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Total Synthesis of Antibiotic Varacins B and C, Design and Synthesis of the Core Functionalities of Leinamycin and Investigation of Their Biological Properties

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Ph. D.

THE HONG KONG
POLYTECHNIC UNIVERSITY
2003

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Total Synthesis of Antibiotic Varacins B and C, Design and Synthesis of the Core Functionalities of Leinamycin and Investigation of Their Biological Properties

LEE HO FAI

A Dissertation Submitted in Partial Fulfillment of the Requirements For the Degree of the Doctor of Philosophy

In

Chemistry

May, 2003.

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ACKNOWLEDGEMENT

During the three and a half years of my Ph. D. training, several interesting research works and ideas have come to light that were not available earlier. All kinds of scientific research combine with human being's determination, diligent, fascinating idea, knowledge and a little bit luck. Independent thinking and helpful discussion are the keys of success. I would like to make this opportunity to express my most truly gratitude to my supervisor, Prof. Albert Sun-chi Chan (Member of The Chinese Academy of Science) for his invaluable advice, guidance, kindness and unlimited supports to my works. I thank my co-supervisor, Dr. Tianhu Li for his guidance, helpful discussion on the research everyday. I should also like to thank him for his aid in preparation of a lot of our ambitious publications. I also particularly grateful to Dr. Tze-lock Chan (CUHK) for his warmly care and enlightenment since my first degree study. In assembling materials for this dissertation, Dr. Tianhu Li and Dr Yeung Chi-hung checked the manuscript and suggested many improvements.

Financial supports from The Hong Kong Polytechnic University Area of Strategic Development Fund, the University Grants Committee of Hong Kong (Areas of Excellence Scheme, AoEP/10-01) and The Hong Kong Research Grant Council research grant (B-Q534) are gratefully acknowledged. I also thank The Hong Kong Polytechnic University Tuition Scholarship for financial support of my Ph. D.

Prof. Eric T. Kool at Stanford University and The Croucher Foundation (Hong Kong) are gratefully acknowledged for their generous supports to secure my Postdoctoral Training in United States in coming years.

Alex Ho Fai Lee, May, 2003.

Department of Applied Biology

and Chemical Technology

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ABBREVIATIONS

Ac	acetyl	DNA	deoxyribonucleic acid
Boc	*t*-butoxycarbonyl	A, G, T, C	adenine, guanine, thymine, cytosine
Bp.	boiling point	DTT	dithiothreitol
^{t}Bu	*tert*-Butyl	2D COSY	Two-dimensional Correlation Spectroscopy
calcd.	calculated	δ	chemical shift
cm^{-1}	wavenumber	EI	electron impact
CCD	Charge Coupled Device	equiv.	equivalence
CI	chemical ionization	ESI	electrospray ionization
cat.	catalytic	Et	ethyl
d	doublet	FAB	fast atom bombardment
dd	doublet and doublet	FT	Fourier Transform
DBDM	dibenzoyldiazomethane	GC	Gas chromatography
DBU	1,8-diazabicyclo[5.4.0]-undec-7-ene	h	hour(s)
DCC	dicyclohexylcarbodiimide	HPLC	High performance liquid chromatography
DETAPAC	diethylenetriaminepentaacetic acid	HR	high resolution
DHU	dicyclohexylurea	Hz	hertz
DIBAL	diisobutylaluminium hydride	IC_{50}	cytotoxic parameter
DMF	dimethylformamide	IR	Infrared
DMAD	dimethylacetylene dicarboxylate	ISC	Intersystem crossing
DMAP	4-dimethylaminopyridine	*J*	coupling constant
DMSO	dimethyl sulphoxide	LAH	lithium aluminium hydride

lit.	literature	RNA	ribonucleic acid
M^+	molecular cation	RSSH	hydrodisulfides
*m*CPBA	*m*-chloroperoxybenzoic acid	RSS_xSR	polysulfides
m	multiplet	r.t.	room temperature
M	molarity	s	singlet
Me	methyl	SOD	superoxides dismutase
m/z	mass to charge ratio	10X TAE	400 mM Tris-Acetate/ 100 mM EDTA
min.	minute(s)	TBDMSCl	*tert*-butyldimethylsilyl chloride
mp.	melting point	TEMPO	2,2,6,6-tetramethyl piperidine 1-oxyl
MS	mass spectrometry	TFA	trifluoroacetic acid
μ	micro 10^{-6}	THF	tetrahydrofuran
μM/bp	micromole per base pair	TLC	thin layer chromatography
n	nano 10^{-9}	TMS	tetramethylsilane
NBS	*N*-bromosuccinimide	Tris·HCl	triphosphate hydrogenchloride
NHS	*N*-hydroxysuccinimide	UV	ultraviolet
NMR	nuclear magnetic resonance	V	volt
NTS	*N*-thiocycanatosuccinimide	*vacuo*	vacuum
PDC	pyridinium dichromate	υ_{max}	maximum absorption
PDT	photodynamic therapy	W	watt
ppm	part per million		
psi.	pound per square inch		
q	quartet		

