA, C17 does not inhibit ChEH (Figure 54).[170] In addition, C17 was found to be only weakly cytotoxic and DDB was not toxic to cells up to 20 μM for 72 h, indicating the nature of the amine side chains play an important role in the biological properties of these cholesterol derivatives.[189]

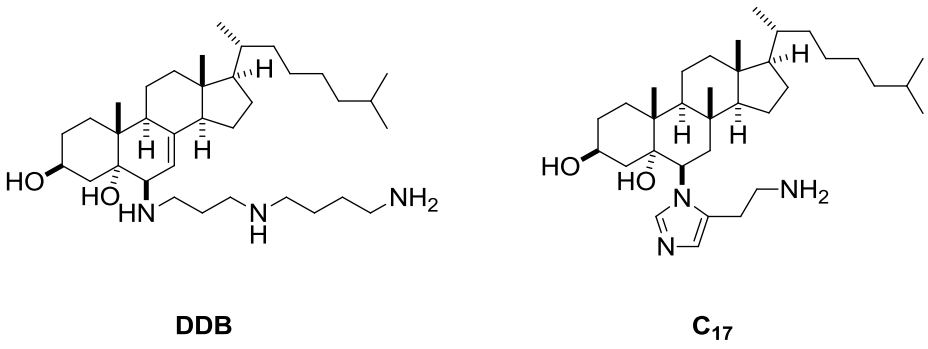


Figure 54: The structures of DDB and C_{17}

Although DDB was found not to be cytotoxic against tumour cells at low concentration, it was shown to induce dendrite outgrowth and cell differentiation of the P19 into neurons at as low as 100 nM concentration.[189] The ability of DDB to induce neutrite outgrowth was also tested in SH-SY5Y and U87 human cells as well as mouse Neuro2A, in all of which DDB was shown to induce comparable neurite outgrowth at a concentration approximately 20-fold and 400-fold lower than that of ATRA and docosahexaenoic acid (DHA), respectively.[189] These results demonstrate

the potential use of DDB as a neurotropin for the treatment of neurodegenerative disorders such as Alzheimer's and Parkinson's diseases.[189]

5.2 Summary for our understanding of Tamoxifen and AEBS/ChEH and the important findings in our project.

In various mice models, our collaborators, Grainger *et al.* have demonstrated that Tamoxifen inhibited atherosclerosis by suppressing the diet-induced formation of lipid lesions in the aorta.[48, 55] Tamoxifen is a potent inducer of autophagy and autophagy is believed to have beneficial effects for atherosclerosis and plague progression, possibly by degrading damaged intracellular organelles, thereby preventing oxidative injuries and cellular distresses.[57] However, Tamoxifen is a well-known multi-targeting drug and it is not clear which molecular targets of Tamoxifen is/are responsible for the induction of autophagy and the cardioprotective effects of Tamoxifen. In addition, the estrogenic activity of Tamoxifen in the endometrium cells is linked to an increased risk of ulcerous cancers.[19] Therefore, the development of more selective autophagy inducers is needed if they are ever to become medicinally useful in the treatment or prevention of cardiovascular diseases.

We started our project based on the extensive research and findings of Magarian *et al.* who had demonstrated that the dichlorocyclopropyl analogues of Tamoxifen were pure antiestrogens, with very interesting anti-cancer and anti-tubulin properties.[75-77, 79, 81-83, 149, 204]

A number of those diaryldichlorocyclopropanes had been synthesized in racemical form and as we attempted to develop a synthetic route for their asymmetric synthesis, we found out the diaryldichlorocyclopropanes were not particularly stable and degraded easily under normal handling conditions. As a result, we abandoned the asymmetric synthesis of diaryldichlorocyclopropane and moved on to try to synthesize the difluorocyclopropyl analogues instead since it was believed that the relative stronger C-F bond may offer greater stability.

Methods for the synthesis of *gem*-difluorocyclopropanes are extremely limited, with the vast majority of the reactions reported involving the [2+1] cycloaddition of difluorocarbene to alkenes.[86] Additionally, the difluorocarbene is the least reactive dihalocarbene and thus the synthesis of difluorocyclopropyl analogues of Tamoxifen from unreactive olefins, such as stilbenes, is particularly challenging. Having attempted to generate the difluorocarbene using various methods, we found that $TMSCF_3$ is probably one of the most effective difluorocarbene precursors reported to date. Not only does $TMSCF_3$ offer many advantages such as low toxicity, commercial availability and mild reaction conditions, but the catalyst NaI is also inexpensive, reliable and easy to handle. However, the difluorocarbene generated under the various reaction conditions reported by Hu *et al.*[205] is only of moderate reactivity and does not transform the electron-deficient diarylethenes to their prospective difluorocyclopropanes at all. In our laboratory, we have developed two alternative methods based on Hu *et al*.'s original finding which provides the desired diaryldifluorocyclopropanes without the use of highly toxic $Cd(CF_3)_2$, the only reagent ever reported to date to convert *cis*-stilbene to its difluorocyclopropane analogue. We discovered that microwave-assisted difluorocyclopropanation can

enhance the reactivity of the difluorocarbene generated using $TMSCF_3$ and allows rapid transformation of various olefins to their corresponding difluorocyclopropanes in just under 20 minutes. Although the conversion was not particularly impressive for stilbenes (~20%), it was very effective for more reactive and electron-rich olefins, such as styrene, with conversion as high as >95% (as determined by ^1H NMR spectroscopy of the crude reaction mixtures). We also accidently discovered that the iodine ion can attack $TMSCF_3$, giving $^-CF_3$ which subsequently decomposes to $:CF_2$ and reacts with alkenes slowly at room temperature. We observed that large amount of gases were produced, leading us to believe that $^-CF_3$ can deprotonate CH_3CN to give HCF_3 as a gaseous by-product as the reactions proceeded. This belief is supported by the findings of other researchers who reported the extremely basic nature of trifluoromethyl anion and its ability to abstract a proton from CH_3CN.[135] The loss of trifluoromethyl anions and formation of HCF_3 can be limited by performing the difluorocyclopropanation in sealed pressure tubes. Although slow, the room-temperature difluorocyclopropanation method is particularly suitable for unstable difluorocyclopropanes such as those derived from *cis*-stilbene in which the energy barrier for isomerisation is lower than that for difluorocyclopropanation. Any input of energy would cause the *cis*-stilbene and its analogues to isomerise to their *trans*-counterpart, making the stereoselective synthesis of diaryldifluorocyclopropanes very difficult to achieve at elevated temperatures. Both microwave-assisted and room-temperature difluorocyclopropanation have their own advantages and disadvantages and it would be interesting to try these methods on a wider range of alkenes and determine their efficacies and potential applications.

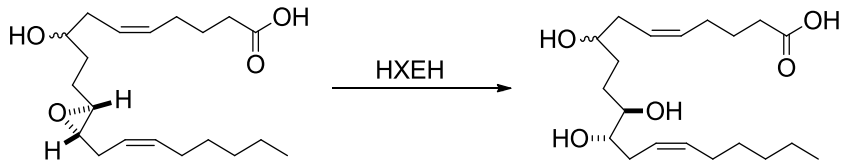
A number of derivatives of Tamoxifen have been synthesized and tested for this project, yet only compounds whose structures were similar to selective ligands of AEBS, such as DPPE and PBPE, induced autophagic responses. DPPE and PBPE have been demonstrated to induce autophagy potentially through disruption in cholesterol biosynthesis pathway and induction of sterol accumulation.[161] Therefore, it appears that our drug candidates are also selective ligands of AEBS and the autophagic effect of our compounds is as a result of inhibition of AEBS and cholesterol synthesis on the basis of high structural similarities between our 1,1-diaryldifluorocyclopropyl and 1,1-diarylmethylene compounds with those well-known AEBS ligands.

Furthermore, we found that the presence of an aminoethoxy basic side chain is crucial for induction of autophagic activities and this finding is consistent with the fact that a basic side chain is also important for binding to AEBS.[34] We were able to confirm that DPPE and PBPE were potent autophagic inducers in our independent study, although not analogues with a morpholine moiety attached to the aminoethoxy side chain. The morpholinyl derivatives MBPE should have also induced autophagic activity if they were high-affinity AEBS ligands as reported by Poirot *et al.*[191] and this inconsistency in our findings deserve further investigation. Moreover, our novel aminoethoxy difluorocyclopropanes were found to be consistently more active than any of the derivatives of diarylmethane in our biological assay. Most importantly, we have demonstrated that our drug candidates stimulate autophagic response through an estrogen receptor independent pathway as they do not bind to the ERs.

5.3 Other molecular targets of diphenylmethanes

In addition to cholesterol 5,6-epoxide hydrolase, a number of other mammal epoxide hydrolases and have also been identified and studied which includes hepoxilin epoxide hydrolase (HXEH), soluble epoxide hydrolase (sEH), microsomal epoxide hydrolase (mEH) and leukotriene-A4 hydrolase (LTA_4H).

HXEH catalyses the hydrolysis of hepoxilin A_3 to trioxilin A_3 (Figure 55). HXEH was first reported by Lee *et al.* who isolated the epoxide hydrolase from rat liver cytosol and described it as being different from other epoxide hydrolases based on its apparent molecular weight and specific activity for hepoxilin A_3 over other epoxides such as leukotriene A_4 and styrene oxide. However, a recent study showed that HXEH and sEH are actually the same enzyme.[206]



Hepoxilin A_3 **Trioxilin A_3**

Figure 55: The substrate and product of HXEH hydrolase

sEH is a bifunctional enzyme which carries out epoxide hydrolysis activity in the C-terminal domain and a phosphate ester hydrolysis activity in the *N*-terminal domain (Figure 56). sEH seems to play a major role in the *in vivo* metabolism of endogenous lipid epoxides. Many of these lipids are signaling molecules with various functions in physiological regulations such as cell proliferation, nociception, inflammation and control of blood pressure.[207]

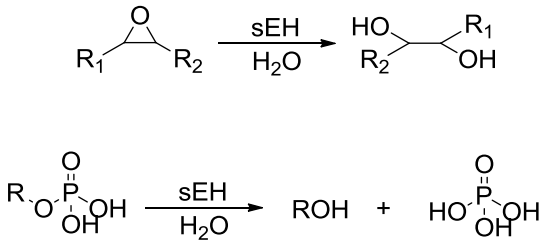


Figure 56: Reactions catalyzed by soluble epoxide hydrolase

sEH hydrolyzes epoxyeicosatrienoic acids (EETs) and to dihydroxyeicosatrienoic acids (DHETs) which are signaling lipids derived from arachidonic acid by the metabolic action of the Cytochrome P450 epoxygenase (Figure 57).[208] EETs exhibit vasorelaxant, anti-inflammatory and cardioprotective properties, but their hydrolytic metabolites, DHETs, are biologically less active.[209] Therefore, sEH is considered a potential therapeutic target for cardiovascular diseases. Indeed, the inhibition of sEH has been demonstrated to have anti-hypertensive and anti-inflammatory and cardioprotective effects.[209] In addition, sEH inhibition also leads to neuroprotection and pain reduction.[176]

Arachidonic acid is the precursor of the leukotrienes that is released from membranes by Cytosolic Phospholipase A2 (cPLA2), and further transformed by 5-lipoxygenase to give epoxide leukotriene A4 (LTA_4), Figure 58. LTA_4 is the substrate of LTA_4H which is a bifunctional enzyme that catalyzes not only the hydrolysis of epoxide LTA_4, but also aminopeptides. However, no natural peptide substrate has been identified yet and it is speculated that LTA_4H may hydrolyze peptides associated to inflammatory and host defense.[211, 212] In respect to its epoxide hydrolase activity, LTA_4H hydrolyses LTA_4 to the respective diol LTB_4 (Figure 58) which is a powerful pro-inflammatory mediator.[213]

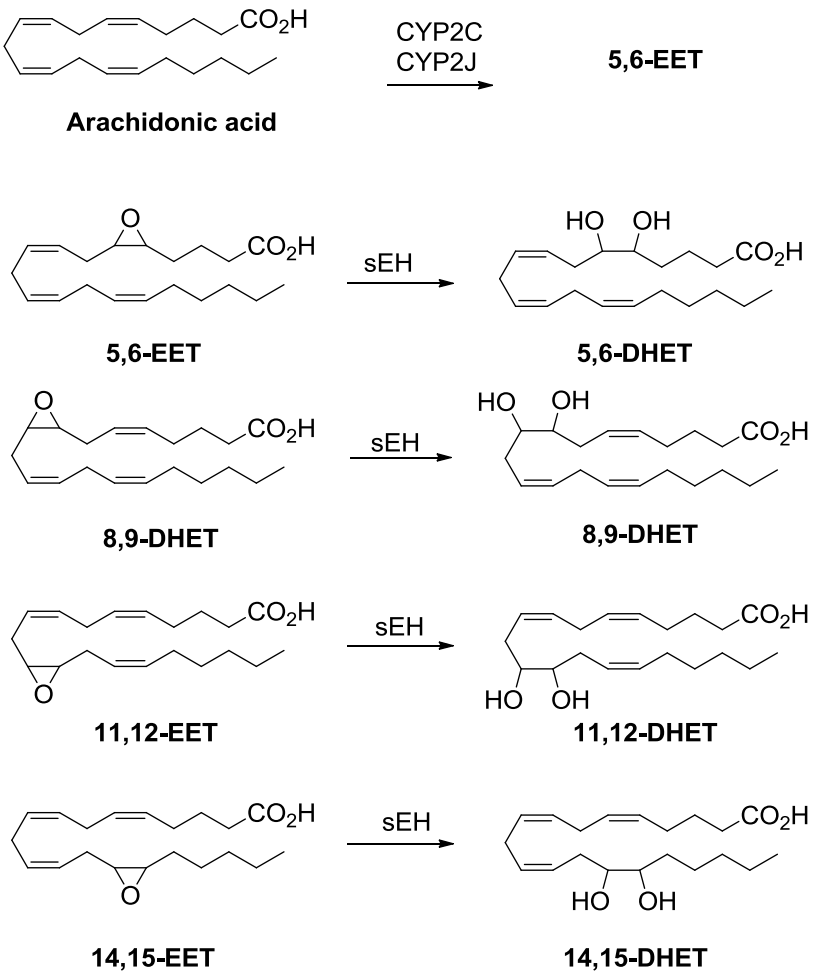


Figure 57: The metabolite of arachidoonic acid, EETs and DHETs

This figure is reproduced from the work of Imig.[210]

LTB_4 is a potent inducer of chemotaxis that induces recruitment and activation of neutrophils to areas of tissue damages, mediating inflammatory responses by binding to BLT1 and BLT2 which are G-protein-coupled receptors.[211] Genetic data in animals and humans have linked the LTB_4 to cardiovascular disease and variants in the LTA_4H gene have been associated with vulnerability to asthma.[214] Thus, compounds inhibiting LTA_4H are potentially beneficial in treatment of chronic, autoimmune-driven inflammatory diseases. Indeed, some selective LTA_4H inhibitors have been demonstrated to be effective in treating asthma, inflammatory bowel

disease and arthritis in various preclinical models, showing promising therapeutic effects and potential use for treating multiple inflammatory indications.[214]

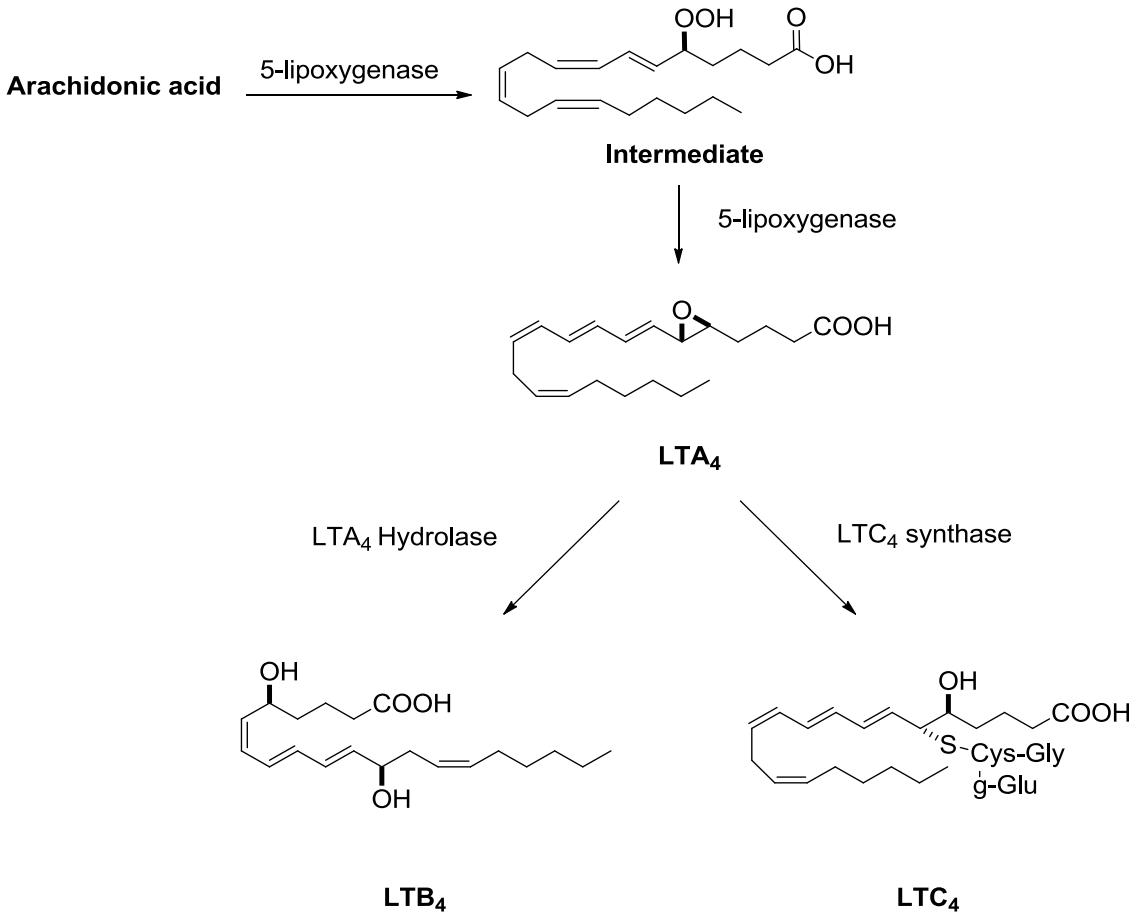


Figure 58: The formation and metabolic fate of LTA_4

Interestingly, many of the aminoethoxy-benzylphenols and aminoethoxy-phenoxyphenols synthesized and tested in our biological assays have also been demonstrated to be potent inhibitors of LTA_4H with IC_{50} in the micro- to nano-molar ranges.[213]

The microsomal epoxide hydrolase (mEH) catalyses the hydrolysis of a large number of structurally different, highly reactive xenobiotic epoxides, such as *cis*- and *trans*-stilbene oxide, butadiene monoxide and benzo[a]pyrene-4,5-oxide to vicinal diols (Figure 59). It is generally regarded as a key detoxifying enzyme since

most of the corresponding diols are less toxic or mutagenic than the epoxide substrates and hence its inhibition is considered as not desired.[176]

Interestingly, MBPE, another AEBS ligand reported by Poirot *et al.* (Figure 44), and Tamoxifen have been reported to inhibit the catalytic activity of mEH, and styrene oxide, a substrate of mEH, was found to be a competitive inhibitor of Tamoxifen binding to AEBS/ChEH.[215] Certain series of substrates of both epoxide hydrolases do share striking resemblances, in particular, the aromatic moiety in the diphenylmethyl series of AEBS/ChEH ligands and some classes of mEH ligands such as stilbene, benzophenone and chalcone.[215] Therefore, it is believed that the aromatic moiety of AEBS ligands may affect the catalytic activity of mEH.[215]

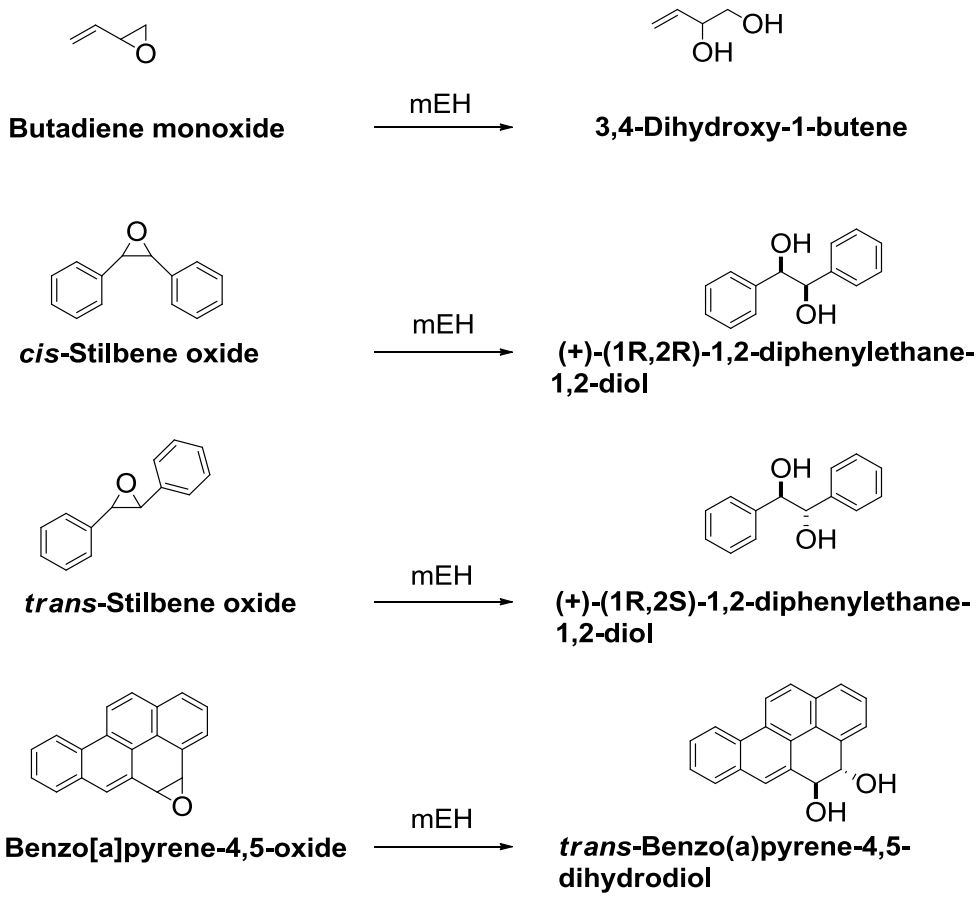


Figure 59: Model substrates and products of microsomal epoxide hydrolase

Tamoxifen has been shown to be a carcinogen in rat tissues by inducing the formation of DNA and protein adducts and this carcinogenicity of Tamoxifen may be related to the inhibition of mEH.[215] It has been speculated that Tamoxifen may inhibit the hydrolysis of its own metabolic epoxide derivatives or other toxic epoxide substrates of mEH.[215]

The fact that MBPE has been demonstrated to bind to mEH,[215] AEBS/ChEH[191] and LTA_4H[213] with various affinities, implies that these EHs may have similar structural characteristics to one other. However, while studies of amino acid sequences of mEH and sEH suggest these two enzymes are distantly related enzymes that have been derived from a common ancestral protein, the LTA_4H hydrolase is distinct.[216] In addition, from the single knockdown experiments of D8D7I and DHCR7, the subunits of AEBS/ChEH, it is believed that AEBS/ChEH is structurally unrelated to the mEH and sEH.[173]

5.4 Future work

Poirot *et al.* are aware of the fact that selective ligands of AEBS/ChEH may also bind to other epoxide hydrolases[215] and this could be the reason why DDA has been tested on both sEH and mEH.[217] In their studies, DDA was found to have no affinity for those epoxide hydrolases.[217] However, DDA is a derivative of 5,6-ECs and 5,6-ECs are the only known natural substrates of cholesterol epoxide hydrolase, hence it is not surprising that DDA binds to AEBS/ChEH with high affinity and specificity.

The aromatic moiety in diarylmethane ligands of AEBS/ChEH is thought to mimic the steroid backbones of 5,6-ECs,[218] but it is also thought to affect the catalytic

activities of mEH.[215] In addition, the diphenylmethane analogues of Tamoxifen have also been reported to bind to the LTA_4H with high potency.

It is not known whether our drug candidates are inhibitors of mEH or sEH or even AEBS/ChEH. We suggest that our drug candidates are inhibitors of AEBS/ChEH based on the large amount of well-performed scientific studies done on the ligands of AEBS/ChEH and the similarities in structures of our compounds with these ligands. In addition, it has been established in our project that our compounds do induce autophagy potently and in a dose-dependent manner. Selective ligands of AEBS/ChEH have also been reported to induce autophagic activities by others,[71, 161, 219] whereas we are not aware of any connection between inhibition of LTA_4H or mEH/sEH/HXEH and induction of autophagy. Moreover, we found that ligands without a basic side chain do not induce autophagy which is consistent with the report that the presence of a basic side chain is also crucial for binding to AEBS/ChEH.[34]

While the inhibition of LTA_4H may offer potential benefits in treating acute and chronic inflammatory diseases such as asthma and cardiovascular diseases, inhibiting the mEH could be extremely detrimental. Thus, further research is needed to find out whether our compounds are potent inhibitors of any other known epoxide hydrolases. This could be achieved by testing the relative binding affinities of our compounds for each epoxide hydrolase. Additionally, high-affinity and high-selectivity ligands of each epoxide hydrolase should also be tested for their ability to induce autophagic activities. Thereby, even if our compounds bind to one or more epoxide hydrolases, we may be able to determine which epoxide hydrolase(s) is (are)

responsible for the autophagic effects observed in our biological assays. If our compounds do bind to more than one epoxide hydrolases, further lead optimization is necessary for high selectivity.

Chapter 6 Experimental

6.1 General Experimental

^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance DPX 300, 400 and 500 MHz spectrometers. Chemical shifts are reported in parts per million (ppm), and coulpling constants (*J*) are given in Hertz to the nearest 0.5 Hz. Multiplicities are given as s = singlet, d = doublet, t = triple, q = quartet, m = multiplet, br = broad.

Infrared spectra were measured neat using a Perkin Elmer Spectrum 100 FT-IR machine.

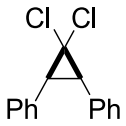
Melting points were obtained using a Stuart STMP10 apparatus and are uncorrected.

Optical rotations were measured on an optical activity AA 1000 polarimeter set at 589 nm. $[\alpha]_D$ values are expressed in units of 10^{-1} degree cm^2 g^{-1}.

High resolution mass spectra (HRMS) data were obtained with the help of Dr Lijang Song and Mr Philip Aston on a Bruker micro-TOF ESI spectrometer.

Typical procedure for the synthesis of *gem*-dichloro-diphenyl/triphenylcyclopropanes

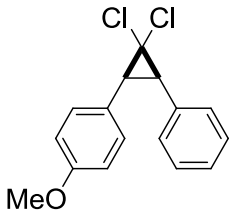
***cis*-1,1-Dichloro-2,3-diphenylcylcopropane (1)**



To a solution of *cis*-stilbene (0.42 g, 2.3 mmol, 1.0 equiv.) in chloroform (4.7 mL, 58 mmol, 25 equiv.) cooled on an ice bath was added benzyltrimethylammonium bromide (12 mg, 52 µmol, ~2 mol%) with stirring. A solution of 50% aqueous NaOH (2.3 mL, 29 mmol, 12.5 equiv.) was then added dropwise under rapid stirring. The reaction mixture was further stirred at room temperature overnight before the solvent was removed in *vacuo* and water was added. The aqueous layer was extracted with diethyl ether (3 x 15 mL), dried over $MgSO_4$, filtered and concentrated to give an oil. The product was purified by standard silica chromatography (eluent: 9:1 hexane:CH_2Cl_2) to give 0.54 g (88%) of **1** as a very pale-yellow oil which solidified upon storage at 0 ºC, mp 49 – 51 ºC (lit.,[75] mp 51 – 52 ºC); IR (neat) υ_{max}/cm^{-1} 3061-3031 (C-H), 1603 (C=C), 1497, 805, 752, 695 (Ar-H); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.35-7.13 (6H, m, Ar-*H*), 7.05-7.00 (4H, m, Ar-*H*), 3.30 (2H, s, C*H*CCl_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 132.1 (*ipso* Ar-*C*), 131.0, 127.9, 127.4 (Ar-C), 65.3 (*C*Cl_2), 39.4 (*C*HCCl_2). Mass spectrometric data were not obtainable via ESI mass spectrometry.

