SYNTHESIS AND APPLICATION OF FLUORESCENTLY LABELED

MONOCYCLIC THIO- β-LACTAM DERIVITIVES

By

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DEDICATION

Dedicated to Dr. Mark S. Erickson. For his enduring chemical and life lessons, which instilled a love of chemistry, learning, and teaching that have been carried into and beyond the laboratory.

SYNTHESIS AND APPLICATION OF FLUORESCENTLY LABELED MONOCYCLIC THIO- β-LACTAM DERIVITIVES

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ABSTRACT

Novel methodologies for the synthesis of fluorescently labeled monobactam derivatives were developed and explored. Two separate ß-lactam derivatives, 4-(4 aminophenylthio)azetidin-2-one and 4-phenylsulflanyl-azetidin-2-one were labeled with several combinations of fluorophores and electron withdrawing groups allowing for these drugs to be observed within a living bacterial cell. These drugs have shown considerable antimicrobial activity, but are not classified as β-lactamase inhibitors or substrates. Observation of the drugs *in vivo* will provide valuable information as to the molecular targets and specific mechanism of antimicrobial action of these compounds.

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CHAPTER 1

INTRODUCTION

Infections caused by antibiotic-resistant bacteria pose a constant challenge both to physicians and researchers. Growing resistance among infectious gram-positive and gram-negative pathogens has forced the scientific community not only to develop new and innovative antibiotics to combat these advancing strains, but new ways of understanding and observing the mechanisms by which these drugs act.

The monobactam class of penicillin drugs belongs to the β-Lactamase inhibitory class of antibiotics as a subset of the β-Lactam class of general antibiotics[1,2]. These antibiotics operate around the key chemical structure of the four membered β-Lactam ring shown in Figure 1[3].

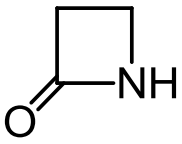


Figure 1: The β-Lactam Ring

These drugs function by inhibiting the production of the bacterial cell wall through competitive inhibition of transpeptidase during cell wall synthesis[4,5,6]. This results in a weak, deformed cell wall that will not survive the regular changes in osmotic cellular pressure. These drugs have shown great success due to their lack of toxicity in humans.

Unfortunately, many pathogens have developed bacterial resistance to β-lactam antibiotics through the production of a β-lactamase enzyme that hydrolyzes the β-lactam unit before it is able to reach the target, rendering the antibiotic inactive[7] (Figure 2).

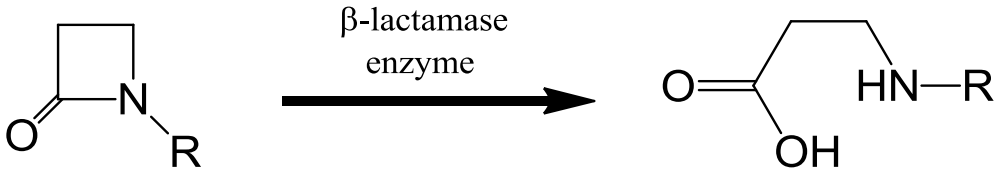


Figure 2: Hydrolysis of a β-lactam by β-lactamase

To contend with this growing multi drug resistant (MDR) machinery, the β-lactamase inhibitory class of drugs was developed[8]. It has been proposed that β-lactamase enzymes all share a common catalytic mechanism within their active sites. A serine residue, which acts as a nucleophile, undergoes acylation followed by deacylation during substrate turnover.[9] β-lactamase inhibitors can be designed in such a manner as to irreversibly inhibit the active serine residue prior to substrate binding (Figure 3), resulting in disruption of enzymatic catalysis[5,10].

Research by Konaklieva et. al. suggests that a leaving group (LG) attached to the 4 Carbon of the ring as well as an electron withdrawing group (EWG) attached at the amide site increases the drug's activity while also providing a certain level of specificity among species of bacteria[11]. For example, C4 thiophenol-substituted β-Lactams have shown high to moderate activity against *Moraxella catarrhalis* and *Mycobacterium tuberculosis*, yet C4 phenol-substituted β-Lactams are inactive against the same two species.

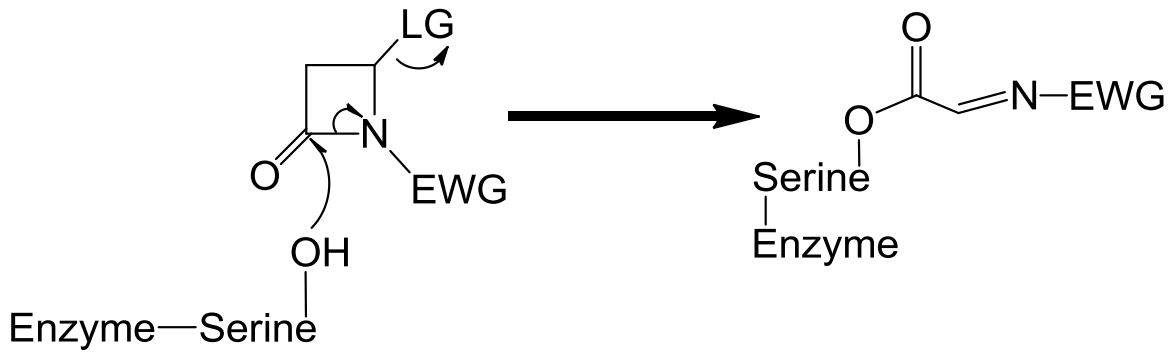


Figure 3: Proposed Inhibitory Mechanism

Taking advantage of this highly conserved mechanism has allowed for the use of β-lactams as serine acetylating agents and excellent models for β-lactamase inhibitory compounds.

Fluorophores as a Method for Observing Drug Reactivity Mechanisms:

We have developed a class of drugs that show considerable antimicrobial activity, but are not classified as β-lactamase inhibitors or substrates. In order to better understand the mechanism of action and molecular target for this new class of drug, fluorescent labels will be attached to the compound to be followed within the cell.

It is believed the EWG serves to increase activity by creating a better electophile at the carbonyl carbon for reaction with the serine hydroxyl group. Physical observation of the EWG and possible leaving group would allow for "tracking" of the molecule once inside the cell as well as after acylation, providing valuable insight into the structure activity relationship and mechanism for these drugs.

Fluorescent labeling is the process of covalently attaching a fluorophore to another molecule, such as a protein or nucleic acid[12]. This is generally accomplished

using a reactive derivative of the fluorophore. Fluorescent labels are commonly used for detection of a protein or other labeled molecule via a fluorescence microscope, flow cytometer or other fluorescence reading instruments. These can be useful in localization of a target within a cell and can be observed in real time[13].

Due to their organic composition and flexibility of functional group attachment, aromatic hydrocarbons provide a readily available library of compounds from which to choose fluorescent labels. One major concern is the ability of the cell to take in the labeled molecule[14,15]. Many fluorescent labels are very large in relation to what a cell would normally consume, some being on the order of 500-600 Da[16,17]. If the labeled molecule is too large to be up taken by the cell, it obviously cannot be tracked.

Objectives:

Novel methodologies for the synthesis of fluorescently labeled monobactam derivatives are developed and explored. Specifically, 4-aminothiophenol is attached to 4-acetoxy-2-azetidinone and used as a "handle" for various combinations of fluorescent compounds and electron withdrawing groups. The compounds are characterized using ^{1}H NMR and ^{13}C NMR spectrometry. Furthermore, the synthesized fluorescent β-lactam derivatives will be sent for biological testing and inhibitory action towards β-lactamases and other serine-containing enzymes.

CHAPTER 2

RESULTS AND DISSCUSION

The goal of the research in this thesis was to develop novel fluorescently labeled monobactam derivatives that could be observed within a living microbial cell. As stated earlier, it is believed that the mechanism of action for this class of drug involves a possible leaving group from the 4 carbon of the lactam ring (Figure 3).

In order to test this hypothesis, both the predicted leaving group and original lactam ring would have to be tagged. Upon acylation both the leaving group and amide will remain tagged, allowing them to be followed throughout the cell (Figure 4).

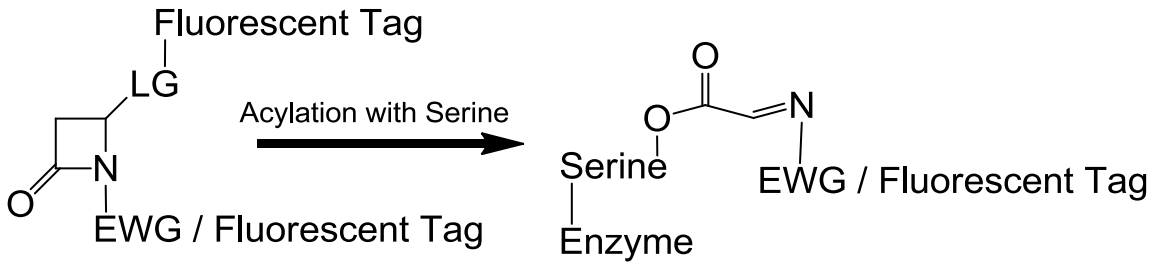
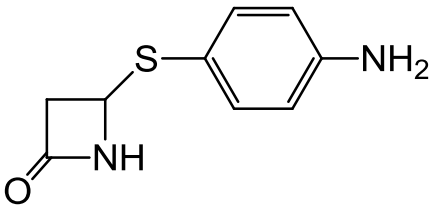
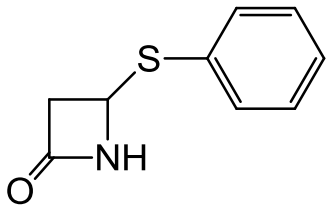


Figure 4: Fluorescently Tagged Lactam Before and After Acylation with Serine Residue

Preparation of a doubly tagged molecule is no trivial matter and novel reaction schemes and methodologies were required. Two separate lactam derivatives, 4-(4 aminophenylthio)azetidin-2-one **(1)** and 4-phenylsulflanyl-azetidin-2-one **(2)**, were chosen to be labeled (Figure 5).



4-phenylsulflanyl-azetidin-2-one **(1)**



4-(4 aminophenylthio)azetidin-2-one **(2)**

Figure 5: Chosen Lactam Derivatives

Using Scheme 1 (Figure 6) both **(1)** and **(2)** were synthesized using previously adapted methodology[18,19]. Both products were purified before being used for further reactions.

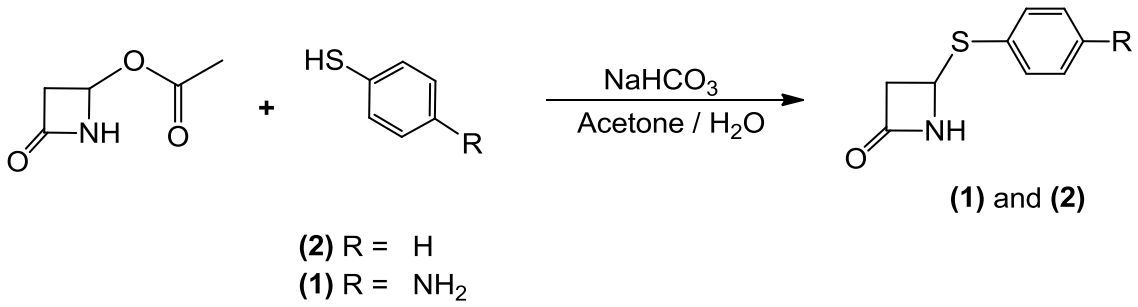
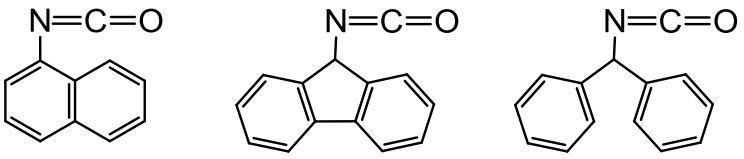


Figure 6: Scheme 1

Preparation of Fluorescently Mono-labeled Lactam Derivatives:

Previous research by Konaklieva et al. has shown that the attachment of an EWG to the secondary amide of **(2)** resulted in significantly increased antimicrobial activity from the lactam alone[7]. The usefulness of **(2)** as a starting material was then three fold. First, **(2)** was used to determine if it was indeed possible to label the secondary amide with a fluorophore. Second, the reaction was used to observe if this attachment could act as an EWG to increase the antimicrobial activity similarly to the EWGs previously mentioned. And third, if this attachment was successful, whether or not the new compound could enter and be traced inside the cell would be assessed.

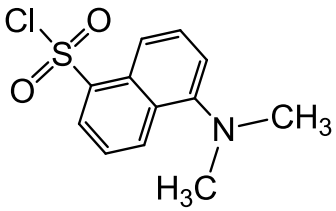
Four unique fluorescent tags were chosen to be attached. It was known that several isocyanate derivatives attach readily to the secondary amide of the lactam ring[2,7]. To this end, the fluorescently active isocyanates1-naphthyl isocyanate **(3)**, 9H-fluoren-9-yl isocyanate **(4)**, and diphenylmethyl isocyanate **(5)** were chosen in an attempt to label the secondary amide (Figure 6).



