Synthesis of Fluorescent Phosphonium Salts for Mitochondrial Imaging

-This project is a dive into the ongoing investigation of phosphonium salts acting as medical agents to highlight mitochondrial disfunction involved in certain diseases such as Cancer, Alzheimer's and Parkinson's.

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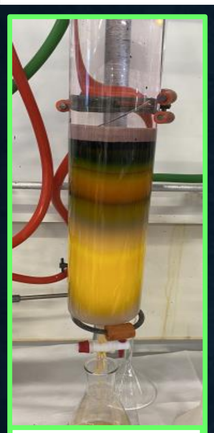
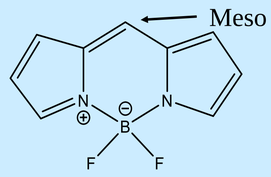


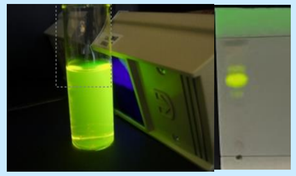
(Fig 7) Large Column chromatography displaying the colours present when separating BODIPY compounds.

In life and death, mitochondria play an important role as the battery of the human cell controlling the release of energy for metabolic reactions, or the natural breakdown of cells (apoptosis) with regards to aging.[1] The regulation of mitochondria relies on certain proteins (PHB) that participate in function.[2] They have multiple roles and functions and with that, they are a target for many therapeutic approaches for instance the three-parent baby technology pioneered at Newcastle University. One exciting strategy for response to a drug is to establish how the mitochondria respond to a given treatment and for this we first need to assess whether a mitochondria is performing correctly.
Mitochondria have a negatively charged membrane containing charged groups/channels influencing the transference of ions.[3] Which is why a positive phosphonium salt can be used to specifically target them. Malfunctioned mitochondria, which are present in many diseases, have their membrane potential is altered and thus we observe a change in cellular uptake of the phosphonium cation which can indicate the presence of the disease. Existing strategy for this approach uses triaryl-based phosphonium salts which cannot be visualised easily and their accumulation in the organelle must be measured by other means. This project aims instead to make fluorescent P-salt analogues.

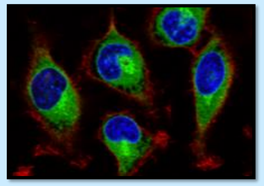
Within this project, we aim to develop medical probes with fluorescent characteristics to again specifically target mitochondria but also allow easy visualisation of its uptake. BODIPY compounds (left) are commonly used for optical imaging, because they have very useful fluorescent properties and are highly stable molecules due to extensive conjugation. The Meso position indicated on the BODIPY is ideal for incorporating groups with a different purpose without altering the fluorescence significantly and this is the location for the phosphorus functionality that will target the mitochondria.



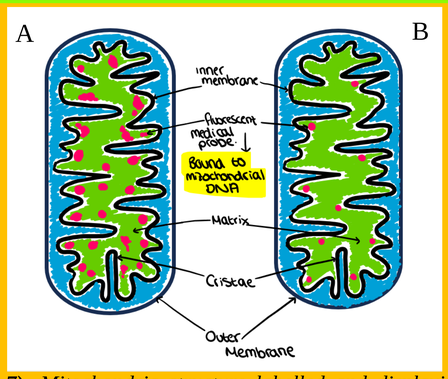
(Fig 4) *BODIPY Molecule, stable, conjugated, cyclic compound. Indicating the meso position, the target for the phosphorus substituent.*



(Fig 5) *BODIPY compound under UV light as solution and through TLC Plate.*



(Fig 6) *Visual imaging of BODIPY uptake into cells.*

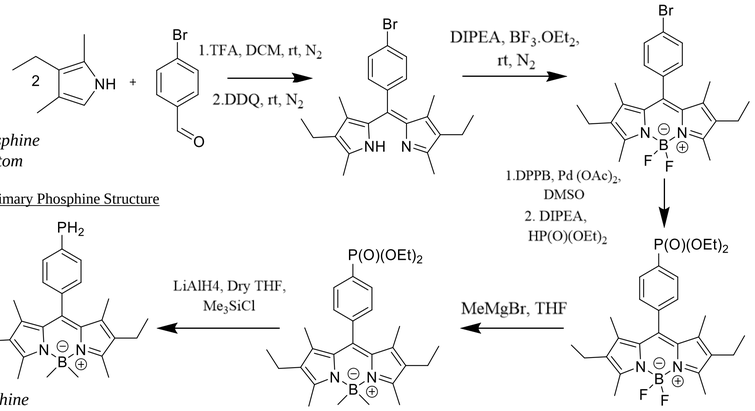


(Fig 7)- *Mitochondria structure labelled and displaying two different uptakes of the fluorescent probe. A displays a normally functioning mitochondria, where B displays one with a malfunction.*

