MISSOURI SOUTHERN STATVE UNIVERSITY HONORS PROGRAM

A Small Molecule Synthesis of Oxazole Cores

With possible applications of the Dakin-West Reaction and the Robinson-Gabriel Synthesis

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Protection of the sulfur atom of L-cysteine with 4-bromobenzylbromide in the presence of methanol/ammonia gave 109% yield. Subsequent reaction of the product 4-bromobenzyl cysteine with acetic anhydride in the presence of triethylamine gave 19.5% yield. Product was confirmed by IR and 1H NMR spectra.

1) Background.

1.1) Ras proteins

Cellular proliferation, survival, and differentiation are controlled by a variety of hormones, growth factors, and cytokines present in the extracellular environment of organisms. These molecules serve as ligands for cellular receptors on the exterior of cells and regulate cellular processes through intricate 2nd messenger system signaling pathways.[1] In tumor cells, these cellular functions and signaling pathways are altered through activation of oncogenes and inactivation of tumor suppressor genes. One of the most frequently activated oncogenes in human cancer is the Ras gene family which has been found to be mutated in 20 to 30% of all tumors (see Figure 1.1).[2 3]

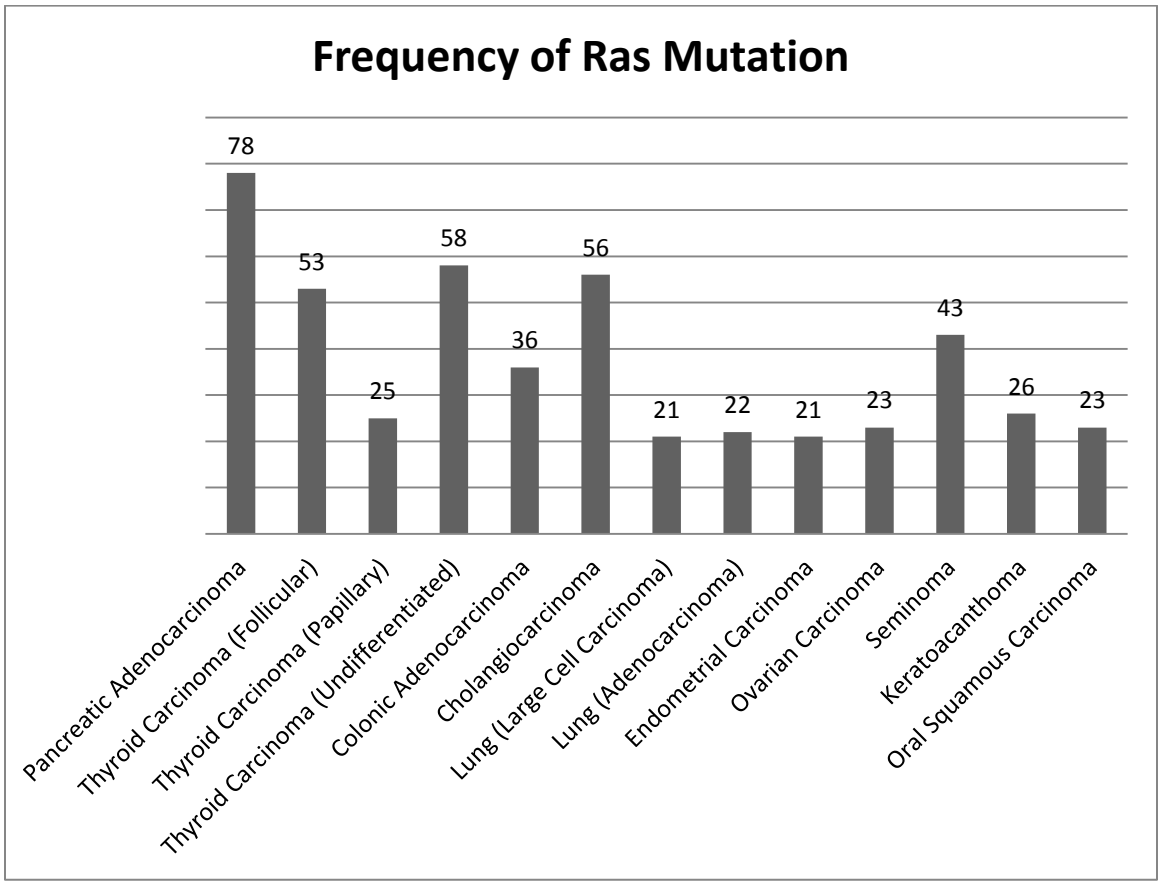

Figure 1.1 – Frequency of Ras mutation in percentage by cancer type.