This compound is known and has previously been reported with ^{1}H NMR spectroscopic data consistent with those reported here.[75]

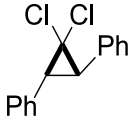
(±)-*cis*-1,1-Dichloro-2-(4-methoxyphenyl)-3-phenylcyclopropane (2)



Prepared in a similar manner to compound **1**, the reaction of *cis*-4-methoxystilbene (0.51 g, 2.4 mmol, 1.0 equiv.) with $CHCl_3$ (4.9 mL, 60 mmol, 25 equiv.) and a solution of 50% aqueous NaOH (2.4 mL, 30 mmol, 12.5 equiv.) in the presence of benzyltrimethylammonium bromide (13 mg, 56 µmol, 2 mol%) gave 0.39 g (55%) of **2** as a yellow oil which solidified upon storage at 0 ºC, mp 56 – 58 ºC (lit.,[220] mp 59 – 60 ºC); IR (neat) υ_{max}/cm^{-1} 3032 – 2836 (C-H), 1611 (C=C), 1512, 802, 739, 697 (Ar-H), 1246 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.29-7.16 (3H, m, Ar-*H*), 7.08-6.98 (2H, m, Ar-*H*), 6.96-6.89 (2H, m, Ar-*H*) , 6.79-6.73 (2H, m, Ar-*H*), 3.75 (3H, s, O*CH*$_3$), 3.24 (2H, s, C*H*CCl_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 158.9 (Ar*C*OMe), 132.1, 123.9 (*ipso* Ar-*C*), 132.0, 131.0, 128.0, 127.3, 113.4, (Ar-*C*H), 65.5 (*C*Cl_2), 55.3 (O*C*H_3), 39.2, 38.9 (*C*HCCl_2).

This compound is known and has previously been reported with ^{1}H NMR spectroscopic data consistent with those reported here.[220]

(±)-*trans*-1,1-Dichloro-2,3-diphenylcylcopropane (3)

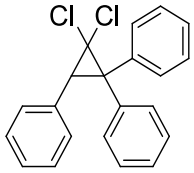


Prepared in a similar manner to compound **1**, the reaction of *trans*-stilbene (0.32 g, 1.8 mmol, 1.0 equiv.) with $CHCl_3$ (3.6 mL, 45 mmol, 25 equiv.) and a solution of

50% aqueous NaOH (1.8 mL, 23 mmol, 12.5 equiv.) in the presence of benzyltrimethylammonium bromide (9 mg, 39 µmol, ~2 mol%) gave 0.39 g (69%) of **3** as a yellow oil which solidified upon standing for overnight, mp 38 – 40 ºC, (lit.,[221] mp 39 – 40 ºC); IR (neat) υ_{max}/cm^{-1} 3062-3031 (C-H), 1497 (Ar); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.43-7.26 (10H, m, Ar-*H*), 3.25 (2H, s, C*H*CCl_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 134.6 (*ipso* Ar-*C*), 129.0, 128.6, 127.9 (Ar-*C*H), 65.4 (*C*Cl_2), 39.8 (*C*HCCl_2). Mass spectrometric data were not obtainable via ESI mass spectrometry.

These data were consistent with those previously reported.[221]

(±)-1,1-Dichloro-2,2-diphenyl-3-phenylcylcopropane (4)

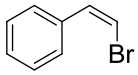


Prepared in a similar manner to compound **1**, the reaction of triphenylethylene (0.51 g, 2.0 mmol, 1.0 equiv.) with $CHCl_3$ (4.0 mL, 40 mmol, 25 equiv.) and a solution of 50% aqueous NaOH (2.0 mL, 25 mmol, 12.5 equiv.) in the presence of benzyltrimethylammonium bromide (9 mg, 39 µmol, 2 mol%) gave 0.55 g (81%) of **4** as a pale-yellow solid, mp 102 - 103 ºC, (lit.,[222] mp 105 – 107 ºC); IR (neat) υ_{max}/cm^{-1} 3062-3030 (C-H), 1601 (C=C), 1493, 784-746, 694 (Ar-H); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.45-7.43 (2H, m, Ar-*H*), 7.24-7.09 (11H, m, Ar-*H*), 6.92-6.90 (2H, m, Ar-*H*), 3.48 (1H, s, C*H*CCl_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 143.6, 136.5, 133.4 (*ipso*, Ar-*C*) 131.3, 130.9, 128.8, 128.8, 128.3, 127.8, 127.5, 127.3 (Ar-*C*H), 69.9 (*C*Cl_2), 49.2 (Ph_2*C*CCl_2CH), 46.0 (Ph_2CCCl_2*C*H).

Mass spectrometric data were not obtainable via ESI mass spectrometry.

This compound is known and has previously been reported with ^{1}H NMR spectroscopic data consistent with those reported here.[222]

***cis*-*β*-Bromosytrene (6)**

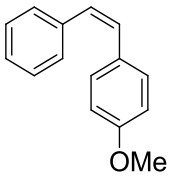


Cinnamic acid (7.53 g, 50 mmol, 1.0 equiv.) was dissolved in chloroform (70 mL), and a solution of bromine (3.0 mL, 0.59 mmol, 1.2 equiv.) in chloroform (5 mL) was added dropwise with stirring. The reaction mixture was stirred overnight before the solvent was evaporated to dryness. To the residues, acetone (90 mL) and K_2CO_3 (10.83 g, 78 mmol, 1.5 equiv.) were added and the reaction mixture was heated at reflux for four hours. After cooling to room temperature, the solvent was removed in *vacuo* and water (30 mL) was added. The product was extracted with diethyl ether (3 x 20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude material was purified by standard silica chromatography using hexane to give 6.57 g (71%) of **6** as a pale-yellow oil; IR (neat) υ_{max}/cm^{-1} 3025 (C-H), 1613 (C=C), 1490, 690 (Ar-H), 678 (C-Br); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.65 (2H, d, *J* = 7.0, *ortho* Ar-*H*), 7.35-7.27 (3H, m, *meta*, *para* Ar-*H*), 6.99 (1H, d, *J* = 8.0, C*H*=CH), 6.36 (1H, d, *J* = 8.0, C*H*=CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 135.1 (*ipso* Ar-*C*), 132.6 (*C*H=CHBr), 129.2, 128.5, 128.4 (Ar-*C*H), 106.6 (CH=*C*HBr);

Mass spectrometric data were not obtainable via ESI mass spectrometry.

These data were consistent with those previously reported.[223]

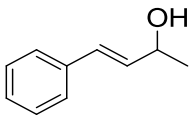
***cis*-4-Methoxystilbene (7)**



cis-β-Bromosytrene **(6)** (1.15 g, 6.3 mmol, 1.0 equiv.) and KOH aq. (3.0 M, 6.3 mL, 19 mmol, 3.0 equiv.) was introduced to a two neck round bottom flask and the solution was degassed for 15 minutes before 4-methoxybenzene boronic acid (1.23 g, 8.1 mmol, 1.3 equiv.) and $Pd(Ph_3)_4$ (0.42 g, 0.36 mmol, 6 mol%) were subsequently added under a nitrogen atmosphere. The reaction mixture was heated at 100 °C for 36 hours in the dark. The solvent was evaporated before the reaction mixture was diluted with water (20 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL), dried over $MgSO_4$ and concentrated to give a brownish residue. The residue was purified by standard silica chromatography using 99:1 hexane:EtOAc to give 0.64 g (49%) of **7** as a light orange oil; IR (neat) υ_{max}/cm^{-1} 3006 (C-H), 1605 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.40 - 7.15 (7H, m, Ar-*H*), 6.94 – 6.70 (2H, m, Ar-*H*), 6.56 (2H, s, C*H*=C*H*), 3.80 (3H, s, OC*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 158.2 (*C*OMe), 137.1 (*ipso* Ar-*C*), 129.6, 129.2, (Ar-*C* or *C*H=CH) 129.1 (*ipso* Ar-*C*), 128.3, 128.2, 127.7, 126.4, 113.0 (Ar-*C* or *C*H=CH), 54.6 (O*C*H$_3$); HRMS (+ESI) *m/z* for ($C_{15}H_{15}O$) [M + H] calculated 211.1117 found 211.1115.

These data were consistent with those previously reported.[224]

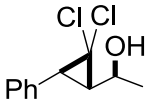
(±)-*trans*-4-Phenyl-3-buten-2-ol (12)



trans-4-Phenyl-3-buten-2-one (1.49 g, 10 mmol, 10 eq,) and cerium (III) chloride heptahydrate (0.38 g, 1 mmol, 1 equiv.) were dissolved in methanol (10 mL) cooled in an ice bath and $NaBH_4$ (0.57 g, 1.5 mmol, 15 equiv.) was added portionwise with stirring. The reaction mixture was further stirred at 0 ºC for 2 hours. After that, the solvent was evaporated and water (30 mL) was added. The aqueous layer was extracted with ethyl acetate (3 x 20 mL), dried over $MgSO_4$, filtered and concentrated to give 1.24 g (83%) of **12** as a pale-yellow oil; IR (neat) υ_{max}/cm^{-1} 3334 (O-H), 3084-3027 (C-H), 1598, (C=C), 1493, 745.61, 691 (Ar-H); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.31-7.15 (5H, m, Ar-*H*), 6.48 (1 H, d, *J* = 16.0, C*H*-Ar), 6.19 (1 H, dd, *J* = 16.0, 6.5, C*H*CHOHCH$_3$), 4.40 (1 H, qd, *J* = 6.5, 1.0, C*H*OHCH$_3$), 3.32 (1 H, s, O*H*), 1.31 (3 H, d, *J* = 6.5, C*H*$_3$); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 136.7 (*ipso* Ar-*C*), 133.6, 129.0, 128.5, 127.4, 126.4 (Ar-*C*H and H*C*=*C*H), 68.5 (*C*OHCH$_3$), 23.3 (*C*H$_3$); HRMS (+ESI) *m/*z for ($C_{10}H_{12}NaO$) [M+Na$^+$] calculated 171.0780 found 171.0780.

These data were consistent with those previously reported.[225]

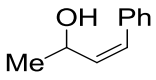
(1*S*, 1'*S*, 3'*S*)-(±)-1-2,2-Dichloro-3-phenylcyclopropylethanol (13)



To a solution of *trans*-4-phenyl-3-buten-2-ol **(12**) (0.21 g, 1.4 mmol, 1.0 equiv.) in chloroform (2.8 mL, 34.7 mmol, 25 equiv.) cooled on an ice bath was added benzyltrimethylammonium bromide (7 mg, 50 µmol, 2 mol%) with rapid stirring. A solution of 50% aqueous NaOH (1.4 mL, 26.7 mmol, 19.1 equiv.) was then added dropwise over a period of 10 minutes. The reaction mixture was further stirred for 2

hours before being diluted with water and extracted 3 times with diethyl ether. The combined organic extracts were dried over $MgSO_4$ and concentrated to give a residue. The residue contained a mixture of diastereoisomers with a *dr* of ~5:1 as determined by ^{1}H NMR spectroscopy. The major diastereoisomer was successfully isolated by standard silica chromatography using 99:1 hexane: ethyl acetate to give 0.16 g (49%) of **13** as a pale-yellow oil; IR (neat) υ_{max}/cm^{-1} 3330 (O-H), 3028 (C-H), 1601 (C=C), 1493, 746, 694 (Ar-H), 1076 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.40 – 7.27 (3H, m, *meta* and *para* Ar-*H*), 7.23 (2H, d, *J* = 7.5, *ortho* Ar-*H*), 3.83 (1H, dq, *J* = 8.5, 6.5 C*H*OH), 2.61 (1H, d, *J* = 8.5, Ar-C*H*CCl_2 or O*H*), 2.59 (1H, d, *J* = 8.5, Ar-C*H*CCl_2 or O*H*), 2.10 (1H, t, *J* = 8.5, C*H*CHOHCH_3), 1.44 (3H, d, *J* = 6.5 *C*H_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 134.1 (*ipso* Ar-*C*), 129.0, 128.7, 128.1 (Ar-*C*H), 70.0 (*C*-OH), 64.8 (*C*Cl_2), 42.1, 39.6 (*CHC*HCCl_2), 21.8 (*C*H_3); HRMS (+ESI) *m/*z for ($C_{11}H_{12}Cl_2NaO$) [M+Na^+], calculated 253.0157 found 253.0159.

(±)-*cis*-4-Phenyl-3-buten-2-ol (15)

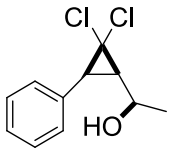


A round bottom flask was charged with hexane (1 mL), 4-phenyl-3-butyn-2-ol (0.5 g, 3.4 mmol), ~5% palladium on calcium carbonate (0.017 g) and quinoline (3 drops). The air inside the round bottom was evacuated and hydrogen was admitted via a balloon. The reaction mixture was stirred at room temperature for 3 hours before filtered through a pad of celite and concentrated to give a residue. The crude material was purified by silica chromatograph to give 0.23 g (44%) of **15** as a colourless oil; IR (neat) υ_{max}/cm^{-1} 3332 (O-H), 3057-2928 (C-H) 1600 (C=C), 1494, 767, 697 (aromatic C-H), 1049 (C-O), 1050 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.39-7.31 (2H, m, Ar-*H*), 7.31-7.23 (3H, m, Ar-*H*), 6.50 (1H, d, *J* = 11.5 Ar-

C*H*=CH), 5.69 (1H, dd, *J* = 11.5, 9.0 Ar-CH=C*H*), 4.79 (1H, dq, *J* = 9.0, 6.0, C*H*OH), 1.57 (1H, s, O*H*), 1.36 (3H, d, *J* = 6.0, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 136.9 (*ipso* Ar-*C*), 136.1, 129.8, 129.0, 128.5, 127.3 (Ar-*C*H and Ar-*C*H=*C*H), 64.2 (*C*HOHCH$_3$), 23.7 (*C*H$_3$); HRMS (+ESI) *m/*z for ($C_{10}H_{12}NaO$) [M+Na$^+$], calculated 171.0780 found 171.0776.

These data were consistent with those previously reported. [226]

(1*R*, 1'*R*, 3'*S*)-(±)-1-2,2-Dichloro-3-phenylcyclopropylethanol (16)



Prepared in a similar manner to compound **13**.

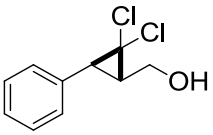
Method A: (±)-*cis*-4-Phenyl-3-buten-2-ol (**15**) (0.52 g, 3.5 mmol, 1.0 equiv.) was allowed to react with chloroform (7.0 mL, 87.1 mmol, 25 equiv.) and a solution of 50% aqueous NaOH (3.49 mL, 50 mmol, 19.1 equiv.) for 2 hours to give a residue that seemed to only contain one diastereoisomer (>95:5) as determined by ^{1}H NMR spectroscopy. The crude product was purified by silica chromatograph (eluent: 98:2 hexane:EtOAc) to give 0.22 g (28%) of **16** as a very pale-yellow oil; IR (neat) υ_{max}/cm^{-1} 3397 (O-H), 2978 (C-H), 1603 (C=C), 1497, 730, 694 (Ar-H), 1075 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.33 – 7.15 (5H, m, Ar-*H*), 3.62 (1H, dq, *J* = 10, 6.0, C*H*OH), 2.95 (1H, d, *J* = 11.5, Ar-C*H*CCl_2), 2.12 (1H, s, O*H*), 2.06 (1H, dd, *J* = 11.5, 10, C*H*CHOHCH$_3$), 1.37 (3H, d, *J* = 6.0, *CH*$_3$); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 132.9 (*ipso* Ar-*C*), 123.0, 128.7, 127.8 (Ar-*C*H), 66.2 (*C*-OH), 63.7 (*C*-OH), 42.3 (*C*HCHOH), 38.2 (Ar-*C*HCCl_2), 22.4 (*C*H$_3$); HRMS (+ESI) *m/*z for ($C_{11}H_{12}Cl_2NaO$) [M+Na$^+$], calculated 253.0157 found 253.0158.

Method B: *cis*-4-Phenyl-3-buten-2-ol (**15**) (0.3 g, 2.0 mmol, 1.0 equiv.) was protected with dihyropyran (0.28 mL, 3 mmol, 1.5 equiv.) by stirring with a little toluene *p*-toluenesulfonic acid in diethyl ether (4 mL) at room temperature for overnight. The solvent was evaporated *in vacuo* and the reaction mixture was washed with $NaHCO_3$ (15 mL). The residue was dissolved in chloroform (4.0 mL, 50.0 mmol, 25.0 equiv.) and benzyltrimethyl ammonium bromide (10 mg, 40 µmol, 2 mol%.) were added with rapid stirring, followed by adding a solution of 50% aqueous NaOH (2.0 mL, 25.0 mmol, 12.5 equiv.) dropwise over a period of 10 minutes. The reaction mixture was further stirred at room temperature for 2 days before diluted with water (15 mL) and extracted with diethyl ether (3 x 10 mL), dried over $MgSO_4$ and concentrated to afford an oil. The oil was dissolved in a solution containing a little of *p*-toluenesulfonic acid in methanol (4 mL) and stirred for overnight. After that, the reaction mixture was treated with sat. $NaHCO_3$ (10 mL) and the solvent was evaporated *in vacuo* before water was added (20 mL). The organic material was extracted with diethyl ether (3 x 15 mL), dried over Na_2SO_4 and filtered to afford a crude oil which contained a mixture of diastereoisomers with *dr* of ~1.5:1 as determined by ^{1}H NMR spectroscopy. The two diastereoisomers was subsequently separated by silica chromatograph to give 0.13 g (29%) yield of the major isomer and 0.05 g (12%) of the minor isomer, with a combined yield of 41%. The spectrometry of the major diastereoisomer is consistent to the data of the product isolated using **method A.**

Minor isomer: IR (neat) υ_{max}/cm^{-1} 3369 (O-H), 3061-2929 (C-H), 1604, 1498, 730, 698 (aromatic C-H), 1075 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.50 – 7.40 (2H, m, *ortho* Ar-*H*), 7.31 – 7.10 (3H, m, *meta*, *para* Ar-*H*), 3.48 (2H, ddq, *J* = 10.0, 6.0, 5.0, C*H*OH), 2.92 (1H, d, *J* = 11.0, Ar-C*H*CCl_2), 1.94 (1H, dd, *J* = 11.0, 10.0,

$CCl_2CHCHOH$), 1.58 (1H, d, *J* = 5.0, O*H*), 1.38 (3H, d, *J* = 6.0, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 133.0 (*ipso* Ar-*C*), 130.5, 128.6, 127.6 (Ar-*C*), 65.8 (*C*-OH), 62.1 (CCl_2), 41.1, 37.5 ($CHCHCCl_2$), 23.4 (CH_3); HRMS (+ESI) *m/*z for ($C_{11}H_{12}C_{l2}NaO$) [$M+Na^+$], calculated 253.0157 found 253.0159.

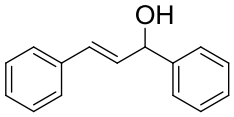
(1*S*, 3*S*)-(±)-2,2-Dichloro-3-phenylcyclopropylmethanol (23)



Prepared in a similar manner to compound **13** using method B, cinnamyl alcohol (0.98 g 7.3 mmol 1.0 equiv.) protected with 3,4-dihydro-2H-pyran (1 mL, 11 mmol, 1.5 equiv.) by stirring with a little (+) camphorsulfonic acid in diethyl ether (15 mL) at room temperature for overnight. The solvent was removed *in vacuo* and the reaction mixture was washed with $NaHCO_3$ (20 mL). The residue was dissolved in chloroform (15 mL, 125 mmol, 17.0 equiv.) and benzyltrimethyl ammonium bromide (84 mg, 0.36 mmol, 5 mol%.) were added with rapid stirring, followed by adding a solution of 50% aqueous NaOH (5.8 mL, 75 mmol, 10 equiv.) dropwise over a period of 10 minutes. The reaction mixture was further stirred at room temperature for 2 days before diluted with water (50 mL) and extracted with CH_2Cl_2 (3 x 25 mL). The organic extracts were combined, washed with water (2 x 25 mL) and brine (1 x 20 mL), dried over $MgSO_4$ and concentrated to afford a pale-yellow oil. The oil was dissolved in a solution containing a little of *p*-toluenesulfonic acid in methanol (15 mL) and stirred for overnight. After that, the reaction mixture was treated with sat. $NaHCO_3$ (10 mL) and the solvent was evaporated *in vacuo* before water was added (20 mL). The organic material was extracted with diethyl ether (3 x 10 mL), dried over $MgSO_4$, filtered and concentrated to give a crude oil. The product

was purified by standard silica chromatography using 95:5 hexane:EtOAc to give 0.50 g (32%) of **21** as a very pale-yellow oil; IR (neat) υ_{max}/cm^{-1} 3330 (O-H), 3032 (C-H), 1603 (C=C), 1047 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.39 – 7.27 (3H, m, *meta*, *para* Ar-*H*), 7.28-7.20 (2H, m, *ortho* Ar-*H*), 4.06 (1H, dd, *J* = 12.0, 5.5, C*H*$_2$OH), 3.85 (1H, dd, *J* = 12.0, 8.5, C*H*$_2$OH), 2.66 (1H, d, *J* = 8.5, Ar-C*H*CCl_2), 2.28 (1H, td, *J* = 8.5, 5.5, C*H*CH_2OH), 2.17 (1H, s, O*H*); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 134.1 (*ipso* Ar-*C*), 129.0, 128.6, 128.0 (Ar-*C*H), 64.7 (*C*Cl_2), 63.0 (*C*H_2OH), 39.2, 36.4 (*C*H*C*HCCl_2); HRMS (+ESI) *m/*z for ($C_{10}H_{12}C_{l2}NaO$) [M+Na^+], calculated 239.0001 found 238.9997.

(±)-*trans*-1,3-Diphenylprop-2-en-1-ol (27)

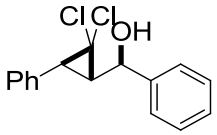


Prepared in a similar manner to compound **12**, *trans*-chalcone (10.41 g, 50 mmol, 1.0 equiv.) and cerium (III) chloride heptahydrate (1.87 g, 5 mmol, 1 equiv.) were dissolved in methanol (100 mL) cooled in an ice bath and $NaBH_4$ (2.85 g, 75 mmol, 15 equiv.) was added portionwise with stirring. After the reaction was completed, the solvent was evaporated *in vacuo* and water (100 mL) was added. The aqueous layer was extracted with ethyl acetate (3 x 50 mL), dried over $MgSO_4$, filtered and concentrated to give a solid. The crude product was purified by silica chromatograph to give 10.41 g (69%) of **27** as a white solid. mp 55 – 56 ºC, (lit.,[227] mp 58 - 59 ºC); IR (neat) υ_{max}/cm^{-1} 3342 (br OH) 3027 (C-H), 1598 (C=H), 1491, 1447, 964, 743 (Ar-H), 1011 (O-H); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.57 – 7.28 (10H, m, Ar-*H*), 6.74 (1H, d, *J* = 16, Ar-C*H*=CH), 6.45 (1H, dd, *J* = 16, 6.5 Ar-CH=C*H*), 5.42 (1H, dd, *J* = 6.5, 3.5, C*H*OH), 2.52 (1H, d, *J* = 3.5 CHO*H*); ^{13}C NMR (101 MHz,

$CDCl_3$) δ ppm 143.1, 136.8 (*ipso* Ar-*C*), 131.8, 130.8, 128.9, 128.8, 128.1, 126.9, 126.7 (Ar-*C*H and *C*H=*C*H), 75.3 (*C*HOH); HRMS (+ESI) *m*/*z* for ($C_{15}H_{14}NaO$) [M+Na^+] calculated 233.0937 found 233.0942.

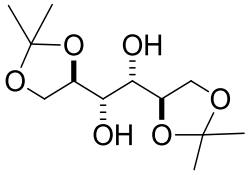
These data were consistent with those previously reported.[227]

(1*R*, 1'*S*, 3'*S*)-(±)-1-(2,2-Dichloro-3-phenylcyclopropylbenzenemethanol (28)



Prepared in a similar manner to compound **13** using method A, the reaction of *trans*-1,3-diphenyl-2-propen-1-ol with CCl_2 derived from $CHCl_3$/NaOH gave a mixture of diastereoisomers with a *dr* of ~6.1 : 1. The major diastereoisomer was successfully isolated by standard silica chromatography using 1:99 EtOAc:hexane as eluent to give **28** as a pale-yellow oil (0.12 g, 39% yield). Upon standing, the product solidified, mp 94 - 95 ºC; IR (neat) υ_{max}/cm^{-1} 3296 (O-H), 2995-2925 (C-H), 1600 (C=C), 1050 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.51 (2H, d, *J* = 7.0, Ar-*H*), 7.47-7.26 (6H, m, Ar-*H*), 7.14-7.05 (2H, m, Ar-*H*), 4.74 (1 H, dd, *J* = 9.0, 3.0, C*H*OH), 2.86 (1H, d, *J* = 8.5, Ar-C*H*), 2.54 (1H, d, *J* = 3.0, OH), 2.42 (1H, t, *J* = 9.0, C*H*CHOH); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 141.3, 133.8 (*ipso*, Ar-*C*), 129.0, 128.9, 128.5, 128.5, 127.9, 126.1 (Ar-*C*), 75.8 (*C*HOH), 42.1, 39.8 (*C*HCCl_2); HRMS (+ESI) *m*/*z* for ($C_{16}H_{14}Cl_2NaO$) [M+Na^+] calculated 315.0310 found 315.0314.

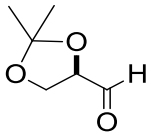
1,2:5,6-Diisopropylidene-*D*-mannitol (31)



2,2-Dimethoxypropane (56 mL, 455 mmol, 2.1 equiv.) was added to a suspension of *D*-mannitol (40.0 g, 219 mmol, 1.0 equiv.) in dry DMF (200 mL). The mixture was stirred well before *p*-toluene-sulfonic acid monohydrate (0.52 g, 2.6 mmol, ~0.6 mol%) was added and the slurry was stirred overnight at room temperature. The reaction mixture was quenched with triethylamine (1.68 mL) and then diluted with EtOAc (1.5 L). The organic layer was washed repeatedly with water (8 x 100 mL). The organic layer was dried over $MgSO_4$ and concentrated to give a residue. Upon standing, a white solid was formed. The solid was suspended in hexane (200 mL) and stirred well for 1 h. The suspension was filtered, the white solid collected and dried to give 25 g (44%) of **31** mp 116-118 ºC, (lit.,[228] mp 58 - 59 ºC); $[\alpha]_D^{28}$ = + 8.3 (c = 1.03, $CHCl_3$); IR (neat) υ_{max}/cm^{-1} 3307 (O-H), 2982 (C-H), 1203 (C-O ether), 1062 (C-O alcohol); ^1H NMR (300 MHz, $CDCl_3$) δ ppm 4.09-4.21 (4H, m, CH_2CHOR and CH_2CHOR), 3.92-3.97 (2H, m, CH_2CHOR), 3.74 (2H, t, *J* = 6.5, *CH*OH), 2.68 (2H, d, *J* = 7.0, O*H*), 1.41 (6H, s, CH_3) 1.35 (6H, s, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 109.5 (CMe_2), 76.4 (CH_2CHOR, 71.3 (*C*HOH), 66.9 (CH_2CHOR), 26.9, 25.3 (CH_3); HRMS (+ESI) *m*/*z* for ($C_{12}H_{22}NaO_6$) [$M+Na^+$] calculated 285.1309 found 285.1315.

These data were consistent with those previously reported.[228]

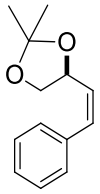
(*R*)-(+)-2,2-Dimethyl-1,3-dioxolane-4-carboxaldehyde (32)



To a solution containing 1,2:5,6-diisopropylidene-D-mannitol (1.4 g, 5.3 mmol, 1.0 equiv.) in CH_2Cl_2 (10 mL) was added sat. $NaHCO_3$ (0.85 mL), $NaIO_4$ (2.25 g, 10.5

mmol, 2.0 equiv.) was then added portion wise over a period of 30 minutes. The resulting suspension was stirred vigorously for 3 hours before $MgSO_4$ was added, and the reaction mixture was filtered and concentrated to give **32** as a colorless which was not purified and used immediately for the next step.

