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SYNTHESIS AND BIOLOGICAL ACTIVITY OF AMINOGLYCOSIDES
AND 1,4-NAPHTHOQUINONE DERIVATIVES

by

Marina Fosso Yatchang

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Chemistry

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2012

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ABSTRACT

Synthesis and Biological Activity of Aminoglycosides
and 1,4-Naphthoquinone Derivatives

by

Marina Fosso Yatchang, Doctor of Philosophy

Utah State University, 2012

Major Professor: Dr. Cheng-Wei Tom Chang
Department: Chemistry and Biochemistry

Aminoglycosides, such as streptomycin, kanamycin and neomycin, are a group of naturally occurring antibiotics that structurally consist of various amino-modified sugars. They have long been used clinically for their broad-spectrum activity against Gram-negative and Gram-positive bacteria. However, the incidence of bacterial resistance has considerably hampered their clinical efficacy, forcing researchers to explore new applications of aminoglycosides.

An aminoglycoside belonging to the class of pyrankancin was identified as the lead compound in the treatment of spinal muscular atrophy, an infantile disease caused by nonsense mutations. To further investigate its therapeutic capabilities, additional batches of this lead compound were prepared and its mode of action study revealed an unprecedented SMN Δ 7 read-through event.

In addition, the chemical derivation of kanamycin B was examined in the aim of developing potential agro fungicides. Indeed, a library of kanamycin B analogs was synthesized to investigate the length of the alkyl chain and its position in kanamycin B that will confer to this latter an optimum antifungal activity. Results of this study revealed that the attachment of an octyl group at the *O*-4'' position of the core structure of the classical aminoglycoside kanamycin B converts this obsolete drug into a broad-spectrum fungicide. Another interesting finding was the simultaneous loss of antibacterial activity usually observed in aminoglycosides. This was essential as it paves the way for the development of a new class of aminoglycoside-based fungicides suitable for use in crop disease application.

Molecules with naphthoquinone scaffolds are also of great interest due to their important biological and pharmaceutical applications. Three synthetic protocols were examined to optimize the production of the 1,4-naphthoquinone derivatives and to conveniently synthesize a library of novel cationic anthraquinone analogs. The antibacterial activities of these compounds were evaluated and they were found to display much higher levels of activities against Gram-positive than Gram-negative bacteria. In addition, with double alkyl chains of various lengths ($C_2 - C_{12}$) at N-1 and N-3 positions, a synergistic effect of the alkyl groups was observed, suggesting the importance of overall lipophilicity in the activity of this class of compounds against Gram-positive bacteria.

PUBLIC ABSTRACT

Synthesis and Biological Activity of Aminoglycosides
and 1,4-Naphthoquinone Derivatives

by

Marina Fosso Yatchang, Doctor of Philosophy

Utah State University, 2012

The research described in this dissertation is at the interface of organic chemistry and biology, and it aimed at designing and synthesizing biologically active molecules for the possible development of therapeutic agents.

Spinal muscular atrophy is an incurable disease that affects 1 in every 6000 babies, making it the leading genetic cause of infant mortality. While no treatment is available, efforts are being taken to solve this issue. Part of the work outlined in this dissertation was carried out in collaboration with researchers from the University of Missouri to investigate a potential therapeutic for this disease.

In addition, the continuous outbreak of diseases caused by bacteria demands for new and improved antibiotics that could help eradicate those pathogens. My research thus allowed me to discover molecules with interesting activity against bacteria for the possible development of potential antibacterial agents.

Finally, my research also allowed me to develop potential agro fungicides, which are still very much needed nowadays. Many crop diseases are due to fungal infections,

which globally cause enormous economic losses. The use of fungicides is still the main strategy to control these diseases. However, current agro fungicides show some limitations. This is illustrated with Fusarium head blight (FHB), a destructive and costly disease of wheat, barley and other small grains, whose economic losses in the Central United States alone were estimated to \$2.7 billion.

DEDICATION

I would like to dedicate this work to my mother, KOUAKEP JOSETTE, for every single sacrifice she has ever made in her life for my well-being.

ACKNOWLEDGMENTS

First and foremost, I would like to express my profound gratitude to my supervisor, Dr. Cheng-Wei Tom Chang, for giving me the opportunity to conduct my doctoral research in his laboratory. He taught me how to work hard and think critically. His guidance, patience, and support throughout my program have been invaluable sources of motivation.

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To my “nounours” Yannick Bidias, you are certainly the one who has experienced my ups and downs during my doctoral program. You offered me your shoulder to cry on when nothing was going on well, and you were there to share every single moment of happiness I had. You are my best friend and I could not think of a better person to share my life with.

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Marina Fosso Yatchang

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LIST OF ABBREVIATIONS

2-DOS: 2-deoxystreptamine

Ac: acetyl

Ac₂O: acetic anhydride

ACOH: acetic acid

APCI: atmospheric pressure chemical ionization

Bn: benzyl

BnBr: benzyl bromide

DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene

DMAP: 4-(dimethylamino)pyridine

DMF: dimethylformamide

DMSO: dimethyl sulfoxide

ESI: electrospray ionization

FAB: fast atom bombardment

FHB: fusarium head blight

G⁻: gram negative

G⁺: gram positive

HTB: hexadecyltrimethylammonium bromide

MALDI: matrix-assisted laser desorption/ionization

MIC: minimum inhibitory concentration

NBS: *N*-bromosuccinimide

NIS: *N*-iodosuccinimide

PBS: phosphate buffer saline

r.t.: room temperature

ROS: reactive oxygen species

SMA: spinal muscular atrophy

SMN: survival motor neuron

S_N²: bimolecular nucleophilic substitution

TBAI: tetrabutylammonium iodide

TBAHS: tetrabutyl ammonium hydrogen sulfate

TEA: triethylamine

Tf₂O: trifluoromethanesulfonyl acid anhydride

TfOH: trifluoromethanesulfonic acid

TFA: trifluoroacetic acid

THF: tetrahydrofuran

TMSOTf: trimethylsilyl trifluoromethanesulfonate

Tr: trityl or triphenylmethyl

Ts: tosyl

TsOH: *p*-toluene sulfonic acid

TsCl: toluenesulfonyl chloride

VPA: valproic acid

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CHAPTER I

GENERAL INTRODUCTION

Between 1940 and 2004, a staggering 335 infectious diseases have been discovered.¹ And with an estimated three new diseases being identified every couple of years, this number is constantly increasing.² Furthermore, diseases that were once treatable are resurging as a threat for a significant portion of the population. Through mutation or genetic exchange, infectious agents are able to develop resistance against available drugs and evolve into new deadly strains.

This alarming prevalence of drug-resistant microorganisms, together with the continuous emergence of infectious diseases, has enabled these pathogens to be two steps ahead of humans and contributed to ranking infectious diseases among the leading causes of mortality in the world.³

In addition to these emerging infectious diseases, which are caused by external factors (bacteria, viruses, and fungi), abnormalities in an individual's genome could also result in disorders called genetic diseases. Genetic diseases can either be inherited from the parents, or they could result from developed mutations or changes on the DNA. Changes that occur on a single gene give rise to Mendelian or single-gene disorders; meanwhile, multifactorial genetic diseases are caused by mutations in several genes, often coupled with environmental factors.

Spinal muscular atrophy (SMA) is an example of Mendelian disorders. It is caused by the homozygous loss of the *Survival Motor Neuron 1 (SMN1)* gene.⁴ It is an incurable neuromuscular disease characterized by the death of motor neurons present in

the anterior horn of the spinal cord.^{5,6} It manifests itself by the progressive weakness and degeneration of the muscles.⁷ Four types of SMA exist and they are categorized based upon the disease severity and the age of onset:⁸

- Type I or Werdnig-Hoffman disease is the most severe form of SMA and manifests itself in the first months of life (0-6 months),
- Type II or Dubowitz disease is the intermediate form with an age of onset between 6 and 18 months,
- Type III or Kugelberg-Welander disease manifests after 18 months (juvenile form),
- Type IV or adult-onset form appears after 35 years.

With an incidence of one in every 5,000 – 10,000 births, SMA is the leading genetic cause of infant mortality.^{9,10}

In light of these observations, the need to develop new and improved drugs that will help treat these diseases and alleviate this global threat becomes obvious. And what better place than nature to find the inspiration! This is clearly evidenced by the number of approved and clinical-trial drugs derived from natural products.^{11,12} For example, 26% of the new drugs approved by the Food and Drug Administration (FDA) in 2009-2010 were derived from nature.¹³

Aminoglycosides and naphthoquinones are two abundant, naturally occurring classes of compounds that have significant pharmacological properties. Anthracyclines, which can be viewed as 1,4-naphthoquinone derivatives, and aminoglycosides have even

been categorized as “drug-productive scaffolds”.¹³ It thus becomes apparent why we have directed our efforts toward these two classes of compounds.

I.1. Aminoglycosides

I.1.1. Classification and traditional mode of action of aminoglycosides

Streptomycin (Figure 1) was the first aminoglycoside to be discovered. Isolated from the actinobacterium *Streptomyces griseus* in 1944,¹⁴ it was the first antibiotic effective in the treatment of tuberculosis. Since then, the broad-spectrum of activity of aminoglycosides against both Gram-negative and Gram-positive bacteria has stimulated multitude interests.

Aminoglycosides are a group of naturally occurring antibiotics that structurally consist of various amino-modified sugars. 2-Deoxystreptamine (2-DOS) has been found to play a pivotal role in the biological activity of aminoglycosides,^{15,16} resulting in its derivatives being among the most studied aminoglycosides. This includes the two major classes of neomycin and kanamycin (Figure 2). Members of the neomycin class can be viewed as 4,5-disubstituted 2-deoxystreptamines, while the kanamycin class encompasses the 4,6-disubstituted 2-deoxystreptamines.

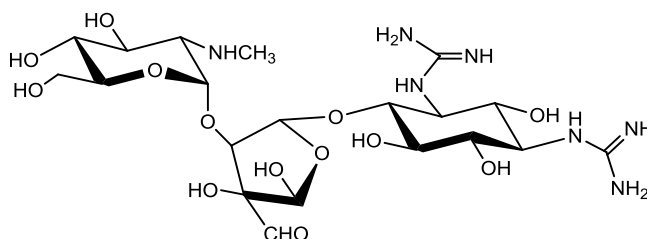
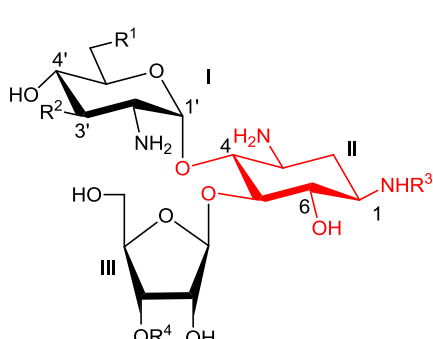
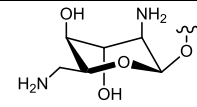
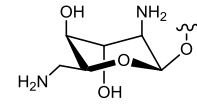
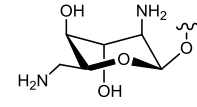
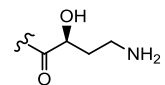


Figure 1: Structure of streptomycin.

4, 5-disubstituted 2-deoxystreptamines – Neomycin class

	R ¹	R ²	R ³	R ⁴	
	Neomycin B	NH ₂	OH	H	
	Paromomycin I	OH	OH	H	
	Lividomycin B	OH	H	H	
	Butirosin B	NH ₂	OH		H
	Ribostamycin	NH ₂	OH	H	H

4, 6-disubstituted 2-deoxystreptamines – Kanamycin class

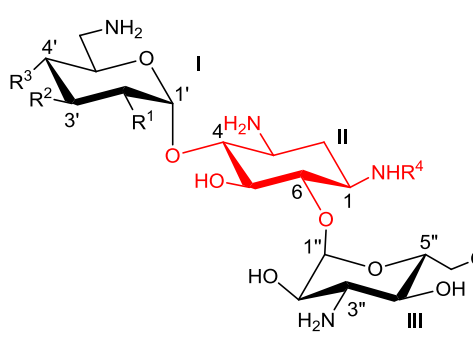
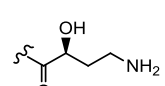
	R ¹	R ²	R ³	R ⁴	
	Kanamycin A	OH	OH	OH	H
	Kanamycin B	OH	NH ₂	OH	H
	Dibekacin	NH ₂	H	H	H
	Arbekacin	NH ₂	H	H	

Figure 2: Structures of 2-deoxystreptamine (2-DOS) aminoglycosides

Aminoglycosides are well known for their traditional role of antibacterial agents. Their mode of action has been extensively studied. They have been found to exert their bactericidal action by selectively binding to the A-site (aminoacyl site) decoding region of the 16S ribosomal RNA (rRNA) of bacteria.^{17,18} During the protein synthesis, binding of the correct tRNA to the mRNA causes conformational changes of two adenine residues (A1492 and A1493) in 16S rRNA, allowing them to contact with the mRNA-tRNA codon-anticodon hybrid.¹⁹ Since mispairing of codon and anticodon cannot induce this conformational change, this “proof-reading” process helps to ensure translational fidelity. However, the binding of the aminoglycosides at the decoding region impacts the conformational changes of A1492 and A1493.^{20,21} As a result, discrimination between cognate and near-cognate tRNA is reduced in the presence of aminoglycosides, enabling codon misreading.²² Misfolded proteins are then produced, some of which are incorporated in the bacterial membrane, leading to the loss of membrane integrity and increased permeability for the antibiotics. As a consequence, aminoglycosides accumulate rapidly in the cytoplasm and saturate all ribosomes, resulting in cell death (Figure 3).²³⁻²⁵

Despite these noticeable advantages, the nephrotoxicity and ototoxicity associated with aminoglycosides have considerably hampered their clinical usefulness.²⁶

Aminoglycosides have also suffered from the emergence of drug-resistant bacteria. Over fifty aminoglycoside-deactivating enzymes have been identified.^{24, 27-30} They act by modifying the structures of aminoglycoside antibiotics. This could be accomplished either through phosphorylation of a hydroxyl group (aminoglycoside

phosphoryltransferases, APH), adenylation of a hydroxyl group (aminoglycoside adenylyltransferases, AAD or ANT), or acetylation of an amino group (aminoglycoside acetyltransferases, AAC). Other mechanisms of resistance include the decrease of drug uptake into bacteria and the alteration of the ribosomal binding sites.

All these phenomena have rendered these once-before-acclaimed drugs obsolete, resulting in a growing interest in the development of new and modified aminoglycosides, with improved antibacterial activity.^{24,31} Despite all the efforts invested, a huge gap is still to be filled. This has thus forced other research groups to explore new applications of aminoglycosides.

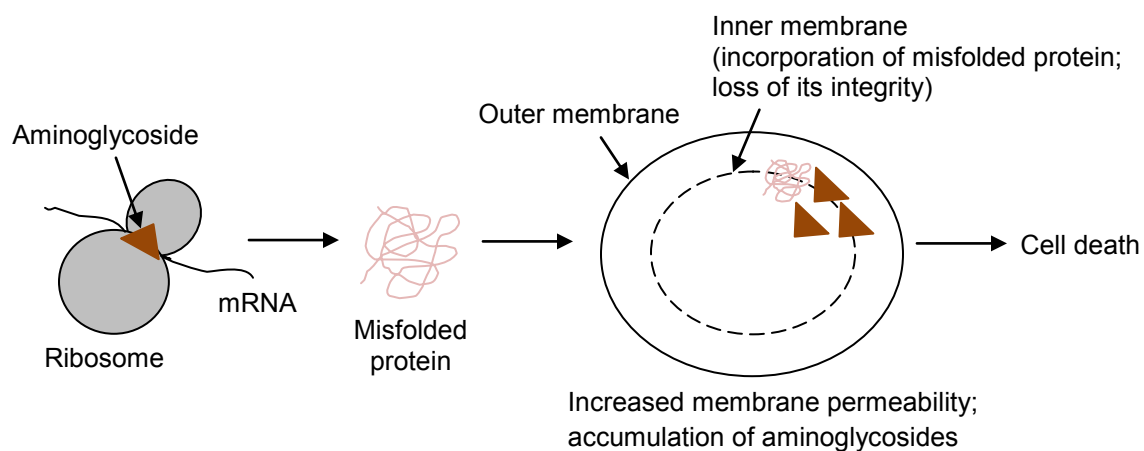


Figure 3: Bactericidal action of aminoglycosides
(Adapted from Kohanski, 2010 [Ref. 25])

I.1.2. Aminoglycosides in the treatment of genetic diseases

The selective binding of aminoglycoside antibiotics toward bacterial ribosome is crucial for their therapeutic use, and this is largely achieved through critical interactions

of the drug with nucleotides of the rRNA that are not similar in bacteria and human.³² For example, studies have demonstrated that A1408 and G1491 of the bacterial decoding site determine the selectivity of aminoglycosides.^{33,34} Eukaryotic cytoplasmic ribosomes are insensitive to aminoglycosides because a guanine residue is found at position 1408 and an adenine residue at position 1491 of 16S rRNA, which are all not able to interact with aminoglycosides.

However, it was found that certain aminoglycosides can bind to the small subunit of bacterial and eukaryotic ribosomes, especially at sites where nucleotides that are conserved between bacteria and eukaryotes are involved.³⁵ For example, apramycin and geneticin (Figure 4) are known to bind to the human decoding site.^{32,36} This disadvantage turned out to be useful in the treatment of genetic diseases caused by nonsense mutations.

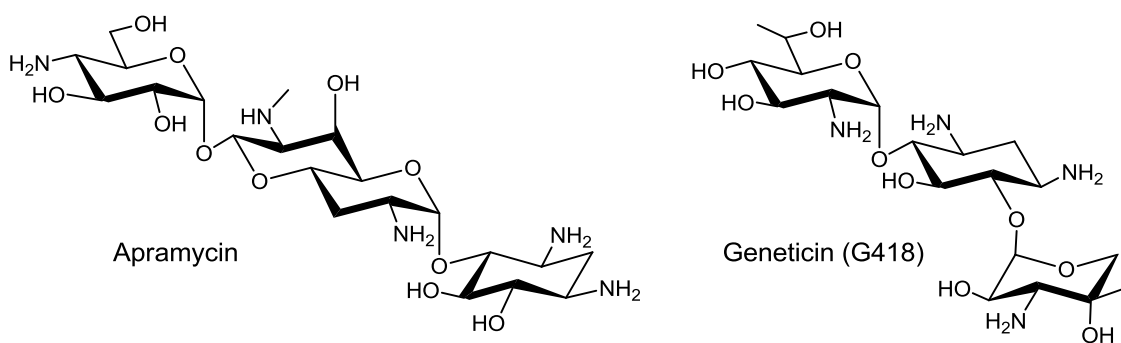
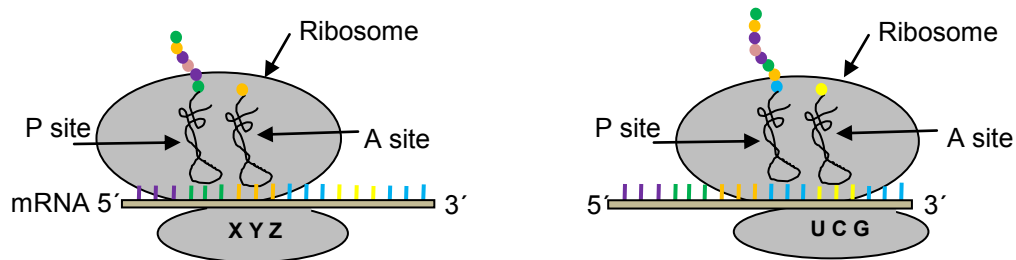


Figure 4: Structures of apramycin and geneticin (G418)

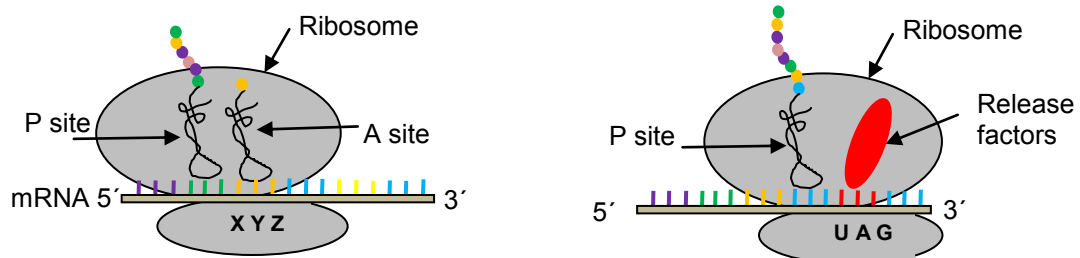
More than 1800 distinct heritable human diseases are caused by nonsense mutations,³⁷ during which a change in a single nucleotide in a DNA sequence converts a codon that specified an amino acid into a stop codon. As a result, protein translation prematurely terminates, leading to the production of non-functional shortened

proteins.^{38,39} In 1985, Burke and Mogg⁴⁰ showed that aminoglycosides can suppress the effect of a nonsense mutation; by binding to the decoding site, aminoglycosides reduce the translation fidelity and allow a random amino acid to be incorporated at a premature-termination codon in mammalian cells. As a result, the protein translation can proceed through the natural stop codon (Figure 5).⁴¹

A) Normal protein translation



B) Nonsense mutation (C→A) - Premature termination of protein translation



C) Aminoglycoside insertion of a near-cognate tRNA - Restored protein translation

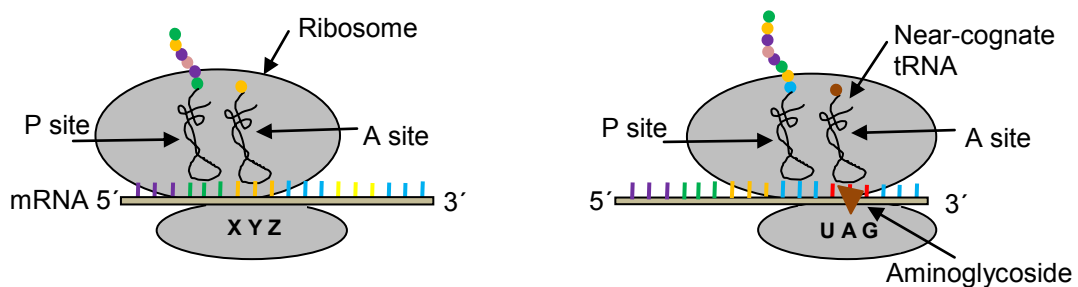


Figure 5: Suppression of nonsense mutation by aminoglycosides
(Adapted from Malik, 2010 [Ref. 41])

This concept has introduced novel research avenues in the field of aminoglycosides, allowing them to emerge as stop codon read-through inducers. This new ability was tested as a therapeutic approach for human genetic diseases, including spinal muscular atrophy.

I.1.3. Aminoglycosides as antifungal agents

Fungal infections are mainly responsible for the huge economic losses generated from crop and turf diseases. Current strategies to control these infections include the direct application of chemical fungicides.⁴² However, their associated toxic side effects toward animals and humans oblige the growers to reduce their dependency on these antibiotics and seek for better alternatives. Unfortunately, while enormous efforts have been devoted to the development of new antibacterial, antiviral, and anticancer therapeutics, only a few new fungicides have been introduced since the mid-1980s.⁴³

Fungi are eukaryotic organisms whose cells contain a nucleus enclosed within a distinct membrane. As in any eukaryote, anionic sphingolipids are found on the outer surface of fungal cell membranes. Therefore, cationic molecules such as aminoglycosides would be expected to interact with the fungal cell walls. As a matter of fact, it was recently reported that certain commercially aminoglycosides are inhibitory to plant pathogenic oomycetes.⁴⁴ However, the major drawback related to the use of aminoglycosides to combat crop diseases is their potential contribution to the propagation of bacterial resistant strains.^{45,46} Therefore, the best aminoglycoside agrofungicide candidates will be those that completely lose their antibacterial capabilities while gaining some antifungal activities.

I.2. 1,4-Naphthoquinone derivatives

I.2.1. History and biological functions

1,4-naphthoquinone belongs to the broad class of compounds called quinones. 1,4-naphthoquinone derivatives are of particular interest because of their large occurrence as natural products.⁴⁷⁻⁵¹ They are found in various parts of plants such as leaves, flowers, roots, bark, and wood. In addition, they exhibit a wide range of interesting biological activities.⁵²⁻⁵⁶ Molecules bearing naphthoquinone scaffold have also been employed as inhibitors against vitamin K dependent carboxylase,⁵⁷ protein kinase,⁵⁸ coenzyme Q,⁵⁹ and as growth stimulator for bifidobacteria.⁶⁰

A representative class of 1,4-naphthoquinone derivatives is the group of fat-soluble compounds called vitamin K. This includes the naturally occurring vitamin K₁, or phylloquinone, required for blood coagulation, and vitamin K₂, or menaquinone, which is of vital importance for bone health (Figure 6). A synthetic form of vitamin K is vitamin K₃ or menadione (Figure 6), which is often used as a quinone model for in vivo studies.

Menadione has been shown to undergo both redox cycling and arylation reactions. This is mainly due to the two carbonyl groups, which give the ability to 1,4-naphthoquinone derivatives to accept one and/or two electrons.⁶¹ 1,4-naphthoquinone derivatives can accept an electron to form the semiquinone radicals upon catalysis by flavoenzymes such as NADPH-cytochrome P-450 reductase (Figure 7).^{52,62,63} The semiquinone radicals can be further reduced to hydroquinones. In aerobic conditions, reactive oxygen species (ROS) are produced by transfer of electrons to oxygen. ROS is

commonly used to refer to superoxide, hydroxyl radical, and hydrogen peroxide, which are all known to break DNA strands.⁶⁴⁻⁶⁶

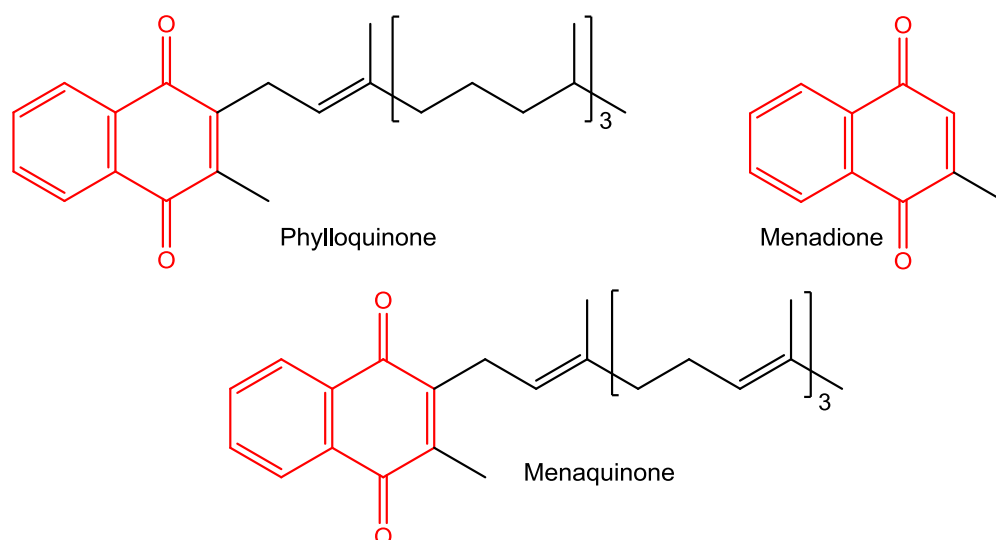


Figure 6: Structures of vitamin K₁ (phylloquinone), vitamin K₂ (menaquinone), and vitamin K₃ (menadione)

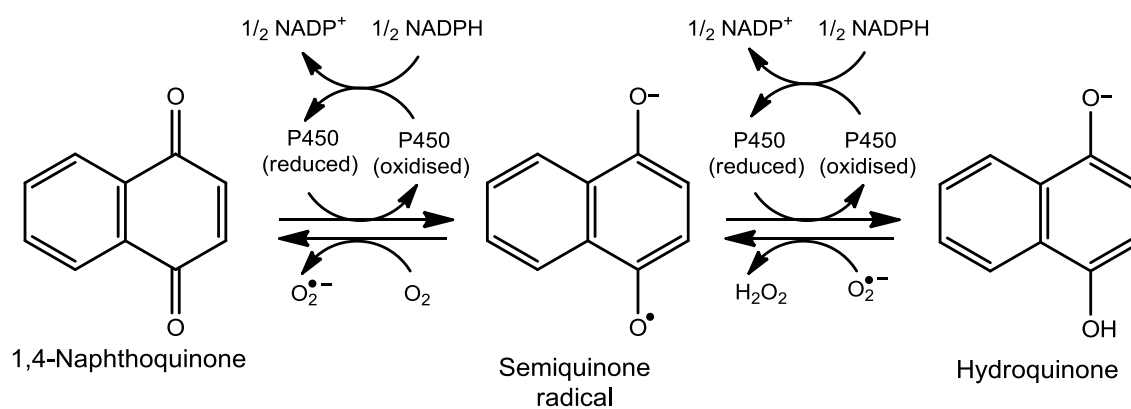


Figure 7: 1,4-Naphthoquinone derivatives as redox cyclers

In addition, 1,4-naphthoquinone derivatives can undergo arylation reactions. They contain electrophilic α,β -unsaturated carbonyl groups, which can react with nucleophilic moieties of proteins, such as thiolate groups, and form covalent bonds. This usually results in the inactivation and loss of protein function.⁶⁷

I.2.2. 1,4-Naphthoquinone derivatives as antibacterial agents

Various 1,4-naphthoquinone derivatives with antibacterial activity are known (Figure 8). This includes plumbagin, juglone and lawsone, which are naturally occurring naphthoquinones of plant origin.⁶⁸ Alkannin, shikonin, and their derivatives are other natural naphthoquinone products whose antimicrobial activity has been widely investigated.⁶⁹ Pleosporone was isolated from a pleosporalean ascomycete.⁷⁰

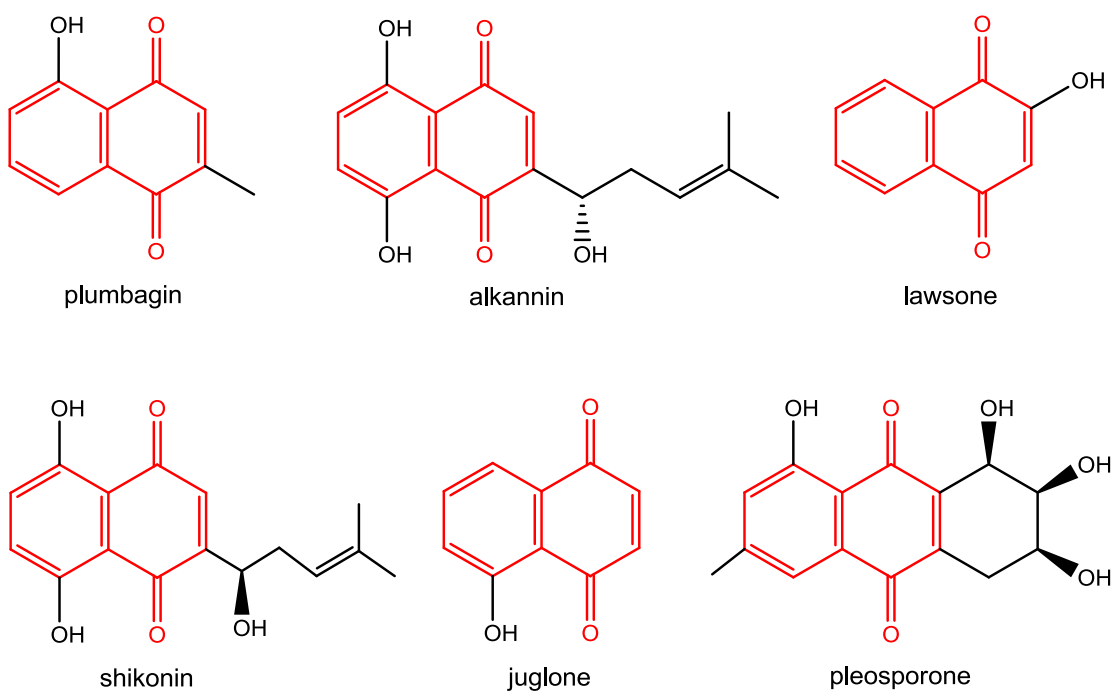


Figure 8: Structures of antibacterial 1,4-naphthoquinone derivatives

1,4-naphthoquinone derivatives can exert their antibacterial activity by decoupling of oxidative phosphorylation, a process essential for ATP synthesis. Because of their ability to accept electrons, they can compete with electron carriers such as coenzyme Q and uncouple the electron transport chain.⁷¹

In addition, 1,4-naphthoquinone derivatives are known to inhibit the growth of Gram-positive bacteria such as *Staphylococcus aureus*, *Enterococcus faecium*, and *Bacillus subtilis*. However, they are ineffective against Gram-negative bacteria such as *Escherichia coli* and *Salmonella typhimurium*.⁶⁹

I.3. Research summary

The aim of this research was to synthesize biologically active molecules. First of all, new therapeutic potentials of antibiotic aminoglycosides were investigated. A pyranmycin compound, an aminoglycoside, was re-synthesized and its mode of action in the treatment of spinal muscular atrophy was studied (chapter II). In addition, synthesis of various kanamycin B analogs revealed that a simple alkylation can convert this well-known antibacterial into an antifungal agent with potential use in agriculture (chapter III). Finally, a library of cationic 1,4-naphthoquinone derivatives was developed and their antibacterial activity studied (chapter IV).

CHAPTER II

AMINOGLYCOSIDES AS THERAPEUTICS

FOR SPINAL MUSCULAR ATROPHY^a

II.1. Rationale

Spinal muscular atrophy (SMA) is an autosomal recessive disease. SMA patients carry a pair of defective chromosomes 5 that both lack the *Survival Motor Neuron-1* (*SMN1*) gene on the long (q) arm, at position 13.2.⁷² *SMN1* produces full-length transcripts that translate into high levels of the survival motor neuron (SMN) protein, essential for the maintenance of motor neurons (specialized nerve cells that control muscle movement). *SMN2*, a nearly identical gene to *SMN1* also found at locus 5q13.2,⁴ exhibits a critical C to T nucleotide variation within the 5' end of exon 7.⁷³ This causes *SMN2*-derived transcripts to undergo alternative splicing at the junction of intron 6 and exon 7.^{74,75} As a result, 90% of the *SMN2*-derived transcripts lack the exon 7 and will therefore code a truncated and unstable SMN Δ 7 protein; only 10% will produce a fully functional SMN protein.^{4,76} Therefore, in the absence of *SMN1*, *SMN2* alone cannot produce enough SMN protein for the maintenance of motor neurons. However, an increase in the number of *SMN2* copies will result in more *SMN2*-derived SMN protein, and thus reduce the severity of SMA.

Dr. Christian Lorson and co-workers (Department of Veterinary Pathobiology, Bond Life Sciences Center, University of Missouri) have previously reported the ability

^a Part of this chapter was coauthored by Virginia B Mattis, Marina Y Fosso, Cheng-Wei Chang and Christian L Lorson. Reproduced with kind permission from *BMC Neurosc.* **2009**, *10*:142. Copyright © 2009, BioMed Central.

of two aminoglycosides, tobramycin and amikacin (Figure 9), to elevate the SMN protein levels in SMA cells.⁷⁷ By employing the capacity of aminoglycosides to read-through ribosomes past stop codons (see chapter I for more details), tobramycin and amikacin enable the incorporation of missing sequences at the C-terminus of SMN Δ 7 protein, thereby restoring the stability and the functionality of this novel SMN protein.

In light of these results, through collaboration with Dr. Lorson, our libraries of previously synthesized aminoglycosides were screened to identify **TC007** (Figure 9) as the lead in the treatment of spinal muscular atrophy.⁷⁸ Therefore, my goal was to prepare more **TC007** in order to study its mode of action in the treatment of spinal muscular atrophy.

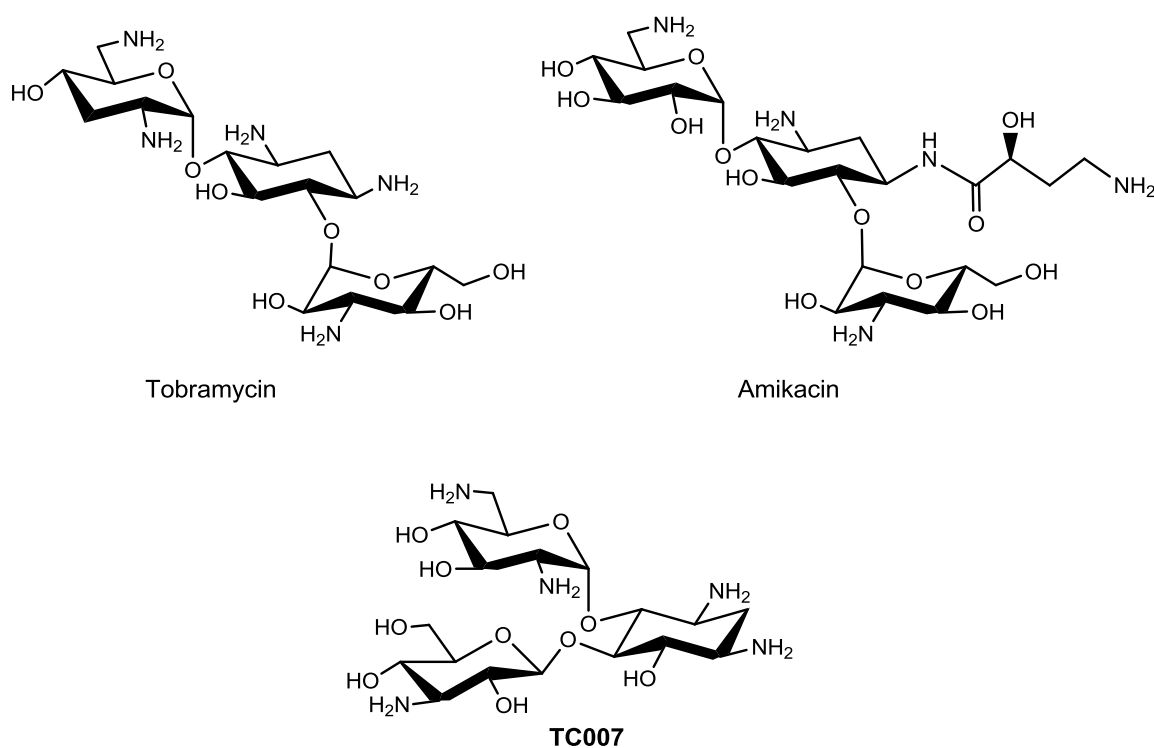


Figure 9: Structures of tobramycin, amikacin, and **TC007**

II. 2. Results and discussion

II.2.1. Synthesis of TC007

TC007 belongs to the class of pyranmycin compounds. These are neomycin analogs and they result from the replacement of the neobiosamine core (rings III and IV) of neomycin with a pyranose (Figure 10).⁷⁹ It has been previously demonstrated that the glycosidic bond of a furanose is more prone to acid cleavage than that of a pyranose.⁸⁰ Pyranmycin could therefore survive harsh acidic conditions that will otherwise degrade neomycin.⁸¹

Following the protocol previously described by Dr. Ravi Rai,⁷⁹ the synthesis of **TC007** will start from the commercially available neomycin B. Conversion of the amino groups into azido groups followed by benzylation afforded compound **1**⁸² (Scheme 1). Cleavage of the glycosidic bond between rings II and III was accomplished by refluxing **1** in the presence of copper (II) chloride, and this gave the known neamine derivative **2**.⁸² The free hydroxyl group at the 5-position is required for the final compound to be a 4,5-disubstituted 2-deoxystreptamine, thus an analog of neomycin.

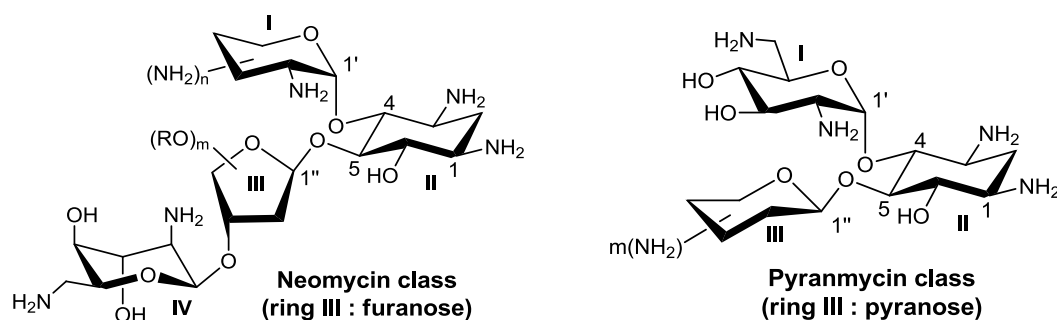
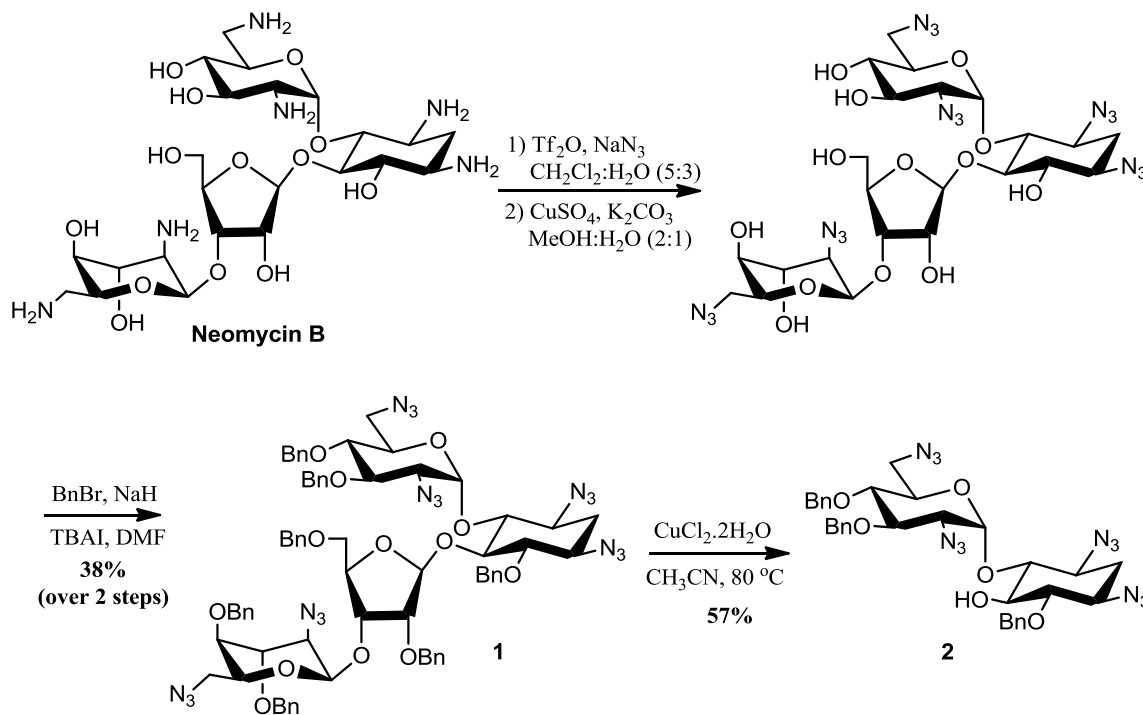


Figure 10: Structures of neomycin and pyranmycin

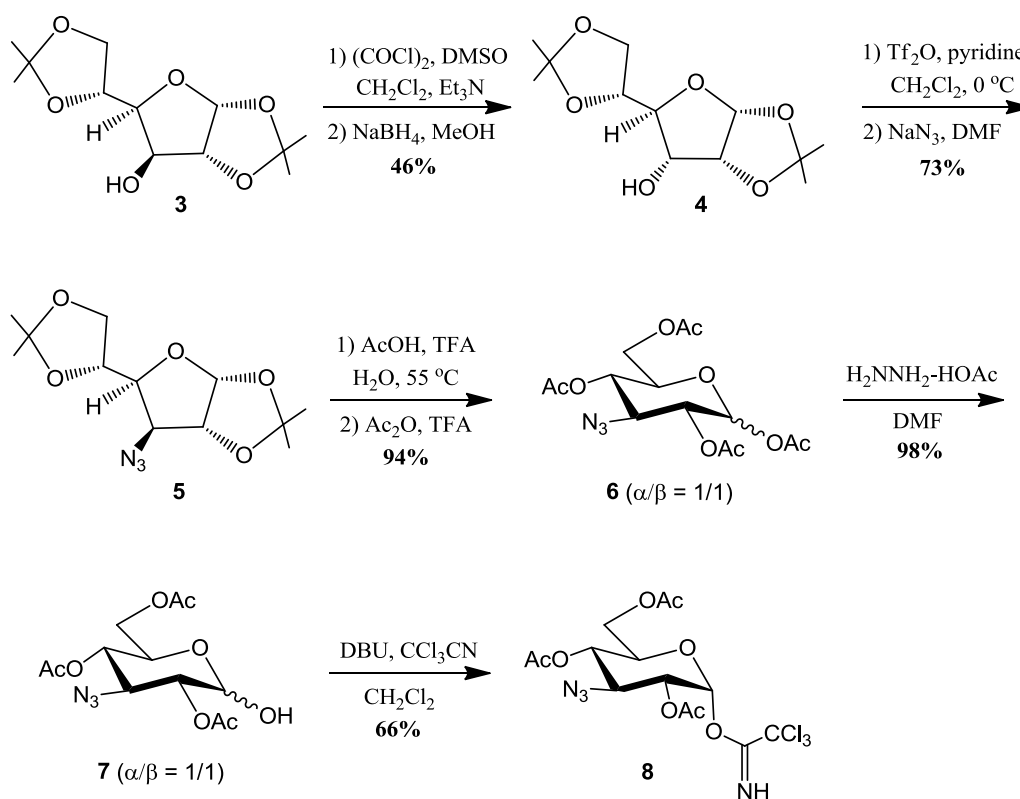


Scheme 1: Synthesis of the neamine derivative acceptor **2**

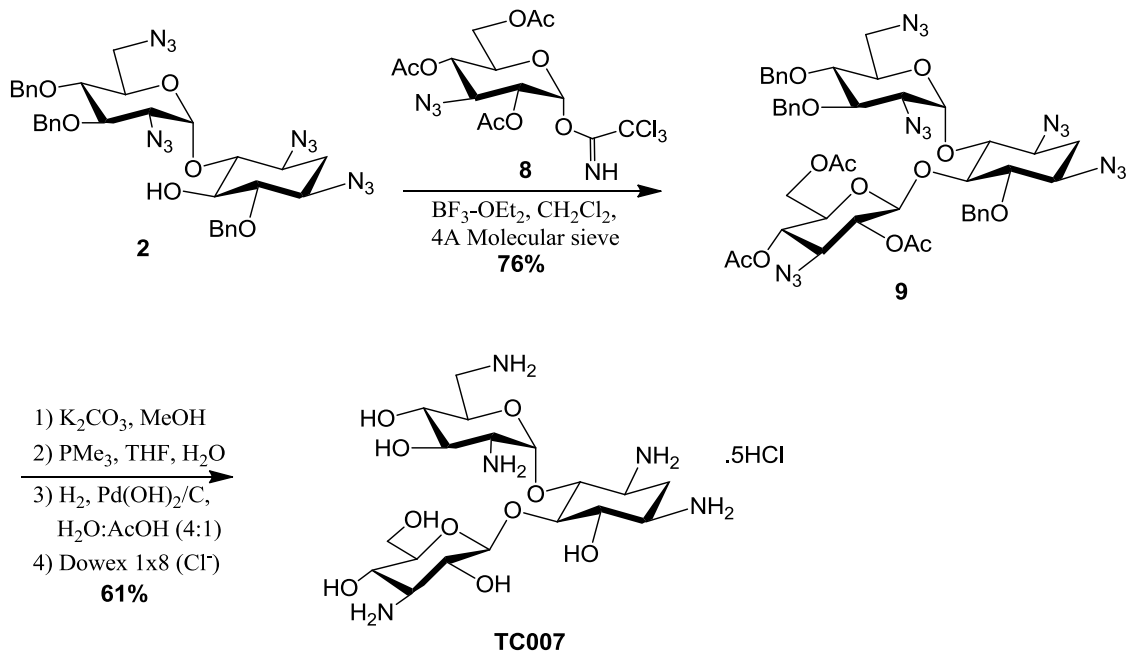
The synthesis of the glycosyl donor **8** started from the commercially available diacetone-D-glucose **3** (Scheme 2). Swern oxidation of **3** and reduction of the corresponding ketone with NaBH_4 gave the epimer alcohol **4**.⁸³ Triflation, which converts the free hydroxyl group into the better triflate leaving group, and $\text{S}_{\text{N}}2$ azido substitution afforded compound **5**.⁸⁴ Acid-catalyzed hydrolysis, followed by acetylation, provided the tetraacetyl pyranose **6**.⁸⁵ Treatment with hydrazine acetate selectively hydrolyzed the acetyl group at the anomeric position to give **7**,⁸⁶ whose free hydroxyl group was then activated in the presence of trichloroacetonitrile to afford our glycosyl donor **8**.⁸⁷

With the neamine acceptor **2** and the glycosyl donor **8** on hand, we were ready to embark on the synthesis of **TC007** (Scheme 3). Glycosylation of **2** and **8** in the presence of the Lewis acid $\text{BF}_3\text{-OEt}_2$ provided compound **9**.⁸⁷ The acetyl group present at C-2 of

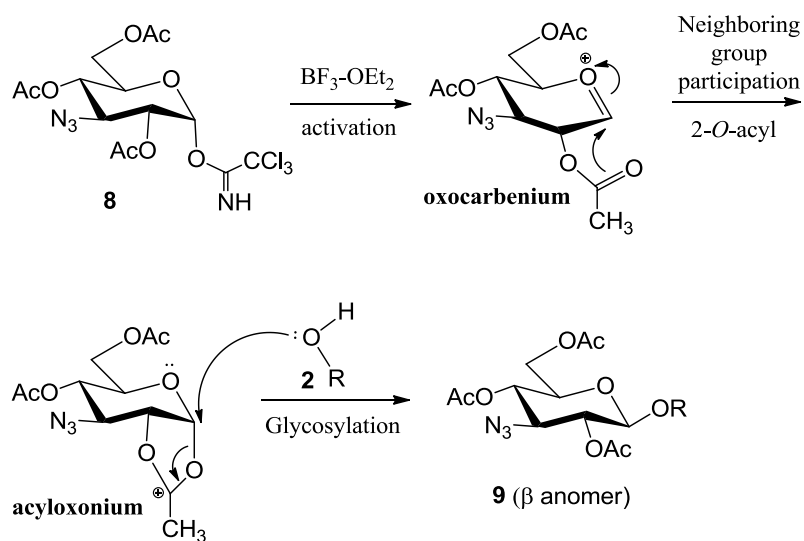
the glycosyl donor **8** controls the formation of the β -glycosidic bond in **9** (Scheme 4). Indeed, as the donor **8** gets activated in the presence of $\text{BF}_3\text{-OEt}_2$, an oxocarbenium intermediate is formed, which in the presence of a 2-*O*-acyl group will give an acyloxonium intermediate. Therefore, the attack by a nucleophile (acceptor **2**) can only happen from the open top face, resulting in the formation of the β -anomer in **9**. The acetyl groups in compound **9** will then be hydrolyzed using K_2CO_3 in methanol. Staudinger reduction of the azide, followed by hydrogenolysis and ion-exchange, provide **TC007** as a chloride salt.



Scheme 2: Synthesis of the glycosyl donor **8**



Scheme 3: Synthesis of TC007

Scheme 4: Formation of the β -glycosidic bond in compound 9

II.2.2. Mode of action of TC007 in the treatment of spinal muscular atrophy

SMN proteins localize in nuclear bodies known as gems.⁸⁸ Gem numbers have been frequently used as a biomarker for total cellular SMN protein levels in SMA patient fibroblasts.^{77,89-93} In a low throughput cell-based screen, **TC007** and other aminoglycosides were found to elevate SMN and gem numbers in SMA type I fibroblasts.⁷⁸ Patient fibroblasts treated for 48 h in 100 $\mu\text{g}/\text{mL}$ of aminoglycoside-media showed a higher amount of SMN nuclear gems (Figure 11).⁷⁸ **TC007** was found to be even more effective than valproic acid (VPA), a previously identified histone deacetylase inhibitor compound known to increase SMN expression by stimulating the SMN2 promoter and SMN exon 7 inclusion.

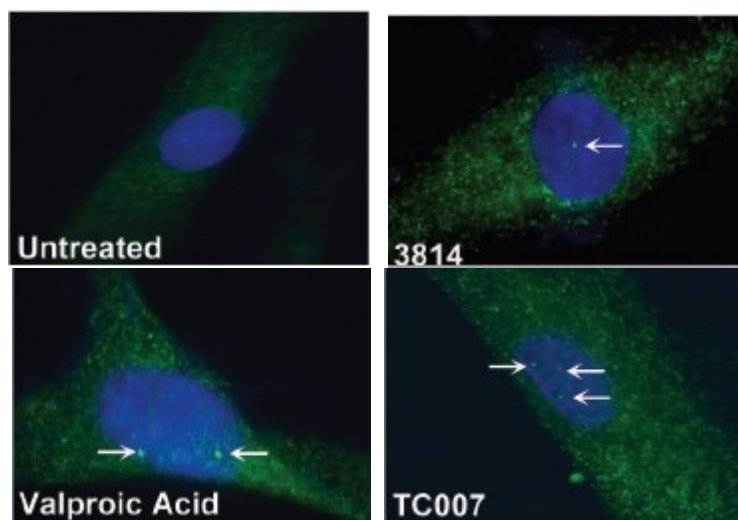


Figure 11: Increase in SMN-positive gems (white arrows) after treatment with **TC007**⁷⁸

TC007 was found to induce SMN protein levels by a novel SMN Δ 7 read-through event. Experiments carried out in Dr. Lorson's laboratory revealed that **TC007** causes the

ribosome to incorporate a tyrosine into the first stop codon of SMN Δ 7 exon 8 and read-through until the second stop, 16 nucleotides downstream (Figure 12).⁷⁸ This allows the translational machinery to elongate the truncated protein by additional five amino acids, length which has been demonstrated sufficient to restore more functionality to the protein. This SMN read-through protein, while in the low level, enters into an SMN complex with the existing full-length protein, resulting into a small increase in SMN-functional (SMN-FI) protein after treatment.

Animal model experiments performed in Dr. Lorson's laboratory have revealed that **TC007** can actually lessen the severity of SMA.⁹⁴ As a result, subcutaneous administration of **TC007** was found to increase myofiber size and gross motor function in SMA mice (Figure 13).⁹⁵

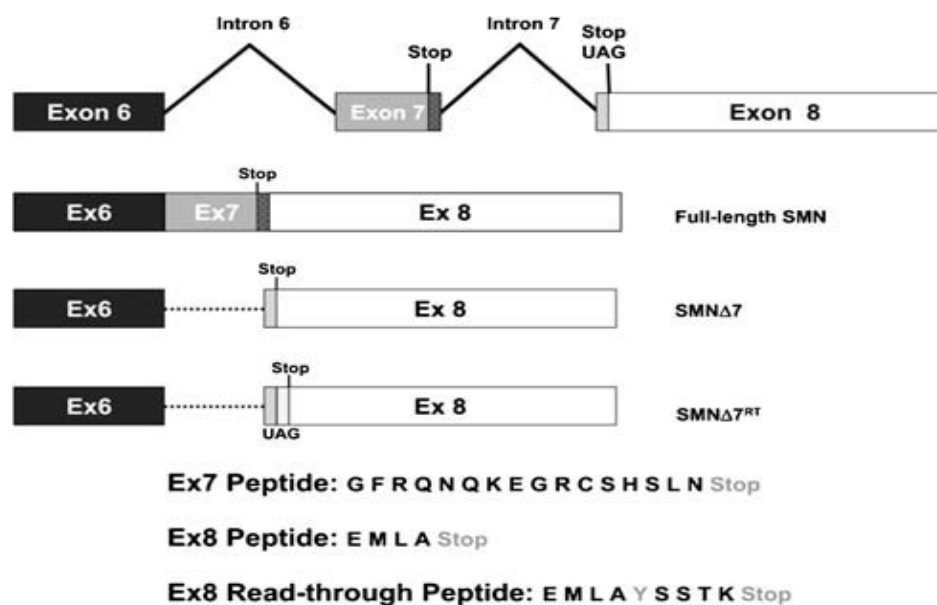


Figure 12: Schematic of SMN C-termini⁷⁸

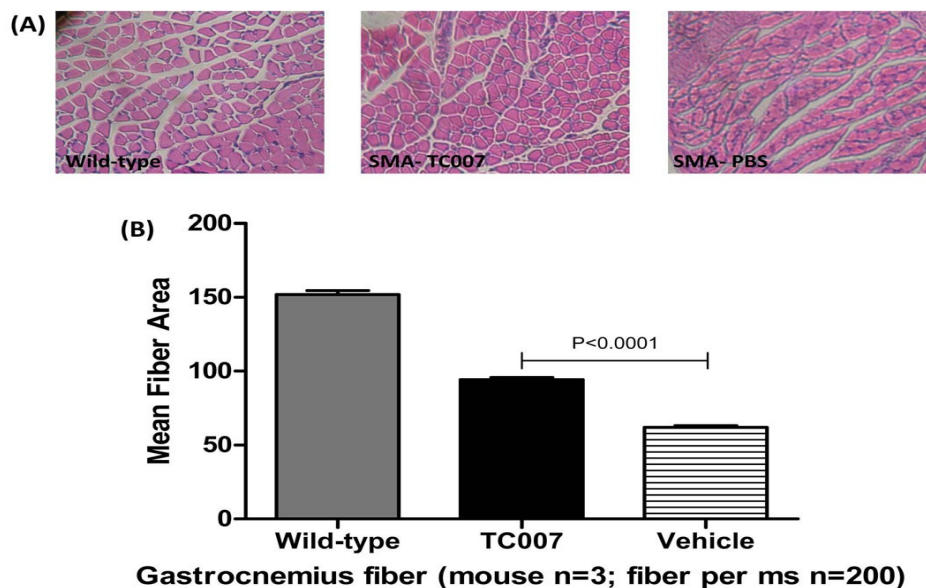


Figure 13: Increase in muscle fiber size of TC007-treated mice⁹⁵

II. 3. Conclusion

The bioactivity screening of a library of previously synthesized aminoglycosides has identified **TC007** as the lead compound in the treatment of spinal muscular atrophy. To further investigate its mode of action, more **TC007** was synthesized from neomycin B. The use of copper (II) chloride in the cleavage of the glycosidic bond between rings II and III of neomycin B was found to be slightly more effective than the previously used MeOH/HCl mixture.

TC007 was found to act through an unprecedented SMN Δ 7 read-through event. **TC007** triggers the insertion of an amino acid such as tyrosine, in the premature stop codon and allows the ribosome to read-through until the following natural stop codon. This elongates the truncated SMN Δ 7 protein by an additional five amino acids, enabling

it to regain functionality. These findings could lead to the development of novel therapeutic approaches for the treatment of spinal muscular atrophy.

CHAPTER III
STRUCTURAL OPTIMIZATION OF ANTIFUNGAL
KANAMYCIN B ANALOGS^b

III.1. Rationale

A recent report has demonstrated that aminoglycosides, including neomycin, paromomycin, ribostamycin, and streptomycin (Figures 1 and 2), can manifest modest to excellent antifungal activity against a panel of pathogenic fungi.⁴⁴ However, all these aminoglycosides are clinically used antibiotics for the treatment of human bacterial pathogens. Therefore, if any of them happened to be involved in plant disease management, microbial resistance could easily be extended to human, leading to an increase of human illnesses and possibly death. This phenomenon has previously been observed with farm animals, when the overuse of in-feed antibiotics in livestock resulted in the detection of a large number of drug-resistant bacteria in human.⁹⁶

In addition, among the two most commonly used classes of aminoglycosides, neomycin and kanamycin, this study limited itself to the neomycin class of aminoglycosides (neomycin, paromomycin, and ribostamycin). No representative member of the kanamycin class was included.

To solve both issues, we decided to investigate the antifungal activity of some kanamycin class aminoglycosides. In addition, these aminoglycosides needed to be inactive against bacteria in order to be the ideal antifungal agent candidates.

^b Part of this chapter was coauthored by Chang, C.-W. T.; Fosso, M.; Kawasaki, Y.; Shrestha, S.; Bensaci, M.; Wang, J.; Evans, C. K.; Takemoto, J. Y. Reproduced with kind permission from *J. Antibiot.* **2010**, 63, 667-672. Copyright © 2010, Nature Publishing Group.

Kanamycin B (Figure 2) is a naturally occurring antibacterial aminoglycoside. As the prototypal model of the kanamycin class of aminoglycosides, its antibiotic efficacy has been excessively used. The net result was the high prevalence of resistant bacteria and thus the loss of its clinical attractiveness. In the effort to restore its antibacterial activity, our group has been involved in the development of new strategies to derivatize this class of compounds and had previously synthesized a library of kanamycin B analogs.⁹⁷⁻⁹⁹ Screening of this library of analogs uncovered few compounds that inhibited the growth of fungi and yeasts. One compound, **FG08**, displayed broad spectrum fungicidal activity coupled with the loss of antibacterial activity. Therefore, more **FG08** had to be prepared to investigate its mechanism of action. In addition, other kanamycin B analogs would be synthesized in the aim of developing potential antifungal agents.

III. 2. Results and discussion

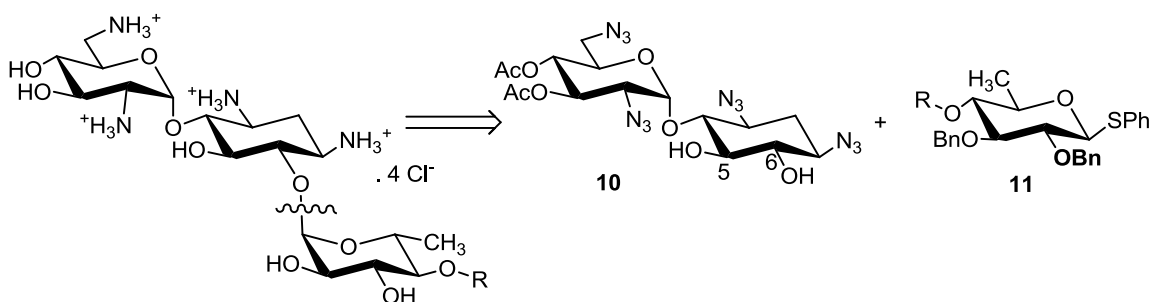
III.2.1. Investigation of the optimum chain length

The surprising antifungal activity of **FG08** was attributed to the attachment of a long (C8) alkyl chain. To investigate the chain length that will confer the optimum antifungal activity, two other kanamycin B analogs were synthesized: **FG01**, with a shorter (C4) alkyl chain, and **FG02**, with a longer (C12) alkyl chain.

a) Retrosynthetic analysis of FG01, FG02 and FG08

FG01, **FG02**, and **FG08** are all kanamycin B analogs and thus share a pseudo-disaccharide core (neamine) substituted at the 6-position. Their syntheses could therefore start from the neamine derivative **10**⁹⁷ and the phenylthioglycosyl donor **11** (Scheme 5).

Unlike the β -glycosidic bond, there is no general and stereospecific protocol for the formation of the α -glycosidic bond. However, a 2-*O*-Bn group does not favor the formation of the acyloxonium intermediate required for β -selectivity. Therefore, the phenylthioglycoside **11** will enable the formation of the α -glycosidic bond between rings II and III due to the anomeric effect. Indeed, upon activation by NIS and TMSOTf, **11** is converted into an oxocarbenium intermediate (Scheme 6). Nucleophilic attack by the acceptor **10** can occur from the top face to give the β -anomer, or from the bottom face to give the α -anomer. Because of the orbital overlap, the β -anomer is less favored than the α -anomer. Therefore, the α -anomer will be obtained as the major product. In addition, since **10** has two free hydroxyl groups, steric hindrance will prevent 5-OH from acting as the nucleophile, allowing glycosylation to happen only at the 6-position.



FG01 (R = C₄H₉), **FG02** (R = C₁₂H₂₅)

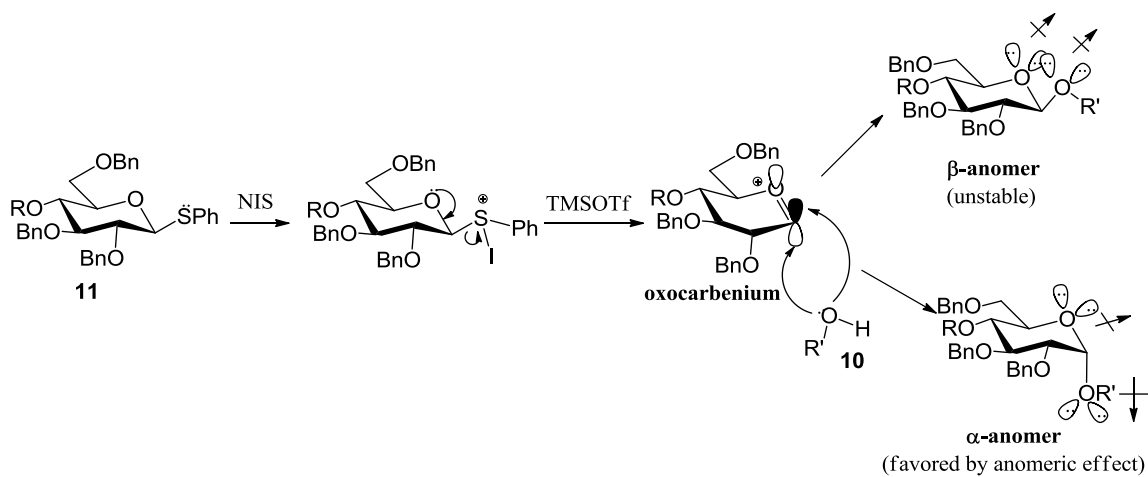
FG08 (R = C₈H₁₇)

Scheme 5: Retrosynthetic analysis of **FG01**, **FG02**, and **FG08**

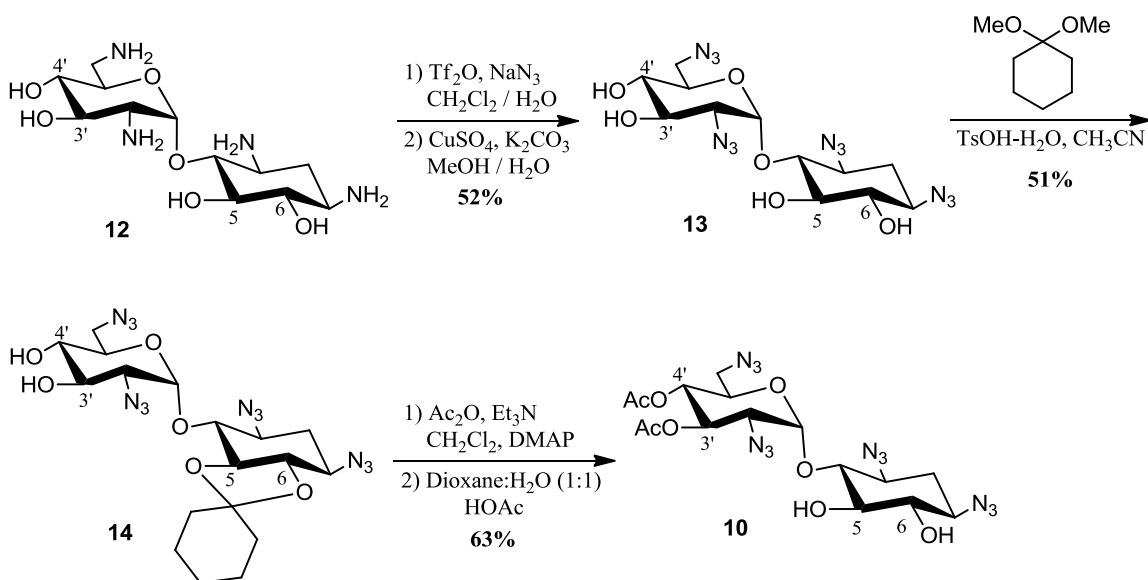
b) Syntheses of **FG01**, **FG02** and **FG08**

Starting from neamine **12**,¹⁰⁰ conversion of the amino groups to azido groups gave **13**¹⁰¹ (Scheme 7). Regioselective protection of the 1,2-diol at positions 5 and 6 gave **14**.⁹⁷

Acetylation of the hydroxyl groups at positions 3' and 4', and acid cleavage of the cyclohexylidene protecting group afforded the neamine derivative **10**.

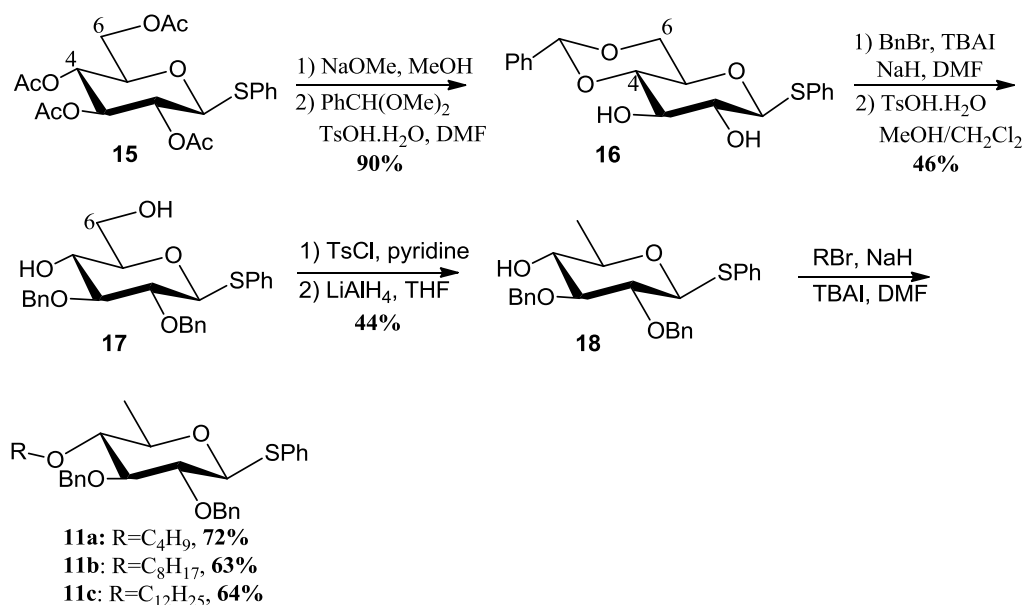


Scheme 6: Anomeric effect



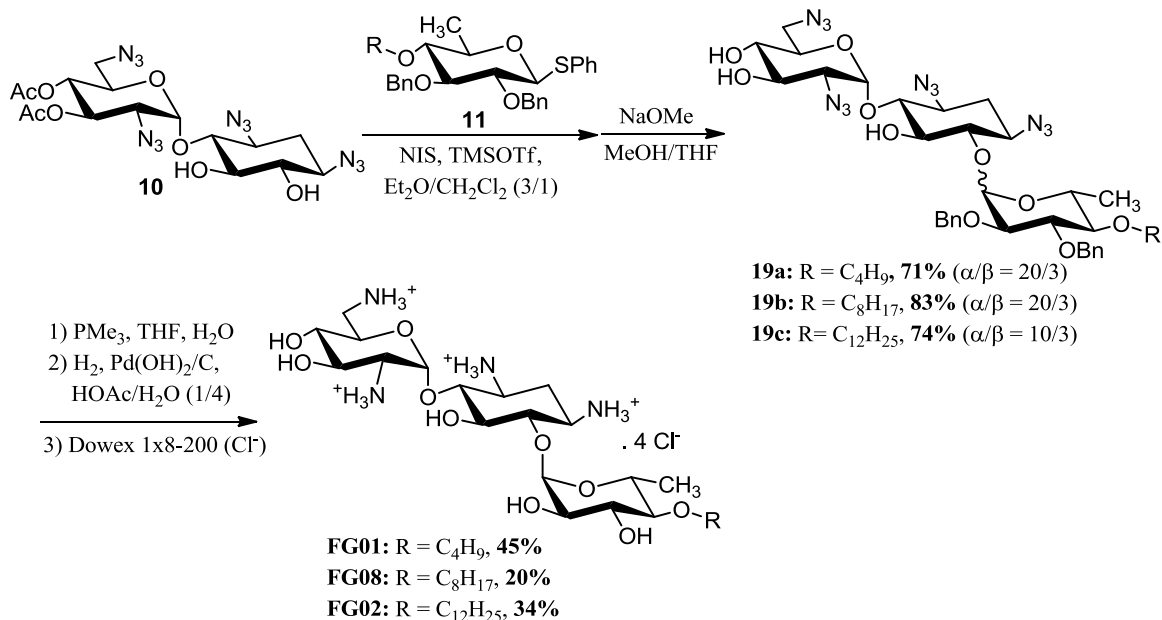
Scheme 7: Synthesis of the neamine derivative acceptor **10**

The synthesis of the glycosyl donors started from the known compound **15**¹⁰² (Scheme 8). Acetonolysis, followed by regioselective protection of the hydroxyl groups at positions 4 and 6 afforded **16**.¹⁰² Benzylolation and acid cleavage of the benzylidene protecting group gave **17**,¹⁰² as a 1,3-diol. Selective tosylation of the primary alcohol and reduction with LiAlH₄ gave **18**.¹⁰³ Alkylation using *n*-butyl bromide, *n*-octyl bromide, and *n*-dodecyl bromide gave the glycosyl donors **11a**, **11b**, and **11c**, respectively.



Scheme 8: Synthesis of the glycosyl donors **11a**, **11b**, and **11c**

With the neamine acceptor **10** and the glycosyl donors **11a**, **11b**, and **11c** on hand, we were ready to embark on the synthesis of **FG01**, **FG02**, and **FG08** (Scheme 9). Glycosylation in the presence of NIS and TMSOTf followed by acetonolysis using NaOMe in MeOH/THF mixture afforded **19**. Staudinger reduction of the azide into an amine, hydrogenation, and ion-exchange provided **FG01**, **FG02**, and **FG08** as chloride salts.



Scheme 9: Synthesis of **FG01**, **FG02**, and **FG08**

c) The C8 alkyl chain confers optimum antifungal activity

Through collaboration with Dr. Jon Takemoto (Department of Biology, Utah State University), the effectiveness of each of the **FG** compounds was evaluated. The synthesized kanamycin B analogs were tested against *Rhodotorula piliminae* (Figure 14).¹⁰⁴ From the disk diffusion growth inhibitory assay, **FG08** gave a larger zone of inhibition and thus is more active than **FG01** and **FG02** against *R. piliminae*.

Further alkyl chain lengths were not investigated because from a similar study of alkyl chain length vs. aminoglycoside bioactivity,¹⁰⁵ it was reported that compounds with C7 and C10 alkyl chains showed reduced activities compared to the parent compound. Therefore, we reasoned that the C8 alkyl chain will still be the best at conferring an optimal antifungal activity.

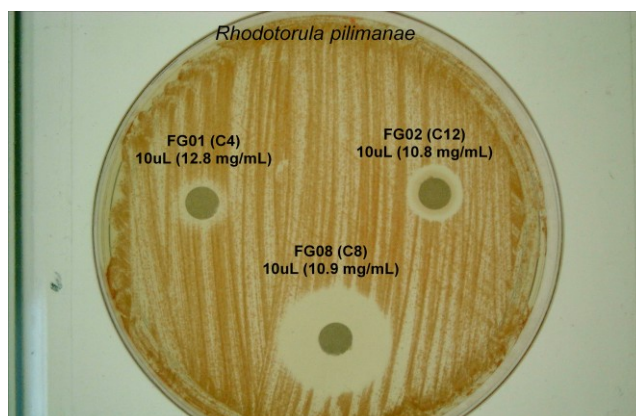


Figure 14: Disk diffusion inhibitory assay of **FG01**, **FG02**, and **FG08**

Based on these results, more emphasis was directed toward the antimicrobial activity of **FG08**. Microbroth dilution assays performed in Dr. Takemoto's laboratory revealed that **FG08** exhibits little to no activity against Gram-positive and Gram-negative bacteria (Table 1). Its MIC values against both types of bacteria were at least 125-fold higher than shown by kanamycin B (Table 1).

In addition, **FG08** was found to inhibit the growth of a wide range of yeasts, oomycetes, and true fungi with MICs ranging between 3.9 and 31.3 $\mu\text{g/mL}$ (Table 1).¹⁰⁴ On the other hand, kanamycin B was not active against those same microbes.

In light of these results, the ability of **FG08** to control Fusarium head blight (FHB) was evaluated.

Table 1: Minimal inhibitory concentrations (MICs) of **FG08** and kanamycin B¹⁰⁴

Organism	MIC ($\mu\text{g/mL}$)	
	FG08	Kanamycin B ^a
Bacteria		
<i>Escherichia coli</i> TG1 ^b	125-250	1.95
<i>Staphylococcus aureus</i> (ATCC 25923) ^c	250	<0.98
<i>Pseudomonas aeruginosa</i> (ATCC 27853) ^b	250	1.95
<i>Enterococcus faecalis</i> (ATCC 29212) ^c	125-250	<0.98
<i>Klebsiella pneumoniae</i> (ATCC 138883) ^b	250	1.95
<i>Klebsiella pneumoniae</i> (ATCC 700603) ^b	250	1.95
Fungi		
<i>Rhodotorula pilimanae</i> (ATCC 26423)	7.8	>250
<i>Candida albicans</i> (ATCC 10231)	31.3	>250
<i>Saccharomyces cerevisias</i> W303	3.9	>250
<i>Fusarium graminearum</i> B-4-5A	31.3	>250
<i>Fusarium oxysporum</i>	7.8	>250
<i>Ulocladium spp.</i>	7.8	ND ^d
<i>Pythium irregular</i>	15.6	ND
<i>Pythium ultimum</i>	15.6	ND
<i>Phytophthora parasitica</i>	15.6	ND
<i>Rhizopus stolonifer</i>	31.3	ND
<i>Cladosporium cladosporioides</i>	31.3	ND
<i>Curvularia brachyspora</i>	31.3	ND
<i>Bortrytis cinerea</i>	31.3	ND
<i>Phoma spp.</i>	31.3	ND

^aMicroboth dilution assays were performed at least twice, and each in triplate

^bGram-negative bacteria

^cGram-positive bacteria

^dNot determined

III.2.2. Antifungal activity of FG08 against *Fusarium graminearum*

F. graminearum is the causative agent of Fusarium head blight (FHB) and affects wheat, barley, and maize. With economic losses averaging \$3 billion annually, FHB is among the most serious plant disease the U.S. has encountered.¹⁰⁶ Efforts to eradicate this crop disease have not been successful yet. Therefore, the development of a fungicide that will inhibit *F. graminearum* is much awaited.

a) Green house experiments

FG08 was investigated for its ability to control FHB of wheat.¹⁰⁴ Leaf infection assays performed by Yukie Kawasaki, a graduate student in Dr. Takemoto's laboratory, revealed the ability of **FG08** to suppress in *planta F. graminearum* infection at its in vitro MIC value. When FHB-susceptible wheat leaves were inoculated with 10 μ L of a mixture of **FG08** (30 μ g/mL) and suspensions of *F. graminearum* macroconidia, not only was mycelial growth prevented (Figure 15, upper panel), but a 5-fold decrease in leaf lesions was also observed (Figure 15, middle panel, white bars). At 180 μ g/mL, **FG08** was found to be phytotoxic (Figure 15, lower panel).

In addition, **FG08** reduced the rate of FHB infection on cultivar Apogee (a rapidly maturing and FHB-susceptible variety of wheat) spikelet florets. Inoculation of **FG08**-pretreated spikelet florets with *F. graminearum* did not result in any of the FHB symptoms (chlorosis and curled spikes) that were noticeable within 4 days on non-pretreated spikelet florets (Figure 16). Therefore, the attachment of the octyl group triggered the loss of the antibacterial activity of **FG08**, while instantly imparting to it a fungicidal activity. This definitely suggested a different mode of action of **FG08**.

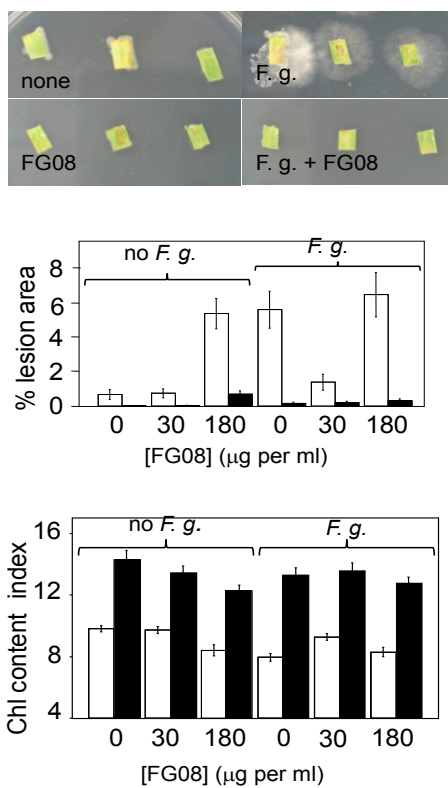


Figure 15: FG08 suppression of wheat leaf infection after exposure to *F. graminearum*¹⁰⁴

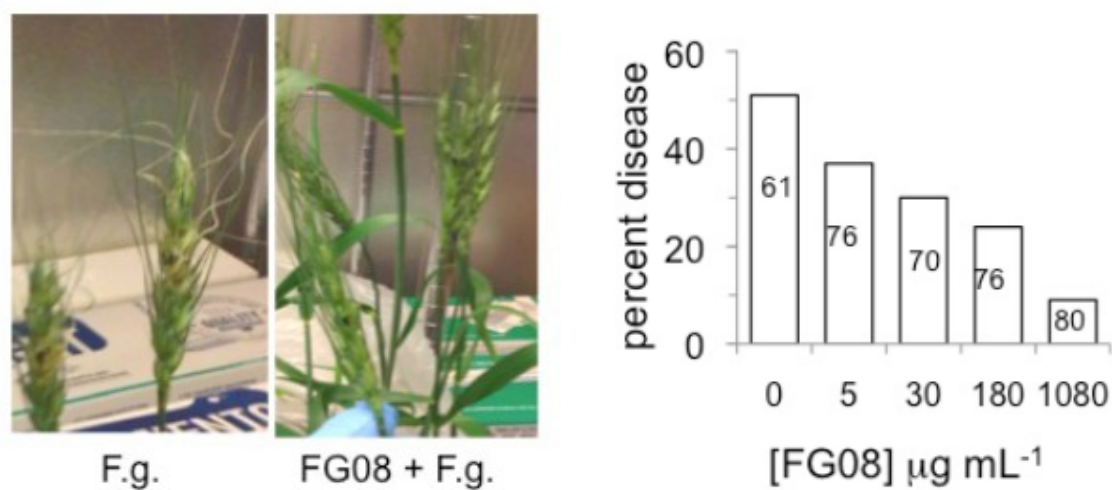


Figure 16: FG08 suppression of FHB disease in wheat spikelet florets¹⁰⁴

b) Mechanism of action of FG08

Aminoglycosides are known to kill bacteria by binding to the ribosome and inhibiting protein synthesis. However, studies with fluorescent dye SYTOX green demonstrated that **FG08** exerts its antifungal activity by perturbation of the membrane function.¹⁰⁴ Upon binding with nucleic acids, SYTOX green will fluoresce when excited at 450-490 nm. Unless the cell membrane is compromised, the dye does not have the ability to cross the membrane. When Mr. Sanjib Shrestha, a graduate student in Dr. Takemoto's laboratory, performed the dye permeation experiment, it was found that **FG08** rapidly influenced the dye permeability of *C. albicans* cells and *F. graminearum* hyphae (10 minutes). In addition, **FG08** increased the membrane permeability of *C. albicans* 12 times better than kanamycin B. Also, **FG08** did not lyse more than 20% of erythrocytes at a concentration 10-fold higher than its fungal MIC. This suggests that **FG08** does not act as a surface-active agent that non-specifically disrupts membranes.

Aminoglycosides are polycationic at physiological conditions. They can then aggregate on the fungal cell membrane by electrostatic interaction with the anionic sphingolipids. Then, the lipophilic alkyl chain found on **FG08** enabled it to insert itself into the membrane bilayer of the fungi and eventually form pores. Therefore, the C8 alkyl chain found in **FG08** confers to it amphipatic properties.

III.2.3. Optimization of FG08

In light of the impressive antifungal activity of **FG08**, we decided to improve on its synthesis by preparing **FG03**. **FG03** differs from **FG08** by the hydroxyl group present at the 6'' position (Figure 17).

a) Synthesis of FG03

Starting from the 1,3-diol **17**,¹⁰² tritylation selectively protects the primary alcohol, leaving a free hydroxyl group at position 4 (Scheme 10). Alkylation of the 4-OH, followed by the acid-catalysed removal of the trityl group revealed the 6-OH in compound **20**. Benzylation afforded the thiophenyl donor **21**. Glycosylation of **2** and **21**, followed by acetonolysis, gave **22**. Staudinger reduction of the azide into amine, hydrogenation, and ion-exchange provided **FG03** as a chloride salt.

b) Antimicrobial activities of FG03

While maintaining its non-antibacterial activity, **FG03** was also found to be effective against a number of fungi (Table 2). In addition, it was even more active than **FG08** against *F. graminearum*.

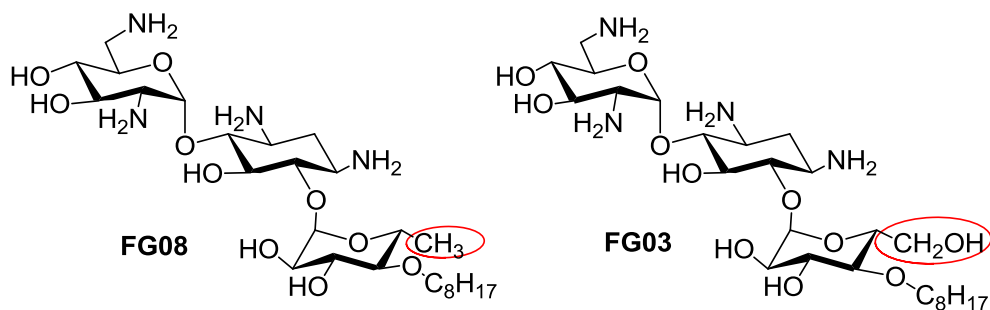
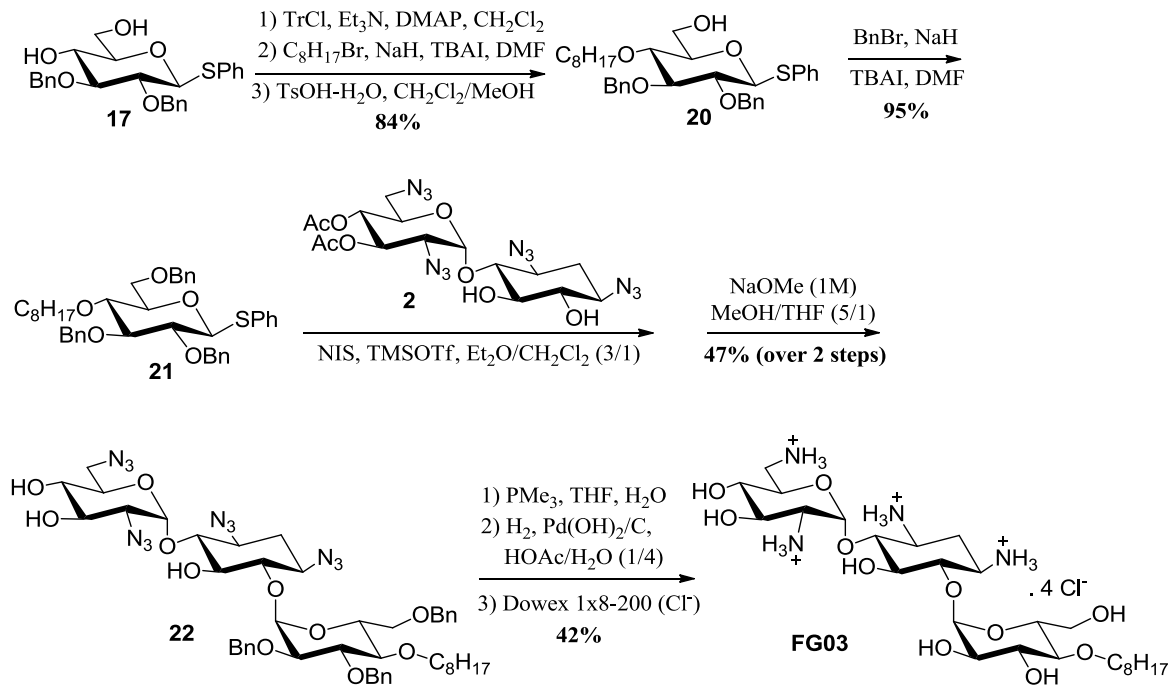


Figure 17: Structures of **FG08** and **FG03**



Scheme 10: Synthesis of FG03

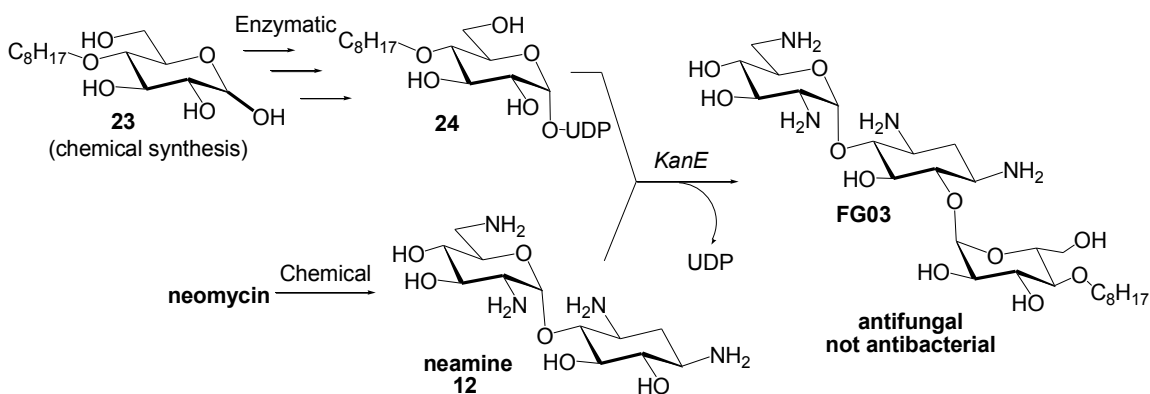
Table 2: MIC values of FG08 and FG03^a

Organism	MIC (μg/mL)	
	FG08	FG03
Bacteria		
<i>Escherichia coli</i> TG1 ^b	125-250	>500
<i>Staphylococcus aureus</i> (ATCC25923) ^c	250	ND ^d
Filamentous fungi		
<i>Fusarium graminearum</i> B-4-5A	31.3	7.8
<i>Pythium ultimum</i>	15.6	62.5
<i>Curvularia brachyspora</i>	31.3	31.3
<i>Bortrytis cinerea</i>	31.3	31.3
Yeasts		
<i>Rhodotorula pilimanae</i> (ATCC26423)	7.8	62.5
<i>Candida albicans</i> (ATCC10231)	31.3	62.5

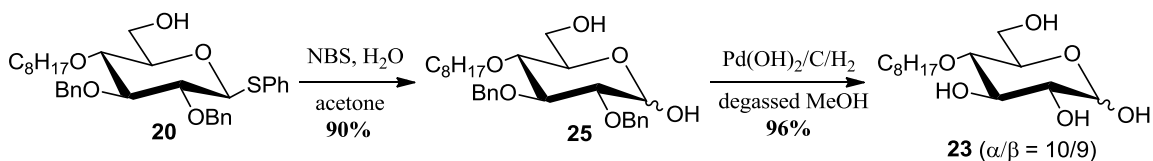
^a Data obtained by Sanjib Shrestha^b Gram-negative bacteria^c Gram-positive bacteria^d Not determined

The scale-up synthesis of **FG03** by a chemoenzymatic approach was attempted in Dr. Takemoto's laboratory (Scheme 11). Although unsuccessful, this approach required 4-*O*-octyl-D-glucopyranoside **23** and neamine **12**, both chemically synthesized in our laboratory.

The synthesis of **23** started from compound **20**. Treatment with *N*-bromosuccinimide gave **35**, whose hydrogenation provided **23** (Scheme 12).



Scheme 11: Proposed chemo-enzymatic synthesis of **FG03**



Scheme 12: Synthesis of compound **23**

III.2.4. Alkyl group mapping

From the promising results of **FG03** and **FG08**, which both have a linear C8 alkyl chain at the *O*-4'' position, we decided to explore the effect of an octyl group at other positions, by synthesizing kanamycin B analogs **FG05**, **FG06**, **FG07**, **FG09**, **FG10**, and **FG11**.

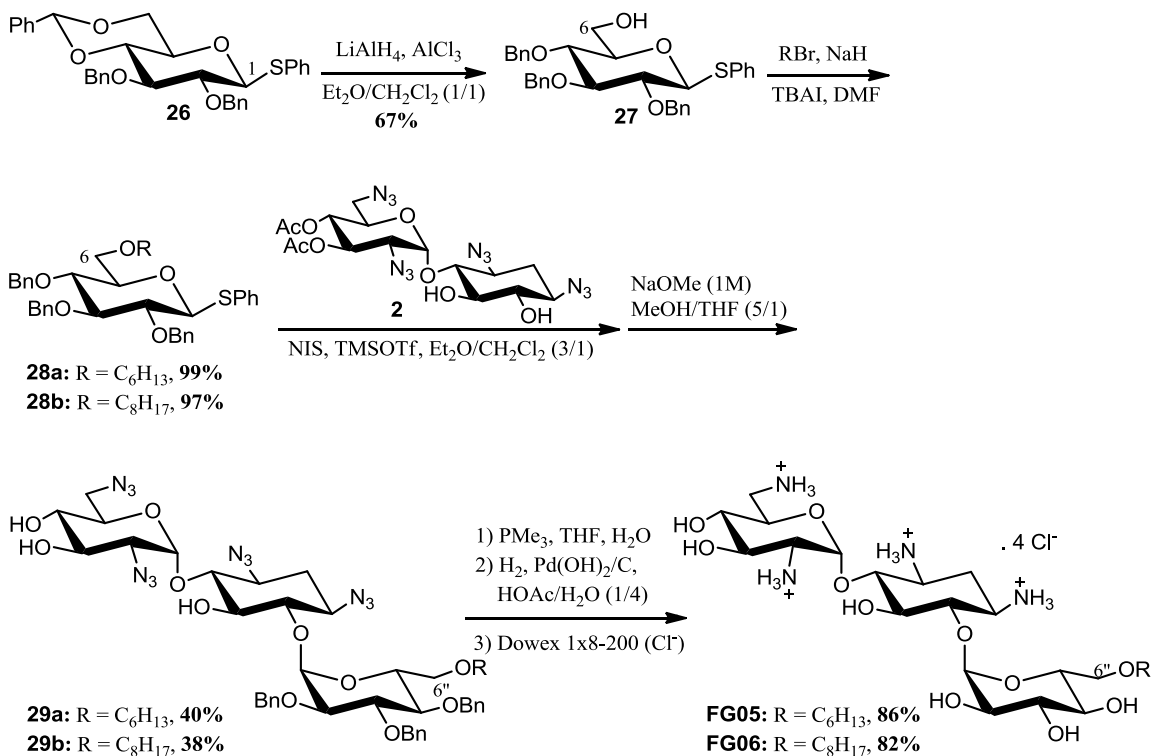
a) Synthesis of **FG05** and **FG06** (alkylation at *O*-6'' position)

The synthesis started with the regioselective ring opening of the known compound **26**¹⁰⁷ to obtain **27**¹⁰⁸ with a free hydroxyl group at the 6-position (Scheme 13). Alkylation using *n*-hexyl bromide and *n*-octyl bromide provided the thiophenyl donors **28a** and **28b**, respectively. Glycosylation followed by acetonolysis gave **29a** and **29b**. Staudinger reaction, hydrogenation, and ion-exchange afforded **FG05** and **FG06**, with C6 and C8 alkyl chain at the *O*-6'' position, respectively.

b) Synthesis of **FG07** (Alkylation at *O*-3'' position)

The synthesis started with the alkylation of diacetone-D-glucose **3** (Scheme 14). This gave the known compound **30**,¹⁰⁹ whose acid-catalysed hydrolysis and acetylation provided **31**. Treatment of **31** with thiophenol in the presence of BF₃-OEt₂ gave **32**. Through neighboring group participation (see Scheme 4), the acetyl group present at position 2 in **32** will favor the formation of a β-anomer after glycosylation. However, a 2-*O*-Bn will provide the required α-glycosidic bond (see Scheme 6). Thus acetonolysis of **32**, followed by benzylation, afforded the thiophenyl donor **33**, with a 2-*O*-Bn. Glycosylation of **2** and **33** in the presence of NIS and TMSOTf, followed by acetonolysis,

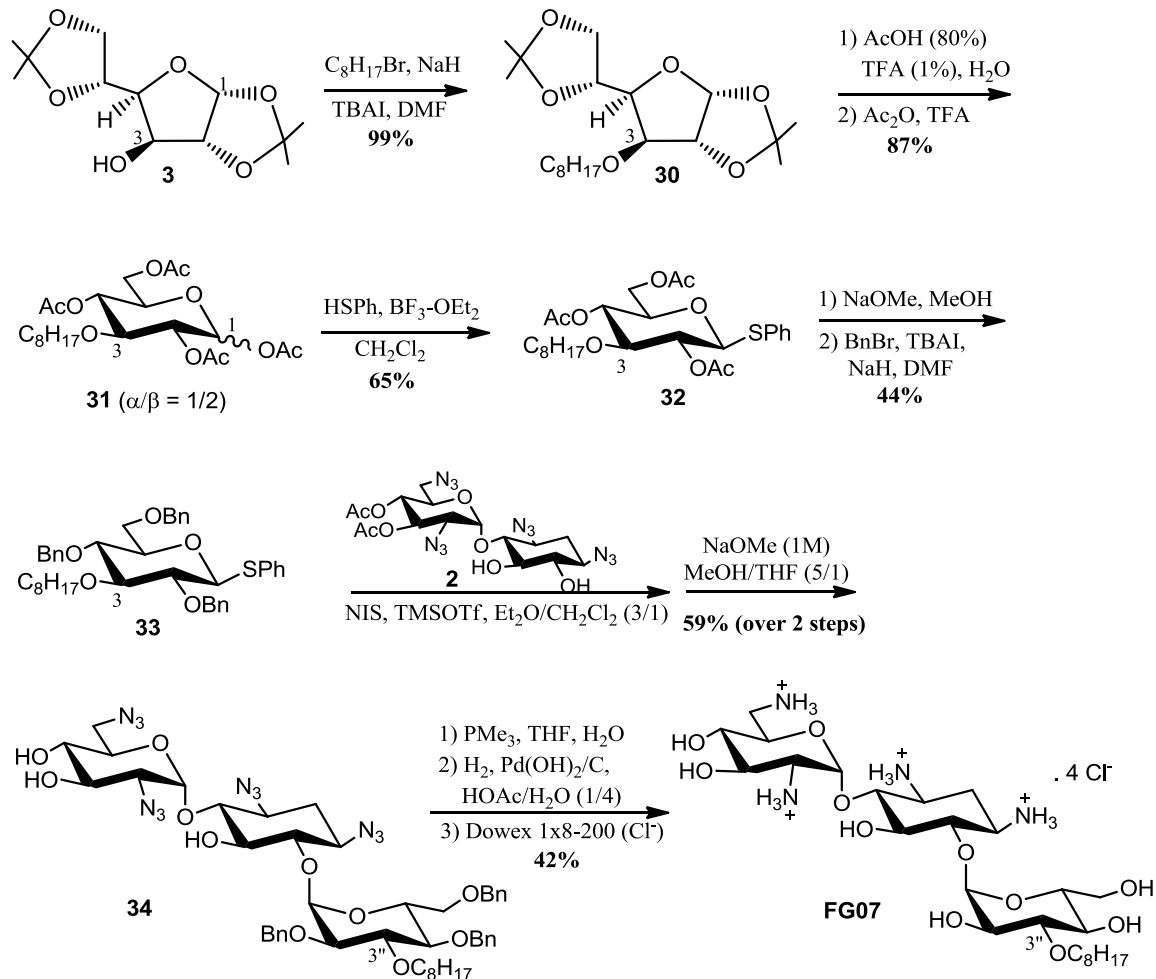
gave **34**. Staudinger reaction, hydrogenation, and ion-exchange afforded **FG07**, which has a C8 alkyl chain at the 3'' position.



