

Supplementary Material

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

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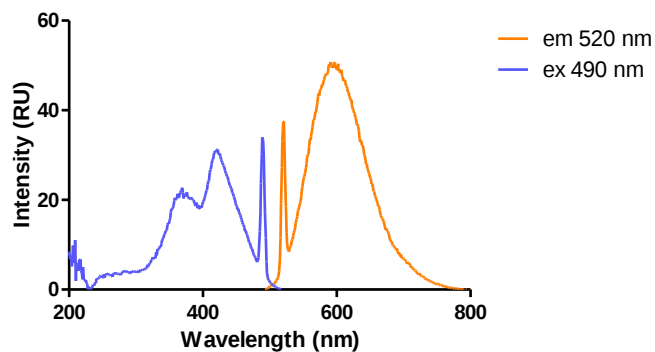
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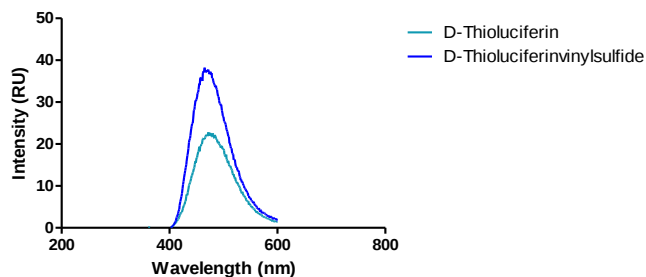
Experimental Procedures

Excitation/Emission Spectra of D-thioluciferin



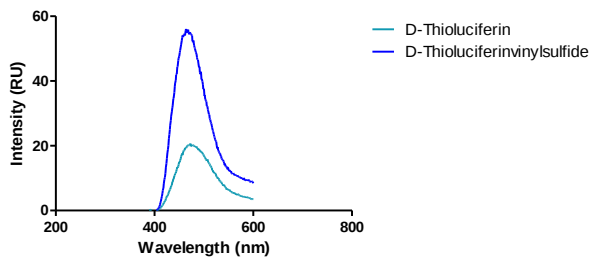
S1. Excitation and emission spectra of D-thioluciferin (3)

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



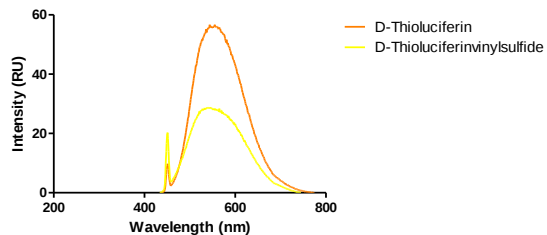
S2. Emission spectra of D-thioluciferin (3) and D-thioluciferin vinylsulfide (IX) at 360 nm

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

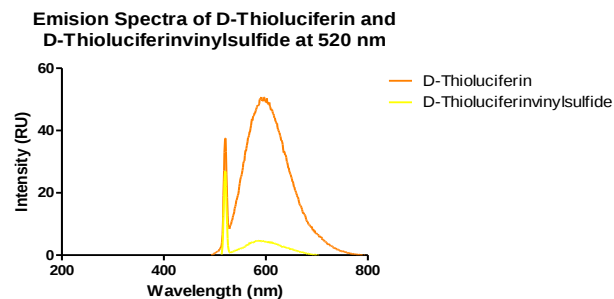


S3. Emission spectra of D-thioluciferin (3) and D-thioluciferin vinylsulfide (IX) at 390 nm

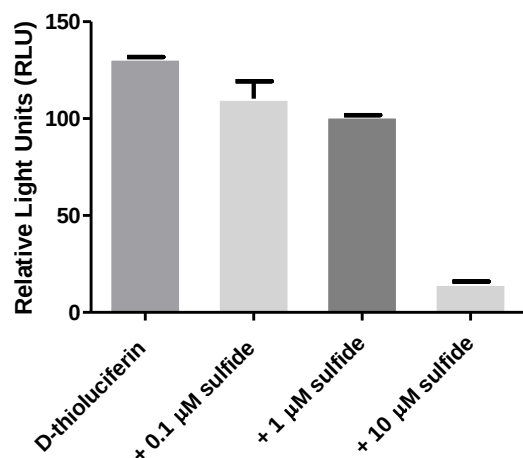
Emission Spectra of D-Thioluciferin and D-Thioluciferinvinylsulfide at 450 nm



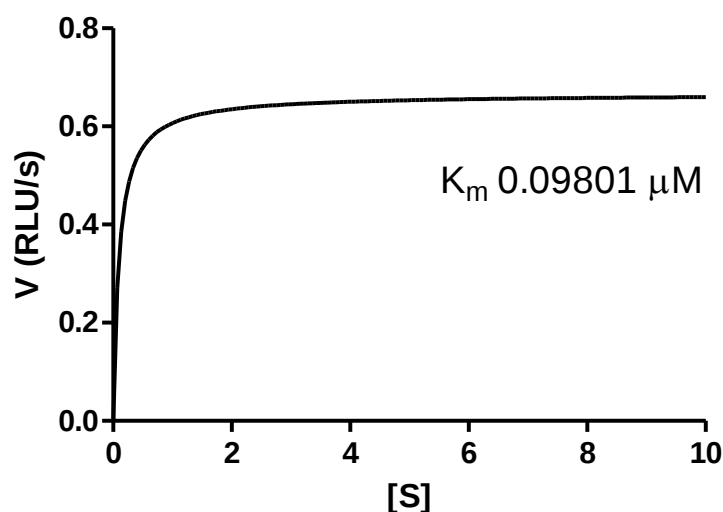
S4. Emission spectra of D-thioluciferin (3) and D-thioluciferin vinylsulfide (IX) at 450 nm