The Ras protein subfamily consists of low molecular weight (21 kDa) monomeric G-proteins (GTPases) bound to the plasma membrane. In normal cells Ras proteins cycle between an inactive GDP-bound state to an active GTP-bound state in response to the binding of growth factors.[4] Following activation, Ras proteins transmit the growth signal to various effectors.[2] Ras must be plasma membrane bound in order for it to carry out these functions. As Ras requires post-translational modification for plasma-membrane binding, enzymes that catalyze reactions to bind Ras to the cell membrane are possible targets for therapeutics.[4]

The four Ras proteins (H-Ras, N-Ras, and K-ras A and B) are translated from three Ras genes (K-Ras A and B are splice variants of a single gene). The majority of Ras proteins comprise 188 amino acids, the first 165 of which are conserved. All Ras proteins have a specific sequence of amino acids located at the carboxyl terminal commonly known as the –CAAX box (where C indicates a cysteine moiety, A a variable amino acid, and X a methionine or serine). Post-translational modification of Ras proteins allows their embedding in the plasma membrane and thus their ability to function in growth signaling. The first primary step involves the attachment of farnesyl lipid molecules (from FDP) at the -CAAX box catalyzed by farnesyl protein transferase (FPTase). At this point the final three amino acids are removed leaving a farnesylated cysteine which then undergoes methylation catalyzed by isoprenylcysteine methyl transferase (ICMT). By inhibiting this final step involving the methylation of the isoprenylcysteine moiety of the Ras protein, it is hoped that the binding of Ras to the plasma membrane will be disrupted thus abrogating its effects. Three options for inhibition exist: 1) analogues of FDP which compete as a substrate for FTPase, 2) molecules that are structurally similar to the –CAAX box or the peptide chain, and 3) compounds that involve some degree of both FDP analogues and –CAAX mimetics or peptidomimetics.[4]

1.2) Oxazole Synthesis

Oxazoles (Figure 1.2a) show structural similarity to isoprenyl cysteine (see Figure 1.2b) and may be synthesized by several methods. The methods of synthesis are from 1) α-acylaminocarbonyl compounds (the Robinson-Gabriel synthesis), 2) acyl derivatives of α-amino acid esters, 3) α-amino acids, 4) benzalaminoacetals, 5) desyl esters and ammonia, 6) aromatic aldehydes and aromatic aldehyde cyanohydrins, 7) amides and α-haloketones and α-haloaldehydes, 8) benzyl, 9) reduction of oxidoöxazoles, and 10) benzoin and nitriles.[5] Traditionally, the Robinson-Gabriel reaction is used for the synthesis of oxazoles as the α-acylaminocarbonyl compounds may be synthesized from α-amino acids via the Dakin-West reaction and the solvents are readily available.

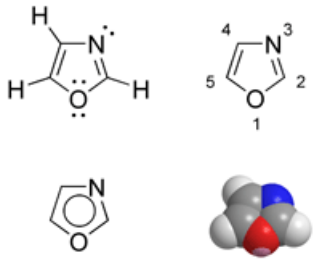

Figure 1.2a – Various representations of 1,3-oxazole.

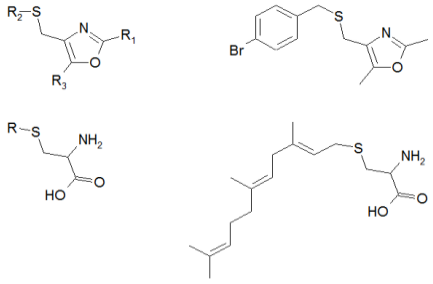

Figure 1.2b – Structural similarity of oxazole cores (top left) to ispoprenyl cysteine (bottom left) and gallemoxazole (top right) to farnesyl cysteine (bottom right).

