CHEMOENZYMATIC SYNTHESIS OF SELECT INTERMEDIATES AND PRODUCTS OF THE DESFERRIOXAMINE E SIDEROPHORE PATHWAY

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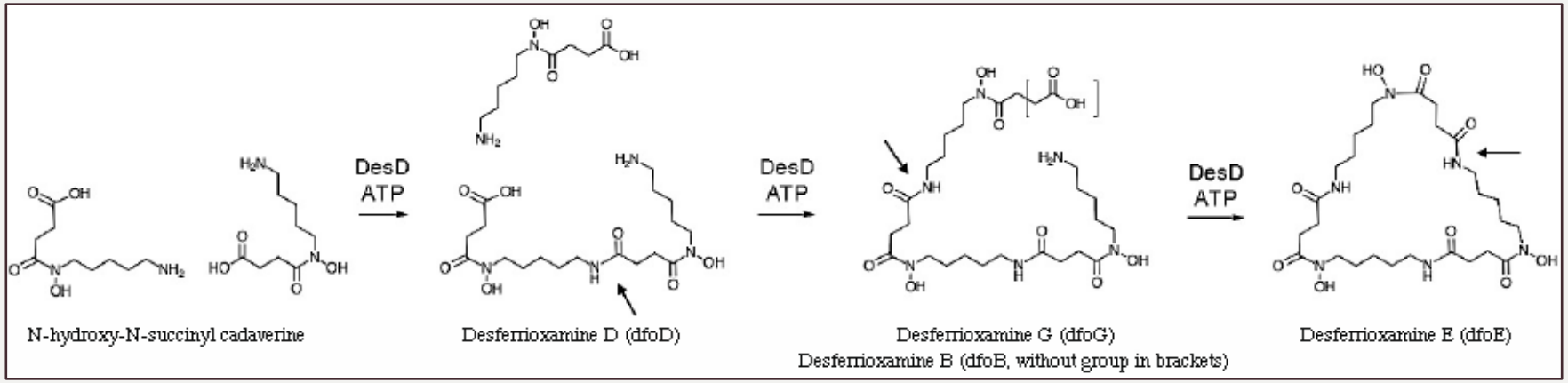
Desferrioxamine E synthesis pathway

- DesD : catalyzes iterative amide bond formations with
  - N-hydroxy, N-succinyl cadaverine (HSC)
  - Desferrioxamine D (dfoD)
  - Desferrioxamine G (dfoG)
- Desferrioxamine E (dfoE) is a clinically useful iron-chelating agent

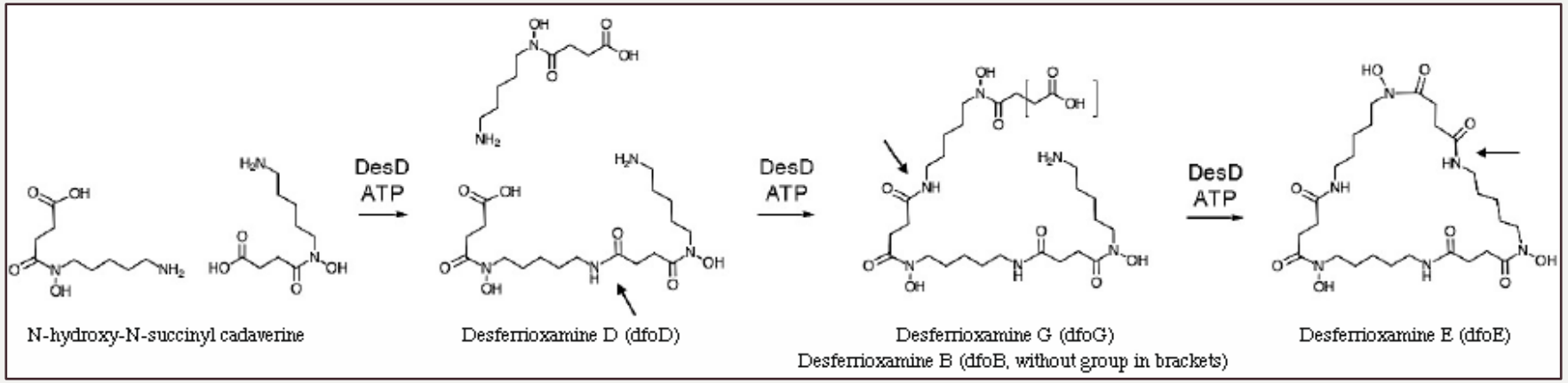


The importance of Iron

- A cofactor in metabolic processes such as aerobic and anaerobic ATP biosynthesis
- Scarcity of ferric iron
  - Commonly complexed with hydroxide and insoluble in aqueous solutions
- As a result, acquiring iron is key to establishing an infection

Why study DesD?

