

## Supplementary Material

### Synthesis and evaluation of D-thioluciferin, a bioluminescent 6'-thio analog of D-luciferin

Marwaan Rylands and Anwar Jardine\*

*Department of Chemistry, University of Cape Town, Rondebosch 7701, Cape Town, South Africa*

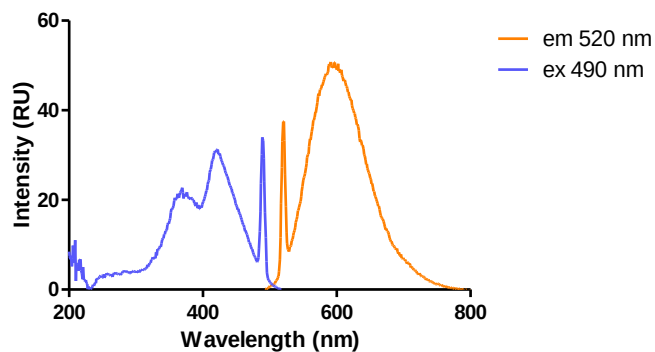
*Email: [anwar.jardine@uct.ac.za](mailto:anwar.jardine@uct.ac.za)*

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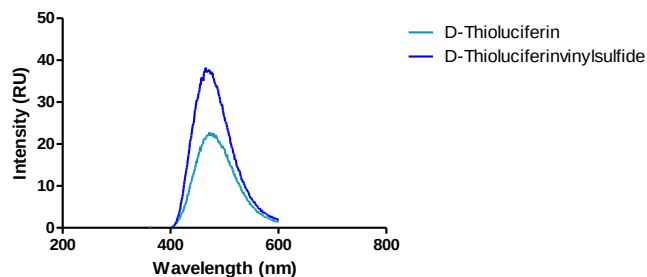
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## Experimental Procedures

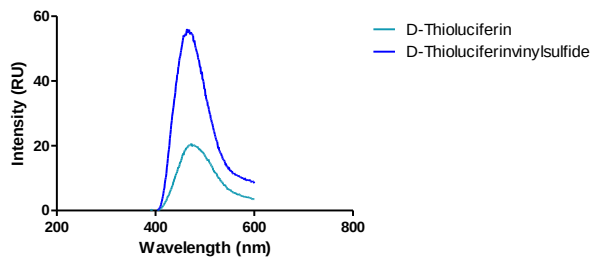
## Excitation/Emission Spectra of D-thioluciferin

S1. Excitation and emission spectra of D-thioluciferin (**3**)

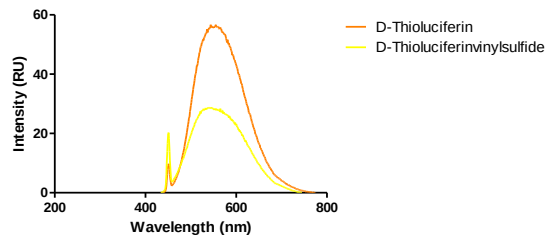
## Emission Spectra of D-Thioluciferin and D-Thioluciferinvinylsulfide at 360 nm

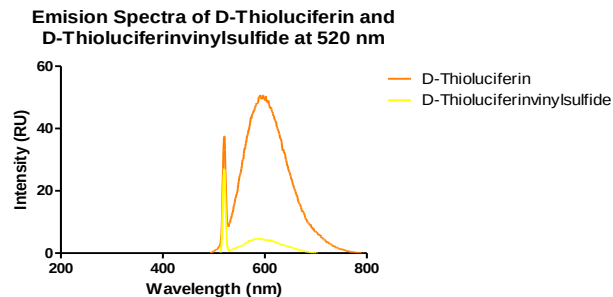
S2. Emission spectra of D-thioluciferin (**3**) and D-thioluciferin vinylsulfide (**IX**) at 360 nm

## Emission Spectra of D-Thioluciferin and D-Thioluciferinvinylsulfide at 390 nm

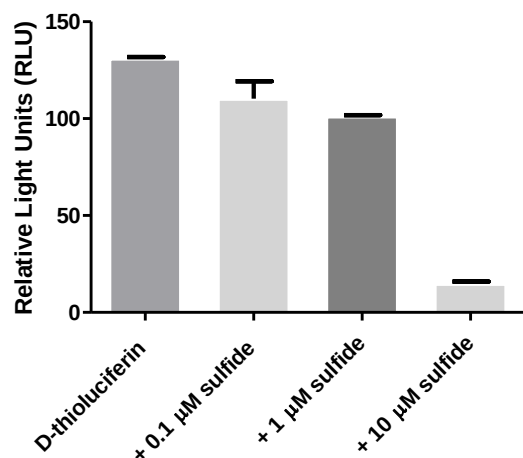
S3. Emission spectra of D-thioluciferin (**3**) and D-thioluciferin vinylsulfide (**IX**) at 390 nm

