Investigating the use of a fluorescent dye for the detection and diagnosis of disease

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An investigation into the use of a fluorescent dye (BODIPY) as the backbone of fluorescent phosphonium salts which can be used to detect mitochondrial dysfunction and thus provide an early diagnosis into diseases such as Alzheimer's and Parkinson's.

Fluorescent phosphonium salts

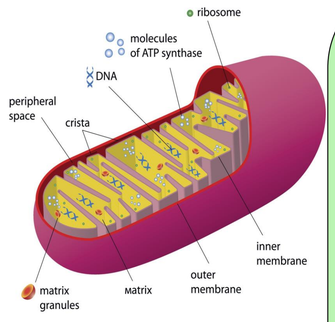

Figure 1: Labelled diagram of a mitochondrion, showing the negatively charged inner membrane.

Phosphonium salts, PR_4^+ (the phosphorus analogues of ammonium salts) can be incorporated into probes which are able to enter the body intravenously. Most cells in the body contain mitochondria which provide energy for the cells. When mitochondrial function is normal, the negatively charged membrane is attracted to the positively charged phosphonium salt. This causes an accumulation of the probe within the mitochondrial membrane, which can be detected by an imaging technique such as PET (positron-emission tomography). This is possible due to the fluorescent dye (BODIPY) being the backbone of the salt. Therefore, in a healthy mitochondrion, fluorescence would be detected by the imaging technique.

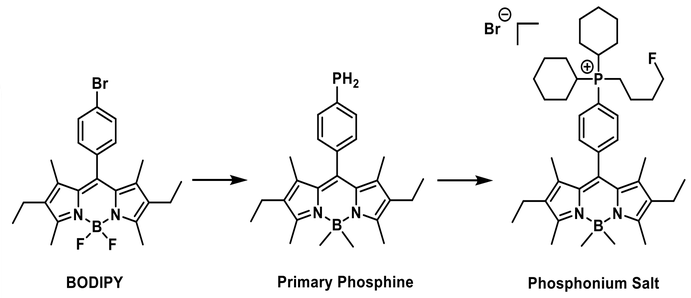

Figure 2: General reaction scheme showing the synthesis of a phosphonium salt starting from the fluorescent dye, BODIPY. As you can see the basic backbone of the structure does not change, it is only the groups attached that differ. This allows the final Phosphonium salt to maintain similar fluorescent properties to the original dye.

If mitochondrial function is abnormal, due to cell apoptosis for instance, the charge balance between the phosphonium salt and the mitochondrial membrane would be disrupted. Thus, the probe would no longer accumulate within the mitochondrion, leading to a decrease in fluorescence intensity being observed by medical imaging tools. The use of fluorescent probes could allow for early detection of diseases which are caused by or involve mitochondrial dysfunction. Alzheimer's and Parkinson's are both examples of diseases that impair or destroy nerve cells within the brain, which would lead to mitochondrial dysfunction. Sufferers of these diseases would benefit from the use of fluorescent probes as it would allow early detection of both diseases without the use of invasive surgery. Early detection could also lead to more successful treatment. Further experimental research could also lead to developments within cancer research, as the use of fluorescent probes could allow doctors to accurately locate tumours, and so provide more precise and effective treatment.

Benefits of the incorporation of BODIPY into a primary phosphine

BODIPY (shorthand for 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) is a fluorescent dye which comprises the backbone of both the primary phosphine and the phosphonium salt. Incorporation of this dye into the probe confers fluorescence properties and allows it to be used as an imaging agent. BODIPY-based compounds are known as fluorophores which are simply a type of fluorescent chemical compound which can re-emit light upon light excitation.

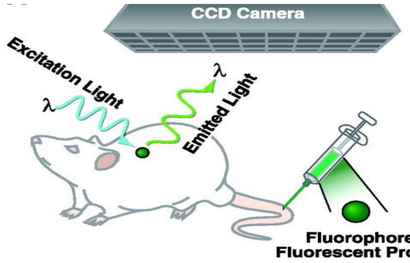

Figure 3: Diagram illustrating how a fluorophore or a probe containing a fluorophore can be injected into an organism, migrate through the body and be detected using an imaging agent (CCD camera in this case). A CCD camera is a charged coupled device which detects movement of electrical charge.

A primary phosphine is used as the starting material to prepare the phosphonium salt. Previously, all primary phosphines were thought to be highly air-sensitive and some are indeed flammable if exposed to air. It was recently proven by calculations performed by the Higham group that the incorporation of BODIPY into the primary phosphine confers air-stability, due to the highly conjugated structure of BODIPY. Experimental studies have shown that a sufficient amount of π-conjugation leads to an increase in air-stability.