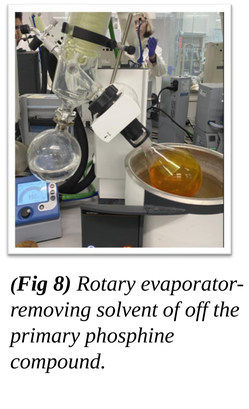
The Synthesis of our Primary Phosphine

(Fig 9) *The formed primary phosphine before extraction from round bottom flask.*

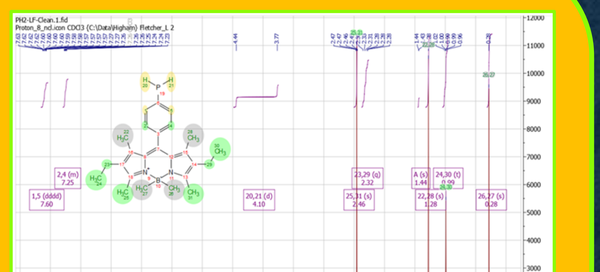
(Fig 10) *Crude Primary Phosphine within sample vial.*



Primary Phosphine Structure



(Fig 8) *Rotary evaporator-removing solvent of off the primary phosphine compound.*



(Fig 11) *displays a proton NMR, proving the presence of the desired primary phosphine whilst displaying any impurities within the sample.*

Synthesis of Phosphonium Salts

Following the route 'A' you can see has lower temperature requirements. This is due to the greater positive delta positive carbon on the Iodomethane which allows for a lower activation energy for the substitution. But following route B has cause for higher temperature conditions. The extended alkane chain provides an electron donating environment, lessening the delta positive carbon-I. Thus, increasing the energy requirements for substitution of iodine for the phosphine lone pair and requiring a solvent with a higher boiling point to meet the temperature requirements. Reaction B was ongoing for the final stage of my project. The formation of product within the first 3 days was deemed insufficient and therefore was left for a longer period. Both novel molecules were successfully synthesised.

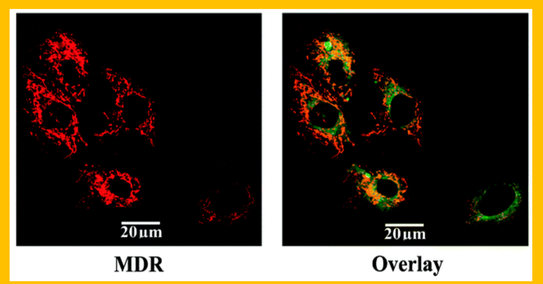
(Fig 12) *Novel Molecule 1 under UV*

(Fig 13) *Reaction set up for the formation of the phosphonium cation under nitrogen.*

Novel Molecule 1

Novel Molecule 2

Both MDR and the BODIPY compound showed similar capabilities for mitochondria localisation as dyes, but BODIPY showed higher coefficient values for uptake, which indicated with comparison a greater potential as an imaging agent.[3] Once the phosphonium salts were explored within membrane dependency, considerations for radiolabelling for future PET Imaging agents via substitution began.



(Fig 14) *(Deep Red) Mito tracker, alongside its overlaid image with fluorescent BODIPY derivative compound.*

CONCLUSION

Within the exploration of this project, information regarding the mitochondrial membrane potential, its dependencies/ required structural features necessary for mitochondrial uptake where acquired, fundamentally furthering our knowledge for optimisation. We aim to develop the medical imaging agent world to create multi-modal imaging agents by combining radiolabelling and localisation to gain insight about cellular/organ health and condition. By introducing radiolabelling, we create a PET active, fluorescent, mitochondria specific multi-modal imaging probe.

Acknowledgements

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REFERENCES

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