Design, Synthesis, and Characterization of a Threonine-Rich Macrocycle; A Review of "Introduction to Research"

by

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Submitted in partial fulfillment of the

requirements for Departmental Honors in

the Department of Chemistry and Biochemistry

Texas Christian University

Fort Worth, Texas

May 2, 2022

Design, Synthesis, and Characterization of a Threonine-Rich Macrocycle; A Review of "Introduction to Research"

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ACKNOWLEDGEMENTS

I would like to thank Professor Eric Simanek for providing me the initial opportunity to work on research with his laboratory group as well as assisting and guiding me on this project. Additionally, I would like to thank graduate students Casey Gardner and Alex Menke for their work in assisting me with laboratory techniques as well as the creation of this thesis. This work was supported by the National Institute of Health and the Robert A. Welch Foundation. Material in this thesis is also presented in a paper that will be submitted for publication in the *Journal of Organic Chemistry* (see reference below).

"The Emergence of Persistent Isomers by Increasing Steric Bulk: Synthesis and Analysis of Triazine Macrocycles Containing β-Branched Amino Acids" Manuscript in Preparation: Alexander Menke, Camryn Gloor, Liam Claton and Eric E. Simanek

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TERMINOLOGY AND ABBREVIATIONS

T-T - threonine-rich macrocycle

DMA – dimethylamine

PPI – protein-protein interactions

CSA – cyclosporin A

NMR – nuclear magnetic resonance spectroscopy

TLC – thin layer chromatography

MeOH – methanol

DCM – dichloromethane

BOC – boc-hydrazine

DMSO – dimethyl sulfoxide

THF – tetrahydrofuran

HOBT – hydroxybenxotriazole

EDC – 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide

DIPEA – N,NDiisopropylethylamine

TFA – trifluoroacetic acid

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CHAPTER 1: SYNTHESIS OF A THREONINE-RICH MACROCYCLE

ABSTRACT

This work describes the synthesis of a 24-atom, threonine-rich macrocycle homodimer, **T-T**. Syntheses of macrocycles are of interest due to their potential applications as drugs. If the synthesis design allows for a wide variety of different groups to be incorporated without affecting the structure itself, classical drug design strategies can be adopted. The benefits of macrocycles derive from their large and flexible structures that can adopt different conformations. This flexibility is important when the macrocycle is required to present either hydrophilic or hydrophobic surfaces when it is inside and outside the cell or crossing the membrane, respectively.

The synthesis of **T-T** is done in three steps and relied on making changes to a previously studied macrocycle synthesis pathway. First, a threonine acid intermediate is prepared by substituting a triazine ring with dimethyl amine, a *t*-butyl protected threonine and a BOC-protected hydrazine. Then, the acid is reacted to create the threonine acetal monomer. The final step involves treating the monomer with acid to yield the homodimer, **T-T**.

The macrocycle and its intermediates were purified through column chromatography and characterized via ^{1}H NMR, ^{13}C NMR, COSY NMR, rOesy NMR, and HSQC NMR. What emerges from these studies is the three-dimensional shape of **T-T.** ^{1}H NMR and ^{13}C NMR were also used to characterize the acid intermediate and the acetal monomer. Mass spectrometry also corroborated these assignments. This research adds to a growing library of similar macrocycles that vary in amino acid in the position of threonine with the eventual goal of creating a library of macrocycles for future research in the area of synthetic drug design.

INTRODUCTION

Macrocycles are cyclical compounds of increasing interest in the field of synthetic drug design. These molecules are typically large flexible structures and have the potential to exist in multiple conformations [1]. The macrocycle that is the target of interest for this research is a threonine-rich macrocycle. It is a homodimer synthesized over the course of multiple days using a three-step synthesis. This macrocycle joins a growing library of similar compounds with different amino acids substituted in the position of threonine. This growing library of macrocyclic compounds seeks to provide an alternative approach to drug discovery compared to the current system being used by the pharmaceutical companies.

Macrocycles break at least one rule within the established Lipinski's "Rule of 5" criteria which are used to predict the ability of drugs to passively diffuse through cell membranes. These rules include a molecular weight of less than 500 Da, a partition coefficient (log P) of less than 5, fewer than 5 hydrogen bond donors, and fewer than 10 hydrogen bond acceptors [2]. While macrocycles do not fit into the standard requirements of Lipinski's rules, certain macrocycles have been shown to demonstrate conformational flexibility [3]. Compounds with the ability to change conformation in different conditions are referred to as "molecular chameleons" which is a term used to highlight the way the molecule behaves depending on if it is in an aqueous or nonpolar environment [4]. Behaving as a molecular chameleon is required when large drugs are faced with the problem of cell permeability. Drugs must be able to pass through the hydrophobic phospholipid bilayer and be soluble in the hydrophilic extra- and intracellular milieu. Passive diffusion is the standard mode of cellular entry for compounds that are less than 1000 Da in mass [3]. If a large molecule, a macrocycle, is able to change its conformation to one that decreases water solubility

when crossing the cell membrane, then it will demonstrate an increased ability to penetrate that cell and maintain its overall aqueous solubility.

Macrocycles are specifically being looked at to target intracellular protein-protein interactions (PPI) [5]. The goal of these macrocycle drugs is to bind to the areas of PPI and disrupt the interaction or binding site. Traditionally drugs have been engineered using the enzyme binding pocket model as the drug target [6]. This phenomenon has led to a large number of structurally small drugs to be designed. The target of macrocyclic drugs are PPIs involving large, dynamic surfaces that are formed by secondary and tertiary protein structures as opposed to primary structure targets [7]. These surfaces are characterized as being between 1500-3000 $Å^2$[3].

Macrocycles have an advantage in targeting these protein surfaces due to their size. A smaller drug has a decreased surface area of possible interaction compared to a larger macrocycle which accounts for their success within the enzyme pocket biding model, however the specificity becomes a disadvantage when the site of binding is less specific [7]. Macrocycles with their larger size are able to form a greater number of contact points with the protein of interest. Macrocycles have been also been shown to demonstrate an antibody-like affinity when binding to these surfaces [7]. The characteristics of these compounds contribute to a growing amount of research on macrocycles for synthetic drug design.

Macrocycles are commonly found within nature and have already been utilized as drugs. Cyclosporin A (CSA) is an example of an orally bioavailable macrocyclic drug acting as a calcineurin inhibitor and used as an immunosuppressant medication [8]. CSA is a molecular chameleon in its ability change is conformation in a way that hides polarity when passing through the phospholipid bilayer and then changes its conformation by re-exposing its hydrophilic amide backbone [3]. While the ability to behave as a molecular chameleon is extremely beneficial when

designing larger drug compounds, the ability to predict chameleonic behavior is extremely difficult. This fact contributes to the need for a library of macrocyclic compounds in order to evaluate potential drug candidates. Another important characteristic of macrocyclic drugs being used today is the reliance on naturally derived macrocycles. For example, CSA is produced by the aerobic fungus *Tolypocladium inflatum*. There have been historic challenges with synthesis of macrocycles within the area of drug design due to their larger more complex structures [9]. It has been more commercially beneficial to design smaller synthetic drug compounds based on this fact. The use of microbes for production of complex naturally occurring compounds has proven beneficial historically, but this technique is not efficient for the continued exploration into macrocycles as drugs due to limitations in synthetic intractability and non-drug-like properties [10]. Development of a simplified synthesis process with the ability to produce a wide range of compounds is therefore critical in the continued search for new drugs.

Incorporating the amino acid threonine within the macrocycle target is the goal of this research. The use of the amino acid threonine within the macrocycle target is important due to the chemical characteristics threonine individually possesses along with its relevance within nature and more specifically proteins. Threonine possesses the chemical characteristics of an aliphatic and polar amino acid and is found at a higher distribution within naturally occurring proteins compared to over half of the other amino acids [11]. The significance of the increase in prevalence of threonine within peptides is unclear but is an important characteristic to acknowledge when looking to target PPIs. Threonine is additionally one of three β-branched amino acids along with valine and isoleucine although it differs from the other two because it contains a hydroxyl group which provides the possibility for additional hydrogen bonding either within the macrocycle or with protein targets.

Both valine and isoleucine have been previously incorporated into macrocycles through the same synthesis pathway described in this paper. When valine is substituted within the macrocycle, it was found to exist as one structural isomer species in DMSO-d_6. Isoleucine was found to exist in two conformations at a ratio of 6:4 between the two isomers. It is of interest to characterize the different structural isomers present within the threonine-rich macrocycle in order to better understand the effects of different amino acid substitution within the growing library of similarly derived macrocycles. β-branched amino acids are of additional interest when compared to other options because the branching structure contributes greater steric bulk to the overall macrocycle in comparison to other amino acids such as glycine or alanine. Chart 1 shows this paper's macrocycle target, referred to as **T-T**.

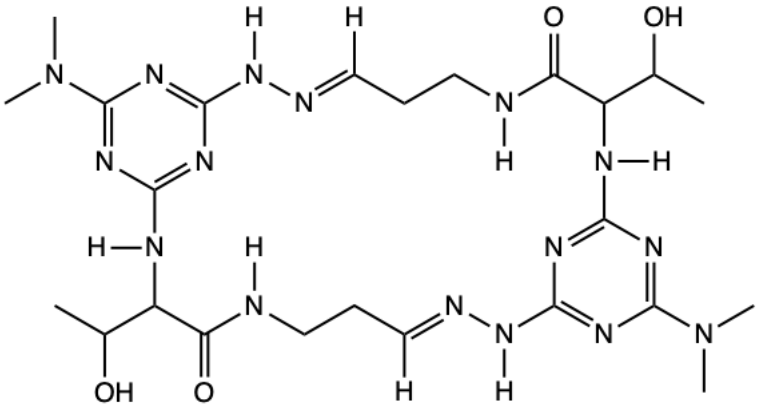


Chart 1 - The **T-T** macrocycle

The research contributes a compound to a growing library of similarly derived macrocycles produced by the Simanek laboratory. This library will be used to explore an alternative approach to drug discovery that differs from the current model which pharmaceutical companies currently use. The types of pharmaceutical drugs that would be investigated through this method would be those which are able to inhibit protein-protein interactions. This alternative model seeks to

generate a large library of similar compounds which can then be evaluated in protein binding assays in order to discover new potential drugs.

