4-1-2022

Microwave-Assisted Synthesis of Quinoxaline Derivatives

Eric Horsting
University of Dayton

Microwave-Assisted Synthesis of Quinoxaline Derivatives

Honors Thesis

Eric Horsting

Department: Chemistry

Advisor: Judit Beagle, PhD

April 2022

Microwave-Assisted Synthesis of Quinoxaline Derivatives

Honors Thesis

Eric Horsting

Department: Chemistry

Advisor: Judit Beagle, PhD

April 2022

Abstract

Quinoxaline and its derivatives have been studied extensively for their relevant biological activity and transition metal selectivity. These compounds are commonly used for their antimicrobial, antifungal, antiparasitic activity, and relevance in the treatment of metabolic diseases [6]. More recently, quinoxaline's ability to inhibit gram-positive bacterial growth has been found especially in oxygen-containing compounds, yielding promising candidates to prevent cancerous tumor growth [5]. The breadth of quinoxaline makes it a valuable tool within the research community However, its synthesis requires complex solvents, extended reaction times, and often produces a low yield. By using a microwave-assisted synthesis, this novel methodology offers high yields in a solvent-free environment, with only 5 minutes gestation.

University *of* Dayton

Table of Contents

Introduction

Quinoxaline and its derivatives have been studied extensively for their relevant biological activity and transition metal selectivity. Some applications for quinoxaline derivatives in industrial settings include agriculture, fluorescent materials, dyes, electroluminescent materials, organic semiconductors, organic light-emitting devices [3]. Further, quinoxaline derivatives are of particular interest for their biological applications. Oxygen-containing quinoxaline derivatives have been used to prevent the growth of cancerous tumors [2]. Finally, researchers have also identified quinoxaline derivatives as promising candidates for the inhibition of RhoA proteins. These proteins are synthesized in the body and responsible for a diverse cellular signaling pathway linked with the development of cardiovascular disease. Thus, quinoxaline derives are a promising therapeutic against cardiovascular disease due to their ability to inhibit the synthesis of RhoA proteins [1].

Quinoxaline derivatives have been synthesized via multiple pathways. However, this experiment will explore their synthesis specifically through the nucleophilic aromatic substitution (NAS) pathway with chloride leaving groups. Literature indicates that this NAS step is a frequent pain point when developing novel quinoxaline derivatives for relevant applications. These reactions require substantial time, energy, and complex catalysts to effectively produce the desired product. This project addresses and improves upon this method by utilizing a microwave methodology which requires only 5 minutes at 160 °C, with no catalysts. Using both oxygen and nitrogen containing nucleophiles, this microwave methodology seeks to show the general use of these conditions, while

improving the yields [1]. A summary of benefits of this microwave-assisted synthesis is given in table 1.

Table 1: Comparison of Microwave-Assisted Synthesis and Literature Methodologies

Microwave-Assisted Synthesis	Literature Methodologies
● Reaction occurs in a solvent-free environment, reducing cost and mitigating environmental burden associated with waste ● Takes only 5 minutes to perform synthesis ● Produces higher yields than other methods in the literature	● Often requires hazardous chemicals, which can be expensive, dangerous to handle, and harmful to the environment ● Cited as having a 30-minute gestation in literature methods [1] ● Lower yields

Methods

Compound **3** is the starting material for the microwave assisted synthesis. Before the microwave-assisted synthesis could be performed using nuanced substituents, a sufficient stockpile of staring material was created using the synthetic pathway outlined in in Figure 1.

