TANDEM REACTIONS OF 3-AMINO-1,2,4-TRIAZOLE WITH
TRANS-CINNAMOYL CHLORIDE AND TRANS-CROTONYL CHLORIDE

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Lou Thao

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by

Lou Thao

Approved by:

________________________, Committee Chair
Cynthia Kellen-Yuen

________________________, Second Reader
Jacqueline Houston

________________________, Third Reader
Mary McCarthy Hintz

________________________
Date
Student:  Lou Thao

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___________________________, Graduate Coordinator
Susan Crawford

Department of Chemistry
Abstract

of

TANDEM REACTIONS OF 3-AMINO-1,2,4-TRIAZOLE WITH
TRANS-CINNAMOYL CHLORIDE AND TRANS-CROTONYL CHLORIDE

by

Lou Thao

Triazoles are heterocyclic, aromatic compounds used as intermediates for the
synthesis of dyes, lubricants, and anticorrosion products. They are found in all aspects of
drug discovery, ranging from pharmaceutical and agriculture industries to proteomic and
DNA studies. Derivatives of triazole have been found to have biological activities such as
anti-HIV, anti-allergenic, anti-microbial, anti-inflammatory, anti-bacterial, and anti-
fungal applications. Triazoles are unique in that the molecule is an aromatic, five-
membered ring containing three nitrogens. The reactivity of each nitrogen in the ring
appears to be dependent upon different reaction conditions; however, the mechanism
leading to the reactivity is unclear.

In the literature, regioisomers result from the reaction of 3-amino-1,2,4-triazole
and bi-functional electrophiles such as the conjugated acid halides. However, the
products reported in the literature were not well characterized and often the issue of
alternate isomers was not even investigated. This study focuses on reactions to
understand both the reactivity of this interesting nucleophile and the mechanism by which products are formed (and possibly thereby leading to some level of control over reaction orientation). Reactions of 3-amino-1,2,4-triazole with two different conjugated acid halides (trans-cinnamoyl chloride and trans-crotonyl chloride) are investigated in this study under different synthetic conditions, varying solvent, reaction time, and temperature to provide a better understanding of the reactivity of this system. Isolated products were purified using flash column chromatography and fully analyzed by GC-MS, $^1$H NMR, $^{13}$C NMR, $^1$H-$^{13}$C HSQC, $^{15}$N NMR, and $^1$H-$^{15}$N HMBC analysis. The full characterization of the products will allow for a better understanding of the reactivity of the 3-amino-1,2,4-triazole nucleophile.

_____________________________, Committee Chair
Cynthia Kellen-Yuen

_____________________________
Date
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Last but not least, a big thanks goes out to my family. Seng Vang, thanks for being such a loving and supportive husband. Thanks Mom, Dad, Chong, Pang, Mai Nhia, Doua, Cheng, Ka Bao and Yia, for all the unconditional love, support, and believing in me. Also, thanks to all my in-laws for all the love and support you have given me.

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DEDICATION

Mom (Xai Chang) and Dad (Fue Thao), this thesis is dedicated to you in honor of all the sacrifices you have made to support the family. You gave up your dreams so my siblings and I could achieve ours. You have always said that your children are your future and have always been the backbone in everything we do. You encouraged us to aim high and hoped that one day we could fulfill the dream you once desired to achieve (to pursue higher education and continue practicing medicine in the US), so today I stand proud and dedicate this work in honor of you. Thank you so much for all your love and support. Both of you are my inspiration and I love you.

(Translation in Hmong)

Nam (Ntxhais Tshaab) hab Txiv (Chaiv Fwj Thoj), kuv muab phau ntawv nuav ua meb tug. Ua tsaug ntau ntau heev rua meb txuj kev ai kev khws ua meb tau ua pub rua peb tsev tuaj neeg es ua rua meb tsis tau nrug luas tej moog kawm ntaub kawm ntawv. Vim muaj peb, meb txhaj le tau muab meb txuj kev npau suav tso pov tseg yuav ua txhua txhua yaam kuam peb, meb cov miv nyuas, moog kuam txus peb lub hom phaj. Meb yeej ib txwm has tas meb cov miv nyuas yog meb txuj kev npau suav hab meb lub hom phaj. Meb txuj kev npau sauv yog kuam peb txhau txhau tus miv nyuas kawm kuam taag qab sab vim le nuav kuv txhaj le xaav muab kuv phau ntawv nuav sau lug ua meb tug. Kuv hlub meb heev hab thov ua meb tsaug ntau ntau rua meb txuj kev hlub hab kev txhawb rua kuam kuv moog kuam txus kuv lub hom phaj.
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LIST OF ABBREVIATIONS

d$_6$-DMSO = universally-deuterated dimethylsulfoxide
ACN = acetonitrile
DCM = dichloromethane
DMF = dimethylformamide c
EtOAc = ethyl acetate
Hz = hertz
MeOH = methanol
Rf = retention factor in chromatography
RT = room temperature
THF = tetrahydrofuran
TMS = tetramethylsilane
NOE = Nuclear Overhauser Effect
COSY = Correlation spectroscopy
HSQC = Heteronuclear single quantum correlation
HMBC = Heteronuclear multiple bond correlation
Chapter 1

INTRODUCTION

1.1 Background

Triazoles are aromatic, five-membered ring compounds with three nitrogen atoms present in the ring (Figure 1). The use of triazoles has been found in various fields and is continuously growing. Triazoles can be used as building blocks for more complex chemical compounds. For example, triazoles can be used as intermediates for the synthesis of dyes, lubricants, anticorrosion products and pharmaceuticals.¹ In pharmaceuticals, the applications of triazoles are found in all aspects of drug discovery, ranging from cutting edge research through combinatorial chemistry and target-templated \textit{in situ} chemistry, to proteomics and DNA research using bioconjugation reactions.² Triazoles are more than just passive linkers; they readily associate with biological targets through hydrogen bonding and dipole interactions.² Derivatives of triazoles have been found to have anti-HIV,³ anti-allergenic,⁴ antimicrobial,⁵ cytostatic,⁵ virostatic,⁵ anti-inflammatory,⁶ anti-bacterial,⁷ and antifungal⁸ activities. In addition, triazoles are being studied for the treatment of obesity⁹ and osteoarthritis.¹⁰ Triazoles are particularly interesting for medicinal use because they are more likely to be water soluble than normal aromatic compounds, and are stable in biological systems.¹¹
A broad spectrum of triazole-based drugs now exist. Drugs such as fluconazole (Figure 1), itraconazole, voriconazole and posaconazole are used to treat cancer and to prevent superficial and systemic fungal infections like athlete's foot and ringworm. These triazole derivatives slow the biosynthesis of ergosterol by inhibiting the cytochrome-dependent enzyme lanosterol 14-alpha-demethylase, which converts lanosterol to ergosterol. Ergosterol is an essential component of the fungal cell membrane. Depletion of ergosterol damages the cell membrane, resulting in cell death.

Besides drug applications, some heterocyclic derivatives of triazole contain a functional structure similar to the nitrogenous bases adenine (Figure 1) and guanine. Studies have shown that triazole derivatives can be incorporated into the DNA strand as artificial 1,2,4-triazole nucleosides, which can be used to study DNA structure and conformational changes. The application of triazole as a nucleoside has opened a broader spectrum of studies regarding the structure, function, and regulation of DNA, RNA, and proteins, as well as the metabolic pathways of cells. Some triazole derivatives
have been shown to mimic the functions of adenine and guanine, which can further be used to study protein folding in DNA and RNA.\textsuperscript{15}

Applications for the use of triazoles are also found in other industrial fields. Various forms of triazoles are found in hydraulic fluids, agrochemicals (fungicides) and photochemical products.\textsuperscript{16} They have also been used as herbicides, light stabilizers, fluorescent whiteners, optical brightening agents, pigments and corrosion retardants.\textsuperscript{17-19} Aminotriazole, amitrole, and amitrol are commonly used as herbicides to control annual grasses and broadleaf and aquatic weeds. However, they are mainly used on non-food croplands, due to their carcinogenic properties.\textsuperscript{20} Herbicide compounds, such as 3-amino-1,2,4-triazole, can have multiple functions; for example, they can also be used as competitive inhibitors for the product of the HIS3 gene product (imidazoleglycerol-phosphate dehydratase), which is an enzyme that catalyzes the sixth step of histidine production.\textsuperscript{20}

1.2 Background of 3-Amino-1,2,4-Triazoles

3-Amino-1,2,4-triazoles belong to a class of compounds called azoles.\textsuperscript{21} The azole structure contains a five-membered aromatic ring with at least one nitrogen atom as well as other heteroatoms such as a nitrogen, sulfur or oxygen. Imidazoles, for example, contain two nitrogens within the five-membered ring, whereas triazoles contain three nitrogens in the ring. An example of the numbering system for the triazole ring can be seen in Figure 2, which shows the structure for 3-amino-1,2,4-triazole. The three
nitrogen heteroatoms are found at positions 1 (N₁), 2 (N₂) and 4 (N₄) in the aromatic ring along with an amine substituent group attached to position 3.

![Diagram of 3-amino-1,2,4-triazole]

**Figure 2.** The structure of 3-amino-1,2,4-triazole

For 3-amino-1,2,4-triazole, three tautomeric amino-containing structures and two imino-containing structures can theoretically exist (Scheme 1). Literature references have shown that each different structure has its own unique name, which is quite different from the IUPAC naming system. For instance in both 3-amino-1H-1,2,4-triazole and 5-amino-1H-1,2,4-triazole, the hydrogen in the ring is bonded directly to N₁, but the nitrogen labeled as N₁ has changed and the direction in which the ring is numbered has been reversed.
Scheme 1. The systematic names and isomeric structures of the tautomers of 3-amino-1,2,4-triazole

Although there are five possible tautomeric structures, only the three amino forms have been isolated. From a research study done by Fritz et al.,\textsuperscript{22} calculations at a semi-empirical level on the amino-imino tautomeric forms have shown that 3-amino-1H-1,2,4-triazole and 5-amino-1H-1,2,4-triazole are the most stable in comparison to 3-amino-4H-1,2,4-triazole. In addition, in Fritz’s study involving aqueous solutions using $^{15}$N NMR spectroscopy, 5-amino-1H-1,2,4-triazole was proven to be the more dominant form over 3-amino-1H-1,2,4-triazole, by a ratio of 2:1.\textsuperscript{22} Unfortunately, within the literature, the compound drawn to correspond to the IUPAC name “5-amino-1H-1,2,4-triazole” is the same compound, which goes by the name “3-amino-1,2,4-triazole” in other papers. Since the structure for the 5-amino tautomer is generally accepted to dominate in solution but the name “3-amino-1,2,4-triazole” is the most common name used for this exact same
structure in the literature, the remainder of this paper will use the more commonly accepted 3-amino-1,2,4-triazole name for this compound.

As shown in Scheme 1, a hydrogen can attach to any of the three nitrogen heteroatoms within the aromatic ring. With so many nitrogen atoms and the possibility of tautomeric forms for the molecule, there are several nucleophilic sites for possible substitution reactions, namely the N₁, N₂, and N₄ positions (Figure 3). The external amino nitrogen (N₃) is also known to behave as a nucleophile, adding a fourth reactivity site to this molecule. With multiple substitution positions available, many product isomers could be expected from nucleophilic substitution reactions.

![Substitution Positions](image)

**Figure 3.** Possible substitution sites on 3-amino-1,2,4-triazole

### 1.3 3-Amino-1,2,4-Triazole Reactions

An acyl halide reaction involving 3-amino-1,2,4-triazole has been studied by Rzeszotarsha et al. (Scheme 2), in which there were two different products formed, depending on the solvent used.²³ Using THF at room temperature, N₁ was the most favorable substitution site, giving rise to product 1. On the other hand, by changing the solvent to ACN and increasing the reaction temperature to 63°C, both product 1 and 2
were formed. Product 2 was proposed to be the major product isolated (product ratio 2:1), suggesting that N₂ became the more favorable substitution position under these conditions.

![Scheme 2. Acylation reactions involving 3-amino-1,2,4-triazole and acetyl chloride](image)

A similar acylation reaction involving benzoyl chloride (3) has been studied by Kellen-Yuen et al.²⁴ The only product for this study was proposed to be (5-amino-[1,2,4]-triazole-1-yl)(phenyl)methanone (4) where substitution occurred at the N₂ position in hot acetone solvent (Scheme 3). The different substitution outcomes for these acylation reactions suggested that the reactive site might be governed by some property of the solvent. Properties such as boiling points and polarity could be affecting the selectivity of the reactivity site. It is unclear which solvent property is mainly responsible for driving the reaction to a specific site. In a reaction where only one single-substitution can occurred, such as the reaction shown in Scheme 3, it appears that lower temperature
reactions tends to favor the \( N_1 \) position and higher temperature reactions favor the \( N_2 \) position.

\[
\begin{align*}
\text{Scheme 3. Reaction of 3-amino-1,2,4-triazole with benzoyl chloride}^{24}
\end{align*}
\]

Since there are multiple nucleophiles within the 3-amino-1,2,4-triazole molecule, it is also possible to do multiple substitution reactions in tandem. If the triazole is allowed to react with a compound that contains two electrophilic sites, for example, the potential exists to create bicyclic reaction products. Lipson et al studied reactions between 3-amino-1,2,4-triazole and \( \alpha \)-haloacid halides (\textbf{Scheme 4}).\textsuperscript{25} During the reaction, Lipson proposed the initial formation of intermediate \( 6 \) which underwent rearrangement to intermediate \( 7 \) before cyclization to give product \( 8 \).
Scheme 4. Proposed reaction of 3-amino-1,2,4-triazole with an α-haloacid halide

In a specific example from this study, 3-amino-1,2,4-triazole reacted with \textit{trans}-cinnamoyl chloride 9 to give the products reported in Scheme 5. In the proposed reaction, product 10 and 11 are formed during the first step of the reaction, which occurred at lower temperature. The ratio of product 10 to product 11 was 1:2, suggesting that the N$_2$ nucleophilic site was more reactive. Product 11 was isolated and then heated for an additional 6 hours in DMF, giving two isolated products: a new mono-substituted molecule 12 and the bicyclic product 13 in a ratio of 1:2. If the structure of 13 is correct, it suggests that 12 is an intermediate which forms before ring closure to the heterocyclic product 13. This suggests that heat and/or DMF solvent causes a migration of the acyl group; however, the reason for the migration step was not explored or further explained.
in this study, and absolute proof of the reaction forming this single regioisomer was not presented.

Scheme 5. Reaction of 3-amino-1,2,4-triazole with trans-cinnamoyl chloride

In fact, in most published studies products are only characterized based on one dimensional (1-D) $^1$H NMR and $^{13}$C NMR. With complex nitrogen-containing compounds like the triazole products formed in these reactions, it is very difficult to fully characterize the isolated product using only 1-D $^1$H NMR and $^{13}$C NMR, since similar structures have similar chemical shifts. Thus, simple 1-D NMR analyses often do not have the ability to distinguish among different regioisomeric compounds. For example, in Lipson’s study (Scheme 5), there are 3 proposed regioisomeric, single-substitution products (Products 10-12) isolated. The differences in their 1-D NMR spectra would be
subtle and very difficult to distinguish, and the tentative identity of only compound 11 and 12 are reported in the literature.

1.4 Other Tandem Reactions Involving 3-Amino-1,2,4-triazole

Other interesting studies have been performed by other groups in an attempt to form bicyclic structures through other tandem reactions. Allen et al performed a cyclization reaction using a β-ketoester as the di-electrophile. As shown in Scheme 6, acidic conditions allow the ketone of ethyl acetoacetate (14) to bind to the amino nitrogen of 3-amino-1,2,4-triazole, and ring closure via an ester to amide substitution then goes through the N₂ position to form product 15. This suggests that the amino nitrogen is the most favorable nucleophilic substitution site in an acidic reaction environment. As reported by Allen, the reaction tended to close in the N₂ direction rather than the N₄ because N₂ has greater negative charge in comparison to N₄. However, the isolated products were not fully characterized by ¹⁵N NMR.

![Scheme 6](image)

\[ \text{Scheme 6. Reaction of 3-amino-1,2,4-triazole with a β-ketoester}^{26} \]

In other cyclization reactions, the N₂ position and the N₄ position are both found to be active nucleophilic sites. A reaction studied by Langer et al demonstrates this
concept.\textsuperscript{27} Langer’s proposed reaction of 3-amino-1,2,4-triazole with oxaliimidoyl dichlorides (16) is shown in Scheme 7. This reaction yields the bicyclic products 17 and 18, with 17 forming in a 6:1 ratio over 18. The authors did not isolate an intermediate; therefore, it is not known whether reactions began with nucleophilic attack by the free amine or by one of the ring nitrogen atoms (N\textsubscript{2} or N\textsubscript{4}). Both products 17 and 18 were assigned based on proton and carbon NOE measurement, but without \textsuperscript{15}N NMR data.

