# Identifying mechanisms of photocapture of proteins

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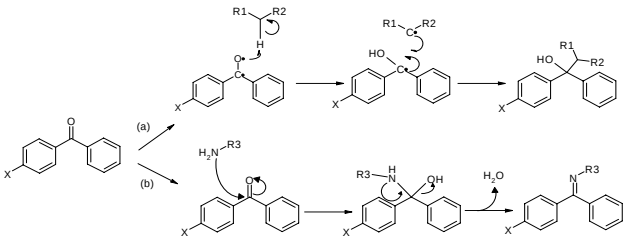
## 1. Introduction

The aim of the research project was to identify a mechanism (the path a reaction takes) for the photocapture of proteins using benzophenones. There were two possible mechanisms that the reaction could follow:

- A radical mechanism
- An imine forming mechanism




A selection of benzophenones (Fig., left) were reacted with a selection of peptides. 'X' represents different groups for each of the different benzophenones.

Pathway (a) is the radical mechanism.

Pathway (b) is the imine-forming mechanism

The main difference between the products is that the imine-forming mechanism releases water (last step) and therefore is eighteen mass units lighter than the radical mechanism.

## 2. Approach taken

•Solubility of the peptides and benzophenones was assessed by taking a portion of each in different solvents. A methanol : water mix was found to be optimal.

•Reactions were carried out in daylight for five hours at 2M, then diluted to a concentration of 500nM for mass spectroscopic analysis. Mass spectroscopy is a very sensitive method that allows us to identify the products of the reaction.

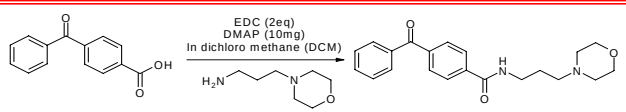
•The radical forming mechanism (a) gives a product with the combined mass of the starting materials.

•The imine product from (b) would be eighteen mass units less due to the loss of water.

• From previous work in the Marsh group, it was suggested that amides benzophenones may favour the radical-forming mechanism. In order to test this, several amides where prepared from the 4-benzyol benzophenone. Water solubility was also sought by using polar substituents (compounds **8** and **9**).

• It was possible that the presence of methanol in the solvent system was quenching the radical forming process and therefore favouring the imine forming mechanism. This was the main reason for attempting to prepare a water soluble derivative.

• In an attempt to avoid solubility problems, experiments were performed at much lower concentration to allow direct injection into the mass spectrometer. Hence only tiny amounts of co-solvent where required to keep the benzophenones in solution.



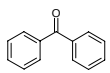

The synthesis of the 3-morpholino propylamine derivative of 4-benzyol benzophenone using the activating agent EDC. This particular reaction achieved a yield of 33%.

## 3. Results

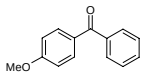
Imine product | Radical product | No reaction | Three ticks – large amounts of product | One tick – small amounts of product

* Could not analyse reaction due to solubility problems | Two ticks – medium amounts of products | Cross – no products detected

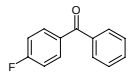
| Benzophenone | Octylamine | Dialanine | Glutathione | Aspartame |
|---|---|---|---|---|
| 1 | ✓✓✓ | ✗ | ✗ | ✗ |
| 2 | ✓✓ | ✗ | ✗ | ✗ |
| 3 | ✓✓✓ | ✗ | ✗ | ✗ |
| 4 | ✗ | ✗ | ✓ | ✓ |
| 5 | ✓✓✓ | ✓✓✓ | ✗ | ✓✓ |
| 6 | ✓ | * | * | * |
| 7 | ✓ | ✓✓✓ | ✗ | ✗ |
| 8 | ✓ | ✗ | ✗ | ✗ |
| 9 | ✓ | ✓ | ✗ | ✓✓ |



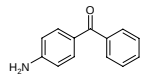
**1** Benzophenone



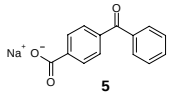
**2** 4-methoxy benzophenone



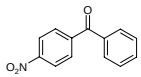
**3** 4-fluoro benzophenone



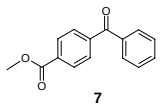
**4** 4-amino benzophenone



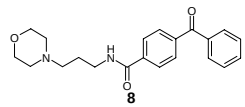
**5** 4-benzyol benzophenone sodium salt



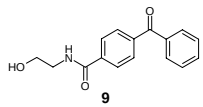
**6** 4-nitro benzophenone



**7** Methyl ester derivative



**8** 3-morpholino propyl-amine derivative



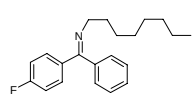
**9** Ethanolamine derivative

## 4. Conclusion

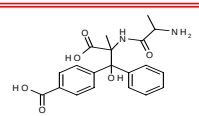
The results of my experiments shows that mostly, there was no reaction between the benzophenones and the peptides (represented as red cross above). This was apparent as there were no peaks present in the mass spectra's obtained that could correspond to a product being formed.

The reactions with octylamine showed that the main product was the imine and over half of them produced large amounts of the imine. The only benzophenone that gave a radical product with octylamine was the ethanolamine derivative. For the reactions with dialanine, a lot of the reactions did not occur. However, dialanine with 4-benzyol benzophenone gave large amount of the radical product. This experiment was repeated and the results confirmed. The ethanolamine and the methyl ester also gave small amounts of the radical product. All of the reactions with glutathione failed, except with 4-amino benzophenone in which a small amount of radical product was detected. Again, reactions with ethanolamine and aspartame produced radical product.

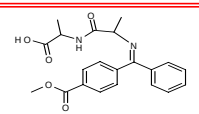
It was also shown that the presence of methanol in the reaction mixture can hinder and even stop radical formation. This was found out when dialanine and 4-benzyol benzophenone were reacted together again but with methanol present as well. In the absence of methanol, this reaction produced large amount of the radical product, however with methanol present this reaction did not occur and no radical product was detected.



Expected product from 4-fluoro benzophenone and octylamine



Expected product between 4-benzyol benzophenone dialanine



Expected imine product between the methyl ester and dialanine

## 5. Acknowledgments

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