SYNTHETIC STUDIES AND BIOLOGICAL EVALUATION OF IMIDAZOLE-CONTAINING MARINE ALKALOIDS

by

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ABSTRACT

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This dissertation discusses the synthetic methods towards and the preliminary biological studies of an unnamed, imidazole-containing alkaloid, derived from the marine species *Oceanapia*. This alkaloid was first discovered in 1996 by researchers at the NIH as a possible inhibitor of the key enzyme in the detoxification cycle of *Mycobacterium tuberculosis*.

Chapter 1 provides an overview of tuberculosis, which is currently the second highest cause of death worldwide, with one third of the world's population infected. In recent years, the emergence of multi-drug resistant strains has created new interest and funding for tuberculosis research. New treatments like the alkaloid described above are

of special interest because they attack unique enzymatic pathways of the bacterium species that are not found in the human host.

Chapter 2 of this dissertation describes a potential synthetic route to this marine alkaloid which is predicated on the assemble and union of three key fragments: a spiro-isoxazoline, an imidazole, and a quiniolone. The spiro-isoxazoline fragment was synthesized using a minor modification of a previously described synthetic route involving an oxidative dearomatization. The imidazole fragment began with the oxidation of L-histidine into 4-cyanomethylimidazole, which was further elaborated into highly functionalized ester and amine intermediates suitable for use in cross-coupling chemistry. The quinolone fragments were synthesized by rearrangement of Meldrum's acid or cyanoacetate adducts. In the course of this chemistry an interesting chemoselectivity issue was uncovered relating to the selective N- vs O- protection. The key cross-coupling of the imidazole-quinolone fragment was attempted by several methods, but unfortuently this proved to be inefficient, these studies are described in detail.

A disk diffusion method was used to evaluate the activity of the main anti-tuberculosis drugs in a non-virulent model system, *Mycobacterium smegmatis*. Pre-loaded disks were purchased from VWR and placed onto petri dishes with the model bacteria. Plates were incubated for 24-48 hrs and the zones of inhibition were recorded. Several advanced intermediates were also screened for activity against the growth of the bacterial model system. Several of the intermediates tested had zone of inhibition ranges similar to the antimycobacterial agents isonazid and rifampin.

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INTRODUCTION

1.1 Tuberculosis

1.1.1 Background Information

Globally, there are 2 billion people, one third of the world's population, infected with the bacterium that causes the lung disease tuberculosis (TB), with at least 15 million active TB cases in the US[1]. Approximately 10% of all those infected will develop active TB infections and go on to infect an additional 20 people. Two million people die each year from a disease that is completely preventable.

According to a 2006 Center for Disease Control (CDC) Report,[2] there are 4.6 cases of active TB per 100,000 people in the United States. Among the US-born population, there are 2.3/100,000, and 22.0/100,000 for the foreign-born population. Less than 1% of all cases had primary drug resistance to the two main anti-TB drugs, isonazid (INH) and rifampin (RIF). Only 3 cases were considered extremely drug resistant to INH, RIF and several second-line drugs. 48% of all cases in the United States occurred in California, New York, Texas and Florida. 13% of cases among 25-44 year olds were dually infected with HIV.

Tuberculosis is an ancient disease. A common ancestor to the mycobacterial diseases *M. tuberculosis*, *M. Africans*, *M. canettii* and *M. bovis* most likely originated in

Africa 15,000-35,000 years ago.[3] Mummified remains and early Egyptian art show the skeletal deformities of the tuberculosis bone disease called Pott's disease. The ancient Greeks called TB "phthisis" (pronounced TEE-sis) after the Greek word meaning wasting or decay. The earliest written texts describing tuberculosis disease came from India 3300 years ago and China 2300 years ago.

TB was a major illness during the Industrial Revolution in Europe in the late 17th century, and then later in the US. This was partly due to the living conditions in the new cities. In rural areas, it was uncommon for the infection to travel outside the infected individual's village. Signs of TB infection include weight loss, a pale complexion, exhaustion, and a cough with blood-streaked phlegm. The eyes would seem to sink in as the face became skeletal, and it has been described as though the body was being consumed from the inside, leading to the term "consumption" and its association with infected individuals. Before antibiotics were discovered, about half of those infected would die within 5 years. Some died peacefully as the infection took over their bodies, but many more drowned painfully as sudden hemorrhages in the lungs occurred.

On March 24, 1882, Robert Koch read his famous paper to the Physiological Society in Berlin. He claimed that TB was an infectious disease that was caused by a bacterium.[4] This was the first proof that the scientific community had that TB was not hereditary, and that it may be cured.

Koch used four postulates to prove the TB infection: 1) he isolated the bacteria from an animal with the disease; 2) he grew the bacteria in culture; 3) he inoculated

healthy animals with the bacteria and watched for signs of infection; and 4) he recovered bacteria from the animals that had died.

In the 19th century, so many young and talented people became infected with TB that it was considered stylish. It was also thought to be a hereditary disease, since usually family members would die of the disease. Some famous people who died from TB were: John Keates, the entire Bronte family, Henry David Thoreau, Frederic Chopin, Franz Kafka, George Orwell and former First Lady Elenanor Rooseveldt.

1.1.2 TB Infection

One of the reasons TB is so infectious, is that it spreads through the air in small moisture droplets a person expels when they laugh, cough, sneeze or talk. TB infection can occur when one bacterium travels through the circulatory system to the alveoli of the lungs. The body's immune cells, macrophages, try to encapsulate the bacteria, but the hard, waxy coating makes the bacteria difficult to penetrate. The macrophages may encapsulate the bacteria and prevent it from multiplying, but eventually the TB can overcome and kill the macrophages. More macrophages rush into the lungs and try to kill the multiplying TB bacteria. However, the bacteria forms small clumps, and the macrophages cannot get to the center of the infection. After several weeks, a tumor-like module, a tubercle, forms.

The tubercles send out daughter cells through the bloodstream and lymph system, spreading the infection as they go. After 3-4 weeks, a healthy person's immune system can overtake the bacteria. The bacteria is not killed, but it is walled off in scar

tissue, and in most people the infection can remain latent, or inactive, for the remainder of their lives.

In about 10% of TB cases, the infection cannot be controlled. In about half of these cases, the infection progresses immediately to an active, infectious disease. In the other half, these packets of encapsulated TB bacteria remain latent for up to several decades, usually until the person becomes immunocompromised.

In active TB infections, the bacteria eat away and destroy healthy lung tissue, creating pus-filled, ulcer-like cavities, and impairing the body's ability to breathe. The damaged lung tissue does not grow back without scarring. Although TB is primarily a lung infection, in active cases, the infection can move to other parts of the body, such as the bone and organs.

1.1.3 TB, Global Travel and Immigration

Approximately 50 million international travelers enter the US every year, including 660,000 legal immigrants.[5] It is estimated that there are between 7-9 million illegal immigrants, often coming from countries with high levels of TB infection (e.g. Mexico, the Philippines, the former Soviet Union, Vietnam, Haiti, El Salvador, India, the Dominican Republic and mainland China). 46% of all active TB cases in the US are found in people who were not born in this country.

All documented legal immigrants to the US over the age of 15 are required to have a chest x-ray, taken within 12 months of entering the country, to show they do not have active TB infection. If an immigrant's x-ray shows active TB infection, they are labeled Class A, and forbidden to enter the US. If there is evidence of infection, the

immigrant is placed into Class B. All Class B immigrants are allowed to enter the US, but they must undergo further testing and ongoing monitoring once they reach their destination. This procedure is not required for business travelers and tourists. TB infection can also pose a risk on international flights over 8 hours long.

1.1.4 Current TB Drug Therapy and DOTS

Streptomycin, **1**, isolated from the throat of a sick chicken in 1943, was one of the first compounds that showed antibiotic activity against TB. Later that same year, the Mayo Clinic in Minnesota was the first institution to administer streptomycin to a human patient: a 21 year old, female patient with severe TB infection, who had already had one lung removed, but the infection had spread to the other lung. With no other treatment options, she was treated with streptomycin from 1944-45, cured of active infection and eventually left the hospital, married and had children.[6,7]

It was soon noticed, however, that streptomycin was not a miracle drug. Many patients got better, but then relapsed. Doctors quickly learned that a combination of drugs, one to kill the primary infection, and 2-3 others to kill the drug-resistant bacteria, was the best treatment option. Other drugs that were discovered soon after were: isonazid (INH, **3**, 1952, Figure 1.1), pyrazinamide (PZA, **4**, 1952), and ethambutol (EMB, **5**, 1963). The first drugs were given as a combination of 3 pills a day for 18-24 months.

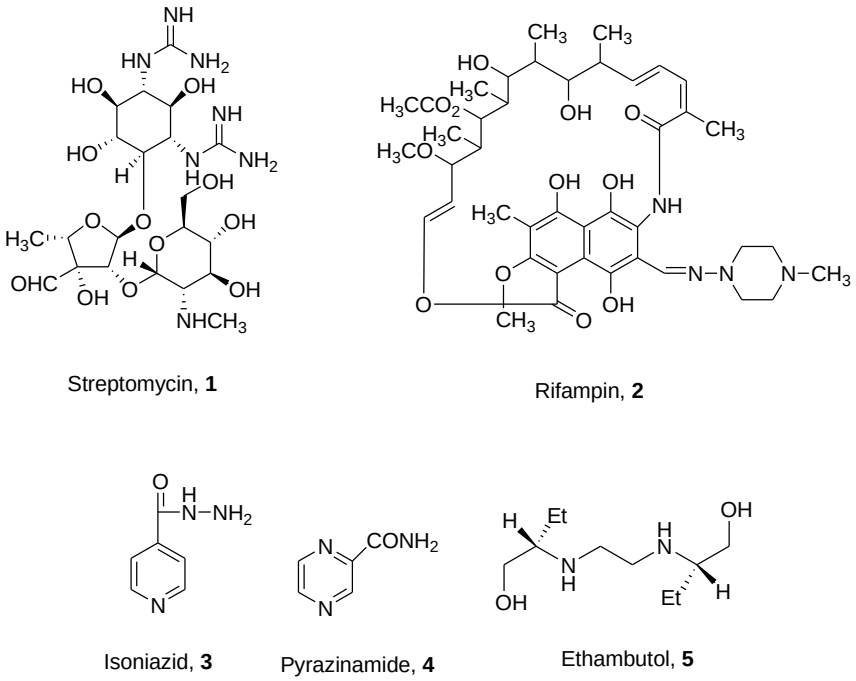


Figure 1.1 First-Line Anti-TB Drugs

In the mid 1960's Dr. Piero Sensi of the Lepetit Research Laboratories in Milan asked colleagues to bring back herb, fungal, algae and soil samples from wherever they were going on vacation. He believed he could create new anti-TB drug with a natural product fermentation, like penicillin. The successful candidate, rifampin (RIF, **2**, Figure 1.1) came from a sample of the bacterium *Nocardia Mediterranei* from the French coast. Rifampin was introduced in Europe in 1968 and in the US in 1974, 10 years after it was first isolated.[8]

The introduction of rifampin shortened the length of treatment to 9 months, but the amount of pills increased to 5 a day. Doctors soon realized that a combination of

PZA/INH/RIF for the first 2 months would shorten the length of treatment to 6 months. This was referred to as short course therapy.

Current therapy for drug susceptible infections involves two possible regimens. The first two-months are a combination of isonazid (**3**), rifampin (**2**), pyraninamide (**4**) and either streptomycin (**1**) or ethambutol (**5**). This is followed by four months of isonazid and rifampin. With this regimen, patients will become non-infectious after the first few weeks, but the long regimen is needed to kill the remaining slow growing bacteria that are not killed with the initial treatment.

Combination drugs, those that combine anti-TB drugs in a single pill, are popular outside of the US. The pills are more convenient for patients and health care providers, and it makes it impossible for a patient to skip one drug, decreasing the risk of drug resistance.

Since TB is a threat to public health, health care providers are often held responsible for proper treatment, not the individual patients.[9] Drug therapy must be monitored through a program called Directly Observed Therapy (DOTS).[10] In areas with low compliance, incentives, such as food, transportation vouchers and free medical care can be offered to encourage patients to take their medications. The WHO claims countries that offer DOTS have a lower level of drug resistant cases and can cure up to 98% of drug sensitive cases. Even with DOTS, a patient with an active case of MDR-TB has only a 50% chance of surviving 5 years.

In 1994, the WHO defined five essential elements of TB control:[11] (1) government commitment of sustainable TB control; (2) diagnosis by sputum-smear

microscopy; (3) standardized short-course chemotherapy, including DOTS; (4) a functional drug supply; (5) a recording and reporting system that allows assessment of treatment results.

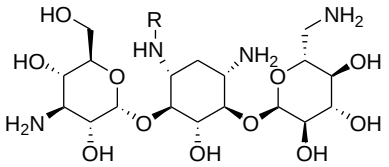
1.1.5 MDR-TB, XDR-TB and Second Line Antibiotics

The World Health Organization defines any case that is resistant to the top two first line drugs, isonazid and rifampin, as multi-drug resistant (MDR-TB). Extremely drug resistant (XDR-TB) cases are defined as strains that are resistant to both isonazid and rifampin, at least one of the injectable second line drugs kanamycin (**6**), amikacin (**7**), and capreomycin (**8**) (Figure 1.2), plus at least one fluoroquinolone (**10**-**17**, Figure 1.3). The drug-resistant cases are not more infectious than drug-susceptible cases, but they are much more difficult and much more expensive to treat. Treatment rates for MDR-TB are approximately 50% and for XDR-TB about 30%.

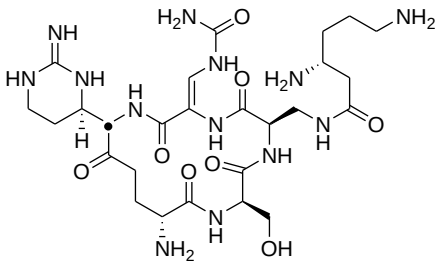
For drug-resistant cases of TB, the WHO classifies second-line drugs (SLDs) according to efficacy, experience of use, and drug class.[12] These drugs are usually not as well tolerated, have serious side effects, are expensive, and can be difficult to obtain outside the developed world, where many of the drug resistant cases are found. Some significant side effects of these drugs include: severe gastrointestinal intolerance, including nausea, vomiting, diarrhea, abdominal pain, nephrotoxicity, pain at injection site, reversible renal failure, hearing loss, headache, insomnia, dizziness, photosensitivity, and depression.

The injectable antituberculosis drugs (Figure 1.2): kanamycin (**6**), amikacin (**7**), capreomycin (**8**), viomycin (**9**), as well as first-line drug streptomycin (**1**, Figure 1.1),

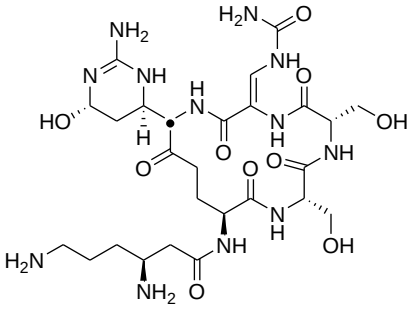
are the most effective and best tolerated SLDs. The aminoglycosides, kanamycin and amikacin, are bactericides that inhibit protein synthesis, and are useful in cases resistant to streptomycin. Capreomycin is more expensive, but since it is has a differenct mechanism than the aminoglycosides, it can be used in cases where there is resistance to kanamycin, amikacin and streptomycin.



R = H, Kanamycin, **6**
R = $COCH(OH)CH_2CH_2NH_2$, Amikacin, **7**



Capreomycin, **8**



Viomycin, **9**

Figure 1.2 Injectable Anti-TB Drugs

Three fluoroquinolones (Figure 1.3), bactericides that inhibit DNA reproduction, are: ofloxacin (**10**), ciprofloxacin (**11**), and levofloxacin (**12**), which have low bactericidal activity, but can be useful when used in combination with other drugs. Due

to adverse side effects or the lack of long-term safety and efficacy studies, the WHO does not recommend the use of sparfloxacin (**14) (**photo-sensitivity),[13] norfloxacin (**15**) (low serum levels), moxifloxacin (**16),** or gatifloxacin (**17).**

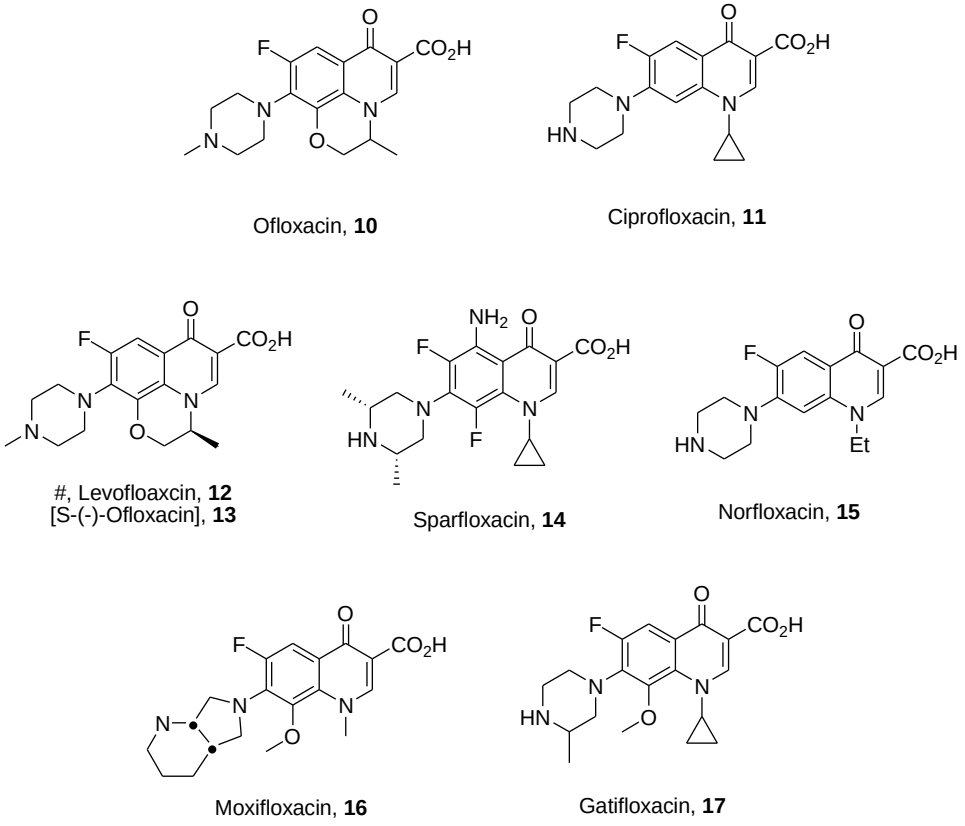


Figure 1.3 Second-Line Anti-TB Drugs: Fluoroquinolones

Second-line oral bacteriostatic agents, which inhibit mycolic acid synthesis (Figure 1.4), a main component of the mycobacterial cell wall, include: ethionamide (**17**), prothionamide (**18**), cycloserine (**19**), p-aminosalicyclic acid (PAS, **20**), and thioacetazone (**21**). Cycloserine, or the derivative terizidone (**22**), are useful to prevent resistance to other active drugs, but is not used often due to its high toxicity. Para-

aminosalicyclic acid is a bacteriostatic agent that is useful for preventing drug resistance to INH and streptomycin.

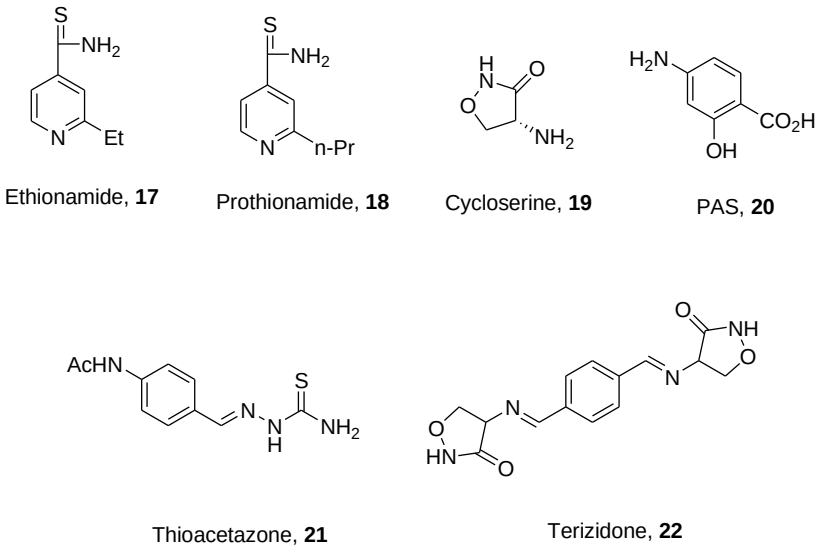


Figure 1.4 Second-Line Anti-TB Drugs: Oral Bacteriostatic Agents

For the approximately 3% of active TB cases each year that are multi-drug resistant (185,000-414,000 cases)[14] it is recommended that at least a five-drug regimine is followed. The preference order of the drugs are as follows: (1) first-line agents that are still active, (2) Any injectable drug (streptomycin, kanamycin, amikacin, capreomycin, or viomycin), (3) One of the fluoroquinolones, (4) Second line bacteriostatics (PAS, ethionamide or prothionamide, or cycloserine), and finally (5) other drugs (rifampin derivatives, amoxicillin, clavulanic acid, clarithromycin).

1.1.6 The Mycobacterium bovis BCG (Bacillus Calmette-Guérin) Vaccine

The BCG vaccine is the most commonly used vaccine in the world with an estimated 3 billion doses administered. It was developed in 1900 by Albert Calmette, the director of the Pasteur research Institute in Lille, France, and his research assistant, Camille Guerin. The vaccine has been proven successful against miliary infections and TB meningitis in children, but it has little effect over pulmonary infections in adults. Because the efficacy of the vaccine depends on the initial replication of the viable organism, it cannot be used as a booster in the adult population that has already been exposed to the bacteria.[15]

Although widely used in developing countries, the US and the Netherlands are the only two countries that have never used the vaccine routinely, and today they are among the countries with the lowest incidence of TB infection. There are several factors that affect the efficacy of the vaccine: virulence differences among *M. tuberculosis* strains, differences in the genetics and malnutrition level of the host population, and effects of environmental mycobacteria on the host immune response.[16] In addition, there are currently six different sub-strains used to administer the vaccine in different geographical regions, which may explain why the vaccine is more or less effective in different countries.

The vaccine can cause redness and hardening of the skin at the injection site, and about 1/3 of patients develop ulceration and persistent drainage. The most severe problem is that the vaccine also causes a positive skin test response, making diagnosis of active TB infection difficult.

Some scientists still believe that a vaccine would be the most successful and cost-efficient way to control a TB pandemic worldwide, since the current combination of the BCG vaccine and available drug therapy is not successful against the most drug-resistant cases. In the last 10 years, there have been more than 200 potential TB vaccines tested in animal models. Two strategies being investigated are replacing the BCG or developing an efficient booster vaccine to protect adult populations. Several new pre-exposure vaccines, ones which stimulate a stronger immune response than is seen with natural infection, are currently being tested, however, it is not known if these will be effective in patients that have already been exposed to the bacteria.

The safest delivery of the new vaccines may be intranasally.[17] Since the most common route of TB infection is inhalation leading to a primary infection in the lung, it is hypothesized that immunizing with this method would be advantageous over both orally or subcutenous methods as long as there are no potential toxic material that could travel to the central nervous system through the roof of the nasal canal.

1.1.7 Dual TB/HIV Cases in the 1980's and The MDR-TB Epidemic

In the early 1980s, a series of patients developed unusual TB-like infections.[18-20] When TB infection spreads to organs outside the lungs, including the brain, it is called extrapulmonary disease. These patients were among the first patients to be diagnosed with a new disease, HIV, and it was soon noted that AIDS could allow TB organisms to behave in a more virulent manner and that the mobidity and mortality rates were much higher in patients dually infected with HIV.

At the same time, there were also a large number of drug-resistant cases emerging in hospitals and prisons in NYC. The worst year of the epidemic in NYC was 1992. There were 3811 cases in NYC's 7½ million people. 441 of these cases were MDR-TB, and at least one third were HIV positive.[21] A few hundred patients died, usually those dually infected with HIV and TB. The outbreaks that occurred in NYC hospitals were up to 80% fatal.

In 2000, the UN program on HIV/AIDS (UNAIDS) declared 34.6 million people were infected with HIV worldwide. One third of these patients die from TB, the leading killer of HIV positive patients. About 5-15% of all HIV positive patients will develop active TB, despite it being completely curable in HIV positive patients.

1.1.8 Development of New Anti-TB Drugs

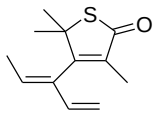
Since TB cases in the US were rapidly decreasing in the 1970's, much federal TB funding was eliminated. There was almost a 30 year period in which no new drugs for TB had been developed. During this time, primary drug resistance was seen in all first-line anti-TB drugs, and in many second-line drugs.

One main issue keeping TB drugs from being developed is drug companies. TB is considered an orphan disease; too few people are infected in developed nations to create the market needed to fuel the drug development. Drug companies often sell drugs for more in industrializes countries so they can subsidize the costs in poorer countries. With few patients in richer countries, TB drugs are not an attractive target for pharmaceutical companies. In the US, treatment for a full course of anti-TB drugs costs $900, whereas in developing countries, only $11.

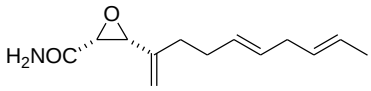
1.1.9 Target-Based Antimycobacterial Natural Products

Since there is existing resistance to all first and second-line anti-TB drugs, researchers are looking at new pathways for fighting the disease. Jachak and Jain reviewed the recent literature about anti-mycobacterial agents.[22] Many of the newly discovered natural products were being tested on new enzymatic pathways that targeted the synthesis of the antimycobacterial cell wall component mycolic acid or antibiotic detoxification. The waxy outer layer of the bacteria is made mostly of mycolic acids, which control the permeability and fluidity of the cell wall. These drugs are attractive towards both the treatment of drug-resistant strains of *M. tuberculosis* and emerging mycobacterium pathogens, such as *M. avium* complex.

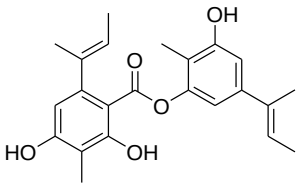
Some of the natural products that are currently being tested as anti-TB drugs are: thiolactomycin (**23,** Figure 1.5), and analogs, cerulein (**24**), agonodepside A (**25**), and myxopyronin A (**26**), and B (**27**).



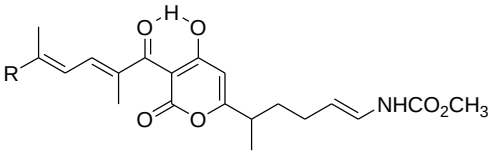
Thiolactomycin, **23**



Cerulenin, **24**



Agonodepside A, **25**



R = n-C_3H_7, Myxopyronin A, **26**
R = n-C_4H_9, Myxopyronin B, **27**

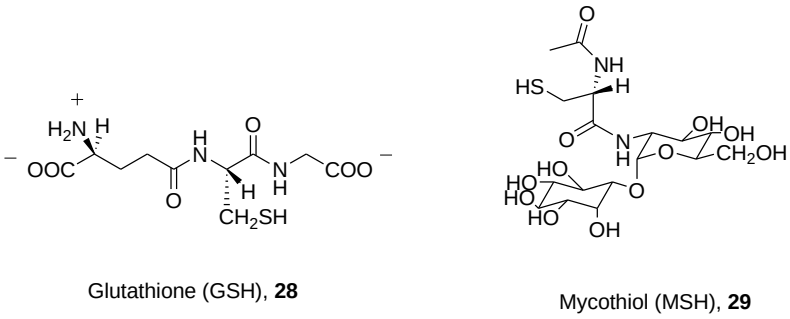
Figure 1.5 Target-Based Antimycobacterial Natural Products

1.2 Mycothiol

1.2.1 The Discovery of Mycothiol

Glutathione (GSH, **28**, Figure 1.6) is a low molecular weight thiol found in millimolar concentrations in most eukaryotic cells and many gram-negative bacteria. GSH plays a detoxification role by protecting the cell against oxygen-toxicity and was thought to be the major detoxification thiol in all living organisms until it was discovered that many gram-positive bacterium did not contain GSH. Quantification of thiols in five *Streptomycetes* species, using a fluoresence-labelled HPLC experiment, determined that the major thiol containing compounds found in GSH-lacking cells were cysteine, CoA, thiosulfate, sulfide, and an unknown thiol, named U17, in larger concentrations.[23] This compound proved to be the same as a previously isolated thiol

from *Micrococcus roseus* and *Streptomycetes griseus*, U25, and was eventually named mycothiol (MSH, **29**).



Glutathione (GSH), **28**

Mycothiol (MSH), **29**

Figure 1.6 Thiols Involved in Detoxification

The structure of mycothiol was first established in 1994 as the oxidized disulfide.[24] In 1996, it was determined that mycothiol was found in all seven major subgroups of the actinomycetes, but not present in actinobacteria. The largest concentrations of mycothiol were found in mycobacteria and streptomycetes.

1.2.2 Mycothiol Biosynthesis

The original work on MSH biosynthesis (Figure 1.7) was done by Steenkamp and coworkers,[25] but they could not determine the enzyme required for the initial step. The initial steps of mycothiol biosynthesis have recently been elucidated by Newton and Fahey.[26] They determined that there were two enzymes needed for the assembly of the mycothiol precursor GlcNAc-Ins (**33**): MshA, a glycosyltransferase, and MshA2, a phosphatase. The biosynthesis begins with *myo*-inositol (**30**) which is phosphorylated to 1L-Ins-1-P (**31**) by an inositol kinase, Inok. In the next step, MshA catalyzes the production of the disaccharide GlcNAc-Ins-P (**32**), 1-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-D-*myo*-inositol 3-phosphate. In this reaction, the *N*-acetylglucosamine

donor is UDP-*N*-acetylglucosamine (UDP-GlnNAc) and the N-acetylglucosamine acceptor is 1L-myo-inositol 1-phosphate (1L-Ins-1-P). The MshA2 enzyme is required to dephosphorylate GlcNAc-Ins-P. The disaccharide is deacetylated by MshB (GlcN-Ins, **34**), then cystein is added with the ligase MshC (Cys-Glc-N-Ins, **35**), and finally, the cystein is acetylated with the acetyltransferase MshD to give mycothiol (**29**).

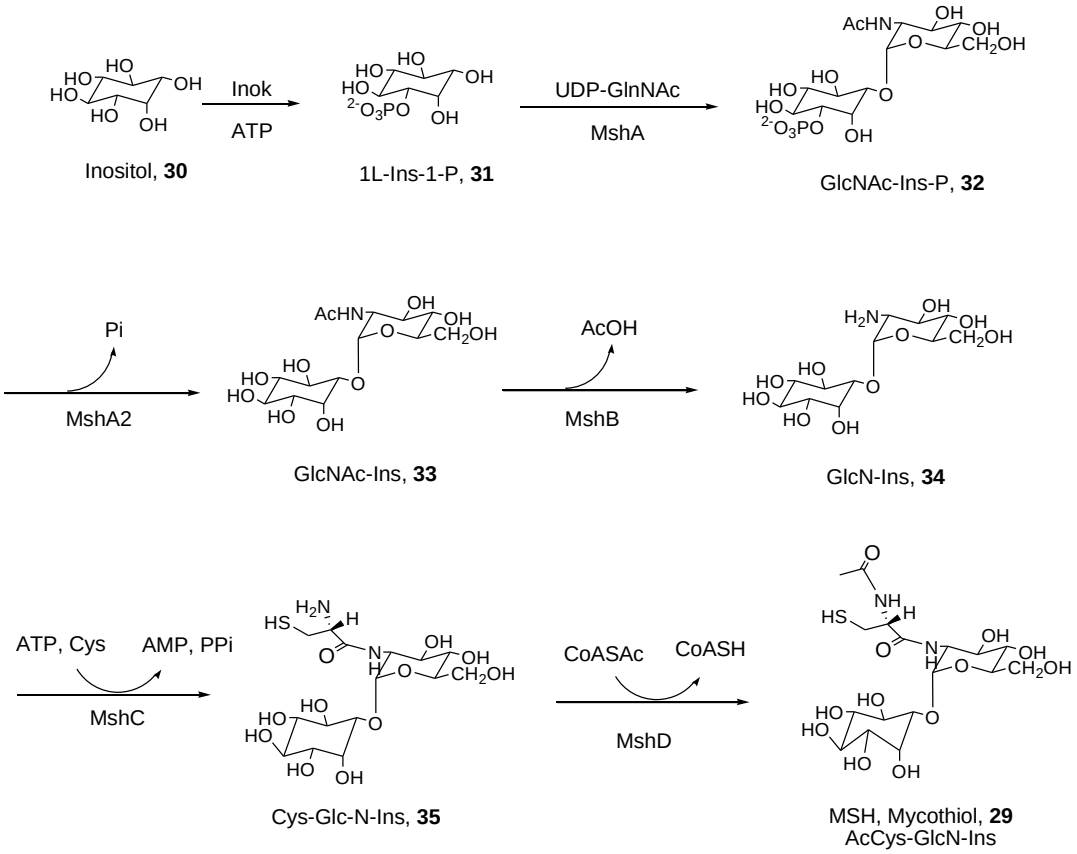
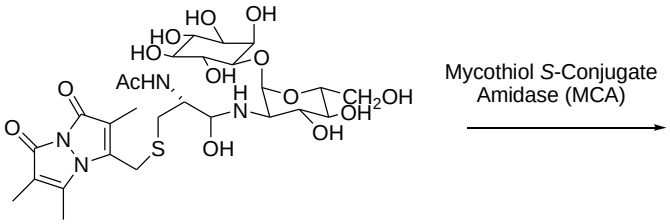


Figure 1.7 Mycothiol Biosynthesis

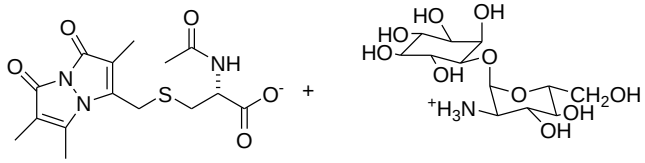
1.2.3 Mycothiol-Dependent Detoxification Cycle

Since mycobacteria do not contain glutathione, researchers were looking for an alternate detoxification cycle involving the low molecular weight thiol, mycothiol. In

2000,[27,28] Newton and others developed a fluorescence-detected assay (Figure 1.8) based on the cleavage and excretion of bimane S-conjugates of MSH. *Mycothiol smegmatis* mc^{2}155, grown in 7H9 Middleborook medium, was incubated with monobromobimane (mBBr) to construct the mycothiol bimane derivative MSmB, **36**. The bimane derivative was treated with purified mycothiol *S*-conjugate amidase (MCA) and the concentration of the resulting bimane derivative of *N*-acetylcysteine (AcCySmB, **37**) excreted in the cell broth was quantitated using HPLC. Mycothiol *S*-conjugates of several other amidase substrates were constructed in an analogous fashion, including ones of known antibiotics, and tested in the same manner.



Mycothiol Bimane (MSmB), **36**



AcCySmB, **37**

GlcN-Ins, **38**

Figure 1.8 MCA Assay

The general detoxification pathway (Figure 1.9) involves the elaboration of mycothiol (**29**) into an S-conjugate (**39**) which is then cleaved to release a mercapturic

acid (**40**) that can be excreted from the cell. The disaccharide portion (**41**) can be retained inside the cell and be recycled to maintain the intracellular concentration of mycothiol.

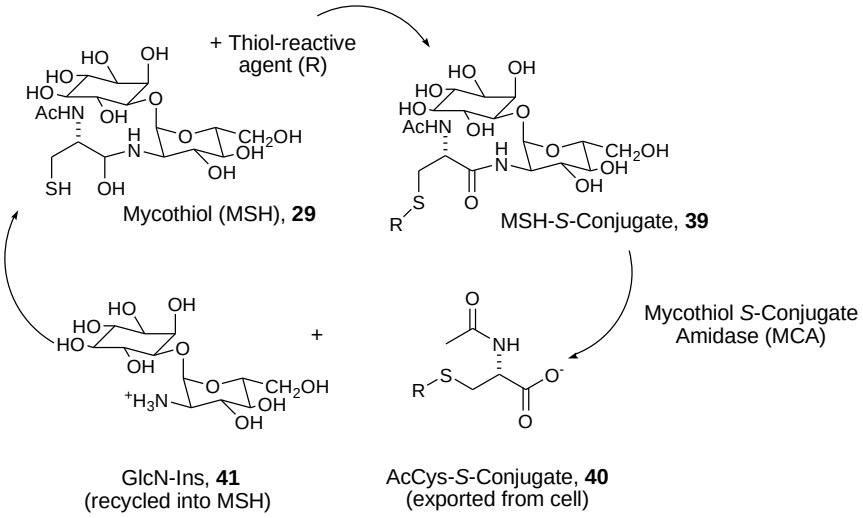


Figure 1.9 Mycothiol-Dependent Detoxification Cycle

1.3 Inhibitors of Mycothiol *S*-Conjugate Amidase

1.3.1 Fluorescence-Detected Assay

From 2001-2003, Bewley *et al*. published a description of a series of natural product MCA inhibitors that were isolated from the marine sponge species *Oceanipia* and *Pseudoceratina* (Figure 1.10).[29-32] The products were screened using a fluorescence-detected assay for MCA inhibition by measuring the extent of cleavage of mycothiol bimane (MSmB). Included in this group of inhibitors were several bromotyrosine-derived natural products which contained three common elements: a

central amide bond, an oxyimine on the carboxy side of the amide, and polar substituents on the amino side.

Further studies confirmed that the distinct structures led to different modes of inhibition for some of the molecules. Molecules that contained the three functional groups described above were determined to be competitive inhibitors, while simpler structures were determined to be non-competitive inhibitors, interacting with both free enzyme or the enzyme-substrate complex.

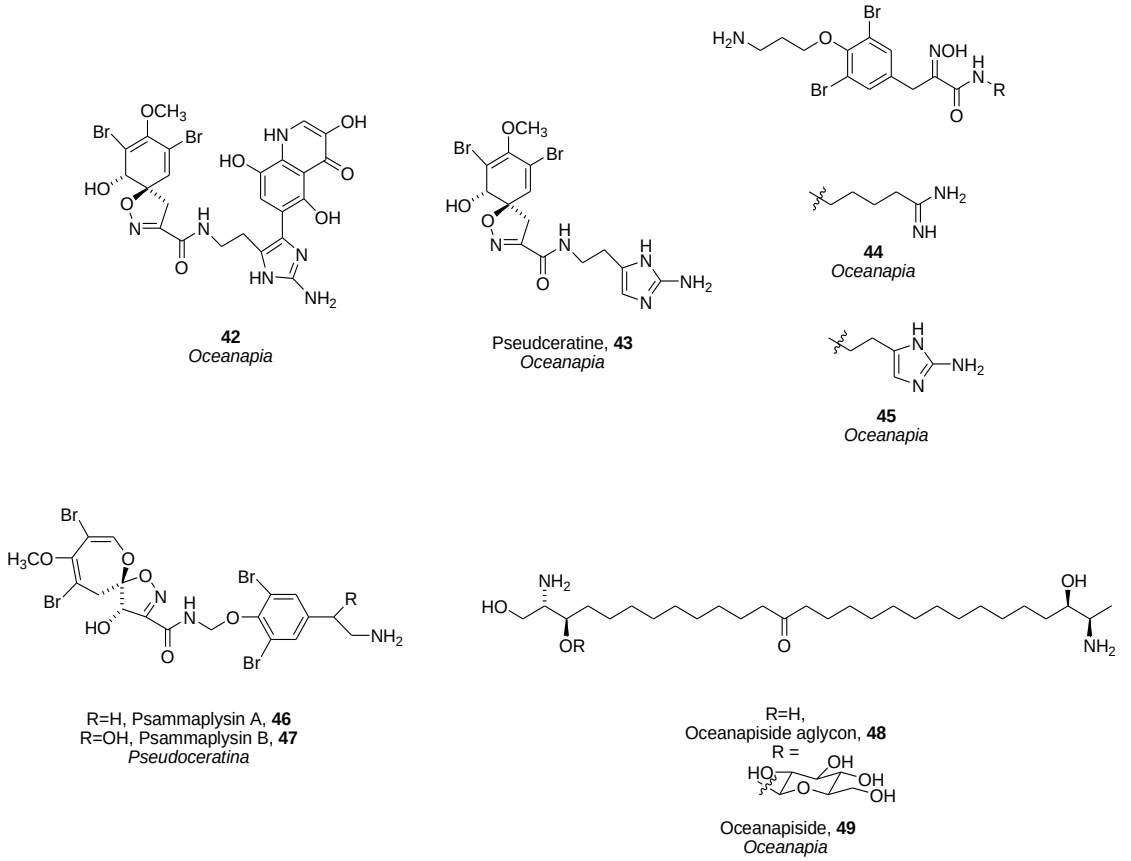


Figure 1.10 MCA Inhibitors

1.3.2 Retrosynthesis of Mycothiol S-Conjugate Amidase (MCA) Inhibitor

Due to the uncommon imidazole-quinolone coupling, our group decided that the unnamed mycothiol *S*-conjugate amidase (MCA) Inhibitor (**42**) would be an exciting synthetic target. The original retrosynthesis is shown in Figure 1.11:

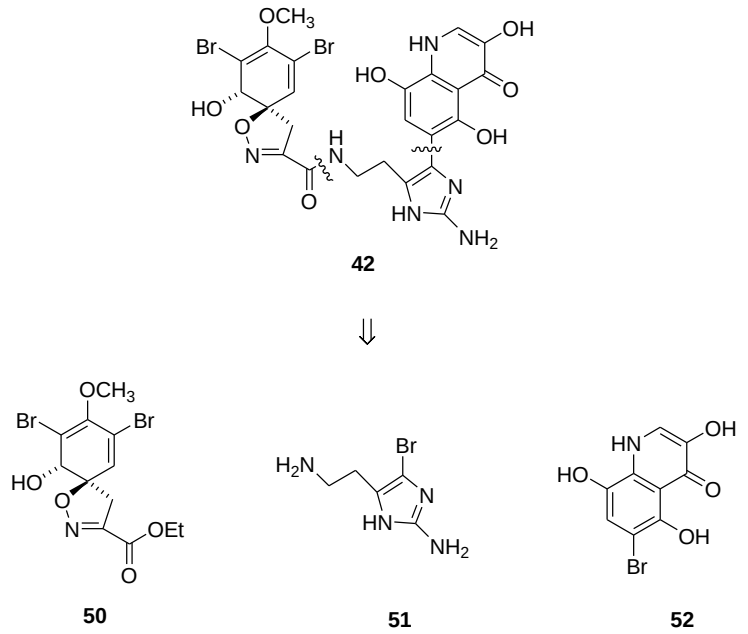


Figure 1.11 Retrosynthesis

The natural product can be broken into three fragments: a spiroisoxazoline (**50**), an imidazole (**51**), and a quinolone (**52**). The spiroisoxazoline and the imidazole can be combined using a condensation reaction. The coupling between the imidazole and the quinolone is quite unique in natural products and is the key step to the total synthesis.

1.3.3 Truncated Analogs

As part of a Structure Activity Relationship (SAR), we propose to synthesize a number of truncated analogs en route to the total synthesis of the natural product. As

stated above, the three common elements needed for activity: (1) a central amide bond; (2) an oxyimine moiety on the carboxy side; and (3) polar substituents on the amino side.

To simplify the synthesis, there are three elements we wish to explore during the SAR. Since the spiro-isoxazoline fragment may not be needed for activity, we suggest removing that fragment and replacing it with a less complicated, commercially available or easily prepared heterocyclic ring. We would also like to investigate removing the 2-amino group in the imidazole fragment. Lastly, the degree of oxidation on the quinolone ring may be reduced. Examples of proposed truncated analogs are shown in Figure 1.12 (**53** and **54**).

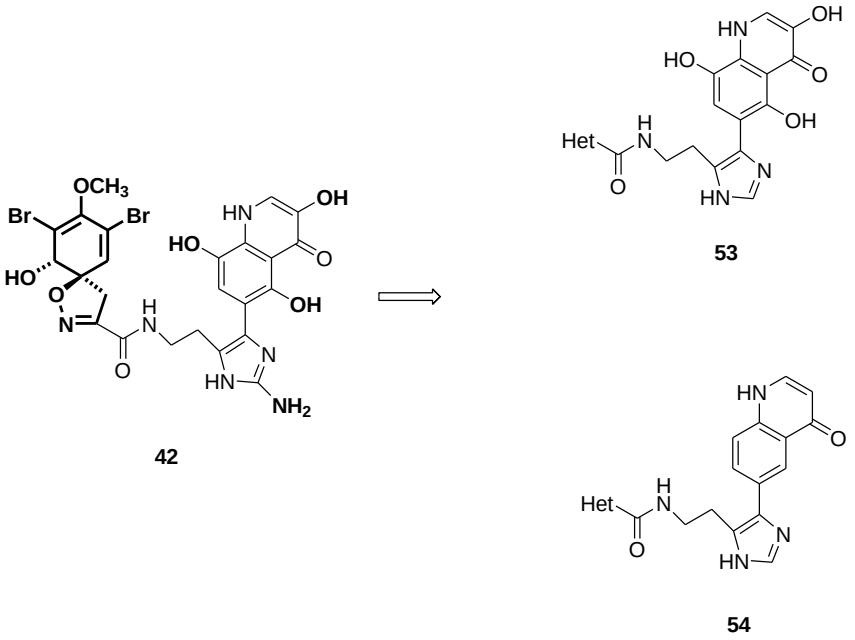


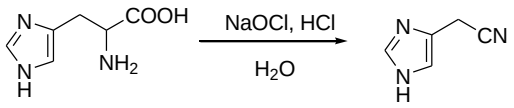
Figure 1.12 Truncated Analogs

1.4 Previous Synthetic Work

1.4.1 Imidazole Fragment

The oxidative decarboxylation of L-histidine (**55**), to 4-cyanomethylimidazole (**56**) using sodium hypochlorite (Scheme 1.1) was first published by Bauer and Tabor in 1957,[33] and the procedure was improved by Hirsch and Richardson in 1969[34] and Prell in 1990.[35]

Scheme 1.1

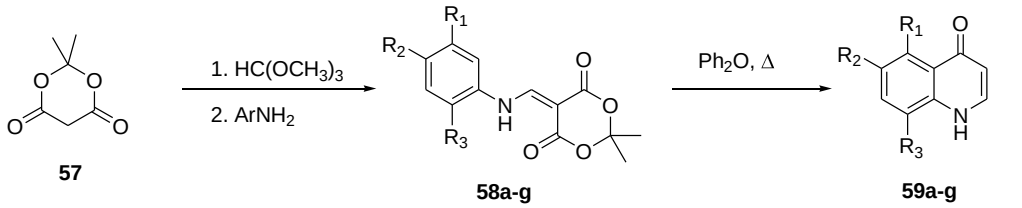


55 **56**

1.4.2 Quinolone Fragment

Valderamma[36] has published a synthesis of several 4(1H)-quinolone skeletons (Scheme 1.2) using a condensation with Meldrum's acid (**57**) as intermediates for the synthesis of quinones. This reaction could be used with several different aniline derivatives to make the quinolone skeletons (**59 a-g**) for analog studies. Although there were previous literature references to synthesize 4(1H)-quinolones by cyclization of 3-aminoacrylates, the yields were only good with electron-donating groups on the aromatic ring. Valderamma's group used several aniline derivatives, with both electron donating and electron withdrawing groups, with no marked difference in yields.

Scheme 1.2



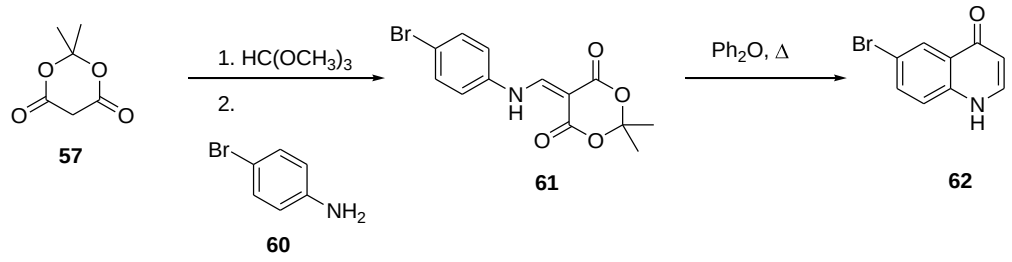
57 **58a-g** **59a-g**

#	R_1	R_2	R_3	Yield (%)
58a	H	H	H	62
58b	H	CH_3	H	74
58c	H	OCH_3	H	80
58d	H	CH_3CO	H	78
58e	H	NO_2	H	79
58f	H	H	NO_2	72
58g	OCH_3	H	OCH_3	74

#	Yield (%)
59a	81
59b	74
59c	71
59d	94
59e	72
59f	96
59g	86

The incorporation of bromine in the 6-position was demonstrated by Beifuss[37] in which the synthesis began with para-bromoaniline (**60**, Scheme 1.3). The Meldrum's acid adduct (**61**) was isolated in 85% yield and the final quinolone (**62**) was isolated in 72% yield.

Scheme 1.3

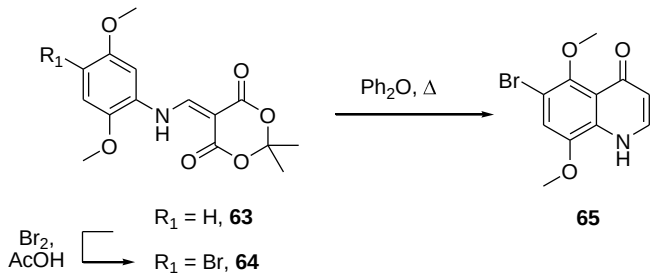


57 **60** **61** **62**

Another method for introducing the bromine in the 6-position was demonstrated by Echavarren[38] as an intermediate in the synthesis of isoascididemin (Scheme 1.4). The addition of bromine in acetic acid to the Meldrum's acid adduct (**63**) gave a yield of

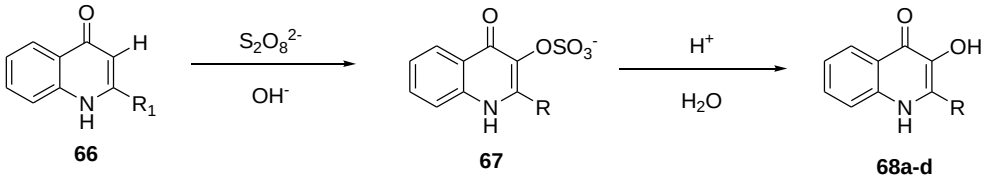
85% of (**64**) after recrystallization from EtOH, and after heating in diphenyl ether, the final quinolone (**65**) was prepared in 81% yield.

Scheme 1.4



A literature search into the introduction of the 3-hydroxyl group on the 4-quinolone resulted in few options. Behrman[39,40] used the peroxodisulfate oxidation (Elb's oxidation, Scheme 1.5) to introduce a hydroxyl group at the 3-position of the quinolone via the sulfate ester. Starting materials could be precipitated out by adjusting the pH to 6, followed by hydrolysis to obtain the 3-hydroxy-4(1H)-quinolone. The yields for this reaction were quite low (23-54%), and are calculated based on the amount of starting material that was not precipitated after the formation of the sulfate ester.

Scheme 1.5



Q#	R_1	Yield (%)
68a	H	23
68b	Me	27
68c	CO_2H	54
68d	Ph	40

An alternate formation of the quinolone fragment could be used by following Zembower's[41] synthesis of 5,8-dimethoxy-3-hydroxy-4-quinolone (**70,** Figure 1.13), an analog of a potent HIV reverse transciptase enzyme, 3,5,8-trihydroxy-4-quinolone (**69**).

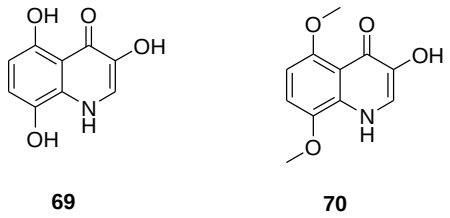
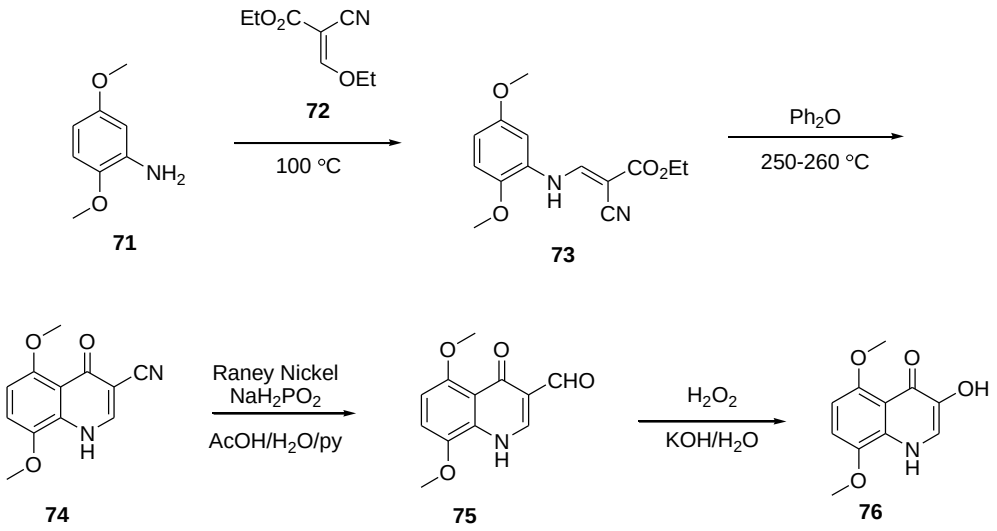


Figure 1.13 HIV Reverse Transcriptase Inhibitors

2,5-Dimethoxyaniline (**71**, Scheme 1.6) was condensed with ethyl 2-cyano-3-ethoxyacrylate (**72**) to form the acetate adduct (**73**), followed by thermal cyclization in diphenyl ether, to produce a 4-(1H)-quinolone (**74**) with functionality in the 3-position. The resulting cyano group was reduced with Raney nickel, and the resulting aldehyde (**75**) was subjected to a Dakin oxidation to give the final product (**76**) in 4 steps and a 5.5% overall yield.

Scheme 1.6



This route would be feasible for our synthesis only if the bromination of the 6-position of the quinolone would be successful. Since the bromination works nicely with Meldrum's acid adducts, we did not anticipate problems introducing the bromine.

1.4.3 Spiro-Isoxazoline Fragment

Although there are several published reports of synthesizing the spiro-isoxazoline fragment,[42-47] we have chosen to follow a combination of Spilling's work[42] with the integration of Nishiyama's asymmetric steps.[48]

Spilling's synthesis began with the MOM-protection of 3,5-dibromo-2-hydroxy-4-methoxybenzaldehyde (**77**, Scheme 1.7), followed by a Wadsworth-Emmons olefination with the phosphonate (**79**) to form (**80**). Deprotection of the silyl enolether and in situ oxime formation provided (**81**). Deprotection of the MOM-group and cyclization with a polymer-supported hypervalent iodobenzene (PSDIB) gave the racemic spiro-isoxazoline (**83**). This procedure was scalable up to 40 g with high

overall yields; 83% for four steps. The report provided a formal synthesis of aerothionin (**84**) (Figure 1.14), homoaerothionin (**85**) and aerophobin-1 (**86**).

Scheme 1.7

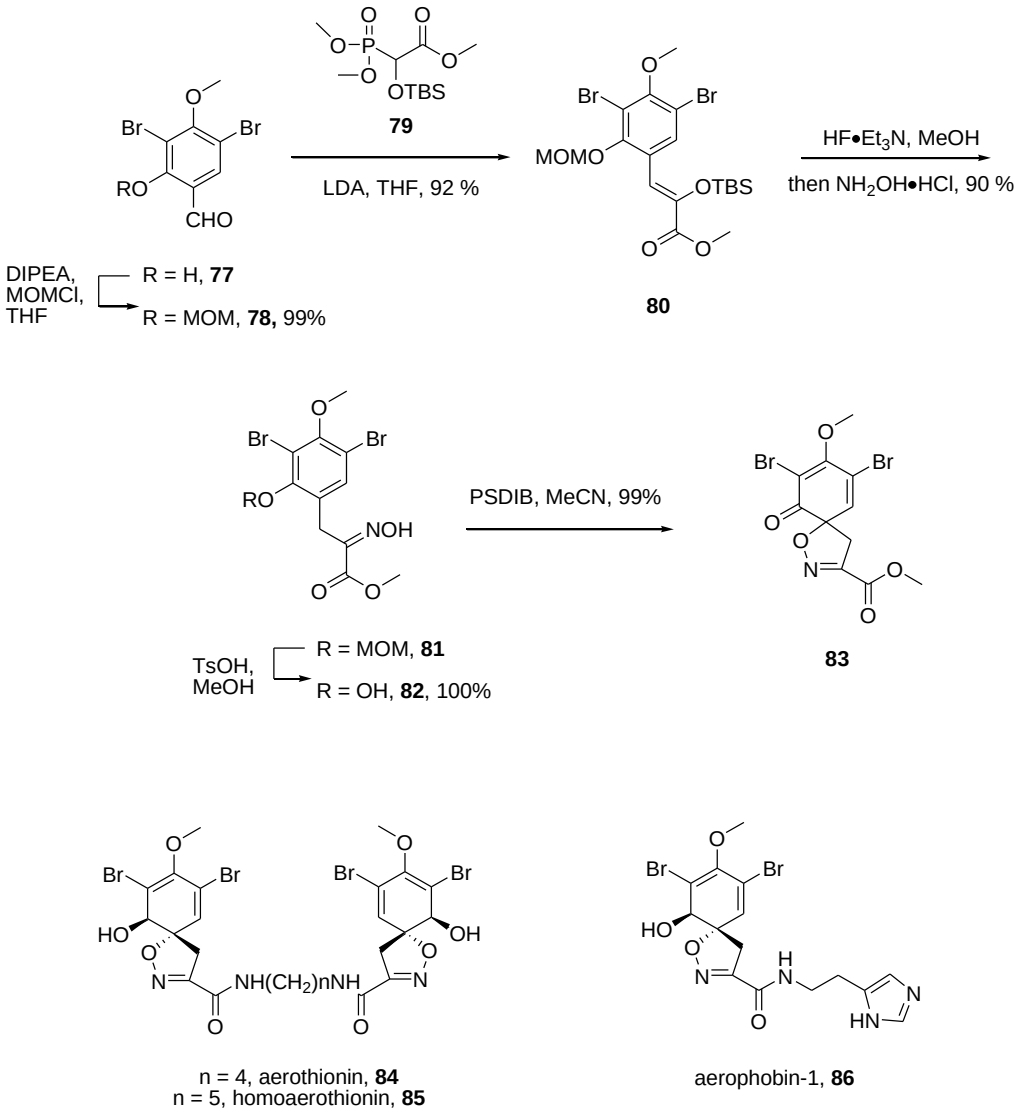
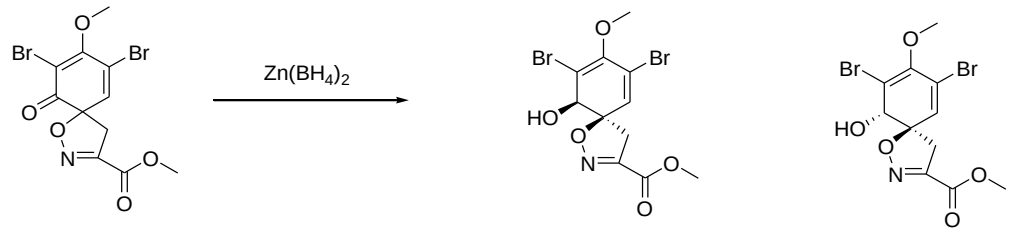


Figure 1.14 Aerothionin, Homoaerothionin, and Aerophobin-1

Nishiyama studied different reducing agents to develop a protocol for the reduction of the ketone. Reduction with $Zn(BH_4)_2$,[49] followed by a work-up with $MgSO_4$, gave the highest yields of the cis (**84**, 39%) and trans (**85**, 41%) products (Scheme 1.8).

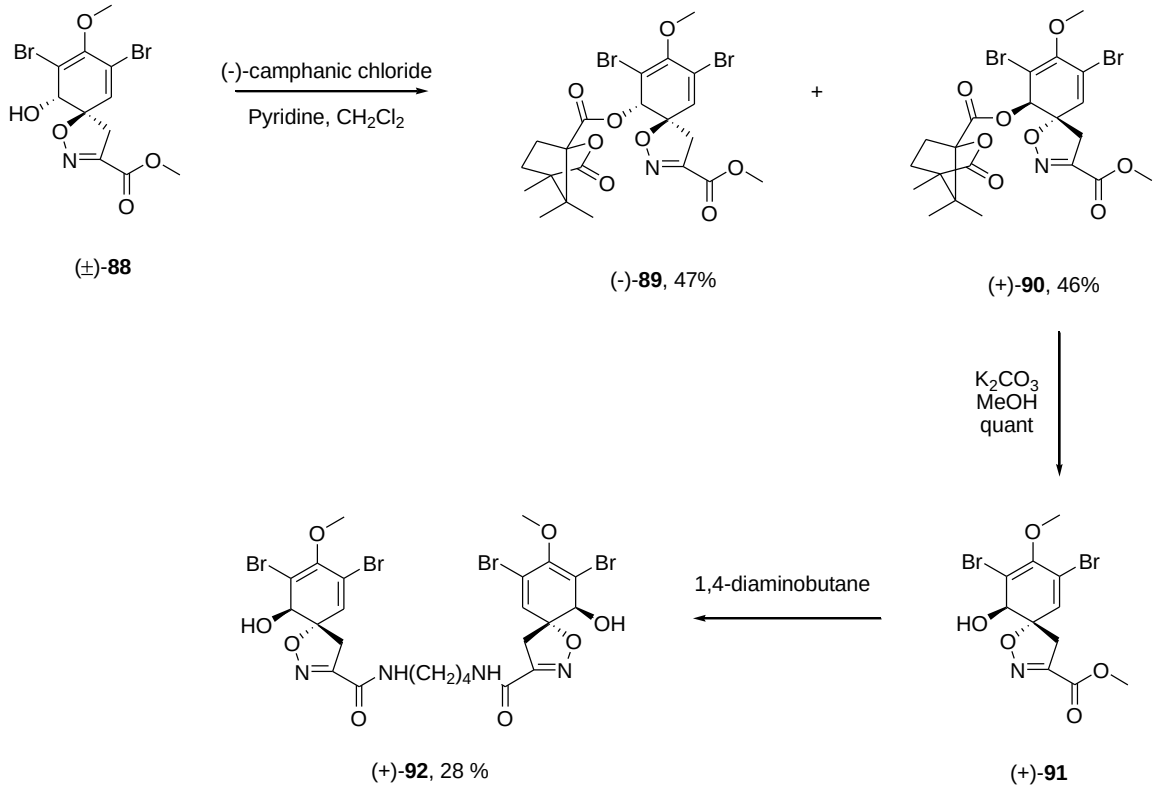
Scheme 1.8



83 cis (39%), **84** trans (41%), **85**

Nishiyama also developed a protocol for the synthesis of the optically pure spiroisoxazoline fragment in route to his formal synthesis of (+)-aerothionin (**92**, Scheme 1.9). Beginning with racemic (±)-spiroisoxazoline (**88**), esterification with (-)-camphanic chloride produced esters (-)-(**89**) and (+)-(**90**), which could be easily separated using silica gel chromatography. Solvolysis of the (+)-ester with K_2CO_3 followed by condensation with 1,4-diaminobutane gave (+)-aerothionin (**92**) in 12.9% overall yield for the three steps. Utilization of this asymmetric synthesis will allow us to confirm the reported stereochemistry for the natural product and compare it with its diastereomer.

Scheme 1.9



1.5 Previous Cross-Coupling Studies

1.5.1 Introduction

There are many examples of natural products that contain imidazole rings, and although there are many established routes for coupling two aromatic moieties together, there are relatively few examples of successful couplings when one of the rings is an imidazole. A literature search lead us to investigate Kumada, Stille, and Suzuki couplings as possibilities to effect this particular bond construction.[50,51]

It is interesting to note that Seley[52] and coworkers investigated a cross-coupling for the construction of a flexible nucleoside analogs containing the 5-iodoimidazole

fragment (Figure 1.15) with a pyrimidine with no success. The group investigated Stille, Kumada and Negishi couplings with various catalysts, solvents and reaction temperatures, finally suggesting that the reaction was unsuccessful due to the electron-deficient pyrimidine moiety being insufficiently activated to participate in the cross-coupling. As a result the group abandoned cross-coupling efforts for a linear synthesis. Although the electronics of these systems compared to ours are different, this publication highlights the need for the development of a successful procedure for cross-couplings of this nature.

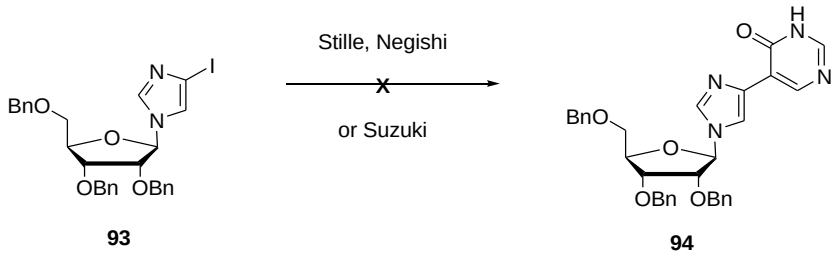


Figure 1.15 Seley's Investigation of Imidazole Cross-Coupling

1.5.2 Kumada Cross-Coupling

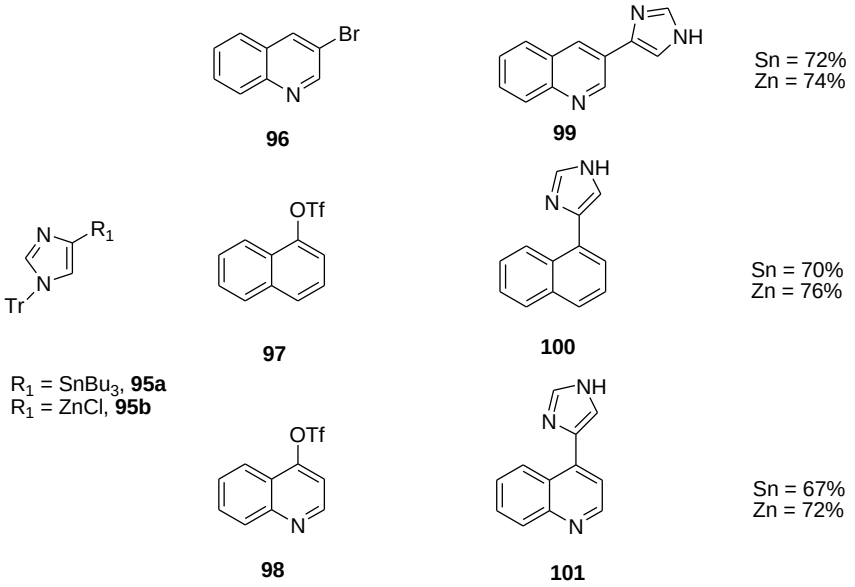
Kumada[53] discovered the cross-coupling of organomagnesium compounds with aryl halides in 1972, which was the first reported example of cross-coupling chemistry in the literature. A Grignard reagent and the aryl halide are reacted in the presence of a nickel or palladium catalyst, and the reaction usually proceeds quickly, even at low temperatures. Although there are no reported examples of this reaction with imidazoles, the Grignard reagents are not difficult to prepare.[54]

1.5.3 Stille Cross-Coupling

Stille[55,56] couplings are defined as palladium catalyzed cross-couplings of an organostannane with an aryl halide. These reactions require elevated temperatures, and the tin materials are more toxic. There are several reports in the literature that use this chemistry with imidazoles.

Jetter[57] published a report where 4-iodo-1-tritylimidazoles were converted to Grignard reagents, then treated with Bu_3SnCl or $ZnCl_2$ to form the corresponding stannane (**95a**) or zinc reagent (**95b**) that could be used in a palladium cross-coupling with aryl or vinyl bromides, iodides, or triflates (Scheme 1.10). Yields for these reactions were generally good (67-76%).

Scheme 1.10



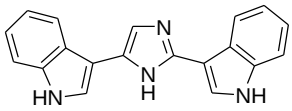
Baldwin and coworkers have developed a method to enhance the Stille reaction by combining copper(I) iodide with cesium fluoride in DMF.[58] This method was

developed to avoid the homocoupling that often occurs with aromatics with electron-withdrawing substitutents. Although the group did apply the reaction conditions to several types of heterocycles, they did not test the reaction on imidazoles.

1.5.4 Suzuki Cross-Couplings

The Suzuki reaction[59] is the coupling of organoboron compounds with carbon electrophiles, such as aryl halides. The addition of a base is required to activate the weakly nucleophilic boron compound for transmetalation, and high temperatures are usually employed. This reaction can be limited by the commercial availability of boron compounds, or the stability of ones made in situ.

Ohta[60-62] has suggested that imidazole boronic acids in particular are not stable enough to undergo Suzuki cross-couplings. Ohta utilized a Suzuki coupling using an indole boronic acid to diarylate the 2- and 4(5)-positions of imidazoles in route of the total synthesis of nortopsentin D (**102,** Figure 1.16).



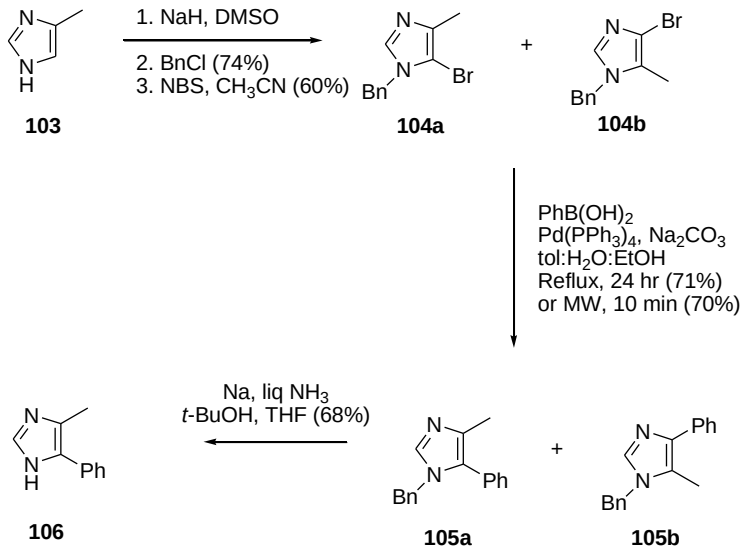
Nortopsentin D, **102**

Figure 1.16 Nortopsentin D

Feliu[63] and coworkers developed a method for the arylation of 5-bromohistidine derivitaives (**104a**) and (**104b**) using microwave irradiation (Scheme 1.11). The model system was a benzyl-protected 4(5)-methylimidazole and 1.1 eq of the boronic acid. Although the regioisomers were not separated for the coupling, the final debenzylation

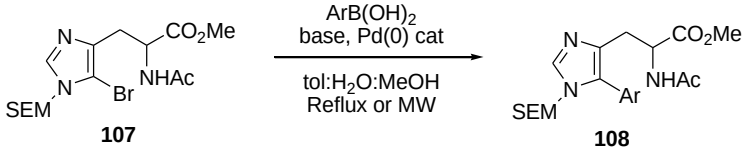
afforded one product (**106**) in 21% overall yield. Microwave irradiation for the last step gave comparable yields to the reflux conditions.

