The Mechanism of Ubiquitin Ligase in Protein Degradation

Taadesha Denson | University of Houston | tdenson@uh.edu

Background

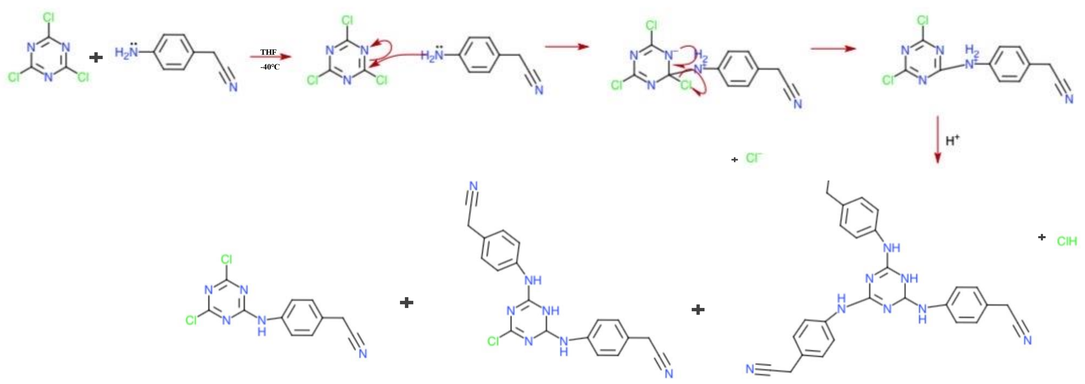
Protein–protein interactions are induced by molecular glue molecules, which lead to protein breakdown in the presence of a ubiquitin ligase. These molecular glue degraders, unlike typical enzyme inhibitors, function sub-stoichiometrically to catalyze the fast depletion of previously inaccessible targets. This eliminates the need for a substrate receptor and enables ubiquitination and degradation of the cyclin. To be active, cyclin subunits must interact with cyclin dependent kinase (CDK) subunits, and when distinct cyclins are stimulated, they oscillate in the cell cycle in different ways. The molecular glue compound attaching to the cyclin dependent kinase will induce an E3 Ligase to be recruited. A complex between CDK-cyclin and an adaptor protein termed DDB1 forms as a result of the CDK inhibitor. After the kinase has been destroyed, the created compound can bind to a new kinase and use enzymes to repeat the degradation process.

Objectives

To create a non-molar binding affinity molecule (being able to have a binding affinity for all cyclin-dependent kinases).
Having the molecular glue compound bind to two cyclin molecules promiscuously.

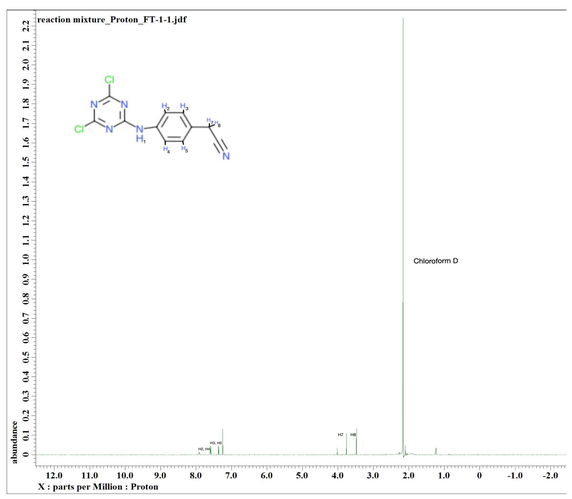
Experimental

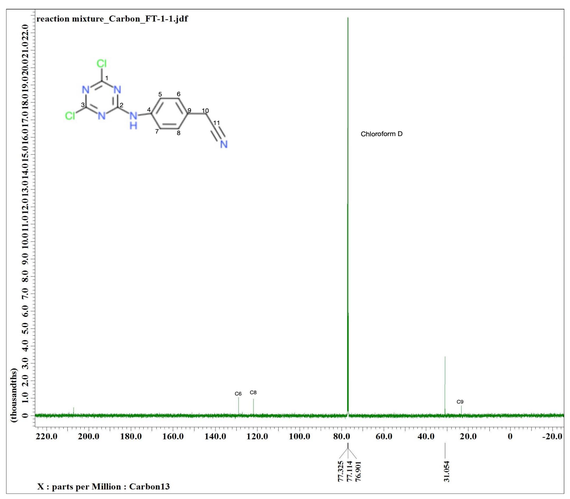
In a liquid nitrogen and acetonitrile bath, 4-amino benzyl cyanide (0.2002 grams, 1.514 mmol) in tetrahydrofuran (10 mL) was added over the course of five minutes to a solution of cyanuric chloride (2.793 grams, 15.148 mmol) in tetrahydrofuran (40 mL). The reaction mixture was stirred at -40℃ for 1 hour and was monitored by thin-layer chromatography (TLC). The eluent for TLC was a 4:1 ratio of Hexane and Ethyl Acetate. After one hour, the reaction was cooled to room temperature (25℃), gravity filtered, and the solvent was removed under vacuum. The crude residue was purified by flash chromatography to give the desired compound(0.33 grams, 11.02% yield).



Mechanism 1: The mechanism for the addition of 4-amino benzyl cyanide to cyanuric chloride under -40℃ conditions.

Results





Conclusion

Considering the aromatic peaks from the 4-amino benzyl cyanide shows up between 6.5 and 8.0 ppm; it was expected to show up close to that range for the product. The 4-amino benzyl cyanide reacted fully, and the excess amount of cyanuric chloride was visible. The amino group (-NH-) has a proton that is exchanged. Exchangeable protons produce broad signals in NMR, and if the concentration is low, the peak in the NMR spectrum may not be visible. Furthermore, carbon NMR is not as sensitive as proton NMR, therefore most the carbons did not appear.

Future Work

Decreasing the amount of cyanuric chloride to reveal the aromatic hydrogens
Increasing the number of scans for carbon NMR to increase the chances of more carbons showing
Continue to research about the numerous cyclins involved in Mitosis

Acknowledgements

Alexander Statsyuk, PhD and The University of Houston Honors College (SURF Grant) provided generous support for this research project.

Reference

Słabicki, M., Kozicka, Z., Petzold, G. *et al.* The CDK inhibitor CR8 acts as a molecular glue degrader that depletes cyclin K.*Nature* **585,** 293–297 (2020). https://doi.org/10.1038/s41586-020-2374-x