MODIFIED BODIPY PROBES TO EXPLORE
PEROXISOME FUNCTION

LIN, YING-CHUN

How to cite:
LIN, YING-CHUN (2010) MODIFIED BODIPY PROBES TO EXPLORE PEROXISOME FUNCTION, Durham theses, Durham University. Available at Durham E-Theses Online: http://etheses.dur.ac.uk/647/

Use policy
The full-text may be used and/or reproduced, and given to third parties in any format or medium, without prior permission or charge, for personal research or study, educational, or not-for-profit purposes provided that:
• a full bibliographic reference is made to the original source
• a link is made to the metadata record in Durham E-Theses
• the full-text is not changed in any way

The full-text must not be sold in any format or medium without the formal permission of the copyright holders.

Please consult the full Durham E-Theses policy for further details.
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.
# Content

## 1 INTRODUCTION

1.1 GENERAL INTRODUCTION ................................................................. 1
1.2 BODIPY DYES ............................................................................. 2
  1.2.1 Introduction to Organic dyes ......................................................... 2
  1.2.2 Introduction to BODIPY dyes ......................................................... 3
    1.2.2.1 BODIPY properties ................................................................. 3
    1.2.2.2 BODIPY Synthesis ................................................................. 9
      1.2.2.2.1 From pyroles and acid chlorides ........................................... 9
      1.2.2.2.2 From pyroles and aldehydes ............................................... 10
      1.2.2.2.3 From ketopyroles ............................................................ 12
  1.3 PHOTOAFFINITY LABELS .............................................................. 14
    1.3.1 Diazirine ............................................................................... 17
      1.3.1.1 The early synthesis .............................................................. 17
      1.3.1.2 The Graham Reaction ......................................................... 18
      1.3.1.3 The Trifluoromethyl Diazirine ........................................... 19
      1.3.1.4 Aromatic Substituted Diazirine .......................................... 21
    1.3.2 Benzophenone ........................................................................ 24
  1.4 PROJECT STRATEGY .................................................................... 25

## 2 RESULTS AND DISCUSSION

2.1 INTRODUCTION ............................................................................ 28
2.2 RETROSYNTHESIS ANALYSIS ....................................................... 28
2.3 SYNTHESIS OF BODIPY ............................................................... 30
  2.3.1 One- pot reaction .................................................................. 30
  2.3.2 Two steps process ................................................................. 31
  2.4 SYNTHESIS OF DIAZIRINE ......................................................... 35
2.5 SYNTHESIS STRATEGIES FOR CROSS COUPLING PRECURSOR .......... 39
  2.5.1 Strategy 1 ............................................................................. 39
  2.5.2 Strategy 2 ............................................................................. 44
  2.5.3 Strategy 3 ............................................................................. 47
  2.5.4 Strategy 4 ............................................................................. 52
  2.5.5 Strategy 5 ............................................................................. 54

Abstract

Introduction

Synthesis Strategies

Synthesis of Diazirine

Photoaffinity Labels

Project Strategy

Results and Discussion

Synthesis of BODIPY

Synthesis of Diazirine

Synthesis Strategies for Cross Coupling Precursor

Abbreviations
2.6 Palladium-catalysed cross coupling reactions ........................................ 57

3 Conclusion and future work ........................................................................ 59
  3.1 Conclusion ............................................................................................... 59
  3.2 Future work ............................................................................................. 60

4 Procedure ...................................................................................................... 61

5 References ..................................................................................................... 86
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-Butyloxy carbonyl
Bpin pinacolborane
bp boiling point
BODIPY Borondipyrromethane
°C 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
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>(p)</td>
<td>(para)</td>
<td>Parts per million</td>
</tr>
<tr>
<td>ppm</td>
<td></td>
<td>room temperature</td>
</tr>
<tr>
<td>rt</td>
<td></td>
<td>room temperature</td>
</tr>
<tr>
<td>(R_f)</td>
<td></td>
<td>Retention factor in chromatography)</td>
</tr>
<tr>
<td>s</td>
<td></td>
<td>second</td>
</tr>
<tr>
<td>TFA</td>
<td></td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td></td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TMS</td>
<td></td>
<td>Trimethylsilyl</td>
</tr>
<tr>
<td>TLC</td>
<td></td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>UV</td>
<td></td>
<td>Ultra violet</td>
</tr>
<tr>
<td>(\Phi_i)</td>
<td></td>
<td>Fluorescent quantum yield</td>
</tr>
</tbody>
</table>
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](image-url)
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. Reflecting this, there are many organic dyes in common use including fluorescein, rhodamine, cyanine, alexa and ethidium, 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, BODIPY](image)}

Figure 1.2 Organic fluorescent dye structures
1.2.2 Introduction to BODIPY dyes

Now the trademark of Molecular Probes, Inc. BODIPYs,4,8 4,4-difluoro-4-borato-3a-azonia-4a-aza-s-indacenes were first discovered in 1968 by Treibs and Kreuzer9 (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

![Basic BODIPY core structure](image)

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 422 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.
In contrast to the simple alkyl substituted BODIPYs the meso-phenyl compound 12 can be synthesized without $\alpha$-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 1.4** Different alkyl substitution patterns on BODIPY dye
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,\textsuperscript{25,26} pH probes,\textsuperscript{27} metal-chelators,\textsuperscript{28,29} and biomolecule conjugating group can be incorporated,\textsuperscript{30,31} Figure 1.8.