(3) **(4)** **(5)**

Figure 7: Fluorescent Isocyanates

Additionally, the fluorophore dansyl chloride **(6)** has been shown to readily bind to primary and secondary amines as well as exhibit enzymatic inhibitory characteristics so it was examined as well[8,9,20]. It was our hope that a secondary amide would prove a strong enough nucleophile for reaction. Structure of the dansyl fluorophore is shown in Figure 7.



(6)

Figure 8: Dansyl Chloride Flouorphore

The aromaticity of these compounds provided visible florescence under a 364 nm UVC lamp in methylene chloride solution and on TLC plates. In order to ensure no side

products would form during reaction, the compound **(2)** was used as the starting lactam derivative. Using Scheme 1 **(2)** was synthesized from 4-Acetoxy-2-azetidinone and thiophenol and purified via recrystalization before attachment was attempted. The attachment of the florescent isocyanate was completed using previously adapted methodology [21]. Labels **(3)**, **(4)**, and **(5)** were successfully attached and purified via column chromatography giving the products 1-(2,2-diphenylacetyl)-4-(phenylthio)azetidin-2-one **(7)**, 1-(2,2-diphenylacetyl)-4-(phenylthio)azetidin-2-one **(8)**, and 1-(2,2-diphenylacetyl)-4-(phenylthio)azetidin-2-one **(9)** respectively as shown in Scheme 2 (Figure 8).

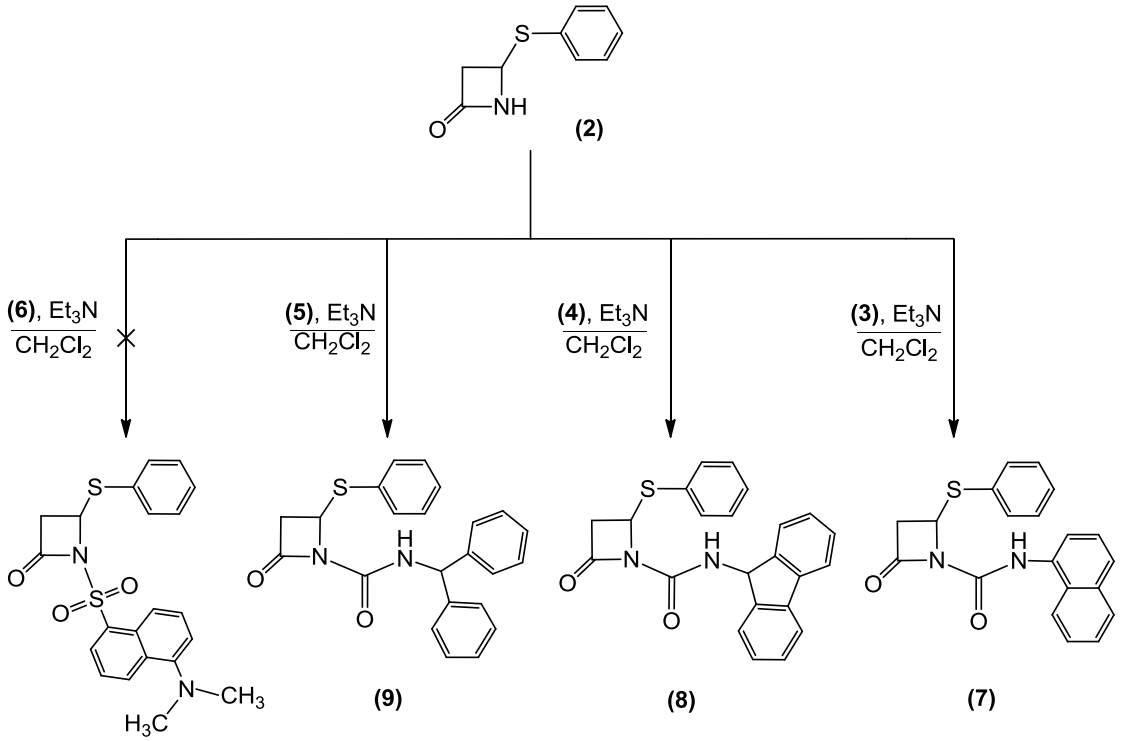


Figure 9: Scheme 2

All attempts to react **(6)** and **(2)** were unsuccessful. It may be that the secondary amide is simply not a strong enough nucleophile for the reaction to take place.

Table 1 shows the observed maximum wavelength intensity (λ_{max}) of each fluorophore and its respective product in a solution of methylene chloride. A slight shift was seen in the λ_{max} each time a fluorophore was attached to the amide of **(2)**. Dansyl chloride was not observed to fluoresce at 364 nm before attachment to **(2)**. This is due to the chlorine still attached to the flouorophore which causes fluorescence at other wavelengths[22].

Table 1: Observed λ_{max} at 365 nm for standard fluorophores and synthesized fluorescent compounds

Compound (#)	**λ max (nm)**
3	392
7	422
4	400
8	491
5	475
9	489
6	-
11	529

Preparation a Di-substituted Dansyl Product:

In order to bolster the ever-expanding library of antimicrobial candidates, **(1)** was synthesized using previously adapted methodology[18]. This compound contains both a secondary amide and secondary amine, effectively giving the "handles" on which to place fluorescent labels both on the proposed leaving group and the lactam amide.

In order to test the reactivity of **(1)** towards substitution at these sites, benzyl isocyanate **(10)** was reacted with **(1)** in attempts to populate only the lower amide in Scheme 3. However upon reaction, the primary amine proved to be a superior nucleophile and a mixture of mono-, di-, and tri-substituted products was obtained.

While it is believed that each of these products was synthesized the mixture proved very difficult to separate into the substituted products. It was determined that they were of little interest to this study and therefore were not purified.

We hypothesize that once the electrophile is attached to the primary amine, the reaction becomes competitive between the amide and newly formed secondary amine, resulting in the multi-substituted mixture.

Taking advantage of the fact that **(6)** was shown to be un-reactive with the secondary amide, Scheme 3 (Figure 9) was attempted with the fluorophore as the new electrophile. As expected, **(6)** was doubly attached to the primary amine of **(1)** while remaining un-reactive with the amide to give the product 6-(dimethylamino)-N-(6-(dimethylamino)naphthalen-2-ylsulfonyl)-N-(4-(4-oxoazetidin-2-ylthio)phenyl) naphthalene-2-sulfonamide **(11)**.

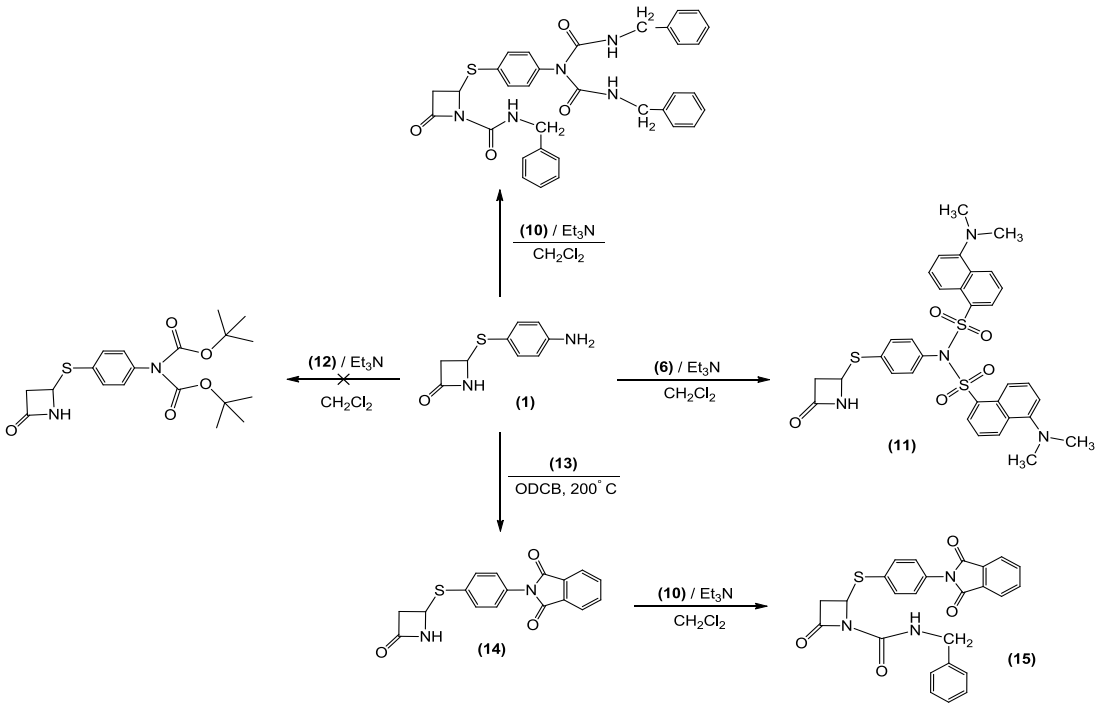


Figure 10: Scheme 3

Preparation of Protected Lactam Derivatives:

The competitive reaction between the nucleophilic amine and amide sites of **(1)** with isocyanates could be remedied by the use of protective groups. If the secondary amine could be protected prior to isocyanate reaction, then the amide can be populated independently of the more reactive amine and competition avoided. The amine could then be de-protected and populated with a second group.

Initial attempts to protect with di-*tert*-butyl dicarbonate **(12)** were unsuccessful, yielding starting materials and a mixture of partially protected products. Formation of an *N*-Phthalamide from phthalic anhydride **(13)** proved successful with adapted methodology from Sasaki et al. in Scheme 3 [23]. Product **(13)** was reacted with an equimolar amount of **(1)** to yield 2-(4-((4-oxoazetidin-2-yl)thio)phenyl)isoindoline-1,3-dione **(14)** in good yields. Compounds **(14)** and **(10)** were successfully reacted to give a protected amide and the product 2-(4-((4-oxo-1-(2-phenylacetyl)azetidin-2-yl)thio)phenyl) isoindoline-1,3-dione **(15)** as shown in Figure 9.

Anti-Microbial Testing

In order to determine if cellular uptake of the labeled compound can occur, all compounds were tested against M. cat and MTb to obtain minimum inhibitory concentrations. Data is shown in Table 1. Most compounds required high concentration to show any activity with the cell. However, compound **(7)** showed excellent activity against MTb at concentrations of 25μM. This may be due to the flat naphthyl structure of (**3)** having less difficulty upon entering the cell that other non-planar groups.

Additionally, **(11)** was effective at low concentrations against M. cat. The planarity of the attached dansyl groups may again account for this ability to enter the cell.

Table 2: Minimum inhibitory concentrations against M. cat and MTb with or without the presence of Clavulanic acid

Comp. #	*M. cat*[*]	MTb (H37Rv)[**]	MTb (H37Rv)[*]
1	>500	>100	>100
7	NT	25	25
8	NT	>100	>100
9	NT	25	50
11	25	>100	>100
14	NT	>300	>300
15	NT	>150	>150
Penicillin	3.1	3.1	3.1

NB: Penicillin is a culture standard

[a] NT = Not Tested

[*] Without Clavulanic acid

[**]With Clavulanic acid

Further Research:

Further research opportunities are now readily available for these compounds. The isocyanates **(3)**, **(4)**, and **(5)** can now be attached to the amide on compound **(14)**. Subsequent de-protection of these compounds as well as **(15)** will allow for new groups such as dansyl chloride or another fluorescent label to be attached doubly to the amine. Additionally, electron withdrawing groups known to increase antimicrobial activity can now be selectively added to either the amide or amine to further expand the library of antimicrobial candidates.

CHAPTER 3

EXPERIMENTAL

All reactions were carried out at room temperature under normal atmospheric pressure unless otherwise noted. Microwave irradiation took place using a CEM *Discover* laboratory microwave. All reagents and solvents were obtained from commercial suppliers and used without any further purification. Unless otherwise noted, reaction mixtures were magnetically stirred and reactions were monitored by thin layer chromatography (TLC) using glass-backed analytical TLC plates coated with silica G with a UV254 indicator (Sorbent Technologies Silica G TLC plates); the chromatograms were visualized under ultraviolet light and/or by staining with an iodine silica chamber.

Product yields refer to chromatographically and spectroscopically pure compounds, unless otherwise noted. All NMR spectra (25°C) were obtained at 400 MHz for proton NMR spectra and 125 MHz for carbon-13. NMR were recorded with the use of an internal deuterium lock at ambient temperature with a Bruker 400 spectrometer (Billerica, MA) in $CDCl_3$. Chemical shifts are given in d units, using the signal at d = 7.27 for residual $CHCl_3$ in $CDCl_3$ as an internal standard. Chemical shifts are reported in δ units (δTMS = 0 ppm to downfield), with the signal multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bds = broad), coupling constant(s) (J in Hz) and integration area in parentheses. ^{13}C NMR spectra were determined using the signal for residual $CHCl_3$ in $CDCl_3$ at δ = 77.16 as an internal standard.