There is a need for an alternative approach to drug design based on two major issues facing pharmaceutical companies today. The first issue is the amount of money which drug makers are required to invest in order to develop a new medication that has market approval. A study from 2019 conducted by Tufts Center for the Study of Drug Development found that on average it was costing pharmaceutical companies $2.6 billion dollars in order to develop new prescription medication, with an annual inflation rate of 8.5% [12]. This continued increase in cost to produce new drugs means that pharmaceutical companies have to get a return on their investment for the medications they are developing. They are able to do this in two ways with the first being increased development in prescription medication that a large number of individuals will use, and the second being increased cost of drugs [13]. This still creates a barrier to drug development that is only readily accessible to pharmaceutical companies. The second important characteristic of pharmaceutical drug development is that a protein of interest is first identified relating to a condition, and then a potential drug is designed based around that protein [14]. While this approach to drug design is effective and efficient when there is a known protein of interest, the process does not inherently allow for these potential drugs to be evaluated against a large number of different protein targets in order to broaden the possibilities for drug discovery. These problems with the current system of pharmaceutical drug design raise the need for an alternative approach which is able to provide a large number of potential drug candidates at a lower expense which can be evaluated against thousands of protein targets. The synthetic procedure outlined in this paper takes the opposite approach to aspects of pharmaceutical companies by emphasizing the expansion of a potential drug class instead of focusing on the protein of interest.

Overall, the goal of this research is to synthesize and characterize of a threonine-rich macrocycle and to explore its 3-D shape. This work adds to a growing understanding of similar homodimeric macrocycles in hopes for their future assessment as potential new drugs and completes the study of the naturally occurring β-branched amino acids.

EXPERIMENTAL

General Experimental Details

NMR Spectroscopy: ^{1}H NMR spectra were recorded on a 400 MHz Bruker Avance spectrometer. Chemical shifts for ^{1}H NMR spectra (in parts per million) referenced to a corresponding solvent resonance (e.g. DMSO-d_6, δ = 2.52 ppm). $^{13}C\{^{1}H\}$ NMR spectra were recorded on the same 400 MHz Bruker spectrometer referenced to corresponding solvent resonance. All 2D spectra were taken on the 400 MHz Bruker Avance relative to corresponding solvent resonances. Identification of NMR signals are as follows: s = singlet, d = doublet, t = triplet, and m = multiplet. NMR solvents were deuterated and purchased as a bottle or ampule.

General Chemistry: Flash chromatography experiments were carried out on silica gel with a porosity of 60Å, particle size 50–63 μm, surface area 500 – 600 m^2/g, a bulk density of 0.4 g/mL and a pH range of 6.5 – 7.5. Dichloromethane/methanol was used as the eluent for chromatographic purification. Thin-layer chromatography experiments were carried out in sealed chambers and visualized with UV or submersion in ninhydrin (1.5g ninhydrin in 100mL of *n*-butanol and 3.0mL acetic acid) followed by heating. Excess solvents were removed via rotary evaporation on a Buchi Rotavapor RII with a Welch Self-Cleaning Dry Vacuum System. All workup and purification procedures were carried out with reagent-grade solvents under ambient atmosphere.

Synthesis of Threonine Acid Intermediate

While continuously stirring, cyanuric chloride (1.0 g, 5.4 mmol) was added rapidly as a solid and dissolved in 25 mL of THF that was previously cooled to –10 °C using a dry ice and acetone bath. The temperature was maintained at –10 °C while a 25 mL solution of BOC-hydrazine (0.72 g, 5.4 mmol) in THF (0.2 M) was added dropwise over 2 minutes via a pressure equilibrium funnel. Over the course of the addition, the solution turned a very pale yellow. After the addition was complete, 1 equivalent of 5 M NaOH (5.4 mmol) was added over 1 minute via pipette. After 30 minutes, thin layer chromatography (10% methanol in ethyl acetate) confirmed that a single product was observed under short wave UV irradiation ($R_f = 0.8$) or using ninhydrin (yellow spot). At this time, the ice bath was removed, and the solution allowed to slowly warm to room temperature.

Next, a solution of D-BOC protected threonine (1.7928 g, 10.8 mmol) in 10.8 mL of 1 M NaOH (to dissolve the threonine) was added dropwise over 2 min while at room temperature. The reaction mixture was measured to a pH of 7 immediately after the addition and brought to a pH of 8 with 4 more mL of 1M NaOH. The solution started a pale yellow and after the addition turned a strong yellow in color. After 2.5 hr, the yellow color slowly changed to a cloudy white. Thin layer chromatography showed the starting material ($R_f = 0.7$) disappeared and a new spot at $R_f = 0.05$ appeared in 10% MeOH in DCM.

Then, dimethylamine (1.22 g, 10.9 mmol) was added dropwise over three minutes (dimethylamine is used as 40% aqueous solution). Immediately following, the pH was measured to be 9. The reaction was stirred for another 3 h at room temperature. Thin layer chromatography in 10% MeOH in DCM showed a new spot at $R_f = 0.5$. The reaction was acidified to pH 4 with 1 M HCl. 100 mL of brine was added to the reaction flask and the organic layer was separated from

the aqueous layer using a separatory funnel. The aqueous layer was then washed 3 times with 25-50 mL of ethyl acetate. All of the organic layers were combined and dried with magnesium sulfate. Once the magnesium sulfate was filtered off via vacuum filtration, the organic layer was dried down to provide a crude yield of 2.28 g of material. Column chromatography was run in 5% MeOH in DCM to yield 0.753 g of acid intermediate.

^{1}H NMR (DMSO-D_6, 400 MHz): 12.70 (s, 1H), 8.55 – 8.54 (s, 1H), 8.42 – 8.32 (t, 1H), 5.45 – 5.35 (m, 1H), 4.56 – 4.38 (m, 1H), 4.36 – 4.15 (m, 1H), 3.01 – 2.99 (s, 6H), 1.40 – 1.28 (d, 9H), 1.14 – 1.08 (m, 12H).

^{13}C{^{1}H} NMR (DMSO-D_6, 100 MHz): δ 173.4, 168.0, 166.4, 165.7, 156.4, 79.0, 73.7, 67.4, 59.1, 35.9, 28.6, 21.7, 21.5.

Synthesis of Threonine Acetal Intermediate

While continuously stirring, threonine acid (0.400 g, 0.939 mmol), diethoxypropyl amine (0.138 g, 0.939 mmol), and HOBT (0.173 g, 1.127 mmol) were dissolved in 22.2 mL of DCM at room temperature. DIPEA (0.308 g, 2.379 mmol) and EDC.HCl (0.216 g, 1.127 mmol) were added neat, separately immediately following the other for an overall 0.25 M solution. After 3 hours, thin layer chromatography (10% methanol in dichloromethane) confirmed the single spot starting material (R_f = 0.30) evolved into new spots with a single ninhydrin-stained yellow spot at an R_f of 0.6 in 7.5% MeOH in DCM. The reaction was dried down via rotary evaporation or airstream and columned using a gradient from 2.5% to 5%. Purified acetal intermediate was initially confirmed via TLC in 19:1 MeOH in DCM with an Rf = 0.4. The product was identified in 4 consecutive fractions from the column, giving a recovered yield of 0.02 g of acetal intermediate.

^{1}H NMR (DMSO-D_6, 400 MHz): 8.54 – 8.26 (m, 2H), 7.90 – 7.68 (m, 1H), 6.41 – 6.24 (m, 1H), 4.50 – 4.48 (m, 1H), 4.31 (m, 1H), 3.92 (s, 1H), 3.54 – 3.52 (m, 2H), 3.41 – 3.39 (m, 2H), 3.02 (s, 6H), 1.66 – 1.65 (m, 2H), 1.40 – 1.30 (m, 9H), 1.10 (m, 18H).

^{13}C{^{1}H} NMR (DMSO-D_6, 100 MHz): δ 171.8, 171.1, 168.0, 167.5, 166.1, 165.8, 165.6, 156.3, 101.0, 79.0, 78.9, 73.9, 67.8, 61.2, 60.5, 55.4, 36.1, 35.8, 35.3, 33.7, 31.1, 28.6, 28.5, 20.7, 19.7, 15.8.

Macrocyclization of the Threonine Acetal Monomer to T-T Macrocycle

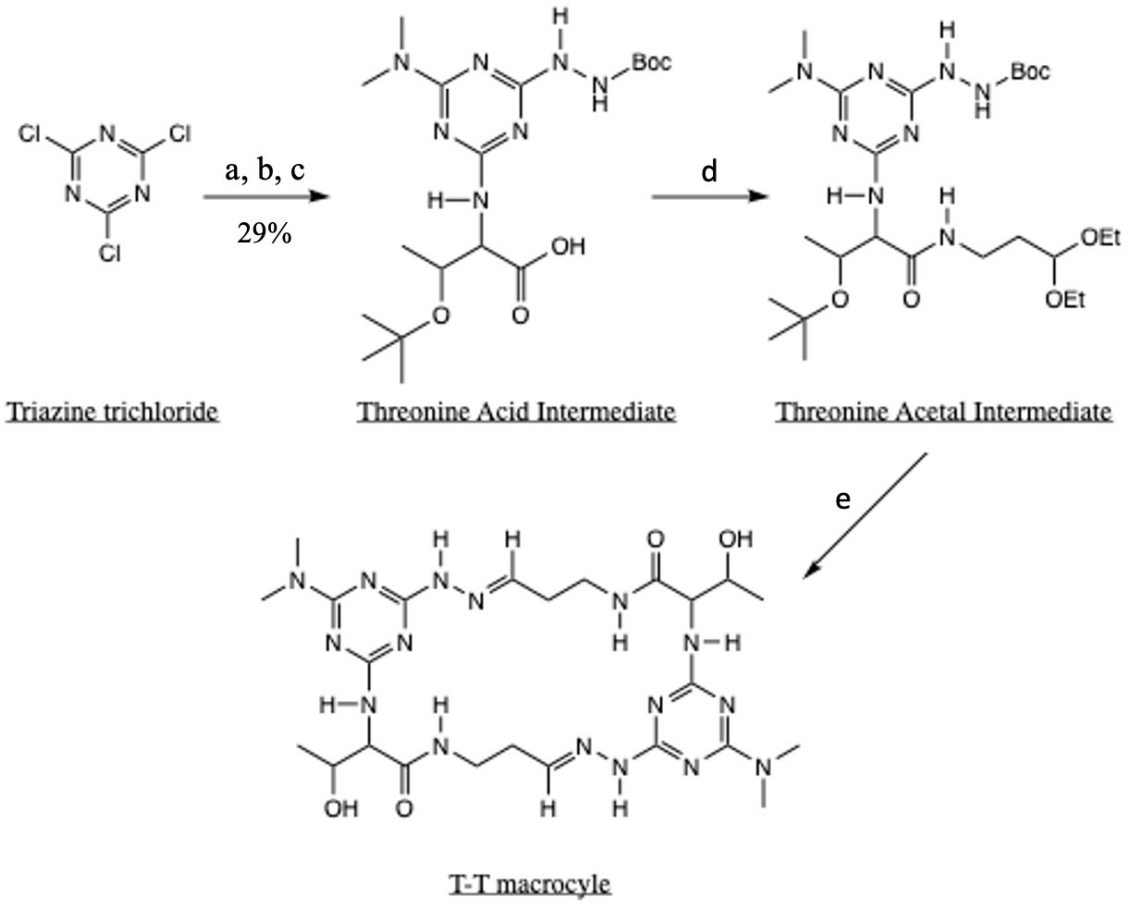
The threonine acetal intermediate (0.010 g) was dissolved in 1 mL of MeOH in a 3 mL vial equipped with a mini stir bar. TFA (1 mL) was added to the reaction vial over 1 minute via pipette. The vial was uncapped to allow for slow evaporation. Evaporation occurred over the course of 2 days before confirming full cyclization via ^{1}H NMR, ^{13}C NMR, COSY NMR, rOesy NMR, and HSQC NMR to confirm the macrocycle structure.

