

# Synthesis and Investigations into the Cytotoxicity of Garlic-Inspired Benzyl Tri- and Tetrasulfides in Cancer Cells

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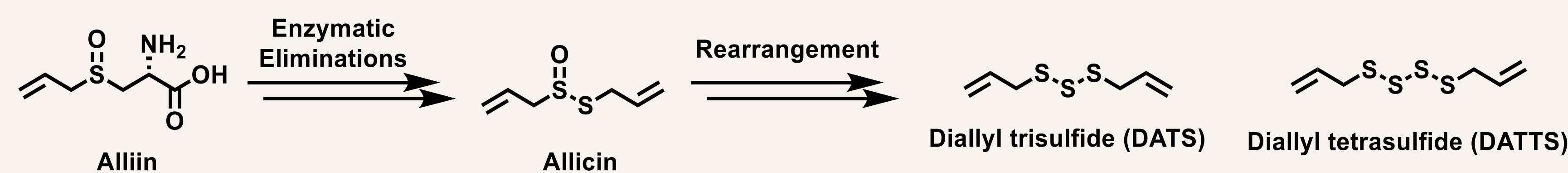
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## Introduction and Aims

*Allium sativum* (also known as garlic), contains more than 30 organosulfur compounds (OSCs) that are produced after crushing and heating of the clove. Many of these compounds possess anticancer activity.<sup>1</sup> The OSC of interest in this project are diallyl trisulfide (DATS) and diallyl tetrasulfide (DATTS), owing to their superior anticancer activity. Previous structure-activity relationship studies in our lab on DATS against WHCO1 cancer cells showed that analogues containing a benzyl side group are more active than the parent DATS. Benzyl groups modulated for their electronic effects were also investigated, and it was found that a methoxy *para*-substituted benzyl substituent was the most active, returning a nanomolar IC<sub>50</sub> value, approximately 200-fold more active than the parent DATS.<sup>2</sup>



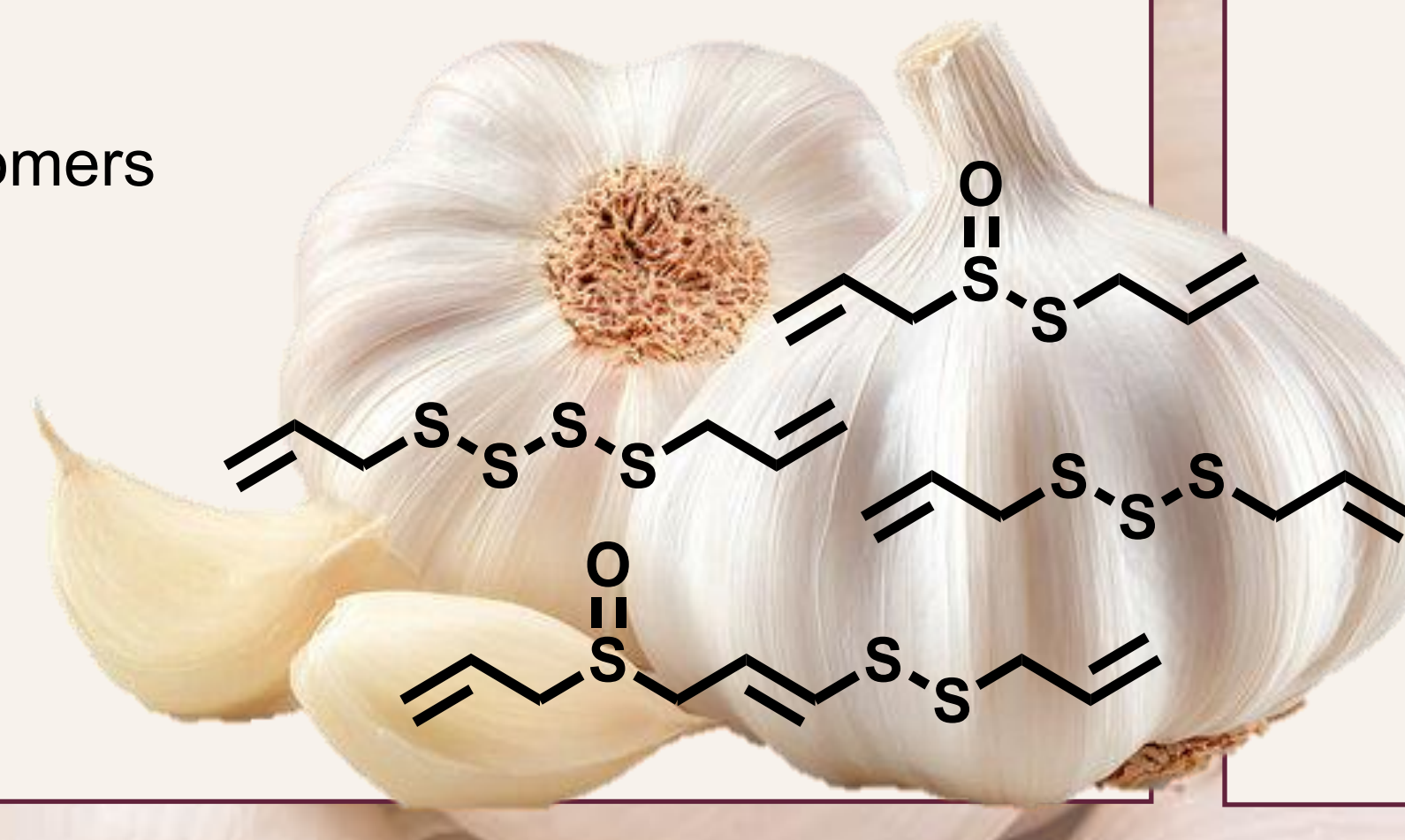
**Figure 1:** Generation of DATS and DATTS in garlic after crushing and heating.<sup>1</sup>