Abstract of thesis entitled "Total Synthesis of Antibiotic Varacins B and C, Design and Synthesis of the Core Functionalities of Leinamycin and Investigation of Their Biological Properties" **sumitted by Alex Ho-Fai Lee for the degree of the Doctor of Philosophy In Chemistry at The Hong Kong Polytechnic University in May, Two Thousand And Three.**

ABSTRACT

Part I. Synthesis of Leinamycin's Core Functionalities as Anti-Cancer Drug Candidates and Study of Their Thiol-Activated DNA-Cleaving Activities

Leinamycin, discovered in 1989, is the only antibiotic ever found in Nature possessing 1,2-dithiolan-3-one 1-oxide moiety which embedding a spiro fashion to an 18-membered lactam. This natural product displays potent antitumor activities and has been examined clinically as an anticancer drug candidate. In the first place of our research, we have attained the synthesis of two Molecules **1** and **2**, the core functionalities of leinamycin, via a 15-plus synthetic operations pathway. The detailed synthesis of leinamycin's cores, **1** and **2**, as well as their biological properties will be presented in the text accordingly. Secondly, we have attempted to employ our cores to mimic leinamycin and study their DNA-cleaving mechanisms. It is the first time the existence of hard evidence that has been proven beyond reasonable doubt if 1,2-dithiolan-3-one 1-oxide functionality existed alone, the thiol-mediated DNA-cleavage was through a radical mechanism, if there is 1,2-dithiolan-3-one 1-oxide and C6-C7 olefinic double bond co-existed in the molecule, both the alkylation and radical mechanisms prevailed. In addition, we have engaged our two Molecules to the linkers. This is an approach headed for linking our mimics covalently to antisense oligonucleotides. In such a way, the mimics are not only brought closely to target DNA but also confined to a specific region within the duplex. This part of our study has paved the road for the future works of further evaluation of the leinamycin's DNA-cleaving

mechanisms and exploration of modified antibiotic for the candidates of anticancer drugs.

Part II. Exploring the Antibiotics Varacin, Varacin B, Varacin C and the Structural Related Entities for Anti-Cancer Drug Candidates

Varacins B and C, isolated from the Far Eastern ascidian *Polycitor* sp. in 1995, are the first antibiotics discovered to date that having the unique structural feature of trithiole 1-oxide as their core functionality. Although biological examinations on these two natural products revealed that both of them exhibit potent antifungal and antimicrobail activities against *Candida albicans* and *Bacillus subtilus*, the modes of their actions at the molecular level have not yet been understood. Moreover, conduction of the total synthesis of varacins B and C as well as the designe and synthesis of their structural analogs have not yet been reported in literature. With the aim of clarifying the mode of actions, a set of structural analogs of varacins B and C were designed and synthesized. Furthermore, we demonstrated for the first time that these synthetic analogs could cleave DNA efficiently under physiological condition. Based upon the encouraging results, the research focuses were turned on the total syntheses of varacins B and C, the designs and synthesis of structural derivatives of the family of varacin. The objectives of our works are to characterize the precise DNA-cleaving mechanisms of varacins B and C and to evaluate the suitability of those title compounds, the structural related derivatives as the candidates of anticancer drugs or lead drugs. It is believed that

our endeavors of this project is not only providing chemists with a new insight into the chemistry that has been long utilized in nature, but also ultimately lay a theoretical foundation for the scientific contributions in the field of cancer chemotherapy and integrity in the study of varacin's family.

Part III. Varacins B, C and Benzotrithiole Oxides Structural Analogs as A Novel Family of Photoinducible DNA Cleaving- Agents

The design and synthesis of DNA cleaving agents are of great interest as a workhorse in chemistry, biology and medicine. During our elaborated research on the antibiotics varacin B, varacin C and the structural related moieties as the thiol-mediated DNA-cleaving candidates, we have accidentally uncovered the photoinduced DNA-cleaving properties of the benzotrithiole oxide, and varacin B, varacin C which incorporated benzotrithiole oxide entity in the molecules. Based on the preliminary results from the mechanical studies and DNA cleavage assays, we proposed the photochemical cleavage of DNA by the candidates was originated from the photolysis of benzotrithiole oxide to yield a singlet excited state, which was rapidly transformed to the strained intermediate then backed to ground state with intramolecular oxygen migration. Meanwhile, intersystem crossing $D^* + A \rightarrow D + A^*$ (one of the quenching mechanism) then generated the hydroxyl radical in aqueous medium and cleaved the DNA strands in the system. This field of study constitutes a timely and challenging topic and leads to the

development of pageantic applications including DNA footprinting techniques and anti-cancer research.

