Synthesis of Chalcones for Analysis as Anti-microbial Agents

DISSERTATION

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By

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List of Abbreviations

- ATP – adenosine triphosphate
- *B. subtilis* – *Bacillus subtilis*
- CFU – colony-forming unit
- COSY – correlation spectroscopy
- DCC – dicyclohexylcarbodiimide
- DCM – dichloromethane
- DDD – defined daily dose
- DMF - N,N-dimethylformamide
- DNA – deoxyribose nucleic acid
- DOSY – diffusion-ordered spectroscopy
- *E.coli* – *Escherichia coli*
- EHEC – Enterohaemorrhagic *Escherichia coli*
- EU – European Union
- EUCAST – European Committee on Antimicrobial Susceptibility Testing
- FDA – Food and Drug Administration
- GCMS – gas chromatography mass spectrometry
- HMBC – heteronuclear multiple bond correlation spectroscopy
- HOBt – 1-hydroxybenzotriazole
- IUPAC – International Union of Pure and Applied Chemistry
- MBC - minimum bactericidal concentration
- MIC – minimum inhibitory concentration
- MRSA – Methicillin-resistant *Staphylococcus aureus*
- MS – Mass Spectroscopy
- NMR – Nuclear Magnetic Resonance
- NOESY – nuclear Overhauser effect spectroscopy
- *P. aeruginosa* – *Pseudomonas aeruginosa*
- PBPs – penicillin binding proteins
- PKS – polyketide synthase
- ppm – parts per million
- *S. epidermidis* – *Staphylococcus epidermidis*
- THF – tetrahydrofuran
- TLC – thin layer chromatography
- UV – ultraviolet
- VTEC – Verocytotoxin-producing *Escherichia coli*

Abstract

Chalcones are diphenyl α-β-unsaturated compounds that are present in a wide variety of plants. Naturally occurring chalcones as well as synthetic chalcones have demonstrated an assortment of biological activity, including anti-inflammatory, anti-viral and antimicrobial activity. The need for new antimicrobially active agents is becoming more imperative as antimicrobial resistance continues to present a large threat. In this project, a series of chalcones were synthesised via a Claisen-Schmidt condensation reaction. The compounds were analysed using NMR spectroscopy and Gas-Chromatography Mass Spectrometry. An attempt was made to convert four of the chalcones to thiochalcones using Lawesson's Reagent. The compounds were tested against *E. coli*, *P. aeruginosa* and *S. epidermidis*. None of the synthesised chalcones displayed any antimicrobial activity but the thiochalcones showed activity against all bacterial strains. Upon further analysis, the thiochalcones appeared to have decomposed in situ, with the major product being the oxa-thio-phosphorane compound. This compound could have been the cause behind the activity seen in compound **11** against *S. epidermidis* and compound **14** against all bacterial strains studied. Compound **11** produced a zone of inhibition of 10mm against *S. epidermidis* whilst compound **14** produced zones of inhibition against *S. epidermidis*, *P. aeruginosa* and *E. coli* of 16mm, 10.3mm and 15.3mm respectively. The MICs and MBCs of the compounds were not greater than Ciprofloxacin, the antibiotic used in this project as the positive control, as shown by the results.

1. Introduction

Antimicrobial resistance is defined as the ability of a microorganism such as bacteria to grow in the presence of antimicrobial agents designed to hinder or halt their growth and reproduction. This resistance usually develops as a result of continuous exposure to said agents leading to selective pressure: either the population dies from contact or a mutation arises in a population, allowing for resistance (Amábile-Cuevas, 2010). It should be noted that some microbes that have never come into contact with antibiotics can also be resistant depending on the extremity of the environments in which they are found (D'Costa et al, 2011). This has become more of a problem in recent times due to the rise of not only more antibiotic-resistant bacteria but also multi-drug resistance. The efficacy of available antibiotics is becoming increasingly less effective. Dr Margaret Chan, former Director-General of the World Health Organisation, believes the world is moving to a "post-antibiotic era", in which "things as common as strep throat or a child's scratched knee could once again kill" (Public Health England, 2016).

Economist Jim O'Neill chaired the 2016 Review of Antimicrobial Resistance. Within his report, he stated that not only does this phenomenon present a risk to the quality and longevity of life but there is also an economic impact. He stated that not acting could cost the global economy $100 trillion. Out of a ten-point intervention plan, he pointed out four of high importance: the need to develop a global public awareness campaign, development of new antibiotics to replace those that are no longer effective, reduction of the unnecessary prescription of antibiotics for both humans and animals and a decrease in the extensive antibiotic use in agriculture (AMR, 2016). Antibiotic consumption in England had increased by 6.5% from 2011-2014, with the defined daily dose (DDD) per 1000 people rising from 21.6 to 23 in that time frame (Public Health England, 2015).

There has been a sharp decline in the development of new antibiotics in recent years and a large contributing factor is the departure of large pharmaceutical companies from antibiotic development, supposedly due to the lack of incentive. Antibiotics are expensive to discover and develop, require an overly complex but necessary regulatory procedure and return less revenue compared to medication for chronic conditions such as cancer and diabetes (Sabtu et al, 2015). Nowadays, due to antibiotic resistance, they are used as a last resort medication, meaning the companies often lose patency of the antibiotic before the cost incurred to develop

the drug can be recovered. The last class of antibiotics to be discovered was lipopeptides by Eli Lilly and Company in the early 1980s, with daptomycin (figure 1), a Gram-positive bactericidal, approved by the Food and Drug Administration (FDA) in 2003 (Pirri et al, 2009).

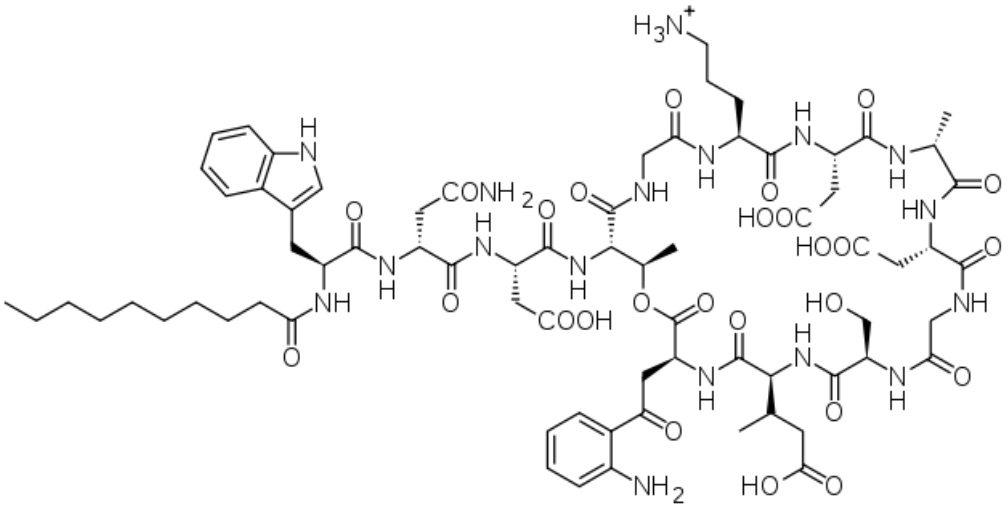


Figure 1: **Daptomycin**
Image source: Micklefield (2004)

The need to develop new classes of antibiotics that are able to act against both Gram-positive and Gram-negative bacteria is becoming increasingly important. This need coincides with an increase in the occurrence of multi-drug resistance bacterial strains. In Europe, an estimated 25,000 people die every year as a result of nosocomial infections caused by the following bacteria: *Escherichia coli, Klebsiella pneumoniae, Enterococcus faecium, Pseudomonas aeruginosa and* Methicillin-resistant *Staphylococcus aureus* (Public Health England, 2015). However, the issue lies particularly with Gram-negative bacteria as reports of multi-drug resistance are occurring at a faster rate in these bacteria, especially *Enterobacteriacecae* and *P. aeruginosa* (Sabtu et al, 2015). Efforts are being made to increase awareness of the issue as well as implementation of programmes like Infectious Disease Society of America's 10x'20 initiative, aimed at developing ten antibacterial agents by 2020, effective against the "ESKAPE" group of microbes: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* species (IDSA, 2010). In addition, grants such as the Longitude Prize, where a public vote determined the outcome of

directed research, was dedicated to overcoming antimicrobial resistance (Longitude Prize, N.D).

Historically, infections have been treated using natural products such as bark and leaves from trees with reports of ancient civilizations such as the Greeks and Egyptians using mixtures containing milk, honey and plant resins as wound dressings (Shah, 2011). Natural products have been the inspiration behind many antibiotics. Arsphenamine (figure 2), the first synthetic antibiotic, was produced by Paul Ehrlich and Sahachiro Hata in 1909 but it was derived from arsenic, a chemical element and a naturally occurring metalloid (Frith, 2013).

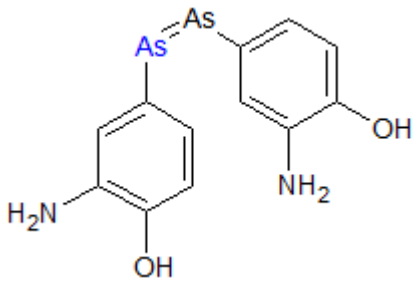


Figure 2: **Arsphenamine**
Image source: author's own

Nature is once again becoming the source of inspiration behind the development of new medication, including novel antibacterial agents. In recent times, chalcones have been the subject of increasing interest in the realm of medicinal chemistry as they have been discovered to display a plethora of biological potential including antimicrobial activity. Chalcones are well-known molecules in natural product chemistry as they have been repeatedly isolated and examined in a bid to understand the chemical basis for herb-based medication. Their synthesis has been proven to be relatively straightforward and able to produce a range of biologically active molecules that can be modified, furthering the understanding of the pharmacophore of the compound.

Chalcones are molecules consisting of two phenyl rings held together by a 3-carbon ketone moiety bearing an α-β unsaturated bond (figure 3).

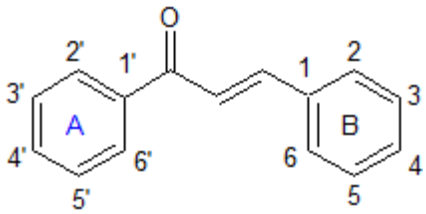


Figure 3: **Chalcone** (IUPAC: 1,3-diphenyl-prop-2-en-1-one)
Image Source: author's own

They were first discovered by Albert-Szent-Gyorgyi after he discovered "vitamin P", a term formally used to describe flavonoids (Grzybowski et al, 2013). Flavonoids are a family of polyphenolic compounds that are responsible for a number of plant-based activities such as regulating cell growth (Higdon, 2016). Chalcones are interesting compounds due to their highly conjugated electron system and the presence of the unsaturated ketone moiety, which is believed to be the sole provider of a chalcone's biological activity.

In this project, various parts of the chalcone were modified in order to establish what exact substituent the compound must bear in order to be biologically active. Varying functional groups were added to the chalcone's structure in order to ascertain whether electron density has a part in its efficacy. The seemingly essential ketone was also altered to bear a sulphur instead of an oxygen. Sulphonamides are already an established class of antibiotics and so the effectiveness of the sulphonamide functional group is well known but this does not apply directly to the possible functionality of a thiochalcone or more specifically, the thione functional group. The findings of these alterations will be discussed further in this dissertation.

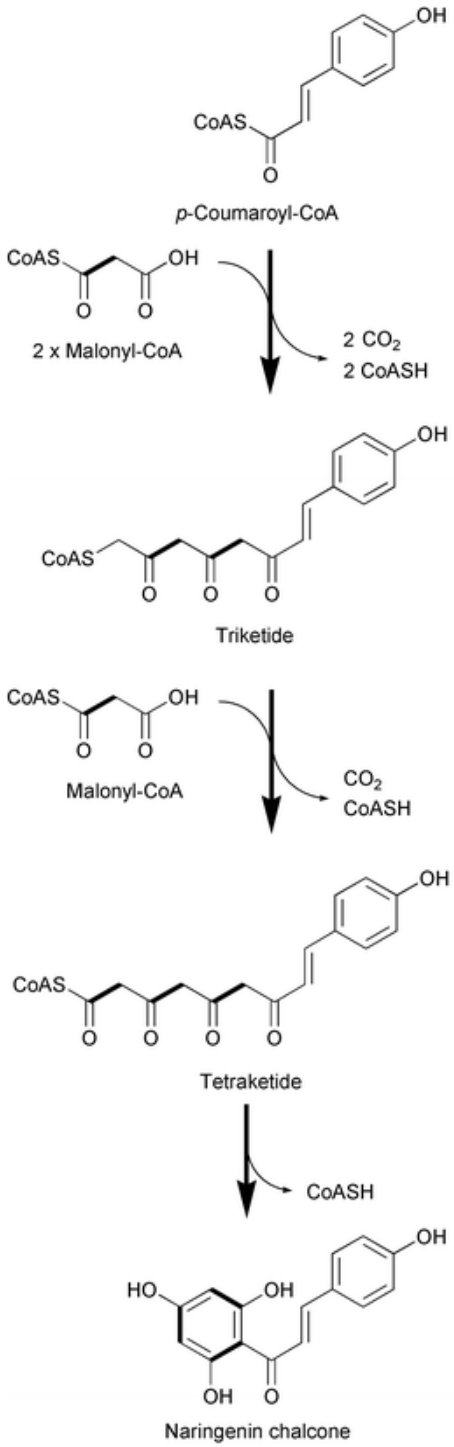
1.1 Literature Review

This review aims to shed light on the two subject areas this research project examines: the synthesis of biologically active chalcones and the need for the development of new antimicrobial agents to combat antimicrobial resistance. The literature discusses varying techniques to synthesise chalcones as well as the effect of different substituents on the compounds' antimicrobial activity. In this research project, modification attempts were made to the pharmacophore of the chalcone. The oxygen atom of the ketone moiety was converted to a sulphur atom by way of

thionation using Lawesson's Reagent. This review also briefly discusses Lawesson's Reagent and its previous use in the conversion of chalcones to thiochalcones. There is a limited array of literature available in regards to the synthesis of thiochalcones but there is little to no data available regarding the biological activity of these compounds. However, in a bid to help understand the possible activity of these compounds, sulphur-containing compounds that are of a similar structure to thiochalcones have been studied. Also, by-products of the use of Lawesson's Reagent are usually phosphorus-containing compounds. Such compounds have been reviewed for the purpose of this literature review.

1.1.1 Biosynthesis of Naturally Occurring Chalcones

Chalcones are the biosynthetic precursors of flavonoids, a family of polyphenolic compounds involved in a number of cell-signalling cascades in plants. The biosynthesis of chalcones is conducted via the polyketide pathway. This begins with the condensation of one molecule of 4-coumaroyl-CoA and three molecules of malonyl-CoA to form an intermediate. This intermediate undergoes a Claisen-like cyclization to give naringenin chalcone (scheme 1) (Zhuang et al, 2017). This reaction is catalysed by chalcone synthase (Dao et al, 2011). Chalcone synthase is classed as a polyketide synthase enzyme (PKS).



Scheme 1: **Biosynthesis of naringenin chalcone**

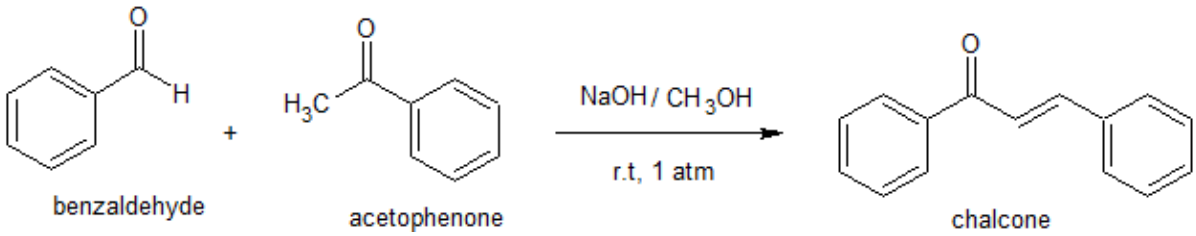
Image source: Springbob et al, 2003

PKSs are classed into three different groups based on their sequences, primary structures and catalytic mechanisms (Yu et al, 2012). Chalcone synthase belongs to the superfamily PKS III. This enzyme is omnipresent in the plant kingdom, proving

the importance of its products to the functionality of plants. Chalcones are normally distributed within various fruits and vegetables especially apples, citrus fruits, potatoes, tomatoes and bean sprouts (Orlikova, 2011), providing insight for the success of herbal medicine in the treatments of various ailments including infections. Chalcones have also been linked to providing the yellow pigment in flowers from the genera *Dahlia*, *Coreopsis* and *Cosmos* (Iwashina, 2000) and the families *Asteraceae*, *Moraceae*, *Fabaceae* and *Aristolochiaceae* (Díaz-Tielas et al, 2016).

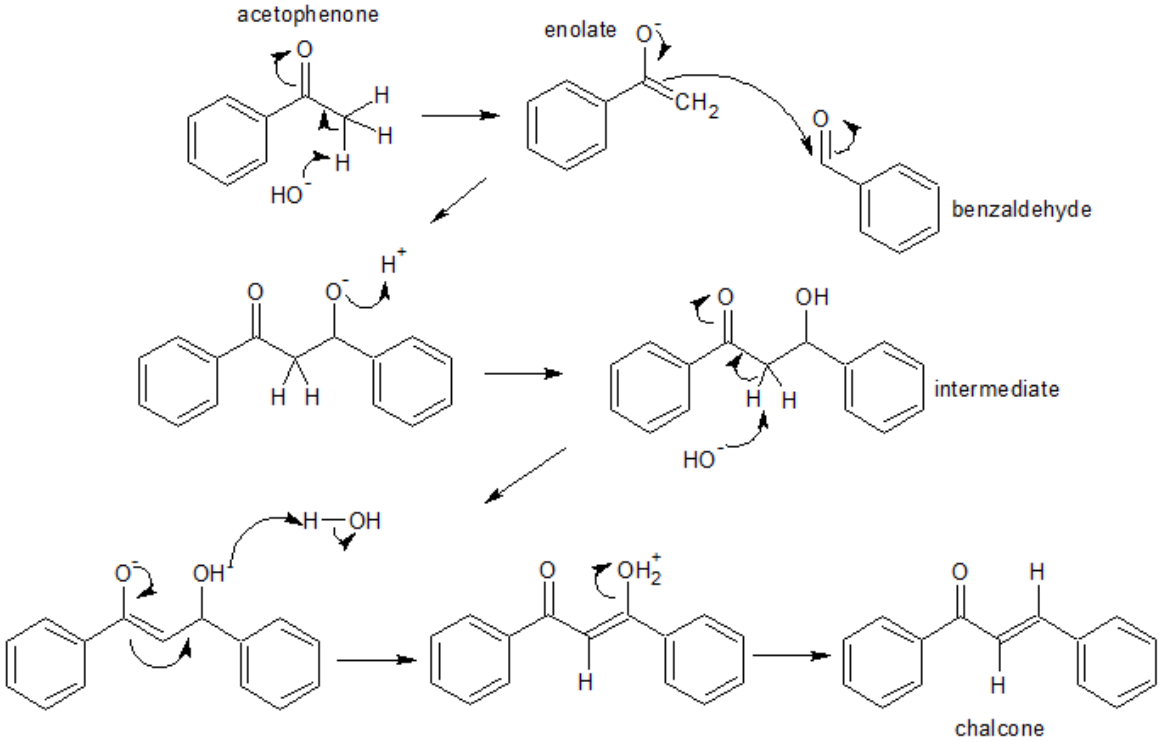
1.1.2 Synthesis

Chalcone synthesis can be achieved using a variety of techniques including Suzuki coupling, Friedal-Crafts acylation and Claisen-Schmidt condensation. Claisen-Schmidt condensation is the most commonly used method. This reaction is well-known in organic chemistry. It involves a suitable benzaldehyde and acetophenone using a strong acid or base catalyst in a polar solvent, typically methanol (scheme 2).



Scheme 2: **Claisen-Schmidt Condensation Reaction of Chalcones**
Image Source: author's own

This reaction is known to produce a mixture of the desired product, by-products and starting material, causing variations in the yields of the reactions. However, it is the most popular method for chalcone synthesis due to its straightforward procedure and relatively efficient formation of the carbon-carbon double bond. The mechanism for the base-catalysed reaction is as follows: an enolate ion is formed from the removal of an α-hydrogen by a base. This enolate, formed from the reacting ketone, reacts with the aldehyde as ketones are less reactive toward nucleophilic addition. This intermediate will undergo dehydration which results in the chalcone (scheme 3).



Scheme 3: **Chalcone Synthesis Mechanism**
Image Source: author's own

Reaction conditions are usually room temperature and 1 atmosphere but the reaction can also be conducted under warmer conditions such as 40-60°C. There have been many reports of synthesis using microwave irradiation. Ahmad et al reported the synthesis of a series of 2-acetyl hetero chalcones using microwave radiation under 180 watts (Ahmad et al, 2016).

In terms of the conversion of chalcones to thiochalcones, the most commonly used methodology to date requires the use of Lawesson's Reagent (figure 4). This reagent was developed in 1978 by Lawesson and his co-workers (Krstić et al, 2010).

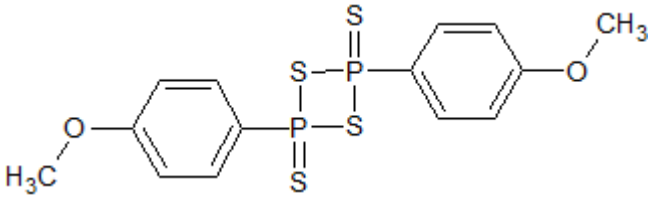
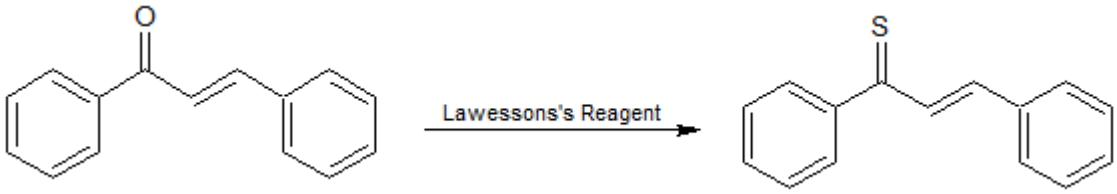


Figure 4: **Lawesson's Reagent** (IUPAC: 2,4-Bis(4-methoxyphenyl)-2,4-dithioxo-1,3,2,4-dithiadiphosphetane).
Image Source: author's own

The reaction is typically carried out under nitrogen and refluxed or in open air without using reflux but at warm temperatures in anhydrous toluene or tetrahydrofuran (THF) (scheme 4). Its use in the conversion of chalcones to thiochalcones is underreported and available data varies greatly in their reports of yield and purity. However, it is often seen that the reaction produces a mixture of the desired product, sulphur and phosphorous-containing by-products as well as starting material (Krstić et al, 2010).



Scheme 4: **Conversion of Chalcone to Thiochalcone using Lawesson's Reagent**
Image source: author's own

Previous procedures to thionate organic compounds utilised phosphorous pentasulfide (P_4S_{10}), first reported in 1869 by Henry and Wislicenus (Cava, 1985) but its procedure required very large amounts of this reagent and long reaction times to produce low yields. Lawesson's Reagent is a more favourable converting reagent due to its availability, ease of use and a relatively straightforward reaction.

1.1.3 Effects of Substituents on Activity & Pharmacophore

It has been suggested that adding substituents on either the A or B ring of the chalcone may influence its biological activity. However, the α-β unsaturated ketone moiety is regarded to be the main pharmacophore of a chalcone as its removal has resulted in either a major reduction or complete loss of antimicrobial activity (Batovska et al, 2010). In regards to antimicrobial activity, certain substituents are favoured more than others. As seen in the Tran et al study, chalcones with two or three methoxy groups on the B ring are inactive regardless of substituents on the A ring (refer to figure 3 for labelling and positioning) (Tran et al, 2012). It is suggested that more than two methoxy groups on the B ring eradicate the hydrophilic property of a chalcone which allows for penetration through the bacterial cell wall (Tran et al, 2012). This was reflected in results obtained by Baba et al, who reported

synthesising a series of trimethoxy-substituted chalcones but when tested, no antimicrobial activity was shown (Baba et al, 2013) (figure 5).

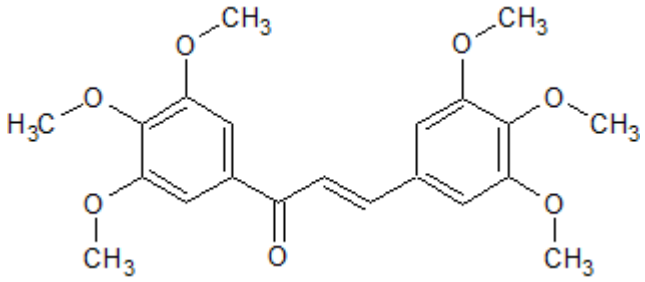


Figure 5: **1-(3', 4', 5'-trimethoxyphenyl)-3-(3, 4, 5-trimethoxyphenyl)-prop-2-ene-1-one**
Image source: Baba et al (2013)

Electron-donating groups such as a hydroxyl group have been seen to increase the antimicrobial activity of a chalcone, but hydroxyl groups are also hydrophilic. A free hydroxyl group on the B ring at position 4 or 6 is important for methicillin-resistant *Staphylococcus aureus* (MRSA) activity (Ávila et al, 2008) (figure 6).

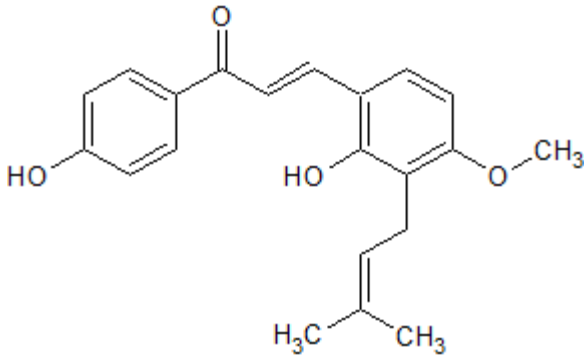


Figure 6: **4-Hydroxyderricin**
Image source: Ávila et al (2008)

A degree of importance is attached to the positioning of the hydroxyl group: the meta position brought about a diminished level of activity (100μg/ml against *B.subtilis*) when compared to the ortho position (50μg/ml against *B.subtilis*) (Silva et al, 2013) (figure 7).

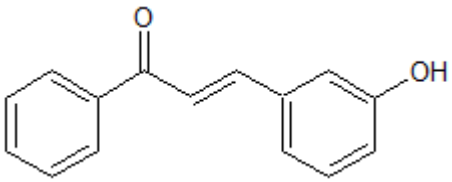


Figure 7: **1-phenyl-3-(3-hydroxyphenyl)-prop-2-ene-1-one** (hydroxyl group shown in ortho position)
Image source: Silva et al (2013)

Avila et al concluded from their study that a free hydroxyl group on the B ring is necessary for any antimicrobial activity as this activity is lost once it is substituted (Ávila et al, 2008). Weakly electron-donating groups such as halogens (chlorine, fluorine, bromine) have been seen to positively contribute to the antimicrobial activity of a chalcone as well. Halogens are naturally electron-withdrawing by way of induction but can also be electron-donating due to the lone pair of electrons they all bear.

Nielsen et al synthesised a series of compounds, including a pair of chalcones bearing a (3, 5)-dibromo substituent and (2, 4, 6)-trifluoromethyl substituents on the B rings of their respective compounds, shown below (Nielsen et al, 2004) (figure 8).

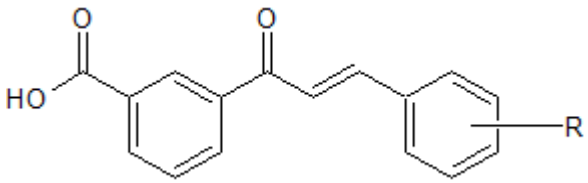


Figure 8: **substituted hydroxychalcone (R representing dibromo or trifluoromethyl substituents)**
Image source: Yazdan et al (2015)

These compounds showed promising activity as did a chalcone synthesised by Karthikeyen et al (2007), which had (4, 6)-dichloro and (3)-fluoro substituents on the A ring (figure 9).

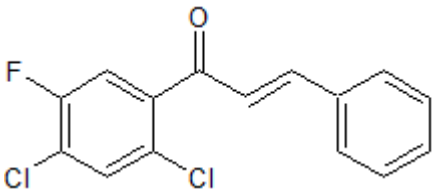


Figure 9: **3-aryl-1-(2',4'-dichloro-5'-fluorophenyl)-2-propen-1-one**
Image source: Khadar-Yazdan et al (2015)

Mohamed et al (2003) converted a series of steroidal ketones to their thioxo equivalents using Lawesson's Reagent and tested the compounds for antimicrobial and antifungal activity (figure 10). They followed a standard procedure of using anhydrous toluene under reflux and isolated the compound using column chromatography. Four of the compounds displayed activity against both Gram-positive and Gram-negative bacteria including *B. subtilis*, *P. aeruginosa* and *E.coli* at 200µg/ml.

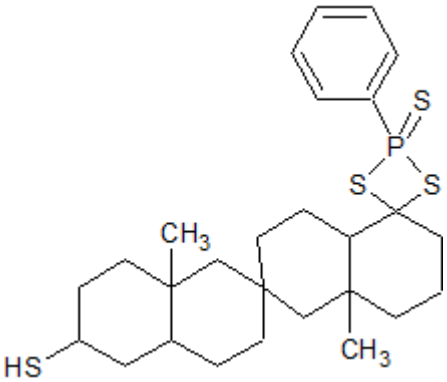


Figure 10: **3β-mercaptospiro(androstan-17, 4-dithiaphosphetan)thione**
Image source: Mohamed et al

Ahmed et al (2015) reported the synthesis of a series of sulfides, their corresponding bis-sulfides and sulfones. They were tested for their antimicrobial activity and they all displayed activity against a variety of Gram-negative and Gram-positive bacteria including *B. subtilis* and *S. aureus* at varying concentrations of 3.9-31.25µg/ml. A particular sulfone with a 3-NH_2 position on the A ring (figure 11) displayed a higher level of activity compared to chalcones with chloro, methyl and trimethoxy substituents against *S. aureus.*

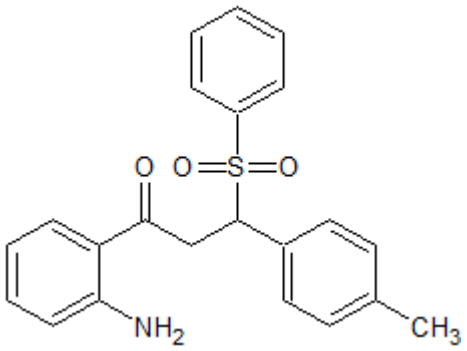


Figure 11: **1-(2'-aminophenyl)-3-(4-methylphenyl)-3 (phenylsufonyl)propan-1-one**
Image source: Ahmed et al (2015)

However, the study failed to state whether this higher level of activity was due to the presence of the sulphur-containing groups or substituents on the rings of the compounds.

Vanangamudi et al (2013) synthesised a series of 2,5-dimethyl-3-thienyl chalcones via a Claisen-Schmidt condensation reaction. All the compounds showed activity against the bacteria tested including *P. aeruginosa*, *B. subtilis* and *E. coli*. In this study, they attributed the improved activity of the compounds to the methoxy, methyl and nitro substituents in the 3rd position on the B ring (figure 12).

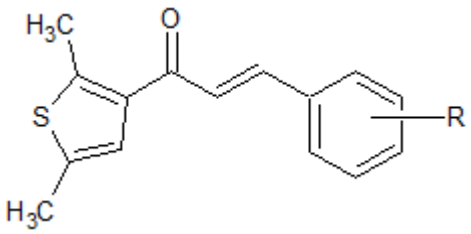


Figure 12: **substituted styryl 2',5'-dimethyl-3-thienyl ketone (R representing methoxy, methyl and nitro substituents)**
Image source: Vanangamudi et al (2017)

In all this, structure-activity relationships still fail to adequately identify what exact part of a chalcone is responsible for its biological activity and the notion that the α-β unsaturated ketone system is the sole proprietor of this activity is challenged in this research project by the exchange of the ketone's oxygen with a sulphur atom.

1.1.4 Antimicrobial Activity of Phosphorous-Containing Compounds

As mentioned before, the use of Lawesson's reagent to convert chalcones to thiochalcones produces a mixture of compounds including phosphorous-containing by-products. It is important to understand the effect these compounds may have on antimicrobial activity as they may alter that of the synthesised compounds.

Moenomycins are a family of phosphorous-containing antibiotics (Ostash et al, 2010). They are produced by *Streptomyces*, a genus of bacteria that produces a majority of antibiotics of natural origin such as chloramphenicol and neomycin (Aínsa et al, 2000). They are classed as phosphoglycolipids due to their chemical composition and have been known to have a very complex structure with an even more complex synthesis (Ostash et al, 2010). The class was first discovered in the 1960s (figure 13) and was shown to have high levels of activity against Gram-positive bacteria (Halliday et al, 2006) but interest in its potential as antibiotics dwindled due to its poor pharmacokinetic properties – it was found to be cytotoxic at concentrations of 10µg/ml and higher (Ostash et al, 2010). Instead, it was commercialised as an antimicrobial growth promoter in animal feeds (Halliday et al, 2006). However, a European Union (EU)-wide ban on antimicrobial growth promotors came into effect on 1 January 2006 (European Commission, 2005).

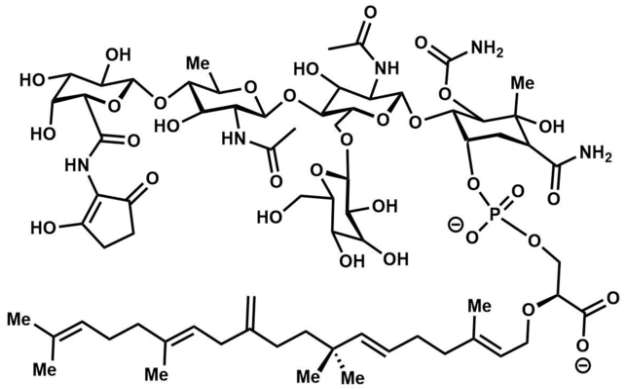


Figure 13: **Moenomycin A**
Image Source: Ostash et al, 2010

Reddy et al synthesised a series of phosphorous heterocycles with an exocyclic P-C link. 2-(20-Chloroethyl)-2,3-dihydro-5-benzoyl-1H-1,3,2-benzodiazaphosphole-2-Oxide (figure 14) was found to be active against both *S. aureus* and *E. coli* at 250µg and 500µg, producing zones of inhibition of 6-9mm and 4-8mm respectively (Reddy et al, 2004).

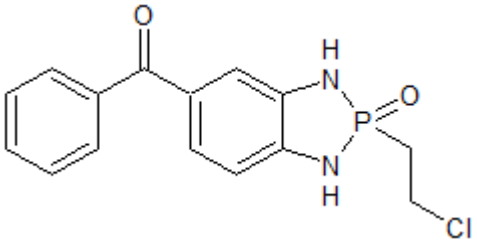


Figure 14: **2-(20-Chloroethyl)-2,3-dihydro-5-benzoyl-1H-1,3,2-benzodiazaphosphole-2-Oxide**
Image Source: Reddy et al, 2004

Polyphosphates are salts formed from repeating units of phosphate units. They are utilised as a food additive by the FDA to prevent food spoilage (Moon et al, 2011). Inorganic polyphosphate is found widespread in all organisms as it can serve as an adenosine triphosphate (ATP) source. However, an external source of polyphosphate has shown antimicrobial activity against many Gram-positive bacteria including *S.aureus*. Its mode of action is thought to include the inhibition of certain stages of cell wall synthesis (Moon et al, 2011).

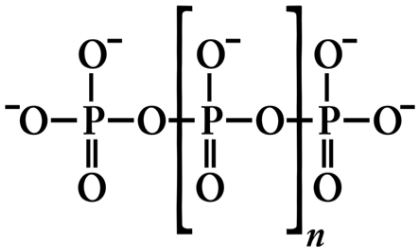


Figure 15: **Polyphosphate**
Image source: Morrisey et al, 2012

1.1.5 Antimicrobial Resistance

Microorganisms can either be inherently resistant to an antibiotic or develop resistance after persistent exposure, this is known as acquired resistance. This acquired resistance could arise by either mutation or a transfer of a resistance mechanism programmed on genes (Sabtu et al, 2015). This horizontal gene transfer can occur through three different mechanisms: conjugation, transformation or bacteriophage-mediated transduction (Arber, 2014). Antimicrobial resistance could be viewed as an inevitable result of antibiotic use but the issue has been exacerbated due to the systematic abuse of this medication both in and outside healthcare settings (Sabtu et al, 2015). Also, in this era of modern travel and the inevitability of human contact, an antimicrobial-resistant strain could be easily spread in a very short amount of time. For example, the New Delhi metallo-β-lactamase-1 (NDM-1) carbapenamese enzyme found in some species of *Enterobacteriaceae* was first detected in Sweden after a patient from India had been transferred for medical reasons (Kumarasamy et al, 2010).

As mentioned earlier, the ESKAPE pathogens are the leading cause of nosocomial infections such as blood infections, urinary tract infections and wound infections (Higuita et al, 2014) and largely contribute to the issue of antimicrobial resistance due to the scale of their combined multi-drug resistance. The organisms include both Gram-negative and Gram-positive whose main structural difference is the makeup of their cell envelopes. They both possess peptidoglycans in their cell walls which provide their characteristic cell shape; Gram-positive bacteria has a thick cell wall whilst Gram-negative bacteria has a thin cell wall. Despite having lower levels of peptidoglycan, Gram-negative bacteria possess a more complex cell envelope structure (Salton et al, 1996). This complexity makes Gram-negative bacteria harder to penetrate, thus more difficult to target using antimicrobial agents.

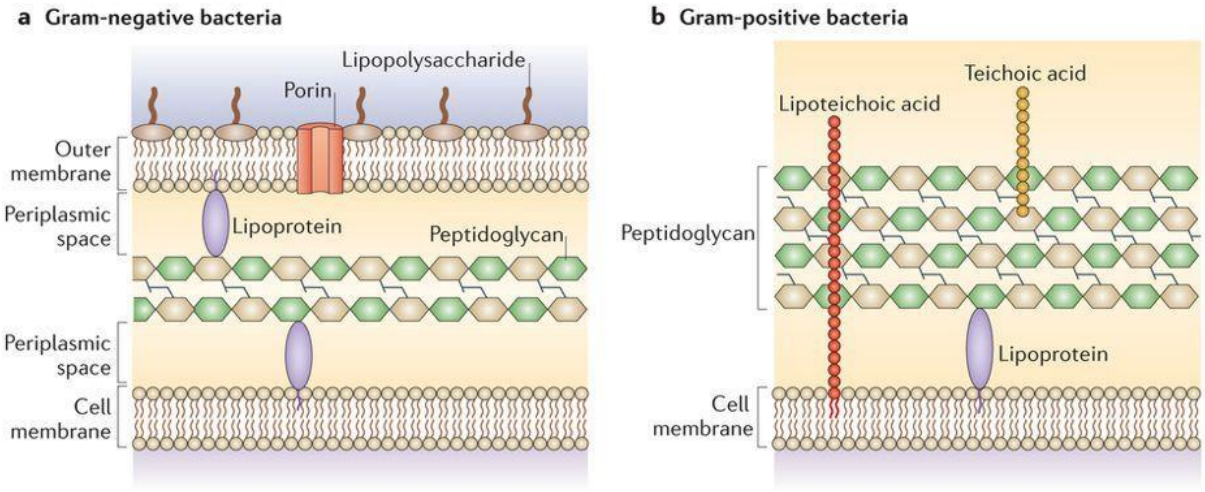


Figure 16: **Differences in Cell Envelope Structure – Gram-negative vs Gram-positive bacteria**
Image Source: Brown et al, 2015

The outer membrane of Gram-negative bacteria contains a high lipopolysaccharide complex content (Rietschel et al, 1994). This lipopolysaccharide complex is multifunctional: it acts as a barrier to lysosomes and to certain antimicrobial agents as well as acting as an adhesive agent in some Gram-negative bacteria to ensure colonization of its host (Wang et al, 2010). When a bacterial cell is lysed by the immune system through phagocytosis, the polysaccharide fragments. These sections bear Lipid A, a moiety that acts as an endotoxin, initiates an inflammatory response in animals that could result in fatal endotoxic shock, also known as septic shock (Tzeng et al, 2002). Therefore, besides the initial difficulty of attacking the cell wall of Gram-negative bacteria, attacking the cell may result in the release of the endotoxin whose action could be fatal.

In this project, the bacteria *Escherichia coli (E. coli)*, *Pseudomonas aeruginosa (P. aeruginosa)* and *Staphylococcus epidermidis (S. epidermidis)* were utilised to test the synthesised compounds' activity against both Gram-negative and Gram-positive bacteria.

E. coli is a rod-shaped, Gram-negative bacterium of the *Enterobactericeae* family. It is a facultative anaerobic bacteria, meaning it produces ATP for respiration in the presence of oxygen but has the ability to respire anaerobically in the absence of oxygen (Stieglmeier et al, 2009). This bacteria is typically found in the lower intestine of warm-blooded organisms but some strains such as Verocytotoxin-producing *E. coli* (VTEC) or enterohaemorrhagic *E. coli* (EHEC) are pathogenic, causing illnesses

associated with food and water contamination (Karmali et al, 2010). In Germany of 2011, there was an outbreak of a food-borne infection caused by EHEC, leading to large numbers of people falling ill with cases of bloody diarrhoea and haemolytic-uremic syndrome (Burger, 2012), a disease that causes severe inflammation of blood vessels and formation of blood clots throughout the body (NHS England, 2017). During the 5-month long outbreak, approximately 3000 cases of EHEC-associated infections were seen with 855 of these cases developing to haemolytic-uremic syndrome. Prior to this outbreak, there were approximately 1000 cases of EHEC-associated infections per year with 70 of these cases developing to haemolytic-uremic syndrome (Burger, 2012). Upon investigation by the German authority for food safety, it was found that the origin of this outbreak was in a contaminated batch of sprouts, with EHEC isolated in the stool of infected patients (Burger, 2012).

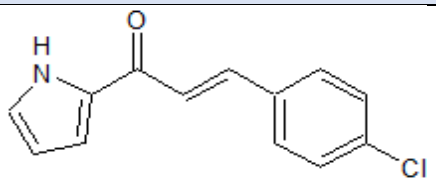
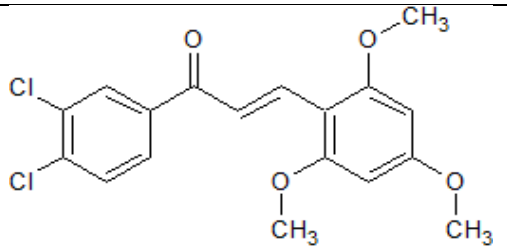
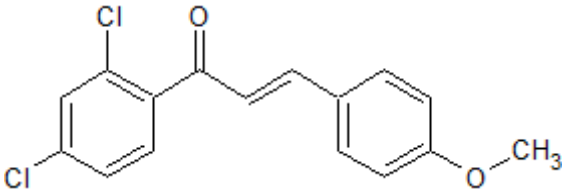
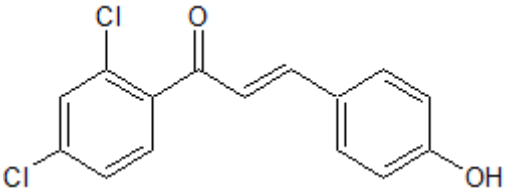
P. aeruginosa is a Gram-negative, rod-shaped bacterium of the *Pseudomonadaceae* family. It is also a facultative anaerobe with the ability to survive in an array of environmental conditions (Wu et al, 2015). It is an opportunistic pathogen as it tends to exploit a host that is in a weakened state i.e. immune-deficient, wounded and ageing (Brown et al, 2015). *P. aeruginosa* is known to cause chronic lung infections in patients with cystic fibrosis and skin infections in severely burned patients (Colmer-Hamood et al, 2016). In the summer of 2018, a human milk bank in Northern Ireland had to be suspended as *P. aeruginosa* was found in the water supply of the milk bank's hospital (BBC, 2018). Previously, four premature babies died in the Royal Maternity Hospital in Belfast due to an infection caused by the bacteria that was later found to be borne in the hospital's water supply as well (BBC, 2012).

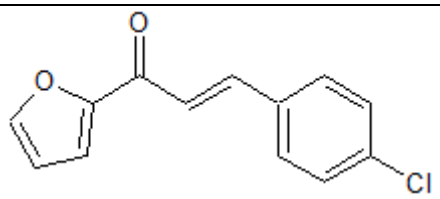
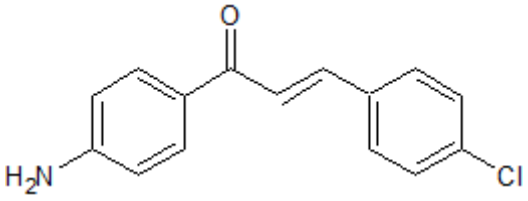
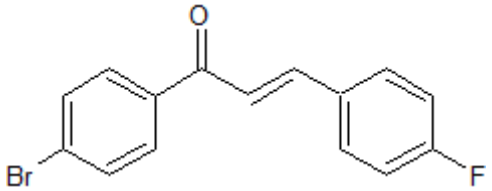
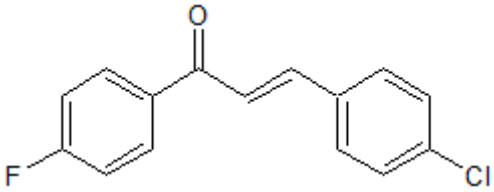
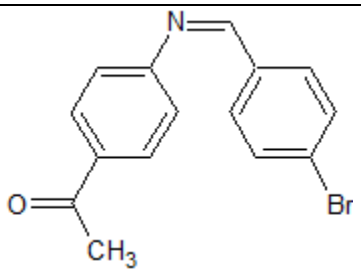
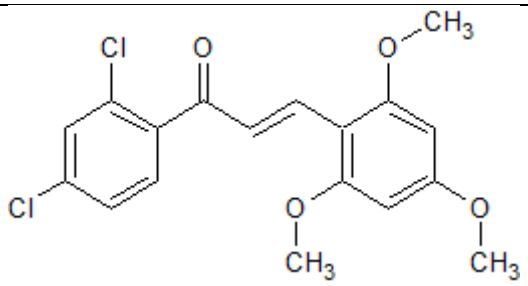
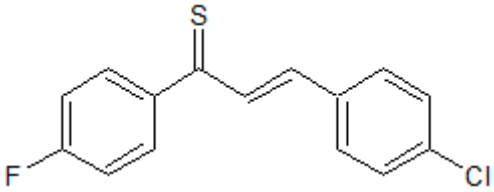
S. epidermidis is a Gram-positive, spherical bacterium of the *Staphylococcaceae* family. It is also facultatively anaerobic and is a member of human skin flora but still has the ability to become pathogenic, being the cause of a significant number of nosocomial infections (Namvar et al, 2014). It is known that infections caused by this bacterium begin with its introduction during device insertion such as needles breaking the surface of the skin (Otto, 2009). In the United States, *S. epidermidis* is the cause of 22% of blood infections (NNIS, 2004). Even though *S. epidermidis* causes infections that are not easy to treat, they do not cause life-threateningly dangerous infections (Otto, 2009) such as the aforementioned bacteria.

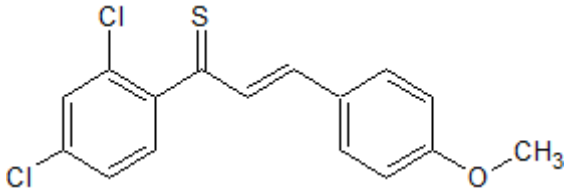
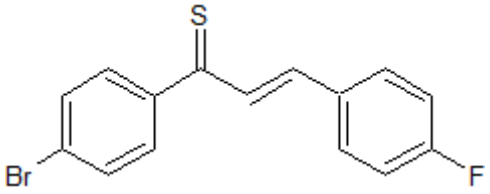
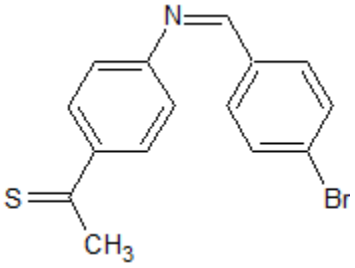
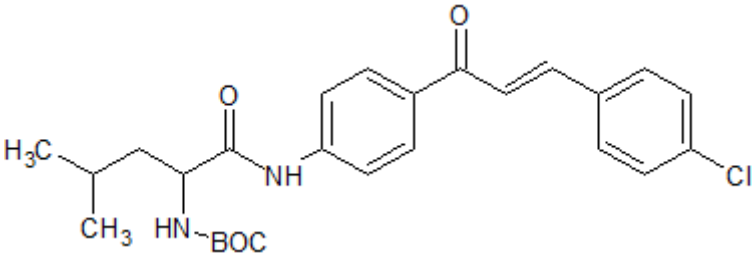
1.2 Aim

The aim of this project was to synthesise a series of chalcones from a combination of substituted acetophenones and benzaldehydes using a reliable methodology, including the use of Lawesson's Reagent to convert these chalcones to thiochalcones, in an attempt to alter the pharmacophore of the compound. The aim also includes establishing any antimicrobial activity of the compounds and investigating said activity further. The synthesis and biological activity of thiochalcones is not heavily reported. The following table itemises the structures of the compounds this project aimed to synthesise:

Table 1: IUPAC Names and Chemical Structures of Proposed Compounds

IUPAC Name/Code	Chemical Structure
1 - 1-(2'-acylpyrrole)-3-(4-chlorophenyl)-prop-2-ene-1-one	
2 - 1-(3',4'-dichlorophenyl)-3-(2,4,6-trimethoxyphenyl)-prop-2-ene-1-one	
3 - 1-(2',4'-dichlorophenyl)-3-(4-methoxyphenyl)-prop-2-ene-1-one	
4 - 1-(2',4'-dichlorophenyl)-3-(4-hydroxyphenyl)-prop-2-ene-1-one	

5 - 1-(2'-acetylfuran)-3-(4-chlorophenyl)-prop-2-ene-1-one	
6 - 1-(4'-aminophenyl)-3-(4-chlorophenyl)-prop-2-ene-1-one	
7 - 1-(4'-bromophenyl)-3-(4-chlorophenyl)-prop-2-ene-1-one	
8 - 1-(4'-fluorophenyl)-3-(4-chlorophenyl)-prop-2-ene-1-one	
9 - N-(4-bromobenzylidene)-4'-acetoaniline	
10 - 1-(2',4'-dichlorophenyl)-3-(2, 4, 6-trimethoxyphenyl)-prop-2-ene-1-one	
11 - 1-(4'-fluorophenyl)-3-(4-chlorophenyl)-prop-2-ene-1-thione	

12 - 1-(2',4'-dichlorophenyl)-3-(4-methoxyphenyl)-prop-2-ene-1-thione	
13 - 1-(4'-bromophenyl)-3-(4-chlorophenyl)-prop-2-ene-1-thione	
14 - N-(4-bromobenzylidene)-4'-thioaniline	
15 - 1-(tert-butoxycarboxylleucine)-3-(4-chlorophenyl)-prop-2-ene-1-one	

2. Methodology

2.1 Materials

All chemical reagents and solvents utilised were sourced from both Sigma-Aldrich and Alfa-Aesar unless otherwise stated. All agar powders and antibiotics used during the microbiology section of this project were sourced from Thermo Fischer Scientific.

Table 2: List of reagents available for synthesis of chalcones and thiochalcones

Reagents	
2-hydroxy-4-methoxybenzaldehyde	4-chloroacetophenone
2,4,6-trimethoxybenzaldehyde	3,4-dichloroacetophenone
Salicylaldehyde	2,4-dichloroacetophenone
4-bromobenzaldehyde	Gallacetophenone
4-hydroxybenzaldehyde	2-acetylfuran
4-chlorobenzaldehyde	2-acetylpyrrole
5-bromo-3-nitrobenzaldehyde	2-hydroxy-3-nitrobenzaldhyde
3,4-dihydroxybenzaldehyde	5-bromo-3-flurosalicyladehyde
3,4-dihydroxybenzaldehyde	4-methoxybenzaldehyde
Sodium/lithium/potassium hydroxide	Lawesson’s reagent

Table 3: List of solvents used during synthesis of chalcones and thiochalcones

Solvents	
Dichloromethane (DCM)	Methanol
Anhydrous N,N-dimethylformamide (DMF)	Deuterated dimethyl sulfoxide (DMSO)
Ethyl acetate	Deuterated chloroform

Anhydrous tetrahydrofuran (THF)	Deuterated methanol
Anhydrous toluene	Distilled water
Hexane	

Table 4: List of materials used in microbiology testing

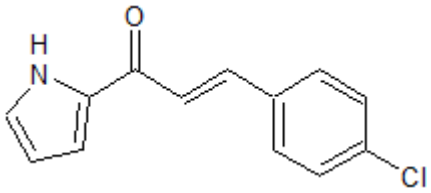
Materials	
Nutrient agar (Oxoid, CM0003)	Distilled water
Mueller-Hinton agar (Oxoid, CM0337)	Ciprofloxacin discs (10µg) (Oxoid, CT1615)
Mueller-Hinton broth (Oxoid, CM0405)	Ringer's Solution Tablets (Oxoid, BR0052)
Dimethyl sulfoxide (DMSO) (molecular biology grade) (Sigma-Aldrich, D8418)	

Chemical reagents were used without purification. A 1 M solution of sodium hydroxide was made by dissolving 4g of sodium hydroxide pellets in 100ml of distilled water. ^{1}H and ^{15}P Nuclear Magnetic Resonance (NMR) were recorded at 400MHz and 162 MHz respectively and ^{13}C at 100MHz on a Varian 400MHz system with Oxford NMR As400 Magnet in deuterated chloroform, DMSO or methanol at room temperature. Infra-red (IR) spectroscopy data was recorded on a Thermo-Scientific Nicolet iS5 FTIR Spectrometer. Gas Chromatography Mass Spectrometry (GCMS) data was recorded on an Agilent 5975C Series GC/MSD System. Reactions were monitored by thin layer chromatography (TLC) using EMD Millipore TLC Silica gel 60 plates and a solvent system consisting of various ratios of hexane, dichloromethane and ethyl acetate. The plates were visualised under an ultraviolet (UV) lamp at 254nm. Column chromatography was carried out using either silica, Sephadex™ or alumina. Mobile phase systems were mixtures of laboratory grade solvents utilised during TLC. All agars, solutions and broths used were prepared according to the manufacturer's instructions and autoclaved before their use. Petri

dishes and 96-well plates sourced from Thermo-Scientific were sterile upon purchase.

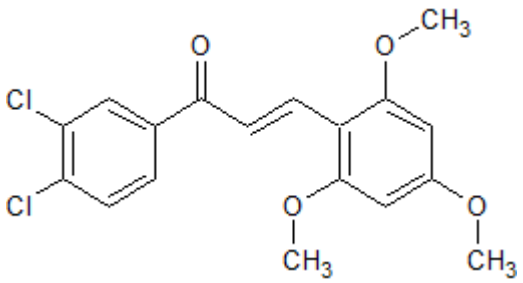
2.2 Chemistry – Experimental procedures

2.2.1 Synthesis of 1-(2'-acylpyrrole)-3-(4-chlorophenyl)-prop-2-ene-1-one (1)



4-chlorobenzaldehyde (0.54 g, 3. mmol) and 2-acetylpyrrole (0.74 g, 6.8 mmol) were dissolved in methanol (10 ml) in a round-bottomed flask and stirred at 65°C until the dissolution of the reagents. A solution of sodium hydroxide (1 M, 2-3 ml) was added and the solution slowly became bright yellow. This was left to stir for 40 minutes. The colour of the solution was yellow. The solution was left to cool before water was added (approx. 5 ml), resulting in the precipitation of the compound. This precipitate was washed with water and filtered under vacuum. Pale yellow crystals were isolated. The crystals were dried in a vacuum oven at 60°C.

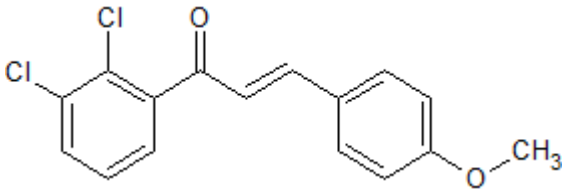
2.2.2 Synthesis of 1-(3', 4'-dichlorophenyl)-3-(2, 4, 6-trimethoxyphenyl)-prop-2-ene-1-one (2)



2, 4, 6-trimethoxybenzaldehyde (1.04 g, 5.3 mmol) and 3,4-dichloroacetophenone (0.95g, 5.04 mmol) were dissolved in methanol (10 ml) in a round-bottomed flask and stirred at 65°C until the dissolution of the reagents. A solution of sodium hydroxide (1 M, 2-3 ml) was added and an instantaneous colour change was noticed, with the solution becoming bright yellow. The solution was left to stir for 30 minutes. A precipitate had already formed upon returning. The mixture was taken off heat and left to cool. Water (approx. 5 ml) was added into the mixture and it was noted that more precipitate seemed to form. The precipitate was filtered under

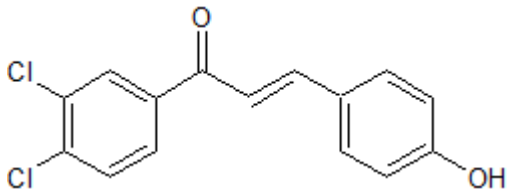
vacuum, resulting crystals were neon yellow. They were dried in a vacuum oven at 60°C for 30 minutes.

2.2.3 Synthesis of 1-(2', 3'-dichlorophenyl)-3-(4-methoxyphenyl)-prop-2-ene-1-one (3)



4-methoxybenzaldehyde (0.74 g, 5.3 mmol) and 2, 4-dichloroacetophenone (0.95g, 5.0 mmol) were dissolved in methanol (10 ml) in a round-bottomed flask and stirred at 65°C until the dissolution of the reagents. A solution of sodium hydroxide (1 M, 2-3 ml) was added and the solution turned yellow. It was left to stir for 30 minutes. A precipitate had already formed without the addition of water and no more formed when approximately 5 ml was added. The resulting precipitate was filtered under vacuum and dried in a vacuum oven at 60°C. The crystals were yellow in colour.

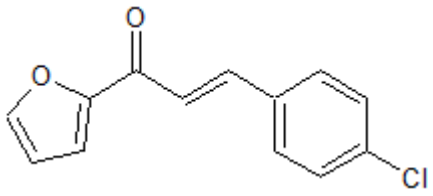
2.2.4 Synthesis of 1-(3',4'-dichlorophenyl)-3-(4-hydroxyphenyl)-prop-2-ene-1-one (4)



4-hydroxybenzaldehyde (0.64 g, 5.3 mmol) and 3, 4-dichloroacetophenone (0.95 g, 4.9 mmol) were dissolved in methanol (10 ml) in a round-bottomed flask and stirred at 65°C until the dissolution of the reagents. A solution of sodium hydroxide (1 M, 2-3 ml) was added but no colour change was noticed at this point. The solution was left to stir for 30 minutes. Upon returning, the solution remained unchanged and was left to stir for an extra 30 minutes. At this point, the solution appeared yellow but translucent. It was taken off the heat and left to cool. Concentrated sulphuric acid was added (2-3 ml) which resulted in the solution turning red but after continuous stirring, it returned to its yellow colour. The product was extracted with dichloromethane (10 ml) and the solvent was removed under reduced pressure. Not all the solvent was removed but a precipitate had begun to form. This mixture was cooled on ice and a very deep yellow precipitate formed. The mixture was placed

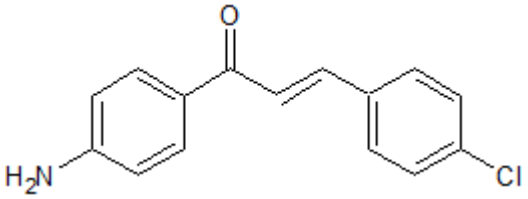
on the vacuum line to be dried for a total of 5 hours. The precipitate was analysed using ^{1}H NMR and was seen to be starting material. However, the aqueous layer had been retained and upon removal of the solvent, a dark yellow solid was obtained. This solid was dried in a vacuum oven for 3 hours at 30°C.

2.2.5 Synthesis of 1-(2'-acetylfuran)-3-(4-chlorophenyl)-prop-2-ene-1-one (5)



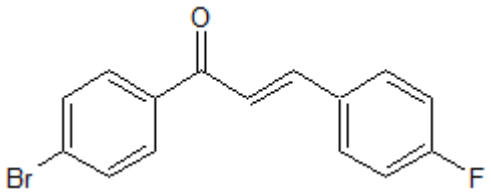
4-chlorobenzaldehyde (0.57g, 4.1 mmol) and acetylfuran (0.43g, 3.9 mmol) were dissolved in methanol (10 ml) in a round-bottomed flask and stirred at 45°C until the dissolution of the reagents. A solution of sodium hydroxide (1 M, 2-3 ml) was added and the solution turned pale red. It was left to stir for 15 minutes and a precipitate formed. The resulting precipitate was washed with water, filtered under vacuum left to air dry in a locker overnight. The crystals were off-white and flaky.

2.2.6 Synthesis of 1-(4'-aminophenyl)-3-(4-chlorophenyl)-prop-2-ene-1-one (6)



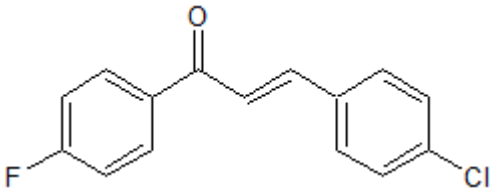
4-chlorobenzaldehyde (0.71g, 5.1 mmol) and 4-aminoacetophenone (0.72 g, 5.3 mmol) were dissolved in methanol (10 ml) in a round-bottomed flask and stirred at 65°C until the dissolution of the reagents. A solution of sodium hydroxide (1 M, 2-3 ml) was added and an immediate colour change was noticed with the solution turning deep orange before slowly becoming bright yellow. It was left to stir for 30 minutes and a precipitate crashed out without the addition of water. Water (10 ml) was added but it did not appear to affect the precipitate nor encourage more of its formation. This was filtered under vacuum. The crystals formed were bright yellow. They were dried in a vacuum oven at 60°C.

2.2.7 Synthesis of 1-(4’-bromophenyl)-3-(4-fluorophenyl)-prop-2-ene-1-one (7)



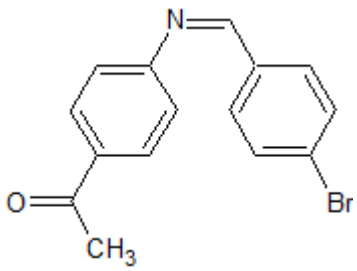
4-fluorobenzaldehyde (0.8 ml, 6.4 mmol) and 4-bromoacetophenone (0.99 g, 4.9 mmol) were dissolved in methanol (10ml) in a round-bottomed flask and stirred at 50°C until the dissolution of the reagents. A solution of sodium hydroxide (1 M, 2-3 ml) was added and a slight colour change was noticed, the solution became a very pale yellow. A few moments later, a precipitate had formed without the addition of water. This was filtered under vacuum. The resulting crystals were pale yellow. They were dried in a vacuum oven at 50°C.

2.2.8 Synthesis of 1-(4’-fluorophenyl)-3-(4-chlorophenyl)-prop-2-ene-1-one (8)



4-chlorobenzaldehyde (0.75 g, 5.3 mmol) and 4-fluoroacetophenone (0.78 ml, 5.6 mmol) were dissolved in methanol (10 ml) in a round-bottomed flask and stirred at 50°C until the dissolution of the reagents. A solution of sodium hydroxide (1 M, 2-3 ml) was added and the solution became pale yellow. A few moments later, a precipitate formed without the addition of water. This was filtered under vacuum and the white crystals were dried in a vacuum oven at 50°C.

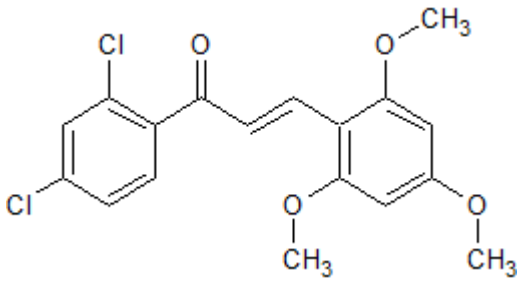
2.2.9 Synthesis of N-(4-bromobenzylidene)-4’-acetoaniline (9)



4-bromobenzaldehyde (2.8 g, 15.1 mmol) and 4-aminoacetophenone (2.1 g, 15.5 mmol) were dissolved in methanol (10 ml) in a round-bottomed flask and stirred at

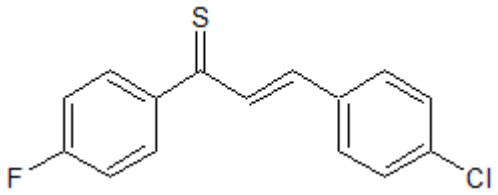
60°C until the dissolution of the reagents. Without the addition of the sodium hydroxide solution, the precipitate began to form. The reaction mixture was left to stir for 30 minutes. Water (10 ml) was added to see if this would alter anything but no change was noticed. The precipitate was filtered under vacuum. Resulting crystals were pale yellow and powdery. Instead of drying them in a vacuum oven, they were left to air dry in a dark locker.

2.2.10 Synthesis of 1-(2',4'-dichlorophenyl)-3-(2,4,6-trimethoxyphenyl)-prop-2-ene-1-one (10)



2, 4, 6-trimethoxybenzaldehyde (0.73g, 3.7 mmol) and 2, 4-dichloroacetophenone (1.01g, 5.3 mmol) were dissolved in methanol (10 ml) in a round-bottomed flask and stirred at 65°C until the dissolution of the reagents. A solution of sodium hydroxide (1 M, 2-3 ml) was added and a precipitate began to form immediately. This was filtered under vacuum. The crystals were left to air dry in a dark locker.

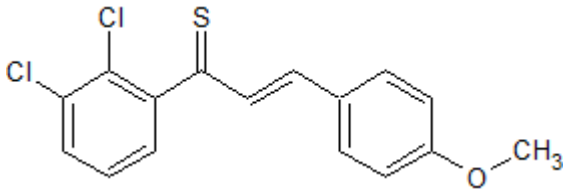
2.2.11 Synthesis of 1-(4'-fluorophenyl)-3-(4-chlorophenyl)-prop-2-ene-1-thione (11)



Compound **8** (0.78g, 0.50 mmol) and Lawesson's Reagent (0.23g, 0.56 mmol) were dissolved in anhydrous THF (30 ml, 0.4 mol) in a round-bottomed flask and stirred under nitrogen whilst heated at 45°C. The reaction was monitored using TLC until the reaction was complete, after approximately 2 hours. The solution became orange upon stirring. The solvent was evaporated off using a rotary evaporator, resulting in an orange, thick substance. Column chromatography was performed using Sephadex™ and a mobile phase of DCM and methanol in a 1:1 ratio. The product, a thick orange liquid was left to air dry in a dark locker overnight. This

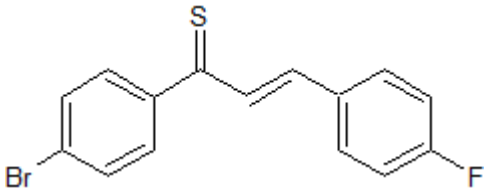
resulted in flaky orange crystals forming. These crystals were stored in a sealed glass tube in a cool and dark area until needed.

2.2.12 Synthesis of 1-(2',3'-dichlorophenyl)-3-(4-methoxyphenyl)-prop-2-ene-1-thione (12)



Compound **3** (0.16 g, 0.52 mmol) and Lawesson's Reagent (0.24 g, 0.059 mmol) were dissolved in anhydrous THF (30 ml) in a round-bottomed flask and stirred under nitrogen whilst heated at 60°C and left to stir at 30 minutes. The remaining THF was evaporated off using a rotary evaporator, resulting in a dark yellow, thick oil-like substance. Column chromatography was performed using Sephadex™ and a mobile phase of DCM and methanol in a 1:1 ratio. The product, a thick yellow liquid was left to air dry in a dark locker overnight. No change was noticed but the sample was stored in a glass tube and kept in a dark and cool area until needed.

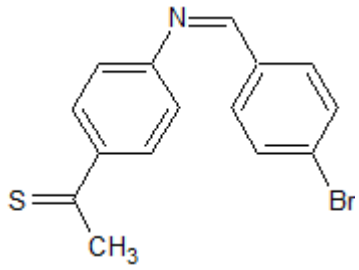
2.2.13 Synthesis of 1-(4'-bromophenyl)-3-(4-chlorophenyl)-prop-2-ene-1-thione (13)



Compound **7** (0.24 g, 0.81 mmol) and Lawesson's Reagent (0.24 g, 0.059 mmol) were dissolved in anhydrous THF (30 ml) in a round-bottomed flask and stirred under nitrogen whilst heated at 40°C. This was left to stir for 2 hours and the solution appeared slightly yellow. This was taken off the heat and the remaining solvent was evaporated using a rotary evaporator. This resulted in a pale yellow solid. This was left to dry overnight. The next day, it was noticed the solid had become a yellow thick liquid. This was washed with water (10 ml) and extracted using ethyl acetate (10 ml). The organic layer was kept and the solvent was evaporated. This step produced a yellow thick liquid. Column chromatography was performed using Sephadex™ and a mobile phase of 30% dichloromethane, 65% hexane and 5% ethyl acetate. The product, also a thick yellow liquid was left to air dry in a dark

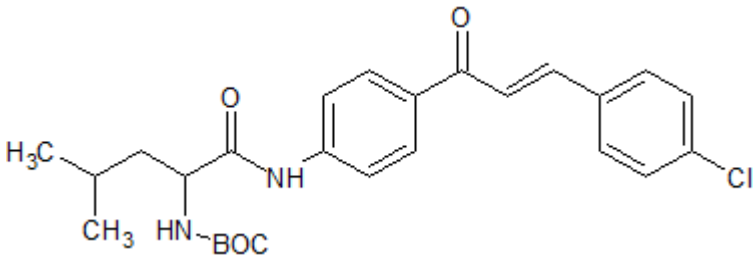
locker overnight. This was stored in a sealed glass tube in a cool and dark area until needed.

2.2.14 Synthesis of N-(4-bromobenzylidene)-4'-acetothioaniline (14)



Compound **9** (0.15 g, 0.49 mmol) and Lawesson's Reagent (0.20 g, 0.049 mmol) were dissolved in anhydrous toluene (30 ml) in a round-bottomed flask and stirred under nitrogen whilst heated at 55°C. The solution became red upon stirring and was left stirring for a total of 8 hours. Every hour, a TLC was done to monitor the progress of the reaction. After 8 hours, there were 2 spots on the TLC meaning starting material was still present. The reaction was left to stir overnight. After an additional 12 hours, the TLC showed one spot. Remaining solvent was removed on the rotary evaporator resulting in a deep purple thick liquid. Column chromatography was performed using alumina and a mobile phase of 30% dichloromethane, 65% hexane and 5% ethyl acetate. The product, a thick purple liquid was left to air dry in a dark locker overnight. This was stored in a sealed glass tube in a cool and dark area until needed.

2.2.15 Synthesis of 1-(tert-butoxycarboxylleucine)-3-(4-chlorophenyl)-prop-2-ene-1-one (15)



An attempt to carry out a DCC/HOBt-mediated coupling of a chalcone with an amino acid was made.

Compound **6** (1.0 g, 3.9 mmol) and HOBt (0.54 g, 4.0 mmol) were added to chilled DCM (30 ml) and stirred on ice for 30 minutes. To this, Boc-Leu-OH (0.93 g, 4.0 mmol) and DCC (1 ml, 4.4 mmol) were added and the reaction mixture was taken off the ice. At room temperature, this mixture was left to stir for 2 hours. This was decanted into glass tubes and stored in a freezer overnight. 4% hydrochloric acid (30 ml), saturated sodium carbonate solution (25 ml) and brine (30 ml) were added consecutively to the reaction mixture. Bubbles evolved at each addition. Calcium sulphate, a desiccating agent (approx. 20 g) was added to this mixture and this formed clumps at the bottom of the round-bottomed flask. Once the clump forming had stopped, it was filtered off from the mixture, leaving a clear solvent with a yellow solid at the bottom of the conical flask. The solvent and the solid were separated and the solid was oven-dried at 50°C for 30 minutes. This produced a yellow sticky solid. Column chromatography was performed using Sephadex™ and a mobile phase of DCM and methanol in a 1:1 ratio. The product, also a yellow sticky solid was stored in a sealed glass tube in a cool and dark area until needed.

2.3 Microbiology – Experimental

The microbial strains *Escherichia coli* (NCTC 7928)*, Pseudomonas aeruginosa* (NCTC 6749) and *Staphylococcus epidermidis* (NCTC 9865) were utilised.

The initial screening of the synthesised compounds was performed according to The European Committee on Antimicrobial Susceptibility Testing (EUCAST) methodology using disk diffusion testing. The bacterial strains were obtained from the microbial bank of the Microbiology department of Kingston University. The bacteria were grown for 24 hours at 37°C on nutrient agar. The inocula for the assays were prepared by cell suspensions in Ringer's solution (Oxoid UK, Ltd.) to give a concentration of approximately $1.5x10^8$ CFU/ml and used within 15 minutes of preparation.

2.3.1 Establishing antimicrobial activity

The compounds were dissolved in DMSO at a concentration of 10 mg/ml. From the prepared cultures, the bacteria were spread onto Mueller-Hinton agar plates (4 mm depth) using a sterile swab and the Petri dishes were divided into three sections: a positive control, a negative control and the synthesised compound. The positive control was ciprofloxacin (10 µg), the negative control was 10 µl DMSO and the

compound was used in 10 µl doses. Each test was done in duplicates and incubated at 37°C for 24 hours. The zones of inhibition produced by the compounds and the antibiotic discs were measured in millimetres (mm) and recorded. Once the active compounds had been identified, repeat tests were done in triplicate to ensure reliability of results.

2.3.2 Minimum inhibitory concentration (MIC)

This was determined using the microtitre broth dilution method. 96-well microtitre plates were used in this procedure. Mueller-Hinton broth was prepared at double and single strength whilst a stock solution of 10 mg/ml of the active compound was prepared. A 100 µl aliquot of the double concentration broth was dispensed to the first 3 rows of the 96-well plate, followed by 200 µl of the active compound's stock solution into the first well of the first 3 rows. The first well was mixed carefully using the pipette before 100 µl was transferred to the next well. Using a sterile tip each time, this was repeated a further four times, making a dilution series of the compound under test as follows: 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, 0.625 mg/ml and 0.3125 mg/ml. The inocula for the wells were prepared as above and a 5 µl aliquot of bacteria dispensed in each well using a sterile tip for each addition. The positive control was the double concentrated Mueller Hinton broth inoculated with 5 µl of bacteria, giving a concentration of approximately $5x10^5$ CFU/ml. The negative controls were the double concentrated Mueller Hinton broth with 5 µl DMSO as well as single concentrated Mueller Hinton broth on its own. The plates were incubated at 37°C for 24 hours. The plates were read by eye and the results were recorded. Repeat tests were done in triplicates to ensure reliability.

2.3.3 Minimum bactericidal concentration (MBC)

Nutrient agar was used in this procedure and each plate was divided into 8 sections, section 1-5 dedicated to the wells of the plates used for determining MIC containing the active compound and bacteria whilst section 6-7 were dedicated to the positive and negative controls. A sterile swab was used to transfer a small amount from each well after the MIC incubation to its corresponding section on the agar. The plates were incubated at 37°C for 24 hours. The plates were read and the results were recorded. Repeat tests were done in triplicates to ensure reliability.

3. Results

A total of fifteen compounds were synthesised: nine chalcones, four thiochalcones, a diphenyl-imine compound and a chalcone-amino acid conjugate. Out of these fifteen compounds, two of the thiochalcones showed antimicrobial activity whilst none of the chalcones showed activity. The chalcone-amino acid conjugate was not tested for any activity. The certainty of the synthesis of the thiochalcones is addressed in the discussion.

Table 5: Masses, Melting Points & Percentage Yields of the Synthesised Compounds

Code	Mass (g)	Melting Point (°C)	Percentage yield (%)
1	1.52	158.8-163.0	100.7
2	0.96	185.7-187.2	52.3
3	0.47	84.1-88.7	30.7
4	2.01	168.2-171.9	138.4
5	0.51	139.0-143.2	40.2
6	0.67	-	51.9
7	1.04	142.9-143.5	64.9
8	1.23	137.0-141.2	89.5
9	4.23	144.1-147.6	93.3
10	0.76	146.1-148.7	55.6
11	0.45	liquid at r.t	-
12	0.47	liquid at r.t	-
13	1.0	liquid at r.t	-
14	0.33	liquid at r.t	-
15	-	-	-

Melting points were recorded on a Stuart™ Digital Melting Point Apparatus and were uncorrected.

Table 6: Molecular Formula, Molecular Weight & Mass Spectral Data of the Synthesised Compounds

Code	Molecular Formula	Molecular Weight (Mw)	Mass (m/z)
1	$C_{13}H_{10}ClNO$	231.67	$[M^+]$ 231 (Abundance – 2400000). $[M^{+2}]$ 233 caused by ^{37}Cl isotope.
2	$C_{18}H_{16}Cl_2O_4$	367.22	$[M^+]$ 367 (Abundance – 100000). $[M^{+2}]$ 369 caused by ^{37}Cl isotope. Base peak is 335, shows the loss of a methoxy group. Other peaks seen: 366 (loss of H^+).
3	$C_{16}H_{12}Cl_2O_4$	307.17	$[M^+]$ 307 (Abundance – 1200000). $[M^{+2}]$ 309 caused by ^{37}Cl isotope. Base peak is 306 (loss of H^+)
4	$C_{15}H_{13}Cl_2O_2$	292.14	Base peak is 149. 295 is closest fragment.
5	$C_{13}H_9ClO_2$	232.66	$[M^+]$ 232 (Abundance – 26800). Base peak is 231 (loss of H^+). $[M^{+2}]$ 234 caused by ^{37}Cl isotope.
6	$C_{15}H_{12}NClO$	257.71	No MS obtained as sample had run out before testing took place.
7	$C_{15}H_{10}BrFO$	305.13	$[M^+]$ 305 (Abundance – 190000). $[M^{+2}]$ 307 caused by ^{37}Cl isotope. Base peak is 225 caused by the loss of a bromine atom.
8	$C_{15}H_{10}ClFO$	260.68	$[M^+]$ 260 (Abundance – 100000). $[M^{+2}]$ 262 caused by ^{37}Cl isotope. Base peak is 75.
9	$C_{15}H_{12}NBrO$	302.16	[M+] 302 (Abundance – 76000). $[M^{+2}]$ 304 caused by Br isotope.

			Base peak is 287/286 (loss of methyl group).
10	$C_{18}H_{16}Cl_2O_4$	367.22	366 is closest fragment with 368 seen as the peak caused by ^{81}Cl isotope. Base peak is 335, possibly caused by the loss of a chlorine atom.
11	$C_{15}H_{10}ClFS$	276.75	267 is closest fragment with 269 seen as the peak caused by ^{37}Cl isotope. Base peak is 207, also peak 209 caused by isotope.
12	$C_{16}H_{12}Cl_2OS$	323.23	320 is closest fragment, $[M^{+2}]$ peak not seen. Base peak is 306, same m/z as starting reagent (**3**)
13	$C_{15}H_{10}BrFS$	321.21	Base peak is 75. 260 peak seen, same m/z as starting reagent (**7**) with 261 seen as $[M^{+2}]$ peak.
14	$C_{15}H_{12}NBrS$	318.22	318 was seen. Base peak is 303, same m/z as starting reagent (**9**). $[M^{+2}]$ 304 (starting material) and 319 $[M^{+1}]$ seen.
15	$C_{26}H_{31}CN_2O_4$	470.98	MS not obtained.

Table 7: Infra-Red Spectroscopy Data of Synthesised Compounds

Code	Absorption (cm^{-1})	Appearance	Group
1	3268	Medium	N-H stretch
	1643	Medium	C=C stretch
	817	Strong	C-Cl stretch
2	1645	Medium	C=C stretch
	840	Strong	C-Cl stretch
3	1653	Medium	C=C stretch
	823	Strong	C-Cl stretch
4	1645	Medium	C=C stretch
	1206	Strong	C-O (OH) stretch
	819	Strong	C-Cl stretch
5	2032	Weak	C-H aromatic bend
	1653	Medium	C=C stretch
	778	Strong	C-Cl stretch
6	-	-	-
7	2032	Weak	C-H aromatic bend
	1699	Strong	C=O conjugated ketone stretch
	668	Strong	C-Br stretch
8	1662	Medium	C=C stretch
	1005	Strong	C-F stretch
	813	Strong	C-Cl stretch
9	1618	Weak	C-H aromatic bend
	1688	Strong	C=O Conjugated ketone stretch
	573	Strong	C-Br stretch
10	1650	Medium	C=C stretch
	1445	Medium	CH (methyl) bend
	821	Strong	C-Cl stretch
11	-	-	-

12	-	-	-
13	-	-	-
14	-	-	-
15	-	-	-

Table 8: ^{1}H NMR Data of Synthesised Compounds

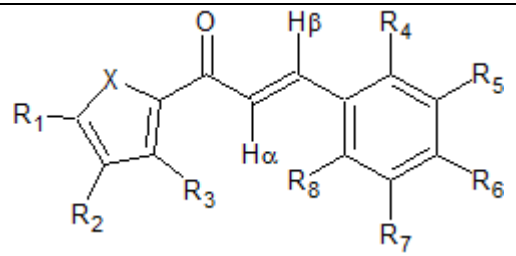


Image 1

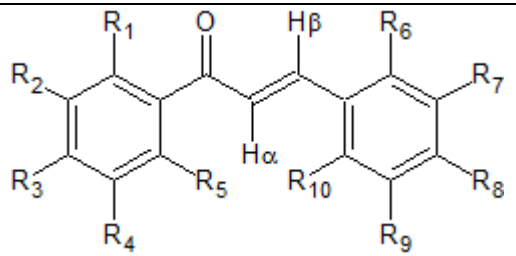


Image 2

^ - see image 1 for reference, • - see image 2 for reference

*** - thiochalcone**

R = H unless otherwise stated

Code	Hα	Hβ	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
1^ X = NH, R6 = Cl	7.33, d, 16Hz, 1H	7.80, d, 16Hz, 1H	7.39, m, 1H	6.28, m, 1H	7.17, m, 1H	7.59, d, 8Hz, 1H	7.41, d, 8Hz, 1H	-	7.41, d, 8Hz, 1H	7.59, d, 8Hz, 1H	-	-
2• R2 = Cl, R3 = Cl, R6, R8, R10 = OCH_3	6.53, d, 14Hz, 1H	6.88, d. 14Hz, 1H	7.69, s, 1H	-	-	7.70, d, 8Hz, 1H	7.86, d, 8Hz, 1H	3.93, s, 3H	6.16, s, 1H	3.89, s, 3H	6.16, s, 1H	3.93, s, 3H
3• R1 = Cl, R3 = Cl, R8 = OCH_3	7.00, d, 16Hz, 1H	7.44, d, 16Hz, 1H	-	7.50, d, 2Hz, 1H	-	7.37, dd, 2Hz,8Hz, 1H	7.42, d, 8Hz, 1H	7.54, d, 8Hz, 1H (overlap with R10)	6.94, d, 8Hz, 1H	3.87, s	6.94, d, 8Hz, 1H	7.54, d, 8Hz, 1H (overlap with R6)

Key: chemical shift (δ), peak splitting pattern, J coupling constant (Hz), integration
s – singlet, d – doublet, dd – doublet of doublets, t – triplet, d of t – doublet of triplets, m – multiplet

4• R1 = Cl, R3 = Cl, R8 = OH	7.82, d, 8Hz, 1H	7.75, d, 8Hz, 1H	-	8.36, d, 2Hz, 1H	-	7.37, dd, 2Hz, 8Hz, 1H	7.43, d, 8Hz, 1H	7.54, d, 9Hz, 1H	6.94, d, 9Hz,1H	-	6.94, d, 9Hz, 1H	7.54, d, 9Hz,1H
5^ X = O, R6 = Cl	7.36, d, 16Hz, 1H	7.68, d, 16Hz, 1H	7.84, d, 16Hz, 1H	6.63, m, 1H	7.45, d, 4Hz, 1H	7.60, d, 8Hz, 2H	7.41, d, 9Hz, 2H	-	7.41, d, 9Hz, 1H	7.60, d, 8Hz, 2H	-	-
6• R3 = NH_2, R8 = Cl	7.75, d, 16Hz, 1H	7.81, d, 16Hz, 1H	-	-	NH_2 at 8.45, s, 2H	6.73, d, 9Hz, 1H	7.89, d, 9Hz, 1H	8.11, d, 8Hz, 1H	7.30, d, 8Hz, 1H	-	7.30, d, 8Hz,1H	8.11, d, 8Hz, 1H
7• R3 = Br, R8 = F	7.50, d, 16Hz, 1H	7.79, d, 16Hz, 1H	7.60, d, 8Hz, 1H	7.43, d, 8Hz, 1H	-	7.43, d, 8Hz, 1H	7.60, d, 8Hz, 1H	8.08, dd, 8Hz, 5Hz, 1H	7.21, t, 8Hz, 1H	-	7.21, t, 8Hz, 1H	8.08, dd, 8Hz, 5Hz, 1H
8• R3 = F, R8 = Cl	7.42, d, 16Hz, 1H	7.81, d, 16Hz, 1H	7.67, dd, 9Hz, 4Hz, 1H	7.15, t, 9Hz, 1H	-	7.15, t, 9Hz, 1H	7.67, dd, 9Hz, 4Hz, 1H	7.67, d, 9Hz, 1H	7.91, d, 9Hz, 1H	-	7.91, d, 9Hz, 21	7.67, d, 9Hz, 1H
9•	-	-	-	-	-	-	-	-	-	-	-	-
10• R1 = Cl, R3 = Cl, R6, R8, R10 = OCH_3	7.48, d, 16Hz, 1H	7.95, d, 16Hz, 1H	-	7.47, d, 2Hz, 1H	-	7.33, dd, 8Hz, 2Hz, 1H	7.43, 8Hz, 1H	3.87, s, 3H	6.12, s, 1H	3.87, s, 3H	6.12, s, 1H	3.87, s, 3H
11* R3 = F, R8 = Cl	-	-	-	-	-	-	-	-	-	-	-	-
12* R2 = Cl, R3 =	6.83, d, 16Hz, 1H	7.43, d, 16Hz, 1H	-	-	-	7.31, d, 8Hz, 1H	7.00, d, 8Hz, 1H		7.36, dd, 16Hz, 1H	3.70, s, 3H	7.25, dd, 8Hz, 1H	-

Cl, R8, = OCH_3												
13* R3 = Br, R8 = F	-	-	-	-	-	-	-	-	-	-	-	-
14* R3 = NH_2, R8 = Br	-	-	-	-	-	-	-	-	-	-	-	-

Peaks for compounds **9, 11, 13** and **14** were not assigned in this table. Compound **9**'s spectra is explained further in table 13 and the discussion whilst compound **11, 13** and **14**'s spectra is explained in table 12 and the discussion.