^{1}H NMR (DMSO-D_6, 400 MHz): 12.58 (m, 1H), 11.62 (m, 1H), 9.17 – 9.15 (m, 1H), 7.47 (s, 2H), 5.48 (m, 1H), 4.25 – 4.22 (m, 2H), 4.01 – 3.98 (m, 1H), 3.10 – 3.07 (s, 6H), 3.02 – 2.99 (m, 1H), 2.60 (s, 2H), 1.24 – 1.22 (m, 3H).

^{13}C{^{1}H} NMR (DMSO-D_6, 100 MHz): δ 172.4, 161.9, 153.8, 153.6, 148.4, 65.8, 60.7, 37.3, 37.1, 33.4, 32.1, 20.6

RESULTS AND DISCUSSION

The general synthesis of the target T-T macrocycle is shown in Scheme 1. The synthesis is completed in three steps. The first step is the synthesis of the threonine acid intermediate. The second step is the synthesis of the threonine acetal monomer. The third step is the dimerization of the acetal monomer into the T-T macrocycle homodimer.



Scheme 1. Synthesis of T-T Macrocycle - a. $BocNHNH_2$ (1 eq), 5M NaOH (1eq) THF, -10ºC, 30 minutes; b. t-Bu-Thr (2 eq),1 M NaOH (2 eq), RT, 2.5 h; c. Dimethylamine (3 eq), 3h; d. EDC.HCl (1 eq), HOBT (1.2 eq), DIPEA (2.5 eq)DMF, RT, 3 h; e. 1:1 DCM:TFA.

Synthesis and Characterization of the Acid Intermediate

The synthesis of the acid intermediate starts with the addition BOC-hydrazine to the starting material of cyanuric chloride. A second addition to the triazine ring is performed with D-t-butyl-threonine, followed by a final addition of dimethyl amine to the triazine ring. After each individual addition a small portion of the intermediate was saved in a 3 mL vial as a TLC standard. TLC is performed after each addition and co-spotted with the standard from the previous addition to the triazine ring in order to confirm successful synthesis. Extraction of the acid monomer in ethyl acetate is the final step in the synthesis of the threonine acid monomer. The acid monomer

is purified by column chromatography. The threonine acid intermediate's structure was confirmed via ^{1}H NMR and ^{13}C NMR.

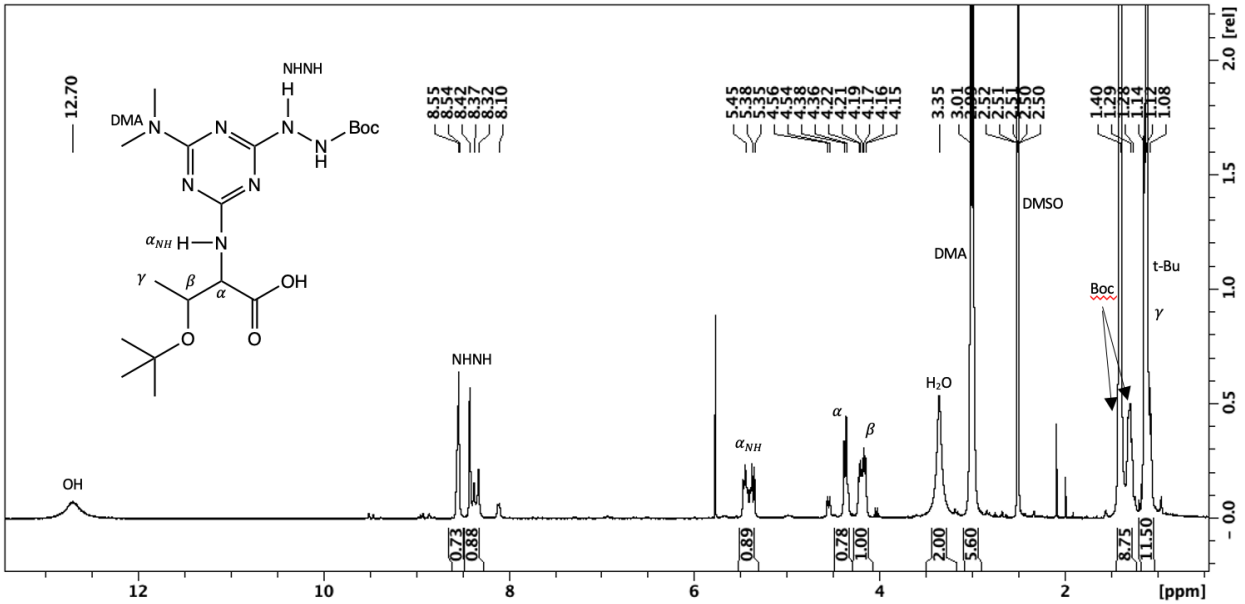


Figure 1 - ^{1}H NMR of Threonine Acid Intermediate

The ^{1}H NMR spectra in Figure 1 was taken of the threonine acid monomer in DMSO-d_6. The inset shows the key used to indicate the NMR signals. Importantly, it is possible to see all the expected resonances that integrate for the correct number of hydrogens. Moving upfield, the carboxylic acid OH appears a 12.7 ppm and confirms the presence of threonine as do the a, b and g resonances at ~4.5 ppm, ~4.2 ppm and ~1.1 ppm, respectively. The BOC-hydrazine group is revealed in the NNH resonances at ~8.5 ppm and the BOC group at 1.40 and 1.28 ppm. Finally, the third substitution with DMA is corroborated by the resonances at 3.00 ppm.

The spectrum also reveals that multiple conformations exist. That is, there are multiple resonances associated with each proton. This situation arises due to hindered rotation about the triazine-N bonds. Of the four isomers that are possible, the NMR clearly shows at least three of them are present. These isomers are best identified in the series of sharp signals at 8.4 ppm.

The ^{13}C NMR spectra in Figure 2 was taken of the threonine acid in DMSO-d_6. and the NMR signals have been tentatively assigned to the chemical structure in the inset. It is possible to visualize and assign all of the carbons present in the acid intermediate. The degenerate methyl groups of the t-butyl substituents appear as one resonance each.

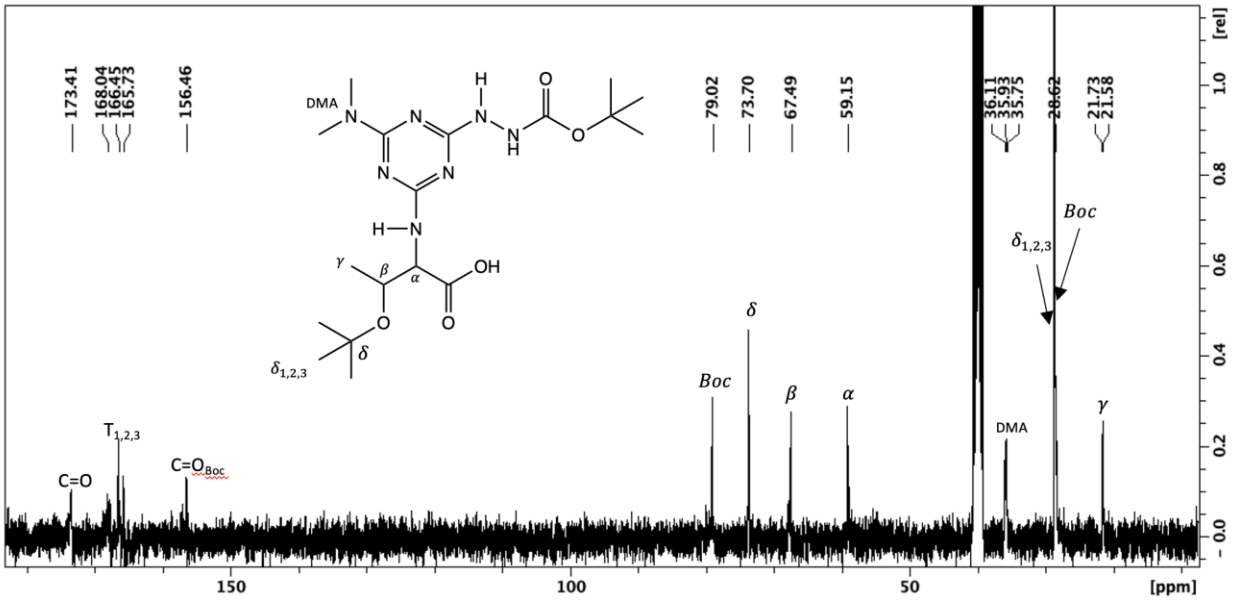


Figure 2 - ^{13}C NMR of Threonine Acid Intermediate

The upfield region is difficult to interpret due to what appears to be low signal-to-noise. While this is part of the explanation, the other is that the ^{13}C NMR also reveals the presence of the four rotational isomers of the triazines. The appearance of multiple lines for each resonance is best observed in this region, although expanding the upfield region reveals that the resonances that appear broad in the figure are indeed multiple peaks.

In summary, the NMR spectra confirm synthesis of the acid intermediate.

Synthesis and Characterization of the Acetal Monomer

The second stage of synthesis to form the acetal monomer starts by combining the acid monomer, HOBT and diethoxypropyl amine in DCM. DIPEA and EDC-HCL are immediately

added and the reaction is allowed to run until completion. The acetal product is then purified via column chromatography and the structure is confirmed with ^{1}H NMR and ^{13}C NMR.

The ^{1}H NMR spectra in Figure 3 was taken of the threonine acetal monomer in DMSO-d_6, and the NMR signals have been assigned based on labels in the inset. It is possible to see the appearance of the C_{NH} hydrogen, both of the ethyl group hydrogens, and the A, B, and C hydrogens in the spectra. These new peaks confirm the structure of the acetal monomer due to the presence of these added signals and the disappearance of the OH signal found in Figure 1.

