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Partial Preparation of 1-Anilino-8-(Mercaptoamino)-1,8-Octadione as a Potential Therapeutic Agent for the Treatment of Huntington's Disease and Selected Cancers

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Partial Preparation of 1-Anilino-8-(Mercaptoamino)-1,8-Octadione as a Potential Therapeutic Agent for the Treatment of Huntington's Disease and Selected Cancers

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ABSTRACT

We are proposing 1-anilino-8-(mercaptoamino)-1,8-octadione as a compound of potential pharmaceutical value. The addition of carbon disulfide to sodium trimethylsilanolate to yield a xanthate product has been analyzed and refined to a 70% yield. Several methods of aminating the xanthate product to yield a sulfenamide have been attempted and analyzed. Multiple attempts have been made to benzoylate the sulfenamide product with little success, with many concerns stemming from the unsuccessful amination reactions.

Keywords: Organic synthesis, sulfenamide, Huntington's Disease, xanthate

INTRODUCTION

Current literature has led to designing the synthesis of a thiol analog of SAHA. The specific analog replaces the oxygen atom in the hydroxyl group with a sulfur atom (see ***Figure 1***) creating a strategic sulfur-nitrogen bond. The IUPAC name for the proposed structure is 1-anilino-8-(mercaptoamino)-1,8-octadione (AMAO). To the best of our knowledge, this derivative of SAHA, or any hydroxamic acid, has not been prepared before. Challenges in this preparation include, but are not limited to, creating the sulfur-nitrogen bond and maintaining this acid-labile bond through other reactions (Craine & Raban, 1989). ***Figure 2*** shows the four reactions of our proposed complete preparation, three of which are addressed in this work.

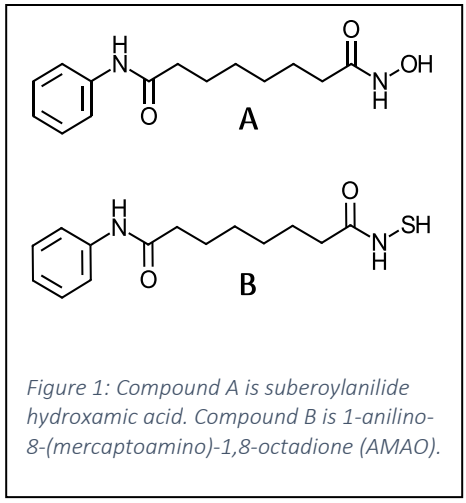


Figure 1: Compound A is suberoylanilide hydroxamic acid. Compound B is 1-anilino-8-(mercaptoamino)-1,8-octadione (AMAO).

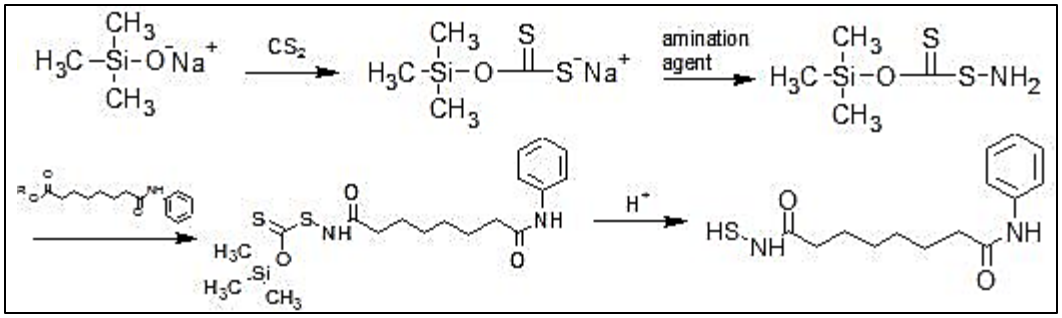


Figure 2: Overall scheme of complete preparation.

In the initial attempts at the third reaction, the benzoyl chloride and 3,5-dinitrobenzoyl chloride were used as place holders for what will eventually become the drug sidearm based on SAHA. These benzoyl chlorides were being used first to investigate and improve the reaction due to their higher reactivity and cheaper expense.

Upon improvement of this reaction, the benzoyl chlorides will be replaced with esters and then the methyl ester of the SAHA sidearm, as indicated in *Figure 2*.

REVIEW OF LITERATURE

A class of compounds called histone deacetylase (HDAC) inhibitors have anticancer potential as they upregulate the genes for apoptosis and downregulate the genes that work against apoptosis, causing cell-cycle arrest, terminal differentiation, apoptosis, and/or autophagic cell death in transformed cells (Sung, Ververis, & Karagiannis, 2014). Suberoylanilide hydroxamic acid (SAHA) was approved by the Food & Drug Administration under the commercial name Zolinza in 2006 for the treatment of cutaneous T-cell lymphoma (2015, Dec 29). This approval came despite some setbacks such as broad spectrum effects, insolubility in water, a half-life of approximately 1.5 hours in patients receiving a 400 mg dose, and some serious side effects such as fatigue, gastrointestinal issues, hematologic complications, and cardiotoxicity (Daniel et al., 2015). It is unsure if SAHA works by epigenetic modifications or another mechanism (Fischer, Sananbenesi, Mungenast, & Tsai, 2010), but it has been found to cross the blood-brain barrier (Hockly, *et. al*, 2003). Non-selective HDAC inhibitors such as SAHA, phenylbutyrate, and sodium butyrate can be effective in *Drosophila* and mice models of Huntington's Disease, but are limited for clinical use by high toxicity levels (Jia, *et. al*, 2012).

Prodrugs often create a safer delivery method of a compound, as they transform into their activated structure once delivered. Sulfenamides, characterized by the sulfur-nitrogen bond, are good candidates for prodrugs because the sulfenamide bond would be cleaved *in vivo* by free thiols such as cysteine or glutathione (Guarino, Karunaratne, & Stella, 2007). By including a sulfenamide bond in an analog of SAHA, it is hoped that

toxicity levels would decrease. The short half-life of SAHA seems to be due to the hydroxamate chelating group, which is also less than optimal as a coordinator of enzymatic zinc (Gu, Nusinzon, Smith, Horvath, & Silverman, 2006). By modifying the hydroxamate group to a thiol derivative, it is expected that the coordination would be much higher, acting more strongly on the HDAC (Go, Chandler, & Jones, 2015). If the drug is more strongly chelated on the zinc ion of the HDAC, a smaller percentage of the drug is available to be metabolized and the half-life would increase. An increased half-life would be very beneficial for clinical useage.

Other groups have realized these flaws in SAHA and synthesized derivatives to attempt to circumvent the faults. Suzuki, *et. al* synthesized a series of non-hydroxamate derivatives of SAHA that had good rates of inhibition but were less effective than SAHA (2005). Gu, Nusinzon, Smith, Horvath, and Silverman created a library of twelve sulfur analogs of SAHA (2006). None of the twelve compounds were an exact match for AMAO, but the work showed that sulfur-containing groups were more successful at chelating zinc than the hydroxamate groups (Gu, Nusinzon, Smith, Horvath, & Silverman, 2006).

The proposed synthesis is unlike any that are currently found in literature. Methodology was developed by finding works in the literature that are similar to each individual step of the proposed synthesis. The first step of the proposed synthesis involves adding carbon disulfide to sodium trimethylsilanolate. The carbon disulfide group would provide a sulfur atom from which the desired compound will be built. The sodium trimethylsilanolate will serve as a protecting group which would get cleaved off at the end of the synthesis. Cleavage of the carbon-oxygen bond in the trimethyl silyl group

will yield the prodrug, which would have the sulfur-nitrogen bond cleaved *in vivo* to produce the activated drug. The protecting group cleavage may be challenging because Craine and Raban describe that sulfenamide bonds are acid-labile and potentially thermally-labile (1989). While adventitious acid can be used to cleave off the trimethylsilyl protecting group (Denmark, & Bui, 2005), Pilcher, Hill, Shimshock, Waltermire, and DeShong recommend fluorosilicic acid as a desilyating agent because it requires less acidic conditions (1992). Less acidic conditions would be beneficial because the sulfenamide bond would hopefully be less favorable to be cleaved than the carbon-oxygen bond of the trimethylsilyl group.

Adding carbon disulfide to the sodium trimethylsilanolate would create a xanthate product (RO-CSS-M^{+1}), the preparation of which is readily available. Barton, Parekh, and Tse produce a tertiary xanthate by adding carbon disulfide to a deprotonated tertiary alcohol and stabilizing by methylation with iodomethane (1993). Davy, Mason, Moreau, and Wulff also describe this reaction with the same results (2012). Carta, Akdemir, Scozzafava, Masini, and Supuran describe the attack of carbon disulfide on methanol, resulting in a pale yellow solid (2013). The parallel reaction in our synthesis also results in a pale yellow solid (see ADDITION OF CS_2 (RUN #2) below). The purification process of xanthate products has been developed (Friebolin, Schilling, Zoller, & Amtmann, 2004). However, dithiolates are hygroscopic and difficult to purify. In most cases, it is best to carry out a reaction without purifying the dithiolate product (Jensen & Henriksen, 1968). Due to hygroscopicity, our reactions are carried out in a nitrogen gas environment and not purified before the next reaction of the synthesis.

Dimethylformamide has been found to increase the rate of breakdown of xanthates (Millican & Sauers, 1979); therefore, its use will be avoided throughout the synthesis. The realization of the detrimental effects of dimethylformamide raised concerns about the validity of samples from the work of Brandon Hamm. These samples were prepared in dimethylformamide and had been stored in trace amounts of dimethylformamide for several years.

The second reaction of the proposed synthesis is to attack one of the sulfur atoms with an amino group to create the sulfur-nitrogen, or sulfenamide, bond. Analysis of the resulting compounds will be challenging because there is no characteristic IR peak for the molecular sulfenamide bond (Hassanzadeh & Andrews, 1992). Heimer and Field confirmed this when the Raman spectra lacked counterparts for the common bands of sulfenamides in the infrared spectra (1970). Davis, Slegeir, Evans, Schwartz, Goff, and Palmer found describe the IR peak of an amino group in a sulfur-nitrogen bond, which is in the typical amino group region (1973).

S-amination of thioureas was accomplished using aqueous alkali hypochlorite in the presence of secondary or cyclic primary amines (Ley, & Eholzer, 1966). The alkali hypochlorite reacts with the amines *in situ* to form chloramines, which attack the sulfur as an aminating agent. The product of the addition of ammonia and hypochlorite depends upon the pH of the solution, with monochloramine being produced at a pH above 8 (Colton & Jones, 1955). Barton, Hesse, O'Sullivan, and Pechet used chloramide under basic conditions in a nonpolar medium to aminate a thiol to produce a sulfenamide bond (1991). Smith, Alliger, Carr, and Young use in situ production of monochloramine to

aminate a diotholate (1949). Our reaction of monochloramine with the xanthate product is modeled after the work of Viswanadhan, et. al (1991), which was the first amination method attempted in our synthesis. Due to apparent lack of success, other amination methods were pursued.

Numerous papers describe the reaction of hydroxylamine-*O*-sulfonic acid to produce an aminated product in various ratios of reactants (Brown, Heydkamp, Breuer, & Murphy, 1964; Campbell & Rees, 1969; Raap, 1969; Rathke, Inoue, Varma, & Brown, 1966; Sisler, Bafford, Omietanski, Rudner, & Drago, 1958). Since hydroxylamine-*O*-sulfonic acid can decompose in water, several nonaqueous buffer systems were proposed to protect the sulfenamide bond from acid cleavage (Matysik, 2000; Sanders, Burka, Shelby, Newbold, & Cunningham, 1997; Stauffer & Weber, 1999). These buffer systems were not viable choices for the amination reaction due to activated nitrogen atoms within the solvent molecules.

Through a modification of Zinner's method, *O*-mesitylenesulfonylhydroxylamine was prepared (Tamura, Minamikawa, Sumoto, Fujii, & Ikeda, 1973). As a result of its relative stability and solubility in organic solvents, some recommend this compound as a convenient reagent for general amination (Tamura, Minamikawa, Sumoto, Fujii, & Ikeda, 1973). Although this research group states that the compound is stable at room temperature (Tamura, Minamikawa, & Ikeda, 1977), other publications stated concerns of overnight decomposition at ambient temperatures (Mendiola, et. al, 2009; Carpino, 1960). While this amination agent was very enticing, it was ruled out due to safety concerns.

A stronger amination agent was thought to be found in of *O*-(trifluoromethansulfonyl)hydroxylamine, but the only publication found describing its preparation raised many concerns (Kellner & Bliepert, 1978). The synthesis was said to be conducted with methanol as a solvent, but this would result in the strong aminating agent vigorously reacting with the activated oxygen. The data presented in the work of Ganswein did not offer definitive proof of the synthesis of *O*-(trifluoromethansulfonyl)hydroxylamine with many concerns remaining (1977). The work of Shiba and Iwadate involves synthesis of *O*-trifluoromethanesulfonic hydroxylamine, $F_3CSO_3NH_2$, but with an ionic bond between the nitrogen and oxygen rather than a covalent bond (2011).

Yet another aminating agent is prepared in the work of Andreae and Schmitz who were able to produce sulfenamide bonds in high yields through amination with oxaziridine (1991). This success was dependent upon reactivity of the substrate and stability of the sulfenamide bond. With all the challenges of finding an appropriate aminating agent, another strategy was considered to move the reaction forward. Microwave-assisted organic synthesis is a promising technique that can be employed on compounds with a dipole moment to quicken reactions using extreme conditions (Listrom, Tierney, Wathey, & Westman, 2001). Successful acylation of amines is described by Petricci, Maugnaini, Radi, Corelli, and Botta using a microwave instrument, which indicates that amination of the xanthate group could be successful (2004).

After the successful production of a xanthate product, many challenges have presented themselves in the face of the amination reaction. Many ideas and techniques

have been considered across the discipline of organic chemistry but a solution has yet to be found. When a successful amination is conducted to yield a stable sulfenamide product, the results will offer significant contributions to the field of synthetic organic chemistry.

EXPERIMENTAL

All procedures were completed in a lab hood. All drying tubes were packed with Drierite (anhydrous $CaSO_4$). All infrared (IR) spectrum were created on a Perkin Elmer Spectrum Two IR Spectrometer. In reference to thin layer chromatography (TLC), each Rf value is representative of a single spot on a plate.

