STRUCTURE ACTIVITY RELATIONSHIP (SAR) OF FLUORINATED AND CARBAMYLATED BETA-LACTAM DERIVATIVES

By

Dina Lloyd

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Chair:

Monika Konaklieva, Ph.D.

Douglas Fox, Ph.D.

James Girard, Ph.D.

Dean of the College of Arts and Sciences

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DEDICATION

This thesis is dedicated to my mother, who taught me that the path laid before me can be grasped and reshaped into my ideal. It is also dedicated to my father and stepmother, for their unwavering supported in my pursuit of life direction and meaning.

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ABSTRACT

While drug-resistance exists in a wide range of clinically important microorganisms, new drug development has significantly lagged behind the need. We are directly addressing this gap in antimicrobial development by synthesizing a novel class of antimicrobials that are not inactivated by the microbial β-lactamases. To date, we have synthesized cadres of compounds with demonstrated good activity (minimum inhibitory (MIC) and minimum bactericidal concentration, MBC, <15 μg/ml) against Mycobacterium tuberculosis (Mtb) or Moraxella catarrhalis (M.cat.).

The focus of my research is to prepare a second generation of these drugs by building a library of compounds containing fluorinated derivatives. The position and multitude of the Fluorine atoms in the compounds' scaffold is expected to improve overall efficacy of these compounds. In addition, preparation of compounds with differently substituted carbamyl groups has been accomplished. The Structure Activity Relationship (SAR) of these two types of compounds will be presented.

ACKNOWLEDGMENTS

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INTRODUCTION

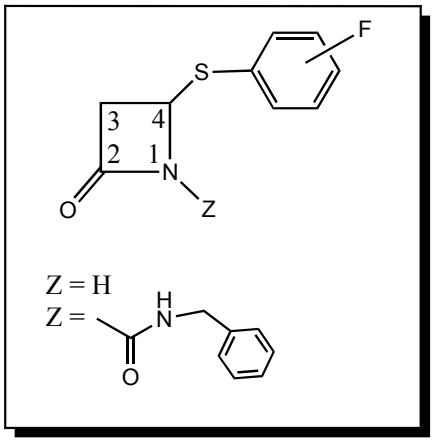
Antimicrobial resistance is a global threat to healthcare and becoming more prevalent due to over-prescriptions of and indiscriminate use of antibiotics. Modes of bacterial resistance include efflux pumps, altered peptidoglycan and expression of β-lactamases.[1-5] The latter is by far the predominant type of resistance mechanisms across different bacterial types. While drug-resistance exists in a wide range of clinically important microorganisms, new drug development has significantly lagged behind.[6-8] There has been a movement in the Infectious Diseases Society of America (IDSA) for the development of 10 new antibiotics by the year 2020, by setting focus on novel classes and exploring modifications to established classes.[6, 8]

The accidental discovery of *Penicillin* mold, by Alexander Flemming in 1928, lead to discovery of the β-lactam class of antibiotics, i.e. penicillins and cephalosporins, composed around a four-member β-lactam (cyclic amide) ring.[9] The lactam inhibits the ability of the bacteria to divide or to support the cytoplasmic pressure.[9] The increasing number of β-lactamase enzymes in numerous strains of bacterium has reduced the effectiveness of β-lactam class as antimicrobials.[10] β-lactamases hydrolyze the lactam antibiotics, thus rendering the antibiotics inactive before they can reach their molecular target – the transpeptidases. This resistance calls for the production of innovative antibiotics that will be resilient to the hydrolysis of lactam amide bond of the lactam ring by bacterial lactamases.[10]

Objective

In order to combat the bacterium resistance, a library of novel monocyclic β-lactams is in preparation in our laboratories. Monocyclic lactams containing hydroxylphenols and thiophenols at the C4 position of the lactam ring has demonstrated antimicrobial activity against two very different Gram(+) organisms. The library of our novel monocyclic β-lactams is tailored to specific bacteria rather than broad-spectrum antimicrobials. The additions of various arylthiols to C4 and the carbamylation of the lactam nitrogen are altered. The antimicrobial testing of our compounds have shown promising antimicrobial activity with the alkylthio-, and arylthio- C4 substitution, with the latter leading to considerably more active compounds. The antimicrobial studies of the compounds having arylthio- groups at C4 demonstrated very good MIC and MBC against M. cat. In order for a drug to be considered as a promising antimicrobial, it should have a minimum bactericidal concentration, MBC, <15μg/mL.

The focus of my research is the addition of fluorinated thiophenols at C4 and different carbamyl groups to the lactam nitrogen, Scheme 1, in order to determine the



Scheme 1. β-Lactam Scaffolding and Alterations with Fluorinated Thiophenols

most promising candidates as antimicrobials. The varying functional groups attched to the aromatic ring give diverse properties to the β-lactam, which allows for Structure Activity Relationship (SAR) determination. The modifications are aimed at lowering the minimum inhibitory (MIC) and minimum bactericidal concentration (MBC) against *Staphylococcus aureus, Mycobacterium tuberculosis* (Mtb) and *Moraxella catarrhalis* (M. cat.).

RESULTS AND DISCUSSION

Design and Synthesis

The antimicrobial activity of the fluorinated monocyclic lactams against *Mycobacterium tuberculosis* (*Mtb*) has been evaluated in the presence and absence of clavulanic acid, a β-lactamase inhibitor. Clavulanic acid inhibitor has been isolated from *Streptomyces clavuligerus*, and is being used as an inhibitor towards various strains of bacterium expressing β-lactamases.[11, 12] The clavulanic acid doesn't appear to alter the inhibitory activity of our compounds. This indicates that that our compounds are not hydrolyzed by the β-lactamases, an evidence of that these lactams could have different mode of action as compared to the "traditional" β-lactams.[13, 14]

In addition to being a part of a library of a fluorinated arylthio- β-lactams for determination of the SAR against Mtb, these lactams could be used as probes for obtaining the identity of the molecular target of our lactams by using ^{19}F Nuclear Magnetic Resonance (NMR). Fluorination has been shown to increase anticancer activity of other compounds such as taxoids.[15-18] Similarly to fluorescent and biotinylation tagging, the fluorinated tags are used on drugs and synthesized proteins, which are used to track the propagation of activity to and within the bacterium.[19-23] The latter could be confirm through isolation of antigens assays of biotin-compound complex. This can be achieved by using streptavidin columns with the bound to the inhibited protein or other molecular target of the bacterium.[20, 21]

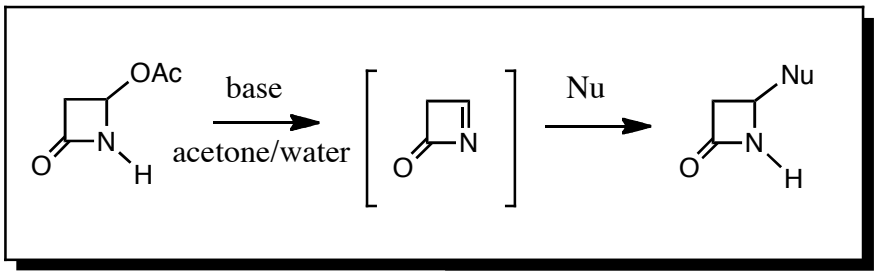
The Fluorine tag will be dramatically less intrusive due to its smaller size, high lipophilicity when attached to monocyclic β-lactam derivatives as compared to that of the much larger biotin or fluorescent probes used in biological testing.[20-22]

The preparation of arylthio-β-lactam scaffolds, having different carbamyl groups at N1, are designed to examine the stability and reactivity of these novel compounds in order to determine the structures having the lowest MIC and MBC. Our β-lactams appear to be nontoxic as it has been shown (unpublished data) that they were neuroprotective against glumatate excitotoxicity in cerebellum prenatal rat cells.[5, 13, 14, 24-32] Glutamate is crucial neurotransmitter for normal neuronal excitability, but high doses cause glumatate cytotoxicity.[24-30] In addition, β-lactams having a carbamyl group at N1 are Human Leukocyte Enzyme (HLE) and human cytomegalovirus protease inhibitors.[33]

The novel class of β-lactams are prepared from commercially available β-lactam **1** following the procedures of Grimme et. al.[34] and Wasserman et. al.[35], where the nucleophile (Nu) is a thiol group, Scheme 2. Currently, it is accepted that the nucleophile could play a role of a leaving group at C4, and its function as such is of importance for the inhibition of essential bacterial serine- and cysteine-containing enzymes.[5, 13, 14]

Specifically, we set to explore the effect of the Fluorine substituent(s) at various positions at the aromatic ring, namely at *o-, m-, p-* positions as well as mono-, di- and pentafluoro, at the C4 arylthio- substituent. The Fluorine additions are particularly interesting since it will keep the size of the formed compound small enough for easy accessibility through the cell wall, but could have a different electronic effect on the activity monocyclic β-lactam, as compared to a hydrogen.

The striking effect of the addition of N1 carbamylation seen in the preliminary

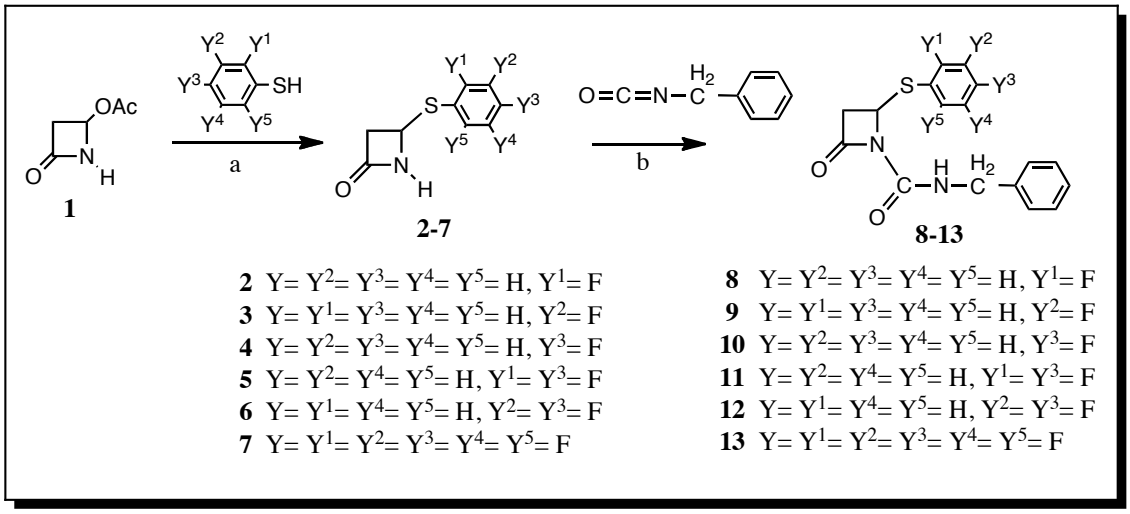


Scheme 2. Synthesis of Monocyclic β-Lactam Substituted C4

tests, led the principle project to expand to not only the addition of differently fluorinated arylthiols at C4, but also modification of the carbamyl group at N1. In addition, the stereochemistry (*cis*- vs. *trans*-) or how the derivatives are aligned on the compound was explored. The stereochemistry of the isocyanate or the thiophenol can change the ability of the drug to interact and inhibit the bacteria. The modifications could also alter the specificity of the drugs, which in turn can evaluate the role of the stereochemistry on the Structure Activity Relationships (SAR). These novel compounds represent the possibility of a one drug-one hit approach, which will enable targeting of specific individual pathogens and again minimizing the MIC/MBC.

β-Lactam with Mono-, Di-, Penta- Fluorinated Thiophenols at C4 and Carbamylation at N1

The C4 substituted β-lactams were prepared from commercially available 4-acetoxy-2-azetidinone **1** following the procedures of Grimme et. al.[34] and Wasserman et. al.[35], where the acetoxy group is replace with a fluorinated arylthiol producing mono-, di- and pentafluorothiophenol compounds **2-7,** Scheme 3a. These mono-fluorinated thiols β-lactam **2-4** were initially synthesized to test the affect of the Fluorine subsituents on unsubstituted arylthiol previously found to have antimicrobial affects.



Reagents and conditions: (a) $NaHCO_3$ in acetone/water, rt, 12h, 45-70%; (b) Et_3N in CH_2Cl_2, rt, 4h, 60-90%.

Scheme 3. Synthesis of N-Carbamylated β-Lactams having a Fluorothiopheno-Groups at C4

Initial antimicrobial testing of the C4 mono-arylthiofluorinated β-lactams **2-4** has revealed that the fluorination of the aromatic ring has a positive effect on the activity of these lactams as compared to the unsubsituted counterparts (unpublished data). The positions of the Fluorine (*para*-, *ortho*-, *meta*-) does not appear to have an effect on the activity. It appears that multi-position fluorination does not have a substantial effect (or has a marginal effect) on antimicrobial activity. Addition of more than one Fluorine atom in the aromatic ring at C4 will allow the use of the fluorinated thiophenol as a fluorinated tag within the bacterial cell. This could lead to opportunity for obtaining information about the molecular target of our lactams by using ^{19}F NMR. In theory, the molecular target can be found by centrifuging the bacterium and based off of densities of organelles in the supernate locate the region being affected should the fluorothiophenol bind covalently to its target.

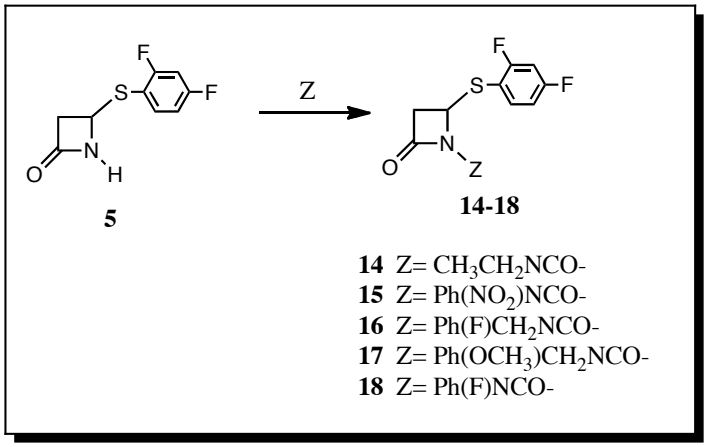
Carbamylation at N1 of the lactam was considered, in order to increase the β-lactam ability to be taken up by the bacterium cell, Scheme 3b. Lactams carbamylated at the lactam N1 are seen in similar structures that are Human Leukocyte Enzyme (HLE) inhibitors.[13, 33] The aim was to take this known HLE inhibition active group and translate it into the scaffold of the monocyclic β-lactam ring.[1-3, 5] The carbamylated N1 also draws similar structure to that of peptidoglycan LD-transpeptidase inhibitors, which causes the malformation of the cell wall of bacterium.[1-3]

β-Lactam with Difluorothiophenol at C4 and Carbamylation at N1 Using Different Isocyanates

Carbamylated N1 derivatives were synthesized from lactam **5** and several commercially available isocyanates with various degrees of electron withdrawing properties following the procedures of Mulchande et. al.[5] or the irradiation method (microwave, MW) developed in our laboratory, Scheme 4.

In order to determine whether or not the electron donating or withdrawing effect of a carbamylated N1 alters the antimicrobial activity of the parent compound, (the fluorinated thiophenyl lactam), differently substituted at the aromatic ring, benzyl isocyanates were used. The lactams **15-18** were synthesized utilizing aryl isocyanates having at the *ortho*- position of the aromatic ring: a) nitro group (**15)**; b) Fluorine (**16**, **18)**; and c) methoxy group (**17)**.

The nitro group was to increase the electron withdrawing properties of the carbamyl group as compared to the ones having Fluorine. The Fluorine, in the carbamyl group, was used to mirror the Fluorine on the arylthiol, lactams **16**, **18**.



Reagent and conditions: a) Et_3N in CH_2Cl_2, rt, 4h, 40-90%, b) Et_3N in dichloromethane, irradiated at 300W, 35°C for 45min, 20-40%

Scheme 4. Carbamylation at N1 of Lactam **5** with Various Isocyanates using MW Irradiation

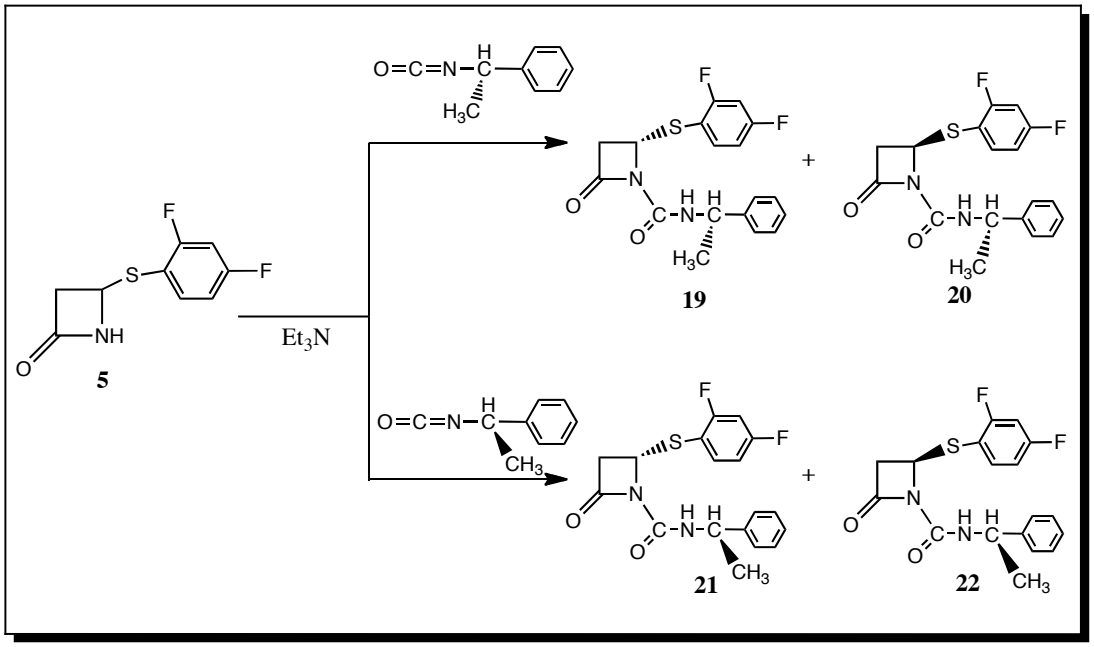
It is conceivable that the Fluorine substituted carbamyl group at N1 could become a probe for locating lactam's metabolic products through NMR analysis after isolation from the bacterium. The electron donating effect of the methoxy group in the carbamyl group was expected to increase the overall stability of this type of compounds, lactam **17**. In addition to the use of benzyl isocyanate, we have also synthesized carbamylated N1 lactams with ethyl isocyanate, **14,** as well as phenyl isocyanates, **15**, **18**. This was done in order to determine the best carbamyl substituent for the antimicrobial activity of our compounds. Lactam **14** was synthesized to evaluate the effect of an alkyl carbamylation on biological activity. Substituting the aryl with an ethyl group was used to indicate the electron withdrawing inductive effect of the carbamyl (NCO-group) without the stabilization of the aromatic ring.

The aryl benzyl was then replaced with aryl phenyl, compounds **15** and **18**. The direct attachment increase the strain of the NCO-group on the lactam ring as well as the

substituent attached to the aryl. Due to the instability of the four member ring **5** with the attachment of these isocyanates, we were unable to further purify lactams **15** and **18**, since hydrolysis products were observed during purification on silica gel using Thin-layer chromatography (TLC). Initially we wanted to follow the progress of the reactions by NMR. Lactam **15** was synthesized within minutes of the addition triethylamine to the NMR tube containing the starting lactam **5** and the corresponding isocyanate, Scheme 4, but when removed from the solvent, the lactam ring was destroyed. The aryl group is directly attached to the nitrogen in the isocyanate, which causes the hydrolysis of the lactam ring due to the powerful electron withdrawing effect of the nitro group. Lactam **18** would not react under the Mulchande et. al.[5] conditions and a new methodology was developed for the carbamylation through the use of irradiation. Lactam **18** was synthesized within 10 minutes of irradiated and followed by NMR, Scheme 4. Again, the fact that the phenyl was directly attached to the neighboring carbamyl N caused **18** to disintegrate before further characterization. Therefore, phenyl carbamylation appears to led to unstable compounds which are unsuitable for antimicrobial testing, due to their unpredictable shelf life.

β-Lactam with Difluorothiophenol at C4 and Carbamylation at N1 Using Chiral Isocyanate

Chirally carbamylated N1 derivatives were synthesized from lactam **5** and commercially available *R*- or *S*- methylated isocyanates following the procedures of Mulchande et. al.[5] or the irradiation method developed in our laboratory, Scheme 5. The stereochemistry of the isocyanate or the thiophenol could change the ability of the drug to interact and



Reagents and conditions: Et_3N in dichloromethane, irradiated at 300W, 35°C for 45min, 20-40%.

Scheme 5. Synthesis of Diastereomers of Fluorinated Lactams

inhibit the bacteria. This idea prompted the synthesis of β-lactams **19-24**. The effect of the Sulfur orientation at the lactam's C4 on the biological activity has yet to be determined. Previously synthesized carbamylated arylthiol lactams **2-18** were racemic mixtures at C4.

