Title: Synthesis and chemical validation of small molecule probes to detect and manipulate species involved in redox signalling.

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Reactive oxygen species play an important physiological role and, at the same time, can have toxic effects towards the human body. Within the cell, mitochondria are the main producers of ROS, being the main source of mitochondrial ROS the respiratory chain. MitoParaquat was developed to allow a further study of how mitochondrial ROS acts within our body, as it accumulates within the mitochondrial matrix and accepts an electron from the flavin site of complex 1, reacting rapidly with molecular oxygen to generate superoxide.

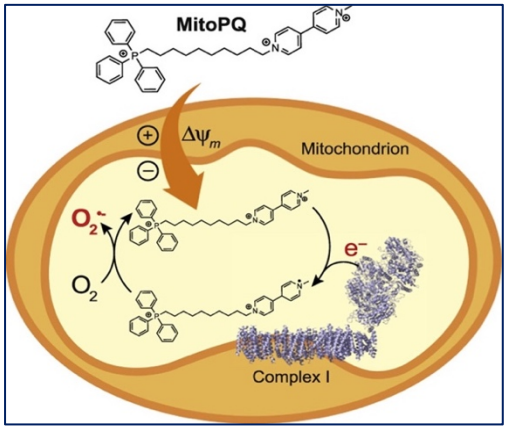
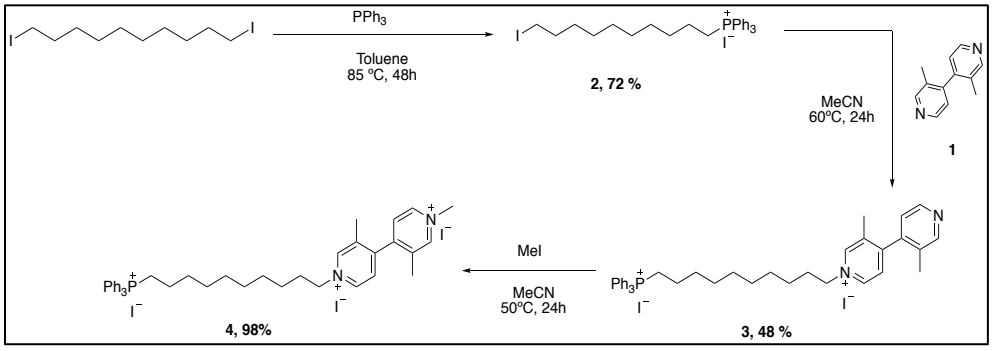


Figure 1. MitoParaquat generation of superoxide within mitochondria.

Due to the success of MitoPQ in the study of superoxide production within mitochondria, it has become necessary to synthetize a control compound. This control should act as MitoPQ, entering the mitochondrial matrix and accumulating within it, but it should not produce superoxide once inside.

Within the Hartley group, they managed to synthesize a new control compound (Scheme 1, compound 4). However, they faced a problem when it came to synthesize the 3,3'-dimethyl-4,4'-bipyridine (Scheme 1, compound 1), as they only got a 6 % yield.



Scheme 1. Synthesis of MitoParaquat control.

The aim of my project was to improve the synthesis of the bipyridine, which was improved up to a 38 %. MitoParaquat control 4 was also synthetized yielding 98%.

The second part of my project was to synthesize a new fluorescent probe that allows us to detect superoxide O_2^- within the mitochondrial matrix. The Hartley group had previously developed MitoNeoD (Figure 2), a phenanthridinium-based $O_2^{\cdot -}$ probe that reacts with superoxide inside the mitochondria to give a specific 2-hidroxyphenanthridinium product.

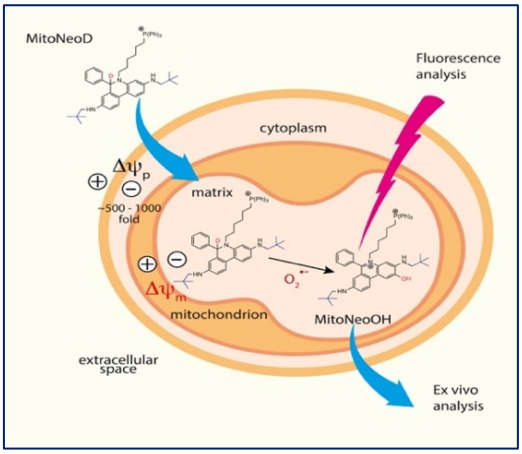
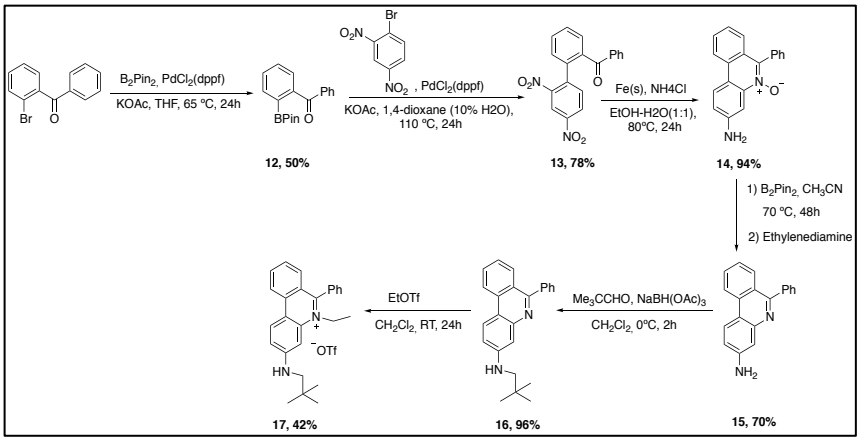


Figure 2. MitoNeoD uptake by mitochondria and reaction with O_2^-.

HaloTag technology will be used to detect superoxide through this new fluorescent probe. HaloTag will form a covalent bond with the HaloTag ligand, which will have the fluorescent probe attached. Its detection is possible because when the fluorophore is in contact with the HaloTag protein present in the mitochondria, it expresses fluorescence, thus being able to be detected within the organelle.

Following the synthetic route of Scheme 2, the new fluorescent probe was synthesized with a 42 % yield.

To be able to see if the phenanthridinium salt 17 had a significant fluorescence to be used in the future as a fluorescent probe, a fluorescence assay was conducted comparing the new probe with MitoNeo, a fluorescent probe previously used within the group.
The spectra proved that MitoNeo had a more intense fluorescence than the new fluorescent probe 17.



Scheme 2. Synthesis of a new mitochondria-targeted superoxide probe.

As a conclusion, the synthesis of 3,3'-dimethyl-4,4'-bipyridine was improved to a 38 %, meaning that a further study of the followed reactions could possibly lead to a better understanding and improvement of the synthesis. Another possible way of improving the synthesis of 3,3'-dimethyl-4,4'-bipyridine could be using a Suzuki coupling of two halide-substituted pyridines.

In the other part of the project, a new fluorescent probe for the detection of superoxide was synthetized, even though the previous fluorescent probe MitoNeo has a better fluorescence. Despite not having improved the fluorescence, the new fluorescent compound 17 has a different excitation wavelength from MitoNeo, thus making it possible to use both at the same time to carry out different studies.