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



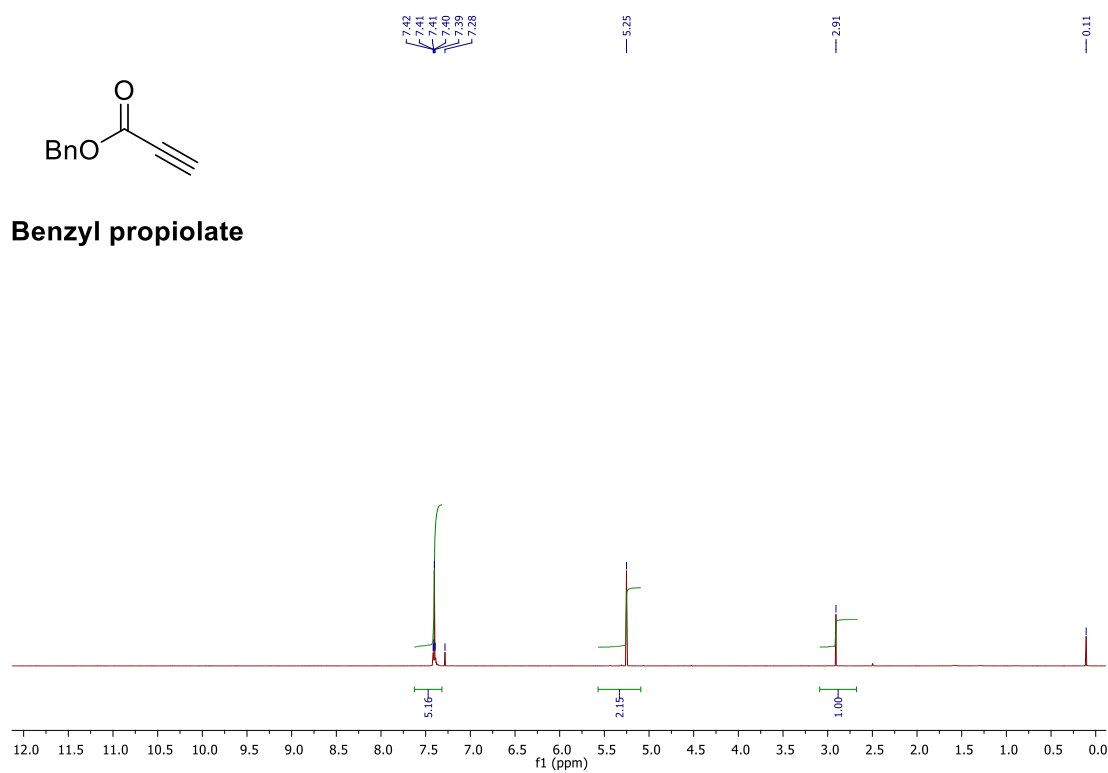
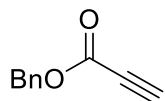
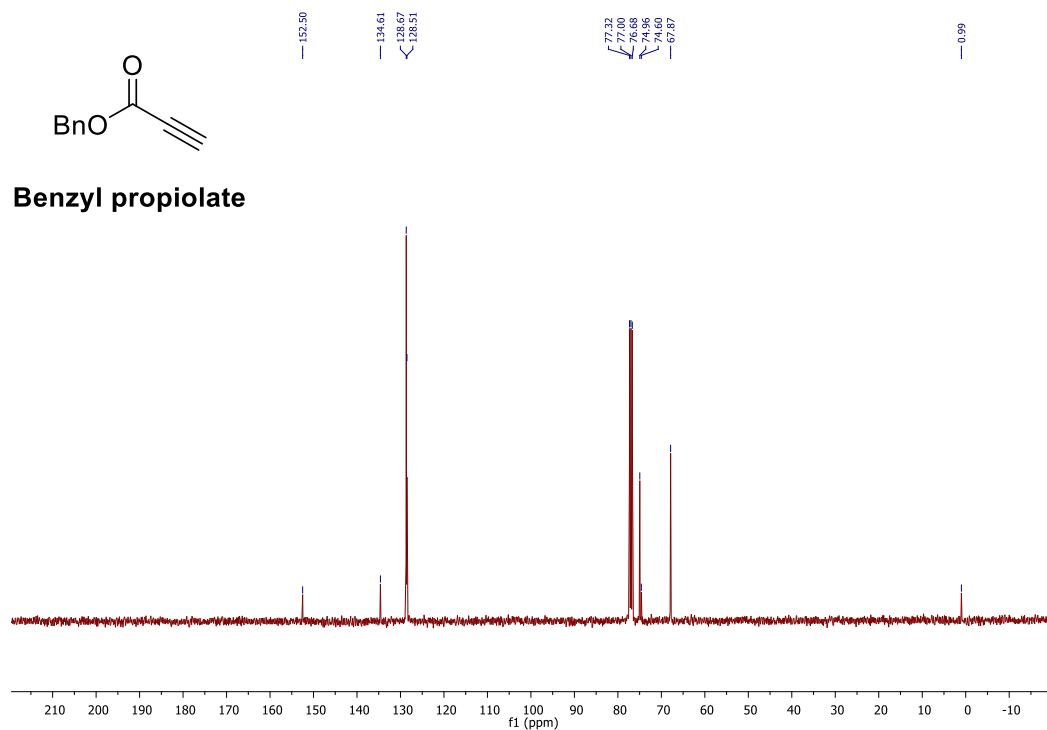
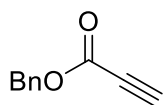
S6. Inhibition study. Inhibition of firefly luciferase by D-thioluciferin thioacrylate (sulfide) (**IX**). D-thioluciferin **3** (10 μM) was treated with purified firefly luciferase (10 nM) expressed from *E. coli* and the luminescence recorded in the absence and presence of (**IX**).