## Emission Spectra of D-Thioluciferin and D-Thioluciferinvinylsulfide at 450 nm

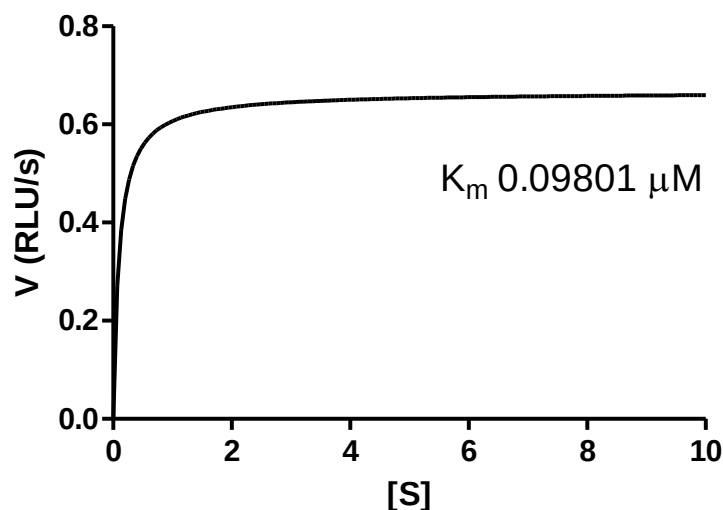
S4. Emission spectra of D-thioluciferin (**3**) and D-thioluciferin vinylsulfide (**IX**) at 450 nm



S5. Emission spectra of D-thioluciferin (**3**) and D-thioluciferin vinylsulfide (**IX**) at 520 nm



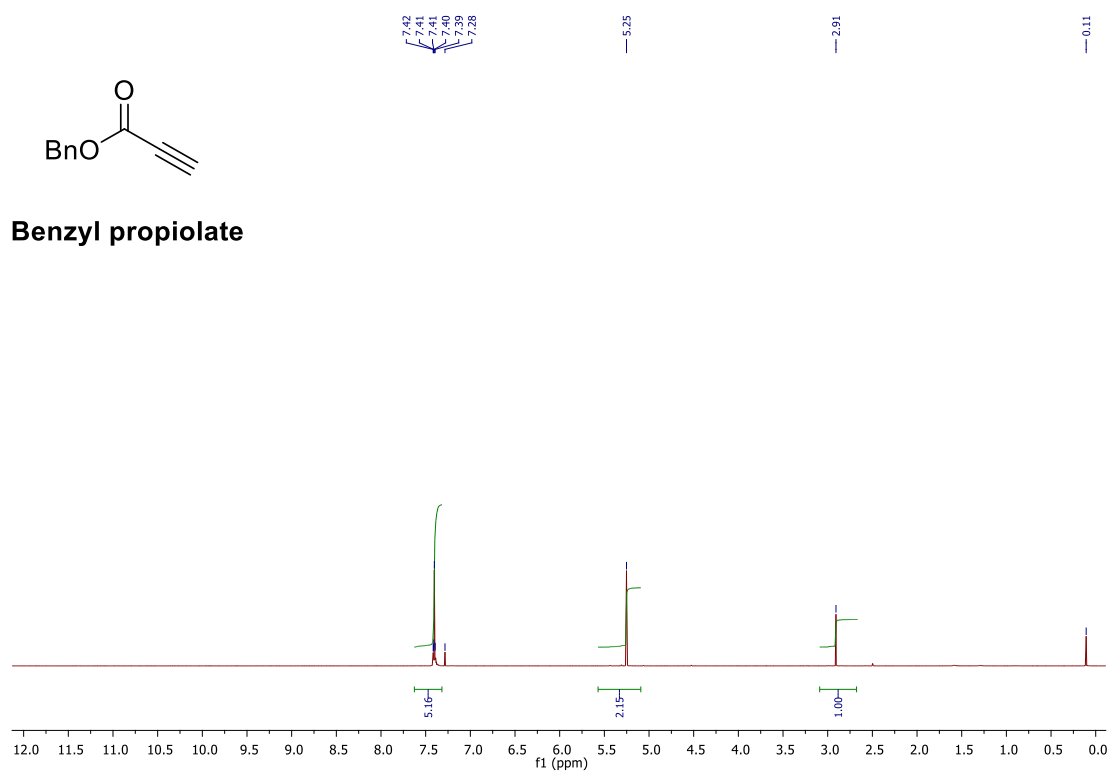
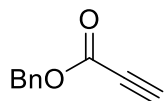
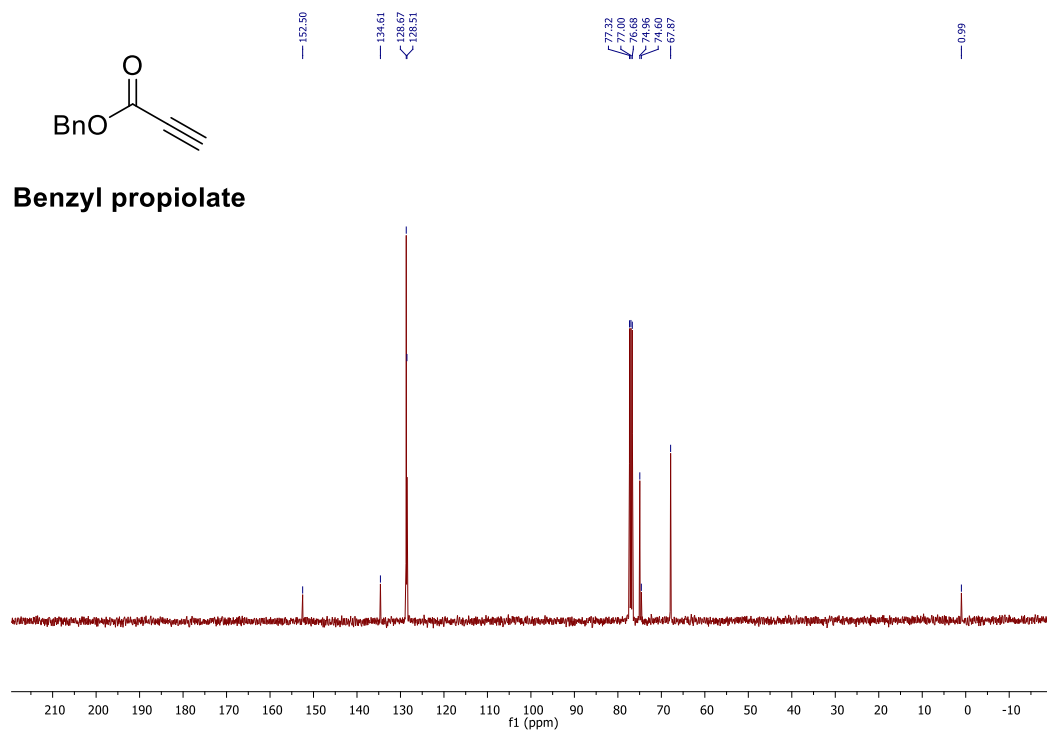
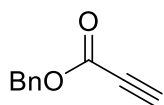
S6. Inhibition study. Inhibition of firefly luciferase by D-thioluciferin thioacrylate (sulfide) (**IX**). D-thioluciferin **3** (10  $\mu\text{M}$ ) was treated with purified firefly luciferase (10 nM) expressed from *E. coli* and the luminescence recorded in the absence and presence of (**IX**).