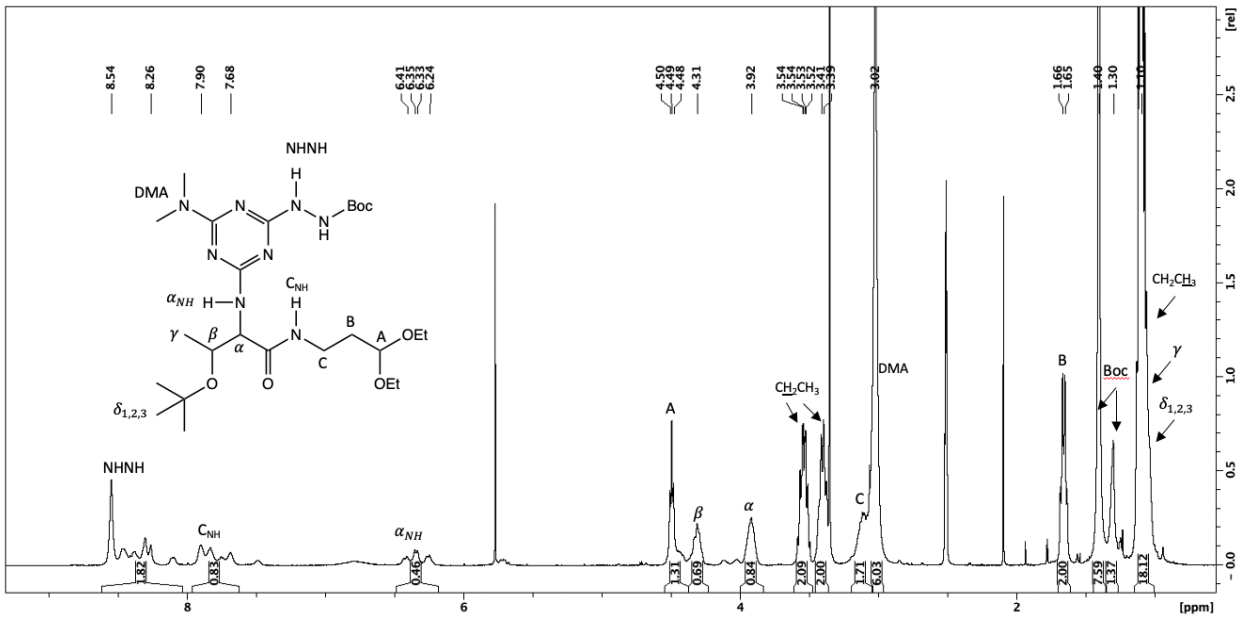


Figure 3 - ^{1}H NMR of Threonine Acetal Derivative

The appearance of a triplet at ~5 ppm confirms successful reaction as do the signals for ethyl groups of the acetal at 1.2 ppm, 3.4 ppm and 3.6 ppm. The existence of rotamers is revealed throughout the spectrum from multiple resonances for the NNH, CNH and α-NH protons as well as the α protons. The ^{13}C NMR shown in Figure 4 corroborates this conclusion.

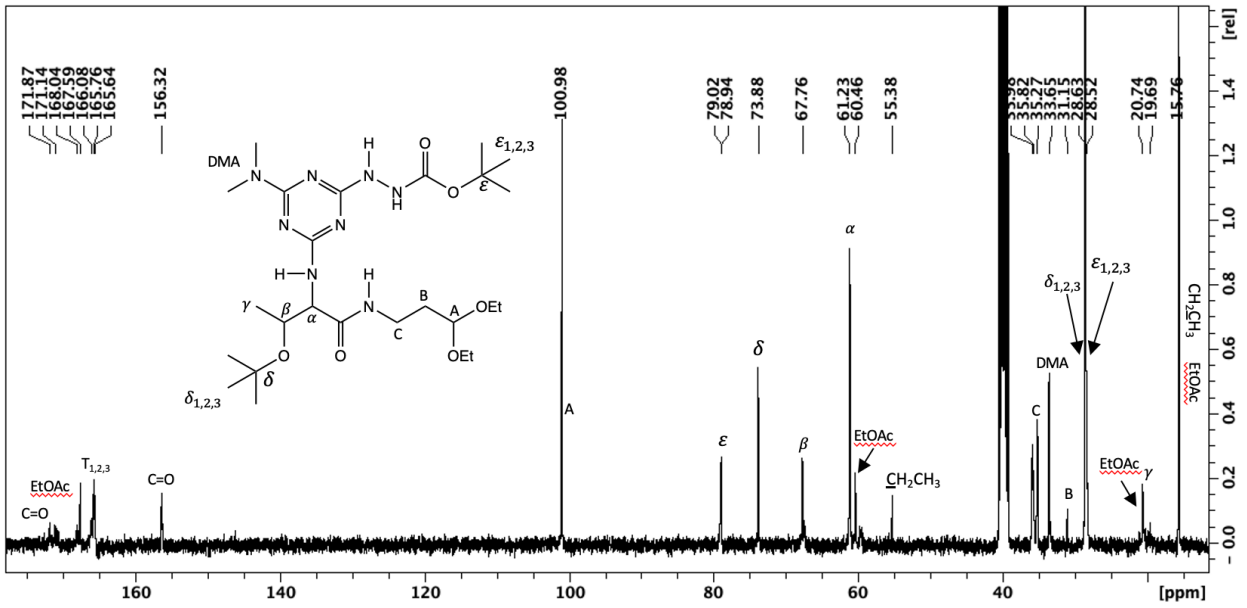


Figure 4 - ^{13}C NMR of Threonine Acetal intermediate

The ^{13}C NMR spectra in Figure 4 was taken in DMSO-d_6. The NMR signals have been assigned to the chemical structure as shown in the inset. There are new signals corresponding to the ethyl groups and the A, B and C carbons. The presence of these resonances in comparison with the carbon spectra of the acid intermediate confirm successful synthesis of the acetal monomer. The α, β, δ, and ε signals are in very similar positions as they were in the acid intermediate, although there is a change in peak intensity. The alpha peak becomes the most intense while the other intensities do not change. The reason for this behavior is unknown.

T-T Macrocyclization and Conformational Analysis

The final stage of synthesis requires dissolving the acetal monomer in methanol and adding TFA to the reaction. The reaction is allowed to evaporate over the course of 2 days while stirring. The final macrocycle, **T-T**, is an amorphous solid. Macrocyclization is best accomplished using smaller portions of the acetal monomer. The acetal monomer is collected from test tube fractions derived from column chromatography. Certain test tube fractions were chosen to cyclize. The advantage to this procedure is the purest fractions of acetal monomer can be used for

macrocyclization. The purity of these acetal fractions was confirmed by the presence of only 1 spot when run on TLC. The **T-T** macrocycle structure is characterized via ^{1}H NMR, ^{13}C NMR, COSY, rOesy, and HSQC. If the macrocycle structure cannot be confirmed after allowing evaporation of the solvent, then the solvent can be re-added to the reaction and the system can be allowed to evaporate again over the course of 2 days. Subsequent redissolving of the product for evaporation increases yield of **T-T** as visualized in the spectra.

The ^{1}H NMR spectra in Figure 5 was taken of the **T-T** macrocycle in DMSO-d_6, and the NMR signals have been assigned to the chemical structure as it is labeled in the figure. There are signals that correspond with every available hydrogen in the structure that confirm the successful dimerization of the acetal monomer to form **T-T**. Of note is that the ethyl group hydrogens are no longer present as see in Figure 3 along with the BOC group hydrogens confirming that the cyclization has been successful. The α signal is at the same location as found in the acetal monomer spectra. The A hydrogen signal has moved from 4.5 ppm to 7.47 ppm when compared to the acetal. It is also apparent that there are two positions in the macrocycle structure accounting for each signal visualized in the spectra. For example, there are two different DMA groups on opposite sides of the macrocycle however there is only 1 corresponding DMA peak at 3.10 ppm integrating to 6. The same can be said for every other identifiable group that produces a signal.

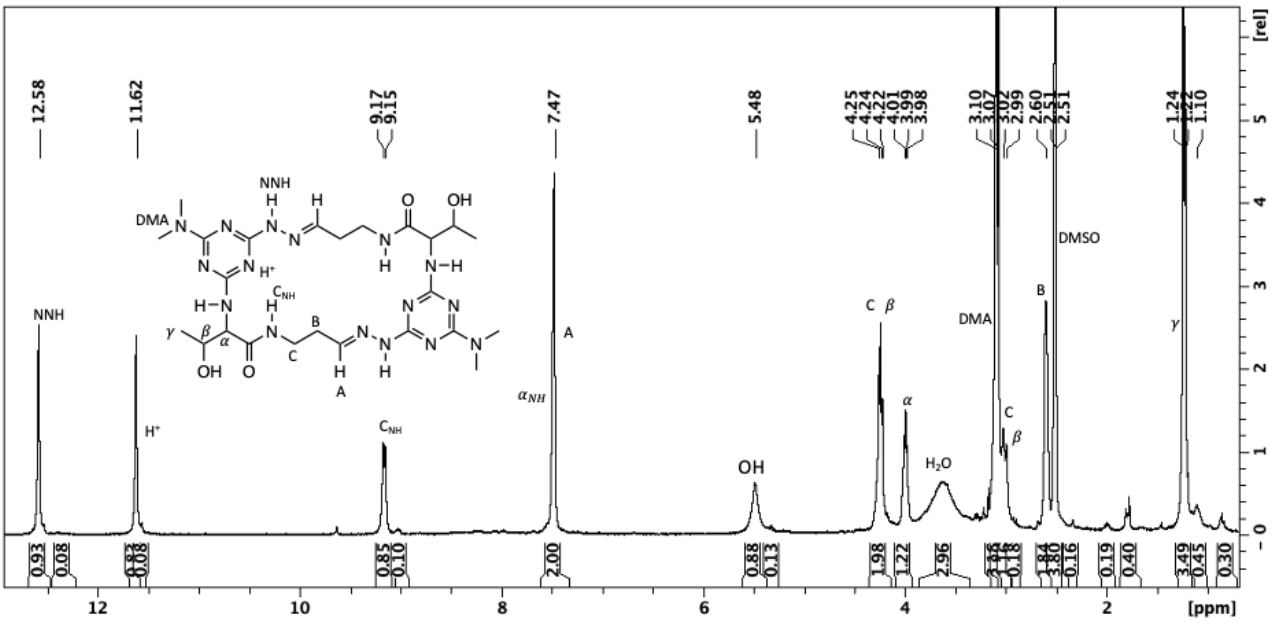


Figure 5 - ^{1}H NMR of T-T macrocycle

What this pattern of resonances demonstrates is that **T-T** is indeed a homodimer where both sides of the macrocycle are identical. The appearance of the H+ signal is due to the TFA used in cyclization. Its position within the structure has been assigned and confirmed by ROESY NMR. At 7.47 ppm, the α-NH hydrogen and the A hydrogen overlap. COSY NMR confirms the overlap. An area of interest is around the β hydrogen as it gives a signal both at 4.22 ppm and 2.99 ppm. This phenomenon also occurs in respect to the C hydrogens when compared with the acetal monomer as it also gives two signals with one appearing at 4.25 ppm and another appearing at 3.02 ppm.

