MODIFIED BODIPY PROBES TO EXPLORE PEROXISOME FUNCTION

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Abstract

This research aims to use fluorescent BODIPY probes to measure biological function within a plant cell. In particular developing BODIPY fluors with emission at near-infrared and long wavelength that provides considerable benefit for specific organelle monitoring will be explored.

The work described in this thesis was mainly focused on the synthesis of the BODIPY and the photoaffinity labelling group. We present an effective synthesis of several BODIPY analogues with different *meso*-substituents. 3-Methoxy trifluoromethyl diazirine was successfully synthesised with the aim at coupling to the BODIPY dyes. However, although many borylation and bromination reactions on *meta* and *para* methoxy substituted trifluoromethyl diazirines, were attempted these were not successful. In an alternative approach, 4-benzoylphenylboronic acid was used as the pro-photoaffinity labelling residue. This could successfully be coupled with iodo-BODIPY for future use as cell-labelling fluorophore.

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Acknowledgements

I would like to thank my supervisor Dr. Patrick Steel who made this thesis possible. Many ideas and advice were supporting me to develop an understanding of the project. I am grateful to John S. for guidance in the lab helping me to learn to think critically and independently. It is a pleasure to thank all friends in CG1, past and present: Kathryn, Mavis, Hazmi, Peter, John M., Michal, and Yu for all their help. I would also like to thank my family for the support and encouragement I received during the course of my education. Lastly, I would like to thank God for giving me the wisdom and strength to complete this project.

Abbreviations

The following abbreviations appear in this thesis:

Å	Angstrom(s)
aq	aqueous
Ar	Aryl
atm	atmosphere(s)
Bn	Benzyl
Boc	*tert*-Butyloxycarbonyl
Bpin	pinacolborane
bp	boiling point
BODIPY	Borondipyrromethane
℃	Degree Celsius
cat	catalytic
cm^{-1}	wavenumber(s)
COSY	Correlation spectroscopy
δ	Chemical shift in parts per million
d	day(s)
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyanobenzoquinone
DIPEA	Diisopropylethylamine (Hunig's base)
DMA	Dimethylacetamide
DMAP	Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethylsulphoxide
$ES^{+}MS$	Positive charge electrospray mass spectrometry
$ES^{-}MS$	Negative charge electrospray mass spectrometry
Et	ethyl
EtOAc	Ethyl acetate
EtOH	Ethanol

eq	equivalents
g	gram(s)
GC	Gas Chromatography
h	hour(s)
HPLC	High performance liquid chromatography
Hz	Hertz
IBX	iodoxybenzoic acid
^{i}Pr	*Iso*-propyl
IR	Infra-red spectroscopy
J	Coupling constant (in NMR spectroscopy)
L	Litre(s)
µ	micro
M	Molar
m/z	Mass to charge ratio
m	*meta*
max	maximum
MeCN	Acetonitrile
MeOH	Methanol
mg	milligram
min	minute
mL	millilitre
mmol	millimole
m.p.	melting point
MS	mass spectrometry
mw	molecular weight
m/z	Mass-to-charge ratio
n-Bu	*n*-butyl
NMR	Nuclear magnetic resonance
NOESY	Nuclear Overhauser enhancement spectroscopy
Nu	Nucleophile

p	*para*
ppm	Parts per million
rt	room temperature
R_f	Retention factor in chromatography)
s	second
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TMS	Trimethylsilyl
TLC	Thin layer chromatography
UV	Ultra violet
Φ_f	Fluorescent quantum yield

1 Introduction

1.1 General Introduction

This project aims to use fluorescent BODIPY probes to measure biological function within cells. In particular the project aims to understand why certain BODIPY dyes specifically localize within the peroxisome of plant cells. This will be achieved by preparing BODIPY fluors coupled with known photoaffinity agents e.g. Figure 1.1. This thesis describes the synthetic work undertaken towards this objective. The thesis is composed of three chapters. The remainder of this chapter provides a short introduction to BODIPY fluors, photoaffinity agents and the background to the project. Chapter two contains a description of the results and associated discussion whilst Chapter three provides the detailed experimental procedures.

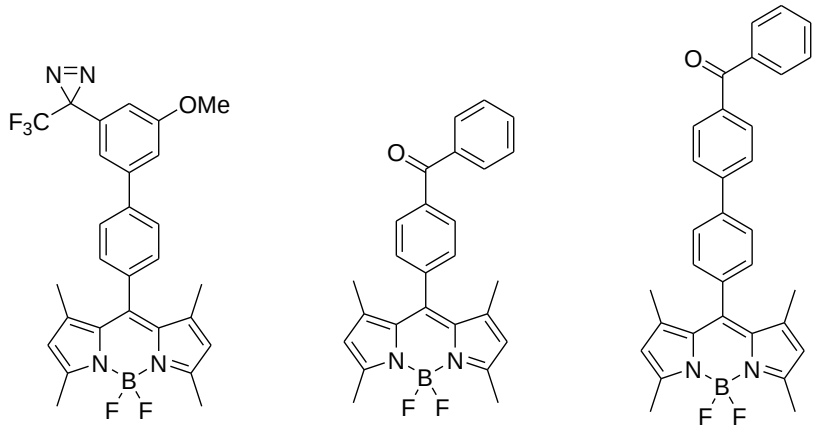
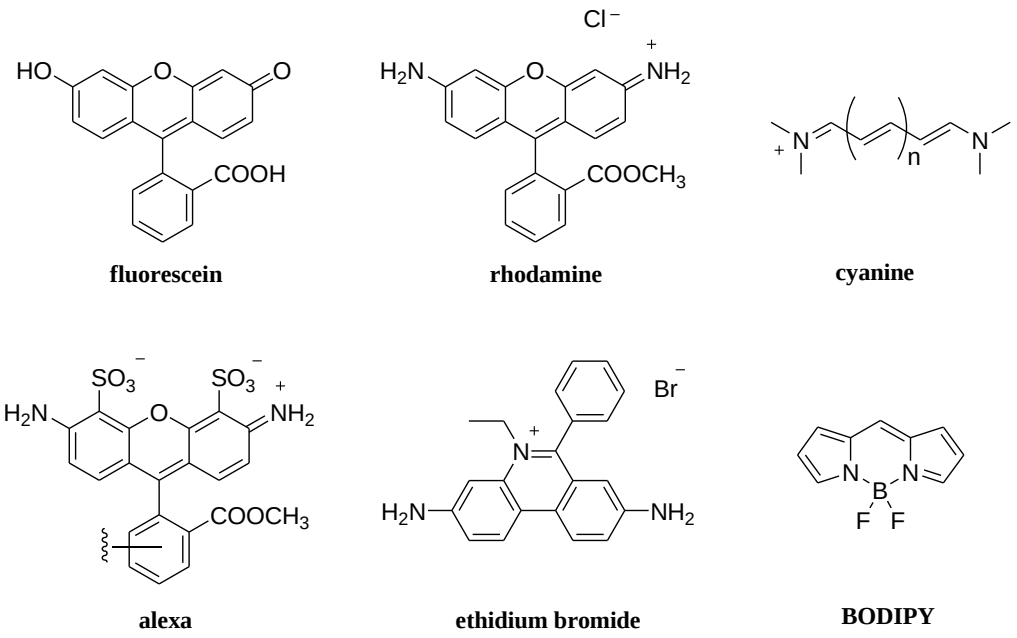


Figure 1.1

1.2 BODIPY Dyes

1.2.1 Introduction to Organic dyes

Bright colours and wide range of wavelengths make fluorescent dyes of interest in many fields of study such as biological chemistry, photochemistry, physical chemistry, and optical engineering. In particular, fluorescence labelling is commonly used for bio-analytical purposes. In most cases this is achieved through the use of an organic fluorescent dye. Many such dyes are known. There are diversity of structures, spectroscopic properties, and chemical reactivities differentiating the dyes providing a huge variation of photochemical properties which can be used to select a dye for a particular purpose.[1] Reflecting this, there are many organic dyes in common use including fluorescein,[2] rhodamine,[3] cyanine,[4] alexa[5] and ethidium,[6] and BODIPYs, Figure 1.2. These all tend to be relatively small molecules with absorption and emission bands in the range 300-800 nm. Given the nature of this project the remainder of this review focuses on BODIPY dyes.



fluorescein **rhodamine** **cyanine**

alexa **ethidium bromide** **BODIPY**

Figure 1.2 Organic fluorescent dye structures

1.2.2 Introduction to BODIPY dyes

Now the trademark of Molecular Probes, Inc. BODIPYs,[7,8] 4,4-difluoro-4-borata-3a-azonia-4a-aza-*s*-indacenes were first discovered in 1968 by Treibs and Kreuzer[9] (Figure 1.3). Since then, the BODIPY dye has become one of the most versatile organic fluorophore labels in use. They have been widely used for monitoring biomolecules in living cells.[10] For instance, BODIPYs have been attached to both proteins,[11,12] and viruses.[13] Other uses include roles in laser dyes,[14,15] nanocrystals,[16] fluorescent switches,[17] and chemosensors.[18]

1.2.2.1 BODIPY properties

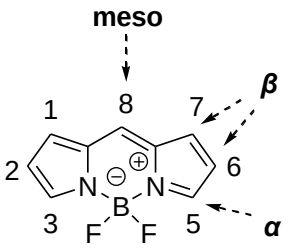


Figure 1.3 Basic BODIPY core structure

BODIPY dyes have found roles in biological labelling due to their strong UV-absorption, high photochemical stability,[19] and high emission quantum yields.[20,21] A number of different substitution patterns can be envisaged, Figure 1.4 which affect their photophysical properties. Whilst the parent BODIPY **4**[22] as well as the simple analogues BODIPYs **5** and **6**, which lack a substituent at C 3 and 5 have not been reported in the literature other more highly substituted (di-, tetra-, and hexa-alkylated) systems, e.g. **7**, **8**, and **9** have been synthesized including those with cycloalkyl substituents, e.g. **10** and **11**.[23,24] Comparing compound **7**, **8** with compound **9**, alkylation at the 2,6-position generally leads to lower quantum yield but with higher absorption and emission wavelengths.

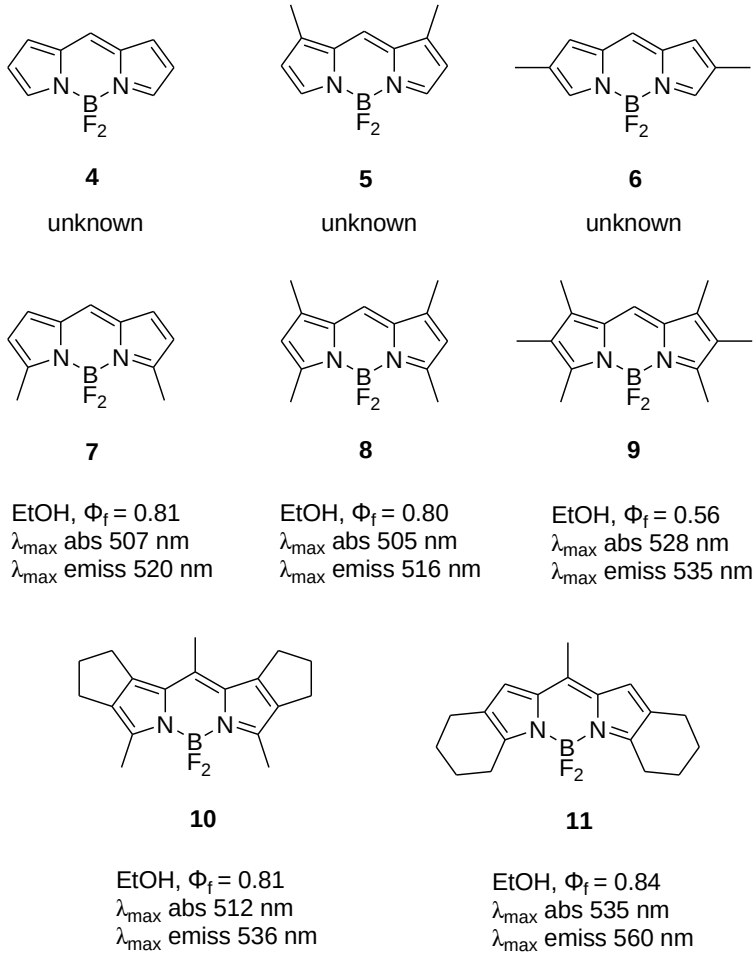


Figure 1.4 Different alkyl substitution patterns on BODIPY dye

In contrast to the simple alkyl substituted BODIPYs the *meso*-phenyl compound **12** can be synthesized without *α*-substituents, Figure 1.5. It has a broader wavelength range than the di- and tetra methyl- substituted analogues albeit with a much reduced quantum yield. The quantum yield of compounds **12** and **13** are much lower than the 1,7- dimethyl substituted analogue, Figure 1.6, suggesting that the BODIPY core and phenyl ring can adapt a co-planar arrangement forming a conjugated system enabling internal quenching mechanisms to occur. In **14**, the 1,7-substituents inhibit free rotation of the phenyl group, and thus prevents this loss of energy from the excited states through non-irradiative processes.

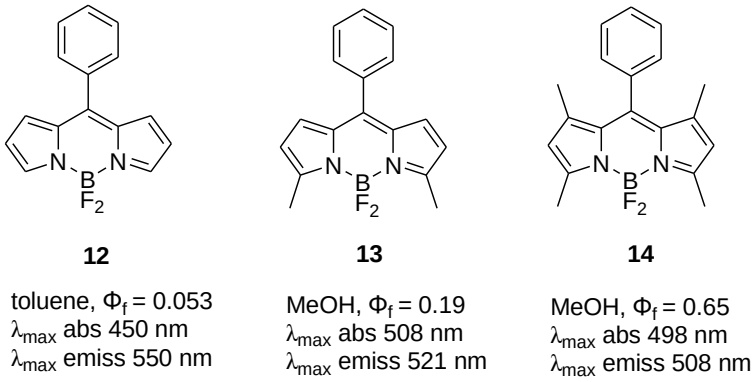


Figure 1.5 Different alkyl substitution patterns on BODIPY dye

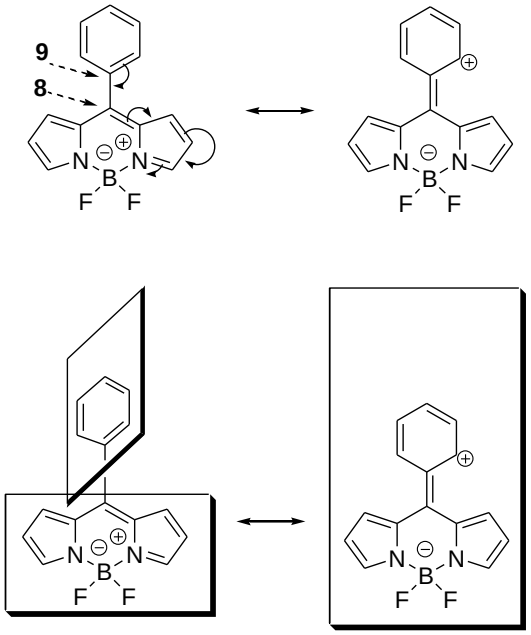


Figure 1.6

Apart from the effect on quantum yield of the BODIPY core, the 8-position, often referred to as the *meso* site, has no significant effect on the absorption and emission wavelengths, Figure 1.7. As a result BODIPYs can be designed for specific function by simple modification of the *meso* substituent. For example, selective sensors of redox active molecules,[25,26] pH probes,[27] metal-chelators,[28,29] and biomolecule conjugating group can be incorporated,[30,31] Figure 1.8.

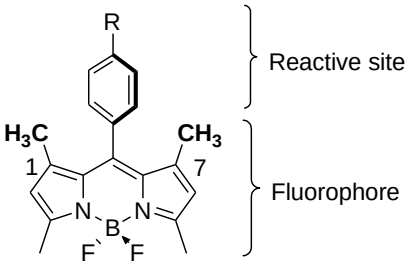


Figure 1.7 Structure of BODIPY with an aryl moiety.

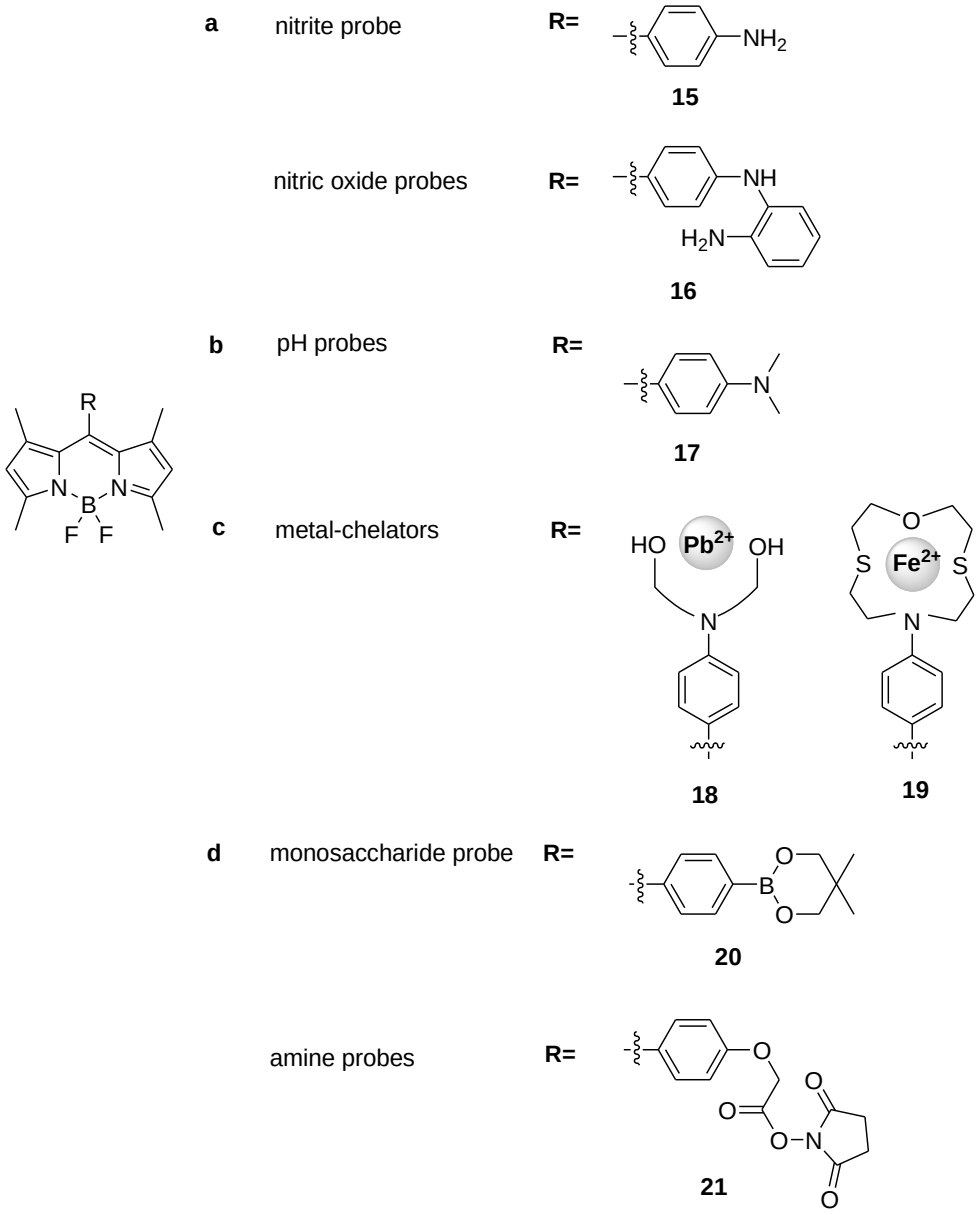


Figure 1.8 Selected BODIPYs with meso-modifications: (a) selective sensors of particular redox active molecules, (b) pH probes, (c) metal-chelators, and (d) biomolecule conjugating group.

Introduction of electron donating substituents on the α-position of the BODIPY core can extend the conjugation of the BODIPY systems, Figure 1.9. For example, **22** and **25** show a green-fluorescent BODIPY fluorophore excitation/emission maximum at 500-537 nm. Compared to **23**, **24**, **26**, and **27**, the extended 3,5-diaryl substituted BODIPYs are shifted to longer wavelengths (545-626 nm).[32] The directing effects of *para*-electron donating group in **24** gives a longer bathochromic shift when compared with that to the *ortho*-substituted analogue **27**. Similarly, the naphthalene extended aromatic substituted example **26** also has red-shifted emission. As discussed above, those examples lacking 1,7 substitution are **23**, **24**, **26**, and **27** have lower fluorescence quantum yields because of nonradiative loss of energy through the potential for conjugation at the β-aryl substituent with the BODIPY core.

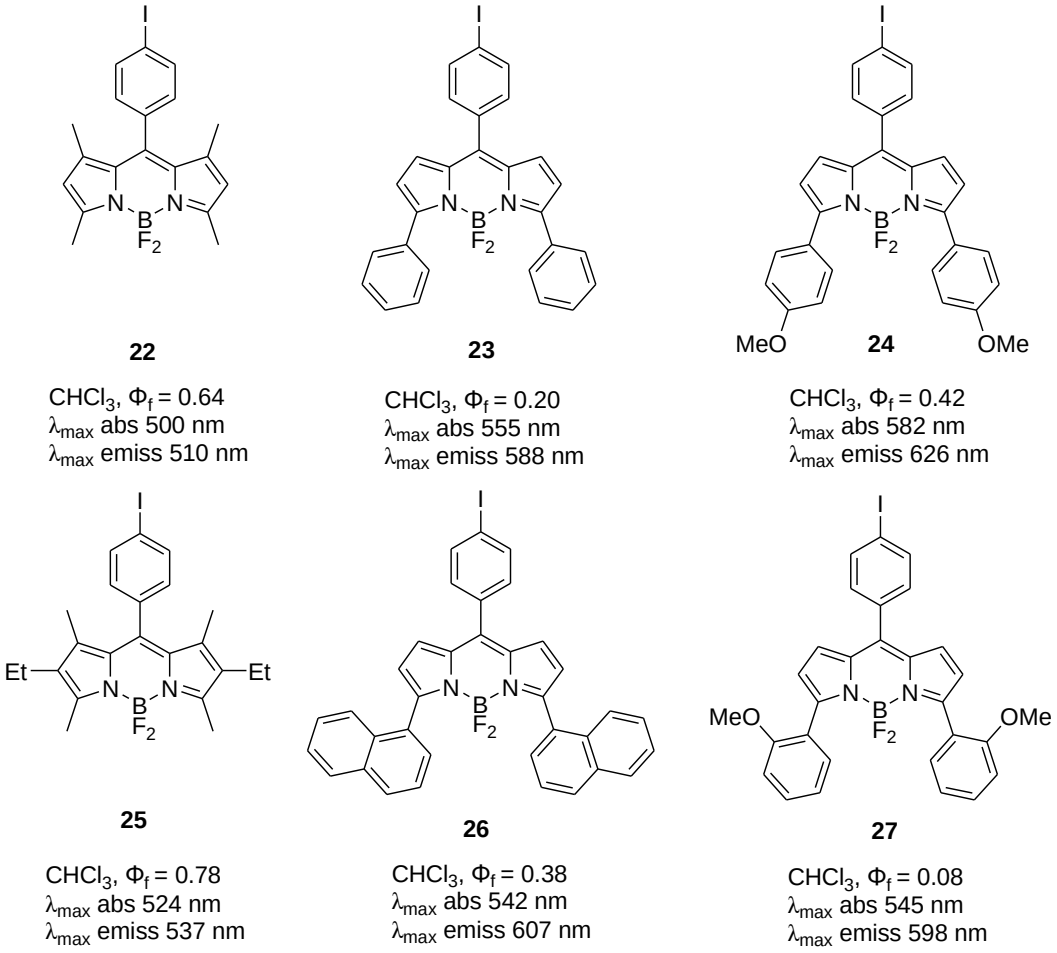
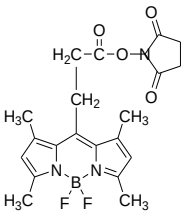
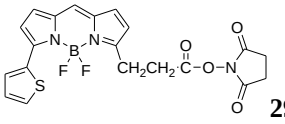
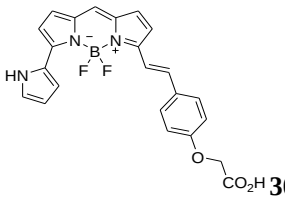
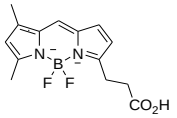
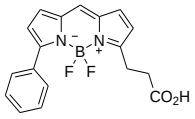
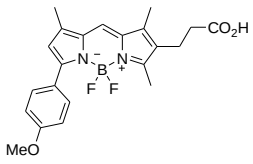
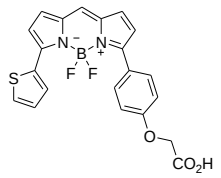


Figure 1.9 Aryl-Substituted BODIPYs

Reflecting these sample structural variations many research groups have reported BODIPYs derivatives with fluorescence emission ranging from 500 to 700 nm.[33] Table 1.1 shows some of the commercial BODIPY fluorophores which have been developed in this respect.

structure	BODIPY	absorption	emission	e [$cm^{-1}M^{-1}$]
 **28**	493/503	500 nm	509 nm	79000
 **29**	558/568	559 nm	568 nm	97000
 **30**	650/665	646 nm	660 nm	102000
 **31**	FL	502 nm	510 nm	82000
 **32**	R6G	528 nm	540 nm	70000
 **33**	TMR	544 nm	570 nm	56000
 **34**	TR	588 nm	616 nm	68000

All BODIPY fluorophores from Molecular Probes (Invitrogen Crop.), Inc.