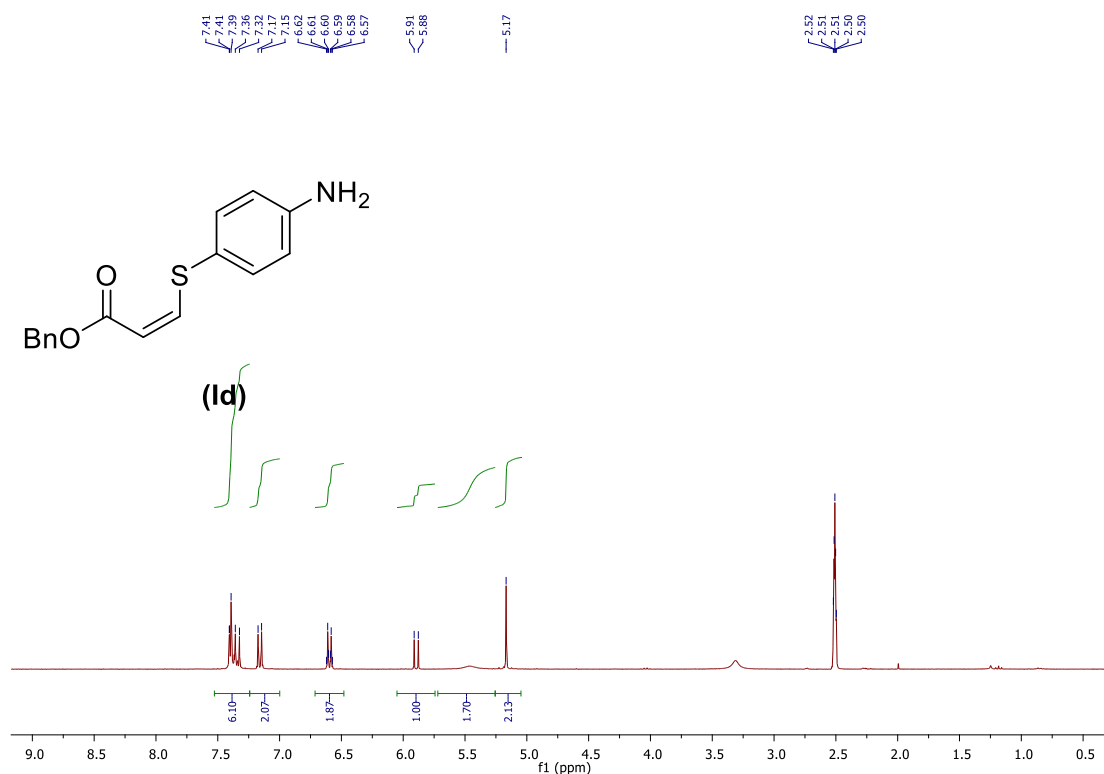
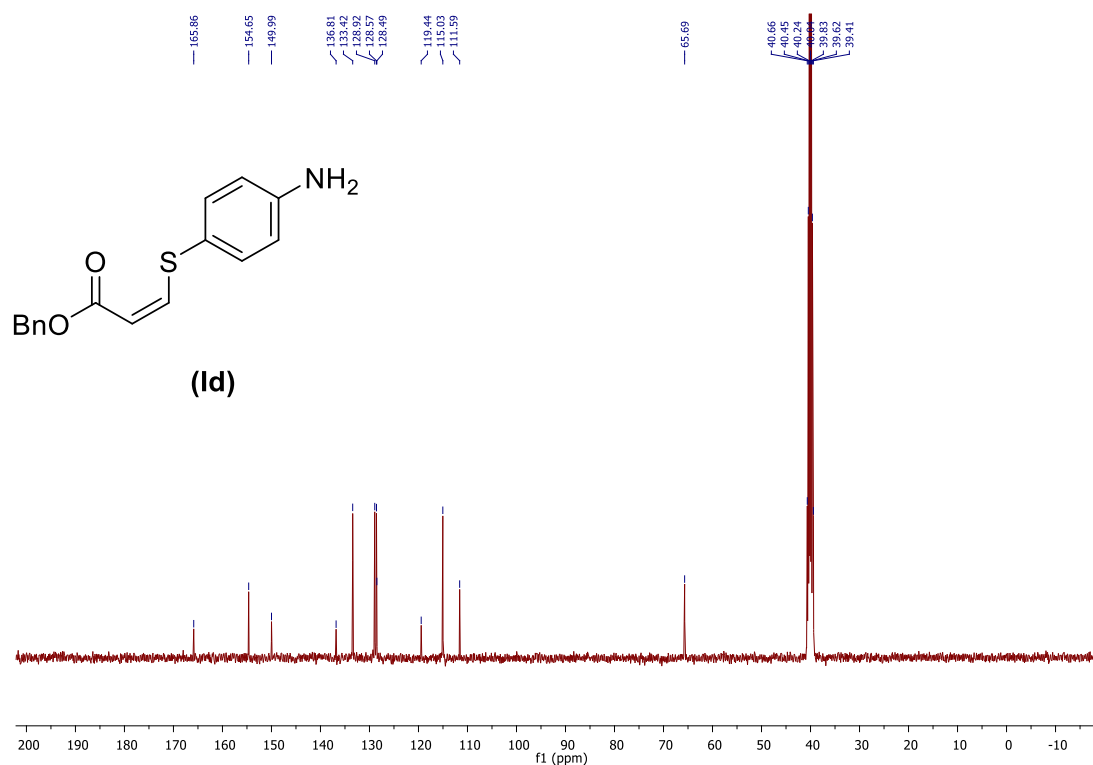