ADDITION OF CS_2 (RUN #1)

The reaction apparatus was a 200 mL, three-neck, round-bottom flask equipped with a dropping funnel, drying tube, ground glass stopper, and magnetic stir bar. Sodium trimethylsilanolate (50 mL of 1.0 M or 50 mmol, Aldrich 335738) in tetrahydrofuran (THF) and 15-crown-5-ether (0.0128 g or 0.058 mmol, Aldrich 188832-1G) were placed in the flask. Carbon disulfide (5.711 g of ≥99% or 75 mmol, Aldrich 335266) was diluted to 50 mL with 99.9% THF (Aldrich 301757-1L) and placed in the dropping funnel. The mixture in the reaction flask was clear golden yellow before dropping began. The carbon disulfide solution was added dropwise at room temperature to the flask for 102 minutes with stirring. The solution immediately became cloudy upon addition of CS_2, indicating the presence of a precipitate. As dropping continued, the solution became cloudier and gradually turned orange. At the end of the addition, the reaction mixture was a bright orange homogeneous thick paste.

The reaction mixture was then filtered and the solid was saved (HD-2-6-1). The filtrate was refluxed in the reaction flask for sixty minutes, after which the mixture was again vacuum filtered. The solid caught on the filter was saved (HD-2-6-3) as well as the filtrate (HD-2-6-2). Both filtrates were transparent golden yellow. Both solid products

were multicolor (yellow and bright orange) and laid on watch glasses to dry. After some time on the watch glasses, both products became a uniform darker red-orange color and were moist when touched with a spatula. Upon this realization, the solids were transferred to tightly capped vials.

HD-2-6-1 weighed 6.12 grams and HD-2-6-3 weighed 0.063 grams. These combined weights gave a 64.99% yield. An IR spectrum of HD-2-6-1 was generated (see APPENDIX A – *HD-2-IR-1*). An IR spectrum was also generated from sodium trimethylsilanolate (see APPENDIX A – *HD-2-IR-2*).

AMINATION OF XANTHATE PRODUCT VIA CLOROX/AMMONIA

Molar ratios of reactants were based on the work of Viswandhan (1991). NH_4OH (11.73 mL of 29% or 88.3 mmol) and 103 mL of deionized (DI) water were placed in a 250 mL Erlenmeyer flask in an ice bath with stirring to cool to 0°C. “Clorox” (28 mL of 8.25% NaOCl or 34.40 mmol, commercial product) and 16.4 mL of DI water were placed in a separate 250 mL Erlenmeyer flask in an ice bath with stirring to cool to 0°C. When both solutions were chilled, they were combined in one of the flasks on ice and stirring for 15 minutes. The first five of these minutes were used to adjust the pH from ≥13 down to 8 using a large amount of 3.0 M HCl (approximately 50 mL).

HD-2-6-1 (5.4 g or 28.67 mmol) was dissolved in 14.3 mL of DI water and placed in a 250 mL, round-bottom flask with an ice bath and stirring. When HD-2-6-1 was dissolved in water, the solution was very dark orange, nearly red. After the $NaOCl/NH_3$(aqueous) solution had chilled for 15 minutes, it was placed in a dropping funnel (excess solution kept on ice). The solution was added dropwise to the reaction

flask over ten minutes. By halfway through the addition, the reaction mixture was still cloudy and bright yellow but had several orange chunks floating around. The reaction mixture was stirring and on ice through the entire addition.

After the addition, the reaction flask was stoppered and removed from the ice bath. It was allowed to warm to room temperature with stirring over 45 minutes. The reaction flask was unstoppered and placed in a 37-45°C water bath for 20 minutes with stirring. The solution became a paler yellow and the solid became a clumpy/sticky solid stuck on the bottom of the flask with the coloring of peanut butter. The reaction flask was stoppered and left stirring at room temperature overnight, after which the solution was bright transparent yellow. The solid was a cream color and stuck to the bottom and sides of the flask.

The mixture in the reaction flask was placed in a 500 mL separatory funnel with 40 mL of ethyl acetate (BDH-1123-4LP). An extraction was performed to separate the organic and aqueous layers. The aqueous layer was extracted four more times with additional 40 mL aliquots of ethyl acetate. During the extractions, a small amount of solid was caught at the interface of the organic and aqueous layers. The solid was removed from the organic layer by vacuum filtration and IR analysis showed no major peaks (see APPENDIX A *– HD-2-IR-3*). The aqueous layer was saturated with sodium chloride. Three additional extractions took place with 40 mL aliquots of ethyl acetate for a total of eight extractions. The combined organic layers had taken on most of the yellow coloring throughout the extractions and were saved (HD-2-14-2).

The organic layer was dried with anhydrous sodium sulfate, which was then removed by gravity filtration. The filtrate was placed on the rotary evaporator at 55°C, resulting in a clear distillate and a yellow solid that was capped under N_2 gas (HD-2-14-4). IR analysis was conducted on HD-2-14-4 (see APPENDIX A – *HD-2-IR-4*). HD-2-14-4 was found to weigh 0.43 grams, giving 8.3% yield.

BENZOYLATION OF SULFENAMIDE PRODUCT VIA BENZOYL CHLORIDE (RUN #1)

HD-2-14-4 (0.43 g or 2.37 mmol) was dissolved in 12 mL of toluene and transferred to a 50 mL, three-neck, round-bottom flask equipped with a stir bar, drying tube, dropping funnel, and stopper. With stirring, pyridine (0.2269 g or 2.84 mmol, Aldrich 36,057-0) was added to the reaction flask. In a 25 mL Erlenmeyer flask, benzoyl chloride (0.3366 g or 2.37 mmol, Aldrich 25,995-0) was swirled with 5 mL of toluene and then placed in the dropping funnel.

Before the addition, the reaction mixture was transparent bright yellow and the solution in the dropping funnel was clear. The benzoyl chloride solution was added dropwise with stirring to the reaction flask over ten minutes and left stirring for 45 minutes. No immediate changes were observed during addition. Within ten minutes of stirring at room temperature, the solution started to become cloudy, and a white, pasty solid began to deposit on the inside surfaces of the flask. The reaction mixture was heated in an oil bath at 60°C for thirty minutes with stirring. The reaction mixture was left stirring at room temperature overnight. After overnight stirring, the solution was transparent yellow with a build-up of grayish-white solid on the inside surfaces.

The reaction mixture was separated by gravity filtration. Trapped solid was laid out on filter paper to dry. While the solvent quickly evaporated off, the solid became more wet and turned into a paste. This led to capping the solid under N_2 gas (HD-2-18-2). The filtrate was placed on the rotary evaporator at 70°C resulting in a bright yellow solid with a bit of oil.

This solid/oil mixture was dissolved in 15 mL of DI water and 15 mL of ethyl acetate (BDH 1123-4LP). This mixture was placed in a 50 mL separatory funnel. The aqueous layer was extracted with three more aliquots of ethyl acetate. The clear aqueous layer was saved (HD-2-19-1) while the cloudy bright yellow organic layer underwent gravity filtration. The solid caught by the filter was saved (HD-2-19-2) with a light yellow appearance and mass of 0.051 grams. The filtrate was dried with anhydrous sodium sulfate. After filtering out the sodium sulfate, the filtrate from the organic layer was placed on the rotary evaporator at 45°C. This resulted in a darker yellow solid with clinging liquid which was capped under N_2 gas after evaporation (HD-2-19-3).

To remove the lingering liquid, a recrystallization of HD-2-19-3 was necessary. The contents of the flask were dissolved in toluene in a 70°C water bath. It was then stoppered at room temperature overnight. No crystals were formed, and the stoppered flask was placed in an ice bath, upon which crystals still did not form. The flask was placed on the rotary evaporator at 75°C until a few crystals appeared. Using the hot water bath at 70°C, toluene was added dropwise until all crystals dissolved. The flask was stoppered and left at room temperature. After three hours, crystals were evident. Both crystals and solution were golden yellow. The amount of crystals was small enough that

no attempt was made to obtain a mass to calculate percent yield. An IR spectrum was generated of the crystals in which toluene contamination was likely (see APPENDIX A – *HD-2-IR-5*).

BENZOYLATION OF SULFENAMIDE PRODUCT VIA BENZOYL CHLORIDE (RUN #2)

HD-1-90-2 (0.43 g or 2.37 mmol) was dissolved in 14 mL of toluene and placed in a 50 mL, three-neck, round-bottom flask equipped with stir bar, condenser, dropping funnel, and drying tube. The flask was submerged in an oil bath heated to 90-95°C. Pyridine (0.2269 g of 99%+ or 2.84 mmol, Aldrich 36,057-0) was also added to the reaction vessel. Benzoyl chloride (0.3366 g or 2.37 mmol, Aldrich 25,995-0) was mixed with 5 mL of toluene in a 25 mL Erlenmeyer flask, swirled for several moments, and then placed in the dropping funnel. Before addition, the reaction mixture was transparent with sticky, undissolved, tan/brown solid stuck to the bottom of the flask. Once the oil bath reached 90°C, the benzoyl chloride solution was added dropwise to the reaction vessel with stirring over a period of 13 minutes. After the addition, the reaction mixture was transparent dark rusty orange with very dark or black solid on the bottom. The reaction vessel continued to stir at 90-95°C for 30 minutes. Ten minutes into the continued heated stirring, the reaction mixture became cloudy and developed a dark red-brown color. The heat source was removed and the condenser and dropping funnel were replaced with stoppers. The flask was left stirring overnight.

The flask contained a solution that was decanted from the solid. This solution was placed on the rotary evaporator at 70-75°C. The solution became cloudy pale orange while a white precipitate formed as a result of the heating. Then both solution and solid

became golden yellow. As the solvent evaporated off, a thick brown liquid with a bit of solid was left in the flask. The distillate was slightly cloudy and saved (HD-2-26-2).

The solid from the rotary evaporator and the solid from the reaction vessel were dissolved in 15 mL of DI water and 15 mL of ethyl acetate. The aqueous layer was extracted a total of four times with 15 mL aliquots of ethyl acetate and remained clear. Sodium chloride was added to the aqueous layer until saturation before the third extraction to prevent emulsion. The combined organic extracts were transparent rusty orange, dried with anhydrous sodium sulfate, and filtered. The filtrate was placed on the rotary evaporator at 45-50°C, resulting in a distillate tinged yellow (HD-2-27-1) and a thick dark brown liquid that became solid upon cooling to room temperature (HD-2-27-2).

An IR spectrum was generated of HD-2-27-2 (see APPENDIX A – ***HD-2-IR-7***). Recrystallization was attempted with HD-2-27-2 with cyclohexane. After 10 mL of cyclohexane had been added in a 55°C, the solution was orange/peach-colored and contained a sticky black solid that would not dissolve. The solution was decanted off (HD-2-29-1) and the solid was set aside (HD-2-29-2). Recrystallization was attempted with toluene on HD-2-29-2. This resulted in a dark orange-brown solution with a sticky tar-like black solid (HD-2-29-3) that would not dissolve and clung to the spatula when attempted to break up. The solution was decanted off and placed on the rotary evaporator at 75°C. This resulted in a pink/peach-colored distillate (HD-2-30-1) and a dark solid with some brown liquid (HD-2-30-2). Methanol was added to HD-2-30-2 and HD-2-29-3, both of which dissolved in the methanol mixture. This was placed on the rotary evaporator at

45°C, yielding a clear distillate and a very dark brown substance that appeared wet and gel-like but was immobile (HD-2-30-3). An IR spectrum was generated of HD-2-30-3 (see APPENDIX A – *HD-2-IR-8*).

HD-2-29-1 was placed on the rotary evaporator at 55°C and recrystallization was attempted again with cyclohexane. A considerable amount of golden yellow solid appeared in the transparent golden yellow solution (HD-2-32-1). An IR was generated of HD-2-32-1 by pulling crystals out of the solution with a microspatula and then allowing the cyclohexane to volatilize before placing it on the diamond of the spectrophotometer (see APPENDIX A – *HD-2-IR-10*).

HD-2-32-1 went through vacuum filtration and rinsing with cyclohexane in a Hirsch funnel at room temperature. The filter held a fluffy yellow gold crystal that was immediately placed in a capped vial (HD-2-34-1). The filtrate was colorless and clear (HD-2-34-3). An IR spectrum was generated of HD-2-34-1 (see APPENDIX A – *HD-2-IR-11*).

TLC was used to analyze the success of the reaction. With an eluent of 20% cyclohexane/80% ethyl acetate, HD-1-90-2 gave Rf values of zero, 0.033, and 0.538 while HD-2-34-1 gave Rf values of 0.764 and zero. A second plate with 100% ethyl acetate as the eluent showed HD-1-90-2 with Rf values of 0.639, 0.124, 0.089, 0.005, and zero while HD-2-34-1 had Rf values of 0.823 and zero.

BENZOYLATION OF SULFENAMIDE PRODUCT VIA BENZOYL CHLORIDE (RUN #3)

HD-1-90-2 (0.43 g or 2.37 mmol) was dissolved in 16 mL of *o*-xylene in a 50 mL, three-neck, round-bottom flask equipped with stir bar, condenser, dropping funnel, and drying tube in an oil bath heated to 120°C. Pyridine (0.2269 g or 2.84 mmol, Aldrich

36,057-0) was also added to the reaction vessel. Benzoyl chloride (0.3366 g or 2.37 mmol, Aldrich 25,995-0) was placed in a 25 mL Erlenmeyer flask with 5 mL of *o*-xylene, swirled for several moments, and placed in the dropping funnel. Before addition, the reaction vessel contained an orange solution and clumpy red solid. Once the oil bath had reached 120°C, the benzoyl chloride solution was added dropwise with stirring to the reaction mixture over 7 minutes. During addition, the reaction vessel mixture darkened from orange to dark brown. Dark solid deposited on the bottom half of the flask. Refluxing deposited a white solid above the solution line in the flask. The reaction vessel was left stirring on the heat for 30 minutes. Then the flask was removed from the heat, stoppered, and left stirring at room temperature overnight. After overnight stirring, a similar appearance of a transparent orange-red solution with black precipitate in the solution and yellow precipitate above the solution remained in the reaction flask.