### AIM

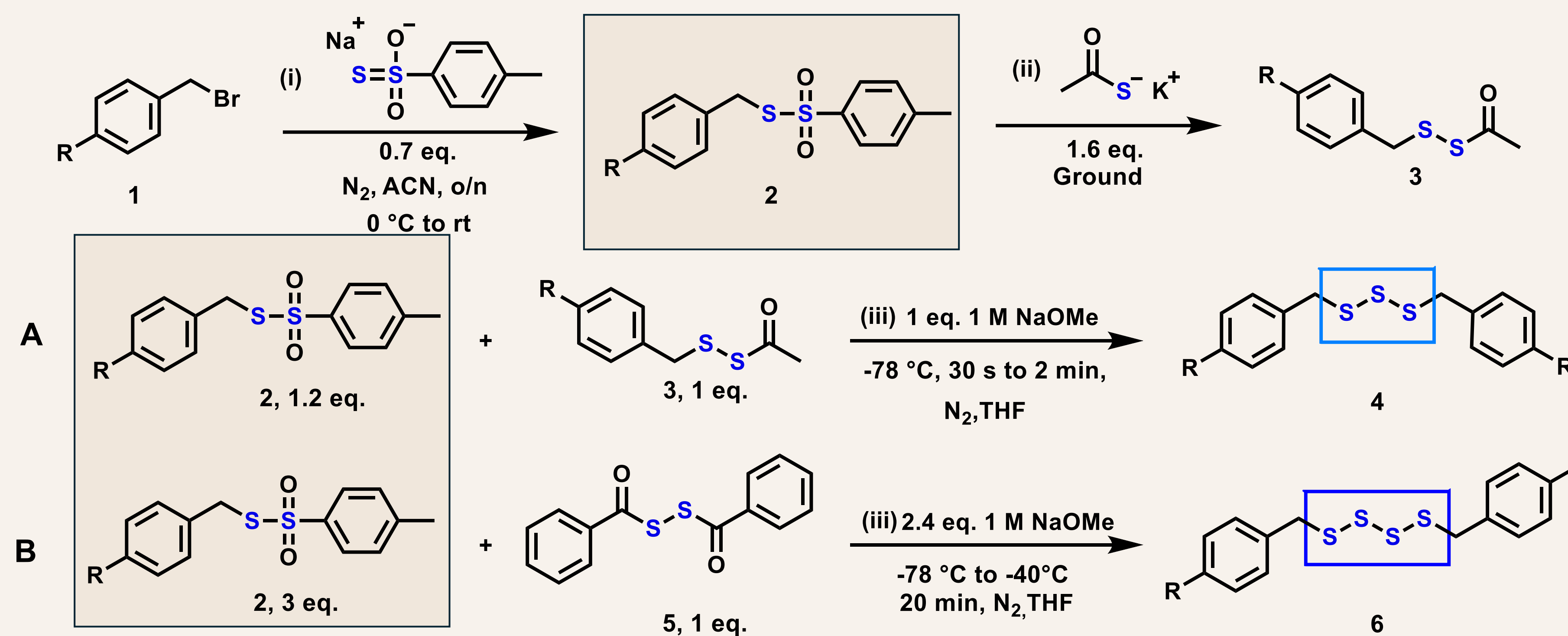
To further investigate other methoxy benzyl trisulfide regioisomers and to expand this library to include tetrasulfide analogues.

