Optimisation studies for the synthesis of coumarin photocages with biomedical applications

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1. Introduction

- ***What?*** To facilitate the controlled absorption of growth factors into stem cells with the aim of growing them into tissues for the treatment of tissue degeneration.
- ***Why?*** Finding accessible technologies to tailor and repurpose cells to our liking is essential to treat severe tissue damage or tissue degeneration so designing systems to grow cells to build custom tissues or even full organs could potentially save millions of lives.
- ***How?*** Using a series of molecules called photocages which act as a lid that block the entry of growth factors and that can be 'opened' and 'closed' with light.

2. *In vitro* application

A cell's destiny depends on specific proteins called **growth factors**, and the time at which they are fed to the cell colony and in what concentrations.

Stem cells would be embedded into the **hydrogel** which would act as a **3D scaffold** into which the cells and tissues are free to grow.

In order to 'feed' the growth factors into the hydrogel, we aim to use a series of molecules called **photocages** which act as a lid that block the entry of growth factors and can be 'opened' and 'closed' with light.

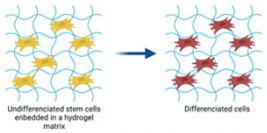


Figure 1. Stem cells embedded in the hydrogel matrix before and after irradiating with the selected wavelengths of light.

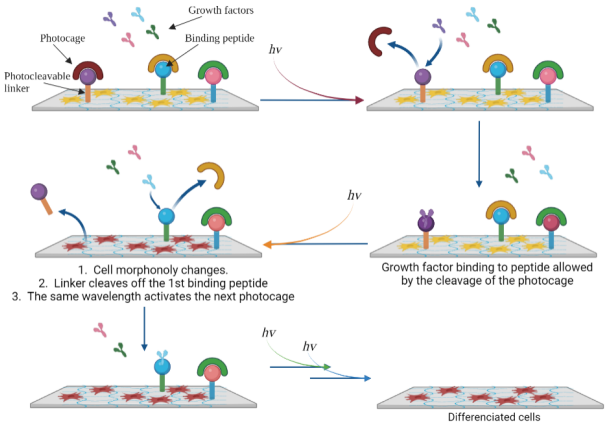
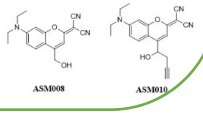


Figure 1b. Process mimicking biological cascades by which irradiating with a wavelength of light aids the binding of a growth factor to a peptide resulting in a change of the cell morphology

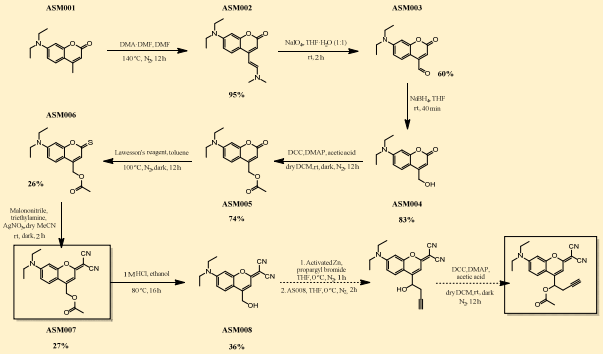
Two molecules were chosen for this study, these being **ASM008** (as the **photocage**) and **ASM010** (as a **photocleavable linker**) **(Figure 2).**

They were chosen due to their **responsiveness to light**.

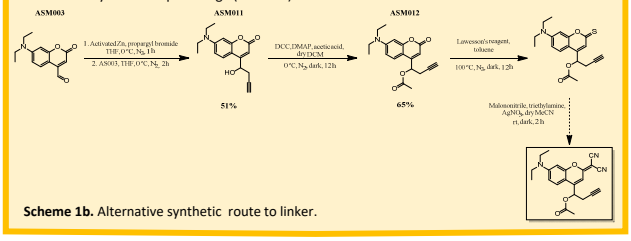


3. Chemical Synthesis

Two routes for the synthesis of the photocage (ASM008) and the photocleavable linker. The latter was not synthesised in during the research period however a route is proposed (dashed arrows).



Scheme 1a. Synthesis of photocage (ASM004) and linker.

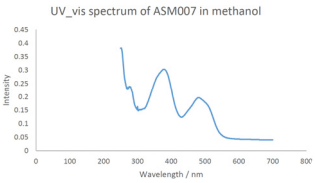


Scheme 1b. Alternative synthetic route to linker.

4. Uncaging studies

The photophysical properties of two synthesised compounds (ASM005 and ASM007) were studied to prove that these compounds could be uncaged with light.

The experiment (**Figure 2.**) involved irradiating a 5 mg of the compound dissolved in methanol with the maximum absorption wavelength (~350 nm) which was determined with UV_vis spectroscopic studies. **(Graph 1a and 1b)**



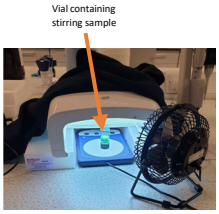
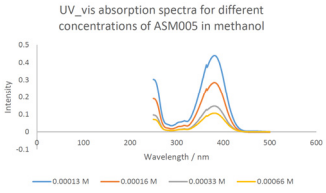
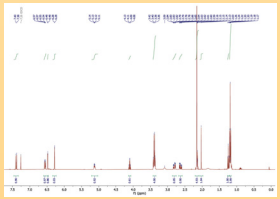


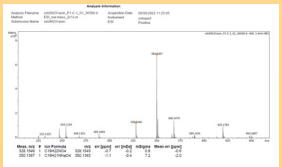
Figure 2. Set up for uncaging studies



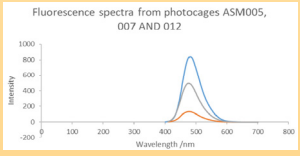
Graph 1a (top) Shows the UV_vis absorption spectrum of ASM005 in methanol. **2b (bottom)** Shows the UV_vis absorption spectrum of ASM007.

5. Results and Future Work





1. Characterisation of a novel compound, using 1H, 13C, HMBC, HSQC, DEPT-135 and COSY NMR, Mass Spectrometry



2. Recording of **fluorescence data** shows that the three caged coumarins (ASM005, ASM007 and ASM012) fluoresce at the **same wavelength**. This is expected as the resonance forms in these compounds is virtually the same.

3. A full **synthetic route** for the photocage was developed. Time did not allow for the complete synthesis of the linker but a route Is proposed **(Scheme 1a and 1b. Dashed arrows)**

4. LCMS results for ASM010 were inconclusive but this could be due to the impurity of the product. However, LCMS results for the uncaging experiment of ASM005 showed clear signs of uncaging.

6. Conclusions

- A novel photocage (ASM012) was synthesised and characterised.
- LCMS data for ASM005 showed proof of uncaging, meaning this family of compounds would be useful for this technology.
- Although the project was not completed in the research period, useful synthetic routes and conclusions can be drawn. For example, routes that involve malononitrile reactions earlier in the synthesis tend to have much lower yields. When possible, do this reaction the last.

Read full report:

Acknowledgements

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7. References

Broguiere, N.; Lüchtefeld, I.; Trachsel, L.; Mazunin, D.; Rizzo, R.; Bode, J.; Lutolf, M.; Zenobi-Wong, M. Morphogenesis Guided By 3D Patterning Of Growth Factors In Biological Matrices. *Advanced Materials* 2020, *32* (25), 1908299.