The solution was decanted from the deposited solid (HD-2-39-1) in the reaction flask and placed on the rotary evaporator at 95°C. The yellow tinged distillate (HD-2-39-2) and thick dark brown liquid (HD-2-39-3) were saved. HD-2-39-1 was washed with cyclohexane at 55°C to remove desired product from the undesired material. The remaining dark brown-red solid was saved (HD-2-39-4) after the cyclohexane was decanted into HD-2-39-3. Ethyl acetate (10 mL) was also added to HD-2-39-3. The combined organic solvents were placed in a 50 mL separatory funnel and washed four times with 10 mL aliquots of DI water. The aqueous layers were transparent and pale yellow (HD-2-40-1) and the combined organic layers were transparent vibrant red-

orange. The organic layer was placed on the rotary evaporator at 100°C, resulting in a dark copper-colored distillate and a dark red-brown liquid (HD-2-40-2).

PURIFICATION OF SULFENAMIDE PRODUCT

HD-1-90-2 and HD-1-90-3 were dissolved in 75 mL of ethyl acetate and 15 mL of DI water. This mixture was placed in a 500 mL separatory funnel. Undissolved sandy-tan solid was removed by gravity filtration and saved (HD-2-42-1). The cloudy golden yellow organic layer was washed with four aliquots of DI water, dried with anhydrous sodium sulfate, filtered, and placed on the rotary evaporator at 40°C. This left a distillate (HD-2-43-3) and an orange-yellow solid (HD-2-43-4). This solid was found to be slightly moist when pressed with a spatula. An IR was generated of HD-2-43-4 (see APPENDIX A – *HD-2-IR-12*).

BENZOYLATION OF SULFENAMIDE PRODUCT VIA 3,5-DINITROBENZOYL CHLORIDE (RUN #1)

Preparations for this reaction were conducted in an Atmosbag filled with N_2 gas. HD-2-43-4 (0.196 g or 1.081 mmol) was placed in a 200 mL, three-neck, round-bottom flask equipped with stir bar, condenser connected to a drying tube, stoppered dropping funnel, and a ground glass stopper. Approximately half of the solid dissolved when 12 mL of *o*-dichlorobenzene was added to the flask. Pyridine (0.1297 g or 1.622 mmol, Aldrich 36,057-0) was also added to the reaction flask. 3,5-Dinitrobenzoyl chloride (0.249 g or 1.081 mmol, Aldrich 156272-25G) was dissolved in 6 mL of *o*-dichlorobenzene in a 25 mL Erlenmeyer flask, placed in the dropping funnel, and stoppered. The reaction apparatus was completely sealed, removed from the Atmosbag, and placed in an oil bath heated to

90°C. Upon heating all solid dissolved in the reaction mixture, leaving a transparent golden yellow solution. The 3,5-dinitrobenzoyl chloride solution was added dropwise to the reaction mixture over 23 minutes. An orange precipitate formed during the addition as well as the development of cloudiness. The contents of the reaction flask remained stirring on the heat for 20 additional minutes, then left stirring at room temperature overnight. Upon removal from the heat, the reaction vessel had a transparent orange solution with some solid deposited in the bottom. After stirring overnight, the reaction vessel contained a transparent orange solution with many small particles of off-white solid.

TLC is used to analyze the presence of reactants in the reaction mixture. With an eluent of 50% cyclohexane/50% ethyl acetate, HD-2-43-4 gave Rf values of 0.959, 0.885, 0.804, 0.374, 0.135, 0.068, and zero; *o*-dichlorobenzene gave Rf values of 0.284 and 0.201; and the reaction mixture gave Rf values of 0.948, 0.073, and zero. The same compounds were run on another plate with an eluent of 100% ethyl acetate to analyze spots with a higher Rf value than 0.900; by changing the solvent, these spots were able to be analyzed in a clear region on the plate. HD-2-43-4 gave Rf values of 0.825 and zero; *o*-dichlorobenzene gave one spot of Rf value zero; and the reaction mixture gave Rf values 0.815 and zero.

The solution from the reaction vessel was poured on a large watch glass to volatilize off the *o*-dichlorobenzene. This left a yellow film which was collected (0.45 g) and dissolved in toluene at 70°C in a 50 mL Erlenmeyer flask for recrystallization. A tan solid would not dissolve and was filtered out (HD-2-51-2). An IR spectrum was generated

from HD-2-51-2 (see APPENDIX A – *HD-2-IR-14*). It was placed on a watch glass and weighed 0.13 grams. The transparent golden yellow filtrate was placed on the rotary evaporator at 70°C yielding a clear distillate and pale yellow solid (HD-2-51-4). An IR spectrum was generated from HD-2-51-4 (see APPENDIX A – *HD-2-IR-15*).

Further TLC testing was conducted to analyze the presence of sodium trimethylsilanolate in the reaction mixture. An eluent of 25% cyclohexane/75% ethyl acetate was used. Sodium trimethylsilanolate gave one spot at the origin; HD-2-43-4 gave Rf values of 0.982, 0.895, 0.528, 0.232, and zero; 3,5-dinitrobenzoyl chloride gave one smear of 0.257 Rf value; and the reaction mixture gave Rf values of 0.986, 0.911, 0.823, 0.523, 0.118, and a clear origin.

ADDITION OF CS_2 (RUN #2)

Carbon disulfide (3.807 g of ≥99% or 50 mmol, Aldrich 335266) was diluted to 50 mL using THF (Aldrich 401757-1L) and placed in a capped dropping funnel. The reaction apparatus of a 200 mL, three-neck, round-bottom flask equipped with magnetic stir bar, drying tube, dropping funnel containing CS_2 solution, and venting hose was placed in an Atmosbag. The Atmosbag was sealed and a N_2 gas environment was established. Sodium trimethylsilanolate in THF (50 mL of 1.0 M or 50 mmol, Aldrich 335738) and 15-crown-5-ether (0.013 g or 0.058 mmol, Aldrich 188832-1G) were placed in the reaction flask. Before addition, the reaction mixture was transparent pale yellow. The carbon disulfide solution was added dropwise with stirring to the reaction flask over 75 minutes. After the addition, the reaction mixture was creamy pastel orange, stirred for an additional sixty minutes, and then separated by gravity filtration. The clear pale yellow filtrate was saved

(HD-2-56-1) and the filter paper was spread out on a watch glass to dry. The filter paper had a large amount of pastel orange solid that became yellow as it dried in the Atmosbag. The solid weighed 6.670 grams, giving 70.8% yield. The solid was placed in a tightly capped vial (HD-2-57-3) before opening the Atmosbag. A small sample of the solid was placed on a watch glass (HD-2-57-2) for exposure to the atmosphere. At this point, the Atmosbag was unsealed, upon which HD-2-57-2 was yellow. After 15 minutes of exposure, the edges of the crystals were turning orange. After an additional 15 minutes of exposure, the entire crystals were orange and appeared wet.

HD-2-57-3 (a dry sample) was used to generate an IR spectrum (see APPENDIX A – *HD-2-IR-16*). HD-2-57-2 (a wet sample from exposure) was used to generate another IR spectrum (see APPENDIX A – *HD-2-IR-17*).

A TLC plate was run with 50% ethyl acetate/50% acetone. 15-crown-5-ether gave one spot with 0.507 Rf value; sodium trimethylsilanolate gave one spot at the origin; and HD-2-57-2 (wet sample from exposure) gave Rf values of 0.741, 0.429, 0.311, and zero.

AMINATION OF XANTHATE PRODUCT VIA HYDROXYLAMINE-O-SULFONIC ACID

A 75 mL solution of saturated sodium bicarbonate (Fisher S233) was made using DI water. This solution was used to dissolve HD-2-57-3 (2.979 g or 15.8 mmol), requiring approximately 25 mL of the saturated solution. This mixture was contained in a 200 mL, three-neck, round-bottom flask equipped with magnetic stir bar, condenser, dropping funnel, and stopper. This reaction apparatus was placed on an oil bath heated to 60-65°C. The reaction flask contained a dark vibrant orange solution. Hydroxylamine-*O*-sulfonic acid (2.144 g or 18.96 mmol, Aldrich 480975-5G) was dissolved in 50 mL of DI water and

stored on an ice bath. The hydroxylamine-*O*-sulfonic acid solution was added to the dropping funnel in small increments to keep it on ice as long as possible. Addition took place dropwise with stirring over 1 hour and 55 minutes. Acidity of the reaction mixture was checked frequently and maintained a pH of 8 without any adjustments being made. During the addition, the solution became gradually lighter and cloudier. After the addition, the reaction flask was removed from the heat. Some cream-colored solid had formed on the bottom of the flask (HD-2-60-1) inside of the pale transparent yellow solution. At this point, TLC was attempted to determine the success of the reaction. All results were inconclusive due to the use of water as a solvent.

HD-2-60-1 was extracted with three 20 mL aliquots of ethyl acetate in a separatory funnel. The transparent yellow ethyl acetate layers were saved (HD-2-61-1) and then three 20 mL aliquots of dichloromethane were used to extract the aqueous layer. The cloudy dirty yellow aqueous layer (HD-2-61-3) and combined transparent dirty yellow dichloromethane layers (HD-2-61-2) were saved. HD-2-61-2 was dried with anhydrous sodium sulfate, filtered, and put on the rotary evaporator at 30-35°C. The pale yellow solid was saved (HD-2-61-4) and used to generate an IR spectrum (see APPENDIX A – ***HD-2-IR-20***). HD-2-61-1 was dried with anhydrous sodium sulfate, filtered, and placed on the rotary evaporator at 40-45°C to yield a yellow solid that was saved (HD-2-61-5) and used to generate an IR spectrum (see APPENDIX A – ***HD-2-IR-21***). An IR spectrum was also generated from hydroxylamine-*O*-sulfonic acid (see APPENDIX A – ***HD-2-IR-22***).

BENZOYLATION OF SULFENAMIDE PRODUCT VIA 3,5-DINITROBENZOYL CHLORIDE (RUN #2)

HD-2-61-4 (0.208 g or 1.15 mmol) was dissolved in 14 mL of *o*-dichlorobenzene in a 50 mL, three-neck, round-bottom flask equipped with magnetic stir bar, condenser attached to a drying tube, dropping funnel, and stopper. Pyridine (0.137 g or 1.725 mmol, Aldrich 36,057-0) was also added to the reaction flask, which was then heated to 90°C in an oil bath. The reaction mixture was transparent pale yellow. 3,5-Dinitrobenzoyl chloride (0.265 g or 1.15 mmol) was dissolved in 6 mL of *o*-dichlorobenzene and placed in the dropping funnel. Addition with stirring took place dropwise over 25 minutes with no change in appearance. The reaction flask was removed from the heat after the addition. A TLC plate ran with 50% cyclohexane/50% ethyl acetate resulted in HD-2-61-4 giving Rf values of 0.988 and 0.911; 3,5-dinitrobenzoyl chloride giving Rf values of 0.480 and zero; pyridine giving an Rf value of 0.493; and the reaction mixture giving Rf values of 0.962, 0.915, 0.402, and zero. A second TLC was run with 100% cyclohexane resulting in HD-2-61-4 giving Rf values of 0.905 and 0.812; 3,5-dinitrobenzoyl chloride giving a spot at the origin; pyridine giving an Rf value of 0.159; and the reaction mixture giving Rf values of 0.895, 0.783, and zero. Since this data showed evidence of reactant in the product, further heating was applied to the reaction mixture.

The reaction vessel was heated at 90°C for an additional 20 minutes. The reaction mixture was analyzed using TLC. A TLC plate ran with 50% cyclohexane/50% ethyl acetate resulted in HD-2-61-4 giving Rf values of 0.945 and 0.877; 3,5-dinitrobenzoyl chloride giving Rf values of 0.877, 0.557, and 0.346; pyridine giving Rf values of 0.868 and 0.445;

and the reaction mixture giving Rf values of 0.932, 0.861, 0.315, and zero. A second TLC was run with 100% cyclohexane resulting in HD-2-61-4 giving Rf values of 0.932, 0.812, and 0.644; 3,5-dinitrobenzoyl chloride giving Rf values of 0.869 and 0.083; pyridine giving Rf values of 0.847 and 0.087; and the reaction mixture giving Rf values of 0.957, 0.845, and zero. Since this data showed evidence of reactant in the product, further heating was applied to the reaction mixture.

The reaction mixture sat overnight with no stirring, after which the reaction mixture was transparent pale yellow with a small amount of solid formed on the bottom. The reaction mixture was heated at 120°C for 15 minutes and then analyzed using TLC. A TLC plate ran with 50% cyclohexane/50% ethyl acetate resulted in HD-2-61-4 giving an Rf value of 0.994; 3,5-dinitrobenzoyl chloride giving Rf values of 0.592 and zero; and the reaction mixture giving Rf values of 0.975, 0.404, 0.211, and zero. A second TLC was run with 100% cyclohexane resulting in HD-2-61-4 giving an Rf value of 0.955; 3,5-dinitrobenzoyl chloride giving a spot at the origin; and the reaction mixture giving Rf values of 0.920, and zero. Since this data showed evidence of reactant in the product, further heating was applied to the reaction mixture.

The reaction mixture was heated at 150°C for 15 minutes and analyzed using TLC. A TLC plate ran with 50% cyclohexane/50% ethyl acetate resulted in HD-2-61-4 giving an Rf value of 0.943; 3,5-dinitrobenzoyl chloride giving Rf values of 0.547 and zero; and the reaction mixture giving Rf values of 0.943, 0.399, 0.223, and zero. A second TLC was run with 100% cyclohexane resulting in HD-2-61-4 giving an Rf value of 0.866; 3,5-dinitrobenzoyl chloride giving a spot at the origin; and the reaction mixture giving Rf

values of 0.869 and zero. Since this data showed evidence of reactant in the product, further heating was applied to the reaction mixture.

The reaction mixture was refluxed at 180°C for 30 minutes and then analyzed using TLC. A small amount of reaction solution was placed on a watch glass to volatilize the solvent and create a solid (HD-2-68-1). The solution was a darker transparent yellow with miniscule amounts of solid, mostly above the solution. Upon cooling, white crystals formed on the bottom of the vessel. A TLC plate ran with 50% cyclohexane/50% ethyl acetate resulted in HD-2-61-4 giving an Rf value of 0.961; 3,5-dinitrobenzoyl chloride giving Rf values of 0.409 and zero; and the reaction mixture giving Rf values of 0.931, 0.249, 0.157, and zero. A second TLC was run with 100% cyclohexane resulting in HD-2-61-4 giving an Rf value of 0.892; 3,5-dinitrobenzoyl chloride giving a spot at the origin; and the reaction mixture giving Rf values of 0.887 and zero. This data showed evidence of reactant in the product, but further heating was not applied to the reaction mixture.