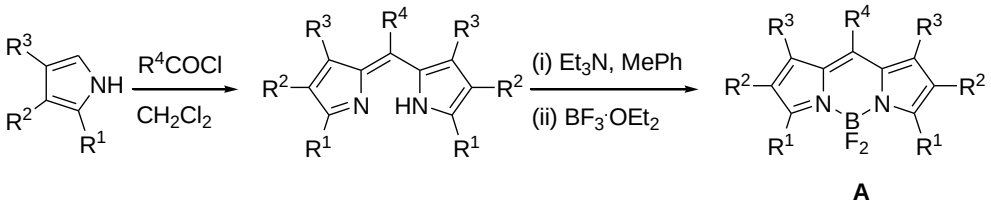
Table 1.1 The different BODIPY absorption and emission wave length

1.2.2.2 BODIPY Synthesis

Given the role found by BODIPY dyes many methods for their synthesis have been described. The construction of the basic procedure of the BODIPY core usually starts from a simple pyrrole condensation with a highly electrophilic carbonyl compound, e.g. aldehyde, acid anhydride, and acyl chloride. Due to the instability of an unsubstituted dipyrromethene intermediate, which can undergo rapid polymerization or porphyrin formation; most routes involve 2-substituted pyrroles. With this in mind, Burgess and collaborators[22] have described the three major routes of BODIPY synthesis: from pyrroles and acid chlorides, from pyrroles and aldehydes, and from ketopyrroles. These are described below.

1.2.2.2.1 From pyrroles and acid chlorides

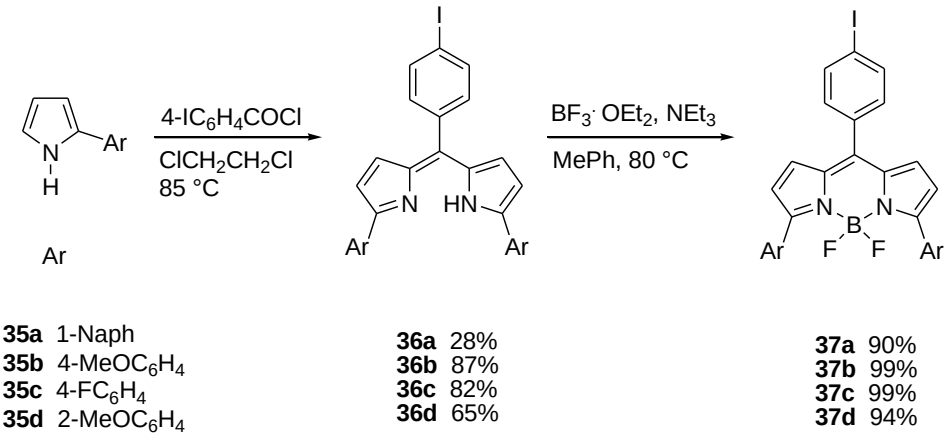
This method of formation involves the condensation of acyl chlorides with pyrroles to give the dipyrrin core of dipyrromethenes, and then subsequent reaction of this with $BF_3 \cdot OEt_2$ in the presence of Et_3N to afford the BODIPY core. The first method used to synthesis a BODIPY derivative followed such a strategy.[22] This process was undertaken in a one-pot, two-step procedure to give the products in 18- 86 % overall yield (Scheme 1.1).[34]



Scheme 1.1 Synthesis of symmetric F-BODIPY dyes from acyl chloride derivatives

The advantage of this method is in the formation of the dipyrromethene in a single process. However, conversions are not always complete and this can complicate

purification. One example of this method is a report by Burgess *et al.* describing the synthesis of BODIPYs from acyl chloride derivatives. Intermediate dipyrromethenes **36** were obtained from pyrroles **35** on reaction with 4-iodobenzoyl chloride in 1,2-dichloroethane. After purification via flash chromatography, the compound **36** was treated with NEt_3 and PhMe before adding boron trifluoride etherate. The reaction mixture was then heated at 80 ℃ for 20 min. The final BODIPYs **37** were isolated following chromatography on alumina (Scheme 1.2).[9]

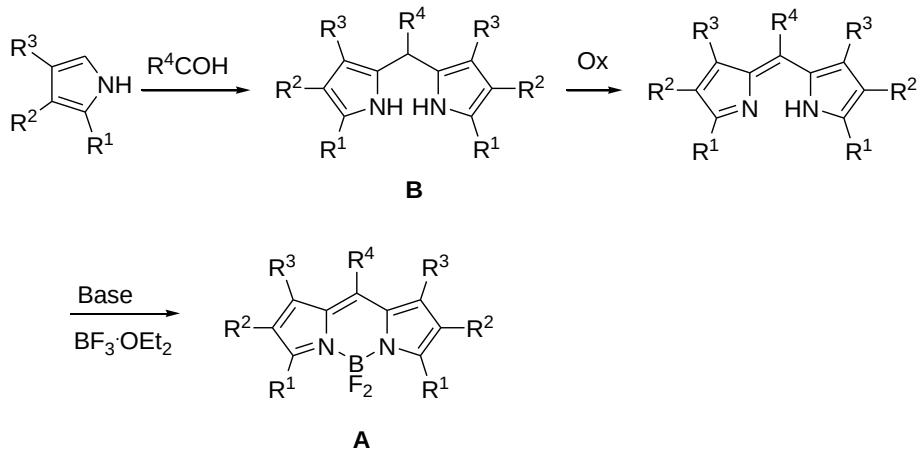


Scheme 1.2 Synthesis of symmetric F-BODIPY dyes

1.2.2.2.2 From pyrroles and aldehydes

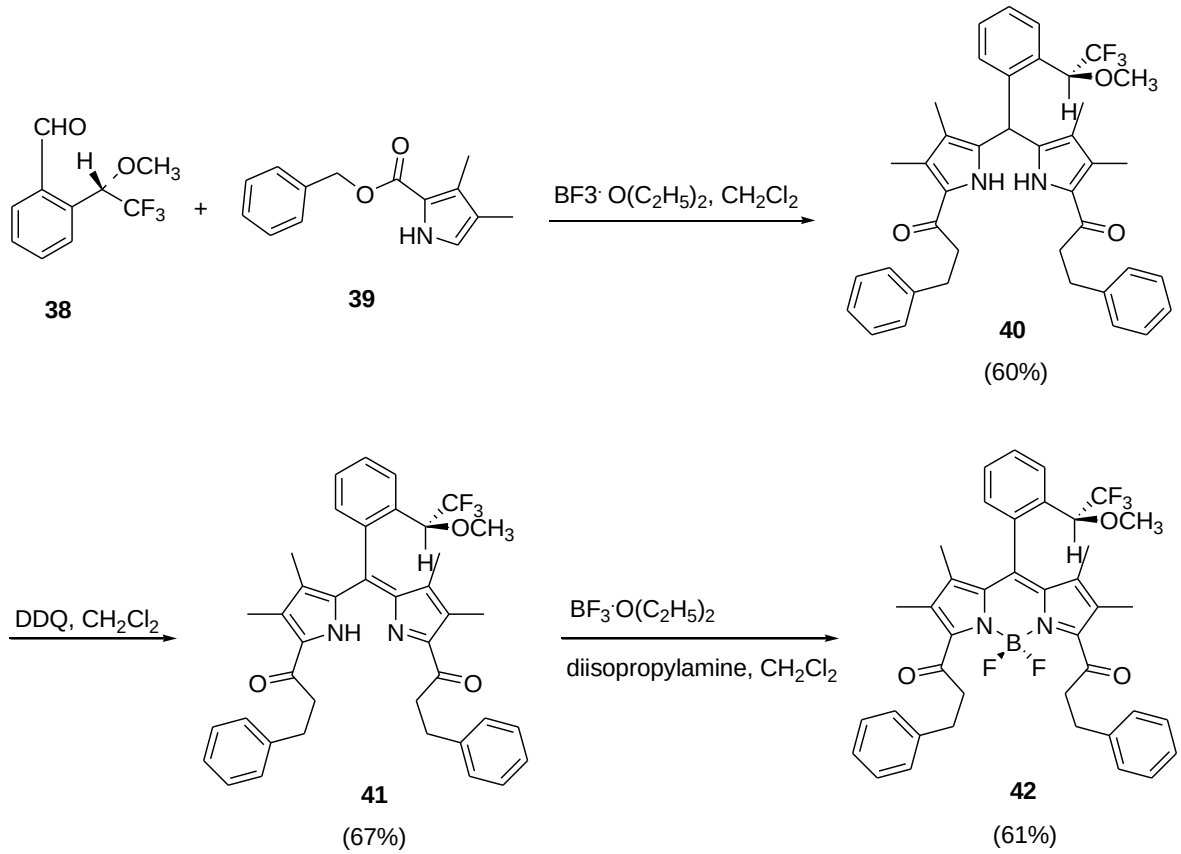
The second method of formation of BODIPYs involves the condensation of aromatic aldehydes with pyrroles to give, after oxidation, the dipyrromethene intermediate. Reaction of the dipyrromethene precursor with boron trifluoride etherate in the presence of a tertiary amine then forms the BODIPYs. Several groups have performed this pathway in a one-pot process. Due to instability of the required unsubstituted dipyrromethene precursor, the condensation usually needs an electron rich substituent, either on the pyrrole R^1 or aldehyde R^4 position. As stated above, the distinction of this method is the requirement for the oxidation of the first formed dipyrromethane **B**.

In most examples 2,3-dichloro5,6-dicyano-*p*-benzoquinone (DDQ) was used as the oxidizing agent.[35,36,37] The overall yield of this reaction varied from 18-43 % depending on the substrate (Scheme 1.3).[38,39,40]



Scheme 1.3 Synthesis of symmetric F-Bodipy dyes from aldehyde derivatives

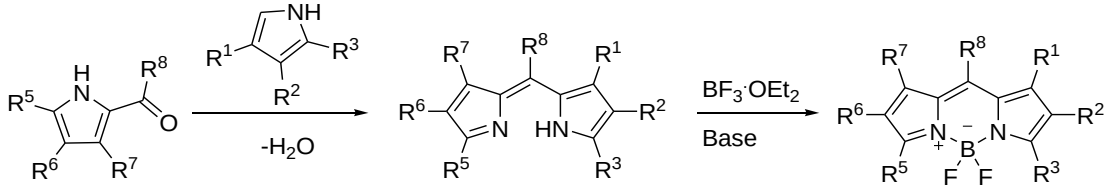
This strategy of condensation of pyrroles with benzaldehyde derivatives is direct and convenient. However, the need for the oxidation means that one more step is required before complexation with boron component. Despite this, many reports have used pyrroles and aldehydes as the starting material, than follow the alternative approach from acid chlorides. For example, Gossauer *et al*.[35] synthesised BODIPY fluorophores from aldehydes with chiral substituents at the α-positions (Scheme 1.4).



Scheme 1.4 Synthesis of BODIPY from aldehydes

1.2.2.2.3 From ketopyrroles

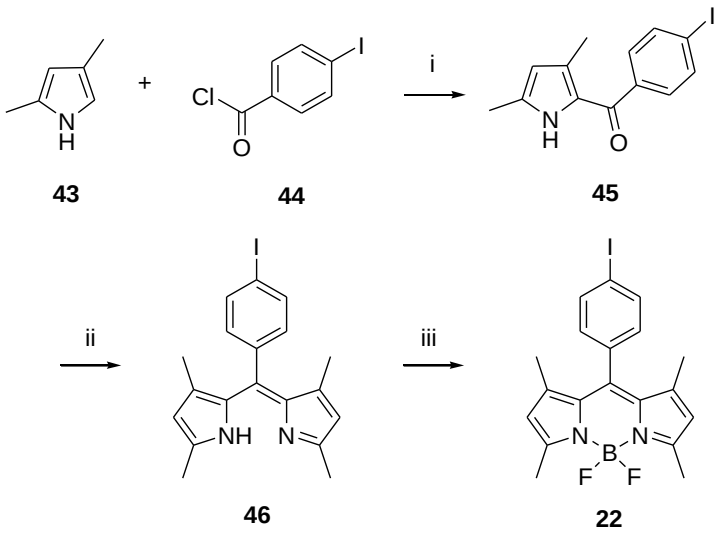
In the previous two methods using acid chlorides or aldehydes, condensation with pyrrole occurs to form symmetrically substituted BODIPY dyes. However, asymmetrically substitute BODIPY dyes cannot be prepared by these routes. Generation of asymmetric BODIPYs can be achieved through condensation of ketopyrrole with a second pyrrole molecule to give the intermediate dipyrromethene followed by reaction with BF_3· OEt and base (Scheme 1.5).[22]



Scheme 1.5 Synthesis from ketopyrroles

The main advantage of this method is in its application to incorporate diverse substituents on the pyrrole rings. However, isolation of the unstable dipyrromethene hydrochloride salt intermediates can be difficult. Overall, although this method needs one more step to isolate the ketopyrrole intermediate, the final yields are still good.

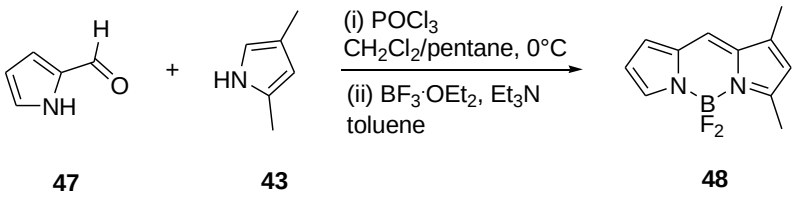
In Scheme 1.6 and 1.7 give examples of BODIPY synthesis from ketopyrroles relevant to this project. Work by Tahtaoui *et al*. had shown that the synthesis of BODIPY **22** could be achieved in a three-step process.[41] Firstly, 2-ketopyrrole **45** was prepared through the reaction of 4-iodobenzoyl chloride **44** with the magnesium salt of pyrrole **43** made by deprotonation with a Grignard reagent. In the second step, the hydrochloride salt of the dipyrromethene **46** is produced from the condensation of pyrrole **43** and ketopyrrole **45** using phosphoryl chloride as the dehydrating agent. Dipyrromethene **46** can be converted directly to the target materials **22** after complexation with boron trifluoride etherate in the presence of triethylamine (Scheme 1.6).



(i) CH_3MgBr, ether, 81%; (ii) **43**, $POCl_3$, CH_2Cl_2/pentane, 0°C, 46 %; (iii) $BF_3.OEt_2$, NEt_3, toluene, 80 %.

Scheme 1.6 Synthesis of symmetrical BODIPY from ketopyrroles

Similarly, unsymmetrical substituted BODIPY **48** was also prepared by this method approach (Scheme 1.7).[22]



47 **43** **48**

Scheme 1.7 Synthesis of unsymmetrical BODIPY from ketopyrroles

1.3 Photoaffinity Labels

Photoaffinity labels (PAL) are molecules which on activation by light covalently bind to biomolecules identifying specific targets associated with a particular function, e.g. proteins.[42] The covalent binding with such fragment of a biomolecule is achieved through a highly reactive intermediate, e.g. carbene or nitrene, generated by irradiation with ultraviolet light.

Many novel photoaffinity labelling groups have been discovered,[43] Figure 1.10. Among various photophores for photoaffinity labelling, the first reported example was in 1962. In this, Westheimer described the use of p-nitrophenyldiazoacetate to covalent label chymotrypsin on photolysis in aqueous solution at low temperatures.[44] Evidence for cross linking was deduced from the fact that the enzymatic activity was not regenerated by the action of hydroxylamine on the modified chymotrypsin. A few years later, aryl azides were used as PAL acting as a nitrene precursor through loss of N_2. During the 1960s, the benefits of the aryl azide as PAL were reported. Subsequently in the 1970s, other groups were developed individually, e.g.

benzophenone, aryldiazonium salts and the diazirines. The basic photochemical reactions of these reagents are shown in Figure 1.10.

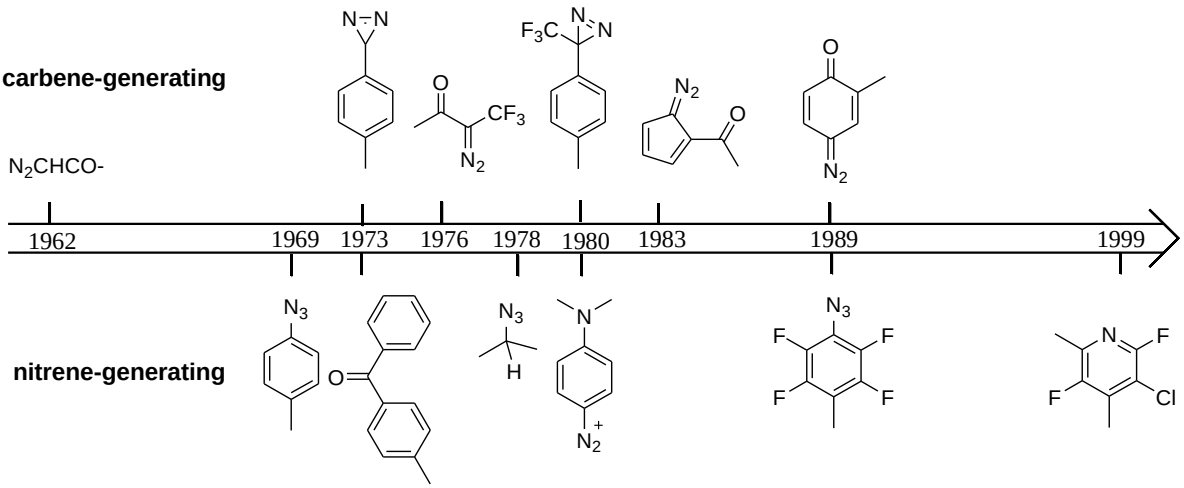


Figure 1.10 Chronology of reports on different functional groups for photoaffinity labelling

Most studies have concentrated on a few types of photoaffinity reagents. These are the aryl azides, benzophenones, aryldiazirines, and alkyl diazirine (Figure 1.11). Such photoreactive probes allow crosslinking among cellular components in biological use with minimal side reactions. Comparing all these, reflecting its stability under the irradiation condition, the 3-aryl-3-trifluoromethyl-diazirines appear to have the greatest potential for PAL. In contrast, aryl azides require shorter photoactivation wavelengths than benzophenone, aryl, and alkyl diazirines. This higher energy can damage biomolecular structure and generate high energy, highly reactive nitrenes that react non-selectively. Whilst benzophenone is potentially a more selective photophore, its bulk means that reactivity is often influenced by geometric/steric constraints. This affects the efficiency and specificity of interaction and binding with the target molecule.

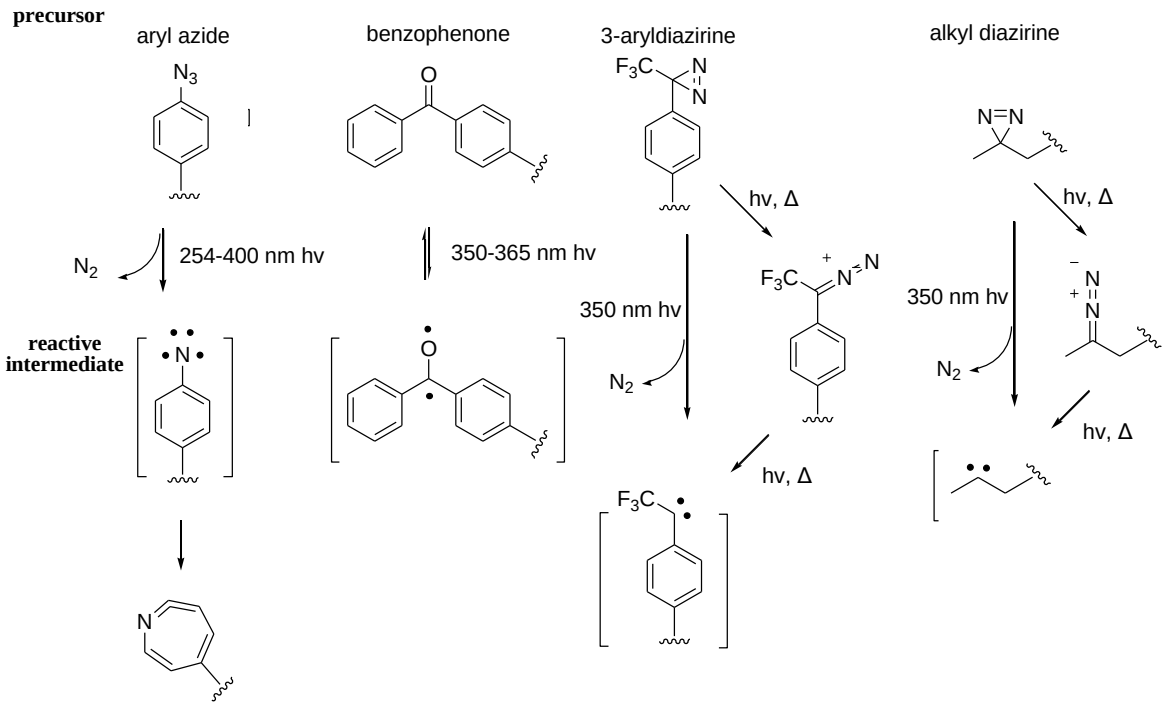


Figure 1.11 Photochemical reactions of major photophores: The top structures are photochemical precursors. The wavelength ranges for photoactivation are shown on the arrows and the structures of the highly reactive intermediate molecules, carbene or nitrene, are shown in the brackets.

In order to label the target structure with a photoaffinity probing, some requirements are needed. The photoreactive groups should have the following characteristics:

(1) The probes are stable in the absence of light.

(2) The probes easy to prepare and handle.

(3) Once activated the lifetime of the reactive photoprobe is shorter than dissociation of the probe from the target molecule.

(4) The product of the photo labelling experiments is stable.

(5) The activation probe is selective for the probe and does not affect the target molecule indirectly.

At present, no photoaffinity label probe fits all the above requirements. Consequently as described in Figure 1.10, a large number of structures have been reported. A detailed structure of all these is beyond the scope of this report which will focus on those used in this project.

1.3.1 Diazirine

Diazirines are molecules characterised by the presence of a 3-membered CNN ring that contains a N=N double bond. Diazirines are common reagents for carbene generated photoaffinity labelling as the radicals formed react with nanosecond or picoseconds time scales under laser flash photolysis.[45] The following section provides the background into the development and application of diazirines as PAL.

1.3.1.1 Early syntheses

The development of diazirines has involved improving the stability through modification of the substituents. In early 1960s, the first reported diazirine was alkyl-3*H*-diazirine **49**. Shortly after dialkyl diazirines **50** were discussed; however, all these molecules were sensitive to heat, UV light and commonly decomposed in the gas phase (Figure 1.12).[46,47]

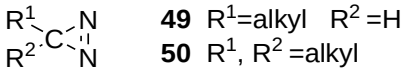


Figure 1.12

For example, 3,3-diethyldiazirine **51** is not a stable compound. On heating to a temperature in the range of 118-149 ℃, this undergoes thermal decomposition to give diazo and carbene compounds. Ultimately, the carbenes undergo intramolecular

rearrangements formed a mixture of *cis*- and *trans*-2-pentenes **52** and ethylcyclopropane **53** (Figure 1.13).[48]

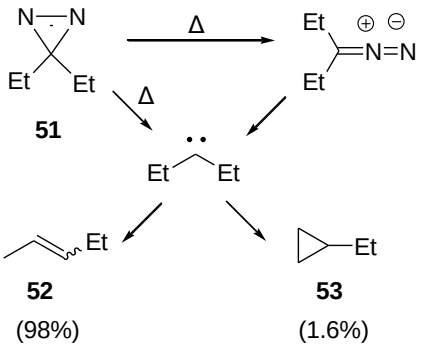


Figure 1.13

The carbene may also be generated by irradiation by UV light. For example, photolysis of diazirine was believed to occur with formation of diazomethane as detected by the chromatographic analyses by using light of 3200 Å (Bausch and Lomb grating monochromator light) (Figure 1.14).[49]

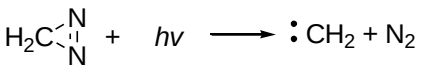


Figure 1.14 Photoisomerisation of diazirine

1.3.1.2 The Graham Reaction

In 1965, Graham reported the one-pot hypohalite oxidation of amidines to 3-halodiazirines,[50] Figure 1.15. The products include either bromine or chlorine atom at the 3-position of the diazirinyl ring. However, such molecules are latently explosive and have not been significantly used as PAL.