### RESEARCH QUESTION

What are the contributing roles of the modified S-thiolated proteins, perthiol generation and H<sub>2</sub>S release to the overall cell cytotoxicity of tri- and tetrasulfides in cancer cells?



## Synthesis – Via a Common Thiotosylate Intermediate

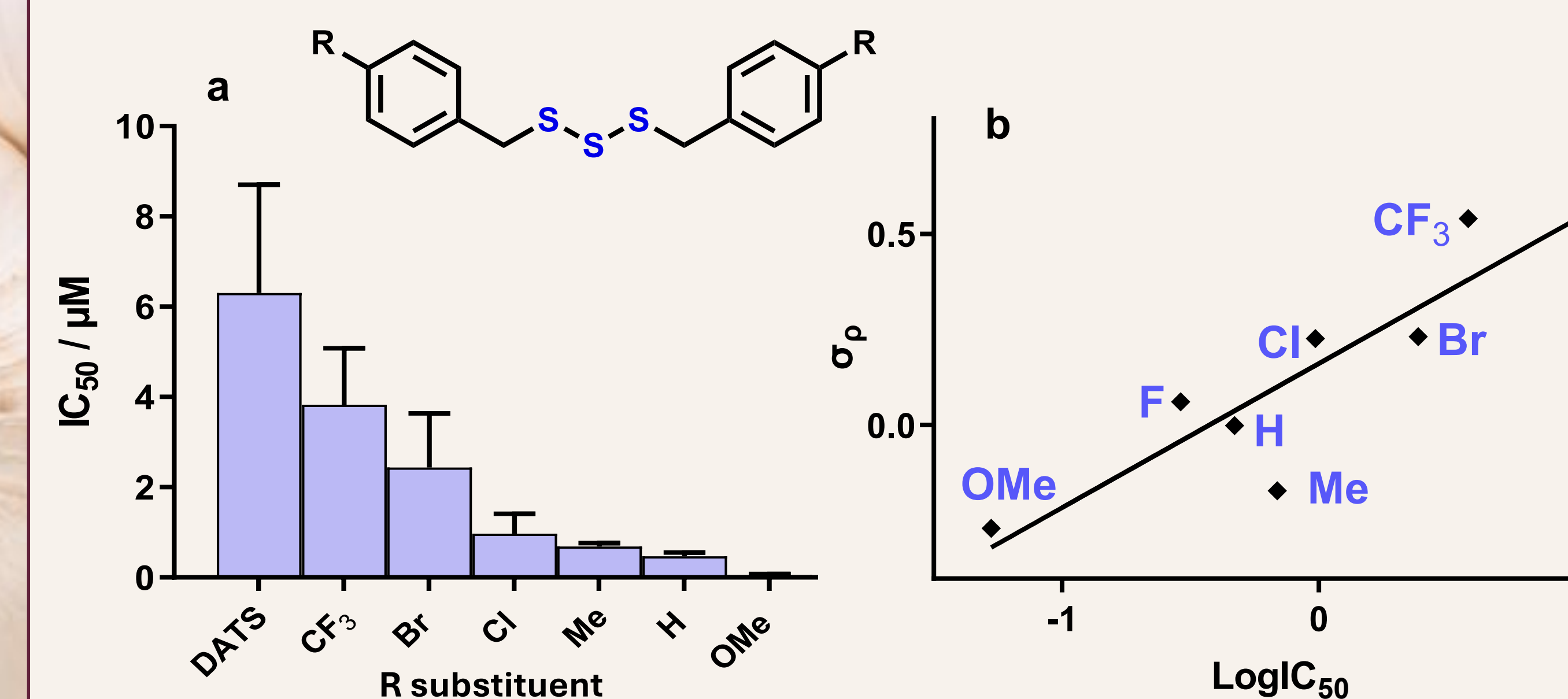


**Scheme 1:** The synthetic route to (A) the benzyl trisulfides and (B) the benzyl tetrasulfides via a common intermediate, **2**. Procedure B performed by Prof Roger Hunter at UCT. The key steps (iii) and (iv) involve low temperature deprotection of the disulfanyl acetate (**4**) or dibenzoyl disulfide (**5**) with sodium methoxide which is rapidly quenched with the thiotosylate (**2**) to generate the trisulfide in 30 s to 2 min, and 20 min for the tetrasulfides.<sup>3</sup> Characterisation: <sup>1</sup>H and <sup>13</sup>C NMR and HPLC. <sup>a</sup> Over two steps, <sup>b</sup> determined by rp-HPLC.

|                      | Yield  | Purity <sup>b</sup> |
|----------------------|--------|---------------------|