Scheme 1.11



Similar reaction conditions were employed to introduce boronic acid derivatives to brominated histidine derivatives (Scheme 1.12). The imidazole nitrogens were protected using a SEM group to aid in the removal of the protecting group after the coupling. Under reflux conditions, low yields (26%) were obtained using 20 mol% $PdCl_2$(dppf), 2.1 equivalents boronic acid, Na_2CO_3 as base, and a reaction time of 24 hours. When applying microwave irradiation (110 °C, 10 min) the optimum reaction conditions were $Pd_2(dba)_3$, 2.1 eq of boronic acid, KF as base, obtaining 62% yield.

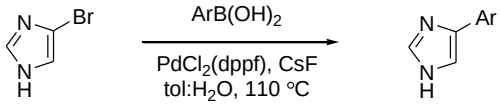
Scheme 1.12



107 **108**

Bellina and Rossi[64] developed an efficient method for preparing 4(5)-aryl-1H-imidazoles using a $PdCl_2$(dppf)-catalyzed Suzuki-Miyaura reaction with unprotected imidazoles under phase transfer conditions (Scheme 1.13). This procedure uses two equivalents of the boronic acid and 5 mol% of $BnEt_3NCl$ as a phase transfer catalyst. The group found that when they replaced $PdCl_2(PPh_3)_2$ with $PdCl_2$(dppf), the undesired homocoupled product of the boronic acids decreased, there were higher yields and lower reaction times (although still long on a relative scale). However, the group was not able to couple the electron-deficient 2-formylboronic acid using this method, only isolating the protodeboronation product, benzaldehyde.

Scheme 1.13

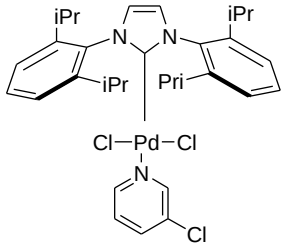


109 **110a-g**

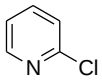
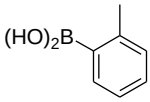
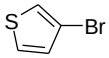
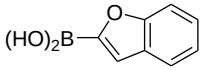
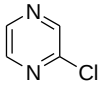
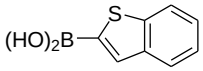
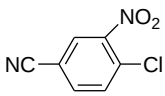
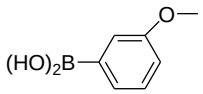
#	Ar	Time (h)	Yield (%)
110a	C_6H_5	65	66
110b	4-ClC_6H_4	48	82
110c	4-$MeOC_6H_4$	66	91
110d	4-AcC_6H_4	72	83
110e	2-naphthyl	72	90
110f	2,5-$(MeO)_2C_6H_3$	72	76
110g	2-$(CHO)C_6H_4$	72	0

Organ and coworkers[65,66] have developed an air- and moisture-stable Pd-NHC (N-heterocyclic carbene) complex as an active pre-catalyst for the Suzuki reaction (Scheme 1.14). The PEPPSI^{TM}-IPr (**111**, pyridine-enhanced precatalyst preparation, stabilization and initiation) precatalyst has been used in various coupling reactions, such as the Suzuki,[67,68] Negishi,[69] and Kumada[70] reactions, with chlorides, bromides, iodides, triflates, tosylates and mesylates, resulting in high yields. As a precatalyst for the Suzuki reaction, the complex has been successful in coupling various heterocyclic halides and boronic acids in high yields. Since the PEPPSI complex is a precatalyst, the Pd(II) must first be reduced to Pd(0). This is usually accomplished under the reaction conditions, as long as an organometallic or β-hydride containing material is available to undergo ligand exchange with the complex.

Scheme 1.14



R^1-X + M-R^2 —(**111**, 1-2 mol %; base, rt or 60 °C)→ R^1-R^2 **112a-d**

#	R^1	R^2	Base	Solvent	Temp (°C)	Time (hr)	Yield (%)
112a			K*t*OBu	*i*PrOH	rt	2	93
112b			KtOBu	*i*PrOH	rt	2	88
112c			K_2CO_3	dioxane	60	2	96
112d			K_2CO_3	dioxane	60	2	77

CHAPTER 2

RESULTS AND DISCUSSION

2.1 Retrosynthesis of the Natural Product

As previously described in Section 1.3.2, the retrosynthesis involves the natural product being broken into three fragments: a spiroisoxazoline (**50**), an imidazole (**51**) and a quinolone (**52**). The spiroisoxazoline and the imidazole can be combined using a condensation reaction. The union between the imidazole and the quinolone is quite unique in natural products and is the key step to the total synthesis.

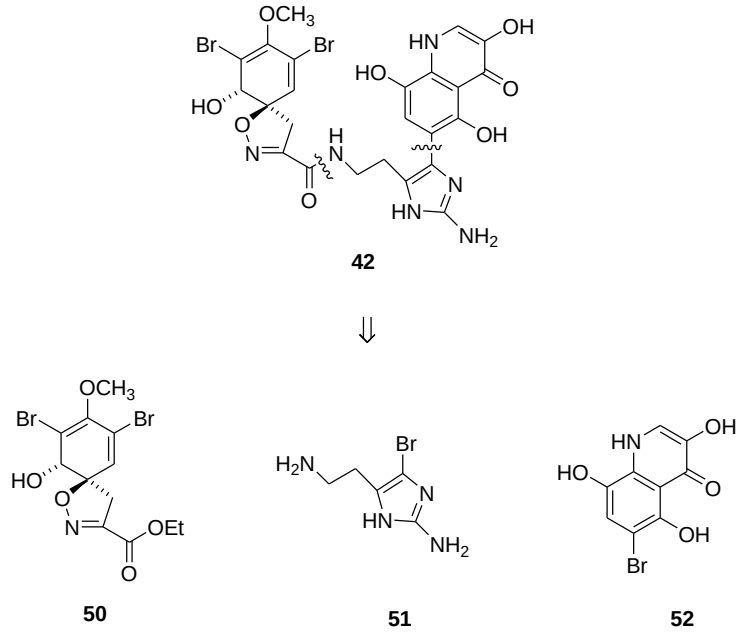


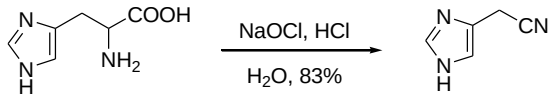
Figure 2.1 Retrosynthesis of Natural Product

2.2 Synthesis of the Imidazole Fragment

2.2.1 Synthesis of 4-Cyanomethylimidazole

The initial synthesis of the imidazole fragment began with the amino acid, L-histidine (**55**), which was oxidized with NaOCl in the presence of HCl to give 4-cyanomethylimidazole (**56**) after Soxhlet extraction with EtOAc (Scheme 2.1).[33-35] We discovered that better yields and purity were obtained with 10% NaOCl bought from Aldrich than with commercial household brands which contain additives that may influence the yields.

Scheme 2.1

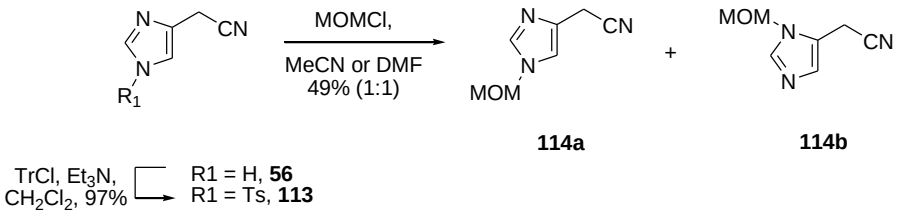


55 **56**

2.2.2 Protection of the Imidazole Nitrogen

4-Cyanomethylimidazole was protected with TrCl to give the 4-protected imidazole **116** exclusively. However, subsequent deprotection and reprotection with MOMCl in either MeCN or DMF gave low yields of approximately (1:1) mixtures of the 4- and 5-MOM protected imidazoles, **114a,b**.[71-74]

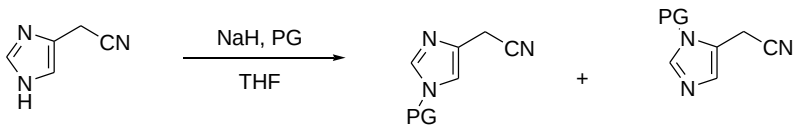
Scheme 2.2



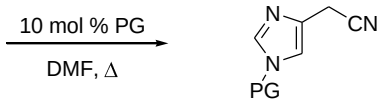
114a **114b**

In response to this and related problems, our lab has developed a method to prepare 4-substituted imidazoles with a two-step procedure (protection-isomerization sequence) using an excess of protecting group, NaH as the base and DMF as the solvent.[75] This procedure was used to obtain 4-cyanomethyl-1-methoxymethylimidazole (**114a**), as well as the benzyl and SEM (2-(trimethylsilyl)ethoxymethyl)-protected derivatives, **115a** and **116a** respectively. We discovered during this series of experiments that DMAS-protection (Dimethylaminosulfonyl) did not proceed as well as the other protecting groups although no obvious explanation was evident. Repeated attempts to perform this reaction gave a black, oily mixture of the DMAS-protected and unprotected materials in low yields. This protecting group has been used routinely by us and others fro protecting imidazoles.

Scheme 2.3



56 **114-116a** **114-116b**



114-116a

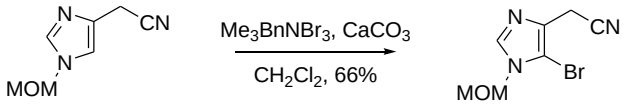
Table 2.1
Protection and Isomerization of 4-Imidazoles

entry	PG-X	Yield (4:5-Isomers) After Initial Protection	Yield (4:5-Isomers) After Isomerization
114	MOMCl	85 (1:0.8)	78 (1:0)
115	BnBr	85 (1:0.6)	85 (1:0.08)
116	SEMCl	91 (1:0.5)	85 (1:0)

2.2.3 Bromination of the 5-Position in Imidazoles

Once we had the N-protected material in hand, the 5-position could be brominated (**117**, Scheme 2.4**)** with Me_3BnNBr_3.[76] This reaction proceeds without bromine insertion at the 2-position, which can be seen using Br_2 as the brominating agent. Although this reaction proceeds nicely to near completion as shown by TLC, the compound is water soluble and must be isolated using silica gel chromatography. Me_3BnNBr_3 can be bought commercially, but it can also be easily prepared in high yields (90%) and excellent purity in the lab by brominating the less expensive Me_3BnNBr.

Scheme 2.4



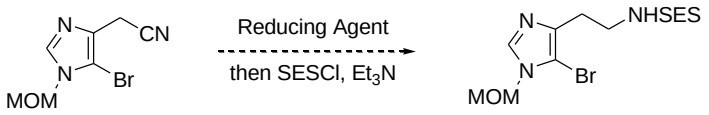
114a **117**

2.2.4 Reduction of the Nitrile

Once the brominated imidazole was obtained, the next step was to reduce the cyano group to a primary amine and then protect with β-trimethylsilylethanesulfonyl

chloride (SESCl, Scheme 2.5).[77] Several problems were encountered with the reduction step, and neither DIBAL-H or $LiAlH_4$[78] gave the desired product in pure form.

Scheme 2.5

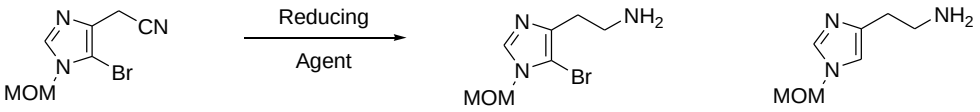


117 **118**

The best yields of the desired product were obtained when 2.0 equivalents of DIBAL-H were used in hexanes at reflux temperatures; ca. 46% of a 4:1 mixture of the desired product and unreacted starting material (Scheme 2.6; Table 2.2, Entry 1). The low recovery may have been due to the solubility of the imidazoles in water during the aqueous work-up. When a large excess of DIBAL-H (6.0 equivalents, Entry 2) was used, even less material was recovered from the work-up, and yields of the desired product were very low. Only ca. 21% of a (1:1:2) mixture of the desired amine **119**, the debrominated amine **120**, and recovered starting material was recovered.

When $LiAlH_4$ was used in diethyl ether, only 83% of the starting material was recovered (Entry 3). Since this may have been due to insolubility of the starting material, we chose to explore THF as the solvent. In this case, a (1:1:2) mixture of the desired amine **119**, the debrominated amine **120**, and recovered starting material was obtained in ca. 39% overall yield (Entry 4). These materials were very polar and all attempts at separating them using silica gel chromatography were unsuccessful. If a reasonably clean conversion to the primary amine could have been achieved, the amine could have been protected in situ to facilitate the purification.

Scheme 2.6



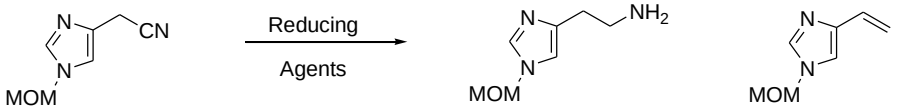
117 **119** **120**

Table 2.2
Reduction of the Nitrile on the Brominated Imidazole

Reducing agent	Solvent	Temp (°C)	Ratio of amine (**119**)	Ratio of amine (**120**)	Ratio of recovered starting material (**117**)	Approximate Yield (%)
2 eq DIBAL-H	Hexanes	reflux	4	0	1	46
6 eq DIBAL-H	Hexanes	rt	1	1	2	21
$LiAlH_4$	Et_2O	rt	0	0	1	83
$LiAlH_4$	THF	rt	1	1	2	39

Since a significant amount of the debrominated amines was obtained using the above sequence, at this point, the reverse sequence of the reduction and bromination steps was investigated (Scheme 2.7). When DIBAL-H was used with hexanes as the solvent at room temperature, a low conversion of the nitrile **114a** to the desired amine **120** was seen (Table 2.3, Entry 1). At elevated temperatures (Entry 2), there was no conversion to the primary amine which was evident by inspection of the NMR spectrum of the crude mixture. However, an unexpected product, the vinyl imidazole **121** was seen in low yields.[79] Changing the reducing agent to $LiAlH_4$ (Entry 3) slightly increased the ratio of the desired amine obtained, however, at this point the use of aluminum reducing agents was abandoned.

Scheme 2.7



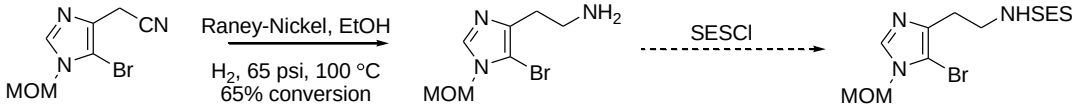
114a **120** **121**

Table 2.3
Reduction of the Nitrile on the Unbrominated Imidazole

Reducing agent	Solvent	Temp (°C)	Ratio of amine (**120**)	Ratio of vinyl imidazole (**121**)	Ratio of Recovered starting material (**114a**)	Approximate Yield (%)
4 eq DIBAL-H	Hexanes	rt	1	1	3	65
4 eq DIBAL-H	Hexanes	reflux	0	1	4	42
$LiAlH_4$	THF	rt	1	0	1	39

The reduction of the 4-cyanomethylimidazole moieties can also be achieved using a Raney-nickel catalyzed hydrogenation (Scheme 2.8).[80] This reaction requires elevated temperatures (up to 100 °C) and high pressures (100 bar) for complete reduction to the primary amine. In our lab, the hydrogenation equipment has a maximum pressure of 65 psi (approximately 4.5 bar), and with these conditions, about 65% of the material could be reduced to the primary amine **119**, without loss of the bromine. However, the polarity of the material caused problems with column purification. To avoid difficulties in purification, the free amine could be protected *in situ* to give the imidazole fragment **122** ready for cross-coupling.

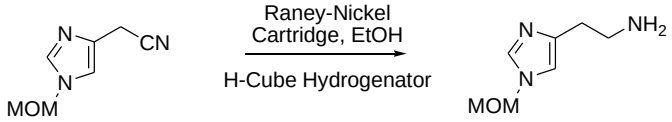
Scheme 2.8



117 **119** **122**

The hydrogenation of 4-cyanomethylimidazole was also studied using an H-Cube hydrogenator, in which the Raney-nickel catalyst was contained in a cartridge (Scheme 2.9). Several reaction conditions were tested before complete conversion to the primary amine **120** was obtained. At 95 °C and 80 bar (Table 2.4, Entry 4), there was almost complete conversion to the primary amine (1:<0.05). One advantage of this type of hydrogenation was the clean conversion to the amine, eliminating the need for column chromatography.

Scheme 2.9



20 **123**

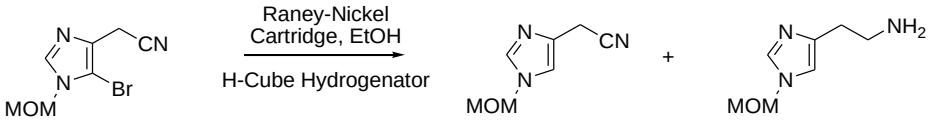
Table 2.4
Reduction of the Nitrile using an H-Cube Hydrogenator

Trial	Temp (°C)	Pressure (bar)	Conversion (Reduced Product :Starting Material)
1	95	40	1:0.60
2	95	50	1:0.25
3	95	60	1:0.20
4	95	80	1:<0.05

Although this was an encouraging result, the reaction did not proceed smoothly with the functionalized imidazole **117** (Scheme 2.10). The material recovered from the

hydrogenator was a (1:0.7) ratio of the debrominated nitrile **114a** and the debrominated amine **120**. This result suggests that the removal of the bromine occurs at a much faster rate than the reduction of the nitrile, therefore, the reaction sequence would have to be adjusted, possibly by attempting the reversal of the reduction and bromination steps. It should be noted that these experiments were conducted some time after the initial investigation.

Scheme 2.10

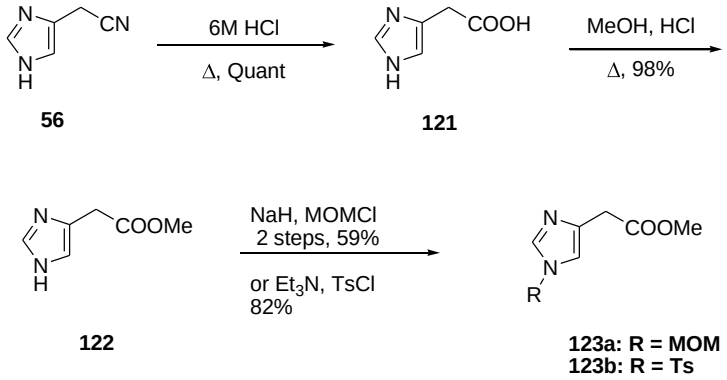


117 **114a** **120**

2.2.5 Alternate Route to the Imidazole Fragment

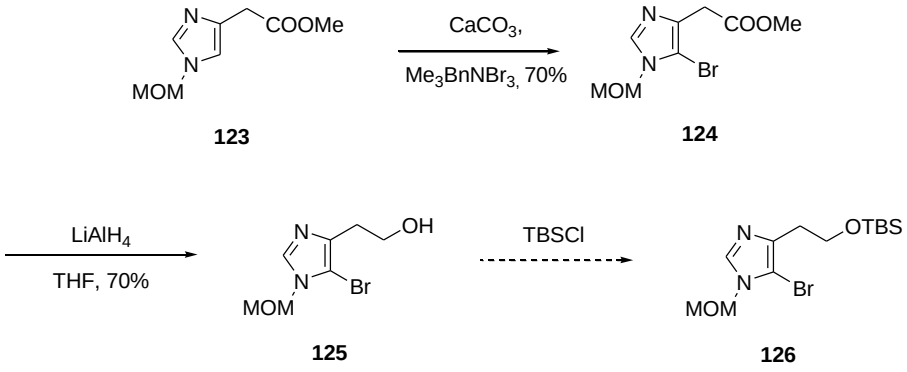
Since the reduction of the cyano moiety proved difficult, an alternative route to the imidazole fragment was investigated (Scheme 2.11). 4-Cyanomethylimidazole was hydrolyzed to imidazole acetic acid (**121**), followed by Fischer esterification to obtain the methyl ester **122** as the hydrochloride salt. The ester was isolated as the free amine, and protected with MOMCl using the same two step sequence that was used for the protection of the 4-cyanomethyimidazole. The MOM-protected ester **123a** was obtained in 59% after the two step protection sequence and the Ts-protected ester **123b**[88] was obtained in 82% yield after treatment with Et_3N and TsCl.

Scheme 2.11



Bromine was introduced to the 5-position **124**, using Me_3BnNBr_3 as the brominating agent and the procedure described above. The protected ester was reduced to the primary alcohol **125** with $LiAlH_4$ in good yield, however, the purification of this product was also problematic. To aid in purification, the alcohol could be protected with TBSCl in situ to give the final cross-coupling product **126**, however, this was not attempted due to loss of the crude material during the purification process.

Scheme 2.12

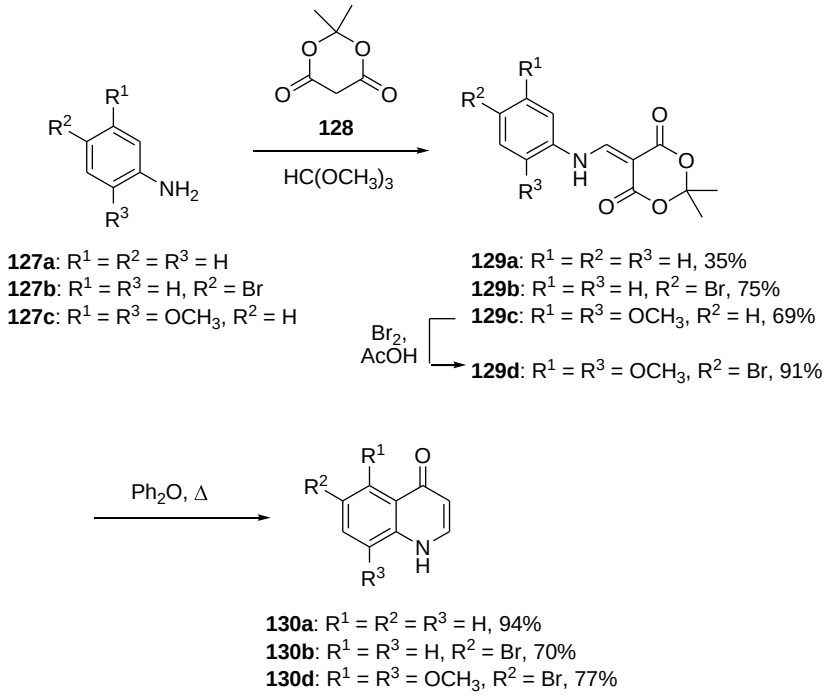


2.3 Synthesis of the Quinolone Fragment

2.3.1 Preparation of the Basic Quinolone Skeleton

The synthesis of the basic quinolone fragment was accomplished using Valderamma's protocol (Scheme 2.13). Commercially available aniline derivatives **127a-c** were heated at reflux temperatures with a mixture of methyl orthoformate and Meldrum's acid (**128**) to obtain the adducts **129a-c** in 35-69% yields. A bromine atom was introduced in excellent yield in the 6-position of the dimethoxy-adduct (**129d**) with Br_2 in AcOH.[38] The adducts were heated in Ph_2O for 4-8 hours at 260 ºC to form the basic quinolone skeletons **130a,b,d** in good yields (70-94%).

Scheme 2.13



127a: $R^1 = R^2 = R^3 = H$
127b: $R^1 = R^3 = H$, $R^2 = Br$
127c: $R^1 = R^3 = OCH_3$, $R^2 = H$

129a: $R^1 = R^2 = R^3 = H$, 35%
129b: $R^1 = R^3 = H$, $R^2 = Br$, 75%
129c: $R^1 = R^3 = OCH_3$, $R^2 = H$, 69%
129d: $R^1 = R^3 = OCH_3$, $R^2 = Br$, 91%

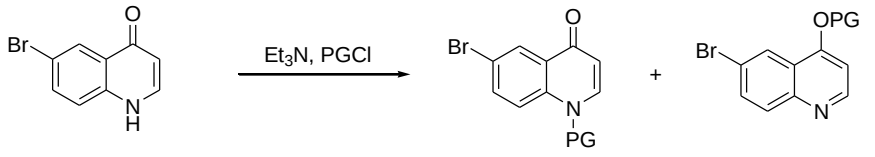
130a: $R^1 = R^2 = R^3 = H$, 94%
130b: $R^1 = R^3 = H$, $R^2 = Br$, 70%
130d: $R^1 = R^3 = OCH_3$, $R^2 = Br$, 77%

2.3.2 Protection of the Quinolone Nitrogen

To prepare the quinolone fragments for cross-coupling studies, the protection of the nitrogen was investigated (Scheme 2.14).[81,82] When 6-bromoquinolone (**130b**) was treated with Et_3N and a slight excess (1.1 equivalents) of tosyl chloride, a quantitative yield of the *N*-tosylquinolone **131a** was isolated. However, upon sitting at room temperature for extended periods of time, a small amount, about 10%, of the product had rearranged to the *O*-tosylquinolone **131b**.

The same effect was more pronounced when using MOMCl as the protecting group and Et_3N as the base. Although the yield was still high, 94%, the O-protected quinolone **132b** was the favored product (63%). Although the protection location was not important, the successful transformation to one isomer was necessary for ease in characterization and the elimination of column chromatography for purification.

Scheme 2.14



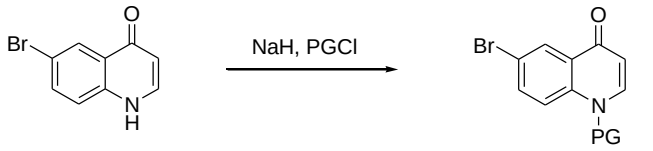
130b **131a, 132a** **131b, 132b**

Q#	PG	Yield (%)	Ratio (a:b)
131	Tos	100	1:0
132	MOM	94	1:2

The problem was solved by replacing Et_3N with NaH as the base (Scheme 2.15). In the case of MOMCl as the protecting group, a yield of 71% of the desired N-protected product **132a** was isolated. When DMASCl was used, a much lower yield

(38%) of the desired quinolone **133a** was isolated. Although the yield of this reaction was quite low, the reaction was still beneficial because the desired product was isolated as only one isomer.

Scheme 2.15

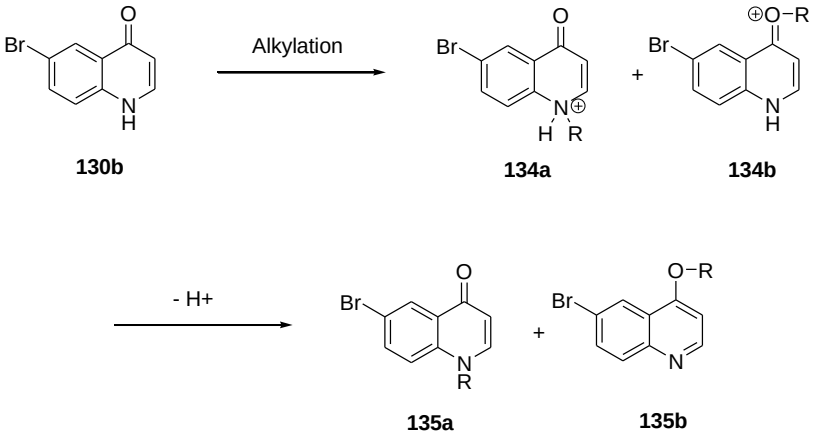


130b **132a, 133a**

Q#	PG	Yield (%)
132a	MOM	71
133a	DMAS	38

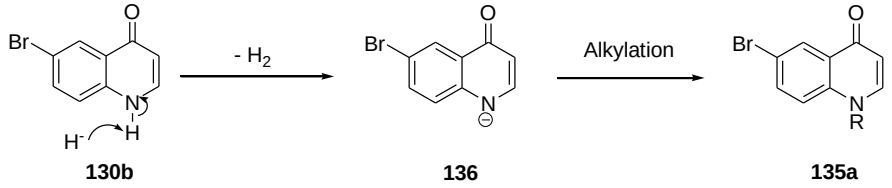
In the first series of reactions, Et_3N is not a strong enough base to deprotonate the quinolone, so alkylation must occur first (Scheme 2.16). With a tosyl group, the formation of the N-S bond must be stronger due to delocalization of the nitrogen lone pair than the O-S bond, therefore alkylation occurs solely at the quinolone nitrogen, followed by deprotonation. When Et_3N is used with MOMCl as the protecting group, there is not as large of a difference in the strength of O-C bond vs the N-C bond, therefore, alkylation can and does occur at both positions. This results in mixtures of the O- and N-protected quinolones **135a,b**.

Scheme 2.16



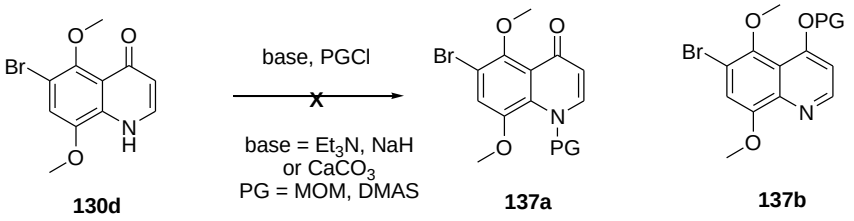
When the base is NaH, the pKa is high enough to deprotonate the quinolone nitrogen prior to substitution (Scheme 2.17). This creates a discrete anion **136**, which can then react with the protecting group to form only the nitrogen-protected materials.

Scheme 2.17



When the same reaction conditions were applied to the highly substituted quinolone **130d**, no protection occurred. This perhaps reflects a steric effect; although H^- is small, and the removal of the proton may be possible, the adjacent methoxy groups could be hindering of the substitution step. Protection of either nitrogen or oxygen does not occur using TsCl, MOMCl, or DMASCl as protecting groups, and NaH, Et_3N or $CaCO_3$ as the base. Given this relative unreactivity, it may be possible to use these more substituted systems in the cross-coupling chemistry without protection.

Scheme 2.18



2.3.3 Introduction of the 3-Hydroxyl Group

The Ebb's persulfate oxidation (Scheme 2.19) was used to introduce the 3-hydroxyl group on several quinolone derivatives. The reported literature yields of this reaction were quite low (20-40%), but it was the only known oxidation that would introduce the functional group at the 3-position of the quinolone.

When the simple quinolone **130a** was treated with potassium persulfate in aqueous KOH, a (2:1) mixture of the 3-hydroxylated product **138** and recovered starting material was isolated in approximately 42% yield. A lower conversion occurred with the heavily functionalized quinolone **130d**. A (1:2) mixture of the 3-hydroxylated product **139**, and starting material were recovered in approximately 60% yield. Although the literature suggests the 3-hydroxylated compounds crystallize out of solution, only mixtures were recovered. Due to the polarity of the compounds, purification using silica gel was difficult, and the hydroxylated compounds **138** and **139** could not be purified from the starting material. Therefore an alternate approach was pursued and is described in Section 2.3.5.

Scheme 2.19

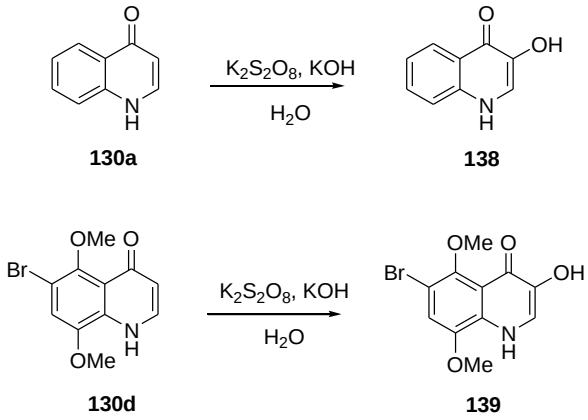


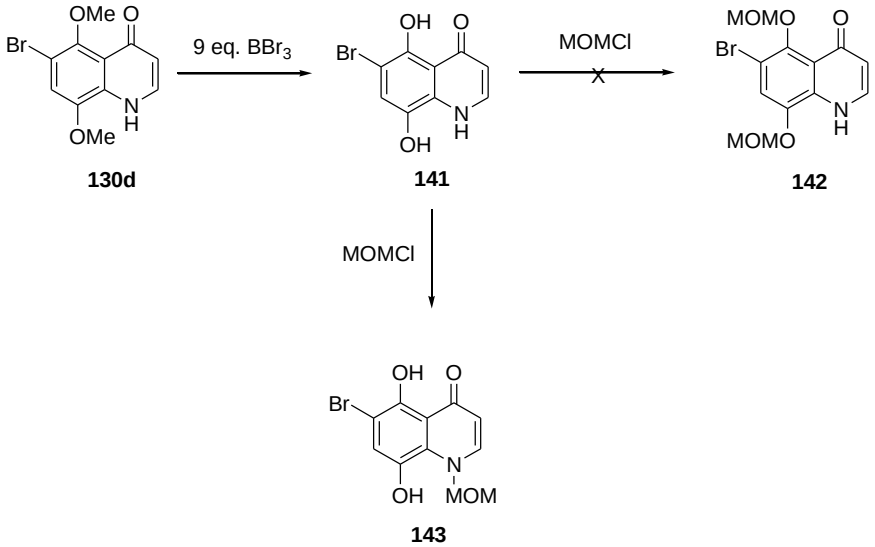
Table 2.5
Introduction of the 3-Hydroxyl Group in Quinolones

Quinolone	Ratio of Hydroxylated Product: Recovered Starting Material	Total Yield (%)
138	2:1	42%
139	1:2	60%

2.3.4 Deprotection of Methyl Ethers and Protection of Alcohols

Deprotection of the methyl ethers was carried out using a large excess (9 equivalents Scheme 2.20) of BBr_3. Subsequent treatment with 2.5 equivalents of MOMCl did not give the anticipated O-protected material **141**, but instead gave a product that had only one MOM-protection. From analysis of the NMR spectrum, it appears that the nitrogen becomes protected and the alcohols do not, with **143** being the possible structure.

Scheme 2.20



130d **141** **142** **143**

The ^{1}H NMR of the unprotected quinolone **130b** clearly shows a broad singlet at δ = 11.5-12 Hz, corresponding to the hydrogen at the 1-position. In the MOM-protected quinolone **132a**, the peak disappears. For the highly functionalized quinolone **130d**, there is also a broad singlet corresponding to the hydrogen at the 1-position, which is not apparent in the NMR spectrum of the crude material obtained from the deprotection/reprotection scheme.

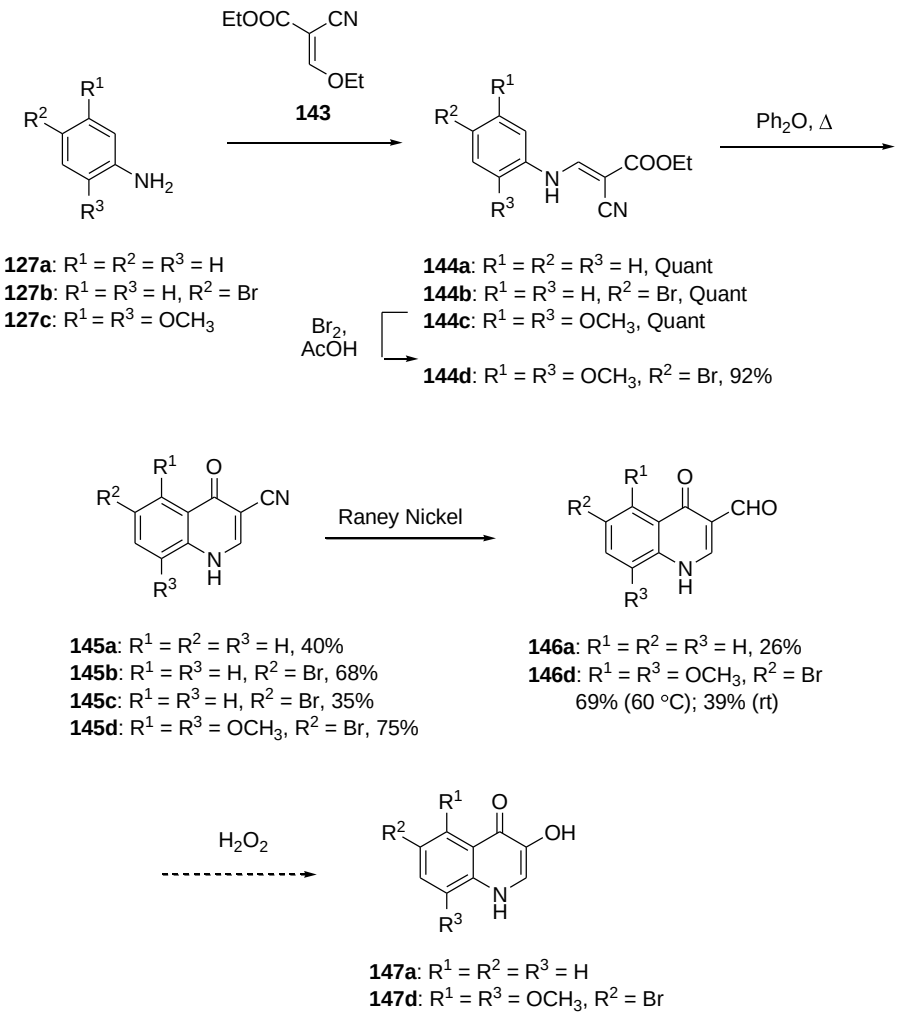
2.3.5 3-Functionalized Quinolone Starting Materials

Since the introduction of the 3-hydroxy group was proving problematic, an investigation into derivatives with the 3-position already functionalized was considered. The same aniline starting materials **127a-c** were condensed with ethyl (ethoxymethylene) cyanoacetate (**143**) to form the adducts **144a-c** in quantitative yields (Scheme 2.21).[41] Generally, the materials were isolated as a mixture of the *E* and *Z* isomers, and could be cyclized in Ph_2O without further purification. The *E*-isomer of

the dimethoxy adduct **144c** could be isolated by recrystallization from EtOH or by column chromatography, and further brominated in AcOH to give **144d** in 92% yield.

Cyclization in Ph_2O at 260 °C, provided quinolones **145a-d** with a 3-cyano moiety in 35-75% yield. The cyano group was then reduced with Raney nickel. Higher yields were obtained when the reaction was done at elevated temperatures (69% of the aldehyde **146d** at 60 °C versus 39% at room temperature). Oxidation with H_2O_2 would give the hydroxylated quinolones that could be used in cross-coupling studies. Preliminary attempts of this reaction were not successful, and more work is necessary to determine the exact reaction conditions needed for the reaction to proceed to completion.

Scheme 2.21



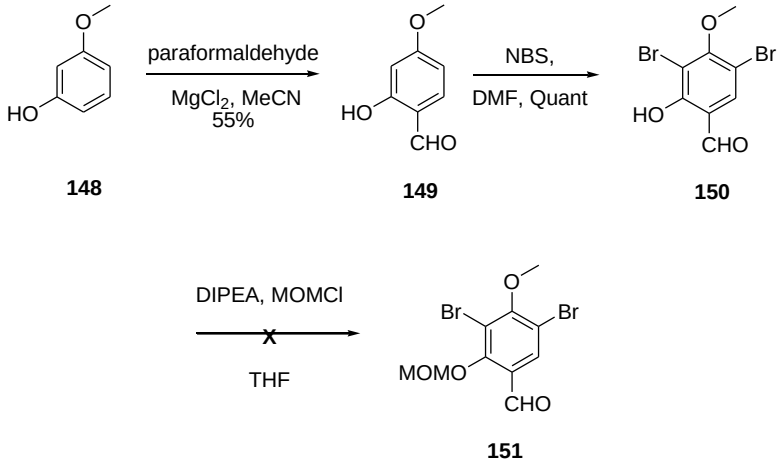
2.4 Synthesis of the Spiro-Isoxazoline Fragment

There are several published syntheses to the spiro-isoxazoline fragment. The synthesis published by Spilling was followed with the exception that the ethyl phosphonate **153** was employed in exchange of the methyl phosphonate used in the published account.[42]

2.4.1 Ortho-formylation of 3-Methoxyphenol

3-Methoxyphenol (**148)** was treated with paraformaldehyde, magnesium dichloride and triethylamine in acetonitrile (Scheme 2.22).[83] The reaction proceeded to give low yields (55%) of the desired aldehyde **149**. The dibromination, with NBS, gave a quantitative amount of the dibrominated aldehyde **150**.[42] However, the MOM-protection did not proceed smoothly, and only starting material was obtained after work-up. A later published review of the reaction reported that anhydrous beads of magnesium dichloride were needed for the reaction to be successful.[84] Since these beads are expensive, this procedure was abandoned the commercially available dibrominated aldehyde **150** was purchased.

Scheme 2.22



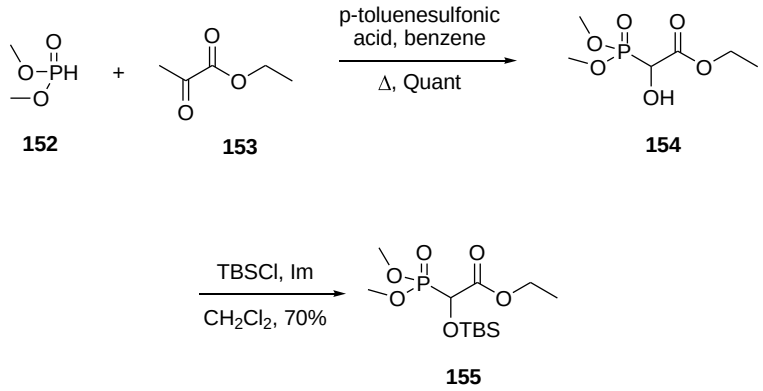
148 **149** **150** **151**

2.4.2 Synthesis of Phosphonate

To prepare the TBS-protected phosphonate **154**,[85] dimethyl phosphite (**152**) and a 50% solution of ethyl glyoxalate (**153**) in benzene were treated with *p*-toluenesulfonic acid and heated to reflux for 5 hours. The solvent was removed and the product was

purified by column chromatography to give a quantitative yield of the phosphonate as a white, waxy solid. The TBS-protection proceeded smoothly. It should be noted that the ethyl ester was prepared instead of the methyl ester since ethyl glyoxalate was commercially available.

Scheme 2.23

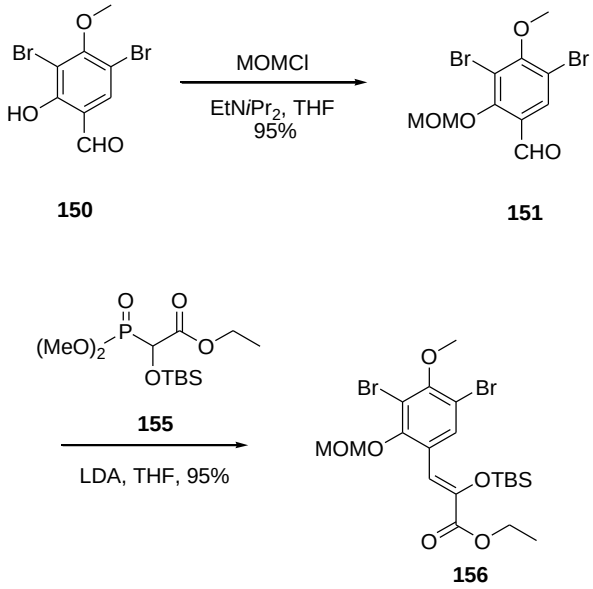


152 **153** **154** **155**

2.4.3 Synthesis of (±)-Spiro-isoxazoline Fragment

Following Spilling's synthesis (Scheme 2.24), the dibrominated aldehyde **150** was protected with MOMCl and then condensed with that anion of the phosphonate **155** to give the silyl enol ether **156** in 95% yield.[42]

Scheme 2.24



150 **151** **156**

TBS deprotection with HF•Et_3N and subsequent treatment with NH_2OH•HCl gave the oxime **157** as an approximately (1:1) mixture of *syn*/*anti* isomers in 83% yield. One stereoisomer could be purified by recrystallization with MeOH to facilitate characterization, and simplify further reactions. Although we thought the mixture of isomers could be used to increase yields, when the mixture was carried through the remaining reactions, there soon became a complex mixture that was difficult to work with. To simplify the remaining reactions, the recrystallized material carried through to the next reaction. The MOM-group could then be removed using *p*-TsOH (**158**), and the ring cyclized using a polymer-supported hypervalent iodine reagent, PSDIB (Figure 2.1) to obtain the spiro-isoxazoline **(±)159**.[42,86,87]

Scheme 2.25

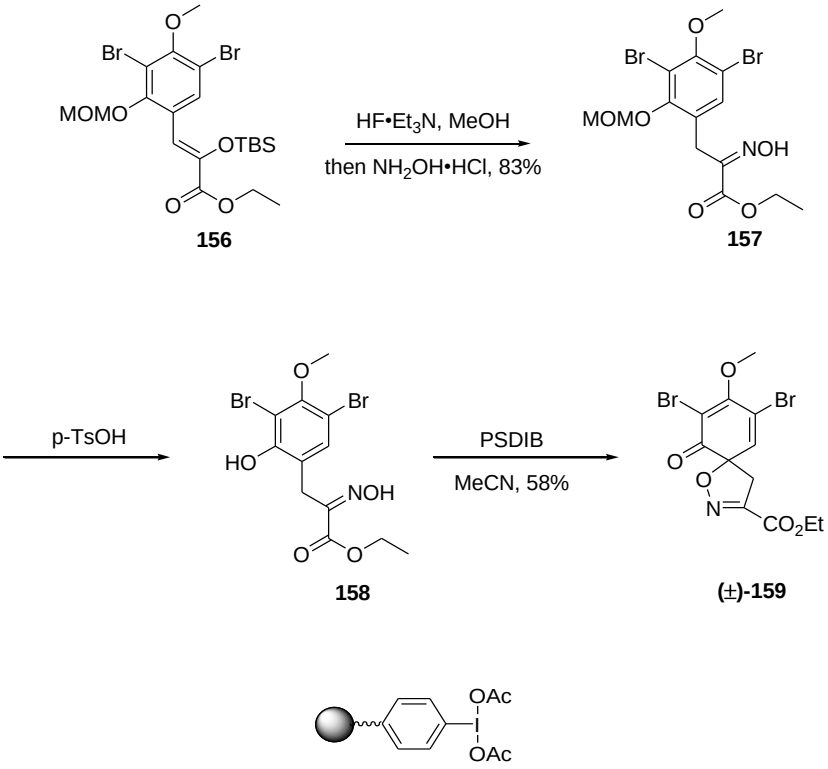


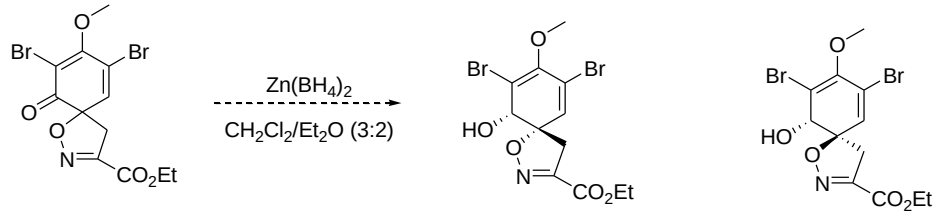
Figure 2.2 PSDIB

2.4.4Future Work on the Spiro-isoxazoline Fragment

2.4.4.1 $Zn(BH_4)_2$ Reduction of the Spiro Ketone

The racemic spiro-isoxazoline can be reduced with excess $Zn(BH_4)_2$, which can be prepared from $ZnCl_2$ and $NaBH_4$ as a 1.0 M solution in diethyl ether, to give a mixture of the *cis* and *trans* alcohols, (Scheme 2.26, **160a,b**) which can be separated using column chromatography.[48]

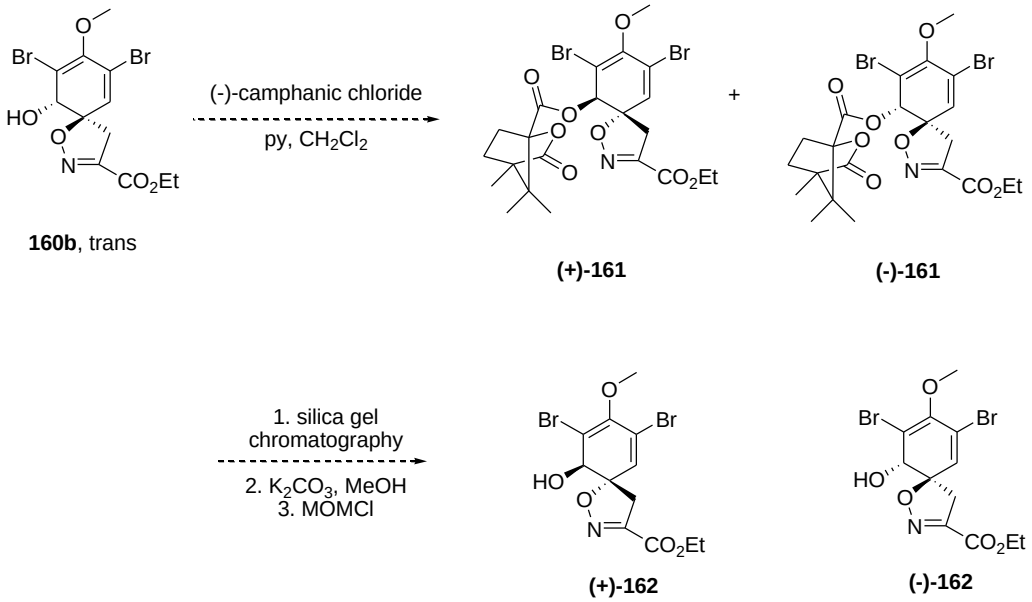
Scheme 2.26



2.4.4.2 Asymmetric Synthesis of the Spiro-isoxazoline Fragment

Since the absolute stereochemistry of the isolated natural product needs to be confirmed, the fragment must be synthesized asymmetrically. Nishiyama's procedure, using (-)-camphanic chloride as the resolving agent (Scheme 2.27) can be used to prepare the asymmetric fragment. The *trans* alcohol **160b** can be treated with (-)-camphanic chloride to provide two diasterometric esters (+)-**161** and (-)-**161**, which can be separated by column chromatography. Removal of the camphonyl group with base hydrolysis will provide two distinct enantiomers (+)-**162** and (-)-**162**. Each of these can be condensed with the imidazole-quinolone fragment to verify that the original stereochemistry is correct.

Scheme 2.27



160b, trans

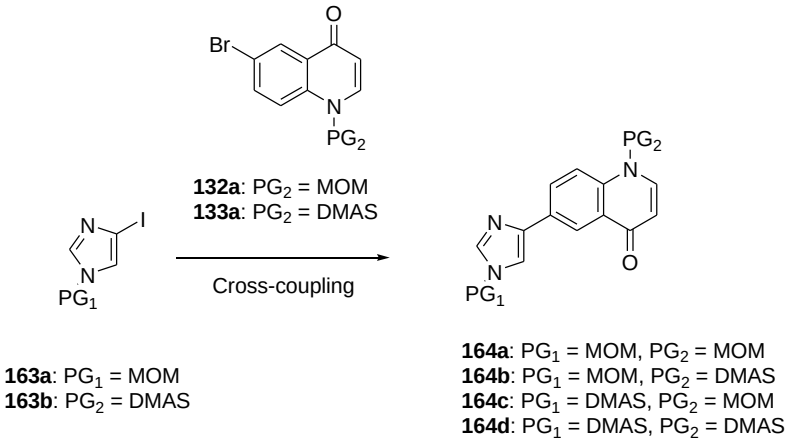
(+)-161 **(-)-161**

(+)-162 **(-)-162**

2.5 Cross-Coupling Reactions

Since at this point all of the fragments or advanced intermediates were prepared, we turned our attention to the cross-coupling chemistry. To evaluate a series of cross-coupling reaction conditions, a model study was carried out using N-protected-4-iodoimidazoles **163a,b** and brominated quinolone fragments **132a** and **133a** (Scheme 2.28). In previous reported literature, the electronic nature of the protecting group on both fragments has been shown to enhance or hinder the reaction, so both fragments were synthesized with several protecting groups.

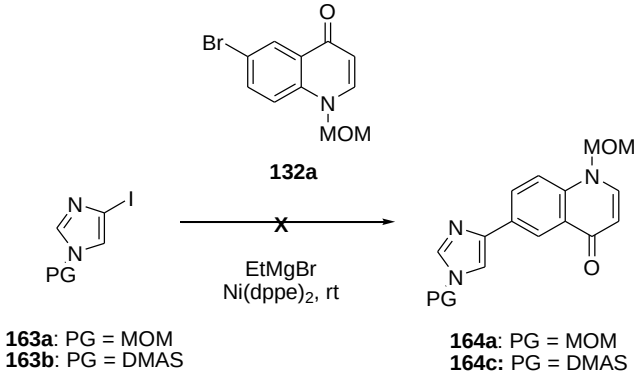
Scheme 2.28



2.5.1 Kumada Couplings

Protected imidazoles **163a,b** were treated with EtMgBr in Et_2O in order to prepare the Grignard reagent for the Kumada cross-coupling (Scheme 2.25).[53,54] 6-Bromo-1-methoxymethyl-4-quinolone **132a** was added, along wth $NiCl_2(dppe)_2$. Although no cross-coupled material was obtained, there was evidence that the starting materials were dehalogenated.

Scheme 2.29



2.5.2 Stille Couplings

To attempt the Stille cross-couplings, the stannane materials **165a,b** were prepared by treating 4-iodo-1-methoxymethylimidazole **163a** or 4-iodo-1-dimethylsulfonylimidazole **163b** with EtMgBr and quenching with Bu_3SnCl (Scheme 2.30).[57]

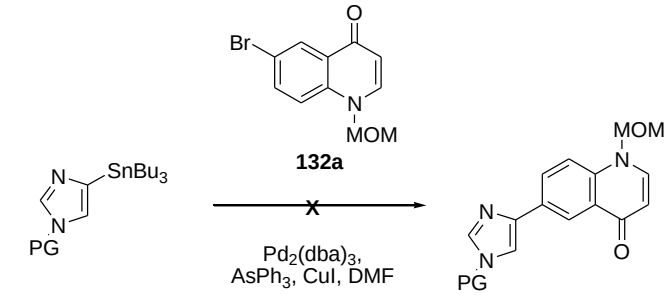
Scheme 2.30

163a: PG = MOM
163b: PG = DMAS

165a: PG = MOM, 65%
165b: PG = DMAS, quant

The 4-imidazolyl stannanes were then treated with 6-bromo-1-methoxymethyl-4-quinolone and $Pd_2(dba)_3$, $AsPh_3$ and CuI under a nitrogen atmosphere (Scheme 2.31). Again, only dehalogenated starting materials were isolated, with no evidence of any cross-coupling products.

Scheme 2.31



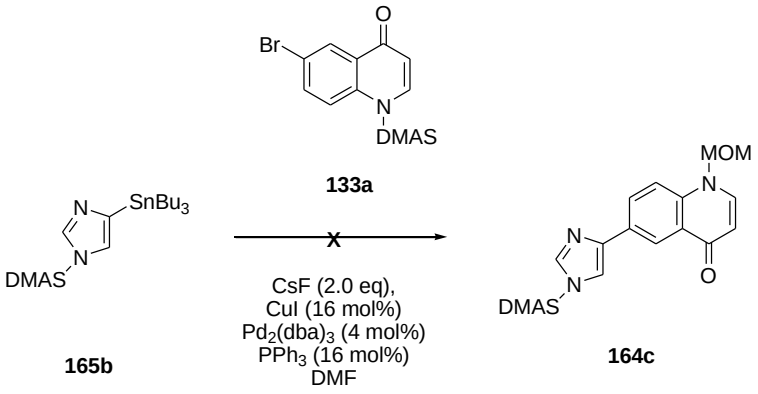
165a: PG = MOM
165b: PG = DMAS

164a: PG = MOM
164c: PG = DMAS

Baldwin and coworkers published a paper using a CuI/CsF system, which significantly enhanced the yields of Stille cross-coupling (Scheme 2.32).[58] The paper

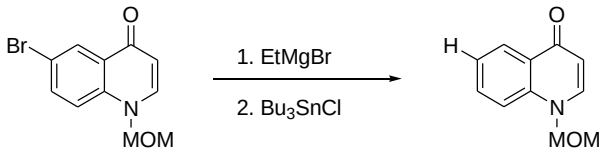
suggested that the reaction worked best with an electron-deficient stannane, so we chose to use the DMAS-protected imidazole. This reaction was also attempted with TBAF as the fluoride source, but no reaction occurred. We have found these conditions to be successful with other imidazoles in our lab.

Scheme 2.32



Since the imidazole stannanes were not giving the desired products, the synthesis of the quinolone stannane was attempted (Scheme 2.33). Only the non-brominated quinolone **167** was isolated from this reaction. It appears that the Grignard reagent is formed, however the transmetalation does not occur, and the Grignard is quenched with water during work-up. Elevating the reaction temperature did not provide the desired stannane.

Scheme 2.33

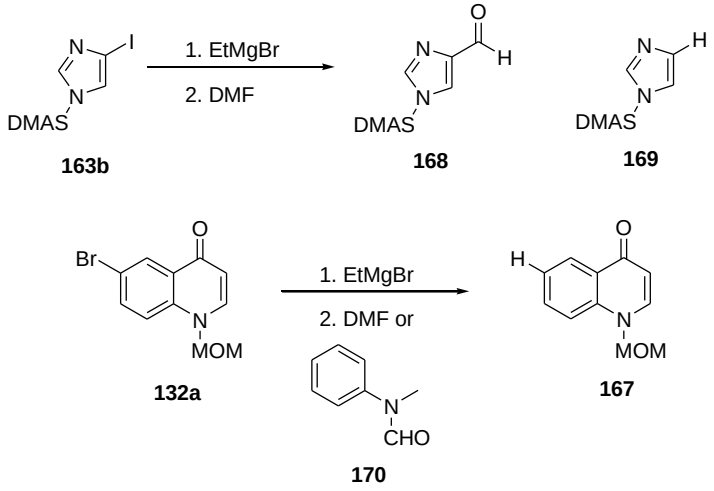


132a **167**

2.5.3 Formylation of the Imidazole and Quinolone

To evaluate the reactivity of the imidazole and quinolone fragments for cross-coupling, the formylation of both fragments was attempted by preparing the Grignard reagents and then quenching the reaction with DMF (Scheme 2.34). The formylation of the imidazole proceeded with modest yield (57%), and resulted in a (1:4) mixture of the formylated product **168** and the deiodinated product **169**. The reaction of the quinolone **135a** with EtMgBr followed by DMF only resulted in the debrominated quinolone **167**. Similar results were found using *N*-methylformanilide **170** as the formylating agent.

Scheme 2.34



163b **168** **169**

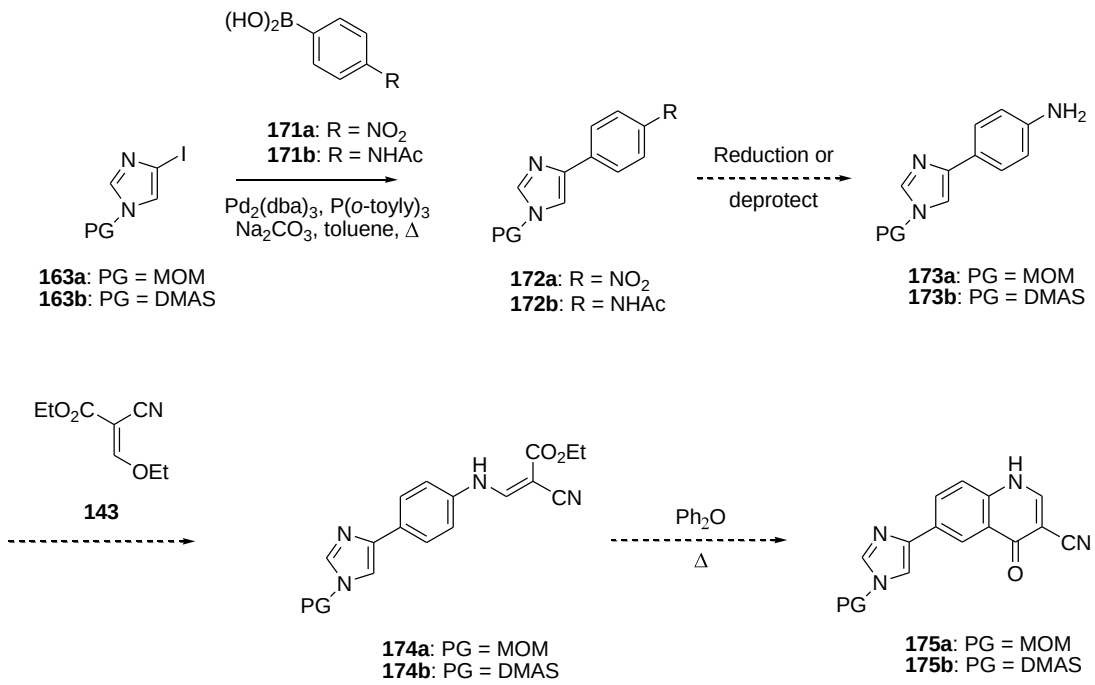
132a **170** **167**

2.5.4 Suzuki Couplings with $Pd_2(dba)_3$

Since the cross-coupling of the imidazole and quinolone fragments had proved to be more difficult than expected, a new approach to the imidazole-quinolone fragment was investigated. The Suzuki cross-coupling reaction is limited by the availability of the boronic acid. Although simple imidazole boronic acids are available, the highly substituted ones needed for this synthesis would need to be prepared. There are no commercially available quinoline boronic acids, and the lack of success in functionalizing the 6-position of quinolone **132a** suggests that these materials might be difficult to prepare, even in situ.

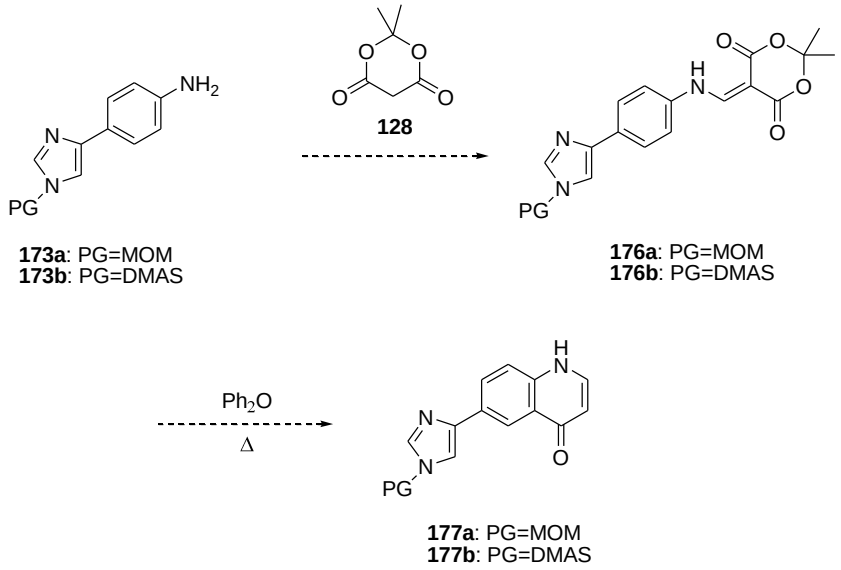
In this approach (Scheme 2.35), the protected imidazoles **163a,b** would be cross-coupled with a boronic acid functionalized in the *para* position with a group that could easily be converted to an amine by reduction or deprotection (**172a,b**). The resulting aniline **173a,b** could be condensed with ethyl (ethoxymethylene) cyanoacetate to obtain the adduct **174a,b**, followed by cyclization in Ph_2O (**175a,b**).

Scheme 2.35



One potential problem with the above scheme is the tendency to obtain mixtures of *E* and *Z* isomers from the condensation with ethyl (ethoxymethylene)-cyanoacetate, which decreases the yields of the cyclization step. Alternatively, the reduced cross-coupled product could be condensed with Meldrum's acid **128**, followed by Ph_2O cyclization, to produce a simpler quinolone fragment (**177a,b**) without functionalization in the 3-position (Scheme 2.36).

Scheme 2.36



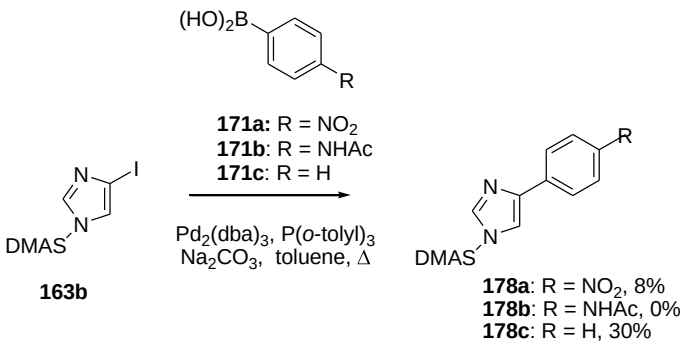
173a: PG=MOM
173b: PG=DMAS

176a: PG=MOM
176b: PG=DMAS

177a: PG=MOM
177b: PG=DMAS

To test the activity of the Suzuki reaction, 4-iodo-1-dimethylsulfonylimidazole was treated with phenylboronic acid in the presence of $Pd_2(dba)_3$ and aqueous Na_2CO_3 in toluene at reflux temperatures (Scheme 2.37). A low yield (10%) of the desired cross-coupling product was obtained from this reaction. To enhance the yields, the reaction was attempted a second time with the use of P(*o*-tolyl)$_3$ as a ligand, resulting in about 30% yield. When attempting the reaction using the functionalized boronic acids **171a,b**, it was discovered that the DMAS-protecting group was removed upon the prolonged heating required for the cross-coupling to take place. Therefore, the reaction was allowed to proceed for only 24 hours. After testing several reaction conditions, a very small yield (8%) of **178a** was obtained. In the case of boronic acid **171b**, no product was ever seen from any reaction conditions employed.

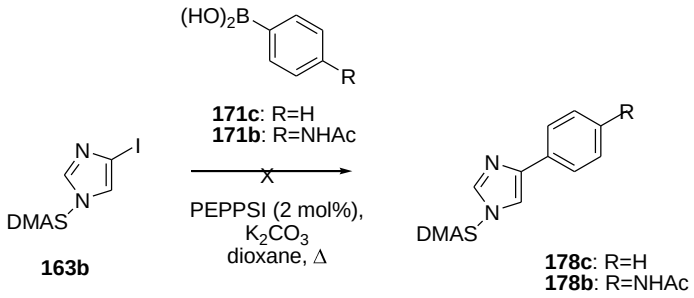
Scheme 2.37



2.5.5 Suzuki Couplings using PEPPSI Catalyst

In order to increase yields of cross-coupled product, we decided to investigate the use of the PEPPSI precatalyst (Scheme 2.38).[65-70] The reactions were carried out using anhydrous dioxane at 60 °C, for a period of 24 hours. Although the cross-coupling between the DMAS-protected imidazole and phenyl boronic acid was not successful, we decided to attempt the coupling with the functionalized boronic acid **174b** as well. No product was recovered from either reaction.

Scheme 2.38

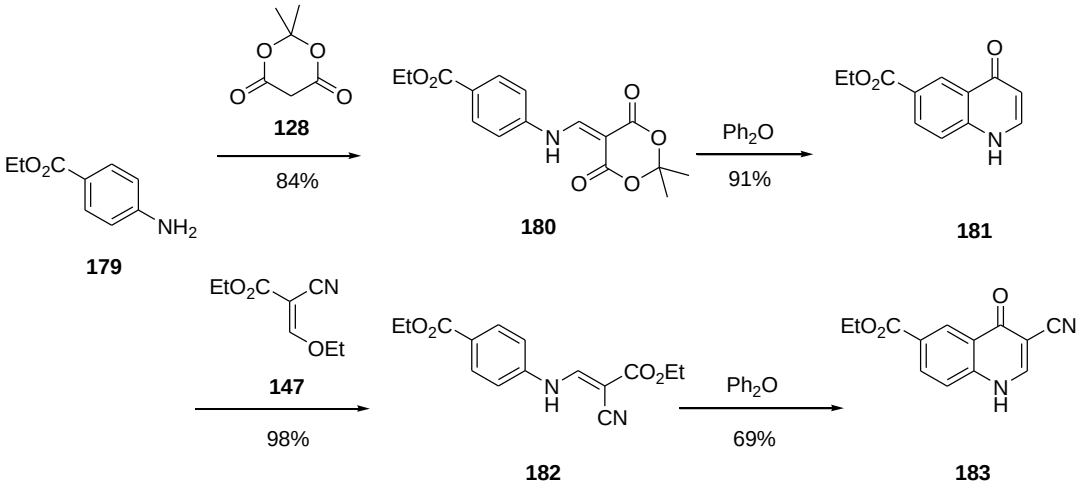


2.6 Construction of the Imidazole Ring from a Functionalized Quinolone

2.6.1. Construction of 6-Functionalized Quinolones

Since the cross-coupling studies were not very successful, an alternate method for constructing the key imidazole-quinolone fragment was investigated. The imidazole fragment can be constructed from a quinolone functionalized with an ester in the 6-position. The functionalized quinolones, using the general quinolone synthesis with ethyl 4-aminobenzoate **179** as the starting material were synthesized. The condensation was attempted with both Meldrum's acid[36] and ethyl (ethoxymethylene) cyanoacetate.[41] Using Meldrum's acid (**128**), 84% of the condensed adduct **180** was obtained, and cyclization in Ph_2O gave 91% of the 6-functionalized quinolone **181**. With ethyl (ethoxymethylene) cyanoacetate, 98% of the adduct **182** was obtained, and 69% of the 6-functionalized quinolone **183** was obtained. These results suggest that this route must be looked at carefully for further synthetic studies.

Scheme 2.39



CHAPTER 3

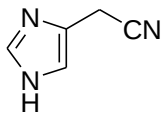
EXPERIMENTAL

3.1 General

All chemicals and solvents were purchased from commercial vendors and were used as received unless indicated otherwise. All reactions involving air- or water-sensitive compounds were conducted in oven-dried glassware under an atmosphere of dry argon or nitrogen. Tetrahydrofuran and diethyl ether were distilled from sodium/benzophenone ketyl and benzene and dichloromethane were distilled from CaH under a nitrogen atmosphere. Additionally, acetonitrile, benzene, dichloromethane, diethyl ether, tetrahydrofuran and toluene were purified using an Innovative Technologies Inc. Pure Solv SPS-400-05 solvent purification system. ^{1}H and ^{13}C NMR spectra (δ in ppm) were recorded using either a JEOL Eclipse+ 500 MHz (500 MHz and 125.8 MHz) or 300 MHz (300 MHz and 75 MHz) spectrometer. NMR spectra were obtained in deuteriochloroform (unless otherwise indicated) using residual $CHCl_3$ (δ = 7.26) as a reference for ^{1}H NMR spectra and carbon absorption of $CDCl_3$ (δ = 77.0) as an internal reference for ^{13}C NMR spectra. Infrared spectra were recorded either as neat films on NaCl plates for liquids or oils or KBr pellets for solids using a Bruker Vector 22 FT-IR spectrometer. Electron ionization mass spectra (EI-MS) were recorded using a Thermo Electron Corporation LCQ Deca XP. Elemental analyses were performed

using a Perkin-Elmer 2400 CHN Analyzer. Melting points were recorded on a Thomas Hoover Scientific capillary tube melting point apparatus and were uncorrected. Analytical thin layer chromatography (TLC) was performed on Whatman silica gel 60_{F254} aluminum precoated plates (0.25 mm layer). All chromatographic purifications were performed using ICN silica gel (200-400 mesh).

4-Cyanomethyl-(*1H*)-imidazole (56)[33-35]



L-Histidine (7.76 g, 0.05 mol) and water (50 mL) were mixed in a slurry. Concentrated hydrochloric acid (4.0 mL, 0.05 mol) was added to the mixture. Sodium hypochlorite (154 mL, 6% solution, 0.11 mol) was added dropwise with vigorous stirring at 0 °C. The mixture was stirred at 0 °C until CO_2 evolution stopped (about 2 hours). The mixture was brought to room temperature and stirred overnight. Solid sodium carbonate was added until the pH = 8. The solvent was removed under reduced pressure and the resulting solid was extracted with ethyl acetate with a Soxhlet apparatus. The solvent was removed under reduced pressure, yielding light orange crystals (2.97 g, 70%). mp. 134-135 °C (lit. 134-137 °C). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.63 (s, 1H), 7.05 (s, 1H), 3.83 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 135.4, 124.0, 118.2, 117.2, 14.1.