PREFACE

The aim of this project reported in this dissertation is to demonstrate the usage of small molecules to trigger damages in the supramolecular scaffold of DNA. DNA-damaging agents have historically played a central part in cancer therapeutic study and medicinal chemistry. Although there is myriad of known DNA-damaging agents, they are classified into few categories if based on the criteria of molecular functionalities and chemical reactions involved in their DNA-cleaving properties. Our project is divided into three major parts here: **Part I.** Synthesis of Leinamycin's Core Functionalities as Anti-Cancer Drug Candidates and Study of Their Thiol-Activated DNA-Cleaving Activities; **Part II.** Exploring the Antibiotics Varacin, Varacin B, Varacin C and the Structural Related Entities for Anti-Cancer Drug Candidates; and **Part III.** Varacins B, C and Benzotrithiole Oxides Structural Analogs as A Novel Family of Photoinducible DNA Cleaving-Agents. Those three individual sections illustrated the total synthesis and the studies of these small molecules as the candidates of DNA-damaging agents and their DNA cleaving properties. The parts of project were designed and accomplished in different stages during my Ph.D. studies. They however, share a similarity in the aspect of DNA-damaging agents' functionality. The agents studied in the project are classified as organic polysulfane. It is well-known that polysulfane-containing compounds, such as proteins, hormones, lipoic acids, enzymes and other species occur naturally and are well-documented for their biological roles. In contrary to our long-held expectation, neither the DNA-cleaving properties nor the interwound relationships with those supramolecular carriers of genetic information was broadly published. Thereby the success of limited examples, such as the antibiotic leinamycin DNA-cleaving studies intrigued me. Soon

we are able to demonstrate the very first time that our proposed simple structural analogs could trigger DNA cleavage as if the structurally complicated parent molecule.

In the **Part I** work, the key findings are as follows: (i) development of effective and concise synthetic pathway for the surrogates of the natural product leinamycin which is only available in meagre yields from extraction or very long total synthesis pathway; (ii) using structurally simple analogs to demonstrate and explain some untold leinamycin DNA-cleaving mechanisms; (iii) opening the new window for future bio-organic study of DNA-damaging agents with manifold biological utilities by tethered linking the agents with antisense oligonucleotides.

In **Part II** and **III**, DNA-damaging agents studied are the members belonging to the family of antibiotic varacin. Varacin, varacins B, C and their structural analogs had been demonstrated to possess DNA-cleaving ability either mediated with *in vitro* thiols, or mediated with ultraviolet radiation. These two parts describe our recent and unpublished discovery that antibiotics varacin and its family members are capable of causing highly efficient DNA cleavage promoted by their acidic surroundings with added thiols or by ultraviolet radiation, and exhibit potent antitumor activities. The first total syntheses of these antibiotics are also reported herein. As the extracellular pH in most tumor cells is lower than that in normal tissue and the antibiotics possessing acid-triggered functionalities are rare in nature, the activating mode of antibiotics described in this report could consequently offer helpful hints to chemists with respect to how to design the acid-activated architectures of molecules as the candidates of tumor cell-

activated prodrugs. In addition, varacin and its related antibiotics with their acid-activated mode of action could themselves have potential applications in tumor chemotherapy. On the other hand, we proposed in **Part III** that the photochemical cleavage of DNA by the candidates which originated from the benzotrithiole oxide singlet excited state in photolysis. The excited moieties passed along a chain of reactions to generate the hydroxyl radical in aqueous medium and cleave the DNA strands in the system. In consideration of the originality and theoretical significance of this research topic and the potential applications of these antibiotics as antitumor drug candidates, we would suggest this piece of works would benefit the general audiences who are interested in synthetic organic chemistry, the chemistry of natural products and antibiotics as well as nucleic acid chemistry.

In short, our works reported herein are the exploration of effective total syntheses to afford naturally occurred antibiotics and their structural analogs aimed to secure a reliable source of compounds for further biological evaluations. Nevertheless, we also explored the applications of our compounds as the candidates of DNA-damaging agents. The corresponding results provided us with the precious data of unprecedented chemical mechanisms involving acid-triggered thiol mediated DNA cleavage and pH-independent ultraviolet radiation induced DNA damaging. The DNA-cleaving processes are proved to be the free radical pathway. In which molecular oxygen presents a paradox to aerobic organisms: it is both essential (in ground state) and potentially toxic (in excited state). The reactive oxygen species including the superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and the most potent hydroxyl radical ($HO^{\cdot}$) are thought to coexist in our

experiments and are responsible for DNA oxidative scissoring. These three parts of my work revealed additional secrets that afforded us a better understanding and knowledge on the complicated but vague mechanisms by which small organic molecules could efficiently and irreversibly modify cellular DNA. More importantly, this report represented a mean to contribute to the active and exciting area of investigations in cancer therapy and medicinal chemistry.