1.3) Protecting Groups and Nucleophilicity

Thiols (R-SH) are more nucleophilic than amines (R-NH_2) or hydroxyl (R-OH) groups due to the greater size of the sulfur atom. Due to this sulfur atoms are less electronegative than oxygen or nitrogen atoms (see Figure 1.3.1).

Atom	Electronegativity Value (Pauling)
Hydrogen	2.20
Carbon	2.55
Nitrogen	3.04
Oxygen	3.44
Sulfur	2.58

Figure 1.3a – Pauling electronegativity values of selected elements.

As the first step of the Dakin-West reaction to be discussed later involves nucleophilic addition/elimation with an acid anhydride to acylate the amino group of an amino acid, the sulfur group must be protected in order to prevent reactions with the thiol. As alkyl halides are electrophilic and react directly with nucleophiles various alkyl halides were selected to protect the nucleophilic sulfur atom from acylation (see Figure 1.3.2). Bromobenzyl bromide may be further reacted following the Robinson-Gabriel synthesis to act as a farnesyl analog.

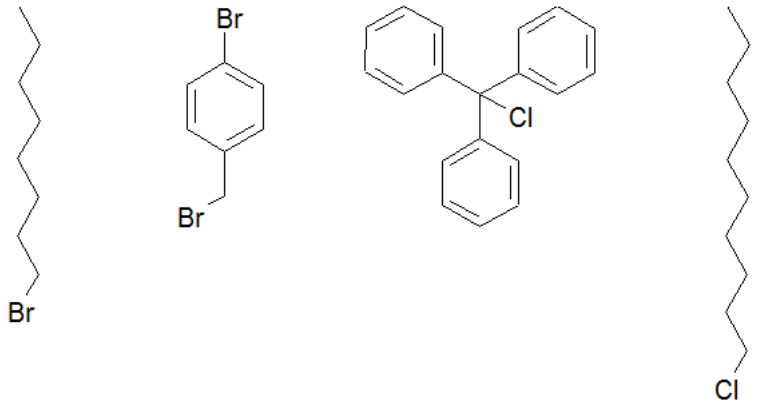

Figure 1.3b – Alkyl halides selected for use as alkylating agents. From left: 1-bromooctane, bromobenzyl bromide, trityl chloride, and 1-chlorodecane.

1.3) The Dakin-West Reaction

Conversion of an α-amino acid into a corresponding α-acetylaminoalkyl methyl ketone (also keto-amide) through the action of an acid anhydride in the presence of pyridine (or another base) occurs with the evolution of carbon dioxide and is commonly known as the Dakin-West reaction (Figure 1.3a).[6]

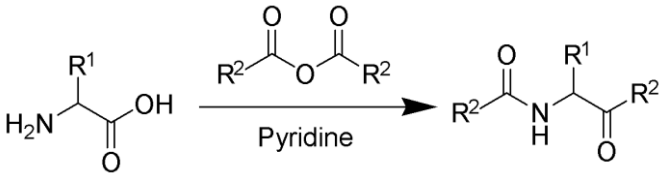

Figure 1.3a – Scheme for the Dakin-West reaction of an amino acid with an amino acid in the presence of pyridine.

This reaction was discovered in 1928 by Henry Drysdale Dakin and Randolph West who subsequently carried out a study of the reactions mechanism and scope (Figure 1.2).[7 8] Several mechanisms for this reaction have been proposed, but the most commonly encountered and favored involves the conversion of an azlactone (Figure 1.2).