3.2 General Procedure for the *N*-Protection of 4- and 5-Imidazoles

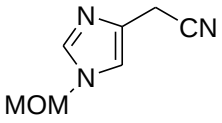
4-Cyanomethylimidazole (300 mg, 2.8 mmol) was dissolved in dry THF (9.0 mL) at 0 °C. NaH (132 mg, 3.08 mmol, 1.1 equivalents) was added portionwise and

stirred 30 minutes. The reaction was brought to room temperature and stirred 1 hour. The mixture was recooled to 0 °C and the protecting group (1.15 equivalents) was added dropwise. The mixture was brought back to room temperature and stirred overnight, quenched with a small amount of water, and the solvent removed. The residue was dissolved in a small amount of water, extracted with CH_2Cl_2, dried (Na_2SO_4), and concentrated.

3.3 General Procedure for the Isomerization of 4- and 5-Substituted Imidazoles

The crude mixture of two isomers was dissolved in freshly distilled DMF (0.3 M). An additional 10 mol% of the protecting group was added and the mixture was heated to reflux overnight. The solvent was removed and the resulting residue was dissolved in a small amount of water, extracted with CH_2Cl_2, dried (Na_2SO_4), and concentrated.

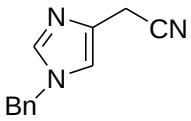
1-Methoxymethyl-4-cyanomethyl-(*1H*)-imidazole (114a)



The crude product was isolated as a (1:0.8) mixture of isomers (378 mg, 83%). After isomerization, the 4-protected product was purified using column chromatography (1:4:5 MeOH/Hexanes/EtOAc), yielding a brown oil (356 mg, 78%). ^{1}H NMR (500 MHz) δ 7.52 (s, 1H), 7.04 (s, 1H), 5.17 (s, 2H), 3.67 (s, 2H), 3.25 (s, 3H). ^{13}C NMR (125 MHz) δ 137.9, 132.6, 117.5, 116.8, 77.9, 56.4, 17.8. FT-IR (neat, cm^{-1}): 3116, 2951, 2256, 1695, 1493, 1404. EI-MS (*m/z*): 151.1 (M^+, 100%), 121.1 (96%). HRMS (ESI) Calcd. for $C_7H_9N_3O$ (*m/z*): 152.0818. Found:

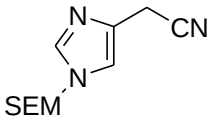
152.0827 $[M+H]^+$. HRMS (ESI) Calcd. for $C_7H_9N_3O$+Na (*m/z*): 174.0638. Found: 174.0639 $[M+Na]^+$.

1-Benzyl-4-cyanomethyl-(*1H*)-imidazole (114b)



The crude product was isolated as a (1:0.6) mixture of isomers (498 mg, 85%). After isomerization, the 4-protected product (1:0.08) was purified using column chromatography (1:4:5 MeOH/Hexanes/EtOAc), yielding a brown oil (504 mg, 85%). ^{1}H NMR (300 MHz) δ 7.64 (s, 1H), 7.37 (m, 3H), 7.28 (m, 2H), 6.94 (s, 1H), 5.10 (s, 2H), 3.73 (s, 2H). ^{13}C NMR (125 MHz) δ 137.7, 135.7, 132.2, 129.2, 128.6, 127.5, 117.7, 117.1, 51.2, 17.9. FT-IR (neat, cm^{-1}): 3144, 3112, 3082, 3067, 3034, 2953, 2251, 1559, 1503. EI-MS (*m/z*): 197.1 (M^+, 100%), 179.1 (32%), 121.1 (47%), 91.1 (76%). HRMS (ESI) Calcd. for $C_{12}H_{11}N_3$+Na (*m/z*): 220.0845. Found: 220.0823 $[M+Na]^+$.

1-2-(Trimethylsilyl)ethoxymethyl-4-cyanomethyl-(*1H*)-imidazole (114c)



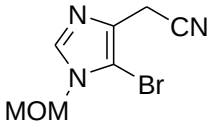
Crude product was isolated as a (1:0.5) mixture of isomers (783 mg, 91%). After isomerization, the 4-protected product was purified using column chromatography (1:4:5 MeOH/Hexanes/EtOAc), yielding a brown oil (581 mg, 85%). ^{1}H NMR (500 MHz) δ 7.52 (s, 1H), 7.04 (s, 1H), 5.20 (s, 2H), 3.68 (s, 2H), 3.45 (t, *J* = 8.3 Hz, 2H), 0.87 (t, *J* = 8.3 Hz, 2H), -0.05 (s, 9H). ^{13}C NMR (125 MHz) δ 137.7, 132.5, 117.5, 116.7, 76.2, 66.7, 17.8, 17.7, -1.35. FT-IR (neat, cm^{-1}): 2953, 2925, 2895, 2251, 1500. EI-MS (*m/z)*: 72.9 (40%), 121.0 (71%), 164.0 (28%),

179.0 (100%), 194.0 (42%). HRMS (ESI) Calcd. for $C_{11}H_{19}N_3OSi$ (*m/z*): 238.1370. Found: 238.1384 $[M+H]^+$. HRMS (ESI) Calcd. for $C_{11}H_{19}N_3OSi+Na$ (*m/z*): 260.1190. Found: 260.1201 $[M+Na]^+$.

3.4 General Procedure for the Bromination of the 5-Position of Imidazoles

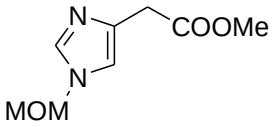
The imidazole (0.66 mmol) was dissolved in 7 mL of a 1:1 mixture of CH_2Cl_2 and MeOH at 0 °C. $BnEt_3NBr_3$ (0.66 mmol, 258 mg) and $CaCO_3$ (1.1 eq., 0.72 mmol, 73 mg) were added and the mixture was stirred until the reaction was complete as monitored by TLC (5:4:1, EtOAc/hexanes/MeOH, about 5 hours). The reaction mixture was filtered through Celite and concentrated. The final products were purified using column chromatography.

5-Bromo-1-methoxymethyl-4-cyanomethyl-(*1H*)-imidazole (117)



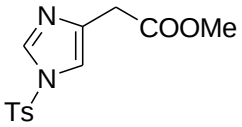
The product was prepared using General Method 3.4 and purified using column chromatography (5:4:1, EtOAc/hexanes/MeOH), yielding a brown oil (100 mg, 66%). ^{1}H NMR (300 MHz) δ 7.70 (s, 1H), 5.23 (s, 2H), 3.66 (s, 2H), 3.32 (s, 3H). ^{13}C NMR (75 MHz) δ 138.7, 130.8, 116.23, 116.22, 76.9, 56.6, 17.1. FT-IR (neat, cm^{-1}): 3113, 2934, 2252, 1684, 1501, 1408, 1190, 1151, 1107, 919, 427, 414. EI-MS (*m/z*): 72.0 (68%), 73.1 (52%), 121.1 (91%), 132.1 (100%), 179.1 (49%), 185.0 (28%), 228.9 (M^+, 82%), 230.9 ($M+2^+$, 89%). HRMS (ESI) Calcd. for $C_7H_8BrN_3O$ (*m/z*): 229.9923, 231.9903. Found: 229.9931 $[M+H]^+$, 231.9910 $[M+2+H]^+$.

Methyl 1-methoxymethyl-4-imidazoleacetate (123a)



Methyl 4-imidazole acetate (633 mg, 4.5 mmol) was protected using the general two-step protection/isomerization method described above. Crude product was isolated as a (1:1) mixture of isomers, yielding a brown oil (637 mg, 77%). After isomerization (330 mg, mmol) the 4-protected product was purified using column chromatography (5:4:1, EtOAc/hexanes/MeOH), yielding brown oil (254 mg, 76%). ^{1}H NMR (300 MHz) δ 7.47 (s, 1H), 6.95 (s, 1H), 5.12 (s, 2H), 3.65 (s, 3H), 3.60 (s, 2H), 3.21 (s, 3H). ^{13}C NMR (75 MHz) δ 171.6, 137.1, 135.9, 117.0, 77.8, 56.2, 52.1, 34.3. FT-IR (neat, cm^{-1}): 3853, 3388, 2953, 2360, 1559, 1501, 1438, 1406, 1363, 1271, 1192, 1155, 1106, 1033, 1012, 917, 833, 735. HRMS (ESI) Calcd. for $C_8H_{12}N_2O_3$-MOM (*m/z*): 141.1399. Found: 141.1154 $[M+H\text{-}MOM]^+$.

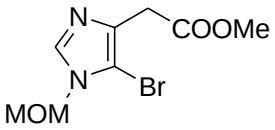
Methyl 1-toluenesulfonyl-4-imidazoleacetate (123b)[88]



Methyl 4(*1H*)-imidazoleacetate (500 mg, 2.35 mmol) was dissolved in 20 mL CH_2Cl_2. Triethylamine (4 eq, 1.31 mL, 9.38 mmol) and tosyl chloride (448 mg, 2.35 mmol) were added and the reaction was stirred for 30 minutes. The solvent was removed to obtain a yellow oil. Product was purified by trituration with H_2O, yielding an off-white solid (485 mg, 70%). mp. 124-5 °C. ^{1}H NMR (300 MHz) δ 8.64 (s, 1H), 7.75 (d, *J* = 7.9 Hz, 2H), 7.17 (m, overlapping signals, 3H), 3.77 (s, 2H), 3.64 (s, 3H), 2.34 (s, 3H). ^{13}C (75 MHz) δ 168.9, 141.6, 140.8, 134.0, 129.2, 126.4, 125.9, 117.6, 52.7, 30.1, 21.4. FT-IR (KBr, cm^{-1}): 3141, 1741, 1679, 1631, 1606, 1583, 1499, 1460, 1436, 1407, 1356, 1313, 1274, 1178, 1124, 1034, 1010,

917, 858, 814, 768, 710. HRMS (ESI) Calcd. for $C_{13}H_{14}N_2O_4S$+Na (*m/z*): 295.0747. Found: 295.0745 $[M+Na]^+$.

Methyl 5-bromo-1-methoxymethyl-4-imidazoleacetate (124)

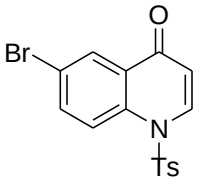


The product was made using General Procedure 3.4 and purified using column chromatography (5:4:1, EtOAc/hexanes/MeOH), yielding a lt. brown oil (122 mg, 70%). ^{1}H NMR (300 MHz) δ 7.42 (s, 1H), 5.20 (s, 2H), 3.69 (s, 2H), 3.68 (s, 3H), 3.21 (s, 3H). ^{13}C NMR (75 MHz) δ 169.6, 136.1, 130.4, 119.5, 77.5, 56.0, 52.5, 28.7. FT-IR (neat, cm^{-1}): 3116, 3001, 2955, 2832, 2016, 1575, 1491, 1438, 1408, 1363, 1337, 1242, 1188, 1164, 1106, 1047, 1018, 995, 919, 817, 785, 737. HRMS (ESI) Calcd. for $C_8H_{11}BrN_2O_3$-MOM (*m/z)*: 218.9769. Found: 219.0543 $[M+H\text{-}MOM]^+$. HRMS (ESI) Calcd. for $C_8H_{11}BrN_2O_3$-MOM+Na (*m/z)*: 240.9589. Found: 241.0358 $[M+Na\text{-}MOM]^+$.

3.5 General Procedure for the Protection of Quinolones using Et_3N as Base

The quinolone was dissolved in dry CH_2Cl_2 (0.15 M). Et_3N (2.0 equivalents) and protecting group chloride (1.0 equivalent) were added and the mixture was stirred at room temperature overnight. The solvent was removed to obtain crude product.

1-*N*-Tosyl-6-bromo-4-(*1H*)-quinolone (131a)



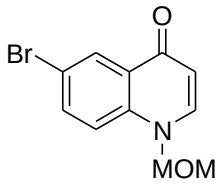
The product was isolated as a tan solid (511 mg, quant). mp: 219-221 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.44 (d, J = 7.1 Hz, 1H), 8.29

(d, *J* = 2.2 Hz, 1H), 7.98 (dd, *J* = 8.9, 2.2 Hz, 1H), 7.75 (d, *J* = 8.9 Hz, 1H), 7.46 (d, *J* = 7.9 Hz, 2H), 7.09 (d, *J* = 7.9 Hz, 2H), 6.64 (d, *J* = 7.1 Hz, 1H), 2.26 (s, 3H). A broad peak can be seen between 4.5-5.5. ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.8, 145.6, 144.5, 138.9, 138.6, 136.9, 128.7, 126.6, 126.0, 124.0, 122.5, 119.6, 107.6, 21.3. FTIR (KBr, cm^{-1}): 3058, 2758, 1716, 1643, 1590, 1561, 1502, 1481, 1416, 1353, 1310, 1232, 1138, 1117, 1030, 1007, 891, 845, 813, 709. HRMS (ESI) Calcd. for $C_{16}H_{12}BrNO_3$ (*m/z*): 377.9794, 379.9774. Found: 377.9808 $[M+H]^+$, 379.9780 $[M+2+H]^+$. HRMS (ESI) Calcd. for $C_{16}H_{12}BrNO_3$+Na: 399.9613, 401.9594. Found: 399.9636 $[M+Na]^+$, 401.9611 $[M+2+Na]^+$.

3.6 General Procedure for the Protection of Quinolones using NaH as Base

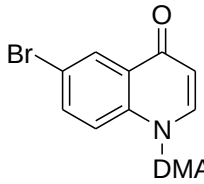
The quinolone was dissolved in dry THF (0.25 M) at 0 °C. NaH (1.1 equivalents, 60% oil dispersion) was added portionwise and stirred 30 minutes. The reaction was brought to room temperature and stirred 1 hour. The mixture was recooled to 0 °C and the protecting group (1.15 equivalents) was added dropwise. The mixture was brought back to room temperature and stirred overnight, quenched with a small amount of water, and the solvent removed. The residue was dissolved in a small amount of water, extracted with CH_2Cl_2, dried (Na_2SO_4), and concentrated.

1-*N*-Methoxymethyl-6-bromo-4(*1H*)-quinolone (132a)



The product was isolated as a brown solid (2.50 g, 71%). mp: > 260 ºC. ^{1}H NMR (500 MHz) δ 8.55 (d, *J* = 2.4 Hz, 1 H), 7.72 (d, *J* = 2.2 Hz , 1H), 7.57-7.52 (overlapping signals, 2H), 6.26 (d, *J* = 7.8, 1H), 5.37 (s, 2H), 3.36 (s, 3H). ^{13}C NMR (75 MHz) δ 177.2, 142.9, 138.5, 135.4, 129.5, 128.4, 119.0, 118.4, 110.2, 84.5, 56.3. FT-IR (KBr, cm^{-1}): 3050, 2924, 1803. ESI-MS (*m/z*): 268.13 $[M+H]^+$, 270.13 $[M+2+H]^+$, 534.80 $[2M+H]^+$, 536.80 $[2M+2+H]^+$, 538.87 $[2M+4+H]^+$. Anal. Calc. for $C_{11}H_{10}NO_2Br$: C, 49.28, H, 3.76, N, 5.22. Found: C, 48.92, H, 3.53, N, 5.07.

1-N-Dimethylaminosulfonyl-6-bromo-4(*1H*)-quinolone (133a)



The product was isolated as a brown solid (58 mg, 38%). mp: > 260 ºC. ^{1}H NMR (500 MHz) δ 8.50 (d, *J* = 2.1 Hz, 1H), 8.28 (d, *J* = 9.2 Hz, 1 H), 8.00 (d, *J* = 9.2 Hz, 1H), 7.76 (dd, *J* = 9.2, 2.1 Hz, 1H), 6.27 (d, *J* = 9.3 Hz, 1H), 2.92 (s, 6H). ^{13}C NMR (75 MHz) δ 177.1, 140.0, 136.3, 135.7, 129.9, 128.0, 120.6, 119.8, 111.5, 38.4. FTIR (KBr, cm^{-1}): 3139, 3077, 2916, 1811, 1628, 1595, 1550, 1466, 1449, 1369, 1327, 1315, 1284, 1261, 1186, 1159, 1108, 1066, 966, 907, 829, 820, 760, 729. ESI-MS (*m*/z): 331.2 $[M+H]^+$, 333.1 $[M+2+H]^+$. HRMS (ESI) Calcd. for $C_{11}H_{11}N_2O_3SBr$: 330.9747, 332.9727. Found: 330.9770 $[M+H]^+$, 332.9751 $[M+2+H]^+$.

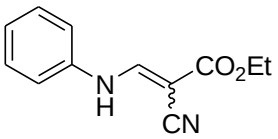
3.7 General Procedure for Cyano Acetate Adducts

Ethyl (ethoxymethylene) cyanoacetate and aniline (1 equivalent) were melted at 100 °C. The product crystallized out of solution, yielding a mixture of *E*/*Z* isomers. In some cases, the product could be purified to a single isomer by crystallization or column chromatography. Alternatively, the *E*/*Z* mixture could be subjected to cyclization without purification.

3.8 General Procedure for Bromination of Meldrum's Acid or Acetate Adducts

Starting material was dissolved in AcOH (0.33 M). Br_2 (1 equivalent) is dissolved in AcOH (0.66 M) and added dropwise at rt. The resulting mixture was stirred overnight, and poured slowly into an equal amount of ice-water. The precipitated product was isolated by filtration.

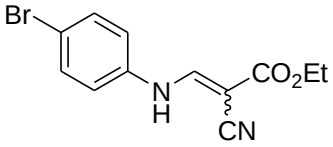
Ethyl 3-[(phenyl)amino]-2-cyano-2-propenoate (144a)



2.55 g of crude product was purified by column chromatography, yielding a mixture of E/Z isomers (1:0.6), as an off-white solid. (2.17 g, 97%) mp: 101-103 °C. ^{1}H NMR (300 MHz) δ 10.72 (d, *J* = 13.4 Hz, 1H), 8.75 (d, *J* = 14.8 Hz, 0.6H), 8.36 (d, *J* = 14.8 Hz, 0.6H), 7.88 (d, *J* = 13.8 Hz, 1H), 7.38-7.31 (m, overlapping signals, 3.2H), 7.18-7.06 (m, overlapping signals, 4.8H), 4.26-4.20 (m, overlapping signals, 3.2H), 1.34-1.29 (m, overlapping signals, 4.8H). ^{13}C NMR (75 MHz) δ 167.6, 164.9, 152.22, 152.17, 139.0, 138.4, 130.1, 129.9, 125.8, 125.5, 118.1, 117.5, 117.2, 115.9, 76.63, 75.4, 61.27, 61.21, 14.5, 14.4.

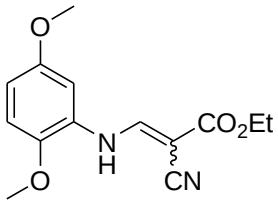
FT-IR (KBr, cm^{-1}): 3194, 3113, 3054, 2990, 2943, 2907, 2872, 2213, 1706, 1671, 1630, 1602, 1588, 1495, 1469, 1434, 1415. ESI-MS (*m/z)*: 217.0 $[M+H]^+$, 234.0 $[M+NH_4]^+$. Anal. Calcd. for $C_{12}H_{12}N_2O_2$: C, 66.65; H, 5.59; N, 12.96. Found: C, 66.75; H, 5.72; N, 12.97.

Ethyl 3-[(4-bromophenyl)amino]-2-cyano-2-propenoate (144b)



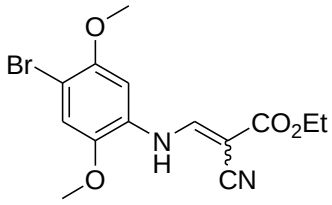
The product was isolated as a 0.75:1 ratio of E/Z isomers, yielding a white solid (1.59 g, 92%). mp: 134-136 °C. ^{1}H NMR (500 MHz) δ 10.73 (d, *J* = 12.8 Hz, 0.75H), 8.78 (d, *J* = 14.7 Hz, 1H), 8.32 (d, *J* = 14.7 Hz, 1H), 7.85 (d, *J* = 13.3 Hz, 0.75 H), 7.49-7.46 (m, signals overlapping, 3.5H), 7.06 (d, *J* = 8.7 Hz, 2H), 6.97 (d, *J* = 8.3 Hz, 1.5H), 4.27-4.26 (m, signals overlapping, 3.5H), 1.34-1.30 (m, signals overlapping, 5.25H). ^{13}C NMR (75 MHz) δ 167.5, 164.6, 151.9, 151.8, 138.1, 137.5, 133.1, 133.0, 119.0, 118.7, 118.6, 118.4, 77.4, 76.3, 61.5, 61.4, 14.5, 14.4. FT-IR (KBr, cm^{-1}): 3188, 3085, 2982, 2900, 2211, 1896, 1708, 1641, 1583, 1488, 1463, 1441, 1380, 1325, 1254, 1179, 1151, 1119, 1072, 1021, 1002, 974, 854, 819, 766, 747. ESI-MS (*m/z*): 293.1 $[M_1+H]^+$, 295.0 $[M_2+H]^+$. HRMS (ESI) Calcd. for $C_{12}H_{11}N_2O_2Br$: 295.0077, 297.0057. Found: 295.0093 $[M+H]^+$, 297.0074 $[M+2+H]^+$.

Ethyl 3-[(2,5-dimethoxylphenyl)amino]-2-cyano-2-propenoate (144c)



The crude product was isolated as a 1:0.5 ratio of E/Z isomers. 2.80 g of crude product was purified by column chromatography (1:4→1:1 EtOAc/Hex), yielding a single isomer as a light yellow solid. (1.87 g, 67%) mp: 129-131 °C. ^{1}H NMR (300 MHz) δ 10.97 (d, *J* = 13.8 Hz, 1H), 7.84 (d, *J* = 13.8 Hz, 1H), 6.86 (d, *J* = 8.9 Hz, 1H), 6.70-6.63 (m, overlapping signals, 2H), 4.29 (q, *J* = 7.0 Hz, 2H), 3.89 (s, 3H), 3.79 (s, 3H), 1.36 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz) 167.2, 154.4, 150.4, 143.1, 128.3, 118.3, 112.5, 109.8, 101.2, 75.7, 61.2, 56.5, 55.9, 14.4. FT-IR (KBr, cm^{-1}): 3180, 3043, 2838, 2223, 1638, 1593, 1569, 1537, 1462, 1429, 1347, 1292, 1257, 1202, 1102, 1067, 979, 934, 886, 797, 714. ESI-MS (*m/z*): 277.1 [M+H]$^{+}$, 294.0 [M+Na]$^{+}$, 574.9 [2M+Na]$^{+}$. Anal. Calcd. For $C_{14}H_{16}N_2O_4$: C, 60.86; H, 5.84; N, 10.14. Found: C, 60.46; H, 5.73; N, 10.12.

Ethyl 3-[(4-bromo-2,5-dimethyoxyphenyl)amino]-2-cyano-2-propenoate (144d)



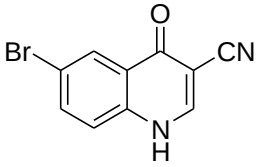
Ethyl 3-[(2,5-dimethoxyphenyl)amino]-2-cyano-2-propenoate (1.83 g, 6.62 mmol) was brominated using the general bromination method, yielding a bright yellow solid (2.15 g, 91%) mp: 207-210 °C. ^{1}H NMR (300 MHz) δ 10.95 (d, *J* = 13.8 Hz, 1H), 7.86 (d, *J* = 13.7 Hz, 1H), 7.14 (s, 1H), 6.70 (s, 1H), 4.30 (q, *J* = 7.1 Hz, 2H), 3.90 (s, 6H), 1.36 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz) δ 167.2, 150.9, 150.3, 143.2, 127.5, 118.2, 117.0, 99.4, 76.3, 61.4, 57.2, 56.8, 14.4. FT-IR (KBr, cm^{-1}): 3206, 3066, 2982, 2832, 2209, 1682, 1636, 1605, 1513, 1458, 1422, 1399, 1379, 1355, 1337, 1296, 1271, 1240, 1215, 1169, 1053, 1032, 987, 912, 849, 793, 727. ESI-

MS (*m/z*) 355.1 $[M+H]^+$, 357.1 $[M+2+H]^+$, 371.9 $[M+NH_4]^+$, 373.9 $[M+2+NH_4]^+$. Anal. Calc. for $C_{14}H_{15}N_2O_4Br$: C, 47.34, H, 4.26, N, 7.89. Found: C, 47.44, H, 4.14, N, 7.90.

3.9 General Procedure for the Cyclization of Quinolones

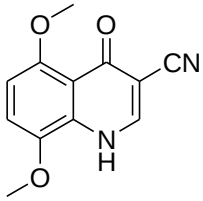
Meldrum's acid adduct or cyanoacetate derivative was added to diphenyl ether (0.13 M) at 250-260 °C and heated 2-6 hours. The product crystallized out of solution once cooled to 0 °C.

6-Bromo-3-cyano-4(*1H*)-quinolone (145b)



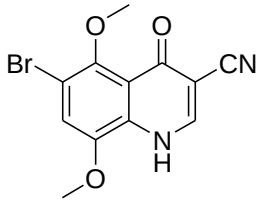
The product was isolated by filtration, yielding a tan powder (58 mg, 35%). mp: > 260 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.76 (s, 1H), 8.17 (s, 1H), 7.92 (d, J = 8.9 Hz, 1H), 7.58 (d, J = 8.9 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 173.8, 147.7, 138.7, 136.5, 127.7, 127.1, 122.4, 118.8, 117.0, 94.5. FT-IR (KBr, cm^{-1}): 3247, 3208, 3161, 3113, 3072, 3037, 2998, 2952, 2910, 2868, 2235, 1792, 1626, 1612, 1584, 1562, 1523, 1470, 1400. HRMS (ESI) Calcd. for $C_{10}H_5BrN_2O$ (*m/z*): 248.9658, 250.9638. Found: 248.9666 $[M+H]^+$, 250.9646 $[M+2+H]^+$. HRMS (ESI) Calcd. for $C_{10}H_5BrN_2O$+Na (*m/z*): 270.9477, 272.9458. Found: 270.9483 $[M+Na]^+$, 272.9465 $[M+2+Na]^+$.

3-Cyano-5,8-dimethoxy-4(*1H*)-quinolone (145c)



The product was isolated by filtration, yielding a tan powder (158 mg, 38%). mp: > 260 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.94 (br s, 1H), 8.30 (s, 1H), 7.30 (d, J = 8.9 Hz, 1H), 6.86 (d, J = 8.9 Hz, 1H), 3.92 (s, 3H), 3.76 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 174.3, 153.0, 145.4, 142.6, 131.9, 117.4, 116.5, 114.5, 107.2, 96.9, 57.1, 56.8. FTIR (KBr, cm^{-1}): 3180, 3044, 2223, 1714, 1681, 1637, 1593, 1569, 1537, 1462, 1428, 1346, 1291, 1256, 1201, 1101, 1066, 979, 934, 885, 796, 713. HRMS (ESI) Calc. for $C_{12}H_{10}N_2O_3$ (*m/z*): 231.0764. Found: 231.0775 $[M+H]^+$. HRMS (ESI) Calc. for $C_{12}H_{10}N_2O_3$+Na (*m/z*): 253.0584. Found: 253.0596 $[M+Na]^+$.

6-Bromo-3-cyano-5,8-dimethoxy-4(*1H*)-quinolone (145d)

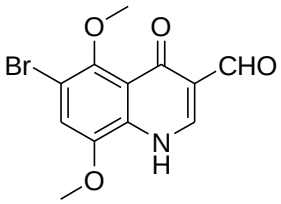


The product was isolated by filtration, yielding a tan powder. (130 mg, 75%). mp: > 260 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.24 (br s, 1H), 8.37 (s, 1H), 7.55 (s, 1H), 3.95, (s, 3H), 3.69 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.1, 148.8, 146.0, 145.9, 131.5, 121.1, 117.4, 117.0, 113.5, 97.1, 61.8, 57.6. FTIR (KBr, cm^{-1}): 3227, 3030, 2929, 2223, 1714, 1676, 1633, 1602, 1571, 1522, 1462, 1426, 1386, 1360, 1339, 1294, 1248, 1220, 1186, 1105, 1062, 980, 941, 913, 841, 804, 765, 725, 714. HRMS (ESI) Calcd. for $C_{12}H_9BrN_2O_3$ (*m/z*): 308.9869, 310.9850. Found: 308.9880 $[M+H]^+$, 310.9857 $[M+2+H]^+$.

3.10 General Procedure for the Reduction of 3-Cyanoquinolone Derivatives to 3-Formylquinolones

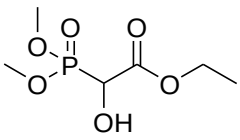
Sodium hypophosphite monohydrate (7.9 equivalents) in H_2O (0.5M), pyridine (2X vol), and acetic acid (1X vol) were added to 3-cyanoquinolones at 5 °C. The solution was warmed to room temperature, and Raney nickel (11.5 equivalents) was added. The reaction was stirred at room temperature or elevated temperatures for 6-24 hours. The cooled solution was filtered through Celite and concentrated. The resulting residue was dissolved in H_2O and filtered.

6-Bromo-5,8-dimethoxy-3-formyl-4(*1H*)-quinolone (146d)



Product was isolated by filtration, yielding a greenish-brown solid (39 mg, 39% at rt; 70 mg, 69% at 60 °C). mp > 260 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.08 (br s, 1H), 10.11, (s, 1H), 8.08 (s, 1H), 7.55 (s, 1H), 3.97 (s, 3H), 3.73 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) 189.4, 175.5, 149.3, 146.1, 141.9, 131.7, 123.5, 118.6, 117.3, 113.6, 61.8, 57.5. FT-IR (KBr, cm^{-1}): 2933, 1523, 1443, 1381, 1338, 1294, 1253, 1094, 1057, 977, 938, 916, 844, 805, 767, 717, 649, 621, 539. HRMS (ESI) Calcd. for $C_{12}H_{10}NO_4Br$ (*m/z*): 311.9866, 313.9846. Found: 311.9883 $[M+H]^+$, 313.9866 $[M+2+H]^+$.

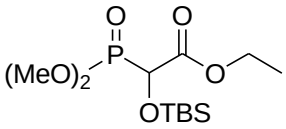
Ethyl (dimethyoxyphospinyl)hydroxy ethanoate (154)



Ethyl glyoxalate (20 g, 50% in toluene, 98 mmol), dimethyl phosphate (10.78 g, 98 mmol), and *p*-toluenesulfonic acid (0.046g, 0.27 mmol) were dissolved in benzene (75 mL) and heated to reflux for 5 hours. All

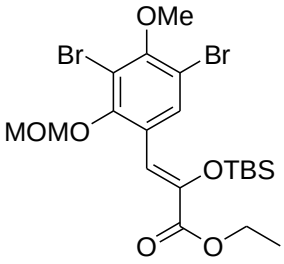
solvent was removed to give a light yellow oil. Product was purified by column chromatography (1:4:5, MeOH/Hex/EtOAc), yielding a white, waxy solid (14.46 g, 76%). mp: 44-46 °C. ^{1}H NMR (500 MHz) δ 4.56 (d, *J* = 16.0 Hz, 1H), 4.34-4.31 (m, 2H), 3.85-3.82 (m, 6H), 1.33-1.30 (m, 3H). ^{13}C NMR (75 MHz) δ 169.1, 69.7, 67.6, 62.7, 54.5, 54.4, 54.3, 54.2, 14.1. FT-IR (KBr, cm^{-1}): 3437, 3264, 2964, 2860, 1725, 1633, 1200, 1050. ESI-MS (*m/z*) 213.1 $[M+H]^+$, 230.0 $[M+NH_4]^+$, 424.9 $[2M+H]^+$, 441.7 $[2M+NH_4]^+$. HRMS (ESI): Calcd. for $C_6H_{13}O_6P$: 213.0523. Found: 213.0521 $[M+H]^+$.

Ethyl (2-(dimethoxyphosphinyl)-2-[[1,1-dimethylethyl)dimethylsilyl]oxy]ethanoate (155)



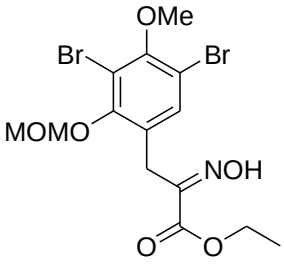
Ethyl (dimethyoxyphospinyl)hydroxy ethanoate (**154,** 5.16 g, 24.3 mmol) was dissolved in CH_2Cl_2 (50 mL) and mixture was cooled to 0 °C. Imidazole (2.23 g, 1.35 equivalents, 32.8 mmol) and TBDMSCl (4.40 g, 1.2 equivalents, 29.2 mmol) were added and the mixture was warmed to rt and stirred for 5 hours. The solvent was removed and the product was purified with column chromatography (1:1 EtOAc/Hex), yielding a light yellow oil. (7.78g, 98%) ^{1}H NMR (300 MHz) δ 4.55 (d, *J* = 17.9 Hz, 1H), 4.26-4.16 (m, 2H), 3.80-3.75 (m, 6H), 1.25 (t, *J* = 7.2 Hz, 3H), 0.87 (s, 9H), 0.06 (d, *J* = 3.1 Hz, 3H), 0.05 (s, 3H). ^{13}C NMR (75 MHz) δ 168.4, 71.7, 69.5, 61.8, 54.16, 54.10, 54.0, 25.6, 18.4, 14.1, -5.3, -5.5. FT-IR (neat, cm^{-1}): 3486, 2959, 2896, 2859, 2711, 1756, 1638, 1465. ESI-MS (*m/z*): 327.3 $[M+H]^+$, 349.3 $[M+Na]^+$, 674.8 $[2M+Na]^+$. Anal. Calcd. For $C_{12}H_{27}O_6PSi$: C, 44.16, H, 8.34. Found: C, 43.93, H, 8.23.

Ethyl 2-(*tert*-butyldimethylsilyloxy)-3-{3,5-dibromo-4-methoxy-2-(*O*-methoxymethylenoxy) phenyl}-prop-2-enoate (156)



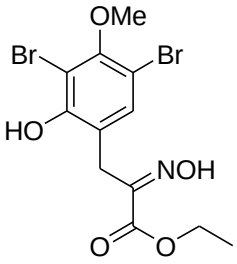
Freshly distilled diisopropylamine (1.29 mL, 9.2 mmol) was dissolved in 10 mL dry THF and cooled to -78 °C. A 0.5M *n*-butyl lithium solution (18.83 mL, 9.41 mmol) was added and the solution was stirred for 1 hour. The phosphonate (**155**, 2.0g, 6.12 mmol) in 10 mL THF was added over ten minutes and stirred 30 minutes. The aldehyde (**151**, 2.38g, 6.74 mmol) in 10 mL THF was added and the reaction was monitored by TLC. The reaction mixture was warmed to rt and quenched with 8 mL saturated NH_4Cl, diluted with 80 mL EtOAc, washed with saturated NH_4Cl (3 X 50 mL), H_2O (3 X 50 mL), dried (Na_2SO_4) and concentrated, yielding a red oil. The product was purified using column chromatography (9:1 hexanes/EtOAc), yielding a colorless oil. (3.08 g, 93%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (d, *J* = 0.7 Hz, 1H), 6.32 (d, *J* = 0.7 Hz, 1H), 5.03 (s, 2H), 4.11 (q, *J* = 7.0 Hz, 2H), 3.87 (s, 3H), 3.59 (s, 3H), 1.11 (t, *J* = 7.0 Hz, 3H), 0.97 (s, 9H), 0.22 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.4, 154.2, 152.7, 144.0, 133.0, 128.1, 114.2, 112.3, 99.8, 61.2, 60.7, 58.2, 25.6, 18.3, 13.8, -4.6. FT-IR (neat, cm^{-1}): 2931, 2898, 2858, 1724, 1632, 1578, 1529. ESI-MS (*m/z*): 575.3 $[M+Na]^+$, 577.2 $[M+2+Na]^+$, 579.1 $[M+4+Na]^+$. Anal. Calcd. For $C_{20}H_{30}O_6Br_2Si$: C, 43.33, H, 5.45. Found: C, 43.70, H, 5.38.

Ethyl 3-{3,5-Dibromo-4-methoxy-2-(*O*-methoxymethylenoxy)phenyl}-2(*E*)-(hydroxyimino) propanoate (157)



Silyl enol ether (**156**, 1.50 g, 2.71 mmol) was dissolved in 5 mL MeOH and Et_3N•HF (0.75 mL) was added, and a white solid precipitated from solution. The reaction was monitored by TLC (hexanes/EtOAc/CH_2Cl_2, 5:5:1, about 10 minutes). When complete, a white solid precipitated out of the solution. NH_2OH•HCl (1.1 equivalents, 0.23 g, 3.45 mmol) was added. When the reaction was complete, as monitored by TLC (Et_2O, overnight), the solvent was removed to give a white solid. The solid was dissolved in EtOAc (70 mL), washed with H_2O (2X50 mL), washed with saturated $NaHCO_3$ (50 mL), dried (Na_2SO_4) and concentrated to give a white solid, yielding a (1:0.25) mixture of two isomers. (1.02g, 83%). The product was purified by recrystallization from MeOH, yielding a white solid. (79 mg, 64%). mp: 123-124 ºC. ^{1}H NMR (300 MHz) δ 10.18 (br s, 1H), 7.21 (s, 1H), 5.14 (s, 2H), 4.28 (q, *J* = 7.8 Hz, 2H), 4.05 (s, 2H), 3.85 (s, 3H), 3.64 (s, 3H), 1.29 (t, *J* = 7.8 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 163.1, 154.0, 153.4, 150.4, 131.8, 128.5, 114.6, 113.2, 99.9, 62.3, 60.7, 58.2, 25.4, 14.1. FT-IR (KBr, cm^{-1}): 3316, 2938, 1727, 1466, 1405, 1375, 1328, 1301, 1204, 1168, 1148, 1128, 1096, 1057, 1015, 973, 936, 920, 784, 762, 725, 661, 521. Anal. Calc. for $C_{14}H_{17}Br_2NO_6$+Na (*m/z*): 431.9053, 433.9033, 435.9015. Found: 431.9077 $[M+Na]^+$, 433.9055 $[M+2+Na]^+$, 435.9043 $[M+4+Na]^+$.

Ethyl 3-(3,5-dibromo-2-hydroxy-4-methoxyphenyl)-2(*E*)-(hydroxyimino)propanoate (158)



Propenoate (**157**, 390 mg, 1.32 mmol) was dissolved in MeOH (mL). One to two crystals of TsOH were added and the mixture was stirred overnight. The solvent was removed to obtain the product as a white solid. mp. 127-128 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (s, 1H), 4.35, (q, *J* = 7.2 Hz, 2H), 3.91, (s, 2H), 3.85 (s, 3H), 1.36 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.2, 153.9, 151.8, 149.8, 133.7, 119.5, 107.8, 107.7, 63.0, 60.7, 25.6, 14.1. FT-IR (KBr, cm^{-1}): 3649, 3503, 3281 (br), 3054, 2986, 2942, 2305, 2254, 1726, 1692, 1597, 1549, 1467, 1435, 1302, 1266, 1204, 1157, 1114, 1057, 1021, 968, 909, 738. HRMS (ESI) Calcd. for $C_{12}H_{13}Br_2NO_5$+Na (*m/z*): 431.9053, 433.9033, 435.9015. Found: 431.9077 $[M+Na]^+$, 433.9055 $[M+2+Na]^+$, 435.9043 $[M+4+Na]^+$.

3.11 General Procedure for Kumada Coupling

The imidazole (1 eq) was dissolved in dry THF (0.5M) and treated with EtMgBr (1.3 eq, 3.0M in Et_2O). The quinolone (1.5 eq) and $Ni(dppe)_2$ (10 mol%) were added and the reaction was stirred at rt (or elevated temperatures) overnight. The solvent was removed and the resulting residue was dissolved in water, extracted with CH_2Cl_2, dried with Na_2SO_4 and concentrated. The crude material was purified by column chromatography.

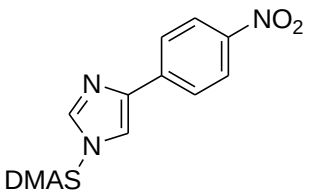
3.12 General Procedure for Stille Coupling

The imidazole stannane (1 eq), the quinolone (1.5 eq), $Pd_2(dba)_3$ (5 mol%), $AsPh_3$ (10 mol%) and CuI (10 mol%) were dissolved in dry THF (1.0M). The reaction was heated to reflux and stirred overnight. The reaction mixture was filtered through Celite and concentrated. The resulting residue was dissolved in water, extracted with CH_2Cl_2, dried with Na_2SO_4 and the solvent was removed. The resulting crude material was purified by column chromatography.

3.13 General Procedure for Suzuki Coupling

The imidazole (1 eq), $Pd_2(dba)_3$ (10 mol%), and P(*o*-tolyl)$_3$ (1 eq) were degassed in dry toluene (0.5 M) for 10 minutes. The boronic acid (2.1 eq) and Na_2CO_3 (2M aq solution, 2 eq) were added and the mixture was heated to 110 °C for 24 hours. The reaction mixture was filtered through Celite and the solvent was removed under reduced pressure. The resulting residue was dissolved in EtOAc, washed with water, washed with brine, dried (Na_2SO_4) and concentrated. The resulting crude material was purified by column chromatography.

1-*N*-Dimethylsulfonyl-4-(4-nitrophenyl)-(*1H*)-imidazole (178a)



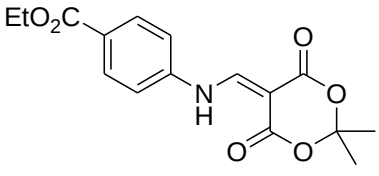
The product was purified using silica gel chromatography (1:9, EtOAc/Hexanes), yielding a dark reddish-brown oily solid (10 mg, 10%). mp 222-224 °C. ^{1}H NMR (300 MHz) δ 8.22 (d, *J* = 8.3 Hz, 2H), 7.96 (s, 1H), 7.89 (d, *J* = 8.6 Hz, 2H), 7.66 (s, 1H), 2.89 (s,

6H). ^{13}C NMR (125 MHz) δ 147.4, 141.2, 138.8, 137.5, 125.9, 124.3, 114.9, 38.4. HRMS (ESI) Calcd. for $C_{11}H_{12}N_4O_4S$ (*m/z*): 297.0652. Found: 297.0655 $[M+H]^+$. HRMS (ESI) Calcd. for $C_{11}H_{12}N_4O_4S$+Na (*m/z*): 319.0471. Found: 319.0478 $[M+Na]^+$.

3.14 General Procedure for Meldrum's Acid Adducts

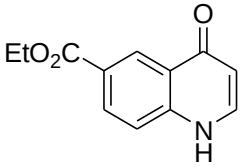
A 0.5 M solution of Meldrum's acid and methyl orthoformate was heated to reflux for two hours. The aniline derivative (0.7 equivalents) was added after cooling the mixture to room temperature and the resulting mixture was heated to reflux for an additional 6 hours. The mixture was cooled to 0 °C and the precipitate was collected by filtration.

5-[[(4-Carboxyethylphenyl)amino]methylene]-2,2-dimethyl-1,3-dioxane-4,6-dione (180)



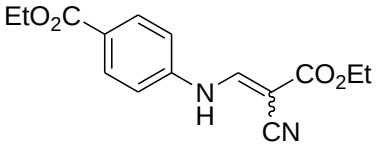
The product was made using General Pocedure 3.12. The product was isolated by filtration, yielding a light yellow crystal (4.68 g, 84%). ^{1}H NMR (300 MHz) δ 11.30 (d, *J* = 14.1 Hz, 1H), 8.69 (d, *J* = 14.1 Hz, 1 H), 8.10 (d, *J* = 8.8 Hz, 2H), 7.29 (d, *J* = 8.8 Hz, 2H), 4.38 (q, *J* = 7.2 Hz, 2H), 1.75 (s, 6H), 1.39 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (75 MHz) δ 165.5, 163.3, 152.1, 141.3, 131.8, 128.7, 117.4, 105.6, 88.7, 70.7, 61.4, 27.2, 14.4. FT-IR (KBr, cm^{-1}): 3213, 3073, 2979, 1943, 1679, 1605, 1582, 1460, 1415, 1381, 1267, 1107, 1013, 932, 862, 817, 767, 730. HRMS (ESI) Calcd. for $C_{16}H_{17}NO_6$+Na (*m/z*): 342.0948. Found: 342.0962 $[M+Na]^+$.

6-Carboxyethyl-4(*1H*)-quinolone (181)



The product was isolated by filtration, yielding an off-white solid (4.68 g, 84%). mp > 260 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.99 (br s, 1H), 8.66 (d, *J* = 2.0 Hz, 1H), 8.10 (dd, *J* = 8.9, 2.0 Hz, 1H), 7.93 (d, *J* = 7.2 Hz, 1H), 7.58 (d, *J* = 8.9 Hz, 1H), 6.08 (d, *J* = 7.2 Hz, 1H), 4.30 (q, *J* = 7.2 Hz, 2H), 1.31 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (300 MHz, DMSO-d_6) δ 177.4, 165.8, 143.4, 140.7, 131.9, 127.9, 125.5, 124.7, 119.4, 110.4, 61.4, 14.7. FT-IR (KBr, cm^{-1}): 3224, 3137, 3065, 2888, 2653, 2216, 1939, 1711, 1638, 1600, 1562, 1501, 1412, 1375, 1280, 1246, 1208, 1151, 1134, 1021, 916, 856, 810, 758, 740. HRMS (ESI) Calcd. for $C_{12}H_{11}NO_3$ (*m/z*): 218.0812. Found: 218.0820 $[M+H]^+$. HRMS (ESI) Calcd. for $C_{12}H_{11}NO_3$ (*m/z*): 240.0631. Found: 240.0636 $[M+Na]^+$.

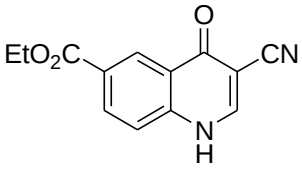
Ethyl 3-[(4-carboxyethylphenyl)amino]-2-cyano-2-propenoate (182)



Crude product was isolated as a 1:0.5 ratio of *E*/*Z* isomers, yielding a light yellow solid (0.50 g, 95%). mp: 172-176 °C. ^{1}H NMR (300 MHz) δ 10.87 (d, *J* = 12.7 Hz, 1H), 8.45 (d, *J* = 14.8 Hz, 0.5H), 8.20 (d, *J* = 14.8 Hz, 0.5H), 8.07 (d, *J* = 8.6 Hz, 3H), 7.92 (d, *J* = 13.4 Hz, 1H), 7.20 (d, *J* = 8.9 Hz, 1H), 7.13 (d, *J* = 8.6 Hz, 2H), 4.40-4.25 (m, overlapping signals, 6H), 1.45-1.30 (m, overlapping signals, 9H). ^{13}C NMR (75 MHz) δ 167.2, 165.7, 165.6, 164.2, 151.2, 151.0, 142.4, 141.8, 131.8, 131.7, 127.5, 127.2, 117.4, 116.5, 116.4, 115.3, 114.1, 78.9, 77.4, 61.6, 61.4, 61.3, 14.4, 14.3. FT-IR (KBr, cm^{-1}): 3221, 3082, 2986, 2212, 1714, 1637, 1606, 1518, 1473, 1445, 1411, 1369,

1247, 1182, 1107, 1021, 847, 786, 766. Anal. Calcd. for $C_{15}H_{16}N_2O_4$: C, 62.49; H, 5.59, N, 9.72. Found: C, 62.40; H, 5.41; N, 9.63. HRMS (ESI) Calcd. for $C_{15}H_{16}N_2O_4$ (*m/z*): 289.1183. Found: 289.1196 $[M+H]^+$. HRMS (ESI) Calcd. for $C_{15}H_{16}N_2O_4$+Na (*m/z*): 311.1002. Found: 311.1016 $[M+Na]^+$.

6-Carboxyethyl-3-cyano-4(*1H*)-quinolone (183)



The product was isolated by filtration, yielding a lt. brown solid (98 mg, 69%). mp: > 260 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.76 (s, 1H), 8.58 (d, *J* = 1.9 Hz, 1H), 8.16 (dd, *J* = 8.7, 1.9 Hz, 1H), 7.65 (d, *J* = 8.7 Hz, 1H), 4.30 (q, *J* = 7.0 Hz, 2H), 1.32 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.7, 165.3, 148.2, 142.4, 133.4, 127.4, 126.9, 125.0, 120.5, 116.8, 95.3, 61.7, 14.7. FT-IR (KBr, cm^{-1}): 2909, 2232, 1939, 1636, 1593, 1566, 1486, 1410, 1349, 1284, 1208, 1171, 1087, 1028, 1013, 932, 907, 846, 817, 792, 763, 750. HRMS (ESI) Calcd. for $C_{13}H_{10}N_2O_3$ (*m/z*): 243.0764. Found:243.0764 $[M+H]^+$. HRMS (ESI) Calcd. for $C_{13}H_{10}N_2O_3$+Na (*m/z*): 265.0584. Found: 265.0590 $[M+Na]^+$.

CHAPTER 4

BIOLOGICAL STUDIES INTRODUCTION

4.1 Growth of Mycobacteria

There are many factors to take into consideration when attempting to grow mycobacteria in the laboratory.[89] All mycobacteria multiply very slowly, even in enriched media. The age of the cells in any culture can vary greatly, and older cultures can be contaminated with a large percentage of dead cells. Quantitative bacteriological methods based on plating or dilution of living cells can be difficult to interpret.

The first media used to routinely grow mycobacteria was egg yolk constitutes, in which unheated sterile egg yolk was diluted in 10 volumes of water. Early studies of mycobacterial media showed the addition of asparagine, $Na_2HPO_4 \cdot 12H_2O$, KH_2PO_4, Na_3 citrate$\cdot 2H_2O$ and $MgSO_4 \cdot 7H_2O$ was beneficial. Asparagine or glutamine was used as an added nitrogen source, but it was also noted that enzymatic hydrolysates of casein could be used. Glucose or glycerol could also be used to increase the total cell growth, but they were not required for growth. The suggested pH range was 6.4-7.5, with no inhibition of growth seen in that range.

Phospholipids, which could be extracted from egg yolk, soy beans, or brain tissue, were used as early mycobacterial growth promoters. The phospholipids were laborious to prepare, had only a slight growth effect on several bacterial strains, and

increased the presence of large clumps of bacteria. Extended periods of standing would render the phospholipids inhibitory, and it was thought this was due to the possible formation of organic peroxides or hydrolysis of the free fatty acids.

Synthetic water-soluble lipids soon replaced phospholipids. The most successful were esters of long chain fatty acids, used primarily for detergency and wetting properties. The compounds include both lipophilic properties due to long aliphatic chains of lauric, palmitic, stearic, oleic, or ricinoleic acid. Hydrophillic properties were due to the polyhydric alcohols and ethylene oxide chains.

The esters of lauric and palmitic acid (G-2124, Tween 20 and Tween 40) proved to be inhibitory to the growth of mycobacteria. Esters of stearic acid (Tween 60) were varied. However, esters of oleic acid (G-2144, Tween 80) had a favorable effect, but the G-2144 formed precipitates when added to serum proteins. Tween 80 is a polyoxyalkylene derivative of sorbitan monooleate. It is a viscous liquid that is soluble in water and miscible with all other media components. Tween 80 can be autoclaved, but solutions stored at room temperature over several weeks can become inhibitory due to the possible release of free fatty acids or production of toxic peroxides. For these reasons, Tween 80 should only be added to freshly prepared media and used immediately.

Glucose or glycerol have been used as energy sources by tubercle bacilli, but they can give off toxic products when autoclaved in the presence of Tween. Therefore, the mixture of the two components becomes inhibitory. This effect is noticed especially

when using small inocula. If glucose or glycerol are to be used, they should only be added aseptically after the media is autoclaved.

Today, media can be commercially obtained that contains specific growth requirements especially tailored to the growth of mycobacteria. We have chosen to use the Middlebrook series.

4.2 Antibiotic Susceptibility Testing by Disk Diffusion

Antibiotic susceptibility testing was developed in the mid 1940's as scientists were discovering that the increasing number of available antibiotics had different inhibitory activity against different bacterial strains. Since the methods required isolation of the pure culture and subsequent susceptibility testing, the tests had a delay of 2 days from receipt of the specimen. Bondi[90] and coworkers developed an assay that was simpler and quicker, using saturated paper disks on blood agar plates, immediately after inoculation with the specimen. Measurement after overnight incubation could evaluate the susceptibility of an organism within 24 hours, regardless if the sample had one organism or several. The first testing used only two antibiotics: penicillin and streptomycin.

The streak method involved three subsequent parallel streaks on veal-infused agar with added tryptose and defibrinated horse blood. Disks made from Whatman filter paper were precut with a paper-punch and sterilized in a hot oven before being saturated with a solution of the known antibiotic. The disks were placed at intervals of 20 mm apart, on a line parallel to the initial streak. The group analyzed the susceptibility of 12 different organisms, including several Streptococci strains,

Staphylococci, Pneumococci, *E. coli*, and *H. influenza*. Organisms were determined very susceptible to penicillin if the zone of inhibition was greater than 20 mm, moderately susceptible with a zone of 10-20 mm, and resistant if the zone was less than 10 mm. The values for streptomycin were greater than 15 mm for very susceptible, 10-15 mm for moderately susceptible and less than 10 if resistant. The group also noticed that occasionally a colony of the bacteria would grow in the zone of inhibition of a susceptible organism. They considered these colonies resistant, and determined that the presence of these colonies should be taken into consideration when antibiotic treatment was being developed.

The diffusion of streptomycin was considered poor compared to penicillin, so greater concentrations were used in the streptomycin testing, and anaerobic conditions gave more consistent results. The stability of each antibiotic was also assessed, and it was determined that fresh solutions of streptomycin needed to be prepared weekly and stored at low temperatures, while solutions of penicillin could be stored for long periods of time at low temperatures with no loss of activity. The method was suggested to be used on antibiotics that were soluble in water, and diffused well on agar. Due to the insolubility, a third available antibiotic at the time, tyrothricin, was not tested using this method.

This method was not developed to replace the earlier, more sensitive methods, but it was developed to be used as a routine test to guide doctors in antibiotic treatment.

A standardized single disk method for rapidly growing bacteria was developed by Bauer et al. in 1965.[91] In this method, only one strength of antibiotic was tested at a

time. Bauer's approach was to inoculate the original culture in a tryptose phosphate or trypticase soy broth to produce a moderately cloudy suspension and use a cotton swab to evenly streak three planes of the dish. Large dishes were used to accommodate up to 12-13 disks per plate. The zones of inhibition were measured after overnight incubation. A series of 20 different antibiotics on commercially available preloaded disks were tested using this method, and bacteria were determined sensitive, intermediate, or resistant based on the size of the zone of inhibition (Table 4.1). The authors explained that the zones were unique for each antibiotic due to differences in disk-potency, and diffusion and solubility of the drugs on Mueller-Hinton medium. Therefore, the largest zone of inhibition does not clearly correlate to the most susceptible antibiotic. This method was not suggested to be used on slow-growing bacteria, any organism that required greater than 24 hours to incubate.

Table 4.1
Zone Sizes and Interpretation for Chemotherapy

Antibiotic	Disk Potency	Inhibition Zone Diameter (mm)		
		Resistant	Intermediate	Sensitive
Ampicillin				
S. aureua	10 μg	< 20	21-28	> 29
other organisms	10 μg	< 11	12-13	> 14
Bacitrancin	10 units	< 8	9-12	> 13
Cephalothin	30 μg	< 14	15-17	> 18
Chloramphenicol	30 μg	< 12	13-17	> 18
Colistin	10 μg	< 8	9-10	> 11
Erythromycin	15 μg	< 13	14-17	> 18
Kanamycin	30 μg	< 13	14-17	> 18
Lincomycin	2 μg	-	-	> 17
Methicillin	5 μg	< 9	10-13	> 14
Nalidixic acid	30 μg	< 13	14-18	> 19
Neomycin	30 μg	< 12	13-16	> 17
Nitrofurantoin	300 μg	< 14	15-16	> 17
Novobiocin	30 μg	< 17	18-21	> 22
Oleandomycin	15 μg	< 11	12-16	> 17
Penicillin-G	10 units	< 20	21-28	> 29
Polymyxin B	300 units	< 8	9-11	> 12
Streptomycin	10 μg	< 11	12-14	> 15
Sulfonamides	300 μg	< 12	13-16	> 17
Tetracycline	30 μg	< 14	15-18	> 19
Vancomycin	30 μg	< 9	10-11	> 12

In the late 1970's, two groups studied the correlation between disk diffusion and minimal inhibitory concentration (MIC). Welch and Kelly[92] tested strains of *M. fortuitum* and *M. chelonei*, both considered rapidly-growing mycobacteria, with several antibiotics (Table 4.2) on Mueller-Hinton agar plates. They plotted the MICs vs zone of inhibition to determine if the simpler disk diffusion method was an accurate screening

technique (Table 4.3). They determined that disk diffusion results could predict *in vitro* susceptibility of certain antimicrobial agents.

Table 4.2
Zone of Inhibition for *M. fortuitum* and *M. cheloni*

Antimicrobial agent	Organism	Range (mm)	Mean (mm)	Organism	Range (mm)	Mean (mm)
Chloramphenicol	*M. fortuitum*	6-36	12	*M. cheloni*	6-22	12
Tetracycline		11-46	23		6-41	20
Kanamycin		24-39	31		27-38	31
Polymyxin B		13-19	16		6-7	6
Gentamicin		16-32	22		12-21	16
Amikacin		30-44	37		11-32	21
Tobramycin		10-26	16		14-29	19

Table 4.3
MIC Values for *M. fortuitum* and *M. cheloni*

Antimicrobial agent	% of strains inhibited by antimicrobial agent concentration (μg/mL)									
	0.25	0.5	1	2	4	8	16	32	64	132
M. fortuitum										
Amikacin	29	94	100	100	100	100	100	100	100	100
Tetracycline	0	0	24	35	47	76	100	100	100	100
Chloramphenicol	0	0	0	10	20	20	50	70	90	90
Gentamicin	0	10	20	50	70	90	100	100	100	100
M. cheloni										
Amikacin	0	0	14	43	43	57	86	100	100	100
Tetracycline	0	0	29	29	29	29	29	43	86	100
Chloramphenicol	0	0	0	0	0	0	0	100	100	100
Gentamicin	0	0	0	0	67	100	100	100	100	100

Later that same year, Wallace and coworkers[93] also used disk diffusion to test susceptibility of *M. fortuitum* and *M. chelonei*, in order to determine whether disk diffusion was an acceptable method for predicting susceptibility. The results mirrored those found by Welch and Kelly. However, they did discover that *M. chelonei* grew

better on 7H10 agar, and concluded that it was a better medium for testing the faster growing mycobacterial species.

Cynamon and Patapow[94] compared the in vitro susceptibility of *M. fortuitum* to cefoxitin by agar dilution, broth dilution and disk diffusion. Although it was known that most mycobacteria were resistant to β-lactam antibiotics due to inherent β-lactamase activity, it was also noted that cefoxitin had increased resistanace to β-lactamases over other cephalosporins. Of 13 isolated starins, 11 had zones of inhibition greater than 18 mm (Table 4.4). It was determined that strains with zones of inhibition greater than 14 mm were susceptible to cefoxitin. They also found a correlation between disk diffusion results and both broth and agar dilution methods.

Table 4.4
Antibiotic Susceptibility of cefoxitin against *M. fortuitum*

Strain	Disk diffusion zone (mm)	Broth dilution MIC (μg/mL)	Agar dilution MIC (μg/mL)
1260	22	12.5	12.5
1261	19	12.5	25
1263	19	25	25
1045	12	12.5	25
1047	21	12.5	12.5
1048	22	25	12.5
1049	18	12.5	25
1059	11	12.5	50
5-4	20	12.5	25
A05	19	12.5	25
914-8	30	6.25	6.25
1004-8	21	12.5	12.5
1030-8	23	6.25	12.5
S. aureus	29	1.56	3.13

Although the disk diffusion method is quite old, it is still being used today as a routine method for quick and efficient antibiotic susceptibility testing of mycobacteria. Mycobacterial diseases have never been treated with either penicillin-type or cephalosporin-type β-lactam antibiotics because the organisms are intrinsically resistant to these antibiotics. The main resistance mechanism results from the production of β-lactamases. However, a combination of β-lactam antibiotics and β-lactamase inhibitors was a possible treatment method for resistant strains of mycobacteria. Pavelka and coworkers[95] used a disk diffusion method to test the antibiotic susceptibility of *M. tuberculosis* and *M. smegmatis* mutants that had their β-lactamase activity deleted by allelic exchange.

The disk diffusion method was chosen for initial screening due to ease of use and the commercial availability of preloaded antibiotic disks. The group tested a wild-type strain of *M. smegmatis* (Table 4.5) and *M. tuberculosis* (Table 4.6), plus three mutants of each in which the β-lactamase had been deleted. The disk diffusion testing showed an overall increase in the susceptibility of the mutant strains over the wild-type, with a more pronounced difference seen in the penicillin-type antibiotics.

Table 4.5
Antibiotic Susceptibility for *M. smegmatis* and Mutant Strains

	Zone of Inhibition (mm) for strain:			
Antibiotic	PM274	PM759	PM791	PM876
Oxacillin (10 μg)	0	0	0	0
Ampicillin (10 μg)	0	17	0	17
Ampicillin (100 μg)	0	43	0	43
Piperacillin (100 μg)	0	14	0	11
Mezlocillin (75 μg)	0	12	0	10
Carbenicillin (100 μg)	0	13	0	14
Amoxicillin (20 μg)	0	24	0	22
Amoxicillin/clavulanic acid (20 μg/10 μg)	22	27	21	23
Cefoxitin (30 μg)	0	11	0	11
Cefoxitin (100 μ)	22	20	22	23
Ceftriaxone (30 μg)	0	0	0	0
Cefixime (5 μg)	0	0	0	0
Imipenem (10μg)	23	25	27	26

Table 4.6
Antibiotic Susceptibility for *M. tuberculosis* and Mutant Strains

	Zone of Inhibition (mm) for strain:			
Antibiotic	H37Rv	PM638	PM669	PM670
Oxacillin (10 μg)	0	0	0	0
Ampicillin (10 μg)	0	25	0	25
Piperacillin (100 μg)	0	40	0	15
Mezlocillin (75 μg)	0	55	0	30
Carbenicillin (100 μg)	0	60	0	50
Amoxicillin (20 μg)	0	45	0	45
Amoxicillin/clavulanic acid (20 μg/10 μg)	15	60	20	40
Cefoxitin (30 μg)	0	20	0	0
Ceftriaxone (30 μg)	15	20	20	10
Cefixime (5 μg)	0	0	0	0
Imipenem (10μg)	25	45	25	25

4.3 *Mycobacterium smegmatis* strain mc^2155

Although mycobacteria diseases had been known for many years, there was a basic lack of genetic knowledge collected due to the slow growth rates, clumping tendencies, and inefficient gene-transfer systems. In 1990, the Jacobs group developed the *M. smegmatis* strain mc^2155 as a cloning host due to its' efficient transformation levels; 10^4 to 10^5 times greater than the parent strain.[96] *M. smegmatis* was chosen because it is fast growing and non-pathogenic.

They first attempted to optimize the efficiency of wild-type *M. smegmatis* strain mc^26 by varying electoporation parameters. When this failed, they hypothesized that the wild-type had a mechanism that inhibited plasmid transformation. They isolated strains of mc^26 that had spontaneously lost a resident plasmid and tested them for ability to be transformed by plasmid DNA. Isolated strains were transformed with a shuttle plasmid, pYUB12 and three kanamycin-sensitive colonies were isolated and named mc^2155, mc^2230 and mc^2235. When tested with numerous transformation plasmids, mc^2155 proved to be the most efficient. This strain is now routinely used for testing in most biochemical laboratories, and is the general approach we have used to test the biological activity of our compounds against *M. smegmatis*.

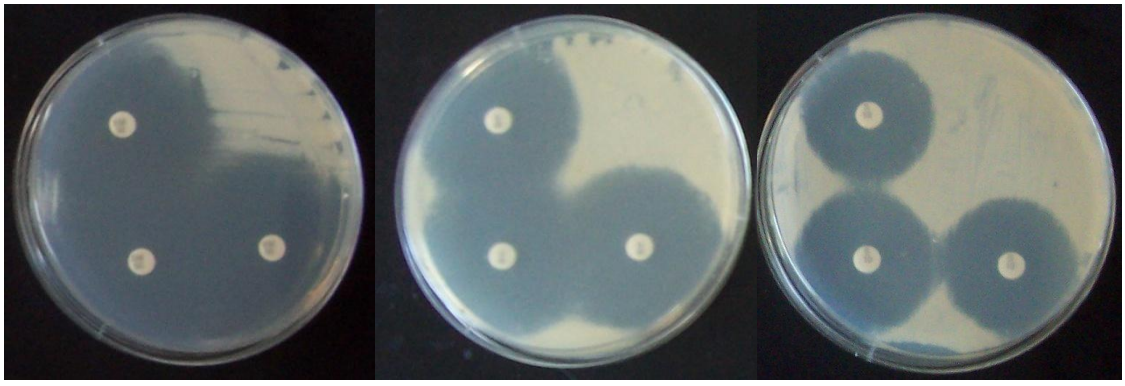
CHAPTER 5

RESULTS AND DISCUSSION – BIOLOGICAL SCREENING

5.1 Model Disk Diffusion Testing with Known Antibiotics

The disk diffusion method was used to determine the zones of inhibition for 5 known antibiotics that have activity against *M. smegmatis* strain mc^2155. Six antibiotic disks were purchased for these tests: Ethambutol (50 μg), Streptomycin (50 μg), Streptomycin (10 μg), Rifampin (5 μg), Isonazid (5 μg) and Isonazid (1 μg).

The broad spectrum antibiotics, ethambutol and streptomycin, had the largest zones of inhibition, as seen in Figure 5.1. Plates were measured after 48 hours, and then again after 96 hours to determine the zones of inhibition.



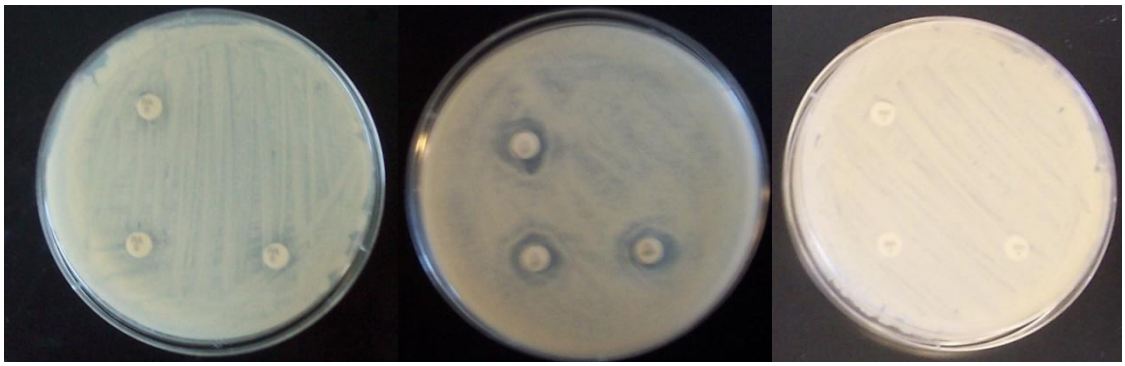
Ethambutol 50 μg	Streptomycin 50 μg	Streptomycin 10 μg

Figure 5.1 Disk Diffusion Results of Broad Spectrum Antibiotics:
Ethambutol and Streptomycin

Table 5.1
Zones of Inhibition: Ethambutol and Streptomycin

Antibiotic	Zone of Inhibition at 48 hr (mm)	Zone of inhibition at 96 hr (mm)
Ethambutol (50 μg)	48 ± 1	50 ± 1
Streptomycin (50 μg)	44 ± 2	44 ± 2
Streptomycin (10 μg)	36 ± 2	36 ± 1

Ethambutol was the most active antibiotic, with a zone = (50 ± 1) mm after 96 hours. Two strengths of streptomycin were tested. The 50 μg strength was more active than the 10 μg disks; (44 ± 2) compared to (36 ± 1) mm at 96 hours. The zones of inhibition for these antibiotics were very large, and often ran into each other, making precise measurement difficult.



Rifampin 5 μg	Isonazid 5 μg	Isonazid 1 μg

Figure 5.2 Disk Diffusion Results of Specific Antimycobacterial Agents:
Rifampin and Isonazid

Table 5.2
Zones of Inhibition: Rifampin and Isonazid

Antibiotic	Zone of Inhibition at 48 hr (mm)	Zone of inhibition at 96 hr (mm)
Rifampin (5 μg)	12 ± 2	12 ± 1
Isonazid (5 μg)	14 ± 1	14 ± 1
Isonazid (1 μg)	No activity	No activity

The zones of inhibition with rifampin and isonazid did not appear as clear as the zones for the broad spectrum antibitoics. Prevous literature reports measured the faded areas around the disks. For rifapmin, there was a faded zone = (12 ± 2) mm. The 5 μg strength of Isonazid had a similar zone = (14 ± 1) mm. The 1 μg strength had no noticeable zone.

A direct comparison of the specific antibiotics to the broad spectrum antibiotics is difficlut, since the commercially available disks varied in strength. However, we can see large, clear zones of inhibiton for the broad spectrum antibiotics, where the specific drugs had only faded areas of growth around the disks. Another problem with this type of testing is that it is dependent on solubility of the antibiotics and their diffusion rate. These parameters cannot easily be tested with paper disks.

5.2 Disk Diffusion Testing of Synthetic Products

5.2.1. Preparation of Disks

To prepare disks for antibiotic susceptibility testing, a 10M solution was made by dissolving 4-6 mg of each compound in 0.4-0.6 mL MeOH. For disk strength of 50 μg, 5 μL of solution was added to blank disks, and dried overnight.

5.2.2 Results of Preliminary Testing

For a negative control test 5 μL of MeOH were loaded onto paper disks and dried overnight. The disks were placed on a petri dish pre-swabbed with the bacterial growth broth and incubated for 48 hours. The absence of a zone of inhibition verifies that the zones measured from each individual sample were from the activity of the target compounds, and not the solvent used to dissolve them.