The C hydrogens and the β hydrogens are not equivalent and appear as separate resonances. The spectrum shows that a second set of resonances appear immediately downfield to each identified signals and these resonances integrate to roughly 1/8th of the primary signal. This phenomenon is of extreme interest because it could be explained by the presence of different isomeric states of **T-T** that exist at a ratio of 8:1 between the primary isomer and the secondary isomer. These secondary signals are very weak in intensity, but their presence is enough evidence

to confirm the existence of multiple isomers. The structural differences between these isomers are not able to be identified due to how weak the secondary signals for each hydrogen appear on the spectra.

The ^{13}C NMR spectra in Figure 6 was taken of the threonine acid monomer in DMSO-d_6. The NMR signals have been assigned to the chemical structure as it is labeled in the figure. As stated in reference to Figure 5, the disappearance of the BOC carbons and the acetal carbons in coordination with the appearance of only one peak for each homodimer carbon that appears twice in the **T-T** structure confirms the synthesis of **T-T**. The overlap of T_3 and T_2 makes it difficult to differentiate those two signals with the signal found in T_1. The β, α, DMA, C, B, and γ carbon signals are very similar to their position found in the acetal monomer, however the A carbon has a large change in position from 100.98 ppm to 148.35 ppm. This shift upfield can be explained by the loss of the acetal carbons and the joining of both monomers to form a homodimer which brings the A carbon to the position of where the BOC group would have been.

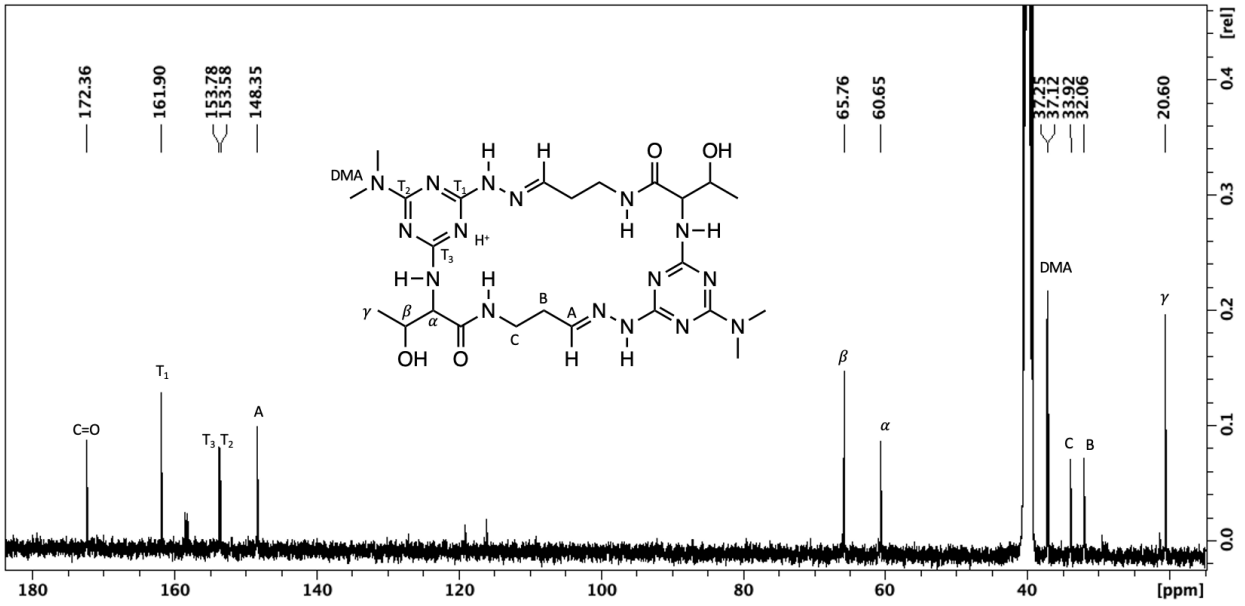


Figure 6 - ^{13}C NMR of T-T macrocycle

CONCLUSION

The synthesis of this threonine rich macrocycle is of importance due to the interest in macrocycles in their potential as drugs. Macrocycles exhibit large and flexible structures that can exist in different conformations which is a reason why they are being looked at within the field of synthetic drug design. Additionally, macrocycles demonstrate the ability to have a wide variety of different groups to be attached without affecting the cyclical structure itself. Indeed, the presence of a competing hydrogen bonding group—the hydroxyl—could have interfered with the structure or led to an asymmetric macrocycle.

The synthesis of **T-T** demonstrates that it is possible to synthesize a large macrocycle in three steps within a short period of time. The first step consists of synthesizing a threonine acid intermediate through three separate additions of BOC-hydrazine, D-t-butyl-threonine, and dimethylamine to a triazine ring. The second step of synthesis is formation of the threonine acetal monomer from the acid intermediate. The third step is the macrocyclization of the acetal monomer to form the **T-T** macrocycle homodimer. Significant importance is given to purity at the end of each stage and is accomplished through column chromatography and TLC in order to ensure a pure intermediate or monomer before moving to the next stage. Conformational analysis at the end of each stage was also characterized via ^{1}H and ^{13}C NMR for all three stages with additional characterization by COSY NMR, rOesy NMR, and HSQC NMR for additional structural analysis of **T-T**.

Synthesis of this threonine rich macrocycle adds to a growing library of similar macrocycles varying in the amino acid in position of threonine. This synthesis contributes to the eventual goal of creating a library of characterized macrocycles for future research in the area of synthetic drug design.

Future areas of exploration lie in improving the synthesis pathway with the goal of production on a greater scale which is important for the potential of **T-T** like macrocycles to be utilized as drugs. Currently this synthesis has been done on an extremely small scale as a proof of concept that the synthesis procedure is successful which has been demonstrated by the results of this study. Improvements in synthesis could be made by increasing the scale of the reaction or by alternative mode of synthesis such as solid phase synthesis. One of the most limiting factors in scaling the production of **T-T** is the need for column chromatography purification after each individual stage of the synthesis. Eliminating this need after each stage would significantly increase the ability to produce larger amounts of **T-T** while cutting down on the time it takes to perform the synthesis.

Future directions for this research lie in the area of three-dimensional characterization of the macrocycle. Accomplishing this goal is of great importance for future applications in synthetic drug synthesis. Knowing the three-dimensional structure allows for predictions and understanding in how the drug will behave and bind within biological systems. The next step in order to understand three-dimensional structure is to perform computational modeling and using the results obtained from COSY and rOesy NMR as a starting point. Once the three-dimensional structure is understood, protein binding assays can be performed with the macrocycle to evaluate its ability to bind to specific proteins of interest.

Additionally, there is evidence that **T-T** exists in multiple conformations and understanding how those conformations differ within space provides a greater range of applications for the potential drug. If multiple conformations do exist, then research is needed into what conditions favor each individual conformation and if conformational changes can take place when conditions of the system are changed. Another area of future research if different

conformations of **T-T** are identified is the changing of those conformations with different substituted groups in place of the dimethylamine.

It is also of interest to evaluate the significance of the alcohol group present on threonine and its contribution to structure. Synthesis of the same macrocycle with serine in place of threonine would allow for accurate analysis of the significance of the methyl group of the sidechain while alanine probes the role of the hydroxyl. It will be important to look at whether the overall conformation of the macrocycle is affected by the presence or absence of the alcohol group on threonine and whether different conformations are more likely to occur. With the knowledge of how conformation is affected by subtle differences in the amino acid choice, better design rules can be articulated that describe the effects of different amino acids and groups substituted onto the macrocycle.

Building a library of compounds brings the focus to developing synthesis pathways which can be easily varied to produce a large variety of macrocycles. These macrocycles can then be analyzed for protein binding affinity and the results of those studies can then be used to explore drug targets that contain proteins used in the assay. This system of building a library is tailored to research groups like the Simanek laboratory because they continually are developing and characterizing new compounds to add to scientific literature while creating a bank of potential drug candidates at the same time.

Chapter 2: Introduction to Research: A Review

ABSTRACT

During the spring semester of the 2020-2021 academic year at Texas Christian University a group of 12 undergraduate students participated in a group project given the title *Introduction to Research*. These students chose to participate in this project after their organic chemistry II laboratory course was moved to an online format two weeks into the start of the semester. The goal of the project was to provide undergraduate students the opportunity to participate in faculty led organic chemistry research which would supplement their laboratory course being moved online. This review seeks to cover what the undergraduate students were able to accomplish over the course of the semester while meeting weekly to work on this project.

Additionally, a survey was given to the students after they completed the semester in order to get their opinion on how the structure of the course operated as well as areas which could be improved if the opportunity was offered to students again in the future. The opinions received from the survey demonstrated that the undergraduate students felt that the experience was extremely positive with only a few areas in which the operations could have been improved. This review takes the summary of student activities through this project as well as their perspectives gained through the survey in order to provide guidelines on how a similarly styled opportunity could be offered in the future as a class course at TCU.

Disclaimer: This review was commissioned by Dr. Simanek and written by Liam Claton who acted as one of the undergraduate teaching assistants during this project as well as actively participating in the research and has continued to work with the Simanek laboratory over the past year. In doing the work to produce this review, Liam Claton received one chemistry credit hour.

INTRODUCTION

In the spring semester of 2020-2021 academic year at Texas Christian University the organic chemistry II laboratory class was moved from in person to an online format by the instructor on record after 2 weeks of the semester starting due to COVID-19 concerns stemming from noncompliance of students with university policies for quarantine protocols. A group of 12 undergraduate students accepted the opportunity to continue their development of organic chemistry laboratory skills in an in-person setting by participating in research facilitated by Dr. Eric Simanek. The name "Introduction to Research" was given to this project. There were additionally two undergraduate teaching assistants from the organic II lab who agreed to oversee and assist this group of students. Beyond the undergraduate teaching assistants, there was involvement of multiple graduate students from Dr. Simanek's laboratory group and the TCU chemistry department. Dr. Simanek and his research group were working on the synthesis of newly developed macrocycles for potential future applications as drugs. At the time when the organic lab went online, their lab group was at a stage where there was need for a large variety of macrocycles synthesized with differing amine group additions in order to begin development of a library of new macrocyclic compounds.

This review attempts to serve two purposes. Its first purpose is to outline what was accomplished over the course of the semester by this group of undergraduate students. The sections pertaining to this purpose will cover the synthesis pathway used by the undergraduate students, the laboratory techniques that were expanded upon or newly taught, and how the project was facilitated throughout the semester. The second purpose of this review serves as a potential guide to the TCU chemistry department if there is ever a decision to formally offer a course titled Introduction to Research. Undergraduate students pursuing a degree in chemistry are required to

perform 3 hours of undergraduate research in order to obtain their degree. These hours have been historically accomplished by a student working in the laboratory of one of the TCU chemistry faculty actively pursuing research. There are future concerns with the growth in the number of TCU students pursuing a degree in chemistry that there may not be enough research positions in faculty laboratory groups for all of the students. “Introduction to Research,” a potentially new course would provide an option to faculty members conducting research to utilize a larger group of undergraduate chemistry students working on the same project over the course of a semester. This course would then be an option for undergraduate students to fulfill their research requirement. Supporting data in this section of the review derives from a survey that was given to the undergraduate students who participated in the original project.