Figure 1.5 Different alkyl substitution patterns on BODIPY dye

Figure 1.6
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). 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.\textsuperscript{33} Table 1.1 shows some of the commercial BODIPY fluorophores which have been developed in this respect.

<table>
<thead>
<tr>
<th>structure</th>
<th>BODIPY</th>
<th>absorption</th>
<th>emission</th>
<th>$e$ [cm$^{-1}$M$^{-1}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Structure 28" /></td>
<td>493/503</td>
<td>500 nm</td>
<td>509 nm</td>
<td>79000</td>
</tr>
<tr>
<td><img src="image" alt="Structure 29" /></td>
<td>558/568</td>
<td>559 nm</td>
<td>568 nm</td>
<td>97000</td>
</tr>
<tr>
<td><img src="image" alt="Structure 30" /></td>
<td>650/665</td>
<td>646 nm</td>
<td>660 nm</td>
<td>102000</td>
</tr>
<tr>
<td><img src="image" alt="Structure 31" /></td>
<td>FL</td>
<td>502 nm</td>
<td>510 nm</td>
<td>82000</td>
</tr>
<tr>
<td><img src="image" alt="Structure 32" /></td>
<td>R6G</td>
<td>528 nm</td>
<td>540 nm</td>
<td>70000</td>
</tr>
<tr>
<td><img src="image" alt="Structure 33" /></td>
<td>TMR</td>
<td>544 nm</td>
<td>570 nm</td>
<td>56000</td>
</tr>
<tr>
<td><img src="image" alt="Structure 34" /></td>
<td>TR</td>
<td>588 nm</td>
<td>616 nm</td>
<td>68000</td>
</tr>
</tbody>
</table>

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

\textbf{Table 1.1} The different BODIPY absorption and emission wave length
1.2.2 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\(^2\) 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.

1.2.2.1 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\(_3\) OEt\(_2\) in the presence of Et\(_3\)N to afford the BODIPY core. The first method used to synthesis a BODIPY derivative followed such a strategy.\(^2\) This process was undertaken in a one-pot, two-step procedure to give the products in 18- 86 % overall yield (Scheme 1.1).\(^3\)

![Scheme 1.1 Synthesis of symmetric F-BODIPY dyes from acyl chloride derivatives](image)

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 dipyrrromethenes 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 NEt3 and PhMe before adding boron trifluoride etherate. The reaction mixture was then heated at 80 °C for 20 min. The final BODIPYs 37 were isolated following chromatography on alumina (Scheme 1.2).

![Scheme 1.2 Synthesis of symmetric F-BODIPY dyes](image)

1.2.2.2 From pyrroles and aldehydes

The second method of formation of BODIPYs involves the condensation of aromatic aldehydes with pyrroles to give, after oxidation, the dipyrrromethene intermediate. Reaction of the dipyrrromethene 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 dipyrrromethene precursor, the condensation usually needs an electron rich substituent, either on the pyrrole R1 or aldehyde R4 position. As stated above, the distinction of this method is the requirement for the oxidation of the first formed dipyrrromethane B.
In most examples 2,3-dichloro5,6-dicyano-\textit{p}-benzoquinone (DDQ) was used as the oxidizing agent.\textsuperscript{35,36,37} The overall yield of this reaction varied from 18-43 \% depending on the substrate (Scheme 1.3).\textsuperscript{38,39,40}

![Scheme 1.3 Synthesis of symmetric F-Bodipy dyes from aldehyde derivatives](image)

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 \textit{et al.}\textsuperscript{35} synthesised BODIPY fluorophores from aldehydes with chiral substituents at the \textit{\alpha}-positions (Scheme 1.4).
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 1.4 Synthesis of BODIPY from aldehydes

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 dipyrrromethene 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.\textsuperscript{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 dipyrrromethene 46 is produced from the condensation of pyrrole 43 and ketopyrrole 45 using phosphoryl chloride as the dehydrating agent. Dipyrrromethene 46 can be converted directly to the target materials 22 after complexation with boron trifluoride etherate in the presence of triethylamine (Scheme 1.6).

\[ \text{p}{\text{H}} + \text{Cl}{\text{O}} \rightarrow \text{p}{\text{O}}{\text{Cl}} \]

(i) CH\textsubscript{3}MgBr, ether, 81%; (ii) 43, POCl\textsubscript{3}, CH\textsubscript{2}Cl\textsubscript{2}/pentane, 0°C, 46%; (iii) BF\textsubscript{3}.OEt\textsubscript{2}, NEt\textsubscript{3}, toluene, 80%.

\textit{Scheme 1.6} Synthesis of symmetrical BODIPY from ketopyrroles
Similarly, unsymmetrical substituted BODIPY 48 was also prepared by this method approach (Scheme 1.7).  

\[
\text{H} + \text{HN} \xrightarrow{(i) \text{POCl}_3, \text{CH}_2\text{Cl}_2/\text{pentane, } 0^\circ\text{C}} \xrightarrow{(ii) \text{BF}_3\text{OEt}_2, \text{Et}_3\text{N}} \text{toluene} \xrightarrow{\text{47}} 48
\]

**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. 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. 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. 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.