The small amount of reaction solution on a watch glass volatilized overnight to give a white film of solid (HD-2-68-1). A small amount of this solid was dissolved in 50% cyclohexane and 50% ethyl acetate to analyze by TLC. A TLC plate ran with 50% cyclohexane/50% ethyl acetate resulted in HD-2-61-4 giving an Rf value of 0.886; 3,5-dinitrobenzoyl chloride giving Rf values of 0.357 and zero; the reaction mixture giving Rf values of 0.867 and 0.151; and HD-2-68-1 gave Rf values of 0.845 and 0.149. A second TLC was run with 100% cyclohexane resulting in HD-2-61-4 giving an Rf value of 0.756; 3,5-dinitrobenzoyl chloride giving a spot at the origin; the reaction mixture giving Rf values of 0.821, and zero; and HD-2-68-1 giving Rf values of 0.811, 0.732, and zero.

HD-2-68-1 was used to generate an IR spectrum (see APPENDIX A – ***HD-2-IR-23***). 3,5-Dinitrobenzoyl chloride (Aldrich 156272-25G) was used to generate another IR spectrum (see APPENDIX A – ***HD-2-IR-24***).

BENZOYLATION OF SULFENAMIDE PRODUCT WITH 3,5-DINITROBENZOYL CHLORIDE VIA MICROWAVE PROMOTION

HD-2-61-5 (0.039 g or 0.215 mmol) was placed in a 0.5-2.0 mL microwave conical vial. Pyridine (0.017 g or 0.215 mmol, Aldrich 36,057-0), approximately 1 mL of *o*-dichlorobenzene, and 3,5-dinitrobenzoyl chloride (0.049 g or 0.215 mmol, Aldrich 156272-25G) were also added to the vial along with a stir vane. A few drops of the reaction solution were removed before heating for TLC (HD-2-72-1). The reaction mixture was a yellow solid in solution. The vial was capped and run at 70°C for 5 minutes in a Biotage Microwave Instrument, "Initiator" model. The reaction mixture had developed on tan solid with the yellow solid remaining. A syringe was used to pull a few drops of reaction mixture out of the vial for TLC (HD-2-73-1). The first TLC analysis was run on 50% cyclohexane/50% ethyl acetate. HD-2-72-1 gave Rf values of 0.901, 0.428, 0.343, 0.084, and zero while HD-2-73-1 gave Rf values of 0.878, 0.469, 0.381, 0.321, 0.195, and zero. TLC suggests the presence of reactants in the vial.

The reaction vial was run again on the Biotage Initiator, this time at 70°C for 20 minutes. The reaction mixture had developed more tan solid but still contained a considerable amount of yellow solid. A few drops were removed (HD-2-73-2) and analyzed by TLC. The second TLC analysis was run on 50% cyclohexane/50% ethyl acetate. HD-2-72-1 gave Rf values of 0.960, 0.549, 0.486, 0.194, and zero while HD-2-73-

2 gave Rf values of 0.977, 0.596, 0.511, 0.414, 0.108, and zero. Again, the TLC suggested that reactants were still present, so additional microwave runs were done, as follows.

The reaction vial was placed again in the Biotage Initiator, this time for 15 minutes at 100°C. The reaction mixture had a large increase in tan solid near the stir vane, a large decrease in yellow solid, and still contained a yellow solution. A few drops were removed (HD-2-73-3) and analyzed by TLC. The third TLC was run with 50% cyclohexane/50% ethyl acetate. HD-2-72-1 gave Rf values of 0.966, 0.620, 0.242, 0.164, and zero while HD-2-73-3 gave Rf values of 0.982, 0.824, 0.656, 0.554, 0.496, 0.220, and zero. The TLC suggests the presence of reactants in the vial.

The reaction vial was placed yet again in the Biotage Initiator, this time at 130°C for 20 minutes. The reaction mixture had a darker tan solid that was all stuck to the sides of the vial rather than free-floating. Some yellow solid was still present and the solution was still yellow. A small amount was removed (HD-2-74-1) and analyzed by TLC. The fourth TLC was run on 50% cyclohexane/50% ethyl acetate. HD-2-72-1 gave Rf values of 0.968, 0.544, 0.303, 0.168, and zero while HD-2-74-1 gave Rf values of 0.985, 0.817, 0.585, 0.475, 0.301, 0.280, and zero. This TLC still suggests the presence of reactants in the vial but further efforts were abandoned.

DISCUSSION

In the ADDITION OF CS_2 (RUN #1), the sodium trimethylsilanolate acts like a deprotonated alcohol which is attacked by the electrophilic carbon of CS_2 to create a dithiolate or xanthate product (Barton, Parekh, & Tse, 1993; Davy, Mason, Moreau, & Wulff,2012; Sawant, Kovalev, Klug, & Efrima, 2001). Trimethylsilanolate is a protecting group that can be selectively cleaved when desired by certain reagents such as adventitious acid (Denmark & Bui, 2005), hydrofluoric acid, or fluorosilic acid (Pilcher, Hill, Shimshock, Waltermire, & DeShong, 1992).

Comparison of ***HD-2-IR-1*** and ***HD-2-IR-2*** suggest that a reaction took place during the ADDITION OF CS_2 (RUN #1). However, due to the seemingly hygroscopic nature of the product, further evaluation could not take place to determine if the desired xanthate ($RO\text{-}CSS\text{-}M^{+1}$) was obtained, where M^{+1} is usually a metal cation (Jensen & Henriksen, 1968). Concerns were raised by the presence of a broad peak in ***HD-2-IR-1***. Such a peak typically suggests -OH or -NH stretching (Colthup, Daly, & Wiberley, 1964) which would not be present if the desired product was obtained.

Several amination methods have been considered to form the S-N bond characteristic of a sulfenamide. One option previously explored by Brandon Hamm (a student of Dr. Armstrong who formerly conducted research in this area) was reacting pyridine with hydroxylamine-*O*-sulfonic acid and hydriodic acid to produce N-aminopyridinium iodide, which was attempted as an aminating agent (Gilman & Blatt, 1932). Another method is direct amination by hydroxylamine-*O*-sulfonic acid (Brown, Heydkamp, Breuer, & Murphy, 1964; Rathke, Inoue, Varma, & Brown, 1966). The third

method is amination by monochloramine, which is generated *in situ* by reaction of NaOCl and NH_4OH (aqueous) as used by Viswandhan (1991). Another option is to react a thiol with an amine to form a sulfenamide bond (Barton, Hesse, O'Sullivan, & Pechet, 1991).

In AMINATION OF XANTHATE PRODUCT VIA CLOROX/AMMONIA, the mixing of NaOCl and NH_4OH (aq.) is expected to form monochloramine *in situ* (Smith, Alliger, Carr, & Young, 1949). Monochloramine would then act as the aminating agent and attack the dithiolate to form a sulfenamide. By maintaining a pH around or above 8, the formation of monochloramine is favored over the formation of dichloramine or trichloramine (Colton & Jones, 1955).

HD-2-IR-4 was analyzed closely to determine if the desired product was obtained. 3192.06 cm^{-1} is assigned to NH_2 stretch (Davis, et al., 1973); 2957.12 or 2926.46 cm^{-1} suggest nitrogen; lack of a peak between 2590 and 2540 cm^{-1} suggests the lack of a sulfhydryl group; 1723.95 cm^{-1} may be due to C=S; 1658.05 cm^{-1} may be assigned to NH_2; 1458.71 cm^{-1} may be assigned to methyl groups of trimethylsilanolate; and peaks 1267.93, 1119.19, and 1070.54 cm^{-1} suggest the xanthate group (Colthup, Daly, & Wiberley, 1964). While all of this shows that the xanthate product is present as well as an amine, none of these peaks prove that the S-N bond was formed. It was found that an S-N radical has an IR peak at 1209.4 cm^{-1} but a molecular S-N bond has no characteristic peak (Hassanzadeh & Andrews, 1992; Heimer & Field, 1970).

In BENZOYLATION OF SULFENAMIDE PRODUCT VIA BENZOYL CHLORIDE (RUN #1), at one point the solid was suspected to be a mixture of pyridinium chloride and our desired product. Extractions were used to remove pyridinium chloride based on

assumptions that it is more soluble in water than our desired product. *HD-2-IR-5* was compared to *HD-2-IR-4* to analyze the success of the reaction. The following peaks were seen in both spectra: 2858.66 cm^{-1} suggests silicon methyl groups, 1121.22 cm^{-1} suggests xanthate, 1071.43 cm^{-1} suggests C=S, and 1272.89 cm^{-1} suggests silicon methyl groups (Colthup, Daly, & Wiberley, 1964). The notable absence of a broad band near 3200 cm^{-1} suggests the lack of NH_2 stretching. A peak observed at 1725.05 cm^{-1} suggests a carbonyl peak. The following peaks suggest aromaticity: 1599.78 cm^{-1}, 464.18 cm^{-1}, 1494.62 cm^{-1}, 1450.89 cm^{-1}, 995.69 cm^{-1}, 776.60 cm^{-1}, and 694.72 cm^{-1}. The peak at 1210.83 cm^{-1} suggested a phenyl-carbon bond. Peaks 2957.73 and 2927.00 cm^{-1} suggested an amide. A peak at 1379.20 cm^{-1} suggests toluene methyl, 1788.57 cm^{-1} suggests benzoyl chloride (Colthup, Daly, & Wiberley, 1964).

From this point, more work was needing to be done on the benzoylation reaction to learn more about what occurred in earlier reactions. To save time from making starting materials, a large amount of materials saved from Brandon Hamm's work was used as the starting material for benzoylation, a sulfenamide product (HD-1-90-2).

In BENZOYLATION OF SULFENAMIDE PRODUCT VIA BENZOYL CHLORIDE (RUN #2), *HD-2-IR-7* was found to be a mixture of substances in subsequent procedures. *HD-2-IR-7* showed every major peak suggesting aromaticity while *HD-2-IR-5* did not. *HD-2-IR-7* showed a peak at 1682.77 cm^{-1} while *HD-2-IR-5* did not. This peak may be the expected carbonyl group in the desired compound. Finally, a large difference was seen in the possible silicon methyl groups (1288.35 cm^{-1} vs. 1272.89 cm^{-1}) for which no reasonable

explanation can be found. Based on these facts, it is unsure if HD-2-20-1 is the desired benzoylated compound.

HD-2-IR-8 did not show the expected peaks related to silicon or sulfur as seen in earlier IR spectra. Peaks shown in the spectrum suggest aromaticity, NH_2, a benzoyl chloride carbonyl, and phenyl-carbon bond (Colthup, Daly, & Wiberley, 1964).

HD-2-IR-10 showed a broad band from 3100 to 2500 cm^{-1} which is probably due to N-H stretching. The peak at 1784.38 cm^{-1} represents the benzoyl chloride carbonyl. This peak is used to analyze the presence of benzoyl chloride in the product. The spectrum shows all expected peaks for silicon, sulfur, xanthate group, aromaticity, and carbonyls (Colthup, Daly, & Wiberley, 1964). A conclusion is drawn that the recrystallization increased the purity of the product.

After rinsing with cyclohexane, the peak at 1784.38 cm^{-1} in *HD-2-IR-11* is decreased in relative size, showing that benzoyl chloride had been removed from the product. It is necessary to remove benzoyl chloride due to its high reactivity which could compromise future reactions.

Multiple spots in the TLC suggest that neither reactant nor product is very purified. Concerns are raised by this because without a pure reactant, there is no guarantee what reaction is taking place.

The third attempt at BENZOYLATION OF SULFENAMIDE PRODUCT VIA BENZOYL CHLORIDE (RUN #3) seemed less than successful as well. The products from this reaction were never fully worked up due to the appearance of side reactions indicated by the dark colors. One suspected reason for the lack of success is impurities in the sulfenamide

reactant. Further evaluation of Brandon Hamm's sample (HD-1-90-2) brought suspicion of the presence of dimethylformamide (DMF). DMF is particularly harmful in this case because it has been found to lead to the decomposition of xanthates (Millican & Sauers, 1979). This lead to PURIFICATION OF SULFENAMIDE PRODUCT. After purification, *HD-2-IR-12* was analyzed for the presence of DMF. Literature shows that the carbonyl peak of DMF is around 1680 cm^{-1} (Colthup, Daly, & Wiberley, 1964). Due to the lack of a peak in this region of *HD-2-IR-12*, it is concluded that the DMF was removed.

With a purer reactant, another benzoylation is attempted. To ensure a better reaction, benzoyl chloride is replaced with its more reactive derivative, 3,5-dinitrobenzoyl chloride. While conducting TLC in BENZOYLATION OF SULFENAMIDE PRODUCT VIA 3,5-DINITROBENZOYL CHLORIDE (RUN #1), it was found that a spotted plate must be left standing for approximately an hour to allow *o*-dichlorobenzene (used as solvent) to volatilize. Without this waiting period, a broad band would impair the reading of the plate. It was also found that plates give better reading when washed with ethyl acetate and cyclohexane before being spotted. Washing the plate removes impurities that can impair the interpretation of data.

The first set of TLC plates were an attempt to determine if reactants were still present in the reaction mixture. If it was found that reactants were present, the reaction mixture would have been subjected to more heating to continue the reaction. Unfortunately, results were rather inconclusive because the spots were comparable but not exact (0.815 vs 0.825), which meant they could be the result of identical compounds or different compounds. It is more likely that these are the result of the same compound.

The second attempt at TLC raised concerns. The multitude of spots from the reaction mixture raised concerns of multiple compounds in the reactants. One considerable possibility is that sodium trimethylsilanolate could have been attacked multiple times by carbon disulfide, resulting in compounds representing attack by no carbon disulfide, or one molecule of carbon disulfide, or two molecules of carbon disulfide. Since an excess of 1.5 molar equivalents of carbon disulfide were used in the reaction, the possibility of multiple attacks is very plausible, but the excess was used because of the extremely high volatility of CS_2.

HD-2-IR-14 and *HD-2-IR-15* are closely analyzed to determine which is the desired compound. No clear results could be found. Both spectra suggest aryl nitro- group peaks at 1340 cm^{-1} and 720 cm^{-1} as well as xanthate peaks at 1244.45 cm^{-1} and 1223.93 cm^{-1}. Neither spectra show a strong carbonyl peak between 1680 and 1630 cm^{-1}. *HD-2-IR-14* had peaks suggesting a monosubstituted amide (trans) at 3380.25 cm^{-1}, 3103.28 cm^{-1}, and 1536.17 cm^{-1} whereas *HD-2-IR-15* only shows the peak at 1534.60 cm^{-1}. *HD-2-IR-15* suggests silicon methyl peaks at 1260.23 cm^{-1} and 787.42 cm^{-1} as well as a Si-O-C peak at 1077.28 cm^{-1} but *HD-2-IR-14* does not display any expected silicon peaks (Colthup, Daly, & Wiberley, 1964).