Table 9: ^{13}C NMR Data of the Synthesised Compounds

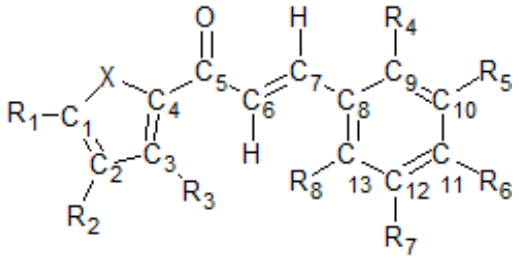


Image 1

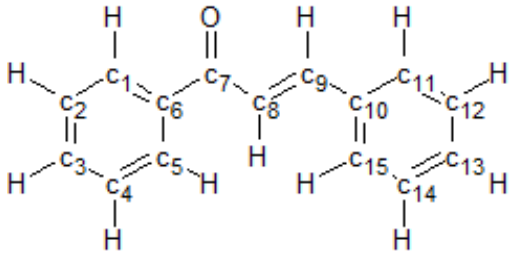


Image 2

^ - see image 1 for reference, • - see image 2 for reference

*** - thiochalcone**

Code	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15
1^ X = NH, R6 = Cl	129.2	111.1	116.4	136.3*	178.4	125.4	140.9	125.7 *	129.6	128.9	132.5*	128.9	129.6	-	-
2• R2 = Cl, R3 = Cl, R6, R8, R10=O CH_3	129.6	136.1*	136.3*	126.3	131.5	155.3*	196.5	126.7	131.5	106.3*	163.-	91.5	166.9	91.5	163.0
3• R1 = Cl, R3 = Cl,	130.3*	123.7	130.1*	127.2	123.2	130.5*	187.6	131.5	146.3	141.0*	130.7	114.5	OCH_3 at 55.4	114.5	130.5

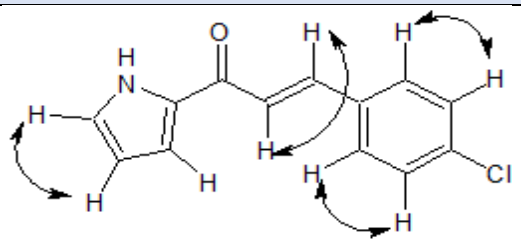
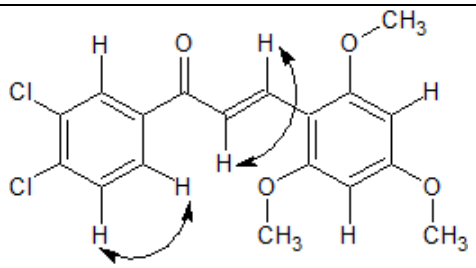
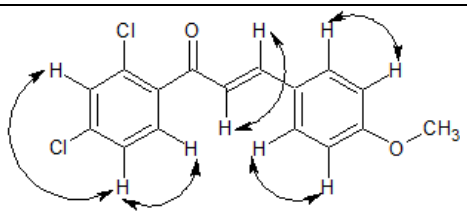
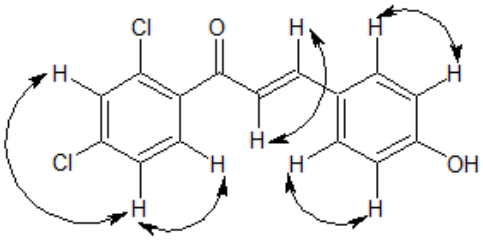
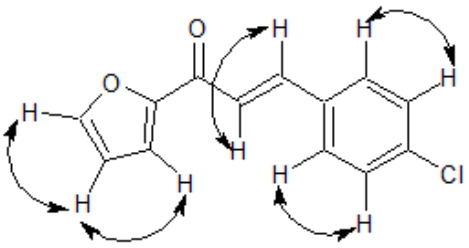
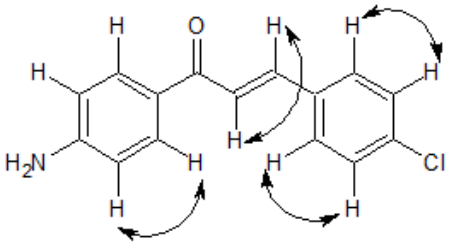
Key: chemical shift (δ), peak splitting pattern, J coupling constant (Hz), integration
s – singlet, d – doublet, dd – doublet of doublets, t – triplet, d of t – doublet of triplets, m – multiplet

R8 = OCH_3															
4• R1 = Cl, R3 = Cl, R8 = OH	131.0	132.2*	136.1*	129.6	132.0	138.5*	187.2	128.9	146.8	126.1*	131.8	116.3	160.3	116.3	131.8
5^ X = O, R6 = Cl	148.5	113.3	131.0	-	178.5	121.5	142.3	-	130.0	129.0	-	129.0	130.0	-	-
6• R3 = NH_2, R8=Cl	130.0	113.9	133.4*	113.9	130.0	134.2*	189.2	129.1	130.5	-135.6*	130.6	130.2	138.1*	130.2	130.6
7• R3 = Br, R8 = F	129.6	129.4	131.1	129.4	129.6	133.5*	189.5	121.9	131.1	136.5*	130.1	116.1	134.1*	116.1	130.1
8• R3 = F, R8 = Cl	130.2	116.1	165.4	116.1	130.2	131.0*	189.6	121.3	144.1	136.5*	130.1	132.2	134.1*	132.2	130.1
9•	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10• R1 = Cl, R3 = Cl, R6, R8,R10 =OCH_3	132.6*	129.9	133.1*	126.9	125.6	106.1*	194.4	130.4	138.3	136.3*	OCH_3 at 55.8	90.5	OCH_3 at 55.4	90.5	OCH_3 at 55.8
11* R3 = F, R8 = Cl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

12* R2 = Cl,R3 =Cl,R6, R8,R10 =OCH_3	-	126.9	-	126.9	123.6	-	200.8				132.0	130.5	-	130.5	132.0
13* R3 = Br, R8 = F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14* R3 = NH_2, R8 = Br	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Peaks for compounds **9, 11, 13** and **14** were not assigned in this table. Compound **9**'s spectra is explained further in table 13 and the discussion whilst compound **11, 13** and **14**'s spectra is explained in table 12 and the discussion.

Table 10: ^{1}H-^{1}H COSY Correlations for Synthesised Compounds

Code	COSY Coupling
1	
2	
3	
4	
5	
6	

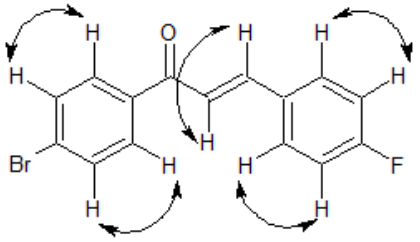
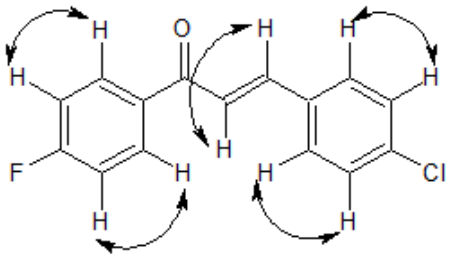
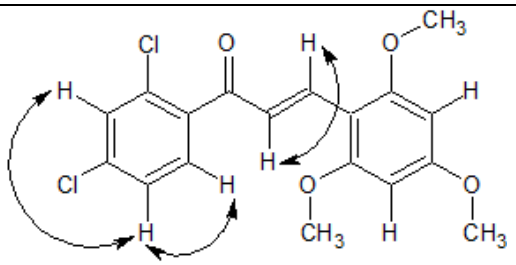
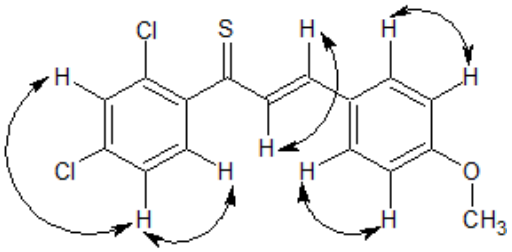
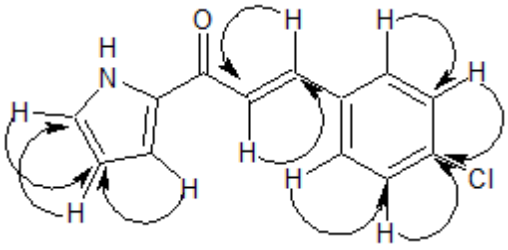
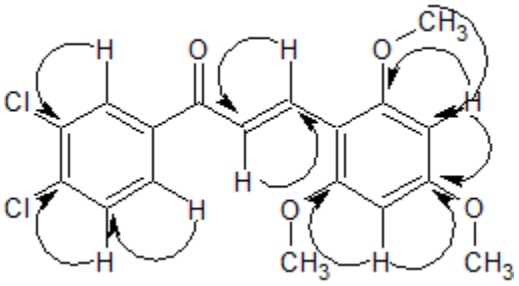
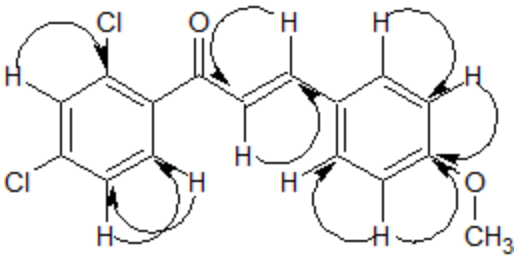
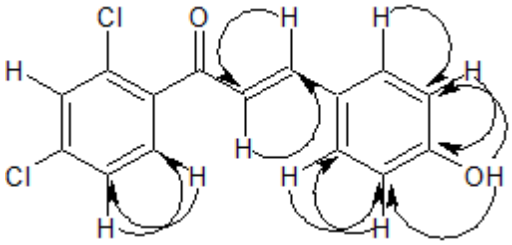
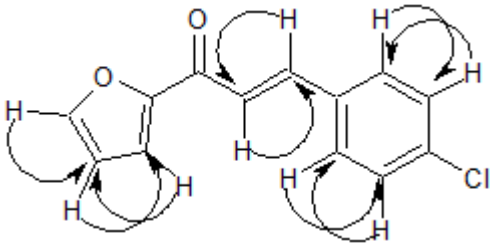
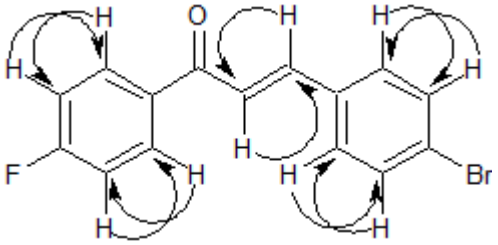
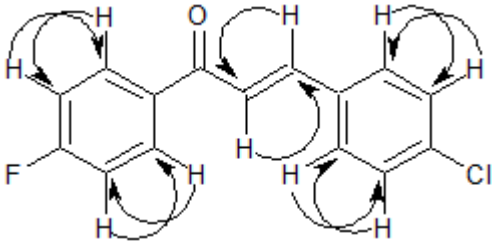
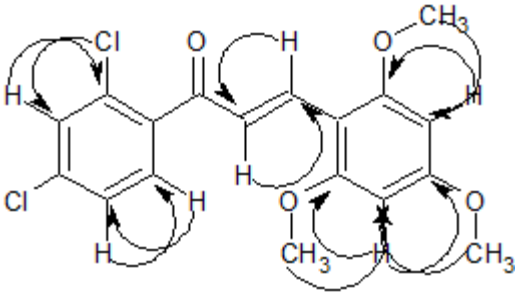
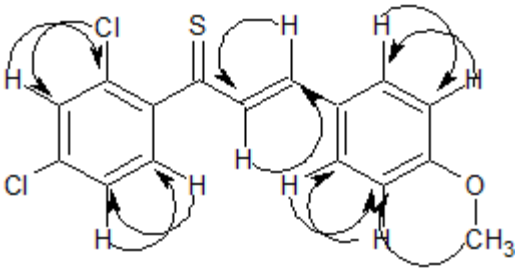
7	
8	
9	-
10	
11	-
12	
13	-
14	-

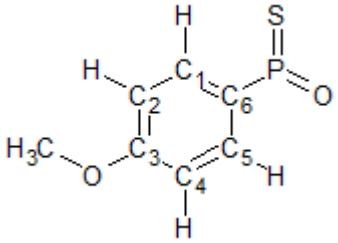
Table 11: ^{1}H-^{13}C HMBC Correlations of Synthesised Compounds

Code	HMBC Coupling
1	
2	
3	
4	
5	
6	-

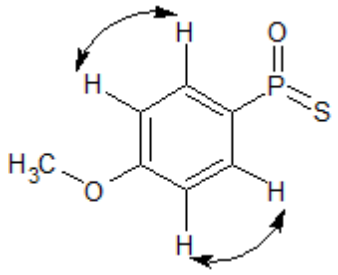
7	
8	
9	-
10	
11	-
12	
13	-
14	-

Regarding the ^{1}H and ^{13}C NMR of compounds **11**, **13** and **14**, refer to the table below. The spectra for compound 14 showed that the expected compound was not isolated. Instead, the oxa-thio-phosphorane compound, a breakdown product from the Lawesson's Reagent mechanism was found to be the major product isolated after column chromatography.

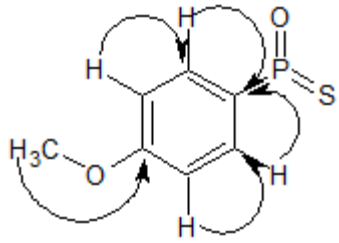
Table 12: ^{1}H, ^{13}C and ^{1}H-^{1}H COSY NMR Data for oxa-thio-phosphorane using spectra of compound **14**



^{1}H NMR Data					
Key: chemical shift (δ), peak splitting pattern, J coupling constant (Hz), integration					
C1	**C2**	**C3**	**C4**	**C5**	**C6**
7.6, d, 12Hz, 1H	7.04, d, 9Hz, 1H	methoxy group: 3.8, s, 3H	7.04, d, 9Hz, 1H	7.6, d, 12Hz, 1H	-
^{13}C NMR Data					
C1	**C2**	**C3**	**C4**	**C5**	**C6**
132.9	113.9	163	113.9	132.9	123.8
^{1}H-^{1}H COSY Data					

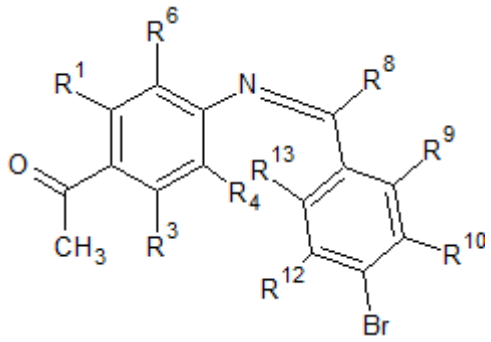


^{1}H-^{13}C HMBC Data



Whilst analysing the data for compound **9** and consolidating the synthetic workup of this compound, it is clear that its structure is not a chalcone but the structure below. For further explanation, see discussion (4.2).

Table 13: ^{1}H, ^{13}C and ^{1}H-^{1}H COSY NMR Data for compound **9**



R = H

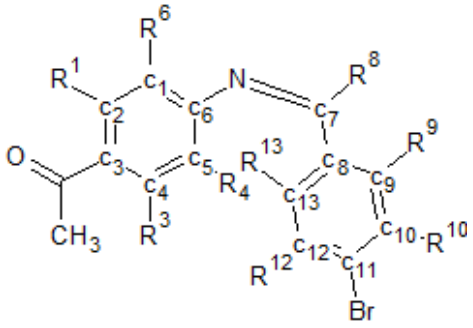
^{1}H NMR Data

Key: chemical shift (δ), peak splitting pattern, J coupling constant (Hz), integration

R1	R3	R4	R6	R8	R9	R10	R12	R13
8.03, d, 8Hz, 1H	8.03, d, 8Hz, 1H	7.80, d, 8Hz, 1H	7.80, d, 8Hz, 1H	8.41,s 1H	7.65, d, 8Hz, 1H	7.24, d, 7Hz, 1H	7.24, d, 7Hz, 1H	7.65, d, 8Hz, 1H

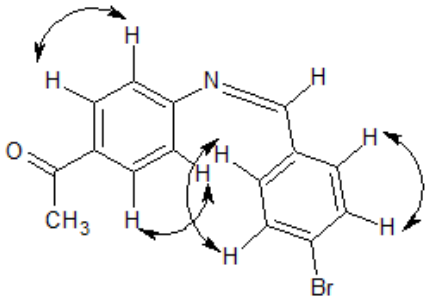
Methyl group of acetophenone: 2.64, s

^{13}C NMR Data

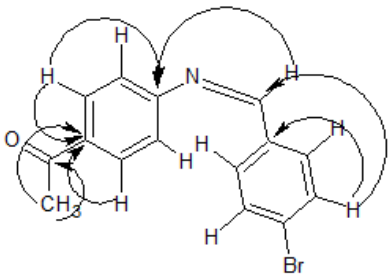


C1	C2	C3	C4	C5	C6	C7	C8	C9
129.9	132.4	129.9	121.0	-	121.0	156.1	134.7	130.1
C10	**C11**	**C12**	**C13**	Methyl group of acetophenone: 26.4, carbonyl group – 191.1				
131.9	126.6	131.9	130.1					

^{1}H-^{1}H COSY NMR Data

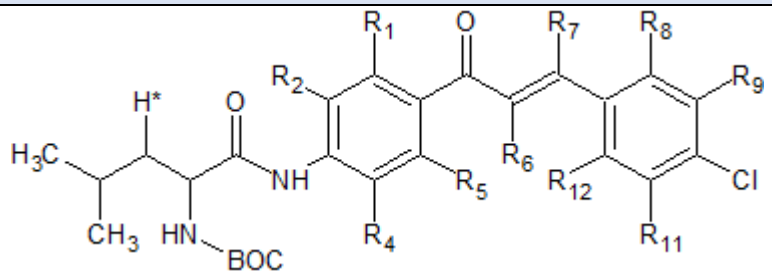
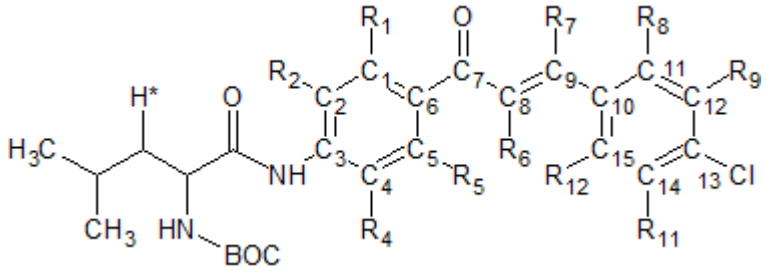


^{1}H-^{13}C HMBC



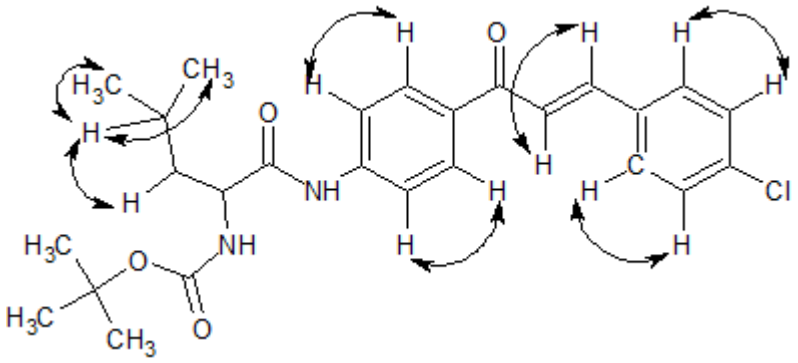
Compound **15** proved difficult to isolate clean with column chromatography. The peak assignments have been done with extensive use of ^{1}H, ^{13}C and ^{1}H-^{1}H COSY spectra.

Table 14: ^{1}H, ^{13}C and ^{1}H-^{1}H COSY NMR Data for Compound **15**

^{1}H NMR Data										
 **R = H**										
R1	**R2**	**R4**	**R5**	**R6**	**R7**	**R8**	**R9**	**R11**	**R12**	**H***
7.40, d, 8Hz, 1H	7.60, d, 9Hz, 1H	7.60 , d, 9Hz, 1H	7.40, d, 8Hz, 1H	7.33, d, 8Hz, 1H	7.88 , d, 8Hz, 1H	7.72 , d, 8Hz, 1H	7.99 , d, 7Hz, 1H	7.99 , d, 7Hz, 1H	7.72 , d, 8Hz, 1H	3.89 , m, 1H
Methyl groups of amino acid – 0.81, 2 x d, 12Hz										
^{13}C NMR Data										
										
C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**	**C11**
130.9	119.05	-	119.05	130.9	-	-	-	-	-	-
C13	**C14**	**C15**	Carbonyl of amino acid – 175.3, methyl groups of amino acid – 28.5, CH* – 52.4							
-	-	-								
A majority of the peaks were not assigned as the resolution of the spectra did not allow for the peaks to be seen. An increased number of scans whilst recording the spectra would have alleviated the issue. Also, the Boc protecting										

group appears to have been removed as its methyl peaks are missing on both ^{1}H and ^{13}C data.

^{1}H-^{1}H COSY NMR Data



^{1}H-^{13}C HMBC

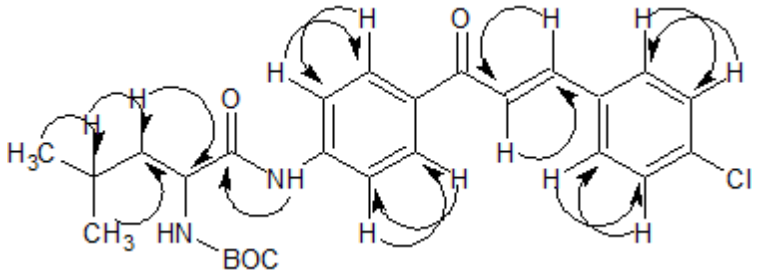


Table 15: In-vitro Antimicrobial Activity of the Synthesised Compounds

Code	Zone of Inhibition (mm)*			
	Concentration (mg/ml)	Bacteria		
		S. epidermidis	*P. aeruginosa*	*E. coli*
1	10	-	-	-
2	10	-	-	-
3	10	-	-	-
4	10	-	-	-
5	10	-	-	-
6	10	-	-	-
7	10	-	-	-
8	10	-	-	-
9	10	-	-	-
10	10	-	-	-
11	10	10	-	-
12	10	-	-	-
13	10	-	-	-
14	10	16	10.3 $\pm$ 0.334	11.3 $\pm$ 0.334
Ciprofloxacin	10µg	15.3 $\pm$ 0.334	18	15.3 $\pm$ 0.334
* mean value given (n=3)				

Table 16: Minimum Inhibitory Concentration (MIC) of the biologically active compounds

Code	MIC (mg/ml)		
	S. epidermidis	*P. aeruginosa*	*E. coli*
11	1.25	-	-
14	0.3125	1.25	1.25
Ciprofloxacin*	1	0.5	0.25-0.5
*(mg/L), as reported by EUCAST (n=3)			

Table 17: Minimum Bactericidal Concentration (MBC) of the biologically active compounds

Code	MBC (mg/ml)		
	S. epidermidis	*P. aeruginosa*	*E. coli*
11	2.5	-	-
14	1.25	2.5	2.5
(n=3)			

Discrepancies between the MICs and MBCs are further discussed in the discussion (4.4). The activity of the synthesised compounds was adequately recorded and noted.

4. Discussion

4.1 Data Analysis

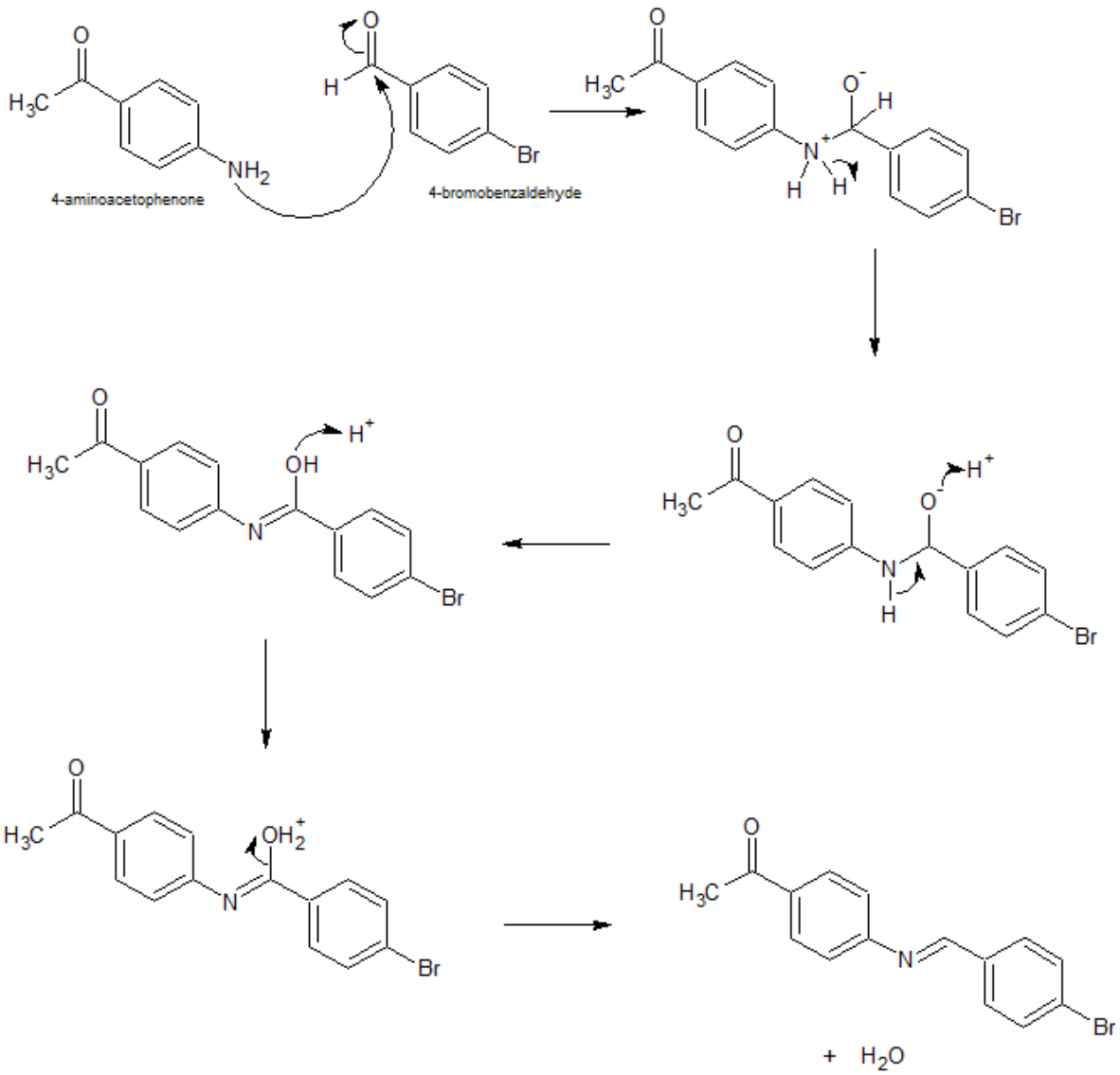
This project is made up of two key aspects: the synthesis of chalcones and thiochalcones followed by the determination of their activity as antimicrobial agents. In order to prove the existence of the compounds synthesised, the data procured from their analytical examination had to be studied.

As seen in tables 8, 9, 10 and 11, each synthesised compound has had their ^{1}H and ^{13}C NMR analysed. The spectra derived from these analytical experiments including ^{1}H-^{1}H Correlation Spectroscopy (COSY) and ^{1}H-^{13}C Heteronuclear Multiple Bond Correlation Spectroscopy (HMBC) can be found in the appendix. Diagnostically, the most important protons to be identified in a chalcone are the protons on the α-β unsaturated bond as these only arise from a successful Claisen-Schmidt condensation and complete conjugation of the electron system.

The synthesised compounds have several substituents, causing the magnetic equivalency of the protons to change. Compounds **1** and **5** had 5-membered A rings. Compound **1** showed coupling of its α-β unsaturated protons at δ7.33 and δ7.80 with J coupling constants of 16Hz as did compound **5** with coupling seen at δ7.41 and δ7.69 with J coupling constants of 16Hz. Compounds **2, 3, 4** and **10** have dichloro substituents on the A ring with their α-β unsaturated protons around δ6.53-7.95 with coupling constants around 14-16.8Hz, with the exception of compound **4**, whose α-β unsaturated protons have a coupling constant of 16 Hz. Compounds **2** and **10** have trimethoxy substituents on the B ring. The remaining compounds had either a halogen (Cl or Br), methoxy or hydroxyl group in the 4th position on the B ring. Peaks were typically seen in the δ7-8 region which is where aromatic protons usually occur (see appendix 1.1.1 for ^{1}H NMR spectra of compound **1**, 1.2.1 for compound **2** and so on). Infra-red spectroscopy showed weak peaks at 1645 cm^{-1} across all the derived spectra, correlating to the C=C bond stretch in the aromatic ring. The C-F and C-Cl stretch around 800 cm^{-1} and 1000 cm^{-1} were seen across all halogenated compounds such as **1, 2, 3, 4, 5, 8, 9** and **10**.

Compound **9**'s synthesis did not require the addition of sodium hydroxide to induce a colour change or precipitation. The amino group on the 4-aminoacetophenone bears a lone pair of electrons on the nitrogen. This could have facilitated the

nucleophilic addition of the benzaldehyde with the acetophenone in solution (scheme 5).



Scheme 5: **Mechanism of Reaction between 4-aminoacetophenone & 4-bromobenzaldehyde**
Image source: author's own

The TLC of the compound showed one spot (solvent system: hexane & DCM, 1:1). The ^1H spectrum supports the proposed structure. The aromatic protons formed doublets at δ8.03, δ7.80, δ7.65 and δ7.24 respectively whilst the benzylidene proton can be seen as a singlet at δ8.41. The GCMS data of the compound produced a pair of M+ peaks at *m/z* 303/305 indicative of bromine being present in the molecule. HMBC correlations were used to assign the aromatic doublets as belonging to ring A or ring B.

Also, the NOESY spectra for compound **9** does not show a correlation between the protons with asterisks (see figure 17) suggesting the compound is of this shape.

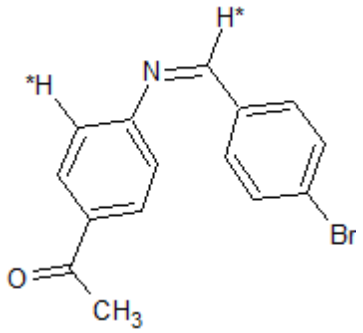


Figure 17: Shape of Compound **9**
Image source: author's own

The use of Lawesson's Reagent to convert the carbonyl to a thiol in this particular compound would have been possible (see scheme 6). However, the spectra for the attempted conversion, compound **14**, was consistent with the oxa-thio-phosphorane compound which is further discussed in the next section.

Upon analysing Nuclear Overhauser Effect Spectroscopy (NOESY) data, a cross peak correlating the α-β unsaturated proton was seen across a majority of the spectra (see appendix 1.1.6 for compound **1** spectrum), which suggests the cis (Z) isomer of the chalcones was synthesised. However, the high J coupling constants of these protons, typically seen at 16Hz in this project, (see table 7) indicate the conformation of the chalcones to be trans (E) isomers as well as geometrically pure (Tran et al, 2012). These are the thermodynamically most stable form (Aksöz et al, 2011).

In any chemical reaction in which two isomers can be formed, there is always a kinetic product and a thermodynamic product. The kinetic product is usually formed when the reaction time is short, not giving the thermodynamic product time to form. The use of a strong base minimises the chances of reversibility at the intermediate step, enhancing the formation of the thermodynamic product – the trans (E) isomer (see scheme 2).

A possible explanation for the appearance of this cross peak was the interaction of a chalcone molecule with another molecule by way of pi stacking. Seeing as NOESY data aims to show signals that arise from protons that are of close proximity in space, this theory fits. The formation of aggregates in solutions is a naturally

occurring phenomena that occurs with supramolecules (Perez et al, 2013). It is likely that a trans (E) isomer was in close contact with a neighbouring trans (E) isomer aggregate, resulting in a strong signal on the NOESY data. The high coupling constants of the α-β protons still suggest that the trans (E) isomer is the abundant product. So, it became imperative to understand why this peak was being shown.

It was hypothesised that the use of an aprotic solvent as well as a dilute sample for NMR would reduce the formation of aggregates and this would result in the loss of the cross peaks in the NOESY spectrum. The ^{1}H NMR data of aggregates are different from those of monomeric species depending on the concentration and temperature that the data is acquired (Perez et al, 2013).

A suggestion was made to acquire new NOESY data and diffusion-ordered spectroscopy (DOSY) NMR data to make a comparison. DOSY separates the NMR signals of different compounds according to their diffusion coefficient. Ideally, signals arising from the same compound should appear in one consecutive row. By acquiring NOESY data from both a concentrated and dilute sample, this was meant to show the cross peak caused by pi stacking of trans (E) isomers due to the concentrated sample and its disappearance with a dilute sample. In terms of DOSY data, two rows of peaks were expected from the concentrated sample – one caused by the pi stacked aggregate and the by the free trans (E) isomer. In the dilute sample, only a row caused by the trans (E) isomer was expected.

Compound **10** was used to acquire this new data. The new DOSY data from both its concentrated and dilute sample showed the expected set of peaks.

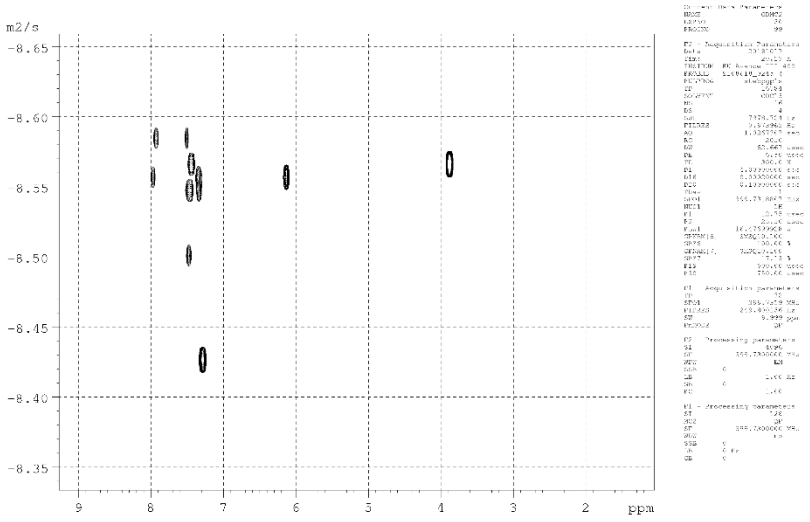


Figure 18: **DOSY spectrum of Compound 10 (concentrated in DMSO)**
Image source: author's own

The concentrated sample showed two rows of signals. The first row showed two peaks, most likely corresponding with the α-β unsaturated protons of the pi stacked aggregates of the trans (E) isomer. The second row showed a number of peaks, corresponding with the free trans (E) isomer.

The dilute sample of **10** showed a single row of peaks caused by the trans (E) isomer.

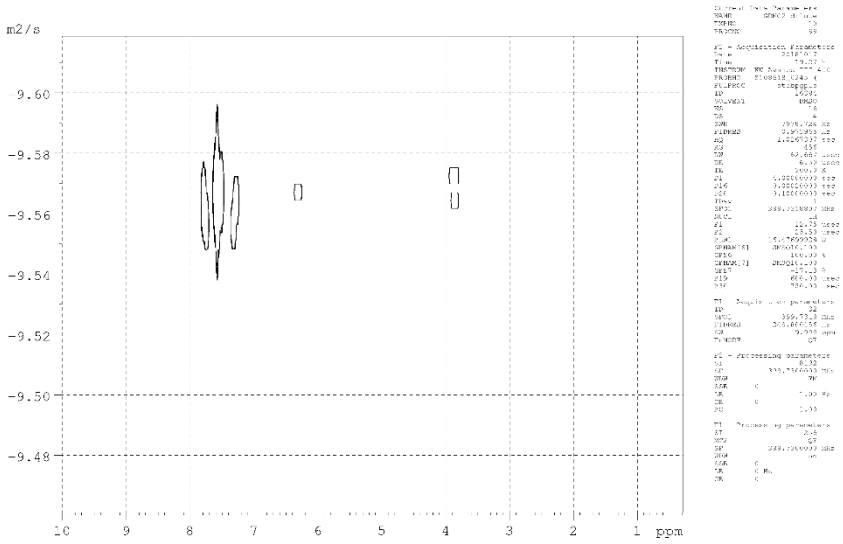


Figure 19: **DOSY spectrum of Compound 10 (dilute in DMSO)**
Image source: author's own

Also, as a standard experiment, new ^{1}H proton data was derived. There was a considerable difference between the original (concentrated) data derived in chloroform compared to the new (dilute) data derived in DMSO. The peaks of the dilute sample had shifted down the ppm scale, giving the peaks new chemical shift values (see appendix 1.1.18). The shift was considerably large, too large to be caused solely by solvent alterations. This was thought to support the hypothesis that the aromatic stacking of the trans (E) aggregates and free trans (E) conformations was at play.

However, the large coupling constants of the α-β unsaturated protons highly suggest the presence of the exclusively trans (E) isomer. Further reading suggested that the cross peaks seen on the NOESY spectra could be caused by the strong COSY correlation between the protons, resulting in the false NOESY peak (Claridge, 2009). In a third attempt to confirm whether this cross peak was a result of pi stacking or a strong COSY correlation, new NOESY and DOSY data was obtained (concentrated and dilute samples in both deuterated chloroform and DMSO) and it was clear that the cross peak was false. The new data was obtained in both chloroform and DMSO to negate the differing effects across the solvents. It was also taken at 600MHz for increased sensitivity. From the DOSY data (see figures 20 and

21), it is clear that a single compound is present under both the concentrated and the dilute sample conditions.

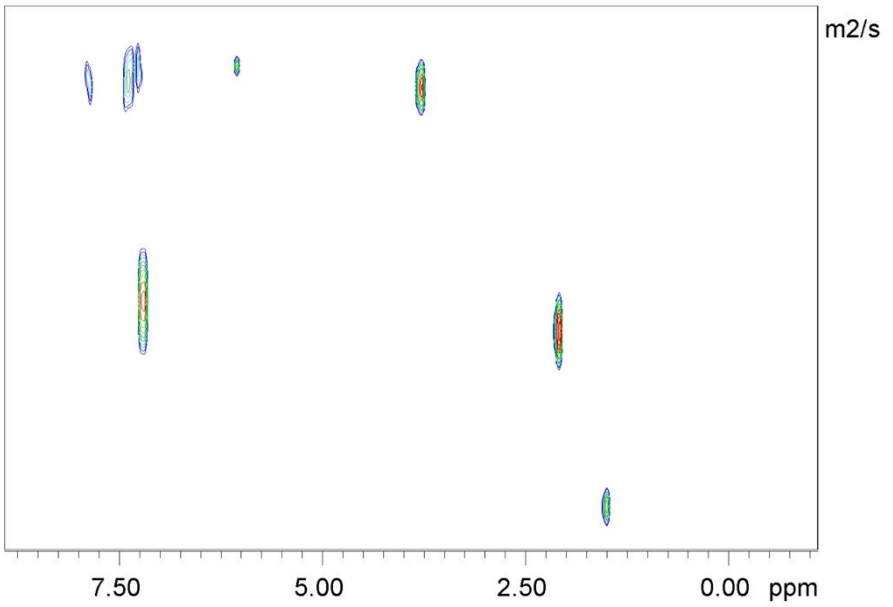


Figure 20: **DOSY spectrum of Compound 10 (dilute in chloroform) – 600MHz**
Image source: author's own

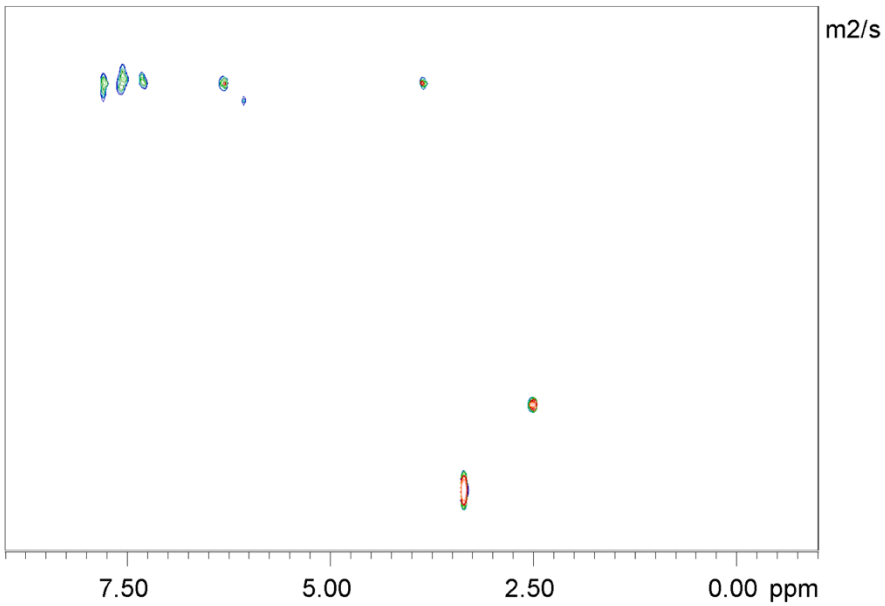


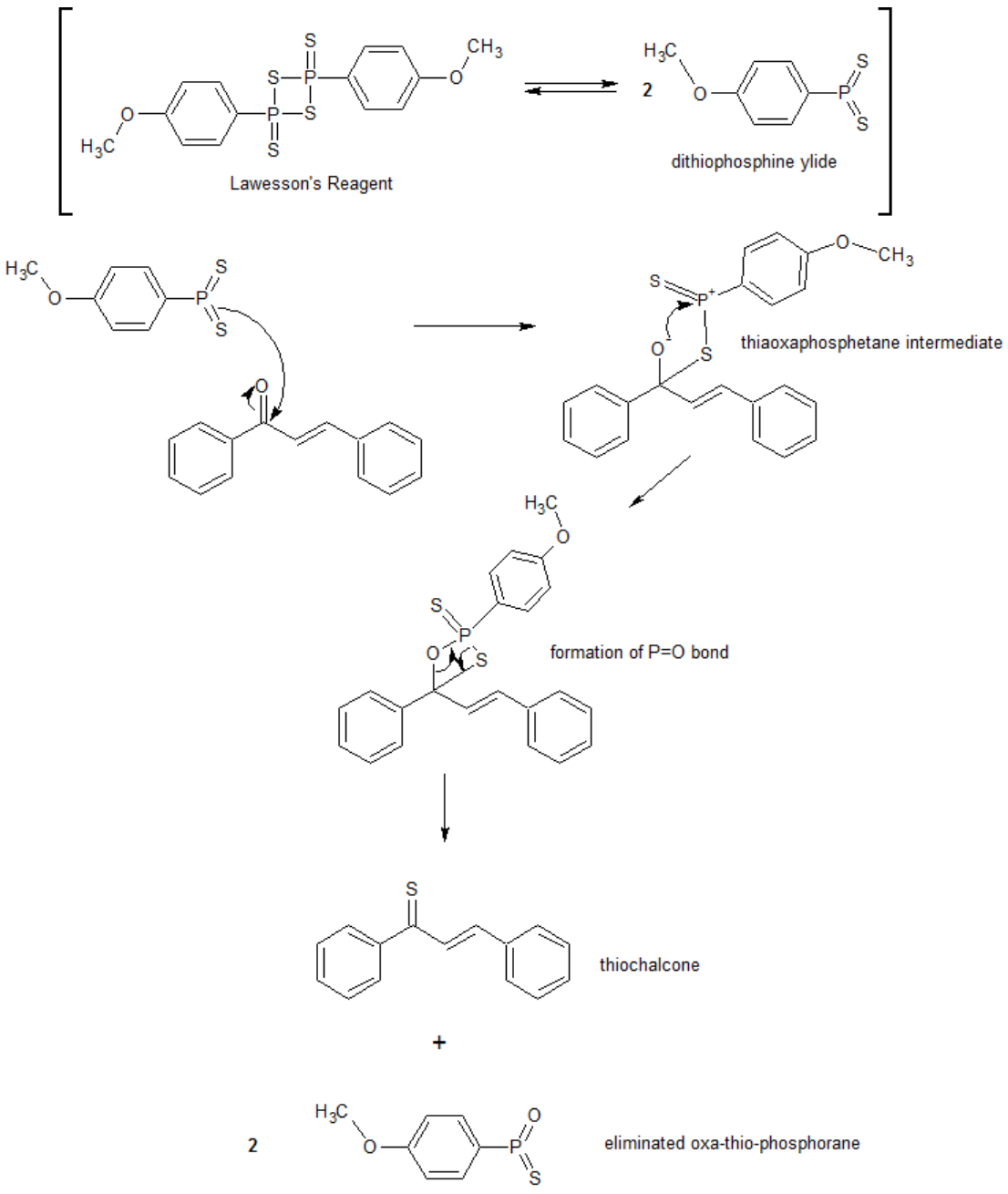
Figure 21: **DOSY spectrum of Compound 10 (dilute in DMSO) – 600MHz**
Image source: author's own

In a former project, previous attempts to synthesise chalcones using the Claisen-Schmidt condensation method using similar reagents produced a two-step synthesis: the reaction of the benzaldehyde and acetophenone to give an aldol which was further dehydrated to form the required chalcone. It was suggested that using a stronger base such as potassium or lithium hydroxide to catalyse the benzaldehyde and acetophenone reaction would have avoided the synthesis of the aldol. During this project, sodium hydroxide was the base used and it was not changed as it facilitated the reactions without forming an aldol; it was not detected during analysis. The expected colour changes (clear-yellow) were seen in all the chalcone reactions. If this was not seen after the addition of the sodium hydroxide solution, this would have given a reason to either make use of a stronger base or allow for longer reaction times.

A typical Claisen-Schmidt condensation is carried out at room temperature but the reactions in this project were carried out at 50-65°C, maximising the chance for the thermodynamic product to be made in situ. The synthesis of exclusively cis-chalcones is underreported in literature but they are also the thermodynamically less stable isomer and so tend to decompose before their presence can be denoted (Zhuang et al, 2017). There has been a report of isomerisation of the trans (E) chalcone to the cis (Z) chalcone by exposing the methanolic solution of the chalcone to visible white light (Iwata et al, 1997). The chances of this photo-isomerisation occurring to the synthesised chalcones was small as they were kept in a dark place until required.

4.2 Thiochalcones

A mechanism of the conversion of chalcones to thiochalcones is as follows: Lawesson's reagent in solution exists in equilibrium as two molecules of dithiophosphine ylide. A single molecule of the ylide attacks the carbon of the chalcone's carbonyl bond (C=O) via the P=S bond, producing a thiaoxaphosphetane intermediate. This intermediate undergoes an intramolecular attack forming the P-O bond, which is the driving force of this reaction (similar to the Witting Reaction). This produces the thiochalcone and two molecules of oxa-thio-phosphorane.



Scheme 6: **Mechanism for Conversion of Chalcones to Thiochalcones Using Lawesson's Reagent**
Image source: author's own

As stated in the literature review (1.1.3), the thionation reaction can produce a mixture of products. Krstić et al utilised Lawessons's Reagent to convert α-β unsaturated steroidal ketones to their thio- equivalents. They noticed that the type of products and their ratio was dependant on reaction conditions. When using anhydrous toluene for 8 hours at refluxing temperature, the reaction produced a mixture of the steroidal ketones' corresponding dimer-sulfides and the starting

ketone. The desired product was not seen after the 8-hour reaction but was isolated when the reaction time was 25-45 minutes (Krstić et al, 2010). Even though reaction times did not reach 8 hours (excluding compound **14** that had a reaction time of 12 hours), they did exceed 25-45 minutes, so it is assumed this was the case with compounds **11, 13** and **14.** Upon analysing their analytical data, it is clear that the eliminated oxa-thio-phosphorane is the abundant product (see table 10). Compound **12** is the exception as a mixture of the thiochalcone, starting chalcone and oxa-thio-phosphorane compound is present upon analysis of ^{1}H, ^{13}C and ^{31}P NMR data (see tables 7, 8 and 9)**.**

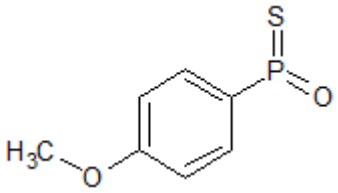


Figure 22: **oxa-thio-phosphorane**
Image source: author's own

The ^{1}H and ^{13}C NMR of **11**, **13** and **14** (see appendices 1.11, 1.12 and 1.13) resembles that of the predicted NMR of the oxa-thio-phosphorane compound (see appendices 1.1.16 and 1.1.17). The spectra share the following factors in common: the presence of a doublet of doublets at both δ7.6 - δ7.8 and δ7 - δ6.7 as well as a singlet at δ3.8 corresponding with the methoxy group on the ring. The protons are also coupled on their respective ^{1}H-^{1}H COSY spectra. The C=S bond that should appear around 200ppm, is missing on the ^{13}C NMR of all the compounds as well as the C=O bond that should appear around 180ppm, but a number of aromatic carbons appear in the region of 110-130ppm. The α-β unsaturated carbon peaks, that should appear around 130ppm and 110ppm respectively, are also not seen on ^{13}C data. It should also be noted that a peak seen at 193.2ppm on the ^{13}C of **12** could correspond with the C=O bond whilst a peak at 200.8ppm could be the C=S peak (see appendix 1.12.7). No I.R data was obtained as it was clear from the NMR data that the expected products were not formed.

The GCMS data of some of the thionated compounds shows that their most abundant peak corresponds to the M+ peak of their corresponding starting chalcones (see table 6). Compound **12**'s M+ peak is the same M+ peak as its starting chalcone, compound **3** (*m/z* – 306) as is the case with compound **14**'s M+ peak and compound **9** (*m/z* – 303). This evidence suggests that the thiochalcones

were made but possibly decomposed, as its monomeric species is unstable (Krstić et al, 2010).

Another method of ascertaining the presence of thionated products is a colour change. A purple colour is associated with the presence of a thionated product and this was reflected in the synthesis of compound **14**, which retained this colour after the completion of the reaction whilst the remaining compounds had a colour change of bright orange to yellow. Compound **11** maintained a bright orange colour.

^{15}P NMR was conducted to ascertain the presence of phosphorous in all the thiochalcone samples. The peaks seen on their respective spectra (see appendix 1.11.8 for ^{15}P spectrum of compound **11**) also support the presence of oxa-thio-phosphorane.

4.3 Effect of Substituents Positions on Aromatic Rings on the Reaction

Aldehydes bearing electron-withdrawing groups favour base-catalysed condensations whilst aldehydes bearing electron-donating groups favour acid-catalysed condensations (Zhuang et al, 2017). The below average yields of the compounds could be explained by this fact. 2, 4, 6-trimethoxybenzaldehyde was used to synthesise compounds **2**, **3** and **10**. The methoxy group is electron-donating. The use of sodium hydroxide to catalyse its reaction suggests the aldehyde combining with the acetophenone was the rate-determining step of this reaction, resulting in the average yields of 52.37%, 30.72% and 55.65% respectively.

Halogen groups are strongly electronegative and withdraw electron density from the ring via induction. They are ortho/para-directors when it comes to electrophilic substitution, due to the ability of the lone pairs to stabilise the carbocation formed during the substitution process when in the ortho and para positions. The overriding effect on the aromatic ring in the chalcones studied here, would be one of electron-withdrawing induction as we are not looking at electrophilic substitution (see figure 23).

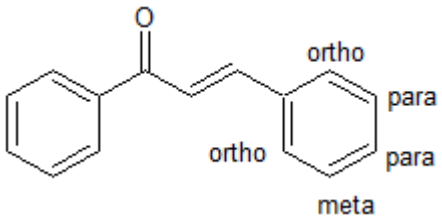


Figure 23: **Non-hydrogen positioning on aromatic ring relative to ketone moiety**
Image source: author's own

4-chlorobenzaldehyde, 4-fluorobenzaldhyde and 4-bromobenzaldehyde were used in the synthesis of compounds **1**, **5**, **6, 7, 8** and **9** that produced yields of 100.7%, 40.28%, 51.97%, 64.92%, 89.51% and 93.32% respectively. These compounds produced a mixture of good and average yields.

The electron-withdrawing effects of the halogens in the para position means the reactions would have produced good yields under basic conditions. The below-average yields of compound **6** could be due to an inadequate reaction time whilst the higher yields of compounds **1** and **9** could mean the products were a mixture of starting material and desired product.

Compound **4** was synthesised using sodium hydroxide, but the precipitate did not form until the use of concentrated sulphuric acid followed by a liquid-liquid extraction. Its uncharacteristically high yield of 138% is most likely due to the presence of water. Further drying would be needed for an accurate yield.

4.4 Antimicrobial Activity

Out of all the synthesised products, only two supposed thiochalcones displayed any antimicrobial activity. The remaining compounds, chalcones especially, did not display any activity. The reasons for this could be due to a number of factors but it is most likely the substituents on the A and B rings.

A free hydroxyl group on the B ring of a chalcone is necessary for biological activity, regardless of the substituents on the A ring (Ávila et al, 2008). Compound **4** bears a free hydroxyl group on the B ring, but no antimicrobial activity was noticed when the compound was tested. A hydroxyl group in the meta position has been noted to bring about a diminished level of antimicrobial activity previously (Silva et al, 2013) but compound **4**'s hydroxyl group was in the para position.

More than 2 methoxy groups on the B ring have been seen to eradicate any biological activity of a chalcone regardless of the substituents on the A ring (Baba et al, 2013) as is reflected in the results of compounds **2** and **10**, which are structural isomers. Compound **3** has a methoxy group in the para position. Such a group in the meta position proved useful in the Ávila et al study but the success of their compounds was attributed to hydrophobic groups such as prenyl or geranyl groups on the A ring (Ávila et al, 2008).

The lack of activity from the synthesised halogen-bearing chalcones was unexpected. Halogens such as chlorine, bromine and fluorine are universally known to increase the biological activity of chalcones. The most prominent presumption to explain this feat is the negation of the halogens, present on both the A and B rings of the chalcone. Chalcones with halogens present on only one ring of the structure are seen to be antimicrobially active, as documented in studies conducted by Osório et al (2010), Burmaoglu et al (2017) and Karthikeyan et al (2007).

Fundamentally, there are two pathways by which antibiotics can enter a bacterial cell: via a lipid-mediated pathway for hydrophobic antibiotics or through diffusion porins for hydrophilic antibiotics (Delcour, 2009).
Bacterial membranes have a high phospholipid content which is negatively charged (Osório et al, 2010). Lipophilicity is an important factor of antimicrobial agents as they have to infiltrate the cell wall envelope of bacteria. Chlorine and bromine are rather lipophilic, but their electronegativity does not differ much compared to carbon. However, fluorine is more hydrophilic than other halogens so the presence of fluorine on the aromatic rings could have caused this lack of activity.

There is a difference between the MIC and MBC values of compound **11** and **14**. The compounds were very brightly coloured in nature, causing the determination of inhibition or otherwise difficult to distinguish. The determination of MBC requires the MIC results. When recording the MBC, the section with the smallest amount of growth prior to the section with zero growth was used to confirm the MIC. For instance, with compound **11**, the MBC was recorded as 2.5 mg/ml but there was turbidity across all the wells during the MIC experiment. The section of the petri dish prior to 2.5 mg/ml had a very small amount of growth, meaning this correlates with the MIC of compound **11**.

Existing antibiotics are split into classes: Beta-Lactams, Sulfonamides, Aminoglycosides, Tetracyclines, Chloramphenicol, Macrolides, Glycopeptides, Oxazolidinones, Ansamycins, Quinolones, Streptogramins and Lipopepties. The groups classify available antibiotics based on their target bacteria (Gram-negative or Gram-positive), mode of action (bactericidal or bacteriostatic) and chemical structure (Brunning, 2014).

Antibiotics have five basic mechanisms of action against bacteria (Rollins and Joseph, 2000):

1. Inhibition of cell wall synthesis
2. Inhibition of protein synthesis
3. Alteration of cell membranes
4. Inhibition of nucleic acid synthesis
5. Antimetabolite activity

Peptidoglycan, a component of the cell walls of both Gram-positive and Gram-negative bacteria, is made up of long, cross-linked polymers of B-N-acetyl-D-glucosamine and N-acetylmuramic acid (Volmer et al, 2008).

It is the D-alanyl-D-alanine portion of a peptide chain that is cross-linked in the presence of penicillin binding proteins (PBP). Beta-lactam and glycopeptide antibiotics target PBPs. Beta-lactam rings are thought to mimic the D-alanyl-D-alanine portion of the peptide chain. They bind strongly with PBPs, thus weakening the peptidoglycan layer, resulting in the lysis of the bacterial cell (Tipper, 1985). Glycopeptides bind to the D-alanyl-D-alanine portion of the peptide chain, which disallows PBPs from binding, leading to the inhibition of cell wall synthesis (Nagarajan, 1991).

Protein synthesis requires the action of DNA being used to synthesise mRNA via transcription. The bacterial 70S ribosome, a macromolecule required to translate mRNA to amino acids is made up of two subunits: 30S and 50S subunits. Aminoglycosides, chloramphenicol, tetracycline, oxazolidinones and macrolides work by affecting the process of translation by inhibiting certain parts of the ribosomal subunits. The inhibition of protein synthesis leads to a disruption of a

number of bacterial processes required for life, resulting in the death of the bacterial cell (Sigma-Aldrich, 2006).

Quinolones inhibit the bacterial enzyme (deoxyribose nucleic acid) DNA gyrase, which is responsible for the ATP-dependent negative super-coiling of double-stranded DNA. This is necessary to prevent the excessive positive supercoiling of the DNA strands when they separate for the commencement of replication or transcription. DNA gyrase is also made up of subunits, two A subunits and two B subunits. Quinlonines bind to the A subunits, disallowing the cutting and resealing of the DNA strands (Aldred et al, 2014).

In terms of antimetabolite activity, sulfonamides, the “cousin” of the synthesised thiochalcones, inhibit bacterial folic acid production. They inhibit dihydropteroate synthase competitively, thus blocking the enzyme’s regular substrate, *p*-aminobenzoic acid from binding. This prevents the production of folic acid in the bacterial cell. All living cells require folic acid for growth but it cannot cross bacterial cell walls via diffusion, so it is synthesised from *p*-aminobenzoic acid within the bacterial cell (Walzer et al, 1988).

The varying antibiotic mechanisms of action shed light on the importance of enzymes in their activity. No concrete link has been made to define how chalcones work as antimicrobial agents. More studies would need to be carried out to determine the mode of action of chalcones and ascertain whether enzymes are involved in this process.

At present, there are two theories that describe how enzymes bind to substrates: Lock and Key Theory and Induced Fit Theory. The conventional Lock and Key theory was introduced by Emil Fisher in 1894, postulating that a substrate was a key and the enzyme was the lock and that only the correctly shaped substrate would fit into the active site of the enzyme, thus activating it (Ophardt, 2003). The Induced Fit theory was suggested by Daniel Koshland in 1958 as experimental evidence of enzymatic activity could not be fully explained by the Lock and Key theory. Koshland suggested that it was the substrate that determined the final shape of the enzyme, meaning an enzyme’s active site is flexible in nature (Koshland, 1958). This means, a substrate would need to be recognisable to the active site in terms of functional groups, side chains or structure. This partially explains how enzymes can bind to competitive molecules that permanently block its active site.

A hypothesis developed in this study is that chalcones may follow a similar mode of action to generate antimicrobial activity. Chalcones could irreversibly block the active site of some bacterial enzymes, thus preventing its necessary action from being carried out, causing either bactericidal or bacteriostatic consequences.

Sivakumar et al synthesised a series of substituted chalcones and tested their activity against *S. aureus* and *E. coli*. The study found that the active compound, with a methoxy group in the para position on the A ring and a hydroxyl group in the ortho position on the B ring, damaged the cell walls of the bacteria, reminiscent of the mechanism of action of glycopeptide antibiotics (Sivakumar et al, 2009). This mechanism involves blocking the active site of the enzyme D-alanyl-D-alanine carboxypeptidase/transpeptidase (Goodsell, 2002). This provides evidence that the mode of action chalcones undertake to behave as antimicrobial agents could possibly involve enzymatic inhibition.

Relating back to the lipophilicity of the compounds, compounds **11** and **14** were the only biologically active compounds. To an extent, their activity could be caused by the presence of oxa-thio-phosphorane (see figure 22). If the activity shown was entirely due to this compound, activity would have been seen in compounds **12** and **13** as well. Compound **11** does bear a fluorine atom on its B ring but it showed activity against the Gram-positive *S. epidermidis*. Gram-positive bacteria have a large peptidoglycan content in their cell wall (see figure 25). It may have been easier for this compound to cross the cell wall of *S. epidermidis*, supposedly boosted by the lipophilic sulphur atom on the thione moiety. With compound **14**, it showed activity against both Gram-positive and Gram-negative bacteria: *S. epidermidis, P. aeruginosa* and *E. coli*. The compound contains nitrogen, sulphur and bromine. Nitrogen is lipophobic whilst sulphur and bromine are more lipophilic. The presence of these atoms in the compound may be the reason for the compound's dual activity, allowing for passage across both cell walls, via a lipid-mediated pathway and through porins (Delcour, 2009), increasing the likelihood of the compound infiltrating the bacteria.

The solubility of chalcones proved problematic during the bacterial screening of this project. An ideal solvent to work with in microbiology is either water or a physiological diluent as this would eliminate the possible effect of solvent interaction on any seen antimicrobial activity. DMSO was used as a compromise: it was the least bacterially disruptive solvent that the chalcones were soluble in. It was noticed

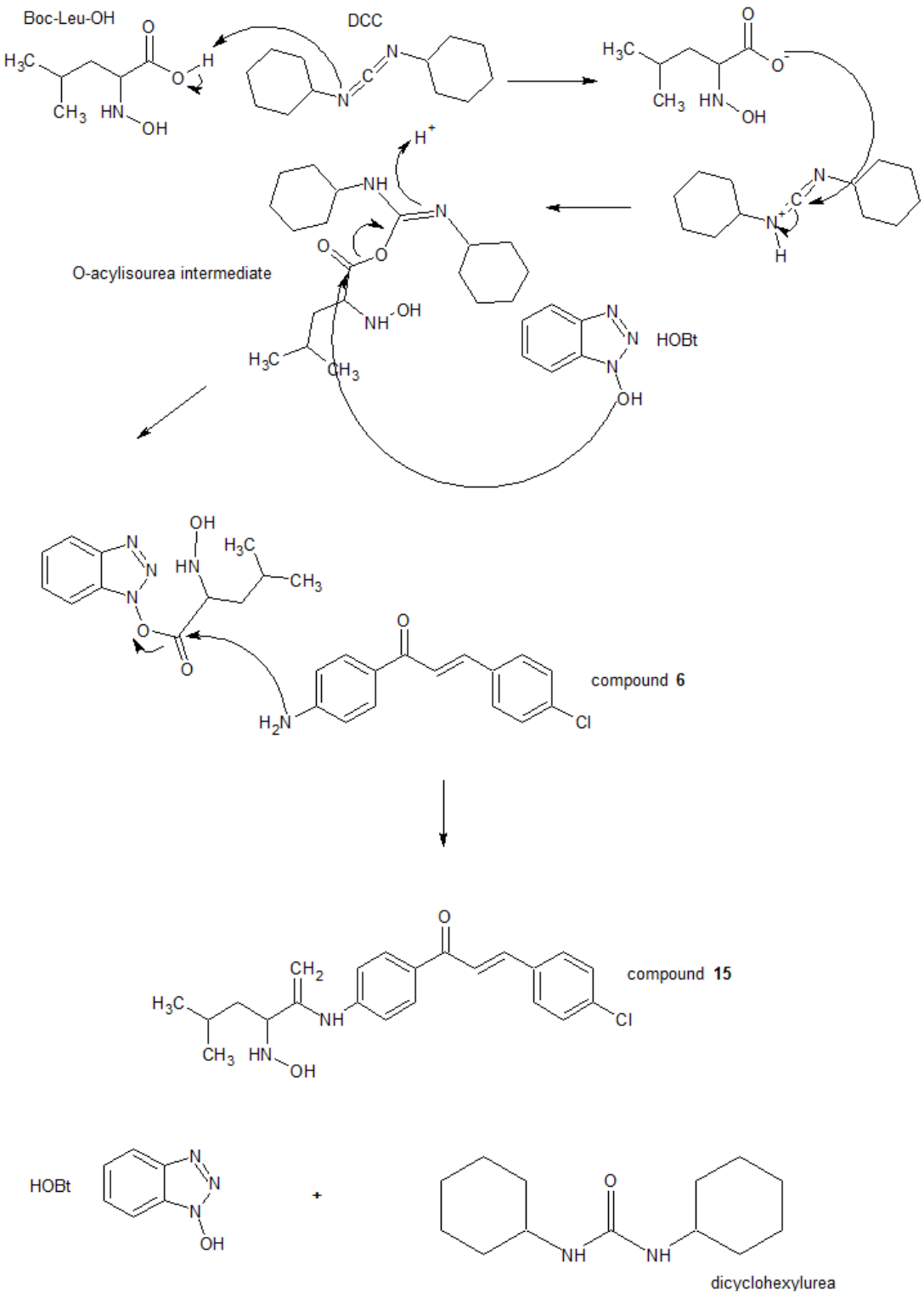
that when the chalcone solutions were introduced to the inoculated agar plates, there was a degree of precipitation of the chalcones. The most likely cause for this is the presence of water in the agar. Water is used during chalcone synthesis to initiate its precipitation. Antimicrobial susceptibility is usually tested using broth dilution, disk diffusion or gradient method (Reller et al, 2009). All these methods still utilise either agar or broth which requires water for its preparation. This antagonism between the chalcones and agar could have affected any activity or lack thereof.

The synthesis of compound **15** was attempted in a bid to increase the solubility of chalcones in water, in turn, improving its bioavailability. Peptide couplings are usually facilitated by coupling reagents such as DCC and 4-Dimethylaminopyridine (DMAP). This synthesis made use of DCC and HOBt. DCC activates the carboxyl group of the amino acid by forming an O-acylisourea intermediate that forms a complex with HOBt. This complex couples with the amino end of compound **6,** forming the desired product, the reformed catalyst HOBt and the by-product dicyclohexylurea (scheme 7). The reaction is usually carried out at low temperatures, facilitated by a mixture of dry ice and ether, to minimise the formation of this by-product. Freezing it overnight prompts its precipitation which can be filtered off before the work-up stage of the reaction. The strong smell of urea was used as an indicator of its presence throughout the reaction.

It appears the Boc protecting group had been removed during the synthesis of compound **15** as the expected peak around δ3.5 on the ^{1}H spectra correlating with the group's methyl substituents is missing. Usually, this removal is facilitated by the use of a strong acid such as trifluoroacetic acid. The workup section of the synthesis utilised 4% hydrochloric acid which would seem too weak to remove the protecting group but there was no other acid use in the synthesis.

Compound **6** was chosen because of the presence of chlorine, with hopes of this compound being soluble in water but also antimicrobially potent due to the halogen. The ^{1}H NMR of **15** (see table 11) showed that there was a mixture of starting chalcone (see appendix 1.15) and compound **15**. However, starting reagent is evident on the ^{1}H spectrum of **6** (see appendix 1.6) so this error was carried forward.

It was difficult to clean compound **15** during column chromatography. Using a silica column still produced a very sticky compound. Its complete synthesis became secondary in a bid to continue synthesising chalcones with an increased premise of biological activity.



Scheme 7: **DCC/HOBt amino acid-chalcone coupling mechanism**
Image source: author's own

5. Conclusion

The aim of this project was to synthesise a series of chalcones from a variety of substituted benzaldehydes and acetophenones. The prospect of a biologically active chalcone with a carbon-sulphur bond prompted the use of Lawesson's Reagent in a bid to convert these chalcones to their thio-equivalents. To an extent, this was achieved upon analysis of the obtained NMR and GCMS data. The conformation of the synthesised chalcones has been confirmed as trans (E) from derived NOESY and DOSY data.

The lack of activity from the synthesised chalcones could be caused by a multitude of reasons including the substituents made to their structures and the methodology selected to test their activity.

The activity seen by thiochalcones **11** against *S. epidermidis* and **14** against *E.coli*, *P. aeruginosa* and *S. epidermidis* could be attributed to the eliminated oxa-thio-phosphorane molecule, an inevitable by-product of the use of Lawesson's Reagent. Compound **12**, shown to have a higher ratio of the desired product did not show any activity against the bacteria used. However, the compounds could still be biologically active but towards other organisms such as viruses, protozoa and fungi (Zhuang et al, 2017).

It has been repeatedly reported that modifications made to the chalcone structure enhances its biological activity. The chalcones synthesised during this study bore a variety of substituents including methoxy, hydroxyl and halogenic groups. The positioning of these groups has been linked to the biological activity and lack thereof in chalcones, as is reflected by the results of this study.

The data from this project could contribute to the ever-expanding field of chalcone synthesis and their biological activity. It could also add to what is known of their structure-activity relationships, especially the importance of substituents and their positioning on the aromatic rings.

6. Future Works

A bid to separate and isolate the products within compounds **11-14** could be undertaken to better understand the dynamics of a Lawesson's Reagent-mediated thionation and to further understand what was displaying antimicrobial activity during this project. To expand on the biological activity of the synthesised chalcones, their cytotoxicity could be determined by running bio-assays on mammalian cells both normal and cancerous, possibly to reveal any anti-tumour behaviour.

Reports of synergy between chalcones and antibiotics have been made (Belofsky et al, 2004). The possibility of this could be determined using the synthesised compounds, testing their efficacy with a variety of antibiotics and seeing whether it is worth further investigating in the use of chalcones in medicine as synergistic agents.

Also, to understand the exact mode of action chalcones use to act as antimicrobial agents, a modelling/docking study in the active sites of key enzymes could be carried out. This would indicate whether enzymatic interaction is a possible cause for a chalcone's antimicrobial activity.

The purification of compound **15** should be undertaken. Solubility tests should also be conducted to ascertain whether the addition of an amino acid improves the solubility of a chalcone and whether it has an effect on its antimicrobial activity, if it displays any.