| <b>4<sup>a</sup></b> | 70-90% | 85-95%              |
| <b>6</b>             | 80-90% | 90-95%              |

**R = OMe, H, CH<sub>3</sub>, Br, CF<sub>3</sub>**  
**Other trisulfides include *o*OMe and *m*OMe**

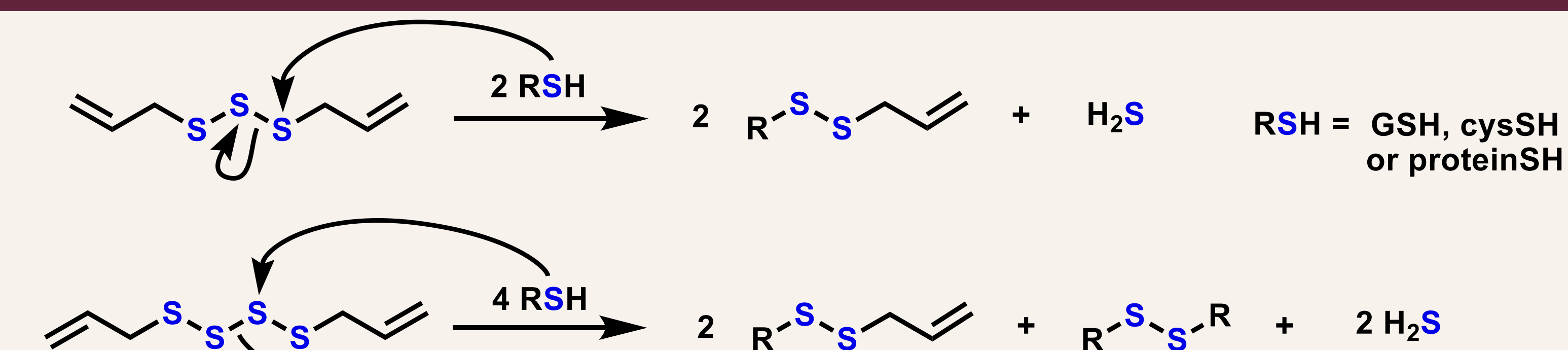
## Anticancer Effects in Trisulfides: The Effect of The Substituent on the Cytotoxicity



**Figure 2:** (a) Cancer cell cytotoxicity of *para*-substituted benzyl trisulfides against WHCO1 cancer cells and (b) the relationship between the cytotoxicity IC<sub>50</sub> and the *para*-Hammett constant of the substituent. <sup>2</sup>

It has been found that the structure-activity relationship of several symmetrical *para*-substituted benzyl trisulfides is modulated by the electronic effects of the substituent.<sup>2</sup> The data shows that ring activating groups, such as OMe, with a negative Hammett constant shows enhanced cytotoxicity towards WHCO1 cancer cells. Conversely, ring deactivating groups with positive Hammett constants, such as CF<sub>3</sub> and Br, showed reduced cytotoxicity.<sup>2</sup>