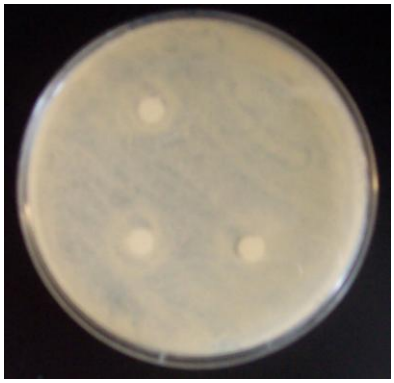
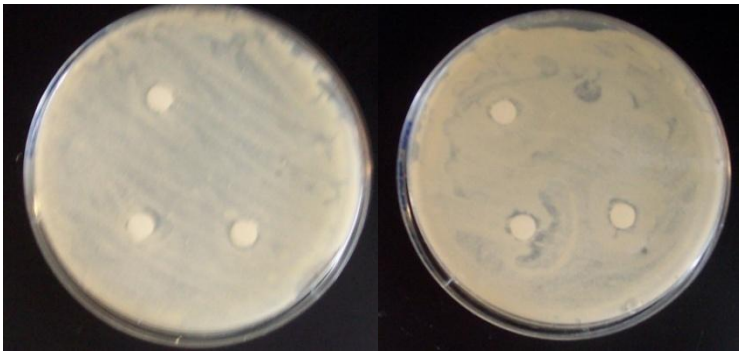
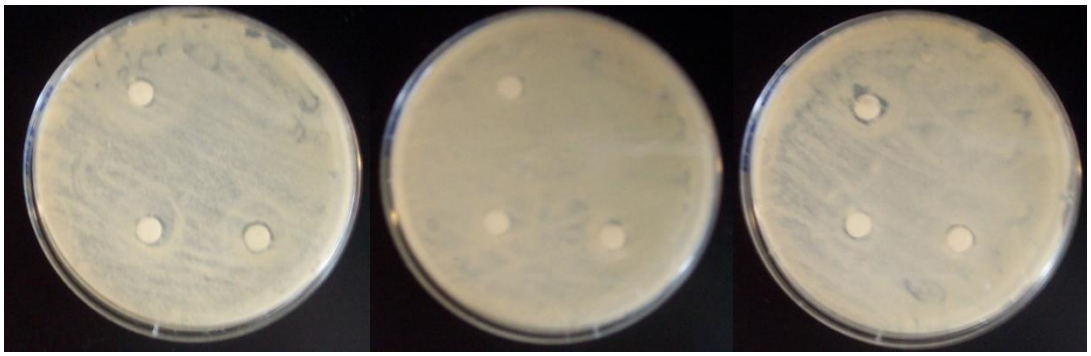


Figure 5.3 Negative Control Disks

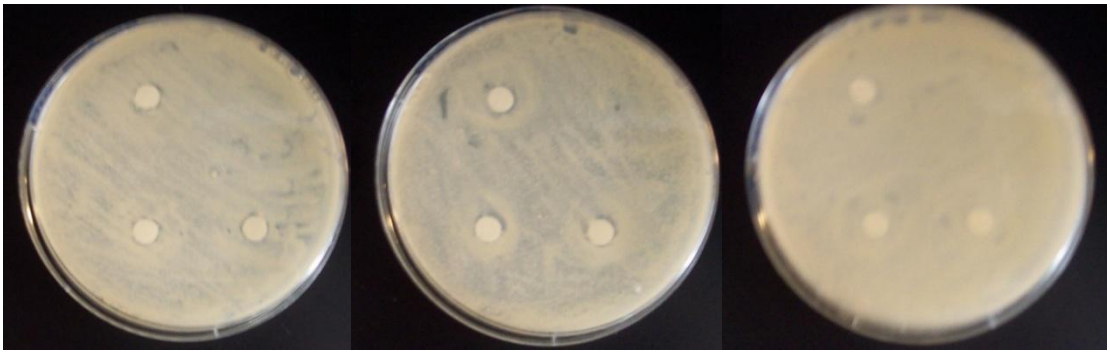
Nine samples were tested in the first round of antibiotic susceptibility testing. For each run, three paper disks were used on one agar plate. The plates were incubated at 37 °C, and measurements were taken at 24 hr and 48 hr. Seven of the samples were quinolone derivatives, and the remaining two were amides that were synthsized from another project.



QMA_001a, 50 µg Zone = (9 ± 1) mm	QMA_001a, 150 µg Zone = (8 ± 1) mm



QMA_002b, 50 µg Zone = (9 ± 1) mm	QMA_002c, 50 µg Zone = (0) mm	QMA_007a, 50 µg Zone = (8 ± 2) mm



QCN_001a, 50 µ Zone = (8 ± 1) mm	QCN_002a, 50 µg Zone = (9 ± 1) mm	QCN_003a, 50 µ Zone = (0) mm

Figure 5.4 Disk Diffusion Results of Quinolone Derivatives

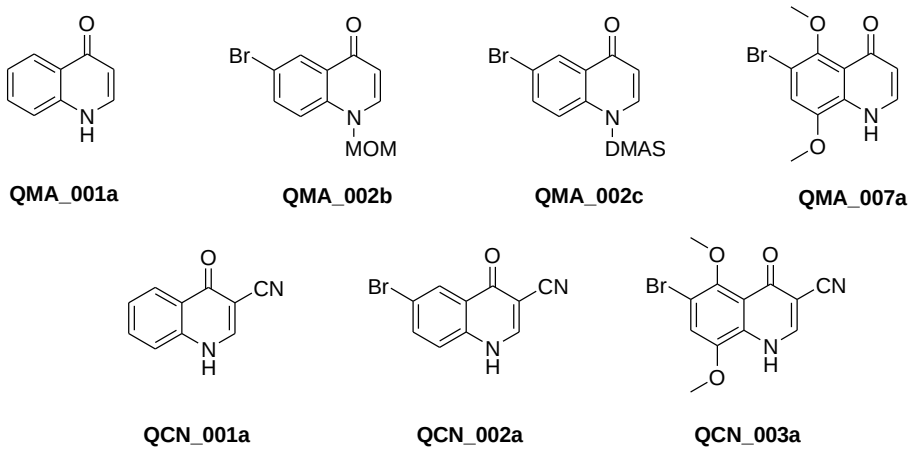
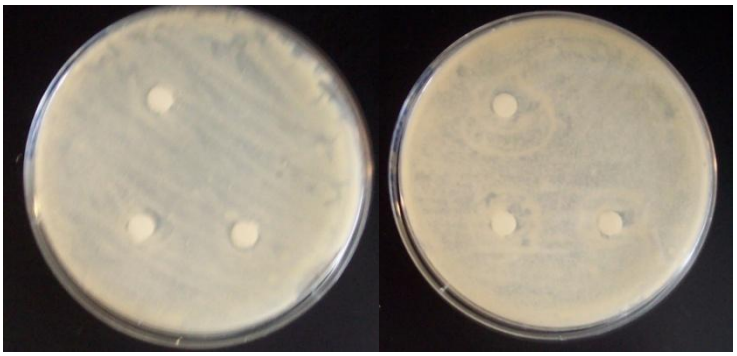


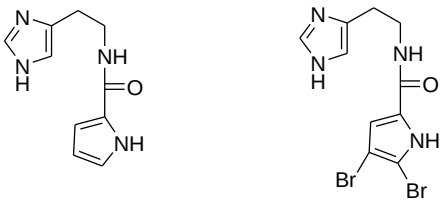
Figure 5.5 Quinolone Derivatives

The majority of the quinolone derivatives, with the exception of QMA_002c and QCN_003a, had zones of inhibition of (8-9) mm. The results suggest that a quinolone skeleton does indeed have a modest inhibitory effect on the growth of *M. smegmatis*. Slight errors in the loading of the samples onto the disks may correspond to less activity on the plates. The other possibility is the solubility of some of the samples may decrease the diffusion of the drug from the disk to the agar. Further studies on these compounds are planned.



AMA_001a, 50 μg Zone = (8 ± 1) mm	AMA_002a, 50 μg Zone = (0) mm

Figure 5.6 Disk Diffusion Results of Amide Derivatives



AMA_001a **AMA_002a**

Figure 5.7 Amide Derivatives

Surprisingly, the two amides tested gave very different zones of inhibition, even though they only differed structurally by the extent of bromination. Since the MCA inhibitor is a competitive inhibitor of amide cleavage, it was hypothesized that these compounds, although structurally different than the target molecule, would exhibit inhibitory activity.

Several of the truncated analogs and amides tested exhibited biological activity comparable to the specific antimycobacterial agents isonazid and rifampin. The smaller zones of inhibition were characteristically similar to the fading seen in those types of

drugs, and unlike the large zones seen with the broad spectrum antibiotics streptomycin and ethambutol. Since it is hypothesized that these drug targets may be potential co-drugs that could be administered to aid in the efficacy of the front line anti-tuberculosis drugs, the additive effect of the two types of drugs should be tested.

CHAPTER 6

BIOLOGICAL – MATERIALS AND METHODS

6.1 General

Mycobacterium smegmatis strain mc^2155 was purchased from ATCC (American Type Culture Collection) and stored at -70 °C. Middlebrook 7H9 Broth and 7H10 Agar were purchased from VWR and prepared according to product literature. All bacterial cultures were grown in either Middlebrook 7H9 broth or 7H10 media on 4 inch petri dishes. Preloaded antibiotic disks and blank disks were purchased from VWR and used as directed. All cell growth was carried out at 37 °C. Cell cultures were autoclaved at 121 °C before disposal. All handling of cell cultures were done using Class 2 Biological Safety procedures.

6.2 Preparation of Middlebrook Media

6.2.1 Preparation of Middlebrook 7H9 Broth (BD 271310)

4.7 g of dehydrated Middlebrook 7H9 broth was dissolved in 900 mL deionized water. 2.0 mL glycerol was added and the solution was stirred until all solid dissolved. The broth was autoclaved at 121 °C for 10 minutes. 100 mL of Middlebrook ADC Enrichment was added aseptically.

6.2.2 Preparation of Middlebrook 7H10 Agar (BD 262710)

19g of dehydrated Middlebrook 7H10 medium was dissolved in 900 mL deionized water. 5.0 mL glycerol was added and the solution was heated to a boil to dissolve all solids. The media was autoclaved at 121 °C for 10 minutes. The media was cooled to 50-55 °C and 100 mL of Middlebrook OADC Enrichment was added aseptically.

6.3 Propagation of *Mycobacterium smegmatis*

Procedure was adapted from ATCC Product Information Sheet

The bacterium was streaked on a Middlebrook 7H10 agar plate. Colonies were small, irregular, raised and a light yellow in color, which darkened upon aging. Plates were incubated aerobically at 37 °C for 5-7 days. Single colonies were picked and grown in Middlebrook 7H9 broth for 48 hours.

6.4 Disk Diffusion Testing

6.4.1 Disk Diffusion Testing with Preloaded Disks

Petri dishes were prefilled with Middlebrook 7H10 agar. The dishes streaked with *M. smegmatis* broth using a cotton swab. The dishes were separated into 4 zones and one preloaded antibiotic disk was placed in the center of three zones. The final zone was left as a cell growth blank. Plates were incubated at 37 °C for 24-72 hours. Zones of inhibition were measured at predetermined time intervals.

6.4.2 Disk Diffusion Testing with Blank Disks

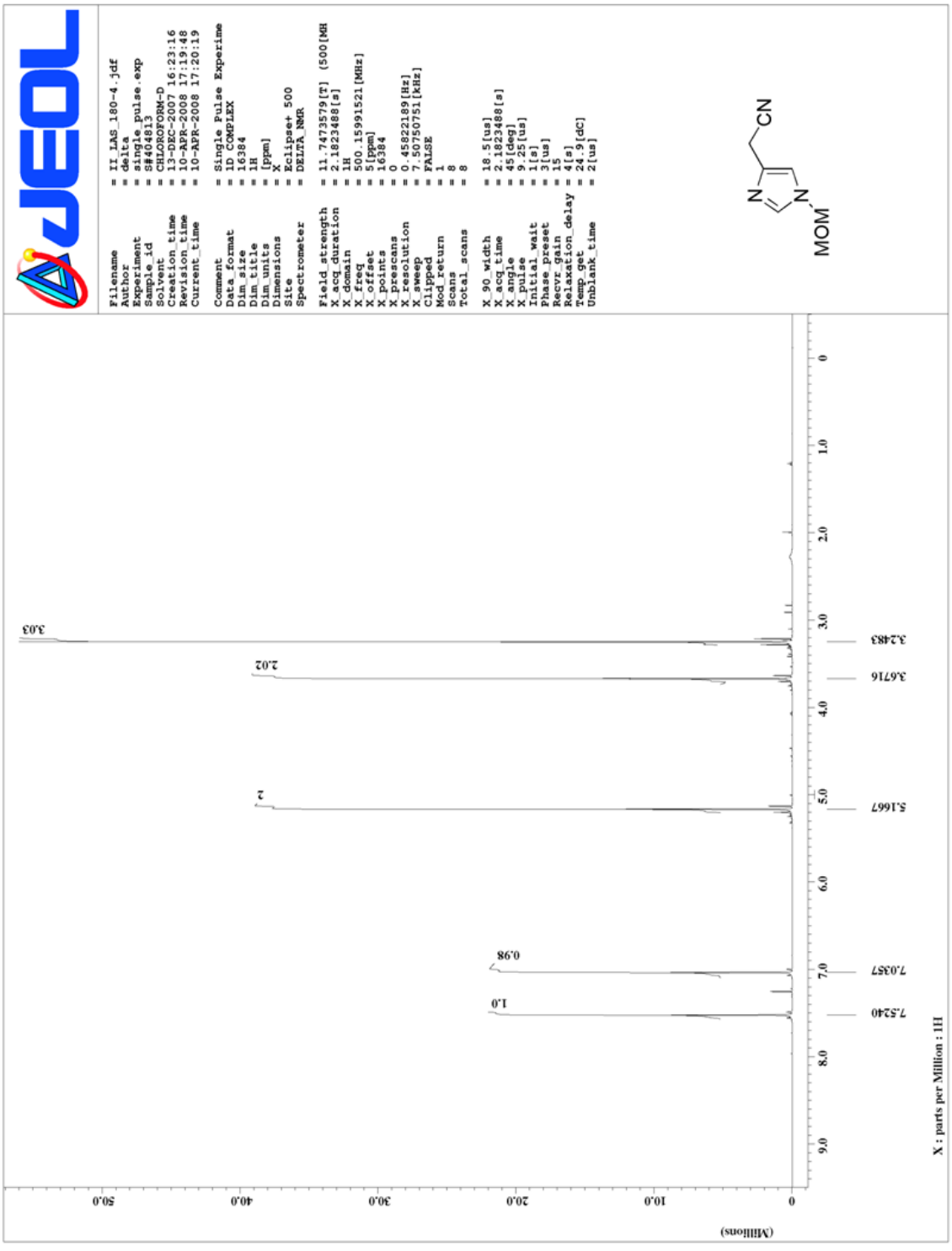
The blank disks were loaded with a predetermined volume of a stock solution made by dissolving each synthetic antibiotic in either MeOH. Petri dishes were

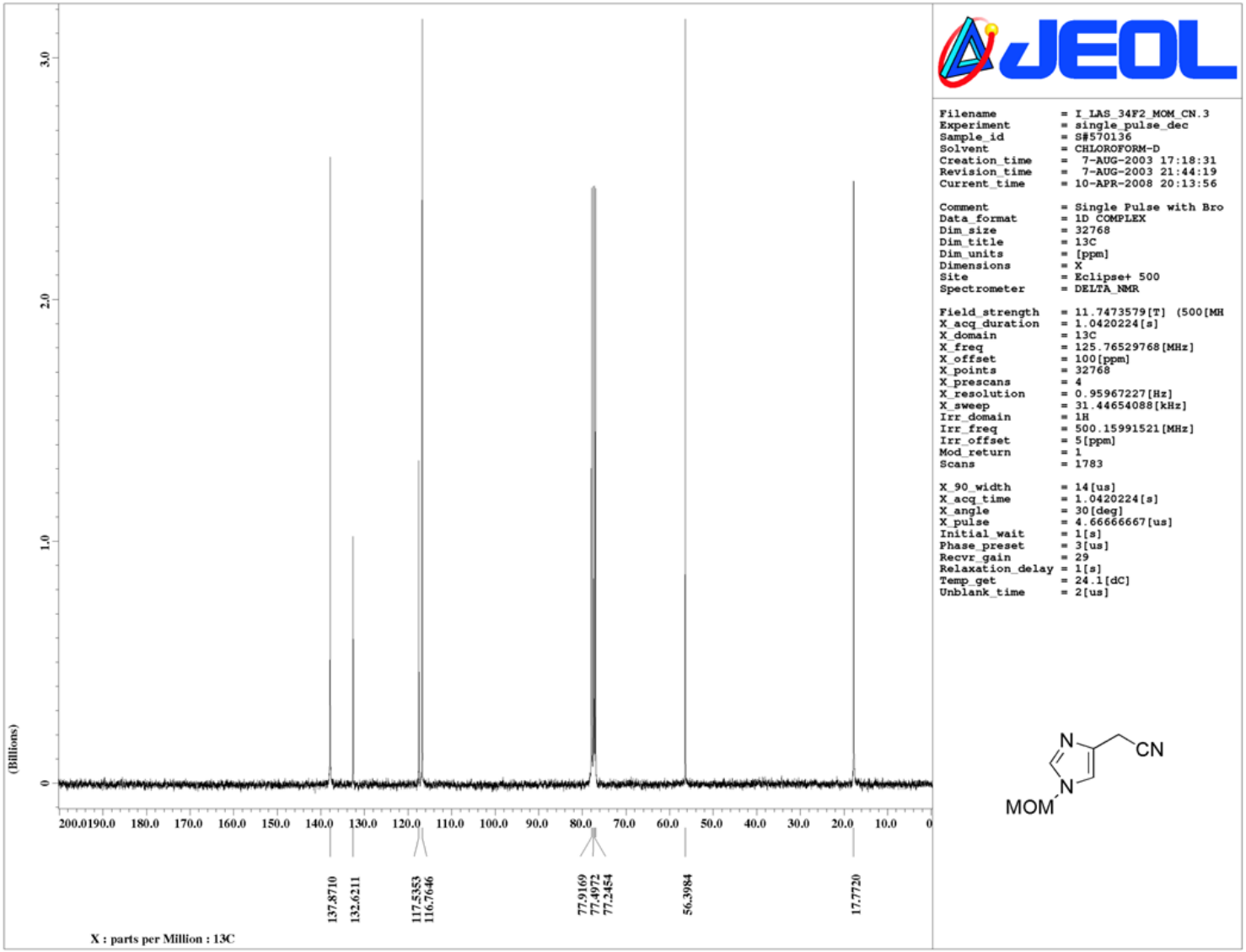
prefilled with Middlebrook 7H10 agar. The dishes streaked with *M. smegmatis* broth using a cotton swab. The dishes were separated into 4 zones and one preloaded antibiotic disk was placed in the center of three zones. The final zone was left as a cell growth blank. Plates were incubated at 37 °C for 24-72 hours. Zones of inhibition were measured at predetermined time intervals.

APPENDIX A

^{1}H AND ^{13}C NMR SPECTRA OF

1-METHOXYMETHYL-4-CYANOMETHYL-(*1H*)-IMIDAZOLE (114a)

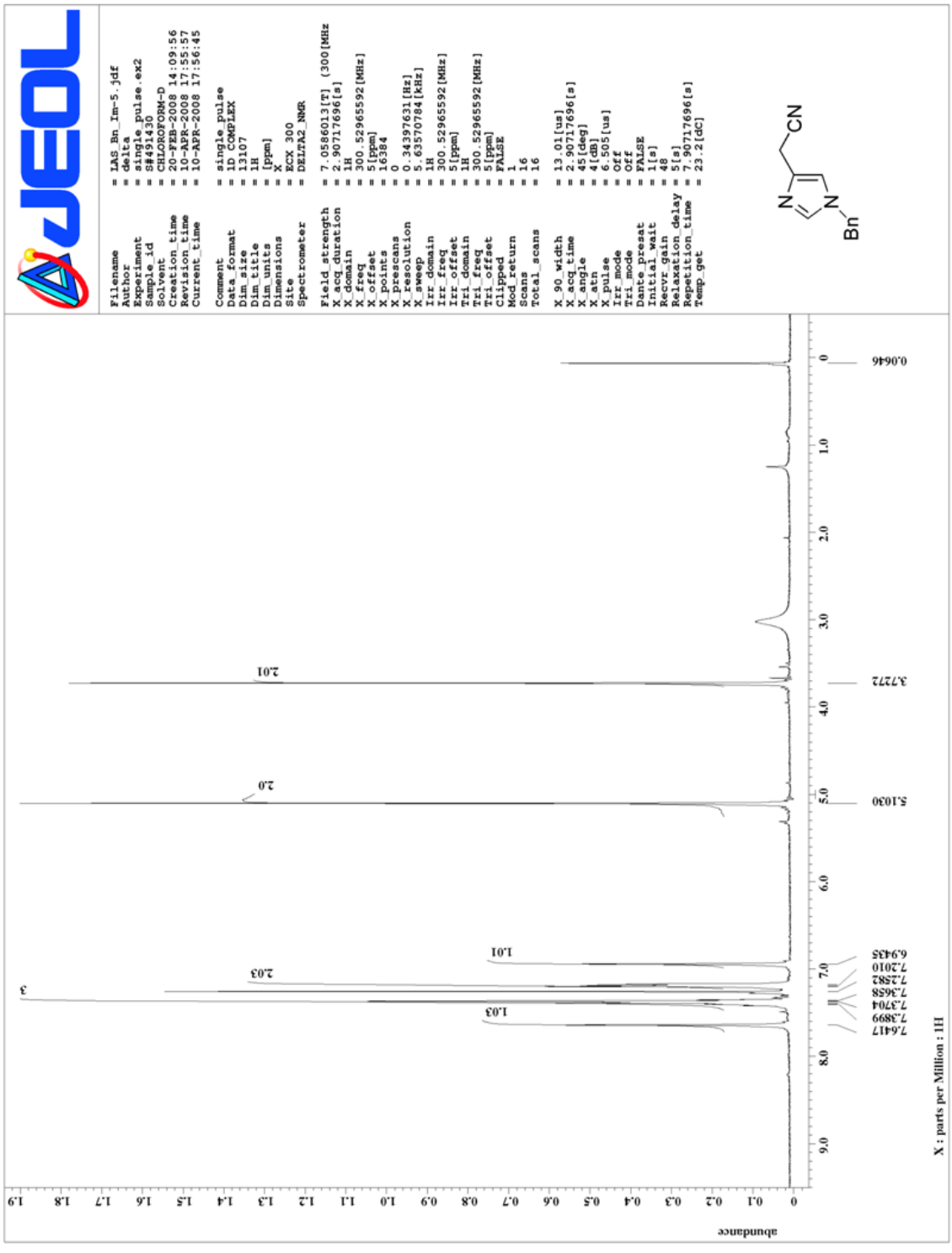

JEOL
Filename = II_IAS_180-4.jdf
Author = delta
Experiment = single_pulse.exp
Sample_id = SM40483
Solvent = CHLOROFORM-D
Creation_time = 13-DEC-2007 16:23:16
Revision_time = 10-APR-2008 17:19:48
Current_time = 10-APR-2008 17:20:19
Comment = Single Pulse Experime
Data_format = 1D COMPLEX
Dim_size = 16384
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = Eclipse+ 500
Spectrometer = DELTA_NMR
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 2.1823488[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.45822189[Hz]
X_sweep = 7.50750751[kHz]
Clipped = FALSE
Mod_return = 1
Scans = 8
Total_scans = 8
X_90_width = 18.5[us]
X_acq_time = 2.1823488[s]
X_angle = 45[deg]
X_pulse = 9.25[us]
Initial_wait = 1[s]
Phase_preset = 3[us]
Recvr_gain = 15
Relaxation_delay = 4[s]
Temp_get = 24.9[dC]
Unblank_time = 2[us]
CN
N
N
MOM
3.03
2.02
2
0.98
1.0
3.2483
3.6716
5.1667
7.0357
7.5240
(Millions)
X : parts per Million : 1H

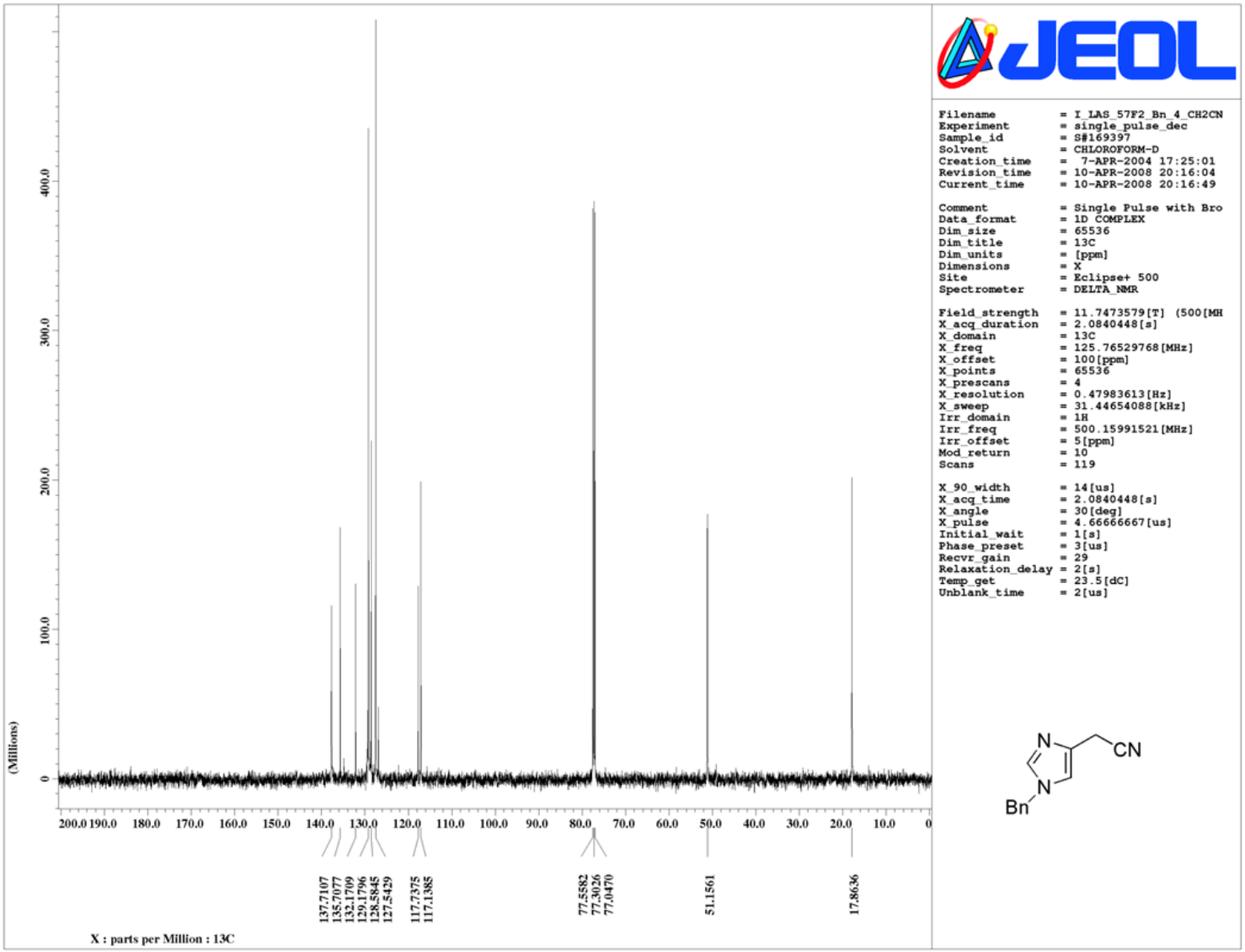

(Billions)
3.0
2.0
1.0
0
200.0190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0
137.8710
132.6211
117.5353
116.7646
77.9169
77.4972
77.2454
56.3984
17.7720
X : parts per Million : 13C
Filename = I_LAS_34F2_MOM_CN.3
Experiment = single_pulse_dec
Sample_id = S#570136
Solvent = CHLOROFORM-D
Creation_time = 7-AUG-2003 17:18:31
Revision_time = 7-AUG-2003 21:44:19
Current_time = 10-APR-2008 20:13:56
Comment = Single Pulse with Bro
Data_format = 1D COMPLEX
Dim_size = 32768
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = Eclipse+ 500
Spectrometer = DELTA_NMR
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 1.0420224[s]
X_domain = 13C
X_freq = 125.76529768[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.95967227[Hz]
X_sweep = 31.44654088[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5[ppm]
Mod_return = 1
Scans = 1783
X_90_width = 14[us]
X_acq_time = 1.0420224[s]
X_angle = 30[deg]
X_pulse = 4.66666667[us]
Initial_wait = 1[s]
Phase_preset = 3[us]
Recvr_gain = 29
Relaxation_delay = 1[s]
Temp_get = 24.1[dC]
Unblank_time = 2[us]
N
N
CN
MOM

APPENDIX B

^{1}H AND ^{13}C NMR SPECTRA OF

1-BENZYL-4-CYANOMETHYL-(*1H*)-IMIDAZOLE (114b)

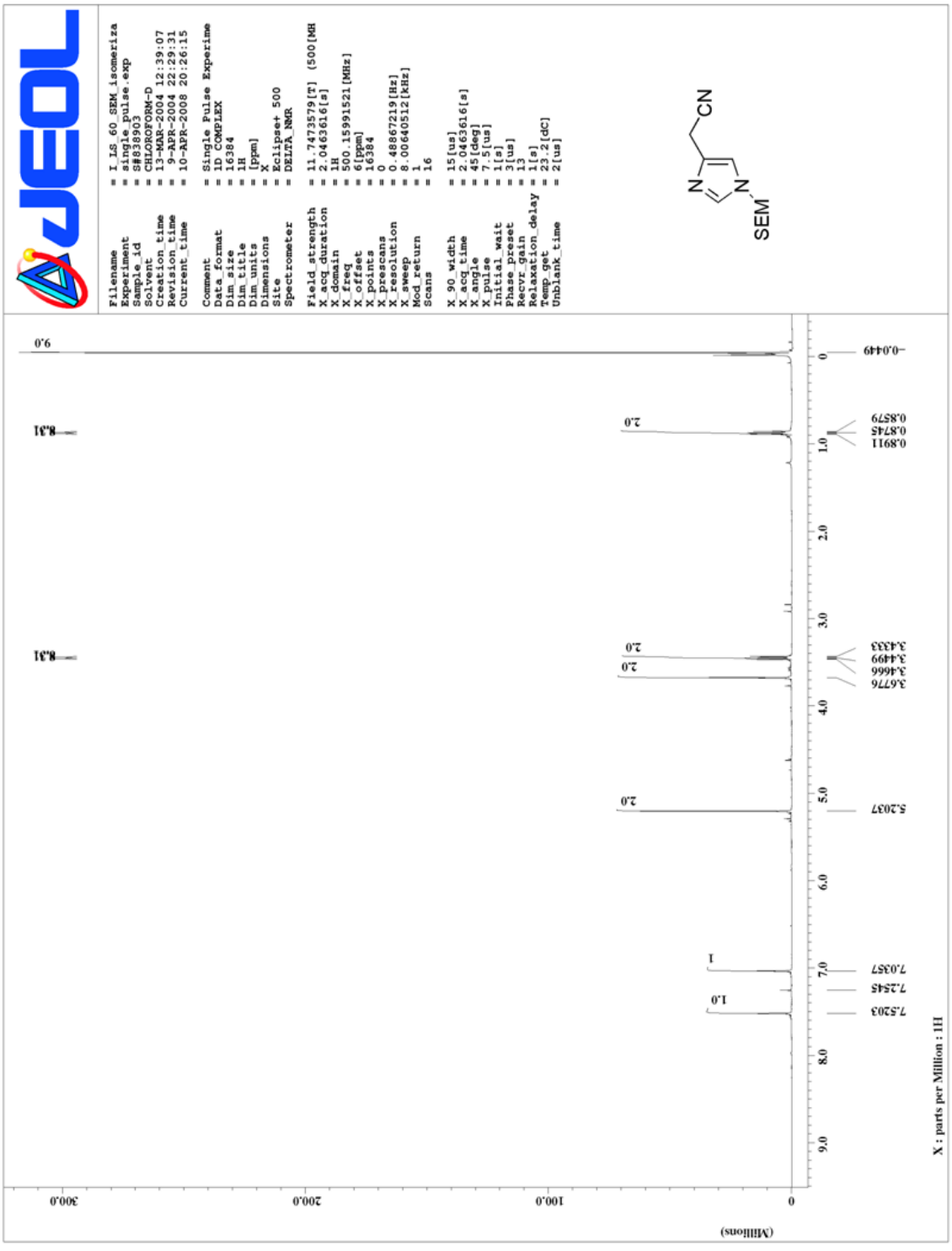

Filename = LAS_Bn_Im-5.jdf
Author = delta
Experiment = single_pulse.ex2
Sample_id = S#491430
Solvent = CHLOROFORM-D
Creation_time = 20-FEB-2008 14:09:56
Revision_time = 10-APR-2008 17:55:57
Current_time = 10-APR-2008 17:56:45
Comment = single_pulse
Data_format = 1D COMPLEX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 2.90717696[s]
X_domain = 1H
X_freq = 300.52965592[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.34397631[Hz]
X_sweep = 5.63570784[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Tri_domain = 1H
Tri_freq = 300.52965592[MHz]
Tri_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 16
Total_scans = 16
X_90_width = 13.01[us]
X_acq_time = 2.90717696[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 6.505[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Recvr_gain = 48
Relaxation_delay = 5[s]
Repetition_time = 7.90717696[s]
Temp_get = 23.2[dC]
CN
N
N
Bn
abundance
X : parts per Million : 1H
3
2.03
2.03
1.03
1.01
2.01
2.0
1.01
7.6417
7.3899
7.3704
7.3658
7.2582
7.2010
6.9435
5.1030
3.7272
0.0646

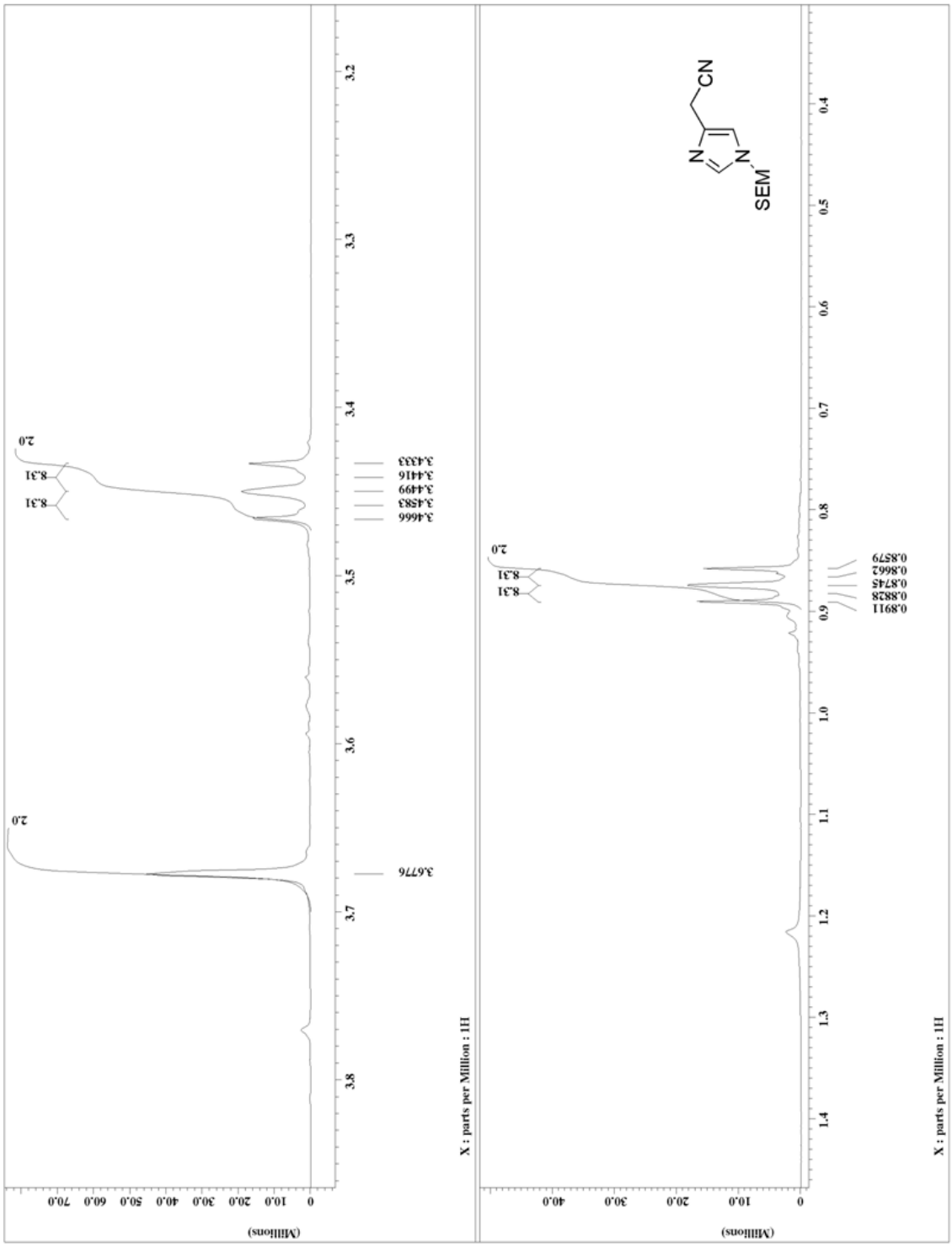

(Millions)
400.0
300.0
200.0
100.0
0
200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0
137.7107
135.7077
132.1709
129.1796
128.5845
127.5429
117.7375
117.1385
77.5582
77.3026
77.0470
51.1561
17.8636
X : parts per Million : 13C
Filename = I_LAS_57F2_Bn_4_CH2CN
Experiment = single_pulse_dec
Sample_id = S#169397
Solvent = CHLOROFORM-D
Creation_time = 7-APR-2004 17:25:01
Revision_time = 10-APR-2008 20:16:04
Current_time = 10-APR-2008 20:16:49
Comment = Single Pulse with Bro
Data_format = 1D COMPLEX
Dim_size = 65536
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = Eclipse+ 500
Spectrometer = DELTA_NMR
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 2.0840448[s]
X_domain = 13C
X_freq = 125.76529768[MHz]
X_offset = 100[ppm]
X_points = 65536
X_prescans = 4
X_resolution = 0.47983613[Hz]
X_sweep = 31.44654088[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5[ppm]
Mod_return = 10
Scans = 119
X_90_width = 14[us]
X_acq_time = 2.0840448[s]
X_angle = 30[deg]
X_pulse = 4.66666667[us]
Initial_wait = 1[s]
Phase_preset = 3[us]
Recvr_gain = 29
Relaxation_delay = 2[s]
Temp_get = 23.5[dC]
Unblank_time = 2[us]
N
CN
N
Bn

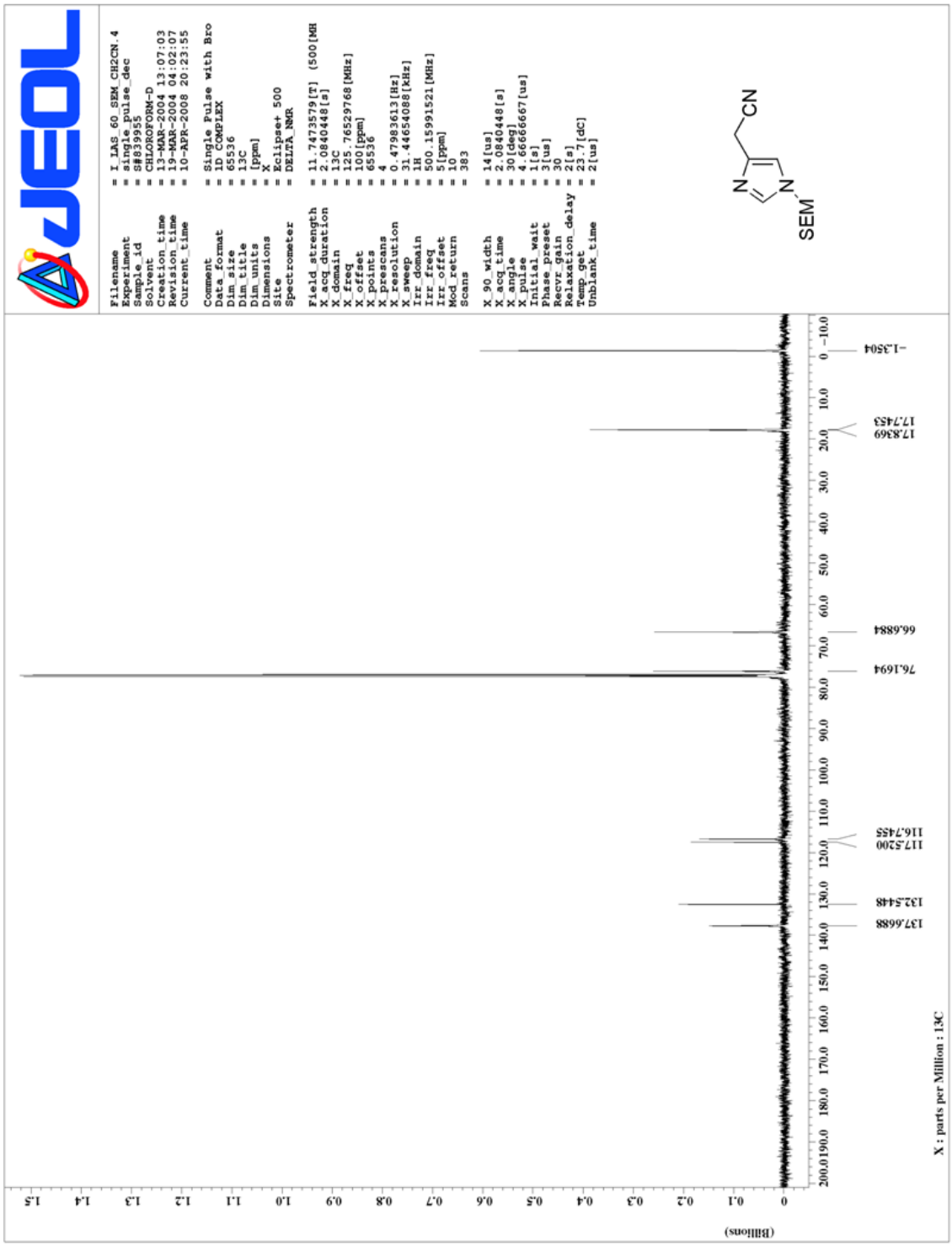
APPENDIX C

^{1}H AND ^{13}C NMR SPECTRA OF

1-2(TRIMETHYLSILYL)ETHOXYMETHYL-4-CYANOMETHYL-(*1H*)-IMIDAZOLE (114c)


JEOL
Filename = I_LS_60_SEM_isomeriza
Experiment = single_pulse.exp
Sample_id = ##03903
Solvent = CHLOROFORM-D
Creation_time = 13-APR-2004 12:39:07
Revision_time = 9-APR-2004 22:29:31
Current_time = 10-APR-2008 20:26:15
Comment = Single Pulse Experime
Data_format = 1D COMPLEX
Dim_size = 16384
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = Eclipse+ 500
Spectrometer = DELTA_NMR
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 2.0463616[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 6[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.48867219[Hz]
X_sweep = 8.00640512[kHz]
Mod_return = 1
Scans = 16
X_90_width = 15[us]
X_acq_time = 2.0463616[s]
X_angle = 45[deg]
X_pulse = 7.5[us]
Initial_wait = 1[s]
Phase_preset = 3[us]
Recvr_gain = 13
Relaxation_delay = 1[s]
Temp_get = 23.2[dC]
Unblank_time = 2[us]
CN
N
N
SEM
9.0
8.31
8.31
2.0
2.0
2.0
2.0
1
1.0
-0.0449
0.8911
0.8745
0.8579
3.4333
3.4499
3.4666
3.6776
5.2037
7.0357
7.2545
7.5203
(Millions)
300.0
200.0
100.0
0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
X : parts per Million : 1H

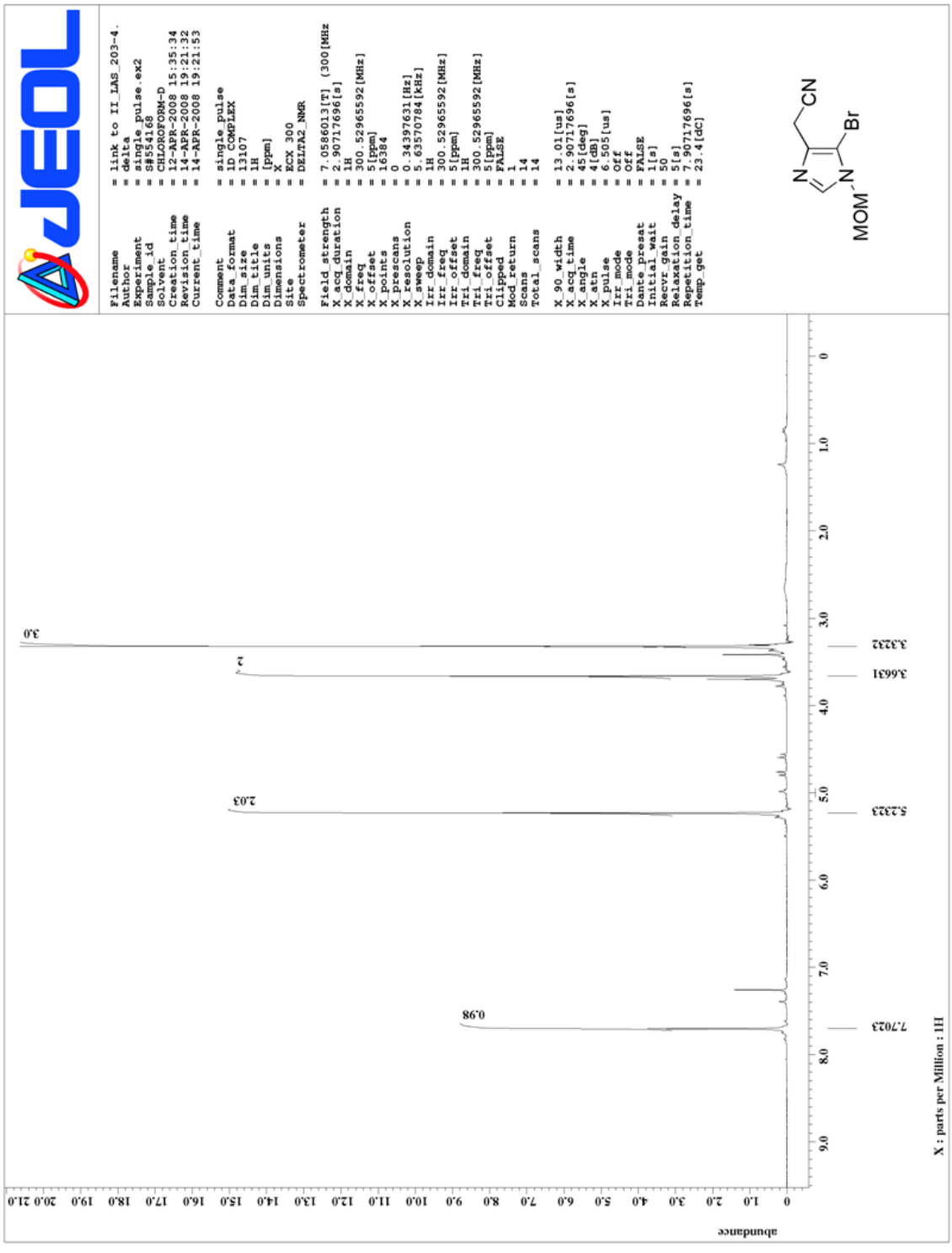

CN
N
N
SEM
0.8911
0.8828
0.8745
0.8662
0.8579
8.31
8.31
2.0
3.6776
3.4666
3.4583
3.4499
3.4416
3.4333
8.31
8.31
2.0
X : parts per Million : 1H
(Millions)
X : parts per Million : 1H
(Millions)

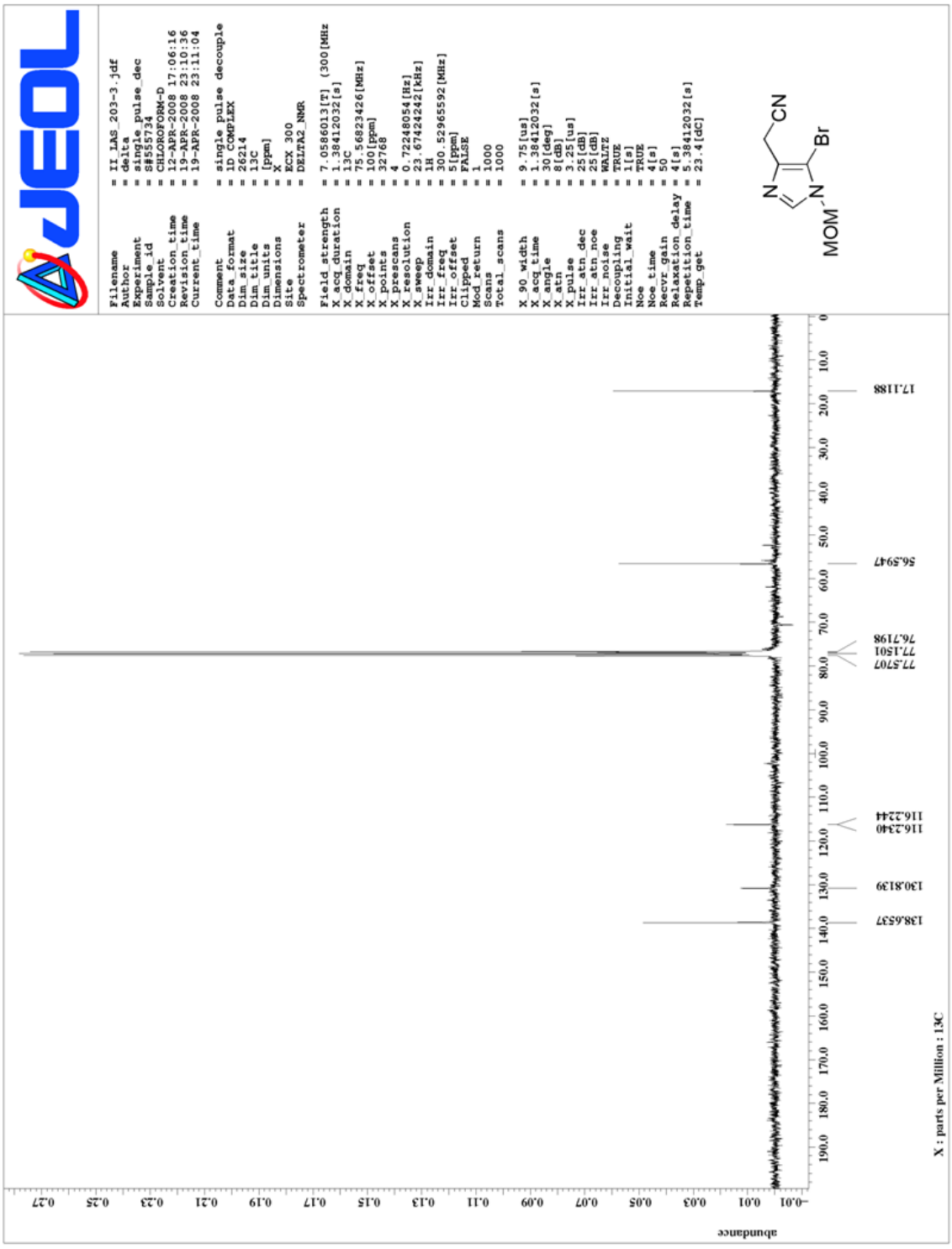

Filename = I_IA8_60_SEM_CH2CN.4
Experiment = single_pulse_dec
Sample_id = S#839955
Solvent = CHLOROFORM-D
Creation_time = 13-MAR-2004 13:07:03
Revision_time = 19-MAR-2004 04:02:07
Current_time = 10-APR-2008 20:23:55
Comment = Single Pulse with Bro
Data_format = 1D COMPLEX
Dim_size = 65536
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = Eclipse+ 500
Spectrometer = DELTA_NMR
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 2.0840448[s]
X_domain = 13C
X_freq = 125.76529768[MHz]
X_offset = 100[ppm]
X_points = 65536
X_prescans = 4
X_resolution = 0.47983613[Hz]
X_sweep = 31.44654088[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5[ppm]
Mod_return = 10
Scans = 383
X_90_width = 14[us]
X_acq_time = 2.0840448[s]
X_angle = 30[deg]
X_pulse = 4.66666667[us]
Initial_wait = 1[s]
Phase_preset = 3[us]
Recvr_gain = 30
Relaxation_delay = 2[s]
Temp_get = 23.7[dC]
Unblank_time = 2[us]
CN
N
N
SEM
-1.3504
17.8369
17.7453
66.6884
76.1694
117.5200
116.7455
132.5448
137.6688
X : parts per Million : 13C
(Billions)

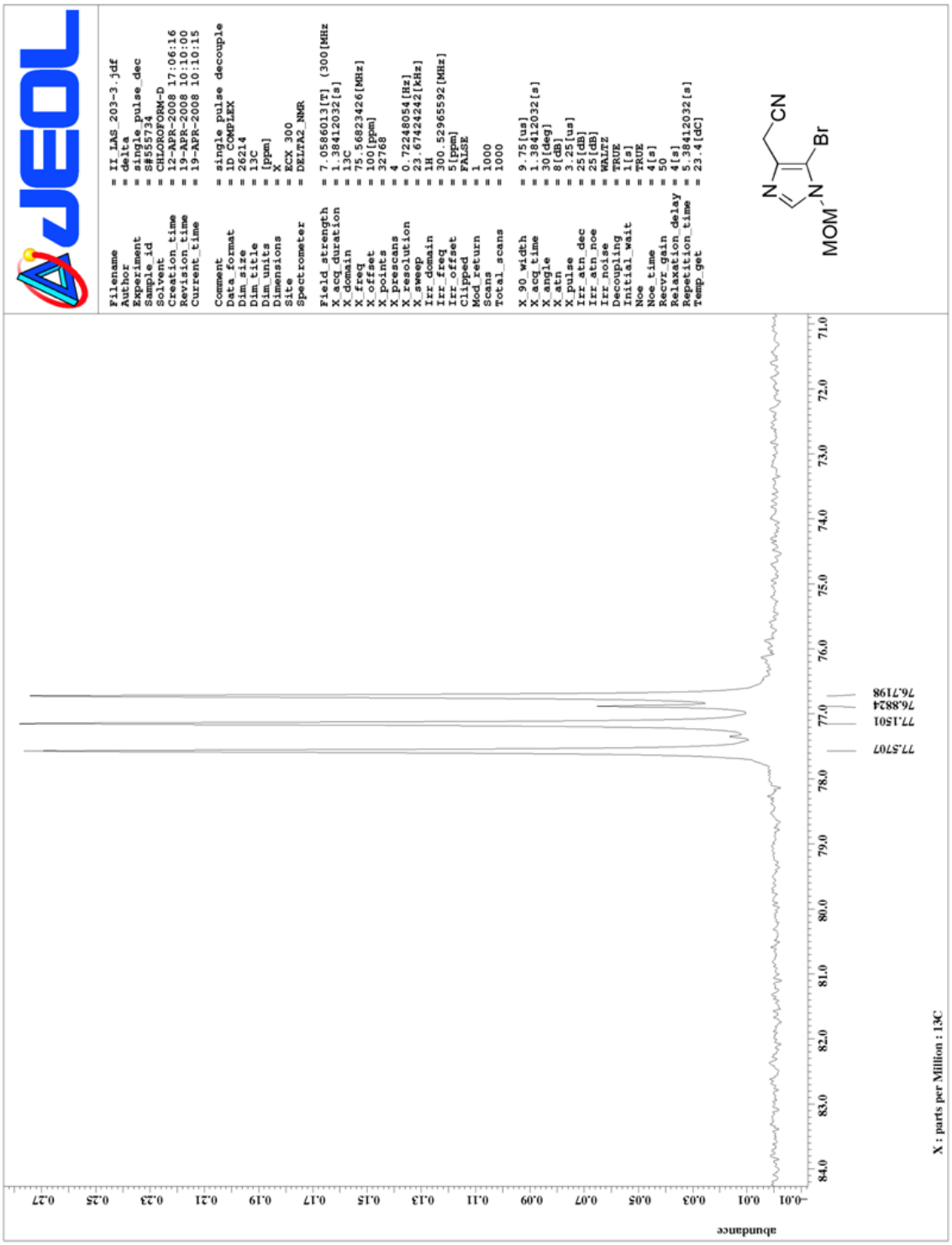
APPENDIX D

^{1}H NMR AND ^{13}C NMR SPECTRA OF

5-BROMO-1-METHOXYMETHYL-4-CYANOMETHYL-(1H)-IMIDAZOLE (117)


JEOL
Filename = link to II_LAS_203-4.
Author = delta
Experiment = single_pulse.ex2
Sample_id = S#554168
Solvent = CHLOROFORM-D
Creation_time = 12-APR-2008 15:35:34
Revision_time = 14-APR-2008 19:21:32
Current_time = 14-APR-2008 19:21:53
Comment = single_pulse
Data_format = 1D COMPLEX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = ECX_300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 2.90717696[s]
X_domain = 1H
X_freq = 300.52965592[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.34397631[Hz]
X_sweep = 5.63570784[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Tri_domain = 1H
Tri_freq = 300.52965592[MHz]
Tri_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 14
Total_scans = 14
X_90_width = 13.01[us]
X_acq_time = 2.90717696[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 6.505[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Recvr_gain = 50
Relaxation_delay = 5[s]
Repetition_time = 7.90717696[s]
Temp_get = 23.4[dC]
CN
Br
N
N
MOM
3.0
2
2.03
0.98
7.7023
5.3323
3.6631
3.3232
abundance
X : parts per Million : 1H


JEOL
Filename = II_LAS_203-3.jdf
Author = delta
Experiment = single_pulse_dec
Sample_id = S#555734
Solvent = CHLOROFORM-D
Creation_time = 12-APR-2008 17:06:16
Revision_time = 19-APR-2008 23:10:36
Current_time = 19-APR-2008 23:11:04
Comment = single_pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 1000
Total_scans = 1000
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 4[s]
Recvr_gain = 50
Relaxation_delay = 4[s]
Repetition_time = 5.38412032[s]
Temp_get = 23.4[dC]
CN
Br
N
N
MOM
17.1188
56.5947
77.5707
77.1501
76.7198
116.2340
116.2244
130.8139
138.6537
abundance
X : parts per Million : 13C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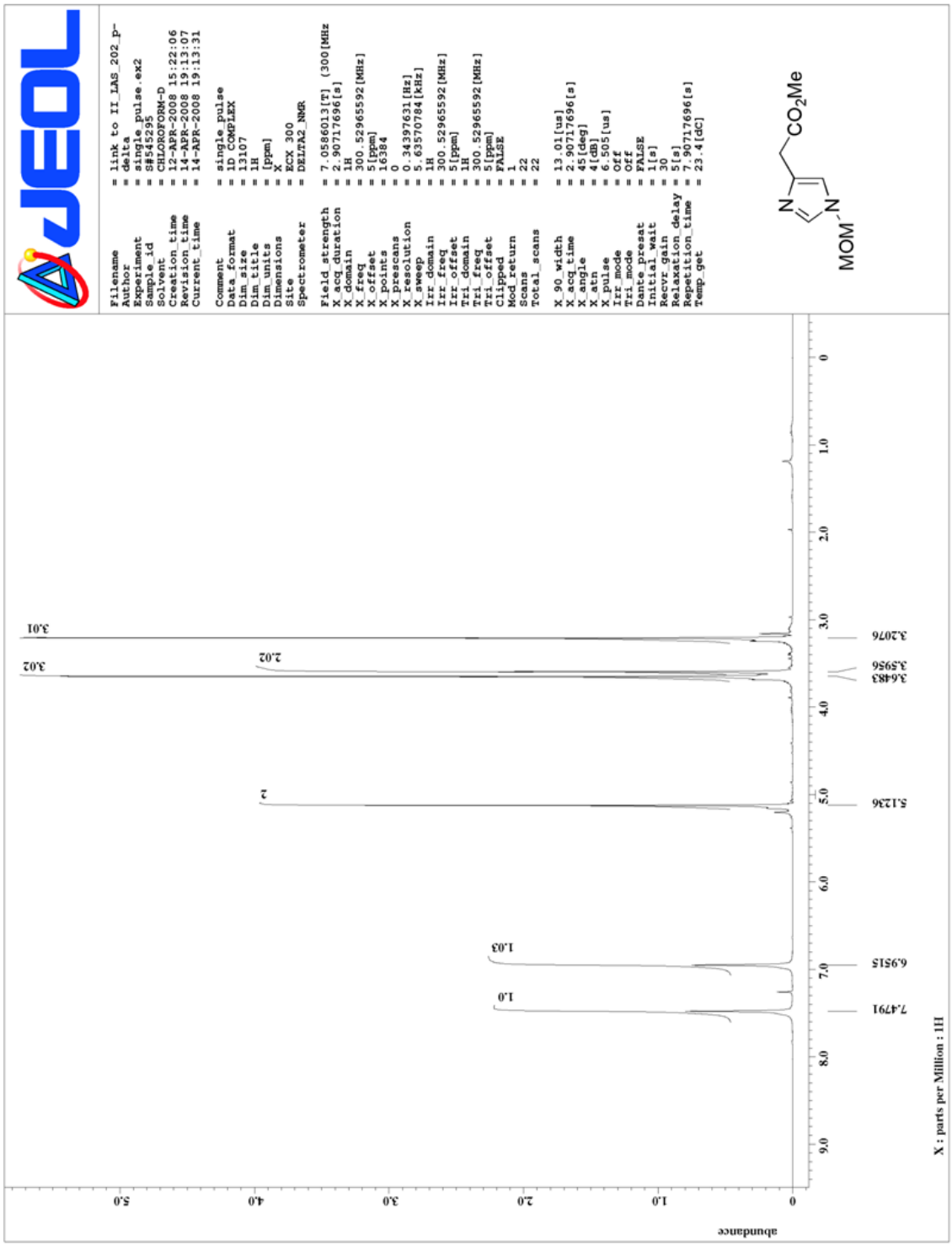
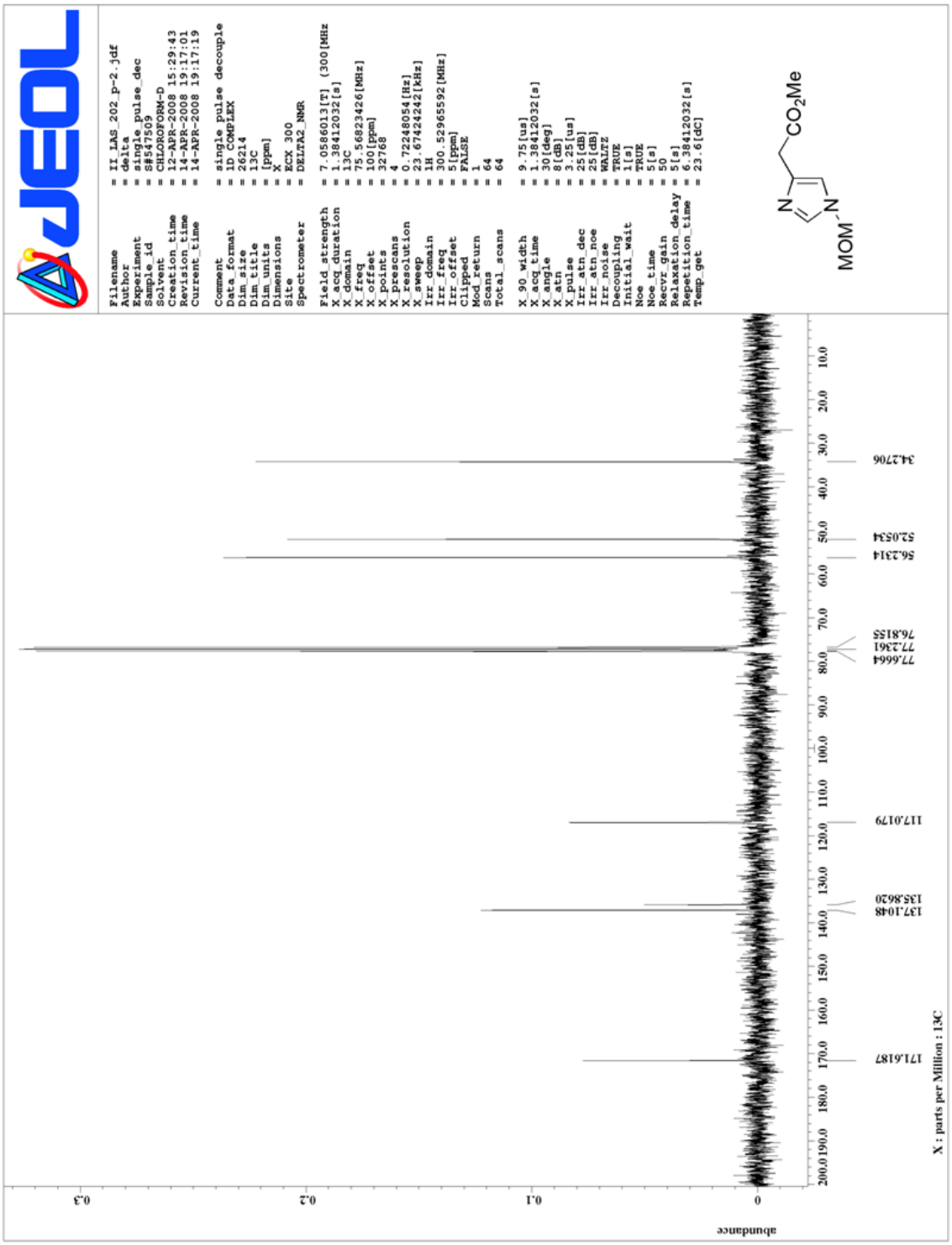
Author = delta
Experiment = single_pulse_dec
Sample_id = S#555734
Solvent = CHLOROFORM-D
Creation_time = 12-APR-2008 17:06:16
Revision_time = 19-APR-2008 10:10:00
Current_time = 19-APR-2008 10:10:15
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.05860013[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 1000
Total_scans = 1000
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 4[s]
Recvr_gain = 50
Relaxation_delay = 4[s]
Repetition_time = 5.38412032[s]
Temp_get = 23.4[dC]
CN
Br
N
N
MOM
77.5707
77.1501
76.8824
76.7198
abundance
X : parts per Million : 13C

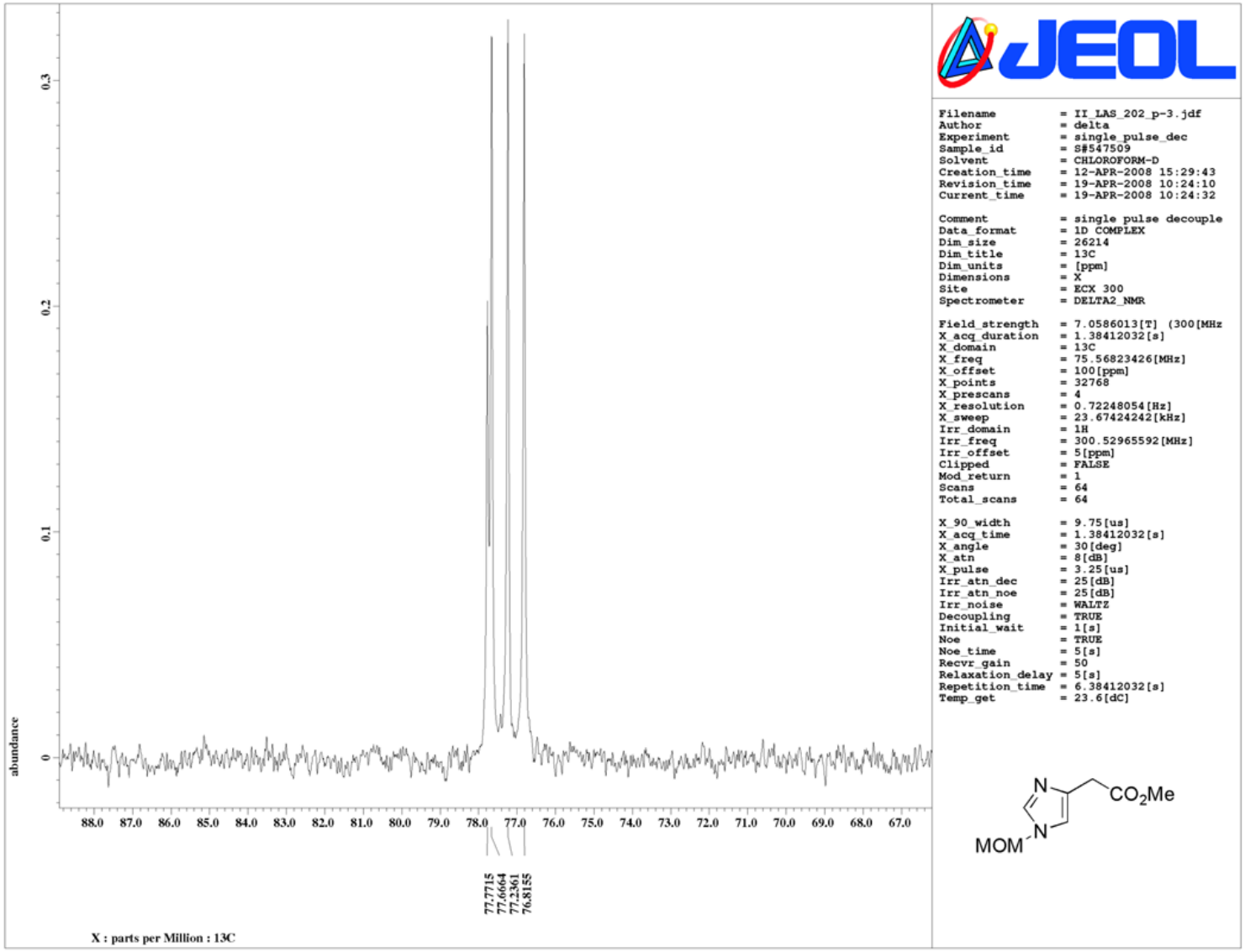
APPENDIX E

^{1}H NMR AND ^{13}C NMR SPECTRA OF

METHYL 1-METHOXYMETHYL-4(*1H*)-IMIDAZOLEACETATE (123a)


JEOL
Filename = link to II_LAS_202_p-
Author = delta
Experiment = single_pulse.ex2
Sample_id = S#545295
Solvent = CHLOROFORM-D
Creation_time = 12-APR-2008 15:22:06
Revision_time = 14-APR-2008 19:13:07
Current_time = 14-APR-2008 19:13:31
Comment = single_pulse
Data_format = 1D COMPLEX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 2.90717696[s]
X_domain = 1H
X_freq = 300.52965592[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.34397631[Hz]
X_sweep = 5.63570784[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Tri_domain = 1H
Tri_freq = 300.52965592[MHz]
Tri_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 22
Total_scans = 22
X_90_width = 13.01[us]
X_acq_time = 2.90717696[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 6.505[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Recvr_gain = 30
Relaxation_delay = 5[s]
Repetition_time = 7.90717696[s]
Temp_get = 23.4[dC]
CO2Me
MOM
N
N
abundance
X : parts per Million : 1H
3.01
3.02
2.02
2
1.03
1.0
3.2076
3.5956
3.6483
5.1236
6.9515
7.4791