Scheme 7. Reaction of 3-amino-1,2,4-triazole with oxaliimidoyl dichlorides\textsuperscript{27}

In summary, a large number of reactions have been done to studied nucleophilic substitution reactions involving 3-amino-1,2,4-triazole. In single substitution reactions (e.g. acylations), N\textsubscript{2} appeared to be the more favorable nucleophilic substitution site, although N\textsubscript{4} and the free amine were also proposed to have good reactivity based on predicted reaction outcomes. In tandem reactions all three of these sites are believed to show reactivity towards substitution. The complexity of the problem of regioselectivity in these nucleophilic sites is only truly appreciated when viewing all of the possible outcomes which might be considered. For example, in one of the tandem cyclization reactions discussed previously (Scheme 5), 3-amino-1,2,4-triazole has multiple available
nucleophilic sites for acid halide substitution reaction. It is possible to have four regioisomer products formed from the initial substitution, reaction as shown in Scheme 8 (Compounds A-D), as well as four bicyclic products after the ring closure step (Compounds E-H). These products each contain the same functional groups with only slight potential differences in polarities. It is easy to imagine that only very slight chemical shift differences might be present in their $^1$H and $^{13}$C NMR spectra, but the number of signals and their splitting patterns would be unchanged. With this many possible regioisomers that can form during a reaction with only subtle differences in their 1-D spectra, a more reliable characterization system would require heteronuclear multiple bond correlation (HMBC) to study the $^1$H-$^{13}$C or $^1$H-$^{15}$N bond correlation interactions to distinguish between these subtly different molecules.
Scheme 8. Overview of all the possible isomeric products which could form from a tandem cyclization reaction of 3-amino-1,2,4-triazole with trans-cinnamoyl chloride.

Although reactions described in the literature have shown interesting results, the isolated products were not analyzed thoroughly, since assignments were often made...
based on 1-D $^1$H and $^{13}$C NMR data alone, and definitive proof of isomer structures was rarely investigated. In addition to $^1$H and $^{13}$C NMR, $^{15}$N NMR is a powerful tool for investigating compounds containing multiple nitrogens. With the combination of $^1$H, $^{13}$C, and $^{15}$N NMR, and the availability of 2-D analyses (COSY, HSQC, HMBC, etc), it should be possible to thoroughly characterized these isomeric compounds and prove definitively their exact structures.

1.5 Background of Nitrogen NMR

Nitrogen has two NMR active isotopes, $^{15}$N and $^{14}$N, which can be distinguished by their peak shape. The peak shape for the $^{15}$N nucleus is usually a very sharp line in comparison to $^{14}$N nuclei, whose peak shape is much broader. The broadening of the peak is due to quadrapolar interactions. When the quadrapolar interaction is high, the peaks become broad and sometimes result in an unobservable peak on a high resolution NMR spectrometer. Figure 4 illustrates the peak shape of an $^{15}$N nucleus versus an $^{14}$N nucleus for the compound urea. Since both nitrogen atoms in urea have the same chemical shift, only one peak is seen in the spectrum.

![Figure 4. The nitrogen NMR spectrum of urea](image)
Due to the differences in abundance among the different NMR-active isotopes, the acquisition time for each isotope must be different. The hydrogen isotope ($^1\text{H}$) has a high natural abundance of 99.99%, therefore the acquisition time to generate a usable NMR spectrum only takes about 1-2 minutes. Since the NMR active carbon isotope ($^{13}\text{C}$) has a lower natural abundance of 1.1%, the acquisition time often takes about 45 minutes. In fact, lower natural abundance isotopes are harder to detect using NMR. In comparison, the natural abundance for $^{15}\text{N}$ isotope is even lower than $^{13}\text{C}$, about 0.36%, meaning it will be much harder to detect the $^{15}\text{N}$ signal; therefore, a longer delay time is required to detect the signals. NMR analyses using low natural abundance generally require the sample to be more concentrated when tested and more pulses will be required to increase the signal intensity. On average, the acquisition time to generate a usable spectrum for $^{15}\text{N}$ varies from about two hours to two days, depending on the concentration of the sample.

NMR analysis is not just a matter of dealing with its natural abundance; the spin state of each active nuclei is also important. In order to have a good signal, the nuclear spin state must be greater than zero. $^{12}\text{C}$ nuclei have a zero net spin therefore the isotope is not detectable by NMR. $^{14}\text{N}$ nuclei have a net spin of one, therefore they are detectable by NMR but limited by the effects of quadrapolar interactions they encounter.

Quadrapolar interaction is the result of an electric interaction occurring from a nucleus that is interacting with an electric field and often performing a circular motion. The electric field gradient is provided by an asymmetric distribution of electron density around the nucleus. This effect allows the degeneracy of the nuclear energy levels to be
lifted and causes the peak shape to become broader; sometime so broad that the peak can
disappear into the baseline. Quadrapolar interactions are often not seen in symmetrical
molecules because not much of an electric field is generated around the nucleus. In
theory, quadrapolar interactions are often seen in nuclei that have a spin \( I \) greater or less
than 1/2, but are not observed when the nuclear spin is 1/2. **Figure 5** illustrates the
electrostatic interactions of the different nuclear spin states; \( I = 1/2 \) and \( I > 1/2 \). The
electrostatic interactions for \( I < 1/2 \) is similar to \( I > 1/2 \) as shown in **Figure 5**.30,31

**Figure 5.** The shape of electrostatic quadrupole interaction in spin \( I > 1/2 \) and \( I = 1/2 \).31

For nuclei that have nuclear spins which are detectable, the sensitivity of the peak
signal depends on the gyromagnetic signal ratio. Gyromagnetic (\( \gamma \)) signals are measured
by the ratio of the magnetic dipole moment to the angular momentum as shown in **Figure
6.**32 The magnetic dipole moment of a nucleus can align with or against the applied
magnetic field, which allows the nucleus to spin clockwise or counter-clockwise.32
Figure 6: A demonstration of a magnetic dipole moment and angular momentum found in $^{13}$C and $^{15}$N nuclei.

The gyromagnetic signal ratio can be mathematically determined by using Equations 1 and 2. Equation 1 calculates the maximum ratio enhancement of the gyromagnetic irradiating nucleus and gyromagnetic observed nucleus. For example, in a $^1$H analysis, the measurement of the nuclear enhancement of the irradiated proton is compared to the nuclear enhancement of the observed proton. If the observed enhancement signal is less than 50% of the total relaxation rate, the observed signal is too weak, and appears as noise in the spectrum. The maximum total predicted intensity can be calculated by Equation 2.

$$\text{NOE}_{\text{max}} = \frac{1}{2}(\gamma_{\text{irr}}/\gamma_{\text{obs}})$$ \hspace{1cm} (Eq. 1)

$$\text{Total Predicted Intensity}_{\text{max}} = 1 + \text{NOE}_{\text{max}}$$ \hspace{1cm} (Eq. 2)
Unlike $^1$H and $^{13}$C NMR, $^{15}$N NMR has a much wider spectral range, from 0 to 850 ppm. The approximate chemical shift for a particular type of nitrogen can be found in Figure 7. For example, the range for an amine nitrogen is more upfield (0-150 ppm) in comparison to azo- or nitroso-nitrogens, which are more downfield in the region of 500-800 ppm.$^{34}$

**Figure 7.** Approximate $^{15}$N chemical shift range of different types of nitrogen group.$^{34}$

Despite the spectral range differences, $^{15}$N NMR can also generate 2-D NMR analyses similar to $^1$H and $^{13}$C nuclei. For example, HSQC (Heteronuclear Single Quantum Correlation) and HMBC (Heteronuclear Multiple Bond Correlation) analyses can be used to measure the coupling between a nitrogen and a hydrogen. HSQC is based on transferring magnetization from the proton to the second nucleus ($^{13}$C or $^{15}$N) via an “insensitive nuclei enhanced by polarization transfer” (INEPT) step and vice versa, after
a time delay, via a retro-INEPT step.\textsuperscript{35} The analysis mention here can gives information about two nuclei which are directly bonded. HMBC, on the other hand, has the ability to also measure multiple bond systems. HMBC can sometimes measure the interaction of nuclei up to four bonds away by coupling through space via an NOE (Nuclear Overhauser Effect). However, in some cases when the molecule of interest is linear or symmetrical, both HSQC and HMBC analyses provide similar spectra. In other cases, where the compound of interest is non-linear or asymmetrical, more cross peaks are likely to show up in the HMBC spectrum than are seen in the HSQC spectrum. The presence of the extra cross peaks usually is an indication of possible coupling associating with an NOE effect. This information can be very helpful to identify the three-dimensional structure of an unknown compound or distinguish between isomers.

Other information that can be used to help identify isomeric compounds is the value of the chemical shift signal observed. Different chemical shift signals appear to be associated with the different types of bond positions between a nitrogen and hydrogen atom. As shown in \textbf{Figure 8}, by using \textsuperscript{15}N NMR, it can be seen that having a proton attached to a nitrogen will significantly affect the observed position of a nitrogen signal. Studies have shown that nitrogen chemical shift between isomeric or tautomeric structures can be affected by the corresponding electron charge densities on the nitrogen atom that is attached to the proton (\textbf{Figure 8a}). Studies also show that in an aromatic ring, the nitrogen signal of a molecule with an \textit{1,2} substitution is different from a \textit{1,3} or \textit{1,4}substitution (\textbf{Figure 8b}). Overall, different types of nearby carbons or hydrogens can
affect the nitrogen signal, resulting in different chemical shifts for what seem to be very similar nitrogens.

![Diagram](image)

**Figure 8.** $^{15}$N NMR chemical shift changes for tautomeric isomers (a) and interaction effects for aromatic ring systems (b). $^{36}$

Substitution pattern can impact the position of the observed NMR signal, but studies also show that changing the group bonded directly to the nitrogen atom may also impact the nitrogen signal. Similar to $^{13}$C NMR, nitrogen’s signal shifts more downfield as the bonded substituent group is replaced by alkyl groups. An example of this is a sequence of alkyl substituent like -CH$_3$ < -CH$_2$R < -CHR$_2$ < -CR$_3$. The chemical signal for a nitrogen bonded to a -CR$_3$ group is slightly more down field compared to when the same nitrogen is bonded to a -CH$_3$ group. For example the signal of a -CHR$_2$ substitution versus a -CH$_3$ may change by 5-10 ppm more downfield.$^{36}$
By contrast, Varela et al discovered that undisturbed corresponding nitrogens maintain their chemical signal position even as substituent groups elsewhere in the molecule are changed, as shown in Scheme 9. Changing the substituent groups on the R positions shown in structures 19 and 20 (particularly position R₆ in compound 19 and R₃ in compound 20), results in very little changes in chemical shift for the corresponding nitrogens in these compounds. The difference in chemical shift only varies by 4-10 ppm for these different compounds with undisturbed nitrogens (i.e. the nitrogen substituents are unchanged).
Scheme 9. $^{15}$N chemical shifts of compounds containing a triazole structure $^{37}$
Marek et al studied the chemical shift environment of the nitrogen found in compound 21-24 by comparing the chemical shift of a non-substituent nitrogen versus a substituent nitrogen.\textsuperscript{38} Scheme 10 shows the observed $^{15}$N chemical shifts for compound 21-24. Note that compounds 21 and 22 and compounds 23 and 24 are sets of regioisomers. In this study, Marek et al studied the chemical shifts for the undisturbed or non-substituent N$_7$ present in compound 22 and 24 and N$_9$ present in compound 21 and 23. They also studied the disturbed N$_7$ present in compound 21 and 23 and the disturbed N$_9$ present in compound 22 and 24. It can be seen that the chemical shift for the nitrogen attached to a substituent group appears lower than the same nitrogen without a substituent due to a change in the nitrogen’s chemical environment. As a result, a non-substituted aromatic nitrogen would be expected to have a higher chemical shift value and be seen more downfield than an aromatic nitrogen attached to a carbon chain, which is more shielded and results in a lower chemical shift. As a comparison from compounds 21 and 22, the observed chemical shift for the non-substituted N$_9$ and N$_7$ are 246.2 and 239.4 ppm, while the substituted N$_7$ and N$_9$ are only 144.2 and 153.6 ppm respectively.\textsuperscript{38} A similar trend is seen in the N$_7$/N$_9$ groups on isomers 23 and 24.
Another group found very similar effects on disturbed and undisturbed nitrogen shifts. Vaughan et al.\textsuperscript{39} have further investigated the chemical shift of a set of nitrogen atoms when different substituents are attached to one of three possible nitrogen sites.

Table 1 shows an example of one of the compounds studied, where the substituent is
attached to N₃ and the difference in chemical shifts for each derivative is shown. Similar to the previous nitrogen study, the chemical shift position of undisturbed nitrogens (N₁ and N₂) only varies slightly (0.5 to 10 ppm), regardless of the substituent attached to N₃. Unlike the undisturbed atoms, the disturbed nitrogen (N₃) can be dramatically affected by the nature of the substituent. The change in chemical shift for an N-H vs N-C bond is found to be small ~1-10 ppm, compared to the shift associated with forming a much more polar N-O bond (~40-50 ppm).

**Table 1.** \(^{15}\)N chemical shift for compounds with various groups at the N₃ position.\(^{39}\)

<table>
<thead>
<tr>
<th>Compound</th>
<th>N₁ (ppm)</th>
<th>N₂ (ppm)</th>
<th>N₃ (ppm)</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) H</td>
<td>-16.5</td>
<td>29.3</td>
<td>-152.5</td>
<td>—</td>
</tr>
<tr>
<td>b) CH₃</td>
<td>-16.0</td>
<td>32.6</td>
<td>-153.8</td>
<td>—</td>
</tr>
<tr>
<td>c) CH₂Ph</td>
<td>-13.5</td>
<td>32.4</td>
<td>-143.8</td>
<td>—</td>
</tr>
<tr>
<td>d) CH₂CH₂Cl</td>
<td>-14.0</td>
<td>31.8</td>
<td>-150.3</td>
<td>—</td>
</tr>
<tr>
<td>e) CH₂CONH₂</td>
<td>-14.7</td>
<td>33.1</td>
<td>-151.3</td>
<td>-274.6</td>
</tr>
<tr>
<td>f) OH</td>
<td>-23.8</td>
<td>24.7</td>
<td>-113.4</td>
<td></td>
</tr>
<tr>
<td>g) OCH₂Ph</td>
<td>-17.1</td>
<td>25.2</td>
<td>-104.1</td>
<td></td>
</tr>
</tbody>
</table>

It would be easy to distinguish between nitrogen atoms that are in very different environments (i.e. the amide N₃ and the pyridine-like N₁ in **Table 1**) using 1D NMR. However, this may not be the best approach to differentiate among very similar nitrogen
environments (N₁ and N₃ in Scheme 10) or among different regioisomers or tautomers that contain multiple nitrogens (like structures 21 and 22). For this reason, a 2D ¹H-¹⁵N HMBC NMR analysis for studying long range coupling would have more potential to differentiate between different isomers.

The ¹H-¹⁵N HMBC spectrum of 6-(3-acetylphenyl)-3-benzylamino-[1,2,4]triazolo[4,3-a]pyrimidine (Compound 25) is shown in Figure 9. Each spot in the spectrum corresponds to a correlation between a nitrogen and hydrogen which is 2-4 bonds away. For example, there are three peaks in this spectrum that show correlation to the only N-H nitrogen, indicating that this nitrogen can interact or is in close proximity to three different protons. Indeed, looking at the structure of the molecule, this amino nitrogen interacts with the benzylic CH₂ protons as well as two protons in the aromatic ring (the ortho hydrogens). It can also be seen that nitrogens N₄ and N₂ are interacting with the N-H proton. The nitrogens at N₈ and N₁ both interact with H₇, while H₅ interacts with N₂ and N₈, although the interactions are not as strong. As a result, ¹H-¹⁵N NMR analysis can be used to clearly establish the position of substituents in the molecule.
Figure 9. $^1\text{H}^{15}\text{N}$ HMBC spectrum of 6-(3-acetylphenyl)-3-benzylamino-[1,2,4]triazolo[4,3-a]pyrimidine

Another technique that could help to resolve this problem of distinguishing between isomeric compounds and understanding long-rang NMR couplings is to conduct X-ray crystallographic analyses. X-ray crystallographic techniques can visually display the compound of interest in a 3D format. To investigate the HMBC results of compound 25 further, Valera et. al compared their results to those of a similar compound (compound 26, Scheme 11) where the X-ray crystallographic structure is known. Based on the skeletal drawing of compound 25, the amino nitrogen appears to be quite far from the...
protons present in the phenyl group attached to the benzylic moiety (4 bonds apart). The crystallographic structure shows that the benzylic moiety is bent in a way which allows the phenyl group to lean toward the amino nitrogen. This allows the amino nitrogen to interact with the protons in the phenyl ring because they are now closer in proximity. This helps to explain how the amino nitrogen and the protons in the phenyl ring in compound 25 can interact as shown in the 2-D spectrum.