(4*S*)-(-)-2,2-Dimethyl-4-[(*Z*)-phenylethenyl]-1,3-dioxolane (33)

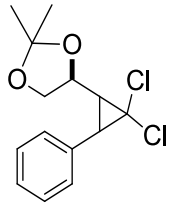


n-BuLi (2.5 M in hexane, 4.3 mL, 10.5 mmol, 2.0 equiv.) was added dropwise at 0 ºC to a white suspension of the benzyltriphenylphosphonium bromide (4.63 g, 10.5 mmol, 2.0 equiv.) in dry THF (10 mL); the resulting mixture immediately became orange. The temperature was allowed to rise to room temperature over a period of 30 minutes and then all of (*R*)-(+)-2,2-dimethyl-1,3-dioxolane-4-carboxaldehyde (**32)** from the previous step was added. The reaction mixture was further stirred until TLC analysis indicated the reaction was complete (approx. 2 hours). The solvent was removed and the reaction mixture was diluted with water and extracted 3 times with EtOAc, dried over $MgSO_4$ and concentrated to give a crude oil. The *cis* and *trans* geometric ratio was about 72:28 as determined by ^{1}H NMR spectroscopy. The crude material was purified by standard silica chromatography using a 1:9 diethyl ether: 40-60 hexane as eluent to give 0.659 g (60% overall yield from 1,2:5,6-di-O-isopropylidene-*D*-mannitol) of **33** as a colorless oil; $[\alpha]_D^{28}$ = -42.1 (c = 1.28, $CHCl_3$); IR (neat) υ_{max}/cm^{-1} 2985-2867 (C-H), 1498 (C=C), 1055 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.42-7.30 (5H, m, Ar-*H*), 6.76 (1H, d, *J* = 11.5, HC=C*H*Ar), 5.75 (1H, dd, *J* = 11.5, 9.0, *H*C=CHAr), 4.95 (1H, ddd *J* = 9.0, 8.0, 6.0, C*H*HCOR), 4.20

(1H, dd, *J* = 8.0, 6.0, CH*H*CHOR), 3.72 (1H, t, *J* = 8.0, CH_2CHOR), 1.53 (3H, s, CH_3) 1.44 (1H, s, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 136.3 (*ipso* Ar-*C*), 134.0, 129.3, 128.8, 128.4, 127.6 (Ar-*C* and C*H*=CH), 109.5 (*C*Me_2), 72.5 (*C*HOR), 69.8 (*C*H_2CHOR), 27.0, 26.0 (*C*H_3); HRMS (+ESI) *m*/*z* for ($C_{13}H_{16}NaO_2$) [M+Na^+] calculated 227.1043 found 227.1046.

These data were consistent with those previously reported.[229]

(4*S*)-(-)-(6',6'-dichloro-7'-phenylcyclopropyl)-2,2-dimethyl-1,3-dioxolane (34)



Prepared in a similar manner to compound **13**, (4*S*)-2,2-dimethyl-4-[(*Z*)-phenylethenyl]-1,3-dioxolane **(33)** (2.0 g 9.8 mmol, 1.0 equiv.) was treated with $CHCl_3$ (7.0 mL, 87.1 mmol, 25 equiv.) and a solution of 50% aqueous NaOH (3.49 mL, 50 mmol, 19.1 equiv.) for 3 days to give a mixture with *dr* ~ 6:1 as determined by ^{1}H NMR spectroscopy. The two diastereoisomers were separated by standard silica chromatography to give 1.71 g (61%) of the major isomer as a pale-yellow oil and 0.23 g (8%) of the minor isomer as a white solid.

Major isomer: $[\alpha]_D^{28}$ = -5.3 (c = 1.075, $CHCl_3$); IR (neat) υ_{max}/cm^{-1} 2987-2873 (C-H), 1604, 1499 (C=C), 1064 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.44-7.42 (2H, m, Ar-*H*), 7.32-7.18 (3H, m, Ar-H), 4.12 (1H, dd, *J* = 8.5, 6.5, C*H*HCHOR), 3.87 (1H, dd, *J* = 8.5, 6.5, CH*H*CHOR), 3.69 (1H, dt, *J* = 9.5, 6.5, CH_2C*H*OR), 3.02 (1H, d, *J* = 11.0, ArC*H*CCl_2), 2.10 (1H, dd, *J* = 11.0, 9.5, CHOC*H*CCl_2), 1.46 (3H, s, CH_3), 1.26 (3H, s, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 132.2 (*ipso* Ar-*C*),

130.8, 128.6, 127.8 (Ar-*C*), 109.6 (*C*Me_2), 73.4 (CH_2*C*HO), 68.7 (*C*H_2CHO), 61.1 (*C*Cl_2), 37.8, 37.2 (*C*HCHCCl_2 and CH*C*HCCl_2), 27.0, 25.6 (*C*H_3); HRMS (ESI) *m*/*z* for ($C_{14}H_{16}{}^{35}Cl_2NaO_2$) [M+$Na^+$] calculated 309.0420 found 309.0419.

Minor isomer: mp 74 – 75 ºC; $[\alpha]_D^{28}$ = -15.8 (c = 1.14, $CHCl_3$); IR (neat) υ_{max}/cm^{-1} 2991-2938 (C-H), 1601, 1499 (C=C), 1021 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.32-7.14 (5H, m, Ar-*H*), 4.08 (1H, dd, *J* = 8.0, 6.0, C*H*$_2$CHOR), 3.90 (1H, dd, *J* = 8.0, 6.0, C*H*$_2$CHOR), 3.78 (1H, dt, *J* = 10.0, 6.0, CH_2C*H*OR), 2.95 (1H, d, *J* = 11.5, ArC*H*CCl_2), 2.10 (1H, dd, *J* = 11.0, 10.0, CHORC*H*CCl_2), 1.46 (3H, s, C*H*$_3$), 1.24 (3H, s, C*H*$_3$); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 132.6 (*ipso* Ar-*C*), 129.9, 128.7, 127.8 (Ar-*C*), 109.8 (*C*Me_2), 74.4 (CH_2*C*HO), 68.3 (*C*H_2CHO), 62.7 (*C*Cl_2), 37.8, 37.3 (*C*HCHCCl_2 and CH*C*HCCl_2), 27.2, 25.5 (*C*H_3); HRMS (+ESI) *m*/*z* for ($C_{14}H_{16}Cl_2NaO_2$) [M+Na^+] calculated 309.0420 found 309.0420.

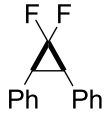
General procedure for microwave assisted difluorocyclopropanation of stilbenes:

NaI (1.25 mmol, 2.5 equiv.) and $TMSCF_3$ (1.25 mmol, 2.5 equiv.) were added to a microwave reaction tube containing the alkene (0.5 mmol, 1.0 equiv.) in 1 mL of CH_3CN. The reaction tube was sealed and the reaction mixture was heated with stirring at 80 ºC for 20 minutes in a microwave reactor. The solvent was removed *in vacuo* before water was added and the aqueous solution was extracted 3 times with ethyl acetate. The organic extracts were combined, dried over $MgSO_4$ and evaporated under vacuum to give a dark-brown mixture which was not purified and directly used for the next step.

General procedure for the oxidative cleavage of stilbenes and isolation of diphenyldifluorocyclopropanes:

Oxone (4 equiv.) was added to potassium osmate(VI) dihydrate (0.01 equiv.) in DMF (0.2 M based on olefin) and the resulting mixture was stirred for 30 minutes, followed by addition of the olefin and difluorocyclopropane mixture in DMF (1 ~ 2 mL). The stirring was continued for overnight, after which Na_2SO_3 (4.2 equiv.) was added and stirred for an additional hour. The resultant mixture was diluted with ethyl acetate and stirred for 10 minutes. The solid was filtered and the filter cake was washed with ethyl acetate. The organic exact was washed 3 times with 1 M HCl and twice with diluted $NaHCO_3$ solution, dried over $MgSO_4$ and concentrated under vacuum to give an oil. The crude material was purified by standard silica chromatography to give diphenyldifluorocyclopropanes as a white solids.

***cis*-1,1- Difluoro-2,3-diphenylcyclopropane (52)**

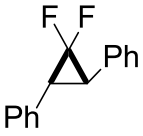


cis-Stilbene (0.9 g, 5 mmol, 1.0) was allowed to react with $TMSCF_3$ (1.85 mL, 12.5 mmol, 2.5 equiv.) and NaI (1.87 g, 12.5 mmol, 2.5 equiv.) in CH_3CN (10 mL) using the microwave-assisted difluorocyclopropanation method, isolated by the oxidative cleavage described above and purified by standard silica chromatography using hexane as eluent to give **52** (0.21 g, 18%) as a mixture of *cis*- and *trans*-isomers with a ratio of 96:4, mp 64 - 66 ºC; IR (neat) υ_{max}/cm^{-1} 3050 (C-H), 1496 (Ar), 1140 (C-F); ^1H NMR (300 MHz, $CDCl_3$) δ ppm 7.29 – 7.16 (6H, m, Ph-*H*), 7.10 – 7.01 (4H, m, Ph-*H*), 3.22 (2H, dd, *J* = 14.5, 2.5, C*H*CF_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 130.8 (*ipso* Ph-*C*), 130.1, 128.2, 127.1 (Ph-*C*), 114.0 (dd, $^1J_{C\text{-}F}$ = 291.5, 288.0, *C*F_2),

32.2 (dd, $^{2}J_{C\text{-}F}$ = 12.0, 9.5, *C*HCF$_2$); ^{19}F NMR (282 MHz, CDCl$_3$) δ ppm -117.0 (dt, $^{2}J_{F\text{-}F}$ = 157.0, $^{3}J_{H\text{-}F}$ = 14.0), -146.6 (d, $^{2}J_{F\text{-}F}$ = 157.0).

This compound is known and has previously been reported with ^{19}F NMR spectroscopic data consistent with those reported here.[125]

***trans* -(±)-1,1- Difluoro-2,3-diphenylcyclopropane (53)**

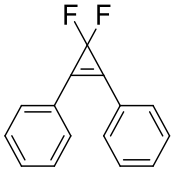


trans-Stilbene (0.9 g, 5 mmol, 1.0) was allowed to react with TMSCF$_3$ (1.85 mL, 12.5 mmol, 2.5 equiv.) and NaI (1.87 g, 12.5 mmol, 2.5 equiv.) in CH$_3$CN$_3$ (10 mL) using the microwave-assisted difluorocyclopropanation method, isolated by the oxidative cleavage described previously and purified by standard silica chromatography using hexane as eluent to give **53** (0.22 g, 19%) as a mixture of *cis*- and *trans*-isomers with a ratio of 4:96, mp 50 - 51 ºC, (lit.,[230] mp 43 ºC); IR (neat) υ_{max}/cm^{-1} 3050 (C-H), 1498 (Ar), 1270 (C-F); ^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 7.34 – 7.13 (10H, m, Ph-*H*), 2.93 (2H, t, *J* = 7.5, C*H*CF$_2$); ^{13}C NMR (75 MHz, CDCl$_3$) δ ppm 132.9 (*ipso* Ph-*C*), 128.1, 127.5, 126.8 (Ph-*C*), 112.2 (t, $^{1}J_{C\text{-}F}$ = 292, *C*F$_2$), 33.9 (t, $^{2}J_{C\text{-}F}$ = 10.5, *C*HCF$_2$); ^{19}F NMR (282 MHz, CDCl$_3$) δ ppm -134.6 (t, $^{3}J_{H\text{-}F}$ = 7.5).

Mass spectrometric data were not obtainable via ESI mass spectrometry.

This compound is known and has previously been reported with ^{1}H and ^{19}F NMR spectroscopic data only which were consistent with those reported here.[126, 230]

1,1-Difluoro-2,3-diphenylcyclopropene (58)



This compound was synthesized according to Hu's *et al.* method. Diphenylacetylene (0.18 g, 1 mmol, 1.0 equiv.), $TMSCF_3$ (0.3 mL, 2 mmol, 2.0 equiv.), NaI (0.33 g, 2.2 mmol, 2.2 equiv.) and THF (3 mL) were added to a pressure tube equipped with a magnetic stirrer and the tube was sealed. The resulting mixture was heated on an oil bath at 80 ºC for 2 hours. The solvent was evaporated and the crude product was purified by standard silica chromatography using hexane as elute to give **58** (0.19 g, 83%) as a white solid; mp 57 - 59 ºC; (lit.,[128] mp 58 - 59 ºC); IR (neat) υ_{max}/cm^{-1} 3001 (C-H), 1263 (C-F); ^1H NMR (400 MHz, $CDCl_3$) δ ppm 7.84 – 7.76 (4H, m, Ar-*H*), 7.61 – 7.41 (6H. m, Ar-*H*); ^{19}F NMR (282 MHz, $CDCl_3$) δ ppm -112.4. Mass spectrometric data were not obtainable via ESI mass spectrometry.

These data were consistent with those previously reported.[128]

General procedure for Horner-Wadsworth-Emmons reaction:

A few *trans*-diarylethenes was prepared according to the procedure of Lion *et al.*[231] Briefly, $NaBH_4$ (0.5 equiv.) was added slowly to a solution of substituted benzaldehyde (1.0 equiv.) in isopropyl alcohol. The resulting mixture was stirred at room temperature for 15 minutes followed by heating at reflux for 10 minutes. After the addition of dilute aqueous HCl (10%) until the solution was slightly acidic, the solvent was evaporated under vacuum. Water was added and the solution was extracted with CH_2Cl_2. The organic extracts were combined and washed with a solution of 5% aqueous $NaHCO_3$, dried over $MgSO_4$ to give the substituted aryl

alcohol as a viscous oil. The crude material was used directly for the next step without any purification.

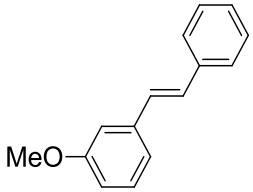
To a solution of mono- or dimethoxy substituted aryl alcohol (1.0 equiv.) in CH_2Cl_2 (10 mL/mol) phosphorus tribromide (1.2 equiv.) was added slowly at 0 ºC, and stirring was continued overnight. Dilute sodium dicarbonate solution was added cautiously, followed by extraction of the organic material with CH_2Cl_2. The organic extracts were combined, dried over $MgSO_4$ and the solvent was evaporated under vacuum to give substituted aryl bromide as a colorless which was used without purification for the next transformation.

Mono- or dimethoxy substituted benzyl bromide (1.0 equiv.) was heated with triethylphosphite (1.5 equiv.) at 140 ºC until completed dissolution occurred and the evolution of bromoethane had ceased. The excess triethylphosphite was subsequently removed by concentration of the reaction mixture under vacuum to afford the substituted phosphonic acid diethyl ester as a viscous oil which was used for the next step without any purification.

To a solution of mono- or dimethoxy substituted phosphonic acid diethyl ester (1.0 equiv.) in dry DMF was added sodium methoxide (2.0 equiv.) and 18-crown-6 ether (0.2 equiv.). The resulting mixture was stirred at room temperature for 5 minutes before benzaldehyde (1.5 equiv.) in dry DMF was added dropwise at 0 ºC. The stirring was continued for an hour followed by heating to 120 ºC for 5 hours. After cooling to room temperature, water was added to quench the reaction and the organic material was exacted with ethyl acetate. The organic solvent was evaporated under

vacuum to give a residue which was then redissolved in CH_2Cl_2 and acethydrazide trimethylammonium chloride (0.6 equiv.) was added. The reaction mixture was stirred for 2 hours before water was added and the layers were separated. The organic layer was collected, washed successively with brine and aqueous Na_2CO_3, dried over $MgSO_4$, and evaporated under vacuum to give the crude product as a solid which was purified either by recrystallization or silica chromatography.

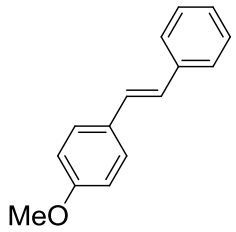
***trans*-3-Methoxystilbene (63)**



63 was synthesized from 3-methoxy benzyl bromide (6.0 g, 30 mmol) using Horner-Wadsworth-Emmons reaction and purified by standard silica chromatography using hexane as eluent to give the title compound (6.03 g, 96%) as a pale-yellow solid, mp 34 – 35 ºC, (lit.,[232] mp 34 – 35 ºC); IR (neat) υ_{max}/cm^{-1} ~3013 (C-H), 1584 (C=C); ^1H NMR (300 MHz, $CDCl_3$) δ ppm 7.66 (2H, m, Ar-*H* or C*H*=CH), 7.55 – 7.45 (2H, m, Ar-*H* or C*H*=CH), 7.45 – 7.35 (2H, m, Ar-*H* or C*H*=CH), 7.31 – 7.15 (4H, m, Ar-*H* or C*H*=CH), 6.97 (1H, ddd, *J* = 8.0, 2.5, 1.0, C*H*CHCHMe or C*H*OMe), 3.94 (3H, s, OC*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 160.1 (*C*OMe), 138.9, 137.4 (*ipso* Ar-*C*), 129.8, 129.1, 128.9, 128.8, 127.9, 126.8, 119.4, 113.4, 112.0 (Ar-*C* or *C*H=CH), 55.3 (O*C*H$_3$); HRMS (+ESI) *m/z* for ($C_{15}H_{15}O$) [M + H] calculated 211.1117 found 211.1119.

These data were consistent with those previously reported.[233]

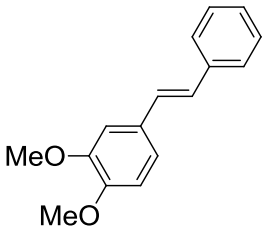
***trans*-4-Methoxystilbene (64)**



64 was synthesized from 4-methoxybenzaldehyde (5.46 g 20 mmol) using Horner-Wadsworth-Emmons reaction and purified by recrystallization to give the title compound (3.52 g, 56%) as a pale-yellow solid, mp 136 - 137 ºC (MeOH), (lit.,[232] mp 135 – 136 ºC); IR (neat) υ_{max}/cm^{-1} 1666 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.30 – 7.18 (4H, m, Ar-*H*), 7.17 – 7.07 (2H, m, Ar-*H*), 7.05 – 6.96 (1H, m, *para* Ar-*H*), 6.84 (1H, d, *J* = 16.5, C*H*=CH), 6.74 (1H, d, *J* = 16.5, C*H*=CH), 6.70 – 6.63 (2H, m, Ar-H), 3.59 (3H, s, OC*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 158.8 (*C*OMe), 137.1 (*ipso* Ar-*C*), 129.6, 128.1, 127.7, 127.2, 126.7, 126.0, 125.7, 113.6 (Ar-*C* or *C*H=CH), 54.7 (O*C*H$_3$); HRMS (+ESI) *m/z* for ($C_{15}H_{15}O$) [M + H] calculated 211.1117 found 211.1115.

These data were consistent with those previously reported.[234]

***trans*-3,4-Dimethoxystilbene (65)**

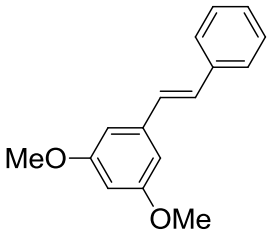


65 was synthesized from 3,4-dimethoxybenzaldehyde (5.0 g, 30 mmol) using Horner-Wadsworth-Emmons reaction and purified by recrystallization to give the title compound (4.10 g, 57%) as a pale-yellow solid, mp 109 - 110 ºC (MeOH), (lit.,[235] mp 108 - 109 ºC); IR (neat) υ_{max}/cm^{-1} 2963 (C-H), 1590 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.49 (2H, d, *J* = 7.5, *ortho* Ph-*H*), 7.34 (2H, *J* = 7.5, *meta*

Ph-*H*), 7.24 (1H, m, *para* Ph-*H*), 7.13 – 7.01 (3H, m, C*H*=CH and *ortho* Ar-*H*), 6.96 (1H, d, *J* = 16.5, C*H*=CH), 6.85 (1H, d, *J* = 8.0, CHC*H*COMe), 3.94 (3H, s, OCH_3), 3.89 (3H, s, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 148.5, 148.3 (*C*OMe), 136.9, 129.8 (*ipso* Ar-*C*), 128.1, 127.9, 126.7, 126.2, 125.7, 119.3 (Ar-*C*H and *C*H=CH), 110.6 (CH*C*HCOMe), 108.1 (*C*HCOMe), 55.3, 55.3 (O*C*H_3); HRMS (+ESI) *m/z* for ($C_{16}H_{17}O_2$) [M + H] calculated 241.1223 found 241.1227.

These data were consistent with those previously reported.[236]

trans-3,5-Dimethoxystilbene (66)

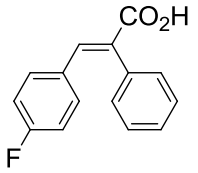


66 was synthesized from 3,5-dimethoxybenzaldehyde (2.0 g, 11 mmol) using Horner-Wadsworth-Emmons reaction and purified by standard silica chromatography using hexane as eluent to give the title compound (1.28 g, 48%) as a pale-yellow solid, mp 55 - 56 ºC, (lit.,[233] mp 53 - 55 ºC); IR (neat) υ_{max}/cm^{-1} 2954 (C-H), 1590 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.44 – 7.33 (2H, m, Ar-*H*), 7.30 – 7.19 (2H, m, Ar-*H*), 7.19 – 7.08 (1H, m, Ar-*H*), 6.98 (1H, d, *J* = 16.5, C*H*=CH), 6.91 (1H, d, *J* = 16.5, C*H*=CH), 6.57 (2H, d, *J* = 2.5, COMeC*H*COMe), 6.29 (1H, t, *J* = 2.0, C*H*COMe), 3.70 (6H, s, O*C*H_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 161.0 (*C*OMe), 139.4, 137.2 (*ipso* Ar-*C*), 129.2 (*C*H=CH), 128.8 (2 x Ar-*C*H), 127.8, 126.7 (*C*H=CH and Ar-*C*H), 104.6 (*C*HCOMe), 100.0 (COMe*C*HCOMe), 55.4 (O*C*H_3); HRMS (+ESI) *m/z* for ($C_{16}H_{17}O_2$) [M + H] calculated 241.1223 found 241.1229.

These data were consistent with those previously reported.[233]

General procedure for the preparation of substituted diphenylpropenoic acid:

***trans*-[(4-Fluorophenyl)methylene]phenylacetic acid (67)**

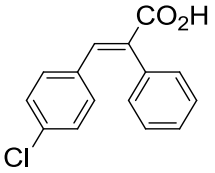


A stirred mixture of phenylacetic acid (5.45 g, 40 mmol, 1.0 equiv.) and 4-fluorobenzaldehyde (4.96 g, 40 mmol, 1.0 equiv.) in triethylamine (4 mL, 29 mmol, 0.7 equiv.) and acetic anhydride (8 mL, 85 mmol, 2.1 equiv.) were heated at reflux for 5 hours. To the cooled reaction mixture, concentrated HCl (9 mL) and water (40 mL) were added. The precipitate was filtered and washed with cold water (2 x 20 mL). The filter cake was mixed with water (50 mL) and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were extracted with 1 M aqueous NaOH solution (3 x 50 mL) and the alkaline solutions were acidified to pH 2 with 1 M hydrochloric acid. After that, the aqueous solution was extracted with CH_2Cl_2 (3 x 50 mL) and the organic extracts were combined, dried over $MgSO_4$ and concentrated under vacuum to give a crude mixture. The crude mixture was purified by recrystallization to give **67 (**5.40 g, 56%) as an intense yellow needle-like crystal, mp 187 - 188 ºC (CH_3CN), (lit.,[237] mp 192 – 195 ºC); IR (neat) υ_{max}/cm^{-1} 2795 (br OH), 1668 (C=O), 1157 (C-F); ^{1}H NMR (300 MHz, d_6-DMSO) δ ppm 12.77 (1H, br, s, COO*H*), 7.78 (1H, s, C*H*CCO_2H), 7.45 – 7.29 (3H, m, Ar-*H*), 7.22 – 7.14 (2H, m, Ar-*H*), 7.14 – 6.96 (4H, m, Ar-*H*); ^{13}C NMR (75 MHz, d_6-DMSO) δ ppm 168.3 (*C*OOH), 162.1 (d, $^1J_{C\text{-}F}$ = 248.0, *C*F), 137.8 (*C*HCCO_2H), 136.1, 133.1 (*ipso* Ar-*C* or CH*C*CO_2H), 132.3 (d, $^3J_{C\text{-}F}$ = 8.5, *C*HCHCF), 131.0 (d, $^4J_{C\text{-}F}$ = 3.0, *ipso* Ar-*C*),

129.4, 128.6, 127.6 (Ar-*C*H), 115.3 (d, $^{2}J_{C\text{-}F}$ = 21.5, *C*HCF); HRMS (-ESI) *m*/*z* for ($C_{15}H_{10}FO_2$) [M-H] calculated 241.0670 found 241.0672.

These data were consistent with those previously reported.[237]

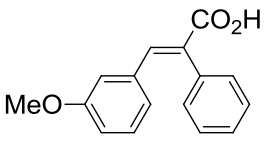
***trans*-[(4-Chlorophenyl)methylene]phenylacetic acid (68)**



Prepared in a similar manner to compound **67**, 4-chlorobenzaldehyde (5.62 g, 40 mmol, 1.0 equiv.) was reacted with phenylacetic acid (5.45 g, 40 mmol, 1.0 equiv.) in triethylamine (4.0 mL, 29 mmol, 0.7 equiv.) and acetic anhydride (8.0 mL, 85 mmol, 2.1 equiv.) to give a crude material. The crude product was purified by recrystallization to give **68** (7.0 g, 68%) as a pale-yellow needle-like crystal, mp >300 ºC (CH_3CN), (lit.,[238] mp 323 – 326 ºC); IR (neat) υ_{max}/cm^{-1} 3340 (br OH), 1653 (C=O), 1632 (C=C), 774 (C-Cl); ^{1}H NMR (300 MHz, d_6-DMSO) δ ppm 7.47 (1H, s, C*H*CCO_2H), 7.34 – 7.16 (3H, m, Ar-*H*), 7.16 – 7.07 (4H, m, Ar-*H*), 6.97 – 6.89 (2H, m, Ar-*H*); ^{13}C NMR (75 MHz, d_6-DMSO) δ ppm 171.2 (*C*OOH), 143.1, 139.8, 135.8, 131.3 (*ipso* Ar-*C*, *C*Cl or CH*C*CO_2H), 130.9, 130.6, 129.3, 127.8, 127.7, 126.1 (Ar-*C*H and CH*C*HCHCOMe); HRMS (-ESI) *m*/*z* for ($C_{15}H_{10}{}^{35}ClO_2$) [M-H] calculated 257.0375 found 257.0379.

This compound is known, but no spectroscopic data were reported.[239]

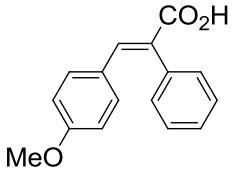
***trans*-[(3-Methoxyphenyl)methylene]phenylacetic acid (69)**



Prepared in a similar manner to compound **67**, 3-anisaldehyde (2.72 g, 20 mmol, 1.0 equiv.) was reacted with phenylacetic acid (2.72 g, 20 mmol, 1.0 equiv.) in triethylamine (2.0 mL, 14 mmol, 0.7 equiv.) and acetic anhydride (3.8 mL, 40 mmol, 2.0 equiv.) to give a crude material. The crude product was purified by recrystallization to give **69** (2.0 g, 39%) as a pale-yellow needle-like crystal, mp 189 - 190 ºC (CH_3CN), (lit.,[240] mp 191 - 192 ºC); IR (neat) υ_{max}/cm^{-1} 3057 (C-H), 2832 (O-CH_3), 1660 (C=O), 1609 (C=C); ^{1}H NMR (300 MHz, d_6-DMSO) δ ppm 12.77 (1H, s, CO_2H), 7.77 (1H, s, C*H*CCO_2H), 7.46 – 7.30 (3H, m, Ar-*H*), 7.24 – 7.16 (2H, m, Ar-*H*), 7.12 (1H, t, *J* = 8.0, CHC*H*CHCOMe), 6.80 (1H, dd, *J* = 8.0, 2.5, C*H*COMe or C*H*CHCHCOMe), 6.73 (1H, d, *J* = 8.0, 1H, C*H*COMe or C*H*CHCHCOMe), 6.54 (1H, s, MeOCC*H*CCHCCO_2H), 3.46 (3H, s, OCH_3); ^{13}C NMR (75 MHz, d_6-DMSO) δ ppm 168.2 (*C*OOH), 158.7 (*C*OMe), 138.9 (*C*HCCO_2H), 136.4, 135.6, 133.5 (*ipso* Ar-C or CH*C*CO_2H), 129.4, 129.3, 128.5, 127.5, 123.2 (Ar-*C*H and CH*C*HCHCOMe), 116.5 (*C*HCOMe or *C*HCHCHCOMe), 114.5 (MeOC*C*HCCHCCO_2H), 54.4 (O*C*H_3); HRMS (-ESI) *m*/*z* for ($C_{16}H_{13}O_3$) [M-H] calculated 253.0870 found 253.0864.