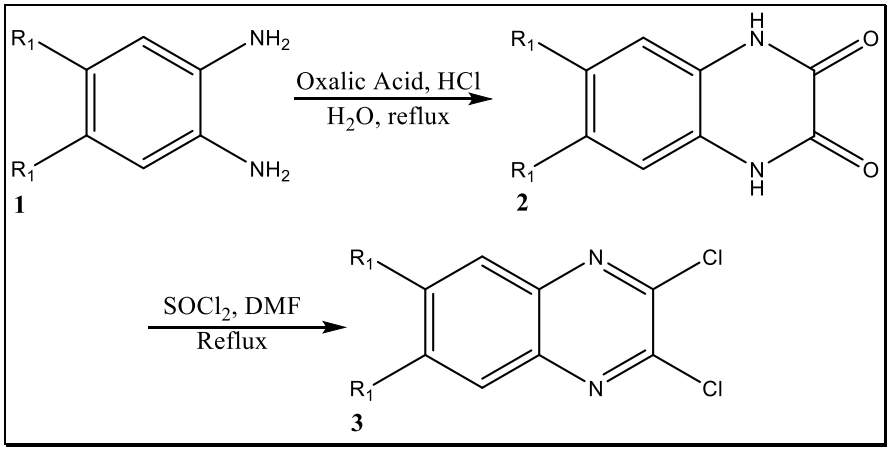


Figure 1: Synthesis of Starting Material

Figure 2 shows the primary synthetic pathway for the synthesis of quinoxaline derivatives using a microwave-assisted synthesis methodology.

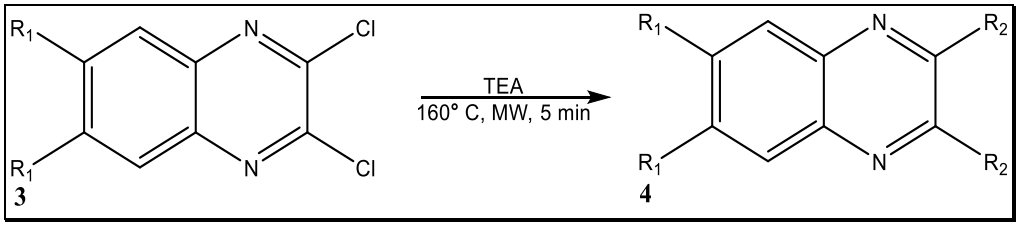


Figure 2: Microwave-Assisted Synthesis

The reaction shown in Figure 2 was trials for many different R2 substituents. Table 2 shows generalized reagent table in molar equivalent.

Table 2: Microwave-Assisted Synthesis Reagents

Compound	**Molar Equivalents**
Compound **3**	1 mmol
R2	2 mmol
Triethylamine	3 mmol

Using a stockpile of Compound **3,** the microwave-assisted synthesis was repeated with a variety of oxygen and nitrogen-containing compounds. These reactions were run in molar quantities as shown in Table 1. After the reaction, TLC was used to determine whether the product still had some of the starting material. If so, another quantity of the substituent was added and microwaved again at 160 °C for 5 minutes. The product was later worked up and verified using an NMR. Further investigations with primary amine substituents were also explored. These experiments are further discussed in future work.

Results

Table 3 depicts all synthesized compounds produced using nitrogen nucleophiles. These compounds were all found in relatively high yields.

Table 3: Nitrogen Results

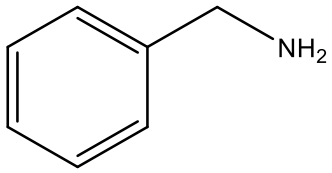
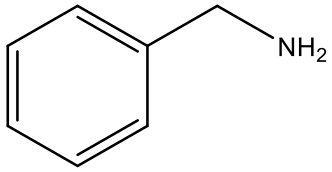
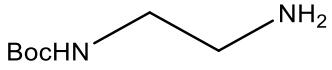
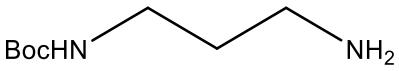
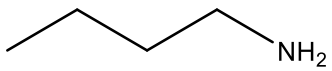
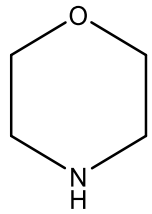
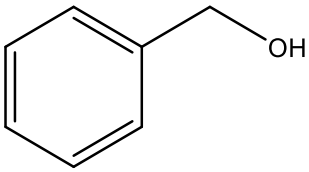
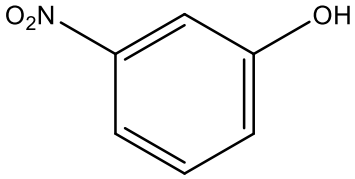
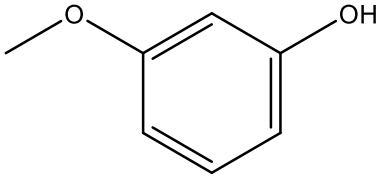
Component	R1	R2	Yield
Benzylamine a	H		69%
Benzylamine b	Methyl		46%
Tert-Butyl 3-Aminoproanoate c	Methyl		69%
Tert-Butyl (3-Aminopropyl) Carbamate d	H		49%
Butylamine e	H		81%