Scheme 13: Synthesis of FG05 and FG06

c) Synthesis of **FG09** (alkylation at *O*-2'' position)

The synthesis of **FG09** started from the known compound **35**¹¹⁰ (Scheme 15). Alkylation of the 2-OH gave **36**, which upon treatment with Ac₂O/AcOH/H₂SO₄ provided **37**. Reaction with thiophenol in the presence of BF₃-OEt₂ gave **38**. Acetonolysis, followed by benzylation gave **39**. Glycosylation of **2** and **39** in the presence of NIS and TMSOTf, followed by acetonolysis provided **40**. Staudinger reaction, hydrogenation, and ion-exchange gave **FG09** with the C8 alkyl chain at position 2''.

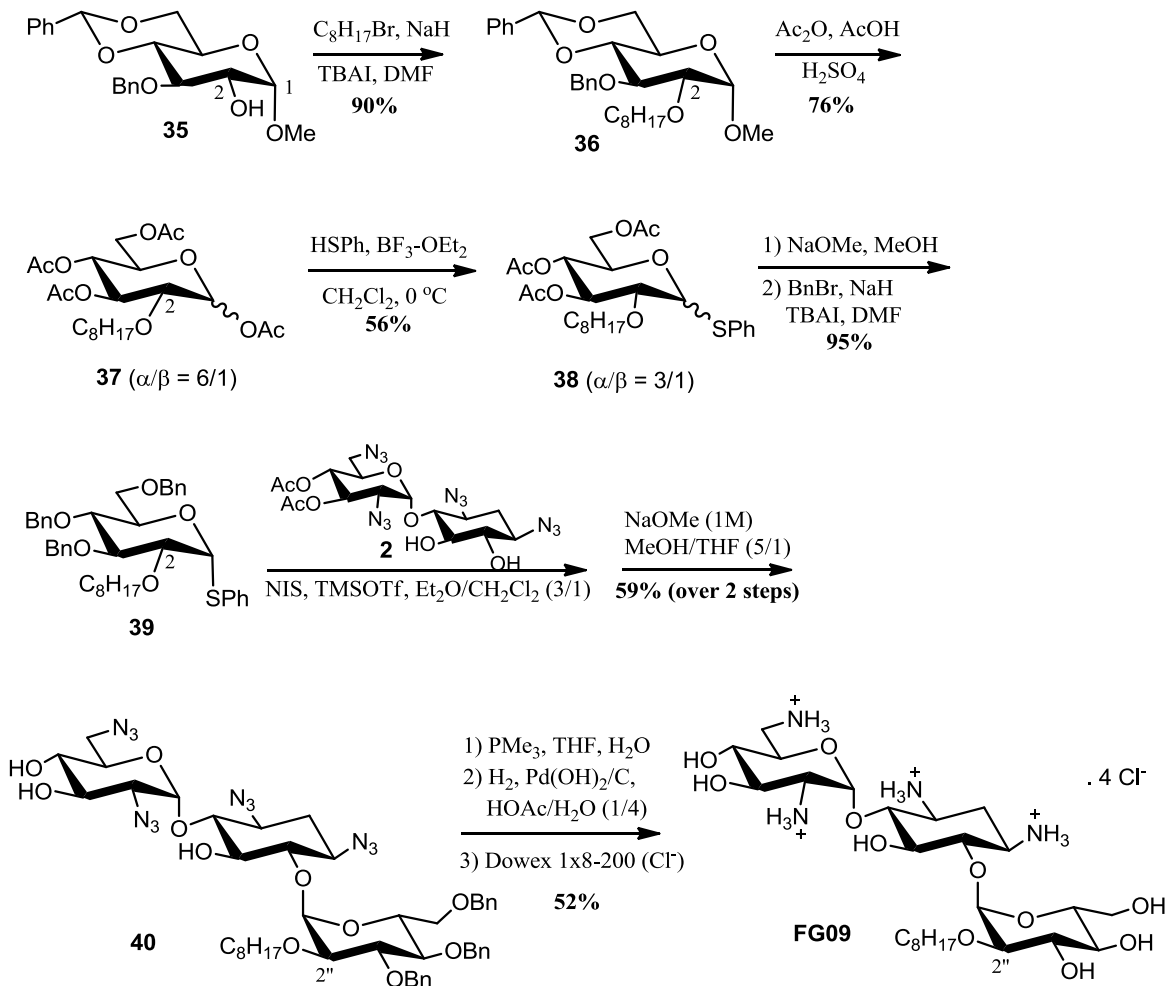


Scheme 14: Synthesis of FG07

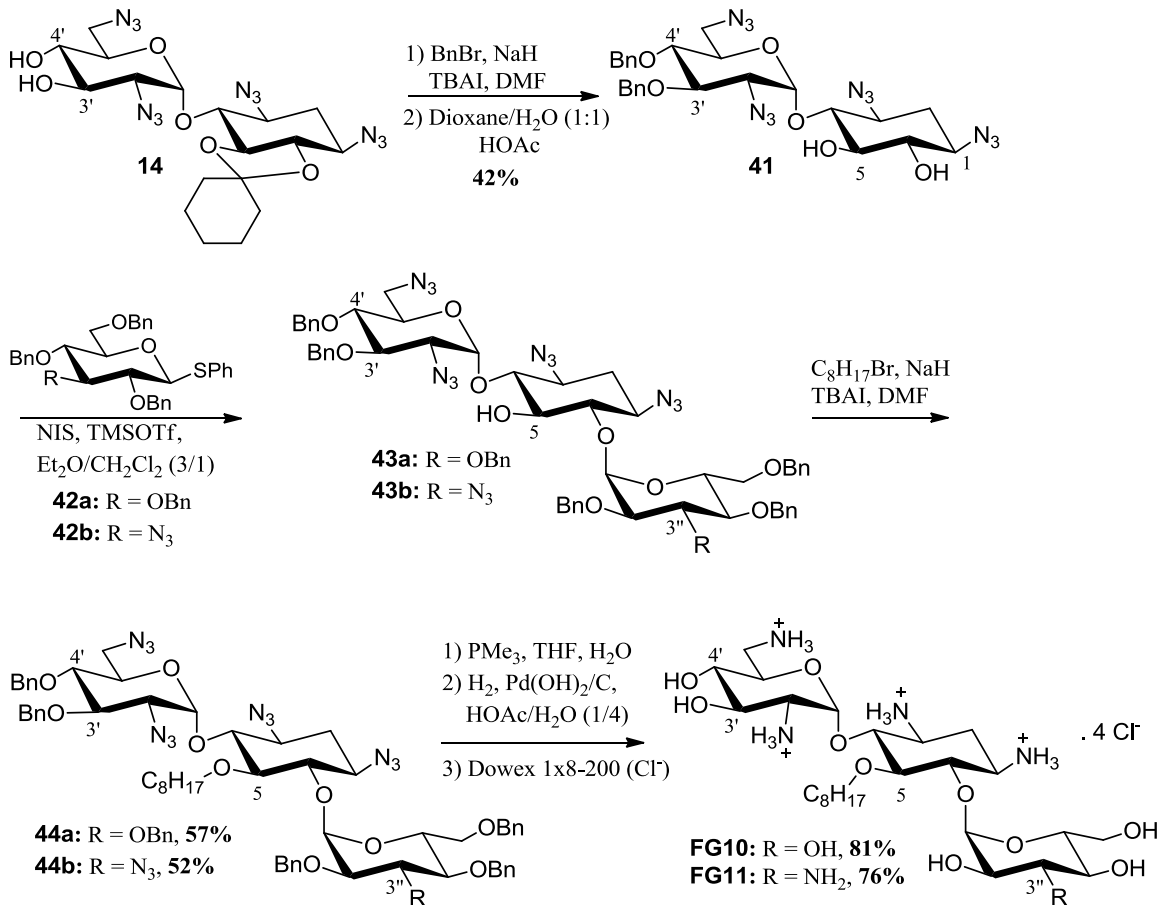
d) Synthesis of FG10 and FG11 (alkylation at O-5 position)

Benzoylation at the 3' and 4' positions of the neamine derivative 14,⁹⁷ followed by the acid-catalyzed cleavage of the cyclohexylidene protecting group gave the glycosyl acceptor 41⁹⁸ (Scheme 16). Glycosylation of 41 with the known thiophenyl donors 42a¹¹¹ and 42b¹¹² gave the compounds 43a and 43b, respectively. Both compounds have a free hydroxyl group at position 5 which will be alkylated to provide 44a and 44b, respectively. Staudinger reaction, hydrogenation, and ion-exchange afforded FG10 and

FG11, respectively, with the C8 alkyl chain at *O*-5 position. **FG10** has a free hydroxyl (OH) group at position 3'', while **FG11** has an amino (NH₂) group at position 3''. **FG10** is thus an analog of **FG08** and **FG11** looks more to kanamycin B.



Scheme 15: Synthesis of **FG09**

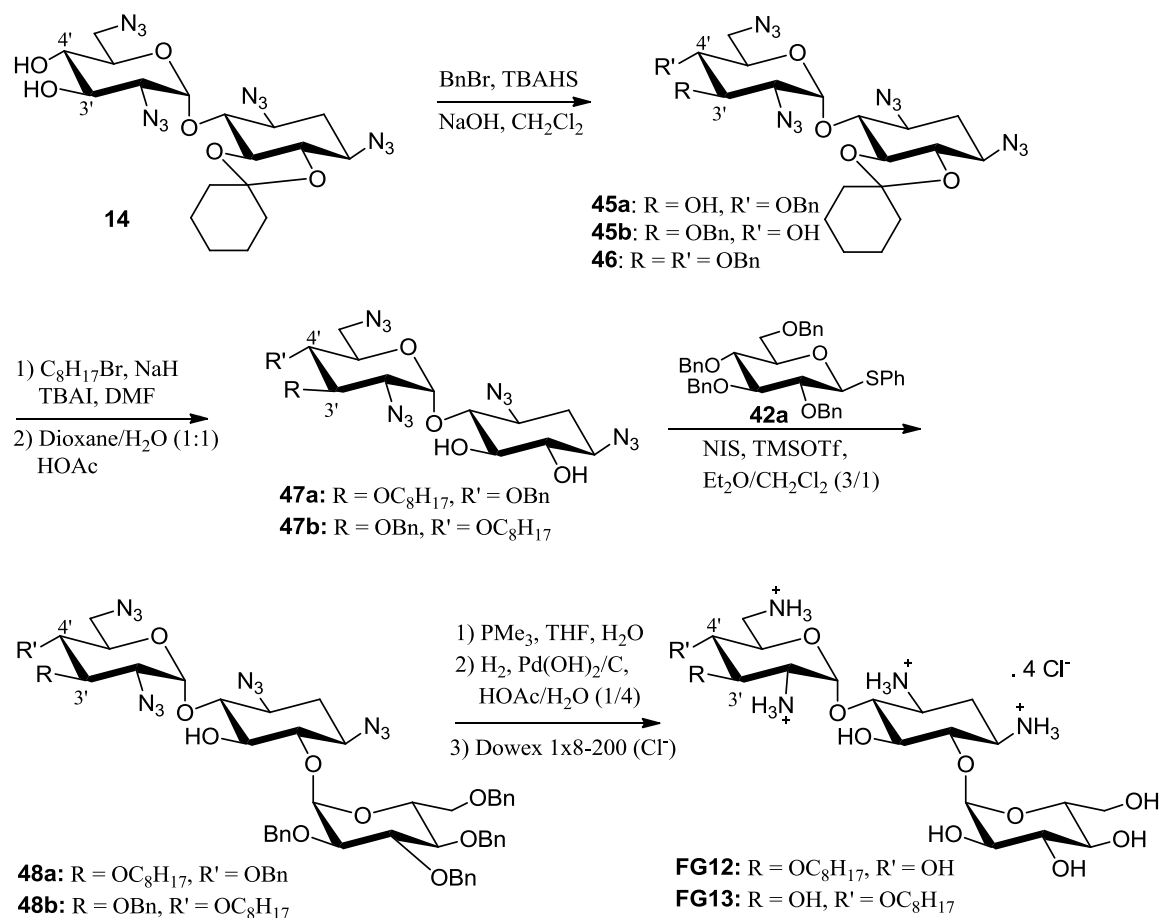


Scheme 16: Synthesis of FG10 and FG11

e) Synthesis of FG12 and FG13 (alkylation at *O*-3' and *O*-4' positions, respectively)

The neamine derivative **14**⁹⁷ has two free hydroxyl groups at position 3' and 4'. Selective benzylation of **14** afforded a mixture of regioisomers (**45a** and **45b**), along with the dibenzylated compound **46** (Scheme 17). The regioisomer **45a** has a Bn group at the 4' position while the regioisomer **45b** has the Bn group at the 3' position. Attempts to separate **45a** and **45b** were unsuccessful. That mixture of **45a** and **45b** was then used as so. Alkylation of the free hydroxyl group in each regioisomer, followed by the acid-

cleavage of the cyclohexylidene protecting group gave compounds **47a** and **47b** as an inseparable mixture. Glycosylation of the acceptors **47a** and **47b** with the donor **42a** afforded **48a** and **48b**, which upon Staudinger reduction, hydrogenolysis, and ion exchange gave a mixture of **FG12** and **FG13**.



Scheme 17: Synthesis of FG12 and FG13

f) Alkylation at O-4'' position confers optimum antifungal activity

The effectiveness of the **FG** compounds was evaluated. The synthesized kanamycin B analogs were tested against the fungus *F. graminearum*. Microbroth

dilution assays performed in Dr. Takemoto's laboratory revealed that **FG08** and **FG03**, which both have an octyl group at the *O*-4'' position, were the most active (Table 3). Indeed, **FG08** and **FG03** were found to inhibit the growth of *F. graminearum* at the minimum concentration of 7.8 µg/mL.

Table 3: MIC values of **FG** compounds against *F. graminearum*^a

Alkylation site	Compound	MIC
O-2''	FG09	20
O-3''	FG07	62.5
O-4''	FG03 (6''-OH)	7.8
O-4''	FG08 (6''-H)	7.8
O-6''	FG05	125
O-6''	FG06	31.3
O-5	FG10 (3''-OH)	<500
O-5	FG11 (3''-NH ₂)	31.3
O-3' & O-4'	FG12 & FG13	≤500

^a Data obtained by Yuki Kawasaki

On the other hand, the mixture of **FG12** and **FG13** showed no activity against *F. graminearum*. Since each compound constituted 50% of the mixture, we can therefore conclude that any of them, by itself, would be inactive against fungi.

From the MIC values, it is therefore obvious that a linear C8 alkyl chain imparts optimum antifungal activity when it is attached at the *O*-4'' position.

III.3. Conclusion

The presence of a linear C8 alkyl chain was found to induce an antibacterial to antifungal transformation to kanamycin B. Indeed, **FG08**, which has a linear C8 alkyl chain at the *O*-4'' position, displayed impressive antifungal activity against a wide range

of crop disease pathogens (fungi). **FG03**, which also has a linear C8 alkyl chain at the *O*-4'' position, was synthesized with the intention to scale up the synthesis of **FG08** by a chemo-enzymatic approach, but this route turned out unsuccessful.

By employing glycodiversification, various sites in kanamycin B have successfully been alkylated to give new analogs. The antifungal activity results indicate the importance of the *O*-4'' position. Indeed, **FG08** and **FG03**, which are both alkylated at the *O*-4'' position, were the most potent antifungal agents.

In light of all these results, a structure-activity relationship can be drawn: attachment of a C8 alkyl chain at the *O*-4'' position of kanamycin B converts this obsolete drug into a potent agro fungicide, with simultaneous loss of antibacterial activity.

CHAPTER IV
SYNTHESIS AND ANTIBACTERIAL STUDY OF CATIONIC
1,4-NAPHTHOQUINONE DERIVATIVES^c

IV.1. Rationale

With the growing rate of bacterial infections and antibiotic-resistance, there have been continuous calls for new antibacterial agents and natural products usually provide resourceful scaffolds. 1,4-naphthoquinone derivatives are ubiquitous in nature⁴⁷⁻⁵¹ and display a wide range of biological activities.⁵²⁻⁵⁶ Our group has thus recently invested some efforts in the development of interesting molecules derived from 1,4-naphthoquinone.¹¹³⁻¹¹⁵ We have synthesized a class of 1-alkyl-1*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-diones.¹¹⁴ These heterocyclic compounds combine two pharmacologically important moieties (1,4-naphthoquinone and 1,2,3-triazole), and were thus expected to exhibit unique biological activities. However, their poor solubility in aqueous media rendered them unavailable for biological testing. Nevertheless, methylation helped to solve this issue and led us to the discovery of a new series of anthraquinone analogs.¹¹⁵ Many of these anthraquinone analogs happened to exhibit impressive antibacterial activity, notably against G⁺ bacteria, which might somewhat be related to the alkyl chain length at N-1 position.

The aim of this project was therefore to optimize the production of 1-alkyl-1*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-diones. In addition, we decided to synthesize a library of 1,3-dialkyl-4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3] triazol-3-ium chloride

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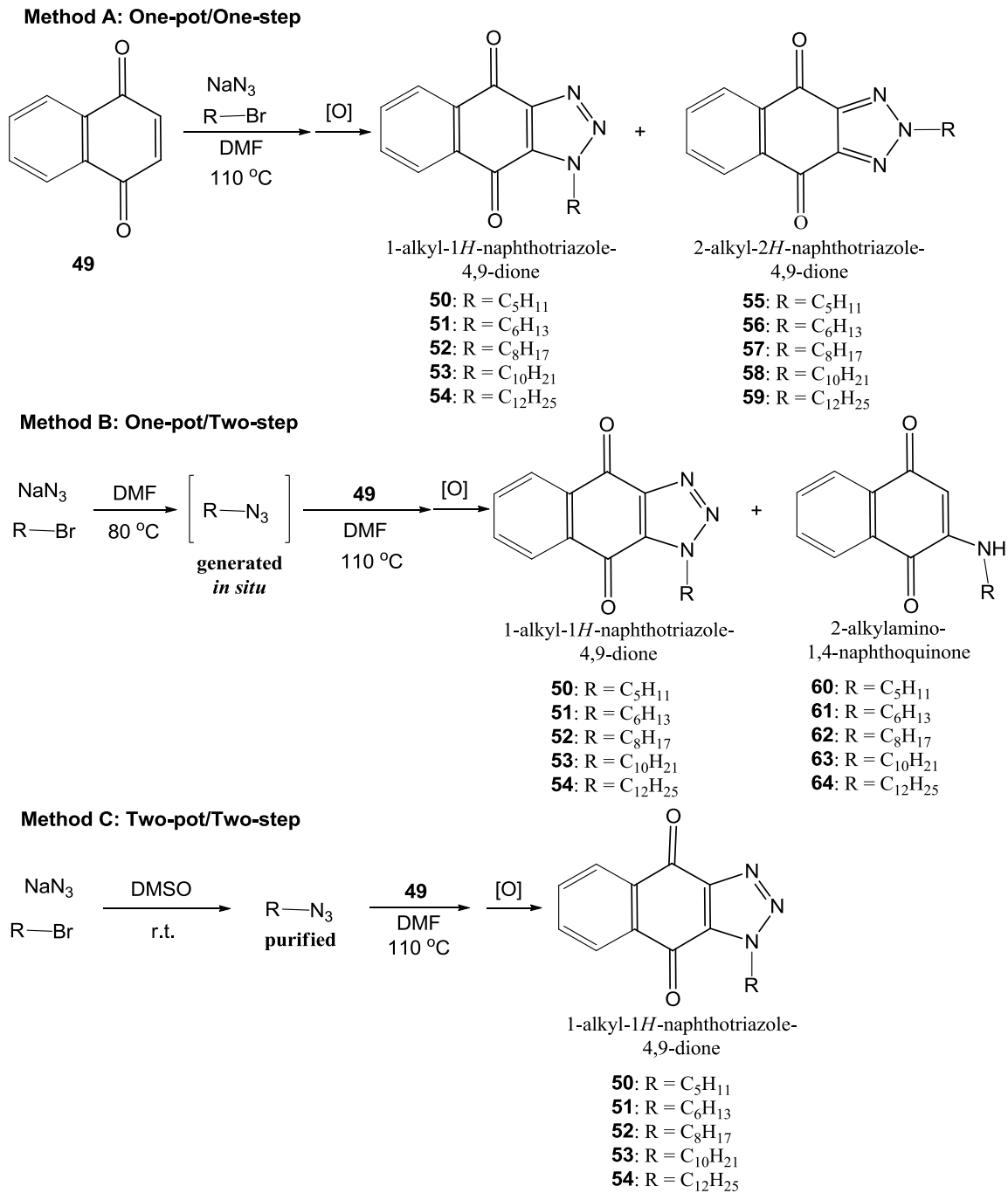
salts, which are cationic 1,4-naphthoquinone derivatives. The study of their antibacterial activity will enable us to elucidate the structure-activity relationship that will result from the incorporation of various alkyl chains at both N-1 and N-3 positions.

IV.2. Results and discussion

IV.2.1. Optimization of the production of 1-alkyl-1*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-diones

The reaction between 1,4-naphthoquinone and azido compounds has been known to occur either via a [2+3] cycloaddition,^{915,116-118} or through a Michael addition and/or oxidation process.^{118,119} Our group has recently reported the synthesis of 1-alkyl-1*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-diones.¹¹⁴ This involves a thermodynamically-controlled cycloaddition of 1,4-naphthoquinone **49** with alkyl azides, followed by an oxidation. Interestingly, this simple but versatile reaction was found to provide structurally diverse molecules, depending on the order of addition of the different reagents or the reaction conditions.

For example, a one-pot/one-step [3+2] cycloaddition in which **49**, sodium azide, and alkyl bromides were allowed to react in DMF provided our expected products **50-54** (Scheme 18, Method A).¹¹⁴ This method also afforded the byproducts 2-alkyl-2*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-diones **55-59**. Although this protocol provided a one-pot divergent synthesis of both classes of compounds in a unique and simple fashion, difficulty in separating them arose. Indeed, they displayed almost identical *R_f* values on TLC plate rendering it very difficult to scale up this protocol.



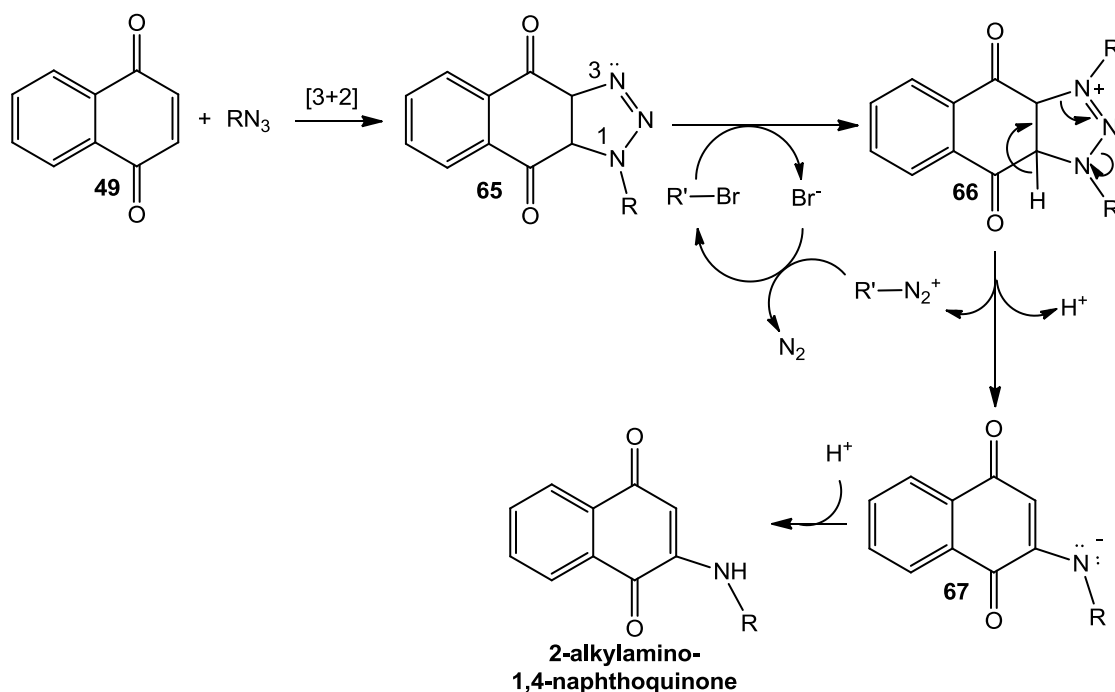
Scheme 18: Protocols for the preparation of compounds **50-54**

To circumvent this problem, we decided to approach the synthesis of compounds **50-54** in a one-pot/two-step fashion: the alkyl azides are first prepared *in situ* by reaction of sodium azide and alkyl bromides, before being allowed to react with 1,4-naphthoquinone **49** (Scheme 18, Method B). To our surprise, this also gave the byproducts 2-alkylamino-1,4-naphthoquinones **60-64**. Even though this class of compounds has been extensively studied for their pharmacological activities (antimycobacterial agents¹²⁰ and inhibitors of coenzyme Q⁵⁷), it was important to understand their formation.

We suggested that the formation of 2-alkylamino-1,4-naphthoquinones **60-64** results from the presence of an excess amount of alkyl bromides in the reacting vessel (Scheme 19). Following the initial cycloaddition of **49** with the alkyl azide, it is possible to have an S_N² nucleophilic substitution via N-3 of the triazoline adduct **65** toward the alkyl bromide. The unstable molecule **66** can undergo decomposition to give the intermediate species **67**. Re-protonation of **67** affords the byproduct 2-alkylamino-1,4-naphthoquinone. In this proposed mechanism, the remaining alkyl bromide from the previous step can actually function as a *catalyst* that facilitates the formation of 2-alkylamino-1,4-naphthoquinone.

In light of these results, we expected that a third alternative, a two-pot/two-step synthesis, whereby the alkyl azides were prepared separately and allowed to react with **49** in another reacting vessel, would only provide our desired compounds **50-54** (Scheme 18, Method C). As expected, Method C generated only **50-54** with yields comparable to the other two methods (Table 4). More importantly, purification of compounds **50-54**

produced in Method C was much easier as they could be isolated by precipitation in diethyl ether, avoiding the use of a column chromatography.



Scheme 19: Mechanistic explanation for the formation of compounds **60-64**

Table 4: Comparison of the different methods for the preparation of compounds **50-54**

Alkyl bromides	1-alkyl-1 <i>H</i> -naphtho[2,3- <i>d</i>][1,2,3] triazole-4,9-dione	Yield (%)		
		Method A ^a	Method B ^b	Method C
1 <i>n</i> -pentyl bromide	50^a	41	53	40
2 <i>n</i> -hexyl bromide	51	n.a.	66	49
3 <i>n</i> -octyl bromide	52^a	52	63	62
4 <i>n</i> -decyl bromide	53^a	64	33	54
5 <i>n</i> -dodecyl bromide	54^a	68	49	68

^a: Ref. 114 ^b: obtained as inseparable mixtures of 1-alkyl-1*H*-naphtho[2,3-*d*][1,2,3] triazole-4,9-dione and 2-alkylamino-1,4-naphthoquinone. The yields of **50-54** are estimated from the integral ratio of the ¹H NMR.

IV.2.2. Synthesis of novel cationic 1,4-naphthoquinone derivatives

Our initial class of cationic 1,4-naphthoquinone derivatives was obtained by methylation at the N-3 position of the triazole motif of compounds **50-54**.¹¹⁵ In order to investigate the effect of the chain length at N-3 position, we synthesized analogs with various chain lengths at both nitrogen atoms (N-1 and N-3) using alkyl triflates (ROTf) prepared *in situ* from the corresponding alcohol (**a-f**) (Scheme 20). After alkylation, the TfO⁻ anion was exchanged with Cl⁻ anion using ion-exchange resin to yield our library of cationic 1,4-naphthoquinone derivatives. This protocol enabled the parallel synthesis of 24 novel 1,3-dialkyl-4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride salts.

IV.2.3. Antibacterial study

Similarly to the series of previously synthesized cationic anthraquinone analogs,¹¹⁵ each member of our library bears the structural scaffolds of naphthoquinone, cation and lipophilic alkyl chain, and was therefore expected to show similar biological activity.

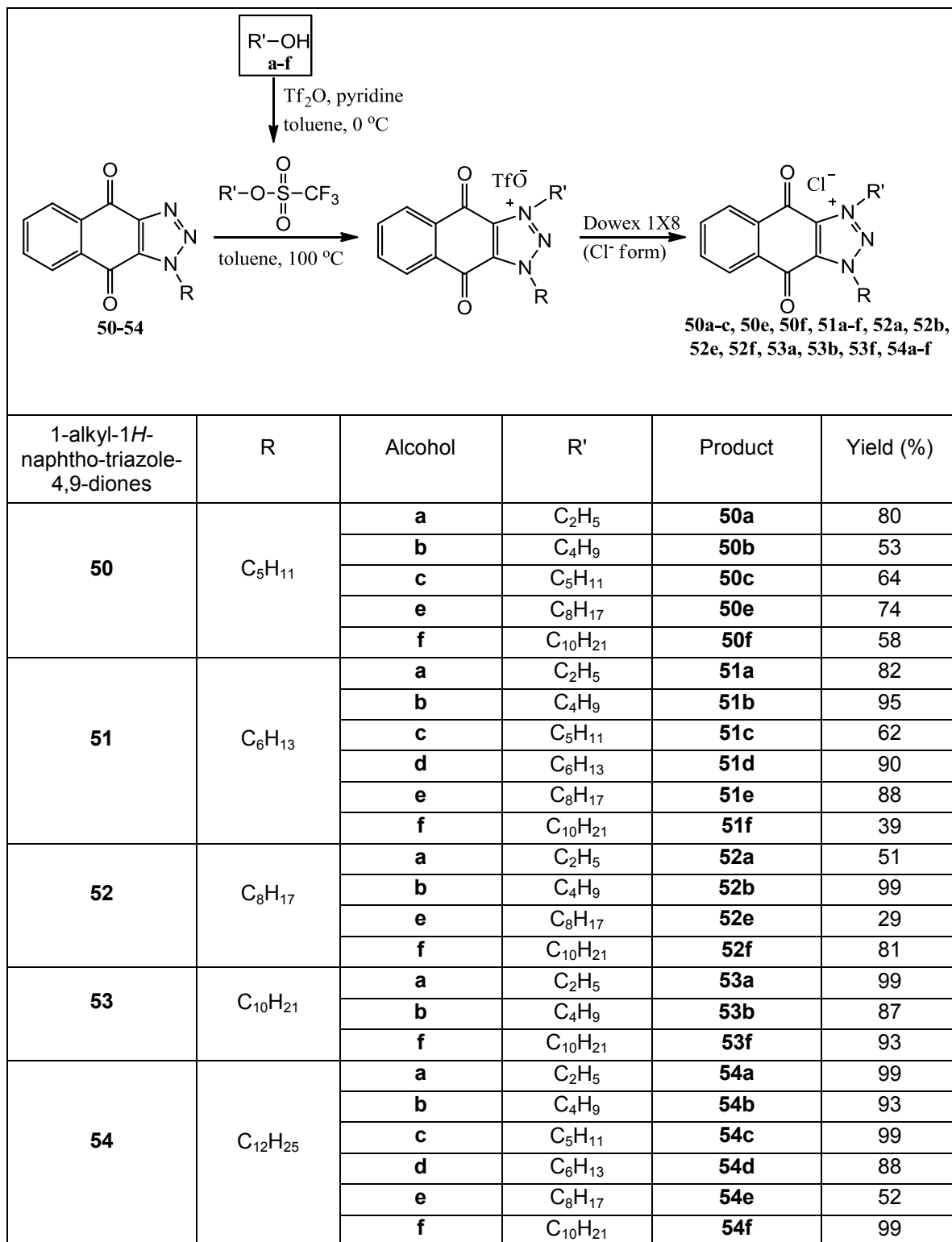
The 1,3-dialkyl-4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*] [1,2,3]triazol-3-ium chloride salts were tested against *E. coli* (ATCC 25922, G-) and *S. aureus* (ATCC 25923, G+) using neomycin, kanamycin, vancomycin, amikacin and hexadecyltrimethylammonium bromide (HTB) as the controls. The MIC values determined in standard fashion using serial 2-fold dilutions are listed in Table 5. The results show that these cationic compounds are more active against G+ bacteria than G-

bacteria, which is consistent with the antibacterial profile of naphthoquinone⁶³ and cationic antiseptic agents such as HTB and cetrimonium bromide.¹²¹

For cationic 1,4-naphthoquinone derivatives with a methyl group at N-3 position, we had previously observed that the antibacterial activity against *S. aureus* slightly increased with the number of carbon atoms in the alkyl group at N-1, reaching a maximum with the octyl group and then decreasing as the chain length was extended to 16 carbons.¹¹⁵

The presence of a different alkyl group at N-3 was however found to have a profound influence on antibacterial activity (Figure 18a). In general, compounds with MIC values below or equal to 1 µg/mL against *S. aureus* were obtained when the total number of carbon atoms of the alkyl groups on both nitrogen atoms was between 9 and 16. This synergistic effect of alkyl group suggests that overall lipophilicity is an important factor in the antibacterial activity. In fact, antiseptic agents with lipophilic alkyl chains have been noted for their ability to disrupt the bacterial membrane of *S. aureus*.¹²² It should also be noted that those cationic antiseptic agents generally have a C₁₂ or longer hydrophobic tail length. This new library therefore combines shorter-chain and longer-chain compounds.

On the other hand, no general trend could be deduced from the MIC values based on the chain length against *E. coli* suggesting that lipophilicity might not be a prerequisite for the antibacterial activity of this library against G- bacteria (Figure 18b).



Scheme 20: Synthesis of cationic 1,4-naphthoquinone derivatives

Table 5: MIC values of cationic 1,4-naphthoquinone derivatives ($\mu\text{g/mL}$)

Compound	R	R'	<i>E. coli</i>	<i>S. aureus</i>
Neomycin B	-	-	8	1
Kanamycin	-	-	4	1-2
Vancomycin	-	-	64-125	0.5
Amikacin	-	-	0.125	0.5
HTB	-	-	1	0.5-1
68^a	C ₅ H ₁₁	CH ₃	8-16	2
50a	C ₅ H ₁₁	C ₂ H ₅	≥ 250	2-4
50b	C ₅ H ₁₁	C ₄ H ₉	≥ 250	0.5
50c	C ₅ H ₁₁	C ₅ H ₁₁	≥ 250	1
50e	C ₅ H ₁₁	C ₈ H ₁₇	32-64	0.125
50f	C ₅ H ₁₁	C ₁₀ H ₂₁	8-16	1-2
69^b	C ₆ H ₁₃	CH ₃	125-250	1-2
51a	C ₆ H ₁₃	C ₂ H ₅	125-250	1
51b	C ₆ H ₁₃	C ₄ H ₉	125-250	1
51c	C ₆ H ₁₃	C ₅ H ₁₁	125	1-2
51d	C ₆ H ₁₃	C ₆ H ₁₃	32-64	0.5-1
51e	C ₆ H ₁₃	C ₈ H ₁₇	4-8	0.5-1
51f	C ₆ H ₁₃	C ₁₀ H ₂₁	2	0.25-0.5
70^a	C ₈ H ₁₇	CH ₃	16-32	0.032-0.064
52a	C ₈ H ₁₇	C ₂ H ₅	≥ 250	0.25-0.5
52b	C ₈ H ₁₇	C ₄ H ₉	64	1-2
52e	C ₈ H ₁₇	C ₈ H ₁₇	≥ 250	2-4
52f	C ₈ H ₁₇	C ₁₀ H ₂₁	32-64	1-2
71^a	C ₁₀ H ₂₁	CH ₃	32	0.032
53a	C ₁₀ H ₂₁	C ₂ H ₅	≥ 250	0.125-0.25
53b	C ₁₀ H ₂₁	C ₄ H ₉	64-125	0.25
53f	C ₁₀ H ₂₁	C ₁₀ H ₂₁	125-250	16-32
72^a	C ₁₂ H ₂₅	CH ₃	16-32	0.064-0.125
54a	C ₁₂ H ₂₅	C ₂ H ₅	32	0.125
54b	C ₁₂ H ₂₅	C ₄ H ₉	≥ 250	0.5-1
54c	C ₁₂ H ₂₅	C ₅ H ₁₁	125-250	0.25-0.5
54d	C ₁₂ H ₂₅	C ₆ H ₁₃	125	0.5-1
54e	C ₁₂ H ₂₅	C ₈ H ₁₇	125-250	2-4
54f	C ₁₂ H ₂₅	C ₁₀ H ₂₁	>250	16-32

^a: Ref. 115; ^b: Compound **69** was synthesized according to the protocol described in reference 115.

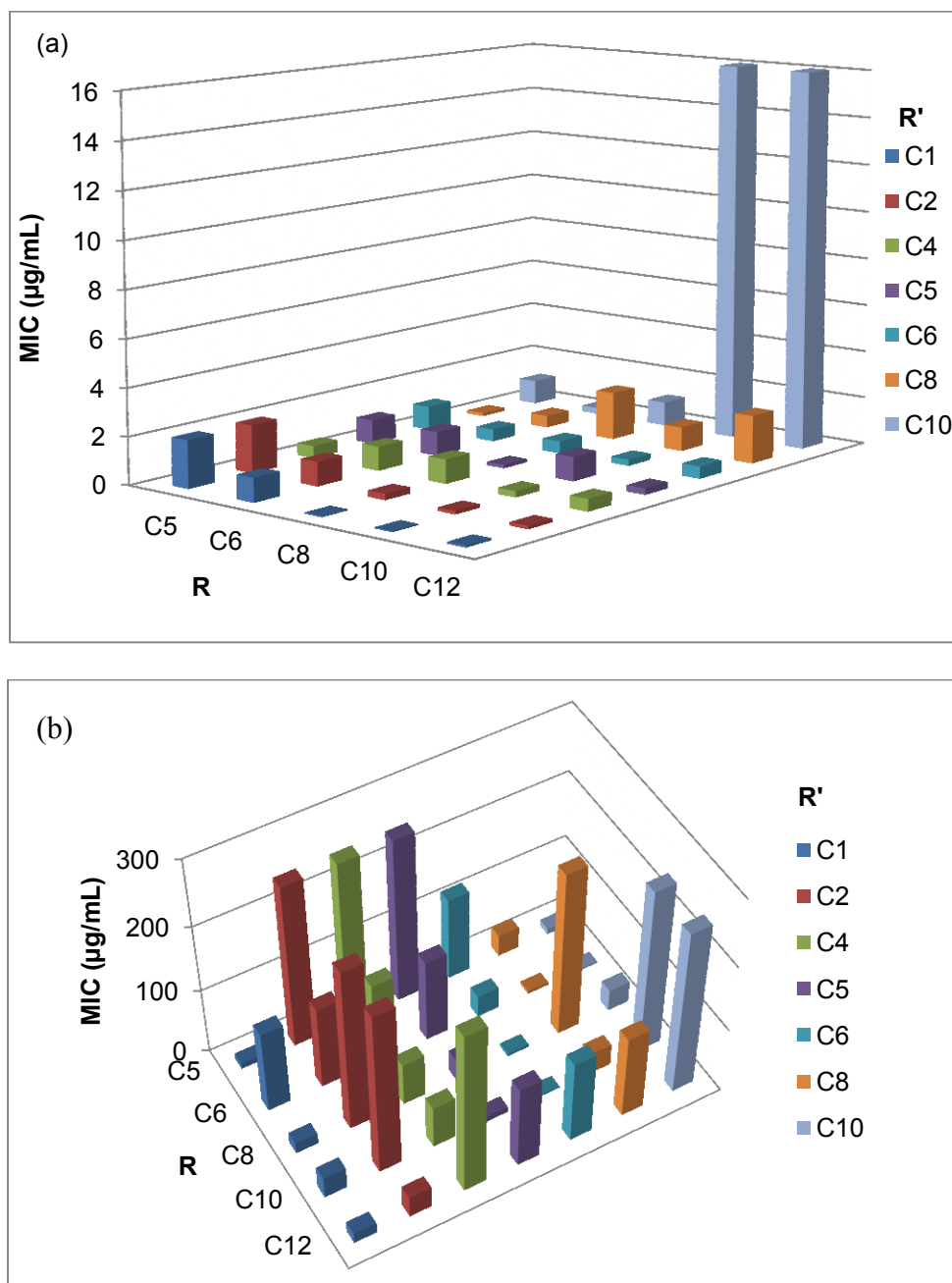


Figure 18: Effect of the alkyl chain length on the MIC values of the cationic 1,4-naphthoquinone derivatives against (a) *S. aureus* and (b) *E. coli*

IV.3. Conclusion

We have developed a new and improved protocol for the synthesis of 1-alkyl-1*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-diones. To further investigate the effect of alkyl substitution at N-3 position, we constructed a library of 4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride salts and tested them against a representative G⁺ and G⁻ bacterium. When the total number of carbon atoms of the alkyl groups at both N-1 and N-3 ranged between 9 and 16, these cationic 1,4-naphthoquinone derivatives will exhibit nanomolar-level antibacterial activity against *S. aureus*, suggesting a synergistic effect of the alkyl group. However, they showed little or no activity against *E. coli*.

CHAPTER V

CONCLUSIONS AND SIGNIFICANCE

Aminoglycosides and 1,4-naphthoquinone derivatives are two classes of naturally occurring compounds that have long attracted interest due their important biological and pharmaceutical applications, earning them the title of “drug-productive scaffolds.”

Aminoglycosides are clinically used antibiotics with a broad-spectrum of activity against Gram-negative and Gram-positive bacteria. However, the continuous emergence of bacterial resistance has seriously hampered their efficacy. While many efforts have been devoted to reviving their antibacterial activity, novel avenues have also been explored in the field of aminoglycosides. Their ability to bind to the ribosome has been exploited in the development of new therapeutic approaches to treat genetic diseases caused by premature nonsense mutations. Our laboratory has previously synthesized libraries of aminoglycosides and the bioactive screening of these libraries has enabled the identification of a lead compound, **TC007**, in the treatment of spinal muscular atrophy. By slightly modifying the original protocol, more **TC007** was prepared and it was found to restore the functionality of the truncated and unstable SMN Δ 7 protein by allowing the incorporation of a near-cognate amino acid at the premature stop codon.

Another screening of our libraries of aminoglycosides has revealed **FG08**, a kanamycin B analog, as a potential antifungal agent with application in agriculture. **FG08** was found to inhibit the growth of several pathogenic fungi that are responsible for a large number of crop diseases. In particular, **FG08** was found to suppress Fusarium head blight, a crop disease that has incurred huge economic losses to the U.S. government.

More interesting, unlike other antibiotics used in plant disease control, **FG08** did not show any activity against bacteria. As a result, **FG08** will unlikely contribute to the transfer of bacterial resistance. The main chemical feature of **FG08** that enabled it to “switch” from an antibacterial agent (kanamycin) to a fungicide was found to be the C8 alkyl chain present at *O*-4'' position. Novel kanamycin B analogs were then synthesized to investigate the alkyl chain length and the position of its attachment that will confer optimum fungicidal activity. First, two different alkyl groups (*n*-butyl and *n*-dodecyl) were inserted at the *O*-4'' position of ring III, which was later on attached by regio- and stereoselective glycosylation at the *O*-6 position of neamine. Second, an *n*-octyl group was introduced at various positions of ring I (*O*-2'', *O*-3'', *O*-4'', and *O*-6''), ring II (*O*-5), and ring III (*O*-3' and *O*-4') to afford seven additional kanamycin B analogs. A bioactive screening of these analogs allowed us to draw a SAR for the optimization of kanamycin B analogs as potential agro fungicides.

Finally, a protocol was developed to improve the production of 1-alkyl-1*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-diones and a library of cationic 1,4-naphthoquinone derivatives was synthesized. Unlike the previously reported one-pot/one-step [3+2] cycloaddition that gives an inseparable mixture of 1-alkyl-1*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-diones and its byproduct,¹¹⁵ a two-pot/two-step method provided only the desired compound upon precipitation in diethyl ether, avoiding the use of a column chromatography. This enabled the facile synthesis of a library of cationic 1,4-naphthoquinone derivatives whose several members were found to exhibit antibacterial activity in the nanomolar range. More importantly, these compounds were more active

against Gram-positive bacteria than Gram-negative. They could be of great importance when antibiotics with narrow-spectrum activity are required. For example, *Clostridium difficile* is a Gram positive bacterium responsible for clodistrium difficile infection (CDI), which is a severe inflammation of the colon. CDI is usually observed following surgery, when the gut flora has been eradicated by the use of antibiotics. The human body lacking the ability to defend itself, invasion of *C. difficile* is now inevitable, unless a drug with specific activity against Gram-positive bacteria is used.

This research has therefore contributed to the investigation of new applications of aminoglycosides, and developed novel cationic 1,4-naphthoquinone derivatives. However, more work is still to be done to get as close as possible to the development of a new drug. With the finding of the conversion of the antibacterial kanamycin B to an agro fungicide as a result of the attachment of a C8 alkyl chain at the 4'' position, an appropriate 4-*O*-octyl glucopyranose derivative needs to be developed for a facile chemoenzymatic synthesis of **FG08** or **FG03**. In addition, novel cationic 1,4-naphthoquinone derivatives with aryl groups at N-1 position could be synthesized.

CHAPTER VI

EXPERIMENTAL SECTION

Chemical reagents and chromatography solvents were purchased from Aldrich Chemical Co. or Acros Chemical Co. and were used without purification unless otherwise noted. Dichloromethane was freshly distilled from calcium hydride. Pyridine and triethylamine were stored over 4 Å molecular sieves. Column chromatographic purifications were carried out on silica gel 230x450 mesh, Sorbent Tech. Analytical TLC was performed on Sorbent Technologies silica gel glass TLC plates. Visualization was accomplished with UV light (254 nm) followed by staining with diluted sulfuric acid (5% in methanol) solution and heating.

Proton magnetic resonance spectra were recorded using JEOL 300 or Bruker ARX 400 spectrometers. Chemical shifts were reported as parts per million (ppm) downfield from tetramethylsilane in δ unit and coupling constants were given in cycles per seconds (Hz). Signal multiplicities were indicated by s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). ^{13}C NMR spectra were obtained using JEOL 300 at 75 MHz, or Bruker ARX 400 at 100 MHz. Routine ^{13}C NMR spectra were fully decoupled by broad-band WALTZ decoupling. All NMR spectra were at ambient temperature. High-resolution fast-atom bombardment (HRFAB), high-resolution MALDI, chemical ionization (CI), atmospheric pressure chemical ionization (APCI) or electrospray ionization (ESI) were provided by the Mass Spectrometry Facilities, University of California, Riverside.

General Procedure for Aminoglycoside Treatment of SMA (performed in Dr. Lorson's laboratory). 3,813 SMA type I patient fibroblasts cells were plated on cover slips and grown in Dulbeccos's modified Eagle's medium (DMEM) containing 10% (v/v) fetal bovine serum and antibiotics for 24 h. Cells were washed three times with phosphate-buffered saline (PBS) and re-fed with DMEM containing the aminoglycoside diluted to the indicated concentration. In prolonged experiments, the medium containing freshly diluted aminoglycoside was changed every 24 h for the indicated duration (up to 96 h). For cells used in Western blot analysis, cells were plated at ~80% confluence in six-well dishes and treated for 48 h. Fresh drug-containing media was replaced every 24 h, diluted to 100 µg/mL. Cells were initially identified by DAPI staining, not by the presence or the absence of SMN and gems. Only after obtaining a field of view, the SMN/FTIC channel was observed. The DAPI field was done randomly across a large number of treated cells, providing an unbiased assessment of gem numbers throughout the cell population.

General Procedure for Mice and TC007 Treatment (performed in Dr. Lorson's laboratory). All animal experiments were carried out in accordance with protocols approved by the Animal Care and Use Committee of the University of Missouri. Mice were genotyped and litters excluded. **TC007** was initially resuspended in distilled water, further diluted in PBS, and administered by subcutaneous injection (10 µL/gram of body weight) on post-natal days 2 through 15. PBS (vehicle) was injected as a negative control. To assess gross motor function, righting reflex was measured starting at post-natal day 5.

General Procedure for MIC Determination. A solution of selected bacteria was inoculated in the Trypticase Soy broth at 35 °C for 1-2 h. The bacteria concentration was found and diluted with broth, if necessary, to an absorption value of 0.08 to 0.1 at 625 nm. The adjusted inoculated medium (100 µL) was diluted with 10 mL of broth and then applied to a 96-well microtiter plate (50 µL). A series of solutions (50 µL each in 2-fold dilution) of the tested compounds was added to the testing wells. The 96-well plate was incubated at 35 °C for 12-18 h. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of compound needed to inhibit the growth of bacteria. The MIC results are repeated at least three times.

General Procedure for Leaf Infection Assay (performed by Ms. Yukie Kawasaki, a graduate student of Dr. Jon Takemoto. All the figures/data related to leaf infection assay remain her sole propriety). Suspensions of *F. graminearum* macroconidia ($2.0 \times 10^4 \text{ mL}^{-1}$) were prepared in sterile solution of 0.25% (wt vol⁻¹) agar and 0.20% (by volume) of Tween 20 and mixed with equal volumes of aminoglycoside made in the same solution.

FHB Disease Suppression (performed by Ms. Yukie Kawasaki, a graduate student of Dr. Jon Takemoto. All the figures/data related to FHB disease suppression remain her sole propriety). Rapid-maturing cultivar Apogee was grown for 5-6 weeks in a greenhouse to the flowering stage. Florets (one per spikelet) were treated with a solution of aminoglycoside at the indicated concentration, and then inoculated with suspension of *F. graminearum* macroconidia ($10 \mu\text{L}, 10^5 \text{ conidia mL}^{-1}$). After 4 days, the spikelets were visually inspected for disease symptoms (chlorosis, spikelet curling, and dehydration).

General Procedure for *O*-Alkylation of Sugars. To a solution of starting material in anhydrous DMF, alkyl bromide (2.0 equivalents), NaH (2.0 equivalents), and catalytic amount of TBAI were added. The reaction was stirred overnight. When complete, the reaction was quenched by addition of MeOH (5 mL) and was slowly poured into a mixture of ice and EtOAc. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with 1 N aqueous HCl, water, saturated aqueous NaHCO₃ and brine, and then dried over solid Na₂SO₄. After removal of the solvent and purification with gradient column chromatography (hexane:EtOAc = 100:0 to 60:40), the product was obtained.

General Procedure for the Glycosylation using Thiophenyl donor, and Hydrolysis. A solution of glycosyl donor, neamine derivative (1.2 equivalents), and activated powder 4 Å molecular sieve was stirred at room temperature for 2 h in 12 mL of a mixed anhydrous solution Et₂O:CH₂Cl₂ = 3:1. The mixture was cooled to -70 °C and *N*-iodosuccinimide (1.2 equivalents) was quickly added. After the temperature has warmed up to -40 °C, trifluoromethanesulfonic acid (0.15 equivalents) was added. The solution was stirred at low temperature till the complete consumption of the glycosyl donor. The reaction mixture was quenched by addition of solid NaHCO₃, Na₂S₂O₃, and Na₂SO₄. After being stirred for 15 minutes, the reaction mixture was filtered through celite. The residue was washed thoroughly with EtOAc. After removal of the solvents, the crude product was purified with gradient column chromatography. The glycosylated compounds were often mixed with inseparable impurities, and were therefore fully characterized after hydrolysis. The glycosylated product was dissolved in THF (1 mL)

and MeOH (5 mL), and 1M NaOMe in MeOH (0.5 mL) was added. The mixture was stirred at room temperature until TLC analysis indicated completion of the reaction (about 30 minutes). The reaction was neutralized with Amberlite IR-120 (H⁺), and filtered through celite. After removal of the solvents, the crude product was purified with gradient column chromatography (Hexane:EtOAc = 100:0 to 50:50) to afford the expected product.

General Procedure for Cycloaddition of 1,4-Naphthoquinone

Method A. is described in ref 114.

Method B. A solution of NaN₃ (~0.1 g) and alkyl bromide (2 equivalents) in DMF (10 mL) was stirred at 80 °C for one day in a sealed vial. Then naphthoquinone (2 equivalents) was added and the mixture was heated for another day at 110 °C. The solvent was evaporated and the crude product was purified by column chromatography (eluted from hexane:EtOAc = 100:0 to 50:50) to afford a mixture containing both 1-alkyl-1*H*-naphtho[2,3-*d*]triazole-4,9-diones and 2-alkylamino-1,4-naphthoquinones. The 2-alkylamino-1,4-naphthoquinones were recovered after N-3 alkylation.

Method C. A solution of alkyl azide (~0.3 g), which was obtained using the method described in reference 124, and naphthoquinone (2 equivalents) in DMF (10 mL) was stirred at 110 °C overnight in a sealed vial. The solvent was evaporated and cold diethyl ether (50 mL) was added. The solid that precipitated was collected by filtration through a Hirsh funnel and washed with more diethyl ether to afford the expected product as a pale brown solid.

General Procedure for N-3 Alkylation. The alcohol (2 equivalents) and pyridine (4 equivalents) were dissolved in anhydrous toluene (10 mL) and cooled in an ice-water bath before TiF_4 (4 equivalents) was slowly added. The mixture was stirred at 0 °C for 2 h and the triazole (0.11 g, 1 equivalent) was then added. This mixture was then refluxed at 110 °C for 6-8 h. After completion of the reaction, the solvent was removed and the crude product was purified by column chromatography (eluted with 300 mL Hexane:EtOAc = 50:50, 200 mL pure EtOAc, and finally 100 mL EtOAc:MeOH = 80:20) to afford the expected product, which was then eluted through a small column packed with Dowex 1x8 (Cl^-) resin for ion exchange.

Hexaazido-hepta-O-benzyl Neomycin (1).⁸² NaN_3 (54.8 g, 842.2 mmol) was first dissolved with distilled water (75 mL) in a 1L round-bottomed flask. Dichloromethane (125 mL) was then added and the flask was transferred in an ice-water bath. TiF_4 (28.4 mL, 168.5 mmol) was slowly added and the mixture was stirred at low temperature. Two hours later, the reaction mixture was transferred into a 1L separatory funnel and a saturated aqueous NaHCO_3 solution was added. The funnel was shaken to release CO_2 gas and the CH_2Cl_2 phase was separated. The aqueous phase was extracted with an additional 75 mL CH_2Cl_2 . The organic layers were then combined and washed with saturated NaHCO_3 solution until no more gas was produced. This freshly prepared dichloromethane solution of triflic azide was slowly added to a mixture of neomycin trisulfate (10.0 g, 14.04 mmol), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.35 g, 1.40 mmol), K_2CO_3 (15.5 g, 112.3 mmol) in H_2O (150 mL) and MeOH (300 mL). The mixture was stirred at room temperature overnight until TLC analysis showed complete reaction. The solvent was

then removed under reduced pressure, and the residue was redissolved in EtOAc. The filtrate obtained following filtration through a celite bed was subsequently extracted with 1N aqueous HCl, saturated aqueous NaHCO₃, water and brine. The organic layer was dried over Na₂SO₄, filtered and concentrated under reduced pressure to provide a greenish crude product. After being dried under vacuum pump for a few hours, the crude product (14.07 g, 18.3 mmol) was dissolved in DMF (100 mL), and BnBr (32.8 mL, 274.1 mmol) and a catalytic amount of TBAI were added. The mixture was then transferred in an ice-water bath and NaH (11.0 g, 274.1 mmol) was slowly added. When TLC analysis performed the following day indicated completion, the reaction was quenched with MeOH (20 mL) and poured over ice. The mixture was diluted with EtOAc, extracted with 1N aqueous HCl, saturated aqueous NaHCO₃, water and brine, dried over Na₂SO₄, filtered, and concentrated. Purification of the crude product by gradient column chromatography (hexane:EtOAc = 100:0 to 50:50) provided **1** (7.44 g, 5.3 mmol, 38% from neomycin sulfate).

1,3,2',6'-Tetraazido-6,3',4'-tri-O-benzyl Neamine (2).⁸² Compound **1** (7.44 g, 5.3 mmol) was dissolved in CH₃CN (100 mL) and CuCl₂·2H₂O (1.81 g, 10.6 mmol) was added. The mixture was stirred at 80 °C overnight until TLC analysis showed completion of the reaction. The solvent was then removed under reduced pressure. The residue obtained was redissolved in EtOAc and filtered through a celite bed. The filtrate was then extracted with water and brine, dried over Na₂SO₄, filtered, and concentrated. Purification by gradient column chromatography (hexane:EtOAc = 100:0 to 20:80) provided **2** (2.10 g, 3.0 mmol, 57%).

1,2:5,6-Di-*O*-isopropylidene- α -D-allofuranose (4).⁸³ To a sealed round-bottomed flask containing anhydrous CH₂Cl₂ (400 mL) at -78 °C, oxalyl chloride (5.03 mL, 57.6 mmol) and anhydrous DMSO (8.2 mL, 115.3 mmol) were added dropwise. When the temperature warmed up to -65 °C, a solution of diacetone-D-glucose **3** in anhydrous CH₂Cl₂ (100 mL) was added, and the reaction was allowed to stir until the temperature reaches -45 °C. At that moment, anhydrous Et₃N (32.3 mL, 230.6 mmol) was added and the reaction mixture was stirred until room temperature. The mixture was diluted with CH₂Cl₂ and washed with 1N aqueous HCl, pH 7 buffer (3 times), and brine. The organic layer was dried over Na₂SO₄, filtered, and concentrated. The crude product obtained was then dissolved in anhydrous MeOH and the solution was cooled down to 0 °C. NaBH₄ (4.36 g, 115.3 mmol) was then slowly added and the reaction was allowed to stir overnight till room temperature. The reaction was quenched by adding HCl dropwise until the solution reaches pH 8. Removal of the solvents gave a syrup-like residue that was diluted with EtOAc. Filtration through layers of silica gel and celite provided a solution that was concentrated and purified by gradient column chromatography (hexane:EtOAc = 100:0 to 40:60) to afford **4** (4.62 g, 17.7 mmol, 46%).

3-Azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene- α -D-glucopyranose (5).⁸⁴ To a solution of **4** (4.59 g, 17.6 mmol) in anhydrous CH₂Cl₂ (100 mL), pyridine (4.2 mL, 51.1 mmol) was added and the mixture was cooled down to 0 °C in an ice-water bath. Tf₂O (7.5 mL, 44.1 mmol) was then added dropwise and the reaction was allowed to stir for 2 h, during which time the temperature reached 20 °C. The reaction mixture was diluted with CH₂Cl₂ and washed with water, saturated aqueous NaHCO₃ (twice), and brine. The

organic layer was dried over Na₂SO₄, filtered, and concentrated. The crude triflate was added to a solution of NaN₃ (3.44 g, 52.9 mmol) in DMF (50 mL) and the reaction was stirred at room temperature overnight until TLC analysis confirmed completion of the reaction. The solvent was then removed to afford a residue that was dissolved in EtOAc. The organic layer was washed with water and brine, dried over Na₂SO₄, filtered, and concentrated. Purification by gradient column chromatography (hexane:EtOAc = 100:0 to 20:80) provided **5** (3.68 g, 12.9 mmol, 73%).

1,2,4,6-Tetra-O-acetyl-3-azido-3-deoxy-D-glucopyranose (6).⁸⁵ A solution of **5** (4.40 g, 15.4 mmol) in 150 mL of a mixed solution of AcOH/TFA/H₂O (80/1/19) was stirred at 55 °C overnight. When TLC analysis indicated completion of the reaction, the solvents were removed. After being dried *in vacuo* for a few hours, the crude product was dissolved in Ac₂O (50 mL) and TFA (5 mL), and the mixture was stirred at room temperature overnight. Solid NaHCO₃ was then added to neutralize the excess acid. EtOAc was added to dilute the solution and the organic layer was washed with water, saturated aqueous NaHCO₃ (3 times), and brine. The organic layer was then dried over Na₂SO₄, filtered, and concentrated. Purification by gradient column chromatography (hexane:EtOAc = 100:0 to 40:60) provided **6** (5.42 g, 14.5 mmol, 94%) as a mixture of α/β anomers in a 1/1 ratio.

2,4,6-Tri-O-acetyl-3-azido-3-deoxy-D-glucopyranose (7).⁸⁶ To a solution of **6** (0.37 g, 0.99 mmol) in anhydrous DMF (5 mL) was added hydrazine acetate (0.11 g, 1.2 mmol). The reaction mixture was stirred at room temperature for 6 h when TLC analysis indicated completion of the reaction. The reaction mixture was then filtered through a

short column packed with layers of silica gel and celite. The column was eluted thoroughly with EtOAc. After removal of the solvents, the crude product was purified by gradient column chromatography (hexane:EtOAc = 100:0 to 0:100) to afford **7** (0.32 g, 0.97 mmol, 98%) as a mixture of α/β anomers in a 1/1 ratio.

3-Azido-2,4,6-tri-O-acetyl-3-deoxy- α -D-glucopyranosyl trichloroacetimidate (8).⁸⁷ To a solution of **7** (0.66 g, 2.0 mmol) and trichloroacetonitrile (1.0 mL, 10.0 mmol) in anhydrous CH₂Cl₂, DBU (0.08 mL, 0.50 mmol) was added dropwise. The solution was stirred at room temperature until TLC analysis indicated completion of the reaction, sometimes as fast as 10 minutes. Then charcoal was added to the reaction mixture. This was then filtered through a short column packed with celite and the column was thoroughly eluted with EtOAc. After removal of the solvents, the crude product was loaded in a column that has been pretreated with triethylamine. Purification by gradient column chromatography (hexane:EtOAc = 100:0 to 50:50) provided **8** (0.63 g, 1.3 mmol, 66%). This was kept in the fridge until needed to prevent it from degrading at room temperature.

5-O-(3''-Azido-2'',4'',6''-tri-O-acetyl-3-deoxy- β -D-glucopyranosyl)-1,3,2',6'-tetrazido-6,3',4'-tri-O-benzyl neamine (9).⁸⁷ A solution of neamine derivative **2** (0.20 g, 0.29 mmol), glycosyl trichloroacetimidate **8** (0.16 g, 0.34 mmol), and activated powder 4 Å molecular sieve was stirred in anhydrous diethyl ether (10 mL) at room temperature for 2 h, then cooled to -50 °C. BF₃-OEt₂ (0.05 mL) was then added. The solution was stirred till the complete consumption of **2**. The reaction mixture was quenched by the addition of powder NaHCO₃. After being stirred for 15 minutes, the reaction mixture was filtered

through celite. The residue was washed thoroughly with EtOAc. After removal of the solvents, the crude product was purified with gradient column chromatography (hexane:EtOAc = 100:0 to 50:50) to afford **9** (0.22 g, 0.22 mmol, 76%).

5-O-(3-Amino-3-deoxy- β -D-glucopyranosyl)neamine (TC007).⁸⁷ A solution of **9** (0.33 g, 0.33 mmol) and K₂CO₃ (0.41 g, 2.94 mmol) was stirred in MeOH (10 mL) at room temperature overnight until TLC analysis indicated completion of the reaction. The solvent was removed, and the reaction mixture was diluted with EtOAc and filtered through a short column packed with layers of silica gel and celite. The column was eluted with EtOAc and MeOH. After removal of the solvents, the crude product was dissolved in THF (5 mL) and the solution was transferred in a reaction flask equipped with a reflux condenser. Then H₂O (0.6 mL) and PMe₃ (1M in THF, 1.36 mL, 1.36 mmol) were added. The reaction mixture was stirred at 50 °C for 2 h until completion of the reaction. Removal of the solvents afforded a crude product that was dissolved in 5 mL of degassed AcOH/H₂O (1/4). Then a catalytic amount of Pd(OH)₂/C (20% Degussa type) was added and the reaction mixture was further degassed. The reaction mixture was then stirred at room temperature under atmospheric H₂ pressure for one day. The reaction mixture was then filtered through celite. The residue was washed with water, and the combined solutions were concentrated, affording a crude product that was eluted through an ion-exchange column packed with Dowex 1X8 resin (Cl⁻ form). Removal of the solvents afforded **TC007** as a chloride salt (136.3 mg, 0.20 mmol, 61% over 4 steps).

1,3,2',6'-Tetraazidoneamine (13).¹⁰¹ NaN₃ (50.0 g, 768.9 mmol) was first dissolved with distilled water (46 mL) in a 1L round-bottomed flask. Dichloromethane

(77 mL) was then added and the flask was transferred in an ice-water bath. Tf₂O (26.0 mL, 153.8 mmol) was slowly added and the mixture was stirred at low temperature. Two hours later, the reaction mixture was transferred into a 1L separatory funnel and saturated aqueous NaHCO₃ was added. The funnel was shaken to release CO₂ gas and then the CH₂Cl₂ phase was separated. The aqueous phase was extracted with an additional 75 mL CH₂Cl₂. The organic layers were then combined and washed with saturated aqueous NaHCO₃ solution until no more gas was produced. (Even though an explosion never happened whenever I had to prepare triflic azide, extra precaution should be taken throughout its synthesis as it is known to be very explosive). This freshly prepared dichloromethane solution of triflic azide was slowly added to a mixture of neamine hydrochloride **12** (9.0 g, 19.2 mmol), CuSO₄·5H₂O (0.48 g, 1.92 mmol), and K₂CO₃ (21.2 g, 153.8 mmol) in H₂O (150 mL) and MeOH (300 mL). The mixture was stirred at room temperature overnight until TLC analysis showed complete reaction. The solvent was then removed under reduced pressure, and the residue was redissolved in EtOAc. The filtrate obtained following filtration through a celite bed was subsequently extracted with 1N aqueous HCl, saturated aqueous NaHCO₃, water and brine. The organic layer was dried over Na₂SO₄, filtered and concentrated. Purification with gradient column chromatography (hexane:EtOAc = 100:0 to 0:100) afforded **13** (4.26 g, 10.0 mmol, 52%).

1,3,2',6'-Tetraazido-5,6-O-cyclohexylideneamine (14).⁹⁷ To a solution of **13** (7.95 g, 18.6 mmol) and *p*-toluenesulfonic acid monohydrate (1.77 g, 9.32 mmol) in anhydrous CH₃CN (100 mL), cyclohexanone dimethyl ketal (12.8 mL, 83.9 mmol) was added, and the mixture was stirred overnight at room temperature. The reaction mixture

was quenched by addition of Et₃N (2.6 mL) and was concentrated. The residue obtained was redissolved in EtOAc, washed with water and brine, dried over Na₂SO₄, and concentrated. Purification with column chromatography provided **14** (4.8 g, 9.5 mmol, 51%).

1,3,2',6'-Tetraazido-3',4'-di-O-acetylneamine (10).⁹⁷ To a solution of **14** (3.20 g, 6.32 mmol) in anhydrous CH₂Cl₂ (50 mL), Et₃N (6.2 mL, 44.3 mmol) and DMAP (0.31 g, 2.53 mmol) were slowly added, followed by Ac₂O (3.0 mL, 32.6 mmol). The reaction was stirred at room temperature for 3 h. When complete, the reaction mixture was quenched with saturated aqueous NaHCO₃ and extracted with EtOAc. The organic layer was washed with 1N aqueous HCl, water, saturated aqueous NaHCO₃, brine, and dried over solid Na₂SO₄. After removal of the solvent, a brownish, oily crude product was obtained, to which 80 mL of a mixed solution of dioxane:H₂O = 1:1 was added, followed by 50 mL glacial acetic acid. The resulting mixture was refluxed at 60~65°C overnight. When complete, the reaction mixture was quenched with saturated aqueous NaHCO₃ and extracted with EtOAc. The organic layer was washed with 1N aqueous HCl, water, saturated aqueous NaHCO₃, brine, and dried over solid Na₂SO₄. After removal of the solvent followed by purification with a gradient column chromatography (hexane:EtOAc = 100:0 to 40:60), **10** was obtained (2.03 g, 4.0 mmol, 63%).

Phenyl 4,6-O-(phenylmethylene)-1-thio-β-D-glucoopyranoside (16).¹⁰² To a solution of **15** (10.0 g, 22.7 mmol) in anhydrous MeOH (300 mL), 5 mL of a 1M solution of NaOMe in MeOH was added and the mixture was stirred at room temperature for 2 h. When complete, the reaction was quenched by adding Amberlite IR 120 H⁺ resin to the

mixture, followed by filtration through celite and concentration of the filtrate. The crude product obtained was diluted in anhydrous DMF (50 mL), and TsOH.H₂O (2.23 g, 11.7 mmol) and benzaldehyde dimethyl acetal (3.53 mL, 23.4 mmol) were added. The reaction flask was then attached to a rotavapor and rotated at 60 °C for 1 h. The temperature of the water bath was then raised to 100 °C and most of the DMF was removed. The reaction mixture was cooled down to room temperature and saturated aqueous NaHCO₃ (60 mL) was added. Lots of white precipitates were formed. The solution was then stirred at 90 °C and cooled down again to room temperature. The solids were filtered through a Buchner funnel, washed with plenty of water, and dried under reduced pressure to give **16** (7.4 g, 20.4 mmol, 90%).

Phenyl 2,3-di-O-benzyl-1-thio-β-D-glucopyranoside (17).¹⁰² To a solution of **16** (2.24 g, 6.2 mmol) in DMF (40 mL), BnBr (3.0 mL, 24.9 mmol) and a catalytic amount of TBAI were added. The mixture was then transferred in an ice-water bath and NaH (1.00 g, 24.9 mmol) was slowly added. When TLC analysis performed the following day indicated completion, the reaction was quenched with MeOH (5 mL) and poured over ice. The mixture was diluted with EtOAc. The organic layer was washed with 1N aqueous HCl, saturated aqueous NaHCO₃, water and brine, dried over Na₂SO₄, filtered, and concentrated. The obtained crude product was dissolved in 30 mL of a mixed solution of MeOH:H₂O = 1:1, and *p*-toluenesulfonic acid monohydrate (0.59 g, 3.1 mmol) was added. The reaction mixture was stirred at room temperature overnight. When complete, the reaction was quenched by addition of Et₃N (1.3 mL) and concentrated. The residue obtained was redissolved in EtOAc, washed with water and brine, dried over

Na₂SO₄, and concentrated. Purification with gradient column chromatography (hexane:EtOAc = 100:0 to 50:50) provided **17** (1.29 g, 2.9 mmol, 46%).

Phenyl 2,3-di-O-benzyl-6-deoxy-1-thio-β-D-glucopyranoside (18).¹⁰³ To a solution of **17** (5.13 g, 11.3 mmol) in anhydrous pyridine was slowly added TsCl (2.59 g, 13.6 mmol) at 0 °C. The reaction mixture was stirred overnight allowing the reaction to warm to room temperature. After completion of the reaction, the reaction mixture was extracted with EtOAc. The combined organic layer was washed with aqueous 1 N HCl (3 times), saturated aqueous NaHCO₃, and brine, and dried over Na₂SO₄. After removal of the solvent, the tosylated crude product was dissolved in anhydrous THF (100 mL) and LiAlH₄ (0.99 g, 26.0 mmol) was added. The reaction was stirred at room temperature overnight and then refluxed for 2 h. When complete, the reaction mixture was quenched by slow addition to ice then filtered through celite. The filtered residue was eluted with more EtOAc. The combined organic layers were washed with 1 N HCl, water, saturated aqueous NaHCO₃ and brine, and dried over Na₂SO₄. Removal of the solvent followed by purification with gradient column chromatography (hexane: EtOAc = 90:10 to 40:60) afforded **18** (2.17 g, 4.97 mmol, 44%).

Phenyl 2,3-di-O-benzyl-6-deoxy-4-O-n-butyl-1-thio-β-D-glucopyranoside (11a). Please refer to the general procedure for *O*-alkylation of sugars. Compound **11a** was obtained with 72% yield. ¹H NMR (CDCl₃, 400 MHz) δ 7.2 - 7.8 (m, 15H), 5.01 (d, *J* = 10.3 Hz, 1H), 4.9 - 5.0 (m, 2H), 4.85 (d, *J* = 10.3 Hz, 1H), 4.77 (d, *J* = 9.8 Hz, 1H, H-1), 3.9 (m, 1H, H-4), 3.7 (m, 2H), 3.57 (dd, *J* = 9.0, 9.5 Hz, 1H, H-2), 3.4 - 3.5 (m, 1H, H-5), 3.13 (t, *J* = 9.2 Hz, 1H, H-3), 1.6 - 1.7 (m, 2H), 1.48 (d, *J* = 6.1 Hz, 3H, H-6), 1.4 -

1.6 (m, 2H), 1.01 (t, $J = 7.4$ Hz, 3H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 138.9, 138.5, 134.3, 132.2 (2 carbons), 129.2 (2 carbons), 128.7 (4 carbons), 128.5 (2 carbons), 128.1 (3 carbons), 128.0, 127.7, 87.7, 86.2, 84.1, 81.5, 76.1 (2 carbons), 75.8, 73.6, 32.9, 19.7, 18.5, 14.3; ESI/APCI calcd for $\text{C}_{30}\text{H}_{36}\text{O}_4\text{SNa}$ ($[\text{M}+\text{Na}]^+$) m/z 515.2232; measured m/z 515.2231.

Phenyl 2,3-di-*O*-benzyl-6-deoxy-4-*O*-*n*-octyl-1-thio- β -D-glucopyranoside (11b).¹⁰³ Please refer to the general procedure for *O*-alkylation of sugars. Compound **11b** was obtained with 63% yield.

Phenyl 2,3-di-*O*-benzyl-6-deoxy-4-*O*-*n*-dodecyl-1-thio- β -D-glucopyranoside (11c). Please refer to the general procedure for *O*-alkylation of sugars. Compound **11c** was obtained with 64% yield. ^1H NMR (CDCl_3 , 400 MHz) δ 7.3 - 7.7 (m, 15H), 5.0 (m, 3H), 4.83 (d, $J = 10.3$ Hz, 1H), 4.74 (d, $J = 9.7$ Hz, 1H, H-1), 3.9 (m, 1H, H-4), 3.7 (m, 2H), 3.4 - 3.6 (m, 2H), 3.12 (t, $J = 9.2$ Hz, 1H, H-3), 1.6 - 1.7 (m, 2H), 1.46 (d, $J = 6.1$ Hz, 3H, H-6), 1.4 (m, 18H), 0.99 (t, $J = 6.6$ Hz, 3H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 138.9, 138.5, 134.3, 132.1 (2 carbons), 129.2 (2 carbons), 128.7 (4 carbons), 128.5 (2 carbons), 128.1 (3 carbons), 127.9, 127.7, 87.7, 86.8, 84.0, 81.4, 76.1 (2 carbons), 75.7, 73.9, 32.2, 30.8, 30.0 (2 carbons), 29.9 (2 carbons), 29.8, 29.7, 26.5, 23.0, 18.4, 14.5; ESI/APCI calcd for $\text{C}_{30}\text{H}_{36}\text{O}_4\text{SNa}$ ($[\text{M}+\text{Na}]^+$) m/z 627.3484; measured m/z 627.3478.

6-*O*-(2,3-Di-*O*-benzyl-6-deoxy-4-*O*-*n*-butyl-D-glucopyranosyl)-1,3,2',6'-tetraazidoneamine (19a). Please refer to the general procedure for glycosylation using thiophenyl donor, and hydrolysis. Compound **19a** was obtained with 71% yield. ^1H NMR (CDCl_3 , 300 MHz) δ 7.2 - 7.4 (m, 10H), 5.69 (d, $J = 3.8$ Hz, 1H, H-1'), 4.92 (d, $J = 3.4$

Hz, 1H, H-1''), 4.6 - 4.9 (m, 4H), 4.2 (m, 1H), 3.8 - 4.0 (m, 4H), 2.9 - 3.6 (m, 12H), 2.3 (m, 1H), 1.3 - 1.6 (m, 5H), 1.24 (d, $J = 6.2$ Hz, 3H, H-6''), 0.88 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 138.8, 138.2, 128.7, 128.5 (2 carbons), 128.4 (2 carbons), 128.2 (2 carbons), 128.0 (2 carbons), 127.7, 98.5, 98.2, 86.2, 83.6, 81.0, 79.8, 79.7, 75.9, 75.5, 73.7, 73.5, 71.6, 71.3, 71.1, 68.8, 63.2, 59.4, 59.1, 51.3, 32.6, 32.4, 19.4, 17.8, 14.0; ESI/APCI calcd for $\text{C}_{36}\text{H}_{48}\text{N}_{12}\text{O}_{10}\text{Na}^+$ ($[\text{M}+\text{Na}]^+$) m/z 831.3509; measured m/z 831.3500.

6-O-(2,3-Di-O-benzyl-6-deoxy-4-O-n-octyl- α -D-glucopyranosyl)-1,3,2',6'-tetraazidoneamine (19b).¹⁰³ Please refer to the general procedure for glycosylation using thiophenyl donor, and hydrolysis. Compound **19b** was obtained with 83% yield.

6-O-(2,3-Di-O-benzyl-6-deoxy-4-O-n-dodecyl- α -D-glucopyranosyl)-1,3,2',6'-tetraazidoneamine (19c). Please refer to the general procedure for glycosylation and hydrolysis. Compound **19c** was obtained with 74% yield. ^1H NMR (CDCl_3 , 300 MHz) δ 7.2 - 7.4 (m, 10H), 5.67 (d, $J = 3.8$ Hz, 1H, H-1'), 4.94 (d, $J = 3.8$ Hz, 1H, H-1''), 4.6 - 4.9 (m, 4H), 4.2 (m, 1H), 3.8 - 4.0 (m, 4H), 2.9 - 3.6 (m, 12H), 2.3 (m, 1H), 1.5 - 1.6 (m, 2H), 1.33 (d, $J = 6.2$ Hz, 3H, H-6''), 1.2 - 1.3 (m, 19H), 0.88 (t, $J = 6.9$ Hz, 3H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 138.8, 138.2, 128.6 (2 carbons), 128.5 (2 carbons), 128.1 (2 carbons), 128.0 (2 carbons), 127.8, 127.7, 98.4, 98.2, 85.9, 83.7, 81.0, 79.8, 79.7, 75.9, 75.7, 74.0, 73.5, 71.6, 71.3, 71.2, 68.7, 63.2, 59.4, 59.1, 51.3, 32.4, 32.0, 30.5, 29.8 (2 carbons), 29.7 (2 carbons), 29.6, 29.5, 26.2, 22.8, 17.8, 14.2; ESI/APCI calcd for $\text{C}_{44}\text{H}_{64}\text{N}_{12}\text{O}_{10}\text{Na}^+$ ($[\text{M}+\text{Na}]^+$) m/z 943.4761; measured mze 943.4751.

6-O-(6-Deoxy-4-O-n-butyl- α -D-glucopyranosyl)neamine (FG01). Please refer to the general procedure for the final synthesis of kanamycin B analogs. **FG01** was

obtained with 45% yield as a chloride salt. ^1H NMR (D_2O , 300 MHz) δ 5.84 (d, $J = 4.1$ Hz, 1H, H-1'), 4.84 (d, $J = 3.1$ Hz, 1H, H-1''), 3.2 - 4.0 (m, 15H), 3.15 (dd, $J = 6.9, 13.4$ Hz, 1H), 2.89 (t, $J = 9.3$ Hz, 1H), 2.4 (m, 1H), 1.7 - 1.8 (m, 1H), 1.4 (m, 2H), 1.2 (m, 2H), 1.15 (d, $J = 6.5$ Hz, 3H, H-6''), 0.73 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (D_2O , 75 MHz) δ 101.6, 95.8, 83.6, 83.0, 77.4, 74.1, 73.2, 72.5, 71.9, 70.6, 69.2, 68.21, 68.17, 53.4, 49.9, 48.3, 40.1, 31.4, 28.0, 18.6, 16.9, 13.1; ESI/APCI calcd for $\text{C}_{22}\text{H}_{45}\text{N}_4\text{O}_{10}^+$ ($[\text{M}+\text{H}]^+$) m/z 525.3130; measured m/z 525.3140.

6-O-(6-Deoxy-4-O-n-octyl- α -D-glucopyranosyl)neamine (FG08)¹⁰³ Please refer to the general procedure for the final synthesis of kanamycin B analogs. **FG08** was obtained with 20% yield as a chloride salt.

6-O-(6-Deoxy-4-O-n-dodecyl- α -D-glucopyranosyl)neamine (FG02). Please refer to the general procedure for the final synthesis of kanamycin B analogs. **FG02** was obtained with 34% yield as a chloride salt. ^1H NMR (CDCl_3 , 300 MHz) δ 5.84 (s, 1H, H-1'), 4.86 (s, 1H, H-1''), 3.2 - 4.0 (m, 17H), 2.3 (m, 1H), 1.7 (m, 2H), 1.46 (d, $J = 7.5$ Hz, 3H, H-6'), 1.1 (m, 19H), 0.7 (m, 3H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 101.6, 95.9, 83.8, 83.6, 77.9, 74.1, 73.9, 72.7, 72.1, 70.8, 69.2, 68.5, 68.1, 53.6, 50.0, 48.4, 40.2, 31.7, 29.6, 29.3 (4 carbons), 29.1 (2 carbons), 28.6, 25.5, 22.4, 17.1, 13.8; ESI/APCI calcd for $\text{C}_{30}\text{H}_{61}\text{N}_4\text{O}_{10}^+$ ($[\text{M}+\text{H}]^+$) m/z 637.4382; measured m/z 637.4395.

Phenyl 2,3-di-O-benzyl-4-O-n-octyl-1-thio- β -D-glucopyranoside (20). To a solution of **17** (1.80 g, 3.98 mmol) in anhydrous CH_2Cl_2 were added TrCl (1.77 g, 6.36 mmol), Et_3N (1.12 mL, 7.95 mmol) and a catalytic amount of DMAP. The reaction mixture was stirred overnight at room temperature. When complete, the reaction was

quenched by addition of MeOH (5 mL). Then the mixture was washed with water, saturated aqueous NaHCO₃, and brine, dried over Na₂SO₄ and concentrated. The tritylated crude product was then dissolved in anhydrous DMF, and octyl bromide (1.7 mL, 9.79 mmol), NaH (0.39 g, 9.79 mmol) and a catalytic amount of TBAI were added. The reaction was stirred overnight. When complete, the reaction was quenched by addition of MeOH (5 mL) and was slowly poured into a mixture of ice and EtOAc. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with water, saturated aqueous NaHCO₃ and brine, and then dried over Na₂SO₄. After removal of the solvent, the obtained crude product was dissolved in 50 mL of a mixed solution of CH₂Cl₂:MeOH = 1:1 and *p*-toluenesulfonic acid monohydrate (0.61 g, 3.20 mmol) was added. The resulting mixture was stirred at room temperature overnight. When complete, the reaction mixture was quenched with Et₃N (1.35 mL) and extracted with EtOAc. The organic layer was washed with 1N aqueous HCl, water, saturated aqueous NaHCO₃, brine, and dried over Na₂SO₄. After removal of the solvent and purification with a gradient column chromatography (hexane:EtOAc = 100:0 to 40:60), **20** was obtained as a white solid (1.84 g, 3.26 mmol, 84%). ¹H NMR (CDCl₃, 300 MHz) δ 7.5 (m, 2H), 7.2 – 7.4 (m, 13H), 4.88 (d, *J* = 10.3 Hz, 1H), 4.85 (s, 1H), 4.84 (s, 1H), 4.74 (d, *J* = 10.3 Hz, 1H), 4.70 (d, *J* = 10.0 Hz, 1H), 3.9 (m, 1H), 3.5 – 3.8 (m, 4H), 3.43 (t, *J* = 9.6 Hz, 1H), 3.3 (m, 2H), 1.94 (t, *J* = 6.9 Hz, 1H, OH), 1.5 (m, 2H), 1.2 (m, 10H), 0.87 (t, *J* = 6.9 Hz, 3H); ¹³C NMR (CDCl₃, 75 MHz) δ 138.5, 138.0, 133.5, 131.9 (2 carbons), 129.1 (2 carbons), 128.5 (4 carbons), 128.3 (2 carbons), 128.0, 127.9, 127.82 (2 carbons), 127.75,

87.5, 86.5, 81.0, 79.5, 78.2, 75.9, 75.6, 73.6, 62.3, 31.9, 30.5, 29.6, 29.3, 26.2, 22.7, 14.2; ESI/APCI calcd for C₃₄H₄₄O₅SNa ([M+Na]⁺) *m/z* 587.2802; measured *m/z* 587.2803.

Phenyl 2,3,6-tri-*O*-benzyl-4-*O*-*n*-octyl-1-thio-β-D-glucopyranoside (21). To a solution of **20** (1.15 g, 2.04 mmol) in DMF (40 mL) were added BnBr (0.49 mL, 4.07 mmol) and a catalytic amount of TBAI. The mixture was then transferred in an ice-water bath and NaH (0.16 g, 4.07 mmol) was slowly added. When TLC analysis performed the following day indicated completion, the reaction was quenched with MeOH (2 mL) and poured over ice. The mixture was extracted with EtOAc. The organic layer was washed with 1N aqueous HCl, saturated aqueous NaHCO₃, water and brine, and then dried over Na₂SO₄. After removal of the solvent and purification with a gradient column chromatography (hexane:EtOAc = 100:0 to 50:50), **21** was obtained (1.26 g, 1.92 mmol, 95%). ¹H NMR (CDCl₃, 300 MHz) δ 7.6 (m, 2H), 7.2 – 7.4 (m, 18H), 4.9 (m, 3H), 4.6 – 4.8 (m, 4H), 3.7 – 3.9 (m, 3H), 3.4 – 3.7 (m, 5H), 1.5 (m, 2H), 1.3 (m, 10H), 0.91 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (CDCl₃, 75 MHz) δ 138.6, 138.5, 138.2, 134.0, 132.0 (2 carbons), 129.2 (2 carbons), 128.5 (4 carbons), 128.4 (2 carbons), 128.3 (2 carbons), 127.9 (2 carbons), 127.8, 127.7 (3 carbons), 127.6, 127.5, 87.5, 86.8, 80.8, 79.4, 78.2, 75.9, 75.6, 73.5, 73.4, 69.2, 32.0, 30.5, 29.6, 29.4, 26.3, 22.8, 14.3; ESI/APCI calcd for C₄₁H₅₀O₅SNa ([M+Na]⁺) *m/z* 677.3271; measured *m/z* 677.3280.

6-*O*-(2,3,6-Tri-*O*-benzyl-4-*O*-*n*-octyl-α-D-glucopyranosyl)-1,3,2',6'-tetraazidoneamine (22). Please refer to the general procedure for glycosylation and hydrolysis. Compound **22** was obtained with 47% yield. ¹H NMR (CDCl₃, 300 MHz) δ 7.2 – 7.4 (m, 15H), 5.63 (d, *J* = 3.4 Hz, 1H, H-1'), 5.02 (d, *J* = 3.8 Hz, 1H, H-1''), 4.92

(d, $J = 11.0$ Hz, 1H), 4.75 (d, $J = 12.4$ Hz, 1H), 4.72 (m, 2H), 4.64 (d, $J = 12.0$ Hz, 1H), 4.51 (d, $J = 12.4$ Hz, 1H), 4.1 – 4.2 (m, 1H), 4.0 – 4.1 (m, 1H), 3.96 (d, $J = 10.3$ Hz, 1H), 3.89 (d, $J = 10.0$ Hz, 1H), 3.2 – 3.8 (m, 18H), 2.31 (ddd, $J = 13.1, 4.5, 4.1$ Hz, 1H, H-2eq), 1.51 (ddd, $J = 13.0, 12.4, 12.4$ Hz, 1H, H-2ax), 1.4 – 1.5 (m, 2H), 1.2 (m, 10H), 0.88 (t, $J = 6.9$ Hz, 3H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 138.8, 138.1, 137.8, 128.55 (2 carbons), 128.49 (2 carbons), 128.4 (2 carbons), 128.13 (2 carbons), 128.06 (2 carbons), 128.0 (3 carbons), 127.9, 127.7, 98.6, 98.2, 86.3, 86.1, 81.4, 79.6, 78.0, 75.9, 75.7, 73.7, 73.5 (2 carbons), 71.6 (2 carbons), 71.4, 71.1, 68.5, 62.9, 59.6, 59.2, 51.3, 32.4, 31.9, 30.4, 29.6, 29.3, 26.2, 22.8, 14.2; ESI/APCI calcd for $\text{C}_{47}\text{H}_{62}\text{N}_{12}\text{O}_{11}\text{Na}$ ($[\text{M}+\text{Na}]^+$) m/z 993.4553; measured m/z 993.4563.

6-*O*-(4-*O*-*n*-Octyl-*D*-glucopyranosyl)neamine (FG03). Please refer to the general procedure for the final synthesis of kanamycin B analogs. **FG03** was obtained with 42% yield as a chloride salt. ^1H NMR (D_2O , 300 MHz) (chloride salt) δ 5.81 (d, $J = 3.8$ Hz, 1H, H-1'), 4.93 (d, $J = 3.8$ Hz, 1H, H-1''), 3.3 - 4.0 (m, 17H), 3.1 – 3.2 (m, 2H), 2.4 (m, 1H), 1.7 - 1.9 (m, 1H), 1.4 – 1.5 (m, 2H), 1.1 - 1.2 (m, 10H), 0.71 (t, $J = 6.9$ Hz, 3H); ^{13}C NMR (D_2O , 100 MHz) (chloride salt) δ 101.7, 96.1, 83.8, 77.8, 77.6, 74.3, 73.7, 72.9, 72.3, 71.8, 70.9, 69.4, 68.4, 60.4, 53.7, 49.9, 48.5, 40.3, 31.3, 29.4, 28.7, 28.6, 28.2, 25.4, 22.2, 13.7; ESI/APCI calcd for $\text{C}_{26}\text{H}_{53}\text{N}_4\text{O}_{11}^+$ ($[\text{M}+\text{H}]^+$) m/z 597.3705; measured m/z 597.3708.

2,3-Di-*O*-benzyl-4-*O*-*n*-octyl-*D*-glucopyranose (25). Compound **20** (0.90 g, 1.59 mmol) was dissolved in a mixture of acetone (35 mL) and CH_2Cl_2 (10 mL). Distilled water (3.44 mL, 191.2 mmol) was added and the mixture was cooled down to 0 °C. *N*-

bromosuccinimide (0.68 g, 3.82 mmol) was added and the reaction mixture was stirred overnight till room temperature. When complete, the solvent was evaporated and the residue was redissolved in EtOAc. The organic layer was washed with water and brine, and dried over Na₂SO₄. After removal of the solvent, purification by gradient column chromatography (Hexane:EtOAc = 100:0 to 0:100) afforded **25** (0.68 g, 1.44 mmol, 90%) as a mixture of α/β anomers in a 1/1 ratio. ¹H NMR (α -anomer) (CDCl₃, 300 MHz) δ 7.2 – 7.4 (m, 10H), 5.16 (dd, J = 3.1, 3.1 Hz, 1H, H-1), 4.6 - 4.9 (m, 4H), 3.3 - 4.0 (m, 7H), 3.17 (d, J = 2.4 Hz, 1H), 2.28 (dd, J = 7.2, 5.8 Hz, 1H), 1.94 (dd, J = 7.9, 4.8 Hz, 1H), 1.5 (m, 2H), 1.2 (m, 10H), 0.87 (t, J = 6.9 Hz, 3H); ¹³C NMR (CDCl₃, 75 MHz) δ 138.7, 137.9, 128.6, 128.5 (3 carbons), 128.2, 128.1 (2 carbons), 128.0, 127.9, 127.7, 91.4, 83.1, 80.0, 78.0, 75.6, 75.0, 73.5, 71.2, 62.0, 31.9, 30.5, 29.6, 29.4, 26.2, 22.7, 14.2; ESI/APCI calcd for C₂₈H₄₀O₆Na ([M+Na]⁺) m/z 495.2717; measured m/z 495.2720.

4-*O*-*n*-Octyl-D-glucopyranose (23). Compound **25** (0.20 g, 0.42 mmol) was dissolved in a degassed mixture MeOH:H₂O (1:1) and a catalytic amount of Pd(OH)₂/C was added. The vial was then sealed and freed of air before H₂ balloon was loaded. The reaction was stirred overnight under H₂ atmosphere. The reaction was filtered through a short column packed with celite and eluted with water. Removal of the solvent afforded **23** as a mixture of α/β anomers in a 10/9 ratio. (0.12 g, 0.41 mmol, 96%). ¹H NMR (α -anomer) (CD₃OD, 300 MHz) δ 5.07 (d, J = 3.8 Hz, 1H, H-1), 3.0 – 4.0 (m, 8H), 1.5 – 1.6 (m, 2H), 1.3 (m, 10 H), 0.88 (t, J = 6.9 Hz, 3H); ¹³C NMR (CD₃OD, 100 MHz) δ 92.6, 78.3, 76.0, 73.8, 72.7, 71.0, 61.1, 31.2, 30.2, 29.5, 29.3, 26.0, 22.5, 13.3; ESI/APCI calcd for C₁₄H₂₈O₆Na ([M+Na]⁺) m/z 315.1778; measured m/z 315.1780.

Phenyl 2,3,4-tri-*O*-benzyl-1-thio- β -D-glucopyranoside (27).¹⁰⁸ **26** (2.00 g, 3.70 mmol) was dissolved in 40 mL of a mixed solution of anhydrous Et₂O:CH₂Cl₂ (1:1) and LiAlH₄ (0.66 g, 17.4 mmol) was slowly added. The mixture was then gently heated. Then a solution of AlCl₃ (1.97 g, 14.8 mmol) in anhydrous CH₂Cl₂ (20 mL) was added to the hot reaction mixture over a 1 h period. The combined solutions were then refluxed at 40 °C. Two hours later, the reaction was complete as confirmed by TLC analysis. The reaction was quenched by transferring it to flask containing ice and EtOAc. The organic layer was washed with 1 N HCl, water, saturated aqueous NaHCO₃, and brine, and dried over Na₂SO₄. Removal of the solvent gave a crude product that was recrystallized in diethyl ether and hexane to give **27** (1.34g, 2.47 mmol, 67%).

Phenyl 2,3,4-tri-*O*-benzyl-6-*O*-*n*-hexyl-1-thio- β -D-glucopyranoside (28a).

Please refer to the general procedure for *O*-alkylation of sugars. Compound **28a** was obtained with 99% yield. ¹H NMR (CDCl₃, 300 MHz) δ 7.6 – 7.7 (m, 2H), 7.2 – 7.5 (m, 18H), 4.9 – 5.0 (m, 4H), 4.77 (d, *J* = 10.0 Hz, 1H), 4.70 (d, *J* = 10.0 Hz, 2H), 3.6 – 3.8 (m, 4H), 3.4 – 3.6 (m, 4H), 1.6 (m, 2H), 1.2 – 1.5 (m, 6H), 0.93 (t, *J* = 7.1 Hz, 3H); ¹³C NMR (CDCl₃, 75 MHz) δ 138.6, 138.3, 138.2, 134.1, 132.0 (2 carbons), 129.0 (2 carbons), 128.60 (5 carbons), 128.57 (2 carbons), 128.4 (2 carbons), 128.03 (2 carbons), 127.97 (3 carbons), 127.9, 127.5, 87.6, 86.9, 81.0, 79.3, 78.0, 76.0, 75.6, 75.2, 71.9, 69.7, 31.9, 30.0, 26.0, 22.3, 14.3; ESI/APCI calcd for C₃₉H₄₆O₅S Na ([M+Na]⁺) *m/z* 649.2958, measured *m/z* 649.2971.

Phenyl 2,3,4-tri-*O*-benzyl-6-*O*-*n*-octyl-1-thio- β -D-glucopyranoside (28b).

Please refer to the general procedure for *O*-alkylation of sugars. Compound **28b** was

obtained with 97% yield. ^1H NMR (CDCl_3 , 300 MHz) δ 7.6 – 7.7 (m, 2H), 7.2 – 7.5 (m, 18H), 4.9 – 5.0 (m, 4H), 4.79 (d, $J = 10.3$ Hz, 1H), 4.71 (d, $J = 10.0$ Hz, 2H), 3.7 – 3.8 (m, 4H), 3.4 – 3.6 (m, 4H), 1.6 (m, 2H), 1.2 – 1.5 (m, 10H), 0.94 (t, $J = 6.9$ Hz, 3H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 138.6, 138.4, 138.2, 134.2, 132.0 (2 carbons), 129.0 (2 carbons), 128.63 (5 carbons), 128.64 (2 carbons), 128.4 (2 carbons), 128.1 (2 carbons), 128.0 (3 carbons), 127.9, 127.5, 87.7, 86.9, 81.0, 79.4, 78.0, 76.0, 75.6, 75.2, 71.9, 69.8, 32.1, 30.1, 29.7, 29.5, 26.4, 22.9, 14.3; ESI/APCI calcd for $\text{C}_{41}\text{H}_{50}\text{O}_5\text{SNa}$ ($[\text{M}+\text{Na}]^+$) m/z 677.3271, measured m/z 677.3280.

6-*O*-(2,3,4-Tri-*O*-benzyl-6-*O*-*n*-hexyl- α -D-glucopyranosyl)-1,3,2',6'-tetraazidoneamine (29a). Please refer to the general procedure for glycosylation and hydrolysis. Compound **29a** was obtained with 40% yield. ^1H NMR (CDCl_3 , 300 MHz) δ 7.2 - 7.4 (m, 15H), 5.69 (d, $J = 3.8$ Hz, 1H, H-1'), 5.05 (d, $J = 3.8$ Hz, 1H, H-1''), 4.97 (d, $J = 11.0$ Hz, 1H), 4.88 (d, $J = 10.7$ Hz, 1H), 4.82 (d, $J = 11.0$ Hz, 1H), 4.75 (s, 1H), 4.74 (s, 1H), 4.59 (d, $J = 10.7$ Hz, 1H), 4.48 (d, $J = 2.4$ Hz, 1H), 4.1 – 4.2 (m, 1H), 3.9 – 4.1 (m, 3H), 3.1 – 3.7 (m, 15H), 2.97 (d, $J = 3.4$ Hz, 1H), 2.92 (d, $J = 4.1$ Hz, 1H), 2.32 (ddd, $J = 13.1, 4.5, 4.1$ Hz, 1H, H-2eq), 1.6 (m, 2H), 1.50 (ddd, $J = 13.1, 12.7, 12.7$ Hz, 1H, H-2ax), 1.2 – 1.4 (m, 6H), 0.88 (t, $J = 6.9$ Hz, 3H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 138.8, 138.16, 138.07, 128.61 (2 carbons), 128.58 (2 carbons), 128.51 (2 carbons), 128.16 (2 carbons), 128.10 (4 carbons), 128.04 (2 carbons), 127.8, 98.6, 98.2, 85.9, 81.5, 79.7, 79.6, 75.8 (2 carbons), 75.4, 73.5, 71.9 (2 carbons), 71.7, 71.6, 71.4, 71.1, 69.1, 63.0, 59.6, 59.2, 51.3, 32.4, 31.7, 29.4, 25.8, 22.7, 14.2 ; ESI/APCI calcd for $\text{C}_{47}\text{H}_{62}\text{N}_{12}\text{O}_{11}\text{Na}$ ($[\text{M}+\text{Na}]^+$) m/z 965.4240; measured m/z 965.4255.

