Development of Small Molecule Activators of Caseinolytic Protease P

by

Alan Jay Nhieu

A thesis submitted in conformity with the requirements
for the degree of Master of Science

Graduate Department of Chemistry
University of Toronto

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Abstract

Caseinolytic protease (ClpP) is a cylindrical protease that degrades proteins in the presence of ATPase chaperones. On its own, bacterial ClpP can only degrade small peptides; however, the addition of a novel class of antibiotics, ADEPs, can cause unregulated proteolysis leading to bacterial cell death.

Bacterial ClpP is an attractive target for antibiotic development. A high-throughput screen of small molecules identified a group of compounds which are termed Activators of Self-Compartmentalizing Proteases (ACP). A collection of ACP3 and ACP4/5 analogs was synthesized and investigated for biological activity. The project resulted in compounds with greater activity than the lead structures against isolated *E. coli* ClpP. Also, several analogs possessed bacteriostatic activity against *N. meningitidis* and *S. aureus* cell lines.

Acknowledgments

I wish to express my deepest regards and sincere gratitude to my supervisor Professor Robert Batey. I would like to thank him for all the encouragement, patience, guidance and constant support. His teachings of both knowledge in organic chemistry and problem-solving skills have made my thesis project a great learning experience.

I would like to thank Elisa Leung for her patience and time in attaining the necessary biological data. I would like to also express my gratitude to the Batey group for their time, invaluable discussion and help. I would especially like to thank Jordan Goodreid for his insight and contributions to the research project. I would like to also thank Simon Kim for his help with editing.

Lastly, I give special thanks to my parents and my sister for all their love and support.

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List of Abbreviations

^{1}H NMR	proton nuclear magnetic resonance spectroscopy
^{13}C NMR	carbon-13 nuclear magnetic resonance spectroscopy
ADEP	acyldepsipeptide
ClpAP	caseinolytic protease AP
ClpC	caseinolytic protease C
ClpE	caseinolytic protease E
ClpP	caseinolytic protease P
ClpXP	caseinolytic protease XP
DCC	*N,N′*-dicyclohexylcarbodiimide
DIEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EDC·HCl	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
ESI MS	electrospray ionization mass spectroscopy
EtOAc	ethyl acetate
EtOH	ethanol

FT-IR	fourier transformed infrared spectroscopy
FITC	fluorescein isothiocyanate
HATU	2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HBTU	2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate
HOAc	acetic acid
HPLC	high pressure liquid chromatography
HRMS	high resolution mass spectroscopy
MBC	minimum bactericidal concentrations
mp	melting point
MS	mass spectroscopy
NMM	*N*-Methylmorpholine
NMP	*N*-methylpyrrolidone
PyBOP	benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
R_f	retention factor
RD_{25}	relative degradation at 25 μM
rt	room temperature
RNA	ribonucleic acid
SAR	structure activity relationship
SD	standard deviation

TBTU	*O*-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate
TLC	thin layer chromatography

Chapter 1

Introduction

1.1 Emergence of Antibiotic Resistant Bacteria

Antibiotics, the miracle drugs of the 20th century, have revolutionized treatment of routine or life-threatening bacterial infections.[1,2] However, in recent years, more and more bacterial infections have evaded standard treatment and are difficult, if not impossible, to treat. There has been an increased frequency of drug resistant gram-positive pathogens: methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, and penicillin-resistant *Streptococcus pneumonia.* Similarly, a rise in resistant gram-negative bacteria: *P. aeruginosa* and *A. baumanii*, have extended the resistance problem beyond the confines of the hospital to community settings.[3] The previous last line of defense drugs – vancomycin, quinupristin/dalfopristin, and linezolid, have increasingly become first-line therapies.

Antibiotic resistance is the result of both antibiotic misuse and bacterial adaptive evolution. Development of resistance is a natural consequence of evolution conferred by a variety of mechanisms (e. g., modification of target molecules, repression of uptake systems, activation of efflux pumps, inactivation of the antibiotic, and chromosomal mutations).[4] These mechanisms limit the efficacy and life span of every antibiotic.

Recent practices incorporating appropriate antibiotic use, quarantining infected individuals and rapid diagnostics have all merely slowed the rise of antibiotic resistance.[2,4] It is only through persistent discovery and development of new antibiotic agents, with novel mechanisms of action, that this problem will be curtailed.

1.2 The Acyldepsipeptide Antibiotics

In 1985, researchers from Eli Lilly isolated a complex A54556 which consisted of eight related depsipeptidic factors A-H. These acyldepsipeptides (ADEPs), of the enopeptin family, were produced by aerobic fermentation of *Streptomyces hawaiiensis* NRRL 15010.[5,6,7] These compounds were found to have significant antibiotic properties. As the original patent had been abandoned, it was later discovered that the main components of the eight factors were factor A and B, labeled ADEP1 and 2 respectively.[8] The enopeptin structure consists of a 16-membered lactone ring made up of five (S)-amino acids and a lipophilic acylated phenylalanine side chain attached via the serine nitrogen.[9,10]

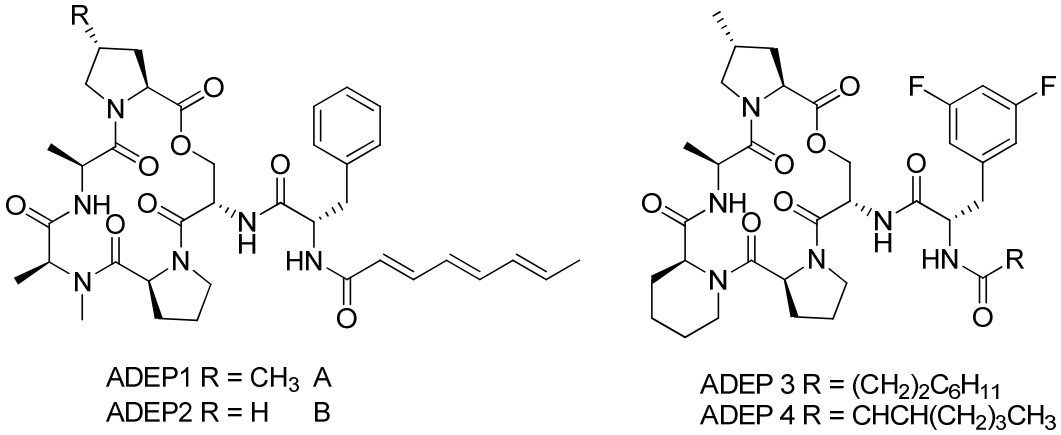

Figure 1: Structure of Natural and Unnatural Acyldepsipeptide (ADEP) Antibiotics

The initial complex A54556 and its separated factors demonstrated *in vitro* activity against gram-positive penicillin-resistant staphylococci and streptococci.[5] Preliminary mode of action studies with *B. subtilis* revealed impaired cell division, and the induction of filamentation, as the underlying causes of antibacterial activity.[2] Further studies conducted on congeners (ADEP3 and 4) of the naturally occurring ADEPs, produced by *de novo* synthesis, showed potent activity against gram-positive bacteria and multidrug-resistant clinical isolates. Against gram-negative bacteria, efflux pump deletions and the addition of permeabilizing agents were required for activity. *In vivo* investigations demonstrated that synthetic ADEP3 and 4 were a viable treatment for bacterial infections in rodents, even surpassing the activity of the marketed competitor linezolid. Treatment of mice separately infected with *E. faecalis* and *S. aureus* with

synthetic ADEPs resulted in high survival rates. Moreover, these particular ADEPs were superior to linezolid in treating rats with *S. pneumoniae* bacteria.

1.3 Novel Antibacterial Target

Applying reversed genomics techniques, Brötz-Oesterhelt and co-workers discovered that ADEPs bound to and activated a new antibacterial target, caseinolytic protease P (ClpP).[8] Initially, a genomic library from an ADEP-resistant mutant was constructed. Plasmids in bacteria colonies with high-levels of resistance to ADEPs were sequenced and identified to be inactive ClpP. An induced point mutation in the ClpP active center resulted in reduced ADEP-mediated cell death. ClpP deletion mutants of several bacterial strains caused complete resistance to ADEPs, confirming that ClpP is indeed the target of ADEPs for antibacterial activity. Routine incorporation assays that track molecules of interest showed that in *B. subtilis*, ADEP1 did not hinder the biosynthesis of either DNA, RNA, proteins, cell wall or fatty acids. These results signify a mechanism of action that did not fall into one of the classical target areas and showed promise for further development of ClpP as an antibacterial target.

1.4 Caseinolytic Protease P, ClpP

Proteases play a critical role in protein quality control by removing short-lived regulatory proteins, as well as misfolded or damaged proteins, thus maintaining cellular homeostasis.[11,12] Several proteases involved in protein degradation such as ClpXP, ClpAP and HsIV are oligomeric, cylindrical and self-compartmentalized. ClpP is a representative member of these cylindrical proteases, sharing a similar mechanistic process, but differing in substrate recognition, activation, subunit architecture and active-site environment.

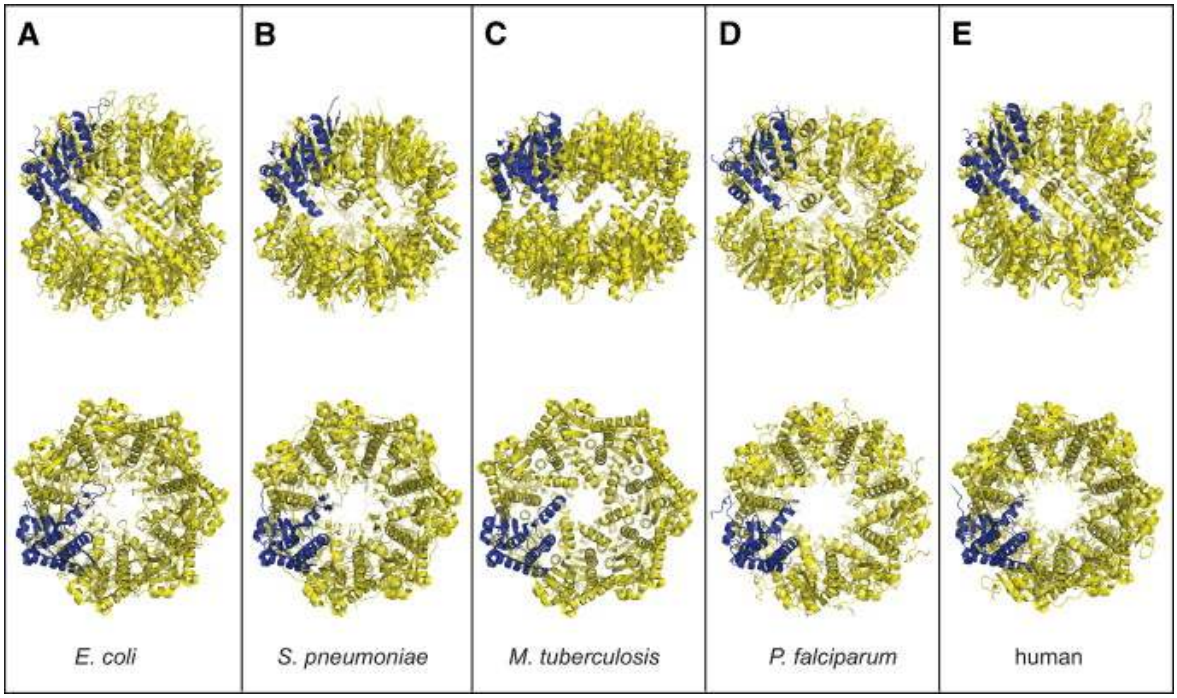

Figure 2: Side and top views of the X-ray structures of tetradecameric ClpPs from *E. coli, S. pneumoniae, M. tuberculosis, P. falciparum*, and human.*

* Reprinted from FEBS Letters, 581, Angela Yeou Hsiung Yu and Walid A. Houry, ClpP: A distinctive family of cylindrical energy-dependent serine proteases, 3751, (2007), with permission from Elsevier

ClpP is a conserved serine protease present throughout bacteria and eukaryotes. Differences include additional residues at the N and C-termini of human ClpP and an improper orientation of the catalytic triad compared to its bacterial counterparts. These differences are sufficient to selectively target bacterial ClpP over human ClpP, an obvious concern for the development of antibiotics.

ClpP is found in all bacteria sequenced to date except for *Mollicutes* and is mostly studied in *Escherichia coli*. It is comprised of 14 subunits arranged into two heptameric rings, forming a cylindrical structure. The cylinder encloses a large chamber containing the protease active sites with axial pores that allow entrance into the chamber. It has been observed in *E. coli* that ClpP forms complexes with AAA+ (ATPases associated with various cellular activities) chaperones, ClpX and ClpA. These hexameric chaperones weighing 46 kDa and 83 kDa respectively, can stack onto one or both ends of ClpP to form ClpAP or ClpXP complexes.

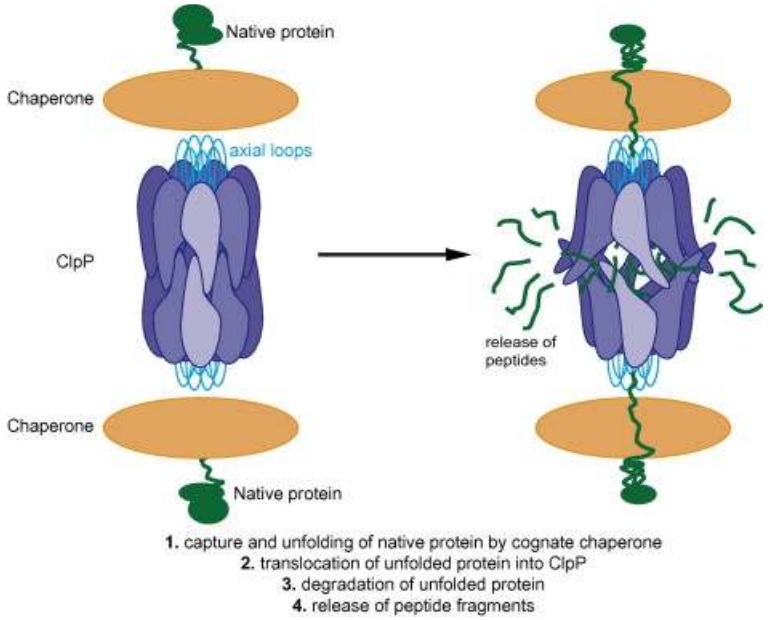

Figure 3: Model of ClpP mechanism of function. Shown is a cartoon model of the ClpP tetradecamer. The chaperones bound to ClpP are drawn as simple ellipses.[†]

[†] Reprinted from FEBS Letters, 581, Angela Yeou Hsiung and Yu,Walid A. Houry, ClpP: A distinctive family of cylindrical energy-dependent serine proteases, 3754, (2007), with permission from Elsevier.

The process of protein degradation by ClpP is outlined in Figure 3. Substrates are recognized, unfolded by the chaperone proteins, and then fed through the axial pores into the proteolytic chamber for degradation. Polypeptides and proteins are normally degraded into peptides of 7-8 amino acids which are eventually released. Only the unfolding and threading by the chaperones require ATP, while proteolysis by ClpP does not. Without the ATPase components, ClpP has only limited degradative activity against small peptides.

1.5 Activation and Deregulation of ClpP by ADEPs

ADEPs act via an unprecedented mechanism, by binding and deregulating ClpP, causing the degradation or cleavage of folded proteins in the absence of the necessary Clp ATPases.[11,13] As a consequence, this unregulated proteolysis by ClpP triggers cell death in gram-positive bacteria.[12] Cell death is initiated by binding of ADEPs to the interphase of two adjacent ClpP monomers. A crystal structure of an ADEP1-*B. subtilis* ClpP complex illustrated the tetradecameric protease binding to 14 ADEP molecules in a 1:1 ratio at the hydrophobic pockets on the apical surfaces.[11] This binding event triggers the oligomerization process that is a prerequisite of forming a functional proteolytic core. Consequently, an important contact site by ATPases is blocked, thereby preventing the interaction between the two partners and the normal functions of ClpP. More importantly, binding induces a conformational change in ClpP that widens the pore and allows larger proteins to be degraded.

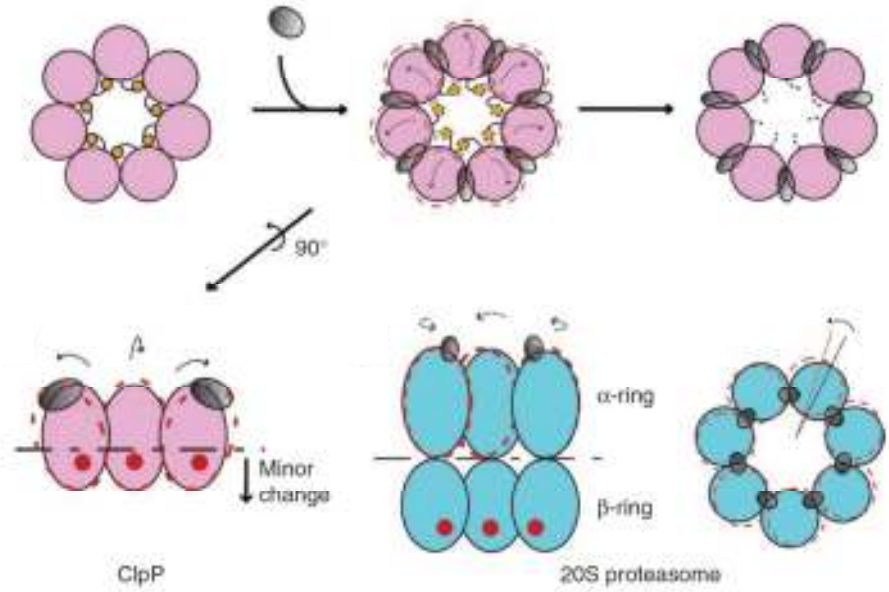

Figure 4: Conformation of the ADEP-ClpP complex. The activator ADEP is depicted as a transparent gray oval. Activator binding triggers outwards movement of individual subunits of the ClpP body as indicated by arrows.‡

Recently, Brötz-Oesterhelt and co-workers have identified the exclusive cause of bacterial death to be the degradation of the essential cell division protein FtsZ.[13] This tubulin-like protein forms the cytoskeletal framework for cell division in all bacteria.[14] During cell division, FtsZ localizes to the cell midpoint very early in cytokinesis and then polymerizes to form a circumferential ring associated with the cytoplasmic membrane.[15] Several other division proteins involved in producing a new cell between the dividing cells, are recruited to the FtsZ ring. The degradation of this protein by ClpP, inhibits septum formation, a crucial process in cell division, and causes the delocalization of central cell division proteins from the mid cell positions. By preventing cell division, this class of antibiotics inhibits a vital cellular process of bacteria that is not targeted by any therapeutic antibiotic so far.

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1.6 Small Molecule Activators of Bacterial ClpP

The unprecedented mode of action, which is unaffected by resistance mechanisms compromising current antibiotic therapeutics, make ClpP an attractive target for antibiotic development. However, the synthetic complexities of ADEPs, and concerns about intellectual property rights, have driven our focus towards the discovery of small molecule activators.[16]

By switching to the development of small molecules, a high-throughput screening approach incorporating libraries of drug-like compounds can be utilized to identify leads. In collaboration with a team led by Professor Walid Houry, a high-throughput screening assay with a fluorescence-based readout was developed. The assay employed fluorescein isothiocyanate (FITC)-labeled casein (casein-FITC) which served as the proteolytic target of *E. coli* ClpP. When casein-FITC was intact, FITC fluorescence was quenched. Protease-catalyzed hydrolysis of casein-FITC relieves this quenching, yielding highly fluorescent dye-labeled peptides. The principle of the screen was to select for compounds that resulted in increased fluorescence upon incubation of casein-FITC with ClpP. Using this approach, a chemical screen of the Maybridge (50,000 compounds) and Chembridge (10,000) libraries resulted in the identification of several structurally diverse non-ADEP compounds that activated *E. coli* ClpP.[17] The five confirmed hits were designated Activators of Self-Compartmentalizing Proteases (ACPs) 1-5, and are illustrated in Figure 5. The revisions of the chemical structure of ACP4 will be discussed later.

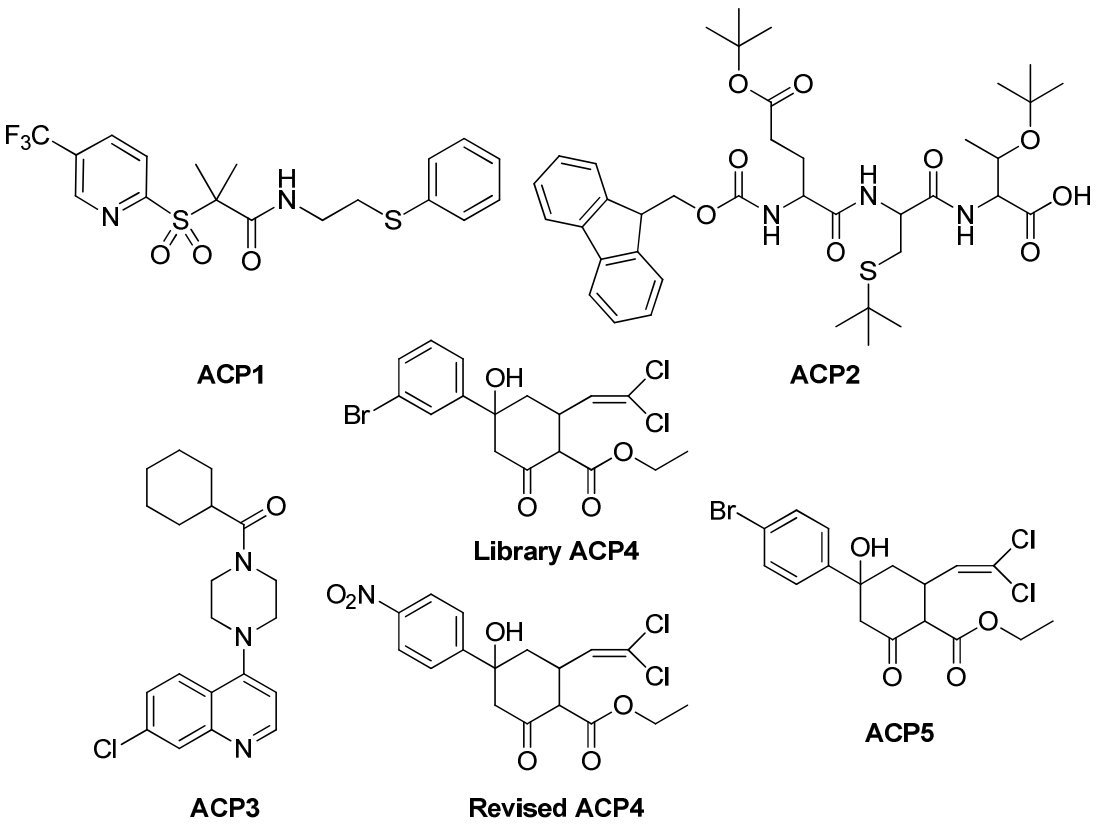

Figure 5: Chemical Structures of ACP1-5

To assess and compare the potency of hits, an *in vitro* assay using isolated *E. coli* ClpP was employed. After treatment of ClpP with varying concentrations of the ACPs, the degradations of casein-FITC were observed through fluorometric measurements after 6 hours. The results were evaluated using a quantitative measure, the relative degradation index (RD), which is defined as follows:

$$RD = \frac{(\Delta\varphi_{\text{ClpP + compound}})_{\text{after 6 hrs}} - (\Delta\varphi_{\text{ClpP}})_{\text{after 6 hrs}}}{(\Delta\varphi_{E.\ coli\ \text{ClpAP}})_{\text{after 6 hrs}} - (\Delta\varphi_{E.\ coli\ \text{ClpP}})_{\text{after 6 hrs}}}$$

$\Delta\varphi$ was the change in fluorescence after 6 hours of starting the reaction. ClpAP, an ATPase, was a benchmark for maximum ClpP proteolytic activity. RD_{25} values, which refer to measurements in the presence of 25 μM of compound, were used to compare the potencies of the ACPs to one another; values closer to 1 imply a more active compound (Table 1).

Compound	RD_{25}	SD
ACP1	0.53	0.04
ACP2	0.20	0.01
ACP3	0.10	0.04
ACP4	0.37	0.05
ACP5	0.39	0.04

Table 1: Comparison of the RD_{25} Values for ACP1-5.
Data Shown Represent the Average of Three Repeats. SD is Standard Deviation[17]

Previous reports by Lee and coworkers had shown ADEPs bind to the hydrophobic pocket (H pocket) on the ClpP apical surface. This binding event interferes with chaperone binding to ClpP, resulting in reduced ATPases-mediated degradation of GFP-ssRA peptide.[11] A similar effect, shown in Figure 6, was also seen for ACPs, suggesting that these compounds bind to, or allosterically alter, the H pocket of ClpP.[17] Computational studies using DOCK6.3 software predicted with equal probability that ACPs bind either to the H pocket or a separate pocket that featured a large number of charged residues, aptly named the C pocket. The H and C pockets are separated by residues near the C terminus of ClpP, corresponding to amino acids 203-207. While ADEPs co-crystallized with ClpP were found to be bound to the H pocket exclusively, ACP1-5 docked well to both pockets, and their docking scores were similar for the two pockets.

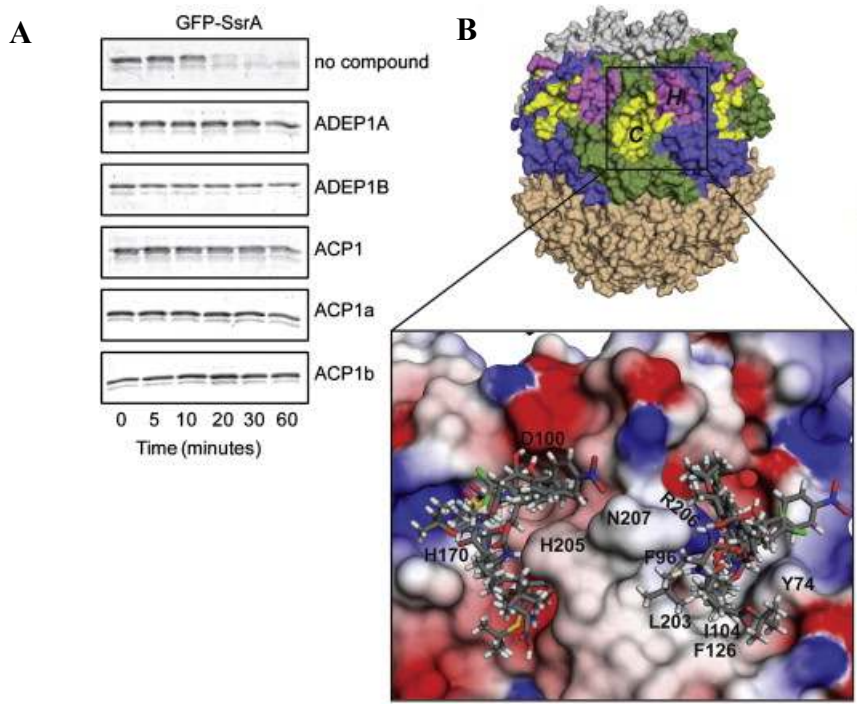

Figure 6: (A) Shown is the inhibition of ClpXP-mediated GFP-ssrA degradation by ACPs and ADEPs on SDS-PAGE gels. (B) Surface model of ClpP is shown on the top. The bottom panel shows a close up view of the predicted binding conformations of the five ACPs in the two ClpP binding pockets.[§]

When tested against ten different bacteria, several of the ACPs showed low minimum bactericidal concentrations (MBC in μg/mL). It was observed by Houry and co-workers that gram-negative bacteria were generally more sensitive to these compounds than gram-positive bacteria.

Efforts towards the development of antibiotics based on ACP1 due its drug-like properties, and its high biological activity, have been an ongoing project in our lab since 2008. ACP2 is a relatively straightforward core to evaluate in SAR studies, however, it was not considered optimal due to its protected tripeptide framework which we believed would not survive enzymatic degradation *in vivo*. Based on the drug-like and relatively straightforward

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[§] Reprinted from Chemistry & Biology, 18, Walid A. Houry e*t al*., Activators of Cylindrical Proteases as Antimicrobials: Identification and Development of Small Molecule Activators of ClpP Protease, 1176, 2011, with permission from Elsevier.

cores, we launched synthetic studies to further explore the biological activity around the ACP3 and ACP4/5 scaffolds.

Chapter 2

Results and Discussion

2.1 Synthesis of ACP3 and Its Analogs

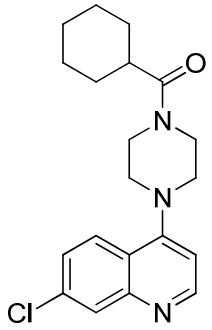
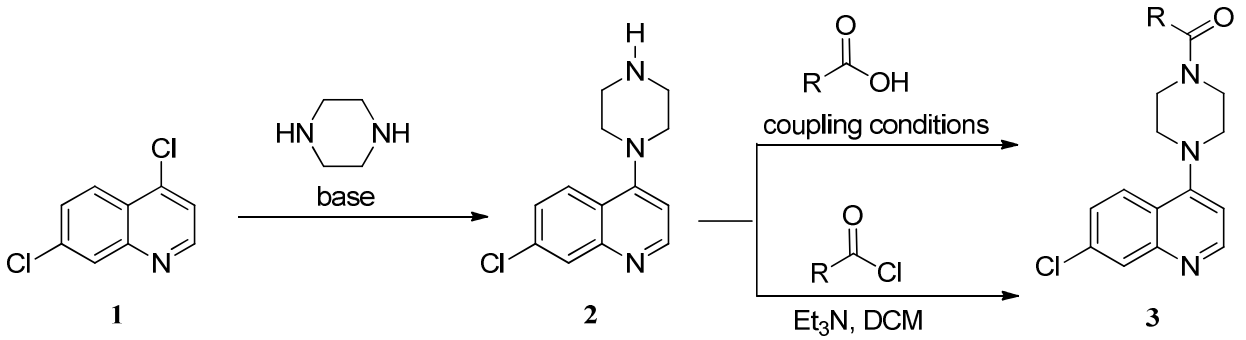


Figure 7: Structure of ACP3

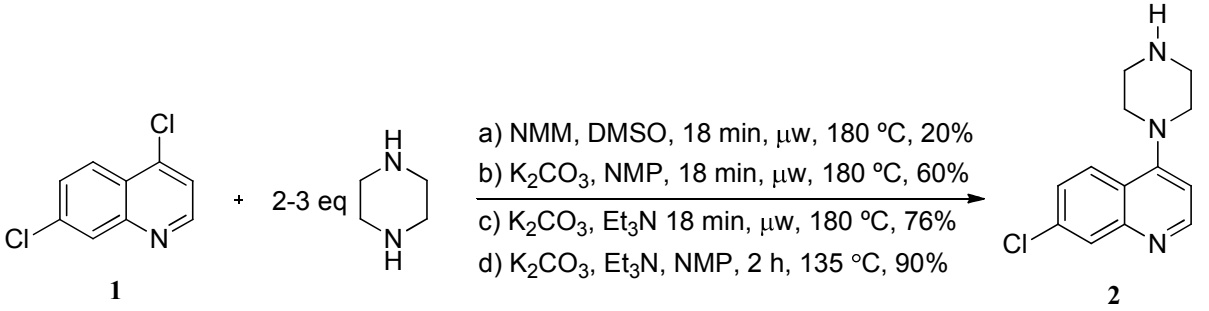
The structure of ACP3 consists of a central piperazine core substituted with cyclohexylamide and 7-chloroquinoline groups. Quinoline derivatives of this type are an important class of heterocyclic compounds found in many synthetic and natural products of biomedical interest.[18,19,20] Specifically, anti-viral, anti-cancer, anti-bacterial, anti-fungal, anti-obesity, and anti-inflammatory activities. As a drug lead, ACP3 satisfies Lipinski's rule of five: it has less than five hydrogen donors, less than ten hydrogen bond acceptors, and a molecular mass less than 500 Daltons.[21]

Our planned synthetic route to the desired target and its analogs is outlined in Scheme 1. We reasoned that the most direct strategy to generate a library of analogs was to attach a large number of different groups at the "northern" amine of intermediate **2**. We believed secondary amine **2** would in turn be generated from the union of 4,7-dichloroquinoline with piperazine.



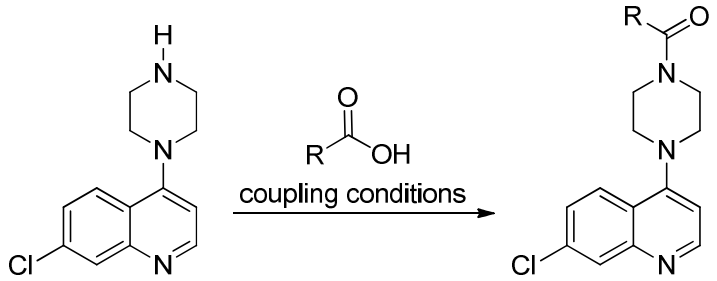
Scheme 1: General Synthetic Route to ACP3 Analogs

While high yielding (> 90%) reactions to furnish **2** are known, literature procedures require a large excess of piperazine (> 4 eq).[22] Therefore we first attempted to develop a microwave procedure to reduce the requisite amounts of piperazine used, as displayed in Scheme 2. We found a decrease in reaction times and amount of piperazine required for conversion. However, the yields of **2** were lower, and perhaps most limiting, we found that the restricted microwave vessel size (< 20 mL) allowed only small scale reactions. In this regard, we abandoned microwave conditions in favour of conventional heating as per literature procedures. Thus, **2** was prepared in gram quantities by the nucleophilic aromatic substitution of 4,7-dichloroquinoline **1** with piperazine (5 eq) under basic conditions, in 90% yield.



Scheme 2: Initial Attempts to Form Intermediate **2** via Microwave or Conventional Heating

With the piperazinyl core **2** in hand, we next set forth to find appropriate conditions to couple carboxylic acids to the free secondary amine. Our attempts are illustrated in Table 2. Of various amide coupling reagents tested, uronium (TBTU) and phosphonium (PyBOP) based coupling reagents provided the greatest yields. However, due to the absence of DMF and faster reaction times, benzotriazole/$SOCl_2$ was chosen as our standard procedure for carboxylic acids.[23]

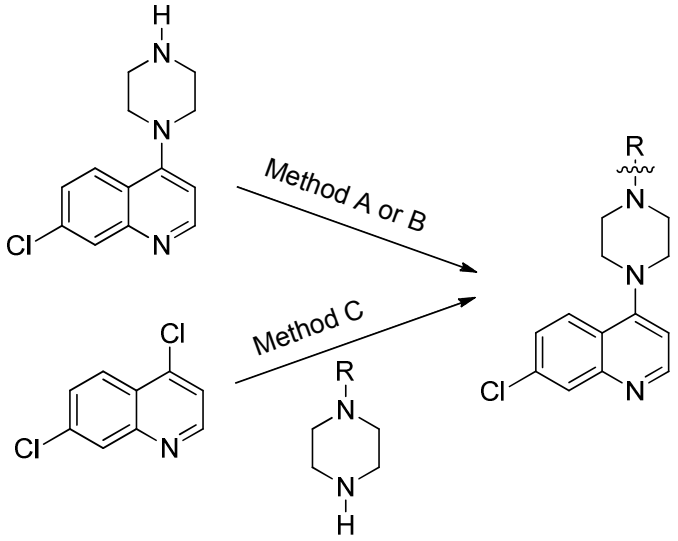


2 **3**

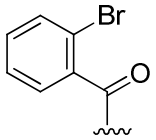
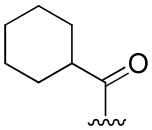
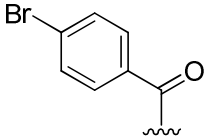
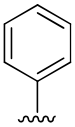
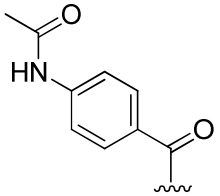
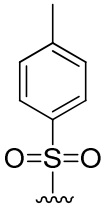
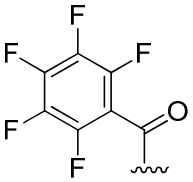
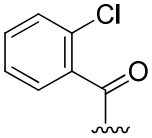
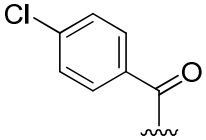
Entry	Coupling conditions	Yield (%)
1	PyBOP, DIEA, DMF, 2 h, rt	95
2	DCC, DMAP, DMF, 6 h, 0 °C to rt	42
3	EDC·HCl, DMAP, DMF, 6 h, 0 °C to rt	65
4	Benzotriazole, $SOCl_2$, Et_3N, 1 h, rt	78
5	TBTU, DIEA, DMF, 2 h, rt	>99

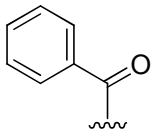
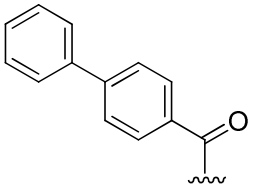
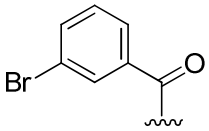
Table 2: Amide Coupling Conditions for ACP3 Synthesis

When the acid chlorides were commercially available, simple treatment under basic conditions afforded ACP3 analogs. We then coupled various carboxylic acids and acid chlorides with differing aromatic rings, such as substituted phenyl groups and naphthyl groups. Moreover, when aryl piperazines were available, microwave heating with dichloroquinoline afforded analogs with deletion of the carbonyl group. The results of these efforts are summarized in Table 3.



Method A = carboxylic acid, benzotriazole, $SOCl_2$, Et_3N, DCM, 1 h, rt
Method B = acyl chloride, Et_3N, DCM 1 h, rt
Method C = NMM, DMSO, 18 min, 180 °C μw

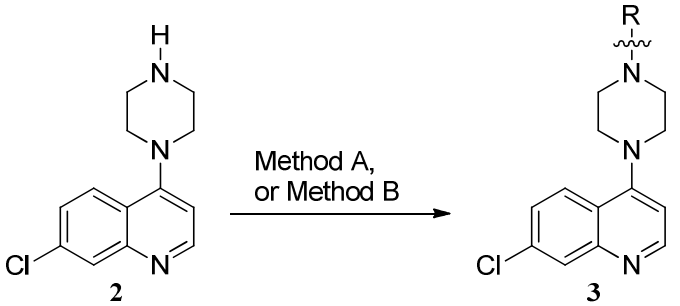
Compound	R	Yield (%)	Compound	R	Yield (%)
2	H	90	**10**		99[b]
4 (ACP3)		77[a]	**11**		86[b]
5		72[c]	**12**		81[a]
6		90[c]	**13**		67[a]
7		78[b]	**14**		95[b]

8		63[a]	**15**		82[a]
9		99[b]			

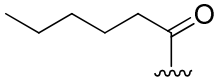
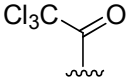
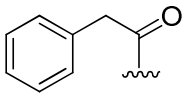
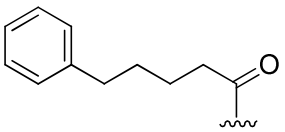
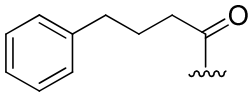
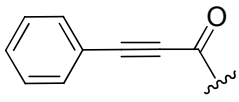
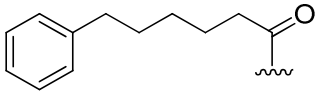
[a] Prepared via Method A. [b] Prepared via Method B. [c] Prepared via Method C.

Table 3: Structures of ACP3 and Acyl Modified Analogs

Moreover, we were interested in testing alkyl substituted groups between the carbonyl and aromatic rings. These products are displayed in Table 4.



Method A = carboxylic acid, benzotriazole, $SOCl_2$, Et_3N, DCM, 1 h, rt
Method B = acyl chloride, Et_3N, 1 h

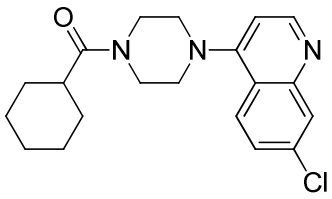
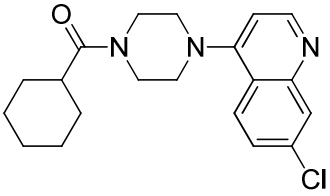
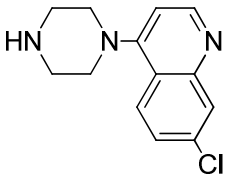
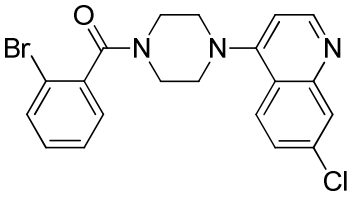
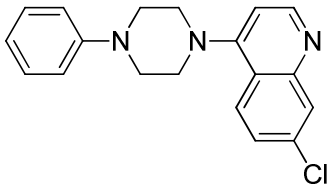
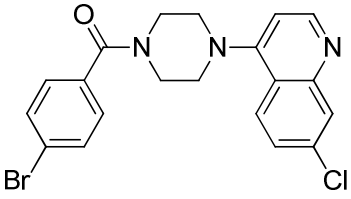
Compound	R	Yield (%)	Compound	R	Yield (%)
16		64[a]	**20**		89[b]
17		50[a]	**21**		69[a]
18		65[a]	**22**		45[a]
19		70[a]			

[a] Prepared via Method A. [b] Prepared via Method B.

Table 4: Structures of ACP3 Analogs with Acyl Modifications

2.2 Structure Activity Relationship Studies for ACP3

The biological activity of ACP3 analogs were evaluated by Elisa Leung at the University of Toronto, Biochemistry Department using the *in vitro* assay developed for the initial leads. The RD_{25} measurements from the assay refer to the measurement of *E. coli* ClpP activity in the presence of 25 μM of the analog to be tested. The bacterial *E. coli* ClpAP was once again used as a standard for maximum ClpP proteolytic activity with an RD_{25} equal to 1. ADEP1 and 2, used as references, and ACP3 analogs with modifications to the acyl end, were initially studied. The biological testing values of our synthetic ACP3 analogs are shown in Table 5 below.

Structure	RD_{25} (SD)	Structure	RD_{25} (SD)
ADEP1	0.78 (0.01)	**ADEP2**	0.60 (0.12)
 **Library ACP3**	0.10 (0.04)	 **4 (Synthetic ACP3)**	0.00 (0.00)
 **2**		 **10**	0.04 (0.08)
 **5**	0.00 (0.00)	 **11**	0.04 (0.01)

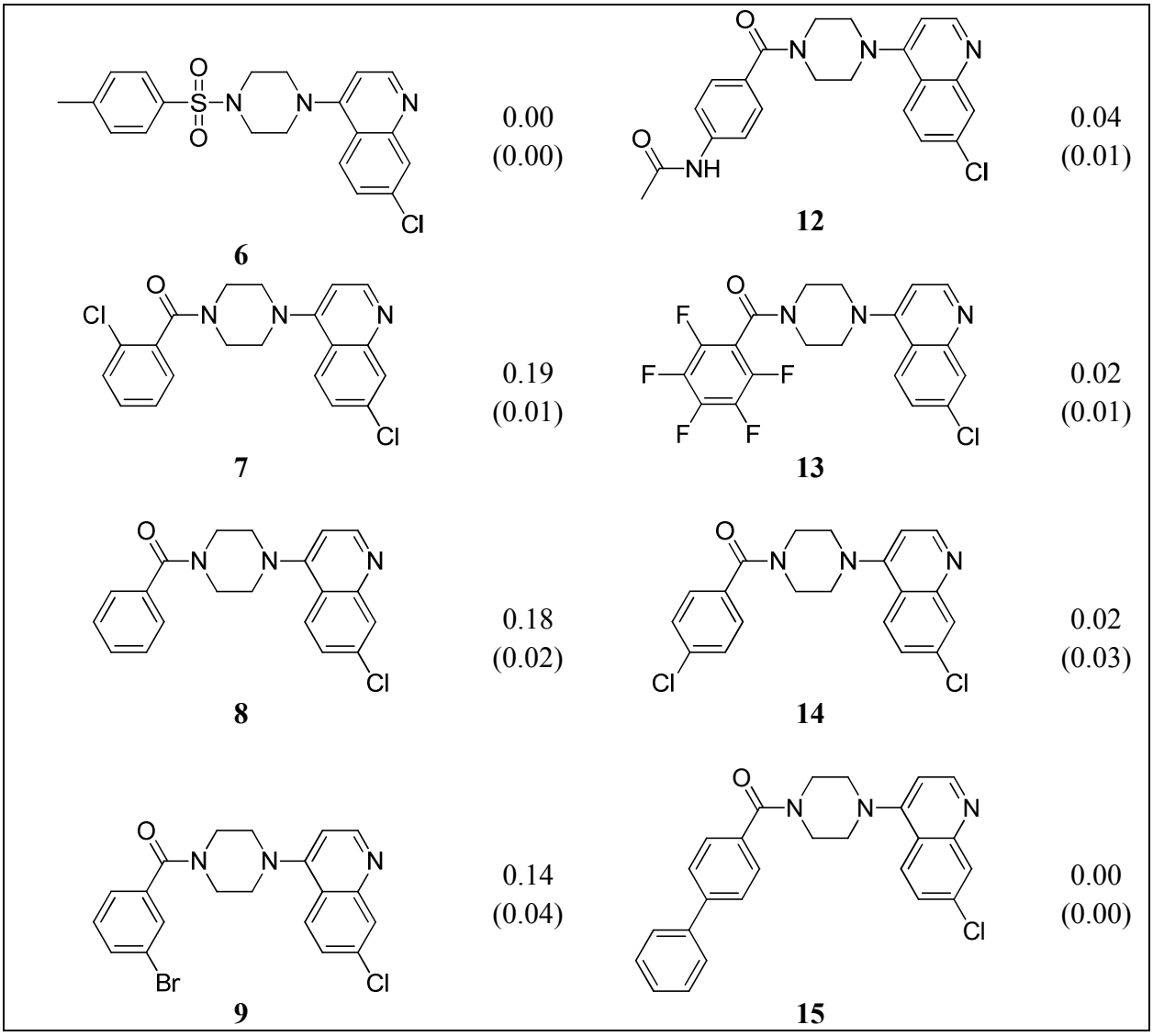


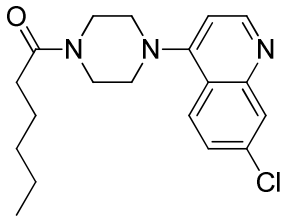
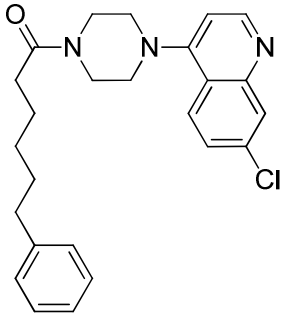
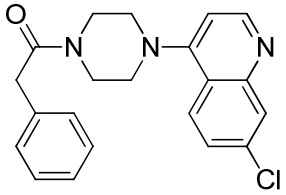
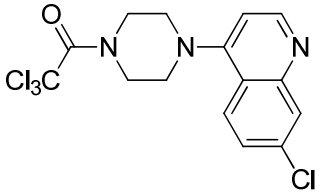
Table 5: RD_{25} Values of the Natural ADEPs, ACP3 and Analogs

The immediate objective was to identify synthetic variants with an *in vitro* activity comparable to ADEP1. Initial ClpP activation experiments with the natural products and ACP3 had the following order: ADEP1 > ADEP2 > ACP3. Our synthetic analog of ACP3, **4**, failed to demonstrate any biological activity, having a RD_{25} value of zero. This raised concerns over the correct identity of ACP3 from the Maybridge library or if ACP3 was a false-positive in the assay. Pressing forward, the deletion of the carbonyl functionality in analogs **5** and **6** resulted in loss of activity; thus, indicating the necessity of a carbonyl functionality in the ACP3 core.

Substitutions to the cyclohexyl fragment of the core were made in favour of more electronically stable phenyl groups. From the onset, analogs **7** – **9**, which possessed an *ortho*-

chlorophenyl, unsubstituted phenyl and a *meta*-bromophenyl group, respectively, displayed slightly higher activities compared to ACP3. Although analog **7** gave the highest activity in this set, it was still considerably less than ADEP level. It did dismiss the false-positive concern, supporting that ACP3 is of the piperazinyl quinolone nature, however, most likely not substituted with a cyclohexyl group. Aromatic modifications involving different halogens (analogs **10**, **11**, and **14**), an electron deficient penta-flouro moiety (analog **13**), and an acetamide functionality (analog **12**), resulted in reduced to no activity.

Next, a pentyl hydrocarbon chain in **16** was substituted for the cyclohexyl group of ACP3, giving a slight increase in activity. As this demonstrated that an extended alkyl functionality was tolerated, several analogs **17**, **18**, **19**, **20**, and **22** incorporated varying methylene lengths in between the phenyl moiety and the carbonyl. These analogs, unfortunately, resulted in decreased activity towards ClpP. Analog **20**, which possessed a smaller trichloroacetyl appendage compared to ACP3 also displayed no activity. Lacking promising biological activity around the ACP3 core, we then refocused our efforts towards new ACP4/5 analogs due to their more promising RD_{25} values.

Structure	RD_{25} (SD)	Structure	RD_{25} (SD)
 **16**	0.16 (0.01)	 **20**	0.02 (0.01)
 **17**	0.05 (0.01)	 **21**	0.01 (0.01)

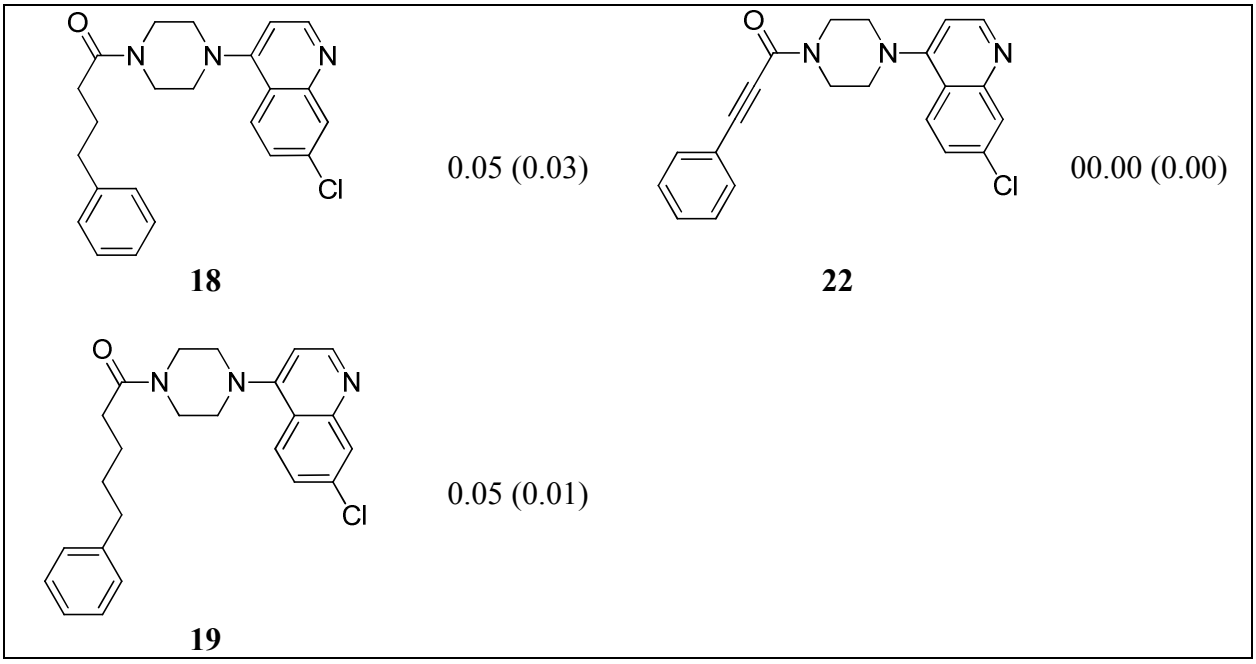


Table 6: RD_{25} Values of ACP3 Analogs

2.3 Unforeseen Activation of Human ClpP

Human ClpP, located in the mitochondrial matrix, shares a (71%) similarity with *E. Coli* ClpP, although its role is still not known.[12] The human ClpP has a very low peptidase activity compared to *E. coli* ClpP. Unlike ADEPs, ACP1-5 studies show minimal activity in activating human ClpP, a requirement for our antibiotic program for obvious biomedical reasons.

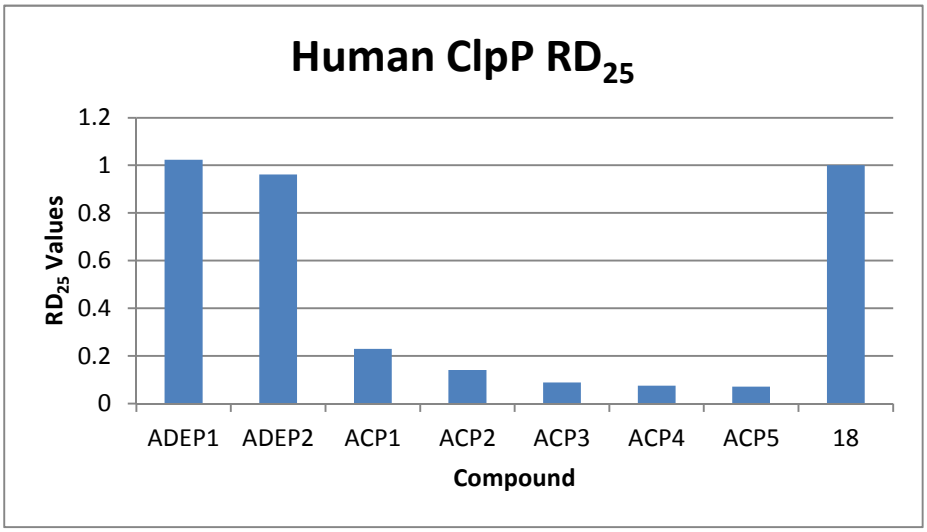
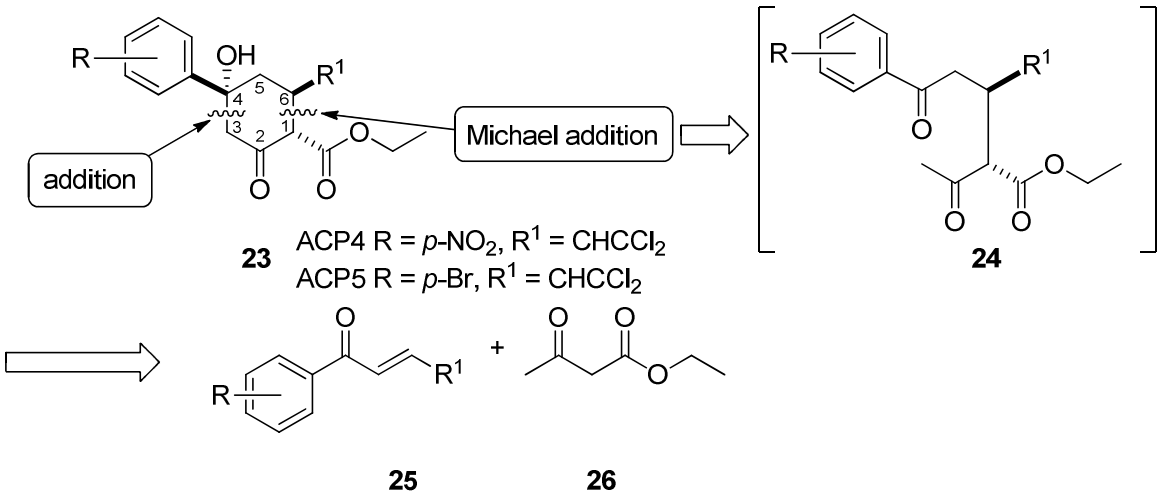


Figure 8: Human ClpP RD_{25} Values of ADEP1-2, ACP1-5, and **18**

In this connection, all compounds were cross tested for human ClpP activity, the values of which are displayed in Figure 8. Analog **18**, which displayed minimal activity in *E. coli* (*c.f.* Table 6), possessed potent activity in activating human ClpP with a $RD_{25} \approx 1$. Even at lower concentrations of 5 μM, **18** displayed a high RD_5 value of 0.63 (0.04). However, we were immediately excited by the potential for anti-cancer activity. As previously mentioned above, the exact role of human ClpP in healthy cells is not known, however it is known that cancerous human cells up regulate glucose metabolism for energy.[24] We speculate that other molecules than glucose present near the cancer cell can also be absorbed at a faster rate. The activator, in turn, would activate a cancer cell's ClpP towards uncontrollable proteolysis, leading to cell death. To our delight, preliminary studies upon analog **18** demonstrated cytotoxic activity to leukemia cell lines. In this connection, we are currently considering serious research efforts to develop **18** as an anti-cancer lead.

2.4 Synthesis of ACP4/5 and Its Analogs

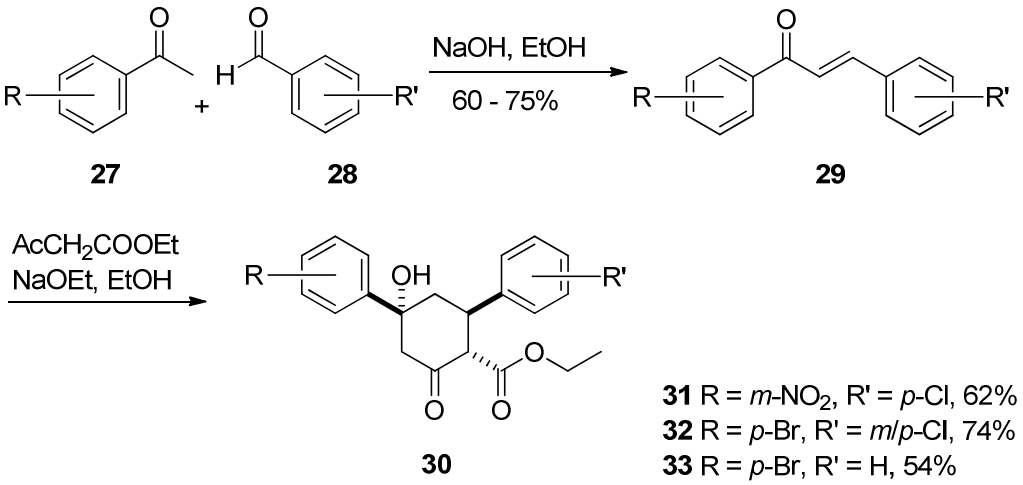


Scheme 3: Retrosynthetic analysis of ACP4/5

As displayed in Scheme 3, the core of the ACP4 and 5 lead structures resembles cyclic chalcones, a class whose members occasionally display anticancer activity.[25] Structurally, this core consists of a cyclohexanone ring substituted with an ethyl ester at the C-1 position, a hydroxyl group and an aromatic ring at the C-4 position, as well as an unusual dichlorovinyl moiety at the C-6 position. As drug targets, ACP4/5 satisfies Lipinski's rule of five.[21] There are, however, some undesirable characteristics of this structure including the labile hydroxyl group that is prone to dehydration. Moreover, the dichlorovinyl residue presents synthetic challenges for diversification and, we speculate, may be unstable for *in vivo* therapeutic applications. In spite of these aspects, their modest biological activity, second only to ACP1 in RD_{25} measurements, and its specificity in activating bacterial ClpP prompted further investigation.