Scheme 11. The skeletal and crystallographic structure of compound 26.

Based on the 3D structure of 26, the main parent structure (fused 5 and 6 membered aromatic rings) appears not to bend at all, i.e. it remains in a flat plane as shown in the crystallographic structure. It should be noted that in the study reported herein, the compounds also contain fused 5 and 6 membered rings; however, only one is aromatic, therefore the structure is not as likely to remain flat. For this reason, an alternative route to fully characterize and distinguish the exact structure of the products isolated from this study will require 1-D $^{15}$N NMR and 2-D $^1$H-$^{15}$N HMBC analyses.
1.6 Statement of Problem:

Since literature studies seem to show a variability in the reactivity the various nitrogens of the triazole ring, the general purpose of this study is to examine several factors to develop an understanding of how to predict or control the reactivity of 3-amino-1,2,4-triazole in a particular nucleophilic cyclization reaction. These factors will include solvent, time and temperature studies. The reaction being studied is that between 3-amino-1,2,4-triazole and a conjugated acid halide, (cinnamoyl chloride and trans-crotonyl chloride). The solvent study includes reagents shown to give differing outcomes in previous studies: acetone, dimethylformamide (DMF), tetrahydrofuran (THF), and acetonitrile (ACN). In addition to the nature of the solvent, studies were undertaken to determine if temperature or reaction time causes any differences in the product outcomes. Products were isolated, purified, and characterized by spectroscopy to try to determine their exact structure, as this may help to determine the exact mechanism for the reactions and lead to information on the relative reactivities of the various nitrogen nucleophiles.

In addition, as an interesting side investigation, the unusual migration step proposed in the Lipson study, where the nucleophilic substrate apparently jumps from the N₂ to the amino nitrogen prior to cyclization²⁵ was also investigated in these studies.²⁵ Attempts were made to isolate intermediates in the reaction and then cyclize each compound separately to see if both intermediates close to form the same cyclic compound. In all studies, a major focus lies in the spectral analysis of the products. Of particular interest is establishing both the 1D ¹⁵N and the 2D ¹H-¹⁵N HMBC analyses.
2.1 Reaction Overview Studies

In order to understand the mechanism of the reaction of 3-amino-1,2,4-triazole with trans-cinnamoyl chloride or with trans-crotonyl chloride, it is important to understand the variables that can affect the reaction outcome. Solvent, reagents, temperature, and time can all be variables that can control the outcome of an organic synthesis. Scheme 12 is a general overview of the conditions used for each reaction in this study.

Scheme 12. A brief overview of the reactions studied

Schemes 13 and 14 show all of the possible mono-substitution and 6-membered ring products which might be isolated from the two reactions studied. Both reactions are run under similar conditions, with variations in solvents and temperatures. There is a possibility of 8 different products that can be formed from each reaction. With the assumption that all N₁-N₄ site are reactive for substitution, four of the products are
regioisomers formed from the initial amide formation (Products A-D and Products I-L) and four cyclization regioisomers (Products E-H and Products M-P) are formed from the cyclization of Product A-C or Product I-K.

Scheme 13: Overview of the possible isomeric products which might form in a tandem cyclization reaction of 3-amino-1,2,4-triazole with trans-cinnamoyl chloride
Scheme 14: Overview of all the possible isomeric products which might form in a tandem cyclization reaction of 3-amino-1,2,4-triazole with trans-crotonyl chloride.

Non-bicyclic products such as A-D and I-L are formed by nucleophilic substitution reaction mechanisms. In this case, one of the nitrogens from 3-amino-1,2,4-triazole acts as the nucleophile, reacting with the acid halide electrophile. Since there are four potentially nucleophilic nitrogen atoms, there are four possible amide products that can be formed from the first step in this reaction.
Cyclization products such as E-H and M-P are formed by a subsequent Michael addition reaction. The activated double bond adjacent to the carbonyl acts as a Michael acceptor, while a second nitrogen atom from the 3-amino-1,2,4-triazole acts as the electron donor. Because of steric constraints, only selected nitrogen atoms can form the 6-membered ring products after the initial amide reaction takes place. This means that there are only four possible tandem cyclization products possible in each reaction.

Products D and L are not able to go on to form a 6-membered ring; they can bind with N$_2$ to form a 5-membered ring, but this is less likely to occur due to steric effects. Since the literature has shown that solvent, reagents, temperature, and time can all be variables that can control the outcome of an organic synthesis; these conditions have been the focus of the present studies.

### 2.1.1 Solvent Studies

The solvents selected in this study were acetone, dimethylformamide (DMF), tetrahydrofuran (THF), and acetonitrile (ACN). Each of these solvents was chosen because they have shown some impact in a similar reaction in past literature studies as discussed in the Introduction. Each solvent’s boiling point and polarity are different from one another. These properties can influence the outcome of the reaction, and an understanding of how this influence occurs can bring more knowledge about the mechanism of the reaction. For example, the literature boiling points for acetone, DMF, THF, and ACN are: 56 °C, 153 °C, 66 °C, and 82 °C respectively. Since the reactions are performed at reflux, this boiling point dictates the temperature of the reaction.
Studies were conducted by heating a 1:1 molar ratio of reagents in 15 mL of the chosen solvent in the presence of a pyridine base. Reactions were heated for 7 days to ensure the reaction had reached completion and to provide time for all of the reactive sites to have the opportunity to form products wherever possible. Results for the reaction of 3-amino-1,2,4-triazole with \textit{trans}-cinnamoyl chloride are shown in Table 2. Two cyclization products (\textbf{Products E} and \textbf{F}) and a non-cyclized product (\textbf{Product B}) were isolated from these reactions. The identification of \textbf{Products B, E,} and \textbf{F} is very complex, therefore the NMR data used to prove the identities of each product will be discussed in \textit{Sections 2.3.2 - 2.3.4.}

\textbf{Table 2.} Solvent studies from reaction of 3-amino-1,2,4-triazole and \textit{trans}-cinnamoyl chloride using pyridine catalyst and refluxed for 7 days.

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Product B</th>
<th>Product E</th>
<th>Product F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>85.75%</td>
<td>10.05 %</td>
<td>N/A</td>
</tr>
<tr>
<td>ACN</td>
<td>54.41%</td>
<td>16.38%</td>
<td>N/A</td>
</tr>
<tr>
<td>DMF</td>
<td>N/A</td>
<td>90.35 %</td>
<td>N/A</td>
</tr>
<tr>
<td>THF</td>
<td>N/A</td>
<td>64.38 %</td>
<td>8.92 %</td>
</tr>
</tbody>
</table>
The results from the reactions studied in Table 2 showed that the non-bicyclic product B was only isolated in acetone and ACN and the cyclization product F was only isolated in THF, while the cyclization product E was isolated from all four solvents. It should be noted that Product B precipitated out of the solutions in the reactions where it was isolated, and that this remained as the major product even after a 7 days reflux time. This suggests that Product B is less soluble in acetone and ACN than in DMF or THF. If the solid dropped out of solution it was less likely to be converted into the cyclized product. As shown in Table 2, the formation of Product E is slightly better in ACN than acetone, suggesting a slightly better solubility of Product B in this solvent. Product E does not precipitate out in the solution in any of the solvents studied, therefore the isolation process required additional separation steps to extract Product E from the oily yellow crude mixture. During the separation steps, evidence was seen to suggest small amounts of other products were produced, however isolation of these products was problematic due to low yields, and only the cleanly isolated products are reported here.

By comparison, in the reaction of 3-amino-1,2,4-triazole and trans-crotonyl chloride under the same reaction conditions, the cyclization product M is the only major product formed from all four solvents (Table 3). It is surprising to note that no single-reaction amides were isolated from the reaction with trans-crotonyl chloride, even though an uncyclized amide (Product B) was isolated in the reaction with trans-cinnamoyl chloride. However, no products precipitated out of these solutions, which may again suggest that solubility is a factor in product outcomes. The identification of Product M is
very complex, therefore the NMR data used to prove the identity of this product will be covered in a separate section (2.3.5).

**Table 3.** Solvent studies from reaction of 3-amino-1,2,4-triazole and trans-crotonyl chloride using pyridine catalyst and refluxed for 7 days

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Percent Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>21.47%</td>
</tr>
<tr>
<td>ACN</td>
<td>95.40%</td>
</tr>
<tr>
<td>DMF</td>
<td>38.29%</td>
</tr>
<tr>
<td>THF</td>
<td>30.65%</td>
</tr>
</tbody>
</table>

While the major cyclization product (**Product E** and **Product M**) did not change in these studies, the solvent choice did show an impact on the yield of each product. As seen in **Table 2**, the percent yields for **Product E** using acetone, DMF, THF, and ACN are 10.05%, 90.35%, 64.72%, and 16.38%, respectively. Acetone and ACN, with their lower boiling temperatures, allow the reaction to produce both a cyclization and non-bicyclic product, while a higher-boiling solvent like DMF forms only the cyclization product. Although it appears that higher temperatures drive the reaction to completion, it is much more likely that this is a solubility effect than a temperature effect in reactions.
with trans-cinnamoyl chloride. In general, the relative polarity index of acetone, ACN, DMF, and THF are as follow: 5.1, 5.8, 6.4, and 4.0. Based on the relative polarity indexes, it appears that polarity does seem to have a small effect, in that DMF is more polar than THF, and that, in these two solvents (when the compounds remained in solution), product yields are higher in the more polar solvent. On the other hand, as shown in Table 3, the yield from the medium polarity solvent (ACN) was 95.40%, while the yield with the greatest polarity, DMF, was only 38.29% in the reaction with trans-crotonyl chloride. Here, solvent temperature or polarity showed no direct correlations with product yield, suggesting that the chemistry of a trans-cinnamoyl chloride group is different from trans-crotonyl chloride. This might reflect on the differences in chemistry or reactivity of a phenyl group when compared to a methyl.

2.1.2. Base Studies

Pyridine is often used as a catalyst in acylation reactions, and it can also be used as a weak organic base to neutralize an acidic reaction environment. Since literature reactions of aminotriazoles were conducted both with and without an added base, a study was conducted to determine the effect of pyridine on these tandem cyclization reactions, first focusing on its effect on the initial amide formation reaction. This was done by using acetone as a solvent, which had been shown to produce mostly the non-cyclized amide products in the reaction using trans-cinnamoyl chloride (Table 2). The results of this study are shown in Table 4. In this study, the reaction was only run for 1.5 hours because Product B precipitated as soon as the acid halide was introduced into the reaction
mixture and remained unchanged over time. Therefore the yield isolated from a 1.5 hour reaction was almost identical to the yield of a 7 day reaction (Table 2 and Table 4). It is interesting to note that while maintaining all of the same reaction conditions, the experiment with pyridine appeared to have only a slightly higher percent yield (85.16%) in comparison to the reaction without pyridine (79.11%). This was unexpected, since a base is needed to neutralize the product of the amide formation reaction. It is possible that the large number of available nitrogens atoms in the starting material might serve as an alternate base to help with the neutralization.

Table 4. Base studies from reaction of 3-amino-1,2,4-triazole and trans-cinnamoyl chloride in acetone and refluxed for 1.5 hours

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Percent Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/A</td>
<td>79.11%</td>
</tr>
<tr>
<td>pyridine</td>
<td>85.16%</td>
</tr>
</tbody>
</table>

To further investigate the affect of pyridine on product yield, the isolated non-cyclized product (Product B) was used as a starting material to study the cyclization process. The solvent was switched from acetone to DMF for this study, since DMF was
shown to give high amounts of ring closure according to the solvent studies (Table 2). In this study, two cyclization products—Products E and F—were isolated from the reaction of 1-(5-Amino-[1,2,4]triazol-1-yl)-3-phenylpropenone (Product B) with and without pyridine after refluxing for 7 days (Table 5). This time it was shown that the presence of pyridine had a large effect on the reaction. The yield for Product E isolated in a reaction with pyridine was twice the amount of Product E isolated from a reaction without pyridine. The yield for the minor product, Product F, isolated in the reaction with pyridine was ten times the amount of Product F isolated from a reaction without pyridine. The reason for this increase in yields in the presence of the pyridine base is unknown, since a Michael reaction should not require any neutralization.

**Table 5.** Base studies from ring closure of 1-(5-Amino-[1,2,4]triazol-1-yl)-3-phenylpropenone (Product B) in DMF

<table>
<thead>
<tr>
<th>Base</th>
<th>Product E Percent Yield (%)</th>
<th>Product F Percent Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/A</td>
<td>29.46 %</td>
<td>2.50 %</td>
</tr>
<tr>
<td>pyridine</td>
<td>67.02 %</td>
<td>30.51 %</td>
</tr>
</tbody>
</table>

In summary, the use of a pyridine base was shown to have a small effect on the amide formation reaction and dramatic effect on the Michael condensation reaction,
although the reasons for these effects are not clearly understood. Since there is a definite increase in yields, all subsequent studies were performed using the added pyridine base.

2.1.3 Time studies

2.1.3.1 Effect of reaction time on product formation based on yields of isolated products

Time is another factor that can influence product yields. If a reaction is stopped too early, the reaction may have not gone to completion, which might lower yields and also affect which products are isolated when the reaction is stopped. A time study was first conducted to determine the optimum time to run these tandem cyclization reactions. The first approach was to isolate one major product from each reaction and compare these yields to see how they varied over time. In this study only one isomer (Product M) was isolated and quantified from the reaction of 3-amino-1,2,4-triazole and trans-crotonyl chloride in acetone at reaction times varying from 4 hours to 7 days (Table 6). When the reaction was stopped at 4 hrs, the yield of Product M was only 5%. The product yield increased slowly over time to a maximum when the same reaction was run for 7 days (21.47%).
Table 6. Time studies from reaction of 3-amino-1,2,4-triazole and trans-crotonyl chloride in refluxing acetone

<table>
<thead>
<tr>
<th>Time Studies</th>
<th>Percent Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 hours</td>
<td>5.00 %</td>
</tr>
<tr>
<td>1.5 days</td>
<td>8.29 %</td>
</tr>
<tr>
<td>2.0 days</td>
<td>8.93 %</td>
</tr>
<tr>
<td>2.5 days</td>
<td>9.41 %</td>
</tr>
<tr>
<td>7.0 days</td>
<td>21.47 %</td>
</tr>
</tbody>
</table>

Another time study was conducted on the reaction of 3-amino-1,2,4-triazole and trans-cinnamoyl chloride in DMF. The results are shown in Table 7. Product E was the only cyclization product isolated from this reaction. It can be seen that the yields for the 7 or 10 day reactions are significantly better than the 1 day yield. Although the 10 day yield was slightly better than the 7 day reaction, the effect was small, and not dramatic enough to justify even longer reaction times. As a result, a 7 day reaction is treated as the optimized reaction time for all solvents. However, Table 8 has shown that some solvents may reach completion before the 7 days time.
**Table 7.** Time studies from reaction of 3-amino-1,2,4-triazole and *trans*-cinnamoyl chloride in refluxing DMF

<table>
<thead>
<tr>
<th>Time Studies</th>
<th>Percent Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 day</td>
<td>32.60%</td>
</tr>
<tr>
<td>7.0 days</td>
<td>90.35%</td>
</tr>
<tr>
<td>10.0 days</td>
<td>93.48%</td>
</tr>
</tbody>
</table>

**Table 8.** Time studies from the reaction of 3-amino-1,2,4-triazole and *trans*-crotonyl chloride in refluxing ACN

<table>
<thead>
<tr>
<th>Time Studies</th>
<th>Percent Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0 days</td>
<td>95.29%</td>
</tr>
<tr>
<td>7.0 days</td>
<td>95.40%</td>
</tr>
</tbody>
</table>

In some circumstances, a shorter reaction time may give the desired optimized yield of product. For example, the yield from the 3 day reaction of 3-amino-1,2,4-triazole and *trans*-crotonyl chloride in ACN (95.29%) is nearly identical to the 7 day product
yields (95.40%), as shown in Table 8. With a similarity in yields, a 7 day reaction may not be much different from the results generated from a shorter time.

As shown from Tables 6-8, solvent may not have an effect on distribution, but it is clear that some solvents promote quick formation of final product, while others build slowly over time. A solvent like acetone may require a longer reaction time to form the final product since the yield at the 7 day reaction is only 21.47%. The same reaction with ACN can form the final product more quickly because the yield after 3 days has already reached 95.29% and the yield remains relatively the same going from the 3 day to the 7 day reaction. Similar to ACN, the reaction with DMF also showed high final product yield after 7 days of reaction. This emphasizes the fact that a 7 day reaction time is needed in all of the different solvents studied to ensure completion of each reaction.