All fluorescence emission spectra were obtained with an Ocean Optics USB4000-FL-395 Spectrofluorometer with an illumination source of 365 nm using an Entela UVGL-15 compact UV lamp.

Synthesis of 4-(4 aminophenylthio)azetidin-2-one (1)

The synthetic procedure was adopted from Grimm et al. and Wasserman et al. 4-Acetoxy-2-azetidinone (1.00 g, 7.74 mmol) was dissolved into a mixture of deionized water (30 mL) and acetone (50 mL). To this solution was added 4-Aminothiophenol (1.03 g, 8.23 mmol) and sodium bicarbonate (2.40 g, 30.9 mmol) and the mixture was stirred at room temperature for 14 hours. Upon completion of stirring, sodium chloride (~15 g) was gradually added to the solution while stirring resulting in a separation of layers within the reaction vessel. The solution was filtered and organics were extracted with ethyl acetate (50 mL aliquots). Collected organic fractions were dried over anhydrous magnesium sulfate, filtered, and concentrated *in vacuo* to yield a bright yellow gel. The crude product was purified by column chromatography with a linear mobile phase gradient (10:1 hexanes/ethyl acetate to 1:10 hexanes/ethyl acetate) affording **1** (2.37 g, 78.8%) as a light yellow crystal. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.83 (1H, ddd J= 11.41, 3.01, 1.95); 3.28 (1H, ddd, J=8.34, 2.99, 1.95); 3.87 (1H, s) 4.85 (1H, dd, J=2.33, 2.58); 6.01 (1H, s, bds); 6.53 (2H, d, J=8.60); 7.10 (2H, d, J=8.59). ^{13}C NMR (125 MHz, $CDCl_3$): δ 44.62, 54.75, 115.62, 117.02, 131.11, 147.83, 165.94.

Synthesis of 6-(dimethylamino)-N-(6-(dimethylamino)naphthalen-2-ylsulfonyl)-N-(4-(4-oxoazetidin-2-ylthio)phenyl)naphthalene-2-sulfonamide (11)

A solution of **1** (0.101 g, 0.519 mmol) and triethylamine (15.2 µL, 0.109 mmol) in methylene chloride (5 mL) was stirred for 0.5 hours. Dansyl chloride (0.284 g, 1.07 mmol) was added to the solution and was irradiated with microwaves under pressure at 90º C for 20 minutes. The dark yellow solution was washed with 5% HCl (10 mL aliquots) and resulting organics were concentrated *in vacuo* to yield a dark yellow, viscous liquid. The crude product was purified by column chromatography with a linear mobile phase gradient (10:1 methylene chloride/ethyl acetate to 1:10 methylene chloride/ethyl acetate) affording **2** (0.16 g, 45.9%) as a light yellow crystal. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.81 (12H s) 3.35 (1H, ddd, J=10.42, 3.10, 1.87) 4.94 (1H, dd J=4.97, 4.37) 6.37 (1H, s, bds), 7.04 (4H, m); 7.72 (2H, d J=8.55); 8.24 (2H, dd 2.41, 8.59); 8.56 (2H, d J=8.41) NB: 1H peak at 2.81 dd is obscured by large methyl H singlet.^{13}C NMR (125 MHz, $CDCl_3$): δ 44.36, 53.32, 59.47, 114.35, 117.56, 119.29, 122.11, 124.10, 127.65, 128.73, 129.93, 133.20, 134.49, 137.04, 150.82, 165.91.

Synthesis of 2-(4-((4-oxoazetidin-2-yl)thio)phenyl)isoindoline-1,3-dione (14)

A mixture of **1** (0.900 g, 4.63 mmol) and phthalic anhydride (0.755 g, 5.09 mmol) was dissolved in orthodichloro benzene (10 mL). The reaction vessel was set in a silicon oil bath and the solution was allowed to reflux at 200º C for 2 hours. Upon cooling to room temperature the solution was dissolved in ethyl acetate and washed with saturated aqueous sodium bicarbonate solution (30 mL aliquots), deionized water (30 mL aliquots), and brine (30 mL aliquots). The resulting organics were combined and dried over

anhydrous magnesium sulfate, filtered, and concentrated *in vacuo* to yield a chalky white powder. The crude product was purified by column chromatography with a linear mobile phase gradient (10:1 hexanes/methanol to 1:10 hexanes/methanol) affording **3** (1.03 g, 68.7%) as a granular yellow crystal. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.97 (1H, ddd J= 11.65, 1.46, 1.07); 3.28 (1H, ddd, J=3.04, 2.01, 1.92); 5.14 (1H, dd, J= 2.59, 2.37); 6.39 (1H, s, bds); 7.43 (2H, d, J=8.43); 7.51 (2H, d, J= 8.51); 7.82 (2H, dd, J= 2.49, 2.99); 7.93 (2H, dd, J= 2.30, 3.01) ^{13}C NMR (125 MHz, $CDCl_3$)δ 45.74, 54.33, 123.95, 127.16, 131.57, 131.95, 131.99, 133.36, 134.68, 165.58, 167.00.

Synthesis of 2-(4-((4-oxo-1-(2-phenylacetyl)azetidin-2-yl)thio)phenyl)isoindoline-1,3-dione (15)

A solution of **14** (0.101 g, 0. 311 mmol) and triethylamine (0.111 mL, 1.09 mmol) in methylene chloride (5 mL) was stirred for 0.5 hours. Benzyl isocyanate (98.2 µL, 0.795 mmol) was added to the solution and was irradiated with microwaves under pressure at 70º C for 15 minutes. The clear solution was washed with 5% HCl (10 mL aliquots) and resulting organics were concentrated *in vacuo* to yield a clear, viscous liquid. The crude product was purified by column chromatography with a linear mobile phase gradient (10:1 methylene chloride/ethyl acetate to 1:10 methylene chloride/ethyl acetate) affording **4** (0.16 g, 46.1%) as a clear, colorless gel. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.92 (1H, dd J= 13.77, 2.71); 3.44 (1H, dd, J=10.81, 5.71); 4.51 (2H, dt, J= 8.83, 8.15, 5.92); 5.30 (1H, dd, J=2.99, 2.67); 6.79 (1H, s, bds); 7.15 (4H, m); 7.49 (7H, m); 7.60 (2H, dt, J= 5.98, 1.03, 1.94). ^{13}C NMR (125 MHz, $CDCl_3$): δ 45.40, 54.23, 128.76, 129.45, 131.25, 133.60

Synthesis of 4-phenylsulf lanyl-azetidin-2-one (2)

The synthetic procedure was adopted from Grimm et al. and Wasserman et al. 4-Acetoxy-2-azetidinone (1.00 g, 7.74 mmol) was dissolved into a mixture of deionized water (30 mL) and acetone (50 mL). To this solution was added thiophenol (0.95 mL, 9.29 mmol) and sodium bicarbonate (2.40 g, 30.9 mmol) and the mixture was stirred at room temperature for 24 hours. Upon completion of stirring, sodium chloride (~15 g) was gradually added to the solution resulting in a separation of layers within the reaction vessel. The solution was filtered and organics were extracted with ethyl acetate (50 mL aliquots). Collected organic fractions were dried over anhydrous magnesium sulfate, filtered, and concentrated *in vacuo* to yield a bright yellow liquid. The crude product was purified by recrystallization from methylene chloride/hexanes to afford **5** (2.37 g, 78.8%) as a clear yellow crystal. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.92 (1H, ddd J= 11.68, 1.51, 0.57); 3.40 (1H, ddd, J=8.46, 3.07, 1.86); 5.04 (1H, dd, J= 2.26, 2.61); 6.15 (1H, s, bds); 7.29 (1H, s); 7.39 (2H, t, J=2.84); 7.49 (2H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 45.40, 54.23, 128.76, 129.45, 131.25, 133.60 NB: C=O peak obscured by background noise.

1-(2,2-diphenylacetyl)-4-(phenylthio)azetidin-2-one (7)

To a solution of **2** (0.139 g, 0. 775 mmol) and 1-naphthyl isocyanate (0.115 mL, 0.679 mmol) in methylene chloride (5 mL) was added triethylamine (0.111 mL, 1.09 mmol). The solution was irradiated with microwaves under pressure at 70º C for 20 minutes. The solution was then washed with 5% HCl (10 mL aliquots) and resulting organics were purified by column chromatography with a linear mobile phase gradient (10:1 methylene chloride/ethyl acetate to 1:10 methylene chloride/ethyl acetate) affording **6** (0.21 g,

82.5%) as a clear, colorless gel. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.05 (1H, dd J= 13.65, 2.74); 3.56 (1H, dd, J=10.73, 5.68); 5.54 (1H, dd, J= 2.91, 2.76); 7.40 (1H, s); 7.42 (4H, m); 7.56 (2H, m); 7.66 (1H, m); 7.72 (1H, d, J=8.25); 7.93 (1H, dd, J=9.22, 8.39); 8.21 (1H, d, J=7.01); 9.09 (1H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 44.15, 57.24, 118.59, 120.14, 125.20, 125.73, 125.86, 126.18, 126.66, 128.83, 129.34, 129.44, 131.69, 134.01, 135.33, 147.45, 166.30.

1-(2,2-diphenylacetyl)-4-(phenylthio)azetidin-2-one (8)

To a solution of **2** (0.119 g, 0.664 mmol) and 9H-fluoren-9-yl isocyanate (0.141 g, 0.798 mmol) in methylene chloride (5 mL) was added triethylamine (95.3 µL, 0.683 mmol). The solution was irradiated with microwaves under pressure at 70º C for 15 minutes. The solution was washed with 5% HCl (10 mL aliquots) and the resulting organics were purified by column chromatography with a linear mobile phase gradient (10:1 methylene chloride/ethyl acetate to 1:10 methylene chloride/ethyl acetate) which gave the product **7** (0.22 g, 89.7%) as a yellow crystal. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.67 (1H, dd J= 13.66, 2.75); 3.19 (1H, dd, J=10.74, 5.67); 5.18 (1H, dd, J= 2.93, 2.73); 5.95(2H, d, J=8.79); 6.55 (1H, d, J=8.78); 7.18 (8H, m); 7.45 (2H, m); 7.50 (2H, m) ^{13}C NMR (125 MHz, $CDCl_3$): δ 41.81, 52.55, 54.56, 117.89, 122.86, 125.63, 126.69, 127.05, 127.14, 133.07, 138.31, 141.36, 141.66, 148.16.

1-(2,2-diphenylacetyl)-4-(phenylthio)azetidin-2-one (9)

To a solution of **2** (0.107 g, 0. 596 mmol) and diphenylmethyl isocyanate (0.116 mL, 0.615 mmol) in methylene chloride (5 mL) was added triethylamine (85.5 µL, 0.614 mmol). The solution was irradiated with microwaves under pressure at 70º C for 20

minutes. The solution was washed with 5% HCl (10 mL aliquots) and the resulting organics were purified by column chromatography with a linear mobile phase gradient (10:1 methylene chloride/ethyl acetate to 1:10 methylene chloride/ethyl acetate) which gave the product **7** (0.19 g, 86.3%) as a light brown gel. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.91 (1H, dd J= 13.66, 2.74); 3.44 (1H, dd, J=10.77, 5.67); 5.29 (1H, dd, J= 2.86, 2.74); 5.99 (1H, s, bds);7.21 (1H, s); 7.31(10H, m); 7.54 (4H, d, J=7.78).

CHAPTER 4

CONCLUSIONS

Novel methodologies for the synthesis of fluorescently labeled monobactam derivatives were developed and explored. Two separate ß-lactam derivatives, 4-(4 aminophenylthio)azetidin-2-one and 4-phenylsulflanyl-azetidin-2-one were labeled with several combinations of fluorophores and electron withdrawing groups allowing for these drugs to be observed within a living bacterial cell. Observation of the drugs *in vivo* will provide valuable information as to the structure activity relationship and exact mechanism of action of these compounds. The protection strategies developed here will allow for a more diverse library of lactam compounds to be synthesized, further aiding in the ongoing struggle against these rapidly adapting bacteria.