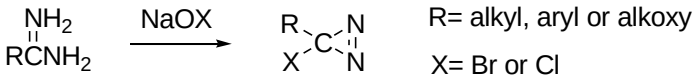


Figure 1.15

However the halogen can be easily replaced by a variety of nucleophiles, Figure 1.16. This gives diazirines of better stability e.g. compound **54**, which have been used for hydrophobic labelling of membrane proteins. However, this approach is limited to simple diazirines (Figure 1.16).[51,52,53,54]

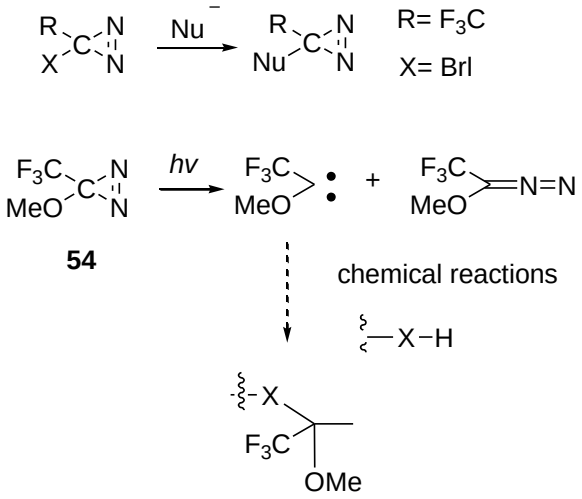
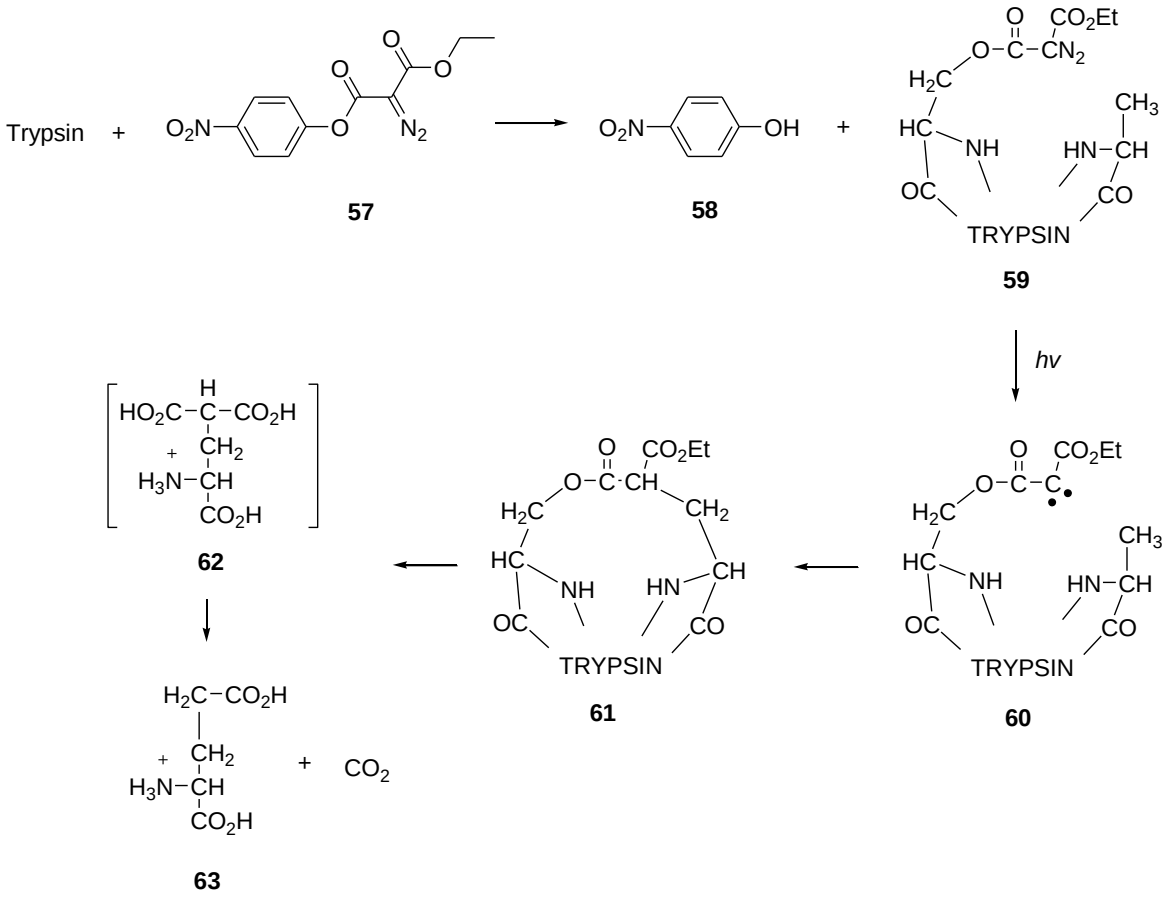


Figure 1.16

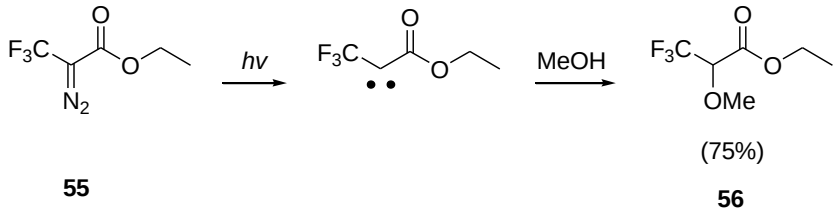
1.3.1.3 The Trifluoromethyldiazirine

Simple carbenes, such as those derived from diazoacetates[55] and diazomalonates,[56] have been used in biological photo-labelling experiments. For example, compound **57** was used to label the active site of trypsin, Scheme 1.8. However, the process was not efficient giving only low yields of labelled product (1-3 %).



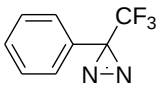
Scheme 1.8

In comparison, trifluoromethylated carbenes are more stable than the equivalent hydrocarbon structures lacking the halogen substituents. This can be exploited in the development of efficient photoaffinity labelling procedures. For example, the carbene formed from 2-diazo-3,3,3-trifluoropropionate **55** does not undergo intramolecular rearrangement by fluorine migration and such trifluoromethyl compounds have much promise for the development of new photoaffinity labels (Scheme 1.9).[57]



Scheme 1.9

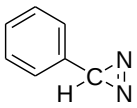
Building on these observations trifluoromethylated diazirines were first reported by Brunner *et al*.[61] In this report the aryl trifluoromethyldiazirine **68** was shown to be more stable for handling under UV irradiation. The development of this concept is discussed in the following section (1.3.1.4).



68

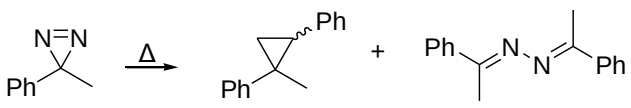
1.3.1.4 Aromatic Substituted Diazirine

The introduction of aryl substituents, e.g. aryl-3*H*-diazirines **64**,[58] seems to provide greater stability than the alkyl substituted analogues. Moreover, in contrast to aryl azide derived nitrenes, the carbene precursor from **64** does not undergo intramolecular rearrangements involving expansion through carbene insertion.



64

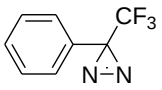
Building upon these observations, Liu *et al*. attempted to develop arylalkyl diazirines **65**.[59] Unhappily, this molecule affords cyclopropanes **66**, and diazo species **67** through intramolecular rearrangement and reaction with second carbene precursor (Scheme 1.10).[60]



65 **66** **67**

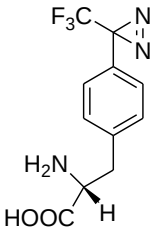
Scheme 1.10

Combining these ideas, in 1980, Brunner's group introduced trifluoromethyl phenyl diazirines **68**. Importantly the carbene generated from this precursor does not undergo intramolecular rearrangements. Furthermore, trifluoromethyl phenyl diazirines can be activated by irradiation at 350 nm well-removed from the wavelength known to cause protein damage and cell death (<250 nm). As a result, 3-aryl-3-(trifluoromethyl)diazirines have found widespread application in biological and synthetic macromolecular studies.[61]



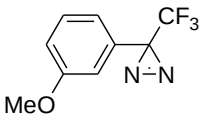
68

For example, *D*-phenylalanine analogue **69** has been used to probe the ligand-binding site of hT1R2 for the sweet taste receptor.[62]



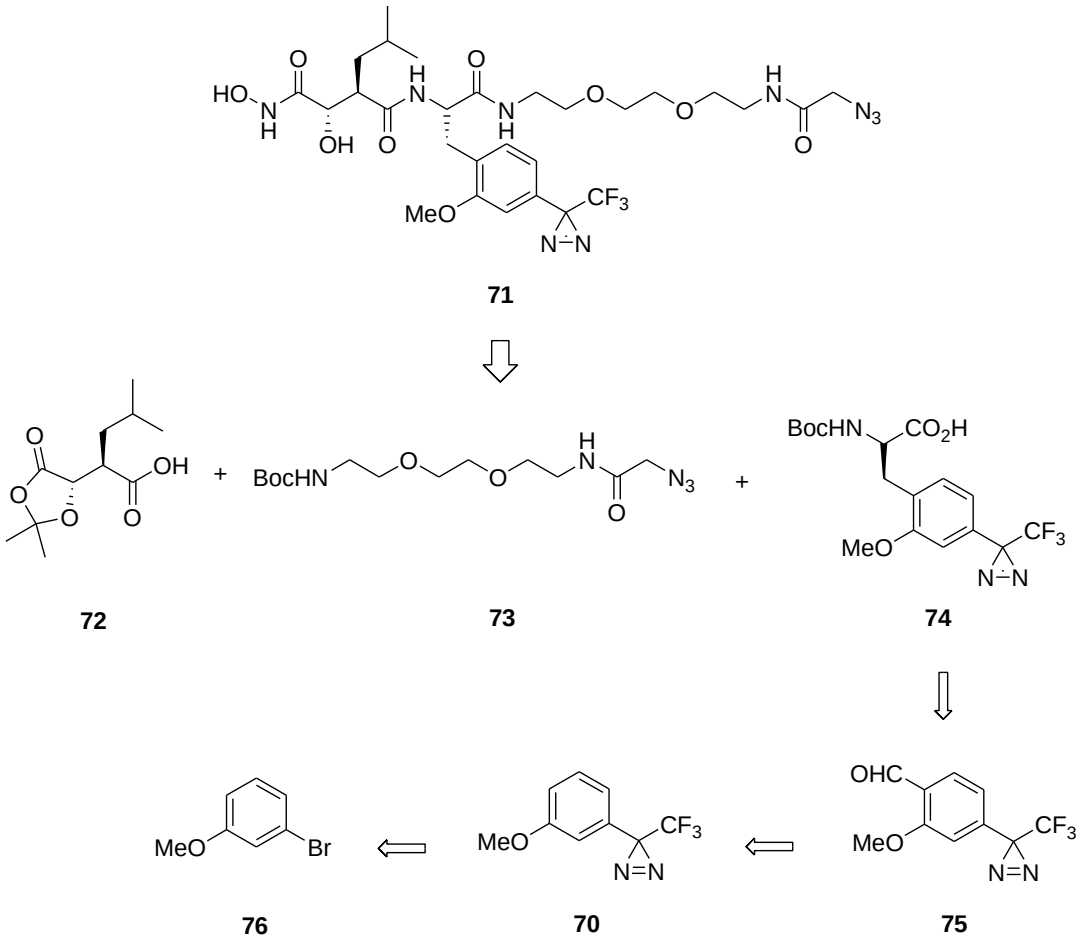
69

The introduction of a methoxy group in the aromatic ring is a common strategy to activate the benzene ring to aromatic substitution e.g. **70**. In this case since the position *para* to the methoxy group is sterically hindered by the 3-(trifluoromethyl)diazirinyl moiety substitution occurs preferentially at the position *ortho* to the methoxy group.[63]



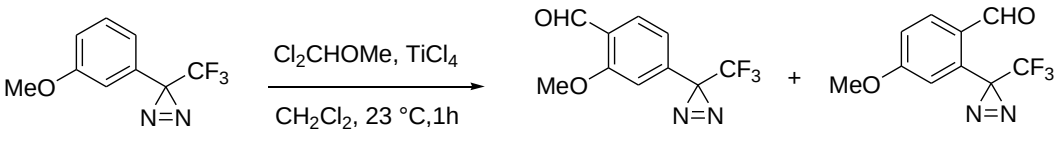
70

For example, Wenwei *et al*. have successfully designed and synthesized a tri-functional photoaffinity probe **71** which labeled matrix metalloproteinases (MMP2-CD) in a B16F10 cell culture. The retrosynthetic analysis of this probe is outlined in Schemes 1.11 and 1.12.[64] As discussed above the *m*-methoxy directing group of the diazirine **70** activates the benzene ring for Friedel-Crafts substitution to give the desired aldehyde **75**.



71 **72** **73** **74** **76** **70** **75**

Scheme 1.11 The retrosynthetic analysis of probe 71.



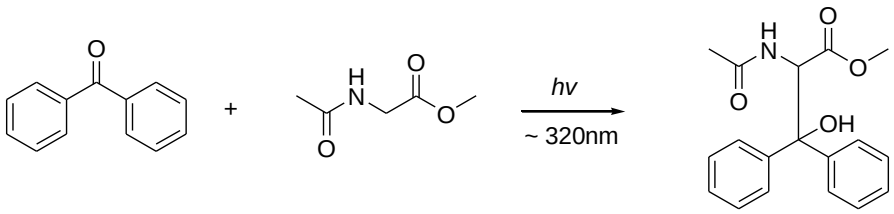
(41%) (9%)

70 **75** **77**

Scheme 1.12

1.3.2 Benzophenone

In 1973, Galardy *et al*. introduced benzophenone (Bp) as a photochemical probe **78**, Scheme 1.13. On photochemical activation the benzophenone derived radical inserted into the methyl ester of acetyl glycine **79** (AcGlyOMe) in water solution to give an amino acid **80**.[65]

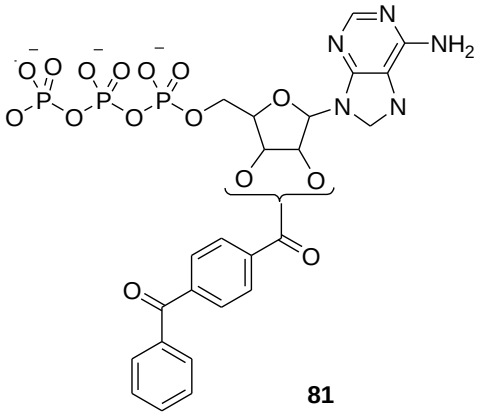


78 **79** **80**

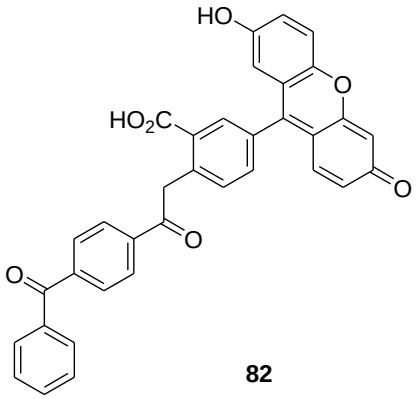
Scheme 1.13

Since then, benzophenone photoprobes have been used in many different biological processes, such as nucleic acid,[66] drugs, proteins,[67,68] and enzymes.[69] One advantage of the benzophenone photolabel is that it can be activated reversibly through excitation-relaxation cycles[70] and so has high labelling effectiveness.[71] Secondly, the required irradiation wavelength of 350 nm does not lead to cell damage.[72] Lastly, Bp has good chemical stability and is able to react with C-H bonds even in presence of water. Reflecting these benefits, Bp has been frequently used for biochemistry research.

Figure 1.17 shows the examples of benzophenones used in biological studies. Compound **81** is used to explore bacterial plasma membrane[73] whilst compound **82** was used as a fluorescent nucleotide photoaffinity label for studies with ATP.[74]



81



82

Figure 1.17 Application of benzophenone photoprobes in biochemistry structures

1.4 Project strategy

Previous work in the group:

Previous work in the group by M. Landrum had involved the preparation of a BODIPY dye with a nitro-aryl group at C-8. As predicted by Density Functional Theory (DFT) calculations this molecule was non-fluorescent. However on incubation into plant cells a fluorescent response was observed. More surprisingly this fluorescence was highly localised to a specific organelle within the plant cell. Subsequent co-incubation experiments with a fluorescent protein containing a known protein targeting sequence (PTS 1) showed that the dye was specifically localising to the peroxisome[75] (Figure 1.18). However the cause of this localisation could not be determined nor could the mode at "switch-on" of fluorescence. One possibility is that the BODIPY is specifically binding to a target biomolecule. Attempts to identify this 'target' by mass spectroscopy were not successful. An alternate approach is to combine the BODIPY core with a secondary label to facilitate target identification. The synthesis of such a functional molecule was the focus of this project.

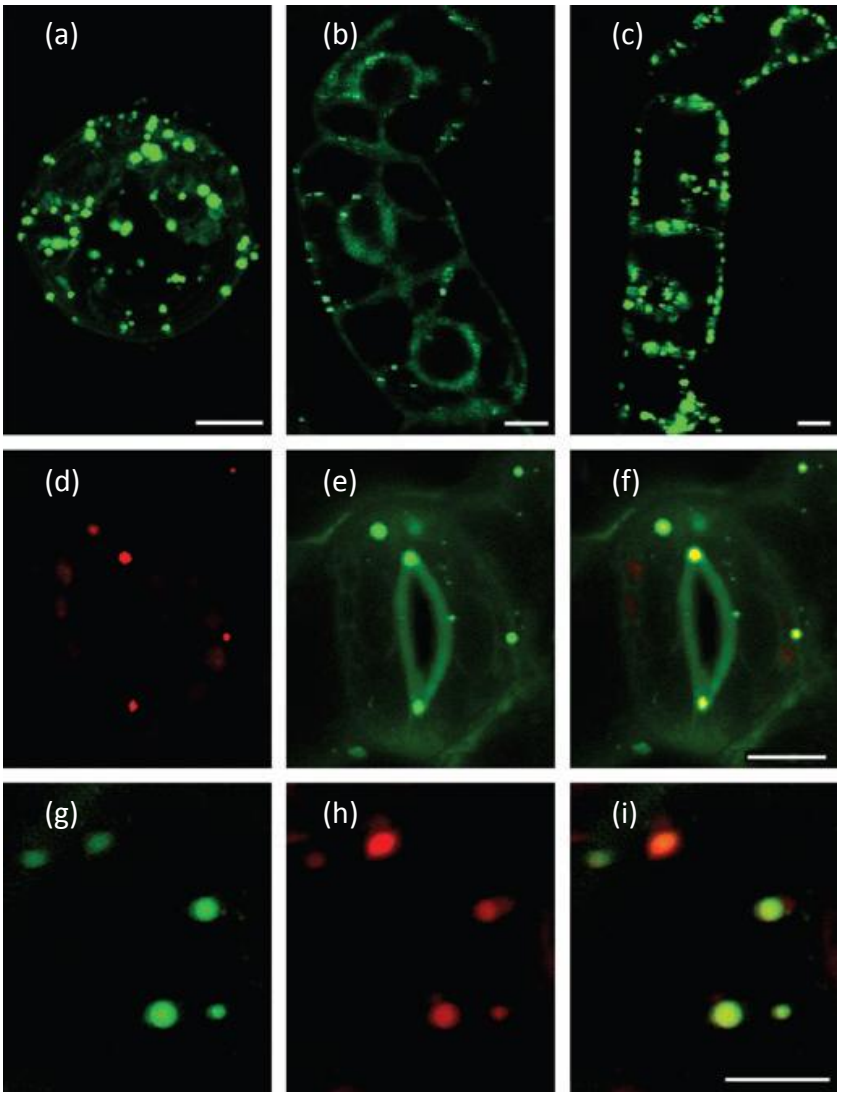
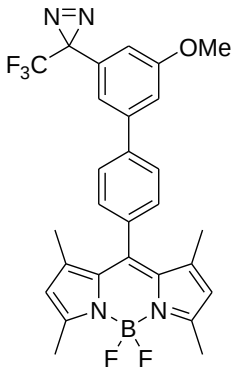


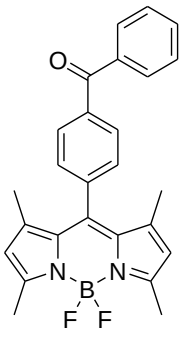
Figure 1.18 nitroBODIPY probe co-localizes with peroxisomes in plant cells. (a) Staining of *A. thaliana* protoplast (b,c) Localization of the probe in control (b) and clofibrate-treated (c) BY-2-cells. (d-i) Co-localization of peroxisome probe PTS1-mCherry (red) and nitroBODIPY probe (green) in *Nicotiana benthamiana* stomata (d-f) and leaf pavement (g-i) cells. In the merged images (f,i), co-localization of the probes appears as yellow staining. Scale bars = 10 μm (Landrum *et al.*, 2010).[75]

Synthesis strategy:

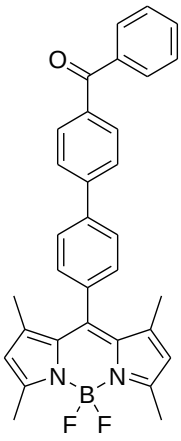
As described above, the goal of the project was to couple a BODIPY core with a second label to identify the target of the BODIPY within the peroxisome. In order to do this a reactive group that could be photoactivated will incorporated into the BODIPY core. Reflecting the precedents for preparing functional used BODIPYs, this would be best achieved at the C-8 position of the BODIPY. Similarly based on the analysis of photoaffinity labelling (PAL) the choice to the synthesis were BODIPY diazirine (**1**) and BODIPY benzophenone (**2**, **3**). The synthetic strategy for probe **1** is based on the aryldiazirine photophore which coupling with the BODIPY substrates, whilst **2** and **3** are based on the benzophenone photophore. The synthesis and analysis of these compounds is described in the next chapter.



1



2



3

2 RESULTS AND DISCUSSION

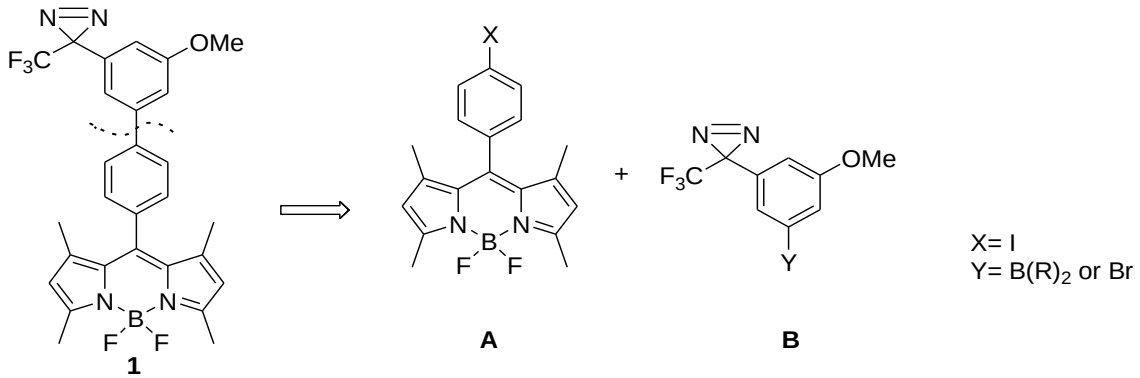
2.1 Introduction

This chapter details the work undertaken in the area of BODIPY dyes and diazirine synthesis. The aim of this work was to synthesise BODIPY dyes conjugated to photoaffinity labelling groups for applications in plant cell biology. Work undertaken on BODIPY dye synthesis will be discussed first, followed by the developments on diazirine synthesis. Lastly, attempts to link these two moieties with palladium-catalysed cross coupling reactions to reach the target compound are described in section 2.6.