6-O-(2,3,4-Tri-O-benzyl-6-O-n-octyl- α -D-glucopyranosyl)-1,3,2',6'-

tetraazidoneamine (29b). Please refer to the general procedure for glycosylation and hydrolysis. Compound **29b** was obtained with 38% yield. ^1H NMR (CDCl_3 , 300 MHz) δ 7.2 - 7.4 (m, 15H), 5.69 (d, $J = 3.8$ Hz, 1H, H-1'), 5.05 (d, $J = 3.8$ Hz, 1H, H-1''), 4.97 (d, $J = 11.0$ Hz, 1H), 4.88 (d, $J = 10.7$ Hz, 1H), 4.82 (d, $J = 11.0$ Hz, 1H), 4.75 (s, 1H), 4.74 (s, 1H), 4.59 (d, $J = 10.7$ Hz, 1H), 4.48 (d, $J = 2.4$ Hz, 1H), 4.1 - 4.2 (m, 1H), 3.9 - 4.1 (m, 3H), 3.1 - 3.7 (m, 15H), 2.97 (d, $J = 3.4$ Hz, 1H), 2.92 (d, $J = 4.1$ Hz, 1H), 2.32 (ddd, $J = 13.1, 4.5, 4.1$ Hz, 1H, H-2eq), 1.6 (m, 2H), 1.50 (ddd, $J = 13.1, 12.7, 12.7$ Hz, 1H, H-2ax), 1.2 - 1.4 (m, 6H), 0.88 (t, $J = 6.9$ Hz, 3H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 138.9, 138.3, 138.2, 128.7 (4 carbons), 128.6 (2 carbons), 128.23 (6 carbons), 128.22 (2 carbons), 127.9, 98.7, 98.3, 85.8, 81.6, 79.8 (2 carbons), 77.7, 75.9 (2 carbons), 75.5, 73.6, 72.0, 71.8, 71.7, 71.6, 71.3, 69.2, 63.2, 59.7, 59.3, 51.4, 32.5, 32.1, 29.7, 29.6, 29.5, 26.3, 22.9, 14.3 ; ESI/APCI calcd for $\text{C}_{47}\text{H}_{62}\text{N}_{12}\text{O}_{11}\text{Na}$ ($[\text{M}+\text{Na}]^+$) m/z 993.4611; measured m/z 993.4578.

6-O-(6-O-n-Hexyl-D-glucopyranosyl)neamine (FG05). Please refer to the

general procedure for the final synthesis of kanamycin B analogs. **FG05** was obtained with 86% yield as a chloride salt. ^1H NMR (D_2O , 300 MHz) (chloride salt) δ 5.85 (d, $J = 4.1$ Hz, 1H, H-1'), 4.89 (d, $J = 3.5$ Hz, 1H, H-1''), 3.2 - 4.0 (m, 18H), 3.1 (m, 1H), 2.4 (m, 1H), 1.8 (m, 1H), 1.3 - 1.5 (m, 2H), 1.0 - 1.2 (m, 6H), 0.69 (t, $J = 6.9$ Hz, 3H); ^{13}C NMR (D_2O , 100 MHz) (chloride salt) δ 101.9, 95.5, 83.8, 77.0, 74.3, 72.9, 72.11, 72.07, 71.7, 70.8, 69.4, 69.3, 68.8, 68.4, 53.6, 49.9, 48.4, 40.4, 31.1, 28.6, 28.1, 25.0, 22.1, 13.6; ESI/APCI calcd for $\text{C}_{24}\text{H}_{49}\text{N}_4\text{O}_{11}$ ($[\text{M}+\text{H}]^+$) m/z 569.3392; measured m/z 569.3408.

6-*O*-(6-*O*-*n*-Octyl-*D*-glucopyranosyl)neamine (FG06). Please refer to the general procedure for the final synthesis of kanamycin B analogs. **FG06** was obtained with 82% yield as a chloride salt. ¹H NMR (D₂O, 300 MHz) (chloride salt) δ 5.92 (d, *J* = 4.1 Hz, 1H, H-1'), 4.95 (d, *J* = 3.8 Hz, 1H, H-1'), 3.3 - 4.0 (m, 18H), 3.2 (m, 1H), 2.4 - 2.5 (m, 1H), 1.9 (m, 1H), 1.4 - 1.5 (m, 2H), 1.1 - 1.2 (m, 10H), 0.74 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (D₂O, 100 MHz) (chloride salt) δ 102.0, 95.6, 83.8, 76.9, 74.3, 72.9, 72.2, 72.1, 71.8, 70.9, 69.5, 69.3, 68.8, 68.4, 53.7, 49.9, 48.6, 40.4, 31.3, 28.8, 28.7, 28.6, 28.0, 25.4, 22.2, 13.7; ESI/APCI calcd for C₂₆H₅₃N₄O₁₁ ([M+H]⁺) *m/z* 597.3705; measured *m/z* 597.3708.

1,2:5,6-Di-*O*-isopropylidene-3-*O*-*n*-octyl- α -*D*-glucopyranose (30).¹⁰⁹ Please refer to the general procedure for *O*-alkylation of sugars. Compound **30** was obtained with 99% yield.

1,2,4,6-Tetra-*O*-acetyl-3-*O*-*n*-octyl-*D*-glucopyranose (31). Please refer to the synthesis of **6**. Compound **31** was obtained with 87% yield as a mixture of α/β anomers in the ratio 1/2. ¹H NMR (CDCl₃, 300 MHz) (α and β anomers) δ 6.27 (d, *J* = 3.8 Hz, 1H, H-1 α), 5.62 (d, *J* = 8.3 Hz, 1H, H-1 β), 4.9 - 5.5.1 (m, 4H), 3.9 - 4.2 (m, 4H), 3.4 - 3.8 (m, 8H), 2.0 - 2.1 (m, 24H), 1.1 - 1.3 (m, 24H), 0.86 (t, *J* = 6.9 Hz, 6H).

Phenyl 2,4,6-tri-*O*-acetyl-3-*O*-*n*-octyl-1-thio- β -*D*-glucopyranoside (32). A solution of **31** (4.04 g, 8.77 mmol) and thiophenol (3.4 mL, 33.3 mmol) in anhydrous CH₂Cl₂ (50 mL) was cooled down to 0 °C and BF₃-OEt₂ was slowly added. The reaction was stirred for 2 days till completion. Solid NaHCO₃, Na₂SO₄ and some few drops of water were then added and the mixture was stirred for 1 h. The solution was then filtered

through a Fritz funnel and the collected solids were washed with EtOAc. After removal of the solvents, purification by gradient column chromatography afforded **32** (2.91 g, 5.70 mmol, 65%). ^1H NMR (CDCl_3 , 300 MHz) δ 7.4 (m, 2H), 7.1 – 7.2 (m, 3H), 4.89 (dd, $J = 9.6, 9.6$ Hz, 1H, H-2), 4.88 (dd, $J = 9.6, 9.6$ Hz, 1H, H-4), 4.56 (d, $J = 10.3$ Hz, 1H, H-1), 3.9 – 4.1 (m, 3H), 3.5 – 3.6 (m, 1H), 3.4 – 3.5 (m, 2H), 2.03 (s, 3H), 2.00 (s, 3H), 1.95 (s, 3H), 1.3 – 1.4 (m, 2H), 1.1 – 1.2 (m, 10H), 0.77 (t, $J = 6.9$ Hz, 3H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 170.6, 169.4, 169.2, 132.9, 132.5 (2 carbons), 129.0 (2 carbons), 128.1, 86.2, 81.9, 76.1, 72.9, 71.5, 69.8, 62.7, 31.9, 30.4, 29.5, 29.4, 26.1, 22.7, 21.1, 20.9, 20.8, 14.2 ; ESI/APCI calcd for $\text{C}_{26}\text{H}_{38}\text{O}_8\text{SNa}$ ($[\text{M}+\text{Na}]^+$) m/z 533.2180; measured m/z 533.2187.

Phenyl 2,3,6-tri-*O*-benzyl-4-*O*-*n*-octyl-1-thio- β -D-glucopyranoside (33**).** To a solution of **32** (2.91 g, 5.70 mmol) in anhydrous MeOH (40 mL), 0.5 mL of a 1M solution of NaOMe in MeOH was added and the mixture was stirred at room temperature for 1 h. When complete, the reaction was quenched by adding amberlite IR 120 H^+ resin to the mixture, followed by filtration through celite and concentration of the filtrate. The obtained crude product was dissolved in DMF (40 mL), and BnBr (10.0 mL, 84.0 mmol) and a catalytic amount of TBAI were added. The mixture was then transferred in an ice-water bath and NaH (3.36 g, 84.0 mmol) was slowly added. When TLC analysis performed the following day indicated completion, the reaction was quenched with MeOH (5 mL) and poured over ice. The mixture was diluted with EtOAc, extracted with 1N aqueous HCl, saturated aqueous NaHCO_3 , water and brine, and dried over Na_2SO_4 . After removal of the solvents, purification by gradient column chromatography

(Hexane:EtOAc = 100:0 to 50:50) gave **33** (2.00 g, 3.05 mmol, 44%). ^1H NMR (CDCl_3 , 300 MHz) δ 7.2 – 7.8 (m, 20H), 5.0 (m, 2H), 4.86 (d, $J = 10.3$ Hz, 1H), 4.6 – 4.8 (m, 4H), 3.8 – 4.0 (m, 4H), 3.5 – 3.7 (m, 4H), 1.7 – 1.8 (m, 2H), 1.3 – 1.5 (m, 10H), 1.01 (t, $J = 6.9$ Hz, 3H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 138.7, 138.65, 138.55, 134.3, 132.3 (2 carbons), 129.2 (2 carbons), 128.75 (4 carbons), 128.67 (2 carbons), 128.5 (2 carbons), 128.3 (2 carbons), 128.1 (2 carbons), 128.0 (2 carbons), 127.9, 127.7, 87.7, 87.1, 81.2, 79.4, 78.1, 75.7, 75.3, 74.4, 73.7, 69.4, 32.2, 31.0, 29.9, 29.6, 26.7, 23.0, 14.5 ; ESI/APCI calcd for $\text{C}_{41}\text{H}_{50}\text{O}_5\text{SNa}$ ($[\text{M}+\text{Na}]^+$) m/z 677.3271; measured m/z 677.3270.

6-O-(2,4,6-Tri-O-benzyl-3-O-n-octyl- α -D-glucopyranosyl)-1,3,2',6'-tetraazidoneamine (34). Please refer to the general procedure for glycosylation and hydrolysis. Compound **34** was obtained with 59% yield. ^1H NMR (CDCl_3 , 300 MHz) δ 7.1 – 7.5 (m, 15H), 5.60 (d, $J = 3.8$ Hz, 1H, H-1'), 5.02 (d, $J = 3.8$ Hz, 1H, H-1''), 4.83 (d, $J = 10.7$ Hz, 1H), 4.76 (d, $J = 12.0$ Hz, 1H), 4.70 (d, $J = 11.7$ Hz, 1H), 4.61 (d, $J = 12.4$ Hz, 1H), 4.54 (s, 1H), 4.53 (s, 1H), 4.49 (d, $J = 12.4$ Hz, 1H), 4.45 (d, $J = 10.7$ Hz, 1H), 4.0 – 4.1 (m, 2H), 3.0 – 4.0 (m, 18H), 2.30 (ddd, $J = 13.1, 4.5, 4.1$ Hz, 1H, H-2eq), 1.6 – 1.7 (m, 2H), 1.49 (ddd, $J = 12.7, 12.7, 12.7$ Hz, 1H, H-2ax), 1.2 – 1.4 (m, 10H), 0.88 (t, $J = 6.9$ Hz, 3H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 138.4, 138.1, 137.9, 128.7 (6 carbons), 128.32 (2 carbons), 128.25 (2 carbons), 128.1 (4 carbons), 128.0, 98.7, 98.4, 85.8, 81.5, 79.9, 79.6, 77.8, 75.9, 75.4, 74.0, 73.6 (2 carbons), 71.7, 71.5 (2 carbons), 71.3, 68.5, 63.0, 59.7, 59.3, 51.4, 32.5, 32.1, 30.9, 29.8, 29.5, 26.5, 22.9, 14.3 ; ESI/APCI calcd for $\text{C}_{47}\text{H}_{62}\text{N}_{12}\text{O}_{11}\text{Na}$ ($[\text{M}+\text{Na}]^+$) m/z 993.4553; measured m/z 993.4564.

6-*O*-(3-*O*-*n*-Octyl-*D*-glucopyranosyl)neamine (FG07). Please refer to the general procedure for the final synthesis of kanamycin B analogs. **FG07** was obtained with 42% yield as a chloride salt. ¹H NMR (D₂O, 300 MHz) (chloride salt) δ 5.81 (d, *J* = 4.1 Hz, 1H, H-1'), 4.93 (d, *J* = 4.0 Hz, 1H, H-1'), 3.3 - 4.0 (m, 18H), 3.16 (dd, *J* = 13.7, 6.9 Hz, 1H), 2.42 (ddd, *J* = 12.4, 4.1, 4.1 Hz, 1H, H-2eq), 1.87 (ddd, *J* = 12.7, 12.4, 12.4 Hz, 1H, H-2ax), 1.4 - 1.5 (m, 2H), 1.0 - 1.3 (m, 10H), 0.71 (t, *J* = 6.9 Hz, 3H); ¹³C NMR (D₂O, 100 MHz) (chloride salt) δ 101.8, 96.2, 83.8, 81.3, 77.7, 74.2, 73.4, 73.2, 71.3, 70.8, 69.4, 68.9, 68.4, 60.5, 53.6, 49.9, 48.4, 40.3, 31.3, 29.5, 28.7, 28.6, 28.1, 25.3, 22.2, 13.6; ESI/APCI calcd for C₂₆H₅₃N₄O₁₁ ([M+H]⁺) *m/z* 597.3705; measured *m/z* 597.3720.

Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-*n*-octyl- α -*D*-glucopyranoside (36).

Please refer to the general procedure for *O*-alkylation of sugars. Compound **36** was obtained with 90% yield. ¹H NMR (CDCl₃, 300 MHz) δ 7.5 (m, 2H), 7.2 - 7.4 (m, 8H), 5.58 (s, 1H), 4.89 (d, *J* = 11.3 Hz, 1H), 4.84 (d, *J* = 3.8 Hz, 1H, H-1), 4.82 (d, *J* = 11.3 Hz, 1H), 4.30 (dd, *J* = 9.6, 4.1 Hz, 1H, H-2), 3.98 (dd, *J* = 9.3, 8.9 Hz, 1H, H-4), 3.6 - 3.9 (m, 6H), 3.46 (s, 3H), 1.6 - 1.7 (m, 2H), 1.2 - 1.4 (m, 10H), 0.90 (t, *J* = 6.9 Hz, 3H); ¹³C NMR (CDCl₃, 100 MHz) δ 139.0, 137.7, 129.1, 128.5 (2 carbons), 128.4 (2 carbons), 128.2 (2 carbons), 127.7, 126.3 (2 carbons), 101.5, 99.2, 82.2, 80.7, 78.6, 75.5, 72.4, 69.3, 62.6, 55.2, 32.1, 30.3, 29.7, 29.5, 26.2, 22.9, 14.4 ; ESI/APCI calcd for C₂₉H₄₀O₆Na ([M+Na]⁺) *m/z* 507.2717; measured *m/z* 507.2723.

1,3,4,6-Tetra-*O*-acetyl-2-*O*-*n*-octyl-*D*-glucopyranose (37). Please refer to the synthesis of **6**. Compound **37** was obtained with 76% yield as a mixture of α/β anomers in a 6/1 ratio. ¹H NMR (α -anomer) (CDCl₃, 300 MHz) δ 6.25 (d, *J* = 3.8 Hz, 1H, H-1), 5.21

(dd, $J = 10.0, 9.6$ Hz, 1H, H-4), 4.94 (dd, $J = 10.3, 9.6$ Hz, 1H, H-3), 4.17 (dd, $J = 13.0, 4.1$ Hz, 1H), 3.9 – 4.0 (m, 2H), 3.4 – 3.6 (m, 2H), 3.3 (m, 1H), 2.05 (s, 3H), 1.95 (s, 3H), 1.92 (s, 3H), 1.91 (s, 3H), 1.3 – 1.4 (m, 2H), 1.1 (m, 10H), 0.75 (t, $J = 6.9$ Hz, 3H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 170.5, 170.1, 169.7, 169.0, 89.3, 76.7, 71.6 (2 carbons), 69.8, 68.1, 61.7, 31.9, 29.8, 29.3 (2 carbons), 25.9, 22.7, 21.0, 20.8, 20.72, 20.66, 14.1 ; ESI/APCI calcd for $\text{C}_{22}\text{H}_{36}\text{O}_{10}\text{Na}$ ($[\text{M}+\text{Na}]^+$) m/z 483.2201; measured m/z 483.2192.

Phenyl 3,4,6-tri-*O*-acetyl-2-*O*-*n*-octyl-1-thio-*D*-glucopyranoside (38). Please refer to the synthesis of **32**. Compound **38** was obtained with 56% yield as a mixture of α/β anomers in a 3/1 ratio. ^1H NMR (α -anomer) (CDCl_3 , 300 MHz) δ 7.5 (m, 2H), 7.3 (m, 3H), 5.77 (d, $J = 5.5$ Hz, 1H, H-1), 5.30 (dd, $J = 9.6, 9.6$ Hz, 1H, H-4), 5.01 (dd, $J = 10.3, 9.3$ Hz, 1H, H-3), 4.54 (ddd, $J = 10.3, 5.2, 2.1$ Hz, 1H, H-5), 4.29 (dd, $J = 12.0, 5.2$ Hz, 1H, H-6), 3.99 (dd, $J = 12.4, 2.1$ Hz, 1H, H-6'), 3.6 – 3.8 (m, 2H), 3.3 – 3.5 (m, 1H), 2.04 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 1.5 (m, 2H), 1.2 – 1.4 (m, 10H), 0.87 (t, $J = 6.9$ Hz, 3H).

Phenyl 3,4,6-tri-*O*-benzyl-2-*O*-*n*-octyl-1-thio- α -*D*-glucopyranoside (39). Please refer to the synthesis of **33**. Compound **39** was obtained with 95% yield. ^1H NMR (CDCl_3 , 300 MHz) δ 7.5 – 7.7 (m, 2H), 7.2 -7.5 (m, 18H), 5.86 (d, $J = 4.8$ Hz, 1H, H-1), 5.10 (d, $J = 11.0$ Hz, 1H), 4.95 (d, $J = 10.7$ Hz, 1H), 4.87 (d, $J = 10.7$ Hz, 1H), 4.68 (d, $J = 11.7$ Hz, 1H), 4.59 (d, $J = 11.0$ Hz, 1H), 4.50 (d, $J = 12.1$ Hz, 1H), 3.5 – 4.0 (m, 8H), 1.6 – 1.8 (m, 2H), 1.3 – 1.5 (m, 10H), 0.95 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 139.2, 138.6, 138.3, 135.1, 132.0, 131.8 (2 carbons), 129.2 (2 carbons), 128.7 (5 carbons), 128.3 (3 carbons), 128.2 (3 carbons), 128.0 (2 carbons), 127.9, 127.3, 87.2,

82.8, 81.0, 77.2, 76.0, 75.4, 73.7, 71.5, 70.7, 68.9, 32.2, 30.4, 29.8, 29.6, 26.5, 23.0, 14.5 ; ESI/APCI calcd for C₄₁H₅₀O₅NaS ([M+Na]⁺) *m/z* 677.3271; measured *m/z* 677.3277.

6-*O*-(3,4,6-Tri-*O*-benzyl-2-*O*-*n*-octyl- α -D-glucopyranosyl)-1,3,2',6'-tetraazidoneamine (40). Please refer to the general procedure for glycosylation and hydrolysis. Compound **40** was obtained with 59% yield. ¹H NMR (CDCl₃, 300 MHz) δ 7.1 – 7.5 (m, 15H), 5.60 (d, *J* = 3.8 Hz, 1H, H-1'), 5.02 (d, *J* = 3.8 Hz, 1H, H-1''), 4.83 (d, *J* = 10.7 Hz, 1H), 4.76 (d, *J* = 12.0 Hz, 1H), 4.70 (d, *J* = 11.7 Hz, 1H), 4.61 (d, *J* = 12.4 Hz, 1H), 4.54 (s, 1H), 4.53 (s, 1H), 4.49 (d, *J* = 12.4 Hz, 1H), 4.45 (d, *J* = 10.7 Hz, 1H), 4.0 – 4.1 (m, 2H), 3.0 – 4.0 (m, 18H), 2.30 (ddd, *J* = 13.1, 4.5, 4.1 Hz, 1H, H-2eq), 1.6 – 1.7 (m, 2H), 1.49 (ddd, *J* = 12.7, 12.7, 12.7 Hz, 1H, H-2ax), 1.2 - 1.4 (m, 10H), 0.88 (t, *J* = 6.9 Hz, 3H); ¹³C NMR (CDCl₃, 100 MHz) δ 138.4, 138.1, 137.9, 128.7 (6 carbons), 128.32 (2 carbons), 128.25 (2 carbons), 128.1 (4 carbons), 128.0, 98.7, 98.4, 85.8, 81.5, 79.9, 79.6, 77.8, 75.9, 75.4, 74.0, 73.6 (2 carbons), 71.7, 71.5 (2 carbons), 71.3, 68.5, 63.0, 59.7, 59.3, 51.4, 32.5, 32.1, 30.9, 29.8, 29.5, 26.5, 22.9, 14.3 ; ESI/APCI calcd for C₄₇H₆₂N₁₂O₁₁Na ([M+Na]⁺) *m/z* 993.4553; measured *m/z* 993.4564.

6-*O*-(2-*O*-*n*-Octyl-D-glucopyranosyl)neamine (FG09). Please refer to the general procedure for the final synthesis of kanamycin B analogs. **FG09** was obtained with 52% yield as a chloride salt. ¹H NMR (D₂O, 300 MHz) (chloride salt) δ 5.81 (d, *J* = 3.8 Hz, 1H, H-1'), 5.06 (d, *J* = 3.4 Hz, 1H, H-1''), 3.0 - 4.0 (m, 19H), 2.4 (m, 1H), 1.4 – 1.5 (m, 3H), 1.1 - 1.2 (m, 10H), 0.72 (t, *J* = 6.5 Hz, 3H); ¹³C NMR (D₂O, 100 MHz) (chloride salt) δ 100.3, 96.4, 83.9, 80.1, 78.0, 74.4, 73.3, 73.2, 72.7, 70.8, 69.5, 69.4,

68.4, 60.7, 53.7, 49.8, 48.4, 40.3, 31.3, 29.3, 28.7, 28.5, 28.0, 25.1, 22.2, 13.6; ESI/APCI calcd for C₂₆H₅₃N₄O₁₁ ([M+H]⁺) *m/z* 597.3705; measured *m/z* 597.3701.

3',4'-Di-O-benzyl-1,3,2',6'-Tetraazidoneamine (41)⁹⁸. To a solution of **14** (3.60 g, 7.11 mmol) in DMF (40 mL) were added BnBr (3.40 mL, 28.5 mmol) and a catalytic amount of TBAI. The mixture was then transferred in an ice-water bath and NaH (1.14 g, 28.5 mmol) was slowly added. When TLC analysis performed the following day indicated completion, the reaction was quenched with MeOH (2 mL) and poured over ice. The mixture was extracted with EtOAc. The organic layer was washed with 1N aqueous HCl, saturated aqueous NaHCO₃, water and brine, and dried over Na₂SO₄. After removal of the solvent, a brownish crude product was obtained, to which 80 mL of mixed solution of dioxane: H₂O = 1:1 was added, followed by 35 mL glacial acetic acid. The resulting mixture was refluxed at 60~65 °C overnight. When complete, the reaction mixture was quenched with saturated aqueous NaHCO₃ and extracted with EtOAc. The organic layer was washed with 1N aqueous HCl, water, saturated aqueous NaHCO₃, brine, and dried over Na₂SO₄. After removal of the solvent followed by purification with a gradient column chromatography (pure hexane to hexane: EtOAc = 40:60), **41** was obtained (2.03 g, 6.62 mmol, 42%).

6-O-(2,3,4,6-Tetra-O-benzyl- α -D-glucopyranosyl)-3',4'-O-dibenzyl-1,3,2',6'-tetraazidoneamine (43a). A solution of **41** (0.20 g, 0.33 mmol), **42a** (0.25 g, 0.40 mmol), and activated powder 4 Å molecular sieve was stirred at room temperature for 2 h in 12 mL of a mixed anhydrous solution Et₂O:CH₂Cl₂ = 3:1. The mixture was cooled to -70 °C and *N*-iodosuccinimide (0.09 g, 0.40 mmol) was quickly added. After the

temperature has warmed up to $-40\text{ }^{\circ}\text{C}$, trifluoromethanesulfonic acid (0.05 mL) was added. The solution was stirred at low temperature till the complete consumption of the glycosyl donor. The reaction mixture was quenched by addition of solid NaHCO_3 , $\text{Na}_2\text{S}_2\text{O}_3$ and Na_2SO_4 . After being stirred for 15 minutes, the reaction mixture was filtered through celite. The residue was washed thoroughly with EtOAc. After removal of the solvents, the crude product was purified with gradient column chromatography (Hexane:EtOAc = 100:0 to 50:50) to afford **43a**. Because it was mixed with inseparable impurities, it was used as so in the next step.

6-O-(3-Azido-3-deoxy-2,4,6-tri-O-benzyl- α -D-glucopyranosyl)-3',4'-O-dibenzyl-1,3,2',6'-tetraazidoneamine (43b). Please refer to the synthesis of **43a**.

Compound **43b** was also obtained mixed with inseparable impurities and was then used as so in the next step.

6-O-(2,3,4,6-Tetra-O-benzyl- α -D-glucopyranosyl)-3',4'-O-dibenzyl-5-O-n-octyl-1,3,2',6'-tetraazidoneamine (44a). Please refer to the general procedure for *O*-alkylation of sugars. Compound **44a** was obtained with 57% yield. ^1H NMR (CDCl_3 , 300 MHz) δ 7.2 -7.5 (m, 30H), 5.72 (d, $J = 3.4$ Hz, 1H, H-1'), 5.62 (d, $J = 3.8$ Hz, 1H, H-1''), 4.8 - 5.0 (m, 8H), 4.67 (d, $J = 11.3$ Hz, 1H), 4.65 (d, $J = 12.0$ Hz, 1H), 4.53 (d, $J = 11.3$ Hz, 1H), 4.47 (d, $J = 12.0$ Hz, 1H), 4.32 (d, $J = 9.6$ Hz, 1H), 4.15 (d, $J = 10.0$ Hz, 1H), 4.04 (dd, $J = 10.3, 8.9$ Hz, 1H), 3.9 - 4.0 (m, 1H), 3.3 - 3.8 (m, 15H), 2.4 (m, 1H), 1.5 - 1.7 (m, 3H), 1.0 - 1.4 (m, 10H), 0.86 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 138.8, 138.7, 138.1 (2 carbons), 137.83, 137.77, 128.64 (3 carbons), 128.57 (3 carbons), 128.49 (4 carbons), 128.25 (3 carbons), 128.20 (4 carbons), 128.1 (3 carbons), 128.0 (3

carbons), 127.9 (2 carbons), 127.8, 127.7, 127.6 (2 carbons), 127.5, 97.5, 96.0, 83.3, 82.1, 80.2, 79.5, 78.8, 77.7, 77.5, 76.1, 75.8, 75.7, 75.5, 75.2, 75.1, 73.5, 73.4, 71.1, 70.2, 68.5, 63.5, 60.6, 60.5, 59.3, 32.1, 31.9, 30.2, 29.7, 29.6, 26.1, 22.8, 14.2; ESI/APCI calcd for $C_{68}H_{80}N_{12}O_{11}Na$ ($[M+Na]^+$) m/z 1263.5962; measured m/z 1263.5961.

6-*O*-(3-Azido-3-deoxy-2,4,6-tri-*O*-benzyl- α -D-glucopyranosyl)-3',4'-*O*-dibenzyl-5-*O*-*n*-octyl-1,3,2',6'-tetraazidoneamine (44b). Please refer to the general procedure for *O*-alkylation of sugars. Compound **44b** was obtained with 52% yield. 1H NMR ($CDCl_3$, 300 MHz) δ 7.2 -7.5 (m, 25H), 5.70 (d, $J = 3.5$ Hz, 1H, H-1'), 5.58 (d, $J = 3.8$ Hz, 1H, H-1''), 4.92 (d, $J = 11.3$ Hz, 1H), 4.91 (s, 2H), 4.82 (d, $J = 12.0$ Hz, 1H), 4.80 (d, $J = 10.6$ Hz, 1H), 4.76 (d, $J = 12.0$ Hz, 1H), 4.65 (d, $J = 11.3$ Hz, 1H), 4.64 (d, $J = 12.0$ Hz, 1H), 4.47 (d, $J = 12.0$ Hz, 1H), 4.3 (m, 1H), 4.11 (d, $J = 10.0$ Hz, 1H), 4.02 (dd, $J = 10.3, 8.9$ Hz, 1H), 3.3 – 3.9 (m, 17H), 2.3 – 2.4 (m, 1H), 1.4 – 1.7 (m, 3H), 1.0 – 1.3 (m, 10H), 0.88 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.2, 138.0, 137.9, 137.8, 137.6, 128.8 (5 carbons), 128.7 (2 carbons), 128.43 (2 carbons), 128.40 (2 carbons), 128.3 (5 carbons), 128.2 (2 carbons), 128.04 (4 carbons), 128.00 (2 carbons), 127.9, 97.6, 95.2, 83.3, 80.3, 78.9, 77.5, 76.5 (2 carbons), 76.3, 75.8, 75.5, 75.3, 75.1, 73.8, 73.1, 71.2, 69.9, 68.3, 65.8, 63.6, 60.5, 59.3, 51.2, 32.1, 32.0, 30.3, 29.7, 29.6, 26.0, 22.9, 14.3; ESI/APCI calcd for $C_{61}H_{73}N_{15}O_{10}Na$ ($[M+Na]^+$) m/z 1198.5557; measured m/z 1198.5527.

6-*O*-(α -D-Glucopyranosyl)-5-*O*-*n*-octylneamine (FG10). Please refer to the general procedure for the final synthesis of kanamycin B analogs. **FG10** was obtained with 81% yield as a chloride salt. 1H NMR (D_2O , 300 MHz) (chloride salt) δ 5.59 (d, $J =$

3.8 Hz, 1H, H-1'), 5.01 (d, $J = 3.4$ Hz, 1H, H-1'), 3.0 - 4.0 (m, 19H), 2.4 (m, 1H), 1.9 (m, 1H), 1.4 - 1.5 (m, 3H), 1.1 - 1.2 (m, 10H), 0.72 (t, $J = 6.5$ Hz, 3H); ^{13}C NMR (D_2O , 100 MHz) (chloride salt) δ 102.1, 93.1, 81.7, 80.7, 73.7, 73.31, 73.30, 72.8, 72.2, 71.3, 69.9, 68.6, 68.4, 59.9, 53.1, 50.2, 48.7, 40.0, 31.2, 29.4, 29.0, 28.5, 28.2, 25.2, 22.2, 13.6; ESI/APCI calcd for $\text{C}_{26}\text{H}_{53}\text{N}_4\text{O}_{11}$ ($[\text{M}+\text{H}]^+$) m/z ; measured m/z .

6-*O*-(3-Amino-3-deoxy- α -D-glucopyranosyl)-5-*O*-*n*-octylneamine (FG11).

Please refer to the general procedure for the final synthesis of kanamycin B analogs.

FG11 was obtained with 28% yield as a chloride salt. ^1H NMR (D_2O , 300 MHz) (chloride salt) δ 5.61 (d, $J = 3.5$ Hz, 1H, H-1'), 5.08 (d, $J = 3.5$ Hz, 1H, H-1'), 3.0 - 4.2 (m, 19H), 2.4 (m, 1H), 1.9 (m, 1H), 1.4 - 1.5 (m, 2H), 1.1 - 1.2 (m, 10H), 0.72 (t, $J = 6.5$ Hz, 3H); ^{13}C NMR (D_2O , 100 MHz) (chloride salt) δ 101.3, 93.0, 82.0, 81.1, 73.7, 73.2, 71.8, 71.1, 69.6, 68.7, 68.2, 64.9, 59.3, 54.9, 53.0, 49.0, 48.7, 39.9, 31.2, 29.4, 29.0, 28.6, 27.9, 25.3, 22.2, 13.6; ESI/APCI calcd for $\text{C}_{26}\text{H}_{54}\text{N}_5\text{O}_{10}$ ($[\text{M}+\text{H}]^+$) m/z 596.3865; measured m/z 596.3865.

4'-*O*-benzyl-5,6-*O*-benzylidene-1,3,2',6'-Tetraazidoneamine (45a). To a solution of **14** (3.72 g, 7.35 mmol) in CH_2Cl_2 (25 mL) was added TBAHS (0.75 g, 2.21 mmol), followed by BnBr (0.97 mL, 8.09 mmol) and NaOH (25 mL, 1N aqueous solution). The mixture was refluxed at 60 °C overnight. When complete, CH_2Cl_2 was removed from the reaction mixture using a rotavapor and the obtained solution was extracted with EtOAc. The organic layer was then washed with 1 N aqueous HCl, water and brine, and then dried over solid Na_2SO_4 . After removal of the solvent and purification with gradient column chromatography (hexane:EtOAc = 100:0 to 40:60), the product **45a**

was obtained mixed with its regioisomer **45b** in a 1/1 ratio (1.97 g, 3.31 mmol, 45%). ¹H NMR (CDCl₃, 300 MHz) (mixture of **45a** and **45b**) δ 7.3 – 7.4 (m, 10H), 5.56 (d, *J* = 3.4 Hz, 1H), 5.52 (d, *J* = 3.8 Hz, 1H), 4.96 (d, *J* = 11.3 Hz, 1H), 4.85 (d, *J* = 11.7 Hz, 1H), 4.70 (d, *J* = 11.7 Hz, 2H), 4.0 - 4.1 (m, 4H), 3.7 – 3.9 (m, 2H), 3.3 – 3.7 (m, 13H), 3.23 (dd, *J* = 10.7, 3.8 Hz, 1H), 2.81 (d, *J* = 3.8 Hz, 1H), 2.50 (d, *J* = 3.8 Hz, 1H), 2.2 -2.4 (m, 2H), 1.3 – 1.8 (m, 24H).

3'-O-benzyl-5,6-O-benzylidene-1,3,2',6'-Tetraazidoneamine (45b). Please refer to the synthesis of compound **45a**.

4'-O-benzyl-3'-O-*n*-octyl-1,3,2',6'-Tetraazidoneamine (47a). To a solution of a mixture of **45a** and **45b** (1.22 g, 2.04 mmol) in anhydrous DMF (50 mL), *n*-octyl bromide (1.42 mL, 8.18 mmol), NaH (0.33 g, 8.18 mmol), and a catalytic amount of TBAI were added. The reaction was stirred at room temperature overnight. When complete, the reaction was quenched by addition of MeOH (5 mL) and was slowly poured into a mixture of ice and EtOAc. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with 1 N aqueous HCl, water, saturated aqueous NaHCO₃ and brine, and then dried over solid Na₂SO₄. After removal of the solvent, a brownish, oily crude product was obtained, to which 70 mL of a mixed solution of dioxane:H₂O = 1:1 was added, followed by 50 mL glacial acetic acid. The resulting mixture was refluxed at 60 °C overnight. When complete, the reaction mixture was quenched with saturated aqueous NaHCO₃ and extracted with EtOAc. The organic layer was washed with 1N aqueous HCl, water, saturated aqueous NaHCO₃, brine, and dried over solid Na₂SO₄. After removal of the solvent followed by purification with a gradient

column chromatography (hexane:EtOAc = 100:0 to 40:60), a mixture of **47a** and **47b** was obtained in a 10/7 ratio (0.92 g, 1.46 mmol, 72%). ^1H NMR (CDCl_3 , 300 MHz) (mixture of **47a** and **47b**) δ 7.3 – 7.4 (m, 10H), 5.12 (d, $J = 3.7$ Hz, 1H), 5.11 (d, $J = 3.4$ Hz, 1H), 4.89 (d, $J = 10.7$ Hz, 1H), 4.87 (d, $J = 10.3$ Hz, 1H), 4.83 (d, $J = 10.3$ Hz, 1H), 4.63 (d, $J = 1.0$ Hz, 1H), 4.0 - 4.2 (m, 4H), 3.7 – 3.9 (m, 5H), 3.3 – 3.6 (m, 16H), 3.2 – 3.3 (m, 4H), 2.8 (m, 1H), 2.3 (m, 2H), 1.4 – 1.7 (m, 6H), 1.2 (m, 20H), 0.87 (t, $J = 7.2$ Hz, 6H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 137.8 (2 carbons), 128.8 (4 carbons), 128.32, 128.27, 128.16 (2 carbons), 128.09 (2 carbons), 99.7 (2 carbons), 84.3 (2 carbons), 81.2 (2 carbons), 80.9 (2 carbons), 79.1, 78.7 (2 carbons), 76.1, 75.8, 75.5, 74.2, 73.9, 71.7, 71.5, 64.4 (2 carbons), 59.9 (2 carbons), 59.0 (2 carbons), 51.1 (2 carbons), 32.2 (2 carbons), 32.0 (2 carbons), 30.6 (2 carbons), 29.7 (2 carbons), 29.4 (2 carbons), 26.3 (2 carbons), 22.8 (2 carbons), 14.3 (2 carbons); ESI/APCI calcd for $\text{C}_{27}\text{H}_{40}\text{N}_{12}\text{O}_6\text{Na}$ ($[\text{M}+\text{Na}]^+$) m/z 651.3086; measured m/z 651.3105.

3'-O-benzyl-4'-O-n-octyl-1,3,2',6'-Tetraazidoneamine (47b). Please refer to the synthesis of compound **47a**.

6-O-(2,3,4,6-Tetra-O-benzyl- α -D-glucopyranosyl)-4'-O-benzyl-3'-O-n-octyl-1,3,2',6'-tetraazidoneamine (48a). A solution of the mixture of **47a** and **47b** (0.20 g, 0.32 mmol), **42a** (0.24 g, 0.38 mmol), and activated powder 4 Å molecular sieve was stirred at room temperature for 2 h in 12 mL of a mixed anhydrous solution $\text{Et}_2\text{O}:\text{CH}_2\text{Cl}_2 = 3:1$. The mixture was cooled to -70 °C and *N*-iodosuccinimide (0.09 g, 0.38 mmol) was quickly added. After the temperature has warmed up to -40 °C, trifluoromethanesulfonic acid (0.05 mL) was added. The solution was stirred at low temperature till the complete

consumption of the glycosyl donor. The reaction mixture was quenched by addition of solid NaHCO_3 , $\text{Na}_2\text{S}_2\text{O}_3$ and Na_2SO_4 . After being stirred for 15 minutes, the reaction mixture was filtered through celite. The residue was washed thoroughly with EtOAc. After removal of the solvents, the crude product was purified with gradient column chromatography (Hexane:EtOAc = 100:0 to 50:50) to afford a mixture of **48a** and **48b**, obtained together with some inseparable impurities that prevented a full characterization.

6-O-(2,3,4,6-Tetra-O-benzyl- α -D-glucopyranosyl)-3'-O-benzyl-4'-O-n-octyl-1,3,2',6'-tetraazidoneamine (48b). Please refer to the synthesis of **48a**.

6-O-(α -D-Glucopyranosyl)-3'-O-n-octylneamine (FG12). Please refer to the general procedure for the final synthesis of kanamycin B analogs. An inseparable mixture of **FG12** and **FG13** was obtained in 35% yield as chloride salts. The spectral information of only one of them (**FG12** or **FG13**) is reported as follows: ^1H NMR (D_2O , 300 MHz) (chloride salt) δ 5.79 (d, $J = 3.8$ Hz, 1H), 4.95 (d, $J = 3.1$ Hz, 1H), 3.3 - 4.0 (m, 19H), 2.4 (m, 1H), 1.7 - 1.9 (m, 1H), 1.4 - 1.5 (m, 2H), 1.1 - 1.2 (m, 10H), 0.71 (t, $J = 6.9$ Hz, 3H); ^{13}C NMR (D_2O , 100 MHz) (chloride salt) 101.8, 96.1, 84.0, 79.2, 77.6, 74.3, 73.0, 72.9 (2 carbons), 71.7, 69.8, 69.3 (2 carbons), 60.6, 52.9, 49.9, 48.4, 40.1, 31.3, 29.5, 28.7, 28.5, 25.3, 25.2, 22.2, 13.6; ESI/APCI calcd for $\text{C}_{26}\text{H}_{53}\text{N}_4\text{O}_{11}$ ($[\text{M}+\text{H}]^+$) m/z 597.3705; measured m/z 597.3716.

6-O-(α -D-Glucopyranosyl)-4'-O-n-octylneamine (FG13). Please refer to the synthesis of **FG12**.

1-Pentyl-1*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-dione (50).¹¹⁴ Please refer to the general procedure for cycloaddition of 1,4-naphthoquinone, Method C. Compound **50** was obtained in 40% yield.

1-Hexyl-1*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-dione (51). Please refer to the general procedure for cycloaddition of 1,4-naphthoquinone, Method C. Compound **51** was obtained in 49% yield. ¹H NMR (CDCl₃, 300 MHz) δ 8.3 – 8.4 (m, 1H), 8.2 – 8.3 (m, 1H), 7.8 – 7.9 (m, 2H), 4.85 (t, *J* = 7.2 Hz, 2H), 2.0 (m, 2H), 1.2 - 1.4 (m, 6H), 0.88 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (CD₃OD, 100 MHz) δ 177.1, 175.7, 145.8, 135.4, 134.5, 133.7, 133.5, 133.1, 128.1, 127.6, 50.9, 31.3, 30.2, 26.2, 22.6, 14.1; ESI/APCI calcd for C₁₆H₁₈N₃O₂⁺ ([M+H]⁺) *m/z* 284.1394; measured *m/z* 284.1390.

1-Octyl-1*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-dione (52).¹¹⁴ Please refer to the general procedure for cycloaddition of 1,4-naphthoquinone, Method C. Compound **52** was obtained in 62% yield.

1-Decyl-1*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-dione (53).¹¹⁴ Please refer to the general procedure for cycloaddition of 1,4-naphthoquinone, Method C. Compound **50** was obtained in 54% yield.

1-Dodecyl-1*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-dione (54).¹¹⁴ Please refer to the general procedure for cycloaddition of 1,4-naphthoquinone, Method C. Compound **50** was obtained in 68% yield.

2-Pentylamino-1,4-naphthoquinone (60).⁵⁷ Please refer to the general procedure for cycloaddition of 1,4-naphthoquinone, Method B. Compound **60** was obtained in 29% yield (estimated from the integral ratio of ¹H NMR).

2-Hexylamino-1,4-naphthoquinone (61). Please refer to the general procedure for cycloaddition of 1,4-naphthoquinone, Method B. Compound **61** was obtained in 18% yield (estimated from the integral ratio of ^1H NMR). ^1H NMR (CDCl_3 , 300 MHz) δ 8.0 – 8.1 (m, 2H), 7.7 (m, 1H), 7.6 (m, 1H), 5.87 (br s, 1H), 5.72 (s, 1H), 3.16 (q, $J = 7.2$ Hz, 2H), 1.7 (m, 2H), 1.2 -1.5 (m, 6H), 0.90 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 183.1, 182.1, 148.2, 134.9, 133.9, 132.1, 130.7, 126.45, 126.39, 100.9, 42.8, 31.6, 28.4, 26.9, 22.7, 14.2. ESI/APCI calcd for $\text{C}_{16}\text{H}_{20}\text{NO}_2^+$ ($[\text{M}+\text{H}]^+$) m/z 258.1489; measured m/z 258.1492.

2-Octylamino-1,4-naphthoquinone (62).¹²⁰ Please refer to the general procedure for cycloaddition of 1,4-naphthoquinone, Method B. Compound **62** was obtained in 11% yield (estimated from the integral ratio of ^1H NMR).

2-Decylamino-1,4-naphthoquinone (63). Please refer to the general procedure for cycloaddition of 1,4-naphthoquinone, Method B. Compound **63** was obtained in 3% yield (estimated from the integral ratio of ^1H NMR). ^1H NMR (CDCl_3 , 300 MHz) δ 8.0 – 8.1 (m, 2H), 7.7 (m, 1H), 7.6 (m, 1H), 5.88 (br s, 1H), 5.73 (s, 1H), 3.16 (q, $J = 7.2$ Hz, 2H), 1.7 (m, 2H), 1.2 -1.4 (m, 14H), 0.88 (t, $J = 6.9$ Hz, 3H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 183.1, 182.2, 148.2, 135.0, 133.9, 132.1, 130.7, 126.5, 126.4, 100.9, 42.8, 32.1, 29.9, 29.7 (2 carbons), 29.5, 28.5, 27.2, 22.9, 14.3 ESI/APCI calcd for $\text{C}_{20}\text{H}_{28}\text{NO}_2^+$ ($[\text{M}+\text{H}]^+$) m/z 314.2115; measured m/z 314.2112.

2-Dodecylamino-1,4-naphthoquinone (64).¹²³ Please refer to the general procedure for cycloaddition of 1,4-naphthoquinone, Method B. Compound **64** was obtained in 4% yield (estimated from the integral ratio of ^1H NMR).

3-Ethyl-1-pentyl-4,9-dioxo-4,9-dihydro-1H-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride (50a). Please refer to the general procedure for N-3 alkylation. Compound **50a** was obtained in 80% yield. ¹H NMR (CD₃OD, 300 MHz) δ 8.36 (dd, *J* = 8.9, 2.7 Hz, 2H), 8.04 (dd, *J* = 9.3, 2.4 Hz, 2H), 5.12 (q, *J* = 7.2 Hz, 2H), 5.06 (t, *J* = 7.2 Hz, 2H), 2.1 – 2.2 (m, 2H), 1.74 (t, *J* = 7.2 Hz, 3H), 1.4 - 1.5 (m, 4H), 0.95 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (CD₃OD, 100 MHz) δ 171.2, 171.1, 134.4 (2 carbons), 134.31, 134.30, 131.2 (2 carbons), 126.3 (2 carbons), 52.8, 48.6, 26.9, 26.5, 20.3, 11.4, 11.3; ESI/APCI calcd for C₁₇H₂₀N₃O₂⁺ ([M]⁺) *m/z* 298.1550; measured *m/z* 298.1543.

3-Butyl-1-pentyl-4,9-dioxo-4,9-dihydro-1H-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride (50b). Please refer to the general procedure for N-3 alkylation. Compound **50b** was obtained in 53% yield. ¹H NMR (CD₃OD, 300 MHz) δ 8.35 (dd, *J* = 9.3, 2.4 Hz, 2H), 8.02 (dd, *J* = 9.3, 2.4 Hz, 2H), 5.07 (t, *J* = 7.2 Hz, 2H), 5.06 (t, *J* = 7.2 Hz, 2H), 2.1 (m, 4H), 1.4 - 1.6 (m, 6H), 1.03 (t, *J* = 7.2 Hz, 3H), 0.95 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (CD₃OD, 100 MHz) δ 172.7 (2 carbons), 135.9 (4 carbons), 132.8 (2 carbons), 127.8 (2 carbons), 54.3, 54.1, 30.6, 28.4, 28.1, 21.8, 19.3, 12.9, 12.4; ESI/APCI calcd for C₁₉H₂₄N₃O₂⁺ ([M]⁺) *m/z* 326.1863; measured *m/z* 326.1856.

1,3-Dipentyl-4,9-dioxo-4,9-dihydro-1H-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride (50c). Please refer to the general procedure for N-3 alkylation. Compound **50c** was obtained in 64% yield. ¹H NMR (CD₃OD, 400 MHz) δ 8.38 (dd, *J* = 9.0, 2.5 Hz, 2H), 8.05 (dd, *J* = 9.0, 2.4 Hz, 2H), 5.08 (t, *J* = 7.2 Hz, 4 H), 2.1 (m, 4 H), 1.2 - 1.6 (m, 8H), 0.98 (t, *J* = 7.1 Hz, 6H); ¹³C NMR (CDCl₃, 100 MHz) δ 172.7 (2 carbons), 135.9 (4 carbons), 132.8 (2 carbons), 127.9 (2 carbons), 54.3 (2 carbons), 28.4 (2 carbons), 28.1

(2carbons), 22.2 (2 carbons), 21.8 (2 carbons), 12.9 (2 carbons); ESI/APCI calcd for $C_{20}H_{26}N_3O_2^+$ ($[M]^+$) m/z 340.2020; measured m/z 340.2028.

3-Octyl-1-pentyl-4,9-dioxo-4,9-dihydro-1H-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride (50e). Please refer to the general procedure for N-3 alkylation. Compound **50e** was obtained in 74% yield. 1H NMR (CD_3OD , 300 MHz) δ 8.37 (dd, $J = 9.3, 2.4$ Hz, 2H), 8.04 (dd, $J = 8.9, 2.4$ Hz, 2H), 5.07 (t, $J = 7.2$ Hz, 4 H), 2.1 (m, 4 H), 1.3 - 1.5 (m, 14H), 0.96 (t, $J = 7.2$ Hz, 3H), 0.89 (t, $J = 6.9$ Hz, 3H); ^{13}C NMR (CD_3OD , 100 MHz) δ 172.7 (2 carbons), 136.0 (2 carbons), 135.9 (2 carbons), 132.8 (2 carbons), 127.9 (2 carbons), 54.3 (2 carbons), 31.7, 29.0, 28.7 (2 carbons), 28.4, 28.1, 26.0, 22.5, 21.8, 13.2, 12.9; ESI/APCI calcd for $C_{23}H_{32}N_3O_2^+$ ($[M]^+$) m/z 382.2489; measured m/z 382.2497.

3-Decyl-1-pentyl-4,9-dioxo-4,9-dihydro-1H-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride (50f). Please refer to the general procedure for N-3 alkylation. Compound **50f** was obtained in 58% yield. 1H NMR (CD_3OD , 400 MHz) δ 8.4 (m, 2H), 8.1 (m, 2H), 5.10 (t, $J = 7.2$ Hz, 4H), 2.1 (m, 4H), 1.2 - 1.6 (m, 18H), 0.8 - 1.0 (m, 6H); ^{13}C NMR (CD_3OD , 100 MHz) δ 172.7 (2 carbons), 136.0 (2 carbons), 135.9 (2 carbons), 132.7 (2 carbons), 127.9 (2 carbons), 54.3 (2 carbons), 31.8, 29.4, 29.2 (2 carbons), 28.7 (2 carbons), 28.5, 28.1, 26.0, 22.5, 21.8, 13.2, 12.9; ESI/APCI calcd for $C_{25}H_{36}N_3O_2^+$ ($[M]^+$) m/z 410.2802; measured m/z 410.2813.

3-Ethyl-1-hexyl-4,9-dioxo-4,9-dihydro-1H-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride (51a). Please refer to the general procedure for N-3 alkylation. Compound **51a** was obtained in 82% yield. 1H NMR (CD_3OD , 300 MHz) δ 8.37 (dd, $J = 8.9, 2.1$ Hz, 2H), 8.04 (dd, $J = 8.9, 2.7$ Hz, 2H), 5.13 (q, $J = 7.2$ Hz, 2H), 5.07 (t, $J = 7.6$ Hz, 2H), 2.1

(m, 2H), 1.73 (t, $J = 7.2$ Hz, 3H), 1.3 - 1.5 (m, 6H), 0.93 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (CD_3OD , 100 MHz) δ 172.73, 172.66, 135.9 (2 carbons), 135.8 (2 carbons), 132.8 (2 carbons), 127.9 (2 carbons), 54.3, 50.2, 31.0, 28.7, 25.7, 22.2, 13.1, 13.0; ESI/APCI calcd for $\text{C}_{18}\text{H}_{22}\text{N}_3\text{O}_2^+$ ($[\text{M}]^+$) m/z 312.1707; measured m/z 312.1710.

3-Butyl-1-hexyl-4,9-dioxo-4,9-dihydro-1H-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride (51b). Please refer to the general procedure for N-3 alkylation. Compound **51b** was obtained in 95% yield. ^1H NMR (CD_3OD , 300 MHz) δ 8.34 (dd, $J = 8.9, 2.4$ Hz, 2H), 8.01 (dd, $J = 8.9, 2.8$ Hz, 2H), 5.05 (t, $J = 7.2$ Hz, 2H), 5.04 (t, $J = 7.2$ Hz, 2H), 2.1 (m, 4H), 1.2 - 1.6 (m, 8H), 1.02 (t, $J = 7.2$ Hz, 3H), 0.91 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (CD_3OD , 100 MHz) δ 172.8 (2 carbons), 135.9 (4 carbons), 132.8 (2 carbons), 127.8 (2 carbons), 54.3, 54.1, 30.9, 30.6, 28.6, 25.7, 22.2, 19.3, 13.1, 12.4; ESI/APCI calcd for $\text{C}_{20}\text{H}_{26}\text{N}_3\text{O}_2^+$ ($[\text{M}]^+$) m/z 340.2020; measured m/z 340.2025.

1-Hexyl-3-pentyl-4,9-dioxo-4,9-dihydro-1H-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride (51c). Please refer to the general procedure for N-3 alkylation. Compound **51c** was obtained in 62% yield. ^1H NMR (CD_3OD , 400 MHz) δ 8.38 (dd, $J = 9.1, 2.4$ Hz, 2H), 8.05 (dd, $J = 9.1, 2.4$ Hz, 2H), 5.08 (t, $J = 7.3$ Hz, 4H), 2.1 (m, 4H), 1.2 - 1.6 (m, 10 H), 0.9 (m, 6H); ^{13}C NMR (CD_3OD , 100 MHz) δ 172.2 (2 carbons), 136.0 (4 carbons), 132.8 (2 carbons), 127.9 (2 carbons), 54.3 (2 carbons), 31.0, 28.7, 28.4, 28.1, 25.7, 22.2, 21.8, 13.1, 12.9; ESI/APCI calce for $\text{C}_{21}\text{H}_{28}\text{N}_3\text{O}_2^+$ ($[\text{M}]^+$) m/z 354.2176; measured m/z 354.2178.

1,3-Dihexyl-4,9-dioxo-4,9-dihydro-1H-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride (51d). Please refer to the general procedure for N-3 alkylation. Compound **51d**

was obtained in 90% yield. ^1H NMR (CD_3OD , 300 MHz) δ 8.34 (dd, $J = 8.9, 2.7$ Hz, 2H), 8.01 (dd, $J = 9.3, 2.4$ Hz, 2H), 5.05 (t, $J = 7.2$ Hz, 4H), 2.1 (m, 4H), 1.3 - 1.6 (m, 12H), 0.92 (t, $J = 7.2$ Hz, 6H); ^{13}C NMR (CD_3OD , 100 MHz) δ 172.8 (2 carbons), 135.9 (4 carbons), 132.8 (2 carbons), 127.8 (2 carbons), 54.3 (2 carbons), 30.9 (2 carbons), 28.6 (2 carbons), 25.7 (2 carbons), 22.2 (2 carbons), 13.0 (2 carbons); ESI/APCI calcd for $\text{C}_{22}\text{H}_{30}\text{N}_3\text{O}_2^+$ ($[\text{M}]^+$) m/z 368.2333; measured m/z 368.2340.

1-Hexyl-3-octyl-4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride (51e). Please refer to the general procedure for N-3 alkylation. Compound **51e** was obtained in 88% yield. ^1H NMR (CD_3OD , 300 MHz) δ 8.35 (dd, $J = 9.3, 2.4$ Hz, 2H), 8.02 (dd, $J = 8.9, 2.4$ Hz, 2H), 5.05 (t, $J = 7.2$ Hz, 4H), 2.1 (m, 4H), 1.3 - 1.6 (m, 16H), 0.9 (m, 6H); ^{13}C NMR (CD_3OD , 75 MHz) δ 172.6 (2 carbons), 135.8 (4 carbons), 132.7 (2 carbons), 127.7 (2 carbons), 54.2 (2 carbons), 31.6, 30.8, 28.8, 28.6, 28.53, 28.52, 25.9, 25.6, 22.4, 22.1, 13.1, 13.0; ESI/APCI calcd for $\text{C}_{24}\text{H}_{34}\text{N}_3\text{O}_2^+$ ($[\text{M}]^+$) m/z 396.2646; measured m/z 396.2650.

3-Decyl-1-hexyl-4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride (51f). Please refer to the general procedure for N-3 alkylation. Compound **51f** was obtained in 39% yield. ^1H NMR (CD_3OD , 300 MHz) δ 8.37 (dd, $J = 8.9, 2.4$ Hz, 2H), 8.04 (dd, $J = 8.9, 2.0$ Hz, 2H), 5.08 (t, $J = 7.2$ Hz, 4H), 2.1 (m, 4H), 1.2 - 1.6 (m, 20H), 0.9 (m, 6H); ^{13}C NMR (CD_3OD , 100 MHz) δ 172.7 (2 carbons), 136.1 (2 carbons), 135.9 (2 carbons), 132.7 (2 carbons), 127.9 (2 carbons), 54.4 (2 carbons), 31.8, 31.0, 29.4, 29.3, 29.2, 28.7 (3 carbons), 26.0, 25.7, 22.5, 22.3, 13.2, 13.1; ESI/APCI calcd for $\text{C}_{26}\text{H}_{38}\text{N}_3\text{O}_2^+$ ($[\text{M}]^+$) m/z 424.2959; measured m/z 424.2962.

3-Ethyl-1-octyl-4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride (52a).¹²⁴ Please refer to the general procedure for N-3 alkylation. Compound **52a** was obtained with 51% yield.

3-Butyl-1-octyl-4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride (52b). Please refer to the general procedure for N-3 alkylation. Compound **52b** was obtained in 99% yield. ¹H NMR (CD₃OD, 300 MHz) δ 8.35 (dd, *J* = 8.9, 2.8 Hz, 2H), 8.02 (dd, *J* = 8.9, 2.4 Hz, 2H), 5.0 - 5.1 (m, 4H), 2.1 (m, 4H), 1.2 - 1.6 (m, 12H), 1.03 (t, *J* = 7.2 Hz, 3H), 0.90 (t, *J* = 6.5 Hz, 3H); ¹³C NMR (CD₃OD, 100 MHz) δ 172.8 (2 carbons), 135.9 (4 carbons), 132.8 (2 carbons), 127.8 (2 carbons), 54.3, 54.1, 31.7, 30.6, 28.9, 28.71, 28.65, 26.0, 22.5, 19.3, 13.2, 12.5; ESI/APCI calcd for C₂₂H₃₀N₃O₂⁺ ([M]⁺) *m/z* 368.2333; measured *m/z* 368.2337.

1,3-Dioctyl-4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride (52e). Please refer to the general procedure for N-3 alkylation. Compound **52e** was obtained in 29% yield. ¹H NMR (CD₃OD, 300 MHz) δ 8.38 (dd, *J* = 8.9, 2.4 Hz, 2H), 8.05 (dd, *J* = 8.9, 2.7 Hz, 2H), 5.10 (t, *J* = 7.2 Hz, 4H), 2.1 (m, 4H), 1.2 - 1.6 (m, H), 0.89 (t, *J* = 6.8 Hz, 6H); ¹³C NMR (CD₃OD, 100 MHz) δ 172.6 (2 carbons), 136.1 (2 carbons), 135.9 (2 carbons), 132.7 (2 carbons), 127.9 (2 carbons), 54.4 (2 carbons), 31.7 (2 carbons), 30.0 (2 carbons), 28.9 (2 carbons), 28.7 (2 carbons), 26.0 (2 carbons), 22.5 (2 carbons), 13.2 (2 carbons); ESI/APCI calcd for C₂₆H₃₈N₃O₂⁺ ([M]⁺) *m/z* 424.2959; measured *m/z* 424.2960.

3-Decyl-1-octyl-4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride (52f). Please refer to the general procedure for N-3 alkylation. Compound **52f**

was obtained in 81% yield. ^1H NMR (CD_3OD , 400 MHz) δ 8.40 (dd, $J = 9.2, 2.4$ Hz, 2H), 8.06 (dd, $J = 9.0, 2.5$ Hz, 2H), 5.10 (t, $J = 7.2$ Hz, 4H), 2.1 (m, 4H), 1.2 – 1.6 (m, 24H), 0.9 (m, 6H); ^{13}C NMR (CD_3OD , 100 MHz) δ 172.7 (2 carbons), 136.0 (2 carbons), 135.9 (2 carbons), 132.8 (2 carbons), 127.9 (2 carbons), 54.4 (2 carbons), 31.8, 31.7, 29.4, 29.3, 29.2, 28.9, 28.7 (4 carbons), 26.0 (2 carbons), 22.52, 22.49, 13.2 (2 carbons); ESI/APCI calcd for $\text{C}_{22}\text{H}_{30}\text{N}_3\text{O}_2^+$ ($[\text{M}]^+$) m/z 452.3280; measured m/z 452.3272.

3-Ethyl-1-decyl-4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride (53a). Please refer to the general procedure for N-3 alkylation. Compound **53a** was obtained in 99% yield. ^1H NMR (CD_3OD , 300 MHz) δ 8.32 (dd, $J = 9.3, 2.4$ Hz, 2H), 8.00 (dd, $J = 8.9, 2.4$ Hz, 2H), 5.08 (q, $J = 7.2$ Hz, 2H), 5.01 (t, $J = 7.6$ Hz, 2H), 2.1 (m, 2H), 1.72 (t, $J = 7.2$ Hz, 3H), 1.2 - 1.5 (m, 14H), 0.87 (t, $J = 6.8$ Hz, 3H); ^{13}C NMR (CD_3OD , 100 MHz) δ 172.9, 172.8, 135.9 (2 carbons), 132.8 (2 carbons), 127.8 (4 carbons), 54.3, 50.2, 31.8, 29.4, 29.2 (2 carbons), 28.7, 28.6, 26.0, 22.5, 13.3, 12.9; ESI/APCI calcd for $\text{C}_{18}\text{H}_{22}\text{N}_3\text{O}_2^+$ ($[\text{M}]^+$) m/z 368.2333; measured m/z 368.2342.

3-Butyl-1-decyl-4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride (53b). Please refer to the general procedure for N-3 alkylation. Compound **53b** was obtained in 87% yield. ^1H NMR (CD_3OD , 300 MHz) δ 8.35 (dd, $J = 8.9, 2.4$ Hz, 2H), 8.02 (dd, $J = 9.3, 2.4$ Hz, 2H), 5.07 (t, $J = 7.2$ Hz, 2H), 5.06 (t, $J = 7.2$ Hz, 2H), 2.1 (m, 4H), 1.2 - 1.6 (m, 16H), 1.03 (t, $J = 7.2$ Hz, 3H), 0.88 (t, $J = 6.9$ Hz, 3H); ^{13}C NMR (CD_3OD , 100 MHz) δ 172.8 (2 carbons), 135.9 (4 carbons), 132.8 (2 carbons), 127.8 (2 carbons), 54.3, 54.1, 31.8, 30.6, 29.4, 29.22, 29.20, 28.73, 28.65, 26.0, 22.5, 19.3, 13.2, 12.4; ESI/APCI calcd for $\text{C}_{24}\text{H}_{34}\text{N}_3\text{O}_2^+$ ($[\text{M}]^+$) m/z 396.2646; measured m/z 396.2651.

1,3-Didecyl-4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride (53f). Please refer to the general procedure for N-3 alkylation. Compound **53f** was obtained in 93% yield. ¹H NMR (CD₃OD, 300 MHz) δ 8.35 (dd, *J* = 9.3, 2.4 Hz, 2H), 8.02 (dd, *J* = 9.3, 2.4 Hz, 2H), 5.06 (t, *J* = 7.2 Hz, 4H), 2.1 (m, 4H), 1.2 - 1.5 (m, 28H), 0.88 (t, *J* = 7.2 Hz, 6H); ¹³C NMR (CD₃OD, 100 MHz) δ 172.7 (2 carbons), 135.9 (4 carbons), 132.8 (2 carbons), 127.9 (2 carbons), 54.3 (2 carbons), 31.8 (2 carbons), 29.4 (2 carbons), 29.3 (2 carbons), 29.2 (2 carbons), 28.8 (2 carbons), 28.6 (2 carbons), 26.0 (2 carbons), 22.5 (2 carbons), 13.3 (2 carbons); ESI/APCI calcd for C₃₀H₄₆N₃O₂⁺ ([M]⁺) *m/z* 480.3585; measured *m/z* 480.3588.

1-Dodecyl-3-ethyl-4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride (54a). Please refer to the general procedure for N-3 alkylation. Compound **54a** was obtained in 99% yield. ¹H NMR (CD₃OD, 300 MHz) δ 8.35 (dd, *J* = 8.9, 2.4 Hz, 2H), 8.02 (dd, *J* = 8.6, 2.4 Hz, 2H), 5.11 (q, *J* = 7.2 Hz, 2H), 5.04 (t, *J* = 7.2 Hz, 2H), 2.1 (m, 2H), 1.73 (t, *J* = 7.2 Hz, 3H), 1.2 - 1.5 (m, 18H), 0.88 (t, *J* = 6.9 Hz, 3H); ¹³C NMR (CD₃OD, 100 MHz) δ 172.8, 172.7, 135.9 (4 carbons), 132.8 (2 carbons), 127.8 (2 carbons), 54.3, 50.2, 31.9, 29.5 (2 carbons), 29.4, 29.3, 29.2, 28.8, 28.7, 26.0, 22.5, 13.2, 12.9; ESI/APCI calcd for C₂₄H₃₄N₃O₂⁺ ([M]⁺) *m/z* 396.2646; measured *m/z* 396.2639.

3-Butyl-1-dodecyl-4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride (54b). Please refer to the general procedure for N-3 alkylation. Compound **54b** was obtained in 93% yield. ¹H NMR (CD₃OD, 300 MHz) δ 8.33 (dd, *J* = 9.3, 2.4 Hz, 2H), 8.00 (dd, *J* = 8.9, 2.4 Hz, 2H), 5.04 (t, *J* = 7.2 Hz, 2H), 5.03 (t, *J* = 7.2 Hz, 2H), 2.1 (m, 4H), 1.2 - 1.6 (m, 20H), 1.01 (t, *J* = 7.2 Hz, 3H), 0.87 (t, *J* = 6.8 Hz, 3H); ¹³C NMR

(CD₃OD, 100 MHz) δ 172.8 (2 carbons), 135.9 (2 carbons), 135.8 (2 carbons), 132.8 (2 carbons), 127.8 (2 carbons), 54.3, 54.1, 31.9, 30.5, 29.5 (2 carbons), 29.4, 29.3, 29.2, 28.7, 28.6, 26.0, 22.5, 19.3, 13.2, 12.5; ESI/APCI calcd for C₂₆H₃₈N₃O₂⁺ ([M]⁺) *m/z* 424.2959; measured *m/z* 424.2958.

1-Dodecyl-3-pentyl-4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride (54c). Please refer to the general procedure for N-3 alkylation. Compound **54c** was obtained in 99% yield. ¹H NMR (CD₃OD, 300 MHz) δ 8.36 (dd, *J* = 9.3, 2.4 Hz, 2H), 8.02 (dd, *J* = 8.9, 2.4 Hz, 2H), 5.07 (t, *J* = 7.2 Hz, 4 H), 2.1 (m, 4 H), 1.2 - 1.6 (m, 22H), 0.95 (t, *J* = 6.8 Hz, 3H), 0.88 (t, *J* = 6.8 Hz, 3H); ¹³C NMR (CD₃OD, 100 MHz) δ 172.7 (2 carbons), 136.0 (2 carbons), 135.9 (2 carbons), 132.8 (2 carbons), 127.9 (2 carbons), 54.3 (2 carbons), 31.9, 29.5 (2 carbons), 29.4, 29.3 (2 carbons), 28.74, 28.69, 28.4, 28.1, 26.0, 22.5, 21.8, 13.2, 12.9; ESI/APCI calcd for C₂₇H₄₀N₃O₂⁺ ([M]⁺) *m/z* 438.3128; measured *m/z* 438.3122.

1-Dodecyl-3-hexyl-4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride (54d). Please refer to the general procedure for N-3 alkylation. Compound **54d** was obtained in 88% yield. ¹H NMR (CD₃OD, 300 MHz) δ 8.35 (dd, *J* = 9.3, 2.4 Hz, 2H), 8.02 (dd, *J* = 9.3, 2.4 Hz, 2H), 5.06 (t, *J* = 7.2 Hz, 4H), 2.1 (m, 4H), 1.2 - 1.6 (m, 24H), 0.8 - 1.0 (m, 6H); ¹³C NMR (CD₃OD, 100 MHz) δ 172.7 (2 carbons), 136.0 (2 carbons), 135.9 (2 carbons), 132.8 (2 carbons), 127.9 (2 carbons), 54.4 (2 carbons), 31.9, 31.0, 29.5 (2 carbons), 29.4, 29.3 (2 carbons), 28.8, 28.7 (2 carbons), 26.0, 25.7, 22.5, 22.3, 13.2, 13.1; ESI/APCI Calcd for C₂₈H₄₂N₃O₂⁺ ([M]⁺) *m/z* 452.3273; measured *m/z* 452.3273.

1-Dodecyl-3-octyl-4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride (54e). Please refer to the general procedure for N-3 alkylation. Compound **54e** was obtained in 52% yield. ¹H NMR (CD₃OD, 300 MHz) δ 8.37 (dd, *J* = 9.3, 2.4 Hz, 2H), 8.05 (dd, *J* = 9.3, 2.4 Hz, 2H), 5.09 (t, *J* = 7.2 Hz, 4 H), 2.1 (m, 4 H), 1.2 - 1.5 (m, 28H), 0.9 (m, 6H); ¹³C NMR (CD₃OD, 100 MHz) δ 172.6 (2 carbons), 136.1 (2 carbons), 135.9 (2 carbons), 132.7 (2 carbons), 127.9 (2 carbons), 54.4 (2 carbons), 31.9, 31.7, 29.5 (2 carbons), 29.4, 29.3 (2 carbons), 29.0, 28.8 (4 carbons), 26.0 (2 carbons), 22.52, 22.48, 13.2 (2 carbons); ESI/APCI calcd for C₃₀H₄₆N₃O₂⁺ ([M]⁺) *m/z* 480.3585; measured *m/z* 480.3580.

3-Decyl-1-dodecyl-4,9-dioxo-4,9-dihydro 1*H*-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride (54f). Please refer to the general procedure for N-3 alkylation. Compound **54f** was obtained in 99% yield. ¹H NMR (CD₃OD, 300 MHz) δ 8.36 (dd, *J* = 8.9, 2.4 Hz, 2H), 8.03 (dd, *J* = 8.9, 2.4 Hz, 2H), 5.07 (t, *J* = 7.2 Hz, 4 H), 2.1 - 2.2 (m, 4 H), 1.2 - 1.5 (m, 32H), 0.88 (t, *J* = 6.9 Hz, 6H); ¹³C NMR (CD₃OD, 100 MHz) δ 172.7 (2 carbons), 136.0 (4 carbons), 132.8 (2 carbons), 127.9 (2 carbons), 54.4 (2 carbons), 31.9 (2 carbons), 29.4 (2 carbons), 29.3 (4 carbons), 28.8 (2 carbons), 28.7 (2 carbons), 26.0 (2 carbons), 22.5 (2 carbons), 13.2 (2 carbons); ESI/APCI calcd for C₃₂H₅₀N₃O₂⁺ ([M]⁺) *m/z* 508.3898; measured *m/z* 508.3896.

3-Methyl-1-hexyl-1*H*-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride (69).

Compound **69** was synthesized according to the protocol described in reference 115. ¹H NMR (CD₃OD, 300 MHz) δ 8.3 - 8.4 (m, 2H), 8.0 (m, 2H), 5.04 (t, *J* = 7.2 Hz, 2H), 4.68 (s, 3H), 2.1 (m, 2H), 1.3 - 1.6 (m, 6H), 0.93 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (CD₃OD, 100

MHz) δ 172.72, 172.65, 136.2, 136.0 (2 carbons), 135.6, 132.9, 132.7, 127.9, 127.8, 54.3, 39.8, 31.0, 28.8, 25.7, 22.2, 13.1; ESI/APCI calcd for $C_{17}H_{20}N_3O_2^+$ ($[M]^+$) m/z 298.1550; measured m/z 298.1560.

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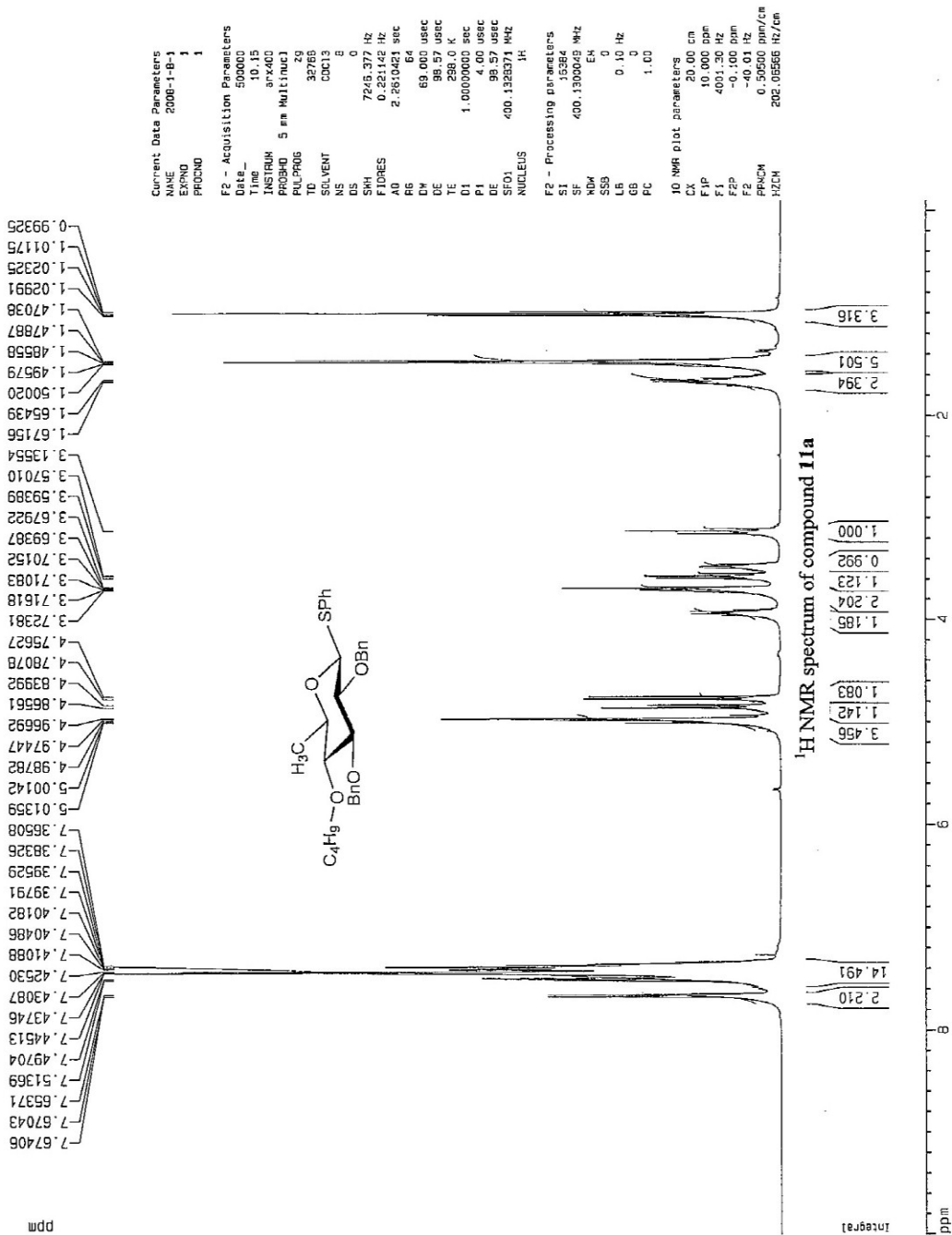
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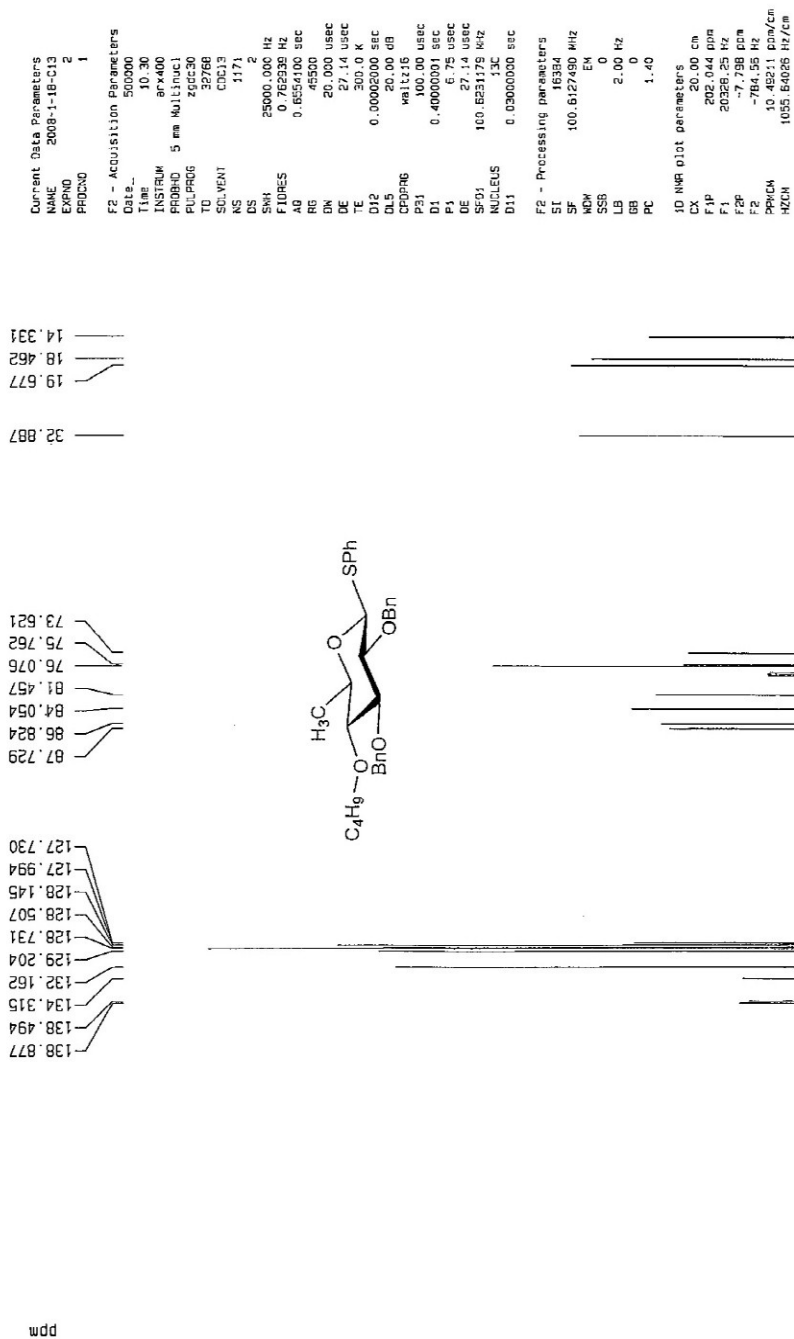
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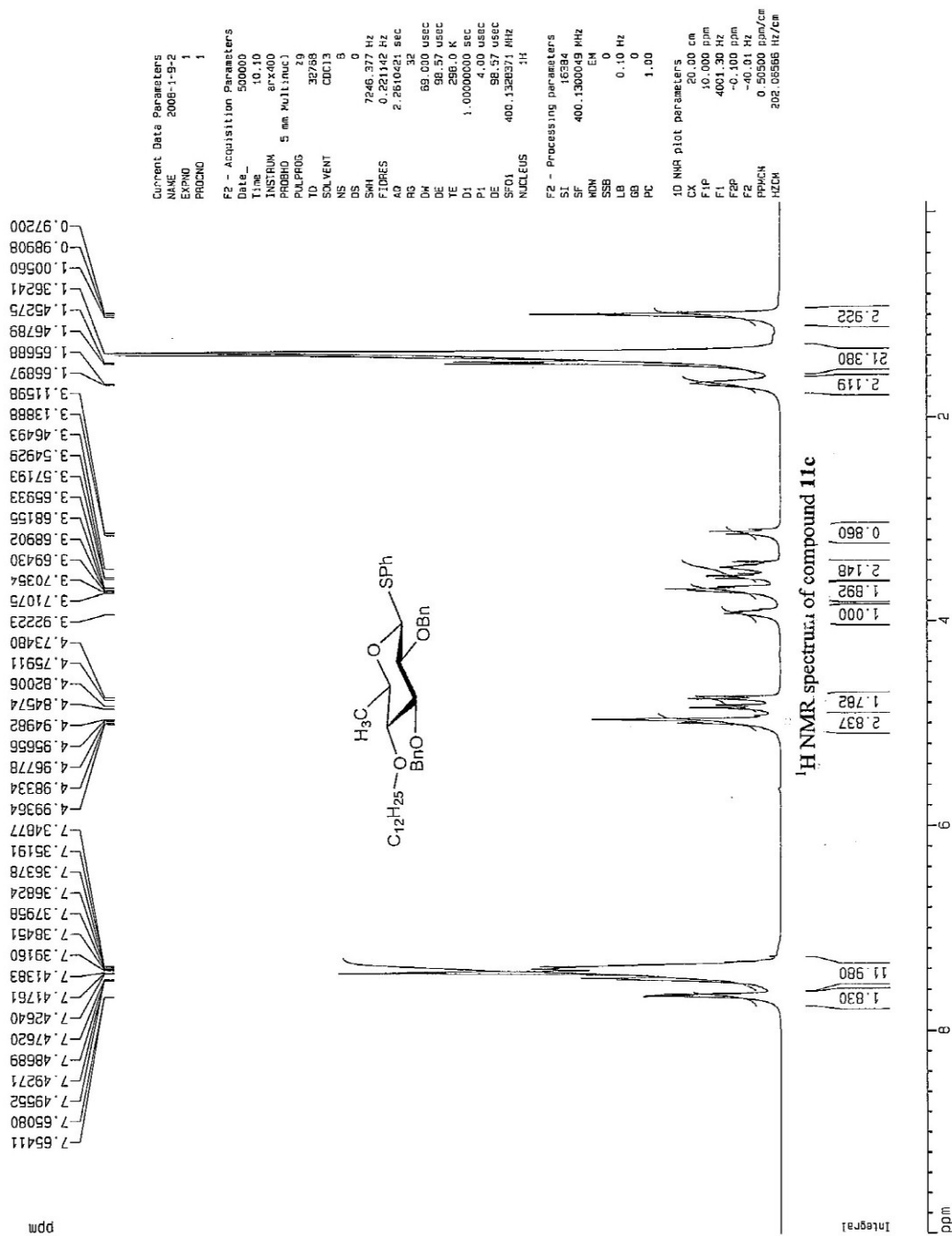
APPENDICES

Appendix A. ^1H NMR and ^{13}C NMR Spectra for Selected Compounds



Standard ¹³C
Experiment





Standard ¹³C
Experiment

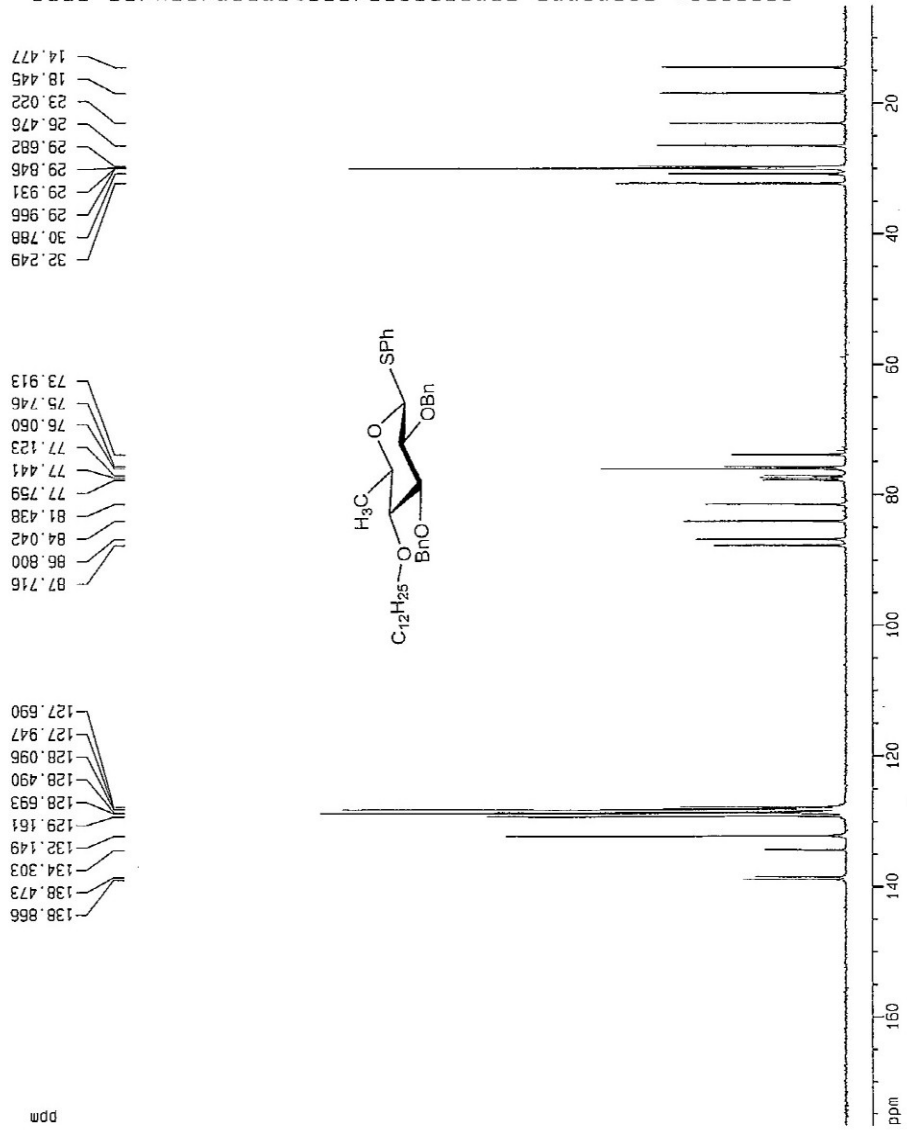
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Current Data Parameters
NAME 2009-1-9-2-C13
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 500000
Time 10.24
INSTRUM ark400
PROBHD 5 mm Multinucl
PULPROG zgpg30
TO 32768
SOLVENT CDCl3
NS 647
DS 2
SWH 25000.000 Hz
FIDRES 0.762938 Hz
AQ 0.6554100 sec
RG 48500
DF 20.000 usec
TE 300.2 K
TE2 300.2 K
D12 0.0002000 sec
DL5 20.00 dB
COPROG waltz16
P31 100.00 usec
O1 0.40000001 sec
P1 5.75 usec
DE 27.14 usec
SFO1 100.6231179 MHz
NUCLEUS 13C
D11 0.03000000 sec

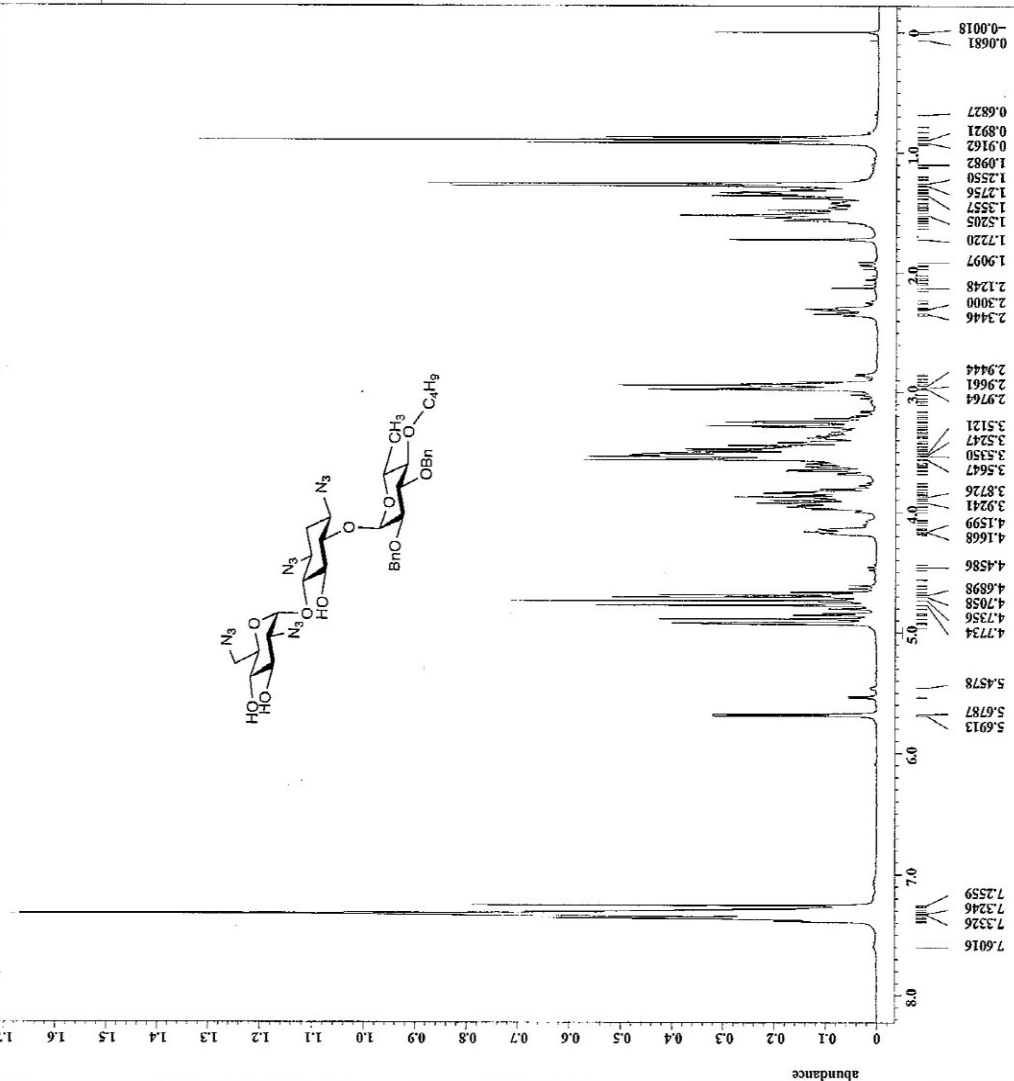
F2 - Processing parameters
SI 16384
SF 100.6127490 MHz
WDW EM
SSB 0
LB 2.00 Hz
GB 0
PC 1.40

1D NMR plot parameters
CX 20.00 cm
FAP 176.793 dB
F1 17767.56 Hz
F2P 5.081 dB
F2 511.17 Hz
PPHCH 0.595560 dB/cx
HZCHK 893.82104 Hz/cx
  
```





Filename = single_pulseNF-148-5
 Author = fasso
 Experiment = single_pulse.ex2
 Sample_id = NF48
 Solvent = CDCl3
 Date_Exp = 4-FEB-2008 10:26:06
 Revision_time = 18-MAY-2009 18:35:59
 Current_time = 18-MAY-2009 18:36:06
 Comment = single_pulse
 Data_format = 1D_COMPLEX
 Dim_size = 13107
 Dim_title = 1H
 Dim_units = [ppm]
 Dimensions = X 300
 Spectrometer = ECTX-300
 Field_strength = 7.0586013 [T] (300 [MHZ])
 X_acq_duration = 2.90717696 [s]
 X_domain = 10.52965592 [MHZ]
 X_offset = 5 [ppm]
 X_points = 16384
 X_prescans = 1
 X_resolution = 0.34397631 [Hz]
 X_sosolve = 1
 Irf_domain = 1H 637.0784 [kHz]
 Irf_freq = 300.52965592 [MHZ]
 Irf_offset = 5 [ppm]
 T1_domain = 1H
 T1_freq = 300.52965592 [MHZ]
 T1_offset = 5 [ppm]
 Clipped = FALSE
 Mod_return = 1
 Scans = 8
 Total_scans = 8
 X_90_width = 13.43 [us]
 X_acq_time = 2.90717696 [s]
 X_angle = 45 [deg]
 X_atn = 3 [dB]
 X_pulse = 6.715 [us]
 T1_mode = OFF
 Dante_Preset = FALSE
 Initial_wait = 1 [s]
 Recvr_gain = 40
 Receiver_delay = 1 [us]
 Repetition_time = 3.90717696 [s]
 Temp_set = 22.6 [degC]



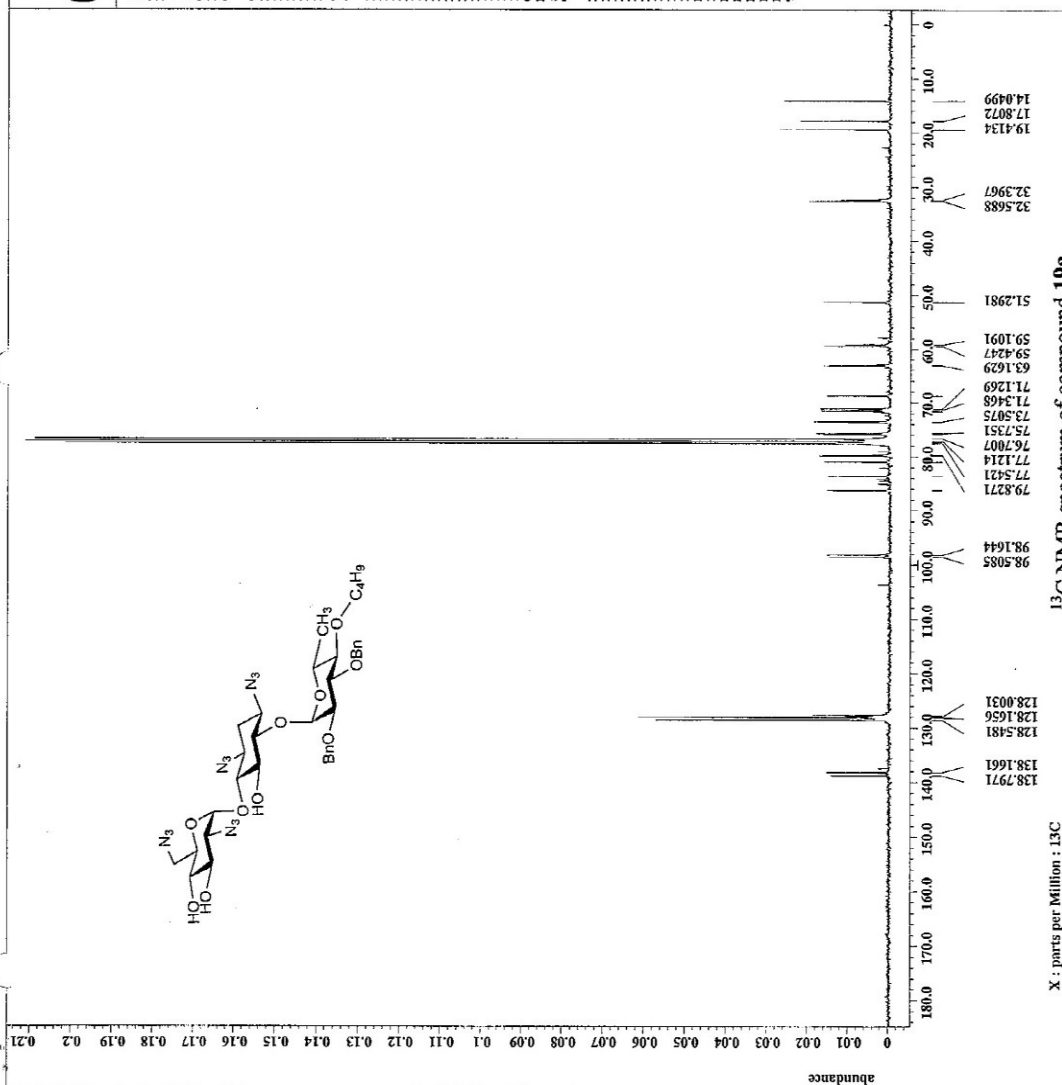
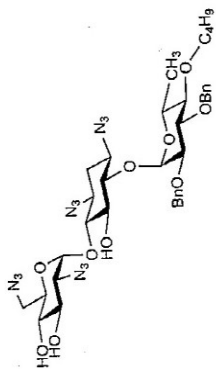
¹H NMR spectrum of compound 19a

abundance



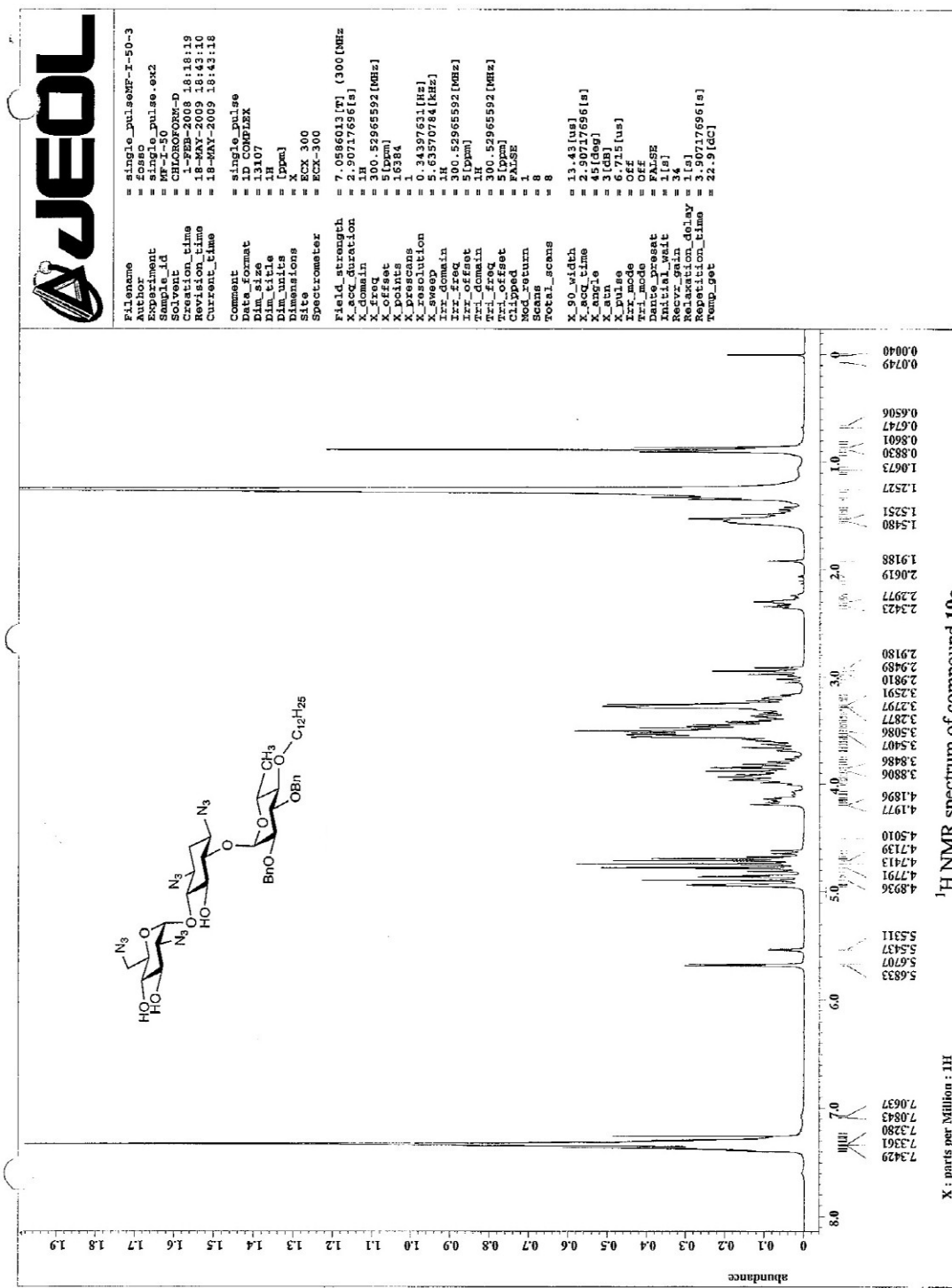
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Experiment = single_pulse_dec
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Pulse Program = zgpg30mf-d
Creation_time = 5-FEB-2008 05:59:18
Revision_time = 18-MAY-2009 15:00:49
Current_time = 18-MAY-2009 15:01:01
Comment = single_pulse decouple
Data Format = 1D_COMPLEX
Dir_size = 26214
Dir_title = 13C
Dir_units = [ppm]
Dimensions = X 300
Spectrometer = ECPX-300
Field_strength = 7.6586013 [T] (300 [MHz])
X_acq_duration = 1.38412032 [s]
X_domain = 75.56823426 [MHz]
X_offset = 100 [ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054 [Hz]
X_sweep_rate = 14.67424242 [kHz]
IR_freq = 300.52965592 [MHz]
IR_offset = 5 [ppm]
Clipped = FALSE
Acq_return = 1
SOLVENT = CDCl3
TOTAL_scans = 10000
X_90_width = 10.12 [us]
X_acq_time = 1.38412032 [s]
X_delay = 6 [ms]
X_atn = 6 [dB]
X_pulse = 3.37333333 [us]
IR_atn_dec = 23 [dB]
IR_atn_rec = 23 [dB]
IR_gain = 1 [dB]
WALTZ = WALTZ
Initial_wait = 1 [s]
Nce = TRU8
Nce_time = 2 [s]
Recvr_gain = 54
Sensitivity_delay = 1 [s]
Repetition_time = 1.38412032 [s]
temp_get = 32.5 [dC]
  