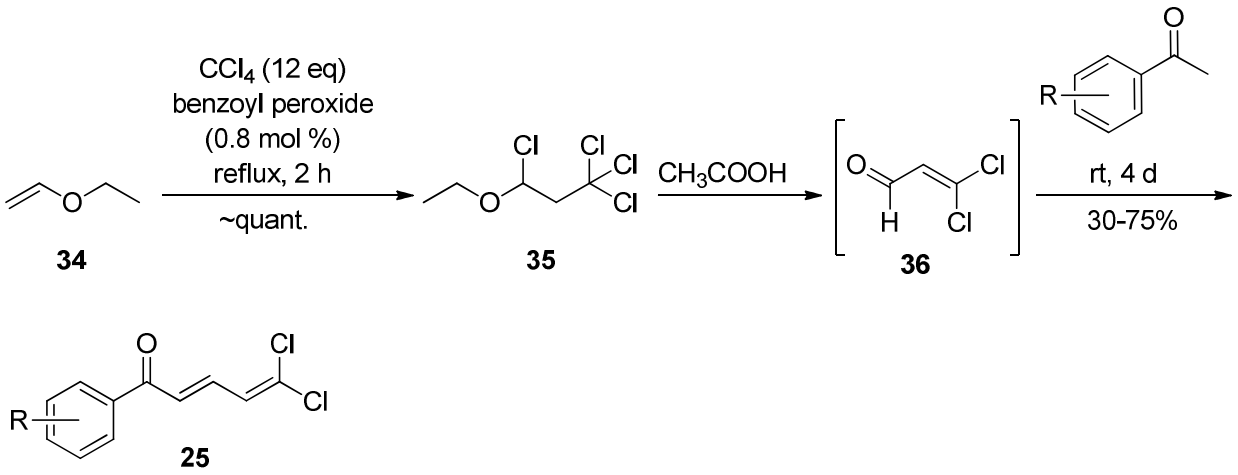
We decided to follow literature precedent and synthesize analogs of ACP4/5 core **23** via a domino Michael addition/ addition reaction of dichlorovinyl chalcones and ethyl acetoacetate. The Michael adduct presumably cannot be isolated, due to the rate of addition.[26,27]

In connection with the aforementioned, we initially pursued the preparation of various β-aryl cyclohexanones **30,** which are analogs of ACP4/5 wherein the dichlorovinyl group is replaced with aromatic substituents. The known synthetic sequence began with the base-catalyzed aldol condensation of appropriately substituted acetophenone **27** with substituted benzaldehydes **28** in ethanol (Scheme 4).[28,29] After crystallization, chalcones **29** were obtained in yields of 60 – 75%. The reaction of the chalcones with ethyl acetoacetate in the presence of sodium ethoxide yielded cycloketols **30** in moderate yields.[27]



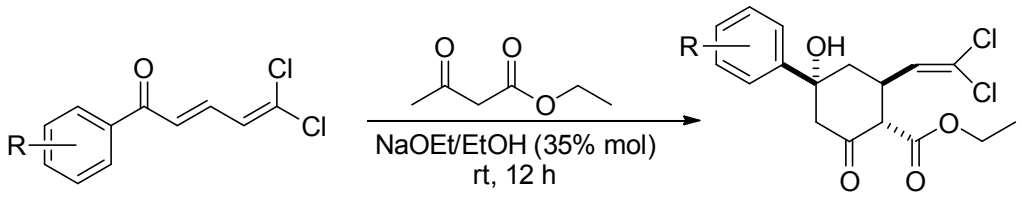
Scheme 4: Synthesis of ACP4/5 Analogs

With the aforementioned analogs **30** completed, we next turned our attention to analogs of ACP4/5 wherein the "left" aromatic ring is substituted. The preparation of the dichlorovinyl chalcones, which is precedented in literature, is shown in Scheme 5.[30] The first step was to prepare the key chloroether **35** intermediate by addition of carbon tetrachloride across vinyl ethyl ether **34** through an atom transfer radical addition mechanism.[31,32] Literature precedents prescribe the isolation of **35** prior to acid-catalyzed fragmentation. However, several attempts that followed this procedure via distillation at reduced pressure led to unusable polymerized product. To remedy this issue, our revised synthesis of the dichlorovinyl chalcones was adapted from a patent,[33] wherein the tetrachloropropyl ether **35** was taken forward without purification. Thus, crude intermediates **35** were treated with the various acetophenones in acetic acid for four days, affording aryldichloropentadienones **25** in varying yields.



Scheme 5: Synthesis of ACP4/5

As shown in Table 7, dichlorovinyl chalcones **25** were coupled with ethyl acetoacetate through the use of NaOEt as a base. By this method, racemic ACP4 and 5 were synthesized in 59% and 53% yields, respectively. Despite the fact that these analogs contain three chirality centres, they are formed as single diastereomers, with equatorial aromatic ring, dichlorovinyl and ester groups (Figure 9). This characterization has been proven by X-ray crystal analyses performed by Woznesensky and co-workers. [34]



Analog	R	Yield (%)	Analog	R	Yield (%)
37 ACP4	*p*-NO_2	59	**45**	*p*-OCH_2CH_3	62
38 ACP5	*p*-Br	53	**46**	*p*-phenyl	30
39	*p*-I	62	**47**	*m*-NO_2	75
40	*p*-CF_3	52	**48**	*m*-Br	60
41	*p*-Cl	51	**49**	*m*-Cl	71
42	*p*-CH_3	42	**50**	*o*-Br	41
43	*p*-CN	57	**51**	H	49
44	*p*-OCH_3	45	**52**	*m*-OCH_3	66

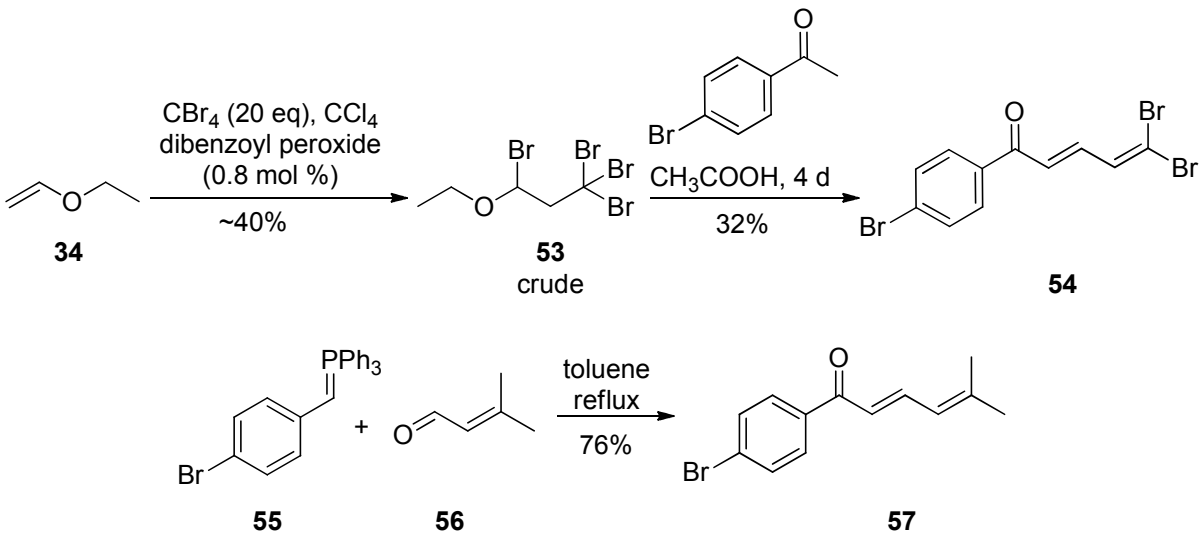
Table 7: Structures of ACP4/5 and Aryl Modified Analogs

Figure 9: Predominant Conformation of ACP4/5

Disappointingly, dichlorovinyl chalcones with electron-deficient aryl rings such monofluorinated aryl rings, pentafluorobenzene and pyridine were either isolated in low yield, or did not undergo an efficient reaction with ethyl acetoacetate.

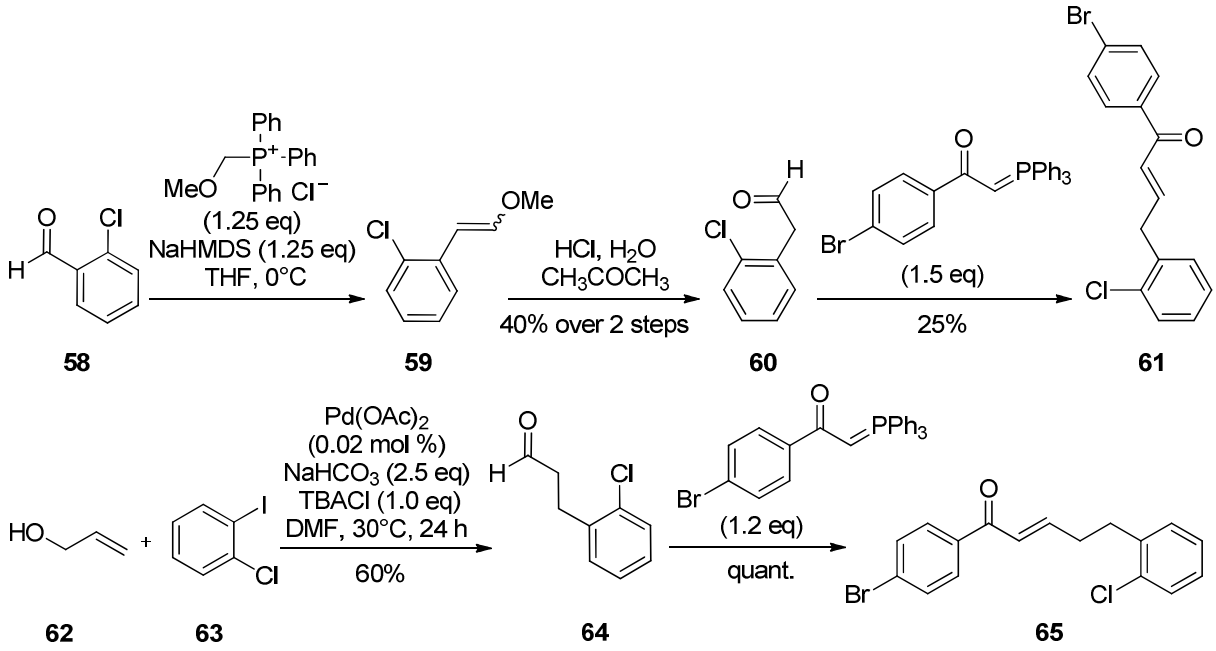
Our next course of action was to prepare analogs of ACP4/5 for which the dichlorovinyl group was substituted by a more sterically hindered dimethylvinyl group, a more electropositive dibromovinyl group, and methylene spacer groups. Dibromovinyl chalcone **54** was prepared in a similar manner to the standard procedure shown previously (*c.f.* Scheme 5), using carbon

tetrabromide rather than carbon tetrachloride. Then we prepared chalcone **57** by the Wittig reaction of phosphonium **55** and 3-methylbut-2-enal **56**.[35]



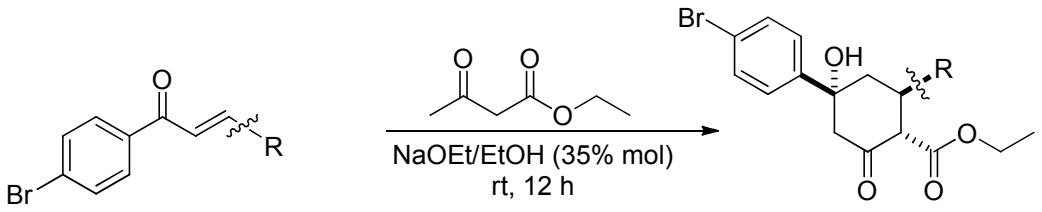
Scheme 6: Synthesis of **54** and **57**

The next synthetic goal was to synthesize the corresponding homologated chalcones **61** and **65**. Relative to analogs **31 – 33** possessing an aryl ring in place of the dichlorovinyl group found in ACP4/5, we envisaged that the addition of a spacer would provide more flexibility. In this connection, 2-chlorobenzaldehyde was subjected to Wittig reaction using phosphonium **58**. Subsequent acid hydrolysis of compound **59** and a second Wittig reaction afforded the product **61**.[36,37] Installation of a two carbon saturated linker was achieved by a Heck reaction developed by Jeffery, which involved coupling of 2-chloroiodobenzene and allyl alcohol.[38] Subsequent Wittig reaction gave chalcone **65**.



Scheme 7: Synthesis of Extended Chalcones

As illustrated below, the final cyclization of ethyl acetoacetate and the aforementioned chalcones proceeded in modest yields relative to that obtained for the previous analogs (*c.f.* Table 7).



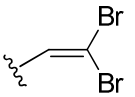
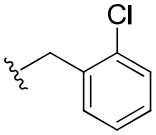
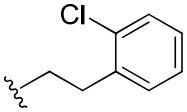
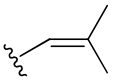
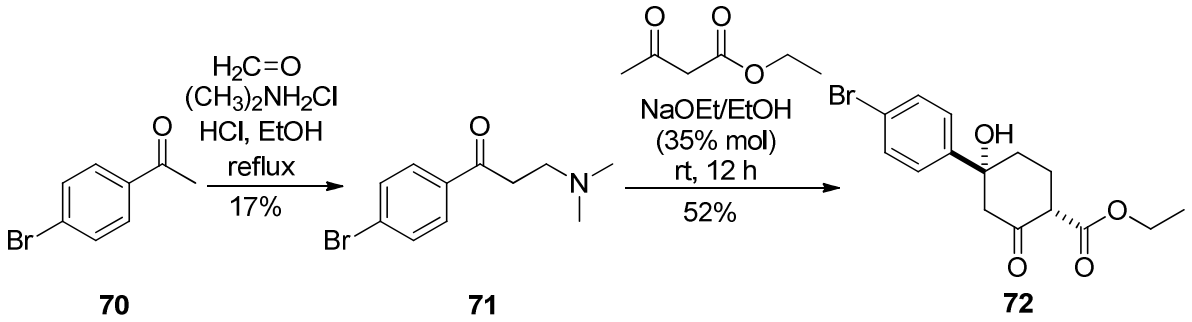
Compound	R	Yield (%)	Compound	R	Yield (%)
66		33	**68**		21
67		25	**69**		35

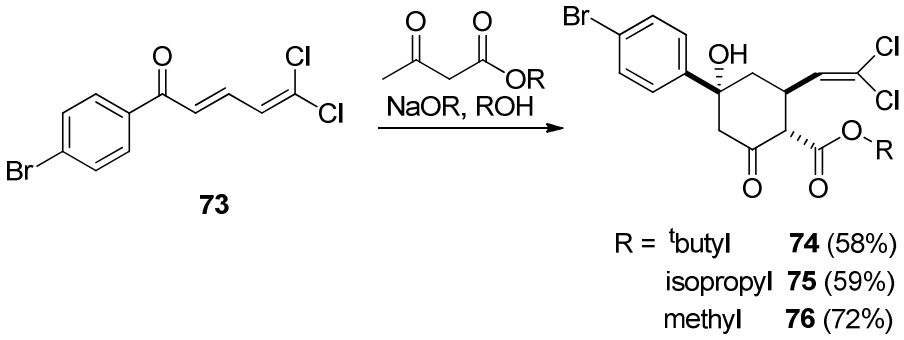
Table 8: Structures of ACP4/5 analogs with various functional groups at the C-6 position

In order to test whether the dichlorovinyl groups in ACP4/5 are important for its activity, we also prepared an analog without this group. Preparing Mannich adduct **71**, followed by standard cyclization conditions with ethyl acetoacetate, afforded analog **72**.[39,40]



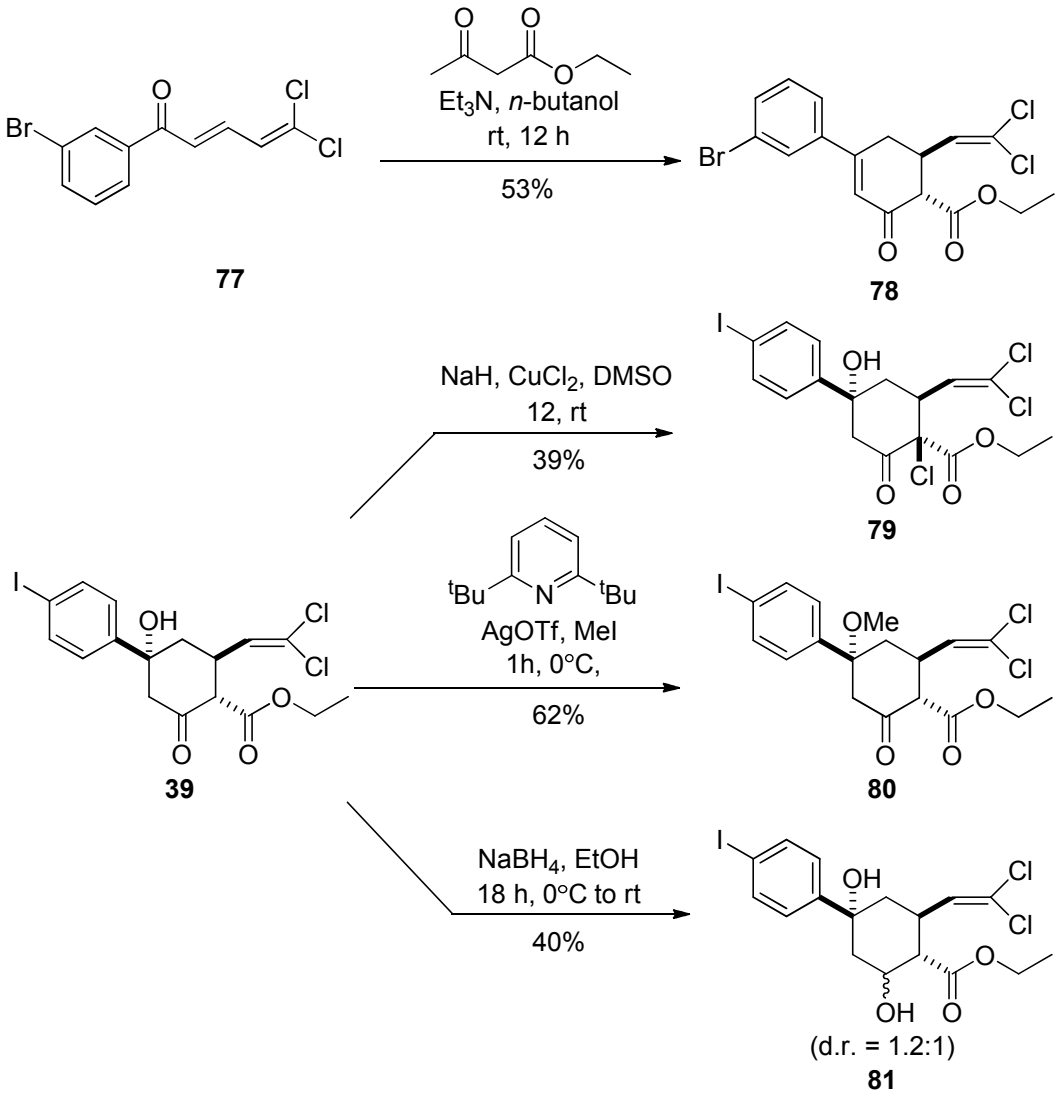
Scheme 8: Synthesis of Analog **72**

With analog **72** in hand, we felt that major structural modifications had been sufficiently explored. Thus, we set forth to prepare analogs with less dramatic structural modifications. As shown in Scheme 9, modifications of the ethyl ester group were achieved by reacting **73** with varying alkyl acetoacetates. The base and solvent were chosen to prevent mixtures of ester products in each case.



Scheme 9: Synthesis of ACP4/5 analogs with various alkyl esters at the C-1 position

Other modifications we explored are shown in Scheme 10. The hydroxyl group of ACP4/5 was eliminated by heating **77** with triethylamine, resulting in compound **78**. Direct modifications to ACP4/5 were restricted due to the labile OH group. Nonetheless, a limited number of straightforward modifications led to chlorinated **79**, a methyl ether **80**, and a reduced *sec*-alcohol **81**.

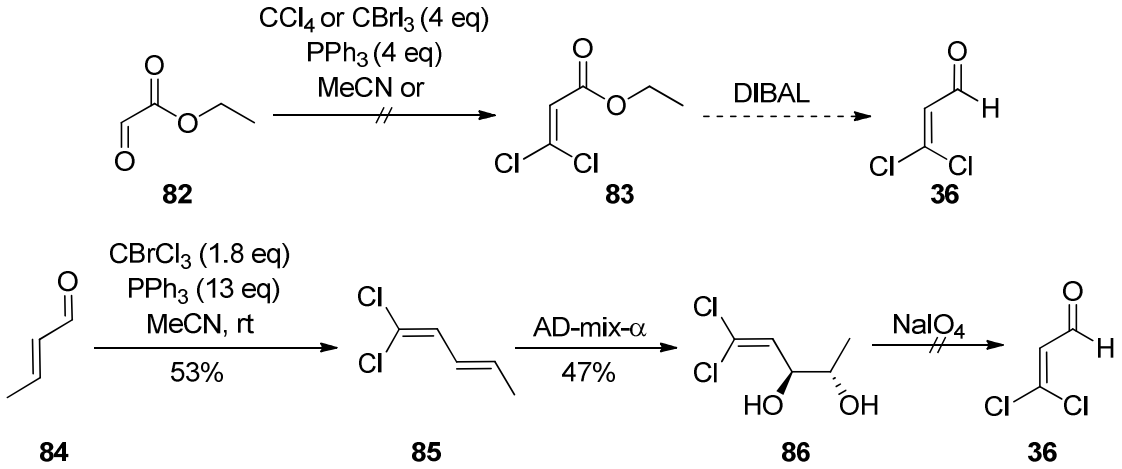


Scheme 10: Synthesis of a conformationally modified analog **78** and analogs **79-81** synthetically diversified from analog **39**

Briefly, we have synthesized over 30 analogs of ACP4/5 with modifications to the aromatic ring, the dichlorovinyl group, the ester, hydroxyl, and ketone groups. The biological activities are discussed in section 2.7 along with future plans.

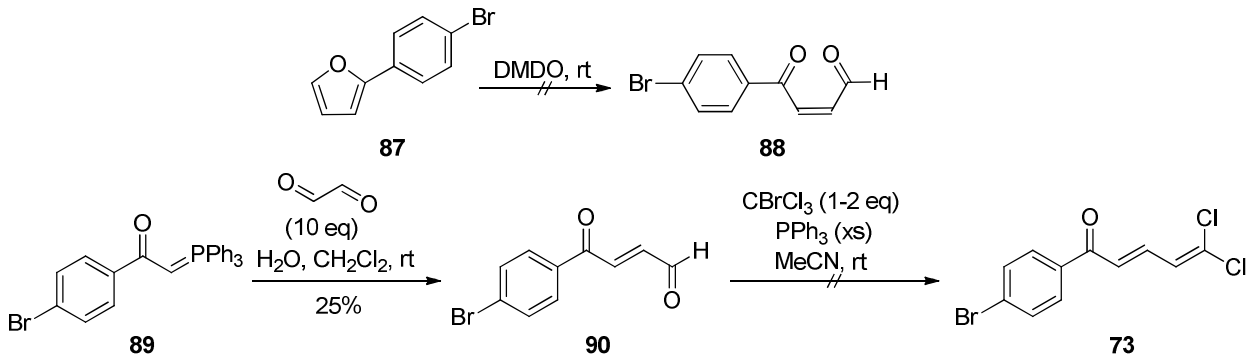
2.5 Alternate Routes to Dichlorovinyl Chalcones

As mentioned earlier, several attempts to isolate tetrachlorinated ether **35** failed, preventing access to the required 3,3-dichloroacrolein **36** (*c.f.* Scheme 5). Prior to the successful preparation of the dichlorovinyl chalcones, several other avenues illustrated in Scheme 11 were investigated to prepare **36**. We envisioned that one possible synthetic route was to perform a Wittig reaction on ethyl glyoxylate **82**, followed by DIBAL reduction of the ethyl ester to the corresponding aldehyde. Unfortunately, the treatment of **82**, which was distilled prior to use, using various conditions resulted in unreacted starting material**.** Next, we decided to modify a procedure that was previously used to synthesize an analogous 3,3-dibromoacrolein.[41] Wittig reaction of crotonaldehyde **84** afforded dichlorodiene **85**. Subsequent dihydroxylation of the more electron-rich double bond led to vincinal diol **86**. The key cleavage step of the diol was low yielding and did not afford an aldehyde that resembled **36**. We speculate that the 3,3-dichloroacrolein was not amenable to the work up conditions in the final step due to its volatility. With no further developments on this front, routes towards the preparation and isolation of 3,3-dichoroacrolein were abandoned.



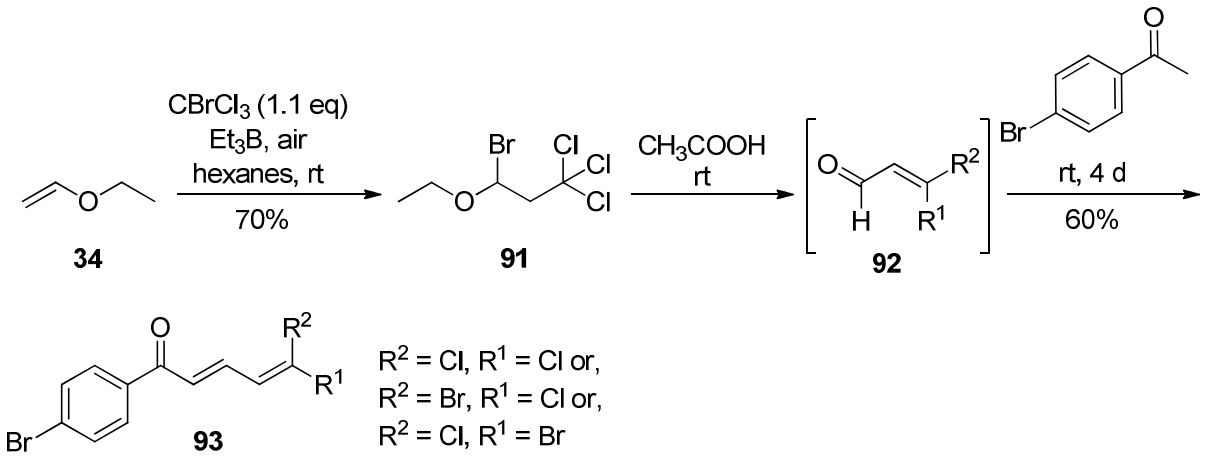
Scheme 11: Attempts to Prepare 3,3-Dichloroacrolein

In an alternate strategy, we planned to install the dichlorovinyl group late stage as opposed to introducing it earlier in the synthesis. In an attempt to synthesize *cis*-precursor **88**, an Achmatowicz reaction was employed.[42] Ring opening of aryl furan **87** with dimethyldioxirane as the oxidant led to a complex mixture of inseparable products. An analogous *trans*-precursor **90** was prepared by the Wittig reaction of glyoxal. Several attempts to introduce the dichlorovinyl group were unsuccessful, resulting in decomposition products. Efforts toward late stage dichlorovinyl group installation were eventually abandoned.



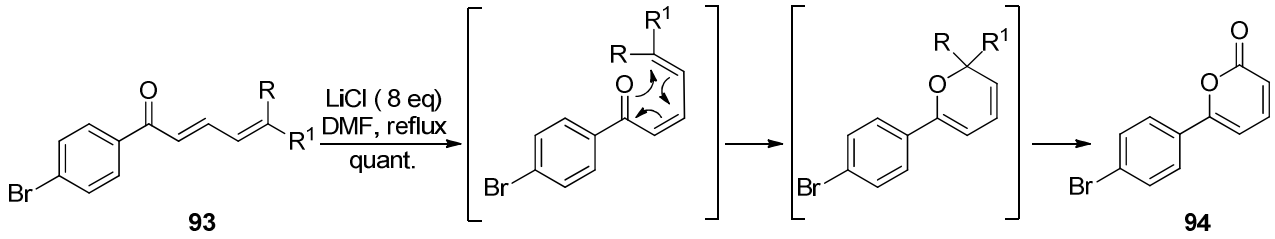
Scheme 12: Attempts to Install a Dichlorovinyl Group

In order to limit the excess use of carbon tetrachloride (> 12 eq) in the synthesis of dichlorovinyl chalcones, a triethylborane-initiated radical reaction with the more reactive bromotrichloromethane was used to prepare halogenated ether **91**.[43] Treatment of **91** with *p*-bromoacetophenone in acetic acid for four days afforded aryldichloropentadienone **93**. We found that **93** was an inseparable mixture of gem-bromochloro and gem-dichlorovinyl compounds. We speculate that the bromide evolved from acid-catalyzed fragmentation of compound **91** undergoes halogen-exchange with either compound **92** or **93**, resulting in a mixture of products.



Scheme 13: Attempted Synthesis of Compound **93**

To remedy this problem, we attempted to convert the brominated impurities to **93** using excess lithium chloride. However, under these conditions, compound **93** underwent an electrocyclization to aryl coumarin **94**. We speculate that refluxing conditions allowed alkene isomerization and subsequent electrocyclization to the coumarin. This protocol involving the use of bromotrichloromethane was therefore abandoned due to the mixture of halogenated chalcones that was produced.



Scheme 14: Electrocyclization of Compound **93**

2.6 Revision of the ACP4 Structure

Initial RD_{25} measurements indicated an order of activity of DEP1 > ADEP2 > ACP4/5. ACP4 and 5 were the only initial hits with obvious structural similarities, differing in the substituents of an aromatic group. There was initial confusion over the chemical structure of ACP4. Based on labels from the Chembridge library, ACP4 was believed to be compound **48**. However, biological studies on authentic **48** synthesized in our lab showed it to possess no activity. Further analysis of the spectral data provided by Chembridge suggested ACP4 to be compound **37**, which possessed a *para*-nitrophenyl group instead of a meta-substituted aryl group as proposed. Our synthetic variant **37** of the revised ACP4 structure displayed similar biological activity, albeit with a lower RD_{25} value compared to the initial hit. Compound **38**, synthetic material corresponding to the structure of ACP5, displayed similar activity to the ACP5 that was initially screened.

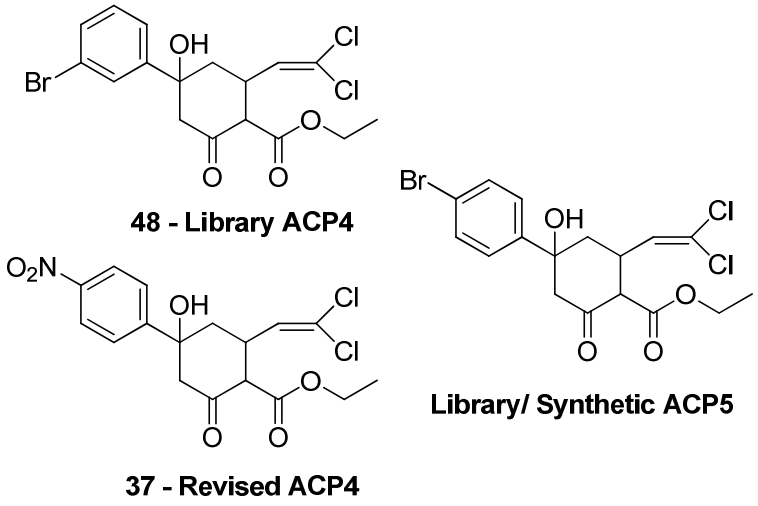
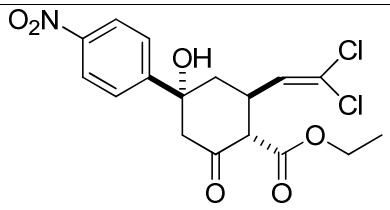
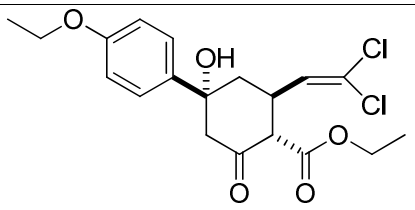
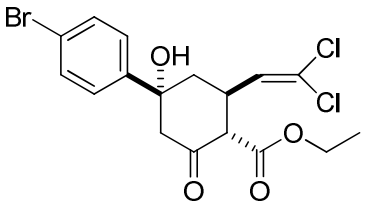
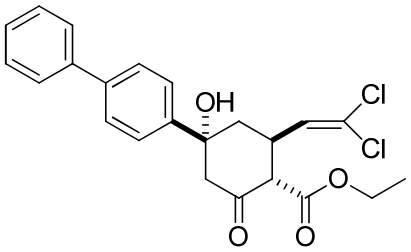
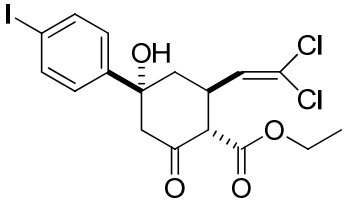
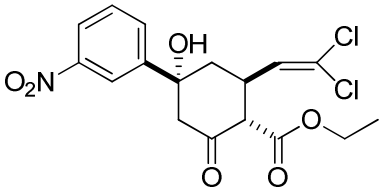
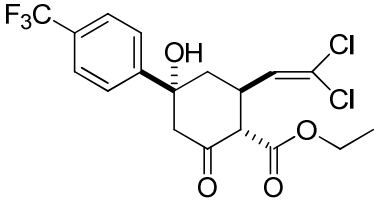
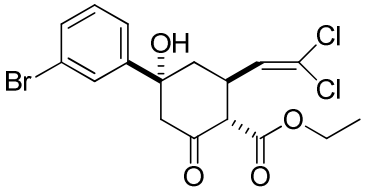
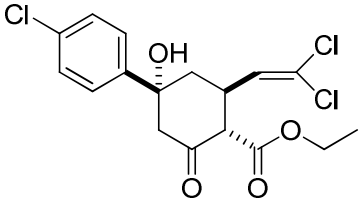
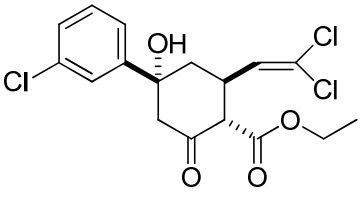
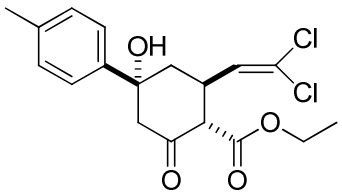
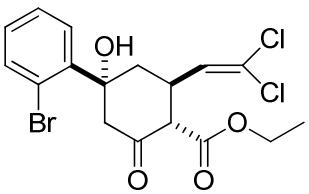


Figure 10: Revision of the Structure of ACP4

2.7 Structure Activity Relationship Studies for ACP4/5

The northwestern aromatic fragment was the starting point for chemical optimization because of the relatively easy access to the aryldichloropentadiones **25**. Deletion of the bromo group (analog **51**) resulted in loss of activity. Activity trends were evident from analogs bearing electron-rich and electron-deficient substituents at different positions on the aromatic group. It was found that para substitution was optimal, highlighted by analogs **38 – 41**. Analogs **47 - 50**, that relocated substituents to the ortho and meta positions led to diminished activity. Replacing the bromo group with a larger iodo group showed an increase in activity. The general trend related to atomic radii of the halogen was I > Br > Cl. However, when an even larger trifluoromethyl group in **40** was used, the activity diminished slightly. Analogs **44** and **45** with more electron rich methoxy and ethoxy aryl groups displayed reduced activity. The naphthyl substituted analog **46** showed no detectable activity, suggesting that larger aromatic rings are not tolerated. Through these findings, the northwestern fragment was optimized to a para-iodosubstituted phenyl group.

Structure	RD_{25} (SD)	Structure	RD_{25} (SD)
 **37 (Synthetic ACP4)**	0.11 (0.03)	 **45**	0.05 (0.04)
 **38 (ACP5)**	0.41 (0.07)	 **46**	0.01 (0.01)
 **39**	0.55 (0.02)	 **47**	0.00 (0.00)
 **40**	0.33 (0.06)	 **48 (Library ACP4)**	0.00 (0.00)
 **41**	0.23 (0.02)	 **49**	0.00 (0.00)
 **42**	0.06 (0.01)	 **50**	0.00 (0.00)

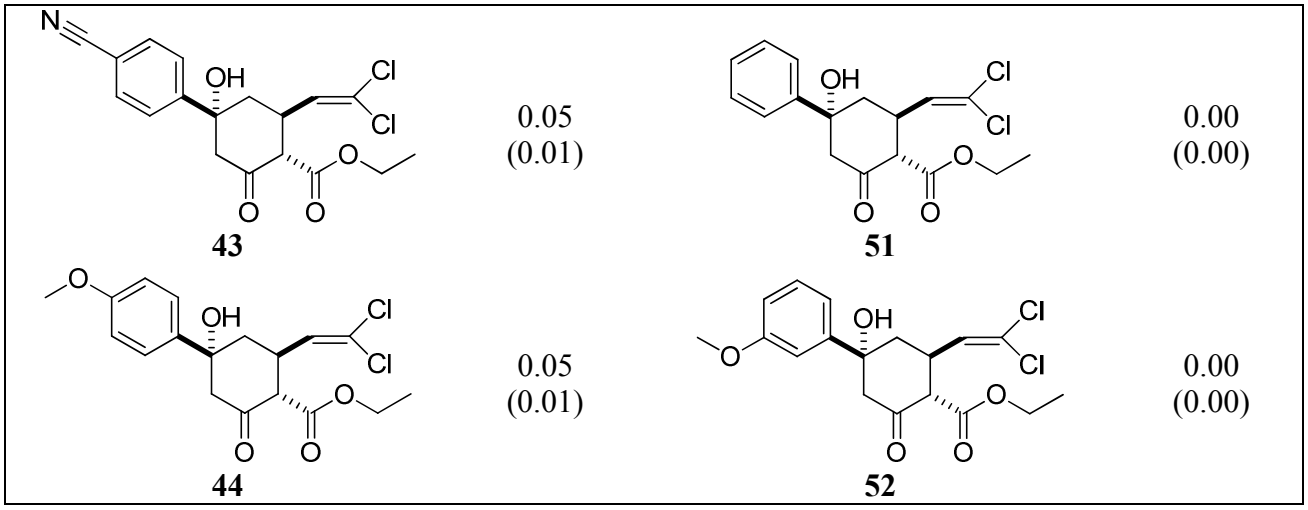
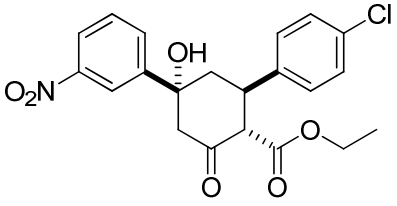
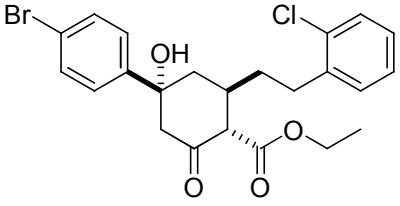
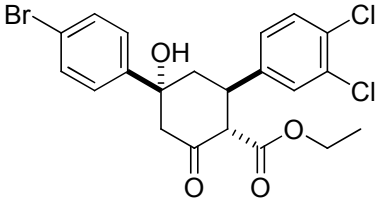
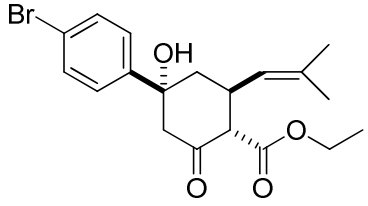


Table 9: RD_{25} Values of ACP4/5 Analogs with Northwestern Modifications

The effects of modification of the dichlorovinyl group on activity are outlined in Table 10. Introduction of aryl groups in compounds **31**–**33** resulted in a loss in activity. Analogs bearing methylene spacers (**67** and **68**) show dramatically reduced activity. Substitution of the *gem*-dichloro groups with bromo and methyl groups (analogs **66** and **69**, respectively) led to more than a twofold decrease in activity. Removal of the dichloro group completely, compound **72** led to no activity towards ClpP. Modifications to the northeastern dichlorovinyl group of ACP4/5 were poorly tolerated, reinforcing the importance of this group for biological activity.

Structure	RD_{25} (SD)	Structure	RD_{25} (SD)
 **31**	0.00 (0.00)	 **67**	0.06 (0.07)
 **32**	0.00 (0.00)	 **69**	0.04 (0.02)

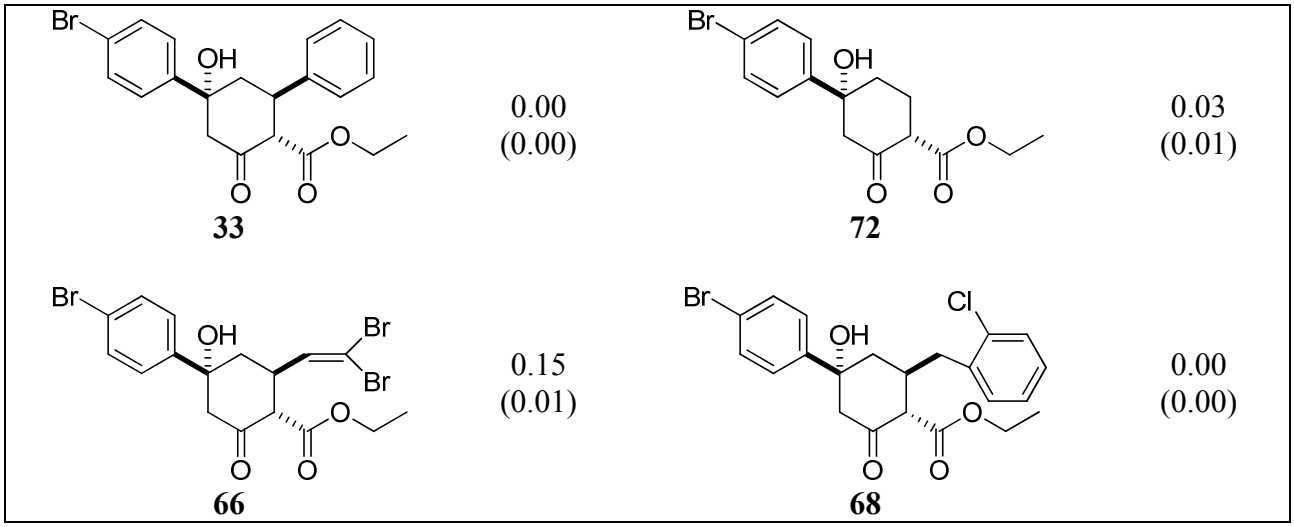


Table 10: RD_{25} Values of ACP4/5 Analogs with Northeastern Modifications

Modifications to the ethyl ester fragment of ACP4/5 resulted in analogs **74 - 76**. The *t*-butyl and isopropyl esters were tolerated, however, with reduced activity. The methyl ester variant showed almost no activity. It is not known how longer linear alkyl esters would affect activity.

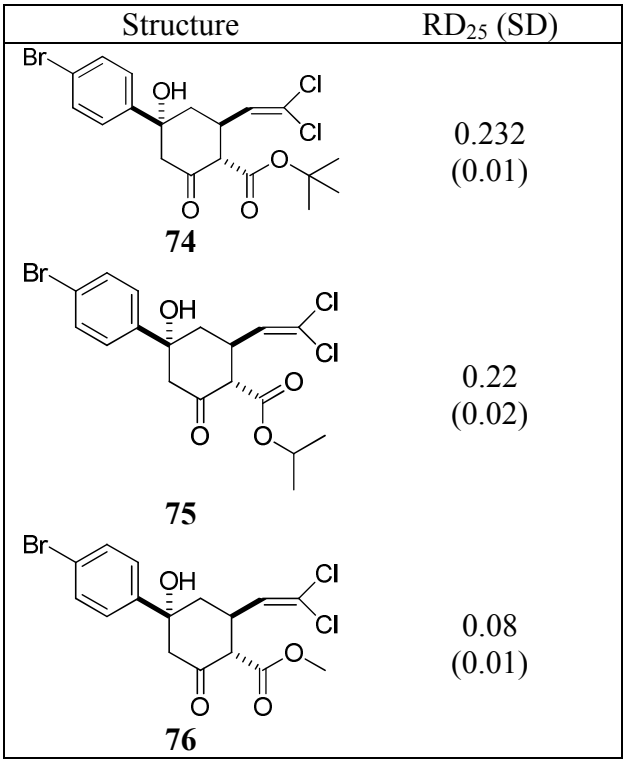


Table 11: RD_{25} Values of ACP4/5 Analogs with Various Alkyl Esters

Conformational change of ACP4/5 to a flatter half-chair structure, **78**, showed no activity, indicating that the chair conformation of ACP4/5 is ideal for ClpP binding. Synthetic diversification of compound **39** led to analogs that displayed interesting aspects. Chlorination of the α-hydrogen of the ketoester led to compound **79**, which showed similar activity to the most potent ACP4/5 analog **39.** This suggests that the α-hydrogen is not involved in any major interactions and also rules out the possibility of the enol form of ACP4/5 analogs being involved in interactions with ClpP. Analog **80**, with a methyl cap of the hydroxyl group, displayed slightly reduced activity. This result opens new avenues for future analogs as the methyl cap provides some stability over the labile OH. Reduction of the ketone carbonyl afforded alcohol analog **81**, which had almost no activity. We speculate that conversion of the carbonyl group to an alcohol changes a hydrogen bond acceptor group to a hydrogen bond donor, hindering binding to ClpP.

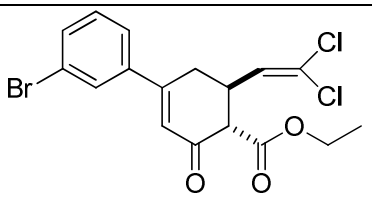
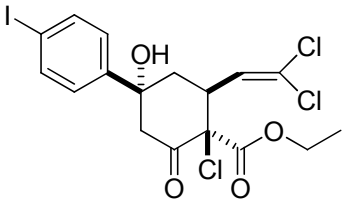
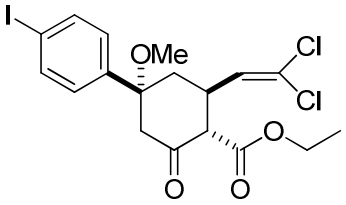
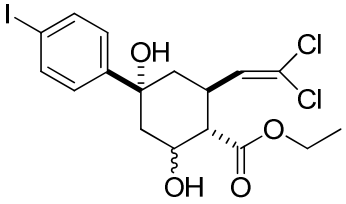
Structure	RD_{25} (S. D)
 **78**	0.00 (0.00)
 **79**	0.52 (0.01)
 **80**	0.45 (0.03)
 **81**	0.02 (0.01)

Table 12: RD_{25} Values of a conformationally modified analog **78** and analogs **79-81** synthetically derived from analog **39**

2.8 Disk Assays

The majority of the ACP3 and ACP4/5 analogs synthesized were evaluated against *N. meningitidis, S. aureus and E. coli* in disk assays performed by Shannon E. McCaw at the University of Toronto Molecular Genetics department. These Kirby-Bauer disk diffusion susceptibility tests determine the sensitivity of pathogenic bacteria to various anti-microbial compounds.[44,45] The presence or absence of growth around the disks allows a visual measure of the ability of our analogs to inhibit intact bacteria. The general protocol involved fresh bacteria being spread onto the surface of standard growth media. A filter disc impregnated with ~2.6 μg of an analog to be tested was then laid on its surface. The diameters of the zones of clearing on the plates were measured with an analytical ruler and reflect inhibition of bacterial growth. The tests were performed in triplicate for each analog.

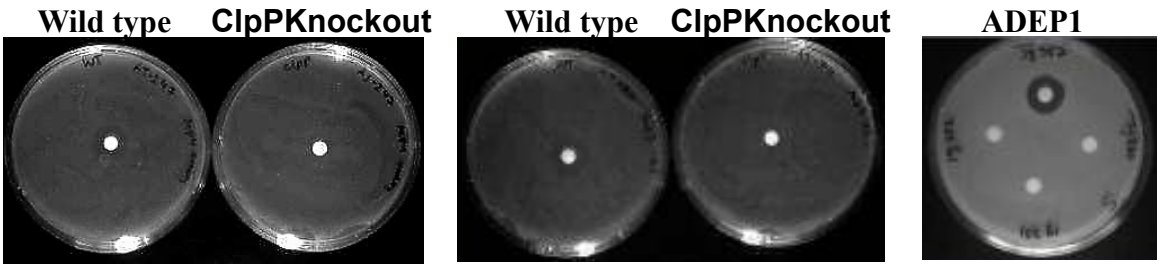
	E. coli (cm)	*N. meningitidis* (cm)						*S. aureus* (cm)		
	Test 1-3	Test 1		Test 2		Test 3		Test 1	Test 2	Test 3
	Wt	Wt	ko	Wt	ko	Wt	ko	Wt	Wt	Wt
37	–	1.0*	–	1.0*	–	1.0*	–	–	-	–
38	–	1.2*	0.65*	1.2*	0.9*	1.2*	0.9*	0.8*	0.65*	–
39	–	1.0*	–	0.9*	–	1.0*	–	–	-	–
41	–	1.2*	1.1*	1.3*	0.8*	1.2*	1.0*	–	0.7*	–
33	–	1.2*	0.7*	1.2*	0.8*	1.4*	1.0*	–	-	–

Wt = wildtype, ko = ClpP knockout, – = no zone of inhibition, *= hazy (zone of inhibition)

Table 13: Disk Assays

Analogs that displayed activity are outlined in Table 13 and representative illustrations are shown in Figure 11. Interestingly, analogs that displayed proteolytic activity with isolated *E. coli* ClpP did not show any activity against *E. coli* in the disk assays. This is likely attributable to the multidrug efflux pumps that *E. coli* is known to possess.[8] Bactericidal activity is determined by defined solid zones of clearing as seen in the plate for ADEP1 (Figure 11). Our lead ACP4 and 5 analogs, compounds **37** and **38** respectively, demonstrated bacteriostatic activity against *N. meningitidis*, conveyed by the hazy zone of clearings (*c.f.* Figure 11). Compound **38** (ACP4) also inhibited *S. aureus*. Lead analog **39** showed bacteriostatic activity against *N. meningitidis*. Surprisingly, analog **33**, which showed no activity as judged by its RD_{25} measurement, was able to inhibit *N. meningitidis*. ClpP knockout studies used bacteria with inactive ClpP. Disk assays

using this type of bacteria can determine whether analogs are selectively targeting ClpP (by the absence of a positive test) or if antibiotic mechanisms other than ClpP activation are at play (by the appearance of zones of clearing). ACP4 and analog **39** showed positive results for only wildtype *N. meningitidis* bacteria, signifying that they are selective for ClpP. In contrast, the other analogs inhibited bacteria in both wildtype and ClpP knockout bacteria, indicating that other mechanisms are present. The reason for this difference in behaviour of such apparently similar structures is not fully understood.



Analog **38** vs. *N. meningitidis*

Analog **33** vs. *N. meningitidis*

S. aureus

Figure 11: Representative Disk Assays

2.9 Conclusion

The ability of ADEPs to activate ClpP towards deregulated proteolysis provided us with an attractive target for developing new antibiotics with a novel mode of action. In collaboration with the Houry group, five ACP compounds were identified as antibiotic leads. A series of ACP3 and ACP4/5 analogs was synthesized. The compounds based on the ACP3 core displayed minimal *in vitro* activity against bacterial ClpP. However, ACP3 analog, **18** from this family displayed potent activity against human ClpP, which may lead to the development of anticancer agents. Of the ACP4/5 analogs, compound **39** was the most promising, showing increased activity towards ClpP relative to initial hits. Moreover, disk assay studies on **39** demonstrated its bacteriostatic activity against *N.meningitidis*. At this point, an X-ray co-crystal structure of ClpP with either compound **39** or another synthetic analog is required for a more rational approach. The lack of a structural model has hampered our effects to design more active analogs, or understand the molecular bases for ClpP activation of either the ACP3 or ACP4/5 families.

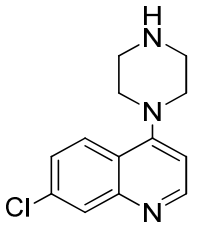
Experimental

3.1 General Experimental

CH_2Cl_2 was distilled from CaH_2 under argon. All other solvents and commercial reagents were used as received (Aldrich and Alfa Aesar). All glassware was oven dried and cooled under a stream of nitrogen, or flame dried under a stream of dry nitrogen. Melting points were obtained with Fisher-Johns melting point apparatus and are uncorrected. Infrared (IR) spectra were obtained on a Perkin-Elmer Spectrum 100 instrument equipped with a single-reflection diamond / ZnSe ATR accessory, either in the solid state or as neat liquids, as indicated and are reported in cm^{-1}. LRMS and HRMS were obtained on a Waters GCT Premier Time of Flight (ToF) mass spectrometer (EI) or an AB/Sciex QStar mass spectrometer (ESI). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Varian Unity 400 MHz spectrometer. Data for ^{1}H-NMR are referenced relative to residual $CDCl_3$ proton signals at δ 7.26 ppm and to DMSO-d_6 δ at 2.50 ppm. Data for ^{13}C-NMR are referenced relative to $CDCl_3$ at δ 77.16 ppm and to DMSO-d_6 δ at 39.51 ppm. Data for ^{1}H are reported as follows: chemical shift (ppm), integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, h =heptet, m = multiplet, dd = doublet of doublets, ddd = doublet of doublet of doublets, td = triplet of doublets), and coupling constants. Flash chromatography on silica gel (60 Å, 230 – 400 mesh, obtained from Silicycle® Inc.). Analytical thin-layer chromatography (TLC) was performed on precoated silica gel plates (SiliaPlate™Aluminium with F245 indicator purchased from Silicycle® Inc.).

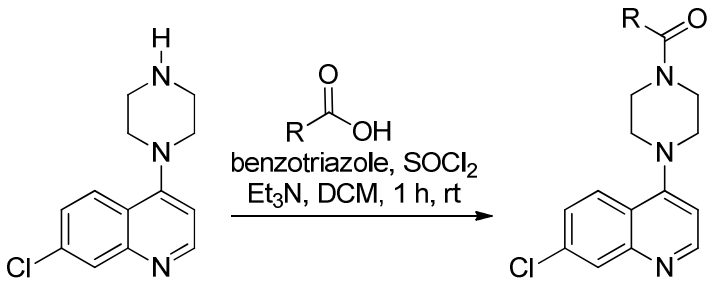
3.2 Synthesis of ACP3 Analogs

7-Chloro-4-(piperazin-1-yl)quinoline (1):



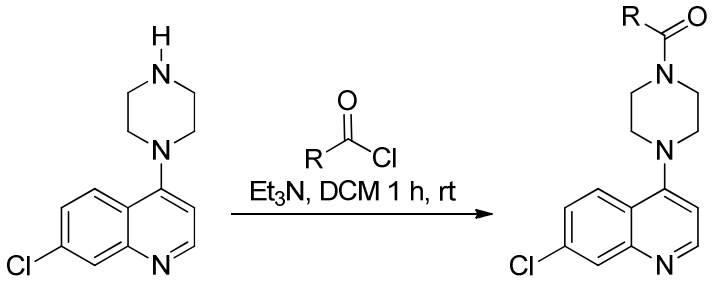
The title compound was prepared according to a modified literature procedure.[22] A mixture of piperazine (10.9 g, 127 mmol), potassium carbonate (1.05 g, 7.58 mmol), triethylamine (5.28 mL, 37.9 mmol) and 4,7-dichloroquinoline (5.00 g, 25.3 mmol) in *N*-methyl-2-pyrrolidinone (20.0 mL) under nitrogen was stirred at 135 °C for 2 h. After cooling to room temperature, the mixture was diluted with CH_2Cl_2 (200 mL). The organic layer was then washed with brine (2 × 50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting oil was purified by column chromatography on silica gel using CH_2Cl_2/MeOH (4:1 v/v) as the eluent to afford the desired product as a yellow solid (5.60 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (1H, d, *J* = 5.0 Hz), 8.04 (1H, d, *J* = 2.0 Hz), 7.96 (1H, d, *J* = 9.0 Hz), 7.42 (1H, dd, *J* = 9.0, 2.0 Hz), 6.83 (1H, d, *J* = 5.0 Hz), 3.21–2.85 (8H, m), 1.83 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.5, 152.1, 150.3, 135.0, 129.0, 126.2, 125.4, 122.1, 109.1, 53.7, 46.3.

General Procedure A



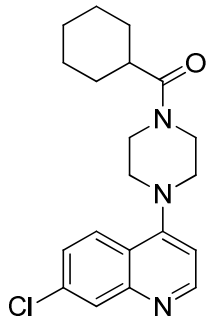
A stock solution (1.5 M) was prepared by making up a volume of thionyl chloride (5.5 mL, 0.075 mol) and benzotriazole (8.9 g, 0.075 mol) with dry CH_2Cl_2 up to 50 mL and transferred to a sealed 100 mL round-bottom flask stored under argon. The reaction was carried out by adding the stock solution (0.39 mmol) intermittently via syringe to a stirred solution of the corresponding carboxylic acid (0.36 mmol) in dry CH_2Cl_2 (7 mL). Before the addition was complete, benzotriazole hydrochloride started precipitating. The reaction mixture was stirred further for 5-10 min. The resulting liquid was cannulated to a second reaction vessel with a solution of 7-chloro-4-(piperazin-1-yl)quinoline (0.30 mmol) and triethylamine (0.38 mmol) in dry CH_2Cl_2 (5 mL) and stirred at ambient temperature for 1 h. The mixture was diluted with CH_2Cl_2 (5 mL). The organic layer washed with 1 N NaOH (5 mL), H_2O (5 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel using 2-10% MeOH in EtOAc as the eluent to afford the coupled product.

General Procedure B



To a stirred solution of 7-chloro-4-(piperazin-1-yl)quinoline (0.3 mmol) and triethylamine (0.45mmol) in dry CH_2Cl_2 (1 mL) was added the corresponding acyl chloride (0.33 mmol) intermittently. The reaction mixture was stirred further at ambient temperature for 2 h. The mixture was diluted with CH_2Cl_2 (10 mL). The organic layer was then washed with 1 N NaOH (2 x 5 mL), H_2O (5 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel using 2-10% MeOH in EtOAc as the eluent to afford the coupled product.

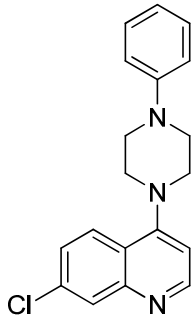
(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)(cyclohexyl)methanone (4):[46]



The title compound was prepared according to General Procedure A in 77% yield. White solid, mp = 161-163 ºC (MeOH/EtOAc); R*f* = 0.32 (2% MeOH/EtOAc); IR (solid): υ 2922, 2858, 2840, 1637, 1579, 1441, 1420, 1245, 1212, 1127, 1008, 881, 863, 823 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.76 (1H, d, *J* = 5.0 Hz), 8.07 (1H, d, *J* = 2.0 Hz), 7.97 (1H, d, *J* = 9.0 Hz), 7.47 (1H, dd, *J* = 9.0, 2.0 Hz), 6.85 (1H, d, *J* = 5.0 Hz), 3.95–3.76 (4H, m), 3.20 (4H, s), 2.54 (1H, tt, *J* = 11.5, 3.5 Hz), 1.95–1.50 (7H, m), 1.38–1.24 (3H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.0, 156.6, 152.1, 150.4, 135.3, 129.3, 126.7, 125.0, 122.0, 109.4, 45.6, 41.7, 40.7, 29.6, 26.1, 26.0;

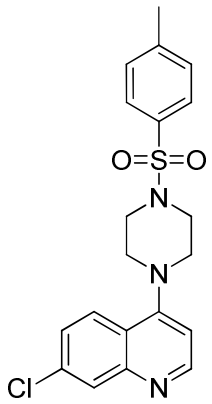
m/e (rel intensity) 358 (100), 248 (2); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{20}H_{24}ClN_3O$) calcd 358.1686, found 358.1692.

7-Chloro-4-(4-phenylpiperazin-1-yl)quinoline (5):



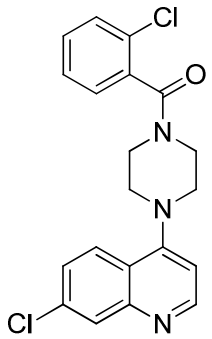
To a stirred solution of 1-phenylpiperazine (0.90 g, 5.5 mmol) and DMSO (2 mL) in a 5 mL microwave reaction vessel equipped with a magnetic stirrer was added 4,7-dichloroquinoline (0.50 g, 2.5 mmol) and *N*-methylmorpholine (0.28 mL, 2.5 mmol). The microwave vessel was sealed, placed in a microwave reactor, and stirred at 180 °C for 18 min under an atmosphere of argon. The reaction was cooled to 0 °C and diluted with diethyl ether (5 mL). The resulting sediment was filtered and purified by column chromatography on silica gel using 8% MeOH/ CH_2Cl_2 as the eluent to afford a yellow solid (0.82 g, 72%), mp = 170-172 ºC (MeOH/EtOAc); R*f* = 0.74 (8% MeOH/CH_2Cl_2); IR (solid): υ 3050, 2842, 1599, 1574, 1562, 1494, 1450, 1424, 1379, 1228, 1016, 939, 867, 823, 765 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.77 (1H, d, *J* = 5.0 Hz), 8.08 (1H, d, *J* = 2.0 Hz), 8.02 (1H, d, *J* = 9.0 Hz), 7.46 (1H, dd, *J* = 9.0, 2.0 Hz), 7.34 (2H, dd, *J* = 9.0, 7.0 Hz), 7.03 (2H, dd, *J* = 9.0, 1.0 Hz), 6.94 (1H, t, *J* = 7.0 Hz), 6.91 (1H, d, *J* = 5.0 Hz), 3.51–3.44 (4H, m), 3.42–3.31 (4H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.0, 152.2, 151.2, 150.4, 135.2, 129.4, 129.2, 126.5, 125.2, 122.1, 120.5, 116.5, 109.3, 52.4, 49.5; *m/e* (rel intensity) 340 (2), 324 (100); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{19}H_{18}ClN_3$) calcd 324.1267, found 324.1264.

7-Chloro-4-(4-tosylpiperazin-1-yl)quinoline (6):



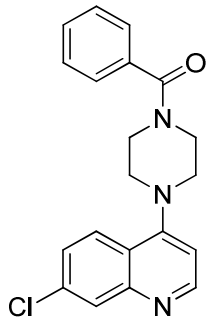
A stirred solution of 1-tosylpiperazine (0.20 g, 0.83 mmol) and DMSO (0.5 mL) in a 2 mL microwave reaction vessel equipped with a magnetic stirrer was added 4,7-dichloroquinoline (75 mg, 0.38 mmol) and *N*-methylmorpholine (42 μL, 0.38 mmol). The microwave vessel was sealed, placed in a microwave reactor, and stirred at 180 °C for 18 min under an atmosphere of argon. The reaction was cooled to 0 °C and diluted with diethyl ether (2 mL). The resulting sediment was filtered and purified by column chromatography on silica gel using 100% EtOAc as the eluent to afford a yellow solid (0.14 g, 90%), mp = 158-160 ºC (MeOH/EtOAc) (MeOH/EtOAc); R*f* = 0.45 (100% EtOAc); IR (solid): υ 2906, 2826, 1598, 1573, 1576, 1563, 1423, 1381, 1346, 1328, 1265, 1164, 1016, 939, 868, 821, 733 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 (1H, d, *J* = 5.0 Hz), 8.05 (1H, d, *J* = 2.0 Hz), 7.76 (3H, dd, *J* = 13.5, 8.5 Hz), 7.42–7.34 (3H, m), 6.85 (1H, d, *J* = 5.0 Hz), 3.30 (8H, s), 2.49 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.3, 152.1, 150.3, 144.3, 135.3, 132.7, 130.1, 129.2, 128.0, 126.7, 124.7, 121.8, 109.6, 51.7, 46.2, 21.8; *m/e* (rel intensity) 418 (4), 402 (100), 246 (4), 210 (2), 157 (3); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{20}H_{20}ClN_3O_2S$) calcd 402.1043, found 402.1050.