To determine the optimal configuration of the Sulfur in relation to the lactam ring in biological activity, N1 carbamylated compounds **19-22** have been formed from lactam **5** with the methylated benzyl methylene isocyanate in either an *R*- or *S*- chirality. The presence of chiral methyl group at the benzyl carbon is used as a reference point to identify the chirality of the group at C4 using X-ray spectroscopy. N1 carbamylated diastereomers were prepared, namely, lactams **19-22**. The *R*- or *S*- carbamyl substituents at N1, allows for preparation and separation of pure diastereomers.

The addition of a second chiral center, onto the lactam carbamyl subsitutent at N1 of **5**, led to the production of diastereomeric pairs **19-24**, Scheme 5. The chemical shifts of the proton (H) at C4 and the proton at the benzyl carbon of the carbamyl group were indicative of the preparation of the corresponding diastereomers, **19-24**. The differences in the NMR spectra coupled with the ease of purification of these diastereomers on silica gel TLC prep plates led to obtaining the pure isomers.

When comparing the NMR spectra of the chiral, derivatized at N1 between compound **19** and **20** the following differences are found: the proton at C4 of lactam **5** has a dd at 4.96ppm, Scheme 5. With the carbamyl attached at N1, the signal for the proton at C4 shifted downfield to 5.14ppm for **19**, while this peak shifted to 5.18ppm for **20**, Scheme 5. In the aromatic region of **19** there is a multiplet present at 7.67ppm, Figure 11, whereas there is no such proton shift downfield in the aromatic region of **20**, Figure 12. This change in the aromatic region is due to the stereochemistry at the N1 subsitutent and the arylthio at C4. Similar shifts of proton at C4 and the aryl group were seen when comparing all pairs of diastereomeric compounds **19-24**, Scheme 5. Thus, compounds **19-24** can be separated and analyzed as individual compounds with specified stereochemistry allowing for a better understanding of SAR associated with their antimicrobial activity. The rational is to find the optimal configuration of the Sulfur in the previously tested lactams **8-13** with relative low MIC/MBC and increase the concentration of the lactam's isomer that will lead to the improved MICs.

If the presence of the methyl group at the benzylic carbon of the carbamyl group at N1 presents steric hindrance, which could diminish the ability of the lactam to bind to its moleculat target, the alternative to the chiral isocyanates are various cinchona bases

(cinchonine and cinchonidine). The latter can be utilized, instead of the sodium bicarbonate, to construct the *R*- or *S*- chirality of the C4 at the time of the nucleophilic fluoroarylthiol substitution, Scheme 3a.

Bacterial Inhibitory Activity

Cation-adjusted Muller-Hinton broth assays were used to screen compounds for antimicrobial activity. Organisms (*Eschericia coli* ATCC 25922*, Pseudomonas aeruginosa* ATCC 27853*, Staphylococcus auerus* ATCC 25923*, Enterococcus faecalis* ATCC 29212) used to screen synthesized compounds represent highly stable quality control strains routinely used for antimicrobial testing. They also represent a range of organisms, both Gram positive and Gram negative, that cause clinically important infections. In addition, clinical isolates of *Moraxella catarrhalis* (M. cat.) a major cause of otits media, sinusitis and acute exacerbation of chronic obstructive pulmonary disease (COPD) were screened. All *M. catarrhalis* strains produced β-lactamase as documented by nitrocefin cleavage studies. None of the synthesized compounds affected the growth of E.*coli, Pseudomonas aeruginosa, Staphylococcus auerus, or Enterococcus faecalis.*

The lactam biological activity was conducted by looking at the minimum inhibitory concentrations against *Moraxella catarrhalis* clinical strain and H37Rv *Mycobacterium tuberculosis* laboratory strain with or without clavulanic acid, Table 1. The activities are compared to penicillin as a standard antimicrobial compound with clavulanic acid. The significance of these strains used in biological activity is that they are known to express β-lactamase. The clavulanic acid, as stated above, is used to inhibit the hydrolytic capabilities of β-lactamases against the β-lactam ring and allow the

Table 1. Minimum Inhibitory Concentrations (MIC) Against M. cat. and Mtb with or without the Presence of Clavulanic Acid

Comp. #	M. cat.*	Mtb (H37Rv)**	Mtb (H37Rv)*
2	100/100	25	>100
3	200/200	25	>100
4	>200	>100	>100
5	NE	25	50
6	50/50***	12.5	25
7	NT	NT	>100
8	12.5/12.5***	3.125	3.125
9	25/50***	NT	NT
10	25/25***	6.25	6.25
11	1.625/1.625***	6.25	6.25
12	12.5/12.5***	100	NT
13	NT	NT	>100
14	NT	NT	>100
15	NT	NT	NT
16	NT	NT	50
17	NT	NT	≥100
18	NT	NT	>100
19	NT	NT	>100
20	NT	NT	>100
21	NT	NT	>100
22	NT	NT	>100
23	NT	NT	>100
24	NT	NT	>100
25	NT	NT	>100
26	NT	NT	>100
Penicillin	3.1/3.1***	3.1	NT

Note. Penicillin is a culture standard.

[a] NE = No Effect

[b] NT = Not Tested

* Without Clavulanic acid

**With Clavulanic acid

*** MIC/MBC in µg/mL cation-adjusted Muller-Hinton broth

β-lactam antibiotics to interact with the bacterium before the lactam cyclic amide can be hydrolyzed by the β-lactamase.

Compounds **6, 9,** and **10** exhibited modest antibacterial activity against M. cat. The compounds with the best activity against M. cat. were N1 carbamylated compounds using benzyl isocyanate, namely lactams **8** (MIC = 12.5 μg/ml), **11**(MIC = 1.625 μg/ml) and **12**(MIC = 12.5 μg/ml). Compound **11**, the most active β-lactam against M. cat., possesses an aromatic ring with a difluorine-substituted arylthiol at the *p*- and *o*-positions and carbamylated N1 using benzyl isocyanate, Scheme 3. Therefore, compound **11** was chosen as the scaffold to prepare the various carbamylated derivatives because of the demonstrated good biological activity. The N-unsubstituted fluroinated arylthiol **5** has no antimicrobial properties, Table 1. However, carbamylation of lactam **5** at N1 (using benzyl isocyanate), led to preparation of the most potent antibacterial active compound, namely lactam **11** (MIC= 1.625μg/mL). The lactams with various carbamyl groups at N1, compounds **13**-**25** were not synthesized at the time M. cat. clinical strains were available during high patient infection rates.

The compounds **2**, **3**, **5**, **6**,and **16** showed modest antibacterial activity against Mtb with clavulanic acid. The compounds that showed potent antimicrobial activity for Mtb with clavulanic acid were **7** (MIC = 12.5 μg/ml), **8** (MIC = 3.125 μg/ml), **10** (MIC = 6.25 μg/ml), and **11** (MIC = 6.25 μg/ml). Specifically, **8, 10, 11**, showed consistent antimicrobial activity without clavulanic acid as compared to clavulanic acid being present. This is evidence of the β-lactamase have no effect on these lactams.

Compound **8**, the most active β-lactam against Mtb, possesses an aromatic ring with a monofluorine-substituted arylthio at the *o*-position at C4 and benzyl isocyanate

carbamylated at N1, Scheme 3. As in M. cat. antibacterial activity of **11**, it held the antibacterial effect against Mtb, but not as effective as its monofluorinated counterpart **8**. The fluorinated carbamyl, **16**, was the only carbamyl lactam outside the benzyl carbamyl lactams **8-13** to show modest activity. The additional electron withdrawing effect of fluorine on the aryl group may not have interfered with the activity of the lactam as much the other carbamyl groups, such as ethyl and phenyl, as mentioned earlier, Table 1.

The modest antibacterial activity of the chiral carbamyl, **19-24**, was unexpected. With the thought that the chiral methylated benzyl would not impede on the overall activity of the original benzyl isocyanate. It appears that both carbamyl modifications, chiral or extreme donating/withdrawing substituted aryl isocyanates, have a negative effect on antibacterial activity when combined with difluroinated arylthio-lactam **5**. The methylated carbamyl may have stericly hindered the active site or diminish the effects of the aryl carbamyl. This provokes thought to the promising activity of the benzyl carbamyl monofluorinated lactam **8**. The future focus is to carbamylate chiral isocyanates of this monofluorine arylthio lactams as well as carbamylate the Fluorine aryl isocyanate for antibacterial activity against Mtb. An alternative to assess the effect of chirality would be to start with utilization of methods leading to preparation of the desired chirality of C4 and derivatization of N1 with benzyl isocyanate, Scheme 3a. This could aid in identifying the favorable chirality to increased antimicrobial activity. Even though the varying carbamyl lactams have not been tested on the M. cat., these same conclusions will be drawn to aid in identifying effective novel β-lactam.

Conclusion

We have designed, synthesized and tested a variety of novel monocyclic β-lactams with fluroinated aryl-thio-groups at C4 and carbamylates at N1. Several compounds have shown specific antibacterial activity against *M. catharralis* and *Mycobacterium tuberculosis*. Compound **11** had the most potent antimicrobial activity for M. cat. with MIC 1.625μg/ml and compound **8** was the most potent for Mtb with MIC 3.125μg/ml.

The most effective for both bacteria were **8**,**10**, and **11**. The chiral carbamyls **19-24** did not prove effective against Mtb, but antibacterial activity has not been tested agains M. cat. The potent activity of **8** has held hope for the monofluorinated arylthio with chiral and fluorinated carbamylation. If those avenues are unsuccessful, then a novel framework will restart with selecting the chirality of the Sulfur at the C4 lactam.

Also, since these novel compounds have activity against β-lactamase producing strains, it is possible that their molecular target is different than the known targets of the β-lactams. Current studies in our laboratories are directed towards a better understanding of the structure-activity relationships and the molecular target of these promising new anti-bacterial β-lactam compounds.

EXPERIMENTAL

Equipment and Materials

All reactions were carried out at room temperature under normal atmospheric pressure. All common 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 on glass-backed analytical TLC plates coated with silica G with UV254 indicator (Sorbent Technologies Silica G TLC plates); the chromatograms were visualized under ultraviolet light and/or by staining with iodine silica chamber. Rf values are reported on silica gel. Flash column chromatography was carried out on Combi flash chromatography silica gel (40 lm) or on AC- ROS aluminum oxide, activated, neutral (50–200 micron). Unless otherwise noted, the compounds were detected under UV light and iodine vapors.

Product yields refer to chromatographically and spectroscopically pure compounds, unless otherwise noted. 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 d units (dTMS = 0 ppm to downfield), with the signal multiplicity (s = singlet, d = doublet, t = triplet, q = quar- tet, quint. = quintuplet, m = multiplet, br = broad, app = apparent),

coupling constant(s) (J in Hz) and integration area in parentheses. 13C NMR spectra were determined using the signal for residual CHCl3 in CDCl3 at d = 77.16 as an internal standard. In most cases, signals due to exchangeable protons have been omitted. Melting points were determined on a fisher-johns melting point apparatus and are uncorrected. IR spectra were obtained as a thin film on NaCl plates and in solid form (KBr standard) on a Shimadzu FT-IR-8300 (Columbia, MD). Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Irradiation reaction performed in CEM Corporation Discover System. Product names were electronically generated used ChemDraw version 12 software.

Synthesis

General Procedure for Synthesis of β-Lactams Containing Difluorothiophenols, and Pentathiolphenol (2-7)

The synthetic procedure was adopted from Grimm et al.[34] and Wasserman et al.[35] and applied to our compounds **2-7**. To a solution of 4-acetoxy-2-azetidinone **1** (1 g, 8 mmol) in acetone (30 mL) and water (20 mL) were added 1.05 mol eq. of the corresponding substituents: 2-fluorothiophenol, 3-fluorothiophenol, 4-fluorothiophenol, 2,4-difluorothiophenol, 2,3-difluorothiophenol and pentafluorothiophenol. 4 mol eq. of sodium bicarbonate were added and the mixture was stirred vigorously for 12h in a closed round bottom flask. Sodium chloride was added to the solution and after formation of two layers the mixture was filtered out and extracted with ethyl acetate (3 x 50 mL). The combined organic layers were dried over magnesium sulfate and concentrated under

vacuum. The crude material was purified by flash chromatography or recrystallized from ethyl acetate/hexanes to give white crystals for all five products in quantitative yield.

4-(2-Fluoro-Phenylsulfanyl)-Azetidin-2-one (**2**)

Purified by flash chromatography from ethyl acetate/hexanes to give 65% yield. 1H NMR (400 MHz, CDCl3): δ_H 2.87 (dd, *J*= 1.38, *J*= 13.86, 1H); 3.36 (dd, *J*=1.85, *J*= 3.12, *J*= 8.48, 1H); 4.99 (dd, *J*= 2.30, *J*= 2.66, 1H); 6.16 (brs, 1H); 6.97-7.30 (m, 4H). 13C NMR (125 MHz, CDCl3): δ 46.04, 54.44, 116.62, 125.06, 131.40, 136.30, 161.57, 164.02, 165.75. IR (neat) υ_{max} (C=O) 1750 cm^{-1}.

4-(3-Fluoro-Phenylsulfanyl)-Azetidin-2-one (**3**)

Purified by flash chromatography from ethyl acetate/hexanes to give 65% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.87 (dd, *J*= 1.38, *J*= 13.86, 1H); 3.36 (ddd, *J*=1.85, *J*= 3.12, *J*= 8.48, 1H); 4.99 (dd, *J*= 2.30, *J*= 2.66, 1H); 6.16 (brs, 1H); 6.97-7.30 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 45.81, 54.21, 115.67, 115.88, 119.70, 119.92, 128.60, 130.93, 165.95. IR (neat) υ_{max} (C=O) 1750 cm^{-1}.

4-(4-Fluoro-Phenylsulfanyl)-Azetidin-2-one (**4**)

Purified by flash chromatography from ethyl acetate/hexanes to give 70% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.87 (d, *J* = 15.27, 1H); 3.37 (ddd, *J* = 1.82, *J* = 3.02, *J* = 8.58, 1H); 4.96 (dd, *J* = 2.19, *J* = 2.66, 1H); 6.11 (1H, brs); 7.01-7.52 (4H, m). ^{13}C NMR

(125 MHz, $CDCl_3$): δ 45.28, 54.78, 116.59, 116.81, 126.13, 136.63, 162.21, 164.70, 166.73. IR (neat) υ_{max} (C=O) 1750 cm^{-1}.

4-(2,4-Difluoro-Phenylsulfanyl)-Azetidin-2-one (**5**)

Purified by recrystallization in ethyl acetate/hexanes to give 70% yield with m.p. 70-73°C. ^1H NMR (400 MHz, $CDCl_3$): δ_H 1.6 (s, 1H); 2.29 (dt *J* = 11.62, *J* =1.88, 1H); 3.42 (dd *J* = 8.531, *J* =3.10, *J* =1.86, 1H); 4.96 (ddd *J* = 2.156, *J* =1.84, *J* =0.44, 1H); 6.13 (brs, 1H), 6.91-6.98 (m, 3H); 7.29 (1H); 7.52 (ddd *J* = 6.29, *J* =5.30, *J* =2.17, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 45.55, 54.38, 104.88, 112.31, 113.36, 161.90, 162.50, 164.37, 165.01, 166.43. IR (neat) υ_{max} (C=O) 1750 cm^{-1}. *Anal.* calcd for $C_9H_7F_2NOS$; C, 50.23 ; H, 3.28 ;N, 6.51. Found: C, 49.55; H, 3.59; N, 6.08.

4-(3,4-Difluoro-Phenylsulfanyl)-Azetidin-2-one (**6**)

Purified by recrystallization in ethyl acetate/hexanes to give 47.1% yield with m.p. 74-77°C. ^1H NMR (400 MHz, $CDCl_3$): δ_H 1.6 (s, 1H); 2.2 (s, 1H); 2.90 (dd *J* = 11.72, *J* =1.3, 1H); 3.3 (dt *J* = 12.64, *J* =1.42, 1H); 3.44 (ddd *J* = 8.215, *J* =2.85, *J* =2.13, 1H); 3.64 (ddd *J* = 9.62, *J* =2.31, *J* =1.74, 1H); 5.00 (dd *J* = 2.64, *J* =2.33, 1H); 5.70 (dd *J* = 2.70, *J* =1.39, 1H); 6.17 (br, 1H); 7.11-7.37 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 45.45, 54.70, 118.33, 123.23, 127.39, 130.77, 149.07, 149.89, 151.87, 152.39, 166.53. IR (neat) υ_{max} (C=O) 1750 cm^{-1}. *Anal.* calcd for $C_9H_7F_2NOS$; C, 50.23; H, 3.28; N, 6.51. Found: C, 50.15; H, 3.26; N, 6.45.

4-Pentafluorophenylsulfanyl-Azetidin-2-one (**7**)

Purified by recrystallization in ethyl acetate/hexanes to give 46% yield with m.p. 80-82°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.18 (s, 1H); 2.05 (t *J* = 26.57, 1H); 2.92 (dd 10.32, *J* =15.52, 1H); 3.40 (ddd *J* =10.84, *J* =3.28, *J* =1.49, 1H); 4.91 (d *J* =3.01, 1H); 6.75 (s, 1H); 7.21 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.6, 29.65, 30.78, 44.73, 46.26, 54.63, 73.01, 104.94, 110.26, 136.65, 139.05, 143.64, 146.59, 148.98, 165.81. IR (neat) υ_{max} (C=O) 1755 cm^{-1}.

General Procedure for the Synthesis of N-Carbamoylazetidin-2-one Derivatives (8-13 from 2-7 respectively)

The synthetic procedure was adopted from Mulchande et al.[5] and applied to our compounds. To a solution of appropriate azetidin-2-one **5** (1.7g, 5.4mmol) in dichloromethane (5 mL) was added to1.2mol eq. of triethylamine and 1.2 mol eq. of the corresponding substituents: benzyl isocyanate, ethyl isocyanate, and 2-nitrophenyl isocyanate. The reaction was stirred at room temperature and monitored by TLC. After completion of the reaction, the solution was evaporated under reduced pressure.

2-(2-Fluoro-Phenylsulfanyl)-4-Oxo-Azetidine-1-Carboxylic acid Benzylamide (**8**)

Purified by flash chromatography from ethyl acetate/hexanes to give 67% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.94 (dd, *J* = 2.60, *J* = 13.92, 1H); 3.47 (dd, *J* = 5.64, *J* = 10.84, 1H); 4.50 (dd+dd, *J* = 5.35, *J* = 6.00, *J* = 8.50, 2H); 5.35 (dd, *J* = 2.60, *J* = 2.99, 1H); 6.77 (1H, brs); 7.12-7.18 (5H, m); 7.30-7.61 (4H, m). ^{13}C NMR (125 MHz, $CDCl_3$):

δ 40.74, 43.85, 56.29, 116.27, 116.49, 127.63, 128.82, 132.04, 137.77, 137.95, 149.64, 160.39, 162.00, 164.46. IR (neat) υ_{max} (C=O) 1774, 1709 cm^{-1}.

2-(3-Fluoro-Phenylsulfanyl)-4-Oxo-Azetidine-1-Carboxylic acid Benzylamide (**9**)

Purified by flash chromatography from ethyl acetate/hexanes to give 70% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.94 (dd, J = 2.75, J = 13.66, 1H); 3.46 (dd, J = 5.70, J = 10.70, 1H); 4.52 (dd+dd, J = 5.93, J = 6.04, J = 6.12, 2H); 5.33 (dd, J = 2.75, J = 2.90, 1H); 6.84 (1H, brs); 7.07-7.40 (9H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 43.82, 44.47, 56.98, 116.18, 116.39, 121.13, 121.35, 127.84, 128.95, 130.13, 130.70, 132.60,138.04, 149.77, 165.44. IR (neat) υ_{max} (C=O) 1774, 1709 cm^{-1}.

2-(4-Fluoro-Phenylsulfanyl)-4-Oxo-Azetidine-1-Carboxylic acid Benzylamide (**10**)

Purified by flash chromatography from ethyl acetate/hexanes to give 60% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.89 (dd, J = 2.74, J = 13.65, 1H); 3.44 (dd, J = 5.66, J = 10.73, 1H); 4.52 (dd+dd, J = 6.05, J = 8.81, J = 11.34, 2H); 5.26 (dd, J = 2.73, J = 2.91, 1H); 6.84 (1H, brs); 7.01-7.58 (9H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 44.07, 57.22, 116.56, 116.77, 124.72, 127.88, 128.96, 137.83, 149.79, 162.52, 165.01, 165.44. IR (neat) υ_{max} (C=O) 1774, 1709 cm^{-1}.

2-(2,4-Difluoro-Phenylsulfanyl)-4-Oxo-Azetidine-1-Carboxylic acid Benzylamide (**11**)

Purified by ethyl acetate/hexane wash (5x 20ml) to give 97.4% yield with m.p. 134-136°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.79 (dd J = 13.84, J = 2.64, 1H); 3.32 (dd

J = 10.79, 5.69, 1H); 4.34 (ddd *J* = 12.04, *J* =8.88, *J* =6.02, 1H); 5.15 (q 2.73, 1H); 6.69-6.80 (m, 3H); 7.14-7.25 (m, 3H); 7.44 (dd *J* = 6.51, *J* =1.80, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 43.84, 44.23, 56.59, 105.05, 112.53, 127.74, 128.84, 137.96, 139.30, 149.64, 162.51, 163.12, 165.24. IR (neat) υ_{max} (C=O) 1774, 1709 cm^{-1}. *Anal.* calcd for $C_{17}H_{14}F_2N_2O_2S$; C, 58.61; H, 4.05; N, 8.04. Found: C, 58.53; H, 4.06; N, 8.03.