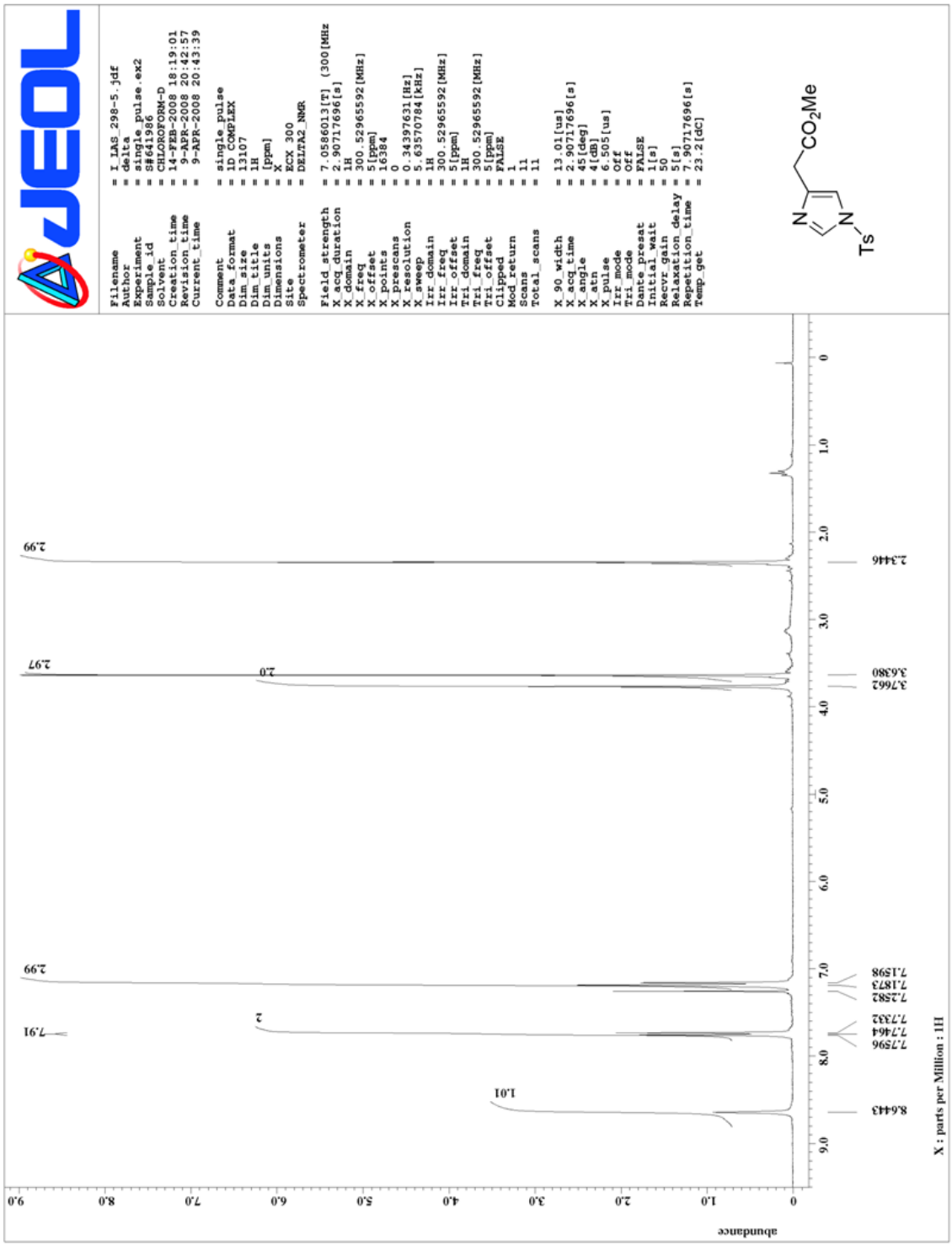

JEOL
Filename = II_LAS_202_p-2.jdf
Author = delta
Experiment = single_pulse_dec
Sample_id = S#547509
Solvent = CHLOROFORM-D
Creation_time = 12-APR-2008 15:29:43
Revision_time = 14-APR-2008 19:17:01
Current_time = 14-APR-2008 19:17:19
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.05860131[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 64
Total_scans = 64
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 5[s]
Recvr_gain = 50
Relaxation_delay = 5[s]
Repetition_time = 6.38412032[s]
Temp_get = 23.6[dC]
CO2Me
N
N
MOM
abundance
0.3
0.2
0.1
0
200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0
X : parts per Million : 13C
171.6187
137.1048
135.8620
117.0179
77.6664
77.2361
76.8155
56.2314
52.0534
34.2706

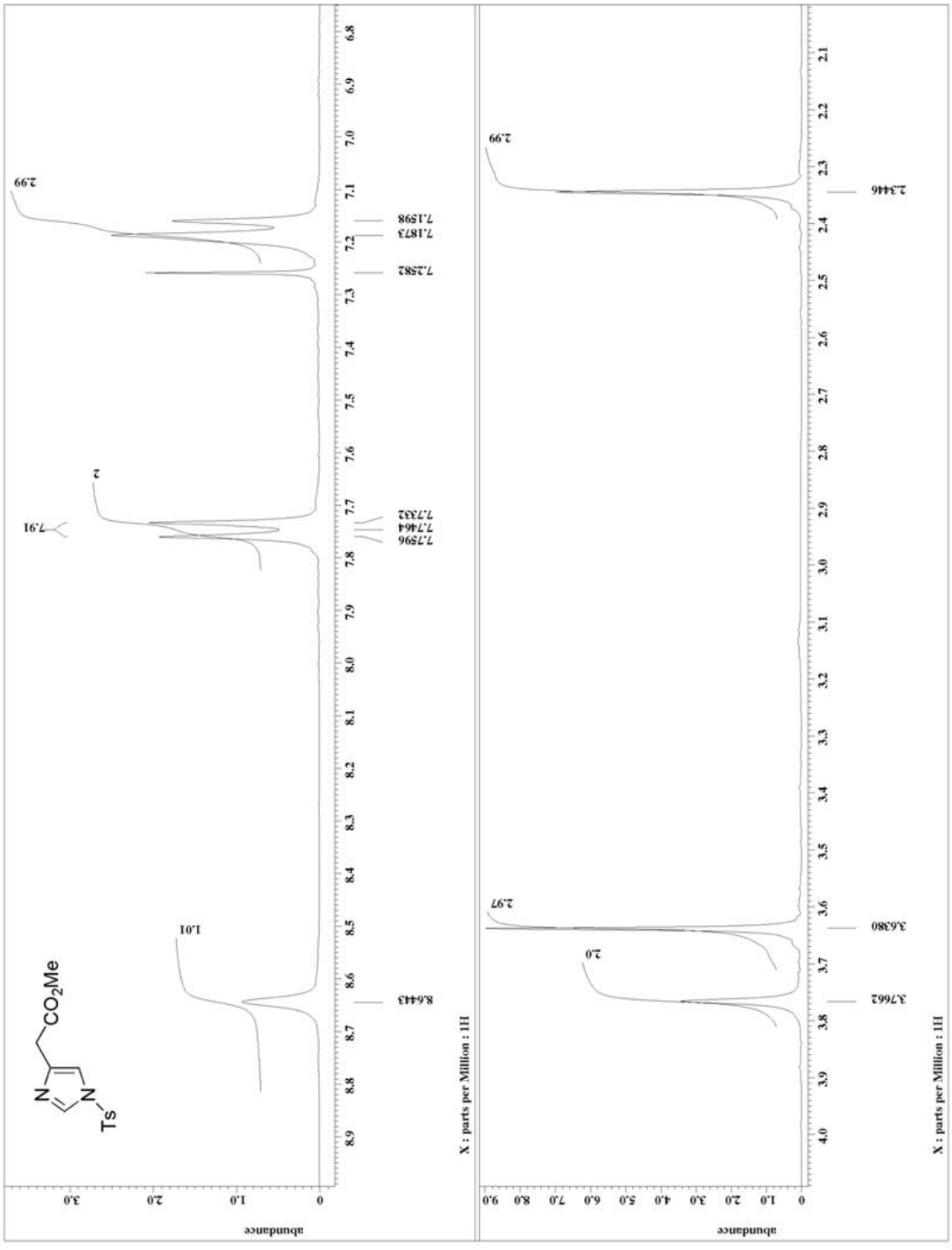

abundance
0.3
0.2
0.1
0
88.0 87.0 86.0 85.0 84.0 83.0 82.0 81.0 80.0 79.0 78.0 77.0 76.0 75.0 74.0 73.0 72.0 71.0 70.0 69.0 68.0 67.0
77.7715
77.6664
77.2361
76.8155
X : parts per Million : 13C
Filename = II_LAS_202_p-3.jdf
Author = delta
Experiment = single_pulse_dec
Sample_id = S#547509
Solvent = CHLOROFORM-D
Creation_time = 12-APR-2008 15:29:43
Revision_time = 19-APR-2008 10:24:10
Current_time = 19-APR-2008 10:24:32
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 64
Total_scans = 64
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 5[s]
Recvr_gain = 50
Relaxation_delay = 5[s]
Repetition_time = 6.38412032[s]
Temp_get = 23.6[dC]
N
N
CO2Me
MOM

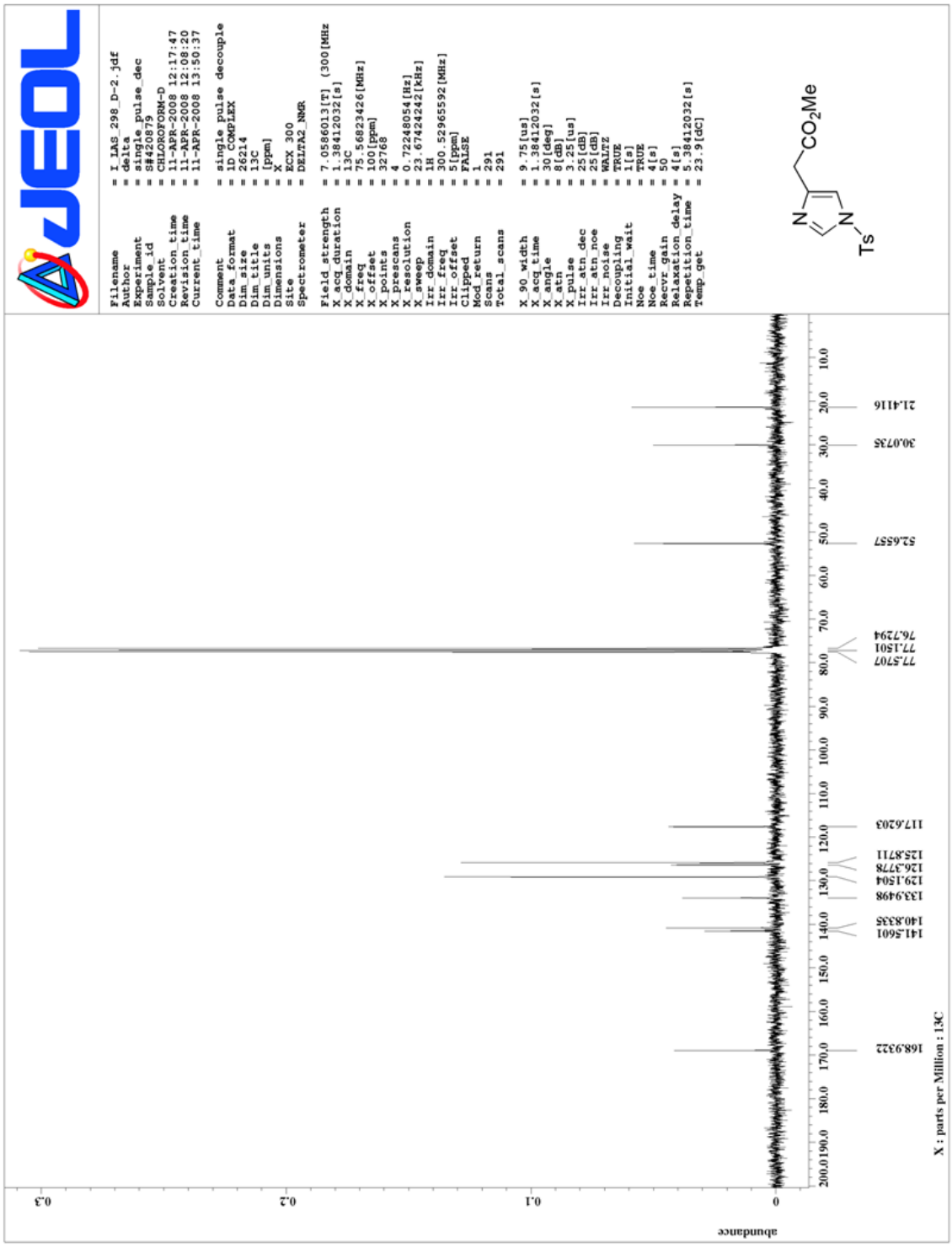
APPENDIX F

^{1}H NMR AND ^{13}C NMR SPECTRA OF

METHYL 1-TOLUENESULFONYL-4(*1H*)-IMIDAZOLEACETATE (123b)


JEOL
Filename = I_IA5_298-5.jdf
Author = delta
Experiment = single_pulse.ex2
Sample_id = S#641986
Solvent = CHLOROFORM-D
Creation_time = 14-FEB-2008 18:19:01
Revision_time = 9-APR-2008 20:42:57
Current_time = 9-APR-2008 20:43:39
Comment = single_pulse
Data_format = 1D COMPLEX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 2.90717696[s]
X_domain = 1H
X_freq = 300.52965592[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.34397631[Hz]
X_sweep = 5.63570784[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Tri_domain = 1H
Tri_freq = 300.52965592[MHz]
Tri_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 11
Total_scans = 11
X_90_width = 13.01[us]
X_acq_time = 2.90717696[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 6.505[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Recvr_gain = 50
Relaxation_delay = 5[s]
Repetition_time = 7.90717696[s]
Temp_get = 23.2[dC]
CO2Me
N
N
Ts
abundance
X : parts per Million : 1H
8.6443
7.7596
7.7464
7.7332
7.2582
7.1873
7.1598
3.7662
3.6380
2.3446
1.01
2
2.99
7.91
2.97
2.0
2.99

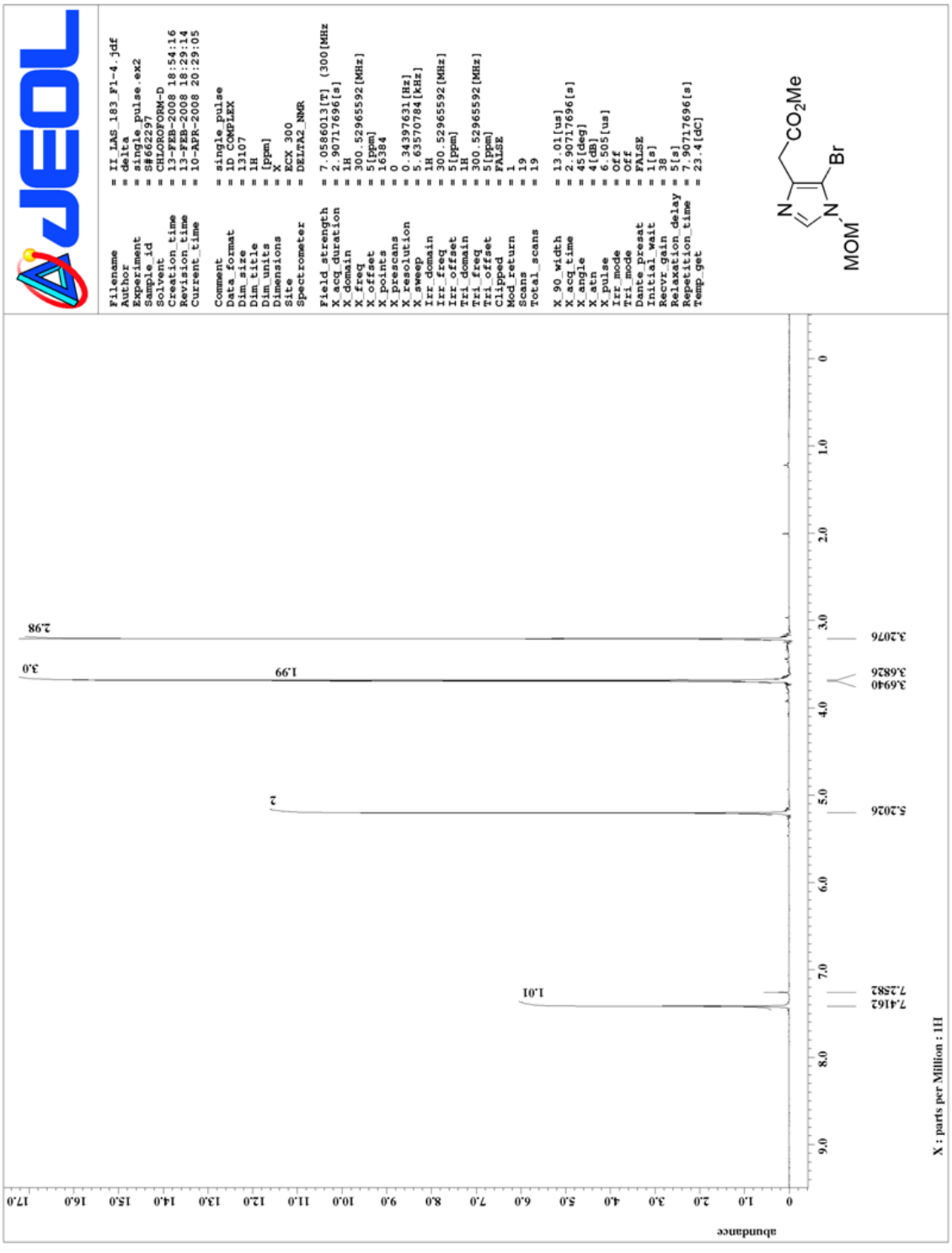

CO2Me
N
N
Ts
1.01
2.99
7.91
2
8.6443
7.7596
7.7464
7.7332
7.2582
7.1873
7.1598
2.99
2.97
2.0
3.7662
3.6380
2.3446
abundance
X : parts per Million : 1H

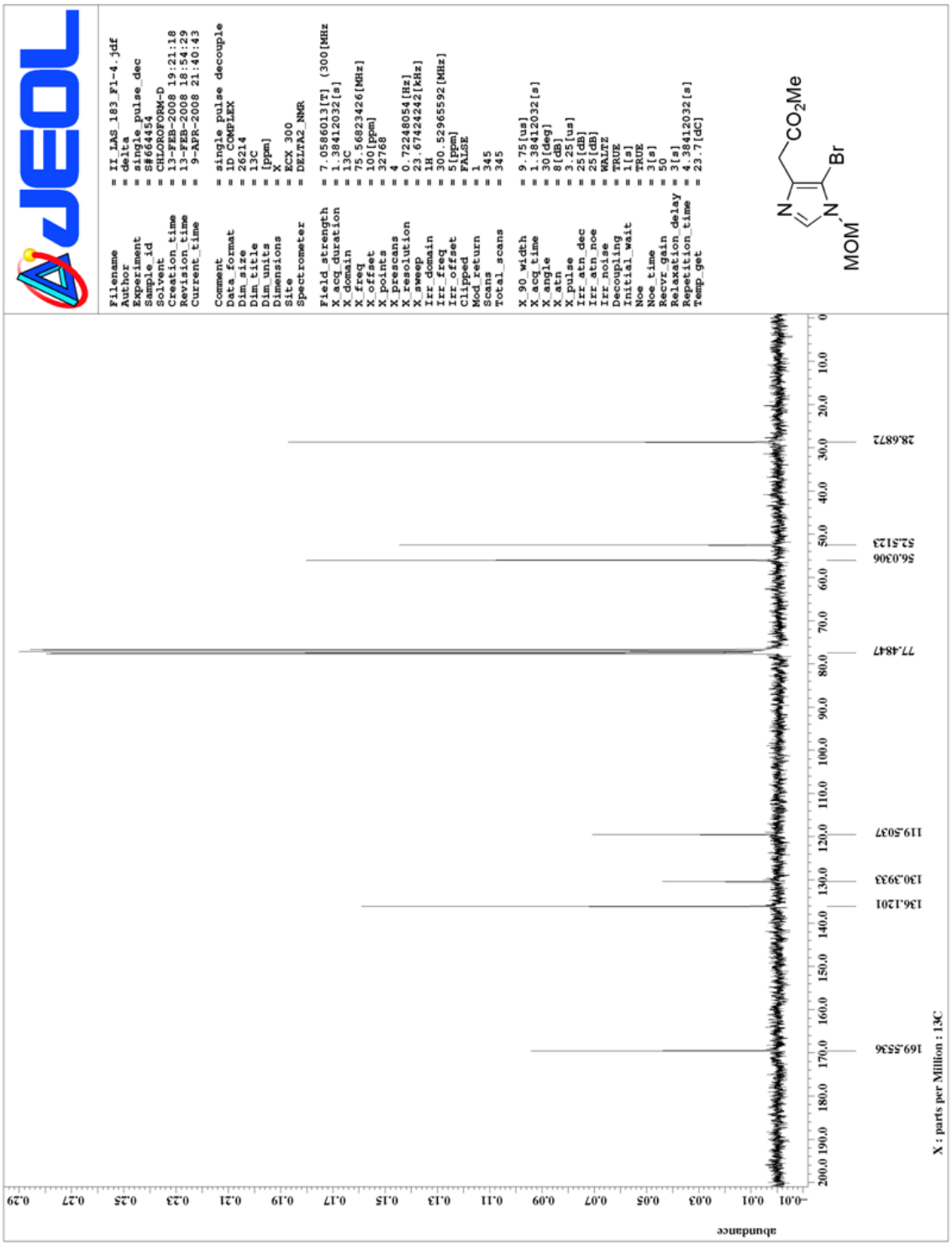

JEOL
Filename = I_IA5_298_D-2.jdf
Author = delta
Experiment = single_pulse_dec
Sample_id = S#420879
Solvent = CHLOROFORM-D
Creation_time = 11-APR-2008 12:17:47
Revision_time = 11-APR-2008 12:08:20
Current_time = 11-APR-2008 13:50:37
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX_300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 291
Total_scans = 291
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 4[s]
Recvr_gain = 50
Relaxation_delay = 4[s]
Repetition_time = 5.38412032[s]
Temp_get = 23.9[dC]
CO2Me
N
N
Ts
abundance
0.3
0.2
0.1
0
200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0
168.9322
141.5601
140.8335
133.9498
129.1504
126.3778
125.8711
117.6203
77.5707
77.1501
76.7294
52.6557
30.0735
21.4116
X : parts per Million : 13C

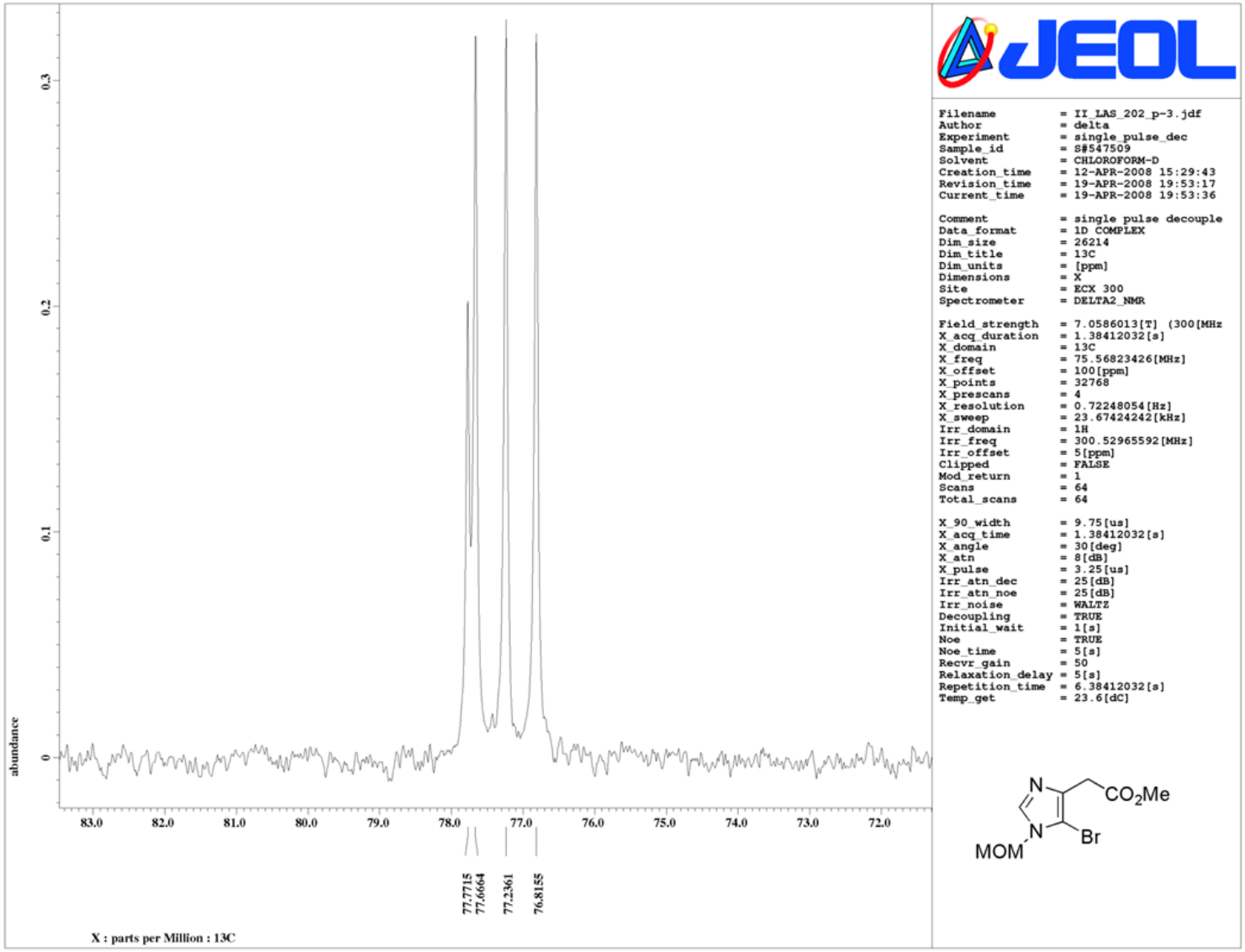
APPENDIX G

^{1}H NMR AND ^{13}C NMR SPECTRA OF

METHYL 5-BROMO-1-METHOXYMETHYL-4(*1H*)-IMIDAZOLEACETATE (124)


JEOL
Filename = II_IAS_183_F1-4.jdf
Author = delta
Experiment = single_pulse.ex2
Sample_id = ##60229
Solvent = CHLOROFORM-D
Creation_time = 13-FEB-2008 18:54:16
Revision_time = 13-FEB-2008 18:29:14
Current_time = 10-APR-2008 20:29:05
Comment = single_pulse
Data_format = 1D COMPLEX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = ECX_300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 2.90717696[s]
X_domain = 1H
X_freq = 300.52965592[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.34397631[Hz]
X_sweep = 5.63570784[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Tri_domain = 1H
Tri_freq = 300.52965592[MHz]
Tri_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 19
Total_scans = 19
X_90_width = 13.01[us]
X_acq_time = 2.90717696[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 6.505[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Recvr_gain = 38
Relaxation_delay = 5[s]
Repetition_time = 7.90717696[s]
Temp_get = 23.4[dC]
CO2Me
Br
N
N
MOM
2.98
1.99
2
1.01
3.0
3.2076
3.6940
3.6826
5.2026
7.4162
7.2582
abundance
X : parts per Million : 1H

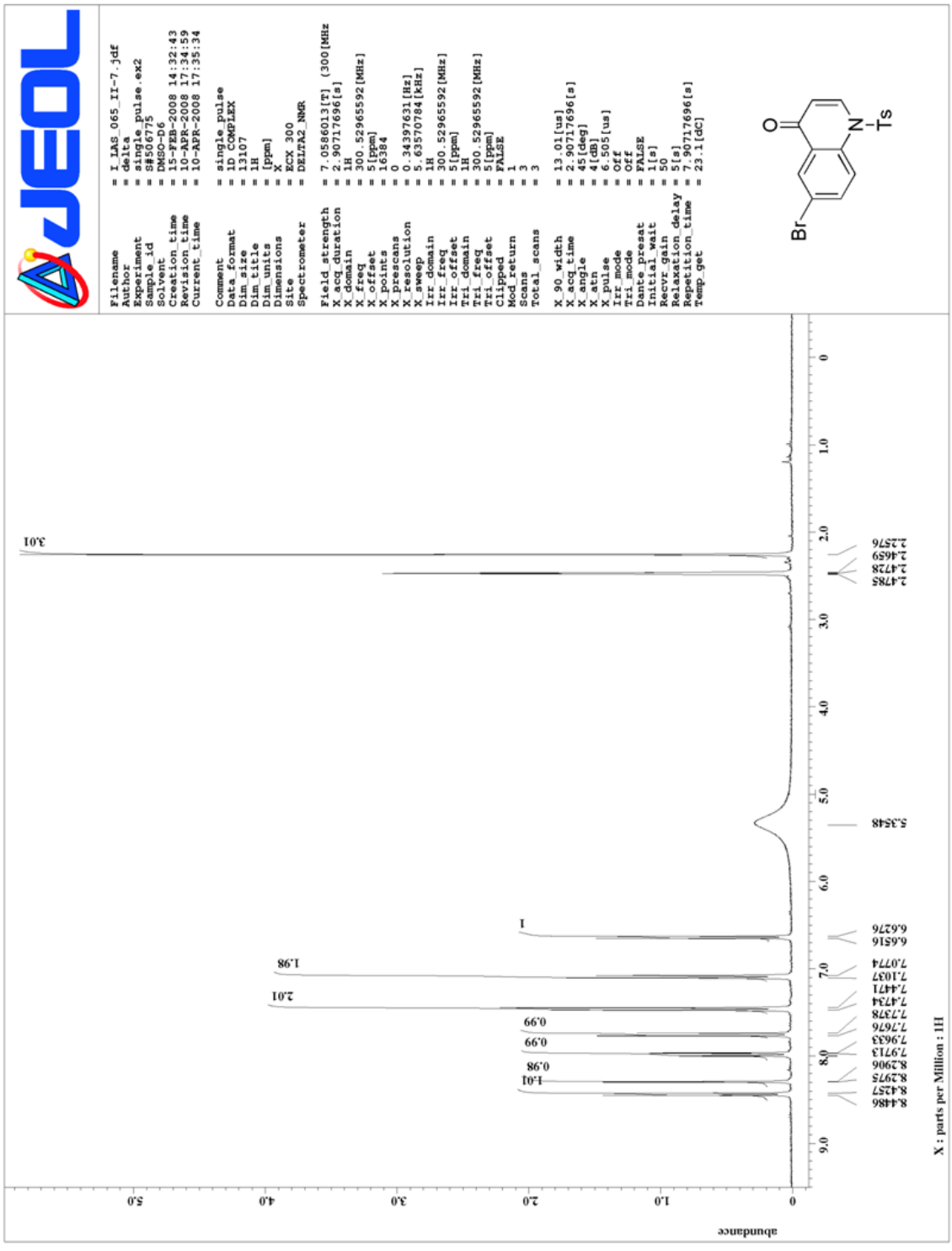

Filename = IT_LAS_183_F1-4.jdf
Author = delta
Experiment = single_pulse_dec
Sample_id = S#664454
Solvent = CHLOROFORM-D
Creation_time = 13-FEB-2008 19:21:18
Revision_time = 13-FEB-2008 18:54:29
Current_time = 9-APR-2008 21:40:43
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 345
Total_scans = 345
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 3[s]
Recvr_gain = 50
Relaxation_delay = 3[s]
Repetition_time = 4.38412032[s]
Temp_get = 23.7[dC]
CO2Me
Br
N
N
MOM
28.6872
52.5123
56.0306
77.4847
119.5037
130.3933
136.1201
169.5536
X : parts per Million : 13C
abundance

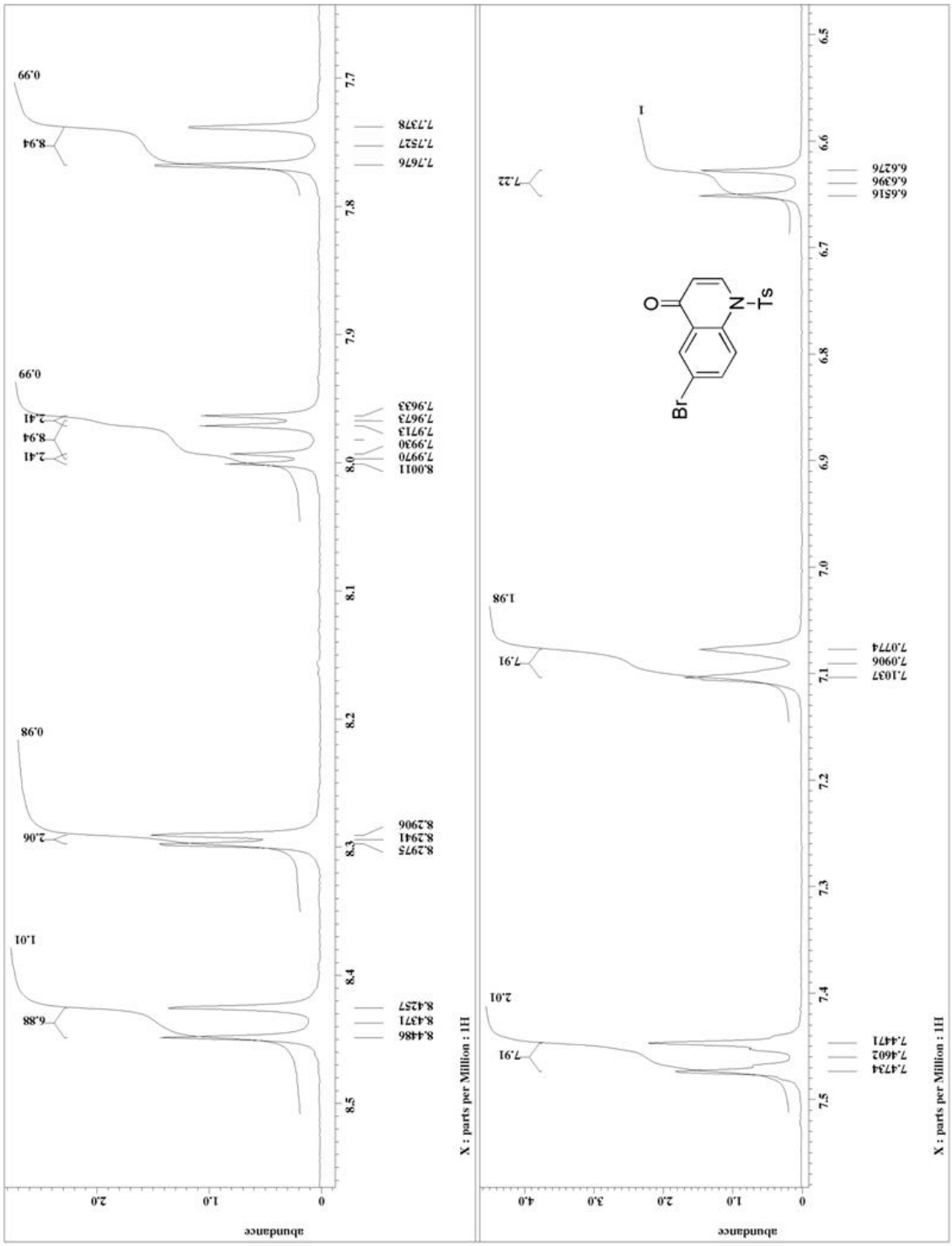

abundance
0.3
0.2
0.1
0
83.0
82.0
81.0
80.0
79.0
78.0
77.0
76.0
75.0
74.0
73.0
72.0
77.7715
77.6664
77.2361
76.8155
X : parts per Million : 13C
JEOL
Filename = II_LAS_202_p-3.jdf
Author = delta
Experiment = single_pulse_dec
Sample_id = S#547509
Solvent = CHLOROFORM-D
Creation_time = 12-APR-2008 15:29:43
Revision_time = 19-APR-2008 19:53:17
Current_time = 19-APR-2008 19:53:36
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 64
Total_scans = 64
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 5[s]
Recvr_gain = 50
Relaxation_delay = 5[s]
Repetition_time = 6.38412032[s]
Temp_get = 23.6[dC]
N
N
CO2Me
Br
MOM

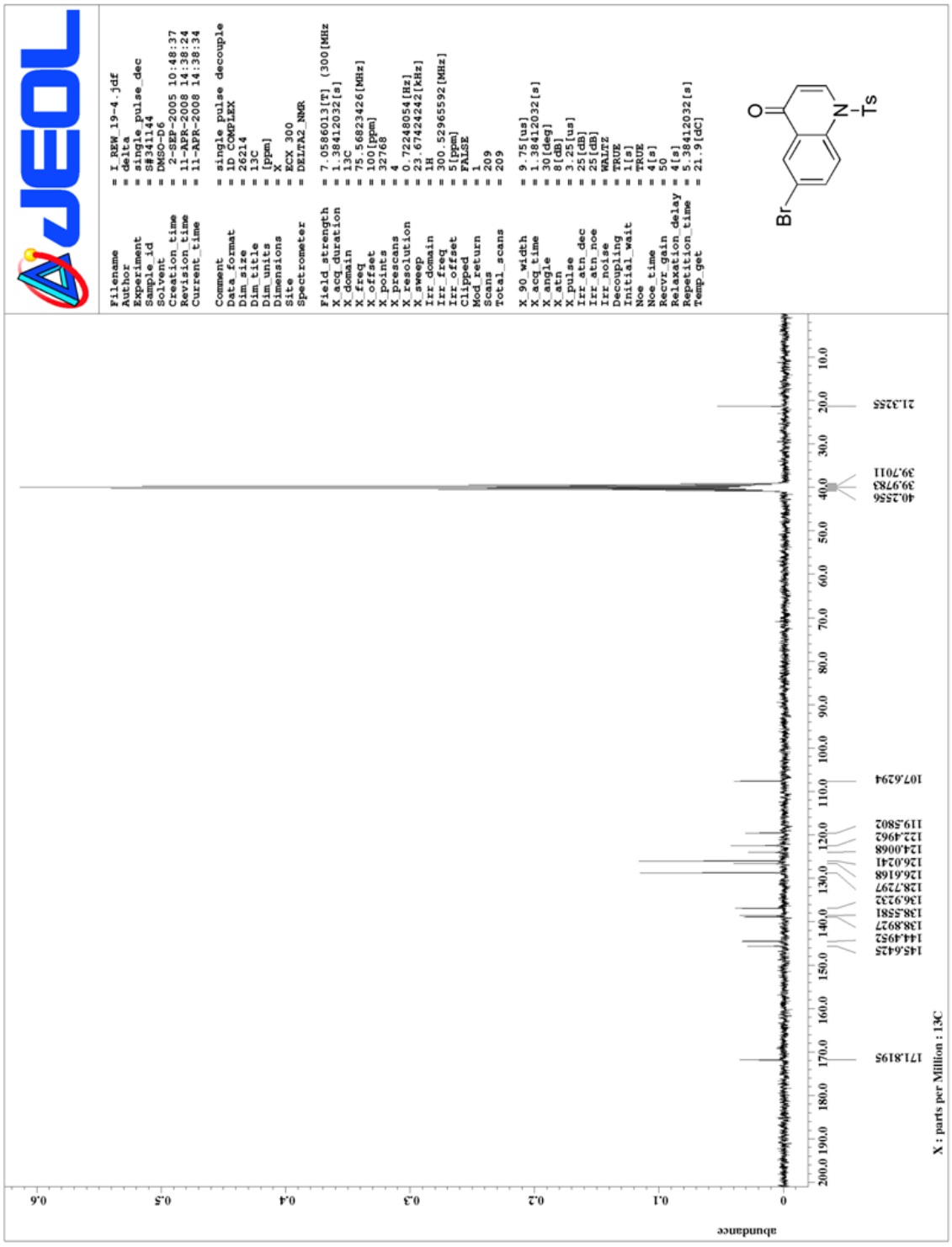
APPENDIX H

^{1}H NMR AND ^{13}C NMR SPECTRA OF

1-*N*-TOSYL-6-BROMO-4-(*1H*)-QUINOLONE (131a)


JEOL
Filename = I_IA5_065_II-7.jdf
Author = delta
Experiment = single_pulse.ex2
Sample_id = S#506775
Solvent = DMSO-D6
Creation_time = 15-FEB-2008 14:32:43
Revision_time = 10-APR-2008 17:34:59
Current_time = 10-APR-2008 17:35:34
Comment = single_pulse
Data_format = 1D COMPLEX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = ECX_300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 2.90717696[s]
X_domain = 1H
X_freq = 300.52965592[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.34397631[Hz]
X_sweep = 5.63570784[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Tri_domain = 1H
Tri_freq = 300.52965592[MHz]
Tri_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 3
Total_scans = 3
X_90_width = 13.01[us]
X_acq_time = 2.90717696[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 6.505[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Recvr_gain = 50
Relaxation_delay = 5[s]
Repetition_time = 7.90717696[s]
Temp_get = 23.1[dC]
O
N
Ts
Br
3.01
1.98
2.01
0.99
0.99
0.98
1.01
1
2.01
1.98
0.99
0.99
0.98
1.01
1
5.3548
2.4785
2.4728
2.4659
2.2576
8.4486
8.4257
8.2975
8.2906
7.9713
7.9633
7.7676
7.7378
7.4734
7.4471
7.1037
7.0774
6.6516
6.6276
abundance
X : parts per Million : 1H

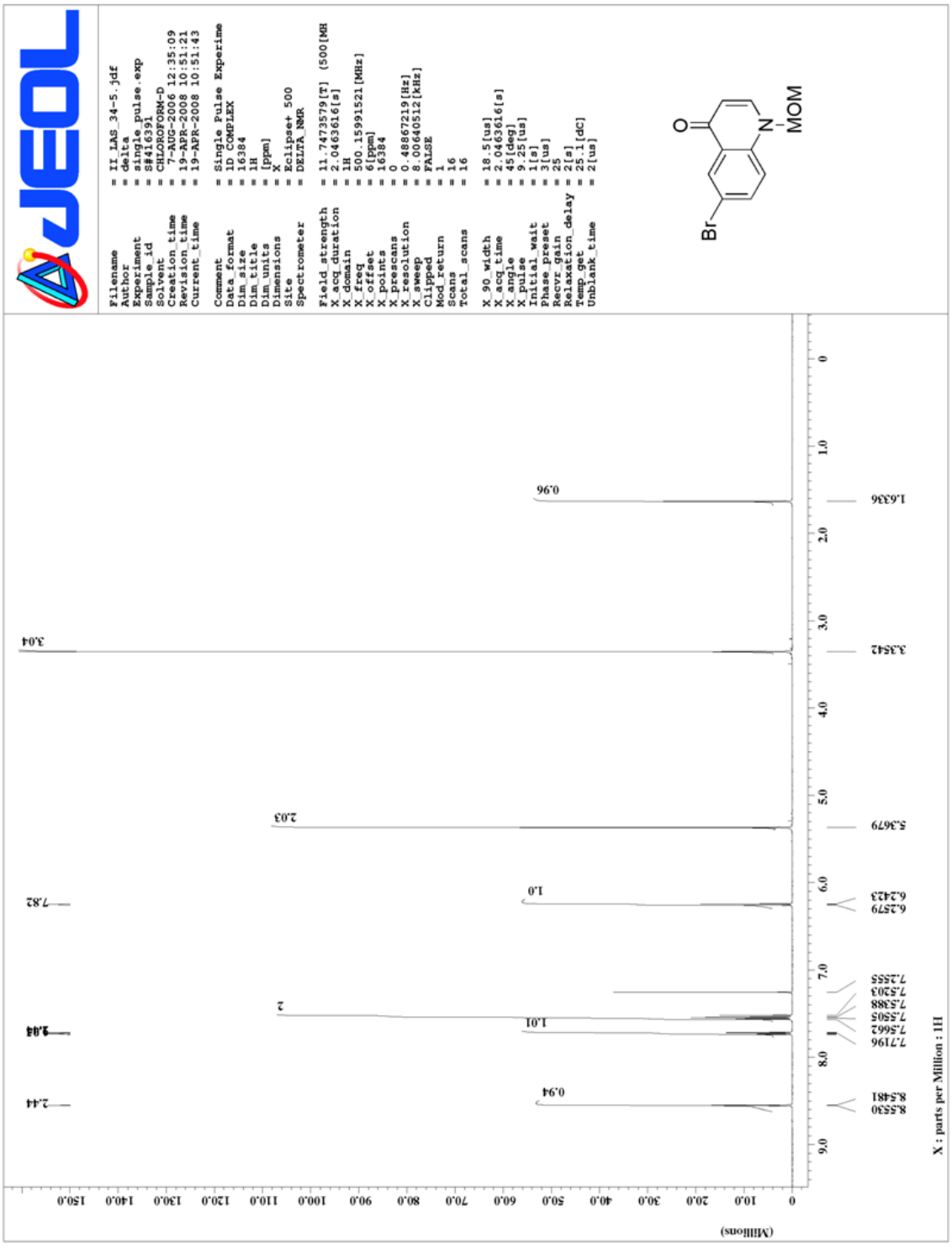

abundance
X : parts per Million : 1H
Br
O
N
Ts
7.4734
7.4602
7.4471
7.1037
7.0906
7.0774
6.6516
6.6396
6.6276
8.4486
8.4371
8.4257
8.2975
8.2941
8.2906
8.0011
7.9970
7.9930
7.9713
7.9673
7.9633
7.7676
7.7527
7.7378


Filename = I_REW_19-4.jdf
Author = delta
Experiment = single_pulse_dec
Sample_id = #341144
Solvent = DMSO-D6
Creation_time = 2-SEP-2005 10:48:37
Revision_time = 11-APR-2008 14:38:24
Current_time = 11-APR-2008 14:38:34
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 209
Total_scans = 209
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 4[s]
Recvr_gain = 50
Relaxation_delay = 4[s]
Repetition_time = 5.38412032[s]
Temp_get = 21.9[dC]
O
N
Ts
Br
abundance
X : parts per Million : 13C
21.3255
39.7011
39.9783
40.2556
107.6294
119.5802
122.4962
124.0068
126.0241
126.6168
128.7297
136.9232
138.5581
138.8927
144.4952
145.6425
171.8195

APPENDIX I

^{1}H NMR AND ^{13}C NMR SPECTRA OF

1-*N*-METHOXYMETHYL-6-BROMO-4(*1H*)-QUINOLONE (132a)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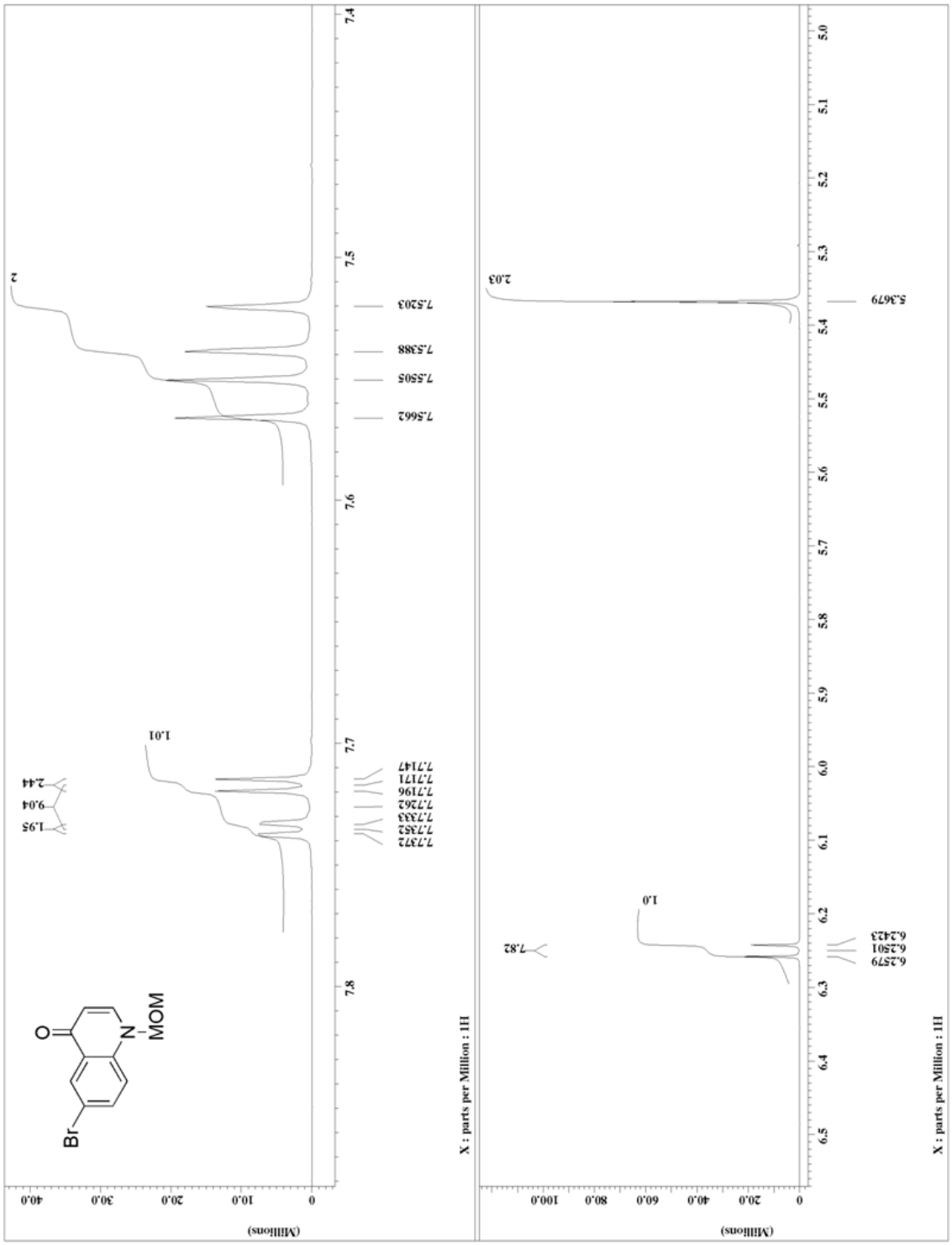
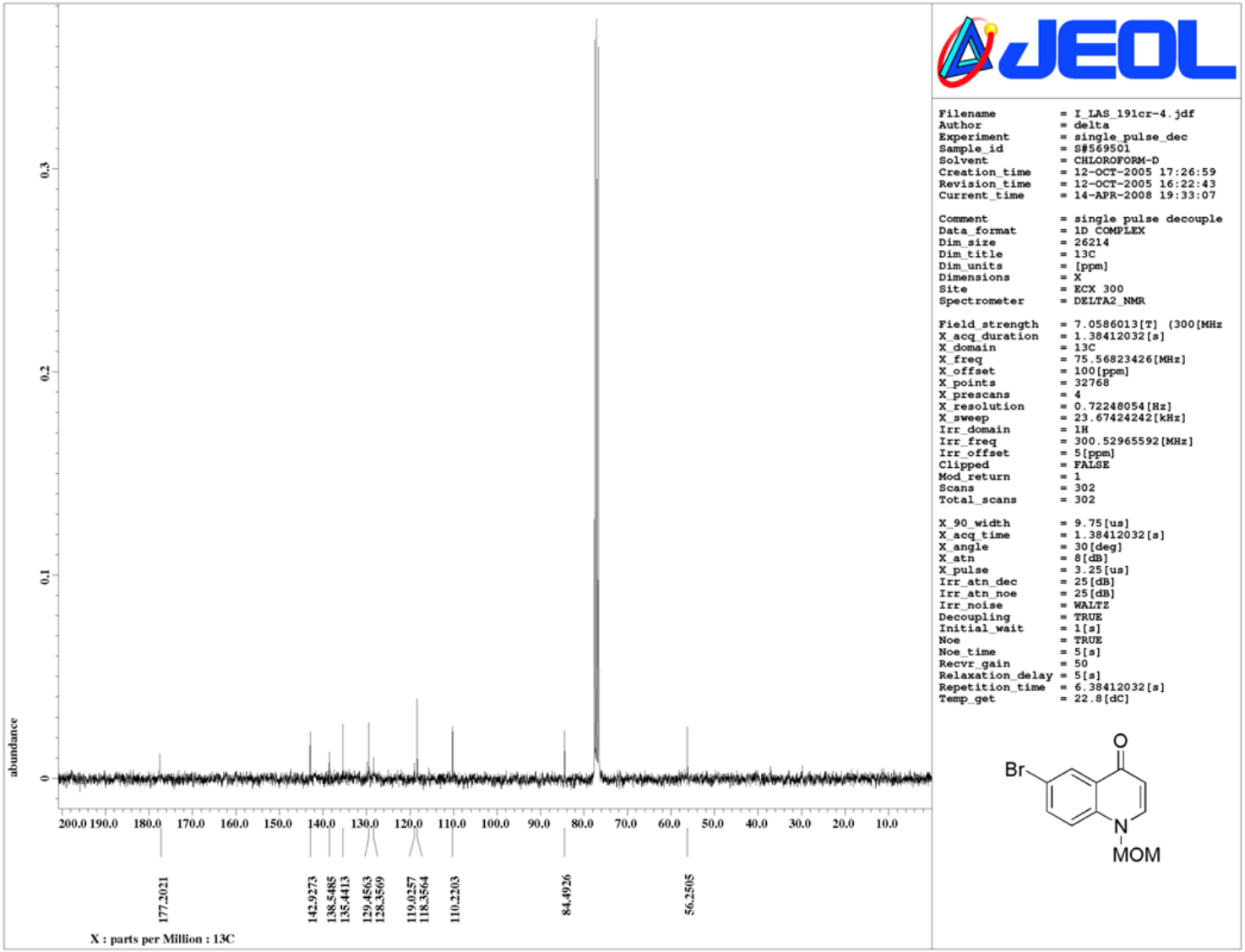
Filename = II_LAS_34-5.jdf
Author = delta
Experiment = single_pulse.exp
Sample_id = S#416391
Solvent = CHLOROFORM-D
Creation_time = 7-AUG-2006 12:35:09
Revision_time = 19-APR-2008 10:51:21
Current_time = 19-APR-2008 10:51:43
Comment = Single Pulse Experime
Data_format = 1D COMPLEX
Dim_size = 16384
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = Eclipse+ 500
Spectrometer = DELTA_NMR
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 2.0463616[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 6[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.48867219[Hz]
X_sweep = 8.00640512[kHz]
Clipped = FALSE
Mod_return = 1
Scans = 16
Total_scans = 16
X_90_width = 18.5[us]
X_acq_time = 2.0463616[s]
X_angle = 45[deg]
X_pulse = 9.25[us]
Initial_wait = 1[s]
Phase_preset = 3[us]
Recvr_gain = 25
Relaxation_delay = 2[s]
Temp_get = 25.1[dC]
Unblank_time = 2[us]
O
N
MOM
Br
(Millions)
X : parts per Million : 1H
8.5530
8.5481
7.7196
7.5662
7.5505
7.5388
7.5203
7.2555
6.2579
6.2423
5.3679
3.3542
1.6336
0.96
0.94
1.01
1.0
2.03
3.04
2.44
2.84
7.82

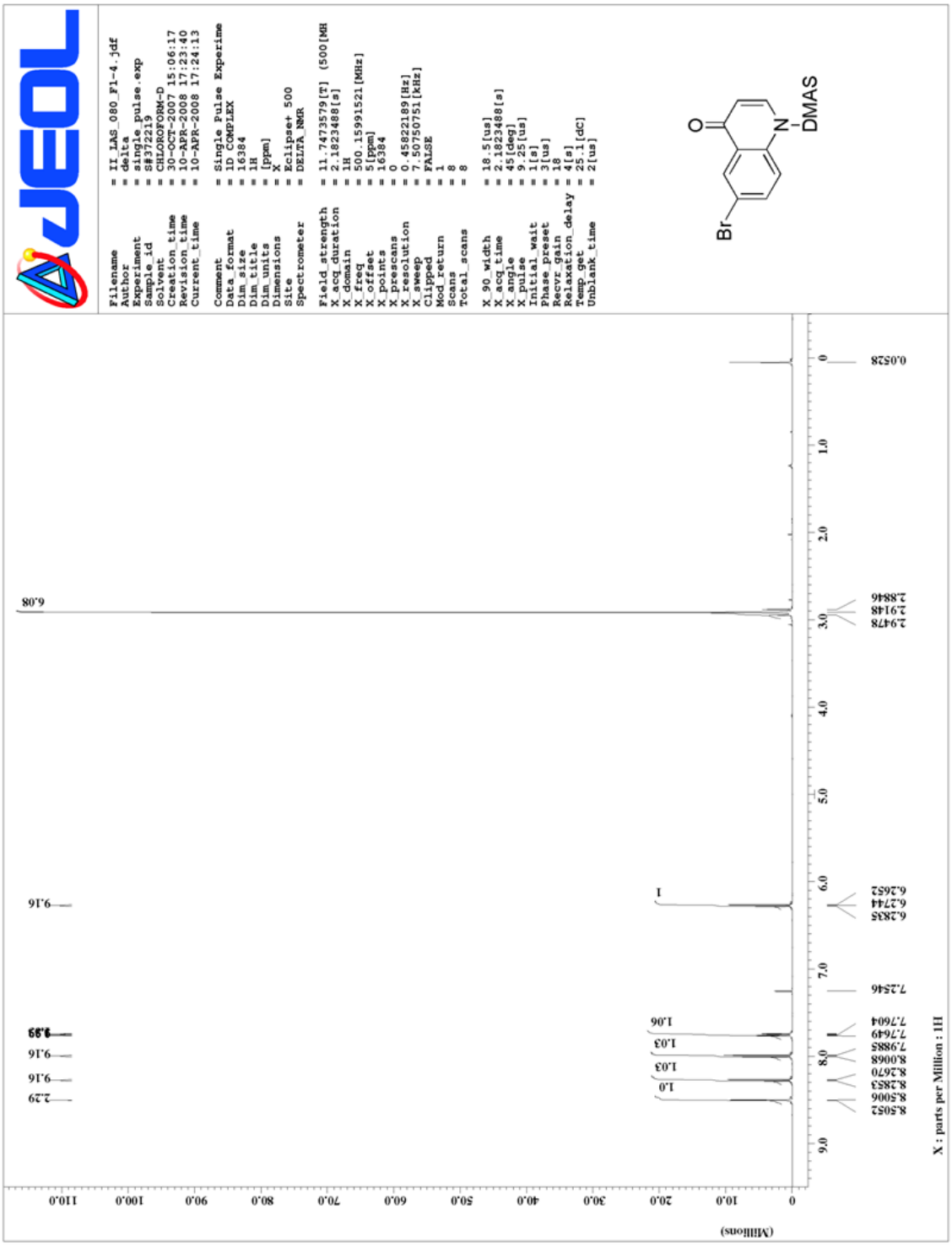

O
N
MOM
Br
1.01
1.95
9.04
2.44
7.3712
7.3562
7.3333
7.2962
7.1796
7.1171
7.1147
7.5203
7.5388
7.5505
7.5662
2.03
5.3679
7.82
1.0
6.2579
6.2501
6.2423
(Millions)
X : parts per Million : 1H

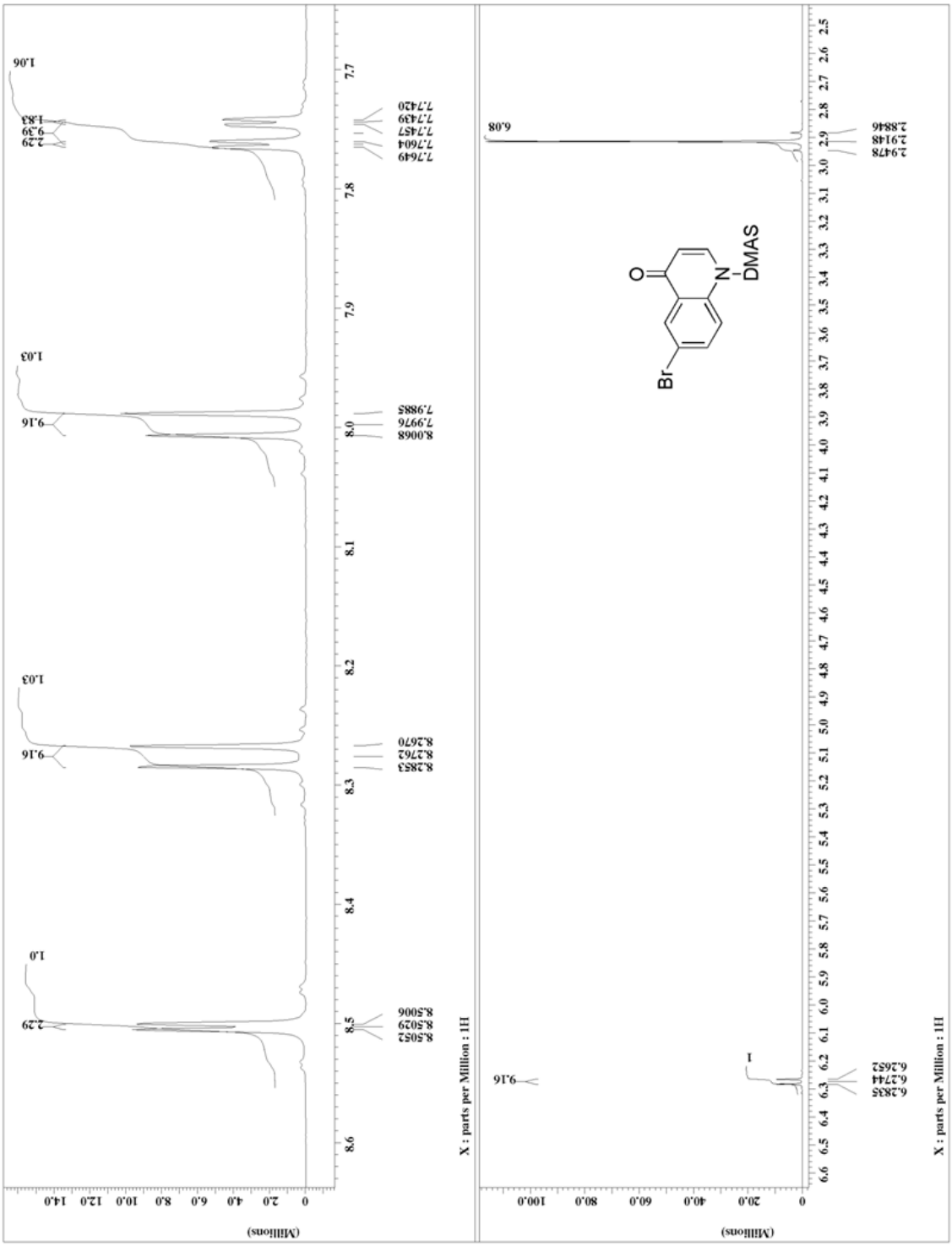

abundance
0
0.1
0.2
0.3
200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0
177.2021
142.9273
138.5485
135.4413
129.4563
128.3569
119.0257
118.3564
110.2203
84.4926
56.2505
X : parts per Million : 13C
JEOL
Filename = I_LAS_191cr-4.jdf
Author = delta
Experiment = single_pulse_dec
Sample_id = S#569501
Solvent = CHLOROFORM-D
Creation_time = 12-OCT-2005 17:26:59
Revision_time = 12-OCT-2005 16:22:43
Current_time = 14-APR-2008 19:33:07
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 302
Total_scans = 302
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 5[s]
Recvr_gain = 50
Relaxation_delay = 5[s]
Repetition_time = 6.38412032[s]
Temp_get = 22.8[dC]
Br
O
N
MOM

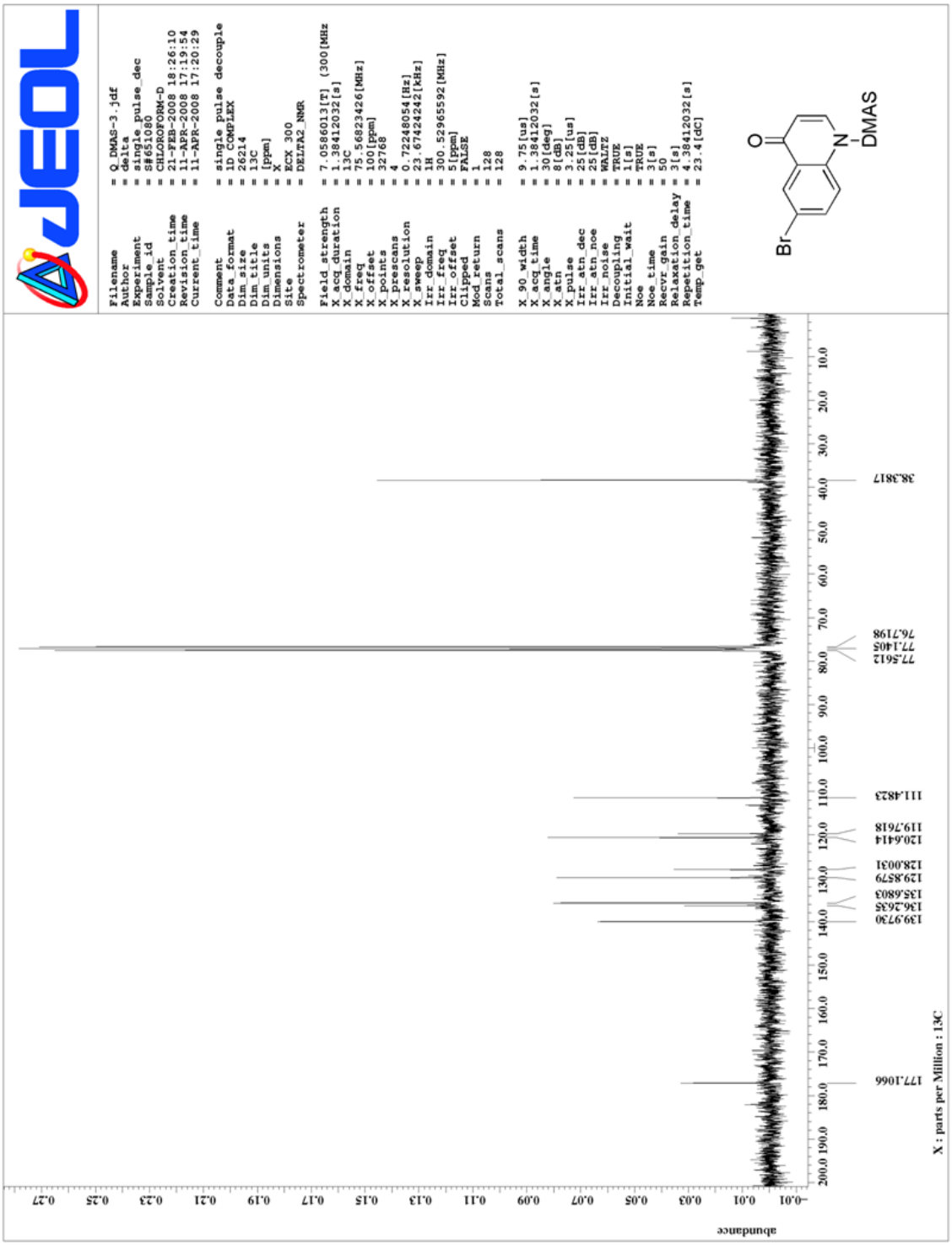
APPENDIX J

^{1}H NMR AND ^{13}C NMR SPECTRA OF

1-*N*-DIMETHYLAMINOSULFONYL-6-BROMO-4(*1H*)-QUINOLONE (133a)


Filename = II_LAS_080_F1-4.jdf
Author = delta
Experiment = single_pulse.exp
Sample_id = S#72219
Solvent = CHLOROFORM-D
Creation_time = 30-OCT-2007 15:06:17
Revision_time = 10-APR-2008 17:23:40
Current_time = 10-APR-2008 17:24:13
Comment = Single Pulse Experime
Data_format = 1D COMPLEX
Dim_size = 16384
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = Eclipse+ 500
Spectrometer = DELTA_NMR
Field_strength = 11.74735579[T] (500[MH
X_acq_duration = 2.18234880[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.45822189[Hz]
X_sweep = 7.50750751[kHz]
Clipped = FALSE
Mod_return = 1
Scans = 8
Total_scans = 8
X_90_width = 18.5[us]
X_acq_time = 2.18234880[s]
X_angle = 45[deg]
X_pulse = 9.25[us]
Initial_wait = 1[s]
Phase_preset = 3[us]
Recvr_gain = 18
Relaxation_delay = 4[s]
Temp_get = 25.1[dC]
Unblank_time = 2[us]
Br
O
N
DMAS
6.08
9.16
9.99
9.16
9.16
2.29
1.0
1.03
1.03
1.06
I
0.0528
2.8846
2.9148
2.9478
6.2652
6.2744
6.2835
7.2546
7.7604
7.7649
7.9885
8.0068
8.2670
8.2853
8.5006
8.5052
(MHHons)
X : parts per Million : 1H

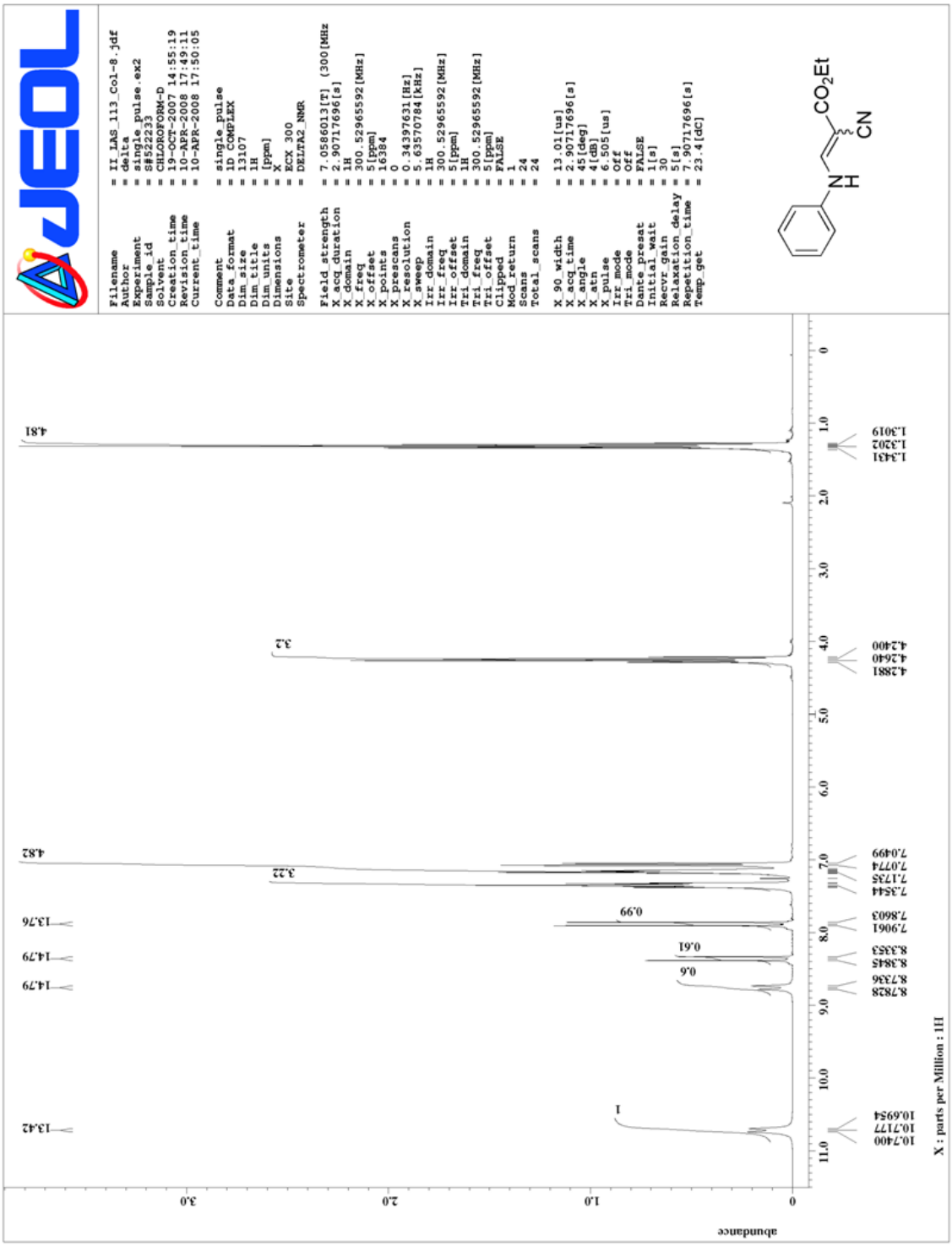

DMAS
Br
O
N
X : parts per Million : 1H
(Millions)
9.16
6.08
6.2835
6.2744
6.2652
2.9478
2.9148
2.8846
8.5052
8.5029
8.5006
8.3853
8.3762
8.2670
8.0068
7.9976
7.9885
7.7649
7.7604
7.7457
7.7439
7.7420
2.29
9.39
1.83
1.06
9.16
1.03
1.0