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 1.1** 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 are 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.\textsuperscript{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\textit{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).\textsuperscript{46,47}

\[
\begin{align*}
\text{49} & : \text{R}^1=\text{alkyl, } \text{R}^2=\text{H} \\
\text{50} & : \text{R}^1, \text{R}^2=\text{alkyl}
\end{align*}
\]

\textbf{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 °C, 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).\textsuperscript{48}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure13.png}
\caption{Figure 1.13}
\end{figure}

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).\textsuperscript{49}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure14.png}
\caption{Figure 1.14 Photoisomerisation of diazirine}
\end{figure}

1.3.1.2 The Graham Reaction

In 1965, Graham reported the one-pot hypohalite oxidation of amidines to 3-halodiazirines,\textsuperscript{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.
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

1.3.1.3 The Trifluoromethylidiazirine

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 %).
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).\(^{57}\)

\[
\text{Scheme 1.8}
\]

\[
\text{Scheme 1.9}
\]
Building on these observations trifluoromethylated diazirines were first reported by Brunner et al.\textsuperscript{61} In this report the aryl trifluoromethyl diazirine 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).

![Image of 68]

1.3.1.4 Aromatic Substituted Diazirine

The introduction of aryl substituents, e.g. aryl-3H-diazirines 64,\textsuperscript{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.

![Image of 64]

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

![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

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

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
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. 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.

Scheme 1.11 The retrosynthetic analysis of probe 71.
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

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 cycles70 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 whilst compound 82 was used as a fluorescent nucleotide photoaffinity label for studies with ATP.

![Figure 1.17 Application of benzophenone photoprobes in biochemistry structures](image)

### 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 (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).
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.
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.
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.

![Scheme 2.4]

This reaction was repeated from Landrum’s thesis. Initial attempts to synthesise 90 began by using benzoyl chloride. This was successfully achieved using two equivalent of 2,4-dimethylpyrrole, Et$_3$N, and three equivalent BF$_3$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 $^1$H 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-benzoylephenyl 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).

![Scheme 2.5](image)

**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
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.

![Chemical Reaction Diagram](image_url)

Scheme 2.6

The intermediate symmetrical dipyromethenes 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.
The isolated yields of dipyromethenes are a compromise between their acidity and stability. Burghart et al. has described the isolation of the intermediate dipyromethenes process as best done using a deactivated alumina column rather than silica gel. Following this idea, alumina oxide based column purification increased the yield from the 10% obtained using silica gel to 80% yield. The intermediate 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).

\[ \text{F} \quad 95: R = H \\
96: R = \text{NO}_2 \\
45: R = \text{I} \]

\[ \text{D} \quad 88: R = H \quad (83\%) \\
89: R = \text{NO}_2 \\
46: R = \text{I} \quad (50\%) \]
In a final step, the dipyrromethenes were complexed with boron trifluoride by treatment with BF$_3$·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).

![Scheme 2.9](image)

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 $^\circ$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).

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

Scheme 2.10 Synthesis of 3-(3-Methoxyphenyl)-3-(trifluoromethyl)-3H-diazirine as reported by Baldwin.76
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 $\delta$ -71.18 and the IR spectrum showed an C=O stretch at 1710 cm$^{-1}$ confirming formation of the trifluoromethyl ketone.

![Scheme 2.11](image)

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.

![Scheme 2.12](image)
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 $\delta$ 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).

\[
\text{Scheme 2.13}
\]

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

\[
\text{Scheme 2.14}
\]
The tosyloxime 82 was then converted to diaziridine 81 by reaction with liquid ammonia at -78 °C for 2 h. A reaction mechanism for this conversion is shown in 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 $\delta$ -75.45, and ES MS showed a molecular ion with m/z 218 (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).

With the photoaffinity group now in hand, the borylation with bis(pinacolato)-diboron ($\text{B}_2\text{Pin}_2$) 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 ($\text{B}_2\text{Pin}_2$) 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 \textit{meta} position only, Scheme 2.18.

\[
\begin{align*}
\text{[Ir(OMe)COD]}_2 \cdot \text{dtbpy} & \quad \text{B}_2\text{pin}_2 \\
& \quad \text{80} \degree \text{C}, \mu \text{W, MTBE} \\
\end{align*}
\]

\textit{Scheme 2.18}

As a result it was our intention to utilise this approach to insert a boronate, \textit{meta} to the diazirine group of 70. The active reagent was prepared in a stock solution containing 1.0 mmol of bis pinacolato-diboron (\text{B}_2\text{Pin}_2, \text{pin}=\text{O}_2\text{C}_2\text{Me}_4), 1.5 \text{ mol } \% \text{ of precatalyst [Ir(OMe)COD]}_2, \text{ and 3 mol } \% \text{ of 4,4'}-\text{di-t-butyl-2,2'}-\text{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).
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 °C 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$_2$pin$_2$, respectively. It was thought that preferential coordination of the NH groups was inhibiting the reactivity of the Iridium catalyst.

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$_2$O) 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 °C to rt. The reaction process was monitored by $^1$H 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).

Furthermore, reaction of diaziridine 81 with Boc$_2$O 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).
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 °C for 10 min, saw only starting material by TLC. The reaction was continued for one more hour and heated up to 100 °C. Disappointingly, only starting material was detected by $^{19}$F NMR. The second experiment of this reaction was using microwave heating at 100 °C 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$_2$Pin$_2$ respectively. As a result of these difficulties, this borylation strategy was then discontinued.

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).

![Scheme 2.24](image)

The reaction was carried out with microwave heating at 80 °C 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 °C for 16 h in 73 % yield, \(^8\) 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 \(n\)-butyllithium with compound 103 was attempted (Scheme 2.25).
Scheme 2.25 Attempted synthesis of 104 from 103

After 20 min. stirring with n-BuLi in THF at -78 °C, 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 nBuLi preferentially underwent nucleophilic attack on the Bpin ring, generating complex mixtures upon work up.