With concerns raised about the addition of carbon disulfide and inability to identify the desired product in the latest reaction, attentions were returned to the first reactions in the sequence. Concerns considered when designing ADDITION OF CS_2 (RUN #2) included the possible double attack by carbon disulfide, picking up moisture from the air, and being oxidized by O_2 gas. These could not all be addressed at once due to safety

concerns. Oxidization by O_2 gas is not supported upon evaluation of ***HD-2-IR-1***. The other concerns are addressed by using an Atmosbag and removing excess carbon disulfide.

The small sample of product from the ADDITION OF CS_2 (RUN #2) that was exposed to the atmosphere quickly turned dark orange and became wet. This supports evidence that the desired xanthate compound is hygroscopic. This claim is further supported upon comparison of ***HD-2-IR-16*** (a dry sample) and ***HD-2-IR-17*** (a wet sample from exposure). All peaks were comparable in wavenumber but different in broadness. The peaks in ***HD-2-IR-17*** were broader, most likely due to the hydrogen bonding from moisture picked up from exposure. This is very strong evidence that the color changes are due to moisture picked up in the air and not from oxidization.

In AMINATION OF XANTHATE PRODUCT VIA HYDROXYLAMINE-O-SULFONIC ACID, hydroxylamine-*O*-sulfonic acid is used to directly aminate the xanthate. Due to the acidity of hydroxylamine-*O*-sulfonic acid, care was taken to protect the xanthate with a buffer, since it is acid-labile. Since hydroxylamine-*O*-sulfonic acid slowly decomposes in water, nonaqueous buffer systems were investigated. Several options were found such as an acetonitrile based nonaqueous buffer of acetonitrile, 1 M acetic acid, and 10 mM sodium acetate (Matysik, 2000); a 1:1 methanol-acetonitrile buffer containing 10 mM ammonium acetate and 1% acetic acid (Sanders, Burka, Shelby, Newbold, & Cunningham, 1997); or a nonaqueous buffer of 1.67×10^{-4} M 2,2,6,6-tetramethylpiperdine / 1.0×10^{-4} M $HClO_4$ (Stauffer & Weber, 1999). However, after considering these nonaqueous buffer systems, it was realized that an active nitrogen would cause a competing reaction. Rather than risk a competing reaction, it was decided to use the aqueous buffer of sodium

bicarbonate and risk the slow decomposition hydroxylamine-*O*-sulfonic acid. This risk seemed lower because reports in literature show amination by hydroxylamine-*O*-sulfonic acid in aqueous systems (Campbell & Rees, 1969; Raap, 1969; Sisler, Bafford, Omietanski, Rudner, & Drago, 1958). Despite the low risk, hydroxylamine-*O*-sulfonic acid solutions were kept on ice as much as possible to slow the process of decomposition.

HD-2-IR-20 and ***HD-2-IR-21*** raised concerns because of small peaks, a small vertical axis range, and a large slant in the spectra. The spectrophotometer was checked with a polystyrene standard and was functioning properly. Since subsequent spectra do not show the same concerns, it appears that something within the sample has caused the strange spectra. When compared to ***HD-2-IR-22***, neither sample shows signs of hydroxylamine-*O*-sulfonic acid. Despite concerns, enough product (HD-2-61-4) was obtained to move on to the next reaction.

In BENZOYLATION OF SULFENAMIDE PRODUCT VIA 3,5-DINITROBENZOYL CHLORIDE (RUN #2), it is quite a mystery as to why the reaction is not taking place. One possibility is that the product is not soluble in *o*-dichlorobenzene and therefore is not appearing in the TLC analyses. When looking at ***HD-2-IR-23***, the lack of a peak at 1760 cm^{-1} suggests a lack of 3,5-dinitrobenzoyl chloride in the product. 1538.26 cm^{-1}, 1342.74 cm^{-1}, and 719.99 cm^{-1} are all shown to be aromatic nitro groups upon comparison to ***HD-2-IR-24***. 1734.55 cm^{-1} is predicted to be the product's carbonyl group since it is slightly lower than the 3,5-dinitrobenzoyl chloride carbonyl group. Typical silicon and xanthate peaks are also present in the spectrum. Concerns are raised by the lack of a broad NH

stretch near 3200 cm^{-1} and the weakness of the proposed carbonyl peak at 1734.55 cm^{-1} (Colthup, Daly, & Wiberley, 1964).

Since BENZOYLATION OF SULFENAMIDE PRODUCT VIA 3,5-DINITROBENZOYL CHLORIDE (RUN #2) was not successful under the extremes of conventional heat, a new technique was employed that used microwave heat. The use of microwave heat can be more effective than the use of conventional heat if the compounds being heated have a dipole moment (Listrom, Tierney, Wathey, & Westman, 2001). Microwave-assisted synthesis has been reported successfully for acylation of amines (Petricci, Mugnaini, Radi, Corelli, & Botta, 2004). In case the problem arises from a competing reaction with pyridine, the excess of pyridine is not being used in BENZOYLATION OF SULFENAMIDE PRODUCT WITH 3,5-DINITROBENZOYL CHLORIDE VIA MICROWAVE PROMOTION. Analysis of the four TLC plates showed a limited success for the reaction. While new spots were present in the heated samples, the spots from the reactants never fully disappear. Again, this could be due to lack of solubility of the product. Continuations of this reaction are postponed until the amination reaction is more successful and refined.

CONCLUSION

Upon identification of problem situations in the addition of carbon disulfide, each problem was investigated and solved. It was found that the product was not being oxidized by the air but rather picking up moisture. It was suspected that double attack by carbon disulfide might have happened, and if so, it may be prevented by removing excess carbon disulfide and conducting the addition very slowly. The addition of carbon disulfide works best in a nitrogen environment, allowing for the obtaining of product weight without any moisture.

Several methods of amination have been attempted with little success, as follows:

1. The work of Brandon Hamm, former research student of Dr. Armstrong, with N-aminopyridinium iodide was not giving good results as those compounds were used for benzoylation.
2. Formation *in situ* of monochloramine resulted in very low yields. While several ideas have been discussed for increasing these yields, the expectations are low.
3. The direct amination by hydroxylamine-*O*-sulfonic acid also gave low yields and compounds that did not read well on the IR spectrophotometer.

Efforts have been made to use a stronger amination agent, O-(trifluoromethanesulfonyl)hydroxylamine. After a thorough search of literature, only one paper was located covering the preparation of the compound (Kellner & Bliepert, 1978). Close inspection of this procedure reveals many concerns and potential flaws preventing the preparation of O-(trifluoromethansulfonyl)hydroxylamine. Further literature

searching must be conducted to locate an amination agent that could be used on the xanthate product. Despite the multitude of attempts, the benzoylation of sulfenamides showed no success. The issues with benzoylation cannot be addressed until the amination reaction has been improved.

A lot of progress has been made towards synthesizing 1-anilino-8-(mercaptoamino)-1,8-octadione with the immediate next steps of research being identified. This data will be presented at ONU's Scholars Week on April 18, 2017 at 5:30 pm in Reed 330. Besides making progress on the synthesis, a considerable amount of information has been obtained about the properties of the novel compounds scattered throughout this work.

WORKS CITED

Andreae, S., and Schmitz, E. (1991). Electrophilic Aminations with Oxaziridines. *Synthesis*, 1991 (5), 327-341.

Barton, D. H. R., Hesse, R. H., O'Sullivan, A. C., and Pechet, M. M. (1991). A One-pot Synthesis of Sulfenamides. *Journal of Organic Chemistry*, 56 (23), 6702-6704.

Barton, D. H. R., Parekh, S. I., and Tse, C. L. (1993). On the Stability and Radical Deoxygenation of Tertiary Xanthates. *Tetrahedron Letters*, 34 (17), 2733-2736.

Brown, H. C., Heydkamp, W. R., Breuer, E., and Murphy, W. S. (1964). The Reaction of Organoboranes with Chloramine and with Hydroxylamine-*O*-sulfonic Acid. A Convenient Synthesis of Amines from Olefins via Hydroboration. *Journal of the American Chemical Society*, 86, 3565-3566.

Campbell, C. D., and Rees, C. W. (1969). Reactive Intermediates. Part I. Synthesis and oxidation of 1- and 2-aminobenzotriazole. *Journal of the Chemical Society* C, 5, 742-747.

Carpino, L. (1960). O-Acylhydoxylamines. II. O-Mesitylenesulfonyl-, O-*p*-Toluenesulfonyl- and O-Mesitoylhydroxylamine. *Journal of the American Chemical Society*, 82(12), 3133-3135.

Carta, F., Akdemir, A., Scozzafava, A., Masini, E., and Supuran, C. T. (2013). Xanthates and Trithiocarbonates Strongly Inhibit Carbonic Anhydrases and Show Antiglaucoma Effects in Vivo. *Journal of Medicinal Chemistry*, 56, 4691-4700.

Colthup, N. B., Daly, L. H., and Wiberley, S. E. (1964). Introduction to Infrared and Raman Spectroscopy. New York: Academic Press.

Colton, E., and Jones, M. M. (1955). Monochloramine. *Journal of Chemical Education*, 32 (9), 485-487.

Craine, L., and Raban, M. (1989). The Chemistry of Sulfenamides. *Chemical Reviews*, 89 (4), 689-712.

Daniel, K. B., Sullivan, E. D., Chen, Y., Chan, J. C., Jennings, P. A., Fierke, C. A., & Cohen, S. M. (2015). Dual-mode HDAC prodrug for covalent modification and subsequent inhibitor release. *Journal of Medicinal Chemistry*, 58(11), 4812.

Davis, F. A., Slegeir, W. A. R., Evans, S., Schwartz, A., Goff, D. L., and Palmer, R. (1973). Chemistry of the Sulfur-Nitrogen Bond. VI. A Convenient One-Step Synthesis of Sulfenimines (S-Aryl Thiooximes). *Journal of Organic Chemistry*, 38 (16), 2809-2813.

Davy, J. A., Mason, J. W., Moreau, B., and Wulff, J. E. (2012). Xanthates as Synthetic Equivalents of Oxyacyl Radicals: Access to Lactones under Tin-Free Conditions. *Journal of Organic Chemistry*, 77, 6332-6339.

Denmark, S. E., and Bui, T. (2005). Lewis Base Catalyzed Enantioselevtive Aldol Addition of Acetaldehyde-Derived Silyl Enol Ether to Aldehydes. *Journal of Organic Chemistry*, 70, 10190-10193.

Fischer, A., Sananbenesi, F., Mungenast, A., & Tsai, L. (2010). Targeting the correct HDAC(s) to treat cognitive disorders. *Trends in Pharmacological Sciences*, 31(12), 605-617.

Food & Drug Administration (2016, Dec 29). FDA approves vorinostat (Zolinza) for the treatment of cutaneous manifestations of cutaneous T-cell lymphoma (CTCL). Retrieved from: http://www.fda.gov/aboutfda/centersoffices/officeofmedicalproductsandtobacco/cder/ucm094952.htm

Friebolin, W., Schilling, G., Zoller, M., and Amtmann, E. (2004). Synthesis and Structure – Activity Relationship of Novel Antitumoral Platinum Xanthate Complexes. *Journal of Medicinal Chemistry*, 47, 2256-2263.

Gänswein, V. B., and Behm, H. (1977). The Problem of the C—S Vibration in Trifluoromethane Sulfonates. Structural Investigations of $H_3NOH^+CF_3SO_3^-$. *Journal of Inorganic and General Chemistry*, 428 (1), 248-253.

Gilman, H., Blatt, A. H. (Eds.) (1932) ORGANIC SYNTHESES. COLLECTIVE VOLUME V. New York: Wiley, 43-45.

Go, Y., Chandler, J. D., & Jones, D. P. (2015). The cysteine proteome. *Free Radical Biology & Medicine*, 84, 227-245.

Gu, W., Nusinzon, I., Smith, R. D., Horvath, C. M., & Silverman, R. B. (2006). Carbonyl- and sulfur-containing analogs of suberoylanilide hydroxamic acid: Potent inhibition of histone deacetylases. *Bioorganic & Medicinal Chemistry*, 14(10), 3320-3329.

Guarino, V. R., Karunaratne, V., & Stella, V. J. (2007). Sulfenamides as prodrugs of NH-acidic compounds: A new prodrug option for the amide bond. *Bioorganic & Medicinal Chemistry Letters*, 17(17), 4910-4913.

Hassanzadeh, P., and Andrews, L. (1992). Infrared Spectra of New Sulfur-Nitrogen Species in Solid Argon. *Journal of American Chemical Society*, 114, 83-91.

Heimer, N. E., and Field, L. (1970). Organic Disulfides and Related Substances. XXIX. Studies in the Chemistry of Sulfenamides. *Journal of Organic Chemistry*, 35 (9), 3012-3022.

Hockly, E., Richon, V. M., Woodman, B., Smith, D. L., Zhou, X., Rosa, E., . . . Bates, G. P. (2003). Suberoylanilide hydroxamic acid, a histone deacetylase inhibitor, ameliorates motor deficits in a mouse model of Huntington's disease. *Proceedings of the National Academy of Sciences of the United States of America*, 100(4), 2041-2046.

Jia, H., Pallos, J., Jacques, V., Lau, A., Tang, B., Cooper, A., . . . Thomas, E. A. (2012). Histone deacetylase (HDAC) inhibitors targeting HDAC3 and HDAC1 ameliorate polyglutamine-elicited phenotypes in model systems of Huntington's disease. *Neurobiology of Disease*, 46(2), 351-361.

Jensen, K. A., and Henriksen, L. (1968). Studies of Thioacids and Their Derivatives. XIV. Reactions of Carbon Disulfides with Active Methylene Compounds. *Acta Chemica Scandinavica*, 22, 1107-1128.

Kellner, V., Bliepert, C. (1978). N- and O-(Trifluormethylsulfonyl) Hydroxylamine. *Journal of Fluorine Chemistry*, 12 (3), 249-252.

Ley, K., and Eholzer, U. (1966). S-Amination of Thioureas and Thiourethanes. *Angew. Chem. Internat. Edit.,* 5(7), 674.