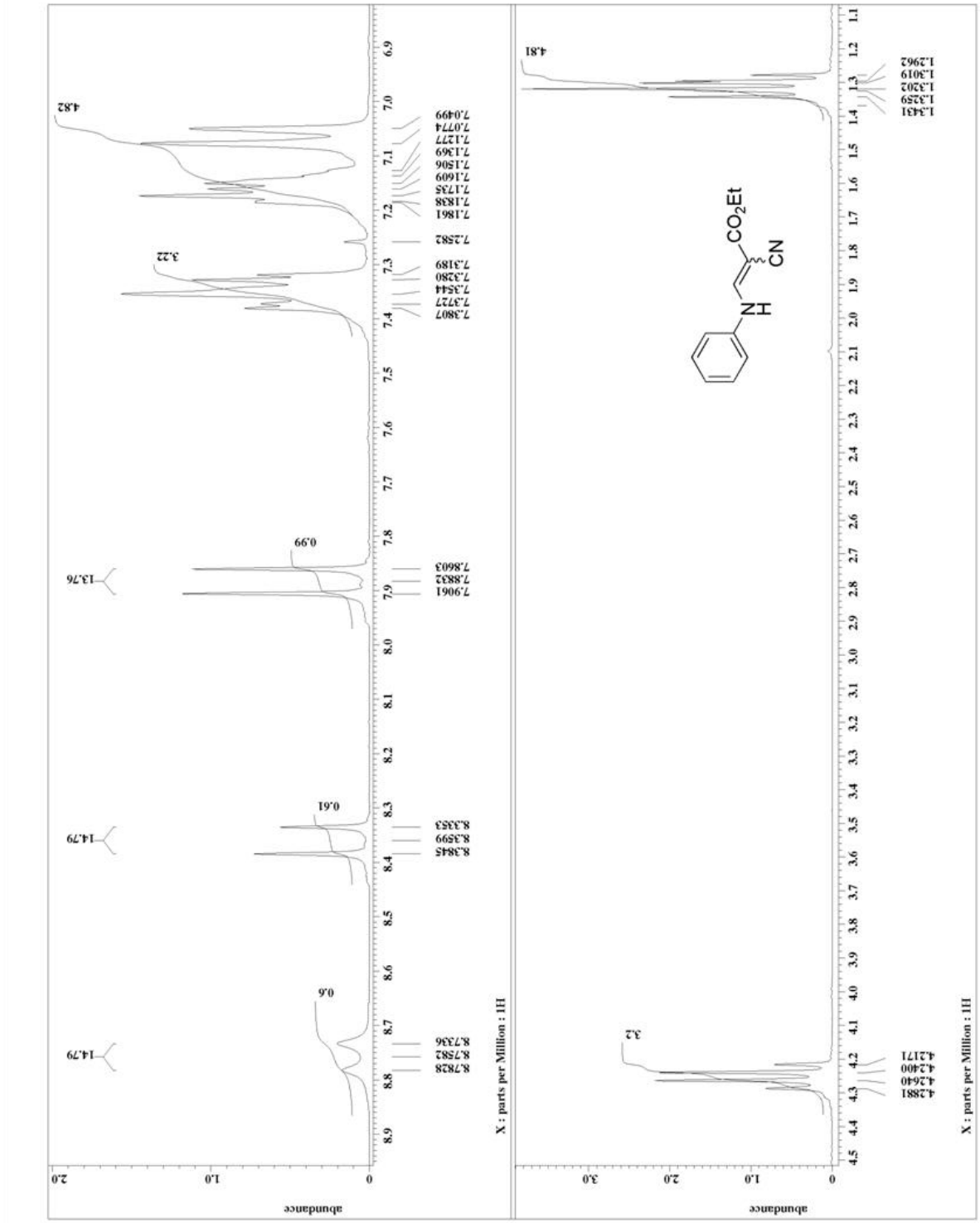

JEOL
Filename = Q_DMAS-3.jdf
Author = delta
Experiment = single_pulse_dec
Sample_id = S#651080
Solvent = CHLOROFORM-D
Creation_time = 21-FEB-2008 18:26:10
Revision_time = 11-APR-2008 17:19:54
Current_time = 11-APR-2008 17:20:29
Comment = single_pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX_300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 128
Total_scans = 128
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 3[s]
Recvr_gain = 50
Relaxation_delay = 3[s]
Repetition_time = 4.38412032[s]
Temp_get = 23.4[dC]
O
N
DMAS
Br
abundance
X : parts per Million : 13C
38.3817
76.7198
77.1405
77.5612
111.4823
119.7618
120.6414
128.0031
129.8579
135.6803
136.2635
139.9730
177.1066

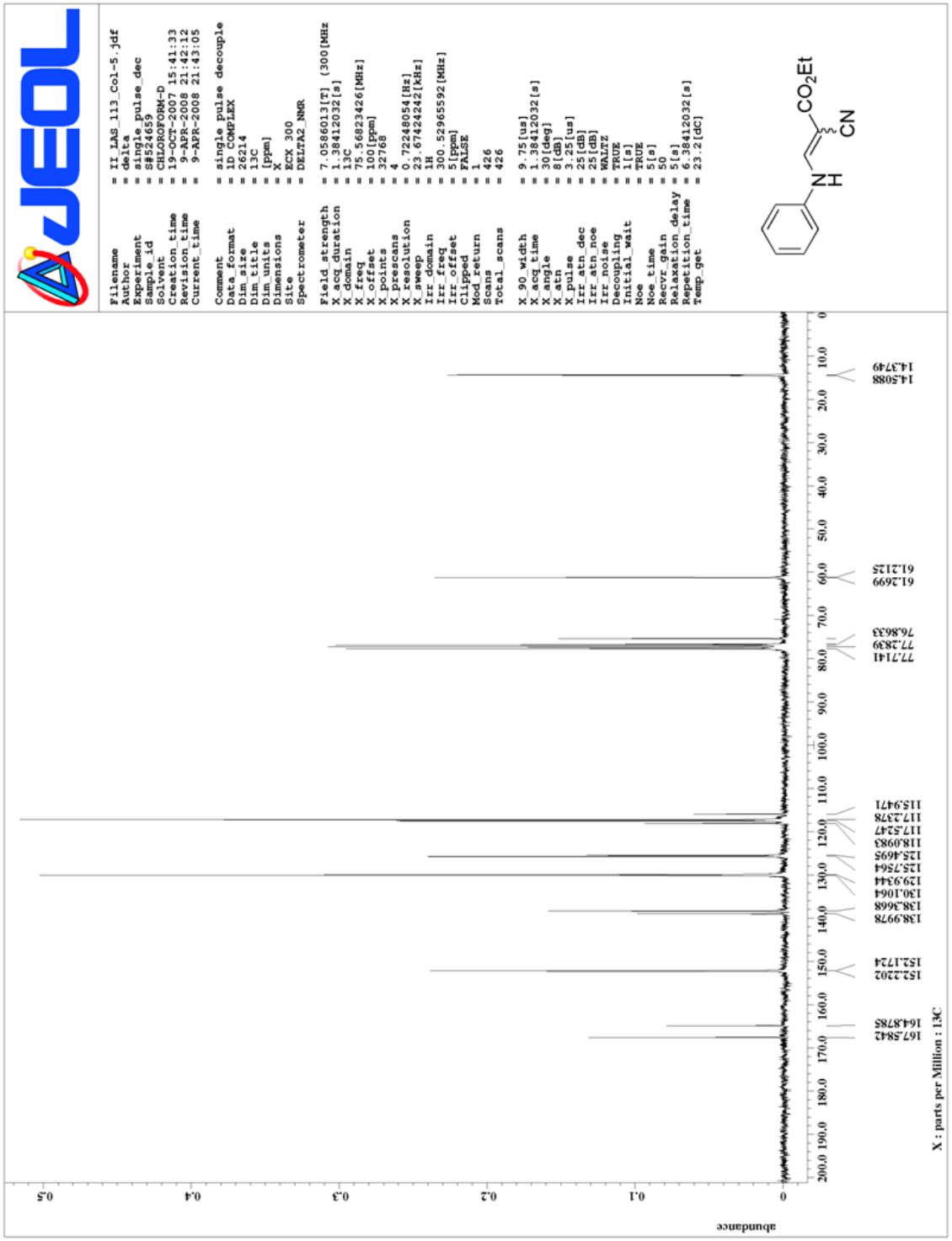
APPENDIX K

^{1}H NMR AND ^{13}C NMR SPECTRA OF

ETHYL 3-[(PHENYL)AMINO]-2-CYANO-2-PROPENOATE (144a)


JEOL
Filename = II_LAS_113_Col-8.jdf
Author = delta
Experiment = single_pulse.ex2
Sample_id = S#522233
Solvent = CHLOROFORM-D
Creation_time = 19-OCT-2007 14:55:19
Revision_time = 10-APR-2008 17:49:11
Current_time = 10-APR-2008 17:50:05
Comment = single_pulse
Data_format = 1D COMPLEX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 2.90717696[s]
X_domain = 1H
X_freq = 300.52965592[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.34397631[Hz]
X_sweep = 5.63570784[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Tri_domain = 1H
Tri_freq = 300.52965592[MHz]
Tri_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 24
Total_scans = 24
X_90_width = 13.01[us]
X_acq_time = 2.90717696[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 6.505[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Recvr_gain = 30
Relaxation_delay = 5[s]
Repetition_time = 7.90717696[s]
Temp_get = 23.4[dC]
CO2Et
CN
NH
abundance
X : parts per Million : 1H
10.7400
10.7177
10.6954
8.7828
8.7336
8.3845
8.3353
7.9061
7.8603
7.3544
7.1735
7.0774
7.0499
4.2881
4.2640
4.2400
1.3431
1.3202
1.3019
13.42
14.79
14.79
13.76
4.82
3.22
4.81
3.2
0.99
0.61
0.6
1


abundance
X : parts per Million : 1H
4.81
3.2
4.2881
4.2640
4.2400
4.2171
1.3431
1.3259
1.3202
1.3019
1.2962
CO2Et
CN
NH
4.82
3.22
0.99
0.61
0.6
13.76
14.79
14.79
7.9061
7.8832
7.8603
8.3845
8.3599
8.3353
8.7828
8.7582
8.7336
7.3807
7.3727
7.3544
7.3280
7.3189
7.2582
7.1861
7.1838
7.1735
7.1609
7.1506
7.1369
7.1277
7.0774
7.0499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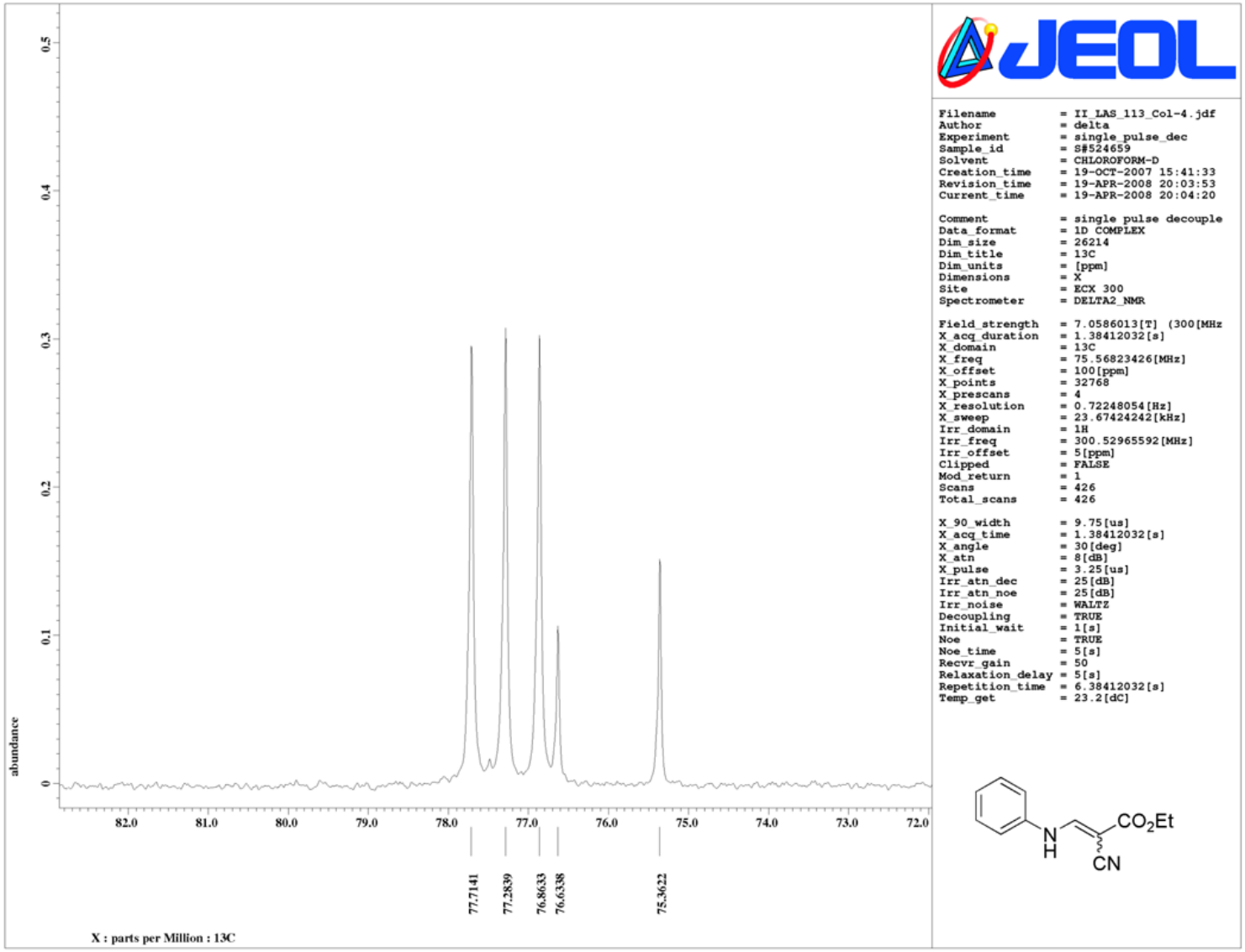
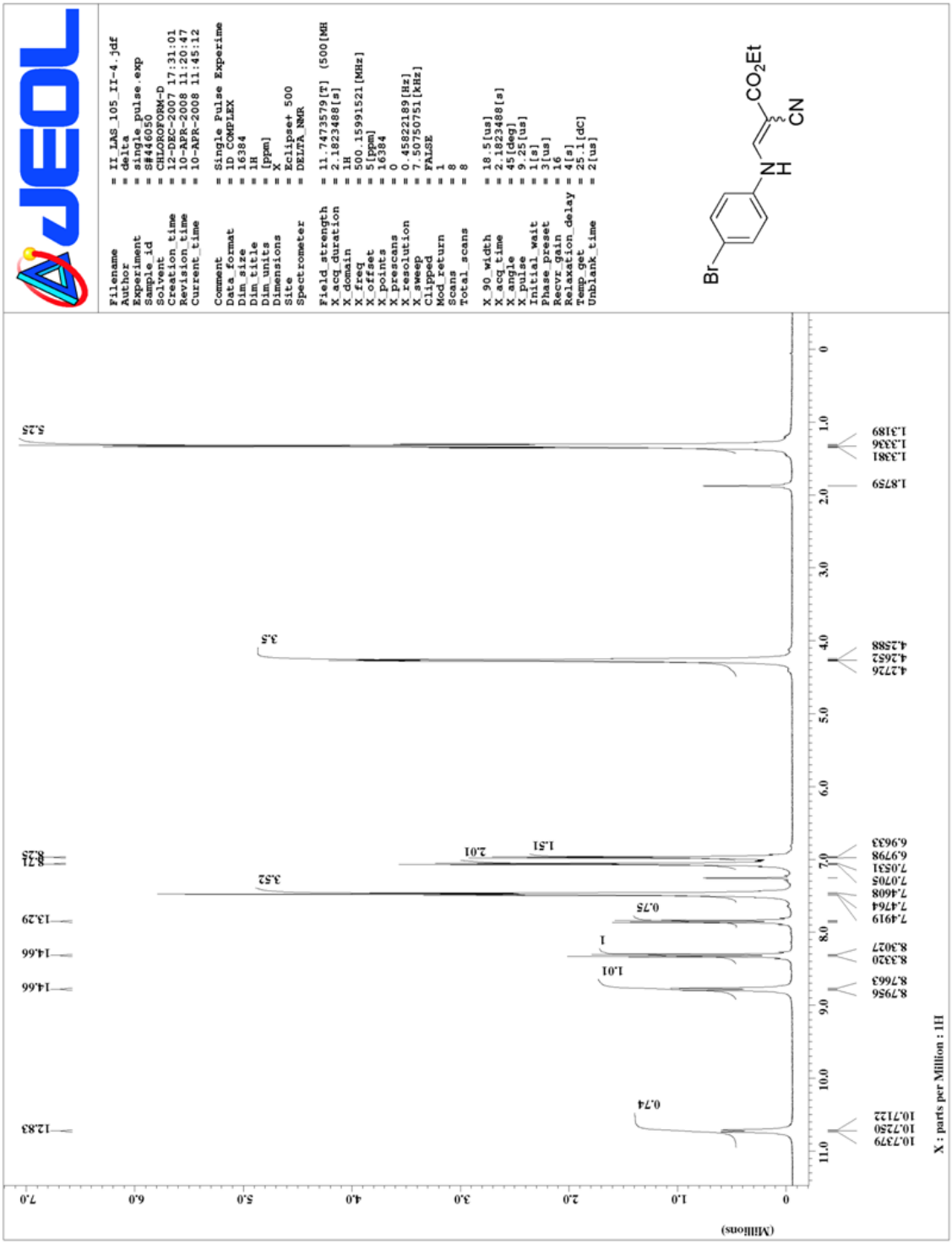
Filename = II_LAS_113_Col-5.jdf
Author = delta
Experiment = single_pulse_dec
Sample_id = SHS24609
Solvent = CDCl3
Creation_time = 19-OCT-2007 15:41:33
Revision_time = 9-APR-2008 21:42:12
Current_time = 9-APR-2008 21:43:05
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 426
Total_scans = 426
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 5[s]
Recvr_gain = 50
Relaxation_delay = 5[s]
Repetition_time = 6.38412032[s]
Temp_get = 23.2[dC]
CO2Et
NH
CN
abundance
X : parts per Million : 13C
14.5088
14.3749
61.2699
61.2125
77.7141
77.2839
76.8633
167.5842
164.8785
152.2202
152.1724
138.9978
138.3668
130.1064
129.9344
125.7564
125.4695
118.0983
117.5247
117.2378
115.9471

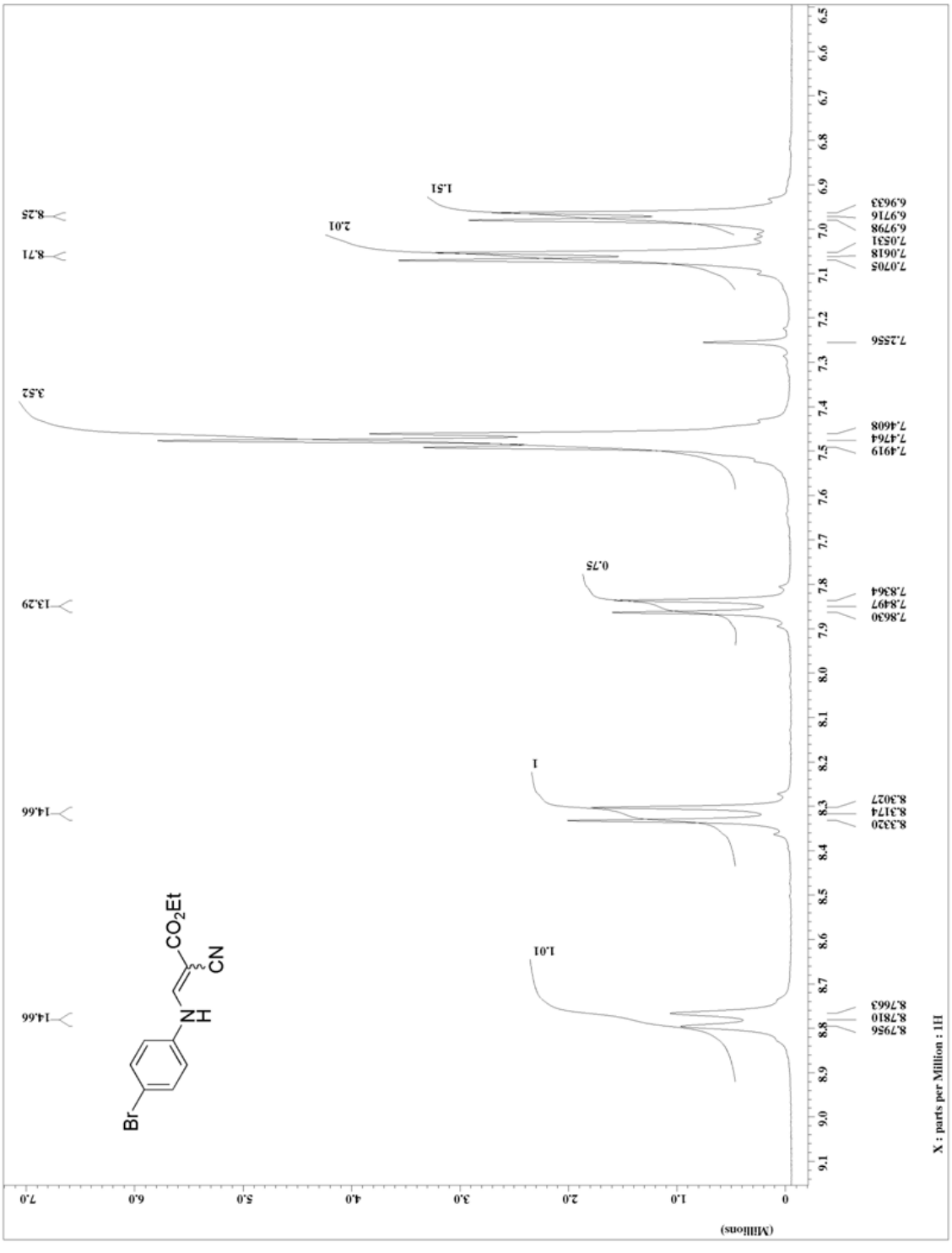

abundance
0.5
0.4
0.3
0.2
0.1
0
82.0
81.0
80.0
79.0
78.0
77.0
76.0
75.0
74.0
73.0
72.0
77.7141
77.2839
76.8633
76.6338
75.3622
X : parts per Million : 13C
JEOL
Filename = II_LAS_113_Col-4.jdf
Author = delta
Experiment = single_pulse_dec
Sample_id = S#524659
Solvent = CHLOROFORM-D
Creation_time = 19-OCT-2007 15:41:33
Revision_time = 19-APR-2008 20:03:53
Current_time = 19-APR-2008 20:04:20
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 426
Total_scans = 426
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 5[s]
Recvr_gain = 50
Relaxation_delay = 5[s]
Repetition_time = 6.38412032[s]
Temp_get = 23.2[dC]
N
H
CO2Et
CN

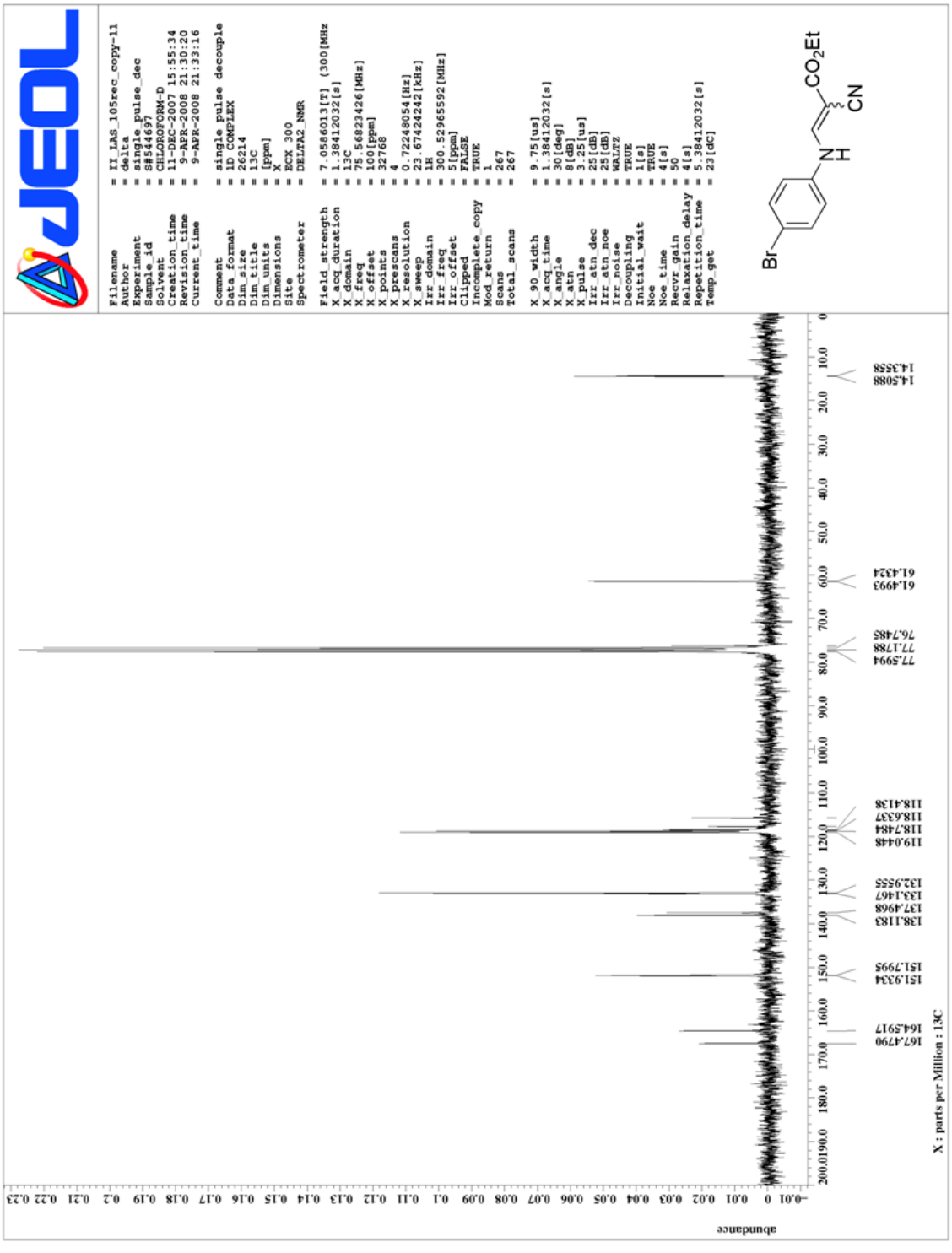
APPENDIX L

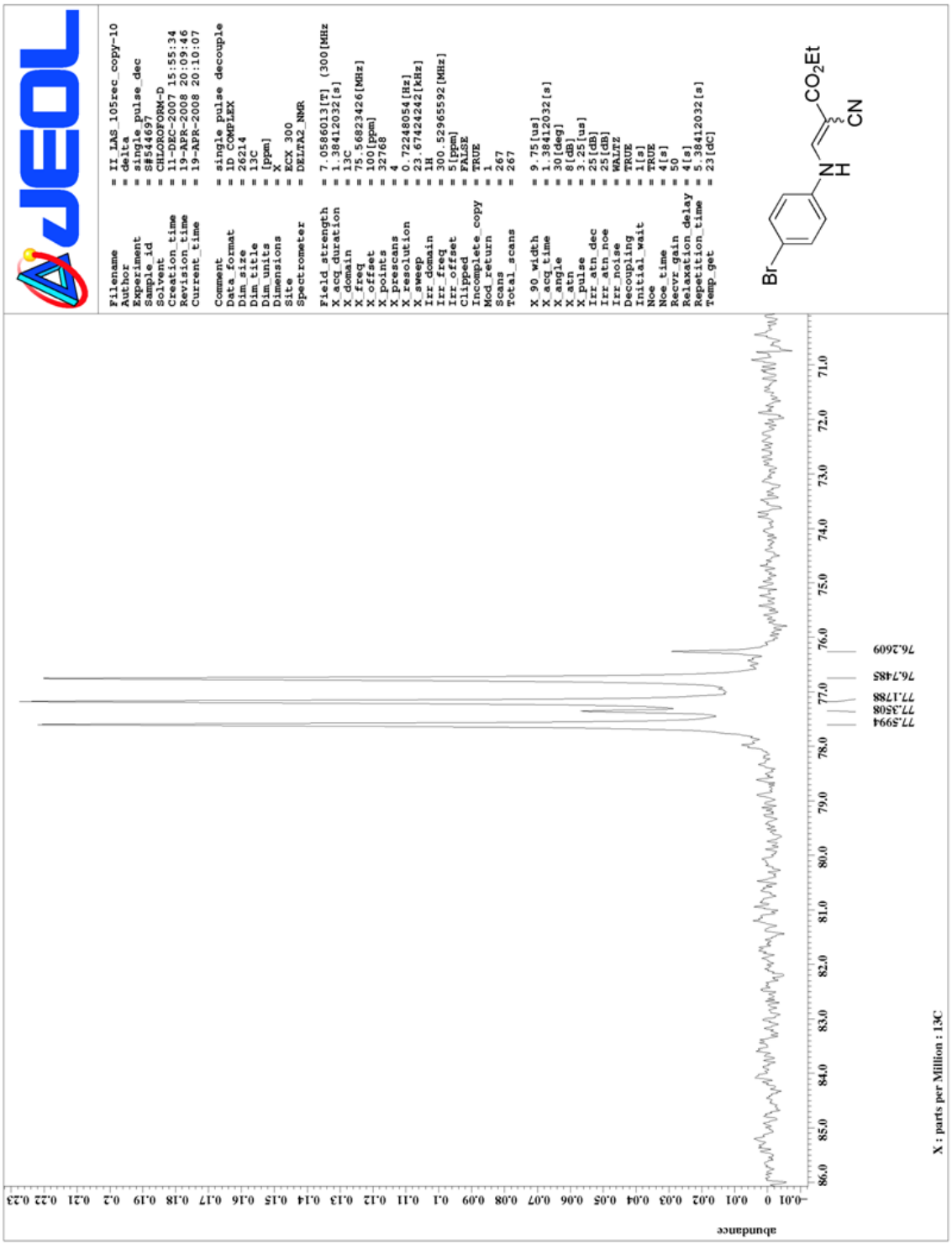
^{1}H NMR AND ^{13}C NMR SPECTRA OF

ETHYL 3-[(4-BROMOPHENYL)AMINO]-2-CYANO-2-PROPENOATE (144b)


JEOL
Filename = II_LAS_105_II-4.jdf
Author = delta
Experiment = single_pulse.exp
Sample_id = S#446050
Solvent = CHLOROFORM-D
Creation_time = 12-DEC-2007 17:31:01
Revision_time = 10-APR-2008 11:20:47
Current_time = 10-APR-2008 11:45:12
Comment = Single Pulse Experime
Data_format = 1D COMPLEX
Dim_size = 16384
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = Eclipse+ 500
Spectrometer = DELTA_NMR
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 2.1823488[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.45822189[Hz]
X_sweep = 7.50750751[kHz]
Clipped = FALSE
Mod_return = 1
Scans = 8
Total_scans = 8
X_90_width = 18.5[us]
X_acq_time = 2.1823488[s]
X_angle = 45[deg]
X_pulse = 9.25[us]
Initial_wait = 1[s]
Phase_preset = 3[us]
Recvr_gain = 16
Relaxation_delay = 4[s]
Temp_get = 25.1[dC]
Unblank_time = 2[us]
Br
N
H
CN
CO2Et
(Millions)
X : parts per Million : 1H


Br
N
H
CN
CO2Et
14.66
14.66
13.29
3.52
8.71
8.25
2.01
1.51
1.01
1
0.75
8.7956
8.7810
8.7663
8.3320
8.3174
8.3027
7.8630
7.8497
7.8364
7.4919
7.4764
7.4608
7.2556
7.0705
7.0618
7.0531
6.9798
6.9716
6.9633
X : parts per Million : 1H
(Millions)

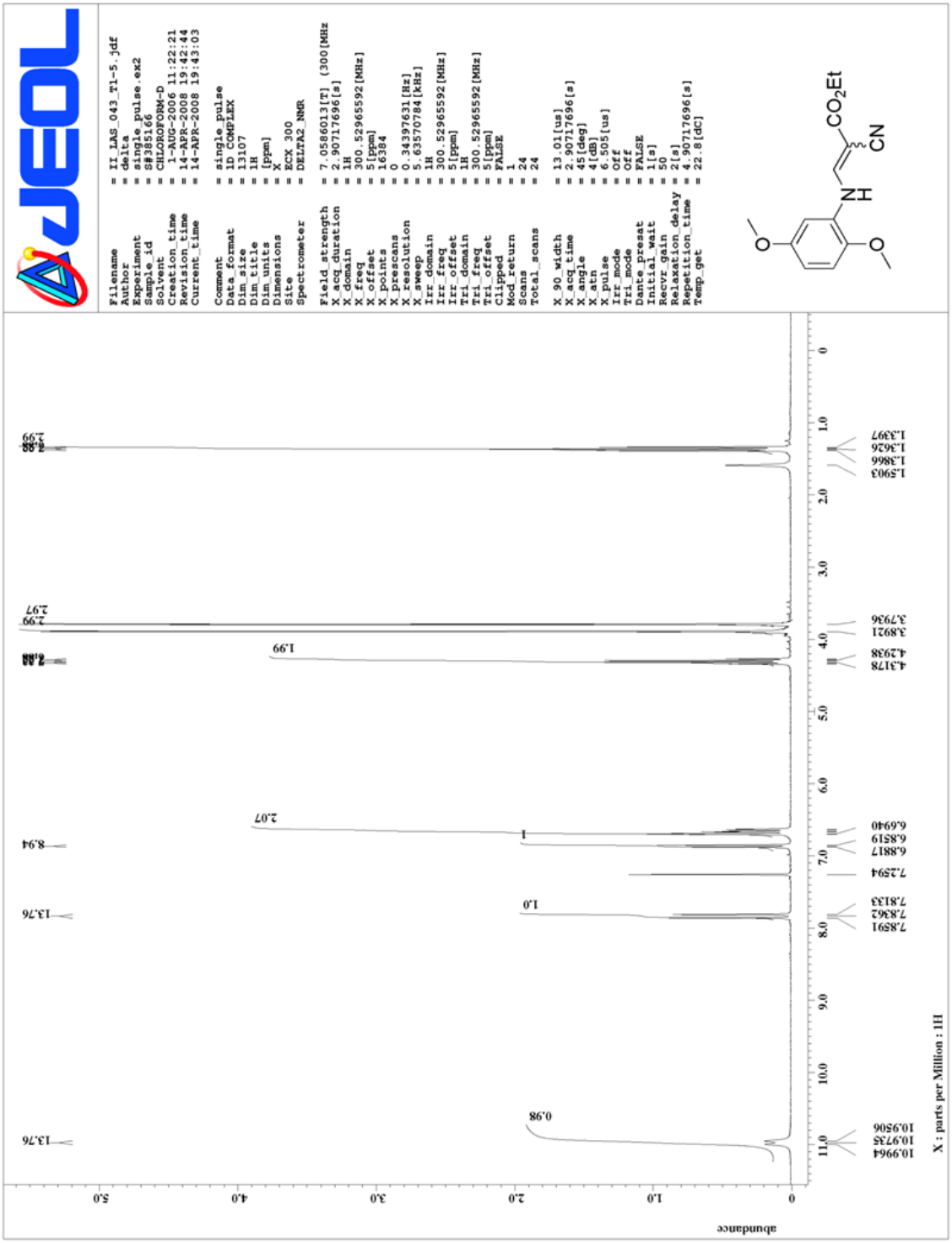

JEOL
Filename = IT_LAS_105rec_copy-11
Author = delta
Experiment = single_pulse_dec
Sample_id = S#544697
Solvent = CHLOROFORM-D
Creation_time = 11-DEC-2007 15:55:34
Revision_time = 9-APR-2008 21:30:20
Current_time = 9-APR-2008 21:33:16
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Incomplete_copy = TRUE
Mod_return = 1
Scans = 267
Total_scans = 267
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 4[s]
Recyc_gain = 50
Relaxation_delay = 4[s]
Repetition_time = 5.38412032[s]
Temp_get = 23[dC]
Br
N
H
CO2Et
CN
abundance
X : parts per Million : 13C
14.5088
14.3558
61.4993
61.4324
77.5994
77.1788
76.7485
119.0448
118.7484
118.6337
118.4138
138.1183
137.4968
133.1467
132.9555
151.9334
151.7995
167.4790
164.5917


JEOL
Filename = II_LAS_105rec_copy-10
Author = delta
Experiment = single_pulse_dec
Sample_id = S#544697
Solvent = CHLOROFORM-D
Creation_time = 11-DEC-2007 15:55:34
Revision_time = 19-APR-2008 20:09:46
Current_time = 19-APR-2008 20:10:07
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Incomplete_copy = TRUE
Mod_return = 1
Scans = 267
Total_scans = 267
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 4[s]
Recvr_gain = 50
Relaxation_delay = 4[s]
Repetition_time = 5.38412032[s]
Temp_get = 23[dC]
Br
N
H
CO2Et
CN
abundance
X : parts per Million : 13C
77.5994
77.3508
77.1788
76.7485
76.2609

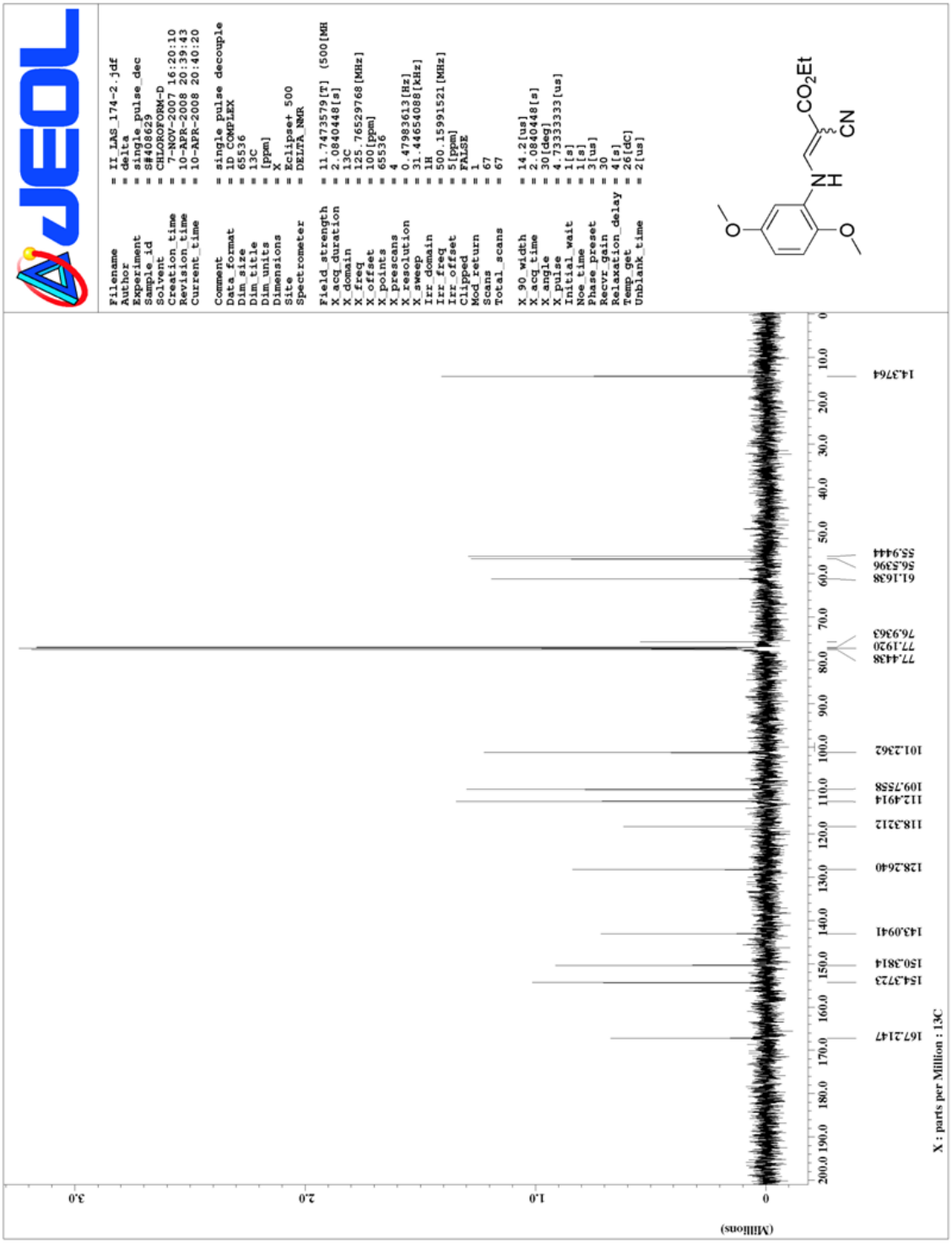
APPENDIX M

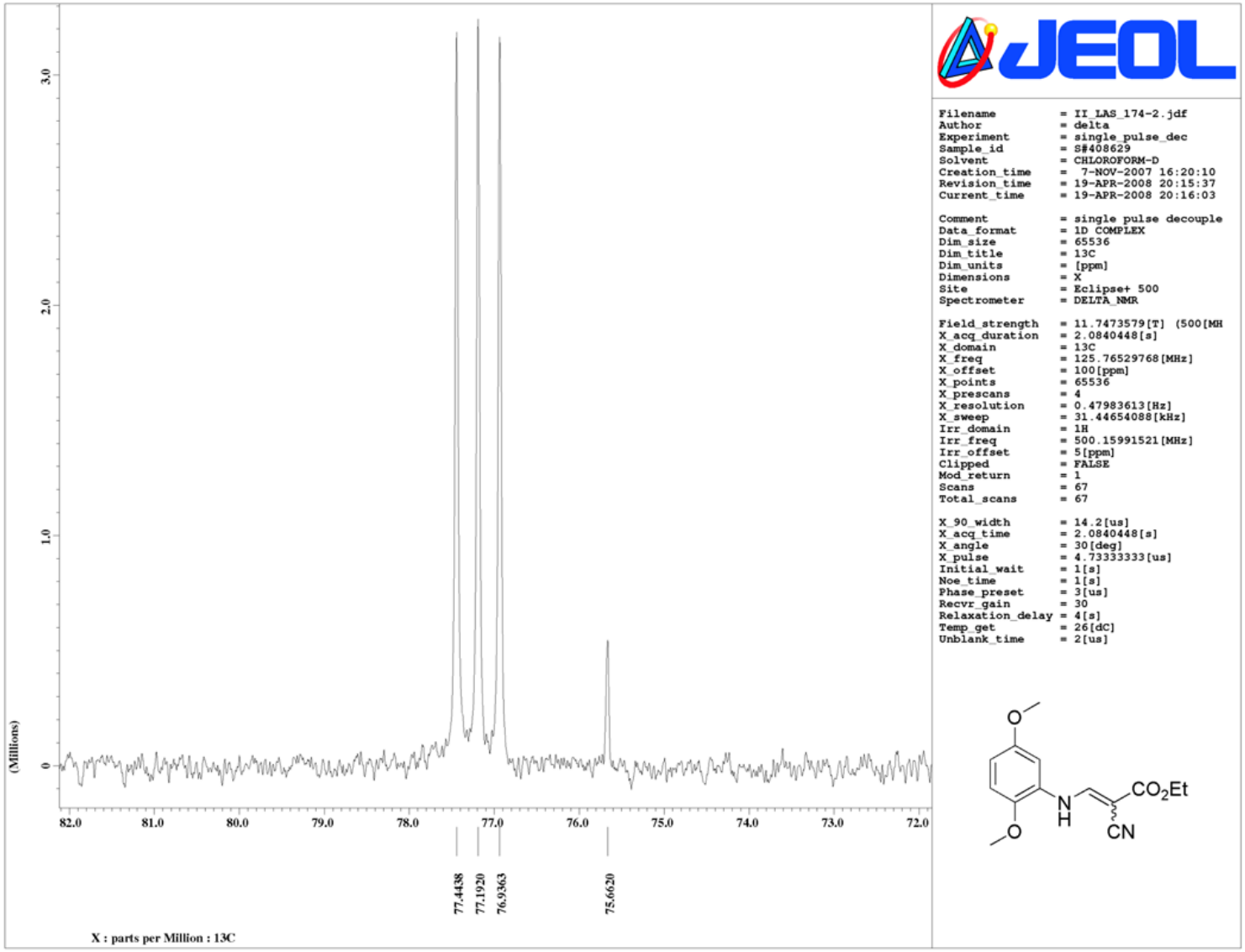
^{1}H NMR AND ^{13}C NMR SPECTRA OF

ETHYL 3-[(2,5-DIMETHOXYPHENYL)AMINO]-2-CYANO-2-PROPENOATE (144c)


JEOL
Filename = II_LAS_043_T1-5.jdf
Author = delta
Experiment = single_pulse.ex2
Sample_id = S#385166
Solvent = CHLOROFORM-D
Creation_time = 1-AUG-2006 11:22:21
Revision_time = 14-APR-2008 19:42:44
Current_time = 14-APR-2008 19:43:03
Comment = single_pulse
Data_format = 1D COMPLEX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 2.90717696[s]
X_domain = 1H
X_freq = 300.52965592[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.34397631[Hz]
X_sweep = 5.63570784[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Tri_domain = 1H
Tri_freq = 300.52965592[MHz]
Tri_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 24
Total_scans = 24
X_90_width = 13.01[us]
X_acq_time = 2.90717696[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 6.505[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Recvr_gain = 50
Relaxation_delay = 2[s]
Repetition_time = 4.90717696[s]
Temp_get = 22.8[dC]
CO2Et
CN
NH
O
O
abundance
X : parts per Million : 1H

CO2Et
CN
NH
O
O
X : parts per Million : 1H
abundance
7.8591
7.8362
7.8133
7.2594
6.8817
6.8668
6.8519
6.7020
6.6940
6.6676
6.6596
6.6379
6.6299
13.76
1.0
8.94
1
2.07
4.3419
4.3178
4.2938
4.2709
3.8921
3.7936
1.5903
1.3866
1.3626
1.3511
1.3397
7.33
7.32
6.88
2.99
2.97
1.99

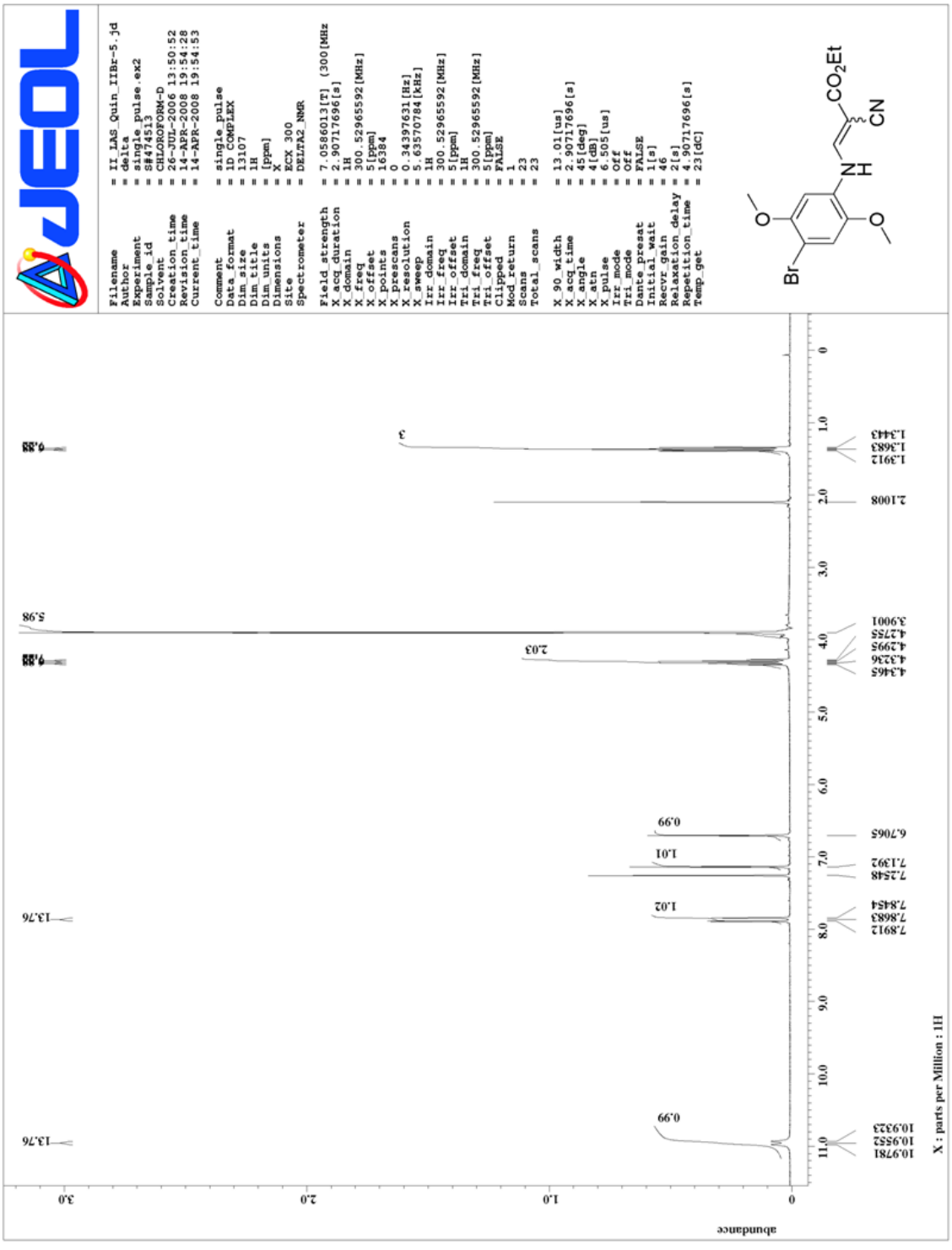

JEOL
Filename = II_LAS_174-2.jdf
Author = delta
Experiment = single_pulse_dec
Sample_id = S#408629
Solvent = CHLOROFORM-D
Creation_time = 7-NOV-2007 16:20:10
Revision_time = 10-APR-2008 20:39:43
Current_time = 10-APR-2008 20:40:20
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 65536
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = Eclipse+ 500
Spectrometer = DELTA_NMR
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 2.0840448[s]
X_domain = 13C
X_freq = 125.76529768[MHz]
X_offset = 100[ppm]
X_points = 65536
X_prescans = 4
X_resolution = 0.47983613[Hz]
X_sweep = 31.44654088[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 67
Total_scans = 67
X_90_width = 14.2[us]
X_acq_time = 2.0840448[s]
X_angle = 30[deg]
X_pulse = 4.73333333[us]
Initial_wait = 1[s]
Noe_time = 1[s]
Phase_preset = 3[us]
Recvr_gain = 30
Relaxation_delay = 4[s]
Temp_get = 26[dC]
Unblank_time = 2[us]
CO2Et
CN
N
H
O
O
(Millions)
3.0
2.0
1.0
0
14.3764
55.9444
56.5396
61.1638
76.9363
77.1920
77.4438
101.3362
109.7558
112.4914
118.3212
128.2640
143.0941
150.3814
154.3723
167.2147
X : parts per Million : 13C


(Millions)
3.0
2.0
1.0
0
82.0
81.0
80.0
79.0
78.0
77.0
76.0
75.0
74.0
73.0
72.0
77.4438
77.1920
76.9363
75.6620
X : parts per Million : 13C
JEOL
Filename = II_LAS_174-2.jdf
Author = delta
Experiment = single_pulse_dec
Sample_id = S#408629
Solvent = CHLOROFORM-D
Creation_time = 7-NOV-2007 16:20:10
Revision_time = 19-APR-2008 20:15:37
Current_time = 19-APR-2008 20:16:03
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 65536
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = Eclipse+ 500
Spectrometer = DELTA_NMR
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 2.0840448[s]
X_domain = 13C
X_freq = 125.76529768[MHz]
X_offset = 100[ppm]
X_points = 65536
X_prescans = 4
X_resolution = 0.47983613[Hz]
X_sweep = 31.44654088[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 67
Total_scans = 67
X_90_width = 14.2[us]
X_acq_time = 2.0840448[s]
X_angle = 30[deg]
X_pulse = 4.73333333[us]
Initial_wait = 1[s]
Noe_time = 1[s]
Phase_preset = 3[us]
Recvr_gain = 30
Relaxation_delay = 4[s]
Temp_get = 26[dC]
Unblank_time = 2[us]
O
O
N
H
CO2Et
CN

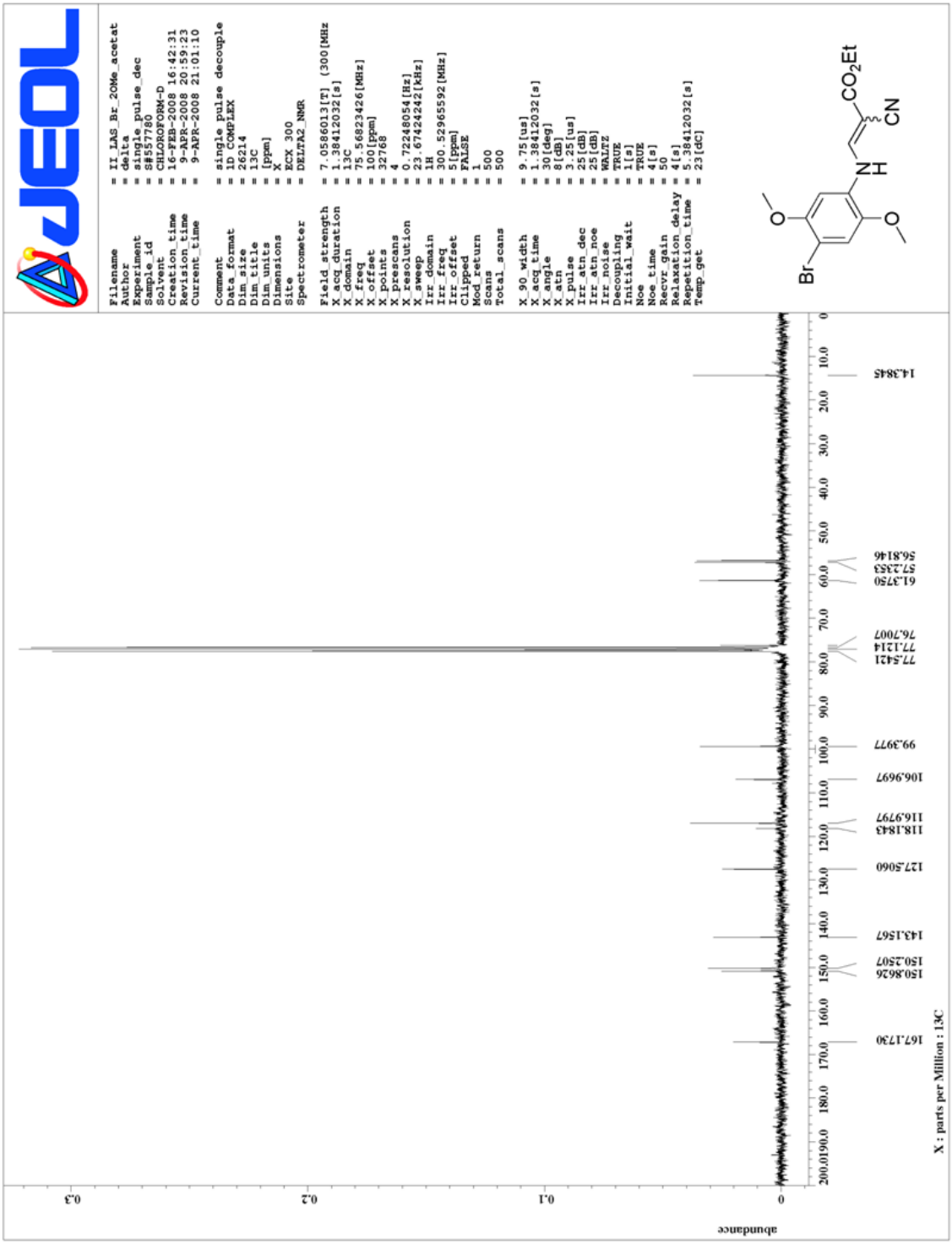
APPENDIX N

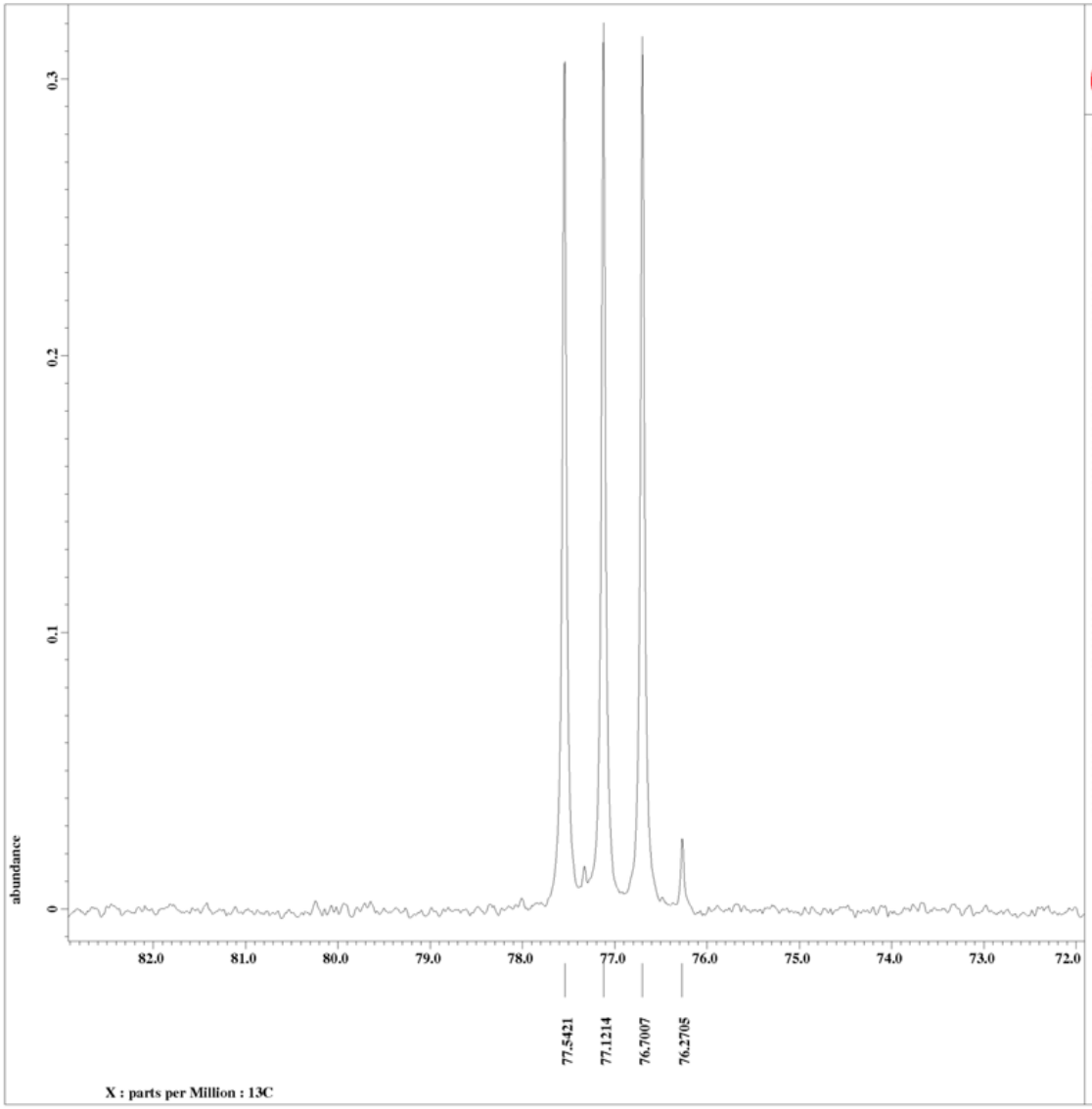
^{1}H NMR AND ^{13}C NMR SPECTRA OF

ETHYL 3-[(4-BROMO-2,5-DIMETHOXYPHENYL)AMINO]-2-CYANO-2-PROPENOATE (144d)


JEOL
Filename = IT_LAS_Quin_IIBr-5.jd
Author = delta
Experiment = single_pulse.ex2
Sample_id = S#474513
Solvent = CHLOROFORM-D
Creation_time = 26-JUL-2006 13:50:52
Revision_time = 14-APR-2008 19:54:28
Current_time = 14-APR-2008 19:54:53
Comment = single_pulse
Data_format = 1D COMPLEX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 2.90717696[s]
X_domain = 1H
X_freq = 300.52965592[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.34397631[Hz]
X_sweep = 5.63570784[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Tri_domain = 1H
Tri_freq = 300.52965592[MHz]
Tri_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 23
Total_scans = 23
X_90_width = 13.01[us]
X_acq_time = 2.90717696[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 6.505[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Recvr_gain = 46
Relaxation_delay = 2[s]
Repetition_time = 4.90717696[s]
Temp_get = 23[dC]
Br
O
N
H
CN
CO2Et
abundance
X : parts per Million : 1H

abundance
X : parts per Million : 1H
7.8912
7.8683
7.8454
7.2548
7.1392
6.7065
13.76
1.02
1.01
0.99
4.3465
4.3236
4.2995
4.2755
2.03
5.98
3.9001
2.1008
1.3912
1.3683
1.3563
1.3443
3
Br
O
O
N
H
CN
CO2Et


JEOL
Filename = II_LAS_Br_2OMe_acetat
Author = delta
Experiment = single_pulse_dec
Sample_id = S#557780
Solvent = CHLOROFORM-D
Creation_time = 16-FEB-2008 16:42:31
Revision_time = 9-APR-2008 20:59:23
Current_time = 9-APR-2008 21:01:10
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 500
Total_scans = 500
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 4[s]
Recyr_gain = 50
Relaxation_delay = 4[s]
Repetition_time = 5.38412032[s]
Temp_get = 23[dC]
Br
N
H
CN
CO2Et
abundance
0
0.1
0.2
0.3
X : parts per Million : 13C
200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0
167.1730
150.8626
150.2507
143.1567
127.5060
118.1843
116.9797
106.9697
99.3977
77.5421
77.1214
76.7007
61.3750
57.2353
56.8146
14.3845

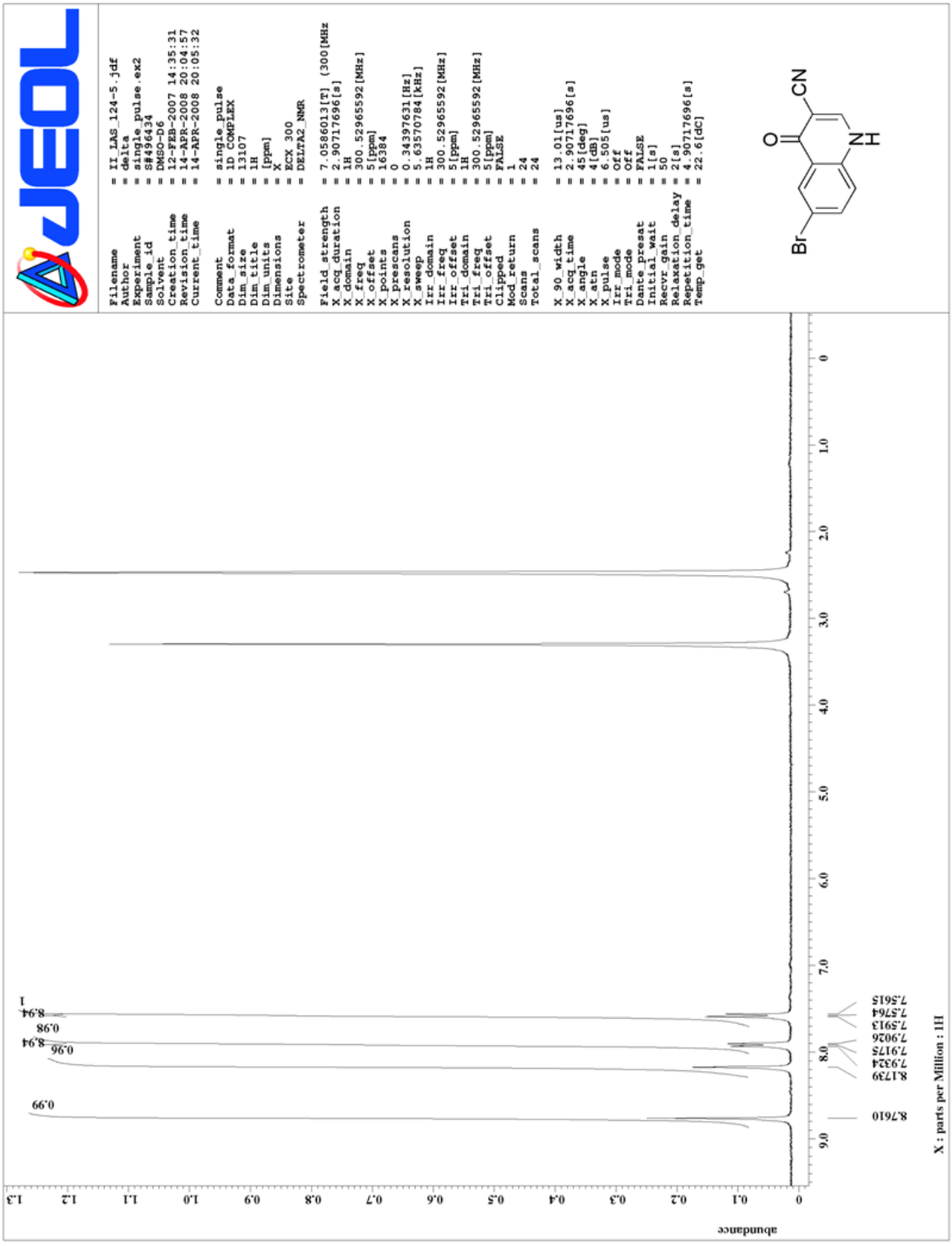

abundance
0.3
0.2
0.1
0
82.0
81.0
80.0
79.0
78.0
77.0
76.0
75.0
74.0
73.0
72.0
77.5421
77.1214
76.7007
76.2705
X : parts per Million : 13C


Filename = II_LAS_Br_2OMe_acetat
Author = delta
Experiment = single_pulse_dec
Sample_id = S#557780
Solvent = CHLOROFORM-D
Creation_time = 16-FEB-2008 16:42:31
Revision_time = 19-APR-2008 20:26:36
Current_time = 19-APR-2008 20:27:09
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 500
Total_scans = 500
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 4[s]
Recvr_gain = 50
Relaxation_delay = 4[s]
Repetition_time = 5.38412032[s]
Temp_get = 23[dC]
Br
O
N
H
O
CO2Et
CN

APPENDIX O

^{1}H NMR AND ^{13}C NMR SPECTRA OF

6-BROMO-3-CYANO-4(*1H*)-QUINOLONE (145b)

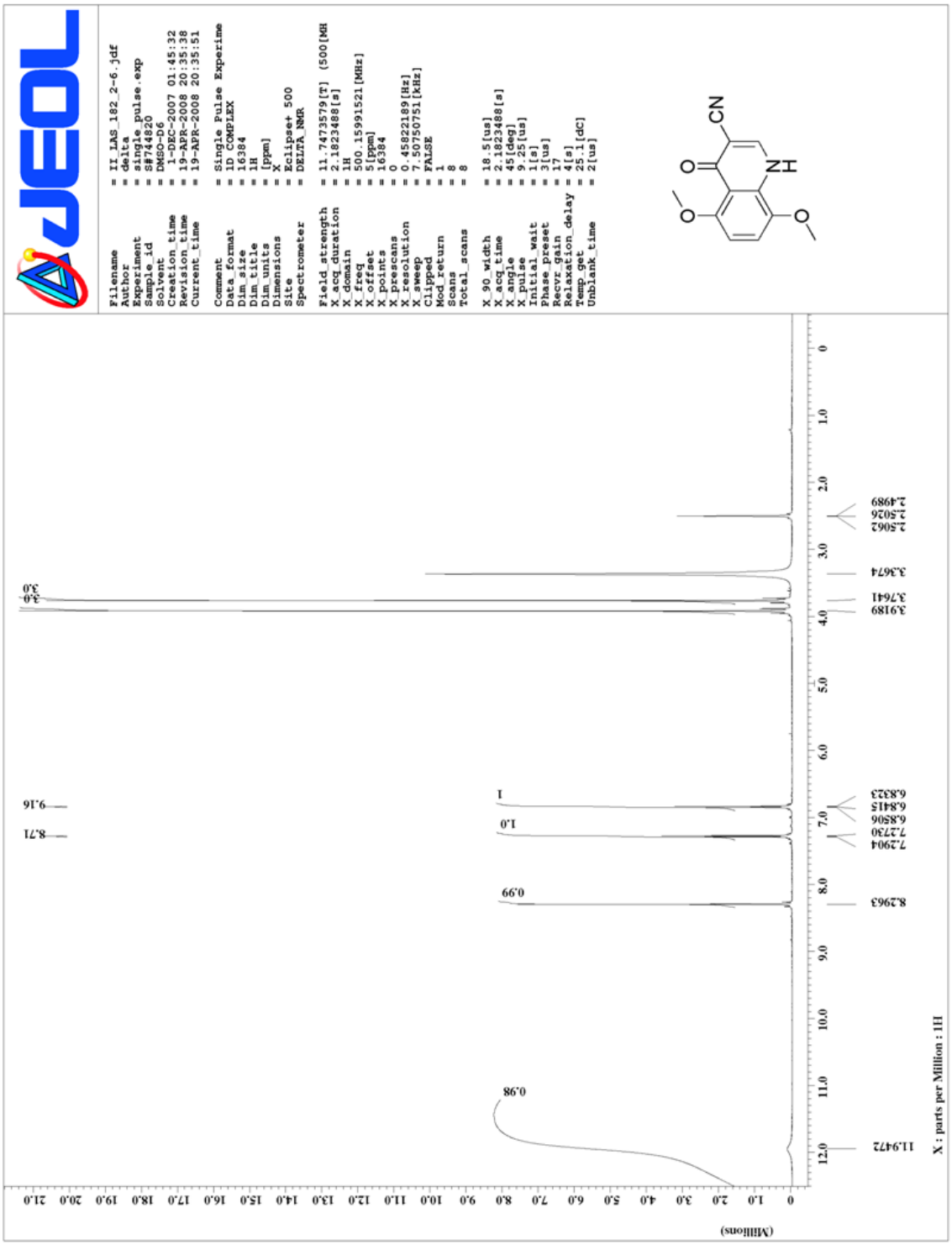

JEOL
Filename = II_LAS_124-5.jdf
Author = delta
Experiment = single_pulse.ex2
Sample_id = S#496434
Solvent = DMSO-D6
Creation_time = 12-FEB-2007 14:35:31
Revision_time = 14-APR-2008 20:04:57
Current_time = 14-APR-2008 20:05:32
Comment = single_pulse
Data_format = 1D COMPLEX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 2.90717696[s]
X_domain = 1H
X_freq = 300.52965592[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.34397631[Hz]
X_sweep = 5.63570784[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Tri_domain = 1H
Tri_freq = 300.52965592[MHz]
Tri_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 24
Total_scans = 24
X_90_width = 13.01[us]
X_acq_time = 2.90717696[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 6.505[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Recvr_gain = 50
Relaxation_delay = 2[s]
Repetition_time = 4.90717696[s]
Temp_get = 22.6[dC]
Br
O
CN
NH
abundance
X : parts per Million : 1H
8.7610
8.1739
7.9324
7.9175
7.9026
7.5913
7.5764
7.5615
0.99
0.96
8.94
0.98
8.94
1