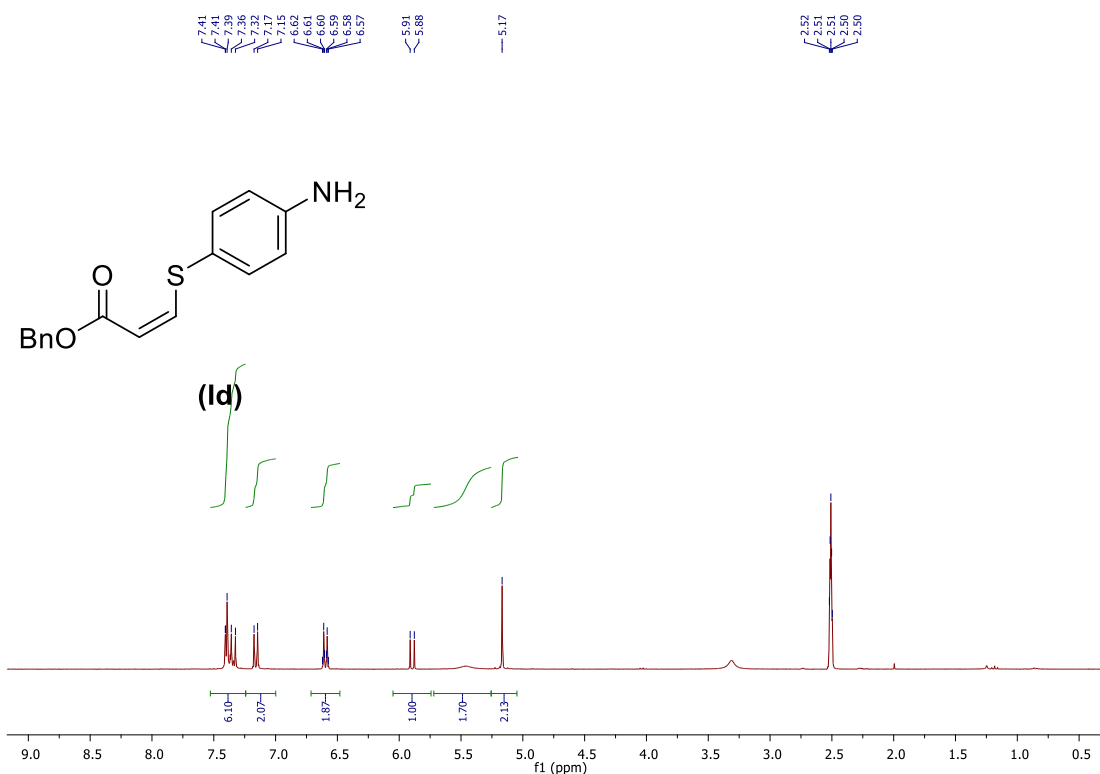
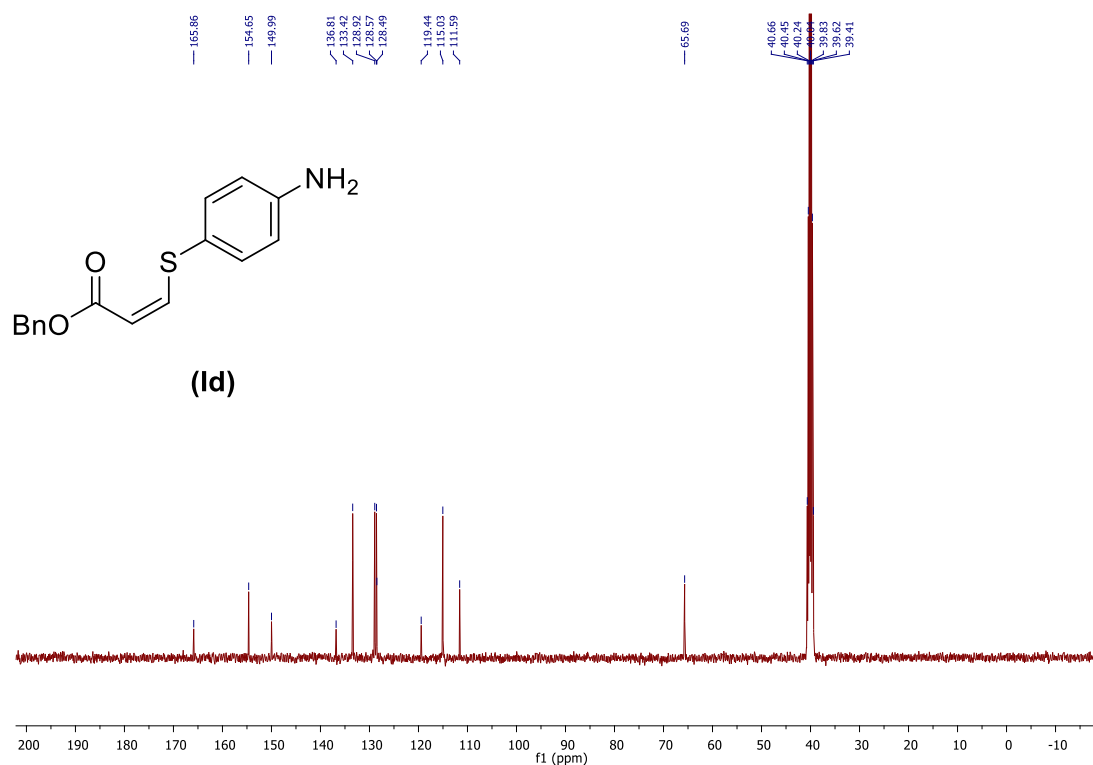