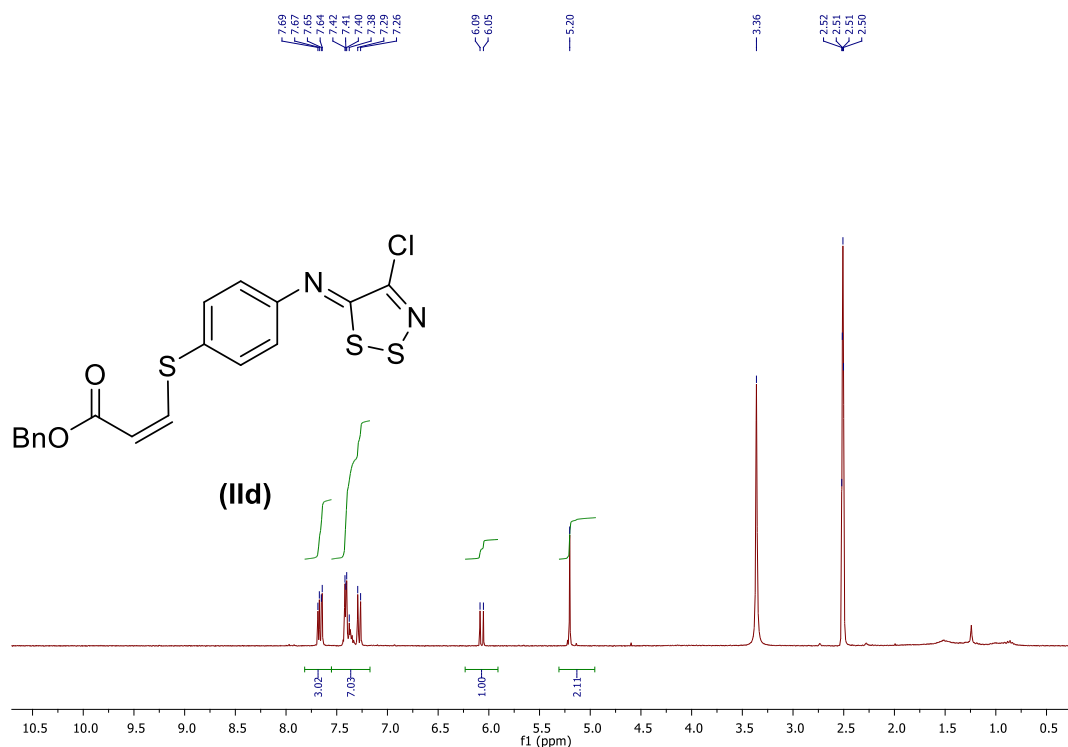
S7. Michaelis-Menton Kinetics Kinetic reaction curves with purified firefly luciferase (luciferase from Photinus Pyralis, Sigma-Aldrich) were recorded at a 10 nM final enzyme concentration. All the assays were performed in triplicate and measurements were taken every 5 s post-addition for 20 min.