2.2 Retrosynthetic Analysis

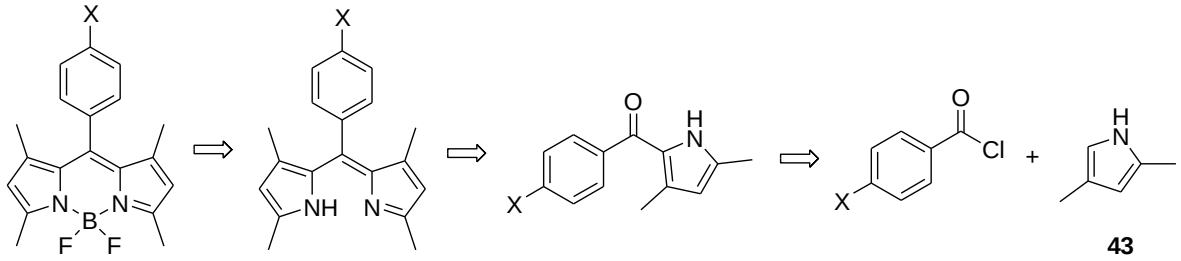
Scheme 2.1 shows the synthetic route proposed for the target molecule **1**. This retrosynthetic analysis disconnects (**1**) at the biaryl bond to give **A** and **B**.

Molecule **A** is a BODIPY derivative which contains a fluorescent core with four methyl groups and an electrophilic residue. Molecule **B** is a known photoaffinity label with its main features being the diazirine on the aryl group for photoisomerization purposes and an organometallic group or a halogen as the other substituent.



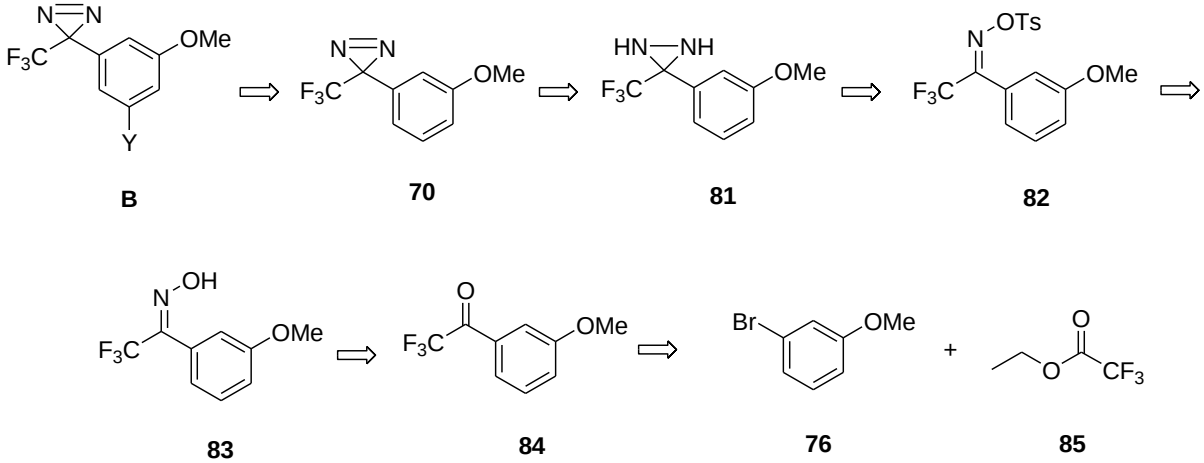
Scheme 2.1

The retrosynthetic analysis of the BODIPY core **A**, basically follows the literature procedure which has already been discussed in the previous chapters. Details of the synthesis are presented in section 2.3.



Scheme 2.2

Scheme 2.3 shows the retrosynthetic analysis of diazirine functional group **B**. This again follows an established literature method for the function of **70**. Introduction of the organometallic group ($B(OR)_2$) would build upon the research in the group using iridium catalysed borylation methods.



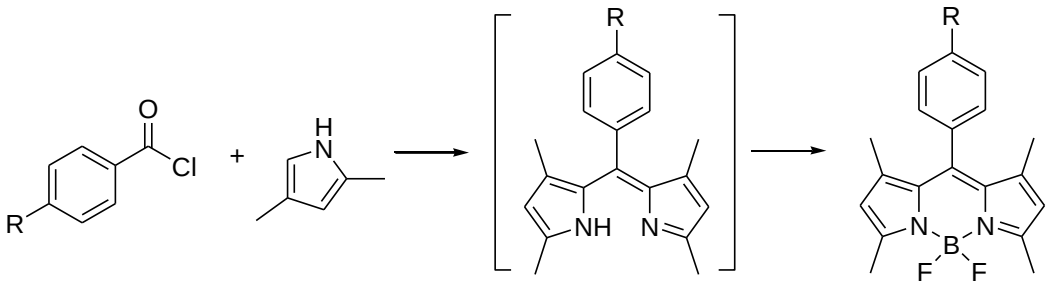
Scheme 2.3

2.3 Synthesis of BODIPY

Based on the analysis described in section 2.2, the first goal of the synthesis was the BODIPY core **A**. This dye was synthesized in two ways building on procedures based on published literature and the previous work within the group.

2.3.1 One-pot reaction

The first attempts to synthesise the BODIPY **90** and **91** involved the one-pot condensation of an acid chloride with two equivalents of the pyrrole **43** as shown in Scheme 2.4.



C **43** **D** **E**

86: R = H
87: R = NO_2

88: R = H
89: R = NO_2

90: R = H
91: R = NO_2

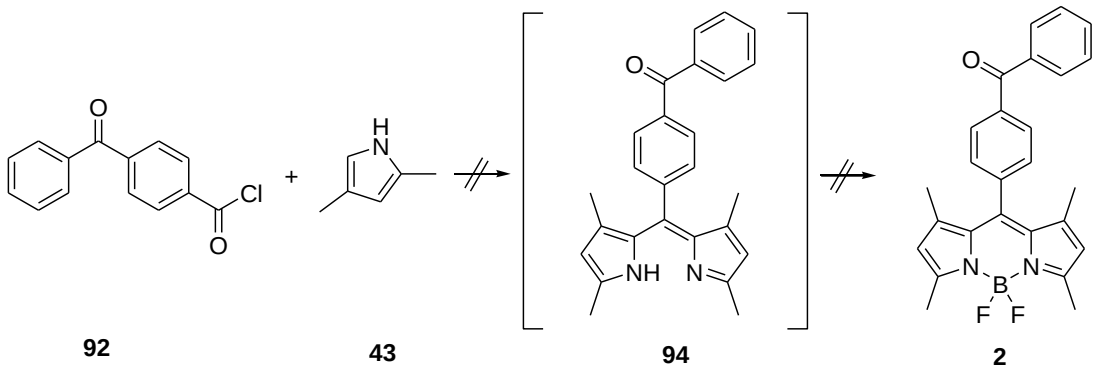
Scheme 2.4

This reaction was repeated from Landrum's thesis.[76] Initial attempts to synthesise **90** began by using benzoyl chloride. This was successfully achieved using two equivalent of 2,4-dimethylpyrrole, Et_3N, and three equivalent $BF_3{\cdot}OEt_2$ base reagent. However, this reaction was very inefficient giving only 1 % isolated yield of the desired BODIPY **90**. The presence of the BODIPY molecule was confirmed by the ^1H NMR spectrum from the appearance of two peaks corresponding to the aryl group and the CH groups (1.56 ppm, 6H; 1.37 ppm, 6H) and the seven protons (7.56-7.43 ppm, 3H;

7.28 ppm, 2H; 5.98 ppm, 2H). This data agrees with that reported by Landrum (Scheme 2.4).

Repeating the same method, BODIPY **91** was successfully isolated, albeit in only 7 % isolated yield initially. Analysis of **91** by ^{1}H NMR spectroscopy recorded four methyl groups (2.57 ppm, 6H; 1.36 ppm, 6H) and six protons (8.42 ppm, 2H; 7.58 ppm, 2H; 6.02 ppm, 2H).

Although low yielding, the successful isolation of the product was encouraging. The attempts to use of other substituent which might afford the desired compound **2** contains a photo benzophenone label. Following the same one-pot procedure, we expected 4-benzoyphenyl acid chloride **92** to react with 2,4-dimethylpyrrole **43**. Unfortunately, no product could be detected by GC-MS or ^{1}H NMR. The reasons for this are not obvious (Scheme 2.5).



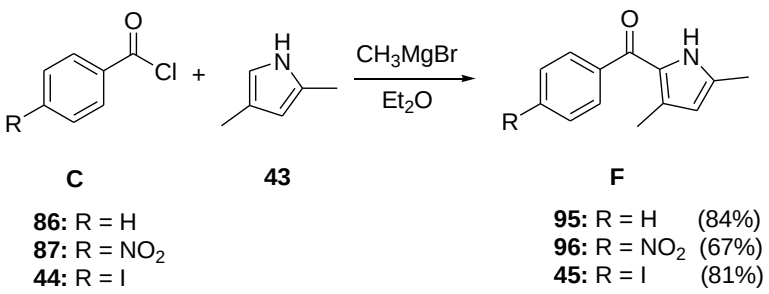
92 **43** **94** **2**

Scheme 2.5

2.3.2 Two step process

Due to the low isolated yield of the final BODIPY product **90** and **91**, the procedure was modified to a two-step process that has been previously described in the

literature.[77] This involved the reaction of the acyl chlorides **C** with the magnesium salt of pyrrole **43**, to afford 2-ketopyrroles **F** in 67-84 % yield, Scheme 2.6. Evidence for the formation of **95** was seen by the presence of a singlet in the ^{1}H NMR spectrum at 9.24 ppm (1H, s) and two further singlets at 2.31 ppm (3H, s) and 1.93 ppm (3H, s) corresponding to an NH group and two methyl groups, respectively. In addition a signal due to a carbonyl carbon could be seen in the ^{13}C NMR spectrum at 185.4 ppm. From the IR spectrum, a peak at 3266 cm^{-1} indicated that an N-H group peak is present. Structurally similar compounds **96** and **45** have similar ^{1}H NMR data to **95** described above.



C

86: R = H
87: R = NO_2
44: R = I

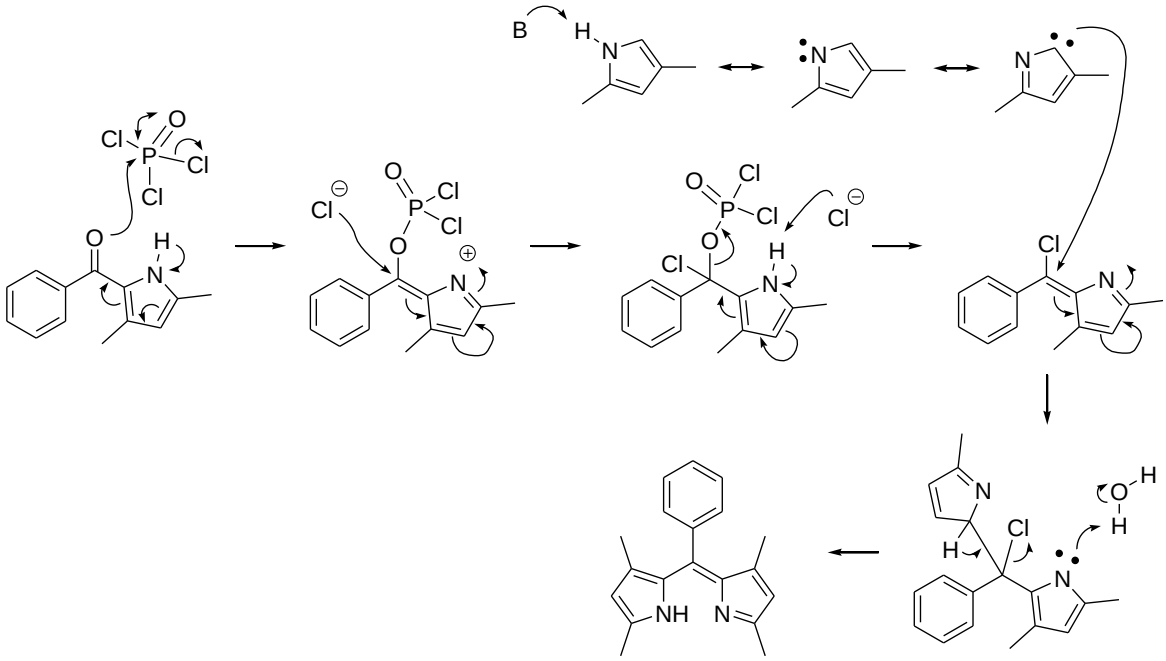
43

F

95: R = H (84%)
96: R = NO_2 (67%)
45: R = I (81%)

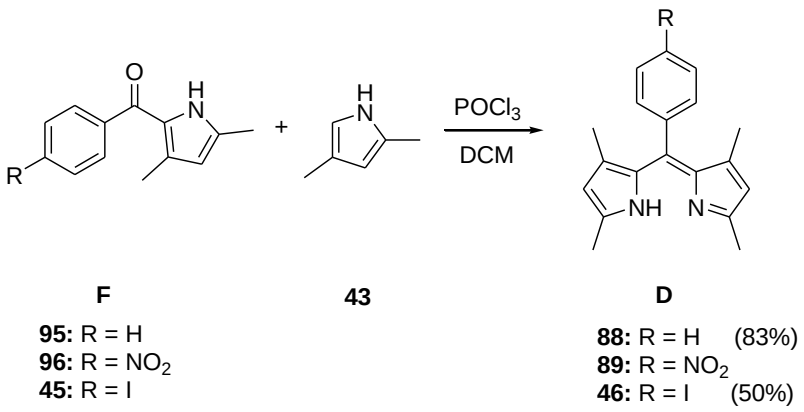
Scheme 2.6

The intermediate symmetrical dipyrromethenes **D** were then obtained through condensation with pyrrole **43** in the presence of $POCl_3$, Scheme 2.8. The reaction mechanism is shown in Scheme 2.7.



Scheme 2.7

The isolated yields of dipyrromethenes are a compromise between their acidity and stability. Burghart *et al*. has described the isolation of the intermediate dipyrromethenes process as best done using a deactivated alumina column rather than silica gel.[78] Following this idea, alumina oxide based column purification increased the yield from the 10 % obtained using silica gel to 80 % yield. The intermediate **88** was confirmed by the appearance of a peak from the pyrrole NH at 11.96 ppm (1H, s) in the ^{1}H NMR spectrum (Scheme 2.8).



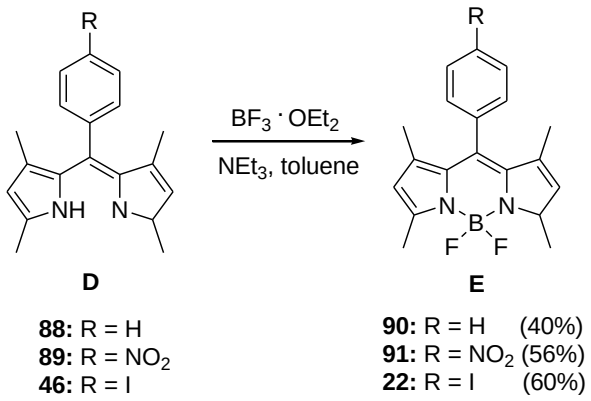
F **43** **D**

95: R = H
96: R = NO_2
45: R = I

88: R = H (83%)
89: R = NO_2
46: R = I (50%)

Scheme 2.8

In a final step, the dipyrromethenes were complexed with boron trifluoride by treatment with $BF_3 \cdot OEt_2$ and triethylamine in dichloromethane. Following the reaction, the desired BODIPY dyes **E** can then be purified by flash column chromatography and crystallization. Repeating this two step process procedure gave **91** in 56 % yield (2.57 ppm, 6H; 1.36 ppm, 6H), and **22** in 60 % yield (2.55 ppm, 6H; 1.42 ppm, 6H) by ^{1}H NMR spectrum (Scheme 2.9).



D

88: R = H
89: R = NO_2
46: R = I

E

90: R = H (40%)
91: R = NO_2 (56%)
22: R = I (60%)

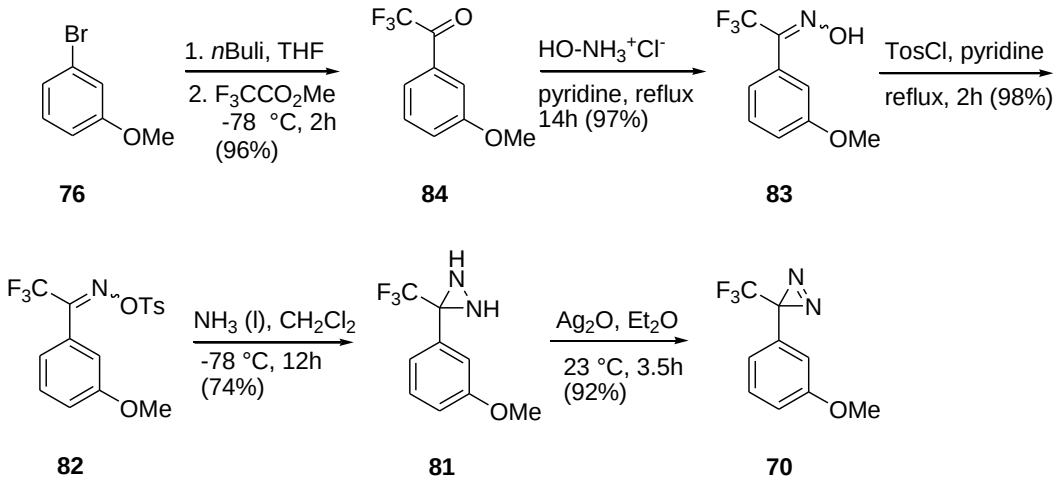
Scheme 2.9

Whilst with this modification of the influence of reaction time, the reaction yield also improves with increasing concentration of the reagent and the temperature. The best method was found to involve the direct conversion to the target BODIPY without purification of the intermediate dipyrromethene **E**. Simply evaporation of the solvent with flowing argon before adding boron trifluoride diethyletherate and triethylamine proved to be sufficient. The conversion was complete within 30 min on heating at 80 °C. As before, the best isolated yields (60 %) were obtained using alumina oxide chromatography instead of silica gel.

2.4 Synthesis of diazirine

Having completed the synthesis of the BODIPY it was necessary to prepare the photocrosslinker partners. As discussed above in section 2.3 the trifluoromethyl phenyl diazirine **B** was selected as the optimal labelling regent. The following section will describe the synthetic approaches explored.

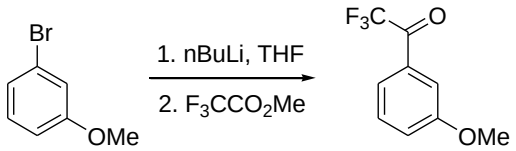
Diazirine **70** previously has been reported,[79] using a five step sequence starting with bromide **76**, Scheme 2.10. Initial metalation of **76** with *n*-butyllithium and subsequent reaction with methyl trifluoroacetate gave ketone **84**. Oxime **83** was obtained through reaction with hydroxylamine hydrochloride in the present of base and then protected with tosyl chloride to give the *p*-tolylsulfonyloxime **82**. Reaction of **81** with ammonia then oxidation with silver oxide gives the desired probe **70** (Scheme 2.10).



Scheme 2.10 Synthesis of 3-(3-Methoxyphenyl)-3-(trifluoromethyl)-3*H*-diazirine as reported by Baldwin.[76]

Following this literature procedure, starting from the commercially available, bromoanisole **76**, metalation with *n*-butyllithium serves to form the carbanion.

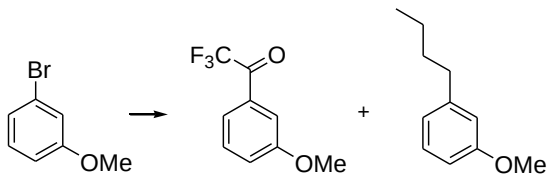
Acylation of the resultant aryllithium with methyl trifluoroacetate then proceeded to give ketone **84** (Scheme 2.11). The synthetic crude product was purified by Kugelröhr distillation at 80 °C and 2 mbar. Analysis by GC-MS successfully detected the molecular ion at *m/z* 204 and ^{19}F NMR spectroscopy revealed a characteristic peak at δ -71.18 and the IR spectrum showed an C=O stretch at 1710 cm^{-1} confirming formation of the trifluoromethyl ketone.



76 **84**

Scheme 2.11

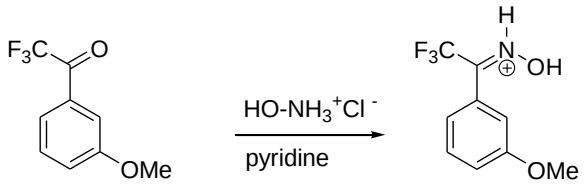
Importantly, if the reaction with *n*-butyllithium was left for a period of time exceeding two hours, a by-product **97** was observed. Analysis of the crude reaction mixture by GC $EI^{+}MS$ revealed a peak for butyl methoxybenzene **97** with *m/z* 164 (Scheme 2.12). Consequently, metalation with *n*-butyllithium needed to be tracked by TLC and the acylating agent added as soon as the halide starting material was consumed.



76 **84** **97**

Scheme 2.12

Ketone **84** was then converted to the corresponding oxime **83** by reaction with hydroxylamine in the presence of pyridine. The analysis of the product by ^{1}H NMR spectroscopy revealed two signals for the OH group probably reflecting low interconversion between *cis* and *trans* oxime isomers at δ 8.86 and 9.08 ppm. Further evidence for the oxime was found in the both the IR spectrum, which showed a broad band consistent with the presence of an OH group at 3328 cm^{-1} together with that for an imine at 1576 cm^{-1}, and the ES^{-}MS which showed a molecular ion peak at *m/z* 219 (Scheme 2.13).

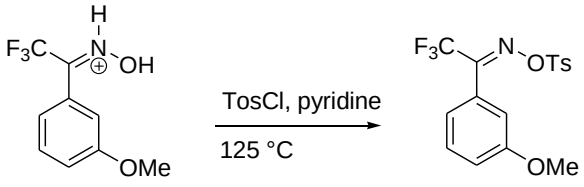


(98%)

84 **83**

Scheme 2.13

Tosylation of the oxime with toluenesulfonyl chloride in the presence of pyridine proceeded smoothly to give tosyloxime **82** in high yield. Compound **82** was identified by ^{1}H NMR spectroscopy which shows a peak for the tolyl methyl group at 2.48 ppm (3H, s). Further evidence included peaks in the ^{19}F NMR spectra at δ -66.9, and ES^{-}MS which showed a molecular ion peak at *m/z* 373 (Scheme 2.14).

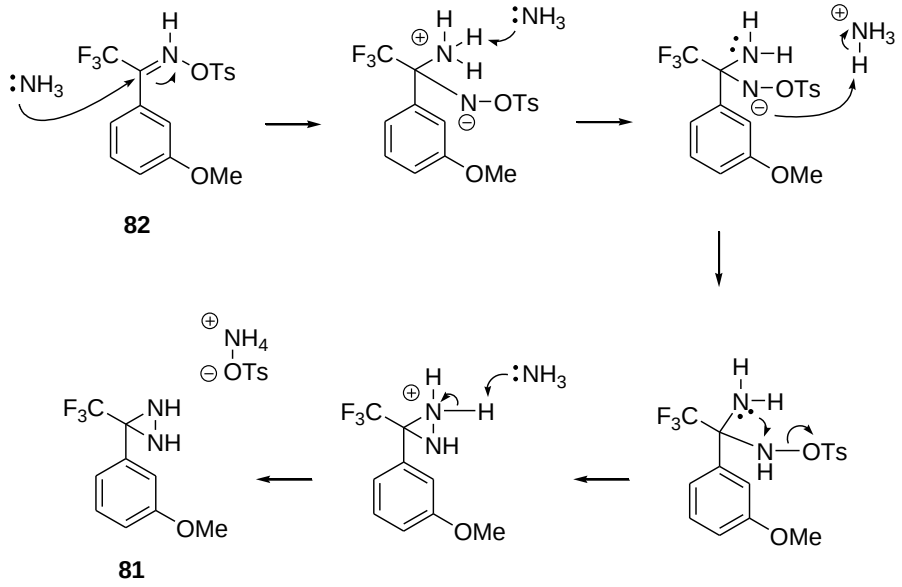


(96%)

83 **82**

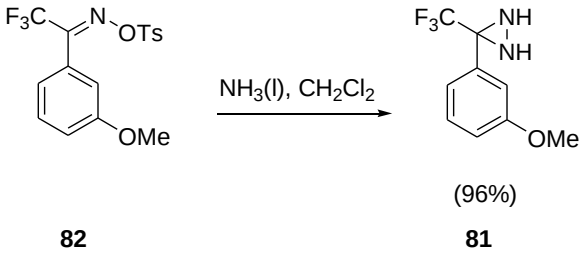
Scheme 2.14

The tosyloxime **82** was then converted to diaziridine **81** by reaction with liquid ammonia at -78 ℃ for 2 h. A reaction mechanism for this conversion is shown in Scheme 2.15.