N-Benzyl-2-((3,4-Difluorophenyl)Thio)-4-Oxoazetidine-1-Carboxamide (**12**)

Purified by ethyl acetate/hexane wash (5x 20ml) to give 80.2% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.80 (1H, dd, *J* =2.69, *J* =13.70); 3.35 (1H, dd, *J* =5.69, *J* =10.69); 4.43 (2H, dd+dd, *J* =5.93, *J* =9.04, *J* =11.40); 5.17 (1H, d, *J* =2.70, *J* =2.96); 6.75 (1H, brs); 6.90-7.49 (8H, m).^{13}C NMR (125 MHz, $CDCl_3$): δ 43.70, 57.06, 116.55, 124.47, 127.74, 128.80, 137.76, 149.60, 162.36, 164.85, 165.28 IR (neat) υ_{max} (C=O) 1775, 1700cm^{-1}.

N-Benzyl-2-Oxo-4-((Perfluorophenyl)Thio)Azetidine-1-Carboxamide (**13**)

Purified by preparatory place on silica gel (3:1 Hexanes: Ethyl acetate) to give 37.5% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H; 2.99 (dd *J* = 13.625, *J* =2.85, 1H); 3.60 (dd *J* = 10.62, *J* =5.85, 1H); 4.5 (,1H);5.46 (dd *J* = 2.96, *J* =2.89, 1H); 6.69 (brs, 1H); 7.39-7.26 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 43.76, 55.66, 77.36, 127.58, 128.78, 149.21, 164.11. IR (neat) υ_{max} (C=O) 1775, 1700 cm^{-1}.

2-((2,4-Difluorophenyl)Thio)-N-Ethyl-4-Oxoazetidine-1-Carboxamide (**14**)

Purified by running a plug of silica gel in buchner funnel with washing with 2:1 Hexanes: Ethyl acetate to give 95% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.20 (1H, d *J* = 7.24); 2.90 (1H, dd *J* = 13.68, *J* = 2.72); 3.33 (1H, pentet *J* = 7.07, *J* =6.27); 3.462 (1H, dd *J* = 10.75, *J* = 5.66); 5.26 (1H, dd *J* = 2.96, *J* =2.70); 6.39 (1H, brs); 6.92 (1H, t *J* = 8.09); 7.25 (1H, s); 7.63 (1H, q *J* = 8.11, 6.79);. ^{13}C NMR (125 MHz, $CDCl_3$): δ 15.08, 34.75, 43.99, 77.05, 104.89, 112.45, 138.91, 149.4,165.10. IR (neat) υ_{max} (C=O) 1775 cm^{-1}.

2-((2,4-Difluorophenyl)Thio)-N-(2-Nitrobenzyl)-4-Oxoazetidine-1-Carboxamide (**15**)

The synthesis was performed as described by Mulchande et al.[5] with the following modifications: 0.04mmol, 10mg of DL01 was dissolved in 1 mL of $CDCl_3$ with 1.5 mol eq. of 2-nitrophenyl isocyanate. Then triethylamine (.001214g, .3 mol eq.) was added dropwise. The reaction was shook, and monitored by NMR. It was confirmed the product was present after 30min, but out of solution product became unstable. No Further analysis took place. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.82 (dd, *J* = 13.712, *J* = 2.93, 1H); 3.39 (dd, *J* = 10.72, *J* = 5.92, 1H); 5.20 (dd, *J* = 2.96, *J* = 2.91, 1H); 7.4 (m, 3H)

General Procedure for the Synthesis of Achiral N-Carbamoylazetidin-2-one Derivatives via Irradiation (16-18, 25)

The synthesis was performed as developed in our laboratory and applied to compounds: To a solution of appropriate azetidin-2-one **5** (0.1g, 0.46mmol) in dichloromethane (4 mL) was added to1.2mol eq. of triethylamine was added to 1.1 mol

eq. of the corresponding substituents: 2-fluorobenzyl isocyanate, 2-fluorophenyl isocyanate, and 2-methoxybenzyl isocyanate. The reaction was irradiated under-pressure in microwave for 10 to 60 minutes at 300W, and 35°C and monitored by TLC and NMR.

2-((2,4-Difluorophenyl)Thio)-N-(2-Fluorobenzyl)-4-Oxoazetidine-1-Carboxamide (**16**)

Irradiated for 45minutes at specifications stated above. Purified by preparatory place on silica gel (2:1 Hexanes: Ethyl acetate) of the filtrate to give 34.3% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.83 (dd *J* = 13.88, *J* =2.62, 1H); 3.4 (dd *J* = 10.81, *J* =5.68, 1H); 4.45 (t *J* = 6.54, 1H); 5.22 (dd *J* = 2.97, *J* =2.62, 1H); 6.69-6.87 (m, 3H); 7.03 (dt *J* = 8.32, *J* =8.3, 2H); 7.44 (dd *J* = 6.51, *J* =1.80, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ IR (neat) υ_{max} (C=O) 1774, 1709 cm^{-1}.

2-((2,4-Difluorophenyl)Tthio)-N-(2-Methoxybenzyl)-4-Oxoazetidine-1-Carboxamide (**17**)

Irradiated for 25minutes at specifications stated above. Purified by running a plug of silica gel in buchner funnel with washing with ethyl acetate to give 62.6% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.19 (s, 1H); 2.79 (dd, *J* =13.71, *J* =2.69, 1H); 3.35 (dd *J* =10.73, *J* =5.69, 1H); 3.78 (s, 1H); 4.38 (dd, *J* =3.44, *J* =2.67, 1H); 5.15 (dd, *J* =2.48, *J* =2.64, 1H); 7-8.23. ^{13}C NMR (125 MHz, $CDCl_3$): δ 30.07, 39.75, 55.28, 56.30, 104.53, 104.80, 105.06, 110.32, 112.23, 112.43, 120.57, 125.86, 129.10, 129.60, 139.19, 149.35, 157.59, 162.24, 162.89, 164.87, 165.35. IR (neat) υ_{max} (C=O) 1775, 1700 cm^{-1}.

2-((2,4-Difluorophenyl)Thio)-N-(2-Fluorophenyl)-4-Oxoazetidine-1-Carboxamide (**18**)

After 10minutes of irradiated at specifications stated above. The product was confirmed by NMR, but once solvent was dried product became unstable. No Further analysis took place. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.90 (dd, *J* = 13.84, *J* = 2.75, 1H); 3.47 (dd, *J* = 10.84, *J* = 5.74, 1H); 5.28 (dd, *J* = 2.92, *J* = 2.75, 1H); 7.5 (m, 3H).

N-Benzyl-2-Oxoazetidine-1-Carboxamide (**25**)

After 10minutes of irradiated at specifications stated above. The product was confirmed by NMR, but once solvent was dried product became unstable. No Further analysis took place. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.187 (s, 1H); 2.95 (t, *J*= 4.77, 1H) 3.55 (t *J*= 4.76, 1H); 4.39 (d *J*= 6.02, 1H); 7.13-7.28 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 30.07, 36.07, 37.23, 43.68, 128.21, 128.50, 137.94, 150.62, 167.05 IR (neat) υ_{max} (C=O) 1760, 1700 cm^{-1}.

General Procedure for the Synthesis of Chiral N-Carbamoylazetidin-2-one Derivatives via Irradiation (19-24)

The synthesis was performed as developed in our laboratory and applied to compounds: To a solution of appropriate azetidin-2-one **5** (0.1g, 0.464mmol) in dichloromethane (4 mL) was added to1.2mol eq. of triethylamine was added to 1.1 mol eq. of the corresponding *S*-methylbenzyl isocyanate, and *R*-methylbenzyl isocyanate. The reaction was heated under-pressure in irradiated for 45 minutes at 300W, and 35°C and monitored by TLC and NMR.

(*R*)-2-((2,4-Difluorophenyl)Thio)-4-Oxo-N-((*S*)-1-Phenylethyl)Azetidine-1-Carboxamide (**19**)

Purified by preparatory place on silica gel (2:1 Hexanes: Ethyl acetate) of the filtrate to give 18.8% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.47 (d, *J* =6.94, 1H); 2.83 (dd, *J* = 13.73, *J* = 2.71, 1H); 3.36 (dd, *J* = 10.77, *J* = 5.68, 1H); 4.96 (pentet *J* = 7.23, 1H); 5.14 (dd, *J* = 2.95, *J* = 2.68, 1H); 6.66 (d, *J* = 7.98, 1H); 7.6 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 22.56, 44.15, 49.58, 56.51, 104.89, 125.99, 127.53, 128.75, 138.91, 142.75, 148.59, 165.15. IR (neat) υ_{max} (C=O) 1775, 1700 cm^{-1}.

(S)-2-((2,4-Difluorophenyl)Thio)-4-Oxo-N-((S)-1-Phenylethyl)Azetidine-1-Carboxamide (**20**)

Purified by preparatory place on silica gel (2:1 Hexanes: Ethyl acetate) of the filtrate to give 20% yield. ^{1}H/C13 NMR (400 MHz, $CDCl_3$): δ_H 1.44 (d *J* = 6.95, 1H); 2.83 (dd *J* = 13.79, *J* =2.68, 1H);3.38 (dd *J* = 10.82, *J* =5.65, 1H); 4.98 (pentet *J* = 7.243, 1H); 5.18 (dd *J* = 2.98, *J* =2.74, 1H); (m,1H,); 6.78 (dt *J* = 6.06, *J* =2.65, 1H); 7.19 (s, 1H); 7.22-7.38 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 22.33, 44.08, 49.40, 56.24, 77.04, 104.81, 112.29, 126.10, 127.57, 128.75, 139.38, 142.84, 148.57, 165.12. IR (neat) υ_{max} (C=O) 1775, 1700 cm^{-1}.

(*R*)-2-((2,4-Difluorophenyl)Thio)-4-Oxo-N-((R)-1-Phenylethyl)Azetidine-1-Carboxamide (**21**)

Purified by preparatory place on silica gel (2:1 Hexanes: Ethyl acetate) of the filtrate to give 28.5% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H check beginning 2.06 (s, 3H); 2.62 (s, 1H);2.92 (dd *J*=13.77, *J* =2.70, 1H); 3.46 (dd *J*= 5.72 *J*= 10.76, 1H); 4.16

(1H, m); 5.05 (pentet *J*= 7.35, 1H);5.23 (dd *J*=2.99, *J* =2.69, 1H); 5.31 (s, 1H); 6.45 (t *J*=9.068, 1H); 6.74 (d *J*= 7.82, 1H); 7.6 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 22.56, 44.16, 49.57, 56.52, 104.62, 104.89, 105.15, 112.33, 112.51, 125.99, 127.53, 128.75, 139.95, 142.73, 148.58, 162.86, 165.14. IR (neat) υ_{max} (C=O) 1774, 1709 cm^{-1}.

(*S*)-2-((2,4-Difluorophenyl)Thio)-4-Oxo-N-((*R*)-1-Phenylethyl)Azetidine-1-Carboxamide (**22**)

Purified by preparatory place on silica gel (2:1 Hexanes: Ethyl acetate) of the filtrate to give 13% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.21 (d *J*=7.15, 1H); missing HZ between here; 2.81 (dd *J*=13.81, *J* = 2.66, 1H); 3.40 (dd *J*= 10.82, *J* =5.65, 1H); 4.06 (dd *J*= 7.13 *J*= 7.15 ,1H,); 4.98 (pentet *J*= 7.34, 1H); 5.18 (dd *J*= 2.95, *J* =2.65, 1H); 5.23 (s, 1H); 6.65 (m, 1H); 6.79 (dt *J*= 2.64, *J*= 4.35, 1H); 7.20 (s, 1H); 7.22-7.38 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 22.34, 44.07, 49.40, 56.24, 104.85, 105.08, 111.61, 111.84, 112.25, 112.50, 126.10, 127.57, 128.75, 139.40, 142.85, 162.42, 162.98, 164.84, 165.25. IR (neat) υ_{max} (C=O) 1785, 1700 cm^{-1}.

(S)-2-((3,4-Difluorophenyl)Thio)-4-Oxo-N-((S)-1-Phenylethyl)Azetidine-1-Carboxamide (**23**)

Purified by preparatory place on silica gel (2:1 Hexanes: Ethyl acetate) of the filtrate to give 40.4% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.56 (d *J*=6.95, 1H); 2.92 (dd *J*= 13.62, *J* =2.78, 1H); 3.47 (dd *J*= 10.70, *J* =5.72, 1H); 5.07 (pentet *J*= 7.35, 1H); 5.21 (dd *J*= 2.92, *J* =2.78, 1H); 6.80 (d *J*=8.13, 1H); 7.90 (m,1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 22.70, 44.31, 49.65, 57.42, 118.05, 123.80, 126.15, 126.55, 126.62, 127.57,

128.55, 128.78, 131.36, 131.41, 131.46, 142.76, 148.85, 148.90, 149.90, 151.30, 151.60, 152.45, 165.21. IR (neat) υ_{max} (C=O) 1775, 1700 cm^{-1}.

(*R*)-2-((3,4-Difluorophenyl)Thio)-4-Oxo-N-((*S*)-1-Phenylethyl)Azetidine-1-Carboxamide (**24**)

Purified by preparatory place on silica gel (2:1 Hexanes: Ethyl acetate) of the filtrate to give 34.3% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.55 (d *J*= 6.96, 1H); 2.91 (dd *J*=13.65, *J* =2.75, 1H); 3.50 (dd *J*=10.69, *J* =5.70, 1H); 5.11 (pentet, *J*= 7.38, 1H); 5.26 (dd *J*= 2.74 *J*= 2.89, 1H); 6.82 (d *J*= 7.90, 1H); 7.90 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 22.44, 44.05, 49.51, 57.05, 118.00, 124.30, 125.66, 125.77, 126.01, 127.62, 128.83, 131.95, 132.00, 142.81, 148.68, 148.85, 149.99, 151.29, 152.59, 165.14. IR (neat) υ_{max} (C=O) 1775, 1700 cm^{-1}.

General Procedure for the Synthesis of Carbamothioates Derivatives (26)

The synthesis was performed as developed in our laboratory and applied to compounds: a solution of of 2,4- difluorothiophenol (0.25g, 1.71mmol) in dichloromethane (4 mL) was added to 1.2mol eq. of triethylamine was added to 1.5 mol eq. of 2-nitrophenyl isocyanate under argon gas. The reaction was stirred at room temperature and monitored by TLC. After completion of the reaction, the solution was evaporated under reduced pressure.

S-(2,4-Difluorophenyl) (3-Nitrophenyl)Carbamothioate (**26**)

The crude material was purified by flash chromatography from ethyl acetate/hexanes to give 9.3% yield with m.p. 70-73°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H HAVE DR. K LOOK AT SPECTRA .^{13}C NMR (125 MHz, $CDCl_3$): δ 29.73, 99.99, 126.27, 127.37, 131.24, 132.02, 132.57, 134.89, 135.52, 145.03, 147.4. IR (neat) υ_{max} (C=O) 1725 cm^{-1}.

APPENDIX A:

NMR SPECTRA

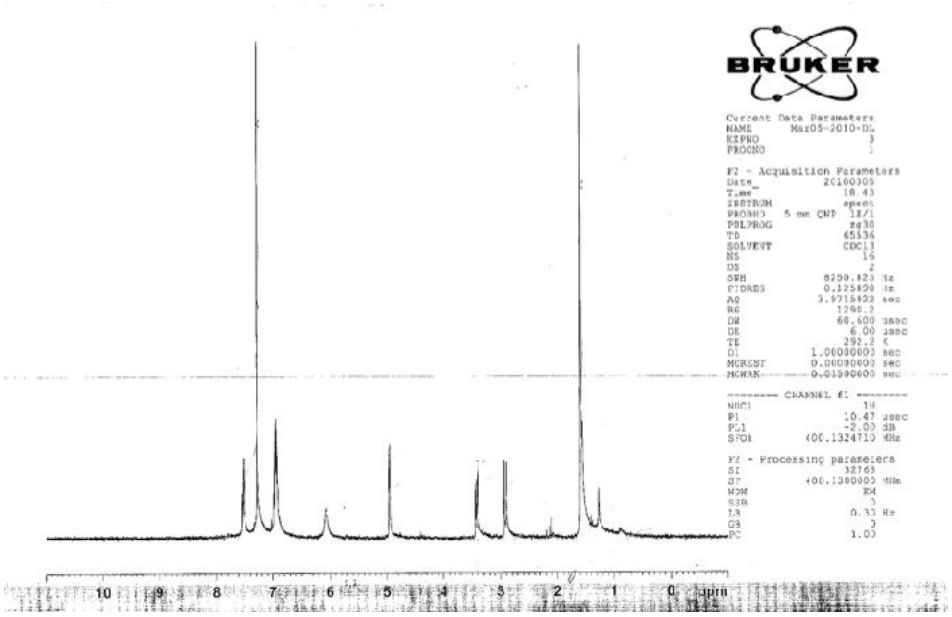


Figure 1. ^{1}H NMR Spectrum of Compound **5**

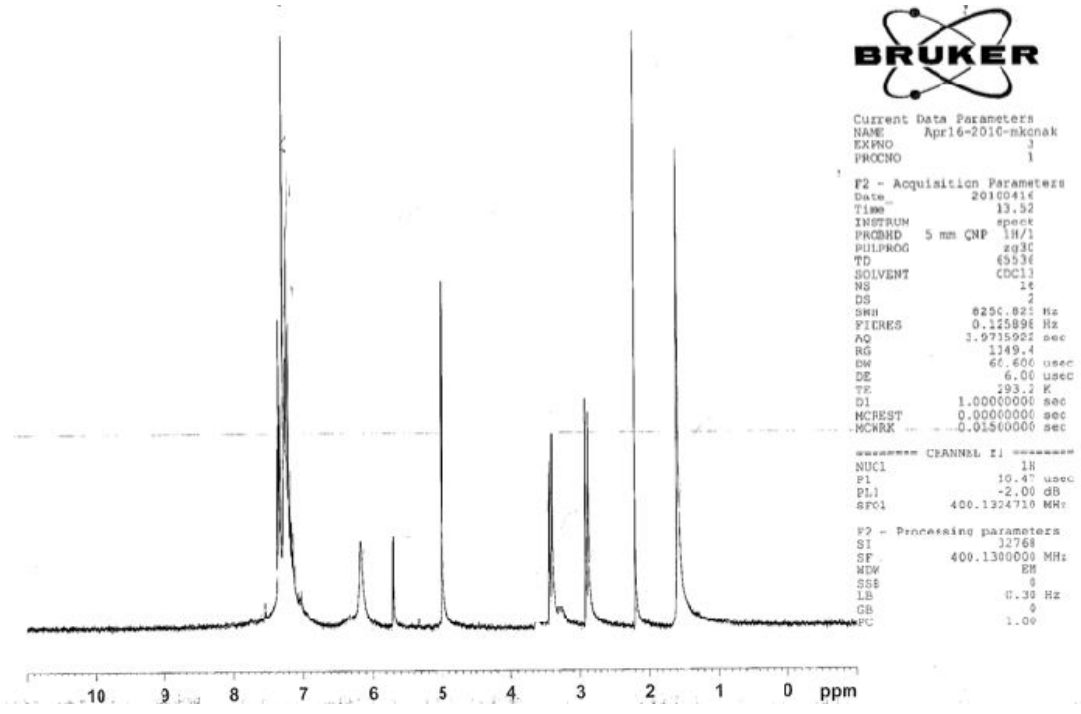


Figure 2. ^{1}H NMR Spectrum of Compound **6**

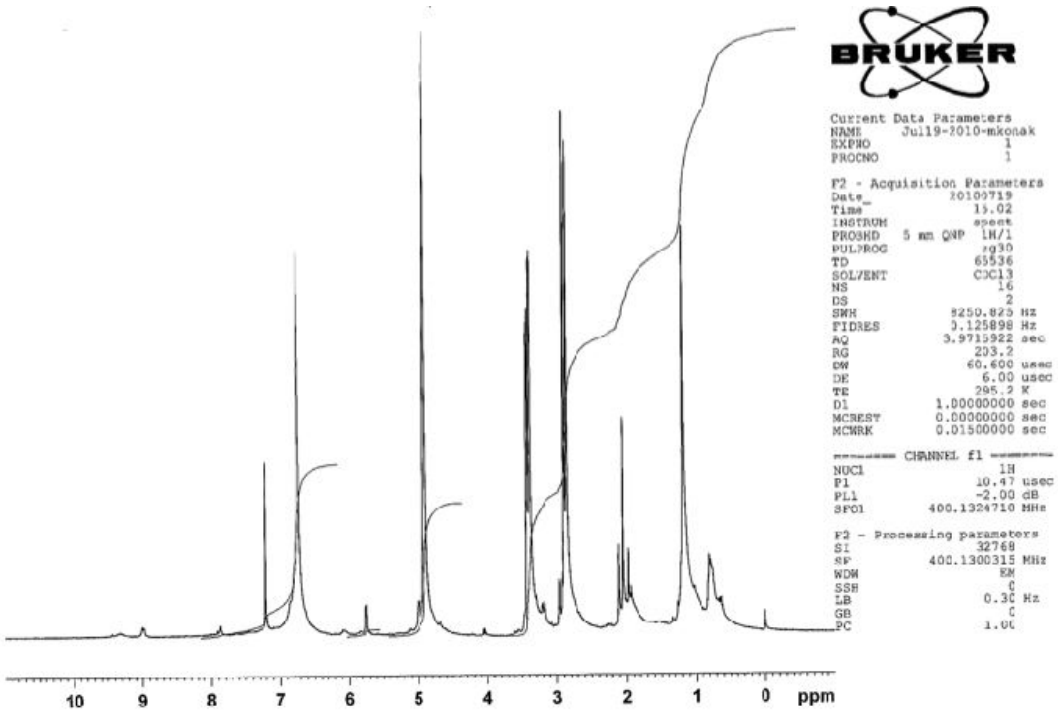


Figure 3. ^{1}H NMR Spectrum of Compound **7**

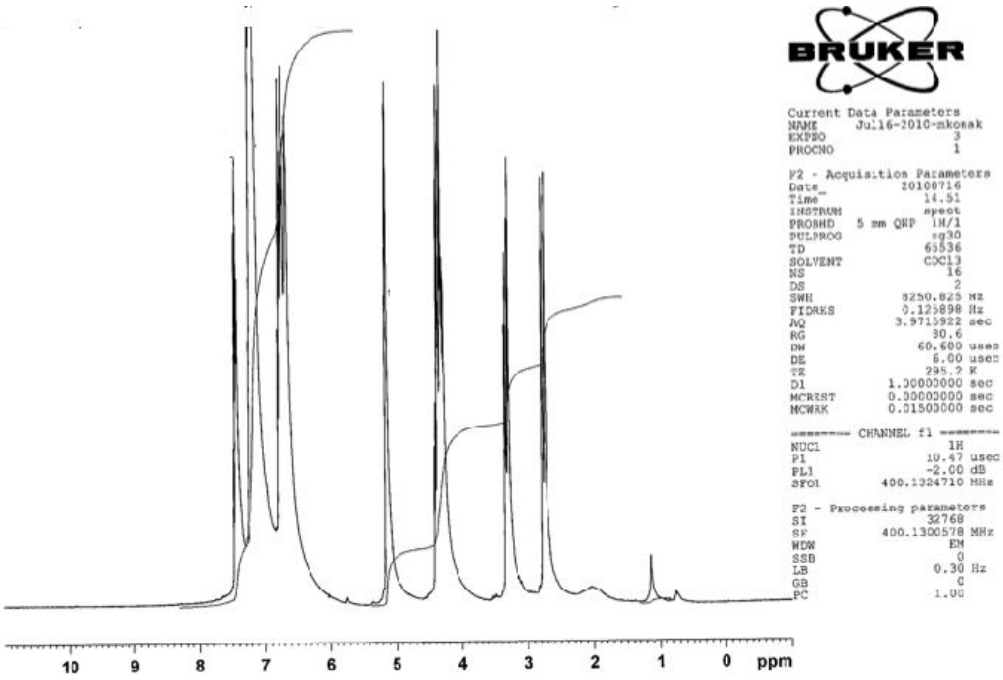


Figure 4. ^{1}H NMR Spectrum of Compound **11**

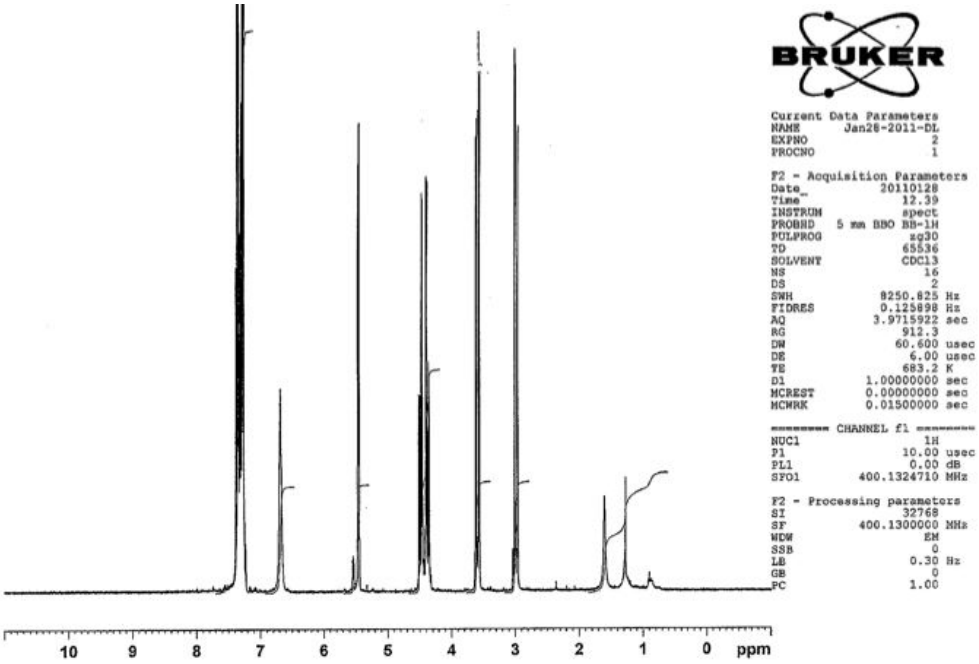


Figure 5. ^{1}H NMR Spectrum of Compound **13**

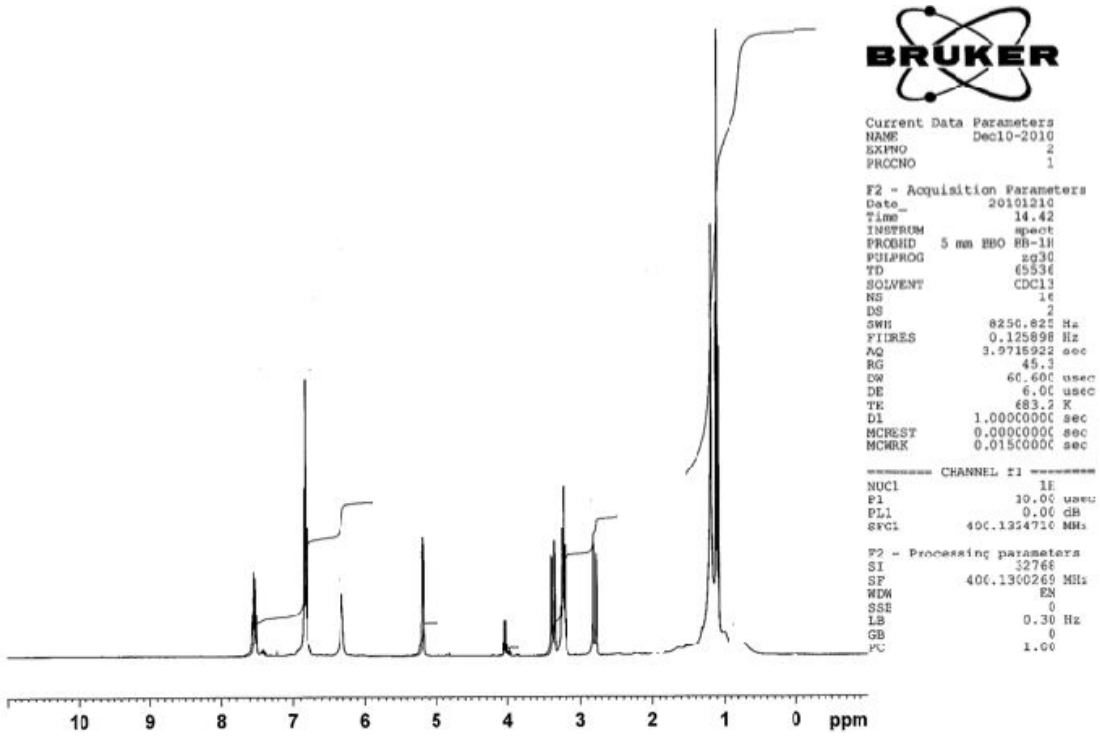


Figure 6. ^{1}H NMR Spectrum of Compound **14**

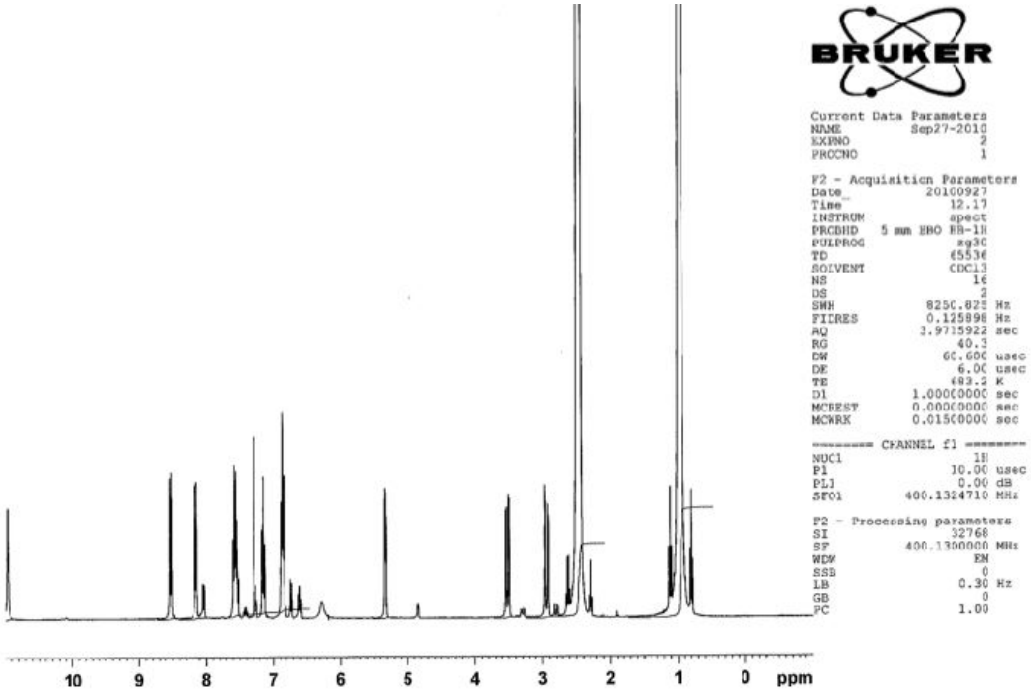


Figure 7. ^{1}H NMR Spectrum of Compound **15**

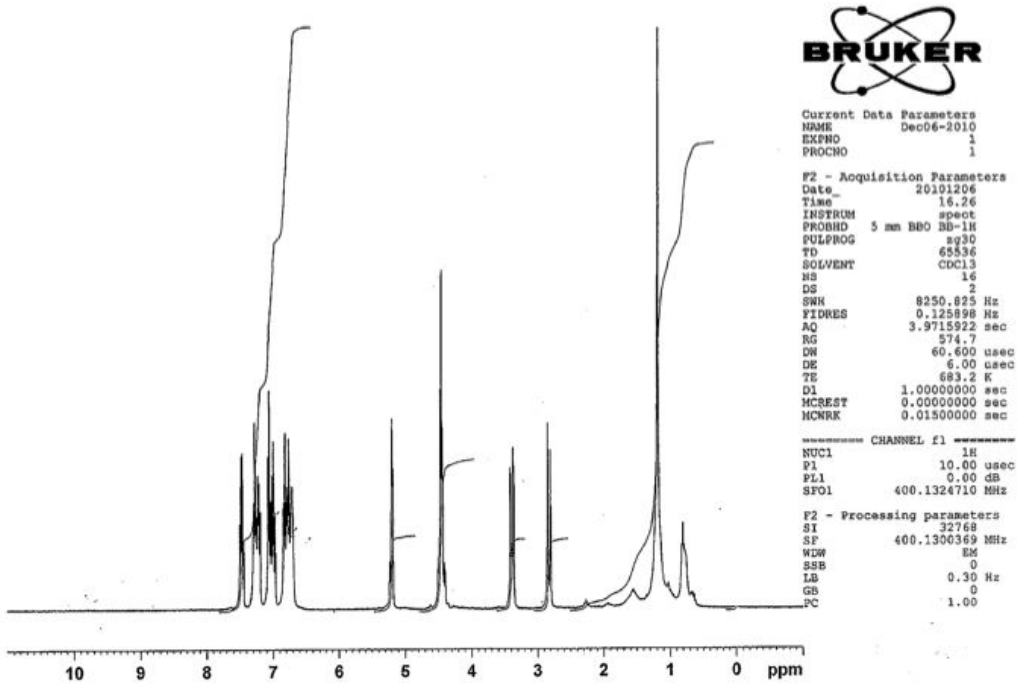


Figure 8. ^{1}H NMR Spectrum of Compound **16**

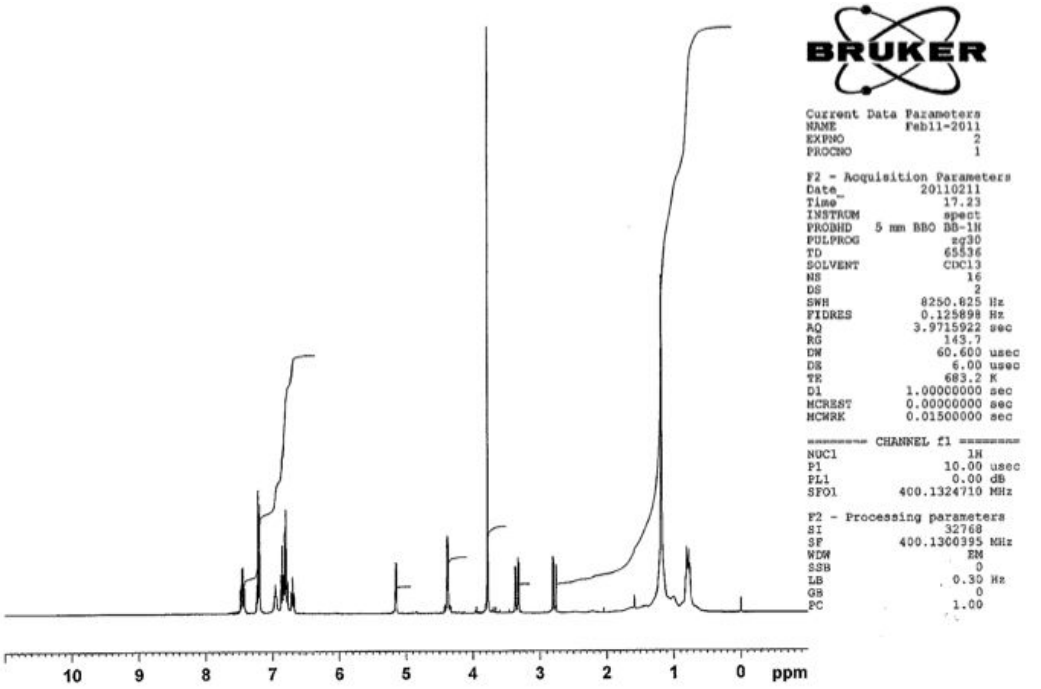


Figure 9. ^{1}H NMR Spectrum of Compound **17**

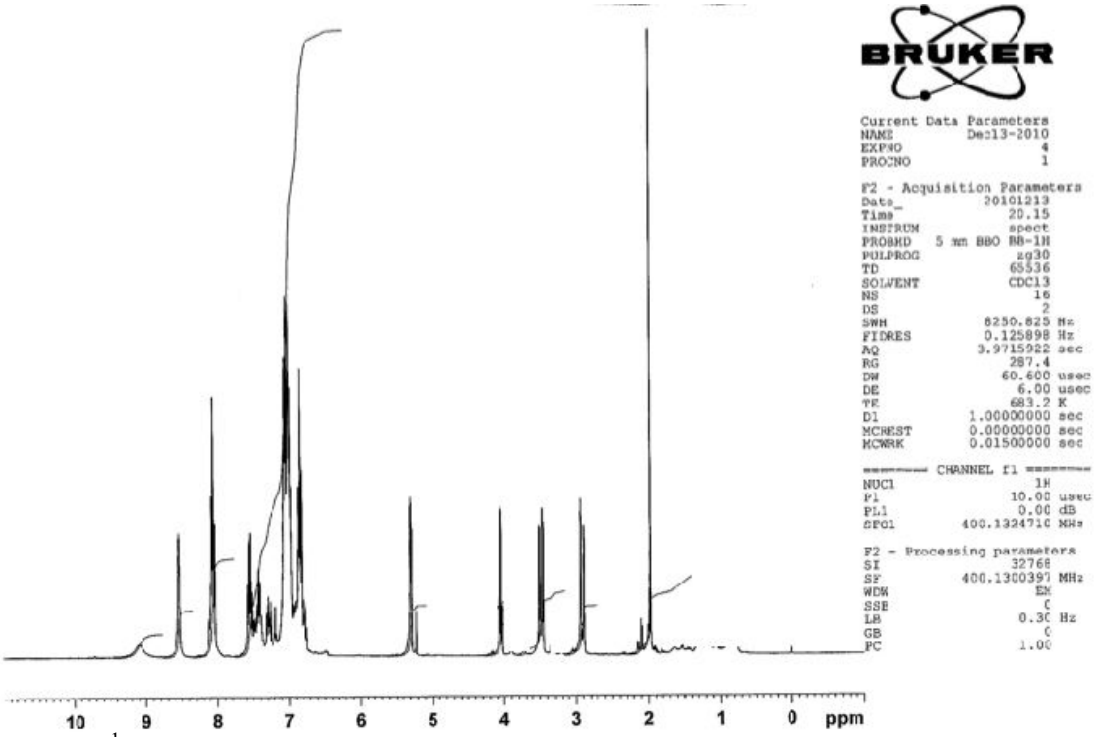


Figure 10. ^{1}H NMR Spectrum of Compound **18**

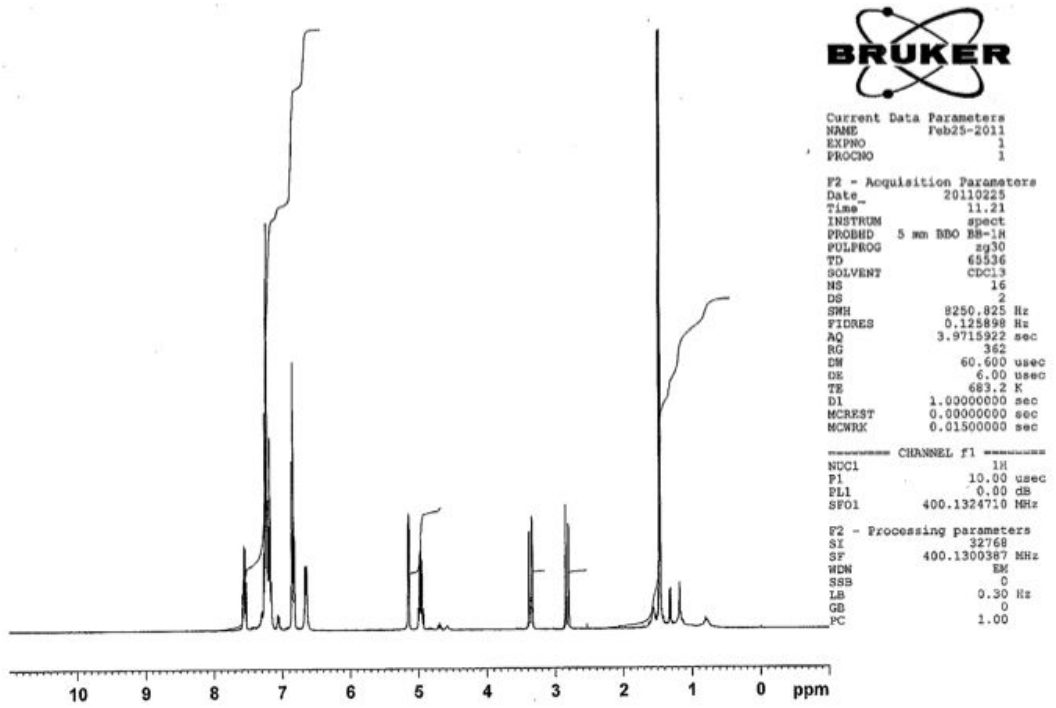


Figure 11. ^{1}H NMR Spectrum of Compound **19**