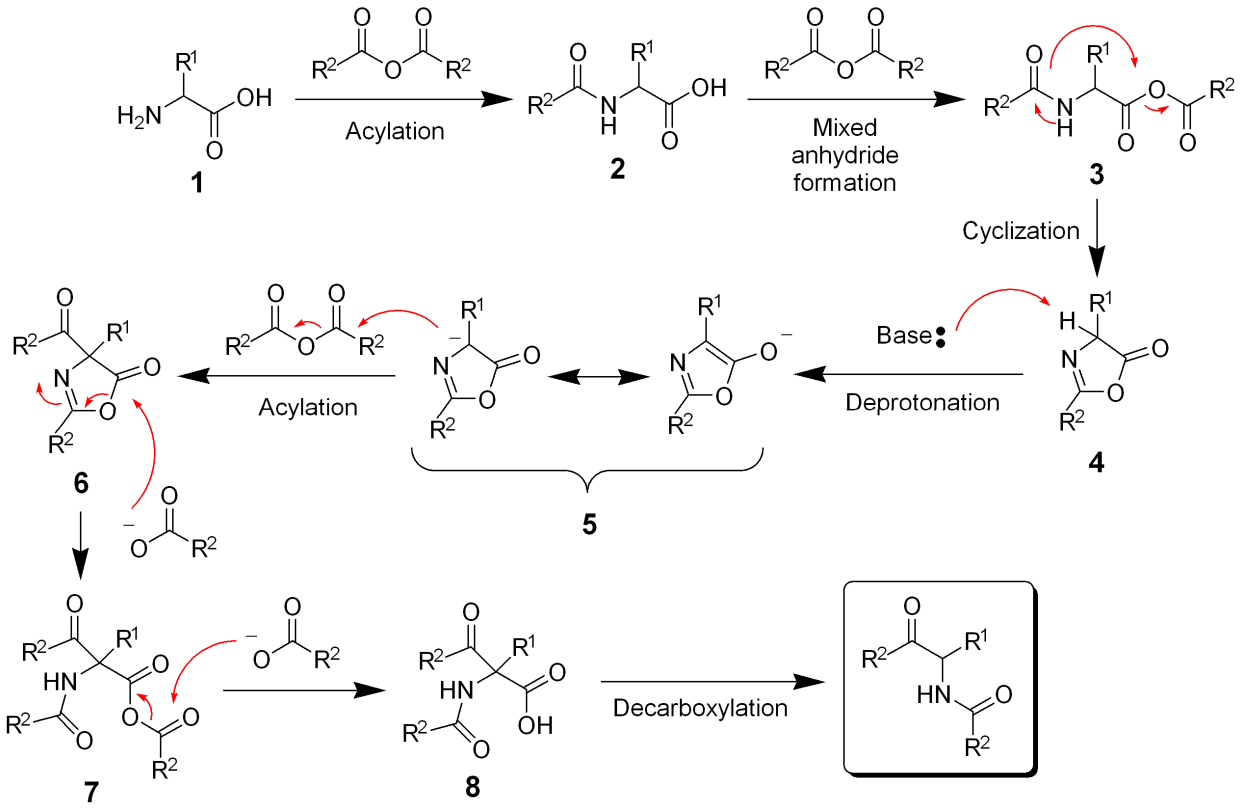

Figure 1.3b – The suggested azlactone mechanism of the Dakin-West reaction.

In this mechanism the reaction proceeds first by acylation and activation of the amino acid **1** to the mixed anhydride **3** followed by the cyclization of the acylated product to the azlactone **4**. The azlactone is deprotonated in the presesnce of pyridine (or another suitable base) giving rise to a resonance stabilized carbanion **5**. The carbanion reacts with the acid anhydride to give a second azlactone product **6** which undergoes ring-opening and conversion to the acetamido ketone **8**. Decarboxylation follows to give the α-acetylaminoalkyl methyl ketone.[9]

1.4) The Robinson-Gabriel Synthesis

The dehydration of the α-acetylaminoalkyl methyl ketone with a strong acid leads to the formation of an oxazole and is known as the Robinson-Gabriel Synthesis (Figure 1.3).

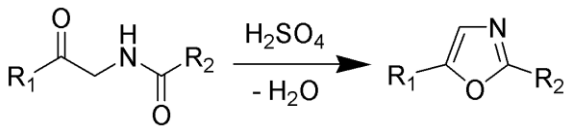

Figure 1.4a – Scheme for the Robinson-Gabriel Synthesis with sulfuric acid as the strong acid.