```



¹³C NMR spectrum of compound 19a

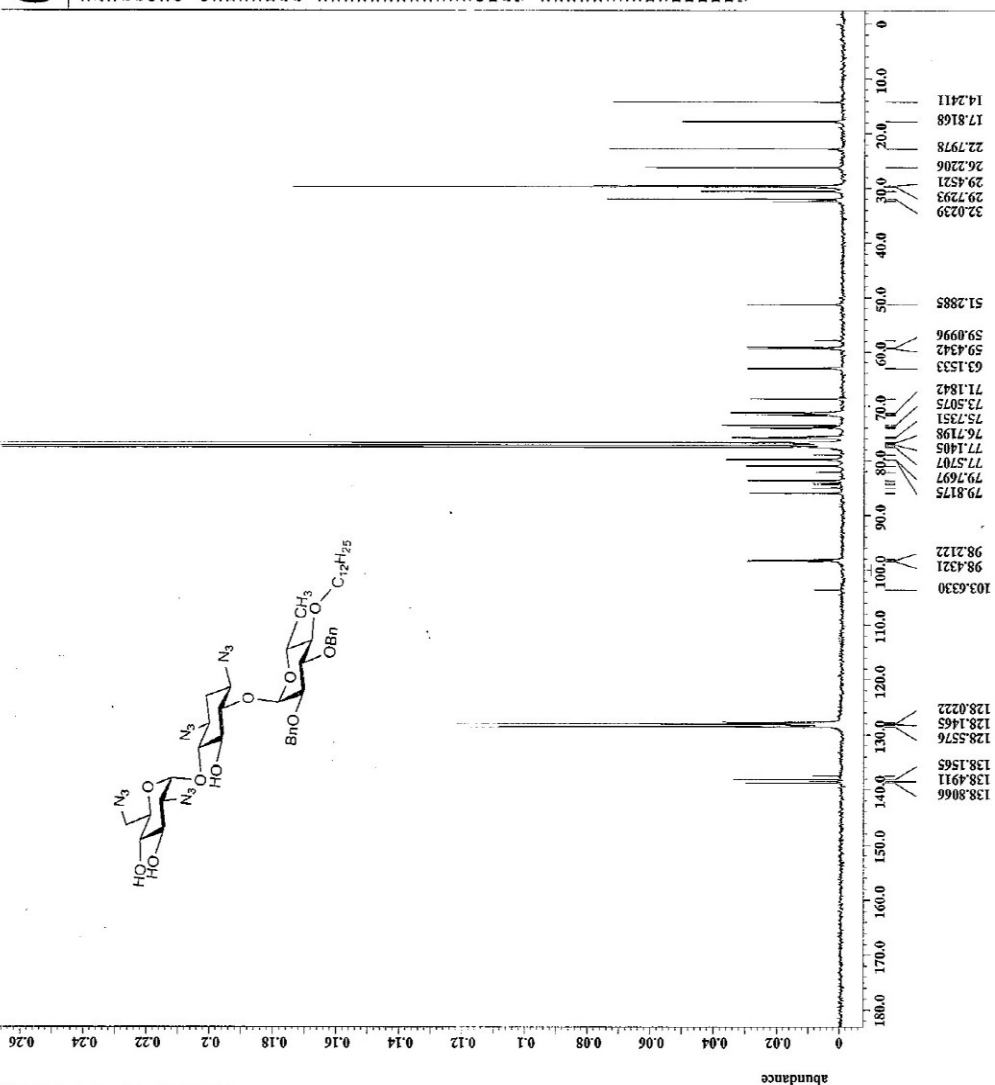
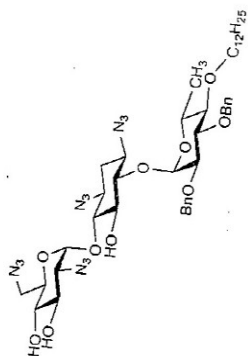
X : parts per Million : 13C





```

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Author = foso
Experiment = single_pulse_dec
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Solvent = CDCl3FORM-D
Creation time = 2-FEB-2009 02:06:10
Revision time = 18-MAY-2009 19:01:34
Current time = 18-MAY-2009 19:01:42
Comment = single_pulse decouple
          ID: COMPLEX
Data format = 46214
Dim_size = 133
Dim_title = [ppm]
Dim_units = X
Dimensions = X 100
Spectrometer = EXX-300
Field_strength = 7.0586013 [T] (300 [MHz]
X_acq_duration = 1.38412032 [s]
X_gain = 1.38412032 [s]
X_freq = 75.56823426 [MHz]
X_offset = 100 [ppm]
X_points = 32758
X_prescans = 4
X_resolution = 0.72248054 [Hz]
X_sensitivity = 14.57424242 [kHz]
Irr_freq = 300.52965592 [MHz]
Irr_offset = 8 [ppm]
Clipped = FALSE
Scan_return = 8000
TOTAL_scans = 8000
X_90_width = 10.12 [us]
X_acq_time = 1.38412032 [s]
X_delay = 8 [ppm]
X_atn = 8 [dB]
X_pulse = 5.37333333 [us]
Irr_atn_dec = 23 [dB]
Irr_atn_noc = 23 [dB]
Pulsprog = WALTZ
Tactical_wait = 1 [s]
Noc = TRUE
Roc_time = 2 [s]
Recvr_gain = 56
Repetition_delay = 3.38412032 [s]
Repetition_time = 3.38412032 [s]
Temp_get = 22.8 [dC]
    
```



¹³C NMR spectrum of compound 19

X : parts per Million : 13C

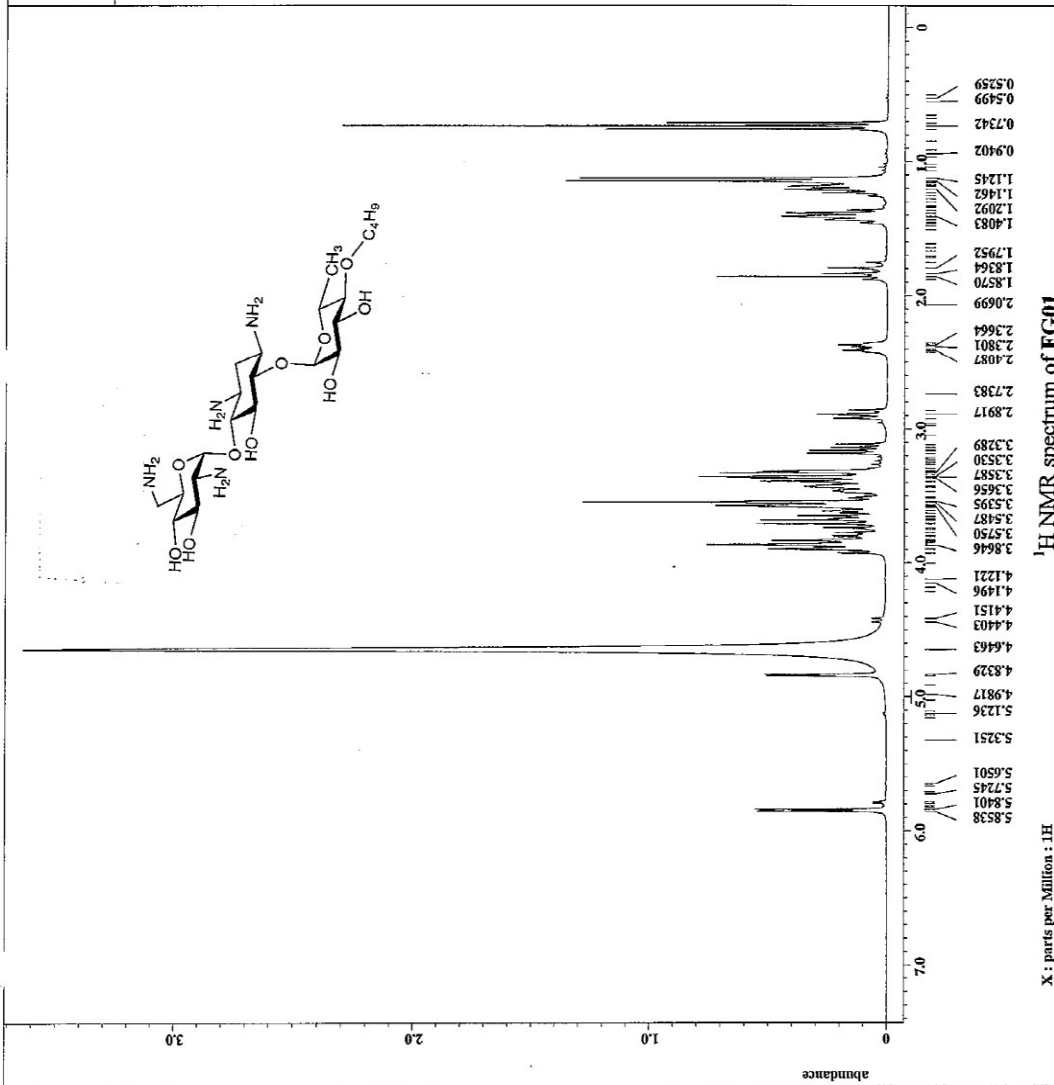
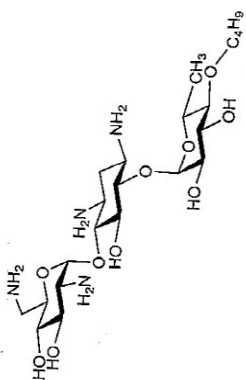


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Relaxation_time = 16-MAR-2008 16:06:55
Current_time = 18-FEB-2010 18:49:30

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Comment = single_pulse
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Pulse_program = zgpg30
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Dim_units = [ppm]
Dimensions = X
Site = ECK 300
Spectrometer = ECK-300

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X_acq_duration = 2.90717696[s]
X_domain = 1H
X_freq = 300.52965592[Mhz]
X_offset = 16284
X_p1 = 16284
X_prescans = 1
X_resolution = 0.34397631[Hz]
X_sweep = 5.63570784[Mhz]
Xr_domain = 1H
Xr_freq = 300.52965592[Mhz]
Xr_offset = 51ppm
Xr_domain = 1H
Xr_freq = 300.52965592[Mhz]
Xr_offset = 51ppm
Mod_return = 1
Scans = 8
Total_scans = 8
X_90_width = 13.43[us]
X_acq_time = 2.90717696[s]
X_angle = 45[deg]
X_atn = 3[db]
X_pulse = 6.715[us]
Xr_mode = Off
Xr_mode = Off
Data_preset = FARGE
Initial_wait = 1[s]
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Relaxation_delay = 1[s]
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=====
  
```



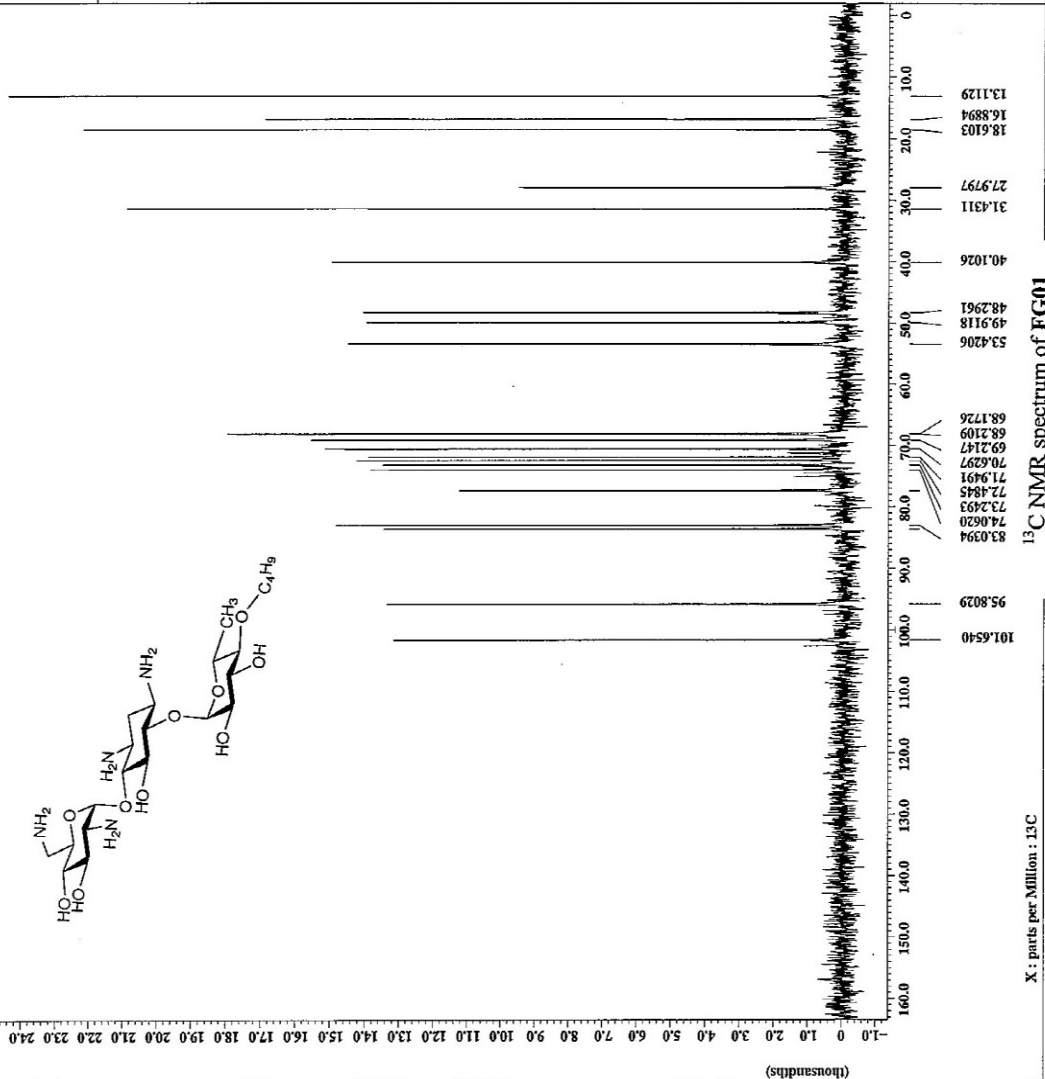
¹H NMR spectrum of FG01

X : parts per Million : 1H



```

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Dim_2 = 1
Dim_units = [ppm]
Dimensions = X
Site = ECK 300
Spectrometer = ECK-300
Field_strength = 7.0586013[G] (300[MHZ]
X_domain = 1.38412032[s]
X_duration = 13C
X_freq = 75.56823426[MHz]
X_offset = 100[ppm]
X_phase = 27.98
X_pulses = 4
X_resolution = 0.72248054[Hz]
X_sweep = 1H
X_domain = 22.67424242[MHz]
X_offset = 0.00000000[MHz]
X_phase = 50.00000000[deg]
X_resolution = 5.00000000[MHz]
Clipped = FALSE
Mod_return = 1
Scans = 7000
Total_scans = 7000
X_90_width = 10.12[us]
X_acq_time = 1.38412032[s]
X_angle = 30[deg]
X_delay = 3.00000000[s]
X_pulses = 313133333[us]
Irr_atn_dec = 23[dB]
Irr_atn_moe = 23[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Attain_wait = 1[s]
Noe_time = 2[s]
Recvr_gain = 50
Relaxation_delay = 2[s]
Relaxation_time = 1.38412032[s]
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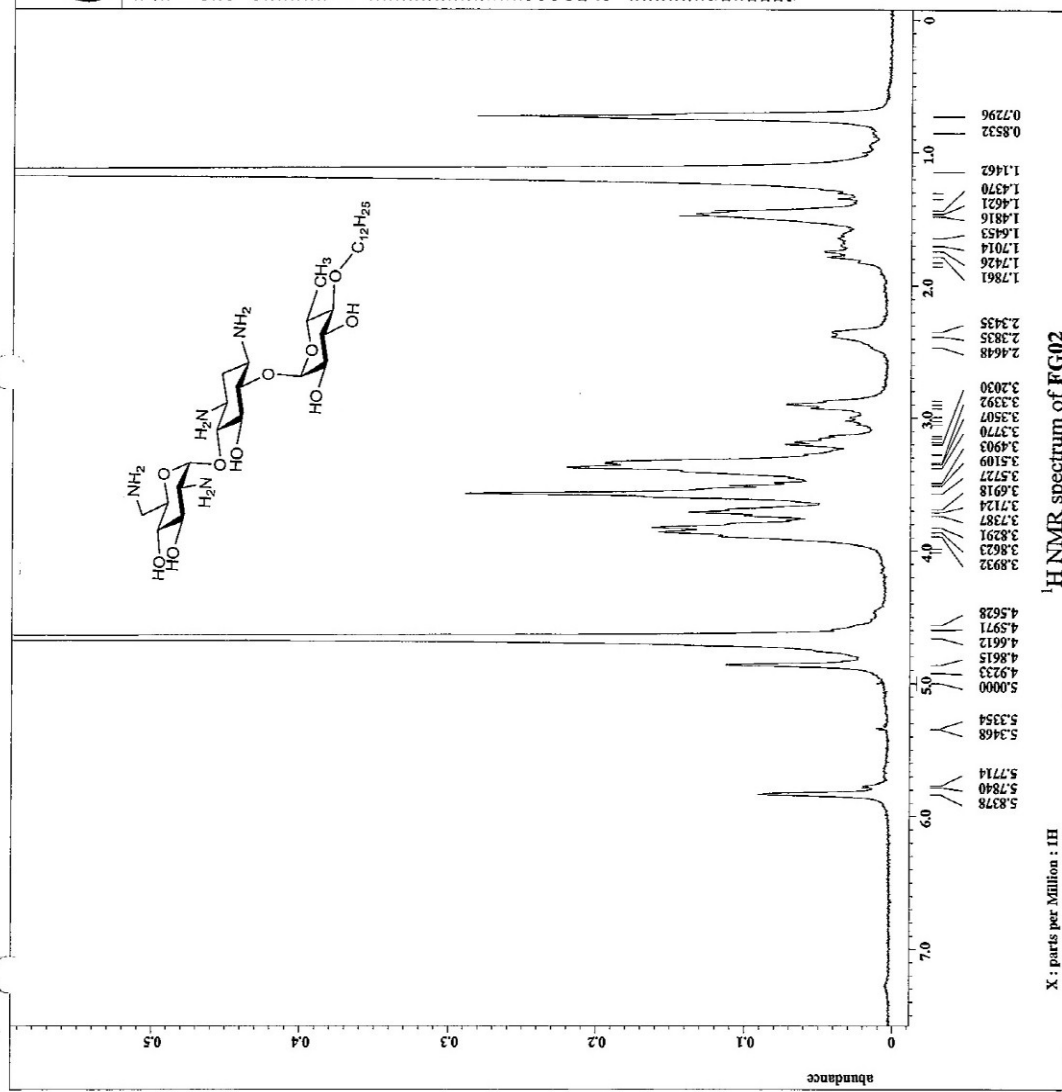
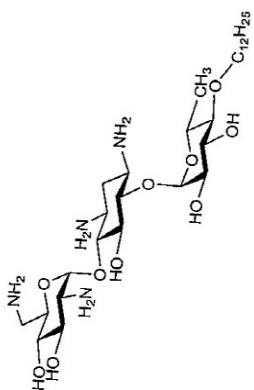
¹³C NMR spectrum of FG01

X : parts per Million : 13C



```

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= 18-FEB-2010 18:53:58
= 18-FEB-2010 18:53:58
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= ID_COMPLEX
= 1107
= 1H
= [ppm]
= X
= EXC 300
= EXC-300
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X_sweep = 5.63570784 [MHz]
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X_stop_freq = 5 [ppm]
X_tri_domain = 1H
X_tri_freq = 300.52965592 [MHz]
X_tri_offset = 5 [ppm]
X_tri_offset = 1
Mod_return = 1
Scans = 8
Total_scans = 8
X_90_width = 13.43 [us]
X_acq_time = 2.90717696 [s]
X_angle = 45 [deg]
X_atn = 3 [dB]
X_pulse = 6.715 [us]
X_pulse_mode = OF
X_tri_mode = OF
Data_preset = FALSE
Initial_wait = 1 [s]
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```





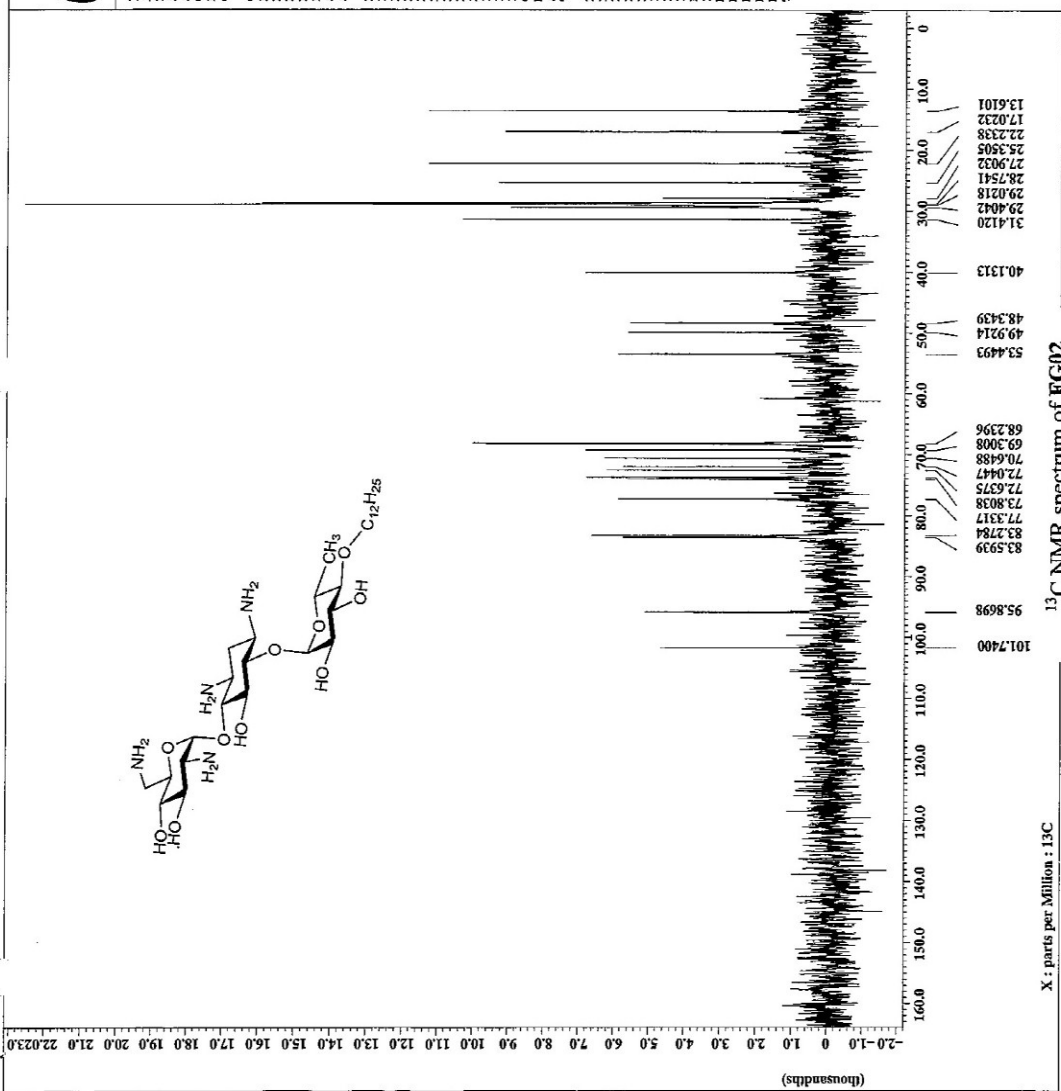
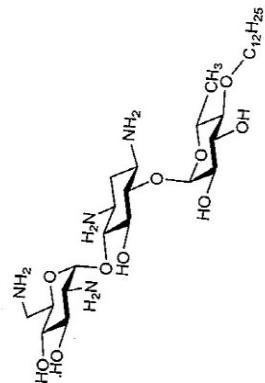
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Acquisition_time = 18-FEB-2010 18:29:33
Current_time  = 18-FEB-2010 18:29:33

Comment       = single pulse decouple
Data_format   = 1D COMPLEX
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Dim_title     = [ppm]
Dim_units     = X
Dimensions    = X
Site          = ECK 300
Spectrometer  = ECK-300

Field_strength = 7.0586013[C] (300[MHz]
X_acq_duration = 1.38412032[s]
X_domain       = 13C
X_freq         = 75.56823436[MHz]
X_offset       = 101[ppm]
X_prescans    = 32788
X_resolution   = 4
X_sweep       = 0.72248054[Hz]
X_sweep_rate  = 22.67424242[MHz]
Irr_domain    = 10
Irr_offset    = 50[ppm]
Clipped       = FALSE
Mod_return    = 1
Scans         = 7000
Total_scans   = 7000

X_90_width    = 10.12[us]
X_acq_time    = 1.38412032[s]
X_angle       = 30[deg]
X_pulses      = 3
X_pulse_prog  = 3233333[us]
Irr_atn_dec   = 23[dB]
Irr_atn_noe   = 23[dB]
Irr_noise     = WALZE
Decoupling    = TRUE
Initial_wait  = TRUE
Noe_time      = 2[s]
Reconv_gain   = 56
Relaxation_delay = 2[s]
Operation_time = 1.38412032[s]
Temp_set      = 23.7[degC]
    
```

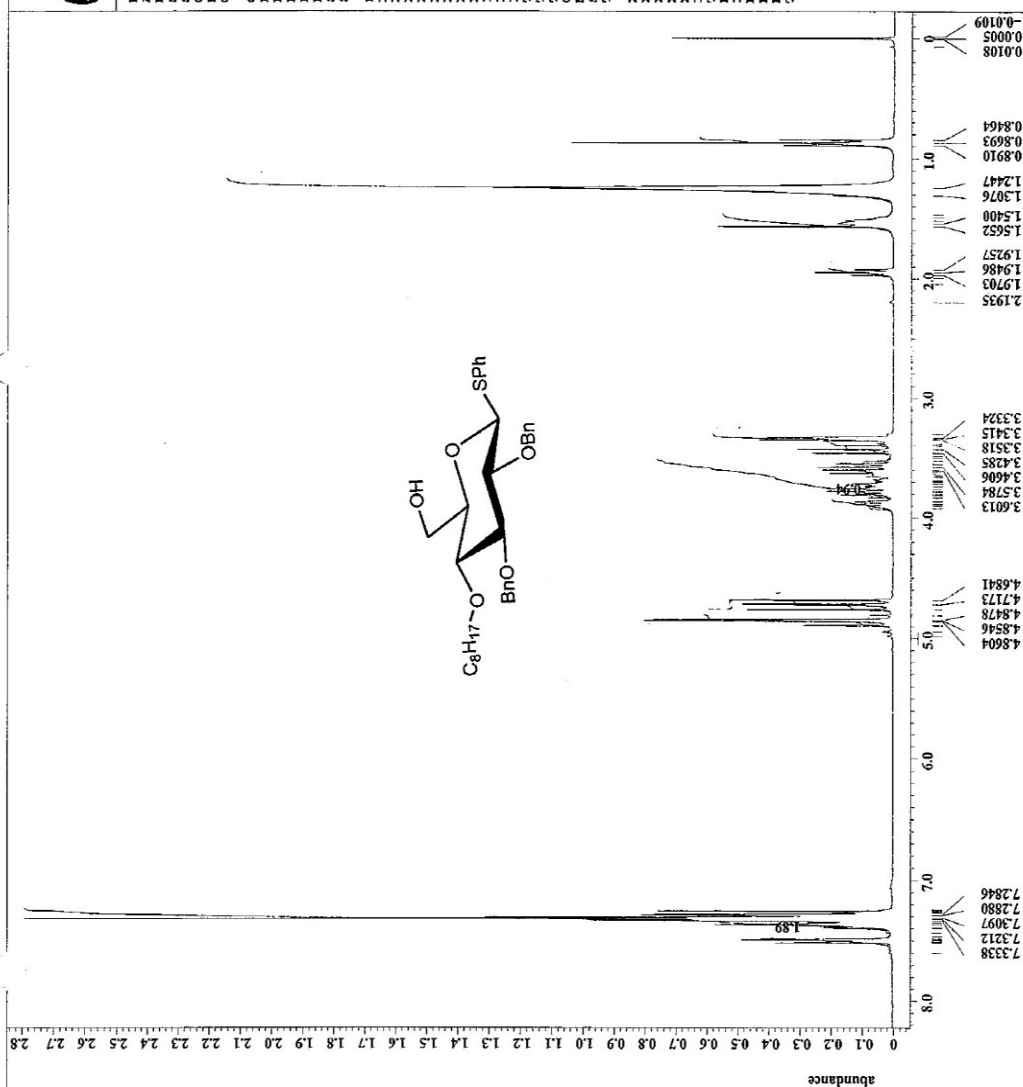


13C NMR spectrum of FG02

X : parts per Million : 13C



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 = CHLOROPROM-D
 = 10-NOV-2009 19:41:01
 = 10-NOV-2009 19:05:34
 = 10-NOV-2009 19:05:47
 = single_pulse
 = ID COMPLEX
 = 13107
 = 1H
 = [ppm]
 = X
 = EXC 300
 = EXC-300
 = EXC-300
 = 7.0586013 [r] (300 [MHz]
 = 2.90717696 [s]
 = 10
 = 300.52965592 [MHz]
 = 5 [ppm]
 = 16384
 = 1
 = 14397631 [Hz]
 = 5.8570784 [MHz]
 = 1H
 = 300.52965592 [MHz]
 = 5 [ppm]
 = 1H
 = 0.0052965592 [MHz]
 = FALSE
 = 1
 = 8
 = 13.43 [us]
 = 2.90717696 [s]
 = 45 [deg]
 = 3 [dB]
 = 6.25 [us]
 = OFF
 = FALSE
 = 1 [s]
 = 44
 = 3.90717696 [s]
 = 23.3 [dC]



¹H NMR spectrum of compound 20

X : parts per Million : 1H

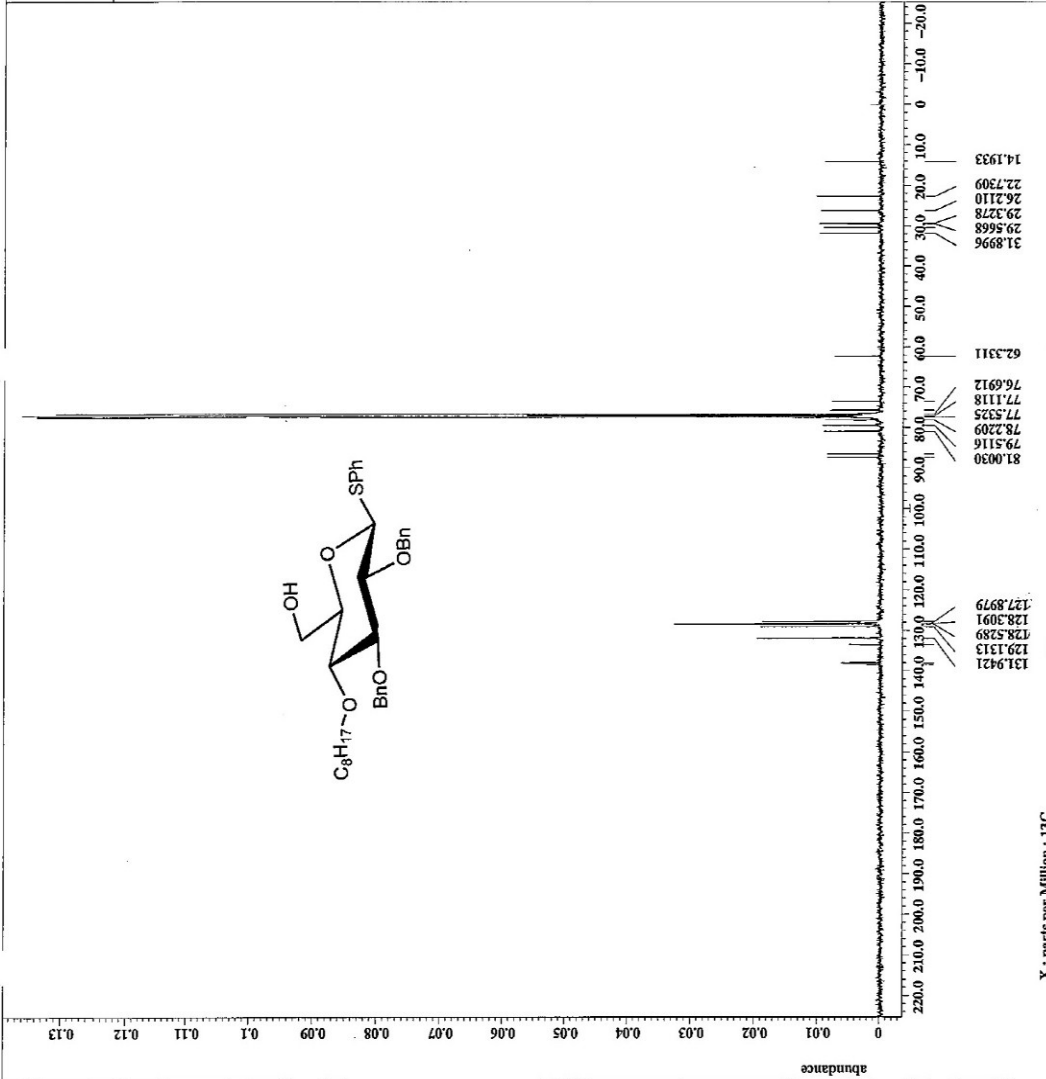
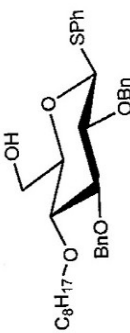


```

File Name      = single_pulse_decoupl-II
Author         = fcaso
Experiment     = single_pulse_dec
Sample ID      = MF-III-59
Solvent        = CHLOROFORM-D
Date_Exp      = 2010.03.25.02
Exp. Time     = 30-APR-2010 18:06:15
Revision Time = 30-APR-2010 18:06:18
Current Time   =

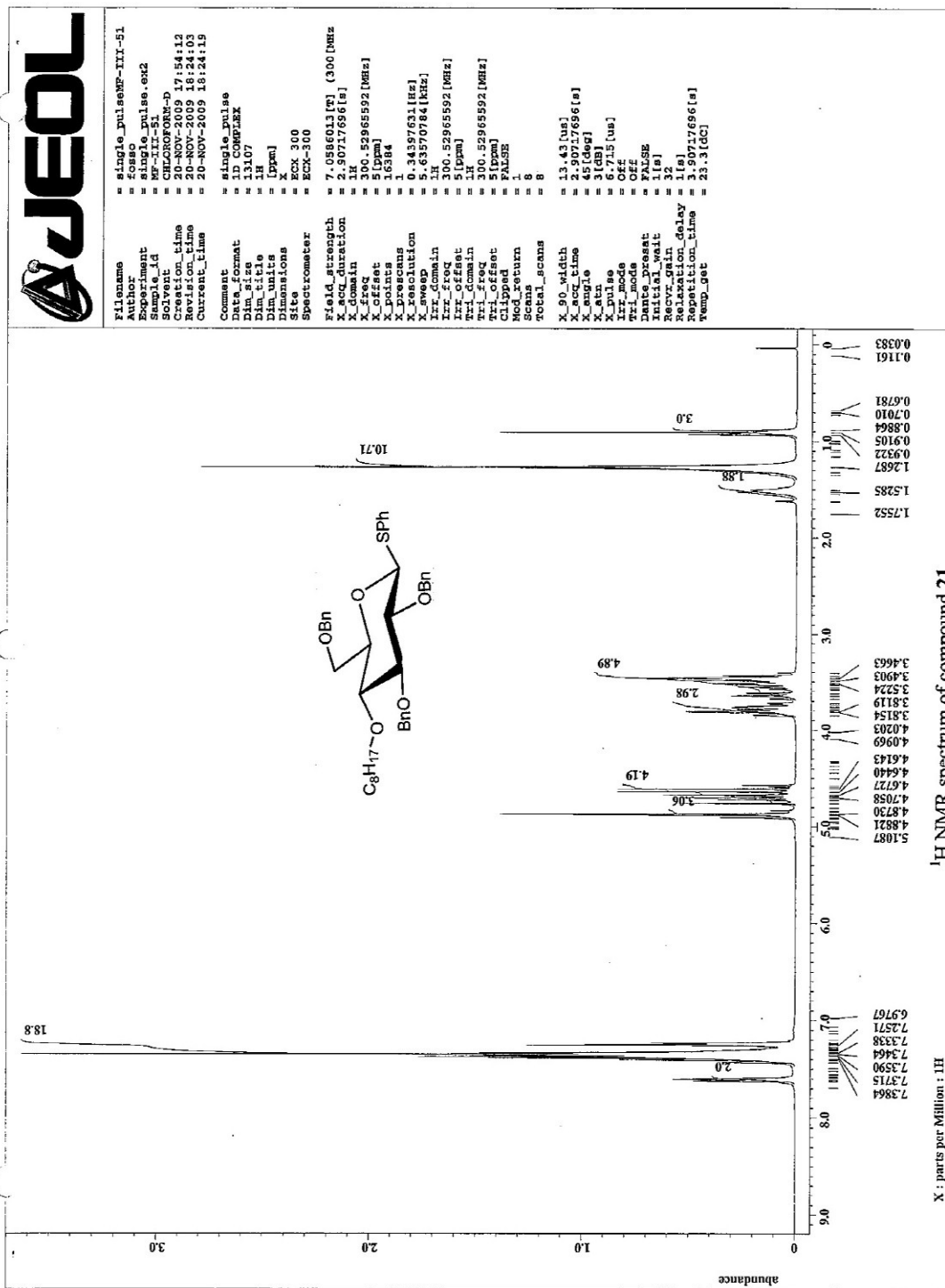
Comment       = single pulse decouple
Proc Format    = 2D COMPLEX
Dim 1         = 13C
Dim 2         = 13C
Dim Units     = [ppm]
Dimensions    = X
Site          = ECK 300
Spectrometer = ECK-300

Field Strength = 7.0586013[T] (300 [MHz]
X_acq_Auration = 1.38412032[s]
X_domain       = 13C
X_freq         = 75.56823426 [MHz]
X_p1          = 180 [ppm]
X_p2          = 32748
X_p3          = 4
X_prescans    = 0.72248054 [Hz]
X_resolution  = 23.67424242 [MHz]
X_sweep       = 300.52965592 [MHz]
X_time        = 5 [Dpm]
X_offset      = FALSE
Clipped       = 1
Mod_return    = 500
Gain          = 5000
Total_scans   = 10.12 [us]
X_90_width   = 1.38412032 [s]
X_acq_time   = 0 [deg]
X_angle      = 3.97333333 [us]
X_pulse      = 23 [dB]
Irr_atn_dec  = 23 [dB]
Irr_atn_pwr = 23 [dB]
Irr_noise    = WALTZ
Prescans     = 1 [s]
Inhibit_wait = TRUE
Noe          = TRUE
Noe_time     = 2 [s]
Recvr_gain   = 50
Relaxation_delay = 2 [s]
Relaxation_time = 23.9 [s]
Temp_rot     = 23.9 [C]
    
```



¹³C NMR spectrum of compound 20

X: parts per Million : 13C

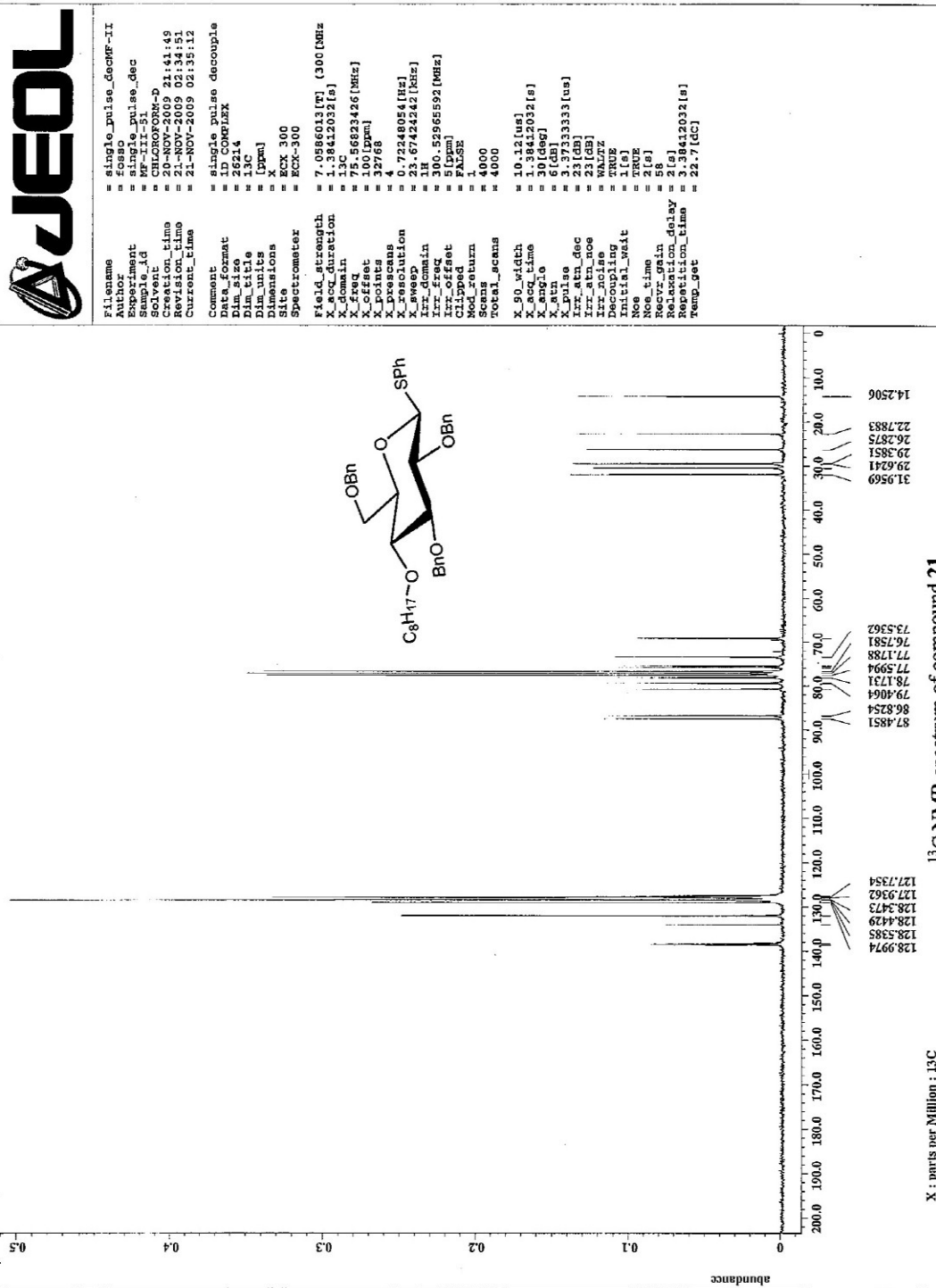


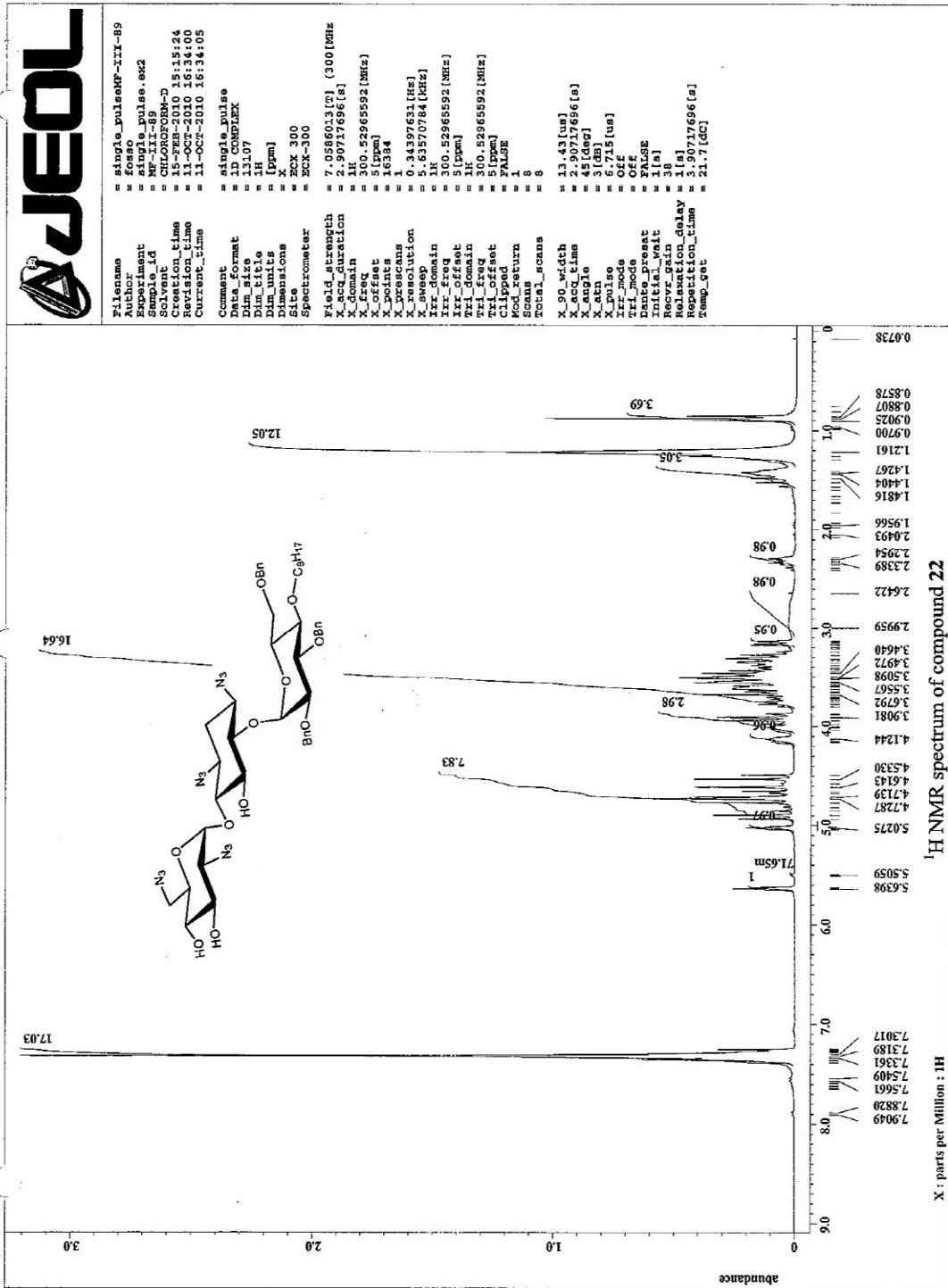
```

=====
Filename      = single_pulseNF-111-51
Author        = fcsso
Experiment    = single_pulse.em2
Sample_ID     = NF-111-51
Date_Exp     = 20-NOV-2009 17:54:12
Creation_time = 20-NOV-2009 17:54:12
Revision_time = 20-NOV-2009 18:24:03
Current_time  = 20-NOV-2009 18:24:15

Comment       = single_pulse
              = INOPREP2X
Data_format   = 13107
Dim_size      = 1H
Dim_title     = [ppm]
Dim_units     = X
Dimensions    = X 300
Spectrometer  = ECK-300

Field_strength = 7.0586013[T] (300 MHz)
X_acq_duration = 2.50717696[s]
X_domain       = 10.52965592 [ppm]
X_offset       = 5 [ppm]
X_points       = 16384
X_prescans     = 1
X_resolution   = 0.34597631 [Hz]
X_sfs          = 51
X1_domain      = 65570784 [kHz]
X2_domain      = 306.52965592 [MHz]
X3_domain      = 5 [ppm]
X4_domain      = 1H
X5_domain      = 300.52965592 [MHz]
X6_domain      = FALSE
X7_domain      = FALSE
X8_domain      = 1
X9_domain      = 8
X10_domain     = 13.43 [us]
X11_domain     = 2.90717696 [s]
X12_domain     = 45 [deg]
X13_domain     = 3 [dB]
X14_domain     = 6.715 [us]
X15_domain     = OFF
X16_domain     = OFF
X17_domain     = FALSE
X18_domain     = FALSE
X19_domain     = 1 [s]
X20_domain     = 1 [s]
X21_domain     = 1 [s]
X22_domain     = 1 [s]
X23_domain     = 1 [s]
X24_domain     = 1 [s]
X25_domain     = 1 [s]
X26_domain     = 1 [s]
X27_domain     = 1 [s]
X28_domain     = 1 [s]
X29_domain     = 1 [s]
X30_domain     = 1 [s]
X31_domain     = 1 [s]
X32_domain     = 1 [s]
X33_domain     = 1 [s]
X34_domain     = 1 [s]
X35_domain     = 1 [s]
X36_domain     = 1 [s]
X37_domain     = 1 [s]
X38_domain     = 1 [s]
X39_domain     = 1 [s]
X40_domain     = 1 [s]
X41_domain     = 1 [s]
X42_domain     = 1 [s]
X43_domain     = 1 [s]
X44_domain     = 1 [s]
X45_domain     = 1 [s]
X46_domain     = 1 [s]
X47_domain     = 1 [s]
X48_domain     = 1 [s]
X49_domain     = 1 [s]
X50_domain     = 1 [s]
X51_domain     = 1 [s]
X52_domain     = 1 [s]
X53_domain     = 1 [s]
X54_domain     = 1 [s]
X55_domain     = 1 [s]
X56_domain     = 1 [s]
X57_domain     = 1 [s]
X58_domain     = 1 [s]
X59_domain     = 1 [s]
X60_domain     = 1 [s]
X61_domain     = 1 [s]
X62_domain     = 1 [s]
X63_domain     = 1 [s]
X64_domain     = 1 [s]
X65_domain     = 1 [s]
X66_domain     = 1 [s]
X67_domain     = 1 [s]
X68_domain     = 1 [s]
X69_domain     = 1 [s]
X70_domain     = 1 [s]
X71_domain     = 1 [s]
X72_domain     = 1 [s]
X73_domain     = 1 [s]
X74_domain     = 1 [s]
X75_domain     = 1 [s]
X76_domain     = 1 [s]
X77_domain     = 1 [s]
X78_domain     = 1 [s]
X79_domain     = 1 [s]
X80_domain     = 1 [s]
X81_domain     = 1 [s]
X82_domain     = 1 [s]
X83_domain     = 1 [s]
X84_domain     = 1 [s]
X85_domain     = 1 [s]
X86_domain     = 1 [s]
X87_domain     = 1 [s]
X88_domain     = 1 [s]
X89_domain     = 1 [s]
X90_domain     = 1 [s]
X91_domain     = 1 [s]
X92_domain     = 1 [s]
X93_domain     = 1 [s]
X94_domain     = 1 [s]
X95_domain     = 1 [s]
X96_domain     = 1 [s]
X97_domain     = 1 [s]
X98_domain     = 1 [s]
X99_domain     = 1 [s]
X100_domain    = 1 [s]
=====
    
```



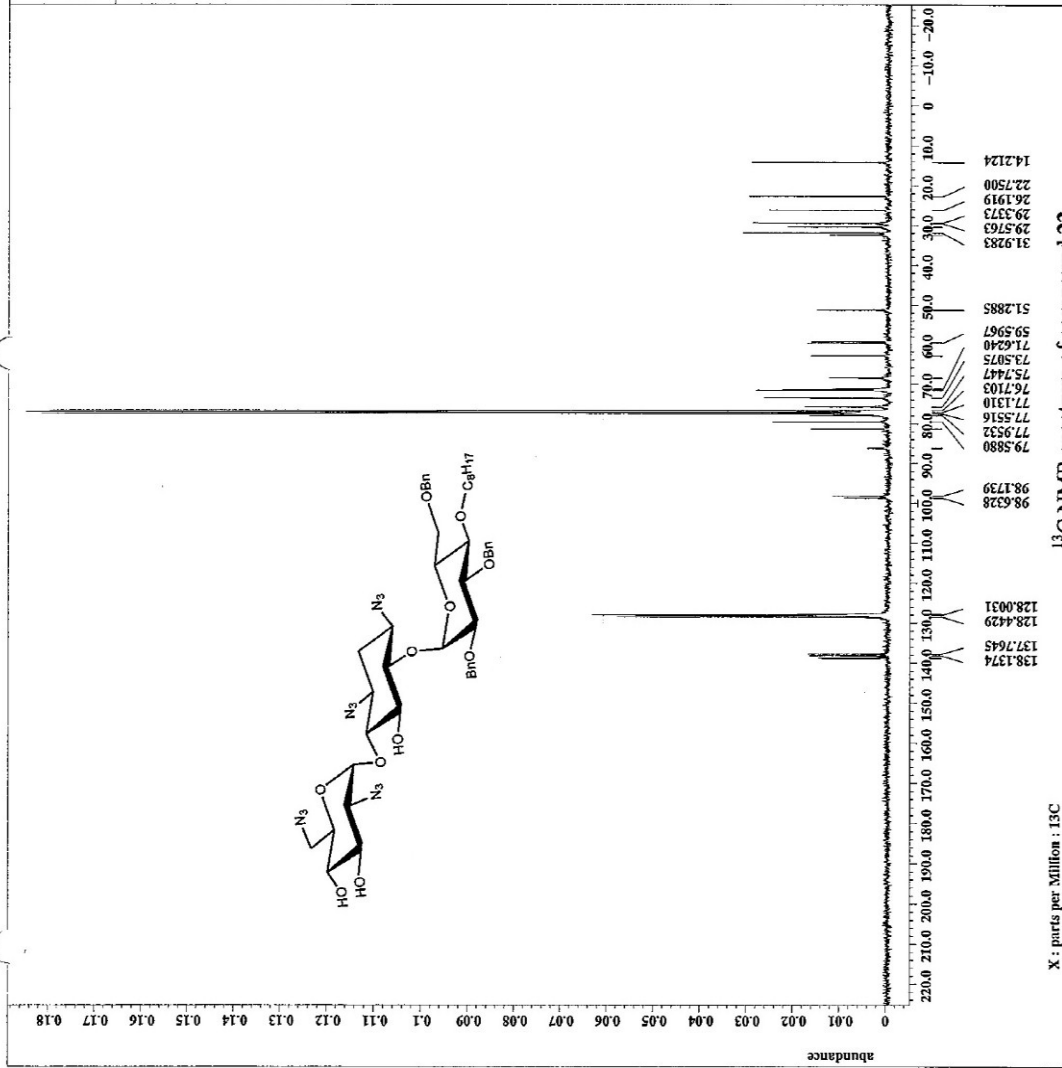
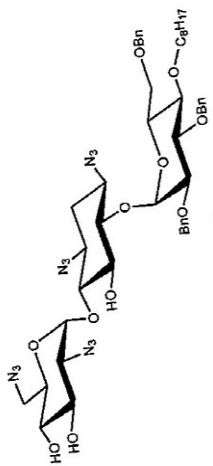
1H NMR spectrum of compound 22

X: parts per Million : 1H



```

Filename = single_pulse_decoupl-II
Author = fasso
Experiment = single_pulse_dec
Date_Exp = 15-SEP-2010
Date_Acq = 15-SEP-2010
Creation_time = 20:55:30
Revision_time = 11-OCT-2010 16:25:27
Current_time = 11-OCT-2010 16:25:32
Comment = single pulse decouple
Data format = 1D COMPLEX
Dim_size = 26214
Dim_title =
Dim_units = [ppm]
Dimensions =
F2 = 300
Spectrometer = ECKX-300
Field_strength = 7.05860137 (300 MHz)
X_acq_duration = 1.38412032 [s]
X_chan = 13C
X_freq = 75.56823426 [MHz]
X_offset = 100 [ppm]
X_points = 32768
X_procs = 4
X_resolution = 0.72248054 [Hz]
X_sfs = 18.07424242 [kHz]
X_t1 = 1.38412032 [s]
X_t1_rho = 18
X_t1_tau = 300.52965592 [MHz]
Irr_offset = 5 [ppm]
Clipped = FALSE
Soc_return = 6000
Total_scans = 6000
X_90_width = 10.12 [us]
X_acq_time = 1.38412032 [s]
X_chan = 13C
X_prg = 6 [dB]
X_prg2 = 6 [dB]
X_pulse = 3.37333333 [us]
Irr_atn_dec = 23 [dB]
Irr_atn_poe = 23 [dB]
Irr_freq = 75.56823426 [MHz]
Decoupling = WALTZ
Initial_wait = 1 [s]
Noe = TRUE
Noe_time = 2 [s]
Recvr_gain = 54
Soc_return = 6000
Repetition_delay = 1 [s]
Repetition_time = 3.38412032 [s]
Temp_get = 23.8 [C]
    
```



¹³C NMR spectrum of compound 22

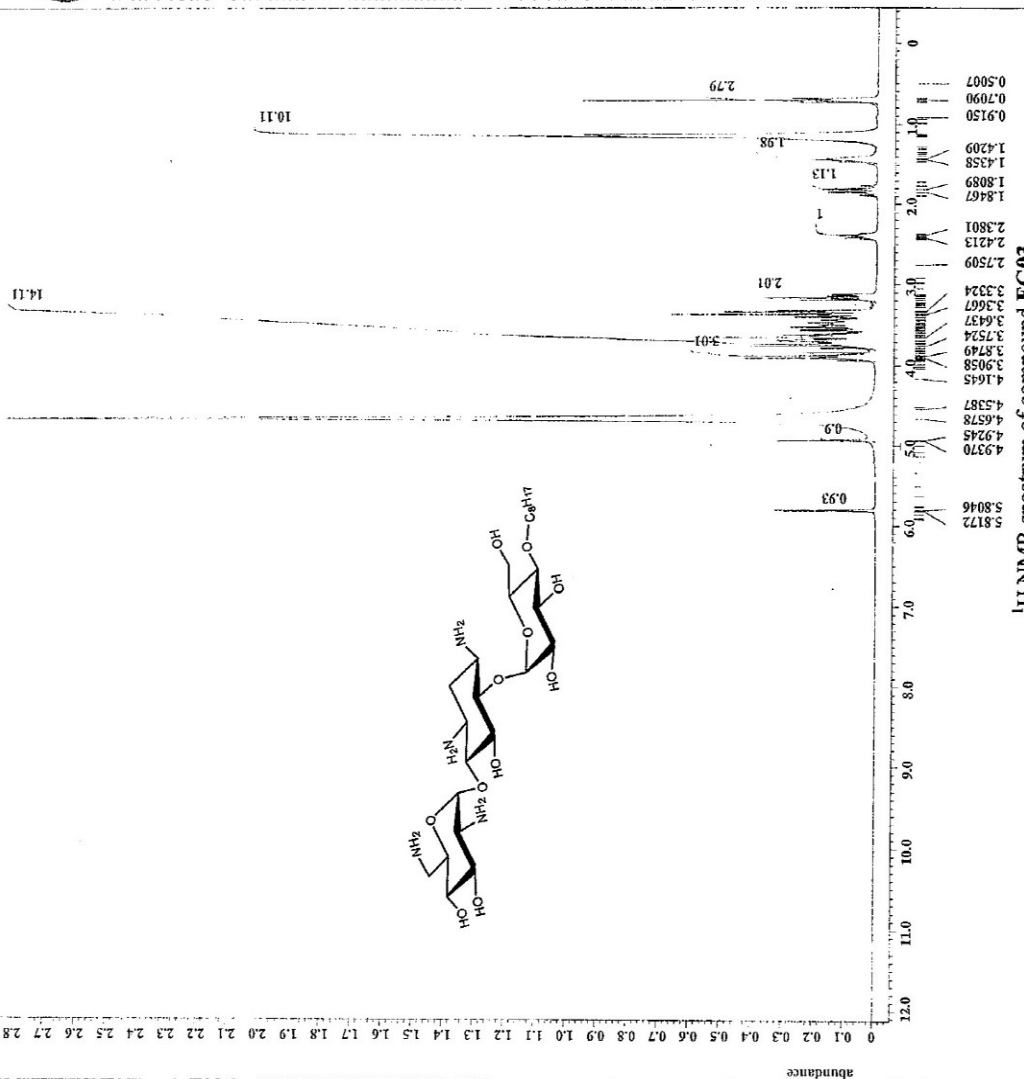
X : points per Million : 15C



```

=====
File Name      = F003_single_pulse-3.j
Author         =
Experiment     = single_pulse.exe
Sample ID     = F003_single_pulse
Solvent       = D2O
Acquisition  = 19-DEC-2009 17:16:24
Relaxation    = 19-DEC-2009 17:16:24
Current Time  = 19-DEC-2009 17:38:20
=====
Comment       = single_pulse
Data Format    = 1D COMPLEX
Pulse Prog    = zgpg30
Date_ Acq    = 12/19/09
Dim Title     =
Dim Units     = [ppm]
Dimensions    = X
Site          = ECK 300
Spectrometer = ECK-300
=====
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_P1           = 1.0384
X_P2           = 1
X_P3           = 1
X_P4           = 1
X_P5           = 1
X_P6           = 1
X_P7           = 1
X_P8           = 1
X_P9           = 1
X_P10          = 1
X_Resolution  = 0.34397631 [Hz]
X_Sweep       = 1H
X_Domain      = 1H
X_Freq        = 5.63570784 [MHz]
X_Offset      = 5 [ppm]
X_P1          = 1
X_P2          = 1
X_P3          = 1
X_P4          = 1
X_P5          = 1
X_P6          = 1
X_P7          = 1
X_P8          = 1
X_P9          = 1
X_P10         = 1
X_P11         = 1
X_P12         = 1
X_P13         = 1
X_P14         = 1
X_P15         = 1
X_P16         = 1
X_P17         = 1
X_P18         = 1
X_P19         = 1
X_P20         = 1
X_P21         = 1
X_P22         = 1
X_P23         = 1
X_P24         = 1
X_P25         = 1
X_P26         = 1
X_P27         = 1
X_P28         = 1
X_P29         = 1
X_P30         = 1
X_P31         = 1
X_P32         = 1
X_P33         = 1
X_P34         = 1
X_P35         = 1
X_P36         = 1
X_P37         = 1
X_P38         = 1
X_P39         = 1
X_P40         = 1
X_P41         = 1
X_P42         = 1
X_P43         = 1
X_P44         = 1
X_P45         = 1
X_P46         = 1
X_P47         = 1
X_P48         = 1
X_P49         = 1
X_P50         = 1
X_P51         = 1
X_P52         = 1
X_P53         = 1
X_P54         = 1
X_P55         = 1
X_P56         = 1
X_P57         = 1
X_P58         = 1
X_P59         = 1
X_P60         = 1
X_P61         = 1
X_P62         = 1
X_P63         = 1
X_P64         = 1
X_P65         = 1
X_P66         = 1
X_P67         = 1
X_P68         = 1
X_P69         = 1
X_P70         = 1
X_P71         = 1
X_P72         = 1
X_P73         = 1
X_P74         = 1
X_P75         = 1
X_P76         = 1
X_P77         = 1
X_P78         = 1
X_P79         = 1
X_P80         = 1
X_P81         = 1
X_P82         = 1
X_P83         = 1
X_P84         = 1
X_P85         = 1
X_P86         = 1
X_P87         = 1
X_P88         = 1
X_P89         = 1
X_P90         = 1
X_P91         = 1
X_P92         = 1
X_P93         = 1
X_P94         = 1
X_P95         = 1
X_P96         = 1
X_P97         = 1
X_P98         = 1
X_P99         = 1
X_P100        = 1
=====
X_90 Width    = 13.43 [us]
X_Acq Time    = 45.0297696 [s]
X_Solvent     = DMS
X_Ana         = 3 [dB]
X_Pulse       = 6.715 [us]
Irr_Mode      = Off
Irr_Freq     = 17.000 [MHz]
Irr_Offset   = 1 [ppm]
Initial Wait  = 1 [s]
Recvr Gain    = 32
Relaxation Delay = 1 [s]
Repetition Time = 3.90717696 [s]
Temp_Set     = 23.3 [C]
=====

```

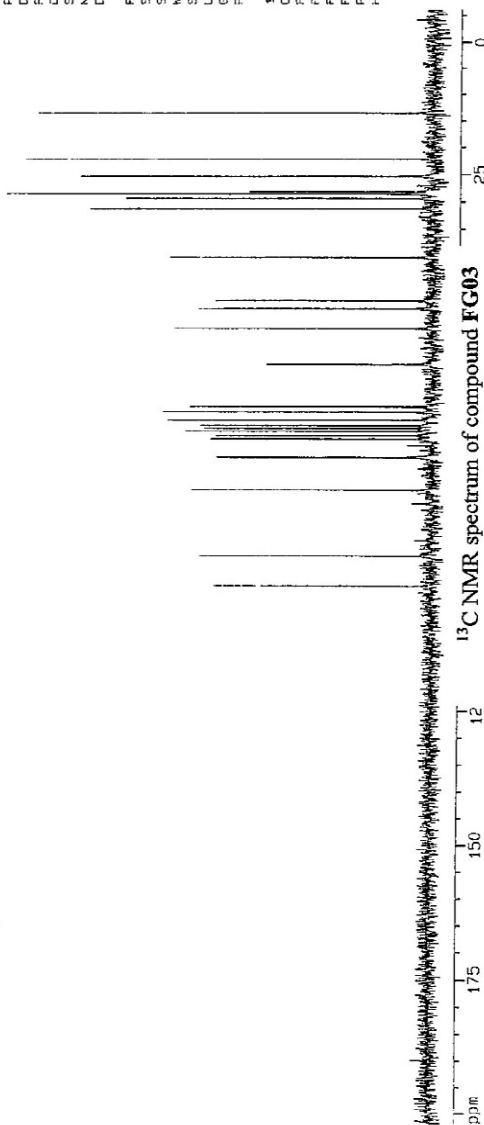
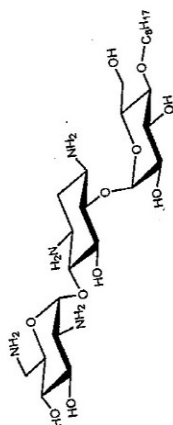
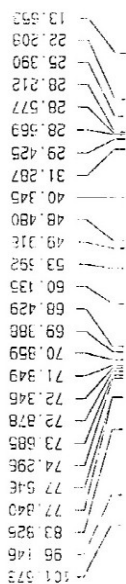


¹H NMR spectrum of compound FG03

X : parts per Million : 1H

Standard ¹³C
Experiment

Current: Data Parameters
 NAME: FG03
 EXPNO: 1
 PROCNO: 1
 F2 - Acquisition Parameters
 Date_ Time: 500000
 18.13
 INSTRUM: mri400
 PULPROG: zgpg30
 TO: 32766
 SOLVENT: D2O
 NS: 2338
 DS: 2
 SWH: 25000.000 Hz
 FIDRES: 0.762939 Hz
 AQ: 0.8524100 sec
 RG: 49300
 CP: 20.000 usec
 PC: 27.14 usec
 VE: 800.0 K
 D12: 0.0000000 sec
 CL5: 26.000 usec
 CPOPRG: hslz15
 P31: 100.00 usec
 D1: 0.4000000 sec
 P1: 6.75 usec
 DE: 27.14 usec
 SF01: 100.6231179 MHz
 NUC1EUS: ¹³C
 D11: 0.0300000 sec
 F2 - Processing parameters
 SI: 16384
 SF: 100.627460 MHz
 KW: EK
 SFO: 0
 LB: 2.00 Hz
 GB: 0
 PC: 1.40
 F0 MMR pilot parameters
 CA: 20.00 cm
 CP: 201.972 ppm
 F1P: 200.00 MHz
 F2P: 0.000000 MHz
 F2: -817.08 Hz
 PPM0M: 10.40556 ppm/cg
 HZCM: 1046.92236 Hz/cg



ppm

¹³C NMR spectrum of compound FG03

12

150

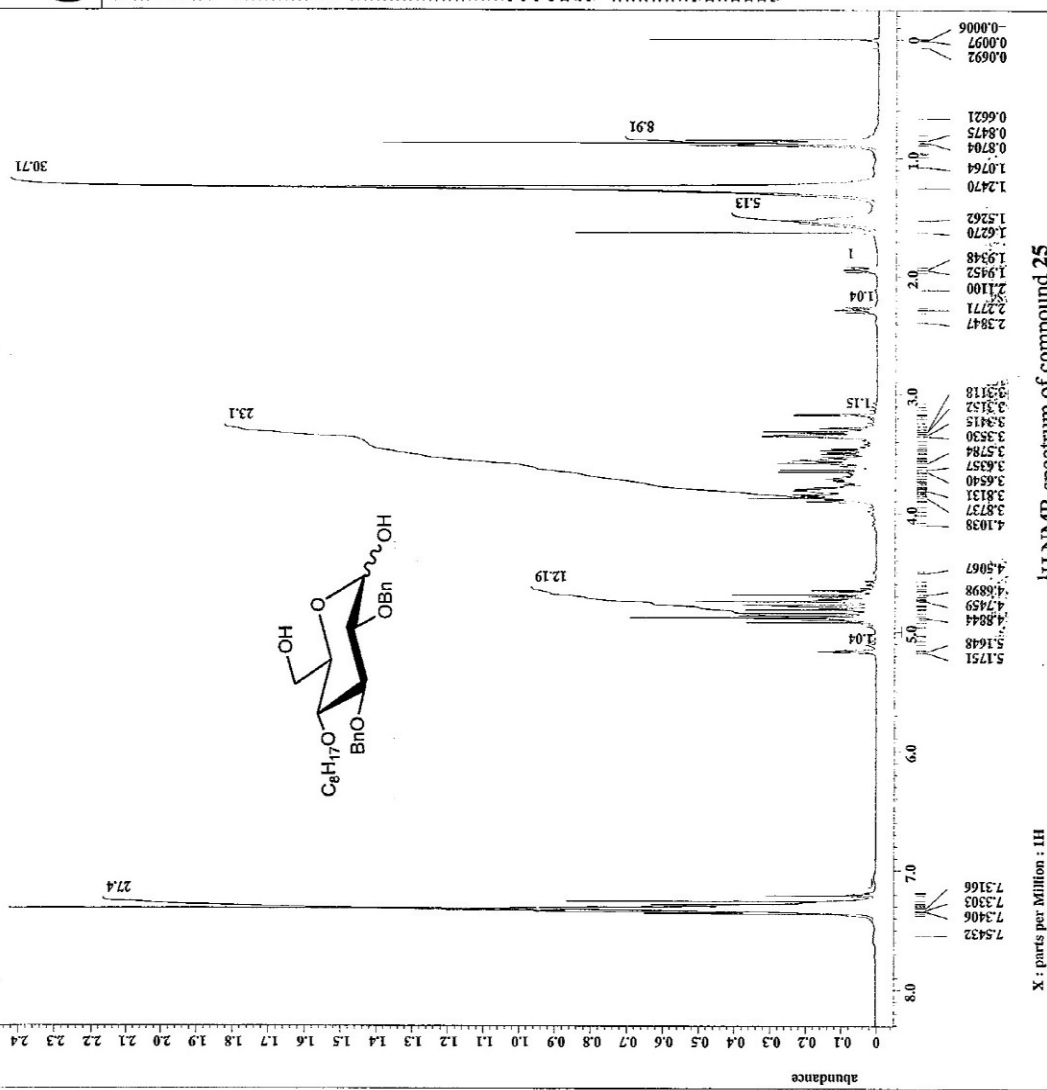
175

ppm



```

File Name = single_pulseNF-III-87
Author = zoso
Experiment = single_pulse.ex2
Sample_ID = NF-III-87
Date_Exp = 11-07-2010
Creation_time = 11-FEB-2010 10:03:10
Revision_time = 11-OCT-2010 16:23:36
Current_time = 11-OCT-2010 16:23:58
Comment = single_pulse
Data Format = 1D-COMPZK
Dim Size = 13107
Dim Title = 1H
Dim Units = [ppm]
Dimensions = X
Spectrometer = ECX-300
Field_strength = 7.6586013 [T] (300 [MHz])
X_acq_duration = 2.90717696 [s]
X_domain = 10.52965592 [MHz]
X_offset = 5 [ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.34397631 [Hz]
X_sweep_rate = 10.52965592 [MHz]
Irr_domain = 1H
Irr_freq = 300.52965592 [MHz]
Irr_offset = 5 [ppm]
Irr_domain = 1H
Irr_freq = 300.52965592 [MHz]
Irr_offset = 5 [ppm]
Clipped = FALSE
Mod_return = 1
Scans = 8
Total_scans = 8
X_90_width = 13.43 [us]
X_acq_time = 2.90717696 [s]
X_angle = 45 [deg]
X_atn = 3 [dB]
X_pulse = 6.715 [us]
X_mode = Off
Dante_Preset = FALSE
Initial_wait = 1 [s]
Recvr_Gain = 44
Sensitivity_delay = 1 [s]
Repetition_time = 3.190717696 [s]
Temp_get = 31.9 [dC]
    
```



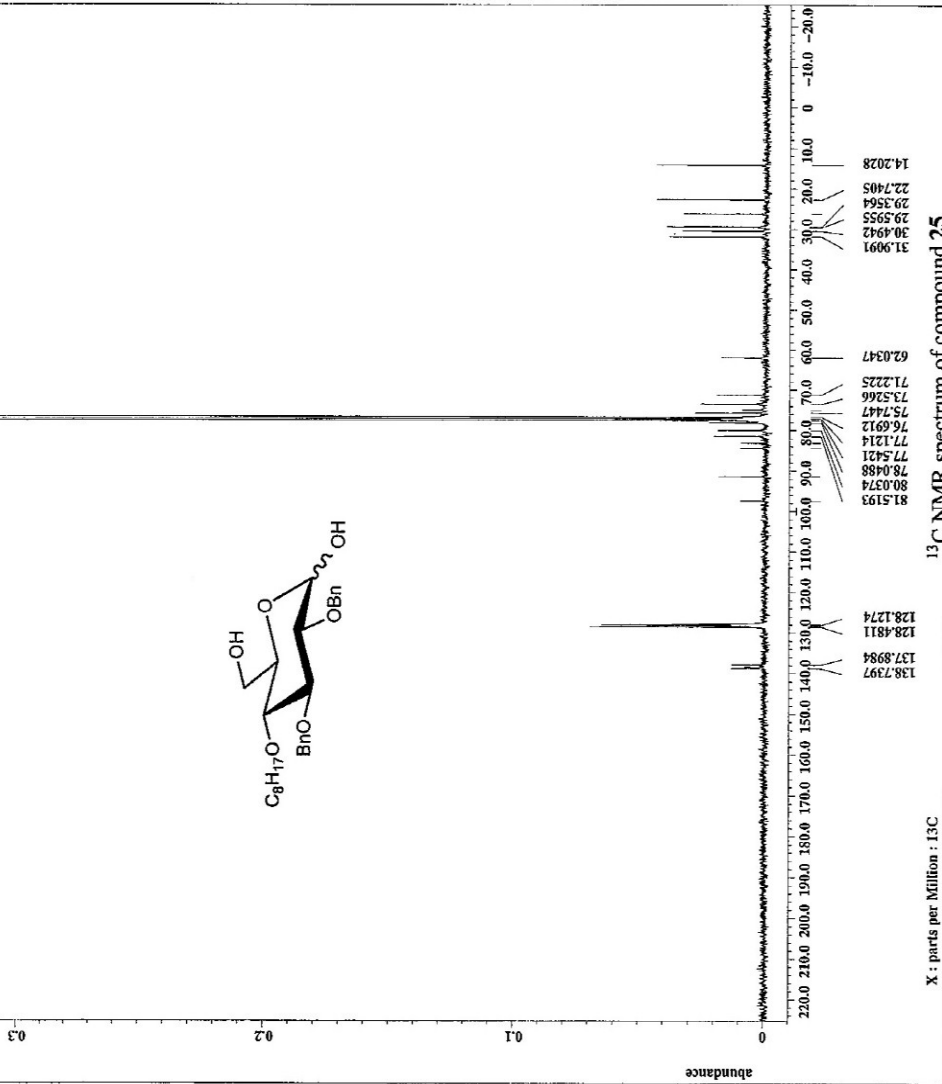
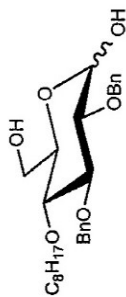
¹H NMR spectrum of compound 25

X : parts per Million : 1H



```

= single_pulse_decouple
= fssso
Author
Experiment = single_pulse_dec
Sample_id = MR-118-87B
Solvent = CDCl3
Creation_time = 8-MAR-2010 19:11:48
Revision_time = 11-OCT-2010 16:27:24
Current_time = 11-OCT-2010 16:27:28
Comment = single_pulse_decouple
ID = COMPLEX
Data_format = 28214
Dim_size = 43C
Dim_title
Dim_units [ppm]
Dimensions
Spectrometer = EXX-300
Field_strength = 7.058601312 (300 MHz)
X_acq_duration = 1.39412032 [s]
X_gain = 10.12 [us]
X_freq = 75.56823426 [MHz]
X_offset = 100 [ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054 [Hz]
Xr_domain = 4K.07424242 [MHz]
Xr_freq = 300.52965592 [MHz]
Xr_offset = 5 [ppm]
Clipped = FALSE
Scan_return = 3500
Total_scans = 3500
X_90_width = 10.12 [us]
X_acq_time = 1.39412032 [s]
X_delay = 6 [s]
X_atn = 6 [dB]
X_pulse = 3.373333333 [us]
Xr_atn_dec = 23 [dB]
Xr_atn_pcc = 23 [dB]
Decoupling = WALTZ
Initial_wait = 1 [s]
Noe_time = TRUE
Noe_time = 2 [s]
Recvr_gain = 56
Xr_gain_delay = 5 [s]
Repetition_time = 3.38412032 [s]
Temp_set = 23.2 [dC]
    
```



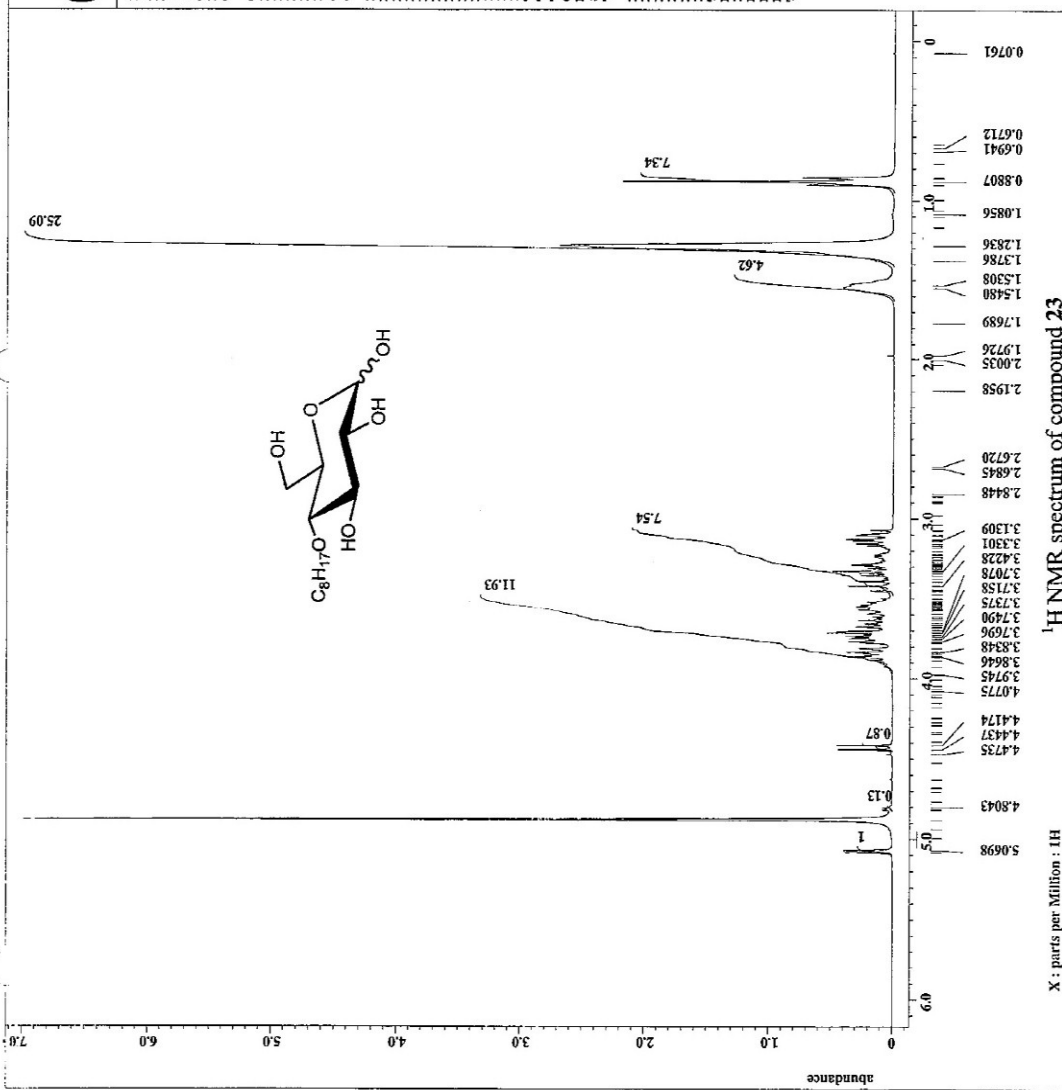
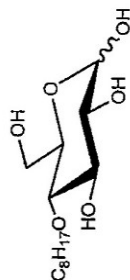
¹³C NMR spectrum of compound 25

X: parts per Million : 13C



```

Filename = 101410NF-3-225-3.jdf
Author = zoso
Experiment = single_pulse.ex2
Sample_id = 101410NF-3-225
Date_1 = 14-08-2010 10:38:32
Date_2 = 14-08-2010 10:38:32
Creation_time = 14-08-2010 10:38:32
Revision_time = 14-08-2010 10:39:47
Current_time = 14-08-2010 10:41:23
Comment =
Data_format = single_pulse
ID_COMMENT = ID_COMMENT
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X 300
Spectrometer = ECA-300
Field_strength = 7.6586013 [T] (300 [MHz])
X_acq_duration = 2.90717696 [s]
X_domain = 10.52965592 [MHz]
X_offset = 5 [ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.34397631 [Hz]
X_swept_freq = 10.52965592 [MHz]
X_t1 = 16.63570784 [MHz]
IRF_domain = 1H
IRF_freq = 300.52965592 [MHz]
IRF_offset = 5 [ppm]
T1_domain = 1H
T1_freq = 300.52965592 [MHz]
T1_offset = 5 [ppm]
Clipped = FALSE
Mod_return = 1
Scans = 8
Total_scans = 8
X_90_width = 13.43 [us]
X_acq_time = 2.90717696 [s]
X_angle = 45 [deg]
X_atn = 3 [dB]
X_pulse = 6.715 [us]
X_mode = Off
Date_preset = FALSE
Initial_wait = 1 [s]
Recvr_gain = 30
Sensitron_delay = 2 [s]
Repetition_time = 22.5 [s]
Temp_set = 22.5 [DC]
    
```



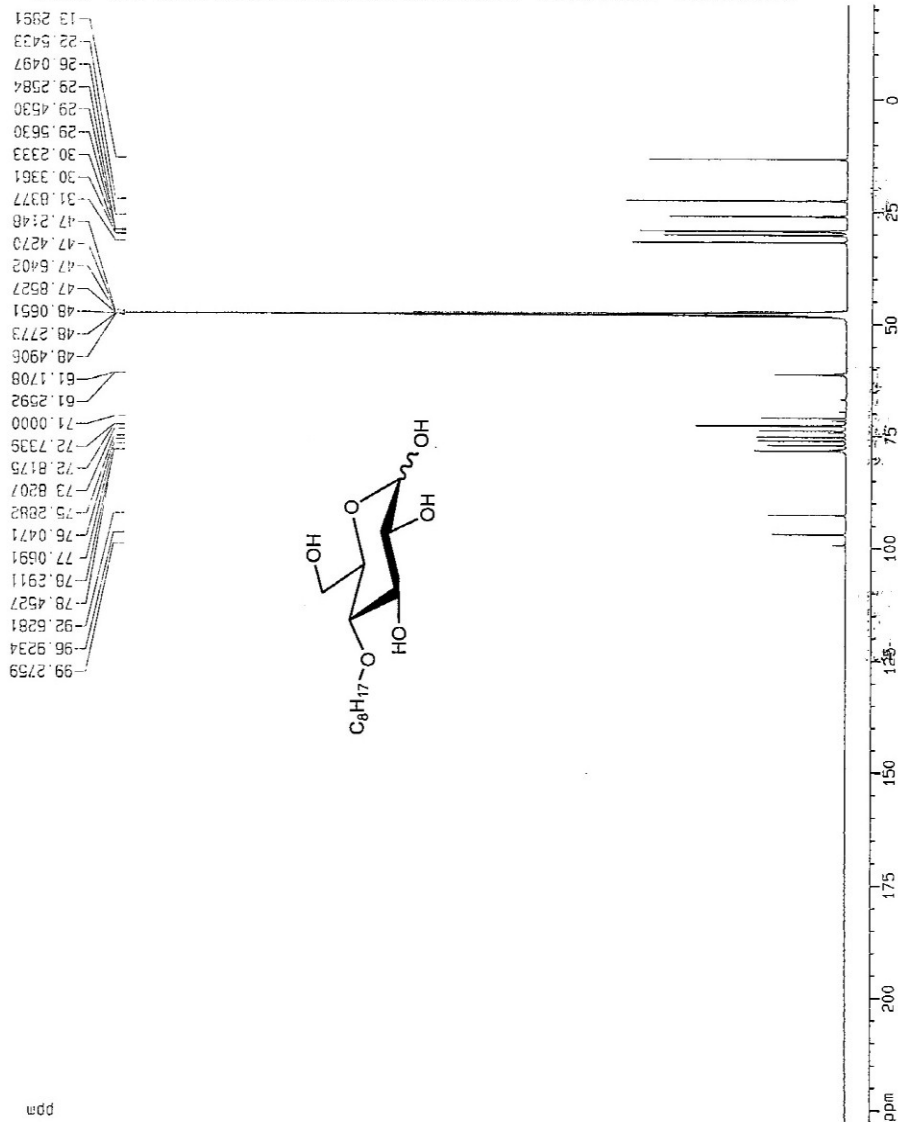
Standard ¹³C
Experiment

Current Data Parameters
 NAME: M-3-229
 EXPNO: 1
 PROCNO: 1

F2 - Acquisition Parameters
 Date_ : 500000
 Time : 20.46
 INSTRUM : BRUKER
 PULPROG : zgpg30
 ID : zgpg30
 SOLVENT : MeOH
 DS : 20000
 SWH : 25000.000 Hz
 FIDRES : 0.763930 Hz
 AQ : 0.6554100 sec
 RG : 45500
 DM : 20.000 usec
 DE : 27.14 usec
 TE : 300.0 K
 D12 : 0.0002000 sec
 SFO1 : 100.00 usec
 P1 : 0.4000000 sec
 P2 : 6.75 usec
 CE : 27.14 usec
 SFO2 : 100.6231175 MHz
 NUC1 : ¹³C
 NUC2 :
 U1 : 0.0300000 sec

F2 - Processing parameters
 SI : 32768
 SF : 100.6127480 MHz
 NMQ : 0
 SSB : 0
 LB : 2.00 Hz
 GB : 0
 PC : 1.40

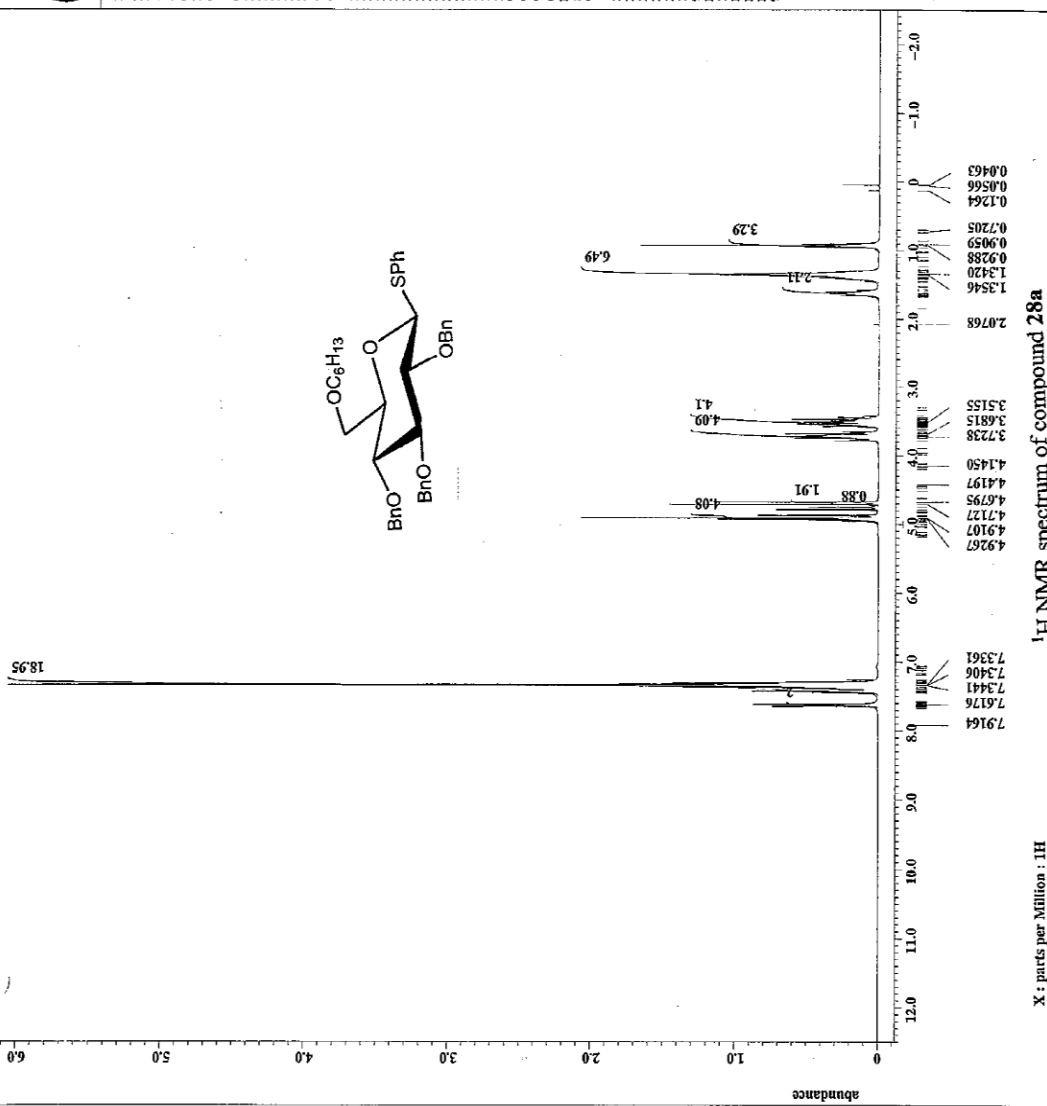
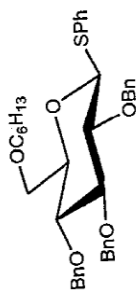
1D NMR plot parameters
 CX : 20.00 cm
 F1 : 227.296 ppm
 F2 : 22869.66 Hz
 F3 : -21.181 ppm
 F4 : -2131.11 Hz
 PPH4 : 12.42397 ppm/cm
 N2CH : 1250.00012 Hz/cm





```

Filename = 1101DMF-3-249-3.jje
Author = fssc
Experiment = single_pulse.ex2
Sample_id = 1101DMF-3-249
SOLVENT = CDCl3
Creation_time = 10-NOV-2010 09:42:01
Revision_time = 10-NOV-2010 23:55:22
Current_time = 10-NOV-2010 23:55:28
Comment = single_pulse
Data_format = 1D COMPLEX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = XCX 300
Spectrometer = ECK-300
Field_strength = 7.0586013 [T] (300 [MHz]
X_acq_duration = 2.90717696 [s]
X_chan = 1H
X_freq = 300.52965592 [MHz]
X_offset = 5 [ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.34397651 [Hz]
X_sfs = 1H
X_sfs_freq = 83970784 [MHz]
X_t1 = 1H
X_t1_delay = 300.52965592 [MHz]
X_t1_offset = 1H
X_t1_offset_freq = 300.52965592 [MHz]
X_t1_offset_offset = 5 [ppm]
X_t1_offset_offset_freq = 300.52965592 [MHz]
X_t1_offset_offset_offset = FALSE
Mod_return = 1
Scans = 8
Total_scans = 8
X_90_width = 13.43 [us]
X_acq_time = 2.90717696 [s]
X_angle = 45 [deg]
X_atn = 3 [db]
X_pulses = 0.715 [us]
X_pulse_offset = 0.715 [us]
X_pulse_offset_offset = OFF
Tri_mode = OFF
Dante_preset = FALSE
Initial_wait = 1 [s]
Recev_gain = 32
Recev_delay = 1 [s]
Relaxation_time = 7.90717696 [s]
Temp_get = 21.5 [dC]
    
```



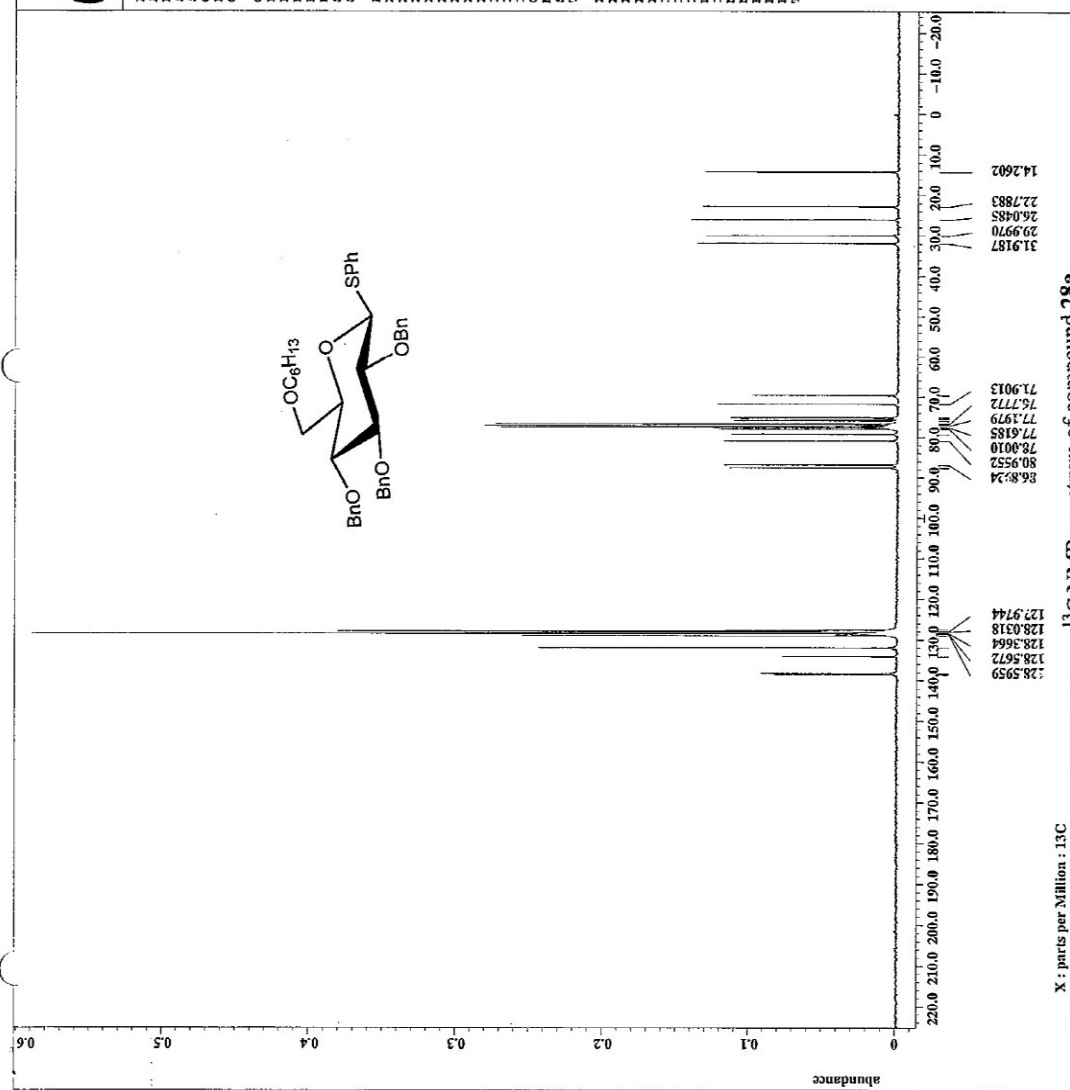
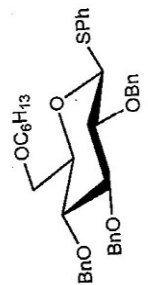


```

Filename = single_pulse_decNF-3
Author = fonsso
Experiment = single_pulse_dec
Date_Exp = 2010-11-10
Solvent = CHLOROFORM-D
Creation_Time = 10-NOV-2010 22:57:15
Revision_Time = 10-NOV-2010 23:51:06
Current_Time = 10-NOV-2010 23:51:11
Comment = single pulse decouple
Data_Format = 1D COMPLEX
Dim_Size = 26214
Dim_Title = 13C
Dim_Units = [ppm]
Dimensions =
Site = ECX 300
Spectrometer = ECX-300

Field_strength = 7.0586013 [T] (300 MHz)
X_acq_duration = 1.38412032 [s]
X_chan = 13C
X_freq = 75.56823426 [MHz]
X_offset = 100 [ppm]
X_points = 22768
X_prescans = 4
X_resolution = 7284.054 [Hz]
X_resolution_ppm = 51.674242 [kHz]
Xr_domain =
Xr_freq = 300.52955592 [MHz]
Xr_offset = 5 [ppm]
Clipped = FALSE
Scan_return =
Scans = 5000
Total_scans = 5000

X_90_width = 10.12 [us]
X_acq_time = 1.38412032 [s]
X_delay = 6 [us]
X_gain = 6 [dB]
X_atn =
X_pulse = 3.37333333 [us]
Xr_atn_dec = 23 [dB]
Xr_atn_poe = 23 [dB]
Xr_atn_ppm = 23 [ppm]
Xr_coupling =
Xr_delay = 1 [us]
Xr_initial_wait =
Xr_noise = TRUE
Xr_noise_time = 2 [s]
Xr_noise_ppm = 5 [ppm]
Xr_noise_delay = 5 [us]
Repetition_time = 3.38412032 [s]
Temp_get = 21.9 [dC]
    
```