APPENDIX A

LIST OF SPECTRA

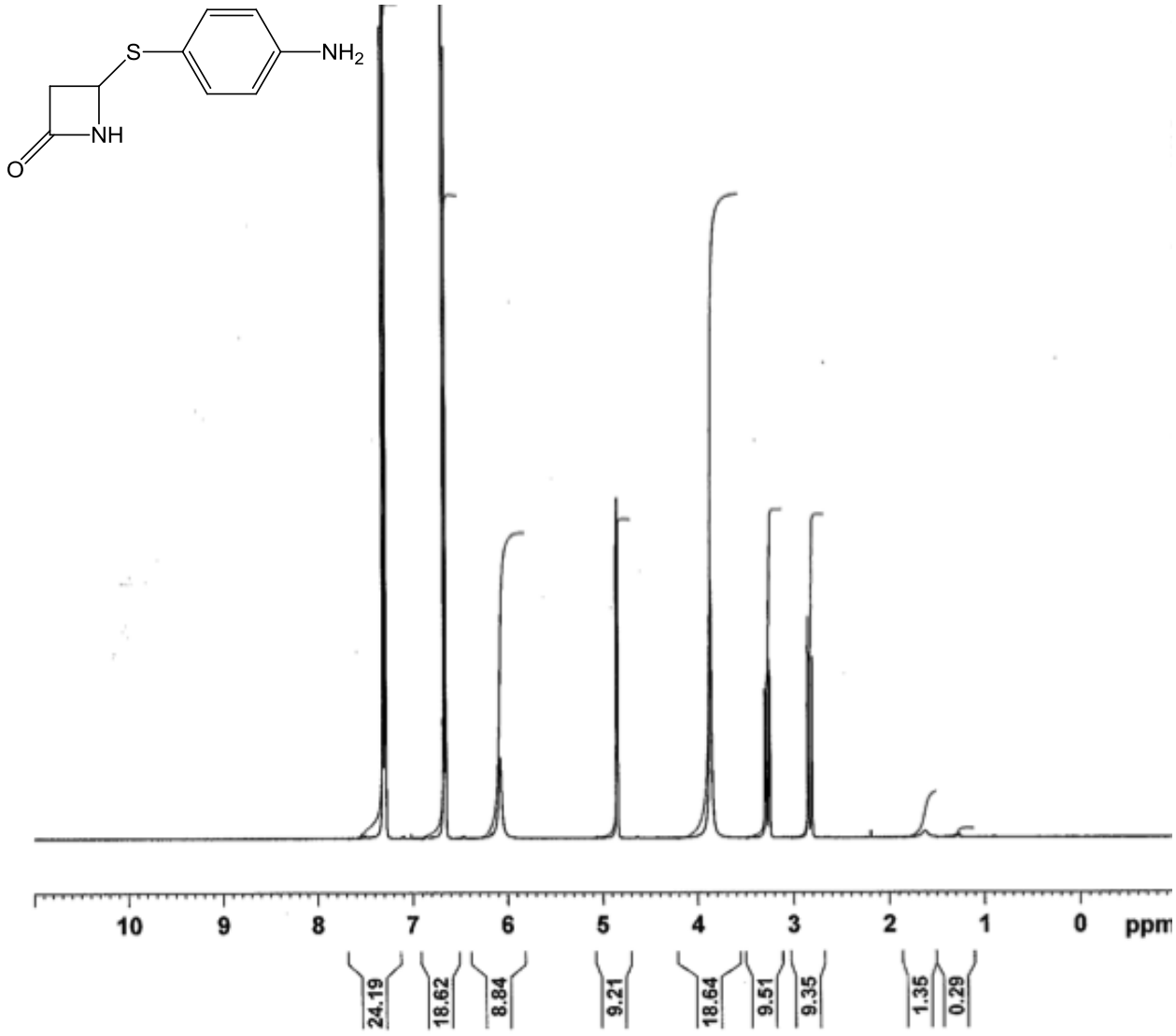


Figure 11: 1H NMR of compound **(1)**

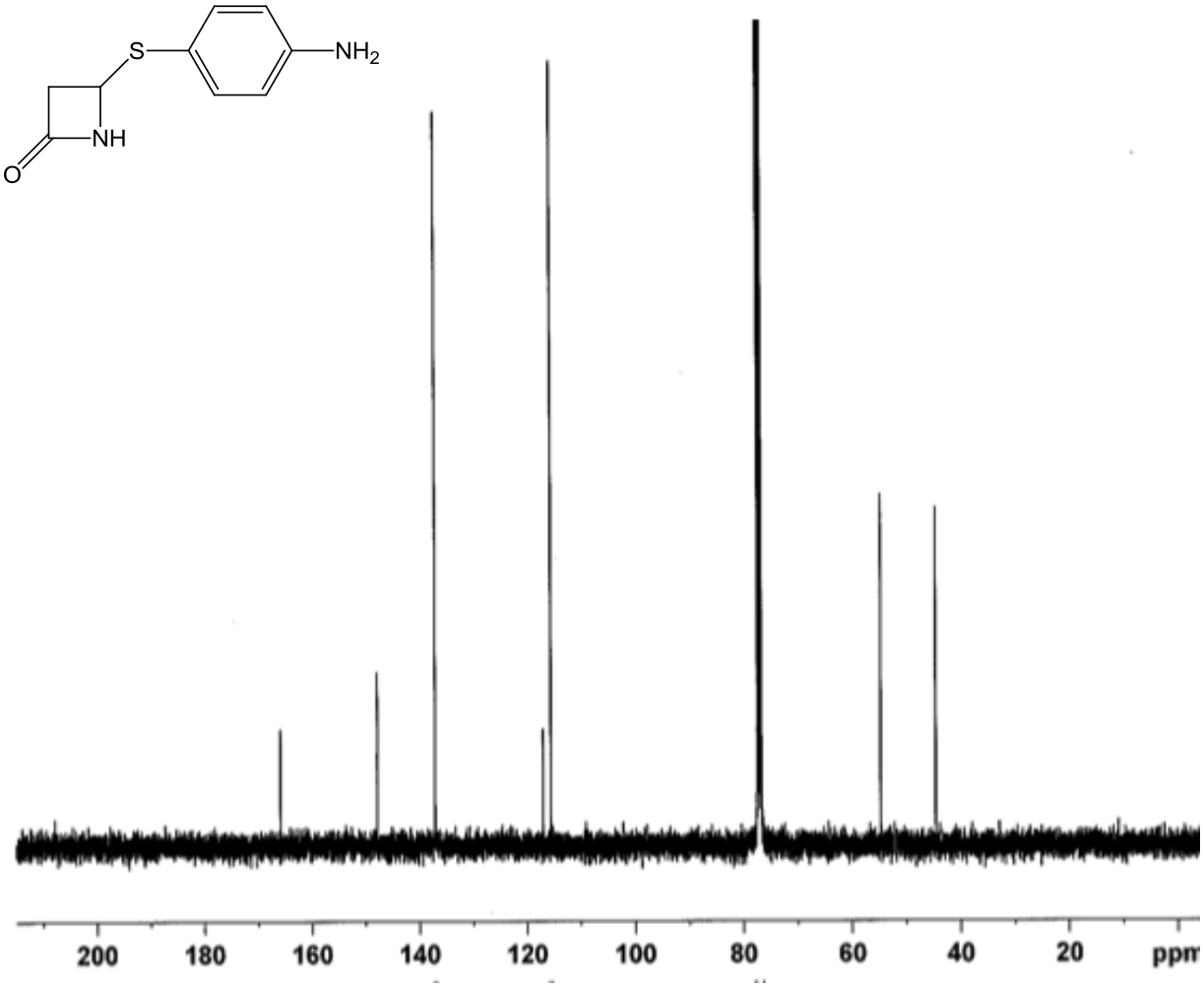


Figure 12 ^{13}C NMR of compound **(1)**

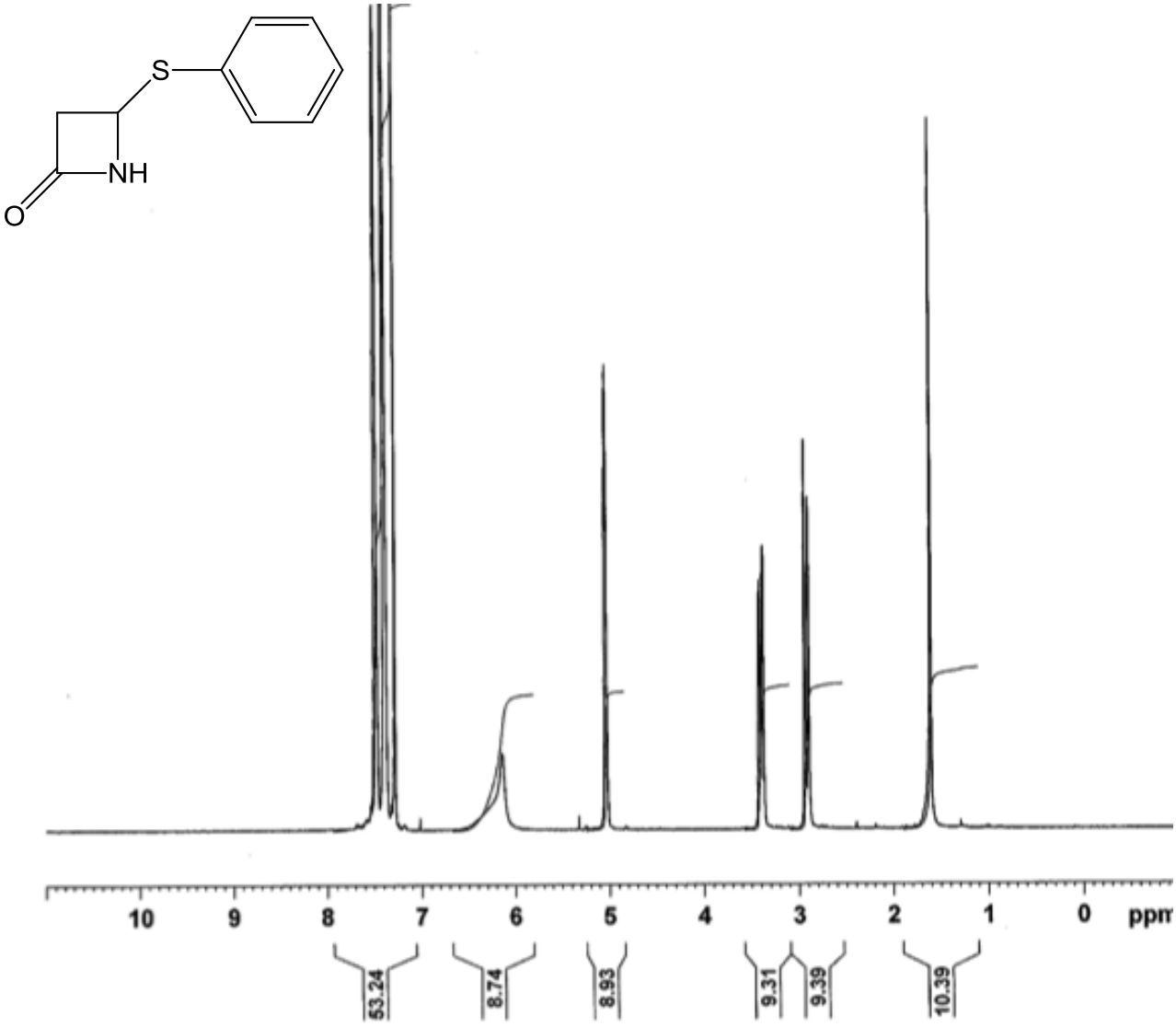


Figure 13: ^{1}H NMR of compound **(2)**

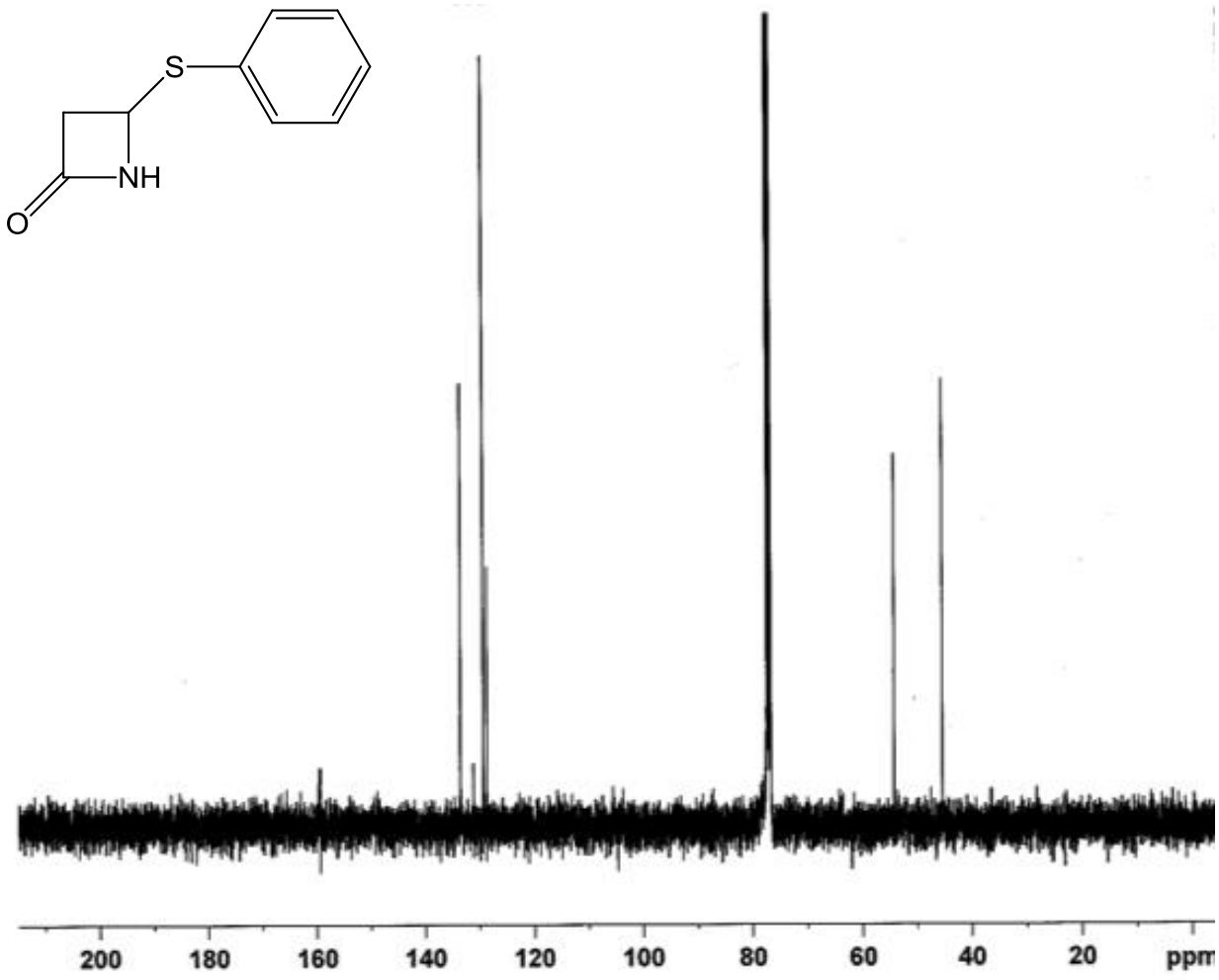


Figure 14: ^{13}C NMR of compound **(2)**