The mechanism for the Robinson-Gabriel Synthesis is simple comparatively to that of the Dakin-West. In **1**, the ketone carbonyl is protonated and the lone pair of electrons on the nitrogen of the amide functional group aids cyclization (**2**) while the proton attached to the nitrogen is removed to neutralize the positive charge on the nitrogen (**3**). In **4**, the hydroxyl group acts as a leaving group giving the oxazole **5**.[10]

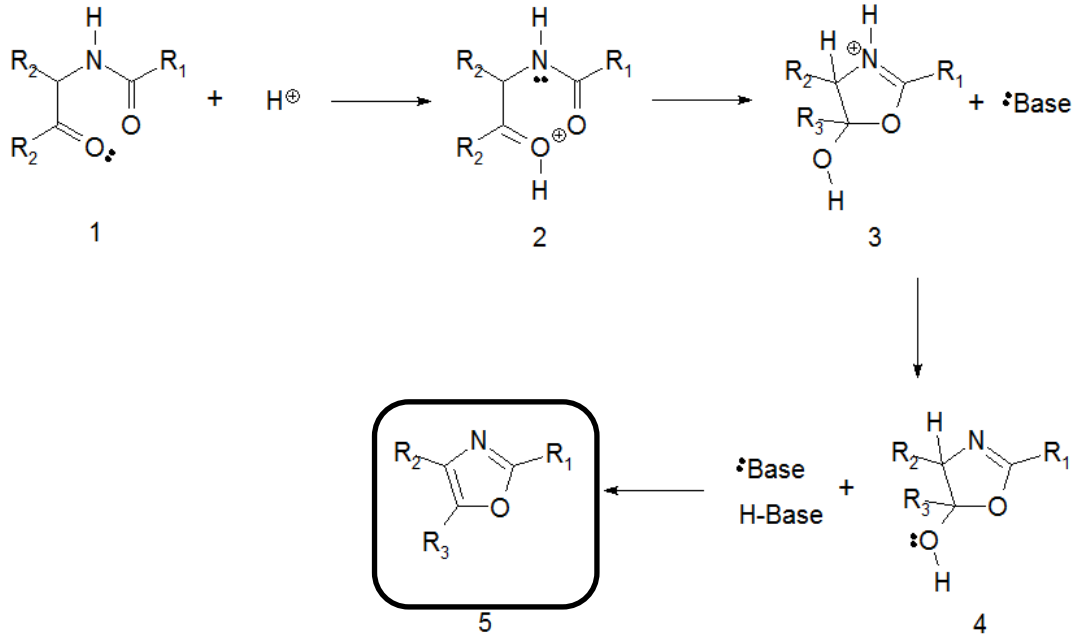

Figure 1.4b – Mechanism for the Robinson-Gabriel Synthesis of oxazoles from α-acetylaminoalkyl methyl ketone in strong acid.

2) Methods.

^{1}H NMR spectra were recorded at 60 MHz with $CDCl_3$ as solvent, TMS was not used in all samples as an internal standard as it interfered with spectra results. IR spectra were obtained with IR Spectrophotometer utilizing CH_2Cl_2 and $CHCl_3$ as standards. Alkylation of cysteine with bromobenzyl bromide in methanol/ammonia as solvent at 0ºC gave 109% yield. Purification of the product could not

be performed due to solubility. The Dakin-West reaction of the product of the alkylation reaction [S-(4-bromobenzyl)cysteine] with acetic anhydride and pyridine and triethylamine as solvent gave 19.5% yield (assuming 100% yield from alkylation) after separation with a solution of citric acid / DI H_2O and dehydration with Na_2SO_4 and purification in an ion exchange column using silica gel as the solid phase and a 5% MetOH / 95% CH_2Cl_2 solution and 4% ethyl acetate / 96% hexane as mobile phases. The Robinson-Gabriel synthesis did not proceed. The reaction scheme for this reaction is shown in Figure 2. Optimal solvents were determined for step **1**, various solvents were attempted (CH_3N/CH_2Cl_2, CH_3OH/NaOH, CH_3N/Lutadine/Imidazole, and CH_3OH/NH_3 as stated above). TLC tests were performed to determine appropriate solvents for all steps as well as to determine mobile phases for column chromatography.