PART I

Synthesis of Leinamycin's Core Functionalities as Anti-Cancer Drug Candidates and Study of Their Thiol-Activated DNA-Cleaving Activities

I. 1 Introduction

DNA is thought to be the principle target of a number of clinically useful antitumor agents including, mitomycin, adriamycin, *cis*-platinum and bleomycin. These compounds interfere with DNA through cross-linkage, strand breakage, intercalation, or other kinds of interactions which result in inhibition of nucleic acid synthesis.[1] The discovery of novel antitumor agents which interact with DNA provided us with an insight into mechanistic principle that can be used to mediate DNA recognition and cleavage.[2]

Among the most advanced cancer chemotherapeutic agents which are widely used in clinical applications today are various categories of organic and organometallic compounds that exert their cytotoxic effects on cancer cells by reacting chemically with the DNA inside the cells' nuclei. These compounds are believed to react with cytosine and guanine bases in DNA, affording a monoadducted product, an intra- or interstand cross-link, or a DNA-protein cross-link. The reaction can also occur at the phosphate moiety, but interference at this site is generally not considered to be of much significance. It is also known that the environmental factor of cytosine and guanine which are the target of DNA-damaging compounds, plays a crucial role. Certain compounds prefer guanine-rich regions, whereas others are less selective. Bonding distances are also important, with some compounds believed to yield adjacent guanine-guanine or guanine-cystosine intrastrand cross-links or complementary guanine-cytosine interstrand cross-links, whereas others are believed to produce guanine-guanine interstrand cross-links. DNA repairing is also one of the considerations for the

compounds that react with DNA, and repair of monoadducts generally occurs readily but repairing of inter- or intrastrand cross-links occurs with difficulty. These compounds are to be distinguished from those that are DNA intercalators and DNA minor groove binding compounds. Whereas intercalators and minor-grove binding compounds are the molecules which are capable of slipping between the layers of nucleic acid base pairs and disrupting the shape of the double helix. As a result, the DNA replication and transcription are interrupted.[3]

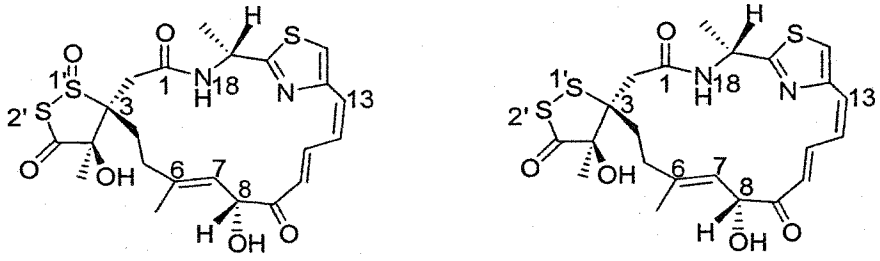
Although new approaches to cancer treatment become available, it seems that there will be a continued enthusiasm for studying and developing of novel DNA-damaging agents. These agents will see continued usage due to their well-established role in treating various types of cancer. Many of the new approaches to cancer therapy such as inhibition of angiogensis, immunotherapy, and modulation of the cell cycle are most effective when used in combination with traditional DNA-damaging agents.[4]

In this part of our research, the current study and results on a novel antitumor antibiotic, leinamycin will be presented.[5] Since the discovery, structural elucidation[6] and synthesis[7] of leinamycin in 1989, it has been demonstrated that this antibiotic exhibits potent activity against murine experimental tumor leukemia P388 and saccoma 180 as well as against Gram-positive bacteria.[8] Subsequent investigation indicated that the latent DNA-cleaving activity of leinamycin accounts for its cytotoxicity.[9,10] However, due to the poor binding affinity between leinamycin and DNA (the target of this antibiotic to deliver its cytotoxicity), the precise DNA-cleaving mechanisms of this antibiotic have not yet been clearly identified.[8,11] The focus of this research project is

therefore on designing and synthesizing mimics of the core functionality of leinamycin and to explore the possibility of introducing a linker to the molecule of mimics. The work leads a way for future development of linking the designed mimics covalently to antisense oligodeoxynucleotides, which will subsequently enable us to characterize the precise DNA-cleavage mechanisms of this natural product.[12,13] Moreover, the action of mimics of leinamycin's core functionality against cancer cell lines, and its possibility as the anticancer drug candidates were evaluated. We agreed that the execution of these works not only provide a new insight for the chemists into the chemistry that has been long utilized in nature, but also lay a theoretical foundation for potential pharmaceutical and biotech companies in Hong Kong for their development of advance anticancer drug candidates.