Development of a primary phosphine

The main focus of this summer project was the synthesis of an air-stable primary phosphine starting with the synthesis of fluorescent dye BODIPY.

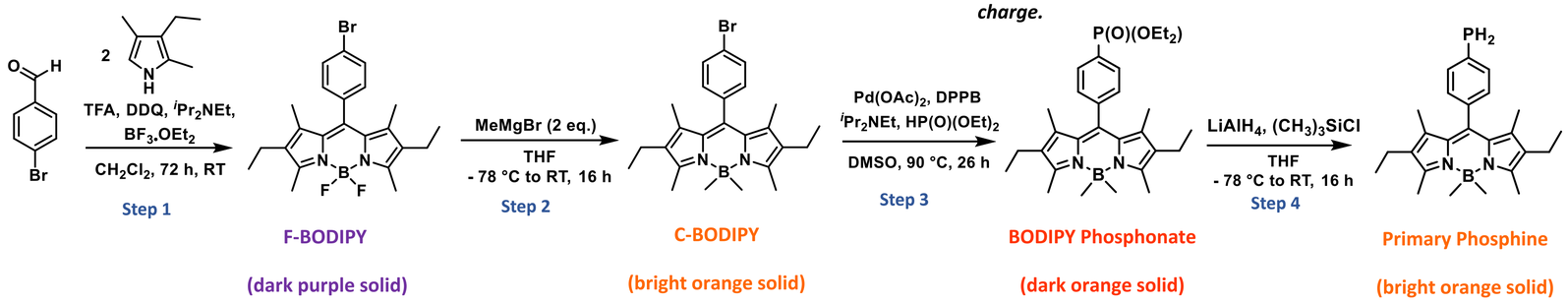

Figure 5: The reaction scheme used to synthesise the primary phosphine. Step 1 indicates the synthesis of the fluorescent starting material F-BODIPY using bromobenzaldehyde, 2 equivalents of 3-ethyl-2,4-dimethylpyrrole, trifluoroacetic acid (TFA), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), boron trifluororide diethyl etherate and N,N-diisopropylethylamine ($^iPr_2NEt$). The resulting product was methylated using methyl magnesium bromide at room temperature in step 2 to produce C-BODIPY. This step was necessary as without it, the boron-fluorine bond would be attacked by the reducing agent in step 4. Step 3 introduces phosphorus onto the BODIPY structure using 1,4-bis(diphenylphosphino)butane (DPPB), N,N-diisopropylamine ($^iPr_2NEt$), diethyl phosphite and palladium acetate which acted as a catalyst. The mixture was stirred for 26 hours at 90 °C, and the resulting phosphonate was reduced to the primary phosphine in step 4 using lithium aluminium hydride and chlorotrimethylsilane ($(CH_3)_3SiBr$).

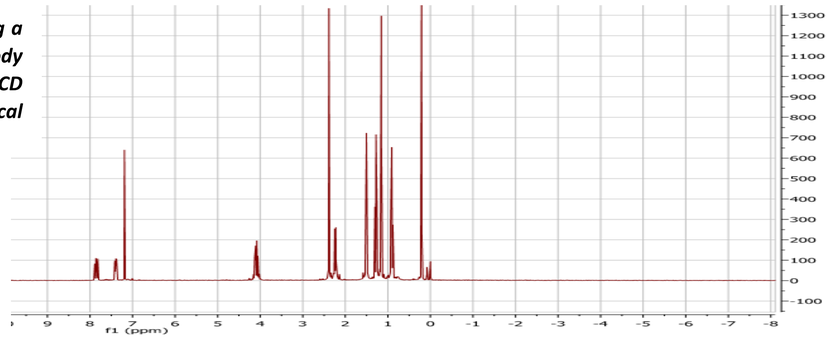

Figure 4: ^1H NMR (Nuclear magnetic resonance) spectrum taken of the phosphonate (product of step 3) synthesised during the 8 week project. This particular spectrum indicates that this product contains very little impurity as it only displays the peaks assigned to the phosphonate. Extra peaks on the spectrum are a sign of an impure product.

References: James and S. Gambhir (2012) A Molecular Imaging Primer: Modalities, Imaging Agents, and Applications. Physiological reviews.92 (2),897-965.

L. H. Davies (2013). 'Air-stable Fluorescent Primary Phosphines and their Potential Applications as Precursors for Disease Imaging Agents', PHD thesis, Newcastle University.

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