(2-Chlorophenyl)(4-(7-chloroquinolin-4-yl)piperazin-1-yl)methanone (7):



The title compound was prepared according to General Procedure B in 78% yield. Brown solid, mp = 174-176 ºC (MeOH/EtOAc); R*f* = 0.29 (2% MeOH/EtOAc); IR (solid): υ 2982, 2911, 2829, 1635, 1574, 1421, 1284, 1246, 1006, 864, 822, 767, 742 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (1H, d, *J* = 5.0 Hz), 8.08 (1H, d, *J* = 2.0 Hz), 7.95 (1H, d, *J* = 9.0 Hz), 7.48–7.42 (2H, m), 7.41–7.35 (3H, m), 6.87 (1H, d, *J* = 5.0 Hz), 4.29–3.84 (2H, m), 3.68–3.41 (2H, m), 3.32 (2H, t, *J* = 5.0 Hz), 3.19 (2H m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.3, 156.5, 152.1, 150.2, 135.6, 135.4, 130.7, 130.5, 129.9, 129.2, 128.0, 127.5, 126.8, 124.8, 122.0, 109.6, 52.6, 52.2, 46.9, 41.8; *m/e* (rel intensity) 404 (1), 402 (4), 386 (100), 225 (5), 179 (2), 157 (9); HRMS (ESI) *m/e* $[M + H]^+$ for $(C_{20}H_{17}Cl_2N_3O)$ calcd 386.0827, found 386.0827.

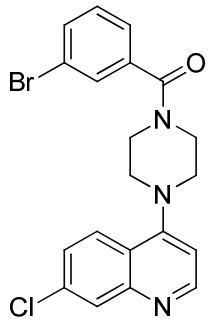
(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)(phenyl)methanone (8):[47]



The title compound was prepared according to General Procedure A in 63% yield. Orange solid, mp = 134-136 ºC (MeOH/EtOAc); R*f* = 0.28 (8% MeOH/CH_2Cl_2); IR (solid): υ 2990, 2858, 2837, 1633, 1573, 1422, 1379, 1367, 1278, 1249, 1009, 864, 814, 703 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (1H, d, *J* = 5.0 Hz), 8.07 (1H, d, *J* = 2.0 Hz), 7.96 (1H, d, *J* = 9.0 Hz), 7.62–7.42 (6H, m), 6.86 (1H, d, *J* = 5.0 Hz), 4.23–3.64 (4H, m), 3.24 (4H, s); ^{13}C NMR (100 MHz, $CDCl_3$)

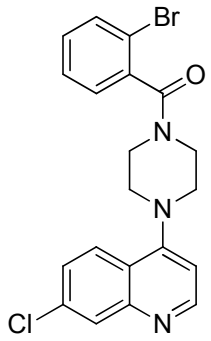
δ 170.8, 156.6, 152.1, 150.3, 135.5, 135.4, 130.2, 129.2, 128.8, 127.3, 126.8, 124.2, 122.3, 109.8, 52.4; *m/e* (rel intensity) 419 (2), 352 (100), 283 (4), 233 (5), 203 (3), 191 (4); HRMS (ESI) *m/e* $[M + H]^+$ for $(C_{20}H_{18}ClN_3O)$ calcd 352.1216, found 352.1215.

(3-Bromophenyl)(4-(7-chloroquinolin-4-yl)piperazin-1-yl)methanone (9):



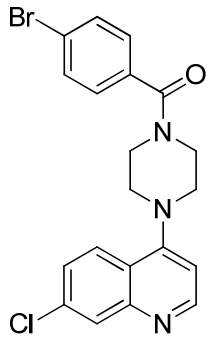
The title compound was prepared according to General Procedure B in 99% yield. Yellow solid, mp = 85-87 ºC (MeOH/EtOAc); R*f* = 0.34 (2% MeOH/EtOAc); IR (solid): υ 3063, 2989, 2829, 1631, 1563, 1421, 1280, 1246, 1006, 865, 822, 797, 735 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.77 (1H, d, *J* = 5.0 Hz), 8.09 (1H, d, *J* = 2.0 Hz), 7.95 (1H, d, *J* = 9.0 Hz), 7.67–7.54 (2H, m), 7.47 (1H, dd, *J* = 9.0, 2.0 Hz), 7.40 (1H, ddd, *J* = 7.5, 1.5, 1.5 Hz), 7.33 (1H, dd, *J* = 7.5, 7.5 Hz), 6.87 (1H, d, *J* = 5.0 Hz), 3.91 (4H, m), 3.24 (4H, br s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.0, 156.5, 152.1, 150.3, 137.4, 135.4, 133.3, 130.4, 130.3, 129.2, 126.9, 125.8, 124.8, 123.0, 122.0, 109.6, 52.6; *m/e* (rel intensity) 448 (1), 432 (100), 430 (78); HRMS (ESI) *m/e* $[M + H]^+$ for $(C_{20}H_{17}BrClN_3O)$ calcd 430.0322, found 430.0324.

(2-Bromophenyl)(4-(7-chloroquinolin-4-yl)piperazin-1-yl)methanone (10):



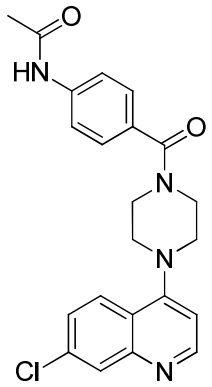
The title compound was prepared according to General Procedure B in 99%, yield. Yellow oil; R*f* = 0.34 (2% MeOH/EtOAc); IR (oil): υ 3048, 2919, 2849, 1634, 1574, 1421, 1379, 1284, 1246, 1006, 865, 823, 797, 732 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 (1H, d, *J* = 5.0 Hz), 8.06 (1H, t, *J* = 2.0 Hz), 7.94 (1H, dd, *J* = 9.0, 2.0 Hz), 7.61 (1H, d, *J* = 8.0 Hz), 7.49–7.36 (2H, m), 7.34–7.28 (2H, m), 6.86 (1H, dd, *J* = 5.0, 2.0 Hz), 4.24–3.93 (2H, m), 3.65–3.41 (2H, m), 3.40–3.17 (3H, m), 3.18–3.04 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.0, 156.4, 152.1, 150.3, 137.8, 135.3, 133.1, 130.7, 129.3, 128.0, 127.9, 126.8, 124.8, 121.9, 119.3, 109.5, 52.5, 52.1, 46.9, 41.7; *m/e* (rel intensity) 448 (1), 432 (100), 430 (78); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{20}H_{17}BrClN_3O$) calcd 430.0322, found 430.0325.

(4-Bromophenyl)(4-(7-chloroquinolin-4-yl)piperazin-1-yl)methanone (11):



The title compound was prepared according to General Procedure B in 86% yield. White solid, mp = 80-82 ºC (MeOH/EtOAc); R*f* = 0.34 (2% MeOH/EtOAc); IR (solid): υ 2967, 2865, 2829, 1635, 1564, 1421, 1248, 1006, 864, 819, 751 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (1H, d, *J* = 5.0 Hz), 8.08 (1H, d, *J* = 2.0 Hz), 7.95 (1H, d, *J* = 9.0 Hz), 7.62–7.57 (2H, m), 7.47 (1H, dd, *J* = 9.0, 2.0 Hz), 7.38–7.33 (2H, m), 6.86 (1H, d, *J* = 5.0 Hz), 4.16–3.60 (4H, m), 3.23 (4H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.7, 156.4, 152.1, 150.4, 135.4, 134.3, 132.1, 129.3, 129.0, 126.9, 124.8, 124.6, 122.0, 109.6, 52.4, 52.3; *m/e* (rel intensity) 432 (100), 430 (78); HRMS (ESI) *m/e* $[M + H]^+$ for $(C_{20}H_{17}BrClN_3O)$ calcd 430.0322, found 430.0331.

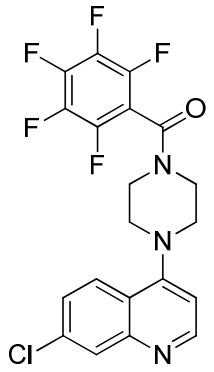
N-(4-(4-(7-Chloroquinolin-4-yl)piperazine-1-carbonyl)phenyl)acetamide (12):



The title compound was prepared according to General Procedure A in 81% yield. Pink solid, mp = 118-120 ºC (MeOH/EtOAc); R*f* = 0.41 (1 MeOH: 9 CH_2Cl_2: 0.1 Et_3N); IR (solid): υ 3570, 3228, 1698, 1626, 1526, 1313, 1249, 1010, 849, 747 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (1H, d, *J* = 5.0 Hz), 8.08 (1H, d, *J* = 2.0 Hz), 7.96 (1H, d, *J* = 9.0 Hz), 7.62 (1H, s), 7.57 (2H, d, *J* = 8.5 Hz), 7.47 (1H, dd, *J* = 9.0, 2.0 Hz), 7.45–7.41 (2H, m), 6.87 (1H, d, *J* = 5.0 Hz), 3.92

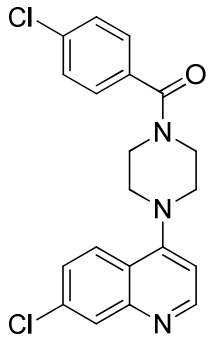
(4H, s), 3.23 (4H, s), 2.21 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 168.8, 156.4, 151.9, 150.0, 139.9, 135.2, 130.4, 128.9, 128.2, 126.7, 124.7, 121.7, 119.7, 109.6, 52.3, 24.8; *m/e* (rel intensity) 423 (4), 409 (100), 248 (9), 246 (2), 196 (2), 180 (10); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{22}H_{21}ClN_4O_2$) calcd 409.1431, found 409.1426.

(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)(perfluorophenyl)methanone (13):



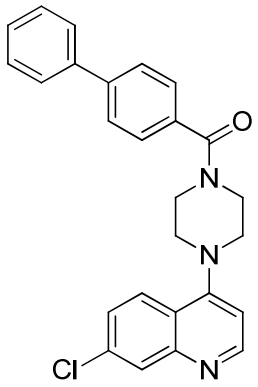
The title compound was prepared according to General Procedure A in 67%, yield. White solid, mp = 164-166 ºC (MeOH/EtOAc); R*f* = 0.48 (100% EtOAc); IR (solid): υ 3063, 2918, 1646, 1580, 1498, 1460, 1422, 1236, 1090, 989, 942, 916, 906, 813, 799 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.78 (1H, d, *J* = 5.0 Hz), 8.09 (1H, d, *J* = 2.0 Hz), 7.95 (1H, d, *J* = 9.0 Hz), 7.48 (1H, dd, *J* = 9.0, 2.0 Hz), 6.88 (1H, d, *J* = 5.0 Hz), 4.13 (2H, dd, *J* = 4.5, 4.5 Hz), 3.65 (2H, dd, *J* = 4.5, 4.5 Hz), 3.32 (2H, dd, *J* = 5.0, 5.0 Hz), 3.22 (2H, dd, *J* = 5.0, 5.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.2, 156.0, 152.0, 150.3, 144.3 (m, Pf), 143.5 (m, Pf), 141.7 (m, Pf), 140.9 (m, Pf), 139.1 (m, Pf), 136.6 (m, Pf), 135.4, 131.9 (m, Pf), 129.3, 127.0, 125.3 (m, Pf), 124.6, 121.9, 109.7, 104.9, 52.5, 52.1, 47.1, 42.5; *m/e* (rel intensity) 442 (100), 284 (7); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{20}H_{13}ClF_5N_3O$) calcd 442.0745, found 442.0752.

(4-Chlorophenyl)(4-(7-chloroquinolin-4-yl)piperazin-1-yl)methanone (14):[47]



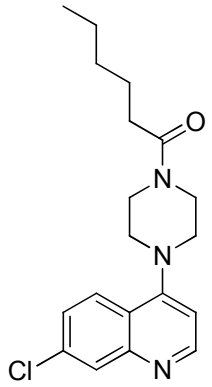
The title compound was prepared according to a General Procedure B in 95% yield. Yellow solid, mp = 167-169 ºC (MeOH/EtOAc); R*f* = 0.22 (2% MeOH/EtOAc); IR (solid): υ 2920, 2866, 2830, 1645, 1573, 1418, 1247, 1008, 865, 829, 755 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (1H, d, *J* = 5.0 Hz), 8.10 (1H, d, *J* = 2.0 Hz), 7.96 (1H, d, *J* = 9.0 Hz), 7.48 (1H, dd, *J* = 9.0, 2.0 Hz), 7.45–7.39 (4H, m), 6.87 (1H, d, *J* = 5.0 Hz), 4.15–3.59 (4H, br m), 3.24 (4H, br s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.7, 156.6, 152.0, 150.1, 136.4, 135.5, 133.8, 131.5, 129.1, 128.9, 126.9, 124.8, 121.9, 109.5, 52.4; *m/e* (rel intensity) 391 (2), 386 (100), 371 (10), 225 (2), 179 (1); HRMS (ESI) *m/e* $[M + H]^+$ for $(C_{20}H_{17}Cl_2N_3O)$ calcd 386.0827, found 386.0827.

[1,1'-Biphenyl]-4-yl(4-(7-chloroquinolin-4-yl)piperazin-1-yl)methanone (15):



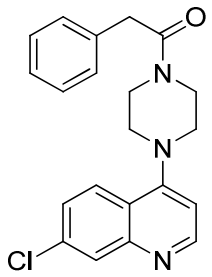
The title compound was prepared according to General Procedure A in 82%, yield. White solid, mp = 168-170 ºC (MeOH/EtOAc); R*f* = 0.33 (2% MeOH/EtOAc); IR (solid): υ 3054, 3827, 1636, 1565, 1435, 1423, 1277, 1008, 862, 842, 814, 744 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (1H, d, *J* = 5.0 Hz), 8.08 (1H, d, *J* = 2.0 Hz), 7.98 (1H, d, *J* = 9.0 Hz), 7.72–7.64 (2H, m), 7.65–7.57 (2H, m), 7.60–7.52 (2H, m), 7.52–7.43 (3H, m), 7.45–7.35 (1H, m), 6.88 (1H, d, *J* = 5.0 Hz), 4.23–3.69 (4H, m), 3.26 (4H, br s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 156.8, 152.5, 150.7, 143.5, 140.6, 135.7, 134.5, 129.6, 129.4, 128.4, 128.2, 127.8, 127.6, 127.1, 125.2, 122.3, 109.9, 52.9, 52.8.; *m/e* (rel intensity) 444 (2), 428 (100), 352 (1), 267 (1); HRMS (ESI) *m/e* $[M + H]^+$ for $(C_{26}H_{22}ClN_3O)$ calcd 428.1529, found 428.1523.

1-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)hexan-1-one (16):



The title compound was prepared according to General Procedure A in 64% yield. White solid, mp = 94-96 ºC (MeOH/EtOAc); R*f* = 0.27 (3% MeOH/EtOAc); IR (solid): υ 2930, 2870, 1645, 1572, 1422, 1245, 1196, 1014, 863, 830, 719 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (1H, d, *J* = 5.0 Hz), 8.08 (1H, d, *J* = 2.0 Hz), 7.97 (1H, d, *J* = 9.0 Hz), 7.47 (1H, dd, *J* = 9.0, 2.0 Hz), 6.85 (1H, d, *J* = 5.0 Hz), 3.97–3.87 (2H, m), 3.81–3.71 (2H, m), 3.26–3.11 (4H, m), 2.40 (2H, t, *J* = 7.5 Hz), 1.77–1.59 (2H, m), 1.36 (4H, ddd, *J* = 7.0, 4.5, 3.0 Hz), 0.96–0.87 (3H, t, 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 156.4, 151.9, 150.1, 135.1, 129.0, 126.6, 124.7, 121.9, 109.3, 52.4, 52.1, 45.6, 41.4, 33.3, 31.6, 25.0, 22.5, 14.0; *m/e* (rel intensity) 362 (1), 346 (100); HRMS (ESI) *m/e* $[M + H]^+$ for $(C_{19}H_{24}ClN_3O)$ calcd 346.1686, found 346.1686.

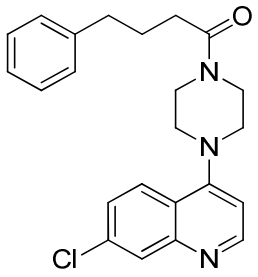
1-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-2-phenylethanone (17):



The title compound was prepared according to General Procedure A in 50% yield. Orange solid, mp = 144-146 ºC (MeOH/EtOAc); R*f* = 0.20 (2% MeOH/EtOAc); IR (solid): υ 2916, 2981, 2815, 1632, 1576, 1422, 1379, 1280, 1245, 1012, 868, 822, 727, 712 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 (1H, d, *J* = 5.0 Hz), 8.06 (1H, d, *J* = 2.0 Hz), 7.91 (1H, d, *J* = 9.0 Hz), 7.44 (1H, dd, *J* = 9.0, 2.0 Hz), 7.39–7.33 (2H, m), 7.32–7.27 (3H, m), 6.78 (1H, d, *J* = 5.0 Hz), 3.95 (2H,

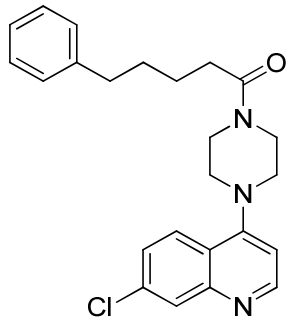
dd, *J* = 5.0, 5.0 Hz), 3.83 (2H, s), 3.73 (2H, dd, *J* = 5.0, 5.0 Hz), 3.17 (2H, dd, *J* = 5.0, 5.0 Hz), 3.00 (2H, dd, *J* = 5.0, 5.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 156.8, 152.4, 150.6, 135.6, 135.2, 129.4, 129.0, 127.5, 127.1, 125.2, 122.3, 109.8, 105.3, 52.7, 52.4, 46.6, 42.2, 41.7; *m/e* (rel intensity) 382 (1), 366 (100), 248 (2); HRMS (ESI) *m/e* $[M + H]^+$ for $(C_{21}H_{20}ClN_3O)$ calcd 366.1373, found 366.1381.

1-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-4-phenylbutan-1-one (18):



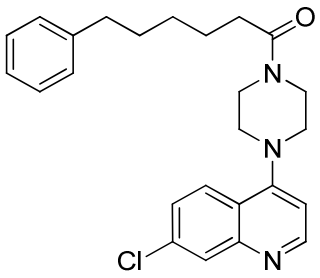
The title compound was prepared according to General Procedure A in 65% yield. Orange oil; R*f* = 0.24 (2% MeOH/EtOAc); IR (oil): υ 3024, 2920, 2854, 1635, 1574, 1421, 1379, 1245, 1230, 1012, 867, 822, 746, 698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 (1H, d, *J* = 5.0 Hz), 8.08 (1H, d, *J* = 2.0 Hz), 7.95 (1H, d, *J* = 9.0 Hz), 7.47 (1H, dd, *J* = 9.0, 2.0 Hz), 7.34–7.28 (2H, m), 7.25–7.18 (3H, m), 6.84 (1H, d, *J* = 5.0 Hz), 3.91 (2H, dd, *J* = 5.0, 5.0 Hz), 3.68 (2H, dd, *J* = 5.0, 5.0 Hz), 3.24–3.08 (4H, m), 2.73 (2H, t, *J* = 7.5 Hz), 2.40 (2H, t, *J* = 7.5 Hz), 2.04 (2H, tt, *J* = 7.5, 7.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 156.6, 152.0, 150.2, 141.7, 135.3, 129.1, 128.7, 128.6, 126.7, 126.2, 124.9, 122.0, 109.4, 52.5, 52.3, 45.6, 41.6, 35.46, 32.5, 26.7; *m/e* (rel intensity) 394 (100), 276 (2), 248 (2), 233 (2), 180 (1); HRMS (ESI) *m/e* $[M + H]^+$ for $(C_{23}H_{24}ClN_3O)$ calcd 394.1686, found 394.1686.

1-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-5-phenylpentan-1-one (19):



The title compound was prepared according to General Procedure A in 69% yield. Yellow oil; R*f* = 0.23 (2% MeOH/EtOAc); IR (oil): υ 3026, 2919, 2854, 1641, 1574, 1420, 1379, 1228, 1012, 867, 823, 745, 699 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.75 (1H, d, *J* = 5.0 Hz), 8.07 (1H, d, *J* = 2.0 Hz), 7.94 (1H, d, *J* = 9.0 Hz), 7.46 (1H, dd, *J* = 9.0, 2.0 Hz), 7.35–7.24 (2H, m), 7.22–7.12 (3H, m), 6.82 (1H, d, *J* = 5.0 Hz), 3.89 (2H, dd, *J* = 5.0, 5.0 Hz), 3.71 (2H, dd, *J* = 5.0, 5.0 Hz), 3.15 (4H, s), 2.67 (2H, t, *J* = 7.0 Hz), 2.41 (2H, t, *J* = 7.0 Hz), 1.82–1.60 (4H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 156.3, 151.1, 149.8, 142.0, 135.0, 128.7, 128.3, 128.2, 126.4, 125.6, 124.7, 121.6, 109.1, 52.1, 51.9, 45.4, 41.3, 35.5, 33.0, 30.9, 24.7; *m/e* (rel intensity) 424 (2), 408 (100); HRMS (ESI) *m/e* $[M + H]^+$ for $(C_{24}H_{26}ClN_3O)$ calcd 408.1843, found 408.1846.

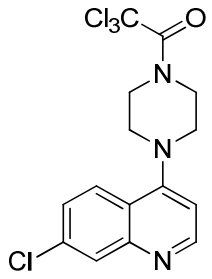
1-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-6-phenylhexan-1-one (20):



The title compound was prepared according to General Procedure A in 70% yield. Yellow oil; R*f* = 0.30 (2% MeOH/EtOAc); IR (oil): υ 3022, 2924, 2854, 1643, 1575, 1421, 1379, 1228, 1013, 868, 824, 734, 700 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.75 (1H, d, *J* = 5.0 Hz), 8.07 (1H, d, *J* = 2.0 Hz), 7.95 (1H, d, *J* = 9.0 Hz), 7.46 (1H, dd, *J* = 9.0, 2.0 Hz), 7.32–7.23 (2H, m), 7.23–7.10 (3H, m), 6.84 (1H, d, *J* = 5.0 Hz), 3.91 (2H, dd, *J* = 5.0, 5.0 Hz), 3.74 (2H, dd, *J* = 5.0, 5.0 Hz), 3.45–3.10 (4H, m), 2.64 (2H, t, *J* = 7.5 Hz), 2.39 (2H, t, *J* = 7.5 Hz), 1.77–1.63 (4H, m), 1.56–

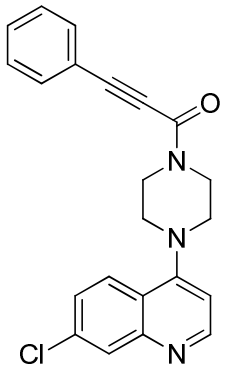
1.34 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 156.5, 152.1, 150.3, 142.6, 135.3, 129.2, 128.5, 128.4, 126.7, 125.8, 124.9, 122.0, 109.4, 52.5, 52.3, 45.7, 41.6, 35.9, 33.4, 31.4, 29.2, 25.3; *m/e* (rel intensity) 422 (100); HRMS (ESI) *m/e* $[M + H]^+$ for $(C_{25}H_{28}ClN_3O)$ calcd 422.2000, found 422.2009.

2,2,2-Trichloro-1-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)ethanone (21):



The title compound was prepared according to General Procedure B in 89% yield. Orange solid, mp = 159-161 ºC (MeOH/EtOAc); R*f* = 0.47 (2% MeOH/EtOAc); IR (solid): υ 2815, 1669, 1577, 1421, 1381, 1232, 1013, 941, 868, 821, 808, 773, 666 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (1H, d, *J* = 5.0 Hz), 8.09 (1H, d, *J* = 2.0 Hz), 7.96 (1H, d, *J* = 9.0 Hz), 7.48 (1H, dd, *J* = 9.0, 2.0 Hz), 6.88 (1H, d, *J* = 5.0 Hz), 4.14 (4H, s), 3.85–2.98 (4H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.7, 156.2, 151.9, 150.1, 135.6, 129.2, 127.0, 124.8, 121.8, 109.5, 92.9, 51.9; *m/e* (rel intensity) 410 (2), 394 (100), 392 (77), 179 (3); HRMS (ESI) *m/e* $[M + H]^+$ for $(C_{15}H_{13}Cl_4N_3O)$ calcd 391.9891, found 391.9895.

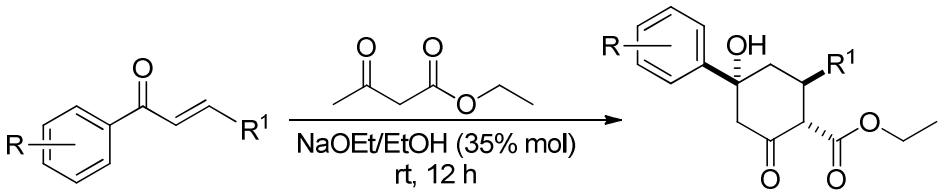
1-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-3-phenylprop-2-yn-1-one (22):



The title compound was prepared according to General Procedure A in 45% yield. Orange solid, mp = 76-78 ºC (MeOH/EtOAc); R*f* = 0.41 (2% MeOH/EtOAc); IR (solid): υ 3068, 2916, 2831, 2208, 1623, 1574, 1489, 1420, 1378, 1282, 1202, 1011, 867, 822, 755, 725, 688 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.78 (1H, d, *J* = 5.0 Hz), 8.09 (1H, d, *J* = 2.0 Hz), 7.98 (1H, d, *J* = 9.0 Hz), 7.58 (2H, dd, *J* = 8.0, 1.5 Hz), 7.49 (1H, dd, *J* = 9.0, 2.0 Hz), 7.46–7.41 (1H, m), 7.41–7.35 (2H, m), 6.88 (1H, d, *J* = 5.0 Hz), 4.15 (2H, dd, *J* = 5.0, 5.0 Hz), 4.00 (2H, dd, *J* = 5.0, 5.0 Hz), 3.31–3.24 (4H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.3, 153.3, 151.4, 150.1, 135.2, 132.4, 130.3, 129.1, 128.6, 126.7, 124.6, 121.8, 120.2, 109.4, 91.4, 80.76, 52.4, 51.8, 47.0, 41.5; *m/e* (rel intensity) 392 (2), 376 (100), 276 (1), 248 (2), 215 (2), 196 (1), 180 (1); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{22}H_{18}ClN_3O$) calcd 376.1217, found 376.1231.

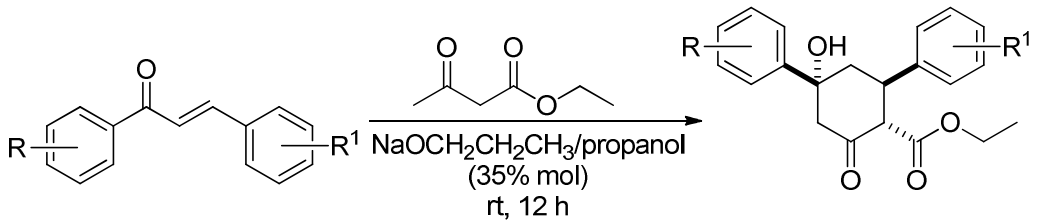
3.3 Synthesis of ACP4/5 Analogs

General Procedure C



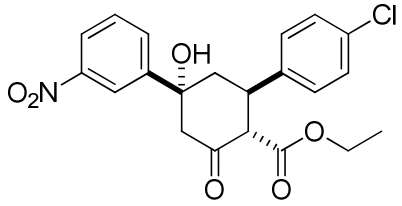
To a stirred solution of ethyl acetoacetate (1.2 mmol) and 21% sodium ethoxide/ethanol (0.14 mmol) in ethanol (1 mL) was added either dichlorovinyl chalcone **25** (0.40 mmol) or chalcones **54**, **57**, **61**, **65** (0.40 mmol) portionwise. The mixture was stirred overnight and then concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel using 15-30% EtOAc in hexane as the eluent to afford the coupled product.

General Procedure D



To a stirred solution of ethyl acetoacetate (1.2 mmol) in propanol (1 mL) was added a solution of sodium propylate (0.1 mmol in 1 mL of propanol). After stirring for 10 min, chalcone **29** (0.40 mmol) was added portionwise. The mixture was stirred overnight. The thickened reaction mass was diluted with hexane and neutralized with acetic acid. The resulting sediment was filtered off to afford the cyclohexanone product.

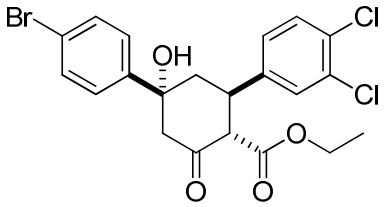
(1S*,2R*,4R*)-Ethyl 2-(4-chlorophenyl)-4-hydroxy-4-(3-nitrophenyl)-6-oxocyclohexanecarboxylate (31):



The title compound was prepared according to General Procedure D in 62% yield. Yellow solid, mp = 68-70 ºC (hexanes); R*f* = 0.25 (50% EtOAc/hexanes); IR (solid): υ 3475, 2972, 1711, 1678,

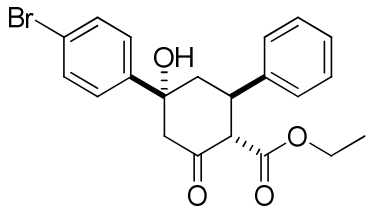
1525, 1422, 1347, 1276, 1232, 1013, 809, 739 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.40 (1H, dd, *J* = 2.0, 2.0 Hz), 8.13 (1H, ddd, *J* = 8.0, 2.5, 1.0 Hz), 8.00 (1H, ddd, *J* = 8.0, 2.0, 1.0 Hz), 7.72–7.60 (1H, m), 7.43–7.33 (2H, m), 7.33–7.12 (2H, m), 6.04 (1H, s), 4.20 (1H, d, *J* = 12.5 Hz), 4.03–3.87 (2H, m), 3.84–3.74 (1H, m), 3.35 (1H, d, *J* = 13.5 Hz), 2.65 (1H, dd, *J* = 13.0, 13.0 Hz), 2.44 (1H, dd, *J* = 13.5, 2.5 Hz), 1.88 (1H, ddd, *J* = 13.5, 3.5, 3.5 Hz), 1.01 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 204.4, 169.2, 150.6, 148.4, 141.9, 132.1, 132.0, 130.5, 130.0, 129.2, 122.6, 120.2, 75.9, 62.2, 60.7, 53.0, 44.7, 42.2, 14.6, *m/e* (rel intensity) 435 (41), 418 (76), 400 (100), 328 (13), 288 (2), 131 (4); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{21}H_{20}ClNO_6$) calcd 418.1057, found 418.1061.

(1S*,2R*,4R*)-Ethyl 4-(4-bromophenyl)-2-(3,4-dichlorophenyl)-4-hydroxy-6-oxocyclohexanecarboxylate (32):



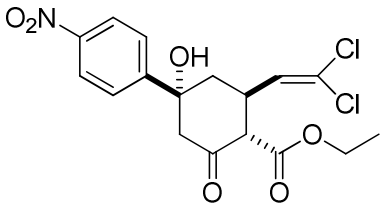
The title compound was prepared according to General Procedure D in 73% yield. White solid, mp = 68-70 ºC (hexanes); R*f* = 0.39 (50% EtOAc/hexanes); IR (solid): υ 3430, 2980, 1710, 1646, 1470, 1371, 1244, 1152, 1131, 1028, 1007, 819, 799 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.69–7.16 (7H, m), 5.76 (1H, s), 4.18 (1H, d, *J* = 12.5 Hz), 4.03–3.90 (2H, m), 3.84–3.67 (1H, m), 3.20 (1H, d, *J* = 13.5 Hz), 2.71–2.49 (1H, m), 2.40 (1H, dd, *J* = 13.5, 2.0 Hz), 1.87 (1H, dd, *J* = 13.5, 13.5 Hz), 1.00 (1H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 204.3, 169.2, 147.6, 144.3, 131.7, 131.6, 131.3, 130.1, 130.0, 128.7, 127.6, 120.7, 75.8, 61.9, 60.8, 53.3, 44.5, 42.0, 14.6; *m/e* (rel intensity) 489 (11), 487 (30), 469 (100), 397 (12), 356 (2); HRMS (ESI) *m/e* $[M + NH_4]^+$ for ($C_{21}H_{19}BrCl_2NO_4$) calcd 484.9922, found 484.9936.

(1S*,4R*,6R*)-Ethyl 4-(4-bromophenyl)-4-hydroxy-2-oxo-6-phenylcyclohexanecarboxylate (33):



The title compound was prepared according to General Procedure D in 54% yield. White solid, mp = 68-70 ºC (hexanes); R*f* = 0.33 (50% EtOAc/hexanes); IR (solid): υ 3380, 2943, 1744, 1730, 1706, 1488, 1373, 1261, 1234, 1154, 1029, 1005, 828, 753, 696 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ (400 MHz, DMSO-d_6) δ 7.62–7.45 (4H, m), 7.38–7.09 (5H, m), 5.74 (1H, s), 4.15 (1H, d, *J* = 12.5 Hz), 4.03–3.86 (2H, m), 3.83–3.68 (1H, m), 3.25 (1H, d, *J* = 13.5), 2.54 (1H, dd, *J* = 13.5, 13.5 Hz), 2.40 (1H, dd, *J* = 13.5, 2.5 Hz), 1.87 (1H, ddd, *J* = 13.5, 4.0, 2.5 Hz), 0.97 (1H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 204.3, 168.7, 147.2, 142.4, 130.8, 128.4, 127.3, 126.9, 126.7, 119.9, 75.1, 61.6, 59.8, 52.6, 44.6, 42.0, 13.8; *m/e* (rel intensity) 434 (11), 417 (30), 399 (100), 289 (1); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{21}H_{21}BrO_4$) calcd 417.0701, found 417.0713.

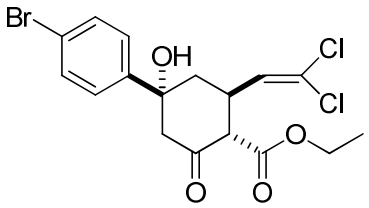
1S*,2R*,4R*)-Ethyl 2-(2,2-dichlorovinyl)-4-hydroxy-4-(4-nitrophenyl)-6-oxocyclohexanecarboxylate (37):



The title compound was prepared according to General Procedure C in 59% yield. Yellow solid, mp = 68- 70 ºC (EtOAc/hexanes); R*f* = 0.20 (25% EtOAc/hexanes); IR (solid): 3462, 2982, 1734, 1712, 1607, 1516, 1344, 1263, 1097, 890, 852, 699 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.24 (2H, d, *J* = 9.0 Hz), 7.76 (2H, d, *J* = 9.0 Hz), 6.11 (1H, d, *J* = 9.0 Hz), 6.05 (1H, s), 4.21–4.07 (2H, m), 3.81 (1H, d, *J* = 12.0 Hz), 3.70–3.59 (1H, m), 3.21 (1H, d, *J* = 13.0 Hz), 2.40 (1H, dd, *J* = 13.0, 2.0 Hz), 2.31 (1H, dd, *J* = 13.0, 13.0 Hz), 1.81–1.75 (1H, m), 1.22 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 202.5, 168.4, 154.5, 146.4, 131.1, 126.1, 123.3, 120.1, 75.1, 60.3, 59.7, 52.0, 40.8, 37.8, 14.0; *m/e* (rel intensity) 419 (41), 402 (100), 384 (45), 312 (4),

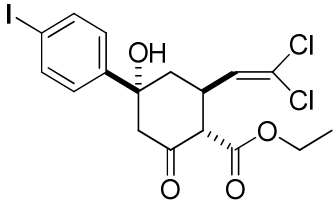
306 (7), 131 (4); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{17}H_{17}Cl_2NO_6$) calcd 402.0511, found 402.0510.

(1S*,2R*,4R*)-Ethyl 4-(4-bromophenyl)-2-(2,2-dichlorovinyl)-4-hydroxy-6-oxocyclohexanecarboxylate (38):



The title compound was prepared according to General Procedure C in 53% yield. Yellow solid, mp = 139- 141 ºC (EtOAc/hexanes); R*f* = 0.22 (15% EtOAc/hexanes); IR (solid): υ 3382, 1739, 1708, 1627, 1488, 1371, 1241, 1171, 1025, 1008, 891, 843, 817 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.55 (2H, d, *J* = 9.5 Hz), 7.76 (2H, d, *J* = 9.5 Hz), 6.09 (1H, d, *J* = 10.0 Hz), 5.79 (1H, s), 4.21-4.07 (2H, m), 3.76 (1H, d, *J* = 12.5 Hz), 3.67–3.55 (1H, m), 3.12 (1H, d, *J* = 13.5 Hz), 2.36 (1H, dd, *J* = 13.5, 2.0 Hz), 2.31 (1H, dd, *J* = 13.5, 13.5 Hz), 1.78–1.70 (1H, m), 1.20 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 202.9, 168.4, 146.7, 131.3, 130.9, 126.9, 120.1, 119.9, 74.8, 60.3, 59.7, 52.4, 40.4, 37.8, 14.0; *m/e* (rel intensity) 454 (29), 437 (49), 419 (100); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{17}H_{17}BrCl_2O_4$) calcd 434.9765, found 434.9759.

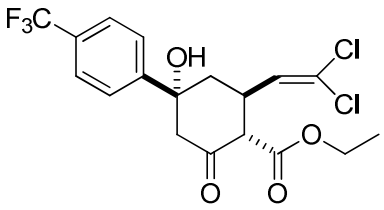
(1S*,2R*,4R*)-Ethyl 2-(2,2-dichlorovinyl)-4-hydroxy-4-(4-iodophenyl)-6-oxocyclohexanecarboxylate (39):



The title compound was prepared according to General Procedure C in 62% yield. Yellow solid, mp = 135-137 ºC (EtOAc/hexanes); R*f* = 0.18 (15% EtOAc/hexanes); IR (solid): υ 3462, 2979, 2930, 1724, 1708, 1615, 1468, 1369, 1262, 1186, 1095, 1025, 1004, 889, 821 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.72 (2H, d, *J* = 8.5 Hz), 7.28 (2H, d, *J* = 8.5 Hz), 6.08 (1H, d, *J* = 10.0 Hz), 5.76 (1H, s), 4.20–4.04 (2H, m), 3.76 (1H, d, *J* = 12.0 Hz), 3.66–3.52 (1H, m), 3.10 (1H, d, *J* = 13.5 Hz), 2.35 (1H, dd, *J* = 13.5, 2.5 Hz), 2.21 (1H, dd, *J* = 13.0, 13.0 Hz), 1.80–1.65 (1H,

m), 1.20 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 203.0, 168.5, 147.2, 136.8, 131.4, 127.1, 119.9, 93.0, 74.9, 60.3, 59.7, 52.4, 40.4, 37.8, 14.0; *m/e* (rel intensity) 500 (37), 483 (34), 465 (100), 388 (5), 371 (7), 221 (1); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{17}H_{17}Cl_2IO_4$) calcd 482.9627, found 482.9631.

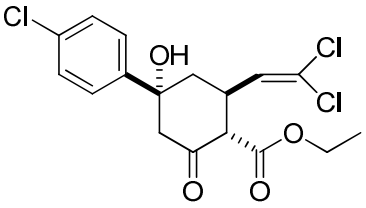
(1S*,2R*,4R*)-Ethyl 2-(2,2-dichlorovinyl)-4-hydroxy-6-oxo-4-(4-(trifluoromethyl)phenyl)cyclohexanecarboxylate (40):



The title compound was prepared according to General Procedure C in 52% yield.

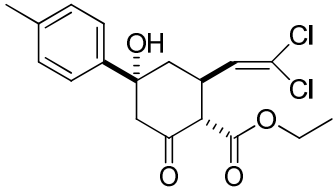
Yellow solid, mp = 120-122 ºC (EtOAc/hexanes); R*f* = 0.31 (15% EtOAc/hexanes); IR (solid): υ 3469, 1725, 1708, 1616, 1369, 1325, 1298, 1264, 1143, 1108, 1068, 1016, 890, 837 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.79–7.65 (4H, m), 6.10 (1H, d, *J* = 10.0 Hz), 5.93 (1H, s), 4.24–4.04 (2H, m), 3.80 (1H, d, *J* = 12.0 Hz), 3.70–3.56 (1H, m), 3.18 (1H, d, *J* = 13.5 Hz), 2.39 (1H, dd, *J* = 13.5, 2.0 Hz), 2.29 (1H, dd, *J* = 12.5, 12.5 Hz), 1.82–1.73 (1H, m), 1.20 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 202.8, 168.4, 151.7, 131.2, 127.6 (q, *J* = 31.5 Hz), 125.5, 125.0 (q, *J* = 3.5 Hz), 124.3 (q, *J* = 254.0 Hz), 120.0, 75.0, 60.3, 59.7, 52.3, 40.3, 37.9, 14.0; ^{19}F NMR (375 MHz, DMSO-d_6) δ -61.2; *m/e* (rel intensity) 442 (42), 425 (100), 407 (38), 329 (5); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{18}H_{17}Cl_2F_3O_4$) calcd 425.0534, found 25.05465.

(1S*,2R*,4R*)-Ethyl 4-(4-chlorophenyl)-2-(2,2-dichlorovinyl)-4-hydroxy-6-oxocyclohexanecarboxylate (41):[26]



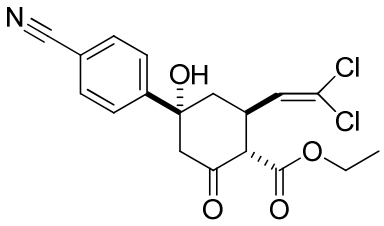
The title compound was prepared according to General Procedure C in 51% yield. White solid, mp = 140-142 ºC (EtOAc/hexanes); R*f* = 0.18 (15% EtOAc/hexanes); IR (solid): υ 3364, 1741, 1711, 1628, 1491, 1370, 1244, 1171, 1091, 1025, 1011, 892, 822 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.49 (2H, d, *J* = 8.0 Hz), 7.42 (2H, d, *J* = 8.0 Hz), 6.09 (1H, d, *J* = 10.0 Hz), 5.79 (1H, s), 4.19–4.08 (2H, m), 3.76 (1H, d, *J* = 12.0 Hz), 3.68–3.56 (1H, m), 3.12 (1H, d, *J* = 13.5 Hz), 2.36 (1H, dd, *J* = 13.5, 2.0 Hz), 2.22 (1H, dd, *J* = 12.5, 12.5 Hz), 1.79–1.71 (1H, m), 1.20 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 202.9, 168.4, 146.3, 131.5, 131.3, 128.0, 126.5, 119.9, 74.8, 60.3, 59.7, 52.5, 40.5, 37.8, 14.0; *m/e* (rel intensity) 426 (7), 408 (52), 391 (60), 373 (100), 240 (4), 195 (4), 151 (4); HRMS (ESI) *m/e* $[M + H]^+$ for $C_{17}H_{17}Cl_3O_4$) calcd 391.0271, found 391.0290.

(1S*,2R*,4R*)-Ethyl 2-(2,2-dichlorovinyl)-4-hydroxy-6-oxo-4-(p-tolyl)cyclohexanecarboxylate (42):[26]



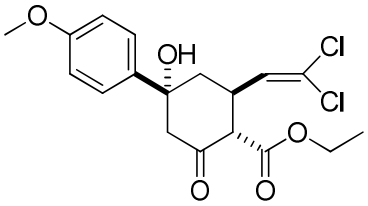
The title compound was prepared according to General Procedure C in 42% yield. White solid, mp = 150-152 ºC (EtOAc/hexanes); R*f* = 0.20 (25% EtOAc/hexanes); IR (solid): υ 3539, 3041, 2979, 1737, 1708, 1620, 1375, 1228, 1175, 1160, 1028, 889, 820 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.35 (2H, d, *J* = 8.0 Hz), 7.15 (2H, d, *J* = 8.0 Hz), 6.09 (1H, d, *J* = 9.5 Hz), 5.61 (1H, s), 4.25–4.03 (2H, m), 3.75 (1H, d, *J* = 12.0 Hz), 3.72–3.53 (1H, m), 3.09 (1H, d, *J* = 13.5 Hz), 2.35 (1H, dd, *J* = 13.5, 1.5 Hz), 2.28 (3H, s), 2.20 (1H, ddd, *J* = 13.5, 12.0, 1.5 Hz), 1.75 (1H, ddd, *J* = 13.5, 4.0, 2.5 Hz), 1.20 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 203.2, 168.5, 144.4, 135.8, 131.5, 128.5, 124.4, 119.7, 74.9, 60.2, 59.8, 52.8, 40.8, 37.9, 20.5, 14.0; *m/e* (rel intensity) 388 (32), 371 (32), 353 (100), 281 (2), 251 (1); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{18}H_{20}Cl_2O_4$) calcd 371.0817, found 371.0828.

(1S*,2R*,4R*)-Ethyl 4-(4-cyanophenyl)-2-(2,2-dichlorovinyl)-4-hydroxy-6-oxocyclohexanecarboxylate (43):



The title compound was prepared according to General Procedure C in 57% yield. White solid, mp = 143-145 ºC (EtOAc/hexanes); R*f* = 0.42 (30% EtOAc/hexanes); IR (solid): υ 3445, 3042, 3061, 2986, 2931, 1726, 1708, 1606, 1369, 1262, 1190, 1021, 881, 837 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.85 (2H, d, *J* = 8.5 Hz), 7.68 (2H, d, *J* = 8.5 Hz), 6.10 (1H, s), 5.96 (1H, s), 4.22–3.98 (2H, m), 3.78 (1H, d, *J* = 12.0 Hz), 3.69–3.56 (1H, m), 3.18 (1H, d, *J* = 14.0 Hz), 2.37 (1H, dd, *J* = 14.0, 2.5 Hz), 2.26 (1H, dd, *J* = 13.0, 13.0 Hz), 1.83–1.66 (1H, m), 1.20 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 202.7, 168.4, 152.5, 132.1, 131.2, 125.7, 120.0, 118.7, 109.8, 75.1, 60.3, 59.7, 52.0, 40.9, 37.8, 14.0; *m/e* (rel intensity) 399 (73), 382 (92), 364 (100), 292 (3), 221 (2), 131 (7); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{18}H_{17}BrCl_2NO_4$) calcd 382.0613, found 382.0623.

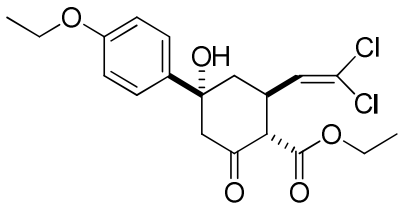
(1S*,2R*,4R*)-Ethyl 2-(2,2-dichlorovinyl)-4-hydroxy-4-(4-methoxyphenyl)-6-oxocyclohexanecarboxylate (44):



The title compound was prepared according to General Procedure C in 45% yield. Yellow solid, mp = 125-127 ºC (EtOAc/hexanes); R*f* = 0.12 (15% EtOAc/hexanes); IR (solid): υ 3462, 2979, 2935, 1725, 1708, 1615, 1486, 1368, 1262, 1186, 1095, 1025, 1004, 889, 821 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.38 (2H, d, *J* = 9.0 Hz), 6.90 (2H, d, *J* = 9.0 Hz), 6.09 (1H, d, *J* = 10.0 Hz), 5.58 (1H, s), 4.26–4.02 (2H, m), 3.88–3.71 (4H, m), 3.69–3.51 (1H, m), 3.08 (1H, d, *J* = 13.5 Hz), 2.36 (1H, dd, *J* = 13.5, 2.5 Hz), 2.25–2.05 (1H, m), 1.76 (1H, ddd, *J* = 13.5, 4.0, 2.5 Hz), 1.20 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 203.3, 168.6, 158.1, 139.5, 131.5, 125.7, 119.7, 113.3, 74.7, 60.2, 59.8, 55.0, 52.9, 40.9, 37.9, 14.0; *m/e* (rel intensity) 404

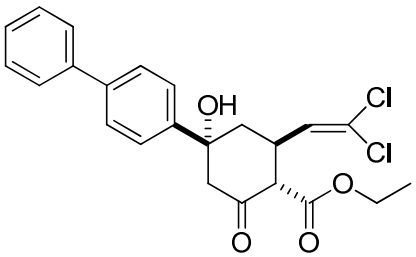
(8), 387 (30), 369 (100), 291 (1); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{18}H_{20}Cl_2O_5$) calcd 387.0766, found 387.0765.

(1S*,2R*,4R*)-Ethyl 2-(2,2-dichlorovinyl)-4-(4-ethoxyphenyl)-4-hydroxy-6-oxocyclohexanecarboxylate (45): [26]



The title compound was prepared according to General Procedure C in 62% yield. Yellow solid, mp = 113-115 ºC (EtOAc/hexanes); R*f* = 0.10 (15% EtOAc/hexanes); IR (solid): υ 3542, 1736, 1708, 1608, 1512, 1309, 1243, 1225, 1175, 1160, 1112, 1025, 890 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.36 (2H, d, *J* = 9.0 Hz), 6.89 (2H, d, *J* = 9.0 Hz), 6.09 (1H, d, *J* = 9.5 Hz), 5.57 (1H, s), 4.23–4.05 (2H, m), 4.01 (2H, q, *J* = 7.0 Hz), 3.74 (1H, d, *J* = 12.0 Hz), 3.67–3.55 (1H, m), 3.08 (1H, d, *J* = 14.0 Hz), 2.36 (1H, dd, *J* = 14.0, 2.0 Hz), 2.20 (1H, dd, *J* = 13.0, 13.0 Hz), 1.80–1.70 (1H, m), 1.32 (3H, t, *J* = 7.0 Hz), 1.20 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 203.3, 168.6, 157.3, 139.3, 131.5, 125.6, 119.7, 113.8, 74.7, 62.9, 60.2, 59.7, 52.9, 40.9, 37.9, 14.6, 14.0; *m/e* (rel intensity) 401 (93), 383 (100); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{19}H_{22}Cl_2O_5$) calcd 401.0922, found 401.0931.

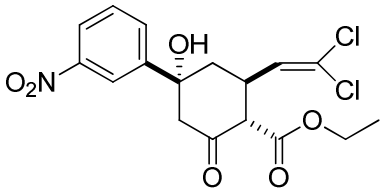
(1S*,2R*,4R*)-Ethyl 4-([1,1'-biphenyl]-4-yl)-2-(2,2-dichlorovinyl)-4-hydroxy-6-oxocyclohexanecarboxylate (46):



The title compound was prepared according to General Procedure C in 30% yield. Yellow solid, mp = 160-162 ºC (EtOAc/hexanes); R*f* = 0.42 (30% EtOAc/hexanes); IR (solid): υ 3543, 3041, 2985, 1743, 1712, 1625, 1375, 1230, 1173, 1162, 1114, 1030, 887, 768, 733, 696 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.71–7.62 (4H, m), 7.60–7.52 (2H, m), 7.50–7.42 (2H, m), 7.39–7.32

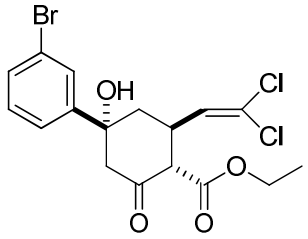
(1H, m), 6.11 (1H, d, *J* = 9.5 Hz), 5.75 (1H, s), 4.22–4.04 (2H, m), 3.79 (1H, d, *J* = 12.0 Hz), 3.67 (1H, ddd, *J* = 12.0, 10.0, 4.0 Hz), 3.17 (1H, d, *J* = 13.5 Hz), 2.42 (1H, dd, *J* = 13.5, 2.5 Hz), 2.34–2.23 (1H, dd, *J* = 13.5, 13.5 Hz), 1.81 (1H, ddd, *J* = 13.5, 4.0, 2.5 Hz), 1.21 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 203.2, 168.5, 146.5, 139.7, 138.7, 131.4, 128.8, 127.3, 126.6, 126.3, 125.1, 119.8, 75.0, 60.3, 59.8, 52.6, 40.7, 37.9, 14.0; *m/e* (rel intensity) 450 (16), 433 (28), 415 (100), 343 (1); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{23}H_{22}Cl_2O_4$) calcd 433.0973, found 433.0961.

(1S*,2R*,4R*)-Ethyl 2-(2,2-dichlorovinyl)-4-hydroxy-4-(3-nitrophenyl)-6-oxocyclohexanecarboxylate (47):



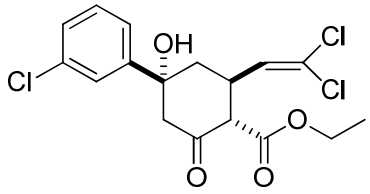
The title compound was prepared according to General Procedure C in 75% yield. Yellow solid, mp = 183-185 ºC (EtOAc/hexanes); R*f* = 0.20 (25% EtOAc/hexanes); IR (solid): υ 3372, 1739, 1710, 1628, 1522, 1348, 1246, 1173, 1025, 891, 868, 858, 811, 739 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (1H, dd, *J* = 2.0, 2.0 Hz), 8.15 (1H, ddd, *J* = 8.0, 2.0, 1.0 Hz), 7.95 (1H, ddd, *J* = 8.0, 2.0, 1.0 Hz), 7.69 (1H, dd, *J* = 8.0, 8.0 Hz), 6.10 (1H, d, *J* = 10.0 Hz), 6.07 (1H, s), 4.21–4.07 (2H, m), 3.81 (1H, d, *J* = 12.0 Hz), 3.69–3.58 (1H, m), 3.25 (1H, d, *J* = 14.0 Hz), 2.40 (1H, dd, *J* = 14.0, 2.5 Hz), 2.34 (1H, dd, *J* = 13.0, 13.0 Hz), 1.82–1.75 (1H, m), 1.20 (3H, t, *J* = 7.5 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 202.6, 149.5, 132.7, 131.4, 131.1, 129.8, 125.9, 122.0, 120.1, 119.5, 74.9, 60.3, 59.7, 52.2, 38.8, 37.8, 14.0; *m/e* (rel intensity) 419 (45), 402 (100), 384 (70), 312 (45), 306 (9); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{17}H_{17}Cl_2NO_6$) calcd 402.0511, found 402.0514.

(1S*,2R*,4R*)-Ethyl 4-(3-bromophenyl)-2-(2,2-dichlorovinyl)-4-hydroxy-6-oxocyclohexanecarboxylate (48):[26]



The title compound was prepared according to General Procedure C in 60% yield. White solid, mp = 139-141 ºC (EtOAc/hexanes); R*f* = 0.35 (30% EtOAc/hexanes); IR (solid): υ 3390, 2973, 2933, 1736, 1707, 1626, 1370, 1244, 1169, 1138, 1024, 890, 876, 846, 786, 711, 692 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.67 (1H, dd, *J* = 2.0, 2.0 Hz), 7.51–7.40 (2H, m), 7.33 (1H, dd, *J* = 8.0, 8.0 Hz), 6.08 (1H, d, *J* = 10.0 Hz), 5.84 (1H, s), 4.28–3.98 (2H, m), 3.76 (1H, d, *J* = 12.0 Hz), 3.67–3.55 (1H, m), 3.16 (1H, d, *J* = 14.0 Hz), 2.36 (1H, dd, *J* = 14.0, 2.5 Hz), 2.25 (1H, dd, *J* = 13.0, 13.0 Hz), 1.74 (1H, ddd, *J* = 13.0, 4.0, 2.5 Hz), 1.20 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 202.8, 168.4, 150.0, 131.3, 130.3, 129.7, 127.5, 123.7, 121.6, 119.9, 74.8, 60.3, 59.7, 52.4, 41.3. 37.9, 14.0; *m/e* (rel intensity) 454 (35), 421 (42), 419 (100), 339 (1), 306 (1), 221 (1), 131 (6); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{17}H_{17}BrCl_2O_4$) calcd 434.9766, found 434.9772.

(1S*,2R*,4R*)-Ethyl 4-(3-chlorophenyl)-2-(2,2-dichlorovinyl)-4-hydroxy-6-oxocyclohexanecarboxylate (49):

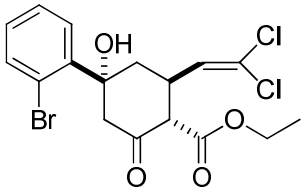


The title compound was prepared according to General Procedure C in 71% yield.

White solid, mp = 140-142 ºC (EtOAc/hexanes); R*f* = 0.17 (15% EtOAc/hexanes); IR (solid): υ 3392, 1738, 1710, 1624, 1371, 1243, 1167, 1137, 1025, 891, 846, 788 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.53 (1H, dd, *J* = 1.5, 1.5 Hz), 7.46–7.42 (1H, m), 7.40 (1H, dd, *J* = 8.0, 8.0 Hz), 7.35–7.30 (1H, m), 6.08 (1H, d, *J* = 10.0 Hz), 5.85 (1H, s), 4.22–4.04 (2H, m), 3.76 (1H, d, *J* = 11.5 Hz),) 3.67–3.54 (1H, m), 3.16 (1H, d, *J* = 14.0 Hz), 2.36 (1H, dd, *J* = 14.0, 2.0 Hz), 2.25 (1H, dd, *J* = 13.0, 13.0 Hz), 1.75 (1H, ddd, *J* = 13.0, 4.0, 2.0 Hz), 1.20 (3H, t, *J* = 7.0 Hz); ^{13}C

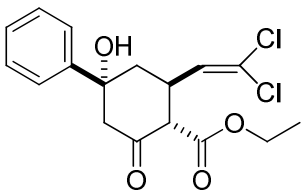
NMR (100 MHz, DMSO-d_6) δ 202.8, 168.4, 149.8, 132.9, 131.2, 130.0, 126.8, 124.6, 123.3, 119.9, 74.9, 60.3, 59.7, 52.4, 40.5, 37.9, 14.0; *m/e* (rel intensity) 426 (5), 408 (80), 391 (67), 373 (100), 240 (4), 196 (7), 179 (5); HRMS (ESI) *m/e* $[M + NH_4]^+$ for ($C_{17}H_{17}Cl_3O_4$) calcd 408.0536, found 408.0545.

(1S*,2R*,4R*)-Ethyl 4-(2-bromophenyl)-2-(2,2-dichlorovinyl)-4-hydroxy-6-oxocyclohexanecarboxylate (50):



The title compound was prepared according to General Procedure C in 41% yield. White solid, mp = 135-137 ºC (EtOAc/hexanes); R*f* = 0.25 (30% EtOAc/hexanes); IR (solid): υ 3470, 2981, 2931, 1711, 1651, 1371, 1273, 1237, 1017, 890, 756 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.70 (1H, dd, *J* = 8.0, 1.5 Hz), 7.63 (1H, dd, *J* = 8.0, 1.5 Hz), 7.41 (1H, ddd, *J* = 8.0, 7.5, 1.5 Hz), 7.22 (1H, ddd, *J* = 8.0, 7.5, 1.5 Hz), 6.20 (1H, d, *J* = 9.5 Hz), 5.94 (1H, s), 4.28–3.98 (2H, m), 3.76 (1H, d, *J* = 12.0 Hz), 3.69–3.57 (2H, m), 2.72 (1H, ddd, *J* = 13.5, 12.0, 1.0 Hz), 2.45 (1H, d, *J* = 2.5 Hz), 1.91–1.71 (1H, m), 1.20 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 202.6, 168.4, 143.8, 135.0, 131.4, 129.4, 127.9, 127.7, 119.8, 119.7, 75.5, 60.3, 59.8, 49.8, 37.5, 37.0, 14.0; *m/e* (rel intensity) 457 (1), 454 (49), 437 (100), 419 (85), 364 (4), 346 (16), 306 (14), 223 (10), 177 (2), 148 (6), 131 (30); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{17}H_{17}BrCl_2O_4$) calcd 434.9765, found 434.9773.