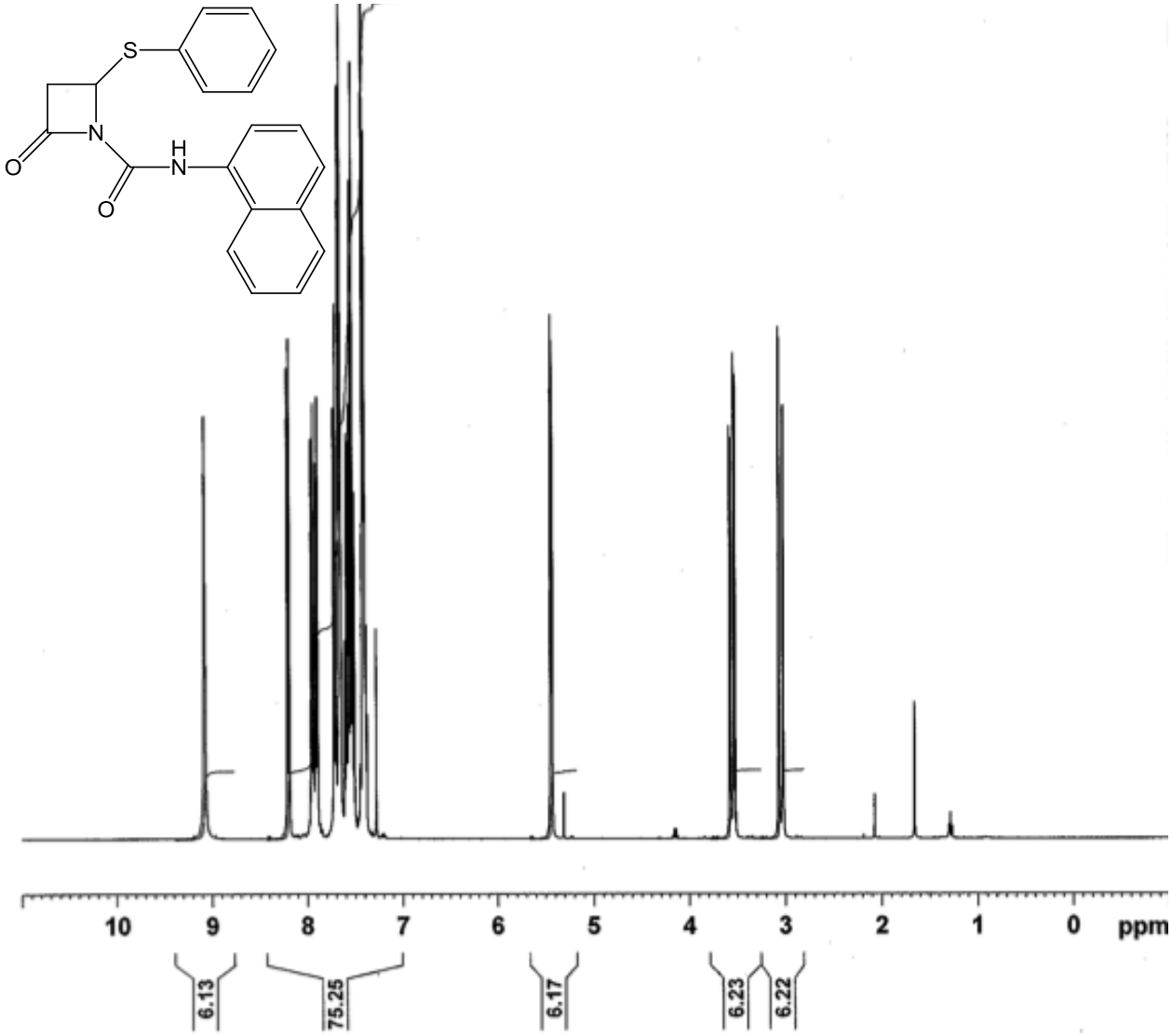


Figure 15: ^{1}H NMR of compound **(7)**

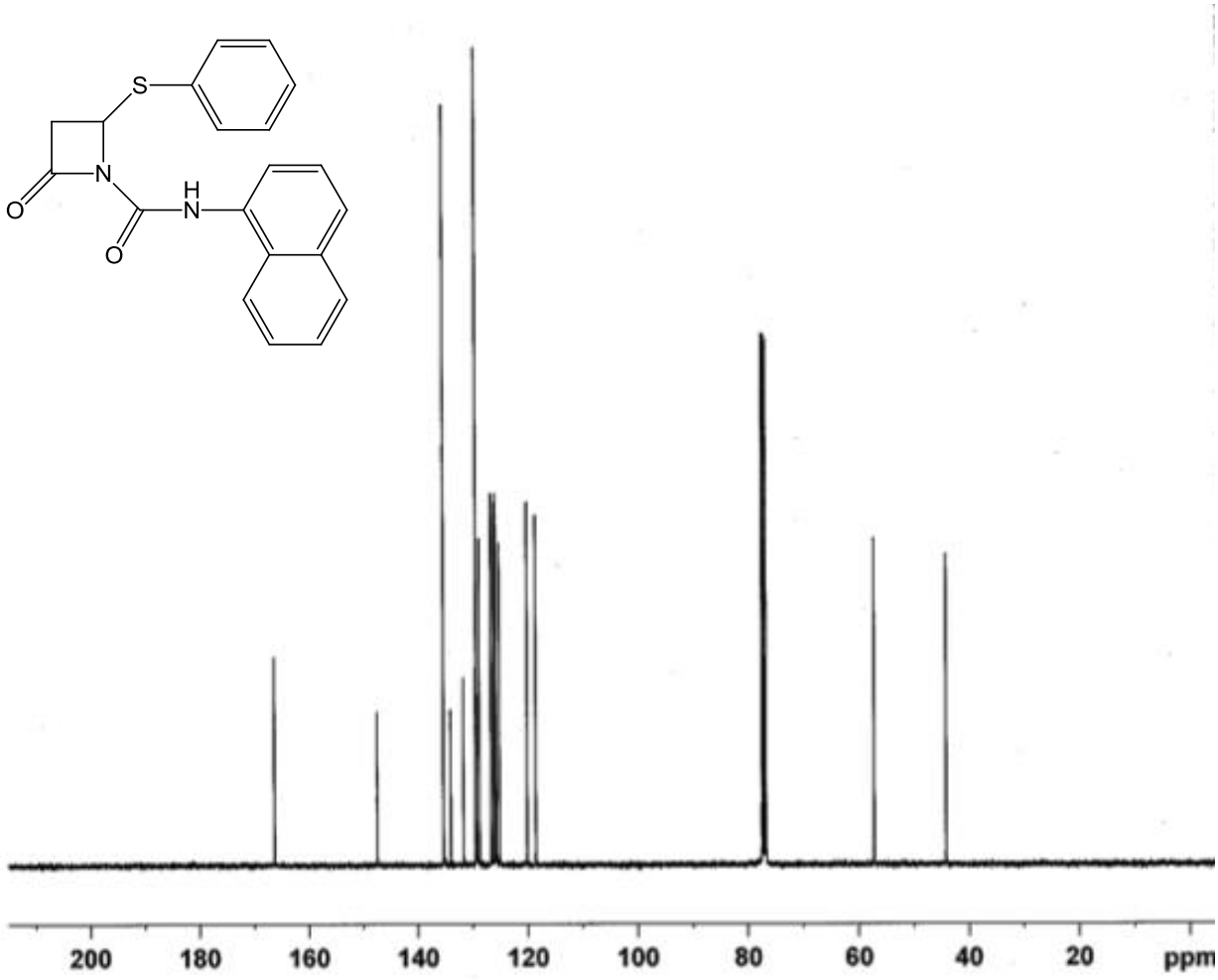


Figure 16: ^{13}C NMR of compound **(7)**

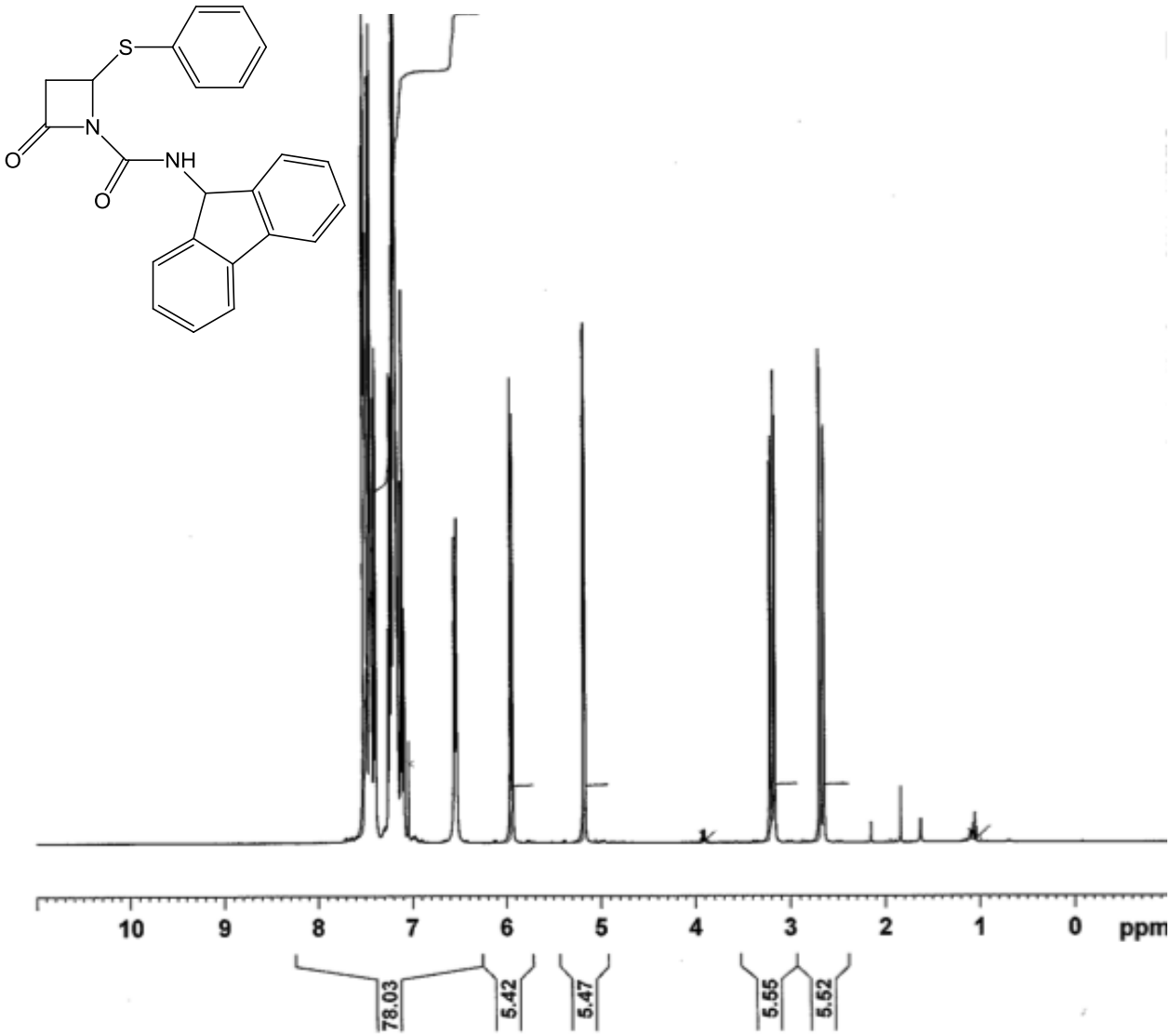


Figure 17: ^{1}H NMR of compound **(8)**

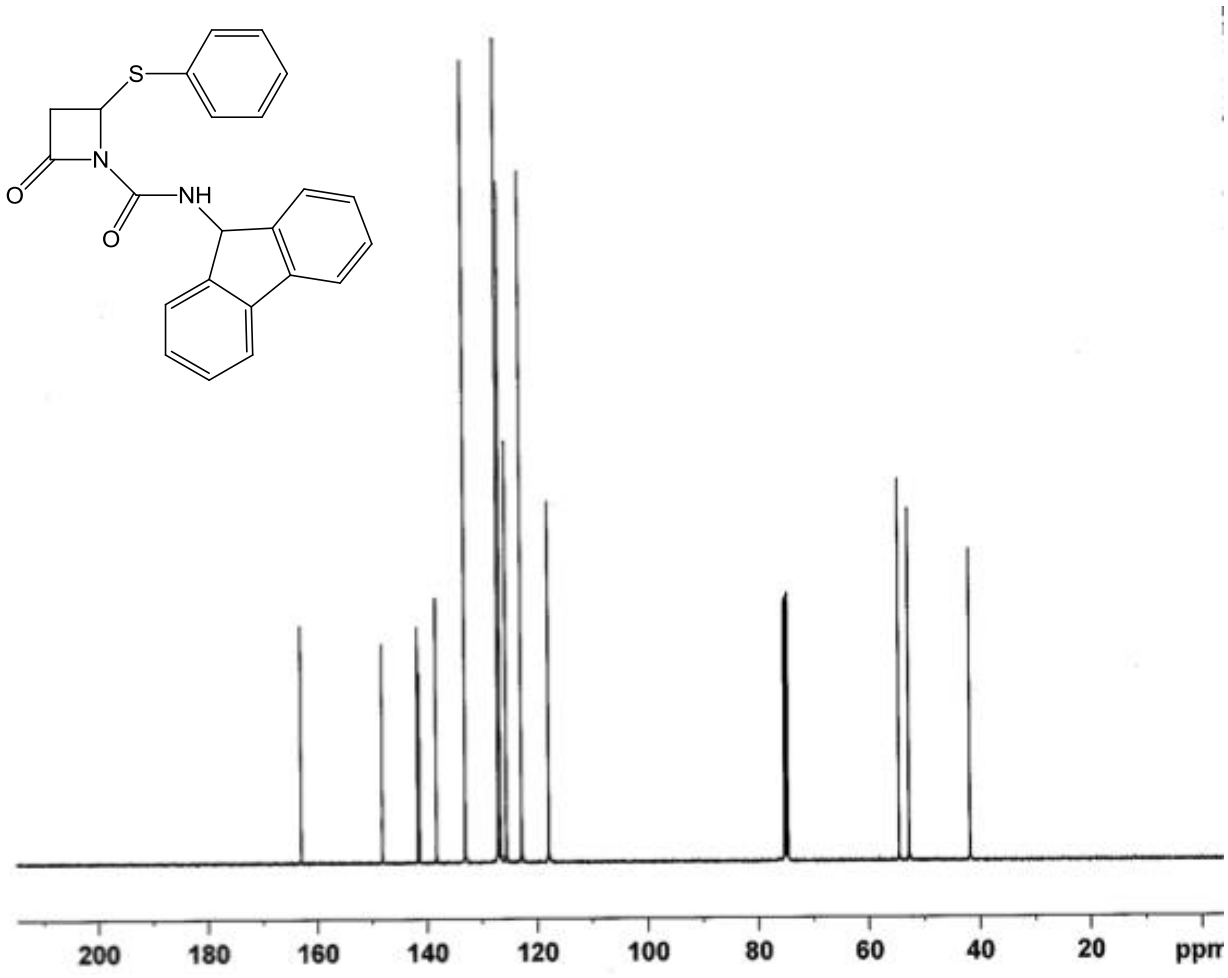


Figure 18: ^{13}C NMR of compound **(8)**