These data were consistent with those previously reported.[241]

***trans*-[(4-Methoxyphenyl)methylene]phenylacetic acid (70)**

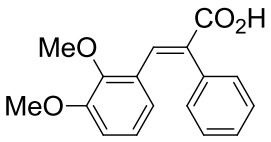


Prepared in a similar manner to compound **67**, 4-anisaldehyde (2.72 g, 20 mmol, 1.0 equiv.) was reacted with phenylacetic acid (2.72 g, 20 mmol, 1.0 equiv.) in triethylamine (2.0 mL, 14 mmol, 0.7 equiv.) and acetic anhydride (4.0 mL, 42 mmol,

2.1 equiv.) to give a crude material. The crude product was purified by recrystallization to give **70 (**2.20 g, 43%) as a pale-yellow needle-like crystal, mp 188 - 189 ºC (CH_3CN), (lit.,[237] mp 188 - 189 ºC); IR (neat) υ_{max}/cm^{-1}: 2940 (C-H), 2842 (O-CH_3), 1666 (C=O), 1600 (C=C); ^{1}H NMR (300 MHz, d_6-DMSO) δ ppm 12.56 (1H, s, CO_2H), 7.74 (1H, s, C*H*CCO_2H), 7.52 – 7.25 (3H, m, Ar-*H*), 7.18 (2H, m, Ar-*H*), 7.00 (2H, d, *J* = 9.0, C*H*CHCOMe), 6.75 (2H, d, *J* = 9.0, C*H*COMe), 3.69 (3H, s, OC*H*$_3$); ^{13}C NMR (75 MHz, d_6-DMSO) δ ppm 168.5 (*C*OOH), 159.9 (*C*OMe), 138.8 (*C*HCCO_2H), 136.8 (*ipso* Ar-*C* or CH*C*CO_2H), 132.0 (*C*HCHCOMe), 130.7 (*ipso* Ar-*C* or CH*C*CO_2H), 129.5, 128.6, 127.4 (Ar-*C*H), 126.7 (*ipso* Ar-*C* or CH*C*CO_2H), 113.8 (*C*HCOMe), 55.1 (O*C*H_3); HRMS (-ESI) *m*/*z* for ($C_{16}H_{13}O_3$) [M-H] calculated 253.0870 found 253.0873.

These data were consistent with those previously reported.[237]

***trans*-[(2,3-Dimethoxyphenyl)methylene]phenylacetic acid (71)**

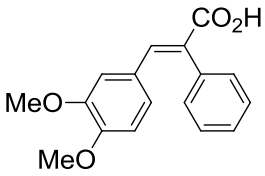


Prepared in a similar manner to compound **67**, 2,3-dimethoxybenzaldehyde (6.65 g, 40 mmol, 1.0 equiv.) was reacted with phenylacetic acid (5.45 g, 40 mmol, 1.0 equiv.) in triethylamine (4.0 mL, 29 mmol, 0.7 equiv.) and acetic anhydride (8.0 mL, 85 mmol, 2.1 equiv.) to give a crude material. The crude product was purified by recrystallization to give **71** (7.98 g, 70%) as a pale-yellow needle-like crystal, mp 156 - 157 ºC (CH_3CN), (lit.,[242] mp 154 - 155 ºC); IR (neat) υ_{max}/cm^{-1} 2923 (C-H), 2824 (O-CH_3), 1667 (C=O), 1572 (C=C); ^{1}H NMR (300 MHz, d_6-DMSO) δ ppm 7.97 (1H, s, C*H*CCO_2H), 7.40 – 7.20 (3H, m, Ar-*H*), 7.23 – 7.04 (2H, m, Ar-*H*), 6.91 (1H, dd, *J* = 8.0, 1.0, C*H*COMe or C*H*CHCHCOMe), 6.74 – 6.63 (1H, t, *J* = 8.0,

C*H*CHCOMe), 6.16 (1H, dd, *J* = 8.0, 1.0, C*H*COMe or C*H*CHCHCOMe), 3.80 (3H, s, OC*H*$_3$), 3.78 (3H, s, OC*H*$_3$); ^{13}C NMR (75 MHz, d_6-DMSO) δ ppm 168.3 (*C*OOH), 152.3, 147.9 (*C*OMe), 136.0 (*ipso* Ar-*C*), 134.0 (CH*C*CO$_2$H), 133.8 (*C*HCCO$_2$H), 129.7 (*ortho* Ph-*C*H), 128.5 (*ipso* Ar-C), 128.1, 127.4 (*meta* and *para* Ph-*C*H), 123.3 (*C*HCHCHCOMe), 121.3 (*C*HCHCOMe), 113.3 (*C*HCOMe), 60.6, 55.6 (O*C*H$_3$); HRMS (-ESI) *m/z* for ($C_{17}H_{15}O_3$) [M-H] calculated 283.1009 found 283.0981.

These data were consistent with those previously reported.[242]

***trans*-[(3,4-Dimethoxyphenyl)methylene]phenylacetic acid (72)**

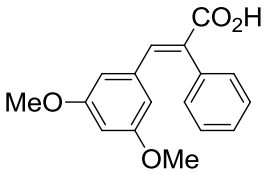


Prepared in a similar manner to compound **67**, 3,4-dimethoxybenzaldehyde (3.32 g, 20 mmol, 1.0 equiv.) was reacted with phenylacetic acid (2.72 g, 20 mmol, 1.0 equiv.) in triethylamine (2.0 mL, 14 mmol, 0.7 equiv.) and acetic anhydride (3.8 mL, 40 mmol, 2.0 equiv.) to give a crude material. The crude product was purified by recrystallization to give **72** (2.13 g, 38%) as a pale-yellow needle-like crystal, mp 224 - 225 ºC (CH_3CN), (lit.,[242] mp 231 – 232 ºC); IR (neat) υ_{max}/cm^{-1} 2935 (C-H), 2800 (O-CH_3), 1664 (C=O), 1574 (C=C); ^{1}H NMR (300 MHz, d_6-DMSO) δ ppm 12.55 (1H, s, CO$_2$*H*), 7.72 (1H, s, C*H*CCO$_2$H), 7.48 – 7.30 (3H, m, Ar-*H*), 7.28 – 7.15 (2H, m, Ar -*H*), 6.87 – 6.77 (2H, m, C*H*COMeCOMe and C*H*CHCOMe), 6.46 (1H, s, CC*H*COMe), 3.73 (3H, s, OC*H*$_3$), 3.29 (3H, s, OC*H*$_3$); ^{13}C NMR (75 MHz, d_6-DMSO) δ ppm 168.5 (*C*OOH), 149.7, 147.8 (*C*OMe), 139.2 (*C*HCCO$_2$H), 136.9 (*ipso* Ar-*C*), 130.6 (CH*C*CO$_2$H), 129.6, 128.6, 127.4 (Ar-*C*H), 126.8 (*ipso* Ar-*C*),

124.8 (*C*HCHCOMe), 112.5 (C*C*HCOMe), 111.1 (CHC*H*COMe), 55.3, 54.5 (O*C*H_3); HRMS (-ESI) *m/z* for ($C_{17}H_{15}O_3$) [M-H] calculated 283.0976 found 283.0975.

These data were consistent with those previously reported.[242]

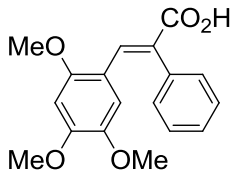
***trans*-[(3,5-Dimethoxyphenyl)methylene]phenylacetic acid (73)**



Prepared in a similar manner to compound **67**, 3,5-dimethoxybenzaldehyde (3.32 g, 20 mmol, 1.0 equiv.) was reacted with phenylacetic acid (2.72 g, 20 mmol, 1.0 equiv.) in triethylamine (2.0 mL, 14 mmol, 0.7 equiv.) and acetic anhydride (3.8 mL, 40 mmol, 2.0 equiv.) to give a crude material. The crude product was purified by recrystallization to give **73** (3.41 g, 60%) as a pale-yellow needle-like crystal, mp 206 - 207 ºC (CH_3CN), (lit.,[242] mp 204 – 206 ºC); IR (neat) υ_{max}/cm^{-1} 2944 (C-H), 2832 (O-CH_3), 1668 (C=O), 1583 (C=C); ^{1}H NMR (300 MHz, d_6-DMSO) δ ppm 12.78 (1H, s, CO_2H), 7.74 (1H, s, C*H*CCO_2H), 7.54 – 7.29 (3H, m, Ar-*H*), 7.29 – 7.10 (2H, m, Ar-*H*), 6.40 (1H, t, *J* = 2.0, COMeC*H*COMe), 6.27 (2H, d, *J* = 2.0, C*H*OMe), 3.52 (6H, s, OCH_3); ^{13}C NMR (75 MHz, d_6-DMSO) δ ppm 168.2 (*C*OOH), 159.9 (*C*OMe), 139.0 (*C*HCCO_2H), 136.5, 136.0 (*ipso* Ar-C), 133.7 (CH*C*CO_2H), 129.4, 128.5, 127.5 (Ar-*C*H), 108.2 (*C*HCOMe), 101.4 (COMe*C*HCOMe), 54.8 (O*C*H_3); HRMS (-ESI) *m/z* for ($C_{17}H_{15}O_3$) [M-H] calculated 283.0976 found 283.0980.

These data were consistent with those previously reported.[242]

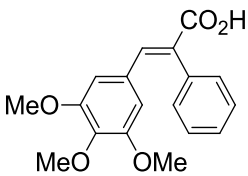
***trans*-[(2,4,5-Trimethoxyphenyl)methylene]phenylacetic acid (74)**



Prepared in a similar manner to compound **67**, 2,4,5-trimethoxybenzaldehyde (7.85 g, 40 mmol, 1.0 equiv.) was reacted with phenylacetic acid (5.45 g, 40 mmol, 1.0 equiv.) in triethylamine (4.0 mL, 29 mmol, 0.7 equiv.) and acetic anhydride (8.0 mL, 85 mmol, 2.1 equiv.) to give a crude material. The crude product was purified by recrystallization to **74** (5.40 g, 48%) as a pale-yellow needle-like crystal, mp 219 - 220 ºC (CH_3CN), (lit.,[243] mp 220 ºC); IR (neat) υ_{max}/cm^{-1} 2945 (C-H), 2850 (O-CH_3), 1666 (C=O), 1581 (C=C); ^{1}H NMR (300 MHz, d_6-DMSO) δ ppm 12.47 (1H, br, s, COO*H*), 8.04 (1H, s, C*H*CCO_2H), 7.49 – 7.24 (3H, m, Ph-*H*), 7.24 – 7.13 (2H, m, Ph-*H*), 6.65 (1H, s, C*H*COMe), 6.11 (1H, s, C*H*COMe), 3.86 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 3.04 (3H, s, OCH_3); ^{13}C NMR (75 MHz, d_6-DMSO) δ ppm 168.6 (*C*OOH), 153.5, 150.9, 141.5, 137.1 (*C*OMe or *ipso* Ar-*C* or CH*C*CO_2H), 133.2 (*C*HCCO_2H), 129.8 (Ph-*C*), 129.7 (*C*OMe or *ipso* Ar-*C* or CH*C*CO_2H), 128.5, 127.2 (Ph-*C*), 113.5 (*C*OMe or *ipso* Ar-*C* or CH*C*CO_2H), 112.5, 97.2 (*C*HCOMe), 56.3, 55.6, 54.6 (O*C*H_3); HRMS (-ESI) *m/z* for ($C_{18}H_{17}O_3$) [M-H] calculated 313.1081 found 313.1079.

This is a known compound and NMR spectroscopic data have previously been reported in $CDCl_3$.[244]

***trans*-[(3,4,5-Trimethoxyphenyl)methylene]phenylacetic acid (75)**

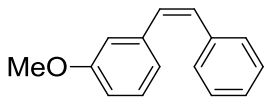


Prepared in a similar manner to compound **67**, 3,4,5-trimethoxybenzaldehyde (7.85 g, 40 mmol, 1.0 equiv.) was reacted with phenylacetic acid (5.45 g, 40 mmol, 1.0 equiv.) in triethylamine (4.0 mL, 29 mmol, 0.7 equiv.) and acetic anhydride (8.0 mL, 85 mmol, 2.1 equiv.) to give a crude material. The crude product was purified by recrystallization to give **75** (7.81 g, 63%) as a pale-yellow needle-like crystal, mp 185 - 186 ºC (CH_3CN), (lit.,[245] mp 186 - 187 ºC); IR (neat) υ_{max}/cm^{-1} 2940 (C-H), 2830 (O-CH_3), 1666 (C=O), 1579 (C=C), 1505 (Ar); ^{1}H NMR (300 MHz, d_6-DMSO) δ ppm 12.67 (1H, br, s, COO*H*), 7.73 (1H, s, C*H*CCO_2H), 7.52 – 7.30 (3H, m, Ar-*H*), 7.26 – 7.16 (2H, m, Ar-*H*), 6.40 (2H, s, C*H*COMe), 3.61 (3H, s, *para* OC*H*$_3$), 3.45 (6H, s, *meta* OC*H*$_3$); ^{13}C NMR (75 MHz, d_6-DMSO) δ ppm 168.3 (*C*OOH), 152.2 (*ipso* Ar-C or COMe or CH*C*COOH), 139.1 (*C*HCCOOH), 138.2, 136.7, 132.3 (*ipso* Ar-C or COMe or CH*C*COOH), 129.5, 128.6, 127.4 (Ar-*C*), 108.0 (*C*HCOMe), 59.9 (*para* O*C*H_3), 55.2 (*meta* O*C*H_3); HRMS (-ESI) *m/z* for ($C_{18}H_{17}O_3$) [M-H] calculated 313.1081 found 313.1091.

This is a known compound and NMR spectroscopic data have previously been reported in $CDCl_3$.[244]

General procedure for decarboxylation of diarylpropenoic acid:

***cis*-3-Methoxystilbene (76)**

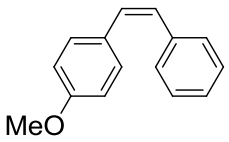


To a solution of *trans*-[(3-methoxyphenyl)methylene]phenylacetic acid (**69)** (1.4 g, 5.5 mmol, 1.0 equiv.) in quinoline (7.2 mL, 61 mmol, 11 equiv.) was added copper chromite (0.11 g, 0.71 mmol, 0.12 equiv.). The resulting mixture was stirred and heated to reflux at 230 - 240 ºC for 2 h. After cooling to room temperature, the

reaction mixture was filtered through Celite and washed with ethyl acetate. The organic layer was repeatedly washed with 2 M HCl, dried over $MgSO_4$ and concentrated under vacuum to give a mixture of *cis*- and *trans*-isomers. Careful separation of the two stereoisomers on silica chromatography using hexane as eluent allowed **76** (0.84 g, 73%) to be isolated as a pale-yellow oil; IR (neat) υ_{max}/cm^{-1} 3008 (C-H), 1576 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.47 – 7.05 (6H, m, Ar-*H*), 6.99 – 6.82 (2H, m, Ar-*H*), 6.82 – 6.71 (1H, m, Ar-*H*), 6.60 (1H, d, *J* = 12.5, C*H*=CH), 6.54 (1H, d, *J* = 12.5, CH=C*H*), 3.63 (3H, s, OC*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 158.9 (*C*OMe), 138.0, 136.8 (*ipso* Ar-*C*), 130.0, 129.7, 128.8, 128.4, 127.7, 126.7, 121.0, 113.3, 112.8 (Ar-*C*H or *C*H=CH), 54.5 (O*C*H$_3$); HRMS (+ESI) *m/z* for ($C_{15}H_{15}O$) [M + H] calculated 211.1117 found 211.1120.

These data were consistent with those previously reported.[246]

cis-4-Methoxystilbene (77)

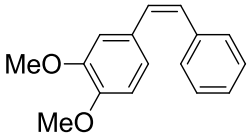


Prepared in a similar manner to compound **76**, the decarboxylation of *trans*-[(4-methoxyphenyl)methylene]-phenylacetic acid **(70)** (1.8 g, 7.0 mmol, 1.0 equiv.) was achieved using quinoline (9.2 mL, 78 mmol, 11 equiv.) and copper chromite (0.12 g, 0.78 mmol, 0.11 equiv.) to give a mixture of *cis*- and *trans*-isomers. Careful separation of the two stereoisomers on silica chromatography using hexane as eluent allowed **77** (1.16 g, 78%) to be isolated as a pale-yellow oil; IR (neat) υ_{max}/cm^{-1} 3006 (C-H), 1605 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.40 - 7.15 (7H, m, Ar-*H*), 6.94 – 6.70 (2H, m, Ar-*H*), 6.60 (1H, d, *J* = 12, C*H*=CH), 6.55 (1H, *J* = 12, CH=C*H*), 3.80 (3H, s, OC*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 158.1 (*C*OMe),

137.1 (*ipso* Ar-*C*), 129.6, 129.3, (Ar-*C*H or *C*H=CH) 129.1 (*ipso* Ar-*C*), 128.3, 128.2, 127.7, 126.4, 113.0 (Ar-*C* or *C*H=CH), 54.6 (O*C*H_3); HRMS (+ESI) *m/z* for ($C_{15}H_{15}O$) [M + H] calculated 211.1117 found 211.1115.

These data were consistent with those previously reported.[246]

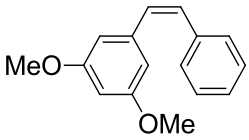
cis-3,4-Dimethoxystilbene (78)



Prepared in a similar manner to compound **76**, the decarboxylation of *trans*-[(3,4-dimethoxyphenyl)methylene]phenylacetic acid (**72)** (1.0 g, 3.5 mmol, 1.0 equiv.) was achieved using quinoline (4.5 mL, 38 mmol, 11 equiv.) and copper chromite (0.06 g, 0.39 mmol, 0.11 equiv.) to give a mixture of *cis*- and *trans*-isomers. Careful separation of the two stereoisomers on silica chromatography using hexane as eluent allowed **78** (0.67 g, 80%) to be isolated as a pale-yellow oil; IR (neat) υ_{max}/cm^{-1} 2954 (C-H), 1599 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$) ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.39 - 7.11 (5H, m, Ph-*H*), 6.84 (1H, dd, *J* = 8.0, 2.0, Ar-*H*), 6.85 – 6.75 (2H, m, Ar-*H*), 6.57 (1H, d, *J* = 12.5, C*H*=CH), 6.52 (1H, d, *J* = 12.5, CH=C*H*), 3.84 (3H, s, OCH_3), 3.57 (3H, s, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 148.3, 148.2 (*C*OMe), 137.7 (*ipso* Ar-*C*), 130.0 (Ar-*C*H or *C*H=CH), 129.8 (*ipso* Ar-*C*), 128.9, 128.3, 127.0, 126.9, 121.9 (Ar-*C*H or *C*H=CH), 111.7, 110.8 (C*H*COMe), 55.8, 55.4 (O*C*H_3); HRMS (ESI) *m/z* for ($C_{16}H_{17}O_2$) [M + H] calculated 241.1223 found 241.1224.

These data were consistent with those previously reported.[247]

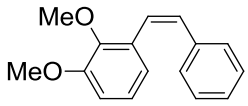
cis-3,5-Dimethoxystilbene (79)



Prepared in a similar manner to compound **76**, the decarboxylation of *trans*-[(3,5-dimethoxyphenyl)methylene]phenylacetic acid **(73)** (1.7 g, 6 mmol, 1.0 equiv.) was achieved using quinoline (7.9 mL, 66 mmol, 11 equiv.) and copper chromite (0.11 g, 0.71 mmol, 0.11 equiv.) to give a mixture of *cis*- and *trans*-isomers. Careful separation of the two stereoisomers on silica chromatography using hexane as eluent allowed **79** (0.81 g, 56%) to be isolated as a pale-yellow oil; IR (neat) υ_{max}/cm^{-1} 2955 (C-H), 1586 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.33 - 7.12 (5H, m, Ar-*H*), 6.62 (1H, d, *J* = 12.0, C*H*=CH), 6.53 (1H, d, *J* = 12.0, CH=C*H*), 6.41 (2H, d, *J* = 2.5, CC*H*COMe), 6.32 (1H, t, *J* = 2.5, COMeC*H*COMe), 3.63 (6H, s, OC*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 159.9 (COMe), 138.5, 136.7 (*ipso* Ar-*C*), 130.1, 129.7, 128.8, 127.6, 126.6 (Ar-*C*H and *C*H=CH), 106.1 (COMe*C*HCOMe), 99.3 (*C*HCOMe), 54.6 (O*C*H$_3$); HRMS (+ESI) *m/z* for ($C_{16}H_{17}O_2$) [M + H] calculated 241.1223 found 241.1226.

These data were consistent with those previously reported.[246]

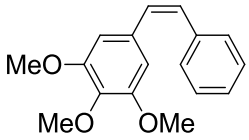
cis-2,3-Dimethoxystilbene (80)



Prepared in a similar manner to compound **76**, the decarboxylation of *trans*-[(2,3-dimethoxyphenyl)methylene]phenylacetic acid **(71)** (9.3 g, 33 mmol, 1.0 equiv.) was achieved using quinoline (43 mL, 0.36 mol, 11 equiv.) and copper chromite (0.56 g, 3.6 mmol, 0.11 equiv.) to give a mixture of *cis*- and *trans*-isomers. Careful separation of the two stereoisomers on silica chromatography using hexane as eluent

allowed **80** (2.1 g, 26%) to be isolated as a pale-yellow oil which solidified upon storage at 0 ºC, mp 47 - 48 ºC; IR (neat) υ_{max}/cm^{-1} 2956 (C-H), 1575 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.33 - 7.09 (5H, m, Ar-*H*), 6.88 – 6.75 (3H, m, Ar-*H*), 6.75 (1H, d, *J* = 12.5, C*H*=CH), 6.66 (1H, d, *J* = 12.5, C*H*=CH), 3.89 (3H, s, OCH_3), 3.87 (3H, s, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 152.2, 146.5 (*C*OMe), 136.5, 131.2 (*ipso* Ar-*C*), 130.2, 128.4, 127.5, 126.5, 125.1, 123.0, 121.4, 110.7 (*C*H=CH or Ar-*C*), 55.2 (2 x O*C*H_3); HRMS (+ESI) *m/z* for ($C_{16}H_{16}NaO_2$) [M + Na] calculated 263.1043 found 263.1042.

***cis*-3,4,5-Timethoxystilbene (81)**



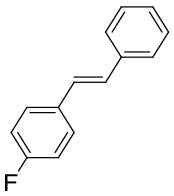
Prepared in a similar manner to compound **76**, the decarboxylation of *trans*-[(3,4,5-trimethoxyphenyl)methylene]phenylacetic acid **(75)** (6.28 g, 20 mmol, 1.0 equiv.) was achieved using quinoline (26 mL, 0.22 mol, 11 equiv.) and copper chromite (0.34 g, 2.2 mmol, 0.11 equiv.) to give a mixture of *cis*- and *trans*-isomers. Careful separation of the two stereoisomers on silica chromatography using hexane as eluent allowed **81** (3.48 g, 64%) to be isolated as a pale-yellow oil; IR (neat) υ_{max}/cm^{-1} 2937 (C-H), 1579 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.31 - 7.07 (5H, m, Ar-*H*), 6.56 (1H, d, *J* = 12.0, C*H*=CH), 6.45 (1H, d, *J* = 12.0, CH=C*H*), 6.42 (2H, s, C*H*COMe), 3.79 (3H, s, *para* OCH_3), 3.60 (6H, s, *meta* OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 152.2, 136.9 (*C*OMe), 136.5, 131.8 (*ipso* Ar-*C*), 129.5, 129.4, 128.3, 127.6, 126.5, (Ar-*C*H and *C*H=CH), 105.5 (*C*HCOMe), 60.2, 55.2 (O*C*H_3); HRMS (+ESI) *m/z* for ($C_{17}H_{18}NaO_3$) [M + Na] calculated 293.1148 found 293.1151.

These data were consistent with those previously reported.[248]

General procedure for iodine-catalysed isomerisation of *cis*- to *trans*-diarylthene:

Iodine (one crystal) was added to a solution of the *Z/E* mixture of diarylethene (5 mmol) in hexane (50 mL), the resulting solution was heated at reflux at 70 ºC for 2 days. After cooling to room temperature, the reaction mixture was diluted with ethyl acetate (50 mL) and washed with saturated aqueous sodium bisulfate. The organic layer was dried over $MgSO_4$ and concentrated under vacuum to give predominated the corresponding *E* isomer which was purified by recrystallization or silica chromatography.

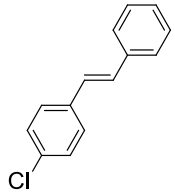
***trans*-4-Fluorostilbene (82)**



82 (1.47 g, 37%) was isolated as a white solid, mp 125 - 126 ºC (MeOH), (lit.,[249] mp 125 ºC); IR (neat) υ_{max}/cm^{-1} 3000 (C-H), 1590 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.30 - 7.16 (4H, m, Ar-*H* or C*H*=CH), 7.16 – 7.07 (2H, m, Ar-*H* or C*H*=CH), 7.06 – 6.96 (1H, m, Ar-H or C*H*=CH), 6.87 – 6.72 (4H, m, Ar-*H* or C*H*=CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 161.7 (d, $^{1}J_{CF}$ = 247.0, *C*F), 137.3 (*ipso* Ph-*C*), 133.6 (d, $^{4}J_{CF}$ = 3.0, *C*CHCHCF), 128.9 (*C*H=CH or Ar-*C*H), 128.6 (d, $^{5}J_{CF}$ = 2.0, *C*HC_6H_4F), 127.4 (d, $^{3}J_{CF}$ = 8.0, *C*HCF), 127.8, 127.6, 126.6, (*C*H=CH or Ar-*C*H), 115.8 (d, $^{2}J_{CF}$ = 21.50, *C*HCF); ^{19}F NMR (282 MHz, $CDCl_3$) δ ppm -114.8.

These data were consistent with those previously reported.[249]

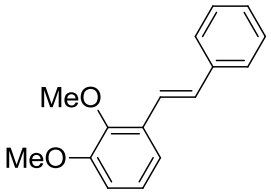
***trans*-4-Chlorostilbene (83)**



83 (1.58 g, 37%) was isolated as a white solid, mp 131 - 132 ºC (MeOH), (lit.,[250] mp 130 - 132 ºC); IR (neat) υ_{max}/cm^{-1} 2950 (C-H), 1576 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.41 – 7.52 (2H, m, Ar-*C*), 7.37 – 7.31 (2H, m, Ar-*C*), 7.31 – 7.13 (5H, m, Ar-*C*), 7.00 (1H, d, *J* = 16.5, C*H*=CH), 6.94 (1H, d, *J* = 16.5, C*H*=CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 137.0, 135.9, 133.1 (*ipso* Ar-*C* or *C*Cl), 129.3, 128.9, 128.8, 127.9, 127.7, 127.4, 126.6 (Ar-*C*H or *C*H=CH).

These data were consistent with those previously reported.[250]

***trans*-2,3-Dimethoxystilbene (84)**

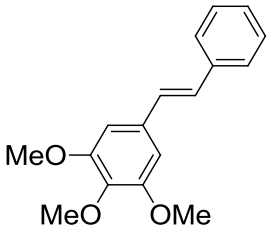


84 (3.12 g, 38%) was purified by standard silica chromatography using 20:80 EtOAc:hexane to give a waxy solid, mp 39 - 40 ºC, (lit.,[235] mp 37 - 39 ºC); IR (neat) υ_{max}/cm^{-1} 2934 (C-H), 1598 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.45 (2H, d, *J* = 7.5, *ortho* Ph-*H*), 7.37 (1H, d, *J* = 16.5, C*H*=CH), 7.31 – 7.20 (2H, m, *meta* Ph-*H*), 7.20 – 7.10 (2H, m, C*H*CHCHCOMe and *para* Ph-*H*), 7.02 (d, *J* = 16.5, CH=C*H*), 6.95 (1H, t, *J* = 8.0, C*H*CHCOMe), 6.73 (dd, *J* = 8.1, 1.3, C*H*COMe), 3.75 (3H, s, OCH_3), 3.73 (3H, s, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 152.5, 146.4 (*C*OMe), 137.1, 131.0 (*ipso* Ar-*C*), 129.3, (*para* Ph-*C*H), 128.1 (*meta* Ph-*C*H), 127.1 (*C*H=CH), 126.1 (*ortho* Ph-*C*H), 123.5 (*C*H=CH), 122.3 (*C*HCHCOMe), 117.3

(*C*HCHCHCOMe), 110.8 (*C*HCOMe), 60.3, 55.4 (OCH_3); HRMS (+ESI) *m/z* for ($C_{16}H_{17}O_2$) [M + H] calculated 241.1223 found 241.1222.

These data were consistent with those previously reported.[251, 252]

***trans*-3,4,5-Trimethoxystilbene (85)**



85 (1.54 g, 29%) was isolated as a white solid. mp 107 - 108 ºC (MeOH), (lit.,[235, 248] mp 107 – 108 ºC); IR (neat) υ_{max}/cm^{-1} 2966 (C-H), 1583 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.48 – 7.31 (2H, m, Ar-*H*), 7.29 – 7.18 (2H, m, Ar-*H*), 7.14 (1H, m, Ar-*H*), 6.94 (1H, d, *J* = 16.5, C*H*=CH), 6.88 (1H, d, *J* = 16.5, CH=C*H*), 6.62 (2H, s, C*H*COMe), 3.79 (6H, s, *meta* OC*H*$_3$), 3.76 (3H, s, *para* OC*H*$_3$); 13C NMR (75 MHz, $CDCl_3$) δ ppm 153.7, 138.2 (*C*OMe), 137.5, 133.3 (*ipso* Ar-C), 129.0, 128.9, 128.4, 127.9, 126.7, 103.8, (*C*HCOMe and Ar-*C*H), 60.6, 56.4 (OCH_3); HRMS (+ESI) *m/z* for ($C_{17}H_{18}NaO_3$) [M + Na] calculated 293.1148 found 293.1149.