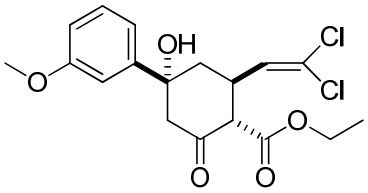
(1S*,2R*,4R*)-Ethyl 2-(2,2-dichlorovinyl)-4-hydroxy-6-oxo-4-phenylcyclohexanecarboxylate (51):[26]



The title compound was prepared according to General Procedure C in 49% yield. White solid, mp = 151-153 ºC (EtOAc/hexanes); R*f* = 0.16 (15% EtOAc/hexanes); IR (solid): υ 3395, 2967, 1740, 1709, 1625, 1242, 1163, 1136, 1024, 890, 844, 748, 691, 666 cm^{-1}; ^1H NMR (400 MHz,

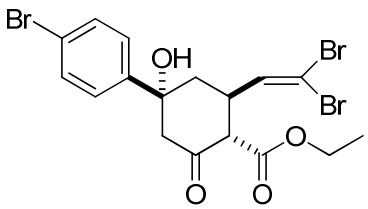
DMSO-d_6) δ 7.53–7.43 (2H, m), 7.36 (2H, t, *J* = 7.5 Hz), 7.26 (1H, t, *J* = 7.5 Hz), 6.10 (1H, d, *J* = 10.0 Hz), 5.69 (1H, s), 4.23–4.01 (2H, m), 3.77 (1H, d, *J* = 12.0 Hz), 3.71–3.54 (1H, m), 3.13 (1H, d, *J* = 14.0 Hz), 2.38 (1H, dd, *J* = 14.0, 2.5 Hz), 2.23 (1H, dd, *J* = 13.0, 13.0 Hz), 1.77 (1H, ddd, *J* = 13.0, 4.0, 2.5 Hz), 1.20 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 203.2, 168.5, 147.3, 131.4, 128.0, 126.8, 124.4, 119.8, 75.0, 60.3, 59.8, 52.7, 40.7, 37.9, 14.0; *m/e* (rel intensity) 390 (2), 374 (51), 357 (38), 339 (100), 267 (7), 261 (1), 221 (2); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{17}H_{18}Cl_2O_4$) calcd 357.0660, found 357.0660.

(1S*,2R*,4R*)-Ethyl 2-(2,2-dichlorovinyl)-4-hydroxy-4-(3-methoxyphenyl)-6-oxocyclohexanecarboxylate (52):



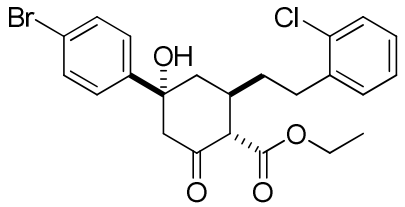
The title compound was prepared according to General Procedure C in 66% yield. Yellow solid, mp = 116-118 ºC (EtOAc/hexanes); R*f* = 0.17 (15% EtOAc/hexanes); IR (solid): υ 3534, 2982, 2840, 1742, 1713, 1608, 1583, 1430, 1328, 1251, 1155, 1048, 1029, 892, 858, 786, 700 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.27 (1H, dd, *J* = 8.0, 8.0 Hz), 7.09–6.96 (2H, m), 6.94–6.67 (1H, m), 6.09 (1H, d, *J* = 10.0 Hz), 5.69 (1H, s), 4.26–4.02 (2H, m), 3.96–3.65 (4H, m), 3.68–3.56 (1H, m), 3.12 (1H, d, *J* = 14.0 Hz), 2.36 (1H, dd, *J* = 14.0, 2.5 Hz), 2.23 (1H, dd, *J* = 13.0, 13.0 Hz), 1.84–1.66 (1H, m), 1.20 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz DMSO-d_6) δ 203.1, 168.5, 159.1, 149.0, 131.4, 129.1, 119.8, 116.7, 112.0, 110.5, 75.1, 60.3, 59.8, 55.0, 52.7, 40.7, 37.9, 14.0; *m/e* (rel intensity) 420 (4), 404 (44), 387 (42), 369 (100), 351 (6), 240 (1), 195 (2); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{18}H_{20}Cl_2O_5$) calcd 387.0766, found 387.0774.

(1S*,2R*,4R*)-Ethyl 4-(4-bromophenyl)-2-(2,2-dibromovinyl)-4-hydroxy-6-oxocyclohexanecarboxylate (66):



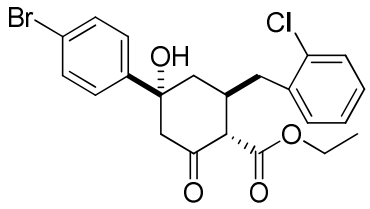
The title compound was prepared according to General Procedure C in 33% yield. Orange solid, mp = 134-136 ºC (EtOAc/hexanes); R*f* = 0.24 (30% EtOAc/hexanes); IR (solid): υ 3478, 2980, 2931, 1713, 1645, 1371, 1223, 1093, 1073, 1026, 1008, 812 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.55 (2H, d, *J* = 8.5 Hz), 7.43 (2H, d, *J* = 8.5 Hz), 6.54 (1H, d, *J* = 9.5 Hz), 5.79 (1H, s), 4.22–4.00 (2H, m), 3.77 (1H, d, *J* = 11.5 Hz), 3.62–3.44 (1H, m), 3.11 (1H, d, *J* = 13.5 Hz), 2.36 (1H, dd, *J* = 13.5, 2.5 Hz), 2.20 (1H, dd, *J* = 13.0, 13.0 Hz), 1.74 (1H, ddd, *J* = 13.5, 4.0, 2.5 Hz), 1.21 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 202.9, 168.5, 147.1, 136.8, 131.3, 127.1, 119.9, 92.9, 74.9, 60.3, 59.7, 52.4, 40.0, 37.9, 14.0; *m/e* (rel intensity) 524 (14), 506 (100), 426 (5), 394 (4), 366 (2), 327 (30), 279 (2), 188 (4), 131 (5); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{17}H_{17}Br_3O_4$) calcd 522.8755, found 522.8768.

(1S*,2R*,4R*)-Ethyl 4-(4-bromophenyl)-2-(2-chlorophenethyl)-4-hydroxy-6-oxocyclohexanecarboxylate (67):



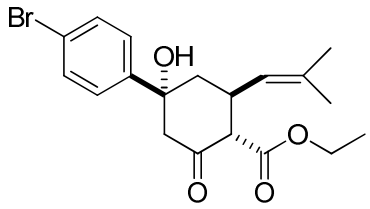
The title compound was prepared according to General Procedure C in 25% yield. Colourless oil; R*f* = 0.45 (30% EtOAc/hexanes); IR (oil): υ 3466, 2931, 1710, 1658, 1371, 1256, 1149, 1030, 1008, 825, 752, 680 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.57 (2H, d, *J* = 8.5 Hz), 7.48 (2H, d, *J* = 8.5 Hz), 7.40–7.35 (1H, m), 7.34–7.16 (3H, m), 5.57 (1H, s), 4.13 (2H, ddd, *J* = 7.0, 4.5, 4.5 Hz), 3.57 (1H, d, *J* = 12.0 Hz), 3.13 (1H, d, *J* = 13.5 Hz), 2.84–2.56 (4H, m), 2.31 (1H, dd, *J* = 13.5, 2.0 Hz), 2.08 (1H, d, *J* = 11.0 Hz), 2.01–1.86 (1H, m), 1.60–1.50 (2H, m), 1.18 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 204.7, 169.5, 147.5, 139.2, 132.6, 130.8, 130.6, 129.2, 127.8, 127.3, 127.0, 119.8, 74.9, 61.5, 52.5, 51.6, 41.2, 36.8, 35.4, 34.4, 29.8; *m/e* (rel intensity) 498 (13), 481 (16), 463 (100), 391 (2), 323 (26); HRMS (ESI) *m/e* $[M + NH_4]^+$ for ($C_{23}H_{24}BrClO_4$) calcd 496.0890, found 496.0898.

(1S*,2R*,4R*)-Ethyl 4-(4-bromophenyl)-2-(2-chlorobenzyl)-4-hydroxy-6-oxocyclohexanecarboxylate (68):



The title compound was prepared according to General Procedure C in 21% yield. White solid, mp = 133-135 ºC (EtOAc/hexanes); R*f* = 0.21 (30% EtOAc/hexanes); IR (solid): υ 3397, 2923, 2853, 1737, 1706, 1372, 1241, 1155, 1031, 1008, 821, 752, 680 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.52 (2H, d, *J* = 8.5 Hz), 7.43–7.33 (3H, m), 7.30–7.00 (3H, m), 5.52 (1H, s), 4.09 (1H, q, *J* = 7.0 Hz), 3.61 (1H, d, *J* = 12.0 Hz), 3.13 (1H, d, *J* = 13.5 Hz), 2.95 (1H, d, *J* = 11.5 Hz), 2.86–2.63 (2H, m), 2.29 (1H, dd, *J* = 13.5, 2.5 Hz), 2.24–1.83 (1H, m), 1.63–1.51 (1H, m), 1.22 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 204.4, 169.3, 147.3, 136.4, 133.3, 131.8, 130.8, 129.3, 128.1, 127.0, 126.9, 119.8, 74.6, 61.9, 60.2, 52.5, 41.2, 38.1, 36.1, 14.0; *m/e* (rel intensity) 485 (14), 465 (35), 449 (100), 419 (2), 377 (2), 339 (2), 319 (2); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{22}H_{22}BrClO_4$) calcd 487.0282, found 487.0261.

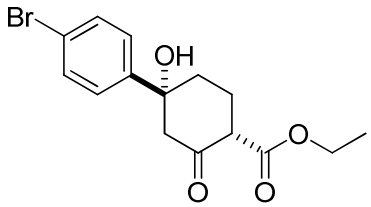
(1S*,2S*,4R*)-Ethyl 4-(4-bromophenyl)-4-hydroxy-2-(2-methylprop-1-en-1-yl)-6-oxocyclohexanecarboxylate (69):



The title compound was prepared according to General Procedure **C** in 35% yield. White solid, mp = 89-91 ºC (EtOAc/hexanes); R*f* = 0.47 (30% EtOAc/hexanes); IR (solid): υ 3378, 2974, 2915, 1743, 1705, 1662, 1370, 1239, 1149, 1027, 1008, 836, 816 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.53 (1H, d, *J* = 7.5 Hz), 7.44 (1H, d, *J* = 7.5 Hz), 5.62 (1H, s), 5.02–4.89 (1H, m), 4.07 (2H, q, *J* = 7.0 Hz), 3.61–3.41 (2H, m), 3.07 (1H, d, *J* = 13.5 Hz), 2.27 (1H, dd, *J* = 13.5, 2.5 Hz), 2.08 (1H, dd, *J* = 13.5, 11.0 Hz), 1.75–1.64 (1H, m), 1.61 (6H, d, *J* = 7.5 Hz), 1.15 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.2, 169.1, 133.3, 132.5, 130.8, 126.9, 126.0, 119.8, 75.1, 61.6, 59.8, 52.4, 42.6, 35.7, 25.5, 17.9, 14.0; *m/e* (rel intensity) 414 (14), 395 (38),

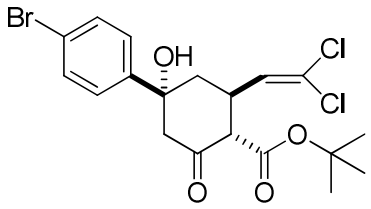
377 (100), 349 (1), 321 (3), 305 (2); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{19}H_{23}BrO_4$) calcd 395.0858, found 395.0875.

(1S*,4R*)-Ethyl 4-(4-bromophenyl)-4-hydroxy-2-oxocyclohexanecarboxylate (72):



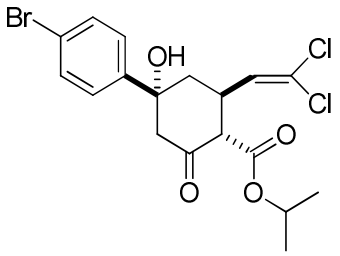
Using a procedure adapted from literature,[40] a stirred solution of ethyl acetoacetate (0.15 mL, 1.2 mmol) and 21% sodium ethoxide/ethanol (73 μL, 0.20 mmol) in ethanol (1 mL) was added 1-(4-bromophenyl)-3-(dimethylamino)propan-1-one **71** (0.10 g, 0.40 mmol) portionwise. The mixture was stirred overnight and then concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel using 15-30% EtOAc in hexane as the eluent to afford a white solid (70 mg, 50%), mp = 108-110 ºC (EtOAc/hexanes); R*f* = 0.42 (30% EtOAc/hexanes); IR (solid): υ 3394, 2976, 2927, 2853, 1709, 1658, 1585, 1488, 1375, 1258, 1228, 1200, 1161, 1036, 1007, 957, 821, 668 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) (enol form) δ 12.17 (1H, s), 7.63–7.68 (2H, m), 7.50–7.41 (2H, m), 5.43 (1 H, s), 4.21–4.15 (2H, m), 3.65 (1H, dd, *J* = 13.0, 6.0 Hz) 2.99–2.93 (1H, m) 2.77–2.63 (1H, m), 2.45–2.39 (1H, m), 2.11–2.04 (1H, m), 1.83–1.66 (1H, m), 1.25 (3H, t, *J* = 7.0 Hz); *m/e* (rel intensity) 361 (4), 358 (36), 341 (76), 323 (100), 295 (1), 251 (7), 240 (1); HRMS (ESI) *m/e* $[M + NH_4]^+$ for ($C_{15}H_{17}BrO_4$) calcd 358.0654, found 358.0659.

(1S*,2R*,4R*)-tert-Butyl 4-(4-bromophenyl)-2-(2,2-dichlorovinyl)-4-hydroxy-6-oxocyclohexanecarboxylate (74):



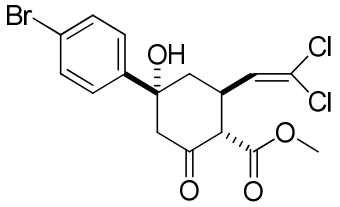
To a stirred solution of *tert*-butyl acetoacetate (0.13 mL, 0.8 mmol) and sodium butoxide (13 mg, 0.13 mmol) in THF (1 mL) was added (E)-1-(4-bromophenyl)-5,5-dichloropenta-2,4-dien-1-one (80 mg, 0.26 mmol) portionwise. The mixture was stirred overnight and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel using 10-40% EtOAc in hexane gradient as the eluent to afford a white solid (72 mg, 59%), mp = 159-161 ºC (EtOAc/hexanes); R*f* = 0.45 (30% EtOAc/hexanes); IR (solid): υ 3500, 2982, 2935, 1717, 1701, 1306, 1148, 1007, 891, 839, 829, 737 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.55 (2H, d, *J* = 8.5 Hz), 7.42 (2H, d, *J* = 8.5 Hz), 6.08 (1H, d, *J* = 9.5 Hz), 5.75 (1H, s), 3.62–3.53 (2H, m), 3.08 (1H, d, *J* = 14.0 Hz), 2.33 (1H, dd, *J* = 14.0, 2.5 Hz), 2.27–2.09 (1H, m), 1.78–1.65 (1H, dd, *J* = 13.5, 13.5 Hz), 1.18 (9H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 203.1, 167.6, 146.8, 131.4, 130.9, 126.9, 120.0, 119.7, 80.5, 74.8, 60.7, 52.4, 40.4, 38.1, 27.6; *m/e* (rel intensity) 482 (12), 465 (4), 408 (14), 390 (100), 311 (2), 293 (1), 243 (1), 192 (2), 169 (2); HRMS (ESI) *m/e* $[M + NH_4]^+$ for ($C_{19}H_{21}BrCl_2O_4$) calcd 480.0344, found 480.0365.

(1S*,2R*,4R*)-Isopropyl 4-(4-bromophenyl)-2-(2,2-dichlorovinyl)-4-hydroxy-6-oxocyclohexanecarboxylate (75):



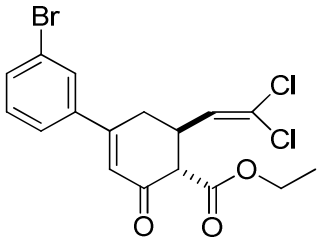
To a stirred solution of isopropyl acetoacetate (0.17 g, 1.2 mmol) in isopropanol (0.70 mL) was added a solution of sodium metal (5.0 mg, 0.20 mmol) in isopropanol (0.7 mL). After stirring for 10 min, (E)-1-(4-bromophenyl)-5,5-dichloropenta-2,4-dien-1-one (0.12 g, 0.34 mmol) was added portionwise. The mixture was stirred overnight and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel using 10-30% EtOAc in hexane gradient as the eluent to afford a white solid (90 mg, 58%), mp = 155-157 ºC (EtOAc/hexanes); R*f* = 0.29 (30% EtOAc/hexanes); IR (solid): υ 3549, 3042, 2982, 1735, 1709, 1375, 1230, 1171, 1105, 1008, 889, 864, 823 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.55 (2H, d, *J* = 8.5 Hz), 7.43 (2H, d, *J* = 8.5 Hz), 6.08 (1H, d, *J* = 9.5 Hz), 5.78 (1H, s), 5.04–4.80 (1H, m), 3.71 (1H, d, *J* = 12.0 Hz), 3.68–3.55 (1H, m), 3.10 (1H, d, *J* = 14.0 Hz), 2.35 (1H, dd, *J* = 14.0, 2.5 Hz), 2.21 (1H, dd, *J* = 12.5, 12.5 Hz), 1.81–1.63 (1H, m), 1.29–1.14 (6H, m); ^{13}C NMR (100 MHz, DMSO-d_6) δ 202.9, 167.9, 146.7, 131.3, 130.9, 126.9, 120.4, 120.1, 74.8, 67.8, 59.8, 52.4, 40.5, 37.9, 21.5; *m/e* (rel intensity) 468 (31), 451 (78), 433 (100), 390 (1), 346 (4); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{18}H_{19}BrCl_2O_4$) calcd 448.9922, found 448.9911.

(1S*,2R*,4R*)-Methyl 4-(4-bromophenyl)-2-(2,2-dichlorovinyl)-4-hydroxy-6-oxocyclohexanecarboxylate (76):



To a stirred solution of methyl acetoacetate (0.041 mL, 0.5 mmol) and sodium methoxide (3.1 mg, 0.06 mmol) in methanol (1 mL) was added (E)-1-(4-bromophenyl)-5,5-dichloropenta-2,4-dien-1-one (50 mg, 0.16 mmol) portionwise. The mixture was stirred overnight and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel using 10-30% EtOAc in hexane gradient as the eluent to afford a yellow solid (50 mg, 72%), mp = 144-146 ºC (EtOAc/hexanes); R*f* = 0.22 (25% EtOAc/hexanes); IR (solid): υ 3392, 2951, 2919, 1746, 1707, 1627, 1362, 1240, 1164, 1141, 1007, 882 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.55 (2H, d, *J* = 8.5 Hz), 7.43 (2H, d, *J* = 8.5 Hz), 6.08 (1H, d, *J* = 9.5 Hz), 5.80 (1H, s), 3.80 (1H, d, *J* = 12.0 Hz), 3.66 (3H, s), 3.64–3.57 (1H, m), 3.13 (1H, d, *J* = 13.5 Hz), 2.36 (1H, dd, *J* = 13.5, 2.5 Hz), 2.23 (1H, ddd, *J* = 13.0, 12.0, 1.5 Hz), 1.76 (1H, ddd, J = 13.5, 4.0, 2.5 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 202.9, 169.0, 146.7, 131.3, 130.9, 126.9, 120.1, 119.9, 74.8, 59.6, 52.4, 51.6, 40.4, 37.8; *m/e* (rel intensity) 440 (73), 423 (40), 404 (100), 325 (3), 307 (1), 240 (2), 195 (2), 151 (2); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{16}H_{15}BrCl_2O_4$) calcd 420.9609, found 420.9626.

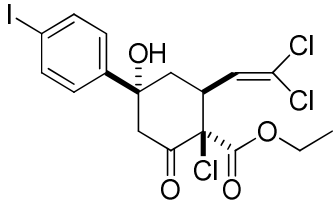
(3R*,4S*)-Ethyl 3'-bromo-3-(2,2-dichlorovinyl)-5-oxo-2,3,4,5-tetrahydro-[1,1'-biphenyl]-4-carboxylate (78):



To a stirred solution of ethyl acetoacetate (0.083 mL, 0.65 mmol) and triethylamine (0.26 mL, 1.8 mmol) in *n*-butanol (1 mL) was added (E)-1-(3-bromophenyl)-5,5-dichloropenta-2,4-dien-1-one (80 mg, 0.26 mmol). The mixture was stirred at 70 ºC (EtOAc/hexanes) for 3 h and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel using 10-30% EtOAc in hexane gradient as the eluent to afford the white solid (58 mg, 53%), mp

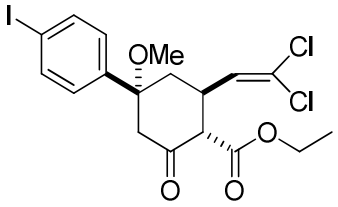
= 99-101 ºC (EtOAc/hexanes); R*f* = 0.55 (30% EtOAc/hexanes); IR (solid): υ 3023, 2986, 2902, 1736, 1647, 1603, 1410, 1262, 1237, 1037, 880, 788, 693 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.92 (1H, dd, *J* = 2.0, 2.0 Hz), 7.76–7.63 (2H, m), 7.41 (1H, dd, *J* = 8.0, 8.0 Hz), 6.54 (1H, d, *J* = 1.5 Hz), 6.24 (1H, d, *J* = 10.0 Hz), 4.14 (2H, q, *J* = 7.0 Hz), 3.71 (1H, d, *J* = 12.5 Hz), 3.70–3.52 (1H, m), 3.00–2.79 (2H, m), 1.20 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 192.7, 168.80, 156.9, 139.5, 133.1, 130.8, 130.3, 129.1, 125.5, 123.9, 122.3, 121.0, 60.5, 56.8, 49.8, 30.7, 14.0; *m/e* (rel intensity) 422 (7), 419 (100), 279 (1), 159 (2); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{17}H_{15}BrCl_2O_3$) calcd 416.9660, found 416.9655.

(1R*,2S*,4R*)-Ethyl 1-chloro-2-(2,2-dichlorovinyl)-4-hydroxy-4-(4-iodophenyl)-6-oxocyclohexanecarboxylate (79):



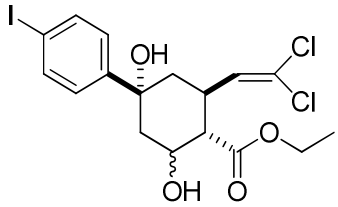
To a stirred solution of (1S,2R,4R)-ethyl 2-(2,2-dichlorovinyl)-4-hydroxy-4-(4-iodophenyl)-6-oxocyclohexanecarboxylate **39** (25 mg, 0.050 mmol) in DMSO (0.5 mL) was added sodium hydride (2.0 mg, 0.050 mmol) at 0 °C for 1 h. Subsequently, $CuCl_2$ (0.15 mmol) was added to the reaction mixture and the mixture was further stirred at ambient temperature for 12 h. The mixture was washed with 5% HCl (10 mL) and extracted with diethyl ether (15 mL). The organic layer was washed with H_2O (5 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel using 30% EtOAc in hexane as the eluent to afford a white solid (10 mg, 39%), mp = 156-158 ºC (EtOAc/hexanes); R*f* = 0.43 (30% EtOAc/hexanes); IR (solid): υ 3457, 2985, 2931, 1757, 1716, 1388, 1248, 1216, 1161, 1093, 1025, 1003, 890, 838, 814 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.73 (2H, d, *J* = 8.5 Hz), 7.32 (2H, d, *J* = 8.5 Hz), 6.14 (1H, d, *J* = 9.5 Hz), 6.04 (1H, s), 4.33–4.15 (2H, m), 3.53 (1H, d, *J* = 14.0 Hz), 2.46 (1H, d, *J* = 2.0 Hz), 2.36–2.20 (1H, m), 1.87–1.69 (1H, ddd, *J* =14.0, 4.0, 2.0 Hz), 1.24 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 197.7, 165.3, 146.1, 136.9, 127.1, 126.5, 122.2, 93.42, 75.4, 73.7, 62.8, 47.5, 41.5, 38.0, 13.8; *m/e* (rel intensity) 533 (73), 517 (24), 498 (100), 464 (5), 428 (4), 392 (2), 371 (2), 352 (1) 279 (1), 223 (2), 172 (3); HRMS (ESI) *m/e* $[M + NH_4]^+$ for ($C_{17}H_{16}Cl_3IO_4$) calcd 533.9502, found 533.9511.

(1S*,2R*,4R*)-Methyl 2-(2,2-dichlorovinyl)-4-(4-iodophenyl)-4-methoxy-6-oxocyclohexanecarboxylate (80):



To a stirred solution of (1S,2R,4R)-ethyl 2-(2,2-dichlorovinyl)-4-hydroxy-4-(4-iodophenyl)-6-oxocyclohexanecarboxylate **39** (25 mg, 0.05 mmol), silver triflate (14 mg, 0.6 mmol), 2,6-di-*t*-butylpyridine (17 μL, 0.08 mmol) in CH_2Cl_2 (0.5 mL) was added methyl iodide (4 μL, 0.06 mmol) at 0 °C and stirred for 1 h. The reaction mixture was diluted with CH_2Cl_2 and filtered through a small plug of celite. The resulting filtrate was washed with 1 N NaOH (2.5 mL) and brine. The crude mixture was concentration *in vacuo* and purified by column chromatography on silica gel using 30% EtOAc in hexane as the eluent to afford a white solid (15 mg, 62%), mp = 137-139 ºC (EtOAc/hexanes); R*f* = 0.80 (30% EtOAc/hexanes); IR (solid): υ 2980, 2927, 1725, 1708, 1617, 1300, 1262, 1004, 889, 821 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.72 (2H, d, *J* = 8.5 Hz), 7.28 (2H, d, *J* = 8.5 Hz), 6.08 (1H, d, *J* = 10.0 Hz), 5.75 (3H, s), 4.24–3.91 (2H, m), 3.76 (1H, d, *J* = 12.0 Hz), 3.71–3.51 (1H, m), 3.10 (1H, d, *J* = 13.5 Hz), 2.35 (1H, dd, *J* = 13.5, 2.5 Hz), 2.21 (1H, dd, *J* = 13.5, 12.0 Hz), 1.74 (1H, ddd, *J* = 13.5, 2.5, 2.5 Hz), 1.19 (3H, dt, *J* = 9.5, 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 202.9, 168.5, 147.1, 136.8, 131.3, 127.1, 119.95, 92.9, 74.9, 60.3, 59.7, 52.4, 40.4, 37.9, 34.6, 13.8; *m/e* (rel intensity) 500 (7), 483 (16), 465 (100), 387 (1), 352 (1), 279 (5), 221 (2); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{18}H_{19}Cl_2IO_4$) calcd 482.9627, found 482.9620.

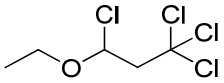
(1S*,2R*,4R*)-Ethyl 2-(2,2-dichlorovinyl)-4,6-dihydroxy-4-(4-iodophenyl)cyclohexanecarboxylate (81):



To a stirred solution of (1S,2R,4R)-ethyl 2-(2,2-dichlorovinyl)-4-hydroxy-4-(4-iodophenyl)-6-oxocyclohexanecarboxylate **39** (50 mg, 0.10 mmol) in EtOH (0.5 mL) was added sodium borohydride (2.0 mg, 0.050 mmol) at 0 °C and stirred for 18 h. The resulting reaction mixture was diluted with CH_2Cl_2 The organic layer was then washed with 5% HCl (2 mL), brine, dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel using 30% EtOAc in hexane as the eluent to afford a white solid (20 mg, 40%), mp = 125-127 ºC (EtOAc/hexanes); R*f* = 0.28 (30% EtOAc/hexanes); IR (solid): υ 3425, 2987, 2930, 1704, 1373, 1183, 1095, 1030, 1003, 890, 876, 817 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.68 (2H, d, *J* = 8.5 Hz), 7.26 (2H, d, *J* = 8.5 Hz), 5.99 (1H, d, *J* = 9.5 Hz), 5.82 (1H, s), 5.50 (1H, d, *J* = 7.0 Hz), 4.34 (1H, dd, *J* = 7.0, 3.0 Hz), 4.19–3.91 (2H, m), 3.43–3.33 (1H, m), 2.71 (1H, dd, *J* = 11.5, 3.0 Hz), 2.03–1.93 (1H, m), 1.89–1.73 (2H, m), 1.64 (1H, ddd, *J* = 13.0, 4.0, 2.5 Hz), 1.19 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.2, 148.6, 137.3, 134.5, 127.7, 118.83, 93.2, 73.6, 68.9, 60.4, 51.2, 42.5, 42.0, 32.1, 14.7; *m/e* (rel intensity) 505 (1), 469 (1), 453 (9), 449 (100), 371 (2); HRMS (ESI) *m/e* $[M + Na]^+$ for ($C_{17}H_{19}Cl_2IO_4$) calcd 506.9597, found 506.9575.

3.4 Synthesis of Various Chalcone Precursors

1,1,1,3-Tetrachloro-3-ethoxypropane (35):



The title compound was prepared according to a modified procedure.[31] A solution of ethyl vinylether (3.0 g, 42.0 mmol) in carbon tetrachloride (4 mL) was added dropwise via syringe pump to a solution of dibenzoyl peroxide (83 mg, 0.34 mmol) in carbon tetrachloride (50 mL, 0.52 mol) at 80 °C over 30 min. After the addition, the reaction mixture was stirred at 80 °C for 2 h. The cooled reaction mixture was concentrated *in vacuo* and sealed under argon. The colourless crude oil was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.96 (1H, dd, J = 7.0, 2.5 Hz), 4.00 (1H, dq, J = 9.5, 7.0 Hz), 3.73–3.35 (3H, m), 1.29 (3H, t, J = 7.0 Hz).

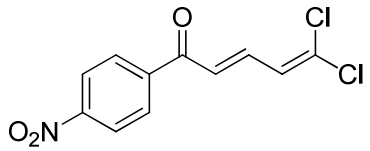
General Procedure E

To a stirred solution of the corresponding acetophenone **27** (1.4 mmol) in acetic acid (2 mL) under argon, was added crude 1,1,1,3-tetrachloro-3-ethoxypropane (17 mmol) via syringe. The reaction mixture was stirred at ambient temperature for 4 days. The resulting mixture was concentrated *in vacuo* and the residue was purified column chromatography on silica gel using 10-25% EtOAc/hexane as the eluent to afford the dichlorovinyl chalcone product.

General Procedure F

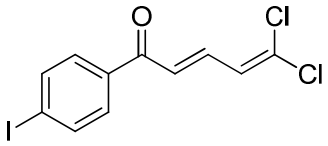
A mixture of substituted acetophenone (3.0 mmol) and substituted benzaldehyde (3.0 mmol), and sodium hydroxide (30 mL, 2N aqueous) in absolute ethanol (50 mL) was stirred at room temperature for 24 h. The resulting solid was filtered, washed with water, dried, and recrystallized from ethanol.

***(E)*-5,5-Dichloro-1-(4-nitrophenyl)penta-2,4-dien-1-one (25a):**



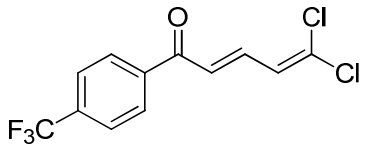
The title compound was prepared according to General Procedure E in 26% yield. Yellow solid, mp = 58-60 ºC (EtOAc/hexanes); R*f* = 0.25 (15% EtOAc/hexanes); IR (solid): υ 3110, 2925, 2857, 1688, 1603, 1520, 1343, 1319, 1254, 1217, 1011, 904, 851, 853, 703 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40–8.26 (2H, m), 8.26–7.92 (2H, m), 7.62 (1H, dd, *J* = 15.0, 11.0 Hz), 7.03 (1H, dd, *J* = 15.0, 1.0 Hz), 6.74 (1H, dd, *J* = 11.0, 1.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.3, 150.4, 139.6, 129.4, 129.0, 126.5, 125.1, 124.1, 123.9; *m/e* (rel intensity) 340 (1), 272 (100), 236 (1); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{11}H_7Cl_2NO_3$) calcd 271.9881, found 271.9884.

***(E)*-5,5-Dichloro-1-(4-iodophenyl)penta-2,4-dien-1-one (25b):**



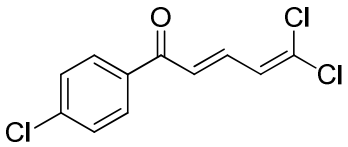
The title compound was prepared according to General Procedure E in 36% yield. Yellow solid, mp = 123-125 ºC (EtOAc/hexanes); R*f* = 0.32 (10% EtOAc/hexanes); IR (solid): υ 3093, 3065, 2920, 1647, 1587, 1558, 1392, 1331, 1292, 1253, 1209, 1024, 1003, 970, 900, 877, 810, 720 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (2H, d, *J* = 8.5 Hz), 7.66 (2H, d, *J* = 8.5 Hz), 7.57 (1H, dd, *J* = 15.0, 11.0 Hz), 7.00 (1H, dd, *J* = 15.0, 1.0 Hz), 6.70 (1H, d, *J* = 11.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.1, 138.2, 137.8, 137.0, 131.3, 130.0, 127.7, 126.9, 101.32; *m/e* (rel intensity) 353 (100); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{11}H_7Cl_2IO$) calcd 352.8996, found 352.8999.

***(E)*-5,5-Dichloro-1-(4-(trifluoromethyl)phenyl)penta-2,4-dien-1-one (25c):**



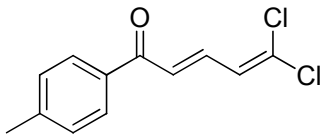
The title compound was prepared according to General Procedure E in 33% yield. Yellow solid, mp = 68-71 ºC (EtOAc/hexanes); R*f* = 0.50 (10% EtOAc/hexanes); IR (solid): υ 2253, 1665, 1594, 1578, 1320, 1175, 1138, 1067, 1014, 905, 732, 651 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (2H, d, *J* = 8.0 Hz), 7.77 (2H, d, *J* = 8.0 Hz), 7.60 (1H, dd, *J* = 15.0, 11.0 Hz), 7.04 (1H, dd, *J* = 15.0, 1.0 Hz), 6.73 (1H, dd, *J* = 11.0, 1.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.9, 140.3, 138.2, 134.3 (d, *J* = 32.8 Hz), 131.7, 128.7, 127.3, 126.7, 125.7 (q, *J* = 3.5 Hz), 123.5 (d, *J* = 273.0 Hz); ^{19}F NMR (375 MHz, $CDCl_3$) δ -63.5; *m/e* (rel intensity) 321 (3), 312 (8), 295 (100), 279 (1), 359 (1), 241 (1), 172 (2); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{12}H_7Cl_2F_3O$) calcd 294.9904, found 294.9914.

***(E)*-5,5-Dichloro-1-(4-chlorophenyl)penta-2,4-dien-1-one (25d):**



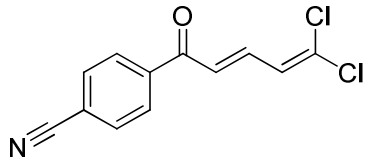
The title compound was prepared according to General Procedure E in 58% yield. Yellow solid, mp = 110-113 ºC (EtOAc/hexanes); R*f* = 0.42 (10% EtOAc/hexanes); IR (solid): υ 3054, 2986, 1662, 1598, 1412, 1270, 1011, 742, 705 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00–7.81 (2H, m), 7.57 (1H, dd, *J* = 15.0, 11.0 Hz), 7.51–7.42 (2H, m), 7.02 (1H, dd, *J* = 15.0, 0.5 Hz), 6.71 (1H, dd, *J* = 11.0, 0.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.7, 139.8, 137.8, 136.1, 131.3, 130.0, 129.2, 127.6, 127.0; *m/e* (rel intensity) 261 (100), 171 (1); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{11}H_7Cl_3O$) calcd 260.9640, found 260.9644.

***(E)*-5,5-Dichloro-1-(p-tolyl)penta-2,4-dien-1-one (25e):**



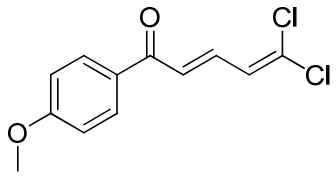
The title compound was prepared according to General Procedure E in 55% yield. Yellow solid, mp = 89-91 ºC (EtOAc/hexanes); R*f* = 0.35 (10% EtOAc/hexanes); IR (solid): υ 3036, 2918, 1653, 1606, 1588, 1572, 1305, 1258, 1180, 1013, 976, 894, 884, 810, 721 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (2H, d, *J* = 8.0 Hz), 7.56 (1H, dd, *J* = 15.0, 11.0 Hz), 7.29 (2H, d, *J* = 8.0 Hz), 7.07 (1H, dd, *J* = 15.0, 1.0 Hz), 6.70 (1H, dd, *J* = 11.0, 1.0 Hz), 2.43 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.4, 144.2, 136.9, 135.2, 130.5, 129.6, 128.8, 127.8, 127.7, 21.9; *m/e* (rel intensity) 251 (4), 241 (100); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{12}H_{10}C_{l2}O$) calcd 241.0187, found 241.0179.

***(E)*-4-(5,5-Dichloropenta-2,4-dienoyl)benzonitrile (25f):**



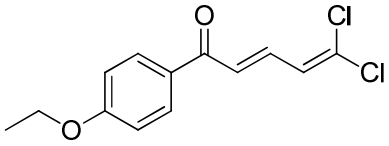
The title compound was prepared according to General Procedure E in 12% yield. Orange solid, mp = 155-157 ºC (EtOAc/hexanes); R*f* = 0.25 (15% EtOAc/hexanes); IR (solid): υ 3079, 3040, 2230, 1692, 1663, 1593, 1309, 1256, 1178, 1026, 972, 909, 893, 803, 790, 710, 673 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08–7.93 (2H, m), 7.84–7.69 (2H, m), 7.61 (1H, dd, *J* = 15.0, 11.0 Hz), 7.01 (1H, dd, *J* = 15.0, 0.5 Hz), 6.72 (1H, dd, *J* = 11.0, 0.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.6, 140.9, 138.9, 132.7, 132.4, 129.0, 127.5, 126.4, 118.1, 116.5; *m/e* (rel intensity) 269 (29), 252 (100), 163 (1), 146 (1); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{12}H_7Cl_2NO$) calcd 251.9982, found 251.9980.

***(E)*-5,5-Dichloro-1-(4-methoxyphenyl)penta-2,4-dien-1-one (25g):**



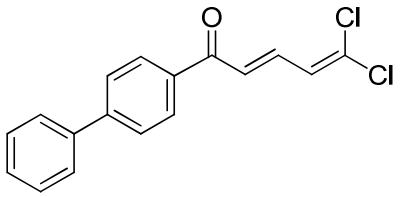
The title compound was prepared according to General Procedure E in 40% yield. Yellow solid, mp = 105-107 ºC (EtOAc/hexanes); R*f* = 0.35 (10% EtOAc/hexanes); IR (solid): υ 2937, 2843, 1650, 1590, 1513, 1454, 1308, 1215, 1024, 978, 895, 887, 823, 737, 676 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (2H, d, *J* = 8.0 Hz), 7.55 (1H, dd, *J* = 15.0, 11.0 Hz), 7.08 (1H, d, *J* = 15.0 Hz), 6.97 (2H, d, *J* = 8.0 Hz), 6.70 (1H, dd, *J* = 11.0, 1.0 Hz), 3.89 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.2, 163.9, 136.6, 131.0, 130.8, 130.3, 128.0, 127.6, 114.2, 55.8; *m/e* (rel intensity) 257 (100); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{12}H_{10}Cl_2O_2$) calcd 257.0136, found 257.0137.

***(E)*-5,5-Dichloro-1-(4-ethoxyphenyl)penta-2,4-dien-1-one (25h):**



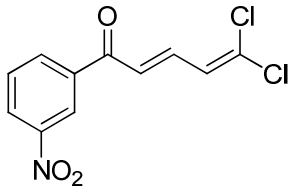
The title compound was prepared according to General Procedure E in 86% yield. Yellow solid, mp = 70-75 ºC (EtOAc/hexanes); R*f* = 0.45 (10% EtOAc/hexanes); IR (solid): υ 2985, 2872, 1649, 1605, 1591, 1513, 1309, 1252, 1176, 980, 870, 821, 676 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (2H, d, *J* = 9.0 Hz), 7.54 (1H, dd, *J* = 15.0, 11.0 Hz), 7.08 (1H, d, *J* = 15.0 Hz), 6.95 (2H, d, *J* = 9.0 Hz), 6.69 (1H, d, *J* = 11.0 Hz), 4.12 (2H, q, *J* = 7.0 Hz), 1.45 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.2, 163.3, 136.5, 131.0, 130.6, 130.2, 128.0, 127.6, 114.6, 64.0, 14.9; *m/e* (rel intensity) 297 (8), 271 (100); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{13}H_{12}C_{l2}O_2$) calcd 271.0293, found 271.0299.

***(E)*-1-([1,1'-Biphenyl]-4-yl)-5,5-dichloropenta-2,4-dien-1-one (25i)**



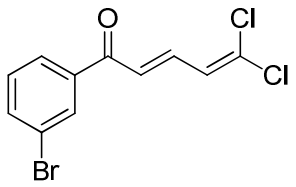
The title compound was prepared according to General Procedure E in 36% yield. Yellow solid, mp = 144-146 ºC (EtOAc/hexanes); R*f* = 0.45 (15% EtOAc/hexanes); IR (solid): υ 3038, 1658, 1597, 1583, 1553, 1344, 1314, 1257, 1207, 1032, 976, 920, 886, 849, 817, 681 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08–8.00 (2H, m), 7.78–7.69 (2H, m), 7.69–7.55 (3H, m), 7.54–7.45 (2H, m), 7.47–7.37 (1H, m), 7.13 (1H, dd, *J* = 15.0, 0.5 Hz), 6.73 (1H, dd, *J* = 11.0, 0.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.4, 146.1, 140.0, 137.3, 136.5, 130.8, 129.3, 129.2, 128.5, 127.9, 127.59, 127.58, 127.5; *m/e* (rel intensity) 303 (100), 259 (1), 197 (2), 181 (2), 131 (1); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{17}H_{12}Cl_2O$) calcd 303.0343, found 303.0356.

***(E)*-5,5-Dichloro-1-(3-nitrophenyl)penta-2,4-dien-1-one (25j):**



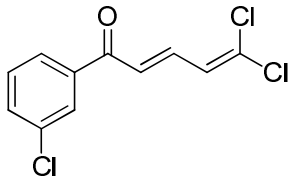
The title compound was prepared according to General Procedure E in 29% yield. Yellow solid, mp = 141-144 ºC (EtOAc/hexanes); R*f* = 0.33 (10% EtOAc/hexanes); IR (solid): υ 2361, 2253, 1665, 1352, 1320, 910, 775, 651 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (1H, ddd, *J* = 2.5, 1.5, 0.5 Hz), 8.45 (1H, ddd, *J* = 8.0, 2.5, 1.0 Hz), 8.29 (1H, ddd, *J* = 7.5, 1.5, 1.0 Hz), 7.72 (1H, ddd, *J* = 8.0, 7.5, 0.5 Hz), 7.66 (1H, dd, *J* = 15.0, 11.0 Hz), 7.09 (1H, dd, *J* = 15.0, 1.0 Hz), 6.76 (1H, dd, *J* = 11.0, 1.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 187.6, 148.7, 139.1, 139.1, 134.2, 132.5, 130.3, 127.6, 127.5, 126.1, 123.5; *m/e* (rel intensity) 289 (5), 272 (100); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{11}H_7Cl_2NO_3$) calcd 271.9881, found 271.9889.

***(E)*-1-(3-Bromophenyl)-5,5-dichloropenta-2,4-dien-1-one (25k):**



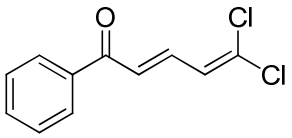
The title compound was prepared according to General Procedure E in 67% yield. Orange solid, mp = 72-74 ºC (EtOAc/hexanes); R*f* = 0.47 (30% EtOAc/hexanes); IR (solid): υ 3074, 1653, 1589, 1561, 1418, 1320, 1244, 1203, 986, 894, 877, 791, 711, 702, 689, 656 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11–8.03 (1H, m), 7.91–7.81 (1H, m), 7.78–7.69 (1H, m), 7.59 (1H, dd, *J* = 15.0, 11.0 Hz), 7.45–7.31 (1H, m), 7.01 (1H, d, *J* = 15.0 Hz), 6.72 (1H, d, *J* = 11.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.6, 140.0, 138.2, 136.2, 131.7, 131.6, 130.5, 127.7, 127.1, 126.9. 123.3; *m/e* (rel intensity) 380 (4), 332 (9), 323 (10), 307 (100); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{11}H_7BrCl_2O$) calcd 304.9135, found 304.9133.

***(E)*-5,5-Dichloro-1-(3-chlorophenyl)penta-2,4-dien-1-one (25l):**



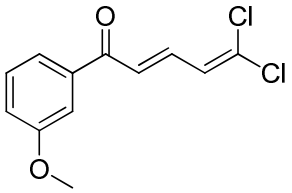
The title compound was prepared according to General Procedure E in 40% yield. Yellow solid, mp = 68-71 ºC (EtOAc/hexanes); R*f* = 0.45 (10% EtOAc/hexanes); IR (solid): υ 3054, 2986, 1663, 1597, 1419, 1238, 908, 859, 728, 705 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (1H, d, *J* = 1.5 Hz), 7.82 (1H, dd, *J* = 7.5, 1.5 Hz), 7.64–7.51 (2H, m), 7.49–7.37 (1H, m), 7.02 (1H, d, *J* = 15.0 Hz), 6.72 (1H, d, *J* = 11.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.6, 139.4, 138.1, 135.3, 133.3, 131.6, 130.3, 128.8, 127.7, 126.9, 126.7; *m/e* (rel intensity) 291 (8), 278 (8), 261 (100), 227 (4), 155(9); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{11}H_7C_{l3}O$) calcd 260.9640, found 260.9642.

***(E)*-5,5-Dichloro-1-phenylpenta-2,4-dien-1-one (25m):**



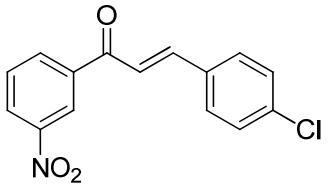
The title compound was prepared according to General Procedure E in 58% yield. Yellow solid, mp = 73-75 ºC (EtOAc/hexanes); R*f* = 0.46 (10% EtOAc/hexanes); IR (solid): υ 3053, 2984, 1658, 1600, 1591, 1448, 1275, 1016, 919, 741, 705, 650 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08–7.88 (2H, m), 7.68–7.41 (4H, m), 7.08 (1H, d, *J* = 15.0 Hz), 6.71 (1H, d, *J* = 11.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.0, 137.8, 137.4, 133.3, 130.9, 128.9, 128.7, 127.8, 127.6; *m/e* (rel intensity) 227 (100), 223 (4); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{11}H_8Cl_2O$) calcd 227.0030, found 227.0030.

***(E)*-5,5-Dichloro-1-(3-methoxyphenyl)penta-2,4-dien-1-one (25n):**



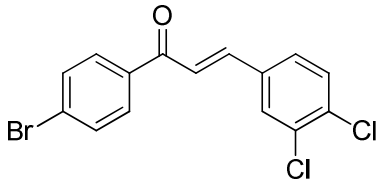
The title compound was prepared according to General Procedure E in 43% yield. Yellow oil; R*f* = 0.30 (10% EtOAc/hexanes); IR (oil): υ 3039, 3027, 2970, 1656, 1590, 1575, 1430, 1338, 1231, 1198, 1016, 982, 885, 868, 782, 711, 668 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (1H, dd, *J* = 15.0, 11.0 Hz), 7.53–7.46 (2H, m), 7.45–7.34 (1H, m), 7.14 (1H, dd, *J* = 8.0, 2.5 Hz), 7.05 (1H, dd, *J* = 15.0, 1.0 Hz), 6.70 (1H, dd, *J* = 11.0, 1.0 Hz), 3.88 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.7, 160.2, 139.2, 137.4, 130.9, 129.9, 127.8, 127.7, 121.2, 119.9, 113.0, 55.7; *m/e* (rel intensity) 274 (2), 257 (100), 135 (1); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{12}H_{10}Cl_2O_2$) calcd 257.0136, found 257.0139.

***(E)*-3-(4-Chlorophenyl)-1-(3-nitrophenyl)prop-2-en-1-one (29a):**[48]



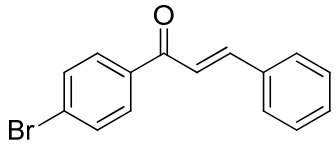
The title compound was prepared according to General Procedure F in 40% yield. Brown solid, mp = 201-203 ºC (EtOH); R*f* = 0.26 (25% EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.84 (1H, s), 8.47 (1H, d, *J* = 7.5 Hz), 8.36 (1H, d, *J* = 7.5 Hz), 7.86 (1H, d, *J* = 16.0 Hz), 7.74 (1H, dd, *J* = 7.5, 7.5 Hz), 7.63 (2H, d, *J* = 8.0 Hz), 7.52 (1H, d, *J* = 16.0 Hz), 7.44 (2H, d, *J* = 8.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 187.7, 145.2, 139.3, 137.2, 134.4, 134.1, 130.0, 129.9, 129.4, 127.2, 124.0, 123.2, 121.0.

***(E)*-1-(4-Bromophenyl)-3-(3,4-dichlorophenyl)prop-2-en-1-one (29b):**[49]



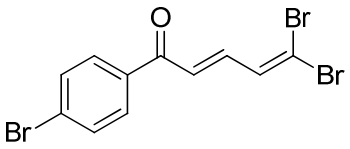
The title compound was prepared according to General Procedure F in 45% yield. White solid, mp = 157-159 ºC (EtOH); R*f* = 0.35 (25% EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91–7.86 (2H, m), 7.74–7.70 (2H, m), 7.69–7.63 (2H, m), 7.52–7.42 (3H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.6, 142.4, 136.4, 134.7, 134.6, 133.3, 132.0, 131.0, 130.0, 129.7, 128.3, 127.5, 122.8.

***(E)*-1-(4-Bromophenyl)-3-phenylprop-2-en-1-one (29c):[50]**



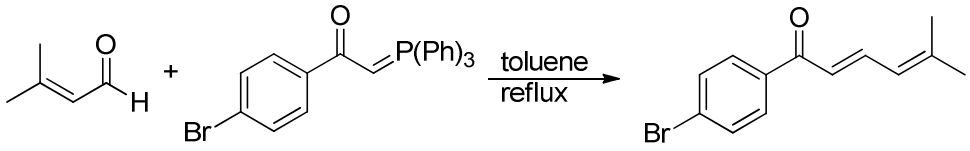
The title compound was prepared according to General Procedure F in 70% yield. White solid. mp = 198-200 ºC (EtOH); R*f* = 0.38 (25% EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94–7.86 (2H, m), 7.74–7.69 (1H, m), 7.70–7.62 (4H, m), 7.53–7.48 (1H, d,), 7.46–7.41 (3H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.8, 142.6, 136.7, 134.9, 133.6, 132.3, 131.2, 130.2, 130.0, 127.8, 123.

***(E)*-5,5-Dibromo-1-(4-bromophenyl)penta-2,4-dien-1-one (54):**



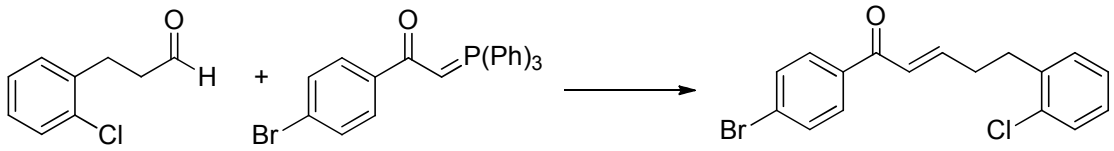
To a stirred solution of the *p*-bromoacetophenone (0.42 g, 2.1 mmol) in acetic acid (5 mL) under argon, was added crude 1,1,1,3-tetrabromo-3-ethoxypropane (0.86 g, 2.1 mmol) via syringe. The reaction mixture was stirred at ambient temperature for 4 days. The resulting mixture was concentrated *in vacuo* and the residue was purified by column chromatography on silica gel using 10-25% EtOAc/hexane as the eluent to afford a yellow solid (0.27 g, 32%), mp = 144-146 ºC (EtOAc/hexanes); R*f* = 0.39 (15% EtOAc/hexanes); IR (solid): υ 3098, 3036, 1650, 1582, 1563, 1397, 1253, 1208, 969, 844, 811, 722, 665 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87–7.77 (2H, m), 7.70–7.58 (2H, m), 7.45 (1H, dd, *J* = 15.0, 11.0 Hz), 7.24 (1H, d, *J* = 11.0 Hz), 7.07 (1H, d, *J* = 15.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.1, 140.2, 136.5, 135.7, 132.3, 130.2, 128.6, 127.3, 102.2; *m/e* (rel intensity) 413 (3), 395 (100), 380 (6), 279 (1); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{11}H_7Br_3O$) calcd 392.8125, found 392.8110.

***(E)*-1-(4-Bromophenyl)-5-methylhexa-2,4-dien-1-one (57):**



Using a procedure adapted from the literature,[51] a solution of 3-methylbut-2-enal (0.4 mL, 4.2 mmol) and [(4-bromobenzoyl)methylene]triphenylphosphorane (0.96 g, 2.1 mmol) in dry toluene (15 mL) was heated at reflux for 24 h. The cooled reaction mixture was concentrated *in vacuo*. The resulting crude product was washed with diethyl ether (2 x 15 mL). The remaining solid triphenylphosphine oxide was filtered off. Evaporation of the washings left a residue which was filtered through a 10 g alumina plug with 30 mL ether:hexane (1:1). Removal of the solvent afforded a yellow solid (0.42 g, 38%), mp = 72-74 ºC (EtOAc/hexanes); IR (solid): υ 2965, 2906, 1655, 1581, 1566, 1352, 1278, 1067, 1005, 989, 811, 722, 666 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.90–7.73 (3H, m), 7.67–7.52 (2H, m), 6.83 (1H, d, J = 14.5 Hz), 6.16 (1H, dd, J = 11.5, 2.0 Hz), 1.96 (6H, d, J = 4.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.9, 149.5, 142.0, 137.5, 132.0, 130.1, 127.7, 124.8, 122.5, 27.1, 19.5; *m/e* (rel intensity) 265 (100); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{13}H_{13}BrO$) calcd 265.0228, found 265.00224.

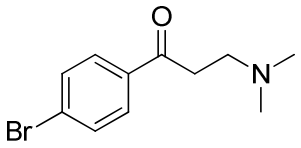
***(E)*-1-(4-Bromophenyl)-5-(2-chlorophenyl)pent-2-en-1-one (65):**



To a solution of [(4-bromobenzoyl)methylene]triphenylphosphorane (0.33 g, 0.72 mmol) in dry CH_2Cl_2 (5 mL) was added 3-(2-chlorophenyl)propionaldehyde (0.10 g, 0.60 mmol) portionwise. The reaction mixture was then stirred for 24 h. The resulting mixture was concentrated *in vacuo* and the residue was purified by column chromatography on silica gel using 10-25% EtOAc/hexanes as the eluent to afford an orange oil (0.20 g, 95%). R*f* = 0.47 (30% EtOAc/hexanes); IR (oil): υ 3054, 3010, 2943, 2892, 1667, 1616, 1582, 1473, 1441, 1397, 1214, 1068, 1050, 1040, 1031, 992, 960, 826, 808, 745, 726, 659 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.84–7.72 (2H, m), 7.64–7.57 (2H, m), 7.40–7.35 (1H, m), 7.24–7.15 (3H, m), 7.14–7.05 (1H, m), 6.82 (1H, dt, J = 15.5, 1.5 Hz), 3.04–2.90 (2H, m), 2.80–2.54 (2H, m); ^{13}C NMR (100 MHz,

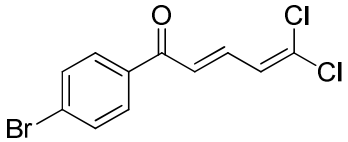
$CDCl_3$) δ 189.7, 148.8, 138.4, 136.6, 134.0, 131.9, 130.6, 130.2, 129.8, 127.9, 127.8, 127.0, 126.3, 32.9, 32.4; *m/e* (rel intensity) 368 (10), 351 (100); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{17}H_{14}BrClO$) calcd 348.9995, found 348.9998.

1-(4-Bromophenyl)-3-(dimethylamino)propan-1-one (71):[39]



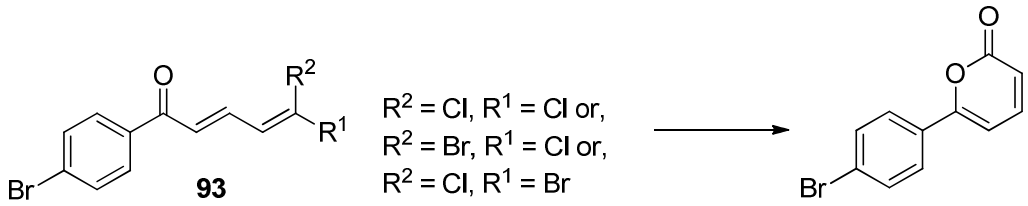
Using a procedure adapted from the literature,[39] a solution of *p*-bromoacetophenone (1.0 g, 5.0 mmol), dimethylamine hydrochloride (0.54 g, 6.5 mmol) and paraformaldehyde (0.20 g, 2.2 mmol) in dioxane (15 mL) was added concentrated HCl (10 μL). The reaction mixture was then heated at reflux for 2 h. The resulting mixture was concentrated *in vacuo* and the residue was purified by column chromatography on silica gel using $MeOH/CH_2Cl_2/Et_3N$ (5:94.9:0.1) as the eluent to afford a yellow solid (0.30 g, 17%), mp = 110-112 ºC ($MeOH/CH_2Cl_2$); R*f* = 0.10 (5% $MeOH/CH_2Cl_2$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.90–7.76 (2H, m), 7.68–7.50 (2H, m), 3.15–2.99 (2H, m), 2.79–2.61 (2H, m), 2.44–2.04 (6H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.2, 135.8, 132.1, 129.8, 128.4, 54.4, 45.7, 37.1;

***(E)*-1-(4-Bromophenyl)-5,5-dichloropenta-2,4-dien-1-one (73):**[52]



The title compound was prepared according to General Procedure E in 36% yield. Yellow solid, mp = 114-117 ºC (EtOAc/hexanes); R*f* = 0.47 (10% EtOAc/hexanes); IR (solid): υ 3054, 2986, 1660, 1595, 1586, 1398, 1276, 1008, 908, 768, 705 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.90–7.74 (2H, m), 7.70–7.51 (2H, m), 7.57 (1H, dd, *J* = 15.0, 11.0 Hz), 7.01 (1H, d, *J* = 15.0 Hz), 6.70 (1H, d, *J* = 11.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.9, 137.9, 136.5, 132.3, 131.4, 130.2, 128.6, 127.7, 127.0; *m/e* (rel intensity) 380 (15), 332 (1), 306 (100); HRMS (ESI) *m/e* $[M + H]^+$ for ($C_{11}H_7BrCl_2O$) calcd 304.9135, found 304.9145.

6-(4-Bromophenyl)-2H-pyran-2-one (94):[52]



A solution of lithium chloride (67 mg, 1.6 mmol) and compound **93** in DMF (5.0 mL) was refluxed for 3 h. The cooled reaction mixture was diluted with ethyl acetate (10 mL). The organic layer was then washed with H_2O (10 mL x 5), brine, dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel using 30-50% EtOAc in hexane as the eluent to afford a yellow solid (40 mg, 81%), mp = 77-79 ºC (EtOAc/hexanes); R*f* = 0.28 (50% EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65–7.73 (2H, m) 7.57–7.62 (2H, m) 7.42 (1H, dd, *J* = 9.5, 7.0 Hz) 6.65 (1H, dd, *J* = 7.0, 1.0 Hz) 6.31 (1H, dd, *J* = 9.5, 1.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.8, 160.2, 143.8, 132.5, 130.5, 127.2, 125.7, 114.7, 101.4.

References

1 Silver, L.; Bostian, K. Eur. J. Clin. Microbiol. Infect. Dis. **1990**, *9*, 455 – 461.

2 Nussbaum, F. V.; Brands, M.; Hinzen, B.; Weigand, S.; Häbich, D. *Angew. Chem. Int. Ed.* **2006***, 45*, 5072-5129.

3 Levy, S. B.; Marshall, B. *Nat. Med.* **2004***, 10*, S122-129.

4 Andersson, D. I. *Curr. Opin. Microbiol.* **2003**, *6*, 452-456.

5 Michel, K. H.; Hastner, R. E. U.S. Patent. 4,492,650. Jan 8, 1985.

6 Hinzen, B.; Raddatz, S.; Paulsen, H.; Lampe, T.; Schumacher, A.; Häbich, D.; Hellwig, V.; Benet-Buchholz, J.; Endermann, R.; Labischinski, H.; Brötz-Oesterhelt, H. *ChemMedChem* **2006***, 1*, 689-693.

7 Cossette, M. MSc. Dissertation, University of Toronto. 2012.

8 Brötz-Oesterhelt, H.; Beyer, D.; Kroll, H.; Endermann, R.; Ladel, C.; Schroeder, W.; Hinzen, B.; Raddatz, S.; Paulsen, H.; Henninger, K.; Bandow, J. E.; Sahl, H.; Labischinski, H. *Nat. Med.* **2005***, 11*, 1082-1087.

9 Koshino, H.; Osada, H.; Yano, T.; Uzawa, J.; Isono, K. *Tetrahedron Lett*. **1991**, *32*, 7707 – 7710.

10 Osada, H.; Yano, T.; Koshino, K.; Isono, J. *J. Antibiotics* **1991***, 44*, 1463-1466.

11 Lee, B.; Park, E. Y.; Lee, K.; Jeon, H.; Sung, K. H.; Paulsen, H.; Rubsamen-Schaeff, H.; Brotz-Oesterhelt, H.; Song, H. K. *Nat Struct Mol Biol* **2010***, 17*, 471-478.