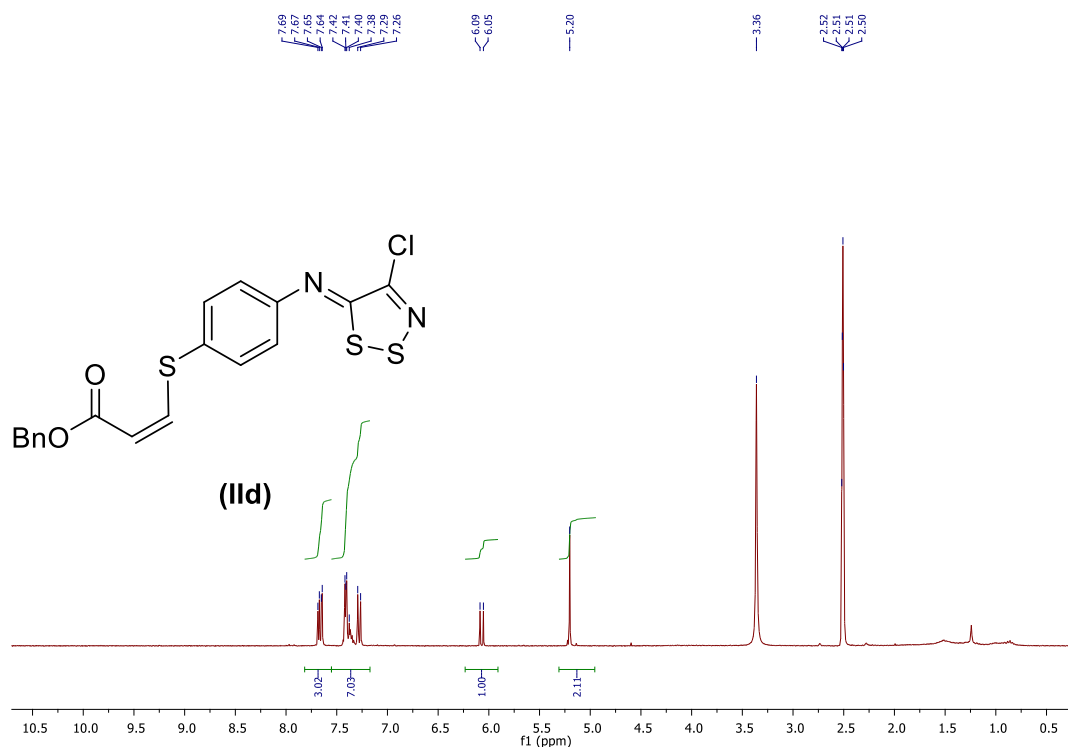
S7. Michaelis-Menton Kinetics Kinetic reaction curves with purified firefly luciferase (luciferase from *Photinus Pyralis*, Sigma-Aldrich) were recorded at a 10 nM final enzyme concentration. All the assays were performed in triplicate and measurements were taken every 5 s post-addition for 20 min.