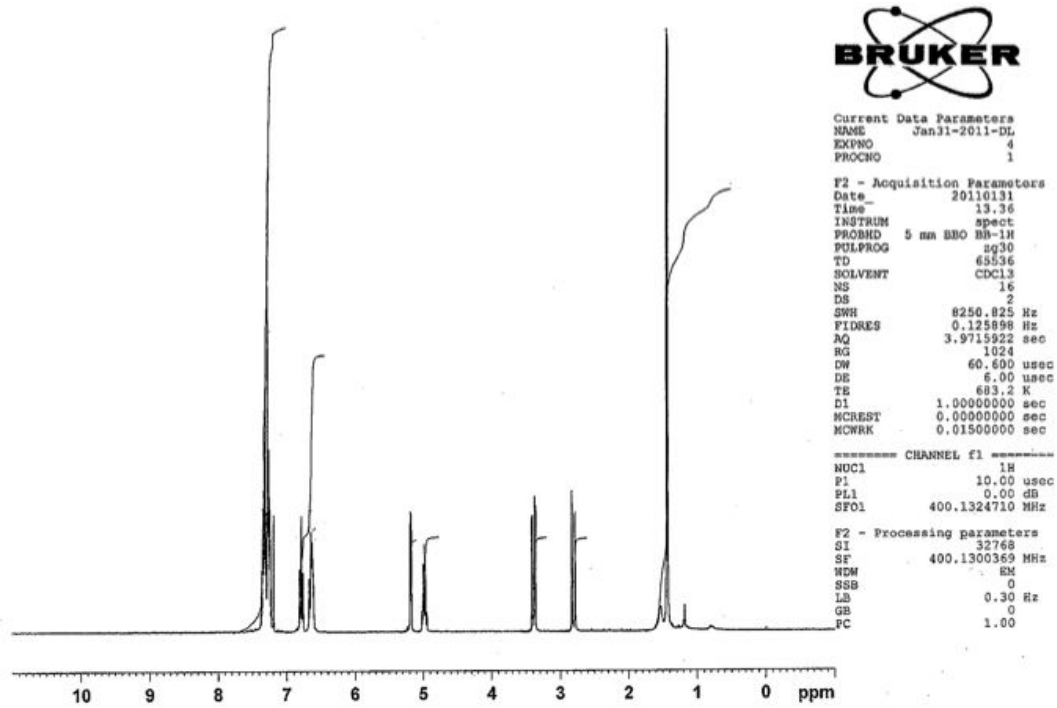


Figure 12. ^{1}H NMR Spectrum of Compound **20**

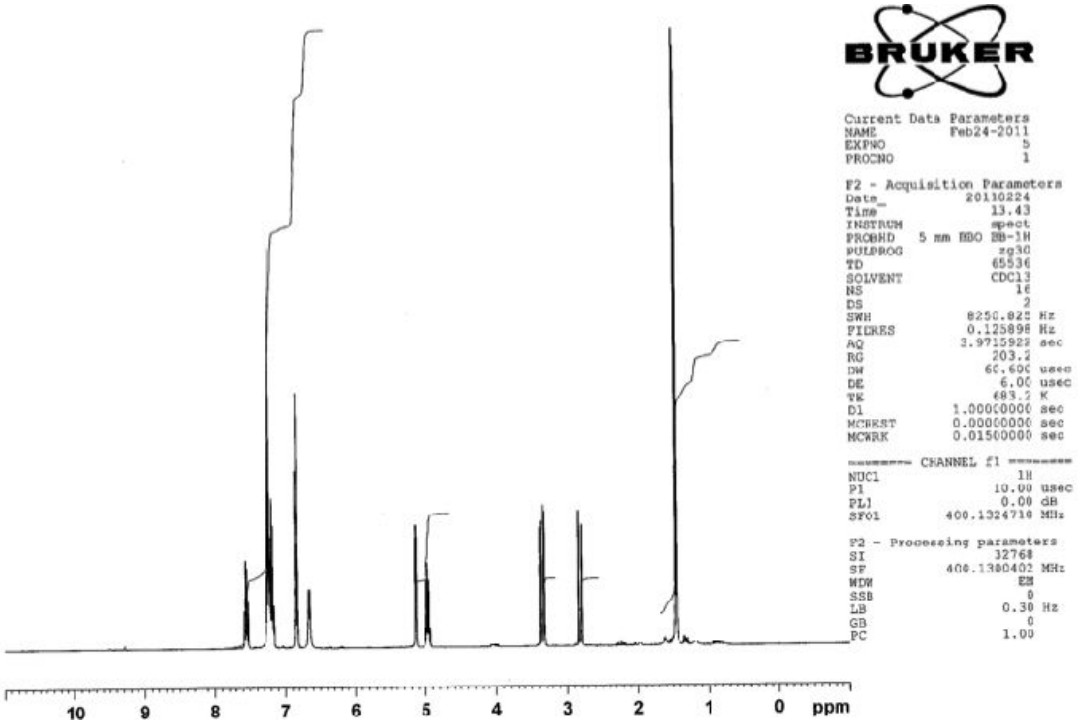


Figure 13. ^{1}H NMR Spectrum of Compound **21**

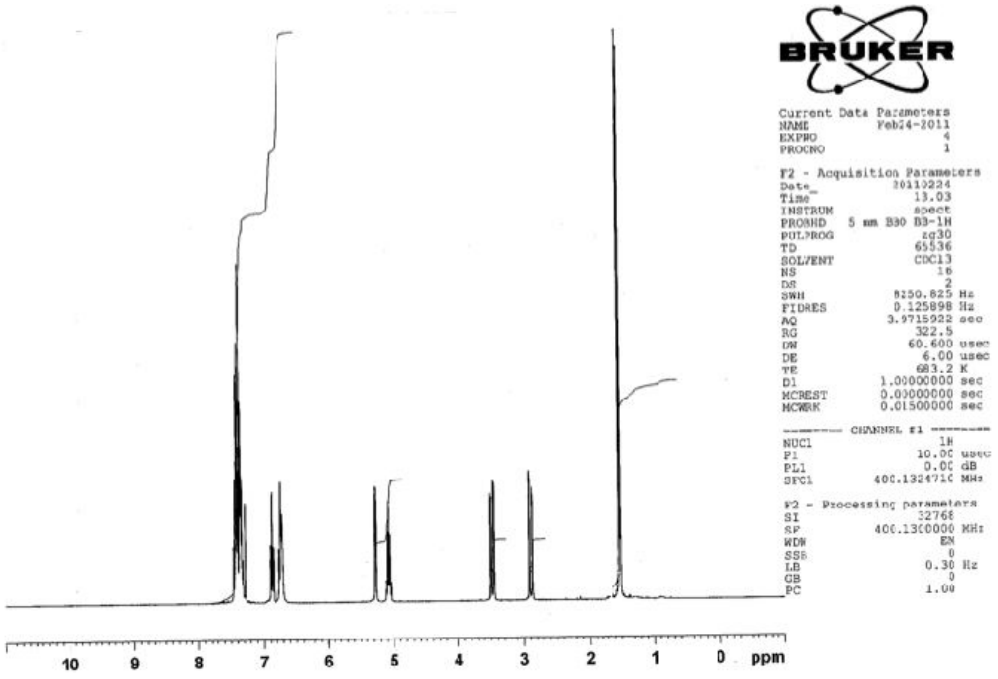


Figure 14. ^{1}H NMR Spectrum of Compound **22**

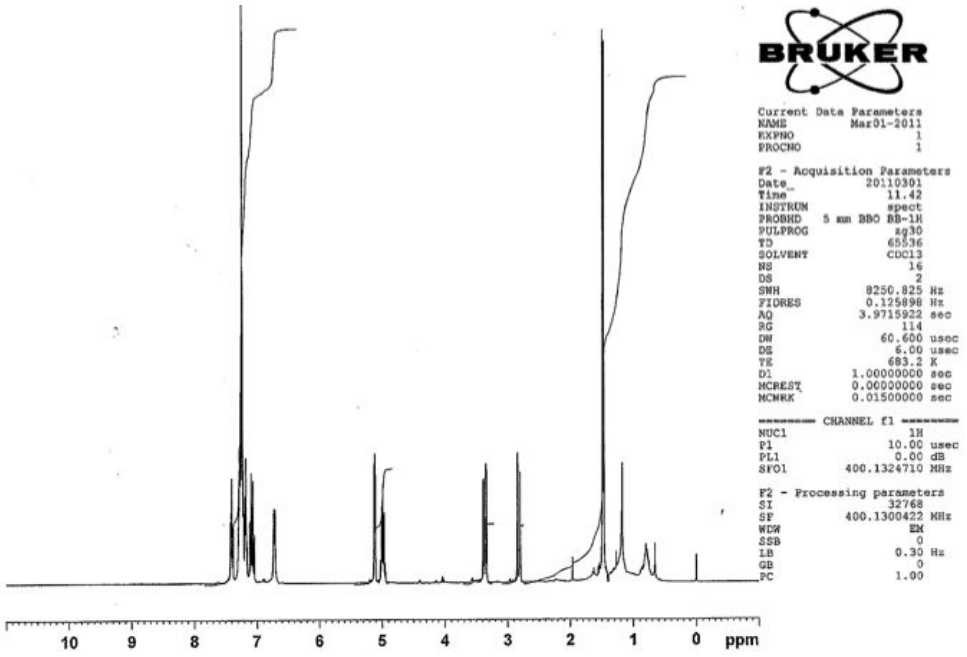


Figure 15. 1H NMR Spectrum of Compound **23**

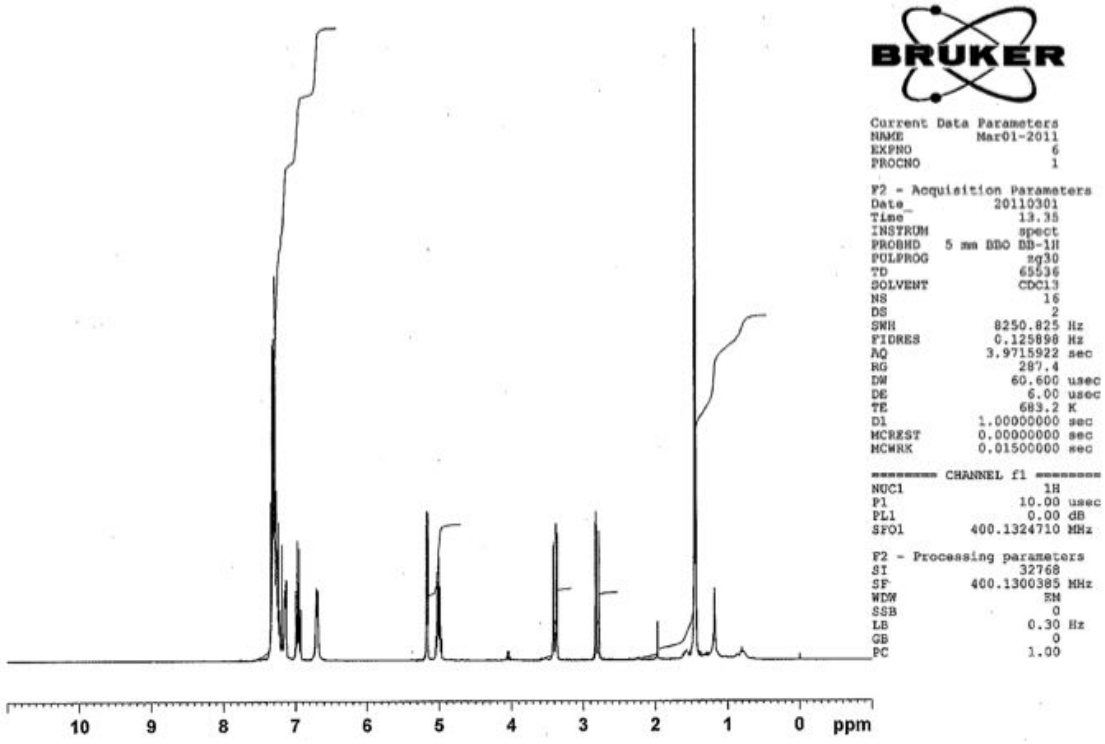


Figure 16. ^{1}H NMR Spectrum of Compound **24**

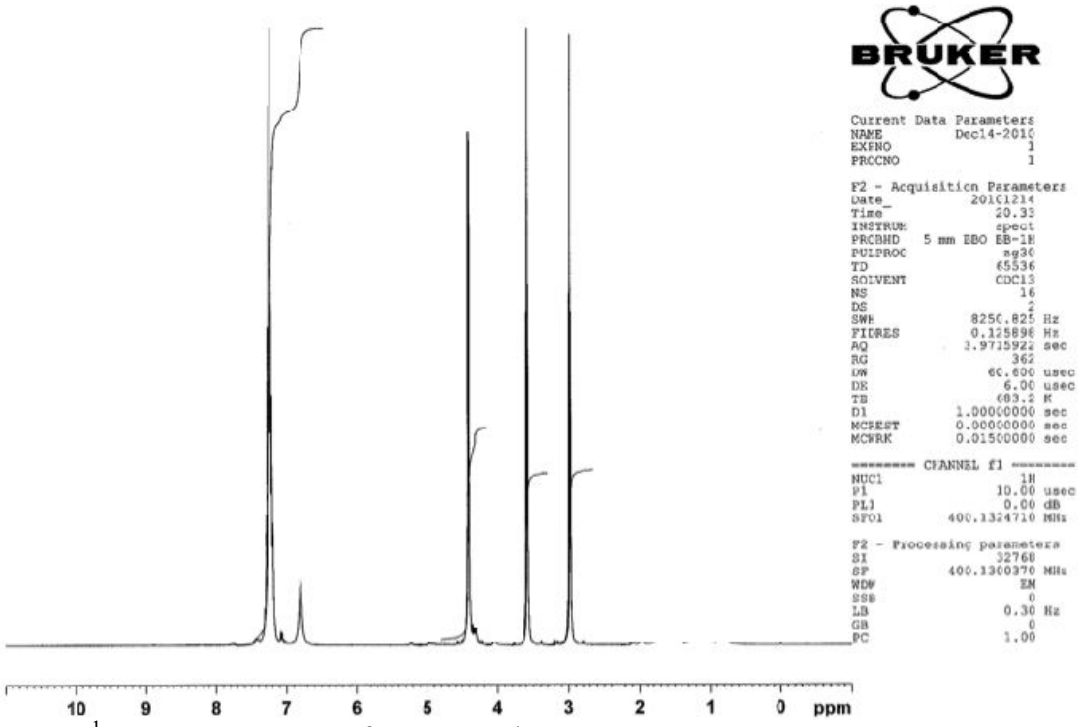


Figure 17. ^{1}H NMR Spectrum of Compound **25**

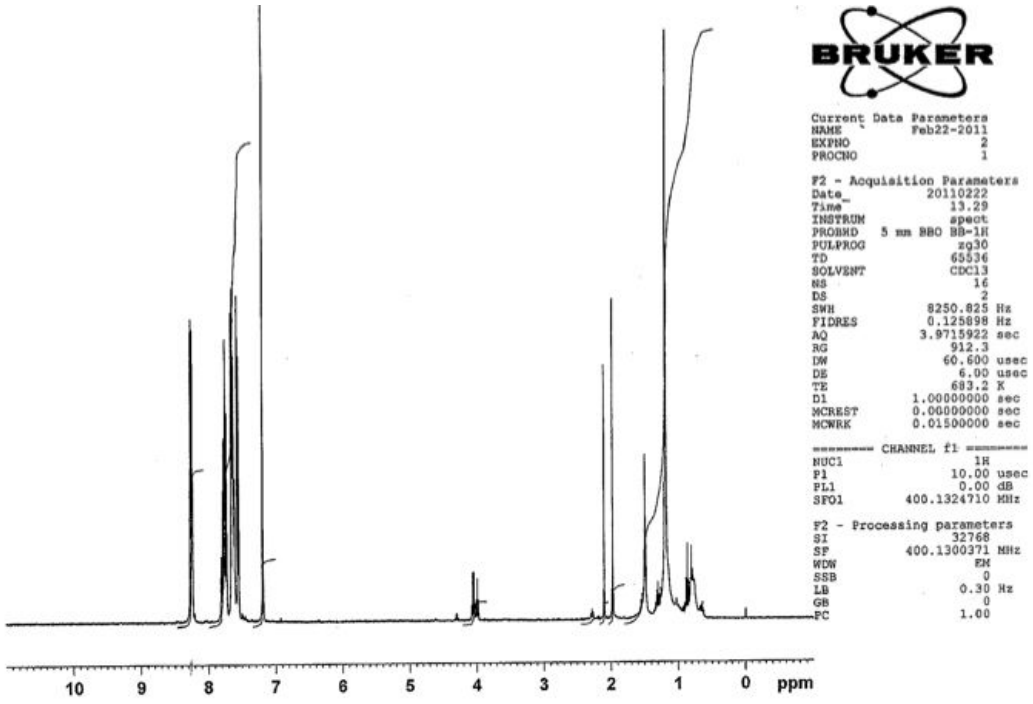


Figure 18. ^{1}H NMR Spectrum of Compound **26**

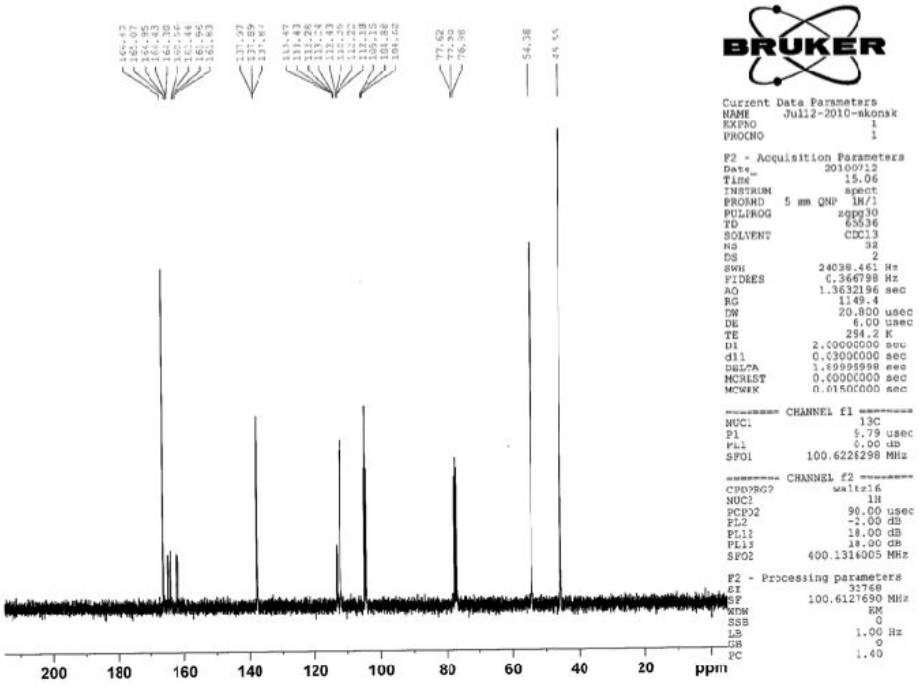


Figure 19. ^{13}C NMR Spectrum of Compound **5**

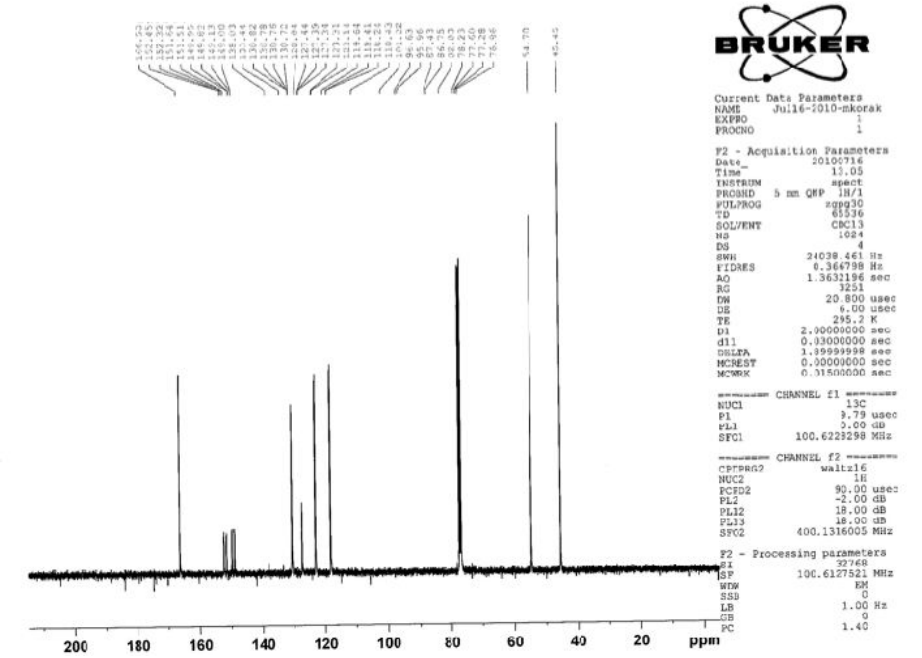


Figure 20. ^{13}C NMR Spectrum of Compound **6**

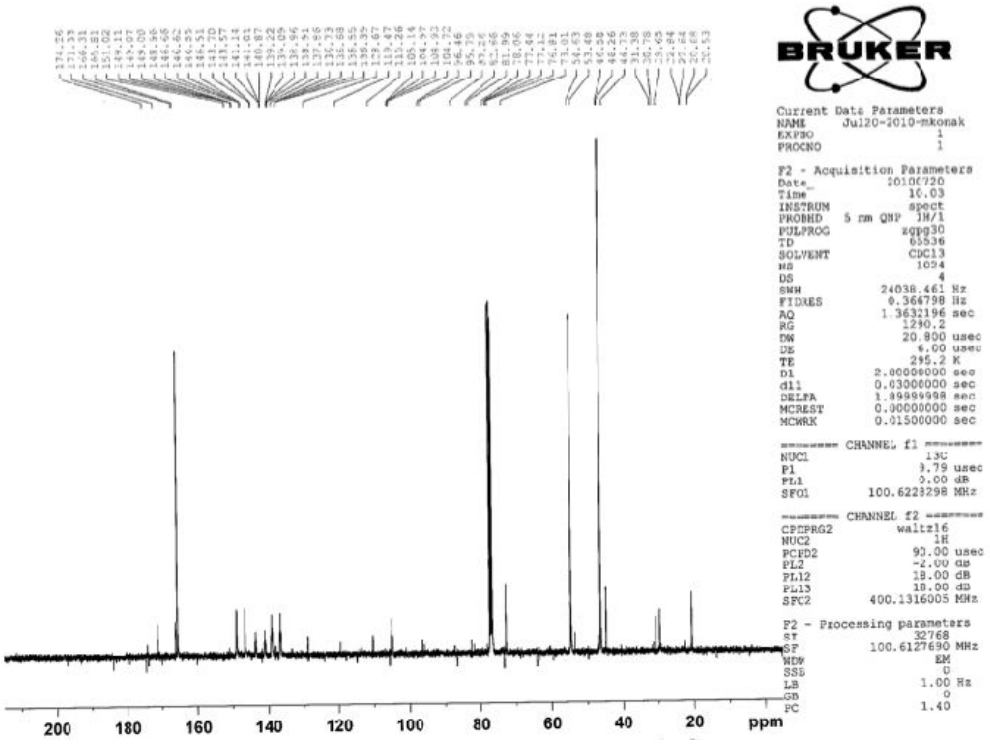


Figure 21. ^{13}C NMR Spectrum of Compound **7**

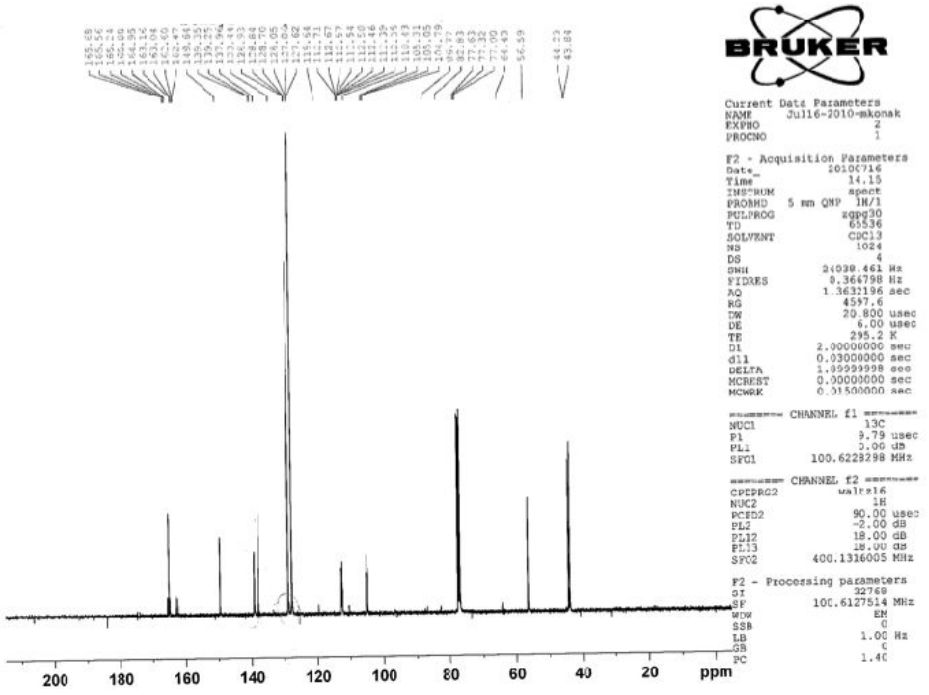


Figure 22. ^{13}C NMR Spectrum of Compound **11**