12 Yu, A. Y. H.; Houry, W. A. *FEBS Letters* **2007***, 581*, 3749-3757.

13 Brötz-Oesterhelt, H.; Hamoen, L.; Sahl, H-G.; Schiffer, G.; Famulla, K.; Josten, M.; Sass, P. *PNAS* **2011**, *108*, 17474-17479.

14 Erickson, H. P.; Taylor, D. W.; Taylor, K. A.; Bramhill, D. *PNAS* **1996**, *93*, 519-523.

15 Ma, X.; Ehrhardt, D. W.; Margolin, W. *PNAS* **1996**, *93*, 12998-13003.

16 Harvey, A. L. *Drug Discov. Today* **2008**, *13*, 895-901.

17 Leung, E.; Datti, A.; Cossette, M.; Goodreid, J.; McCaw, S. E.; Mah, M.; Nakhamchik, A.; Ogata, K.; Bakkouri, M. E.; Cheng, Y. Q.; Wodak, S.J.; Eger, B. T.; Pai, E. F.; Liu, J.; Gray-Owen, S.; Batey, R. A.; Houry, W. A. *Chem Biol.* **2011**, *18*, 1167-1178.

18 Lourenco, M. C. S.; Ferreira, M. L.; Peralta, M. A.; Kaiser, C. R.; Pais, K. C.; Souza, M. V. N. *Bioorg. Med. Chem.* **2009**, *17*, 1474-1480.

19 Kaminsky, D.; Meltzer, R. I. *J. Med. Chem.* **1968**, *11*, 160-163.

20 Font, M.; Monge, A.; Ruiz, I.; Heras, B. *Drug Des. Discovery* **1997**, *14*, 259-264.

21 Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Deliv. Rev.* **2001***, 46*, 3-26.

22 Saaden, H. A.; Mosleh, I, M.; Mubarak, M. S. *Molecules* **2009**, *14*, 1483-1494.

23 Chaudhari, S. S.; Akamanchi, K. G. *Synlett.* **1999,** *11*, 1763-1765.

24 Kroemer, G.; *Oncogene* **2006**, *25*, 4630-4632.

25 Perjesi, P.; Berki, T.; Rozmer, Z. *Toxicol. in Vitro* **2006**, *20*, 1354-1362.

26 Woznesensky, S. A.; Dudinov, A. A.; Belenkii, L. L.; Struchkova, M. L.; Nesterov, K N.; Krayushkin, M. M.; Struchkov, Y. T. *Russ. Chem. Bull.* **1997**, *46*, 501-505.

27 Usova, E. B.; Lysenko, L. I.; Krapivin, G. D.; Kul'nevich, V. G. *Chem. Heterocycl. Compd.* **1996,** *32*, 548-554.

28 Ceylan, M.; Dingil, A.; Gudere, M. B.; Gezegen, H.; Karaman, Isa. *Chem. Biodivers.* **2010**, *7*, 400-403.

29 Wattanasin, S.; Murphy, W. S.; *Synthesis* **1980**, *8*, 647-651.

30 Chukovskaya, E. T.; Kamyshova, A. A.; Freidlina, R . K. *Russ. Chem. Bull.* **1965**, *3*, 445-449.

31 Schroth, W.; Schmiedl, D.; Jahn, U.; Spitzner, R. *Z. Chem.* **1989**, *29*, 419-420.

32 Muñoz-Molina, M.; Belderrain, T. R.; Pérez, P. J. *Eur. Org. Chem.* **2011**, *21*, 3155-3164.

33 Muehlebach, M.; Fisera, R.; Karvas, M.; Rempfler, H.; Brunner, H. Intl. Patent. WO/2003/07640. Sept 18, 1985.

34 Reichardt, C. *Solvents and Sotvenr Effects in Organic Chemistry*.; Verlag-Chemie, Weinheim, 1988.

35 Kluge, A. F.; Lillya, C. P. *J. Org. Chem.* **1971**, *36*, 1988-1995.

36 Lee, C.; Hisa, A.; Nagaraj, H. K. M.; William, A. D.; Williams, M.; Xiong, Z. Intl. Patent. WO/2009/093981. Jul 30, 2009.

37 Schuler, M.; Duvvuru, D.; Retailleau, P.; Betzer, J.; Marinetti, A. *Org. Lett.* **2009**, *11*, 4406-4409.

38 Heim-Reither, A. *Synthesis* **2008,** 883-886.

39 Max, C. E.; *Org. Syn. Coll. Vol. 3*, **1955**, *23*, 305-306.

40 Brahmbhatt, D. I.; Pandya, Urvish R. *Indian J. Chem., Sect B* **2001**, *40*, 419-421.

41 Paterson, I.; Kan, J.; Gibson, L. J. *Org. Lett.* **2010**, *12*, 3724–3727.

42 Adger, B. J.; Barrett, C.; Brennan, J.; McGuigan, P.; McKervey, M. A.; Tarbit, B.; *J. Chem. Soc., Chem. Commun.* **1993**, *13*, 1220-1222.

43 Yorimitsu, H.; Shinokubo, H.; Matsubara, S.; Oshima, K. *J. Org. Chem.*, **200**1, *66*, 7776–7785.

44 Bauer, A. W.; Perry, D. M.; Kirby, V. M. *Arch. Intern. Med.* **1959**, *104*, 208–216.

45 Murray, P. R.; Baron, E. J.; Jorgensen, J. H.; Landry, M. L.; Pfaller, M. A. Susceptibility Test Methods: Dilution and Disk Diffusion Methods. In *Manual of Clinical Microbiology*; Jorgensen, J. H.; Turnidge, J. D., Ed.; ASM Press: Washington, D.C., 2007; p 1152–1172.

46 Agrawal, V. K.; Sharma, S. *Indian J. Chem., Sect B* **1987**, *26*, 550-555.

47 Youngsaye, W.; Hartland, C. L.; Morgan, B. J.; Buhrlage, S. J.; Johnston, S.; Bittker, J. A.; MacPherson, L.; Dandapani, S.; Palmer, M.; Schreiber, S. L.; Munoz, B.; Vincent, B.; Whitesell, L.; Lindquist, S. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5502-5505.

48 Dzurilla, M.; Kristian, P. *Coll. Czech CC.* **1970**, *35*, 417-429.

49 Al-Arab, M. M.; Ghanem, B. S.; Fitton, A. O. *Tetrahedron* **1989**, *45,* 6545-6552.

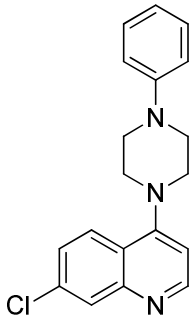
50 Hayat, F.; Salahuddin, A.; Azam, A.; Umar, S**.** *Eur. J. Inorg. Chem.* **2010**, *45*, 4669 – 4675.

51 Lillya, C. P.; Kluge, A. F. *J. Org. Chem*. **1971**, *36*, 1989-1995.

52 Bullot, J. *Bull. Soc. Chim. Fr.* **1960**, 23-26.

Appendix: Spectra of Selected Compounds

5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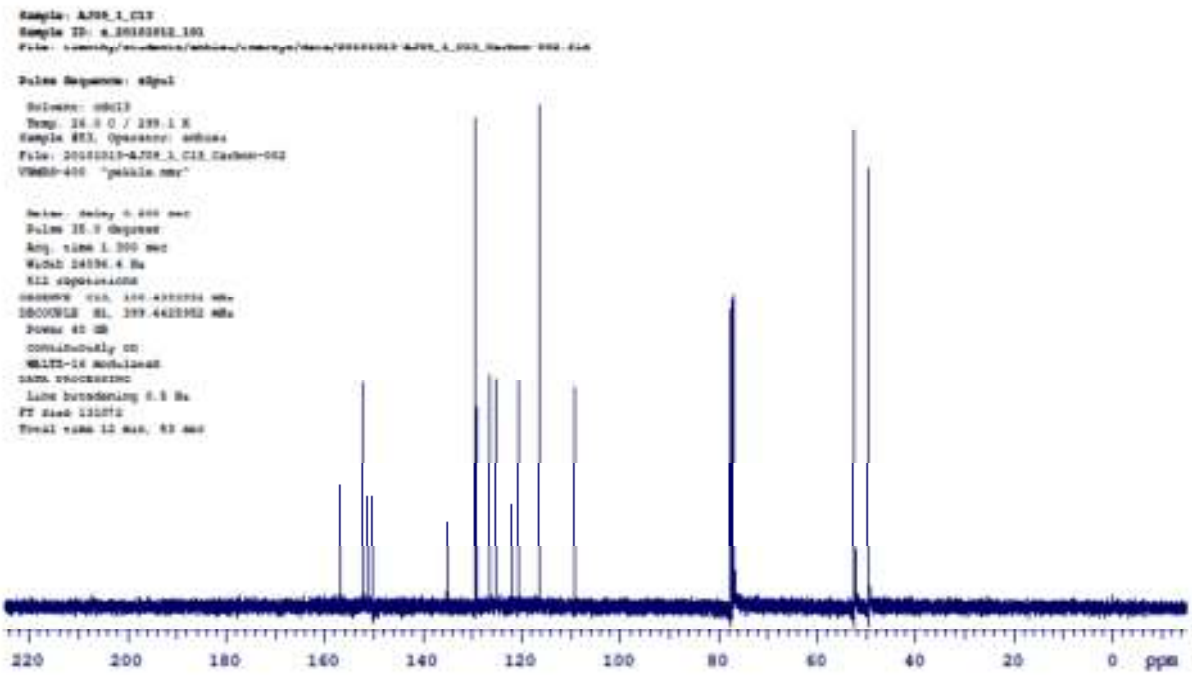
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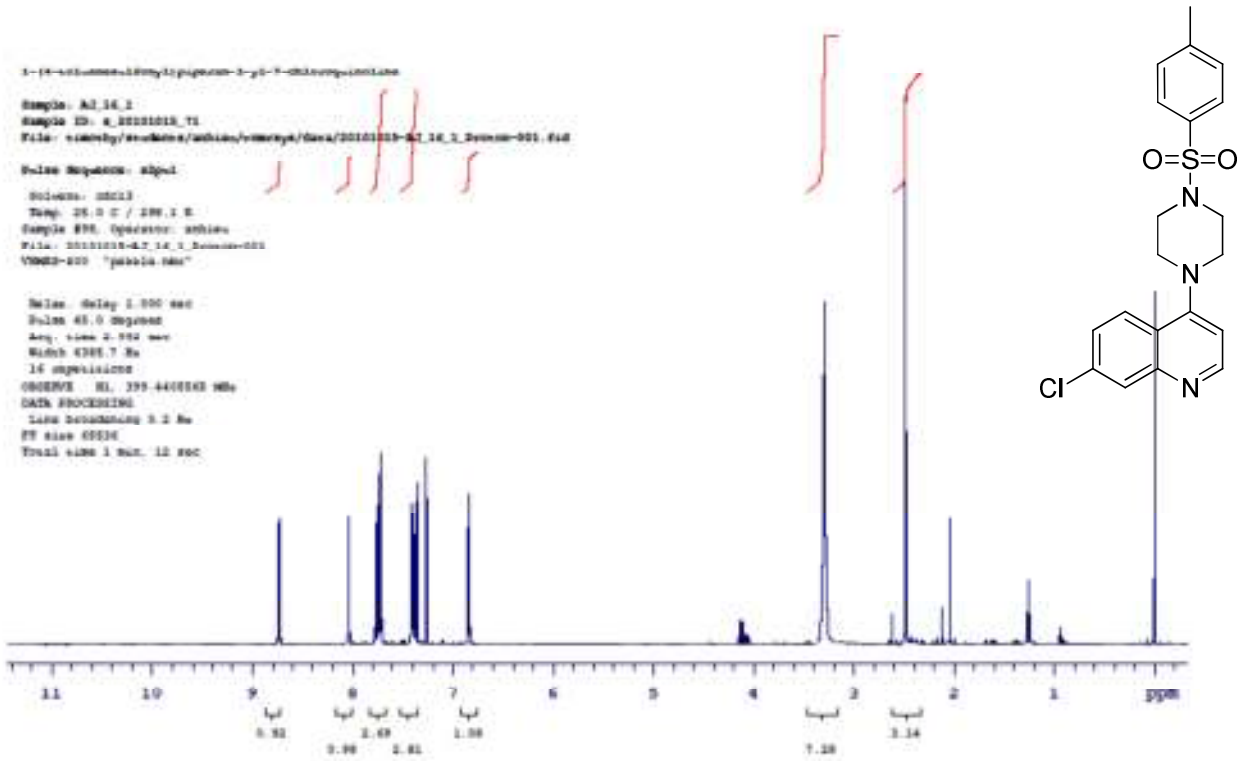

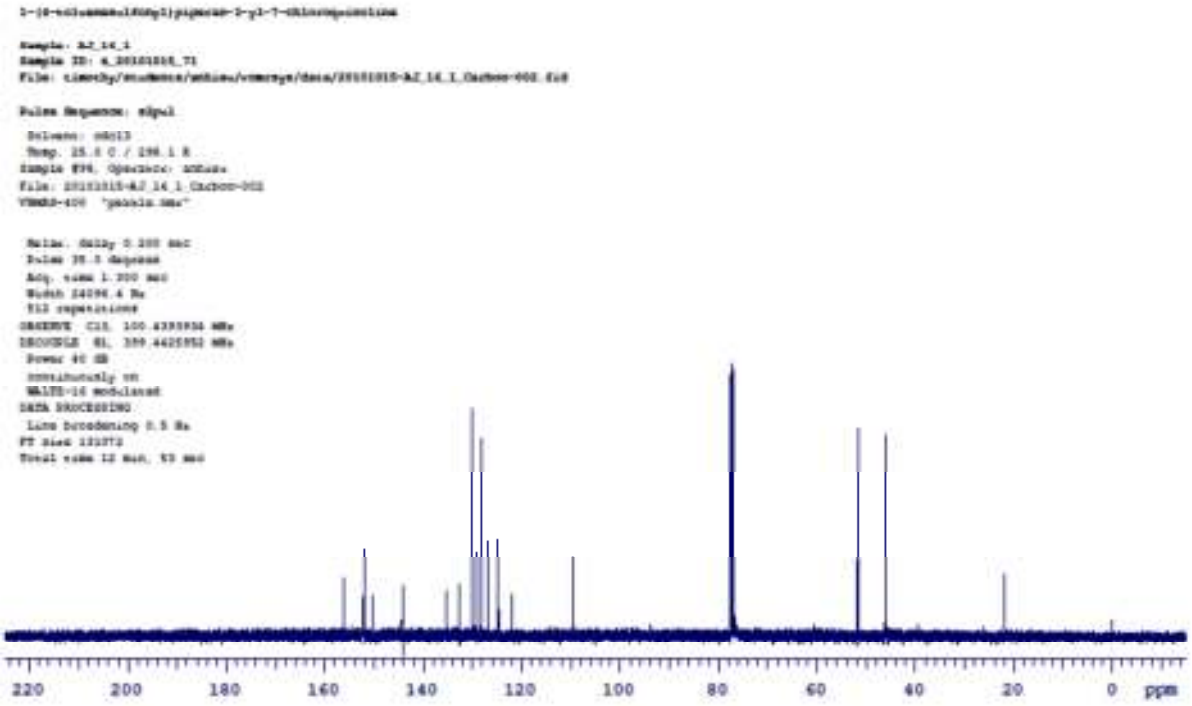

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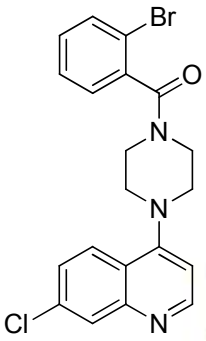
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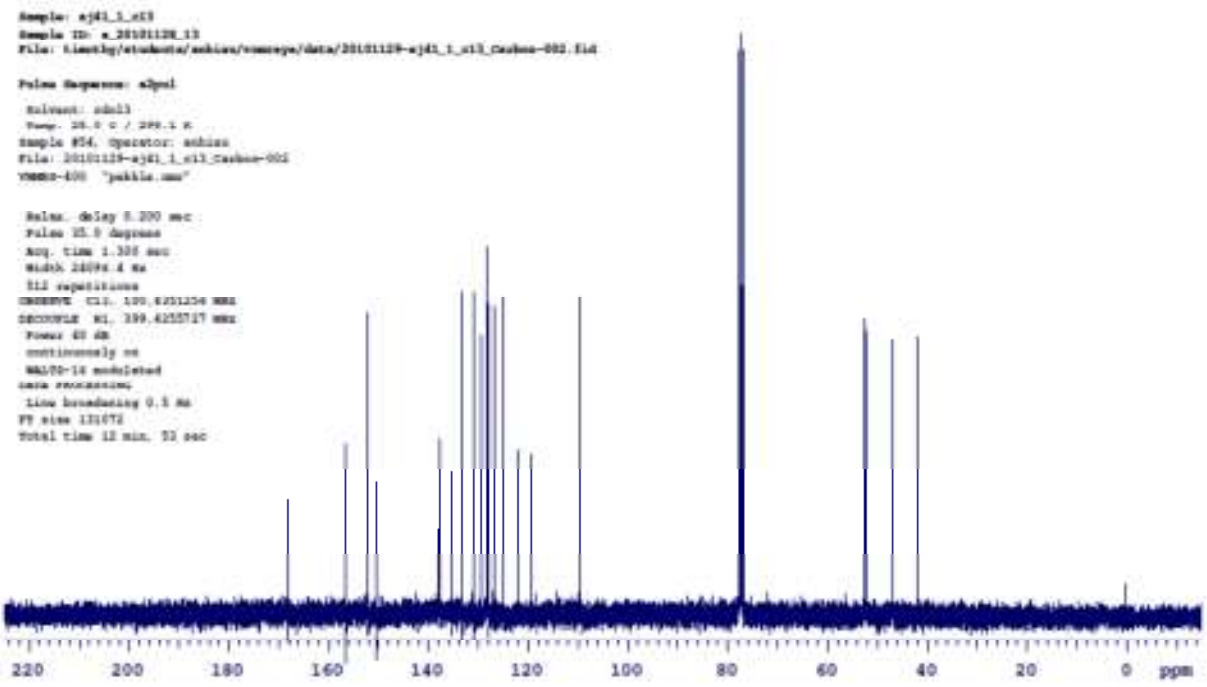
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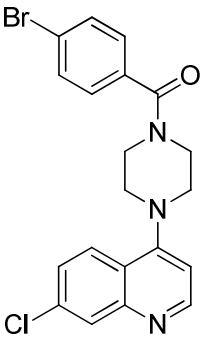
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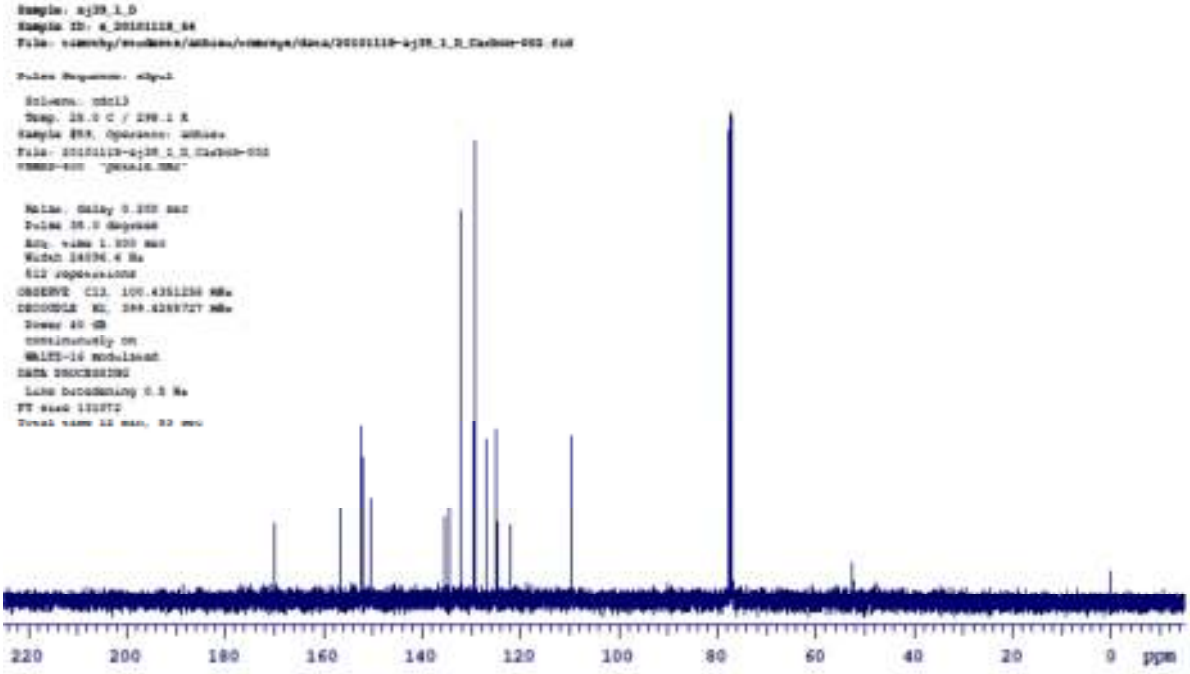
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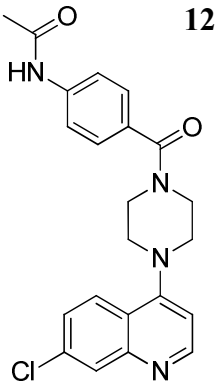
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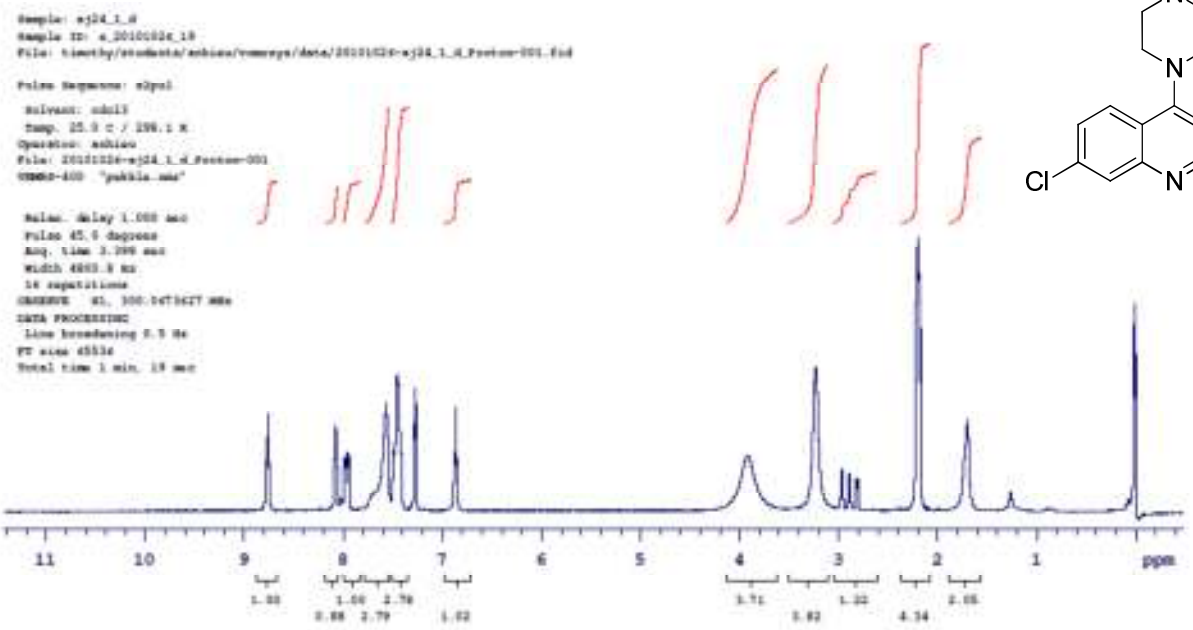
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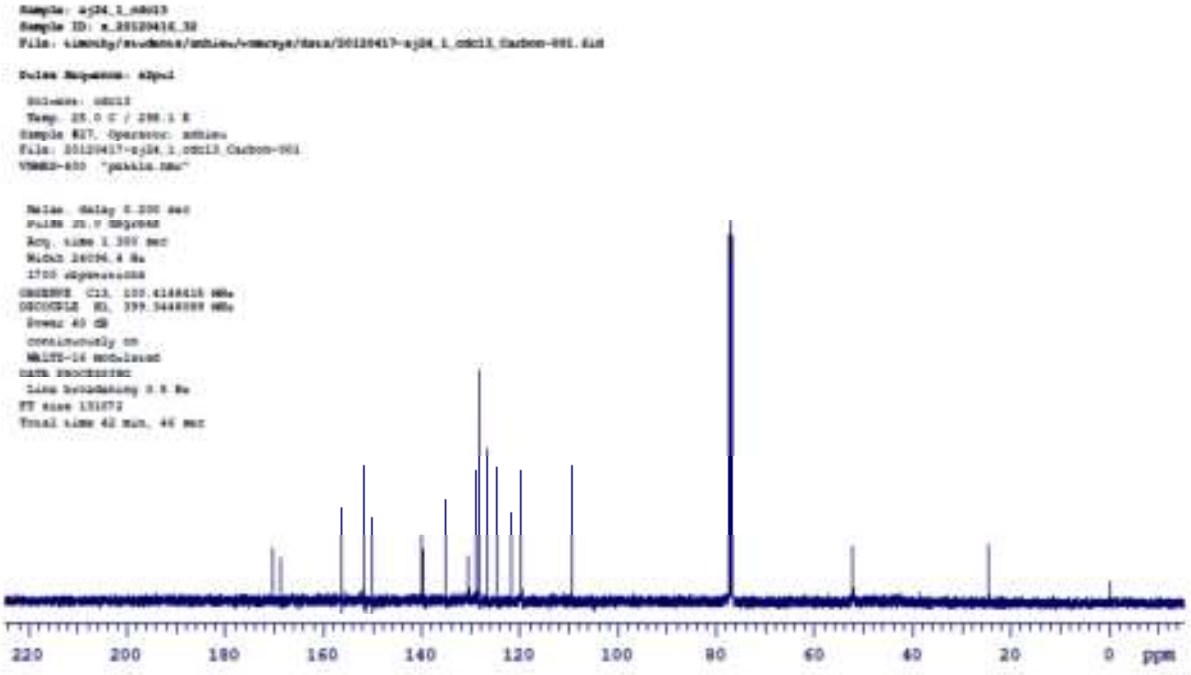
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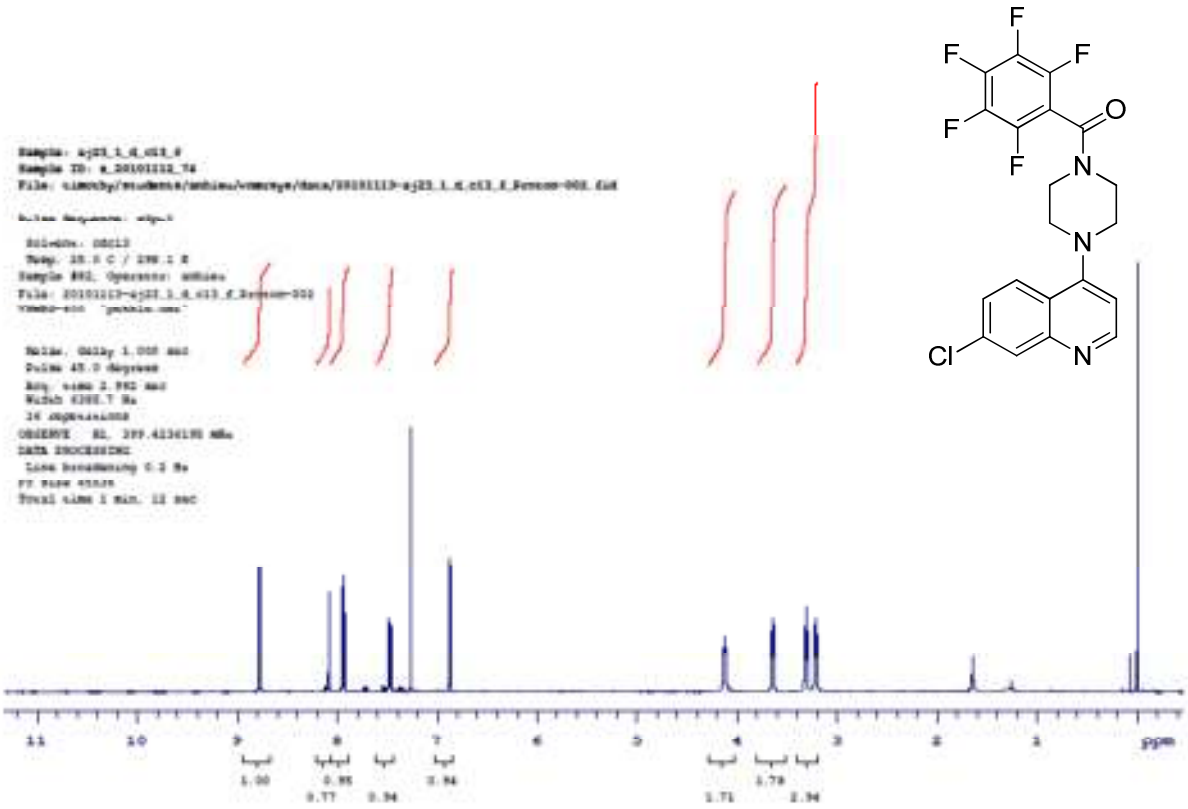

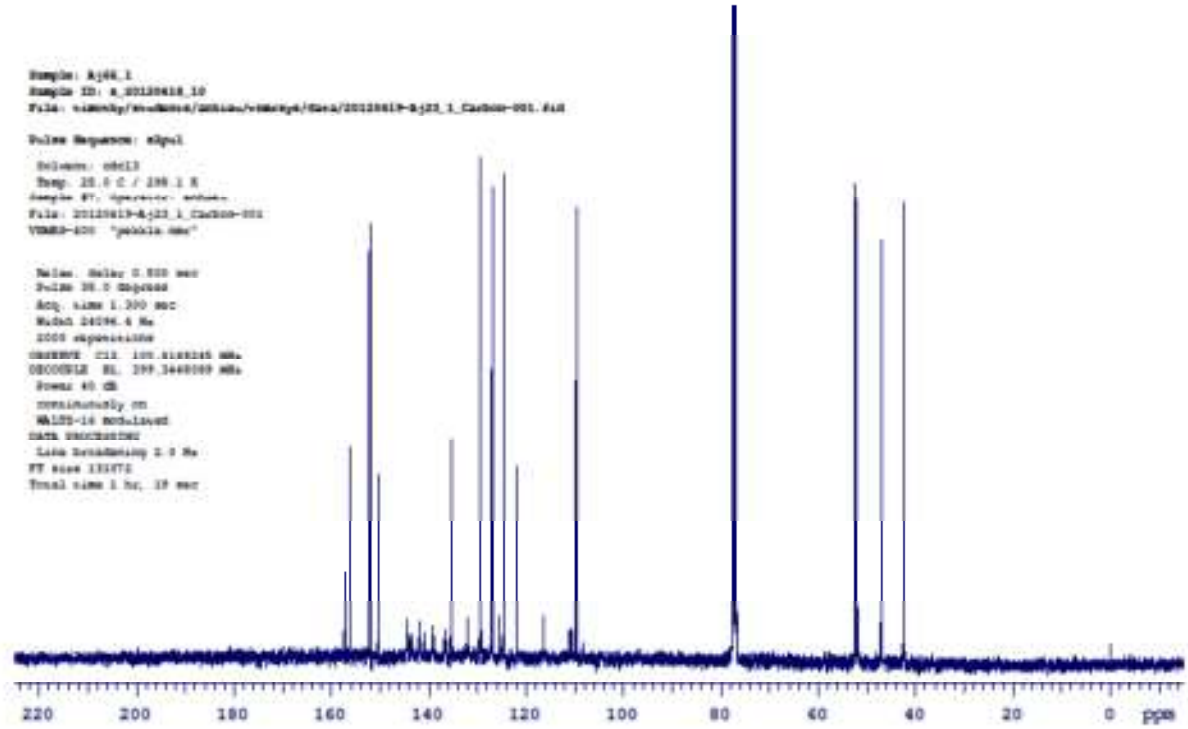

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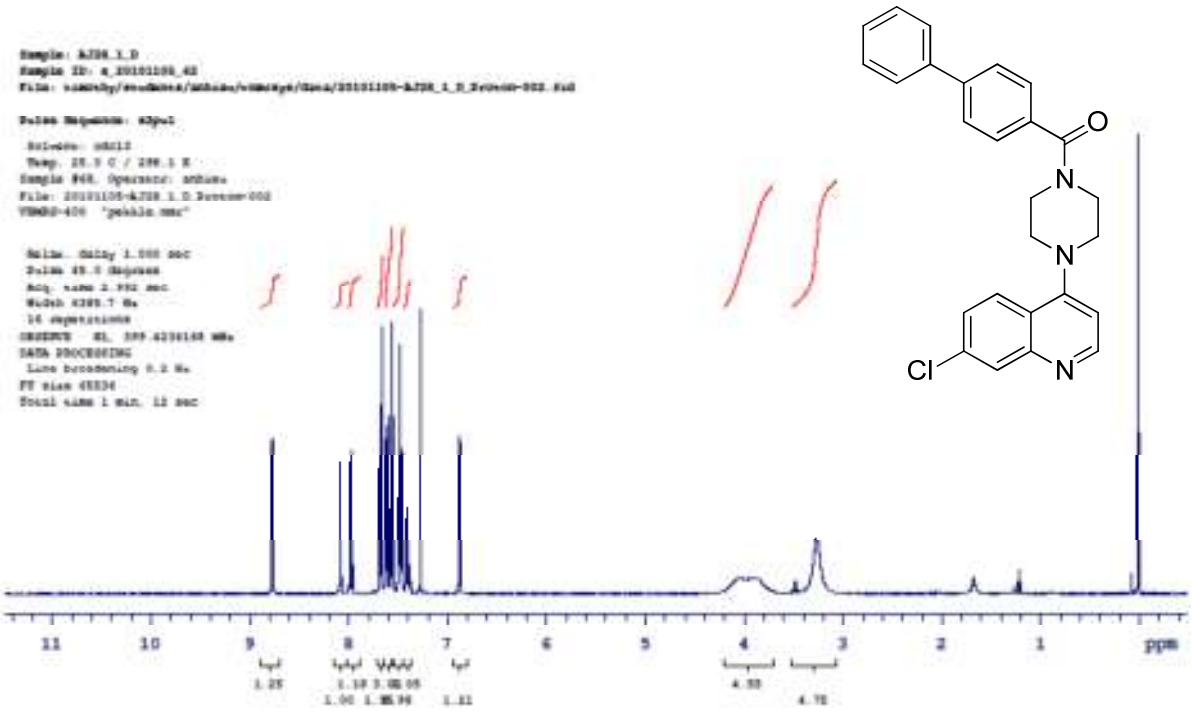
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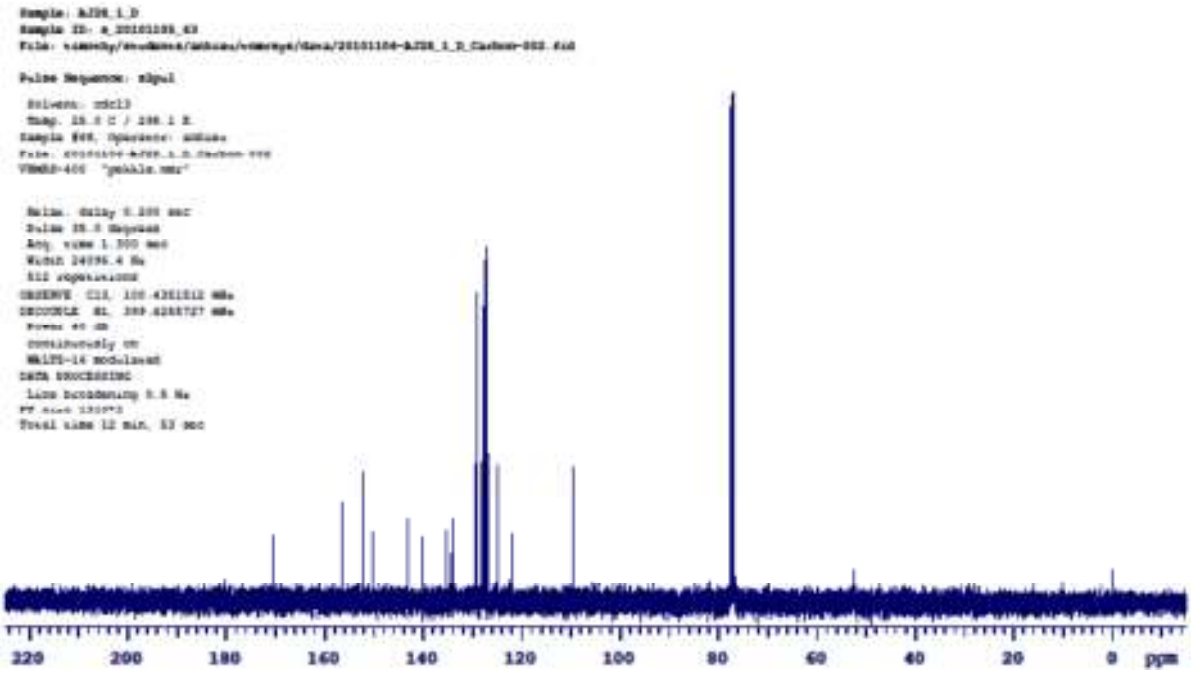
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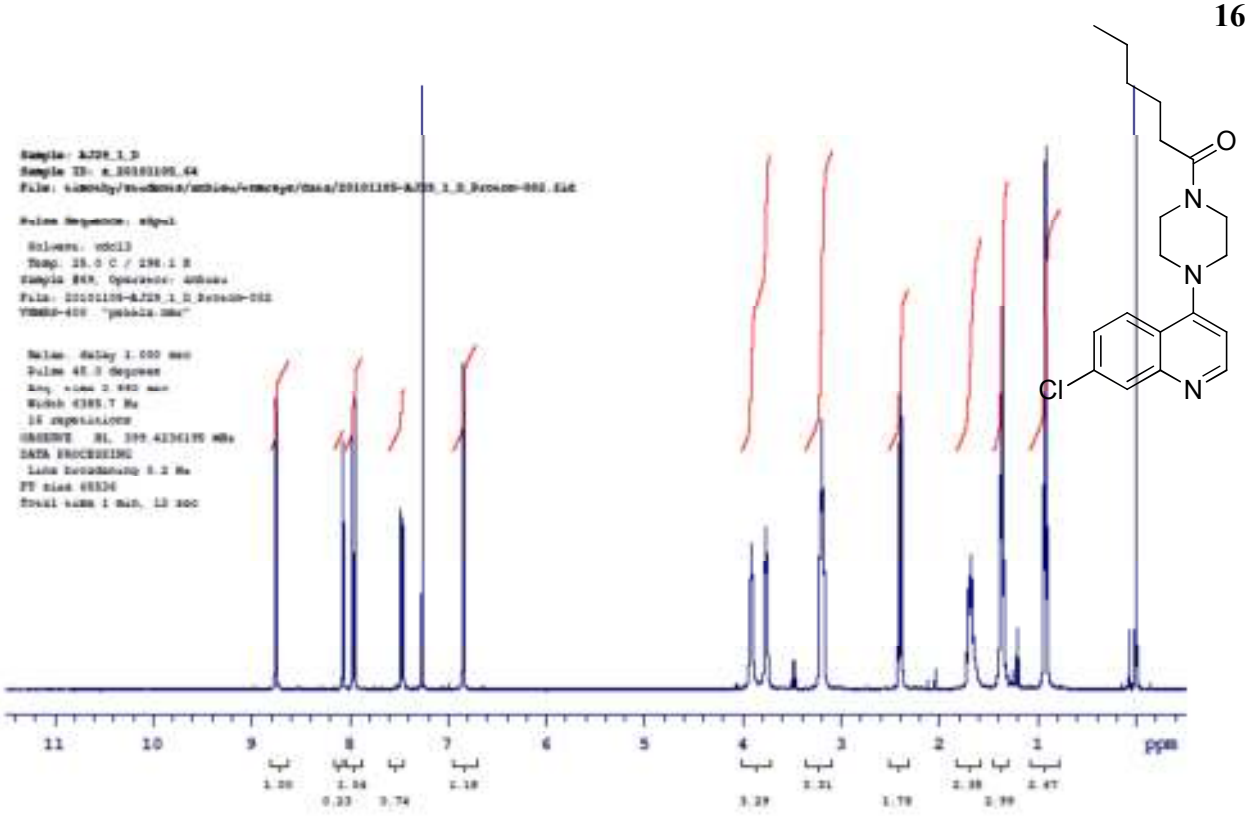

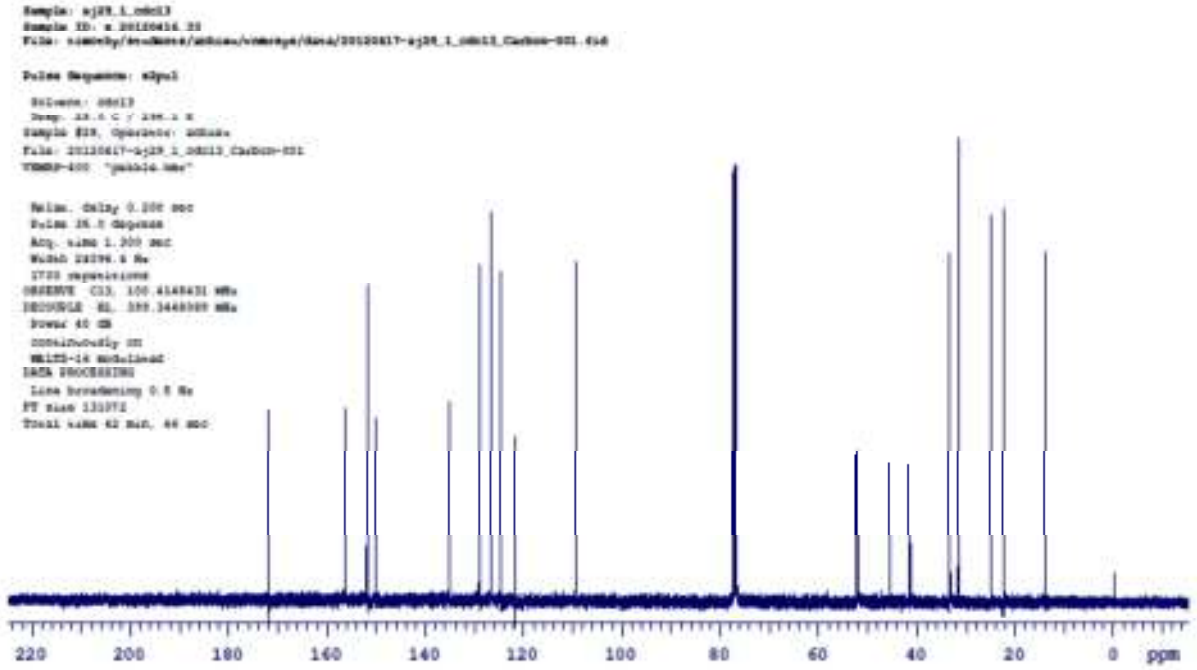

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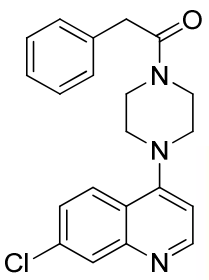
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Cl
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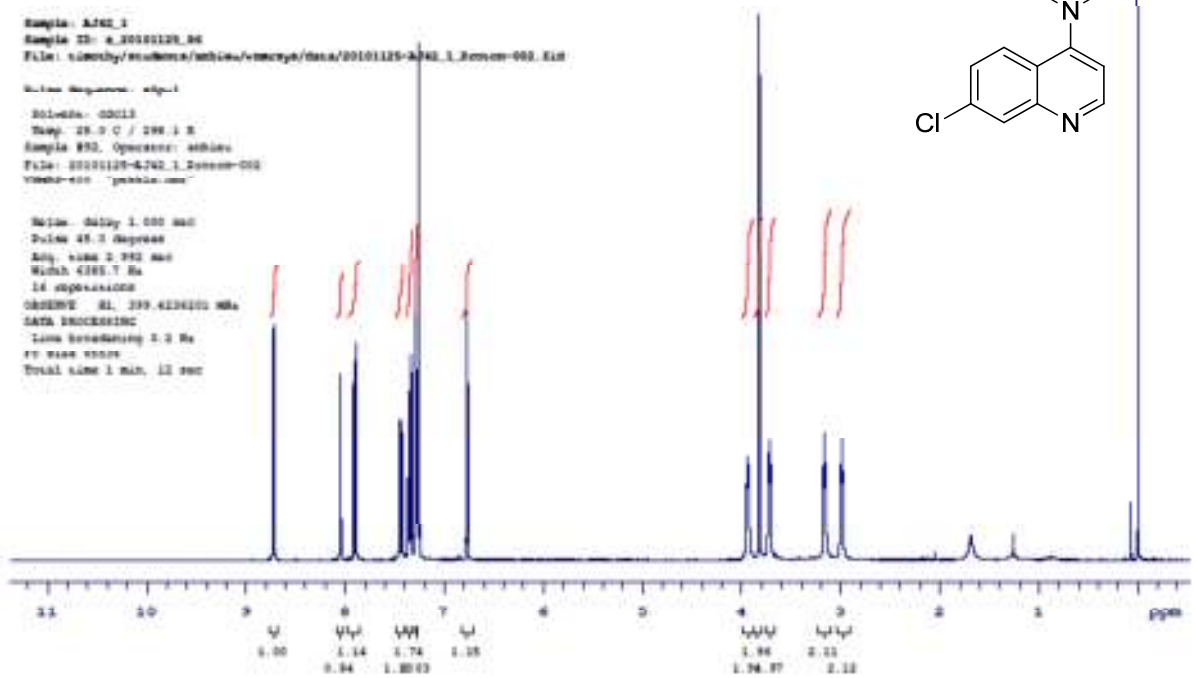
11
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9
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7
6
5
4
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1.14
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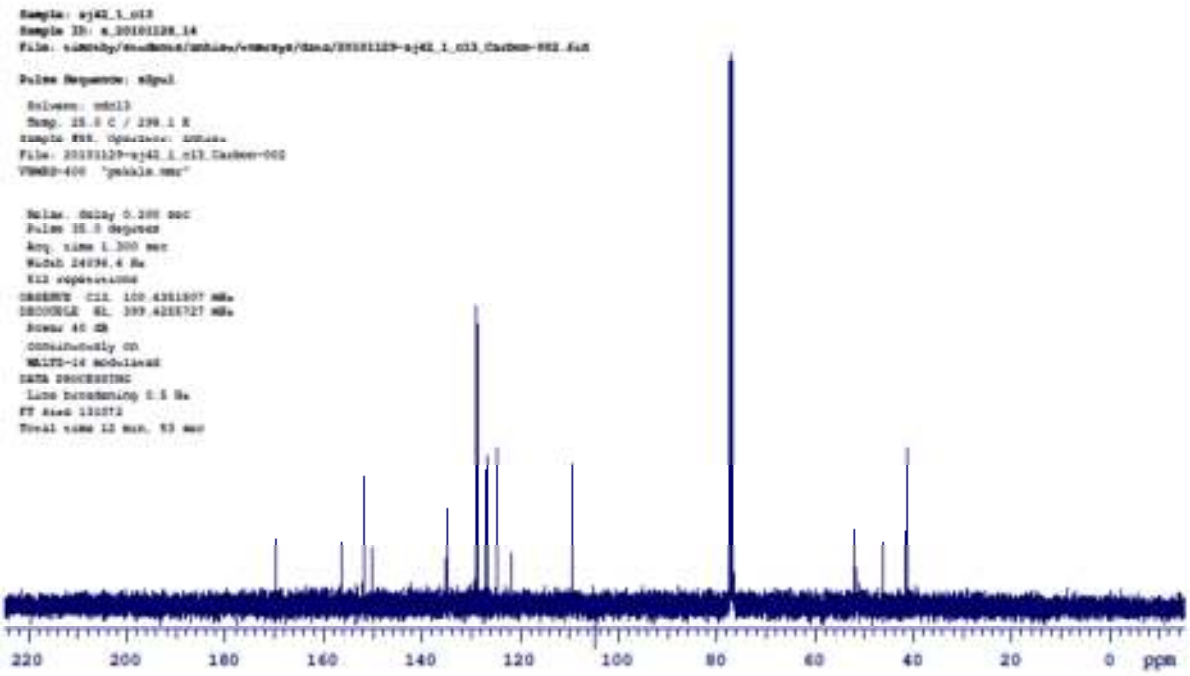
1.74
1.80
1.15
1.96
1.98
2.11
2.12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40
20
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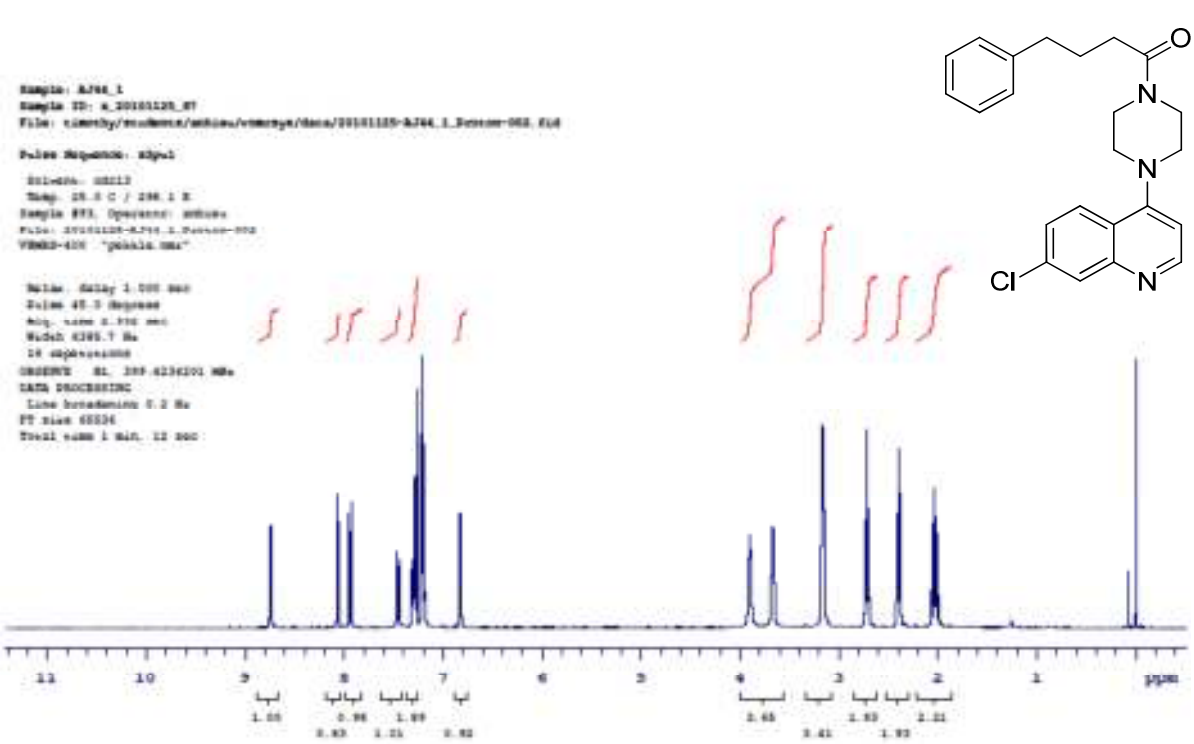
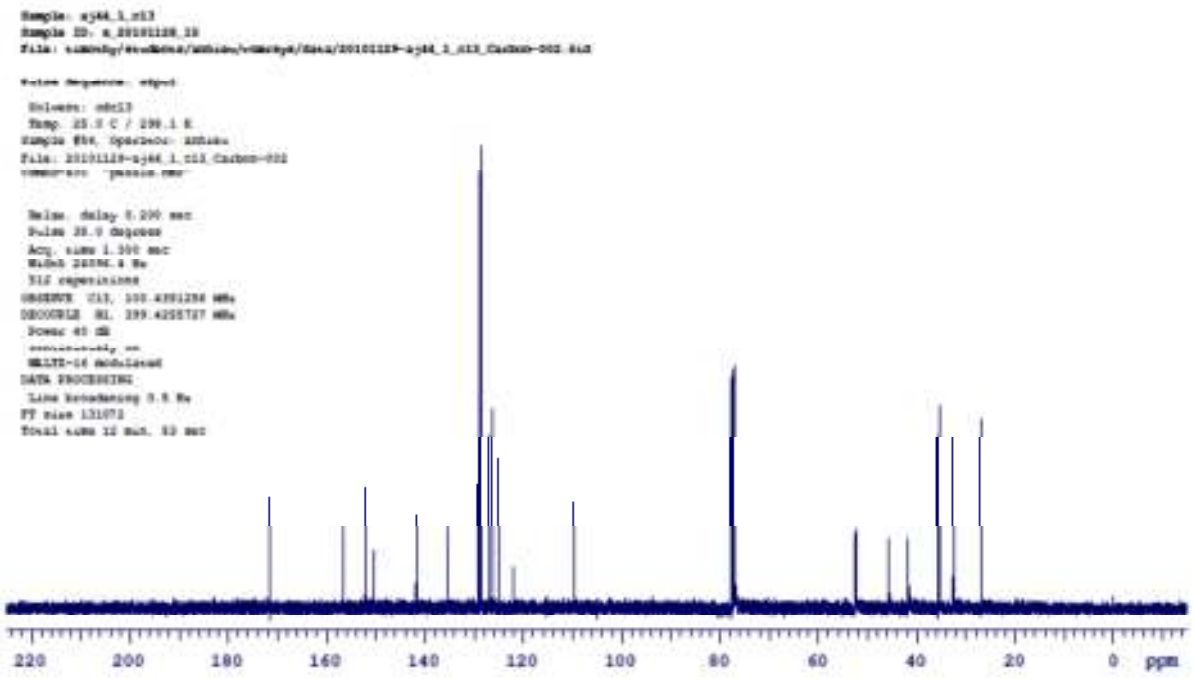
ppm

18





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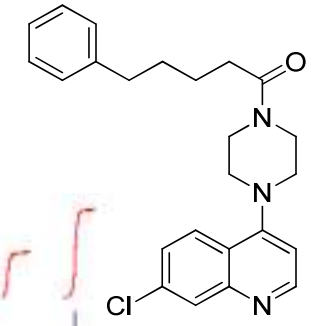
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Cl
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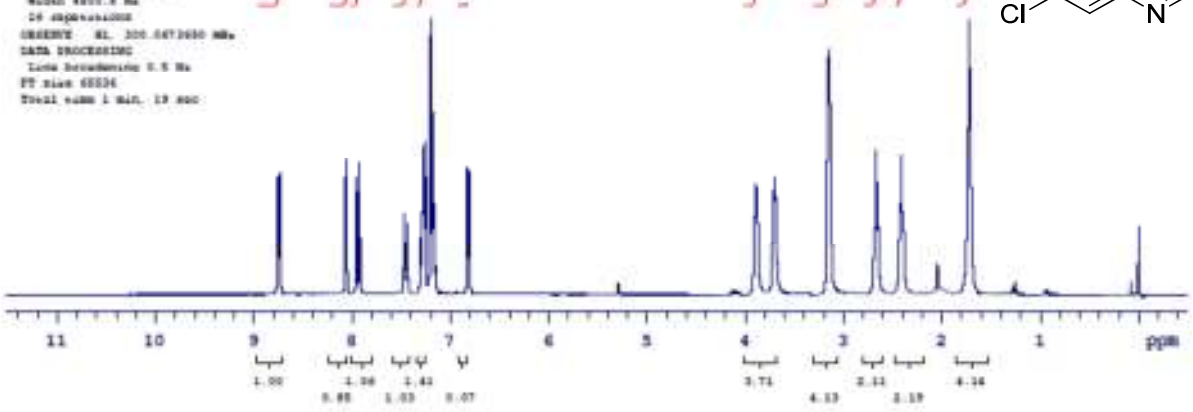
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3
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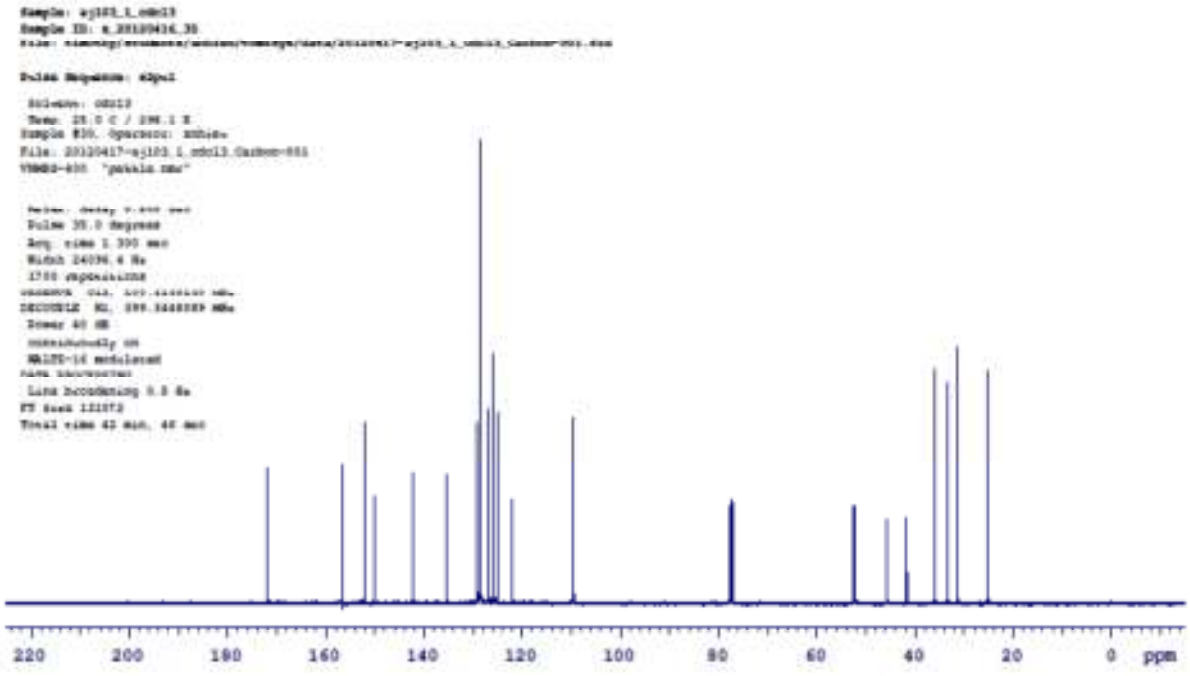
1
ppm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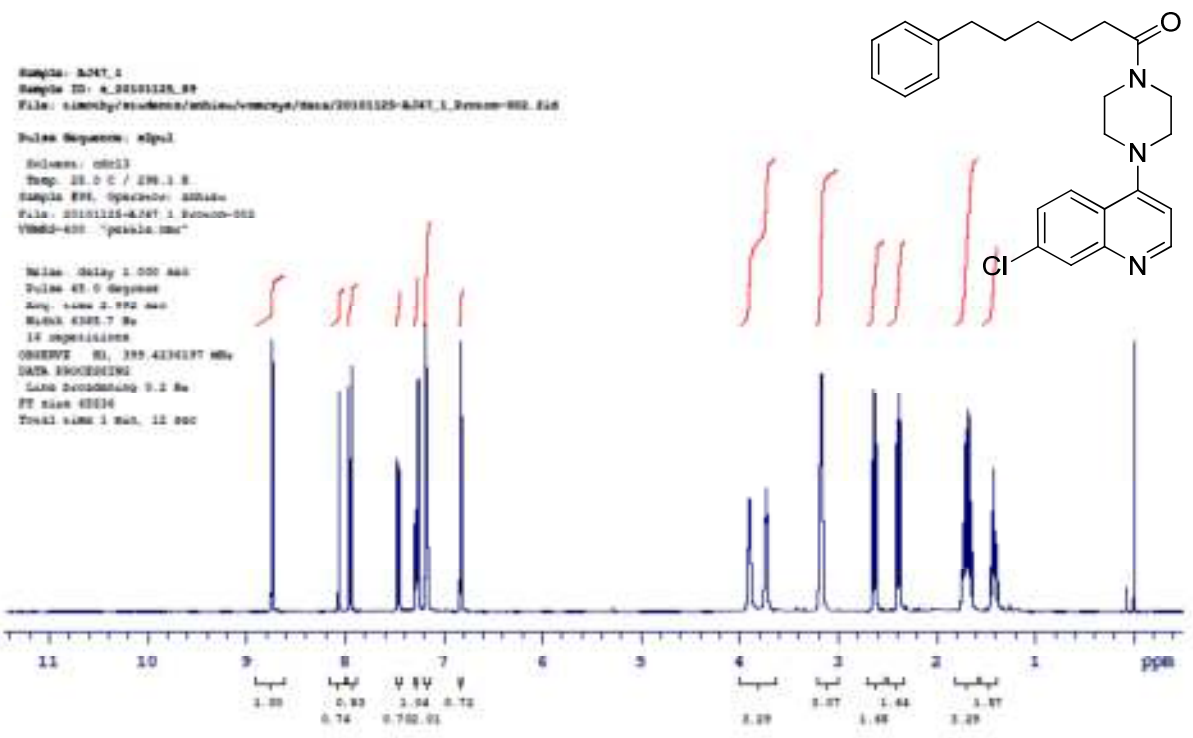
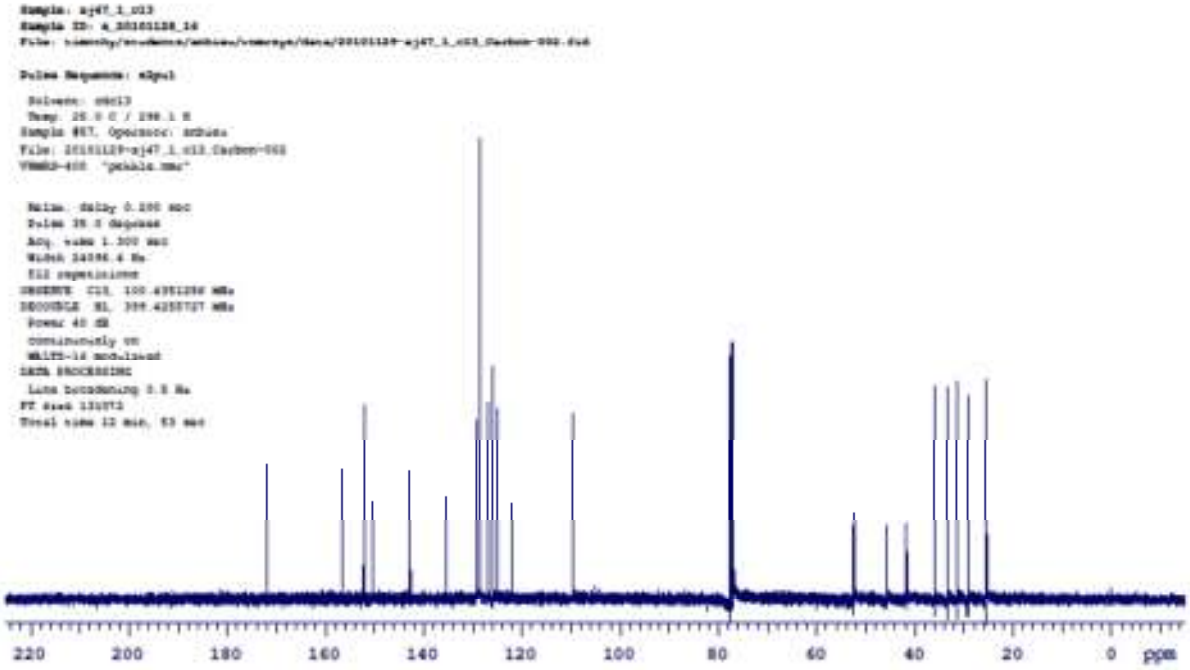
140
120
100
80
60
40
20
0
ppm