2.1.3.2 Effect of time on trans-crotonyl product formation based on GC-MS analysis

Since time can affect not only yield but also the products formed during a reaction, time studies using GC-MS analysis were also undertaken. These studies provide information on other products that were formed during the long reaction times of previous studies but could not be isolated cleanly and quantified. For these studies, a sample was taken directly from the reaction flask from time to time over a period of 7 days via a clean syringe needle, without disrupting or stopping the reaction. The samples were analyzed by GC-MS. For reference, all starting materials and reagent chromatograms can be found in Appendix A.
The first study focused on the reaction of 3-amino-1,2,4-triazole with trans-crotonyl chloride in acetone. The total ion count (TIC) for the region of 5.00-10.0 minutes from a sample collected at 2 hours is shown in Figure 10. Interestingly, a possibility of three cyclization products, Product M (RT = 6.55 minutes), Product N (RT = 6.35 minutes), and a possible third cyclization product (RT = 6.18 minutes), had already been formed within 2 hours. Products M, N, and the unknown cyclization product were isolated via flash column chromatography and the full characterization of each compound is further discussed in Section 2.3. While the peak area for the unknown product was tabulated, the areas for Product M and N did not integrate. Based on the appearance of the peak shape and height, the unknown cyclization product’s peak appears to be ~4X higher than the peaks of Products M and N, indicating it is the major product early in the reaction.
Since the unknown cyclic product, Product N, and Product M seen Figure 10 produced similar mass spectral fragments of 152.1 m/z (molecular peak), 84.1 m/z, and 69.1 m/z (base peak); this evidence strongly suggests that all of these peaks are isomeric products. It is therefore possible that the unknown product with the retention time of 6.18 minutes is one of the non-bicyclic products (Product I-L) or a different cyclization product (Product O or P). To confirm if the unknown product is bicyclic or non-bicyclic, a 1D $^1$H NMR spectrum was obtained, and the details can be found in Section 2.2.7. The
$^1\text{H}$ NMR data collected for the unknown product suggests that the unknown product is a bicyclic product (Product O or P).

To investigate if the unknown product is Product O or P, the full mass spectral fragmentation patterns were compared. The mass spectrum of the unknown cyclization product was almost identical to Product N which suggests that the structure of the unknown cyclization product is more similar to Product N than Product M (Appendix A). This lead to the possibility that the unknown cyclization product could be Product P since the formation of the bicyclic in Product N and P are both formed by the amide linkage (N$_3$) and N$_4$; the bicyclic in Product N is formed by N$_3$ to N$_4$ while Product P is formed from N$_4$ to N$_3$. However, the unknown cyclization product tends to be a sticky product therefore it is very difficult to separate impurities from the isolated unknown product as shown in the 1D $^1\text{H}$ NMR spectrum (Section 2.2.7). Since there was no definite NMR data to support the fact that the isolated unknown cyclization product is Product P, the isolated product with the retention time of 6.18 minutes remained unknown.

To further understand the product’s formation over time, the reaction was then monitored for a total of 7 days and the chromatograms are given in Figure 11. The full GC-MS chromatograms of each time studied and the overlay of all the different time studied are shown in Appendix A. The abundance, y-axis, is different for each chromatogram shown in Figure 11 due to slight differences in concentration of each sample tested; however, the growth or declining trends of each product can easily be associated with the height of the peak over time.
Figure 11. GC-MS chromatograms from the reaction of 3-amino-1,2,4-triazole and \textit{trans}-crotonyl chloride in acetone over time.
As shown in Figure 11, based on the peak heights, the unknown cyclic peak slowly disappears over time while **Product M** grows in over the same time period. **Product N** appears to fluctuate slightly over this time period, but never becomes more than a minor product throughout the study. The declining trends of the unknown cyclic’s peak shape over time suggests that the product is kinetically unstable. It is believed that the unstable isomer can undergoes a ring opening and converts into other more stable isomers over time. The chromatograms shown in Figure 11 clearly support that **Product M** grows steadily over time, which suggests that while it forms more slowly, it is thermodynamically stable.

### 2.1.3.3 Effect of time on *trans*-cinnamoyl product formation based on GC-MS analysis

A second time study was performed, this time using the *trans*-cinnamoyl chloride in THF because this solvent showed good yields and at least two cyclization products were formed during the solvent studies. Similar to the time study with *trans*-crotonyl chloride, samples were taken directly from the reaction flask at various times without stopping the reaction. The sequence of these GC-MS chromatograms are shown in Figure 12 (full chromatograms with mass fragmentation are given in Appendix A).
Figure 12. GC-MS chromatograms from the reaction of 3-amino-1,2,4-triazole and \textit{trans}-cinnamoyl chloride in THF over time.
As shown in Figure 12, the peaks at 8.35 minutes, 8.42 minutes, 8.51 minutes, and 9.20 minutes correspond to Products E, B, A, and F respectively. Each peak was assigned based on the patterns in the mass spectrum and NMR analysis of isolated products. Based on the mass spectra, the non-bicyclic products (Products A and B) and the cyclization products (Product E and F) show nearly identical fragments (51.1, 77.0, 103.1, 131.1 (base peak), and 214.1 m/z) with a distinction of the non-bicyclic product showing a background peak at 186 m/z while the bicyclic products show a peak at 171 m/z in the mass spectrum. Interestingly, the peak with the retention time of 9.95 minutes also contains a similar mass spectral pattern to the bicyclic products, which suggests the possibility of belonging to Product G or H. However, since this product was never isolated for analysis, a positive identification proved impossible.

It can be seen from the peaks shown in Figure 12 that the bicyclic products E and F are gradually increasing over time, while the non-bicyclic products A and B are decreasing. This illustrates the expectation that the open-chain amides will convert into the bicyclic products over time. Based on this study, it can be seen that Product F is stable over time, but relative peak areas suggest that it is not as favorable as Product E.

2.1.4. Temperature Studies

In an effort to try to isolate other isomers besides the bicyclic product M, the reaction of 3-amino-1,2,4-triazole and trans-crotonyl chloride was studied at room temperature and ice bath conditions, using acetone and ACN as solvents. No temperature reaction studies were conducted in THF and DMF, since these solvents only favor the
cyclization product (Product M) over a non-bicyclic product (Product I-L), as shown by the solvent and time studies described above. For the reactions using trans-cinnamoyl chloride, only ACN and acetone have been shown to have the potential to produce a non-cyclization product; however, solubility issues with the products in hot acetone make it a problem for cold temperature studies because once the precipitation product is formed, no other forms of isomers would be formed. This makes all four solvents problematic in reactions using trans-cinnamoyl chloride. Therefore, the temperature studies were focused only on reactions with trans-crotonyl chloride in acetone and ACN (Tables 9 and 10).

**Table 9.** Temperature studies of reaction of 3-amino-1,2,4-triazole and trans-crotonyl chloride in acetone.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Reaction Times</th>
<th>Percent Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reflux</td>
<td>4.0 hrs</td>
<td>5.00 %</td>
</tr>
<tr>
<td>Room Temperature</td>
<td>2.0 hrs</td>
<td>8.12 %</td>
</tr>
</tbody>
</table>

As shown in Table 9, the reflux and the room temperature reactions using acetone yield similar results. It appeared that only Product M is produced at both temperatures.
The yield for the refluxed acetone reaction (5.00%) is similar to the yield produced in the room temperature reaction (8.12%). The similarity in the reaction outcomes from the two different temperature reactions studies suggested that the temperature was not having an effect on the reaction; therefore the temperature study was shifted to focus on ACN (Table 10).

Table 10. Temperature studies of reaction of 3-amino-1,2,4-triazole and trans-crotonyl chloride in ACN

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Reaction Times</th>
<th>Percent Yield (%)</th>
<th>Percent Yield (%)</th>
<th>Percent Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reflux</td>
<td>3.0 days</td>
<td>95.29%</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Room Temperature</td>
<td>2.0 hrs</td>
<td>4.80%</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Ice Bath</td>
<td>2.0 hrs</td>
<td>26.64%</td>
<td>4.25%</td>
<td>4.30%</td>
</tr>
</tbody>
</table>

The results of the temperature study using ACN (Table 10) show that at colder temperatures two more cyclization products, Products N and another potential cyclization product (possibly Product O or P), can be formed in the reactions with trans-crotonyl chloride. Although Products N and the unknown cyclization product can be formed, the low isolation yields (4.25% and 4.30%, respectively) suggest that the two products are not as stable as Product M. It is possible that both Product N and the
unknown cyclization product are the kinetic products which can convert into Product M, therefore suggesting that Product M is the thermodynamic product and more stable over time. This would explain why small amounts of Product N and the unknown cyclization product are only isolated at extremely low temperatures, and their yields quickly decrease with increasing heat. However, it is very interesting that the yield for Product M is higher under colder conditions for ACN. This reaction has been tested twice, and similar yields were obtained both times. It almost appears that the more extreme the conditions are, such as hotter or colder reaction conditions, the more Product M is formed.

Overall, it has been demonstrated that colder temperatures reactions have the potential to slow down the reaction and allow more bicyclic products to be formed. As shown in this study, the major bicyclic product again tends to favor the isomer with the ring closure going from the amino nitrogen to the N$_2$ position similar to the major product formed in the reaction with trans-cinnamoyl chloride. Although the ring closure mechanisms for both the reactions with trans-cinnamoyl chloride and trans-crotonyl chloride may appear to be very similar, it is not possible to assume the temperature results for one starting material will be the same in both.

2.2 NMR Studies

According to the GC-MS analyses of the various reactions of 3-amino-1,2,4-triazole and trans-cinnamoyl chloride, four distinct products (Products A, B, E, and F) were created. On the other hand, only three products (Products M, N, and possibly Product O or P) out of the 8 expected isomeric outcomes have been isolated from the
reactions with trans-crotonyl chloride. In both sets of reactions, the acid form of the acid halide (trans-cinnamic acid and trans-crotonic acid) were also captured after flash column, which would be expected since all work-up procedures included water.

Each product was shown to be distinctly different based on mass spectral analysis, and all were characterized and identified by $^1$H, $^{13}$C, and $^{15}$N NMR studies. In some cases, the total amount of product isolated in one reaction or by combining the products of multiple reactions was below the minimum amount needed to run $^{15}$N and/or $^1$H-$^{15}$N HMBC NMR analyses; therefore, only $^1$H and $^{13}$C NMR data were available to study the structure of that molecule. The full characterization of each compound is discussed in greater detail below.

### 2.2.1 Characterization of 3-Phenyl-N-(2H-[1,2,4]triazol-3-yl)-acrylamide (Product A)

The full $^1$H-NMR spectrum of 3-phenyl-N-(2H-[1,2,4]triazol-3-yl)-acrylamide (Product A) is displayed in Figure 13. The full $^1$H-NMR spectrum with integrals is shown in Appendix B. Proton assignments are based on integral ratios and splitting patterns. The doublet peak at 6.90 ppm shows one proton, which corresponds to H$_a$ as it has a strong trans coupling with H$_b$. The integral ratio for the multiplet peak around 7.50-7.42 ppm is showing three protons, and corresponds to overlapping H$_c$ and H$_d$ protons. The doublet of doublets peak at 7.64 ppm integrates for 2 protons and corresponds to the aromatic proton H$_c$, since it can couple with both H$_c$ and H$_d$. The doublet peak at 7.70 ppm corresponds to the alkene proton H$_b$ proton, since it shows a strong coupling with H$_a$
and an integral of one. The singlet peak at 7.88 ppm corresponds to the triazole hydrogen, \( H_6 \), which is the only aromatic proton, which should have no coupling. The broad singlet peak at 11.56 ppm corresponds to the amine proton, \( H_g \). Although it is difficult to see, integral data (Appendix B) show the amide nitrogen (\( H_h \)) in the broad peak at 13.50 ppm, which is typical of conjugated amides.

![NMR spectrum of 3-phenyl-N-(2H-[1,2,4]triazol-3-yl)-acrylamide](image)

**Figure 13.** \(^1\)H NMR spectrum of 3-phenyl-N-(2H-[1,2,4]triazol-3-yl)-acrylamide (Product A) in d\(_6\)-DMSO

The assignment of the different carbons present in 3-phenyl-N-(2H-[1,2,4]triazol-3-yl)-acrylamide are based on the \(^1\)H and \(^{13}\)C bond correlations generated from the HSQC NMR spectrum, as shown in Figure 14. This spectrum shows a total of six
carbon/hydrogen interactions; therefore, only six carbons (C₂-C₇) were identified and confirmed using this spectral data. Spot 1 corresponds to the interaction between the alkene H₄ and C₇. Spots 2 and 3 correspond to the interactions between the aromatics, Hₑ and C₂ and H₄ and C₄. Spot 4 corresponds to the remaining aromatic interaction between Hₑ and C₃. Spot 5 correlates the interaction of H₆ and C₆. There was no cross peak present for C₅ because there are no hydrogens attached to C₅, therefore no correlation interaction is observed. The full spectrum can go up to 167.80 ppm; however, the HSQC spectrum only shows the regions with interactions therefore the chemical shifts of C₁, C₈, and C₉ are not shown in the spectrum. Ideally, C₁ is expected to couple with H₆; however, no cross peak is observed for the interaction. Interestingly, the absence of the interaction between the triazole carbon (C₁) and hydrogen (H₆) is also observed in another non-bicyclic product (Product B, Figure 18).
Figure 14. HSQC-NMR spectra of 3-phenyl-N-(2H-[1,2,4]triazol-3-yl)-acrylamide (Product A) in d$_6$-DMSO

Figure 15 shows the full $^{13}$C NMR spectrum for 3-phenyl-N-(2H-[1,2,4]triazol-3-yl)-acrylamide (Product A). The chemical shifts for C$_2$-C$_7$ were established from the HSQC spectrum in the order as follows: 129.55 ppm, 128.45 ppm, 130.75 ppm, 134.73 ppm, 142.55 ppm, and 120.45 ppm. The remaining three carbons that need to be assigned are C$_1$, C$_8$, and C$_9$. These other carbons can be assigned based on the typical expected range for the carbon chemical shift as suggested in the literature. A typical chemical shift for a carbonyl carbon can be found in the range between 165.00 to 200.00 ppm, therefore suggesting that the peak furthest downfield at 167.80 ppm corresponds to C$_9$, which
leaves the other two un-assigned peaks at 150.00 ppm and 164.01 ppm to C₁ and C₈. Since C₈ is not attached to any hydrogen, it’s expected to be more downfield compared to C₁ therefore the peak at 150.00 ppm correspond to C₁ and the peak at 164.01 ppm correspond to C₈.

Figure 15. $^{13}$C NMR spectrum of 3-Phenyl-N-(2H-[1,2,4]triazol-3-yl)-acrylamide (Product A) in d₆-DMSO

The NMR spectral interpretation seems to align with what would be expected for compound Product A, particularly the presence of two types of N-H and the alkene signals. However, the isolation of the Product A was extremely difficult to achieve.

Product A formed as tiny crystals which grew slowly out of the crude reaction mixture, in particular in the 5-day THF reaction. Product clean-up proved quite difficult due to the formation of multiple products in this reaction and the low yield for Product A (4.64%).

Natural abundance $^{15}$N NMR analyses requires fairly concentrated samples, therefore the
low yield attained for Product A prevented analysis by 1D $^{15}$N NMR or $^1$H-$^{15}$N HMBC. These analyses were attempted, but the low concentration lead to peaks which were buried within the baseline noise, and therefore difficult to interpret. Due to this issue, no $^{15}$N NMR and $^1$H-$^{15}$N HMBC spectrum are available for this compound.

2.2.2 Characterization of 1-(5-Amino-[1,2,4]triazol-1-yl)-3-phenylpropenone (Product B)

Out of all the various conditions studied for the reaction of 3-amino-1,2,4-triazole with trans-cinnamoyl chloride, 1-(5-amino-[1,2,4]triazol-1-yl)-3-phenylpropenone (Product B) is the only product to precipitate out in solution while refluxing, resulting in moderate to high isolated yields (~ 54.00-85.00%). The $^1$H NMR spectrum of Product B is displayed in Figure 16.