S8. Table (Luminescence data for luciferins)¹⁻⁴

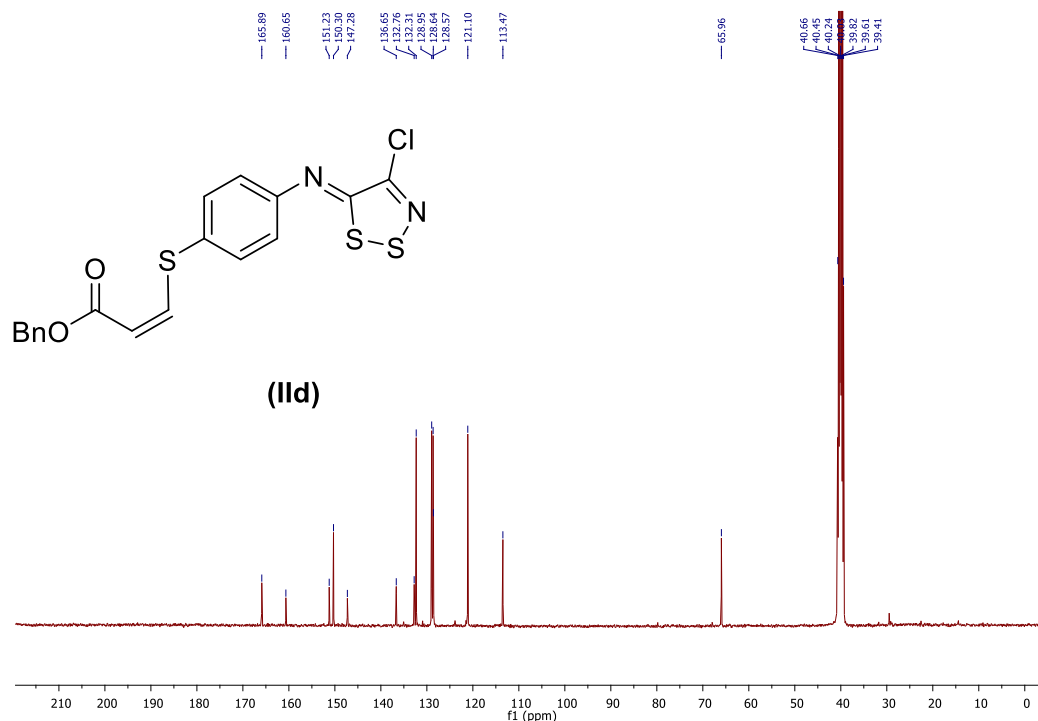
	D-Luciferin	D-Aminoluciferin	D-Thioluciferin
$\lambda_{\max}$	553 nm	597 nm	599 nm
$\lambda_{\max}$ (lit.)	551 nm	594 nm	-
$V_{\max}$	57 ± 13 RLU/s	13 ± 2.2 RLU/s	0.7597 ± 0.1071 RLU/s 0.6661 ± 0.1522 RLU/s
$V_{\max}$ (lit.)	1626 ± 24 photons/min $1.027 \pm 0.036 \times 10^{-5}$ RLU/s	169 ± 2.6 photons/min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

S9. ¹H NMR Spectrum of benzyl propiolateS10. ¹³C NMR Spectrum of benzyl propiolate

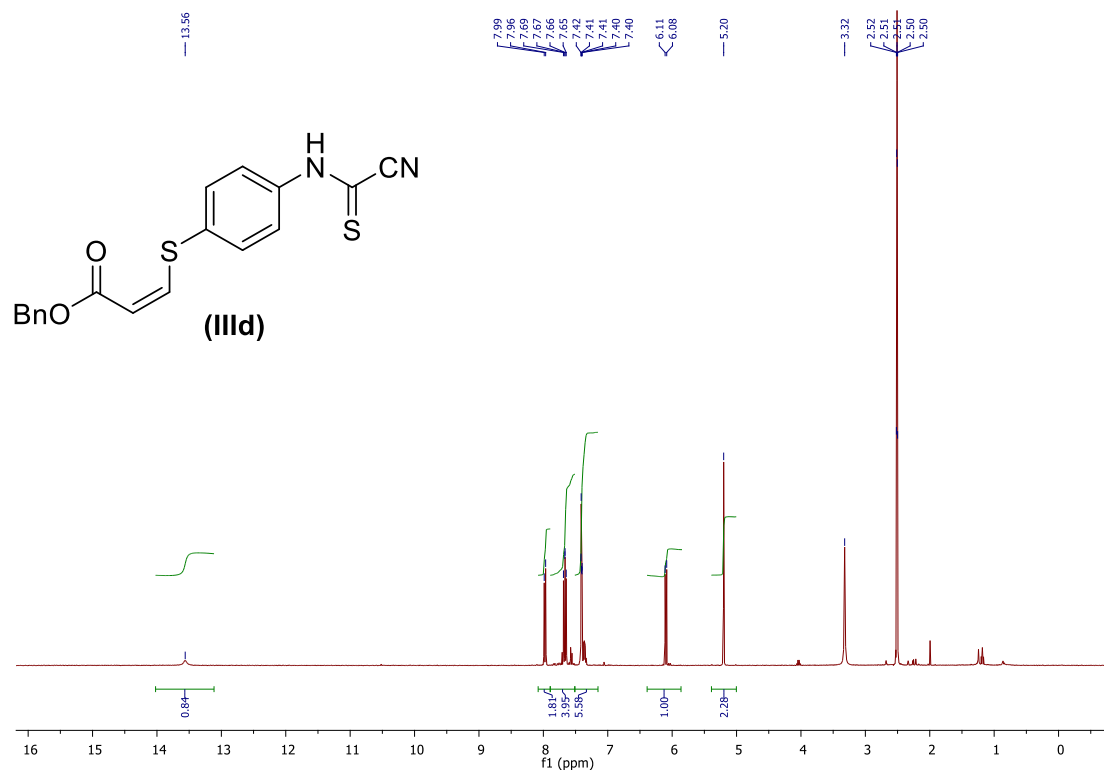
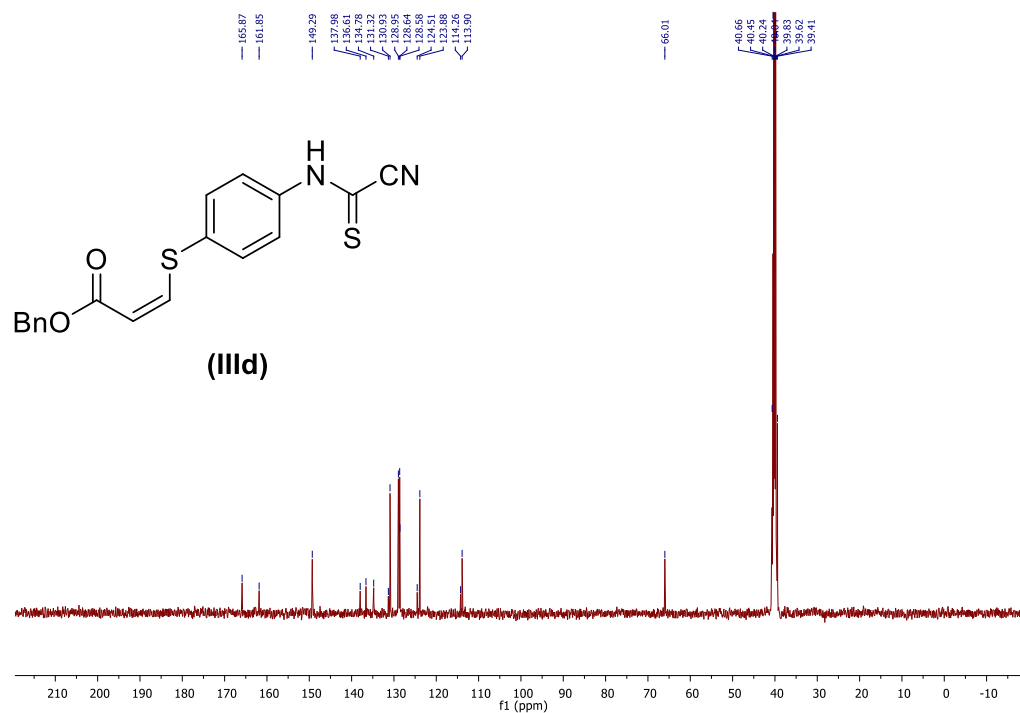
S11. ¹H NMR spectrum of benzyl (Z)-3-((4-aminophenyl)thio)acrylate (**Id**)S12. ¹³C NMR Spectrum of benzyl (Z)-3-((4-aminophenyl)thio)acrylate (**Id**)