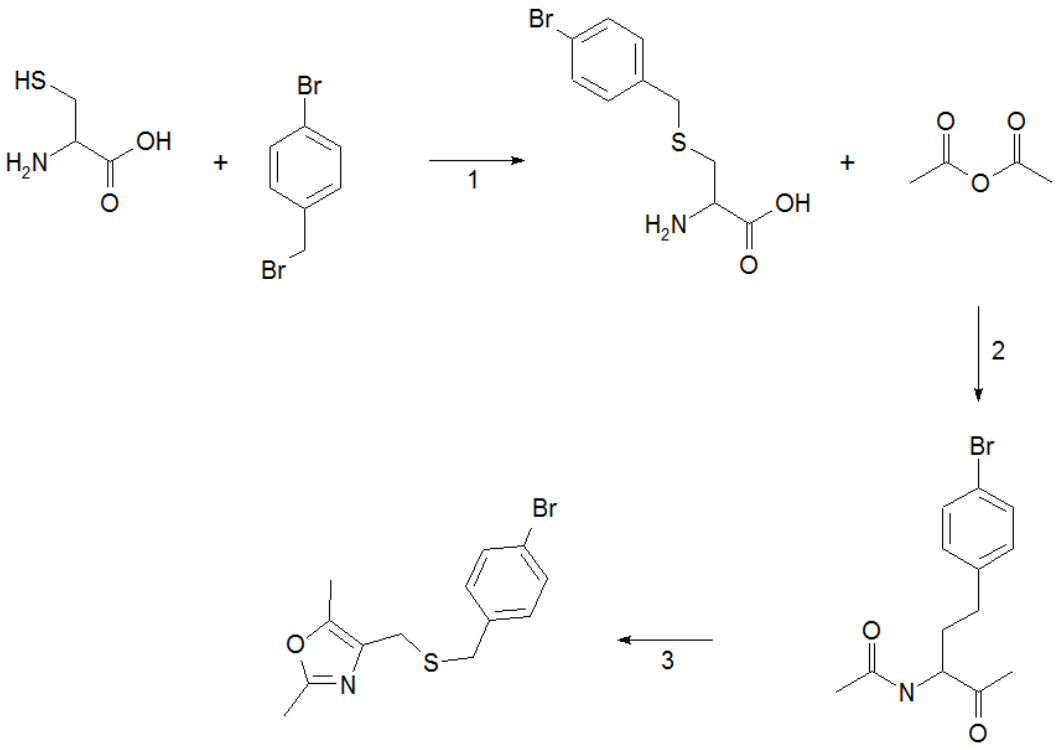

Figure 2 – Reaction scheme for synthesis of 2,4-dimethyl-4-[3-(4-bromobenzyl)-2-thiopropyl]-1,3-oxazole. **1** is CH_3OH/NH_3, **2** is CH_3N, and **3** is H_2SO_4. Pyridine or DMAP may also be used in **2**, however refluxing is required with pyridine.

3) Results and Conclusion

^1H NMR spectra indicates the final product after attempted dehydration with concentrated H_2SO_4 obtained a doublet peak at 7.072 and 7.207 PPM consistent with aromatic protons the peak at 2.210 are

consistent with α-methyl protons (see Figure 3a). The other indicated peaks are consistent with the remaining proton environments of the second product. However a peak is missing for one the -CH_2- proton environment. IR spectra indicates a peak at 1693.20 (see Figure 3b) which is consistent with a carbonyl of an amide functional group, two carbonyl peaks would be expected, however. These two spectra indicate that the compound is most likely *N*-1-[2-(4-bromophenyl)ethyl-2-oxopropyl]acetamide, the product of the Dakin-West reaction.