![Figure 16](https://example.com/figure16.png)

**Figure 16.** The $^1$H NMR spectrum of 1-(5-amino-[1,2,4]triazol-1-yl)-3-phenylpropenone (Product B) in d$_6$-DMSO
Similar to the H₆ alkene proton in **Product A**, the H₆ alkene proton in **Product B** is also shown to be more downfield compare to H₅. With the support of the integral ratio, H₅ is embedded within the multiplet containing H₇ and H₈, which makes the doublet peak hard to see. The two singlet peaks appearing at 7.64 ppm and 7.68 ppm correspond to H₈ (from the amine) and H₇ (from the triazole ring) respectively; H₈ has a wider peak shape due to H-bonding. The aromatic hydrogen, H₅ (7.81 ppm), is a doublet of doublets due to interactions with both H₆ and H₇. The remaining aromatic protons, H₆ and H₇ are overlapping in the multiplet at 7.46-7.50 ppm. Integration of this multiplet showing three hydrogens helps to confirm the assignment (see **Appendix C**). It should be noted that the splitting patterns and integral ratios of **Product B** would also be similar for **Products C** and **D**. It is clear that the spectrum does not correspond to **Product A** due to the absence of the amide peak at the region of 11.00-13.00 ppm and the integration of two for the amine peak. To further investigate the structure of this isolated product, COSY and ¹H-¹³C HSQC analyses have been conducted.

A COSY analysis allows for the study the ¹H-¹H interactions within the molecule by focusing on the off-axis interactions. As shown in **Figure 17**, there are two cross peaks observed for this compound. Cross peak 1 correlates to the coupling interaction between the alkene hydrogens, H₅ and H₆, helping to identify the alkene hydrogen (Ha) hidden amongst the other signals at 7.64 ppm. Cross peak 2 correlates to the coupling interactions between H₆, H₇ and H₈. Since H₇ and H₈ have no nearby protons, no coupling interactions would be expected for these protons. Unfortunately, because of the structures
of **Products C** and **D**, it is possible that both of these isomers can also provide a similar COSY spectrum, therefore further analysis will be require for confirm the structure.

![Chemical structure](image)

**Figure 17.** The $^1$H-$^1$H COSY of 1-(5-amino-[1,2,4]triazol-1-yl)-3-phenylpropenone (**Product B**) in d$_6$-DMSO

To investigate the structure further, an HSQC analysis was done to study the direct $^1$H-$^{13}$C bond correlations within the molecule which helps to identify the carbon assignments. The HSQC spectrum of 1-(5-amino-[1,2,4]triazol-1-yl)-3-phenylpropenone (**Product B**) is displayed in **Figure 18**. The spectrum only shows the region of interest, therefore the carbonyl carbon known as C$_9$ (166.50 ppm) and the carbon at C$_8$, (158.00
ppm) are not present in the figure. The full carbon spectrum can be found in Appendix C.

Figure 18. The $^1$H-$^{13}$C HSQC spectrum of 1-(5-amino-[1,2,4]triazol-1-yl)-3phenylpropenone (Product B) in d$_6$-DMSO.

As shown in the HSQC spectrum, there are 5 peaks corresponding to 5 proton-carbon coupling interactions. Cross peak 1 corresponds to the interaction between the more shielded alkene $C_7$ and $H_a$. Cross peak 2 corresponds to the interaction between the aromatic $C_3$ and $H_c$, the ortho position of the aromatic ring. Cross peak 3 corresponds to
the interaction between C_2 and H_e, the meta position of the aromatic ring. Cross peak 4 corresponds to the interaction between C_4 and H_d, the remaining aromatic position. The light spot known as cross peak 5 corresponds to the interaction between the more electron poor alkene C_6 and H_b. There should also be an interaction for the triazole carbon and proton (C_1 and H_f), but, due to the low intensities, the signals may be too low. Interestingly, no correlation for these atoms appeared in the HSQC for Product A either. Again, the HSQC spectrum cannot be used to eliminate Products C or D, thus even with four types of analyses, it is not enough to differentiate among these isomers. However, ^15N NMR analysis may be an alternative to differentiate isomers with similar structures.\textsuperscript{37,38}

To further confirm the identification of Product B, a ^1H-^15N HMBC analysis was conducted. To assign all four nitrogen (N_1-N_4) in the 1D ^15N NMR spectrum, the starting material of 3-amino-1,2,4-triazole was analyzed by NMR and used as a reference guide to assign the different types of nitrogens present in the isolated product. In this study, the 1D inverse-gated ^15N NMR spectrum of 3-amino-1,2,4-triazole, shown in Figure 19, is used as a baseline for comparisons.\textsuperscript{39} Sometimes the baseline can become very noisy due to a low concentration of sample, and expected peaks would not even be noticeable. For example, Figure 19A only shows the N_3 and N_4 peak in the raw spectrum at 212 and 49 ppm. Ideally, the raw data would most likely show peaks for the nitrogens with hydrogens attached, but in this case the raw spectrum showed N_4 instead of N_2. Fortunately, the same data can be modified so that all the nitrogen peaks are visible in the same spectrum (Figure 19B). This modification can easily be done by an autophase
adjustment of the raw spectrum. Not only are N\textsubscript{4} and the amino nitrogen N\textsubscript{3} observed, the phasing allows the tiny peaks for N\textsubscript{1} and N\textsubscript{2} to be observed at 250 and 189 ppm. The positions of these signal align well with those assigned by Michl et al, for N\textsubscript{1}, N\textsubscript{2}, N\textsubscript{3}, and N\textsubscript{4}: 260.54 ppm, 189.00 ppm, 48.70 ppm, and 216.55 ppm, respectively.\textsuperscript{42} The nitrogen assignments shown in Figure 19 for the starting material 3-amino-1,2,4-triazole will be used for comparison when assigning the isolated product’s nitrogens going forward.

Figure 19. The non-modified (A) and the modified (B) $^{15}\text{N}$ NMR spectra of 3-amino-1,2,4-triazole in d\textsubscript{6}-DMSO

The autophase-adjusted, inverse-gated $^{15}\text{N}$ NMR spectrum of 1-(5-amino-[1,2,4]triazol-1-yl)-3phenyl-propenone (Product B) is shown in Figure 20. Based on the
studies by Varela et al, Merak et al, and Vaughan et al on undisturbed nitrogens having similar chemical shifts to the reference compound, N₁, N₃, and N₄ of **Product B** were assigned to the peaks at 269.38, 68.00, and 210.00 ppm, respectively. The chemical shift of undisturbed nitrogens when a new substitution is bound elsewhere in the molecule only differ by 10.-20 ppm. The chemical shifts for N₁ and N₄ in 3-amino-1,2,4-triazole are 260.54 ppm and 216.55 ppm, while N₁ and N₄ in **Product B** are assigned at 269.38 ppm and 210.00 ppm. As studied by Vaughan et al, a nitrogen environment altered by changing the substitution group from a hydrogen to a carbonyl carbon group can increase the chemical shift up 10.00-50.00 ppm, which is echoed in this study, where the chemical shift of N₂ in **Product B** increased by 9.00 ppm relative to the starting material (189.00 ppm to 199.50 ppm).

![Figure 20](image.png)

**Figure 20.** The inverse-gated $^{15}\text{N}$ NMR spectrum of 1-(5-amino-[1,2,4]triazol-1-yl)-3-phenylpropenone (**Product B**) in d6-DMSO.
To further test these assignments, the $^1$H-$^{15}$N HMBC spectrum was next studied (Figure 21), with the expansion of the two cross peaks shown in Figure 22. Based on the nitrogen assignments from the inverse-gated $^{15}$N analysis, the spots correspond to correlations involving N$_1$ and N$_4$; at 269.38 ppm and 210.00 ppm, respectively. Cross peak 1 corresponds to the bond interaction between N$_4$ and H$_f$ (the triazole ring hydrogen), and cross peak 2 corresponds to N$_1$ interacting with H$_f$. Since HMBC is a long-range coupling analysis, particularly strong for 2-3 bond interactions, the fact that N$_2$ does not couple with H$_f$ is very suggestive of its position far from this hydrogen.

**Figure 21.** The full $^1$H-$^{15}$N HMBC spectra of 1-(5-amino-[1,2,4]triazol-1-yl)-3phenyl-propenone (Product B) in d$_6$-DMSO
Figure 22. The enlarged $^1$H-$^{15}$N HMBC spectrum of the $N_1$ and $N_4$ cross peaks in the region of 7.560-7.710 ppm

The combination of the 2D HMBC (Figure 20) and the 1D $^{15}$N NMR (Figure 20) spectra correspond well with **Product B**. The key to this analysis lies in the appearance of the $N_2$ signal. Similar to the studies done by Vaughan et al, the substitution of the carbonyl group causes the chemical shift of $N_2$ in **Product B** to shift slightly higher (198.00 ppm) than the original chemical shift of $N_2$ found in 3-amino-1,2,4-triazole (189.00 ppm), where $N_2$ is only bonded to a hydrogen. In addition, due to the deshielding...
affect caused by the carbonyl group, N₁ and N₃ in Product B are also slightly shifted
downfield compared to the N₁ and N₃ found in 3-amino-1,2,4-triazole. In comparison, the
chemical shift of N₃ shows an increase of 19.30 ppm therefore suggesting that the
carbonyl chain substitution is attached to N₂ and not on N₁, where N₃ is in closer
proximity to the carbonyl substitution chain to have the increase chemical shift impact.
On the other hand, N₄ is slightly lower in Product B than what is seen in 3-amino-1,2,4-
triazole. This is the only nitrogen to move upfield, suggesting it is farthest away from the
electron-withdrawing effect of the carbonyl attachment. For these reasons, the NMR
spectra have supported the structure of Product B and not Product C or D.

2.2.3 Characterization of 7-phenyl-6,7-dihydro-4H-[1,2,4]triazolo[1,5-α]pyrimidin-
5-one (Product E)

The most commonly isolated product from the reaction of 3-amino-1,2,4-triazole
with trans-cinnamoyl chloride was the bicyclic product 7-phenyl-6,7-dihydro-4H-
[1,2,4]triazolo[1,5-α]pyrimidin-5-one (Product E), a yellow oil-like substance.
Regardless of the solvent used, all of the 7 day reactions give Product E as the major
product. The characterization of Product E is based on a combination of the ¹H, ¹³C, ¹⁵N,
¹H-¹³C HSQC, and ¹H-¹⁵N HMBC NMR analyses.

Figure 23 shows the ¹H-NMR spectrum of Product E, while the full spectrum
with integrations can be found in Appendix D. In contrast to the non-cyclization
products (A and B), Figure 23 shows two doublet of doublets peaks at 3.00 ppm and 3.40
ppm which correspond to the methylene protons, Hₐ1 and Hₐ2, suggesting that the ring
closure process has taken place. Another key to differentiate the analyzed compound as the cyclization product is the multiplet peak (H_b) at 5.75 ppm, which is clearly not in the expected region for the alkene signals observed in the previous molecules, suggesting it belongs to the deshielded alkane of a 6-membered ring system proton. Based on the peak present at 11.75 ppm, a typical amide (H_g) peak, the structure involves an amide link at N_3. If cyclization has occurred, then the ring close process links N_3 and either N_2 or N_4.

![Figure 23. The 1H-NMR spectrum of 7-phenyl-6,7-dihydro-4H-[1,2,4]triazolo[1,5-α]pyrimidin-5-one (Product E) in d_6-DMSO](image)

Similar to the non-cyclization products, the singlet peak at 7.80 ppm corresponds to the triazole hydrogen, H_f. The aromatic protons are found as a doublet of doublets at
7.10 ppm (Hc) and an overlapping multiplet at 7.30 - 7.40 ppm (Hc and Hd), with a total integration of five for these aromatics, in keeping with the mono-substituted aromatic ring. Based on the spectral evidence, it is clear that the alkene signals are gone and a ring has formed, signaling that this is indeed a cyclization product.

To investigate this cyclic product’s structure further, an HSQC analysis was run to study the direct $^1$H-$^{13}$C coupling interactions within the molecule. However, it is also very important to fully understand the $^{13}$C chemical shifts in order to interpretive the HSQC data. The $^{13}$C NMR spectrum is shown in Figure 24. In agreement with the $^1$H NMR data, the peaks present at the upfield region of 0.00-60.00 ppm strongly suggest alkane carbons (C₁ and C₂). In addition, the aromatic carbons, C₃-C₆, all appear in the aromatic region as seen in Products A and B. The triazole carbon C₇, appears to almost overlap the unprotonated carbon C₈. The two unprotonated carbons, C₈ and C₉, are assigned based on the type of carbon group. The carbonyl carbon C₉ would be seen more downfield in comparison to the aromatic carbon C₈. A typical chemical shift for a carbonyl carbon is found within the region of 160.00 ppm to 200.00 ppm therefore the peak at 169.60 ppm is assigned to C₉. The carbon assignments are confirmed by the HSQC analysis.
Figure 24. The $^{13}$C NMR spectrum of 7-phenyl-6,7-dihydro-4H-[1,2,4]triazolo[1,5-$\alpha$]pyrimidin-5-one (Product E) in d$_6$-DMSO

The HSQC spectrum is displayed in Figure 25. The $^{13}$C spectrum, shown on the vertical axis, shows only 8 peaks since C9 did not show any interactions in this spectrum. Cross peaks 1 and 2 correspond to C$_1$ interacting with both H$_{a1}$ and H$_{a2}$, emphasizing the diastereotopic nature of these two alkane protons. Cross peak 3 corresponds to the interaction between the methine C$_2$ and H$_b$. Cross peak 4 corresponds to the interaction between C$_3$ and H$_c$. Cross peak 5 corresponds to two interactions as shown in the expanded spectrum (Figure 26)--C$_4$ with H$_d$ and C$_5$ with H$_e$. In addition, Figure 25 also showed a tiny spot appearing as cross peak 6, which corresponds to the interaction between C$_7$ and H$_f$. This is the first time the triazole interaction has appeared, but it is a very weak interaction. Although the HSQC analysis is useful, it is still unable to allow the differentiation between Product E or F, since the same proton and carbon
environments still exist in each structure. Unfortunately, even a $^1$H-$^{13}$C HMBC analysis would not solve this issue because both isomer structures are so similar.

**Figure 25.** The $^1$H-$^{13}$C HSQC spectrum of 7-phenyl-6,7-dihydro-4H-[1,2,4]triazolo[1,5-α]pyrimidin-5-one (Product E) in d$_6$-DMSO
Figure 26. Enlarged region of HSQC spectrum of 7-phenyl-6,7-dihydro-4H-[1,2,4]triazolo[1,5-α]pyrimidin-5-one (Product E) in d$_6$-DMSO.

To further investigate the structure of this cyclic product, a $^1$H-$^{15}$N HMBC analysis would help to establish the long range (2-4 bond) interactions within the molecule. Similar to the HSQC study, a 1D $^{15}$N NMR would be needed before jumping into the $^1$H-$^{15}$N HMBC study. The $^{15}$N spectrum of Product E is shown in Figure 27. The chemical shift of the nitrogens were first determined and assigned according to the reference chemical shift of 3-amino-1,2,4-triazole. The chemical shifts for N$_1$ and N$_4$ in Product E are very similar to the nitrogens in 3-amino-1,2,4-triazole, since these would
be undisturbed nitrogens. Both move slightly downfield in the product molecule. The chemical shift for N₂ is only slightly different because of the change from a hydrogen-substituent to a carbon-substituent does not drastically change its environment, shifting only ~3 ppm upfield. On the other hand, N₃ has had a major change in its environment, as the nitrogen went from an amine to an amide nitrogen. For reference, an amine nitrogen is likely to be found in the region of 0 to 100 ppm, and a secondary amide would be found in the region of 110.00 ppm to 160.00 ppm. Since N₃ is a secondary amide, N₃ is assigned to the peak at 130.00 ppm (far from the 48.00 ppm signal of the free amine).

Based on the data collected, the N₁-N₄ chemical shifts for Product E are as follows: 283.61 ppm, 186.00 ppm, 130.00 ppm, and 220.38 ppm, respectively.