Table 4 depicts all synthesized compounds produced using oxygen nucleophiles. Generally, the yield for these compounds was lower compared to nitrogen nucleophiles.

Table 4: Oxygen Results

Component	R1	R2	Yield
Morpholine f	H		39%
Benzyl alcohol g	H		NA
3-Nitrophenol h	H		69%
3-Methoxyphenol i	H		14%

These results show that primary nitrogen nucleophiles tend to have the highest yield. Though secondary nitrogen nucleophiles are viable with the methodology, they greatly reduce yield. Results also conclude that functional groups on the R2 substituent have a large impact on the reactivity of the nucleophilic molecule.

Discussion

There were 3 key findings. Key finding **1** is that nitrogen nucleophiles tend to have higher yields than oxygen in this nucleophilic aromatic substitution (NAS) reaction. The average yield for successfully reacted nitrogen nucleophiles was 63%. For oxygen, this yield was only 41%. More specifically, the reaction using a morpholine substituent also demonstrated that nitrogen was a better nucleophile than oxygen. Morpholine contains nitrogen and oxygen; since nitrogen acted as the nucleophile during the NAS reaction (which was validated using NMR), it is concluded that nitrogen is the better nucleophile. The final indicator that nitrogen was a better nucleophile than oxygen using microwave NAS chemistry was that benzylamine reacted in high yield. However, benzyl alcohol which has the same structure with a different nucleophile, did not react at all. This suggests that benzyl alcohol was limited by the nucleophilicity of oxygen.

Key finding **2** is that secondary nitrogen nucleophiles are viable using microwave-assisted NAS chemistry, though yields are reduced due to increased steric hindrance. This was evidenced primarily through morpholine, the only secondary amine which was reacted with the starting material. Morpholine had a 39% yield, which was lower than all primary amines.

Key finding **3** is the importance of functional groups on the nucleophilic substituent. This importance is manifest in the differences between benzyl alcohol and 3-nitrophenol. Each reagent involves a oxygen nucleophile which attaches to dichloroquinoxaline. Yet, benzyl alcohol didn’t react while 3-nitrophenol had a 69% yield. This is due to the resonance stabilization of the 3-nitrophenol’s conjugate base,

which increases the acidity of 3-nitrophenol. As the acidity of a compound increases, so does its reactivity. This explains the differences between the two, yet there's also a stark difference in yield between 3-nitrophenol and 3-methoxyphenol. As shown, both compounds have oxygen nucleophiles; the only difference resides in the functional groups which are not actively participating in NAS chemistry. In the case of 3-nitrophenol, the nitro group acts as an electron withdrawing group. Conversely, the methoxy group on the 3-methoxyphenol acts as an electron donating group. For NAS chemistry to work, compounds require some sort of electron withdrawing group to increase reactivity. Thus, the nitro group enhances the reactivity of the nucleophile while methoxy decreases reactivity. In fact, the only reason that the 3-methoxyphenol nucleophile gave a 14% yield can be attributed to the electron withdrawing nature of nitrogen in the starting material. This reaction is rarely accomplished in literature, so even this relatively low yield is a demonstration that the microwave-assisted synthesis is an effective method for performing this NAS chemistry.

Future Work

The proposed future work is shown in the Figure 3. It is important to recognize that the first two steps of the propose synthetic pathway have already been completed. The first step depicts the microwave assisted synthesis, which seeks to add a diamine nucleophile with a carbon-chain of variable length. Nucleophiles c and d show the yield for both a 2-carbon chain and 3-carbon chain.