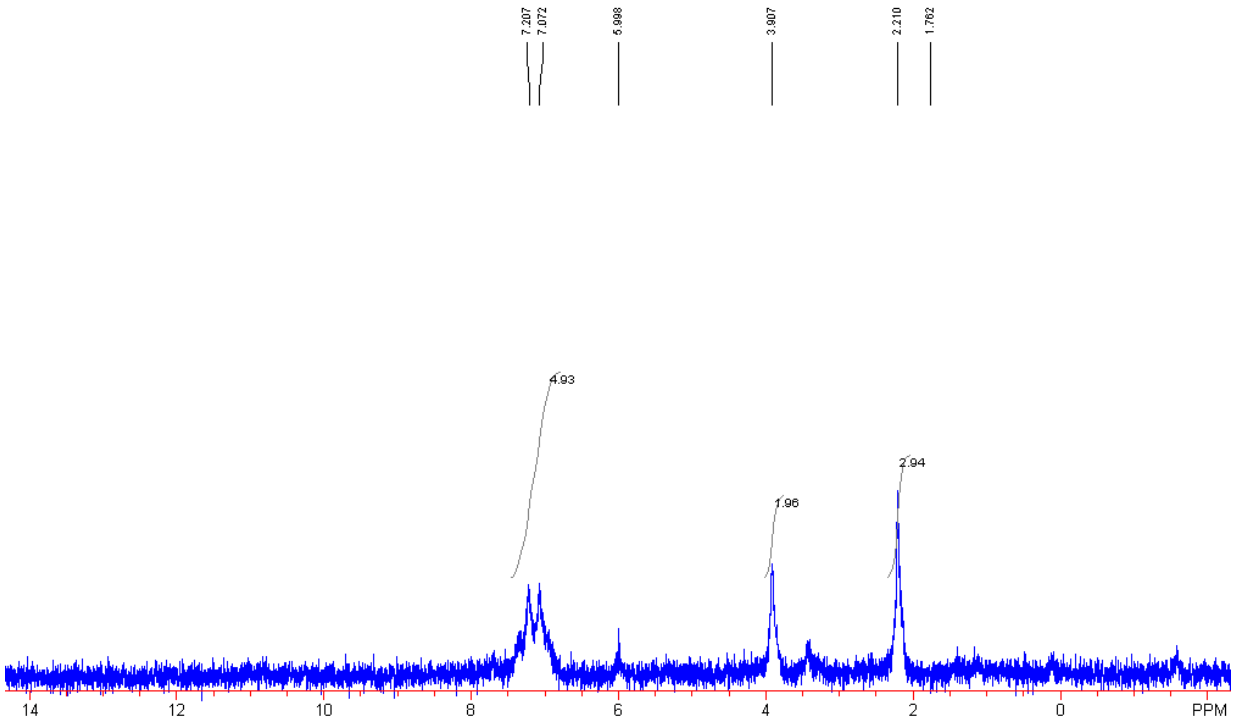

Figure 3a – ^{1}H NMR spectra for product of the Robinson-Gabriel synthesis. The spectra is indicative that this reaction did not proceed due to varying intensities of proton peaks and environments.