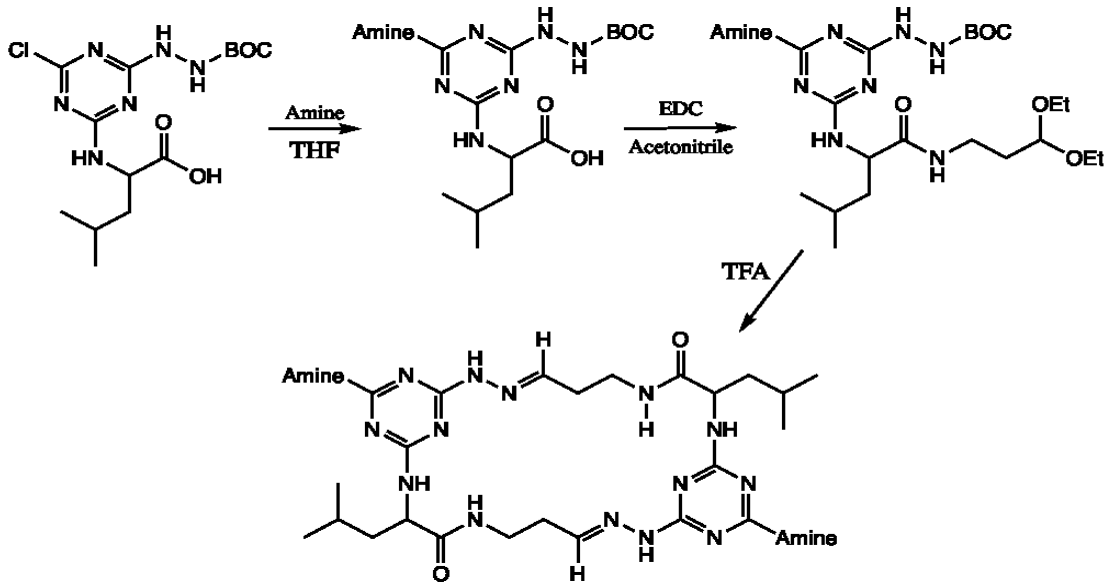
REVIEW of 2020-2021 SPRING SEMESTER

It is important to contextualize the experience level of the majority of the undergraduate students who participated in this project. The majority of students who participated were in the second semester of their sophomore year of college with previous chemistry experience of general chemistry I and II lecture, one condensed general chemistry lab covering two semesters, organic chemistry I and organic chemistry I lab. These previous laboratory courses included hands-on experience where students would meet once a week in lab for 4 hours and perform a provided laboratory procedure.

The research topic they were introduced to was synthesis and characterization of new macrocyclic compounds. Macrocycles are of increasing interest as possible new drug candidates. The students were tasked with synthesis of leucine-rich macrocyclic compounds. Leucine-rich macrocycles are of interest due their potential of disrupting leucine zipper formation within proteins [15]. Leucine zippers have been shown to be able to interact with DNA binding sites

stimulating gene expression [16]. The long-term goal expressed to the students was design of a macrocycle which would be assessed in its ability to block leucine zipper formation in order to affect gene expression within cancer cells.

Students were given a previously synthesized starting material that consisted of a di-substituted monochlorotriazine ring substituted with leucine and BOC-hydrazine substitutions. Students were then assigned a nucleophilic amine group to substitute onto the ring in place of the chlorine atom.



Scheme 1 - Synthetic route to the macrocycles. Students were assigned different amines. Dr. Simanek provided the starting material.

Common protocols were employed. In general, 1 equivalent of starting material was dissolved in THF and then 3 equivalents of the amine group were added. The solution was allowed to stir until thin layer chromatography (TLC) confirmed the reaction was complete. The time it took for the substitution to run to completion varied depending on nucleophile. After confirmation of substitution via TLC, the students purified the now tri-substituted triazine ring using column chromatography. Varying ratios of DCM:MeOH were used by the students for their column

chromatography depending the polarity of their compound as determined by the TLC retention factor.

One cohort of students had an extra week on the project based on the TCU calendar. This group was able to mono-protect and purify diamines for use as nucleophiles. BOC anhydride was the protection reagent. Column chromatography was employed as the method of purification for these materials.

Students then performed an EDC-mediated (or DCC-mediated) coupling reaction on the intermediate in acetonitrile and monitored the reaction via TLC until its completion. Again, the reaction was purified of side products and excess reagents using column chromatography. Once the second intermediate was purified, the resulting monomer was treated with trifluoracetic acid to facilitate the cyclization process to yield the desired macrocycle. Due to the difference in amine additions between students there were different techniques utilized in order to complete the cyclization process.

Macrocycle formation was confirmed via ^{1}H NMR and ^{13}C NMR. In addition to successful macrocycle formation, ^{1}H NMR was performed after addition of the amine group and again after performing the EDC coupling reaction. Students were responsible for preparing their own NMR tubes and then would perform the NMR reading with assistance from a graduate student or Dr. Simanek.

A large number of laboratory techniques were employed by the students throughout the duration of this research project. There were certain techniques that the students had previous experience with and others which were new to the students requiring a certain level of teaching on behalf of those coordinating the project. Some of the techniques that students already had experience with included accurate measurement of materials and reagents, proper utilization of

glassware, and procedures such as extraction and recrystallization. On the other hand, laboratory techniques such as TLC, column chromatography, and utilization of a rotary evaporator were new to the students. Demonstrations of each new technique were performed by those facilitating the class and by the end of the project all students were able to demonstrate confidence in performing these techniques. It should also be noted that the majority of students had yet to be introduced to characterization techniques such as NMR (both practice and theory) before starting the course but were able to quickly pick up on the theory surrounding how NMR functions and apply that theory to assist with their own macrocycle characterization. For the new laboratory techniques there was easily available help for the students in the form of undergraduate teaching assistants, graduate students, and from Dr. Simanek.

Overall, the most difficult of the laboratory techniques that students were introduced to was column chromatography. Issues would commonly be the inability to separate different products through the column or losing samples to the column. A common issue which caused problems with this technique was improper setup of the column by the students. Another common issue included having too much cotton and sand at the base of the column which caused the eluent to move too slowly through the silica. This caused a longer time for the column to finish and there were multiple instances of students stopping their column before all of the sample eluted. Another common mistake in column setup was the loading of silica into the column where students would have a large formation of air bubbles within the silica causing issues with separation of products due to a nonlinear flow of eluant. The final mistake commonly seen surrounding column chromatography stemmed from improper loading of a sample to the column. Common loading mistakes were disturbances in the silica at the top of the column when loading the sample which would cause uneven movement of eluent through the column thus negatively affecting results.

Another loading mistake seen throughout this project was students adding excess eluent before their sample had fully entered into the silica column. The effects of this were a completely diluted sample before it entered the column making it impossible to separate the phases and difficult to reclaim all of the sample. In working with undergraduate students in the future around column chromatography these common mistakes should be kept in mind.

The formatting of the project was planned and facilitated by Dr. Eric Simanek. The space utilized was the same laboratory space previously occupied by the organic chemistry II lab therefore the resources available in the lab were similar to the students. Students would come to the laboratory once a week for typically a time period of 4 hours although a benefit to the formatting of this project was that it was very easy to move time spent in the laboratory to best benefit the schedules of the students. Each meeting period would start with Dr. Simanek giving an overview to the chemistry that the group was about to perform along with an overview to the lab techniques within the procedure. Dr. Simanek would then be present in the laboratory for the entirety of each time period that students were working. This was advantageous because it allowed for students to better contextualize what they were doing rather than just following a procedure. It also allowed students to ask questions or for clarifications as well as allow Dr. Simanek to question the students on what they were doing specifically. By the end of the semester, students were able to provide a satisfactory explanation of what chemistry they were performing as well as why the research being done was important. Students tracked their research within their own laboratory notebook. Students have previous experiences using a laboratory notebook to record results and observations, and they were able to accurately track their research project without the need to have their notebooks regularly checked or turned in.

In order to have a place to share data and information for all the students participating in this research, a Microsoft Box folder was created which all of the participants and facilitators had access to. This shared folder was used to communicate important academic papers relating to the research project as well as report results obtained by individual students such as NMR spectroscopy or photos of TLC plates. Overall communication between those participating in this project was primarily in the form of e-mail. There were no grades or assignments associated with this project. Students were later given the option to write a summary of their experience in order to receive class credit; however, this was not advertised at the beginning of the project to the students. In terms of successful synthesis of new macrocycles, some students were able to complete the synthesis of a macrocycle while others ran into difficulties with their specific amine group in relation to synthesis of the end product. Either way, students were able to make significant progress in the synthesis of new macrocycles to add to a growing library of similar compounds. Students who were unable to get to the final product still were able to practice good research techniques and learn about what might be causing difficulties as well as possible solutions in reference to their specific compound.

COURSE REFLECTION AND FUTURE CONSIDERATIONS

As previously stated at the beginning of this review, there is a potential concern with future growth of the number of students seeking a degree in chemistry or biochemistry in that there will not be enough undergraduate research positions within TCU faculty laboratories in order for all students to complete their undergraduate research requirement. This section of the review will cover how introduction to research could be a new course offered to a larger number of students with facilitation from a TCU faculty member. Specific areas that this section will address include reflections from students who participated in the initial project, course outcomes, research project

criteria to fit the course, and recommended guidelines to facilitate the potential course. The opinions of this review are based on the success of the previous undergraduate group's performance during the spring semester of the 2020-2021 academic year as well as areas where the project could have been improved. It is important to keep in mind that the contents of this review are not by any means a complete formula to run a successful course. There may be problems, challenges, or changes that need to be made in the future which are not addressed in this review; however, consideration has been given to every aspect of the way the original project was facilitated in hopes of providing the most information possible.

Students who participated in the initial project were sent a thirteen-question survey to complete which was designed to gauge student's perspective on the experience, their overall understanding of the chemistry that they performed as well as their opinion on areas which could be improved in the future. Out of the 12 undergraduates who participated in the original project, 6 submitted anonymous responses. All of the full responses to each of the questions will be included in a section at the end of this review but considering the open-ended nature of the reflections their responses will also be summarized for convenience. Overall, the responses were very positive as will be demonstrated in the summary of this survey, but it is important to consider the other 50% of participants who did not submit a response. Because of this it is difficult to accurately assess how reflective these responses are to the entirety of students who participated. The questions of the survey are outlined in figure 1.