These data were consistent with those previously reported.[248]

Typical reaction conditions for room temperature difluorocyclopropanation:

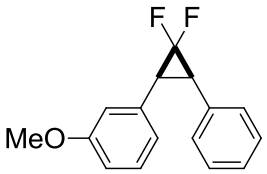
NaI (5.0 equiv.) and $TMSCF_3$ (5.0 equiv.) were added to a pressure tube containing the alkene (1.0 equiv.) in CH_3CN (1 mL per 1.25 mmol of $TMSCF_3$ and NaI). The pressure tube was tightly sealed and the reaction mixture was stirred at room temperature for 14 days. The reaction mixture was filtered and the solid washed with

twice with CH_3CN (10 mL). The filtrate and washings were reduced under vacuum to give a crude product which was not purified and directly used for the next step.

General procedure for the oxidative cleavage of olefins and isolation of diphenyldifluorocyclopropanes:

Oxone (4 equiv.) was added to potassium osmate(VI) dihydrate (0.01 equiv.) in DMF (0.2 M based on olefin) and the resulting mixture was stirred for 30 minutes, followed by addition of the olefin and difluorocyclopropane mixture in DMF (1 ~ 2 mL). The stirring was continued for overnight, after which Na_2SO_3 (4.2 equiv.) was added and stirred for an additional hour. The resultant mixture was diluted with ethyl acetate and stirred for 10 minutes. The solid was filtered and the filter cake was washed with EtOAc. The organic exacts were washed 3 times with 1 M HCl and twice with diluted $NaHCO_3$ solution, dried over $MgSO_4$ and concentrated under vacuum to give an oil. The crude material was purified by standard silica chromatography to give diaryldifluorocyclopropanes most of the time as a white solid.

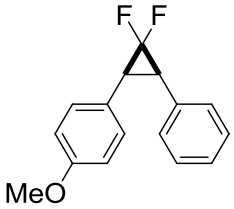
(±)-*cis*-1,1-Difluoro-2-(3-methoxyphenyl)-3-phenylcyclopropane (86)



The reaction of *cis*-3-methoxystilbene **(76)** (0.42, 2 mmol, 1.0 equiv.) with $TMSCF_3$ (1.48 mL, 10 mmol, 5.0 equiv.) and NaI (1.50 g, 10 mmol, 5.0 equiv.) in CH_3CN (8 mL) gave a dark-brown crude product. The crude product was purified by standard silica chromatography using hexane as eluent to give **86** (0.23 g, 44%) a pale-yellow

oil with *cis*- and *trans*-isomeric ratio of ~98:2; IR (neat) υ_{max}/cm^{-1} 3029 (C-H), 1602 (C=C), 1257 (C-F), 1142 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.33 – 7.21 (3H, Ar-*H*), 7.22 – 7.04 (3H, Ar-*H*), 6.80 (1H, m, Ar-*H*), 6.70 (1H, m, Ar-*H*), 6.52 (1H, s, *C*HCOMeCH), 3.63 (3H, s, OC*H*$_3$), 3.33 – 3.13 (2H, m, C*H*CF_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 159.5 (CH*C*OCH_3), 132.5, 131.0 (*ipso* Ar-*C*), 130.4, 129.3, 128.4, 127.4, 122.8, 115.3 (Ar-*C*H), 114.1 (dd, $^{1}J_{C\text{-}F}$ = 291.5, 288.5, *C*F_2), 113.4 (C*H*COMe), 55.3 (OC*H*$_3$), 32.6 (d, $^{2}J_{C\text{-}F}$ = 10, *C*HCF_2), 32.3 (d, $^{2}J_{C\text{-}F}$ = 10, *C*HCF_2); ^{19}F NMR (282 MHz, $CDCl_3$) δ ppm -117.6 (ddd, $^{2}J_{F\text{-}F}$ = 156.5, $^{3}J_{H\text{-}F}$ = 14.0, 5.5), -147.2 (d, $^{2}J_{F\text{-}F}$ = 157.0); HRMS (+ESI) *m*/*z* for ($C_{16}H_{14}F_2NaO$) [M+Na] calculated 283.0905 found 283.0908.

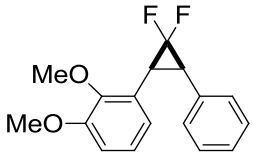
(±)-*cis*-1,1-Difluoro-2-(4-methoxyphenyl)-3-phenylcyclopropane (87)



The reaction of *cis*-4-methoxystilbene **(77)** (0.84, 4 mmol, 1.0 equiv.) with $TMSCF_3$ (2.96 mL, 20 mmol, 5.0 equiv.) and NaI (3.0 g, 20 mmol, 5.0 equiv.) in CH_3CN (16 mL) gave a dark-brown crude product. The crude product was purified by standard silica chromatography using hexane as eluent to give **87 (**0.62 g, 60%) as a pale-yellow solid with *cis*- and *trans*-isomeric ratio of 89:11, mp 37 - 39 ºC; IR (neat) υ_{max}/cm^{-1} 3036 (C-H), 1514 (C=C), 1250 (C-F), 1149 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.23 – 7.13 (3H, m, Ar-*H*), 7.05 – 6.87 (4H, m, Ar-*H*), 6.78 – 6.68 (2H, m, C*H*COMe), 3.74 (3H, s, OC*H*$_3$), 3.12 (2H, dd, $^{3}J_{H\text{-}F}$ = 14.5, 2.0, C*H*CF_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 159.0 (CH*C*OCH_3), 131.5 (Ar-*C*H), 131.3 (*ipso* Ar-*C*), 130.3, 128.4, 127.2 (Ar-*C*H), 122.8 (*ipso* Ar-*C*), 114.3 (dd, $^{1}J_{C\text{-}F}$ = 291.5, 288.0,

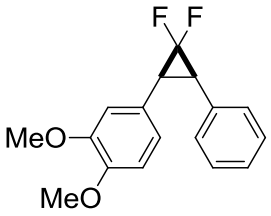
CF_2), 113.9 (*C*HCOCH$_3$), 55.4 (O*C*H$_3$), 32.3 (dd, $^2J_{C\text{-}F}$ = 12.0, 9.5, *C*HCF$_2$), 31.8 (dd, $^2J_{C\text{-}F}$ = 12.0, 9.5, *C*HCF$_2$); ^{19}F NMR (282 MHz, CDCl$_3$) δ ppm -117.5 (dt, $^2J_{F\text{-}F}$ = 156.5, $^3J_{H\text{-}F}$ = 14.5), -147.5 (d, $^2J_{F\text{-}F}$ = 156.5); HRMS (+ESI) *m*/*z* for ($C_{16}H_{14}F_2NaO$) [M+Na] calculated 283.0905 found 283.0906.

(±)-*cis*-1,1-Difluoro-2-(2,3-dimethoxyphenyl)-3-phenylcyclopropane (88)



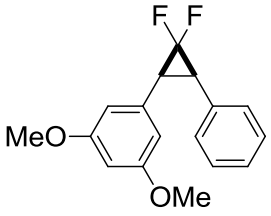
The reaction of *cis*-2,3-dimethoxystilbene **(80)** (0.48, 2 mmol, 1.0 equiv.) with TMSCF$_3$ (1.48 mL, 10 mmol, 5.0 equiv.) and NaI (1.50 g, 10 mmol, 5.0 equiv.) in CH$_3$CN (8 mL) gave a dark-brown crude material. The crude product was purified by standard silica chromatography using 20:80 EtOAc:hexane as eluent to give **88** (0.33 g, 57%) as a pale-yellow oil with *cis*- and *trans*-isomeric ratio of ~95:5; IR (neat) υ_{max}/cm^{-1}: 3001 (C-H), 1442, 1476 (C=C), 1278 (C-F), 1143 (C-O); ^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 7.23 – 7.11 (3H, m, Ar-*H*), 7.04 (2H, m, Ar-*H*), 6.89 – 6.72 (2H, m, Ar-*H*), 6.57 – 6.43 (1H, m, Ar-*H*), 3.81 (3H, s, OC*H*$_3$), 3.70 (3H, s, OC*H*$_3$), 3.44 – 3.12 (2H, m, C*H*CF$_2$); ^{13}C NMR (75 MHz, CDCl$_3$) δ ppm 152.7, 148.9 (COMe), 131.6 (*ipso* Ar-*C*), 130.0, 128.3, 127.2 (Ar-*H*), 125.2 (*ipso* Ar-*C*), 123.5, 122.1 (d, $^3J_{C\text{-}F}$ = 12.5, 8.5, *ipso* Ar-*C*), 114.7 (dd, $^1J_{C\text{-}F}$ = 291.5, 288.5, *C*F$_2$), 111.9 (*C*HCOMe), 55.9 (2 x O*C*H$_3$), 32.4 (dd, $^2J_{C\text{-}F}$ = 11.5, 9.5, *C*HCF$_2$), 27.7 (dd, $^2J_{C\text{-}F}$ = 12.5, 8.5, *C*HCF$_2$). δ ppm -117.1 (dt, $^2J_{F\text{-}F}$ = 157.0, $^3J_{H\text{-}F}$ = 14.0), -147.4 (d, $^2J_{F\text{-}F}$ = 156.5); HRMS (+ESI) *m*/*z* for ($C_{17}H_{16}F_2NaO_2$) [M+Na] calculated 131.1011 found 313.1011.

(±)-*cis*-1,1-Difluoro-2-(3,4-dimethoxyphenyl)-3-phenylcyclopropane (89)



The reaction of *cis*-3,4-dimethoxystilbene **(78)** (0.48, 2 mmol, 1.0 equiv.) with $TMSCF_3$ (1.48 mL, 10 mmol, 5.0 equiv.) and NaI (1.50 g, 10 mmol, 5.0 equiv.) in CH_3CN (8 mL) gave a dark-brown crude product. The crude product was purified by standard silica chromatography using 20:80 EtOAc:hexane as eluent to give **89 (**0.35 g, 60%) as a waxy solid with *cis*- and *trans*-isomeric ratio of 83:17, mp 33 - 36 ºC; IR (neat) υ_{max}/cm^{-1} 3004 (C-H), 1516 (C=C), 1252 (C-F), 1135 (C-O); ^1H NMR (300 MHz, $CDCl_3$) δ ppm 7.22 – 7.12 (3H, m, Ar-C*H*), 7.01 (2H, m, Ar-C*H*), 6.74 – 6.60 (2H, m, Ar-C*H*), 6.26 (1H, s C*H*COMeCOMe), 3.80 (3H, s, OC*H*$_3$), 3.51 (3H, s, OC*H*$_3$), 3.16 – 3.07 (2H, m, $CHCF_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 148.5, 148.3 (CH*C*OMe), 131.2 (*ipso* Ar-*C*), 130.4, 128.4, 127.3 (Ar-*C*H), 123.3 (*ipso* Ar-*C*), 122.9 (Ar-*C*H), 114.2 (dd, $^1J_{C\text{-}F}$ = 291.5, 288.5, *C*F_2), 113.1 (Ar-*C*H), 111.0 (COMe*C*HCOMe), 56.0, 55.8 (O*C*H_3), 32.5 – 31.9 (m, 2 x *C*HCF_2); ^{19}F NMR (282 MHz, $CDCl_3$) δ ppm -117.8 (dt, $^2J_{F\text{-}F}$ = 157.0, $^3J_{H\text{-}F}$ = 14.0), -147.4 (d, $^2J_{F\text{-}F}$ = 156.5); HRMS (+ESI) *m*/*z* for ($C_{17}H_{16}F_2NaO_2$) [M+Na] calculated 131.1011 found 313.1015.

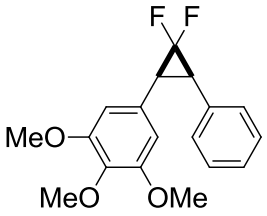
(±)- *cis*-1,1-Difluoro-2-(3,5-dimethoxyphenyl)-3-phenylcyclopropane (90)



The reaction of *cis*-3,5-dimethoxystilbene **(79)** (0.48, 2 mmol, 1.0 equiv.) with $TMSCF_3$ (1.48 mL, 10 mmol, 5.0 equiv.) and NaI (1.50 g, 10 mmol, 5.0 equiv.) in

CH_3CN (8 mL) gave a dark-brown crude product. The crude product was purified by standard silica chromatography using 20:80 EtOAc:hexane as eluent to give **90** (0.19 g, 37%) as a pale-yellow solid with *cis*- and *trans*-isomeric ratio of 95:5, mp 37 - 39 ºC; IR (neat) υ_{max}/cm^{-1} 3021 (C-H), 1608 (C=C), 1204 (C-F), 1146 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.34 – 7.20 (3H, m, Ar-C*H*), 7.20 – 7.03 (2H, m, Ar-C*H*), 6.36 (1H, t, *J* = 2.5, COMeC*H*COMe), 6.17 (2H, d, *J* = 2.0, CHCC*H*COMe), 3.63 (6H, s, OCH_3), 3.33 – 3.08 (2H, m, C*H*CF_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 160.5 (*C*OMe), 133.1, 131.0 (*ipso* Ar-*C*), 130.4, 128.5, 127.4 (Ar-*C*H), 114.1 (dd, $^1J_{C\text{-}F}$ = 291.5, 288.5, *C*F_2), 108.2 (*C*HCOMeCHCOMe), 99.9 (COMe*C*HCOMe), 55.4 (2 x O*C*H_3), 32.7 (dd, $^2J_{C\text{-}F}$ = 12.0, 9.5, *C*HCF_2), 32.3 (dd, $^2J_{C\text{-}F}$ = 12.0, 9.5, *C*HCF_2); ^{19}F NMR (282 MHz, $CDCl_3$) δ ppm -117.5 (dt, $^2J_{F\text{-}F}$ = 157.0, $^3J_{H\text{-}F}$ = 14.0), -147.0 (d, $^2J_{F\text{-}F}$ = 157.0); HRMS (+ESI) *m*/*z* for ($C_{17}H_{16}F_2NaO_2$) [M+Na] calculated 131.1011 found 313.1013.

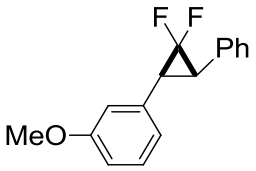
(±)-*cis*-1,1-Difluoro-2-(3,4,5-trimethoxyphenyl)-3-phenylcyclopropane (91)



The reaction of *cis*-3,4,5-trimethoxystilbene **(81)** (1.35, 5 mmol, 1.0 equiv.) with $TMSCF_3$ (3.70 mL, 25 mmol, 5.0 equiv.) and NaI (3.75 g, 25 mmol, 5.0 equiv.) in CH_3CN (20 mL) gave a dark-brown crude product. The crude product was purified by standard silica chromatography using 20:80 EtOAc:hexane as eluent to give **91 (**0.57 g, 36%) as a pale-yellow solid with *ci*- and *trans*-isomeric ratio of 96:4, mp 71 - 73 ºC; IR (neat) υ_{max}/cm^{-1} 3007 (C-H), 1586, 1415 (C=C), 1238 (C-F), 1122 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.37 – 7.21 (3H, m, Ar-*H*), 7.20 – 7.06 (2H, m,

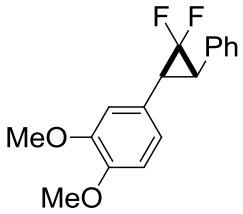
Ar-*H*), 6.18 (2H, s, C*H*OMe), 3.85 (3H, s, *para* OCH_3), 3.64 (6H, s, *meta* OCH_3), 3.33 – 3.07 (2H, m, C*H*CF_2); ^{13}C NMR (75 MHz, CDCl_3) δ ppm 152.8, 137.2 (*para* and *meta* *C*OMe), 130.7 (*ipso* Ar-*C*), 130.3, 128.4, 127.3 (Ar-*C*H), 126.3 (*ipso* Ar-*C*), 114.0 (dd, $^1J_{C\text{-}F}$ = 292.0, 288.0, *C*F_2), 107.3 (*C*HCOMe), 55.9 (2 x O*C*H_3), 32.5 (dd, $^2J_{C\text{-}F}$ = 12.0, 9.5, *C*HCF_2), 32.1 (dd, $^2J_{C\text{-}F}$ = 12.0, 9.5, *C*HCF_2); ^{19}F NMR (282 MHz, CDCl_3) δ ppm -117.6 (dt, $^2J_{F\text{-}F}$ = 157.0, $^3J_{H\text{-}F}$ = 14.0), -147.2 (d, $^2J_{F\text{-}F}$ = 157.0); HRMS (+ESI) *m*/*z* for ($C_{18}H_{18}F_2NaO_3$) [M+H] calculated 343.1120 found 343.1123.

trans-(±)-1,1-Difluoro-2-(3-methoxyphenyl)-3-phenylcyclopropane (92)



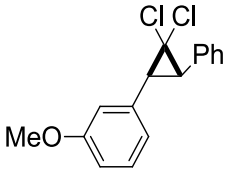
The reaction of *trans*-3-methoxystilbene **(63)** (0.53, 2.5 mmol, 1.0 equiv.) with TMSCF_3 (1.85 mL, 12.5 mmol, 5.0 equiv.) and NaI (1.87 g, 12.5 mmol, 5.0 equiv.) in CH_3CN (5 mL) gave a dark-brown crude product. The crude product was purified by standard silica chromatography using hexane as eluent to give **92** (0.41 g, 64%) as a pale-yellow oil; IR (neat) υ_{max}/cm^{-1} 3029 (C-H), 1602 (C=C), 1274 (C-F), 1154 (C-O); ^{1}H NMR (300 MHz, CDCl_3) δ ppm 7.37 – 7.11 (6H, m, Ar-*H*), 6.89 – 6.70 (3H, m, Ar-*H*), 3.70 (3H, s, OCH_3), 2.92 (2H, t, $^3J_{H\text{-}F}$ = 7.5, C*H*CF_2). ^{13}C NMR (75 MHz, CDCl_3) δ ppm 160.1 (*C*OMe), 135.3, 133.7 (*ipso* Ar-*C*), 130.0, 129.0, 128.4, 127.7, 120.7, 114.3 (Ar-C*H*), 113.1 (t, $^1J_{C\text{-}F}$ = 291.5, *C*F_2), 113.1 (*C*HCOMe), 55.5 (O*C*H_3), 34.8 (t, $^2J_{C\text{-}F}$ = 10.5, *C*HCF_2); ^{19}F NMR (282 MHz, CDCl_3) δ ppm -134.4 (t, $^3J_{H\text{-}F}$ = 7.5); HRMS (+ESI) *m*/*z* for ($C_{16}H_{14}F_2NaO$) [M+Na] calculated 283.0905 found 283.0909.

***trans*-(±)-1,1-Difluoro-2-(3,4-dimethoxyphenyl)-3-phenylcyclopropane (93)**



The reaction of *trans*-3,4-dimethoxystilbene **(65)** (0.60, 2.5 mmol, 1.0 equiv.) with $TMSCF_3$ (1.85 mL, 12.5 mmol, 5.0 equiv.) and NaI (1.87 g, 12.5 mmol, 5.0 equiv.) in CH_3CN (5 mL) gave a dark-brown crude product. The crude product was purified by standard silica chromatography using 20:80 EtOAc:hexane as eluent to give **93** (0.31 g, 43%) as a pale-yellow oil; IR (neat) υ_{max}/cm^{-1} 3004 (C-H), 1517 (C=C), 1256 (C-F), 1157 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.48 – 7.04 (5H, m, Ar-C*H*), 6.97 – 6.59 (3H, m, Ar-C*H*), 3.80 (3H, s, OC*H*$_3$), 3.79 (3H, s, OC*H*$_3$), 2.98 – 2.83 (2H, m, C*H*CF_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 149.3, 148.8 (*C*OMe), 133.8 (*ipso* Ar-*C*), 128.9, 128.4, 127.7 (Ar-*C*H), 126.1 (*ipso* Ar-*C*), 120.6 (Ar-*C*H), 113.2 (t, $^1J_{C\text{-}F}$ = 291.5, *C*F_2), 111.8, 111.5 (*C*HCOMe), 56.2 (2 x O*C*H_3), 34.2 (t, $^2J_{C\text{-}F}$ = 10.5, *C*HCF_2); ^{19}F NMR (282 MHz, $CDCl_3$) δ ppm -134.3 (ddd, $^2J_{F\text{-}F}$ = 152.0, $^3J_{H\text{-}F}$ = 10.5, 4.5), -135.0 (ddd, $^2J_{F\text{-}F}$ = 151.5, $^3J_{H\text{-}F}$ = 10.5, 4.5); HRMS (+ESI) *m*/*z* for ($C_{17}H_{16}F_2NaO_2$) [M+Na] calculated 131.1011 found 313.1014.

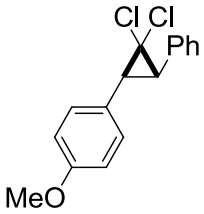
(±)-*trans*-1,1-Dichloro-2-(3-methoxyphenyl)-3-phenylcyclopropane (94)



Prepared in a similar manner to compound **1**, the reaction of *trans*-3-methoxystilbene **(63)** (0.84 g, 4 mmol, 1.0 equiv.) with $CHCl_3$ (8.0 mL, 99 mmol, 25 equiv.) and a solution of 50% aqueous NaOH (4.0 mL, 50 mmol, 12.5 equiv.) in the presence of

benzyltrimethylammonium bromide (20 mg, 86 µmol, ~2 mol%) gave a dark-brown crude product. The crude product was purified by standard silica chromatography using hexane as eluent to give **94 (**1.06 g, 91%) as a pale-yellow oil; IR (neat) υ_{max}/cm^{-1}: 3060 (C-H), 1600 (C=C), 1040 (C-O), 691(C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.35 – 7.10 (6H, m, Ar-*H*), 6.89 – 6.83 (1H, m, Ar-*H*), 6.82 – 6.73 (2H, m, C*H*COMe), 3.71 (3H, s, OC*H*$_3$), 3.10 (2H, s, C*H*CCl_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 160.0 (*C*OMe), 136.4, 134.8 (*ipso* Ar-*C*), 129.8, 129.3, 128.8, 128.2, 121.6 (Ar-C*H*) 115.1, 113.6 (C*H*COMe), 65.6 (CCl_2), 55.6 (OCH_3), 40.1, 40.0 (*C*HCCl_2); HRMS (+ESI) *m/*z for ($C_{16}H_{14}Cl_2NaO$) [M+Na] calculated 315.0315 found 315.0311.

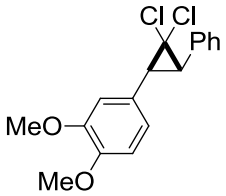
(±)-*trans*-1,1-Dichloro-2-(4-methoxyphenyl)-3-phenylcyclopropane (95)



Prepared in a similar manner to compound **1**, the reaction of *trans*-4-methoxy stilbene **(64)** (0.84 g, 4 mmol, 1.0 equiv.) with $CHCl_3$ (8.0 mL, 99 mmol, 25 equiv.) and a solution of 50% aqueous NaOH (4.0 mL, 50 mmol, 12.5 equiv.) in the presence of benzyltrimethylammonium bromide (20 mg, 87 µmol, ~2 mol%) gave a dark-brown crude product. The crude product was purified by standard silica chromatography using hexane as eluent to gave **95** (1.06 g, 91%) as a pale-yellow solid, mp 53 – 55 ºC; IR (neat) υ_{max}/cm^{-1}: 3032 (C-H), 1517 (C=C), 1253 (C-O), 696 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.44 – 7.23 (7H, m, Ar-*H*), 6.95 – 6.87 (2H, m, C*H*COMe), 3.80 (3H, s, OC*H*$_3$), 3.15 (2H, s, C*H*CCl_2); ^{13}C NMR (101 MHz,

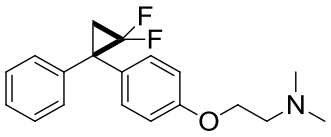
$CDCl_3$) δ ppm 159.5 (*C*OMe), 134.9 (*ipso* Ar-*C*), 130.3 129.2, 128.8, 128.1 (Ar-*C*H), 126.9 (*ipso* Ar-*C*), 114.2 (*C*HCOMe), 65.9 (CCl_2), 55.6 (OCH_3), 40.1, 39.4 ($CHCCl_2$); HRMS (+ESI) *m/*z for ($C_{16}H_{14}Cl_2NaO$) [M+Na] calculated 315.0315 found 315.0313.

(±)-*trans*-1,1-Dichloro-2-(3,4-dimethoxyphenyl)-3-phenylcyclopropane (96)



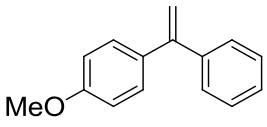
Prepared in a similar manner to compound **1** , the reaction of *trans*-3,4-dimethoxy stilbene **(65)** (0.38 g, 1.6 mmol, 1.0 equiv.) with $CHCl_3$ (3.2 mL, 39 mmol, 25 equiv.) and a solution of 50% aqueous NaOH (1.6 mL, 20 mmol, 12.5 equiv.) in the presence of benzyltrimethylammonium bromide (8 mg, 43 µmol, ~2 mol%) gave a dark-brown crude product. The crude product was purified by standard silica chromatography using hexane as eluent to gave **96** (0.45 g, 88%) as a pale-yellow solid, mp 93 - 94 ºC; IR (neat) υ_{max}/cm^{-1} 3059 (C-H), 1514 (C=C), 1137 (C-O), 702 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.39 – 7.16 (5H, m, Ar-*H*), 6.85 – 6.68 (3H, m, C*H*COMe and Ar-*H*), 3.79 (3H, s, OCH_3), 3.76 (3H, s, OCH_3), 3.13 – 2.96 (2H, m, C*H*CCl_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 149.1, 149.0 (*C*OMe), 134.8 (*ipso* Ar-*C*), 129.2, 128.7, 128.0 (Ar-*C*H), 127.2 (*ipso* Ar-*C*), 121.2 (Ar-*C*H), 112.5, 111.2 (*C*HCOMe), 65.7 (CCl_2), 56.2, 56.1 (OCH_3), 40.1, 39.6 ($CHCCl_2$); HRMS (+ESI) *m/*z for ($C_{17}H_{16}Cl_2NaO_2$) [M+Na^+] calculated 345.0420 found 345.0423.

(±)-1,1-Difluoro-2-(4-(2-(dimethylamino)ethoxy)phenyl)-2-phenylcyclopropane (97)



A mixture of 2-chloro-*N*,*N*-dimethylethylamine hydrochloride (0.43 g, 3 mmol, 0.75 equiv.), **101** (0.98 g, 4 mmol, 1.0 equiv.) and anhydrous K_2CO_3 (1.93 g, 14 mmol, 3.5 equiv.) in acetone (50 mL) was heated at reflux overnight. After cooling to ambient temperature, the solid was filtered and the filtrate was concentrated *in vacuo* to give a crude material. The crude product was purified by standard silica chromatography using 20:80 acetone:hexane as eluent to give **97** (0.74 g, 77%) as a pale-yellow oil which solidified upon storage at 0 ºC, mp 62 – 64 ºC; IR (neat) υ_{max}/cm^{-1} 3012 (C-H), 2756 (N-CH_2) 1608, 1512, 1474 (Ar-H), 1206 (C-F), 977, 796, 696 (Ar-H); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.23 – 6.99 (7H, m, Ar-H), 6.71 – 6.64 (2H, m, CH_2OCCH), 3.83 (2H, t, *J* = 6.0, OCH_2CH_2), 2.50 (2H, t, *J* = 6.0, OCH_2CH_2), 2.12 (6H, s, $N(CH_3)_2$), 1.91 – 1.78 (2H, m,CF_2CH_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 158.4 ($CHCOCH_2$), 139.3, 131.2 (*ipso* Ar-*C*), 130.2, 129.0, 128.9, 127.5 (Ar-*C*H), 115.0 ($CHCOCH_2$), 113.4 (t, $^1J_{C\text{-}F}$ = 288.5, CF_2), 66.2 (OCH_2CH_2), 58.5 (OCH_2CH_2), 46.2 ($N(CH_3)_3$), 39.7 (t, $^2J_{C\text{-}F}$ = 10.5, CH_2CCF_2), 24.0 (t, $^2J_{C\text{-}F}$ = 10.0, CH_2CCF_2); ^{19}F NMR (282 MHz, $CDCl_3$) δ ppm -130.6- -130.8 (m); HRMS (+ESI) *m*/*z* for ($C_{19}H_{22}F_2NO$) [M+H] calculated 318.1664 found 318.1668.