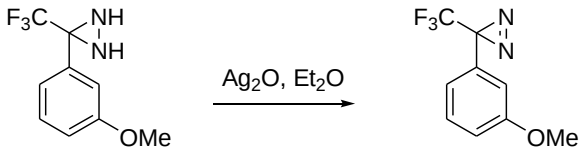
Scheme 2.15

Following a standard work up, the diaziridine **81** was isolated as a pale yellow oil. This was characterised by ^{1}H NMR spectroscopy with peaks at 2.75 ppm (1H, s) and 2.21 ppm (1H, s) being consistent with the two N-H protons. ^{19}F NMR spectroscopy revealed a characteristic peak for the CF_3 group at δ -75.45, and ES$^-$MS showed a molecular ion with *m/z* 218 (Scheme 2.16).



Scheme 2.16

Lastly, oxidation of **81** with silver oxide furnished diazirine **70.** Successful conversion could be seen in the ^{1}H NMR spectrum by the loss of the signal for the NH protons present in the starting material **81**. This was supported by a different molecular ion in the $ES^{-}MS$ spectrum at *m/z* 216 (Scheme 2.17).



81 (95%) **70**

Scheme 2.17

With the photoaffinity group now in hand, the borylation with bis(pinacolato)-diboron (B_2Pin_2) and then coupling with the BODIPY core could be investigated. This is described in the next section.

2.5 Synthetic strategies for cross-coupling reactions

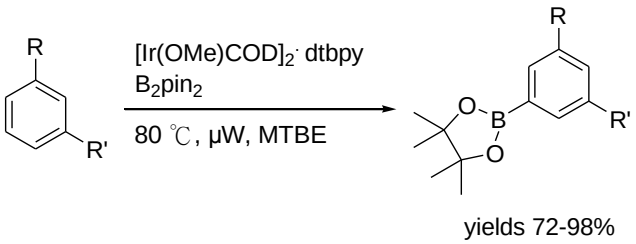
With the successful synthesis of iodo-BODIPY **22**, and *meta* trifluoromethyl diazirine 70. Cross-coupling of these molecules could now be undertaken. BODIPY probe **1** need to be converted to the corresponding boronic acid. To do this, several strategies were explored. The following sections describe each one in detail.

2.5.1 Strategy 1

Iridium-catalyzed arene borylation reactions allows the synthesis of boronates by cross-coupling of bis pinacolato diboron (B_2Pin_2) with C-H bonds. This has become an area of much activity with many recent developments. In particular, work in the group by Harrison who had successfully used a microwave heating to generate

arylboronates from the corresponding unfunctionalised aromatic C-H bond with high efficiency.[80]

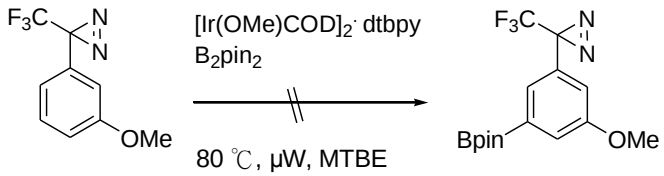
This method for generating aryl boron compounds can be highly regioselective. This selectivity is due to the steric bulk of substituents around the aromatic ring. For example, 1,3-disubstituted arenes undergo borylation at the *meta* position only, Scheme 2.18.



Scheme 2.18

As a result it was our intention to utilise this approach to insert a boronate, *meta* to the diazirine group of **70**. The active reagent was prepared in a stock solution containing 1.0 mmol of bis pinacolato-diboron (B_2Pin_2, pin= $O_2C_2Me_4$), 1.5 mol % of precatalyst $[Ir(OMe)COD]_2$, and 3 mol % of 4,4'-di-t-butyl-2,2'-bipyridine (dtbpy).

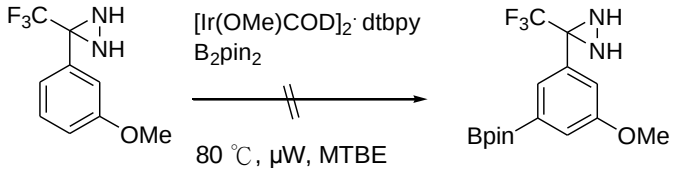
The first attempts to synthesise diazirine **98** began by the borylation of **70**. However, under standard conditions, the reaction produced no product that could be detected by either GC-MS or ^{1}H NMR with the bulk of the starting material being recovered. A possible reason might be that the diazirine moiety is inhibiting the iridium catalytic cycle (Scheme 2.19).



70 **98**

Scheme 2.19

Due to the failure of diazirine **70** as the borylation substrate, we took one step backwards to explore diaziridine **81** as the boronate precursor (Scheme 2.20). Following the standard conditions, diaziridine **81** was borylated with microwave heating at 80 ℃ for 2 min. Analysis of the crude reaction by GCMS shows *m/z* 217 and *m/z* 239, 1: 1.3 peak ratios, which are characteristic of the starting material **81**, and B_2pin_2, respectively. It was thought that preferential coordination of the NH groups was inhibiting the reactivity of the Iridium catalyst.

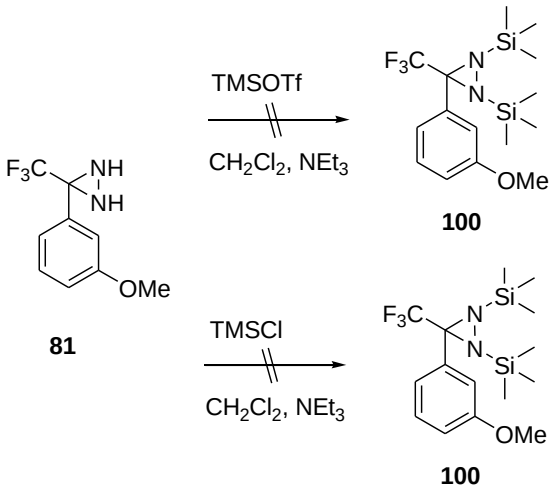


81 **99**

Scheme 2.20

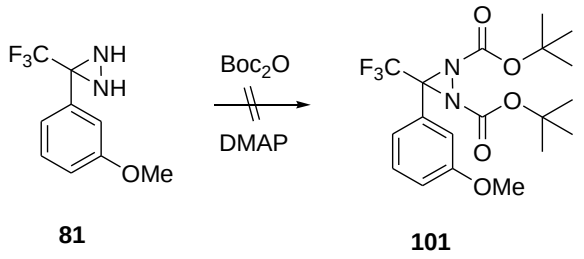
Since the formation of boronate **90** from diaziridine **81** turned out to be incompatible with the borylation conditions, it was decided to examine the reaction with protecting groups on the nitrogen atoms which might prove more effective. On this basis trimethylsilyl chloride (TMSCl), trimethylsilyl trifluoromethanesulfonate (TMSOTf) and di-*tert*-butyl dicarbonate (Boc_2O) group were considered as NH protecting groups.[81,82] Both TMSCl and TMSOTf were tested in the same conditions with triethylamine in DCM at -78 ℃ to rt. The reaction process was monitored by ^1H and

^{19}F NMR analysis. In this 0.1 mL samples were taken from the reaction mixtures and concentrated in vacuum and analysed. After one day, starting material still remained in the reaction mixture with unknown by-products, and no evidence of the desired product (Scheme 2.21).



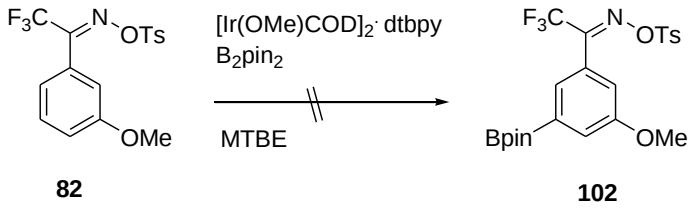
Scheme 2.21

Furthermore, reaction of diaziridine **81** with Boc_2O was examined with 1equiv DIPEA (diisopropylethylamine) at room temperature. After three days stirring, analysis by ^{1}H NMR spectroscopy, ^{19}F NMR spectroscopy and GCMS only revealed a mixture of starting materials and unknown side products. Increasing the number of DIPEA equivalents and using longer reaction times were also unsuccessful. As a result of these disappointing results protection studies were discontinued (Scheme 2.22).



Scheme 2.22

As none of the attempted borylation reactions were successful on **70** and **81** (see page 40), 4-toluenesulfonyl oxime **82** was explored as the borylation precursor. Using the standard conditions as described above the synthesis of iridium-catalysed borylation was attempted, Scheme 2.23. An initial attempt to borylate at 80 ℃ for 10 min, saw only starting material by TLC. The reaction was continued for one more hour and heated up to 100 ℃. Disappointingly, only starting material was detected by ^{19}F NMR. The second experiment of this reaction was using microwave heating at 100 ℃ for 30 min, the crude products were monitored by GCMS showing two peaks at *m/z* 373 and *m/z* 239 which are the starting materials **82** and B_2Pin_2 respectively. As a result of these difficulties, this borylation strategy was then discontinued.

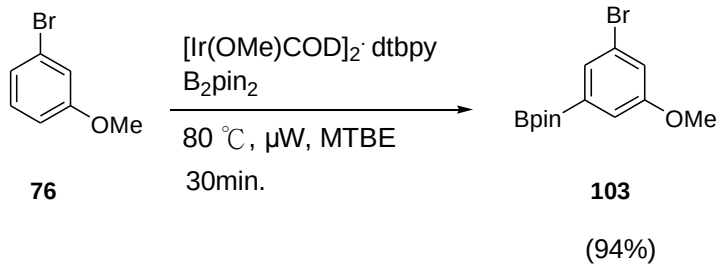


82 **102**

Scheme 2.23

2.5.2 Strategy 2

Since all attempts to borylate **70**, **81** and **82** and to prepare protected variants of diaziridine **81** were unsuccessful, attention turned to introduction of the Bpin group at the start of the diazirine synthesis. Using the standard condition which have been described in the previous section, the synthesis of **103** was attempted (Scheme 2.24).



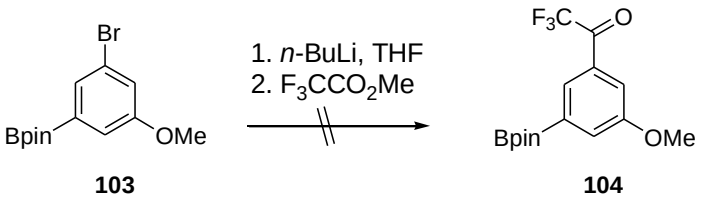
76 **103**

(94%)

Scheme 2.24

The reaction was carried out with microwave heating at 80 ℃ for 30 min and gave good isolated yield. Analysis of **103** by ^{1}H NMR spectroscopy recorded the desired pinacolborane methyl group signals at δ 1.34 (12H, s), and *m/z* 313 shown by GC-MS. Although compound **103** has been synthesized previously in the Ishiyama group, heating at 80 ℃ for 16 h in 73 % yield,[83] Iridium catalyzed borylation with microwave heating took a shorter time and gave a better isolated yield.

Having successfully synthesised **103** using microwave heated iridium-catalyzed borylation of 3-bromoanisole **76**. Attention turned to modifying the metalation acylation steps to give ketone **104**. Following the standard procedure as section 2.4 (see page 35), the attempted metalation with *n*-butylithium with compound **103** was attempted (Scheme 2.25).

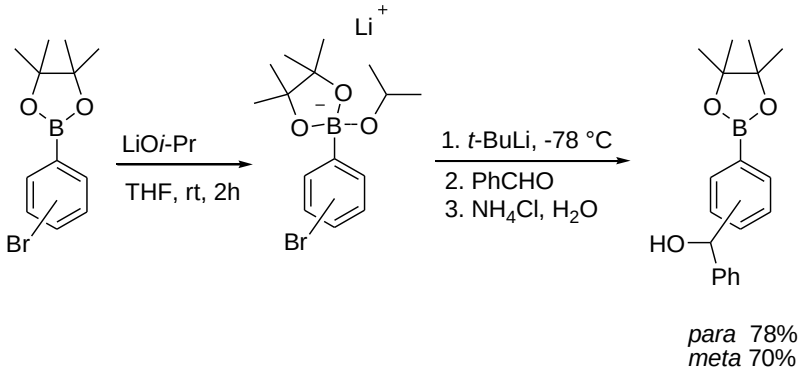


103 **104**

Scheme 2.25 Attempted synthesis of **104** from **103**

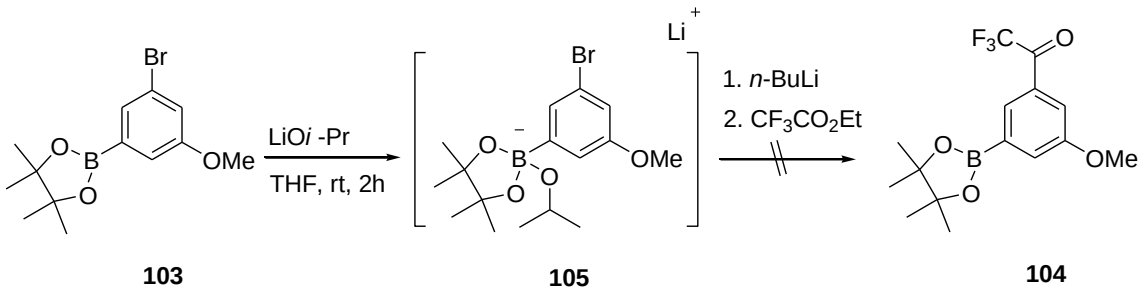
After 20 min. stirring with *n*-BuLi in THF at -78 ℃, the reaction mixture became very gummy, and was hard to stir. Due to this physical change, the reaction was difficult to track by TLC. After the standard work up, analysis of the crude reaction mixtures by GC-FID trace recorded a signal for the ketone **104** as shown by a molecular ion *M/Z* = 330 in the ESMS spectrum at 17.5 min. However, this peak only integrated for 7 % in area percentage. The majority compound in the mixture was the starting material showing 60 % in area percentage, with the rest being unknown by-products. Overall this suggested that the *n*BuLi preferentially underwent nucleophilic attack on the Bpin ring, generating complex mixtures upon work up.

Since reaction of *n*-butylithium with compound **103** appeared to lead to side reactions with the Bpin group, attention was drawn to the results from Jiang *et al*. They reported protection of arylboronates with lithium isopropoxide in a one-pot reaction.[84] These studies showed that the protected intermediate *meta*- and *para*-bromoboronates could undergo metal-halogen exchange with *t*-BuLi, Scheme 2.26.



Scheme 2.26 Protection of Boronates with Lithium Isopropoxide

With the information from this research in hand a synthetic route was proposed. Firstly protection of boronate **103** will generate the desired complex **104** following literature procedure followed by further addition of *n*-BuLi and ethyl trifluoroacetate (Scheme 2.27).



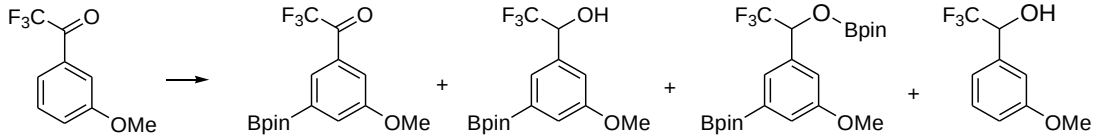
Scheme 2.27 Attempted synthesis of 15 from 14 intermediate

Compound **103** was treated with lithium isopropoxide to give anionic intermediate **105**. Following metal-halogen exchange with *n*-butyllithium ethyl trifluoroacetate was added. Unfortunately, after andard aqueous workup, TLC and analysis by ^{1}H NMR spectroscopy only revealed a mixture of starting materials. Presumably *n*-BuLi causes decomposition of the pinacolatoboronate intermediate.

2.5.3 Strategy 3

From the previous section, although we successfully introduced a boronic acid moiety to **103** (see page 43), it was not possible to achieve the halogen lithium exchange even with lithium isopropoxide protection of the boronate. Consequently, the order of events was reversed and borylation of ketone **84** was investigated.

This synthesis summarized in Table 2.1 shows the reaction carried out under microwave heating at 80 ℃ for various times and equivalents of B_2Pin_2. On following different reaction condition, after microwave borylation, crude products were monitored by GC-MS and got various results (Scheme 2.28 and Table 2.1). As a result the most useful analytical technique was determined to be GC-MS. Although this technique did not provide accurate information regarding the product percentage it did confirm some characteristic peak of **84**, **104**, **106**, and **107**. Overall all compounds purification proved difficult because of the similarity of the physical properties of the isomers.



84 **104** **106** **107** **108**

Scheme 2.28

Entry	B_2Pin_2 eq	Time	Temp. (℃)	104	106	107	108	84
1	1	1h	80	12% [b]	87% [b] (66%) [a]	nd [b]	<1% [b]	nd [b]
2	1	15min	80	45% [b] (8%) [a]	34% [b] (60%) [a]	7% [b]	<1% [b]	nd [b]
3	1	5min	80	34% [b]	47% [b]	8% [b]	<1% [b]	nd [b]
4	0.5	5min	80	88% [b] (35%) [a]	7% [b] (41%) [a]	<1% [b]	<1% [b]	<1% [b]
5	0.5	1min	80	72% [b] (45%) [a]	10% [b] (34%) [a]	5% [b]	2% [b]	11% [b]
6	0.5	1min	80	47% [b] (29%) [a]	22% [b] (38%) [a]	6% [b]	1% [b]	19% [b]

[a] Purified isolated yield. [b] determined by GC-MS analysis of crude product. nd: not detected.

Table 2.1 Comparison of Microwave Borylation with different time and eq.

Initial attempts used 1 eq of B_2Pin_2 for 1 hour microwave heating at 80 °C. Although conversion of Bpin was good, reduction of ketone occurred to give a mixture of **106** and **108** (Entry 1). The formation of **106** was suggested by GC-MS which showed a peak with a molecule ion at *m/z* 332. ^{1}H NMR provided quintet at δ 5.04 – 4.97 (1H) and doublet at δ 2.55 (1H) attributed to the secondary alcohol moiety. In addition analysis of the ^{11}B NMR and ^{19}F NMR spectra showed peaks at 6.87 and -78.07 ppm respectively consistent with the proposed structure.

Next attempts were made to reduce the reaction time also with 1 eq of B_2Pin_2. This produced the desire product **104** albeit in low yield (Entry2 and 3). Evidence for arylboronate **104** was obtained from the ^{1}H NMR spectrum which revealed the presence of the pinacol methyl group signals at δ 1.37 (12H, s), and 180.8 ($CF_3\underline{C}O$, q) for the carbonyl carbon group. GC-MS showing a molecule ion *m/z* 330. The proposed **107** and **108** showed *m/z* 458 and *m/z* 206, respectively.

Reducing the B_2Pin_2 loading to only half equivalent lead to increased yields of the desired ketone **104** (Entry 4). However isolated yields **104** did not equate to those identified from GC-MS analysis.

In addition to this work it has also been shown that reducing the reaction time to 1 min. microwave heating prevent less reduction of ketone (Entry 5). However, attempts to reproduce these conditions were not successful and further work is still needed (Entry 6).

Analysis of **84**, and **104** by ^{19}F NMR spectroscopy proved difficult as these have very similar chemical shifts (Figure 2.1).

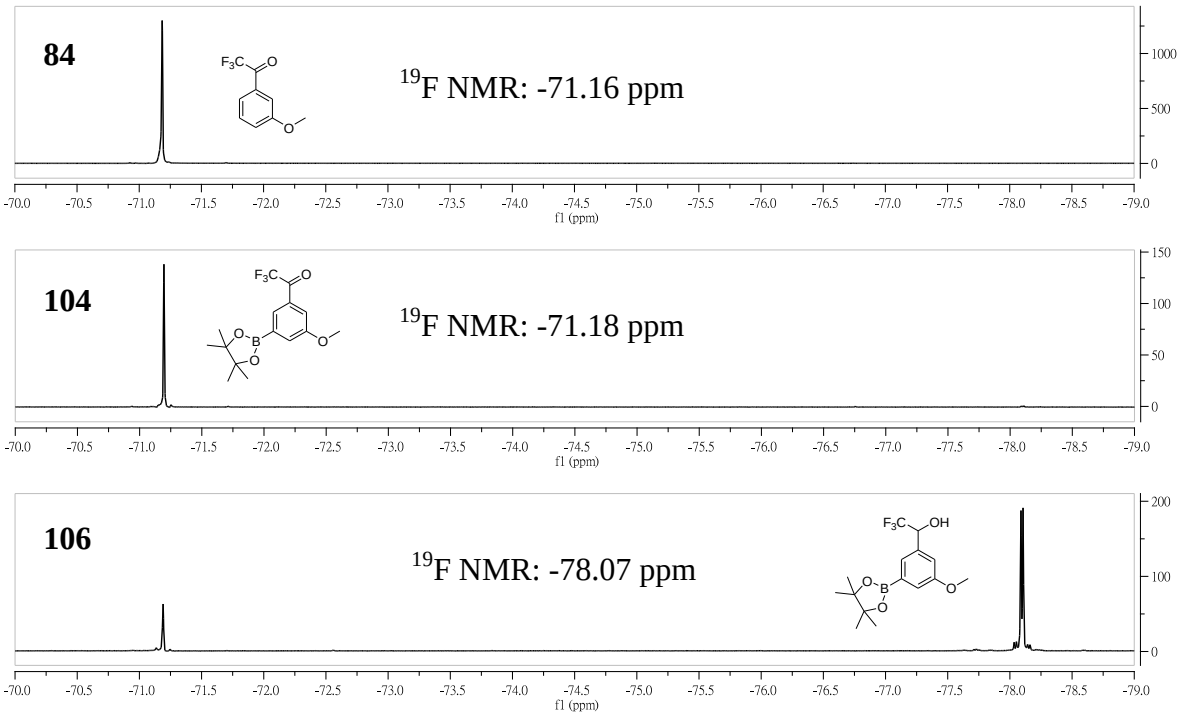


Figure 2.1 ^{19}F NMR spectrum of compound **84**, **104** and **106**

In summary these borylation, reactions lead to a complex mixture of products being observed by GC-MS and ^{19} F NMR. Although reduction of the microwave heating time gave better selectivities, incomplete conversion of the starting material and reduction of ketone still occurred. Several factors are proposed to lead to the formation of alcohol. Most notably the HBpin generated during C-H borylation of arenes could potentially react to reducing the carbonyl group.

In attempts to avoid completing reduction of the ketone during the borylation reaction with ketone, a secondary sacrificial carbonyl compound was added to the reaction mixture. Acetone was selected as a suitable trial additive. In order to evaluate the effect of acetone, three reactions containing different equivalents of acetone were explored using the conditions described below in Table 2.2.