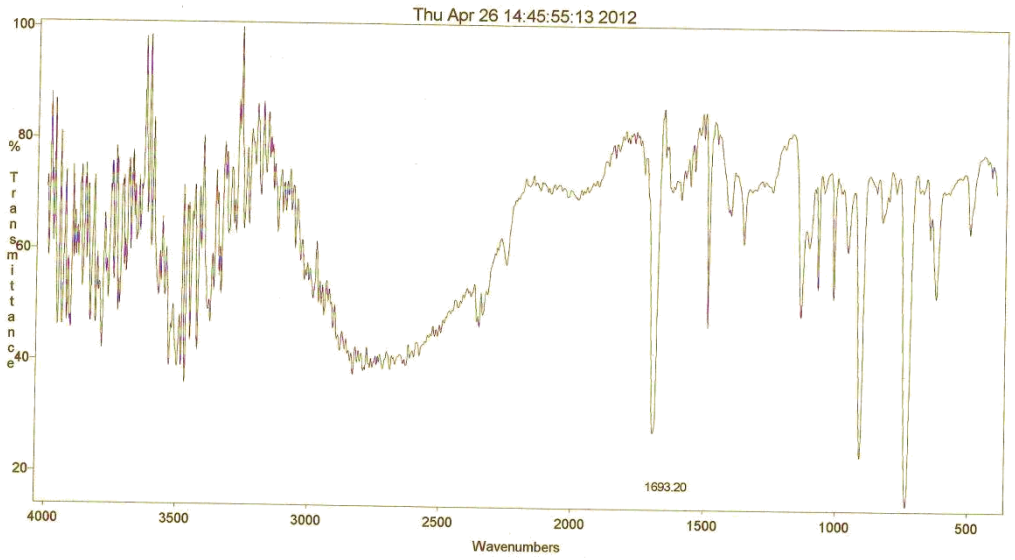

Figure 3a – IR spectra for product of the taken after Robinson-Gabriel synthesis. The peak at 1693.20 is indicative of a carbonyl functional group.

The ^{1}H NMR and IR spectra have perplexing results. Only one carbonyl peak is found on the IR spectra when two were to be expected. The results, however, were subjected to an auto-baseline algorithm and it is possible that this interfered with the results. A second peak does exist at approximately 1500, however this is below the expected range for a carbonyl. The ^{1}H NMR spectra is also perplexing as a protons from a -CH_2- group are not seen. As both of the -CH_2- groups of the compound cannot be lost during these reactions, the absence may be attributable to problems with the ^{1}H NMR. Despite these results the data seems to indicate that likely *N*-1-[2-(4-bromophenyl)ethyl-2-oxopropyl]acetamide was most likely synthesized and that the cyclization did not proceed. Mass spectrometry will be performed to confirm the molecular weight of the compound and thus confirm that likely *N*-1-[2-(4-bromophenyl)ethyl-2-oxopropyl]acetamide was synthesized.

Alkylation of cysteine was successful with bromobenzyl bromide. 1-Bromooctane alkylation of cysteine was unsuccessful as was the alkylation of trityl chloride. In the case of trityl chloride, altering the solvent to DMF and stirring at room temperature for a period of at least 2 days before adding sodium acetate would produce a better outcome; however, solubility would most likely still be an issue. It is unknown why ^{1}H NMR spectra and IR spectra of the alkylation results did not support that S-(4-bromobenzyl)cysteine was synthesized. However, as ^{1}H NMR spectra and IR spectra of the final product indicate that the *N*-1-[2-(4-bromophenyl)ethyl-2-oxopropyl]acetamide was synthesized it is possible that an elimination occurred in the process of heating the product in order to increase its solubility for performing ^{1}H NMR and IR spectrophotometry after the Dakin-West reaction was carried out.

The high yield of the protection step (109%) is assumed to be due to excess solvent. It is likely that solvent molecules became trapped in the product and were unable to be fully evaporated by the rotary evaporator and product weight might thus be greater than the initial weights of 4-bromobenzylbromide and cysteine. The low yield of the Dakin-West reaction is likely due to that column chromatography was

run twice due to inadequate purification of the first purification. Purification entails some loss of product and as it was performed twice, product loss would be greater.

Suitable solvents for the protection reaction (CH_3OH/NH_3) were found as well as for the Dakin-West reaction (CH_3CN). Pyridine was not tested as a solvent as refluxing conditions were required, furthermore DMAP was not used due to toxicity. These solvents will be used by future research students in order to enable more expeditious synthesis of oxazoles. Information obtained through this project will be utilized in future projects which will attempt to utilize the 2,4-dimethyl-4-[3-(4-bromobenzyl)-2-thiopropyl]-1,3-oxazole compound to synthesis farnesyl cysteine analogues by substitution reactions of the 4-bromobenzylbromide substituent group. A second attempt of the Robinson-Gabriel synthesis is being attempted currently. Other future steps would involve synthesis of more oxazole compounds derived from cysteine substituting various alkyl halides in the first step of the reaction scheme as well as substituting other acid anhydrides during the Dakin-West reaction in order to alter the alkyl groups located on the thioether and carbons two and four of the oxazole. These oxazole derivatives may also be tested as possible inhibitors of ICMT or could be plated with cells expressing mutated Ras protein to determine inhibition of growth.

4) Works Cited.

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