20





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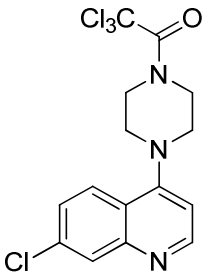
Cl3C
O
N
N
Cl
N

9
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4
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2
1
ppm
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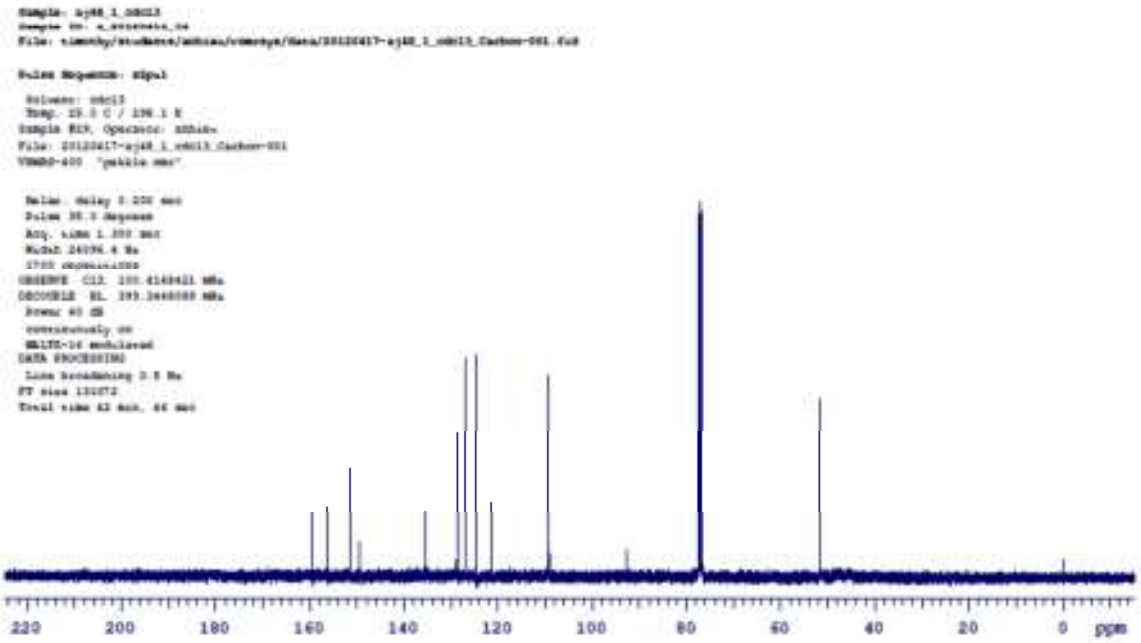
1.07
0.70
1.01
1.31
3.79
3.89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20
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ppm

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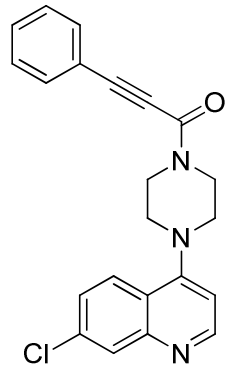
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N
N
Cl
N

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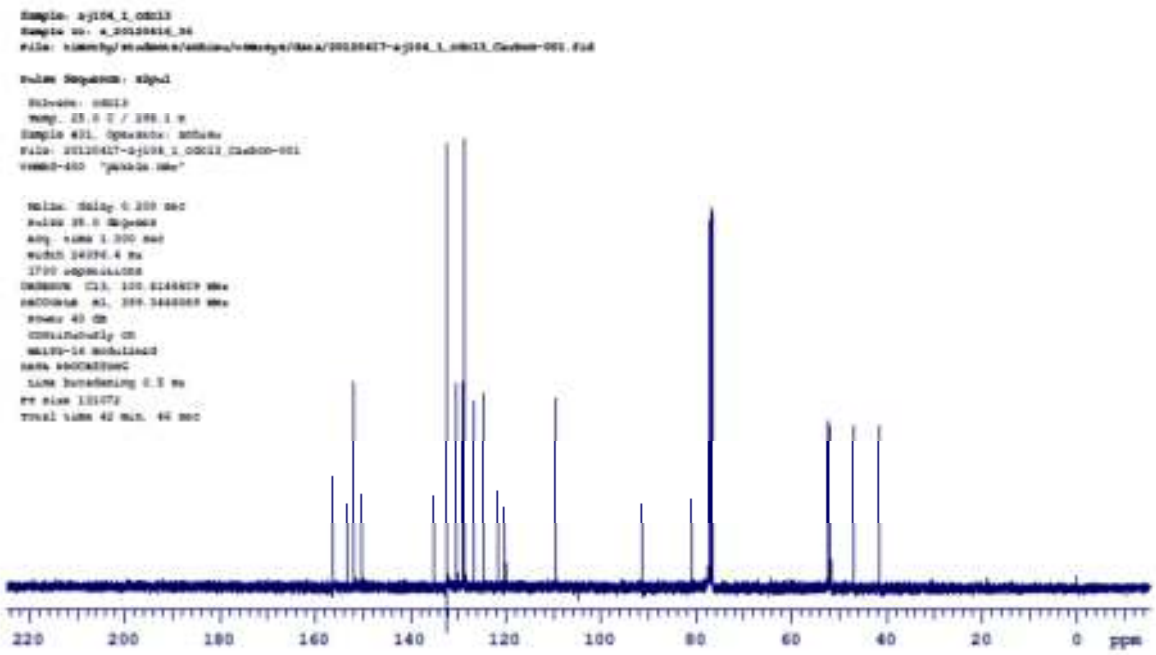
7
6
5
4
3
2
1
ppm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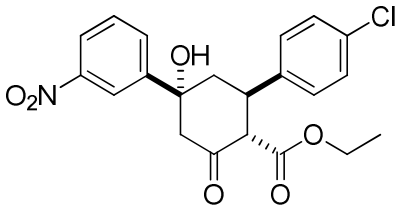
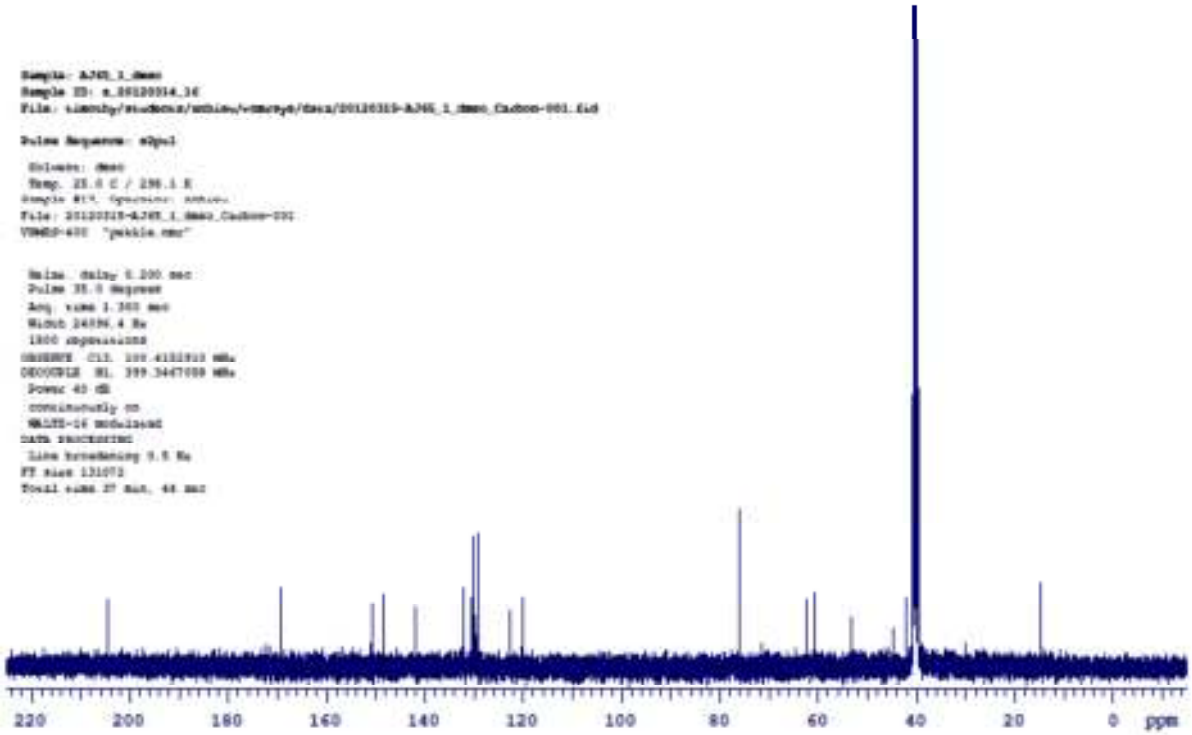
80
60
40
20
0
ppm

31


Cl
OH
O_2N
O
O
O

9
8
7
6
5
4
3
2
1
0 ppm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140
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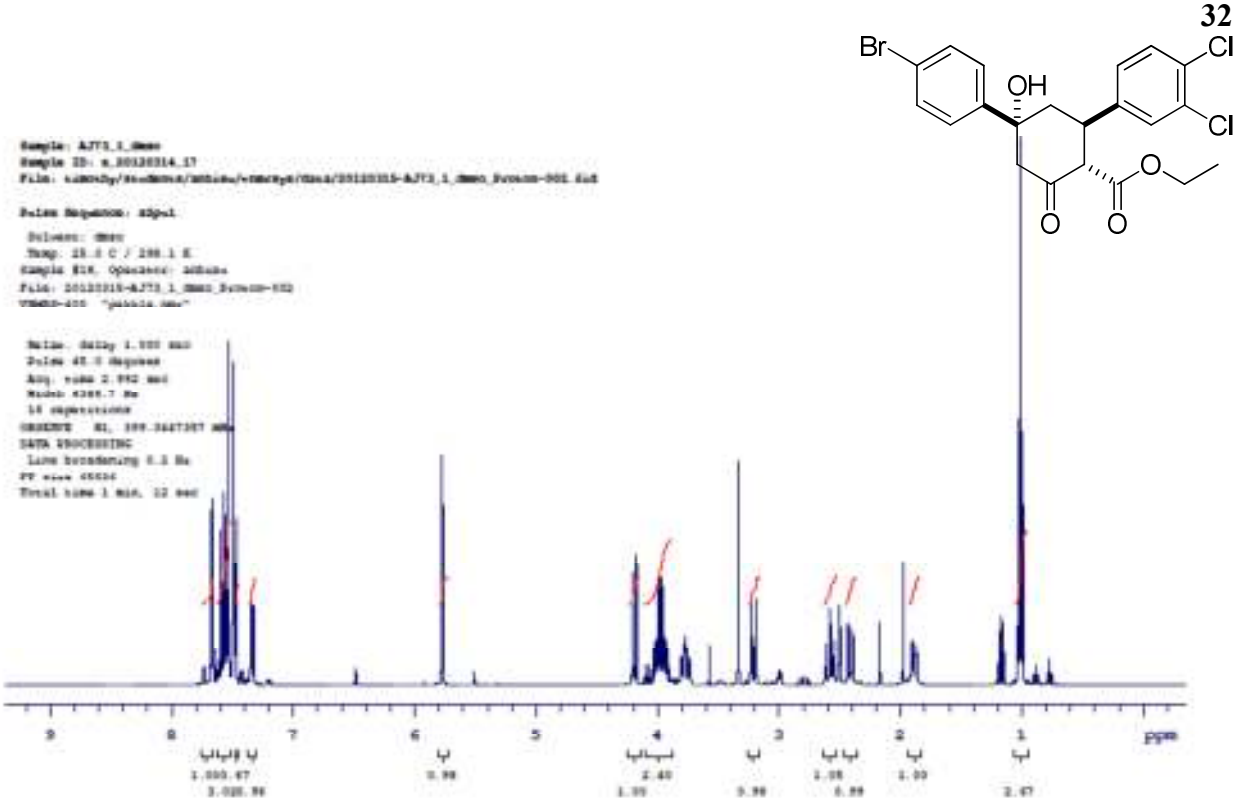
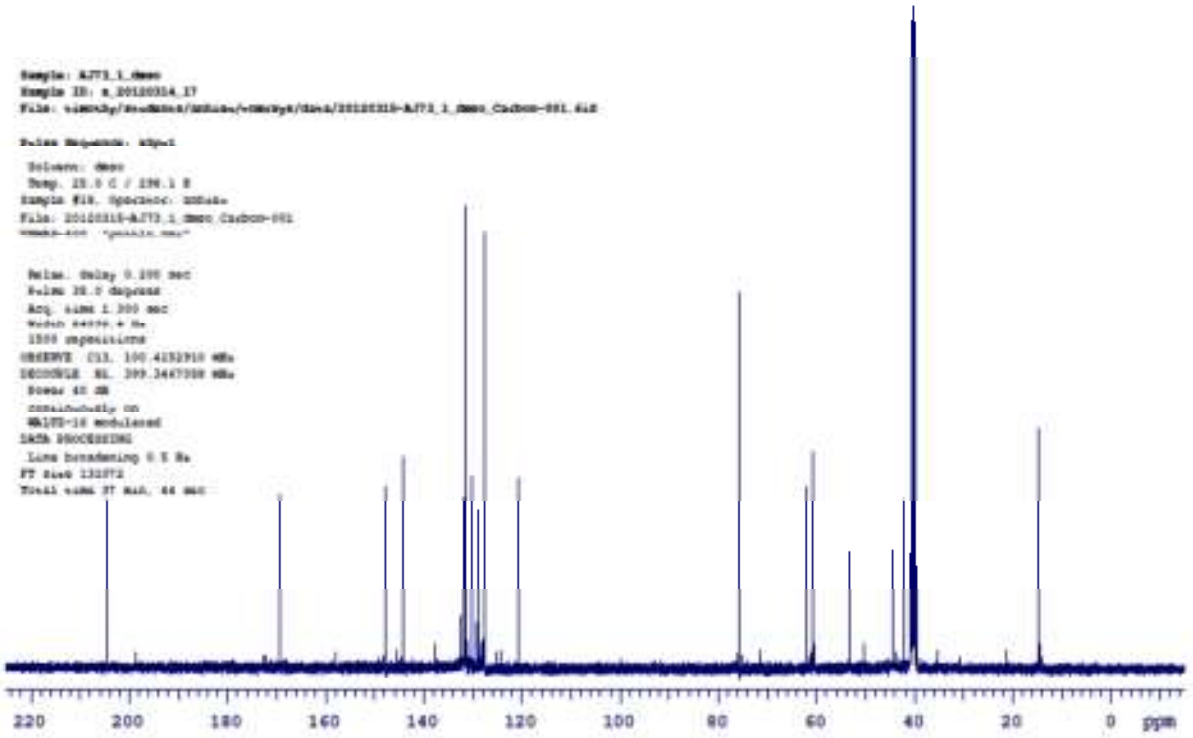
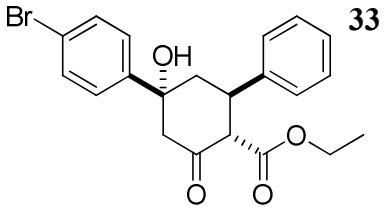
100
80
60
40
20
0 ppm

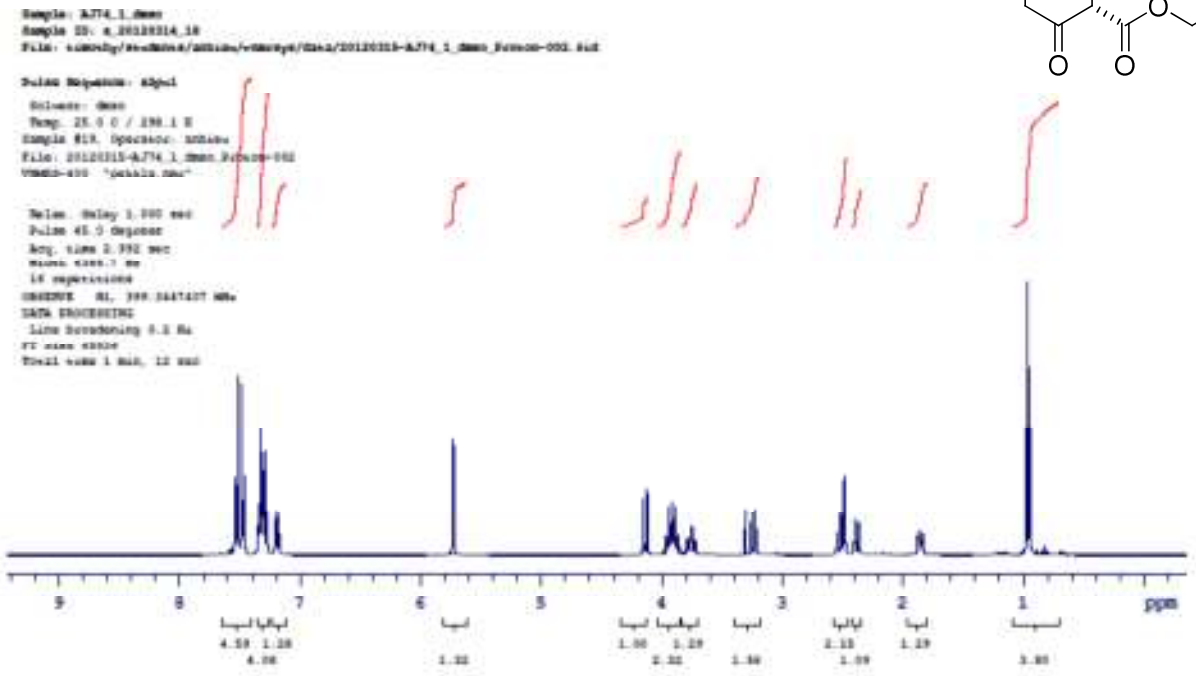
32






Br
OH
O
O
O

33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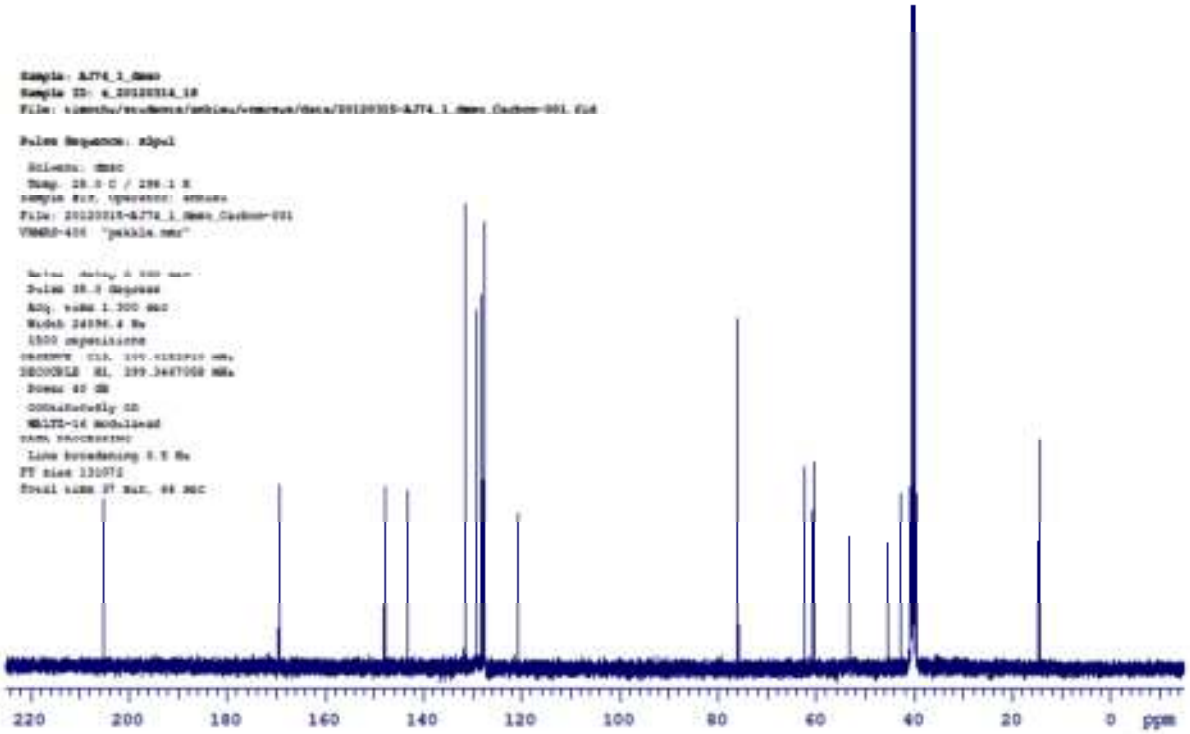
1
ppm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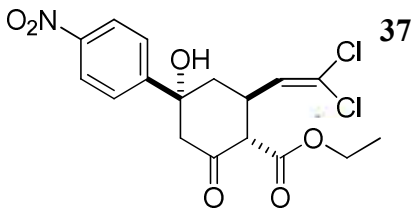

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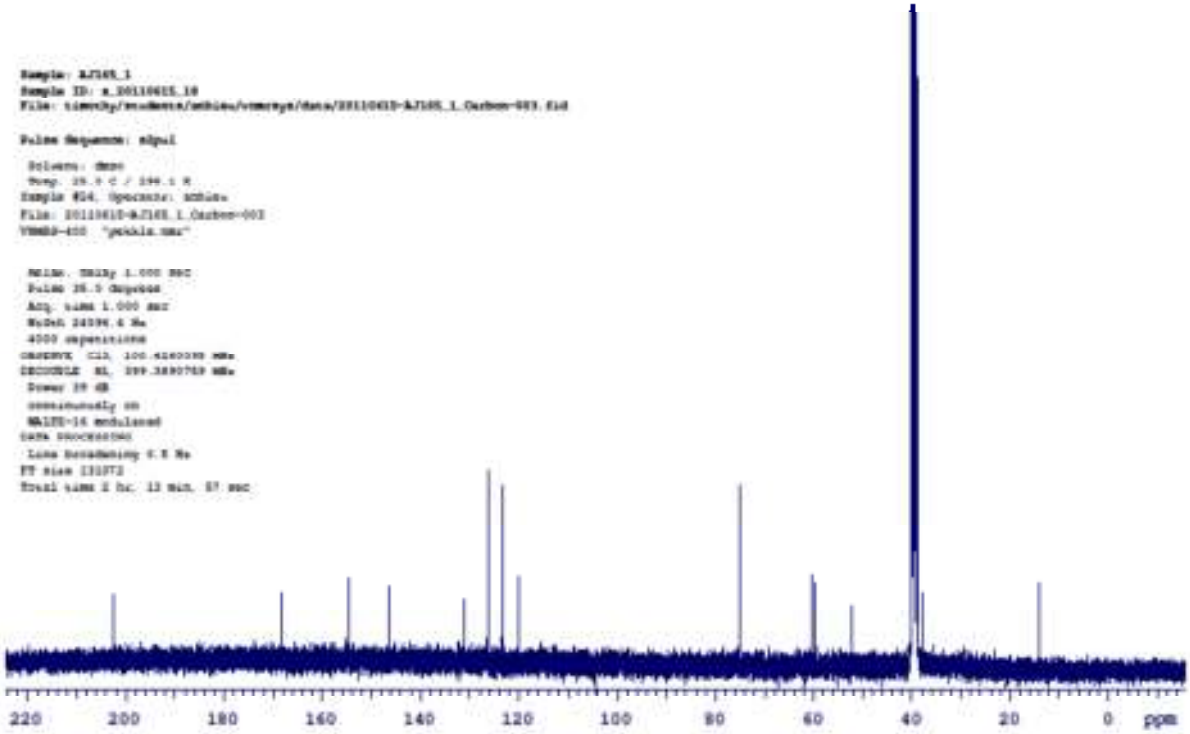

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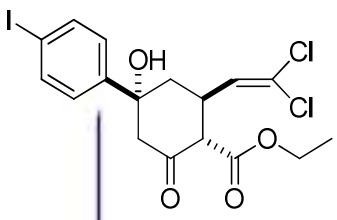
I
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Cl
Cl
O
O
O

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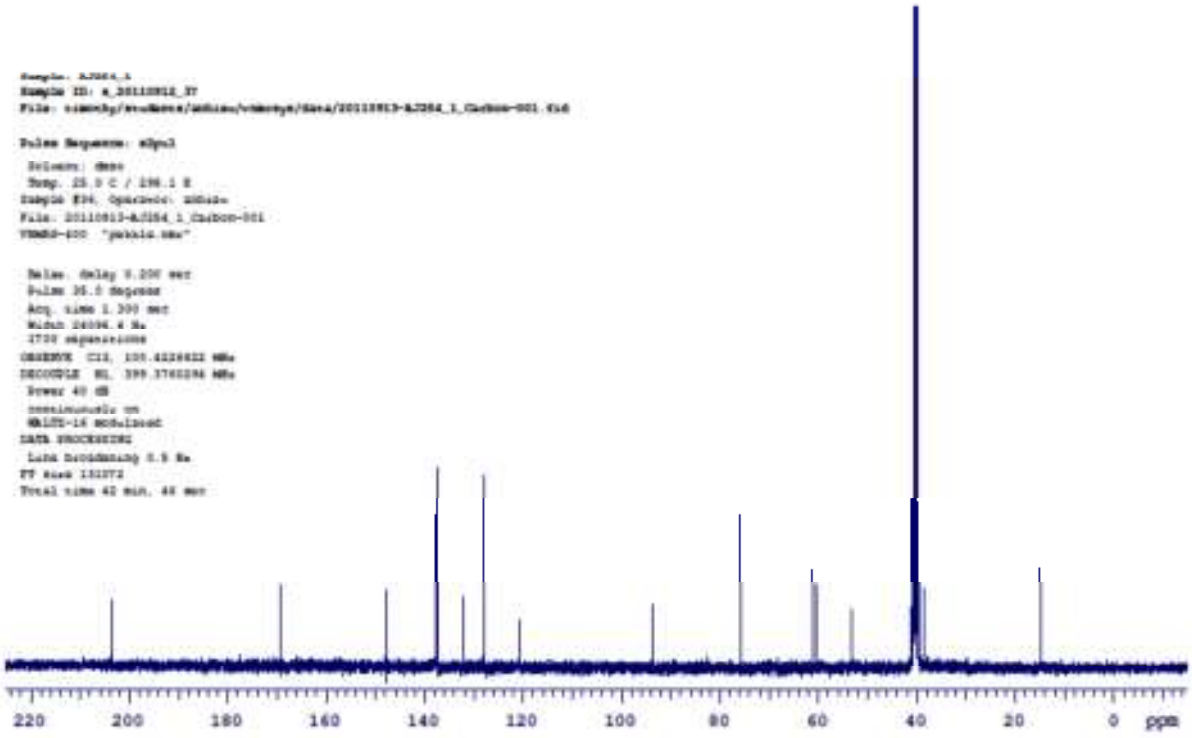
8
7
6
5
4
3
2
1
0
ppm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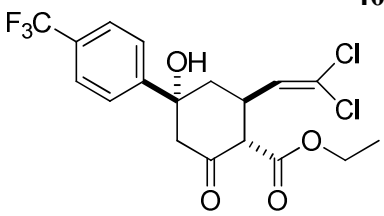

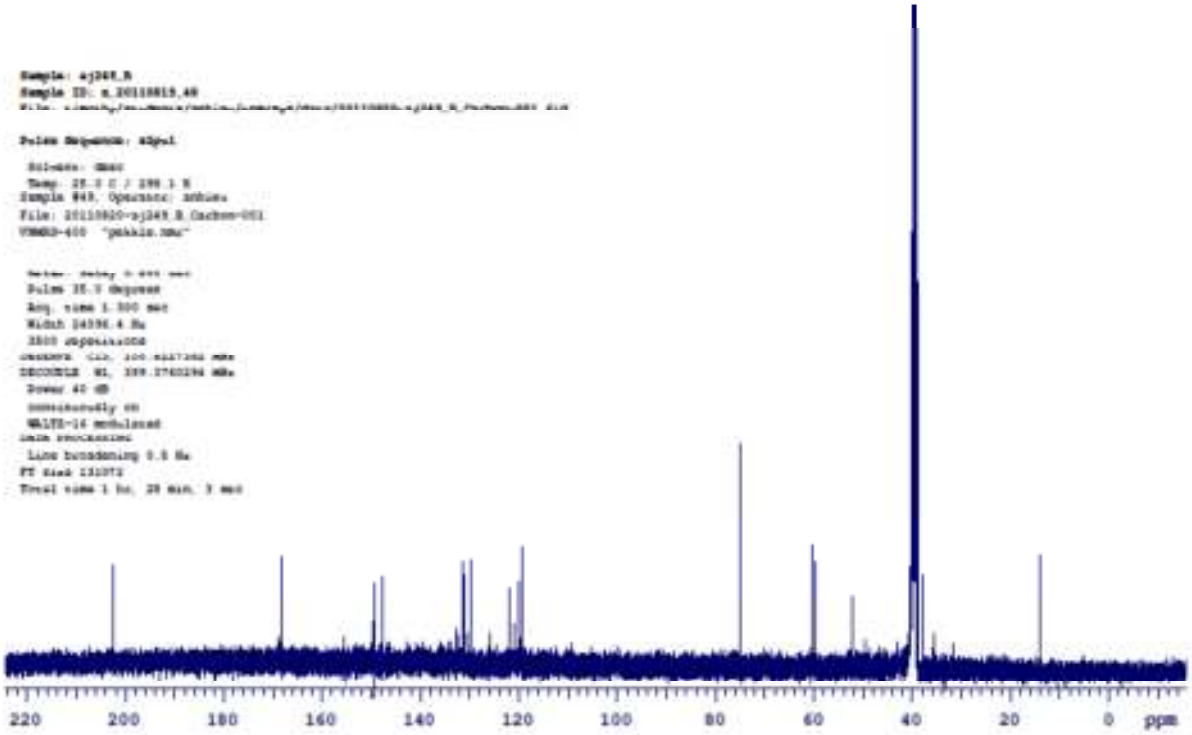

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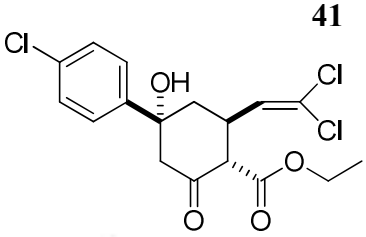

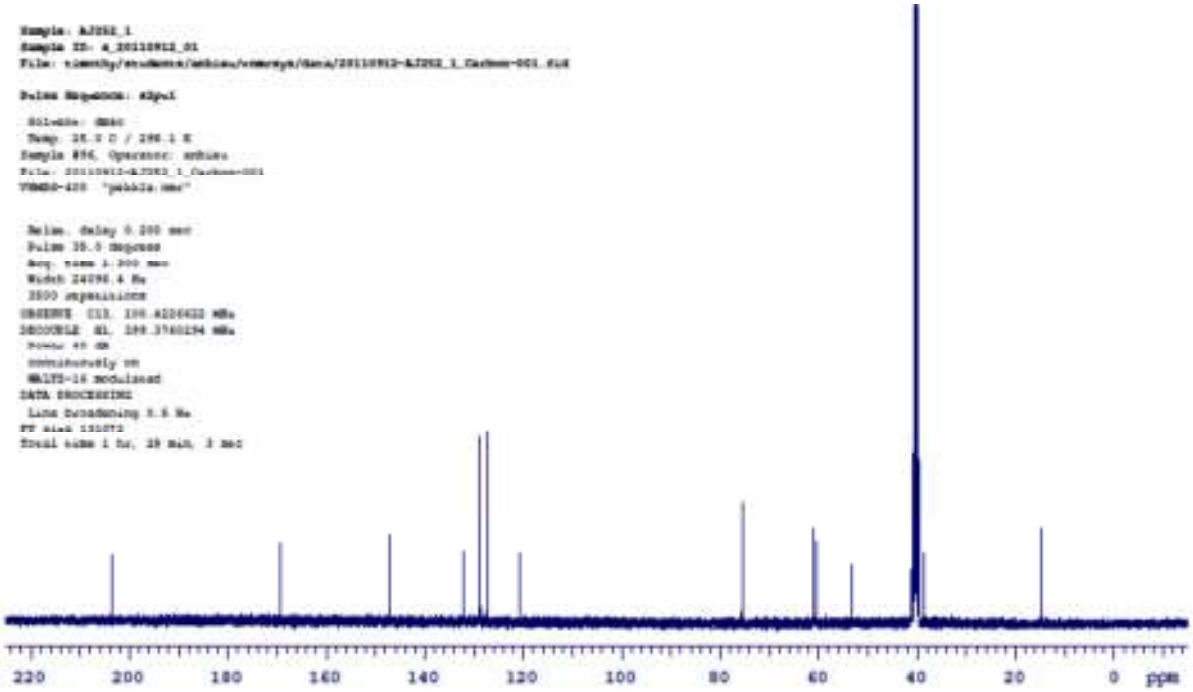

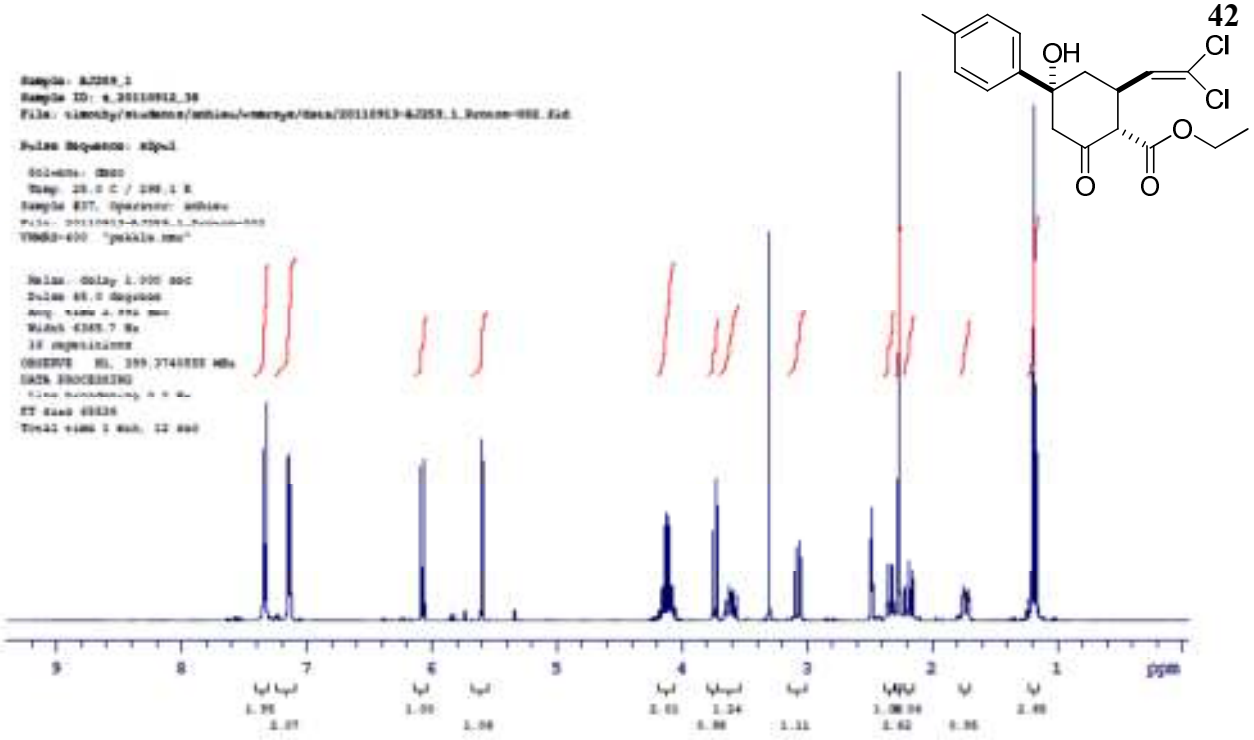
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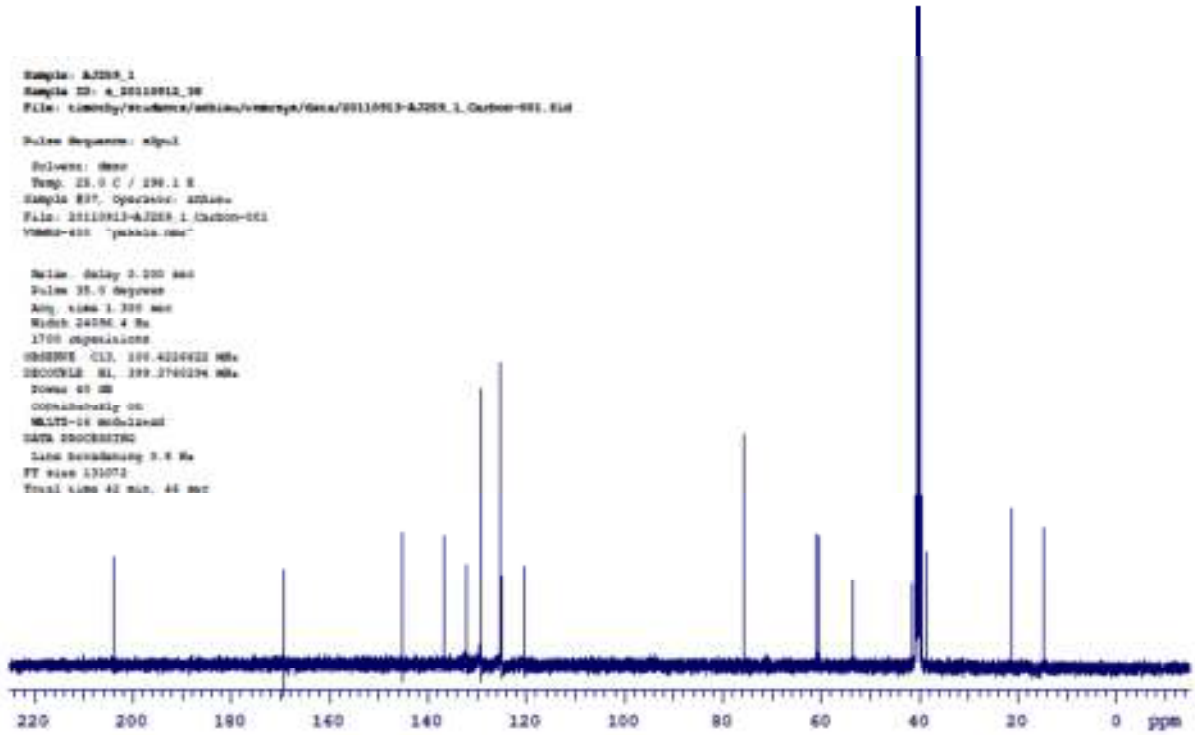
Cl
Cl
O
O
O
9
8
7
6
5
4
3
2
1
ppm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200
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140
120
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60
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20
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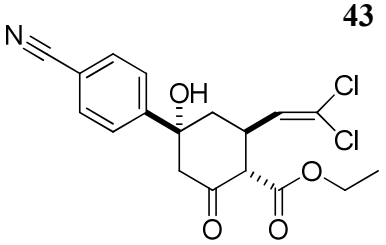
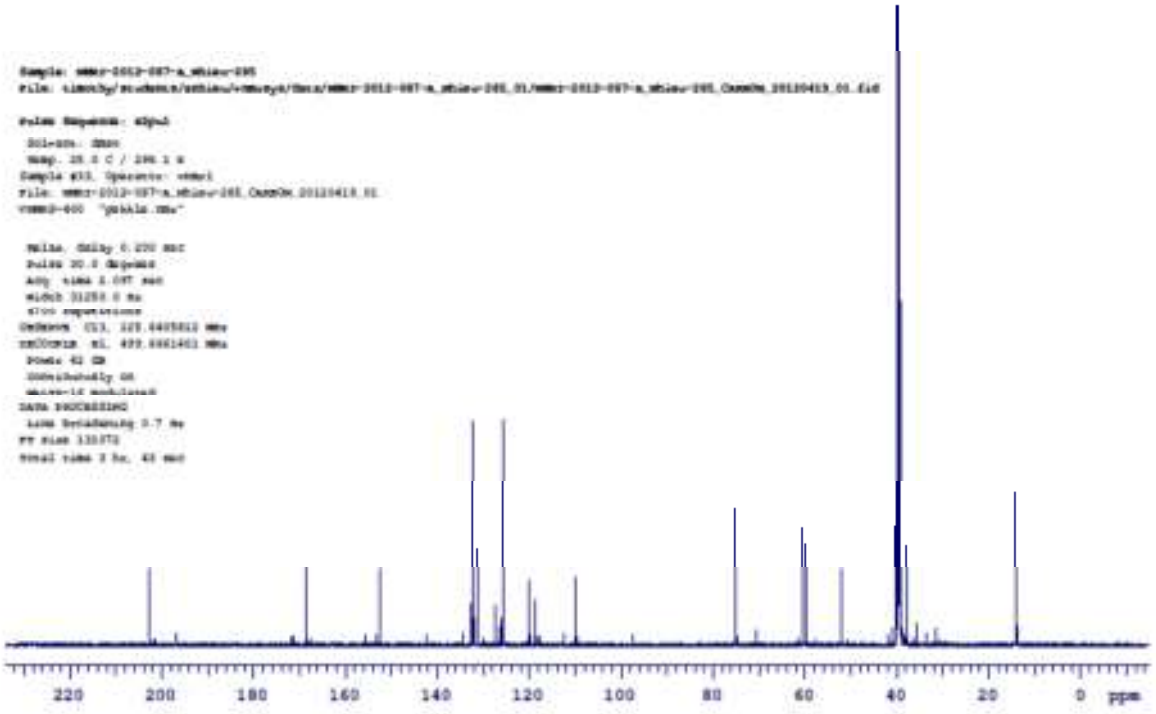
ppm

43





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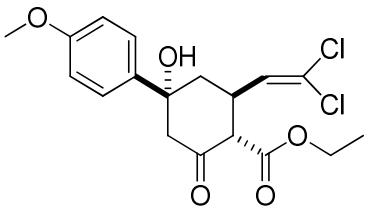

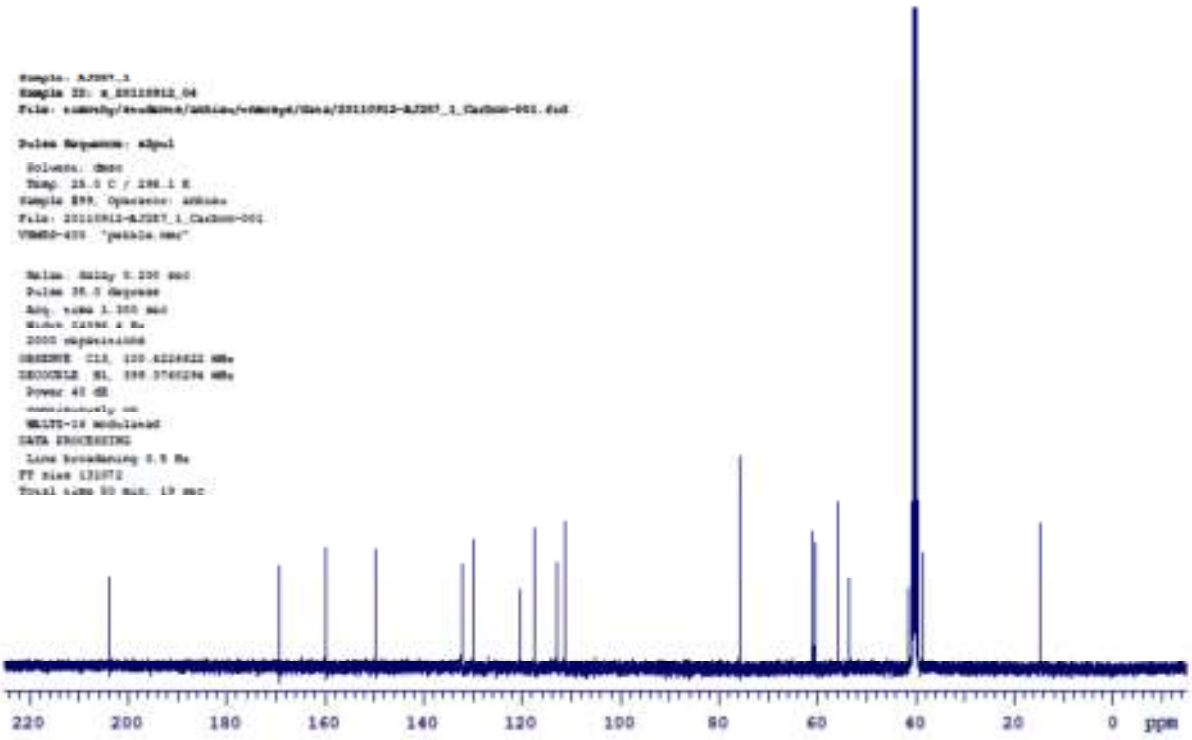

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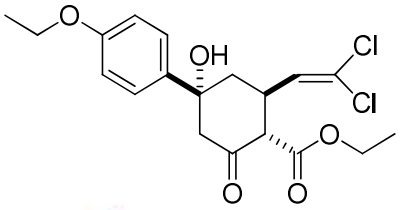
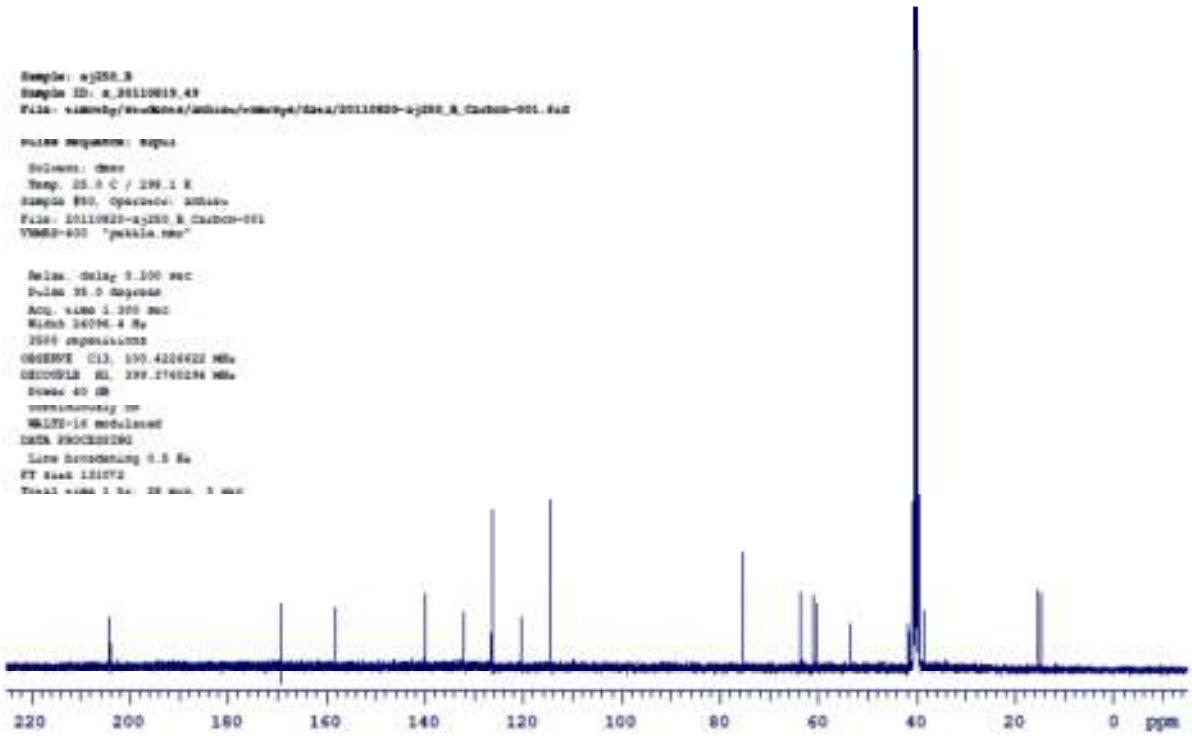

O
OH
Cl
Cl
O
O
O

9
8
7
6
5
4
3
2
1
0
ppm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100
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60
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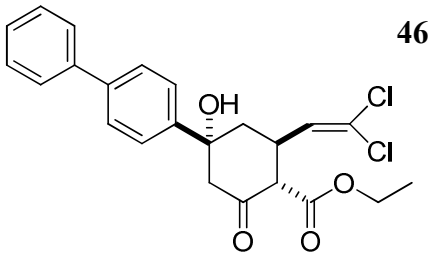
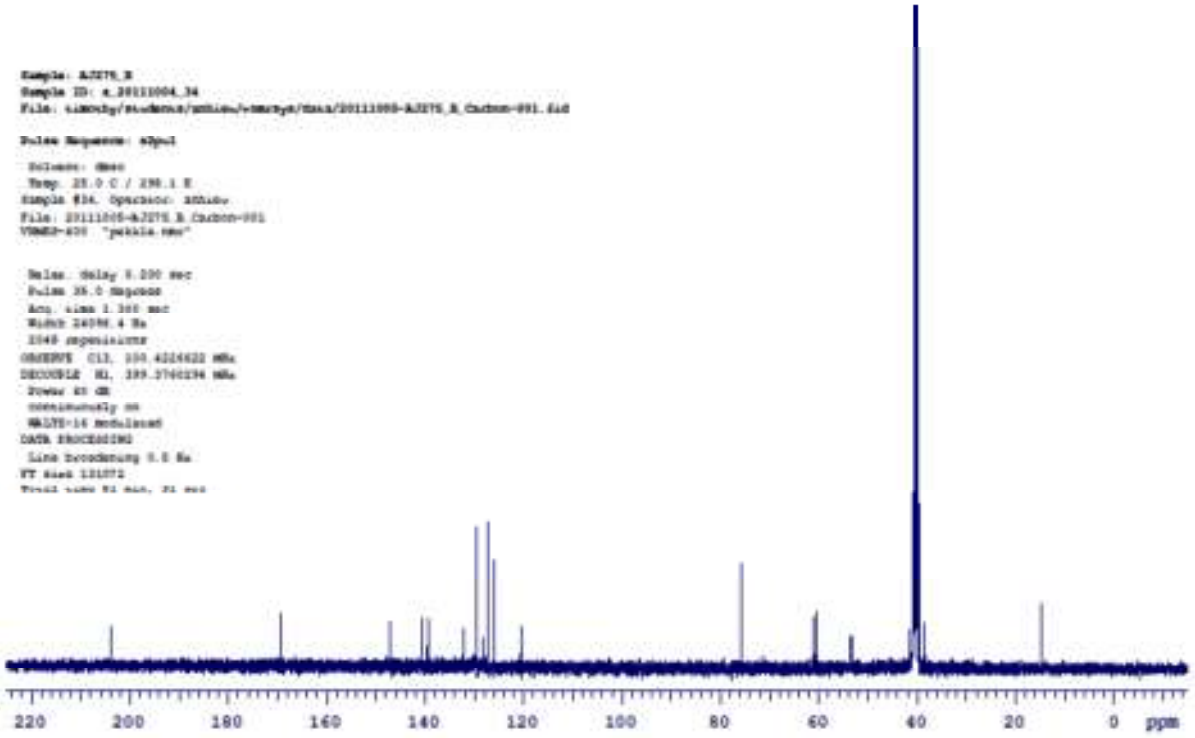
20
0
ppm

46





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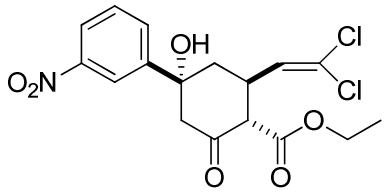

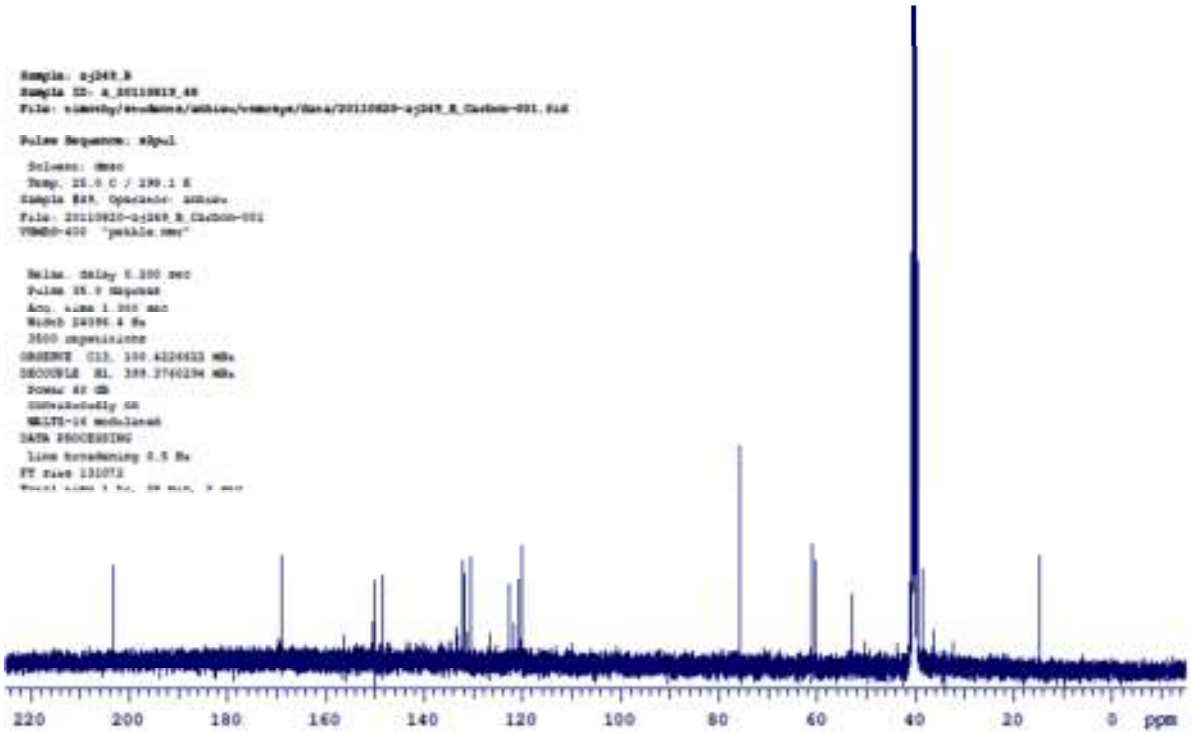

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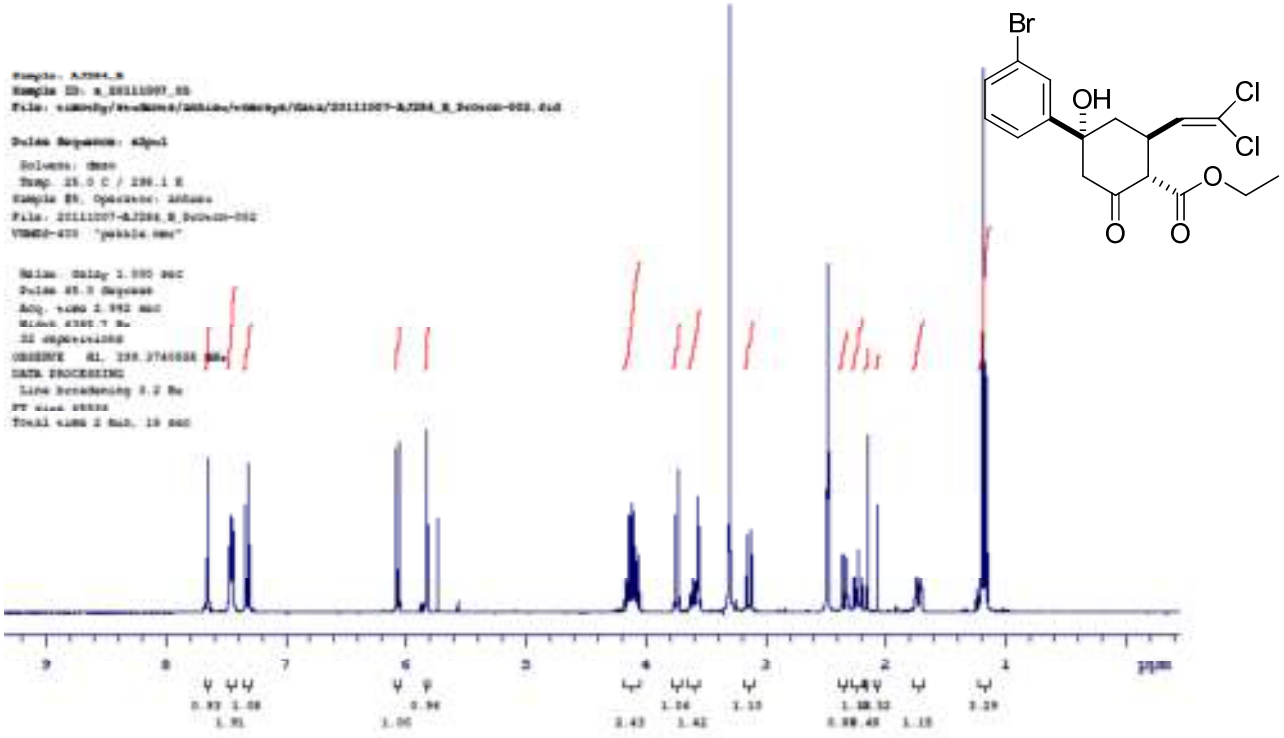

