Fluorescent Phosphonium Salts as Medical Imaging Agents in Cells

An investigation into the synthesis of fluorescent bodipy phosphonium salts to be used as medical imaging agents within cells to identify mitochondrial dysfunction associated with cancer, Alzheimer's & Parkinson's disease

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Why bodipy phosphonium salts? They are fluorescent and highly selective for mitochondria

Bodipy phosphonium salts typically exhibit **fluorescent** properties due to the fact that they absorb and emit photons i.e. particles of energy. The energy of the photons emitted is in the visible region, allowing these compounds to be used as fluorescent probes i.e. medical imaging agents within cells. These probes can be taken up by cells and observed using a technique called fluorescence microscopy.

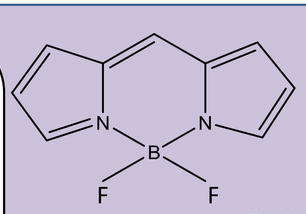
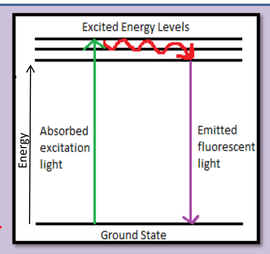


Fig 1.

Fig 2.



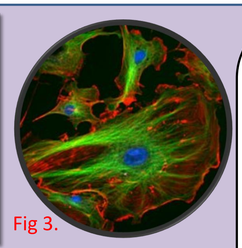


Fig 3.

Fig 1. The bodipy fluorophore which allows the probe to be fluorescent. Fig 2. A diagram illustrating how the energy of the photon absorbed by the probe is greater than that of the photon emitted, resulting in fluorescence. Fig 3. These fluorescent properties allow imaging within cells through fluorescence microscopy, as shown for an endothelial cell.

The phosphonium cation, which has a positive charge, is attracted to the negatively charged outer membranes of the mitochondria which are present in cells, allowing the probe to pass through. This enables the probe to be highly **selective** for the mitochondria, preventing it from interacting undesirably with other targets. Dysfunctional mitochondria are associated with diseases such as Parkinson's, Alzheimer's and cancer, which result in a change in the mitochondrial membrane potential. This affects the ability of the probe to pass through the membrane, resulting in less accumulation of the probe in the cell. Thus the probe's ability to enter the cell can indicate the presence of these diseases.

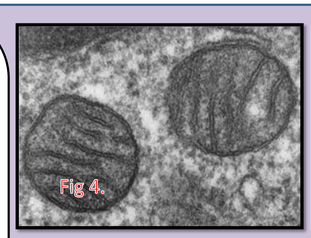


Fig 4.

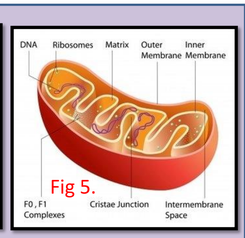


Fig 5.

Fig 4. Mitochondria within a cell shown under electron microscopy. Fig 5[1]. Structural features in a mitochondrion. The outer membrane is negatively charged allowing the positively charged probe to pass through it.

Synthesising the primary phosphine – a precursor to phosphonium salts

Fig 6.

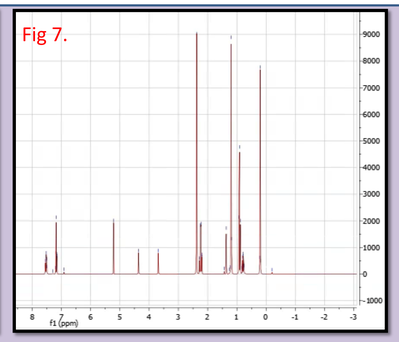


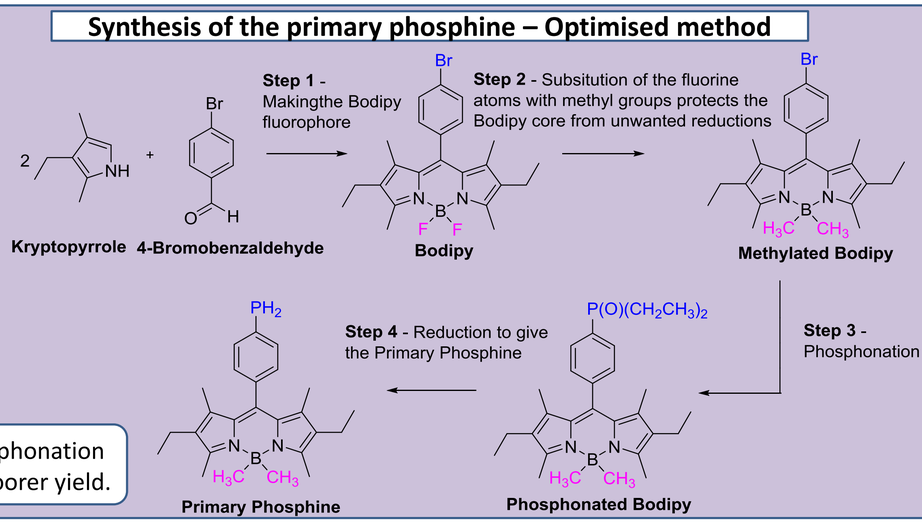
Fig 7.