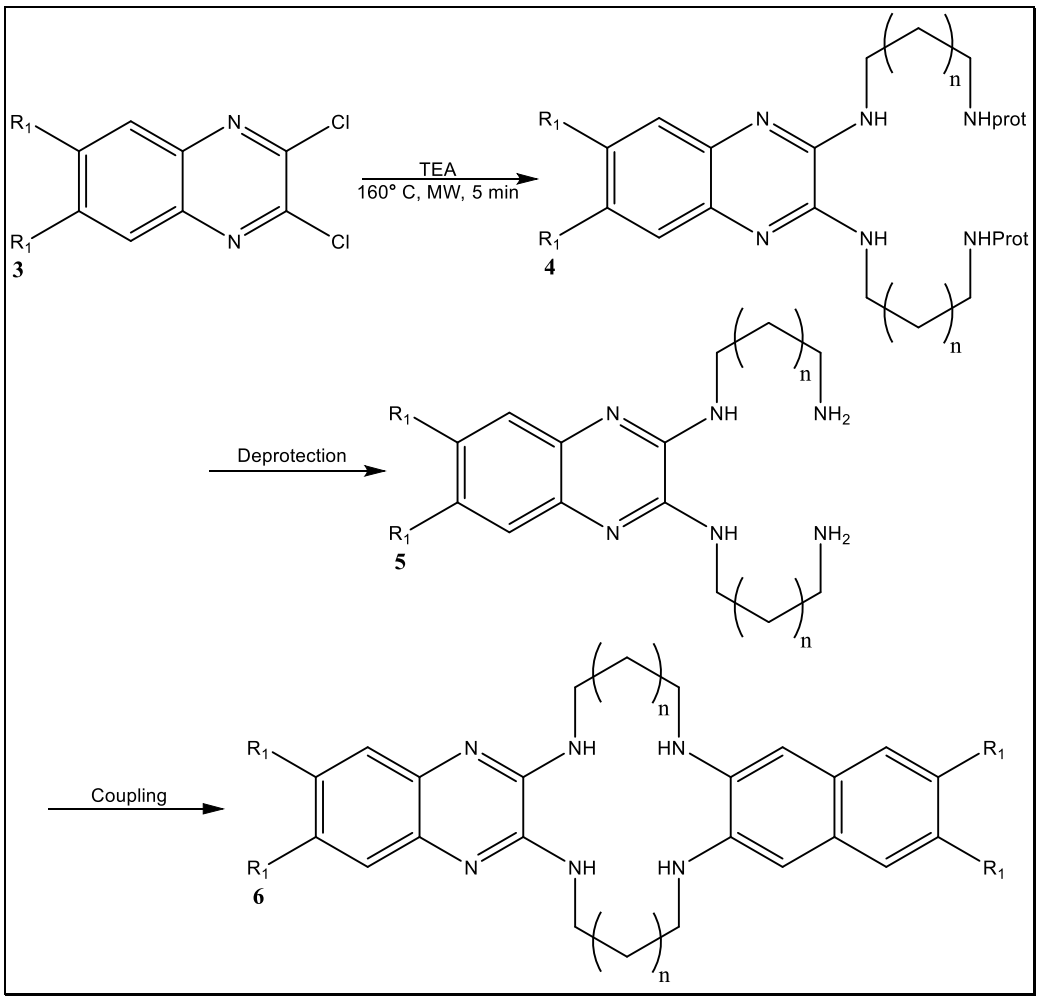
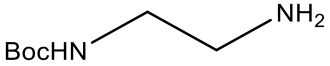
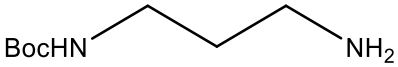


Figure 3: Proposed Synthetic Pathway

The second step is a deprotection of this disubstituted quinoxaline derivative. Yields of this step are given in Table 5.

Table 5: Deprotection Results

Component	R	Yield
Tert-Butyl 3-Aminoproanoate a		100%
Tert-Butyl (3-Aminopropyl) Carbamate b		89%

The only step which has not been completed is the coupling to create compound **6**. The resulting peraza crown unit can create a ligand with transition metal ions in water and soil as a decontaminate without a transition metal template.