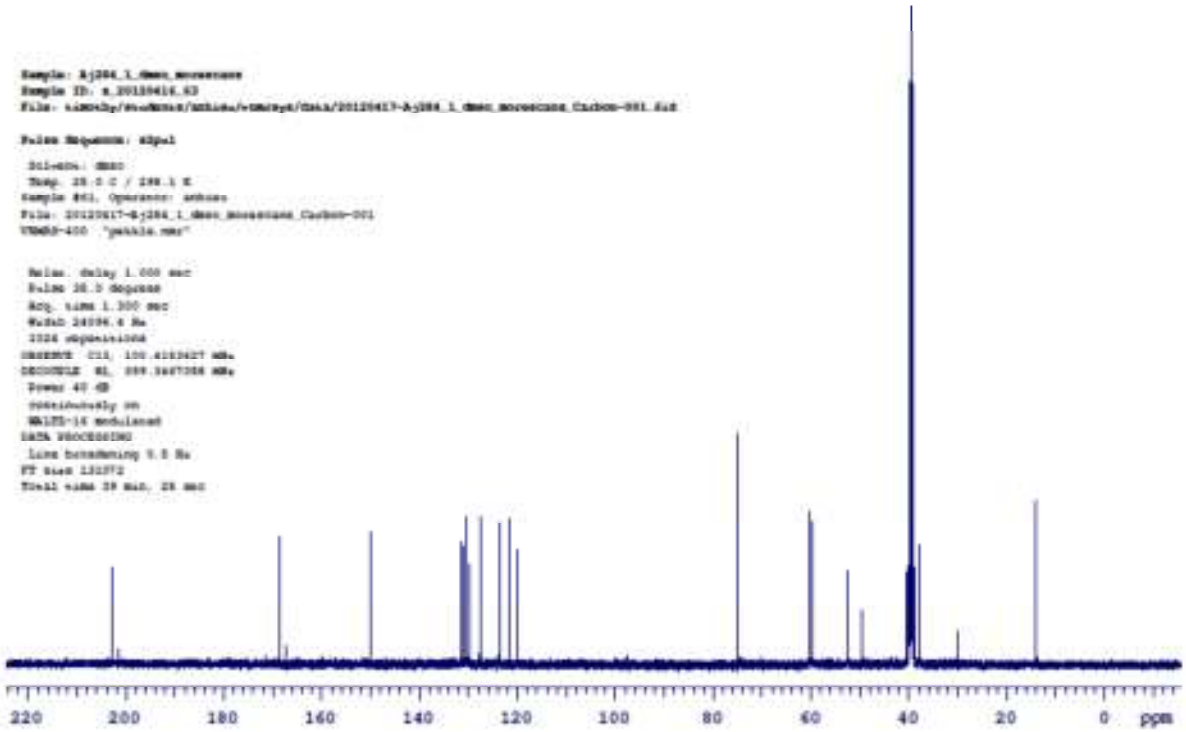

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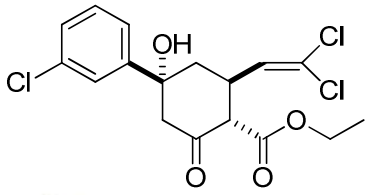
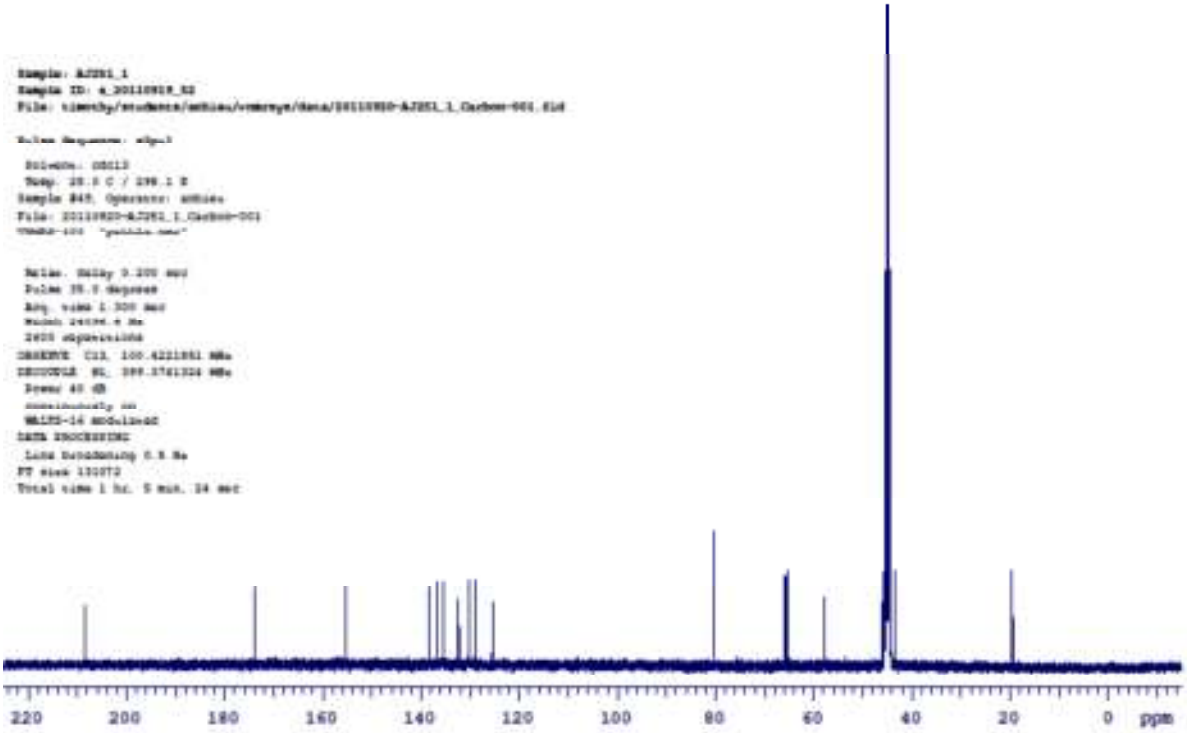

OH
Cl
Cl
Cl
O
O
O

8
7
6
5
4
3
2
1
0 ppm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140
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100
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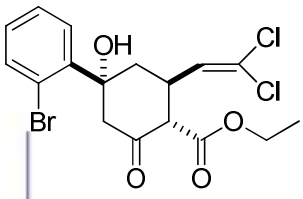
60
40
20
0 ppm

50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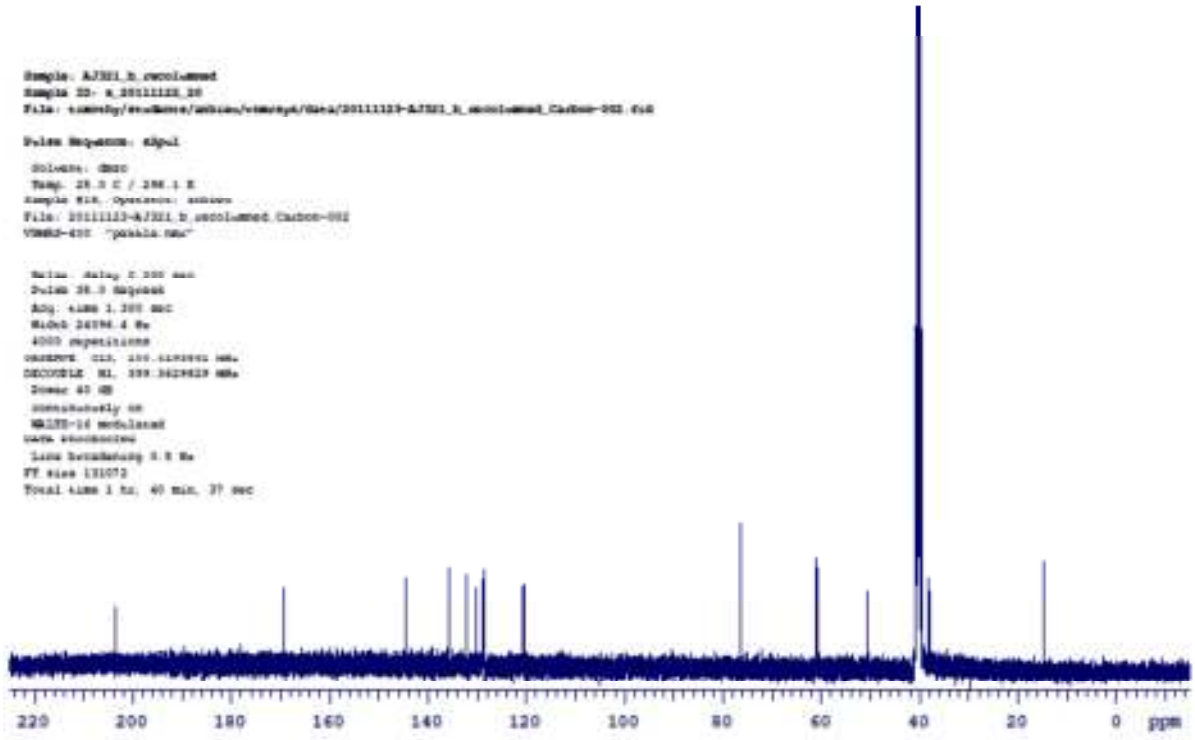
Cl
Cl
Br
O
O
O

9
8
7
6
5
4
3
2
1
0 ppm


220
200
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120
100
80
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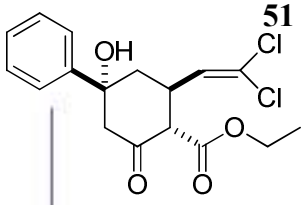
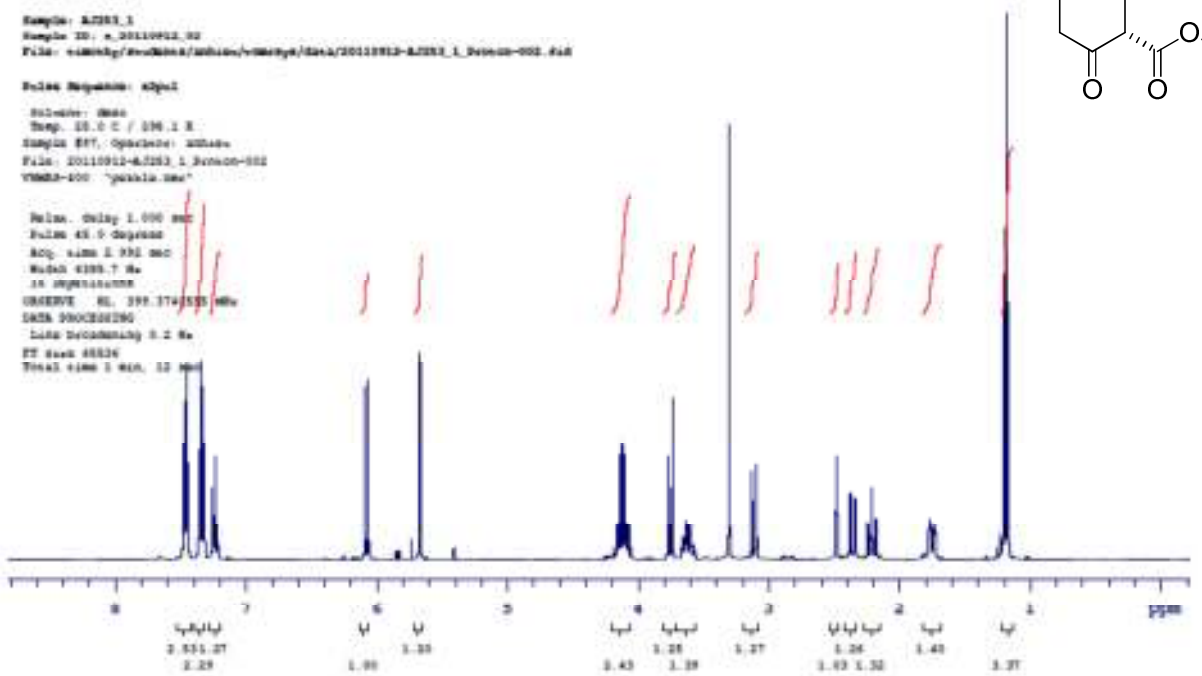
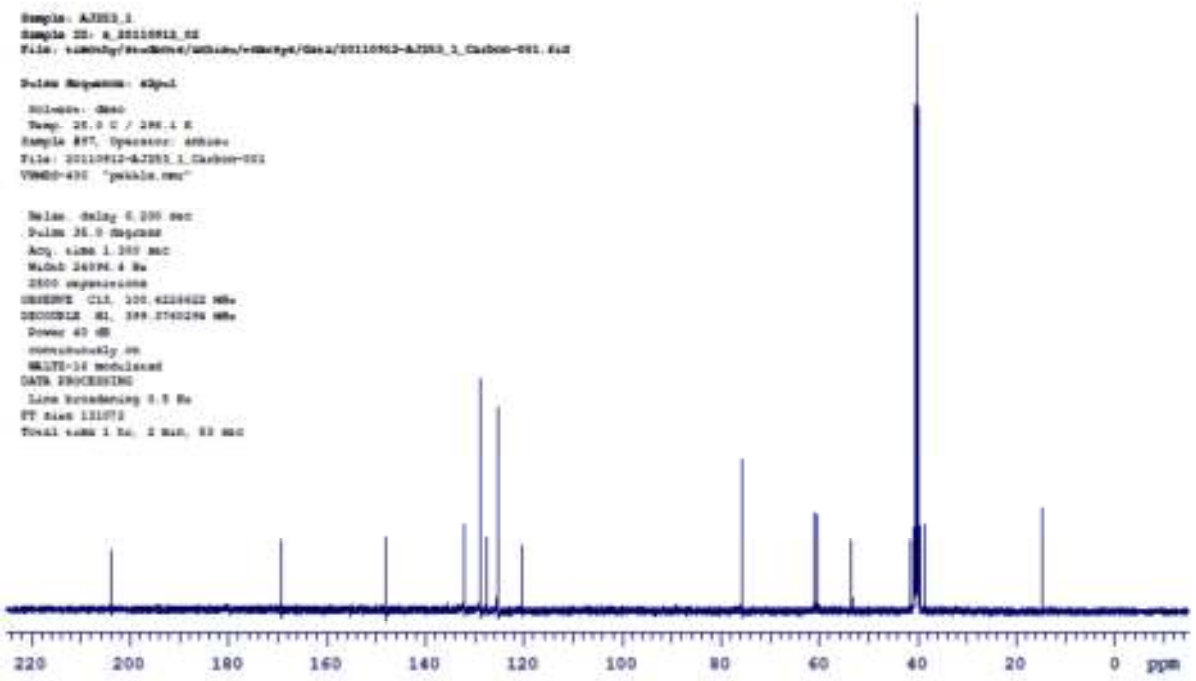
40
20
0 ppm

51







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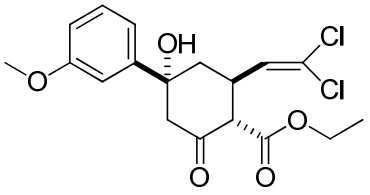
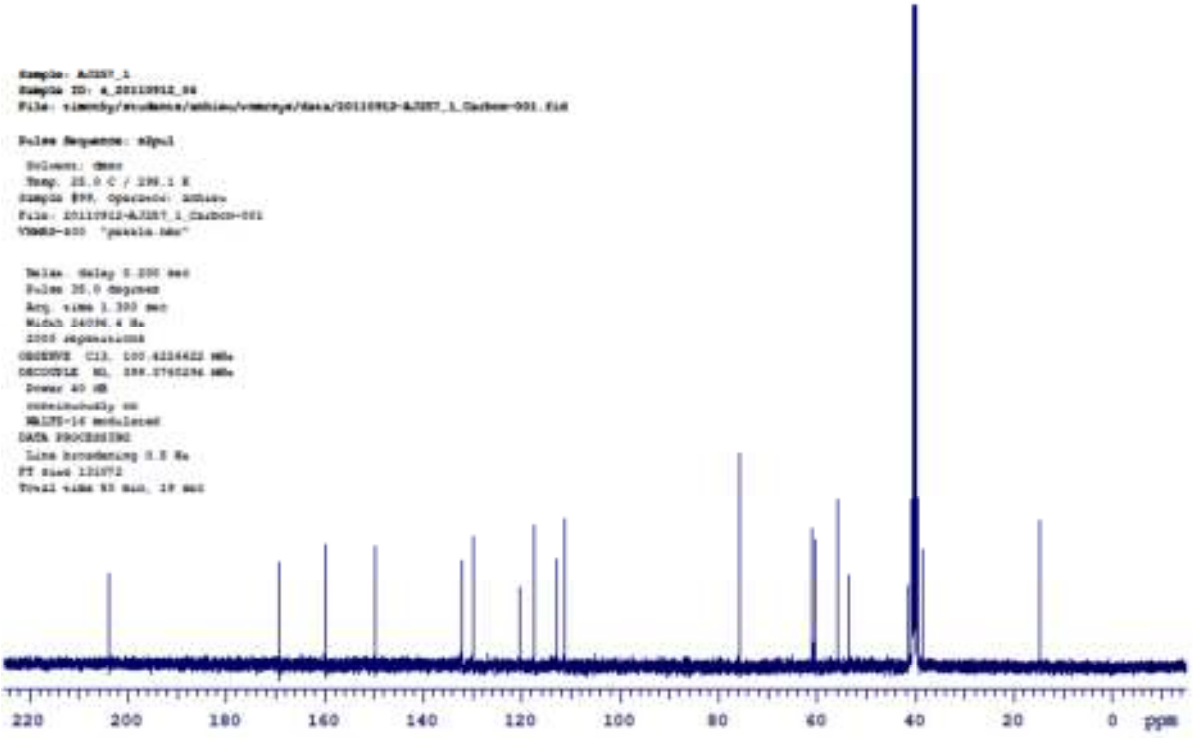

OH
Cl
Cl
O
O
O
O

8
7
6
5
4
3
2
1
ppm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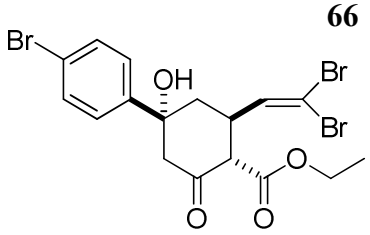
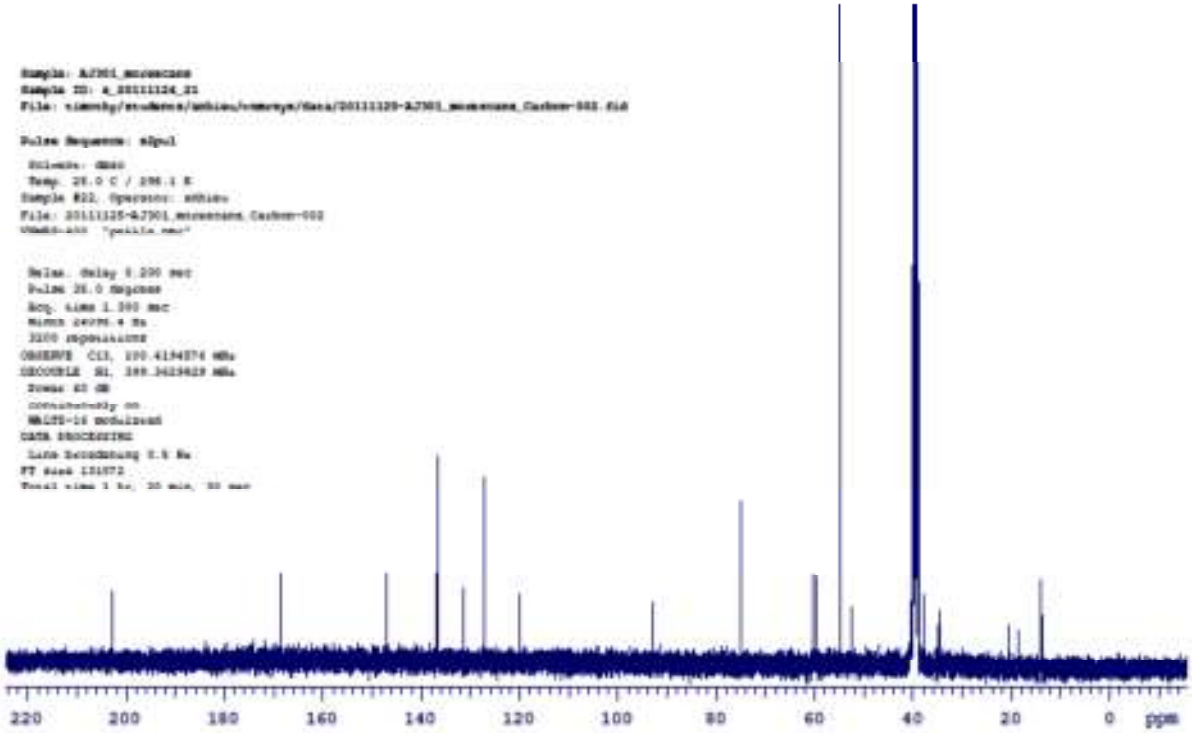
66





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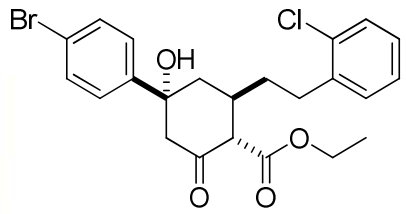

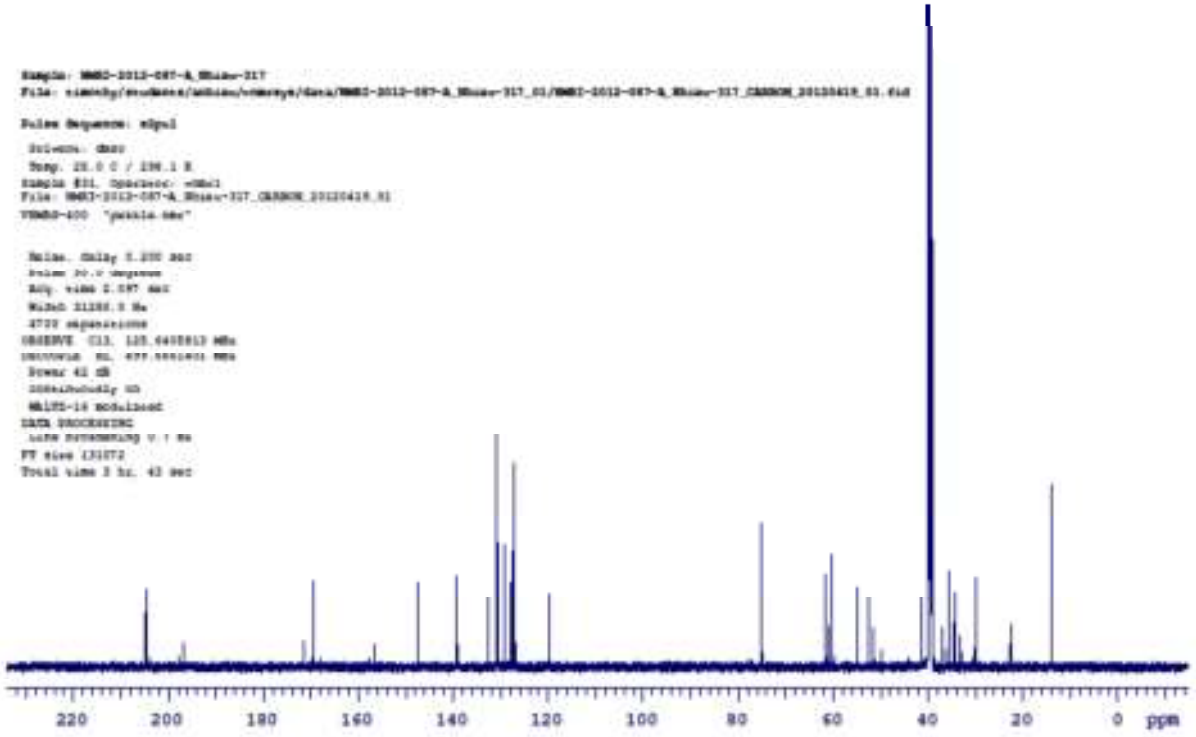

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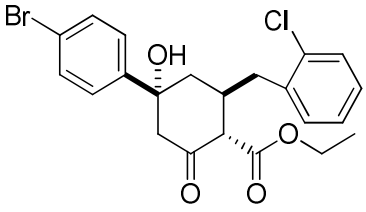
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O

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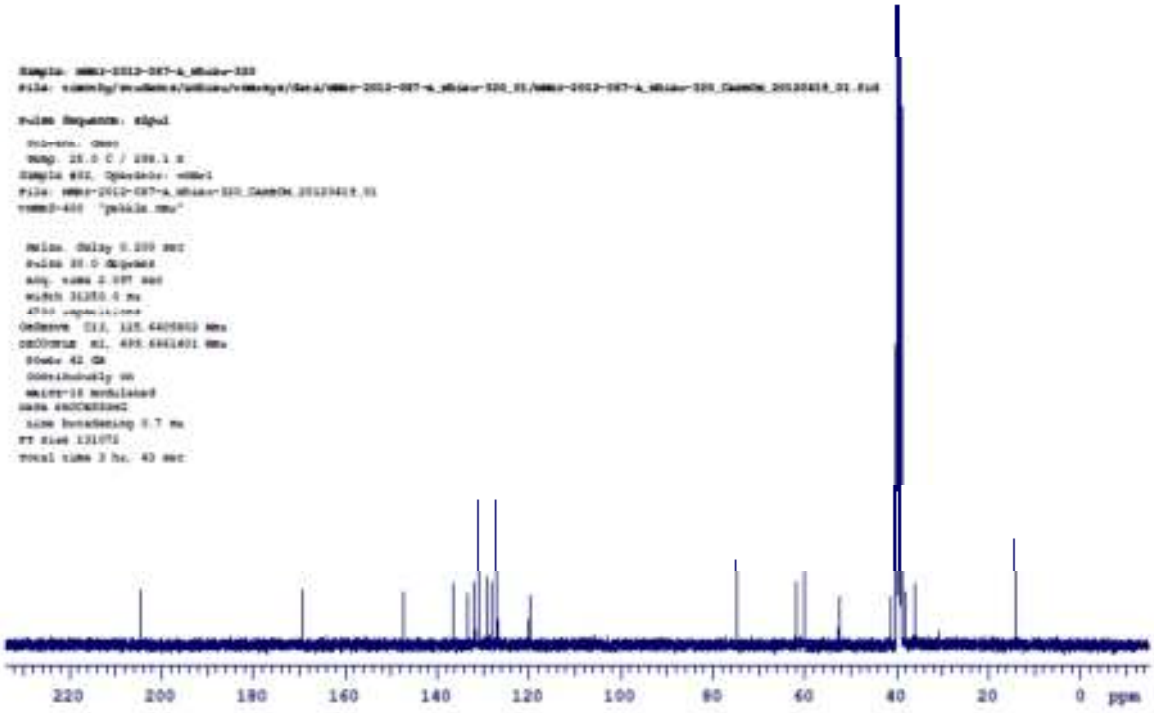
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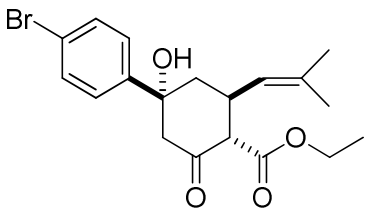

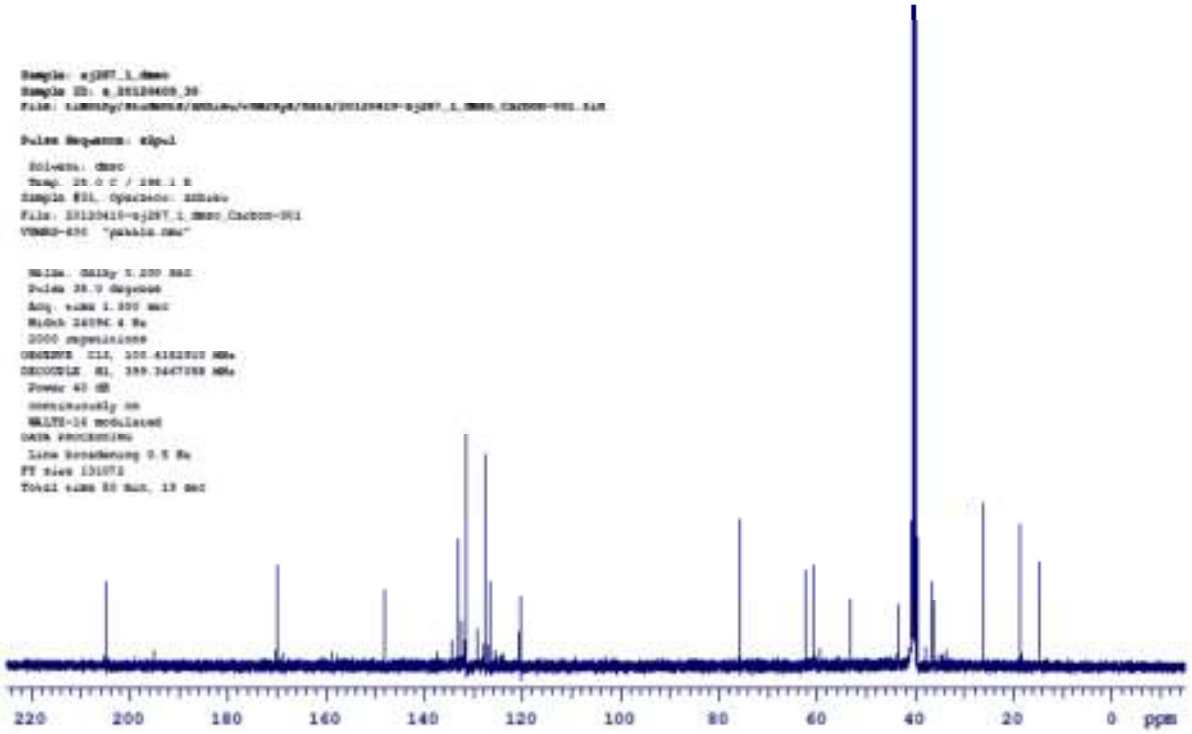

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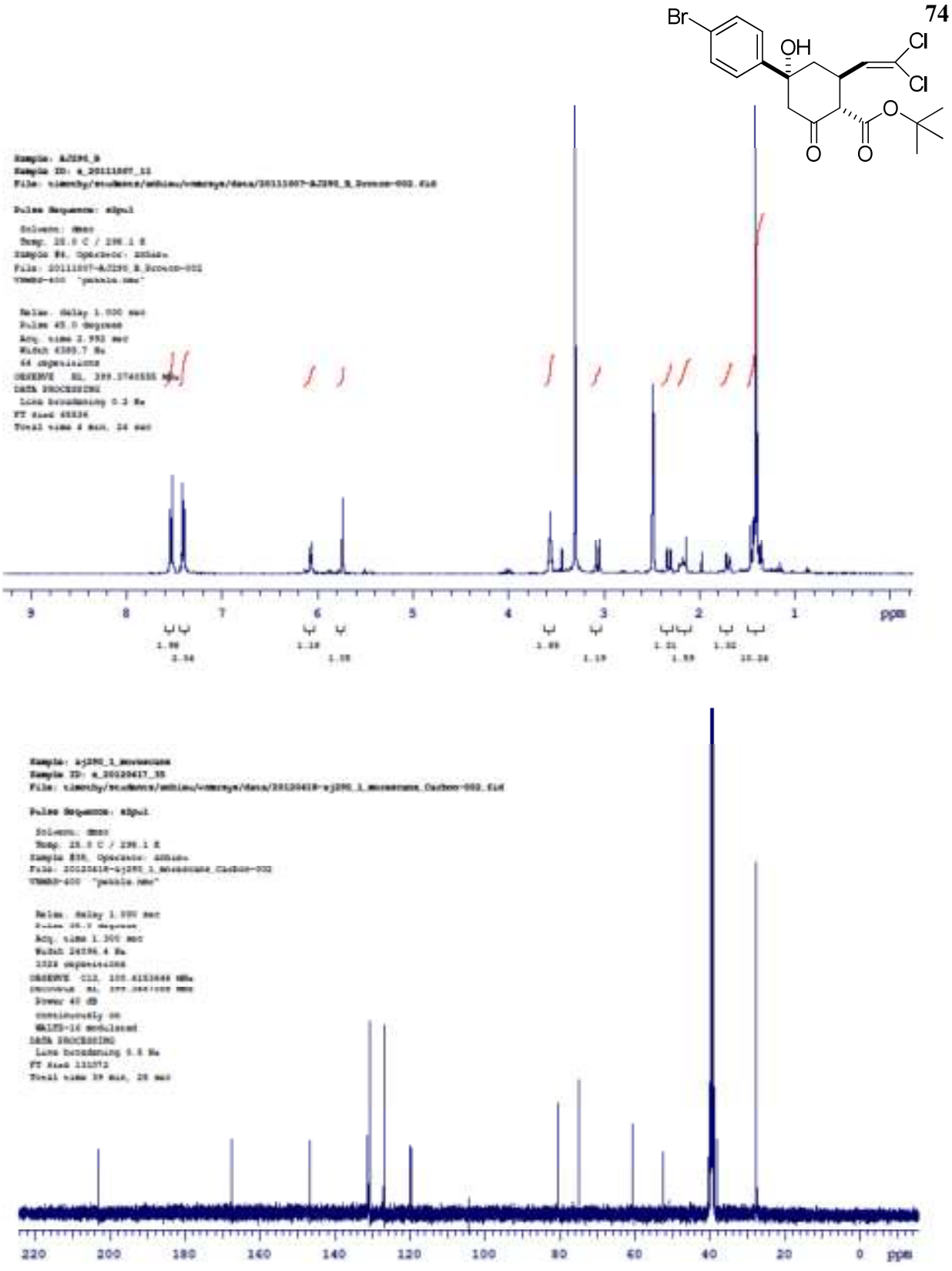
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Cl
O
O
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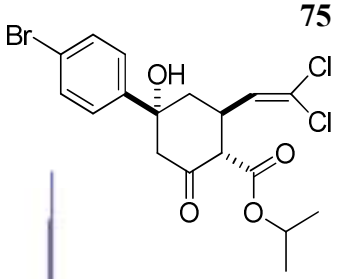
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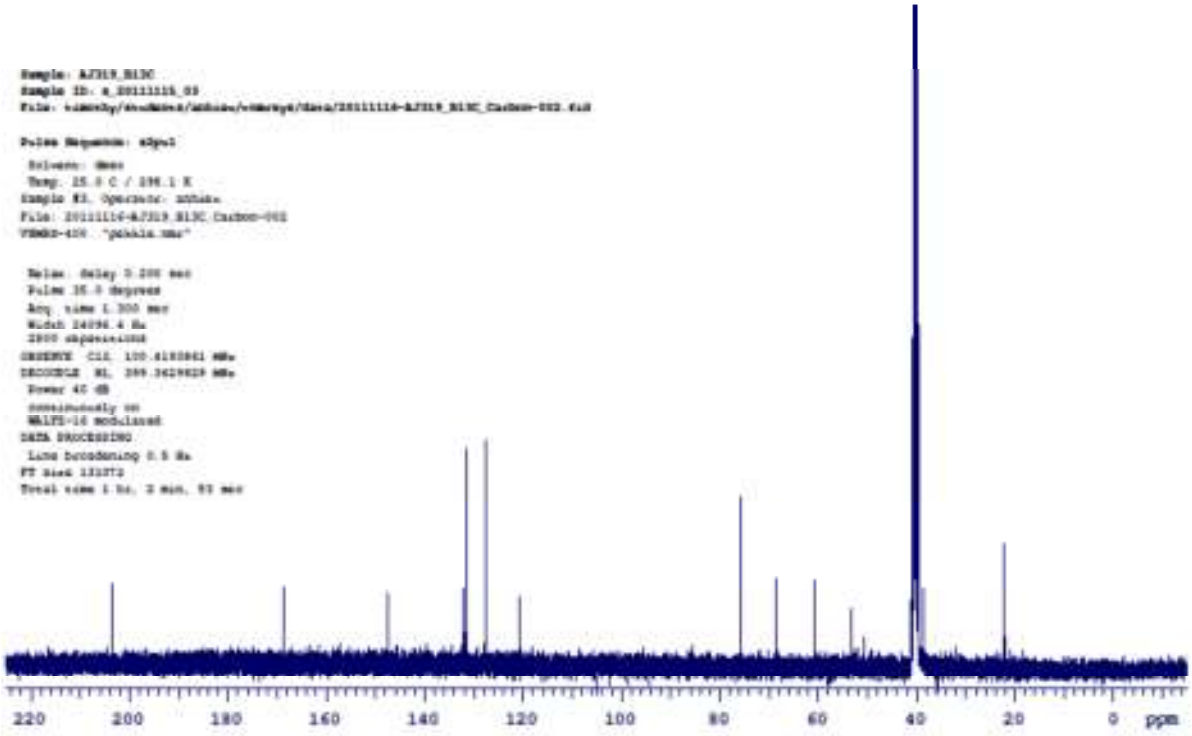
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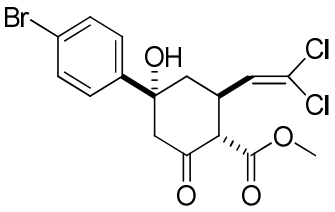
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O
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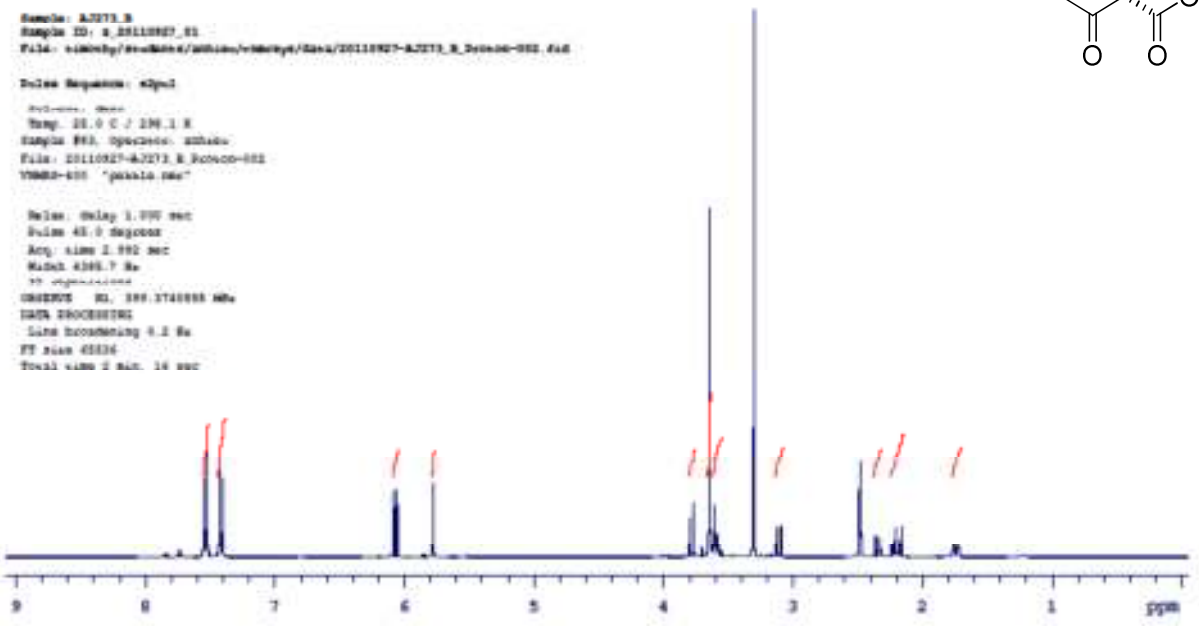
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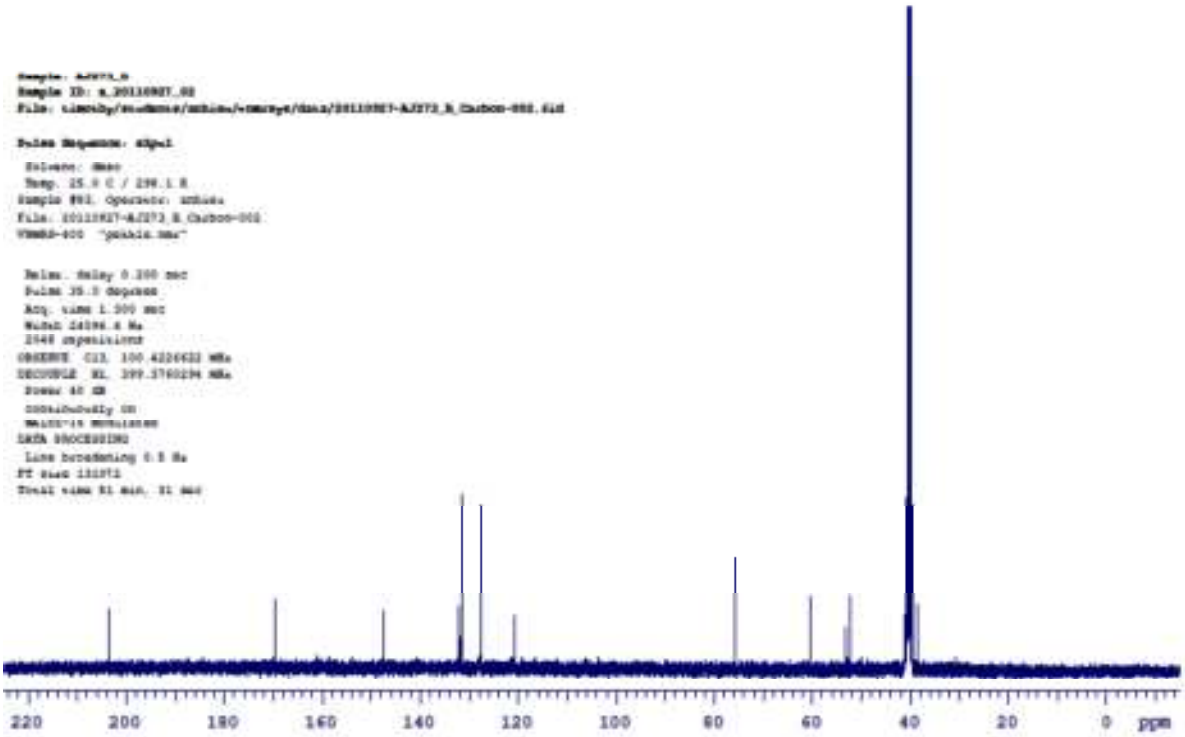
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20
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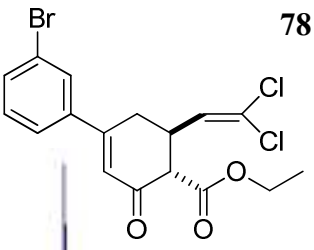
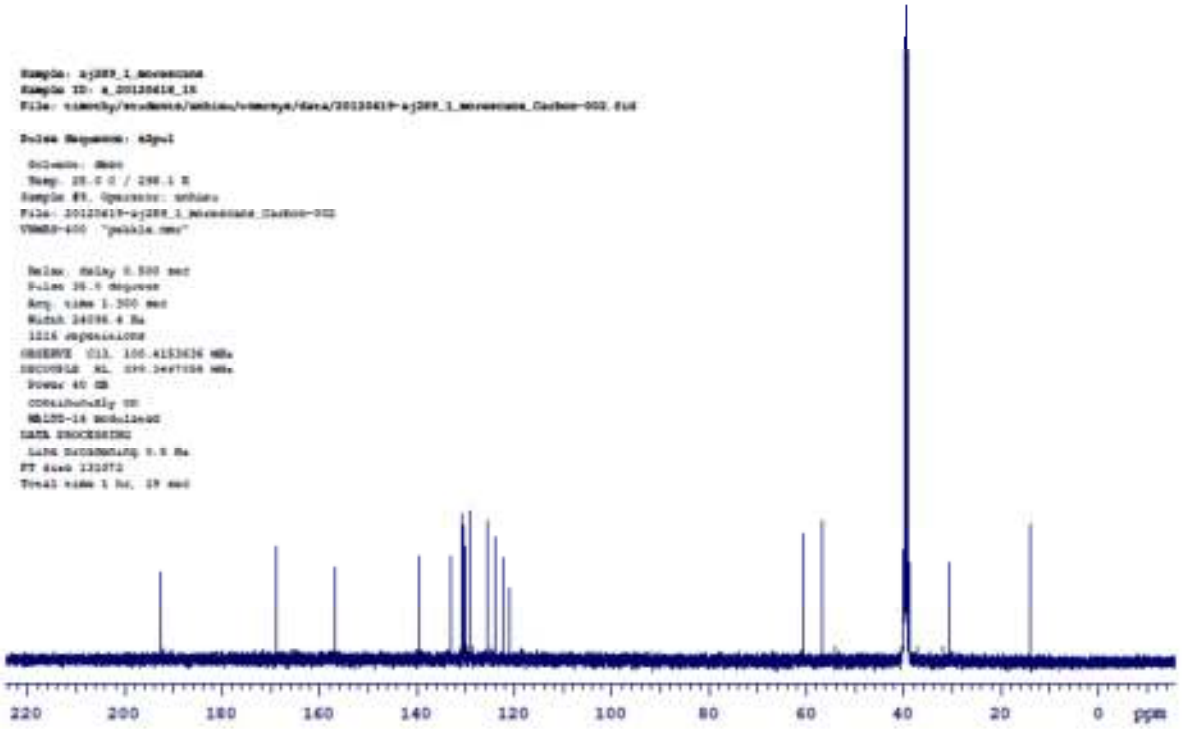
ppm

78





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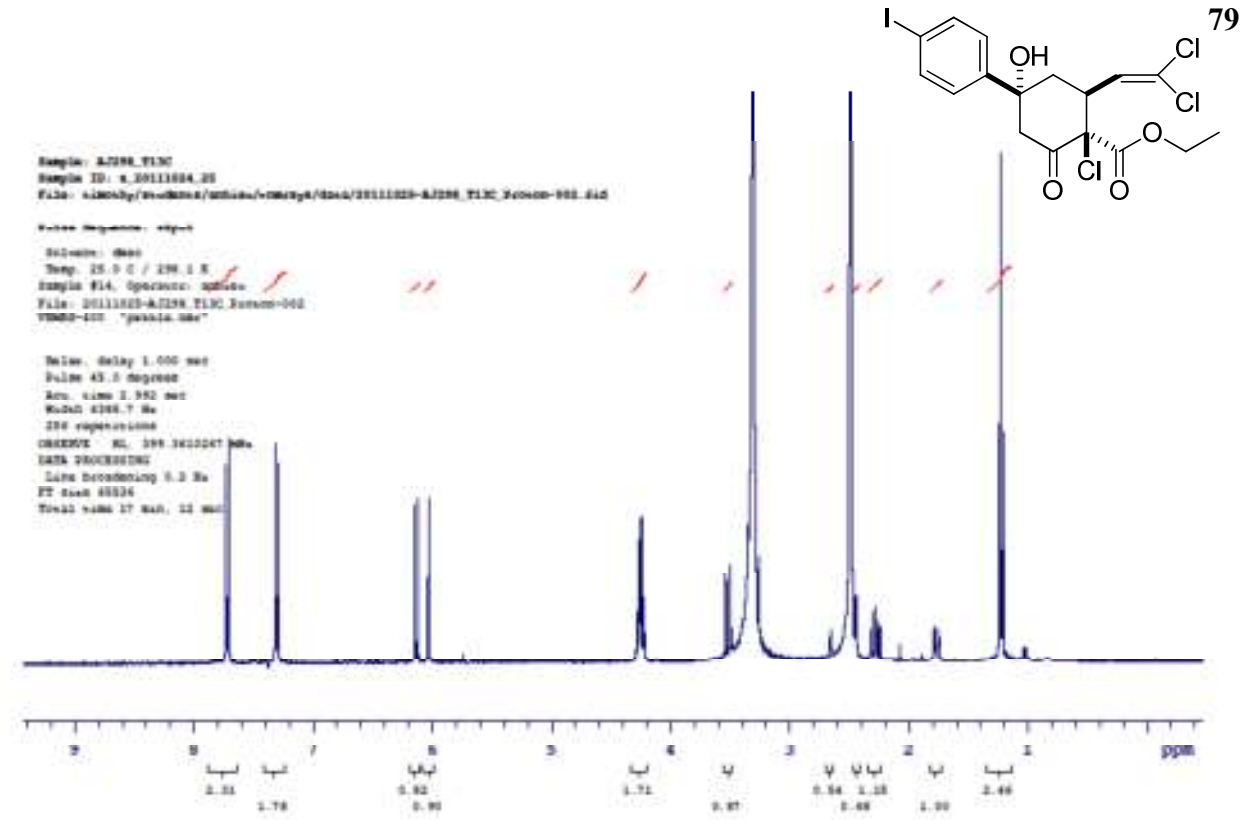

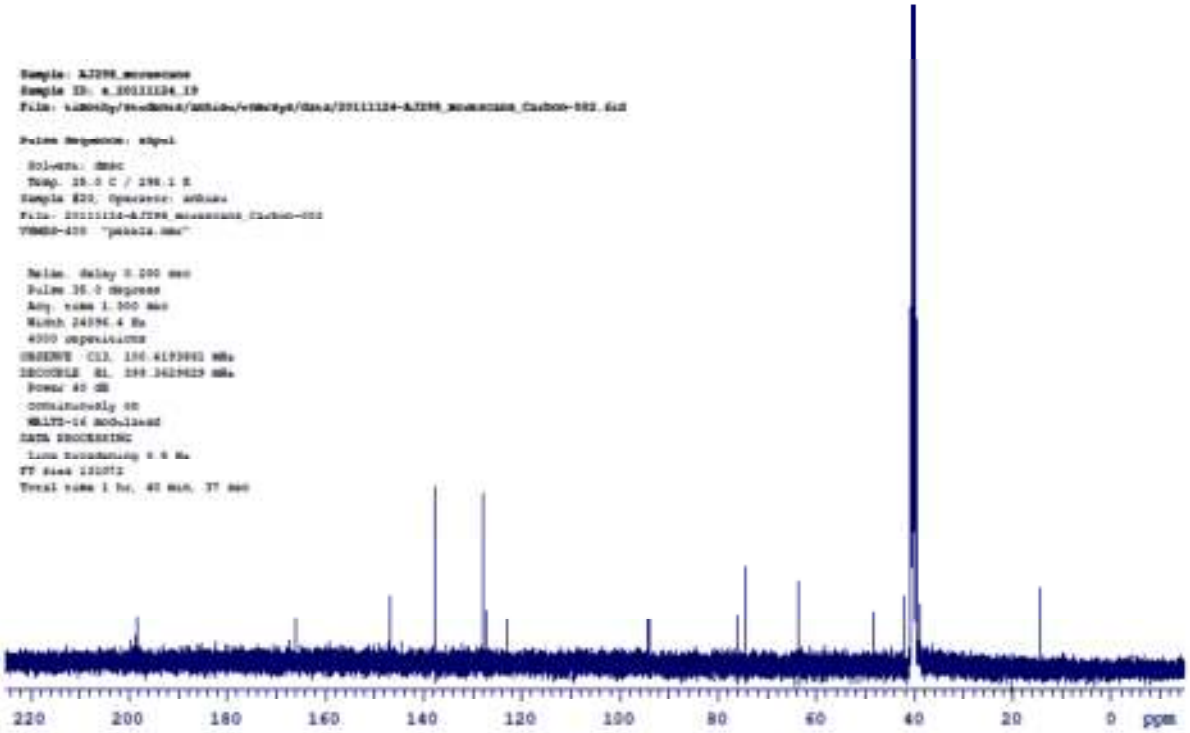

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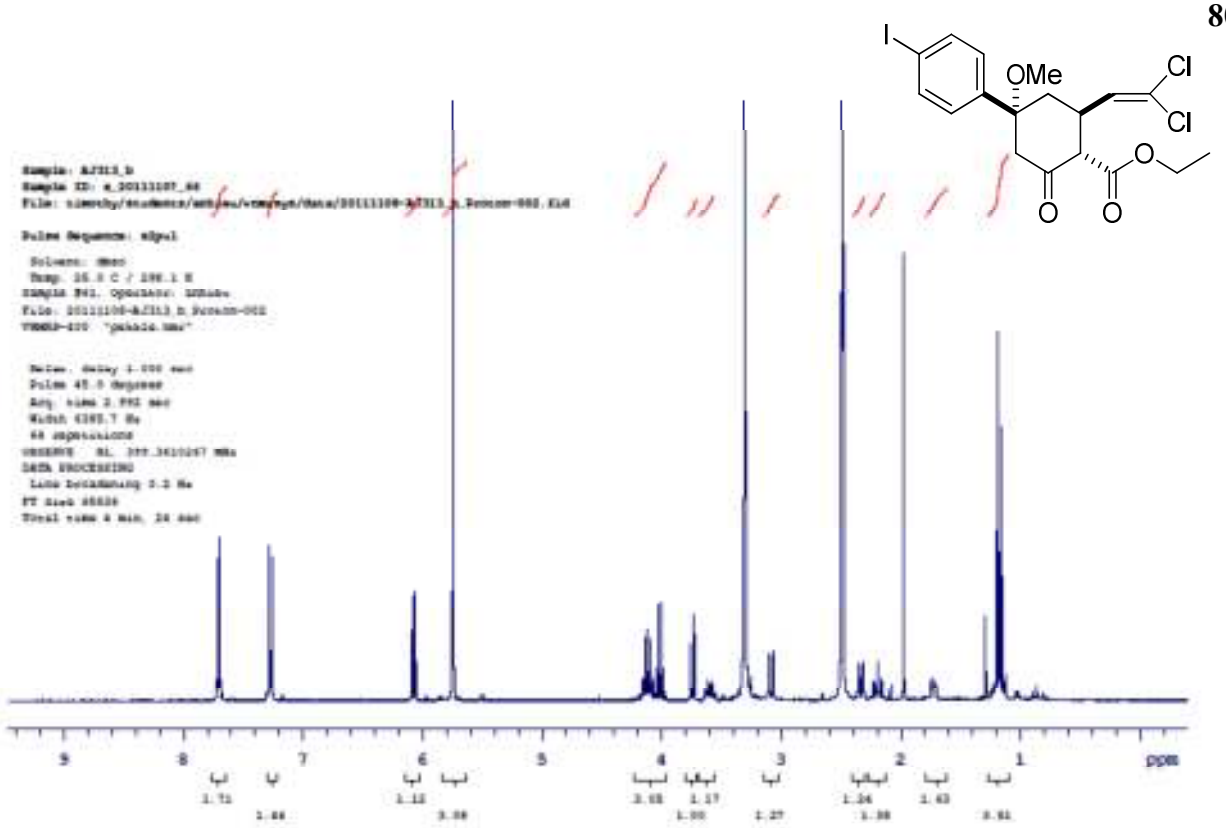

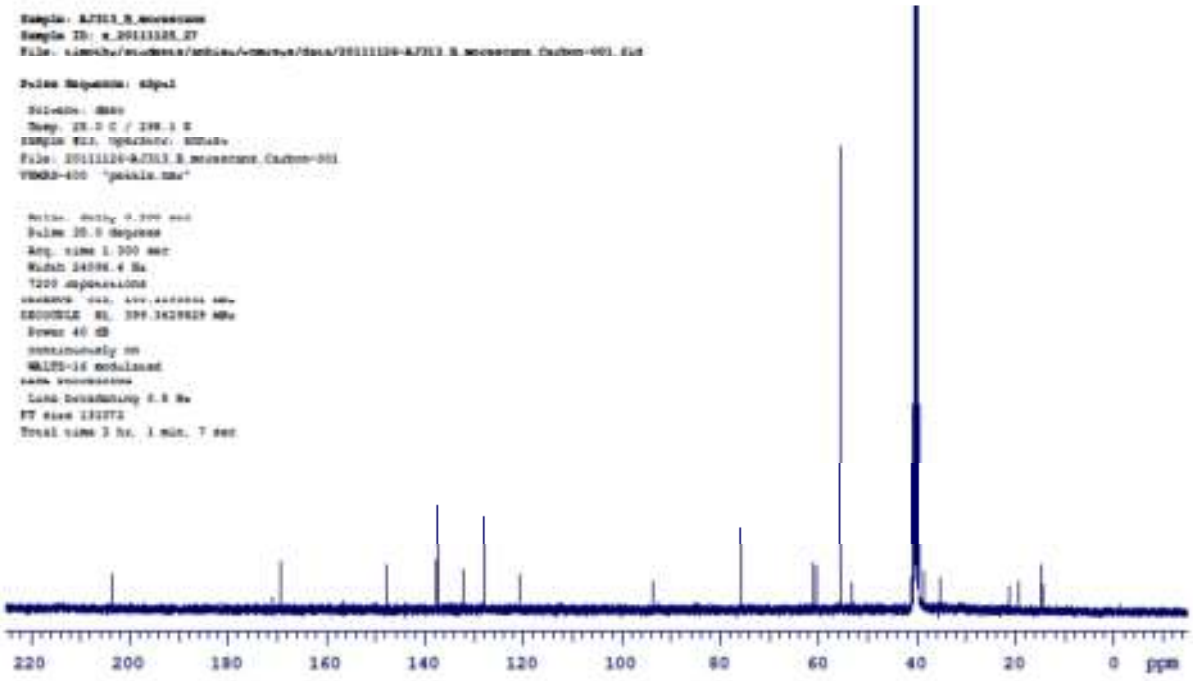

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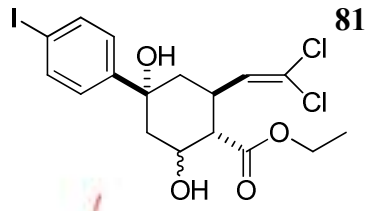
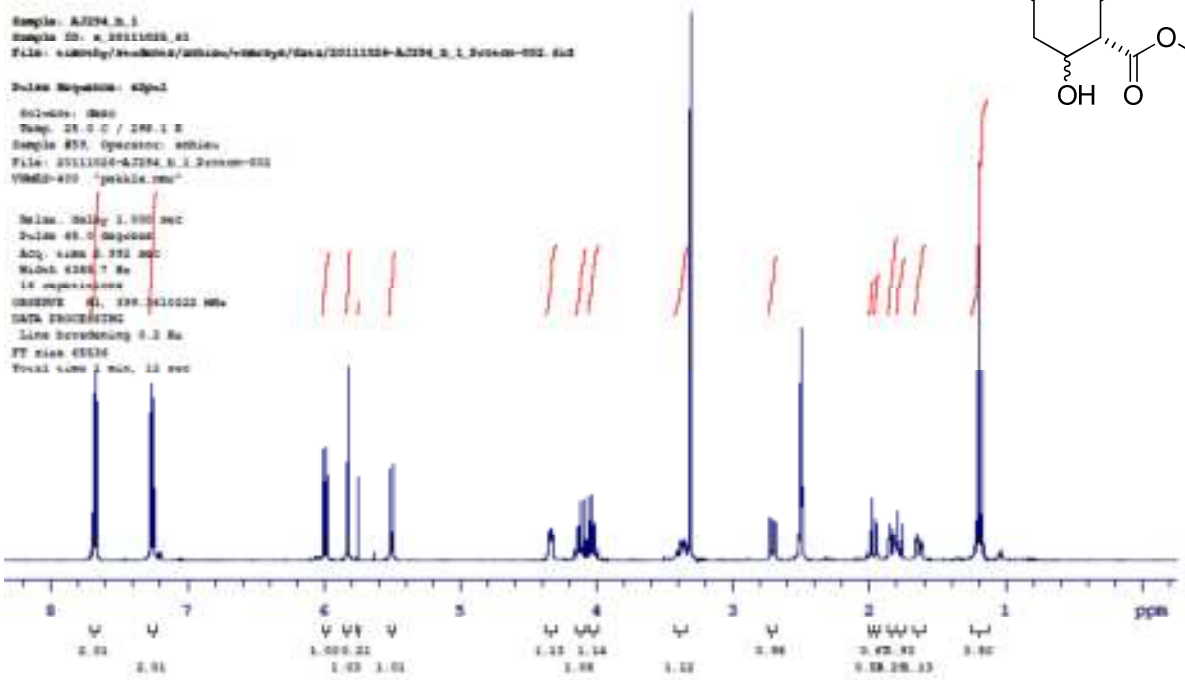

I
OH
Cl
Cl
O
OH
O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3
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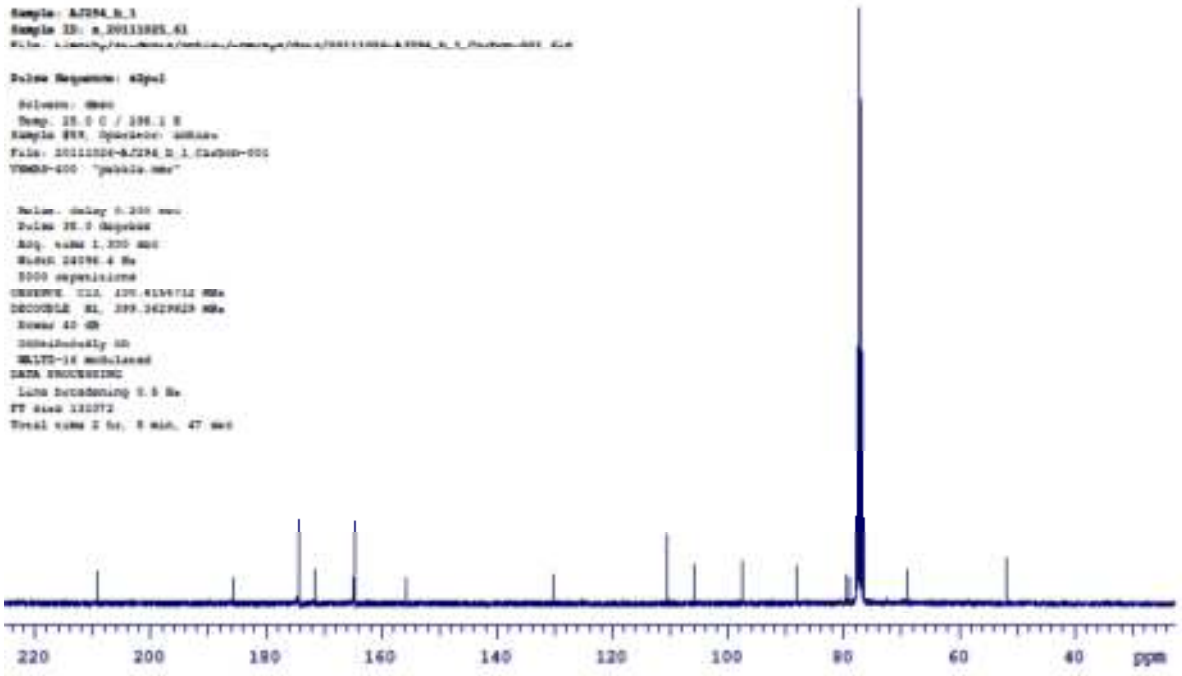
ppm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140
120
100
80
60
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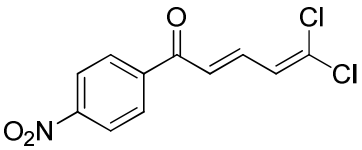
ppm