Since reaction of n-butyllithium 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. These studies showed that the protected intermediate meta- and para-bromoboronates could undergo metal-halogen exchange with i-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 standard 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 °C for various times and equivalents of B₂Pin₂. 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.

Scheme 2.28

<table>
<thead>
<tr>
<th>Entry</th>
<th>B₂Pin₂ eq</th>
<th>Time</th>
<th>Temp. (°C)</th>
<th>104</th>
<th>106</th>
<th>107</th>
<th>108</th>
<th>84</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1h</td>
<td>80</td>
<td>12% b</td>
<td>87% b (66%) a</td>
<td>nd b</td>
<td>&lt;1% b</td>
<td>nd b</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>15min</td>
<td>80</td>
<td>45% b (8%) a</td>
<td>34% b (60%) a</td>
<td>7% b</td>
<td>&lt;1% b</td>
<td>nd b</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>5min</td>
<td>80</td>
<td>34% b</td>
<td>47% b</td>
<td>8% b</td>
<td>&lt;1% b</td>
<td>nd b</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>5min</td>
<td>80</td>
<td>88% b (35%) a</td>
<td>7% b (41%) a</td>
<td>&lt;1% b</td>
<td>&lt;1% b</td>
<td>&lt;1% b</td>
</tr>
<tr>
<td>5</td>
<td>0.5</td>
<td>1min</td>
<td>80</td>
<td>72% b (45%) a</td>
<td>10% b (34%) a</td>
<td>5% b</td>
<td>2% b</td>
<td>11% b</td>
</tr>
<tr>
<td>6</td>
<td>0.5</td>
<td>1min</td>
<td>80</td>
<td>47% b (29%) a</td>
<td>22% b (38%) a</td>
<td>6% b</td>
<td>1% b</td>
<td>19% b</td>
</tr>
</tbody>
</table>

*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$_2$Pin$_2$ 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 $\delta$ 5.04 – 4.97 (1H) and doublet at $\delta$ 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$_2$Pin$_2$. This produced the desire product 104 albeit in low yield (Entry 2 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 $\delta$ 1.37 (12H, s), and 180.8 (CF$_3$CO, 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$_2$Pin$_2$ 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).
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.
<table>
<thead>
<tr>
<th>Entry</th>
<th>Dry acetone (eq)</th>
<th>B$_2$Pin$_2$ (eq)</th>
<th>Time (min)</th>
<th>Temperature (°C)</th>
<th>19$^F$ NMR ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>80</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>80</td>
<td>1.25</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>1</td>
<td>1</td>
<td>80</td>
<td>0.4</td>
</tr>
</tbody>
</table>

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 (MnO₂) 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 ¹H 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).

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

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.
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 $\delta$7.49 (1H, d), $\delta$7.13 (1H, d), $\delta$6.86 (1H, dd) consistent with the proposed substitution pattern. The other di-substituted compound 111 showed peaks at $\delta$7.55 (1H, d), $\delta$6.86 (1H, d) in the $^1$H NMR spectroscopy, and m/z 372/374/376 shown by GC-MS (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 °C 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 $\delta$ -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 $\delta$ -73.18, and ES MS showed a molecule ion m/z 294/296 (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).
The differences came in the synthesis of \textit{para}-methoxyphenyl tosylxime 115 which contrasts with the method used in the preparation of tosylxime 82. A report by Hatanaka \textit{et al.} describing the preparation of oxime 114 at 0 °C while adding p-toluenesulfonyl chloride. 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 $\delta$ -65.98 by $^{19}$F NMR spectroscopy. ES MS showed a molecular ion with $m/z$ 373 (Scheme 2.34).

![Scheme 2.34](image)

In the last section whilst bromination had been possible it was not selective. Consequently we turned to \textit{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
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$_2$Pin$_2$, bromine and NBS. However, a complex mixture of products was formed which were very difficult to separate and identify.

![Scheme 2.36](image)

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.

![Scheme 2.37](image)

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.

![Scheme 2.38](attachment:image.png)

### 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 synthesis on diazirine photoaffinity probes, we decided to explore the cross-coupling with a commercial available compound, 4-benzoylephanyl 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

![Scheme 2.39](attachment:image.png)

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 (PPh₃)₄] were heated reflux in 2M Na₂CO₃ 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).

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 dipyrrromethene 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 treatment with BF₃ OEt₂ 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 Br₂ 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 F254 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 $\delta$ (ppm) (number of protons, multiplicity, coupling constant $J$ (Hz), assignment). The residual protic solvent was used as the internal reference (CHCl$_3$ $\delta_H$ = 7.26 ppm; $\delta_c$ = 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, CI) 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 University of Durham.
General Procedure