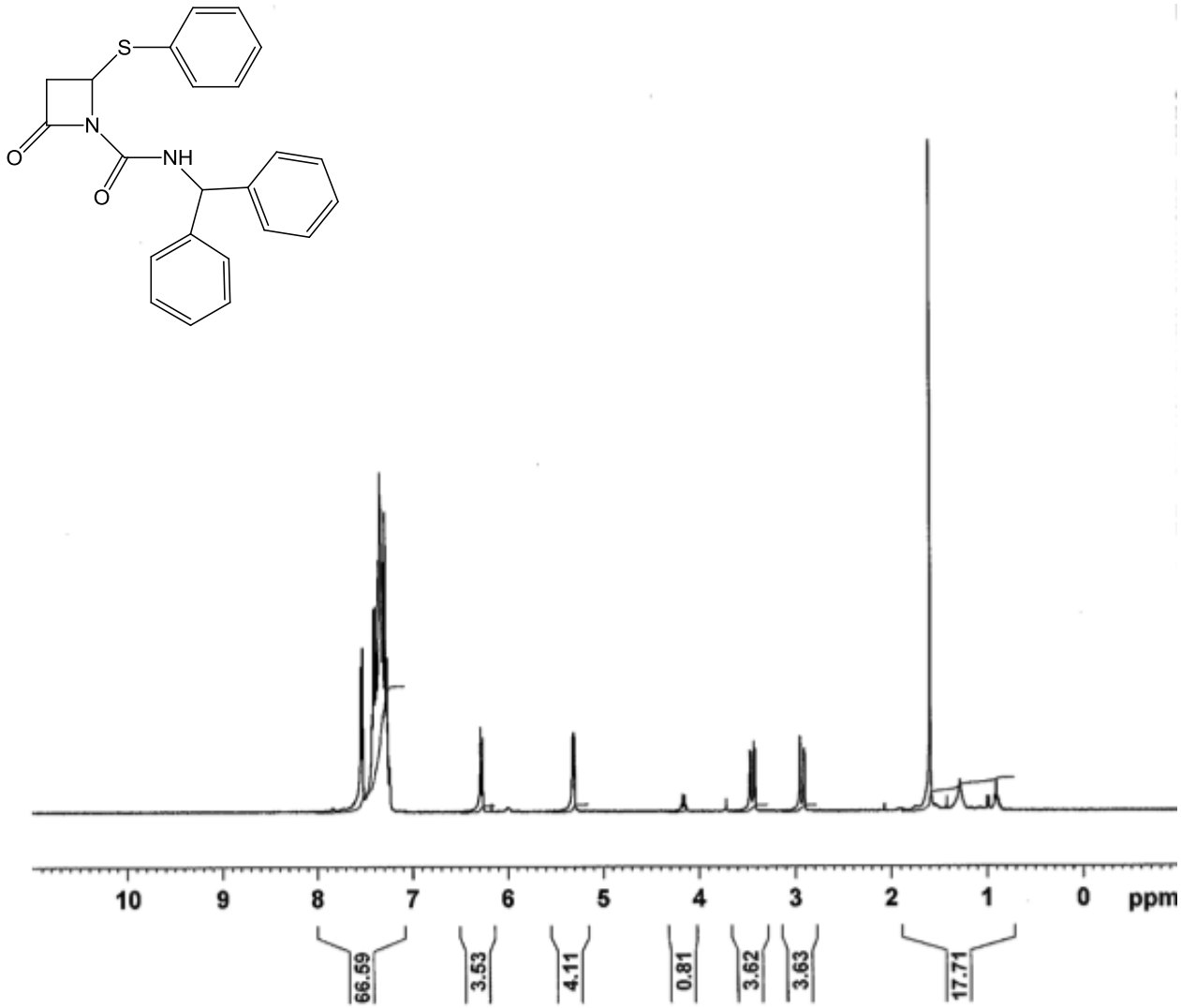


Figure 19: ^{1}H NMR of compound **(9)**

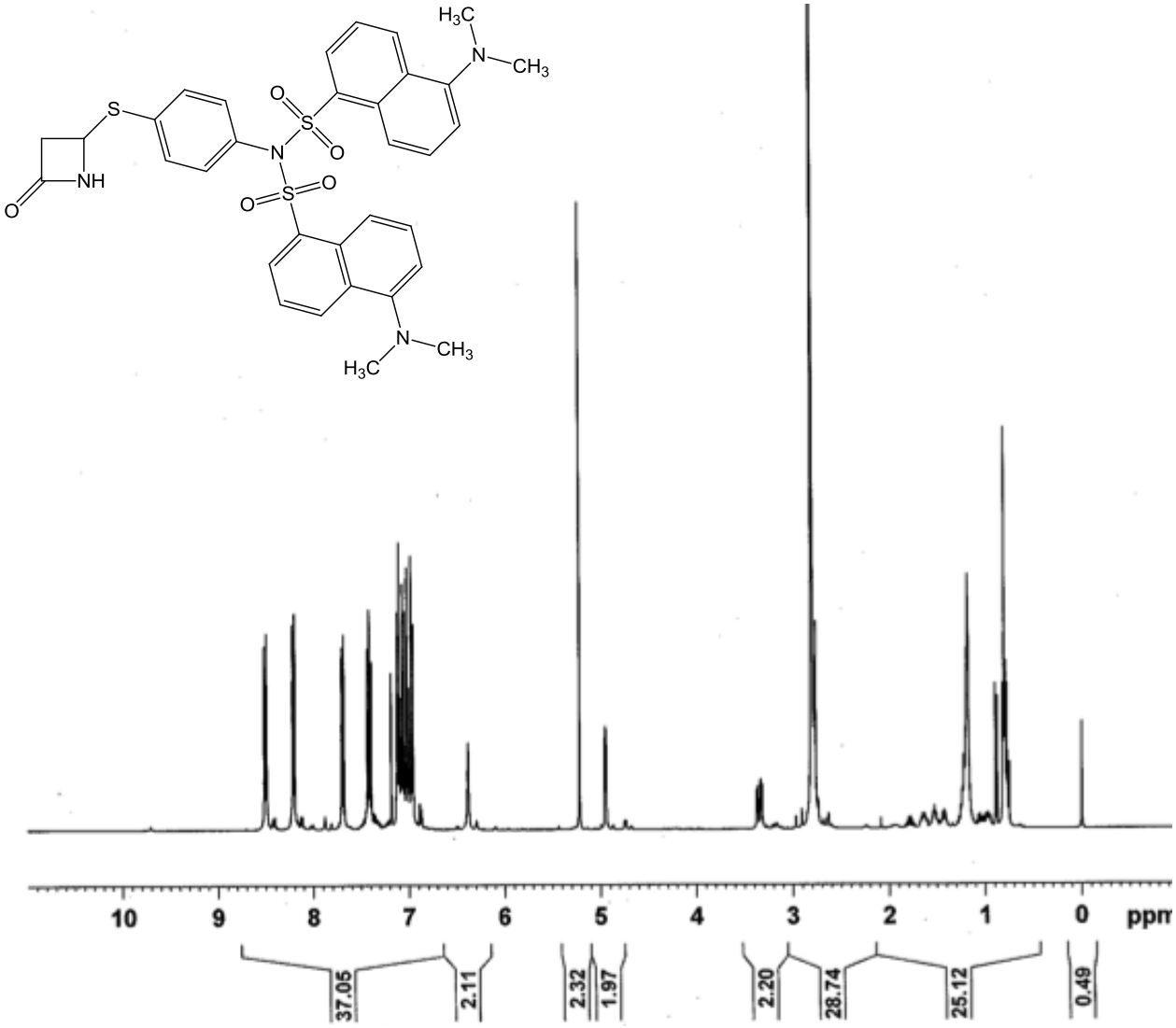


Figure 20: ^{1}H NMR of compound **(11)**

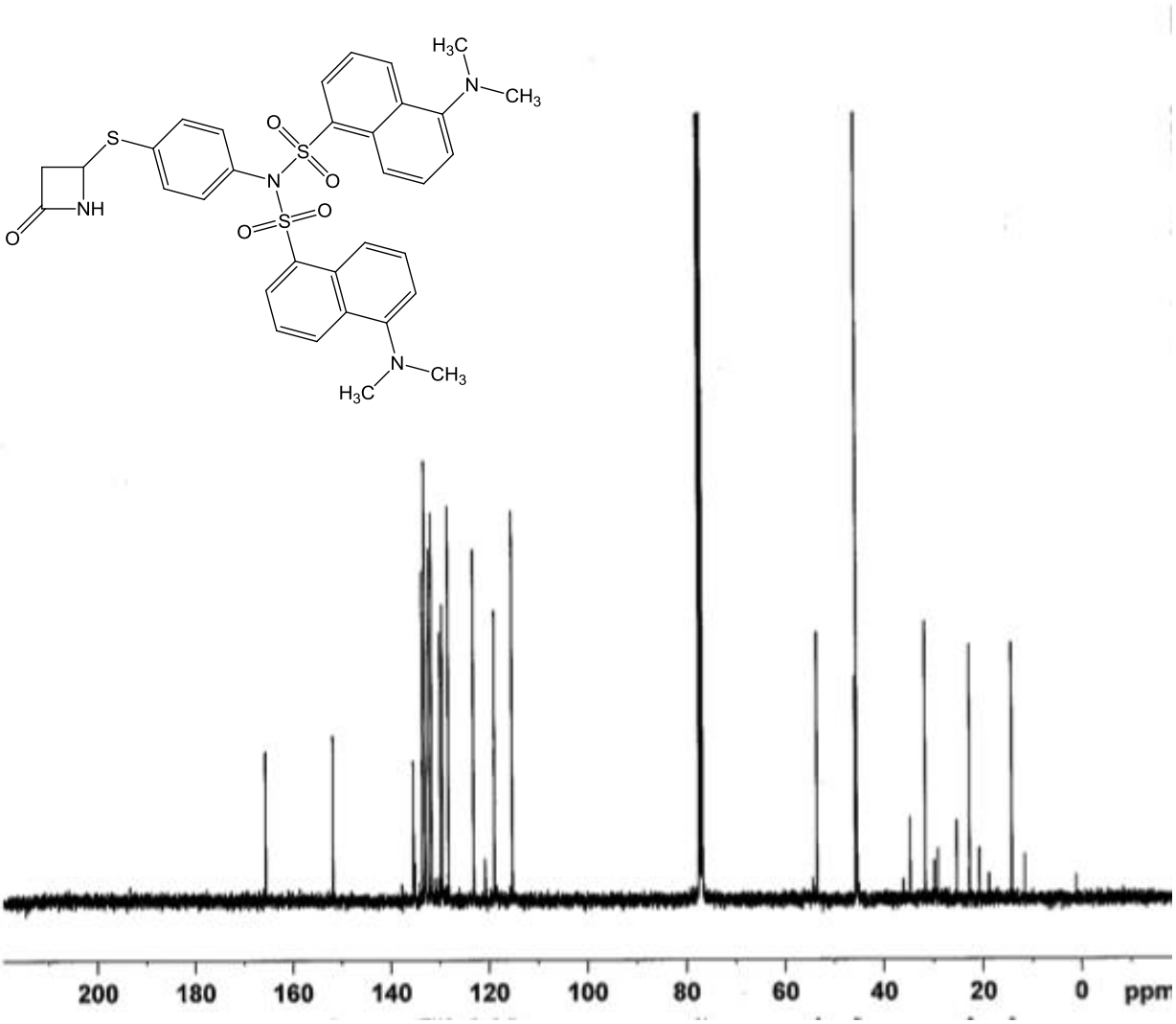


Figure 21: ^{13}C NMR of compound **(11)**

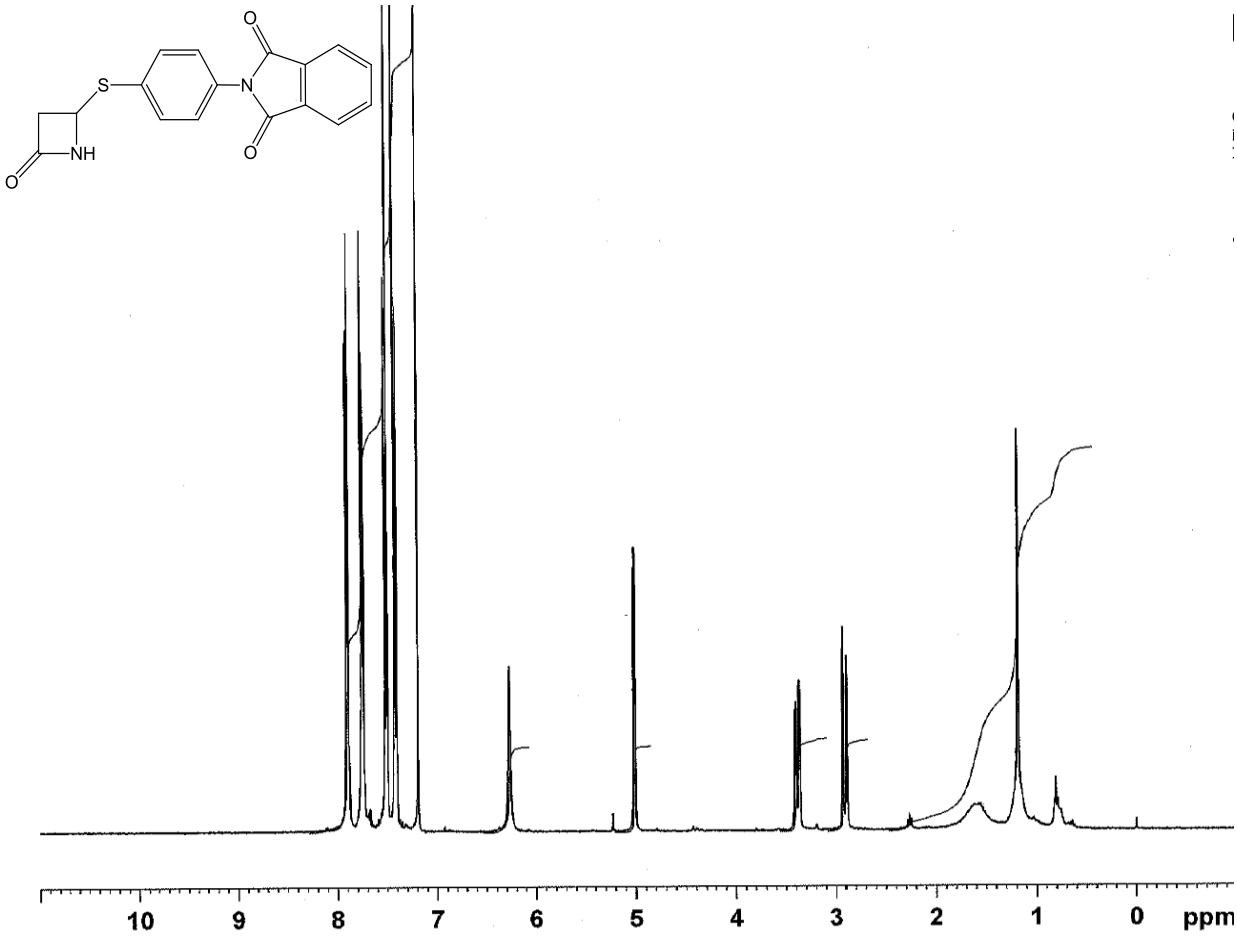


Figure 22: ^{1}H NMR of compound **(14)**