¹³C NMR spectrum of compound 28a

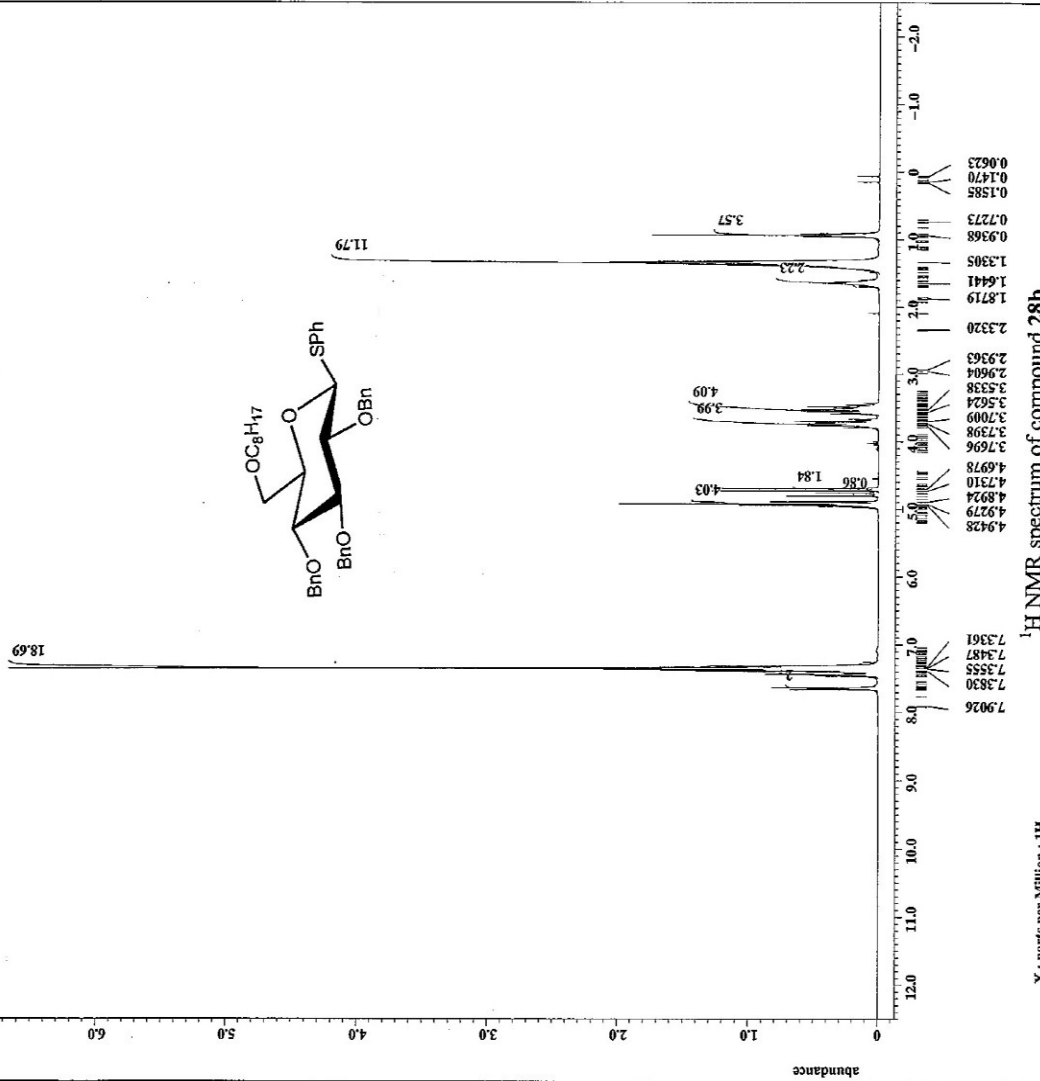
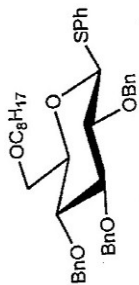
X : parts per Million : 13C



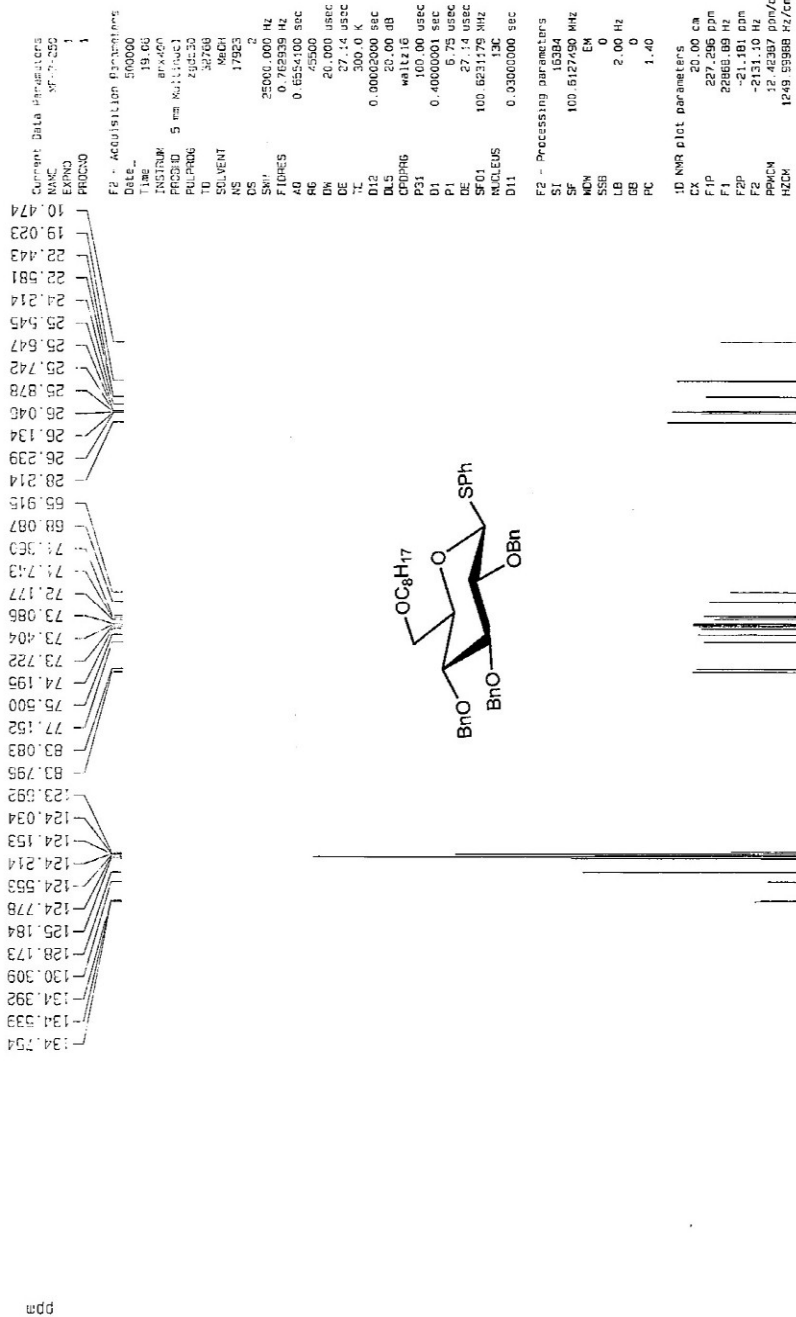
```

Filename = 111010MP-3-250-3-jds
Author = fossco
Experiment = single_pulse.ez2
Sample_id = 111010MP-3-250
Creation_time = 10-NOV-2010 09:49:20
Revision_time = 10-NOV-2010 23:57:16
Current_time = 10-NOV-2010 23:57:23
Comment = single_pulse
Data_format = ID COMPREX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X: 300
Spectrometer = ECK-300

Field_strength = 7.05860131(2) (300 MHz)
X_acq_duration = 2.90717696[s]
X_domain = 16.0
X_offset = 5[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.34397631[Hz]
X_sweep = 1K
X_start = 63270784[Hz]
X_stop = 300.52965592[Mhz]
Xr_freq = 5[ppm]
Xr_offset = 5[ppm]
Xr_domain = 300.52965592[Mhz]
Xr_offset = 5[ppm]
Xr_offset = 300.52965592[Mhz]
Xr_offset = 5[ppm]
Xr_offset = 5[ppm]
Xr_offset = 5[ppm]
Mod_return = FALSE
Scans = 1
Total_scans = 8
X_90_width = 13.43[us]
X_acq_time = 2.90717696[s]
X_angle = 45[deg]
X_atn = 3[db]
X_pulse = 6.715[us]
X_mode = Off
Pulse_program = OFF
Dante_presat = FALSE
Initial_wait = 1[s]
Recur_gain = 30
Relaxation_delay = 7[s]
Relaxation_time = 7.90717696[s]
Temp_get = 21.9[deg]
  
```



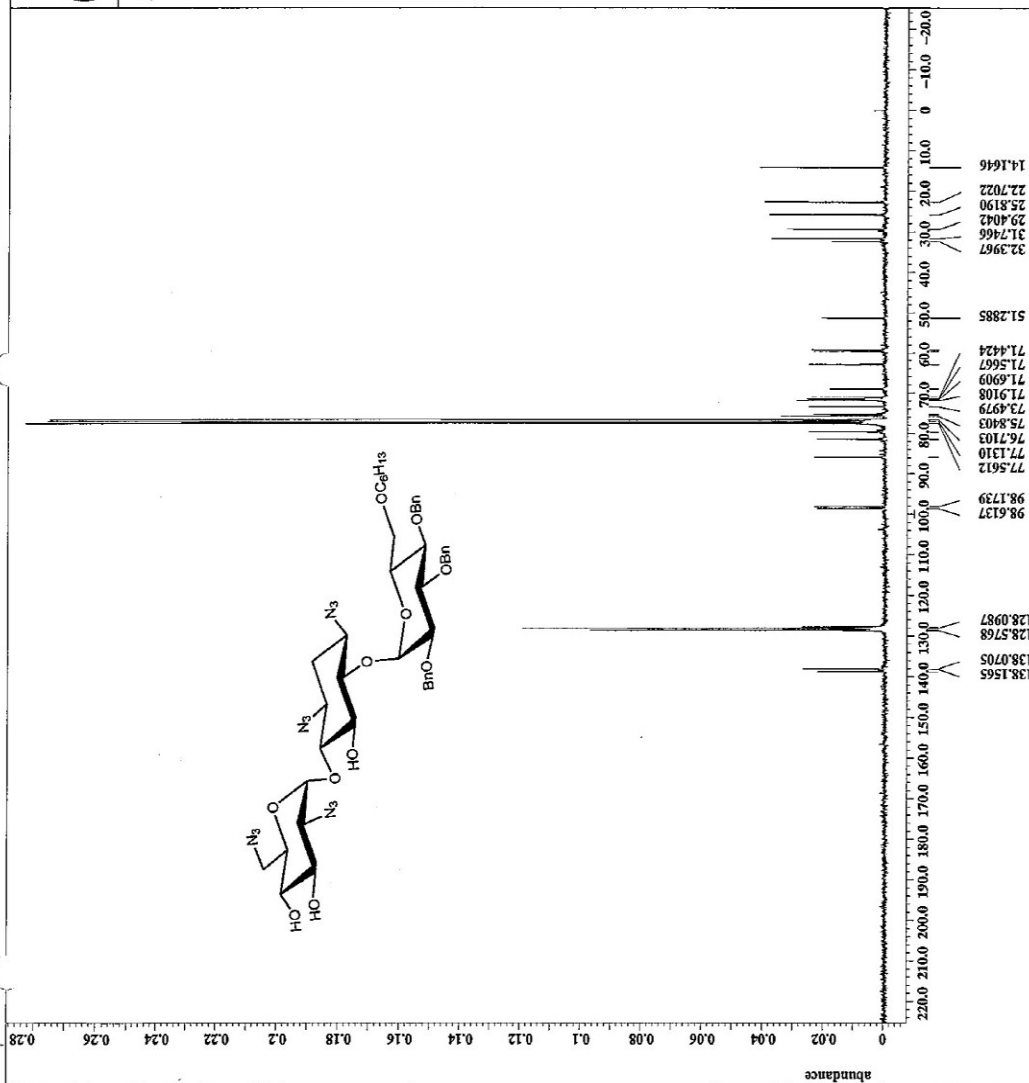
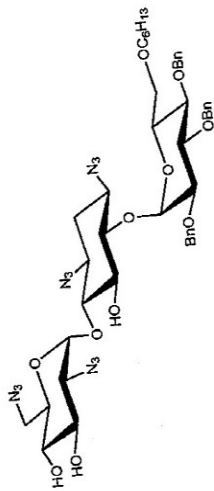
Standard ¹³C
Experiment





```

File Name = MF-3-260dec-2.jdf
Author = fossco
Experiment = single_pulse_dec
Sample_ID = MF-3-260dec
Date_Exp = 30-NOV-2010 22:55:02
Creation_time = 30-NOV-2010 22:55:11
Revision_time = 30-NOV-2010 22:55:11
Current_time = 30-NOV-2010 22:55:20
Comment = single pulse decouple
Data Format = 1D
DirName = 26224
Dir_size = 13C
Dir_title = [ppm]
Dir_units = X
Dimensions = X
Spectrometer = EXC-300
Field_strength = 7.0586013[T] (300[Mhz]
X_acq_duration = 1.38412032[s]
X_chan = 13C
X_freq = 75.56823426[Mhz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 9.72248054[Hz]
X_sweep = 1H
X_t1 = 19.07424242[Mhz]
Irr_freq = 300.52965592[Mhz]
Irr_offset = 5[ppm]
Clipped = FALSE
Acq_return = 5500
Total_scans = 5500
X_90_width = 10.12[us]
X_acq_time = 30.8422032[s]
X_pulse = 6[db]
X_atn = 6[db]
X_pulse = 3.37333333[us]
Irr_atn_dec = 23[db]
Irr_atn_noe = 23[db]
Irr_atn = 23[db]
Decoupling = WALTZ16
Initial_wait = 1[s]
Noe_time = TRUE
Noe_time = 2[s]
Relaxation = 6[s]
Relaxation_delay = 2[s]
Repetition_time = 3.38412032[s]
Temp_get = 22.9[degC]
    
```



¹³C NMR spectrum of compound 29a

X : parts per Million : 13C



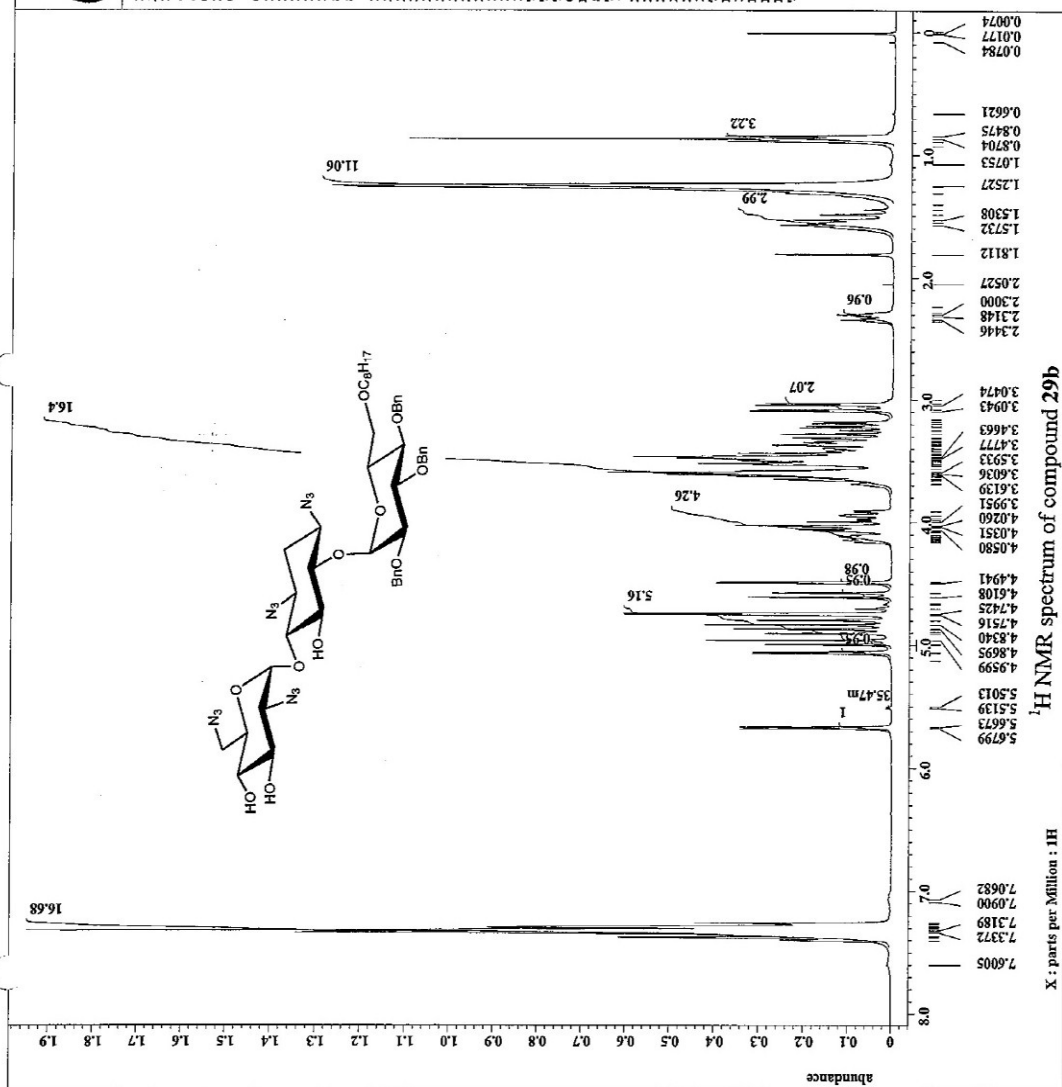
```

=====
File       = 120210MF-3-262B-3-jdf
Author    =
Experiment = single_pulse-ex2
Sample_id = 120210MF-3-262B
Solvent   = CHOROFORM-D
Name      =
Acq_date  = 2-BEC-2010 13:42:23
Acq_time  = 2-BEC-2010 13:46:53
Current_time

Comment   = single_pulse
Date_format = DD COMPLEX
Data_dir   = 120210
Dim_title  =
Dim_units  = [ppm]
Dimensions = X
Site       = ECX 300
Spectrometer = ECX-300

Field_strength = 7.0586013 [T] (300 [MHz]
X_acq_duration = 2.90717696 [s]
X_domain       = 1H
X_freq         = 300.52965592 [MHz]
X_gamma       = 103.84
X_pulses      = 1
X_resolution  = 0.34357631 [Hz]
X_sweep       = 5.63570784 [kHz]
X_t1          = 300.52965592 [MHz]
X_t1_offset   = 1H
X_t2          = 300.52965592 [MHz]
X_t2_offset   = 5 [ppm]
X_t3          = 1
X_t3_offset   = FALSE
Mod_return    = 1
Total_scans   = 8

X_90_width   = 13.43 [us]
X_acq_time    = 2.90717696 [s]
X_angle       = 45 [deg]
X_atn         = 31 [dB]
X_pulse       = 6.715 [us]
X_rf_mode     = OF
X_t1_mode     = OF
Dantco_preset = FALSE
Initial_wait  = 1 [s]
Recvr_gain    = 36
Relaxation_delay = 5 [s]
Spectrum_time = 2.90717696 [s]
Temp_get      = 22.7 [degC]
=====
  
```



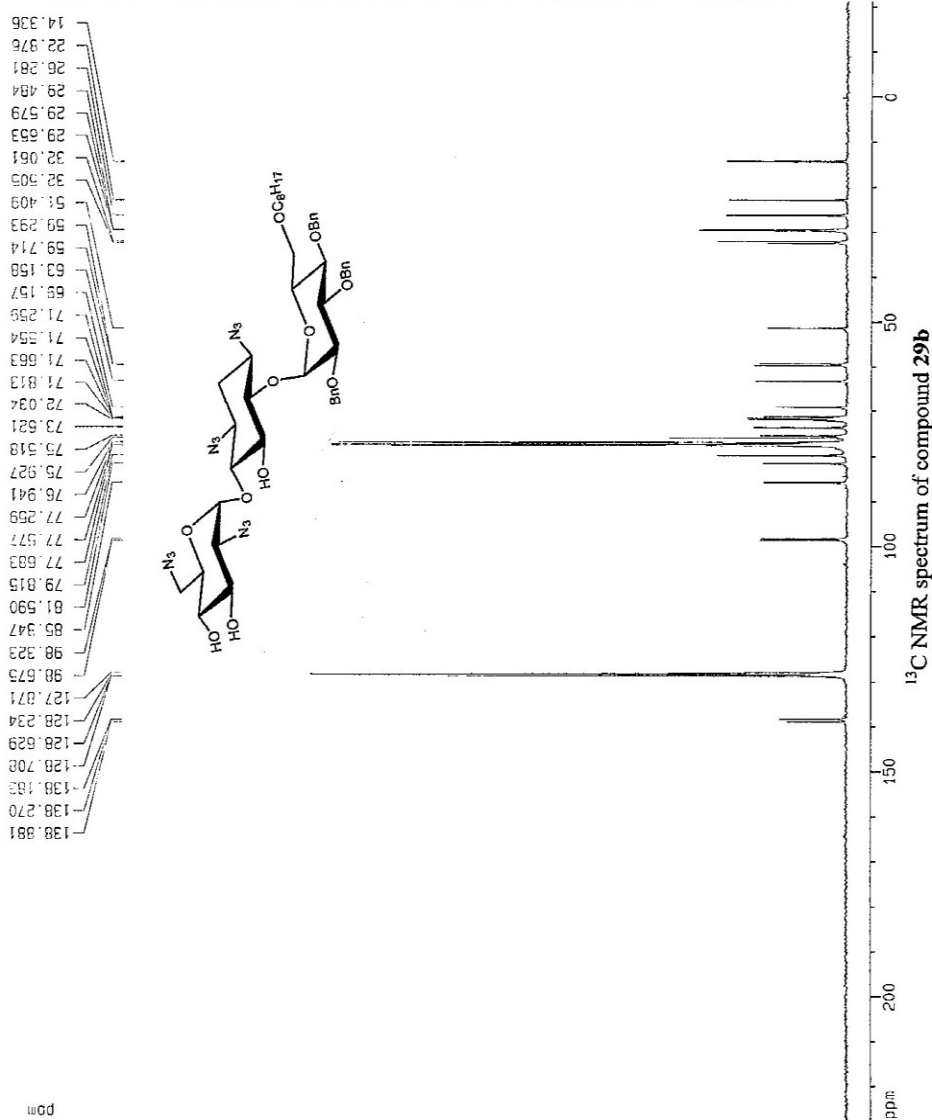
Standard 13C
Experiment

Current Data Parameters
NAME MF-3-2620
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 500000
Time 16:33
INSTRUM brx400
PROBHD 5 mm Multinuc 1
PULPROG zgpg30
TD 32768
SOLVENT CDCl3
DS 16000
SWH 26000.000 Hz
FIDRES 0.762939 Hz
AQ 0.655400 sec
RG 45500
DN 20.000 usec
DE 27.14 usec
TE 300.0 K
D12 0.0002000 sec
EUS 20.00 dB
CPDPRG waltz16
P31 100.00 usec
P1 0.40000001 sec
P2 6.75 usec
DE 27.14 usec
SFO1 100.623179 MHz
NUCLEUS 13C
D11 0.03000000 sec

F2 - Processing parameters
SI 16384
SF 100.6127460 MHz
AQ 4.00000000 sec
RG 45500
WDW EM
SSB 0
LB 5.00 Hz
GB 0
PC 1.40

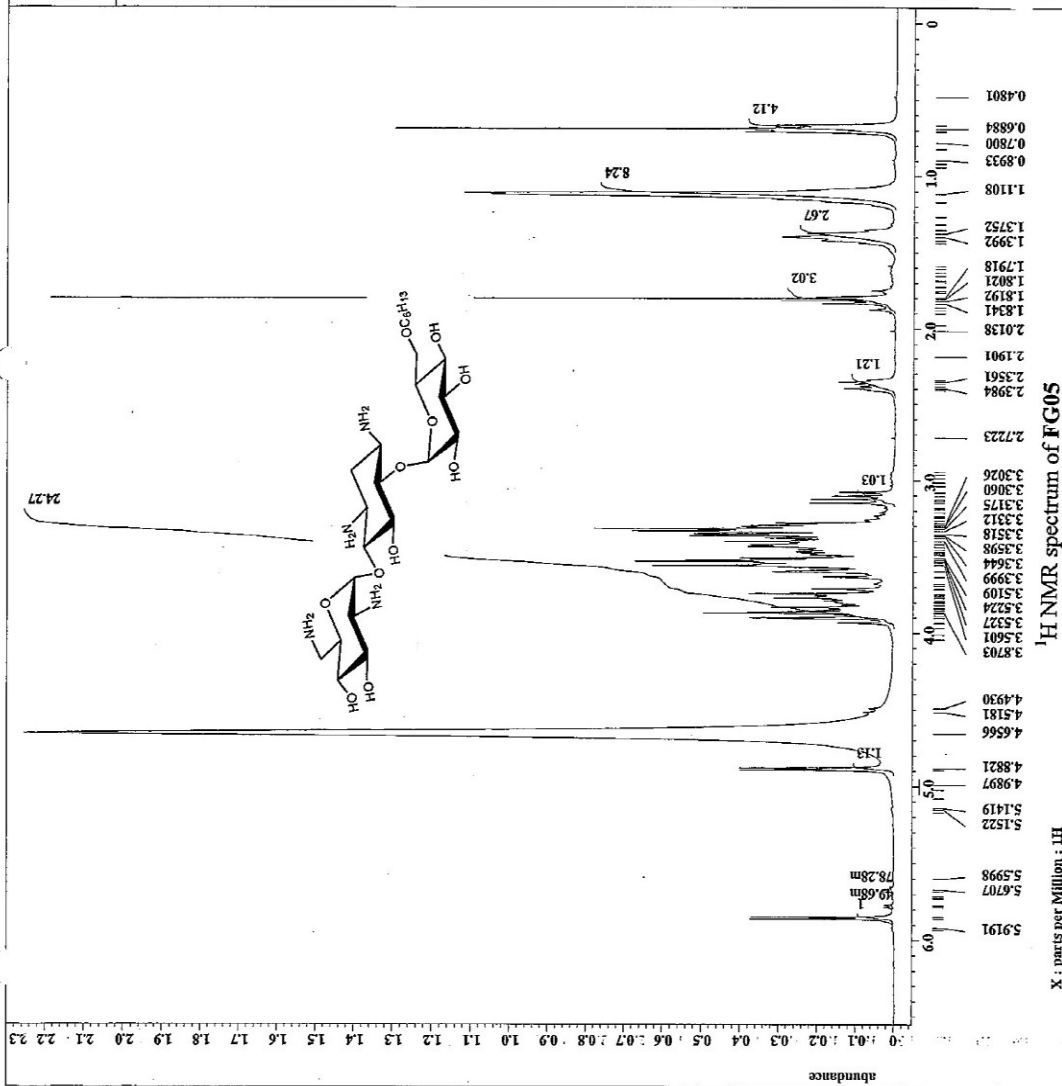
1D NMR plot parameters
CX 20.00 cm
F1P 227.295 ppm
F1 22859.69 Hz
F2P -21.181 ppm
F2 -2131.10 Hz
PPMCM 12.42307 ppm/cm
HZCM 1245.89986 Hz/cm



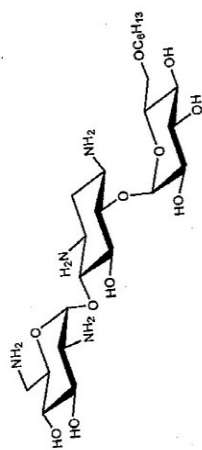
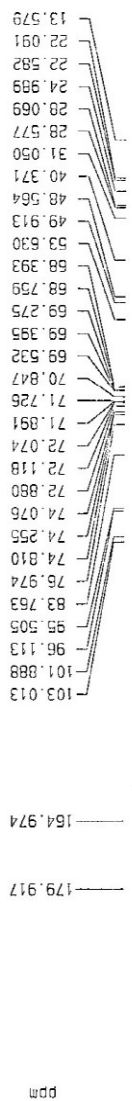


```

file_name = single_pulseFG05 (Cl) -
Author = fasso
Experiment = single_pulse.ex2
Sample_id = FG05 (Cl)
Date_1 = 16-DEC-2010 03:05:44
Date_2 = 16-DEC-2010 03:05:44
Creation_time = 16-DEC-2010 03:07:01
Revision_time = 16-DEC-2010 03:07:01
Current_time = 16-DEC-2010 03:07:14
Comment = single_pulse
Operator = fasso
Dim_size = 13107
Dim_title = IN
Dim_units = [ppm]
Dimensions = X 300
Spectrometer = ECKX-300
Field_strength = 7.0585013 [T] (300 [MHz]
X_acq_duration = 2.90717696 [s]
X_domain = IN 0.5265592 [MHz]
X_offset = 5 [ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.3439761 [Hz]
X_sweep = 5.53570784 [kHz]
Xr_freq = 300.5265592 [MHz]
Xr_offset = 5 [ppm]
Xr_domain = IN
Xr_freq = 300.5265592 [MHz]
Xr_offset = 5 [ppm]
Xr_domain = IN
Mod_return = 1
Scans = 8
Total_scans = 8
X_90_width = 13.43 [us]
X_acq_time = 2.90717696 [s]
X_angle = 45 [deg]
X_atn = 3 [dB]
X_pulse = 6.715 [us]
Xr_mode = OFF
Date_presat = FALSE
Initial_wait = 1 [s]
Recovery_gain = 20
Relaxation_delay = 5 [s]
Relaxation_time = 22.9 [s]
Temp_set =
    
```



Standard ¹³C
Experiment



Current Data Parameters
 NAME: FG05C1
 EXPNO: 2
 PROCNO: 1

F2 - Acquisition Parameters
 Date_: 500000
 Time: 19:59
 INSTRUM: mrx400
 PULPROG: zgpg30
 D1: 2.7000
 SOLVENT: DMS
 NS: 13800
 DS: 4
 SWH: 25000.000 Hz
 FIDRES: 0.765938 Hz
 AQ: 0.656400 sec
 RG: 45500
 CW: 20.000 usec
 DE: 27.14 usec
 TE: 300.0 K
 D12: 0.0000000 sec
 D13: 0.4000000 sec
 CPDPRG: waltz15
 P31: 100.00 usec
 P1: 0.4000000 sec
 P2: 6.75 usec
 P3: 27.14 usec
 SFO1: 100.621179 MHz
 NUC1: 13C
 D11: 0.0300000 sec

F2 - Processing parameters
 SI: 65536
 SF: 100.612760 MHz
 KW: 64
 SSF: 0
 LB: 2.00 Hz
 GB: 0
 PC: 1.40

10 NMR plot parameters
 CX: 20.00 cm
 F1P: 227.255 ppm
 F1: 22860.89 Hz
 F2P: -21.181 ppm
 F2: -2131.10 Hz
 PPMCM: 12.42867 ppm/cm
 HZCM: 1245.99868 Hz/cm

¹³C NMR spectrum of FG05

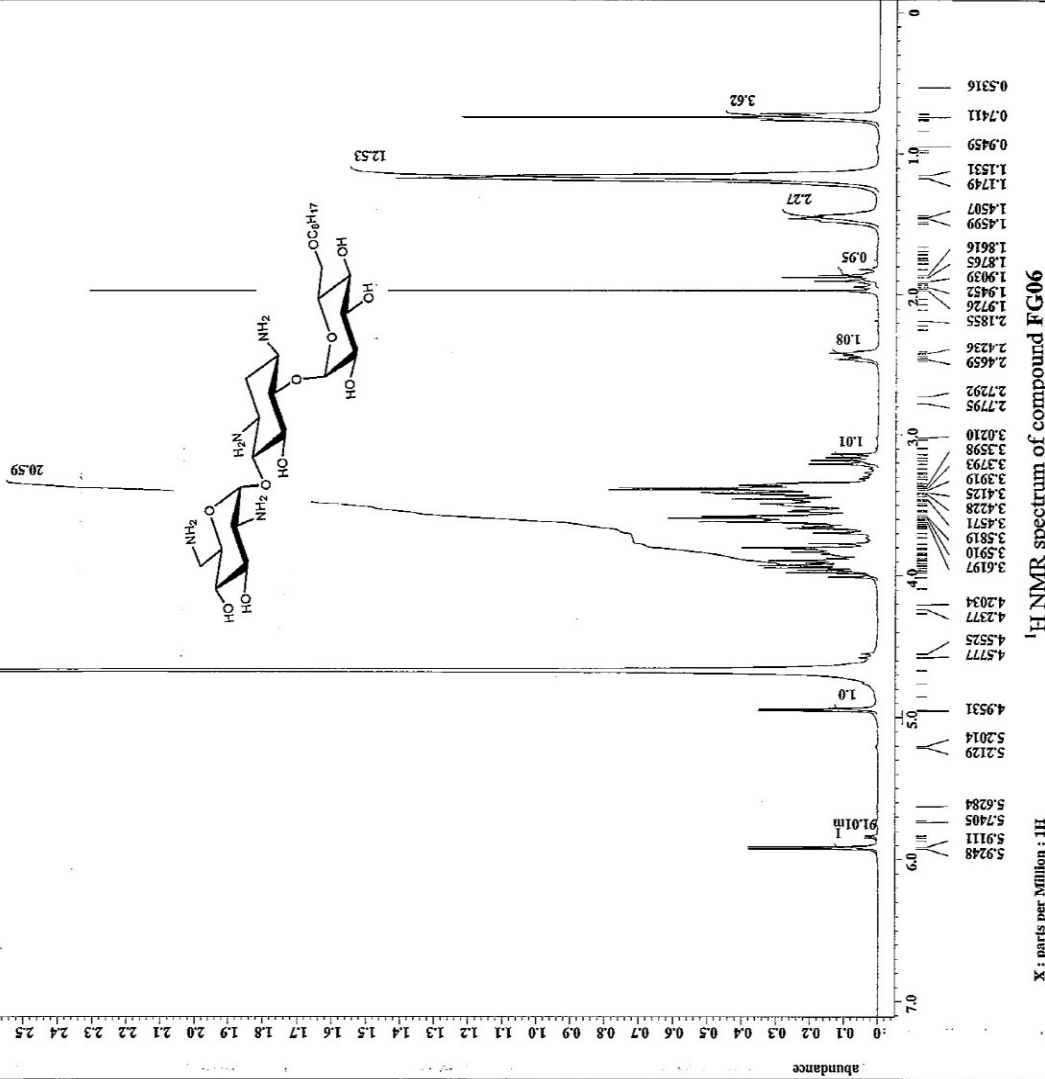
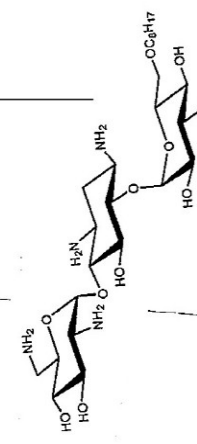


```

File Name      = 121510FG06(C1)-3-jdf
Author        = fossco
Experiment    = single_pulse.exp2
Sample Id     = 121510FG06(C1)
Creation time = 15-DEC-2010 19:30:31
Revision time = 15-DEC-2010 19:33:49
Current time  = 15-DEC-2010 19:34:12

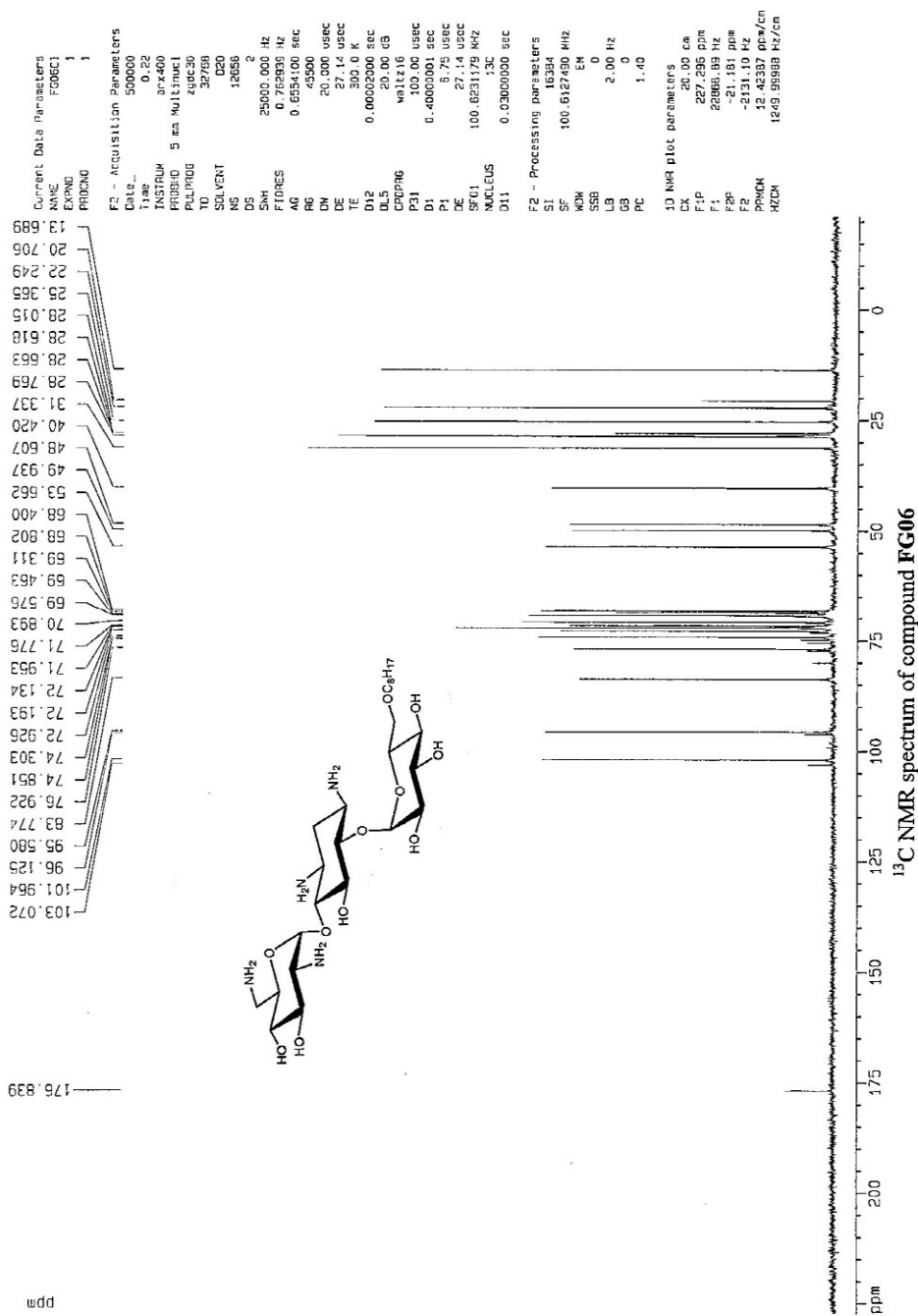
Comment      = single_pulse
              = 1D COMPLEX
Data format  = 13107
Dim_size     = 1H
Dim_title    = [ppm]
Dim_units    = X
Dimensions   = SX-300
Spectrometer = SUX-300

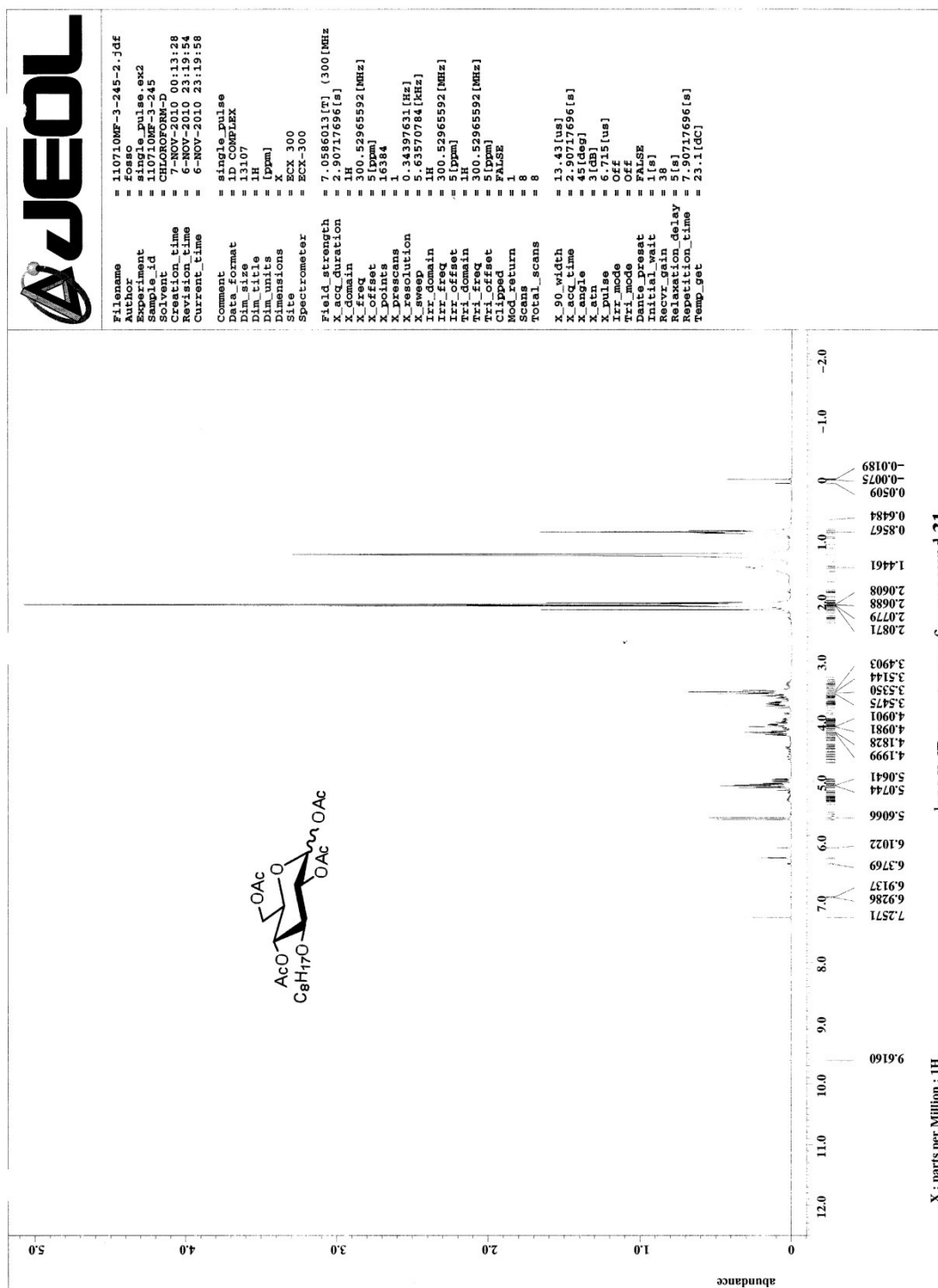
Field strength = 7.0586013 [T] (300 [MHz]
X_acq_duration = 2.90717696 [s]
X_domain       = 1H
X_offset       = 0
X_points       = 16384
X_resolution   = 1
X_sweeps       = 0.3439761 [Hz]
X_version      = 1
F2_domain      = 1H
F2_freq        = 65570784 [MHz]
F2_offset      = 300.52965592 [MHz]
F2_points      = 5 [ppm]
F2_resolution  = 1H
F2_sweeps      = 0.3439761 [Hz]
F2_version     = 1
F1_domain      = 1H
F1_freq        = 300.52965592 [MHz]
F1_offset      = 0
F1_resolution  = 1H
F1_sweeps     = 0.3439761 [Hz]
F1_version     = 1
Mod_return     = FALSE
Total_scans    = 1
              = 8
X_90_width     = 13.43 [us]
X_acq_time     = 2.90717696 [s]
X_angle        = 45 [deg]
X_atn          = 3 [dB]
X_pulse        = 6.715 [us]
X_mode         = Off
Dante_presat   = FALSE
Initial_wait   = 1 [s]
Recvr_gain     = 51 [dB]
Relaxation_delay = 5 [s]
Relaxation_time = 23 [DC]
Temp_set       = 23 [C]
  
```

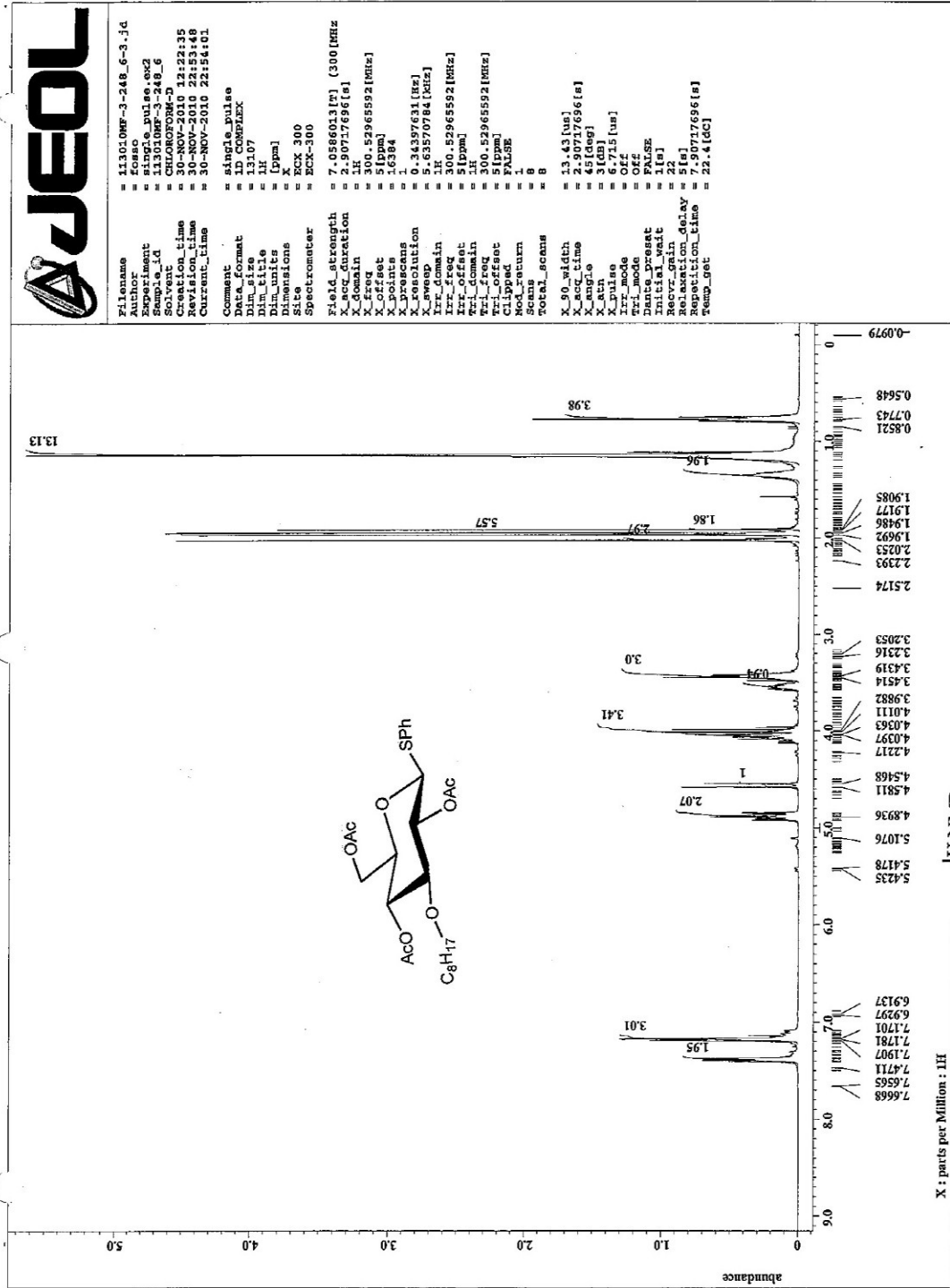


¹H NMR spectrum of compound FG06
X : parts per Million : 1H

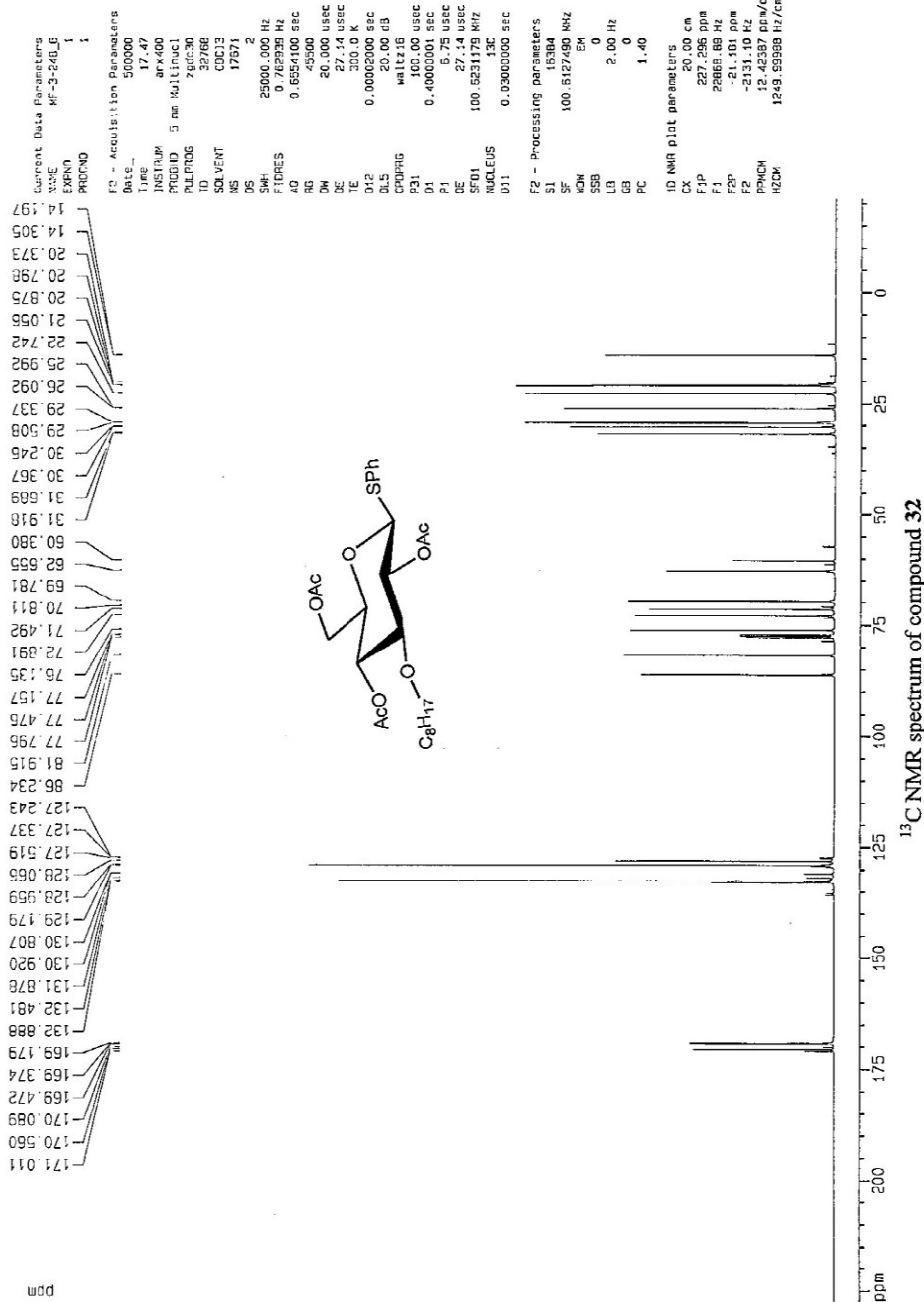
Standard ¹³C
Experiment







Standard ¹³C
Experiment



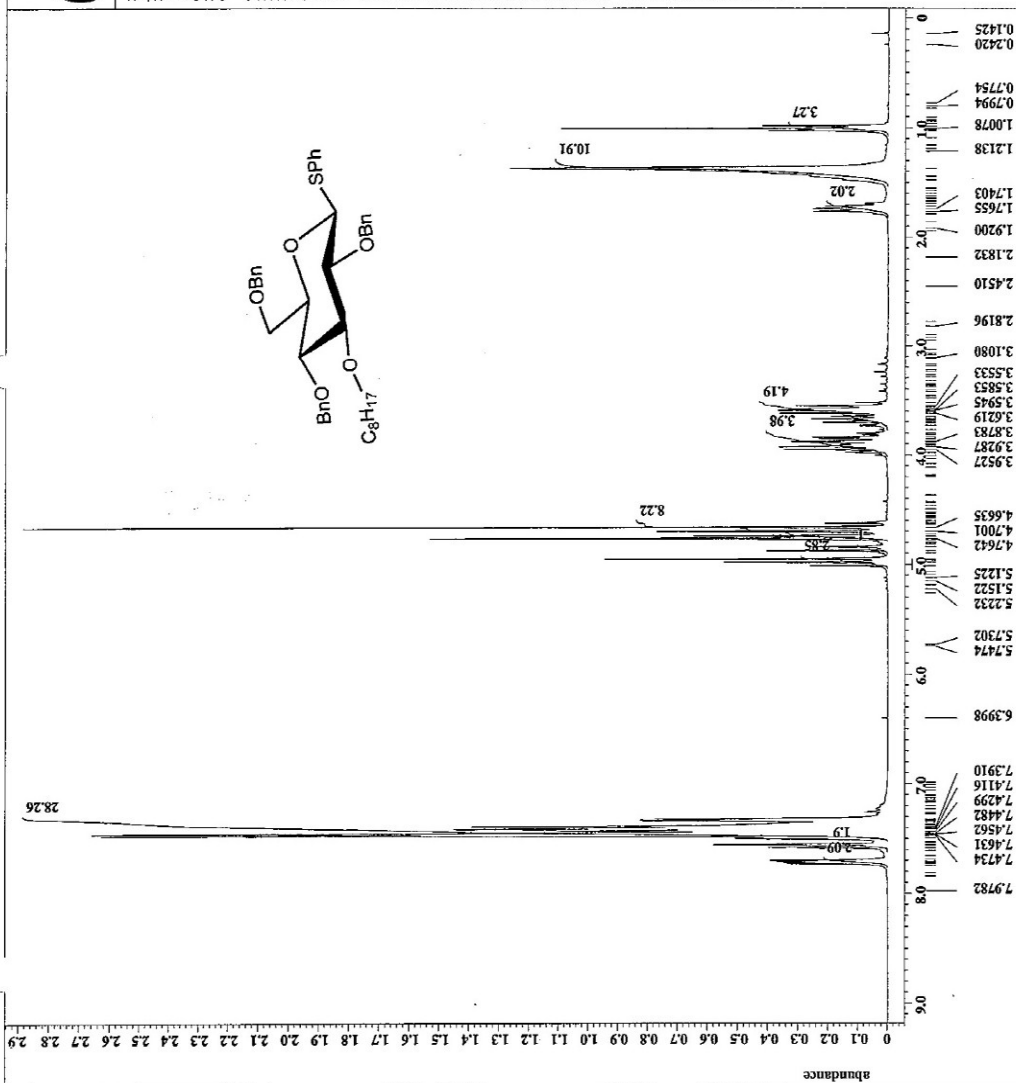


```

Filename = 120710MP-3-265-3.jdf
Author = fossio
Experiment = single_pulse.exe2
Sample_id = 120710MP-3-265
Solvent = CDCl3
Acquisition_time = 8-DEC-2010 17:48:38
Revision_time = 8-DEC-2010 17:48:38
Current_time = 8-DEC-2010 17:48:46

Comment = single_pulse
          CDCl3
          13107
          1H
          [ppm]
          X
          EXC 300
          EXC-300

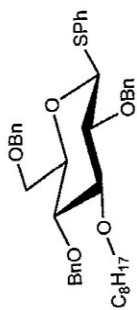
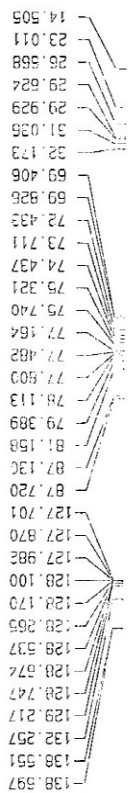
Spectrometer
Field_strength = 7.0586013 [T] (300 [MHz]
X_acq_duration = 2.90717696 [s]
X_domain = 1H
X_offset = 5 [Dppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.34327631 [Hz]
X_sweep = 18
X_sweep_gain = 1.63570784 [MHz]
Irr_freq = 300.52965592 [MHz]
Irr_offset = 5 [Dppm]
Tri_domain = 1H
Tri_freq = 300.52965592 [MHz]
Tri_offset = 5 [Dppm]
Clipped4 = FALSE
Mod_return = 1
Scans = 8
Total_scans = 8
X_90_width = 13.43 [us]
X_acq_time = 2.90717696 [s]
X_angle = 45 [deg]
X_atn = 3 [dB]
X_pulse = 0.2 [us]
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1 [s]
Recev_gain = 24
Recev_delay = 1 [s]
Repetition_time = 7.90717696 [s]
Temp_get = 22.6 [dC]
    
```



¹H NMR spectrum of compound 33

X : parts per Million : 1H

Standard ¹³C
Experiment



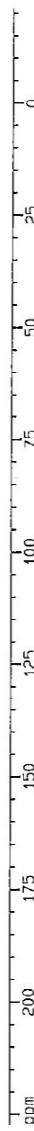
Experiment Parameters
NAME: 3F-3-265
EXPNO: 1
PROCNO: 1

Acquisition Parameters
Date_: 500000
Time: 18.15
INSTRUM: spect
PROBHD: 5 mm Multic1
PULPROG: zgpg30
TO: 32768
SOLVENT: CDCl3
NS: 18191
DS: 2
SWH: 25000.000 Hz
FIDRES: 0.762395 Hz
AQ: 0.625400 sec
RG: 3500
DB: 20.000 usec
DE: 27.14 usec
TE: 300.2 K
D12: 0.0002000 sec
D15: 20.00 usec
wait216
CPDPRG2: waltz16
P31: 100.00 usec
D1: 0.40000001 sec
P1: 6.75 usec
DE: 27.14 usec
SFO1: 100.623175 MHz
NUCLEUS: 13C
D11: 0.0300000 sec

F2 - Processing parameters
SI: 16384
SF: 100.6127950 MHz
WDW: EM
SSB: 0
GB: 0
PC: 1.40

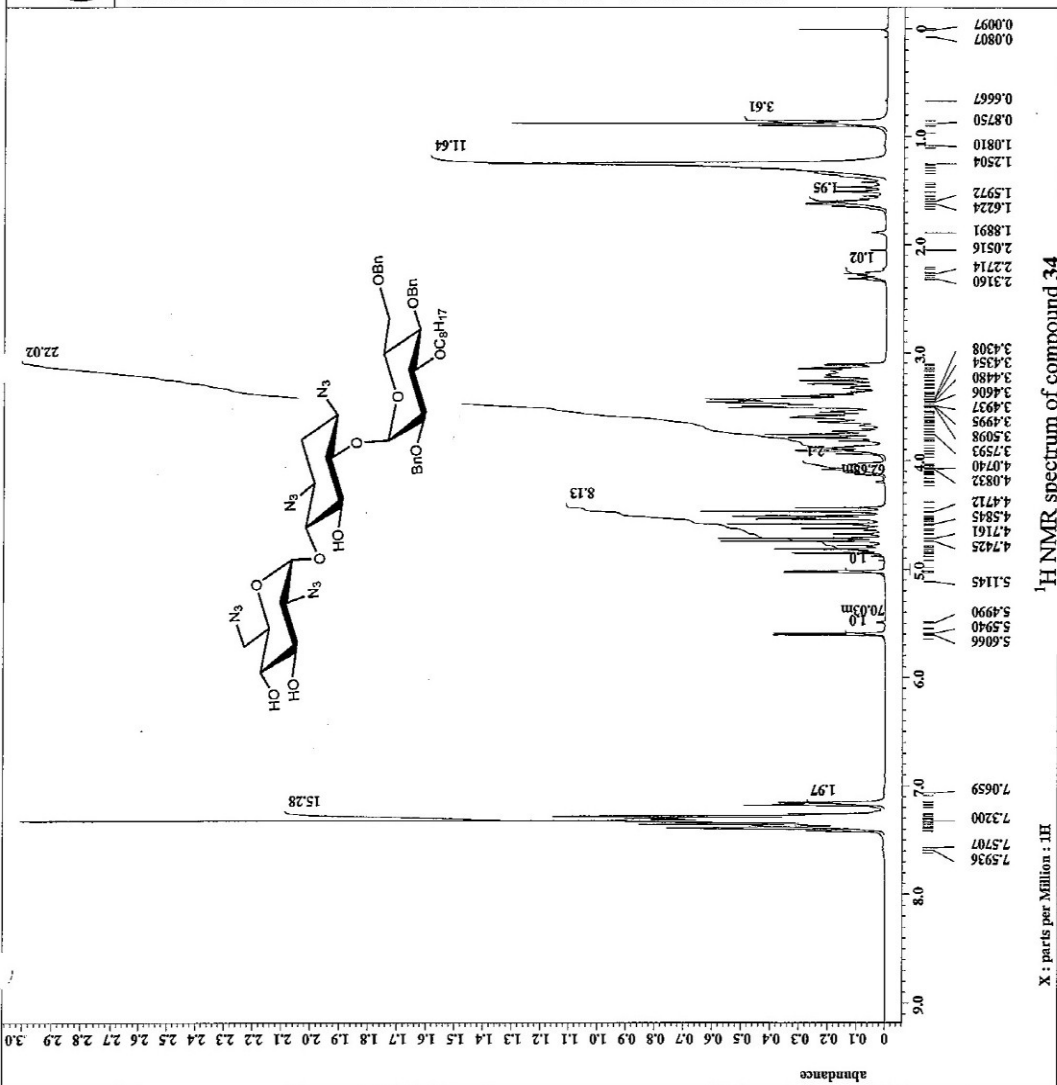
1D NMR plot parameters
CX: 20.00 cm
F1P: 227.256 ppm
F1: 22859.69 Hz
F2P: -21.181 ppm
F2: -2131.10 Hz
PPHOM: 12.42307 ppm/cm
HZCM: 1246.99888 Hz/cm

¹³C NMR spectrum of compound 33





Filename = 012011MF-3-289-3.fid
 Author = fossa
 Date_acq = 20-JAN-2011 16:04:05
 File_name = 012011MF-3-289
 Sample_id = CHLOROFORM-D
 Solvent = CHLOROFORM-D
 Creation_time = 20-JAN-2011 15:55:31
 Revision_time = 20-JAN-2011 16:04:05
 Current_time = 20-JAN-2011 16:04:13
 Comment = single pulse
 Data_format = ID COMPLEX
 Dim_size = 33107
 Dim_time = 1H
 Dim_units = X
 Dimensions = X
 Site = ECK 300
 Spectrometer = ECK-300
 Field_strength = 7.05860137G (300 MHz)
 X_acq_duration = 2.90717696[s]
 X_domain = 1H
 X_freq = 300.52965592 [MHz]
 X_offset = 5 [ppm]
 X_points = 16384
 X_resolution = 0.34397631 [Hz]
 X_sweep = 5.63570784 [kHz]
 Irr_domain = 1H
 Irr_freq = 300.52965592 [MHz]
 Irr_offset = 5 [ppm]
 Clipped = FALSE
 Scans = 8
 Total_scans = 8
 X_90_width = 13.43 [us]
 X_acq_time = 4.0727696 [s]
 X_att = 14.0 [dB]
 X_atn = 3 [dB]
 X_pulse = 6.715 [us]
 Irr_mode = Off
 Irr_offset = 0 [ppm]
 Irr_sweep = 0 [ppm]
 Initial_wait = 1 [s]
 Recvr_gain = 36
 Relaxation_delay = 5 [s]
 Repetition_time = 7.90717696 [s]
 Temp_set = 22.9 [dC]



¹H NMR spectrum of compound 34

X : parts per Million : 1H

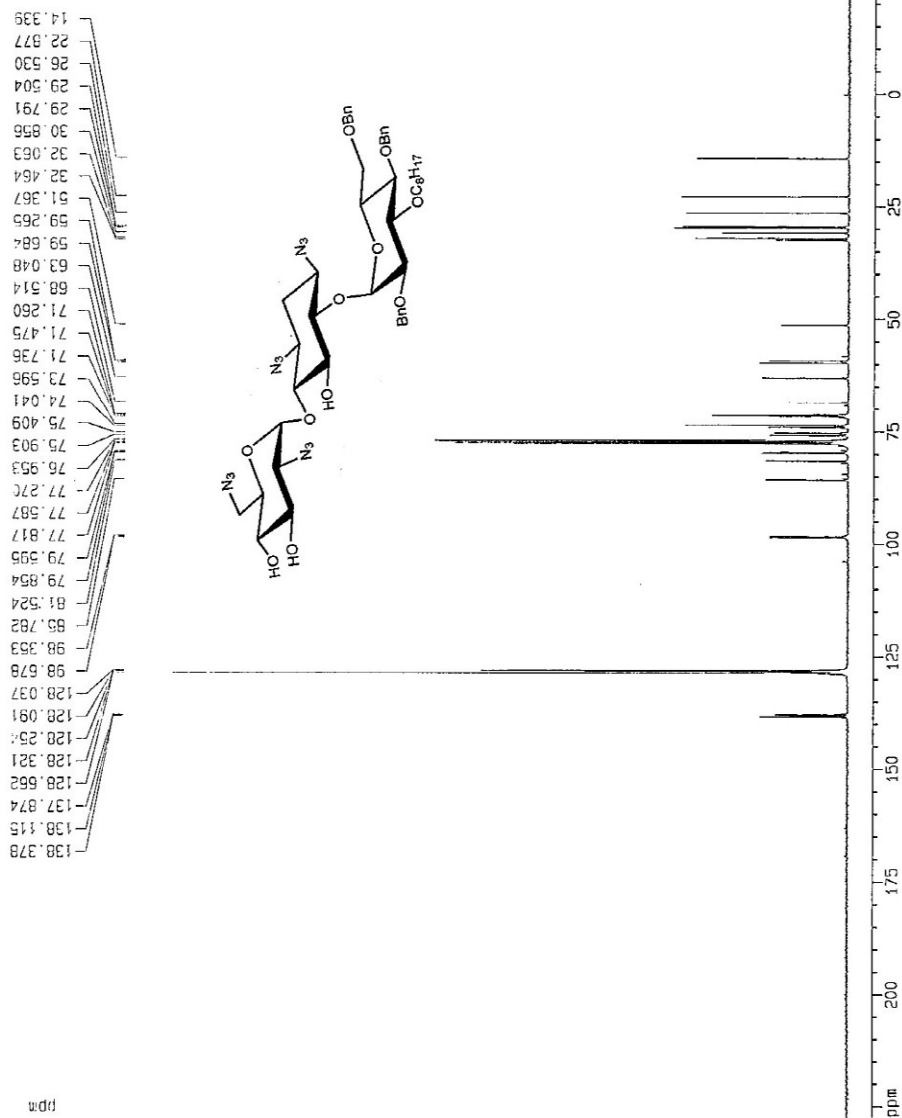
Standard ¹³C
Experiment

Current Data Parameters
NAME MF-3-289
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 500000
Time 18.37
INSTRUM drx400
PROBHD 5 mm Multinucl
PULPROG zgpg30
TD 32768
SOLVENT CDCl3
NS 17680
DS 2
SWH 26000.000 Hz
FIDRES 0.716393 Hz
AQ 0.6554100 sec
RG 45500
DN 20.000 usec
DE 27.14 usec
TE 300.0 K
D12 0.00002000 sec
EL5 20.00 dB
CPDPRG W11215
P31 100.00 usec
D1 0.40000001 sec
P1 6.75 usec
DE 27.14 usec
SF01 100.6231179 MHz
NUCLEUS ¹³C
D11 0.05000000 sec

F2 - Processing parameters
SI 16384
SF 100.6127450 MHz
WDW EK
SSB 0
LB 2.00 Hz
GB 0
PC 1.40

1D NMR plot parameters
CX 20.00 cm
FIP 227.256 pps
F1 22669.89 Hz
F2P -21.181 pps
F2 -2131.10 Hz
PPMCM 12.42387 ppm/cm
HZCM 1246.99986 Hz/cm





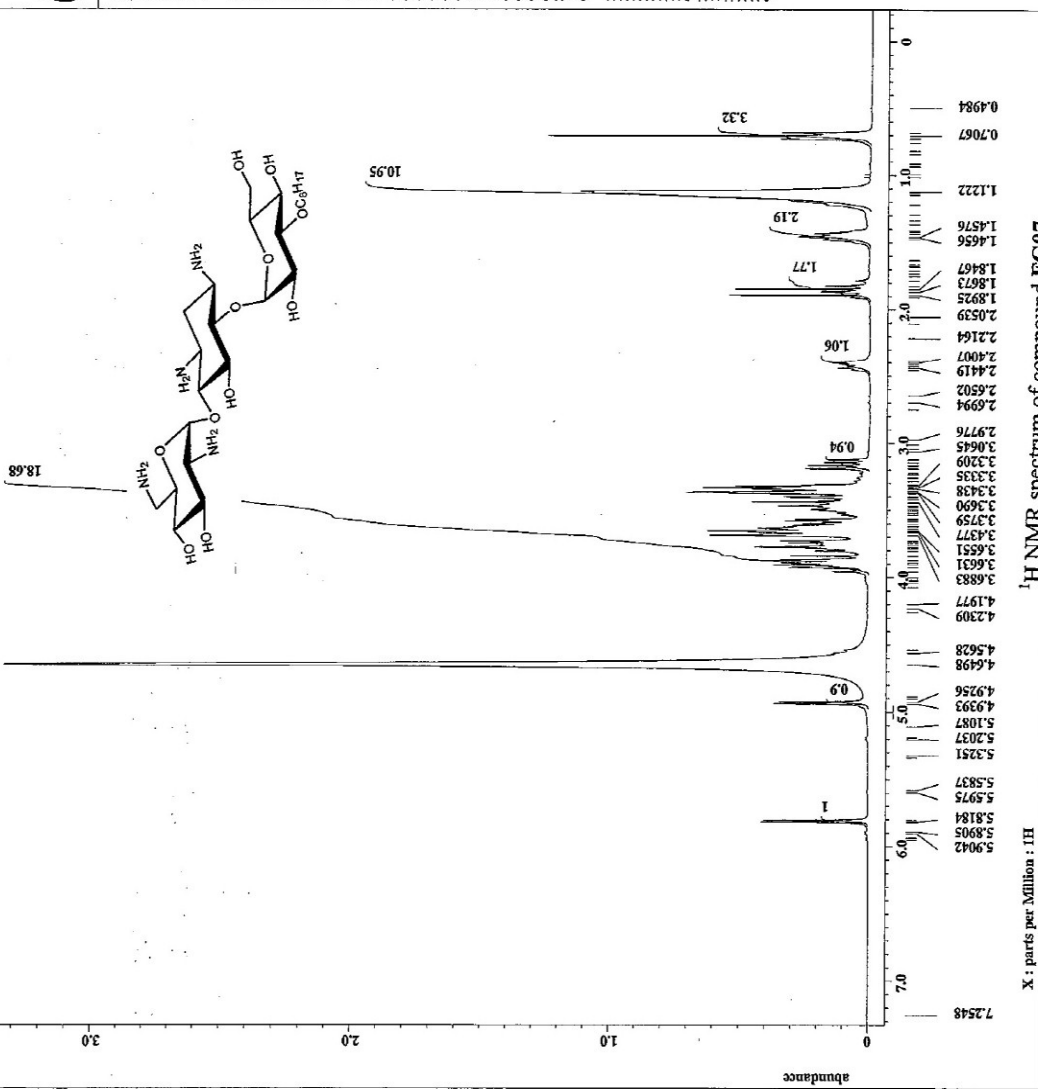
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Filename = 02091F07(C1)-3.jcf
Author = zoso
Experiment = 02091F07(C1)
Sample_id = 02091F07(C1)
Solvent = D2O
Creation_time = 9-FEB-2011 11:25:43
Revision_time = 9-FEB-2011 11:28:21
Current_time = 9-FEB-2011 11:28:54

Comment = single pulse
Data_format = 1D COMPLEX
Dim_size = 13107
Dim_title =
Dimensions = X
Site = ECK 300
Spectrometer = ECK-300

Field_strength = 7.0566013 [T] (300 MHz)
X_acq_time = 13
X_domain = 13
X_freq = 300.52965592 [MHz]
X_offset = 5 [ppm]
X_points = 16384
X_resolution = 0.34397631 [Hz]
X_sweep = 5.63570784 [kHz]
Irr_domain = 13
Irr_freq = 300.52965592 [MHz]
Irr_offset = 5 [ppm]
Irr_sweep = 5.63570784 [kHz]
Tri_offset = 1 [ppm]
Tri_freq = 300.52965592 [MHz]
Tri_offset = 5 [ppm]
Clipped = FALSE
Soft_return = 1
Total_scans = 8

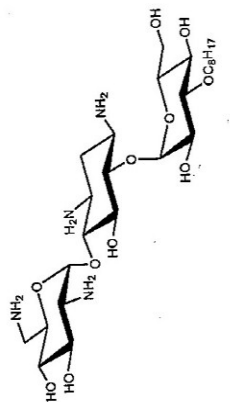
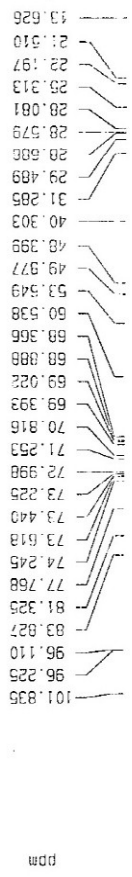
X_90_width = 13.43 [us]
X_acq_time = 2.90717696 [s]
X_p1 = 100
X_p2 = 3 [dB]
X_pulse = 6.715 [us]
Irr_mode = Off
Irr_offset = Off
Irr_sweep = FALSE
Irr_start = 0 [s]
Irr_stop = 34 [s]
Recovery_gain = 1
Relaxation_delay = 5 [s]
Repetition_time = 7.90717696 [s]
Temp_set = 22.4 [C]
    
```



¹H NMR spectrum of compound FG07

X : parts per Million : 1H

Standard 13C
Experiment



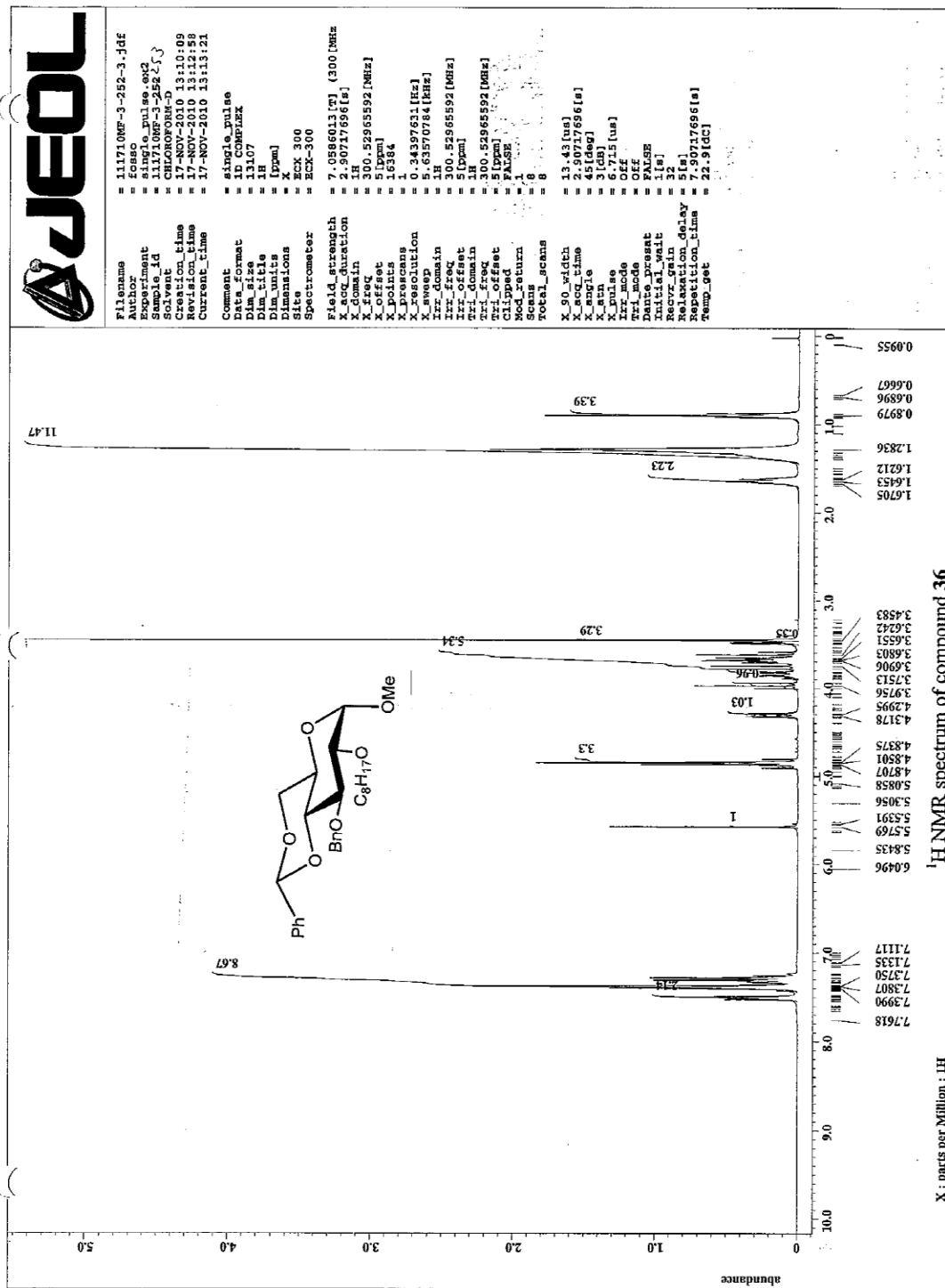
Current Data Parameters
 NAME: FG07
 EXPNO: 1
 PROCNO: 1

F2 - Acquisition Parameters
 Date_: 500000
 Time: 19.15
 INSTRUM: ark400
 PROBHD: 5 mm Multinuc
 PULPROG: zgpg30
 TD: 32768
 SOLVENT: d2o
 NS: 19259
 DS: 2
 SWH: 25000.000 Hz
 FIDRES: 0.762350 Hz
 AQ: 0.6554100 sec
 RG: 45500
 DA: 20.600 usec
 DE: 27.14 usec
 TE: 300.0 K
 D12: 0.0000000 sec
 DL5: 20.00 dB
 CPDPRG: Multiz15
 P31: 100.00 usec
 P1: 0.4000000 sec
 P2: 6.75 usec
 UE: 27.14 usec
 SF01: 100.6231179 MHz
 NUC1EUS: 13C
 D11: 0.0300000 sec

F2 - Processing parameters
 SI: 32768
 SF: 100.6127450 MHz
 AN: EN
 ASB: 0 Hz
 LB: 0 Hz
 GB: 0 Hz
 PC: 1.40

10. NMR plot parameters
 CX: 20.00 cm
 F1P: 125.000 ppm
 F1: 12576.96 Hz
 F2P: -21.181 ppm
 F2: -2131.10 Hz
 PPHCM: 7.30906 ppm/cm
 HZCM: 735.38483 Hz/cm

¹³C NMR spectrum of compound FG07



```

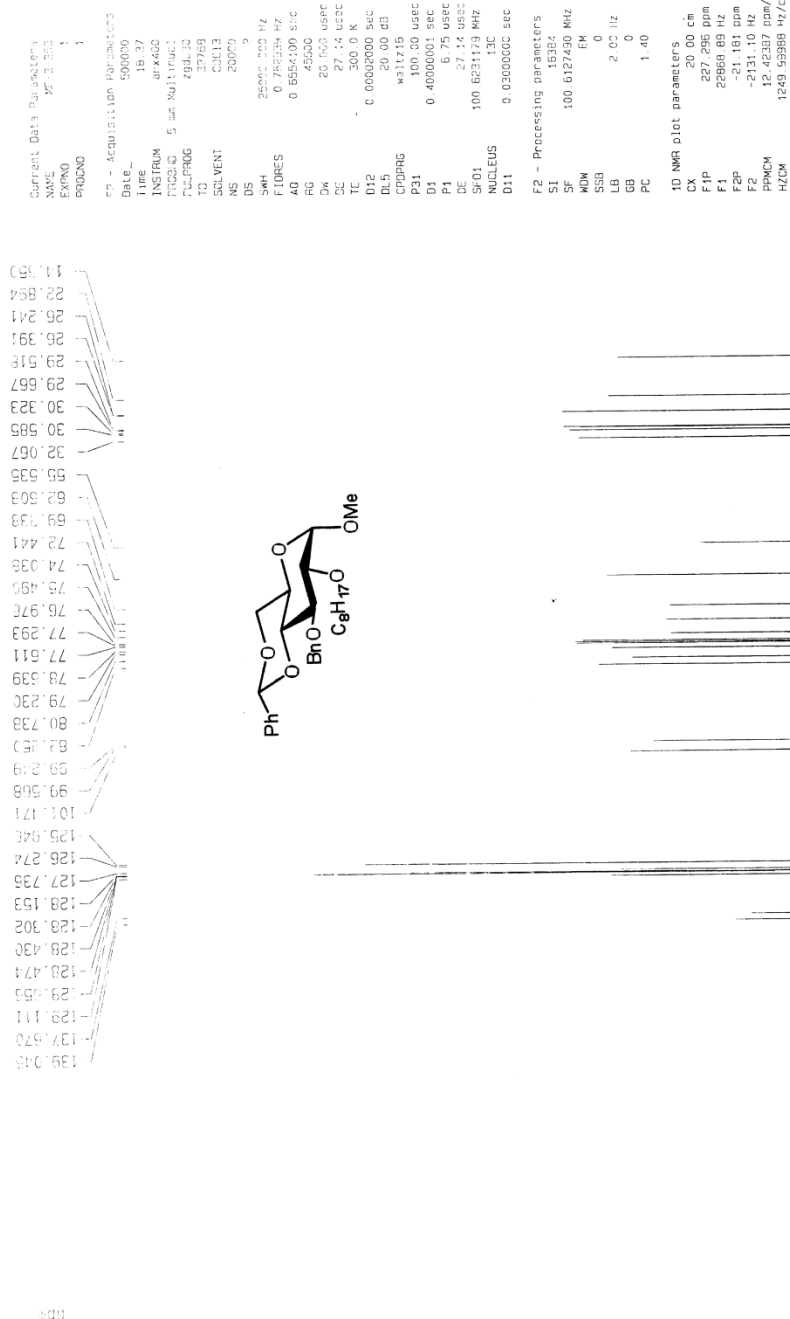
=====
File name      = 111710MF-3-252-3-jdf
Author        = fcaso
Experiment    = single_pulse.ec2
Sample_id     = 111710MF-3-252-3
Solvent      = CDCl3
Acq date     = 17-NOV-2010 13:10:09
Revision time = 17-NOV-2010 13:12:58
Current time  = 17-NOV-2010 13:13:21

=====
Comment       = single_pulse
Dim_1         = 131.07
Dim_2         = 131.07
Dim_title     = 1H
Dim_units     = [ppm]
Dimensions    = X
Spectrometer  = ECK 300
Site          = ECK-300

Field strength = 7.0586013(T) (300 MHz)
X_acq_duration = 2.80717696[s]
X_domain       = 1H
X_freq         = 300.52965592 [MHz]
X_offset       = 0.00000000 [ppm]
X_points       = 16384
X_prescans     = 1
X_resolution   = 0.34397611 [Hz]
X_sweep        = 5.63570784 [kHz]
X_tau         = 100.52865592 [MHz]
Xr1_freq       = 5 [ppm]
Xr1_offset     = 1H
Xr1_domain     = 1H
Xr1_freq       = 300.52965592 [MHz]
Xr1_offset     = 5 [ppm]
Xr1_domain     = 1H
Xr2_freq       = 1
Xr2_offset     = 1
Xr2_domain     = 1
Xr3_freq       = 1
Xr3_offset     = 1
Xr3_domain     = 1
Xr4_freq       = 1
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Xr4_domain     = 1
Xr5_freq       = 1
Xr5_offset     = 1
Xr5_domain     = 1
Xr6_freq       = 1
Xr6_offset     = 1
Xr6_domain     = 1
Xr7_freq       = 1
Xr7_offset     = 1
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Xr100_domain  = 1
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```

Standard ¹³C
Experiment





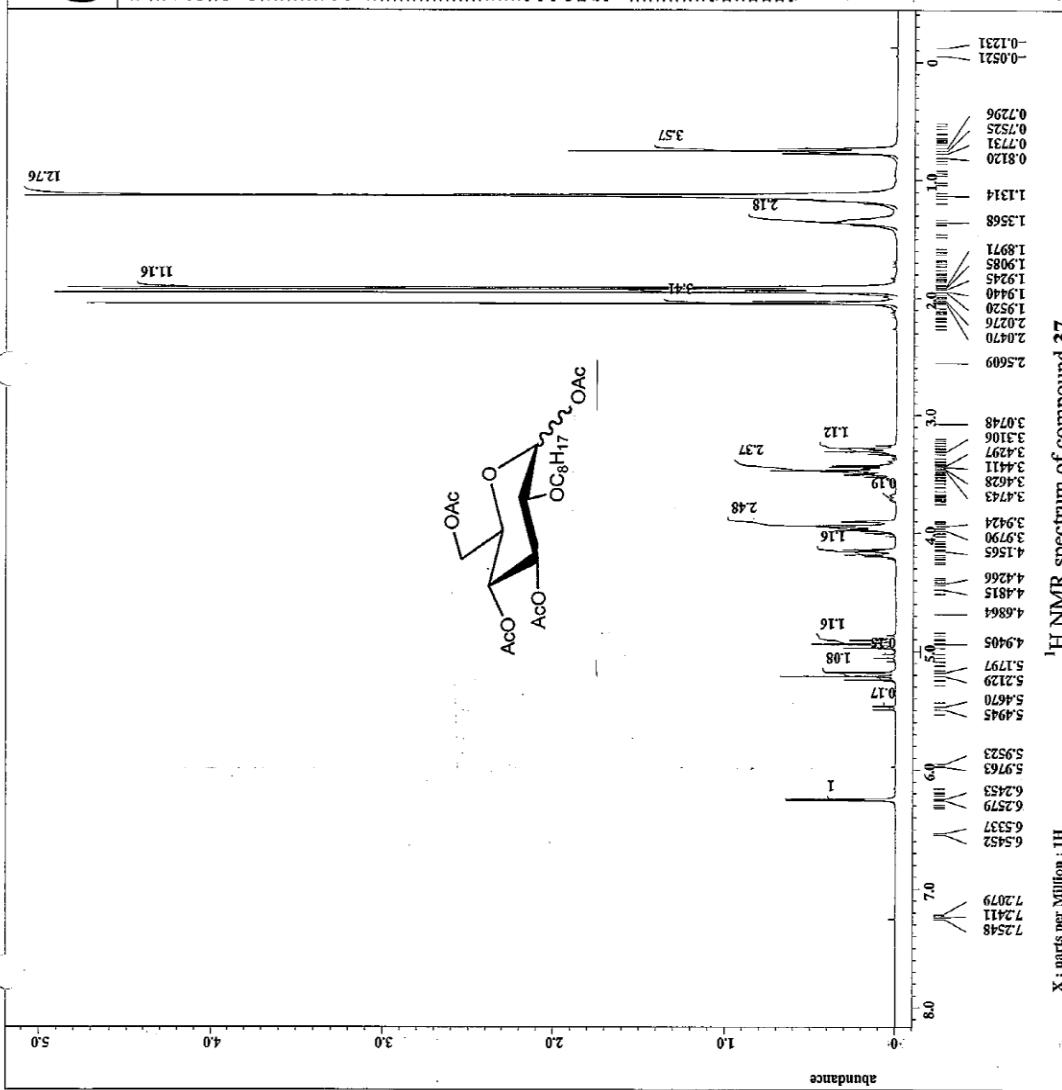
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File name      = 121610MF-3-255-3.jde
Author        = zoso
Experiment    = single_pulse-ex2
Sample Id     = 121610MF-3-255
Date         = 15-DEC-2010 14:17:19
Creation time = 15-DEC-2010 14:17:19
Revision time = 15-DEC-2010 14:19:28
Current time  = 15-DEC-2010 14:19:52

Comment       = single pulse
Data format  = 1D_1HPR2K
Dir          = 13107
Dim_size     = 1H
Dim_title    = [ppm]
Dim_units    = X
Dimensions   = X
Site         = EX-300
Spectrometer = EX-300

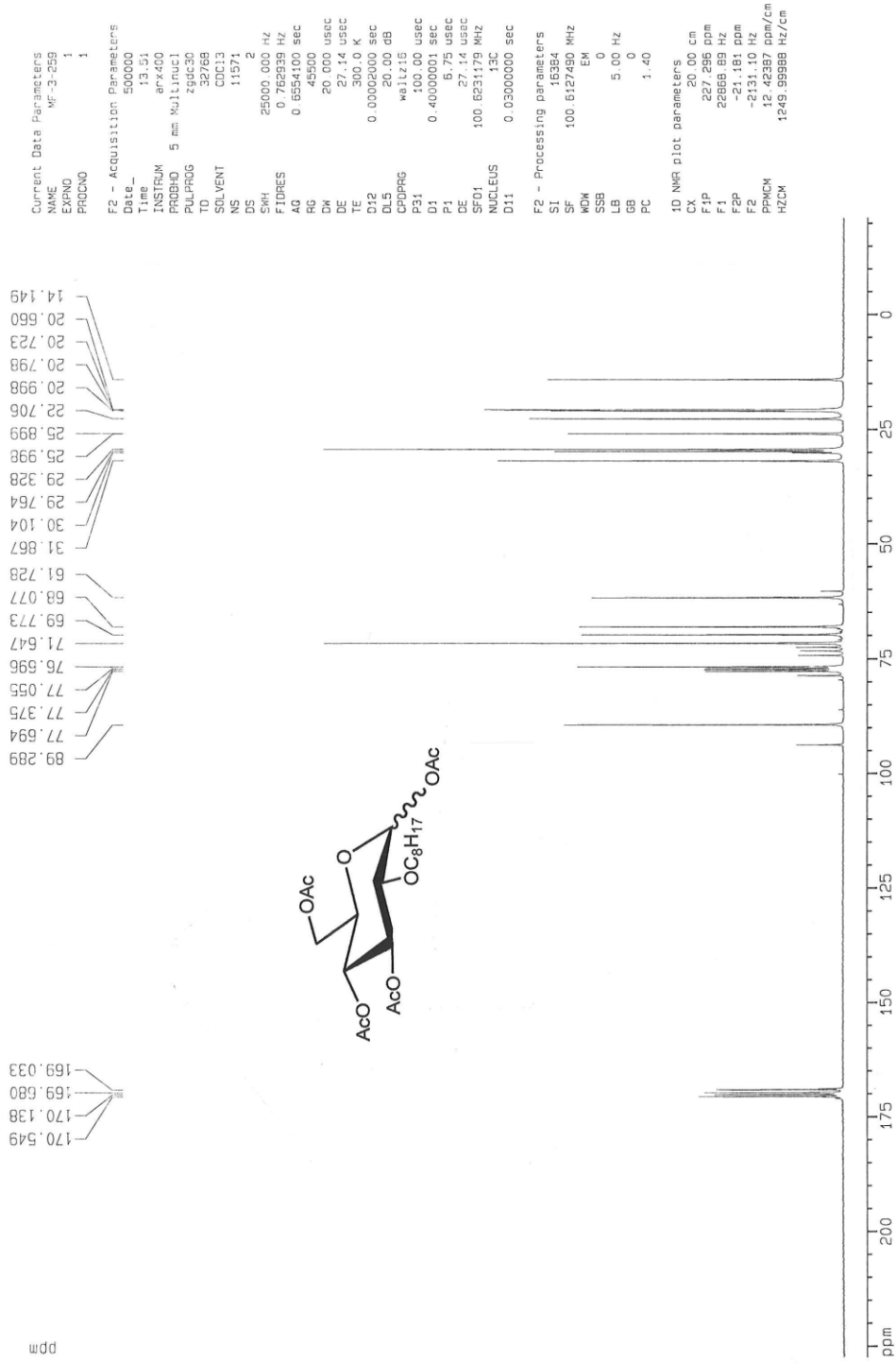
Field strength = 7.058601317 (300) [MHZ]
X_acq_duration = 2.80717696 [s]
X_domain       = 1H
X_offset       = 50.52965592 [MHZ]
X_points      = 16384
X_prescans    = 1
X_resolution  = 0.34397631 [Hz]
X_sweep       = 5.63570784 [MHZ]
X_start       = 300.52965592 [MHZ]
X_stop        = 51 [ppm]
X_t1          = 1H
X_t2          = 1H
X_t3          = 1H
X_t4          = 1H
X_t5          = 1H
X_t6          = 1H
X_t7          = 1H
X_t8          = 1H
X_t9          = 1H
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X_t12         = 1H
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X_t95         = 1H
X_t96         = 1H
X_t97         = 1H
X_t98         = 1H
X_t99         = 1H
X_t100        = 1H
=====

```



X : parts per Million : 1H

Standard ¹³C
Experiment



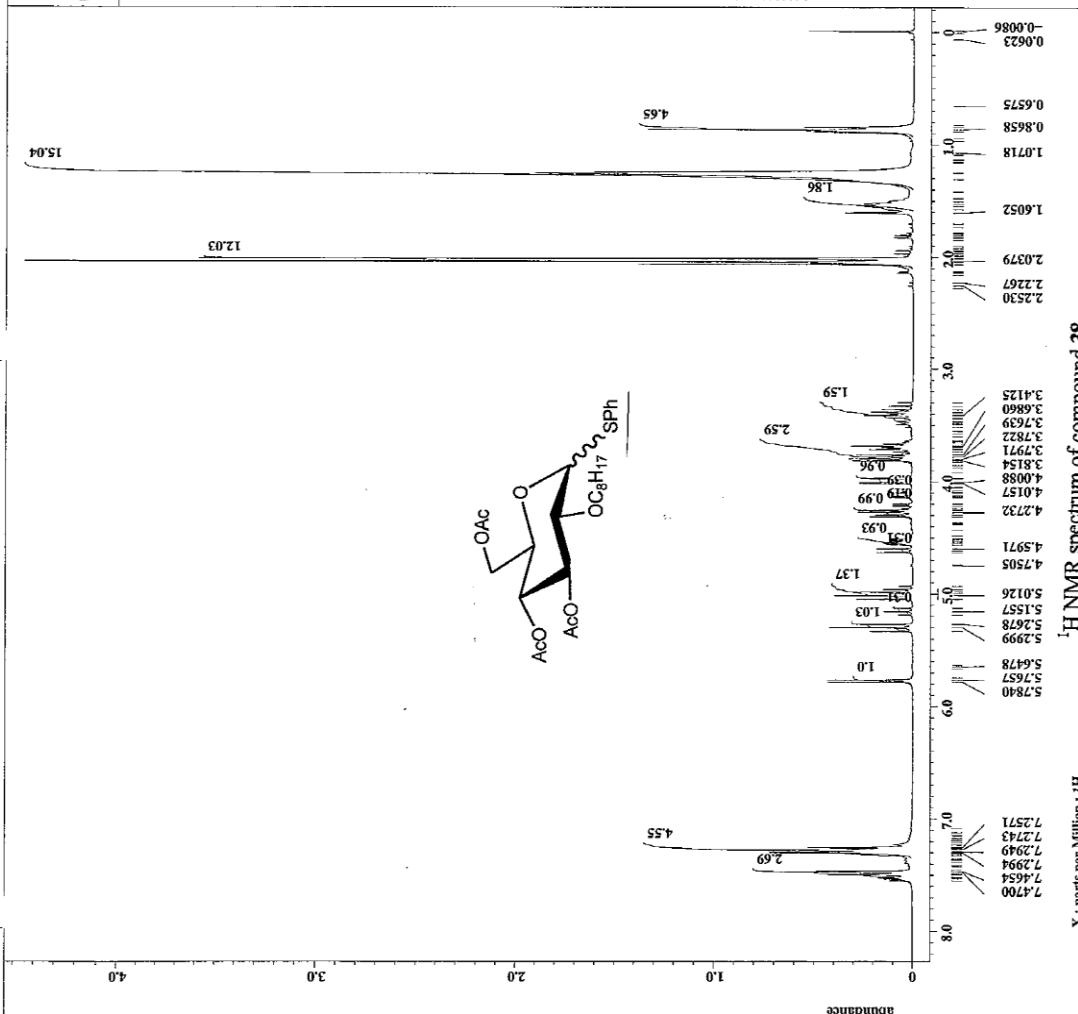


```

Filename = 011711MF-3-277-3-3JGf
Anchor = fosse
Experiment = 1177.pulse.ac2
Sample_id = 011711MF-3-277
Solvent = CHLOROFORM-D
Creation_time = 17-JAN-2011 16:07:24
Revision_time = 4-MAY-2011 17:06:48
Current_time = 4-MAY-2011 17:07:04

Comment = single_pulse
Data_format = ID COMPLEX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Spectrometer = ECX-300

Field_strength = 7.0586013[T] (300[MHz]
X_acq_duration = 2.90717696[s]
X_domain = 1H 0.52965592[MHz]
X_offset = 57093.7
X_points = 16384
X_prescans = 1
X_resolution = 0.34397631[Hz]
X_sweep = 5.63570784[Hz]
Irr_domain = 1H
Irr_offset = 300.52965592[MHz]
Irr_freq = 57093.7
Irr_offset = 5[ppm]
Tri_domain = 1H
Tri_freq = 300.52965592[MHz]
Tri_offset = 5[ppm]
Acquisition = F1JSE
Mode1_return = F1JSE
Mode2_return = F1JSE
Scans = 8
Total_scans = 8
X_90_width = 13.45[us]
X_90_time = 45.917696[s]
X_pulse = 31[us]
X_sfn = 3(GB)
X_sfn = 6.715[un]
Irr_mode = Off
Irr_pulse = Off
Irr_presat = F1JSE
Irr_presat = F1JSE
Irr_presat = F1JSE
Relaxation_delay = 5[s]
Recovery_gain = 40
Repetition_time = 7.90717696[s]
Temp_get = 22.6[dc]
    