S8. Table (Luminescence data for luciferins)<sup>1-4</sup>

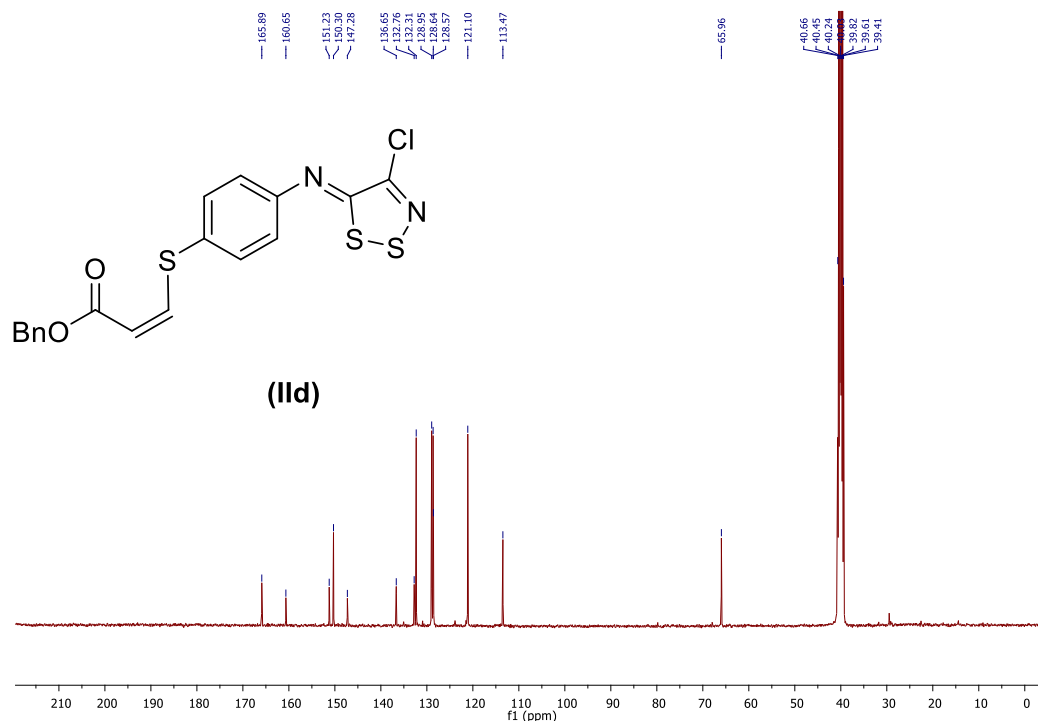
|                         | D-Luciferin                                                             | D-Aminoluciferin          | D-Thioluciferin                                             |
|-------------------------|-------------------------------------------------------------------------|---------------------------|-------------------------------------------------------------|
| $\lambda_{\max}$        | 553 nm                                                                  | 597 nm                    | 599 nm                                                      |
| $\lambda_{\max}$ (lit.) | 551 nm                                                                  | 594 nm                    | -                                                           |
| $V_{\max}$              | $57 \pm 13$ RLU/s                                                       | $13 \pm 2.2$ RLU/s        | $0.7597 \pm 0.1071$ RLU/s<br>$0.6661 \pm 0.1522$ RLU/s      |
| $V_{\max}$ (lit.)       | $1626 \pm 24$ photons/min<br>$1.027 \pm 0.036 \times 10^{-5}$ RLU/s     | $169 \pm 2.6$ photons/min | -                                                           |
| $K_m$ (App.)            | $8.3 \pm 0.5$ $\mu$ M                                                   | $0.39 \pm 0.08$ $\mu$ M   | $0.1169 \pm 0.01163$ $\mu$ M<br>$0.098 \pm 0.01471$ $\mu$ M |
| $K_m$ (lit.)            | $9.15 \pm 1.32$ $\mu$ M<br>$16 \pm 1$ $\mu$ M<br>$11.8 \pm 1.0$ $\mu$ M | $0.62 \pm 0.05$ $\mu$ M   | -                                                           |
| Fluorescence            | -                                                                       | -                         | 500 nm                                                      |
| Fluorescence (lit.)     | 528 nm                                                                  | 517 nm                    | -                                                           |

S9. <sup>1</sup>H NMR Spectrum of benzyl propiolateS10. <sup>13</sup>C NMR Spectrum of benzyl propiolate