Fig 8.

Fig 6. The primary phosphine. Fig 7. A ^{1}H NMR spectrum providing proof that the primary phosphine was made. Fig 8[2]. Bodipy in solution shown under a UV lamp.

Another method of synthesising the primary phosphine involves performing the phosphonation before the methylation. However, this reaction was found to take longer and gave a poorer yield.

Synthesis of the primary phosphine – Optimised method



A cheaper alternative – Because Kryptopyrrole is an expensive starting material, Step 1 was also attempted using pyrrole as a more cost effective option. The bodipy product exhibited less fluorescence when pyrrole was used and had a lower **fluorescence quantum yield** i.e. the ratio of the number of photons emitted compared to the number absorbed was lower.

Fig 9. Emission spectrum showing how the fluorescence intensity of the bodipy product is greater when kryptopyrrole is used as a starting material [red] as opposed to pyrrole [blue].

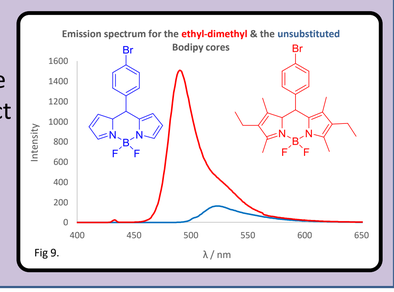


Fig 9.

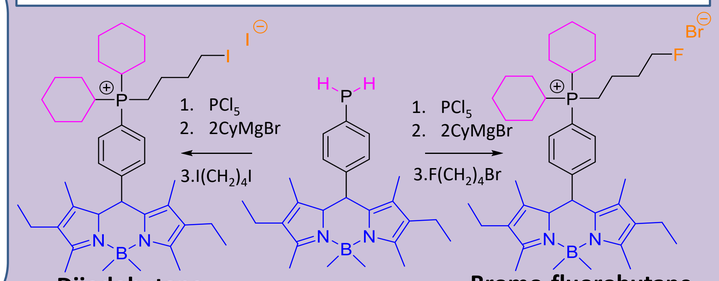
Synthesising phosphonium salts – Medical Imaging Agents to identify mitochondrial dysfunction

Fig 10.

Fig.10 A Schlenk line provides an inert atmosphere.

All reactions were carried out under an inert nitrogen gas atmosphere using a Schlenk line to prevent unwanted oxidation of the product. The primary phosphine was found to be air-stable in the solid state and in chloroform. It could also be functionalised to give phosphonium salts to be used as probes.

The primary phosphine can react to give phosphonium probes



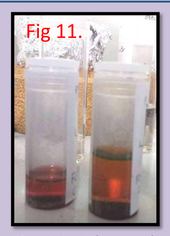


Fig 11.

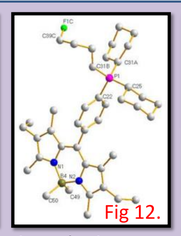
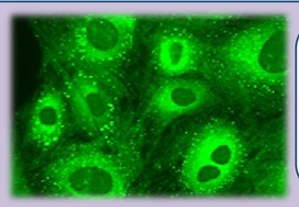


Fig 12.

Fig 11. The salts in solution. Fig 12. Crystal structure of the bromo-fluorobutane probe determined by X-ray crystallography.



These salts will be sent to Wang lab at the University of California in San Diego to be tested as probes for use in cells [Left][3] for medical imaging as a non-invasive technique for identifying mitochondrial dysfunction.

References & Acknowledgements

1. *Structure of mitochondria*, available at http://biology.tutorvista.com/animal-and-plant-cells/mitochondria.html
2. J.G Felber, *Synthesis of fluorescent-labelled Rhodium and Iridium complexes with application in medicinal diagnostics and catalysis*, 2017, Master Report, Newcastle University
3. Dr Lee J. Higham, *LJH Research Group*, available at http://leejohnhighamresearch.co.uk/