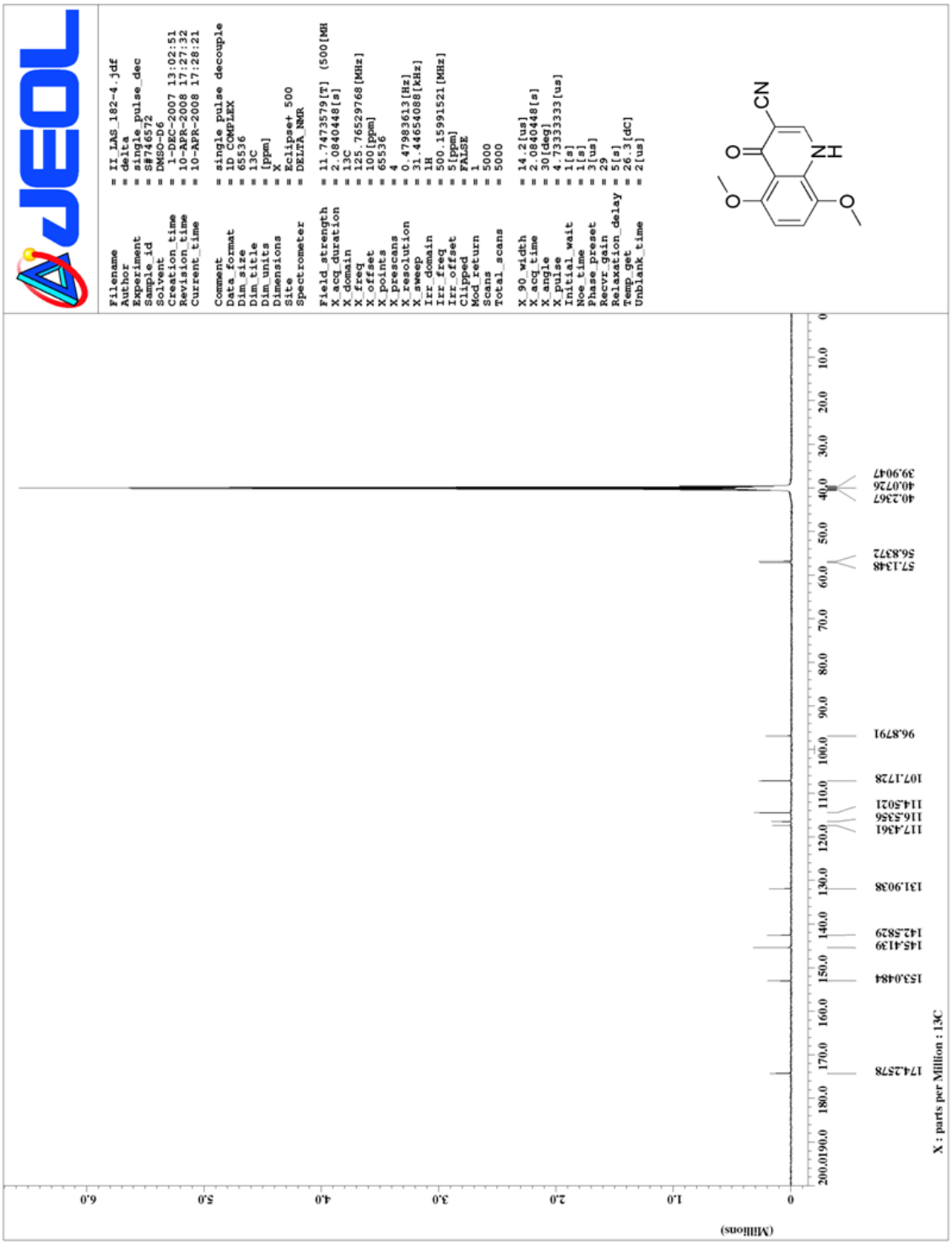
JEOL
Filename = II_LAS_124-6.jdf
Author = delta
Experiment = single_pulse_dec
Sample_id = S#803698
Solvent = DMSO-D6
Creation_time = 27-OCT-2007 09:47:08
Revision_time = 10-APR-2008 17:25:44
Current_time = 10-APR-2008 17:26:52
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 65536
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = Eclipse+ 500
Spectrometer = DELTA_NMR
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 2.0840448[s]
X_domain = 13C
X_freq = 125.76529768[MHz]
X_offset = 100[ppm]
X_points = 65536
X_prescans = 4
X_resolution = 0.47983613[Hz]
X_sweep = 31.44654088[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5[ppm]
Clipped = TRUE
Mod_return = 1
Scans = 3000
Total_scans = 3000
X_90_width = 14.2[us]
X_acq_time = 2.0840448[s]
X_angle = 30[deg]
X_pulse = 4.73333333[us]
Initial_wait = 1[s]
Noe_time = 1[s]
Phase_preset = 3[us]
Recvr_gain = 29
Relaxation_delay = 5[s]
Temp_get = 25.7[dC]
Unblank_time = 2[us]
O
CN
NH
Br
(Millions)
X : parts per Million : 13C
173.7923
147.6649
138.7218
136.5432
127.6688
127.0850
122.4341
118.7638
117.0431
94.5212
40.2634
40.0955
39.9276

APPENDIX P

^{1}H NMR AND ^{13}C NMR SPECTRA OF

3-CYANO-5,8-DIMETHOXY-4(*1H*)-QUINOLONE (145c)

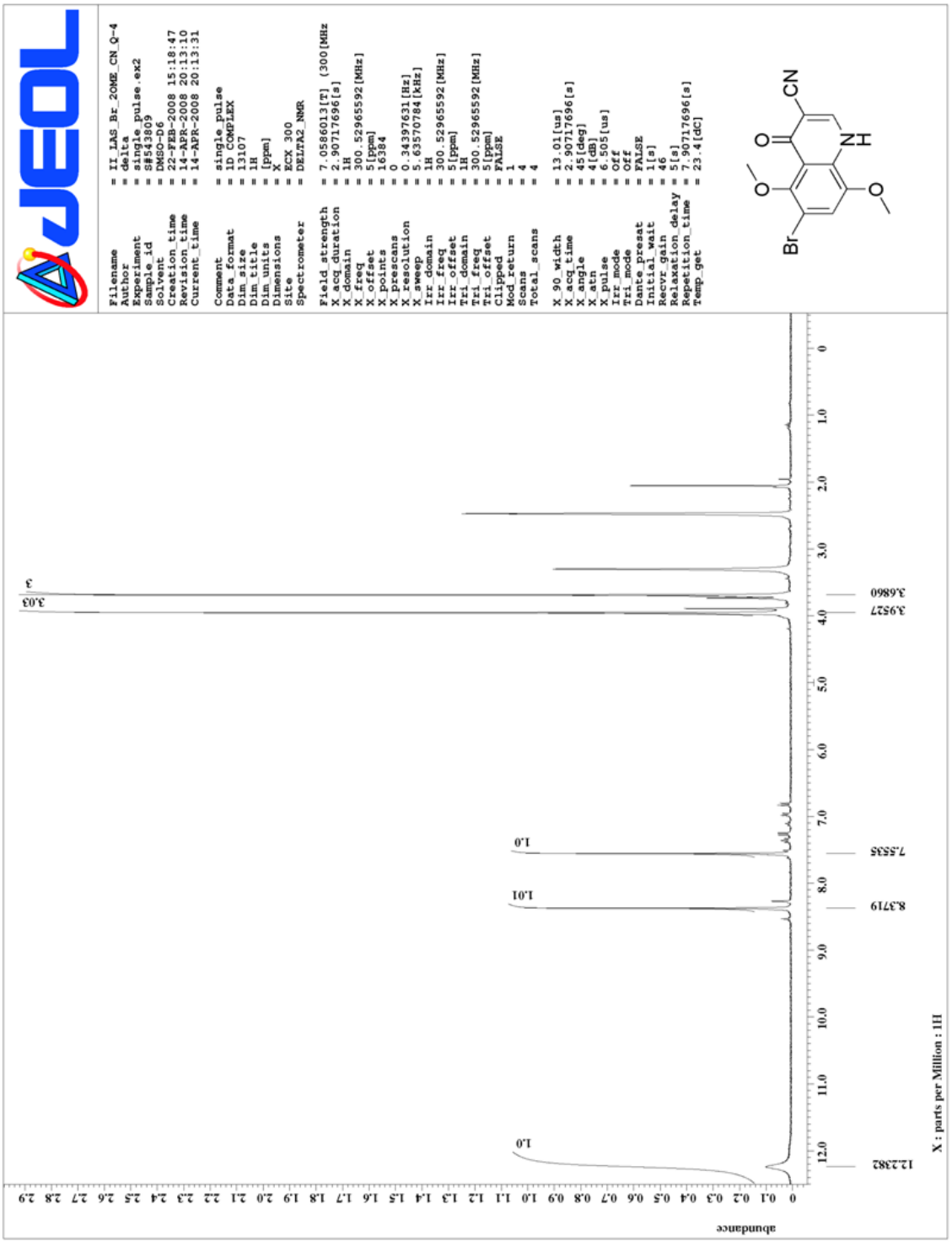

JEOL
Filename = II_LAS_182_2-6.jdf
Author = delta
Experiment = single_pulse.exp
Sample_id = S#744820
Solvent = DMSO-D6
Creation_time = 1-DEC-2007 01:45:32
Revision_time = 19-APR-2008 20:35:38
Current_time = 19-APR-2008 20:35:51
Comment = Single Pulse Experime
Data_format = 1D COMPLEX
Dim_size = 16384
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = Eclipse+ 500
Spectrometer = DELTA_NMR
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 2.1823488[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.45822189[Hz]
X_sweep = 7.50750751[kHz]
Clipped = FALSE
Mod_return = 1
Scans = 8
Total_scans = 8
X_90_width = 18.5[us]
X_acq_time = 2.1823488[s]
X_angle = 45[deg]
X_pulse = 9.25[us]
Initial_wait = 1[s]
Phase_preset = 3[us]
Recvr_gain = 17
Relaxation_delay = 4[s]
Temp_get = 25.1[dC]
Unblank_time = 2[us]
CN
O
NH
O
O
11.9472
8.2963
7.2904
7.2730
6.8506
6.8415
6.8323
3.9189
3.7641
3.3674
2.5062
2.5026
2.4989
9.16
8.71
0.98
0.99
1.0
1
3.0
3.0
(Millions)
X : parts per Million : 1H

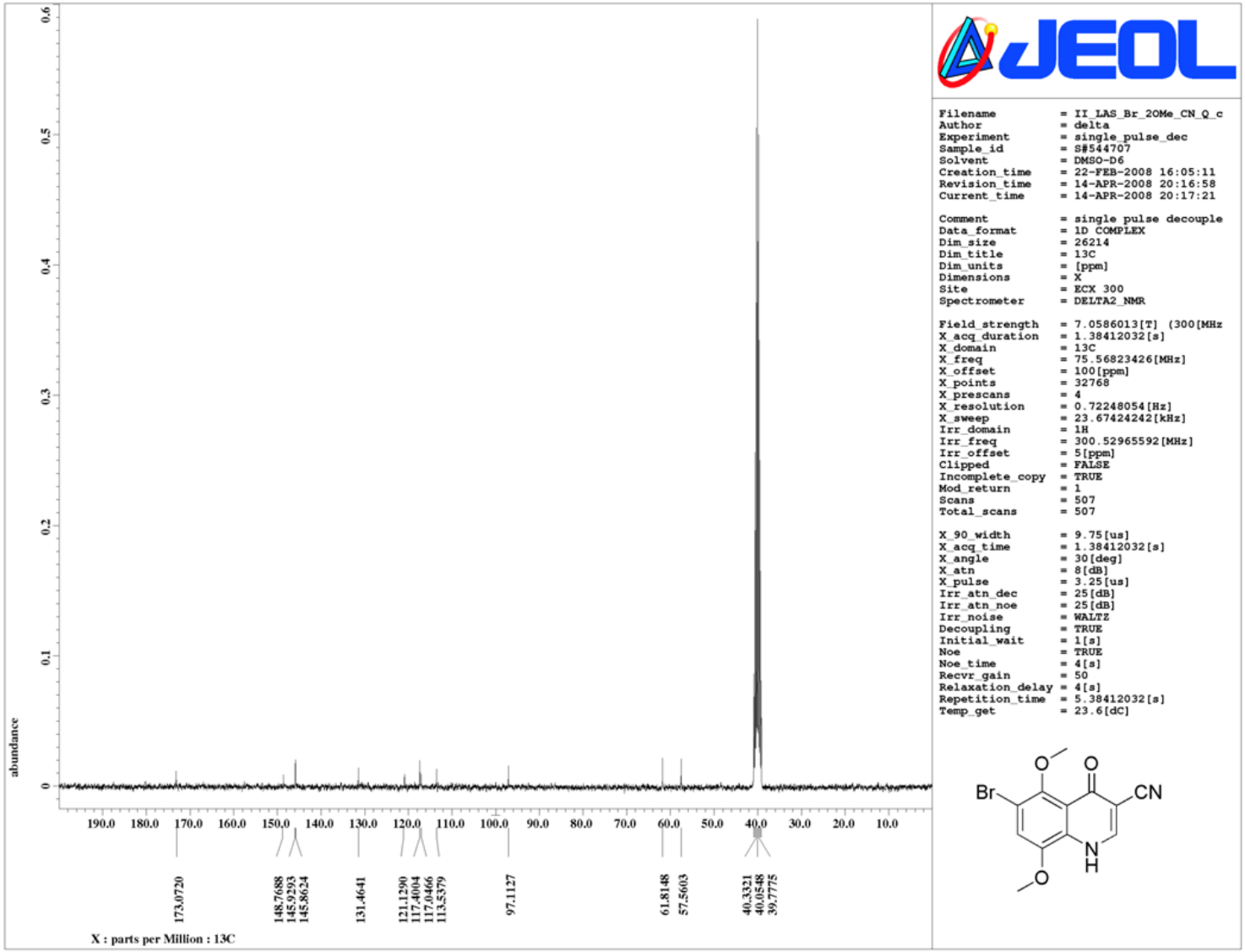

JEOL
Filename = IT_LAS_182-4.jdf
Author = delta
Experiment = single_pulse_dec
Sample_id = S#746572
Solvent = DMSO-D6
Creation_time = 1-DEC-2007 13:02:51
Revision_time = 10-APR-2008 17:27:32
Current_time = 10-APR-2008 17:28:21
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 65536
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = Eclipse+ 500
Spectrometer = DELTA_NMR
Field_strength = 11.74735579[T] (500[MH
X_acq_duration = 2.08400448[s]
X_domain = 13C
X_freq = 125.76529768[MHz]
X_offset = 100[ppm]
X_points = 65536
X_prescans = 4
X_resolution = 0.47983613[Hz]
X_sweep = 31.44654088[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 5000
Total_scans = 5000
X_90_width = 14.2[us]
X_acq_time = 2.08400448[s]
X_angle = 30[deg]
X_pulse = 4.73333333[us]
Initial_wait = 1[s]
Noe_time = 1[s]
Phase_preset = 3[us]
Recvr_gain = 29
Relaxation_delay = 5[s]
Temp_get = 26.3[dC]
Unblank_time = 2[us]
CN
O
N
H
O
O
(Millions)
X : parts per Million : 13C
174.2578
153.0484
145.4139
142.5829
131.9038
117.4361
116.5356
114.5021
107.1728
96.8791
57.1348
56.8372
40.2367
40.0726
39.9047

APPENDIX Q

^{1}H NMR AND ^{13}C NMR SPECTRA OF

6-BROMO-3-CYANO-5,8-DIMETHOXY-4(*1H*)-QUINOLONE (145d)

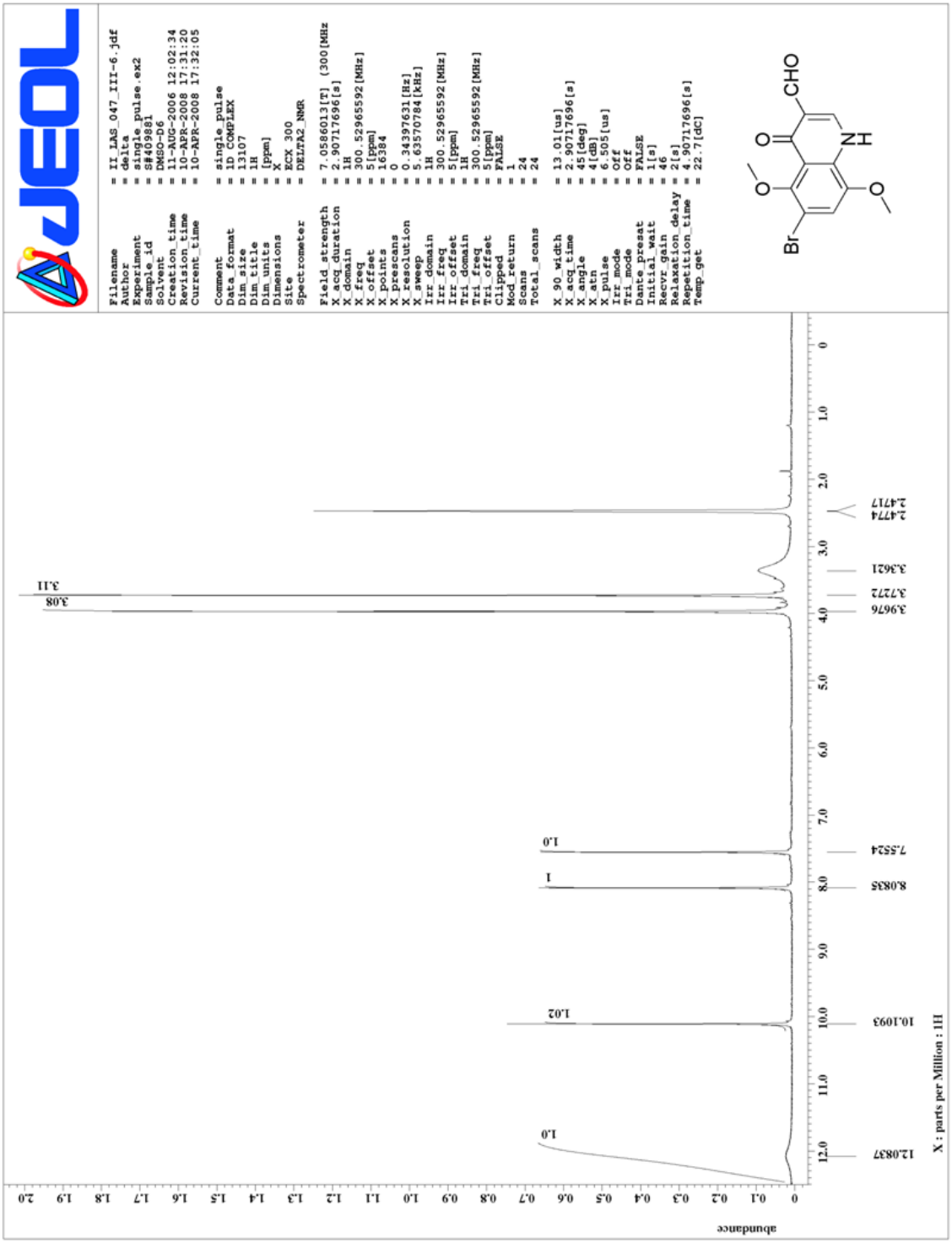

JEOL
Filename = II_LAS_Br_2OME_CN_Q-4
Author = delta
Experiment = single_pulse.ex2
Sample_id = #84809
Solvent = DMSO-D6
Creation_time = 22-FEB-2008 15:18:47
Revision_time = 14-APR-2008 20:13:10
Current_time = 14-APR-2008 20:13:31
Comment = single_pulse
Data_format = 1D COMPLEX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 2.90717696[s]
X_domain = 1H
X_freq = 300.52965592[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.34397631[Hz]
X_sweep = 5.63570784[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Tri_domain = 1H
Tri_freq = 300.52965592[MHz]
Tri_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 4
Total_scans = 4
X_90_width = 13.01[us]
X_acq_time = 2.90717696[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 6.505[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Recvr_gain = 46
Relaxation_delay = 5[s]
Repetition_time = 7.90717696[s]
Temp_get = 23.4[dC]
CN
O
O
NH
O
Br
abundance
X : parts per Million : 1H
12.2382
8.3719
7.5535
3.9527
3.6860
3.03
1.0
1.01
1.0
3

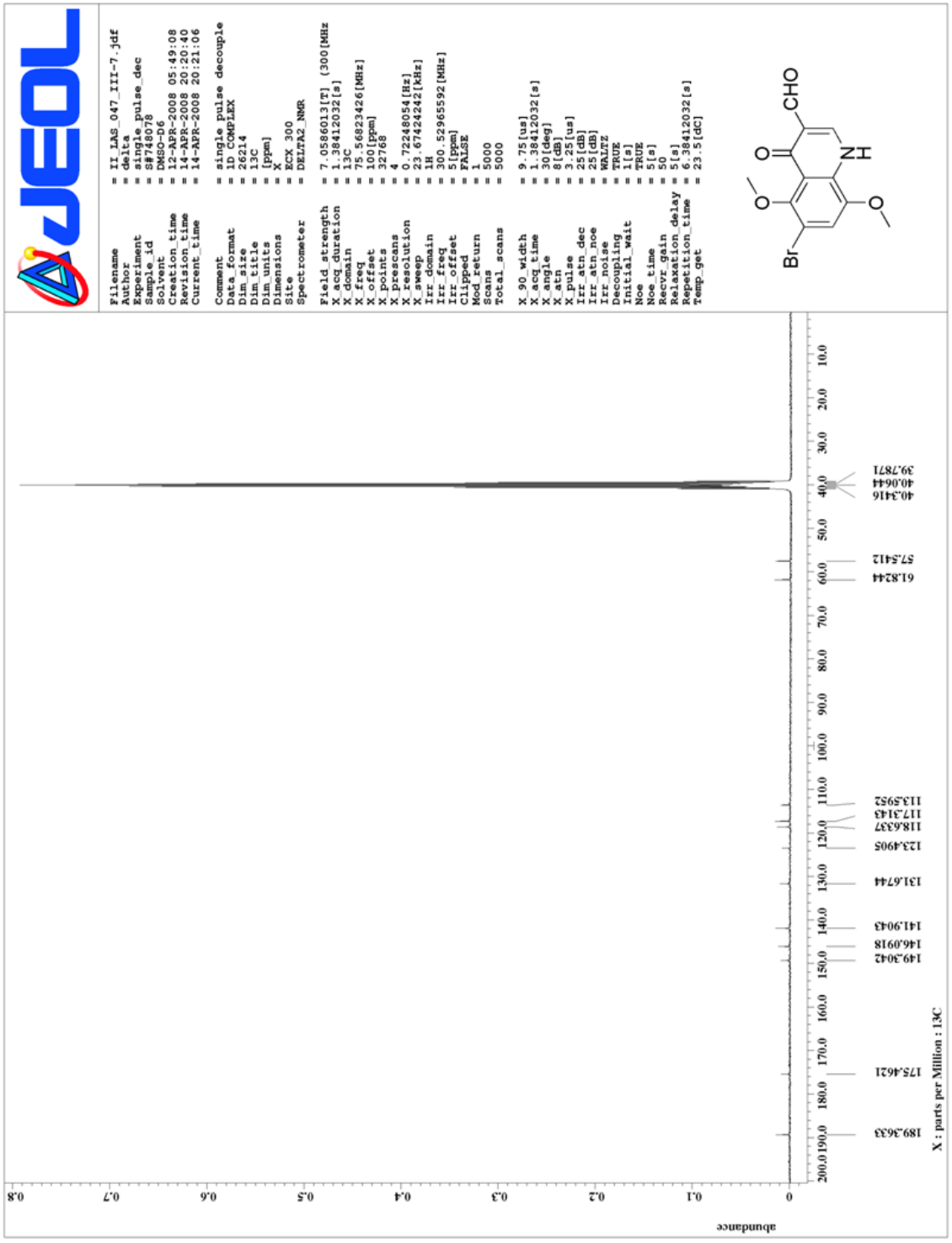

abundance
0.6
0.5
0.4
0.3
0.2
0.1
0
190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0
173.0720
148.7688
145.9293
145.8624
131.4641
121.1290
117.4004
117.0466
113.5379
97.1127
61.8148
57.5603
40.3321
40.0548
39.7775
X : parts per Million : 13C
JEOL
Filename = II_LAS_Br_2OMe_CN_Q_c
Author = delta
Experiment = single_pulse_dec
Sample_id = S#544707
Solvent = DMSO-D6
Creation_time = 22-FEB-2008 16:05:11
Revision_time = 14-APR-2008 20:16:58
Current_time = 14-APR-2008 20:17:21
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Incomplete_copy = TRUE
Mod_return = 1
Scans = 507
Total_scans = 507
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 4[s]
Recvr_gain = 50
Relaxation_delay = 4[s]
Repetition_time = 5.38412032[s]
Temp_get = 23.6[dC]
O
O
Br
CN
N
H
O

APPENDIX R

^{1}H NMR AND ^{13}C NMR SPECTRA OF

6-BROMO-5,8-DIMETHYL-3-FORMYL-4(*1H*)-QUINOLONE (146d)

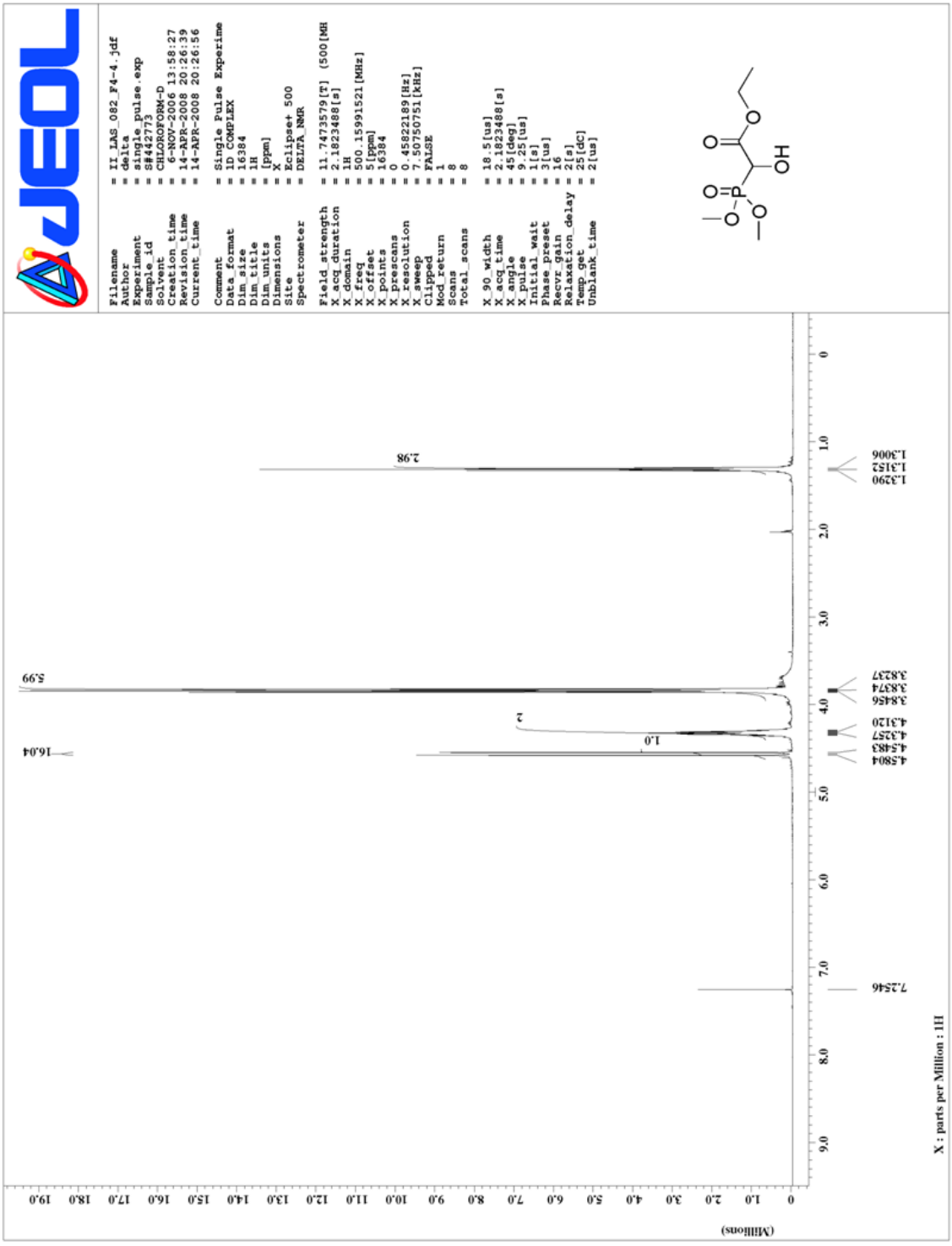

JEOL
Filename = II_LAS_047_III-6.jdf
Author = delta
Experiment = single_pulse.ex2
Sample_id = S#409881
Solvent = DMSO-D6
Creation_time = 11-AUG-2006 12:02:34
Revision_time = 10-APR-2008 17:31:20
Current_time = 10-APR-2008 17:32:05
Comment = single_pulse
Data_format = 1D COMPLEX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 2.90717696[s]
X_domain = 1H
X_freq = 300.52965592[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.34397631[Hz]
X_sweep = 5.63570784[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Tri_domain = 1H
Tri_freq = 300.52965592[MHz]
Tri_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 24
Total_scans = 24
X_90_width = 13.01[us]
X_acq_time = 2.90717696[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 6.505[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Recvr_gain = 46
Relaxation_delay = 2[s]
Repetition_time = 4.90717696[s]
Temp_get = 22.7[dC]
CHO
O
NH
O
O
Br
abundance
X : parts per Million : 1H
12.0837
10.1093
8.0835
7.5524
3.9676
3.7272
3.3621
2.4774
2.4717
3.08
3.11
1.0
1.02
1
1.0
1.0

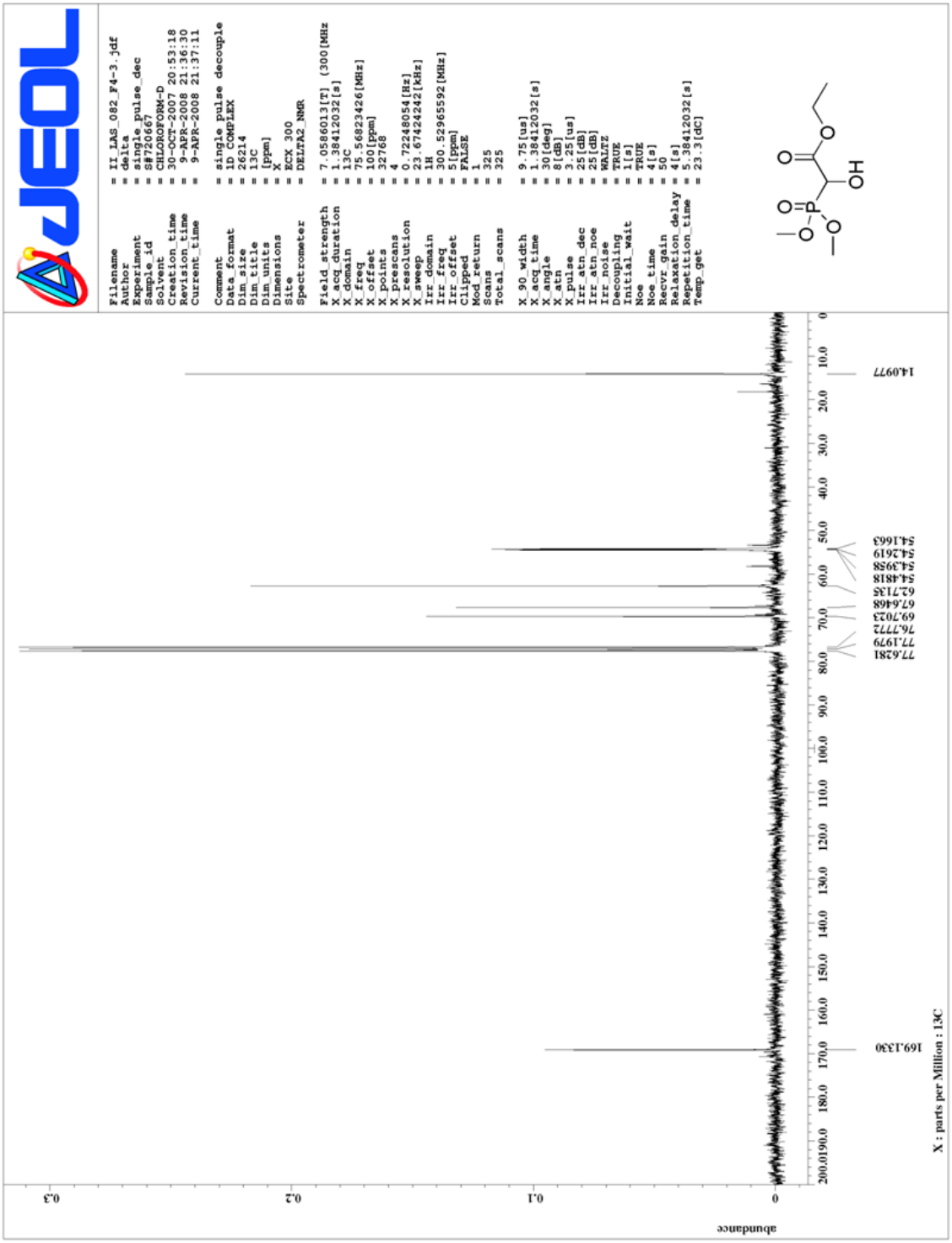

Filename = II_LAS_047_III-7.jdf
Author = delta
Experiment = single_pulse_dec
Sample_id = SM74078
Solvent = DMSO-d6
Creation_time = 12-APR-2008 05:49:08
Revision_time = 14-APR-2008 20:20:40
Current_time = 14-APR-2008 20:21:06
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 5000
Total_scans = 5000
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 5[s]
Recvr_gain = 50
Relaxation_delay = 5[s]
Repetition_time = 6.38412032[s]
Temp_get = 23.5[dC]
CHO
O
N
H
O
Br
O
abundance
X : parts per Million : 13C
189.3633
175.4621
149.3042
146.0918
141.9043
131.6744
123.4905
118.6337
117.3143
113.5952
61.8244
57.5412
40.3416
40.0644
39.7871

APPENDIX S

^{1}H NMR AND ^{13}C NMR SPECTRA OF

ETHYL (DIMETHOXYPHOSPHINYL)HYDROXY ETHANOATE (154)

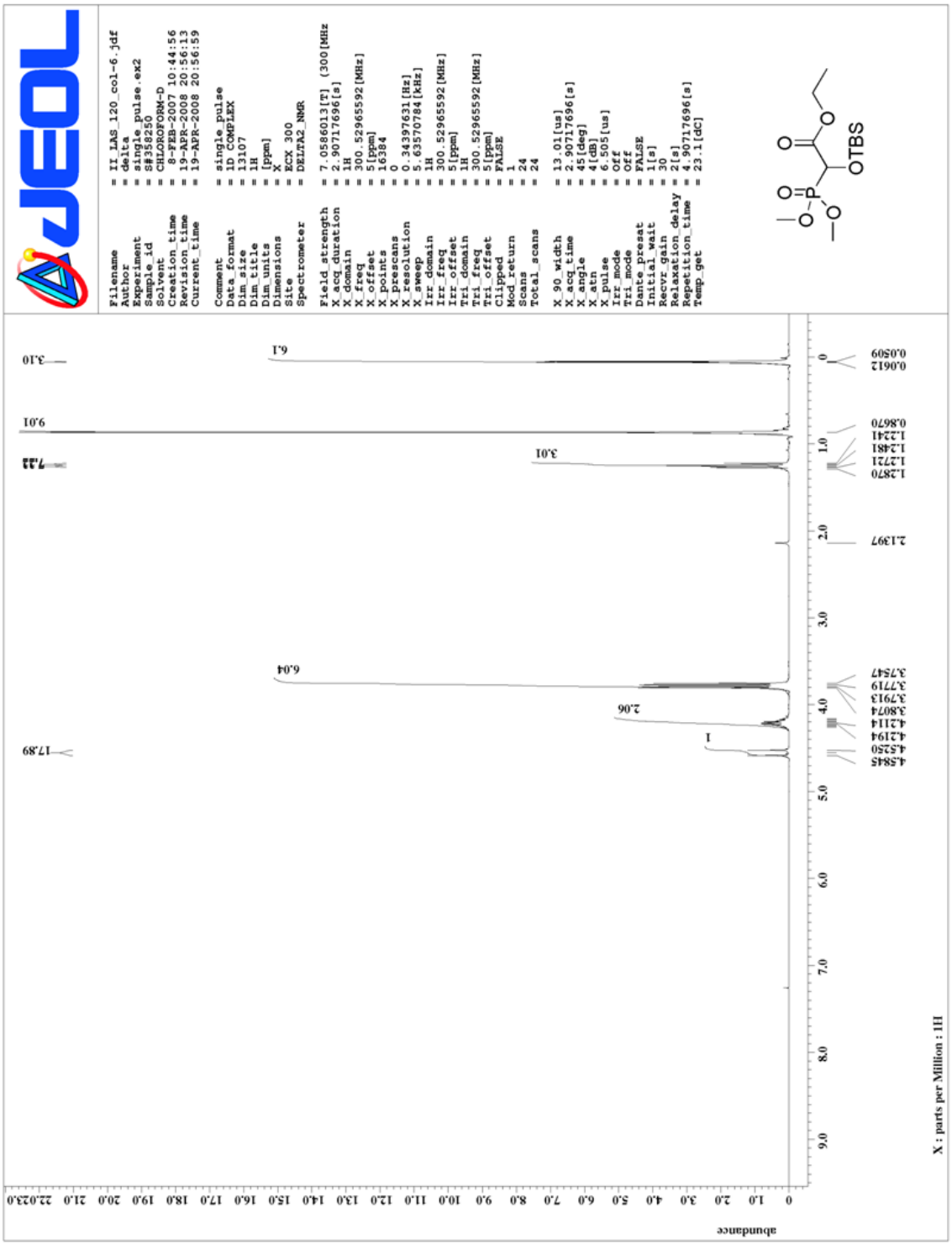

JEOL
Filename = II_LAS_082_F4-4.jdf
Author = delta
Experiment = single_pulse.exp
Sample_id = S#442773
Solvent = CHLOROFORM-D
Creation_time = 6-NOV-2006 13:58:27
Revision_time = 14-APR-2008 20:26:39
Current_time = 14-APR-2008 20:26:56
Comment = Single Pulse Experime
Data_format = 1D COMPLEX
Dim_size = 16384
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = Eclipse+ 500
Spectrometer = DELTA_NMR
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 2.1823488[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.45822189[Hz]
X_sweep = 7.50750751[kHz]
Clipped = FALSE
Mod_return = 1
Scans = 8
Total_scans = 8
X_90_width = 18.5[us]
X_acq_time = 2.1823488[s]
X_angle = 45[deg]
X_pulse = 9.25[us]
Initial_wait = 1[s]
Phase_preset = 3[us]
Recvr_gain = 16
Relaxation_delay = 2[s]
Temp_get = 25[dC]
Unblank_time = 2[us]
OH
16.04
5.99
2.98
2
1.0
7.2546
4.5804
4.5483
4.3257
4.3120
3.8456
3.8374
3.8237
1.3290
1.3152
1.3006
(Millions)
X : parts per Million : 1H

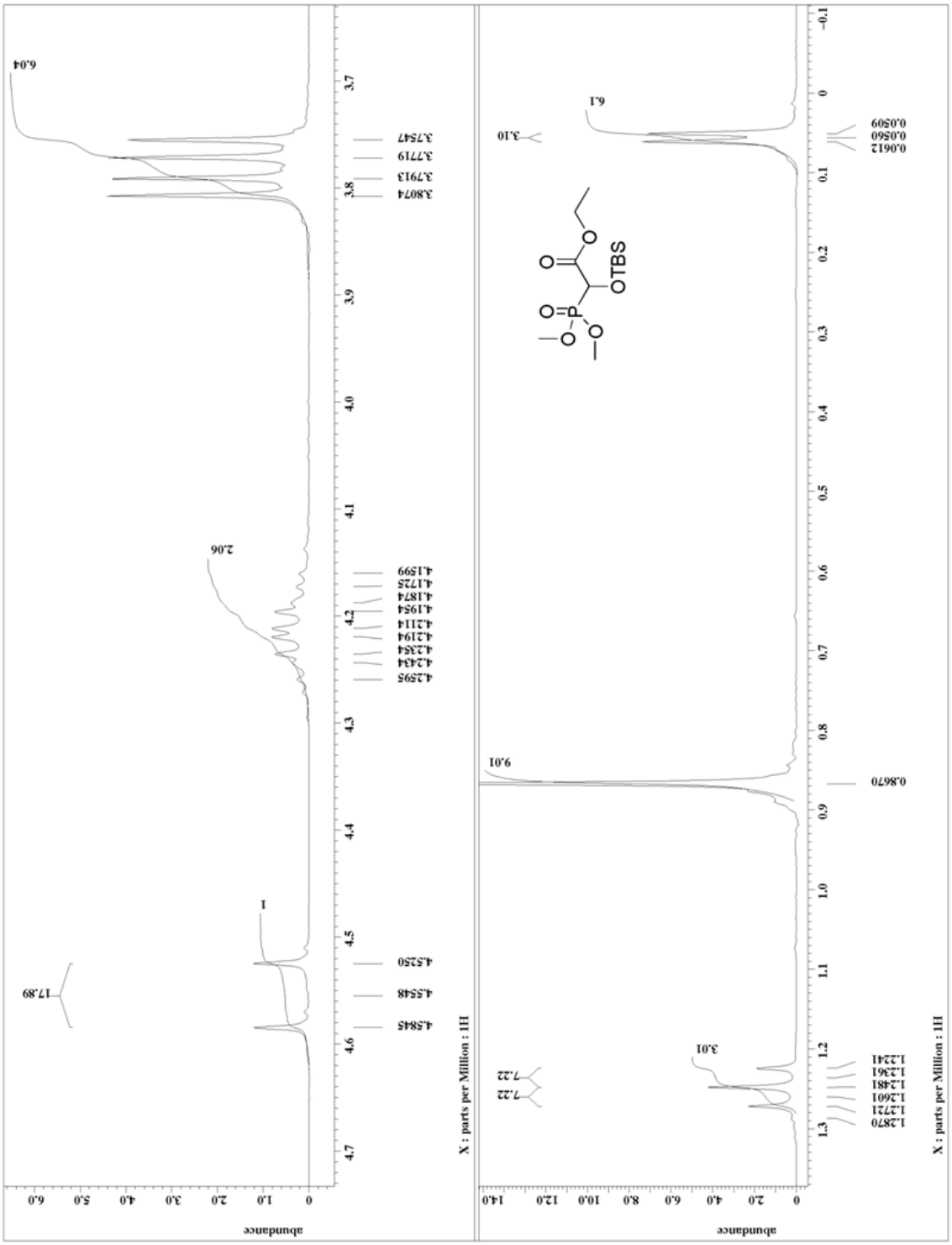

JEOL
Filename = II_LAS_082_F4-3.jdf
Author = delta
Experiment = single_pulse_dec
Sample_id = S#720667
Solvent = CHLOROFORM-D
Creation_time = 30-OCT-2007 20:53:18
Revision_time = 9-APR-2008 21:36:30
Current_time = 9-APR-2008 21:37:11
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 325
Total_scans = 325
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 4[s]
Recvr_gain = 50
Relaxation_delay = 4[s]
Repetition_time = 5.38412032[s]
Temp_get = 23.3[dC]
14.0977
54.1663
54.2619
54.3958
54.4818
62.7135
67.6468
69.7023
76.7772
77.1979
77.6281
169.1330
abundance
X : parts per Million : 13C

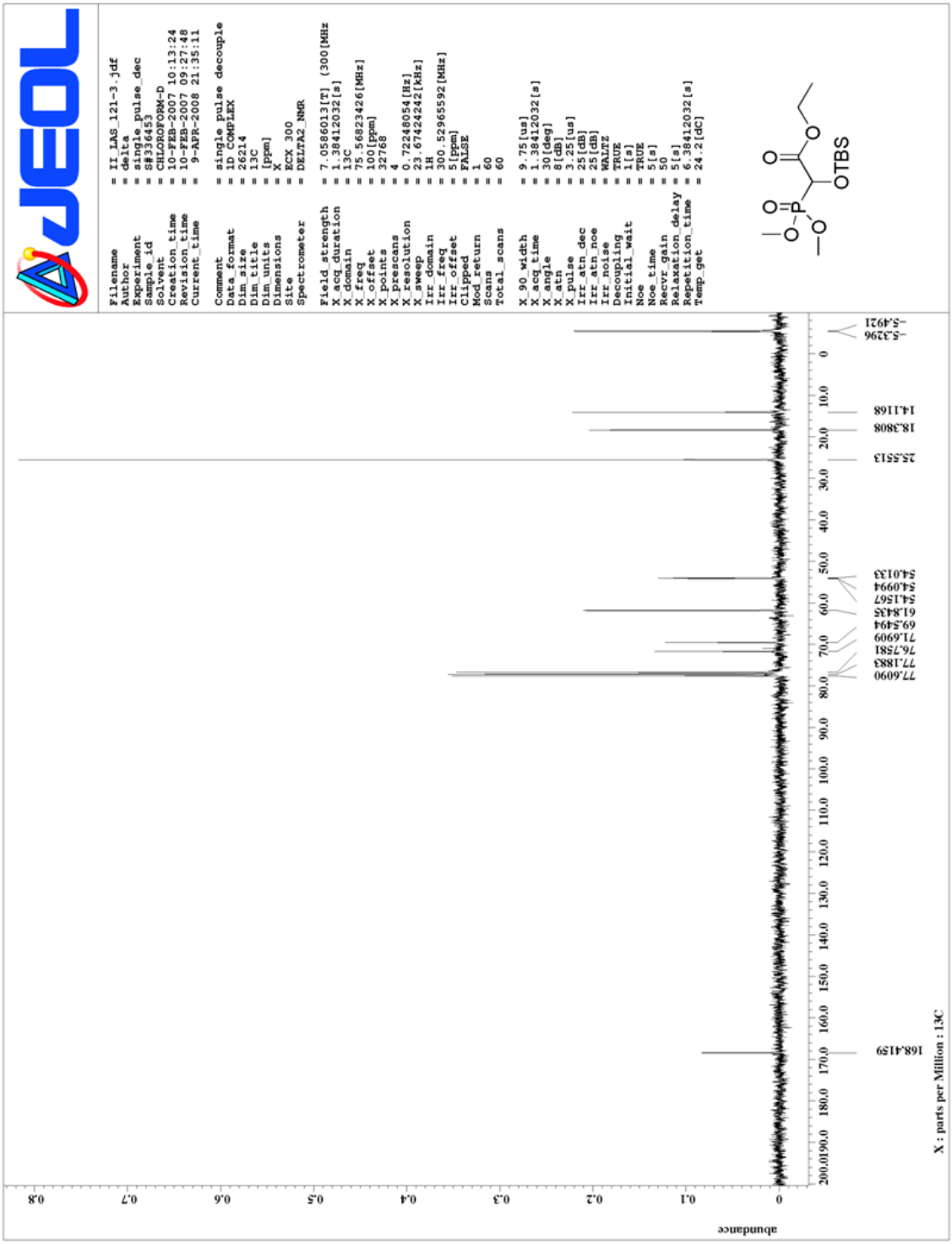
APPENDIX T

^{1}H NMR AND ^{13}C NMR SPECTRA OF

ETHYL (2-(DIMETHOXYPHOSPHINYL)-2-[[1,1-DIMETHYLETHYL)DIMETHYLSILYL]OXY] ETHANOATE (155)


JEOL
Filename = II_LAS_120_col-6.jdf
Author = delta
Experiment = single_pulse.ex2
Sample_id = S#358250
Solvent = CHLOROFORM-D
Creation_time = 8-FEB-2007 10:44:56
Revision_time = 19-APR-2008 20:56:13
Current_time = 19-APR-2008 20:56:59
Comment = single_pulse
Data_format = 1D COMPLEX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.05860131[T] (300[MHz
X_acq_duration = 2.90717696[s]
X_domain = 1H
X_freq = 300.52965592[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.34397631[Hz]
X_sweep = 5.63570784[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Tri_domain = 1H
Tri_freq = 300.52965592[MHz]
Tri_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 24
Total_scans = 24
X_90_width = 13.01[us]
X_acq_time = 2.90717696[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 6.505[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Recvr_gain = 30
Relaxation_delay = 2[s]
Repetition_time = 4.90717696[s]
Temp_get = 23.1[dC]
OTBS
abundance
X : parts per Million : 1H
17.89
7.44
6.04
3.10
9.01
6.1
3.01
2.06
1
4.5845
4.5250
4.2194
4.2114
3.8074
3.7913
3.7719
3.7547
2.1397
1.2870
1.2721
1.2481
1.2241
0.8670
0.0612
0.0509

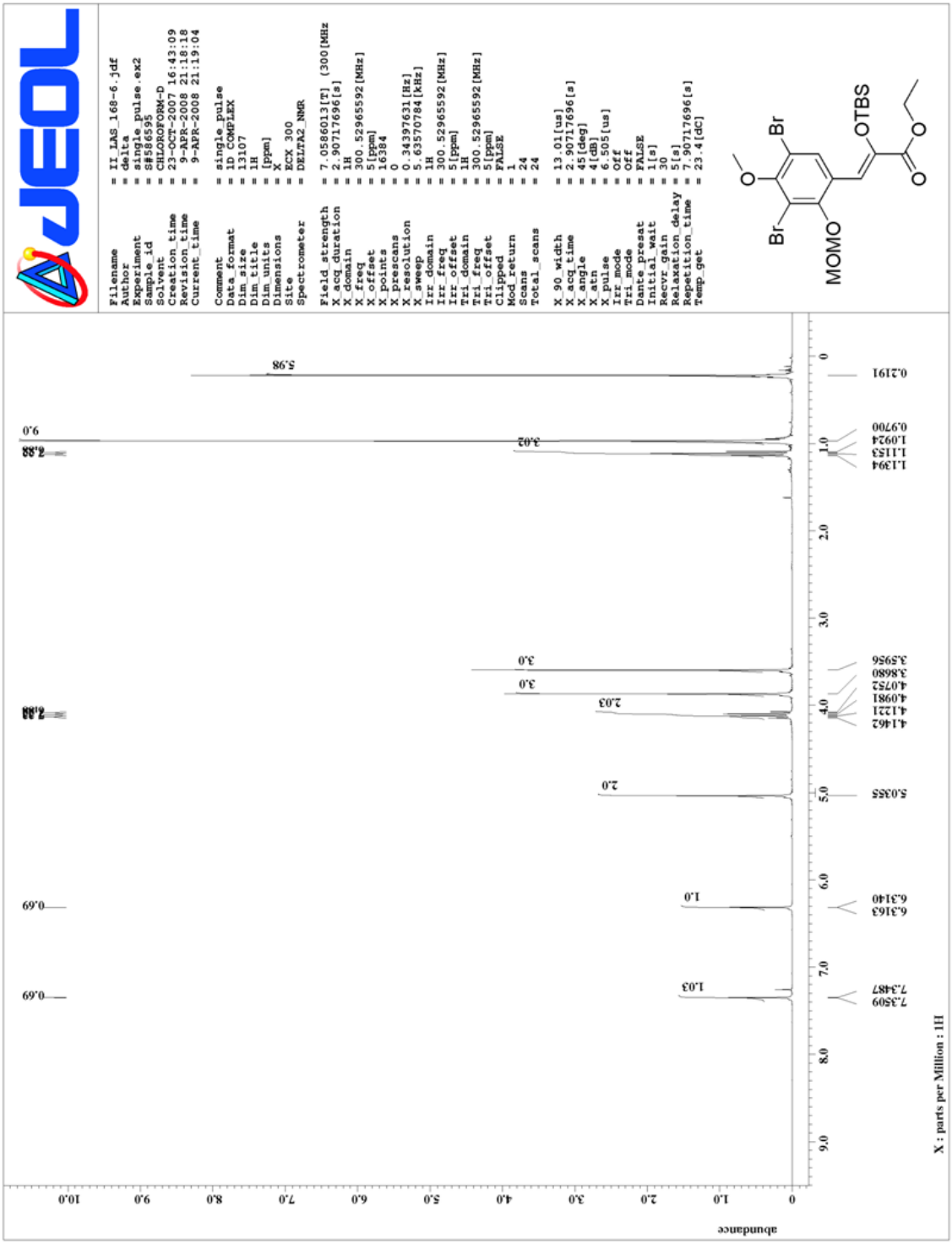

OTBS
X : parts per Million : 1H
abundance
9.01
6.1
3.10
3.01
7.22
7.22
2.06
6.04
17.89
1
0.8670
0.0612
0.0560
0.0509
1.2870
1.2721
1.2601
1.2481
1.2361
1.2241
4.5845
4.5548
4.5250
4.2595
4.2434
4.2354
4.2194
4.2114
4.1954
4.1874
4.1725
4.1599
3.8074
3.7913
3.7719
3.7547

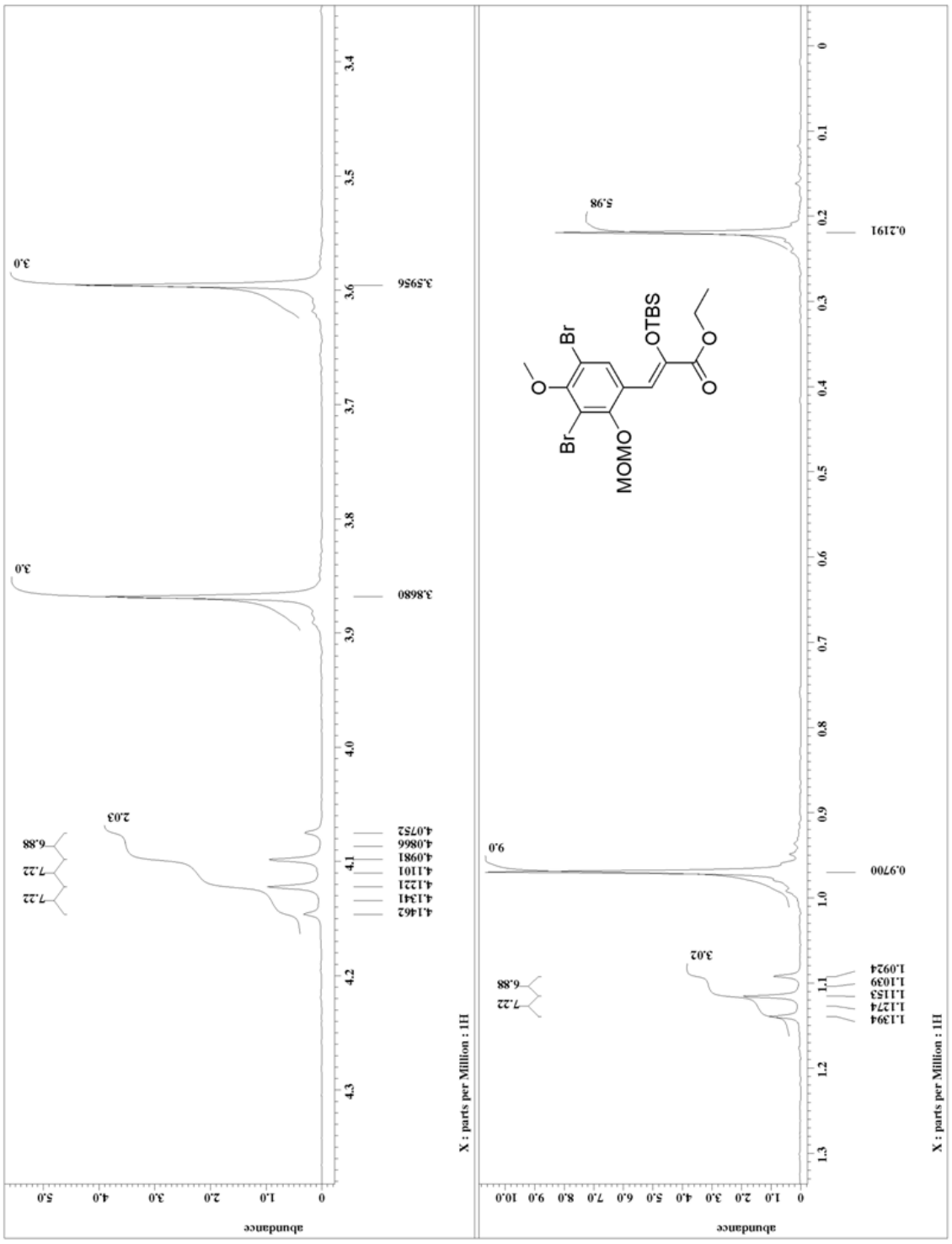

JEOL
Filename = II_LAS_121-3.jdf
Author = delta
Experiment = single_pulse_dec
Sample_id = S#336453
Solvent = CHLOROFORM-D
Creation_time = 10-FEB-2007 10:13:24
Revision_time = 10-FEB-2007 09:27:48
Current_time = 9-APR-2008 21:35:11
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 60
Total_scans = 60
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 5[s]
Recvr_gain = 50
Relaxation_delay = 5[s]
Repetition_time = 6.38412032[s]
Temp_get = 24.2[dC]
OTBS
-5.3296
-5.4921
14.1168
18.3808
25.5513
54.0133
54.0994
54.1567
61.8435
69.5494
71.6909
76.7581
77.1883
77.6090
168.4159
X : parts per Million : 13C
abundance

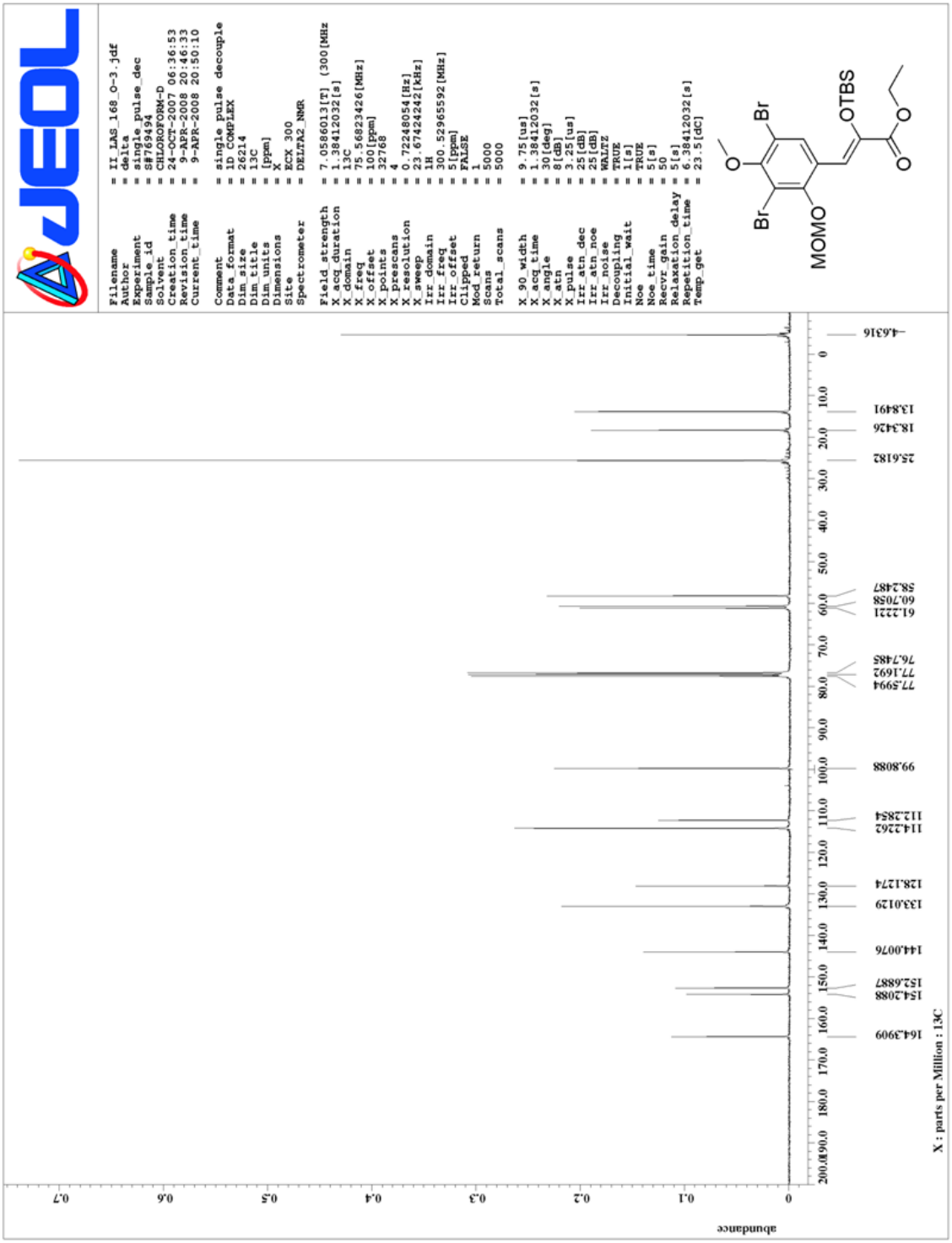
APPENDIX U

^{1}H NMR AND ^{13}C NMR SPECTRA OF

ETHYL 2-(*tert*-BUTYLDIMETHYLSILYLOXY)-3-{3,5-DIBROMO-4-METHOXY-2-(*O*-METHOXYMETHYLENOXY)PHENYL}-PROP-2-ENOATE (156)


JEOL
Filename = II_LAS_168-6.jdf
Author = delta
Experiment = single_pulse.ex2
Sample_id = S#586595
Solvent = CHLOROFORM-D
Creation_time = 23-OCT-2007 16:43:09
Revision_time = 9-APR-2008 21:18:18
Current_time = 9-APR-2008 21:19:04
Comment = single_pulse
Data_format = 1D COMPLEX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 2.90717696[s]
X_domain = 1H
X_freq = 300.52965592[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.34397631[Hz]
X_sweep = 5.63570784[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Tri_domain = 1H
Tri_freq = 300.52965592[MHz]
Tri_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 24
Total_scans = 24
X_90_width = 13.01[us]
X_acq_time = 2.90717696[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 6.505[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Recvr_gain = 30
Relaxation_delay = 5[s]
Repetition_time = 7.90717696[s]
Temp_get = 23.4[dC]
Br
OTBS
O
O
Br
MOMO
O
abundance
X : parts per Million : 1H
0.2191
0.9700
1.0924
1.1153
1.1394
3.5956
3.8680
4.0752
4.0981
4.1221
4.1462
5.0355
6.3140
6.3163
7.3487
7.3509
5.98
3.02
3.0
3.0
2.03
2.0
1.0
1.03
0.69
0.69
2.88
2.88
9.0

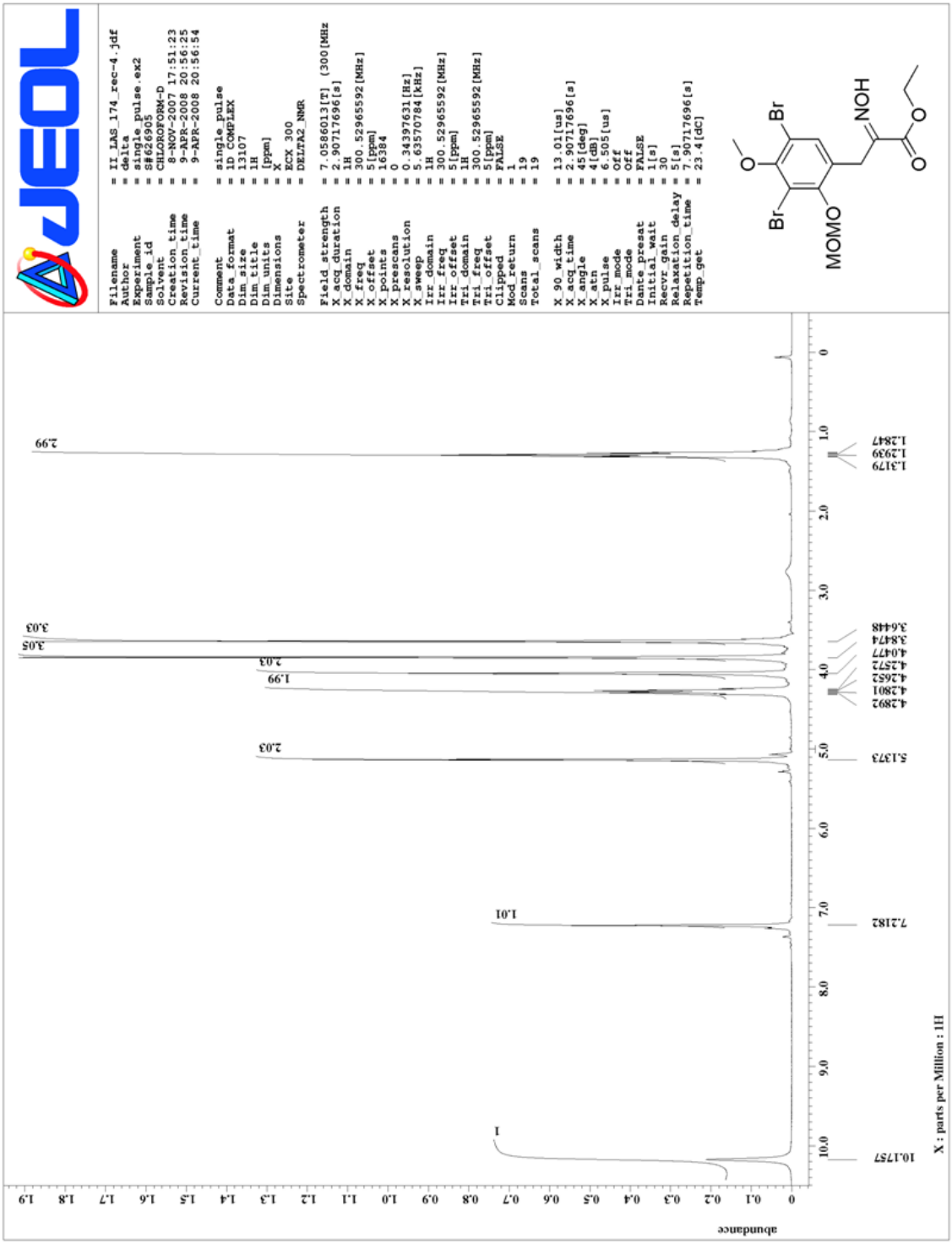

abundance
X : parts per Million : 1H
0.97700
0.21911
5.98
9.0
3.02
6.88
7.22
1.13944
1.12744
1.11553
1.10339
1.09244
Br
OTBS
O
O
Br
MOMO
3.86880
3.59560
3.0
2.03
4.14662
4.13410
4.12210
4.11010
4.09810
4.08660
4.07520

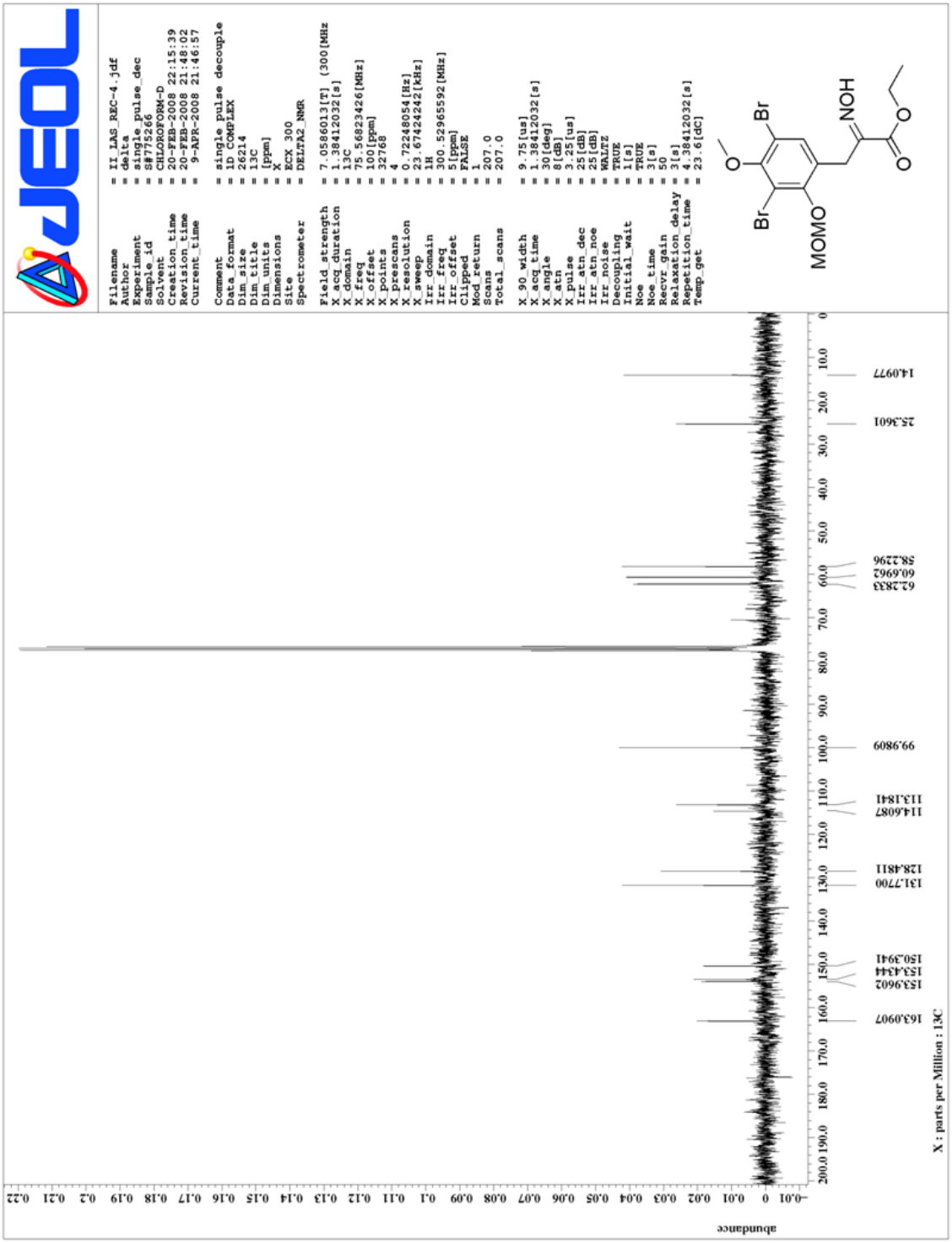

JEOL
Filename = II_LAS_168_O-3.jdf
Author = delta
Experiment = single_pulse_dec
Sample_id = S#769494
Solvent = CHLOROFORM-D
Creation_time = 24-OCT-2007 06:36:53
Revision_time = 9-APR-2008 20:46:33
Current_time = 9-APR-2008 20:50:10
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 5000
Total_scans = 5000
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 5[s]
Recvr_gain = 50
Relaxation_delay = 5[s]
Repetition_time = 6.38412032[s]
Temp_get = 23.5[dC]
Br
Br
OTBS
MOMO
O
O
O
abundance
X : parts per Million : 13C
-4.6316
13.8491
18.3426
25.6182
58.2487
60.7058
61.2221
76.7485
77.1692
77.5994
99.8088
112.2854
114.2262
128.1274
133.0129
144.0076
152.6887
154.2088
164.3909

APPENDIX V

^{1}H NMR AND ^{13}C NMR SPECTRA OF

ETHYL 3-{3,5-DIBROMO-4-METHOXY-2-(*O*-METHOXYMETHYLENOXY)PHENYL}-2(*E*)-(HYDROXYIMINO)PROPANOATE (157)