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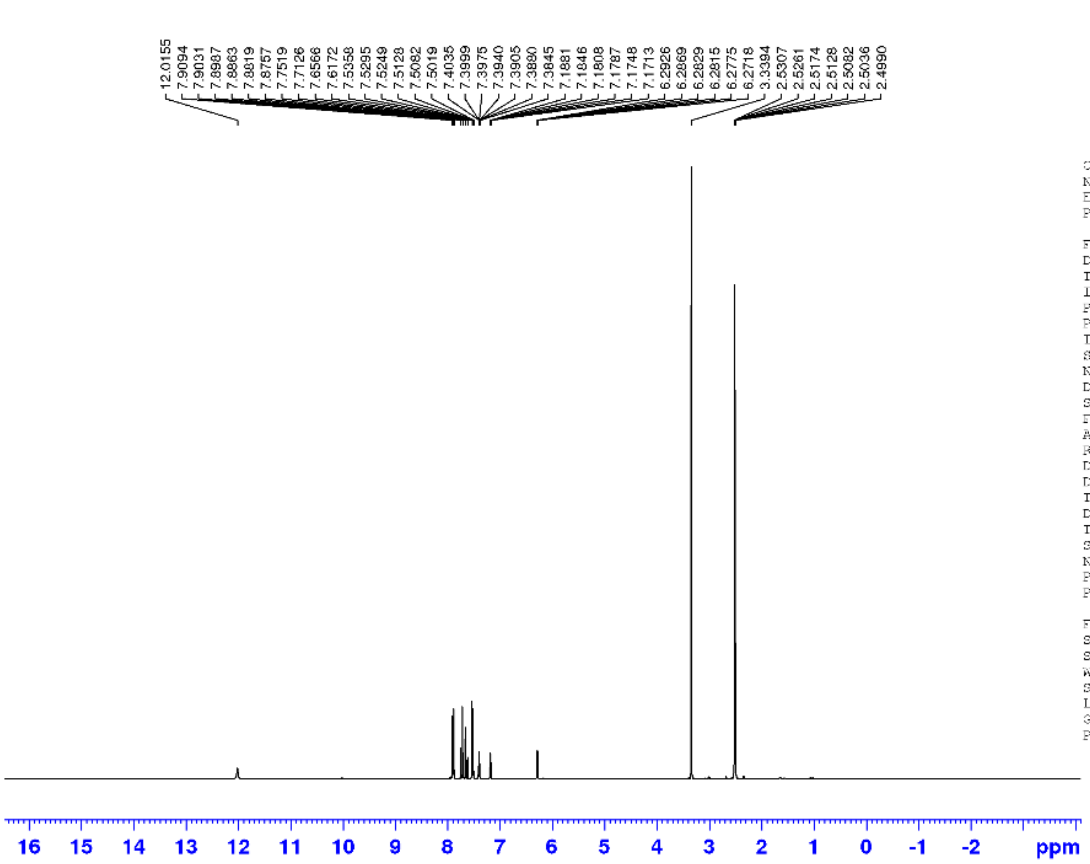
Appendix

1. Compound 1

1.1 ^{1}H NMR

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PROCNO                    1

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PROBHD    2108618_0245 (
PULPROG                zg30
TD                    65536
SOLVENT                DMSO
NS                        16
DS                         2
SWH               8223.685 Hz
FIDRES            0.250967 Hz
AQ                3.9845585 sec
RG                      228
DW                   60.800 usec
DE                   16.30 usec
TE                  296.0 K
D1              1.00000000 sec
TD0                        1
SFO1           399.7324685 MHz
NUC1                    1H
P1                   12.75 usec
PLW1           16.47899528 W

F2 - Processing parameters
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SSB       0
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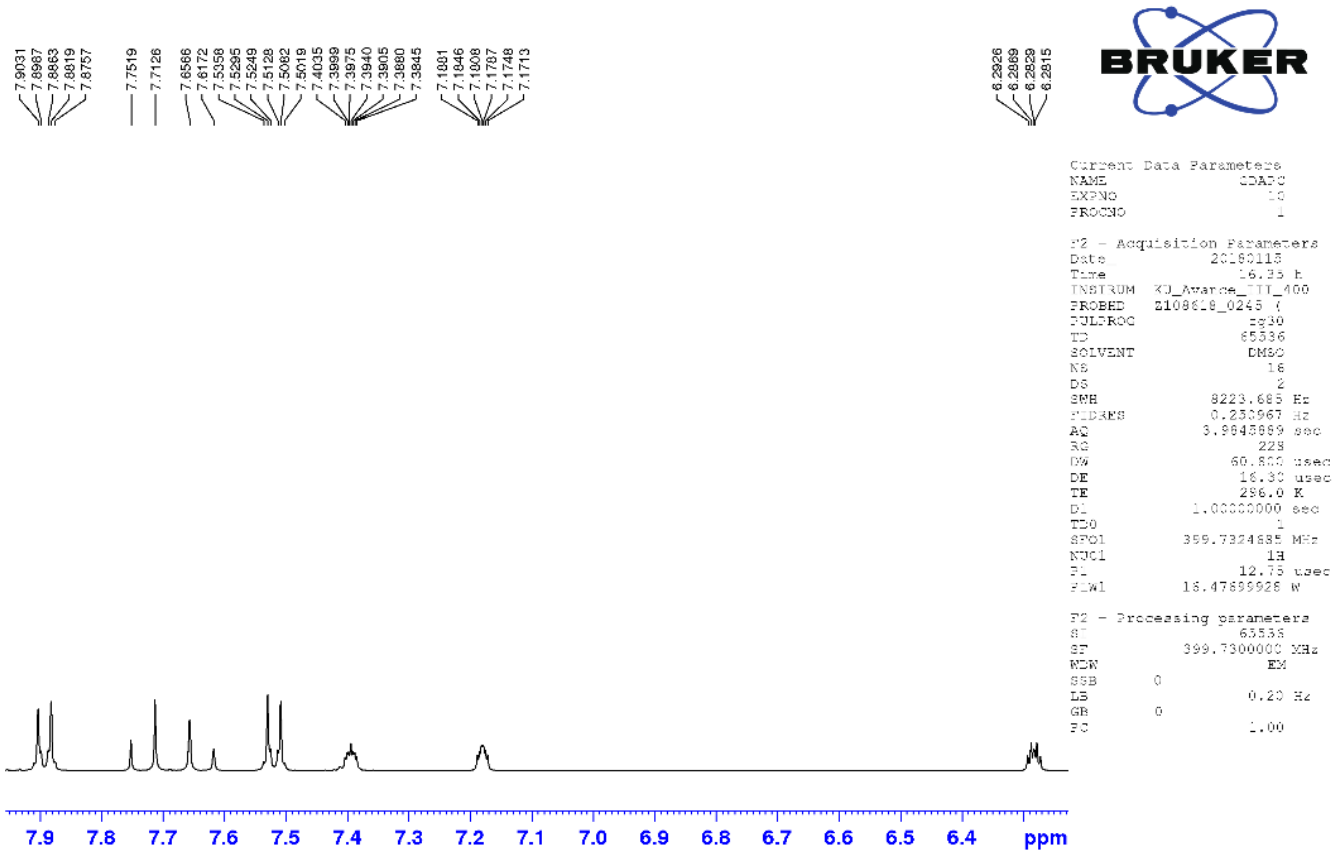
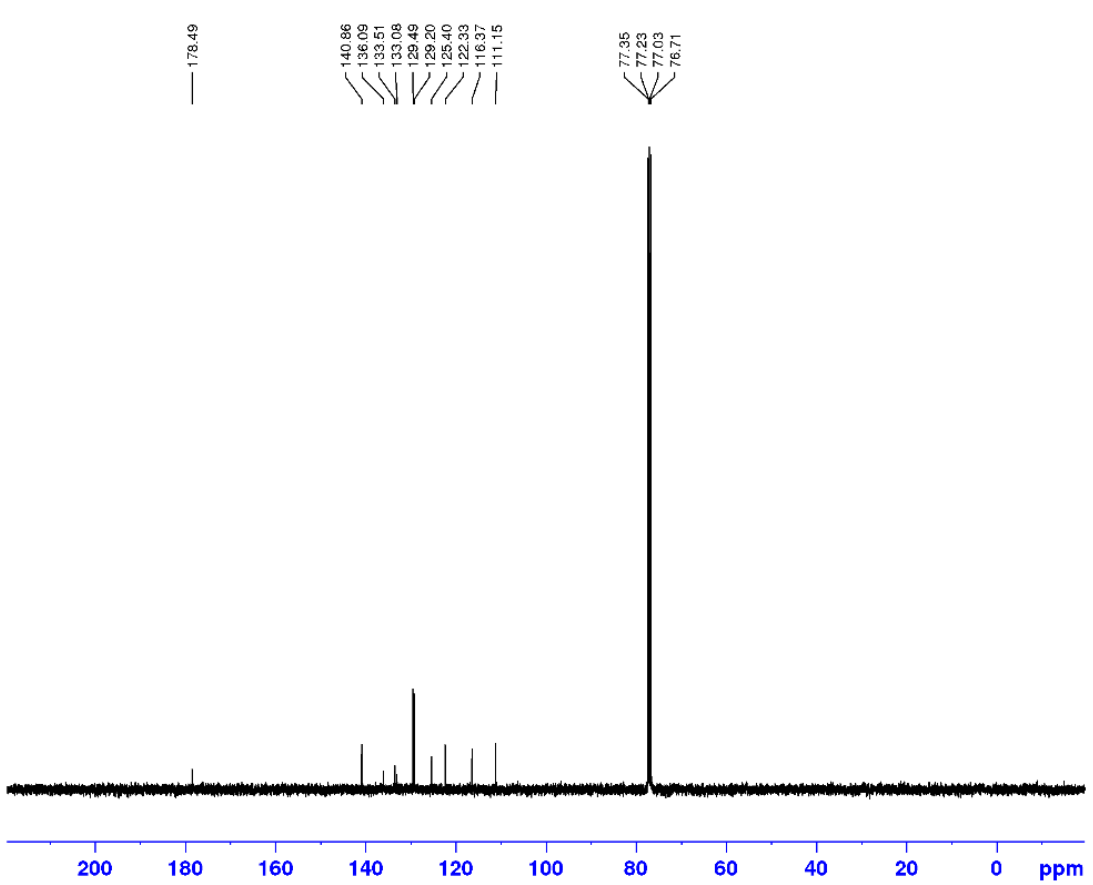
1.1.1 Expansion of aromatic region of ^{1}H



1.1.2 ^{13}C NMR

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PULPROG             zgpg30
TD                   65536
SOLVENT              CDCl3
NS                     256
DS                       4
SWH              24038.461 Hz
FIDRES           0.733596 Hz
AQ               1.3631488 sec
RG                    2050
DW                 20.800 usec
DE                 22.74 usec
TE                 296.1 K
D1               2.00000000 sec
D11              0.03000000 sec
TD0                      1
SFO1           100.5222401 MHz
NUC1                   13C
P1                    9.20 usec
PLW1           49.50000000 W
SFO2           399.7816989 MHz
NUC2                   1H
CPDPRG[2            waltz16
PCPD2                80.00 usec
PLW2           16.47699928 W
PLW12           0.35068001 W
PLW13           0.26785001 W

F2 - Processing parameters
SI                   65536
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SSB       0
LB                    1.00 Hz
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PC                    1.40
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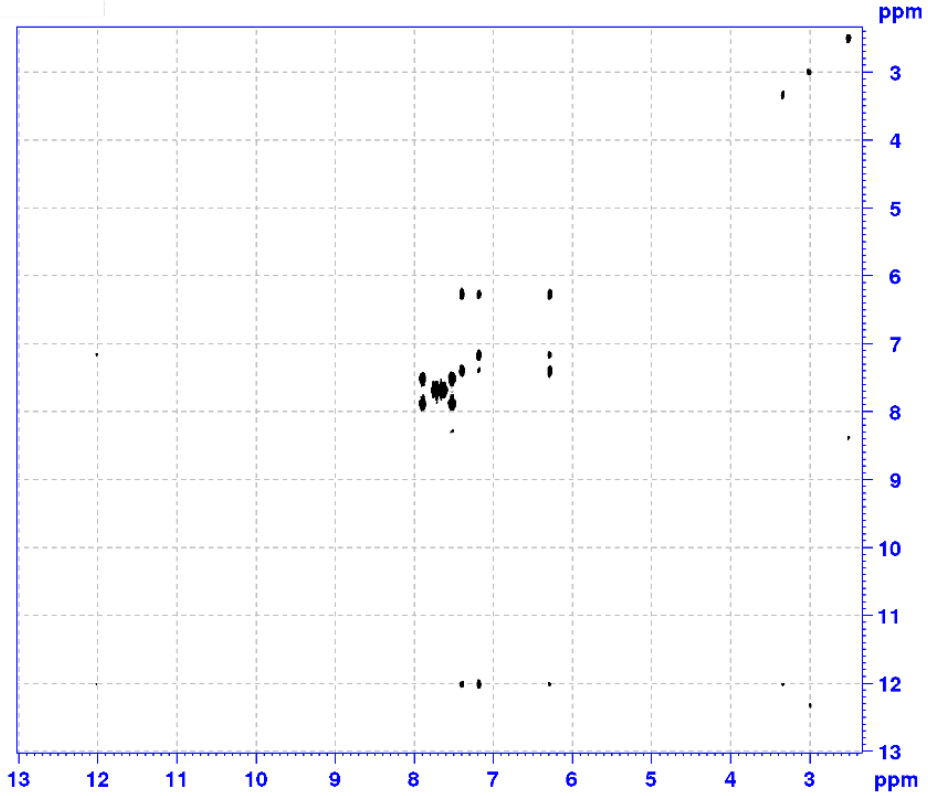
1.1.3 ^{1}H-^{1}H COSY

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PROCNO                   1

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TD                    2048
SOLVENT               DMSO
NS                       2
DS                       8
SWH              4273.504 Hz
FIDRES           4.173344 Hz
AQ               0.2396160 sec
RG                    2030
DW                 117.000 usec
DE                   6.50 usec
TE                  296.1 K
D0               0.00000300 sec
D1               1.95207733 sec
D13              0.03000400 sec
D16              0.00020000 sec
ND0              0.00003400 sec
TD0                      1
SFO1           399.7330832 MHz
NUC1                    1H
P1                   12.75 usec
PLW1           16.47699928 W
GPNAM[1]          SINE.100
GPZ1                 16.00 %
GPNAM[2]          SINE.100
GPZ2                 12.00 %
GPNAM[3]          SINE.100
GPZ3                 40.00 %
P16                1000.00 usec

F1 - Acquisition parameters
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SFO1           399.7330 MHz
FIDRES         66.773606 Hz
SW                 10.691 ppm
FnMODE                 QF

F2 - Processing parameters
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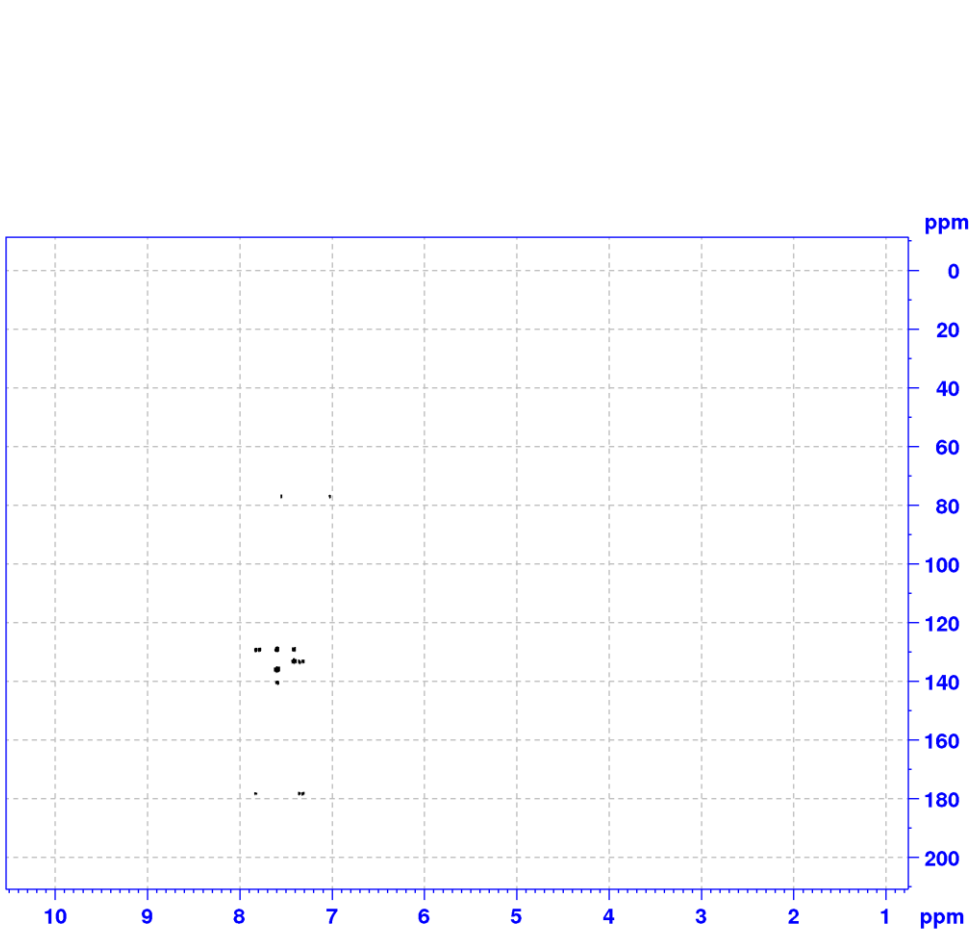
SF           399.7330000 MHz
WDW                    SINE
SSB                      0
LB                    0 Hz
GB                       0
PC                     1.40

F1 - Processing parameters
SI                    1024
MC2                    QF
SF           399.7330000 MHz
WDW                    SINE
SSB                      0
LB                    0 Hz
GB                       0
```

1.1.4 ^{1}H-^{13}C HMBC




```
Current Data Parameters
NAME                  GDAPC
EXPNO                    14
PROCNO                    1

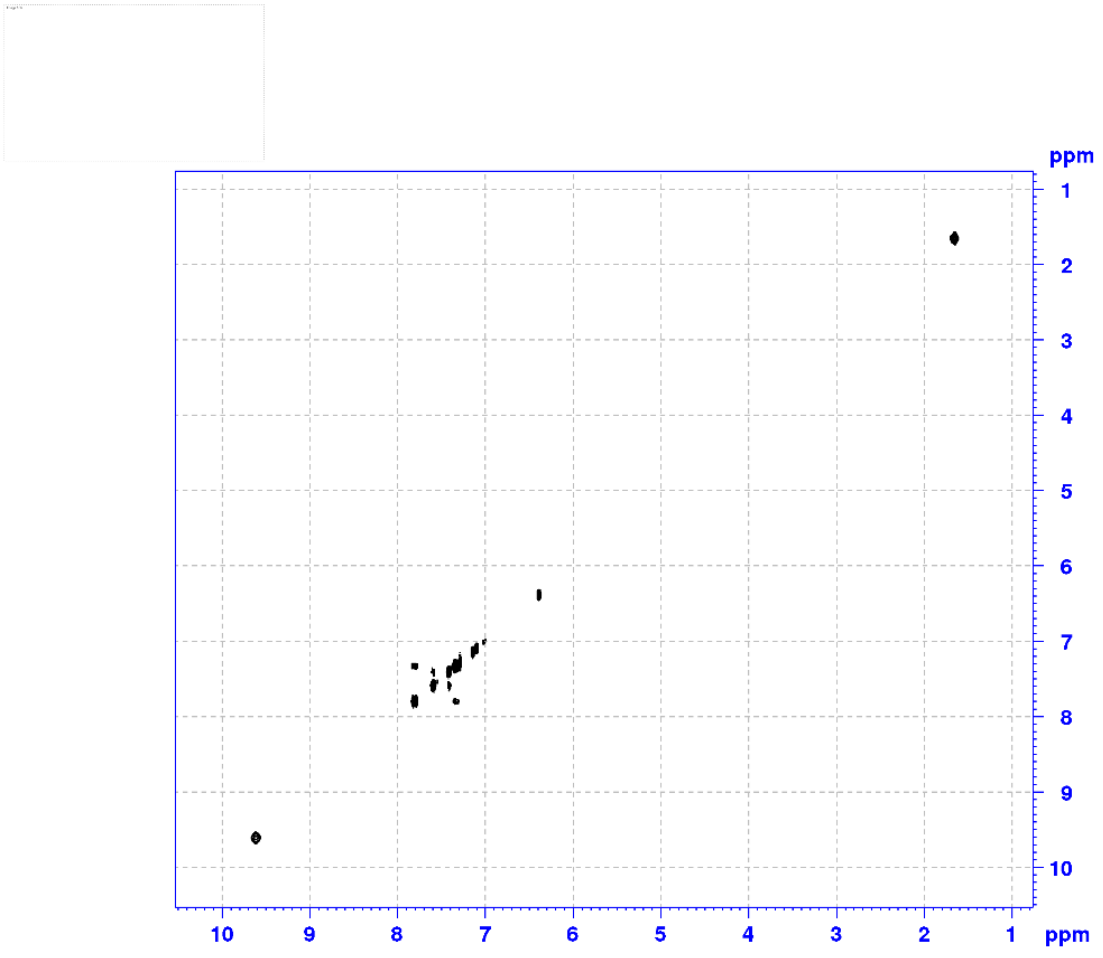
F2 - Acquisition Parameters
Date_              20180620
Time                 20.52 h
INSTRUM   KU_Avance_III_400
PROBHD    2108618_0245 (
PULPROG     hmbcetgp1hnd
TD                     4096
SOLVENT             CDCl3
NS                       16
DS                       16
SWH                3906.250 Hz
FIDRES             1.907349 Hz
AQ                 0.5242880 sec
RG                      2050
DW                  128.000 usec
DE                    6.50 usec
TE                   296.5 K
CNST6            120.0000000
CNST7            165.0000000
CNST13            10.0000000
D0                 0.00000300 sec
D1                 1.36892796 sec
D6                 0.05000000 sec
D16                0.00020000 sec
IN0                0.00002240 sec
TDav                        1
SFO1            399.7322566 MHz
NUC1                      1H
P1                    12.75 usec
P2                    25.50 usec
PLW1              16.47699928 W
SFO2              100.5222241 MHz
NUC2                      13C
P3                     9.20 usec
P24                 2000.00 usec
PLW2              49.50000000 W
SPNAM[7]     Crp60comp.4
SPOAL7                 0.500
SPOFFS7  0 Hz
SPW7               6.40140009 W
GPNAM[1]            SINE.100
GPZ1                   80.00 %
GPNAM[3]            SINE.100
GPZ3                   14.00 %
GPNAM[4]            SINE.100
GPZ4                   -8.00 %
GPNAM[5]            SINE.100
GPZ5                   -4.00 %
GPNAM[6]            SINE.100
GPZ6                   -2.00 %
P16                 1000.00 usec

F1 - Acquisition parameters
TD                      128
SFO1              100.5222 MHz
FIDRES           348.772308 Hz
SW                  222.055 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                     2048
SF              399.7300000 MHz
WDW                    SINE
SSB                       2
LB        0 Hz
GB        0
PC                     1.40

F1 - Processing parameters
SI                     1024
MC2         echo-antiecho
SF              100.5121884 MHz
WDW                    SINE
SSB                       2
LB        0 Hz
GB        0
```

1.1.5 ^{1}H-^{1}H NOESY



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```
Current Data Parameters
NAME                  CEAPC
EXPNO                    15
PROCNO                    1

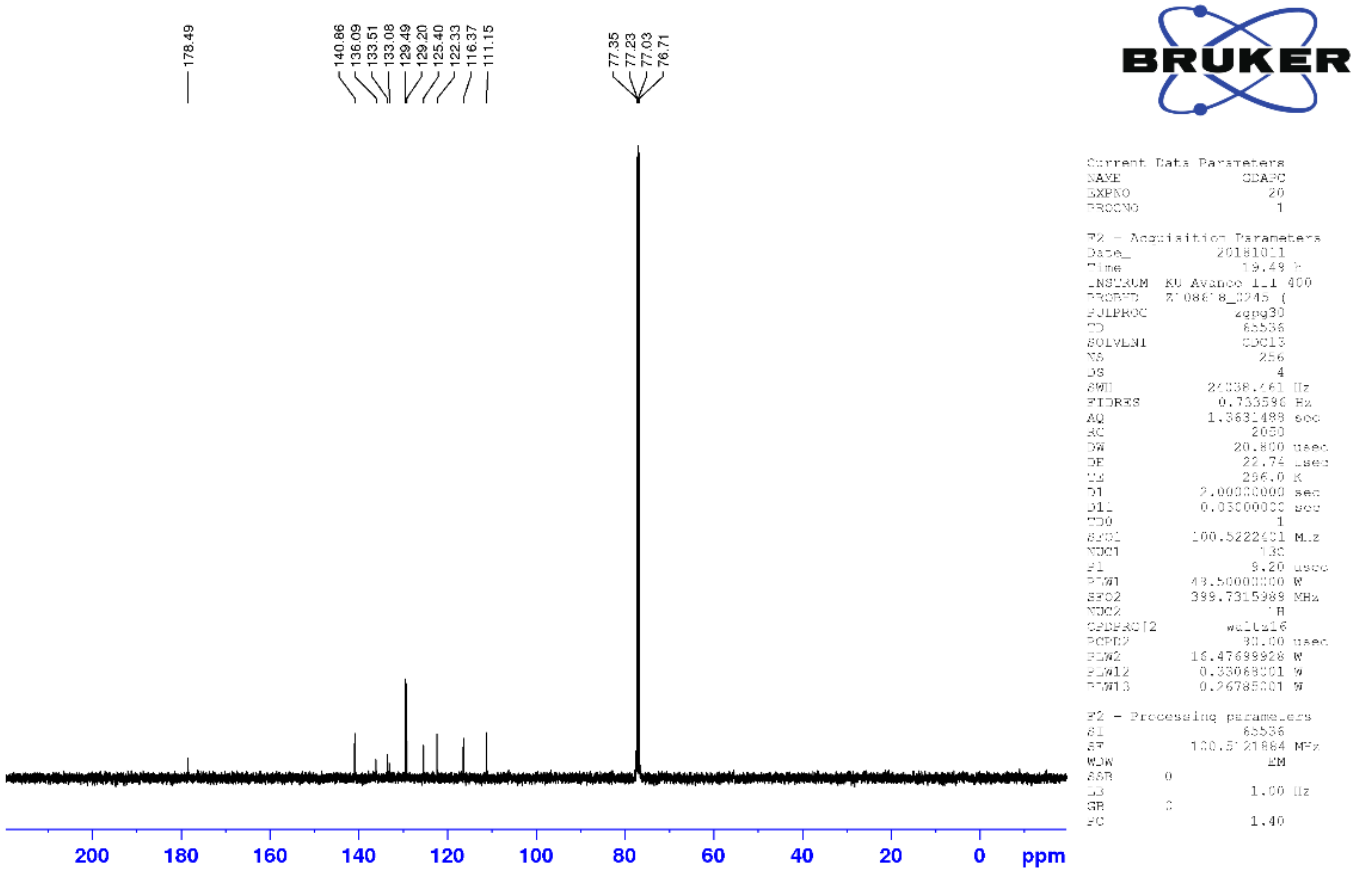
F2 - Acquisition Parameters
Date_           20_030620
Time                 22.2? h
INSTRUM   BL Avance III 400
PROBHD   5 mm BBFO z-
PULPROG         noesygpph
TD                    2048
SOLVENT               CDCl3
NS                       8
DS                       4
SWH                3906.252 Hz
FIDRES             3.814697 Hz
AQ                 0.2621440 sec
RG                     287
DW                  128.000 usec
DE                    6.09 usec
TE                  297.? K
D0                 0.00011177 sec
D1                 1.98853099 sec
D8                 0.30000001 sec
D16                0.00020000 sec
INO                0.00025600 sec
IDAV                     1
SFO1            399.7822566 MHz
NDC1                    17
P1                    12.75 usec
P2                    25.50 usec
PLW1            18.4769992 W
GPNAM[1]          SINE.100
GPZ1                  40.00 %
P16                 1000.00 usec

F1 - Acquisition parameters
TD                       256
SFO1             399.7323 MHz
FIDRES          30.517578 Hz
SW                   9.772 ppm
FnMODE          States-TPPI

F2 - Processing parameters
SI                       1024
SF             399.7300020 MHz
WDW                    QSINE
SSB                       2
LB          0 Hz
GB          0
PC                     1.00

F1 - Processing parameters
SI                       1024
MC2             States-TPPI
SF             399.7300020 MHz
WDW                    QSINE
SSB                       2
LB          0 Hz
GB          0
```

1.1.6 ^{13}C NMR



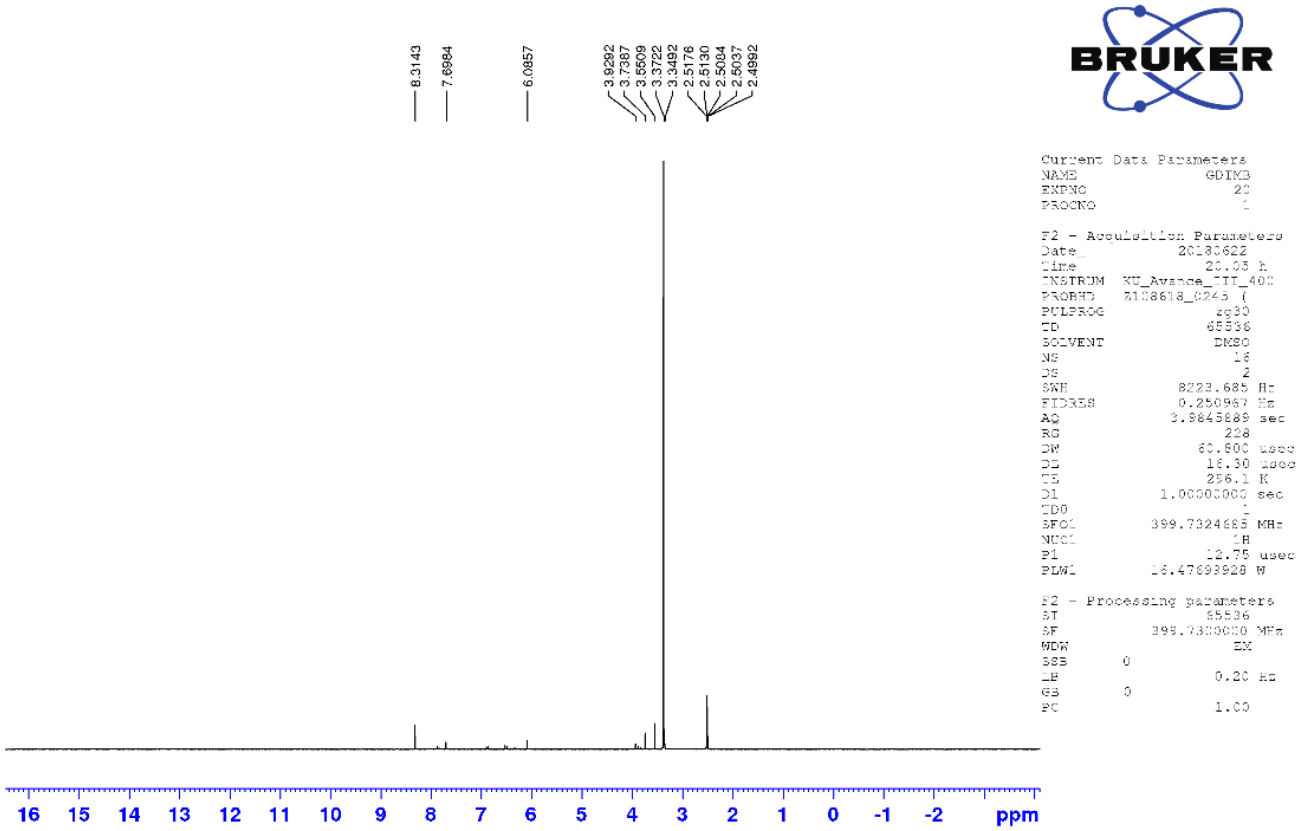

```
Current Data Parameters
NAME                  CDAPC
EXPNO                    20
PROCNO                    1

F2 - Acquisition Parameters
Date_           20181011
Time                19.49 h
INSTRUM  BV Avance III 400
PROBHD   Z108678_0245 (
PULPROG             zgpg30
TD                   65536
SOLVENT             CDCl3
NS                     256
DS                       4
SWH           24038.461 Hz
FIDRES         0.733590 Hz
AQ             1.3631488 sec
RG                    2050
DW                20.800 usec
DE                22.74 usec
TE                256.0 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                      1
SFO1         100.5222631 MHz
NUC1                   13C
P1                    9.20 usec
PLW1         48.50000000 W
SFO2         399.7315989 MHz
NUC2                   1H
CPDPRG[2           waltz16
PCPD2                 90.00 usec
PLW2         16.47699928 W
PLW12         0.33068001 W
PLW13         0.26785001 W

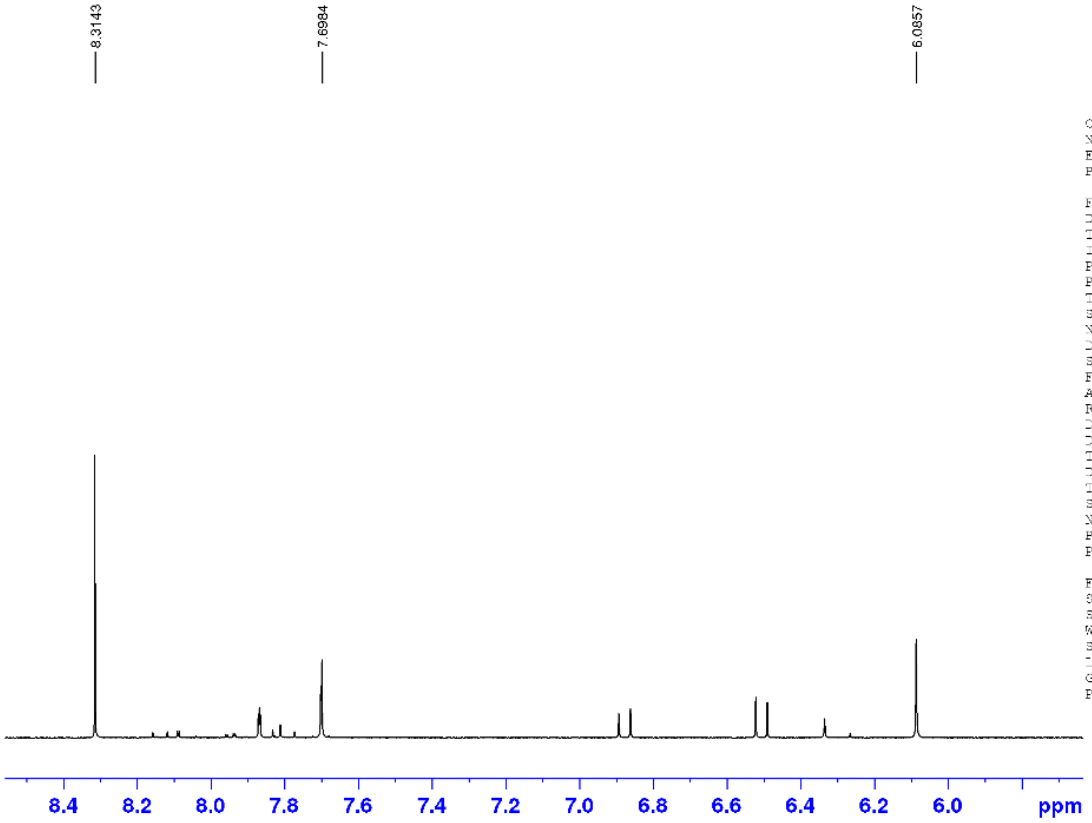
F2 - Processing parameters
SI                   65536
SF          100.5271884 MHz
WDW                    EM
SSB      0
LB                    1.00 Hz
GB       0
PC                    1.40
```

1.2 Compound 2

1.2.1 ^{1}H NMR



1.2.2 Expansion of aromatic region of ^{1}H

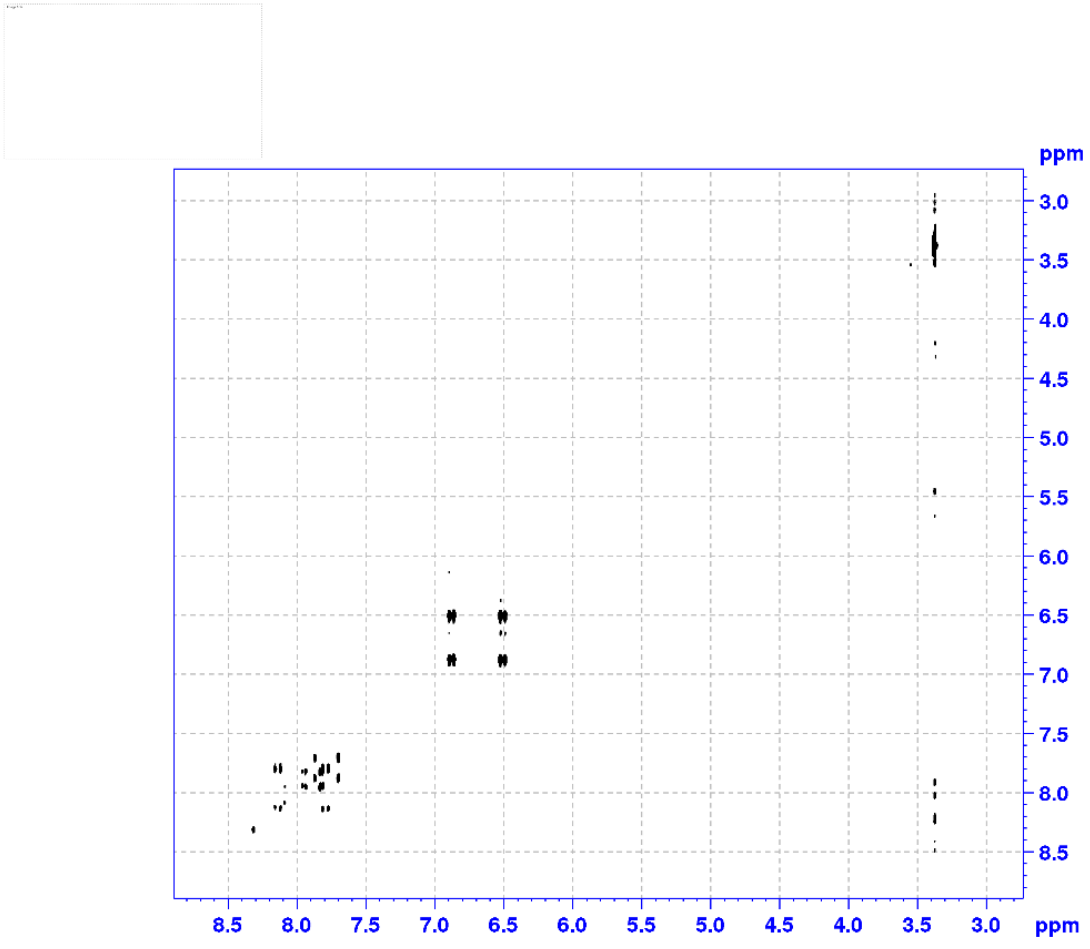



```
Current Data Parameters
NAME                  SDTME
EXPNO                    20
PROCNO                    1

F2 - Acquisition Parameters
Date_              20180622
Time                 20.08 h
INSTRUM  KU_Avance_III_400
PROBHD   Z108618_0243 (
PULPROG                zg30
TD                    65536
SOLVENT                DMSO
NS                       16
DS                        2
SWH                8223.685 Hz
FIDRES             0.230967 Hz
AQ                 3.9845889 sec
RG                       228
DW                    60.800 usec
DE                    16.30 usec
TE                    296.1 K
D1                 1.00000000 sec
TD0                       1
SFO1           399.7324685 MHz
NUC1                     1H
P1                    12.75 usec
PLW1           16.47699928 W

F2 - Processing parameters
SI                    65536
SF             399.7300000 MHz
WDW                      EM
SSB          0
LB                    0.20 Hz
GB           0
PC                    1.00
```

1.2.3 ^{1}H -^{1}H COSY



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```
Current Data Parameters
NAME                 GDIMB
EXPNO                   22
PROCNO                   1

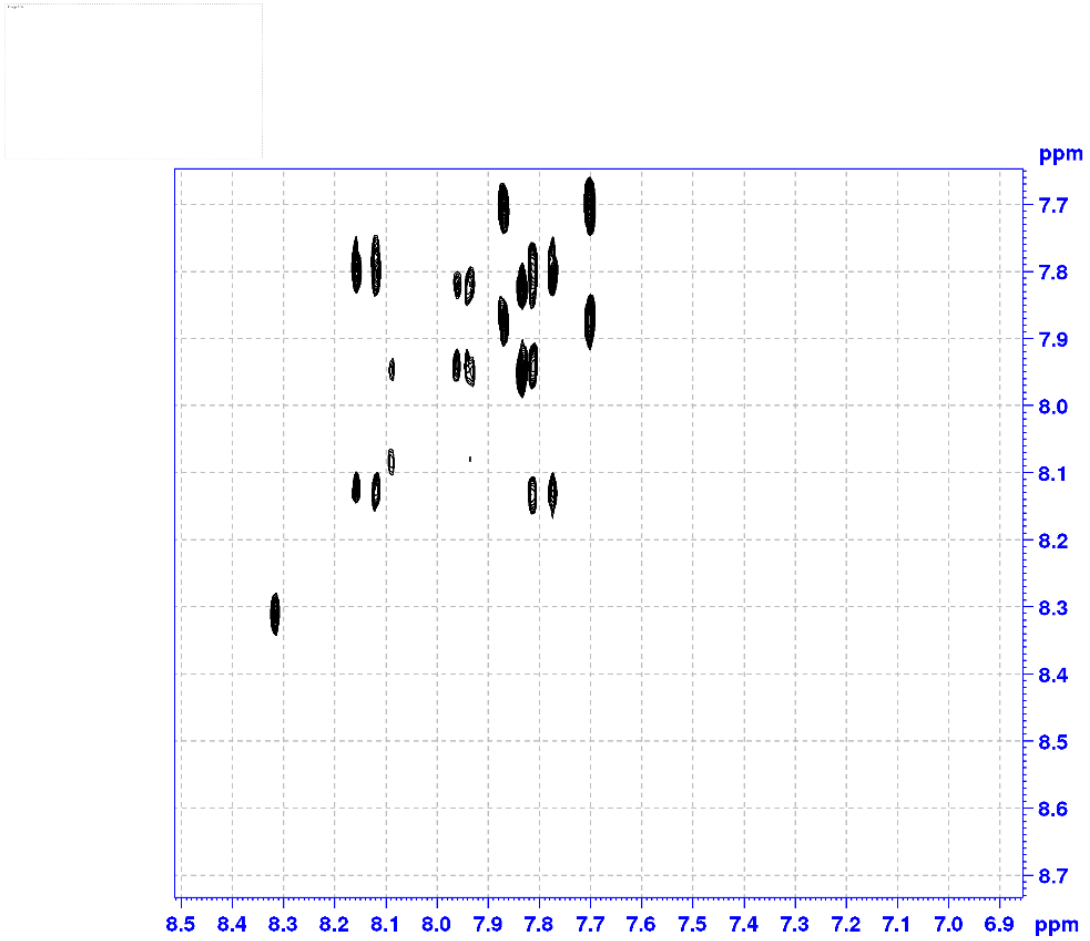
F2 - Acquisition Parameters
Date_              20180622
Time                 20.48 h
INSTRUM  XU_Avance_III_400
PROBHD   Z108618_0246 (
PULPROG       cosygpmfqf
TD                    2048
SOLVENT               DMSO
NS                       2
DS                       8
SWH                2463.054 Hz
FIDRES             2.405326 Hz
AQ                 0.4137440 sec
RG                    2030
DW                  203.000 usec
DE                    6.50 usec
TE                  295.8 K
D0                 0.00000300 sec
D1                 1.77594800 sec
D13                0.00000400 sec
D16                0.00020000 sec
IN0                0.00040800 sec
ZGAIN                    1
SFO1               399.7323231 MHz
NUC1                     1H
P1                    12.75 usec
P_W1              16.47699998 W
GPNAM[1]             SINE.100
GPZ1                    16.00 %
GPNAM[2]             SINE.100
GPZ2                    12.00 %
GPNAM[3]             SINE.100
GPZ3                    40.00 %
D16                  1000.00 usec

F1 - Acquisition parameters
TD                       128
SFO1               399.7323 MHz
FIDRES             38.485222 Hz
SW                    8.162 ppm
FnMODE                   QF

F2 - Processing parameters
SI                       1024
SF              399.7300000 MHz
WDW                     QSINE
SSB          0
LB           0 Hz
GB           0
PC                       1.40

F1 - Processing parameters
SI                       1024
MC2                        QF
SF              399.7300000 MHz
WDW                     QSINE
SSB          0
LB           0 Hz
GB           0
```

1.2.4 Expansion of COSY



BRUKER

```
Current Data Parameters
NAME                  GDYMB
EXPNO                    22
PROCNO                    1

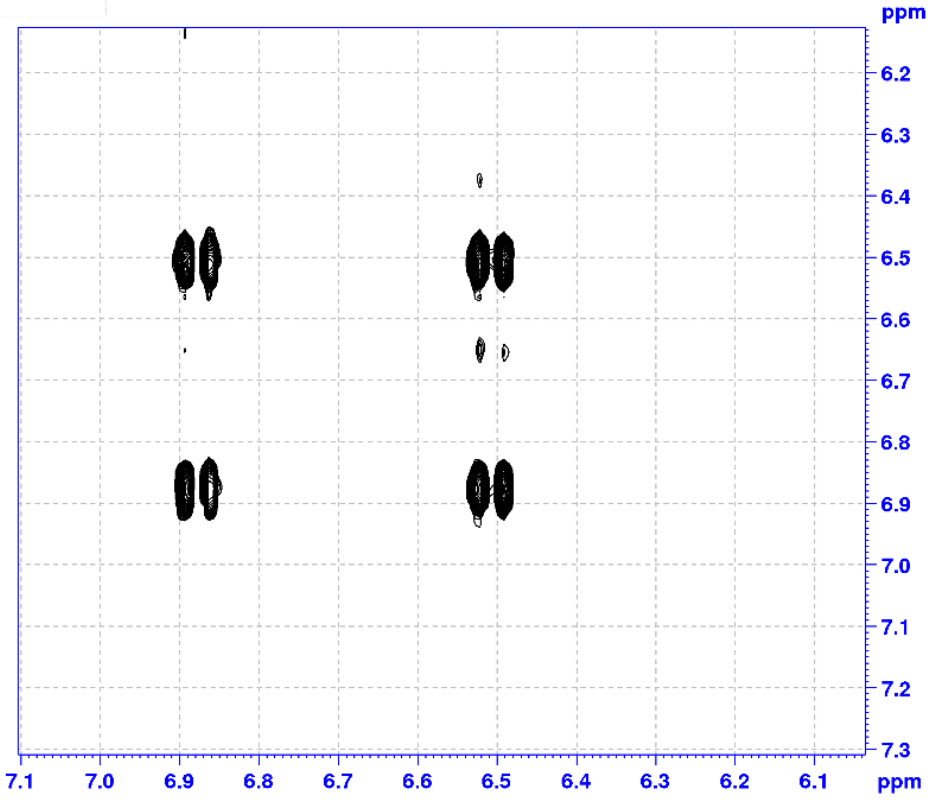
F2 - Acquisition Parameters
Date_              20180622
Time                  20.48 h
INSTRUM   XU_Avance_III_400
PROBHD    Z108618_0246 (
PULPROG       ccayqpnfqf
TD                     2048
SOLVENT                DMSO
NS                        2
DS                        8
SWH                2463.054 Hz
FIDRES             2.405326 Hz
AQ                 0.4157440 sec
RG                      203
DW                 203.000 usec
DE                   6.50 usec
TE                 295.8 K
D0                 0.00000300 sec
D1                 1.77594800 sec
D13                0.00000400 sec
D16                0.00020000 sec
ND0                0.00004800 sec
TD0                       1
SFO1               399.7323231 MHz
NUC1                      1H
P1                      12.75 usec
PLW1               16.47699928 W
GPNAM[1]             SINE.100
GPZ1                    16.00 %
GPNAM[2]             SINE.100
GPZ2                    12.00 %
GPNAM[3]             SINE.100
GPZ3                    40.00 %
P16                   1000.00 usec

F1 - Acquisition parameters
TD                       1024
SFO1               399.7323 MHz
FIDRES             38.485222 Hz
SW                     8.562 ppm
FnMODE                    QF

F2 - Processing parameters
SI                       1024
SF                 399.7330020 MHz
WDW                      EM
SSB                   0
LB                    0 Hz
GB                    0
PC                       1.40

F1 - Processing parameters
SI                       1024
MC2                      QF
SF                 399.7330020 MHz
WDW                      EM
SSB                   0
LB                    0 Hz
GB                    0
```

1.2.5 Expansion of COSY



Current Data Parameters
NAME GCOM3
EXPNO 22
PROCNO 1

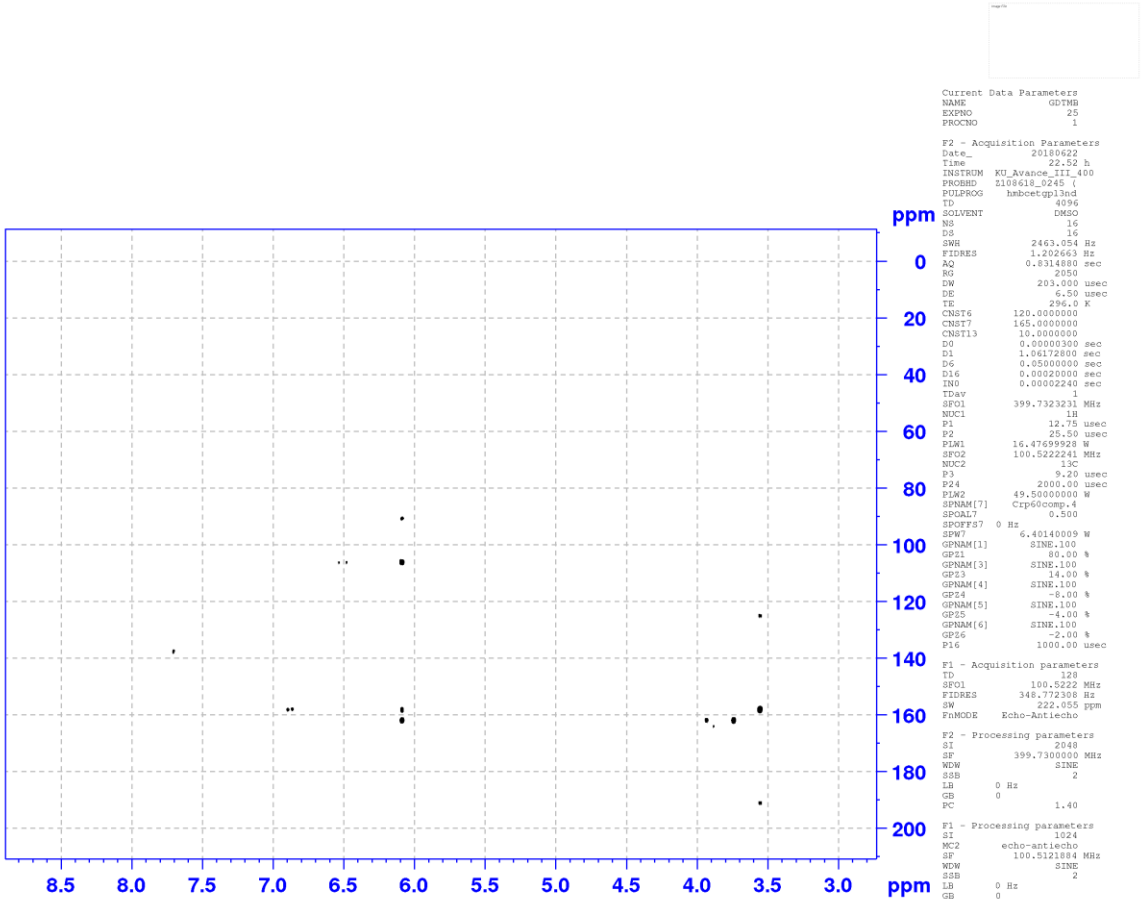
F2 - Acquisition Parameters
Date_ 20180822
Time 20.48 h
INSTRUM AC Avance III 400
PROBHD 5 mm BBFO zg
PULPROG zgcosygpmfqf
TD 2048
SOLVENT CMSO
NS 2
DS 8
SWH 2763.054 Hz
FIDRES 2.700328 Hz
AQ 0.3717470 sec
RG 2050
DW 203.000 usec
DE 6.50 usec
TE 295.8 K
D0 0.00000300 sec
D1 1.7599900 sec
D13 0.00000400 sec
D16 0.00020000 sec
IN0 0.00040600 sec
TD0 1
SFO1 399.7323231 MHz
NUC1 1H
P1 12.75 usec
PL1 18.47999928 W
SPNAM[1] Sine.100
SPOI 18.00 %
SPNAM[2] Sine.100
SPO2 12.00 %
SPNAM[3] Sine.100
SPO3 40.00 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 128
SFO1 399.7323 MHz
FIDRES 38.485222 Hz
SW 6.162 ppm
FnMODE QF

F2 - Processing parameters
SI 1024
SF 399.7300000 MHz
WDW SINE
SSB 0
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 1024
MC2 QF
SF 399.7300000 MHz
WDW SINE
SSB 0
LB 0 Hz
GB 0

1.2.6 ^{1}H-^{13}C HMBC




```
Current Data Parameters
NAME                  GDTMB
EXPNO                    25
PROCNO                    1

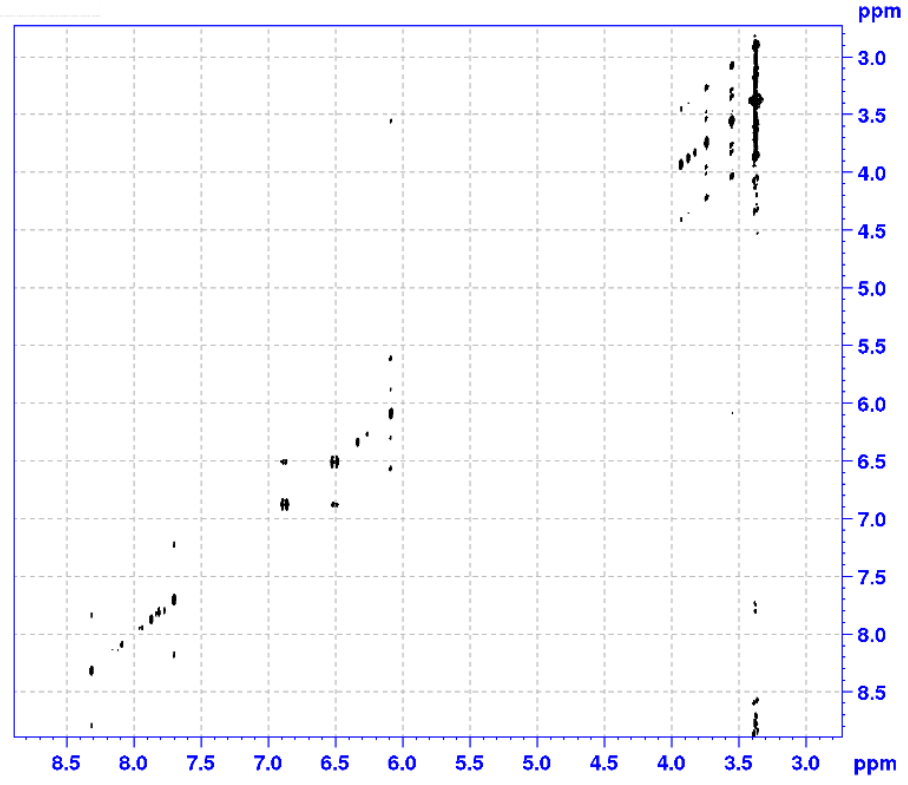
F2 - Acquisition Parameters
Date_              20180622
Time                  22.52 h
INSTRUM  KU_Avance_III_400
PROBHD   Z108618_0245 (
PULPROG    hmbcetgpl3nd
TD                     4096
SOLVENT                DMSO
NS                       16
DS                       16
SWH                2463.054 Hz
FIDRES             1.202663 Hz
AQ                 0.8314880 sec
RG                      2050
DW                  203.000 usec
DE                    6.50 usec
TE                   296.0 K
CNST6            120.0000000
CNST7            165.0000000
CNST13            10.0000000
D0                 0.0000300 sec
D1                 1.06172800 sec
D6                 0.05000000 sec
D16                0.00020000 sec
IN0                0.00002240 sec
TDav                      1
SFO1             399.7323231 MHz
NUC1                     1H
P1                    12.75 usec
P2                    25.50 usec
PLW1             16.47699928 W
SFO2             100.5222241 MHz
NUC2                     13C
P3                     9.20 usec
P24                 2000.00 usec
PLW2             49.50000000 W
SPNAM[7]     Crp60comp.4
SPOAL7                0.500
SPOFFS7  0 Hz
SPW7              6.40140009 W
GPNAM[1]          SINE.100
GP21                 80.00 %
GPNAM[3]          SINE.100
GP23                 14.00 %
GPNAM[4]          SINE.100
GP24                 -8.00 %
GPNAM[5]          SINE.100
GP25                 -4.00 %
GPNAM[6]          SINE.100
GP26                 -2.00 %
P16                1000.00 usec

F1 - Acquisition parameters
TD                       128
SFO1              100.5222 MHz
FIDRES         348.772308 Hz
SW                 222.055 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                       2048
SF             399.7300000 MHz
WDW                      SINE
SSB                         2
LB        0 Hz
GB        0
PC                      1.40

F1 - Processing parameters
SI                       1024
MC2         echo-antiecho
SF              100.5121884 MHz
WDW                      SINE
SSB                         2
LB        0 Hz
GB        0
```

1.2.7 ^{1}H-^{1}H NOESY



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```
Current Data Parameters
NAME                  GUIMB
EXPNO                     24
PROCNO                     1

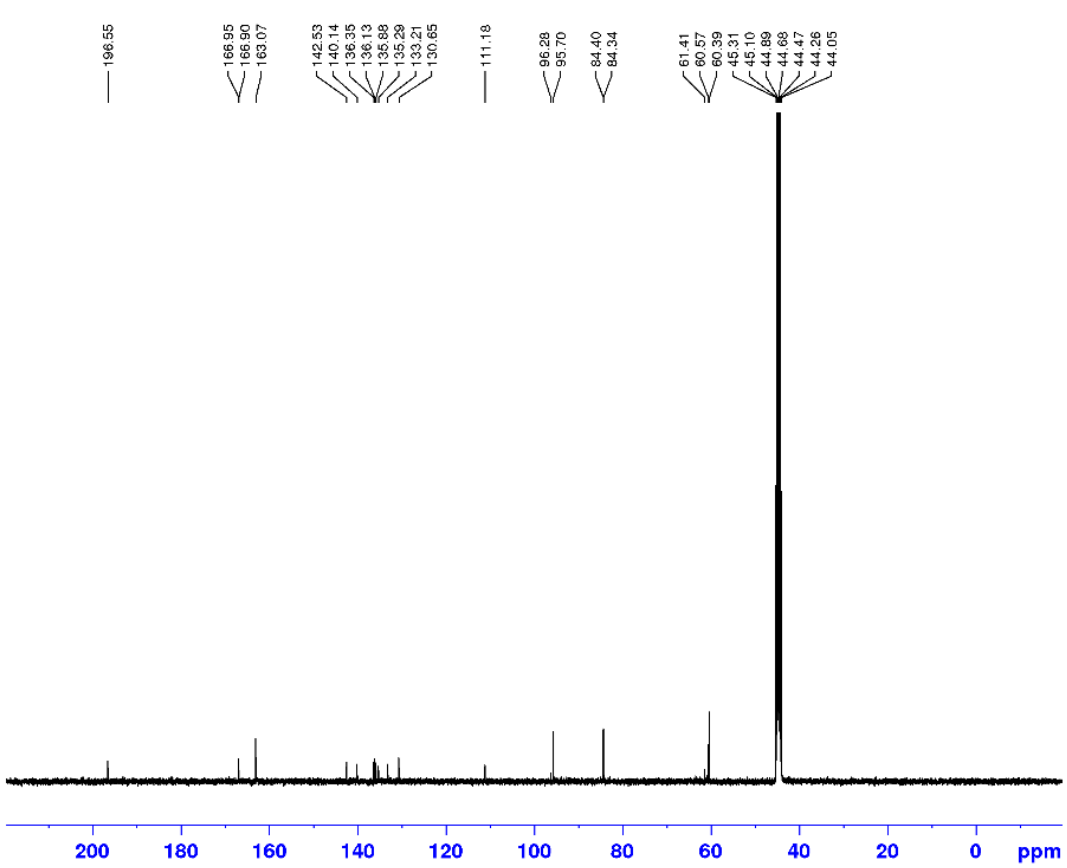
F2 - Acquisition Parameters
Date_          20180822
Time               21.23 h
INSTRUM  KU_Avance_III_400
PROBHD   Z109818_0245 (
PULPROG          noesygpph
TD                    2048
SOLVENT               DMSO
NS                       8
DS                       4
SWH              2463.054 Hz
FIDRES           2.405326 Hz
AQ               0.4157440 sec
RG                     128
DW                 203.000 usec
DE                  30.26 usec
TE                 296.0 K
D0               0.00018677 sec
D1               1.83793 03 sec
D8               0.30000001 sec
D16              0.00020000 sec
IN0              0.00040800 sec
TDav                       1
SFO1           399.7923231 MHz
NUC1                    1H
P1                    12.75 usec
P2                    25.50 usec
PLW1             8.47699928 W
GPNAM[1]           STD?.*.00
GPZ1                  40.00 %
P16                1000.00 usec

F1 - Acquisition parameters
TD                     256
SFO1             399.7923 MHz
FIDRES          19.242611 Hz
SW                 6.162 ppm
FnMODE       States-TPPI

F2 - Processing parameters
SI                    1024
SF             399.7900000 MHz
WDW                  QSINE
SSB                       2
LB         0 Hz
GB         0
PC                     1.00

F1 - Processing parameters
SI                    1024
MC2          States-TPPI
SF             399.7900000 MHz
WDW                  QSINE
SSB                       2
LB         0 Hz
GB         0
```

1.2.8 ^{13}C NMR



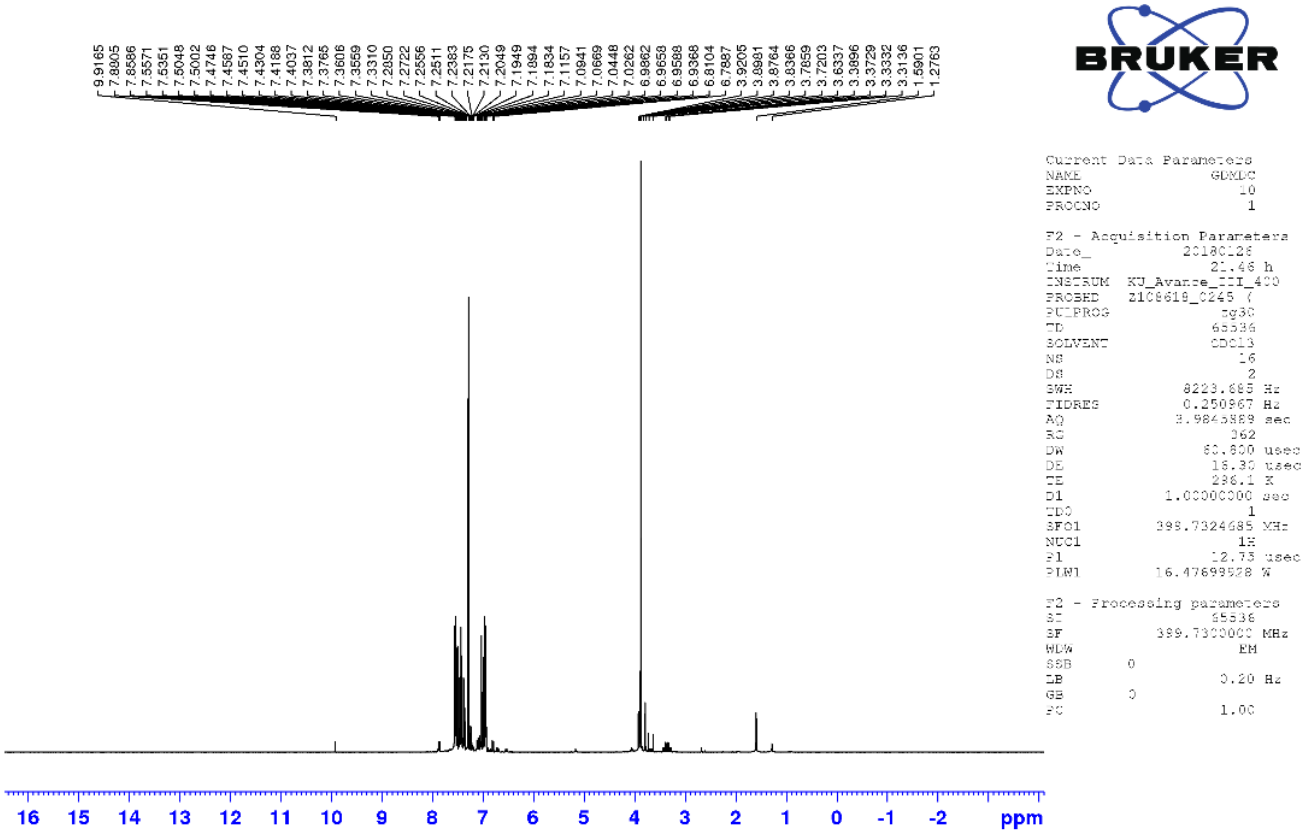

```
Current Data Parameters
NAME                  CD1MB
EXPNO                     10
PROCNO                     1

F2 - Acquisition Parameters
Date_           20_081015
Time                22.33 h
INSTRUM  RU_Avance_III_400
PROBHD   Z108618_0245_(
PULPROG              zgpg30
TD                    65536
SOLVENT                CDCl3
NS                     2560
DS                        4
SWH              24038.461 Hz
FIDRES            0.733596 Hz
AQ                1.3631488 sec
RG                     2050
DW                  20.800 usec
DE                  22.74 usec
TE                  296.0 K
D1               2.00000000 sec
D11              0.03000000 sec
TD0                       1
SFO1         100.5222401 MHz
NUC1                    13C
P1                    9.20 usec
PLW1         49.50003000 W
SFO2         399.7316389 MHz
NUC2                    1H
CPDPRG_2            waltz16
PCPD2                 90.00 usec
PLW2         16.47699928 W
PLW12         0.53066800 W
PLW13         0.26785001 W

F2 - Processing parameters
SI                    65536
SF           100.5122884 MHz
WDW                     EM
SSB       0
LB                    1.00 Hz
GB        0
PC                    1.40
```

1.3 Compound 3

1.3.1 ^{1}H NMR

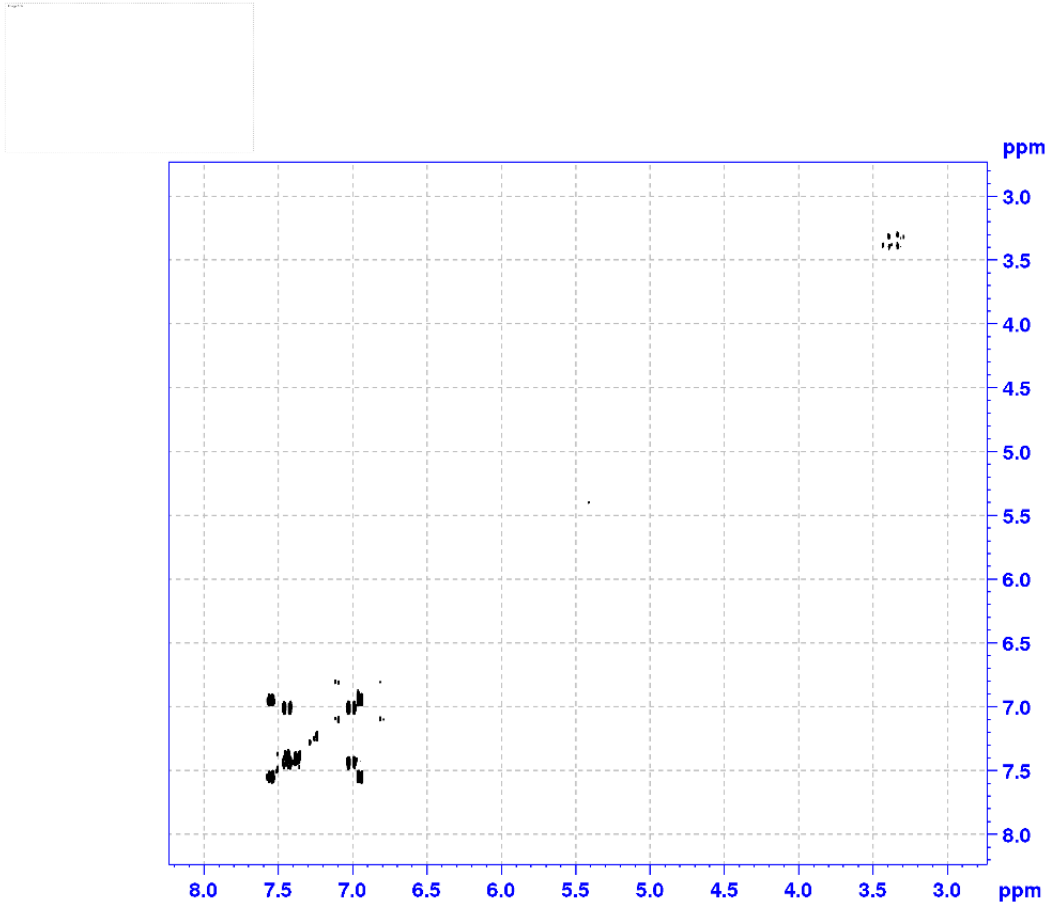



```
Current Data Parameters
NAME                  GUMJC
EXPNO                    10
PROCNO                    1

F2 - Acquisition Parameters
Date_              20180126
Time                 21.46 h
INSTRUM   KU_Avance_III_400
PROBHD    Z108618_0245 (
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                       16
DS                        2
SWH                8223.685 Hz
FIDRES             0.250967 Hz
AQ                 3.9845888 sec
RG                      362
DW                   60.800 usec
DE                   18.30 usec
TE                   296.1 K
D1                1.00000000 sec
TD0                       1
SFO1             399.7324685 MHz
NUC1                     1H
P1                    12.73 usec
PLW1            16.47699528 W

F2 - Processing parameters
SI                    65536
SF               399.7300000 MHz
WDW                      EM
SSB          0
LB                    0.20 Hz
GB           0
PC                    1.00
```

1.3.2 ^{1}H -^{1}H COSY



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```
Current Data Parameters
NAME                 GOMDC
EXPNO                   12
PROCNO                   1

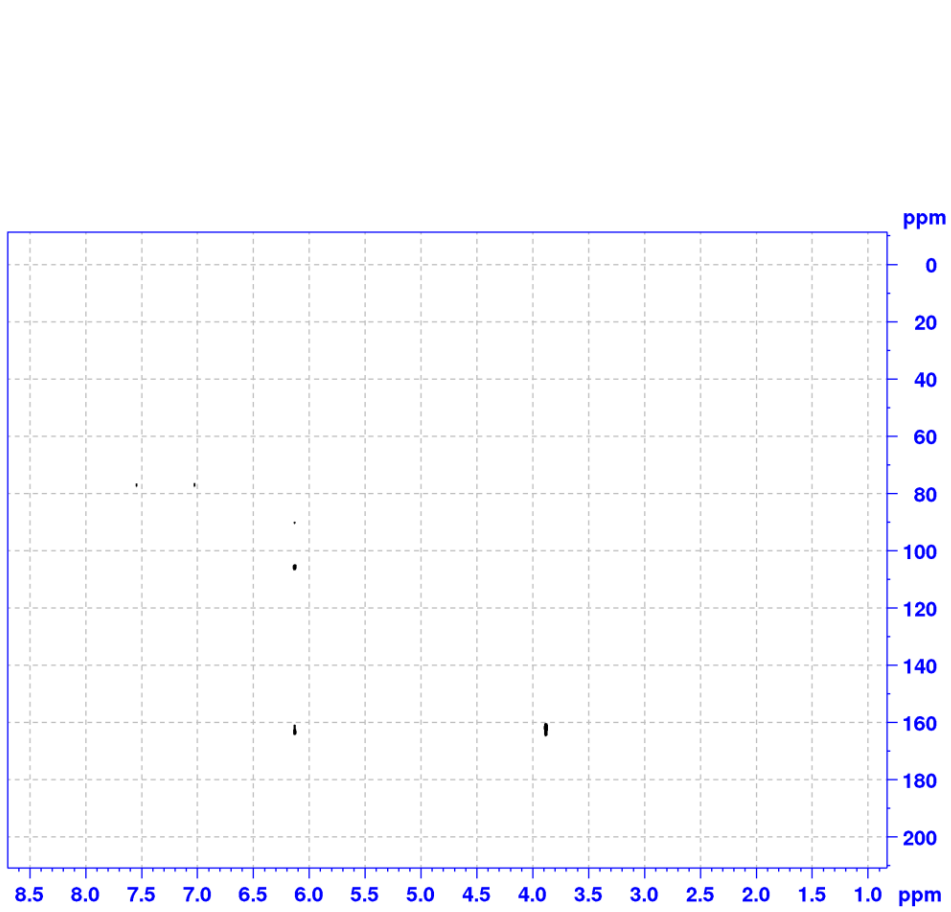
F2 - Acquisition Parameters
Date_              20160620
Time                 14.04 h
INSTRUM  XU_Avance_III_400
PROBHD   Z108618_0260 (
PULPROG      cosygpmfqf
TD                     2048
SOLVENT              CDCl3
NS                        2
DS                        8
SWH                2941.177 Hz
FIDRES             2.872243 Hz
AQ                 0.3481620 sec
RG                      2050
DW                  170.000 usec
DE                    6.50 usec
TE                  296.3 K
D0                 0.00000300 sec
D1                 1.84353904 sec
D13                0.03000400 sec
D16                0.00020000 sec
  NO               0.05093000 sec
CDav                      1
SFO1               399.7318215 MHz
NUC1                      1H
P1                       12.75 usec
PLW1              16.47669928 W
GPNAM[1]             SINE.100
GPZ1                    16.00 %
GPNAM[2]             SINE.100
GPZ2                    12.00 %
GPNAM[3]             SINE.100
GPZ3                    40.00 %
P16                   1000.00 usec

F1 - Acquisition parameters
TD                       1024
SFO1               399.7318 MHz
FIDRES             45.955883 Hz
SW                    7.358 ppm
MCREST                   QF

F2 - Processing parameters
SI                       1024
SF                 399.7320020 MHz
WDW                      SINE
SSB                      0
LB                       0 Hz
GB                       0
PC                       1.40

F1 - Processing parameters
SI                       1024
MC2                      QF
SF                 399.7320020 MHz
WDW                      SINE
SSB                      0
LB                       0 Hz
GB                       0
```

1.3.3 ^{1}H-^{13}C HMBC




```
Current Data Parameters
NAME                  GDMC2
EXPNO                    14
PROCNO                    1

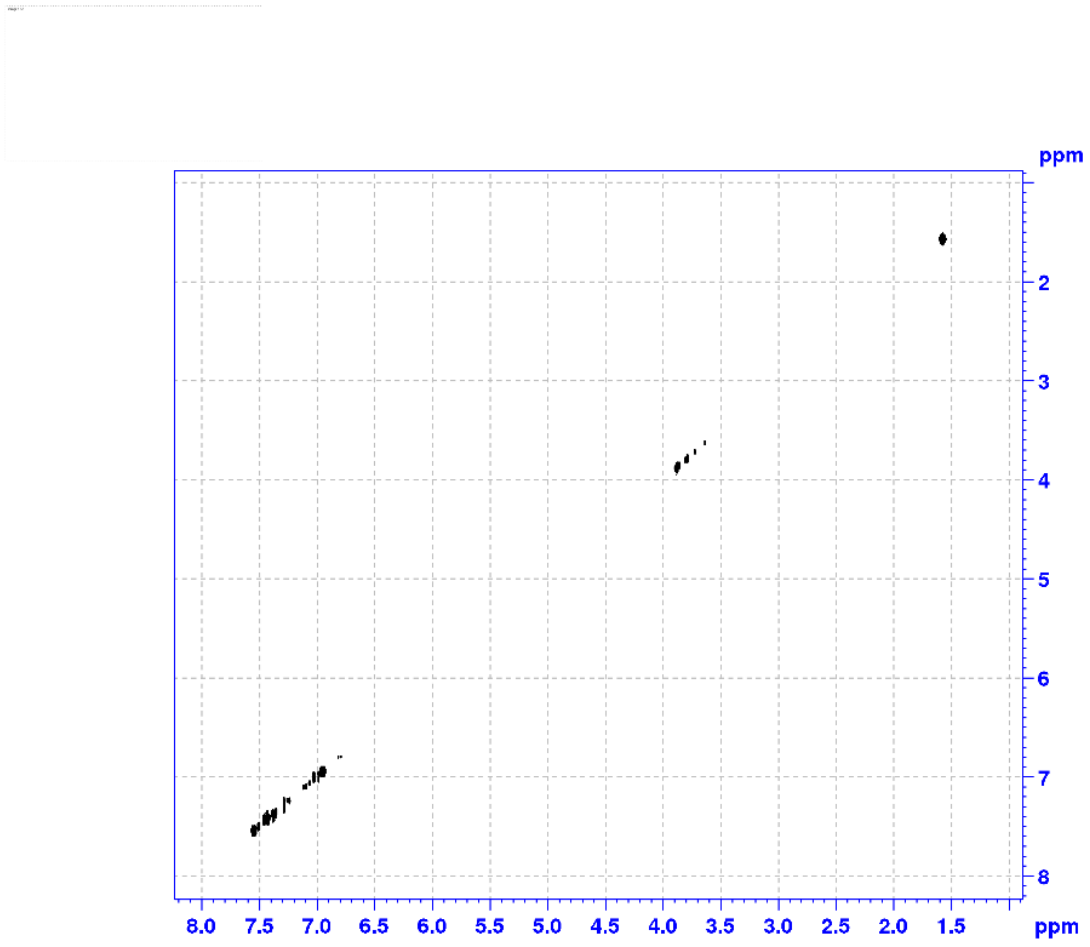
F2 - Acquisition Parameters
Date_              20180719
Time                  19.37 h
INSTRUM  KU_Avance_III_400
PROBHD   2108618_0245 (
PULPROG    hmbcetgpl3nd
TD                     4096
SOLVENT              CDCl3
NS                       16
DS                       16
SWH                3144.654 Hz
FIDRES             1.535476 Hz
AQ                 0.6512640 sec
RG                      2050
DW                   159.000 usec
DE                     6.50 usec
TE                   296.1 K
CNST6           120.0000000
CNST7           165.0000000
CNST13           10.0000000
D0                0.00000300 sec
D1                1.24195194 sec
D6                0.05000000 sec
D16               0.00020000 sec
IN0               0.00002240 sec
TDav                      1
SFO1           399.7319039 MHz
NUC1                    1H
P1                    12.75 usec
P2                    25.50 usec
PLW1           16.47699928 W
SFO2           100.5222241 MHz
NUC2                    13C
P3                     9.20 usec
P24                 2000.00 usec
PLW2           49.50000000 W
SPNAM[7]       Crp60comp.4
SPOAL7               0.500
SPOFFS7  0 Hz
SPW7            6.40140009 W
GPNAM[1]          SINE.100
GPZ1                 80.00 %
GPNAM[3]          SINE.100
GPZ3                 14.00 %
GPNAM[4]          SINE.100
GPZ4                 -8.00 %
GPNAM[5]          SINE.100
GPZ5                 -4.00 %
GPNAM[6]          SINE.100
GPZ6                 -2.00 %
P16                1000.00 usec

F1 - Acquisition parameters
TD                      128
SFO1              100.5222 MHz
FIDRES          348.772308 Hz
SW                222.055 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                     2048
SF             399.7300000 MHz
WDW                    SINE
SSB                       2
LB        0 Hz
GB        0
PC                     1.40

F1 - Processing parameters
SI                     1024
MC2         echo-antiecho
SF              100.5121884 MHz
WDW                    SINE
SSB                       2
LB        0 Hz
GB        0
```

1.3.4 ^{1}H-^{1}H NOESY



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```
Current Data Parameters
NAME                  CDMDC
EXPNO                    15
PROCNO                    1

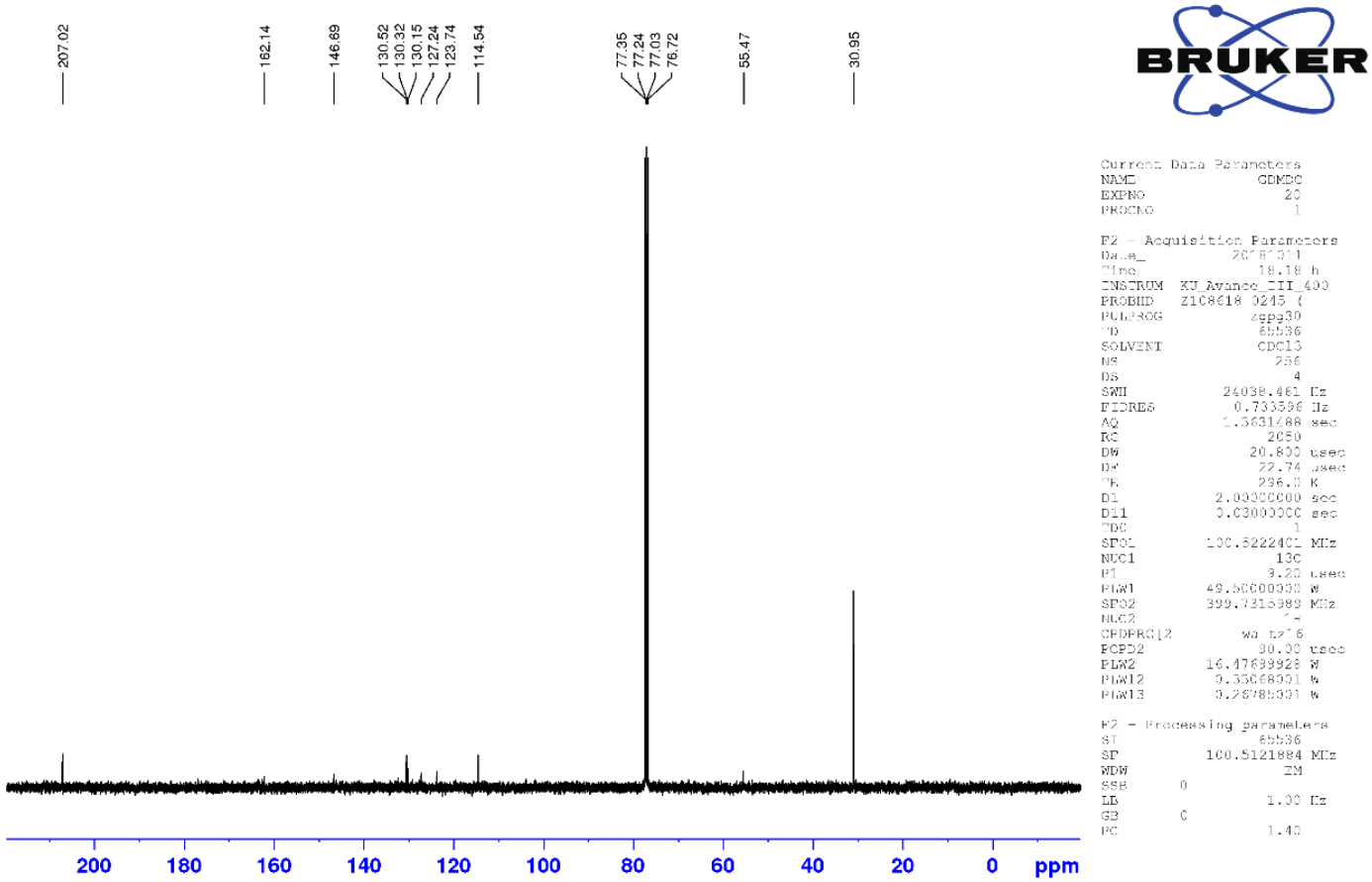
F2 - Acquisition Parameters
Date_              20180620
Time                  16.30 h
INSTRM  400 Avance III 400
PROBHD  5 mm BBFO zgrad z
PULPROG          noesygppp1
TD                     2048
SOLVENT               CDCl3
NS                       8
DS                       4
SWH                2941.177 Hz
FIDRES             2.872243 Hz
AQ                 0.3481600 sec
RG                     406
DW                  170.000 usec
DE                   23.97 usec
TE                  296.9 K
D0                 0.00013377 sec
D1                 1.90000501 sec
D8                 0.30000001 sec
D16                0.00020000 sec
DNC                0.00004000 sec
TDav                       1
SFO1            399.7318215 MHz
NUC1                      1H
P1                      12.35 usec
P2                      25.50 usec
PLW1               16.47839928 W
GPNAM[1]             SINE.100
GPZ1                    40.00 %
P16                   1000.00 usec

F1 - Acquisition parameters
TD                        256
SFO1               399.7318 MHz
FIDRES             22.077962 Hz
SW                  7.358 ppm
FnMODE          States-TPPI

F2 - Processing parameters
SI                       1024
SF              399.7300000 MHz
WDW                     QSINE
SSB                        2
LB          0 Hz
GB          0
PC                      1.00

F1 - Processing parameters
SI                       1024
MC2             States-TPPI
SF              399.7300000 MHz
WDW                     QSINE
SSB                        2
LB          0 Hz
GB          0
```

1.3.5 ^{13}C NMR



BRUKER

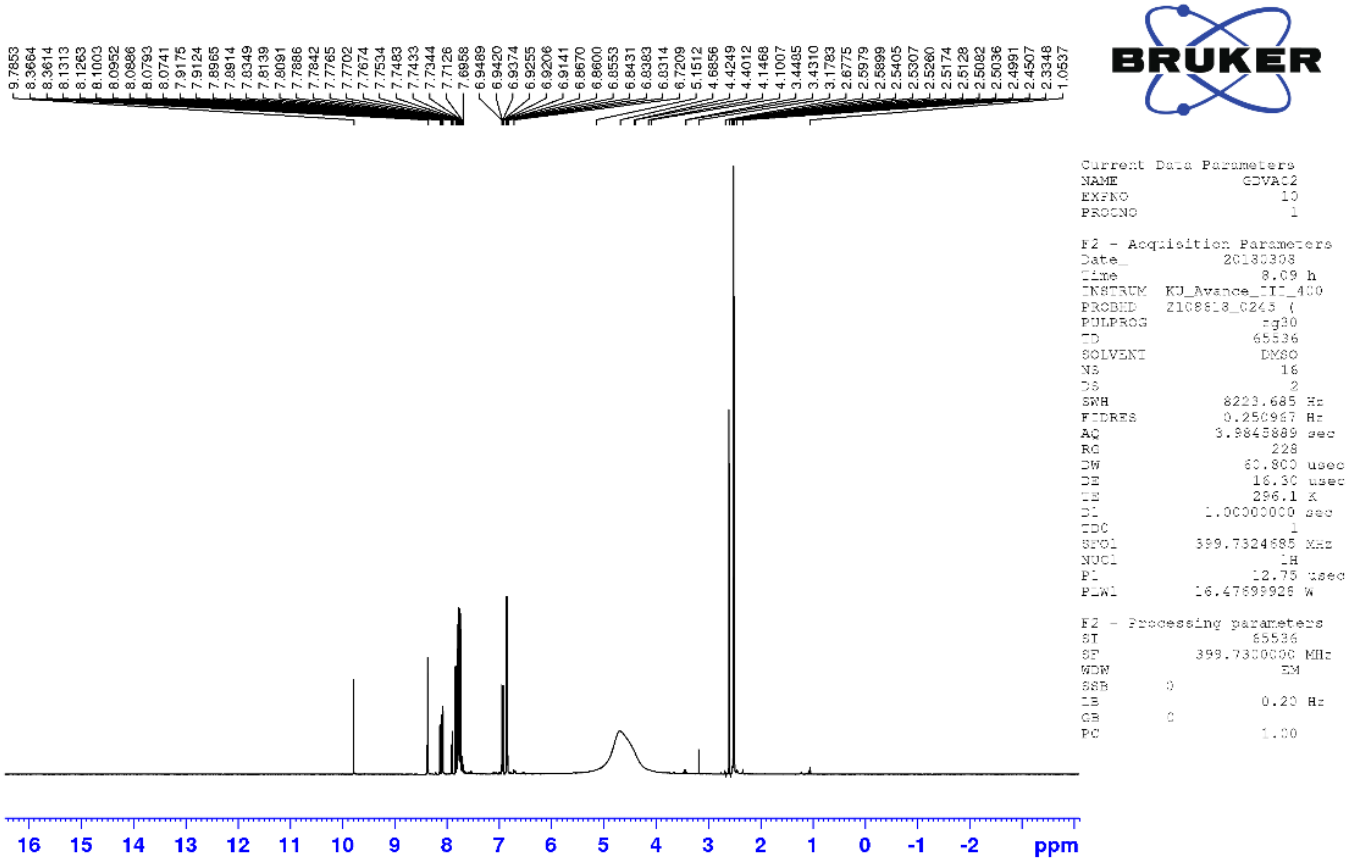
```
Current Data Parameters
NAME                  GDMDC
EXPNO                    20
PROCNO                    1

F2 - Acquisition Parameters
Date_              20[illegible]
Time                 18.18 h
INSTRUM  KJ_Avance_III_400
PROBHD   Z108618_0215_(
PULPROG             zgpg30
TD                  65536
SOLVENT             CDCl3
NS                    256
DS                      4
SWH             24038.461 Hz
FIDRES          0.733596 Hz
AQ              1.3631488 sec
RG                   2050
DW                 20.800 usec
DE                 22.74 usec
TE                 296.0 K
D1              2.00000000 sec
D11             0.03000000 sec
TD0                     1
SFO1          100.5222401 MHz
NUC1                  13C
P1                   9.20 usec
PLW1          49.50000000 W
SFO2          399.7315989 MHz
NUC2                  [illegible]H
CPDPRG[2          wa[illegible]tz16
PCPD2               90.00 usec
PLW2          16.47699928 W
PLW12          0.33068001 W
PLW13          0.26785001 W

F2 - Processing parameters
SI                  65536
SF            100.5121884 MHz
WDW                   EM
SSB       0
LB                   1.00 Hz
GB        0
PC                   1.40
```

1.4 Compound 4

1.4.1 ^{1}H NMR

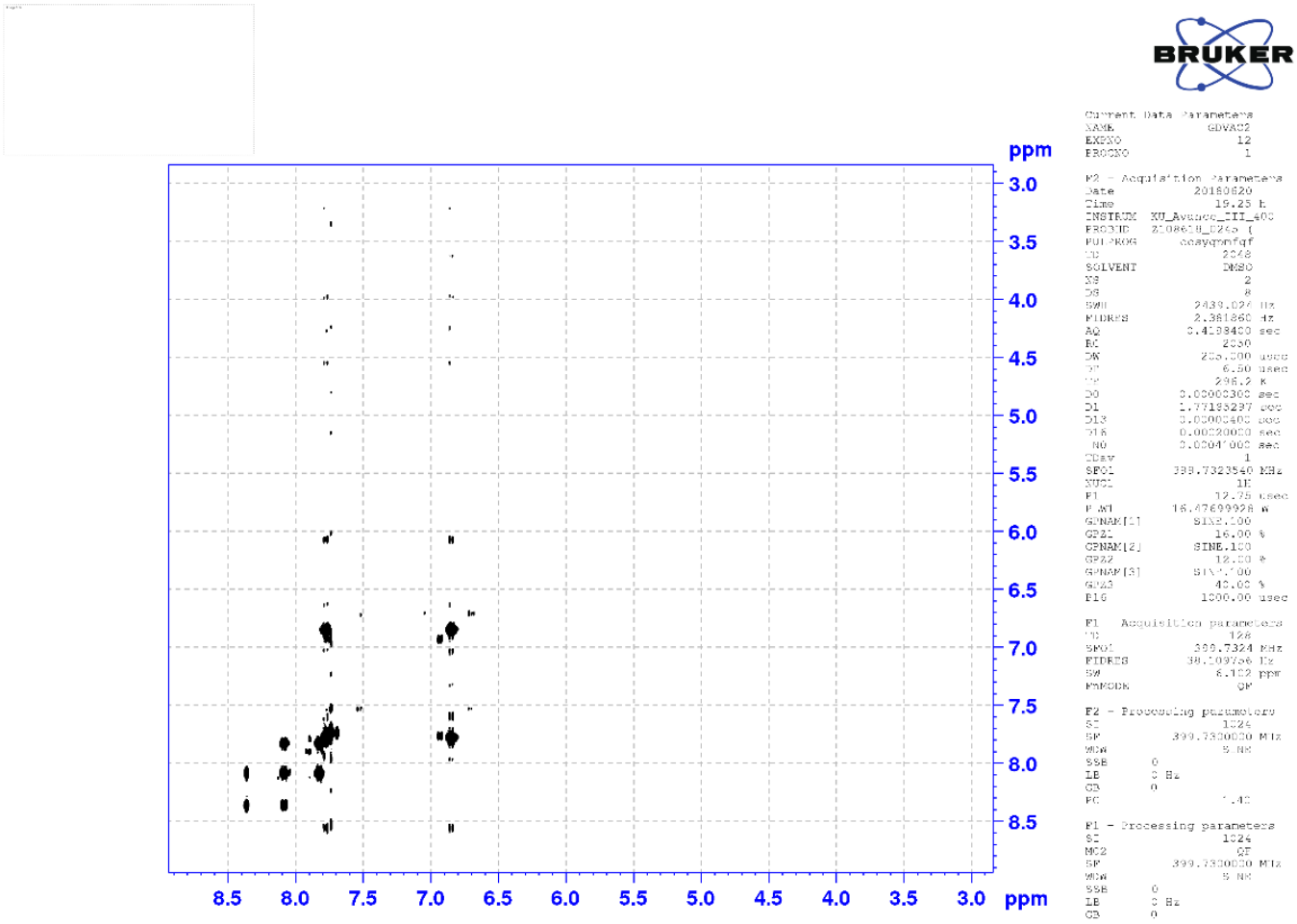



```
Current Data Parameters
NAME                 GDVA02
EXPNO                    10
PROCNO                    1

F2 - Acquisition Parameters
Date_              20180308
Time                  8.09 h
INSTRUM  KU_Avance_III_400
PROBHD   Z108818_0243 (
PULPROG                zg30
TD                    65536
SOLVENT                DMSO
NS                       16
DS                        2
SWH                8223.685 Hz
FIDRES             0.250967 Hz
AQ                 3.9843889 sec
RG                      228
DW                   60.800 usec
DE                   16.30 usec
TE                   296.1 K
D1                1.00000000 sec
TDO                       1
SFO1            399.7324685 MHz
NUC1                      1H
P1                    12.75 usec
PLW1           16.47699928 W

F2 - Processing parameters
SI                    65536
SF              399.7300000 MHz
WDW                      EM
SSB          0
LB                    0.20 Hz
GB           0
PC                    1.00
```