Listrom, P., Tierney, J., Wathey, B., and Westman, J. (2001). Microwave-assisted Organic Synthesis – a review. *Tetrahedron*, 57 (2001), 9225-9283.

Matysik, F. M. (2000). Experimental Characterization of End-Column Electrochemical Detection in Conjunction with Nonaqueous Capillary Electrophoresis. *Analytical Chemistry*, 72 (11), 2581-2586.

Mendiola. J., Rincon, J., Mateos, C., Soriano, J., Frutos, O., Niemeier, J., and Davis, E. (2009). Preparation, Use, and Safety of *O*-Mesitylenesulfonylhydroxylamine. *Organic Process, Research & Development*, 13, 263-267.

Millican, R. J., and Sauers, C. K. (1979). General Acid-Catalyzed Decomposition of Alkyl Xanthates. *Journal of Organic Chemistry*, 44 (10), 1664-1669.

Petricci, E., Mugnaini, C., Radi, M., Corelli, F., and Botta, M. (2004). Microwave-Assisted Acylation of Amines, Alcohols, and Phenols by the Use of Solid-Supported Reagents (SSRs). *Journal of Organic Chemistry*, 69, 7880-7887.

Pilcher, A. S., Hill, D. K., Shimshock, S. J., Waltermire, R. E., and DeShong, P. (1992). Selective Deprotection of Trialkylsilyl Ethers Using Fluorosilicic Acid. *Journal of Organic Chemistry*, 57, 2492-2495.

Raap, R. (1969). Amination of tetrazoles with hydroxylamine-*O*-sulfonic acid: 1- and 2-aminotetrazoles. *Canadian Journal of Chemistry*, 47 (19), 3677-3681.

Rathke, M. W., Inoue, N., Varma, K. R., and Brown, H. C. (1966). A Stereospecific Synthesis of Alicyclic and Bicyclic Amines via Hydroboration. *Journal of American Chemical Society*, 88 (12), 2870-2871. doi: 10.1021/ja00964a057

Sanders, J. M., Burka, L. T., Shelby, M. D., Newbold, R. R., and Cunningham, M. L. (1997). Determination of Tamoxifen and Metabolites in Serum by Capillary Electrophoresis Using a Nonaqueous Buffer System. *Journal of Chromatography*, 695, 181-185.

Sawant, P., Kovalev, E., Klug, J. T., and Efrima, S. (2001). Alkyl Xanthates: New Capping Agents for Metal Colloids. Capping of Platinum Nanoparticles. *Langmuir*, 17, 2913-2917. doi: 10.1021/la0014961

Shiba, T., and Iwadate, H. (2011). *Patent Identifier No. US 7,964,673*. Location: US Patent & Trademark Office.

Sisler, H. H., Bafford, R. A., Omietanski, G. M., Rudner, B., and Drago, R. J. (1958). Amination of Tertiary Amines by Hydroxylamine-*O*-sulfonic Acid. *Journal of Organic Chemistry*, 24 (6), 859-861.

Smith, G. E. P., Alliger, G., Carr, E. L., and Young, K. C. (1949). Thiocarbamylsulfenamides. *Journal of Organic Chemistry,* 14(6), 935-945.

Stauffer, M. T., and Weber, S. G. (1999). Optical Control of Divalent Metal Ion Binding to a Photochromic Catechol: Photoreversal of Tightly Bound Zn^{+2}. *Analytical Chemistry*, 71 (6), 1146-1151.

Sung, J. J., Ververis, K., & Karagiannis, T. C. (2014). Histone deacetylase inhibitors potentiate photochemotherapy in cutaneous T-cell lymphoma MyLa cells. *Journal of Photochemistry and Photobiology*. B, Biology, 131, 104.

Suzuki, T., Matsuura, A., Kouketsu, A., Hisakawa, S., Nakagawa, H., & Miyata, N. (2005). Design and synthesis of non-hydroxamate histone deacetylase inhibitors: Identification of a selective histone acetylating agent. *Bioorganic & Medicinal Chemistry*, 13(13), 4332-4342.

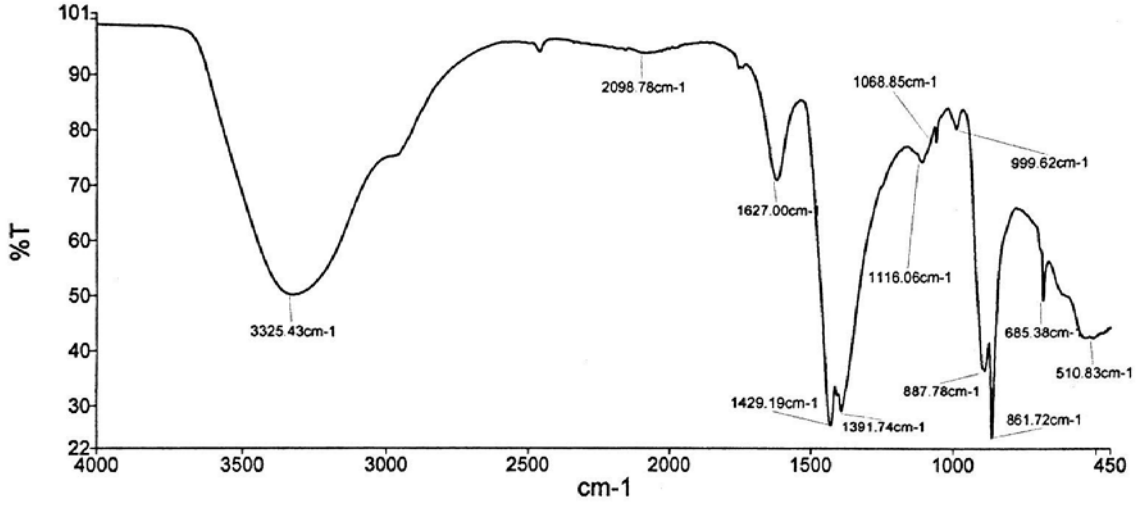
Tamura, Y., Minamikawa, J., Sumoto, K., Fujii, S., and Ikeda, M. (1973). Synthesis and Some Properties of *O*-Acyl- and *O*-Nitrophenylhydroxylamines. *Journal of Organic Chemistry*, 38(6), 1239-1241.

Tamura, Y., Minamikawa, J., and Ikeda, M. (1977). *O*-Mesitylenesulfonylhydroxylamine and Related Compounds – Powerful Aminating Reagents. *Synthesis*, 1977, 1-17.

Viswanadhan, V. N., Ghose, A. K., Hannah, N. B., Matsumoto, S. S., Avery, T. L., Revankar, G. R., and Robins, R. K. (1991). Analysis of the in Vitro Antitumor Activity of Novel Purine-6-sulfenamide, -sulfinamide, and -sulfonamide Nucleosides and Certain Related Compounds Using a Computer-Aided Receptor Modeling Procedure. *Journal of Medicinal Chemistry*, 34 (2), 526-532.

APPENDIX A – INFRARED SPECTRA

HD-2-IR-1



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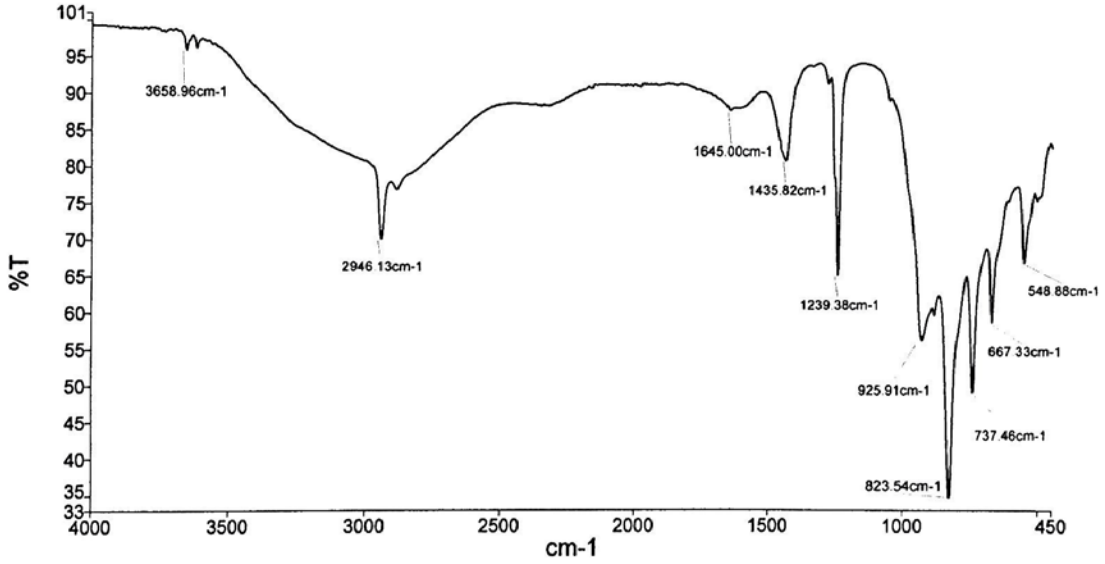

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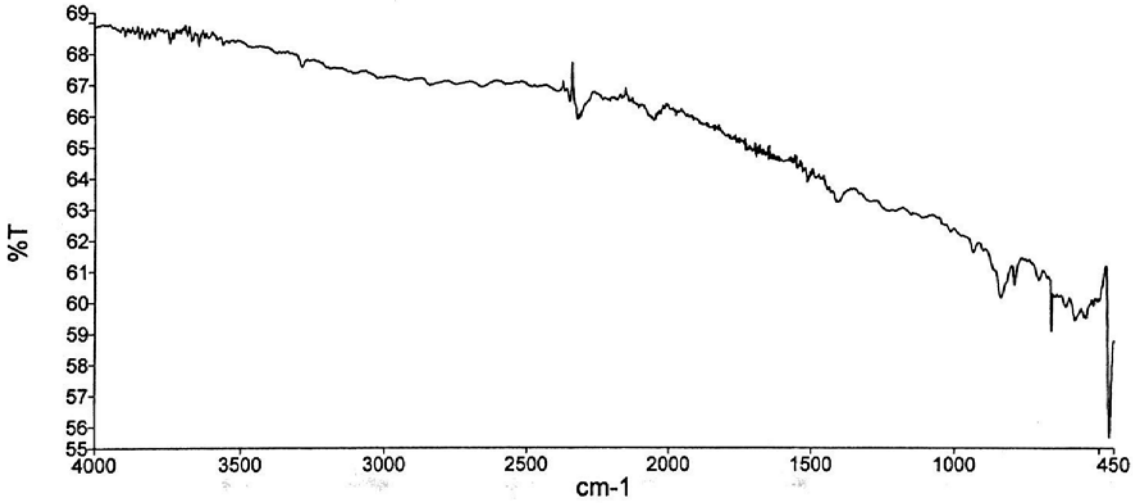

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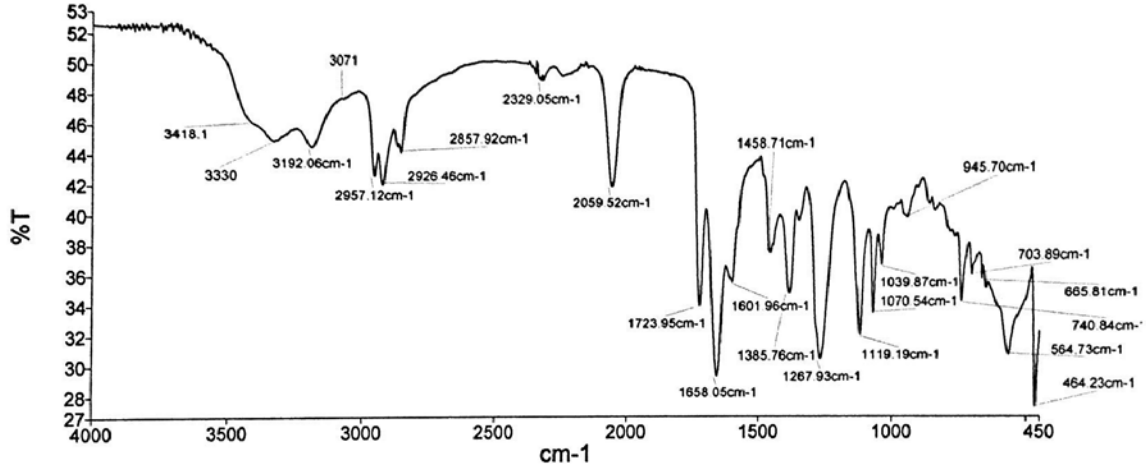

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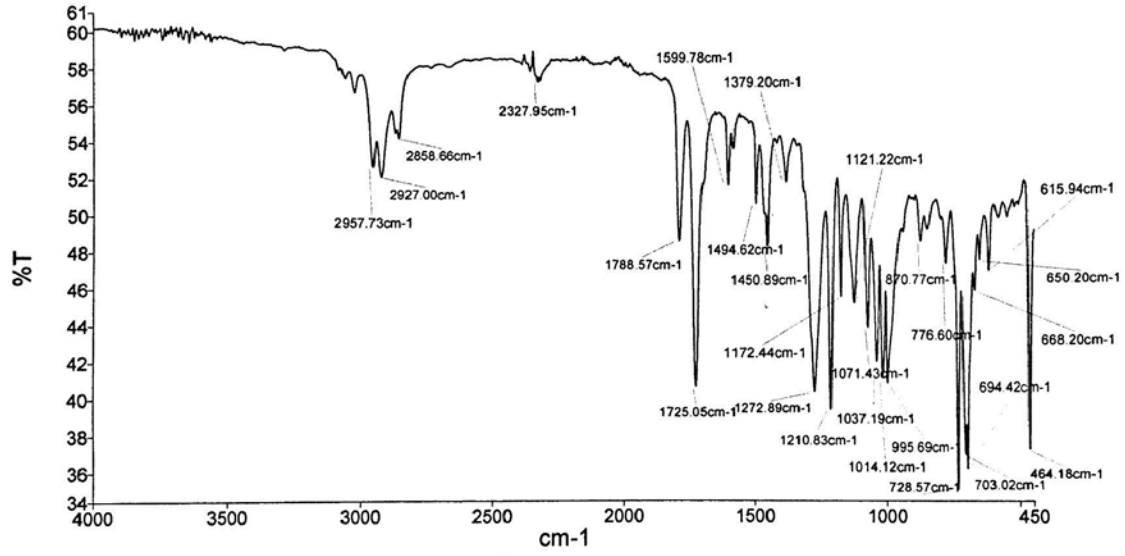