1. Why did you enroll in 'Introduction to Research' that replaced the canceled OChem lab course?
2. What did you hope to learn/accomplish?
3. Please describe what you did during this activity.
4. What did you learn/accomplish as it pertains to techniques?
5. What did you learn/accomplish as it pertains to how research is done?
6. Do you believe the experience was beneficial and if so, why?
7. What were the best elements of the activity?
8. What would you change?
9. How did the experience affect your career plans (and what are they) or future research pursuits?
10. On a scale of 1-5 how likely are you to recommend this activity to others (1=very likely, 3=neutral, 5=unlikely)?
11. Would you make this activity a course offering to satisfy research requirements associated with the major?
12. What role did each person play in your experience (graduate TA, UG TA, Dr. Simanek, classmates)?
13. Please add any additional comments/critiques.

Figure 1 - Survey sent to all undergraduate participants in the initial Introduction to Research. There were six responses out of the twelve people the survey was sent to.

Why did you enroll? All 6 responses conveyed a desire to gain in person lab/research experience. This opinion held by the undergraduate students supports how there is a desire to gain laboratory experience outside of the lab courses that students are required to take. Additionally, these responses demonstrate an interest from students into the process of research itself and how it differs from a laboratory course setting.

What did you hope to learn/accomplish? Responses to what students hoped to learn/accomplish were similar to those of the first response with almost every single participant wanting to learn more organic laboratory techniques as well as learn more about the process of research. While college courses are traditionally designed to teach material and theory, students were looking to learn more real-world applications to the theory they were being taught while experiencing how research differs from coursework.

What did you do? When asked broadly to describe what students did during this project in the third question of the survey the responses were shortened to more of the overall objective which was the synthesis of macrocycles. There would need to be a more in-depth reflection from the participants to accurately gauge overall understanding. If a future survey is given to students in a similar situation in order to assess student understanding it would be recommended to have students write in more of a paragraph form instead of answering a question of a survey.

What techniques did you learn? The fourth question focused specifically on what students learned in regard to laboratory techniques. Students identified thin layer chromatography, column chromatography, how to use a rotary evaporator, and running NMR and the new techniques that they were not experienced with before starting the project. An interesting response of note was that of a student who reported they enjoyed the learning environment because there was no stress of hurting their grade if they got it wrong the first time. It should be considered impressive that students were able to learn all of these techniques and implement them to some degree over the course of only one semester without any previous experience. This is an important fact when considering a future course where the laboratory techniques may include some that the students have not had experience with yet. The project demonstrates that students are able to pick up on new techniques in this setting while building on the ones they have already had experience with.

The Nature of Research. The fifth question of the survey focused on what students learned in relation to how research was done. The responses of the students who completed the survey reflected how students' understanding of the research process was different than what they originally anticipated. Overall themes within responses were how research is a much longer and less straightforward process compared to traditional lab course procedures. Multiple students also highlighted how the process of research often causes the need to start over many times or conduct

multiple attempts to perfect the procedure being done. These responses highlight how the undergraduate student perspective of the way research is conducted is limited without actual lab experience at TCU.

A future course along the lines of this project would be beneficial in providing students with experience into how research is conducted. Outside of the consideration for a new course, these responses can serve to suggest that within undergraduate chemistry courses a greater emphasis could be placed on demonstrating to students what the process of research is like and how it differs from their experiences within laboratory courses. Adding this learning goal of understanding the way research is conducted could be implemented within laboratory courses in a lecture format so that students at least are exposed to the differences between research and coursework.

Was the experience beneficial? Every single student who responded to the survey indicated that the experience was beneficial. This fact alone strongly supports that offering a similar course in the future would be well received by students if conducted in a similar fashion. Multiple individuals commented on how the experience was unlike any other that they had participated in through their coursework within chemistry. One interesting comment was that the experience helped a student have a better idea of what it would be like doing graduate research after completing their undergraduate degree. Providing a similar course to this project that allows students to get a glimpse into what graduate research is like would be extremely beneficial to the TCU chemistry program not only for students who come to college with the plan of attending graduate school but also providing students with different postgraduate plans as future option they might not have considered.

What were the best elements of the activity? The seventh question was also short and open-ended asking students what the best elements of the activity were. Responses ranged from learning to problem solve while working towards an actual research goal to forming new relationships with peers. These responses are varied, however all of them are positive indicating that students were able to get a wide range of positive experiences and interactions through this project.

What would you change? Every single response indicated that there could have been more structure within the project and a better introduction into the project at the beginning. It is to be expected that attempting a new project such as this with little precedence beforehand would give rise to things that could be made better. Overall, it is important to note that there were no complaints in changing the goals of the class or what the actual research was over. Fixing these concerns in the future would be focused around having a cleaner, more direct presentation of the project to be accomplished. A potential solution to this would be a class meeting for an hour or two before the first time being in the lab where more time could be devoted towards explaining the chemistry and research goals. The goals of this project were repeated in the same fashion every time the group met before lab in a 15-30 minute meeting where the research goals and procedure were continually repeated to students. Additional information was also provided in these meetings to students such as x-ray structures of leucine zippers and potential binding conformations of macrocycles through the use of a physical model.

Students were also provided several research papers before the first meeting, but this format of providing information did not give the ability to directly ask questions about the papers nor did it give adequate time for students to process a research concept that they had no prior experience with. Another solution could be having designated lab meeting times once a week in a

similar fashion to other faculty laboratory groups which would allow designated time for discussion of the chemistry and provide an opportunity where students could ask questions.

On a different note, there is something advantageous about providing students an experience that has less structure than a typical laboratory course offered at TCU because it causes students to have to figure things out on their own on a certain level. Struggling at the beginning or having a lack of structure causes students to have to work out certain things individually which is replicative of real-world situations in the academic and professional world. Consideration should be made towards keeping a certain level of ambiguity so that students still have this more real-world experience.

Did the course affect your career plans? All of the students who responded to this question indicated that their plans did not change. While causing the students to change their career plans was not the goal of the project, one student indicated that this experience made research seem not as foreign to them. It would be interesting to continue to ask this question and see if student responses change if a course like this project were instituted in the future

Would you recommend this experience to others? The tenth question asked students to rate the experience in terms of how likely they were to recommend this activity to others with 1 being very likely and 5 being unlikely. The results are presented in chart 1. Every student who filled out the survey responded with either a 1 or a 2 indicating that they would all be likely to recommend this activity to others. This level of response is extremely important in considering whether or not to have a similarly styled course offered to undergraduate students in the future. It can be assumed that a similar course would have a positive impact on students' experience while at TCU and pursuing chemistry through providing them a unique opportunity that is rare for undergrads at other universities to be able to participate in. Something to keep in mind however is that the

students who participated in this activity all chose to do so without knowing that they would have the opportunity to receive credit. This indicates that they are above the type of student who tries to do the minimal level of work in order to pass through the TCU chemistry program.

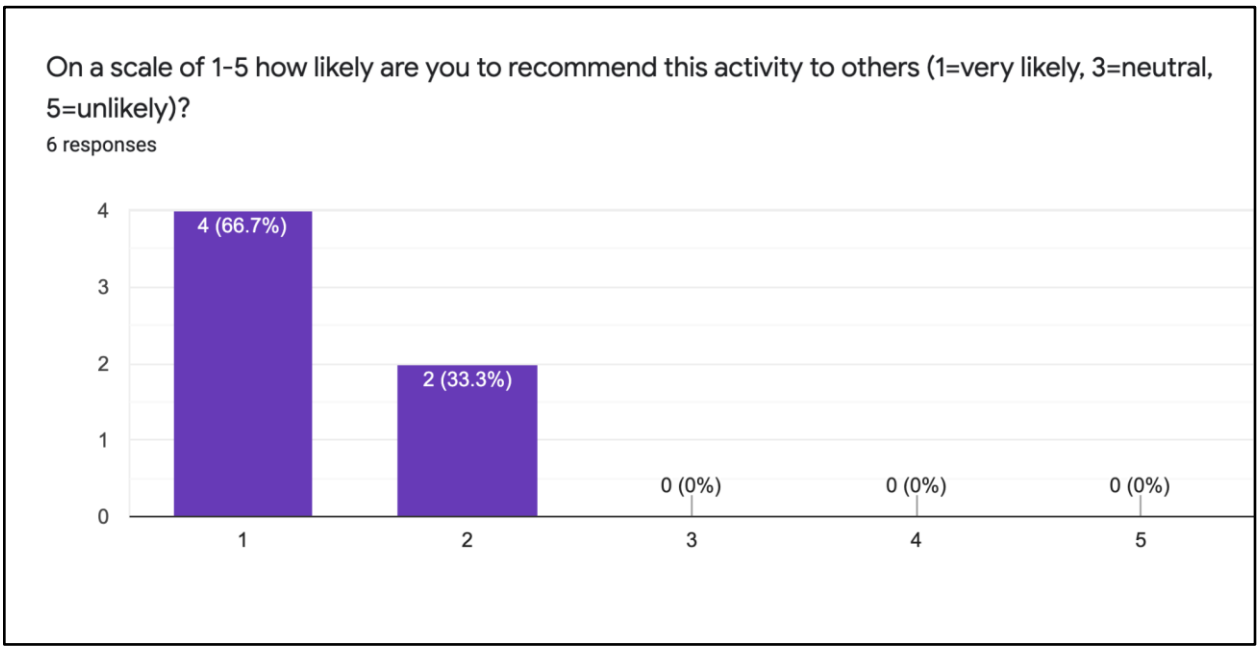


Chart 1 - Question 10 on the survey provided to students participating in the initial Introduction to Research group.

Would you enroll in this course to satisfy a research requirement for the degree? Every student who responded to the question replied yes that they would make the activity a course offered to future students. This demonstrates that students ***believe*** they got the same level of experience out of this project that they would expect to get out of performing undergraduate research in another TCU faculty lab.

What roles did individuals play within the project? Students had positive things to say about the lab director Dr. Simanek, the graduate teaching assistants and the undergrad teaching assistants. Students felt strongly that each level of facilitator played a different, but important, role in making the experience a positive one. Students highlighted that the undergraduate teaching

assistants were beneficial in their ability to answer questions around basic chemistry principles. Graduate teaching assistants were highlighted as being extremely beneficial in contributing to student learning through their ability to explain more complex or new chemical principles being practiced by the students. Dr. Simanek was emphasized specifically on how involved he was in the experience, both in his ability to answer questions and describe concepts. One student commented how they were impressed by the dynamic of working with a professor on research the professor was actually conducting. It can be confidently stated that if this kind of project were offered as a course, having an involved faculty member is beneficial to the students. The students are able to interact with the faculty member in a different context compared to a normal class setting which multiple students commented on as being beneficial while responding to the survey.

Another interesting comment which was outside of the scope of the initial survey question was that multiple students stated how they enjoyed getting to work on this project with their peers and were able to grow closer through working on this project. Having students be able to work around each other on a similar research project is an advantage compared to the normal route of a student joining a faculty laboratory because they have more of their peers to rely on and interact with than they would in the other setting which makes the process of becoming introduced to research easier.