1.4.2 ^{1}H -^{1}H COSY



BRUKER

```
Current Data Parameters
NAME                 GDVAC2
EXPNO                    12
PROCNO                    1

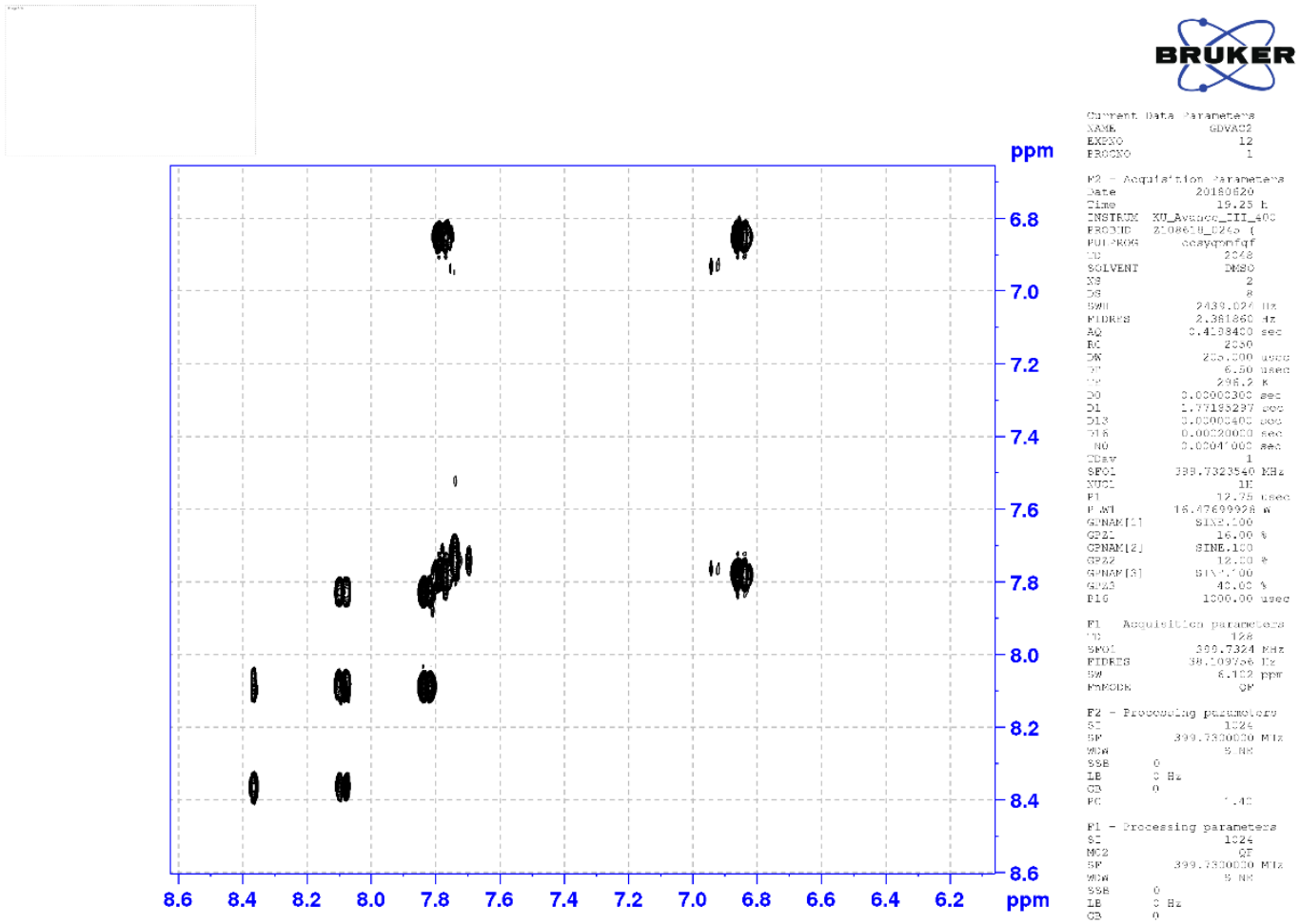
F2 - Acquisition Parameters
Date                20180620
Time                   19.25 h
INSTRUM  KU_Avance_III_400
PROBHD   Z108618_0245 (
PULPROG         zgesyqmnfqf
TD                       2048
SOLVENT                  DMSO
NS                          2
DS                          8
SWH                 2439.027 Hz
FIDRES              2.381860 Hz
AQ                  0.4198400 sec
RG                       2050
DW                   205.000 usec
DE                      6.50 usec
TE                     298.2 K
D0                  0.00000300 sec
D1                  1.77185297 sec
D13                 0.00000400 sec
D16                 0.00020000 sec
 NS                 0.0004000 sec
CDev                        1
SFO1               399.7323540 MHz
NUC1                       1H
P1                       12.75 usec
PLW1               16.47609998 W
GPNAM[1]              SINE.100
GPZ1                     16.00 %
GPNAM[2]              SINE.100
GPZ2                     12.00 %
GPNAM[3]              SINE.100
GPZ3                     42.00 %
P16                    1000.00 usec

F1 - Acquisition parameters
TD                          128
SFO1               399.7324 MHz
FIDRES             38.109756 Hz
SW                      8.102 ppm
FnMODE                     QF

F2 - Processing parameters
SI                         1024
SF                399.7300020 MHz
WDW                        SINE
SSB                 0
LB                  0 Hz
GB                  0
PC                         1.40

F1 - Processing parameters
SI                         1024
MC2                          QF
SF                399.7300020 MHz
WDW                        SINE
SSB                 0
LB                  0 Hz
GB                  0
```

1.4.3 Expansion of COSY



BRUKER

```
Current Data Parameters
NAME                 GDVA02
EXPNO                    12
PROCNO                    1

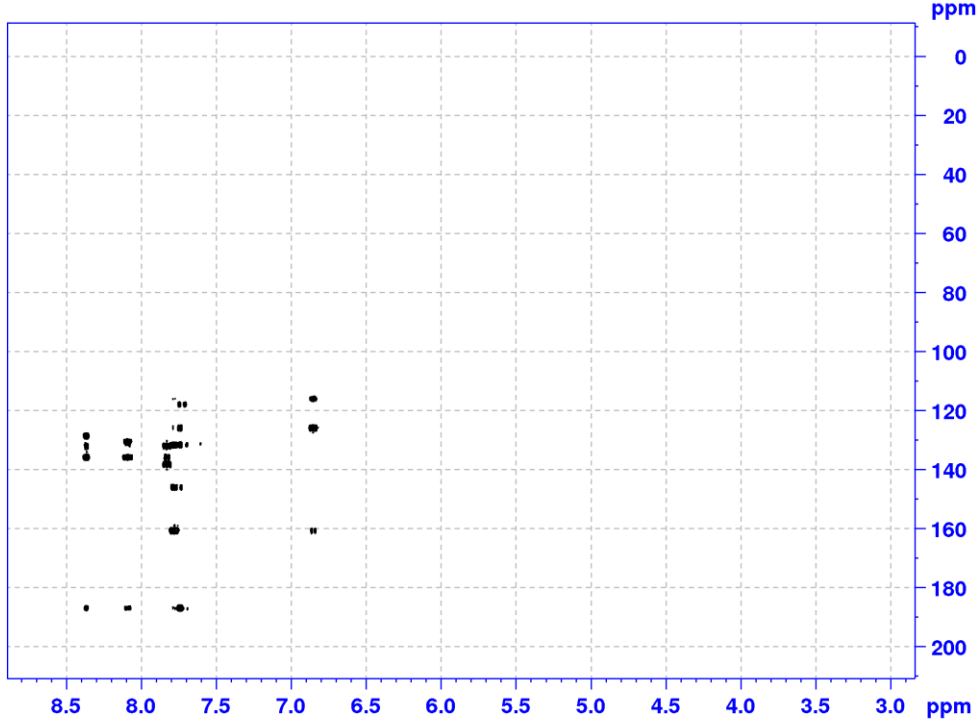
F2 - Acquisition Parameters
Date                20180620
Time                   15.25 h
INSTRUM   XU_Avance_III_400
PROBHD    Z108618_0246 (
PULPROG          cosygpmfqf
TD                       2048
SOLVENT                  DMSO
NS                          2
DS                          8
SWH                 2439.024 Hz
FIDRES              2.381860 Hz
AQ                  0.4198400 sec
RG                       2050
DW                   205.000 usec
DE                     6.50 usec
TE                   296.2 K
D0                  0.00003000 sec
D1                  1.77195297 sec
D13                 0.03000400 sec
D16                 0.00020000 sec
  NO                0.0204'000 sec
CDav                        1
SFO1               399.7323540 MHz
NUC1                       1H
P1                       12.75 usec
PLW1               16.47699928 W
GPNAM[1]              SINE.100
GPZ1                    16.00 %
GPNAM[2]              SINE.100
GPZ2                    12.00 %
GPNAM[3]              SINE.100
GPZ3                    40.00 %
P16                   1000.00 usec

F1 - Acquisition parameters
TD                         128
SFO1               399.7324 MHz
FIDRES             38.109756 Hz
SW                      6.102 ppm
FnMODE                     QF

F2 - Processing parameters
SI                         1024
SF                399.7300020 MHz
WDW                        SINE
SSB                 0
LB                  0 Hz
GB                  0
PC                         1.40

F1 - Processing parameters
SI                         1024
MC2                          QF
SF                399.7300020 MHz
WDW                        SINE
SSB                 0
LB                  0 Hz
GB                  0
```

1.4.4 ^{1}H-^{13}C HMBC




```
Current Data Parameters
NAME                  GDVAC2
EXPNO                     14
PROCNO                     1

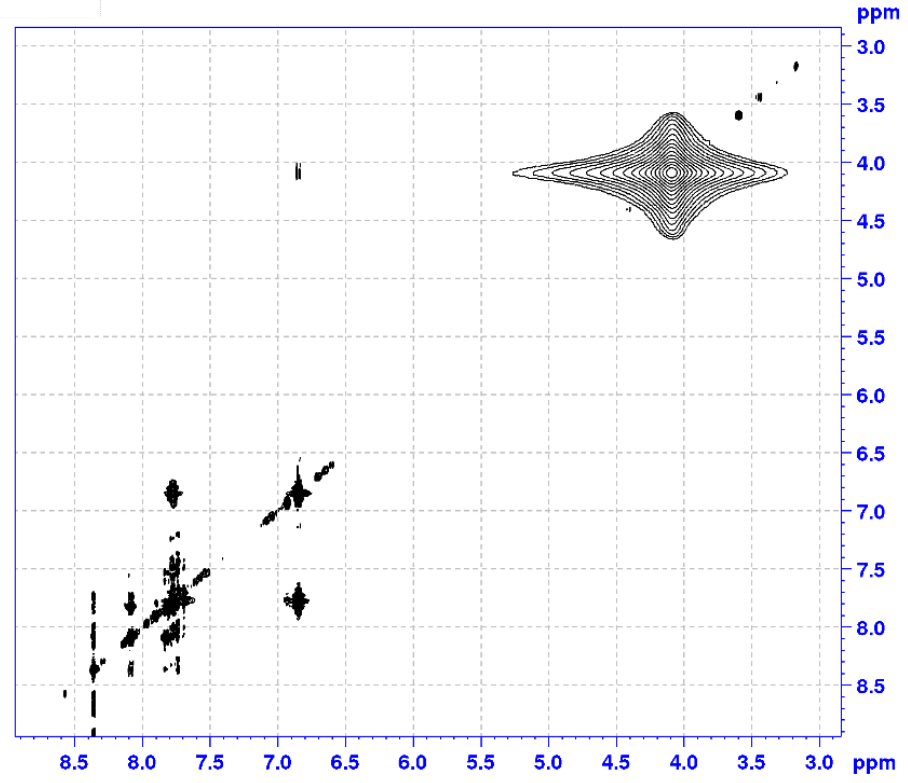
F2 - Acquisition Parameters
Date_              20180620
Time                  23.53 h
INSTRUM   KU_Avance_III_400
PROBHD    Z108618_0245 [
PULPROG     hmbcetgpl3nd
TD                      4096
SOLVENT                 DMSO
NS                        16
DS                        16
SWH                2439.024 Hz
FIDRES             1.190930 Hz
AQ                 0.8396800 sec
RG                      2050
DW                   205.000 usec
DE                     6.50 usec
TE                   296.0 K
CNST6            120.0000000
CNST7            165.0000000
CNST13            10.0000000
D0                 0.00000300 sec
D1                 1.05353606 sec
D6                 0.05000000 sec
D16                0.00020000 sec
IN0                0.00002240 sec
TDav                       1
SFO1             399.7323540 MHz
NUC1                      1H
P1                    12.75 usec
P2                    25.50 usec
PLW1             16.47699928 W
SFO2             100.5222241 MHz
NUC2                      13C
P3                     9.20 usec
P24                 2000.00 usec
PLW2             49.50000000 W
SPNAM[7]         Crp60comp.4
SPOAL7                 0.500
SPOFFS7   0 Hz
SPW7              6.40140009 W
GPNAM[1]             SINE.100
GP21                   80.00 %
GPNAM[3]             SINE.100
GP23                   14.00 %
GPNAM[4]             SINE.100
GP24                   -8.00 %
GPNAM[5]             SINE.100
GP25                   -4.00 %
GPNAM[6]             SINE.100
GP26                   -2.00 %
P16                 1000.00 usec

F1 - Acquisition parameters
TD                        128
SFO1               100.5222 MHz
FIDRES          348.772308 Hz
SW                 222.055 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                       2048
SF              399.7300000 MHz
WDW                      SINE
SSB                         2
LB        0 Hz
GB        0
PC                      1.40

F1 - Processing parameters
SI                       1024
MC2          echo-antiecho
SF              100.5121884 MHz
WDW                      SINE
SSB                         2
LB        0 Hz
GB        0
```

1.4.5 ^{1}H-^{1}H NOESY



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```
Current Data Parameters
NAME                  GDVAC2
EXPNO                     15
PROCNO                     1

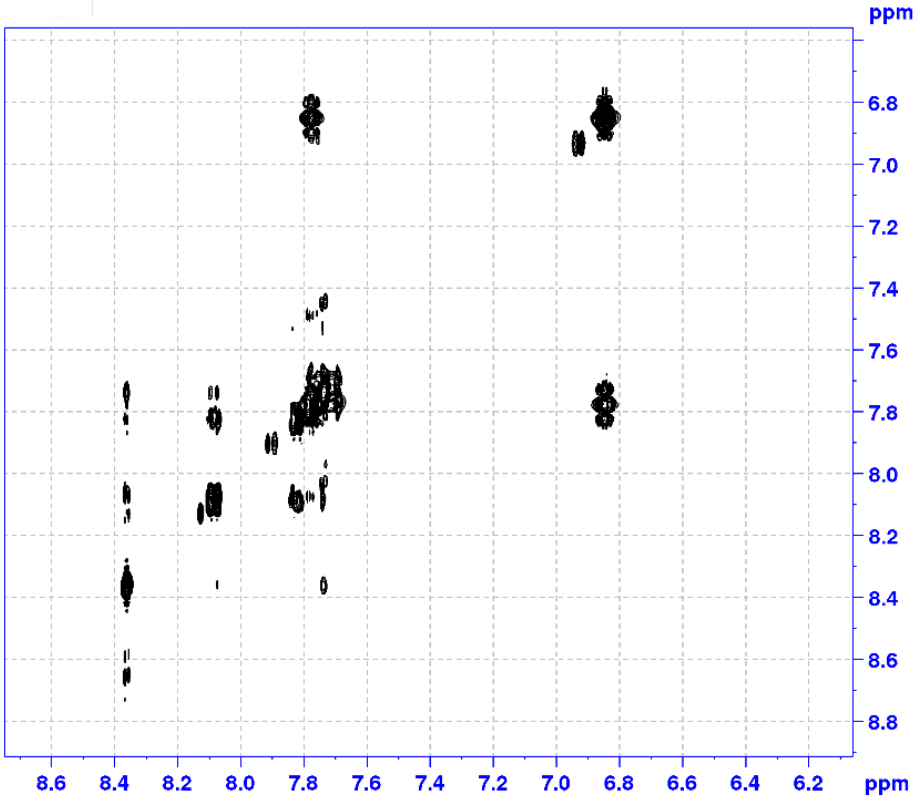
F2 - Acquisition Parameters
Date              20_80621
Time                   1.03 h
INSTRUM   KL Avance III 400
PROBHD    BI38619_0275 (
PULPROG          noesygpph
TD                     2048
SOLVENT                DMSO
NS                        8
DS                        4
SWH               2739.024 Hz
FIDRES            2.381860 Hz
AQ                0.4198400 sec
RG                       67
DW                 255.000 usec
DE                  30.63 usec
TE                  296.1 K
D0                0.00010877 sec
D1                1.93003498 sec
D8                0.30000001 sec
D16               0.00020000 sec
IN0               0.00041000 sec
NDAV                      1
SFO1           399.7323540 MHz
VDLIST                   VT
P1                    12.75 usec
P2                    25.50 usec
PL01             16.47899928 W
SPNAM[1]          Sine.100
SPOI1                 42.20 %
P16                 1000.00 usec

F1 - Acquisition parameters
TD                       256
SFO1              399.7324 MHz
FIDRES           19.054878 Hz
SW                    6.102 ppm
FnMODE        States-TPPI

F2 - Processing parameters
SI                       1024
SF             399.7300000 MHz
WDW                    QSINE
SSB                       2
LB         0 Hz
GB         0
PC                     1.00

F1 - Processing parameters
SI                       1024
MC2           States-TPPI
SF             399.7300000 MHz
WDW                    QSINE
SSB                       2
LB         0 Hz
GB         0
```

1.4.6 Expansion of NOESY



BRUKER

```
Current Data Parameters
NAME                  CDVAC2
EXPNO                     10
PROCNO                     1

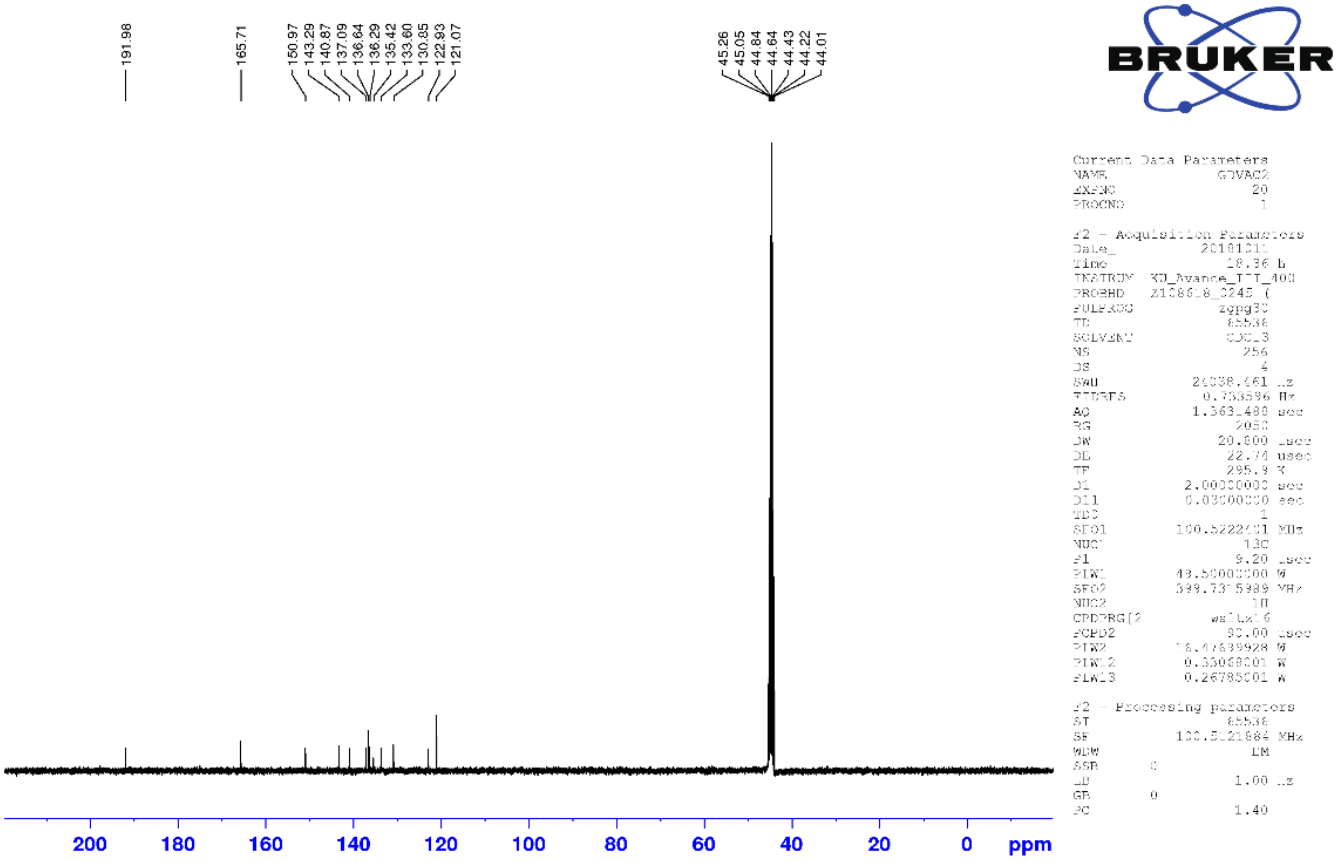
F2 - Acquisition Parameters
Date              20_80621
Time                   1.00 h
INSTRUM   KL Avance III 400
PROBHD    Z138618_0275 (
PULPROG          "noesygpph"
TD                     2048
SOLVENT                DMSO
NS                        8
DS                        4
SWH               2739.024 Hz
FIDRES            2.381860 Hz
AQ                0.4198400 sec
RG                       64
DW                 255.000 usec
DE                  30.63 usec
TE                  296.1 K
D0                0.00218877 sec
D1                1.93063498 sec
D8                0.36000001 sec
D16               0.00220000 sec
INO               0.00041000 sec
TD0                       1
SFO1           399.7323540 MHz
NUC1                      1H
P1                   12.75 usec
P2                   25.50 usec
PL01             16.47699928 W
GPNAM[1]          SINE.100
GPZ1                 45.00 %
P16                1000.00 usec

F1 - Acquisition parameters
TD                      256
SFO1              399.7324 MHz
FIDRES           19.054878 Hz
SW                    6.102 ppm
FnMODE          States-TPPI

F2 - Processing parameters
SI                      1024
SF             399.7300000 MHz
WDW                    QSINE
SSB                       2
LB          0 Hz
GB          0
PC                     1.00

F1 - Processing parameters
SI                      1024
MC2            States-TPPI
SF             399.7300000 MHz
WDW                    QSINE
SSB                       2
LB          0 Hz
GB          0
```

1.4.7 ^{13}C NMR



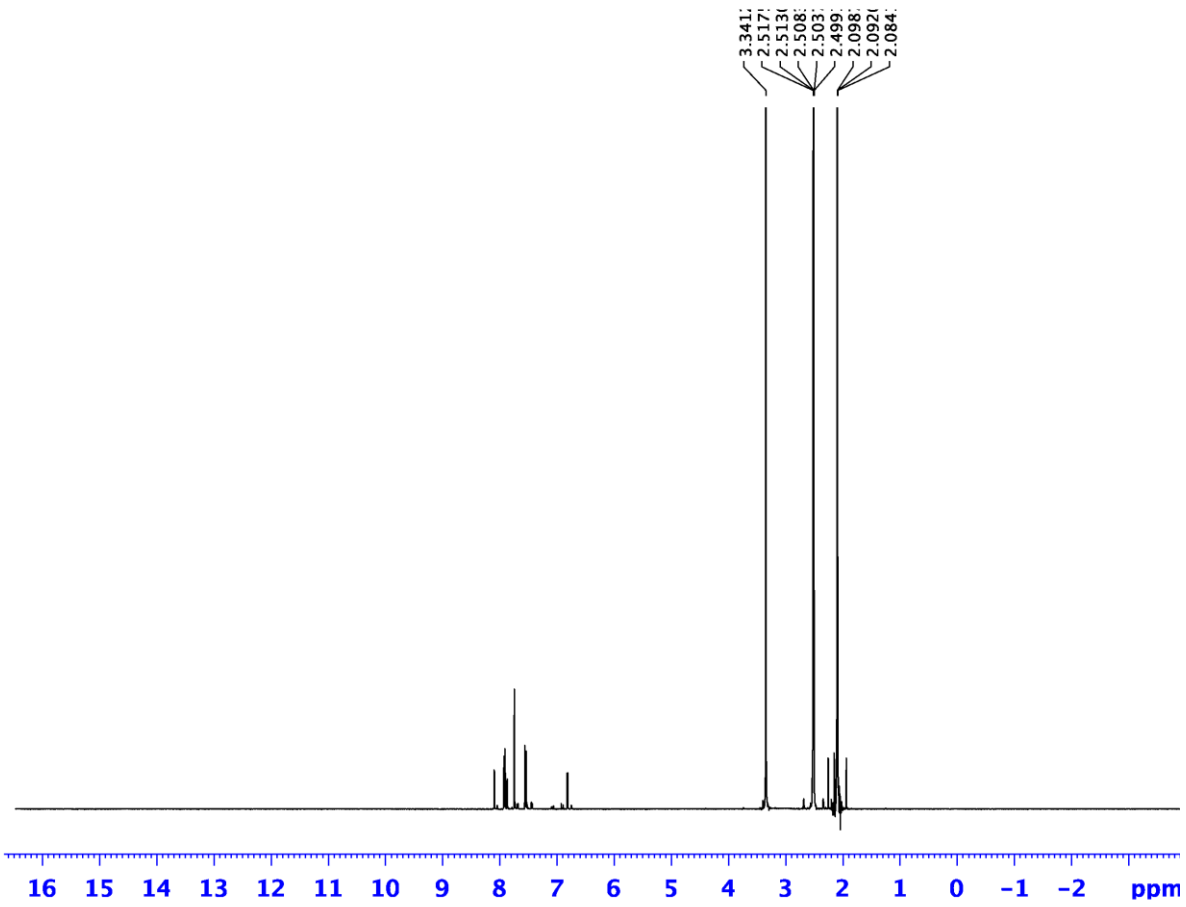

```
Current Data Parameters
NAME                  GDVAC2
EXPNO                     20
PROCNO                     1

F2 - Acquisition Parameters
Date_           20181211
Time                  18.36 h
INSTRUM  XO_Avance_III_400
PROBHD   21086_8_0245 (
PULPROG              zgpg30
TD                    65536
SOLVENT               CDCl3
NS                      256
DS                        4
SWH               24038.461 Hz
FIDRES             0.733596 Hz
AQ                1.3631488 sec
RG                      203
DW                 20.800 usec
DE                  22.74 usec
TE                  295.9 K
D1                2.00000000 sec
D11               0.03000000 sec
TD0                       1
SFO1         100.5222771 MHz
NUC1                    13C
P1                    9.20 usec
PLW1          48.50000000 W
SFO2          399.7375989 MHz
NUC2                    1H
CPDPRG[2          waltz16
PCPD2                80.00 usec
PLW2          16.47639928 W
PLW12          0.33068201 W
PLW13          0.26785001 W

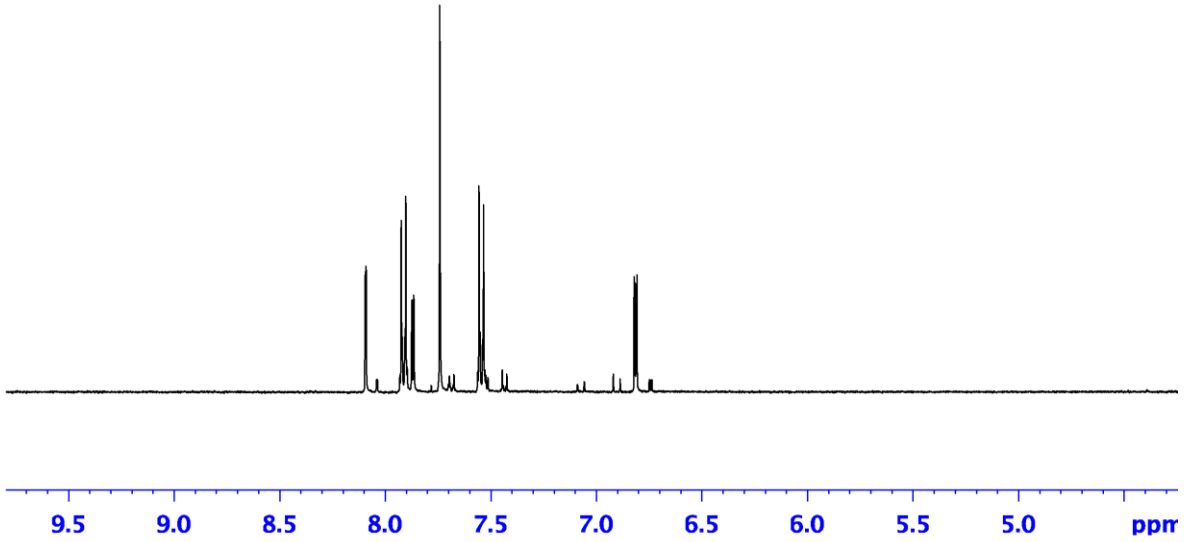
F2 - Processing parameters
SI                    65536
SF            100.5121884 MHz
WDW                      EM
SSB                   0
LB                    1.00 Hz
GB                    0
PC                    1.40
```

1.5 Compound 5

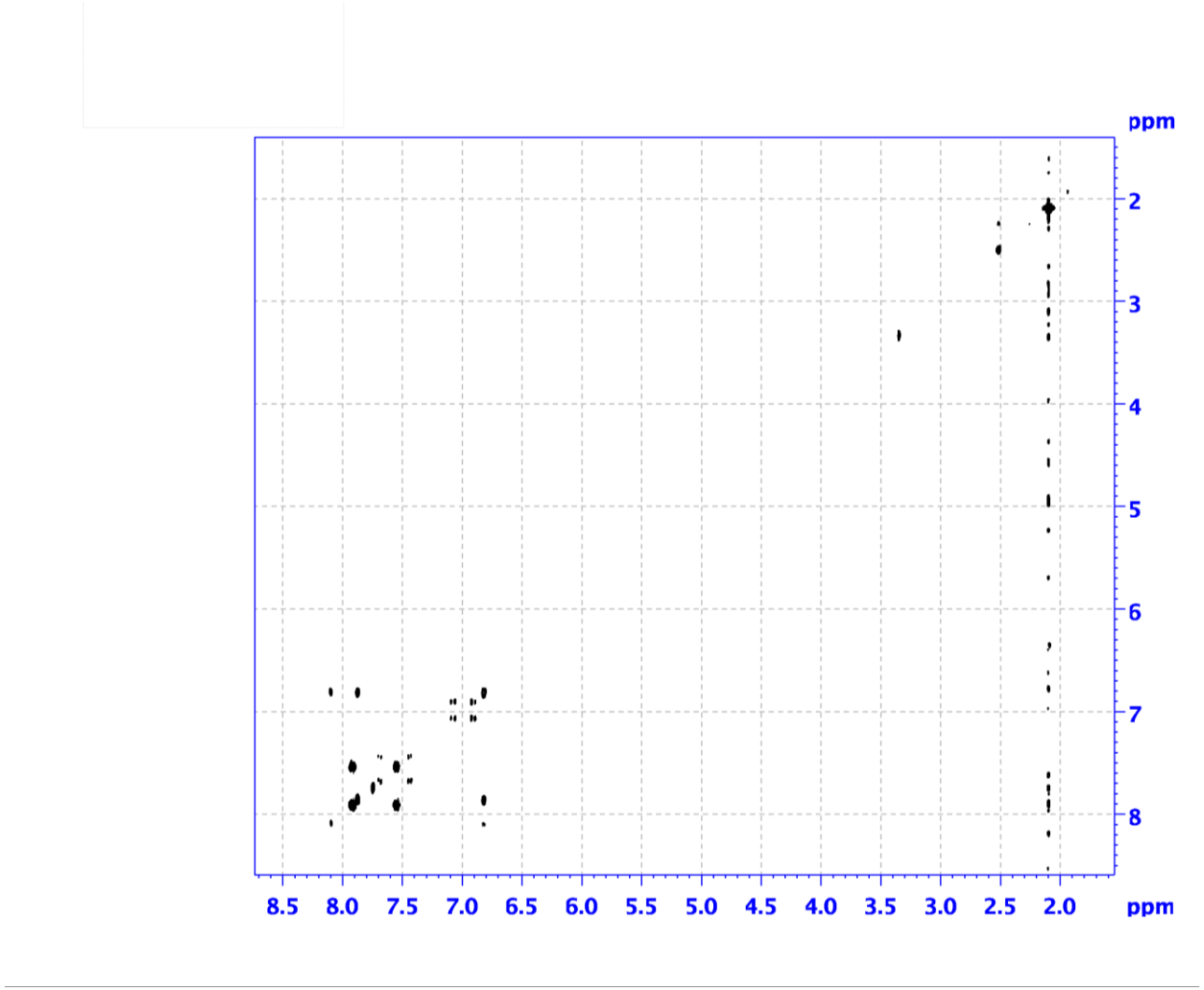
1.5.1 ^{1}H NMR



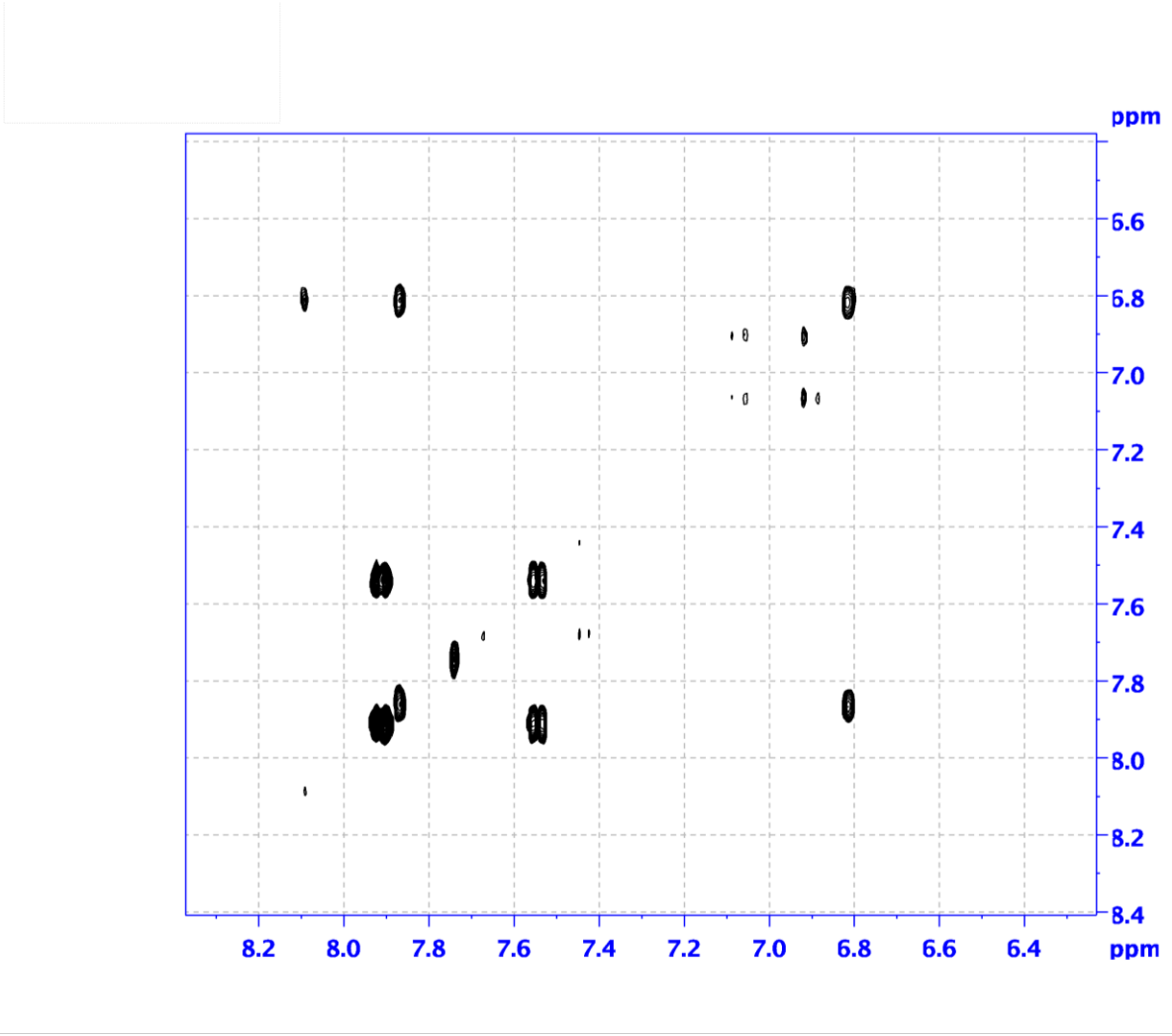
1.5.2 Expansion of aromatic region



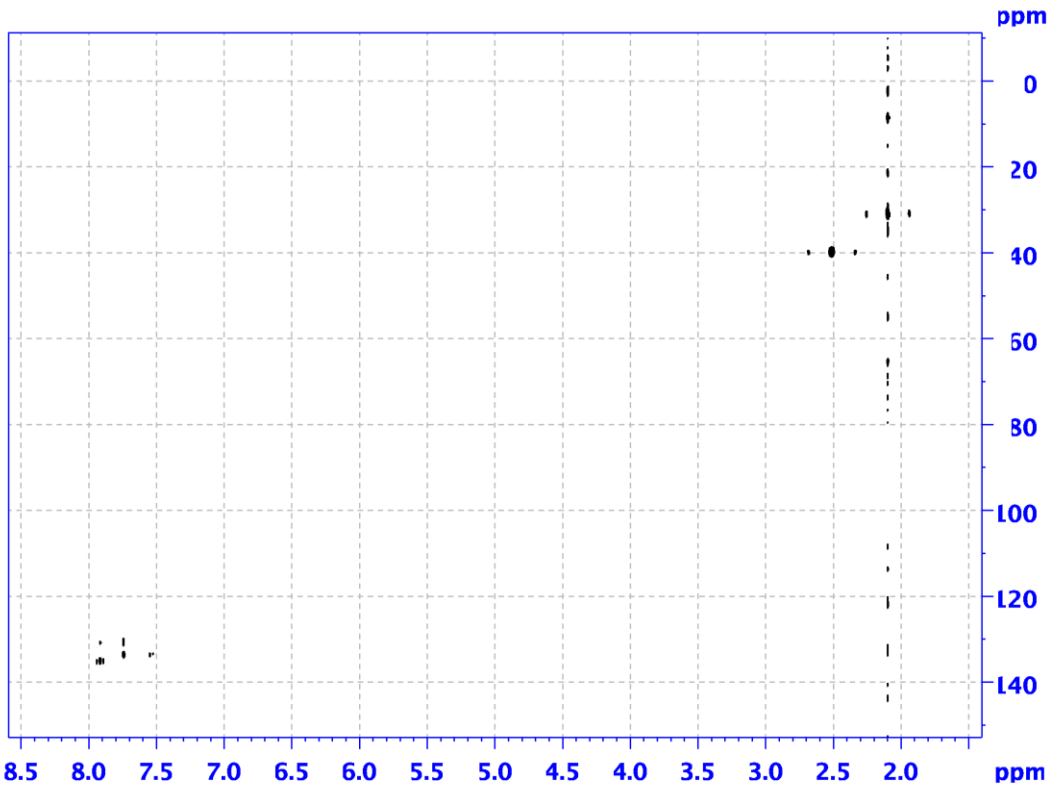
1.5.3 ^{1}H -^{1}H COSY



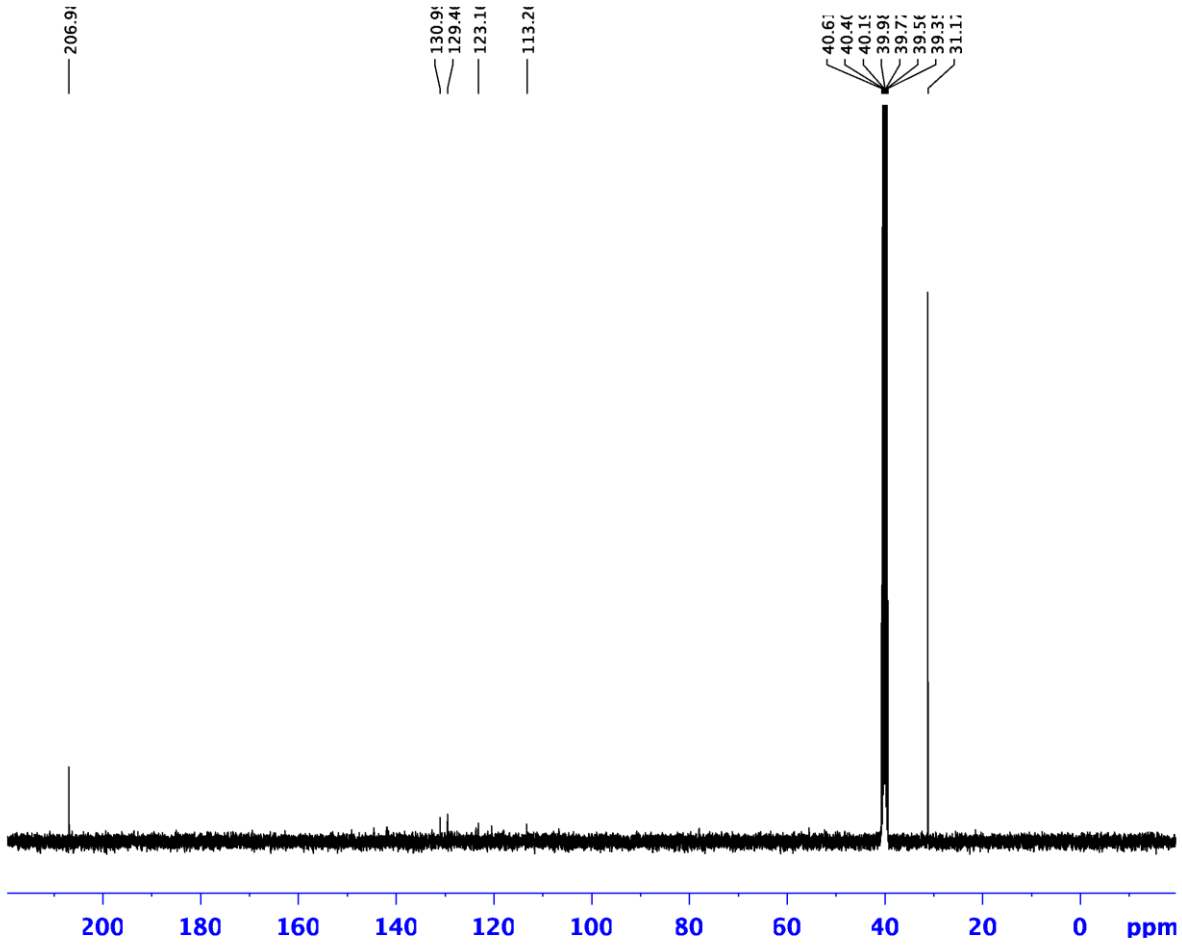
1.5.4 Expansion of COSY



1.5.5 ^{1}H-^{13}C HMBC

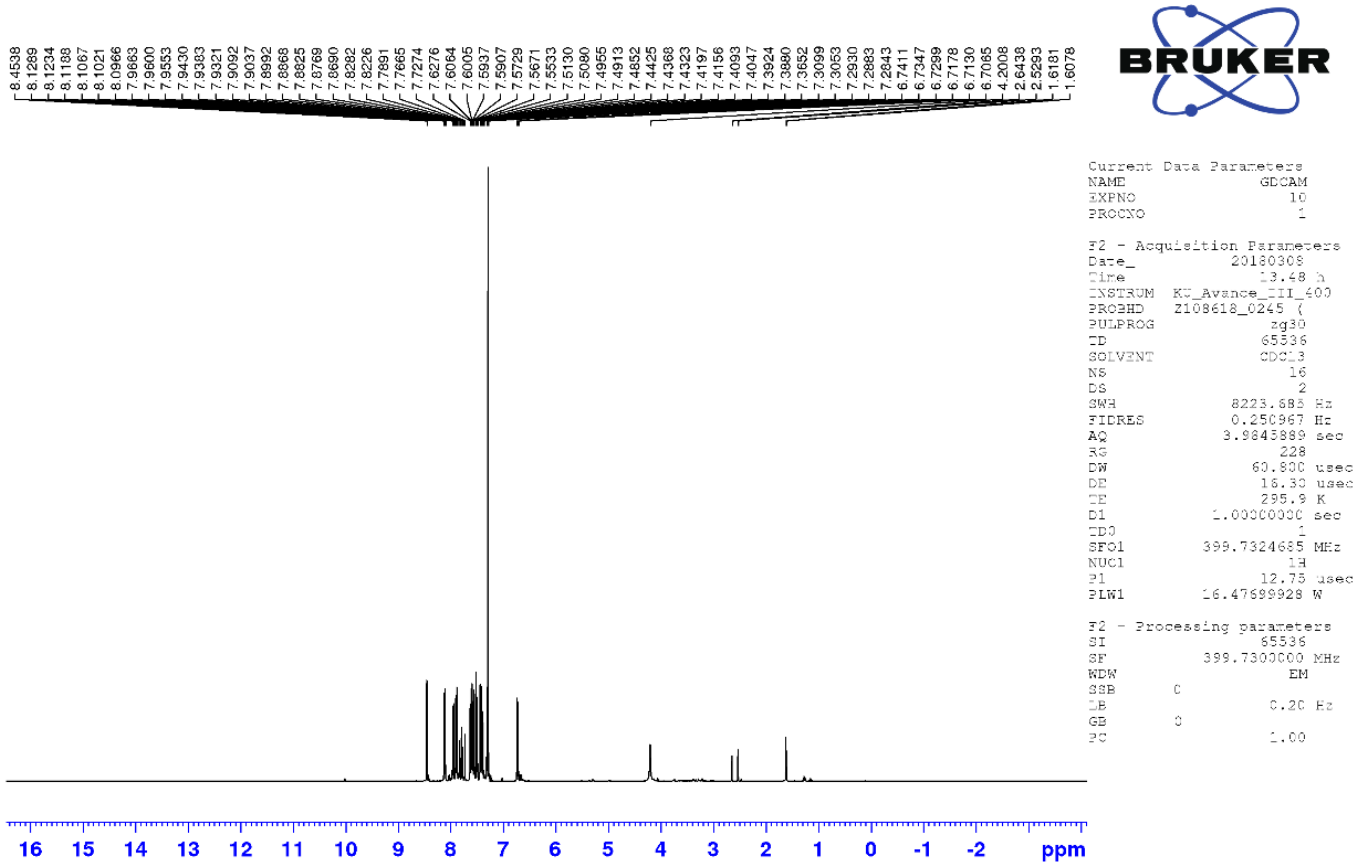


1.5.6 ^{13}C NMR


206.9
130.9
129.4
123.1
113.2
40.6
40.4
40.1
39.9
39.7
39.5
39.3
31.1
200
180
160
140
120
100
80
60
40
20
0
ppm

1.7 Compound 6

1.6.1 ^{1}H NMR

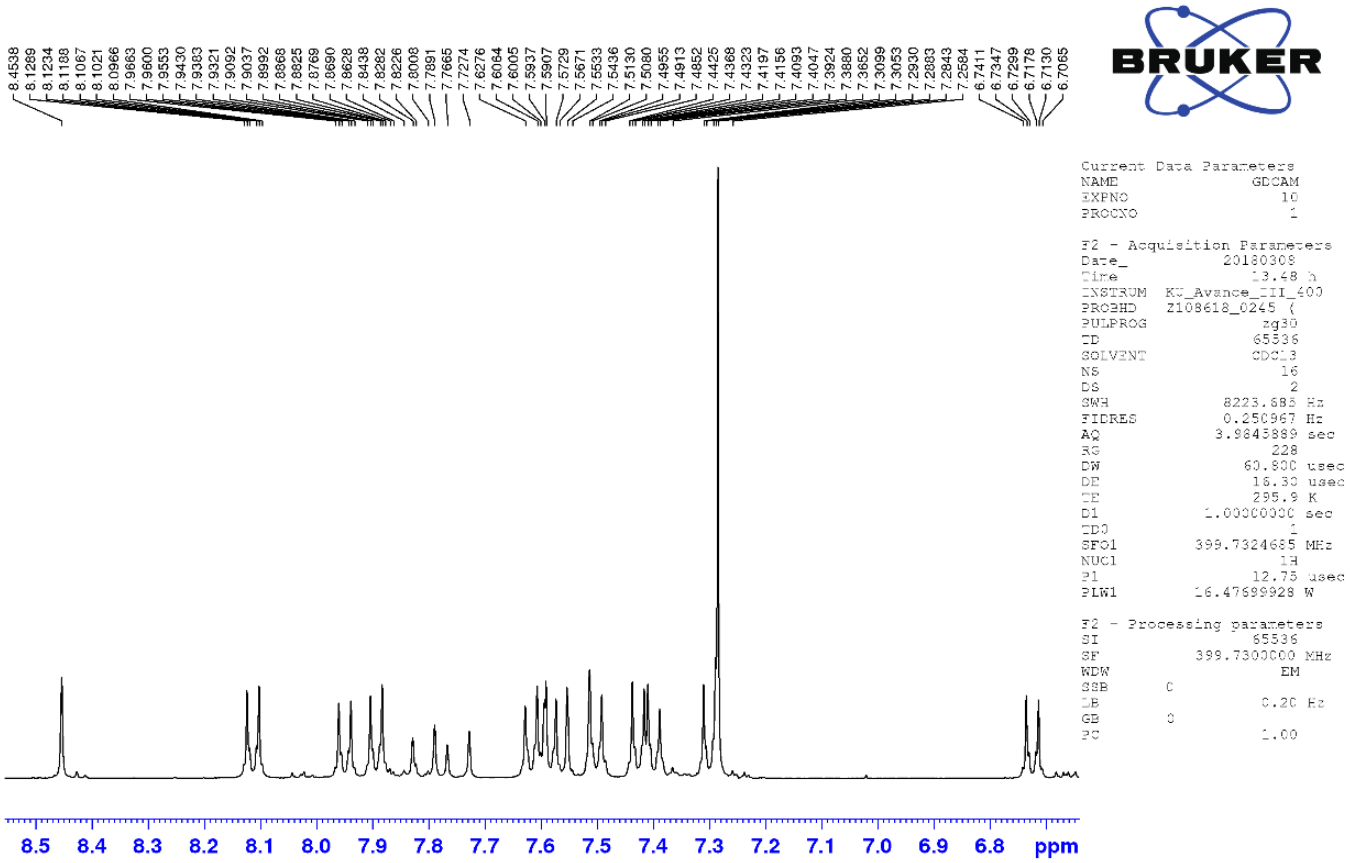



```
Current Data Parameters
NAME                  GDCAM
EXPNO                    10
PROCNO                    1

F2 - Acquisition Parameters
Date_              20180308
Time                 13.48 h
INSTRUM  KC_Avance_III_400
PROBHD   Z108618_0245 (
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                       16
DS                        2
SWH               8223.685 Hz
FIDRES            0.250967 Hz
AQ                3.9845889 sec
RG                      228
DW                 60.800 usec
DE                  16.30 usec
TE                 295.9 K
D1              1.00000000 sec
TD0                       1
SFO1          399.7324685 MHz
NUC1                    1H
P1                   12.75 usec
PLW1          16.47699928 W

F2 - Processing parameters
SI                    65536
SF            399.7300000 MHz
WDW                     EM
SSB       0
LB                  0.20 Hz
GB        0
PC                  1.00
```

1.6.2 Expansion of aromatic region

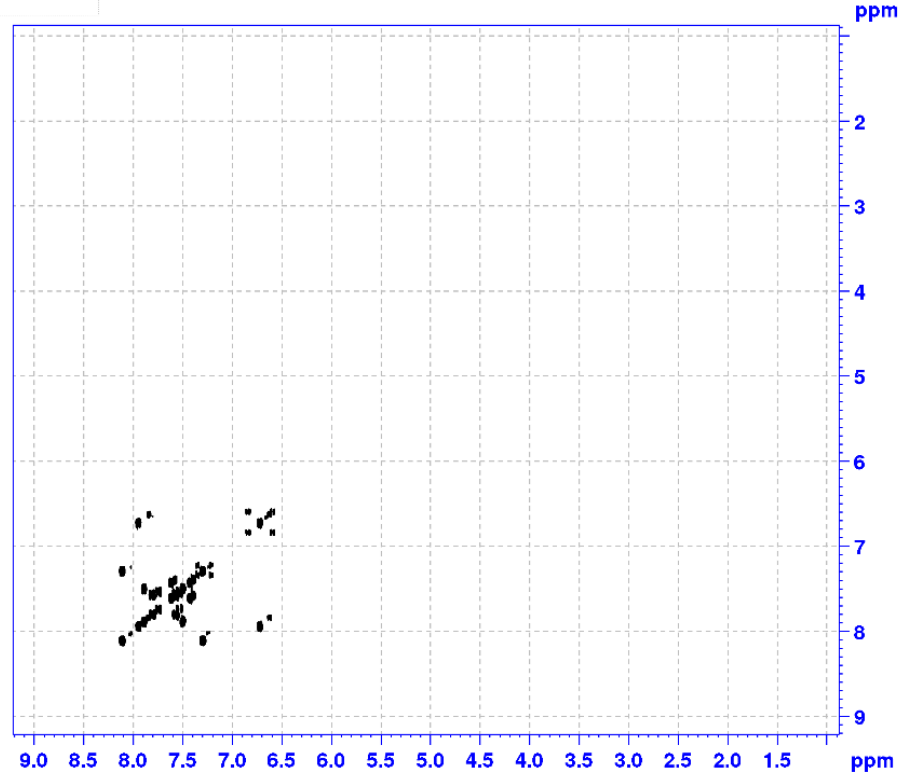



```
Current Data Parameters
NAME                  GDCAM
EXPNO                    10
PROCNO                    1

F2 - Acquisition Parameters
Date_              20180309
Time                 13.48 h
INSTRUM   KU_Avance_III_400
PROBHD    Z108618_0245 (
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                       16
DS                        2
SWH               8223.685 Hz
FIDRES            0.250967 Hz
AQ                3.9845889 sec
RG                      228
DW                   60.800 usec
DE                   16.30 usec
TE                   295.9 K
D1              1.00000000 sec
TD0                       1
SFO1           399.7324685 MHz
NUC1                    1H
P1                   12.75 usec
PLW1           16.47899928 W

F2 - Processing parameters
SI                    65536
SF              399.7300000 MHz
WDW                     EM
SSB          0
LB                    0.20 Hz
GB           0
PC                    1.00
```

1.6.3 ^{1}H -^{1}H COSY



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```
Current Data Parameters
NAME                  GDCAM
EXPNO                    32
PROCNO                    1

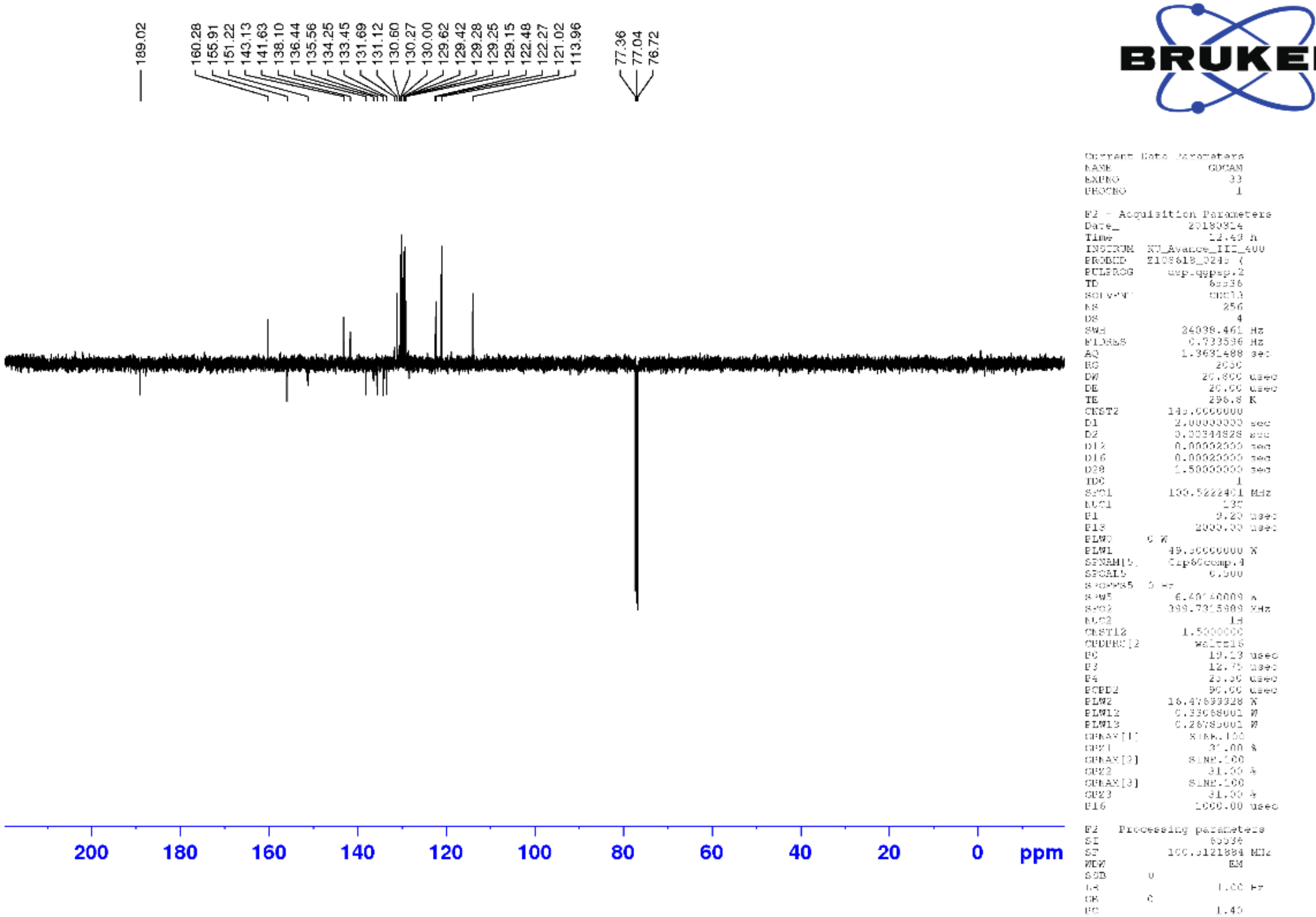
F2 - Acquisition Parameters
Date_              20180314
Time                 12.24 h
INSTRUM  XU_Avance_III_400
PROBHD   Z108618_0246 (
PULPROG      cosygpmfqf
TD                     2048
SOLVENT              CDCl3
NS                        2
DS                        8
SWH                3333.333 Hz
FIDRES            3.255208 Hz
AQ                0.3072000 sec
RG                     2030
DW                 150.000 usec
DE                   6.50 usec
TE                  296.3 K
D0                0.00000300 sec
D1                1.88448299 sec
D13               0.00000400 sec
D16               0.00020000 sec
IN0               0.00030000 sec
CPDPRG                 1
SFO1           399.7320135 MHz
NUC1                   1H
P1                   12.75 usec
PLW1          16.47699928 W
GPNAM[1]         SINE.100
GPZ1                 16.00 %
GPNAM[2]         SINE.100
GPZ2                 12.00 %
GPNAM[3]         SINE.100
GPZ3                 40.00 %
P16                1000.00 usec

F1 - Acquisition parameters
TD                      128
SFO1            399.732 MHz
FIDRES          52.083332 Hz
SW                  8.339 ppm
FnMODE                 QF

F2 - Processing parameters
SI                     1024
SF            399.7300000 MHz
WDW                    QSINE
SSB       0
LB        0 Hz
GB        0
PC                     1.40

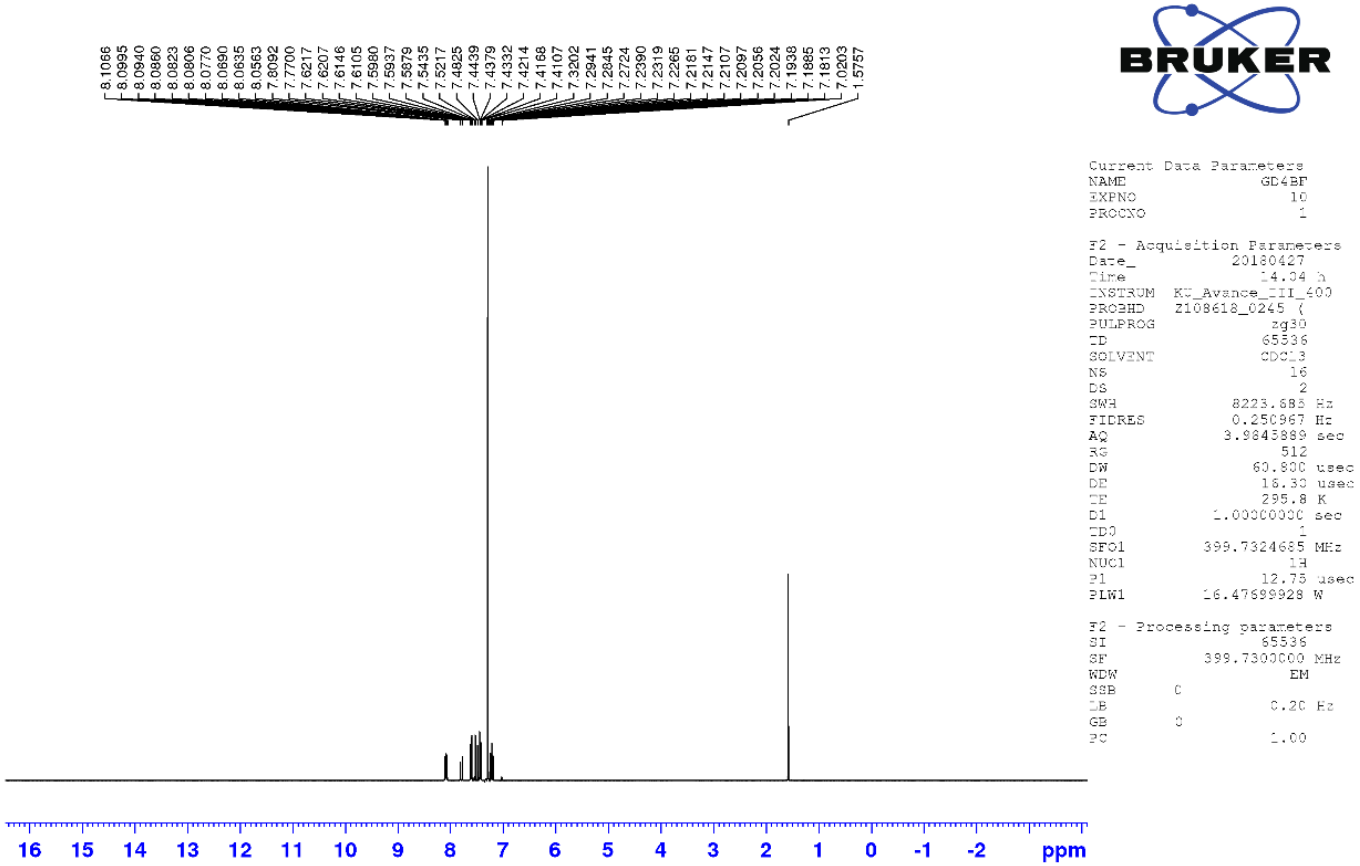
F1 - Processing parameters
SI                     1024
MC2                    QF
SF            399.7300000 MHz
WDW                    QSINE
SSB       0
LB        0 Hz
GB        0
```

1.6.4 ^{13}C NMR


189.02
160.28
155.91
151.22
143.13
141.63
138.10
136.44
135.56
134.25
133.45
131.69
131.12
130.60
130.27
130.00
129.62
129.42
129.28
129.25
129.15
122.48
122.27
121.02
113.96
77.36
77.04
76.72
200 180 160 140 120 100 80 60 40 20 0 ppm

1.7 Compound 7

1.7.1 ^{1}H NMR



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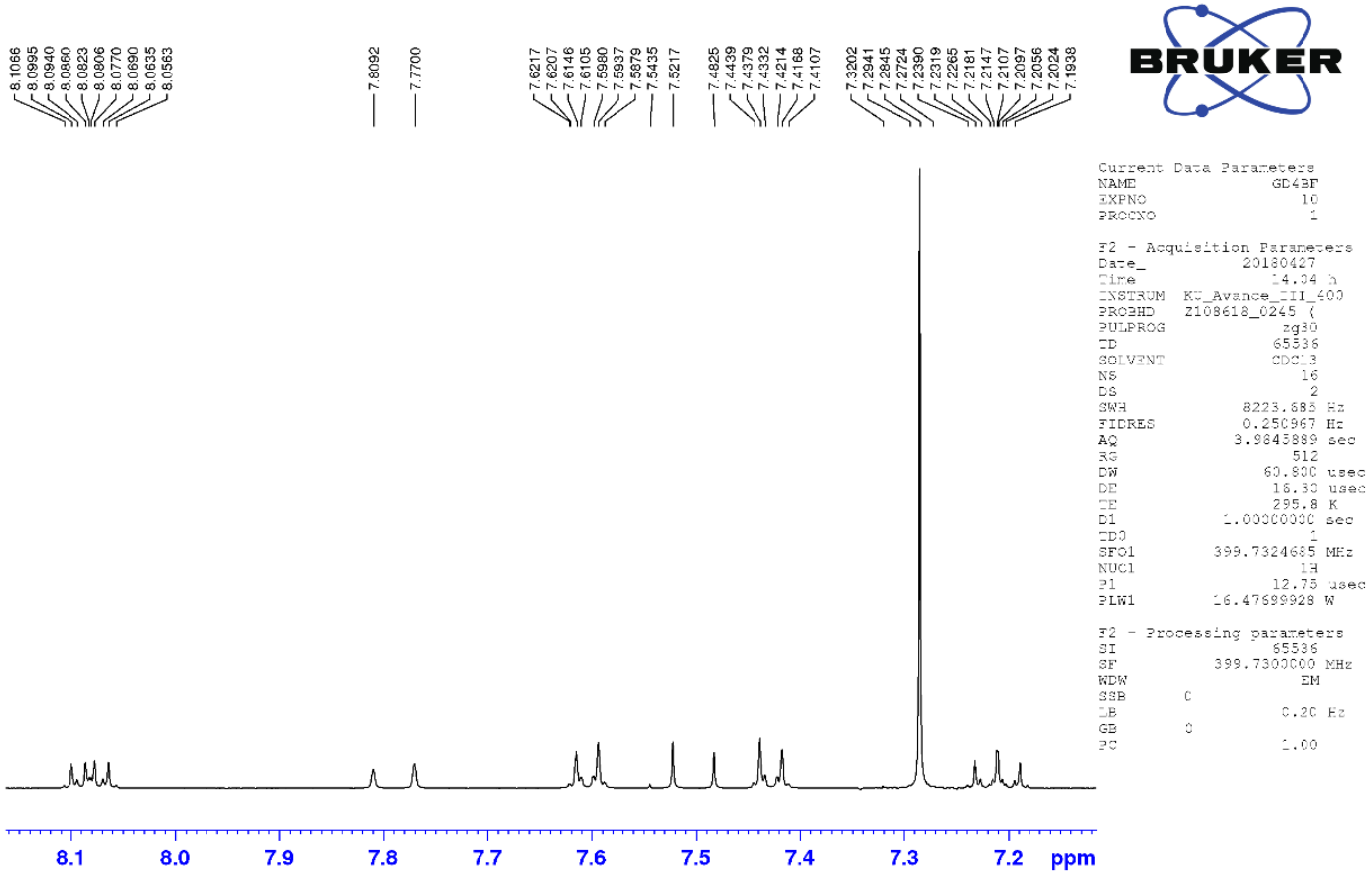
```
Current Data Parameters
NAME                  GD4BF
EXPNO                    10
PROCNO                    1

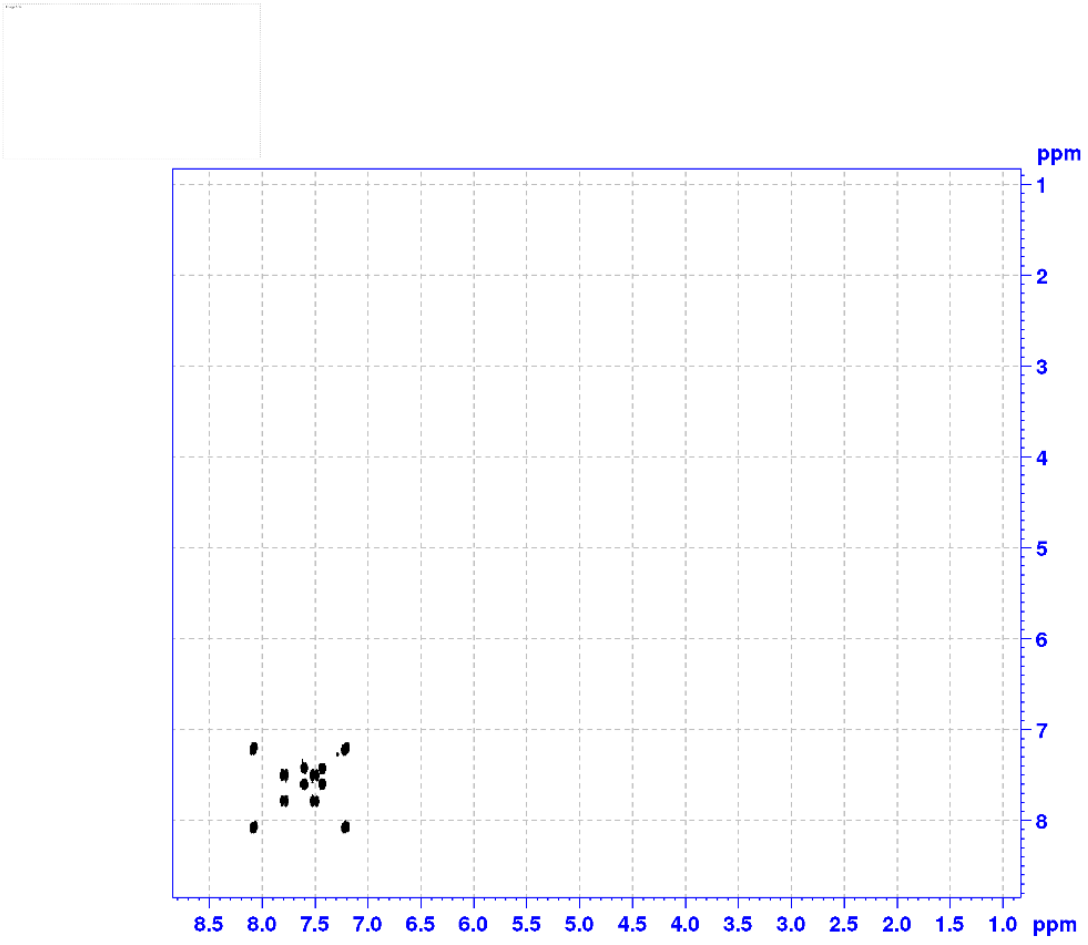
F2 - Acquisition Parameters
Date_              20180427
Time                 14.34 h
INSTRUM  KU_Avance_III_400
PROBHD   Z108618_0245 (
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                       16
DS                        2
SWH               8223.685 Hz
FIDRES            0.250967 Hz
AQ                3.9845889 sec
RG                      512
DW                 60.800 usec
DE                  16.30 usec
TE                 295.8 K
D1              1.00000000 sec
TD0                       1
SFO1           399.7324685 MHz
NUC1                    1H
P1                   12.75 usec
PLW1           16.47699928 W

F2 - Processing parameters
SI                    65536
SF             399.7300000 MHz
WDW                     EM
SSB       0
LB                 0.20 Hz
GB        0
PC                 1.00
```

1.7.2 Expansion of aromatic region



1.7.3 ^{1}H -^{1}H COSY



BRUKER

```
Current Data Parameters
NAME                 GM489
EXPNO                   12
PROCNO                   1

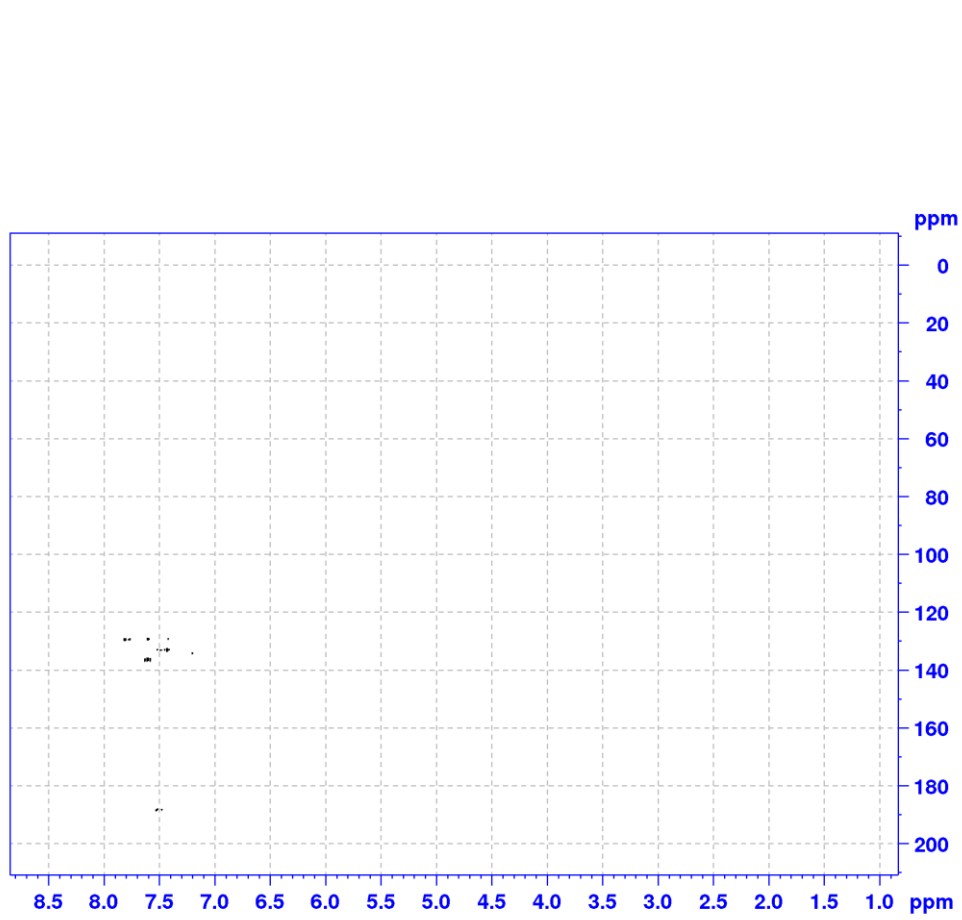
F2 - Acquisition Parameters
Date_              20180427
Time                 14.20 h
INSTRUM   XU_Avance_III_400
PROBHD    Z108618_0264 (
PULPROG       ccayqpnfqf
TD                    2048
SOLVENT               CDCl3
NS                       2
DS                       8
SWH               3205.128 Hz
FIDRES            3.130008 Hz
AQ                0.3194880 sec
RG                     203
DW                 156.000 usec
DE                   6.50 usec
TE                 295.0 K
D0                0.00000300 sec
D1                1.87220502 sec
D13               0.03000400 sec
D16               0.00020000 sec
NO                0.00037200 sec
IDbsv                    1
SFO1           399.7319336 MHz
NUC1                    1H
P1                   12.75 usec
PLW1          16.47699928 W
GPNAM[1]          SINE.100
GPZ1                 16.00 %
GPNAM[2]          SINE.100
GPZ2                 12.00 %
GPNAM[3]          SINE.100
GPZ3                 40.00 %
P16                1000.00 usec

F1 - Acquisition parameters
TD                    1024
SFO1           399.7319 MHz
FIDRES           50.380228 Hz
SW                 8.014 ppm
FnMODE                  QF

F2 - Processing parameters
SI                    1024
SF           399.7330020 MHz
WDW                   EM
SSB          0
LB           0.3 Hz
GB           0
PC                    1.40

F1 - Processing parameters
SI                    1024
MC2                   QF
SF           399.7330020 MHz
WDW                   EM
SSB          0
LB           0.3 Hz
GB           0
```

1.7.4 ^{1}H-^{13}C HMBC




```
Current Data Parameters
NAME                  GD4BF
EXPNO                    14
PROCNO                    1

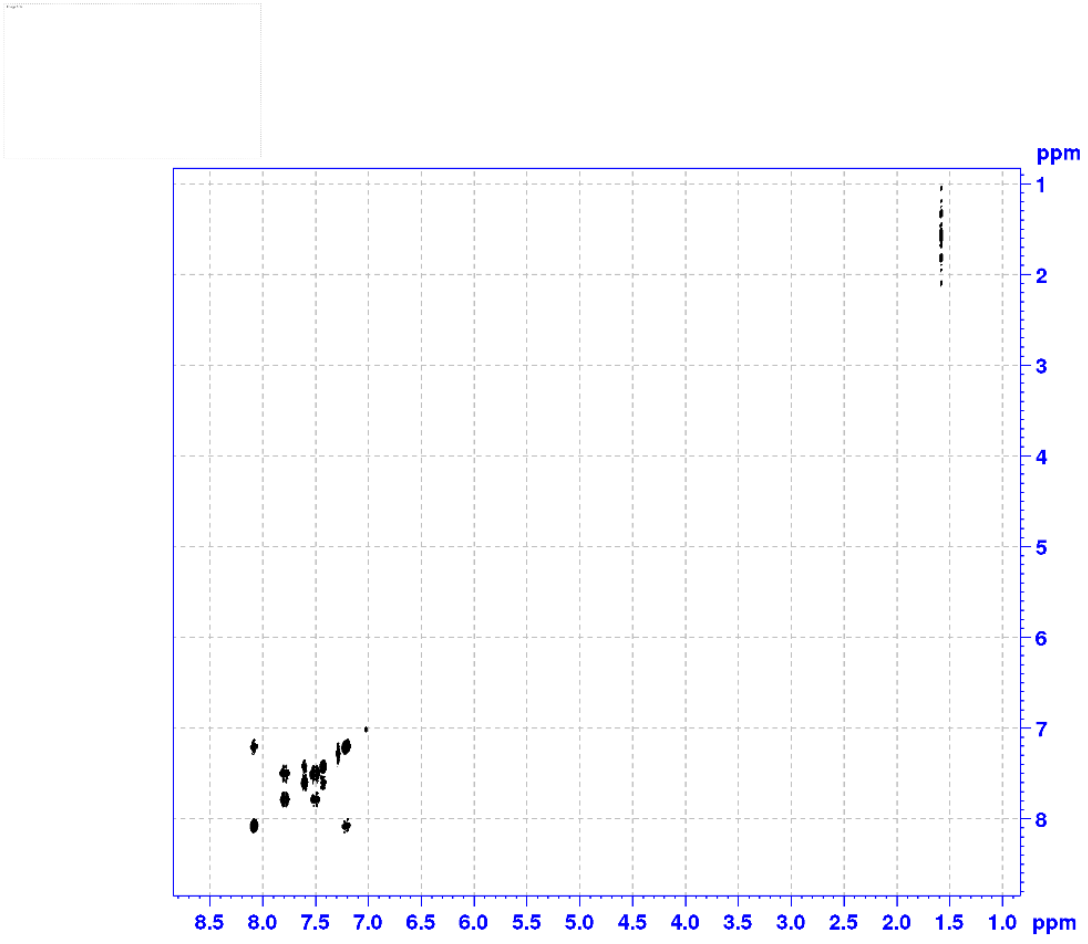
F2 - Acquisition Parameters
Date_              20180427
Time                  15.33 h
INSTRUM  KU_Avance_III_400
PROBHD   Z108618_0245 (
PULPROG     hmbcetgpl3nd
TD                     4096
SOLVENT               CDCl3
NS                        8
DS                       16
SWH                3205.128 Hz
FIDRES             1.565004 Hz
AQ                 0.6389760 sec
RG                      2050
DW                   156.000 usec
DE                     6.50 usec
TE                    296.3 K
CNST6            120.0000000
CNST7            165.0000000
CNST13            10.0000000
D0                 0.00000300 sec
D1                 1.25424004 sec
D6                 0.05000000 sec
D16                0.00020000 sec
IN0                0.00002240 sec
TDav                      1
SFO1              399.7319336 MHz
NUC1                      1H
P1                     12.75 usec
P2                     25.50 usec
PLW1              16.47699928 W
SFO2              100.5222241 MHz
NUC2                      13C
P3                      9.20 usec
P24                  2000.00 usec
PLW2              49.50000000 W
SPNAM[7]      Crp60comp.4
SPOAL7                 0.500
SPOFFS7   0 Hz
SPW7               6.40140009 W
GPNAM[1]            SINE.100
GPZ1                   80.00 %
GPNAM[3]            SINE.100
GPZ3                   14.00 %
GPNAM[4]            SINE.100
GPZ4                   -8.00 %
GPNAM[5]            SINE.100
GPZ5                   -4.00 %
GPNAM[6]            SINE.100
GPZ6                   -2.00 %
P16                  1000.00 usec

F1 - Acquisition parameters
TD                      128
SFO1               100.5222 MHz
FIDRES             348.772308 Hz
SW                 222.055 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                     2048
SF                399.7300000 MHz
WDW                    SINE
SSB                       2
LB          0 Hz
GB          0
PC                     1.40

F1 - Processing parameters
SI                     1024
MC2          echo-antiecho
SF                100.5121884 MHz
WDW                    SINE
SSB                       2
LB          0 Hz
GB          0
```

1.7.5 ^{1}H-^{1}H NOESY



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```
Current Data Parameters
NAME                  CD4BF
EXPNO                    15
PROCNO                    1