Entry	Dry acetone (eq)	B_2Pin_2 (eq)	Time (min)	Temperature (°C) µw	^{19}F NMR ratio		
					84	**104**	**106**
1	0	1	1	80	1	1.5	23
2	5	1	1	80	1	1.25	0.5
3	20	1	1	80	1	0.4	0.3

Table 2.2

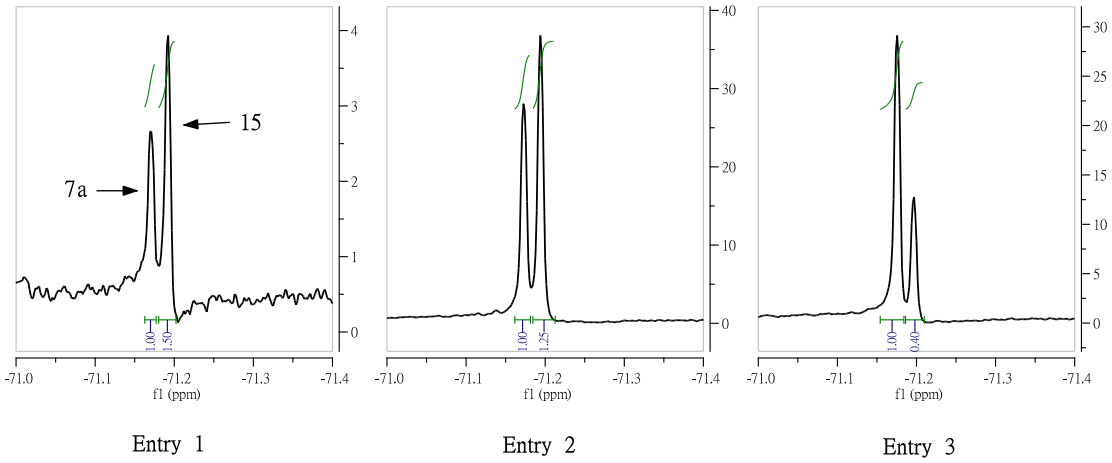
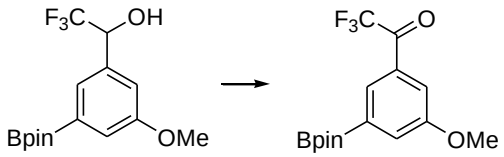


Figure 2.2 The ratio between Starting material **84** and ketone **104**.

Analysis of these three different crude reaction mixtures by ^{19}F NMR spectroscopy using signals for the starting material **84** at -71.16 ppm, product **104** at -71.18 ppm, and peaks for the formation of many side products (-78.07, -78.09, -78.10, -78.12, -78.15, -78.17, -78.20, -78.22) revealed that although increasing the acetone equivalents led to lower ratios of reduced product to the desired ketone **104** this accompanied by lower conversions and increased amounts of unknown side-products. Therefore this approach was abandoned (Figure 2.2).

Due to difficulties in obtaining a good quality yield of ketone **104**, we tried to oxidize alcohol **16** back to ketone **15** with various oxidation reagents. Previous reports in literature have highlighted the use of iodoxybenzoic acid (IBX) for oxidation of alcohols.[85,86] However, on carrying out the reaction with 1.5 equivalents of IBX in

DMSO, alcohol **106** was not oxidized fully. It was thought that due to the mild oxidant, and the deactivating functional group was not compatible with its use. However, subsequent attempts using manganese dioxide (MnO_2) as an oxidizing agent in DCM, stirred for 3 days in room temperature, successfully formed **104** in 40 % isolated yield. Upon investigation of the data it could be seen from the ^{1}H NMR spectrum that there were no alcohol protons present. Furthermore, MS analysis confirmed the product had the expected molecular mass of 330 (Scheme 2.29).



106 **104**

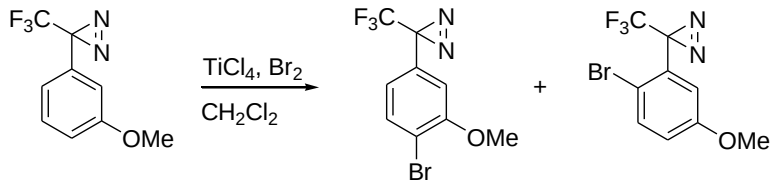
Scheme 2.29

Disappointingly whilst oxidation of alcohol was successfully synthesized twice, further attempts to repeat the synthesis were unsuccessful. The reasons for this are not currently known. Potentially this was because an impurity in the crude alcohol **16** with unknown decomposition pathways complicates the process.

2.5.4 Strategy 4

Previous results have shown that borylation of ketone **84** cause reductions to alcohol and many other side products. Moreover yields were not consistent due to product decomposition or other unknown issues. As a result these methods were not sufficiently robust for the preparation of compounds that we needed as starting points for a 5 step synthesis. With this as a stumbling block in our synthetic path we undertook an re-investigation of the requirement for palladium-catalysed cross-coupling reaction. Since an aryl bromide halogen is required, we proposed bromination of diazirine **70**. The following experiments exploring bromination with bromine and *N*-bromosuccinimide (NBS) were attempted to synthesise suitably modified diaziridines.

In a recent report by Moloney *et al*, the bromination of **70** to give a separable 3.2: 1 mixture of isomeric bromides in an overall yield of 55 % was described.[87]

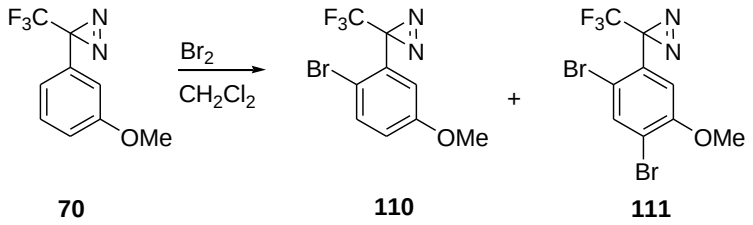


70 **109** **110**

Scheme 2.30

It was our intention to utilise this procedure to provide isomeric **109** with the bromides. Hopefully, bromine would only insert on *para* position to the diazirine group. Using similar conditions to that described above, diazirine **70** was dissolved in DCM, and bromine (1.2 eq) added. This mixture was stirred for three days at room temperature, to afford a separable 1:1.8 mixture of mono and di substitute bromides **110** and **111** in 22 % and 28 % isolated yield after purification by chromatography,

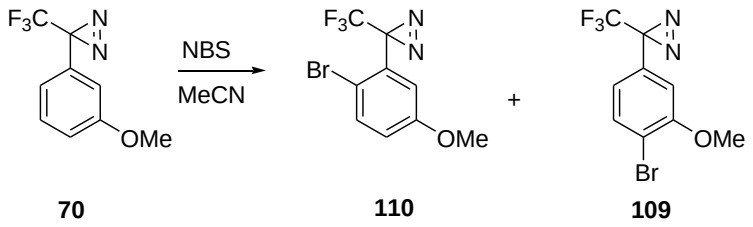
scheme 2.31. The brominated position of 3-methoxy diazirine **110** (GC-MS *m/z* 294/296) was defined using NOESY correlations derived from the methoxy group. Ultimately, ^{1}H NMR spectroscopy enabled the aromatic proton signals to be defined as δ7.49 (1H, d), δ7.13 (1H, d), δ6.86 (1H, dd) consistent with the proposed substitution pattern. The other di-substituted compound **111** showed peaks at δ7.55 (1H, d), δ6.86 (1H, d) in the ^{1}H NMR spectroscopy, and *m/z* 372/374/376 shown by GC-MS (Scheme 2.31).



70 **110** **111**

Scheme 2.31

Due to the undesired *ortho*-selective monobromination and di-substrate bromination, a less reactive brominating reagent (NBS) was then explored. Under similar reaction conditions, **70** was treated with NBS in refluxing MeCN at 95 ℃ for 3 days and gave a mixture of isomeric bromides **110** and **109** in 6.3 % and 11 % isolated yield, respectively. The regiochemical assignment for each of these was determined by ^{1}H NMR and ^{19}F NMR spectroscopy. Diazirine **110** was characterised by ^{1}H NMR spectroscopy at 7.49 ppm (1H, d) 7.13 ppm (1H, d) and 6.86 ppm (1H, dd) revealing aromatic protons. ^{19}F NMR spectroscopy revealed a characteristic peak at δ -68.25, and ES^{-}MS showed with *m/z* 294/296. Diazirine **109** was characterised by ^{1}H NMR spectroscopy at 7.62 ppm (1H, d) 7.17 ppm (1H, d) and 7.00 ppm (1H, dd) revealing aromatic protons. ^{19}F NMR spectroscopy revealed a characteristic peak at δ -73.18, and ES^{-}MS showed a molecule ion *m/z* 294/296 (Scheme 2.32).

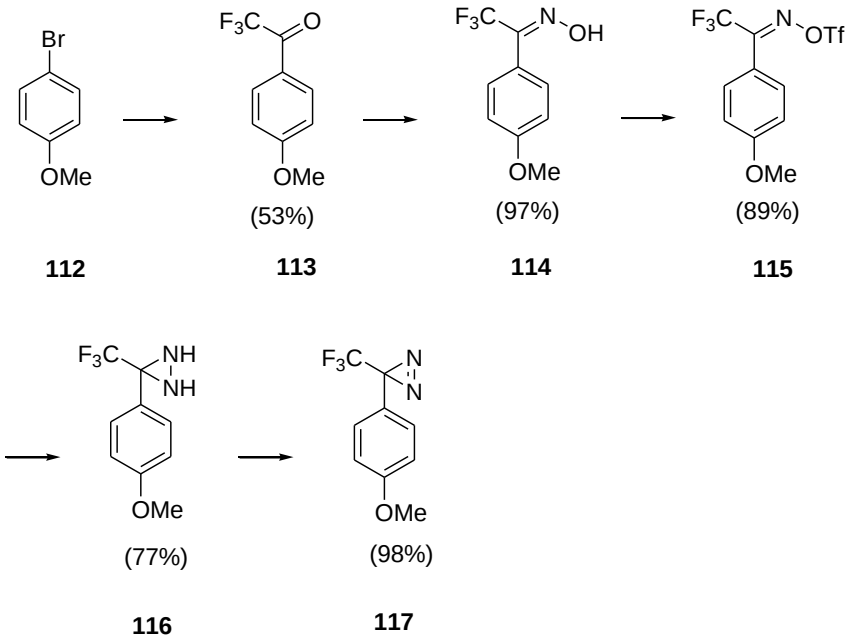


70 **110** **109**

Scheme 2.32

2.5.5 Strategy 5

The primary goal of the project was to borylate or brominate *m*-position of the trifluoromethyl diazirine. However, as covered in the previous sections, this led to many unsuccessful experiments. Therefore, we change the routes to *p*-trifluoromethyl diazirine. With one exception, following the same procedure as described above, we successfully synthesised diazirine **117** (Scheme 2.33).

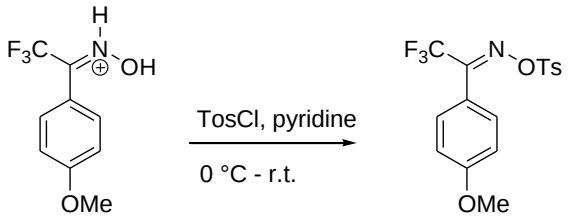


112 **113** **114** **115**

116 **117**

Scheme 2.33

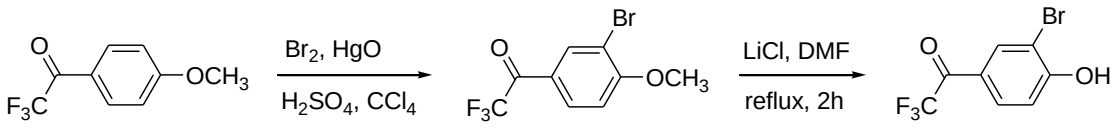
The differences came in the synthesis of *para*-methoxyphenyl tosyloxime **115** which contrasts with the method used in the preparation of tosyloxime **82**. A report by Hatanaka *et al.* describing the preparation of oxime **114** at 0 ℃ while adding p-toluenesulfonyl chloride.[88] Following the procedure, it was successfully isolated **115** in 89 % yield. Analysis by ^{1}H NMR spectroscopy confirmed the methyl groups from tosylate groups at 2.48 ppm (3H, s) and δ -65.98 by ^{19}F NMR spectroscopy. ES^{-}MS showed a molecular ion with *m/z* 373 (Scheme 2.34).



114 **115**

Scheme 2.34

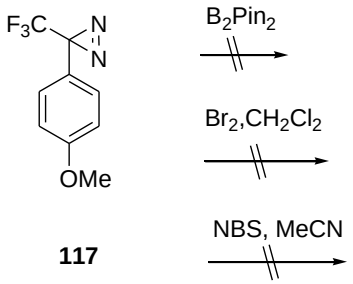
In the last section whilst bromination had been possible it was not selective. Consequently we turned to *p*-methoxy substituted trifluoromethyl diazirine as this is symmetrical thus removing selectivity problems. Bittman and Li have examined the facile bromination of 4-methoxytrifluoroacetophenone **113** using bromine and mercury oxide (Scheme 2.35). [89]



113 **118** (91%) **119** (93%)

Scheme 2.35

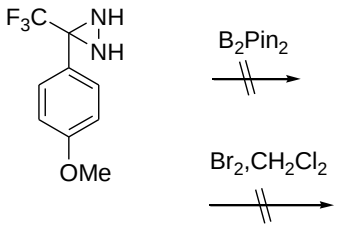
Hence, it was interesting attempt to brominate **117** using the above conditions without the mercury oxide. This diazirine could be made in an analogous way as was used for **70**, Scheme 2.31 and 2.32. Attempts were then made to borylate and brominate diazirine **117** using, in turn, B_2Pin_2, bromine and NBS. However, a complex mixture of products was formed which were very difficult to separate and identify.



117

Scheme 2.36

Similar outcomes were had for the attempted borylation reaction and we took one step backwards to use diaziridine **116** as the borylation and bromination precursor. Using the same condition as described previously, Scheme 2.37. However, this also failed to give the desired product, with only a mixture of decomposition products of starting material being seen.

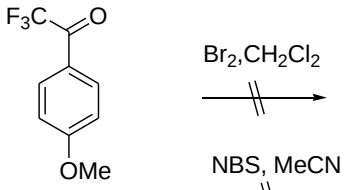


116

Scheme 2.37

Due to the failed use of diazirine **116**, a final attempt was made brominate the *para* trifluoromethyl ketone **113**. Unfortunately, no product was detected by GC-MS, and

^{19}F NMR spectrum shows several unidentifiable peaks. Ultimately, this approach was discontinued and abandoned.

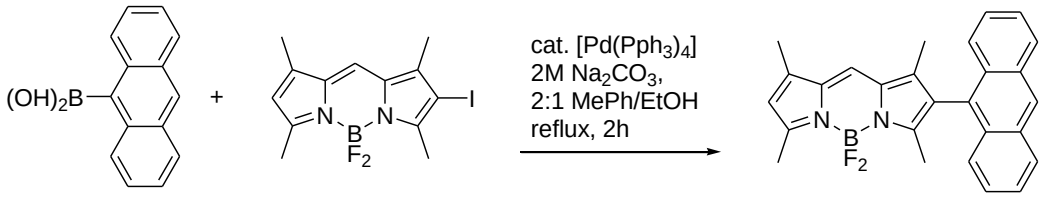


113

Scheme 2.38

2.6 Palladium-catalysed cross coupling reactions

The initial goals of the project were to synthesise a photoaffinity labelling for biological use. Due to many unsuccessful reactions of the borylation and bromination synthesise on diazirine photoaffinity probes, we decided to explore the cross-coupling with a commercial available compound, 4-benzoylphenyl boronic acid. A similar reaction had been described by Wan *et al*. who had reported the cross coupling of 6 position iodo-BODIPY **121** with anthracenyl boronic acid **122** (Scheme 2.39).[90]

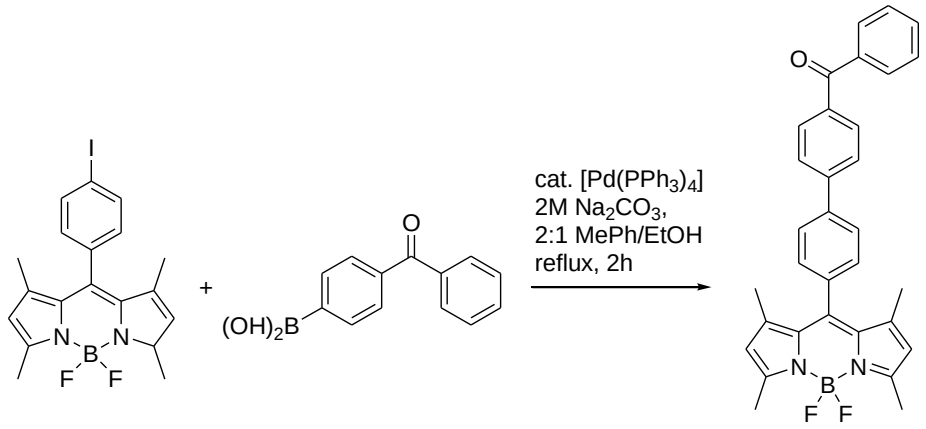


120 **121** **122**

Scheme 2.39

As a result it was our intention to utilise this approach to the palladium-catalysed cross coupling between organoboronic acid and halides moieties. Following this report model studies were undertaken to assess the application of this reaction.

BODIPY **22** and 4-benzoylphenylboronic acid **123** with catalyst [Pd (PPh_3)$_4$] were heated reflux in 2M Na_2CO_3 and toluene/ethanol in 2:1 ratio for five hours. Following a standard work up, the crude reaction was purified by aluminium oxide chromatography because the BODPY frameworks are not stable to silica gel. Although this gave a reasonable yield in 70 %, a mixture of impurity still remained requiring further purified with chromatography and single-solvent recrystallization from methanol. The product **3** was confirmed by the appearance of a peak from GC-MS *m/z* 504 and from IR data, a band at 1650 cm^{-1} consistent with the presence of a C=O group (Scheme 2.40).



22 **123** **3**

Scheme 2.40

Given the difficulties in the purification step, an alternative approach needs investigated in any future study. Whilst this represent a BODIPY treated to a PAL group the difficulties in synthesis and purification has meant that it has not yet been tested in biology.

3 CONCLUSION AND FUTURE WORK

3.1 Conclusion

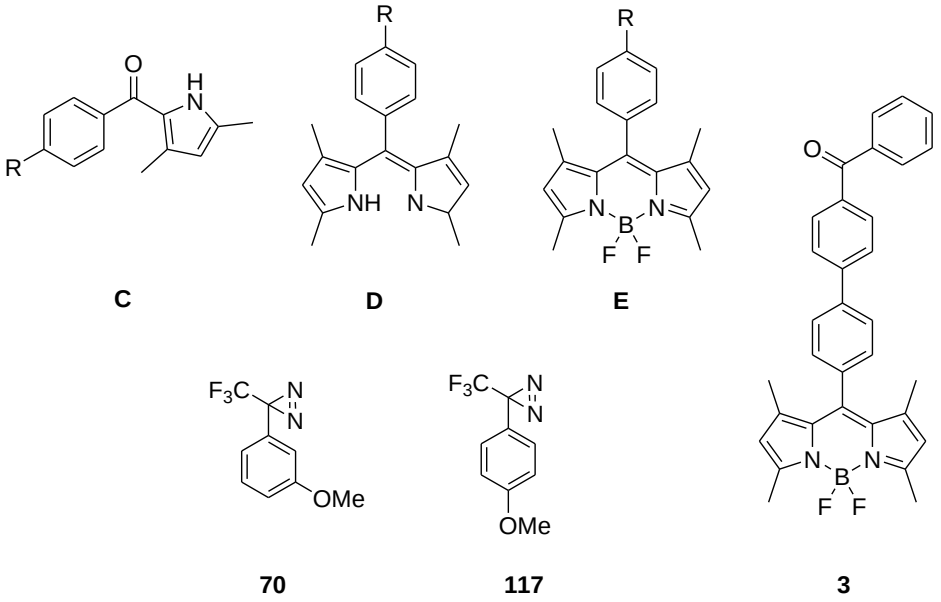
This aim of this project was to design a BODIPY fluorescent probe coupled to photoaffinity labelling group for use in exploring the localization of BODIPY dyes in the peroxisome of a plant cell. Towards this end, this thesis described several strategies that lead to the synthesis of BODIPY fluors and photoaffinity agents.

In respect to BODIPY synthesis, the dipyrromethene precursor **D** could be readily prepared through condensation of pyrrole with ketopyrrole **C**. The optimal method for the formation of the BODIPY proved to be treatement with $BF_3{\cdot}OEt_2$ and triethylamine in a neat reaction. Finally, purification use alumina oxide chromatography instead of silica-gel, enhances the isolated yield. Using this approach BODIPY **E** could be prepared in good yield.

Although *para*- and *meta*-methoxybenzene substituted trifluoromethyl diazirine **70** and **117** were successfully synthesised using a 5-step synthetic route from the literature, further attempts to elaborate the functionality in the benzene ring proved difficult. Whereas functionalisation of the benzene ring of these diazirine compounds as well as the intermediates at the various stages of the 5-step synthetic route using C-H borylation was unsuccessful functionalisation using Br_2 or NBS did work but suffered from poor selectivity.

As a result of difficulty in synthesising in boronates aromatic diazirine series, we decided to explore coupling of BODIPY **3** with a commercially available reagent, 4-benzoylphenylboronic acid. This coupling to a BODIPY dye to give conjugate

probe **3** was successfully achieved. Final purification was challenging and remains to be completed.



3.2 Future work

Following these studies, several possible alternative strategies that we have not used for the synthesis of photoaffinity probe remain to be explored. For example: protection of the ketone **84** before borylation reaction, or the use of *t*-BuLi for the metalation of **103** after protection with lithium isopropoxide. However, this research has led to the synthesis of a potential BODIPY photoaffinity probe **3**. The development of photoaffinity labelling is suitable for the analysis of specific organelle within cells. We plan to incubate the fluorescent label into plant cells and identify peroxisomes. There is a variety of future challenges that require further investigations in use of bio-imaging.

4 PROCEDURE

General Experimental

All sensitive reactions were performed in oven-dried glassware under an inert atmosphere of argon unless otherwise stated.

Solvents

All solvents were obtained dried as per standard procedures within the department and stored under argon before use. In cases where mixtures of solvents were used, the ratios refer to the component volumes.

Reagents

Reagents were used as supplied unless otherwise stated.

Melting points

Melting points were determined using Thermo scientific Electrothermal Digital Melting Point Apparatus (IA9100).

IR Spectroscopy

Infrared spectra were recorded using a Diamond ATR (attenuated total reflection) accessory (Golden Gate) on a Perkin-Elmer FT-IR 1000 spectrometer.

Chromatography

Reactions were monitored using thin layer chromatography (TLC) on aluminium or glass backed sheets of silica gel 60 F_{250} and aluminium backed sheets of aluminium oxide plates (0.2mm layer, N/UV250). Materials were visualized by UV radiation at 254nm, by development in potassium permanganate, in aqueous sodium carbonate, or

in phosphomolybdic acid in ethanol. Purification of products was performed by flash column chromatography using normal phase silica gel 40-63u 60Å or aluminium oxide.

NMR Spectroscopy

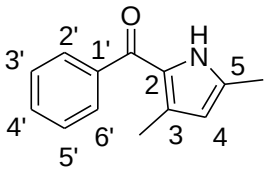
^{1}H and ^{13}C NMR spectra were acquired in $CDCl_3$, unless otherwise stated, on Varian Mercury-200, Bruker Avance-400, Varian Inova-500 or a Varian VNMRS 700, and reported as follows: chemical shift δ (ppm) (number of protons, multiplicity, coupling constant *J* (Hz), assignment). The residual protic solvent was used as the internal reference ($CHCl_3$ δ_H = 7.26 ppm; δc = 77.0 ppm). ^{19}F NMR spectra were recorded at 376 MHz on Varian VCR-400. All chemical shifts are quoted in parts per million relative to the internal reference and coupling constants given in Hertz (Hz). Assignment and determination of stereochemistry were performed using NOESY, COSY, HSQC and HMBC experiments.

Mass Spectrometry

Electrospray mass spectra (ES) were obtained on a Micromass LCT mass spectrometer. Gas Chromatography Mass spectra (GC-MS:EI, Cl) were taken using a Thermo-Finnigan Trace within a 25 cm column connected to a VG Mass Lab Trio 1000. High resolution accurate mass measurement was performed on a LTQFT mass spectrometer (Thermo Finnigan Corporation) using flow-injection electrospray ionization at the Universtiy of Durham.