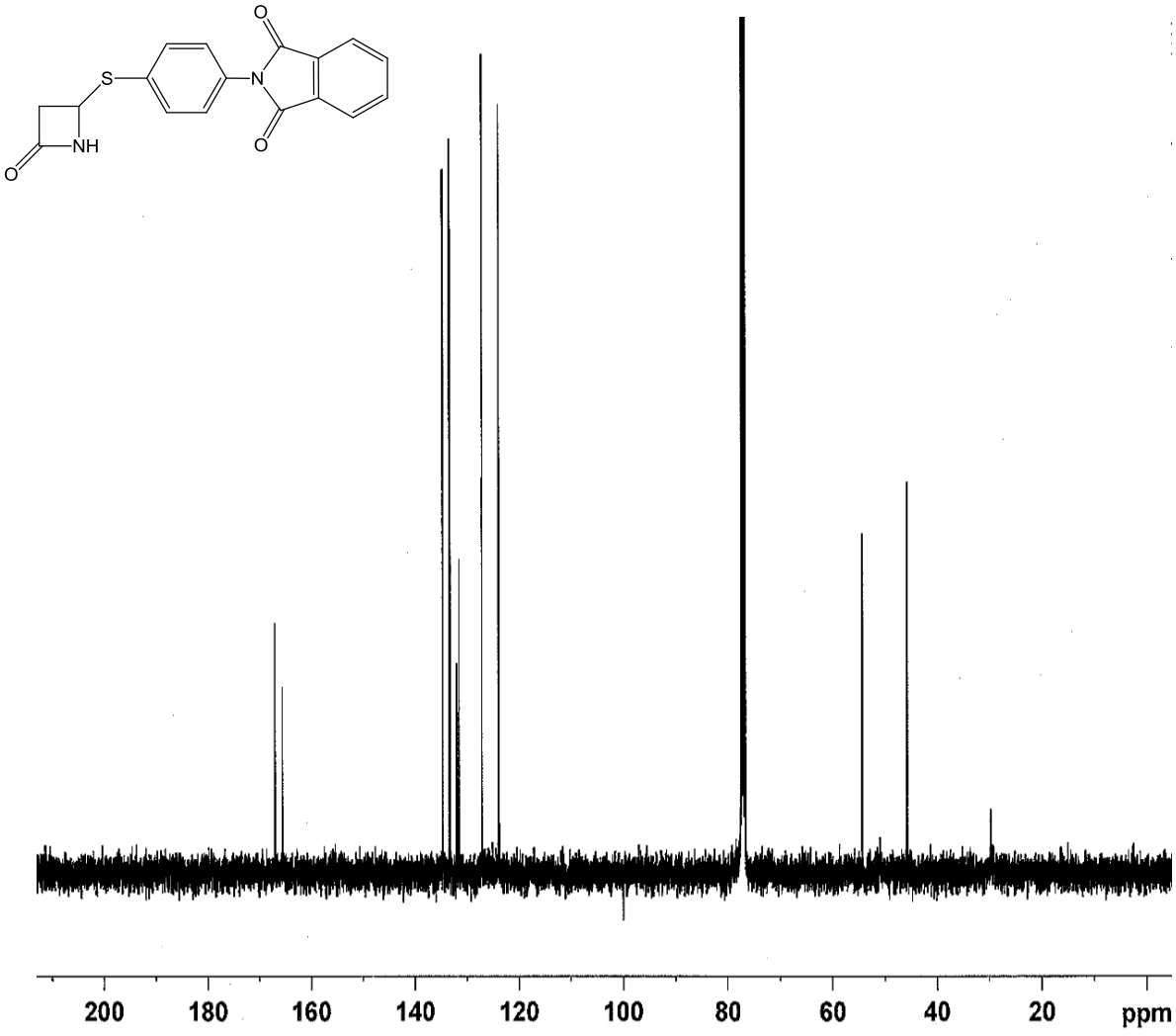


Figure 23: ^{13}C NMR of compound **(14)**

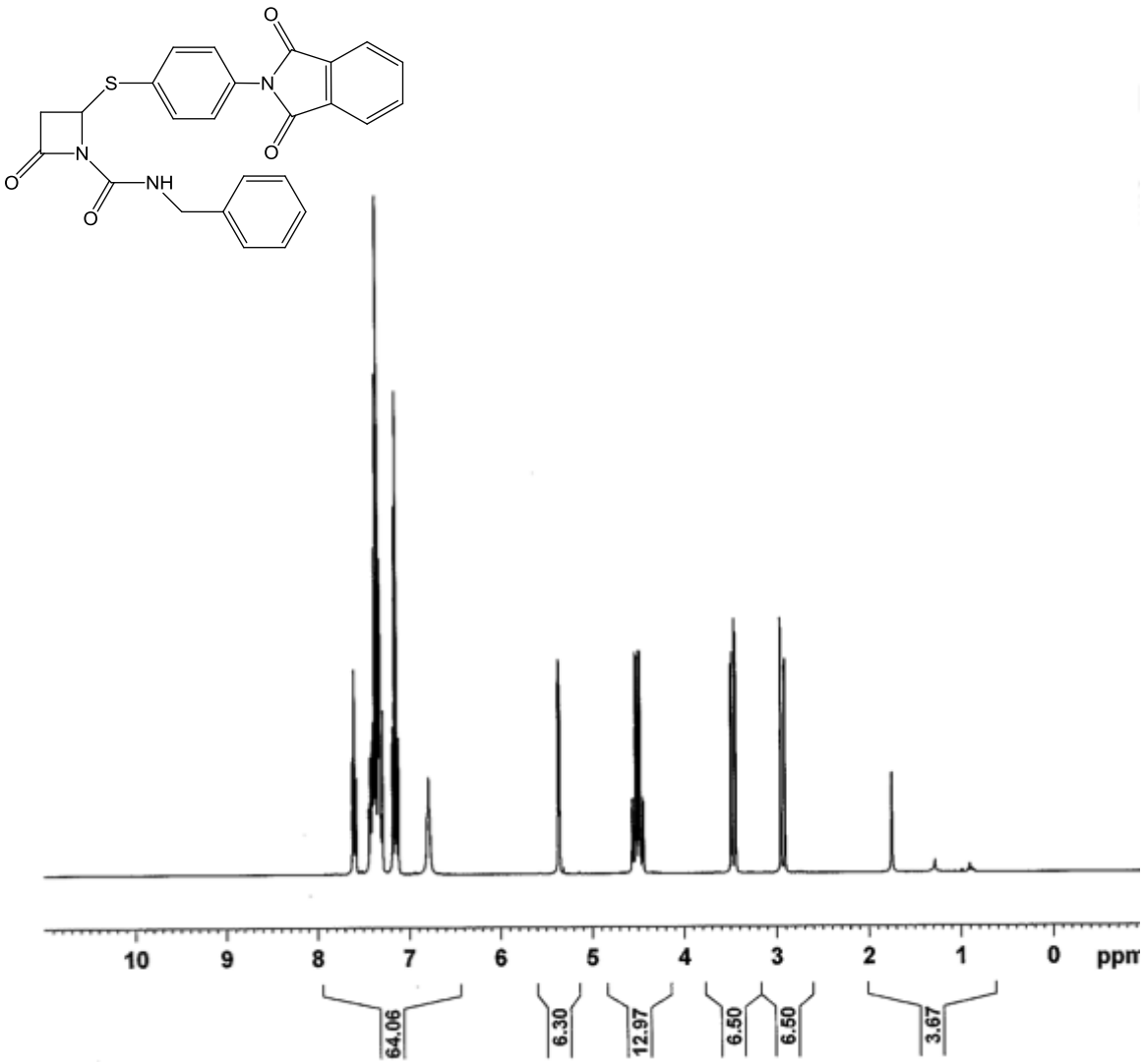


Figure 24: ^{1}H NMR of compound **(15)**

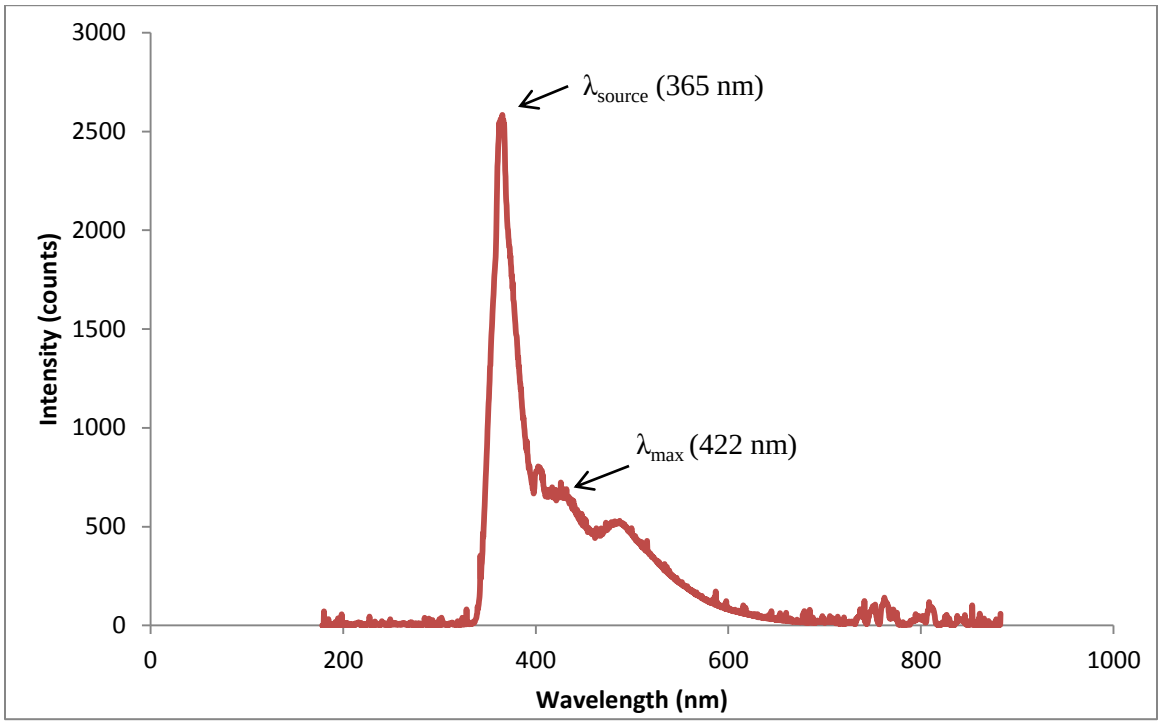


Figure 25: Fluorescence emission spectrum of compound **(7)** in $MeCl_2$

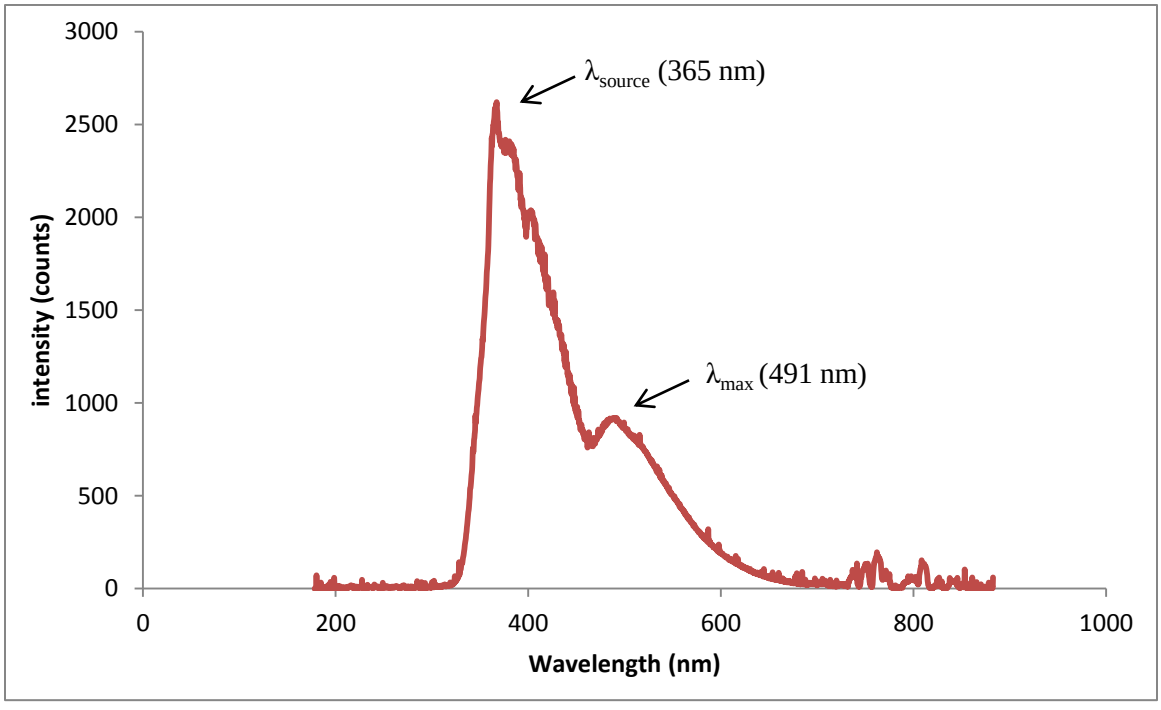