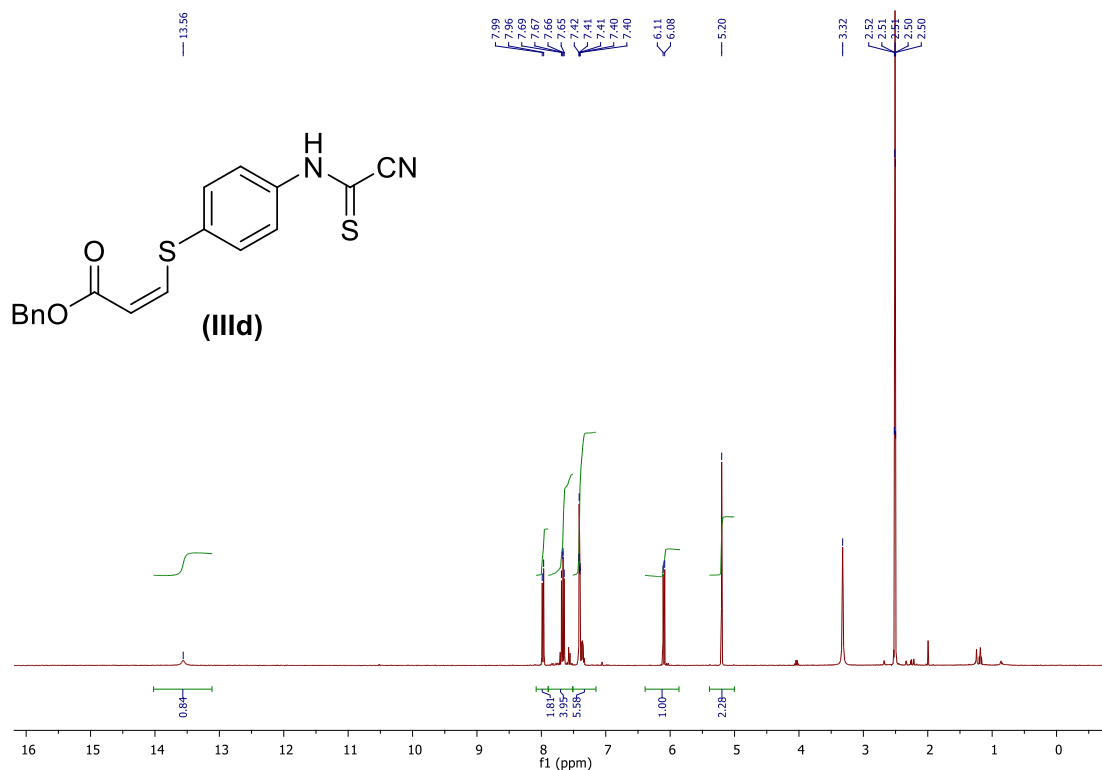
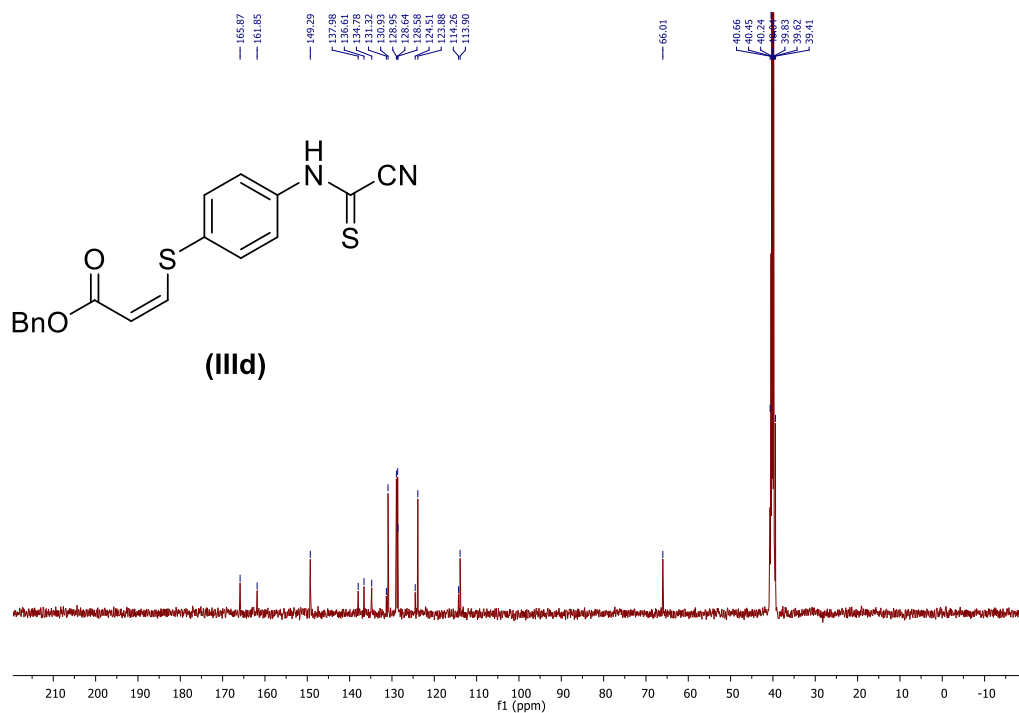
S11. <sup>1</sup>H NMR spectrum of benzyl (Z)-3-((4-aminophenyl)thio)acrylate (**Id**)S12. <sup>13</sup>C NMR Spectrum of benzyl (Z)-3-((4-aminophenyl)thio)acrylate (**Id**)



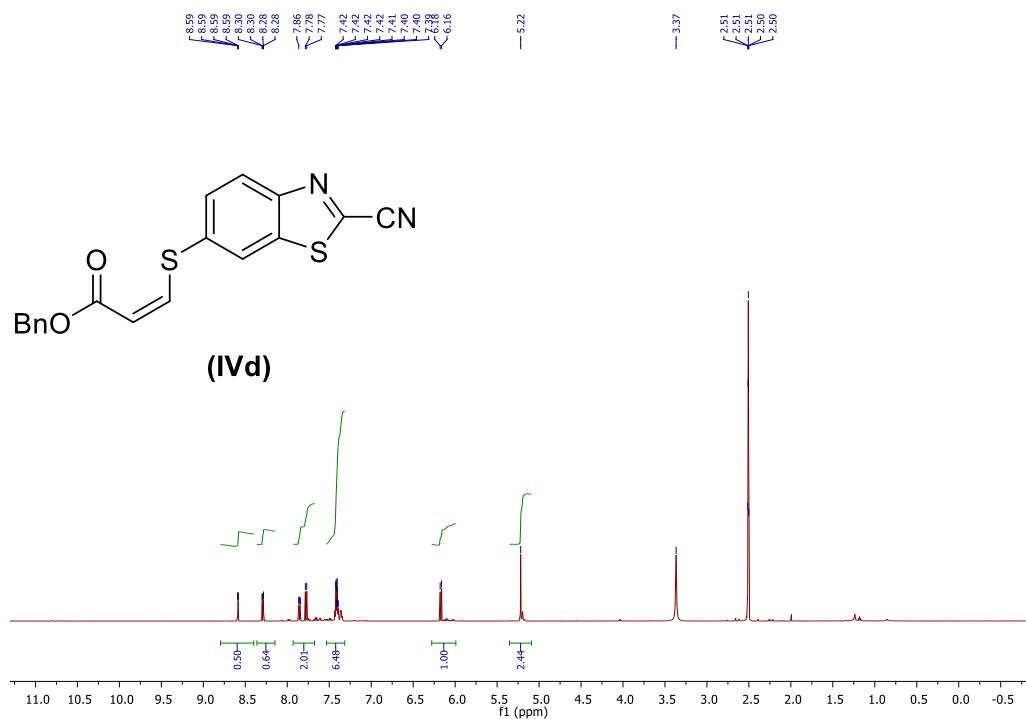
**S13.** <sup>1</sup>H NMR spectrum of benzyl (Z)-3-((4-(((Z)-4-chloro-5H-1,2,3-dithiazol-5-ylidene)amino)phenyl)thio)acrylate (IId)