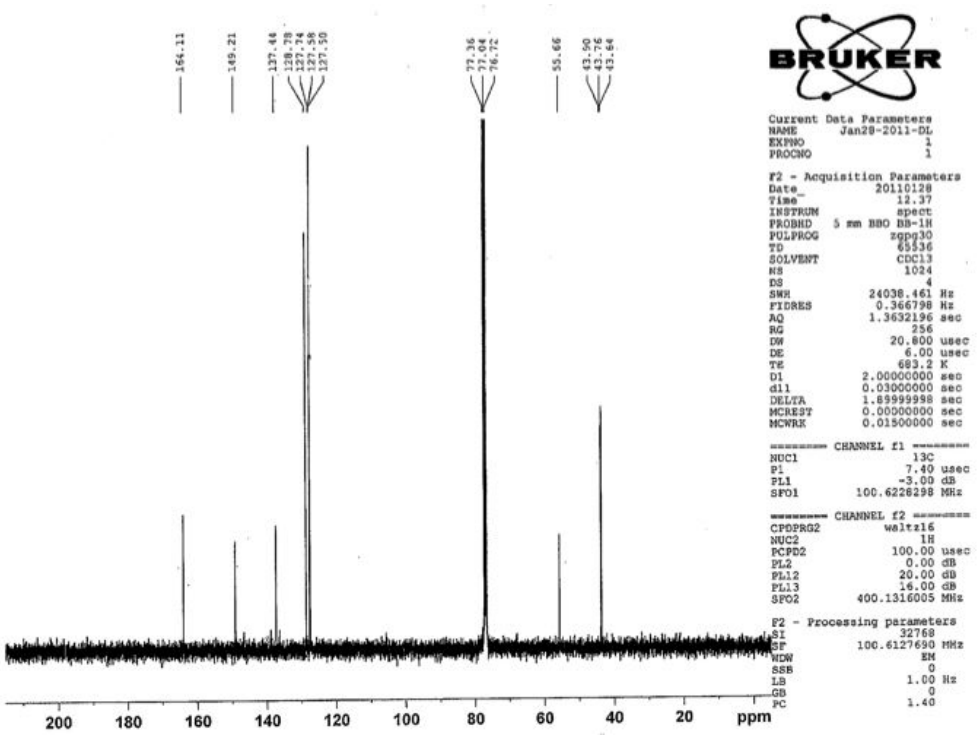


Figure 23. ^{13}C NMR Spectrum of Compound **13**

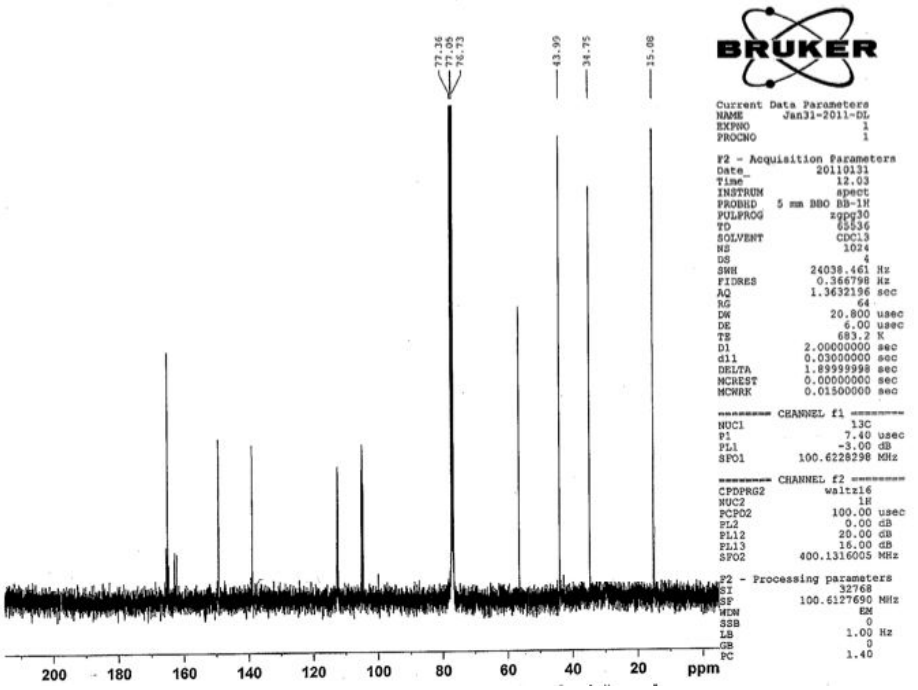


Figure 24. ^{13}C NMR Spectrum of Compound **14**

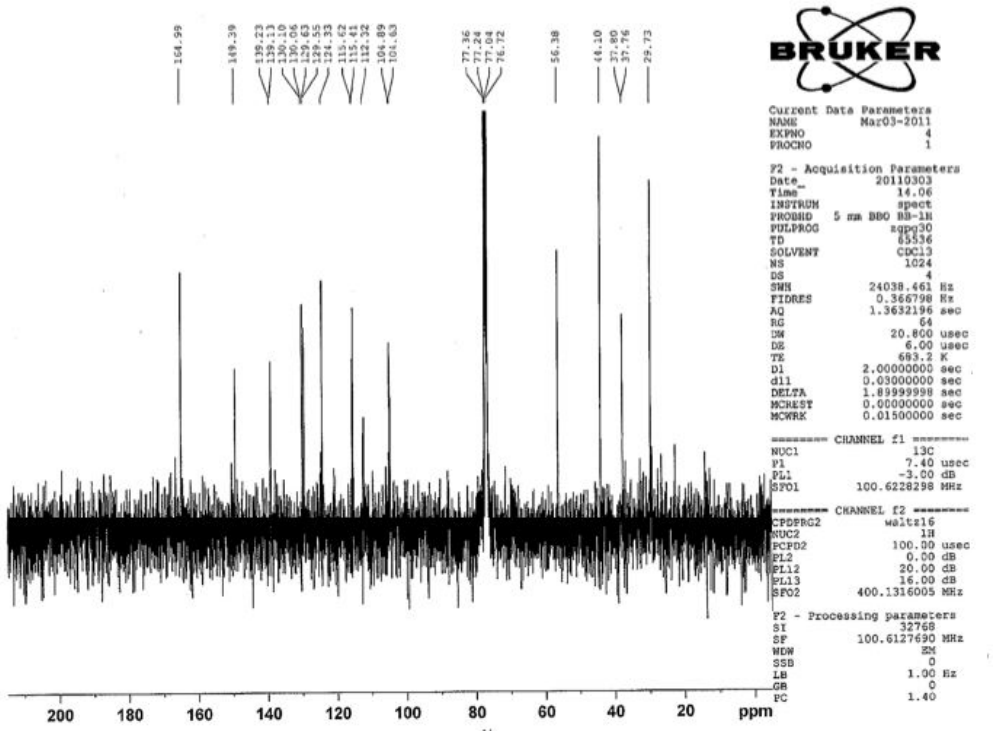


Figure 25. ^{13}C NMR Spectrum of Compound **16**

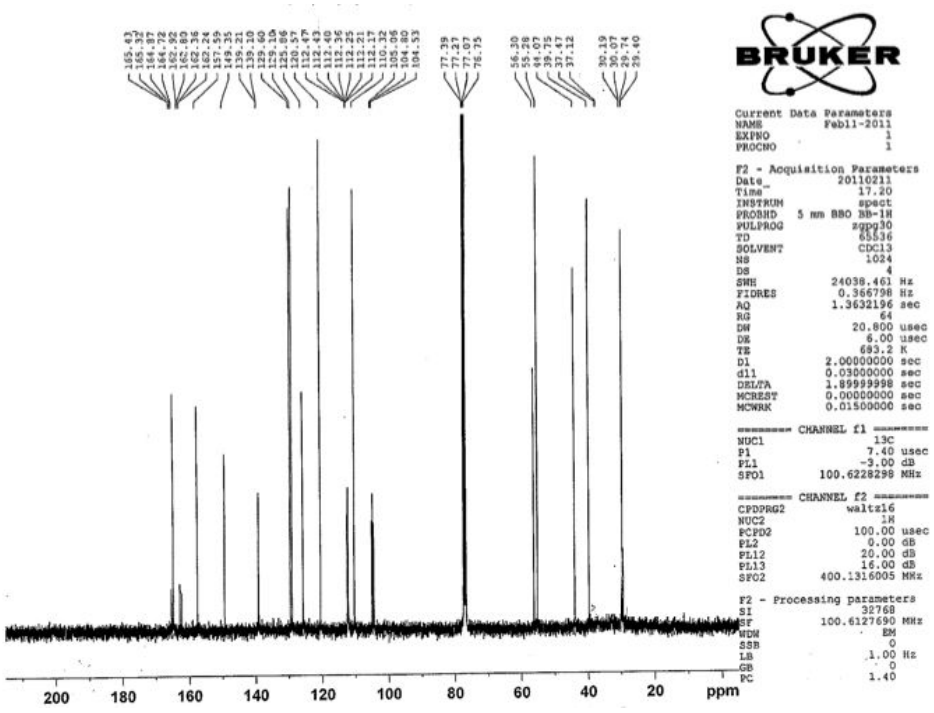


Figure 26. ^{13}C NMR Spectrum of Compound **17**

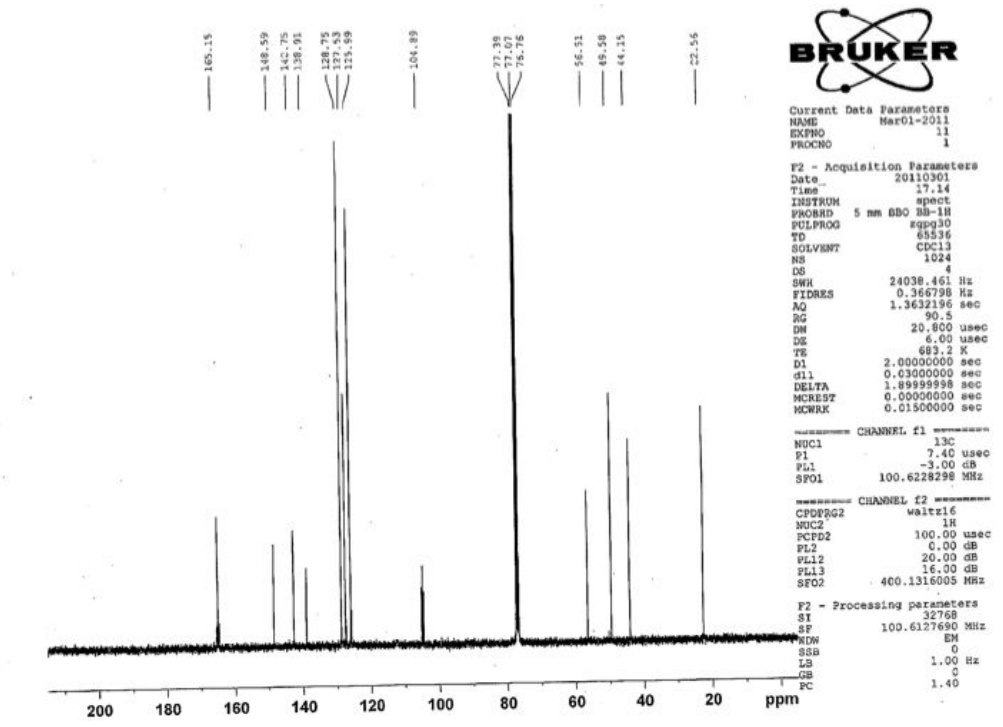


Figure 27. ^{13}C NMR Spectrum of Compound **19**

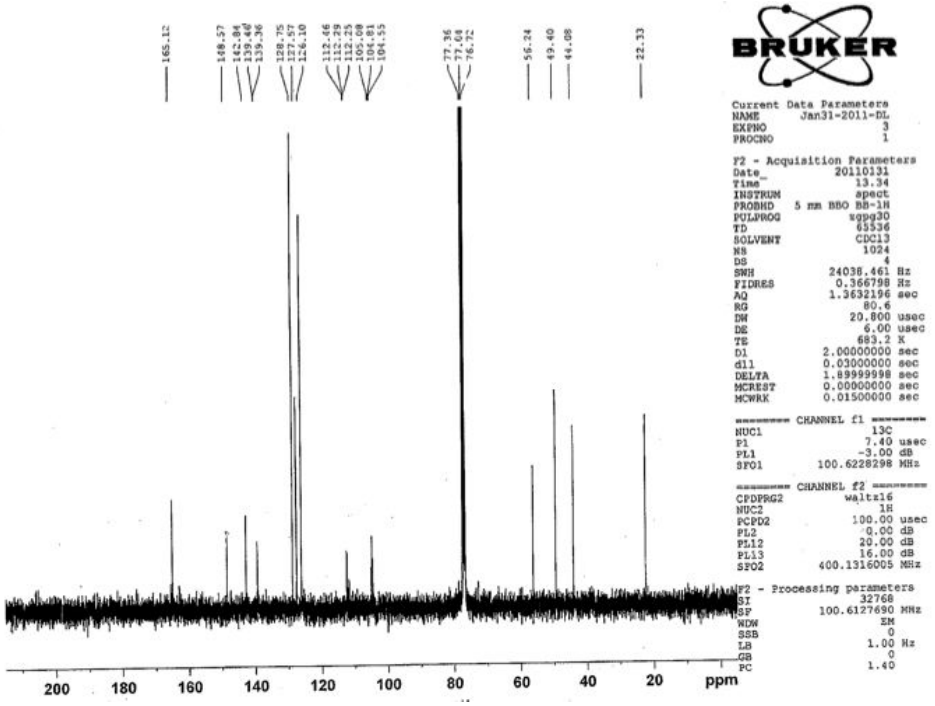


Figure 28. ^{13}C NMR Spectrum of Compound **20**

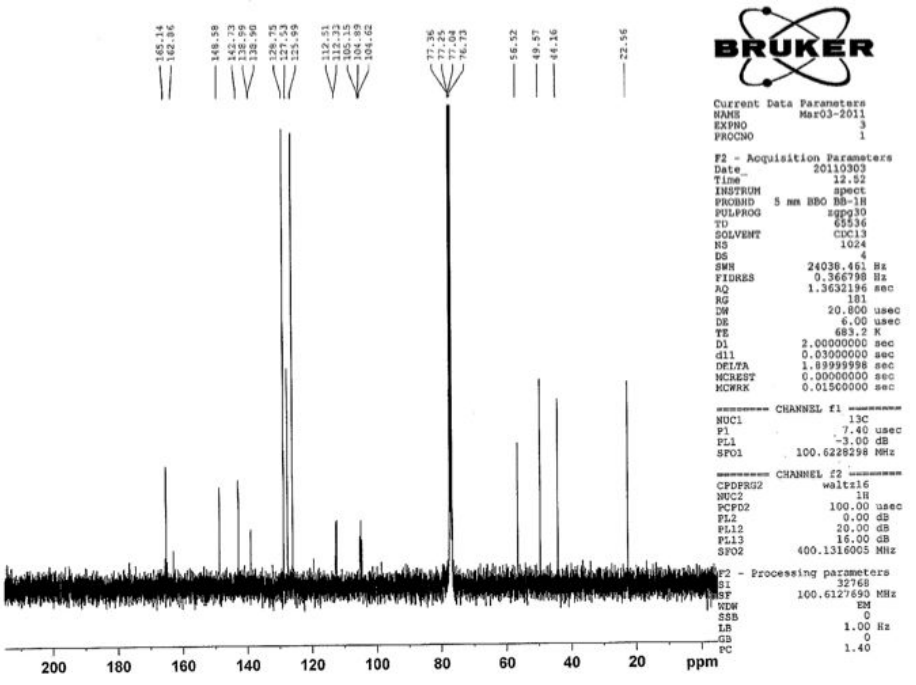


Figure 29. ^{13}C NMR Spectrum of Compound **21**

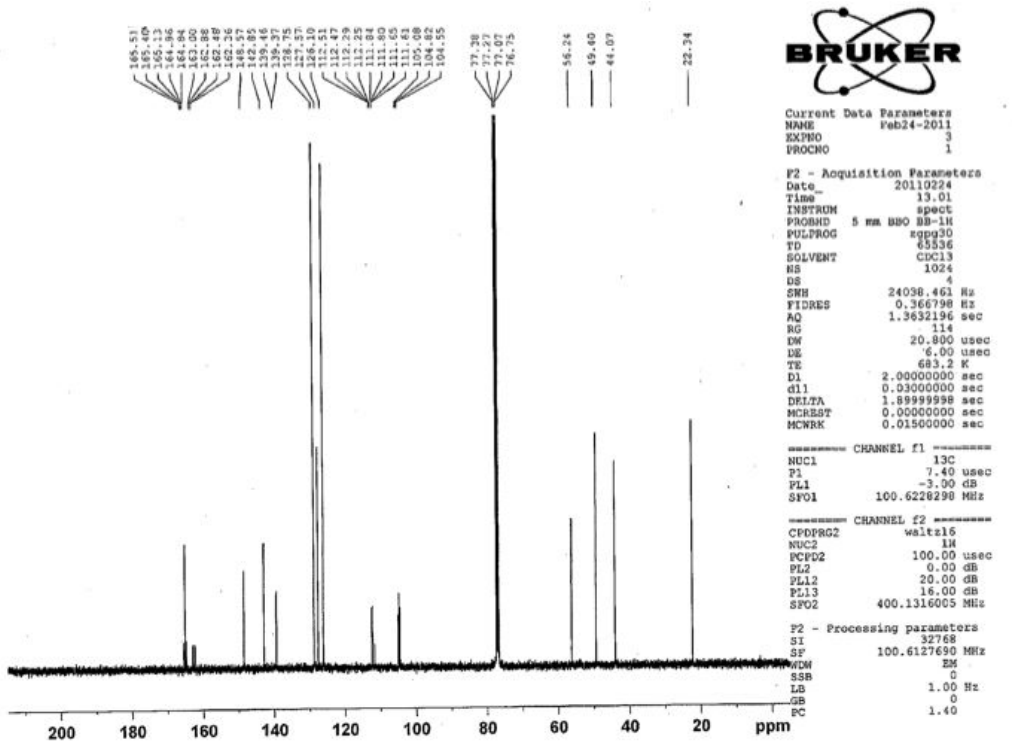


Figure 30. ^{13}C NMR Spectrum of Compound **22**

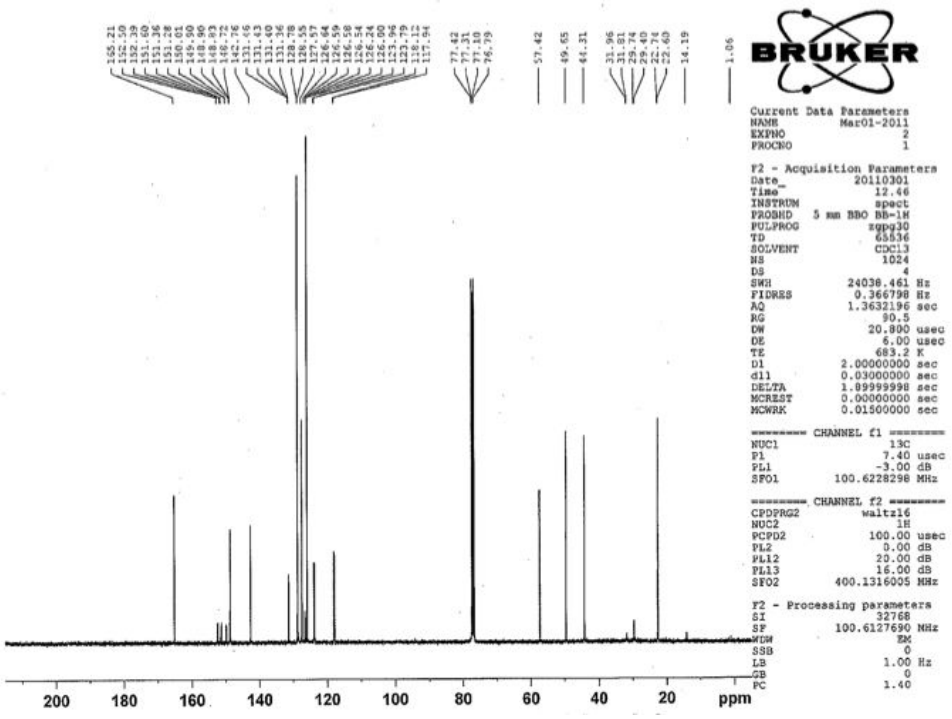


Figure 31. ^{13}C NMR Spectrum of Compound **23**

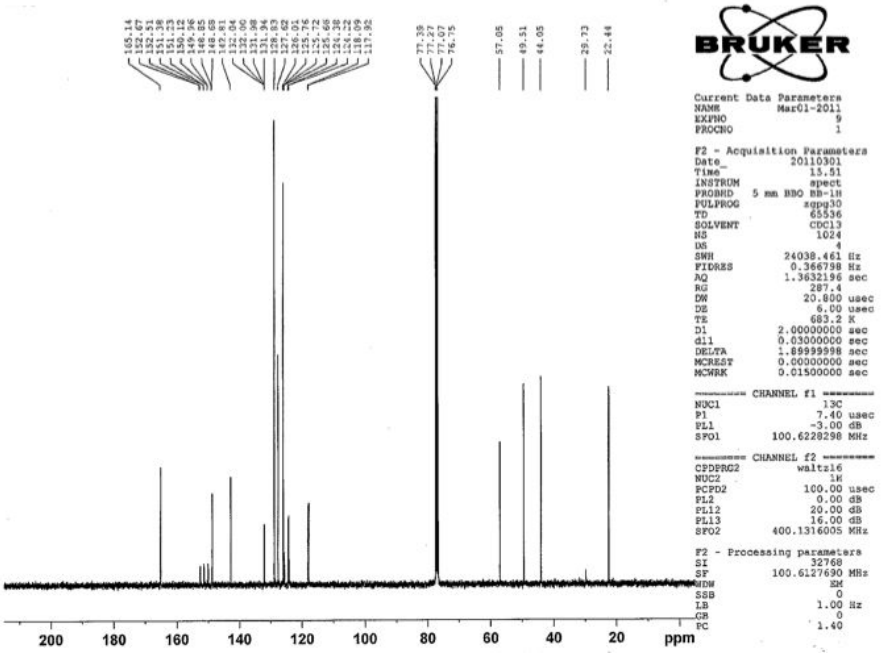


Figure 32. ^{13}C NMR Spectrum of Compound **24**

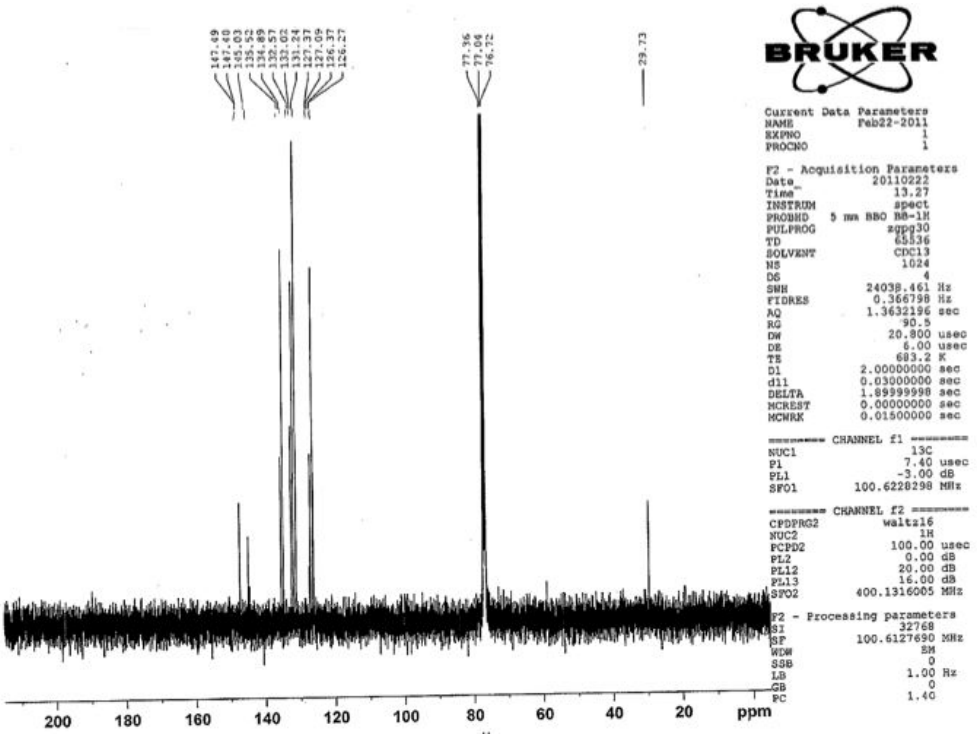


Figure 33. ^{13}C NMR Spectrum of Compound **26**

APPENDIX B:

INFRARED (FT-IR) SPECTRA

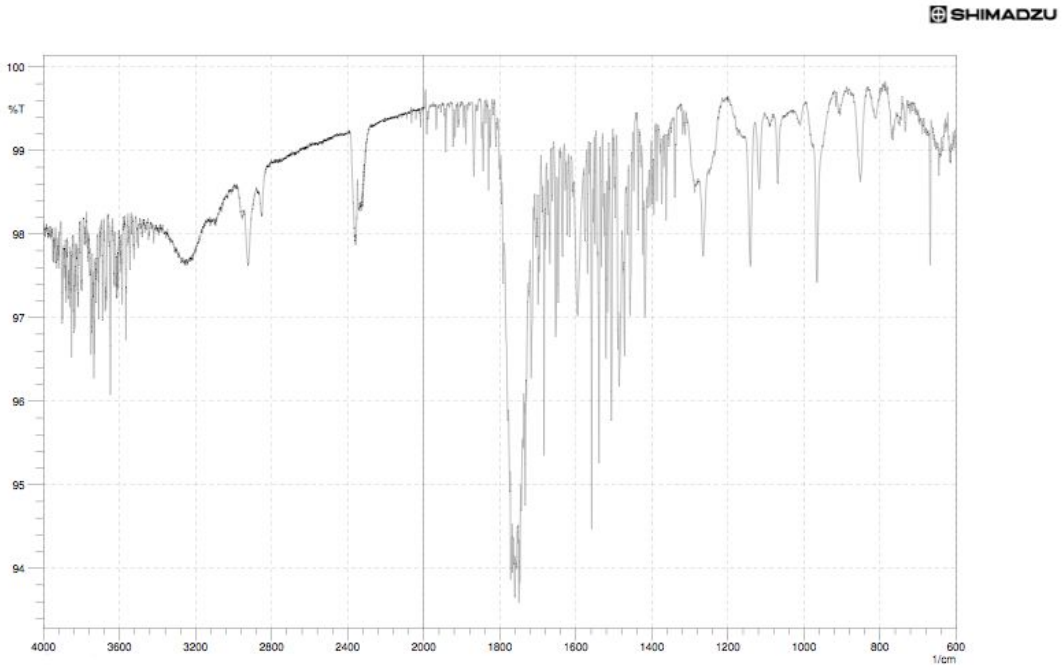


Figure 34. FT-IR spectrum of Compound **5**

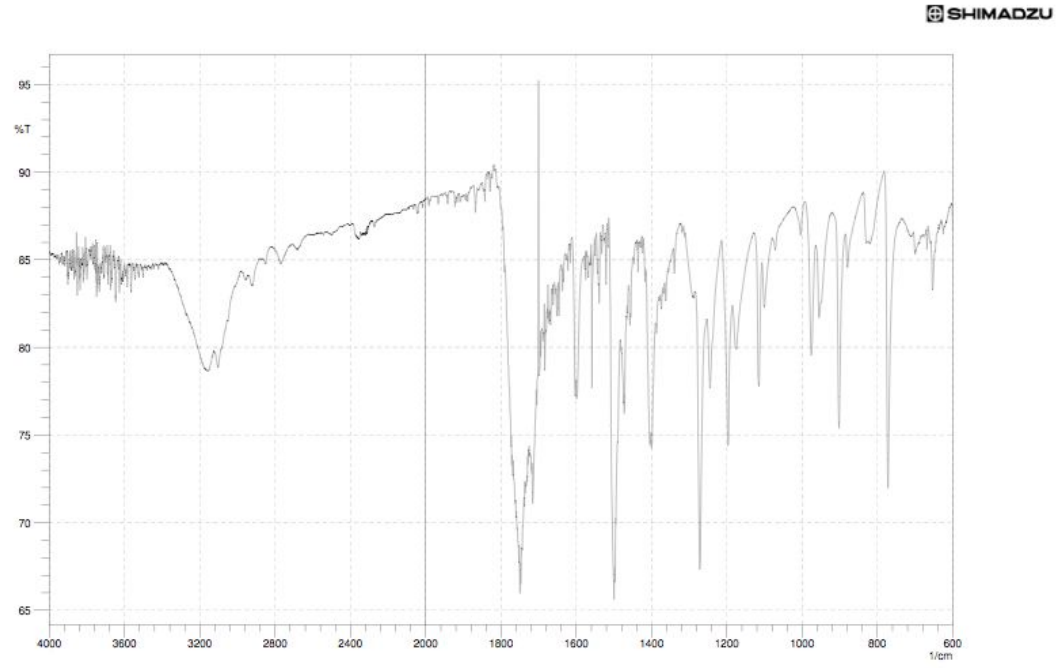


Figure 35. FT-IR spectrum of Compound **6**

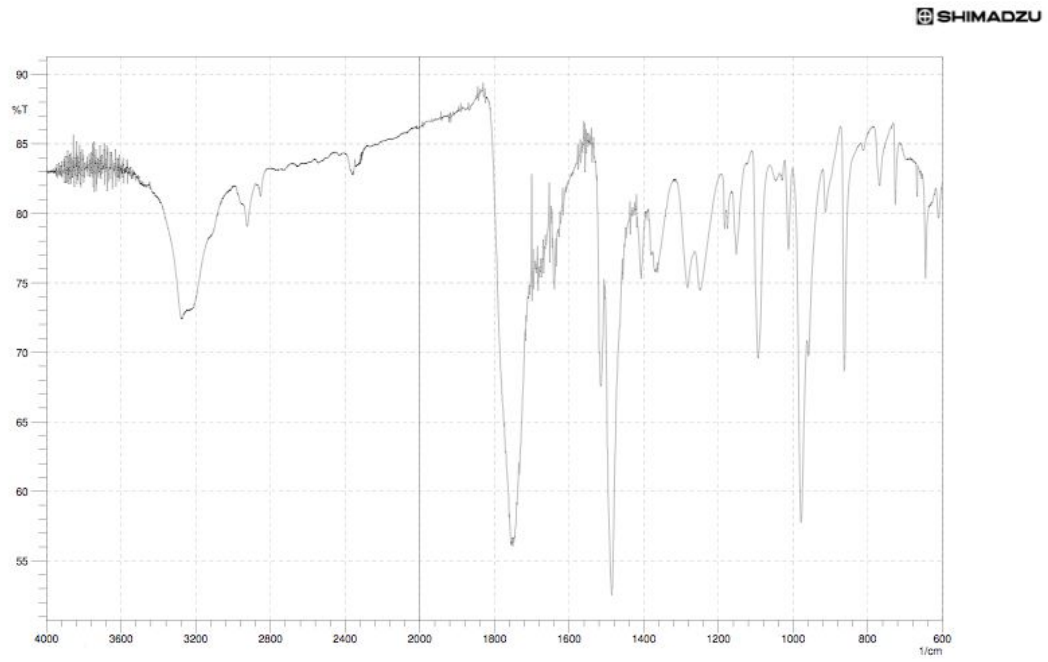


Figure 36. FT-IR spectrum of Compound **7**

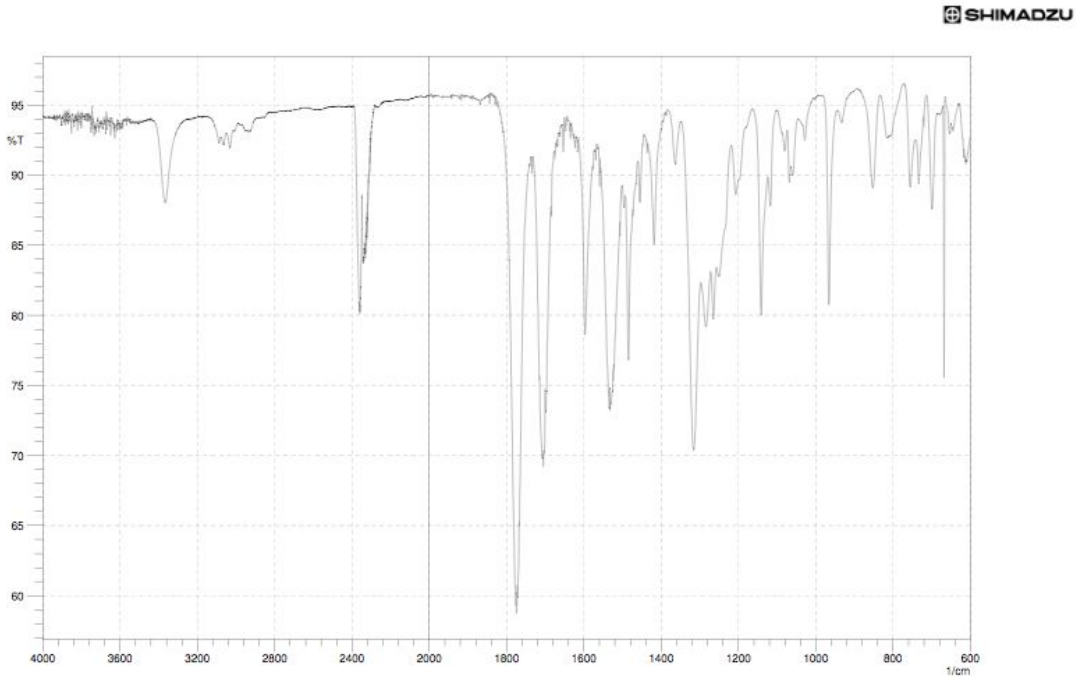


Figure 37. FT-IR spectrum of Compound **11**

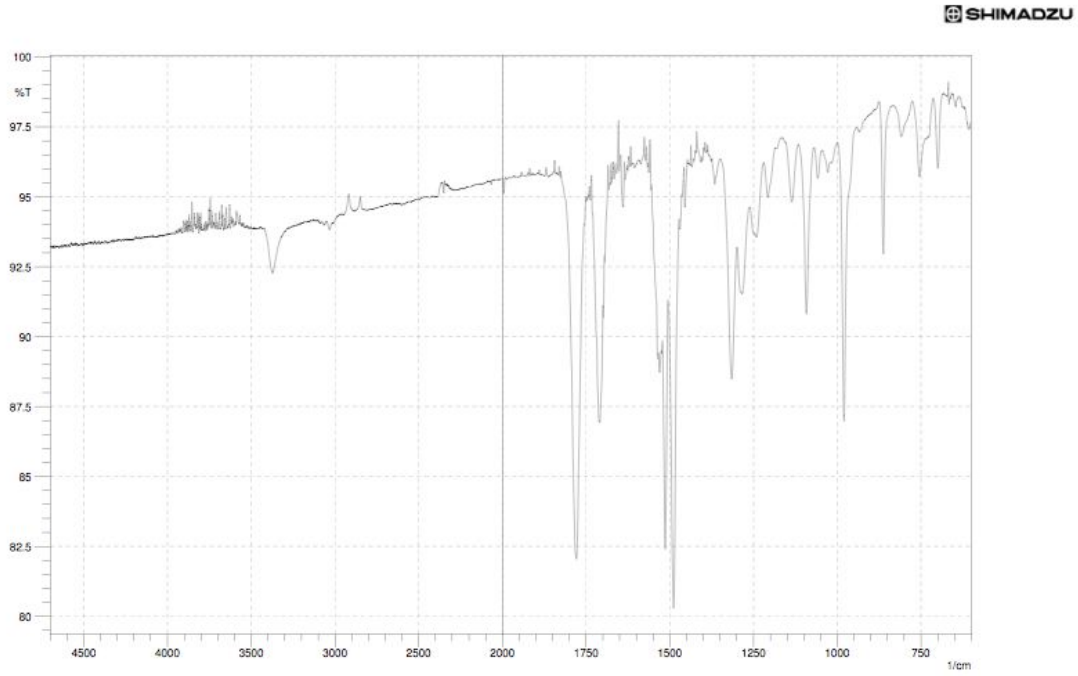


Figure 38. FT-IR spectrum of Compound **13**

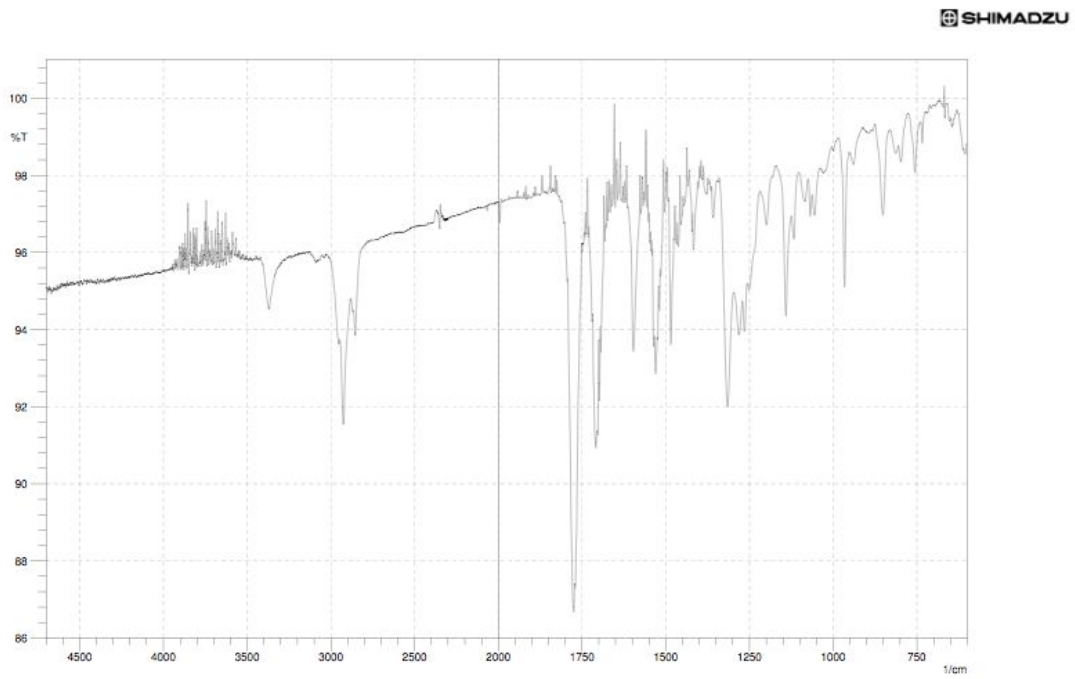


Figure 39. FT-IR spectrum of Compound **14**

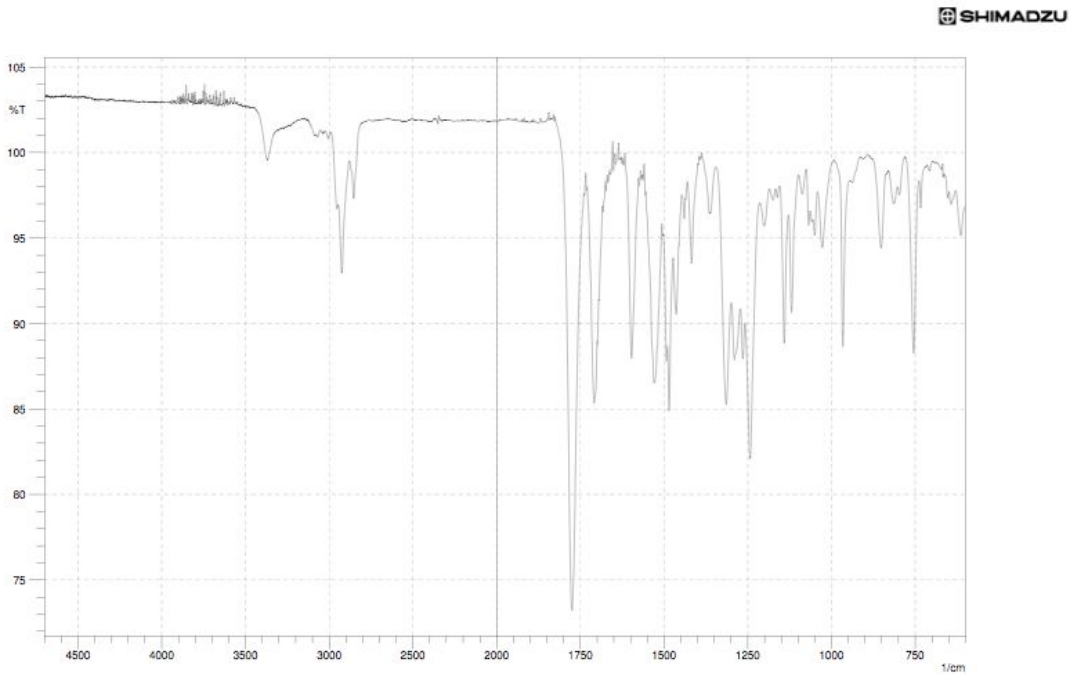


Figure 40. FT-IR spectrum of Compound **16**

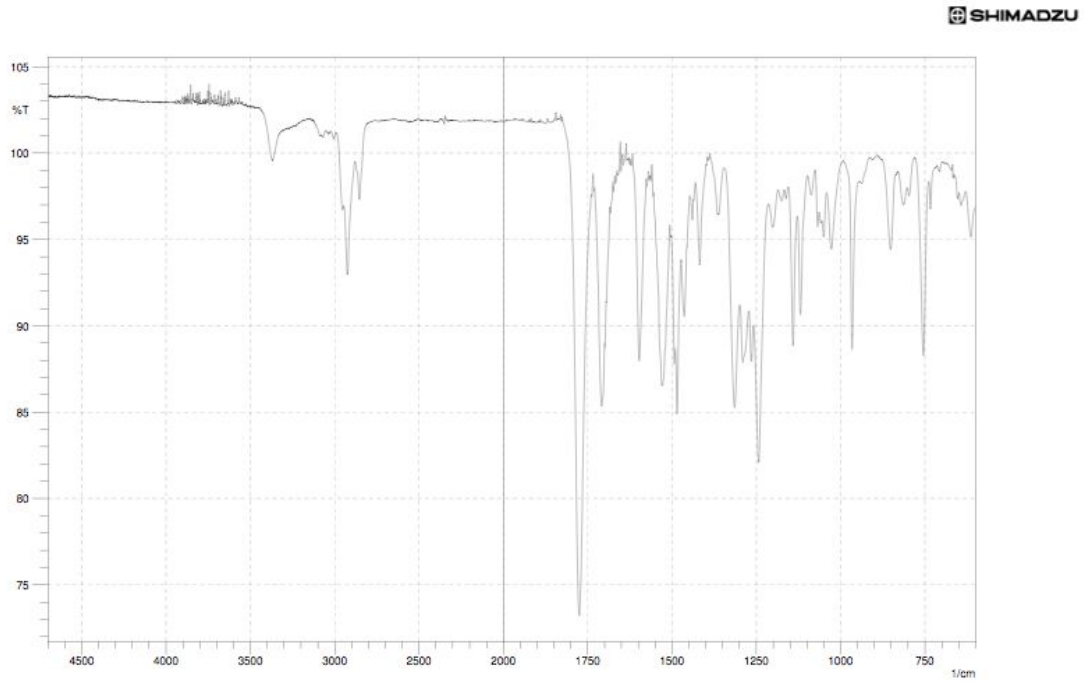