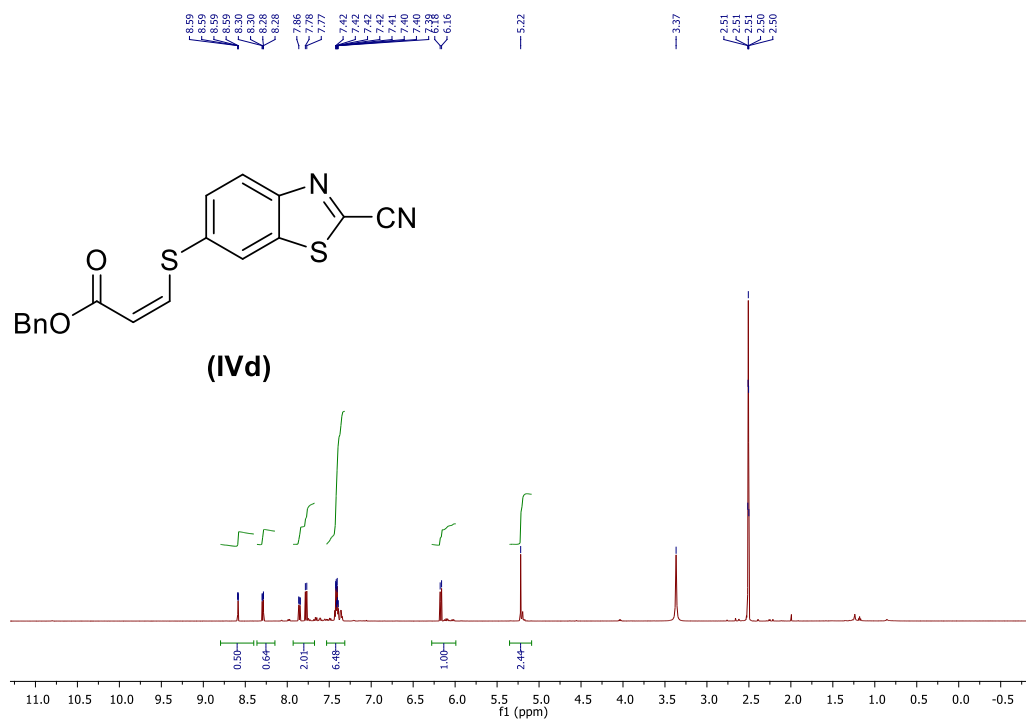
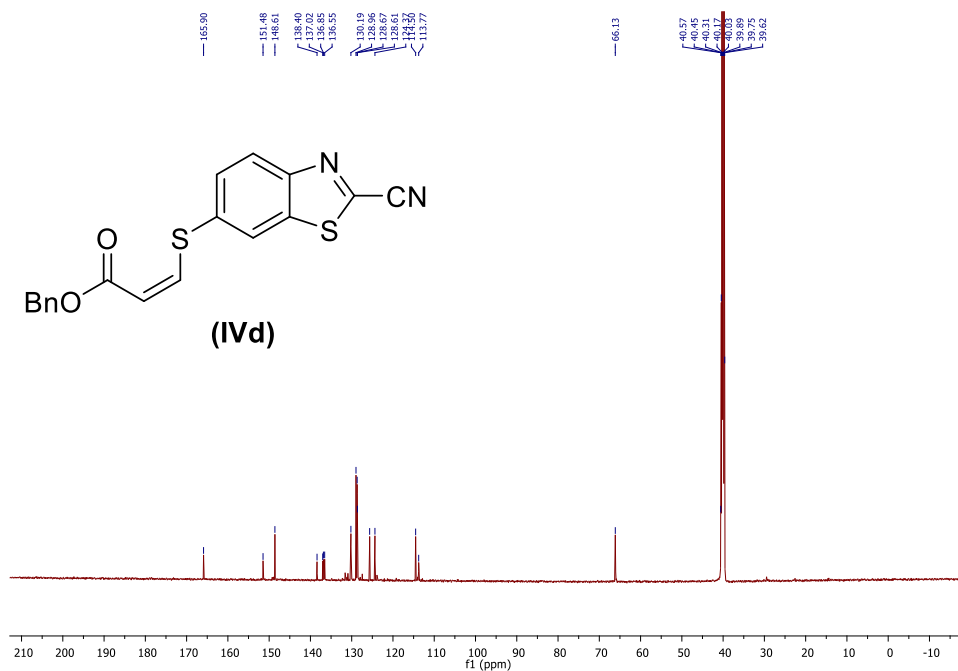