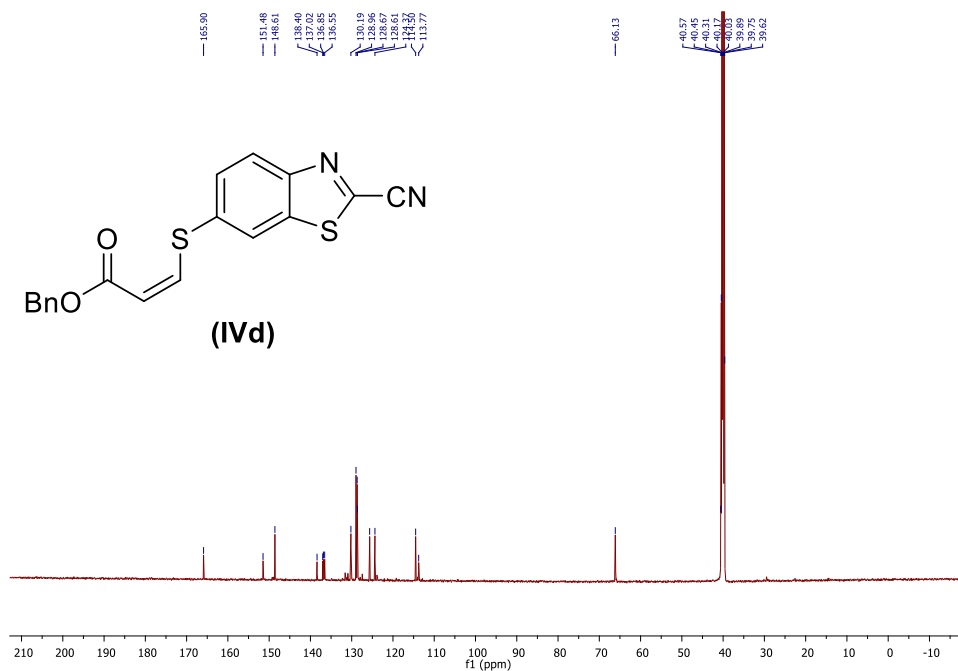
**S14.** <sup>13</sup>C NMR spectrum of benzyl (Z)-3-((4-(((Z)-4-chloro-5H-1,2,3-dithiazol-5-ylidene)amino)phenyl)thio)acrylate (IId)

S15. <sup>1</sup>H NMR Spectra of benzyl (Z)-3-((4-((cyanocarbonothioyl)amino)phenyl)thio)acrylate (**IIIId**)S16. <sup>13</sup>C NMR Spectra of benzyl (Z)-3-((4-((cyanocarbonothioyl)amino)phenyl)thio)acrylate (**IIIId**)