According to Hara *et al*,[5] the 1,2-dithiol-3-ane 1-oxide structure plays an essential role in the leinamycin DNA cleaving activity. With regard to the thiol-mediated activitation of this antibiotic, they predicted that functionality might be the possible target of thiol cofactors (for structures of leinamycin and its deoxy-isomer, see Chart 1.1). They provided further insight into this point by studying the effect of *S*-deoxyleinamycin on the plasmid DNA. Despite the close structural similarity between leinamycin and its *S*-deoxy derivative, the latter entity was found to be biologically inactive; no matter of cytotoxicity on cultured cells and antimicrobial activity against Gram-positive bacteria were less than 1% that of leinamycin, i.e. IC_{50} against HeLa S3 cells was 2.1 μg/mL, and minimum inhibitory concentration against *B. subtilis* was more than 100 μg/mL for *S*-deoxyleinamycin; in contrast, the values for leinamycin were

0.014 and 0.08 μg/mL, respectively. Also, incubation of *S*-deoxyleinamycin with plasmid DNA did not result in any DNA-cleavage even in the presence of thiol cofactors. Those observations indicated that the presence of sulfoxide group in dithiolane ring was crucial for the leinamycin activity and illustrated the role of the core functionality in that antibiotic molecule. Although the full pharmacological profile for leinamycin has not been described, it will not be surprising to find that Hara's proposal for the core functionalty is widely accepted based on other peripheral evidence. Hara's reports provided us the ground of using 1,2-dithiol-3-ane 1-oxide as the core-functionality of leinamycin and refreshed our idea to design leinamycin's mimics in the sense of structural properties.

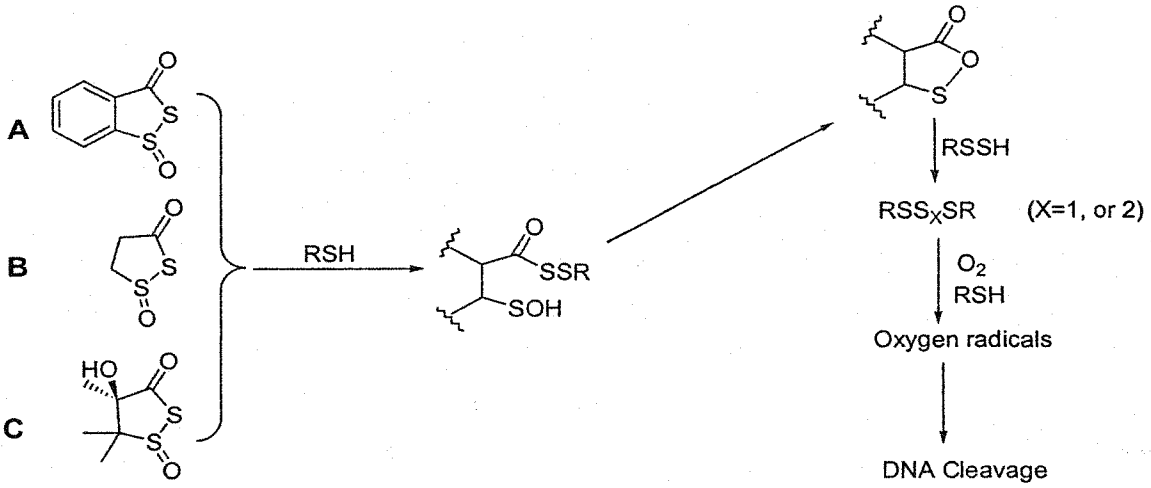


Leinamycin *S*-Deoxyleinamycin

Chart 1.1 Molecular structures of leinamycin and *S*-deoxyleinamycin

As mentioned before, studying the mechanisms of how the drugs interfering on DNA molecules are important. The mechanism of action of many natural products, such as taxol[14] and calicheamicin[15] had been elucidated before they were selected as anti-cancer drug candidates. Unlike taxol and calicheamicin, the precise DNA-cleaving mechanisms of leinamycin have not yet been conclusively confirmed. For example, two

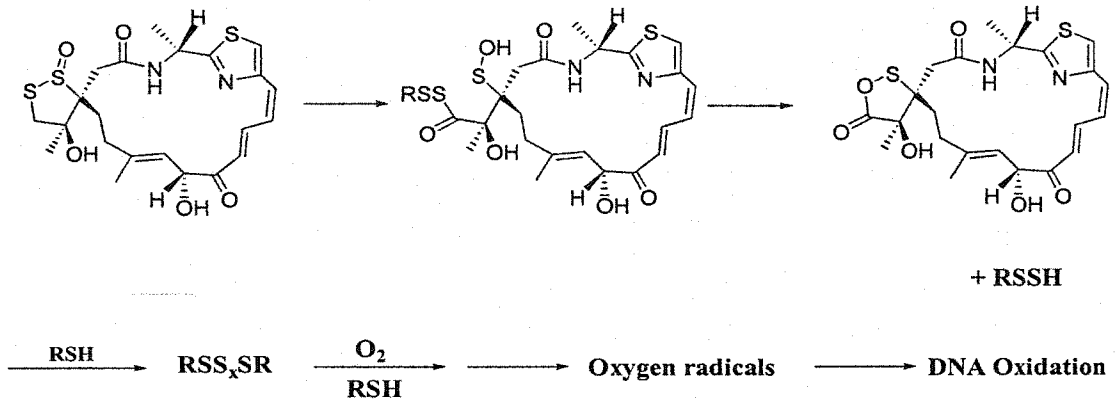
contradicting theories have been proposed as plausible pathways of thiol-mediated DNA cleavage by leinamycin: one via the formation of radical species and the other via a transition of DNA-alkylating intermediate. The major obstacle that prevents experimental confirmation of the mechanism of DNA-cleavage by leinamycin is the poor binding affinity of this antibiotic to its target DNA. The purpose of this research project is therefore to precisely characterize the mechanisms of leinamycin by utilizing the designed mimics containing the core functionality of the antibiotic.