```





```

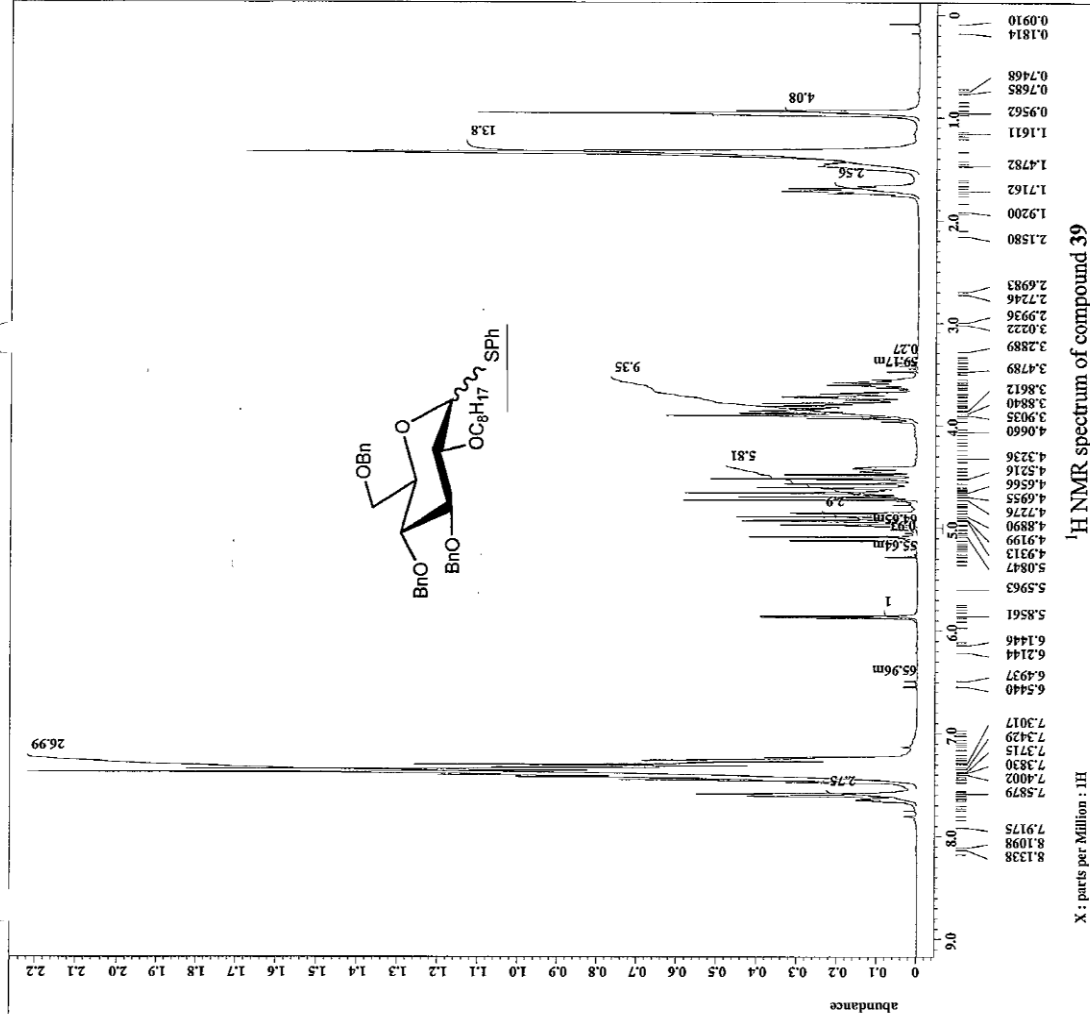
Filename = 012211MF-3-291-3.jcf
Author = fossso
Experiment = 012211mf-3-291-3
PulseProgram = singlepulse
Solvent = CHLOROFORM-D
Creation_time = 22-JAN-2011 11:02:38
Revision_time = 4-MAY-2011 17:10:14
Current_time = 4-MAY-2011 17:10:31

Comment = singlepulse
          ID COMPLEX
Data_format = 13107
Dia_size = 1H
Dia_title = [Dpm]
Dia_units = X
Dimensions = 500
SFO = EXX-300
Spectrometer = EXX-300

Field_strength = 7.0586013 [T] (300 [MHz]
X_acq_duration = 2.90717696 [s]
X_domain = 1H
X_freq = 300.52965592 [MHz]
X_gain = 165884
X_points = 1
X_resolution = 0.34397631 [Hz]
X_sweep = 5.63570784 [Hz]
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
Magnet =
Scan = 8
Total_scans = 8

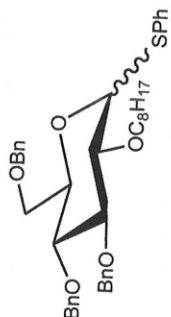
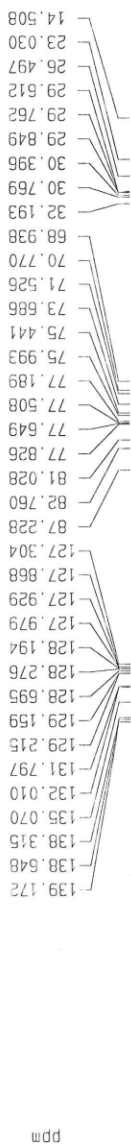
X_90_width = 13.43 [us]
X_acq_time = 2.90717696 [s]
X_gate = 3 [dB]
X_gain = 6.715 [us]
Irr_mode = Off
Tri_mode = Off
Nucleus_presat = FALSE
Recycle_delay = 2.0 [s]
Relaxation_delay = 5 [s]
Repetition_time = 7.90717696 [s]
Temp_get = 22.6 [dC]

```

¹H NMR spectrum of compound 39

X : parts per Million : 1H

Standard ¹³C Experiment

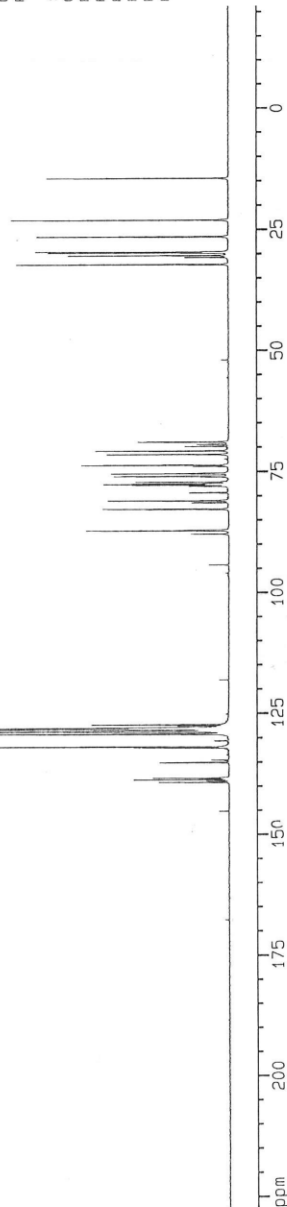


Current Data Parameters
 NAME MF-3-291
 EXPNO 1
 PROCNO 1

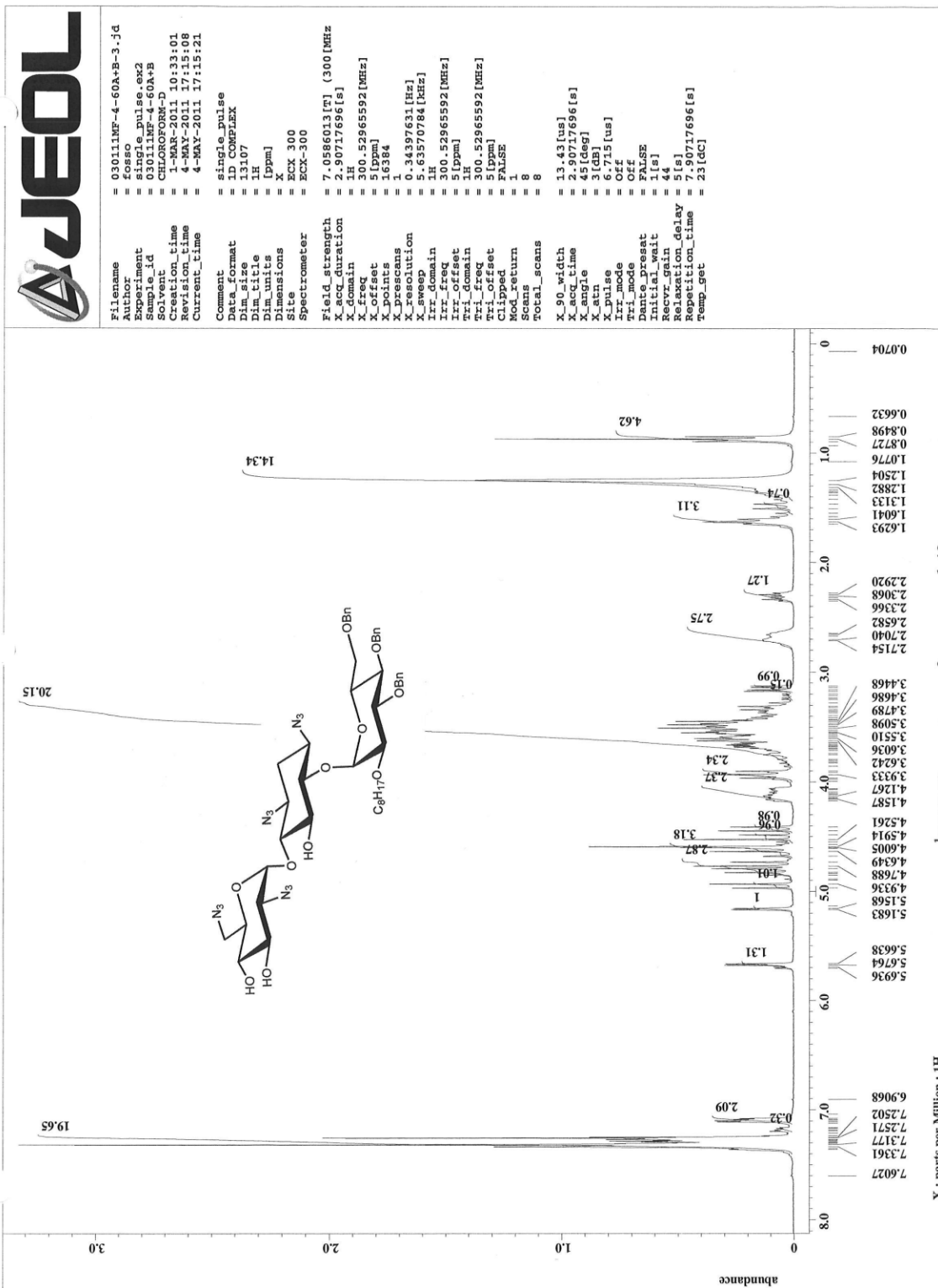
F2 - Acquisition Parameters
 Date_ 500000
 Time 19.04
 INSTRUM arx400
 PROBHD 5 mm Multic1
 PULPROG zgpg30
 TD 32768
 SOLVENT CDCl3
 NS 12576
 DS 2
 SFO1 25000.000 Hz
 FTRES 0.762938 Hz
 AQ 0.1655100 sec
 RG 45300
 DW 20.000 usec
 DE 27.14 usec
 TE 300.0 K
 D12 0.0002000 sec
 DLS 20.00 uS
 CPOPRG waitz16
 P31 100.00 usec
 D1 0.4000001 sec
 P1 6.75 usec
 DE 27.14 usec
 SFO1 100.6231175 MHz
 NUCLEUS 13C
 D11 0.03000000 sec

F2 - Processing parameters
 SI 16384
 SF 100.6127490 MHz
 MDW EM
 SSB 0
 LB 2.00 Hz
 GB 0
 PC 1.40

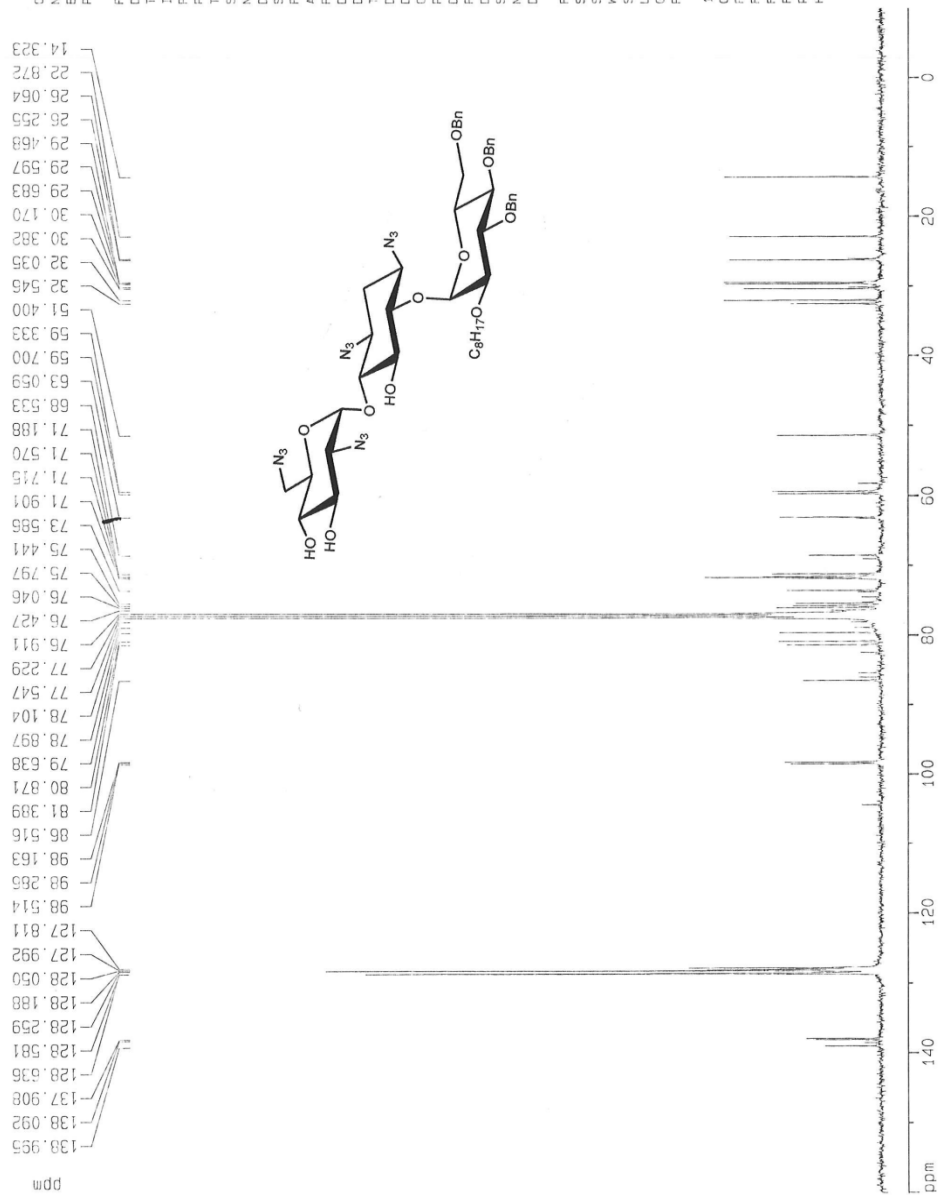
1D NMR plot parameters
 CX 20.00 cm
 F1P 227.295 ppm
 F1 22868.69 Hz
 F2P -21.181 ppm
 F2 -2131.10 Hz
 PPKICK 12.42387 ppm/cm
 HZCM 1249.99868 Hz/cm



¹³C NMR spectrum of compound 39



Standard 13C Experiment



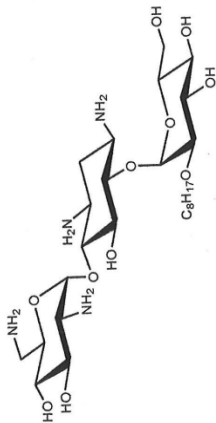
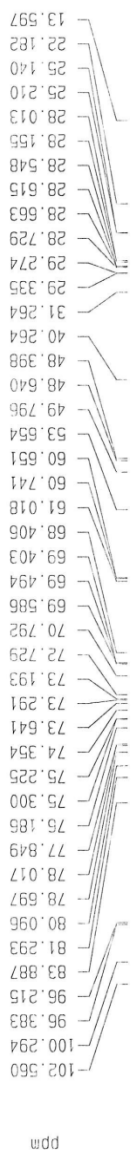
Current Data Parameters
 NAME MF-4-60
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 500000
 Time 1.44
 INSTRUM brx400
 PROBHD 5 mm MSL1001
 PULPROG zgpg30
 TD 32768
 SOLVENT CDCl3
 NS 25000
 DS 2
 SWH 25000.000 Hz
 FIDRES 0.762939 Hz
 AQ 0.6654100 sec
 RG 45500
 DW 20.000 usec
 DE 27.14 usec
 TE 300.0 K
 D12 0.00002000 sec
 DL5 20.00 dB
 CPOPRG waitz16
 P31 100.00 usec
 D1 0.40000001 sec
 P1 6.75 usec
 DE 27.14 usec
 SFO1 100.623179 MHz
 NUCLEUS 13C
 D11 0.03000000 sec

F2 - Processing parameters
 S1 16364
 SF 100.6127490 MHz
 EM
 ADW 0
 SSB 0
 LB 2.00 Hz
 GB 0
 PC 1.40

1D NMR plot parameters
 CX 20.00 cm
 F1 160.000 ppm
 F2 16098.04 Hz
 F3 -10.000 ppm
 F4 -1016.13 Hz
 PPM0H 50000 ppm/cm
 HZCM 865.20837 Hz/cm

Standard ¹³C Experiment

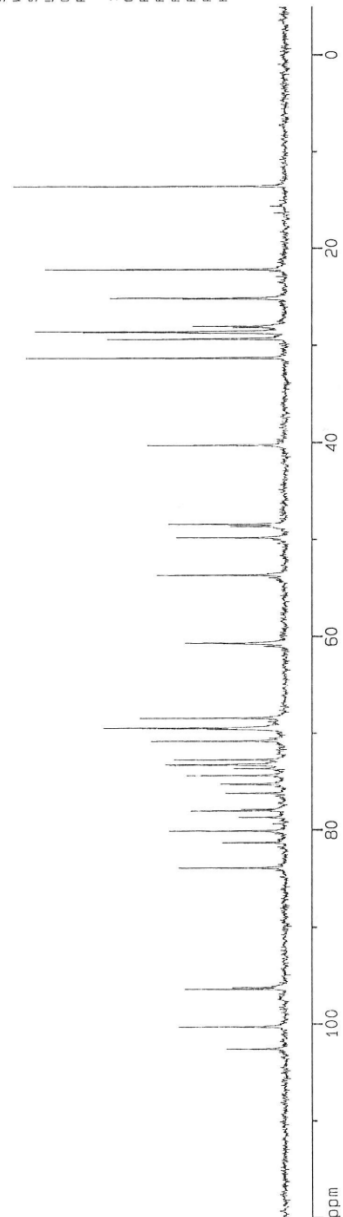


Current Data Parameters
 NAME FG09C1
 EXPNO 3
 PROCNO 1

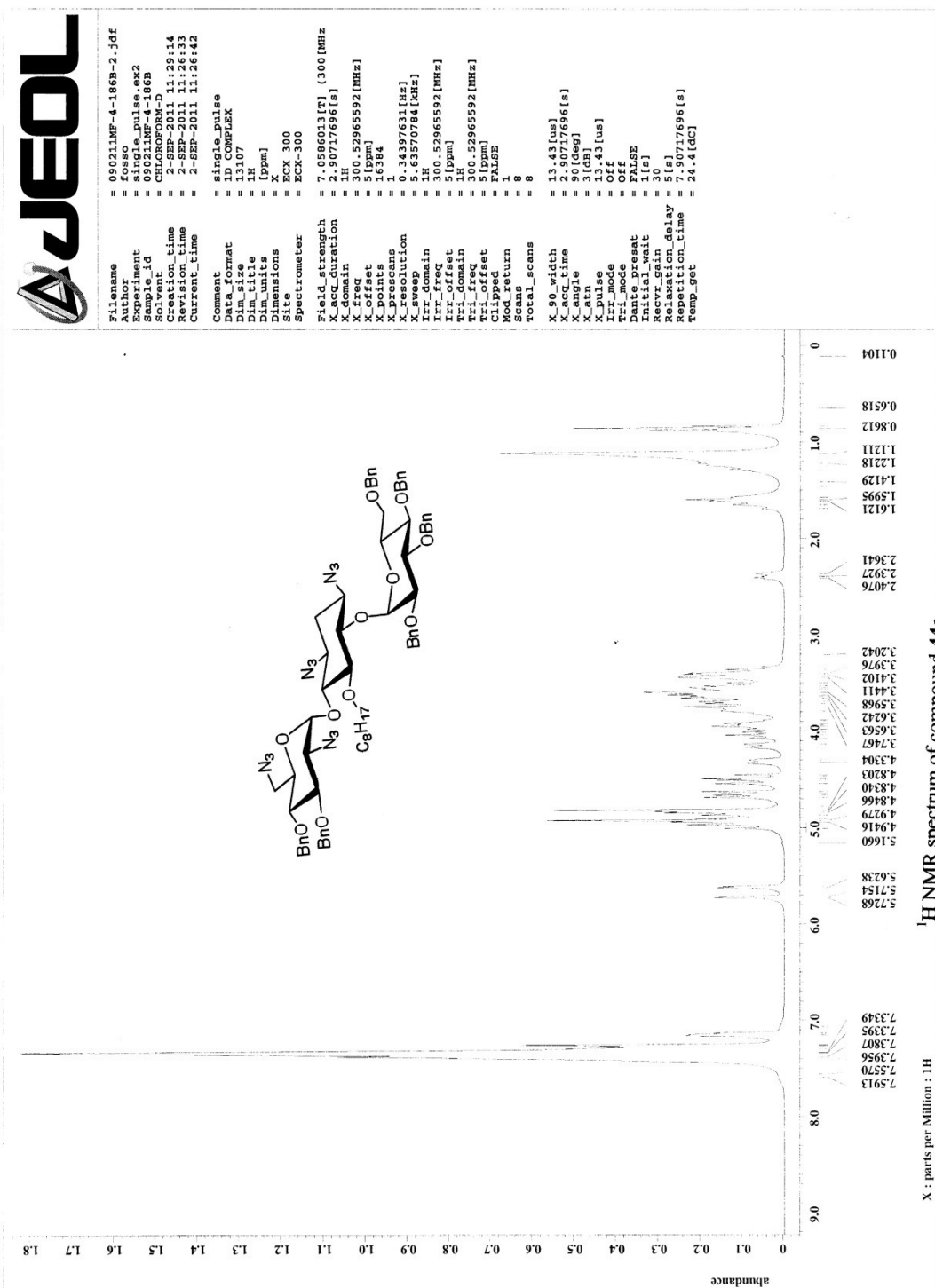
F2 - Acquisition Parameters
 Date_ 500000
 Time 20.58
 INSTRUM air400
 PROCNO 5 mm Multispec1
 PULPROG zgpg30
 TD 32768
 SOLVENT D2O
 NS 20000
 DS 2
 SWH 25000.000 Hz
 FIDRES 0.762939 Hz
 AQ 0.6554100 sec
 RG 45500
 DM 20.000 usec
 DE 27.14 usec
 TE 300.0 K
 D12 0.00002000 sec
 DL5 20.00 dB
 CPOPRG waltz16
 P31 100.00 usec
 D1 0.40000001 sec
 P1 6.75 usec
 DE 27.14 usec
 SFO1 100.6231179 MHz
 NUCLEUS 13C
 D11 0.03000000 sec

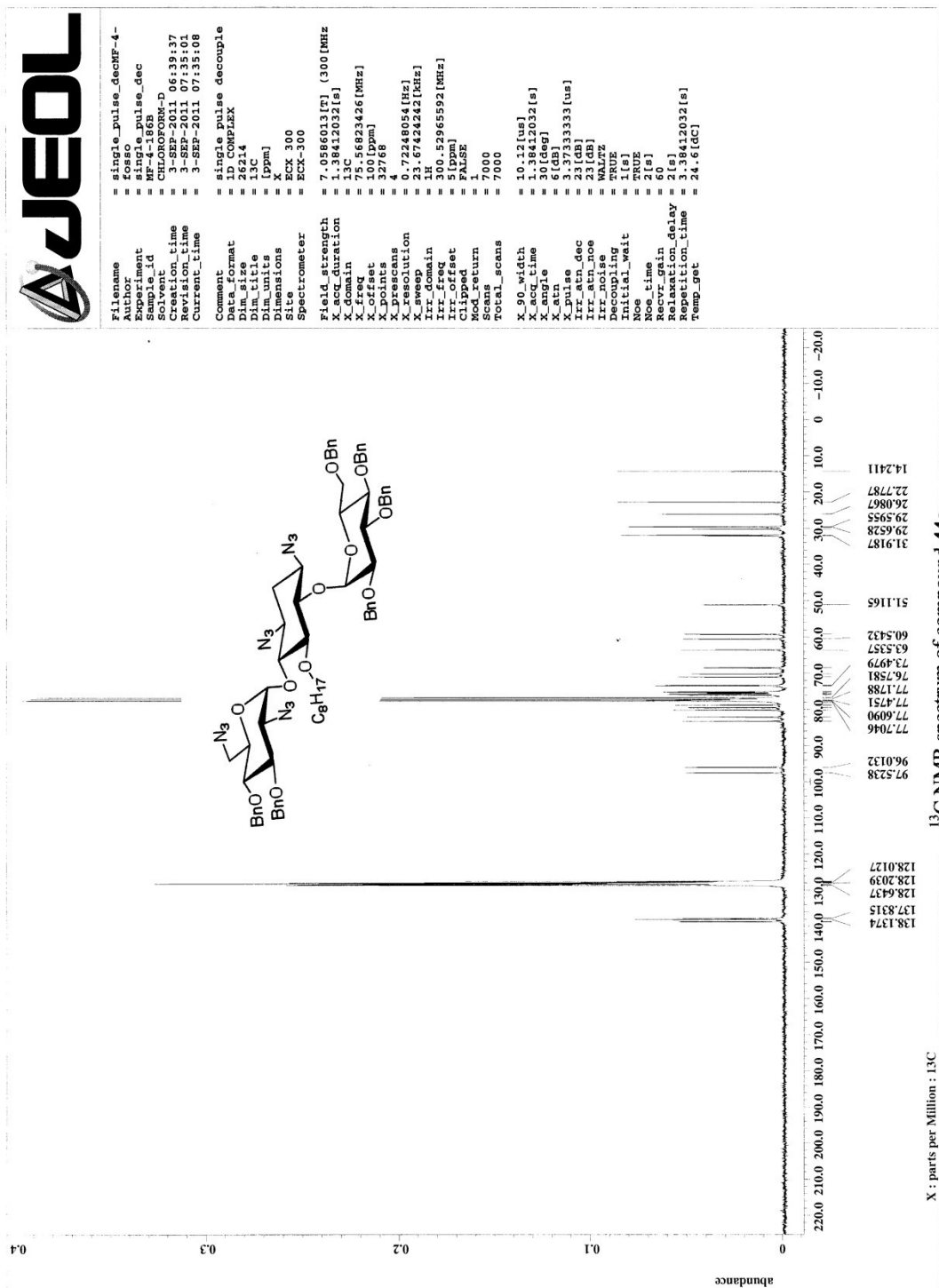
F2 - Processing parameters
 S1 16384
 SF 100.6127490 MHz
 EM
 WDW 0
 SSB 0
 LB 2.00 Hz
 GB 0
 PC 1.40

ID NMR plot parameters
 CX 20.00 cm
 F1P 120.000 ppm
 F1 12073.53 Hz
 F2P -5.000 ppm
 F2 -503.06 Hz
 PPMCM 6.25000 ppm/cm
 KQZ4 628.82965 Hz/cm



¹³C NMR spectrum of compound FG09







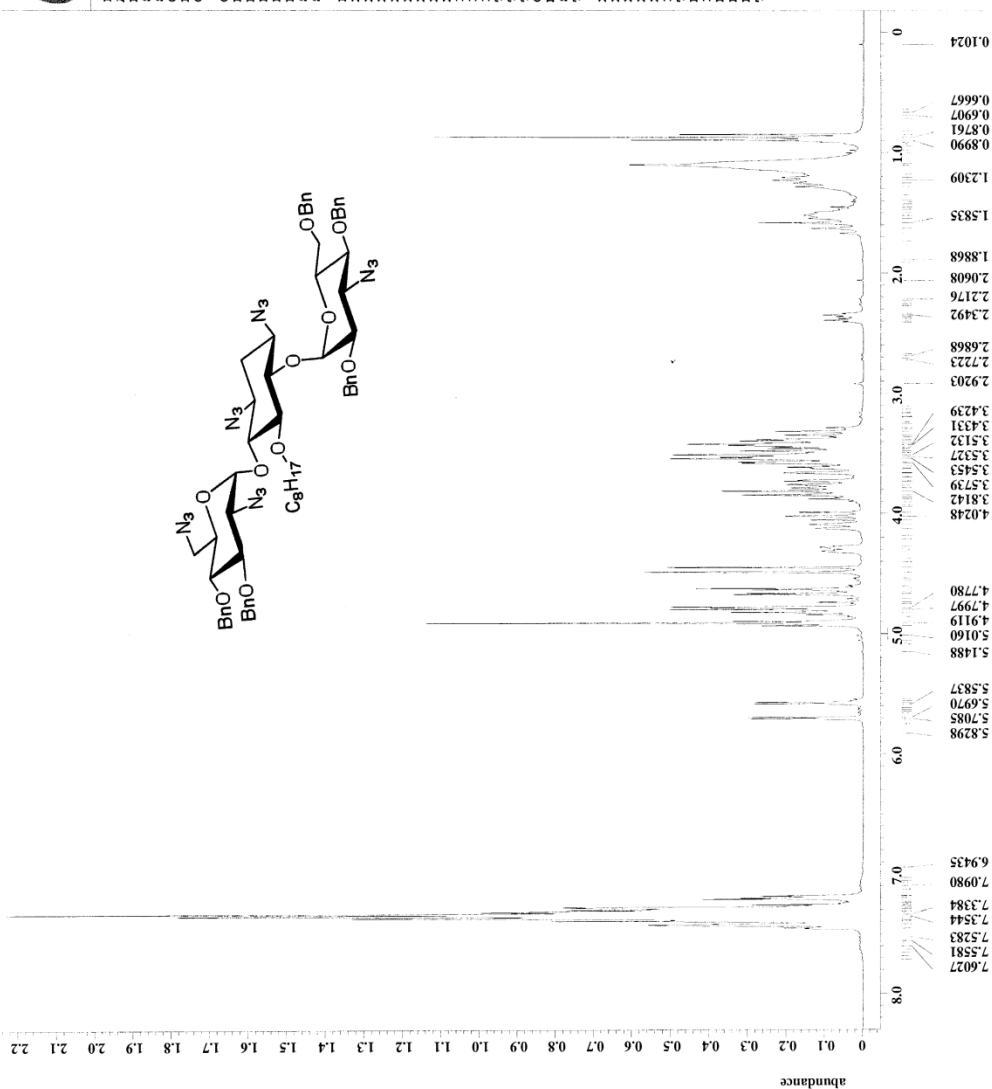
```

= 09031MF-4-187B-2.jdf
File Name
= fssco
Author
= single_pulse.ex2
Experiment
= 09031MF-4-187B
Sample ID
= 3-SEP-2011 07:45:23
Creation Time
= 3-SEP-2011 07:42:06
Revision Time
= 3-SEP-2011 07:42:17
Current Time
= single_pulse
Comment
= 1D COMPLEX
Data Format
= 13107
Dim Size
= 1H
Dim Title
= [ppm]
Dim Units
= [ppm]
Dimensions
= ECX 300
Spectrometer
= ECX-300

Field Strength = 7.0586013 [T] (300 [MHz]
X_acq_duration = 2.90717696 [s]
X_domain = 300.52965592 [MHz]
X_offset = 5 [ppm]
X_points = 16384
X_prescans = 1
X_resolution = 5.34897611 [Hz]
X_resolution_ppm = 0.03570784 [Hz]
Irr_domain = 1H
Irr_freq = 300.52965592 [MHz]
Irr_offset = 5 [ppm]
Tri_domain = H
Tri_offset = 300.52965592 [MHz]
Clipped = FALSE
Mod_return = 1
Total_scans = 8

X_90_width = 13.43 [us]
X_acq_time = 2.90717696 [s]
X_angle = 90 [deg]
X_atn = 1 [db]
X_cpw = 1 [us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1 [s]
Repeat_delay = 5 [s]
Repetition_delay = 7.90717696 [s]
Temp_Get = 24.4 [dc]

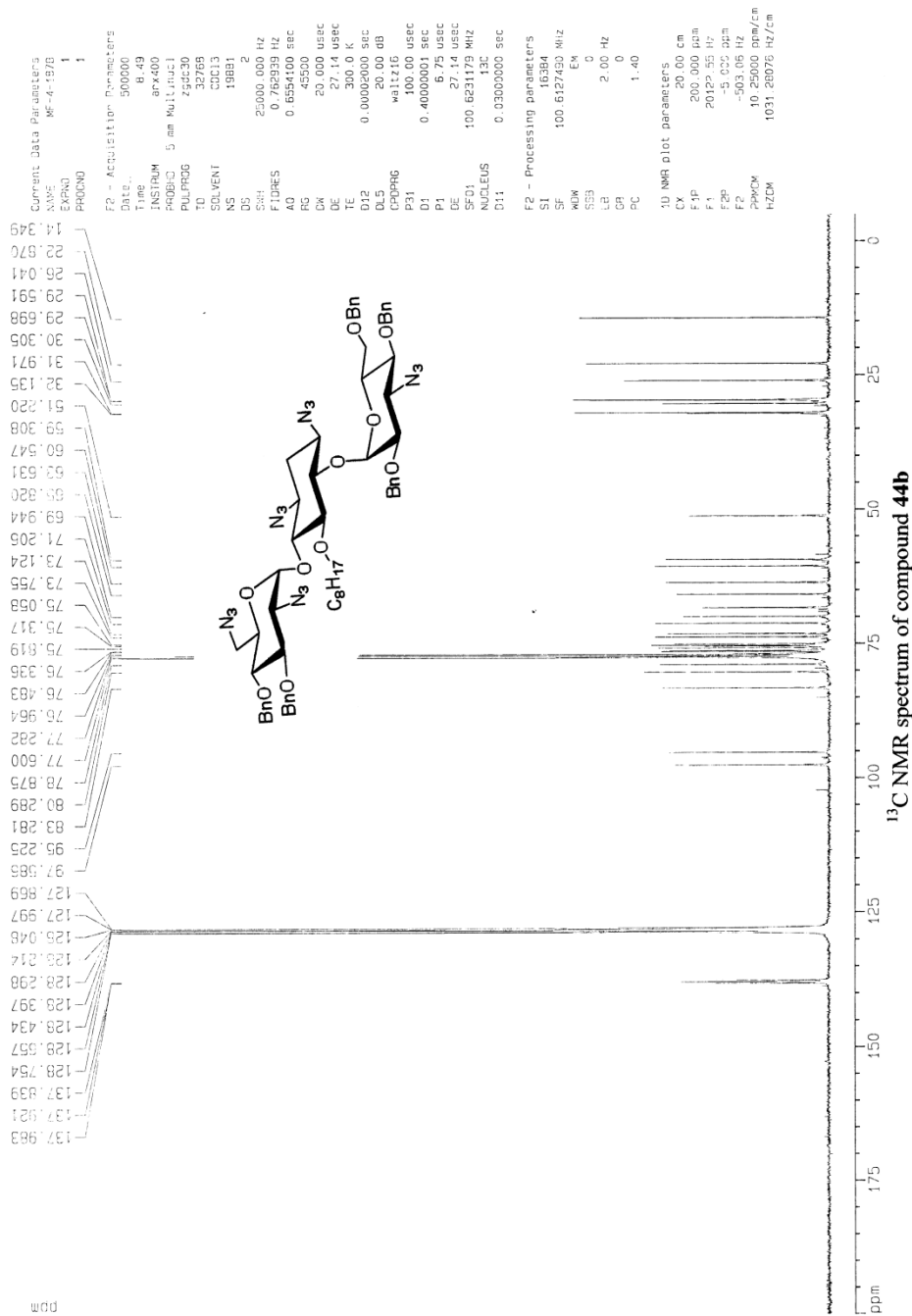
```

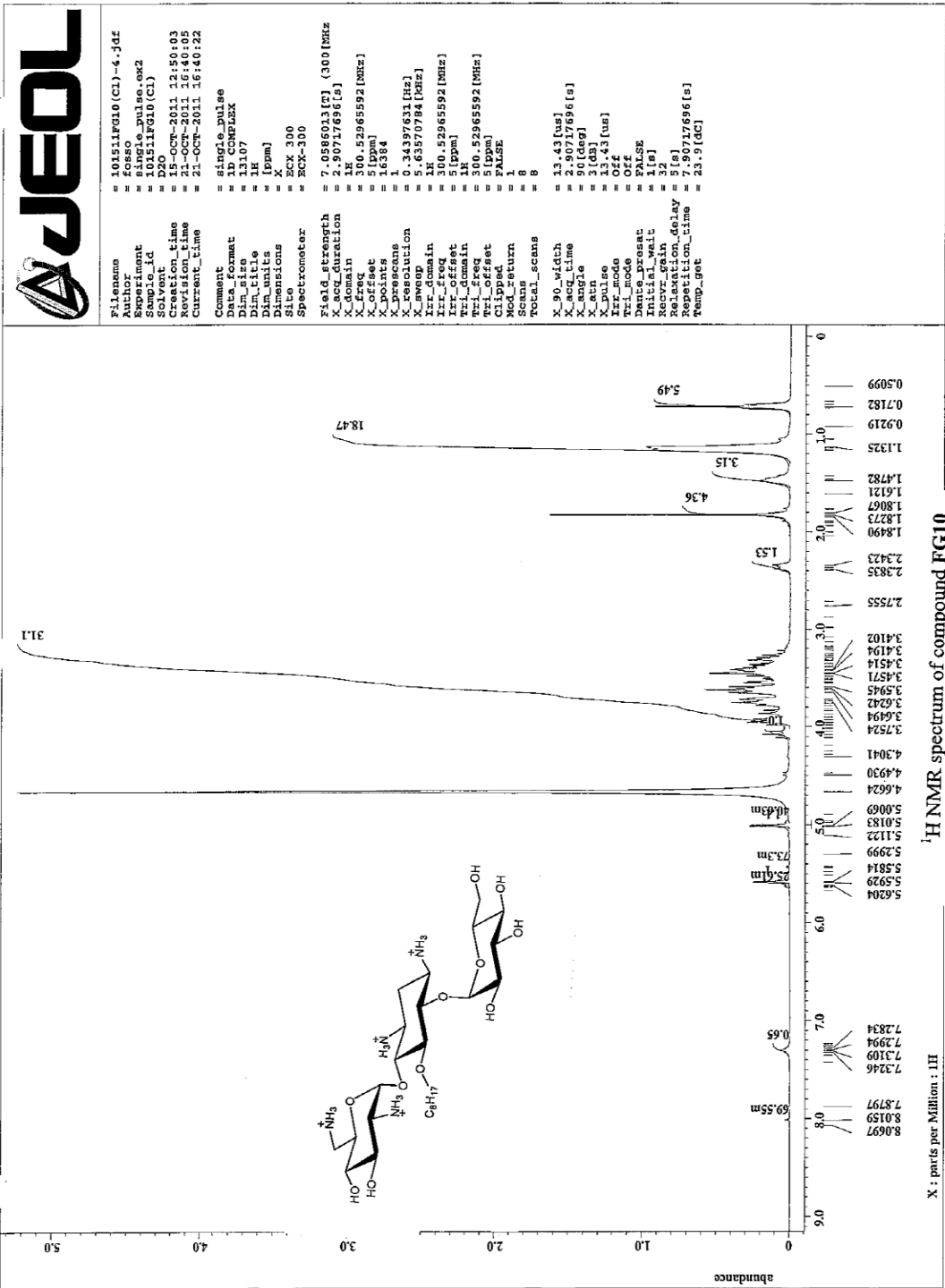


¹H NMR spectrum of compound 44b

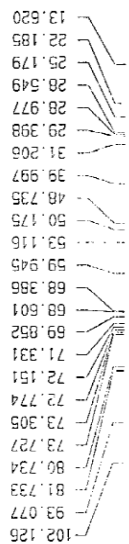
X : parts per Million : 1H

Standard ^{13}C
Experiment





Standard 13C
Experiment

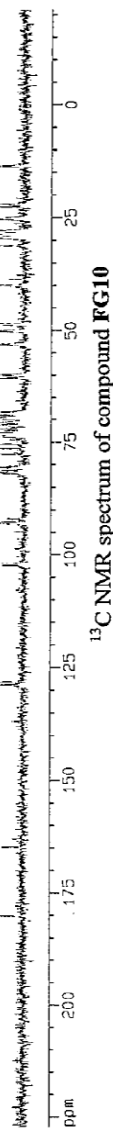
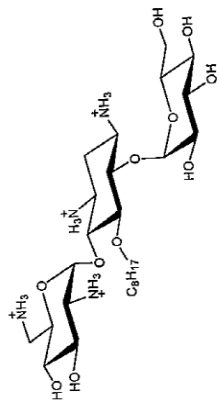


Current Data Parameters
NAME FG10C
EXPNO 1
PROCNO 1

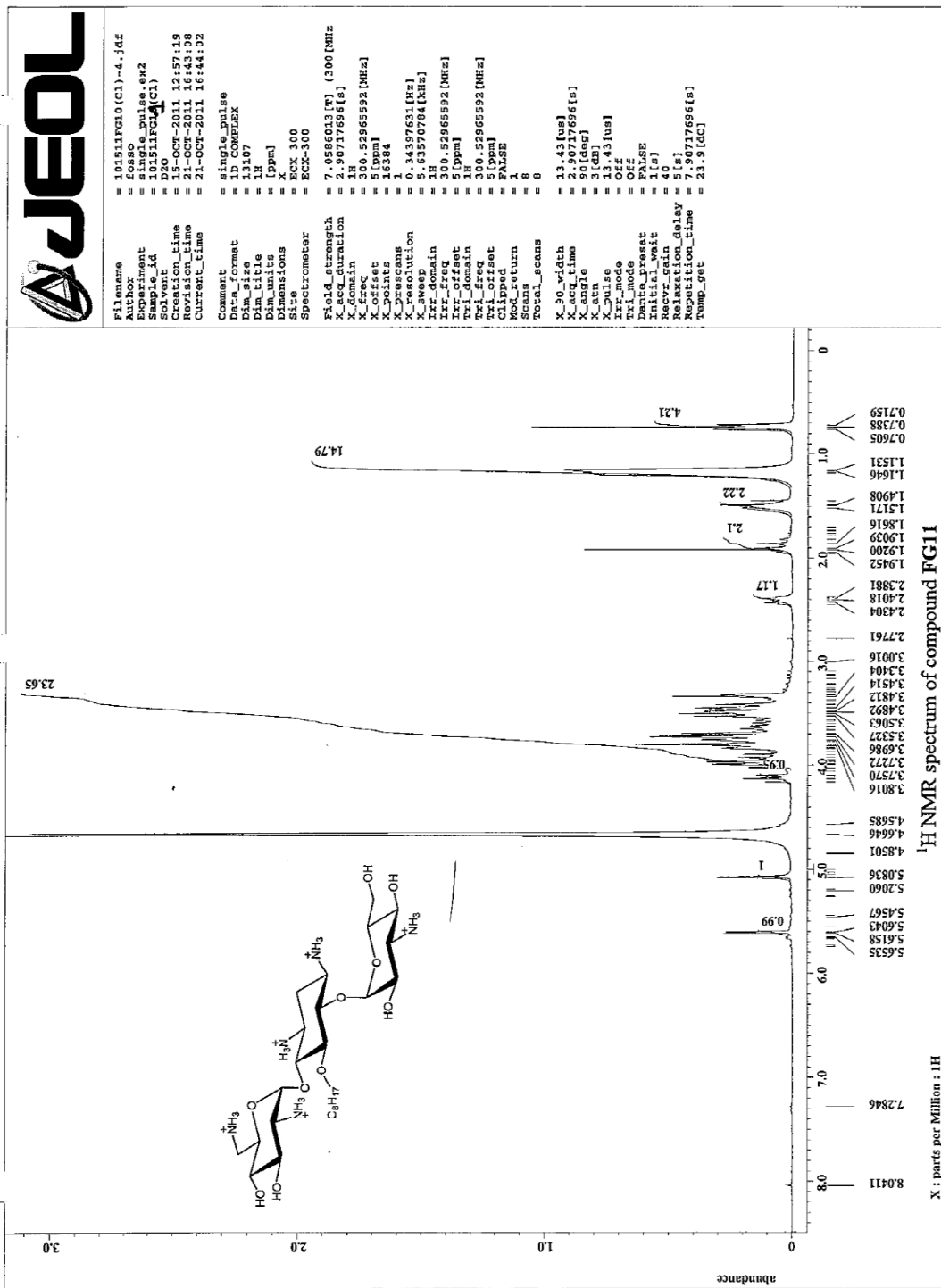
F2 - Acquisition Parameters
Date_ 000000
Time 15.40
INSTRUM spect
PROBHD 5 mm Multispec
PULPROG zgpg30
TD 32768
SOLVENT D2O
NS 2000
DS 2
SWH 25000.000 Hz
FIDRES 0.522025 Hz
AQ 0.565110 sec
RG 45500
DN 20.000 usec
DE 27.14 usec
TE 300.0 K
D1 20.00 usec
D12 0.0003000 sec
D15 20.00 usec
D19 0.0003000 sec
P31 100.00 usec
PRG 100.00 usec
D1 0.40000001 sec
P1 6.75 usec
DE 27.14 usec
SF01 100.623179 MHz
NUCLEUS 13C
D11 0.03000000 sec

F2 - Processing parameters
SI 15384
SF 100.6127450 MHz
WDW EM
SSB 0
LB 2.00 Hz
GB 0
PC 1.40

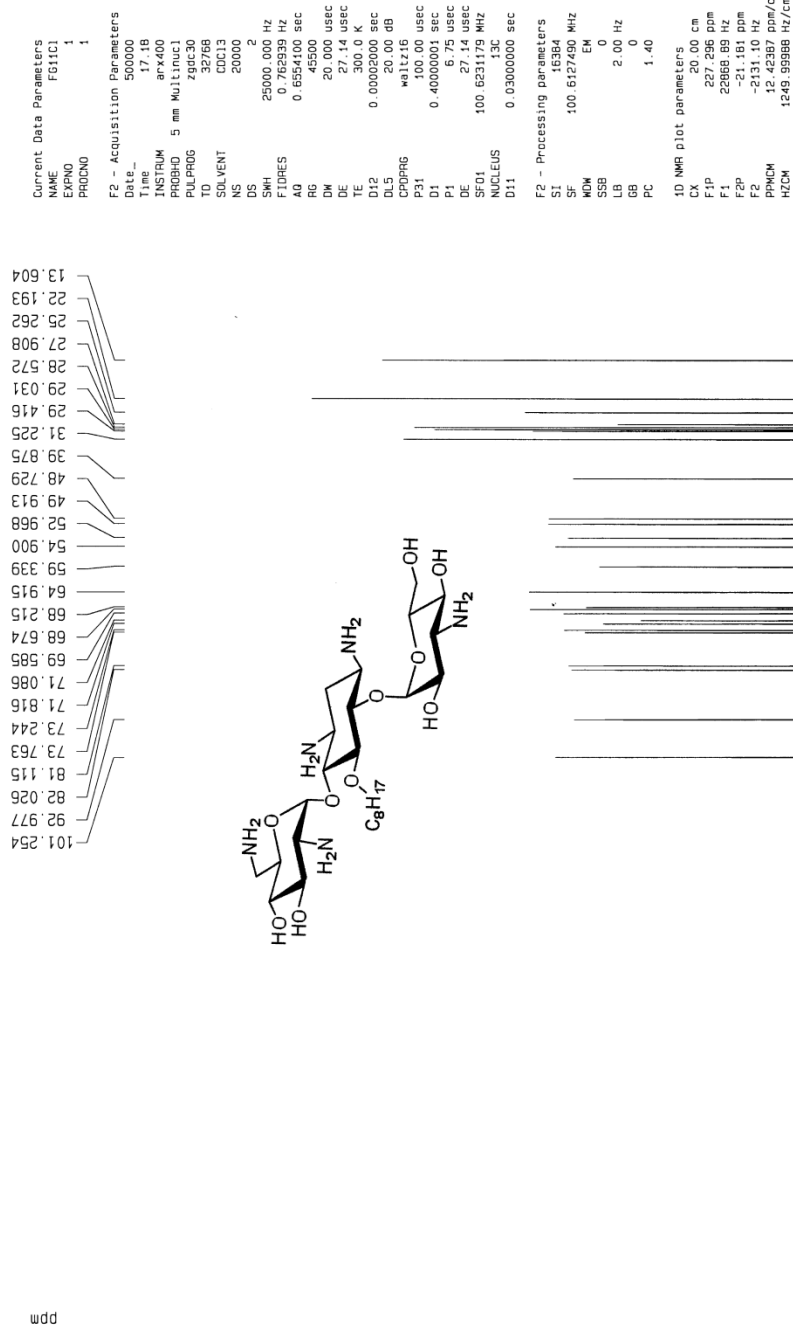
1D NMR plot parameters
CX 20.00 cm
F1P 287.286 ppm
F1 22958.85 Hz
F2P -21.181 ppm
F2 -2131.10 Hz
PRNOM 12.42387 ppm/cm
HZCM 1249.95899 Hz/cm

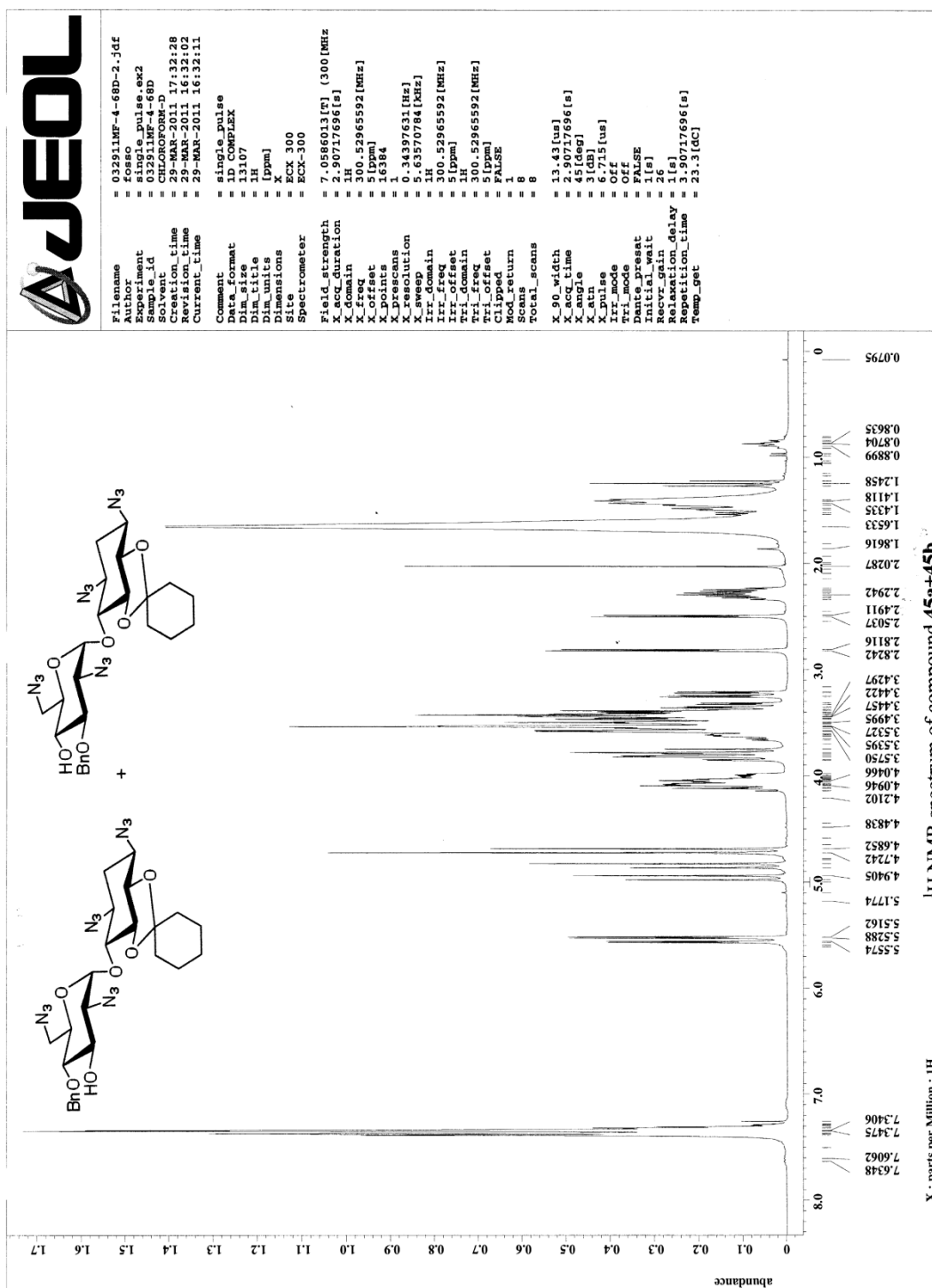


ppm



Standard ¹³C Experiment







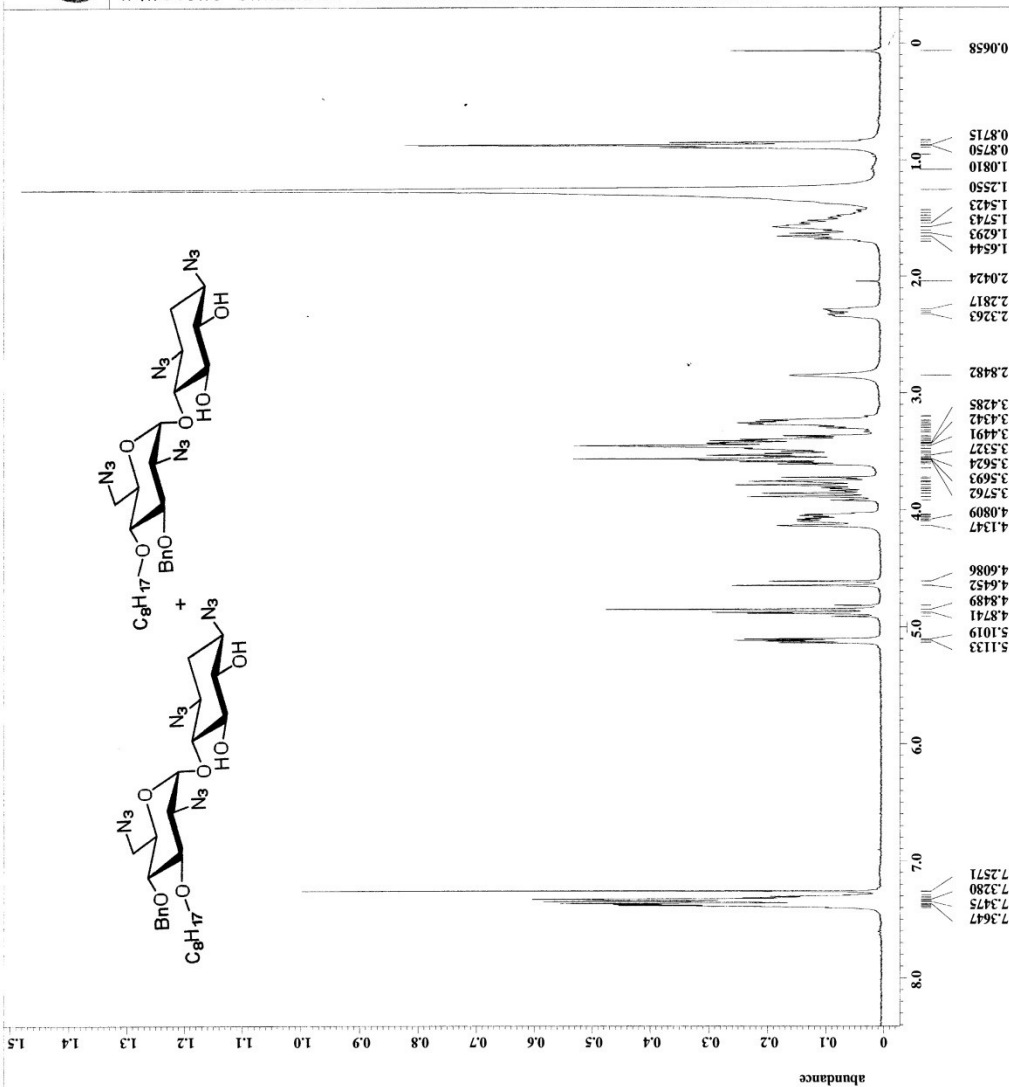
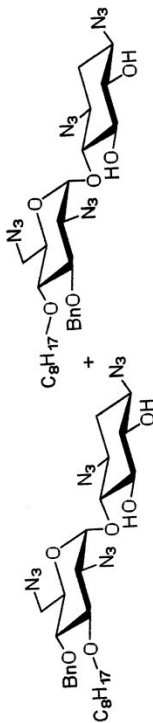
```

File Name = 032612MF-5-44C-2-.jdf
Author = foso
Experiment = single_pulse.ex2
Sample_id = 032612MF-5-44C
Solvent = CHLOROFORM-D
Acq_time = 10:42:35
Revision_time = 26-MAR-2012 10:38:29
Current_time = 26-MAR-2012 10:38:36

Comment = single_pulse
Data_dir = CHMPLX
Dir_name = 13107
Dim_title = [H]
Dim_units = [ppm]
Dimensions = X
Site = ECK 300
Spectrometer = ECK-300

Field_strength = 7.0586013[T] (300[MHz]
X_acq_duration = 2.90717696[s]
X_domain = [H] 0.52965592[MHz]
X_freq = 300.52965592[MHz]
X_points = 16384
X_prescans = 1
X_resolution = 0.34397631[Hz]
X_sweep = 5.6570784[MHz]
X_start = 300.52965592[MHz]
X_stop = 300.52965592[MHz]
Irr_freq = 5[ppm]
Irr_offset = [H]
Irr_domain = 300.52965592[MHz]
Irr_freq = 300.52965592[MHz]
Irr_offset = [H]
Clipped = FALSE
Mod_return = 1
Total_scans = 8

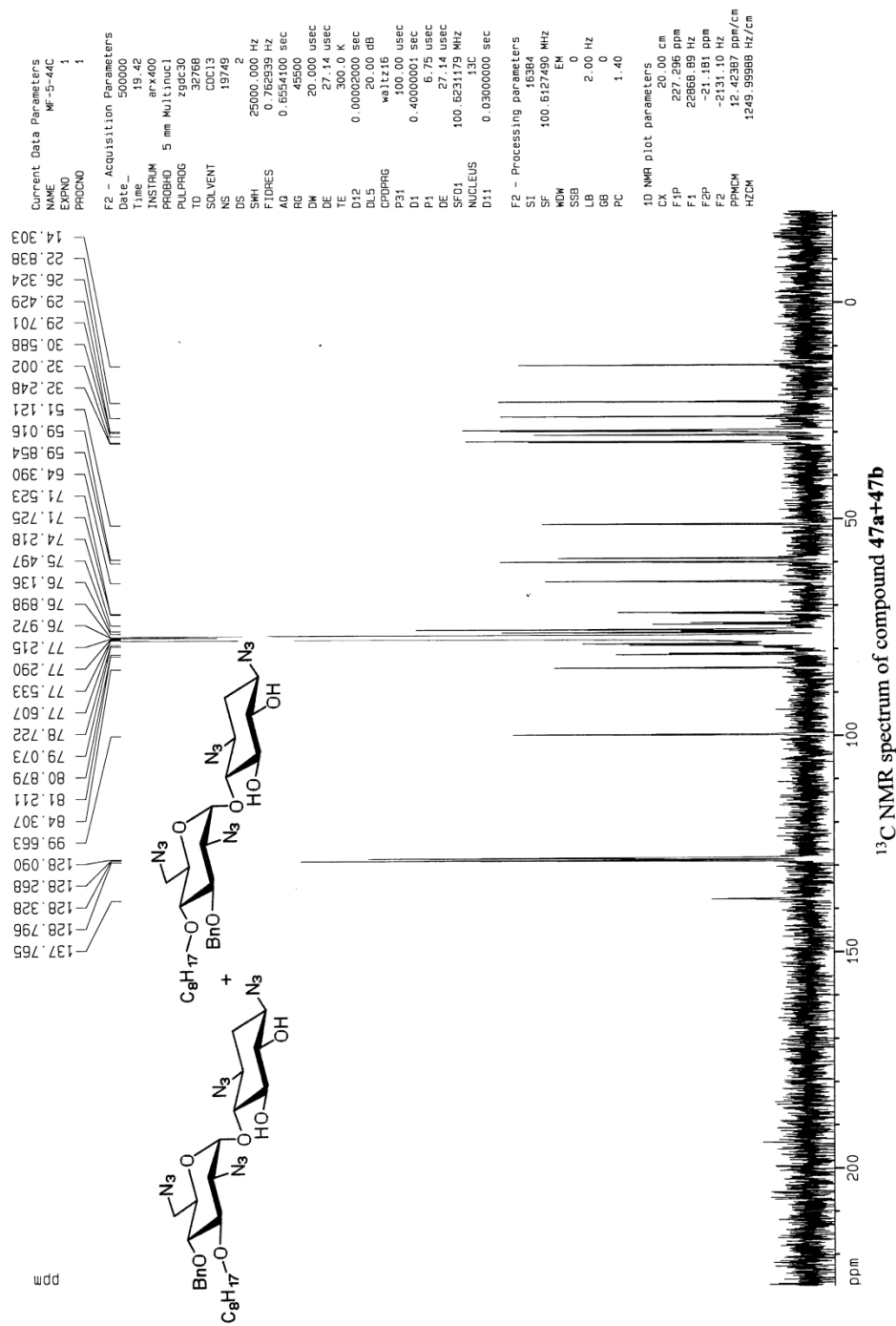
X_90_width = 13.43[us]
X_acq_time = 2.90717696[s]
X_angle = 45[deg]
X_atn = 3[db]
X_pulse = 67.15[us]
X_mode = Off
Dante_Preset = FALSE
Initial_wait = 1[s]
Recvr_gain = 46
Sensitization_delay = 3.15[s]
Repetition_time = 3.90717696[s]
Temp_get = 22.5[dc]
    
```

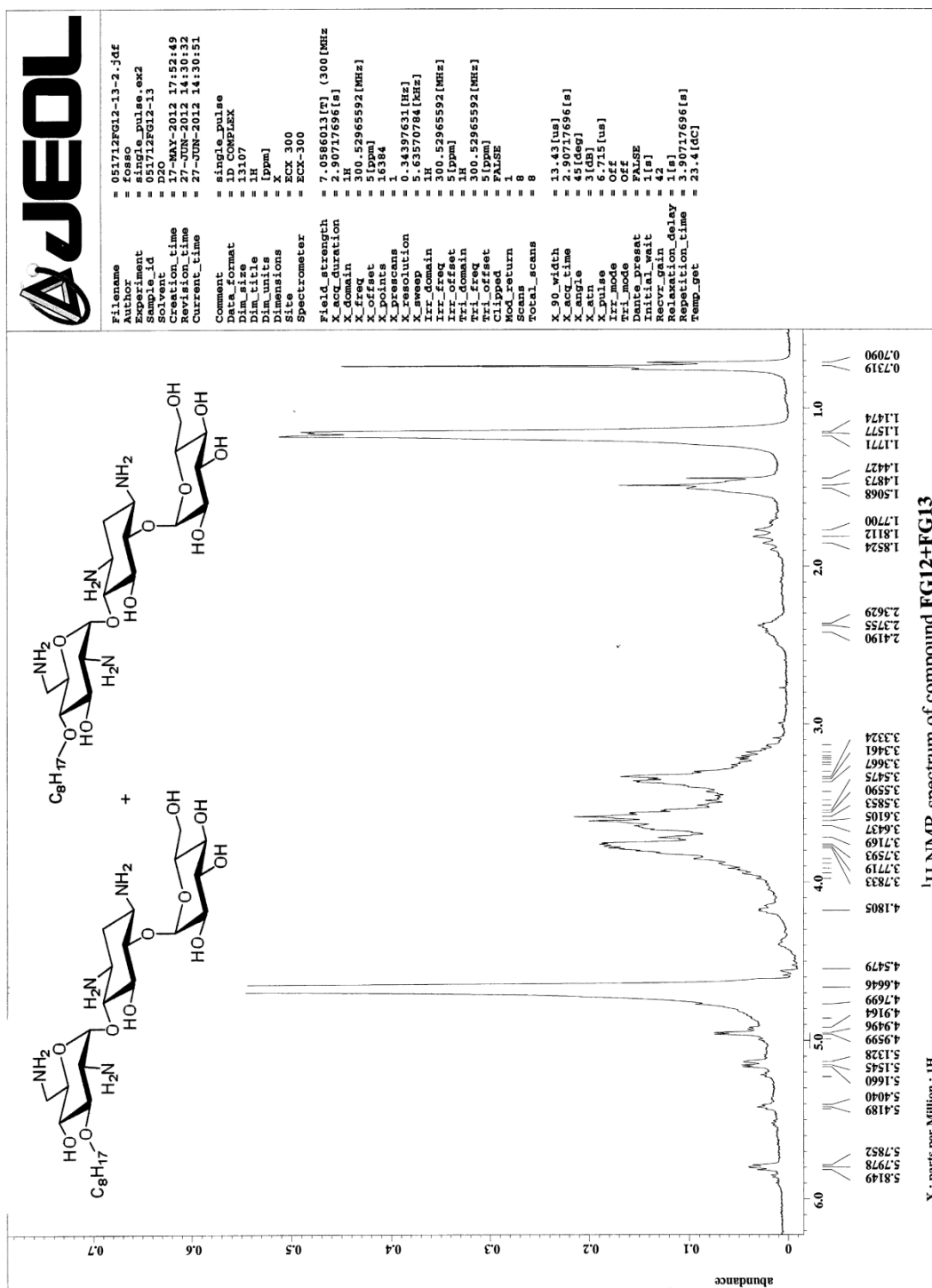


¹H NMR spectrum of compound 47a+47b

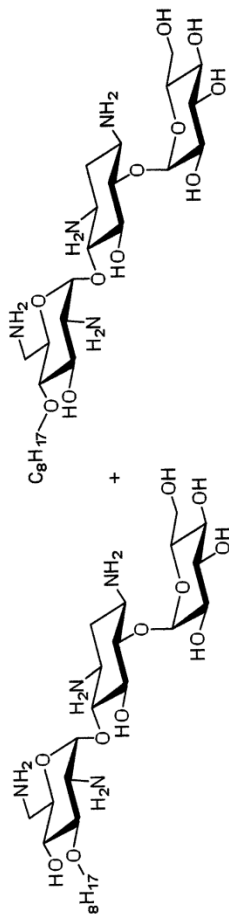
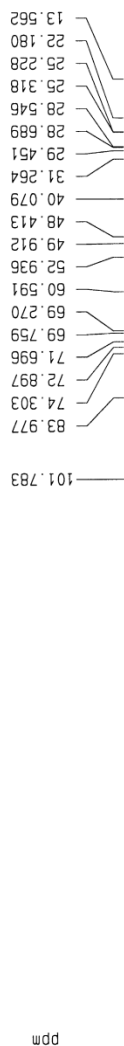
X : parts per. Million : 1H

Standard ¹³C
Experiment





Standard ¹³C
Experiment

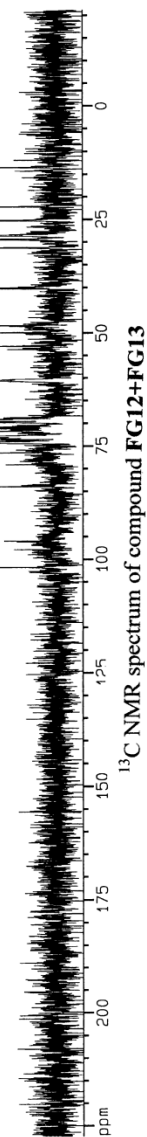


Current Data Parameters
 NAME F612.13
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 500000
 Time 21.28
 INSTRUM arx400
 PROBHD 5 mm Multinucl
 PULPROG zgpg30
 TD 32768
 SOLVENT D2O
 NS 20000
 DS 2
 SMH 25000.000 Hz
 FIDRES 0.762939 Hz
 AQ 0.6554100 sec
 RG 49500
 DM 20.000 usec
 DE 7.14 usec
 TE 300.2 K
 D1 0.00002000 sec
 D12 20.00 usec
 D15 20.00 usec
 CPDPRG waltz16
 P31 100.00 usec
 P1 0.40000001 sec
 P1 6.75 usec
 DE 27.14 usec
 SF01 100.6231179 MHz
 NUCLEUS ¹³C
 D11 0.03000000 sec

F2 - Processing parameters
 SI 16384
 SF 100.6127490 MHz
 WDW EM
 SSB 0
 LB 2.00 Hz
 GB 0
 PC 1.40

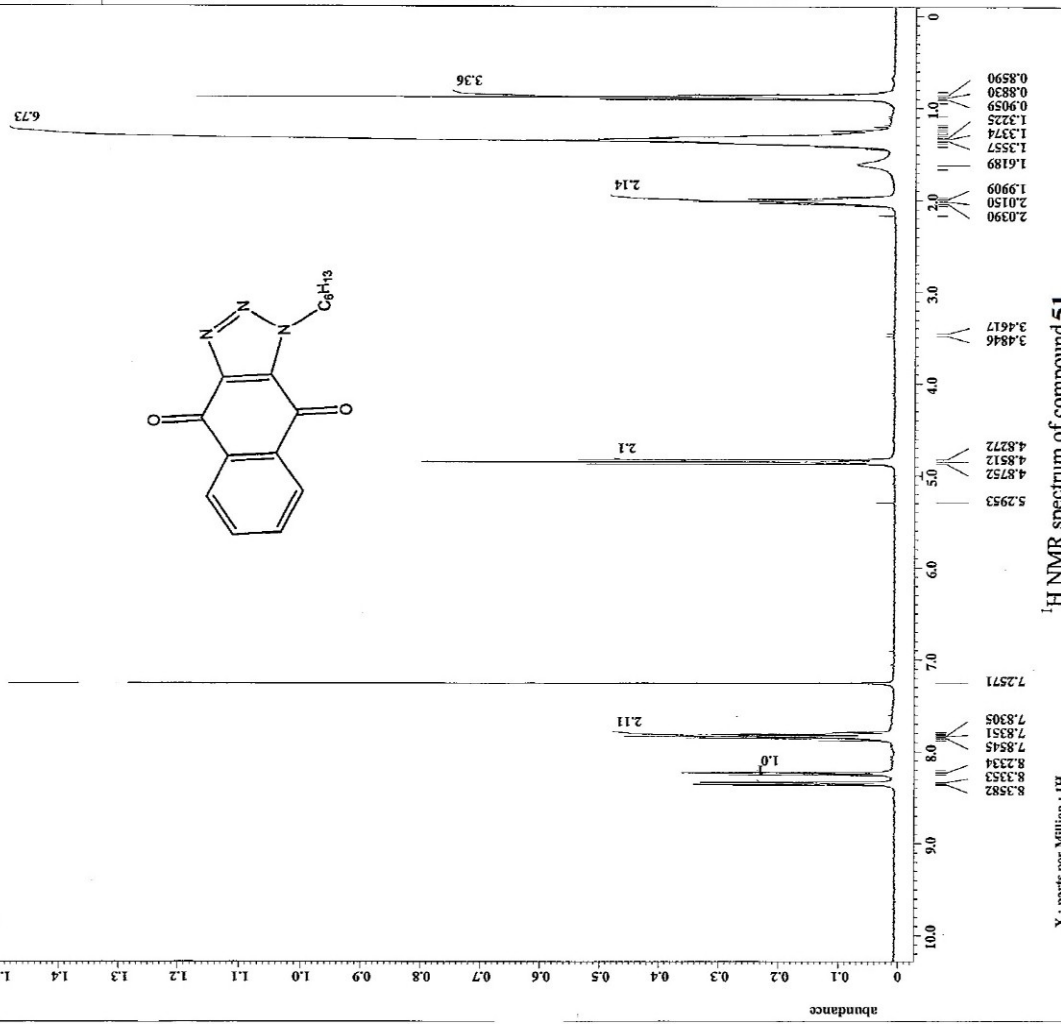
1D NMR plot parameters
 CX 20.00 cm
 F1 207.296 ppm
 F1 22868.89 Hz
 F2 -21.181 ppm
 F2 -2131.10 Hz
 PPMCM 12.42367 ppm/cm
 HZCM 1249.9998 Hz/cm





```

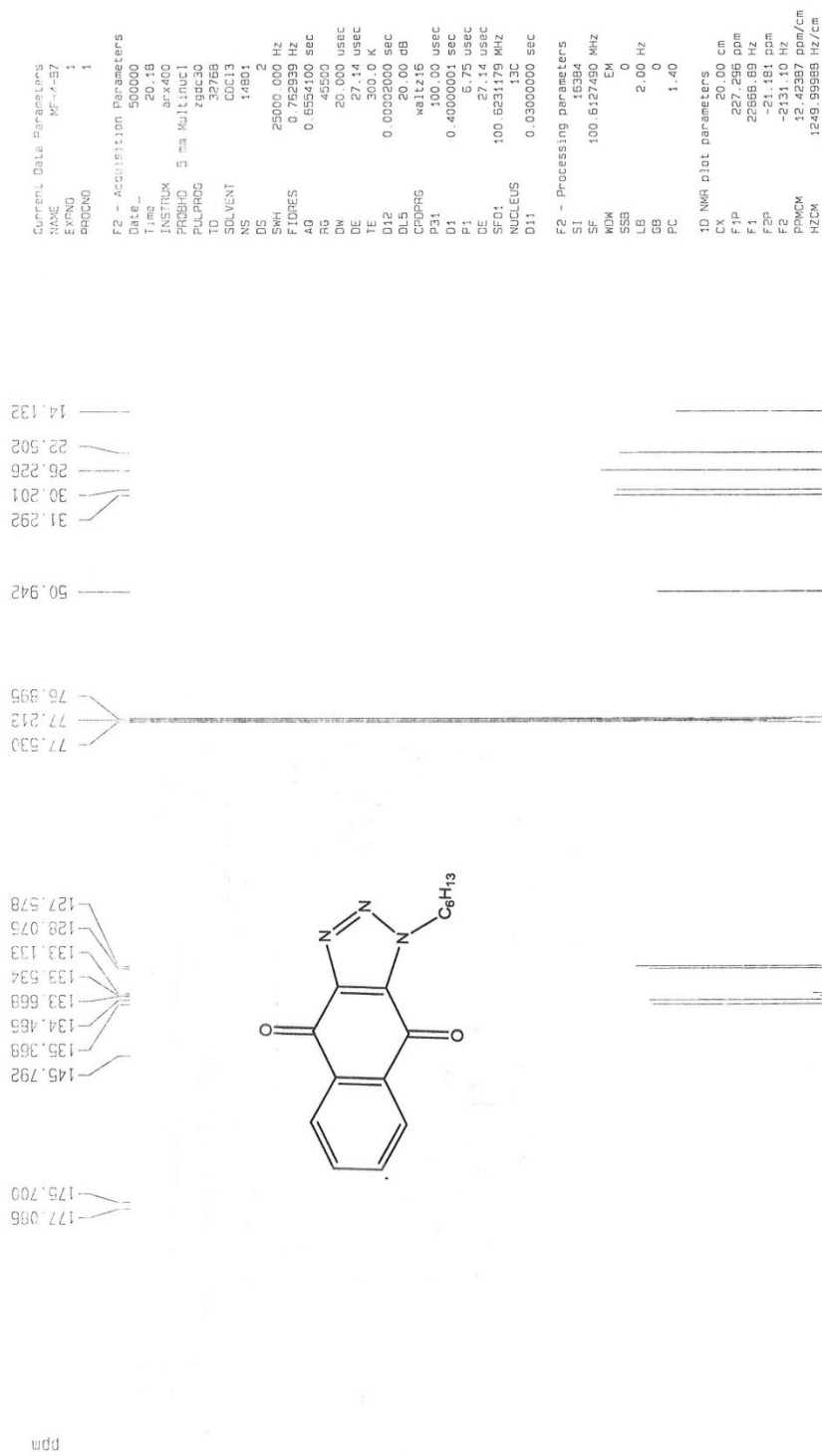
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Filename = 02511MF-4-B7-3-j4f
Author = foso
Experiment = 130
Pulse program = zgpg30
Sample ID = 02511MF-4-B7
Solvent = CHLOROFORM-D
Creation time = 25-MAY-2011 11:47:50
Revision time = 25-MAY-2011 11:47:29
Current time = 25-MAY-2011 11:47:54
=====
Comment = single pulse
Data format = 1D COMPLEX
Dim_size = 13107
Dim_title =
Dim_units = [ppm]
Dimensions = X
Site = EXC 300
Spectrometer = EXC-300
=====
Field_strength = 7.0586013[T] (300[MHz]
X_acq_duration = 2.90717696[s]
X_domain = 1H
X_freq = 300.52965592[MHz]
X_gain = 16384
X_prescans = 1
X_resolution = 0.34397631[Hz]
X_sweep = 5.63570784[MHz]
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
Decoupling =
Return =
Scans = 8
Total_scans = 8
X_90_width = 13.43[us]
X_acq_time = 2.90717696[s]
X_delay = 1.00[s]
X_gain = 31481
X_p1 = 6.715[us]
X_pulse = 6.715[us]
Irr_mode = Off
Tri_mode = Off
DANTE_program = FALSE
DANTE_setup =
DANTE_start = 4[s]
Recovery_time = 4[s]
Relaxation_delay = 5[s]
Repetition_time = 7.96717696[s]
Temp_set = 24.4[degC]
=====
    
```

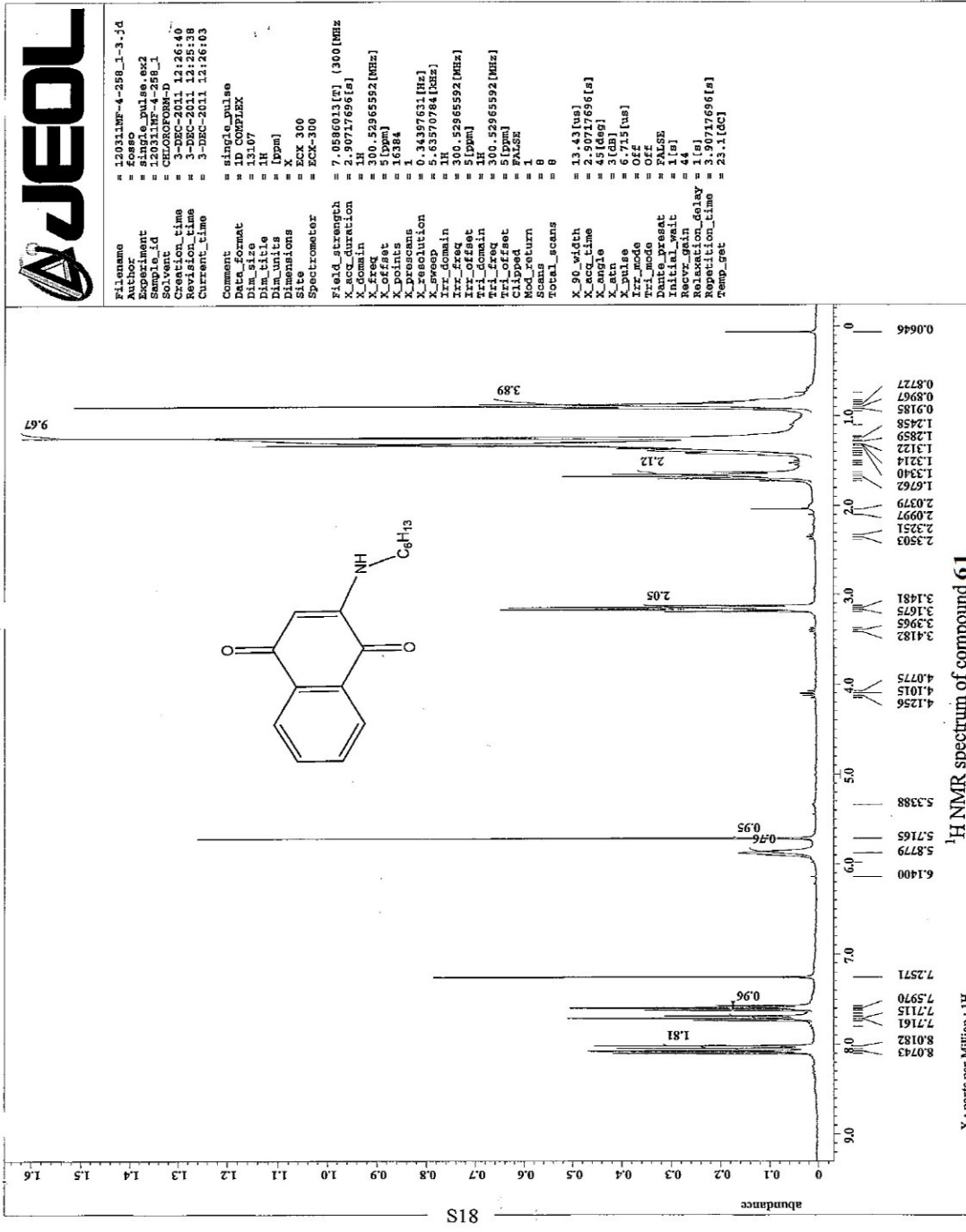


¹H NMR spectrum of compound 5I

X: parts per Million : 1H

Standard ¹³C Experiment





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Standard ¹³C
Experiment

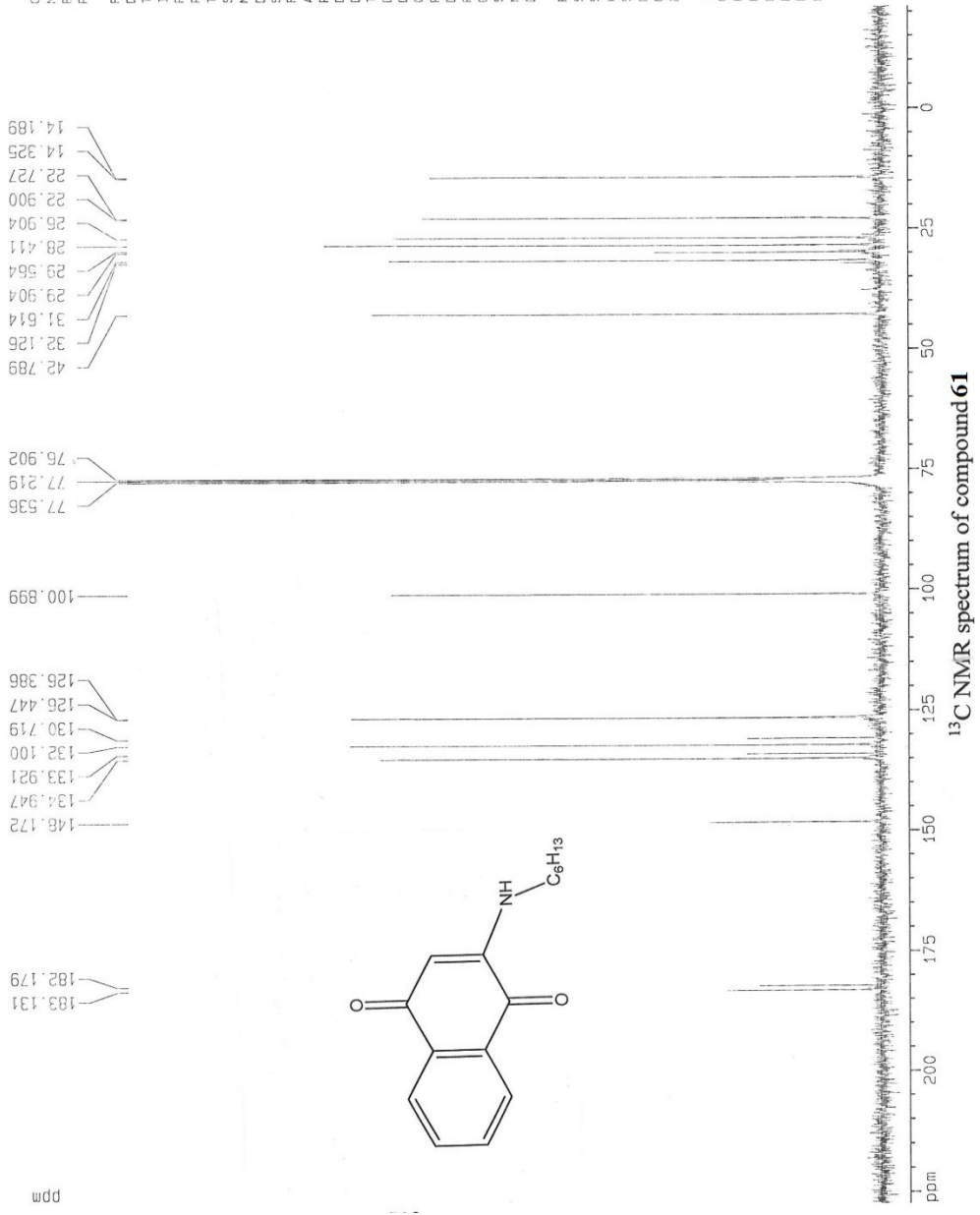
```

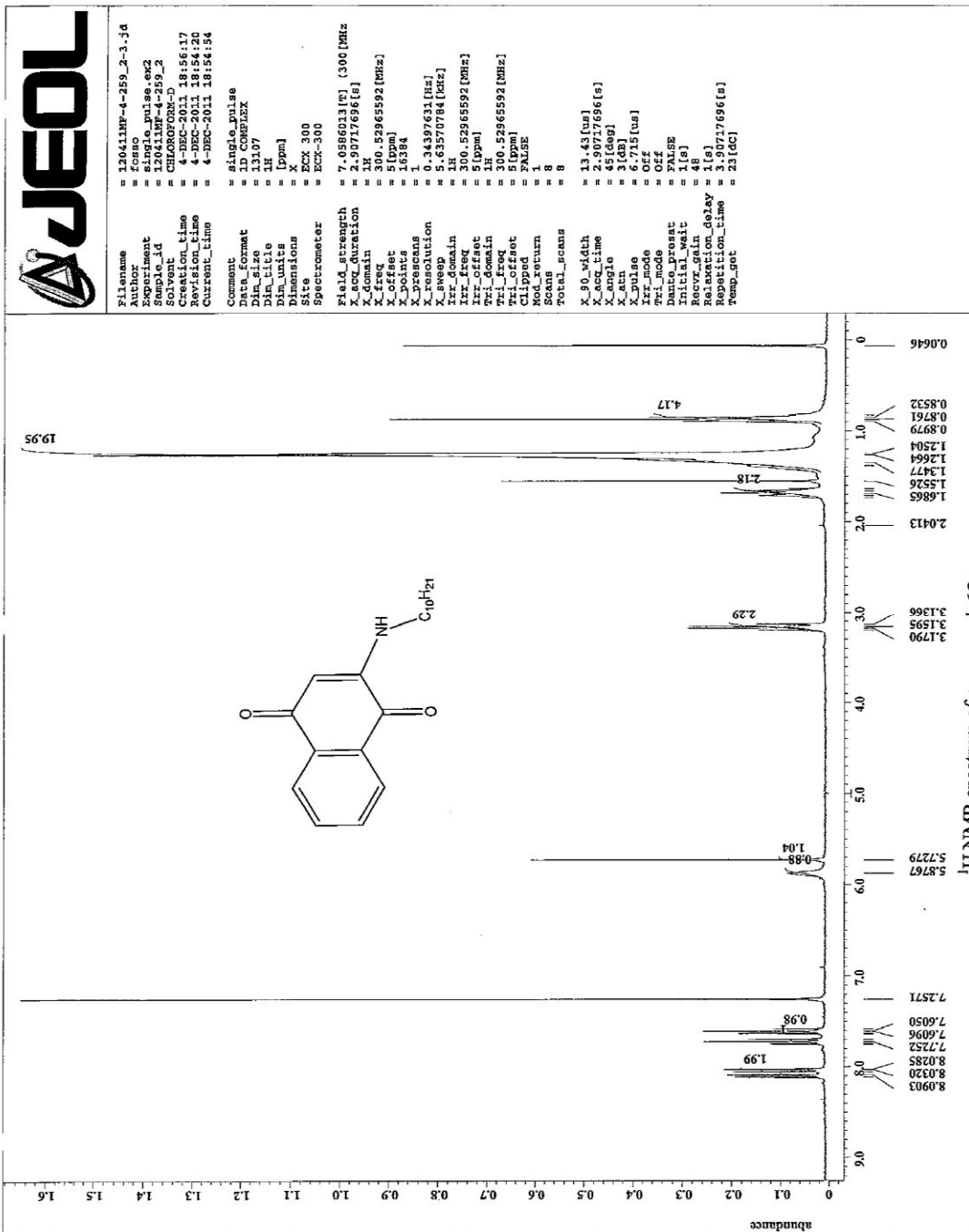
Current Data Parameters
NAME MF-4-20B-1
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 500000
Time 14.58
INSTRUM ark400
PROBHD 5 mm Multinucl
PULPROG zgpg30
TD 32768
SOLVENT CDCl3
NS 16484
DS 2
SWH 25000.000 Hz
FIDRES 0.762939 Hz
AQ 0.6554100 sec
RG 45500
DE 27.14 usec
TE 300.0 K
D12 0.0002000 sec
DL5 20.00 dB
CPDPRG maltz16
P31 100.00 usec
D1 0.40000001 sec
P1 6.75 usec
DE 27.14 usec
SF01 100.6231179 MHz
NUCLEUS 13C
D11 0.03000000 sec

F2 - Processing parameters
SI 16384
SF 100.6127450 MHz
WDW EM
SSB 0
LB 2.00 Hz
GB 0
PC 1.40

1D NMR plot parameters
CY 20.00 cm
F1P 227.286 ppm
F1 22668.89 Hz
F2P -2151.10 ppm
F2 -2151.10 Hz
PRNCH 13.42387 ppm/cm
HZCM 1249.99988 Hz/cm
    
```





Filename = 120411MF-4-259_2-3.jd
 Author = fonsco
 Experiment = single_pulse.ex2
 Sample_id = 120411MF-4-259_2
 Solvent = CHLOROFORM-D
 Creation_time = 4-DEC-2011 18:56:17
 Acquisition_time = 4-DEC-2011 18:58:22
 Current_time = 4-DEC-2011 18:54:54
 Comment = single_pulse
 Data_format = 1D COMPLEX
 Data_size = 11.07
 File_name = 120411MF-4-259_2-3.jd
 Dir_units = [ppm]
 Dimensions = X
 Site = EXC 300
 Spectrometer = EXC-300
 Field_strength = 7.0560031 [T] (300 [MHz]
 X_acq_duration = 2.90717696 [s]
 X_domain = 13
 X_freq = 300.52965592 [MHz]
 X_offset = 5 [ppm]
 X_points = 16384
 X_resolution = 0.34397631 [Hz]
 X_sweep = 5.63570784 [kHz]
 Irr_domain = 1H
 Irr_freq = 300.52965592 [MHz]
 Irr_offset = 5 [ppm]
 Irr_domain = 1H
 T1_domain = 300.52965592 [MHz]
 T1_offset = 5 [ppm]
 Clipped = FALSE
 Mod_return = 1
 Scans = 8
 Total_scans = 8
 X_f0_width = 13.43 [us]
 X_acq_time = 2.90717696 [s]
 X_angle = 45 [deg]
 X_atn = 3 [dB]
 X_pulse = 0.715 [us]
 X_resolution = 0.34397631 [Hz]
 X_sweep = 5.63570784 [kHz]
 Dantle_presat = FALSE
 Initial_wait = 1 [s]
 Recv_gain = 48
 Relaxation_delay = 1.69 [s]
 Repetition_time = 2.31 [s]
 Temp_set =

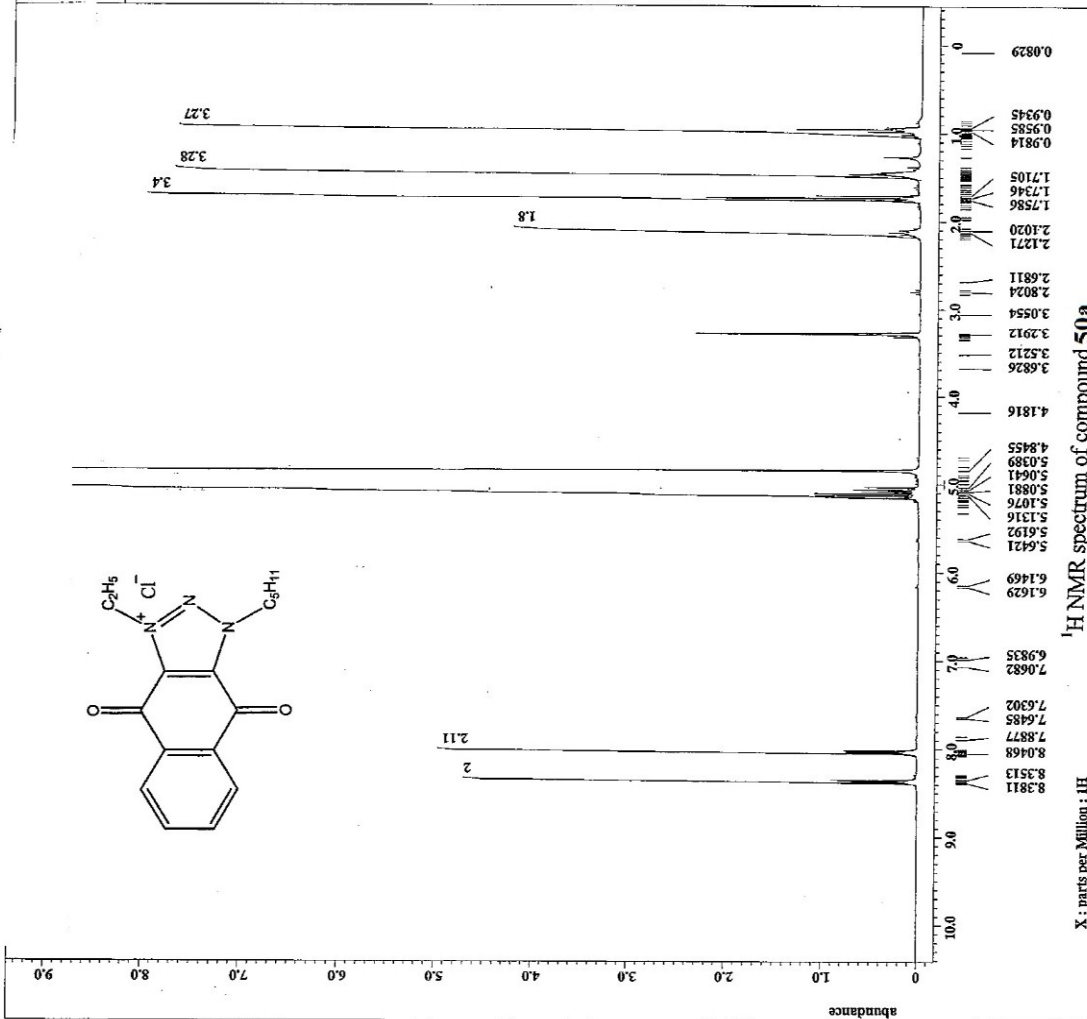
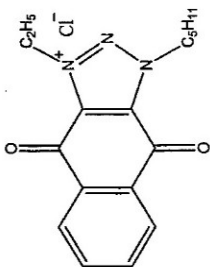
Standard ¹³C
Experiment