Conclusion

The microwave-assisted methodology is an effective method for creating relevant quinoxalines derivatives. Advantages of this method include the absence of costly and harmful solvent, along with the shortened reaction time compared to common practice. Despite these advantages, we report yields like what is obtained in literature.

Experimental Methods and Data

NMR spectra were obtained in $CDCl_3$ or DMSO-d_6. Operating at 400 MHz with TMS as the internal standard. All microwave assisted reactions were carried out with a single mode cavity CEM Discovery Microwave Synthesizer. Purification was accomplished using Teledyne-Isco Combiflash flash chromatography system. All commercially available materials were used without further purification.

Synthesis of Protected Amines

Ethylene diamine was dissolved in 250mL $CHCl_3$. Benzyl chloroformate, 2,2,2-trichloroethoxycarbonyl chloride, or Boc anhydride were dissolved separately in 125mL $CHCl_3$. The protecting group solution was added dropwise into the ethylenediamine, and the resulting mixture was stirred overnight at room temperature in a sealed flask. After stirring overnight, the mixture was extracted three times using water and was dried over sodium sulfate. The product was then dried under a vacuum. The yields can be found in Table 1. Analytical data is consistent with those reported in the literature (Cal, 2013; Holland, 2010)

Synthesis of Compound **2**

Oxalic acid (5.4g, 60mmol) was dissolved in water and was heated to 95°C. 10mL of concentrated HCl were added, and then *o*-phenylenediamine (5.4g, 50mmol) was added to the reaction mixture. The temperature was maintained at 98-100°C for 15 minutes. The mixture was taken off heat, and ~60g of ice were added. The precipitate was filtered, washed with water, and then dried over vacuum. The reaction yielded 6.4711g (39.4mmol)

of 1,4-dihydroquinoxaline-2,3-dione (**2a**) in a 79% yield (6.4711g, 39.4mmol). 6,7-dimethyl-1,4-dihydroquinoxaline-2,3-dione (**2b**) was synthesized using the same procedure to yield 5.8 g (30.8 mmol) product. Analytical data is consistent with those reported in the literature (Komin, 1976)

Synthesis of Compound **3**

Compound **2** (4g, 25mmol) was added to a solution of $SOCl_2$ and DMF (12.5mL $SOCl_2$, 172mmol; 0.25mL DMF). The mixture was refluxed until the solid dissolved (2 hours). Ice was added, and the precipitate was filtered using water and dried under vacuum to give 4.13g (20.7mmol) of 2,3-dichloroquinoxaline at a yield of 83%. Synthesis of 2,3-dichloro-6,7-dimethylquinoxaline (**3b**) was carried out under the same conditions to give the product in 97% yield. Analytical data is consistent with those reported in the literature (Komin, 1976)

General procedure for compound **4**

Dichloroquinoxaline (0.2g, 1mmol) was added to a microwave tube with the nucleophile (2mmol) and triethylamine (0.4mL, 3mmol). The reaction mixture was added to a microwave for 5 minutes at 160°C. The resulting mixture was extracted and dried using solidum sulfate and a rotavapor until it became solid to give compounds 4a-i.

N2,N3-dibenzylquinoxaline-2,3-diamine (4a): yellow solid (69%), 168.8-172.6°C, ^{1}H-NMR ($CDCl_3$) δ: 4.58 (brs, 2H), 4.73 (d, J = 4 Hz, 4H), 7.26-7.44 (m, 12H), 7.65-7.72 (m, 2H). ^{13}C-NMR ($CDCl_3$) δ: 46.04, 125.04, 125.85, 127.66, 128.05, 128.57, 128.73, 137.24, 138.70, 144.15.

N2,N3-dibenzyl-6,7-dimethylquinoxaline-2,3-diamine (4b): dark yellow solid (46%), 107.0-110.0°C, ^{1}H-NMR ($CDCl_3$) δ: 2.38 (d, J = 4.08 Hz, 6H), 4.49 (brs, 1H), 4.70 (d, J = 4.4 Hz, 2H), 4.77 (d, J = 6 Hz, 2H), 5.70 (brs, 1H), 7.27-7.43 (m, 10H), 7.52 (s, 1H), 7.54 (s, 1H). ^{13}C-NMR ($CDCl_3$) δ: 19.82, 19.83, 45.58, 46.06, 125.66, 125.81, 127.26, 128.55, 128.69, 128.77, 135.37, 135.58, 139.89, 140.37.