Figure 26: Fluorescence emission spectrum of compound **(8)** in $MeCl_2$

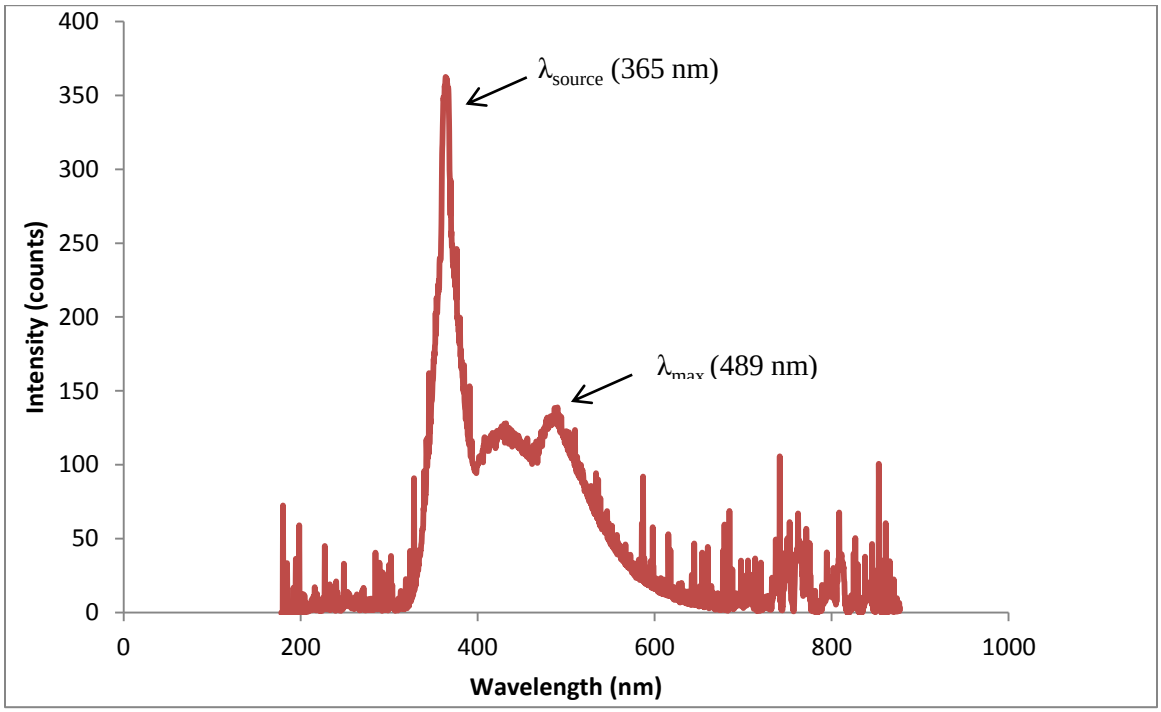


Figure 27: Fluorescence emission spectrum of compound **(9)** in $MeCl_2$

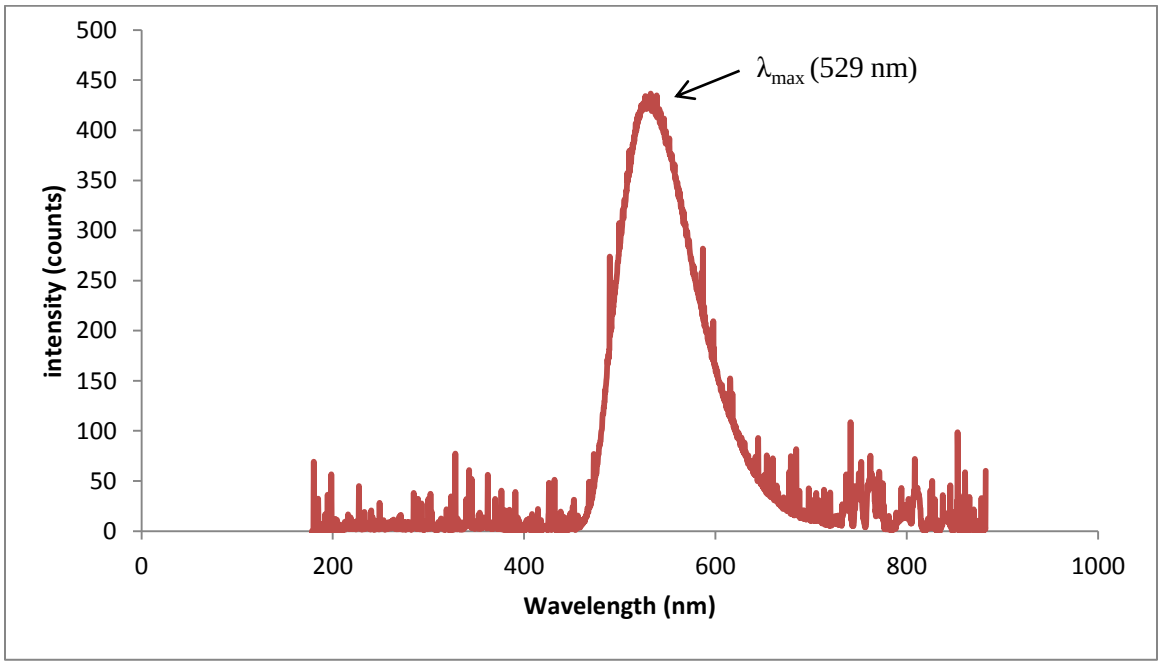


Figure 28: Fluorescence emission spectrum of compound **(11)** in $MeCl_2$

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