4-Methoxy-α-phenylstyrene (99)

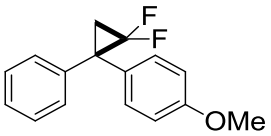


This synthesis of this compound was adopted from the method of Maike *et al.*[253] A solution of acetophenone (2.4 g, 50 mmol, 1.0 equiv.) in THF (150 mL) was added dropwise to 4-(methoxyphenyl) magnesium bromide (prepared by adding 4-bromoanisole (11.2 g, 60 mmol, 1.0 equiv.) in THF (30 mL) to magnesium turning

(1.54 g, 66 mmol, 3.1 equiv.) in THF (50 mL) with a few grains of I_2 crystals] cooled on in ice bath. After the reaction was stirred for 1 hour and the reaction was completed as monitored by TLC. The reaction was quenched by water (100 mL) and neutralized with diluted 1 M HCl solution to pH 7 as determined by indicator paper. The organic material was extracted with diethyl ether (3 x 50 mL), dried over $MgSO_4$ to afford an oil. The crude oil was purified by standard silica chromatography hexane to give **99** (2.56 g, 25%) as a white solid, mp 74 - 75 ºC, (lit.,[255] mp 75.3 – 76.8 ºC); IR (neat) υ_{max}/cm^{-1} 3050 (C-H), 1496 (Ar-H), 1140 (C-F); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.33 – 7.10 (5H, m, Ar-*H*), 6.79 (2H, m, Ar-*H*), 5.36 (1H, d, *J* = 13.0, H_2C=C), 5.30 (1H, d, *J* = 13.0, H_2C=C), 3.82 – 3.67 (3H, s, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 159.7 (*C*OMe), 149.8, 142.1, 134.3 (*ipso* Ar-C), 129.7, 128.6, 128.4, 128.0 (Ar-*C*H), 113.8 (*C*HCOMe), 113.3 (H_2C=C), 55.6 (O*C*H_3); HRMS (+ESI) *m/z* for ($C_{18}H_{22}NO$) [M+H] calculated 268.1701 found 268.1697.

These data were consistent with those previously reported.[255]

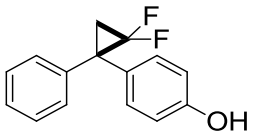
(±)-1,1-Difluoro-2-(4-methoxyphenyl)-2-phenylcyclopropane (101)



To a 5 mL pressure tube containing 1-(4-methoxyphenyl)-1-phenylethylene (**99**) (0.21 g, 1 mmol, 1.0 equiv.) in CH_3CN (2 mL) was added NaI (0.37 g, 2.5 mmol, 2.5 equiv.) and $TMSCF_3$ (0.39 mL, 2.5 mmol, 2.5 equiv.) and the pressure tube was securely sealed. The resulting mixture was heated at 80 ºC for 15 minutes. After that, the mixture was filtered and the solid washed with CH_3CN (2 x 5 mL). The filtrate and washings were evaporated *in vacuo* to give a dark-brown residue. The crude

product was purified by standard silica chromatography using hexane as eluent to give **101** (0.22 g, 84%) as a pale-yellow solid, mp 58 – 59 ºC; IR (neat) υ_{max}/cm^{-1} 3007 (C-H), 1610, 1511, 1443 (Ar-H), 1251 (C-F), 978, 795, 696 (Ar-H); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.37 – 7.10 (7H, m, Ar-*H*), 6.86 – 6.69 (2H, m, C*H*COMe), 3.64 (3H, s, OC*H*$_3$), 2.05 – 1.85 (2H, m, C*H*$_2$CF$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 159.1 (CH*C*OCH$_3$), 139.3, 131.1 (*ipso* Ar-*C*), 130.3, 129.0, 128.9, 127.5 (Ar-*C*H), 114.3 (*C*HCOCH$_3$), 113.5 (t, $^{1}J_{C\text{-}F}$ = 288.5, *C*F$_2$), 55.4 (O*C*H$_3$), 39.7 (t, $^{2}J_{C\text{-}F}$ = 10.5, *C*H$_2$CCF$_2$), 24.0 (t, $^{2}J_{C\text{-}F}$ = 10.0, *C*H$_2$CCF$_2$); ^{19}F NMR (282 MHz, $CDCl_3$) δ ppm -131.2 - -129.8 (m); HRMS (+ESI) *m*/*z* for ($C_{16}H_{14}F_2NaO$) [M+H] calculated 283.0905 found 283.0917.

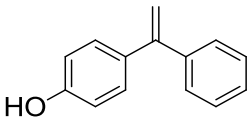
(±)-1,1-Difluoro-2-(4-hydroxyphenyl)-2-phenylcyclopropane (102)



To a 35 mL pressure tube containing 4-hydroxy-α-phenylstyrene (**104**) (1.05 g, 5 mmol, 1.0 equiv.) in CH_3CN (20 mL) was added NaI (3.75 g, 25 mmol, 5.0 equiv.) and $TMSCF_3$ (3.7 mL, 25 mmol, 5.0 equiv.) and the pressure tube was securely sealed. The resulting mixture was heated at 80 ºC for 2 hours. After that, the mixture was filtered and the solid washed with CH_3CN (2 x 10 mL). The filtrate and washings were evaporated *in vacuo* to give a dark-brown residue. The residue was re-dissolved in methanol (15 mL) followed by addition of *N*-bromosuccinimide (0.18 g, 1 mmol, 0.2 equiv.). The resulting mixture was then stirred at room temperature for 2 hours. After that, the methanol was evaporated *in vacuo* to give a crude material. The crude product was purified by standard silica chromatography using 20:80 EtOAc:hexane as eluent to give **102** (0.82 g, 67%) as a pale-yellow oil which solidified on standing, mp 75 – 77 ºC; IR (neat) υ_{max}/cm^{-1} ~3200 br (OH), 3026 (C-

H), 1613, 1513, 1443 (Ar-H), 1205 (C-F), 976, 795, 696 (Ar-H); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.40-7.18 (7H, m, Ar-*H*), 6.77-6.72 (2H, m, HOCC*H*), 5.26 (1H, br s, O*H*), 2.10-1.94 (2H, m, CH_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 154.8 (CH*C*OCH_2), 139.0, 131.1 (*ipso* Ar-*C*), 130.2, 128.7, 128.6, 127.3 (Ar-*C*H), 115.5 (*C*HCOCH_2), 113.1 (t, $^{1}J_{C\text{-}F}$ = 288.5, *C*F_2), 39.4 (t, $^{2}J_{C\text{-}F}$ = 10.5, CH_2*C*CF_2), 23.8 (t, $^{2}J_{C\text{-}F}$ = 10.0, *C*H_2CCF_2); ^{19}F NMR (282 MHz, $CDCl_3$) δ ppm -130.6 - -130.7 (m); HRMS (+ESI) *m*/*z* for ($C_{15}H_{12}F_2NaO$) [M+H] calculated 269.0748 found 269.0748. This compound is known, but no synthesis has been reported and only ^{1}H NMR spectroscopic data were recorded which were consistent with those reported here.[259]

4-Hydroxy-α-phenylstyrene (104)

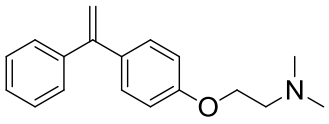


This compound was prepared according to the method of Maike *et al.*[253] A solution of 4-hydroxyacetophenone (2.72 g, 20 mmol, 1.0 equiv.) in THF (150 mL) was added dropwise to PhMgBr (62 mmol, 3.1 equiv.) [prepared by adding PhBr (6.53 mL, 62 mmol, 3.1 equiv.) in THF (30 mL) to Mg turning (1.60 g, 66 mmol, 3.3 equiv.) in THF (30 mL) with a few grains of I_2 crystals] cooled on in ice bath. After the reaction was stirred for 1 hour and the reaction was completed as monitored by TLC. The reaction was quenched by water (100 mL) and neutralized with diluted 1 M HCl solution to pH 7 as determined by indicator paper. The organic material was exacted by diethyl ether (3 x 50 mL), dried over $MgSO_4$ to give afford an oil. The crude oil was purified by standard silica chromatography using 20:80 EtOAc:hexane to give **104** (3.49 g, 89%) as a pale-yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 7.30-7.21 (5H, m, Ar-*H*), 7.17−7.11 (2H, m, HOCCHC*H*), 6.75-6.67 (2H, m,

HOCC*H*), 6.20-5.80 (1H, m, O*H*), 5.35 (1H, d, *J* = 1.2, C*H*$_2$), 5.31 (1H, d, *J* = 1.2, C*H*$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 155.8 (*C*-OH), 149.8, 142.1, 134.3 (*ipso* Ar-*C* and *C*CH_2) 129.9, 128.6, 128.4, 128.0, 115.4 (Ar-*C*H), 113.2 (*C*H_2); HRMS (+ESI) *m*/*z* for ($C_{14}H_{13}O$) [M+H] calculated 197.0961 found 197.0961.

These data were consistent with those previously reported.[254]

1-Phenyl-1-(4-(2-(diethylamino)ethoxy)phenyl)-ethylene (105)

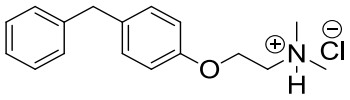


105 was prepared by adapting the method of Sato *et al.*[256] 2-Chloro-*N*,*N*-dimethylethylamine hydrochloride (1.30 g, 9 mmol, 1.8 equiv.) and K_2CO_3 (7.15 g, 51 mmol, 10.0 equiv.) were stirred in acetone/H_2O (80 mL/9 mL) at 0 ºC for 30 minutes, then 4-hydroxy-α-phenylstyrene (**104**) (1.0 g, 5.1 mmol, 1.0 equiv.) and K_2CO_3 (1.95 g, 14 mmol, 2.8 equiv.) were added, and the resulting mixture was heated at reflux for 24 h. After cooling to ambient temperature, the inorganic materials were filtered and the filtrate was evaporated *in vacuo*. The residue was purified by standard silica chromatography using 20:80 acetone:hexane as eluent to give **105** (1.1 g, 81%) as a pale-yellow solid, mp 47 – 48 ºC, (lit.,[257] mp 47 - 48 ºC); IR (neat) υ_{max}/cm^{-1} 2930 (C-H), 2763 (N-CH_2) 1602, 1506, 1442, 1032, 842, 700 (Ar-H); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.40 – 7.04 (7H, m, Ar-*H*), 6.94 – 6.58 (2H, m, C*H*COCH_2), 5.29 (1H, d, *J* = 15.0, PhCC*H*$_2$Ar), 5.27 (1H, d, *J* = 15.0, PhCC*H*$_2$Ar), 3.98 (2H, t, *J* = 5.5, OC*H*$_2$CH_2), 2.64 (2H, t, *J* = 5.5, OCH_2C*H*$_2$), 2.25 (6H, s, N(C*H*$_3$)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 158.8 (CH*C*OCH_2), 149.7, 142.0, 134.2 (*ipso* Ar-*C* and Ar_2C) 129.6, 128.5, 128.3, 127.9 (Ar-*C*H), 114.4 (C*H*OCH_2CH_2), 113.1 (PhC*C*H_2Ar), 66.2 (O*C*H_2CH_2), 58.5 (OCH_2*C*H_2), 46.1

($N(CH_3)_2$); HRMS (+ESI) *m*/*z* for ($C_{18}H_{22}NO$) [M+H] calculated 268.1696 found 268.1697.

This compound is known and has previously been reported with ^{13}C NMR spectroscopic data consistent with those reported here.[258]

[2-(4-Benzyl-phenoxy)-ethyl]-diethyl-amine hydrochloride (111)

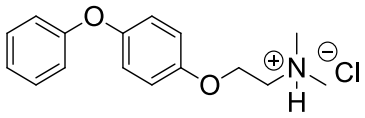


The synthesis of this compound was adopted from the method of Poirot *et al.*[260] 2-Chloro-*N*,*N*-dimethylethylamine hydrochloride (0.72 g, 5 mmol, 1.0 equiv.) was added to a solution of 4-benzylphenol (0.92 g, 5 mmol, 1.0 equiv.) and anhydrous K_2CO_3 (2.07 g, 15 mmol, 3.0 equiv.) in 1:1 DMF:acetone (30 mL). The reaction mixture was heated at 60 ºC for overnight. After cooling to ambient temperature, the solid was filtered and the filtrate was poured into water (100 mL). The organic material was extracted twice with diethyl ether (2 x 100 mL). The organic extracts were combined and washed with 0.1 N sodium hydroxide solution (3 x 15 mL) and then with brine (3 x 15 mL). After that, the organic layer was extracted with a solution of 12 N hydrochloric acid (5 mL). The aqueous layer was collected and evaporated *in vacuo* to give a crude solid. The crude product was recrystallized to give **111** (1.17 g, 80%) as a white solid, mp 175 – 176 ºC (3:1 isopropnaol:acetone), (lit.,[260] mp 178 - 180 ºC); IR (neat) υ_{max}/cm^{-1} 3010 (C-H), 2463 (R_3N-H) 1606, 1509, 1464, 993, 767, 694 (Ar-H); ^1H NMR (300 MHz, D_2O) δ ppm 7.03 – 6.71 (7H, m, Ar-*H*), 6.67 – 6.53 (2H, m,C*H*$COCH_2CH_2$), 3.87 (2H, t, *J* = 5, OCH_2CH_2), 3.47 (2H, s, $PhCH_2Ar$), 3.21 (2H, t, *J* = 5, OCH_2CH_2), 2.65 (6H, s, $N(CH_3)_3$); ^{13}C NMR (75 MHz, D_2O) δ ppm 155.6 ($CHCOCH_2$), 141.7, 134.6 (*ipso* Ar-*C*), 129.8, 128.6,

128.5, 126.0 (Ar-*C*H), 114.7 (*C*HCOCH$_2$), 61.5 (O*C*H$_2$CH$_2$), 56.0 (OCH$_2$*C*H$_2$), 42.7 (N(*C*H$_3$)$_3$), 40.4 (Ph*C*H$_2$Ar); HRMS (ESI) *m*/*z* for ($C_{17}H_{22}NO$) [M-Cl] calculated 256.1696 found 256.1697.

This compound is known, but only ^{1}H NMR spectroscopic data were recorded in *d*$_6$-DMSO and CD_3OD.[191, 213]

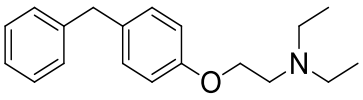
[2-(4-Phenoxy-phenoxy)-ethyl]dimethyl-amine hydrochloride (112)



Prepared in a similar manner to **111,** 2-chloro-*N*,*N*-dimethylethylamine hydrochloride (0.72 g, 5 mmol, 1.0 equiv.) was reacted with 4-phenoxyphenol (0.93 g, 5 mmol, 1.0 equiv.) in the presence of anhydrous K_2CO_3 (2.07 g, 15 mmol, 3.0 equiv.) to give a crude solid. The crude product was recrystallized to give **112** (1.11 g, 75%) as a white solid, mp 136 – 138 ºC (3:1 isopropnaol:acetone), (lit., mp 163 - 164 ºC); IR (neat) υ_{max}/cm^{-1} 3000 (C-H), 2456 (R_3N-H), 1584, 1479, 1396, 992, 750, 691 (Ar-H); ^{1}H NMR (300 MHz, D_2O) δ ppm 7.20 (2H, t, *J* = 8.0, Ar-*H*), 7.02 – 6.81 (7H, m, Ar-*H*), 4.21 (2H, t, *J* = 5, OCH2CH$_2$), 3.5 (2H, t, *J* = 5, OCH$_2$*CH$_2$*), 2.90 (6H, s, N(C*H$_3$*)$_3$); ^{13}C NMR (75 MHz, D_2O) δ ppm 157.6 (*C*OCH$_2$CH$_2$), 153.6, 150.6 (*ipso* Ar-*C*), 129.8, 123.0, 120.6, 117.7, 115.9 (Ar-*C*H), 62.0 (O*C*H$_2$CH$_2$), 56.1 (OCH$_2$*C*H$_2$), 42.7 (N(*C*H$_3$)$_3$); HRMS (ESI) *m*/*z* for ($C_{16}H_{20}NO_2$) [M-Cl] calculated 258.1489 found 258.1490.

This compound is known, but no spectra data were recorded.[261]

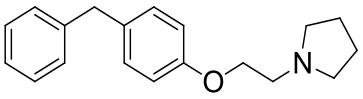
***N*,*N*-Diethyl-2-(4-benzylphenoxy)ethanamine (113)**



A mixture of 2-chloro-*N*,*N*-dimethylethylamine hydrochloride (0.52 g, 3 mmol, 0.75 equiv.), 4-benzylphenol (0.74 g, 4 mmol, 1.0 equiv.) and anhydrous K_2CO_3 (1.93 g, 14 mmol, 3.5 equiv.) in acetone (50 mL) was heated at reflux overnight. After cooling to ambient temperature, the solid was filtered and the filtrate was concentrated *in vacuo* to give a crude residue. The crude product was purified by standard silica chromatography 20:80 acetone:hexane to give **113** (0.70 g, 82%) as a pale-yellow oil; IR (neat) υ_{max}/cm^{-1} 2968 (C-H), 2808 (N-CH_2), 1611, 1509, 1453, 1030, 796, 696 (Ar-H); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.38 – 7.32 (2H, m, *ortho* Ar-*H*), 7.30 – 7.21 (3H, m, *meta*, *para* Ar-*H*), 7.17 (2H, m, *ortho* Ar-*H*), 6.91 (2H, m, C*H*COCH_2), 4.10 (2H, t, *J* = 6.5, OC*H*$_2$CH_2), 4.00 (2H, s, Ph-C*H*$_2$-Ar), 2.95 (2H, t, *J* = 6.5, OCH_2C*H*$_2$), 2.72 (4H, q, *J* = 7.0, NC*H*$_2$CH_3), 1.15 (6H, t, *J* = 7.0, NCH_2C*H*$_3$); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 157.5 (CH*C*OCH_2), 141.8, 133.5 (*ipso* Ar-*C*), 130.1, 129.1, 128.7, 126.2 (Ar-*C*H), 114.7 (CH*C*HCOCH_2), 66.7 (O*C*H_2CH_2), 52.0 (OCH_2*C*H_2), 48.0 (N*C*H_2CH_3), 41.3 (Ph-*C*H_2-Ar), 12.0 (NCH_2*C*H_3); HRMS (+ESI) *m*/*z* for ($C_{19}H_{26}NO$) [M+H] calculated 284.2009 found 284.2008.

The hydrochloride salt form of this compound is known,[191, 213] but the free amine form has never been reported.

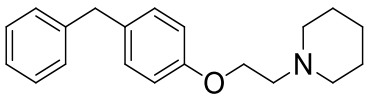
4-(2-(Pyrrolidin-1-yl)ethoxy)benzylbenzene (114)



Prepared in a similar manner to **113,** 1-(2-chloroethyl)pyrrolidine hydrochloride (0.51 g, 3 mmol, 0.75 equiv.) was reacted with 4-benzylphenol (0.74 g, 4 mmol, 1.0 equiv.) in the presence of anhydrous K_2CO_3 (1.93 g, 14 mmol, 3.5 equiv.) to give a crude material. The crude product was purified by silica chromatography using 20:80 acetone:hexane as eluent to give **114** (0.70 g, 83%) ; IR (neat) υ_{max}/cm^{-1} 2961 (C-H), 2779 (N-CH_2), 1611, 1509, 1453, 1031, 796, 696 (Ar-H); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.48 – 7.17 (7H, m, Ar-*H*), 7.09 – 6.89 (2H, m, C*H*COCH$_2$), 4.21 (2H, t, *J* = 6.0, OC*H*$_2$CH$_2$), 4.05 (2H, s, PhC*H*$_2$Ar), 3.02 (2H, t, *J* = 6.0, OCH$_2$C*H*$_2$), 2.78 – 2.70 (4H, m, N(C*H*$_2$CH$_2$)$_2$), 1.93 (4H, m, N(CH$_2$C*H*$_2$)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 157.5 (CH*C*OCH$_2$CH$_2$), 141.8, 133.5 (*ipso* Ar-*C*) 130.0, 129.0, 128.6, 126.2 (Ar-*C*H), 114.8 (*C*HCOCH$_2$CH$_2$), 67.3 (O*C*H$_2$CH$_2$), 55.3 (OCH$_2$*C*H$_2$), 54.9 (N(*C*H$_2$CH$_2$)$_2$), 41.3 (Ph-*C*H$_2$-Ar), 23.7 (N(CH$_2$*C*H$_2$)$_2$); HRMS (+ESI) *m*/*z* for ($C_{19}H_{24}NO$) [M+H] calculated 282.1852 found 282.1853.

This compound is known and has previously been reported with ^{1}H NMR spectroscopic data consistent with those reported here.[213]

4-(2-(Piperidin-1-yl)ethoxy)benzylbenzene (115)

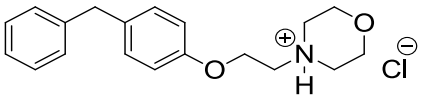


Prepared in a similar manner to **113,** 1-(2-chloroethyl)piperidine hydrochloride (3.59 g, 19.5 mmol, 3.63 equiv.) was reacted with 4-benzylphenol (1.0 g, 5.4 mmol, 1.0 equiv.) in the presence of K_2CO_3 (19.6 g, 0.14 mol, 26.0 equiv.) to give a crude material. The crude product was purified by silica chromatography using 20:80 acetone:hexane as eluent to give **115** (0.74 g, 46%) as a pale-yellow oil; IR (neat) υ_{max}/cm^{-1} 2931 (C-H), 2782 (N-CH_2), 1611, 1509, 1441, 1036, 794, 696 (Ar-H); ^{1}H

NMR (300 MHz, $CDCl_3$) δ ppm 7.41 – 7.11 (7H, m, Ar-*H*), 6.95 – 6.87 (2H, m, C*H*OCH_2CH_2), 4.15 (2H, t, *J* = 6.0, OC*H*$_2$$CH_2$), 3.99 (2H, s, Ph-C*H*$_2$-Ar), 2.83 (2H, t, *J* = 6.0, OCH_2C*H*$_2$), 2.69 – 2.46 (4H, m, CH_2NC*H*$_2$$CH_2CH_2$), 1.74 – 1.62 (4H, m, CH_2NCH_2C*H*$_2$$CH_2$), 1.51 (2H, m, $CH_2NCH_2CH_2$C*H*$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 157.4 (CH*C*OCH_2CH_2), 141.8, 133.5 (*ipso* Ar-*C*), 130.1, 129.0, 128.6, 126.2 (Ar-*C*H), 114.8 (CH*C*HCOCH_2), 66.1 (O*C*H$_2$$CH_2$), 58.2 (O$CH_2$*C*H$_2$), 55.3 ($CH_2$N*C*H$_2$$CH_2CH_2$), 41.3 (Ph-*C*H$_2$-Ar) 26.2 ($CH_2NCH_2$*C*H$_2$$CH_2$), 24.5 ($CH_2NCH$-$_2$$CH_2$*C*H$_2$); HRMS (+ESI) *m*/*z* for ($C_{20}H_{26}NO$) [M+H] calculated 296.2009 found 296.2007.

The hydrochloride salt form of this compound is known,[213] but the free amine form has never been reported.

4-[(2-(4-Benzyl-phenoxy)-ethyl]-morpholin hydrochloride (116)

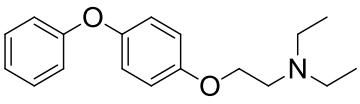


Prepared in a similar manner to **113**, 4-(2-chloroethyl)morpholine hydrochloride (1.86 g, 10 mmol, 3.6 equiv.) was reacted with 4-benzylphenol (0.51 g, 2.8 mmol, 1.0 equiv.) in the presence of anhydrous K_2CO_3 (10.0 g, 72 mmol, 26 equiv.) give a crude oil. The crude oil was purified by standard silica chromatography 20:80 acetone:hexane before a solution of 3N HCl (2 mL) was added and the solvent was evaporated *in vacuo*. The residue was recrystallized to give **116** (0.45 g, 49%) as a white solid, mp 193 – 195 ºC (acetone), (lit.,[191] mp 185 - 186 ºC); IR (neat) υ_{max}/cm^{-1} 2945 (C-H), 2448 (R_3N-H), 1608 1510, 1450, 1020, 856, 728 (Ar-H); ^{1}H NMR (400 MHz, D_2O) δ ppm 8.71 – 8.61 (2H, m, Ar-H), 8.61 – 8.52 (5H, m, Ar-H), 8.40 – 8.31 (2H, m, C*H*OCH_2CH_2), 5.79 (2H, t, *J* = 5.0, ArOC*H*$_2$$CH_2$), 5.55 – 5.18 (6H, m,

Ar-C*H*$_2$-Ph and N(CH$_2$C*H*$_2$)$_2$O or N(C*H*$_2$CH$_2$)$_2$O), 5.09 – 4.59 (6H, m, ArOCH$_2$C*H*$_2$ and N(CH$_2$C*H*$_2$)$_2$O or N(C*H*$_2$CH$_2$)$_2$O); ^{13}C NMR (101 MHz, D$_2$O) δ ppm 157.5 (CH*C*OCH$_2$CH$_2$), 143.0, 136.4 (*ipso* Ar-*C*), 131.1, 129.8, 129.5, 127.1 (Ar-*C*H), 115.8 (CH*C*HCOCH$_2$), 64.9 (N*C*H$_2$CH$_2$O or NCH$_2$*C*H$_2$O), 63.1 (ArO*C*H$_2$CH$_2$), 57.5 (ArOCH$_2$CH$_2$), 53.7 (N(*C*H$_2$CH$_2$)$_2$O or N(CH$_2$*C*H$_2$)$_2$O), 41.9 (Ar-*C*H$_2$-Ph); HRMS (+ESI) *m*/*z* for (C$_{19}$H$_{24}$NO$_2$) [M+H] calculated 298.1802 found 298.1805.

This compound is known, but only ^{1}H NMR spectroscopic data were recorded in *d*$_6$-DMSO and CD$_3$OD.[191, 213]

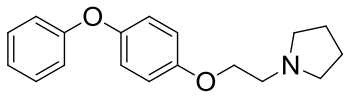
_N_,_N_-Diethyl-2-(4-phenoxyphenoxy)ethanamine (117)



Prepared in a similar manner to **113**, 2-chloro-*N*,*N*-diethylethylamine hydrochloride (0.51 g, 3 mmol, 0.75 equiv.) was reacted with 4-phenoxyphenol (0.74 g, 4 mmol, 1.0 equiv.) in the presence of anhydrous K$_2$CO$_3$ (1.93 g, 14 mmol, 3.5 equiv.) to give a crude material. The crude product was purified by silica chromatography using 20:80 acetone:hexane as eluent to give **117** (0.66 g, 77%) as a pale-yellow oil; IR (neat) υ_{max}/cm^{-1} 2968 (C-H), 2807 (N-CH$_2$), 1589, 1503, 1487, 1008, 840, 690 (Ar-H); ^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 7.29 – 7.16 (2H, m, Ar-*H*), 7.05 – 6.78 (7H, m, C*H*COCH$_2$), 3.98 (2H, t, *J* = 6.5, OC*H*$_2$CH$_2$), 2.83 (2H, t, *J* = 6.5, OCH$_2$C*H*$_2$), 2.60 (4H, q, *J* = 7.0, NC*H*$_2$CH$_3$), 1.03 (6H, t, *J* = 7.0, NCH$_2$C*H*$_3$); ^{13}C NMR (75 MHz, CDCl$_3$) δ ppm 158.8 (CH*C*OCH$_2$), 155.5, 150.4 (*ipso* Ar-*C*), 129.9, 122.7, 121.1, 117.9 (Ar-*C*H), 115.8 (CH*C*HCOCH$_2$), 67.3 (O*C*H$_2$CH$_2$), 52.1 (OCH$_2$*C*H$_2$),

48.1 (N*C*H_2CH_3), 12.2 (NCH$_2$*C*H$_3$); HRMS (+ESI) *m*/*z* for ($C_{18}H_{24}NO_2$) [M+H] calculated 286.1802 found 286.1798.

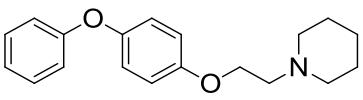
4-(2-(Pyrrolidin-1-yl)ethoxy)phenoxybenzene (118)



Prepared in a similar manner to **113**, 1-(2-chloroethyl)pyrrolidine hydrochloride (0.51 g, 3 mmol, 0.75 equiv.) was reacted with 4-phenoxyphenol (0.74 g, 4 mmol, 1.0 equiv.) in the presence of anhydrous K_2CO_3 (1.93 g, 14 mmol, 3.5 equiv.) to give a crude material. The crude product was purified by silica chromatography using 20:80 acetone:hexane as eluent to give **118** (0.78 g, 92%) as a pale-yellow oil; IR (neat) υ_{max}/cm^{-1} 2960 (C-H), 2781 (N-CH$_2$), 1589, 1503, 1036, 840, 690 (Ar-H); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.30 – 7.16 (7H, m, Ar-*H*), 7.11 – 6.75 (2H, m, C*H*COCH$_2$), 4.03 (2H, t, *J* = 5.5, OC*H*$_2$CH$_2$), 2.84 (2H, t, *J* = 5.5, OCH$_2$C*H*$_2$), 2.73 – 2.41 (4H, m, N(C*H*$_2$CH$_2$)$_2$), 1.90 – 1.59 (4H, m, N(CH$_2$C*H*$_2$)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 158.6 (CH*C*OCH$_2$), 155.4, 150.4 (*ipso* Ar-*C*), 129.8, 122.6, 121.0, 117.8 (Ar-*C*H), 115.8 (CHC*H*COCH$_2$), 67.7 (O*C*H$_2$CH$_2$), 55.3 (OCH$_2$*C*H$_2$), 54.9 (N(*C*H$_2$CH$_2$)$_2$), 23.7 (N(CH$_2$*C*H$_2$)$_2$); HRMS (+ESI) *m*/*z* for ($C_{18}H_{22}NO_2$) [M+H] calculated 284.1645 found 284.1645.