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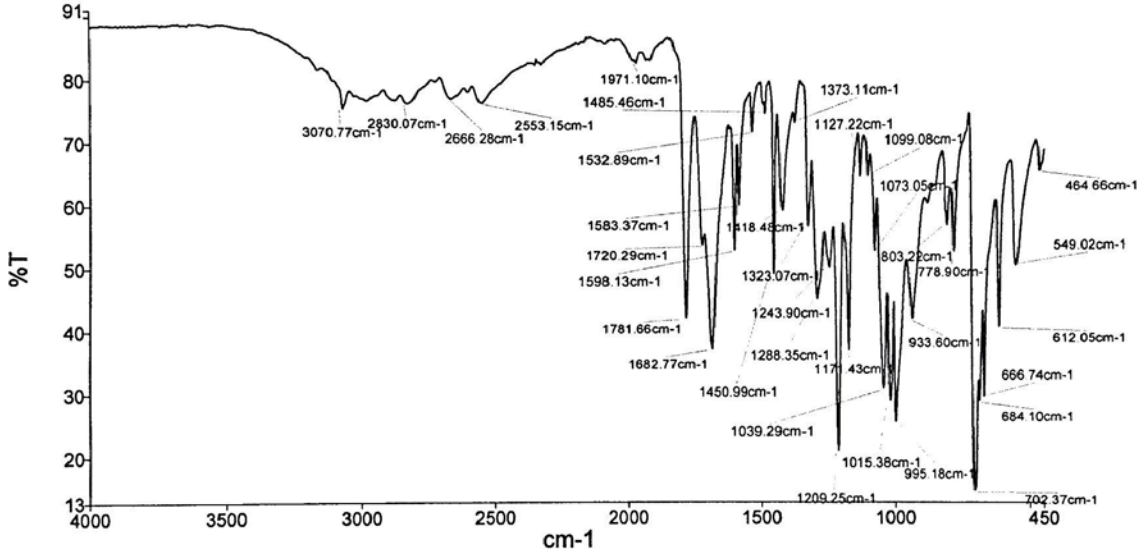

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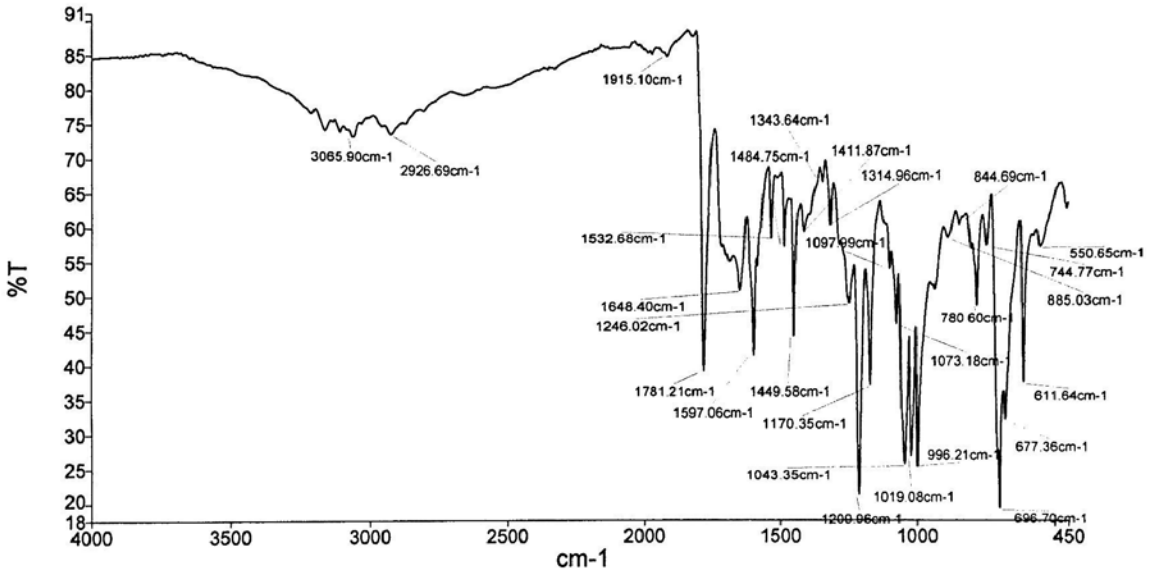

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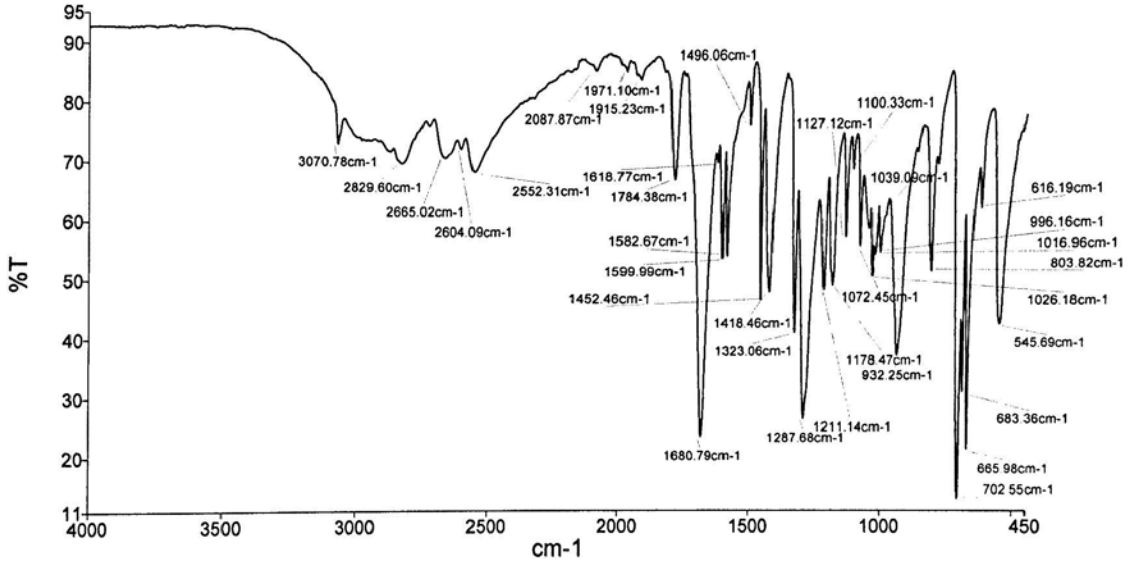

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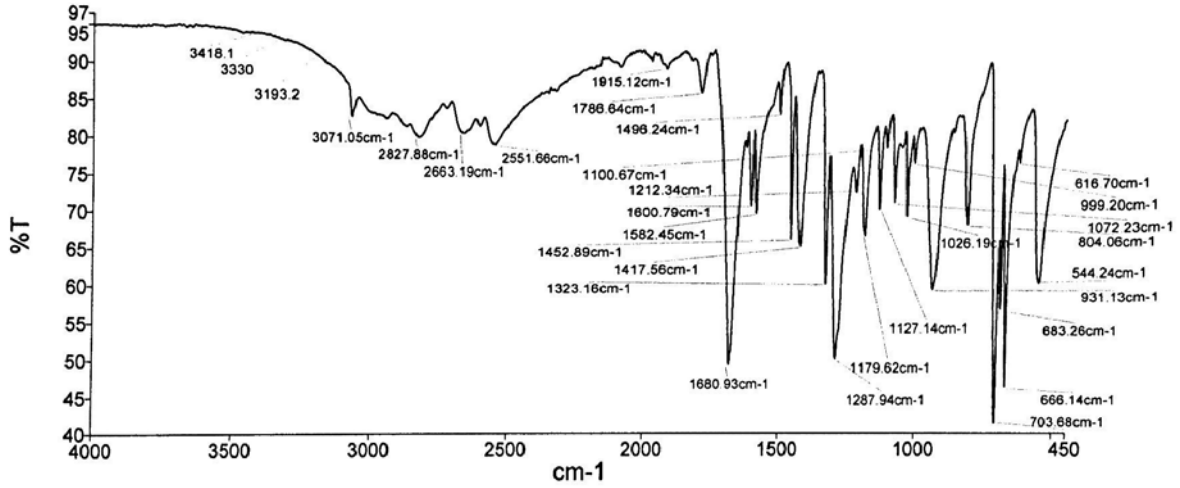

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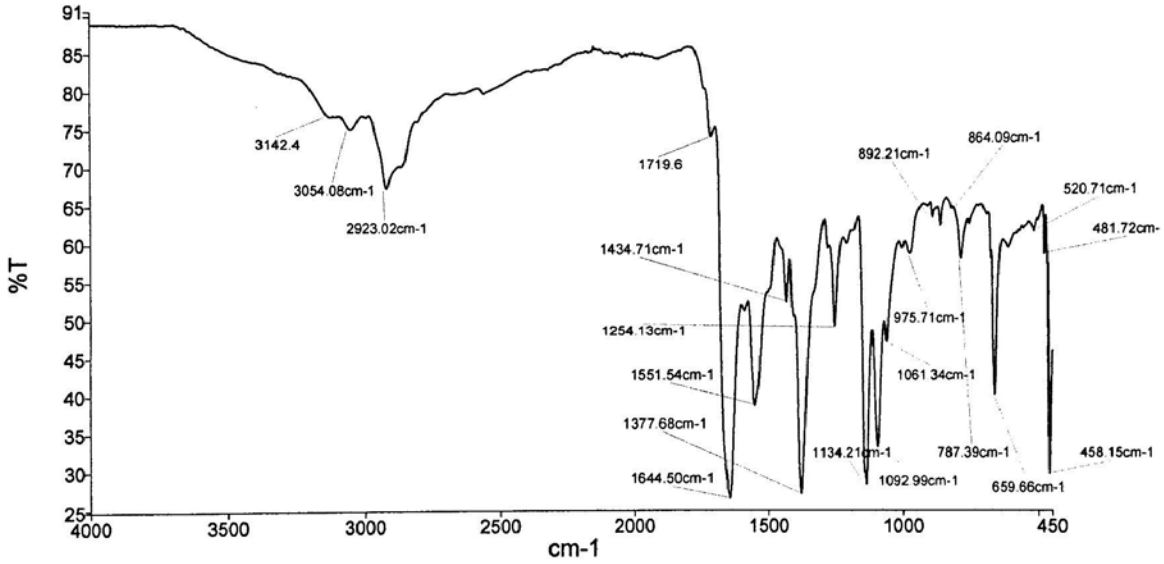

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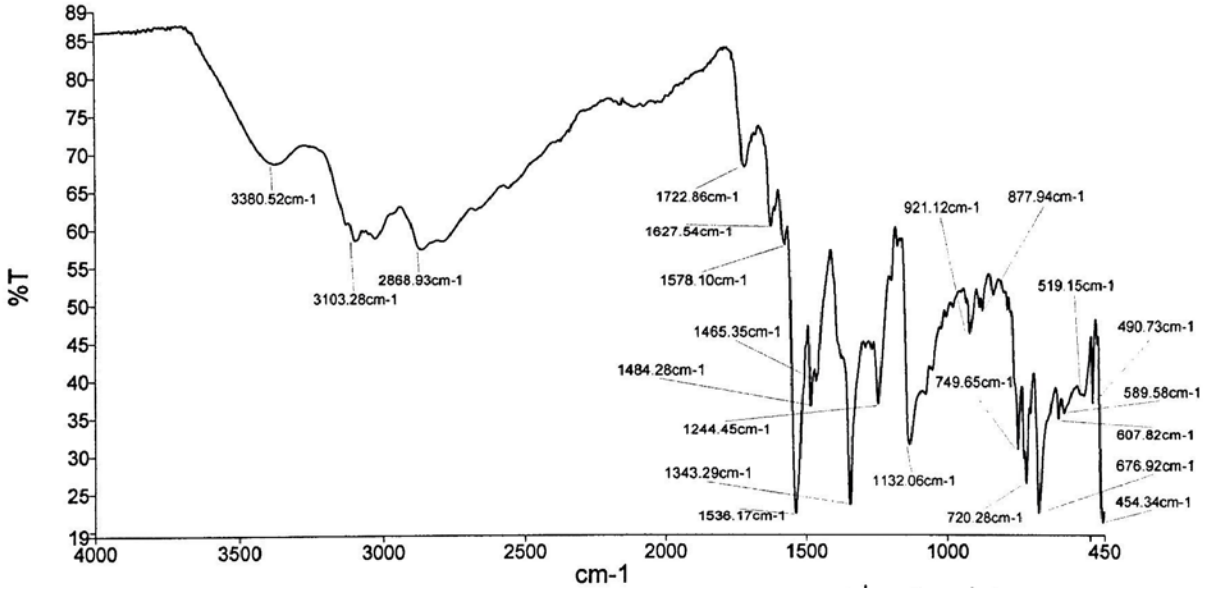

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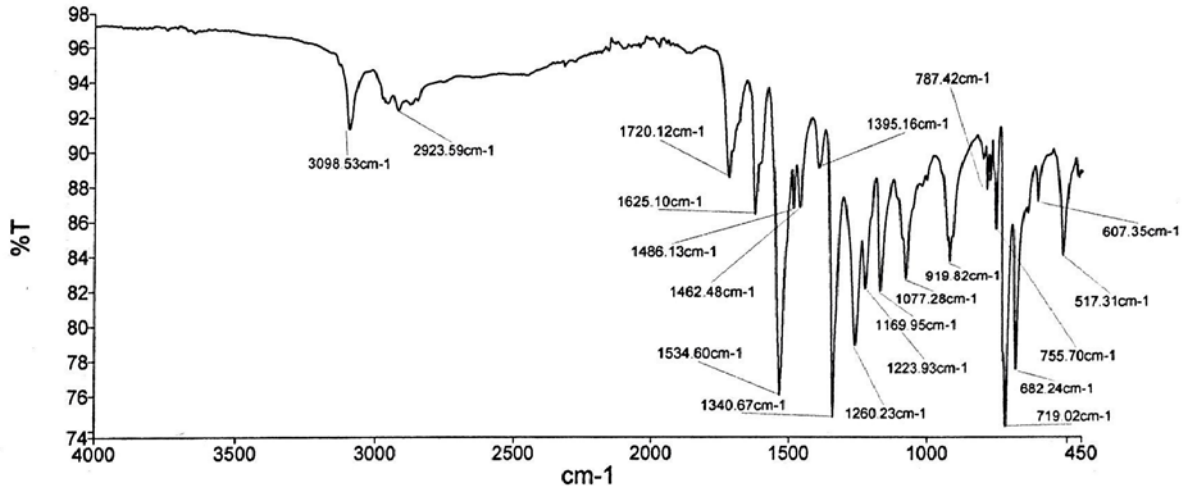