Scheme 1.1 Radical mechanism proposed by Gates' group

Two types of mechanisms concerning the DNA cleavage by leinamycin have been suggested so far: (i) through generation of radical species from Gates[8] and (ii) through a transition of DNA-alkylating process proposed by Asai *et al.*[9] The radical mechanism proposed by the Gates' group was derived from investigating the interactions of calf thymus DNA with analogs **A**, **B** and **C** (Scheme 1.1) and with leinamycin itself. It was demonstrated that the five membered ring, 1,3-dioxo-1,2-

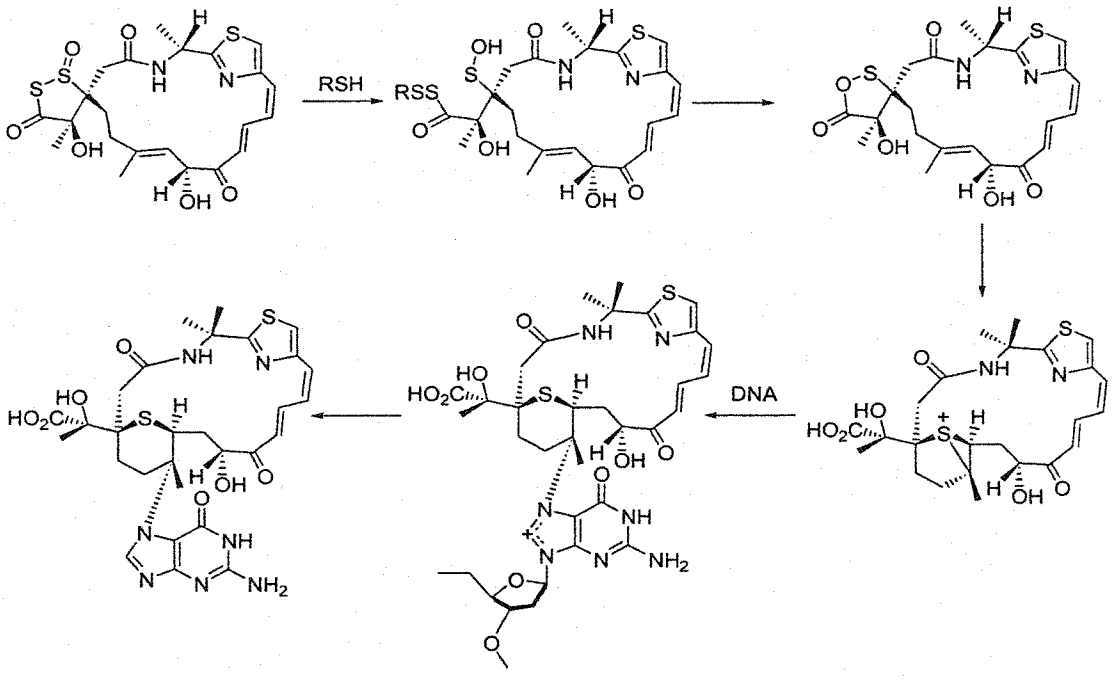
dithiolane moiety alone sufficed for DNA-cleaving activity and the DNA-cleaving reactions were inhibited by radical scavengers.[8a] On the other hand, Asai *et al.* obtained leinamycin-guanine adduct when intact leinamycin was incubated with calf thymus DNA, which led to conclusion of DNA alkylation prior to cleavage by antibiotic.[9] Unlike Gates' radical mechanisms in which 1,3-dioxo-1,2-dithiolane moiety alone was sufficient for its reactivity, Asai's group suggested that the DNA cleavage pre-required the presence of a double bond between C6 and C7 (see Schemes 1.2 and 1.3).



