Synthesis of Disulfonates as DapE Inhibitors Toward the Creation of Novel Antibiotics

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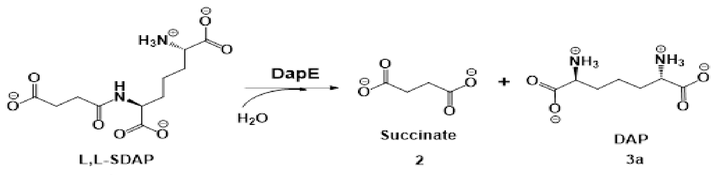
Introduction

In the 21st century, a global antibacterial resistance crisis has risen and is threatening the lives of millions. In addition, it has been determined that antibiotic resistance has added tremendous financial and clinical burdens on the U.S. health care system, patients and their families. The rise of this crisis has in part been attributed to the lack of new drug developments in the field of antibiotics as well as the overuse and misuse of existing medications. A novel bacterial pathway must be discovered and exploited in the fight to combat the antibiotic resistance crisis.

Abstract

DapE is an essential bacterial enzyme in the biosynthetic pathway responsible for the production of meso-diaminopimelic acid (m-DAP) and the amino acid lysine, which are critical to bacterial survival[1]. DapE is an ideal drug target because the human body does not employ the DapE enzyme to manufacture lysine; humans must consume lysine in their diets. Thus, the elimination of mechanism-based toxicity, combined with the discovery of multiple potential inhibitors in our lab for this enzyme, makes DapE a great target to focus on toward the discovery of new antibiotics (Scheme 1). This research has proven to be fruitful, but little is known about DapE and its properties. Synthesis of disulfonates will advance our understanding of the DapE enzyme in exploring the impact of a conformational change triggered by substrate binding means for the catalytic mechanism of the DapE enzyme[2].



Scheme 1: DapE enzymatic reaction.

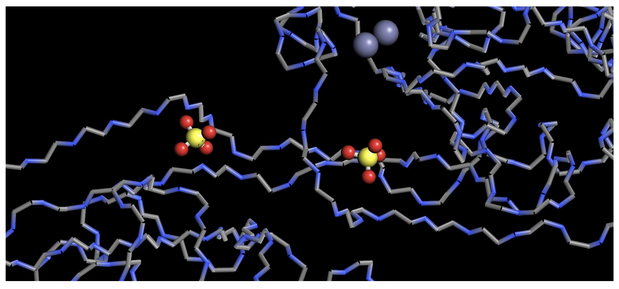
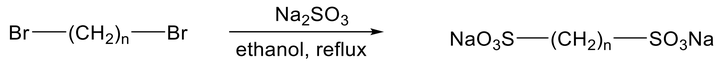


Figure 1: Two sulfates bound to the active site of DapE (PDB 5UEJ). The two zinc atoms in the active site are visible as silver spheres.

We observed the presence of two sulfate groups in the active site of DapE (Figure 1). The distance between the sulfates is 12.3 Å, which is farther than the distance between the negatively charged moieties of the substrate (~10 Å). We hypothesize that the two negative charges anchor the substrate, and that the shorter distance "pulls" the active site from the open into the closed form, enabling the catalytic mechanism to occur. The goal of this research is to mimic these two key negative charges with alkylene-linked disulfonates, varying the distance between them to discover whether the enzyme will be inhibited by these synthetic substrate mimetics, and whether DapE will be inhibited by the analogs.

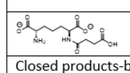
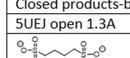
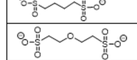
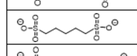
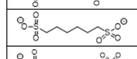
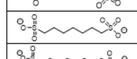
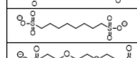
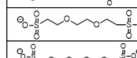
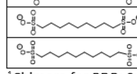
Methodology

The production of disulfonates is in accordance with a published procedure using dibromides as the starting material in a reaction to created disulfonates of varying chain lengths[3].



n = 4,5,6,7,8

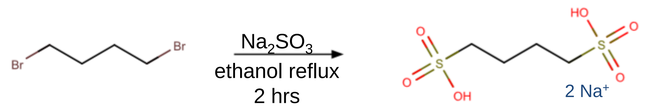
Scheme 2: Synthesis of disulfonates of varying chain lengths.