![Chemical structure and spectrum](image)

**Figure 27.** The $^{15}$N spectrum of 7-phenyl-6,7-dihydro-4H-[1,2,4]triazolo[1,5-$\alpha$]pyrimidin-5-one (Product E) in d$_6$-DMSO.
Further evidence of the assignments is found using the long-range couplings shown in the $^1$H-$^{15}$N HMBC analysis (Figure 28). A coupling, cross peak 1, is clearly present between $N_3$ and $H_{a1}$. Ideally, a cross peak for $N_3$ and $H_{a2}$ would be expected because it is also 3 bonds away, similar to $H_{a1}$. However, even though both $H_{a1}$ and $H_{a2}$ are the same bond distance away from $N_3$, the protons are not projected in the same direction toward $N_3$. $H_{a1}$ may be in closer proximity to $N_3$ than $H_{a2}$, and differences in orientation and distance often affect couplings in NMR analyses. This concept may also apply to $N_1$ and $H_b$, which also do not show the expected coupling. There is only one cross peak visible for the $N_1$ nitrogen, the coupling interaction between $N_1$ and $H_f$ (the triazole hydrogen) as shown by cross peak 7. As expected, both $N_4$ and $N_2$ also show interactions with $H_f$ (cross peaks 6 and 5). Cross peaks 2-5 all correspond to the coupling interaction between $N_2$ and another proton. $N_2$ can interact with all of the alkane protons—$H_{a1}$ (cross peak 2), $H_{a2}$ (cross peak 3), $H_b$ (cross peak 4)—as well as the triazole $H_f$ (cross peak 5).
Figure 28. A $^1$H-$^{15}$N HMBC spectra of 7-phenyl-6,7-dihydro-4H-[1,2,4]triazolo[1,5-\(\alpha\)]pyrimidin-5-one (Product E) in d$_6$-DMSO

Since only $N_2$ and $N_3$ show interactions with the alkane system, this suggests that the ring has closed between these two nitrogens. If Product F was the correct structure, Both $N_3$ and $N_4$ would be expected to couple to these alkane hydrogens, as shown in red in Scheme 17, which highlights all of the possible protons that can interact with $N_4$ in an HMBC analysis for each of the four possible heterocyclic isomers. Therefore, based on
both the 1D and 2D $^{15}$N studies, the assignments more closely match the structure of Product E than Product F. Cyclized Product G would give cross peaks similar to Product E in a $^1$H-$^{15}$N HMBC spectrum, but it can be ruled out by the presence of an amide signal in the proton NMR. Finally, cyclized Product H would be expected to show interactions between both N$_3$ and N$_4$ with the alkane signals of the ring, and would again show no amide in the proton NMR. Based on this analysis, the isolated yellow oil is assigned to 7-phenyl-6,7-dihydro-4H-[1,2,4]triazolo[1,5-$\alpha$]pyrimidin-5-one, Product E.

Scheme 17. Possible long-range coupling interactions for N$_4$ in an HMBC analysis

2.2.4 Characterization of 5-phenyl-5,6-dihydro-8H-[1,2,4]triazolo[4,3-$\alpha$]pyrimidin-7-one (Product F)

Product F is second bicyclic product isolated from the reaction of 3-amino-1,2,4-triazole and trans-cinnamoyl chloride. The labeled $^1$H NMR spectrum is shown in Figure 29, and the full $^1$H NMR spectrum with integrations can be found in Appendix E.

Similar to Product E, the methylene protons, H$_{a1}$ and H$_{a2}$, of Product F appear as two
different signals in the more upfield region (2.94 ppm and 3.21 ppm) but the chemical shift is slightly different from those found in **Product E** (3.00 ppm and 3.40 ppm).

Another interesting difference between **Products E** and **F** is the chemical shift of the triazole proton $H_f$. In **Product F** this signal is found at 8.15 ppm while the chemical shift for $H_f$ in **Product E** is 7.80 ppm. The cause of the increase in the chemical shift of $H_f$ found in **Product F** may be influenced by the close proximity protons of the phenyl group nearby while all the protons in **Product E** are far away or facing the other direction from $H_f$. The slight shifts in all protons for this second bicyclic system suggest that patterns may be drawn for future systems to help distinguish between these different isomers more easily.

![Figure 29](image.png)

**Figure 29.** The $^1$H NMR spectrum of 5-phenyl-5,6-dihydro-8H-[1,2,4]triazolo[4,3-$\alpha$]pyrimidin-7-one (**Product F**) in d6-DMSO
To investigate the structure of **Product F** further, a 2D $^1$H-$^{13}$C HSQC analysis has been studied as shown in **Figure 30**. The observed $^{13}$C chemical shifts for isomer F are also very similar to **Product E**’s. The full $^{13}$C NMR spectrum can be found in **Appendix E**. As shown in **Figure 30**, cross peaks 1 and 2 correspond to the interaction of the diastereotopic hydrogens with most shielded carbon in the molecule, C\textsubscript{1}. Cross peak 3 correlates to the interaction between the remaining alkane carbon and hydrogen (C\textsubscript{2} and H\textsubscript{b}). The expansion of spots 4 and 5 is shown in **Figure 31**, where the aromatic interactions can be clearly seen. As shown in the expanded spectrum, cross peak 4 is the correlation between the aromatic C\textsubscript{3} and H\textsubscript{c}. In addition, spot 5 appears to have two cross peaks which correspond to the correlations for C\textsubscript{4} and H\textsubscript{d} as well as C\textsubscript{5} and H\textsubscript{e}. The faint cross peak 6 corresponds to the interaction between triazole C\textsubscript{7} and H\textsubscript{f}. C\textsubscript{8}, as expected, shows no correlations.
Figure 30. The $^1$H-$^{13}$C HSQC NMR spectrum of 5-phenyl-5,6-dihydro-8H-[1,2,4]triazolo[4,3-α]pyrimidin-7-one (Product F) in d$_6$-DMSO
Figure 31. Expansion $^1$H-$^{13}$C HSQC spectrum of 5-phenyl-5,6-dihydro-8H-[1,2,4]triazolo[4,3-$\alpha$]pyrimidin-7-one (Product F) in d$_6$-DMSO

Since the proton NMR contained an amide nitrogen, the isolated compound could only belong to product E or F. Since the previous compound was identified as Product E, this isomer is most likely to be Product F. Similar to the comparison made in the $^1$H NMR analysis, the carbon spectrum of the two bicyclic products showed several distinctions. The carbon that stood out the most in differentiating isomer F from isomer E is C$_7$, the triazole carbon. The chemical shift of C$_7$ in Product F is more upfield
(139.99 ppm) in comparison to Product E (150.13 ppm). This could also serve as a diagnostic signal in differentiating between isomers in future studies.

Nitrogen NMR studies were also conducted to further confirm the structural assignment. The full $^{15}$N NMR spectrum of Product F is shown in Figure 32. By comparing with the reference $^{15}$N chemical shifts found in 3-amino-1,2,4-triazole and those determined for Product E, the $^{15}$N chemical shift for N$_1$ to N$_4$ found in Product F are determined as 311.32 ppm, 274.80 ppm, 126.28 ppm, and 154.28 ppm, respectively. Like Product E, the chemical environment of N$_3$ found in Product F (126.28 ppm) remain relatively unchanged, therefore yielding similar chemical shifts to those seen in Product E (130.00 ppm). N$_4$, on the other hand, is now the site of an alkyl attachment. Previously, the chemical shift due to the new carbon substituent has resulted in an upfield shift, thus suggesting the assignment of N$_4$ to the signal at 154 ppm. N$_2$ show a dramatic shift to 274.80 ppm, which suggests that the chemical environment of N$_2$ in this new product is very different from that of the previous isomer. Although N$_1$ remain undisturbed, the chemical shift of N$_1$ was also increased as the ring closure is now closed in the opposite side, N$_3$ to N$_4$ respectively, similar to the structures studied by Varela et al.$^{36}$
Figure 32. The $^{15}$N NMR spectrum of 5-phenyl-5,6-dihydro-8H-[1,2,4]triazolo[4,3-$\alpha$]pyrimidin-7-one (Product F) in d$_6$-DMSO

According to the structure drawn for Product F, the chemical environment of N$_2$ is different from the environment found in N$_2$ of 3-amino-1,2,4-triazole or in Product E. The environment of N$_2$ in Product F is now similar to that of an imidazole nitrogen, in which the chemical shift is expected to be higher. On the other hand, the N$_2$ environment found in 3-amino-1,2,4-triazole and Product E are disturbed by either having a hydrogen or carbonyl carbon attached to the nitrogen therefore causing the nitrogen to be observed more further upfield compare to the non-disturbed N$_2$ imidazole nitrogen. Since the assigned N$_1$, N$_3$, and N$_4$ peak seem to fit well with Product F structural arrangement, the last peak present at 274.80 ppm correspond to N$_2$. According to the structure drawn for Product F, the position of N$_2$ within the structure of Product F would have a fully positive resonance structure and it would be expected to have a very large downfield shift, therefore again fitting well with the assignment of this isomer to Product F and not
to **Products E** and **G**. In addition, **Products E** and **G** are also excluded by the amide hydrogen signal given by N3 which is similar to the assigned amide signal seen in **Product E** in the previous studies.

To further confirm the structure of **Product F**, a $^1$H-$^{15}$N HMBC analysis was performed (**Figure 3**). Surprisingly, no cross peak is present for the amide nitrogen in the spectrum as was seen with the previous isomer. Even so, the assignment of N3 can be inferred from the fact that Hf in the structure of **Product F** should be interacting with N1, N2, and N4 in this analysis, therefore N3 is the peak which is not interacting (i.e. 126 ppm). The only nitrogen that interacts with the alkane peaks would therefore would have to be either N2 or N4. Since the data for the previous compound proved conclusive for it being the N2 ring closure, this molecule must therefore be the N4 closure. N4 is therefore assigned to the 154 ppm peak. Cross peak 1 corresponds to the interaction between N4 and H$_{a1}$ and cross peak 2 corresponds to the interaction between N4 and H$_{a2}$. Cross peaks 3 and 4 correspond to the correlation between N4 and H$_b$ and N4 and H$_f$. Cross peak 5 corresponds to the interaction between N2 and H$_f$. Cross peak 6 correspond to the long range coupling between N1 and H$_f$. 
2.2.5 Characterization of 7-methyl-6,7-dihydro-4H-[1,2,4]triazolo[1,5-α]pyrimindin-5-one (Product M)

In all of the refluxed reactions using trans-crotonyl chloride, **Product M** was the dominant product. The $^1$H NMR spectrum of **Product M** is displayed in Figure 34. The proton assignments are based on the integral ratios as shown in Appendix F and the splitting patterns of the peaks. Similar to the phenyl-substituted bicyclic products **Product E** and **F**, the two doublet of doublets peaks appearing at 2.68 ppm and 2.95 ppm
correspond to the inequivalent methylene protons, $H_{a1}$ and $H_{a2}$. The sextet at 4.50 ppm corresponds to the methine hydrogen, $H_b$. As expected, the methyl protons ($H_c$) are more upfield compared to the other type of protons present in the molecule, appearing at 1.45 ppm. The singlet peak at 7.70 ppm corresponds to the triazole $H_d$ and the peak at 11.45 ppm corresponds to the amide proton ($H_e$). Since alkane ring protons are visible, this is clearly one of the bicyclic compounds, Product M, N, O or P. However, the presence of an amide hydrogen signal indicates that the carbonyl must be attached to $N_3$, therefore this must be either Product M or N. Interestingly, the position of the triazole hydrogen, $H_f$, at 7.70 ppm is very similar to that of Product E, which is most similar in structure to Product M.

![NMR spectrum](image)

**Figure 34.** The $^1$H NMR spectrum of 7-methyl-6,7-dihydro-4H-[1,2,4]triazolo[1,5-$\alpha$]pyrimidin-5-one (Product M) in d$_6$-DMSO
To investigate the structure of the isolated product further, a $^1$H-$^{13}$C HSQC analysis was run to establish the $^1$H-$^{13}$C bond coupling interactions within the isolated molecule. The HSQC spectrum is displayed in Figure 35, showing an expansion of the alkane region of the carbon spectrum. To understand the $^1$H-$^{13}$C HSQC analysis, the understanding of a 1D $^{13}$C analysis is vital along with the understanding of a 1D $^1$H analysis which has already been shown in Figure 34. The full $^{13}$C spectrum is given in Appendix F. As shown in Figure 35, there are 4 cross peaks present in the spectrum. Cross peak 1 corresponds to the coupling of the methyl system, C$_1$ and H$_c$. Cross peaks 2 and 3 correspond to the methylene system (C$_2$ interacting with H$_{a1}$ and H$_{a2}$). Cross peak 4 corresponds to the interaction between methine C$_3$ and H$_b$.

Figure 35. Expanded alkyl region of the $^1$H-$^{13}$C HSQC spectrum of 7-methyl-6,7-dihydro-4H-[1,2,4]triazolo[1,5-α]pyrimindin-5-one (Product M) in d$_6$-DMSO
The full $^1$H-$^{13}$C HSQC spectrum, displayed in Figure 36, shows the additional interaction between the triazole C$_4$ and H$_d$ (cross peak 5). Due to the low signal intensity, to see cross peak 5 other noise also appears in the spectrum. C$_5$, which is in the aromatic region but shows no coupling to hydrogen, is assigned to the peak at 149.5 ppm. The carbonyl carbon, C$_6$, is outside of the range of peaks displayed, but is assigned to a peak appearing at 168.00 ppm (Appendix F) As seen in previous analyses, the ability to establish all the $^1$H-$^{13}$C coupling interactions within the isolated product does not definitively prove the identity of the isomer.

![Figure 36](image)

**Figure 36.** The $^1$H-$^{13}$C HSQC spectrum of 7-methyl-6,7-dihydro-4H-[1,2,4]triazolo[1,5-$\alpha$]pyrimidin-5-one (Product M) in $d_6$-DMSO
Due to the fact that the structure of Product M is very similar to the structure in Product E, the nitrogen environment would be expected to be very similar. In fact, the $^{15}$N chemical shifts obtained for Product M are almost identical to Product E’s. The full 1D $^{15}$N NMR spectrum of Product M is shown in Figure 37. The $^{15}$N chemical shifts for N$_{1}$-N$_{4}$ in Product E are assigned to 283.61, 186.00, 130.00, and 220.38 ppm, respectively, while the chemical shifts in Product M were previously assigned at 280.84, 190.00, 129.50, and 220.10 ppm. The difference in chemical shift only varies by 6.00 ppm at its highest, which strongly suggests a similarity in structure. This similarity in nitrogen signals echoes the similarity in hydrogen signals, and thereby supports the assignment of this molecule as also having a ring closure from position N$_{3}$ to N$_{2}$. Notice the chemical shift for N$_{2}$ in this case is slightly upfield compared to the N$_{2}$ in Product E, which is in keeping with a methyl group being slightly more electron donating than a phenyl group.

Figure 37. The full $^{15}$N NMR spectrum of 7-methyl-6,7-dihydro-4H-[1,2,4]triazolo[1,5-$\alpha$]pyrimindin-5-one (Product M) in d$_{6}$-DMSO
To further investigate the structure of **Product M**, a $^1$H-$^{15}$N HMBC analysis was performed to distinguish among the different $^1$H and $^{15}$N bonds correlation within the molecule. The $^1$H-$^{15}$N HMBC spectrum of **Product M** is displayed in Figure 38. Similar to **Product E**’s HMBC spectrum, $N_3$ only shows an interaction with one of the diastereotopic methylene protons ($H_{a2}$), possibly due to the orientational differences of each proton. Another similarity with the spectrum for **Product E** is that again $N_1$ does not show any interaction with the methine proton $H_c$ in this product. Both $N_1$ and $N_4$ appear to interact with the triazole $H_d$ (peaks 7 and 8). It is again notable that $N_2$ shows interactions with almost all of the protons ($H_d$, $H_c$, $H_{a1}$, $H_{a2}$, and $H_b$ as shown in peaks 2-6). This clearly supports the hypothesis that the cyclization of the heterocyclic product is formed from $N_3$ to $N_2$, and therefore provides further confirmation of the assignment of the structure of this isomer to **Product M**.
Figure 38. The $^1$H-$^{15}$N HMBC spectrum of 7-methyl-6,7-dihydro-4H-[1,2,4]triazolo[1,5-$\alpha$]pyrimidin-5-one (Product M) in d$_6$-DMSO

2.2.6 Characterization of 5-methyl-5,6-dihydro-8H-[1,2,4]triazolo[4,3-$\alpha$]pyrimidin-7-one (Product N)

A second bicyclic compound, Product N, was also isolated under several types of reaction conditions. Similar to the study with trans-cinnamoyl chloride, the study with trans-crotonyl chloride also provided the heterocyclic product for ring closure going from the amino nitrogen N$_3$ to N$_4$ (Product N). The full $^1$H NMR spectrum is shown in Figure 39 and the full $^1$H NMR spectrum with integrals is shown in Appendix G. Similar to
what was observed in Product F, the chemical shift for the triazole proton (H\textsubscript{d}) in Product N also appears to be more downfield in comparison to the same proton when the ring is closed from the amino nitrogen to the N\textsubscript{2} position (Product M). Also consistent with the corresponding phenyl isomers, the chemical shifts for the alkyl protons are slightly more shielded in Product N than in Product M. On the other hand, the chemical shift obtained for the amide hydrogen (H\textsubscript{e}) found in both Product M and N are nearly the same which this is also seen in Product E and F comparison.

\textbf{Figure 39.} The \textsuperscript{1}H NMR spectrum of 5-methyl-5,6-dihydro-8H-[1,2,4]triazolo[4,3-\textalpha]pyrimidin-7-one (Product N) in d\textsubscript{6}-DMSO

The chemical shifts for the methylene protons (H\textsubscript{a1} and H\textsubscript{a2}) in Product N are observed at 2.58 ppm and 2.79 ppm which is slightly more upfield in comparison to the
methylenes protons in **Product M** (2.68 ppm and 2.95 ppm). The sextet peak at 4.48 ppm corresponds to the methine hydrogen, H_b. The triazole proton (H_d) in **Product N** is observed at 8.37 ppm which is more downfield than the same proton in **Product M**, and is very similar to the peak found in **Product F**, which is the N_3 to N_4 ring closure product.