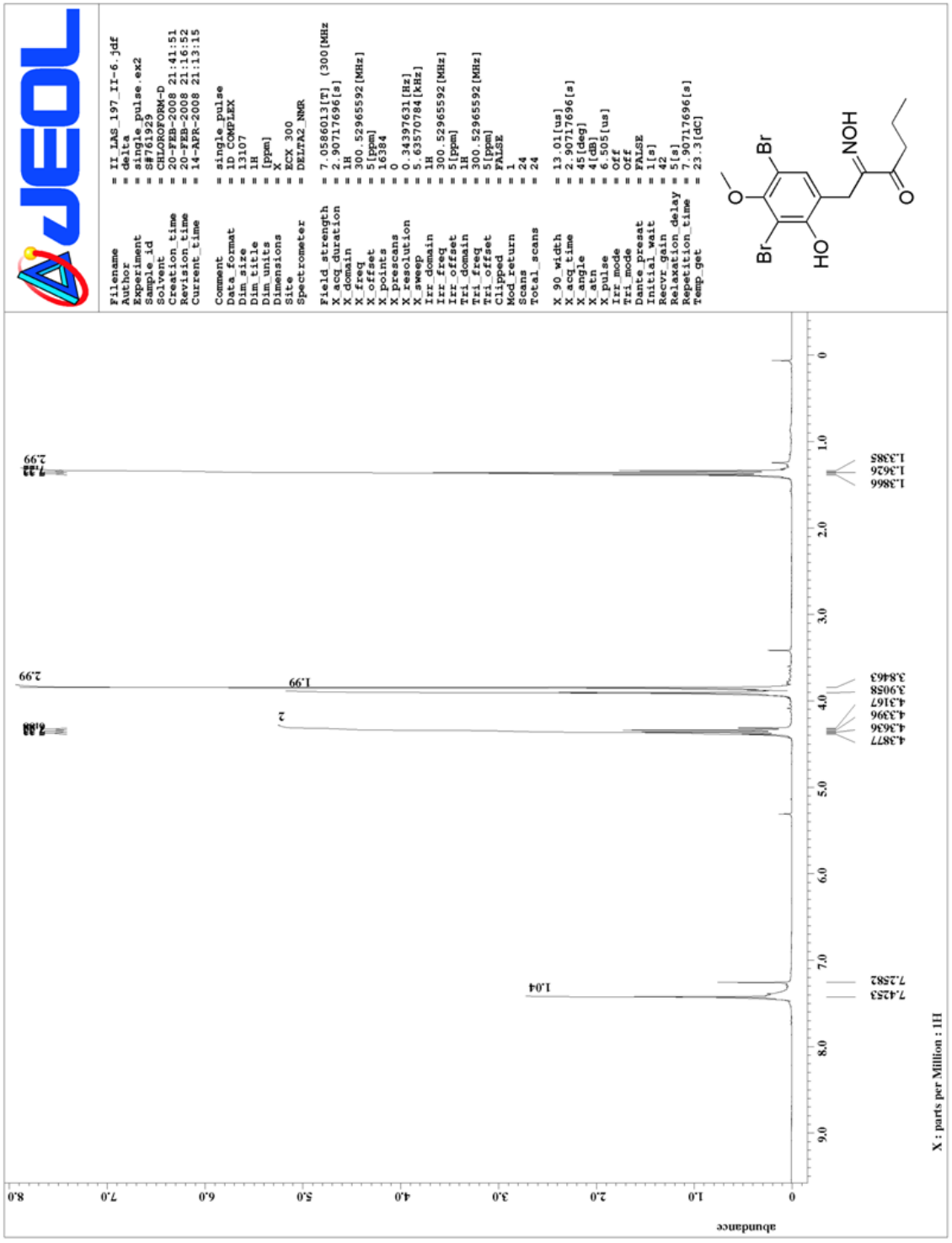

JEOL
Filename = II_LAS_174_rec-4.jdf
Author = delta
Experiment = single_pulse.ex2
Sample_id = S#626905
Solvent = CHLOROFORM-D
Creation_time = 8-NOV-2007 17:51:23
Revision_time = 9-APR-2008 20:56:25
Current_time = 9-APR-2008 20:56:54
Comment = single_pulse
Data_format = 1D COMPLEX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 2.90717696[s]
X_domain = 1H
X_freq = 300.52965592[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.34397631[Hz]
X_sweep = 5.63570784[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Tri_domain = 1H
Tri_freq = 300.52965592[MHz]
Tri_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 19
Total_scans = 19
X_90_width = 13.01[us]
X_acq_time = 2.90717696[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 6.505[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Recvr_gain = 30
Relaxation_delay = 5[s]
Repetition_time = 7.90717696[s]
Temp_get = 23.4[dC]
Br
Br
O
MOMO
NOH
O
O
abundance
X : parts per Million : 1H
10.1757
7.2182
5.1373
4.2892
4.2801
4.2652
4.2572
4.0477
3.8474
3.6448
3.03
3.03
2.03
1.99
2.03
1.01
1
2.99
1.3179
1.2939
1.2847

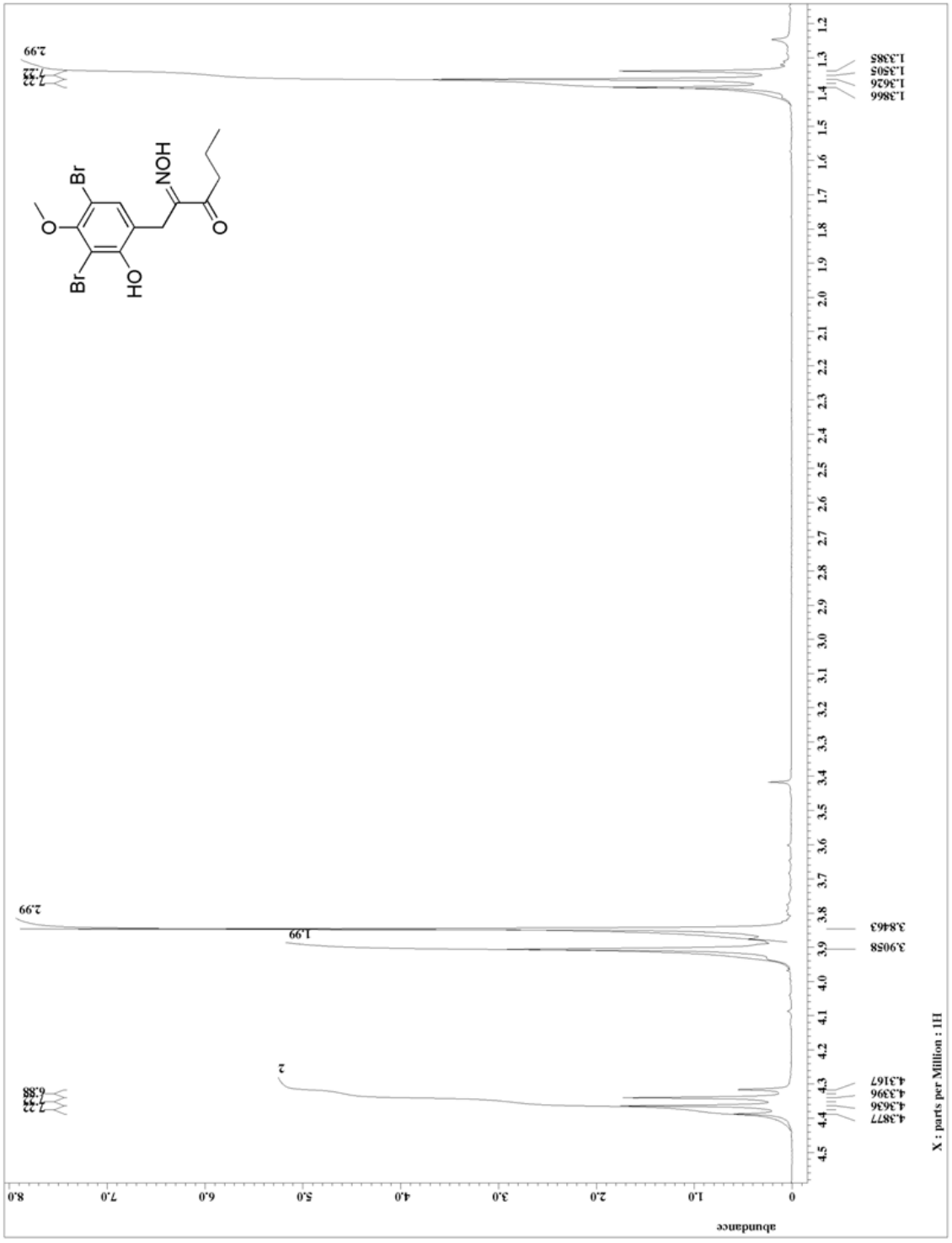

JEOL
Filename = II_LAS_REC-4.jdf
Author = delta
Experiment = single_pulse_dec
Sample_id = S#775266
Solvent = CHLOROFORM-D
Creation_time = 20-FEB-2008 22:15:39
Revision_time = 20-FEB-2008 21:48:02
Current_time = 9-APR-2008 21:46:57
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 207.0
Total_scans = 207.0
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 3[s]
Recvr_gain = 50
Relaxation_delay = 3[s]
Repetition_time = 4.38412032[s]
Temp_get = 23.6[dC]
Br
Br
MOMO
NOH
O
O
O
14.0977
25.3601
58.2296
60.6962
62.2833
99.9809
113.1841
114.6087
128.4811
131.7700
150.3941
153.4344
153.9602
163.0907
abundance
X : parts per Million : 13C

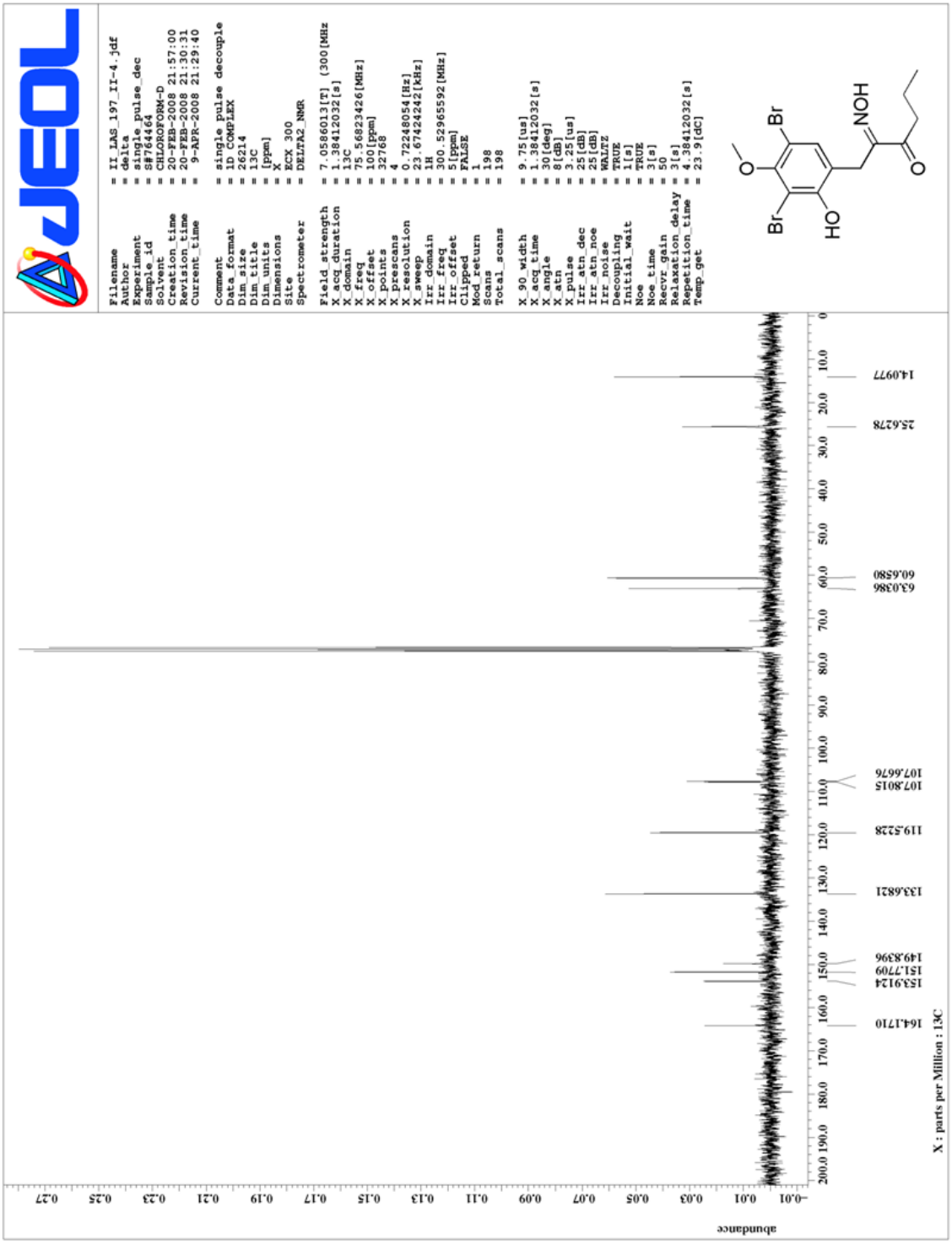
APPENDIX W

^{1}H NMR AND ^{13}C NMR SPECTRA OF

ETHYL 3-(3,5-DIBROMO-2-HYDROXY-4-METHOXYPHENYL)-2(*E*)-(HYDROXYIMINO)PROPANOATE (158)


JEOL
Filename = II_LAS_197_II-6.jdf
Author = delta
Experiment = single_pulse.ex2
Sample_id = SH7682@
Solvent = CHLOROFORM-D
Creation_time = 20-FEB-2008 21:41:51
Revision_time = 20-FEB-2008 21:19:52
Current_time = 14-APR-2008 21:13:15
Comment = single_pulse
Data_format = 1D COMPLEX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = ECX_300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 2.90717696[s]
X_domain = 1H
X_freq = 300.52965592[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.34397631[Hz]
X_sweep = 5.63570784[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Tri_domain = 1H
Tri_freq = 300.52965592[MHz]
Tri_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 24
Total_scans = 24
X_90_width = 13.01[us]
X_acq_time = 2.90717696[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 6.505[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Recvr_gain = 42
Relaxation_delay = 5[s]
Repetition_time = 7.90717696[s]
Temp_get = 23.3[dC]
Br
Br
HO
O
NOH
O
abundance
X : parts per Million : 1H
7.4253
7.2582
4.3877
4.3636
4.3396
4.3167
3.9058
3.8463
1.3866
1.3626
1.3385
2.99
2.99
2
1.99
1.04

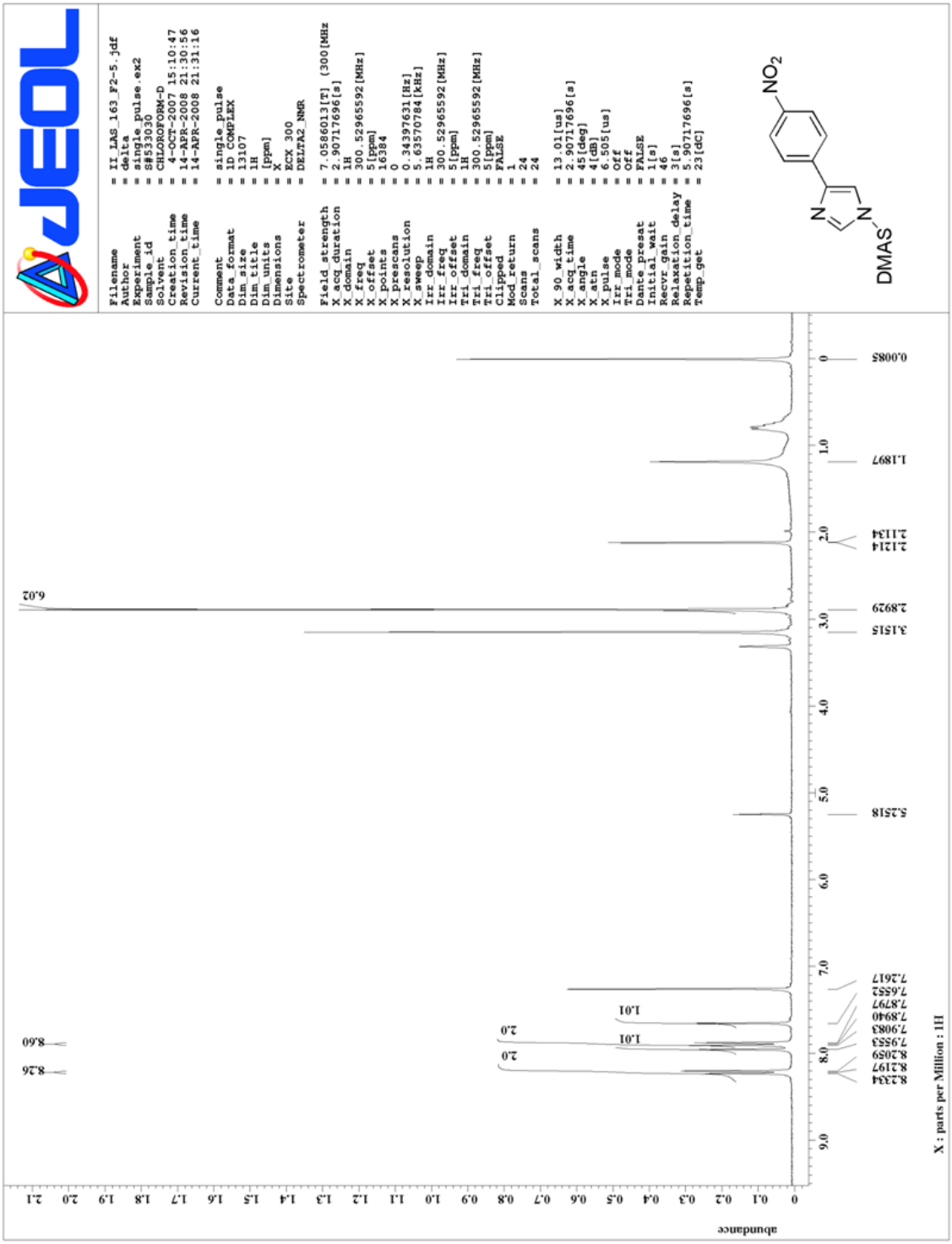

X : parts per Million : 1H
abundance
4.3877
4.3636
4.3396
4.3167
3.9058
3.8463
1.3866
1.3626
1.3505
1.3385
2.99
1.99
2
6.88
7.33
7.44
7.22
2.99
Br
Br
O
HO
NOH
O

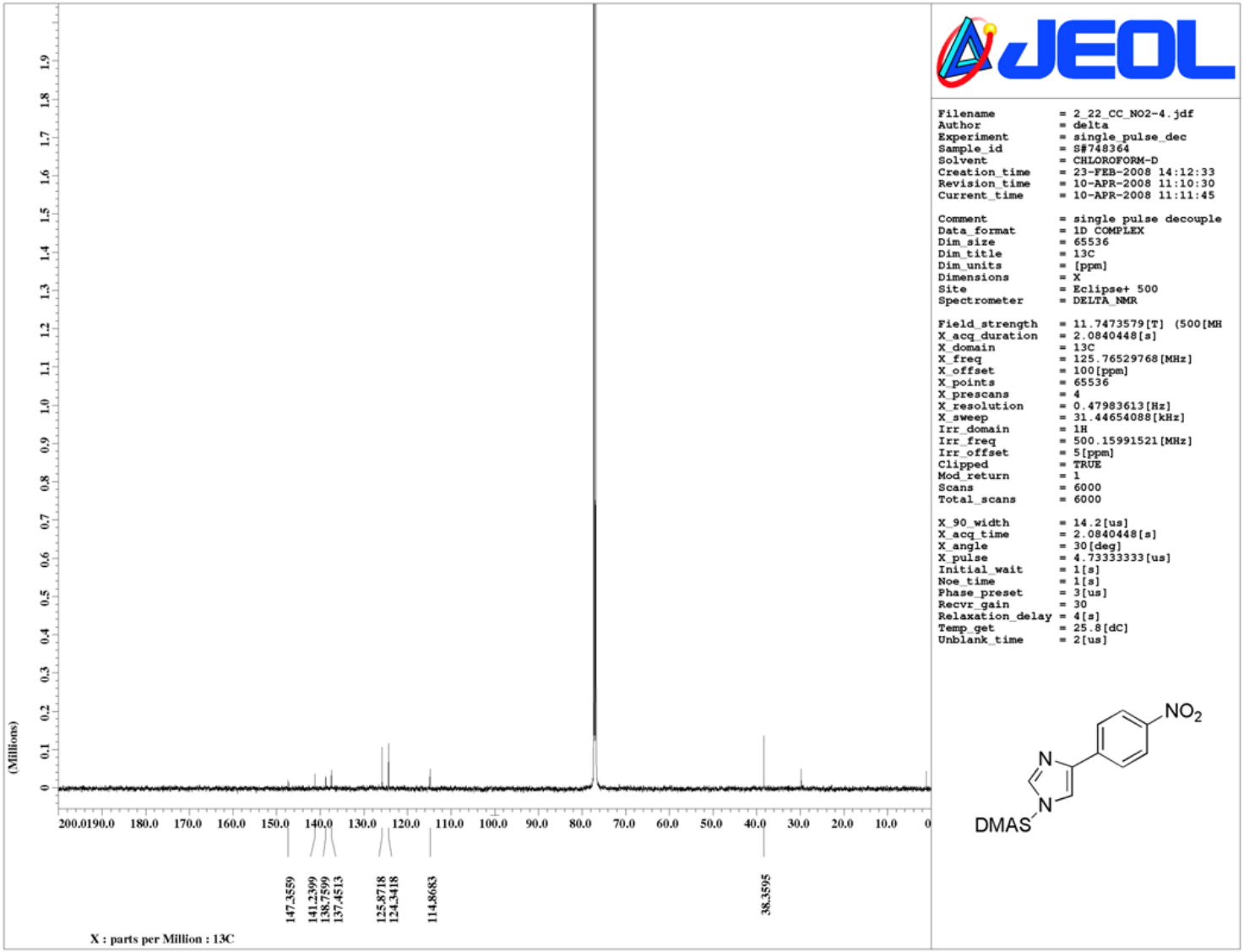

JEOL
Filename = II_LAS_197_II-4.jdf
Author = delta
Experiment = single_pulse_dec
Sample_id = S#764464
Solvent = CHLOROFORM-D
Creation_time = 20-FEB-2008 21:57:00
Revision_time = 20-FEB-2008 21:30:31
Current_time = 9-APR-2008 21:29:40
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 198
Total_scans = 198
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 3[s]
Recvr_gain = 50
Relaxation_delay = 3[s]
Repetition_time = 4.38412032[s]
Temp_get = 23.9[dC]
Br
Br
O
HO
NOH
O
14.0977
25.6278
60.6580
63.0386
107.6676
107.8015
119.5228
133.6821
149.8396
151.7709
153.9124
164.1710
abundance
X : parts per Million : 13C

APPENDIX X

^{1}H NMR AND ^{13}C NMR SPECTRA OF

1-*N*-DIMETHYLSULFONYL-4-(4-NITROPHENYL)-(*1H*)-IMIDAZOLE (178a)

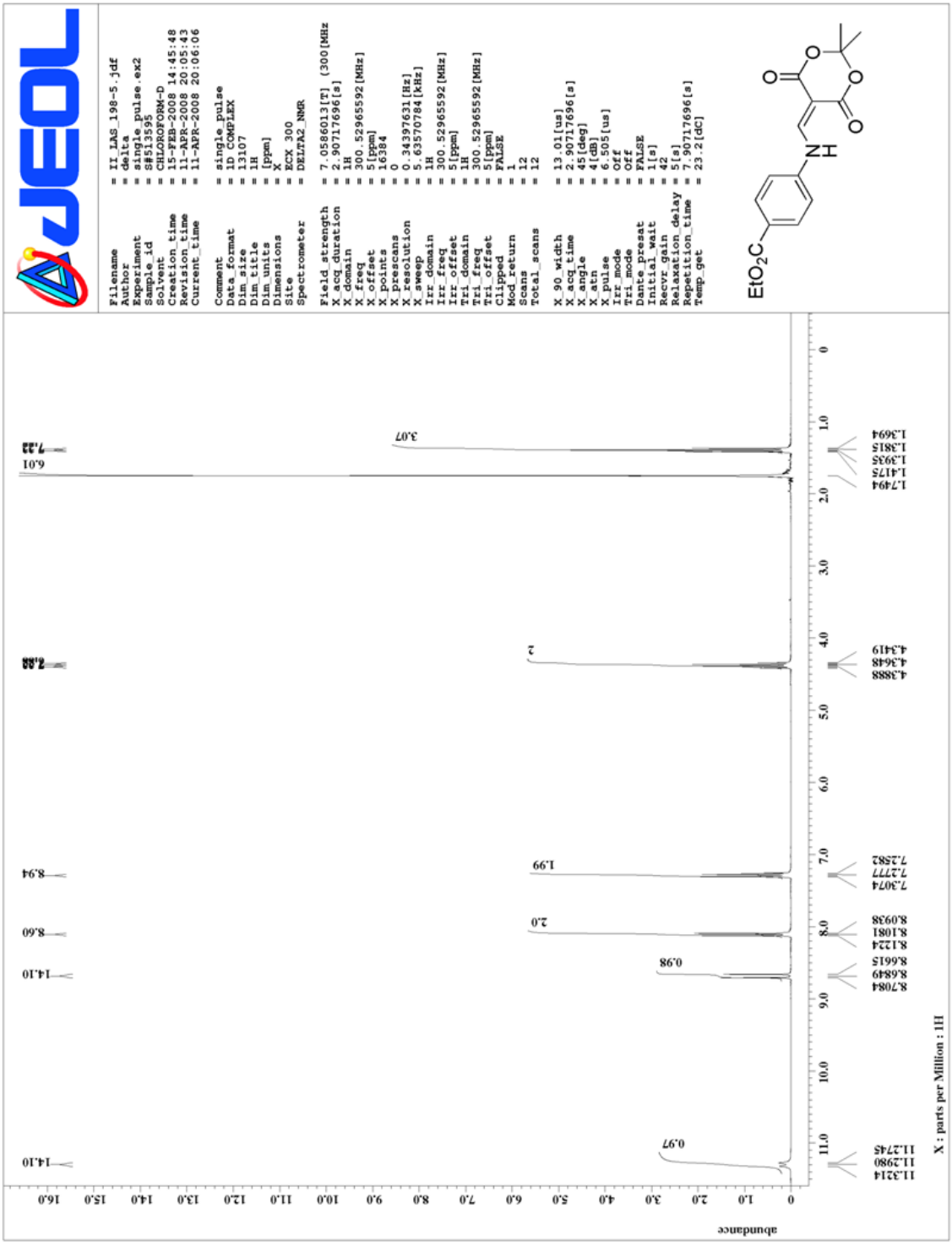

JEOL
Filename = II_LAS_163_F2-5.jdf
Author = delta
Experiment = single_pulse.ex2
Sample_id = S#533030
Solvent = CHLOROFORM-D
Creation_time = 4-OCT-2007 15:10:47
Revision_time = 14-APR-2008 21:30:56
Current_time = 14-APR-2008 21:31:16
Comment = single_pulse
Data_format = 1D COMPLEX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 2.90717696[s]
X_domain = 1H
X_freq = 300.52965592[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.34397631[Hz]
X_sweep = 5.63570784[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Tri_domain = 1H
Tri_freq = 300.52965592[MHz]
Tri_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 24
Total_scans = 24
X_90_width = 13.01[us]
X_acq_time = 2.90717696[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 6.505[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Recvr_gain = 46
Relaxation_delay = 3[s]
Repetition_time = 5.90717696[s]
Temp_get = 23[dC]
NO2
N
N
DMAS
abundance
X : parts per Million : 1H
8.60
8.26
6.02
2.0
2.0
1.01
1.01
8.2334
8.2197
8.2059
7.9553
7.9083
7.8940
7.8797
7.6552
7.2617
5.2518
3.1515
2.8929
2.1214
2.1134
1.1897
0.0085

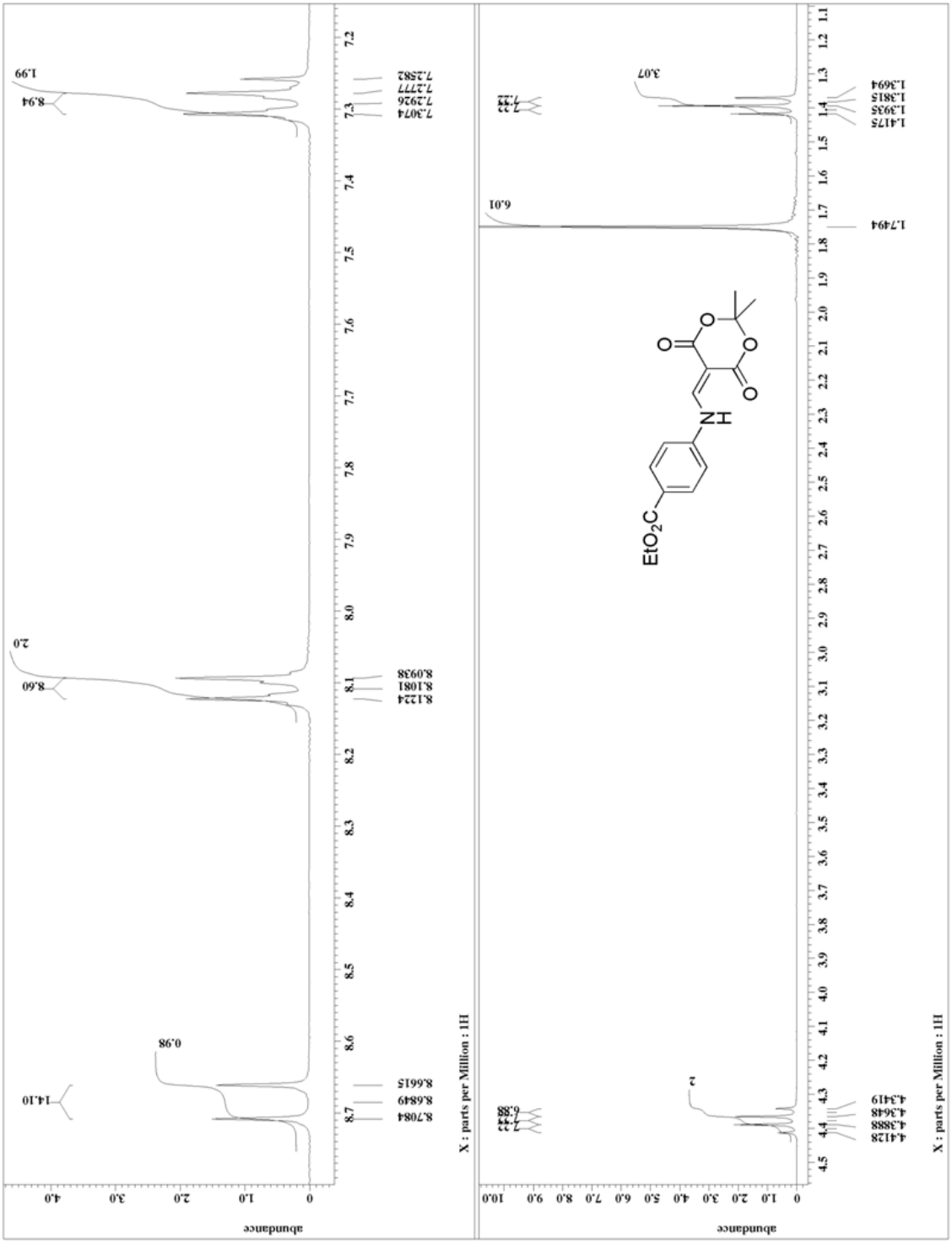

(Millions)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9
200.0190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0
147.3559
141.2399
138.7599
137.4513
125.8718
124.3418
114.8683
38.3595
X : parts per Million : 13C
JEOL
Filename = 2_22_CC_NO2-4.jdf
Author = delta
Experiment = single_pulse_dec
Sample_id = S#748364
Solvent = CHLOROFORM-D
Creation_time = 23-FEB-2008 14:12:33
Revision_time = 10-APR-2008 11:10:30
Current_time = 10-APR-2008 11:11:45
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 65536
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = Eclipse+ 500
Spectrometer = DELTA_NMR
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 2.0840448[s]
X_domain = 13C
X_freq = 125.76529768[MHz]
X_offset = 100[ppm]
X_points = 65536
X_prescans = 4
X_resolution = 0.47983613[Hz]
X_sweep = 31.44654088[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5[ppm]
Clipped = TRUE
Mod_return = 1
Scans = 6000
Total_scans = 6000
X_90_width = 14.2[us]
X_acq_time = 2.0840448[s]
X_angle = 30[deg]
X_pulse = 4.73333333[us]
Initial_wait = 1[s]
Noe_time = 1[s]
Phase_preset = 3[us]
Recvr_gain = 30
Relaxation_delay = 4[s]
Temp_get = 25.8[dC]
Unblank_time = 2[us]
NO2
N
N
DMAS

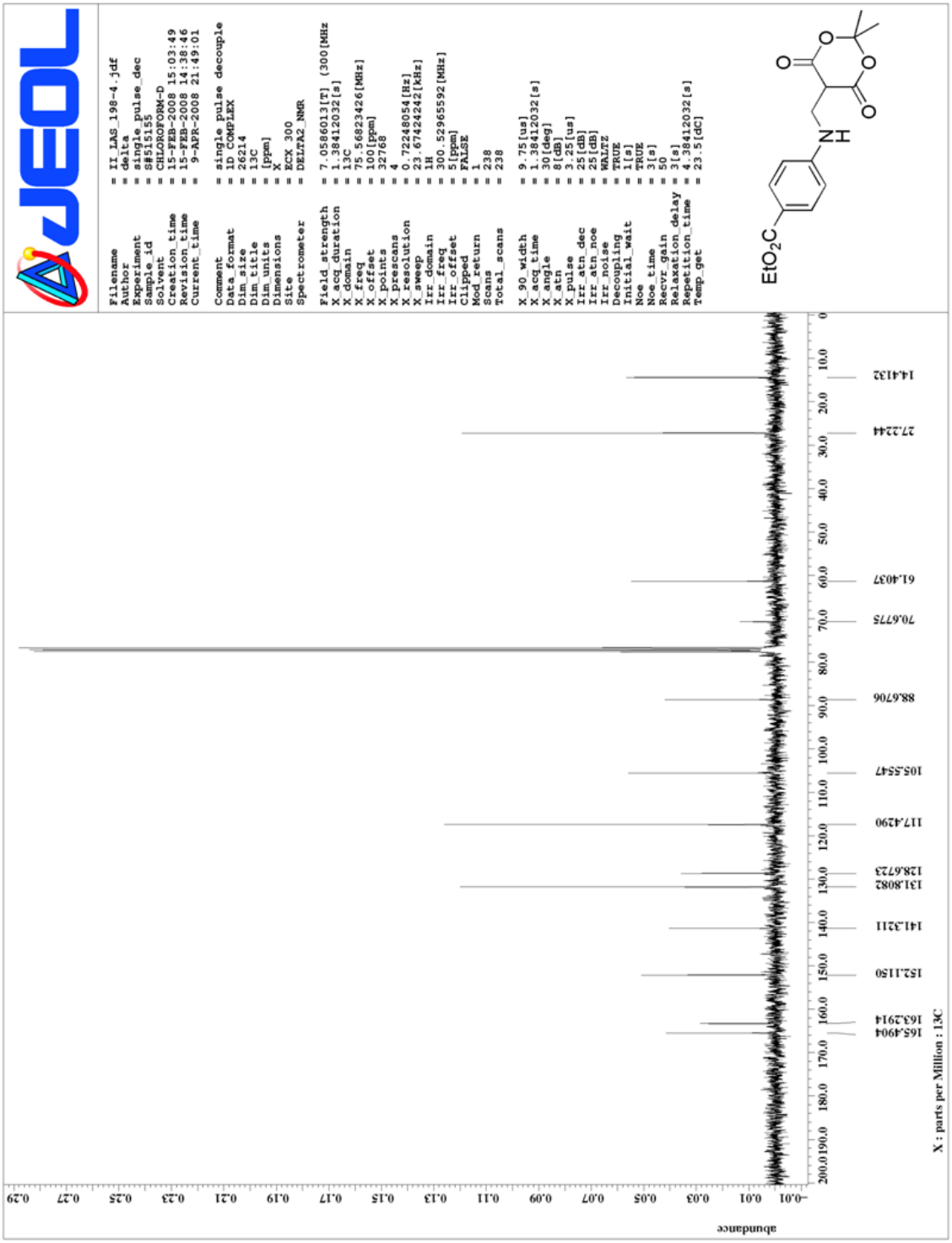
APPENDIX Y

^{1}H NMR AND ^{13}C NMR SPECTRA OF

5-[[(4-CARBOXYETHYLPHENYL)AMINO]METHYLENE]-2,2-DIMETHYL-1,3-DIOXANE-4,6-DIONE (180)


JEOL
Filename = II_LAS_198-5.jdf
Author = delta
Experiment = single_pulse.ex2
Sample_id = S#513595
Solvent = CHLOROFORM-D
Creation_time = 15-FEB-2008 14:45:48
Revision_time = 11-APR-2008 20:05:43
Current_time = 11-APR-2008 20:06:06
Comment = single_pulse
Data_format = 1D COMPLEX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 2.90717696[s]
X_domain = 1H
X_freq = 300.52965592[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.34397631[Hz]
X_sweep = 5.63570784[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Tri_domain = 1H
Tri_freq = 300.52965592[MHz]
Tri_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 12
Total_scans = 12
X_90_width = 13.01[us]
X_acq_time = 2.90717696[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 6.505[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Recvr_gain = 42
Relaxation_delay = 5[s]
Repetition_time = 7.90717696[s]
Temp_get = 23.2[dC]
EtO2C
NH
abundance
X : parts per Million : 1H

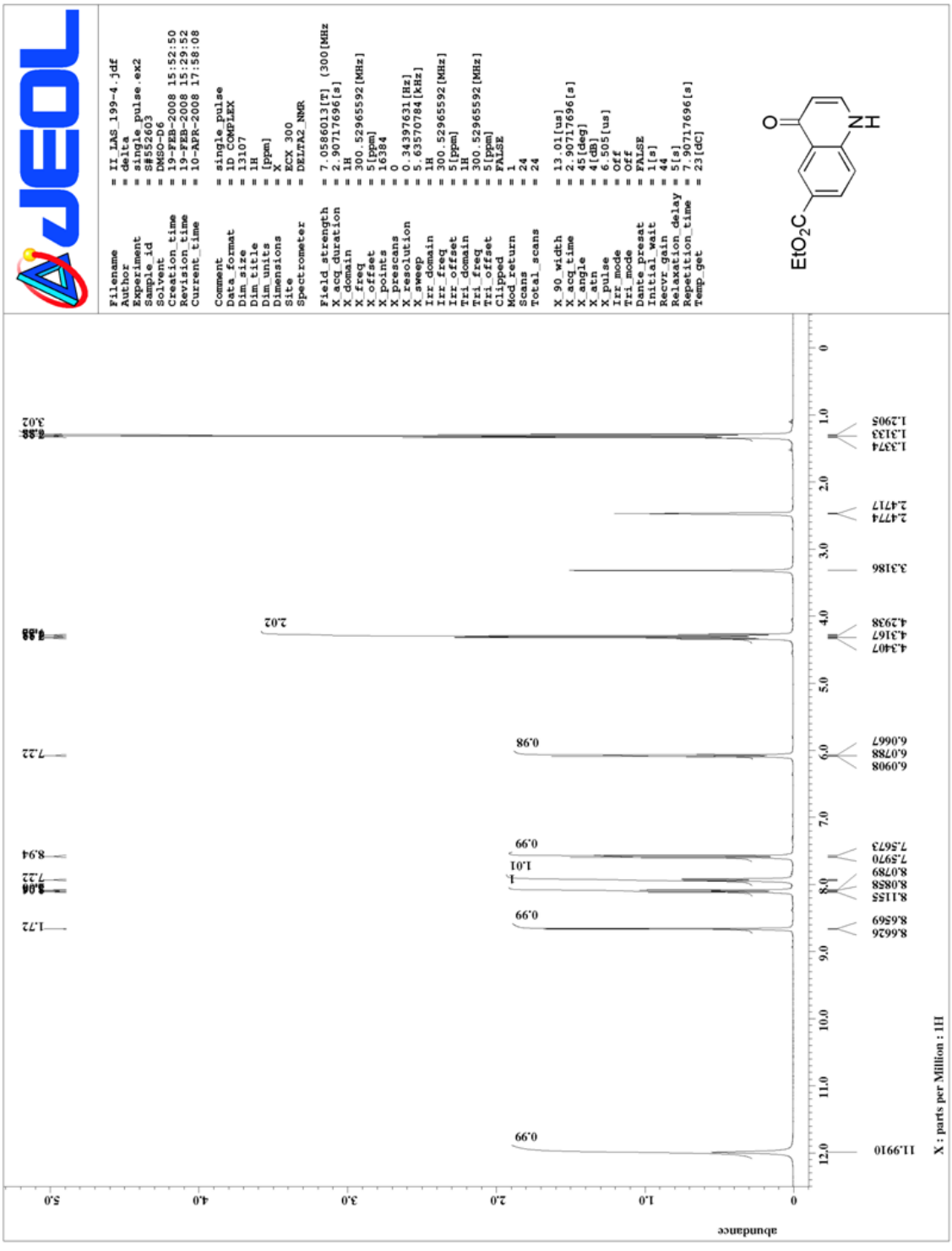

abundance
X : parts per Million : 1H
8.7084
8.6849
8.6615
8.1224
8.1081
8.0938
7.3074
7.2926
7.2777
7.2582
6.88
4.4128
4.3888
4.3648
4.3419
3.07
1.7494
1.4175
1.3935
1.3815
1.3694
0.98
14.10
2.0
8.60
8.94
1.99
6.01
EtO2C
O
O
O
O
NH


JEOL
Filename = II_LAS_198-4.jdf
Author = delta
Experiment = single_pulse_dec
Sample_id = S#51515
Solvent = CHLOROFORM-D
Creation_time = 15-FEB-2008 15:03:49
Revision_time = 15-FEB-2008 14:38:46
Current_time = 9-APR-2008 21:49:01
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 238
Total_scans = 238
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 3[s]
Recyr_gain = 50
Relaxation_delay = 3[s]
Repetition_time = 4.38412032[s]
Temp_get = 23.5[dC]
EtO2C
NH
O
O
O
O
abundance
X : parts per Million : 13C
14.4132
27.2244
61.4037
70.6775
88.6706
105.5547
117.4290
128.6723
131.8082
141.3211
152.1150
163.2914
165.4904

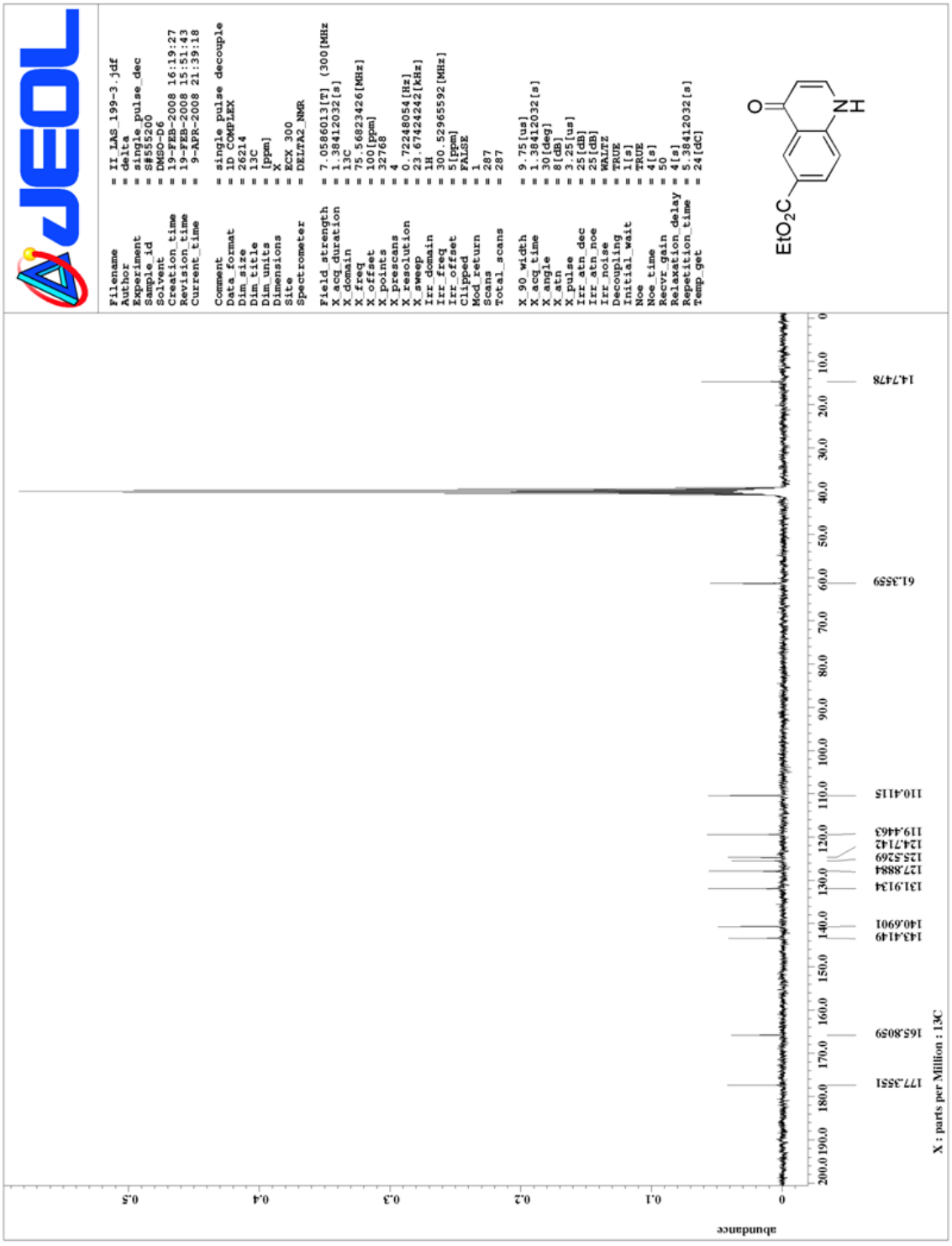
APPENDIX Z

^{1}H NMR AND ^{13}C NMR SPECTRA OF

6-CARBOXYETHYL-4(*1H*)-QUINOLONE (181)


JEOL
Filename = IT_LAS_199-4.jdf
Author = delta
Experiment = single_pulse.ex2
Sample_id = S#552603
Solvent = DMSO-D6
Creation_time = 19-FEB-2008 15:52:50
Revision_time = 19-FEB-2008 15:29:52
Current_time = 10-APR-2008 17:58:08
Comment = single_pulse
Data_format = 1D COMPLEX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 2.90717696[s]
X_domain = 1H
X_freq = 300.52965592[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.34397631[Hz]
X_sweep = 5.63570784[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Tri_domain = 1H
Tri_freq = 300.52965592[MHz]
Tri_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 24
Total_scans = 24
X_90_width = 13.01[us]
X_acq_time = 2.90717696[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 6.505[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Recvr_gain = 44
Relaxation_delay = 5[s]
Repetition_time = 7.90717696[s]
Temp_get = 23[dC]
EtO2C
O
NH
abundance
X : parts per Million : 1H

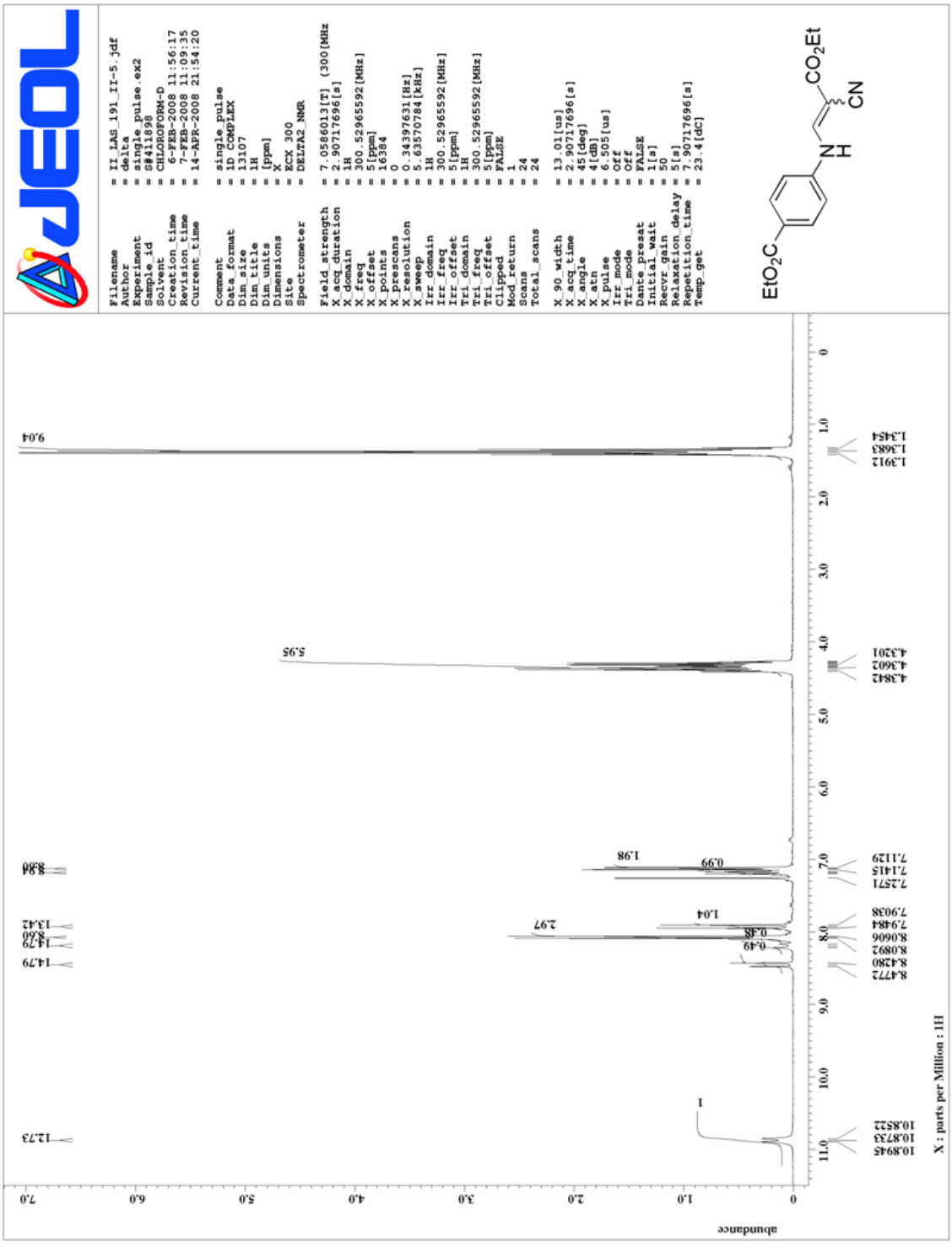
abundance
X : parts per Million : 1H
8.6626
8.6598
8.6569
8.1155
8.1121
8.1086
8.0972
8.0858
8.0789
7.9438
7.9318
7.9198
7.5970
7.5821
7.5673
6.0908
6.0788
6.0667
7.22
1.22
0.99
8.94
1.01
1
4.3407
4.3167
4.2938
4.2698
3.3186
2.4774
2.4717
2.4648
1.3374
1.3133
1.3019
1.2905
3.02
2.02
EtO2C
HN
O


Filename = II_LAS_199-3.jdf
Author = delta
Experiment = single_pulse_dec
Sample_id = S#555200
Solvent = DMSO-D6
Creation_time = 19-FEB-2008 16:19:27
Revision_time = 19-FEB-2008 15:51:43
Current_time = 9-APR-2008 21:39:18
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 287
Total_scans = 287
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 4[s]
Recvr_gain = 50
Relaxation_delay = 4[s]
Repetition_time = 5.38412032[s]
Temp_get = 24[dC]
EtO2C
O
NH
abundance
X : parts per Million : 13C
14.7478
61.3559
110.4115
119.4463
124.7142
125.5269
127.8884
131.9134
140.6901
143.4149
165.8059
177.3551

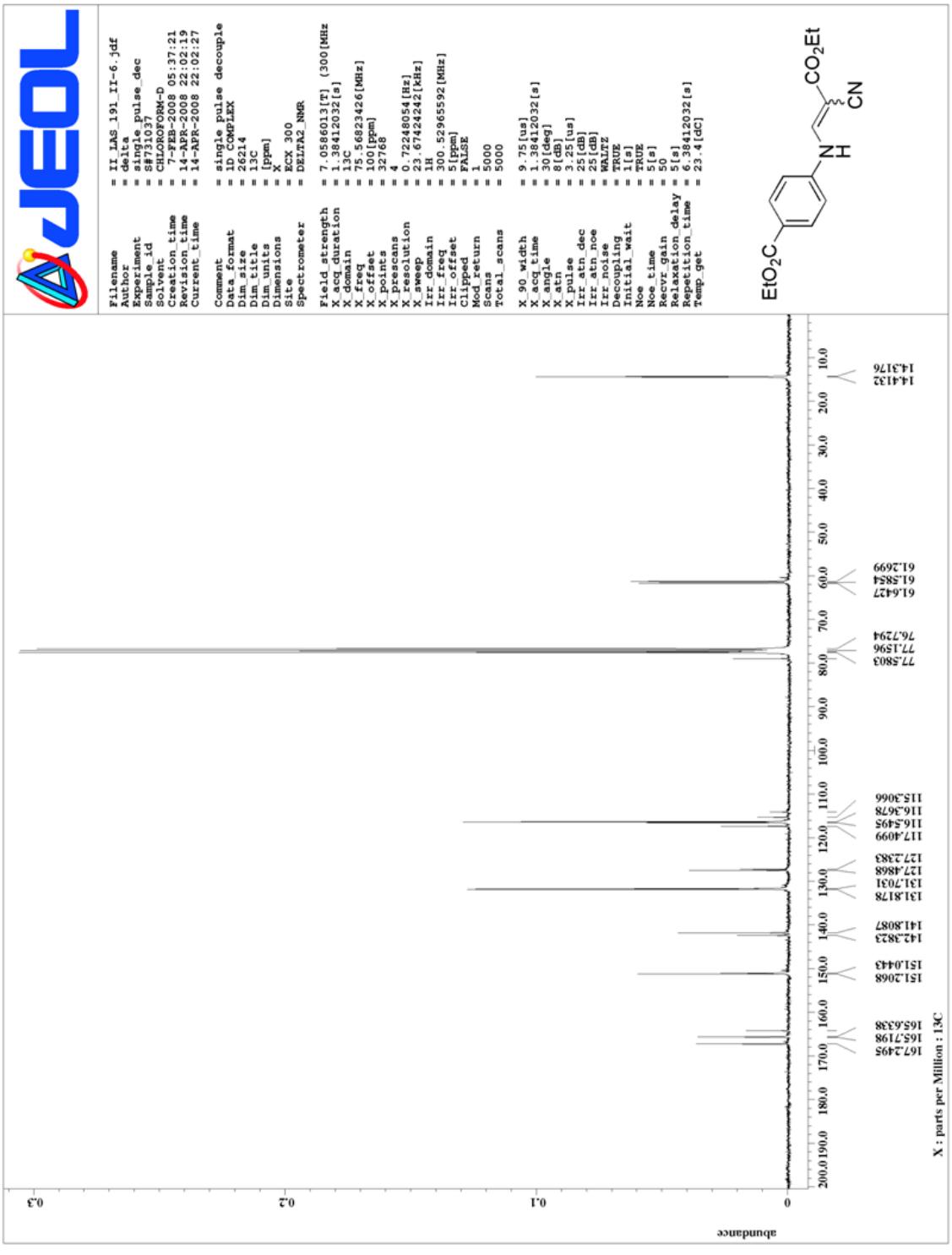
APPENDIX AA

^{1}H AND ^{13}C NMR SPECTRA OF

ETHYL 3-[(4-CARBOXYETHYLPHENYL)AMINO]-2-CYANO-2-PROPENOATE (182)


JEOL
Filename = II_LAS_191_II-5.jdf
Author = delta
Experiment = single_pulse.ex2
Sample_id = S#411898
Solvent = CHLOROFORM-D
Creation_time = 6-FEB-2008 11:56:17
Revision_time = 7-FEB-2008 11:09:35
Current_time = 14-APR-2008 21:54:20
Comment = single_pulse
Data_format = 1D COMPLEX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = RCX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.05860131[T] (300[MHz
X_acq_duration = 2.90717696[s]
X_domain = 1H
X_freq = 300.52965592[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.34397631[Hz]
X_sweep = 5.63570784[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Tri_domain = 1H
Tri_freq = 300.52965592[MHz]
Tri_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 24
Total_scans = 24
X_90_width = 13.01[us]
X_acq_time = 2.90717696[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 6.505[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Recvr_gain = 50
Relaxation_delay = 5[s]
Repetition_time = 7.90717696[s]
Temp_get = 23.4[dC]
EtO2C
CO2Et
N
H
CN
abundance
X : parts per Million : 1H
9.04
5.95
1.98
0.99
2.97
1.04
0.38
0.49
1
12.73
8.84
8.80
13.42
14.29
8.20
14.79
10.8945
10.8733
10.8522
8.4772
8.4280
8.0892
8.0606
7.9484
7.9038
7.2571
7.1415
7.1129
4.3842
4.3602
4.3201
1.3912
1.3683
1.3454

abundance
X : parts per Million : 1H
8.4772
8.4526
8.4280
8.2162
8.1916
8.1670
8.0892
8.0749
8.0606
7.9484
7.9261
7.9038
7.2571
7.1999
7.1850
7.1701
7.1415
7.1272
7.1129
1.98
1.04
0.99
0.49
0.48
2.97
14.79
14.79
13.42
8.60
8.60
8.94
CO2Et
CN
N
H
EtO2C

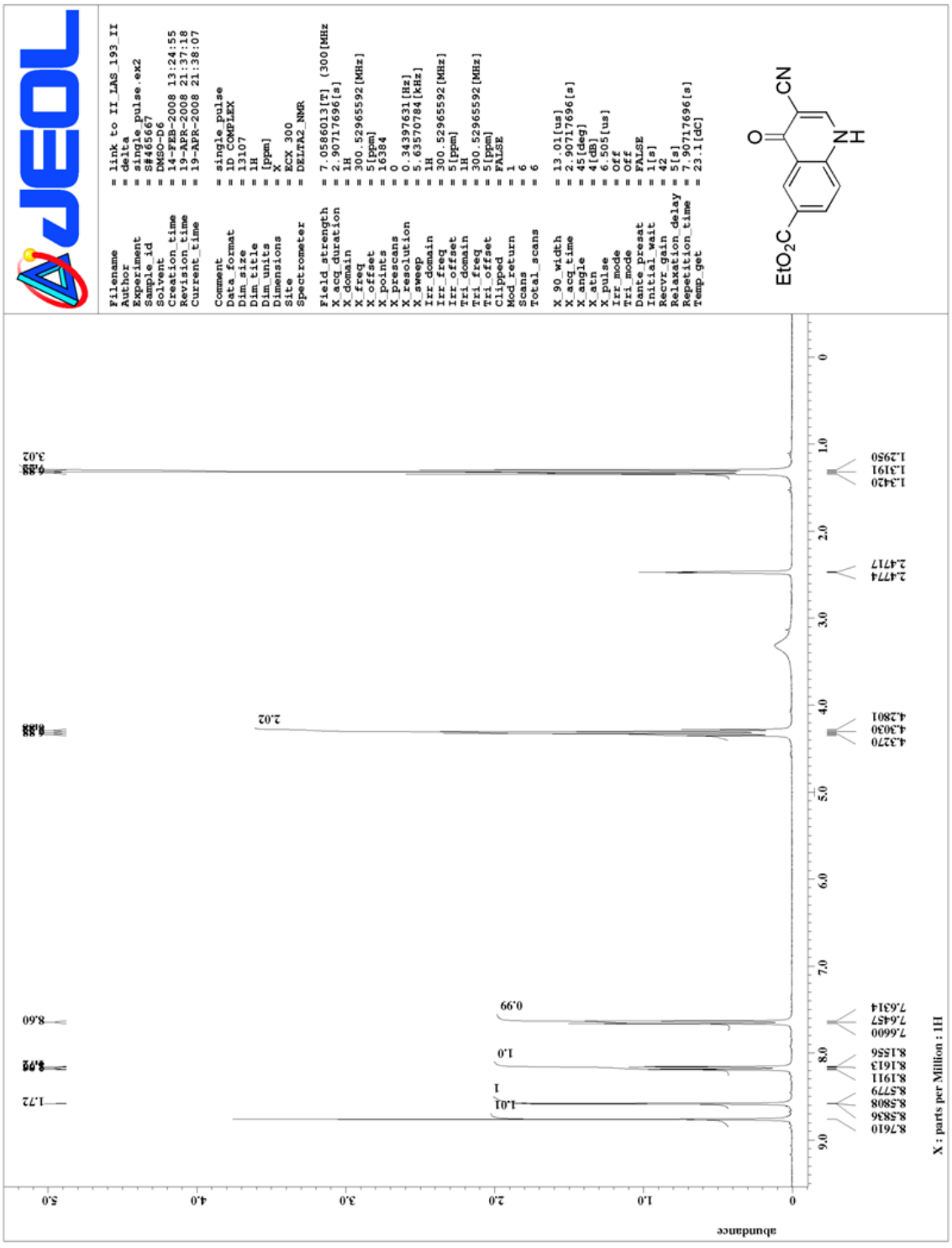

JEOL
Filename = II_LAS_191_II-6.jdf
Author = delta
Experiment = single_pulse_dec
Sample_id = S#731037
Solvent = CHLOROFORM-D
Creation_time = 7-FEB-2008 05:37:21
Revision_time = 14-APR-2008 22:02:19
Current_time = 14-APR-2008 22:02:27
Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
X_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 5000
Total_scans = 5000
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 5[s]
Recyr_gain = 50
Relaxation_delay = 5[s]
Repetition_time = 6.38412032[s]
Temp_get = 23.4[dC]
EtO2C
CO2Et
N
H
CN
abundance
0.3
0.2
0.1
0
200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0
167.2495
165.7198
165.6338
151.2068
151.0443
142.3823
141.8087
131.8178
131.7031
127.4868
127.2383
117.4099
116.5495
116.3678
115.3066
77.5803
77.1596
76.7294
61.6427
61.3854
61.2699
14.4132
14.3176
X : parts per Million : 13C

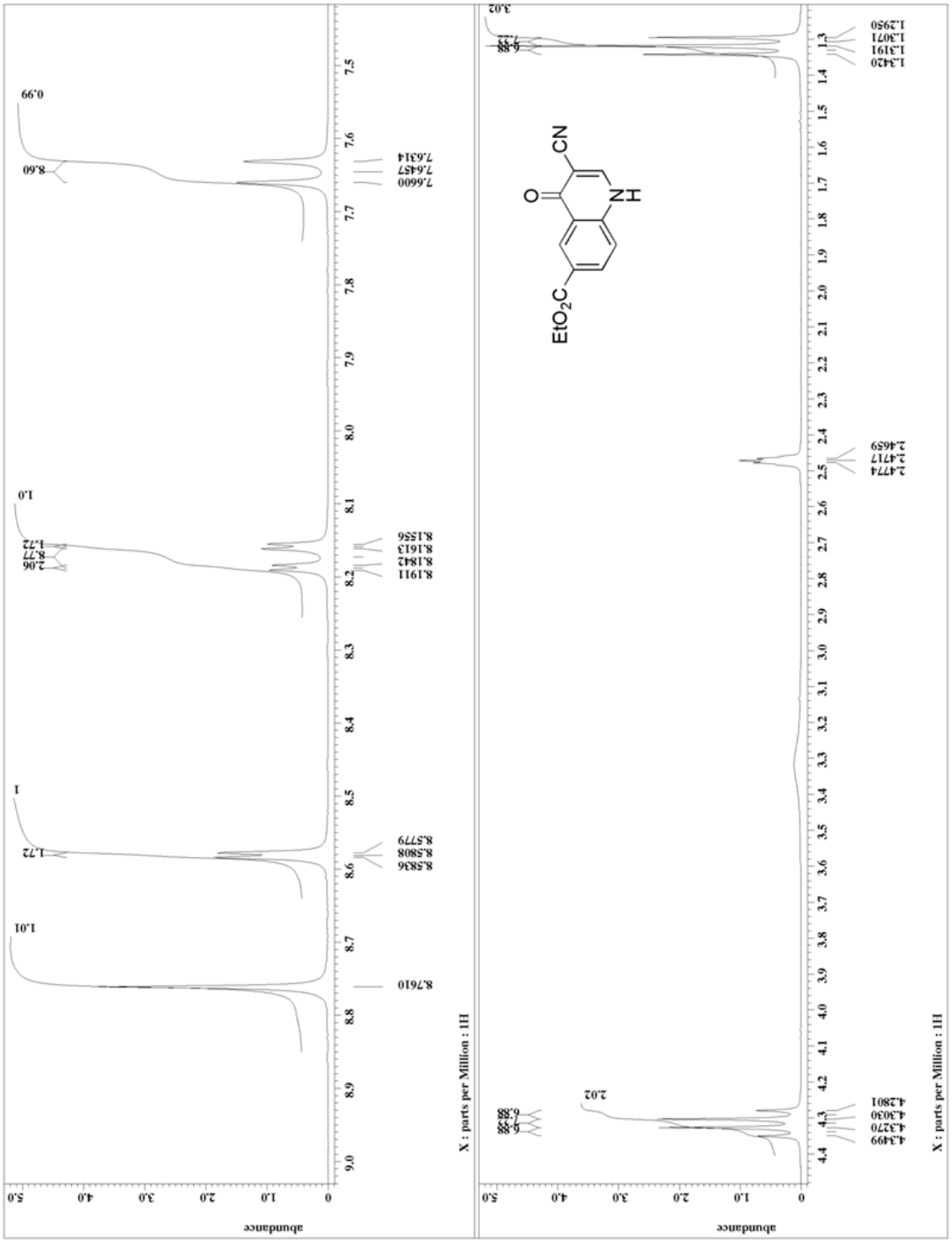
167.2495
165.7198
165.6338
164.2379
151.2068
151.0443
142.3823
141.8087
131.8178
131.7031
127.4868
127.2383
117.4099
116.5495
116.3678
115.3066
114.0924
14.4132
14.3176
61.6427
61.5854
61.2699
78.9857
77.5803
77.4178
77.1596
76.7294
EtO2C
CO2Et
CN
H
N
abundance
X : parts per Million : 13C

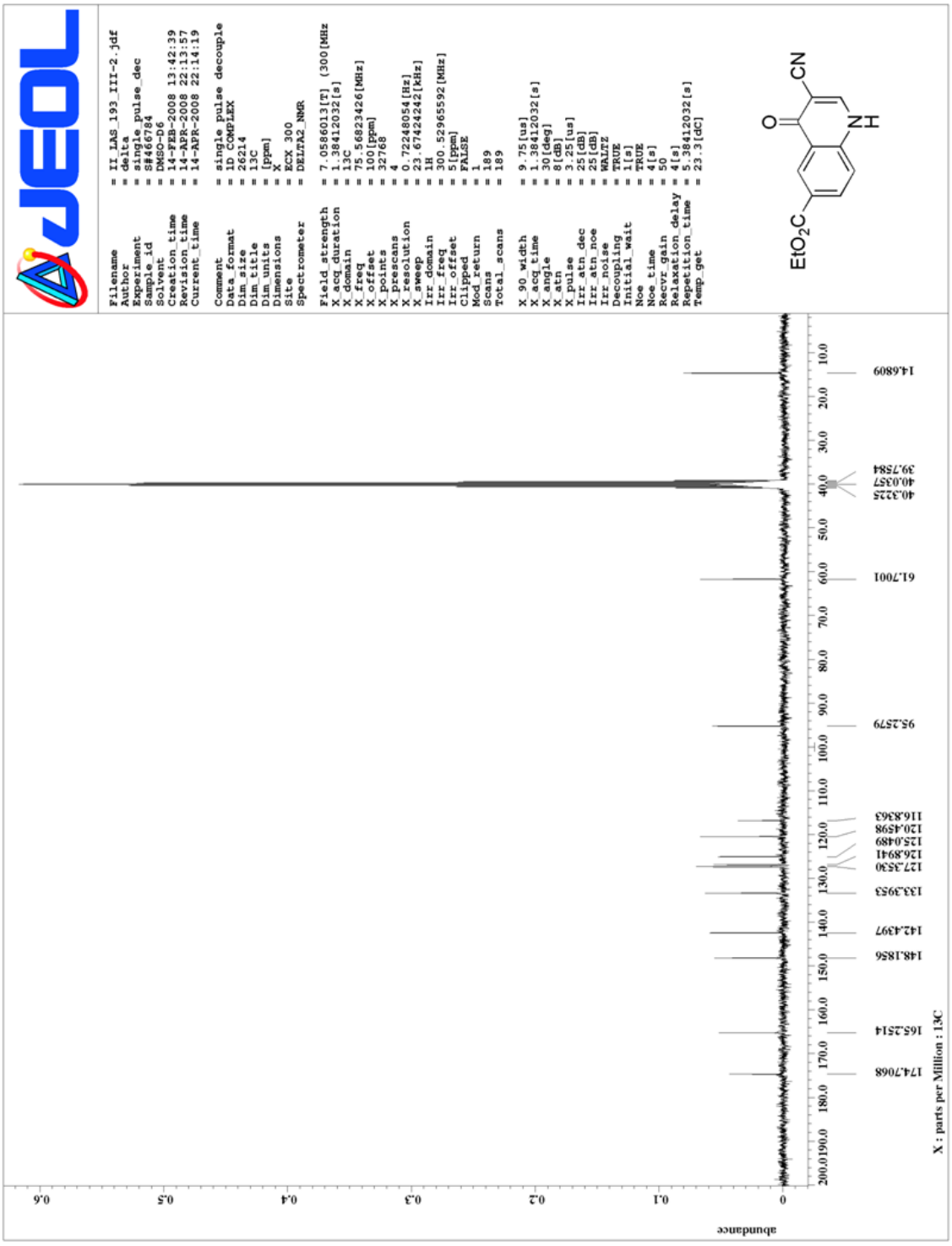
APPENDIX AB

^{1}H AND ^{13}C NMR SPECTRA OF

6-CARBOXYETHYL-3-CYANO-4(*1H*)-QUINOLONE (183)


JEOL
Filename = link to II_LAS_193_II
Author = delta
Experiment = single_pulse.ex2
Sample_id = S#465667
Solvent = DMSO-D6
Creation_time = 14-FEB-2008 13:24:55
Revision_time = 19-APR-2008 21:37:18
Current_time = 19-APR-2008 21:38:07
Comment = single pulse
Data_format = 1D COMPLEX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.05860137[T] (300[MHz
X_acq_duration = 2.90717696[s]
X_domain = 1H
X_freq = 300.52965592[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.34397631[Hz]
X_sweep = 5.63570784[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Tri_domain = 1H
Tri_freq = 300.52965592[MHz]
Tri_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 6
Total_scans = 6
X_90_width = 13.01[us]
X_acq_time = 2.90717696[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 6.505[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Recvr_gain = 42
Relaxation_delay = 5[s]
Repetition_time = 7.90717696[s]
Temp_get = 23.1[dC]
CN
O
NH
EtO2C
abundance
X : parts per Million : 1H
8.7610
8.5836
8.5808
8.5779
8.1911
8.1613
8.1556
7.6600
7.6457
7.6314
4.3270
4.3030
4.2801
2.4774
2.4717
1.3420
1.3191
1.2950
1.72
1.01
1
1.0
0.99
2.02
3.02


abundance
X : parts per Million : 1H
EtO2C
CN
O
NH


JEOL
Filename = II_LAS_193_III-2.jdf
Author = delta
Experiment = single_pulse_dec
Sample_id = S#466784
Solvent = DMSO-D6
Creation_time = 14-FEB-2008 13:42:39
Revision_time = 14-APR-2008 22:13:57
Current_time = 14-APR-2008 22:14:19
Comment = single_pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECX 300
Spectrometer = DELTA2_NMR
Field_strength = 7.0586013[T] (300[MHz
X_acq_duration = 1.38412032[s]
X_domain = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054[Hz]
X_sweep = 23.67424242[kHz]
Irr_domain = 1H
Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 189
Total_scans = 189
X_90_width = 9.75[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_atn = 8[dB]
X_pulse = 3.25[us]
Irr_atn_dec = 25[dB]
Irr_atn_noe = 25[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 4[s]
Recvr_gain = 50
Relaxation_delay = 4[s]
Repetition_time = 5.38412032[s]
Temp_get = 23.3[dC]
CN
O
NH
EtO2C
abundance
X : parts per Million : 13C
14.6809
39.7584
40.0357
40.3225
61.7001
95.2579
116.8363
120.4598
125.0489
126.8941
127.3530
133.3953
142.4397
148.1856
165.2514
174.7068

R

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