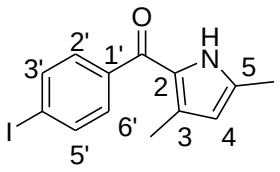
General Procedure

2-benzoyl-3,5-dimethyl pyrrol, 95



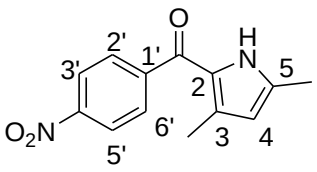
A solution of 2,4-dimethylpyrrole (0.36 mL, 3.55 mmol) and CH_3MgBr 3M in ether (0.41 mL, 1.19 mmol) was refluxed 50 ℃ for 30 min. Benzoyl chloride (0.41 mL, 3.55 mmol) in ether was added to the solution and stirring was continued for 24 h at rt. The mixture was then poured into saturated aqueous NH_4Cl, and the resultant mixture extracted with CH_2Cl_2. The organic layer was washed with water, dried over $MgSO_4$ and concentrated under vacuum to give an yellow oil. The compound was purified by chromatography, eluting with EtOAc/DCM (5-10 %), to furnish the title ketone as a yellowish solid (0.59 g, 84 %); **mp** 107-110 ℃; $\mathbf{R_f}$ 0.58 (10 % EtOAc/DCM); $\boldsymbol{\upsilon_{max}}$ 3266 (NH), 2363, 1682 (C=O), 1564, 1430, 1278, 1095, 1004, 930, 800, 740, 700, 650, 600, 525 cm^{-1}; $\boldsymbol{\delta_H}$ (700 MHz, $CDCl_3$) 9.24 (1H, br s, N<u>H</u>), 7.61 (2H, d, *J* = 1.4, 2'-<u>H</u> and 6'-<u>H</u>), 7.50 (1H, t, *J* = 7.5, 4'-<u>H</u>), 7.44 (2H, t, *J* = 7.5, 3'-<u>H</u> and 5'-<u>H</u>), 5.88 (1H, s, 4-<u>H</u>), 2.31 (3H, s, 3-C<u>H</u>$_3$), 1.93 (3H, s, 5- C<u>H</u>$_3$); $\boldsymbol{\delta_C}$ (176 MHz, $CDCl_3$) 185.4 (C=O), 139.8 (C-3), 130.9 (C-5), 130.1 (C-Ar), 128.4 (C-2), 128.2 (C-Ar), 128.1 (C-Ar), 127.6 (C-Ar), 113.2 (C-4), 14.0 (3-<u>C</u>H$_3$), 13.2 (5-<u>C</u>H$_3$); ***m/z*** (ES^+) 199.2 $[M+H]^+$.

2-(4'-iodobenzoyl)-3,5-dimethylpyrrole, 45



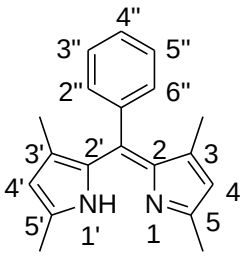
Following the same procedure as described for **95**, 2,4-dimethylpyrrole (1.9 mL, 18.76 mmol), CH_3MgBr 3M (2.2 mL, 6.25 mmol) in ether (5 mL), and 4-iodobenzoyl chloride (5 g, 18.76 mmol) in ether (35 mL), where used to provide, following purification by trituation in 20 % Hexane/ DCM the title ketone **45** (4.96 g, 81 %) as a white yellowish solid. Then the remaining mix compound was isolated again by chromatography on a silica gel flash column (30 % Hexane/EtOAc) to furnish a white solid (0.47 g, 8 %); **mp** 158-159 ℃; $\mathbf{R_f}$ 0.6 (30 % Hexane/EtOAc); $\boldsymbol{\upsilon_{max}}$ 3550, 3448, 3260 (NH), 2912, 2380, 2338, 1734 (C=O), 1687, 1654, 1630, 1494, 1438, 1374, 1290, 1056, 1010, 928, 836, 812, 759, 646, 500 cm^{-1}; $\boldsymbol{\delta_H}$ (400 MHz, $CDCl_3$) 9.03 (1H, br s, N<u>H</u>), 7.80 (2H, d, *J* = 8.4, 2'-<u>H</u> and 6'-<u>H</u>), 7.36 (2H, d, *J* = 8.4, 3'-<u>H</u> and 5'-<u>H</u>), 5.87 (1H, s, 4-<u>H</u>), 2.29 (3H, s, 5-C<u>H</u>$_3$), 1.94 (3H, s, 3-C<u>H</u>$_3$); $\boldsymbol{\delta_C}$ (176 MHz, $CDCl_3$) 184.5 (C=O), 139.4 (C-5), 137.4 (C-3), 135.9 (C-Ar), 130.7 (C-2), 129.8 (C-Ar), 127.5 (C-Ar), 113.2 (C-Ar), 97.7 (C-4), 14.1 (5-<u>C</u>H$_3$), 13.2 (3-<u>C</u>H$_3$); ***m/z*** (ES^+) 325 $[M+H]^+$.

2-(4'-nitrobenzoyl)-3,5-dimethylpyrrole, 96



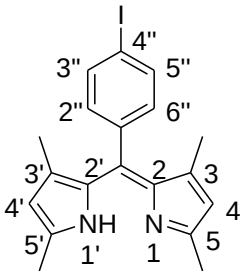
Following the same procedure as describe for **95**, 4-nitrobenzoyl chloride (1 g, 5.39 mmol) in ether, 2,4-dimethypyrrole (0.5 mL, 5.39 mmol) and CH_3MgBr 3M (0.6 g, 1.80 mmol) in ether where used to provide, following purification, by recrystallizing three times with Hexane/DCM to give an yellow solid (0.88 g, 67 %); **mp** 157-160 ℃; $\mathbf{R_f}$ 0.25 (20 % Hexane/EtOAc); $\boldsymbol{\upsilon_{max}}$ 3271 (N-H), 3114, 2922, 2848, 1708 (C=O), 1576, 1530, 1500, 1438, 1344, 1283, 1108, 1016, 931, 851, 803, 741, 506, 460 cm^{-1}; $\boldsymbol{\delta_H}$ (400 MHz, $CDCl_3$) 9.17 (1H, s, N<u>H</u>), 8.33 – 8.30 (2H, m, AA' part of AA'XX' system, 2'-<u>H</u> and 6'-<u>H</u>), 7.78 – 7.73 (2H, m, XX' part of AA'XX' system, 3'-<u>H</u> and 5'-<u>H</u>), 5.91 (1H, s, 4-<u>H</u>), 2.33 (3H, s, 5-C<u>H</u>$_3$), 1.85 (3H, s, 3-C<u>H</u>$_3$); $\boldsymbol{\delta_C}$ (101 MHz, $CDCl_3$) 188.5, 145.9, 137.3, 131.2, 128.9, 123.7, 113.9, 110.2, 108.2, 76.0, 14.1, 13.2; ***m/z*** (ES^+) 244 $[M+H]^+$.

2-[(*Z*)-(3',5'-dimethyl-2*H*-pyrrol-2'-ylidene)phenylmethyl]-3,5-dimethyl-1*H*-pyrrole, 88



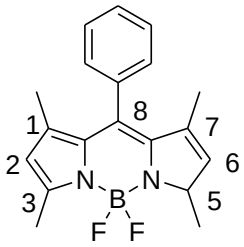
To a solution of pyrrole **95** (0.3 g, 1.51 mmol) in hexane (5 mL) and DCM (2.5 mL) at 0 ℃ was added 2,4 dimethylpyrrole (0.15 mL, 1.51 mmol) and then phosphorus oxychloride (0.14 mL, 1.51 mmol). Stirring was continued for 24 h at rt. The mixture was then treated successively with NEt_3 (0.2 mL, 1.51 mmol), and water (30 mL) and then extracted with CH_2Cl_2. The combined organic extracts were then dried over $MgSO_4$ and concentrated. The crude product was purified by flash chromatography eluting first with 50 % EtOAc/Acetone to remove by-products and then with 20 % DCM/MeOH to give the title pyrrole (**88**) as an orange-brown solid (0.35 g, 83.5 %); **mp** 86-90 ℃; $\boldsymbol{\upsilon_{max}}$ 1521 (NH), 1418, 1355, 1137, 962, 899, 824, 715, 622 cm^{-1}; $\boldsymbol{\delta_H}$ (400 MHz, $CDCl_3$) 11.96 (1H, s, N-H), 7.59 (1H, t, *J* = 7.5, Ar-H), 7.49 (2H, t, *J* = 7.5, 7.0, Ar-H), 7.30 (2H, d, *J* = 7.0, Ar-H), 6.19 (2H, s, 4'-H and 4-H), 2.69 (6H, s, 5'-CH_3 and 5-CH_3), 1.40 (6H, s, 3'-CH_3 and 3-CH_3); $\boldsymbol{\delta_C}$ (126 MHz, $CDCl_3$) 154.4 (C-5' and C-5), 145.4 (C-3' and C-3), 136.8 (C-1''), 131.3 (C-2' and C-2), 129.7 (C-Ar), 129.6 (C-Ar), 129.4 (C-Ar), 129.3 (C-Ar), 129.9 (C-Ar), 121.2 (C-4' and C-4), 51.1 (5'-CH_3 and 5-CH_3), 15.0 (3'-CH_3 and 3-CH_3); ***m/z*** (ES^+) 276 $[M+H]^+$.

2-[(*Z*)-(3',5'-dimethyl-2*H*-pyrrol-2'-ylidene)(4''-iodophenyl)methyl]-3,5-dimethyl-1*H*-pyrrole, 46



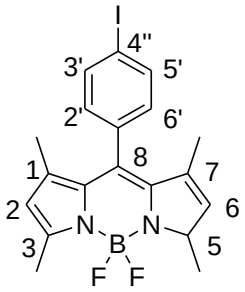
Following the same procedure as describe for **88**, pyrrole **45** (0.36 g, 1.11 mmol) in hexane (20 mL) and DCM (2.6 mL), 2,4 dimethylpyrrole (0.10 mL, 1.11 mmol), phosphorus oxychloride (0.17 mL, 1.11 mmol) and NEt_3 (0.15 mL, 1.11 mmol) where used to provide the title compound **46** following purification by flash chromatography eluting first with 50 % EtOAc/Acetone to remove by-products and then with 20 % DCM/MeOH to give an orange-brown solid (0.22 g, 50 %); **mp** 109-111 ℃; $\boldsymbol{\upsilon_{max}}$ 1540 (N-H), 1410, 1328, 1138, 962, 893, 820, 673, 618 cm^{-1}; $\boldsymbol{\delta_H}$ (400 MHz, $CDCl_3$) 11.19 (1H, s, N-H), 7.78 (2H, d, *J* = 8.4, 4'-H and 4-H), 7.06 (2H, d, *J* = 8.4, 2''-H and 6''-H), 5.90 (2H, s, 3''-H and 5''-H), 2.34 (6H, s, 5'-CH_3 and 5-CH_3), 1.34 (6H, s, 3'-CH_3 and 3-CH_3); $\boldsymbol{\delta_C}$ (126 MHz, $CDCl_3$) 149.8, 144.2, 143.7, 138.6, 114.3, 113.0, 105.3, 100.0, 57.2, 32.8, 10.0; ***m/z*** (ES^-) 402 $[M-H]^-$.

4,4-Difluoro-1,3,5,7-tetramethyl-8-phenyl-4-bora-3a,4a-diaza-s-indacene, 14



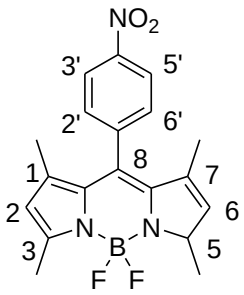
A solution of dipyrromethane **88** (100 mg, 0.36 mmol) in DCM (10 mL) was treated with triethylamine (0.15 mL, 1.09 mmol) and boron trifluoride diethyl etherate ($BF_3.OEt_2$) (0.13 mL, 1.09 mmol). The mixture was stirred at 60 ℃ under argon for 1h. The crude product was filtered through a plug of celite and then concentrated. Purification by silica gel chromatography (20 % Hexane/EtOAc) then gave the title BODIPY as an orange solid (85.9 mg, 73.4 %); **mp** 129-133 ℃; $\boldsymbol{\upsilon_{max}}$ 1542, 1502 (B-F), 1304, 1192, 1185, 1063, 980, 812, 721, 541 cm^{-1}; $\boldsymbol{\delta_H}$ (400 MHz, $CDCl_3$) 7.56 – 7.43 (3H, m, Ar-H), 7.28 (2H, m, Ar-H), 5.98 (2H, s, 2-H and 6-H), 2.56 (6H, s, 3-CH_3 and 5-CH_3), 1.37 (6H, s, 1-CH_3 and 7-CH_3); $\boldsymbol{\delta_C}$ (101 MHz, $CDCl_3$) 155.0, 146.4, 142.3, 138.0, 129.8, 129.5, 129.1, 129.1, 128.9, 127.9, 127.8, 14.3, 14.3; ***m/z*** (ES^+) 324 $[M+H]^+$.

4,4-difluoro-1,3,5,7-tetramethyl-8-(4'-iodophenyl)-4-bora-3a,4a-diaza-*s*-indacene, 22



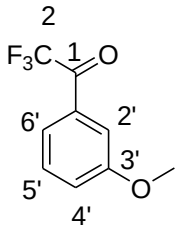
To a solution of pyrrole **45** (0.5 g, 1.54 mmol) in DCM (1 mL) was added 2,4-dimethylpyrrole (0.14 g, 1.54 mmol) and phosphorus oxychloride (0.24 g, 1.54 mmol). The mixture compound was heated up to 60 ℃ for 30 min. After the compound turned dark red-purple colour. The reaction mixture was then concentrated and toluene (4 mL) added. The mixture was then treated with triethylamine (0.52 mL, 3.73 mmol) and boron trifluoride diethyl etherate (0.46 mL, 3.73 mmol). The remaining mixture was heated 100 ℃ for 30min. The crude product was then filtered through a plug of celite, and purified by chromatography on an deactivated basic alumina with 10-20 % Hexane/DCM to give the title BODIPY (**22**) as an orange solid (0.34 g, 60 %); **mp** 225-230 ℃; $\boldsymbol{\upsilon_{max}}$ 2968, 2928, 2854, 2374, 1650, 1536, 1512, 1466, 1310, 1194, 1152, 1056, 1975, 830, 755, 692, 475 cm^{-1}; $\boldsymbol{\delta_H}$ (400 MHz, $CDCl_3$) 7.87 – 7.83 (2H, m, Ar-H), 7.07 – 7.03 (2H, m, Ar-H), 5.99 (2H, s, 2-H and 6-H), 2.55 (6H, s, 3-CH_3 and 5-CH_3), 1.42 (6H, s, 1-CH_3 and 7-CH_3); $\boldsymbol{\delta_C}$ (126 MHz, $CDCl_3$) 156.1, 143.2, 140.3, 138.6, 134.8, 131.4, 130.2, 121.7, 95.0, 14.9, 14.9; ***m/z*** (ES^+) 450.0 $[M+H]^+$.

4,4-difluoro-1,3,5,7-tetramethyl-8-(4'-nitrophenyl)-4-bora-3a,4a-diaza-*s*-indacene, 91



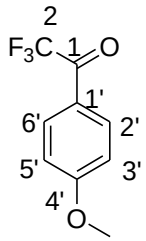
Following the same procedure as described for **22**. Pyrrole **96** (0.79 g, 3.23 mmol) in DCM (2 mL), 2,4-dimethypyrrole (0.23 g, 3.23 mmol), phosphorus oxychloride (0.37 g, 3.23 mmol), toluene (2 mL), triethylamine (0.85 mL, 6.07 mmol) and boron trifluoride diethyl etherate (0.75 mL, 6.07 mmol) was converted to the title BODIPY **91**. Following chromatography on silica gel using 5-30 % Hexane/ DCM the title BODIPY **91** was obtained as an orange solid (0.5 g, 56 %); **mp** 274-275 ℃; $\boldsymbol{\upsilon_{max}}$ 3100, 2970, 2910, 2860, 2395, 1684, 1528, 1500, 1462, 1407, 1345, 1314, 1198, 1159, 1102, 998, 992, 990, 986, 729, 478 cm^{-1}; $\boldsymbol{\delta_H}$ (400 MHz, $CDCl_3$) 8.42 – 8.34 (2H, m, Ar-H), 7.58 – 7.50 (2H, m, Ar-H), 6.02 (2H, s, 2-H and 6-H), 2.57 (6H, s, 3-CH_3 and 5-CH_3), 1.36 (6H, s, 1-CH_3 and 7-CH_3); $\boldsymbol{\delta_C}$ (176 MHz, $CDCl_3$) 156.7 (C-5 and C-3), 148.3 (C-4') 142.5 (C-1 and C-7), 141.9 (C-8), 138.3 (C-1'), 130.6 (C-1a and C-7a), 129.6 (C-2' and C-6'), 124.3 (C-3' and C-5'), 121.8 (C-2 and C-6), 14.7 (3-CH_3 and 5-CH_3), 14.63 (1-CH_3 and 7-CH_3); ***m/z*** (ES^+) 370 $[M+H]^+$.

2,2,2-Trifluoro-1-(3'-methoxyphenyl)ethanone, 84



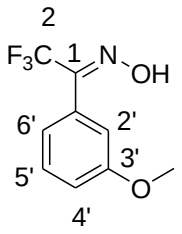
n BuLi (25 mL, 40.1 mmol) was added to a solution of 3 bromoanisole (5 g, 26.73 mmol) in dry THF (10 mL) and the mixture was stirred at -78 ℃ for 35 min. Ethyl trifluoroacetate (4.94 g, 34.75 mmol) in THF (10 mL) was then added dropwise over a period of 15 min to the solution. After the mixture had been stirred for an additional 2 h at -78 ℃, a solution of conc. HCl (60 mL) in methanol (14 mL) was added. The resulting solution was extracted with ether, and the extracts were dried over $MgSO_4$ and concentrated. The residual oil was purified by Kugelrohr distillation (84 ℃, 3 Torr) to give the title product (**84**) as light yellow oil (4.63 g, 85 %); υ_{max} 2839, 1710 (C=O), 1598, 1582, 1490, 1465, 1432, 1342, 1249, 1198, 1137, 1051, 978, 825, 752, 734, 663, 613, 554 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.66 (1H, ddd, *J* = 8.0, 2.4, 1.4, 6'-H), 7.57 (1H, t, *J* = 1.4, 2'-H), 7.46 (1H, t, *J* = 8.0, 5'-H), 7.27-7.24 (1H, ddd, *J* = 8.0, 2.4, 1.4, 4'-H), 3.88 (3H, s, O-CH_3); δ_C (126 MHz, $CDCl_3$) 180.6 (q, $^2J_{CF}$ = 35.1 Hz, CCF_3), 160.2 (C-3'), 131.3 (C-1'), 130.4 (C-5'), 123.0 (q, *J* = 2.6 Hz, C-6'), 122.6 (C-4'), 114.2 (d, *J* = 1.7 Hz, C-2'), 116.8 (q, $^1J_{CF}$ = 291.2 Hz, CF_3), 55.8 (OCH_3); δ_F (376 MHz, $CDCl_3$) -71.18 ppm; ***m/z*** (ES^+) 204 $[M+H]^+$.

2,2,2-trifluoro-1-(4'-methoxyphenyl) ethanone, 113



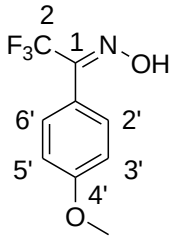
Following the same procedure as described for **84**. *n* BuLi (11 mL, 17.65 mmol), 4-bromoanisole (2.2 g, 11.76 mmol) in THF (5 mL), ethyl trifluoroacetate (2.17 g, 15.29 mmol) in THF (5 mL), conc. HCl (2 mL) in methanol (3 mL) where used to provide into the title compound **113** which was purified by chromatography (0-15 % Hexane/EtOAc) than give the title (**113**) product (1.3g, 53 %) as an yellow oil; $\mathbf{R_f}$ 0.47 (10 % Hexane/EtOAc); $\boldsymbol{\upsilon_{max}}$ 2938, 1702 (C=O), 1598, 1514, 1462, 1428, 1317, 1270, 1161, 1137, 1025, 938, 844, 768, 737, 648, 614 cm^{-1}; $\boldsymbol{\delta_H}$ (400 MHz, $CDCl_3$) 8.06 (2H, d, *J* = 8.5, 2'-H and 6'-H), 7.01 (2H, d, *J* = 8.5, 3'-H and 5'-H), 3.92 (3H, s, OCH_3); $\boldsymbol{\delta_C}$ (101 MHz, $CDCl_3$) 178.9, 165.4, 132.8, 122.8, 118.4, 115.3, 114.4, 60.2, 55.7; $\boldsymbol{\delta_F}$ (376 MHz, $CDCl_3$) -71.18 ppm; ***m/z*** (ES^+) 204 $[M+H]^+$.

2,2,2-Trifluoro-1-(3'-methoxyphenyl)ethanone oxime, 83



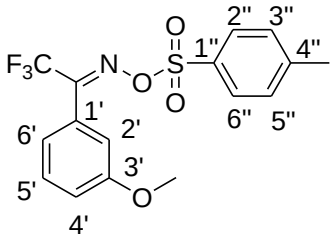
A solution of **84** (2.52 g, 12.34 mmol) and hydroxylamine hydrochloride (1.12 g, 16.04 mmol) in pyridine (10 mL) and dry ethanol (5 mL) was refluxed at 85 ℃ for 17 h. The mixture was concentrated and the residue partitioned between water and Et_2O. The organic layer was washed with 5N HCl (20 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to afford the title oxime as a pale yellow oil which was not purified further (2.65 g, 98 %); $\mathbf{R_f}$ 0.75 (90 % DCM/EtOAc); $\boldsymbol{\upsilon_{max}}$ 3328, 3004, 2942, 2846, 1576, 1492, 1244, 1132, 1008, 958, 846, 733, 704, 625, 544 cm^{-1}; $\boldsymbol{\delta_H}$ (400 MHz, $CDCl_3$) 9.08 (0.4H, s, NO-H), 8.86 (0.6H, s, NO-H), 7.40 (0.6H, t, *J* = 8.3, 5'-H), 7.33 (0.4H, t, *J* = 8.3, 5'-H), 7.09-6.99 (3H, m, 2'-H, 4'-H, 6'-H), 3.83 (3H, s, CH_3); $\boldsymbol{\delta_C}$ (101 MHz, $CDCl_3$) 159.47, 159.46, 159.45, 129.7, 129.5, 129.4, 127.2, 120.77, 120.76, 120.74, 119.2, 116.2, 116.1, 114.3, 113.9, 55.38, 55.35; $\boldsymbol{\delta_F}$ (376 MHz, $CDCl_3$) -66.71 ppm; ***m/z*** (ES^-) 219 $[M-H]^-$.

2,2,2-Trifluoro-1-(4'-methoxyphenyl)ethanone oxime, 114



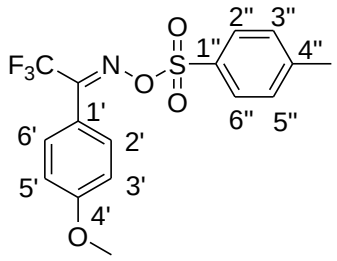
Following the same procedure as described for **83**. A solution of **113** (1.12 g, 5.49 mmol) and hydroxylamine hydrochloride (0.5 g, 7.14 mmol) in pyridine (5 mL) and ethanol (2 mL) was refluxed at 85 ℃ for 5 h. The mixture was concentrated and the residue partitioned between water and Et_2O. The organic layer was washed with 5N HCl (10 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to afford oxime as a pale yellow oil which was not purified further (1.17 g, 97 %); $\mathbf{R_f}$ 0.74 (DCM); $\boldsymbol{\upsilon_{max}}$ 3260 (OH), 2840, 1608, 1515, 1463, 1443, 1340, 1293, 1254, 1208, 1174, 1128, 1021, 1002, 954, 832, 746, 701, 670, 615 cm^{-1}; $\boldsymbol{\delta_H}$ (400 MHz, $CDCl_3$) 8.62 (1H, broad m, O<u>H</u>), 7.55 (2H, d, *J* = 8.8 , 2'-<u>H</u>, 6'-<u>H</u>), 6.98 (2H, d, *J* = 8.8, 3'-<u>H</u>, 5'-<u>H</u>), 3.84 (3H, s, C<u>H</u>$_3$); $\boldsymbol{\delta_C}$ (101 MHz, $CDCl_3$) 160.84, 148.52, 146.58, 146.26, 137.48, 130.45, 129.88, 127.65, 124.28, 118.75, 113.88, 65.95, 55.29; $\boldsymbol{\delta_F}$ (376 MHz, $CDCl_3$) -66.02 ppm; ***m/z*** (ES^-) 220 $[M-H]^-$.