The full $^{13}$C spectrum for this isomer is shown in **Figure 40**. Just as the $^1$H spectrum had hydrogen signals which could suggest the structure of the bicyclic product, the carbons in the triazole ring (C_4 and C_5) are also useful in differentiating between isomers. The chemical shift for C_4 and C_5 appear to be slightly more upfield (136.38 ppm and 139.05 ppm) and separated in **Product N** in comparison to **Product M** (149.47 ppm and 150.00 ppm) where the peaks almost overlap. This distinction clearly shows that the isolated product is not **Product M**. This trend is also in keeping with the same behavior observed for these carbons in **Products E** and **F**. Although both **Product O** and **P** would be expected to provide similar spectra, the presence of an amide signal in the proton NMR strongly rules against these isomers,
Figure 40. The $^{13}$C NMR spectrum of 5-methyl-5,6-dihydro-8H-[1,2,4]triazolo[4,3-$\alpha$]pyrimidin-7-one (Product N) in $d_6$-DMSO

Since the isolated yield of Product N was low, no usable $^{15}$N and $^{15}$N-$^1$H HMBC data could be collected to confirm the structure. However, since the system shows a clear amide hydrogen, and Product M could be very well studied, the only other bicyclic product would have to be Product N. Indeed, the similarity in $^1$H and $^{13}$C NMR studies between Product N and Product F suggest a great similarity in structure, and lead to a reasonable confidence in the assignment of this structure.

2.2.7 Characterization of the Unknown Cyclization Product

As shown in the GC-MS time studies for the reaction of 3-amino-1,2,4-triazole and trans-crotonyl chloride in ACN (Section 2.1.3.2), a third possible bicyclic compound was also isolated in low yields. This product could only be captured from short reflux and
cold temperature reaction conditions with ACN. The integral ratios from the $^1$H NMR spectrum provided an idea about the structure of the unknown product (Figure 41). The peak labeled as $H_1$, at 1.64 ppm, shows 3 protons which correspond to a typical methyl protons. The two doublet of doublet peaks labeled as $H_2$ and $H_3$, at 3.14 ppm and 3.32 ppm, each appears to have an integral ratio of 1 proton which correspond to a methylene proton therefore suggesting that the isolated product is a cyclization product and not a non-bicyclic product. The peak labeled as $H_4$, at 5.24 ppm, appeared to correspond to a methine proton which is similar to what is seen in Product M and N; this reinforces the idea that the isolated product is a cyclization product. Unlike Product M and N, the NH proton ($H_6$) is expected to be more upfield because the deshielding effect cause by the oxygen attached to the carbonyl carbon is now less compare to an amide proton. As a results, the NH proton ($H_6$) is observed at 10.62 ppm in the spectrum which suggest that the NH proton is less likely be an amide proton. On the other hand, it is not easy to assign the indole proton ($H_5$) since there are multiple peaks appear in the expected region. This region is also expanded in the spectrum shown in Figure 41 and the full enlarge $^1$H NMR spectrum can be found in Appendix H.

Since Product M and N has already been established and identified from previous NMR studies, the only 2 possible cyclization products for the isolated unknown cyclization product is Product O or P. In the structure of Product O and P, the carbonyl group is now closer in proximity to the indole proton ($H_5$) which can cause a deshielding effect on $H_5$ therefore it is possible that the $H_5$ proton in the unknown cyclization product would be more downfield compared to the indole proton present in Product M (7.70
ppm) and N (8.37 ppm). In the enlarge spectrum of H₅ (Figure 41), the only peaks that contain only 1 integral proton and the chemical shift is greater than 8.37 ppm is the peak at 8.63 ppm and 8.82 ppm. With two possible peaks for the indole proton (H₅), it’s difficult to assign the correct peak since there are other unknown impurities that are also present in the unknown cyclization product. With a large variation of uncharacterized unknowns, this would be problematic for a $^{15}$N-$^1$H HMBC analysis to differentiate between Product O and P.

Figure 41. The $^1$H NMR spectrum of the unknown cyclization product in d₆-DMSO
Due to the fact that the $^1$H NMR spectrum is not as clean as all the other isolated compounds and the low yields, no further $^{15}$N-$^1$H HMBC analysis was carrying out for this isolated compound. On the other hand, the 1D $^1$H NMR spectrum has provided some evidence that the isolated unknown product is a cyclization product. However, no definite prove have shown the unknown cyclization product to be Product O or Product P.

2.3 Mechanism Study

By being able to isolate and identify several of the products from the tandem cyclization reactions using trans-cinnamoyl chloride or trans-crotonyl chloride, the data can now be used as a guide to further study the mechanism of the reaction. Literature has suggested (and current studies support) that the formation of the bicyclic product is initiated by amide formation on one of four possible nitrogen sites ($N_1$, $N_2$, $N_3$, or $N_4$), and then the ring closure via a Michael addition leading to the bicyclic product. The non-cyclized product's amide linkage is believed to be a strong bond, and yet in at least one paper the author proposes a product which requires a migration of the amide link before ring closure (Scheme 15). To study the mechanism in general, and the possibility of migration specifically, an attempt was made to isolate the open-chain amide products and subject them to ring closure. Unfortunately, the open-chain amide intermediate (Product A) could only be isolated from the reaction with trans-cinnamoyl chloride.
In theory, due to the similarity of the acid halides (trans-cinnamoyl chloride and trans-crotonyl chloride) selected for the study, the acid halide should react similarly when reaction conditions are the same. The only difference between the two acid halides is that trans-cinnamoyl chloride contains a phenyl group at the end of the alkene chain while trans-crotonyl chloride contains a methyl group. It appears that the variation of a phenyl group versus a methyl group does cause a difference in reactivity in these very similar reactions studied. Since the attached group is the only difference between the two selected acid halides, it suggests that bulkiness of the aromatic phenyl group can stabilize the alkene bond more than a less bulky methyl group, or that the extended conjugation of the phenyl group makes the intermediate molecule more stable.

Because there was only one reaction in which stable amides could be isolated, only one reagent could be used in a study of the mechanistic steps involved in the ring closure. In the reaction of 3-amino-1,2,4-triazole and trans-cinnamoyl chloride, two intermediate amide isomers were isolated and fully characterized by NMR (Products A and B). Unfortunately, the isolated yield for Product A (where the amide formed at N₃) was too low to carry out further reactions. This is unfortunate since Product A is the
most direct precursor to the major cyclization products (E and F). The fact that **Product A** was formed in a very low yield that did not improve over time suggests that it is not a product which is stable over the long term. **Product B** (where the amide formed at N₂) was produced in very high yield and was therefore the focus of this study. **Product B** was placed into solution and heated in DMF, a solvent which had been shown to produce cyclized products in initial studies. **Table 5** illustrates the outcomes of the reactions studied. It should be noted that **Product B** remained unchanged when refluxed in DMF and pyridine for 6 hours. This suggested that **Product B** is reasonably stable on its own, and therefore the reactions were run at longer times before any bicyclic products could be isolated.

**Table 5.** Reaction of **Product B** in refluxing DMF, with and without pyridine base

<table>
<thead>
<tr>
<th>Base</th>
<th>Product E Percent Yield (%)</th>
<th>Product F Percent Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/A</td>
<td>29.46 %</td>
<td>2.50 %</td>
</tr>
<tr>
<td>pyridine</td>
<td>67.02 %</td>
<td>30.51 %</td>
</tr>
</tbody>
</table>
As expected from the base studies (Section 2.1.2), the yields for both products from the reaction with pyridine were significantly higher than in the reaction without pyridine. Interestingly, only Products E and F were isolated from these reactions. If the reaction is a straightforward cyclization, Product B would be expected to close to form Product G instead of Products E and F. However, Product G has not been isolated from any of the various reaction condition studied, suggesting that this is not just a simple ring closure, but rather the reaction illustrates that stability issues are also arising during heating. It is possible that Product B did form Product G, a molecule which is not stable under the reaction conditions and therefore it may have converted back into Product B suggesting that N₂ isomers appear to be more stable initially, and seem to resist ring closure as evidenced by their high product yields when isolated. However, N₂ amides appear to be not thermally stable over time. Resonance of the pi aromatic electrons can make the triazole ring a potential leaving group (Scheme 17). On the other hand, the N₃ amide isomer appears to be more thermodynamically stable over N₂ isomer, but it quickly converts to the ring closure products (Product E and F).
Scheme 16. The tautomeric nature of Product B converting to Product A

Scheme 16 illustrated the formation of Product B evolving into Product A. Over time, the newly formed Product A quickly converts into both Products E and F since studies have shown that Product A is not as thermally stable. Based on the GC-MS data as well as the isolated yields, Product E tends to be the more favorable isomer over Product F. This suggests that the ring closure favors the N₂ position over the N₄ position, indicating that N₂ is the more reactive site for this reaction.
3.1 Conclusion

As confirmed by NMR analysis, many isomers have been isolated from the reaction of 3-amino-1,2,4-triazole reacting with trans-cinnamoyl chloride or trans-crotonyl chloride under various solvents, temperatures, times, and conditions. As shown from the times studies using trans-cinnamoyl chloride, the non-bicyclic Product B tends to form in highest yields using acetone and short reaction times, while non-bicyclic Product A can be formed at higher reflux temperatures using THF and longer reaction times. Over time, the disappearance of Product B and the appearance of the bicyclic products E and F suggest a migration of the substituent carbonyl to form Product A during the cyclization process in order to form the bicyclic products. As confirmed by NMR analyses, the amide linkage in Product A seems to be more stable with longer reaction times compared to the linkage found in Product B (the N_2 position). However, Product A is not stable as it reacts further under these conditions to form the bicyclic products (Product E and F). From the solvents studies using trans-cinnamoyl chloride, the non-byecyclic product B tends to form best in acetone or ACN reaction while DMF favors the bicyclic product E under all the reaction conditions studied. This suggests that solvent’s polarity and solubility can play a role in dictating the structure of the product.

Similar to the studies with trans-cinnamoyl chloride, only one bicyclic product (Product M) is formed from the DMF studies using trans-crotonyl chloride. Unlike the reactions with trans-cinnamoyl chloride, no non-bicyclic were produced in any reaction
using trans-crotonyl chloride. Ideally, both reactions containing the trans-cinnamoyl chloride and trans-crotonyl chloride would be expected to react similarly, since the structure of the two starting materials are alike, except for the substituent attached to the alkene. It is interesting that all the reactions studied using trans-cinnamoyl chloride have shown N₂ isomers to be favorable at some point during the reaction, but no products favoring the N₂ position can be identified from the reactions with trans-crotonyl chloride under similar reaction conditions. However, in the reactions with trans-crotonyl chloride a greater variety of cyclic products were observed. The reactions with trans-cinnamoyl chloride produced only two bicyclic products (Products E and F) while three bicyclic products (Products M, N, and a third compound) were isolated from the reactions with trans-crotonyl chloride. With the trans-crotonyl chloride, the major products are still associated with the amide linkage at N₃ with ring closure to both the N₂ and N₄ positions (with N₂ being more favorable).

Overall, in all reactions using conjugated acid chlorides, the most reactive site for ring closure is through the formation of an amide linkage to the amino nitrogen (N₃), followed by ring closure to N₂. These results hold through various solvents, temperatures, and reaction conditions. This suggests that although the literature may show various reactions taking place at different nitrogens around the triazole ring, it may be that the type of reaction may have a stronger influence on determining site reactivity than solvent, temperature, and time variables. Therefore, it can only be concluded that in amide formations, the most reactive site involves N₃.
3.2 Future Work

Since isolation of the non-cyclized products has provided useful insights into reaction mechanism and control, further effort should be used to try to isolate these amides from the trans-crotonyl systems. As shown in the reaction with trans-cinnamoyl chloride, the solvent that gave a non-cyclization product did so because of low product solubility (i.e. acetone and ACN). Future work should include organic solvents like dichloromethane, chloroform, toluene or ethyl acetate to try to drop the solubility of the non-cyclization products.

Further studies should also vary the group attached to the conjugated acid halide in an effort to see if it is an electronic or a steric affect which is influencing the slight differences in products isolated from this reaction. This might shed further light on the mechanism of the reaction, and help to understand any difference in reactivity between the N2 and N4 positions. Other future studies would involve studying other different types of cyclization reactions to elucidate if the type of reaction is a stronger effect on reactive site compare to the effect initiate by the solvent choices, time, and/or temperature variables.
Chapter 4

EXPERIMENTAL

4.1 Abbreviations:

Dimethyl sulfoxide (DMSO); Dimethyl Formamide (DMF); Tetrahydrofuran (THF); Acetonitrile (ACN); Methanol (MeOH); Dichloromethane (DCM); Deuterated-d$_6$ Dimethyl sulfoxide (d$_6$-DMSO).

4.2 Materials:

3-Amino-1,2,4-triazole was purchased from Alfa Aesar. trans-Cinnamoyl chloride, trans-crotonyl chloride, and pyridine were purchased from Acros Organics. THF and DMF solvent were purchased from EMD. All other organic solvents such as acetone, ACN, hexane, ethyl-acetate, DCM, and methanol were purchased from Fisher Scientific. TLC plates was purchased from Whatman (Polyester, UV fluorescence, silica coating, 250 um thickness, and 10 cm x 4 cm plate size). Fluorescent silica gel was prepared in house (2 g 254 nm Indicator / 100 g silica gel). UV light source is generated by a longlife filter flash light purchased from Spectroline. D$_6$-DMSO NMR solvent was purchased from Cambridge Isotope Laboratories, Inc.

4.3 NMR:

NMR spectra were recorded using a Bruker Avance III 500 NMR. All chemical shifts are reported in ppm relative to TMS (0.00 ppm) or DMSO (2.50 ppm) for $^1$H and TMS (0.00
ppm) or DMSO (40.0 ppm) for $^{13}$C. Nitrogen signals ($^{15}$N) are referenced using an external standard (nitromethane) for calibration, 14,000 scans with a delay time of 10-15 seconds, and sample concentration ranging from 15.00-30.00% in d$_6$-DMSO. NMR spectra were analyzed using either Spinworks or Topspin.

4.4 GC-MS:
An Agilent Technologies 7890A GC System containing an Agilent J&W GC Column (stationary phase: HP-5MS, 30 m x 0.250 mm x 0.25 m) with a 5975C inert XL EI/CI MSD with Triple-Axis Detector was used to obtain all MS data. The method used for all runs was: 40 °C for 1 min, 10 °C/min to 150 °C, 30 °C /min to 280 °C , hold for 2 min.

4.5 Procedures:
4.5.1 General Conditions for Reactions of 3-Amino-1,2,4-Triazole and trans-Cinnamoyl Chloride
A 250 mL 2-necked round-bottomed flask was charged with 3-amino-1,2,4-triazole (0.504 g, 6.0 mmol), solvent (acetone, dry DMF, dry THF, or ACN, 5 mL) and dry pyridine (0.5 mL, if incorporated), and equipped with water-cooled condenser, serum stopper and nitrogen inlet. In a separate container, trans-cinnamoyl chloride (1.000 g, 6.0 mmol) and the chosen organic solvent for the reaction (acetone, dry DMF, dry THF, or ACN,5 mL) were combined and stirred with a stir bar. Using a 10 mL syringe, the acid chloride solution was added to the stirred triazole solution dropwise over 15-20 minutes under a positive nitrogen atmosphere. Reaction times varied from 1 hour to 10 days,
depending on study. Temperature conditions of 0 °C, room temperature, and reflux were used depending on study. Once the reaction was ready to be worked up, the mixture was returned to room temperature, and vacuum filtered if there was evidence of products precipitating out of the reaction mixture. The collected precipitate products were washed with cold methanol to remove the yellow color. The filtrate or liquid reaction solution were then diluted with water (150 mL) and extracted with ethyl acetate (2 x 75 mL). The organic layers were dried over sodium sulfate, filtered, and rotovap ed to remove solvent. The crude product was further purified by flash column chromatography on fluorescent silica gel using the indicated solvent systems. All isolated isomers were analyzed with GC-MS and characterized with NMR.

4.5.1.1 3-Phenyl-N-(2H-[1,2,4]triazol-3-yl)-acrylamide (Product A)
Isomer is isolated from a 5 days reaction in THF using the flash column solvent system of 65:35 ACN : DCM to elute out the product (0.059g, 0.275 mmol, 4.64%). \(^1\)H-NMR (500 MHz, d\(_6\)-DMSO): \(\delta 6.90\) (d, 1H), 7.42-7.50 (m, 3H), 7.64 (dd, 2H), 7.70 (d, 1H), 7.88 (sb, 1H), 11.56 (sb, 1H), 13.50 (sb, 1H); \(^13\)C-NMR(500 MHz, d\(_6\)-DMSO): \(\delta 120.45\) (C\(_7\)), 129.55 (C\(_2\)), 128.45 (C\(_3\)), 130.75 (C\(_4\)), 134.73 (C\(_5\)), 150.00 (C\(_1\)), 142.55 (C\(_6\)), 164.01 (C\(_8\)), 167.80 (C\(_9\)); GC-MS: 8.51 minutes (retention time); m/z: 214, 131, 103, 77.