Structure	# Linker atoms	Atoms	Distance (Å)[1]	Effect/Goal
	9	C-to-C	10.601	Substrate inducing close
Closed products-bound	≥9	C-to-C	10.639	Closed but hydrolyzed
5UEJ open 1.3A	NA	S-to-S	12.31	Open relaxed
	4	S-to-S	6.889	
	5	S-to-S	7.850	
	5	S-to-S	8.115	
	6	S-to-S	9.394	Induce open to closed
	7	S-to-S	10.631	Induce open to closed
	8	S-to-S	11.906	Start to close
	8	S-to-S	11.906	Start to close
	9	S-to-S	13.149	Binds to open relaxed
	10	S-to-S	14.422	

[1]Chimera for PDB; Spartan minimized structure for individual small molecules

Table 1: Comparison of carboxylates and sulfonates bound in open and closed forms of DapE, and targeted bis-sulfonates.

Results



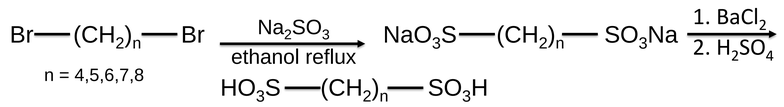
Scheme 3: Synthesis of butane-1,4-disulfonate.

- Butane-1,4-disulfonate was successfully synthesized (Scheme 3).
- Proton NMR demonstrated that the product mixture contains the desired molecule in it but contains some impurities (Table 1).
- The synthesis of disulfonates using dibromides as starting material appears to be a viable synthetic avenue.

Component	Proton	Multiplicity	Peak
Butane-1,4-disulfonate	CH2	3	1.2 ppm
	CH2	3	3.4 ppm

Table 1: ^{1}H NMR Data (D_2O)

- If necessary, we may purify with barium salt as shown in Scheme 4.



n = 4,5,6,7,8

Scheme 4: Synthesis of disulfonate and purification

Future Plans

- Purify product using either a titration with ether, a recrystallization from alcohol, or a barium chloride dihydrate solution washed and treated with sulfuric acid.
- Test the product in the enzymatic assay developed in our lab[4].
- Travel to Argonne National Laboratory in nearby Lemont to work with our collaborators to co-crystalize the molecules to determine their effects on the structure of DapE.
- Run molecular docking simulations using Molecular Operating Environment (MOE) to determine the molecules' exact effects on DapE when binding in the active site, in hopes of determining a possible mechanism for inhibition and for shifting the enzyme from the open conformation to the closed.
- Synthesize five, six, seven, and eight carbon-linked disulfonates using correlating dibromides.
- Initial Molecular Dynamics results suggest that the all-carbon linker sulfonates form poor interactions with intervening amino acid residues between the two charged binding sites. Therefore, we plan to synthesize PEG linked disulfonates.

Acknowledgements

I give sincere thanks and appreciation to Dr. Daniel Becker for giving me the opportunity to join his research lab and for mentoring me. This research is also supported by Thahani S. H. Mohammad, who assisted me. I would also like to show gratitude to Loyola University Chicago and the Department of Chemistry and Biochemistry for funding our research lab and making this project possible.

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2. Gillner D, Becker DP, Holz RC. Lysine biosynthesis in bacteria: a metallodesuccinylase as a potential antimicrobial target. *Journal of Biological Inorganic Chemistry (JBIC)* **2013**, *18*, 155-163.
3. Bharti S, Jacob J, Ghosh AK. Polyaniline doped with α, ω-alkanedisulfonic acids: Preparation and characterization**.** *Polymer International,* **2013**, *62*, 797-803.
4. Heath TK, Lutz Jr MR, Reidl CT, et al. Practical spectrophotometric assay for the dapE-encoded N-succinyl-L,L-diaminopimelic acid desuccinylase, a potential antibiotic target. *PloS one*. **2018**,*13*, e0196010.