## Proposed Cytotoxic Mechanism of Action: is More Sulfur Really...More?



**Figure 3:** The proposed S-thiolysis cytotoxic mechanism of action of DATS (top) and DATTS (bottom, simplified) in cancer cells. A biological thiol (cysSH, GSH or proteinSH) attacks the terminal sulfur of the trisulfide moiety to produce two equivalents of a mixed disulfide (RSSallyl) and one equivalent of H<sub>2</sub>S.<sup>4</sup> DATTS, on the other hand, has the potential to produce two equivalents of H<sub>2</sub>S and RSSallyl and a disulfide following attack at the internal sulfur.<sup>5</sup>

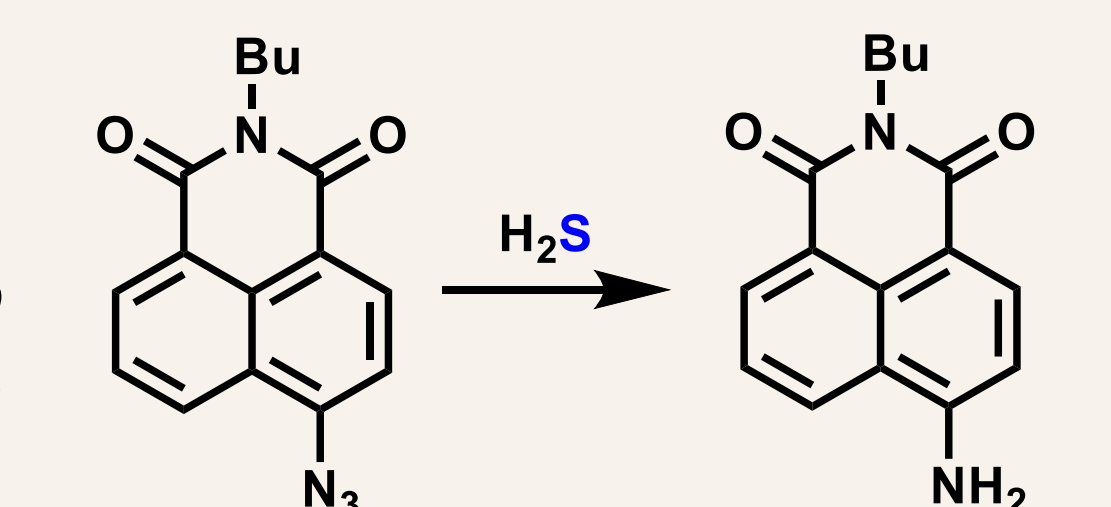
The mixed disulfide RSSallyl has been shown to exert cancer cell cytotoxicity by inducing ER stress, leading to the unfolded protein response (UPR).

H<sub>2</sub>S is a gasotransmitter and its anticancer effects in this context are not so clear.<sup>6</sup> Thus, the two equivalents of H<sub>2</sub>S released by the tetrasulfide moiety could influence the extent of cytotoxicity towards cancer cells.

## Future Work – Biological Investigations

Determine tetrasulfide IC<sub>50</sub> values.

Untangle the mechanism of action with *in vitro* investigations (**Figure 3**) using a probe to capture the H<sub>2</sub>S produced (**Figure 4**).



**Figure 4:** Nathalimide-based probe.

## References

1. D. De Greef and E. M. Barton, *et al.*, *Semin. Cancer Biol.*, 2021, **73**, 219–264.
2. Doaa Ali, PhD Thesis, University of Cape Town, 2024.
3. D. Ali., C. H. Kaschula and R. Hunter, *et al.*, *J. Org. Chem.*, 2019, **84**, 2862–2869.
4. K. G. Fosnacht, M. D. Pluth, *et al.*, *J. Am. Chem. Soc.*, 2024, **146**, 18689–18698.
5. U. Münchberg, C. Jacob, *et al.*, *Org. Biomol. Chem.*, 2007, **5**, 1505–1518.
6. A. Sufian, D. Bhattacharjee, *et al.*, *Chem. Commun.*, 2025, **61**, 4647–4661.