The last question of the survey was an opportunity for students to provide any additional comments or critiques on the project that they were a part of. There were only two students who chose to respond to this question with one indicating that they believe this activity could be a great course offered to students but recommending it be more formalized with clear communication. This critique seems like it would be easily addressed if this kind of project were formalized into a course offering in order to keep in line with TCU course requirements. The other response to the

question was simply to complement the experience and that they would do it again if given the opportunity.

Overall, this survey provides valuable information both supporting the option to include this type of project as a course offering to undergraduate students while also providing valuable feedback about the positive aspects found within the experience and areas that could be improved in the future. If this experience were to be offered as a course, it would be important to continue to give students the opportunity upon completion of the course to fill out a similar survey in order to continually improve the experience. As previously stated, this survey is from a small sample size of dedicated students who chose to continue their in-person laboratory experience after their course was moved into an online format, but the overwhelming positive responses are important to consider when moving forward with this kind of opportunity.

An important point to address when considering this activity for a potential future course offered at TCU would be the articulation of course outcomes or learning objectives for the students. These are the recommendations for potential course objectives based on how the experience was initially conducted. The recommendations provided are subject to change and are only given to provide possible perspective on what a course offered by TCU might look like.

Learning Goals for Introduction to Research:

1. Students will become familiar with the process of academic research
2. Students will utilize previously learned laboratory techniques in addition to learning new laboratory techniques with relevance to their research, of which they are able to demonstrate competency in performing.

3. Students will keep an accurate record of their research in accordance with the standards given by the course facilitator and those standards will be held to the same level as if students were participating in traditional undergraduate research.
4. Students will be able to accurately describe the research goals they are pursuing including relevant theory and techniques being utilized.

Another relevant topic when considering offering this type of experience as a course in the future includes guidelines for the type of project that would be most beneficial to implement in this setting. Qualities that made this initial project successful are that the students were all performing the same overall procedure with only differences in amine group additions. Having a project where students are accomplishing the same goal is beneficial because students are working on similar projects as their peers. If students were all working on different projects or goals without something to tie them together there is greater likelihood of having difficulty in managing everyone.

Projects used for this type of course would ideally be for a faculty member needing a larger amount of compound to be synthesized or a larger amount of data needing to be collected. Undergraduate students have an advantage in these types of projects by providing the faculty member with a larger amount of people to lighten the amount of work intended to be placed on a smaller research group. Faculty members are also then able to continue the work being accomplished by their research group while having an additional group of undergraduates contributing.

Additionally, the project should be one that undergraduate students are able to adequately grasp and understand in order to continue their development as students and contribute to their education. Qualities that can make this course extremely beneficial would be lost if students were

simply performing a procedure without having an understanding of what they were accomplishing. That is not to say that the research needs to be over something that students have already learned, rather it means that equal emphasis should be placed on student learning and growth as it is on producing research results.

Students should be evaluated on the basis of the way they conduct their research and not completely on whether they are successful or not. There is an equal amount learned through failure while practicing good research technique as there is in success. Within the original group who participated in the first semester this was implemented not every student was able to successfully synthesize a macrocycle, but their work equally contributed to the continuation of research for the Simanek group.

CONCLUSION

This review highlights the experience offered to undergraduate organic chemistry students after their organic chemistry II laboratory course was moved to an online format two weeks into the semester. Students were offered the opportunity to work with Dr. Simanek on research his lab group was performing in the synthesis of macrocycles with the future potential to act as drugs. Students were able to begin work immediately on a research topic they had no previous instruction in and perform synthesis of new macrocycle compounds to add to a growing library within the Simanek group.

The experience overall can be described as extremely positive, raising the question of whether a similar course should be implemented in the future for undergraduate students at TCU to take which would fulfill their undergraduate research requirements. Students were able to beneficially participate in a research project without prior experience and accomplish results. Their perspectives give important information in order to provide guidance should TCU consider

implementing a similar course. Major takeaways include that the experience was different from any other chemistry experience offered by TCU and provided students a genuine research experience when they had little to none prior. Further questions surrounding this experience can be directed to Dr. Simanek who is both responsible for facilitating the opportunity and requesting the creation of this review.

SURVEY RESULTS

Why did you enroll in 'Introduction to Research' that replaced the canceled OChem lab course? 6 responses

- Student 1 (S1): To be honest, I didn't know what I was getting myself into. I was I initially told that it would just be an opportunity to learn some of the techniques that we would have learned in class. I wanted to do it because I plan on pursuing a PhD in biology and was looking for as much lab experience as possible.
- Student 2 (S2): I wanted in-person lab experience to learn the techniques. It was also a way to stay connected to peers in an online year
- Student 3 (S3): I had the time and wanted to gain experience. The opportunity presented itself perfectly
- Student 4 (S4): To get experience in a research lab.
- Student 5 (S5): I was interested in continuing my organic chemistry education as well as learning more practical skills in research.
- Student 6 (S6): I felt that getting lab experience was crucial for my learning, and I wanted to expand my learning and try something new.

What did you hope to learn/accomplish? 6 responses

- S1: I wanted more lab experience and to learn new techniques
- S2: Wanted to learn lab techniques and learn more about faculty research.
- S3: I hoped to understand the process of research more, and grow in my personal/professional development
- S4: I hoped to learn more about different techniques used in an organic lab.
- S5: I wanted to learn about the process of research and what is is like to practice science. Furthermore, I felt like I needed more lab experience as many experiences had been cut short due to COVID.
- S6: Lab skills along with thinking skills. I wanted to learn how to examine my work and analyze results.

Please describe what you did during this activity. 6 responses

- S1: 3 step synthesis of marcocycles
- S2: I remember lots of column chromatography, creating compounds, running NMR to see if the compound formed
- S3: We attempted to synthesize macrocycles
- S4: I synthesized a macrocycle from the amino acid 4-aminopiperdine, with the goal of interacting with a leucine zipper or to be used as an antibiotic with the overall charged properties of the compound.
- S5: attempted to synthesize a leucine macrocycle with benzylamine.
- S6: I proceed through a reaction in order to try to form a macrocycle ring compound

What did you learn/accomplish as it pertains to techniques? 6 responses

- S1: I learned thin layer chromatography and Column chromatography. Also learned how to use the RotoVap and run NMR
- S2: I learned a bunch of lab skills without the stress of hurting your grade if you got it wrong the first time.
- S3: Chromatography, interpreting results from NMR, interpreting TLC plates
- S4: I learned how column chromatography and TLC work (that was the main technique I remember doing).
- S5: chromatography, NMR, TLC.
- S6: I learned all of the techniques in a standard ochem lab, coloumn chromatography, NMR, rotevap, and how to follow through experiemntal guidelines.

What did you learn/accomplish as it pertains to how research is done? 5 responses

- S2: I liked how he explained at the beginning of the lab period how the chemistry we were doing that day impacted the compound formation.
- S3: It is not black and white, we start over many times
- S4: That it's a long process but rewarding.
- S5: I learned that research is many times the same things over and over until you perfect the technique
- S6: How to think and draw conclusions on my own, research was about an abstract concept we were trying to reach with no set of instructions, so having to draw conclusions and interpret data on my own was a huge part of that.

Do you believe the experience was beneficial and if so, why? 6 responses

- S1: Yes, allowed me to see another field or research outside bio, provided me with more research experience, Andre me apply what I had learned in class
- S2: Yes, it was my favorite chemistry experience so far. What you were doing actually applied to something in the real world and you were there to gain experience and learn, not be punished for a technique that you just learned going wrong
- S3: YES I learned a lot and grew in my own capabilities. I felt like it was a chance to learn more about chemistry that wasn't an exam with major grades on the line
- S4: Yes, I think it was good experience to gain knowledge from synthesizing molecules/potential drugs, especially since I want to go in to the medical field.
- S5: yes; It taught me what it is like to practice science or what a graduate degree in science might look like.
- S6: Yes, I would never have had the opportunity to learn how to think and trust myself the way I had to do in lab.

What were the best elements of the activity? 5 responses

- S1: Learning to problem solve and troubleshoot
- S2: Working towards an actual faculty research goal
- S3: Interacting with Dr. Simanek and learning something new about research almost every day
- S4: The NMR was cool, but honestly have no clue how to read it (montchamp seemed to think everyone online that semester should've been experts on it tho...).
- S5: forming new relationships with my peers!

What would you change? 5 responses

- S1: There was poor communication and a lack of support at the beginning
- S2: Sometimes I didn't quite understand what each research technique was doing so maybe having clearer explanations would help
- S3: Maybe a little more structure
- S4: Maybe a little more structure because there was a few times when I was lost.
- S5: Possibly a little bit more introduction and background. I left a little confused the first couple weeks with what was going on.

How did the experience affect your career plans (and what are they) or future research pursuits? 5 responses

- S1: Still want to conduct molecular biology research
- S2: Didn't change them, still going to medical school. I was already in a biology research lab at the time but it did show me that a chemistry lab could be fun (I had never considered joining one before)
- S3: Still want to go to med school, but research doesn't seem so foreign to me now which I really appreciate
- S4: I want to go into medicine, specifically psychiatry, so those are my future research pursuits.
- S5: it led into my honors thesis which is a great introduction to publishing for my career in medicine.

On a scale of 1-5 how likely are you to recommend this activity to others (1=very likely, 3=neutral, 5=unlikely)? 6 responses

- See chart 1

Would you make this activity a course offering to satisfy research requirements associated with the major?5 responses

- Yes

What role did each person play in your experience (graduate TA, UG TA, Dr. Simanek, classmates)? 5 responses

- S2: I loved how involved Simanek was. It was nice to get to know a new professor in the department and see how passionate he was about his research. It was helpful to have UG there to help w small procedure questions. And I grew close with my classmates because there was less competitive drive between everyone and we were working towards a common goal
- S3: I was able to grow closer to my classmates. I learned more about graduate school from Alex, basic chemistry principles from Liam, and all sorts of research/chemistry related things from Dr. Simanek
- S4: Dr. Simanek really helped me out a lot along with the TAs. I liked how I was never made to feel stupid, knowing that I'm awful at O-chem especially NMR. My classmates were super helpful too at checking on each other and making sure everyone understood what we had to do.
- S5: I felt like the graduate TA's played the biggest role in my learning followed by Dr. Simanek
- S6: TA was someone I felt comfortable talking to and relying on for help, classmates were friends and also helpers along the way

Please add any additional comments/critiques. 3 responses

- S1: I think that's his would be a great course offering but it needs to be formalized with clear communication.
- S3: n/a
- S4: Overall, great experience (10/10, would do again)

ADDITIONAL INFORMATION

Undergraduate students that participated: Mia Nguyen, Joe Mann, Zach Aldrete, Carl Berghult, Sarah Collins, Gretel Jordan, Alex Caron, Josie Nguyen, Katherine Lester, Nicole Raines, Allison Regan, Morgan Bertrand.

Graduate teaching assistants: Daniel Ta, Timothy Schwartz.

Undergraduate teaching assistants: Annemarie Thompson, Liam Claton

Supervising Faculty Member: Dr. Eric Simanek

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