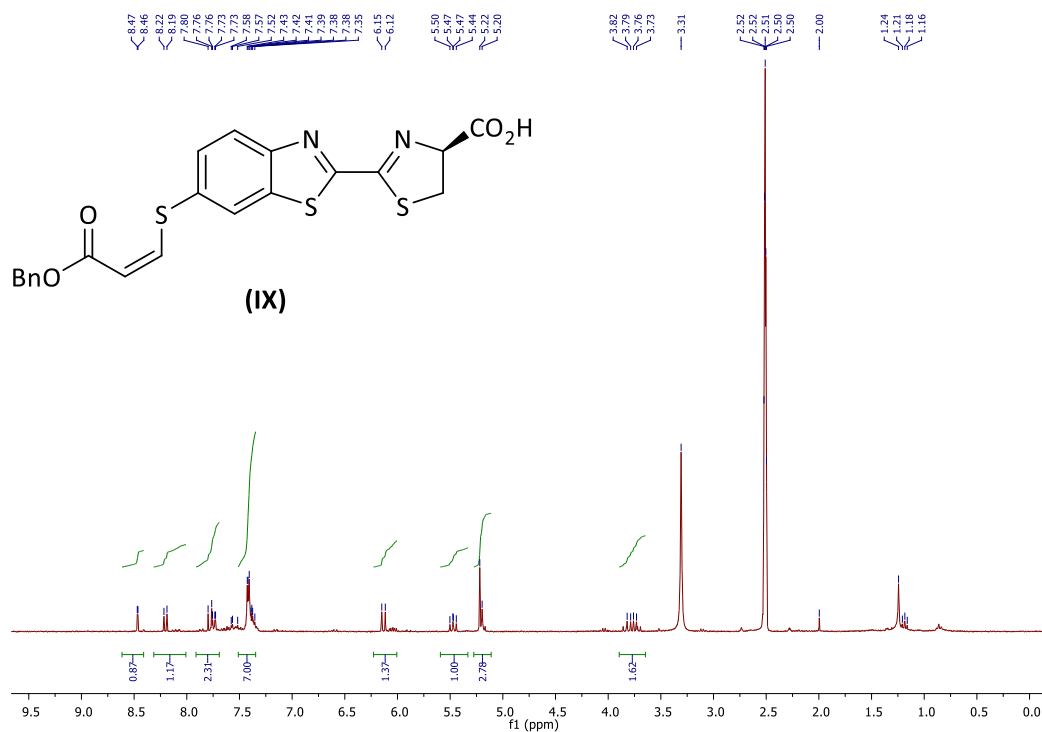
S13. ¹H NMR spectrum of benzyl (Z)-3-((4-(((Z)-4-chloro-5H-1,2,3-dithiazol-5-ylidene)amino)phenyl)thio)acrylate (IId)



