Insight into L-DOPA dioxygenase mechanism with 6-substituted L-DOPA derivatives

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Abstract

Dioxygenase enzymes are essential protein catalysts for the breakdown of catecholic rings, structural components of plant woody tissue. This powerful chemistry is used in nature to make natural products as well as degrade plant material, but we have a limited understanding of substrate space and mechanism across the superfamily. To this end, we report the syntheses, redox potentials and pKas of L-3,4-dihydroxyphenylalanine (L-DOPA) derivatives substituted at the 6-position and their characterization as substrates of L-DOPA dioxygenase from lincomycin biosynthesis in *Streptomyces lincolnensis*. In particular, the spectroscopic properties of 6-nitroDOPA provide insight into the steps of the enzymatic mechanism. The applications for dioxygenase cleavage of 6-substituted L-DOPA derivatives to natural product biosynthesis will be discussed. (Program number 498.13)

Background and Motivation

- The goal of this research is to elucidate the mechanism of L-DOPA dioxygenase (**Fig. 1**), an enzyme from the vicinal oxygen chelate family, which cleaves the aromatic ring of catechols (**Fig. 2**). [1]

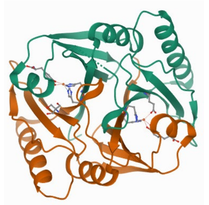

Figure 1. Structure of L-DOPA dioxygenase (*Streptomyces sclerotialus*)

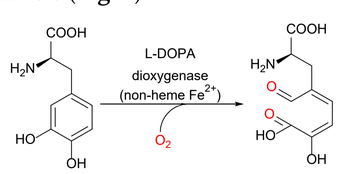

Figure 2. L-DOPA dioxygenase's mechanism on L-DOPA

- Knowledge of this mechanism is limited; dopamine and L-DOPA derivatives (**Fig. 3**) can be used to test L-DOPA dioxygenase interactions with catechol derivatives.

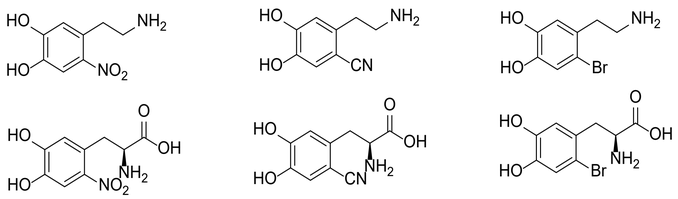

Figure 3. Dopamine and L-DOPA derivatives of interest. All substituents at the 6-position. From left to right: nitro-dopamine, cyano-dopamine, bromo-dopamine, nitroDOPA, cyanoDOPA, bromoDOPA.

- Chemists cannot easily open aromatic rings in the manner L-DOPA dioxygenase can.
- Clarification of the mechanism of action could lead to the use of L-DOPA dioxygenase in bioremediation and lignin breakdown.[2,3]
- Potential applications in natural product biosynthesis include the syntheses of lincosamide antibiotics and secondary metabolites .[4,5]

References

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In Silico Docking of L-DOPA derivatives

L-DOPA derivatives were docked into the active site of L-DOPA dioxygenase (PDB: 6ON3) using UCSF Chimera and AutoDock Vina (**Fig. 4**). Lower docking scores represent higher binding affinities.

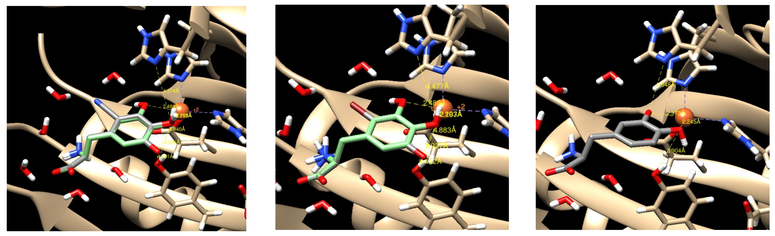

Figure 4. L-DOPA derivatives docked into the L-DOPA dioxygenase active site. L-DOPA amines in ionized form. Water molecules included. From left to right: nitroDOPA, cyanoDOPA, bromoDOPA. Docking scores are -7.3, -7.4, and -6.5 respectively. The control docking score for L-DOPA is -6.8