25a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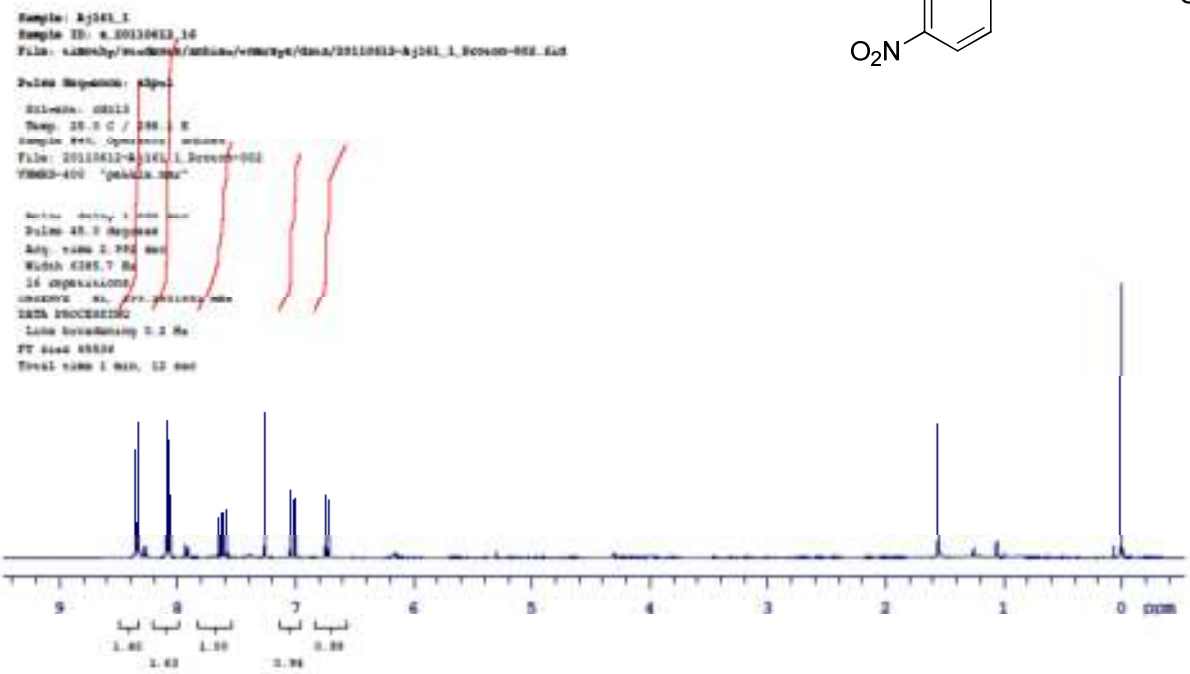
Cl
Cl
O_2N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8
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3
2
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0
ppm
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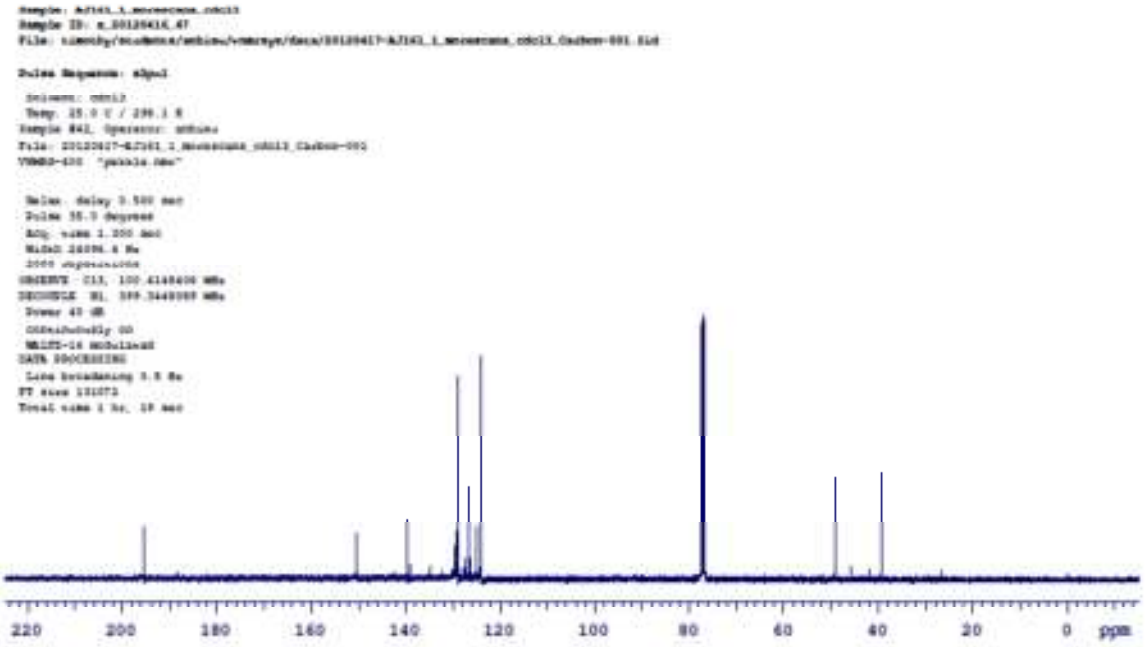
1.03
1.00
0.99
3.96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200
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140
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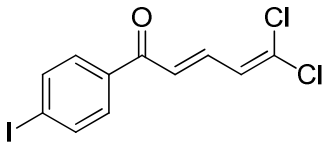
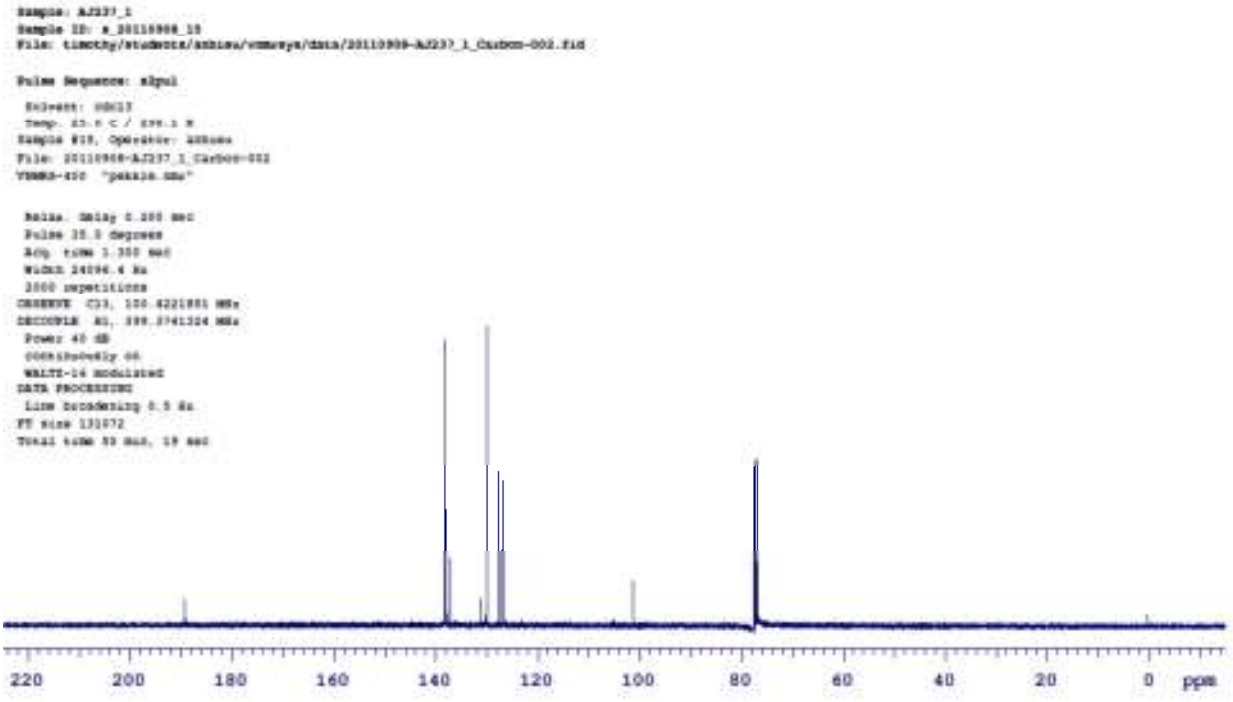
60
40
20
0
ppm

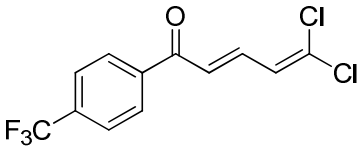
25b

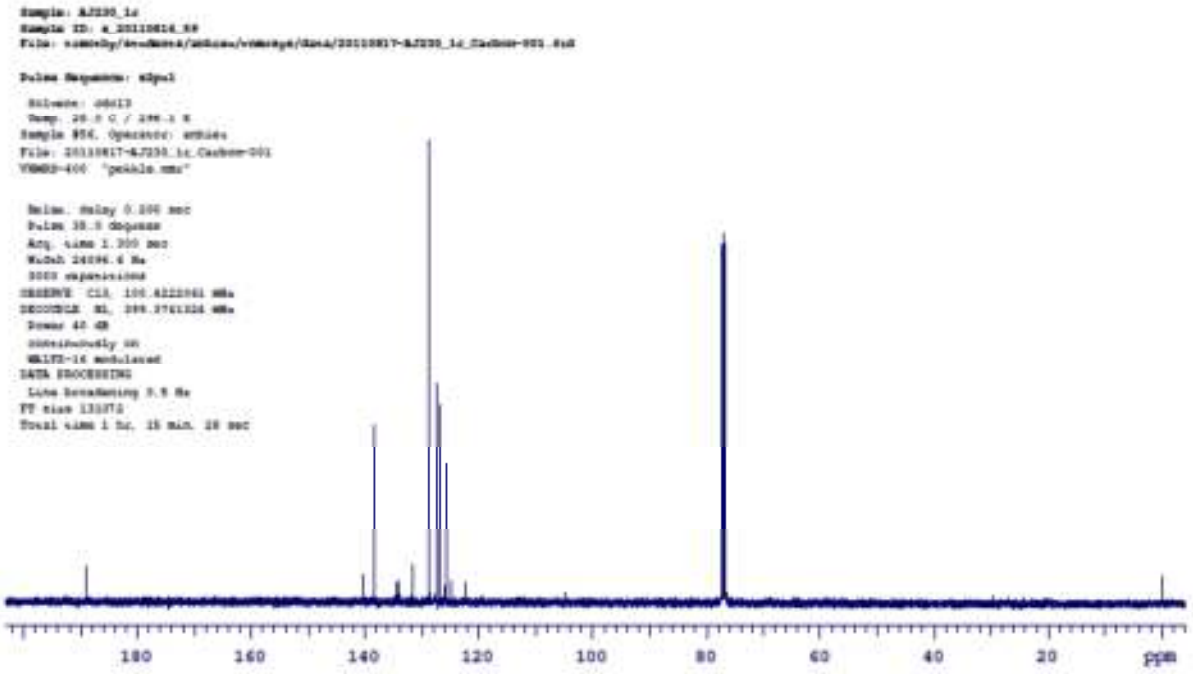

O
Cl
Cl
I

Pulse Sequence: s2pul
Temp. 25.0 C / 298.1 K
Pulse 45.0 degrees
Width 6385.7 Hz
16 repetitions
DATA PROCESSING
Line broadening 0.2 Hz
Total time 1 min, 12 sec
ppm

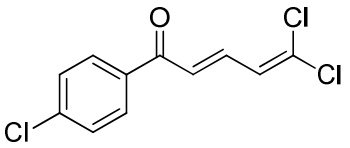

Pulse Sequence: s2pul
Pulse 30.0 degrees
Width 24096.4 Hz
2000 repetitions
Power 40 dB
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 131072
220
200
180
160
140
120
100
80
60
40
20
0
ppm

25c





25d


O
Cl
Cl
Cl

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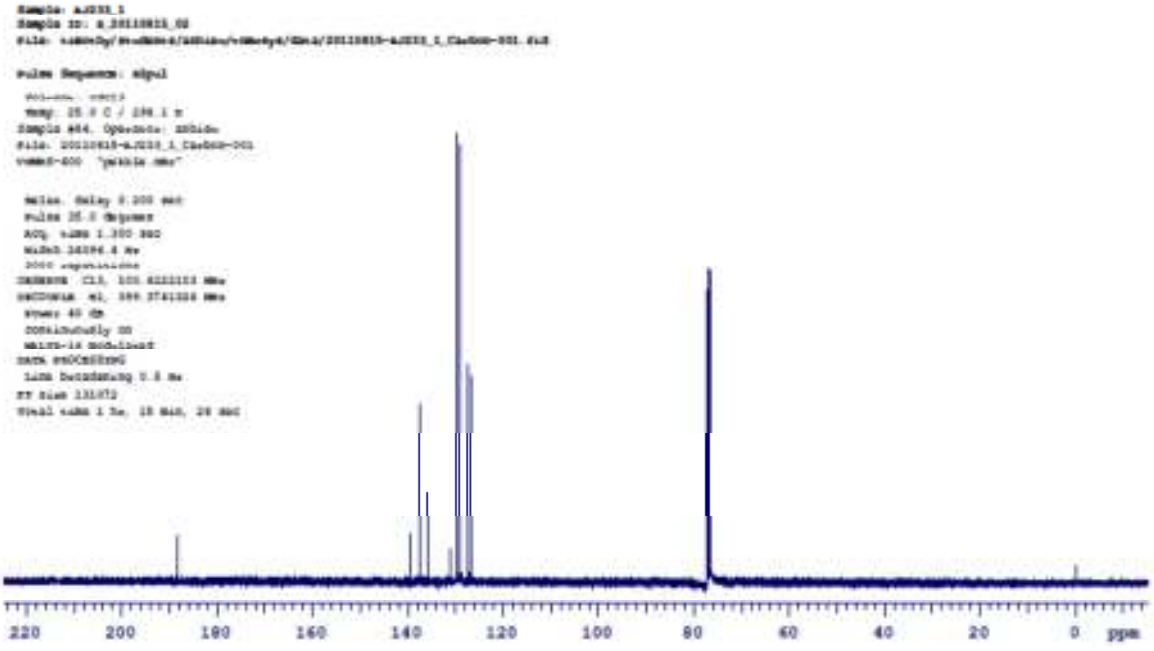
8
7
6
5
4
3
2
1
ppm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60
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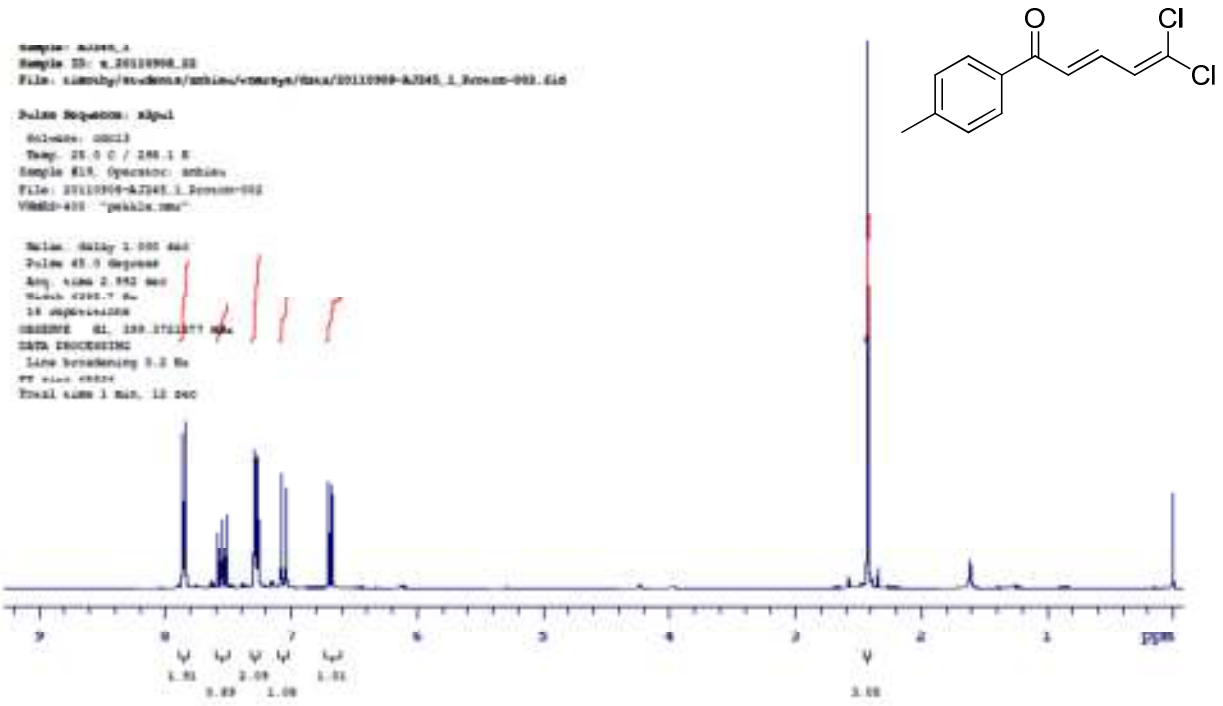
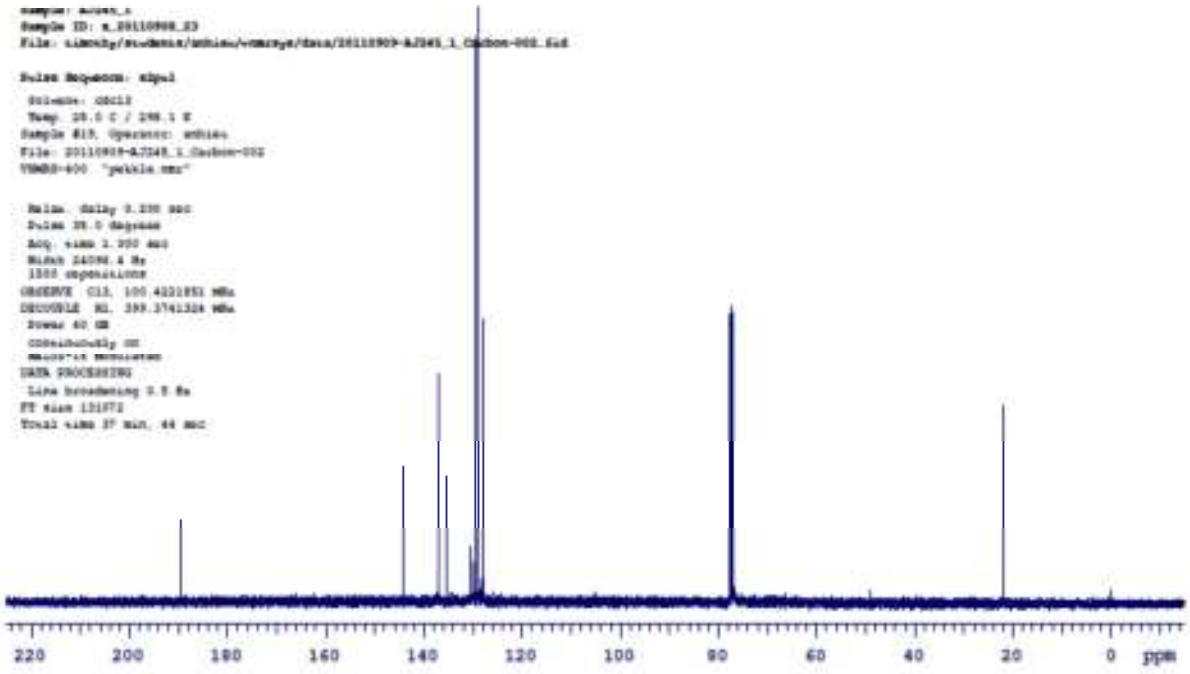
ppm

25e





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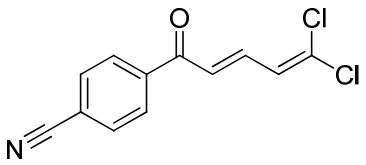
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Cl
Cl
N

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4
3
2
1
ppm
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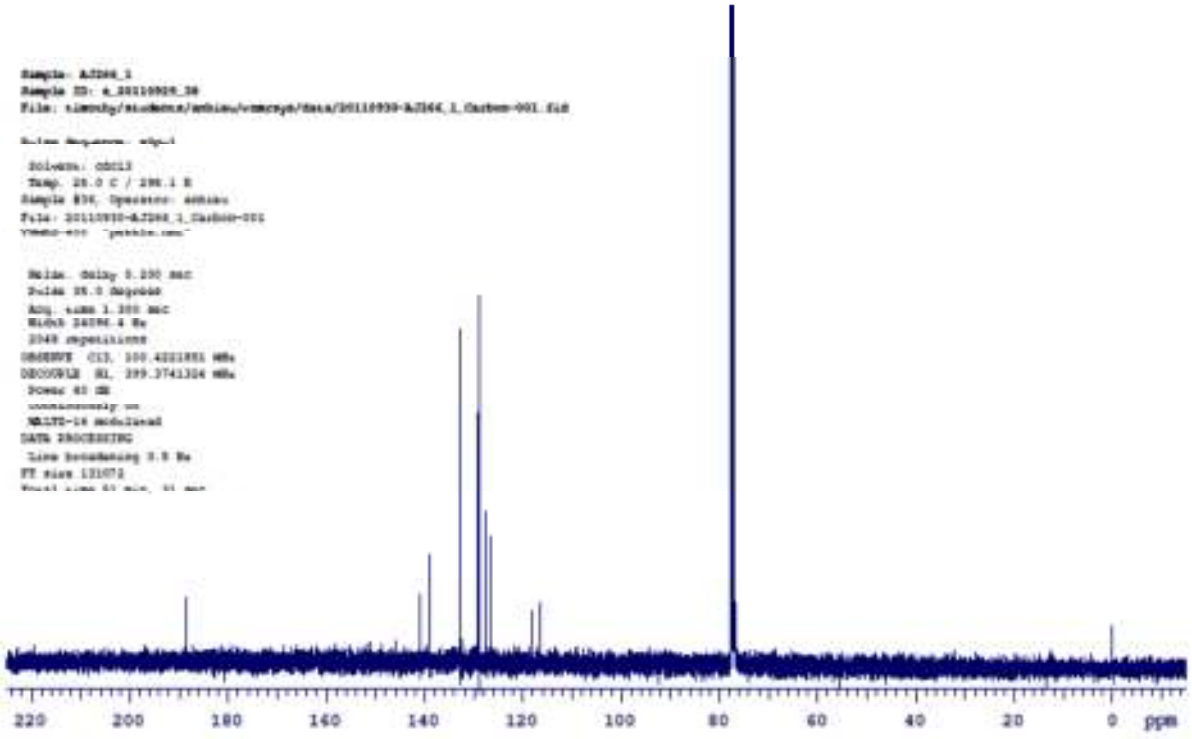
0.98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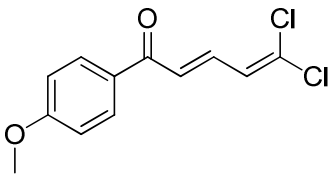
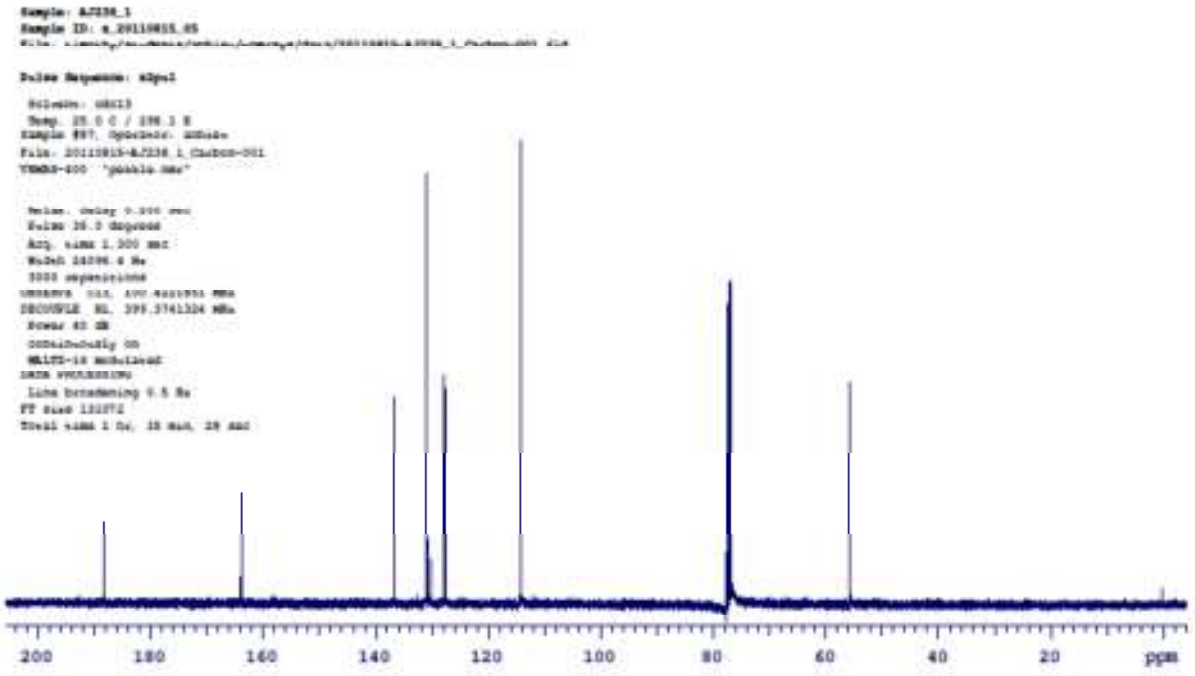
100
80
60
40
20
0
ppm

25g





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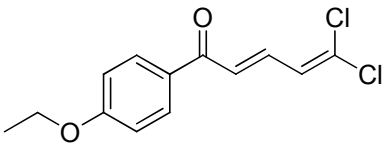

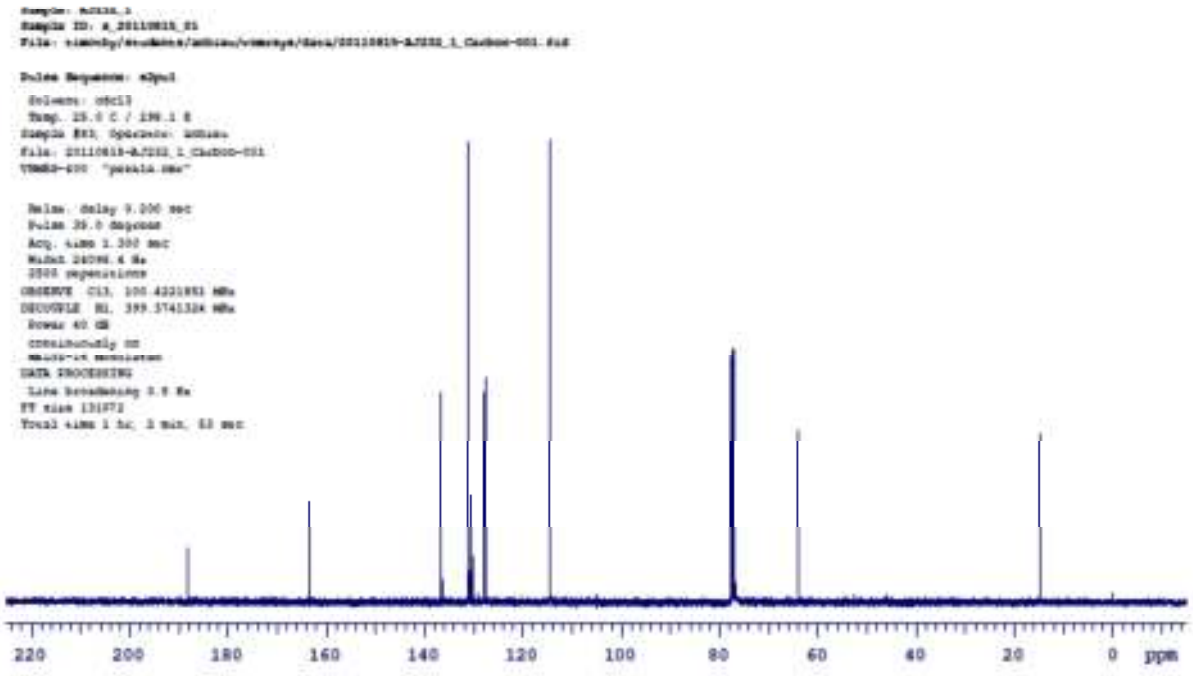

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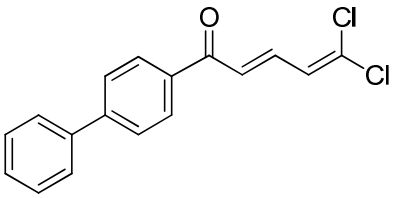
O
Cl
Cl

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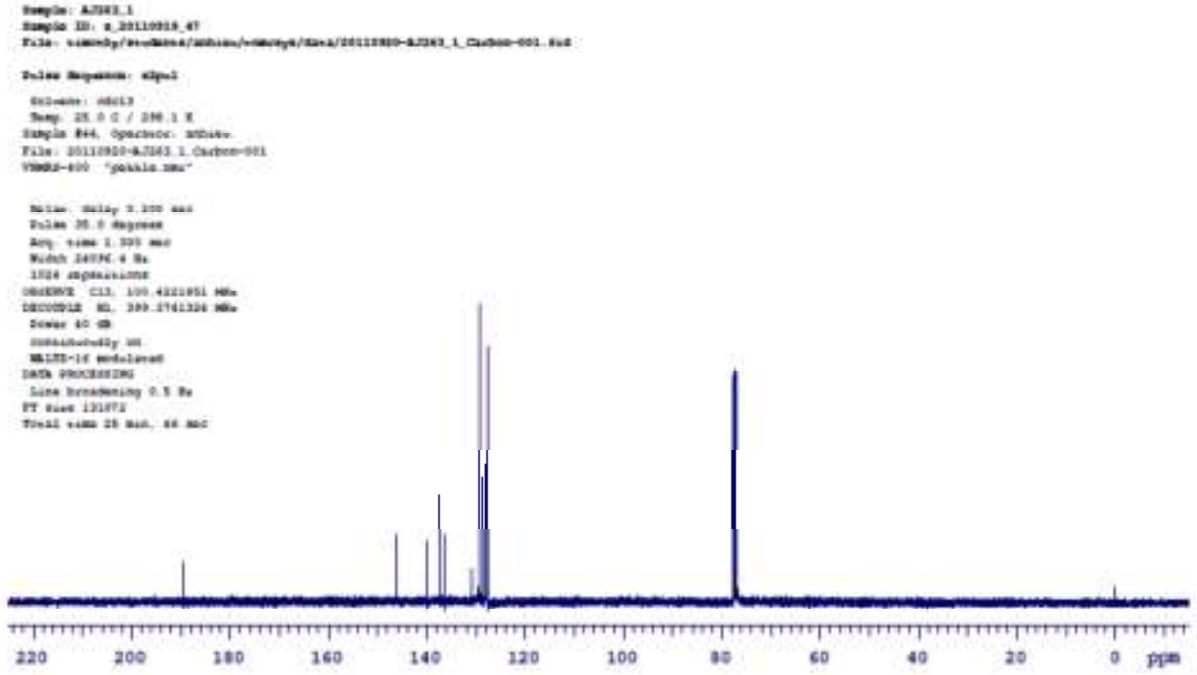
8
7
6
5
4
3
2
1
ppm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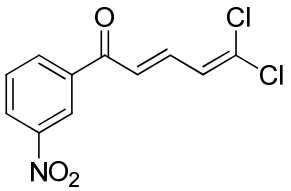
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Cl
Cl
NO2

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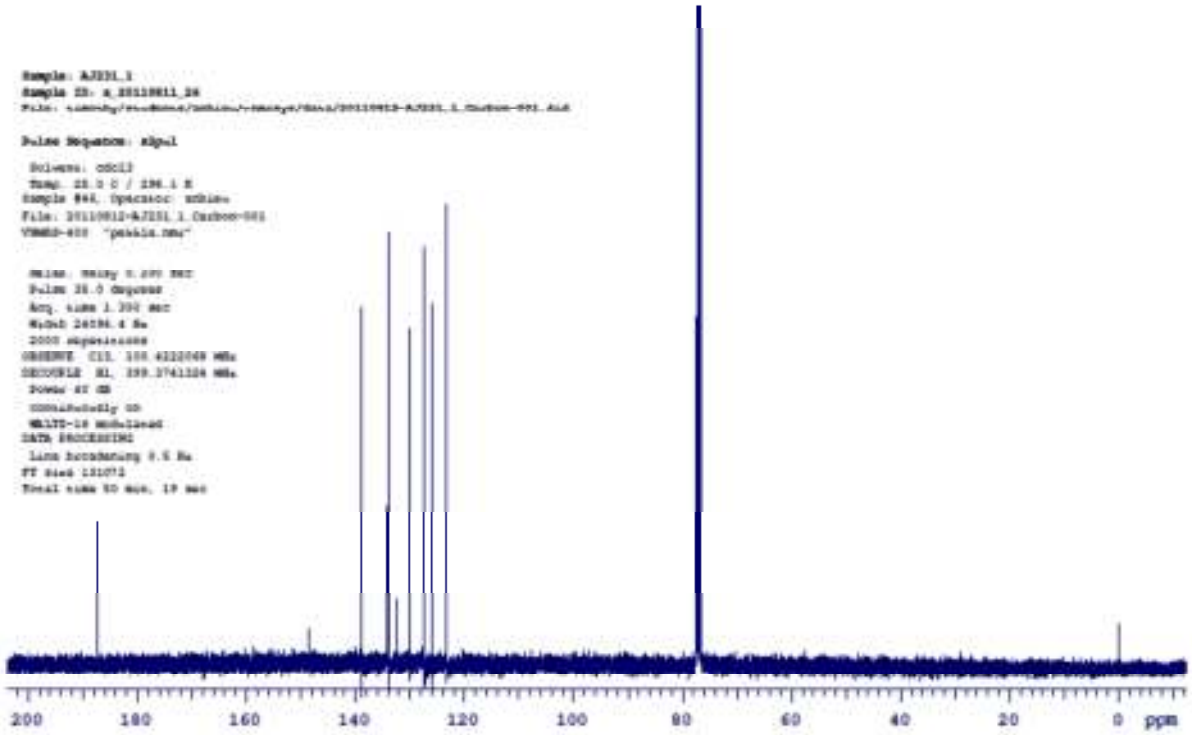
3
2
1
ppm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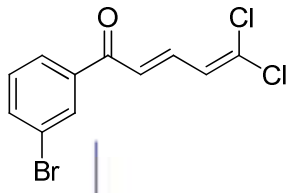
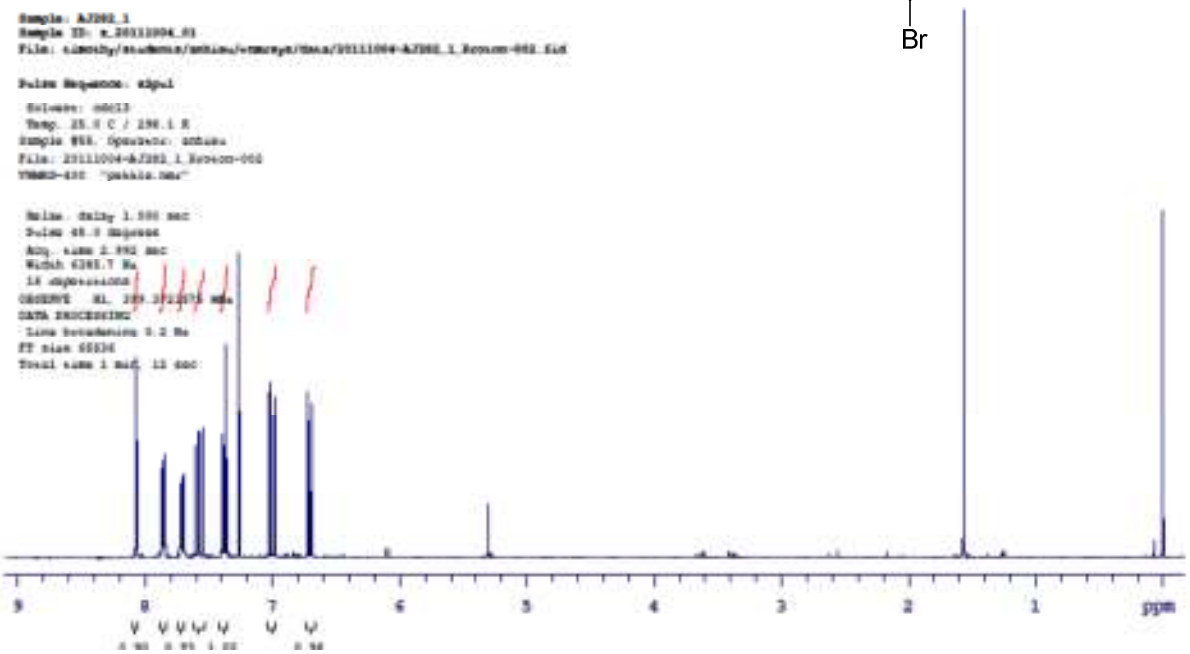
ppm

25k


O
Cl
Cl
Br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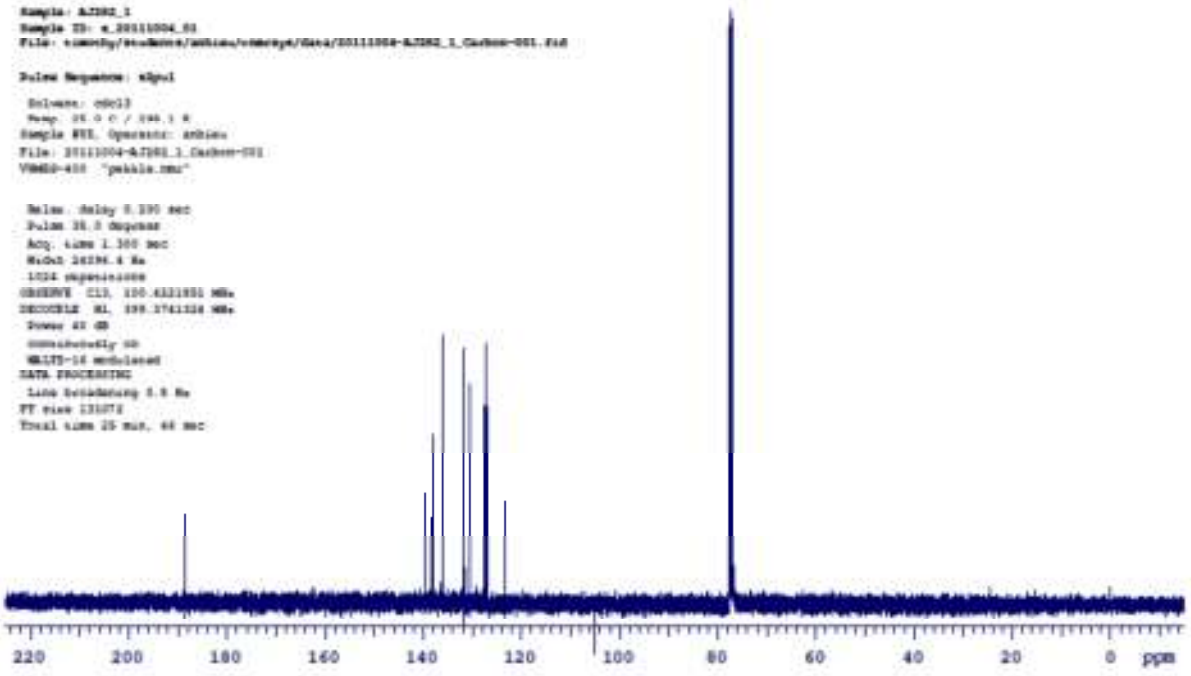
4
3
2
1
ppm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20
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ppm

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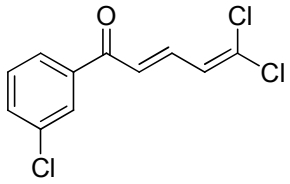

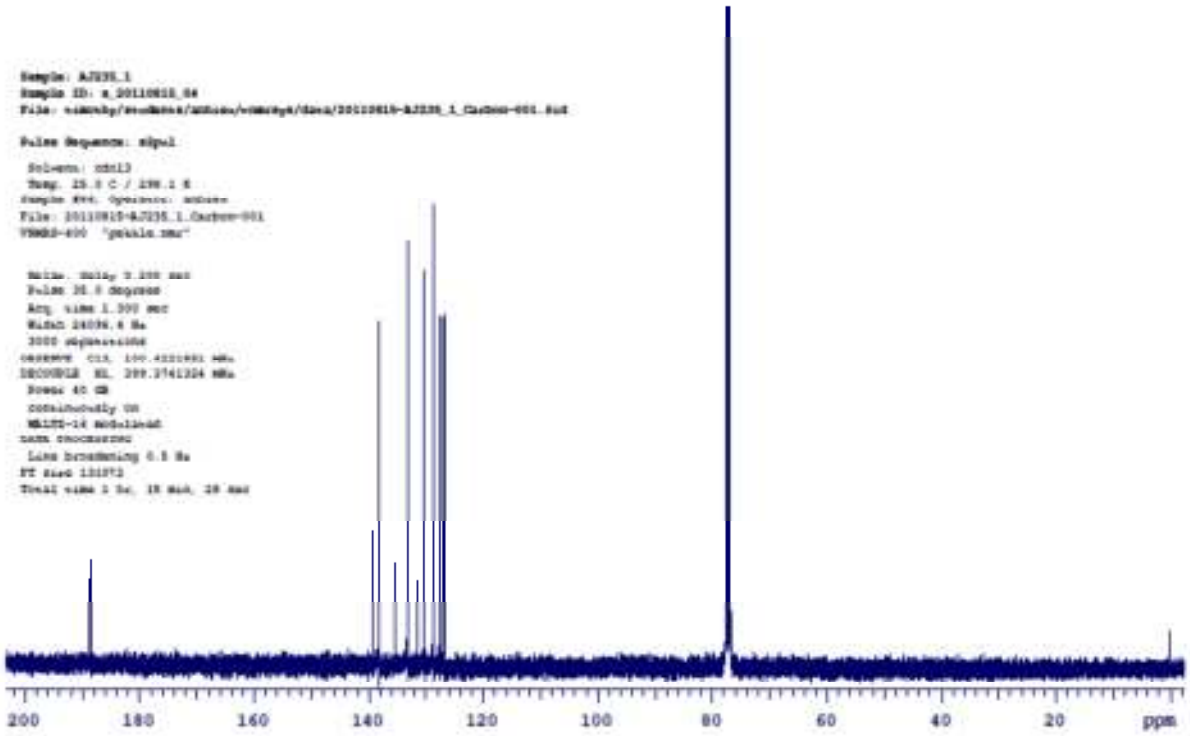

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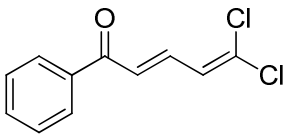
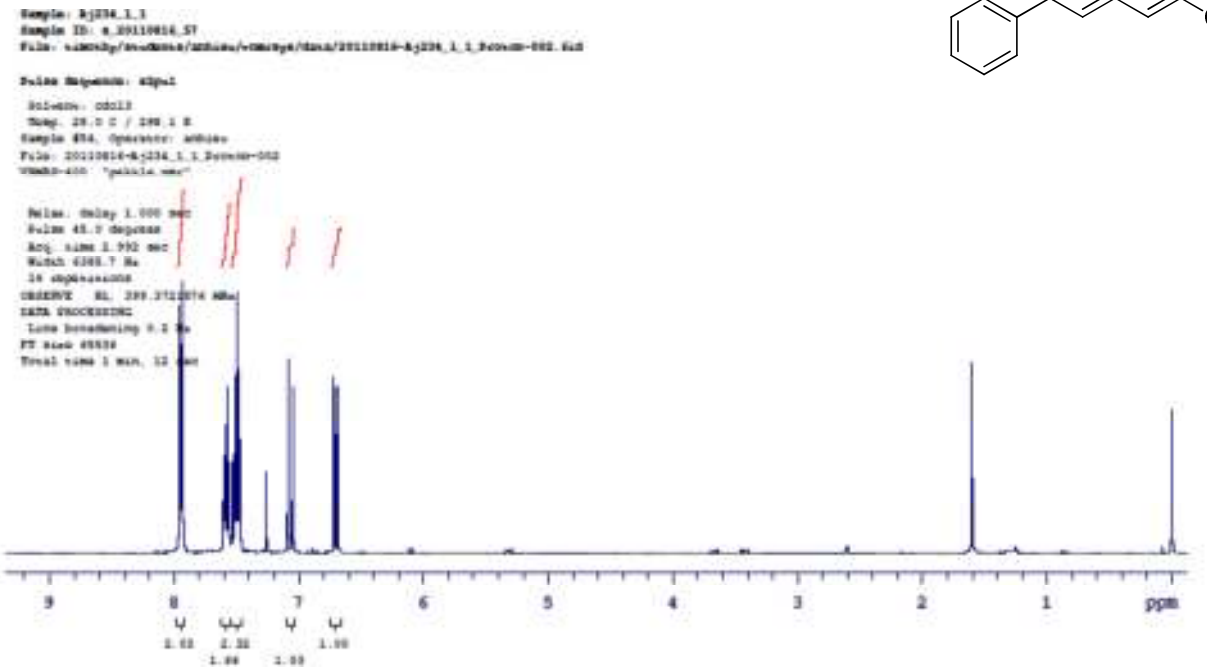
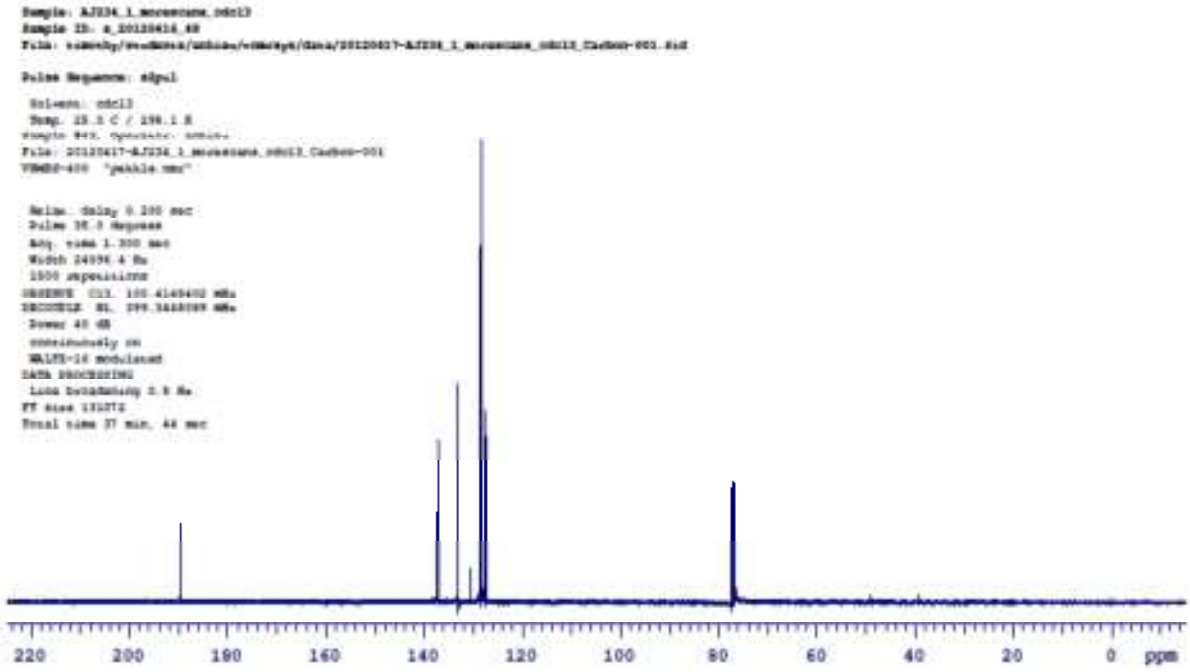

O
Cl
Cl


9
8
7
6
5
4
3
2
1
ppm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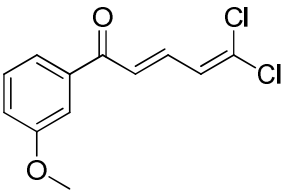
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Cl
O

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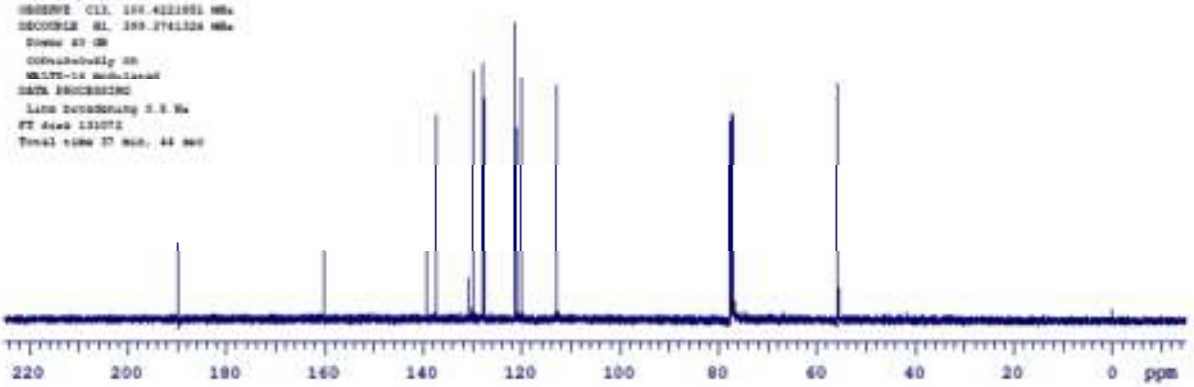
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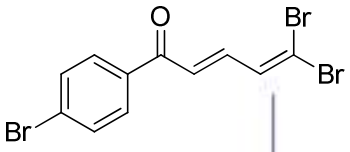
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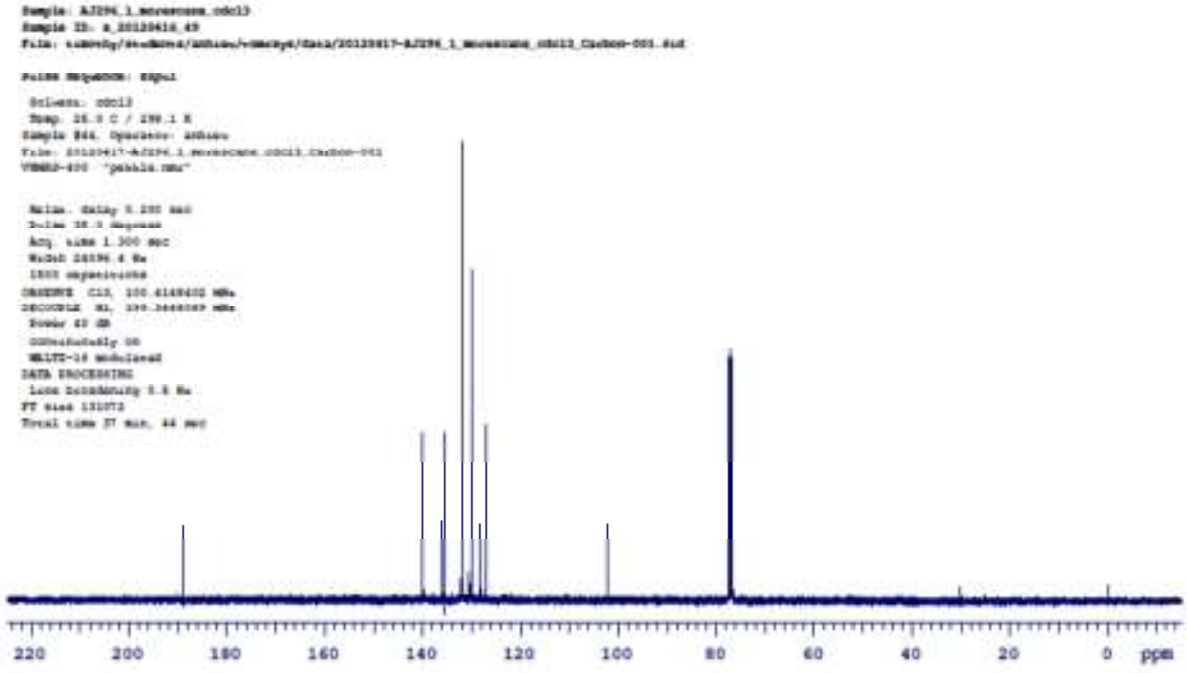
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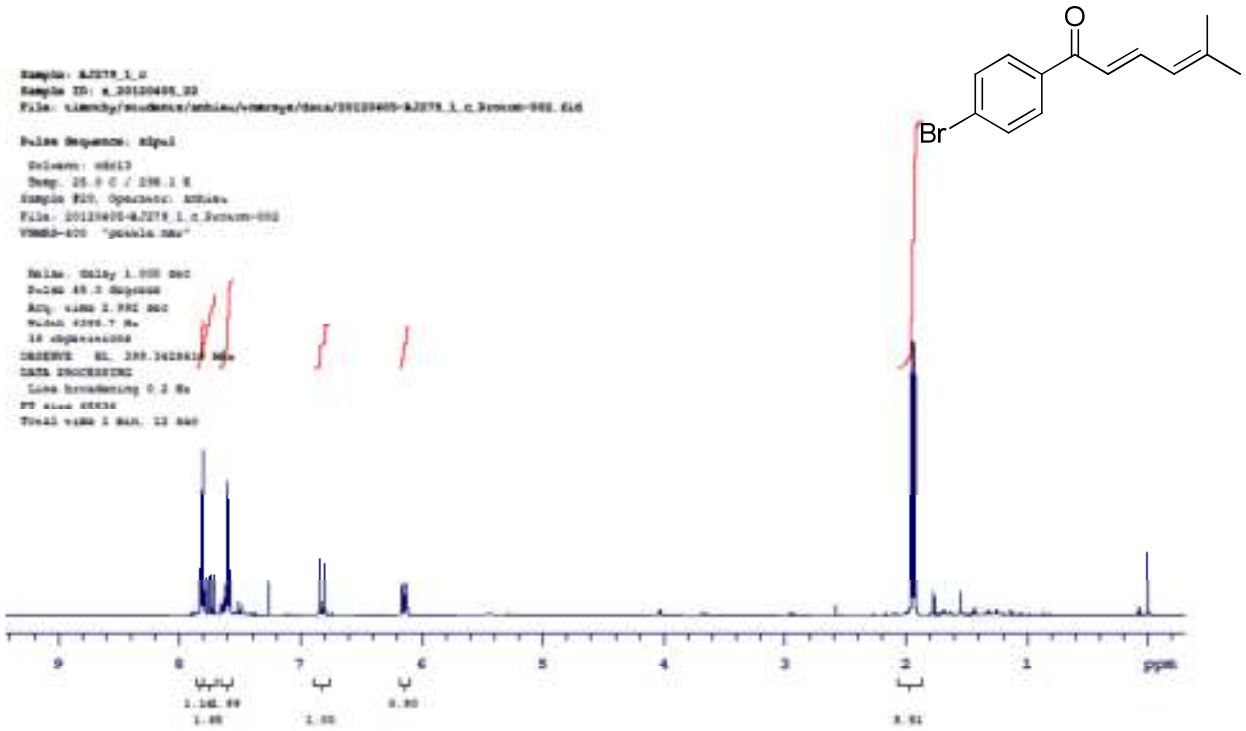

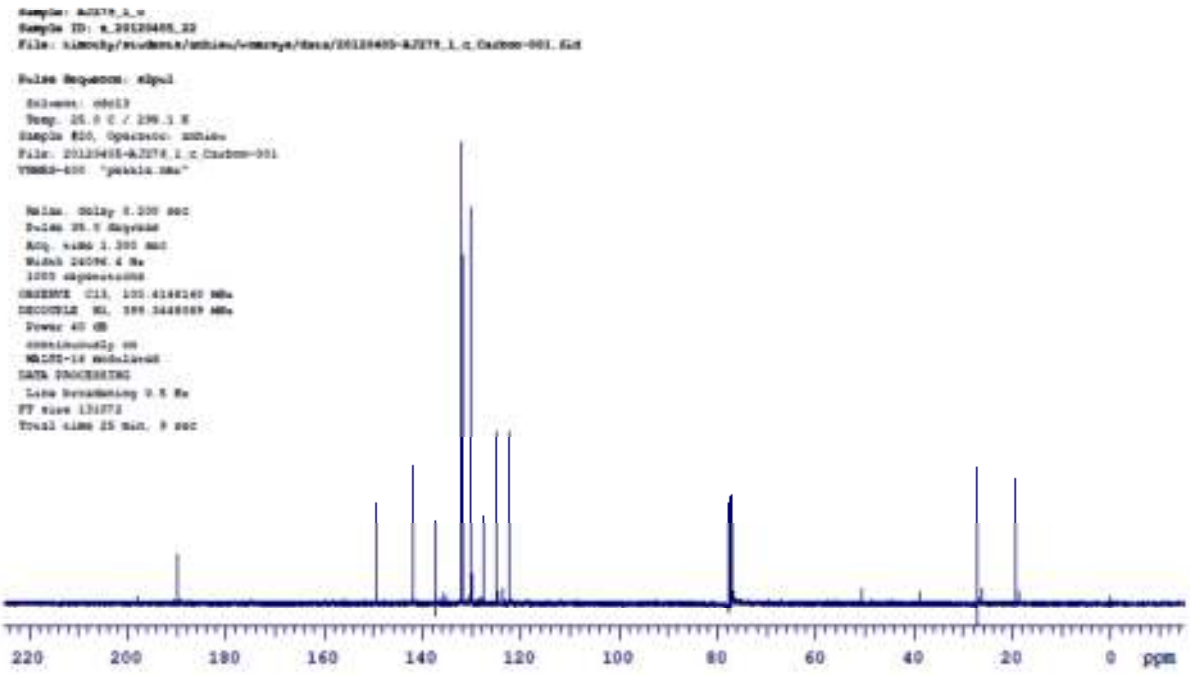

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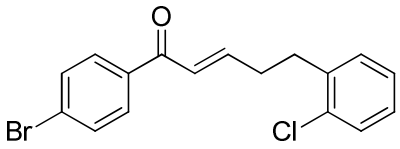
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Cl

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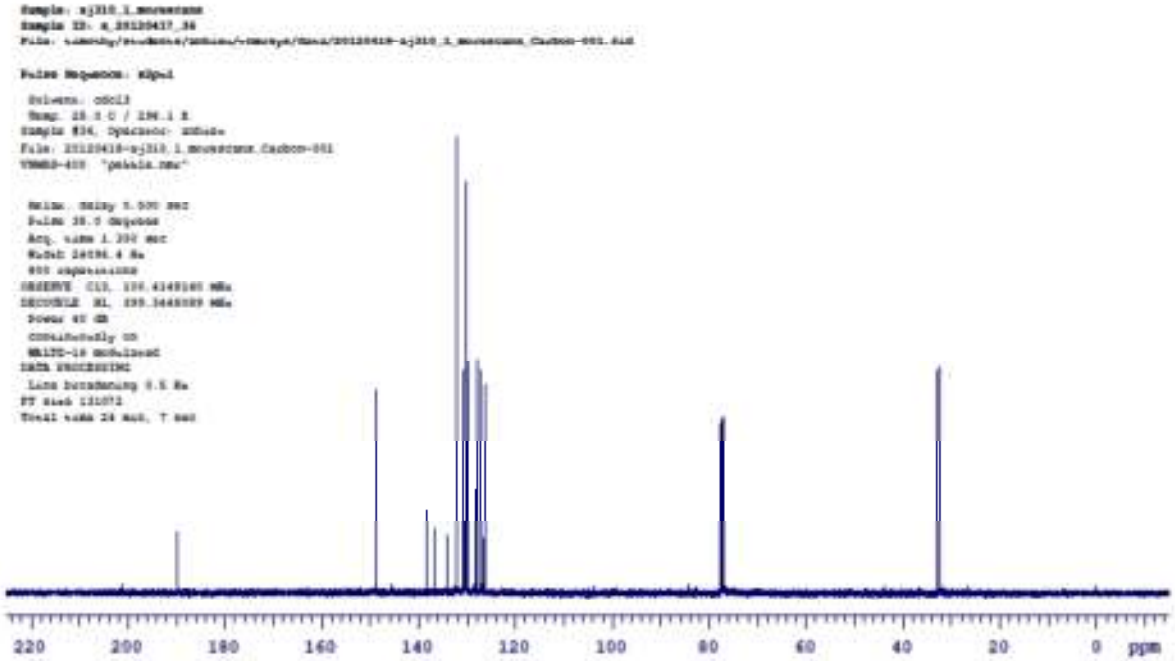
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