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

A solution of 2,4-dimethylpyrrole (0.36 mL, 3.55 mmol) and CH₃MgBr 3M in ether (0.41 mL, 1.19 mmol) was refluxed 50 ºC 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 NH₄Cl, and the resultant mixture extracted with CH₂Cl₂. The organic layer was washed with water, dried over MgSO₄ 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 ºC; Rf 0.58 (10 % EtOAc/DCM); \( \nu_{\text{max}} \) 3266 (NH), 2363, 1682 (C=O), 1564, 1430, 1278, 1095, 1004, 930, 800, 740, 700, 650, 600, 525 cm⁻¹; \( \delta_{\text{H}} \) (700 MHz, CDCl₃) 9.24 (1H, br s, NH), 7.61 (2H, d, \( J = 1.4 \), 2'-H and 6'-H), 7.50 (1H, t, \( J = 7.5 \), 4'-H), 7.44 (2H, t, \( J = 7.5 \), 3'-H and 5'-H), 5.88 (1H, s, 4-H), 2.31 (3H, s, 3-CH₃), 1.93 (3H, s, 5-CH₃); \( \delta_{\text{C}} \) (176 MHz, CDCl₃) 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-CH₃), 13.2 (5-CH₃); \( m/z \) (ES⁺) 199.2 [M+H]⁺.
Following the same procedure as described for 95, 2,4-dimethylpyrrole (1.9 mL, 18.76 mmol), CH$_3$MgBr 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 °C; R$_f$ 0.6 (30 % Hexane/EtOAc); $\nu_{\max}$ 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$^{-1}$; $\delta_H$ (400 MHz, CDCl$_3$) 9.03 (1H, br s, NH), 7.80 (2H, d, $J = 8.4$, 2'-H and 6'-H ), 7.36 (2H, d, $J = 8.4$, 3'-H and 5'-H), 5.87 (1H, s, 4-H), 2.29 (3H, s, 5-CH$_3$), 1.94 (3H, s, 3-CH$_3$); $\delta_C$ (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-CH$_3$), 13.2 (3-CH$_3$); m/z (ES$^+$) 325 [M+H]$^+$. 

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

\[ \text{\includegraphics[width=0.5\textwidth]{image.png}} \]
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-dimethylpyrrole (0.5 mL, 5.39 mmol) and CH$_3$MgBr 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 °C; $R_f$ 0.25 (20 % Hexane/EtOAc); $\nu_{max}$ 3271 (N-H), 3114, 2922, 2848, 1708 (C=O), 1576, 1530, 1500, 1438, 1344, 1283, 1108, 1016, 931, 851, 803, 741, 506, 460 cm$^{-1}$; $\delta_H$ (400 MHz, CDCl$_3$) 9.17 (1H, s, N-H), 8.33 – 8.30 (2H, m, AA’ part of AA’XX’ system, 2’-H and 6’-H ), 7.78 – 7.73 (2H, m, XX’ part of AA’XX’ system, 3’-H and 5’-H), 5.91 (1H, s, 4-H), 2.33 (3H, s, 5-CH$_3$), 1.85 (3H, s, 3-CH$_3$); $\delta_C$ (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-2H-pyrrol-2’-ylidene)phenylmethyl]-3,5-dimethyl-1H-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 °C 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 NEt₃ (0.2 mL, 1.51 mmol), and water (30 mL) and then extracted with CH₂Cl₂. The combined organic extracts were then dried over MgSO₄ 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 °C; v_max 1521 (NH), 1418, 1355, 1137, 962, 899, 824, 715, 622 cm⁻¹; δ_H (400 MHz, CDCl₃) 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’-CH₃ and 5-CH₃), 1.40 (6H, s, 3’-CH₃ and 3-CH₃); δ_C (126 MHz, CDCl₃) 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’-CH₃ and 5-CH₃), 15.0 (3’-CH₃ and 3-CH₃); m/z (ES⁺) 276 [M+H]⁺.
2-[(Z)-(3’,5’-dimethyl-2H-pyrrol-2’-ylidene)(4’’-iodophenyl)methyl]-3,5-dimethyl-1H-pyrrole, 46

Following the same procedure as described 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 NEt₃ (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 °C; νmax 1540 (N-H), 1410, 1328, 1138, 962, 893, 820, 673, 618 cm⁻¹; δH (400 MHz, CDCl₃) 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’-CH₃ and 5-CH₃), 1.34 (6H, s, 3’-CH₂ and 3-CH₂); δC (126 MHz, CDCl₃) 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]⁻.
A solution of dipyrrmethane 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 (BF₃·OEt₂) (0.13 mL, 1.09 mmol). The mixture was stirred at 60 °C 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 °C; ʋₘₐₓ 1542, 1502 (B-F), 1304, 1192, 1185, 1063, 980, 812, 721, 541 cm⁻¹; ǳ_H (400 MHz, CDCl₃) 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-CH₃ and 5-CH₃), 1.37 (6H, s, 1-CH₃ and 7-CH₃); ǳ_C (101 MHz, CDCl₃) 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 ºC 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 ºC 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 ºC; \( \nu_{\text{max}} \) 2968, 2928, 2854, 2374, 1650, 1536, 1512, 1466, 1310, 1194, 1152, 1056, 1975, 830, 755, 692, 475 cm\(^{-1} \); \( \delta_\text{H} \) (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\(_3\)); \( \delta_\text{C} \) (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-dimethylpyrrole (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 ºC; \( \nu_{\text{max}} \) 3100, 2970, 2910, 2860, 2395, 1684, 1528, 1500, 1462, 1407, 1345, 1314, 1198, 1159, 1102, 998, 992, 990, 986, 729, 478 cm\(^{-1}\); \( \delta_{\text{H}} \) (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\(_3\)); \( \delta_{\text{C}} \) (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

\[\text{O} \quad \text{F}_3 \text{C} \quad \text{O} \]

\[\text{F}_3 \text{C} - \text{C} - \text{O} \]

\[6' \quad 2' \quad 5' \quad 3' \quad 4' \]