((3-((3-(tert-butoxy)-3-oxopropyl)amino)-6,7-dimethylquinoxalin-2-yl)amino)methyl 3,3-dimethylbutanoate (4c): dark yellow solid (69%), 147.0-150.0°C, ^{1}H-NMR (DMSO-d_6) δ: 1.44 (s, 18H), 3.46 (q, J = 4.8 Hz, 4H), 3.65 (q, J = 4.7 Hz, 4H), 7.25-7.26 (m, 2H), 7.36-7.38 (m, 2H). ^{13}C-NMR (DMSO-d_6) δ: 19.73, 28.40, 40.99, 43.10, 125.37, 133.69, 143.95.

di-tert-butyl 4,4'-(quinoxaline-2,3-diylbis(azanediyl))dibutyrate (4d): light yellow solid (49%), 156.0-159.2°C, ^{1}H-NMR ($CDCl_3$) δ: 1.48 (s, 18H), 1.84 (quint, J = 6.2 Hz, 4H), 3.24 (q, J = 6.16 Hz, 4H), 3.68 (q, J = 5.84 Hz, 4H), 5.65 (brs, 2H), 5.84 (brs, 2H), 7.25 (dd, J = 3.28, 2.8 Hz, 2H), 7.62 (dd, J = 3.44, 2.6 Hz, 2H). ^{13}C-NMR ($CDCl_3$) δ: 28.48, 29.68, 37.24, 37.66, 79.59, 124.21, 125.14, 136.74, 144.26, 156.93. Anal. Calcd for molecular formula: C, 60.74; H, 8.07; N, 17.71. Found: C, 60.82; H, 8.06; N, 17.46.

N2,N3-dibutyl-6,7-dimethylquinoxaline-2,3-diamine (4e): yellow solid (81%), 102.0-104.4°C, ^{1}H-NMR ($CDCl_3$) δ: 0.99 (t, J = 7.36 Hz, 6H), 1.42-1.51 (m, 4H), 1.64-1.72 (m 4H), 3.53-3.57 (m, 4H), 4.31 (brs, 2H), 7.27-7.33 (m, 2H), 7.60-7.66 (m, 2H). ^{13}C-NMR ($CDCl_3$) δ: 13.86, 20.28, 31.25, 40.95, 114.71, 123.82, 124.64, 125.69, 137.19, 144.68. Anal. Calcd for molecular formula: C 70.55, H 8.88, N 20.57, Found: C 70.43, H 8.79, N 20.36.

2,3-dimorpholinoquinoxaline (4f): tan solid (39%), 220.0-223.9°C, ^{1}H-NMR ($CDCl_3$) δ: 3.59 (t, J = 4.72 Hz, 8H), 3.87 (t, J = 4.64 Hz, 8H), 7.44 (dd, J = 3.44, 2.76 Hz, 2H), 7.73 (dd, J = 3.52, 2.76 Hz, 2H). ^{13}C-NMR ($CDCl_3$) δ: 47.72, 66.74, 126.56, 126.59, 137.89, 148.14.

2,3-bis(3-nitrophenoxy)quinoxaline (4h):brown solid (69%), 198.0-200.8°C, ^{1}H-NMR ($CDCl_3$) δ: 7.55 (m, 2H), 7.66-7.76 (m, 6H), 8.19-8.25 (m, 2H), 8.31 (t, J = 2.2 Hz, 2H). ^{13}C-NMR ($CDCl_3$) δ: 117.54, 120.69, 127.06, 128.20, 128.57, 130.26, 137.34, 147.88, 149.06, 152.82.