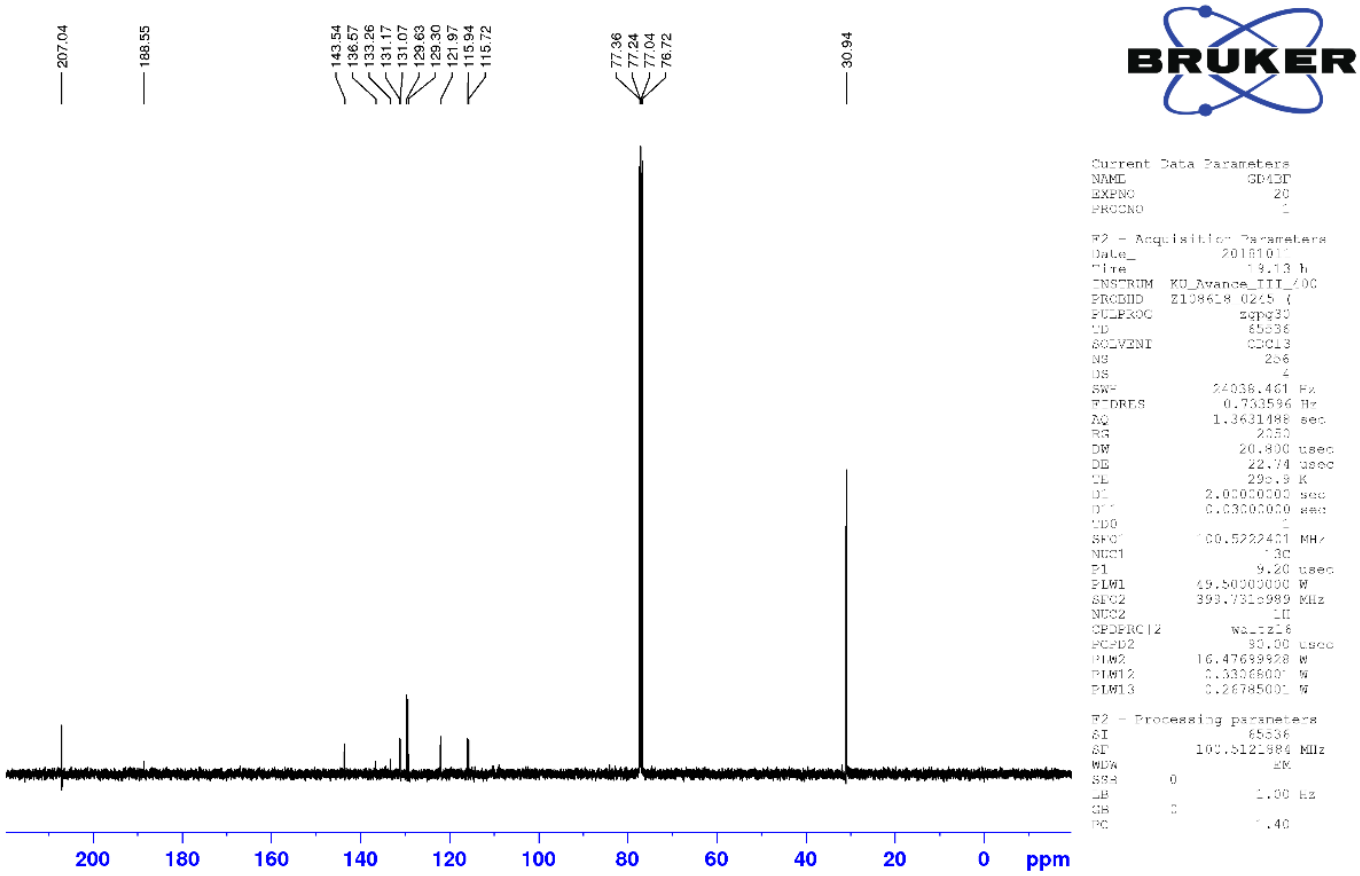
F2 - Acquisition Parameters
Date              20_83427
Time                 19.43 h
INSTRUM  KU Avance III 400
PROBHD   Z138618_0275 (
PULPROG          noesygpph
TD                    2048
SOLVENT               CDCl3
NS                       8
DS                       4
SWH               3205.128 Hz
FIDRES            3.132008 Hz
AQ                0.3194880 sec
RG                     362
DW                156.000 usec
DE                 21.34 usec
TE                 295.9 K
D0                0.00013977 sec
D1                1.93118703 sec
D8                0.35000001 sec
D16               0.00020000 sec
INO               0.00031200 sec
IDAV                     1
SFO1           399.7319336 MHz
NDC                     17
P1                   12.75 usec
P2                   25.50 usec
PL01             16.47899628 W
GPNAM[1]          SINE.100
GPZ1                 40.00 %
P16                1000.00 usec

F1 - Acquisition parameters
TD                     256
SFO1              399.7319 MHz
FIDRES           25.000000 Hz
SW                   8.008 ppm
FnMODE       States-TPPI

F2 - Processing parameters
SI                     1024
SF             399.7300000 MHz
WDW                  QSINE
SSB                       2
LB          0 Hz
GB          0
PC                     1.00

F1 - Processing parameters
SI                     1024
MC2          States-TPPI
SF             399.7300000 MHz
WDW                  QSINE
SSB                       2
LB          0 Hz
GB          0
```

1.7.6 ^{13}C NMR



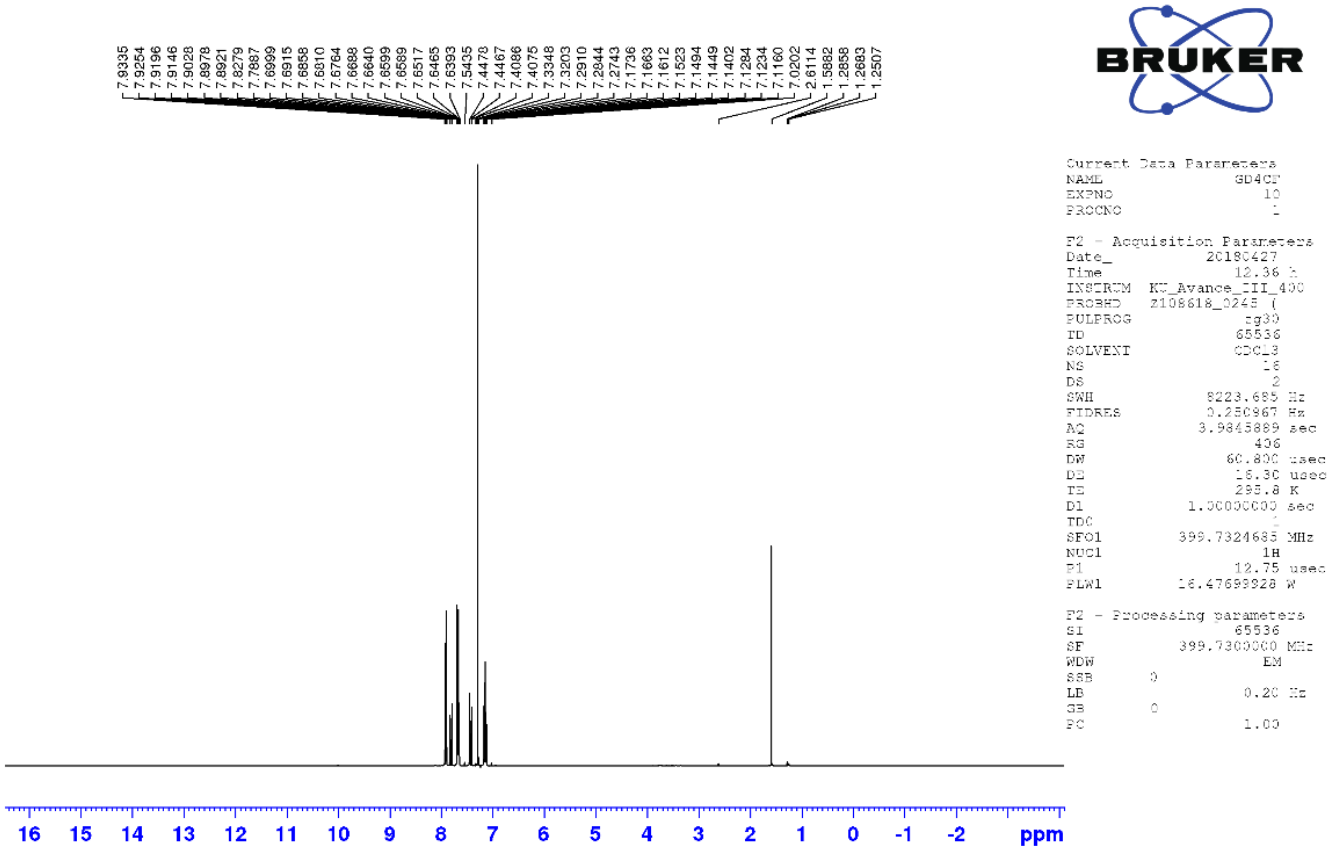

```
Current Data Parameters
NAME                  SD43F
EXPNO                    20
PROCNO                    1

F2 - Acquisition Parameters
Date_              20181011
Time                 19.13 h
INSTRUM  KU_Avance_III_400
PROBHD   Z108618_0225_C
PULPROG            zgpg30
TD                  65536
SOLVENT             CDCl3
NS                    256
DS                      4
SWH              24038.461 Hz
FIDRES           0.733596 Hz
AQ               1.3631488 sec
RG                    2050
DW                  20.800 usec
DE                  22.74 usec
TE                  296.8 K
D1               2.00000000 sec
D11              0.03000000 sec
TD0                     1
SFO1           100.5222401 MHz
NUC1                  13C
P1                   9.20 usec
PLW1          49.50000000 W
SFO2          399.7316989 MHz
NUC2                  1H
CPDPRG[2]          waltz16
PCPD2                80.00 usec
PLW2          16.47699928 W
PLW12          0.33068001 W
PLW13          0.26785001 W

F2 - Processing parameters
SI                   65536
SF            100.5122984 MHz
WDW                     EM
SSB       0
LB                    1.00 Hz
GB        0
PC                    1.40
```

1.8 Compound 8

1.8.1 ^{1}H NMR

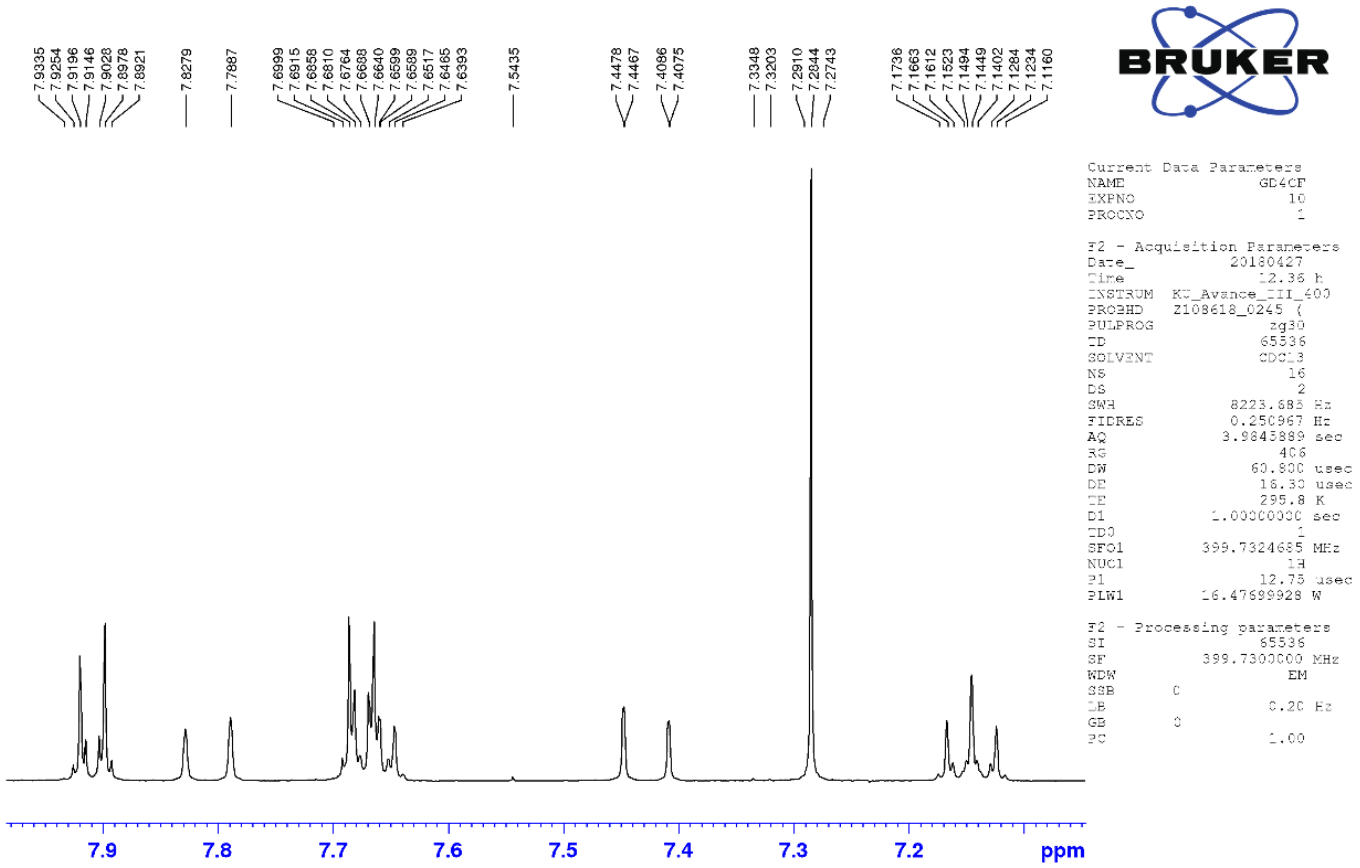



```
Current Data Parameters
NAME                 GD4CT
EXPNO                   10
PROCNO                   1

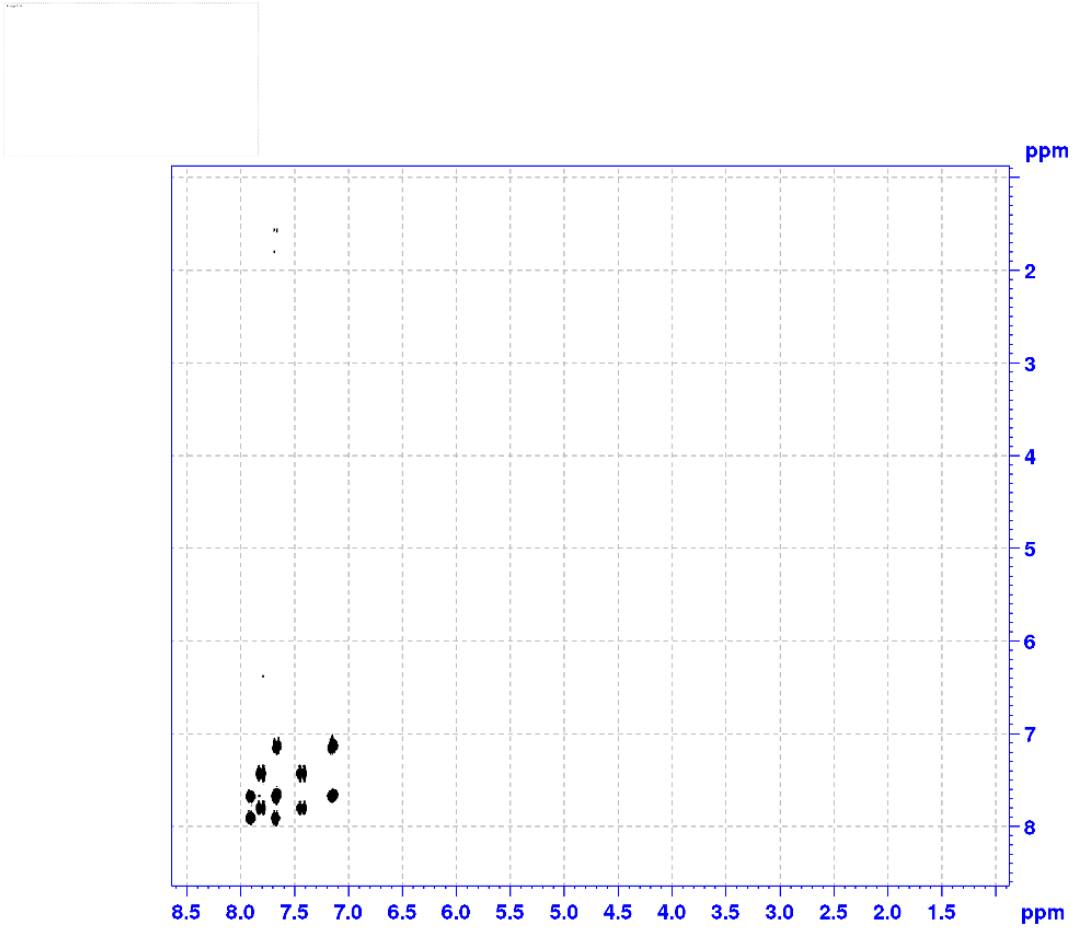
F2 - Acquisition Parameters
Date_           20180427
Time                12.36 h
INSTRUM  KU_Avance_III_400
PROBHD   Z108618_0245 (
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                       16
DS                        2
SWH                9223.685 Hz
FIDRES             0.280967 Hz
AQ                 3.9845889 sec
RG                      406
DW                   60.800 usec
DE                   18.30 usec
TE                   298.8 K
D1              1.00000000 sec
TDC                       1
SFO1           399.7324685 MHz
NUC1                    1H
P1                   12.75 usec
PLW1           16.47699928 W

F2 - Processing parameters
SI                    65536
SF             399.7300000 MHz
WDW                     EM
SSB          0
LB                    0.20 Hz
GB           0
PC                    1.00
```

1.8.2 Expansion of aromatic region



1.8.3 ^{1}H -^{1}H COSY



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```
Current Data Parameters
NAME                 G040F
EXPNO                   12
PROCNO                   1

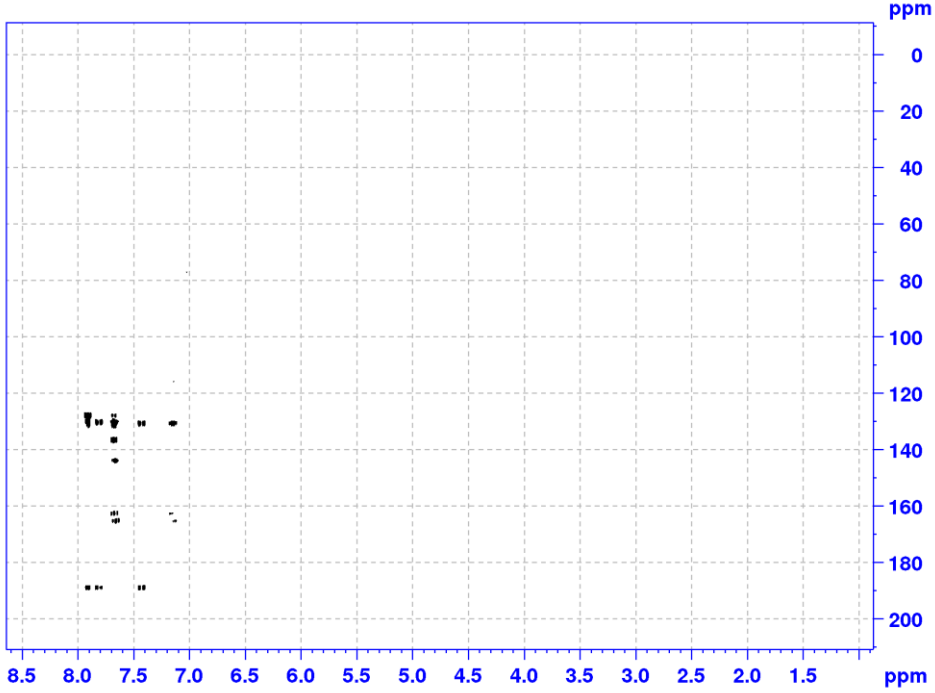
F2 - Acquisition Parameters
Date_             20180427
Time                12.51 h
INSTRUM  XU_Avance_III_400
PROBHD   Z108618_0260 (
PULPROG         cosygpmfqf
TD                    2048
SOLVENT               CDCl3
NS                       2
DS                       8
SWH               3105.590 Hz
FIDRES            3.032803 Hz
AQ                0.3297280 sec
RG                     2050
DW                 161.000 usec
DE                   6.50 usec
TE                  298.0 K
D0                0.00000300 sec
D1                1.86196494 sec
D13               0.00000400 sec
D16               0.00020000 sec
 NS               0.00032200 sec
CDav                     1
SFO1           399.7319010 MHz
NUC1                    1H
P1                   12.75 usec
P.W1              16.4769998 W
GPNAM[1]          SINE.100
GPZ1                 16.00 %
GPNAM[2]          SINE.100
GPZ2                 12.00 %
GPNAM[3]          SINE.100
GPZ3                 40.00 %
P16                1000.00 usec

F1 - Acquisition parameters
TD                     128
SFO1           399.7319 MHz
FIDRES          48.526845 Hz
SW                  7.765 ppm
FnMODE                 QF

F2 - Processing parameters
SI                    1024
SF           399.7300000 MHz
WDW                    SINE
SSB       0
LB        0 Hz
GB        0
PC                    1.40

F1 - Processing parameters
SI                    1024
MC2                     QF
SF           399.7300000 MHz
WDW                    SINE
SSB       0
LB        0 Hz
GB        0
```

1.8.4 ^{1}H-^{13}C HMBC




```
Current Data Parameters
NAME                  GD4CF
EXPNO                    14
PROCNO                    1

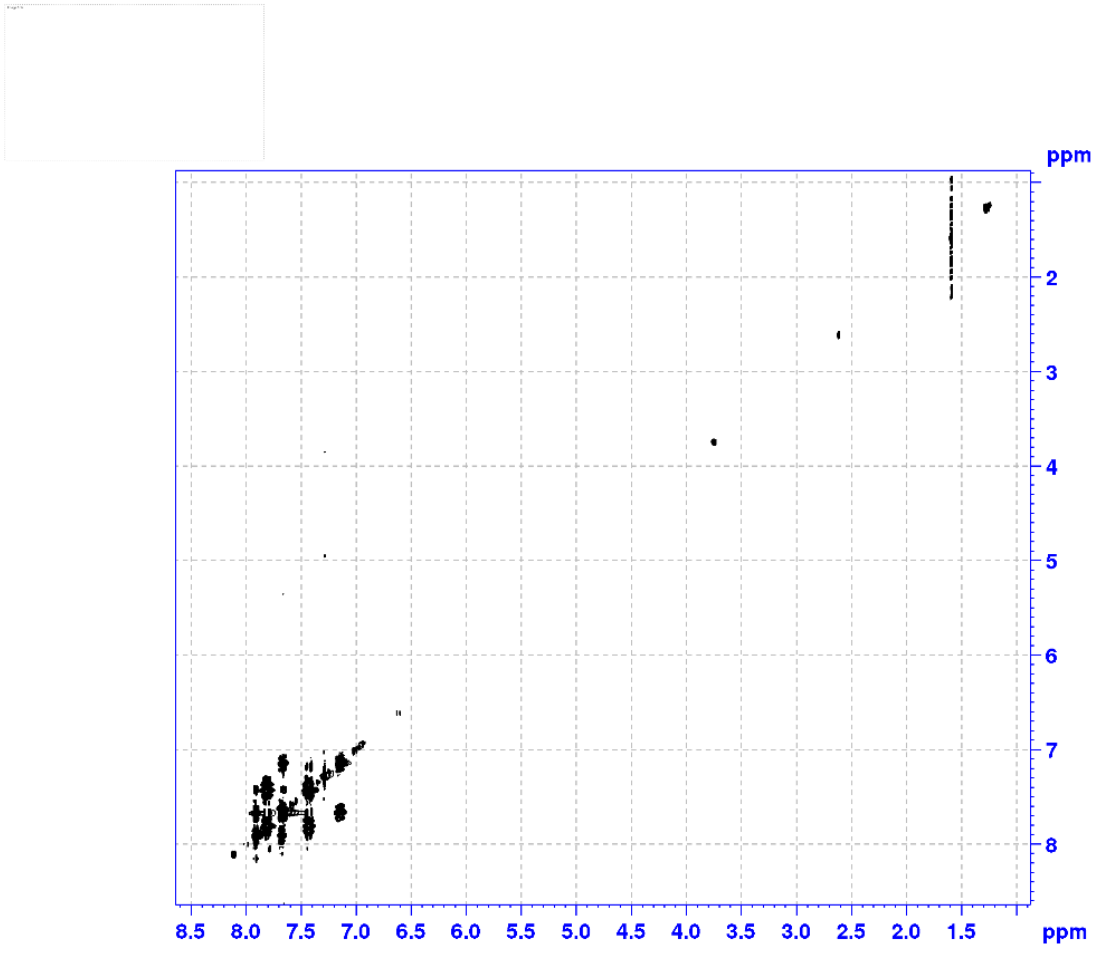
F2 - Acquisition Parameters
Date_              20180427
Time                  13.26 h
INSTRUM  KU_Avance_III_400
PROBHD   Z108618_0245 (
PULPROG    hmbcetgpl3nd
TD                     4096
SOLVENT             CDCl3
NS                        8
DS                       16
SWH                3105.590 Hz
FIDRES             1.516401 Hz
AQ                 0.6594560 sec
RG                      2050
DW                   161.000 usec
DE                     6.50 usec
TE                    296.2 K
CNST6            120.0000000
CNST7            165.0000000
CNST13            10.0000000
D0                 0.00000300 sec
D1                 1.23376000 sec
D6                 0.05000000 sec
D16                0.00020000 sec
IN0                0.00002240 sec
TDav                      1
SFO1             399.7319010 MHz
NUC1                     1H
P1                     12.75 usec
P2                     25.50 usec
PLW1             16.47699928 W
SFO2             100.5222241 MHz
NUC2                    13C
P3                      9.20 usec
P24                  2000.00 usec
PLW2             49.50000000 W
SPNAM[7]     Crp60comp.4
SPOAL7                0.500
SPOFFS7  0 Hz
SPW7              6.40140009 W
GPNAM[1]           SINE.100
GP21                  80.00 %
GPNAM[3]           SINE.100
GP23                  14.00 %
GPNAM[4]           SINE.100
GP24                  -8.00 %
GPNAM[5]           SINE.100
GP25                  -4.00 %
GPNAM[6]           SINE.100
GP26                  -2.00 %
P16                 1000.00 usec

F1 - Acquisition parameters
TD                       128
SFO1              100.5222 MHz
FIDRES         348.772308 Hz
SW                 222.055 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                      2048
SF               399.7300000 MHz
WDW                     SINE
SSB                        2
LB         0 Hz
GB         0
PC                      1.40

F1 - Processing parameters
SI                      1024
MC2          echo-antiecho
SF                100.5121884 MHz
WDW                     SINE
SSB                        2
LB         0 Hz
GB         0
```

1.8.5 ^{1}H-^{1}H NOESY




```
Current Data Parameters
NAME                  CD4CF
EXPNO                    15
PROCNO                    1

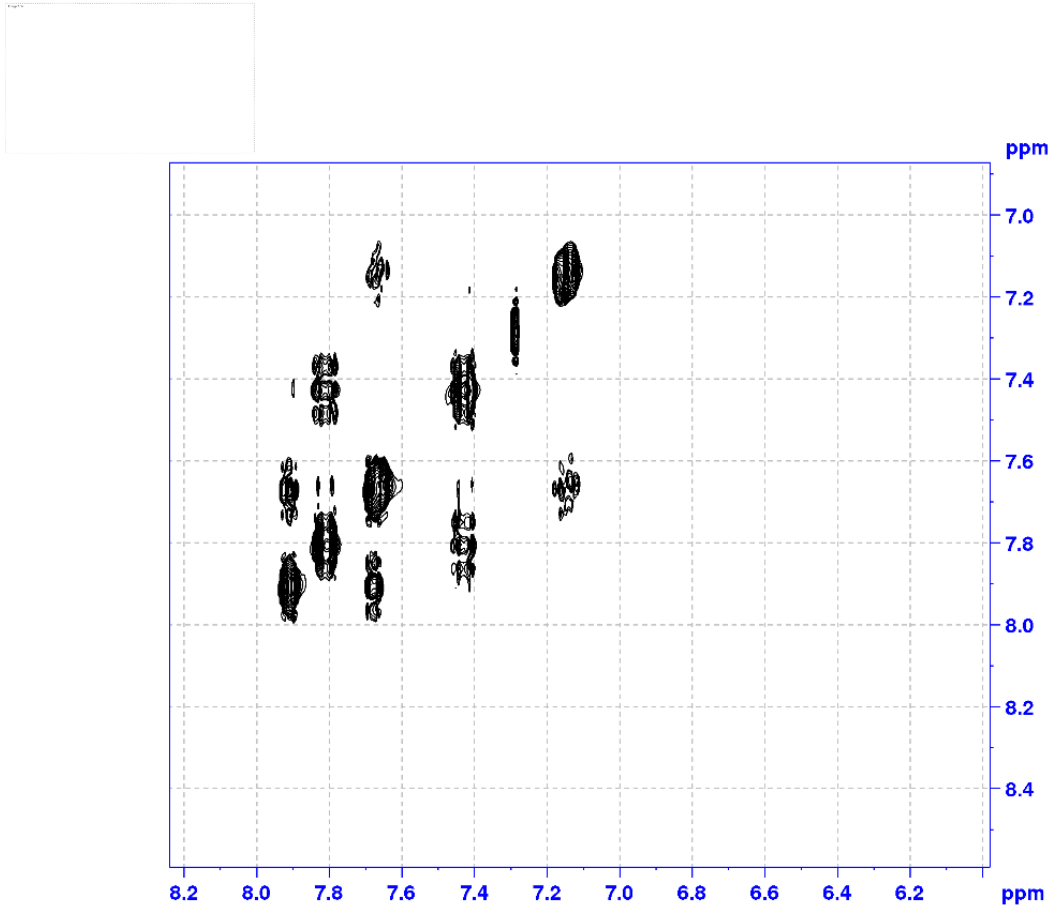
F2 - Acquisition Parameters
Date              20_80427
Time                 18.17 h
INSTRUM  BC Avance III 400
PROBHD   5108618_0215 (
PULPROG          noesygpph
TD                     2048
SOLVENT               CDCl3
NS                        8
DS                        4
SWH               3185.590 Hz
FIDRES               3.032803 Hz
AQ                0.3297290 sec
RG                      287
DW                  157.000 usec
DE                   22.29 usec
TE                  295.7 K
D0                0.00014477 sec
D1                1.92294686 sec
D8                0.30000001 sec
D16               0.00200000 sec
INO               0.00032200 sec
IDAV                      1
SFO1           399.73190_0 MHz
NDC1                     17
P1                   12.75 usec
P2                   25.50 usec
PL01             16.47699928 W
GPNAM[1]          SINE.100
GPZ1                 40.30 %
P16                1000.00 usec

F1 - Acquisition parameters
TD                      256
SFO1              399.7319 MHz
FIDRES           24.262422 Hz
SW                 7.769 ppm
FnMODE         States-TPPI

F2 - Processing parameters
SI                      1024
SF             399.7300000 MHz
WDW                   QSINE
SSB                       2
LB          0 Hz
GB          0
PC                     1.00

F1 - Processing parameters
SI                      1024
MC2            States-TPPI
SF             399.7300000 MHz
WDW                   QSINE
SSB                       2
LB          0 Hz
GB          0
```

1.8.6 Expansion of NOESY



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```
Current Data Parameters
NAME                  CD4CF
EXPNO                    15
PROCNO                    1

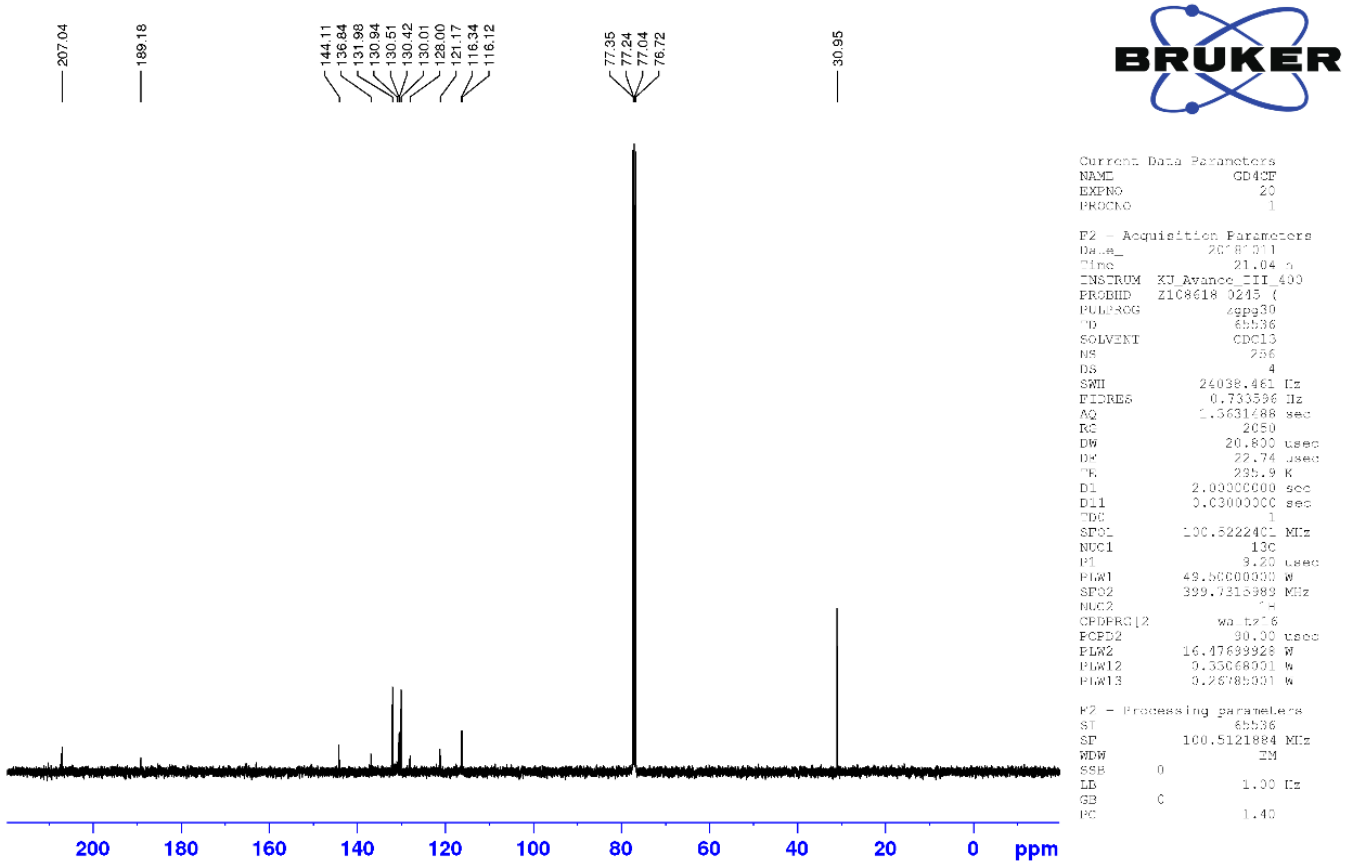
F2 - Acquisition Parameters
Date              20_80427
Time                 8.17 h
INSTRUM   KL Avance III 600
PROBHD    5 mm PABBO BB/
PULPROG          noesygpph
TD                     2048
SOLVENT                CDCl3
NS                        8
DS                        4
SWH               3185.590 Hz
FIDRES               3.6378 Hz
AQ                0.3297280 sec
RG                      287
DW                  157.000 usec
DE                   22.28 usec
TE                  295.7 K
D0                0.00014477 sec
D1                1.92394696 sec
D8                0.30000001 sec
D16               0.00020000 sec
IN0               0.00032200 sec
TD0                       1
SFO1           399.78190_0 MHz
NUC1                    1H
P1                   12.75 usec
P2                   25.50 usec
PL01             16.47899928 W
GPNAM[1]          SINE.100
GPZ1                 40.00 %
P16                1000.00 usec

F1 - Acquisition parameters
TD                      256
SFO1             399.7319 MHz
FIDRES           24.262423 Hz
SW                   7.769 ppm
FnMODE           States-TPPI

F2 - Processing parameters
SI                      1024
SF               399.7300000 MHz
WDW                    QSINE
SSB                        2
LB          0 Hz
GB          0
PC                      1.00

F1 - Processing parameters
SI                      1024
MC2              States-TPPI
SF               399.7300000 MHz
WDW                    QSINE
SSB                        2
LB          0 Hz
GB          0
```

1.8.7 ^{13}C NMR



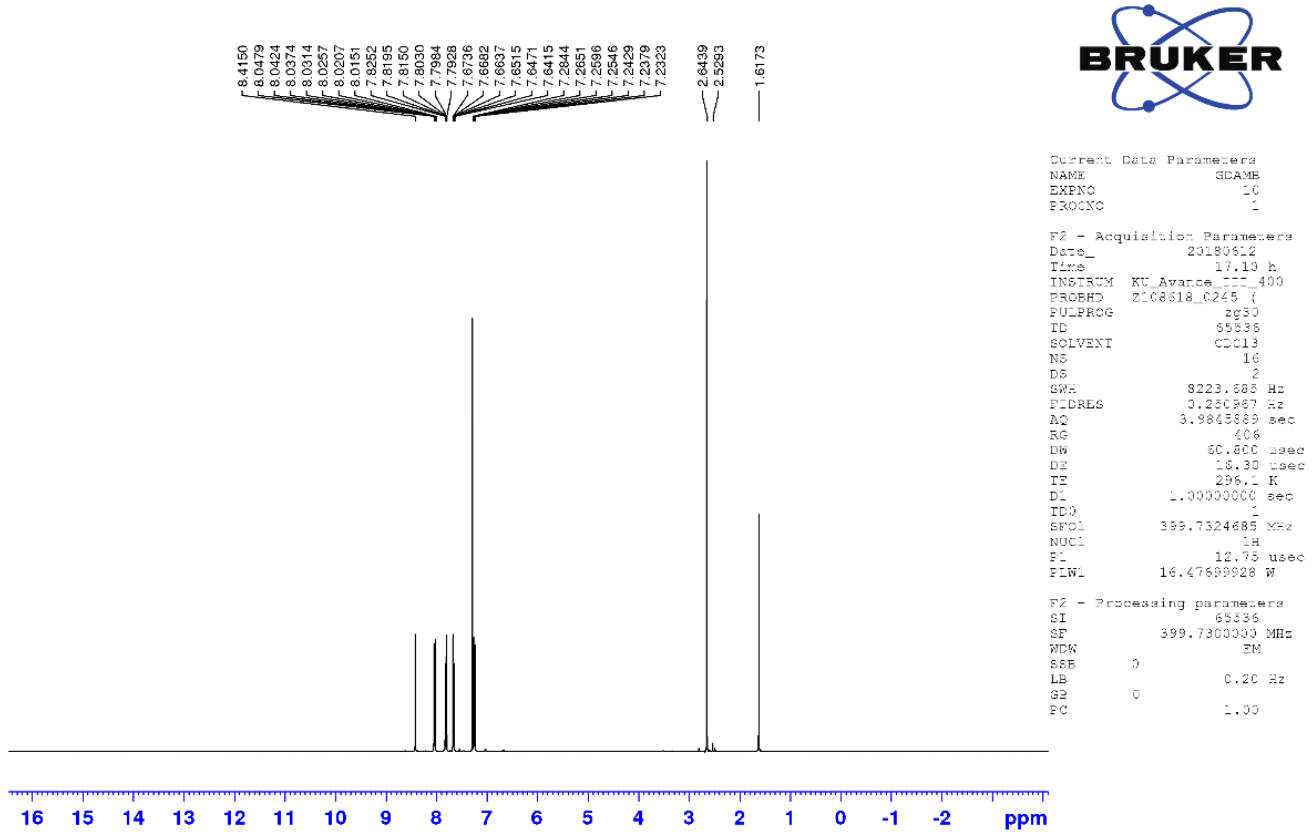

```
Current Data Parameters
NAME                  GD4CF
EXPNO                    20
PROCNO                    1

F2 - Acquisition Parameters
Date_          20?8?011
Time                 21.04 h
INSTRUM  KJ_Avance_III_400
PROBHD   Z108618 0215 (
PULPROG              zgpg30
TD                    65536
SOLVENT               CDCl3
NS                      256
DS                        4
SWH               24038.461 Hz
FIDRES             0.733596 Hz
AQ                 1.3631488 sec
RG                      2050
DW                   20.800 usec
DE                   22.74 usec
TE                   295.9 K
D1                2.00000000 sec
D11               0.03000000 sec
TDC                       1
SFO1         100.5222401 MHz
NUC1                    13C
P1                    9.20 usec
PLW1         49.50000000 W
SFO2         399.7815989 MHz
NUC2                    ?H
CPDPRG[2          wa_tp?6
PCPD2                30.00 usec
PLW2         16.47699928 W
PLW12         0.35068001 W
PLW13         0.26785001 W

F2 - Processing parameters
SI                    65536
SF           100.5121884 MHz
WDW                     EM
SSB       0
LB                    1.00 Hz
GB        0
PC                    1.40
```

1.9 Compound 9

1.9.1 ^{1}H NMR

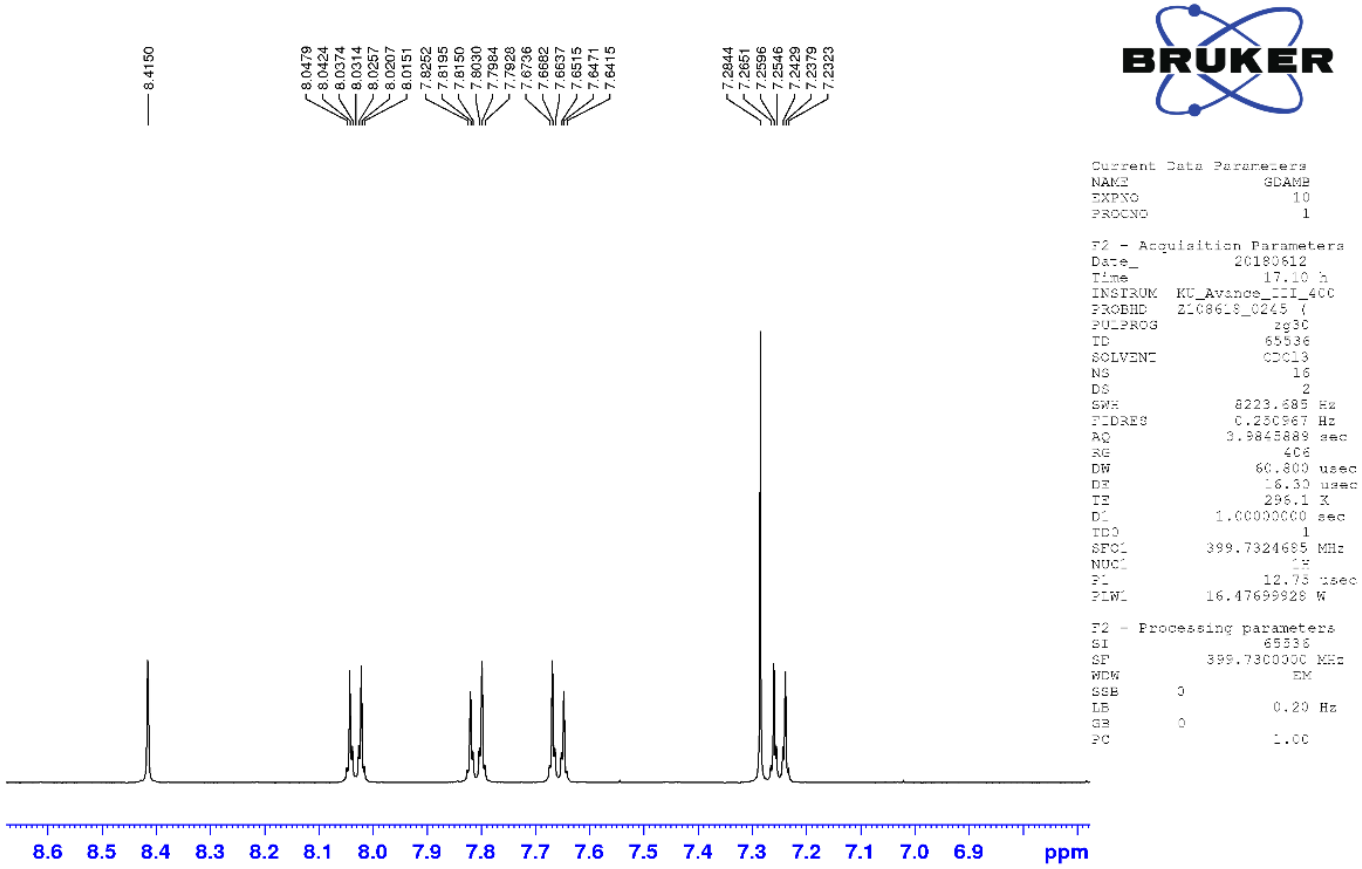



```
Current Data Parameters
NAME                  GDAMB
EXPNO                    10
PROCNO                    1

F2 - Acquisition Parameters
Date_              20180612
Time                  17.10 h
INSTRUM  KU_Avance_III_400
PROBHD   Z108618_0245 (
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                       16
DS                        2
SWH                8223.685 Hz
FIDRES             0.250967 Hz
AQ                 3.9845889 sec
RG                      406
DW                   60.800 usec
DE                   16.30 usec
TE                   296.1 K
D1                 1.00000000 sec
TD0                       1
SFO1           399.7324685 MHz
NUC1                     1H
P1                    12.75 usec
PLW1           16.47699928 W

F2 - Processing parameters
SI                    65536
SF             399.7300000 MHz
WDW                      EM
SSB       0
LB                    0.20 Hz
GB        0
PC                    1.00
```

1.9.2 Expansion of aromatic region



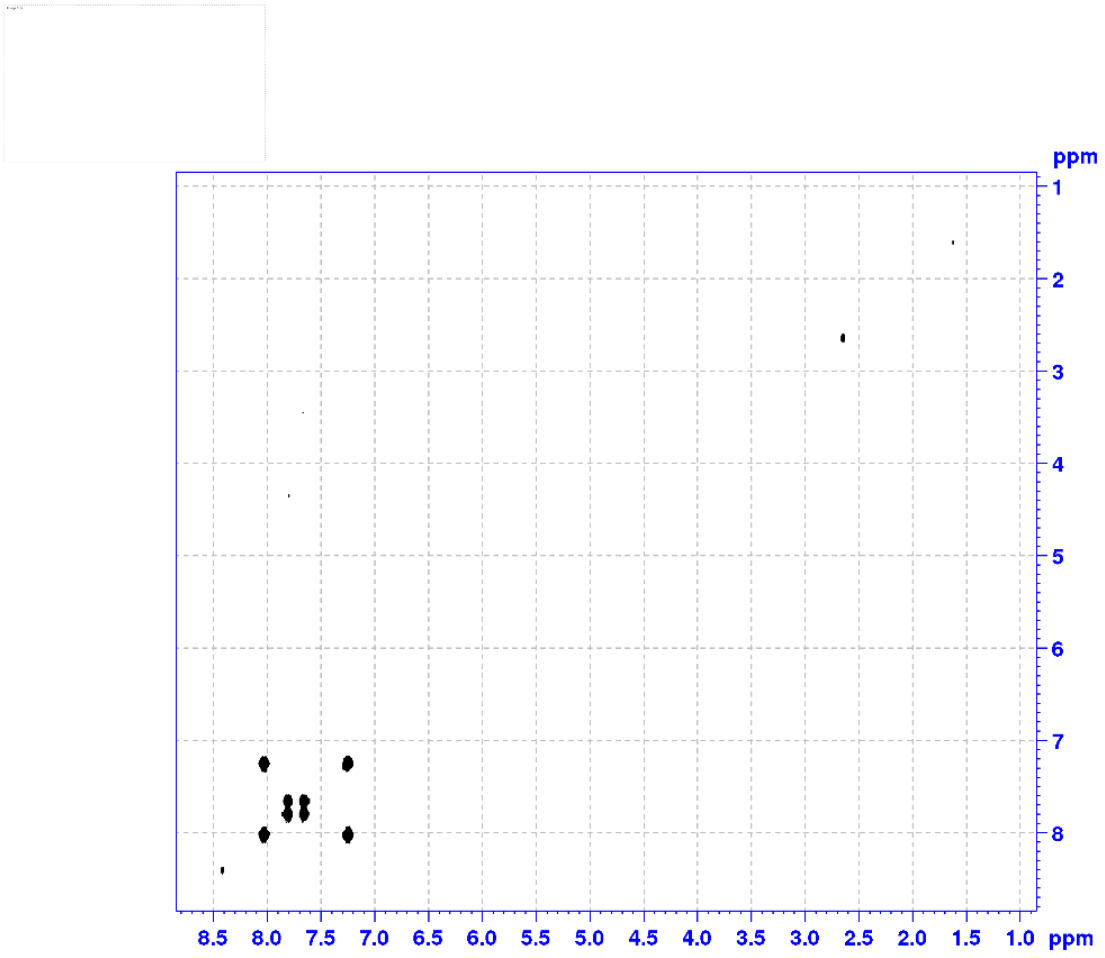
BRUKER

```
Current Data Parameters
NAME                 GDAMB
EXPNO                   10
PROCNO                   1

F2 - Acquisition Parameters
Date_              20180612
Time                 17.10 h
INSTRUM  KU_Avance_III_400
PROBHD   Z108619_0245 (
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                       16
DS                        2
SWH                8223.685 Hz
FIDRES             0.250967 Hz
AQ                 3.9845888 sec
RG                      406
DW                   60.800 usec
DE                    16.30 usec
TE                   296.1 K
D1                1.00000000 sec
TD0                       1
SFO1            399.7324695 MHz
NUC1                      1H
P1                   12.75 usec
PLW1           16.47699928 W

F2 - Processing parameters
SI                    65536
SF              399.7300000 MHz
WDW                      EM
SSB          0
LB                    0.20 Hz
GB           0
PC                    1.00
```

1.9.3 ^{1}H -^{1}H COSY



BRUKER

```
Current Data Parameters
NAME                  GNAME
EXPNO                    12
PROCNO                    1

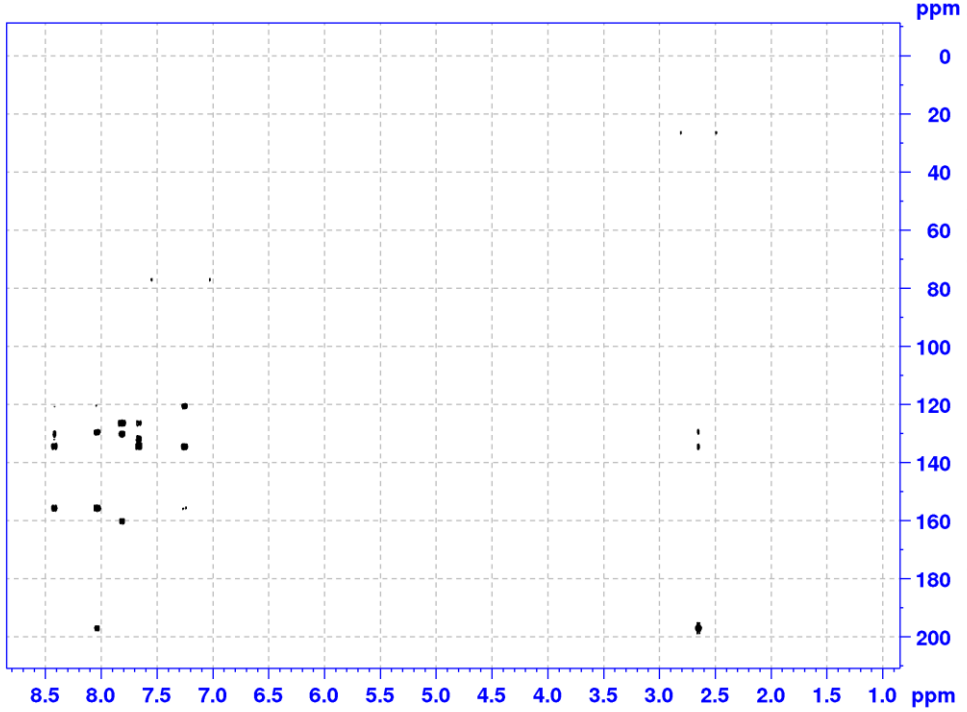
F2 - Acquisition Parameters
Date                20180612
Time                   17.25 h
INSTRUM  XU_Avance_III_400
PROBHD   Z108618_0260 (
PULPROG         cosygpmfqf
TD                      2048
SOLVENT                 CDCl3
NS                         2
DS                         8
SWH                 3333.333 Hz
FIDRES              3.255206 Hz
AQ                  0.3072000 sec
RG                       2560
DW                   150.000 usec
DE                     6.50 usec
TE                   298.1 K
D0                  0.00000300 sec
D1                  1.89449299 sec
D13                 0.00000400 sec
D16                 0.00020000 sec
 NO                 0.00030000 sec
CDav                       1
SFO1               399.7320043 MHz
NUC1                      1H
P1                      12.75 usec
PLW1               16.47699928 W
GPNAM[1]             SINE.100
GPZ1                    16.00 %
GPNAM[2]             SINE.100
GPZ2                    12.00 %
GPNAM[3]             SINE.100
GPZ3                    40.00 %
P16                   1000.00 usec

F1 - Acquisition parameters
TD                       128
SFO1                399.732 MHz
FIDRES             52.083332 Hz
SW                     8.339 ppm
FnMODE                    QF

F2 - Processing parameters
SI                       1024
SF                399.7300020 MHz
WDW                      SINE
SSB                         0
LB                       0 Hz
GB                          0
PC                        1.40

F1 - Processing parameters
SI                       1024
MC2                        QF
SF                399.7300020 MHz
WDW                      SINE
SSB                         0
LB                       0 Hz
GB                          0
```

1.9.4 ^{1}H-^{13}C HMBC




```
Current Data Parameters
NAME                  GDAMB
EXPNO                    14
PROCNO                    1

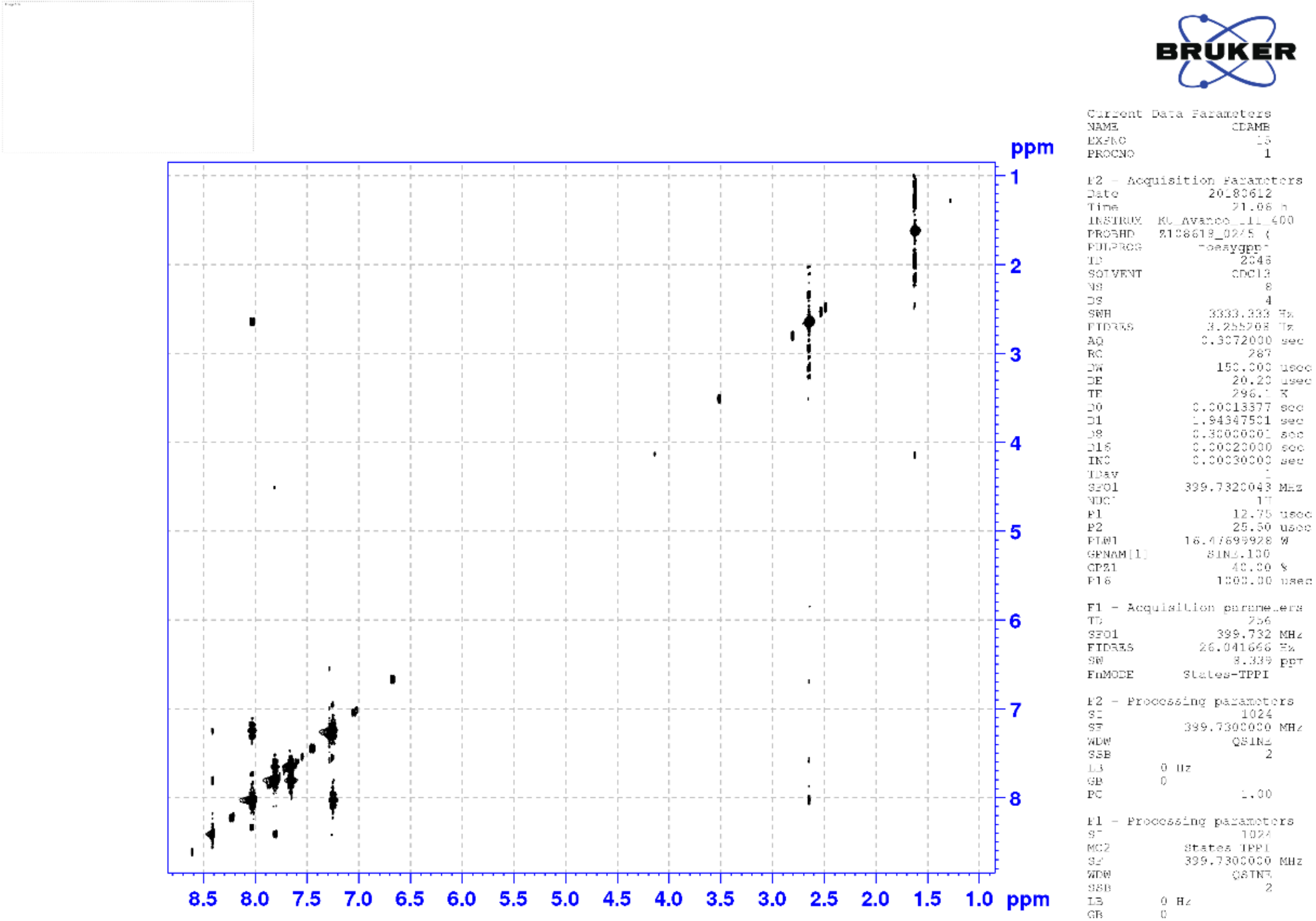
F2 - Acquisition Parameters
Date_              20180612
Time                 19.59 h
INSTRUM  KU_Avance_III_400
PROBHD   z108618_0245 (
PULPROG    hmbcetgpl3nd
TD                     4096
SOLVENT             CDCl3
NS                       16
DS                       16
SWH               3333.333 Hz
FIDRES            1.627604 Hz
AQ                0.6144000 sec
RG                     2050
DW                  150.000 usec
DE                    6.50 usec
TE                   296.4 K
CNST6           120.0000000
CNST7           165.0000000
CNST13           10.0000000
D0                0.00000300 sec
D1                1.27881598 sec
D6                0.05000000 sec
D16               0.00020000 sec
IN0               0.00002240 sec
TDav                     1
SFO1           399.7320043 MHz
NUC1                    1H
P1                    12.75 usec
P2                    25.50 usec
PLW1           16.47699928 W
SFO2           100.5222241 MHz
NUC2                   13C
P3                     9.20 usec
P24                 2000.00 usec
PLW2           49.50000000 W
SPNAM[7]     Crp60comp.4
SPOAL7                0.500
SPOFF27   0 Hz
SPW7           6.40140009 W
GPNAM[1]          SINE.100
GPZ1                 80.00 %
GPNAM[3]          SINE.100
GPZ3                 14.00 %
GPNAM[4]          SINE.100
GPZ4                 -8.00 %
GPNAM[5]          SINE.100
GPZ5                 -4.00 %
GPNAM[6]          SINE.100
GPZ6                 -2.00 %
P16                1000.00 usec

F1 - Acquisition parameters
TD                      128
SFO1             100.5222 MHz
FIDRES          348.772308 Hz
SW                 222.055 ppm
FnMODE     Echo-Antiecho

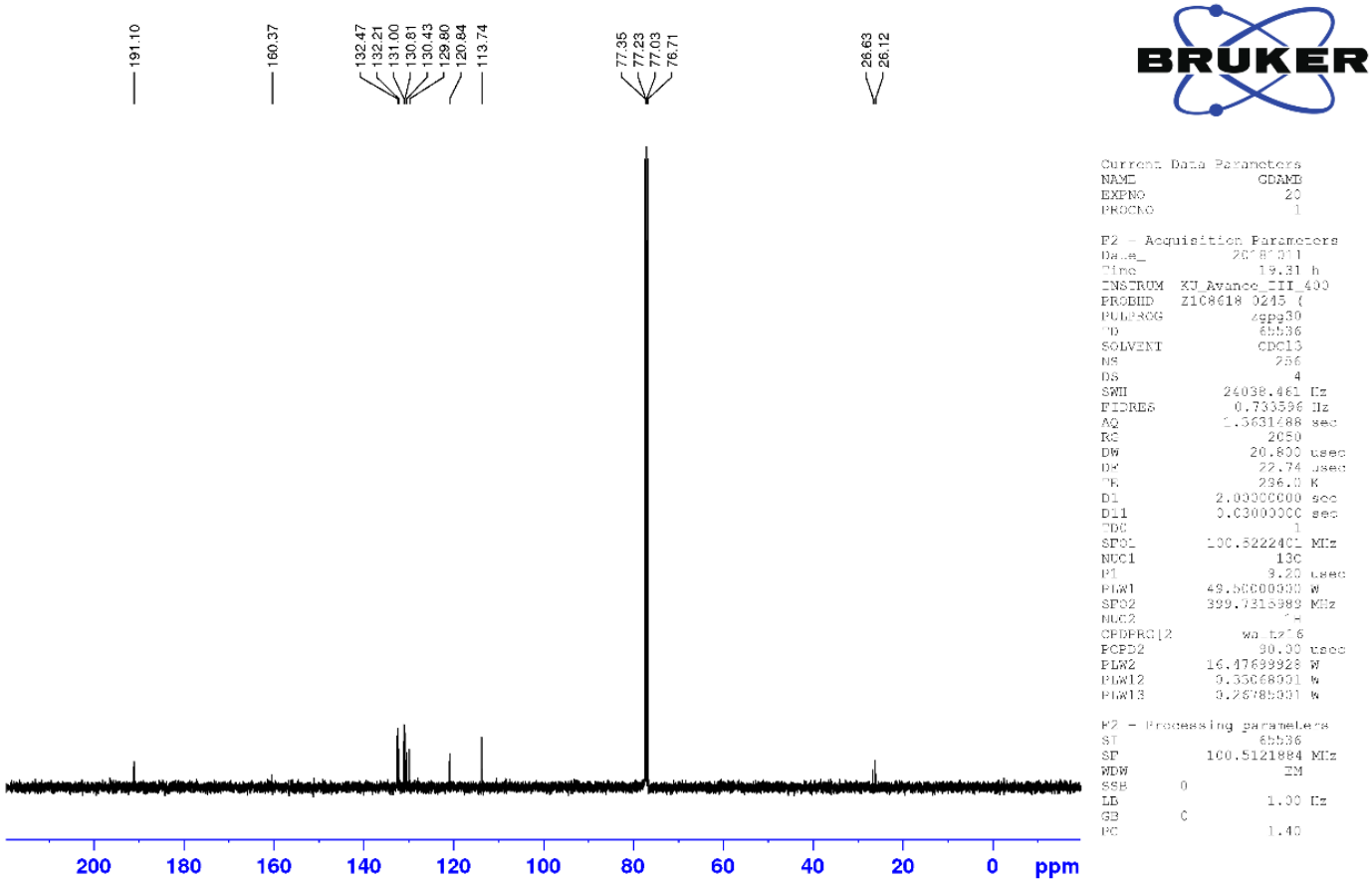
F2 - Processing parameters
SI                     2048
SF             399.7300000 MHz
WDW                    SINE
SSB                       2
LB         0 Hz
GB         0
PC                     1.40

F1 - Processing parameters
SI                     1024
MC2         echo-antiecho
SF               100.5121884 MHz
WDW                    SINE
SSB                       2
LB         0 Hz
GB         0
```

1.9.5 ^{1}H-^{1}H NOESY



1.9.6 ^{13}C NMR



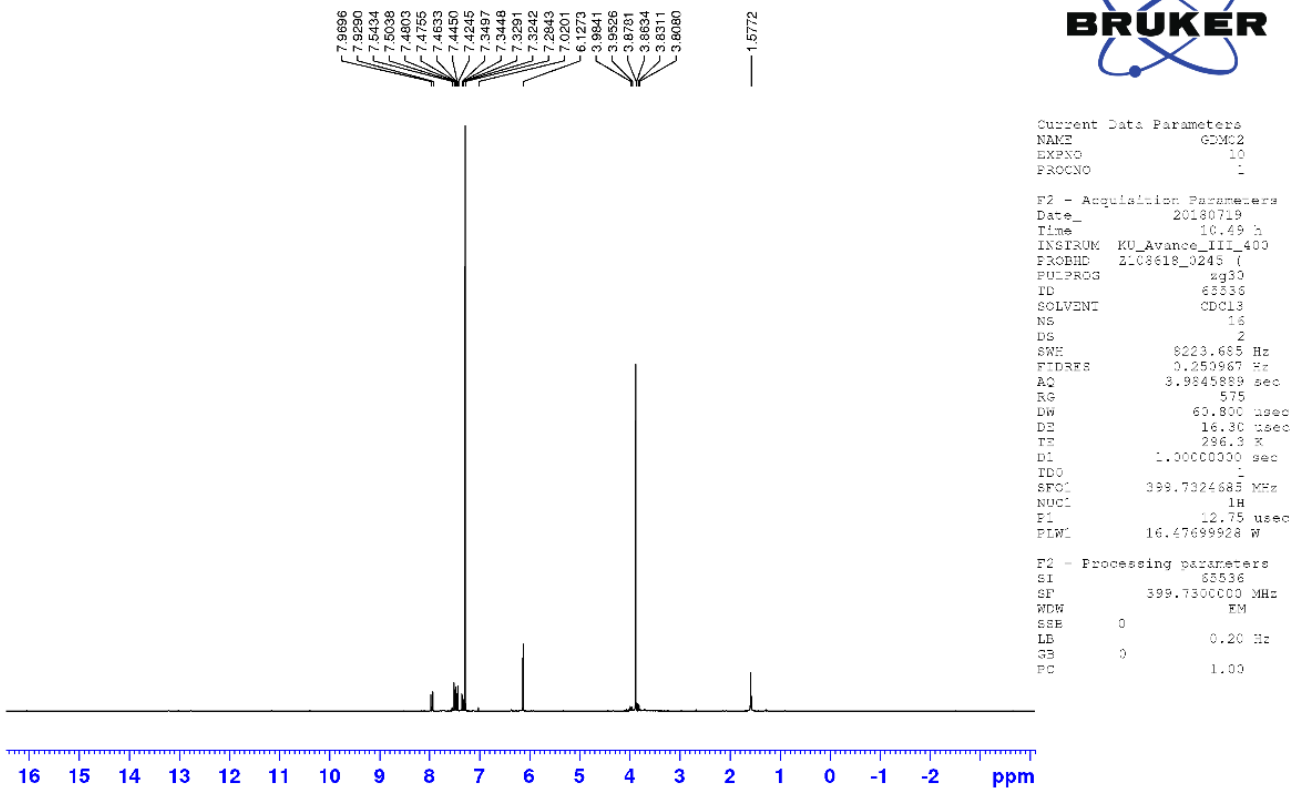

```
Current Data Parameters
NAME                  GDAMD
EXPNO                    20
PROCNO                    1

F2 - Acquisition Parameters
Date_               20180911
Time                   19.31 h
INSTRUM  XU_Avance_III_400
PROBHD   Z108618_0215_(
PULPROG              zgpg30
TD                    65536
SOLVENT               CDCl3
NS                      256
DS                        4
SWH                24038.461 Hz
FIDRES             0.733596 Hz
AQ                 1.3631488 sec
RG                     2050
DW                   20.800 usec
DE                   22.74 usec
TE                   296.1 K
D1                 2.00000000 sec
D11                0.03000000 sec
TD0                       1
SFO1              100.5222401 MHz
NUC1                    13C
P1                      9.20 usec
PLW1              49.56000000 W
SFO2              399.7816989 MHz
NUC2                    1H
CPDPRG[2             waltz16
PCPD2                  80.00 usec
PLW2              16.47699928 W
PLW12              0.33068001 W
PLW13              0.26785001 W

F2 - Processing parameters
SI                    65536
SF                100.5121884 MHz
WDW                      EM
SSB       0
LB                      1.00 Hz
GB        0
PC                      1.40
```

1.10 Compound 10

1.10.1 ^{1}H NMR

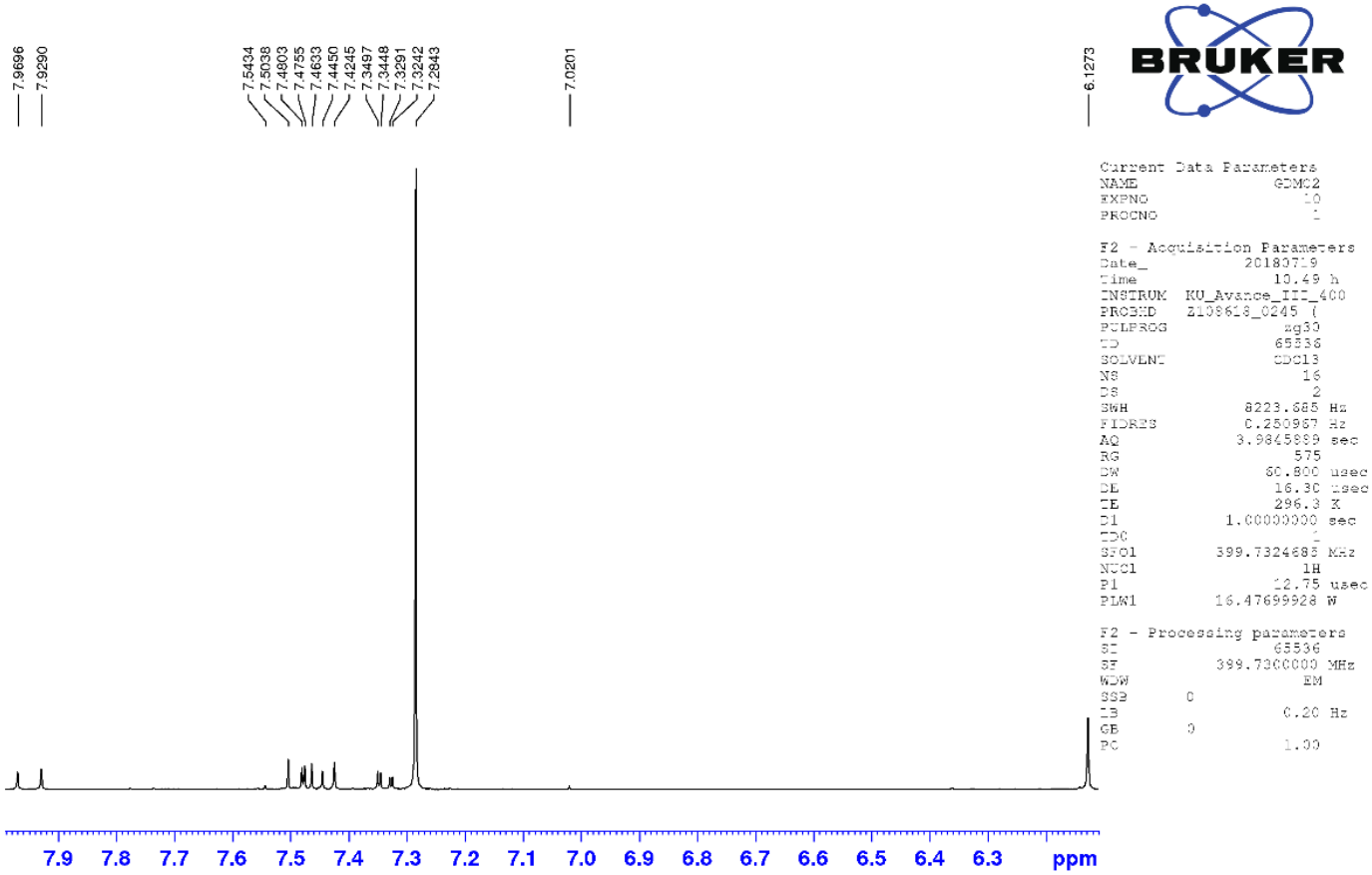



```
Current Data Parameters
NAME                  GDM02
EXPNO                    10
PROCNO                    1

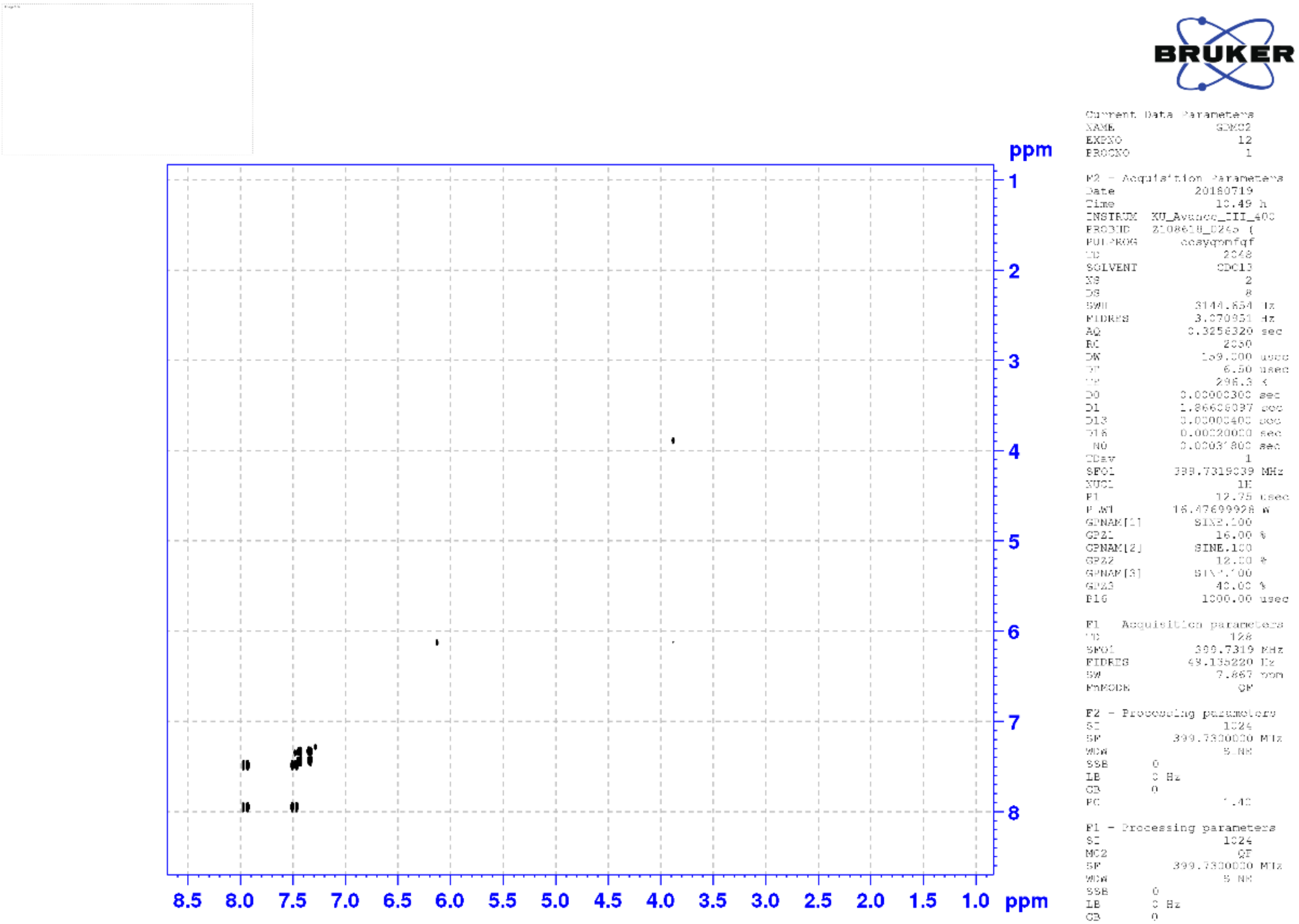
F2 - Acquisition Parameters
Date_              20180719
Time                 10.49 h
INSTRUM  KU_Avance_III_400
PROBHD   2108618_0245 (
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                       16
DS                        2
SWH               8223.685 Hz
FIDRES            0.250967 Hz
AQ                3.9845889 sec
RG                      575
DW                 60.800 usec
DE                 16.30 usec
TE                 296.3 K
D1              1.00000000 sec
TD0                       1
SFO1           399.7324685 MHz
NUC1                    1H
P1                   12.75 usec
PLW1           16.47699928 W

F2 - Processing parameters
SI                    65536
SF              399.7300000 MHz
WDW                     EM
SSB          0
LB                    0.20 Hz
GB           0
PC                    1.00
```

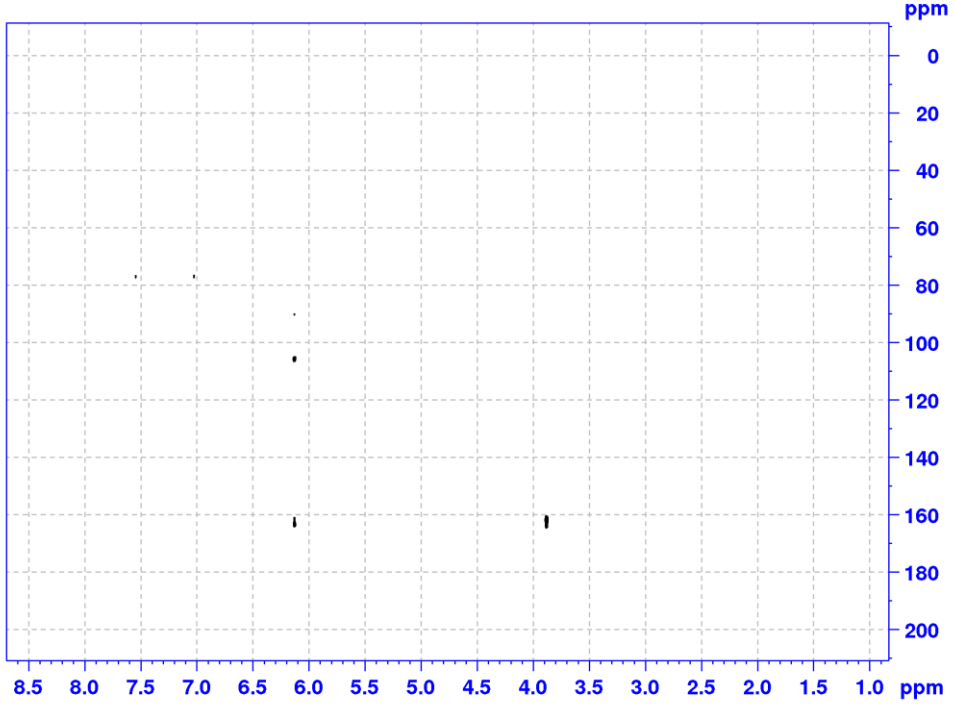
1.10.2 Expansion of aromatic region



1.10.3 ^{1}H -^{1}H COSY



1.10.4 ^{1}H-^{13}C HMBC




```
Current Data Parameters
NAME                 GDMC2
EXPNO                   14
PROCNO                   1

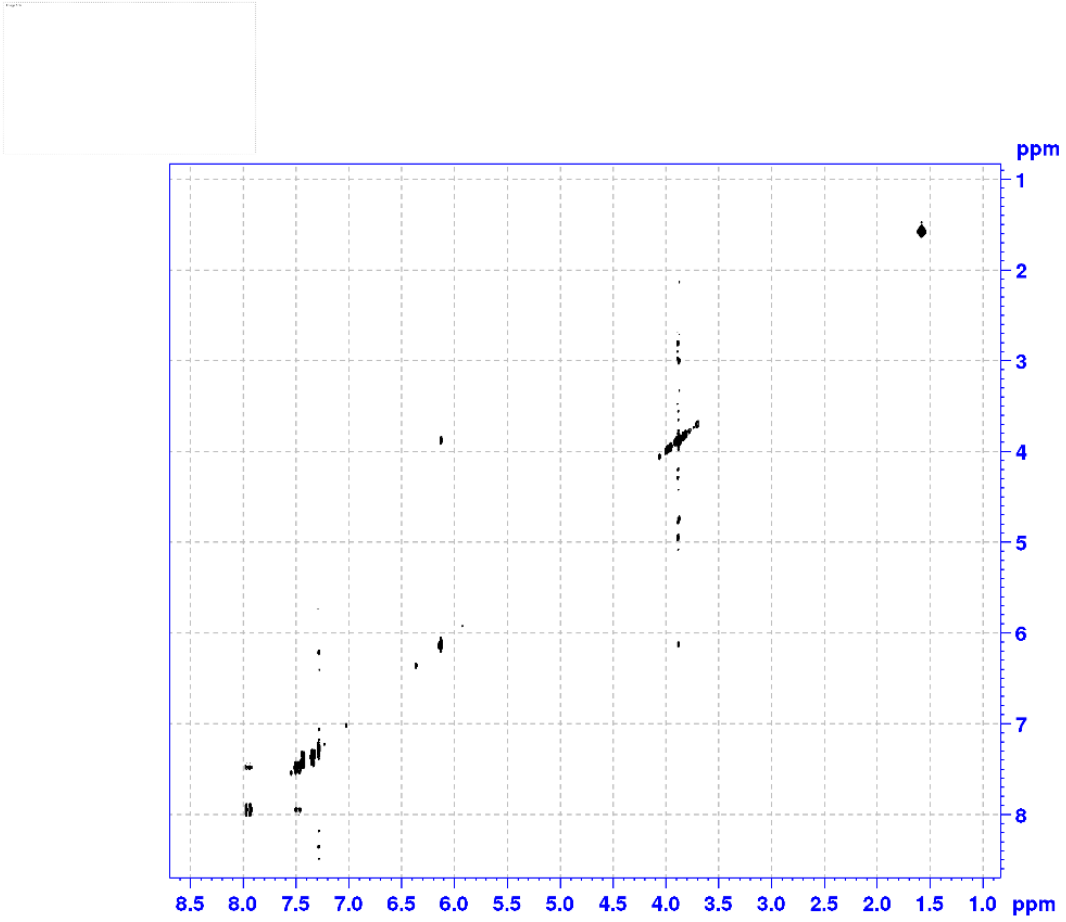
F2 - Acquisition Parameters
Date_              20180719
Time                 19.37 h
INSTRUM  KU_Avance_III_400
PROBHD   2108618_0245 (
PULPROG     hmbcetgpl3nd
TD                    4096
SOLVENT              CDCl3
NS                      16
DS                      16
SWH               3144.654 Hz
FIDRES            1.535476 Hz
AQ               0.6512640 sec
RG                    2050
DW                 159.000 usec
DE                    6.50 usec
TE                  296.1 K
CNST6          120.0000000
CNST7          165.0000000
CNST13          10.0000000
D0              0.00000300 sec
D1              1.24195194 sec
D6              0.05000000 sec
D16             0.00020000 sec
IN0             0.00002240 sec
TDav                    1
SFO1          399.7319039 MHz
NUC1                   1H
P1                   12.75 usec
P2                   25.50 usec
PLW1          16.47699928 W
SFO2          100.5222241 MHz
NUC2                  13C
P3                    9.20 usec
P24                2000.00 usec
PLW2          49.50000000 W
SPNAM[7]      Crp60comp.4
SPOAL7              0.500
SPOFFS7  0 Hz
SPW7           6.40140009 W
GPNAM[1]          SINE.100
GPZ1                80.00 %
GPNAM[3]          SINE.100
GPZ3                14.00 %
GPNAM[4]          SINE.100
GPZ4                -8.00 %
GPNAM[5]          SINE.100
GPZ5                -4.00 %
GPNAM[6]          SINE.100
GPZ6                -2.00 %
P16                1000.00 usec

F1 - Acquisition parameters
TD                     128
SFO1            100.5222 MHz
FIDRES         348.772308 Hz
SW                 222.055 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                    2048
SF              399.7300000 MHz
WDW                   SINE
SSB                      2
LB         0 Hz
GB         0
PC                    1.40