Figure 41. FT-IR spectrum of Compound **17**

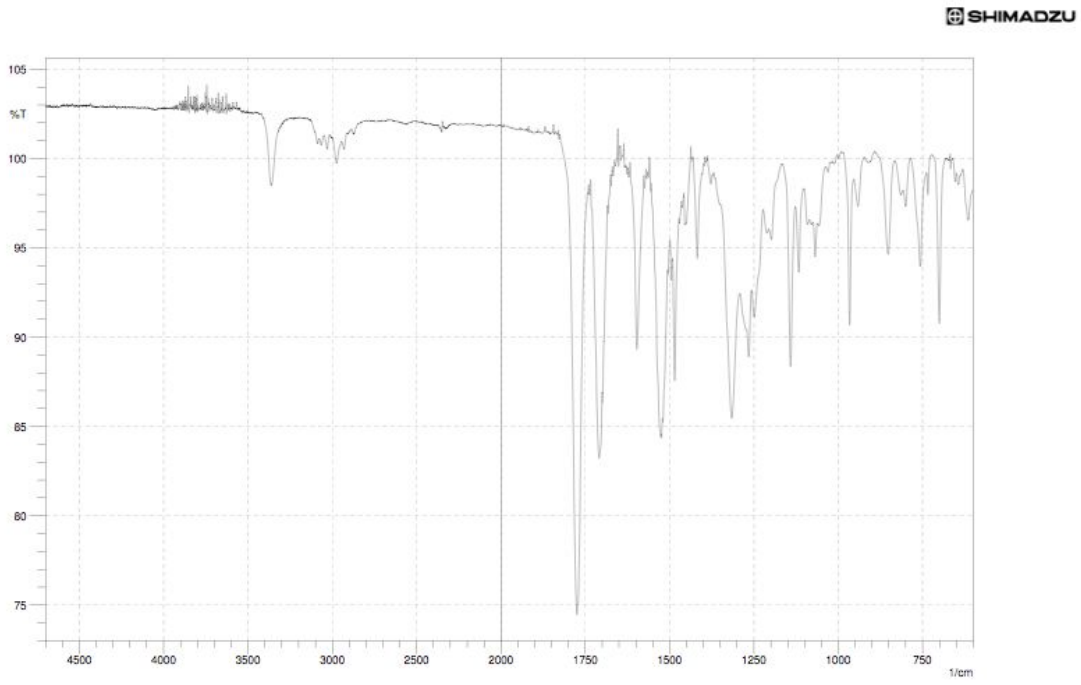


Figure 42. FT-IR spectrum of Compound **19**

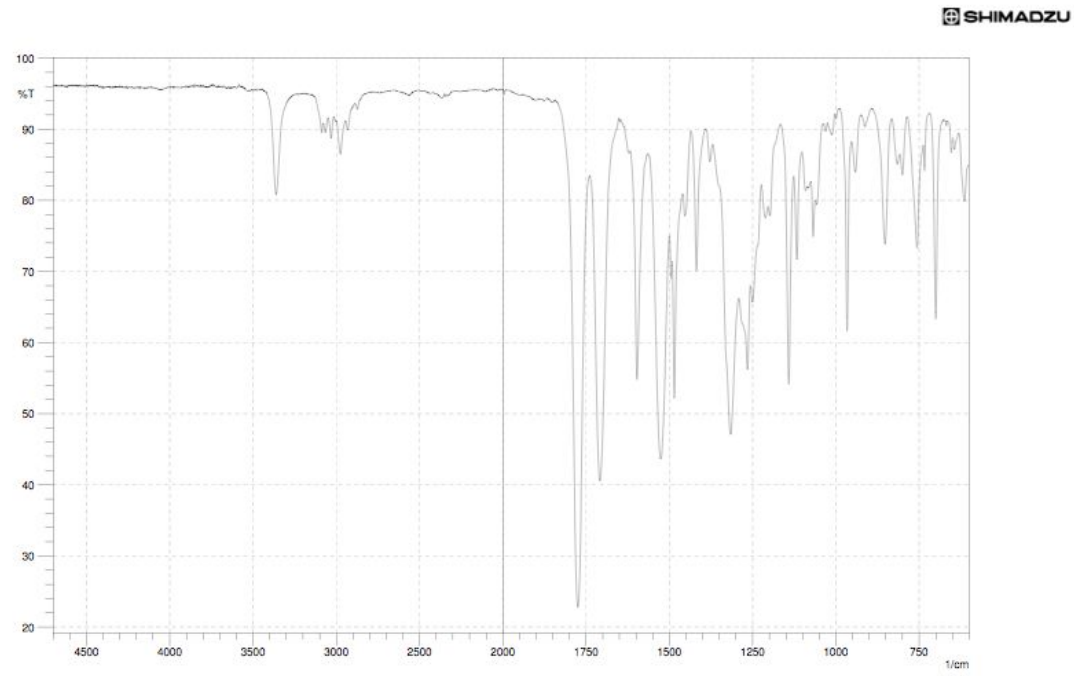


Figure 43. FT-IR spectrum of Compound **20**

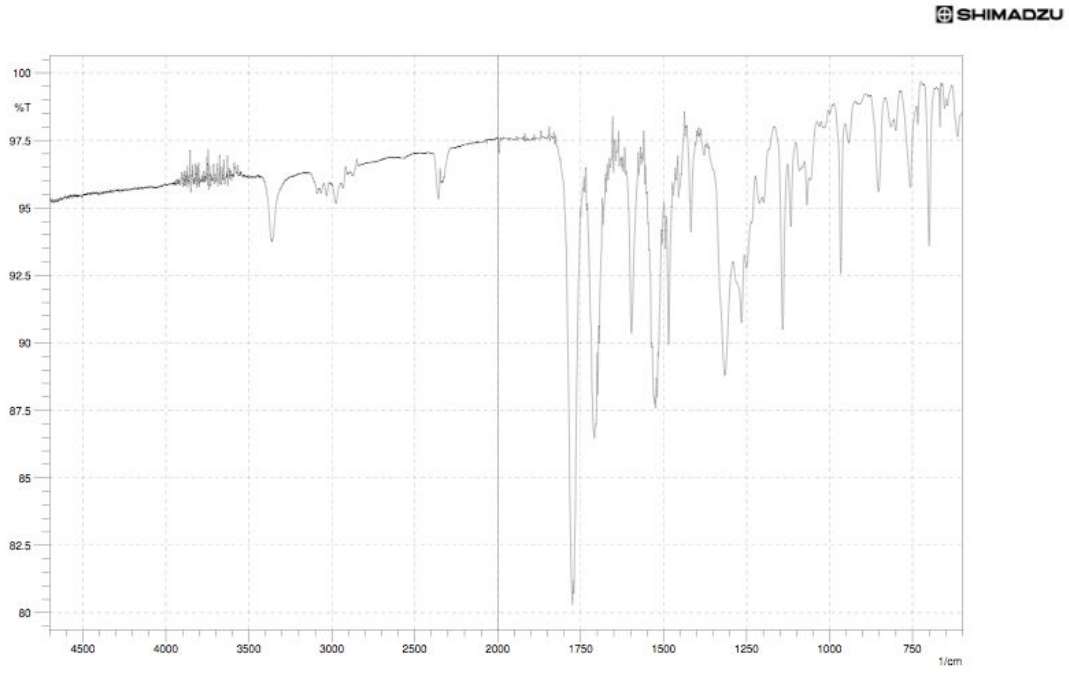


Figure 44. FT-IR spectrum of Compound **21**

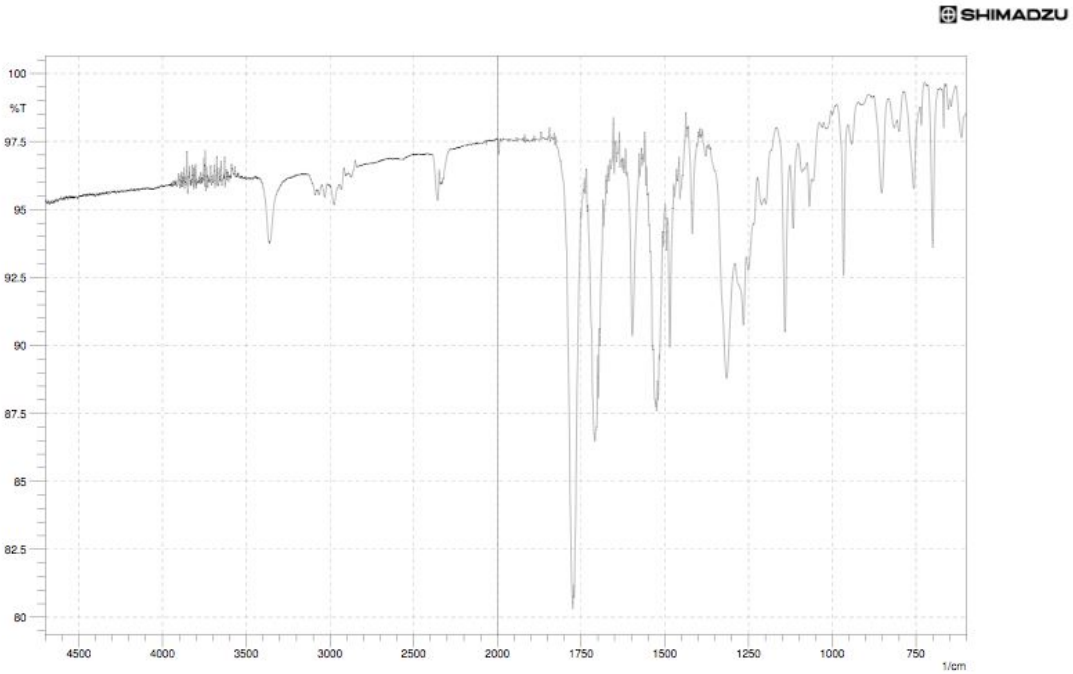


Figure 45. FT-IR spectrum of Compound **22**

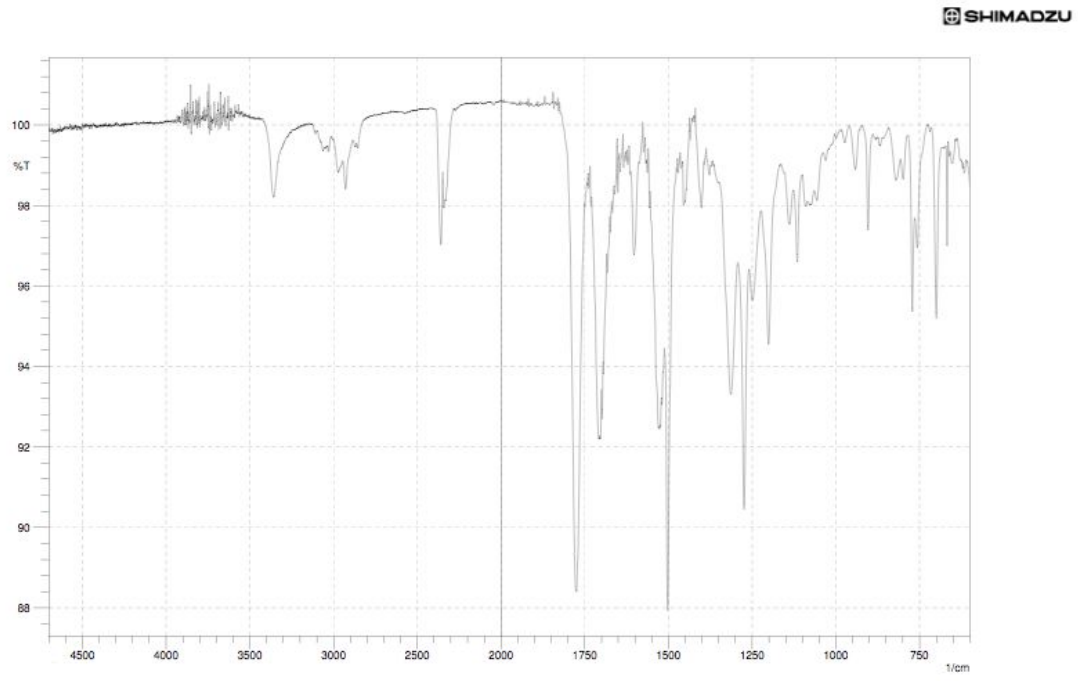


Figure 46. FT-IR spectrum of Compound **23**

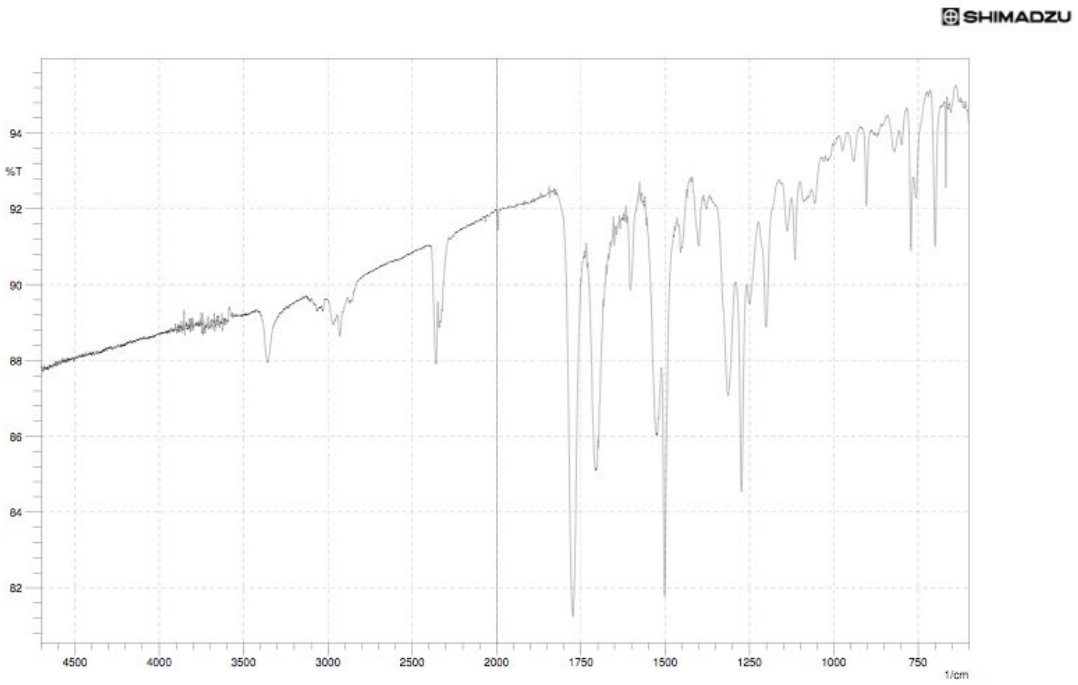


Figure 47. FT-IR spectrum of Compound **24**

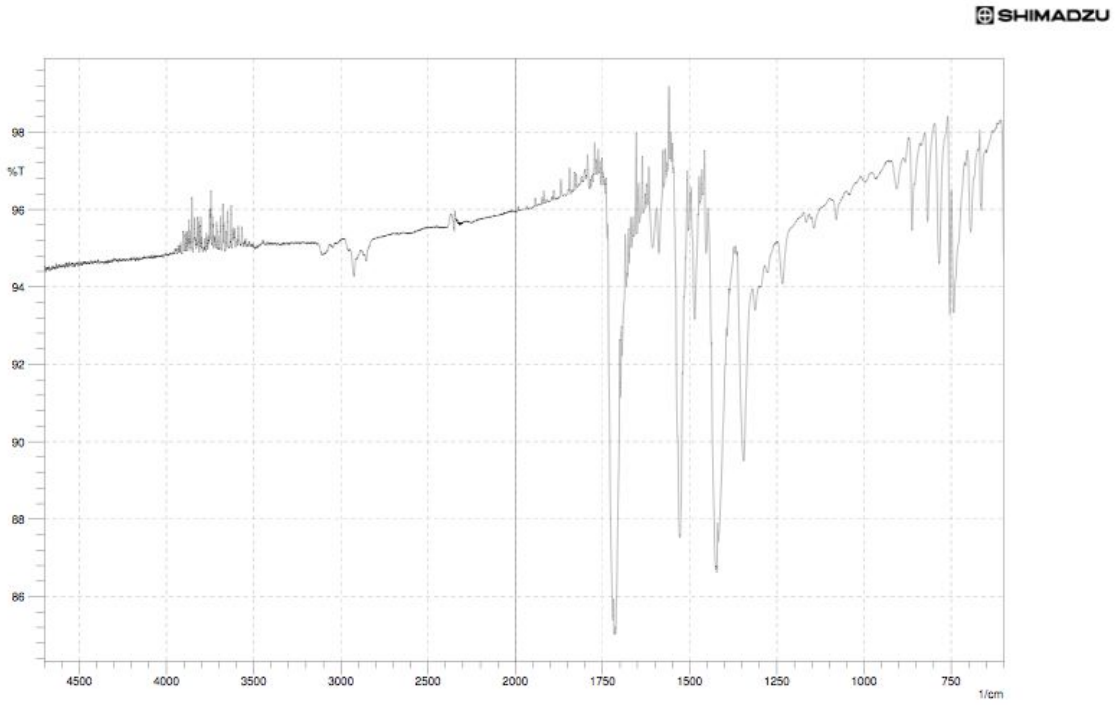


Figure 48. FT-IR spectrum of Compound **26**

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