\[n \text{ 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 °C 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 °C, 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 °C, 3 Torr) to give the title product (84) as light yellow oil (4.63 g, 85 %); \(\nu_{\text{max}}\) 2839, 1710 (C=O), 1598, 1582, 1490, 1465, 1432, 1342, 1249, 1198, 1137, 1051, 978, 825, 752, 734, 663, 613, 554 cm\(^{-1}\); \(\delta_H\) (400 MHz, CDCl\(_3\)) 7.66 (1H, ddd, \(J = 8.0, 2.4, 1.4, 6'-\text{H}\)), 7.57 (1H, t, \(J = 1.4, 2'-\text{H}\)), 7.46 (1H, t, \(J = 8.0, 5'-\text{H}\)), 7.27-7.24 (1H, ddd, \(J = 8.0, 2.4, 1.4, 4'-\text{H}\)), 3.88 (3H, s, O-CH\(_3\)); \(\delta_C\) (126 MHz, CDCl\(_3\)) 180.6 (q, \(^2J_{\text{CF}}\) = 35.1 Hz, C\(_{\text{CF}_3}\)), 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, \(^1J_{\text{CF}}\) = 291.2 Hz, CF\(_3\)), 55.8 (OCH\(_3\)); \(\delta_F\) (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; Rf 0.47 (10 % Hexane/EtOAc); \( \nu_{\text{max}} \) 2938, 1702 (C=O), 1598, 1514, 1462, 1428, 1317, 1270, 1161, 1137, 1025, 938, 844, 768, 737, 648, 614 cm\(^{-1}\); \( \delta_H \) (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\(_3\)); \( \delta_C \) (101 MHz, CDCl\(_3\)) 178.9, 165.4, 132.8, 122.8, 118.4, 115.3, 114.4, 60.2, 55.7; \( \delta_F \) (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 °C for 17 h. The mixture was concentrated and the residue partitioned between water and Et₂O. The organic layer was washed with 5N HCl (20 mL), dried over MgSO₄, filtered, and concentrated in vacuo to afford the title oxime as a pale yellow oil which was not purified further (2.65 g, 98 %); R₇ 0.75 (90 % DCM/EtOAc); νₘₐₓ 3328, 3004, 2942, 2846, 1576, 1492, 1244, 1132, 1008, 958, 846, 733, 704, 544 cm⁻¹; δₜₜ (400 MHz, CDCl₃) 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, CH₃); δₐ (101 MHz, CDCl₃) 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; δₖ (376 MHz, CDCl₃) -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 °C for 5 h. The mixture was concentrated and the residue partitioned between water and Et₂O. The organic layer was washed with 5N HCl (10 mL), dried over MgSO₄, filtered, and concentrated in vacuo to afford oxime as a pale yellow oil which was not purified further (1.17 g, 97 %); Rf 0.74 (DCM); νmax 3260 (OH), 2840, 1608, 1515, 1463, 1443, 1340, 1293, 1254, 1208, 1174, 1128, 1021, 1002, 954, 832, 746, 701, 670, 615 cm⁻¹; δH (400 MHz, CDCl₃) 8.62 (1H, broad m, OH), 7.55 (2H, d, J = 8.8, 2’-H, 6’-H), 6.98 (2H, d, J = 8.8, 3’-H, 5’-H), 3.84 (3H, s, CH₃); δC (101 MHz, CDCl₃) 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; δF (376 MHz, CDCl₃) -66.02 ppm; m/z (ES⁺) 220 [M-H]⁻.
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 °C for 2 h. After cooling, the solvent was evaporated and the residue partitioned between Et₂O, water and 5N HCl (50 mL). The organic layer was dried over MgSO₄, and concentrated under vacuum to 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 °C; Rₚ 0.45 (toluene); νₘₐₓ 1738, 1350, 1234, 1043, 604 cm⁻¹; δ₁ ᵃ ᵃ (400 MHz, CDCl₃) 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, OCH₃), 2.48 (3H, s, 4''-CH₃); δ₁ ᵐ ᵐ (101 MHz, CDCl₃) 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 (OCH₃), 21.8 (CH₃); δᵢ (376 MHz, CDCl₃) -66.9 ppm; m/z (ES') 373 [M-H]⁻.
2,2,2-Trifluoro-1-(4'-methoxyphenyl)-N-[(4''-methylphenyl)sulfonyl]-oxy)ethanmine, 115

\[
\begin{array}{c}
\text{F}_3\text{C} \quad \text{N} \quad \text{O} \quad \text{S} \\
\text{6'} \quad \text{5'} \quad \text{2'} \quad \text{1'} \quad \text{3'} \quad \text{4'} \\
\end{array}
\]

\(p\)-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 °C. 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 °C; \(R_f\) 0.43 (15 % Hexane/EtOAc); \(\nu_{\text{max}}\) 1605, 1511, 1450, 1390, 1343, 1300, 1257, 1192, 1176, 1138, 1089, 1033, 1000, 892, 812, 777, 738, 714, 671, 746, 612 cm\(^{-1}\); \(\delta_H\) (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\(_3\)); \(\delta_C\) (101 MHz, CDCl\(_3\)) 162.1, 146.0, 131.3, 130.7, 129.8, 129.3, 116.5, 114.2, 55.4, 21.8; \(\delta_F\) (376 MHz, CDCl\(_3\)) -65.98 ppm; \(m/z\) (ES\(^{+}\)) 373 [M-H\(^{-}\)].
3-(3’-Methoxyphenyl)-3-(trifluoromethyl)diaziridine, 81

![Chemical Structure]