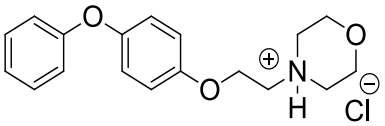
This compound is known, but only ^{1}H NMR spectroscopic data were recorded in d_6-DMSO.[262]

4-(2-(Piperidin-1-yl)ethoxy)phenoxybenzene (119)



1-(2-chloroethyl)piperidine hydrochloride (3.59 g, 19.5 mmol, 3.63 equiv.) and K_2CO_3 (15.4 g, 0.11 mol, 20.7 equiv.) were stirred in acetone/H2O (180 mL/ 20 mL) at 0 ºC for 30 minutes, then 4-phenoxyphenol (1.0 g, 5.4 mmol, 1.0 equiv.) and K_2CO_3 (4.2 g, 30 mmol, 5.6 equiv.) were added, and the resulting mixture was heated at reflux for overnight. After cooling to ambient temperature, the inorganic materials were filtered and the filtrate was concentrated *in vacuo* to give a crude residue. The crude residue was purified by standard silica chromatography using 20:80 acetone:hexane to give **119** (0.93 g, 59%) as a pale-yellow oil; IR (neat) υ_{max}/cm^{-1} 2947 (C-H), 2800 (N-CH_2), 1583, 1502, 1475, 1003, 758, 694 (Ar-H); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.29 – 7.18 (7H, m, Ar-*H*), 7.04 – 6.79 (2H, m, C*H*OCH_2CH_2), 4.04 (2H, t, *J* = 6.0, OC*H*$_2$$CH_2$), 2.76 – 2.68 (2H, t, *J* = 6.0, OCH_2C*H*$_2$), 2.51 – 2.39 (4H, m, CH_2NC*H*$_2$$CH_2CH_2$), 1.66 – 1.49 (4H, m, CH_2NCH-$_2$C*H*$_2$$CH_2$), 1.50 – 1.31 (2H, m, $CH_2NCH_2CH_2$C*H*$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 158.8 (CH*C*OCH_2), 155.4, 150.4 (*ipso* Ar-*C*), 129.9, 122.7, 121.0, 117.9 (Ar-*C*H), 115.9 (CH*C*HCOCH_2), 66.7 (O*C*H$_2$$CH_2$), 58.3 ($OCH_2$*C*H$_2$), 55.4 ($CH_2$N*C*H-$_2$$CH_2CH_2$), 26.1 ($CH_2NCH_2$*C*H$_2$$CH_2$), 24.5 ($CH_2NCH_2CH_2$*C*H$_2$); HRMS (+ESI) *m*/*z* for ($C_{19}H_{24}NO_2$) [M+H] calculated 298.1802 found 298.1803.

1-[2-(4-Phenoxy-phenoxy)-ethyl]-morpholine hydrochloride (120)

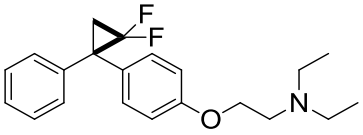


Prepared in a similar manner to **113**, 4-(2-chloroethyl)morpholine hydrochloride (1.86 g, 10 mmol, 3.6 equiv.) was reacted with 4-phenoxyphenol (0.51 g, 2.8 mmol, 1.0 equiv.) in the presence of anhydrous K_2CO_3 (10.0 g, 72 mmol, 26 equiv.) to give a crude oil. The crude oil was purified by standard silica chromatography using

20:80 acetone:hexane as eluent before a solution of 3N HCl (2 mL) was added and the solvent was evaporated *in vacuo* to give a crude residue. The crude residue was purified by recrystallization to give **120** (0.42 g, 46%) as a white solid, mp 177 - 178 ºC (acetone), (lit.,[191] mp 170 - 172 ºC); IR (neat) υ_{max}/cm^{-1} 2961 (C-H), 2470 (R_3N-H), 1611, 1509, 1453, 1031, 796, 696 (Ar-H); ^{1}H NMR (400 MHz, d_6-DMSO) δ ppm 7.43 – 7.35 (2H, m, Ar-*H*), 7.15 – 7.02 (5H, m, Ar-*H*), 7.01 – 6.93 (2H, m, C*H*OCH_2CH_2), 4.50 (2H, t, *J* = 4.5, ArOC*H*$_2$$CH_2$), 4.09 – 3.81 (4H, m, N(CH_2C*H*$_2$)$_2$O or N(C*H*$_2$$CH_2$)$_2$O), 3.55 (4H, m, Ar$OCH_2$C*H*$_2$ and N(CH_2C*H*$_2$)$_2$O or N(C*H*$_2$$CH_2$)$_2$O), 3.36 – 3.14 (2H, m, N(CH_2C*H*$_2$)$_2$O or N(C*H*$_2$$CH_2$)$_2$O); ^{13}C NMR (101 MHz, d_6-DMSO) δ ppm 158.8 (CH*C*OCH_2CH_2), 154.7, 151.0 (*ipso* Ar-*C*), 130.8, 123.6, 121.6 (Ar-*C*H), 118.3 (CH*C*HCOCH_2), 117.0 (Ar-*C*H), 64.0 (N*C*H_2CH_2O or NCH_2*C*H_2O), 63.7 (ArO*C*H_2CH_2), 55.7 (ArOCH_2*C*H_2), 52.5 (N(*C*H_2CH_2)$_2$O or N(CH_2*C*H_2)$_2$O); HRMS (ESI) *m*/*z* for ($C_{18}H_{22}NO_3$) [M-Cl] calculated 300.1594 found 300.1594.

This compound is known and has been previously reported with ^{1}H NMR spectroscopic data consistent with reported here.[191]

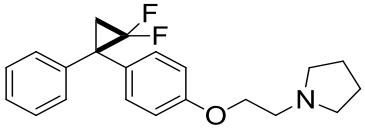
(±)-1,1-Difluoro-2-(4-(2-(diethylamino)ethoxy)phenyl)-2-phenylcyclopropane (121)



Prepared in a similar manner to **113**, 2-chloro-*N*,*N*-diethylethylamine hydrochloride (0.52 g, 3 mmol, 0.75 equiv.) was reacted with **104** (0.98 g, 4 mmol, 1.0 equiv.) and anhydrous K_2CO_3 (1.93 g, 14 mmol, 3.5 equiv.) to give a crude oil. The crude oil was purified by standard silica chromatography using 20:80 acetone:hexane as eluent

to give **121** (0.71 g, 69%) as a pale-yellow oil; IR (neat) υ_{max}/cm^{-1} 2968 (R_3C-H), 2803 (N-CH_2) 1610, 1511, 1454 (Ar-CH), 1211 (C-F), 975, 794, 698 (Ar-H); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.52 – 7.00 (7H, m, Ar-*H*), 6.81 (2H, m, CH_2OCCH), 4.13 (2H, t, *J* = 5.5, OCH_2CH_2), 3.01 (2H, t, *J* = 5.5, OCH_2CH_2), 2.79 (4H, q, *J* = 7.0, CH_2CH_3), 2.05 – 1.87 (2H, m, CF_2CH_2), 1.14 (6H, t, *J* = 7.0, CH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 157.7 ($CHCOCH_2$), 139.1, 131.5 (*ipso* Ar-*C*), 130.3, 128.9, 128.8, 127.5 (Ar-*C*H), 114.8 (*C*HCOCH$_2$), 113.3 (t, $^1J_{C\text{-}F}$ = 288.5, CF_2), 65.3 (OCH_2CH_2), 51.4 (OCH_2CH_2), 47.7 (NCH_2CH_3), 39.5 (t, $^2J_{C\text{-}F}$ = 10.5, CH_2CCF_2), 23.9 (t, $^2J_{C\text{-}F}$ = 10.0, CH_2CCF_2), 10.8 (CH_2CH_3); ^{19}F NMR (282 MHz, $CDCl_3$) δ ppm -129.0 - -132.1 (m); HRMS (+ESI) *m*/*z* for ($C_{21}H_{26}F_2NO$) [M+H] calculated 346.1977 found 346.1976.

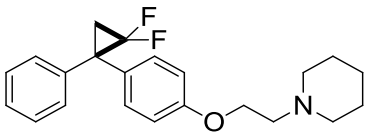
(±)-1,1-Difluoro-2-(4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-2-phenylcyclopropane (122)



Prepared in a similar manner to **113**, 1-(2-chloroethyl)pyrrolidine hydrochloride (0.51 g, 3 mmol, 0.75 equiv.) was reacted with **104** (0.98 g, 4 mmol, 1.0 equiv.) and anhydrous K_2CO_3 (1.93 g, 14 mmol, 3.5 equiv.) to give a crude oil. The crude oil was purified by standard silica chromatography using 20:80 acetone:hexane as eluent to give **122 (**0.78 g, 76%) as an oil which solidified upon storage at 0 ºC, mp 44 – 45 ºC; IR (neat) υ_{max}/cm^{-1} 2959 (C-H), 2816 (N-CH_2) 1610, 1512, 1444 (Ar-CH), 1205 (C-F), 977, 790, 697 (Ar-H); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.38 – 7.11 (7H, m, Ar-*H*), 6.85 – 6.76 (2H, m, CH_2OCCH), 4.04 (2H, t, *J* = 6.0, OCH_2CH_2), 2.84 (2H, t, *J* = 6.0, OCH_2CH_2), 2.67 – 2.45 (4H, m, $N(CH_2)_2$), 2.04 – 1.90 (2H, m,

CF_2CH_2), 1.82 – 1.68 (6H, m, NCH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 158.3 ($CHCOCH_2$), 139.2, 131.2 (*ipso* Ar-*C*), 130.2, 129.0, 128.8, 127.5 (Ar-*C*H), 114.9 ($CHCOCH_2$), 113.4 (t, $^1J_{C\text{-}F}$ = 288.5, CF_2), 67.1 (OCH_2CH_2), 55.2 (OCH_2CH_2), 54.9 ($N(CH_2CH_2)_2$), 39.5 (t, $^2J_{C\text{-}F}$ = 10.5, CH_2CCF_2), 24.0 (t, $^2J_{C\text{-}F}$ = 10.0, CH_2CCF_2), 23.7 ($N(CH_2CH_2)_2$); ^{19}F NMR (282 MHz, $CDCl_3$) δ ppm -129.5 - -130.6 (m); HRMS (+ESI) *m*/*z* for ($C_{21}H_{24}F_2NO$) [M+H] calculated 344.1820 found 344.1819.

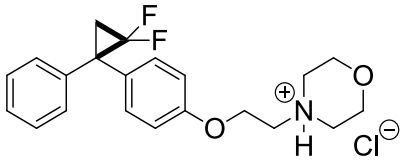
(±)-1,1-Difluoro-2-(4-(2-(piperidin-1-yl)ethoxy)phenyl)-2-phenylcyclopropane (123)



Prepared in a similar manner to **113**, 1-(2-chloroethyl)piperidine hydrochloride (0.55 g, 3 mmol, 0.75 equiv.) was reacted with **104** (0.98 g, 4 mmol, 1.0 equiv.) in the presence of anhydrous K_2CO_3 (1.93 g, 14 mmol, 3.5 equiv.) to give a crude material. The crude product was purified by standard silica chromatography using 20:80 acetone:hexane as eluent to give **123** (0.70 g, 65%) as a pale-yellow solid, mp 80 – 81 ºC; IR (neat) υ_{max}/cm^{-1} 2930 (C-H), 2828 (N-CH_2), 1610, 1512, 1443 (Ar-CH), 1232 (C-F), 977, 790, 698 (Ar-H); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.42 – 7.08 (7H, m, Ar-*H*), 6.88 – 6.71 (2H, m, CH_2OCCH), 4.00 (2H, t, *J* = 6.0, OCH_2CH_2), 2.69 (2H, t, *J* = 6.0, OCH_2CH_2), 2.51 – 2.31 (4H, m, $NCH_2CH_2CH_2$), 2.06 – 1.86 (2H, m, CF_2CH_2), 1.62 – 1.47 (4H, $NCH_2CH_2CH_2$), 1.44 – 1.30 (2H, m, $NCH_2CH_2CH_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 158.3 ($CHCOCH_2$), 139.3, 131.2 (*ipso* Ar-*C*), 130.2, 129.0, 128.9, 127.5 (Ar-*C*H), 114.9 ($CHCOCH_2$), 113.4 (t, $^1J_{C\text{-}F}$ = 288.5, CF_2), 66.1 (OCH_2CH_2), 58.1 (OCH_2CH_2), 55.3 ($NCH_2CH_2CH_2$), 39.7 (t, $^2J_{C\text{-}F}$ = 10.5, CH_2CCF_2), 26.2, 24.4 ($NCH_2CH_2CH_2$ and $NCH_2CH_2CH_2$), 24.0 (t,

$^{2}J_{C\text{-}F}$ = 10.0, CH$_2$CCF$_2$); ^{19}F NMR (282 MHz, CDCl$_3$) δ ppm -130.1 - -131.2 (m); HRMS (+ESI) *m*/*z* for ($C_{22}H_{26}F_2NO$) [M+H] calculated 358.1977 found 358.1982.

(±)-1,1-Difluoro-2-(4-(2-(morpholin-1-yl)ethoxy)phenyl)-2-phenylcyclopropane (124)



Prepared in a similar manner to **113**, 4-(2-Chloroethyl)morpholine hydrochloride (0.55 g, 3 mmol, 0.75 equiv.) was reacted with **104** (0.98 g, 4 mmol, 1.0 equiv.) in the presence of anhydrous K_2CO_3 (1.93 g, 14 mmol, 3.5 equiv.) to give a crude oil. The crude oil was purified by standard silica chromatography using 20:80 acetone:hexane as eluent before a solution of 3N HCl (2 mL) was added and the solvent was evaporated *in vacuo* to give a crude material. The crude product was recrystallized to give **124** (0.52 g, 44%) as a white solid, mp 213 – 214 ºC (acetone); IR (neat) υ_{max}/cm^{-1}~3000 (C-H), 2383 (R$_3$N-H), 1609, 1513, 1444 (Ar-CH), 1207 (C-F), 978, 792, 701 (Ar-H); ^{1}H NMR (400 MHz, CD_2Cl_2) δ ppm 7.48 – 7.04 (7H, m, Ar-*H*), 6.82 (2H, m, CH$_2$OCC*H*), 4.47 – 4.42 (2H, m, ArOC*H*$_2$CH$_2$), 4.26 – 3.70 (4H, m, N(CH$_2$C*H*$_2$)$_2$O), 3.57 – 3.16 (4H, m, ArOCH$_2$C*H*$_2$ and N(C*H*$_2$CH$_2$)$_2$O), 2.98 (2H, br s, N(C*H*$_2$CH$_2$)$_2$O), 1.98 (2H, m, C*H*$_2$CF$_2$); ^{13}C NMR (126 MHz, CD_2Cl_2) δ ppm 157.1 (CH*C*OCH$_2$), 139.3, 132.7 (*ipso* Ar-*C*), 130.7, 129.2, 128.9, 127.9, 116.1 (Ar-*C*H), 113.8 (t, $^{1}J_{C\text{-}F}$ = 288.5, *C*F$_2$), 64.3 (N(*C*H$_2$CH$_2$)$_2$O), 63.3 (Ar-O*C*H$_2$CH$_2$), 56.9 (Ar-OCH$_2$*C*H$_2$), 53.1 (N(CH$_2$*C*H$_2$)$_2$O), 40.0 (t, $^{2}J_{C\text{-}F}$ = 10.5, CH$_2$*C*CF$_2$), 24.0 (t, $^{2}J_{C\text{-}F}$ = 10.0, *C*H$_2$CCF$_2$); ^{19}F NMR (282 MHz, $CDCl_3$) δ ppm -129.2 - -132.0 (m); HRMS (ESI) *m*/*z* for ($C_{21}H_{24}F_2NO_2$) [M-Cl] calculated 360.1770 found 360.1769.

6.2 Reference

1. K. J. Chavez, S. V. Garimella and S. Lipkowitz, *Breast disease*, 2010, 32, 35-48.
2. L. A. Torre, F. Bray, R. L. Siegel, J. Ferlay, J. Lortet-Tieulent and A. Jemal, *CA-Cancer J. Clin.*, 2015, 65, 87-108.
3. K. V. C. Coles, D. Ritchie Clincal and Translational Radiotherapy 2011.
4. G. Litwack, *Hormones and Breast Cancer*, Academic Press, 1st edn., 2013.
5. D. A. Morton, *Medical Issues in Social Security Disability*, James Pub. Inc, 1st edn., 2003.
6. M. Aapro and H. Wildiers, *Ann. Oncol.*, 2012, 23, 52-55.
7. E. M. Fox, M. G. Kuba, T. W. Miller, B. R. Davies and C. L. Arteaga, *Breast Cancer Research*, 2013, 15.
8. F. Lumachi, A. Brunello, M. Maruzzo, U. Basso and S. M. M. Basso, *Curr. Med. Chem.*, 2013, 20, 596-604.
9. H. M. Kronenberg, *Williams Textbook of Endocrinology*, Saunders, 11th edn., 2007.
10. W. Hollander, A. V. Chobanian and R. W. Wilkins, *J. Am. Med. Assoc.* , 1960, 174, 5-12.
11. R. C. Laughlin and T. F. Carey, *J. Am. Med. Assoc.* , 1962, 181, 339-340.
12. J. Avigan, D. Steinberg, H. E. Vroman, M. J. Thompson and E. Mosettig, *J. Biol. Chem.*, 1960, 235, 3123-3126.
13. R. E. M. a. V. C. J. P. Y. Maimov, *Tamoxifen-Pioneering Medicine in Breast Cance*, 2013 edn., 2015.
14. R. O. Kraft, *Cancer Chemoth. Rep. 1*, 1962, 25, 113-115.
15. A. L. Herbst, C. T. Griffiths and R. W. Kistner, *Cancer Chemoth. Rep. 1*, 1964, 43, 39-41.
16. M. J. Harper and A. L. Walpole, *J. Endocrinol.*, 1967, 37, 83-92.
17. Williams.Jg and J. D. Ellis, *J. Obstet. Gynaecol. Br. Commonw.*, 1973, 80, 844-847.
18. M. P. Cole, C. T. A. Jones and I. D. H. Todd, *Brit. J. Cancer*, 1971, 25, 270-&.
19. C. K. Osborne and S. A. W. Fuqua, *J. Clin. Oncol.*, 2000, 18, 3172-3186.
20. N. Heldring, A. Pike, S. Andersson, J. Matthews, G. Cheng, J. Hartman, M. Tujague, A. Stroem, E. Treuter, M. Warner and J.-A. Gustafsson, *Physiol. Rev.*, 2007, 87, 905-931.
21. C. S. Watson, Y. J. Jeng and M. Y. Kochukov, *Faseb J.*, 2008, 22, 3328-3336.
22. S. Turken, E. Siris, D. Seldin, E. Flaster, G. Hyman and R. Lindsay, *J. Natl. Cancer Inst.*, 1989, 81, 1086-1088.
23. C. C. McDonald, F. E. Alexander, B. W. Whyte, A. P. Forrest and H. J. Stewart, *Brit. Med. J.* , 1995, 311, 977-980.
24. K. Yaffe, G. Sawaya, I. Lieberburg and D. Grady, *J. Am. Med. Assoc.*, 1998, 279, 688-695.
25. S. Grilli, *Ann. Ist. Super. Sanita.*, 2006, 42, 170-173.
26. C. K. Osborne, *Breast Cancer Res. Treat.*, 1998, 51, 227-238.
27. B. L. Riggs and L. C. Hartmann, *N. Engl. J. Med.*, 2003, 348, 618-629.
28. C. J. Barnes, R. K. Vadlamudi and R. Kumar, *Cell. Mol. Life Sci.*, 2004, 61, 281-291.
29. V. C. Jordan, *Brit. J. Clin. Pharmacol.*, 1993, 110, 507-517.

30. M. Morrow and V. C. Jordan, *Breast-cancer prevention with antiestrogens*, Humana Press Inc., 2002.
31. S. H. Giordano, *Oncologist*, 2005, 10, 471-479.
32. L. K. Dhaliwal, V. Suri, K. R. Gupta and S. Sahdev, *J. Hum. Reprod. Sci.*, 2011, 4, 76-79.
33. H. N. Khan, R. Rampaul and R. W. Blamey, *Breast*, 2004, 13, 61-65.
34. P. de Medina, G. Favre and M. Poirot, *Curr. Med. Chem. Anticancer Agents*, 2004, 4, 491-508.
35. R. R. Love, R. B. Mazess, H. S. Barden, S. Epstein, P. A. Newcomb, V. C. Jordan, P. P. Carbone and D. L. Demets, *N. Engl. J. Med.*, 1992, 326, 852-856.
36. S. Z. Goldhaber, *Circulation*, 2005, 111, 539-541.
37. A. J. Lusis, *Nature*, 2000, 407, 233-241.
38. A. V. Finn, M. Nakano, J. Narula, F. D. Kolodgie and R. Virmani, *Arterioscler. Thromb. Vasc. Biol.*, 2010, 30, 1282-1292.
39. E. Lutgens, M. Gijbels, M. Smook, P. Heeringa, P. Gotwals, V. E. Koteliansky and M. Daemen, *Arterioscler. Thromb. Vasc. Biol.*, 2002, 22, 975-982.
40. H. L. Kirschenlohr, J. C. Metcalfe, P. L. Weissberg and D. J. Grainger, *Cardiovasc Res.*, 1995, 29, 848-855.
41. D. J. Grainger, J. C. Metcalfe, A. A. Grace and D. E. Mosedale, *J. Cell. Sci.*, 1998, 111, 2977-2988.
42. D. J. Grainger, *Cardiovasc Res.*, 2007, 74, 213-222.
43. V. Ivanovic, A. Melman, B. Davisjoseph, M. Valcic and J. Geliebter, *Nat. Med.*, 1995, 1, 282-284.
44. W. Cui, C. J. Kemp, E. Duffie, A. Balmain and R. J. Akhurst, *Cancer Res.*, 1994, 54, 5831-5836.
45. W. A. Border and N. A. Noble, *N. Engl. J. Med.*, 1994, 331, 1286-1292.
46. N. Sanderson, V. Factor, P. Nagy, J. Kopp, P. Kondaiah, L. Wakefield, A. B. Roberts, M. B. Sporn and S. S. Thorgeirsson, *Proc. Natl. Acad. Sci. U.S.A.*, 1995, 92, 2572-2576.
47. Z. Mallat, A. Gojova, C. Marchiol-Fournigault, B. Esposito, C. Kamate, R. Merval, D. Fradelizi and A. Tedgui, *Circ. Res.* , 2001, 89, 930-934.
48. D. J. Grainger, C. M. Witchell and J. C. Metcalfe, *Nat. Med.*, 1995, 1, 1067-1073.
49. D. J. Grainger, D. E. Mosedale, J. C. Metcalfe and E. P. Bottinger, *J. Cell. Sci.* , 2000, 113, 2355-2361.
50. D. J. Grainger, P. R. Kemp, A. C. Liu, R. M. Lawn and J. C. Metcalfe, *Nature*, 1994, 370, 460-462.
51. G. C. Blobe, W. P. Schiemann and H. F. Lodish, *N. Engl. J. Med.*, 2000, 342, 1350-1358.
52. Y. Vodovotz, C. Bogdan, J. Paik, Q. W. Xie and C. Nathan, *J. Exp. Med.*, 1993, 178, 605-613.
53. D. J. Grainger, P. L. Weissberg and J. C. Metcalfe, *Biochem. J.*, 1993, 294, 109-112.
54. D. J. Grainger and J. C. Metcalfe, *Nat. Med.*, 1996, 2, 381-385.
55. J. Reckless, J. C. Metcalfe and D. J. Grainger, *Circulation*, 1997, 95, 1542-1548.
56. K. Kiyono, H. I. Suzuki, H. Matsuyama, Y. Morishita, A. Komuro, M. R. Kano, K. Sugimoto and K. Miyazono, *Cancer Res.*, 2009, 69, 8844-8852.

57. C. F. Michiels, D. M. Schrijvers, G. R. Y. De Meyer and W. Martinet, *The Role of Autophagy in Atherosclerosis*, Elsevier Academic Press Inc., 2014.
58. S. Verheye, W. Martinet, M. M. Kockx, M. W. M. Knaapen, K. Salu, J. P. Timmermans, J. T. Ellis, D. L. Kilpatrick and G. R. Y. De Meyer, *J. Am. Coll. Cardiol.*, 2007, 49, 706-715.
59. W. Martinet and G. R. Y. De Meyer, *Curr. Atheroscler Rep.* , 2008, 10, 216-223.
60. J. K. Williams, J. D. Wagner, Z. Li, D. L. Golden and M. R. Adams, *Arterioscler. Thromb. Vasc. Biol.*, 1997, 17, 403-408.
61. S. C. Clarke, P. M. Schofield, A. A. Grace, J. C. Metcalfe and H. L. Kirschenlohr, *Circulation*, 2001, 103, 1497-1502.
62. M. B. Buck, J. K. Coller, T. E. Murdter, M. Eichelbaum and C. Knabbe, *Breast Cancer Res. Treat.*, 2008, 107, 15-24.
63. H. Gylling, S. Pyrhonen, E. Mantyla, H. Maenpaa, L. Kangas and T. A. Miettinen, *J. Clin. Oncol.*, 1995, 13, 2900-2905.
64. S. Y. Cho, J. H. Kim and Y. K. Paik, *Mol. Cells.*, 1998, 8, 233-239.
65. A. L. Holleran, B. Lindenthal, T. A. Aldaghlas and J. K. Kelleher, *Metabolism*, 1998, 47, 1504-1513.
66. R. Callaghan and C. F. Higgins, *Brit. J. Cancer*, 1995, 71, 294-299.
67. P. Debry, E. A. Nash, D. W. Neklason and J. E. Metherall, *J. Biol. Chem.*, 1997, 272, 1026-1031.
68. P. de Medina, B. L. Payre, J. Bernad, I. Bosser, B. Pipy, S. Silvente-Poirot, G. Favre, J. C. Faye and M. Poirot, *J. Pharm. Exp. Ther.*, 2004, 308, 1165-1173.
69. V. Guetta, R. M. Lush, W. D. Figg, M. A. Waclawiw and R. O. Cannon, *Am. J. Cardiol.*, 1995, 76, 1072-&.
70. H. Wiseman, G. Paganga, C. Riceevans and B. Halliwell, *Biochem. J.*, 1993, 292, 635-638.
71. P. de Medina, S. Silvente-Poirot and M. Poirot, *Autophagy*, 2009, 5, 1066-1067.
72. I. F. Wang, B.-S. Guo, Y.-C. Liu, C.-C. Wu, C.-H. Yang, K.-J. Tsai and C.-K. J. Shen, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, 109, 15024-15029.
73. A. Brichkina and D. V. Bulavin, *Autophagy*, 2012, 8, 1545-1547.
74. Y.-M. Wei, X. Li, M. Xu, J. M. Abais, Y. Chen, C. R. Riebling, K. M. Boini, P.-L. Li and Y. Zhang, *Cell. Physiol. Biochem.*, 2013, 31, 925-937.
75. R. A. Magarian and E. J. Benjamin, *J. Pharm. Sci.*, 1975, 64, 1626-1632.
76. B. W. Day, R. A. Magarian, P. T. Jain, J. T. Pento, G. K. Mousissian and K. L. Meyer, *J. Med. Chem.*, 1991, 34, 842-851.
77. S. S. Jonnalagadda, E. terHaar, E. Hamel, C. M. Lin, R. A. Magarian and B. W. Day, *Bioorg. Med. Chem.*, 1997, 5, 715-722.
78. L. B. Overacre and R. A. Magarian, *Bioorg. Chem.*, 1998, 26, 15-31.
79. P. T. Jain, J. T. Pento and R. A. Magarian, *Cancer Chemoth. Pharm.*, 1996, 38, 238-244.
80. R. A. Magarian and E. J. Benjamin, *J. Pharm. Sci.*, 1975, 64, 1626-1632.
81. J. T. Pento, R. A. Magarian and M. M. King, *Cancer Lett.*, 1982, 15, 261-269.
82. M. M. King, R. A. Magarian, J. Terao and G. L. Brueggemann, *J. Natl. Cancer Inst.*, 1985, 74, 447-451.
83. P. T. Jain, J. T. Pento and R. A. Magarian, *Breast Cancer Res. Treat.*, 1993, 25, 225-233.