Scheme 1: Single step synthesis of 6-NitroDOPA

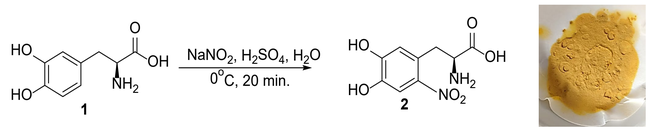

Figure 5. Compound **2** pictured during precipitate filtration

Scheme 2: Proposed multi-step synthesis of 6-CyanoDOPA

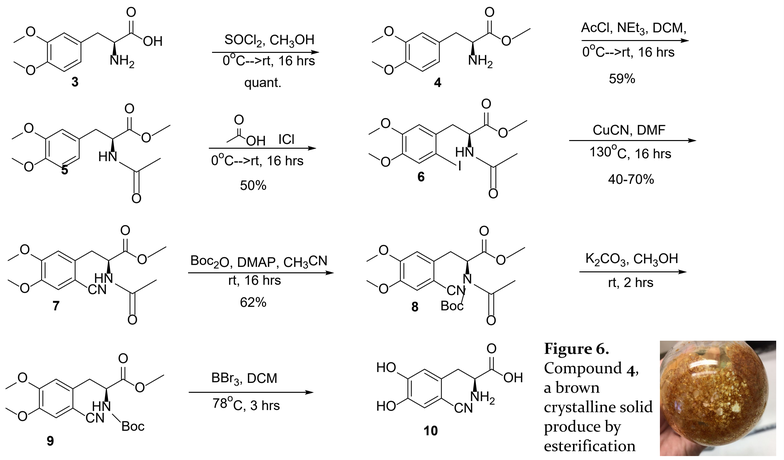

Figure 6. Compound **4**, a brown crystalline solid produce by esterification

Hammett constants

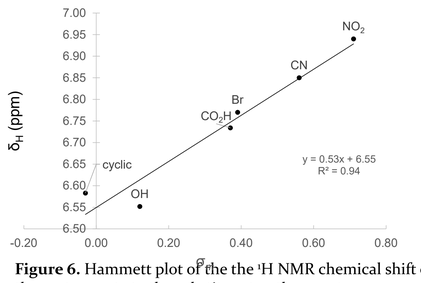

Figure 6. Hammett plot of the the ^{1}H NMR chemical shift of the proton meta to the substituent vs the σ meta

Strong linear correlation between Hammett constants and ^{1}H NMR chemical shift value shows the substituents have the expected electron-donating/ withdrawing effects.

Scheme 3: Proposed multi-step synthesis of 6-BromoDOPA

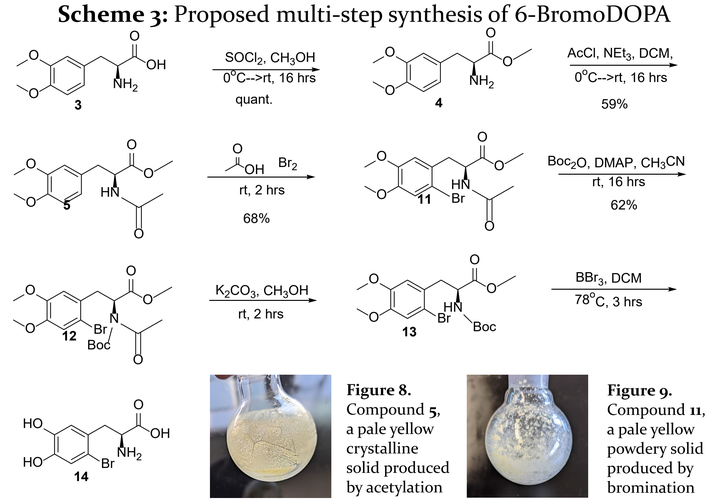

Figure 8. Compound **5**, a pale yellow crystalline solid produced by acetylation

Figure 9. Compound **11**, a pale yellow powdery solid produced by bromination

Redox potentials and pKa

Proton abstraction and oxidation are important mechanistic steps in L-DOPA dioxygenase catalysis.

Table 1. $E_{1/2}$ (mV) and pK_a values of dopamine and dopamine derivatives

Compound	pK_a	$E_{1/2}$ (mV)
Dopamine (DA)	8.99 ± 0.13	115.5
6-bromoDA	8.15 ± 0.11	156
6-cyanoDA	7.23 ± 0.09	259
6-nitroDA	6.32 ± 0.03	-

Conclusions

- Computational docking shows that nitroDOPA and cyanoDOPA are likely to bind to L-DOPA dioxygenase more strongly than L-DOPA.
- Compound **2** synthesis is complete. Ongoing work to improve the yield for this reaction continues.
- Compounds **10** & **14** syntheses are in progress. Methyl ester is lost during deacetylation therefore compounds **9** and **13** were not successfully created. New synthesis attempts involve the creation of different esters to protect the carboxylic acid functional group.
- All completed compounds and intermediates have been confirmed with ^{1}H and ^{13}C NMR.
- Substituent effects confirmed with dopamine derivatives. A similar trend is expected with the L-DOPA derivatives.
- $E_{1/2}$ and pK_a provide insights into the L-DOPA dioxygenase mechanism because deprotonation and oxidation are integral steps.

Acknowledgements

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