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 CH₂Cl₂ (60 mL) was added to liquid ammonia (40 mL) at -78 ºC over a period of 20 min. The solution was stirred at -78 ºC 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 CH₂Cl₂. The organic layer was dried with MgSO₄, filtered, and concentrated in vacuo. The resulting crude product was purified by flash chromatography (0-5 % CH₂Cl₂/EtOAc) to give the diaziridine 81 (2.23 g, 96 %) as a slightly yellow liquid; Rf 0.32 (DCM); vₘₐₓ 3250 (N-H), 1582 (N-H), 1500, 1460, 1390, 1328, 1286, 1246, 1217, 1136, 1044, 958, 922, 784, 715, 692, 645, 443 cm⁻¹; δH (400 MHz, CDCl₃) 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, CH₃), 2.75 (1H, s, NH), 2.21 (1H, s, NH); δC (101 MHz, CDCl₃) 159.7, 133.0, 129.9, 124.9, 120.3, 115.8, 113.6, 57.8, 55.4; δF (376 MHz, CDCl₃) -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 CH₂Cl₂ (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 °C; Rf 0.27 (15 % Hexane/EtOAc); v_max 3214, 3194, 1612, 1518, 1391, 1249, 1027, 952, 886, 836, 821, 801, 733, 700, 592, 569, 528, 427 cm⁻¹; δ_H (400 MHz, CDCl₃) 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, OCH₃) 2.48 (1H, s, NH), 1.25 (1H, s, NH); δ_C (101 MHz, CDCl₃) 160.8, 130.2, 129.5, 123.7, 118.7, 114.9, 114.1, 98.8, 55.4; δ_F (376 MHz, CDCl₃) -75.77; m/z (ES⁺) 218 [M+H]⁺.
3-(3-Methoxyphenyl)-3-(trifluoromethyl)-3H-diazirine, 70

Ag₂O (1.86 g, 8.02 mmol) was added to a solution of diaziridine 81 (0.5 g, 2.29 mmol) in dry Et₂O (20 mL) and stirred at rt for 3.5 h. The dispersion was filtered and washed with Et₂O, and dried over MgSO₄. Evaporation of the solvent gave the pure diazirine 70 (0.47 g, 95 %) of a colourless liquid; Rf 0.84 (95 % DCM/EtOAc); v_max 2938, 1605, 1583, 1493, 1466, 1433, 1343, 1260, 1150, 1042, 996, 979, 964, 872, 821, 778, 727, 690, 648 cm⁻¹; δ_H (400 MHz, CDCl₃) 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, d, J = 0.7, 2-H), 3.80 (3H, d, J = 7.3, CH₃); δ_C (101 MHz, CDCl₃) 159.8 (C-3), 130.5 (C-1), 130.0 (C-5), 122.3 (q, J_CF = 248.3 Hz, C_F₃), 118.7 (C-6), 115.2 (C-4), 112.2 (d, J = 1.4 Hz, C-2), 55.3 (CH₃), 28.27 (m, J_CF = 43.7 Hz, C_F₃); δ_F (376 MHz, CDCl₃) -65.26 ppm; m/z (ES⁺) 216 [M-H]⁺.
3-(4-Methoxyphenyl)-3-(trifluoromethyl)-3H-diazirine, 117

Following the same procedure as describe for 117, Ag₂O (1.4 g, 5.94 mmol), a solution of diaziridine 116 (0.32 g, 1.70 mmol) in dry Et₂O (10 mL) where used to provide the title compound 117 as a yellow oil (0.31 g, 98 %); Rf 0.6 (15 %)

Hexane/EtOAc; υₘₐₓ 2946, 2848, 1613, 1518, 1463, 1343, 1257, 1232, 1177, 1145, 1054, 1032, 936, 823, 730, 593, 540, 402 cm⁻¹; δ_H (400 MHz, CDCl₃) 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, OCH₃); δ_C (101 MHz, CDCl₃) 160.6, 128.8, 128.1, 123.6, 120.9, 120.9, 114.4, 113.5, 103.8, 55.4; δ_F (376 MHz, CDCl₃) -65.64 ppm; m/z (ES⁻) 216 [M-H⁻].
3-(2’-bromo-5’-methoxy-phenyl)-3-trifluoromethyl-3H-diazirine, 110

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

To a solution of 3-(3-Methoxyphenyl)-3-(trifluoromethyl)-3H-diazirine, 70 (67 mg, 0.31 mmol) in DCM (3 mL) at 0 °C was added bromine (59 mg, 0.37 mmol). The mixture was stirred at rt for 24 h and then diluted with CH2Cl2, washed with water. The organic layer was separated, dried with MgSO4 and concentrated in vacuo to yield a yellow oil. Purification by flash column chromatography 5 % Hexane/EtOAc yielded:

**ortho-substitute 110** (20 mg, 22 %); Rf 0.64 (20 % Hexane/EtOAc); v_max 2923, 1643, 1462, 1259, 1146, 637 cm⁻¹; δ_H (700 MHz, CDCl3) 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, OCH3); δ_C (101 MHz, CDCl3) 134.71, 129.02, 123.07, 120.33, 118.42, 117.79, 114.93, 55.67, 29.24 ; δ_F (376 MHz, CDCl3) -68.25 ppm; m/z 296 ([81Br] M-H), 294 ([79Br] M-H).