84. E. terHaar and B. W. Day, *Anticancer Res.*, 1996, 16, 1107-1115.
85. C. A. Thomas, S. G. Grant, B. R. Pflug, R. H. Getzenberg and B. W. Day, *Urol. Oncol. Semin. Ori.*, 2008, 26, 378-385.
86. M. Fedorynski, *Chem. Rev.*, 2003, 103, 1099-1132.
87. D. Seyferth, *Acc. Chem. Res.*, 1972, 5, 65-&.
88. C. J. Ziegler and K. S. Suslick, *J. Organomet. Chem.*, 1997, 528, 83-90.
89. W. Perlikowska, A. M. Modro, T. A. Modro and M. J. Mphahlele, *J. Chem. Soc., Perkin Trans. 2*, 1996, 2611-2613.
90. T. Ohba, N. Tsuchiya, K. Nishimura, E. Ikeda, J. Wakayama and H. Takei, *Bioorg. Med. Chem. Lett.*, 1996, 6, 543-546.
91. C. M. Starks, *J. Am. Chem. Soc.*, 1971, 93, 195-&.
92. M. Makosza, *Pure Appl. Chem*, 1975, 43, 439-462.
93. F. Mohamadi and W. C. Still, *Tetrahedron Lett.*, 1986, 27, 893-896.
94. D. Griller, M. T. H. Liu and J. C. Scaiano, *J. Am. Chem. Soc.*, 1982, 104, 5549-5551.
95. U. H. Brinker, G. Y. Lin, L. X. Xu, W. B. Smith and J. L. Mieusset, *J. Org. Chem.*, 2007, 72, 8434-8451.
96. R. A. Magarian, S. Melton and G. Natarelli, *J. Pharm. Sci.*, 1972, 61, 1216-1219.
97. B. W. Day, R. A. Magarian, P. T. Jain, J. T. Pento, G. K. Mousissian and K. L. Meyer, *J. Med. Chem.*, 1991, 34, 842-851.
98. W. R. Dolbier and M. A. Battiste, *Chem. Rev.*, 2003, 103, 1071-1098.
99. K. B. Wiberg and P. R. Rablen, *J. Am. Chem. Soc.*, 1993, 115, 614-625.
100. K. B. Wiberg and M. Marquez, *J. Am. Chem. Soc.*, 1998, 120, 2932-2938.
101. S. Durmaz and H. Kollmar, *J. Am. Chem. Soc.*, 1980, 102, 6942-6945.
102. T. Taguchi, H. Sasaki, A. Shibuya and T. Morikawa, *Tetrahedron Lett.*, 1994, 35, 913-916.
103. A. Jończyk and G. Kaczmarczyk, *Tetrahedron Lett.*, 1996, 37, 4085-4086.
104. P. R. Schreiner, H. P. Reisenauer, F. C. Pickard Iv, A. C. Simmonett, W. D. Allen, E. Matyus and A. G. Csaszar, *Nature*, 2008, 453, 906-909.
105. D. Bourissou, O. Guerret, F. P. Gabbaï and G. Bertrand, *Chem. Rev.*, 1999, 100, 39-92.
106. R. Gleiter and R. Hoffmann, *J. Am. Chem. Soc.*, 1968, 90, 5457-5460.
107. J. F. Harrison, R. C. Liedtke and J. F. Liebman, *J. Am. Chem. Soc.*, 1979, 101, 7162-7168.
108. W. W. Schoeller, *J. Chem. Soc., Chem. Commun.*, 1980, 124-125.
109. R. A. Moss, L. Wang and K. Krogh-Jespersen, *J. Am. Chem. Soc.*, 2009, 131, 2128-2130.
110. D. L. S. Brahms and W. P. Dailey, *Chem. Rev.*, 1996, 96, 1585-1632.
111. J. M. C. Birchall, G. E.; Haszeldine, R. N. , *Proc. Chem. Soc*, 1960, 81-81.
112. Y. Chang and C. Cai, *Chem. Lett.*, 2005, 34, 1440-1441.
113. K. Oshiro, Y. Morimoto and H. Amii, *Synthesis*, 2010, 2080-2084.
114. C. J. Barnett, B. Huff, M. E. Kobierski, M. Letourneau and T. M. Wilson, *J. Org. Chem.*, 2004, 69, 7653-7660.
115. V. Rautenstrauch, H. J. Scholl and E. Vogel, *Angew. Chem. Int. Ed*, 1968, 7, 288-289.
116. J. M. Birchall, R. Fields, R. N. Haszeldine and R. J. McLean, *J. Fluorine Chem.*, 1980, 15, 487-495.
117. H. Millauer, W. Schwertfeger and G. Siegemund, *Angew. Chem. Int. Ed*, 1985, 24, 161-179.

118. R. A. Mitsch, *J. Am. Chem. Soc.*, 1965, 87, 758-761.
119. P. Balcerzak, M. Fedorynski and A. Jonczyk, *J. Chem. Soc., Chem. Commun.*, 1991, 826-827.
120. D. J. Burton and D. G. Naae, *J. Am. Chem. Soc.*, 1973, 95, 8467-8468.
121. W. R. Dolbier, H. Wojtowicz and C. R. Burkholder, *J. Org. Chem.*, 1990, 55, 5420-5422.
122. D. Seyferth, S. P. Hopper and K. V. Darragh, *J. Am. Chem. Soc.*, 1969, 91, 6536-6537.
123. D. Seyferth, H. Dertouzos, R. Suzuki and J. Y. P. Mui, *J. Org. Chem.*, 1967, 32, 2980-2984.
124. W. R. Cullen and M. C. Waldman, *J. Fluorine Chem.*, 1971, 1, 151-163.
125. R. Eujen and B. Hoge, *J. Organomet. Chem.*, 1995, 503, C51-C54.
126. W. R. Dolbier Jr, F. Tian, J.-X. Duan, A.-R. Li, S. Ait-Mohand, O. Bautista, S. Buathong, J. Marshall Baker, J. Crawford, P. Anselme, X. H. Cai, A. Modzelewska, H. Koroniak, M. A. Battiste and Q.-Y. Chen, *J. Fluorine Chem.*, 2004, 125, 459-469.
127. F. Tian, V. Kruger, O. Bautista, J.-X. Duan, A.-R. Li, W. R. Dolbier and Q.-Y. Chen, *Org. Lett.*, 2000, 2, 563-564.
128. F. Wang, W. Zhang, J. Zhu, H. Li, K.-W. Huang and J. Hu, *Chem. Commun.*, 2011, 47, 2411-2413.
129. F. Wang, T. Luo, J. B. Hu, Y. Wang, H. S. Krishnan, P. V. Jog, S. K. Ganesh, G. K. S. Prakash and G. A. Olah, *Angew. Chem. Int. Ed.*, 2011, 50, 7153-7157.
130. R. Krishnamurti, D. R. Bellew and G. K. S. Prakash, *J. Org. Chem.*, 1991, 56, 984-989.
131. W. R. Dolbier, F. Tian, J. X. Duan, A. R. Lia, S. Ait-Mohand, O. Bautista, S. Buathong, J. M. Baker, J. Crawford, P. Anselme, X. H. Cai, A. Modzelewska, H. Koroniak, M. A. Battiste and Q. Y. Chen, *J. Fluorine Chem.*, 2004, 125, 459-469.
132. T. W. W. R. Roth, M. Boenke, *Liebigs Ann. Recueil*, 1997, 1323-1327.
133. G. Ji, G. Chen, Z. Wu and X. Jiang, *Huaxue Xuebao*, 1987, 45, 904-909.
134. A. de la Hoz, A. Diaz-Ortiz and A. Moreno, *Chem. Soc. Rev.*, 2005, 34, 164-178.
135. D. J. Adams, J. H. Clark, L. B. Hansen, V. C. Sanders and S. J. Tavener, *J. Fluorine Chem.*, 1998, 92, 123-125.
136. G. K. S. Prakash and A. K. Yudin, *Chem. Rev.*, 1997, 97, 757-786.
137. K. O. Christe and W. W. Wilson, *J. Fluorine Chem.*, 1990, 47, 117-120.
138. R. G. Pearson and R. L. Dillon, *J. Am. Chem. Soc.*, 1953, 75, 2439-2443.
139. K. B. Harikumar and B. B. Aggarwal, *Cell Cycle*, 2008, 7, 1020-1035.
140. E. Morselli, M. C. Maiuri, M. Markaki, E. Megalou, A. Pasparaki, K. Palikaras, A. Criollo, L. Galluzzi, S. A. Malik, I. Vitale, M. Michaud, F. Madeo, N. Tavernarakis and G. Kroemer, *Cell Death Dis.*, 2010, 1, 10.
141. E. Morselli, G. Marino, M. V. Bennetzen, T. Eisenberg, E. Megalou, S. Schroeder, S. Cabrera, P. Benit, P. Rustin, A. Criollo, O. Kepp, L. Galluzzi, S. S. Shen, S. A. Malik, M. C. Maiuri, Y. Horio, C. Lopez-Otin, J. S. Andersen, N. Tavernarakis, F. Madeo and G. Kroemer, *J. Cell Biol.*, 2011, 192, 615-629.
142. N. Gurusamy, I. Lekli, S. Mukherjee, D. Ray, M. K. Ahsan, M. Gherghiceanu, L. M. Popescu and D. K. Das, *Cardiovasc Res.*, 2010, 86, 103-112.

143. J.-y. Wu, K.-w. Tsai, J.-j. Shee, Y.-z. Li, C.-h. Chen, J.-j. Chuang and Y.-w. Liu, *Acta Pharmacol. Sin.*, 2010, 31, 81-92.
144. L.-q. He, J.-h. Lu and Z.-y. Yue, *Acta Pharmacol. Sin.*, 2013, 34, 605-611.
145. A. Biederbick, H. F. Kern and H. P. Elsasser, *Eur. J. Cell Biol.*, 1995, 66, 3-14.
146. A. Niemann, A. Takatsuki and H. P. Elsasser, *J. Histochem. Cytochem.*, 2000, 48, 251-258.
147. M. Yamamoto, S. O. Suzuki and M. Himeno, *Oncol. Lett.*, 2010, 1, 489-493.
148. M. Gazvoda, N. Beranič, S. Turk, B. Burja, M. Kočevar, T. L. Rižner, S. Gobec and S. Polanc, *Eur. J. Med. Chem.*, 2013, 62, 89-97.
149. L. B. Overacre and R. A. Magarian, *Bioorganic Chemistry*, 1998, 26, 15-31.
150. K. Iseki, D. Asada, M. Takahashi, T. Nagai and Y. Kobayashi, *Tetrahedron: Asymmetry*, 1996, 7, 1205-1215.
151. H. Burger, R. Eujen and P. Moritz, *J. Organomet. Chem.*, 1991, 401, 249-260.
152. M. Rapp, X. H. Cai, W. Xu, W. R. Dolbier and S. F. Wnuk, *J. Fluorine Chem.*, 2009, 130, 321-328.
153. T. Smail and F. S. Rowland, *J. Phys. Chem.*, 1970, 74, 1866-1871.
154. M. Saunders and R. W. Murray, *Tetrahedron*, 1960, 11, 1-10.
155. I. B. M. Band, D. Lloyd, M. I. C. Singer and F. I. Wasson, *Chem. Commun.*, 1966, 544.
156. G. K. S. Prakash, C. Panja, H. Vaghoo, V. Surampudi, R. Kultyshev, M. Mandal, G. Rasul, T. Mathew and G. A. Olah, *J. Org. Chem.*, 2006, 71, 6806-6813.
157. L. J. Brandes, *Biochem. Biophys. Res.*, 1984, 124, 244-249.
158. R. L. Sutherland and M. S. Foo, *Biochem. Biophys. Res.*, 1979, 91, 183-191.
159. B. Kedjouar, P. de Medina, M. Oulad-Abdelghani, B. Payre, S. Silvente-Poirot, G. Favre, J. C. Faye and M. Poirot, *J. Biol. Chem.*, 2004, 279, 34048-34061.
160. B. Payre, P. de Medina, N. Boubekeur, L. Mhamdi, J. Bertrand-Michel, F. Terce, I. Fourquaux, D. Goudouneche, M. Record, M. Poirot and S. Silvente-Poirot, *Mol. Cancer Ther.*, 2008, 7, 3707-3718.
161. P. de Medina, B. Payre, N. Boubekeur, J. Bertrand-Michel, F. Terce, S. Silvente-Poirot and M. Poirot, *Cell Death Dis.*, 2009, 16, 1372-1384.
162. B. J. Altman and J. C. Rathmell, *Cold Spring Harb Perspect Biol.*, 2012, 4.
163. P. de Medina, M. R. Paillasse, G. Segala, F. Khallouki, S. Brillouet, F. Dalenc, F. Courbon, M. Record, M. Poirot and S. Silvente-Poirot, *Chem. Phys. Lipids*, 2011, 164, 432-437.
164. U. Gundimeda, Z. H. Chen and R. Gopalakrishna, *J. Biol. Chem.*, 1996, 271, 13504-13514.
165. Y.-H. Lee, B. S. Kang and Y.-S. Bae, *Life Sci.*, 2014, 97, 116-122.
166. M. Rosenblat and M. Aviram, *Atherosclerosis*, 2002, 160, 69-80.
167. P. L. H. Hwang and A. Matin, *J. Lipid Res.*, 1989, 30, 239-245.
168. M. Poirot and S. Silvente-Poirot, *Biochimie.*, 2013, 95, 622-631.
169. A. Sevanian and L. L. McLeod, *J. Biol. Chem.*, 1986, 261, 54-59.
170. P. de Medina, M. R. Paillasse, G. Segala, M. Voisin, L. Mhamdi, F. Dalenc, M. Lacroix-Triki, T. Filleron, F. Pont, T. Al Saati, C. Morisseau, B. D. Hammock, S. Silvente-Poirot and M. Poirot, *Nat. Commun.*, 2013, 4.
171. P. de Medina, M. R. Paillasse, G. Segala, M. Poirot and S. Silvente-Poirot, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, 107, 13520-13525.

172. A. J. Fretland and C. J. Omiecinski, *Chem. Biol. Interact.*, 2000, 129, 41-59.
173. S. Silvente-Poirot and M. Poirot, *Curr. Opin. Pharmacol.*, 2012, 12, 696-703.
174. N. T. Nashed, D. P. Michaud, W. Levin and D. M. Jerina, *Arch. Biochem. Biophys.*, 1985, 241, 149-162.
175. C. Morisseau and B. D. Hammock, *Annu. Rev. Pharmacol. Toxicol.*, 2005, 45, 311-333.
176. C. Morisseau, *Biochimie.*, 2013, 95, 91-95.
177. M. R. Paillasse, N. Saffon, H. Gornitzka, S. Silvente-Poirot, M. Poirot and P. de Medina, *J. Lipid Res.*, 2012, 53, 718-725.
178. R. J. Morin, B. Hu, S. K. Peng and A. Sevanian, *J. Clin. Lab. Anal.*, 1991, 5, 219-225.
179. H. S. Black and D. R. Douglas, *Cancer Res.*, 1972, 32, 2630-2632.
180. M. T. Huang, R. C. Smart, R. L. Chang, J. M. Sayer, D. M. Jerina, A. W. Wood and A. H. Conney, *Proc. Am. Assoc. Cancer Res.*, 1986, 27, 122-122.
181. L. L. Smith, V. B. Smart and G. A. S. Ansari, *Mutat. Res.*, 1979, 68, 23-30.
182. Y. W. Cheng, J. J. Kang, Y. L. Shih, Y. L. Lo and C. F. Wang, *Food Chem. Toxicol.* , 2005, 43, 617-622.
183. C. C. Chang, C. Jone, J. E. Trosko, A. R. Peterson and A. Sevanian, *Mutat. Res.*, 1988, 206, 471-478.
184. A. R. Peterson, H. Peterson, C. P. Spears, J. E. Trosko and A. Sevanian, *Mutat. Res.*, 1988, 203, 355-366.
185. A. Sevanian and A. R. Peterson, *Proc. Natl. Acad. Sci. U. S. A.*, 1984, 81, 4198-4202.
186. G. J. Schroepfer, *Physiol. Rev.*, 2000, 80, 361-554.
187. M. A. Lasuncion, C. Martin-Sanchez, A. Canfran-Duque and R. Busto, *Curr. Opin. Pharmacol.*, 2012, 12, 717-723.
188. L. J. Brandes and M. W. Hermonat, *Biochem. Biophys. Res.*, 1984, 123, 724-728.
189. P. de Medina, M. R. Paillasse, B. Payre, S. Silvente-Poirot and M. Poirot, *J. Med. Chem.*, 2009, 52, 7765-7777.
190. L. Reyno, L. Seymour, D. S. Tu, S. Dent, K. Gelmon, B. Walley, A. Pluzanska, V. Gorbunova, A. Garin, J. Jassem, T. Pienkowski, J. Dancey, L. Pearce, M. MacNeil, S. Marlin, D. Lebwohl, M. Voi and K. Pritchard, *J. Clin. Oncol.*, 2004, 22, 269-276.
191. M. Poirot, P. De Medina, F. Delarue, J. J. Perie, A. Klaebe and J. C. Faye, *Bioorg. Med. Chem.*, 2000, 8, 2007-2016.
192. M. G. Rowlands, R. Grimshaw, M. Jarman, A. Bouhoute and G. Leclercq, *Biochem. Pharmacol.*, 1997, 53, 241-244.
193. M. G. Rowlands, I. B. Parr, R. McCague, M. Jarman and P. M. Goddard, *Biochem. Pharmacol.*, 1990, 40, 283-289.
194. L. J. Brandes, J. M. Gerrard, R. P. Bogdanovic, D. W. Lint, R. E. Reid and F. S. Labella, *Cancer Res.*, 1988, 48, 3954-3958.
195. H. Fuda, N. B. Javitt, K. Mitamura, S. Ikegawa and C. A. Strott, *J. Lipid Res.*, 2007, 48, 1343-1352.
196. M. Poirot and S. Silvente-Poirot, *Biochimie*, 2013, 95, 622-631.
197. C. Song, R. A. Hiipakka and S. S. Liao, *Steroids*, 2001, 66, 473-479.
198. M. R. Paillasse, P. de Medina, G. Amouroux, L. Mhamdi, M. Poirot and S. Silvente-Poirot, *J. Lipid Res.*, 2009, 50, 2203-2211.
199. T. Watabe, T. Sawahata and J. Horie, *Biochem. Biophys. Res.*, 1979, 87, 469-475.

200. J. Seidegard and G. Ekstrom, *Environ. Health Perspect.*, 1997, 105, 791-799.
201. L. J. Brandes, L. M. Macdonald and R. P. Bogdanovic, *Biochem. Biophys. Res.*, 1985, 126, 905-910.
202. M. A. Rainey, K. Callaway, R. Barnes, B. Wilson and K. N. Dalby, *J. Am. Chem. Soc.*, 2005, 127, 10494-10495.
203. J. M. Gray and L. Ziemian, *Brain Res.*, 1992, 578, 55-60.
204. M. T. Griffin, R. A. Magarian, P. Jain, J. T. Pento, G. K. Mousissian and D. C. Graves, *Anticancer Drug Des.*, 1992, 7, 49-66.
205. F. Wang, T. Luo, J. Hu, Y. Wang, H. S. Krishnan, P. V. Jog, S. K. Ganesh, G. K. S. Prakash and G. A. Olah, *Angew. Chem. Int. Ed.*, 2011, 50, 7153-7157.
206. A. Cronin, M. Decker and M. Arand, *J. Lipid Res.*, 2011, 52, 712-719.
207. M. Decker, M. Arand and A. Cronin, *Arch. Toxicol.*, 2009, 83, 297-318.
208. A. A. Spector, *J. Lipid Res.*, 2009, 50, S52-S56.
209. H. Qiu, N. Li, J. Y. Liu, T. R. Harris, B. D. Hammock and N. Chiamvimonvat, *Cardiovasc. Ther.*, 2011, 29, 99-111.
210. J. D. Imig, *Physiol. Rev.*, 2012, 92, 101-130.
211. D. R. Davies, B. Mamat, O. T. Magnusson, J. Christensen, M. H. Haraldsson, R. Mishra, B. Pease, E. Hansen, J. Singh, D. Zembower, H. Kim, A. S. Kisclyov, A. B. Burgin, M. E. Gurney and L. J. Stewart, *J. Med. Chem.*, 2009, 52, 4694-4715.
212. A. Rinaldo-Matthis and J. Z. Haeggström, *Biochimie.*, 2010, 92, 676-681.
213. T. D. Penning, N. S. Chandrakumar, B. B. Chen, H. Y. Chen, B. N. Desai, S. W. Djuric, S. H. Docter, A. F. Gasiecki, R. A. Haack, J. M. Miyashiro, M. A. Russell, S. S. Yu, D. G. Corley, R. C. Durley, B. F. Kilpatrick, B. L. Parnas, L. J. Askonas, J. K. Gierse, E. I. Harding, M. K. Highkin, J. F. Kachur, S. H. Kim, G. G. Krivi, D. Villani-Price, E. Y. Pyla, W. G. Smith and N. S. Ghoreishi-Haack, *J. Med. Chem.*, 2000, 43, 721-735.
214. A. M. Fourie, *Curr. Opin. Investig. Drugs*, 2009, 10, 1173-1182.
215. F. Mesange, M. Sebbar, B. Kedjouar, J. Capdevielle, J. C. Guillemot, P. Ferrara, F. Bayard, F. Delarue, J. C. Faye and M. Poirot, *Biochem. J.*, 1998, 334, 107-112.
216. J. W. Newman, C. Morisseau and B. D. Hammock, *Prog. Lipid Res.*, 2005, 44, 1-51.
217. P. de Medina, M. R. Paillasse, G. Segala, M. Voisin, L. Mhamdi, F. Dalenc, M. Lacroix-Triki, T. Filleron, F. Pont, T. Al Saati, C. Morisseau, B. D. Hammock, S. Silvente-Poirot and M. Poirot, *Nat. Commun.*, 2013, 4, 10.
218. P. de Medina, N. Boubekeur, P. Balaguer, G. Favre, S. Silvente-Poirot and M. Poirot, *J. Pharm. Exp. Ther.*, 2006, 319, 139-149.
219. B. Sola, M. Poirot, P. de Medina, S. Bustany, V. Marsaud, S. Silvente-Poirot and J.-M. Renoir, *Oncotarget*, 2013, 4, 911-922.
220. US 5663207, 1997.
221. C.-T. Chien, C.-C. Tsai, C.-H. Tsai, T.-Y. Chang, P.-K. Tsai, Y.-C. Wang and T.-H. Yan, *J. Org. Chem.*, 2006, 71, 4324-4327.
222. L. M. Tolbert and S. Siddiqui, *J. Am. Chem. Soc.*, 1984, 106, 5538-5543.
223. C. Kuang, Q. Yang, H. Senboku and M. Tokuda, *Tetrahedron*, 2005, 61, 4043-4052.
224. D.-J. Dong, H.-H. Li and S.-K. Tian, *J. Am. Chem. Soc.*, 2010, 132, 5018-+.
225. Q. M. Kainz, M. Zeltner, M. Rossier, W. J. Stark and O. Reiser, *Chem. Eur. J.*, 2013, 19, 10038-10045.

226. G.-H. Wang, H.-Y. Bin, M. Sun, S.-W. Chen, J.-H. Liu and C.-M. Zhong, *Tetrahedron*, 2014, 70, 2175-2179.
227. R. Infante, Y. Hernández, J. Nieto and C. Andrés, *Eur. J. Org. Chem.*, 2013, 2013, 4863-4869.
228. Claudia H. Sugisaki, Y. Ruland and M. Baltas, *Eur. J. Org. Chem.*, 2003, 2003, 672-688.
229. M. Tiecco, L. Testaferri, L. Bagnoli, R. Terlizzi, A. Temperini, F. Marini, C. Santi and C. Scarponi, *Tetrahedron: Asymmetry*, 2004, 15, 1949-1955.
230. P. Erbes and W. Boland, *Helv. Chim. Acta*, 1992, 75, 766-772.
231. C. J. Lion, C. S. Matthews, M. F. G. Stevens and A. D. Westwell, *J. Med. Chem.*, 2005, 48, 1292-1295.
232. M. Tsukamoto and M. Schlosser, *Synlett*, 1990, 605-608.
233. S. Mochida, K. Hirano, T. Satoh and M. Miura, *J. Org. Chem.*, 2011, 76, 3024-3033.
234. R. Bandari, T. Hoeche, A. Prager, K. Dirnberger and M. R. Buchmeiser, *Chem. Eur. J.*, 2010, 16, 4650-4658.
235. J. J. Heynekamp, W. M. Weber, L. A. Hunsaker, A. M. Gonzales, R. A. Orlando, L. M. Deck and D. L. V. Jagt, *J. Med. Chem.*, 2006, 49, 7182-7189.
236. J. P. Liu, Y. H. Zhao, Y. Y. Zhou, L. Li, T. Y. Zhang and H. B. Zhang, *Org. Biomol. Chem.*, 2003, 1, 3227-3231.
237. G. Szőllősi, B. Hermán, K. Felföldi, F. Fülöp and M. Bartók, *Adv. Synth. Catal.*, 2008, 350, 2804-2814.
238. Sadiq-ur-Rehman, S. Ali, A. Badshah, M. Mazhar, X. Song, G. Eng and K. M. Khan, *Synth. React. Inorg. Met.-Org. Chem.*, 2004, 34, 1379-1399.
239. Sadiq-ur-Rehman, S. Ali, A. Badshah, M. Mazhar, X. Song, G. Eng and K. M. Khan, *Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry*, 2004, 34, 1379-1399.
240. D. S. Morris, *J. Chem. Soc.*, 1950, 1913-1917.
241. T. Piou, A. Bunescu, Q. Wang, L. Neuville and J. Zhu, *Angew. Chem. Int. Ed.*, 2013, 52, 12385-12389.
242. B. Halton, A. Maidment, D. Officer and J. Warnes, *Aust. J. Chem.*, 1984, 37, 2119-2128.
243. A. S. R. Anjaneyulu, G. S. Rani, U. V. Mallavadhani and Y. L. N. Murthy, *Indian J. Chem. B.*, 1990, 29, 219-223.
244. S. Kumar, S. Sapra, R. Kumar, M. Gupta, S. Koul, T. Kour, A. Saxena, O. Suri and K. Dhar, *Med. Chem. Res.*, 2012, 21, 3720-3729.
245. J. W. Cook and L. L. Engel, *J. Chem. Soc.*, 1940, 198-200.
246. J. C. Roberts and J. A. Pincock, *J. Org. Chem.*, 2004, 69, 4279-4282.
247. D. P. de Lima, R. Rotta, A. Beatriz, M. R. Marques, R. C. Montenegro, M. C. Vasconcellos, C. Pessoa, M. O. de Moraes, L. V. Costa-Lotufo, A. C. H. Frankland Sawaya and M. N. Eberlin, *Eur. J. Med. Chem.*, 2009, 44, 701-707.
248. F. Alonso, P. Riente and M. Yus, *Eur. J. Org. Chem.*, 2009, 2009, 6034-6042.
249. G. Cahiez, O. Gager and F. Lecomte, *Org. Lett.*, 2008, 10, 5255-5256.
250. S.-H. Huang, J.-R. Chen and F.-Y. Tsai, *Molecules*, 2010, 15, 315-330.
251. F. B. Mallory, M. J. Rudolph and S. M. Oh, *J. Org. Chem.*, 1989, 54, 4619-4626.
252. S. Y. Ng, N. Cardullo, S. C. M. Yeo, C. Spatafora, C. Tringali, P.-S. Ong and H.-S. Lin, *Molecules*, 2014, 19, 9577-9590.

253. M. Fischer and P. Wan, *J. Am. Chem. Soc.*, 1999, 121, 4555-4562.
254. D. P. Ojha and K. R. Prabhu, *J. Org. Chem.*, 2012, 77, 11027-11033.
255. Y. Nakao, H. Imanaka, J. Chen, A. Yada and T. Hiyama, *J. Organomet. Chem.*, 2007, 692, 585-603.
256. K. Sato, J. Kuriwaki, K. Takahashi, Y. Saito, J. Oka, Y. Otani, Y. Sha, K. Nakazawa, Y. Sekino and T. Ohwada, *ACS Chem. Neurosci.*, 2012, 3, 105-113.
257. G. R. Bedford, A. L. Walpole and B. Wright, *J. Med. Chem.*, 1974, 17, 147-149.
258. H. A. Elwakil, *Org. Prep. Proced. Int.*, 1991, 23, 754-757.
259. WO2013062079 A1, 2013.
260. M. Poirot, P. De Medina, F. Delarue, J.-J. Perie, A. Klaebe and J.-C. Faye, *Bioorg. Med. Chem.*, 2000, 8, 2007-2016.
261. C. Riffkin and N. Rubin, *J. Am. Pharm. Assoc.*, 1956, 45, 316-320.
262. Z. Chen, Y. Wu, Y. Liu, S. Yang, Y. Chen and L. Lai, *J. Med. Chem.*, 2011, 54, 3650-3660.