4.5.1.2 1-(5-Amino-[1,2,4]triazol-1-yl)-3phenyl-propenone (Product B)
Isomer can be isolated using acetone or ACN solvents using the flash column solvent system of 65:35 ACN : DCM to elute out the product from the various reactions studied:
a 1.5 hours reaction in acetone (0.913g, 4.270 mmol, 85.16%), a 7.0 day reaction in acetone (0.914g, 4.271 mmol, 85.75%) and a 7 day reaction in ACN (0.684g, 3.195 mmol, 54.41%). The characterizations of the product from all reactions are identical. $^1$H-NMR (500 MHz, d$_6$-DMSO): δ 7.48-7.51 (m, 3H), 7.65 (d, J = 16.8, 1H), 7.64 (sb, 1H), 7.68 (s, 1H), 7.81 (dd, 2H), 7.94 (d, J=16.8, 1H); $^{13}$C-NMR (500 MHz, d$_6$-DMSO): δ 117.07 (C$_7$), 129.32 (C$_3$), 129.60 (C$_2$), 131.79 (C$_4$), 134.32 (C$_5$), 147.51 (C$_6$), 151.95 (C$_1$), 158.00 (C$_8$), 166.50 (C$_9$); $^{15}$N-NMR (500 MHz, d$_6$-DMSO): δ 68.00 (N$_3$), 199.50 (N$_2$), 210.00 (N$_4$), 269.38 (N$_1$); GC-MS: 8.42 minutes (retention time); m/z: 214, 131, 103, 77.

4.5.1.3 7-Phenyl-6,7-dihydro-4H-[1,2,4]triazolo[1,5-α]pyrimidin-5-one (Product E)

Isomer can be isolated from reaction in acetone, ACN, dry THF, or dry DMF using the flash column solvent system of 60:40 ACN : DCM to elute out the products from the various reactions studied: a 1.5 hrs reaction in acetone (0.122g, 0.570 mmol, 9.50%), a 7.0 days reaction in acetone (0.128g, 0.840 mmol, 10.05%), a 7 day reaction in ACN (0.210g, 0.981 mmol, 16.38%), a 5 day reaction in dry THF (0.194 g, 0.900 mmol, 15.19%), a 7 days reaction in dry THF (0.836 g, 3.910 mmol, 64.38%), a 3 day reaction in dry DMF (0.078g, 0.360 mmol, 6.09%), a 7 day reaction in dry DMF (0.966g, 5.421 mmol, 90.35%), and a 10 day reaction in dry DMF (1.256g, 5.870 mmol, 99.00%). The characterizations of the product from all reactions are identical. $^1$H-NMR (500 MHz, d$_6$-DMSO): δ 3.00 (dd, 1H), 3.40 (dd,1H), 5.75 (m, 1H), 7.10 (d, 2H), 7.30-7.40 (m, 3H), 7.80 (s, 1H), 11.75 (sb, 1H); $^{13}$C-NMR (500 MHz, d$_6$-DMSO): δ 39.00 (C$_1$), 56.00 (C$_2$), 126.43 (C$_3$), 128.42 (C$_4$), 128.97 (C$_5$), 139.31 (C$_6$), 150.67 (C$_7$), 150.13 (C$_8$), 167.60 (C$_9$);
\[^{15}\text{N}-\text{NMR} (500 \text{ MHz, } d_6-\text{DMSO}): \delta 283.61 \ (N_1), 186.00 \ (N_2), 130.00 \ (N_3), 220.38 \ (N_4)\];

GC-MS: 8.35 minutes (retention time); m/z: 214, 131, 103, 77.

4.5.1.4 5-Phenyl-5,6-dihydro-8H-[1,2,4]triazolo[4,3-\(a\)]pyrimidin-7-one (Product F)

Isomer can be isolated from reaction in a 5 day reaction in dry THF using the flash column solvent system of 80:20 ACN: MeOH to elute out the products (0.114g, 0.530 mmol, 8.90%). \[^{1}\text{H}-\text{NMR} (500 \text{ MHz, } d_6-\text{DMSO}): \delta 2.94 \ (dd, 1H), 3.21 \ (dd, 1H), 5.75 \ (m, 1H), 7.18 \ (d, 2H), 7.40-7.42 \ (m, 3H), 8.15 \ (s, 1H), 11.64 \ (sb, 1H); \]^\[^{13}\text{C} \text{-NMR} (500 \text{ MHz, } d_6-\text{DMSO}): \delta 38.41 \ (C_1), 53.07 \ (C_2), 126.47 \ (C_3), 129.02 \ (C_4), 129.63 \ (C_5), 138.85 \ (C_6), 139.99 \ (C_7), 149.75 \ (C_8), 167.34 \ (C_9); \]^\[^{15}\text{N}-\text{NMR} (500 \text{ MHz, } d_6-\text{DMSO}): \delta 274.80 \ (N_1), 311.32 \ (N_2), 126.00 \ (N_3), 154.28 \ (N_4); \] GC-MS: 9.20 minutes; m/z: 214, 131, 103, 77.

4.5.2 Reaction of 1-(5-Amino-[1,2,4]triazol-1-yl)-3phenyl-propenone (Product B) in DMF

A 250 mL 2-necked round-bottomed flask was charged with 1-(5-Amino-[1,2,4]triazol-1-yl)-3phenyl-propenone (Product B) (0.199g, 1.000 mmol) and dry DMF (10 mL) and dry pyridine (0.5 mL, if incorporated), and equipped with condenser, serum stopper and nitrogen inlet. The reaction was stirred with a stir bar and heated to reflux and for 6 hours, 5 days, or 7 days. The cooled mixture was filtered by a Hirsh funnel to separate the precipitate insoluble product for the 6 hrs reaction. No precipitate insoluble products were formed in the 5 and 7 day reactions therefore the cooled mixture was diluted with 150 mL of water and 2x 75 mL of organic solvent (ethyl acetate) to extracted
the products. The extracted organic layers were dried over sodium sulfate, filtered, and rotovaped to remove the solvent. The extracted water layers were dried by heating to remove the water particles. The crude products isolated from the organic and water layers were further purified by flash column chromatography on fluorescent silica gel using the indicated solvent systems. Both 7-Phenyl-6,7-dihydro-4H-[1,2,4]triazolo[1,5-\(\alpha\)]pyrimidin-5-one (Product E) and 5-Phenyl-5,6-dihydro-8H-[1,2,4]triazolo[4,3-\(\alpha\)]pyrimidin-7-one (Product F) were isolated. The characterization data for Product E and F are shown above in Sections 4.5.1.3 and 4.5.1.4. The yields for Products E and F are as follows: a 6 hrs reaction in dry DMF with pyridine yield 0.00% Product E and F, a 5 day reaction without pyridine Product E (0.063g, 0.290 mmol, 29.46%) and Product F (0.005g, 0.021 mmol, 2.50%), and a 7 day reaction with pyridine Product E (0.143g, 0.668 mmol, 67.02%) and Product F (0.065g, 0.304 mmol, 30.51%).

4.5.3 General Conditions for Reactions of 3-Amino-1,2,4-Triazole and Trans-Crotonyl Chloride

A 250 mL 2-necked, round-bottomed flask was charged with 3-amino-1,2,4-triazole (0.504, 6.0 mmol), dry THF or DMF or acetone or ACN (10 mL), and dry pyridine (0.489 mL, 6.0 mmol) and equipped with a water-cooled condenser, serum stopper, and nitrogen inlet. In a separate container, trans-crotonyl chloride (0.575 mL, 6.0 mmol) and some of the same solvent used in the first step (5 mL) were combined. Using a 10 mL syringe, the acid halide solution was added to the triazole solution dropwise over 20-25 minutes while the mixture is stirred by a stir bar. Reaction times varied from 1
hour to 9 days, depending on study. Temperature conditions of 0 °C, room temperature, and reflux were used depending on study. Once the reaction was ready to be worked up, the mixture was returned to room temperature, diluted with water (150 mL) and extracted with ethyl acetate (2 x 75 mL). The organic layers were dried over sodium sulfate, filtered, and rotovaped to removed the solvent. The crude product was further purified by flash column chromatography on fluorescent silica gel using the indicated solvent systems. **Products M** and **N** were isolated from this reaction and the isomers were analyzed with GC-MS and characterized with NMR.

### 4.5.3.1 7-Methyl-6,7-dihydro-4H-[1,2,4]triazolo[1,5-α]pyrimindin-5-one (Product M)

Isomer can be isolated from all four solvents system, acetone, ACN, dry THF, and dry DMF using a flash column solvent system of 65:35 ACN : DCM to elute out the products. Yields are as follows for different reaction conditions: a 4 hr reaction in acetone (0.046 g, 0.304 mmol, 5.00%), a 1.5 day reaction in acetone (0.077 g, 0.504 mmol, 8.29%), a 2 day reaction in acetone (0.083 g, 0.542 mmol, 8.93%), a 2.5 day reaction in acetone (0.086 g, 0.570 mmol, 9.41%), a 7 day reaction in acetone (0.198 g, 1.305 mmol, 21.47%), a 2 hr room temperature (RT) reaction in acetone (0.074 g, 0.490 mmol, 8.12%), a 7.0 day reaction in ACN (0.881 g, 5.800 mmol, 95.40%), a 2.5 day reaction in ACN (0.881 g, 5.799 mmol, 95.29%), a 2 hr RT reaction in ACN (0.045 g, 0.290 mmol, 4.80%), a 1.5 hr ice bath reaction in ACN (0.204 g, 1.340 mmol, 21.86%), a 1 day reaction in dry THF (0.101 g, 0.670 mmol, 11.13%), a 5.5 day reaction in dry THF (0.230 g, 1.514 mmol,
25.29%), a 7 day reaction in THF (0.279 g, 1.840 mmol, 30.65 %) and a 7 day reaction in dry DMF (0.350g, 2.300 mmol, 38.29%). The characterizations of the product from all reactions are identical. $^1$H-NMR (500 MHz, d$_6$-DMSO): δ 1.45 (d, 3H), 2.68 (dd, 1H), 2.95 (dd, 1H), 4.50 (m, 1H), 7.70 (s, 1H), 11.45 (s, 1H); $^{13}$C -NMR (500 MHz, d$_6$-DMSO): δ 19.47 (C$_1$), 38.25 (C$_2$), 48.81 (C$_3$), 149.47 (C$_4$), 149.90 (C$_5$), 168.00 (C$_6$); $^{15}$N-NMR (500 MHz, d$_6$-DMSO): δ 280.84 (N$_1$), 190.00 (N$_2$), 129.00 (N$_3$), 220.10 (N$_4$); GC-MS: 6.55 minutes; m/z: 152, 84, 69.

4.5.3.2  5-Methyl-5,6-dihydro-8H-[1,2,4]triazolo[4,3-a]pyrimidin-7-one (Product N)

Isomer can be isolated from reactions in acetone, ACN, or dry THF using the flash column solvent system of 65:35 ACN : DCM. The yields are as follows for different reaction conditions: a 7 day reaction in acetone (0.100g, 0.660 mmol, 10.95%), a 1.25 hr ice bath reaction in ACN (0.040g, 0.260 mmol, 4.25%), and a 1 day reaction in dry THF (0.113g, 0.744 mmol, 12.45%). The characterizations of the product from all reactions are identical. $^1$H-NMR (500 MHz, d$_6$-DMSO): δ 1.42 (d, 3H), 2.58 (dd, 1H), 2.79 (dd, 1H), 4.48 (m, 1H), 8.37 (s, 1H), 11.44 (sb, 1H); $^{13}$C -NMR (500 MHz, d$_6$-DMSO): δ 19.87 (C$_1$), 37.86 (C$_2$), 46.34 (C$_3$), 136.83 (C$_4$), 139.05 (C$_5$), 167.86 (C$_6$); GC-MS: 6.35 minutes; m/z: 152, 84, 69.
APPENDICES
Appendix A

The GC-MS chromatogram of 3-amino-1,2,4-triazole.
The GC-MS mass spectrum of 3-amino-1,2,4-triazole.
The GC-MS chromatogram of pyridine.
The GC-MS mass spectrum of pyridine.
The GC-MS chromatogram of *trans*-crotonic acid.
The GC-MS mass spectrum of trans-crotonic acid.
The GC-MS chromatogram of \textit{trans}-cinnamic acid.
The GC-MS mass spectrum of \textit{trans}-cinnamic acid.
The GC-MS chromatogram for the reaction of 3-amino-1,2,4-triazole and \textit{trans}-crotonyl chloride with acetone, 2 hours.
The GC-MS mass spectrum of the unknown cyclization product at the retention time of 6.18 minutes.
The GC-MS mass spectrum of **Product N** at the retention time of 6.35 minutes.
The GC-MS mass spectrum of **Product M** at the retention time of 6.59 minutes.
The overlay GC-MS mass spectrum of **Product O, N, and M** over 2 hours, 1.0 day, 1.5 days, 2.0 days, and 7.0 days reaction time in acetone.
The GC-MS chromatogram of 1-(5-Amino-[1,2,4]triazol-1-yl)-3phenyl-propenone (Product B).
The GC-MS mass spectrum pattern of 1-(5-Amino-[1,2,4]triazol-1-yl)-3-phenyl-propenone (Product B).
The GC-MS chromatogram for reaction of 3-amino-1,2,4-triazole and *trans*-cinnamoyl chloride in THF, 1 hour.
The GC-MS chromatogram for reaction of 3-amino-1,2,4-triazole and \textit{trans}-cinnamoyl chloride in THF, 3 days.
The GC-MS chromatogram for reaction of 3-amino-1,2,4-triazole and trans-cinnamoyl chloride in THF, 4 days.
The GC-MS chromatogram for reaction of 3-amino-1,2,4-triazole and \textit{trans}-cinnamoyl chloride in THF, 5 days.
The overlay GC-MS mass spectrum of Product A, B, E, and F over 1 hours, 3.0 day, 4.0 days, and 5.0 days reaction time in acetone.
Appendix B

The $^1$H-NMR spectra of 3-Phenyl-N-(2H-[1,2,4]triazol-3-yl)-acrylamide (Product A) in $d_6$-DMSO with integral ratio.
Appendix C

The $^1$H-NMR spectrum of 1-(5-Amino-[1,2,4]triazol-1-yl)-3phenyl-propenone (Product B) in d$_6$-DMSO with integral ratio.
The $^{13}$C-NMR spectrum of 1-(5-Amino-[1,2,4]triazol-1-yl)-3phenyl-propenone (Product B) in $d_6$-DMSO.
The $^{15}$N-NMR spectrum of 1-(5-Amino-[1,2,4]triazol-1-yl)-3phenyl-propenone (Product B) in d$_6$-DMSO.
Appendix D

The $^1$H-NMR spectrum of 7-Phenyl-6,7-dihydro-4H-[1,2,4]triazolo[1,5-α]pyrimidin-5-one (Product E) in d$_6$-DMSO with integral ratio.
The $^{13}$C-NMR spectrum of 7-Phenyl-6,7-dihydro-4H-[1,2,4]triazolo[1,5-α]pyrimidin-5-one (Product E) in d$_6$-DMSO.
Appendix E

The $^1$H-NMR spectrum of 5-Phenyl-5,6-dihydro-8H-[1,2,4]triazolo[4,3-α]pyrimidin-7-one (Product F) in d$_6$-DMSO with integral ratio.
The $^{13}$C-NMR spectrum of 5-Phenyl-5,6-dihydro-8H-[1,2,4]triazolo[4,3-$\alpha$]pyrimidin-7-one (Product F) in d$_6$-DMSO
Appendix F

The $^1$H-NMR spectrum of 7-Methyl-6,7-dihydro-4H-[1,2,4]triazolo[1,5-α]pyrimindin-5-one (Product M) in d$_6$-DMSO with integral ratio
The $^{13}\text{C}$-NMR spectrum of 7-Methyl-6,7-dihydro-4H-[1,2,4]triazolo[1,5-$\alpha$]pyrimindin-5-one (Product M) in d$_6$DMSO
Appendix G

The $^1$H-NMR spectrum of 5-Methyl-5,6-dihydro-8H-[1,2,4]triazolo[4,3-$\alpha$]pyrimidin-7-one (Product N) in d$_6$-DMSO with integral ration
Appendix H

The $^1$H-NMR spectrum of the unknown cyclization product in $d_6$-DMSO with integrals
REFERENCES