F1 - Processing parameters
SI                    1024
MC2         echo-antiecho
SF              100.5121884 MHz
WDW                   SINE
SSB                      2
LB         0 Hz
GB         0
```

1.10.5 ^{1}H-^{1}H NOESY



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```
Current Data Parameters
NAME                  CDMC2
EXPNO                    15
PROCNO                    1

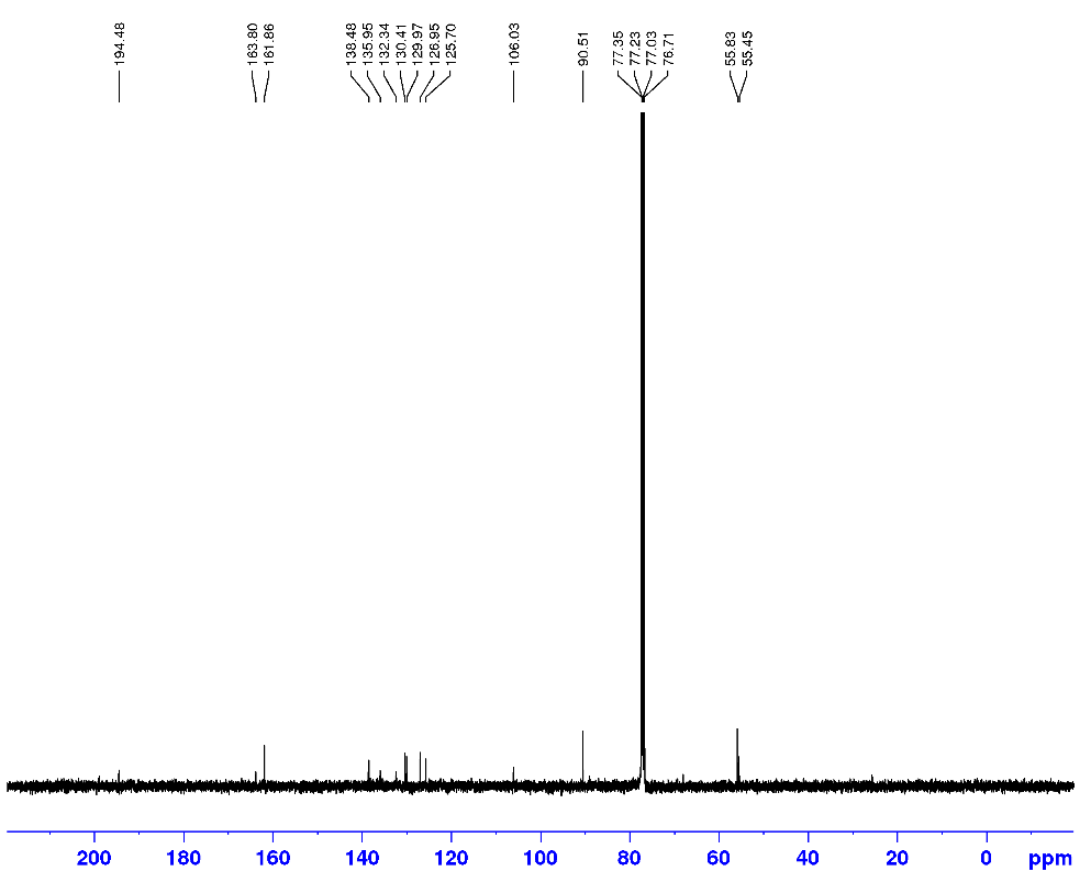
F2 - Acquisition Parameters
Date              20.80719
Time                 8.07 h
INSTRUM   KC_Avance_III_400
PROBHD    BIC8618_0275 (
PULPROG          noesygpph
TD                     2048
SOLVENT               CDCl3
NS                        8
DS                        4
SWH               3174.657 Hz
FIDRES            3.079951 Hz
AQ                0.3256320 sec
RG                      362
DW                  159.200 usec
DE                   22.01 usec
TE                  296.1 K
D0                0.00014277 sec
D1                1.92504298 sec
D8                0.30000001 sec
D16               0.00020000 sec
INO               0.00031800 sec
IDAV                      1
SFO1           399.7319039 MHz
NDC                      1T
P1                    12.75 usec
P2                    25.50 usec
PLW1             16.47899928 W
GPNAM[1]          SINE.100
GPZ1                  40.00 %
P16                 1000.00 usec

F1 - Acquisition parameters
TD                      256
SFO1             399.7319 MHz
FIDRES           24.967610 Hz
SW                 7.967 ppm
FnMODE       States-TPPI

F2 - Processing parameters
SI                      1024
SF            399.7300000 MHz
WDW                   QSINE
SSB                       2
LB         0 Hz
GB         0
PC                     1.00

F1 - Processing parameters
SI                      1024
MC2          States-TPPI
SF            399.7300000 MHz
WDW                   QSINE
SSB                       2
LB         0 Hz
GB         0
```

1.10.6 ^{13}C NMR



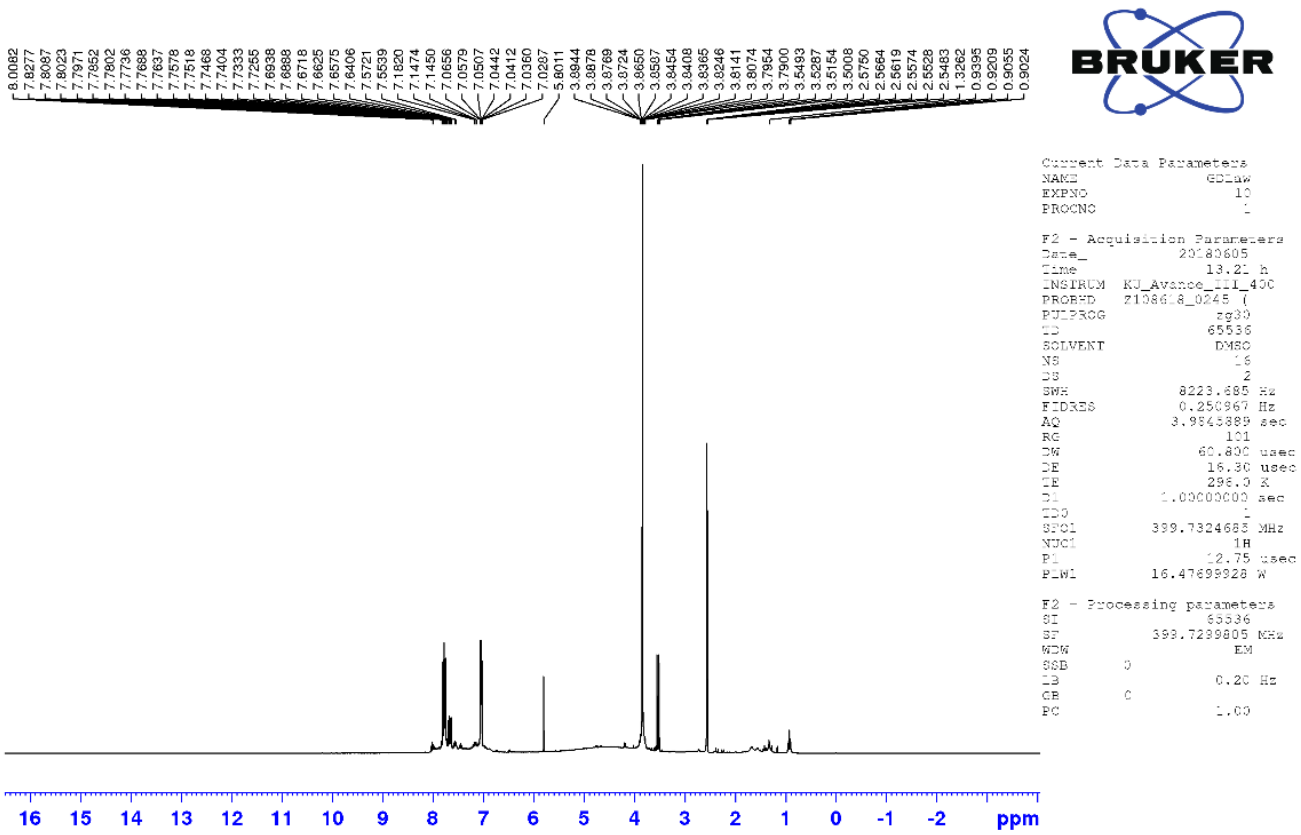

```
Current Data Parameters
NAME                 GDMC2
EXPNO                   10
PROCNO                   1

F2 - Acquisition Parameters
Date_           20180306
Time                 1.03 h
INSTRUM  XJ_Avance_III_400
PROBHD   Z108618_0215_C
PULPROG             zgpg30
TD                   65536
SOLVENT              CDCl3
NS                    2560
DS                       4
SWH              24038.461 Hz
FIDRES           0.733596 Hz
AQ               1.3631488 sec
RG                    2050
DW                 20.800 usec
DE                 22.74 usec
TE                  296.0 K
D1               2.00000000 sec
D11              0.03000000 sec
TD0                      1
SFO1         100.5222401 MHz
NUC1                   13C
P1                   9.20 usec
PLW1         49.50000000 W
SFO2         399.7815988 MHz
NUC2                   1H
CPDPRG[2          waltz16
PCPD2              80.00 usec
PLW2         16.47699928 W
PLW12         0.33068001 W
PLW13         0.25785001 W

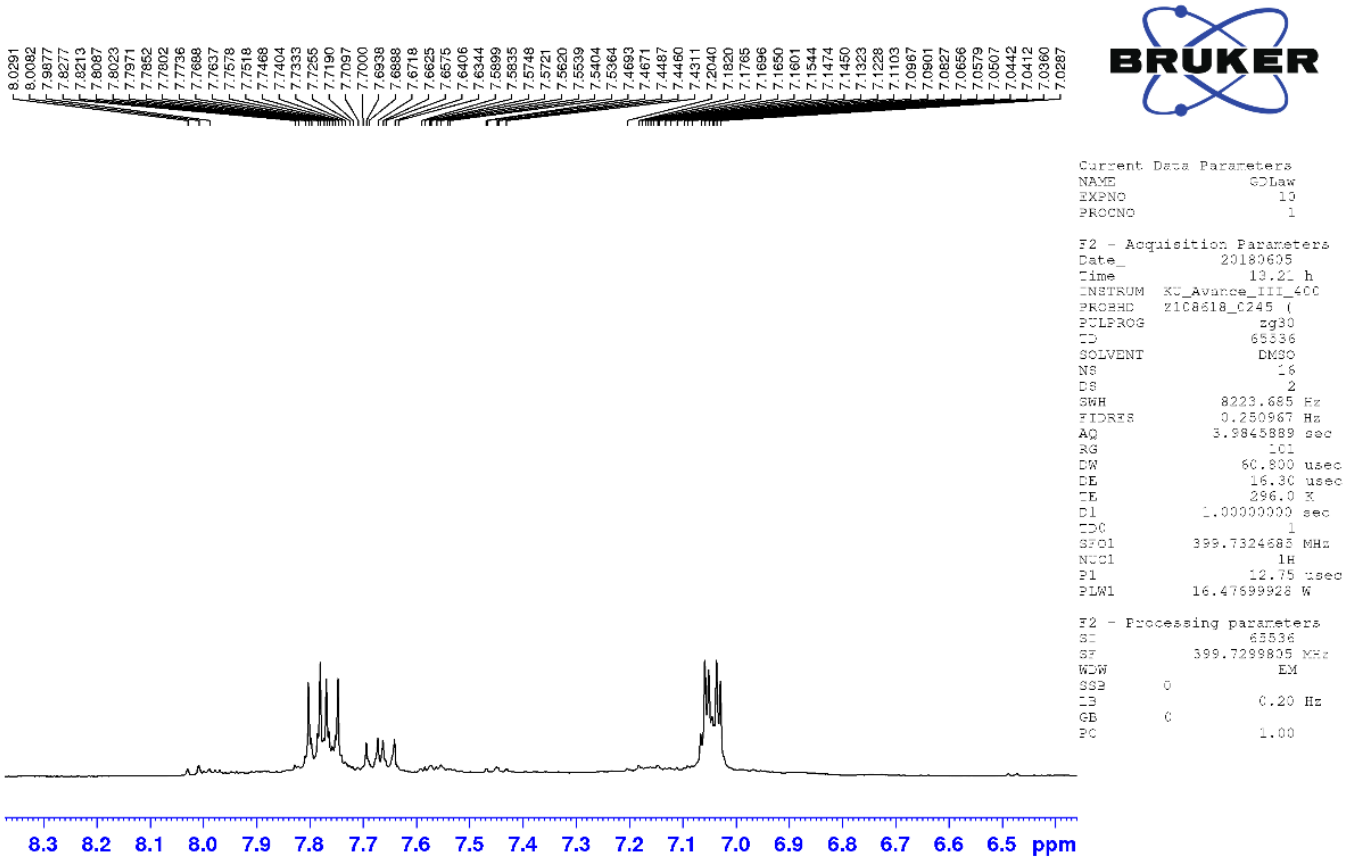
F2 - Processing parameters
SI                   65536
SF           100.5121884 MHz
WDW                    EM
SSB          0
LB                   1.00 Hz
GB           0
PC                   1.40
```

1.11 Compound 11

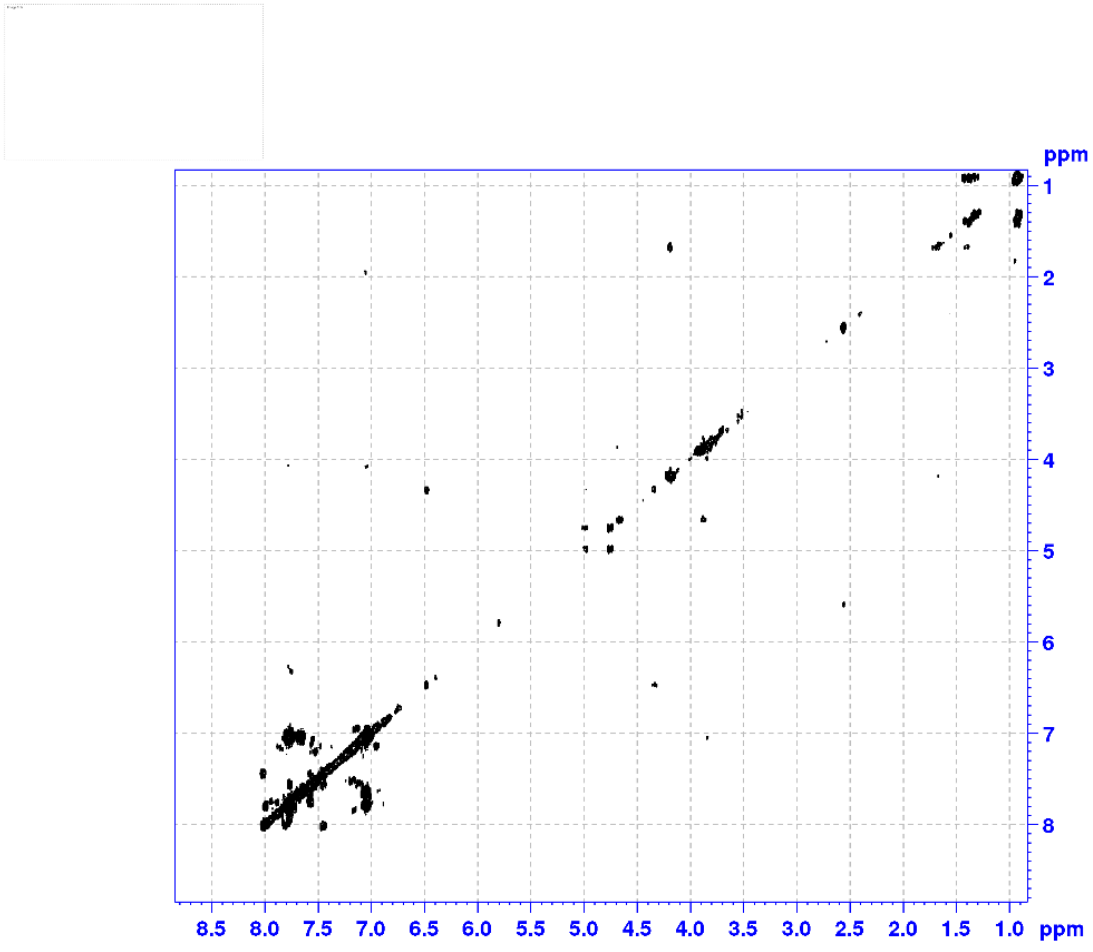
1.11.1 ^{1}H NMR



1.11.2 Expansion of aromatic region



1.11.3 ^{1}H -^{1}H COSY



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```
Current Data Parameters
NAME                  GDiaw
EXPNO                    12
PROCNO                    1

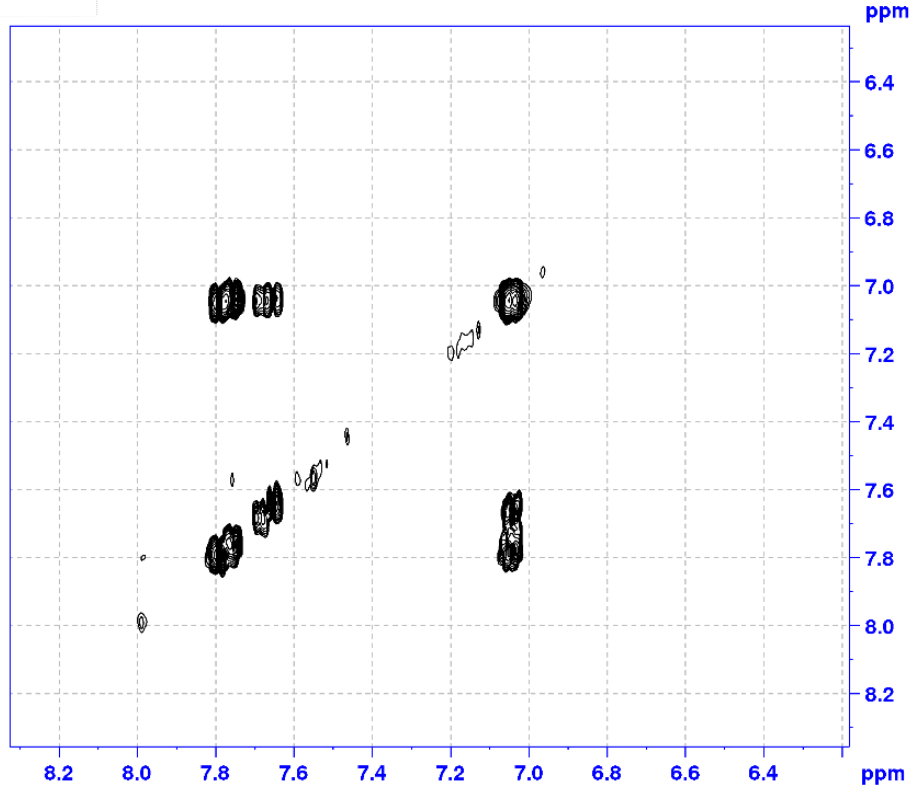
F2 - Acquisition Parameters
Date_              20180605
Time                 13.37 h
INSTRUM   XU_Avance_III_400
PROBHD    Z108610_0246 (
PULPROG       cosygpmfqf
TD                     2048
SOLVENT                DMSO
NS                        2
DS                        8
SWH                3591.127 Hz
FIDRES             3.638600 Hz
AQ                 0.2908160 sec
RG                      2050
DW                  142.000 usec
DE                    6.50 usec
TE                  295.0 K
D0                 0.00000300 sec
D1                 1.90287700 sec
D13                0.00000400 sec
D16                0.00020000 sec
 NO                0.00028800 sec
CDAV                      1
SFO1            399.7317713 MHz
NUC1                      1H
P1                    12.75 usec
PLW1            16.47609928 W
GPNAM[1]           SINE.100
GPZ1                  16.00 %
GPNAM[2]           SINE.100
GPZ2                  12.00 %
GPNAM[3]           SINE.100
GPZ3                  40.00 %
P16                 1000.00 usec

F1 - Acquisition parameters
TD                       128
SFO1            399.7318 MHz
FIDRES            55.817805 Hz
SW                  8.809 ppm
FnMODE                    QF

F2 - Processing parameters
SI                       1024
SF              399.7299825 MHz
WDW                     SINE
SSB                  0
LB                   0 Hz
GB                   0
PC                       1.40

F1 - Processing parameters
SI                       1024
MC2                        QF
SF              399.7299825 MHz
WDW                     SINE
SSB                  0
LB                   0 Hz
GB                   0
```

1.11.4 Expansion of COSY



BRUKER

```
Current Data Parameters
NAME                  SDLaw
EXPNO                    12
PROCNO                    1

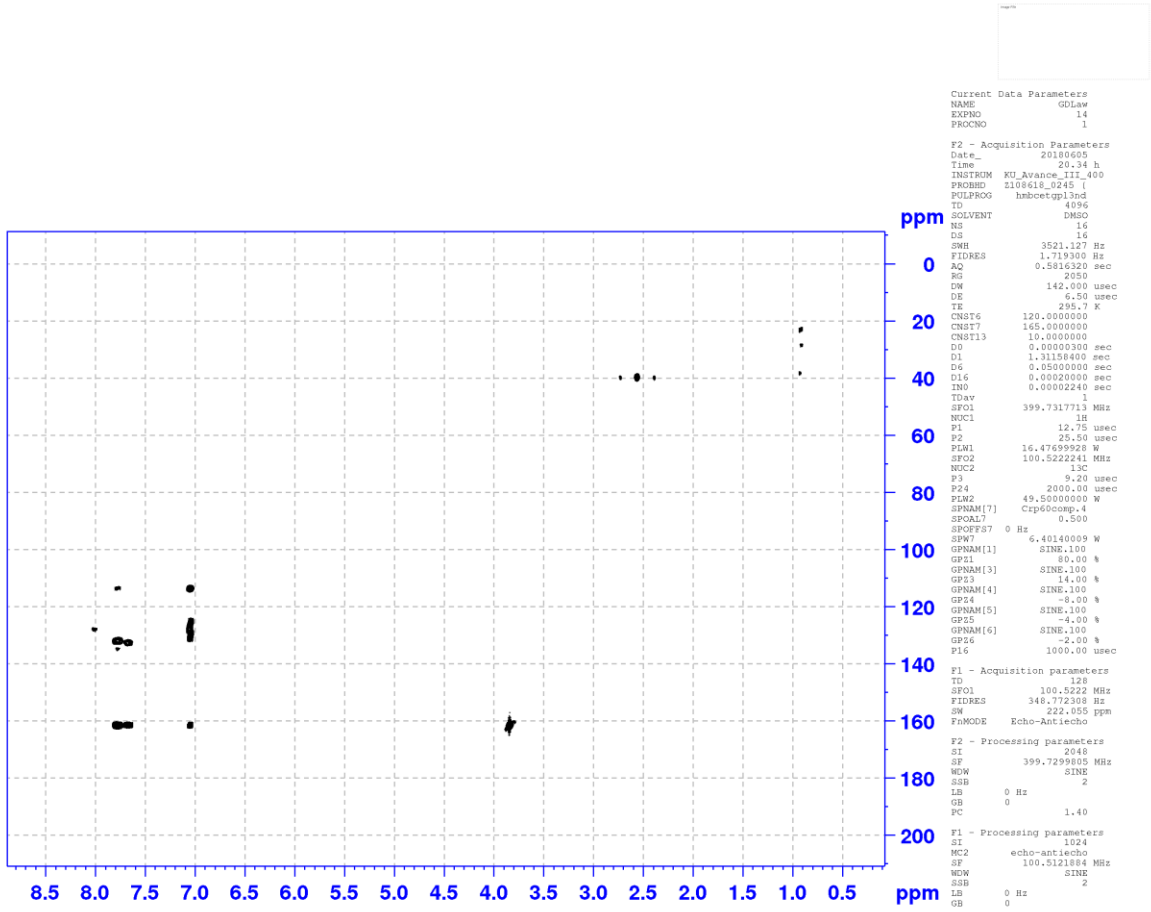
F2 - Acquisition Parameters
Date_              20150605
Time                 13.37 h
INSTRUM  XU_Avance_III_400
PROBHD   Z108630_0260 (
PULPROG        cosygpmfqf
TD                     2048
SOLVENT                DMSO
NS                        2
DS                        8
SWH                3521.127 Hz
FIDRES             3.438600 Hz
AQ                 0.2908160 sec
RG                      2030
DW                  142.000 usec
DE                    6.50 usec
TE                    295.2 K
D0                 0.00003000 sec
D1                 1.90087700 sec
D13                0.00000400 sec
D16                0.00020000 sec
 NS                0.00028400 sec
CDav                      1
SFO1            399.7317713 MHz
NUC1                      1H
P1                     12.75 usec
PLW1             16.47699928 W
GPNAM[1]           SINE.100
GPZ1                  16.00 %
GPNAM[2]           SINE.100
GPZ2                  12.00 %
GPNAM[3]           SINE.100
GPZ3                  40.00 %
P16                 1000.00 usec

F1 - Acquisition parameters
TD                       128
SFO1            399.7318 MHz
FIDRES          55.017605 Hz
SW                   8.809 ppm
FnMODE                    QF

F2 - Processing parameters
SI                       1024
SF              399.7999825 MHz
WDW                     QSINE
SSB          0
LB           0 Hz
GB           0
PC                      1.40

F1 - Processing parameters
SI                       1024
MC2                        QF
SF              399.7999825 MHz
WDW                     QSINE
SSB          0
LB           0 Hz
GB           0
```

1.11.5 ^{1}H-^{13}C HMBC




```
Current Data Parameters
NAME                  GDLaw
EXPNO                    14
PROCNO                    1

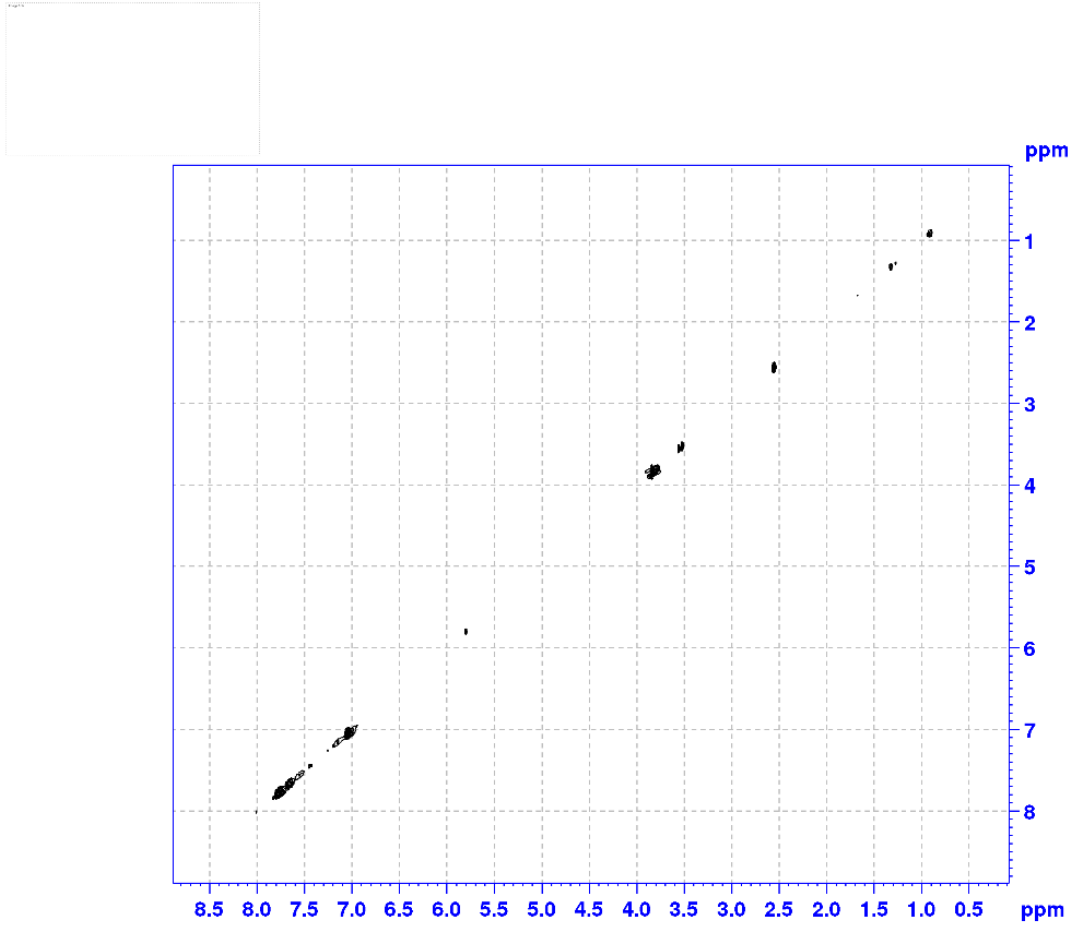
F2 - Acquisition Parameters
Date_              20180605
Time                  20.34 h
INSTRUM   KU_Avance_III_400
PROBHD    Z108618_0245 [
PULPROG     hmbcetgpl3nd
TD                     4096
SOLVENT                DMSO
NS                       16
DS                       16
SWH                3521.127 Hz
FIDRES             1.719300 Hz
AQ                 0.5816320 sec
RG                      2050
DW                   142.000 usec
DE                     6.50 usec
TE                    295.7 K
CNST6            120.0000000
CNST7            165.0000000
CNST13            10.0000000
D0                 0.00000300 sec
D1                 1.31158400 sec
D6                 0.05000000 sec
D16                0.00020000 sec
IN0                0.00002240 sec
TDav                        1
SFO1             399.7317713 MHz
NUC1                      1H
P1                     12.75 usec
P2                     25.50 usec
PLW1             16.47699928 W
SFO2             100.5222241 MHz
NUC2                      13C
P3                      9.20 usec
P24                  2000.00 usec
PLW2             49.50000000 W
SPNAM[7]      Crp60comp.4
SPOAL7                0.500
SPOFFS7  0 Hz
SPW7              6.40140009 W
GPNAM[1]            SINE.100
GP21                  80.00 %
GPNAM[3]            SINE.100
GP23                  14.00 %
GPNAM[4]            SINE.100
GP24                  -8.00 %
GPNAM[5]            SINE.100
GP25                  -4.00 %
GPNAM[6]            SINE.100
GP26                  -2.00 %
P16                 1000.00 usec

F1 - Acquisition parameters
TD                       128
SFO1               100.5222 MHz
FIDRES             348.772308 Hz
SW                 222.055 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                      2048
SF               399.7299805 MHz
WDW                     SINE
SSB                        2
LB        0 Hz
GB        0
PC                      1.40

F1 - Processing parameters
SI                      1024
MC2          echo-antiecho
SF               100.5121884 MHz
WDW                     SINE
SSB                        2
LB        0 Hz
GB        0
```

1.11.6 ^{1}H-^{1}H NOESY



BRUKER

```
Current Data Parameters
NAME                  CDLaw
EXPNO                    15
PROCNO                    1

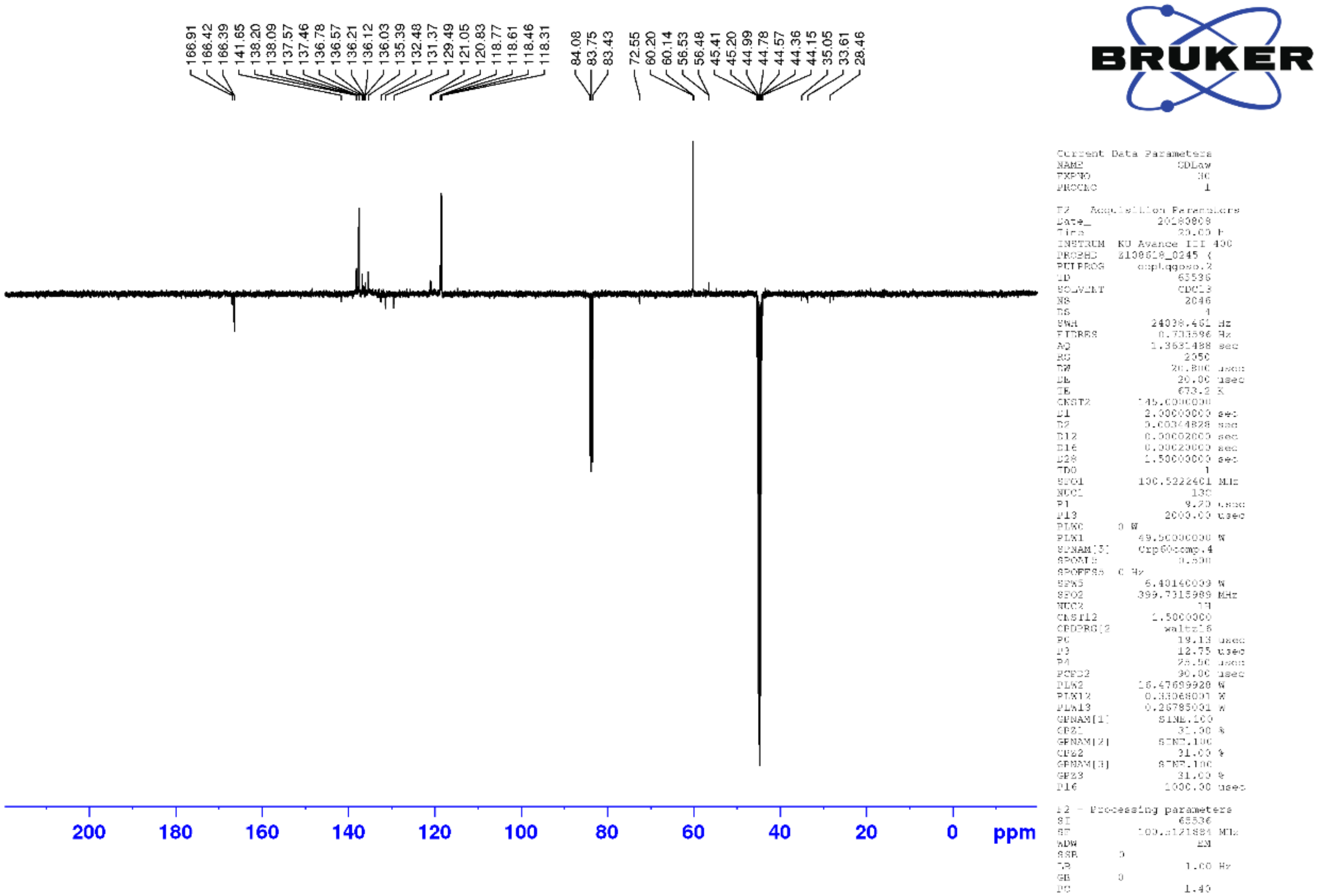
F2 - Acquisition Parameters
Date_             20_80605
Time                21.42 h
INSTRUM   BC_Avance_III_400
PROBHD    Z108618_0275_C
PULPROG          noesygpph
TD                     2048
SOLVENT                DMSO
NS                        8
DS                        4
SWH              3521.127 Hz
FIDRES           3.438600 Hz
AQ               0.2908160 sec
RG                       67
DW                142.000 usec
DE                 18.72 usec
TE                 296.0 K
D0               0.00012577 sec
D1               1.95985901 sec
D8               0.30000000 sec
D16              0.00020000 sec
IN0              0.00228400 sec
IDAV                      1
SFO1           399.73177_3 MHz
NUC1                   1H
P1                   12.75 usec
P2                   25.50 usec
PLW1           16.47699928 W
GPNAM[1]        SINE.100
GPZ1                 40.00 %
P16                1000.00 usec

F1 - Acquisition parameters
TD                     256
SFO1           399.73_8 MHz
FIDRES          27.508802 Hz
SW                 8.809 ppm
FnMODE         States-TPPI

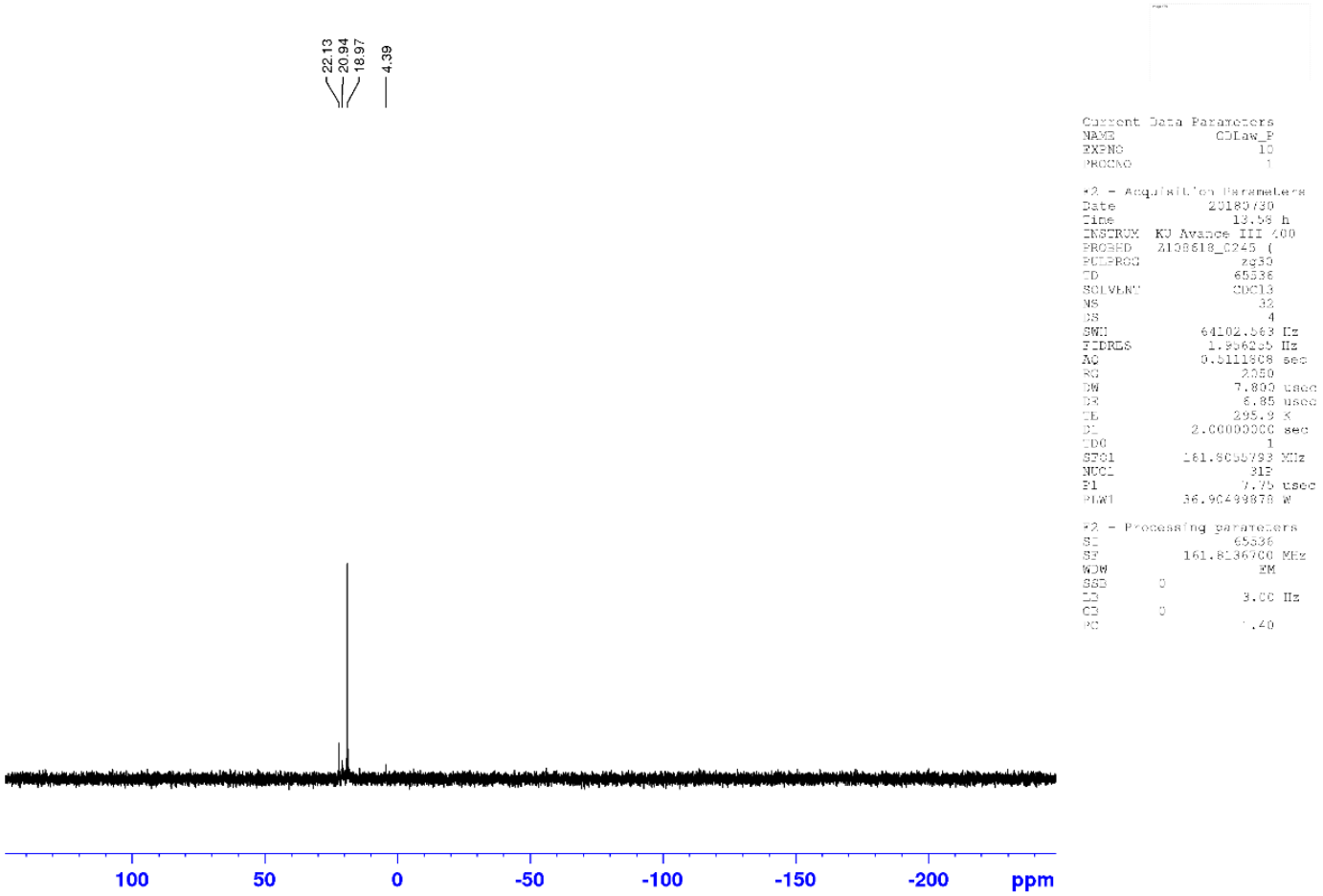
F2 - Processing parameters
SI                    1024
SF             399.7299905 MHz
WDW                   QSINE
SSB                      2
LB          0 Hz
GB          0
PC                    1.00

F1 - Processing parameters
SI                    1024
MC2            States-TPPI
SF             399.7299905 MHz
WDW                   QSINE
SSB                      2
LB          0 Hz
GB          0
```

1.11.7 ^{13}C NMR


166.91
166.42
166.39
141.65
138.20
138.09
137.57
137.46
136.78
136.57
136.21
136.12
136.03
135.39
132.48
131.37
129.49
121.05
120.83
118.77
118.61
118.46
118.31
84.08
83.75
83.43
72.55
60.20
60.14
56.53
56.48
45.41
45.20
44.99
44.78
44.57
44.36
44.15
35.05
33.61
28.46
BRUKER
200
180
160
140
120
100
80
60
40
20
0
ppm

1.11.8 ^{15}P NMR



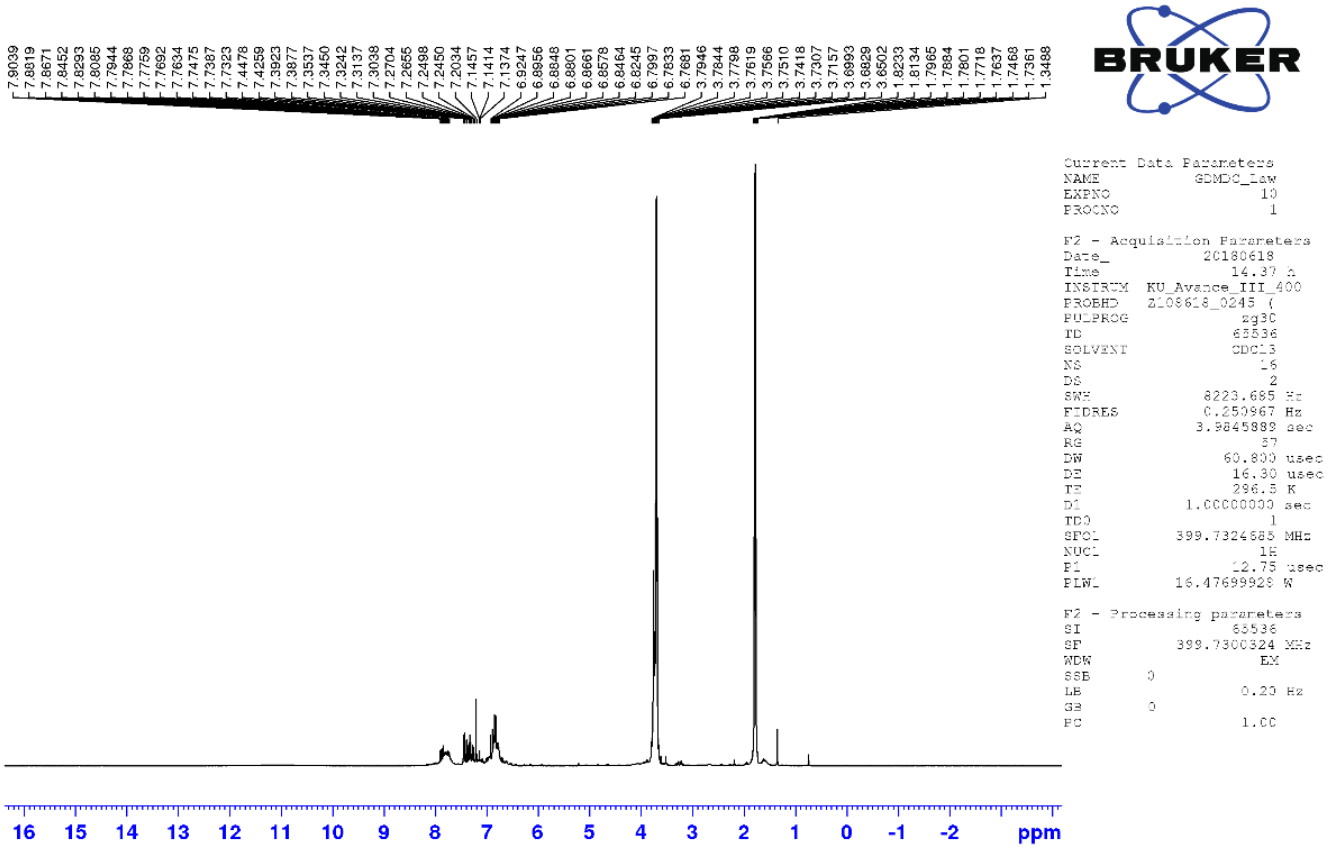

```
Current Data Parameters
NAME                  CDIaw_P
EXPNO                      10
PROCNO                      1

F2 - Acquisition Parameters
Date                20180730
Time                   13.56 h
INSTRUM  KJ Avance III 400
PROBHD   Z108618_C245 (
PULPROG                 zg30
TD                     65536
SOLVENT                CDCl3
NS                        32
DS                         4
SWH                64102.563 Hz
FIDRES              1.956235 Hz
AQ                  0.5111808 sec
RG                       2050
DW                     7.800 usec
DE                     6.85 usec
TE                     295.9 K
D1                2.00000000 sec
TD0                        1
SFO1             161.8655793 MHz
NUC1                     31P
P1                     7.75 usec
PLW1            36.90499878 W

F2 - Processing parameters
SI                       65536
SF               161.8136700 MHz
WDW                        EM
SSB          0
LB                     3.00 Hz
GB           0
PC                     1.40
```

1.12 Compound 12

1.12.1 ^{1}H NMR

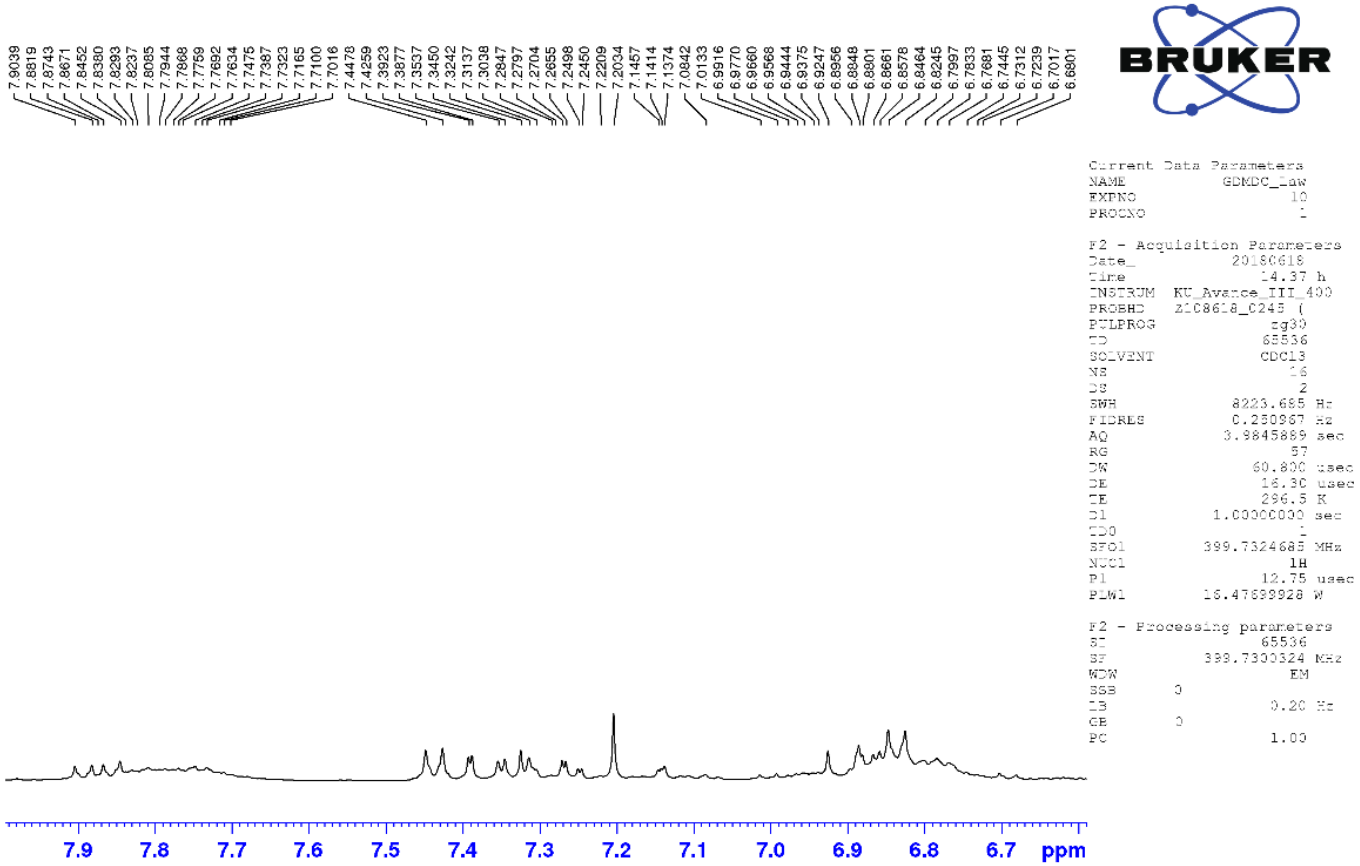



```
Current Data Parameters
NAME                  GDMDC_Law
EXPNO                        10
PROCNO                        1

F2 - Acquisition Parameters
Date_               20180618
Time                     14.37 h
INSTRUM  KU_Avance_III_400
PROBHD   Z108618_0245 (
PULPROG                   zg30
TD                       65536
SOLVENT                  CDCl3
NS                          16
DS                           2
SWH                   8223.685 Hz
FIDRES                0.250967 Hz
AQ                    3.9845888 sec
RG                          57
DW                     60.800 usec
DE                     16.30 usec
TE                     296.5 K
D1                  1.00000000 sec
TD0                          1
SFO1               399.7324685 MHz
NUC1                        1H
P1                       12.75 usec
PLW1               16.47699928 W

F2 - Processing parameters
SI                       65536
SF                 399.7300324 MHz
WDW                         EM
SSB             0
LB                       0.20 Hz
GB              0
PC                       1.00
```

1.12.2 Expansion of aromatic region

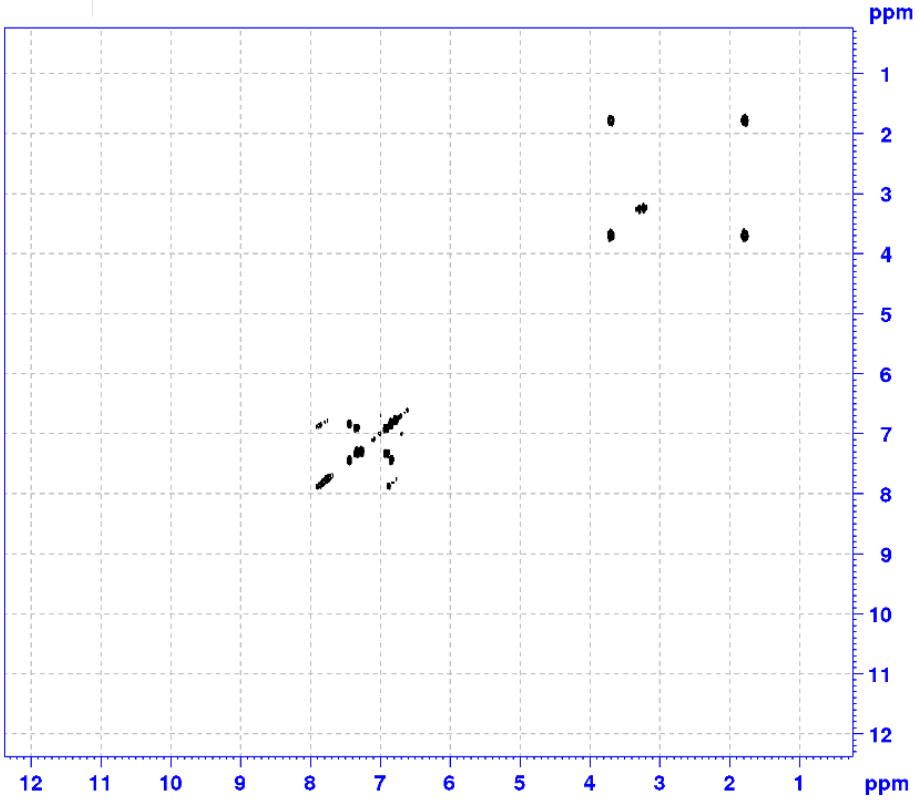



```
Current Data Parameters
NAME            GDMDC_Law
EXPNO                  10
PROCNO                  1

F2 - Acquisition Parameters
Date_            20180618
Time                14.37 h
INSTRUM  KU_Avance_III_400
PROBHD   Z108618_C245 (
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                     16
DS                      2
SWH              8223.685 Hz
FIDRES           0.250987 Hz
AQ               3.9845889 sec
RG                     57
DW                 60.800 usec
DE                 16.30 usec
TE                 296.5 K
D1              1.00000000 sec
TD0                     1
SFO1         399.7324685 MHz
NUC1                   1H
P1                  12.75 usec
PLW1         16.47699928 W

F2 - Processing parameters
SI                  65536
SF           399.7300324 MHz
WDW                    EM
SSB       0
LB                 0.20 Hz
GB        0
PC                 1.00
```

1.12.3 ^{1}H -^{1}H COSY



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```
Current Data Parameters
NAME                 GEMDC Law
EXPNO                       22
PROCNO                       1

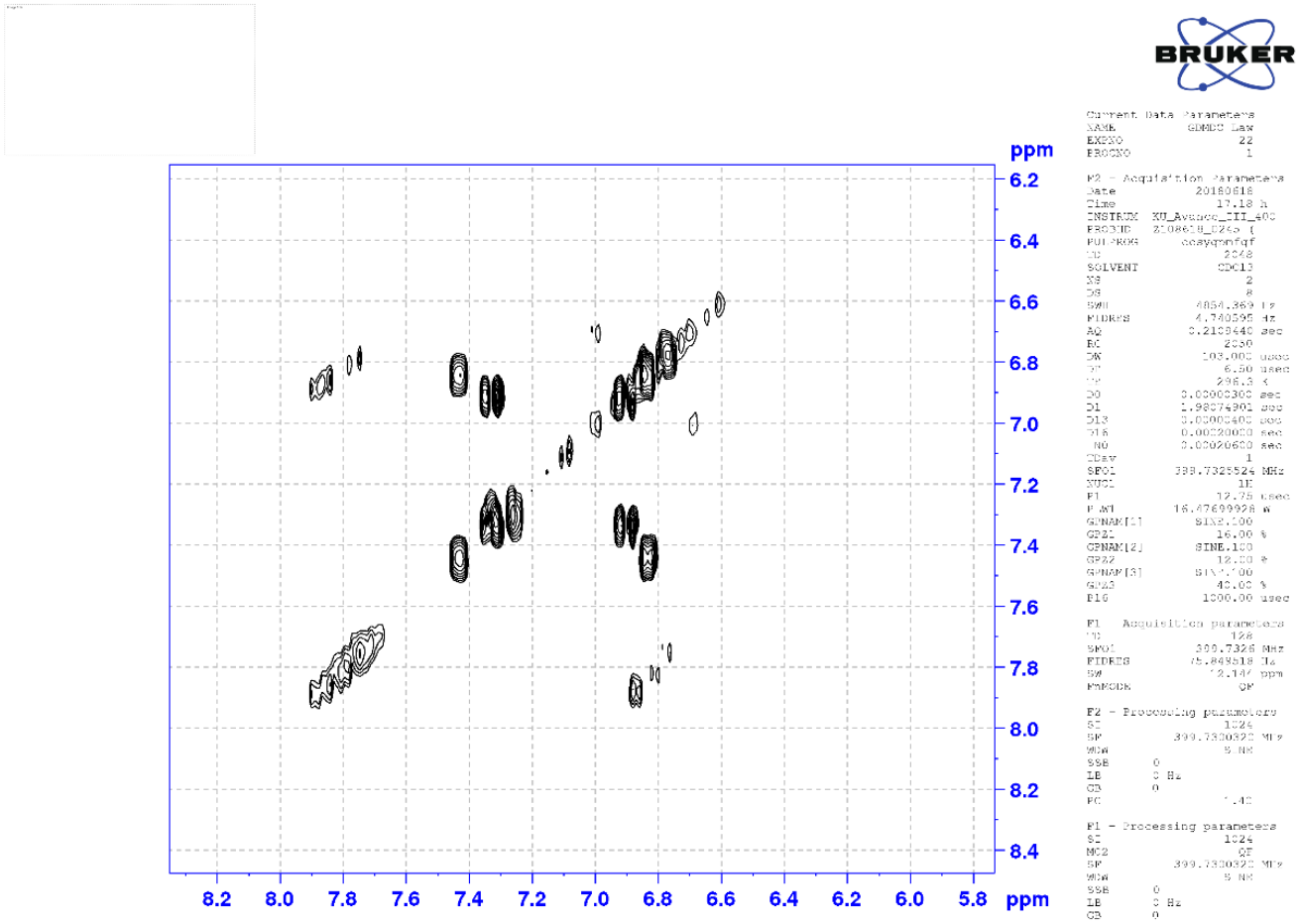
F2 - Acquisition Parameters
Date_              20180618
Time                    17.18 h
INSTRUM  XU_Avance_III_400
PROBHD   Z108618_0246 (
PULPROG         ceayqmqfqf
TD                        2048
SOLVENT                   CDCl3
NS                           2
DS                           8
SWH                  4854.369 Hz
FIDRES               4.740595 Hz
AQ                   0.2113440 sec
RG                        2050
DW                    103.000 usec
DE                      6.50 usec
TE                     296.3 K
D0                   0.00000300 sec
D1                   1.98074901 sec
D13                  0.03000400 sec
D16                  0.00020000 sec
D0                   0.00020600 sec
IDBGV                        1
SFO1               399.7325524 MHz
NUC1                       1H
P1                       12.75 usec
PLW1               16.47699928 W
GPNAM[1]              SINE.100
GPZ1                    16.00 %
GPNAM[2]              SINE.100
GPZ2                    12.00 %
GPNAM[3]              SINE.100
GPZ3                    40.00 %
P16                   1000.00 usec

F1 - Acquisition parameters
TD                         128
SFO1               399.7325 MHz
FIDRES               75.849518 Hz
SW                   12.154 ppm
FnMODE                     QF

F2 - Processing parameters
SI                        1024
SF                399.7300320 MHz
WDW                     SINE
SSB          0
LB           0 Hz
GB           0
PC                       1.40

F1 - Processing parameters
SI                        1024
MC2                         QF
SF                399.7300320 MHz
WDW                     SINE
SSB          0
LB           0 Hz
GB           0
```

1.12.4 Expansion of COSY



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```
Current Data Parameters
NAME              GDMDS Law
EXPNO                    22
PROCNO                    1

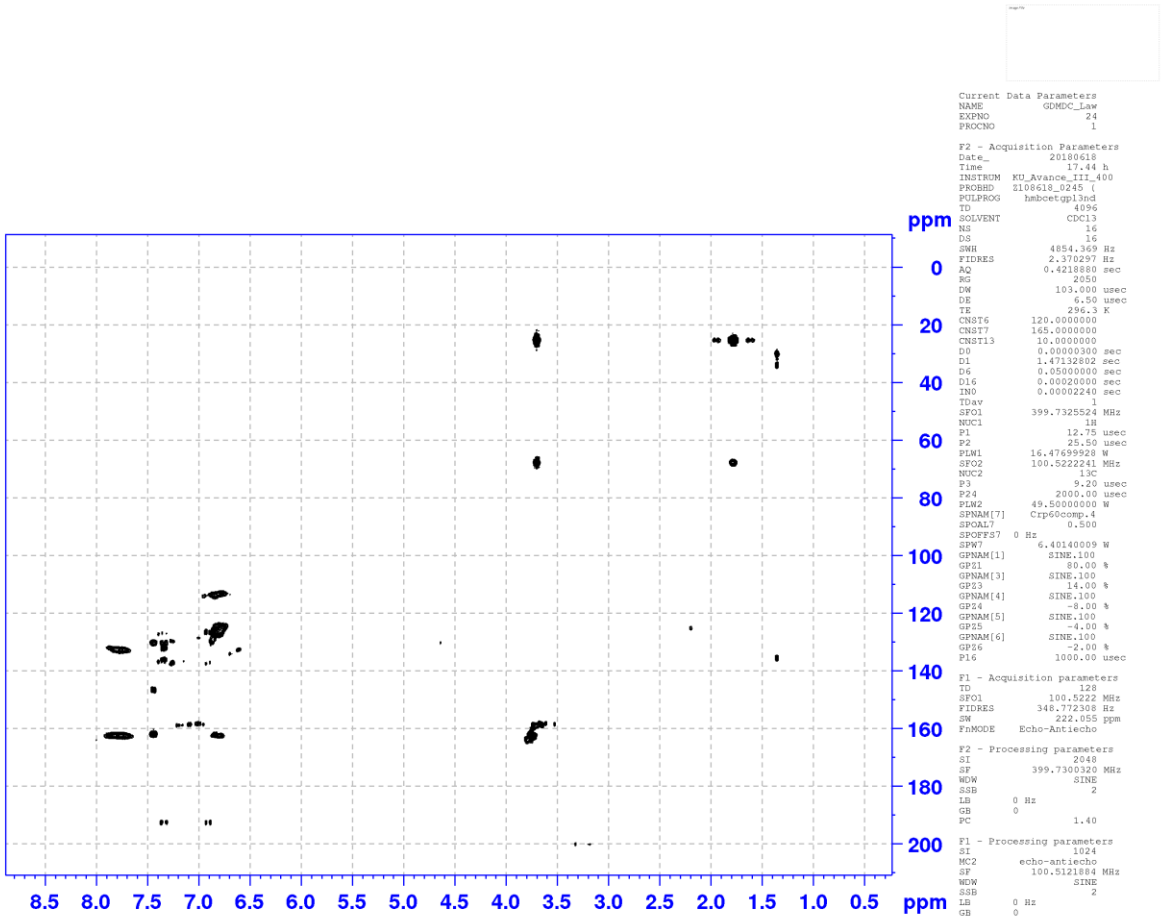
F2 - Acquisition Parameters
Date_           20180618
Time                17.18 h
INSTRUM  XU_Avance_III_400
PROBHD   Z10863(H_BBFo) (
PULPROG         ccsyqmnfqf
TD                    2048
SOLVENT               CDCl3
NS                       2
DS                       8
SWH                4854.369 Hz
FIDRES             4.740595 Hz
AQ                 0.2109440 sec
RG                    2050
DW                  103.000 usec
DE                    6.50 usec
TE                  296.5 K
D0                 3.00000300 sec
D1                 1.98074901 sec
D13                0.03000400 sec
D16                0.00020000 sec
 NO                2.00020600 sec
CDav                     1
SFO1            399.7325524 MHz
NUC1                    1H
P1                    12.75 usec
PLW1              16.4769928 W
GPNAM[1]           SINE.100
GPZ1                  16.00 %
GPNAM[2]           SINE.100
GPZ2                  12.00 %
GPNAM[3]           SINE.100
GPZ3                  40.00 %
P16                 1000.00 usec

F1 - Acquisition parameters
TD                     128
SFO1            399.7328 MHz
FIDRES             75.869518 Hz
SW                 12.744 ppm
FnMODE                   QF

F2 - Processing parameters
SI                     1024
SF              399.7330320 MHz
WDW                    SINE
SSB          0
LB           0 Hz
GB           0
PC                     1.40

F1 - Processing parameters
SI                     1024
MC2                      QF
SF              399.7330320 MHz
WDW                    SINE
SSB          0
LB           0 Hz
GB           0
```

1.12.5 ^{1}H-^{13}C HMBC




```
Current Data Parameters
NAME                  GDMDC_Law
EXPNO                        24
PROCNO                        1

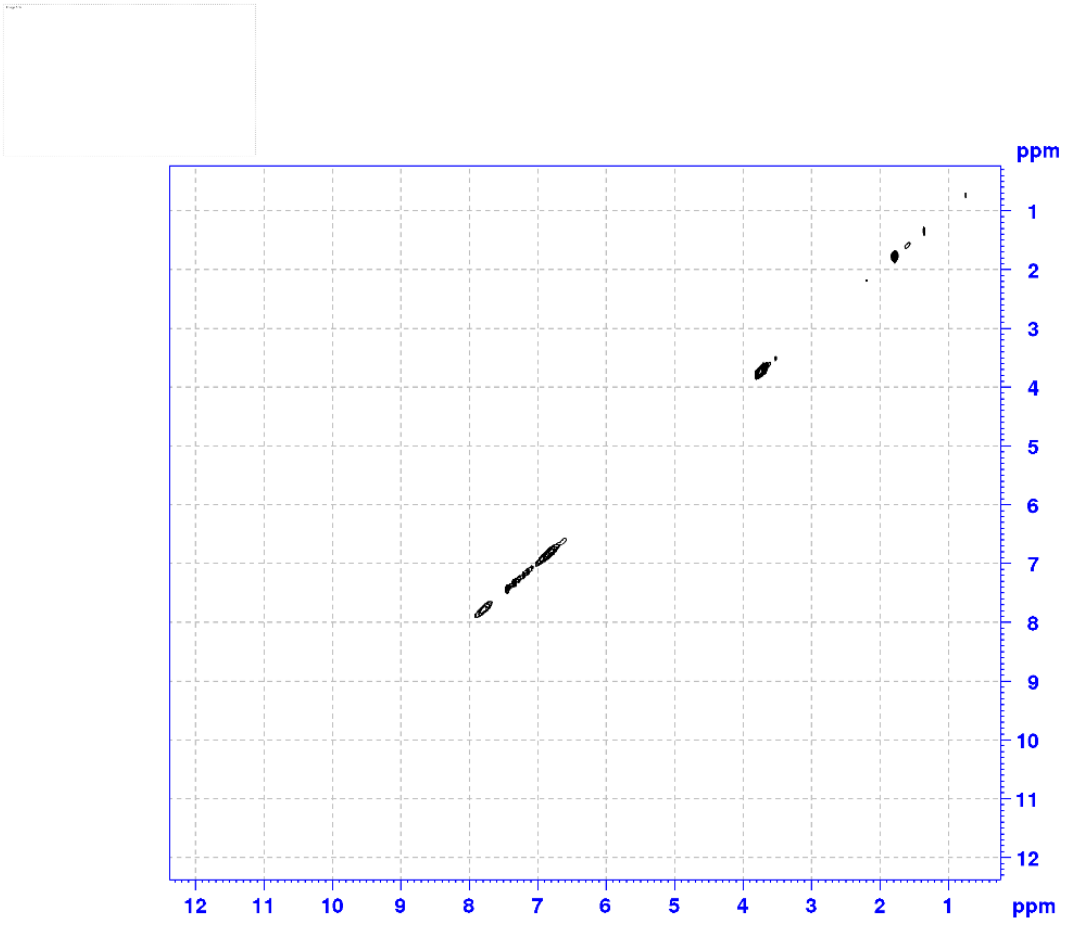
F2 - Acquisition Parameters
Date_               20180618
Time                    17.44 h
INSTRUM   KU_Avance_III_400
PROBHD    Z108618_0245 (
PULPROG     hmbcetgpl3nd
TD                        4096
SOLVENT                 CDCl3
NS                          16
DS                          16
SWH                  4854.369 Hz
FIDRES               2.370297 Hz
AQ                   0.4218880 sec
RG                         2050
DW                      103.000 usec
DE                        6.50 usec
TE                       296.3 K
CNST6               120.0000000
CNST7               165.0000000
CNST13               10.0000000
D0                   0.00000300 sec
D1                   1.47132802 sec
D6                   0.05000000 sec
D16                  0.00020000 sec
IN0                  0.00002240 sec
TDav                          1
SFO1                399.7325524 MHz
NUC1                        1H
P1                       12.75 usec
P2                       25.50 usec
PLW1                16.47699928 W
SFO2                100.5222241 MHz
NUC2                        13C
P3                        9.20 usec
P24                    2000.00 usec
PLW2                49.50000000 W
SPNAM[7]            Crp60comp.4
SPOAL7                    0.500
SPOFFS7   0 Hz
SPW7                 6.40140009 W
SPNAM[1]               SINE.100
GP21                      80.00 %
SPNAM[3]               SINE.100
GP23                      14.00 %
SPNAM[4]               SINE.100
GP24                      -8.00 %
SPNAM[5]               SINE.100
GP25                      -4.00 %
SPNAM[6]               SINE.100
GP26                      -2.00 %
P16                     1000.00 usec

F1 - Acquisition parameters
TD                         128
SFO1                100.5222 MHz
FIDRES               348.772308 Hz
SW                   222.055 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                        2048
SF                  399.7300320 MHz
WDW                       SINE
SSB                          2
LB          0 Hz
GB          0
PC                        1.40

F1 - Processing parameters
SI                        1024
MC2            echo-antiecho
SF                  100.5121884 MHz
WDW                       SINE
SSB                          2
LB          0 Hz
GB          0
```

1.12.6 ^{1}H-^{1}H NOESY



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```
Current Data Parameters
NAME              CEMDC_Lux
EXPNO                    25
PROCNO                    1

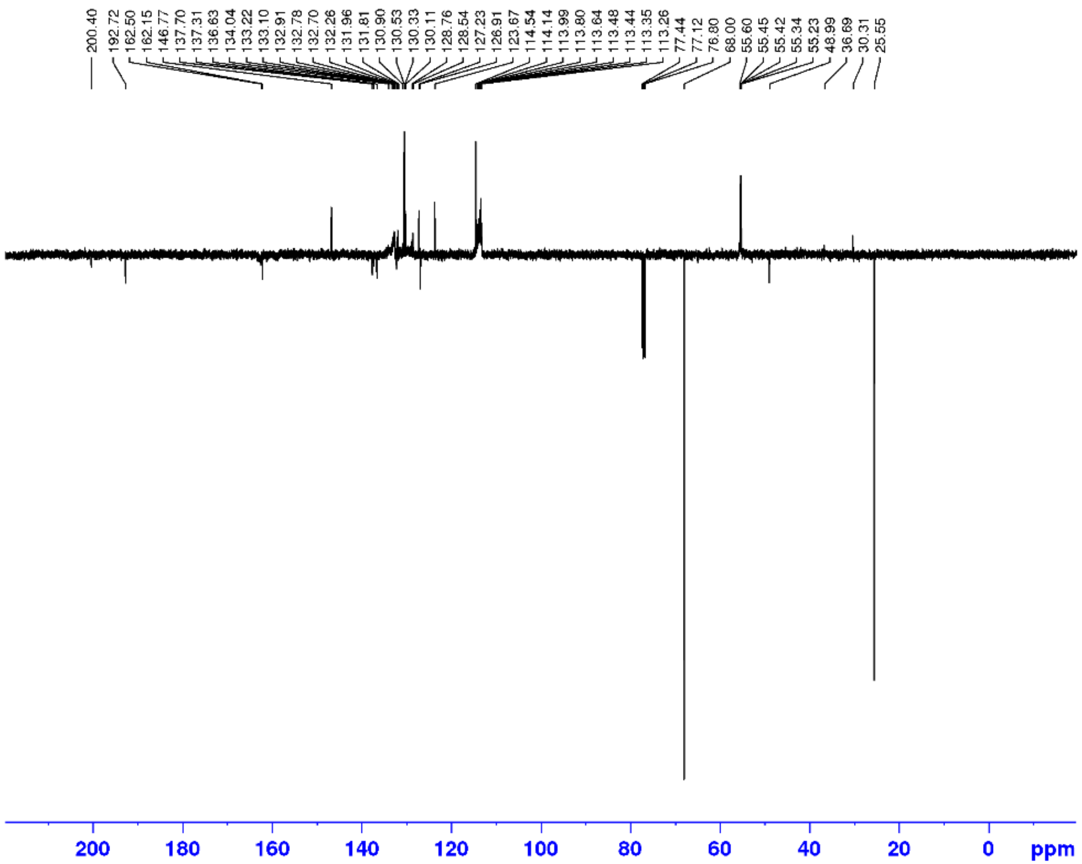
F2 - Acquisition Parameters
Date              20.80618
Time                  8.52 h
INSTRUM  KC_Avance_III_400
PROBHD   5mm_BBO_BB/1H_02/5 (
PULPROG          noesygpph
TD                     2048
SOLVENT               CDCl3
NS                        8
DS                        4
SWH                4954.369 Hz
FIDRES             2.740193 Hz
AQ                 0.2109440 sec
RG                      45.2
DW                   101.000 usec
DE                     1.35 usec
TE                    296.1 K
D0                 0.00003677 sec
D1                 2.00973103 sec
D8                 0.30000000 sec
D16                0.00020000 sec
INO                0.00020000 sec
TD0                       1
SFO1           399.7325524 MHz
NDC                      1.7
P1                    12.75 usec
P2                    25.50 usec
PLW1           16.47899928 W
GPNAM[1]          SINE.100
GPZ1                  40.00 %
P16                 1000.00 usec

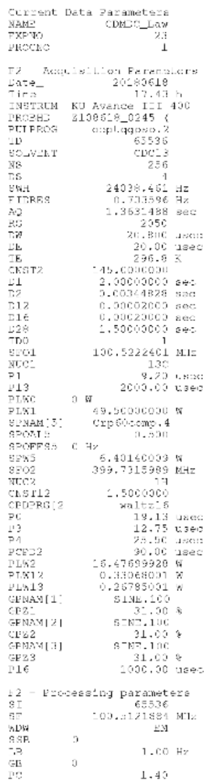
F1 - Acquisition parameters
TD                     256
SFO1           399.7326 MHz
FIDRES          37.924759 Hz
SW                 12.147 ppm
FnMODE       States-TPPI

F2 - Processing parameters
SI                    1024
SF          399.7300320 MHz
WDW                  QSINE
SSB                      2
LB        0 Hz
GB        0
PC                    1.00

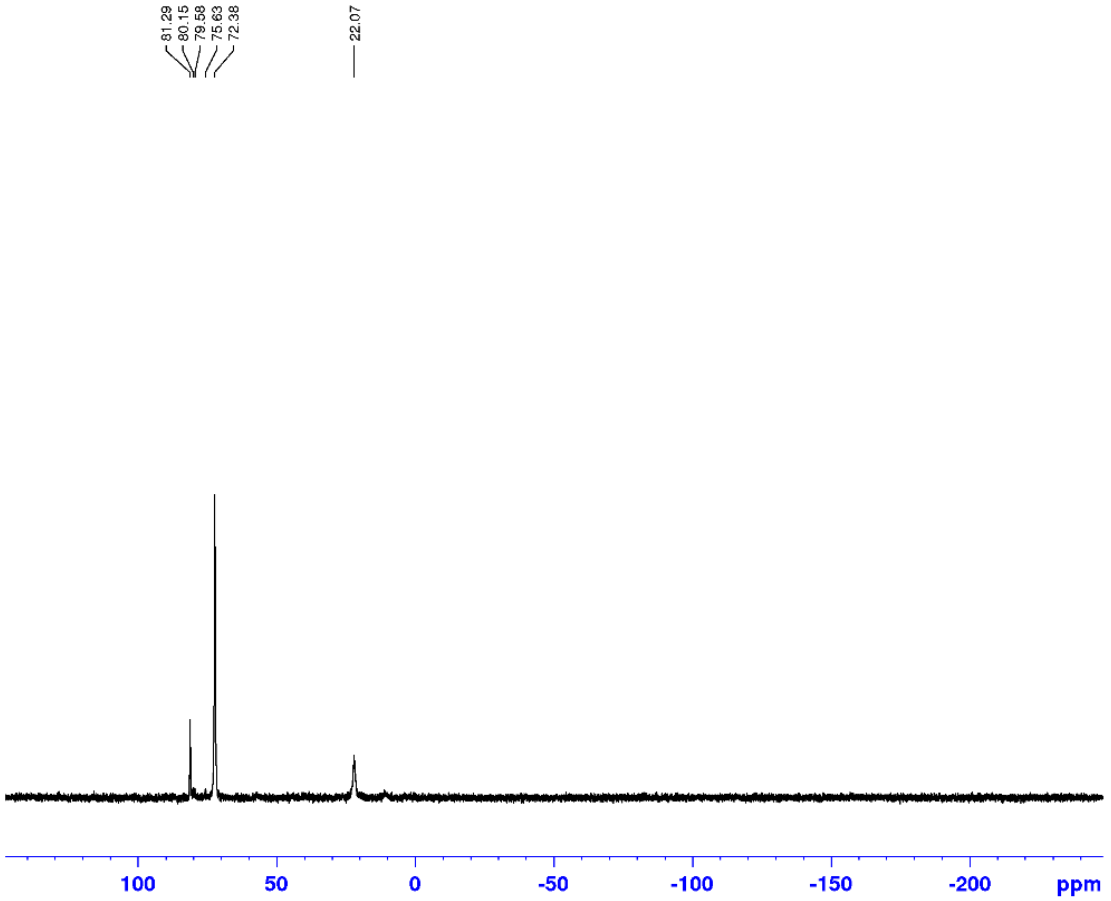
F1 - Processing parameters
SI                    1024
MC2          States-TPPI
SF          399.7300320 MHz
WDW                  QSINE
SSB                      2
LB        0 Hz
GB        0
```

1.12.7 ^{13}C NMR


200.40
192.72
162.50
162.15
146.77
137.70
137.31
136.63
134.04
133.22
133.10
132.91
132.78
132.70
132.26
131.96
131.81
130.90
130.53
130.33
130.11
128.76
128.54
127.23
126.91
123.67
114.54
114.14
113.99
113.80
113.64
113.48
113.44
113.35
113.26
77.44
77.12
76.80
68.00
55.60
55.45
55.42
55.34
55.23
48.99
36.69
30.31
25.55
200
180
160
140
120
100
80
60
40
20
0
ppm


Current Data Parameters
F2 - Acquisition Parameters
INSTRUM KU Avance III 400
SOLVENT CDCl3
NS 256
SWH 24038.461 Hz
AQ 1.3631488 sec
SFO1 100.5222401 MHz
SFO2 399.7315989 MHz
F2 - Processing parameters
SI 65536
WDW EM
LB 1.00 Hz
PC 1.40

1.12.8 ^{15}P NMR



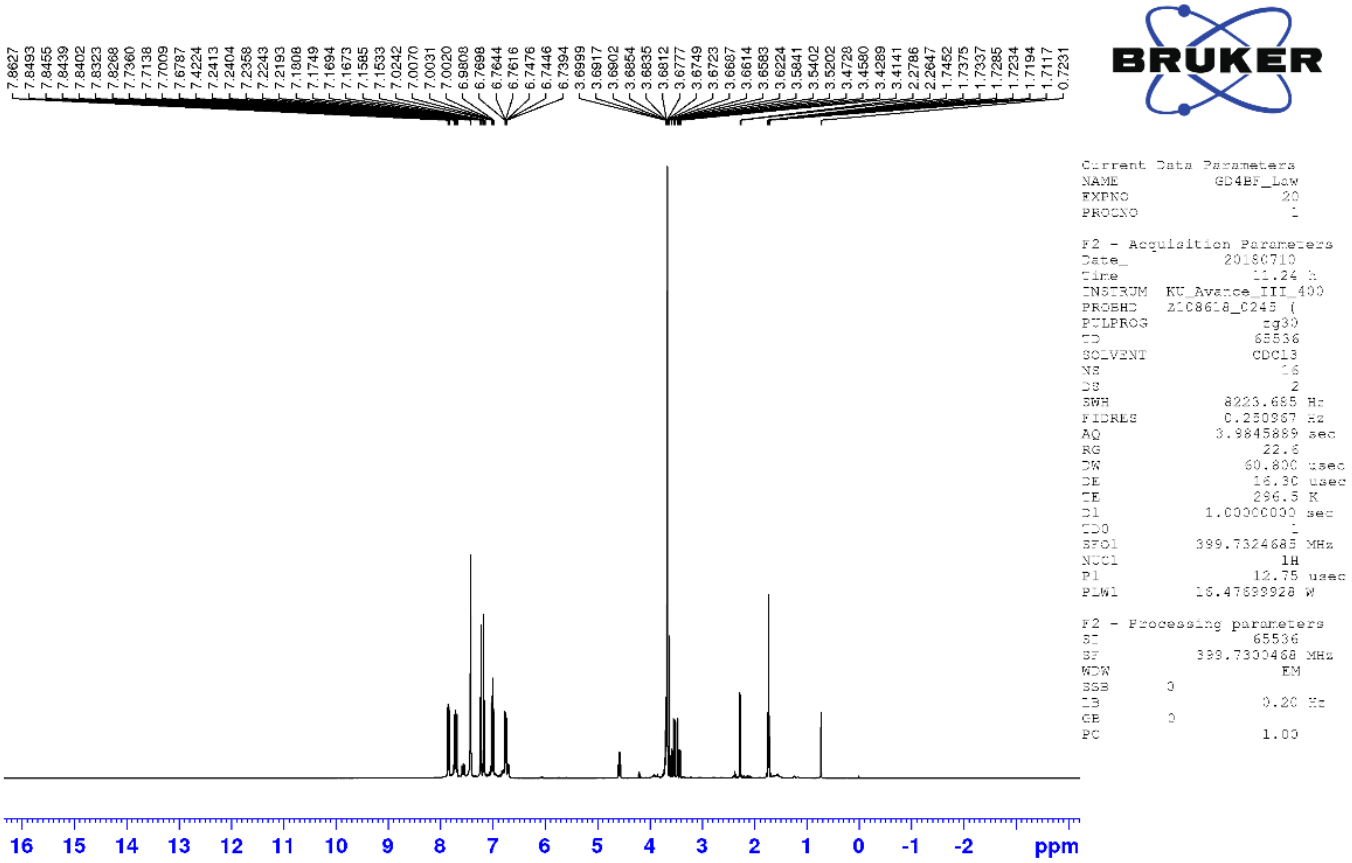

```
Current Data Parameters
NAME          GDYDC_Law_P
EXPNO                  10
PROCNO                  1

F2 - Acquisition Parameters
Date            20180730
Time               13.44 h
INSTRUM  KJ Avance III 400
PROBHD   Z108618_C245 (
PULPROG             zgpg30
TD                  65536
SOLVENT             CDCl3
NS                     32
DS                      4
SWH              64102.563 Hz
FIDRES            1.956235 Hz
AQ               0.5111808 sec
RG                    2050
DW                 7.800 usec
DE                 6.85 usec
TE                 296.0 K
D1              2.00000000 sec
TD0                     1
SFO1          161.8655793 MHz
NUC1                  31P
P1                   7.75 usec
PLW1          36.9049878 W

F2 - Processing parameters
SI                  65536
SF            161.8136700 MHz
WDW                    EM
SSB       0
LB                   3.00 Hz
GB        0
PC                   1.40
```

1.13 Compound 13

1.13.1 ^{1}H NMR

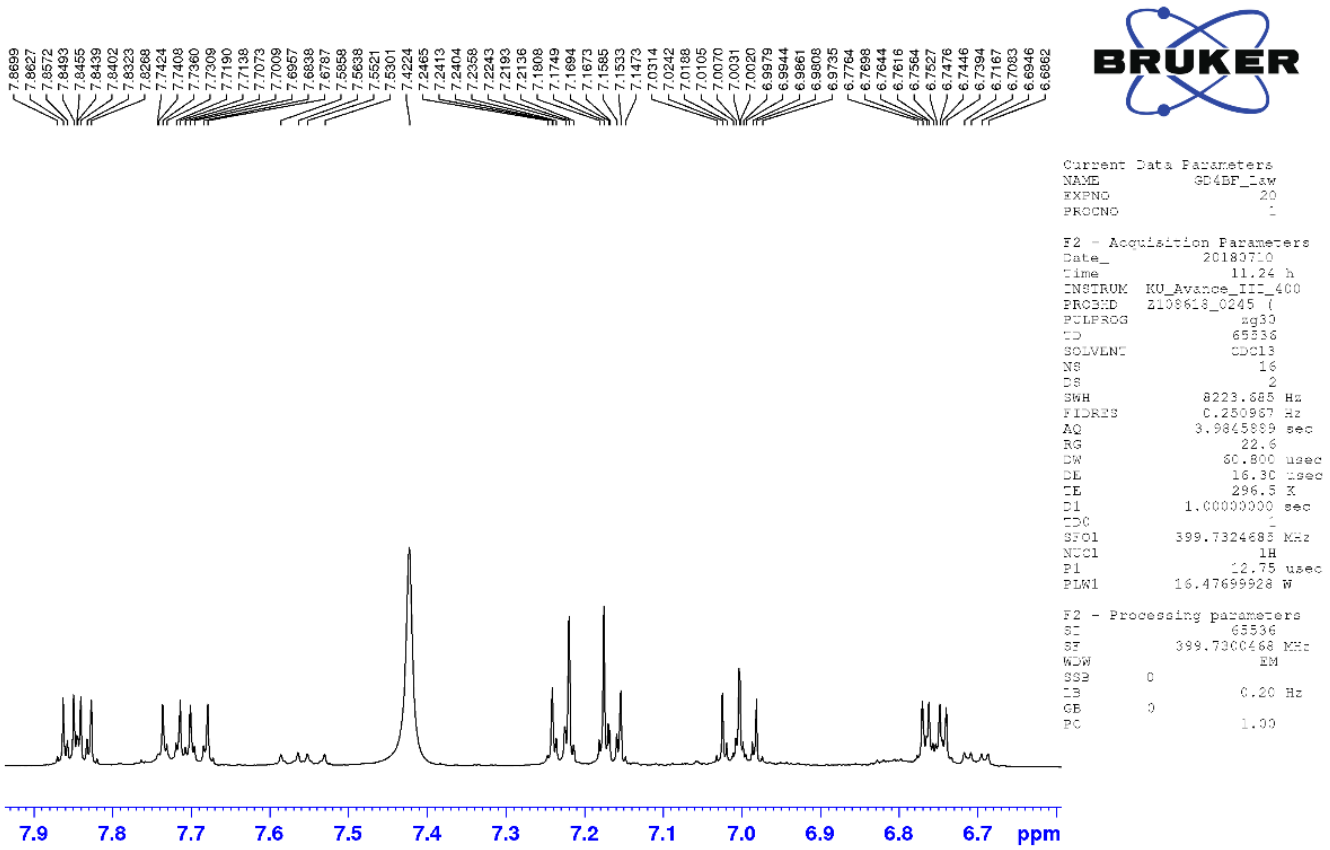



```
Current Data Parameters
NAME            GD4BF_Low
EXPNO                  20
PROCNO                  1

F2 - Acquisition Parameters
Date_            20180710
Time                11.24 h
INSTRUM  KU_Avance_III_400
PROBHD   Z108618_0243 (
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                     16
DS                      2
SWH              8223.685 Hz
FIDRES           0.250987 Hz
AQ               3.9845889 sec
RG                   22.6
DW                 60.800 usec
DE                 16.30 usec
TE                 296.5 K
D1            1.00000000 sec
TD0                     1
SFO1         399.7324685 MHz
NUC1                  1H
P1                 12.75 usec
PLW1         16.47699928 W

F2 - Processing parameters
SI                  65536
SF           399.7300468 MHz
WDW                    EM
SSB       0
LB                  0.20 Hz
GB        0
PC                  1.00
```