```

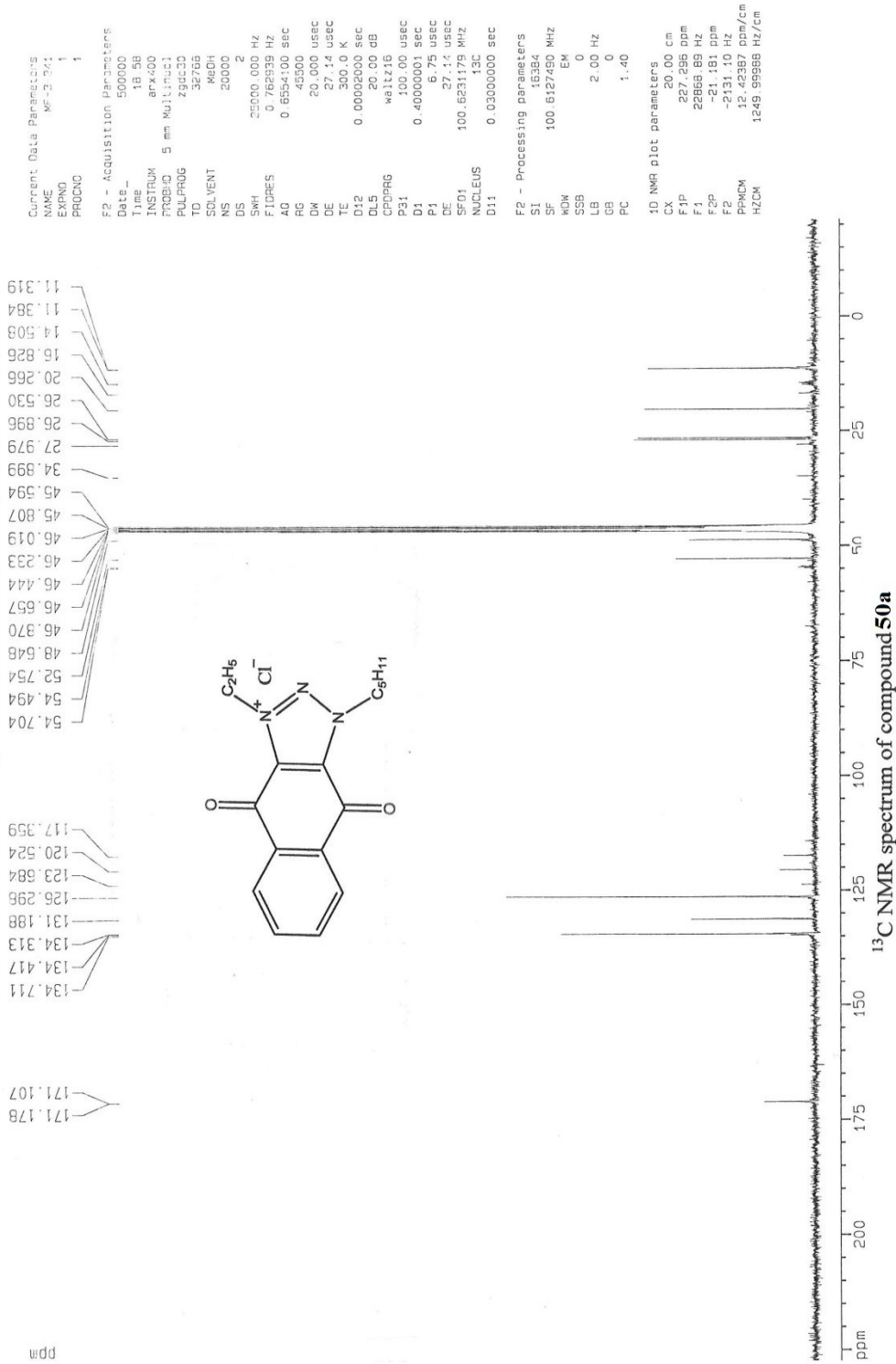
Filename = 020211MR-3-241-3-jdZ
Author = fcsso
Experiment = single_pulse.ex2
Sample_id = 020211MR-3-241
Sample_name = MPTANOL-33
Scan_time = 2-FEB-2011 00:31:45
Current_time = 2-FEB-2011 00:35:05
Revision_time = 2-FEB-2011 00:36:42
Comment = single_pulse
Data_format = ID COMPLEX
Pulse_prog = zgpg30
D1 = 1.07
D11 = 1.07
D12 = 1.07
D13 = 1.07
D14 = 1.07
D15 = 1.07
D16 = 1.07
D17 = 1.07
D18 = 1.07
D19 = 1.07
D20 = 1.07
D21 = 1.07
D22 = 1.07
D23 = 1.07
D24 = 1.07
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D26 = 1.07
D27 = 1.07
D28 = 1.07
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D41 = 1.07
D42 = 1.07
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D47 = 1.07
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D54 = 1.07
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D57 = 1.07
D58 = 1.07
D59 = 1.07
D60 = 1.07
D61 = 1.07
D62 = 1.07
D63 = 1.07
D64 = 1.07
D65 = 1.07
D66 = 1.07
D67 = 1.07
D68 = 1.07
D69 = 1.07
D70 = 1.07
D71 = 1.07
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D77 = 1.07
D78 = 1.07
D79 = 1.07
D80 = 1.07
D81 = 1.07
D82 = 1.07
D83 = 1.07
D84 = 1.07
D85 = 1.07
D86 = 1.07
D87 = 1.07
D88 = 1.07
D89 = 1.07
D90 = 1.07
D91 = 1.07
D92 = 1.07
D93 = 1.07
D94 = 1.07
D95 = 1.07
D96 = 1.07
D97 = 1.07
D98 = 1.07
D99 = 1.07
D100 = 1.07
  
```



¹H NMR spectrum of compound 50a

X : parts per Million : 1H

Standard ¹³C Experiment



Current Data Parameters
 NAME MF-3.74;
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 500000
 Time_ 18.58
 INSTRUM ark400
 PROBHD 5 mm MuIti (unc)
 PULPROG zgpg30
 TD 32768
 SOLVENT MeOH
 NS 20000
 DS 2
 SWH 25000.000 Hz
 FIDRES 0.762938 Hz
 AQ 0.6554100 sec
 RG 45900
 DM 20.000 usec
 DE 27.14 usec
 TE 500.0 K
 D12 0.0002000 sec
 D15 20.00 dB
 CPDPRG Hal1215
 P31 100.00 usec
 P1 0.4000003 sec
 P2 5.75 usec
 P3 5.75 usec
 SFO1 100.623175 MHz
 NUCLEUS ¹³C
 D11 0.03000000 sec

F2 - Processing Parameters
 SI 65384
 SF 100.6127450 MHz
 WDW EM
 SSB 0
 LB 2.00 Hz
 GB 0
 PC 1.40

1D NMR plot parameters
 CX 20.00 cm
 F1P 227.295 ppm
 F1 22866.88 Hz
 F2P -21.181 ppm
 F2 -2131.10 Hz
 PPRDM 12.42387 ppm/cm
 HZDM 1249.95958 Hz/cm

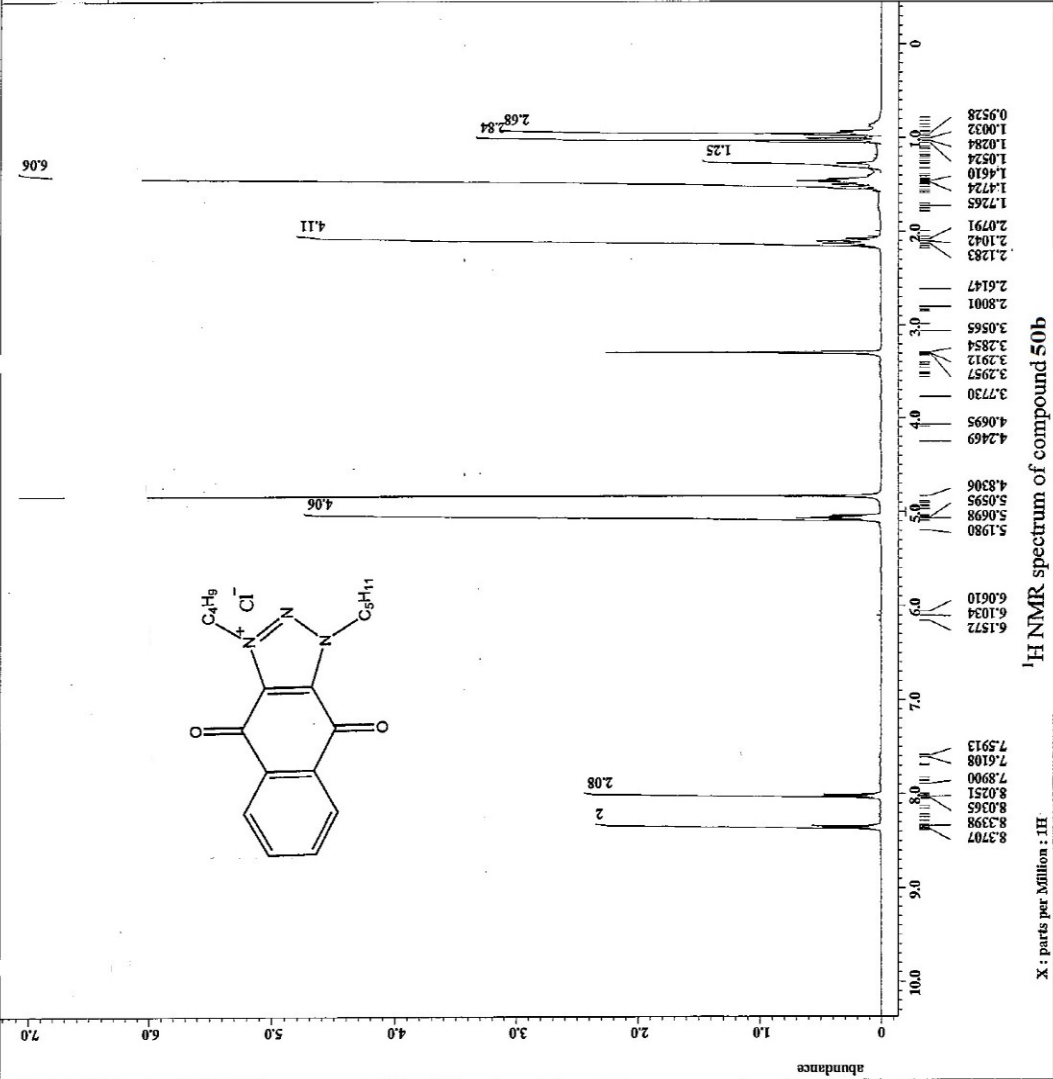


```

Filename = 020711MF-3-215B-4-jdZ
Author = fossco
Experiment = single_pulse.exe2
Sample = M21400F-3-215B
Sample_cd = M21400F-3
Creation_time = 7-FEB-2011 08:57:51
Revision_time = 7-FEB-2011 09:02:16
Current_time = 7-FEB-2011 09:03:01

Comment = single_pulse
Data_format = 1D COMPLEX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X ZK 360
Spectrometer = EXC-300

Field_strength = 7.0586913 [T] (300 [MHz])
X_acq_duration = 2.90717696 [s]
Command = F1
X_freq = 300.52965592 [MHz]
X_offset = 5 [ppm]
X_points = 16384
X_prescans = 1
X_resolution = 5.3437651 [Hz]
X_sweep = 5.0376784 [Hz]
Irr_domain = 1H
Irr_freq = 300.52965592 [MHz]
Irr_offset = 5 [ppm]
Tri_domain = 1H
Tri_offset = 5 [ppm]
Clipped = FALSE
Mod_return = 1
Scans = 8
Total_scans = 8
X_90_width = 13.43 [us]
X_acq_time = 2.90717696 [s]
X_angle = 45 [deg]
X_ph = 6 [ppm]
X_pulse_prog = 6 [ppm]
X_pulse_width = 6 [ppm]
X_pulse_width_us = 6 [ppm]
X_pulse_width_us = Off
X_pulse_width_us = Off
X_pulse_width_us = Off
Dante_presat = FALSE
Initial_wait = 1 [s]
Preparation_delay = 4 [s]
Repetition_delay = 5 [s]
Repetition_time = 7.90717696 [s]
Temp_get = 22.7 [AC]
    
```



Standard ¹³C
Experiment

```

Current Data Parameters
NAME MF-3-2153
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 500000
Time 18.35
INSTRUM ark400
PROBHD 5 mm Multic1
PULPROG zgpg30
TD 32768
SOLVENT MeOH
NS 1825
DS 2
SWH 25000.000 Hz
FIDRES 0.25290 Hz
AQ 0.6554100 sec
RG 45500
CW 20.060 usec
DE 27.14 usec
TE 300.0 K
D12 0.00002000 sec
DL5 20.00 dB
CPDPRG waltz16
P31 100.00 usec
D1 0.40000001 sec
P1 6.75 usec
DE 27.14 usec
SFO: 100.623175 MHz
NUCLEUS 13C
D11 0.00000000 sec

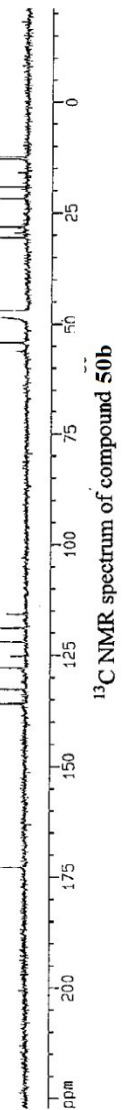
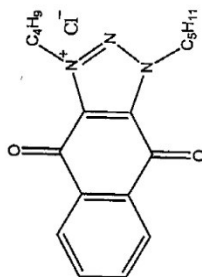
F2 - Processing parameters
SI 16384
SF 100.6127480 MHz
WDW EM
SSB 0
LB 2.00 Hz
GB 0
PC 1.40

ID NMR 0101 parameters
CX 20.00 cm
F1P 287.236 ppm
F1 28868.59 Hz
F2P -21.181 ppm
F2 -2131.10 Hz
P1P104 12.42387 ppm/cm
HZCK 1249.918988 Hz/cm
    
```

12.438
12.896
19.257
21.816
28.109
28.387
30.609
47.173
47.385
47.500
47.813
48.026
48.238
48.454
54.100
54.327

118.931
122.091
127.844
132.794
135.932

172.745



Standard Proton Experiment

Current Data Parameters
 NAME 85-3-22/B
 EXPNO 1
 PROCNO 1

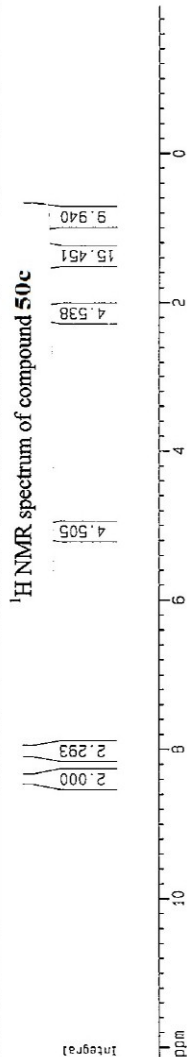
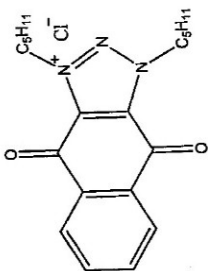
F2 - Acquisition Parameters
 Date_ 50000
 Time 13.51
 INSTRUM spect
 PROBNM 5 ms auto tune
 PULPROG zgpg30
 TO 32768
 SOLVENT MEOD
 NS 8
 DS 0
 SKH 7245.377 Hz
 FIDRES 0.221142 Hz
 AQ 2.2610421 sec
 RG 1024
 DK 69.000 usec
 DE 99.57 usec
 TE 298.0 K
 P1 4.00 usec
 P2 1.0000000 sec
 P3 99.57 usec
 SFO1 400.1328371 MHz
 NUC1US 1H

F2 - Processing parameters
 SI 16384
 SF 400.1300049 MHz
 NMR 0
 LB 0.10 Hz
 GB 0
 PC 1.00

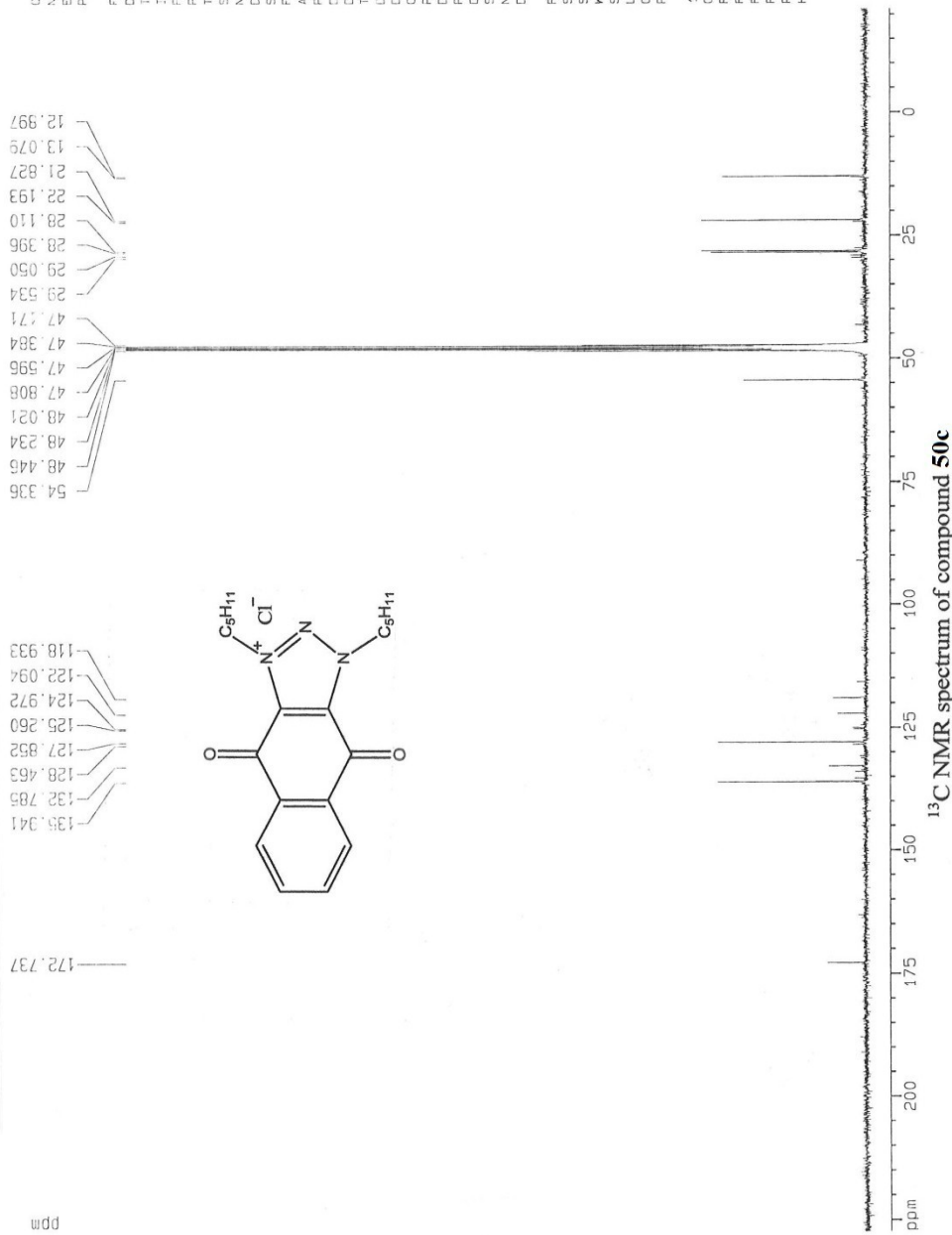
1D NMR plot parameters
 CX 20.00 cm
 F1 16.133 ppm
 F2 4624.014 Hz
 F3 41.977 ppm
 F4 78.00 Hz
 RMWCH 0.70556 ppm/cm
 MICH 282.28304 Hz/cm

5.39369
 5.38553
 5.37936
 5.37120
 5.05931
 5.05106
 5.04505
 5.03676

5.10700
 5.08943
 5.07042
 4.86292
 3.32506
 3.32086
 3.31661
 3.31282
 3.30883
 2.17120
 2.15342
 2.13509
 2.11687
 1.50670
 1.49396
 1.48475
 1.47589
 1.46702
 1.45857
 1.41871
 1.34442
 1.31462
 1.29478
 0.99597
 0.98724
 0.97953
 0.96086

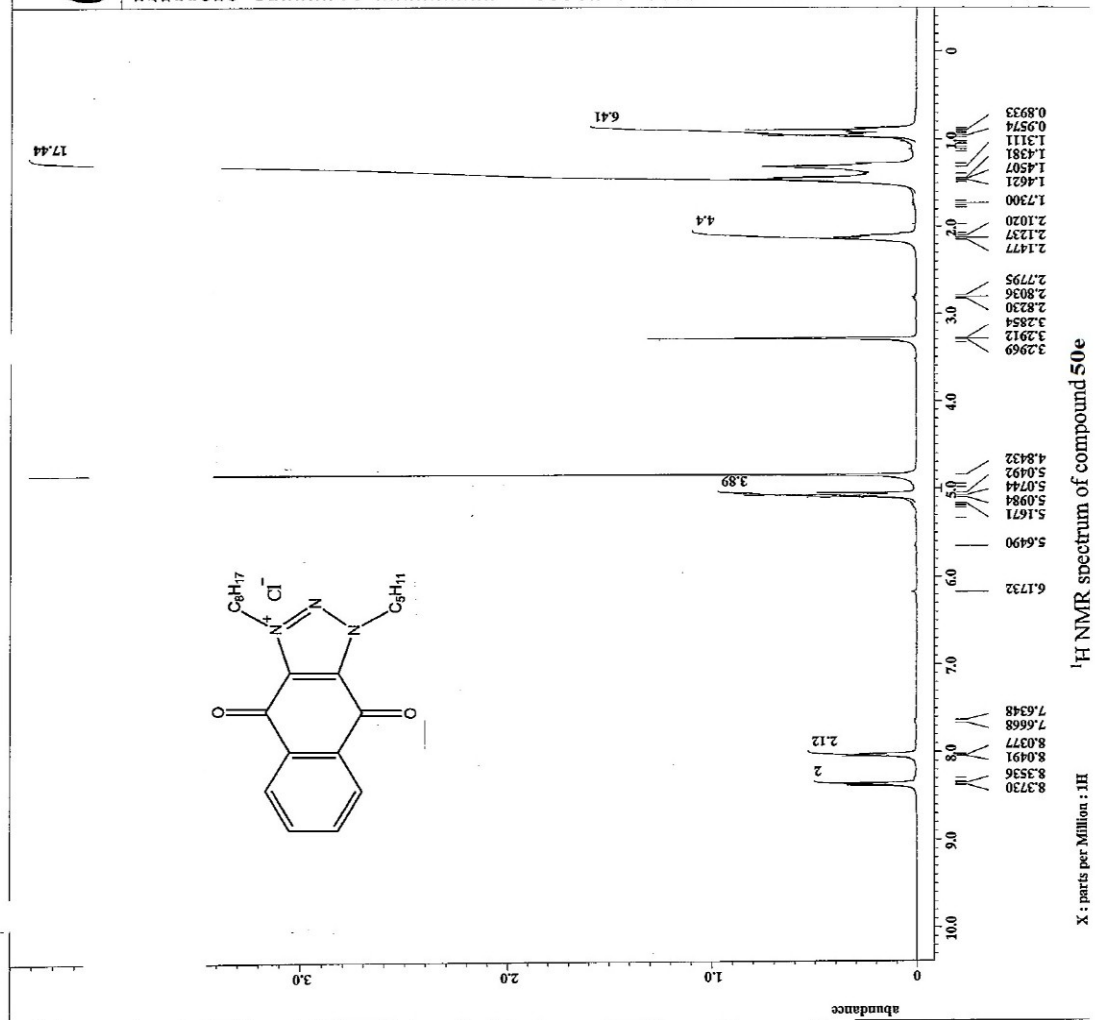


Standard ¹³C
Experiment

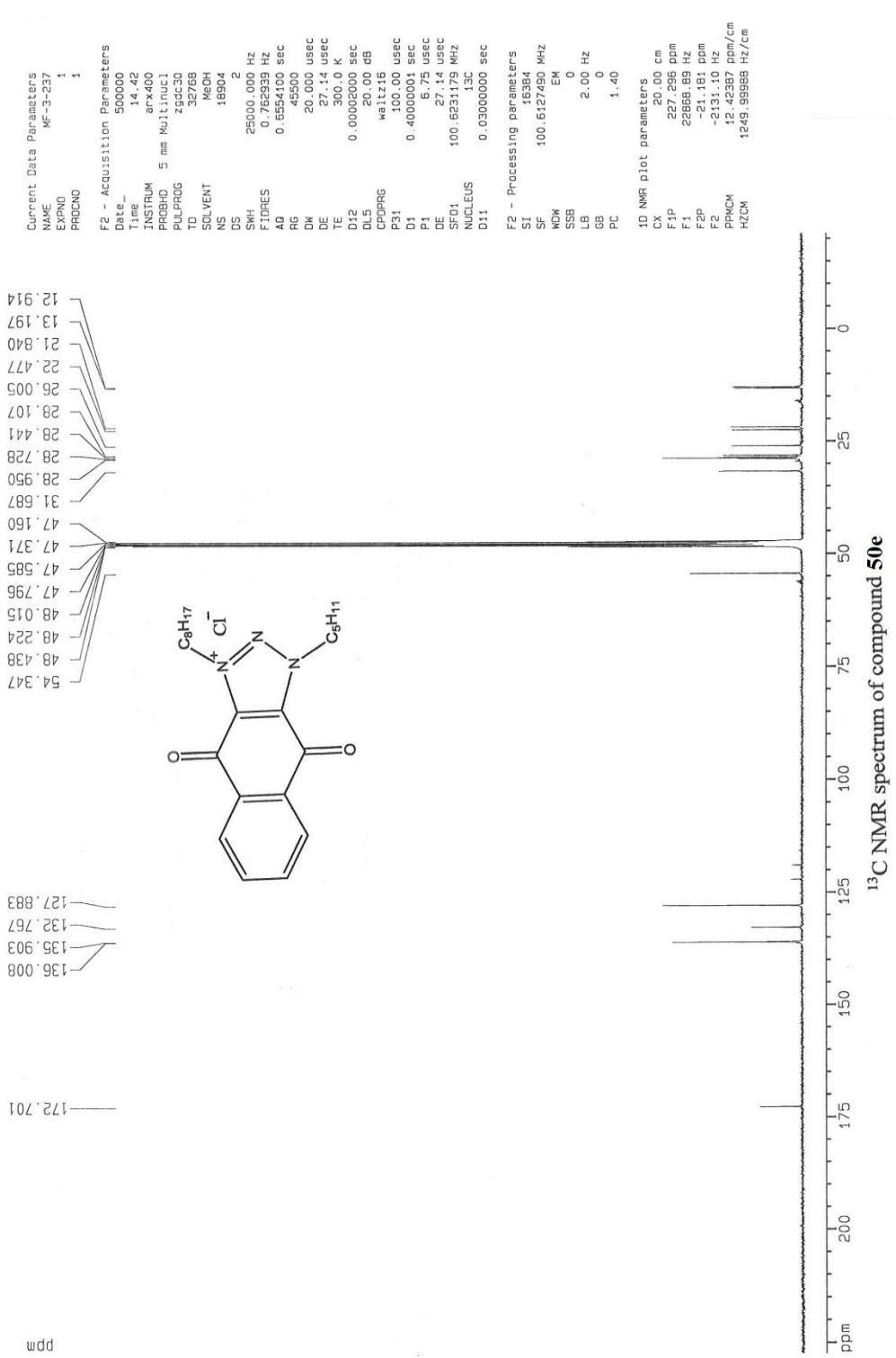




FileNames = 013011MF-3-237-3-J&F
 Author = fossa
 Experiment = single_pulse_0x2
 Date_Exp = 2011-01-30
 Solvent = METHANOL-D3
 Creation_time = 30-JAN-2011 13:40:10
 Revision_time = 30-JAN-2011 13:43:04
 Current_time = 30-JAN-2011 13:44:12
 Comment = single pulse
 Data_format = 1D COMPLEX
 Dim_size = 131.07
 Dim_title = 1H
 Dim_units = [ppm]
 Dimensions = X
 Site = EXX 300
 Spectrometer = EXX-300
 Field_strength = 7.0586013 [T] 300 [MHz]
 X_acc_duration = 2.90717696 [s]
 X_domain = 1H
 X_freq = 300.52965592 [MHz]
 X_gain = 1.6584
 X_prescans = 1
 X_resolution = 0.34397631 [Hz]
 X_sweep = 1H
 X_domain = 5.63570784 [MHz]
 X_freq = 300.52965592 [MHz]
 X_gain = 5.0
 X_prescans = 1H
 X_resolution = 300.52965592 [MHz]
 X_offset = 5 [ppm]
 X_domain = 5 [ppm]
 X_freq = 300.52965592 [MHz]
 X_offset = 5 [ppm]
 Clipped = FALSE
 Scan_return = 8
 Total_scans = 8
 X_90_width = 13.43 [us]
 X_acq_time = 2.90717696 [s]
 X_delay = 3 [s]
 X_atc = 3 [us]
 X_pulse = 6.715 [us]
 X_mode = Off
 X_prescans = Off
 Data_presat = FALSE
 Nuclear_mag = 1
 Relaxation_delay = 4 [s]
 Relaxation_time = 5 [s]
 Repetition_time = 7.90717696 [s]
 Temp_set = 22.5 [C]



Standard ¹³C
Experiment



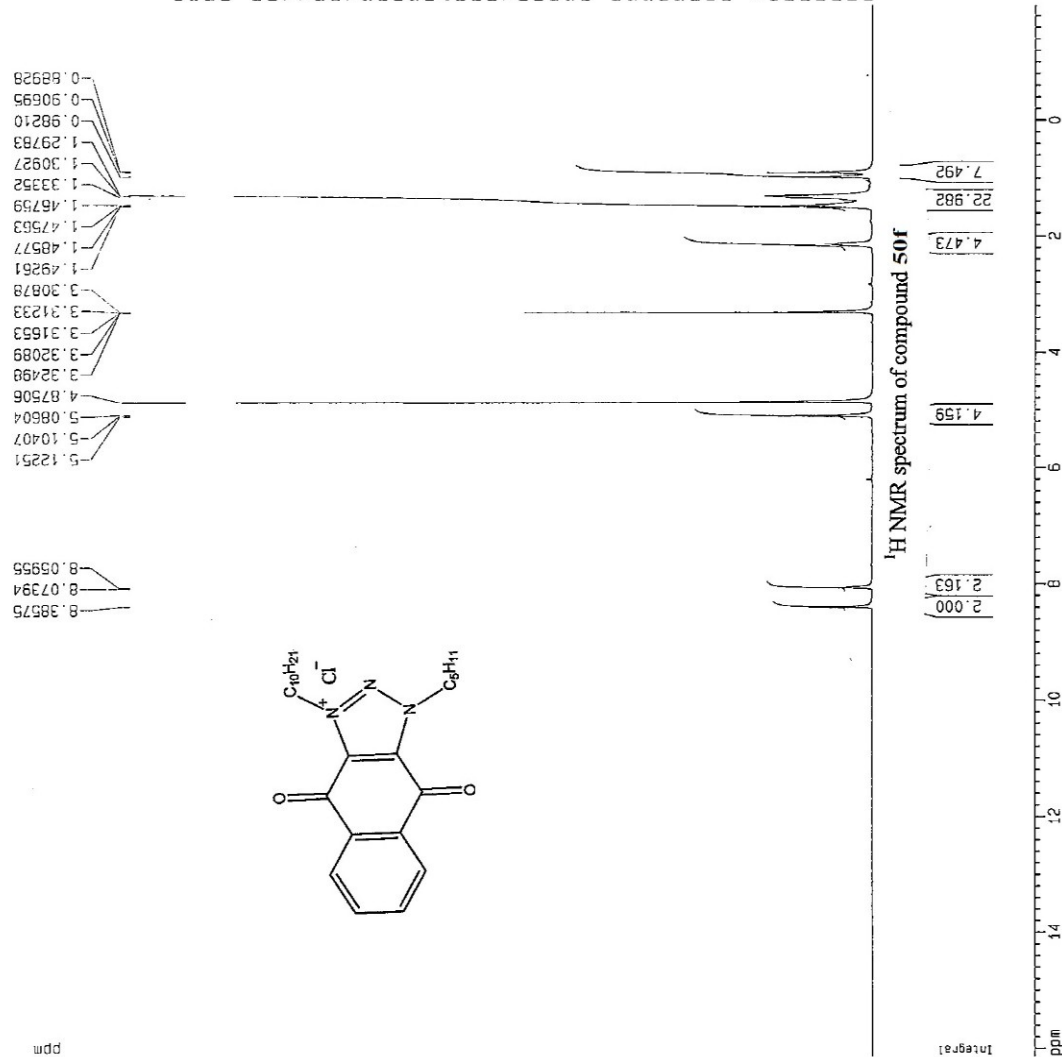
Standard Proton Experiment

Current Data Parameters
 NAME 102410MF-3-208 B
 EXPNO 1
 PROCNO 1

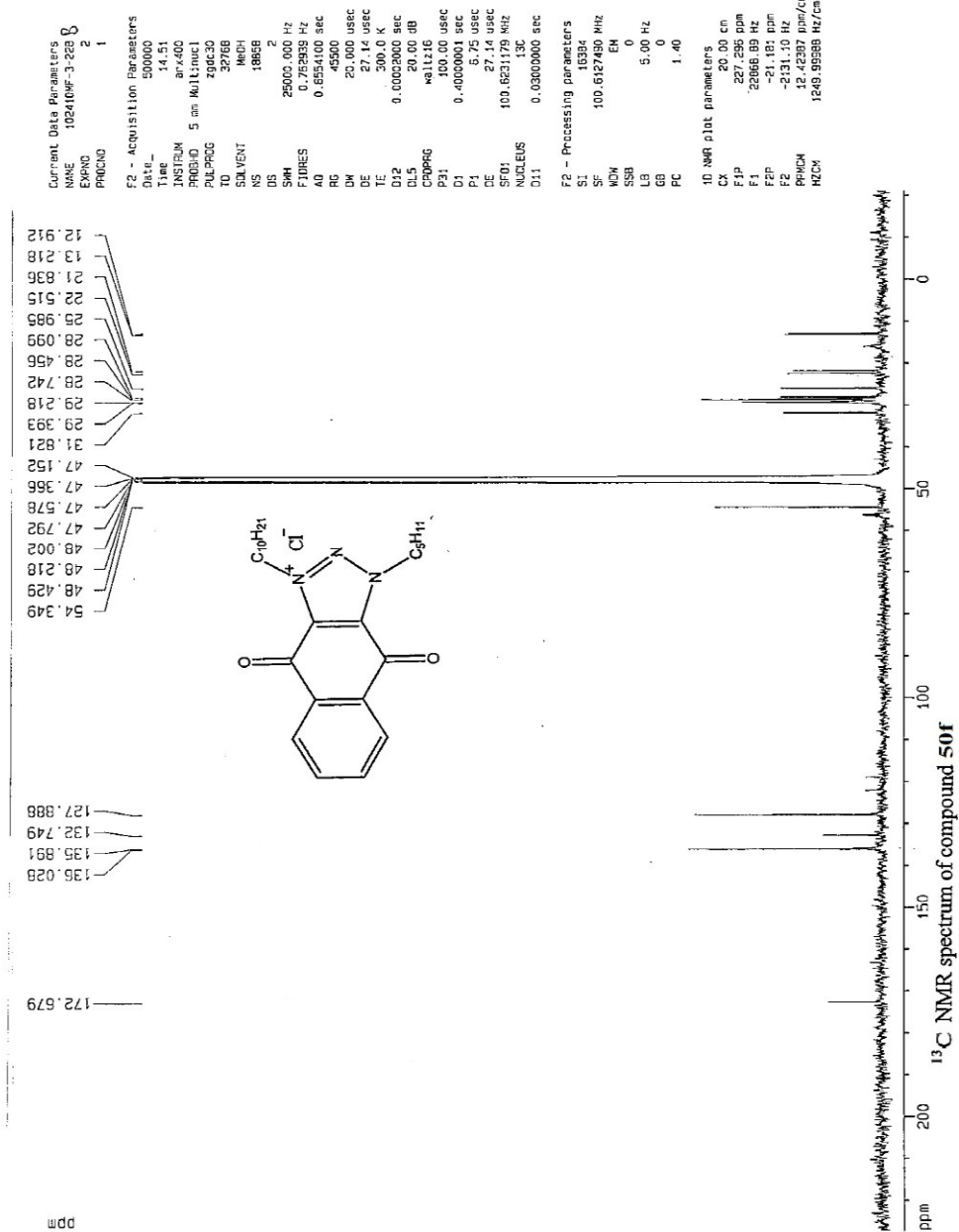
F2 - Acquisition Parameters
 Date_ 500003
 Time 14.10
 INSTRUM brx400
 PROBHD 5 mm Multicore3
 PULPROG zg
 TD 32768
 SOLVENT MeOH
 NS 8
 DS 0
 SWH 7246.377 Hz
 FIDRES 0.221142 Hz
 AQ 2.2610421 sec
 RG 1024
 DM 69.000 usec
 DE 98.57 usec
 TE 298.0 K
 D1 1.0000000 sec
 P1 4.00 usec
 DE 98.57 usec
 SFO1 400.1328371 MHz
 NUCLEUS 1H

F2 - Processing parameters
 SI 16384
 SF 400.1300049 MHz
 NM 8K
 SSB 0
 LB 0.10 Hz
 GB 0
 PC 1.00

1D NMR plot parameters
 CH 20.00 cm
 F1P 15.33 ppm
 F1 6445.35 Hz
 F2P 1977.00 ppm
 F2 -781.03 Hz
 PPGM 0.90550 ppm/cm
 HZCM 362.31885 Hz/cm



Standard ¹³C Experiment

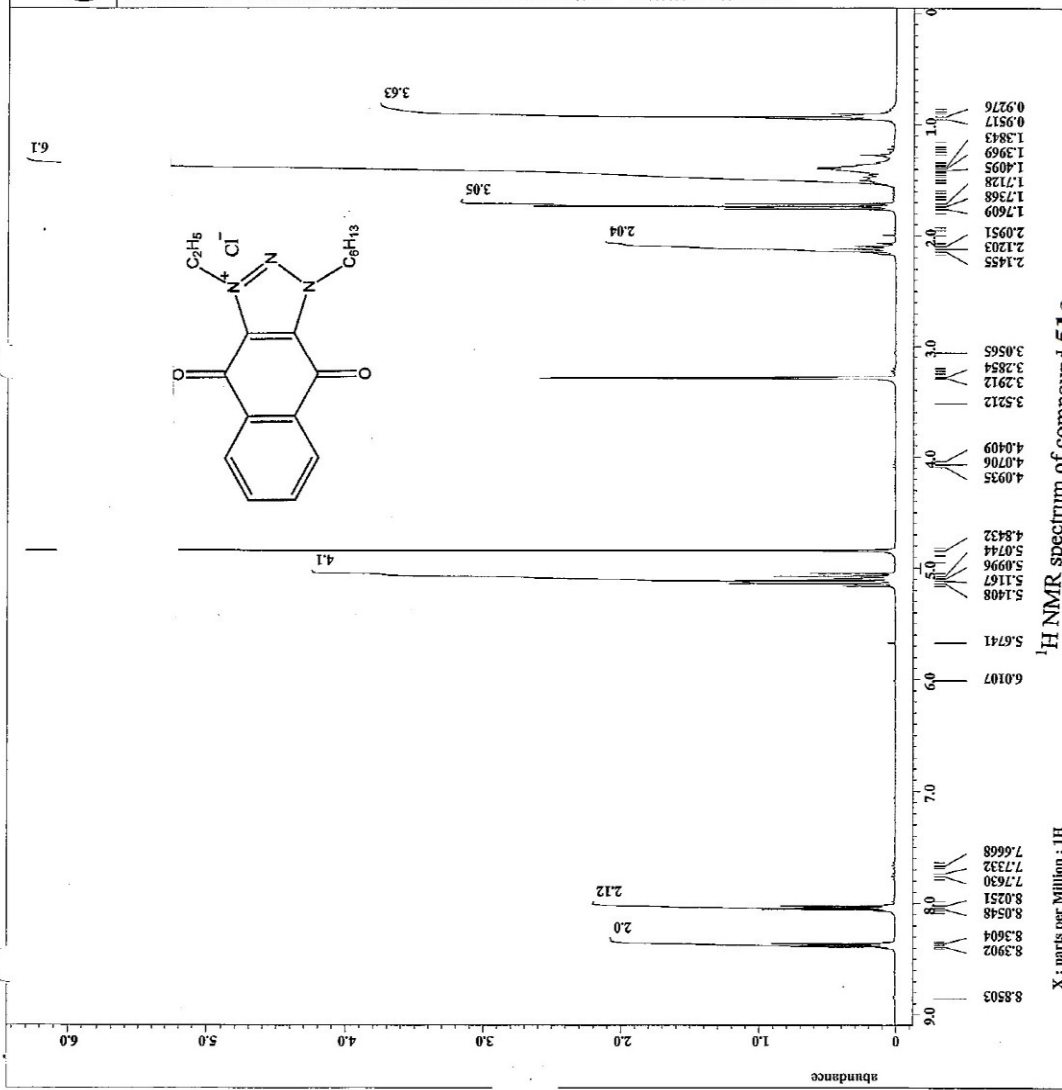
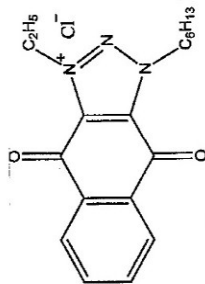




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Filename = 091610MF-3-205B(7)-3.
Author = fcsso
Experiment = single_pulse.exc2
Sample_id = 091610MF-3-205B(7)
Scan_rate = 16-SEP-2010 10:45:09
Creation_time = 16-SEP-2010 10:45:09
Revision_time = 16-SEP-2010 23:11:08
Current_time = 16-SEP-2010 23:11:20
Comment = single_pulse
          1D COMP2K
          13107
          1H
          [ppm]
          X
          EXX-300
          Spectrometer = EXX-300
Field_strength = 7.0586013[T] (300 [MHz]
X_acq_duration = 2.90717696[s]
X_domain = 1H
X_offset = 50.52965592 [MHz]
X_points = 16384
X_prescans = 1
X_resolution = 0.34357631 [Hz]
X_sweep = 1H
X_start = 16.83570784 [MHz]
X_stop = 300.52965592 [MHz]
F1_offset = 5 [ppm]
F1_domain = 1H
F1_freq = 300.52965592 [MHz]
F1_offset = FALSE
Mod_return = 1
Total_scans = 8
X_90_width = 13.43 [us]
X_acq_time = 2.90717696 [s]
X_angle = 45 [deg]
X_atn = 3 [dB]
X_pulse = 6.715 [us]
X1_mode = OF
X2_mode = OF
Dante_present = FALSE
Initial_wait = 1 [s]
Recvr_gain = 46
Relaxation_delay = 5 [s]
Solve_acom_time = 23.1 [DC]
Temp_get =
  
```

61



¹H NMR spectrum of compound 51a

X : parts per Million : 1H

Standard 13C
Experiment

Current Data Parameters
NAME NDC205
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 500000
Time 13.58
INSTRUM gpcx400
PROBHD 5 mm Multispec1
PULPROG zgpg30
TD 32768
SOLVENT MeOH
NS 10711
DS 2
SWH 25000.000 Hz
FIDRES 0.76399 Hz
AQ 0.656400 sec
RG 45900
DM 20.000 uSAC
DE 27.14 uSAC
TE 300.0 K
O12 0.00002000 sec
O13 20.00 dB
DL5 waltz16
P31 100.00 uSAC
D1 0.40000001 sec
P1 5.75 uSAC
DE 27.14 uSAC
SFO1 100.6231179 MHz
NUCLEUS 13C
D11 0.03000000 sec

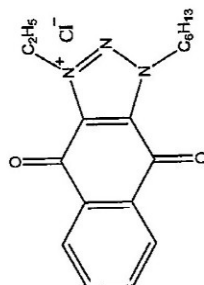
F2 - Processing parameters
SI 15384
SF 100.6127450 MHz
WDW EM
SSB 0
LB 2.00 Hz
GB 0
PC 1.40

1D NMR plot parameters
CX 20.00 cm
F1P 250.000 dpa
F1 25183.19 Hz
F2P -21.181 dpa
F2 -2131.11 Hz
PPM0N 13.55906 ppm/cm
HZ0N 1354.21450 Hz/cm

12.973
13.073
22.226
25.692
28.741
30.956
47.164
47.371
47.585
47.757
48.016
48.226
48.436
50.227
54.342

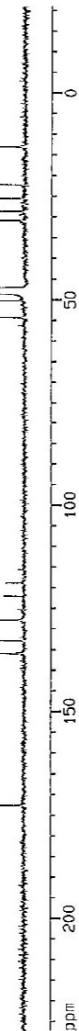
110.906
122.073
127.882
132.751
135.830
135.875
136.016

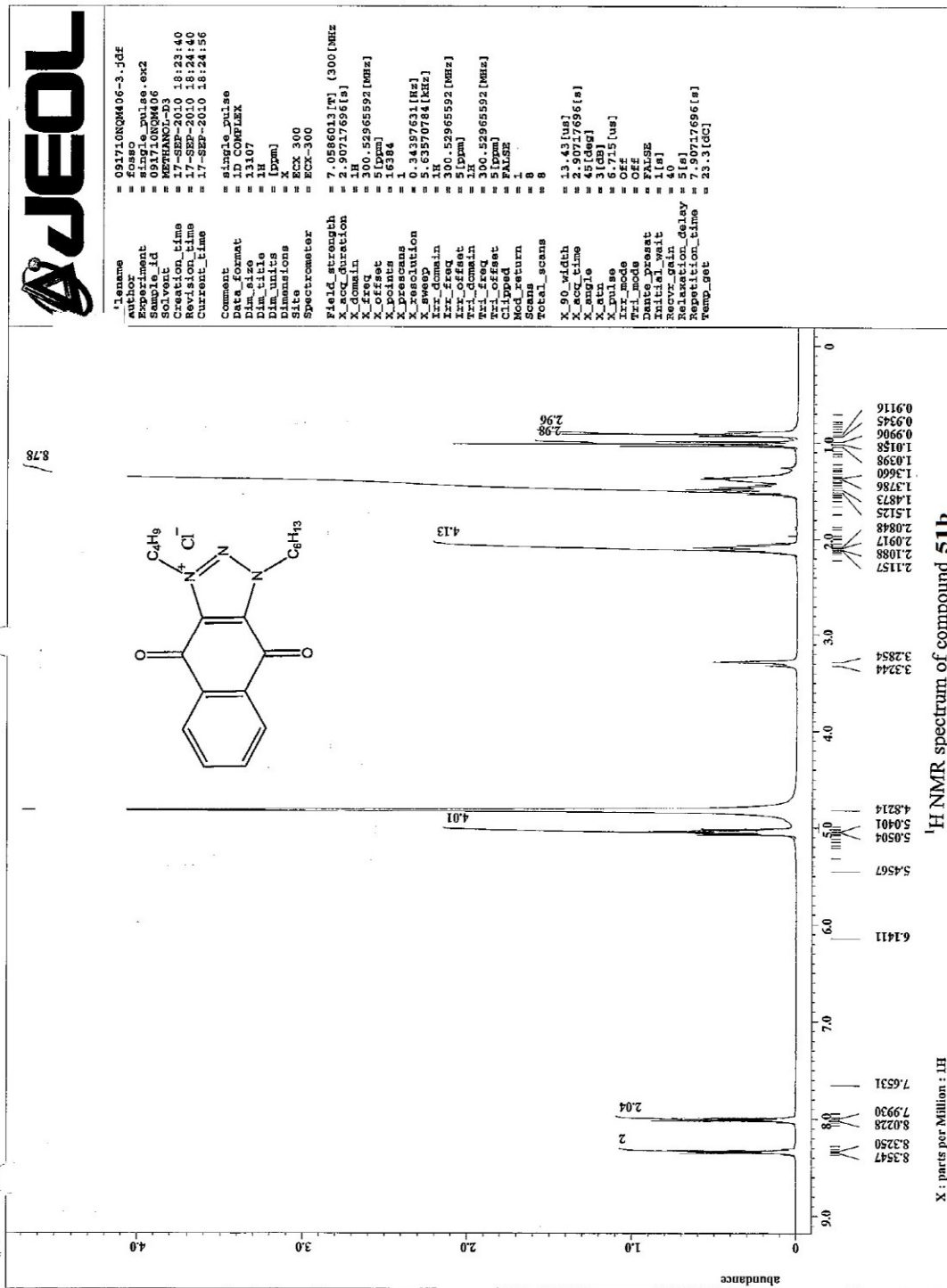
172.659
172.727



ppm

¹³C NMR spectrum of compound 51a



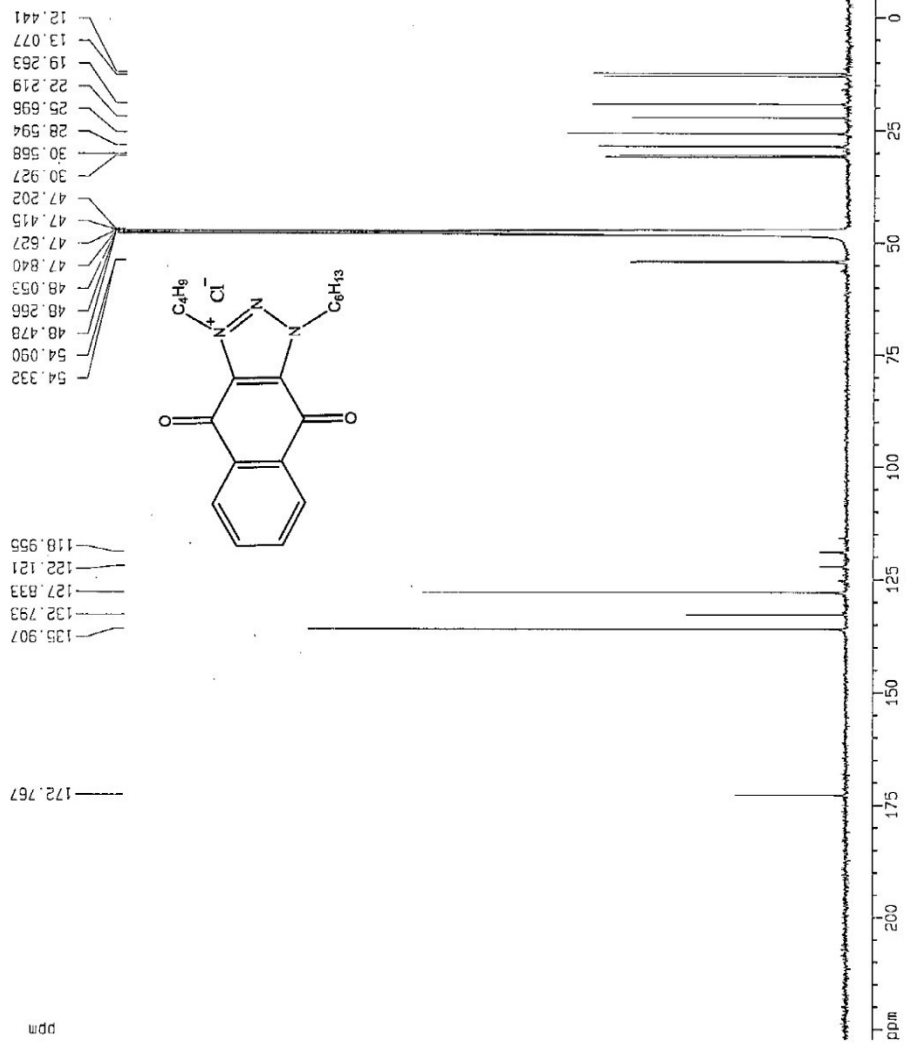


Standard 13C
Experiment

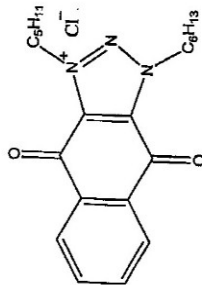
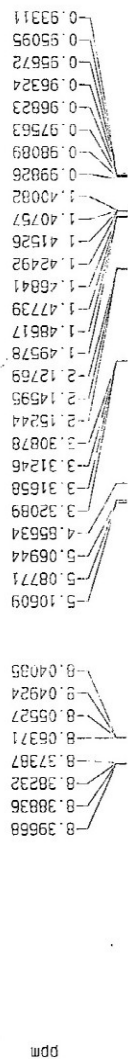
Current Data Parameters
 NAME: NMR405
 EXPNO: 1
 PROCNO: 1

F2 - Acquisition Parameters
 Date_: 500000
 Time: 19.13
 INSTRUM: brx400
 PULPROG: zgpg30
 ID: 32768
 SOLVENT: H2O
 NS: 2
 DS: 2
 SWH: 25000.00 Hz
 FIDRES: 0.752939 Hz
 AQ: 0.6558100 sec
 RG: 45500
 DM: 20.000 usec
 DE: 27.14 usec
 TE: 300.0 K
 D12: 0.0000000 sec
 DL5: 20.00 dB
 CPGPRG: waltz16
 P31: 100.00 usec
 D1: 0.4000000 sec
 F1: 6.75 usec
 DE: 27.14 usec
 SF01: 100.623179 MHz
 NUCLEUS: 13C
 F2 - Processing parameters
 SI: 15364
 SF: 100.6127490 MHz
 EQ: 0
 SSB: 2.00 Hz
 LB: 0
 GB: 0
 PC: 1.40

ID NMR plot parameters
 CX: 20.00 cm
 F1P: 227.236 ppm
 F1: 22666.89 Hz
 F2P: -21.181 ppm
 F2: -2131.11 Hz
 PPMCM: 12.42387 ppm/cm
 INCKM: 1250.00012 Hz/cm



Standard Proton Experiment

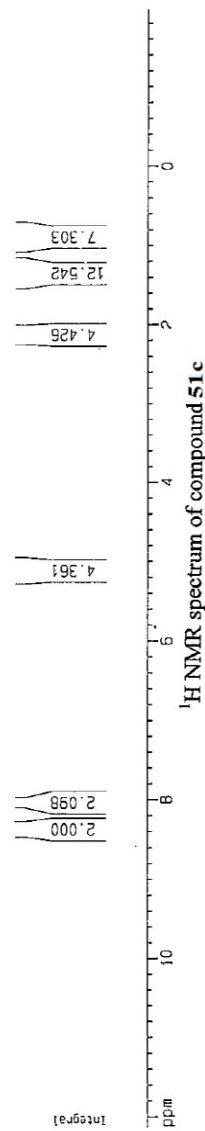


Current Data Parameters
 DATE: 01-2-2009
 EXPRNO: 1
 PROCNO: 1

F2 - Acquisition Parameters
 Date_ 5/20/09
 Time 23:23
 INSTRUM spect
 PULPROG zgpg30
 S as Multirac1
 TD 65536
 FIDRES 0.32778
 AQ 32778
 SOLVENT ACQU
 NS 8
 DS 8
 SM 7246.377 Hz
 F1RES 0.221142 Hz
 AQ 2.3510421 sec
 RG 512
 DM 69.000 usec
 DE 96.57 usec
 TE 296.0 K
 D1 1.0000000 sec
 PE 4.00 usec
 DE 96.57 usec
 SF 400.1326371 MHz
 NUC1E15 1H

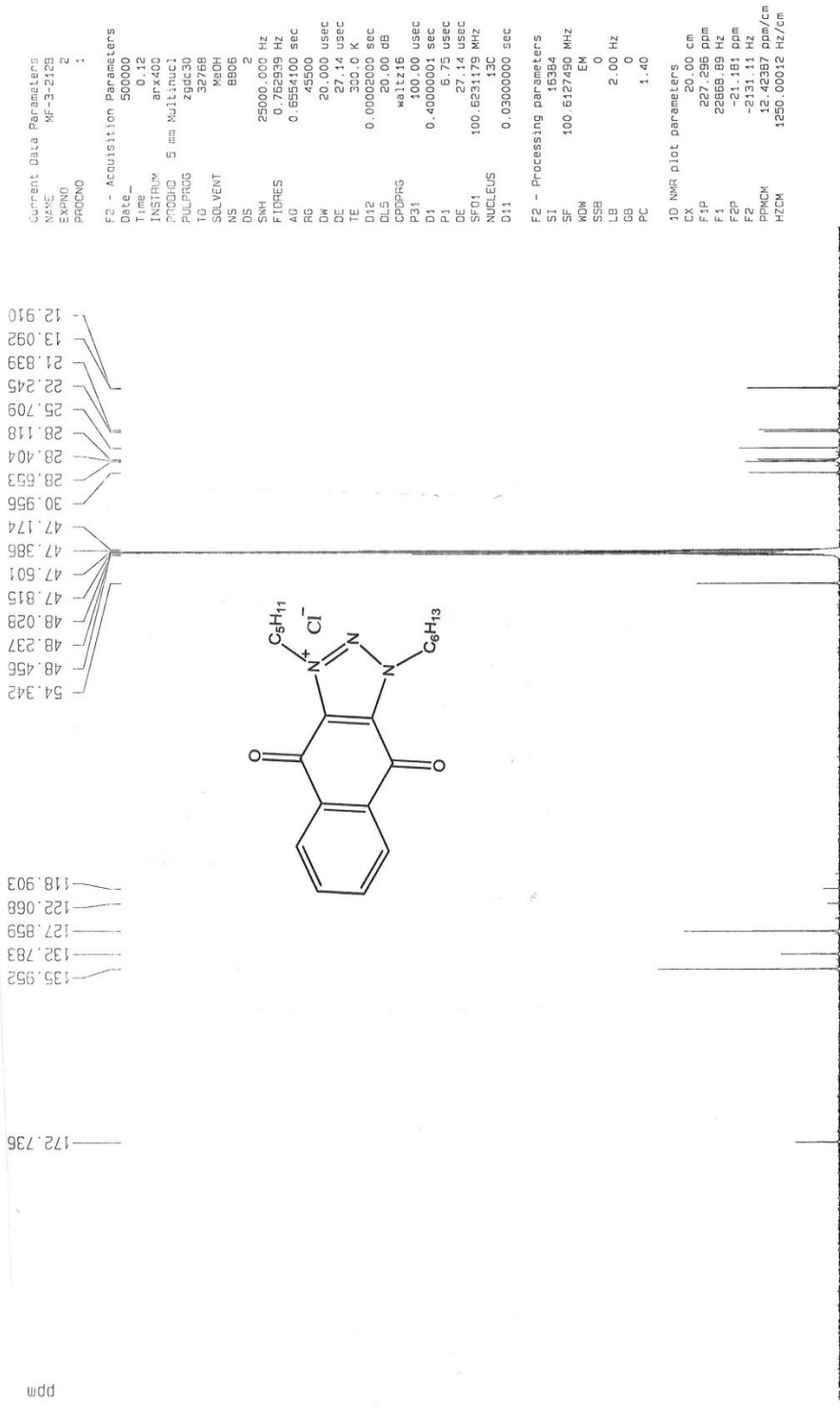
F2 - Processing parameters
 SI 32768
 SF 400.1300000 MHz
 RG 512
 SSF 0
 LB 0.10 Hz
 GB 0
 PC 1.00

1D NMR file parameters
 CX 20.00 cm
 F1P 12.133 ppm
 F1 4854.78 Hz
 F2P -1.577 ppm
 F2 -791.02 Hz
 PPM1C 0.70550 ppm/cm
 HZCM 282.25004 Hz/cm



¹H NMR spectrum of compound 51c

Standard ¹³C
Experiment



Current Data Parameters
 Name MF-3-2128
 EXPNO 2
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 500000
 Time 0.12
 INSTRUM arx400
 PULPROG zgpg30
 TD 32768
 SOLVENT MeOH
 NS 2
 DS 2
 SWH 25000.000 Hz
 FIDRES 0.762939 Hz
 AQ 0.6554100 sec
 RG 45500
 DW 20.000 usec
 DE 27.14 usec
 TE 300.0 K
 D12 0.00002000 sec
 DLS 20.00 dB
 CPDPRG meltz16
 P31 100.00 usec
 D1 0.40000001 sec
 P1 6.75 usec
 DE 27.14 usec
 SFO1 100.6231179 MHz
 NUCLEUS ¹³C
 D11 0.030000000 sec

F2 - Processing parameters
 S1 16384
 SF 100.6127490 MHz
 EM
 MDM 0
 SSB 2.00 Hz
 LB 0
 GB 0
 PC 1.40

1D NMR plot parameters
 CX 20.00 cm
 F1P 227.296 ppm
 F1 22868.89 Hz
 F2P -21.181 ppm
 F2 -2131.11 Hz
 PPMCM 12.42367 ppm/cm
 HZCM 1250.00012 Hz/cm

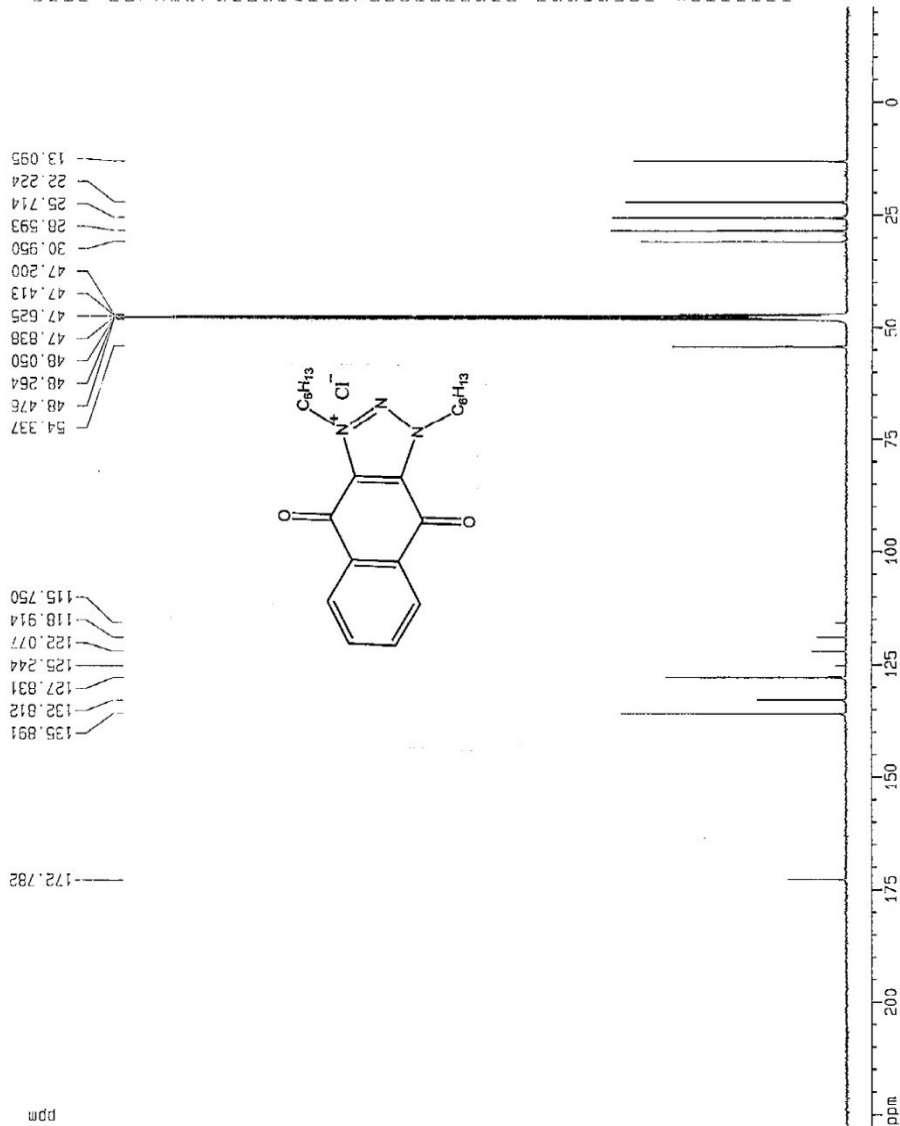
Standard ¹³C
Experiment

Current Data Parameters
 NAME: MFC-3-2085
 EXPNO: 1
 PROCNO: 1

F2 - Acquisition Parameters
 Date_: 500000
 Time: 15.49
 INSTRUM: wxt400
 PROBHD: 5 mm Multic1
 PULPROG: zgpg30
 IC: 32798
 SOLVENT: H2O
 NS: 1539
 DS: 4
 SWH: 25500.000 Hz
 FIDRES: 0.762036 Hz
 AQ: 0.6554100 sec
 RG: 45300
 DM: 20.000 usec
 DE: 27.14 usec
 TE: 300.0 K
 D12: 0.00002000 sec
 DLS: 20.00 dB
 CPOPRG: waitz15
 P31: 100.00 usec
 P1: 0.40000001 sec
 P2: 6.75 usec
 DC: 27.14 usec
 SFO1: 100.623179 MHz
 NUCLEUS: ¹³C
 D11: 0.03000000 sec

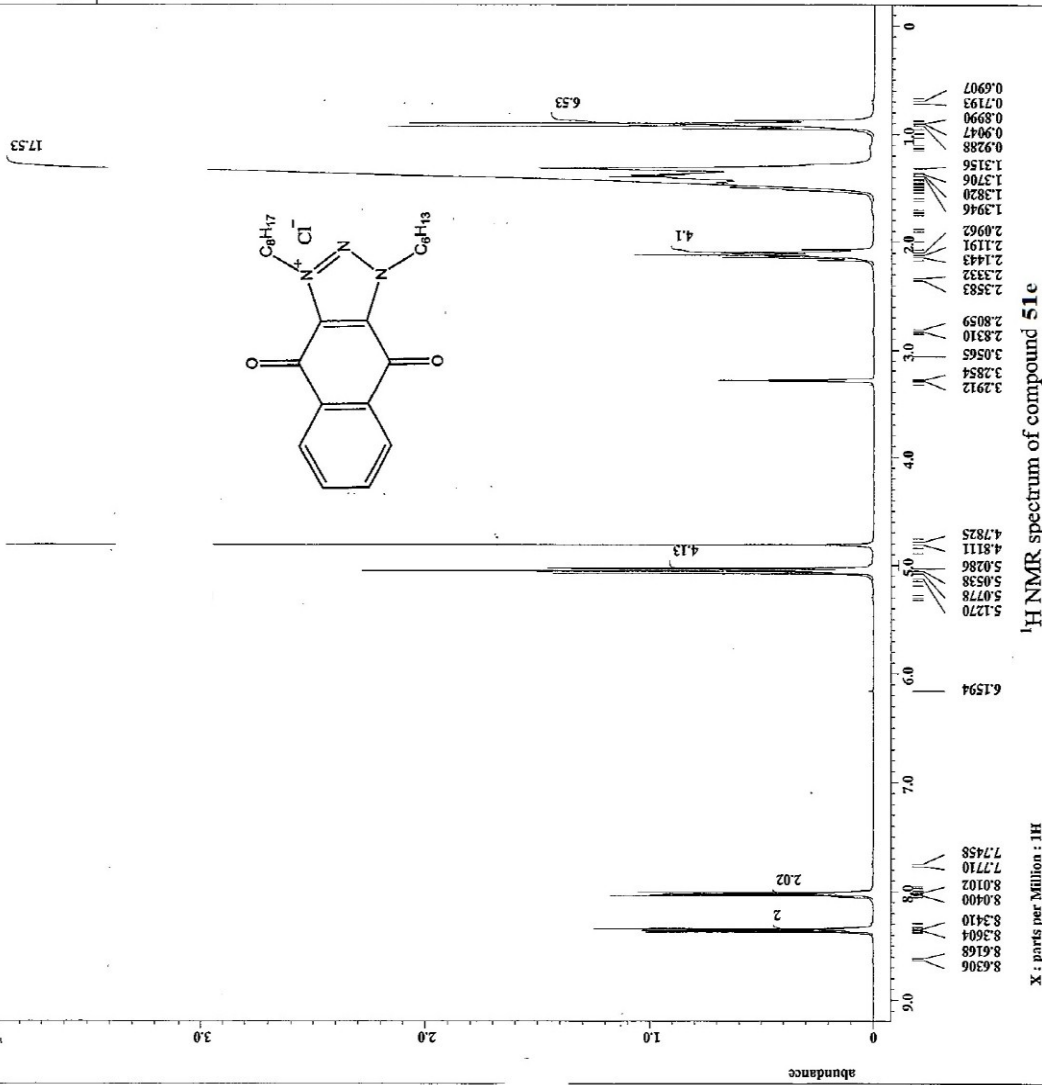
F2 - Processing parameters
 SI: 16384
 SF: 100.6127450 MHz
 XN: 4
 SSB: 0
 LB: 2.00 Hz
 GB: 0
 PC: 1.40

1D NMR plot parameters
 CX: 20.00 ca
 F1P: 227.295 ppm
 F1: 22866.89 Hz
 F2P: -21.181 ppm
 F2: -2131.11 Hz
 PPMCK: 12.42387 ppm/cm
 HZCK: 1250.00012 Hz/cm



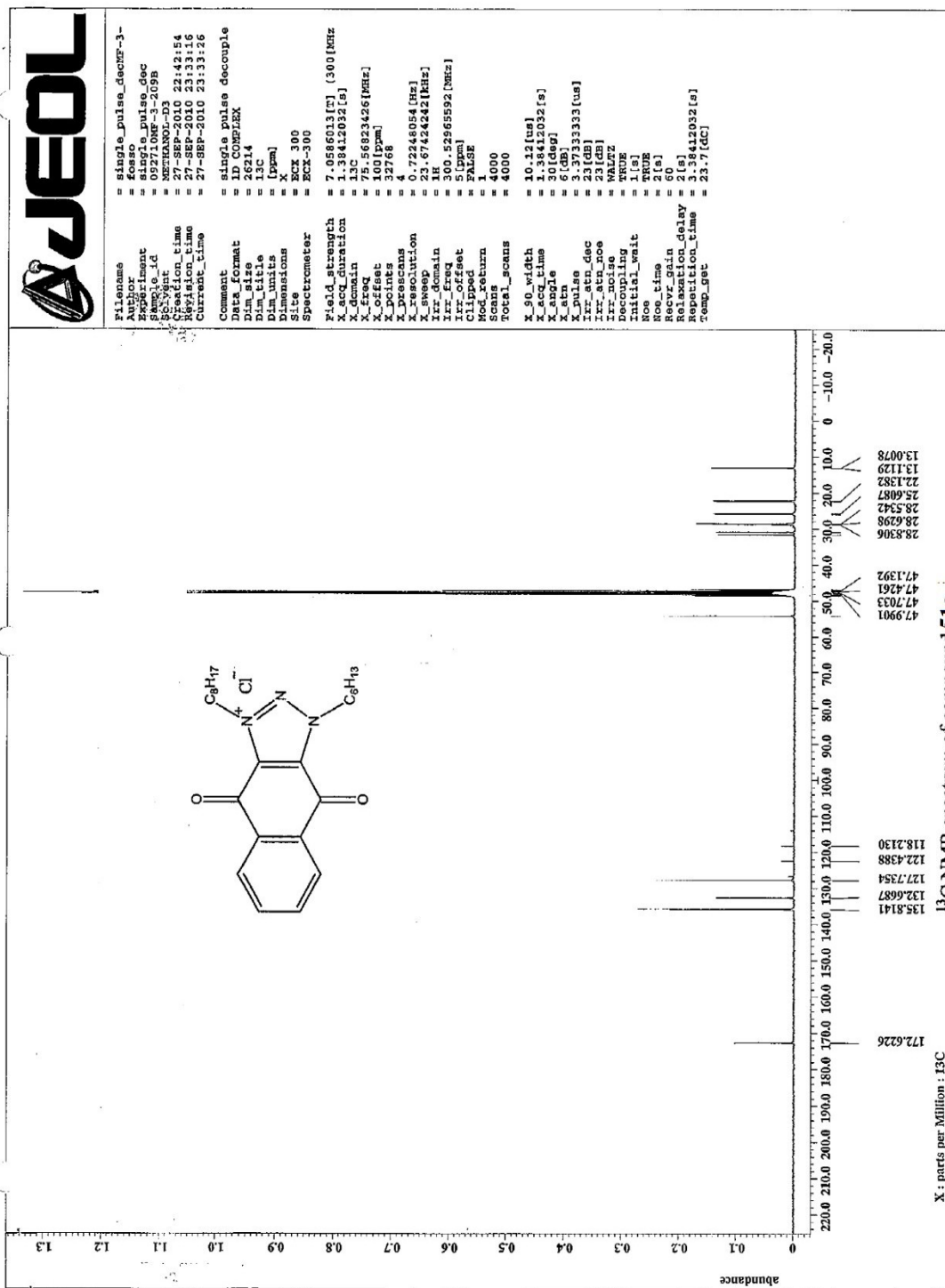


Filename = 09271OMF-3-209B-3.fdf
 Author = fossco
 Experiment = single_pulse.ex2
 Sample_id = 09210MF-3-209B
 Name = MZ10MF-3-209B
 Creation_time = 27-SEP-2010 18:46:48
 Revision_time = 27-SEP-2010 18:46:40
 Current_time = 27-SEP-2010 18:48:06
 Comment = single_pulse
 Data_format = 1D COMPLEX
 Dir_size = 13107
 Dir_title = 1H
 Dir_units = [ppm]
 Dimensions = X 300
 Spectrometer = ECA-300
 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_resolution = 1
 X_resolution = 0.34397631 [Hz]
 X_resolution = 0.83970784 [Hz]
 X_resolution = 1H
 X_resolution = 300.52965592 [MHz]
 X_resolution = 5 [ppm]
 X_resolution = 1H
 X_resolution = 300.52965592 [MHz]
 X_resolution = 5 [ppm]
 X_resolution = 1H
 X_resolution = 300.52965592 [MHz]
 X_resolution = 5 [ppm]
 X_resolution = FALSE
 Mod_return = 1
 Total_scans = 8
 X_90_width = 13.43 [us]
 X_acq_time = 2.90717696 [s]
 X_angle = 45 [deg]
 X_satn = 3 [dB]
 X_pulse = 0.715 [us]
 X_mode = Off
 X_mode = Off
 Dante_preset = FALSE
 Initial_wait = 1 [s]
 Recv_gain_delay = 26
 X_resolution = 1H
 X_resolution = 300.52965592 [MHz]
 X_resolution = 5 [ppm]
 X_resolution = FALSE
 Repetition_time = 23.4 [dc]
 Temp_Get



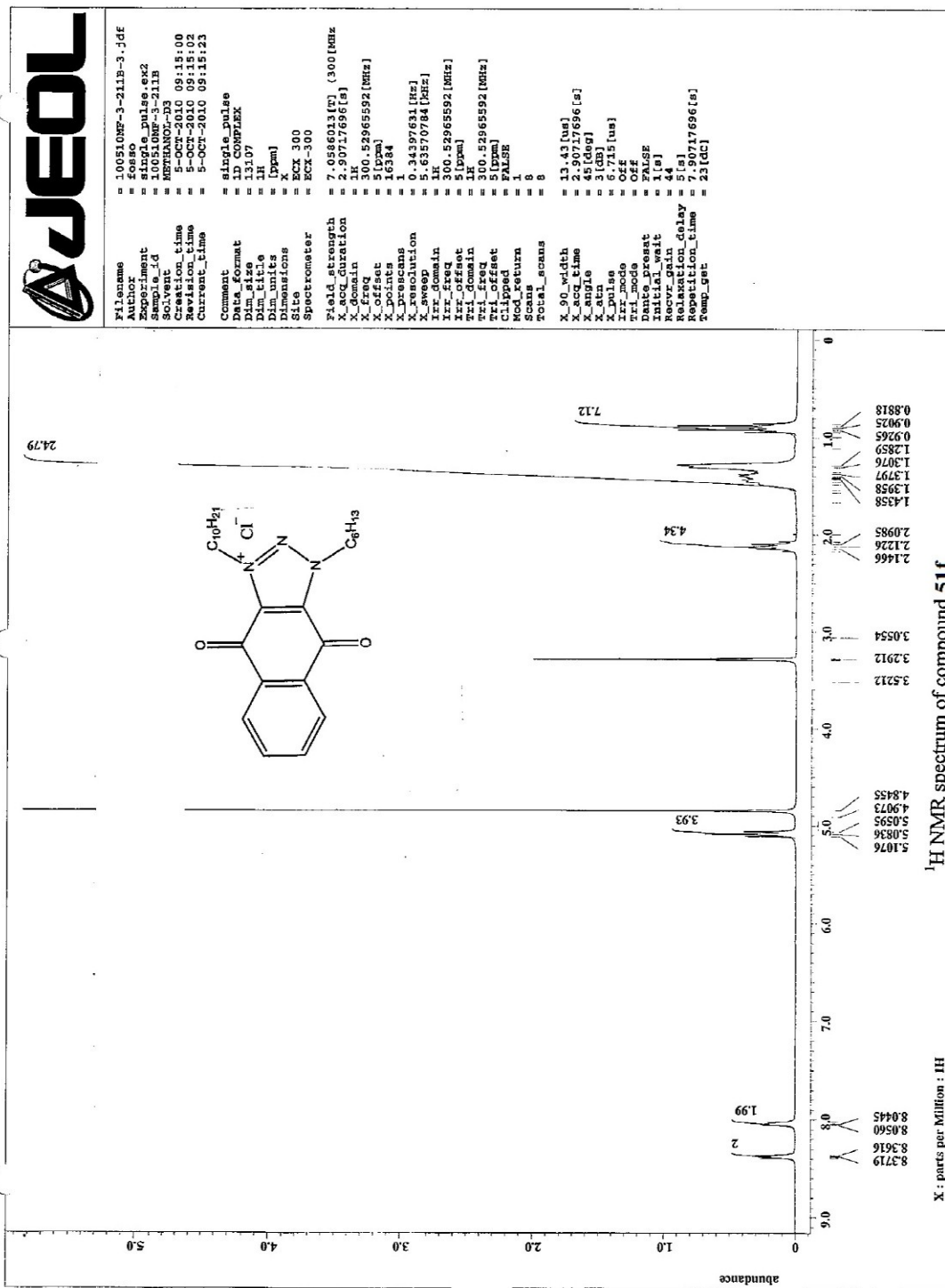
abundance

X : parts per Million : Hz



```

= single_pulse_decouple
= fssco
= single_pulse_dec
= 92710NF-3-209B
= 27-SEP-2010 21:42:14
= 27-SEP-2010 21:42:14
= 27-SEP-2010 23:33:16
= 27-SEP-2010 23:33:26
= single pulse decouple
= 1D COMPLEX
= 26214
= 13C
= [ppm]
= XCP 300
= EXC-300
Spectrometer:
Field_strength = 7.0586013 [T] (300 [MHz]
X_acq_duration = 1.38412032 [s]
X_domain = 7.058623426 [MHz]
X_offset = 100 [ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.72248054 [Hz]
X_sweep = 13.6742442 [MHz]
X_start_freq = 300.52965592 [MHz]
X_end_freq = 5 [ppm]
Irr_offset = FALSE
Clipped =
Xoc_return = 1000
Total_scans = 4000
X_90_width = 10.12 [us]
X_acq_time = 1.38412032 [s]
X_angle = 6 [deg]
X_pulse = 3.37333333 [us]
Irr_atn_dec = 23 [dB]
Irr_atn_hoe = 23 [dB]
Irr_noise = WALTZ
X_pulse_prog = WALTZ
X_pulse_wait = 1 [s]
Noe = TRUE
Noe_time = 2 [s]
Recvr_gain = 60
Relaxation_delay = 3 [s]
Relaxation_time = 31.84412032 [s]
Temp_set = 23.7 [dC]
  
```

```

Filename = 100510MF-3-211B-3.jdf
Author = fesso
Experiment = single_pulse.ex2
Sample_id = 100510MF-3-211B
Number_of_scans = 8
Creation_time = 5-OCT-2010 09:15:00
Revision_time = 5-OCT-2010 09:15:02
Current_time = 5-OCT-2010 09:15:23
Comment = single_pulse
Date_acq = 20100728
Date_mat = 13107
Din_size = 1H
Din_title =
Din_units = [ppm]
Dimensions = X 300
SITS = ECA-300
Spectrometer =
Field_strength = 7.0586013 [T] (300 [MHz]
X_acq_duration = 2.90717696 [s]
X_domain = H
X_offset = 50.52965592 [MHz]
X_points = 16384
X_prescans = 1
X_resolution = 0.34397631 [Hz]
X_sweep = 1.63570784 [MHz]
Xrr_freq = 300.52965592 [MHz]
Xrr_offset = 5 [ppm]
Xrr_domain = 1H
Xrr_freq_offset = 50.52965592 [MHz]
Xrr_offset_offset = 0 [ppm]
Clipped = FALSE
Msd_return = 1
Scans = 8
Total_scans = 8
X_90_width = 13.43 [us]
X_acq_time = 2.90717696 [s]
X_angle = 45 [deg]
X_atn = 3 [dB]
X_pulse = 6.715 [us]
Xrr_mode = Off
Xrr_mods = Off
Dante_presat = FALSE
Initial_wait = 1 [s]
Recovr_gain = 44
Relaxation_delay = 2.00 [s]
Relaxation_time = 2.90717696 [s]
Temp_set = 23 [degC]
    
```

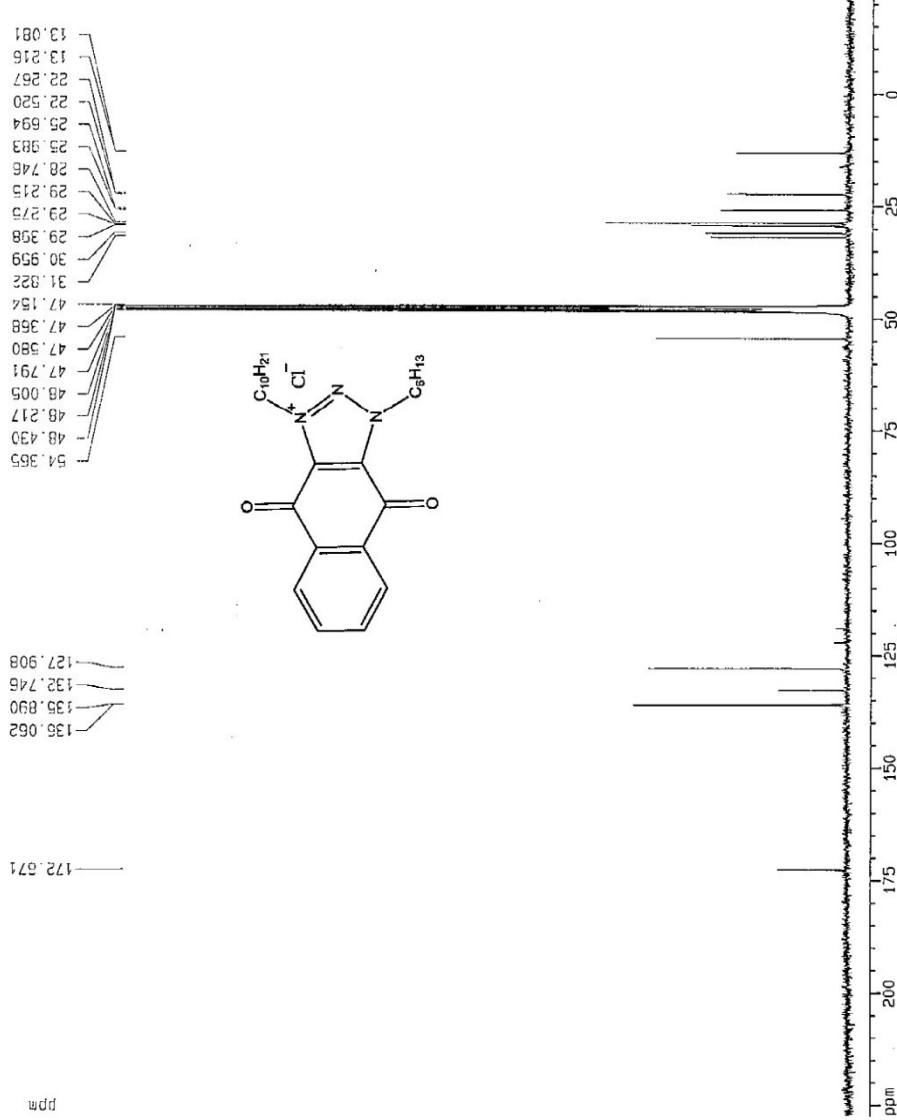
Standard ¹³C
Experiment

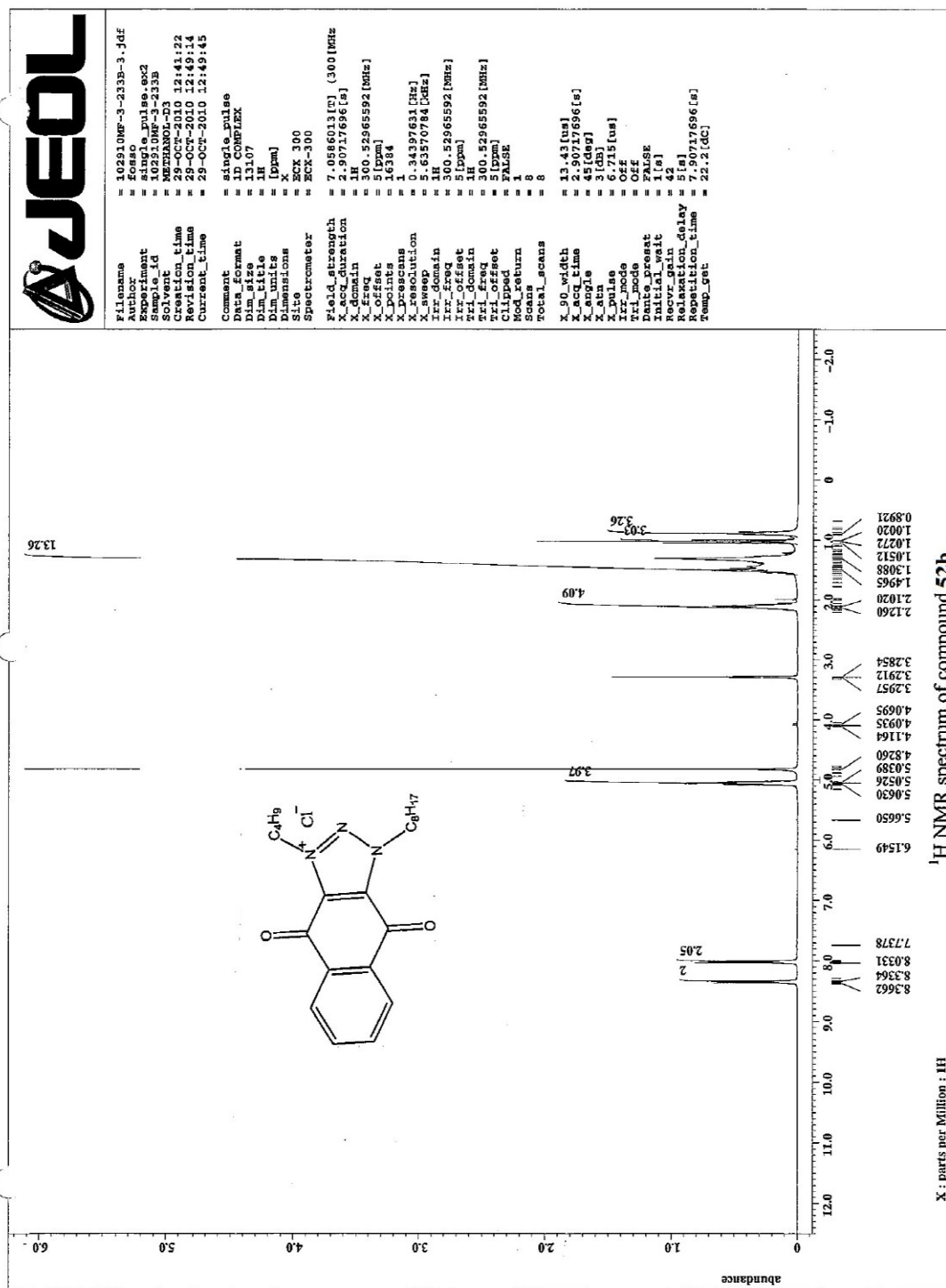
Current Data Parameters
 NAME MF-3-2113
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 500000
 Time 18.59
 INSTRUM mpx400
 PULPROG 9 ma Multiline1
 FULPROG zgpg30
 ID 32768
 SOLVENT MeOH
 NS 16074
 DS
 SWH 25000.000 Hz
 FIDRES 0.762939 Hz
 AQ 0.6554100 Sec
 RG 48500
 Dk 20.000 usec
 DE 27.14 usec
 TE 300.0 K
 D12 0.00000000 sec
 DL5 20.00 dB
 CFDPFG waltz16
 P31 0.40000001 sec
 P1 6.75 usec
 DE 27.14 usec
 SFO1 100.623179 MHz
 ACQLEN 130
 D11 0.03000000 sec

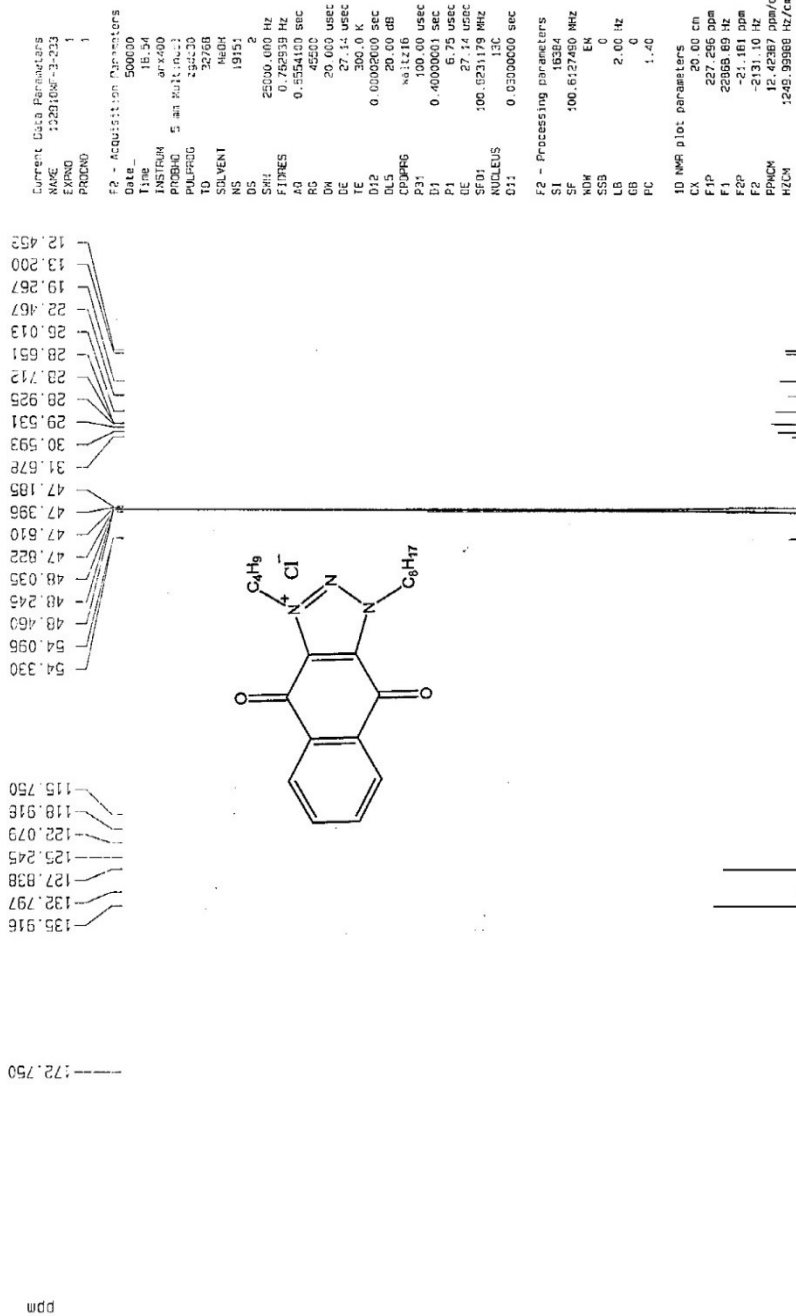
F2 - Processing parameters
 SI 63864
 SF 100.6127450 MHz
 WDW EM
 SSB 0
 LB 2.00 Hz
 GB 0
 PC 1.40

1D NMR 0101 parameters
 CX 20.00 cm
 F1P 227.256 ppm
 F1 22659.85 Hz
 F2P -21.181 ppm
 F2 -2131.11 Hz
 PRICK 12.42367 ppm/cm
 HZCM 1250.00012 Hz/cm





Standard 13C
Experiment



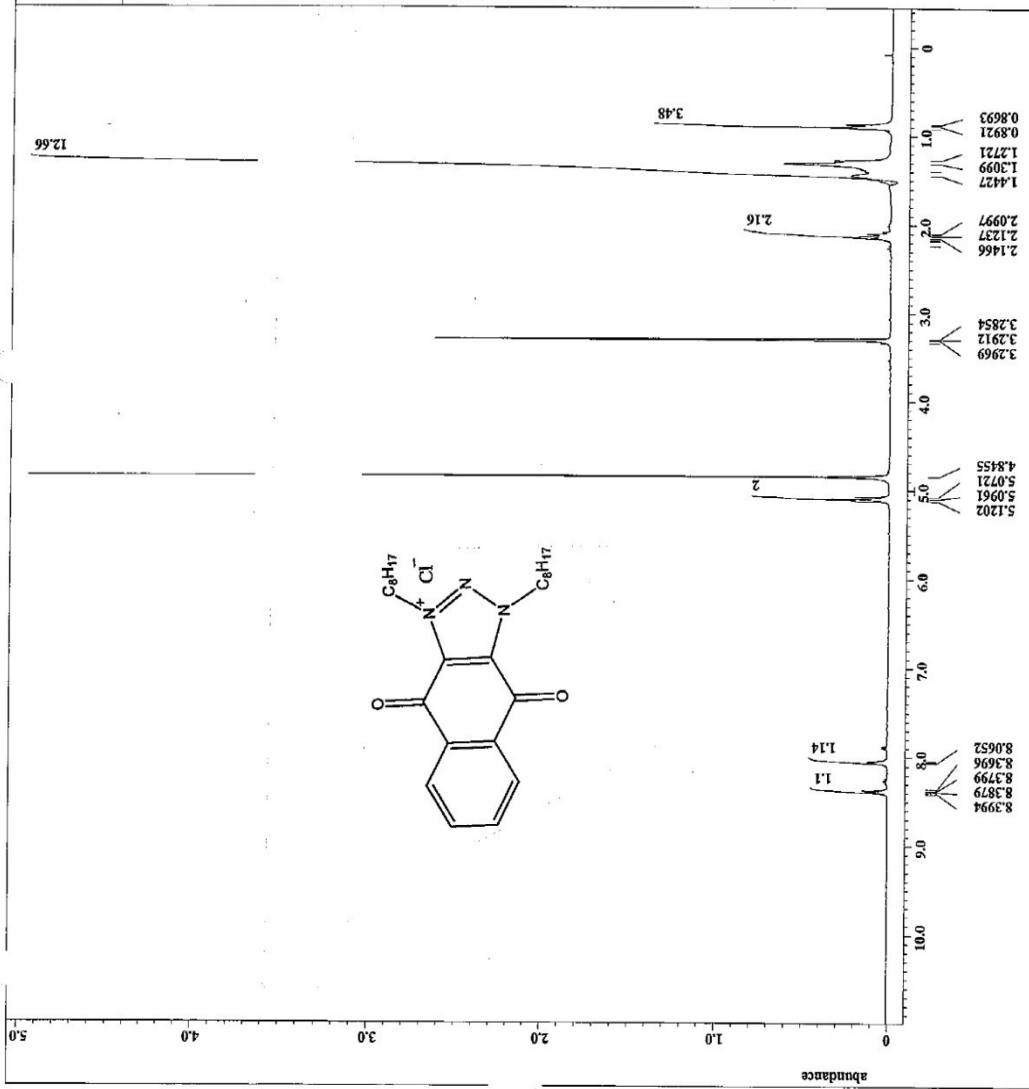


```

Filename = JJ-VI-83N2090(18)-4.
Author =
Experiment = single_pulse.es2
Sample_id = JJ-VI-83N2090(18)
Solvent = METHANOL-D3
Acquisition_time = 27-JUL-2010 11:56:24
Relaxation_time = 27-JUL-2010 11:59:15
Current_time = 27-JUL-2010 11:59:15

Comment = single_pulse
Data_format = ID_COMPLEX
Pulse_program = zgpg30
Dim_units = Hz
DimENSIONS = X
Site = ECK 300
Spectrometer = ECK-300

Field_strength = 7.0586013(T) (300 MHz)
X_acq_duration = 2.90717696[s]
X_domain = 1H
X_freq = 300.52965592 [MHz]
X_gain = 1.984
X_points = 1
X_prescans = 1
X_resolution = 0.34397631 [Hz]
X_sweep = 1
X_time_constant = 5.83570784 [Hz]
Xr1_freq = 300.52965592 [MHz]
Xr1_offset = 1H
Xr1_domain = 1H
Xr1_freq = 300.52965592 [MHz]
Xr1_offset = 1ppm
Xr1_domain = 1
Phase =
Mod_return = 8
Total_scans = 8
X_90_width = 13.43 [us]
X_acq_time = 2.90717696[s]
X_angle = 45 [deg]
X_atn = 3 [dB]
X_pulse = 6.715 [us]
Xr1_mode = Off
Xr2_mode = Off
Dante_preset = FALSE
Initial_wait = 1 [fs]
Recv_gain = 48
Relaxation_delay = 1 [s]
Relaxation_time = 3.0717696[s]
Temp_get = 23.5 [C]
    
```



¹H NMR spectrum of compound 52e

X : parts per Million : 1H

Standard ¹³C
Experiment

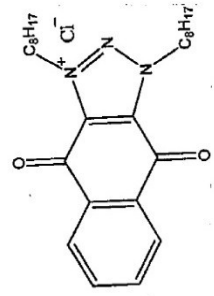
Current Data Parameters
 NAME J1-VI-03X2091
 EXPNO 2091
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 500000
 Time 19:29
 INSTRUM brx400
 PROBHD 5 mm Huj1:nucl1
 PULPROG zgpg30
 TD 32768
 SOLVENT H₂O
 NS 14674
 DS 2
 SWH 25000.00 Hz
 FIDRES 0.762038 Hz
 AQ 0.6554100 sec
 RG 65500
 DM 20.000 usec
 DE 27.14 usec
 TE 300.0 K
 D12 0.0000200 sec
 DL5 20.00 dB
 CHPORG wal1z16
 P31 100.00 usec
 D1 0.40000001 sec
 P1 6.75 usec
 DE 27.14 usec
 SF01 100.6231179 MHz
 NUCLEUS ¹³C
 D11 0.03000000 sec

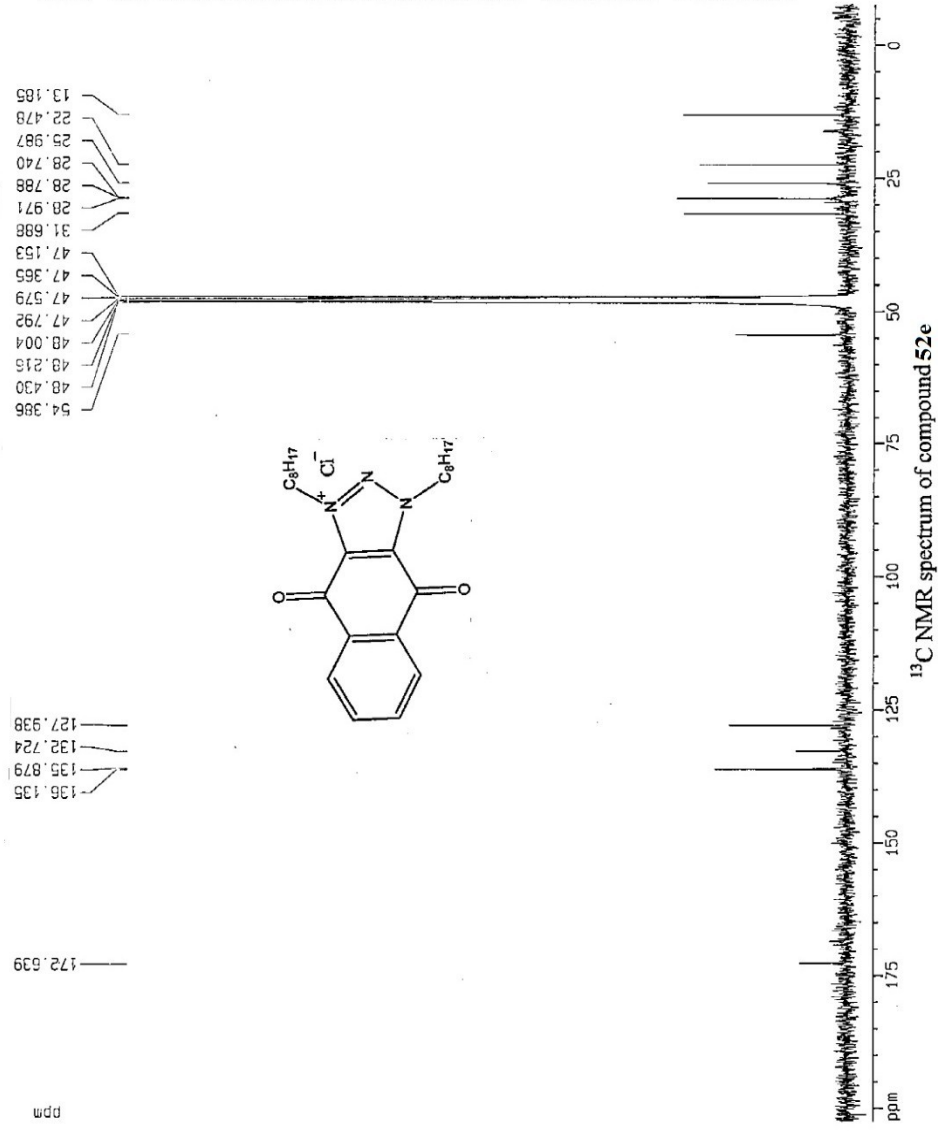
F2 - Processing parameters
 SI 16384
 SF 100.6127490 MHz
 GN 4
 SSB 0
 LB 2.00 Hz
 GB 0
 PC 1.40

ID NMR plot parameters
 CX 20.00 ca
 F1P 202.384 ppm
 F1 20363.45 Hz
 F2P -7.678 ppm
 F2 -772.51 Hz
 PPKM 10.50362 ppm/cm
 HZCM 1056.79773 Hz/cm