2,2,2-Trifluoro-1-(3'-methoxyphenyl)-*N*-{[(4''-methylphenyl)sulfonyl]-oxy}ethanimine, 82



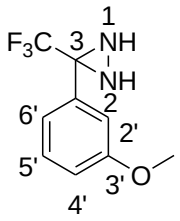
Toluenesulfonyl chloride (3.5 g, 18.34 mmol) was added to a solution of 2,2,2-trifluoro-1-ethanone oxime **83** (0.68 g, 12.23 mmol) in pyridine (23 mL) and the resulting solution was heated 125 ℃ for 2 h. After cooling, the solvent was evaporated and the residue partitioned between Et_2O, water and 5N HCl (50 mL). The organic layer was dried over $MgSO_4$, and concentrated under vacuum is afford title compound **82** (4.39 g, 96 %) as a white solid that was sufficiently pure to use directly in the next step of the synthesis; **mp** 101-102 ℃; $\mathbf{R_f}$ 0.45 (toluene); $\boldsymbol{\upsilon_{max}}$ 1738, 1350, 1234, 1043, 604 cm^{-1}; $\boldsymbol{\delta_H}$ (400 MHz, $CDCl_3$) 7.88 (2H, d, *J* = 8.2, 2''-H and 6''-H), 7.38 (3H, m, *J* = 8.2, 5'-H, 3''-H, 5''-H), 7.05 (1H, ddd, *J* = 8.0, 2.5,0.8, 6'-H), 6.94 (1H, ddd, *J* = 8.0, 2.5, 0.8, 4'-H), 6.88 (1H, t, *J* = 0.8, 2'-H), 3.82 (3H, s, OCH_3), 2.48 (3H, s, 4''-CH_3); $\boldsymbol{\delta_C}$ (101 MHz, $CDCl_3$) 159.6 (C-3'), 146.2 (C-4'), 131.2 (C-Ar), 130.0 (C-Ar), 129.9 (C-2', C-6'), 129.3 (C-3', C-5'), 125.7 (C-5'), 120.5 (C-6'), 117.2 (C-4'), 113.9 (C-2'), 55.4 (OCH_3), 21.8 (CH_3); $\boldsymbol{\delta_F}$ (376 MHz, $CDCl_3$) -66.9 ppm; ***m/z*** (ES^-) 373 $[M-H]^-$.

2,2,2-Trifluoro-1-(4'-methoxyphenyl)-*N*-{[(4''-methylphenyl)sulfonyl]-oxy}ethanimine, 115



p-Toluenesulfonyl chloride (1.05 g, 5.52 mmol) was added to a solution of oxime **114** (1.1 g, 5.02 mmol), triethylamine (0.8 mL, 5.52 mmol) and DMAP (0.04 g, 0.3 mmol) in DCM (4 mL) at 0 ℃. The mixture reaction was then stirred at rt for 2 h. After this time the layer separated, mixture was washed with water, 5N HCl (3 mL) and DCM. Dried with $MgSO_4$, filtered, and concentrated to give the title compound **115** (1.6 g, 89 %) as a yellow solid. No purification was required. **mp** 113-114 ℃; $\mathbf{R_f}$ 0.43 (15 % Hexane/EtOAc); $\boldsymbol{\upsilon_{max}}$ 1605, 1511, 1450, 1390, 1343, 1300, 1257, 1192, 1176, 1138, 1089, 1033, 1000, 892, 812, 777, 738, 714, 671, 746, 612 cm^{-1}; $\boldsymbol{\delta_H}$ (400 MHz, $CDCl_3$) 7.89 (2H, d, *J* = 8.5, 2''-H and 6''-H), 7.45 (2H, d, *J* = 8.9, 2'-H, 6'-H), 7.38 (2H, d, *J* = 8.5, 3''-H, 5''-H), 6.96 (2H, d, *J* = 8.9, 3'-H, 5'-H), 3.86 (3H, s, OCH_3), 2.48 (3H, s, 4''-CH_3); $\boldsymbol{\delta_C}$ (101 MHz, $CDCl_3$) 162.1, 146.0, 131.3, 130.7, 129.8, 129.3, 116.5, 114.2, 55.4, 21.8; $\boldsymbol{\delta_F}$ (376 MHz, $CDCl_3$) -65.98 ppm; ***m/z*** (ES^-) 373 $[M-H]^-$.

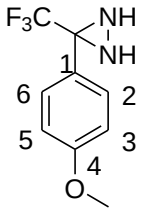
3-(3'-Methoxyphenyl)-3-(trifluoromethyl)diaziridine, 81



A solution of

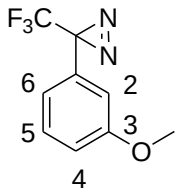
2,2,2-Trifluoro-1-(3-methoxyphenyl)-N-{[(4-methylphenyl)sulfonyl]-oxy}ethanimine (**82**) (4.0 g, 10.72 mmol) dissolved in dry CH_2Cl_2 (60 mL) was added to liquid ammonia (40 mL) at -78 ℃ over a period of 20 min. The solution was stirred at -78 ℃ for 12 h and then warmed up to room temperature overnight during which period the ammonia was allowed to evaporate. The solution was filtered and filtrate between water and CH_2Cl_2. The organic layer was dried with $MgSO_4$, filtered, and concentrated in vacuo. The resulting crude product was purified by flash chromatography (0-5 % CH_2Cl_2/EtOAc) to give the diaziridine **81** (2.23 g, 96 %) as a slightly yellow liquid; $\mathbf{R_f}$ 0.32 (DCM); $\boldsymbol{\upsilon_{max}}$ 3250 (N-H), 1582 (N-H), 1500, 1460, 1390, 1328, 1286, 1246, 1217, 1136, 1044, 958, 922, 784, 715, 692, 645, 443 cm^{-1}; $\boldsymbol{\delta_H}$ (400 MHz, $CDCl_3$) 7.34 (1H, t, *J* = 8.2, 5'-H), 7.20 (1H, ddd, *J* = 8.2, 2.6, 0.97, 6'-H), 7.15 (1H, t, *J* = 0.97, 2'-H), 6.98 (1H, ddd, *J* = 8.2, 2.6, 0.97, 4'-H), 3.83 (3H, s, CH_3), 2.75 (1H, s, NH), 2.21 (1H, s, NH); $\boldsymbol{\delta_C}$ (101 MHz, $CDCl_3$) 159.7, 133.0, 129.9, 124.9, 120.3, 115.8, 113.6, 57.8, 55.4; $\boldsymbol{\delta_F}$ (376 MHz, $CDCl_3$) -75.45 ppm; ***m/z*** (ES^-) 218 $[M-H]^-$.

3-(4-Methoxyphenyl)-3-(trifluoromethyl)diaziridine, 116



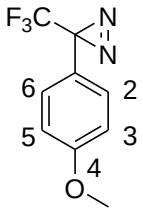
Following the same procedure as describe for **81**, a solution of 2,2,2-Trifluoro-1-(4-methoxyphenyl)-*N*-{[(4-methylphenyl)sulfonyl]-oxy}ethanimine (**115**) (0.3 g, 0.80 mmol) in dry CH_2Cl_2 (10 mL), liquid ammonia (4 mL) where used to provide the title compound. Following the standard work up procedure, the diaziridine **116** (0.16 g, 93 %) as a white solid; **mp** 67 ℃; $\mathbf{R_f}$ 0.27 (15 % Hexane/EtOAc); $\boldsymbol{\upsilon_{max}}$ 3214, 3194, 1612, 1518, 1391, 1249, 1027, 952, 886, 836, 821, 801, 733, 700, 592, 569, 528, 427 cm^{-1}; $\boldsymbol{\delta_H}$ (400 MHz, $CDCl_3$) 7.54 (2H, d, *J* = 8.7, 2-H, 6-H), 6.93 (2H, d, *J* = 8.7, 3-H, 5-H), 3.83 (3H, s, OCH_3) 2.48 (1H, s, NH), 1.25 (1H, s, NH); $\boldsymbol{\delta_C}$ (101 MHz, $CDCl_3$) 160.8, 130.2, 129.5, 123.7, 118.7, 114.9, 114.1, 98.8, 55.4; $\boldsymbol{\delta_F}$ (376 MHz, $CDCl_3$) -75.77; ***m/z*** (ES^+) 218 $[M+H]^+$.

3-(3-Methoxyphenyl)-3-(trifluoromethyl)-3*H*-diazirine, 70



Ag_2O (1.86 g, 8.02 mmol) was added to a solution of diaziridine **81** (0.5 g, 2.29 mmol) in dry Et_2O (20 mL) and stirred at rt for 3.5 h. The dispersion was filtered and washed with Et_2O, and dried over $MgSO_4$. Evaporation of the solvent gave the pure diazirine **70** (0.47 g, 95 %) of a colourless liquid; $\mathbf{R_f}$ 0.84 (95 % DCM/EtOAc); $\mathbf{\upsilon_{max}}$ 2938, 1605, 1583, 1493, 1466, 1433, 1343, 1260, 1150, 1042, 996, 979, 964, 872, 821, 778, 727, 690, 648 cm^{-1}; $\mathbf{\delta_H}$ (400 MHz, $CDCl_3$) 7.31 (1H, t, *J* = 8.1, 5-H), 6.95 (1H, ddd, *J* = 8.1, 1.9, 0.7, 6-H), 6.78 (1H, ddd, *J* = 8.1, 1.9. 0.7, 4-H), 6.69 (1H, t, *J* = 0.7, 2-H), 3.80 (3H, d, *J* = 7.3, CH_3); $\mathbf{\delta_C}$ (101 MHz, $CDCl_3$) 159.8 (C-3), 130.5 (C-1), 130.0 (C-5), 122.3 (q, $^1J_{CF}$ = 248.3 Hz, CF_3), 118.7 (C-6), 115.2 (C-4), 112.2 (d, *J* = 1.4 Hz, C-2), 55.3 (CH_3), 28.27 (m, $^2J_{CF}$ = 43.7 Hz, CCF_3); $\mathbf{\delta_F}$ (376 MHz, $CDCl_3$) -65.26 ppm; ***m/z*** (ES^-) 216 $[M-H]^-$.

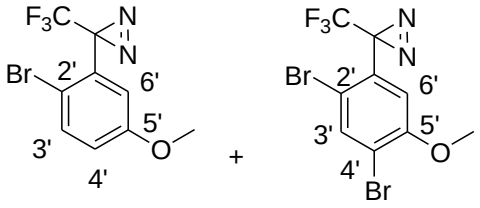
3-(4-Methoxyphenyl)-3-(trifluoromethyl)-3*H*-diazirine, 117



Following the same procedure as describe for **117**, Ag_2O (1.4 g, 5.94 mmol), a solution of diaziridine **116** (0.32 g, 1.70 mmol) in dry Et_2O (10 mL) where used to provide the title compound **117** as a yellow oil (0.31 g, 98 %); $\mathbf{R_f}$ 0.6 (15 % Hexane/EtOAc); $\boldsymbol{\upsilon_{max}}$ 2946, 2848, 1613, 1518, 1463, 1343, 1257, 1232, 1177, 1145, 1054, 1032, 936, 823, 730, 593, 540, 402 cm^{-1}; $\boldsymbol{\delta_H}$ (400 MHz, $CDCl_3$) 7.15 (2H, d, *J* = 8.7, 2-H, 6-H), 6.90 (2H, d, *J* = 8.7, 3-H, 5-H), 3.81 (3H, s, OCH_3); $\boldsymbol{\delta_C}$ (101 MHz, $CDCl_3$) 160.6, 128.8, 128.1, 123.6, 120.9, 120.9, 114.4, 113.5, 103.8, 55.4; $\boldsymbol{\delta_F}$ (376 MHz, $CDCl_3$) -65.64 ppm; ***m/z*** (ES^-) 216 $[M-H]^-$.

3-(2'-bromo-5'-methoxy-phenyl)-3-trifluoromethyl-3*H*-diazirine, 110

3-(2',4'-dibromo-5'-methoxyphenyl)-3-(trifluoromethyl)-3*H*-diazirine, 111



110 **111**

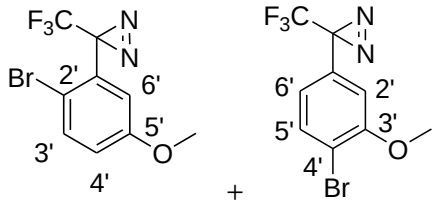
To a solution of 3-(3-Methoxyphenyl)-3-(trifluoromethyl)-3*H*-diazirine, **70** (67 mg, 0.31 mmol) in DCM (3 mL) at 0 ℃ was added bromine (59 mg, 0.37 mmol). The mixture was stirred at rt for 24 h and then diluted with CH_2Cl_2, washed with water. The organic layer was separated, dried with $MgSO_4$ and concentrated in vacuo to yield a yellow oil. Purification by flash column chromatography 5 % Hexane/EtOAc yielded:

ortho-substitute **110** (20 mg, 22 %); $\mathbf{R_f}$ 0.64 (20 % Hexane/EtOAc); $\boldsymbol{\upsilon_{max}}$ 2923, 1643, 1462, 1259, 1146, 637 cm^{-1}; $\boldsymbol{\delta_H}$ (700 MHz, $CDCl_3$) 7.49 (1H, d, J = 8.9 , 3'-H), 7.13 (1H, d, J = 3.0, 6'-H), 6.86 (1H, dd, J = 8.9, 3.0, 4'-H), 3.82 (3H, s, OCH_3); $\boldsymbol{\delta_C}$ (101 MHz, $CDCl_3$) 134.71, 129.02, 123.07, 120.33, 118.42, 117.79, 114.93, 55.67, 29.24 ; $\boldsymbol{\delta_F}$ (376 MHz, $CDCl_3$) -68.25 ppm; ***m/z*** 296 ($[^{81}Br]$ M-H^-), 294 ($[^{79}Br]$ M-H^-).

di-substitute **111** (26 mg, 28 %); $\mathbf{R_f}$ 0.41 (20 % Hexane/EtOAc); $\boldsymbol{\upsilon_{max}}$ 2925, 2853, 1625, 1559, 1455, 1434, 1407, 1313, 1280, 1259, 1218, 1193, 1158, 1061, 975, 911, 808, 746, 693, 638, 607 cm^{-1}; $\boldsymbol{\delta_H}$ (400 MHz, $CDCl_3$) 7.55 (1H, d, J = 8.9 , 3'-H), 6.86 (1H, d, J = 8.9, 6'-H), 3.90 (3H, s, CH_3); $\boldsymbol{\delta_C}$ (101 MHz, $CDCl_3$) 63.11, 61.68, 34.08, 32.81, 29.57, 29.52, 29.41, 28.76, 25.73; $\boldsymbol{\delta_F}$ (376 MHz, $CDCl_3$) -68.158 ppm; ***m/z*** (ES^-) 322/374/376 $[M-H]^-$.

3-(2'-bromo-5'-methoxy-phenyl)-3-trifluoromethyl-3*H*-diazirine, 110

3-(4'-bromo-3'-methoxyphenyl)-3-(trifluoromethyl)-3H-diazirine, 109



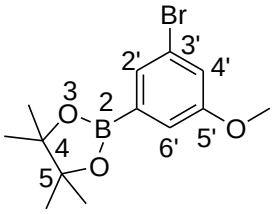
110 **109**

To a solution of 3-(3-Methoxyphenyl)-3-(trifluoromethyl)-3*H*-diazirine, **70** (120 mg, 0.56 mmol) in MeCN (2 mL) was added NBS (130 mg, 0.56 mmol) in MeCN (2 mL) at rt then heated 90 ℃ for 72 h. The organic phase was diluted with CH_2Cl_2, washed with water. The organic layer was separated, dried with $MgSO_4$ and concentrated in vacuo to yield a yellow oil. Purification by flash column chromatography 5 % Hexane/EtOAc to yielded:

ortho-substitute **110** (10.3 mg, 6.3 %); $\mathbf{R_f}$ 0.37 (5 % Hexane/EtOAc); $\boldsymbol{\upsilon_{max}}$ 2922, 1540, 1410, 1330, 1141, 1081, 960, 873, 807, 704, 619 cm^{-1}; $\boldsymbol{\delta_H}$ (400 MHz, $CDCl_3$) 7.49 (1H, d, *J* = 8.9, 3-H), 7.13 (1H, d, *J* = 3.0, 6-H), 6.86 (1H, dd, *J* = 8.9, 3.0, 4-H), 3.81 (3H, s, OCH_3); $\boldsymbol{\delta_C}$ (101 MHz, $CDCl_3$) 134.71, 129.02, 123.07, 120.33, 118.42, 117.79, 114.93, 55.67, 29.24; $\boldsymbol{\delta_F}$ (376 MHz, $CDCl_3$) -68.25 ppm; ***m/z*** 296 ([^{81}Br] M-H^-), 294 ([^{79}Br] M-H^-)..

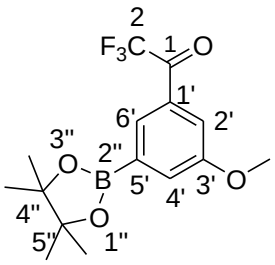
para-substitute **109** (18.2 mg, 11 %); $\mathbf{R_f}$ 0.26 (5 % Hexane/EtOAc); $\boldsymbol{\upsilon_{max}}$ 2979, 2930, 2517, 2448, 2370, 2166, 1981, 1737, 1562, 1240, 1133, 1026, 962, 811, 738, 670 cm^{-1}; $\boldsymbol{\delta_H}$ (400 MHz, $CDCl_3$) 7.62 (1H, d, *J* = 8.9, 5-H), 7.17 (1H, d, *J* = 3.0, 2-H), 7.00 (1H, dd, *J* = 8.9, 3.0, 6-H), 3.84 (3H, s, OCH_3); $\boldsymbol{\delta_C}$ (101 MHz, $CDCl_3$) 156.7, 133.7, 131.7, 117.8, 115.7, 113.7, 98.1, 53.9; $\boldsymbol{\delta_F}$ (376 MHz, $CDCl_3$) -68.25 ppm; ***m/z*** 296 ([^{81}Br] M-H^-), 294 ([^{79}Br] M-H^-).

1,3,2-Dioxaborolane, 2-(3'-bromo-5'-methoxyphenyl)-4,4,5,5-tetramethyl, 103



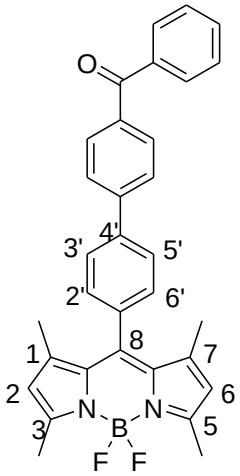
$[Ir(OMe)COD]_2$ (50 mg, 0.075 mmol) and ditertbutyl bipyridine (40 mg, 0.149 mmol) and B_2Pin_2 (1.3 g, 5.12 mmol) dissolve in MTBE (10 mL) in a microwave tube. This procedure was prepared inside the nitrogen hood. The mixture solution was from dark-black turned to dark radish colour. 3-Bromoanisole (0.93 g, 4.97 mmol) was added to the mixture solution and then microwave 80 ℃ for 30 min. The compound was purified by chromatography, eluting with Hexane/EtOAc (5-10 %), to furnish compound **103** as a light-yellow oil (1.46 g, 94 %); $\mathbf{R_f}$ 0.71 (20 % Hexane/EtOAc); $\boldsymbol{\upsilon_{max}}$ 2977, 1559, 1447, 1408, 1344, 1322, 1253, 1229, 1141, 1112, 1045, 964, 903, 851, 812, 701, 664 cm^{-1}; $\boldsymbol{\delta_H}$ (700 MHz, $CDCl_3$) 7.52 (1H, dd, *J* = 1.7, 0.7, 2'-H), 7.24 (1H, dd, *J* = 2.3, 0.7, 4'-H), 7.15 (1H, dd, *J* = 2.3, 1.7, 6'-H), 3.81 (3H, s, OCH_3), 1.34 (12H, s, $(CH_3)_4$); $\boldsymbol{\delta_C}$ (176 MHz, $CDCl_3$) 159.9, 129.7, 122.6, 120.6, 117.9, 84.2, 55.5, 24.8; ***m/z*** (ES^-) 313 $[M-H]^-$.

2,2,2-trifluoro-1-(3'-methoxy-5-(4'',4'',5'',5''-tetramethyl-1'',3'',2''-dioxaborolan -2''-yl)phenyl)ethanone, 104



This reaction was under N_2 used 1.0 mmol of the substrate and 2.4 mL of a stock solution containing $[Ir(OMe)cod]_2$ (104 mg, 0.312 mmol) and ditertbutyl bipyridine (84 mg, 0.312 mmol) and B_2Pin_2 (2644 mg, 010.40 mmol) dissolve in MTBE (25 mL) and shaken in rt developing a deep red colour. The solution was transferred to a vial was sealed with a rubber septum. **103** (0.2 g, 1.0 mmol) was added to 1.2 mL solution of the B_2Pin_2 mixture solution and then microwave heating at 80 ℃ for 1 min. The compound was purified by chromatography, eluting with 30 % Hexane/EtOAc, to furnish compound **104** as a light-yellow oil (147.6 mg, 45 %); $\mathbf{R_f}$ 0.56 (30 % Hexane/Acetone); $\boldsymbol{\upsilon_{max}}$ 2978, 1716, 1587, 1451, 1373, 1317, 1245, 1205, 1187, 1132, 1064, 988, 968, 825, 815, 767, 738, 695, 662, 620 cm^{-1}; $\boldsymbol{\delta_H}$ (500 MHz, $CDCl_3$) 8.08 (1H, s, 2'-H), 7.66 (1H, d, *J* = 2.6, 6'-H), 7.65 (1H, s, 4'-H), 3.90 (3H, s, OCH_3), 1.37 (12H, s, $(C(CH_3)_2)$; $\boldsymbol{\delta_C}$ (176 MHz, $CDCl_3$) 180.8 (q, $^2J_{CF}$ = 25.1 Hz, CCF_3), 159.67 (C-3'), 130.97 (C-Ar), 128.97 (dd, *J* = 4.7, 2.3, C-Ar), 127.46 (C-Ar), 117.56 (d, *J* = 1.8, C-Ar), 116.88 (q, $^1J_{CF}$ = 291.3 Hz, CF_3), 84.67 (C-4'' and C-5''), 55.86 (OCH_3), 25.08 (CCH_3); $\boldsymbol{\delta_F}$ (376 MHz, $CDCl_3$) -71.197 ppm; ***m/z*** (ES^-) 330 $[M-H]^-$.

4,4-difluoro-1,3,5,7-tetramethyl-8-(4'-benzoylphenyl)-4-bora-3a,4a-diaza-*s*-indacen, 3



Iodo-BODIPY **22** (0.2 g, 0.44 mmol), 4-benzoylphenylboronic acid (0.1 g, 0.44 mmol) and [$Pd(PPh_3)_4$] (0.5 g, 0.44 mmol) in toluene (10 mL), ethanol (5 mL) and 2M Na_2CO_3 (2 mL) were reflux for 5 h. After cooling to rt, partitioned between EtOAc and water. The organic layer was dried over Mg_2SO_4, and concentrated under vacuum. It was purified via silica gel chromatography (7-10 % Hexane/EtOAc) to give (0.16 g, 70 %) as a dark-orange solid. This compound was purified again via crystallization with methanol; **mp** 243-245 ℃; $\mathbf{R_f}$ 0.56 (20 % Hexane/EtOAc); $\boldsymbol{\upsilon_{max}}$ 2920, 1736, 1650 (C=O), 1603, 1538, 1510, 1465, 1405, 1366, 1304, 1276, 1188, 1153, 1121, 1073, 1063, 1051, 969, 927, 813, 768, 752, 699, 641 cm^{-1}; $\boldsymbol{\delta_H}$ (700 MHz, $CDCl_3$) 7.93 (3H, d, *J* = 8.2, Ar-H), 7.85 (3H, d, *J* = 6.8, Ar-H), 7.81 (4H, t, *J* = 9.0, Ar-H), 7.76 (1H, dd, *J* = 14.3, 8.1, Ar-H), 7.71 (1H, d, *J* = 8.5, Ar-H), 7.62 (2H, t, *J* = 7.3, Ar-H), 7.52 (3H, t, *J* = 7.6, Ar-H), 7.41 (2H, d, *J* = 8.1, Ar-H), 6.01 (2H, s, 2-H and 6-H), 2.57 (6H, s, 3-CH_3 and 5-CH_3), 1.46 (6H, s, 1-CH_3 and 7-CH_3); $\boldsymbol{\delta_C}$ (176 MHz, $CDCl_3$) 196.2, 155.7, 143.9, 143.0, 141.0, 140.5, 137.6, 136.8, 135.0, 132.7, 132.5, 131.4, 130.8, 130.0, 128.8, 128.4, 128.3, 127.9, 127.8, 127.2, 127.1, 126.9, 121.3, 37.1, 31.9, 29.7, 29.6, 29.3, 22.7; HRMS (ES^-) $C_{32}H_{26}BF_2N_2O$ requires M^- 502.2143, found $[M-H]^-$ 502.2186.

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