1.13.2 Expansion of aromatic region

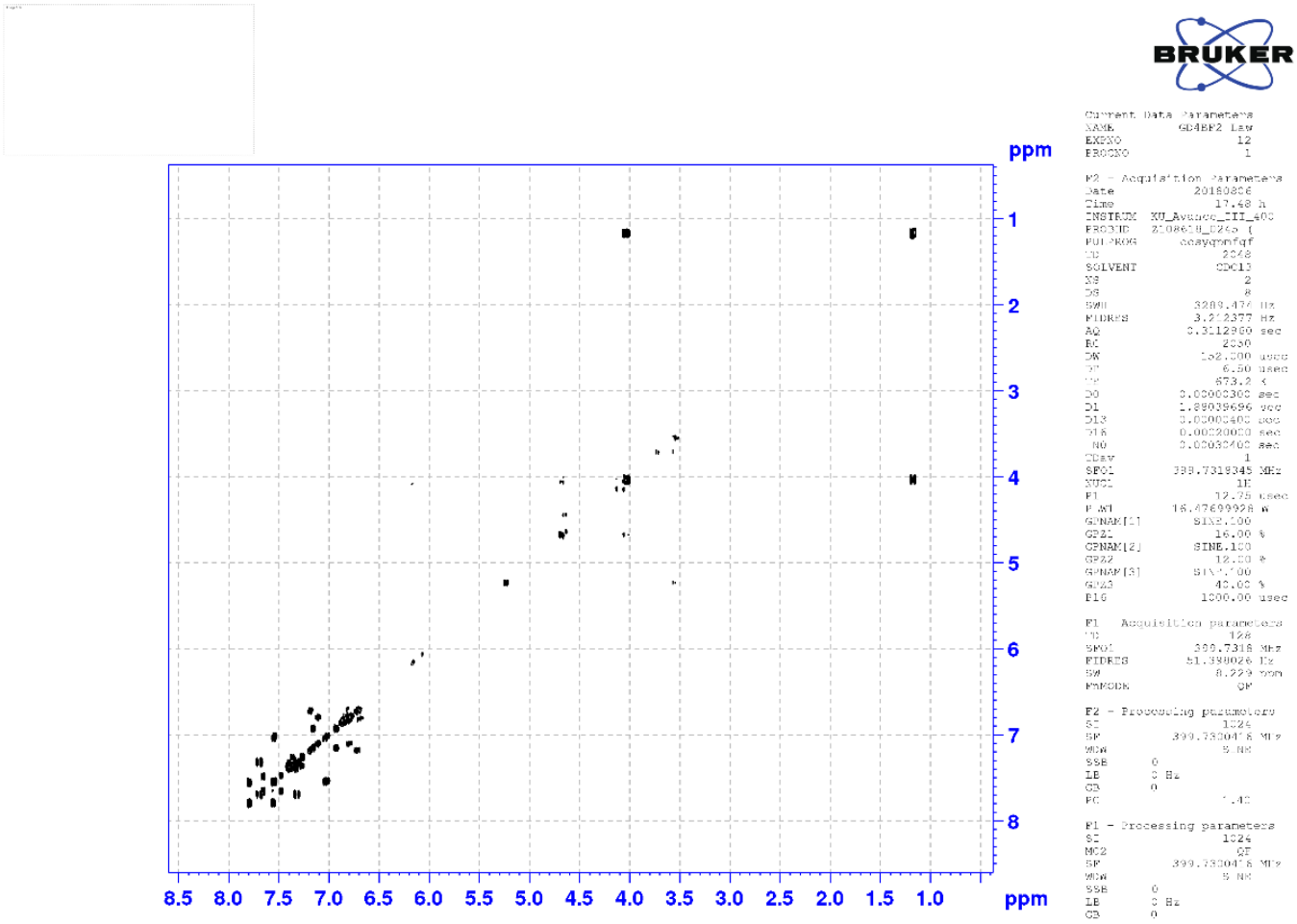



```
Current Data Parameters
NAME                 GD4BF_law
EXPNO                       20
PROCNO                       1

F2 - Acquisition Parameters
Date_               20180710
Time                    11.24 h
INSTRUM  KU_Avance_III_400
PROBHD   Z108618_0245 (
PULPROG                 zg30
TD                     65536
SOLVENT                CDCl3
NS                        16
DS                         2
SWH                 8223.685 Hz
FIDRES              0.250967 Hz
AQ                  3.9845889 sec
RG                       22.6
DW                     60.800 usec
DE                     16.30 usec
TE                     296.5 K
D1                1.00000000 sec
TDC                         1
SFO1            399.7324685 MHz
NUC1                      1H
P1                     12.75 usec
PLW1           16.47699928 W

F2 - Processing parameters
SI                     65536
SF              399.7300468 MHz
WDW                       EM
SSB          0
LB                      0.20 Hz
GB           0
PC                      1.00
```

1.13.3 ^{1}H -^{1}H COSY



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```
Current Data Parameters
NAME              GB4BP2 Law
EXPNO                 12
PROCNO                 1

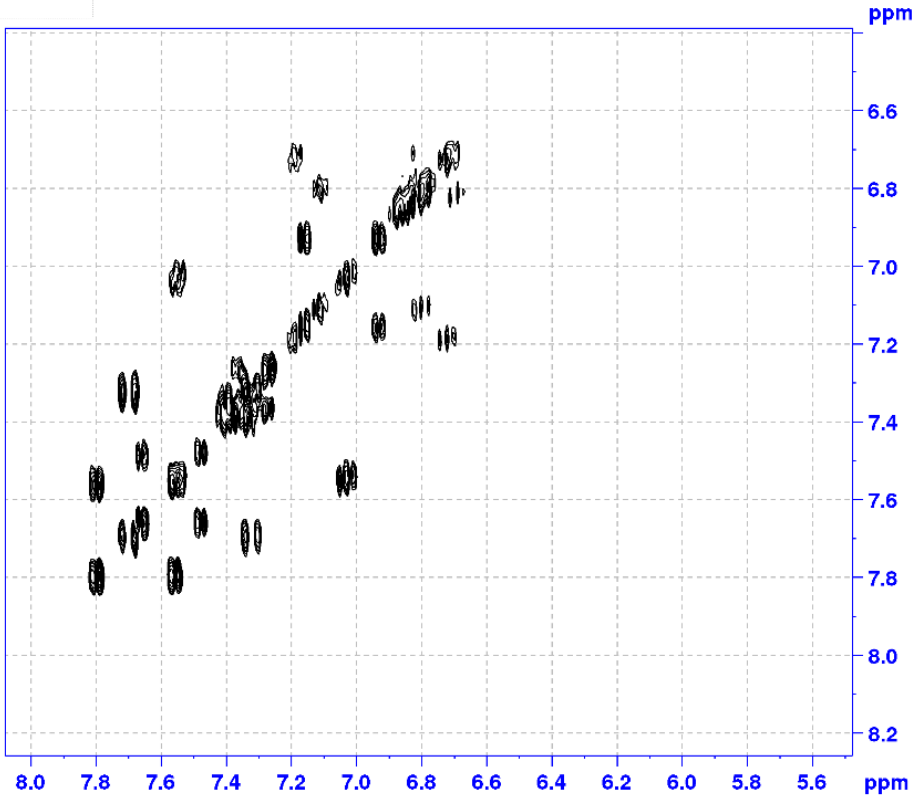
F2 - Acquisition Parameters
Date_          20180806
Time               17.48 h
INSTRUM  KU_Avance_III_400
PROBHD   Z108618_0246 (
PULPROG       ccsyqmfqf
TD                 2048
SOLVENT            CDCl3
NS                    2
DS                    8
SWH             3289.474 Hz
FIDRES          3.212377 Hz
AQ              0.3112960 sec
RG                 2030
DW                 152.000 usec
DE                 6.50 usec
TE                 573.2 K
D0              0.00000300 sec
D1              1.89039696 sec
D13             0.00000400 sec
D16             0.00020000 sec
 NO             0.00030400 sec
CDev                  1
SFO1          399.7819345 MHz
NUC1                  1H
P1                 12.75 usec
PLW1          16.47609928 W
GPNAM[1]         SINE.100
GPZ1                16.00 %
GPNAM[2]         SINE.100
GPZ2                12.00 %
GPNAM[3]         SINE.100
GPZ3                42.00 %
P16              1000.00 usec

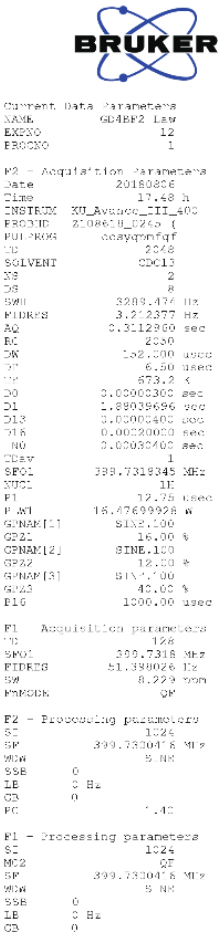
F1 - Acquisition parameters
TD                  128
SFO1           399.7818 MHz
FIDRES         51.398026 Hz
SW                 8.229 ppm
FnMODE               QF

F2 - Processing parameters
SI                 1024
SF           399.7830016 MHz
WDW                  EM
SSB       0
LB        0 Hz
GB        0
PC                 1.40

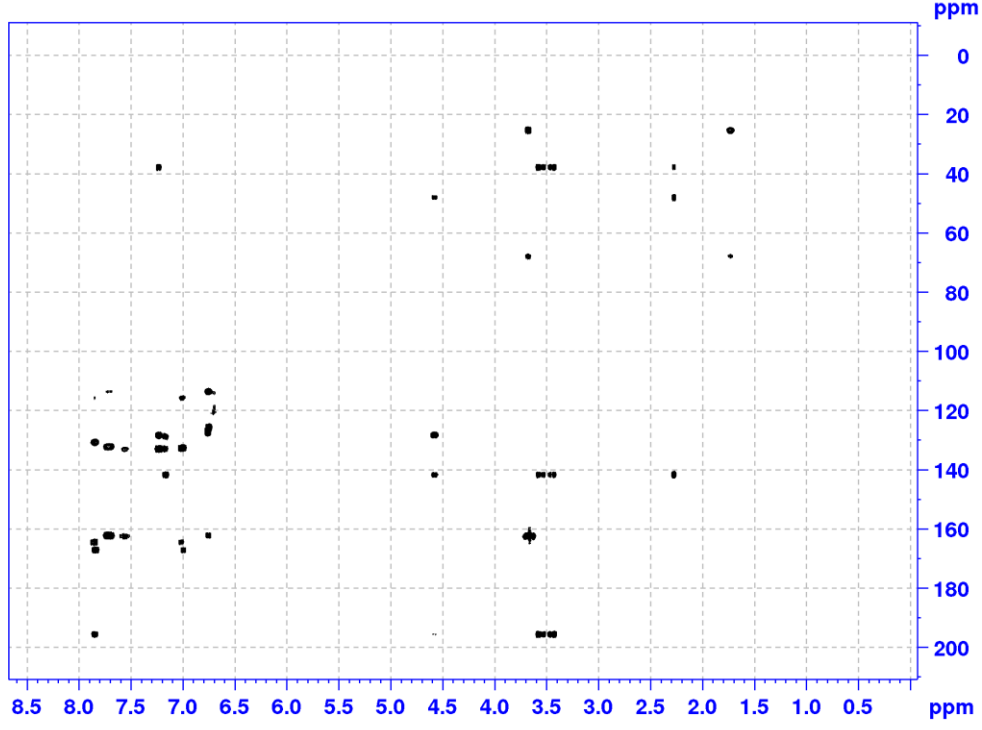
F1 - Processing parameters
SI                 1024
MC2                  QF
SF           399.7830016 MHz
WDW                  EM
SSB       0
LB        0 Hz
GB        0
```

1.13.4 Expansion of COSY





1.13.5 ^{1}H-^{13}C HMBC




```
Current Data Parameters
NAME            GD4BF_Law
EXPNO                  24
PROCNO                  1

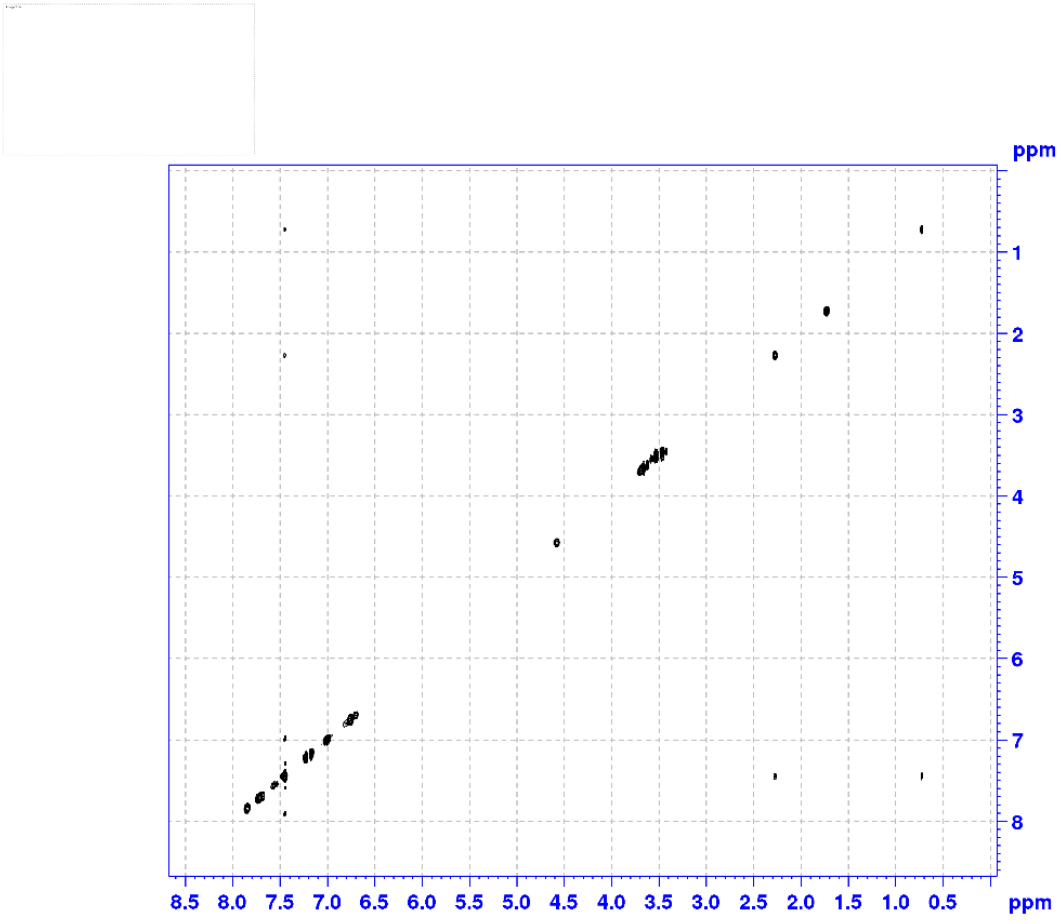
F2 - Acquisition Parameters
Date_          20180710
Time               19.33 h
INSTRUM   KU_Avance_III_400
PROBHD    Z108618_0245 (
PULPROG    hmbcetgpl3nd
TD                   4096
SOLVENT            CDCl3
NS                     16
DS                     16
SWH             3496.503 Hz
FIDRES          1.707277 Hz
AQ              0.5857280 sec
RG                   2050
DW                143.000 usec
DE                  6.50 usec
TE                296.2 K
CNST6         120.0000000
CNST7         165.0000000
CNST13         10.0000000
D0             0.00000300 sec
D1             1.30748796 sec
D6             0.05000000 sec
D16            0.00020000 sec
IN0            0.00002240 sec
TDav                   1
SFO1          399.7317661 MHz
NUC1                  1H
P1                 12.75 usec
P2                 25.50 usec
PLW1          16.47699928 W
SFO2          100.5222241 MHz
NUC2                  13C
P3                  9.20 usec
P24              2000.00 usec
PLW2          49.50000000 W
SPNAM[7]      Crp60comp.4
SPOAL7               0.500
SPOFFS7  0 Hz
SPW7           6.40140009 W
GPNAM[1]          SINE.100
GP21                80.00 %
GPNAM[3]          SINE.100
GP23                14.00 %
GPNAM[4]          SINE.100
GP24                -8.00 %
GPNAM[5]          SINE.100
GP25                -4.00 %
GPNAM[6]          SINE.100
GP26                -2.00 %
P16               1000.00 usec

F1 - Acquisition parameters
TD                   128
SFO1            100.5222 MHz
FIDRES         348.772308 Hz
SW              222.055 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                  2048
SF            399.7300468 MHz
WDW                 SINE
SSB                    2
LB        0 Hz
GB        0
PC                  1.40

F1 - Processing parameters
SI                  1024
MC2         echo-antiecho
SF            100.5121884 MHz
WDW                 SINE
SSB                    2
LB        0 Hz
GB        0
```

1.13.6 ^{1}H-^{1}H NOESY



BRUKER

```
Current Data Parameters
NAME              CD4BF_1ae
EXPNO                    25
PROCNO                    1

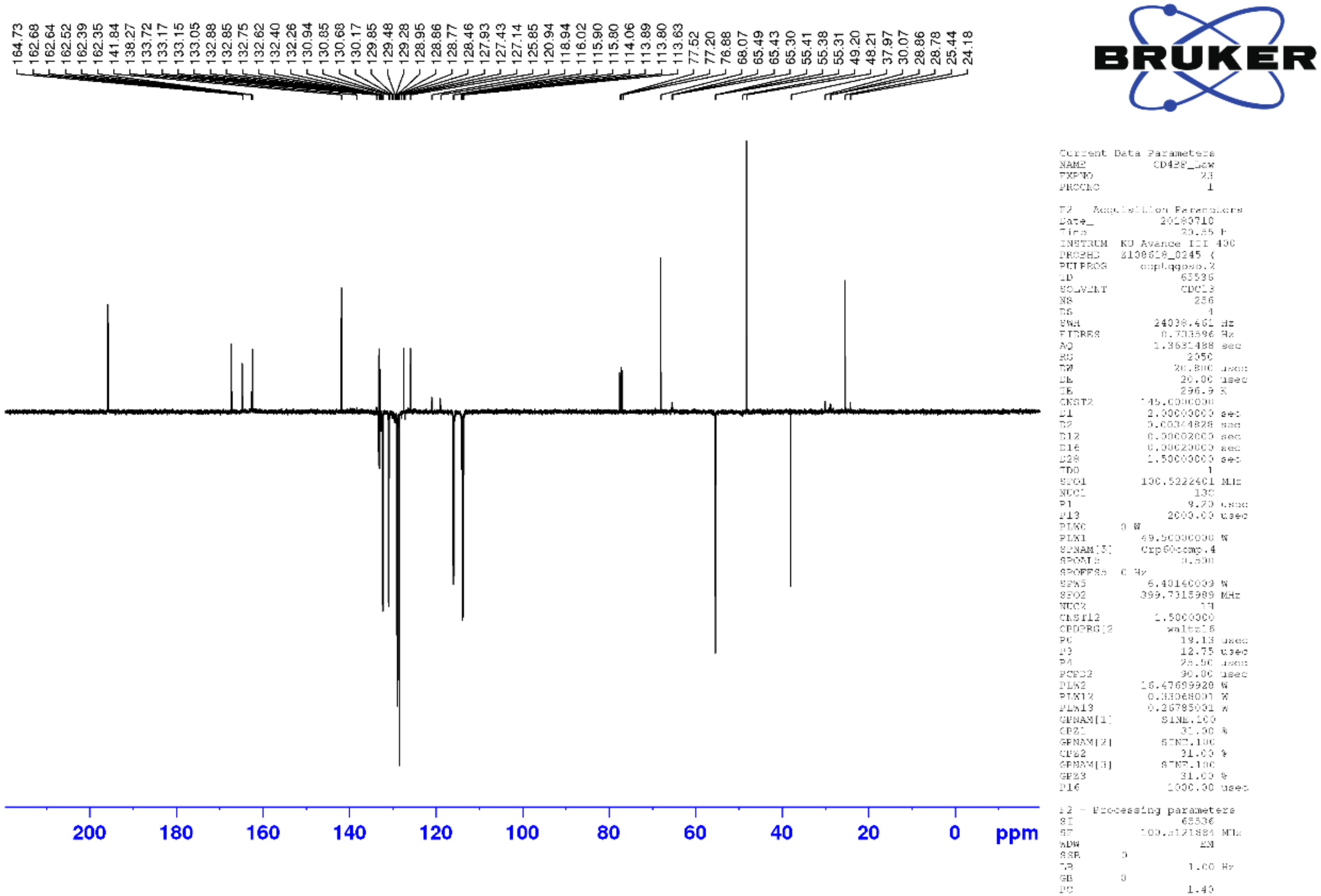
F2 - Acquisition Parameters
Date              20_80710
Time                 18.0? h
INSTRUM   KU_Avance_III_400
PROBHD    BI38618_0275 (
PULPROG          noesygpph
TD                     2048
SOLVENT              CDCl3
NS                        8
DS                        4
SWH               3496.503 Hz
FIDRES            3.414b5? Hz
AQ                0.2928640 sec
RG                       19
DW                 143.000 usec
DE                  19.01 usec
TE                 296.2 K
D0                0.00012677 sec
D1                1.95791100 sec
D8                0.30000001 sec
D16               0.00020000 sec
IN0               0.00029630 sec
IDAW                      -
SFO1           399.7317651 MHz
NUC1                     1H
P1                    12.75 usec
P2                    25.50 usec
PL01           16.47899928 W
GPNAM[1]          SINE.100
GPZ1                 40.00 %
P16                1000.00 usec

F1 - Acquisition parameters
TD                      256
SFO1            399.7318 MHz
FIDRES          27.316?33 Hz
SW                 8.?47 ppm
FnMODE       States-TPPI

F2 - Processing parameters
SI                      1024
SF            399.7300468 MHz
WDW                   QSINE
SSB                        2
LB        0 Hz
GB        0
PC                     1.00

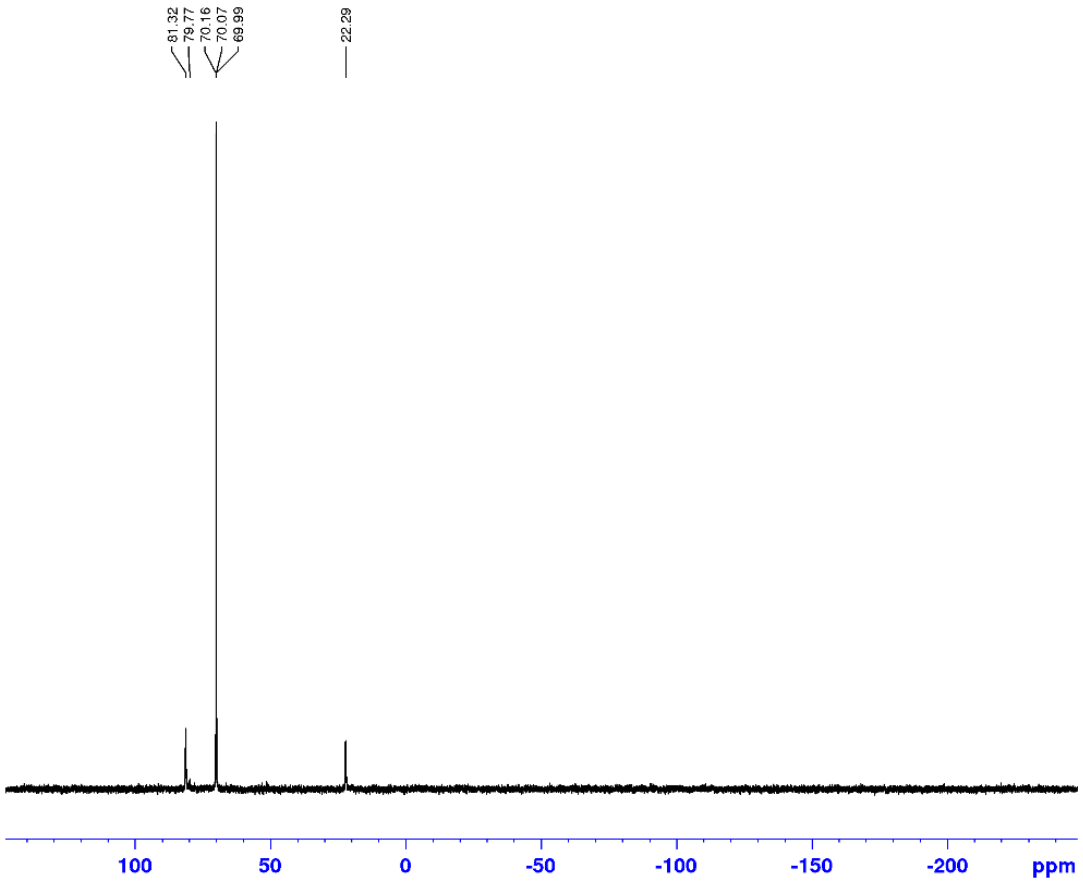
F1 - Processing parameters
SI                      1024
MC2           States-TPPI
SF            399.7300468 MHz
WDW                   QSINE
SSB                        2
LB        0 Hz
GB        0
```

1.13.7 ^{13}C NMR



164.73
162.68
162.64
162.52
162.39
162.35
141.84
138.27
133.72
133.17
133.15
133.05
132.88
132.85
132.75
132.62
132.40
132.26
130.94
130.85
130.68
130.17
129.85
129.48
129.28
128.95
128.86
128.77
128.46
127.93
127.43
127.14
125.85
120.94
118.94
116.02
115.90
115.80
114.06
113.89
113.80
113.63
77.52
77.20
76.88
68.07
65.49
65.43
65.30
55.41
55.38
55.31
49.20
48.21
37.97
30.07
28.86
28.78
25.44
24.18
BRUKER
200
180
160
140
120
100
80
60
40
20
0
ppm

1.13.8 ^{15}P NMR



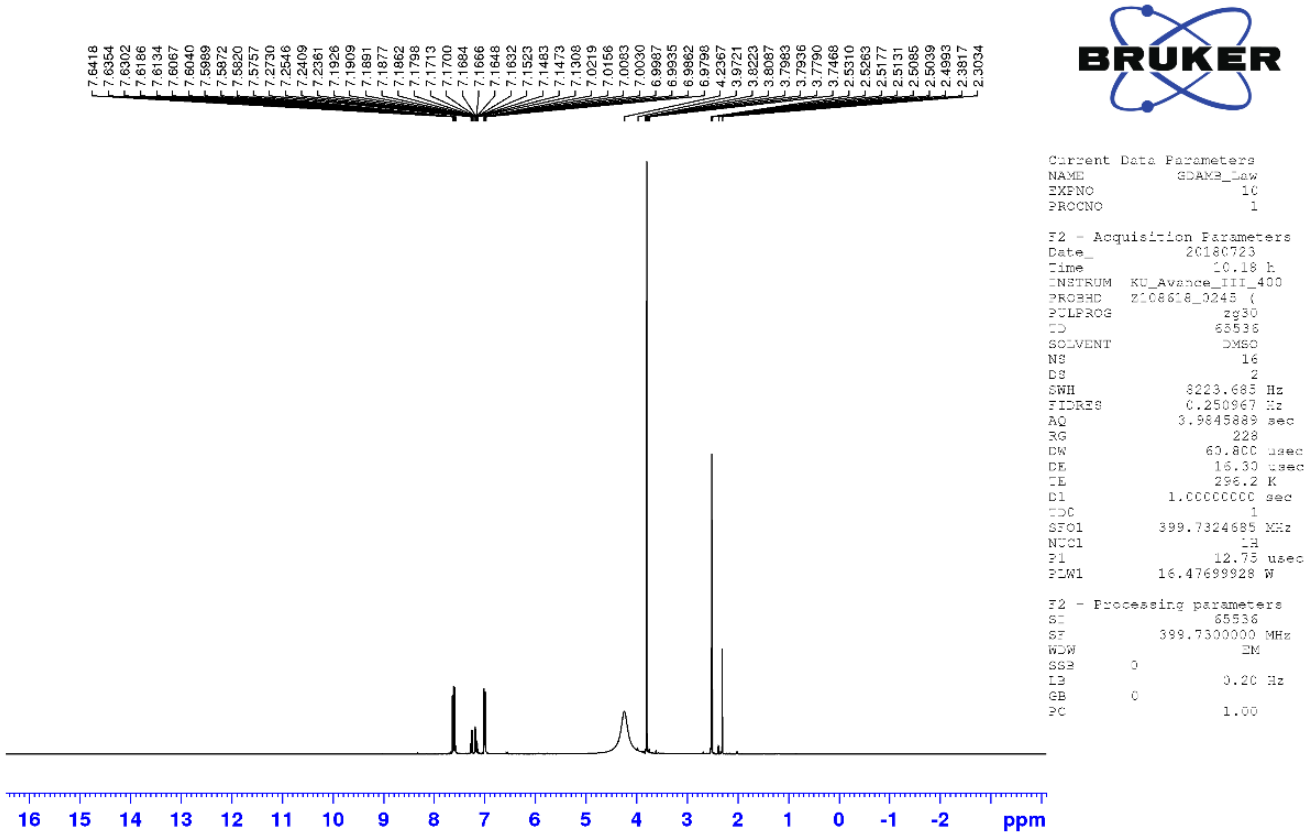

```
Current Data Parameters
NAME         CD4BF_Law_P
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date            20180730
Time                13.49 h
INSTRUM  KU Avance III 400
PROBHD   Z108618_0245 (
PULPROG             zgpg30
TD                  65536
SOLVENT             CDCl3
NS                     32
DS                      4
SWH              64102.563 Hz
FIDRES           0.978625 Hz
AQ               0.5111808 sec
RG                   2050
DW                  7.800 usec
DE                  6.85 usec
TE                  295.9 K
D1               2.00000000 sec
TD0                    1
SFO1          161.9055793 MHz
NUC1                  31P
P1                   7.75 usec
PLW1          36.90499878 W

F2 - Processing parameters
SI                  65536
SF            161.8136700 MHz
WDW                    EM
SSB                    0
LB                   3.00 Hz
GB                     0
PC                   1.40
```

1.14 Compound 14

1.14.1 ^{1}H NMR

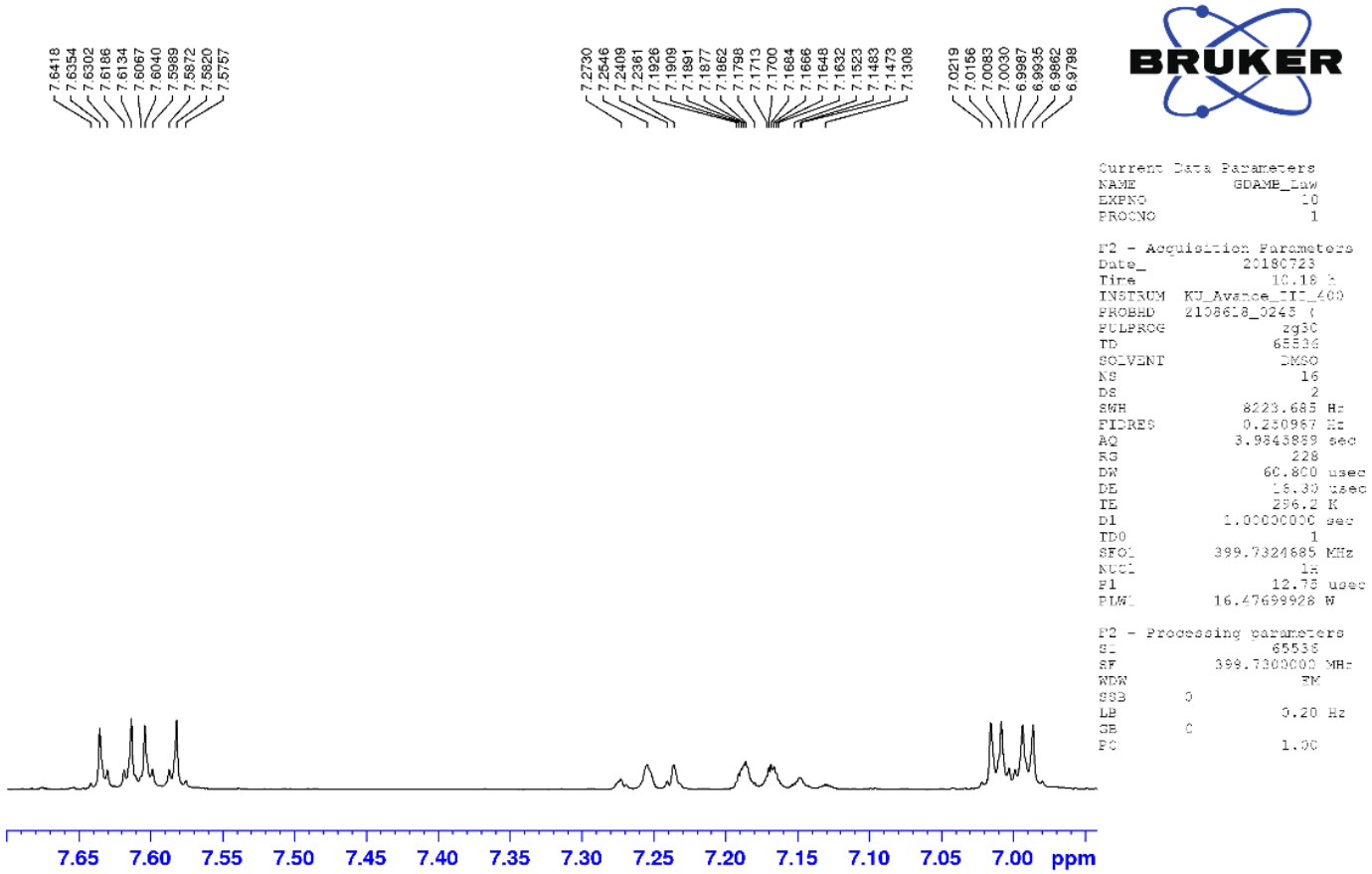



```
Current Data Parameters
NAME            GDAM3_Law
EXPNO                  10
PROCNO                  1

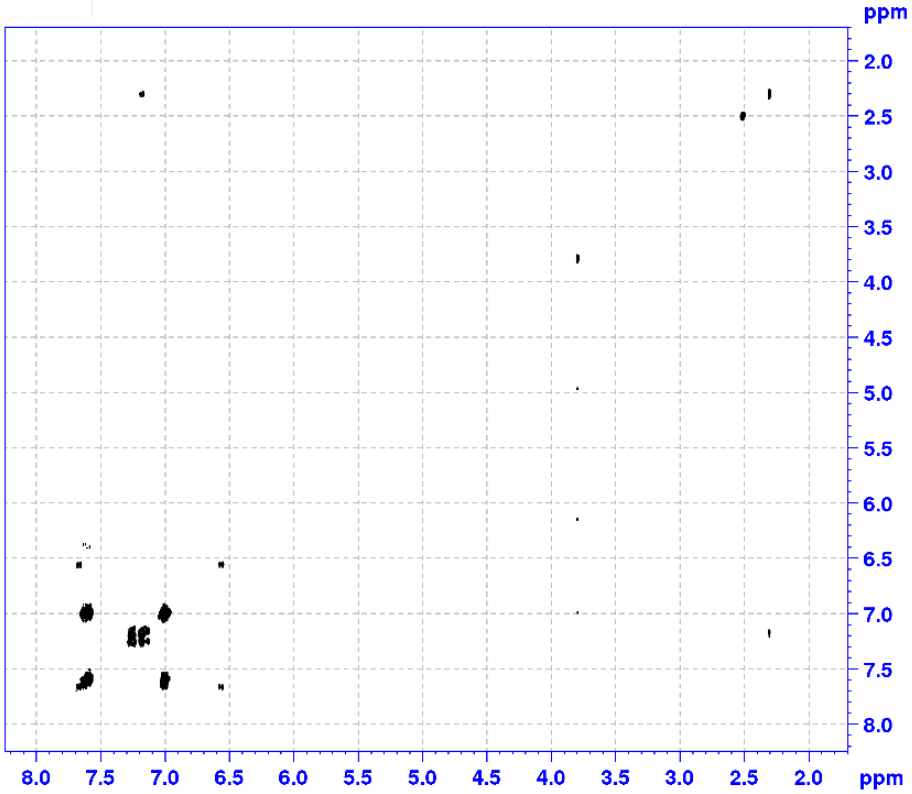
F2 - Acquisition Parameters
Date_           20180723
Time                10.18 h
INSTRUM  KU_Avance_III_400
PROBHD   Z108618_0245 (
PULPROG              zg30
TD                  65536
SOLVENT              DMSO
NS                     16
DS                      2
SWH              8223.685 Hz
FIDRES           0.250967 Hz
AQ               3.9845889 sec
RG                    228
DW                 60.800 usec
DE                 16.30 usec
TE                 296.2 K
D1              1.00000000 sec
TD0                     1
SFO1          399.7324685 MHz
NUC1                   1H
P1                  12.75 usec
PLW1          16.47699928 W

F2 - Processing parameters
SI                  65536
SF            399.7300000 MHz
WDW                    EM
SSB       0
LB                  0.20 Hz
GB        0
PC                  1.00
```

1.14.2 Expansion of aromatic region


7.6418
7.6354
7.6302
7.6186
7.6134
7.6067
7.6040
7.5989
7.5872
7.5820
7.5757
7.2730
7.2646
7.2409
7.2361
7.1926
7.1909
7.1891
7.1877
7.1862
7.1798
7.1713
7.1700
7.1684
7.1666
7.1648
7.1632
7.1523
7.1483
7.1473
7.1308
7.0219
7.0156
7.0083
7.0030
6.9987
6.9935
6.9862
6.9798
BRUKER
Current Data Parameters
NAME GDAME_Law
EXPNO 10
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180723
Time 10.18 h
INSTRUM KU_Avance_III_400
PROBHD 2108618_0243 (
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8223.685 Hz
FIDRES 0.250967 Hz
AQ 3.9845889 sec
RG 228
DW 60.800 usec
DE 16.30 usec
TE 296.2 K
D1 1.00000000 sec
TD0 1
SFO1 399.7324685 MHz
NUC1 1H
P1 12.75 usec
PLW1 16.47699928 W
F2 - Processing parameters
SI 65536
SF 399.7300000 MHz
WDW EM
SSB 0
LB 0.20 Hz
GB 0
PC 1.00
7.65
7.60
7.55
7.50
7.45
7.40
7.35
7.30
7.25
7.20
7.15
7.10
7.05
7.00
ppm

1.14.3 ^{1}H -^{1}H COSY



BRUKER

```
Current Data Parameters
NAME              GDAMS Law
EXPNO                    12
PROCNO                    1

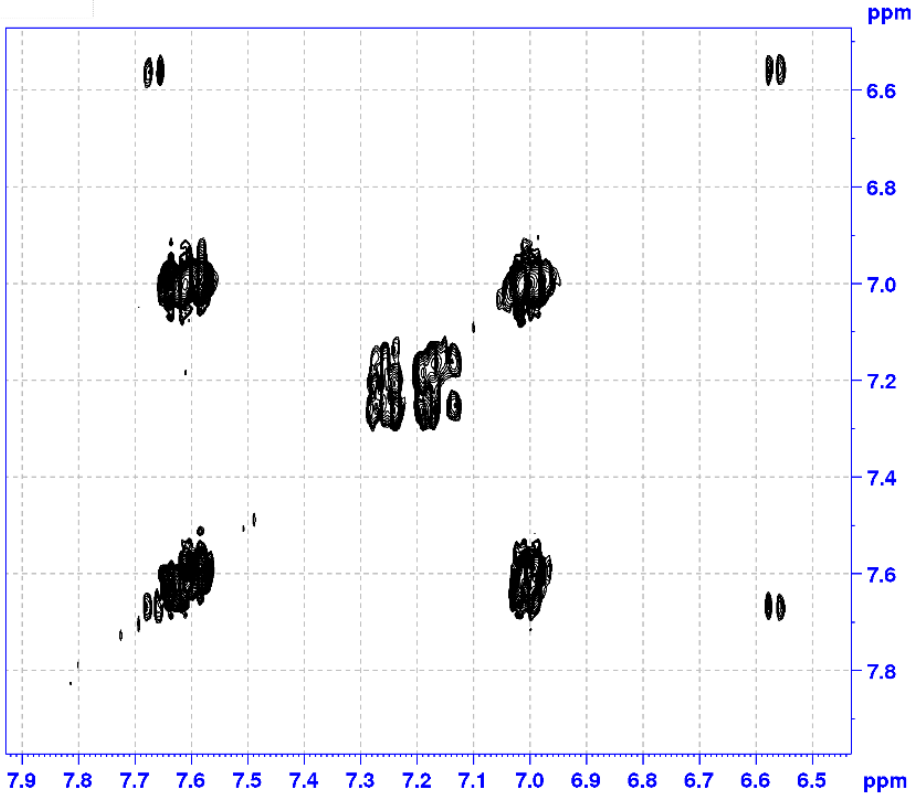
F2 - Acquisition Parameters
Date_            20180723
Time                10.19 h
INSTRUM  XU_Avance_III_400
PROBHD   Z108618_0245 (
PULPROG        cosygpmfqf
TD                   2048
SOLVENT               DMSO
NS                       2
DS                       8
SWH              2617.801 Hz
FIDRES           2.556446 Hz
AQ               0.3911680 sec
RG                    2050
DW                 191.000 usec
DE                   6.50 usec
TE                 296.2 K
D0               0.03000300 sec
D1               1.80052485 sec
D13              0.03000400 sec
D16              0.00020000 sec
  NO             0.02038200 sec
CHsv                     1
SFO1          399.7319885 MHz
NUC1                   1H
P1                   12.75 usec
PLW1          16.47699928 W
GPNAM[1]         SINE.100
GPZ1                16.00 %
GPNAM[2]         SINE.100
GPZ2                12.00 %
GPNAM[3]         SINE.100
GPZ3                40.00 %
P16                1000.00 usec

F1 - Acquisition parameters
TD                    128
SFO1           399.732 MHz
FIDRES          60.933141 Hz
SW                 6.549 ppm
FnMODE                QF

F2 - Processing parameters
SI                   1024
SF            399.7330000 MHz
WDW                  SINE
SSB                     0
LB                   0 Hz
GB                      0
PC                   1.40

F1 - Processing parameters
SI                   1024
MC2                    QF
SF            399.7330000 MHz
WDW                  SINE
SSB                     0
LB                   0 Hz
GB                      0
```

1.14.4 Expansion of COSY



BRUKER

```
Current Data Parameters
NAME                 GDANS Law
EXPNO                     12
PROCNO                     1

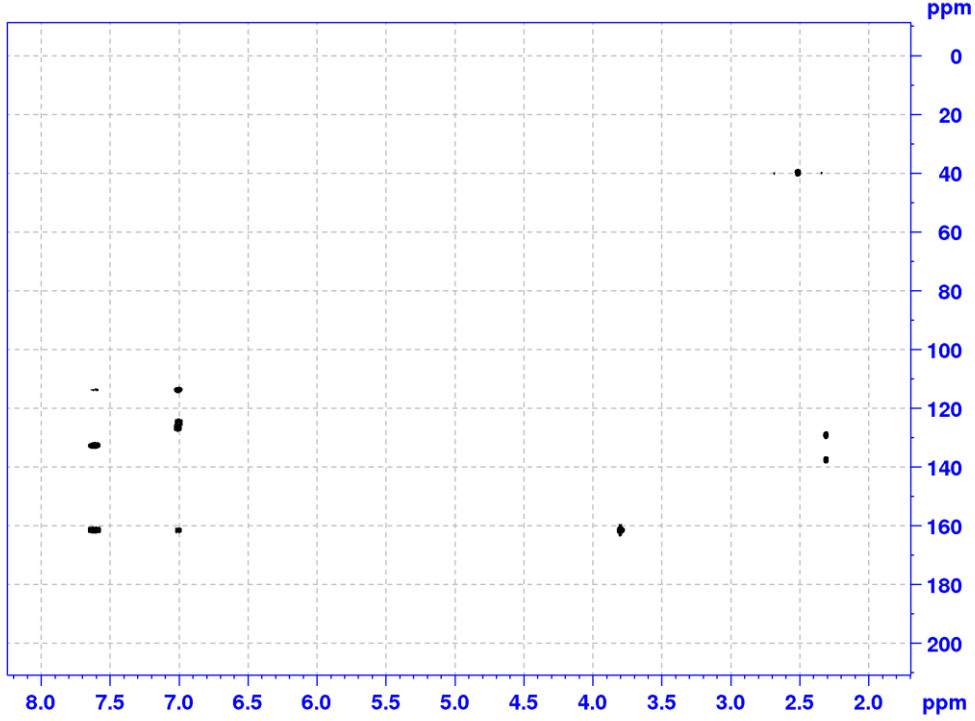
F2 - Acquisition Parameters
Date_              20180723
Time                  10.19 h
INSTRUM  KU_Avance_III_400
PROBHD   2108618_0260 (
PULPROG         cosygpmfqf
TD                      2048
SOLVENT                 DMSO
NS                         2
DS                         8
SWH                2617.801 Hz
FIDRES             2.556446 Hz
AQ                 0.3911680 sec
RG                      2030
DW                   191.000 usec
DE                     6.50 usec
TE                   296.2 K
D0                 0.00000300 sec
D1                 1.80052495 sec
D13                0.03000400 sec
D16                0.00020000 sec
 NO                0.02038200 sec
DSEW                       1
SFO1            399.7319985 MHz
NUC1                      1H
P1                     12.75 usec
PLW1           16.47699928 W
GPNAM[1]          SINE.100
GPZ1                   16.00 %
GPNAM[2]          SINE.100
GPZ2                   12.00 %
GPNAM[3]          SINE.100
GPZ3                   40.00 %
P16                 1000.00 usec

F1 - Acquisition parameters
TD                      1024
SFO1            399.732 MHz
FIDRES            40.903141 Hz
SW                     6.549 ppm
FnMODE                    QF

F2 - Processing parameters
SI                      1024
SF              399.7330020 MHz
WDW                     SINE
SSB         0
LB          0 Hz
GB          0
PC                     1.40

F1 - Processing parameters
SI                      1024
MC2                       QF
SF              399.7330020 MHz
WDW                     SINE
SSB         0
LB          0 Hz
GB          0
```

1.14.5 ^{1}H-^{13}C HMBC




```
Current Data Parameters
NAME          GDAMB_Law
EXPNO                14
PROCNO                1

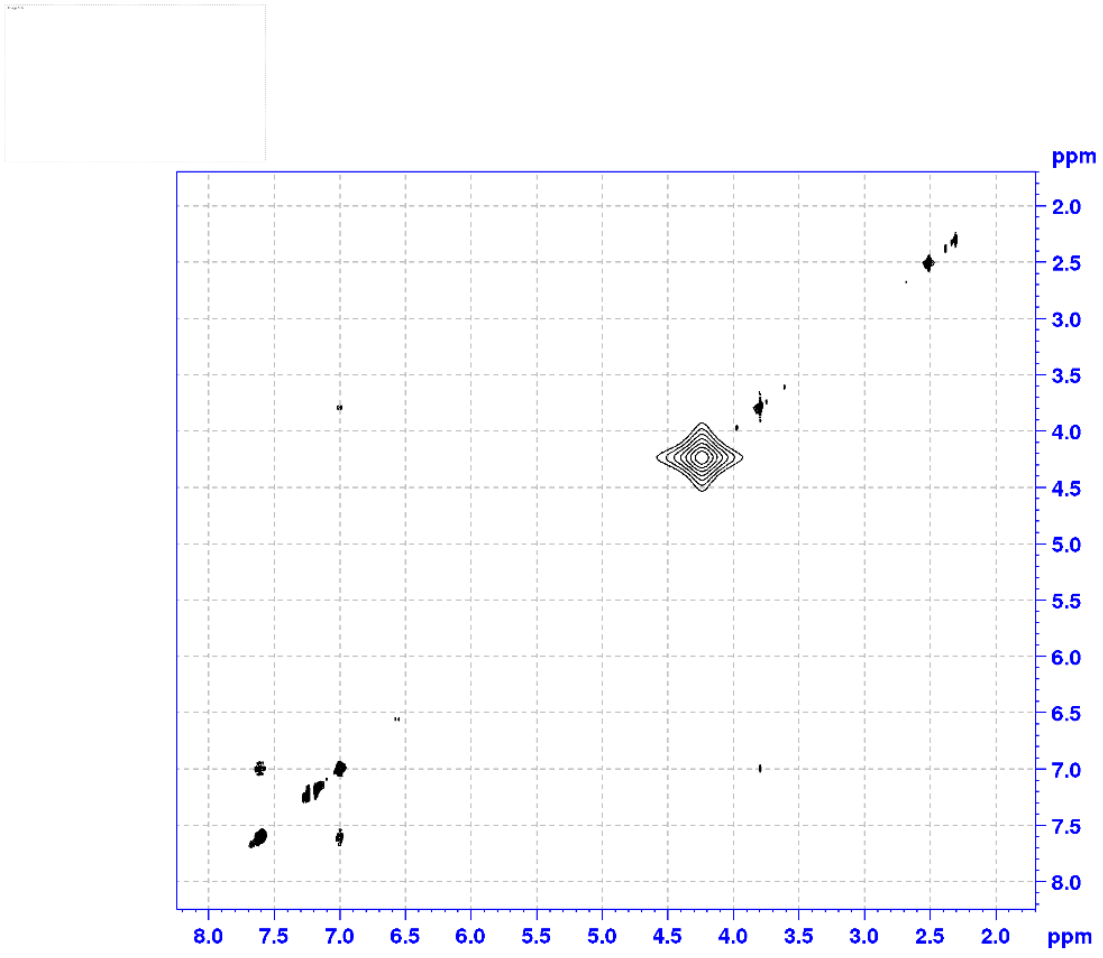
F2 - Acquisition Parameters
Date_          20180723
Time              19.33 h
INSTRUM  KU_Avance_III_400
PROBHD   Z108618_0245 (
PULPROG     hmbcetgpl3nd
TD                4096
SOLVENT            DMSO
NS                   16
DS                   16
SWH            2617.801 Hz
FIDRES         1.278223 Hz
AQ             0.7823360 sec
RG                 2050
DW              191.000 usec
DE                6.50 usec
TE               296.4 K
CNST6        120.0000000
CNST7        165.0000000
CNST13        10.0000000
D0             0.00000300 sec
D1             1.11088002 sec
D6             0.05000000 sec
D16            0.00020000 sec
IN0            0.00002240 sec
TDav                  1
SFO1         399.7319865 MHz
NUC1                 1H
P1                12.75 usec
P2                25.50 usec
PLW1         16.47699928 W
SFO2         100.5222241 MHz
NUC2                13C
P3                 9.20 usec
P24             2000.00 usec
PLW2         49.50000000 W
SPNAM[7]     Crp60comp.4
SPOAL7             0.500
SPOFFS7   0 Hz
SPW7          6.40140009 W
GPNAM[1]         SINE.100
GP21              80.00 %
GPNAM[3]         SINE.100
GP23              14.00 %
GPNAM[4]         SINE.100
GP24              -8.00 %
GPNAM[5]         SINE.100
GP25              -4.00 %
GPNAM[6]         SINE.100
GP26              -2.00 %
P16             1000.00 usec

F1 - Acquisition parameters
TD                 128
SFO1          100.5222 MHz
FIDRES        348.772308 Hz
SW            222.055 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                2048
SF           399.7300000 MHz
WDW               SINE
SSB                  2
LB        0 Hz
GB        0
PC                1.40

F1 - Processing parameters
SI                1024
MC2        echo-antiecho
SF           100.5121884 MHz
WDW               SINE
SSB                  2
LB        0 Hz
GB        0
```

1.14.6 ^{1}H-^{1}H NOESY



BRUKER

```
Current Data Parameters
NAME            CDAMB_law
EXPNO                   15
PROCNO                   1

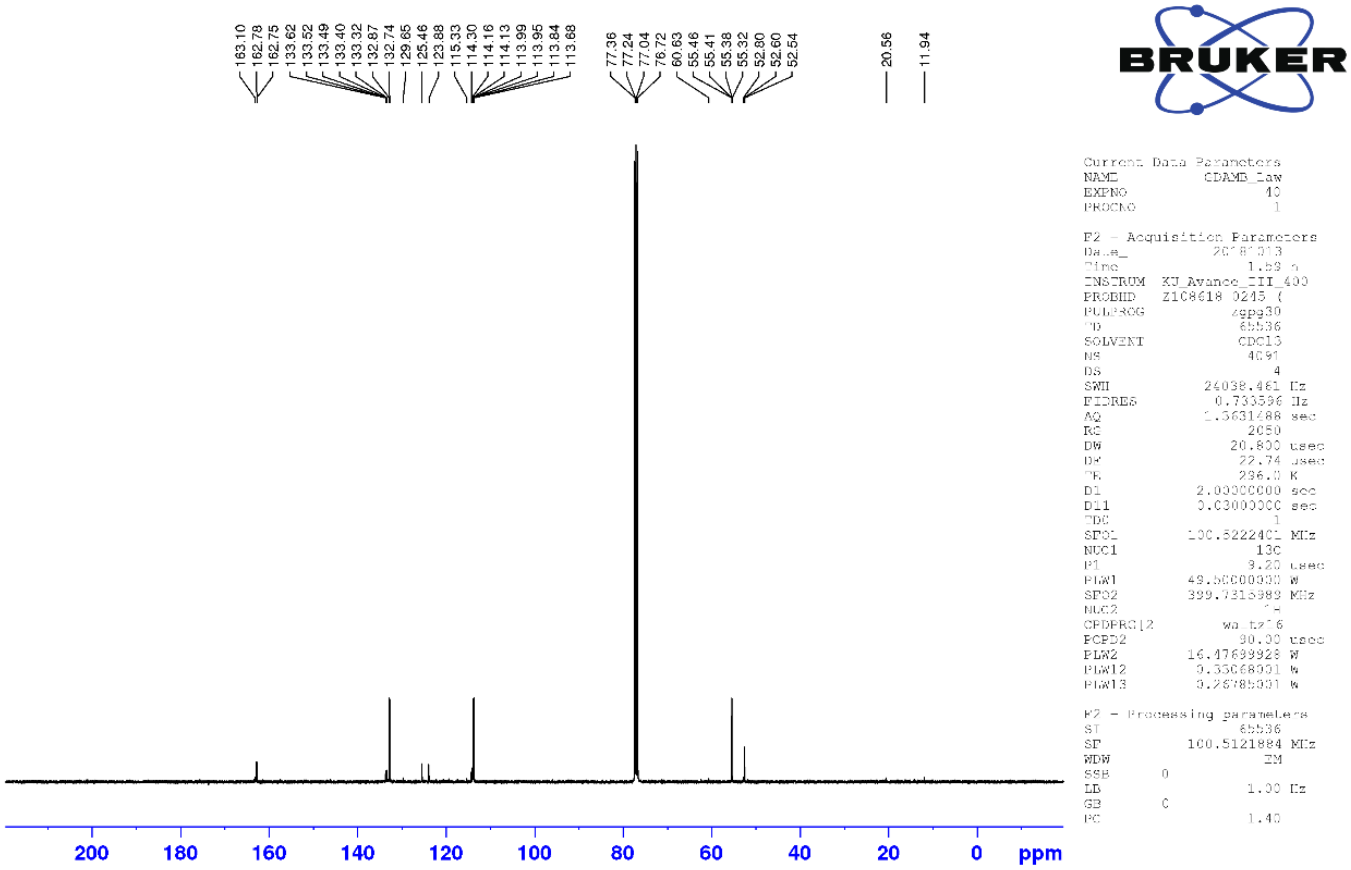
F2 - Acquisition Parameters
Date            20_80723
Time                 8.07 h
INSTRUM  BC Avance III 400
PROBHD   5mm BBO BB-H-D-Z (
PULPROG         noesygpph
TD                    2048
SOLVENT               DMSO
NS                       8
DS                       4
SWH              2617.801 Hz
FIDRES           2.556476 Hz
AQ               0.3911680 sec
RG                     181
DW                191.000 usec
DE                 28.00 usec
TE                 296.6 K
D0               0.00017477 sec
D1               1.85950696 sec
D8               0.30000001 sec
D16              0.00020000 sec
IN0              0.00038202 sec
TD0                      1
SFO1           399.7319865 MHz
NUC1                    1H
P1                   12.75 usec
P2                   25.50 usec
PLW1          16.47699928 W
GPNAM[1]         SINE.100
GPZ1                 40.00 %
P16                1000.00 usec

F1 - Acquisition parameters
TD                     256
SFO1             399.732 MHz
FIDRES          20.451571 Hz
SW                 6.379 ppm
FnMODE         States-TPPI

F2 - Processing parameters
SI                    1024
SF             399.7300000 MHz
WDW                  QSINE
SSB                      2
LB          0 Hz
GB          0
PC                    1.00

F1 - Processing parameters
SI                    1024
MC2            States-TPPI
SF             399.7300000 MHz
WDW                  QSINE
SSB                      2
LB          0 Hz
GB          0
```

1.14.7 ^{13}C NMR



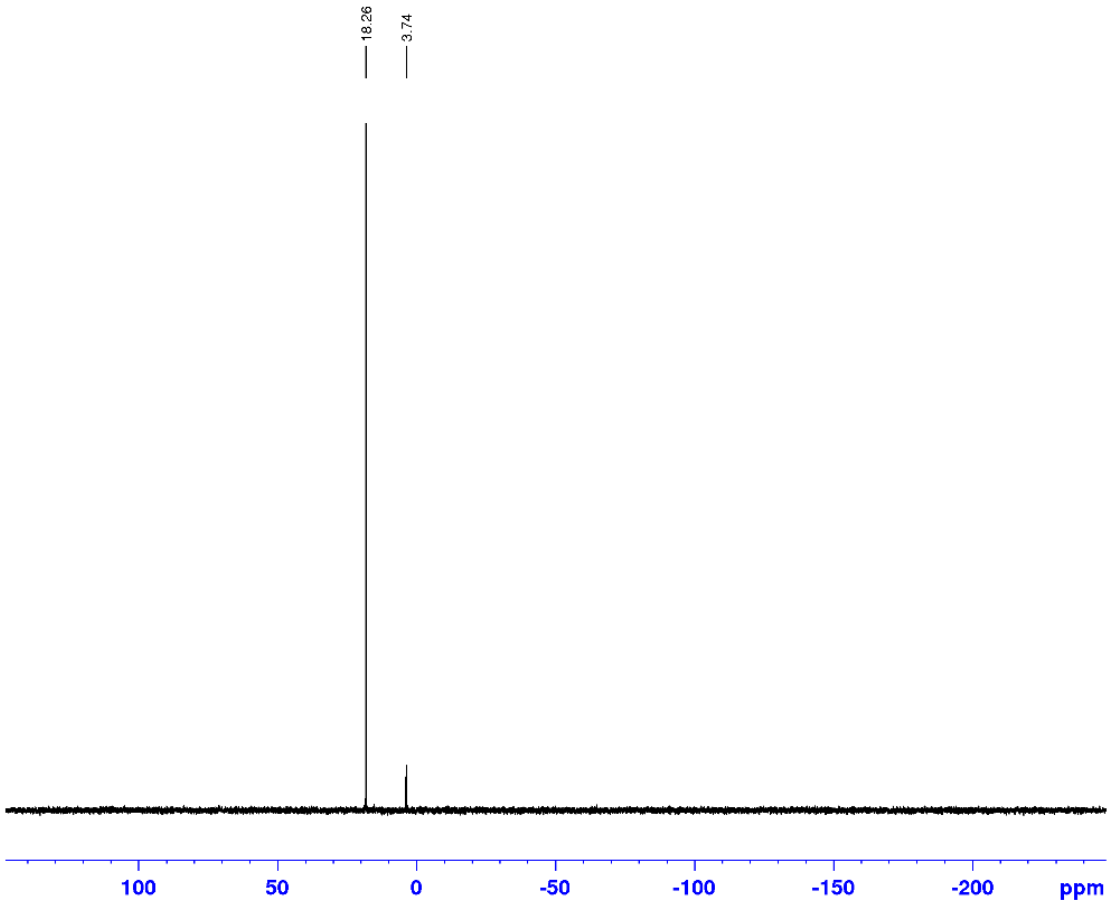
BRUKER

```
Current Data Parameters
NAME            CDAMB_1aw
EXPNO                  10
PROCNO                  1

F2 - Acquisition Parameters
Date_           20[illegible]13
Time                1.53 h
INSTRUM  XU_Avance_III_400
PROBHD   Z108618_0245 (
PULPROG             zgpg30
TD                  65536
SOLVENT             CDCl3
NS                   4091
DS                      4
SWH            24038.461 Hz
FIDRES          0.733596 Hz
AQ              1.3631488 sec
RG                   2050
DW                 20.800 usec
DE                 22.74 usec
TE                 296.1 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                     1
SFO1         100.6222401 MHz
NUC1                  13C
P1                   9.20 usec
PLW1          49.50000000 W
SFO2         399.7316989 MHz
NUC2                  [illegible]
CPDPRG[2          wa[illegible]6
PCPD2               90.00 usec
PLW2          16.47699928 W
PLW12          3.33068001 W
PLW13          3.26788001 W

F2 - Processing parameters
SI                   65536
SF           100.5121884 MHz
WDW                     EM
SSB       0
LB                   1.00 Hz
GB        0
PC                   1.40
```

1.14.8 ^{15}P NMR



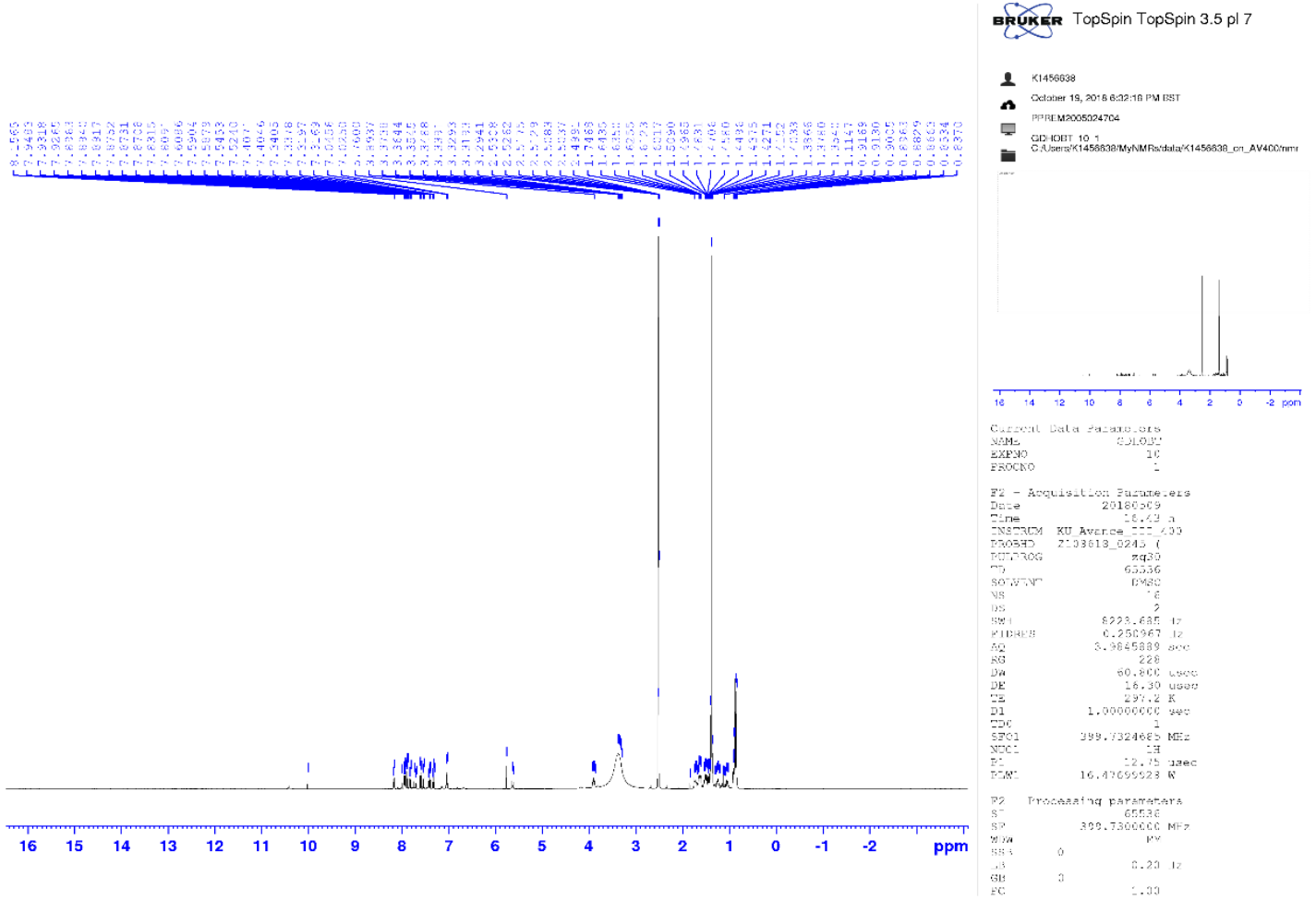

```
Current Data Parameters
NAME          CDAMB_Law_P
EXPNO                  10
PROCNO                  1

F2 - Acquisition Parameters
Date            20180730
Time               18.15 h
INSTRUM  KJ Avance III 400
PROBHD   Z108618_C245 (
PULPROG            zgpg30
TD                  65536
SOLVENT             CDCl3
NS                     32
DS                      4
SWH             64102.563 Hz
FIDRES          1.956235 Hz
AQ              0.5111808 sec
RG                   2050
DW                 7.800 usec
DE                 6.85 usec
TE                 297.5 K
D1              2.00000000 sec
D11             0.03000000 sec
TD0                     1
SF01          161.8066793 MHz
NUC1                  31P
P1                   7.75 usec
PLW1          36.9049987E W
SFO2          399.7315989 MHz
NUC2                  1H
CPDPRG2          waltz16
PCPD2              90.00 usec
PLW2          18.47699928 W
PLW12          0.33068001 W
PLW13          0.26785001 W

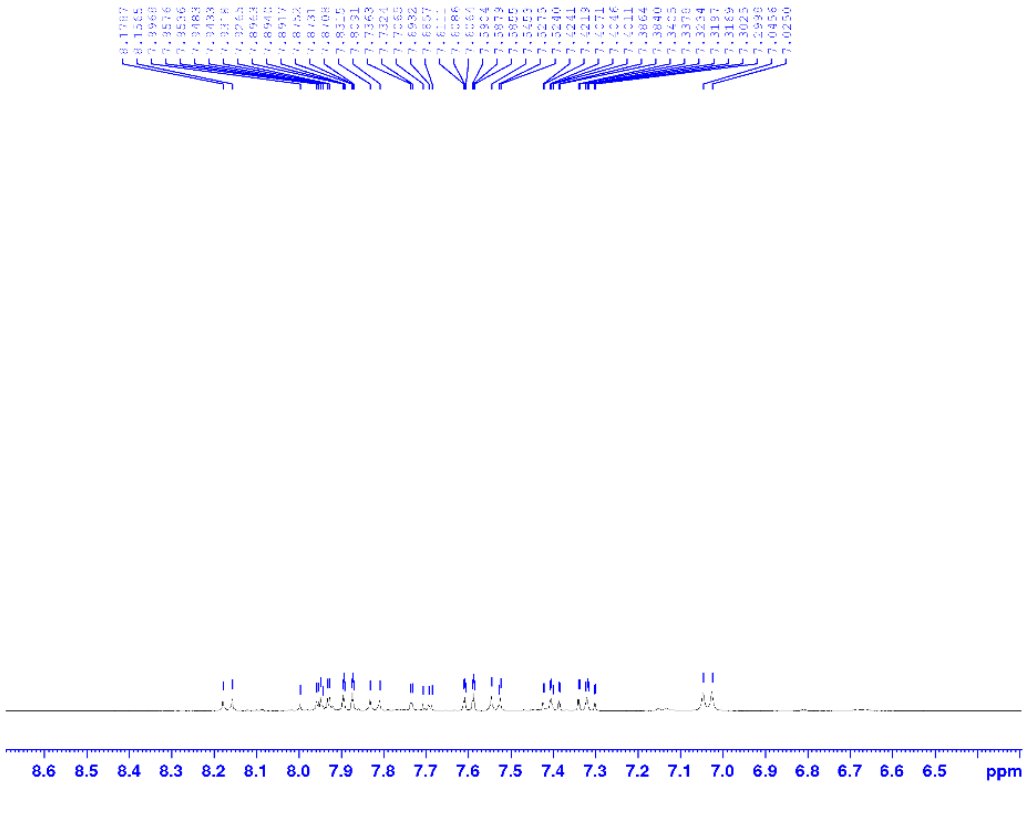
F2 - Processing parameters
SI                  65536
SF            161.8136700 MHz
WDW                    EM
SSB       0
LB                  3.00 Hz
GB        0
PC                  1.40
```

1.15 Compound 15

1.15.1 ^{1}H NMR



1.15.2 Expansion of aromatic region



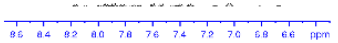
TopSpin TopSpin 3.5 pl 7

K1455638

October 19, 2018 6:32:48 PM BST

PPREM2005024704

GDHOBT_10_1
C:/Users/K1455638/MyNMRs/data/K1455638_on_AV400/nmr

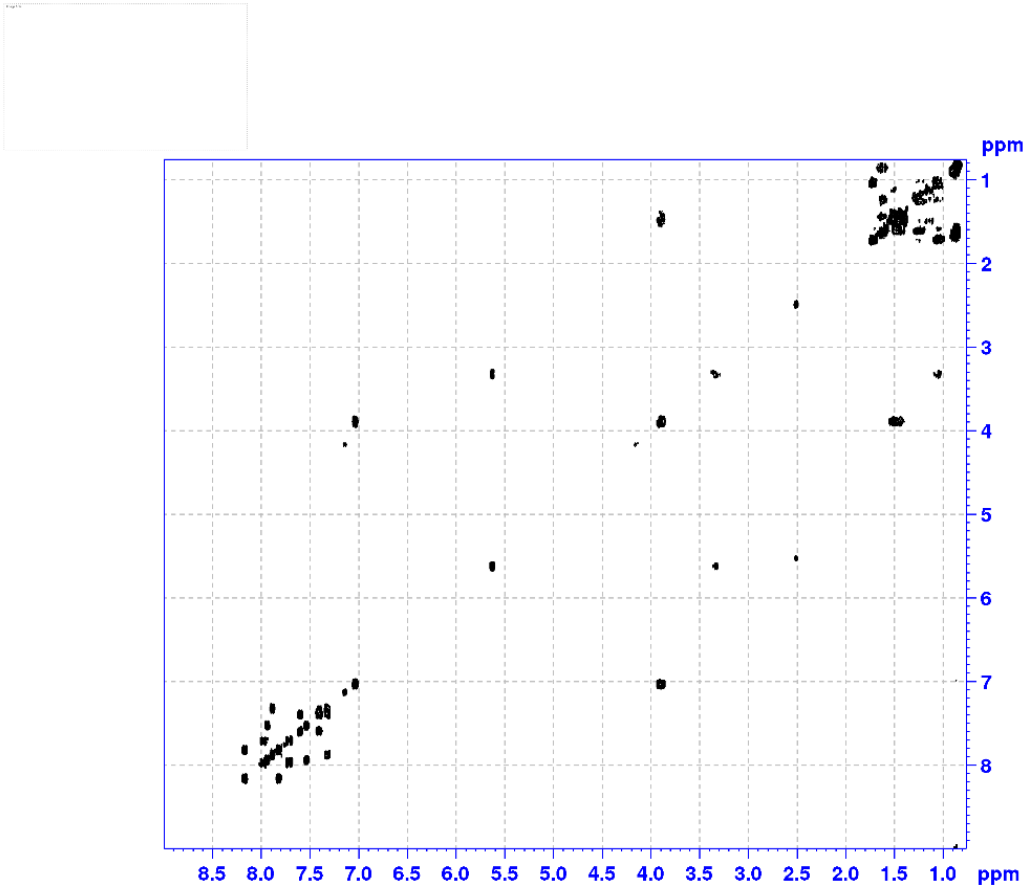



```
Current Data Parameters
NAME                  GDHOBT
EXPNO                     10
PROCNO                     1

F2 - Acquisition Parameters
Date                20180509
Time                   16.43 h
INSTRUM  XC_Avance_III_400
PROBHD   5mm BBFO_0245 (
PULPROG                zg30
TD                    65536
SOLVENT                DMSO
NS                       16
DS                        2
SWH                9223.685 Hz
FIDRES             0.256967 Hz
AQ                 3.9845889 sec
RG                      228
DW                   60.800 usec
DE                    6.50 usec
TE                   287.2 K
D1                1.00000000 sec
TD0                       1
SFO1             399.7324835 MHz
NUC1                     1H
P1                    12.75 usec
PLW1             16.47699928 W

F2 - Processing parameters
SI                    65536
SF               399.7300000 MHz
WDW                      EM
SSB          0
LB                    0.20 Hz
GB           0
PC                    1.00
```

1.15.3 ^{1}H -^{1}H COSY



BRUKER

```
Current Data Parameters
NAME                 GDROBZ
EXPNO                    12
PROCNO                    1

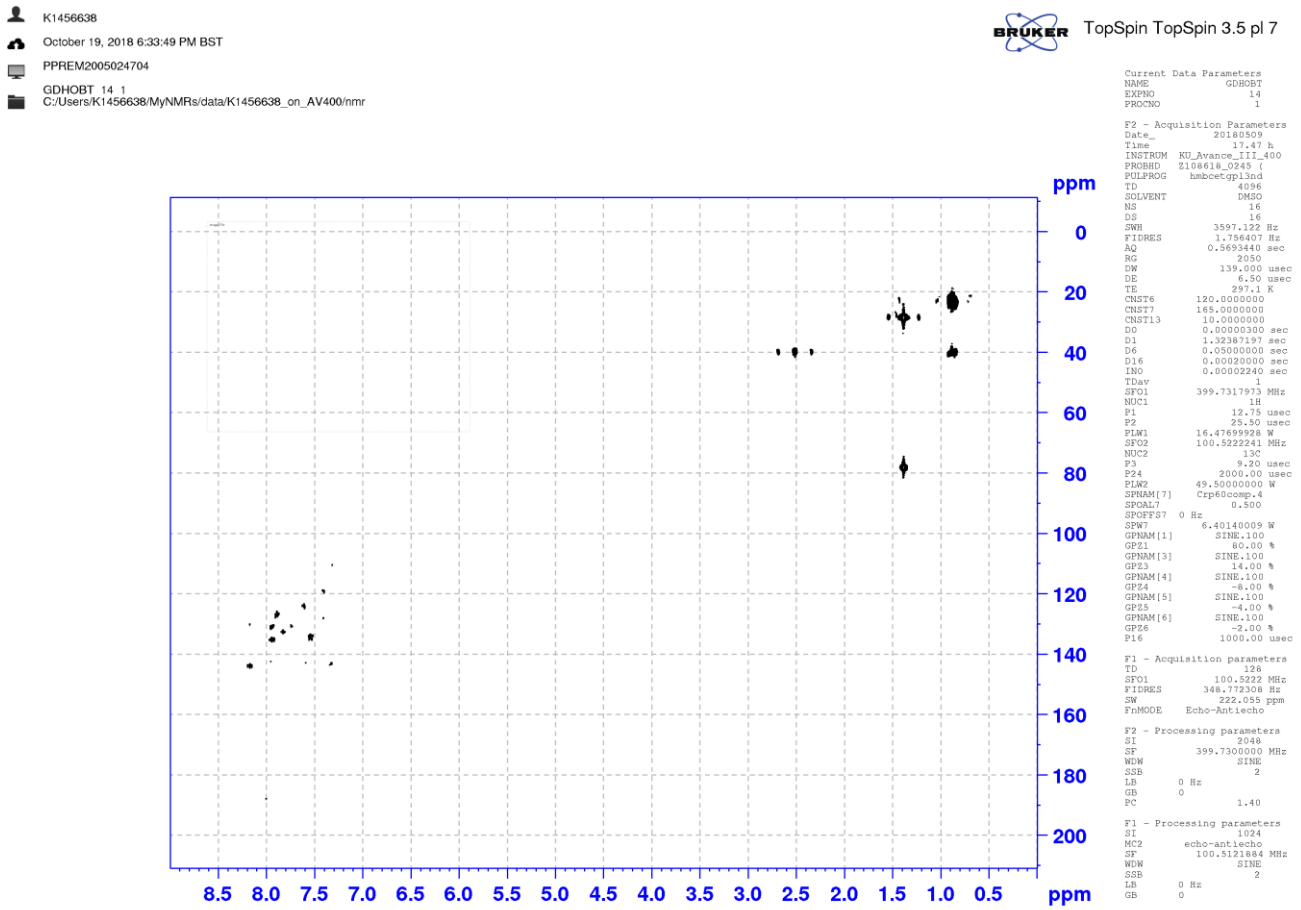
F2 - Acquisition Parameters
Date_             20180309
Time                 16.59 h
INSTRUM  XU_Avance_III_400
PROBHD   Z108638_0245 (
PULPROG       cosygpmfqf
TD                     2048
SOLVENT                DMSO
NS                        2
DS                        8
SWH                3597.122 Hz
FIDRES             3.512815 Hz
AQ                 0.2846720 sec
RG                      2030
DW                  139.000 usec
DE                    6.50 usec
TE                   297.2 K
D0                 0.00000300 sec
D1                 1.90702105 sec
D13                0.00000400 sec
D16                0.00020000 sec
IN0                0.00027800 sec
CDAV                      1
SFO1            399.7317973 MHz
NUC1                    1H
P1                    12.75 usec
PLW1           16.47699928 W
GPNAM[1]          SINE.100
GPZ1                 16.00 %
GPNAM[2]          SINE.100
GPZ2                 12.00 %
GPNAM[3]          SINE.100
GPZ3                 40.00 %
P16                1000.00 usec

F1 - Acquisition parameters
TD                      128
SFO1            399.7316 MHz
FIDRES             56.205036 Hz
SW                  8.999 ppm
FnMODE                   QF

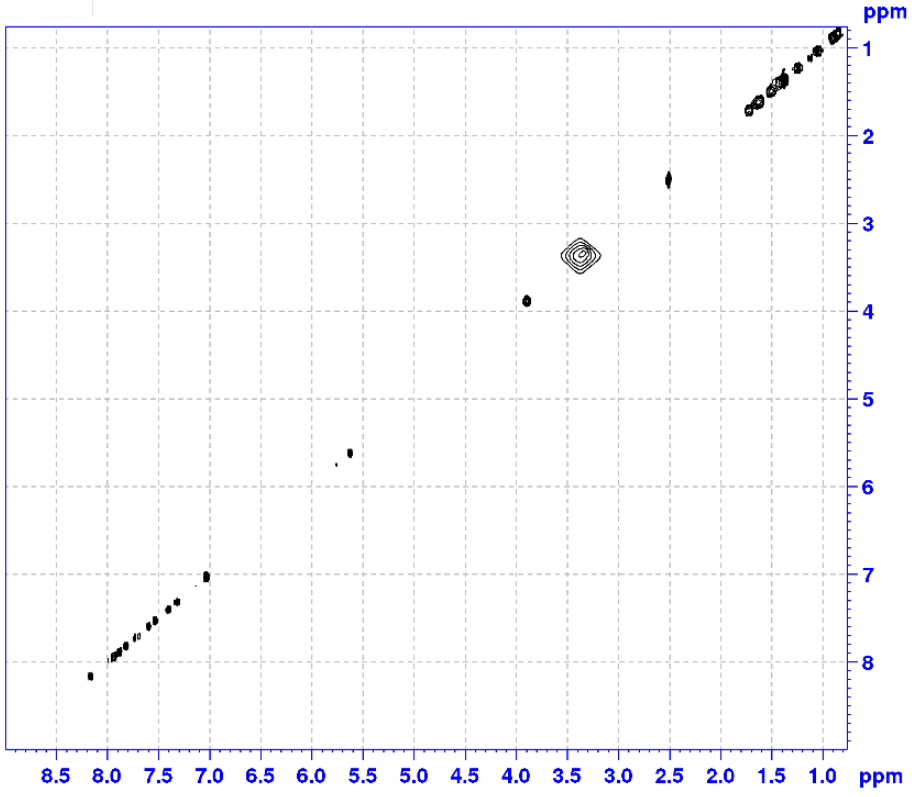
F2 - Processing parameters
SI                      1024
SF              399.7330000 MHz
WDW                     SINE
SSB          0
LB           0 Hz
GB           0
PC                      1.40

F1 - Processing parameters
SI                      1024
MC2                     QF
SF              399.7330000 MHz
WDW                     SINE
SSB          0
LB           0 Hz
GB           0
```

1.15.4 ^{1}H-^{13}C HMBC



1.15.5 ^{1}H-^{1}H NOESY




```
Current Data Parameters
NAME                  GDHOBI
EXPNO                     15
PROCNO                     1