Scheme 1.2 DNA-cleavage by leinamycin via radical pathway

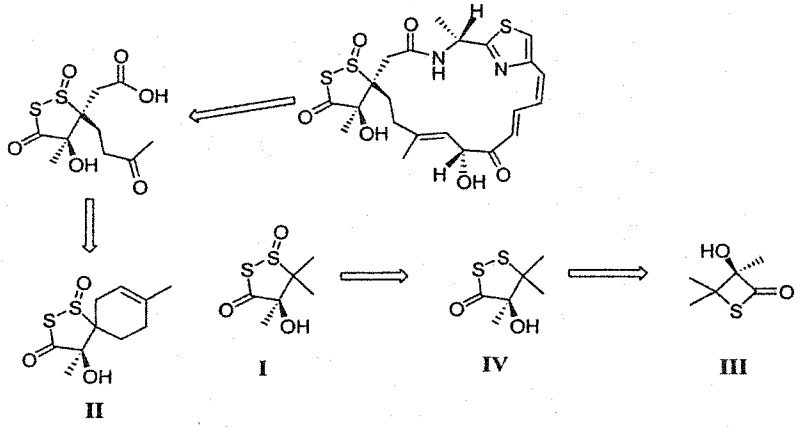
Scheme 1.2 indicates the flow-chart for DNA cleavage in radical mechanism. Both Scheme 1.2 and 1.3 show that leinamycin and its simple analogs react with added thiol to give the sulfenic acid in the course of nucleophilic attack of thiol on the sulfenyl sulfur of the heterocycle, and rupture of sulfur-sulfur bond. Followed by cyclization of resultant sulfenic acid, the unstable oxathiolanone and a persulfide (hydrodisulfide) are afforded. The final products of the reaction derived from the attack of excess thiol on the

oxathiolanone to yield the polysulfides.[8] Then the DNA strands cleavage is caused by hydrodisulfide intermediate (RSSH) and polysulfides (RSS_xSR) which are involved in the conversion of molecular oxygen to DNA-cleaving oxygen radicals. Alkylation pathway explains the DNA-cleavage in a quite different way. The oxathiolanone moiety is produced after the addition of thiol at the sulfenic sulfur atom in the 1,3-dioxo-1,2-dithiolane moiety of leinamycin, giving a hydrodisulfide.[8,9] Intramolecular addition of the oxathiolane moiety to the 6,7-olefin in molecule to give an episulfonium intermediate. The highly electrophilic episulfonium is expected to be nucleophilic attacked by N7 atom of guanine base and yields the adduct compound, then results in DNA cleavage subsequently (see Scheme 1.3).[9]