**di-substitute 111** (26 mg, 28 %); Rf 0.41 (20 % Hexane/EtOAc); v_max 2925, 2853, 1625, 1559, 1455, 1434, 1407, 1313, 1280, 1259, 1218, 1193, 1158, 1061, 975, 911, 808, 746, 693, 638, 607 cm⁻¹; δ_H (400 MHz, CDCl3) 7.55 (1H, d, J = 8.9 , 3’-H), 6.86 (1H, d, J = 8.9, 6’-H), 3.90 (3H, s, CH3); δ_C (101 MHz, CDCl3) 63.11, 61.68, 34.08, 32.81, 29.57, 29.52, 29.41, 28.76, 25.73; δ_F (376 MHz, CDCl3) -68.158 ppm; m/z (ES’) 322/374/376 [M-H]'.

81
3-(2’-bromo-5’-methoxy-phenyl)-3-trifluoromethyl-3H-diazirine, 110

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

To a solution of 3-(3-Methoxyphenyl)-3-(trifluoromethyl)-3H-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 °C for 72 h. The organic phase was diluted with CH₂Cl₂, washed with water. The organic layer was separated, dried with MgSO₄ 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 %); Rf 0.37 (5 % Hexane/EtOAc); υmax 2922, 1540, 1410, 1330, 1141, 1081, 960, 873, 807, 704, 619 cm⁻¹; δH (400 MHz, CDCl₃) 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, OCH₃); δC (101 MHz, CDCl₃) 134.7, 129.0, 123.0, 120.3, 118.4, 117.8, 114.9, 55.7, 29.2; δF (376 MHz, CDCl₃) -68.2 ppm; m/z 296 ([¹⁸¹Br] M-H), 294 ([¹⁷⁹Br] M-H⁻).

para-substitute 109 (18.2 mg, 11 %); Rf 0.26 (5 % Hexane/EtOAc); υmax 2979, 2930, 2517, 2448, 2370, 2166, 1981, 1737, 1562, 1240, 1133, 1026, 962, 811, 738, 670 cm⁻¹; δH (400 MHz, CDCl₃) 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, OCH₃); δC (101 MHz, CDCl₃) 156.7, 133.7, 131.7, 117.8, 115.7, 113.7, 98.1, 53.9; δF (376 MHz, CDCl₃) -68.2 ppm; m/z 296 ([¹⁸¹Br] M-H), 294 ([¹⁷⁹Br] M-H⁻).
1,3,2-Dioxaborolane, 2-(3’-bromo-5’-methoxyphenyl)-4,4,5,5-tetramethyl, 103

![Structural formula of the compound]

[Ir(OMe)COD]₂ (50 mg, 0.075 mmol) and ditertbutyl bipyridine (40 mg, 0.149 mmol) and B₂Pin₂ (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 °C 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 %); \( R_f \) 0.71 (20 % Hexane/EtOAc); \( \nu_{\text{max}} \) 2977, 1559, 1447, 1408, 1344, 1322, 1253, 1229, 1141, 1112, 1045, 964, 903, 851, 812, 701, 664 cm⁻¹; \( \delta_H \) (700 MHz, CDCl₃) 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, OCH₃), 1.34 (12H, s, (CH₃)₄); \( \delta_C \) (176 MHz, CDCl₃) 159.9, 129.7, 122.6, 120.6, 117.9, 84.2, 55.5, 24.8; \( m/z \) (ES⁺) 313 [M-H⁻].
This reaction was under N\textsubscript{2} used 1.0 mmol of the substrate and 2.4 mL of a stock solution containing [Ir(OMe)cod]\textsubscript{2} (104 mg, 0.312 mmol) and ditertbutyl bipyridine (84 mg, 0.312 mmol) and B\textsubscript{2}Pin\textsubscript{2} (2644 mg, 0.1040 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\textsubscript{2}Pin\textsubscript{2} mixture solution and then microwave heating at 80 °C 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 %); R\textsubscript{f} 0.56 (30 % Hexane/Acetone); ν\textsubscript{max} 2978, 1716, 1587, 1451, 1373, 1317, 1245, 1205, 1187, 1132, 1064, 988, 968, 825, 815, 767, 738, 695, 662, 620 cm\textsuperscript{-1}; δ\textsubscript{H} (500 MHz, CDCl\textsubscript{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, OC\textsubscript{H}\textsubscript{3}), 1.37 (12H, s, (C(CH\textsubscript{3})\textsubscript{2}); δ\textsubscript{C} (176 MHz, CDCl\textsubscript{3}) 180.8 (q, \textsuperscript{2}J\textsubscript{CF} = 25.1 Hz, CCF\textsubscript{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, \textsuperscript{1}J\textsubscript{CF} = 291.3 Hz, CF\textsubscript{3}), 84.67 (C-4’’ and C-5’’), 55.86 (OCH\textsubscript{3}), 25.08 (CCH\textsubscript{3}); δ\textsubscript{F} (376 MHz, CDCl\textsubscript{3}) -71.197 ppm; m/z (ES\textsuperscript{-}) 330 [M-H]\textsuperscript{-}.
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$_2$CO$_3$ (2 mL) were reflux for 5 h. After cooling to rt, partitioned between EtOAc and water. The organic layer was dried over Mg$_2$SO$_4$, 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 ºC; R$_f$ 0.56 (20 % Hexane/EtOAc); $\nu_{max}$ 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$^{-1}$; $\delta_{H}$ (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, 3-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$_3$); $\delta_{C}$ (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$_2$N$_2$O requires M$^+$ 502.2143, found [M-H]$^-$ 502.2186.
5 REFERENCES


199-204.


45 Bayley, H. Photogenerated Reagents in Biochemistry and Molecular Biology,