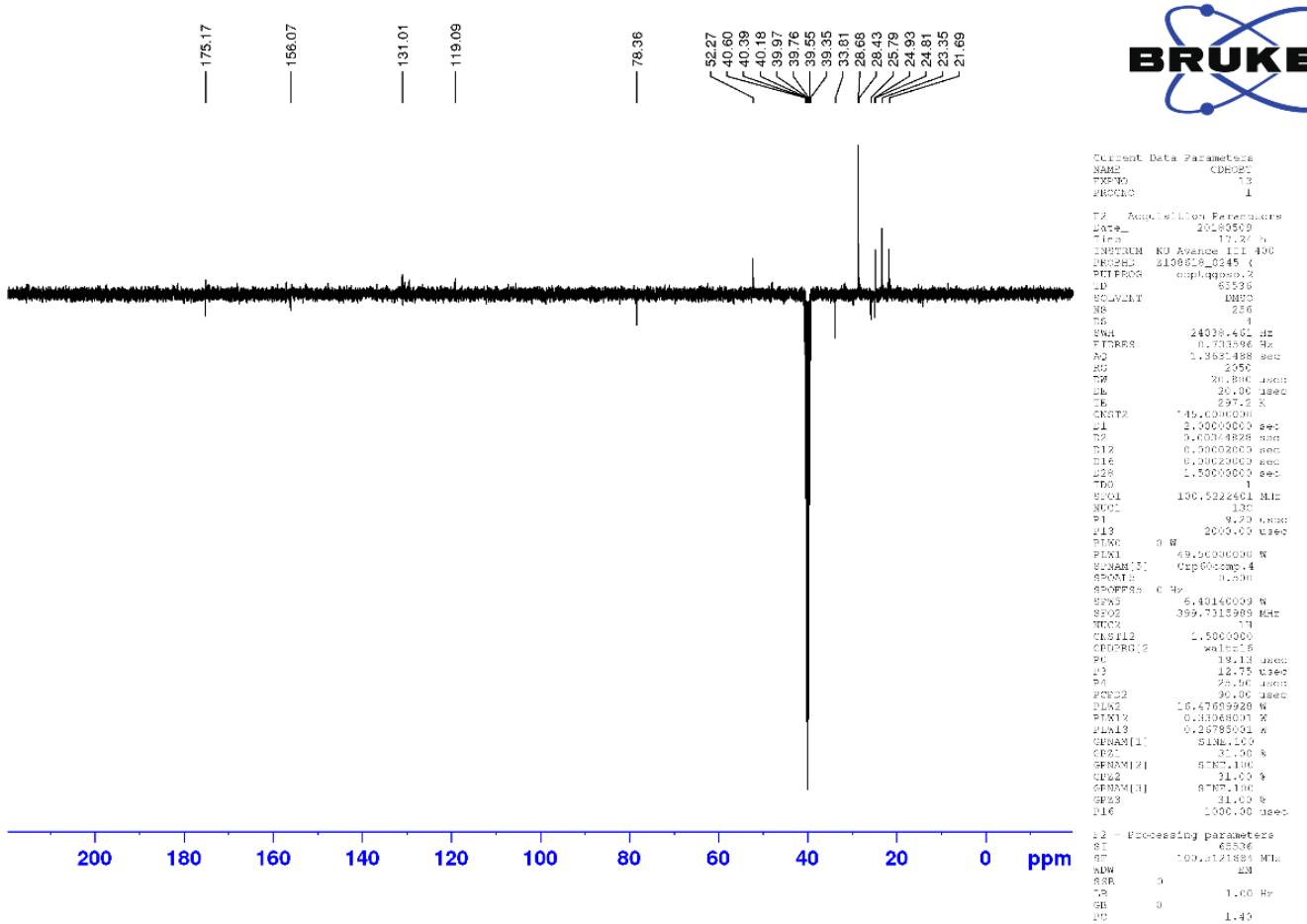
F2 - Acquisition Parameters
Date_              20_80509
Time                  18.54 h
INSTRUM   BC_Avance_III_400
PROBHD    5128618_0275_0
PULPROG          "noesygpp"
TD                      2048
SOLVENT                 DMSO
NS                         8
DS                         4
SWH                3597.122 Hz
FIDRES             3.512815 Hz
AQ                 0.2846720 sec
RG                      71
DW                  139.000 usec
DE                    8.26 usec
TE                  297.1 K
D0                 0.00012277 sec
D1                 1.96600294 sec
D8                 0.30000001 sec
D16                0.00020000 sec
IN0                0.00027800 sec
TD0                     1
SFO1              399.7817973 MHz
NUC1                    1H
P1                     12.75 usec
P2                     25.50 usec
PLW1              16.47899628 W
GPNAM[1]             SINE.100
GPZ1                    40.20 %
P16                  1000.00 usec

F1 - Acquisition parameters
TD                       256
SFO1              399.7818 MHz
FIDRES            28.102518 Hz
SW                     8.999 ppm
FnMODE            States-TPPI

F2 - Processing parameters
SI                       1024
SF                399.7800000 MHz
WDW                     QSINE
SSB                        2
LB          0 Hz
GB          0
PC                      1.00

F1 - Processing parameters
SI                       1024
MC2               States-TPPI
SF                399.7800000 MHz
WDW                     QSINE
SSB                        2
LB          0 Hz
GB          0
```

1.15.6 ^{13}C NMR



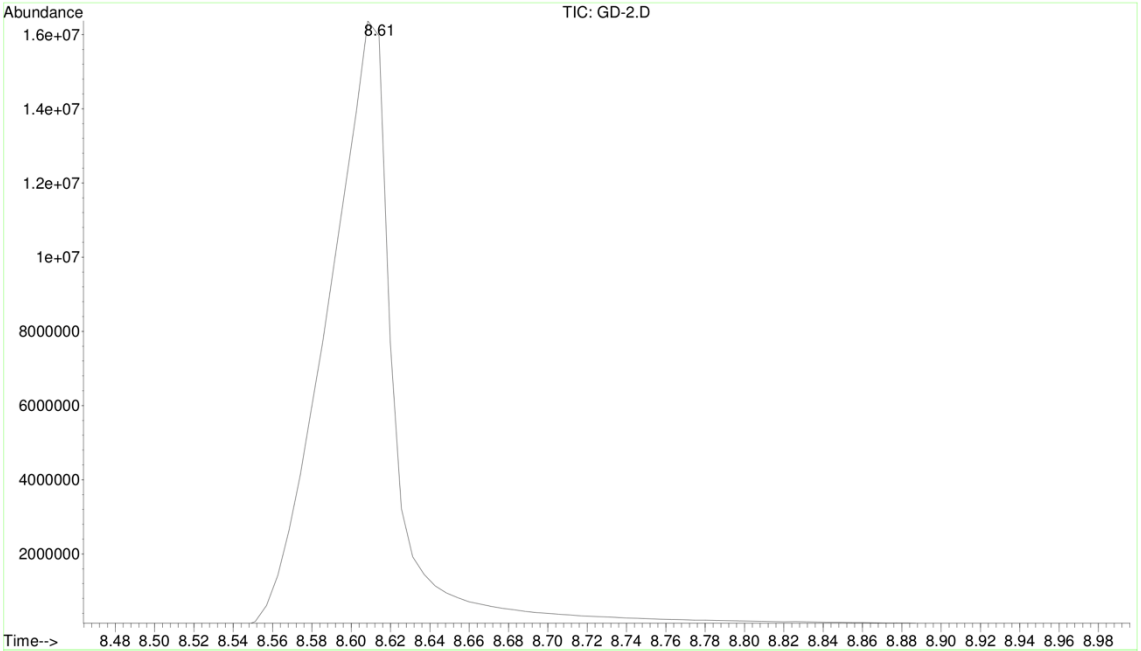

```
Current Data Parameters
NAME                  CDH2BT
EXPNO                     13
PROCNO                     1

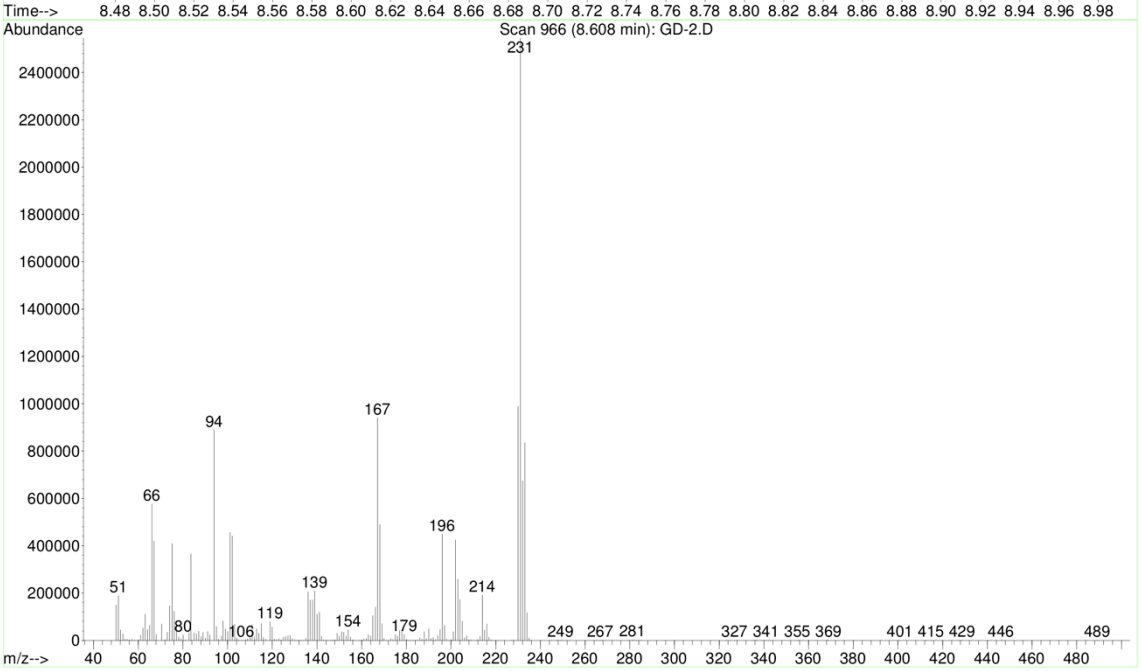
F2 - Acquisition Parameters
Date_              20200509
Time                  17.27 h
INSTRUM  BU Avance III 400
PROBHD   5130618_0245 (
PULPROG      zgpg30pse.2
TD                     65536
SOLVENT                DMSO
NS                       256
DS                         4
SWH              24038.461 Hz
FIDRES            0.733596 Hz
AQ                1.3631488 sec
RG                     2050
DW                  20.800 usec
DE                  20.00 usec
TE                  297.2 K
CNST2           145.0000000
D1                3.00000000 sec
D2                0.00344828 sec
D12               0.00002000 sec
D16               0.00020000 sec
D20               1.50000000 sec
TD0                        1
SFO1           100.5222401 MHz
NUC1                    13C
P1                    9.20 usec
PL13                2000.00 usec
PLW1      0 W
PLW1            49.50000000 W
SPNAM[0]     Crp60comp.4
SPOAL0            0.500
SPOFFS0      0 Hz
SPW0             6.40160000 W
SFO2           399.7815989 MHz
NUC2                    1H
CNST12           2.5000000
CPDPRG[2          waltz16
PCPD2                19.13 usec
PL2                  12.75 dBm
PL12                 23.50 dBm
PCPD2                90.00 usec
PLW2           16.47699928 W
PLW12           0.33068001 W
PLW13           0.26785001 W
GPNAM[1]         SINE.100
GPZ1                 37.00 %
GPNAM[2]         SINE.100
GPZ2                 31.00 %
GPNAM[3]         SINE.100
GPZ3                 31.00 %
P16                1000.00 usec

F2 - Processing parameters
SI                     65536
SF              100.5121884 MHz
WDW                       EM
SSB       0
LB                    1.00 Hz
GB        0
PC                    1.40
```

2. GCMS
2.1 Compound 1

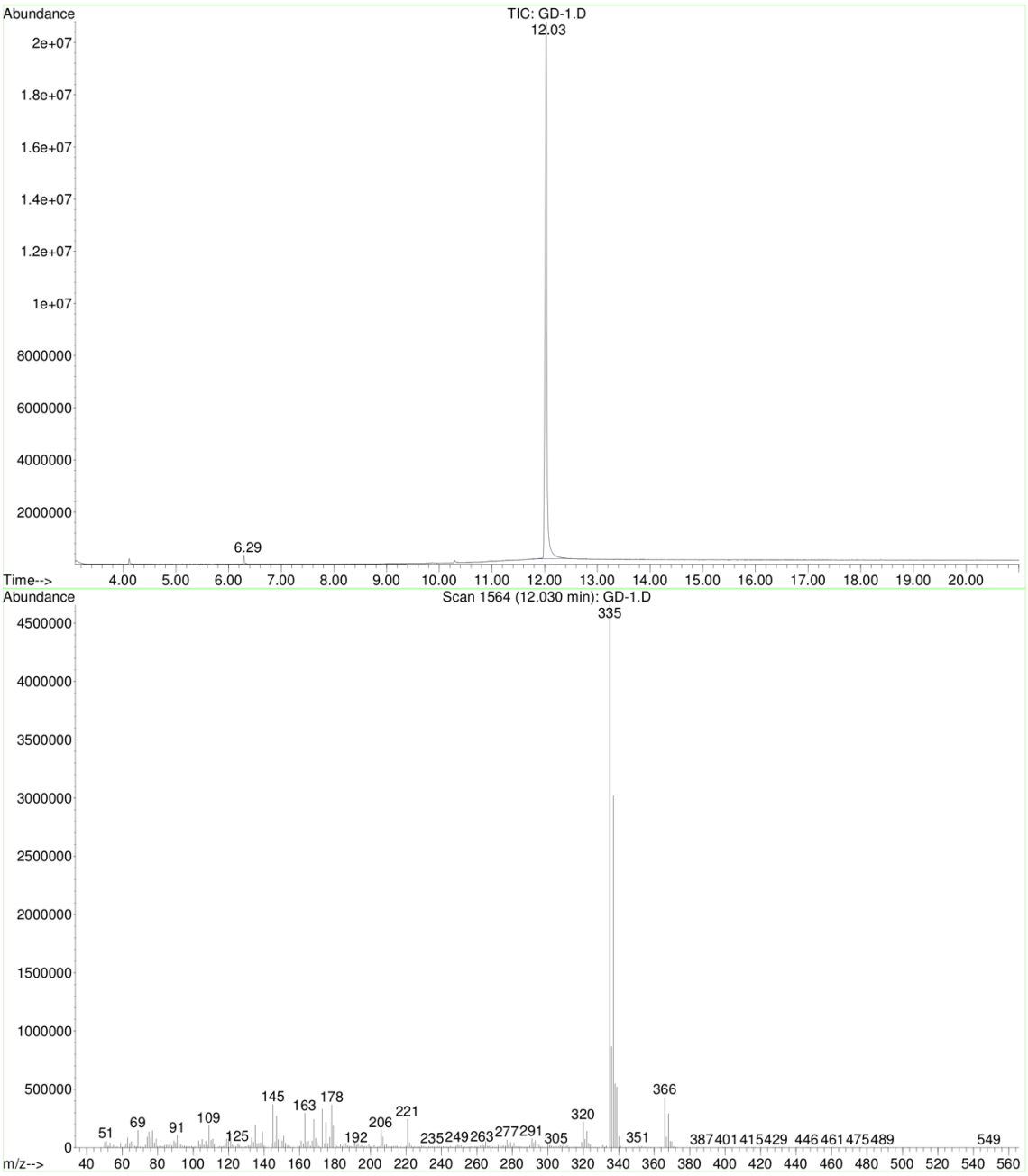
```
File       :E:\DATA\GD-2.D
Operator   :
Acquired   : 20 Feb 2018  23:10     using AcqMethod HIGHTEMPAUTO.M
Instrument :    GC MS 1
Sample Name:
Misc Info  :
Vial Number: 38
```





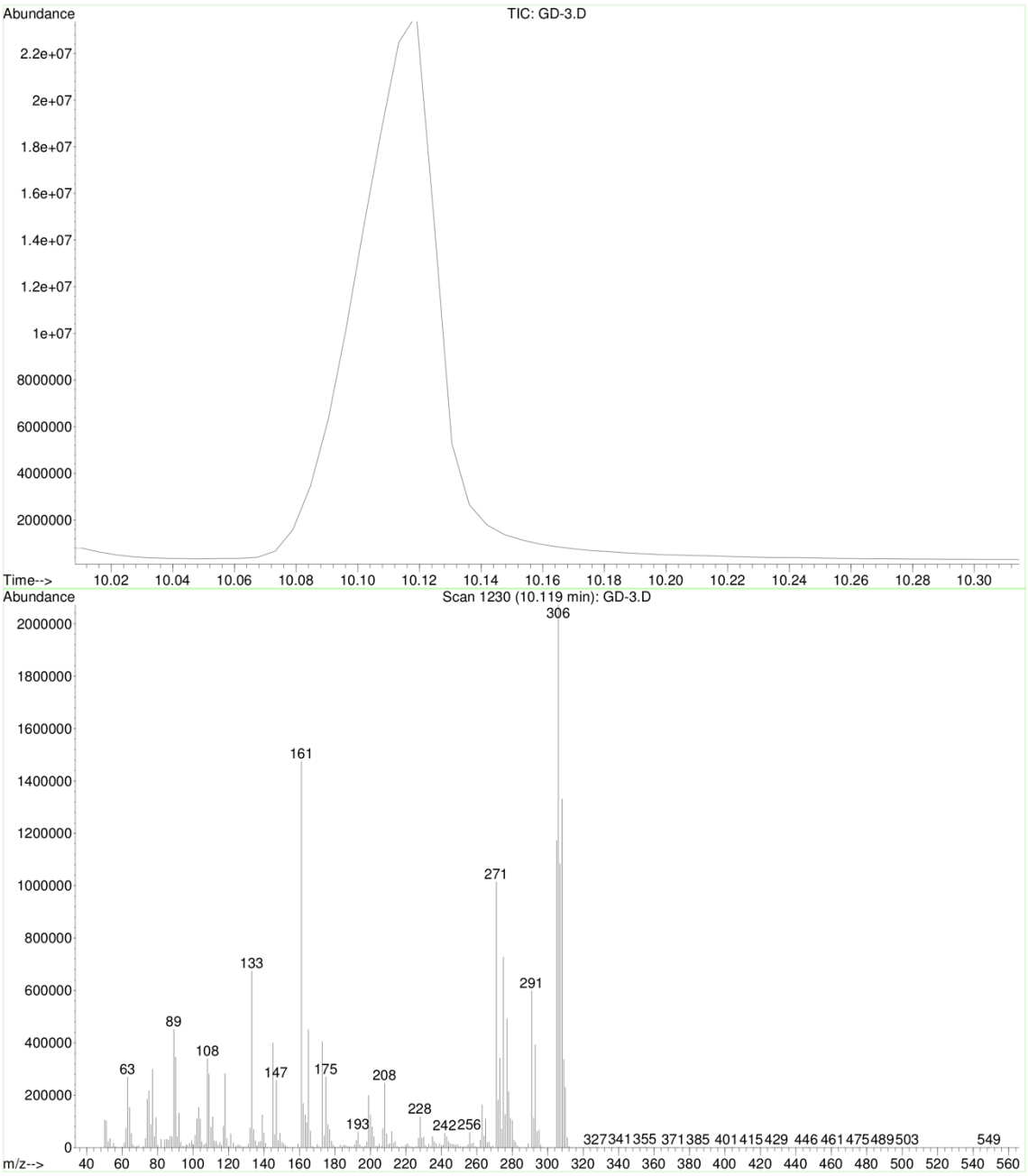
2.2 Compound 2

```
File       :E:\DATA\GD-1.D
Operator   :
Acquired   : 20 Feb 2018  22:45     using AcqMethod HIGHTEMPAUTO.M
Instrument :    GC MS 1
Sample Name:
Misc Info  :
Vial Number: 37
```



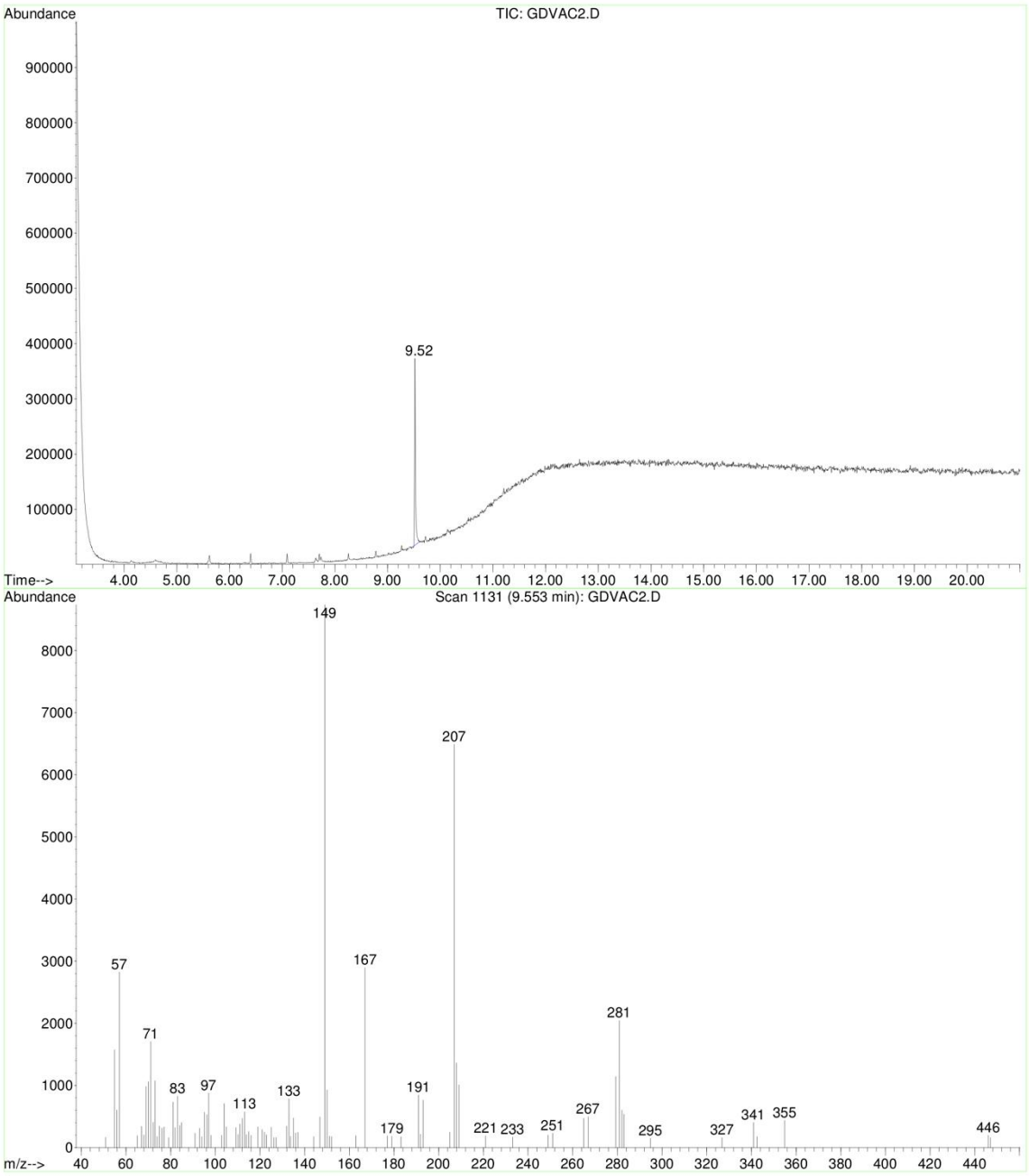
2.3 Compound 3

```
File       :E:\DATA\GD-3.D
Operator   :
Acquired   : 20 Feb 2018  23:36     using AcqMethod HIGHTEMPAUTO.M
Instrument :    GC MS 1
Sample Name:
Misc Info  :
Vial Number: 39
```



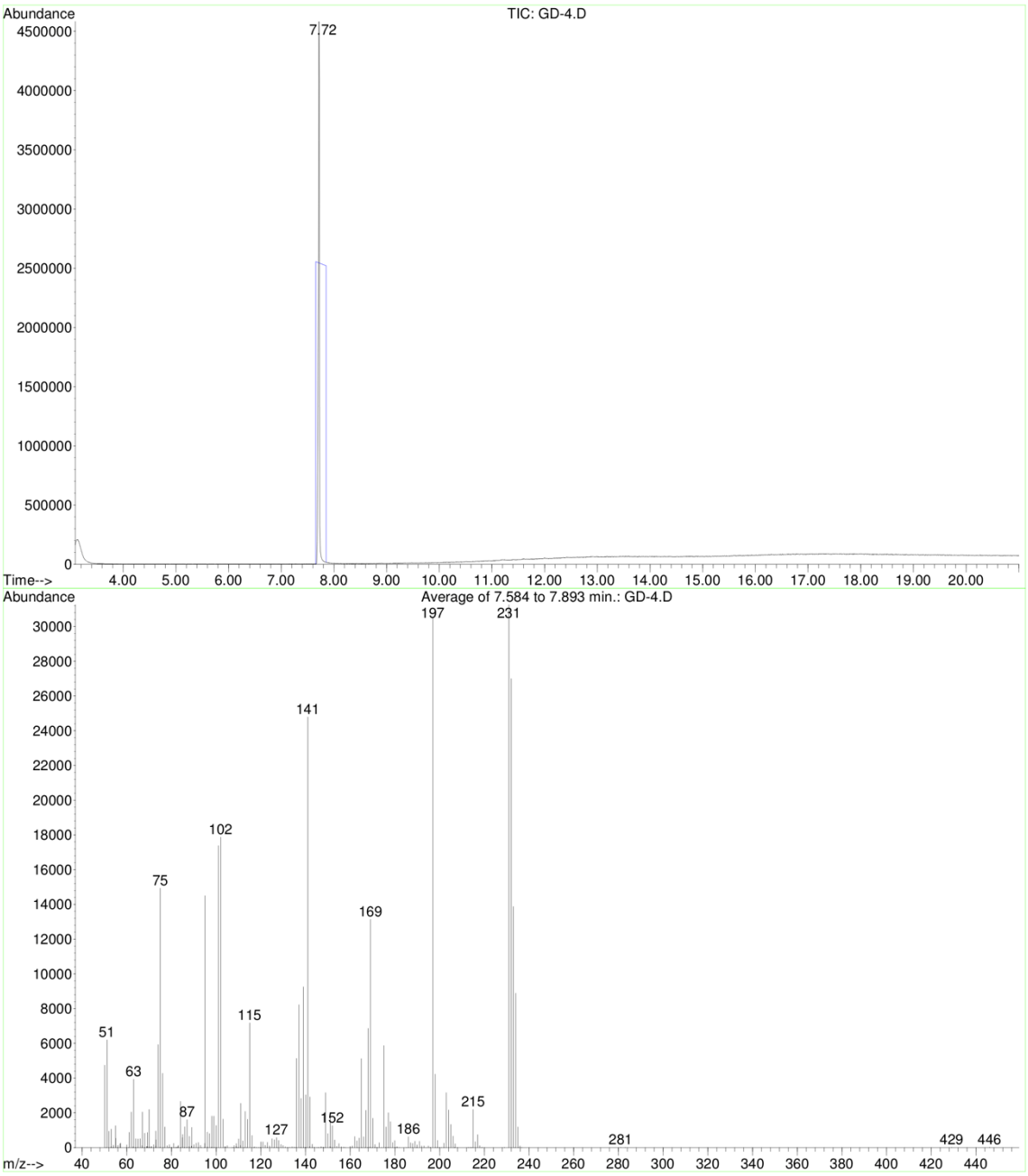
2.4 Compound 4

```
File       :E:\DATA\GDVAC2.D
Operator   :
Acquired   : 19 Oct 2018    9:57      using AcqMethod HIGHTEMPAUTO.M
Instrument :
    GC MS 1
Sample Name:
Misc Info  :
Vial Number: 80
```



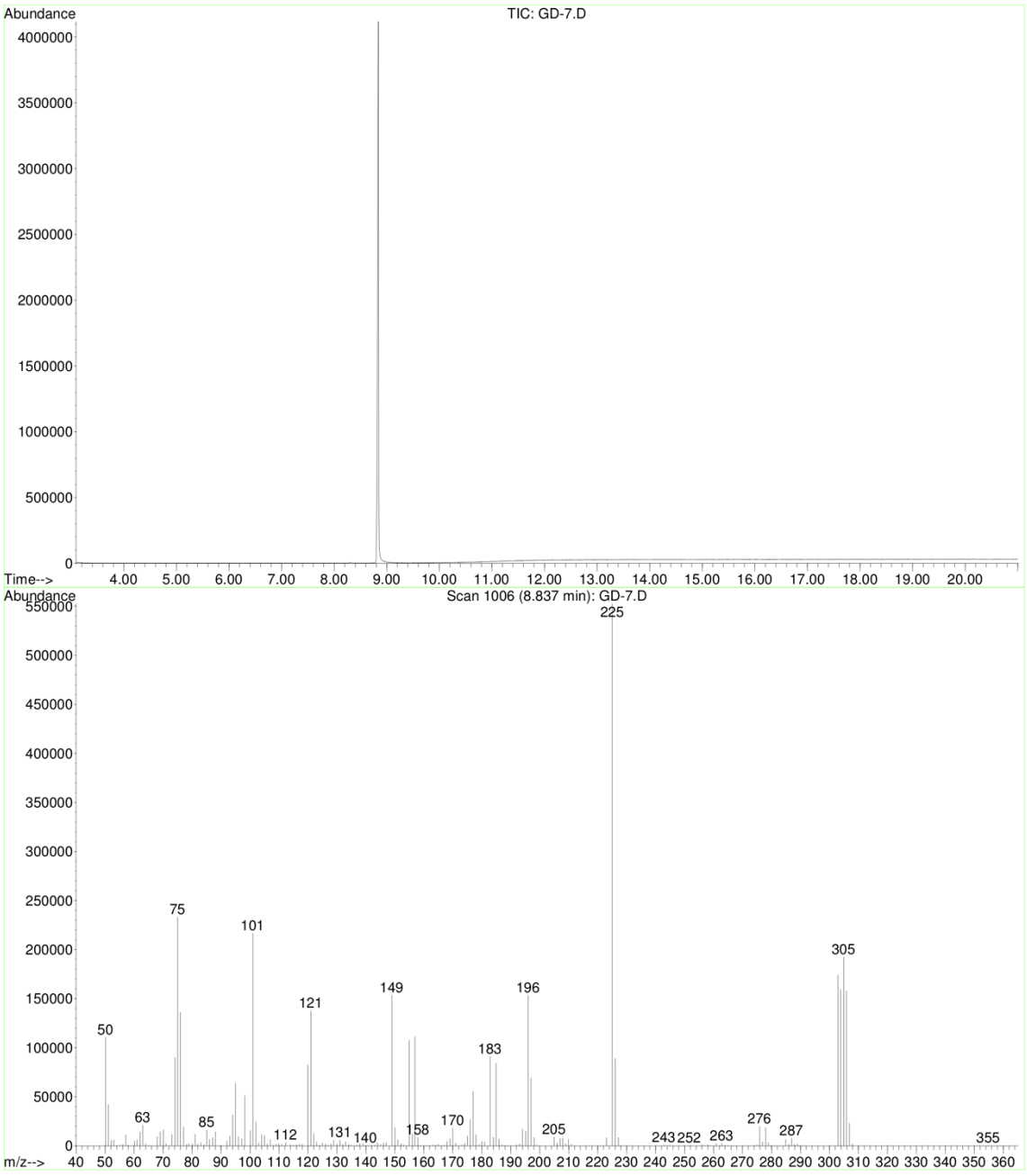
2.5 Compound 5

```
File       :E:\DATA\GD-4.D
Operator   :
Acquired   : 10 Jul 2018  11:26     using AcqMethod HIGHTEMPAUTO.M
Instrument :    GC MS 1
Sample Name:
Misc Info  :
Vial Number: 46
```



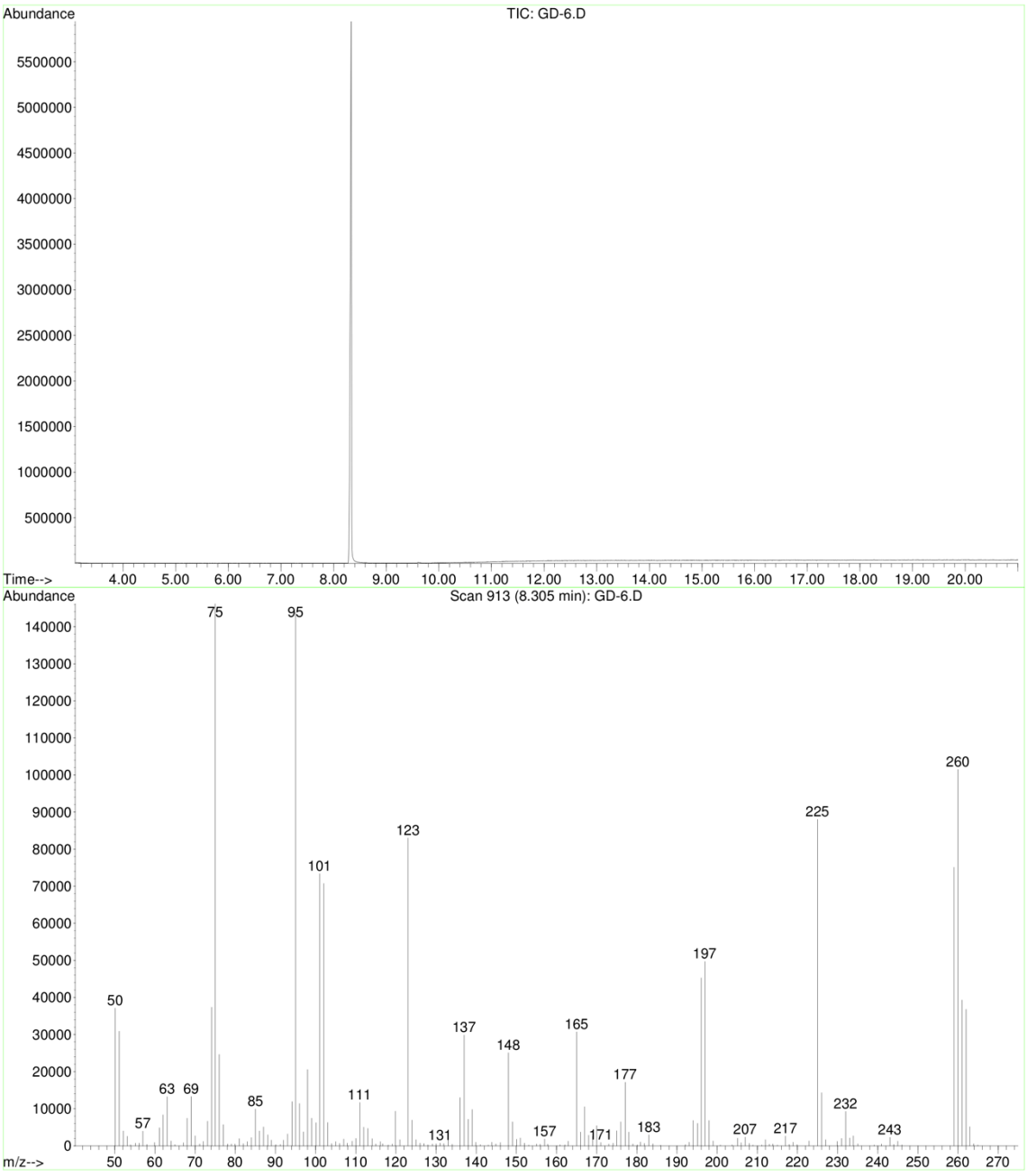
2.6 Compound 7

```
File       :E:\DATA\GD-7.D
Operator   :
Acquired   : 10 Jul 2018  12:39     using AcqMethod HIGHTEMPAUTO.M
Instrument :    GC MS 1
Sample Name:
Misc Info  :
Vial Number: 49
```



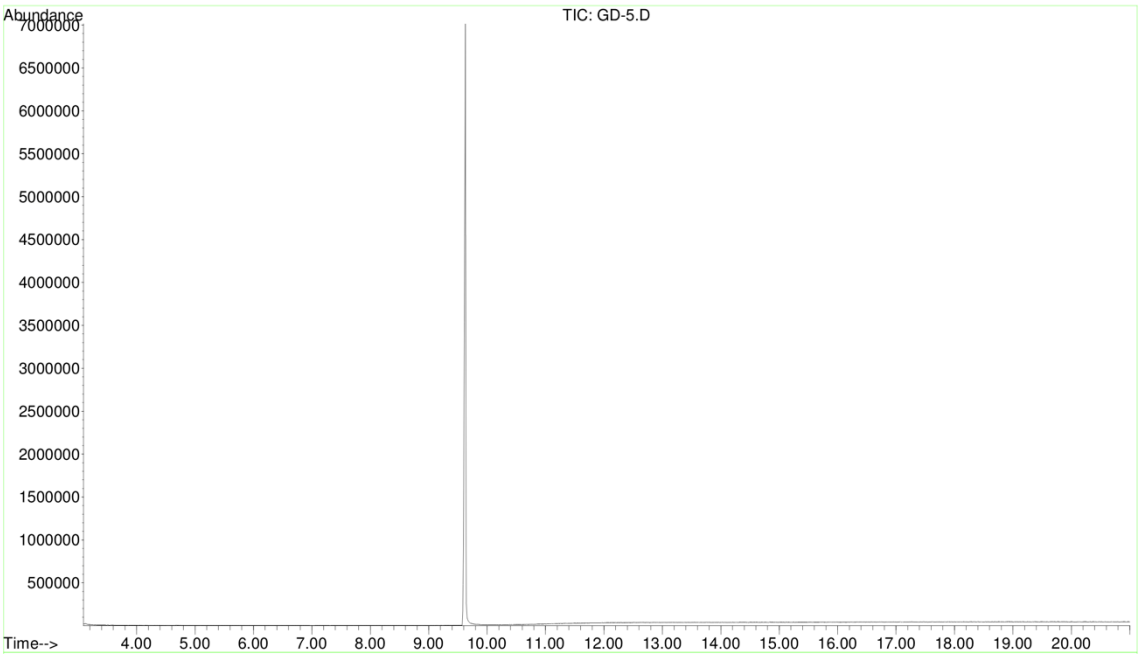
2.7 Compound 8

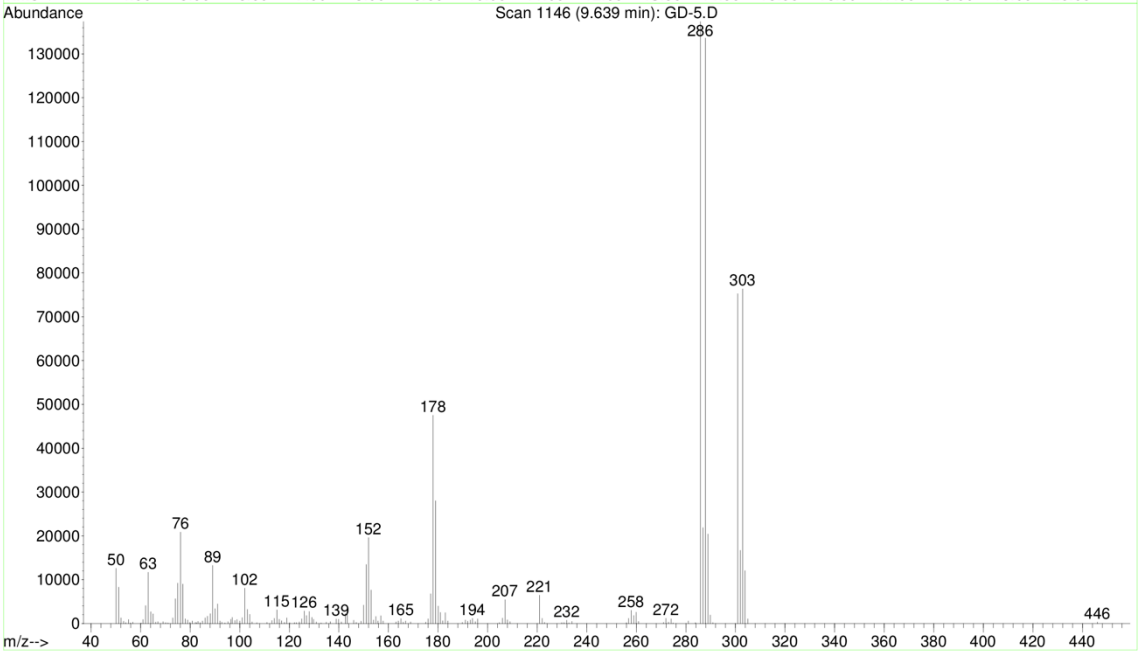
```
File       :E:\DATA\GD-6.D
Operator   :
Acquired   : 10 Jul 2018  12:14     using AcqMethod HIGHTEMPAUTO.M
Instrument :    GC MS 1
Sample Name:
Misc Info  :
Vial Number: 48
```



2.8 Compound 9

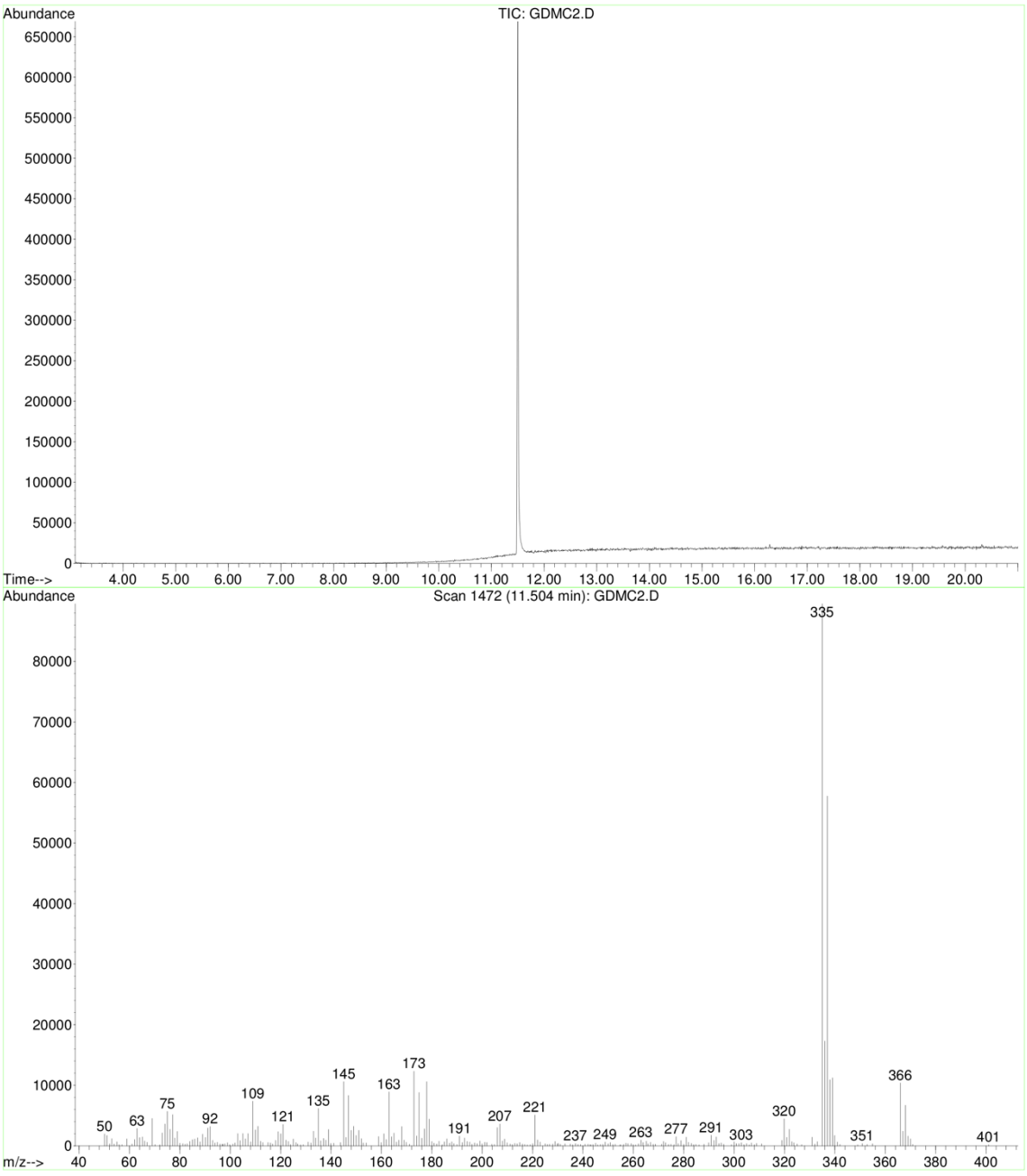
```
File       :E:\DATA\GD-5.D
Operator   :
Acquired   : 10 Jul 2018  11:50     using AcqMethod HIGHTEMPAUTO.M
Instrument :    GC MS 1
Sample Name:
Misc Info  :
Vial Number: 47
```





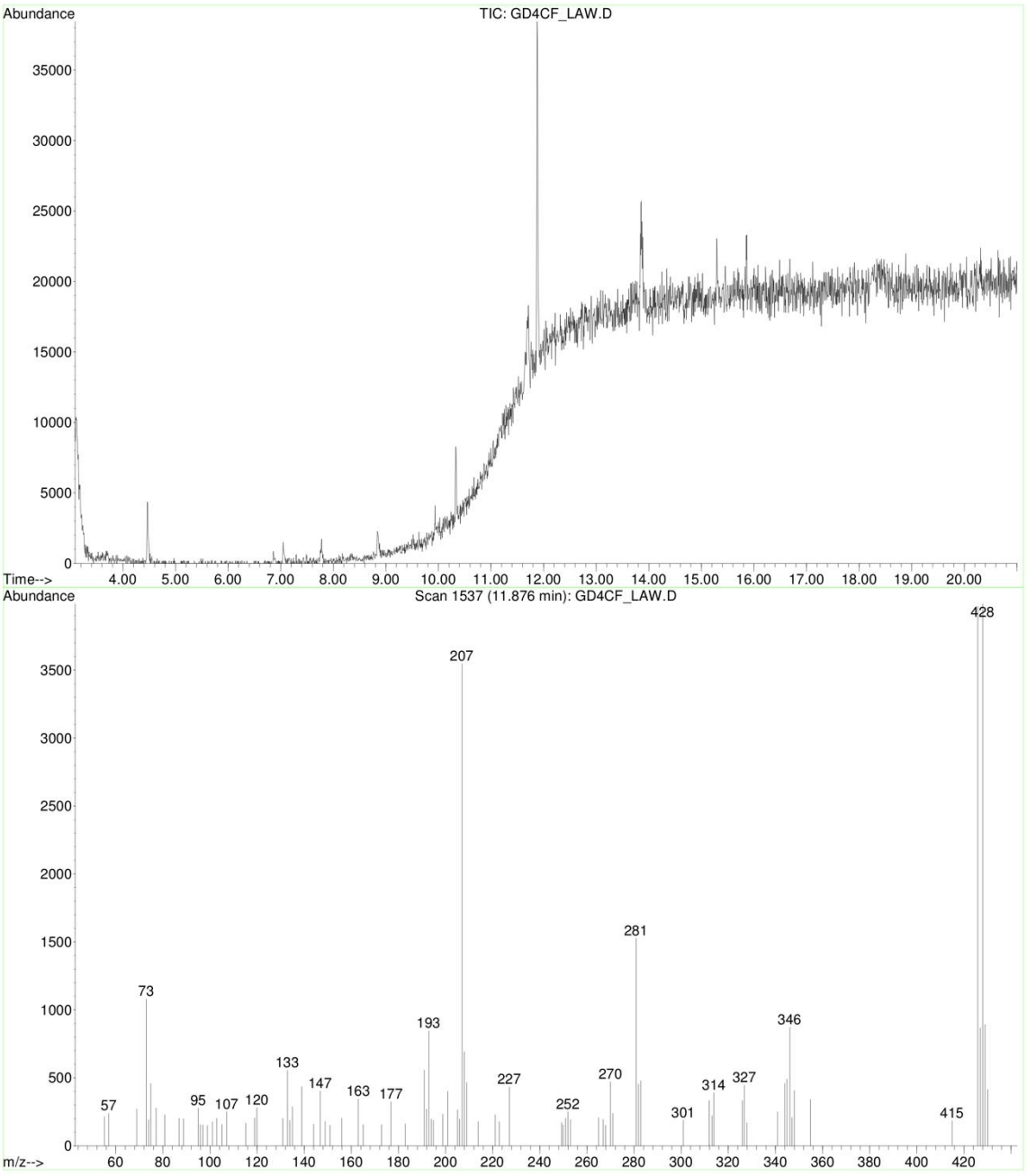
2.9 Compound 10

```
File       :E:\DATA\GDMC2.D
Operator   :
Acquired   : 27 Jul 2018  14:06     using AcqMethod HIGHTEMPAUTO.M
Instrument :    GC MS 1
Sample Name:
Misc Info  :
Vial Number: 82
```



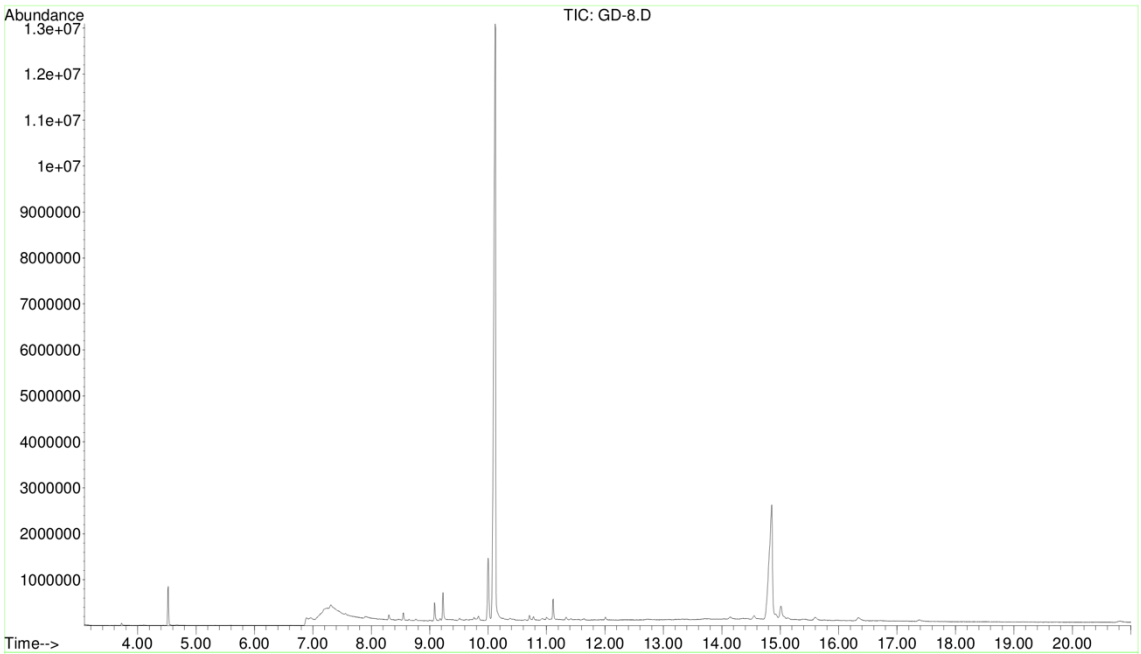
2.10 Compound 11

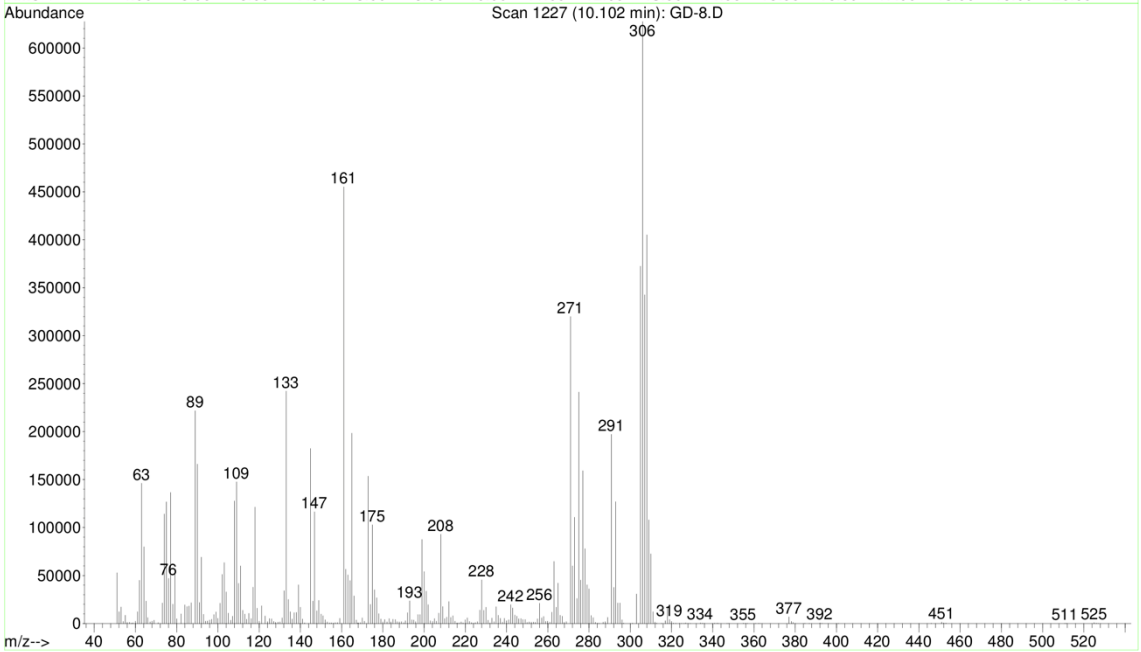
```
File       :E:\DATA\GD4CF_LAW.D
Operator   :
Acquired   : 27 Jul 2018  13:42     using AcqMethod HIGHTEMPAUTO.M
Instrument :    GC MS 1
Sample Name:
Misc Info  :
Vial Number: 81
```



2.11 Compound 12

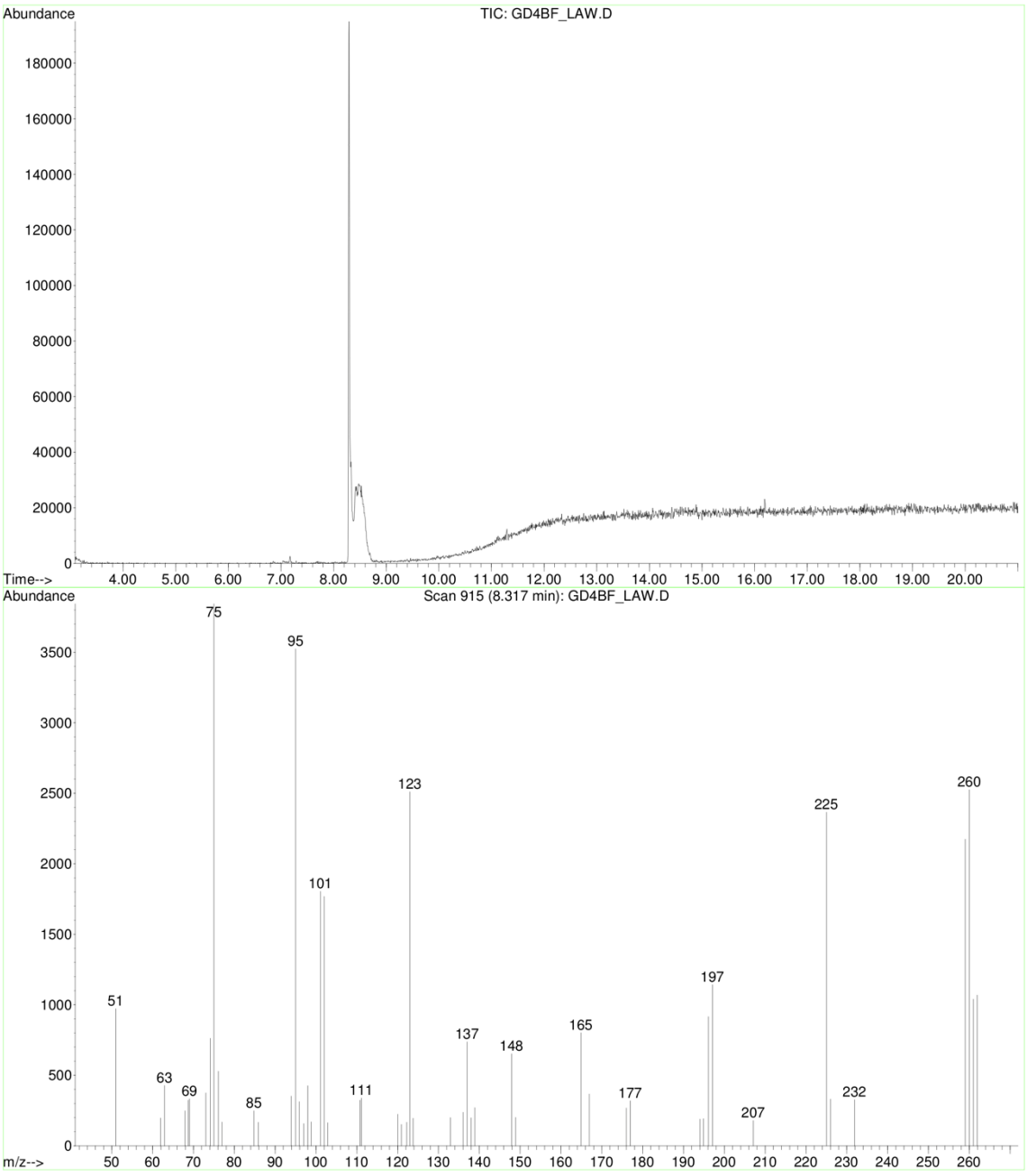
```
File       :E:\DATA\GD-8.D
Operator   :
Acquired   : 10 Jul 2018  13:04     using AcqMethod HIGHTEMPAUTO.M
Instrument :    GC MS 1
Sample Name:
Misc Info  :
Vial Number: 50
```





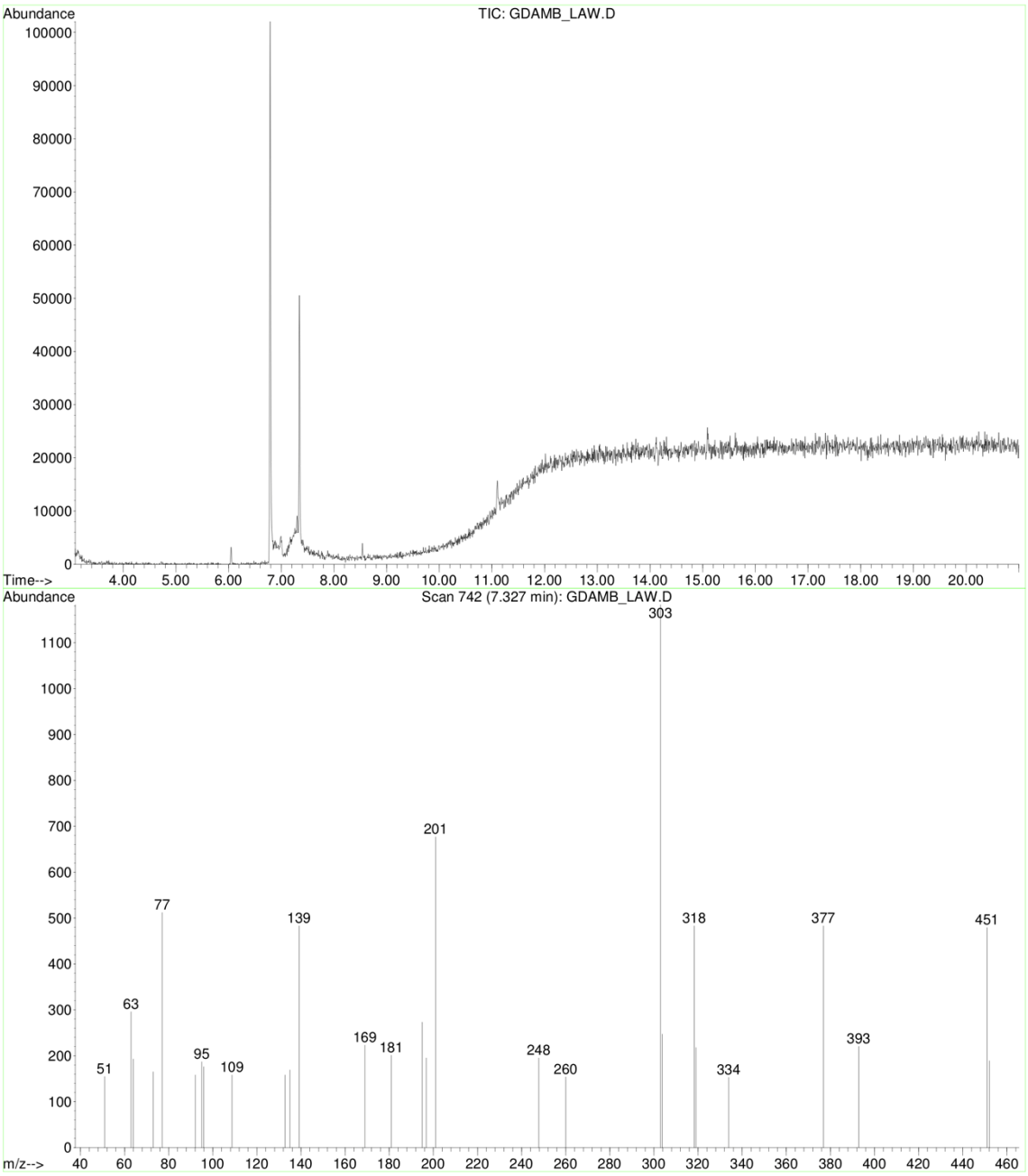
2.12 Compound 13

```
File       :E:\DATA\GD4BF_LAW.D
Operator   :
Acquired   : 27 Jul 2018  14:31     using AcqMethod HIGHTEMPAUTO.M
Instrument :    GC MS 1
Sample Name:
Misc Info  :
Vial Number: 83
```



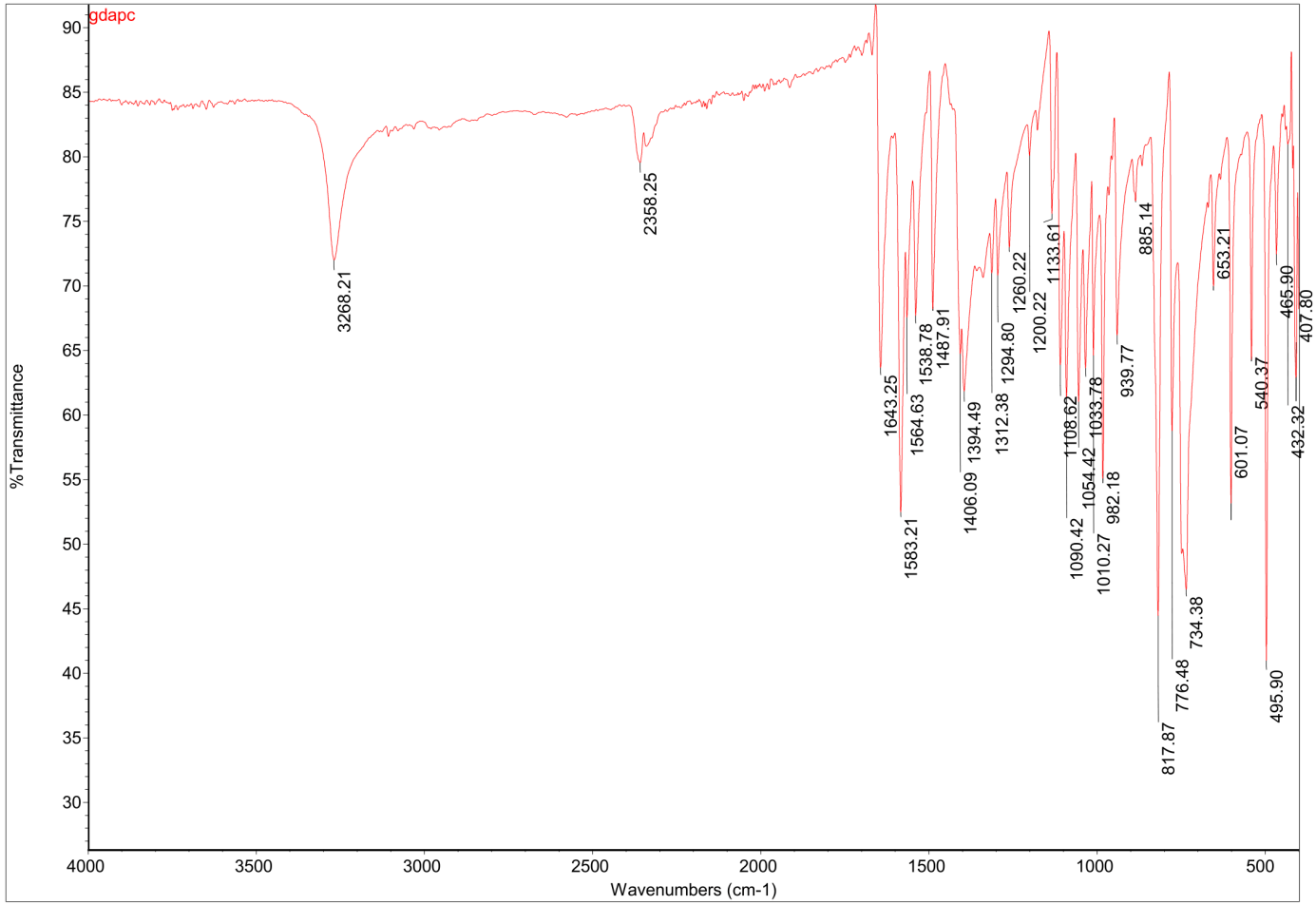
2.13 Compound 14

```
File       :E:\DATA\GDAMB_LAW.D
Operator   :
Acquired   : 27 Jul 2018  15:22     using AcqMethod HIGHTEMPAUTO.M
Instrument :    GC MS 1
Sample Name:
Misc Info  :
Vial Number: 85
```

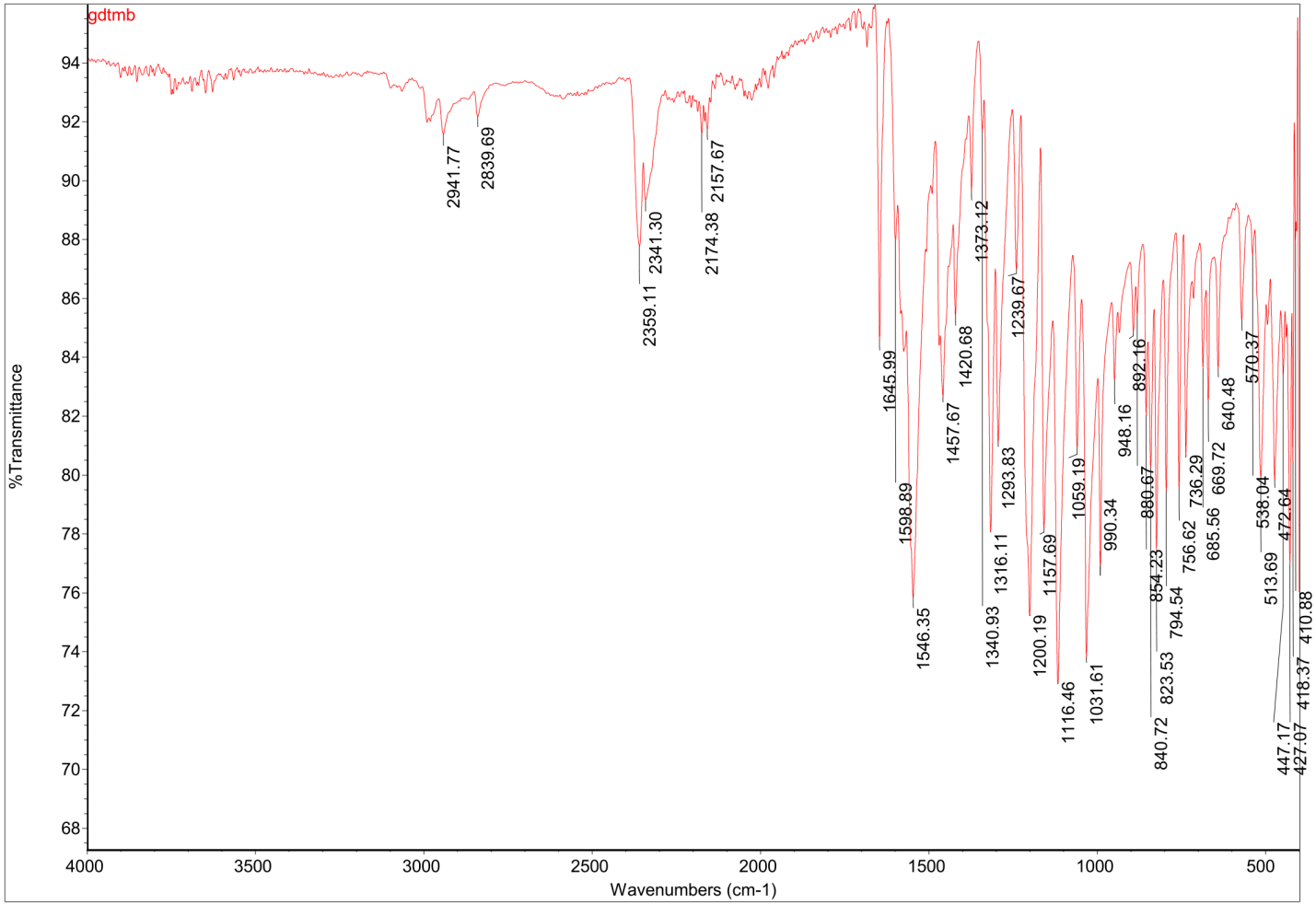


3. IR Data

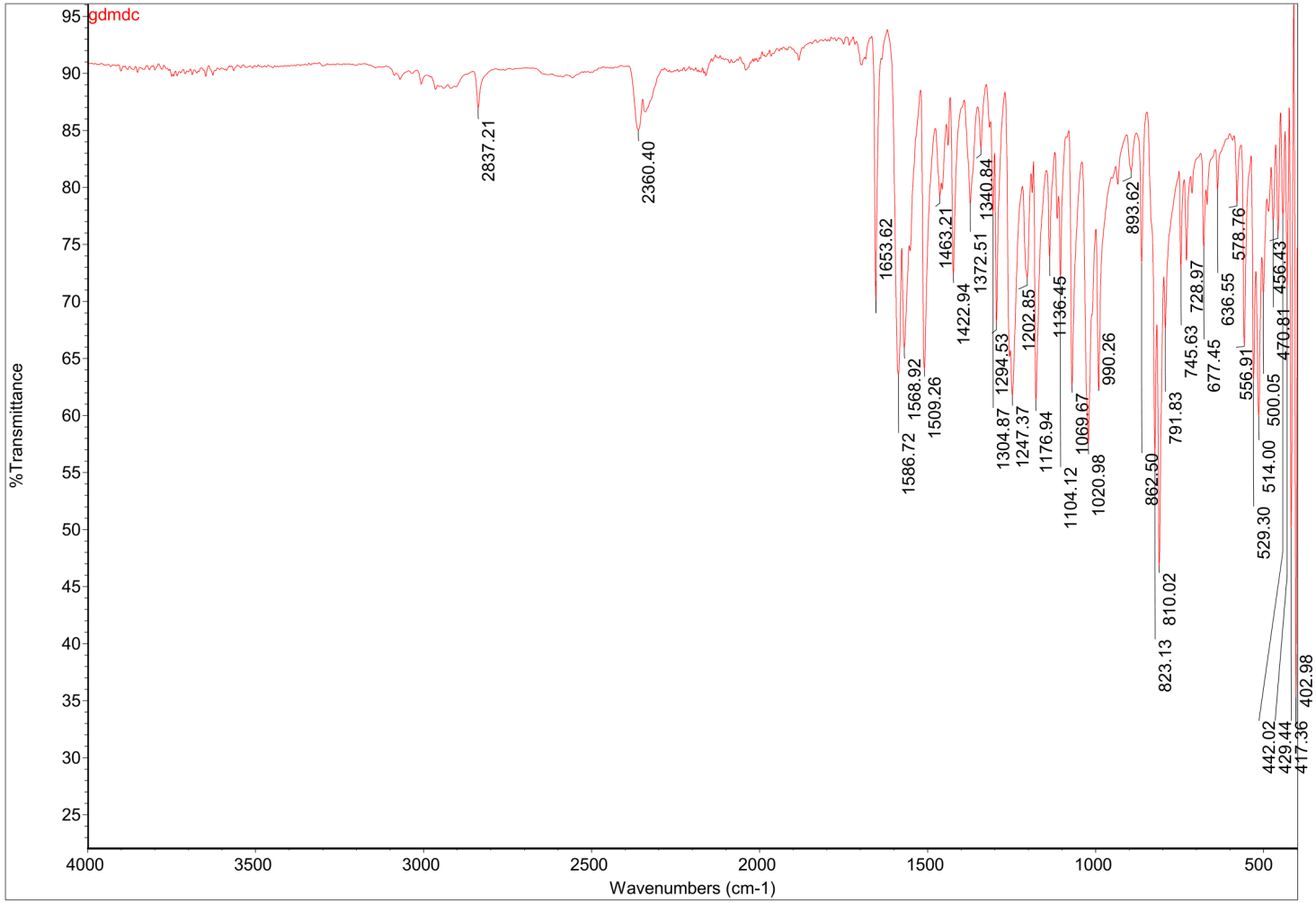
3.1 Compound 1



3.2 Compound 2

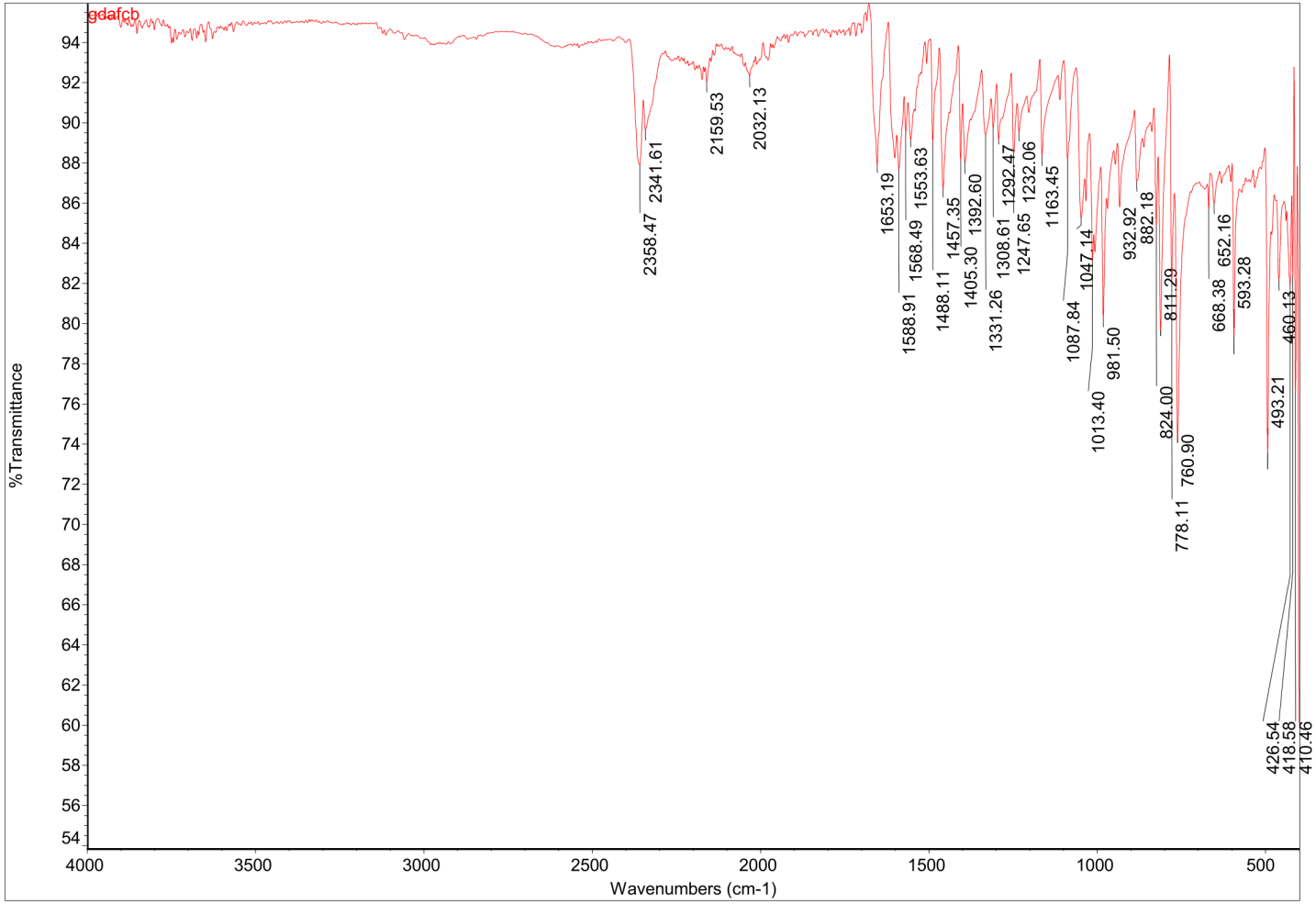


3.3 Compound 3



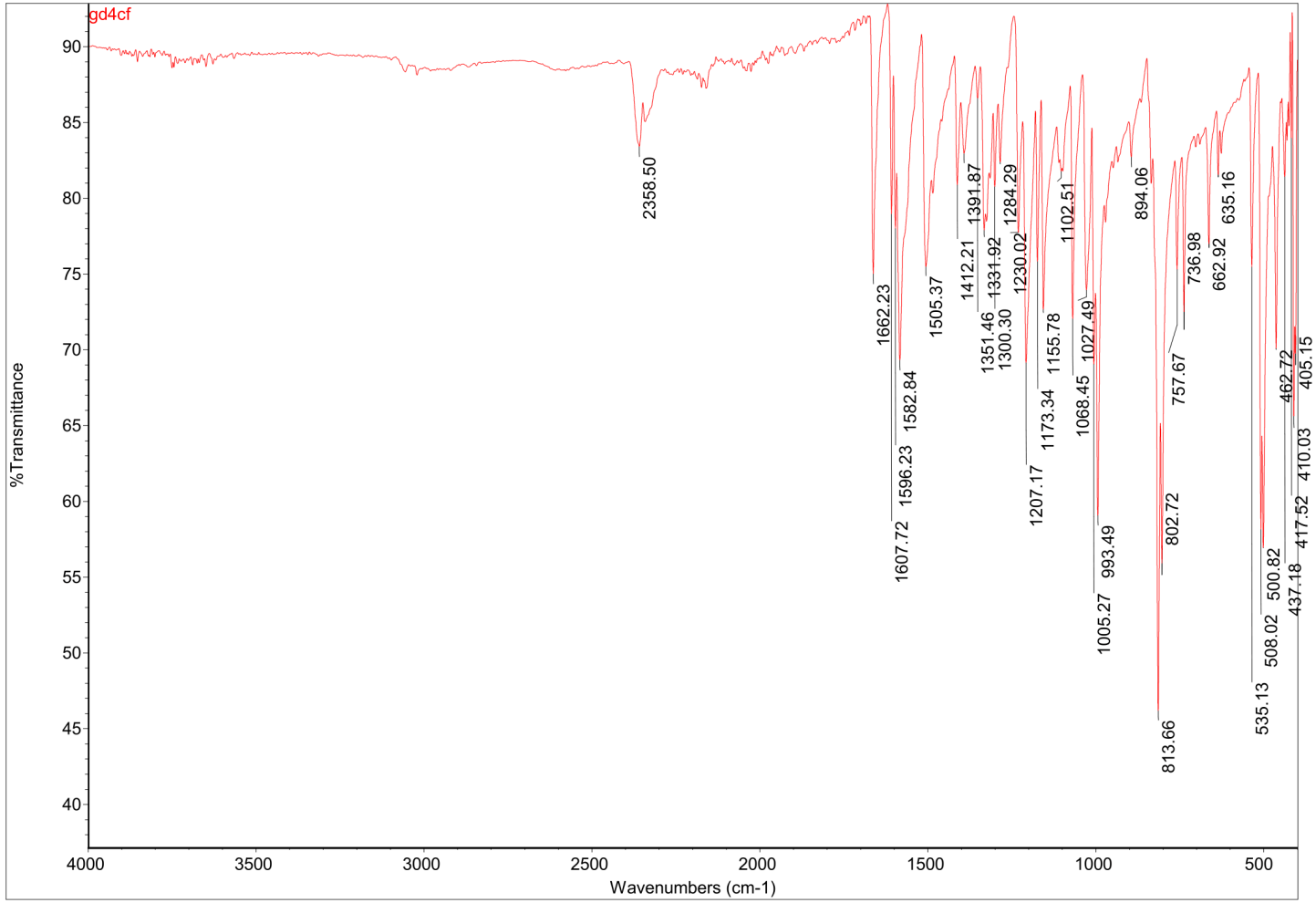
3.4 Compound 4

3.5 Compound 5

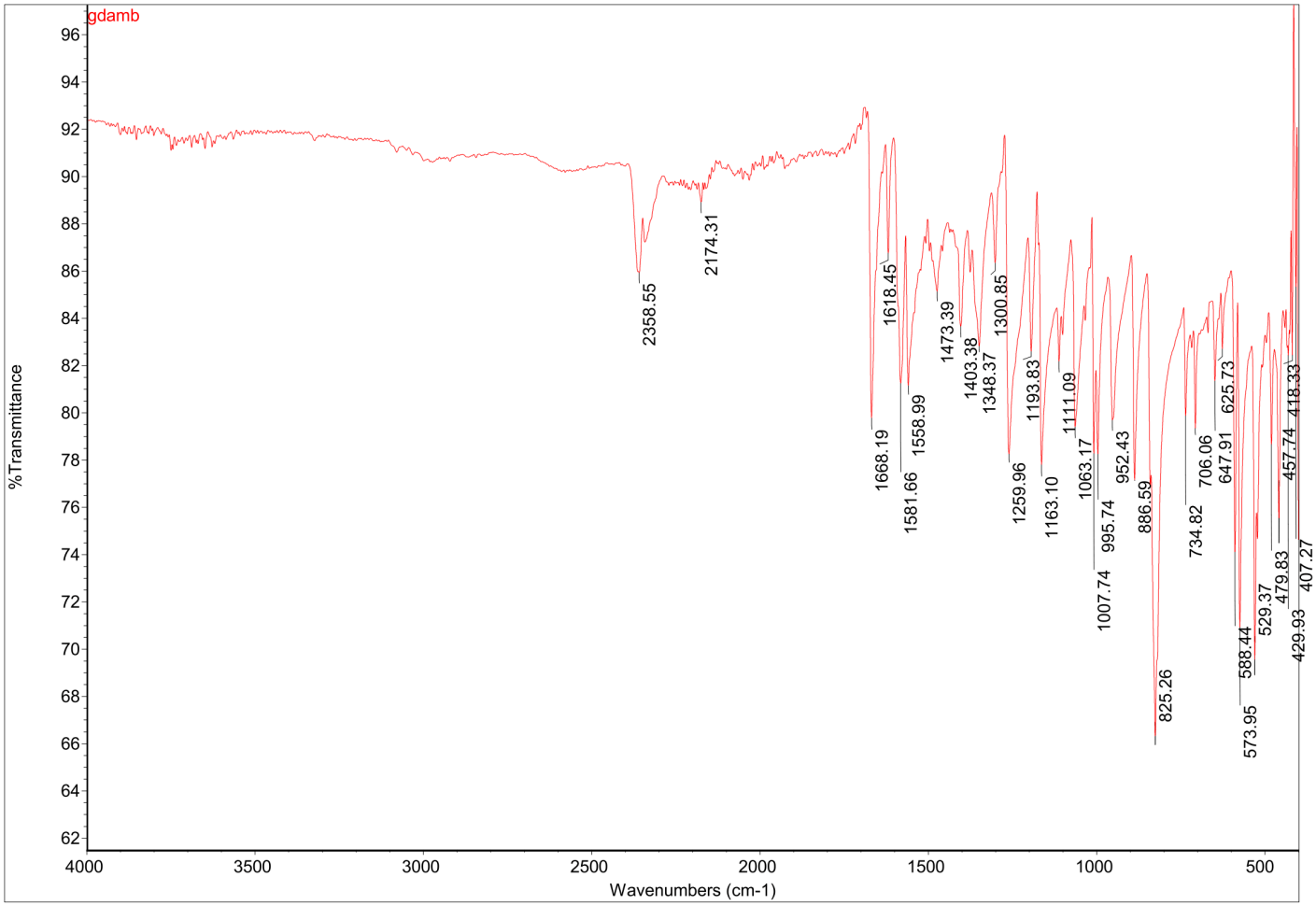


3.6 Compound 7

3.7 Compound 8



3.8 Compound 9



3.9 Compound 10