- Catalyzes iterative amide bond formations in an NIS synthetase mechanism
- Inhibition of DesD is a possible strategy for combating pathogenic bacteria

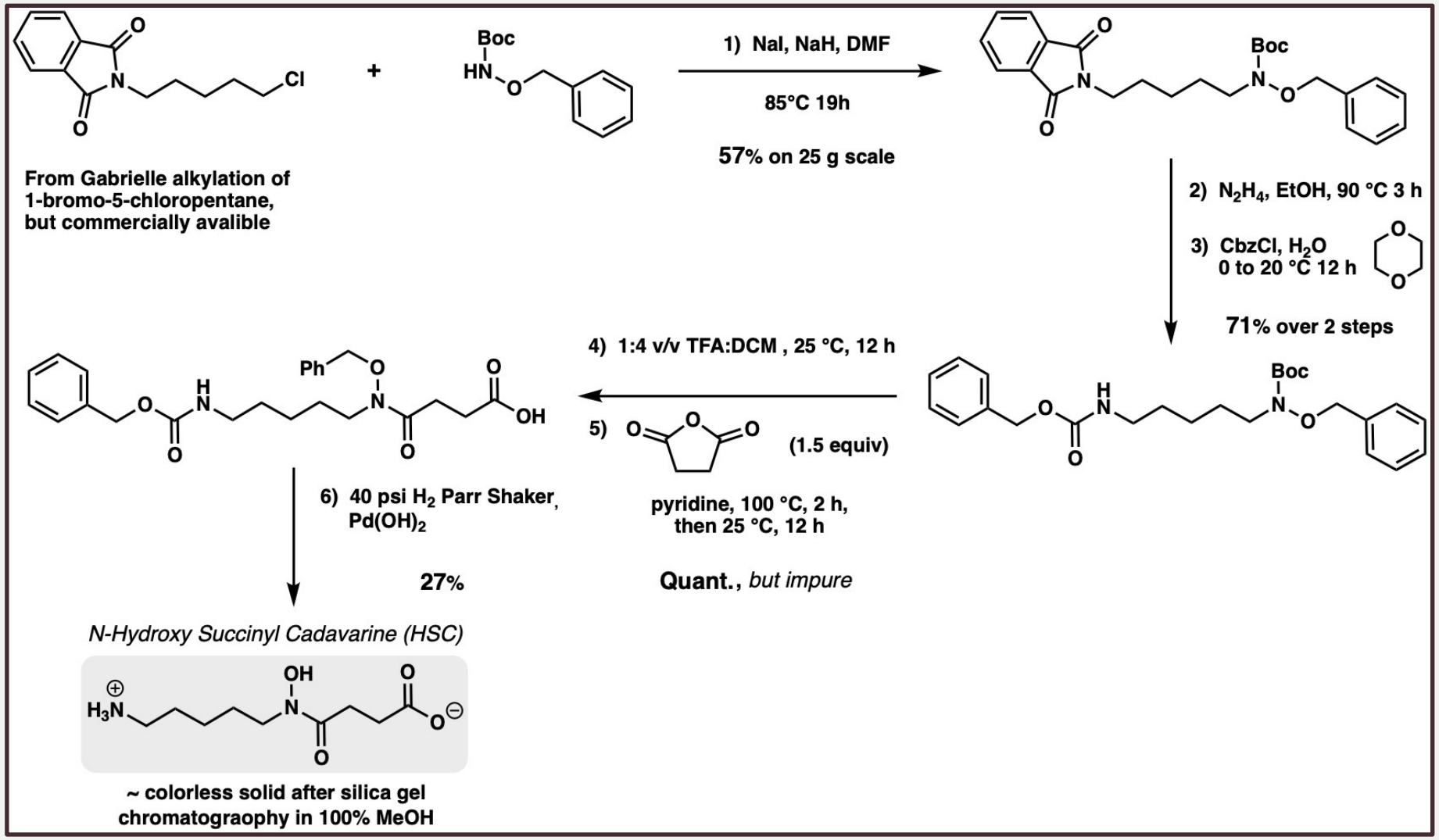


COMMERCIAL AVAILABLILITY

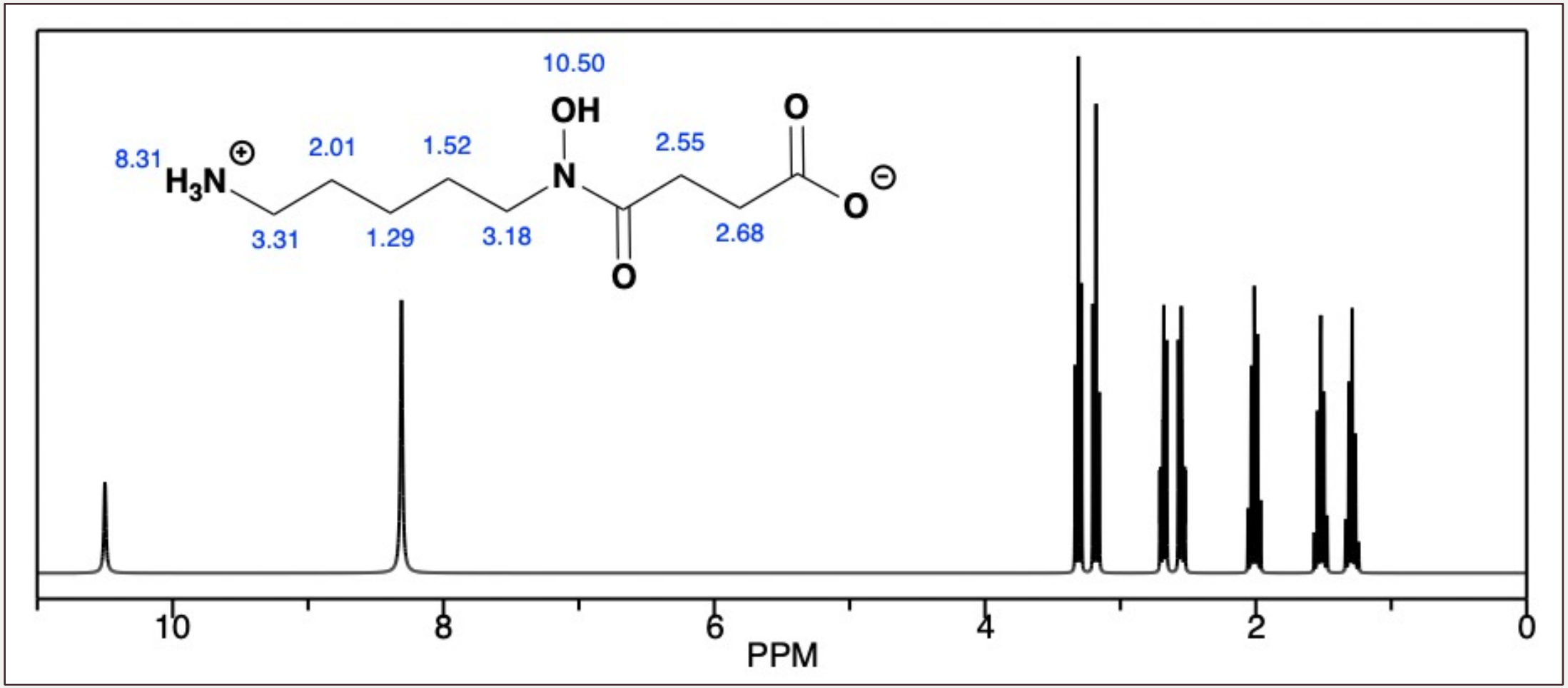
HSC

dfoD

HSC synthesis



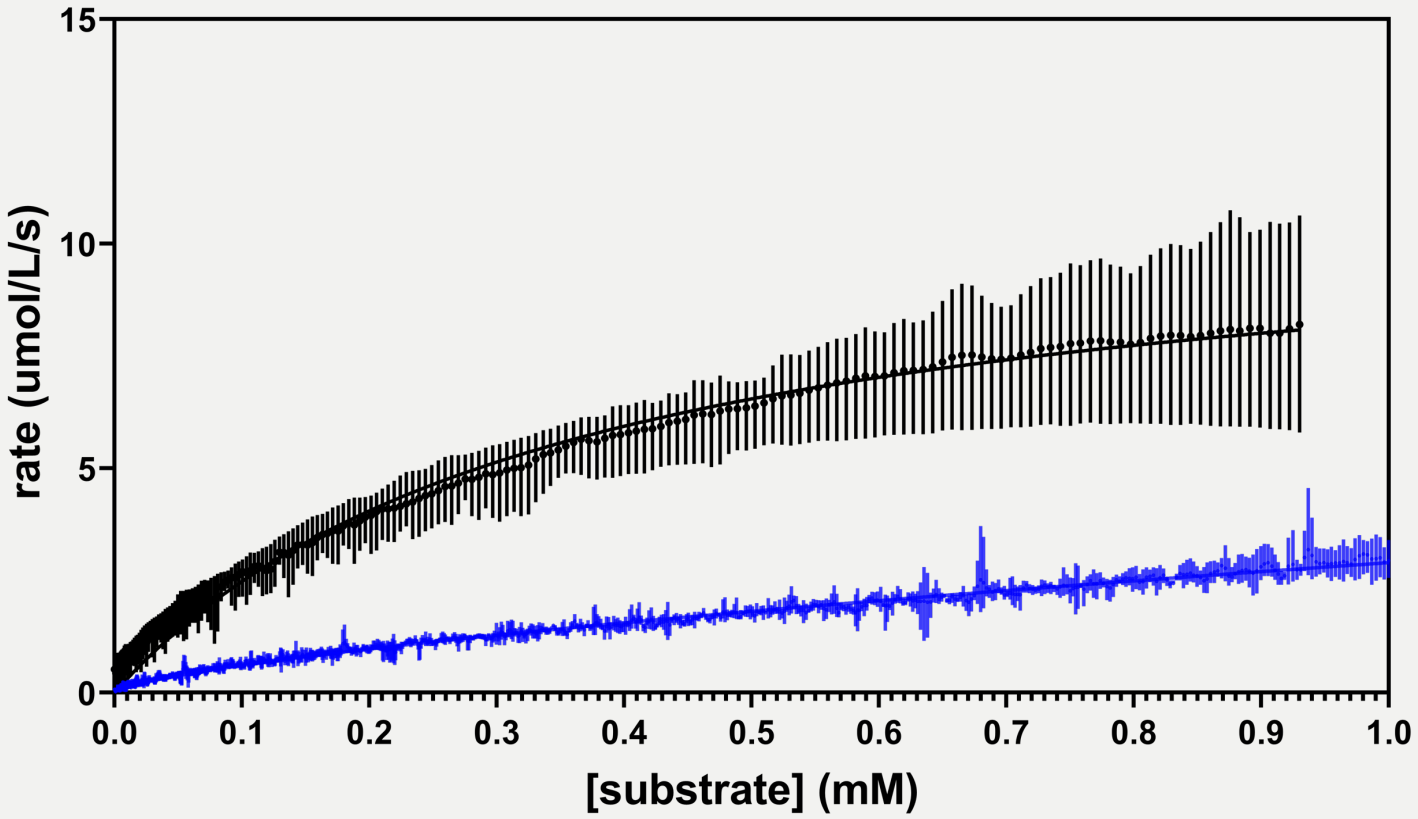
Predicted Proton NMR



Kinetics assay

- Isothermal titration calorimetry (ITC) experiments were performed in
  - *50 mM HEPES buffer at pH 7.5, containing 150 mM NaCl, 5 mM TCEP, 15 mM $MgCl_2$ and 25% glycerol*
  - With care taken to avoid metal contamination from glassware
- Single injection of concentrated DesD enzyme for a final concentration of 1 mM
- The binding cell contained 1 mM substrate and 5 mM ATP, or 1 mM substrate and 0.5 mM ATP
- The difference in thermal power (dQ/dt) was collected continuously as the substrate was fully depleted

Kinetics assays



- wild type kinetics of DesD with 1mM dfoG(black), HSC(blue), and 5 mM ATP.
- dfoG - Michaelis-Menton or hyperbolic curve
- HSC - cooperative curve?

Substrate	K_{cat} / s^{-1}	K_m / mM
dfoG	11.11	0.349
HSC	5.416	1.024

Conclusions

HSC synthesis has been confirmed with NMR

Kinetics of DesD with HSC are significantly different from dfoG

Next Steps

- Completion of gram scale synthesis testing different reaction conditions to increase yield
- Testing of DesD variants with HSC

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References

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