2,3-bis(3-methoxyphenoxy)quinoxaline (4i): light brow solid (14%), 122.0-122.4°C, ^{1}H-NMR ($CDCl_3$) δ: 3.84 (d, J = 1.6 Hz, 6H), 6.83-6.97 (m, 5H), 7.37 (t, J = 9.2 Hz, 1H), 7.47-7.51 (m, 1H), 7.59-7.77 (m, 4H), 7.95-8.00 (m, 1H). ^{13}C-NMR ($CDCl_3$) δ: 55.48, 107.56, 111.38, 113.70, 126.99, 127.58, 128.30, 130.00, 137.54, 139.22, 149.05, 153.52, 160.66.

Synthesis of Product 5

Compounds 4c and 4d was dissolved in 10 mL of acetone with a large stir bar. Concentrated sulfuric acid was added dropwise to form a precipitate. This product was filtered and washed with acetone, and then was dried on a high vacuum to give compounds 5a-b.

***N*^{1}, *N*^{1}'-(quinoxaline-2,3-diyl)bis(ethane-1,2-diamine)** (5a): yellow solid (100%), >260°C, ^{1}H-NMR ($CDCl_3$) δ: 2.10 (Quint, J = 8 Hz, 4H), 3.08 (t, J = 7.6 Hz, 4H), 3.58 (t, J = 7.6 Hz, 4H), 7.28-7.34 (m, 2H), 7.45-7.51 (m, 2H). ^{13}C-NMR ($CDCl_3$) δ: 25.26, 37.05, 120.35, 126.69, 126.97, 127.95, 142.48.

***N*^{1},*N*^{1}-(quinoxaline-2,3-diyl)bis(propane-1,3-diamine)** (5b): tan solid (89%), decomposition at 220°C, 1H-NMR ($CDCl_3$) δ: 2.04 (quint, J = 30 Hz, 5H), 2.99 (d, J = 6 Hz, 4H), 3.67 (t, J = 15 Hz, 4H), 7.42 (quint, J = 12 Hz, 2H), 7.72 (t, J = 6 Hz, 2H). ^{13}C-NMR ($CDCl_3$) δ: 25.59, 26.17, 36.69, 37.28, 126.24, 142.84.

Acknowledgements

I would like to thank Dr. Judit Beagle for her mentorship and friendship over the past four years. She has helped shape my academic interests and professional pursuits. Alex Farmer, Jacob Buechele, and Alexis Smith have also been instrumental in this success of this project. I would also like to thank Dr. Dorf, Gina Lloyd, and the honors program for their support in this thesis process. My final thanks go to my parents, who have always supported me in all my endeavors.

References

[1] Deng, J., Feng, E., Ma, S., Zhang, Y., Liu, X., Li, H., . . . Li, J. (2011). Design and synthesis of small molecule rhoa inhibitors: A new promising therapy for cardiovascular diseases? Journal of Medicinal Chemistry, 54(13), 4508-4522. doi:10.1021/jm200161c

[2] Eissa, I. H., El-Naggar, A. M., El-Sattar, N. E. A. A., & Youssef, A. S. A. (2018). Design and Discovery of Novel Quinoxaline Derivatives as Dual DNA Intercalators and Topoisomerase II Inhibitors. *Anti-Cancer Agents in Medicinal Chemistry*, *18*(2), 195–209. https://doi.org/10.2174/1871520617666170710182405

[3] El-Atawy, M. A., Hamed, E. A., Alhadi, M., & Omar, A. Z. (2019). Synthesis and Antimicrobial Activity of Some New Substituted Quinoxalines. *Molecules*, *24*(22), 4198. https://doi.org/10.3390/molecules24224198

[4] Hancock, R. Macrocycles and their Selectivity for Metal Ions on the Basis of Size. *Pure and Appl. Chem.*, 1986. 58, 1, 1445-1452

[5] Patidar, A. K. (2011). Exploring Potential of Quinoxaline Moiety. International Journal of PharmTech Research, 3(1), 386-392.

[6] Pereira, J. A., Pessoa, A. M., Cordeiro, M. N. D. S., Fernandes, R., Prudêncio, C., Noronha, J. P., & Vieira, M. (2015). Quinoxaline, its derivatives and applications: A State of the Art review. *European Journal of Medicinal Chemistry*, *97*, 664–672. https://doi.org/10.1016/j.ejmech.2014.06.058