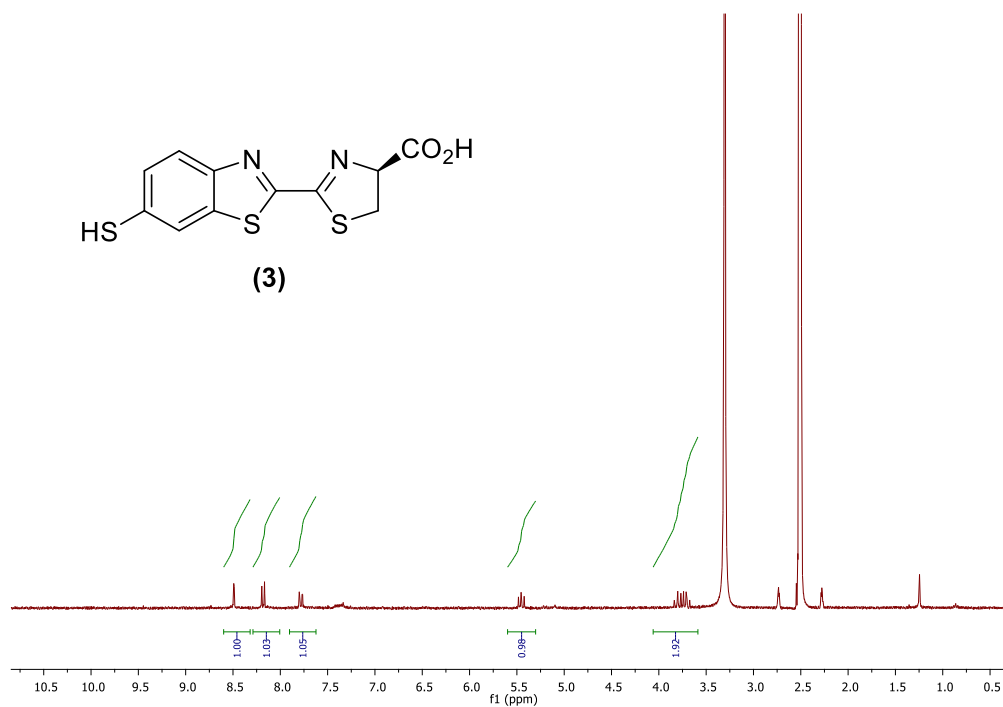
S14. ¹³C NMR spectrum of benzyl (Z)-3-((4-(((Z)-4-chloro-5H-1,2,3-dithiazol-5-ylidene)amino)phenyl)thio)acrylate (IId)

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

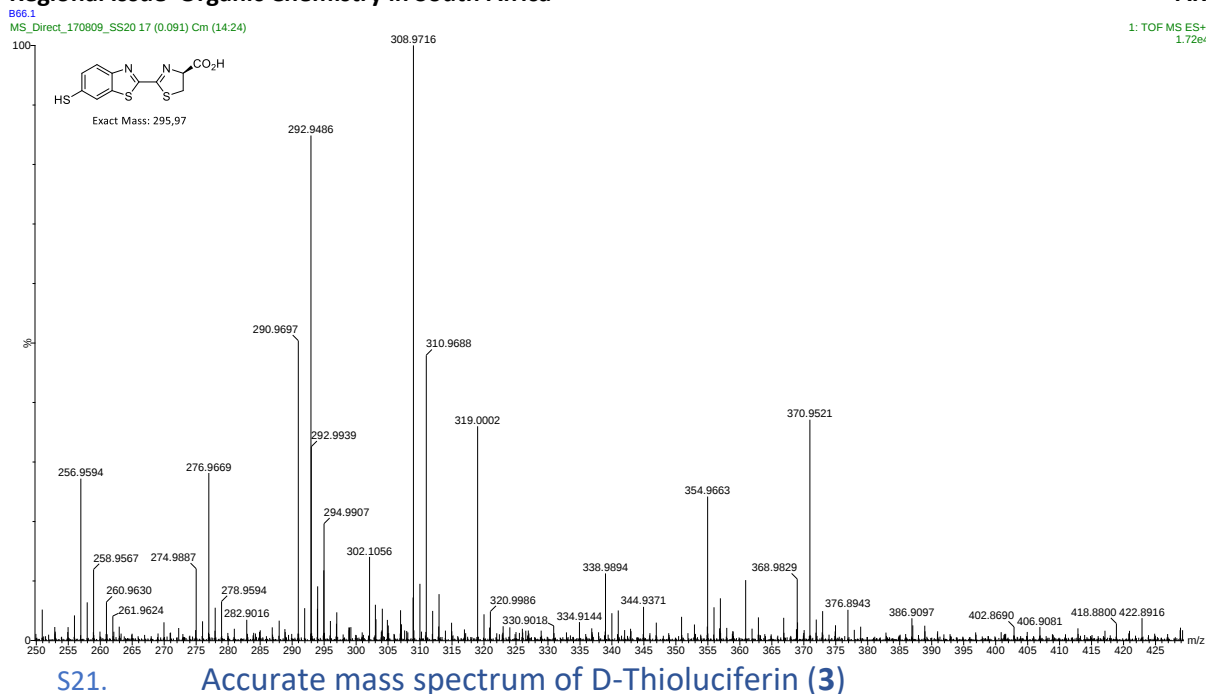
S17. ¹H NMR Spectra of benzyl (Z)-3-((2-cyanobenzo[d]thiazol-6-yl)thio)acrylate (**IVd**)S18. ¹³C NMR Spectra of benzyl (Z)-3-((2-cyanobenzo[d]thiazol-6-yl)thio)acrylate (**IVd**)



S19. ¹H 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. ¹H NMR Spectra of D-thioluciferin (3)



References

- Shinde, R.; Perkins, J.; Contag, C. H., *Biochemistry* **2006**, 45 (37), 11103-12. '10.1021/bi060475o'
- Kiyama, M.; Iwano, S.; Otsuka, S.; Lu, S. W.; Obata, R.; Miyawaki, A.; Hirano, T.; Maki, S. A., *Tetrahedron* **2018**, 74 (6), 652-660.
<https://doi.org/10.1016/j.tet.2017.11.051>
- Pirrung, M. C.; Carlson, A. D.; De Howitt, N.; Liao, J., *Bioorganic & medicinal chemistry letters* **2019**, 29 (19), 126591. '10.1016/j.bmcl.2019.07.050'
- da Silva, L. P.; da Silva, J. C. E., *Photochemical & Photobiological Sciences* **2011**, 10 (6), 1039-1045.