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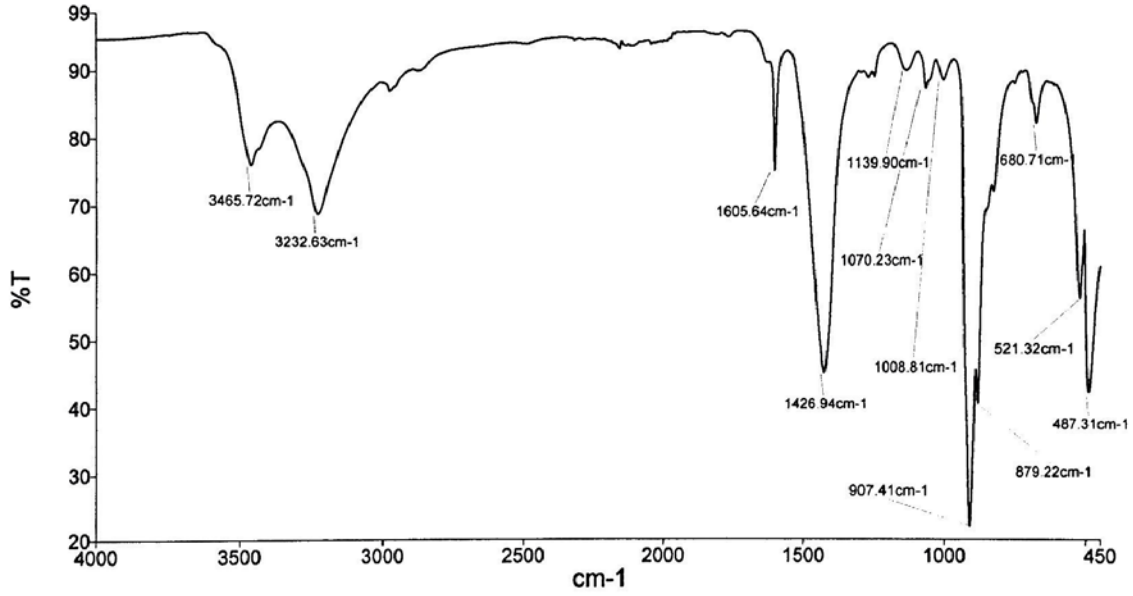

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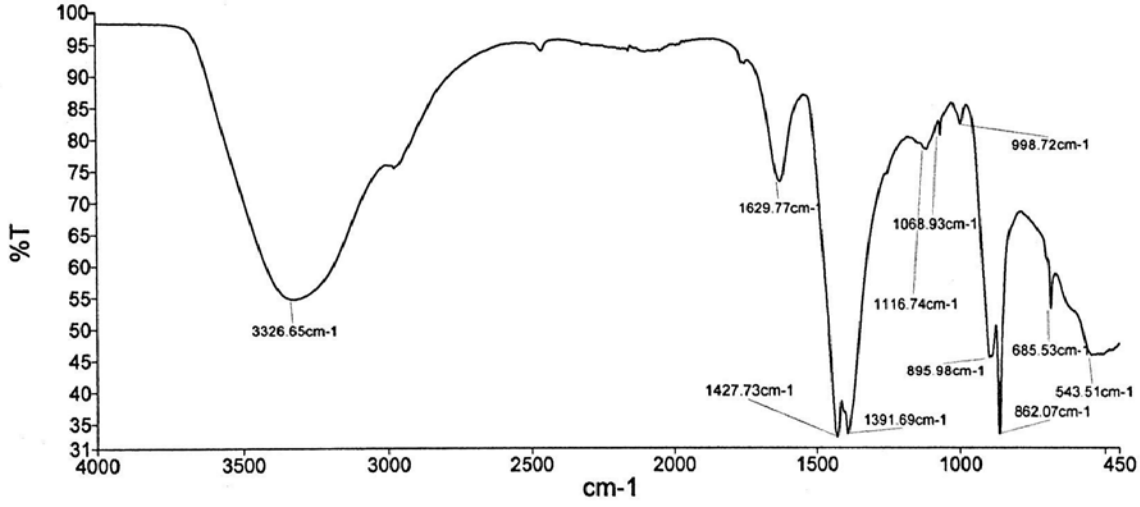

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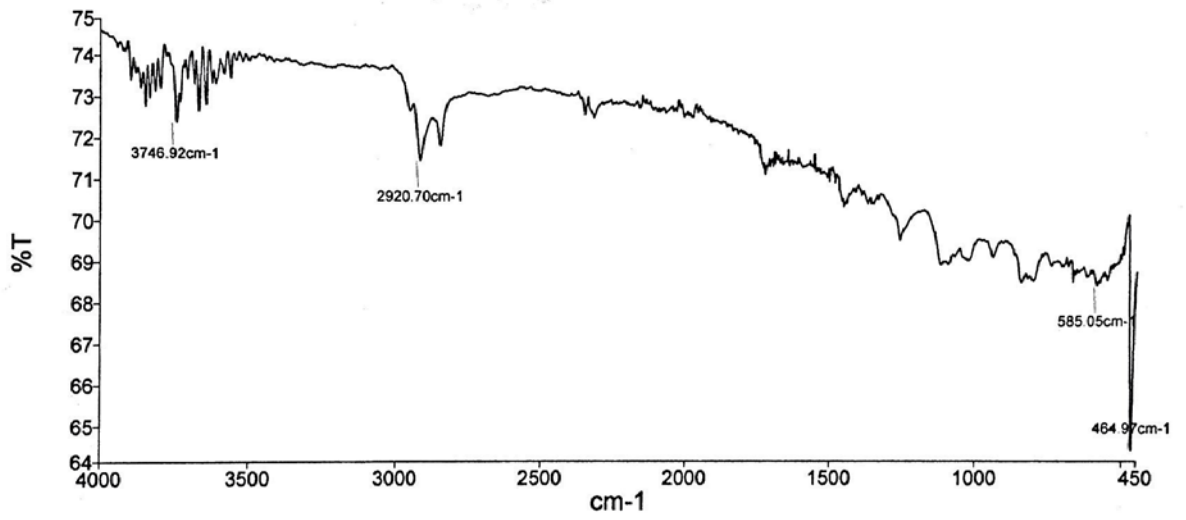

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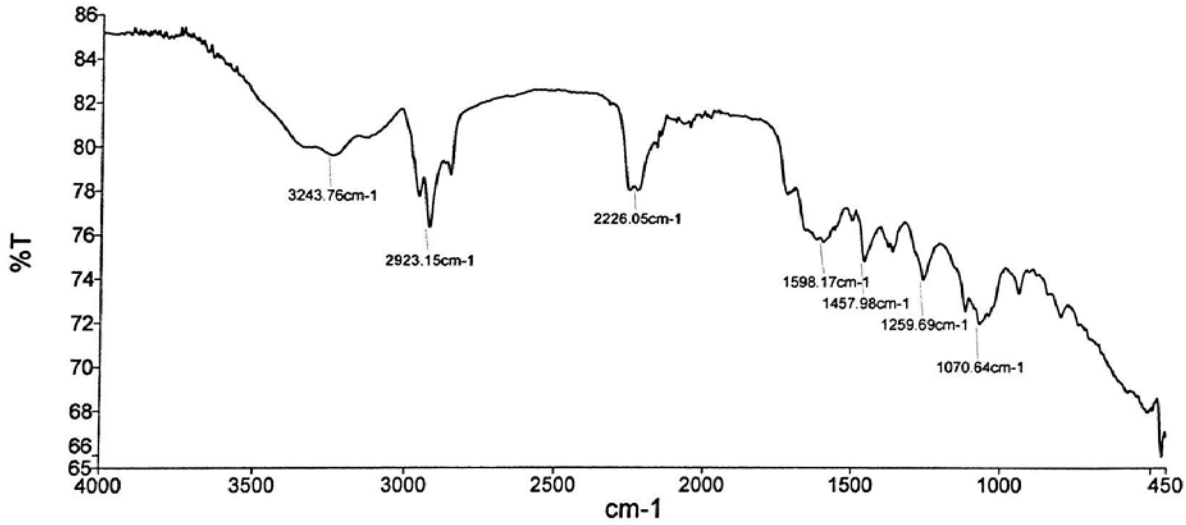

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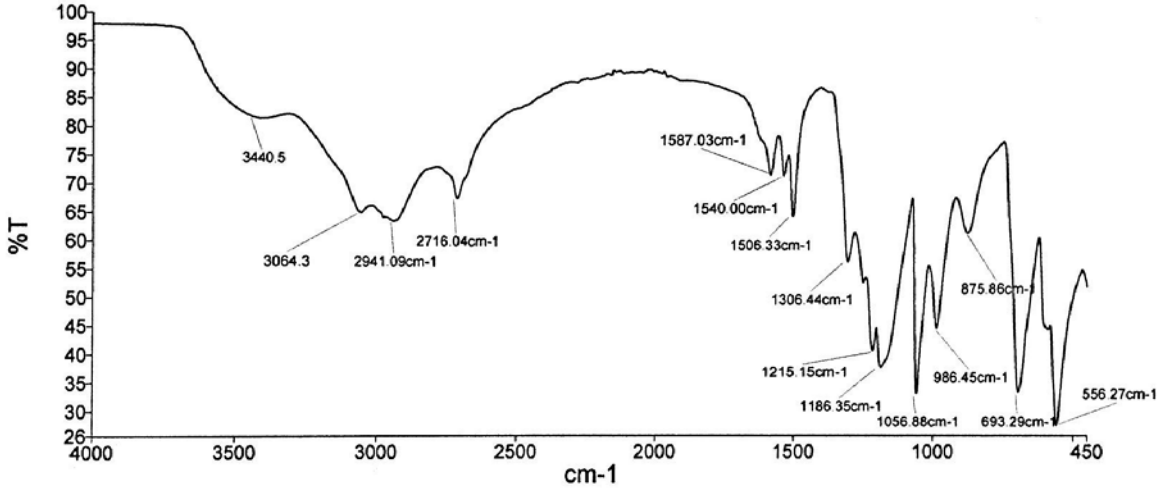

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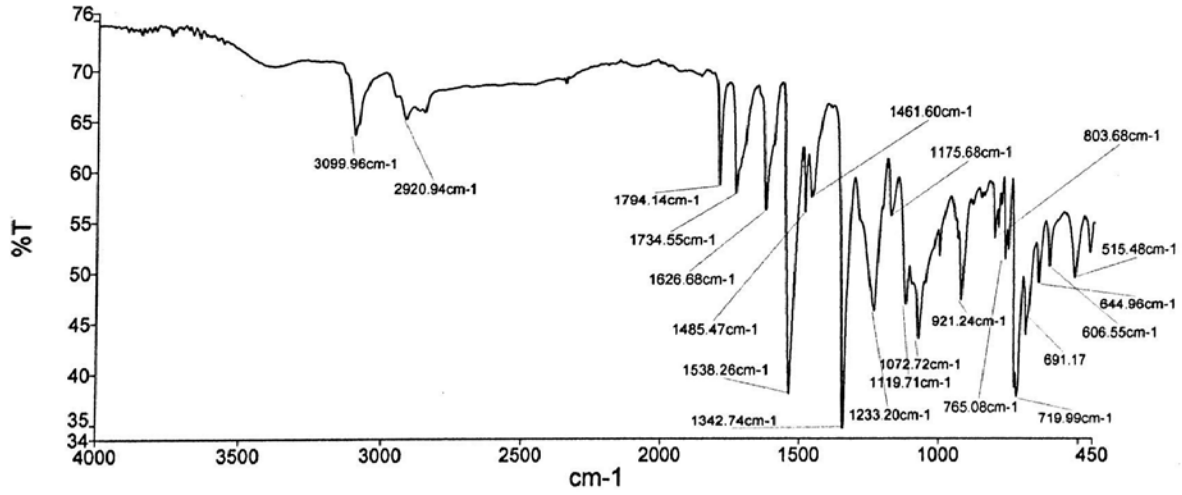

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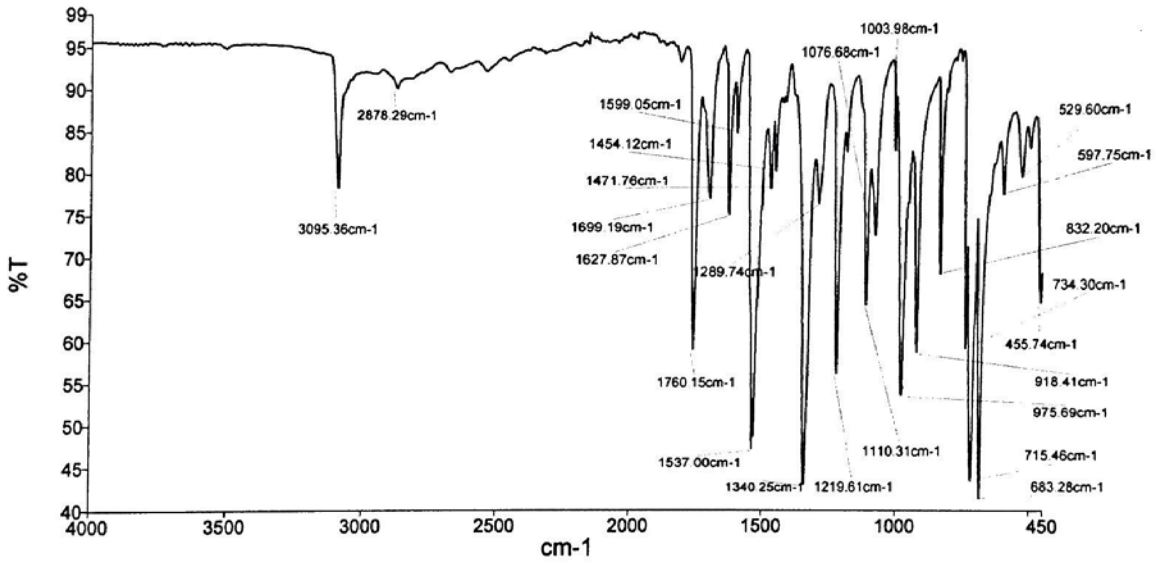
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