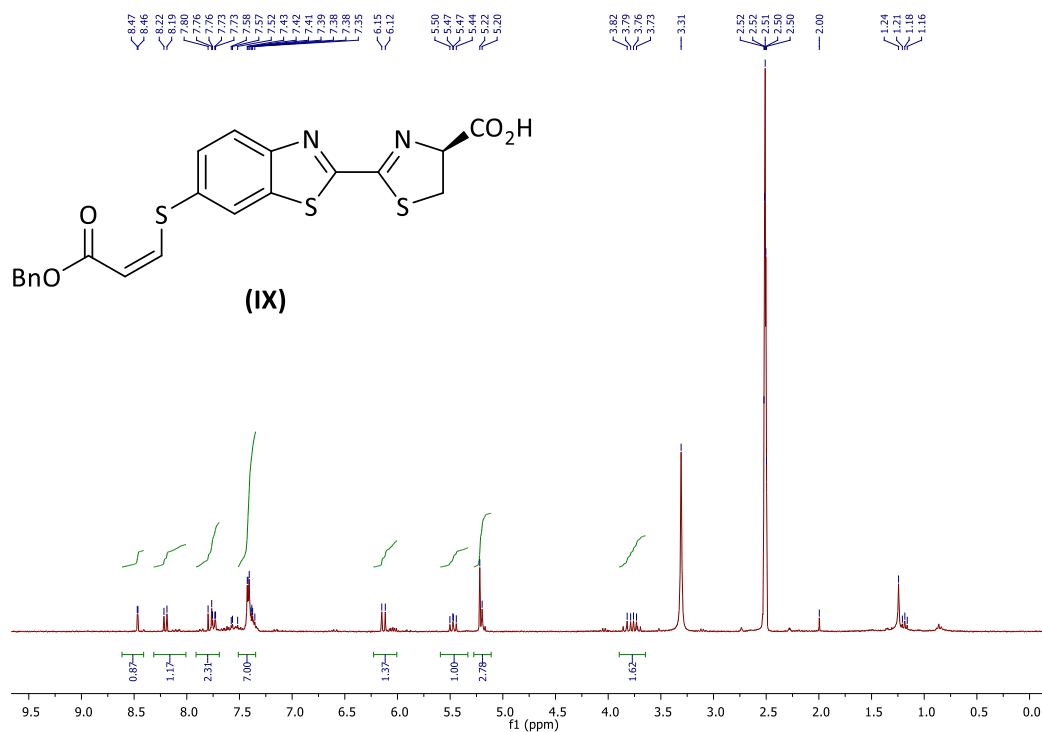




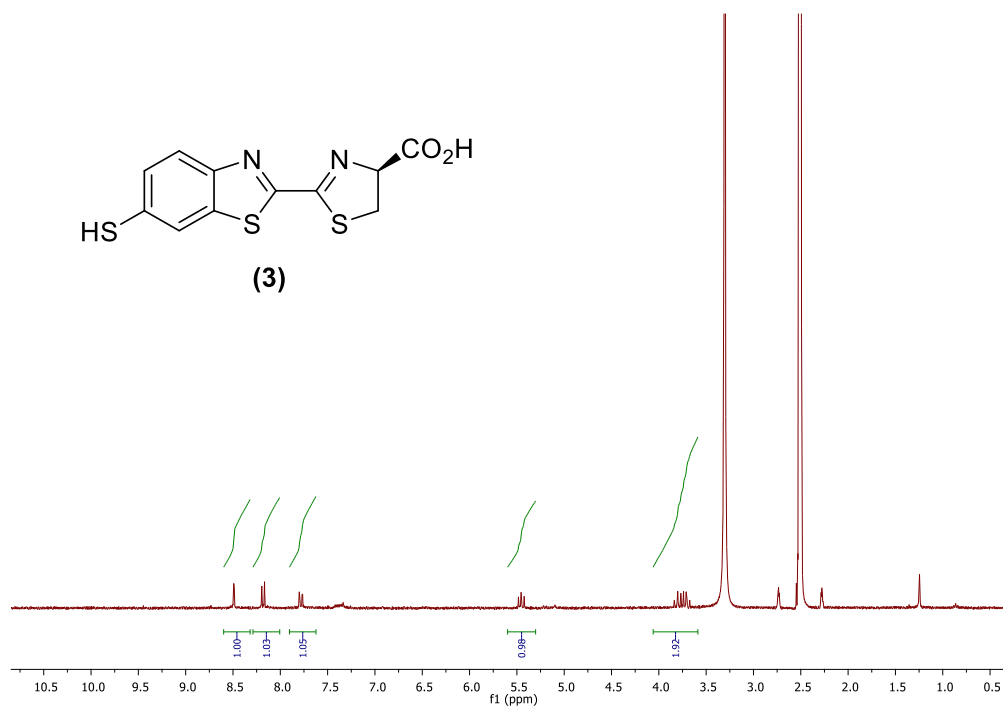
S17. <sup>1</sup>H NMR Spectra of benzyl (Z)-3-((2-cyanobenzo[d]thiazol-6-yl)thio)acrylate (IVd)



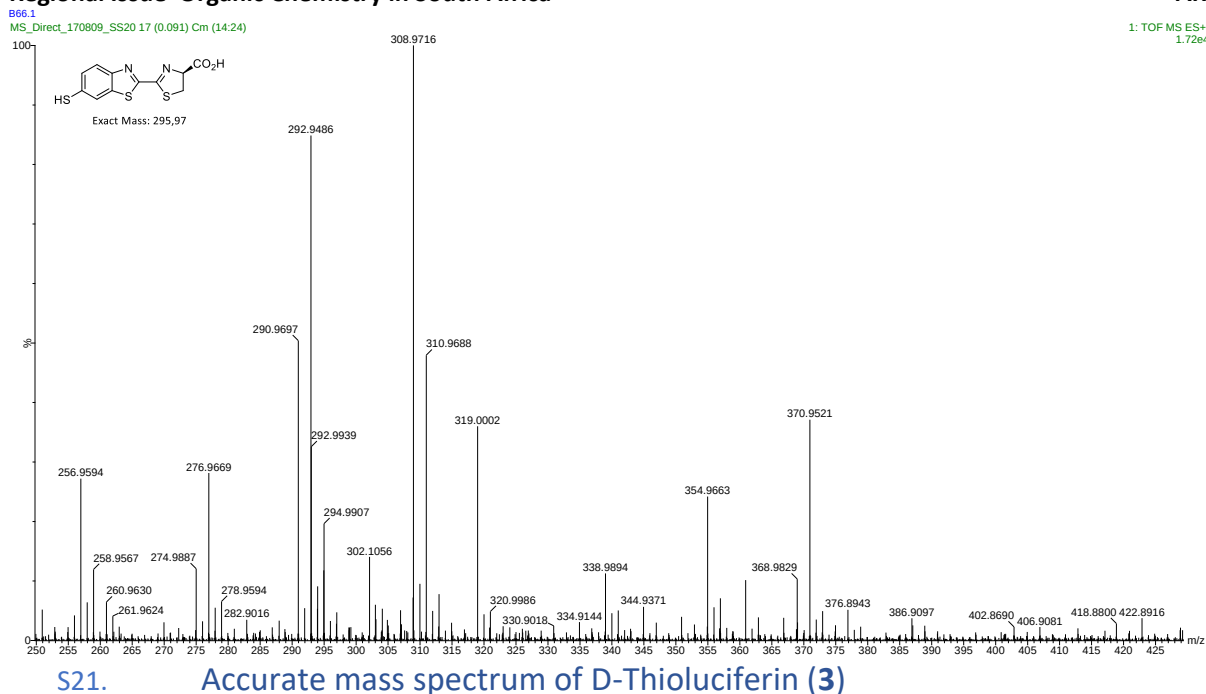
S18. <sup>13</sup>C NMR Spectra of benzyl (Z)-3-((2-cyanobenzo[d]thiazol-6-yl)thio)acrylate (IVd)



S19. <sup>1</sup>H NMR Spectra of (S,Z)-2-(6-((3-(benzyloxy)-3-oxoprop-1-en-1-yl)thio)benzo[d]thiazol-2-yl)-4,5-dihydrothiazole-4-carboxylic acid (IX)



S20. <sup>1</sup>H NMR Spectra of D-thioluciferin (3)



## References

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