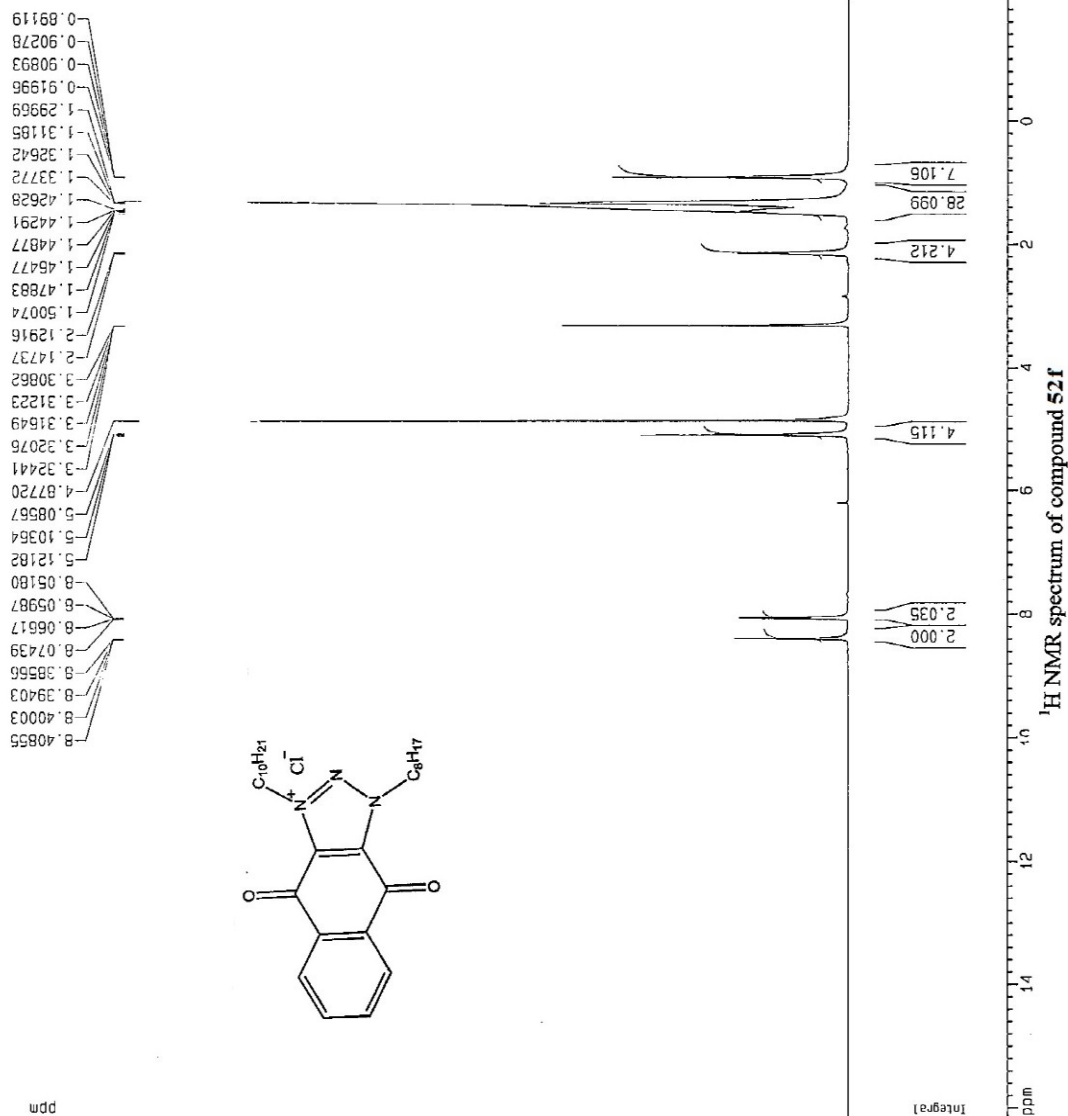
54.430
48.430
48.215
48.004
47.792
47.579
47.365
47.153
31.698
28.971
28.788
28.740
25.987
22.478
13.185



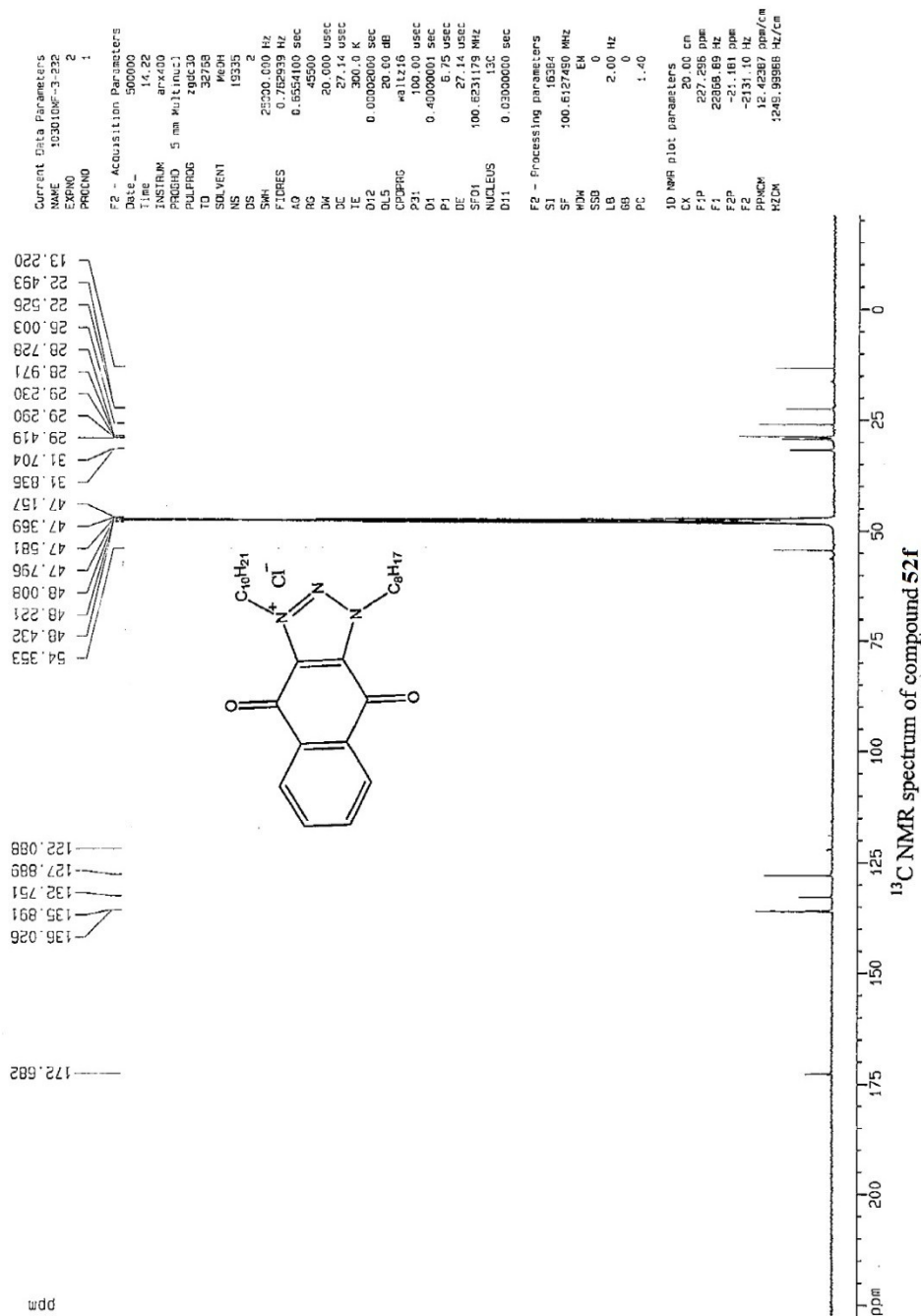
172.639
136.135
135.879
132.724
127.938



Standard Proton Experiment



Standard ¹³C
Experiment



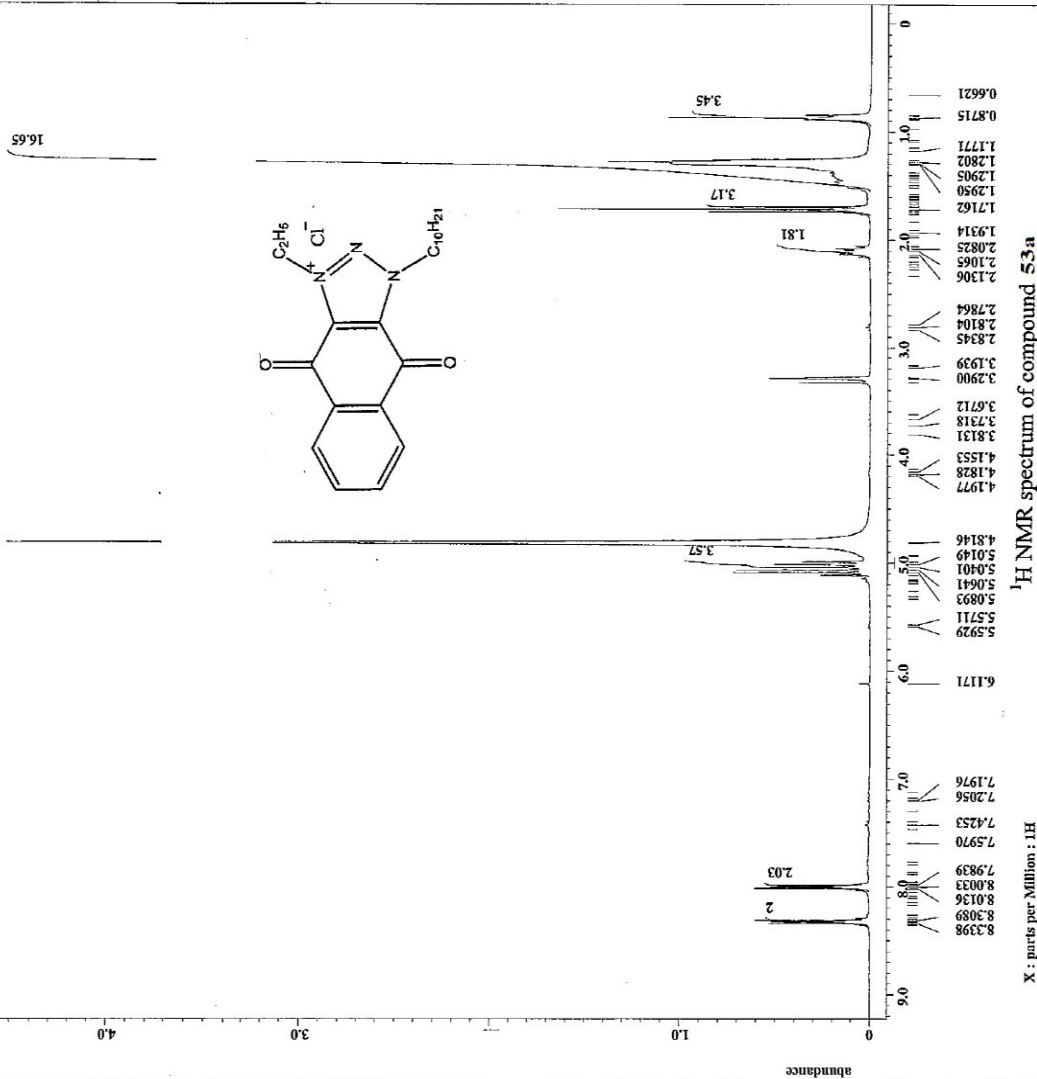


```

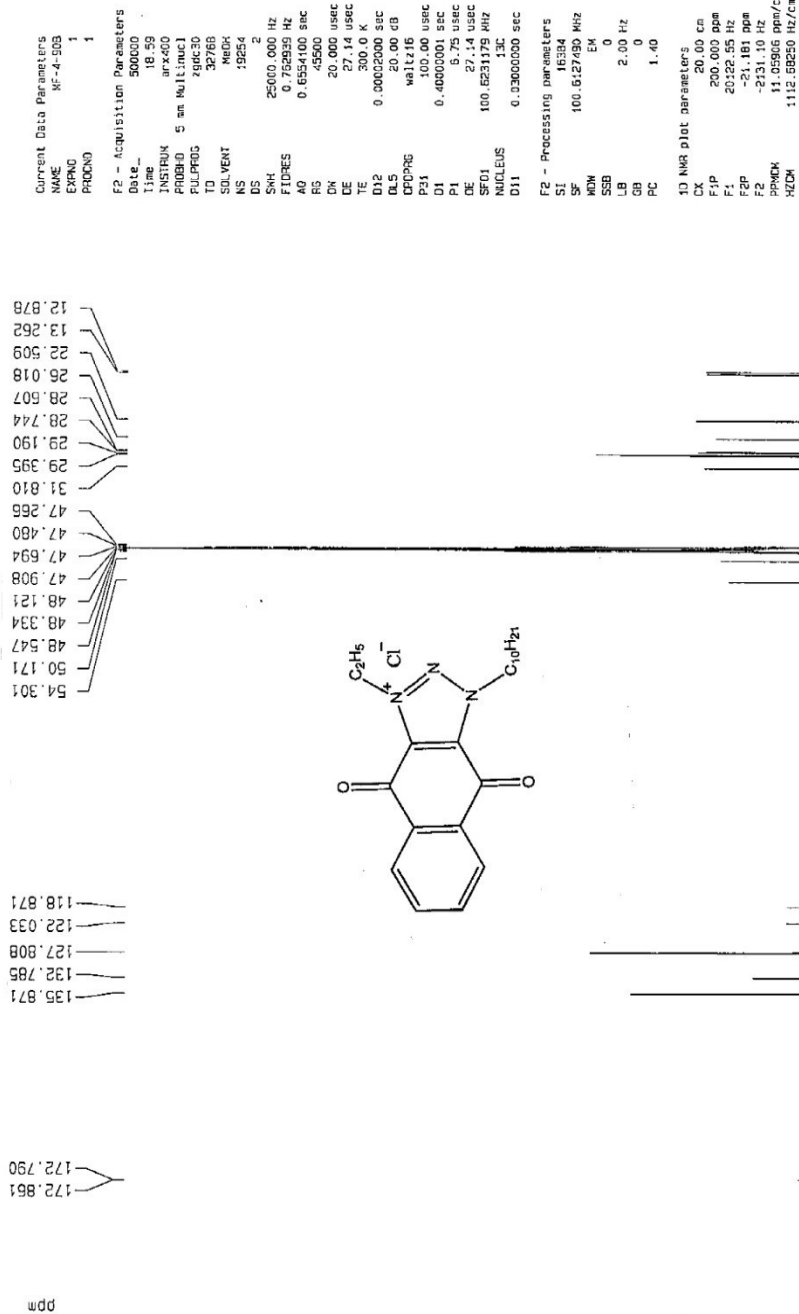
= 05201MF-4-90BEfil1-3.
Filename = 05201MF-4-90BEfil1-3.
Title = single_pulse.exe2
Experiment = 05201MF-4-90BEfil1
Sample_id = METHANOL-D3
Solvent = METHANOL-D3
Creation_time = 20-MAY-2011 12:59:15
Acquisition_time = 20-MAY-2011 13:00:54
Current_time = 20-MAY-2011 13:00:54

Comment = single_pulse
Data_format = 1D COMPLEX
Name = 3.07
Dir = 3.07
Dim_title = [ppm]
Dim_units = X
Dimensions = X
Site = ECK 300
Spectrometer = ECK-300

Field_strength = 7.0586013[T] (300[MHZ]
X_acq_duration = 2.90717696[s]
X_domain = 1H
X_freq = 300.52965592[MHZ]
X_gain = 1.6384
X_offset = 1.6384
X_resolution = 1
X_sweep = 0.34397631[Hz]
X_domain = 5.63570784[MHZ]
X_offset = 10.52965592[MHZ]
X_resolution = 1H
Irr_domain = 1H
Irr_offset = 300.52965592[MHZ]
Tri_domain = 1H
Tri_freq = 300.52965592[MHZ]
Tri_offset = 5[ppm]
Mod_return = 1
Scans = 8
Total_scans = 8
X_90_width = 13.43[us]
X_acq_time = 2.90717696[s]
X_angle = 45[deg]
X_atn = 3[db]
X_pulse = 6.715[us]
Pulse_program = OFF
Pulse_program = PBASE
Dante_preset = PBASE
Initial_wait = 1[s]
Recev_gain = 34
Relaxation_delay = 5[s]
Acquisition_time = 2.90717696[s]
Temp_spc = 22.8[degC]
    
```



Standard ¹³C
Experiment



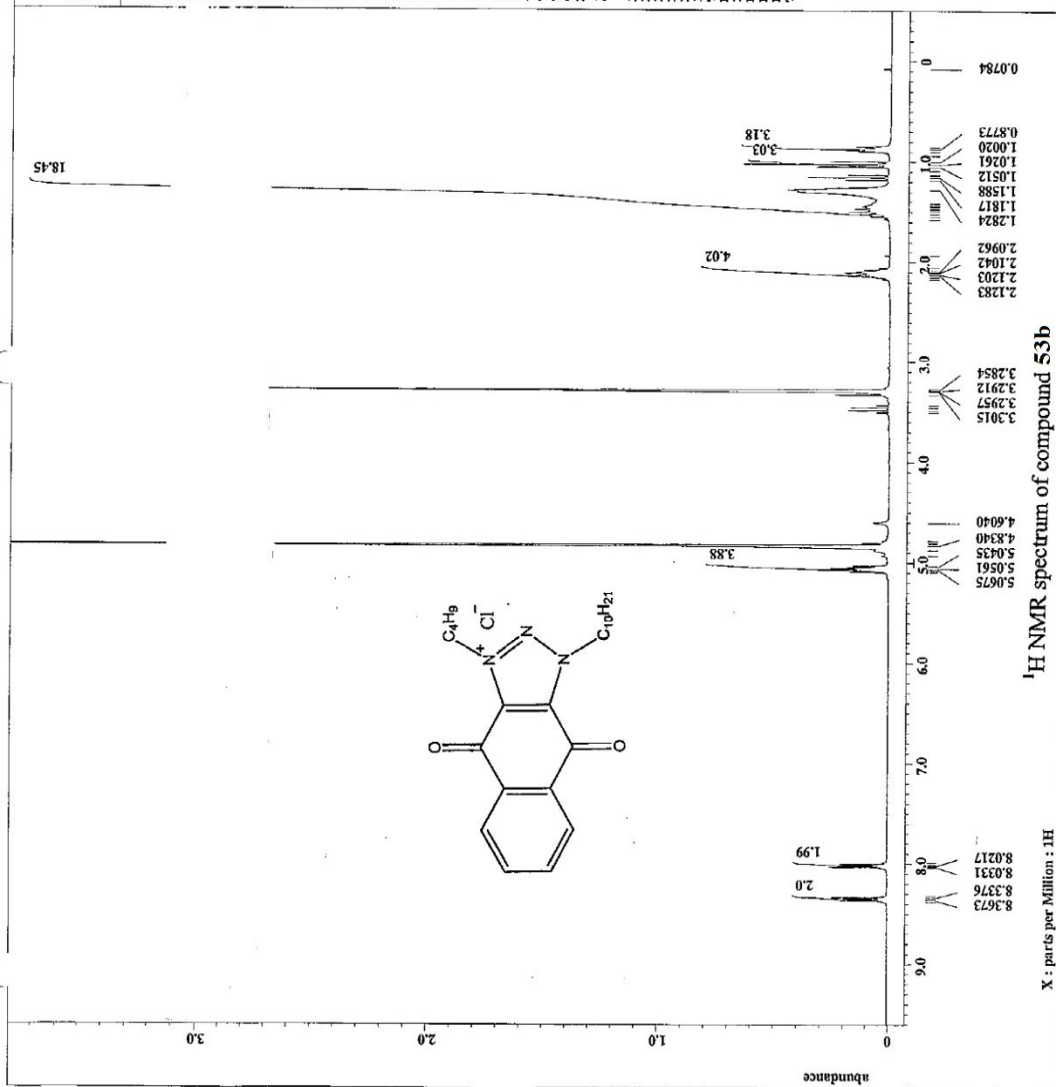


```

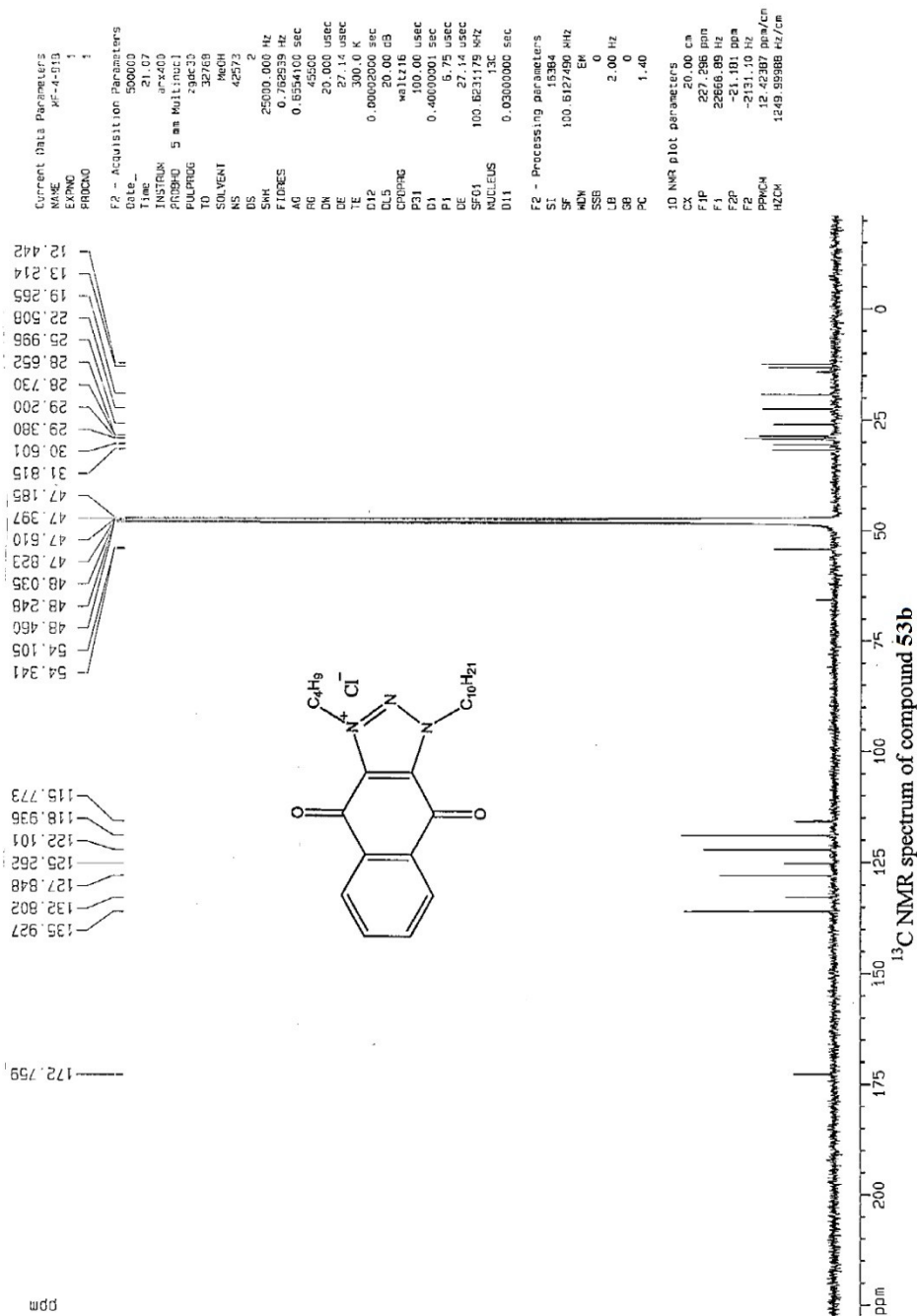
File Name      = 052011MF-4-91B-3_3JGf
Sample         = single_pulse.exe2
Experiment     = 052011MF-4-91B
Sample_ID      = METHANOL-D3
Solvent        = 20-MAY-2011 12:35:19
Revision_Time  = 20-MAY-2011 12:35:49
Current_Time   = 20-MAY-2011 12:37:05

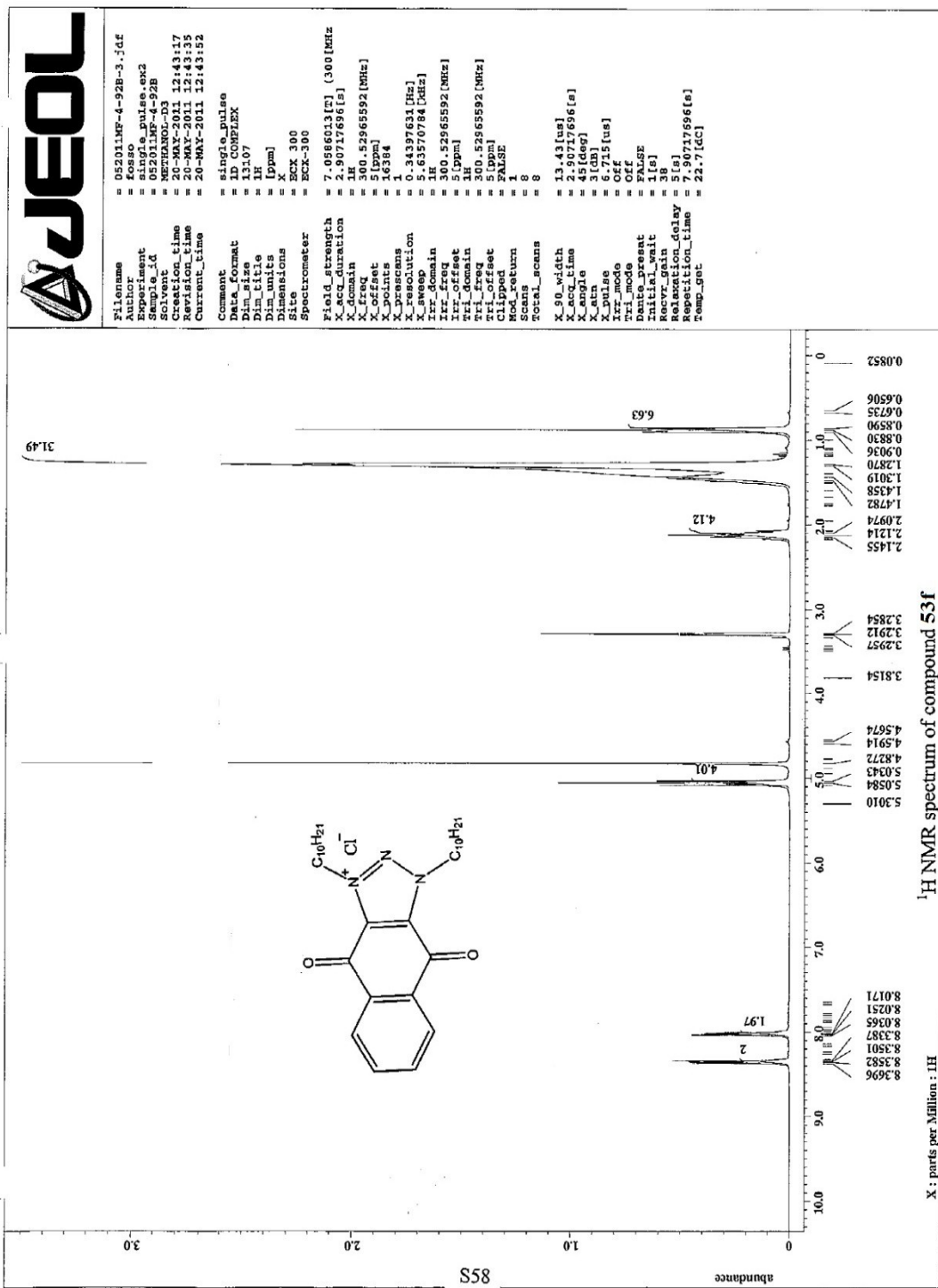
Comment       = single_pulse
Data_Format   = 1D COMPLEX
Dim Size      = 13107
F1 Dim Units  = [ppm]
F2 Dim Units  = X
Dimensions    = ECK 300
Site          = ECK-300
Spectrometer  = ECK-300

Field Strength = 7.0585013 [T] (300 [MHz]
X_eq_duration  = 2.90717596 [s]
X_domain      = 1H
X_freq        = 300.52965592 [MHz]
X_offset      = 5 [ppm]
X_procscan   = 1
X_resolution  = 0.34397631 [Hz]
X_sweep       = 5.63570784 [kHz]
Irr_domain    = 1H
Irr_offset    = 5 [ppm]
Irr_domain    = 1H
Tri_domain    = 300.52965592 [MHz]
Tri_offset    = 5 [ppm]
Modulation    = 1 AUSE
Sweep         = 8
Total_scans   = 8
X_90_width    = 13.43 [us]
X_acquire_time = 45 [sec]
X_angle       = 45 [deg]
X_atn         = 31 [db]
X_pulse       = 6.715 [us]
Irr_mode      = OFF
Pulse_prog    = PMLSE
Dance_poseset = 1 [s]
Initial_wait  = 1 [s]
Recvr_gain    = 46
Relaxation_delay = 5 [s]
Repetition_time = 2.90717596 [s]
Temp_set      = 22.7 [C]
  
```



Standard ¹³C
Experiment





F1: name = 052011MF-4-92B-3.jdf
 Expdate =
 Experiment = single_pulse.exe
 Sample_id = 052011MF-4-92B
 Solvent = METHANOL-D3
 Creation_time = 20-MAY-2011 12:43:17
 Acq_start_time = 20-MAY-2011 12:43:52
 Current_time = 20-MAY-2011 12:43:52
 Comment = single_pulse
 Data_format = 1D COMPLEX
 Dir_name = 110107
 Dir_size = 1H
 Dim_units = [ppm]
 Dimensions = X
 Site = ECK 300
 Spectrometer = ECK-300
 Field_strength = 7.0686013 [T] (300 [MHz]
 X_acq_duration = 2.90717696 [s]
 X_domain = 1H
 X_freq = 300.52965592 [MHz]
 X_offset = 1 [ppm]
 X_prescans = 1
 X_resolution = 0.34397631 [Hz]
 X_sweep = 5 [ppm]
 Irr_domain = H
 Irr_freq = 5.63570784 [MHz]
 Irr_offset = 30.52965592 [MHz]
 Irr_prescans = 9 [ppm]
 Tri_domain = 1H
 Tri_freq = 300.52965592 [MHz]
 Tri_offset = 5 [ppm]
 Mod_return = 1
 ALSE =
 Scans = 8
 Total_scans = 8
 X_90_width = 13.43 [us]
 X_acq_time = 2.90717696 [s]
 X_angle = 45 [deg]
 X_atn = 3 [dB]
 X_pulse = 6.715 [us]
 Irr_mode = Off
 Dantic_present = Off
 Recvr_gain = 1 [s]
 Relaxation_delay = 5 [s]
 Relaxation_time = 22.7 [AC]
 Temp_set = 22.7 [AC]

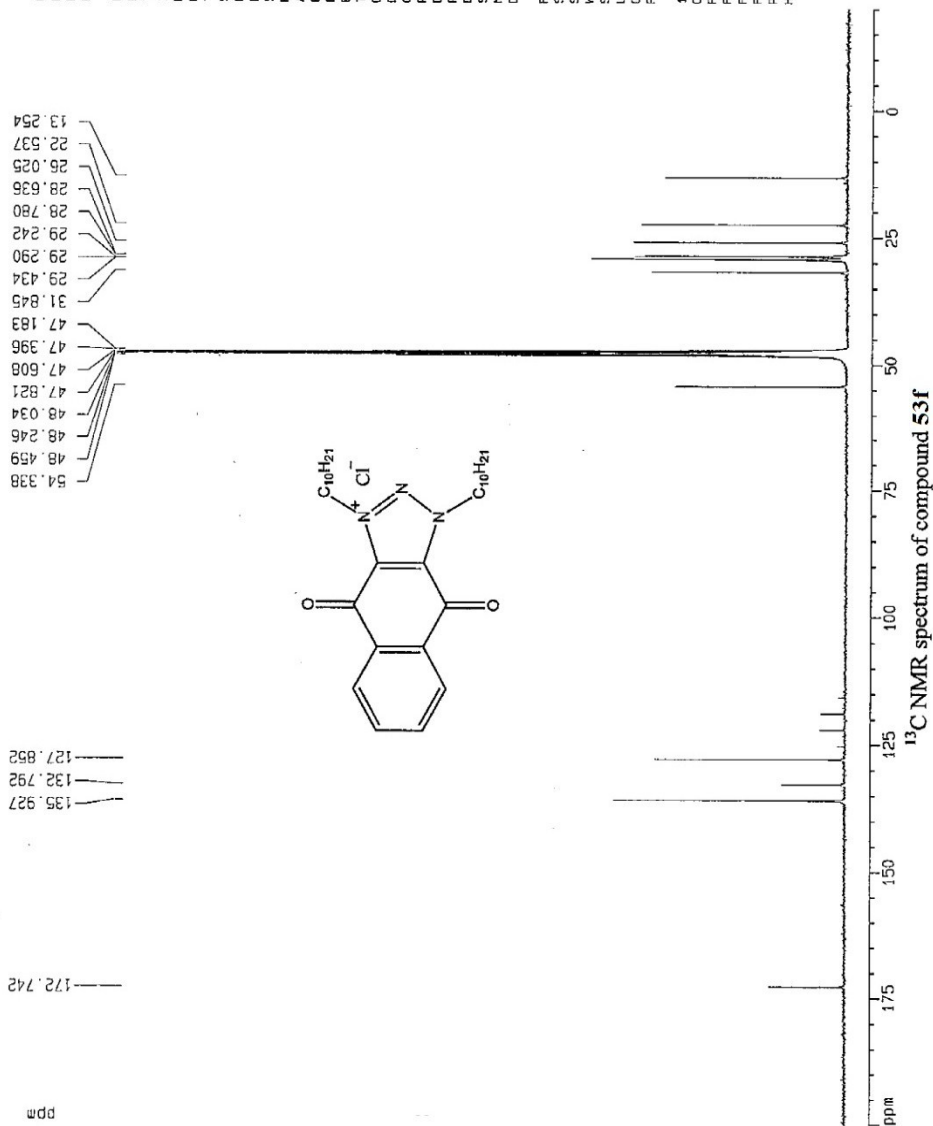
Standard ¹³C Experiment

Current Data Parameters
 NAME MF-4-523
 EXPNO 2
 PROCNO 1

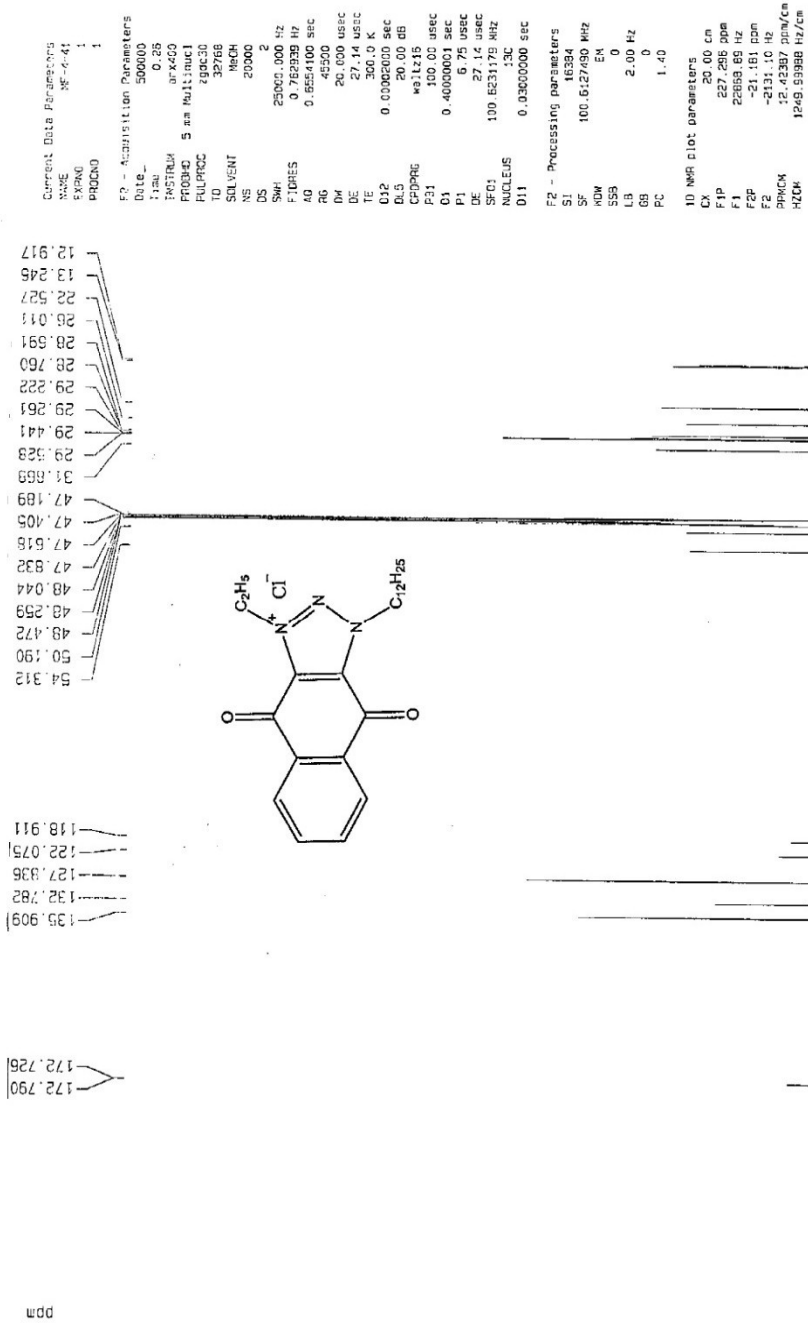
F2 - Acquisition Parameters
 Date_ 500000
 Time 21.32
 INSTRUM ark400
 PROBHD 5 mm Multinucl
 PULPROG zgpg30
 ID 32769
 SOLVENT H₂O
 NS 18645
 DS 2
 SWH 25000.000 Hz
 FIDRES 0.752930 Hz
 AQ 0.6554100 sec
 RG 45500
 DK 20.000 usec
 BE 27.14 usec
 TE 300.0 K
 O12 0.00002000 sec
 DL5 20.00 dB
 CPDPRG waltz16
 P31 0.40000001 sec
 P1 5.75 usec
 DE 27.14 usec
 SFO: 100.623179 MHz
 NUCLEUS ¹³C
 D11 0.03000000 sec

F2 - Processing parameters
 SI 16384
 SF 100.627490 MHz
 WDW EM
 SSB 0
 LB 2.00 Hz
 GB 0
 PC 1.40

1D NMR plot parameters
 CX 20.00 cm
 F1P 200.000 ppm
 F1 20122.55 Hz
 F2P -20.000 ppm
 F2 -2012.26 Hz
 PPMCH 11.00000 ppm/cm
 HZCM 1106.74023 Hz/cm



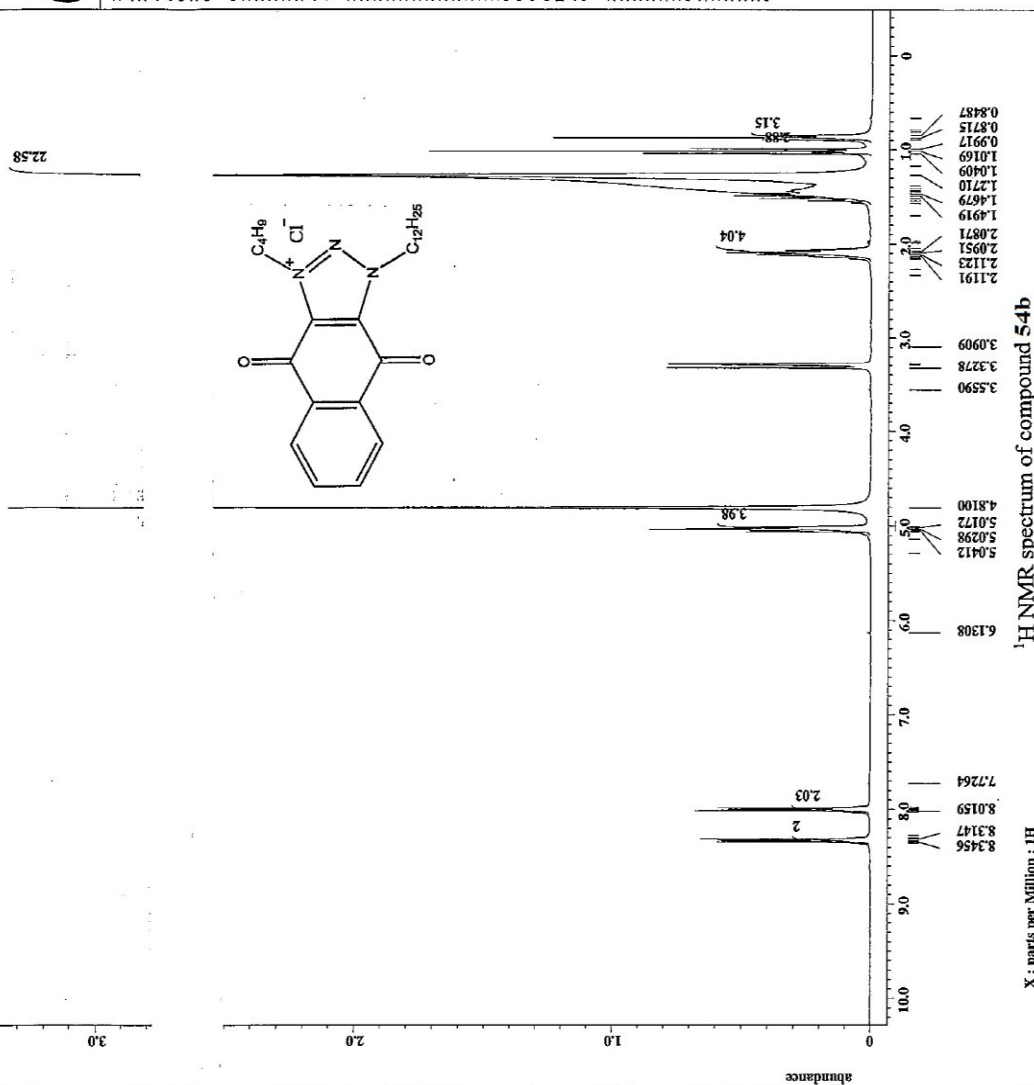
Standard ¹³C
Experiment





```

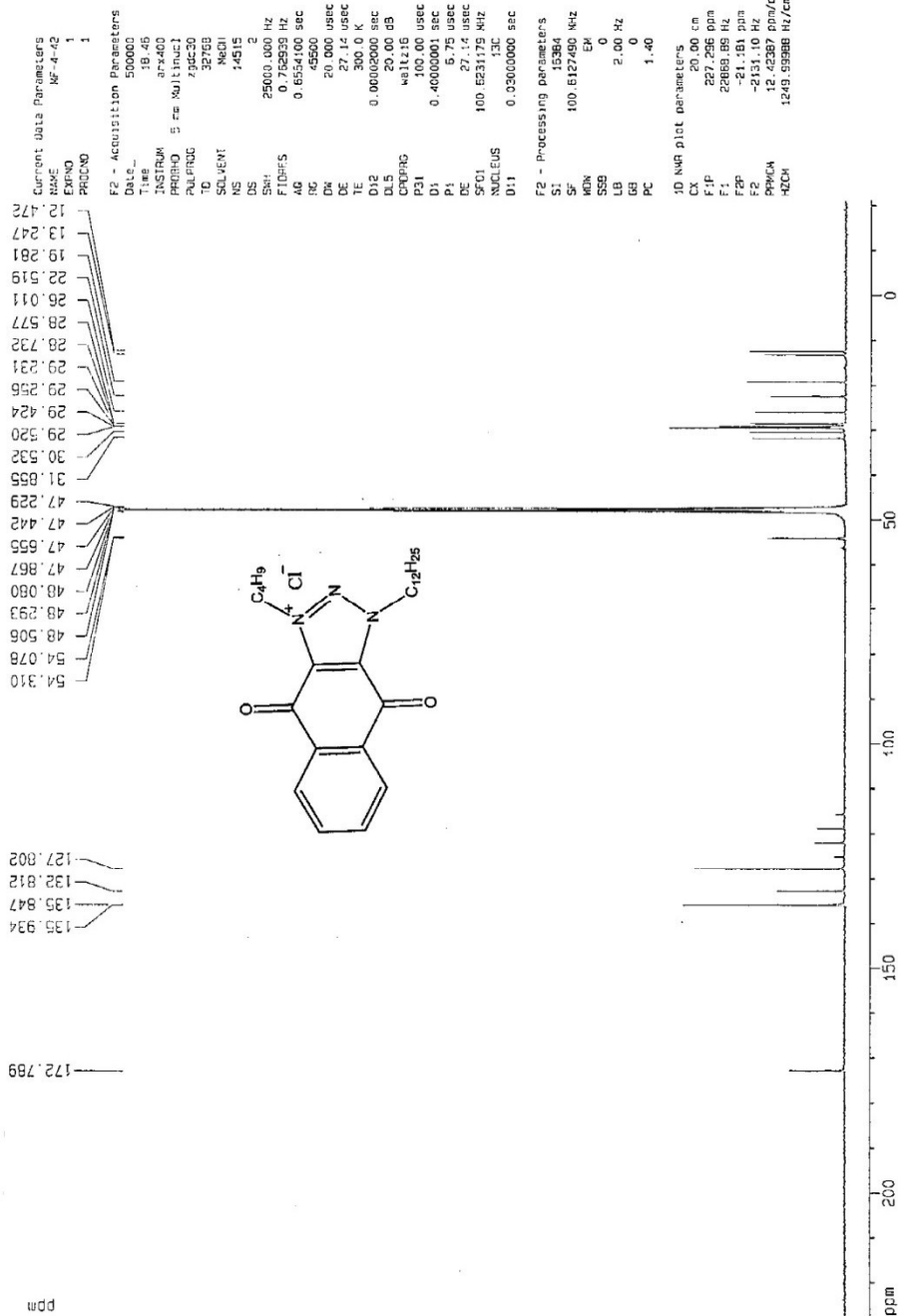
02101MR-4-42-3.jdc
=====
File      = 02101MR-4-42-3.jdc
Author    = zosac
Experiment = single_pulse.exr2
Sample_id = 02101MR-4-42
Date      = 10-FEB-2011 11:39:44
RunName   = 02101MR-4-42
Revision  = 10-FEB-2011 11:44:47
Current_time = 10-FEB-2011 11:45:05
=====
Comment   = single_pulse
Date_acq  = 10-FEB-2011
Dim_size  = 131x17
Dim_title =
Dim_units = [ppm]
Dimensions = X
Site      = EXR 300
Spectrometer = EXR-300
=====
Field_strength = 7.0866013[T] (300[MHz]
X_eq_duration = 2.9071696[s]
X_gain        = 1H
X_offset      = 500.52965592[MHz]
X_points      = 16384
X_rescans     = 1
X_resolution  = 0.34397631[Hz]
X_seg        = 1
X_seg_gain   = 1.63570784[MHz]
X_sweep      = 1
Xr_freq      = 300.52965592[MHz]
Xr_offset    = 5[ppm]
Xr_domain    = 1H
Xr_freq      = 300.52965592[MHz]
Xr_offset    = FALSE
Xr_clip      = FALSE
Mod_return   = 1
Scans        = 8
Total_scans  = 8
X_90_width  = 13.431[us]
X_eq_time    = 2.9071696[s]
X_angle     = 45[deg]
X_atn       = 3[db]
X_pulse     = 0.715[us]
Xr_mode     = Off
Xr_mode     = Off
Date_preset = FALSE
Initial_wait = 1[s]
Reovr_gain  = 3B
Reovr_delay = 7.9071696[s]
Repetition_time = 22.6[s]
Temp_get    =
    
```

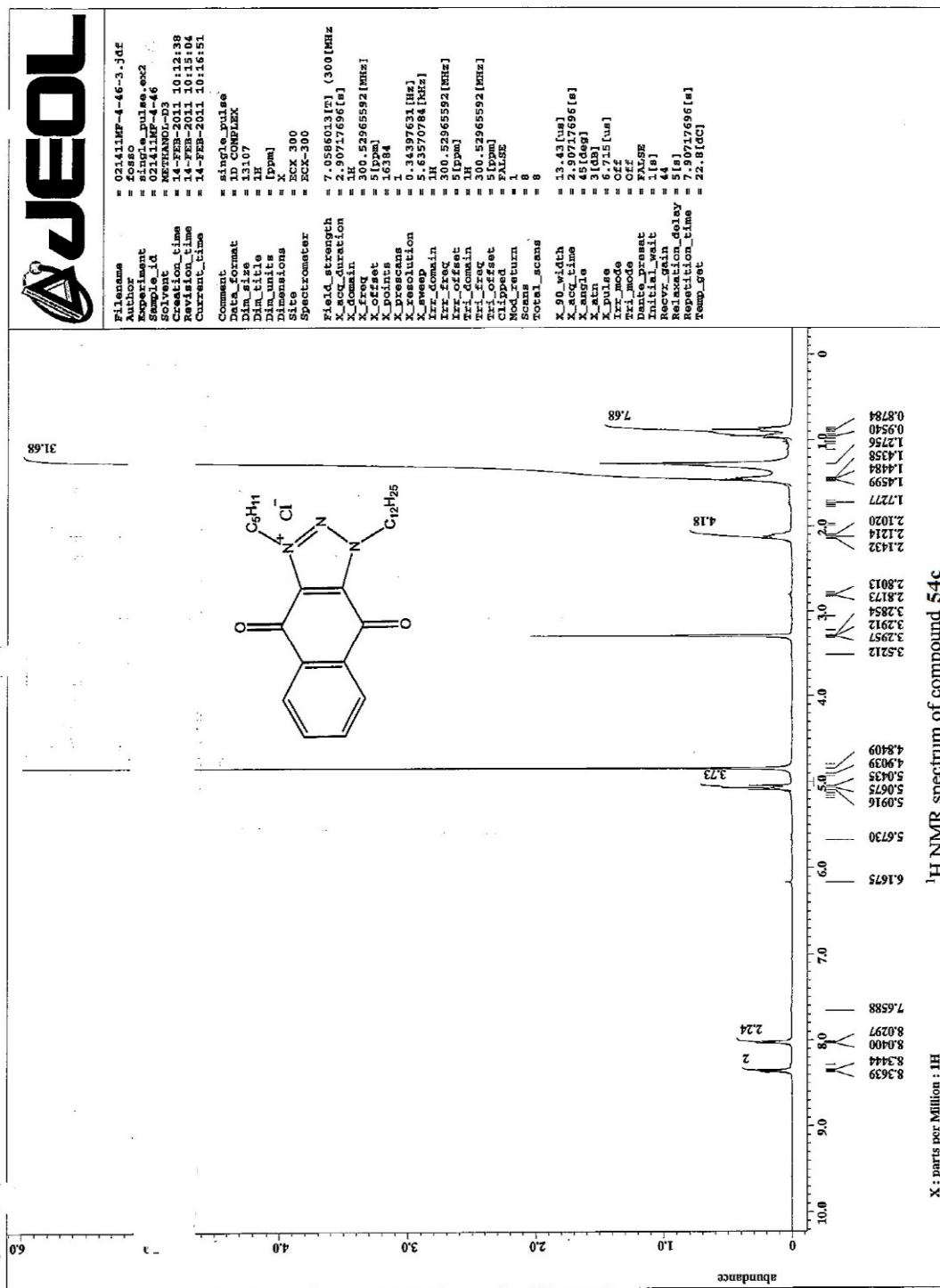


¹H NMR spectrum of compound 54b

X : parts per Million : 1H

Standard ¹³C
Experiment





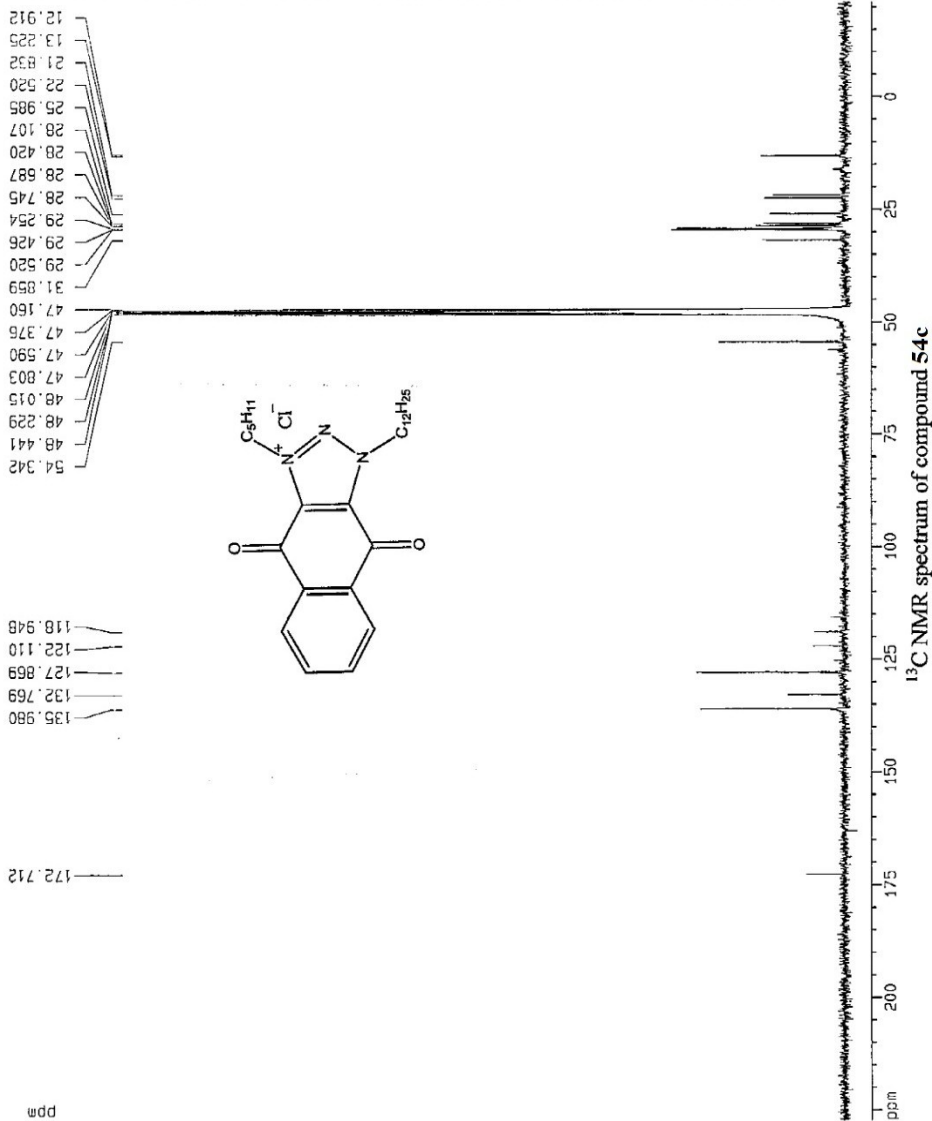
Standard ¹³C
Experiment

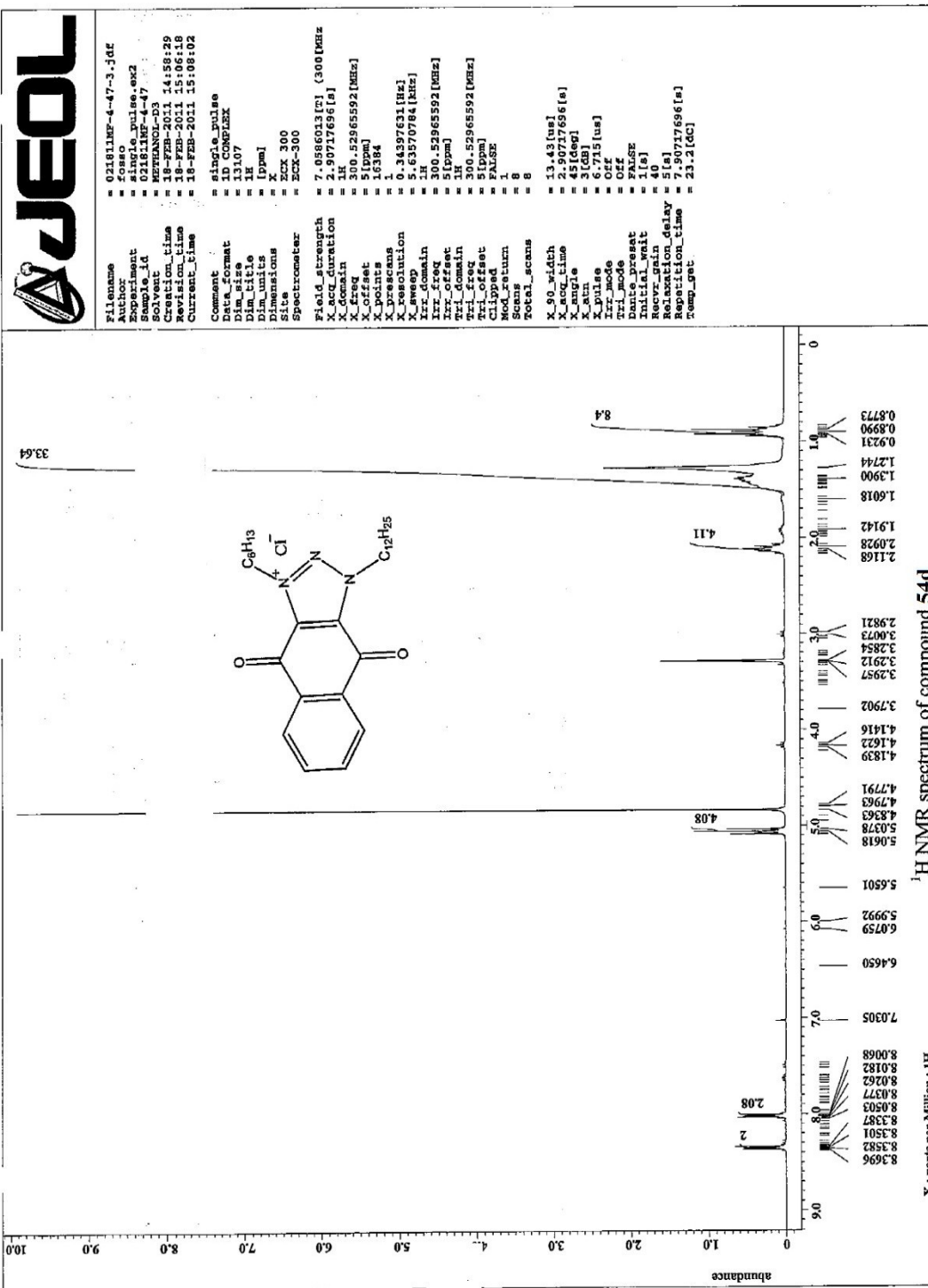
Current Data Parameters
NAME NF-4-46
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 500000
Time_ 15.30
INSTRUM brx400
PROBHD 5 mm Multic1
PULPROG zgpg30
TD 32768
SOLVENT MeOH
AS MeOH
DS 17882
OS 25000.00 Hz
F1 0.762039 Hz
AQ 0.0554100 sec
RG 485000
DM 20.000 usec
DE 27.14 usec
TE 300.0 K
D12 0.0000000 sec
DL5 20.00 dB
CPDPRG waltz15
P31 100.00 usec
D1 0.4000000 sec
P1 6.75 usec
DE 27.14 usec
SFO1 100.6231179 MHz
NUCLEUS ¹³C
D11 0.0300000 sec

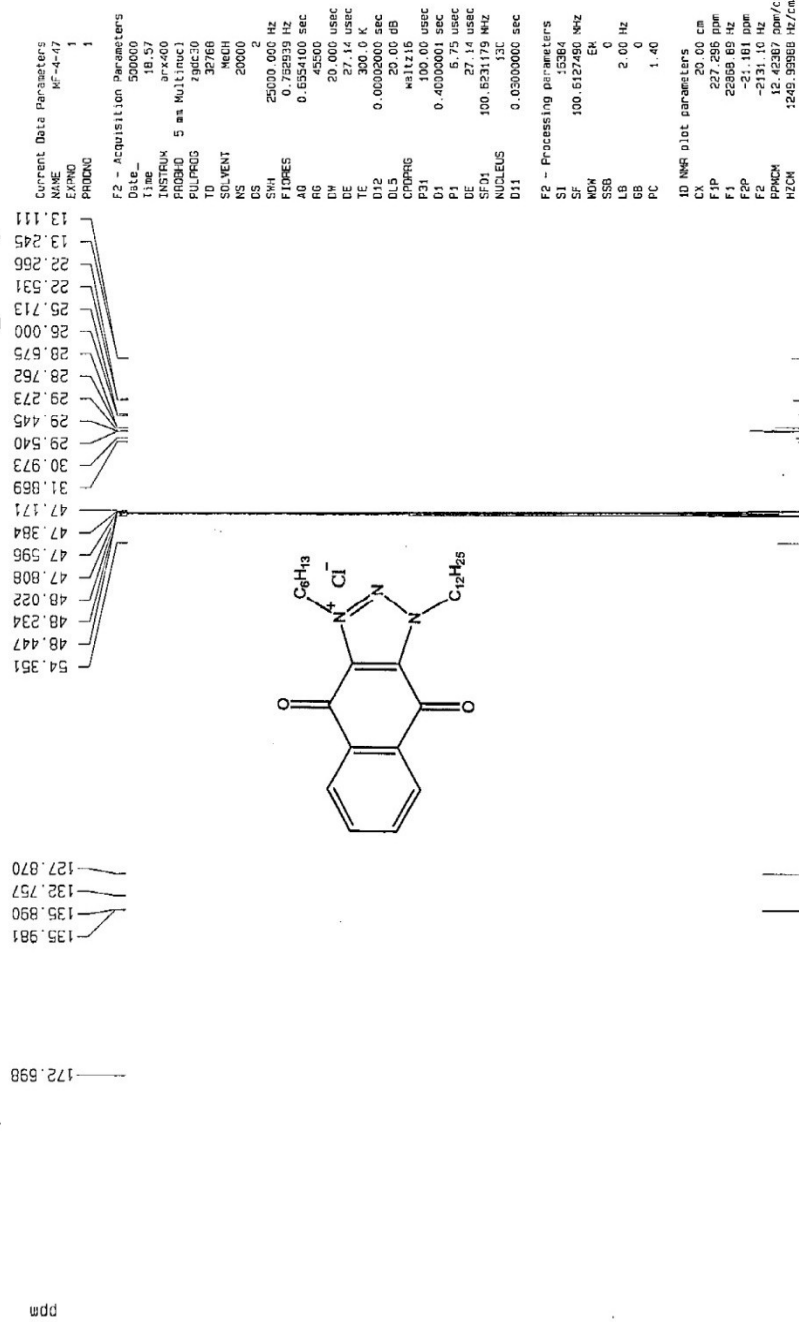
F2 - Processing parameters
SI 16334
SF 100.6127490 MHz
RG 64
SBS 4
GB 0
PC 1.40

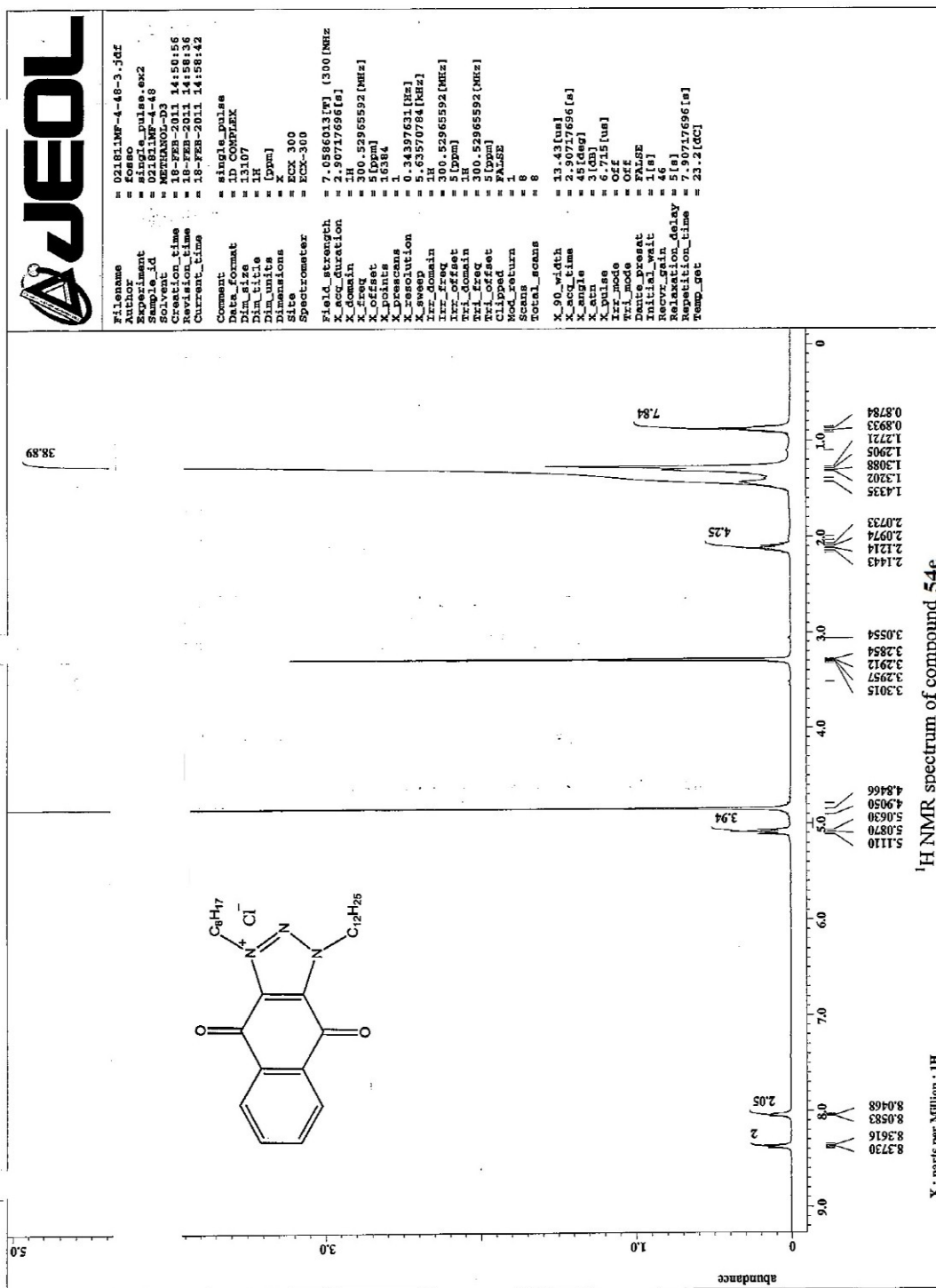
1D NMR plot parameters
CX 20.00 CF
F1P 227.295 00°
F1 22665.89 Hz
F2P -21.181 00°
F2 -2131.10 Hz
PPMCK 12.42357 ppm/CF
HZCK 1249.95988 Hz/CF





Standard ¹³C
Experiment





Standard 13C
Experiment

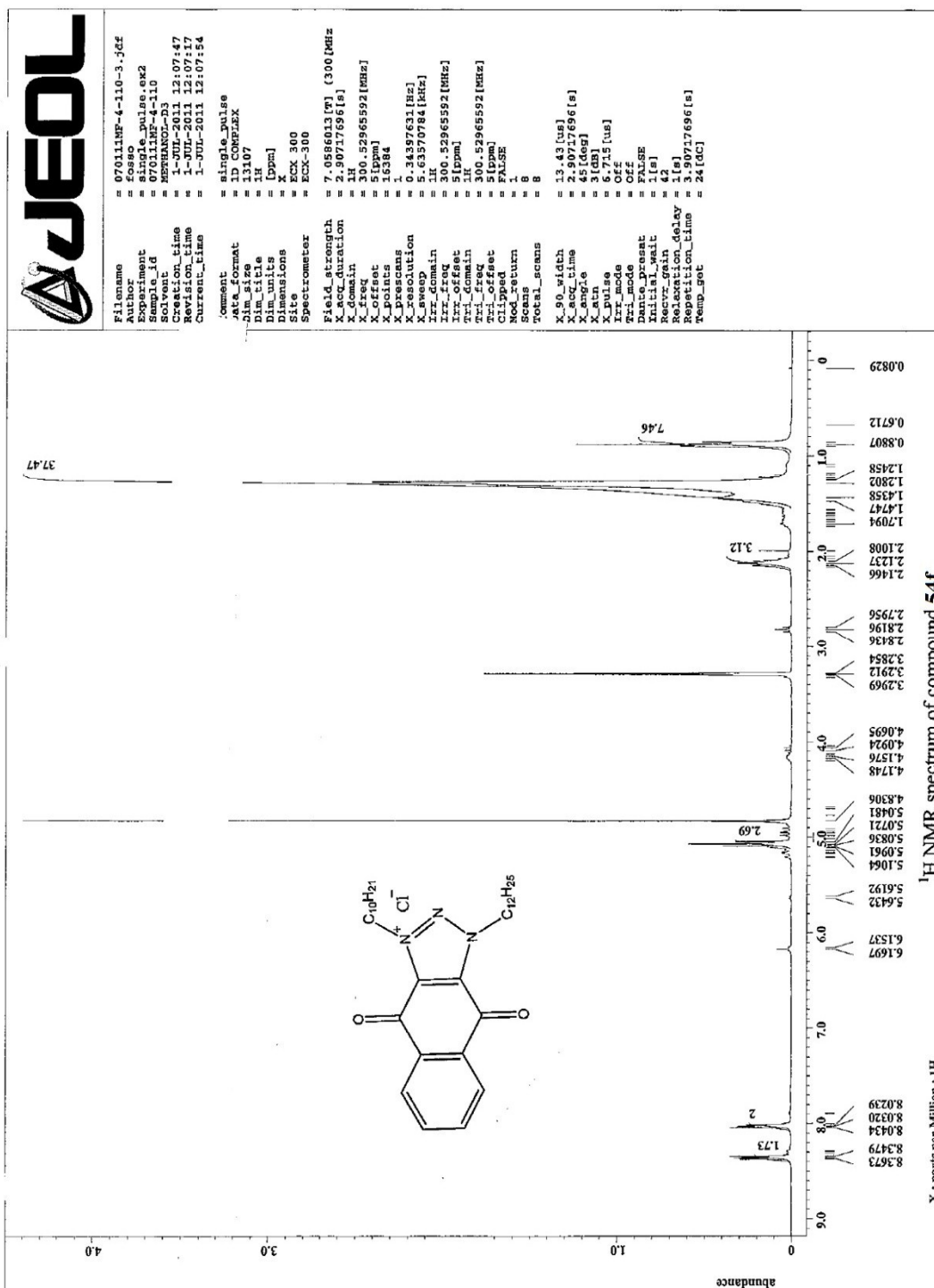


Current Data Parameters
 NAME MF-4-4B
 EXNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 5/20/00
 Time 19:11
 INSTRUM ark400
 PROBP0 5 mm Multic1
 PULPROG zgpg30
 TO 32768
 SOLVENT H2O
 NS 20000
 DS 2
 SWH 25000.000 Hz
 FIDRES 0.762328 Hz
 AQ 0.1654436 sec
 RG 45500
 DW 20.000 USEC
 DE 27.14 USEC
 TE 300.0 K
 D12 0.00002000 sec
 DLS 20.00 CB
 CPROG0 waltz16
 P31 100.00 USEC
 D1 0.00000001 sec
 P1 6.75 USEC
 DE 27.14 USEC
 SFO1 100.623175 MHz
 NUCLEUS 13C
 D11 0.03000000 sec

F2 - Processing parameters
 S1 16894
 SF 100.6127930 MHz
 WDW EM
 SSB 0
 LB 2.00 Hz
 GB 0
 PC 1.40

10 NMR plot parameters
 CX 20.00 cm
 F1P 207.596 ppm
 F1 28669.89 Hz
 F2P -21.181 ppm
 F2 -2131.10 Hz
 PPMCK 12.42397 ppm/cm
 HZCK 1249.69898 Hz/cm



```

File name      = 07011MF-4-110-3.jde
Subst name    = single_pulse.ok2
Experiment    = 07011MF-4-110
Sample ID     = MEXHANO-D3
Solvent       = 
Creation time = 1-JUL-2011 12:07:47
Date_ime     = 1-JUL-2011 12:07:47
Current_time  = 1-JUL-2011 12:07:54

Comment
Data format   = single_pulse
Dim           = ID COMPLEX
Dim 1         = 13
Dim 2         = 13
Dim 3         = 13
Dim units    = [ppm]
Dimensions    = X
Site         = ECX 300
Spectrometer = ECX-300

Field strength = 7.0586013 [T] (300 MHz)
X_acq_duration = 2.90717696[s]
X_domain       = 1H
X_freq         = 300.52965592[MHz]
X_gain         = 1.53594
X_offset       = 0.34397631[Hz]
X_resolution   = 5.63570784[kHz]
X_sweep        = 10.52965592[MHz]
X1_domain     = 1H
X1_freq        = 300.52965592[MHz]
X1_offset      = 51[ppm]
X1_resolution  = 1.7658
X2_domain     = 1H
X2_freq        = 300.52965592[MHz]
X2_offset      = 51[ppm]
X2_resolution  = 1.7658
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X3_freq        = 300.52965592[MHz]
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X4_freq        = 300.52965592[MHz]
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X5_freq        = 300.52965592[MHz]
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X12_freq       = 300.52965592[MHz]
X12_offset    = 51[ppm]
X12_resolution = 1.7658
X13_domain    = 1H
X13_freq       = 300.52965592[MHz]
X13_offset    = 51[ppm]
X13_resolution = 1.7658
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X_p0_width    = 13.43 [us]
X_acq_time    = 2.90717696 [s]
X_angle       = 45 [deg]
X_atn         = 3 [dB]
X_pulse       = 6.715 [us]
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X2_mode       = Off
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X98_mode      = Off
X99_mode      = Off
X100_mode     = Off
Recovery_gain = 42
Recovery_wait = 1[s]
Recovery_delay = 1[s]
Recovery_time = 1[s]
Relaxation_time = 24 [sec]
Temp_set      = 24 [degC]
    
```

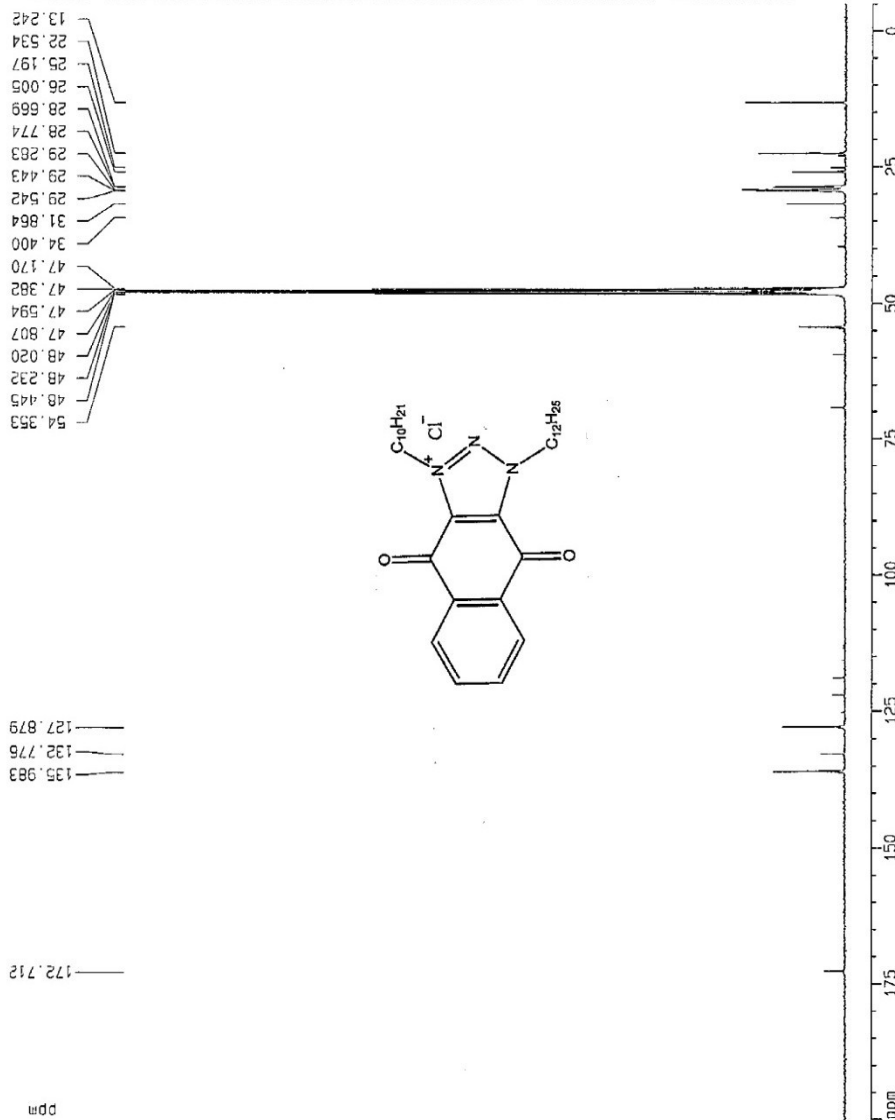
Standard ¹³C
Experiment

Current Data Parameters
 NAME MF-4-110
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 500000
 Time 15.21
 INSTRUM brx400
 PROBHD 5 mm Multinuc1
 PULPROG zgpg30
 TD 32768
 SOLVENT MeOH
 NS 20000
 DS 4
 SWH 25500.015 Hz
 FWHM 0.762038 Hz
 FIDRES 0.6556100 sec
 AQ 4.65500
 RG 20.000 usec
 DE 27.14 usec
 TE 300.0 K
 D12 0.00002000 sec
 DL5 20.00 dB
 CPDPRG waltz15
 P31 100.00 usec
 D1 0.40000001 sec
 P1 6.75 usec
 DE 27.14 usec
 SFO1 100.6231179 MHz
 NUCLEUS ¹³C
 D11 0.03000000 sec

F2 - Processing parameters
 SI 16384
 SF 100.6127490 MHz
 EQ
 SSB 0
 LB 2.00 Hz
 GB 0
 PC 1.40

1D NMR plot parameters
 CX 20.00 cm
 F1P 200.000 ppm
 F1 20122.55 Hz
 F2P -5.000 ppm
 F2 -503.07 Hz
 PPMCH 10.25000 ppm/cm
 HZCH 1031.26076 Hz/cm



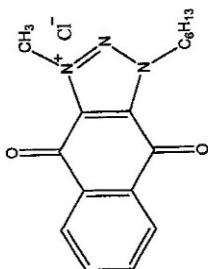
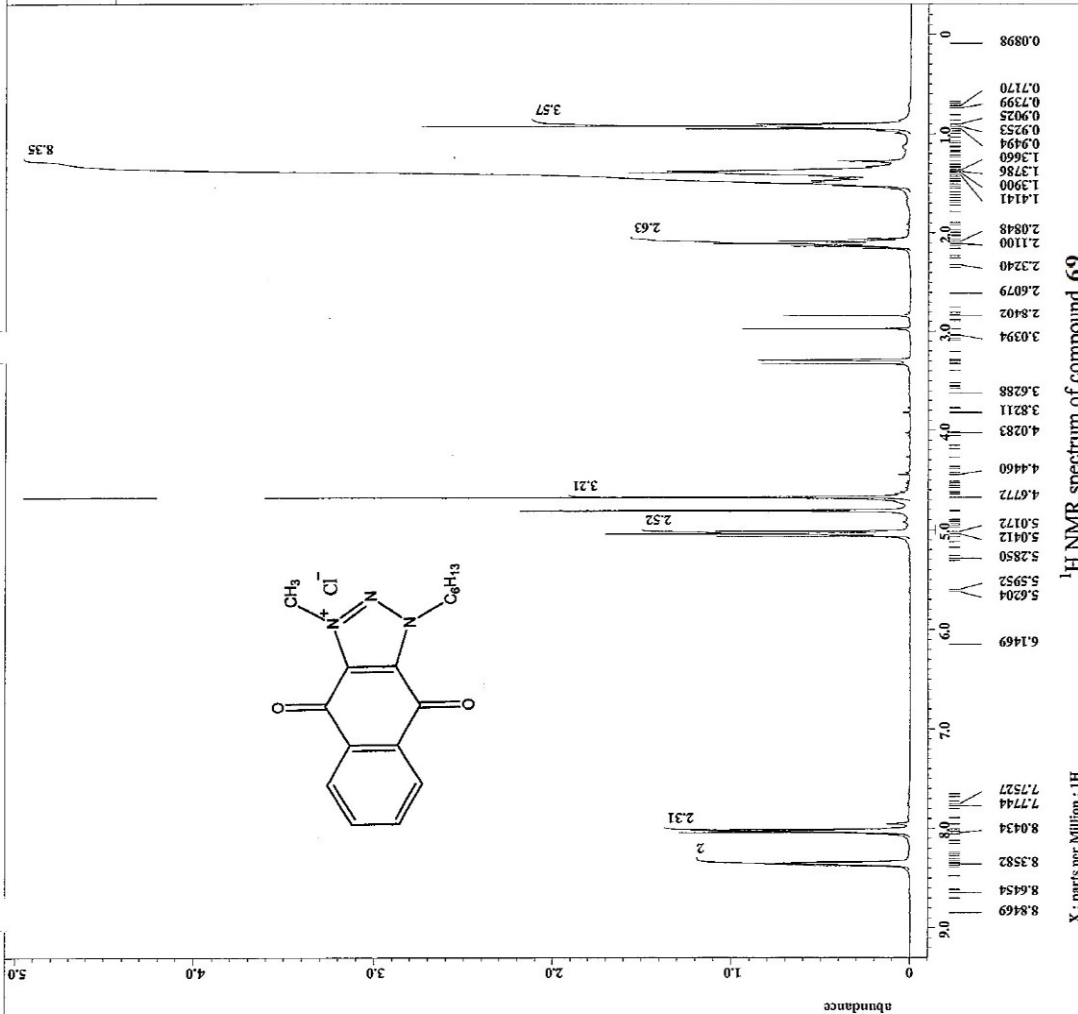


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Solvent = METHANOL-D3
Creation_time = 13-JUL-2011 08:55:49
Revision_time = 27-NOV-2011 18:41:20
Current_time = 27-NOV-2011 18:41:23

Comment = single_pulse
Data_format = ID COMPLEX
Data_size = 13107
Data_title = 1H
Data_units = [ppm]
Dimensions =
Site = ECUX 300
Spectrometer = ECUX-300

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X_acq_duration = 2.90717696 [s]
X_freq = 300.52965592 [MHz]
X_offset = 51 [ppm]
X_points = 16384
X_resolution = 0.34397631 [Hz]
X_sweep_rate = 18.6576784 [kHz]
Xirx_domain =
Xirx_freq = 300.52965592 [MHz]
Xirx_offset = 51 [ppm]
Xirx_domain = 1H
Xirx_freq = 300.52965592 [MHz]
Xirx_offset = 51 [ppm]
Mod_return = 1
Scans = 8
Total_scans = 8
X_90_width = 13.43 [us]
X_acq_time = 2.90717696 [s]
X_angle = 90 [deg]
X_atn = 3 [dB]
X_pulse = 13.43 [us]
Xirx_mode = off
Xirx_offset = 51 [ppm]
Data_presat = NONE
Initial_wait = 1 [s]
Recvz_gain = 40
Relaxation_delay = 1 [s]
Repetition_time = 3.90717696 [s]
Temp_set = 23.2 [dC]
    
```



Standard 13C
Experiment

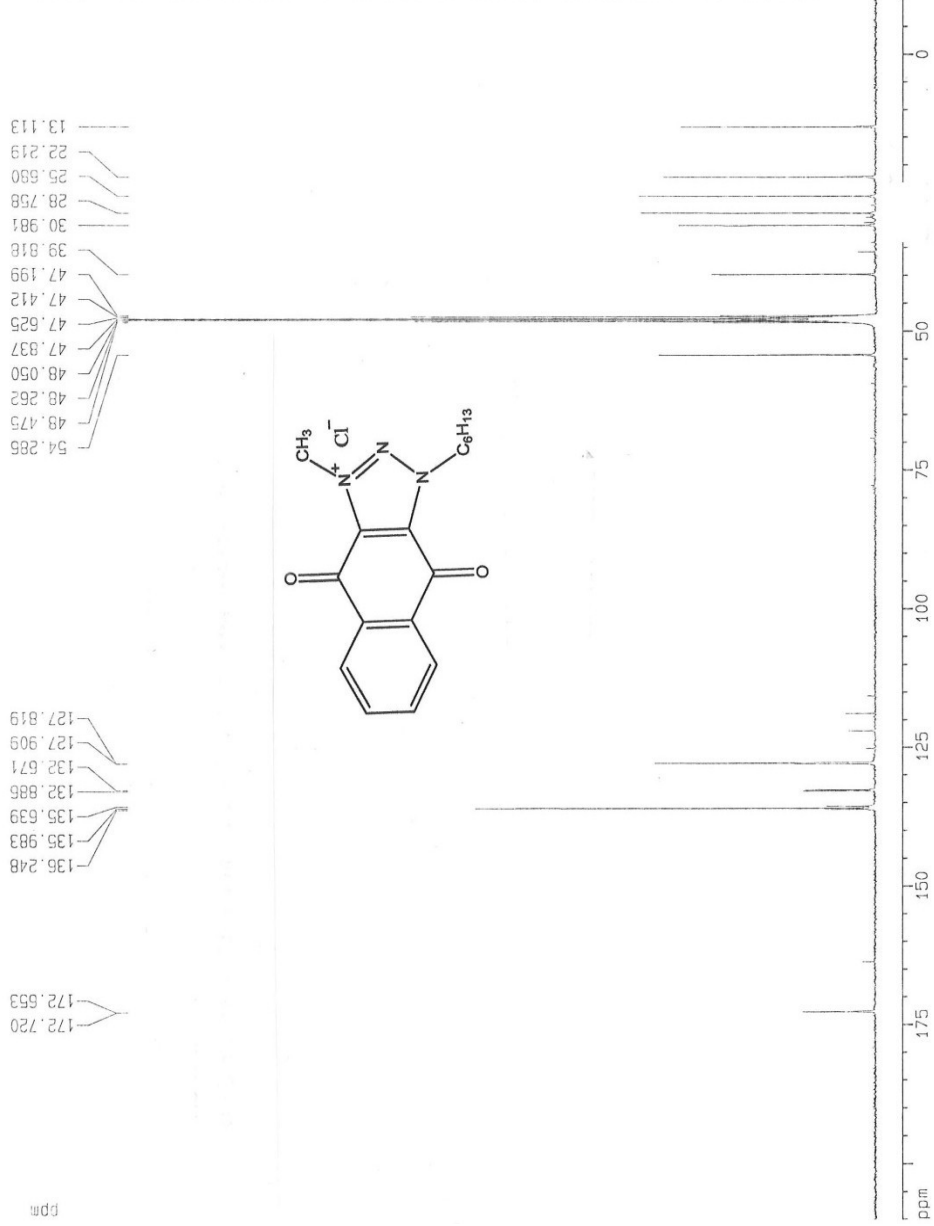
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Current Data Parameters
NAME MF-4-121
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 500000
Time 19.44
INSTRUM ark400
PROBHD 5 mm Multinucl
PULPROG zgpg30
TD 32768
SOLVENT MeOH
NS 19904
DS 2
SWH 25000.000 Hz
FIDRES 0.762939 Hz
AQ 0.6554100 sec
RG 45500
DM 20.000 usec
DE 27.14 usec
TE 300.0 K
D12 0.00002000 sec
D15 20.00 dB
CPDPRG waltz16
P31 100.00 usec
D1 0.40000001 sec
P1 6.75 usec
DE 27.14 usec
SFO1 100.623179 MHz
NUCLEUS 13C
D11 0.03000000 sec

F2 - Processing parameters
SI 16384
SF 100.6127450 MHz
EM
WDW 0
SSB 0
LB 2.00 Hz
GB 0
PC 1.40

ID NMR plot parameters
CX 20.00 cm
F1P 210.000 ppm
F1 21128.68 Hz
F2P -10.000 ppm
F2 -1005.13 Hz
PNUC 11.00000 ppm/cm
HZCK 1105.74023 Hz/cm
    
```



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Utah State University
Department of Chemistry and Biochemistry
0300 Old Main Hill
Logan, UT 84321-0300

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Signed

Virginia B Mattis

Date

6/20/12

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June 20, 2012

Marina Fosso Yatchang
Utah State University
Department of Chemistry and Biochemistry
0300 Old Main Hill
Logan, UT 84321-0300

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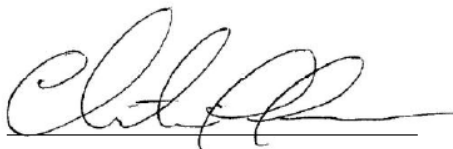
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Author: Cheng-Wei T Chang, Marina Fosso, Yukie Kawasaki, Sanjib Shrestha, Mekki F Bensaci, Jinhua Wang, Conrad K Evans, Jon Y Takemoto

Publication: The Journal of Antibiotics

Publisher: Nature Publishing Group

Date: Oct 6, 2010

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Marina Fosso Yatchang

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Chang, C.-W. T.; Fosso, M.; Kawasaki, Y.; Shrestha, S.; Bensaci, M. F.; Wang, J.; Evans, C. K.; Takemoto, J. Y. "Antibacterial to antifungal conversion of neamine aminoglycosides through alkyl modification. Strategy for reviving old drugs into agrofungicides" *J. Antibiot.* **2010**, *63*, 667-672.

Signed



Date

6/20/12

Permission Letter

June 20, 2012

Marina Fosso Yatchang
Utah State University
Department of Chemistry and Biochemistry
0300 Old Main Hill
Logan, UT 84321-0300

Dear Mr. Shrestha:

I am in the process of preparing my dissertation in the Department of Chemistry and Biochemistry at Utah State University. I hope to complete in the summer of 2012.

I am requesting your permission to include the following paper we co-authored in my doctoral dissertation:

Chang, C.-W. T.; Fosso, M.; Kawasaki, Y.; Shrestha, S.; Bensaci, M. F.; Wang, J.; Evans, C. K.; Takemoto, J. Y. "Antibacterial to antifungal conversion of neamine aminoglycosides through alkyl modification. Strategy for reviving old drugs into agrofungicides" *J. Antibiot.* **2010**, *63*, 667-672.

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Thank you for your cooperation,

Marina Fosso Yatchang

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Signed



Date

06/20/12

Permission Letter

June 20, 2012

Marina Fosso Yatchang
Utah State University
Department of Chemistry and Biochemistry
0300 Old Main Hill
Logan, UT 84321-0300

Dear Dr. Bensaci:

I am in the process of preparing my dissertation in the Department of Chemistry and Biochemistry at Utah State University. I hope to complete in the summer of 2012.

I am requesting your permission to include the following paper we co-authored in my doctoral dissertation:

Chang, C.-W. T.; Fosso, M.; Kawasaki, Y.; Shrestha, S.; Bensaci, M. F.; Wang, J.; Evans, C. K.; Takemoto, J. Y. "Antibacterial to antifungal conversion of neamine aminoglycosides through alkyl modification. Strategy for reviving old drugs into agrofungicides" *J. Antibiot.* **2010**, *63*, 667-672.

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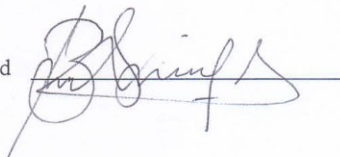
Thank you for your cooperation,

Marina Fosso Yatchang

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Signed



Date

6/21/2012

Permission Letter

June 20, 2012

Marina Fosso Yatchang
Utah State University
Department of Chemistry and Biochemistry
0300 Old Main Hill
Logan, UT 84321-0300

Dear Dr. Wang:

I am in the process of preparing my dissertation in the Department of Chemistry and Biochemistry at Utah State University. I hope to complete in the summer of 2012.

I am requesting your permission to include the following paper we co-authored in my doctoral dissertation:

Chang, C.-W. T.; Fosso, M.; Kawasaki, Y.; Shrestha, S.; Bensaci, M. F.; Wang, J.; Evans, C. K.; Takemoto, J. Y. "Antibacterial to antifungal conversion of neamine aminoglycosides through alkyl modification. Strategy for reviving old drugs into agrofungicides" *J. Antibiot.* **2010**, *63*, 667-672.

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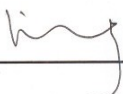
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Signed _____



Date _____

06/24/2012

Permission Letter

June 20, 2012

Marina Fosso Yatchang
Utah State University
Department of Chemistry and Biochemistry
0300 Old Main Hill
Logan, UT 84321-0300

Dear Dr. Evans:

I am in the process of preparing my dissertation in the Department of Chemistry and Biochemistry at Utah State University. I hope to complete in the summer of 2012.

I am requesting your permission to include the following paper we co-authored in my doctoral dissertation:

Chang, C.-W. T.; Fosso, M.; Kawasaki, Y.; Shrestha, S.; Bensaci, M. F.; Wang, J.; Evans, C. K.; Takemoto, J. Y. "Antibacterial to antifungal conversion of neamine aminoglycosides through alkyl modification. Strategy for reviving old drugs into agrofungicides" *J. Antibiot.* **2010**, *63*, 667-672.

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Marina Fosso Yatchang

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Signed C. Kent Evans

Date 6/23/2012



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Publication: ACS Combinatorial Science
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CURRICULUM VITAE

Marina FOSSO YATCHANG

Department of Chemistry and Biochemistry
Utah State University
0300 Old Main Hill
Logan, UT 84322-0300
marina.fosso@aggiemail.usu.edu
(435) 512-6778

CAREER OBJECTIVE

To obtain a research position in a competitive institution that will allow me to apply my extensive knowledge in multi-step synthesis of bioactive molecules. Research interests include: organic synthesis, drug discovery, library synthesis, and methodology development.

EDUCATION

Ph.D., Chemistry

Utah State University (USU)

May 2012
Logan, Utah

Dissertation: Synthesis and Biological Activity of
Aminoglycosides and 1,4-Naphthoquinone Derivatives”

Advisor: Dr. Tom C.-W. Chang

B.S., Chemistry (First Class Honors)

University of Buea (UB)

July 2005
Buea, Cameroon

RESEARCH EXPERIENCE

Research Intern

Phoenix Pharmalabs, Inc

October 2011-August 2012
Logan, UT

- Synthesized four new opioids as potential non-addictive treatments of pain
- Isolated enantiomers from racemic mixtures by column chromatography and diastereoisomeric crystallization

Graduate Research Assistant

Utah State University

December 2007-May 2012
Logan, UT

- Performed the synthesis of a carbohydrate, which was investigated as a potential therapeutic of the infantile genetic disease spinal muscular atrophy
- Explored methods for chemical derivation of the natural product kanamycin B for the development of antifungal agents, with complete loss of antibacterial activity. Results from this work provided general criteria for the design of good agro fungicide candidates

- Developed a methodology for the facile synthesis of libraries of novel antibacterial and anticancer 1,4-naphthoquinone derivatives
- Purified and characterized organic compounds by TLC, column chromatography, recrystallization, NMR (^1H , ^{13}C , COSY, HETCOR) spectroscopy, UV-visible, IR, and mass spectrometry

TEACHING EXPERIENCE

Teaching Assistant

August 2007-December 2011

Utah State University

Logan, UT

- Supervised and instructed 24 students in each of three sections of General and Organic chemistry laboratories for seven semesters. Classes taught include:
 - Chemistry Principles Lab I (CHEM 1215)
 - Chemistry Principles Lab II (CHEM 1225)
 - Organic Chemistry Lab I (CHEM 2315)
 - Organic Chemistry Lab II (CHEM 2325)
- Emphasized keeping complete and accurate scientific notes
- Substituted for major professor to teach General Chemistry II (CHEM 1120) Principles of Organic Chemistry (CHEM 2300)

AWARDS, FELLOWSHIP AND HONORS

- Outstanding Graduate Student in Chemistry, USU 2012
- Dr. Dinesh and Kalpana Patel Doctoral Graduate Fellowship, USU 2011-2012
- Center for Women and Gender Graduate Student Research Grant, USU 2011
- Graduate Student Senate Travel Award, USU 2010
- Teaching Instructor Certificate, USU 2007
- Top Graduating Student in Chemistry, UB 2005
- The Thomas and Janice Huang's Scholarship (Outstanding Student), UB 2004
- Minister of Higher Education Scientific Women Award, UB 2003-2005
- Dean's List Awards, UB 2003-2005

PUBLICATIONS

- **Fosso, M. Y.**; Nziko, V. P. N.; Chang, C.-W. T. "Chemical Synthesis of *N*-Aryl Glycosides" *J. Carbohydr. Chem.* Just accepted
- **Fosso, M. Y.**; Chan, K. Y.; Gregory, R.; Chang, C.-W. T. "Library synthesis and antibacterial study of cationic anthraquinones." *ACS Comb. Sci.* **2012**, *14*, 231
- Chang, C.-W. T.; **Fosso, M. Y.**; Kawasaki, Y.; Shrestha, S.; Bensaci, M. J.; Wang, J.; Evans, C. K.; Takemoto, J. Y. "Antibacterial to antifungal conversion of neamine aminoglycosides through alkyl modification. Strategy for reviving old drugs into agrofungicides." *J. Antibiot.* **2010**, *63*, 667

- Mattis, V. B.; **Fosso, M. Y.**; Chang, C.-W.; Lorson, C. L. “Subcutaneous administration of TC007 reduces disease severity in an animal model of SMA.” *BMC Neurosci.* **2009**, *10*, 142
- Mattis, V. B.; Ebert, A. D.; **Fosso, M. Y.**; Chang, C.-W. T.; Lorson, C. L. “Delivery of a read-through inducing compound, TC007, lessens the severity of a SMA animal model.” *Hum. Mol. Genet.* **2009**, *18*, 3906

PRESENTATIONS

- **Marina Fosso**, Synthetic chemistry of aminoglycolipids. Bioproducts Summit – USU Commercial Enterprises & Synthetic Bioproducts Center. July 19, 2012, Logan, UT (oral)
- **Marina Fosso**, Yukie Kawasaki, Sanjib Shrestha, Jon Takemoto and Tom Chang. Synthesis and antifungal activity of kanamycin B analogs. Gordon Research Conference “Carbohydrates”, June 19-24 2011, Waterville, ME (poster)
- **Marina Fosso**, Yukie Kawasaki, Sanjib Shrestha, Jon Takemoto and Tom Chang. Synthesis and structural optimization of antifungal kanamycin B analogs. 240th ACS National Meeting & Exposition, August 22-26 2010, Boston, MA (poster)
- **Marina Fosso**, Tom Chang, Jon Takemoto, Mekki Bensaci and Yukie Kawasaki, Synthesis of new kanamycin B analogs with surprising antifungal activity Joint 63rd Northwest/ 21st Rocky Mountain (NORM/RMRM), June 17 2008, Park City, UT (poster)

PROFESSIONAL AFFILIATIONS

Memberships

- American Chemical Society 2010-present
- Golden Key International Honor Society 2009-present

Leadership/Service

- Senior graduate student 2008-2011
 - Supervised seven new graduate/undergraduate researchers, training them to perform standard operating procedures and chemical experiments
 - Mentored two high school students during their summer internship in the Chemistry and Biochemistry department at USU, providing them with work directions
 - Managed the laboratory in the absence of the major professor
- Vice-president AFSA (African Students Association), 2009-2010
 - Assisted in the organization of the club’s events to showcase the African culture, attracting more than 300 students

- Volunteer 2008-2009
 - Helped packaging Christmas gifts for kids in the hospitals

SKILLS

Languages: English (fluent), French (native)

Computer skills: Microsoft (Word, Excel, PowerPoint), Chemdraw, Scifinder, ChemsSketch, Discovery Studio, PyMol

REFERENCES

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brad.davidson@usu.edu
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Dr. Alvan C. Hengge

Professor and Department Head
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Logan, UT 84322-0300
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Ph.D. Supervisory Committee Member

Dr. John A. Lawson

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Logan, UT 84332-5604
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jalawson3@comcast.net
Internship Supervisor