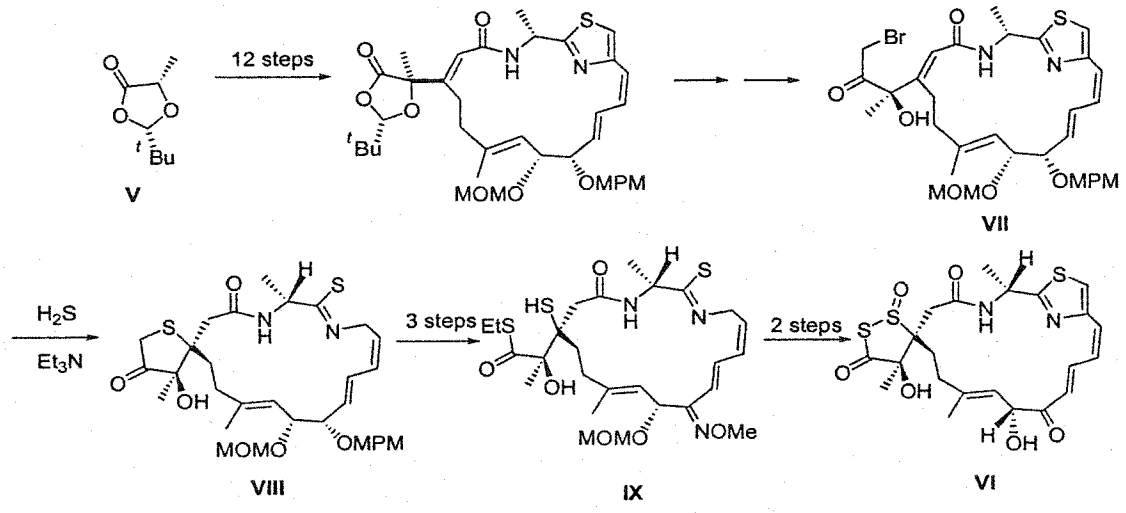


Scheme 1.3 Alkylation mechanism proposed by Asai *et al.*

In addition to the challenges from concluding the mechanism of leinamycin DNA-cleavage, the unique structural features as well as the interesting antitumor activities have prompted several groups to undertake the total synthesis of leinamycin and the structurally related derivatives.[16,17] Pattenden[16] and Kanda[17a] are two of the pioneers in the field of total synthesis of the 1,2-dithiolan-3-one 1-oxide moiety of leinamycin. Pattenden *et al.* have examined a number of complementary routes to the 1,2-dithiol-3-ane 1-oxide ring[18] residue **I** embedded in this unusual structure, and also to the spiro-fused compound **II**, a key intermediate *en route* to leinamycin (see Scheme 1.4). The general strategy they have followed in synthesis of the **I** relied on access to the central β-thiolactone intermediate **III** which was opened, then turned to the 1,2-dithiolane **IV** by oxidative cyclization.[19] On the other hand, Kanda's work[17a] started with readily available dioxolanone **V**.[20] Following by 20 steps of functional group transformations and syntheses, a 4:3 diastereomeric mixture of 1-oxo-1,2-dithiolan-3-one **VI** with undetermined relative stereochemistry was obtained (see Scheme 1.5). The crucial steps in this synthesis were (i) an intramolecular delivery of a sulfur atom (**VII** to **VIII**)[21] and a Beckmann fragmentation (**VIII** to **IX**).



Scheme 1.4 Pattenden's synthesis of leinamycin

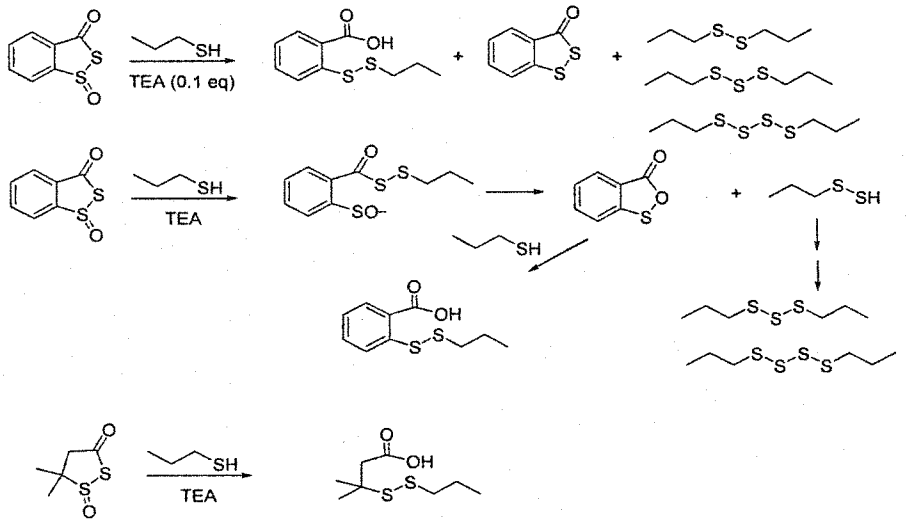


Scheme 1.5 Kanda and Fukuyama's synthesis of leinamycin

Our own synthetic pathways for leinamycin mimics were planned with the bases-catalysed, nucleophilic attack on the carbonyl carbon of β-thiolactone by hydrogen sulfide to yield the ring-opened mercapto thioic acid.[22] Subsequently, it was oxidative cyclized to 1,2-dithiolane by exposure to sodium iodate supported on alumina.[23] Those transformations constitute the backbone of the synthetic routes. Our methodology will be narrated in detail next part.

To our knowledge, several of the simple analogs of leinamycin bearing 1,2-dithiol-3-one 1-oxide core functionality have been employed for studying the single strand DNA cleavage properties of this novel antibiotic.[4,8,9] Gates *et al.* reported a series of detailed researches on the corresponding approach (see Scheme 1.6).[8] However, we find that an urge for the preparation of a pair of leinmaycin mimics with olefinic double bond and without the double bond incorporated into C6-C7 positions of the molecules. It is because Asai suggested that the C6-C7 olefinic double bond in the leinamycin 18-

membered lactam ring served a role in the alkyaltion mechanism of DNA-cleavage.[9] On the other hand, Gates's mimics of leinamyicn do not have any olefinic double bond within the molecules and it may be an incompleteness.[8,10] Moreover, we strongly agree with the widely accepted proposal of 1,2-dithiol-3-ane 1-oxide can act as the core functionality of the antibiotic's DNA cleaving activity. We are also unwilling to eliminate the plausible role played by C6-C7 double bond of the antibiotic molecule in the cytotoxicity. We then designed the two mimics to carry out the experiments of thiol-mediated plasmid DNA cleavage and compared their difference of properties. Our mimics were designed sophisticatedly embedded the hydroxyl group as an anchoring site in their terminals of alkyl chains. This feature leads to an opportunity in modification of the mimics to suit other utilities, such as linkage to antisense oligonucleotides[11-13] which is mentioned before in this part.



Scheme 1.6 Reaction of *n*-propanethiol with benzodithiol-3-one 1-oxide and 5,5-dimethyl-1,2-dithiolan-3-one 1-oxide

I. 2 Results and Discussion

I.2.1 Synthesis of Leinamycin's Mimics

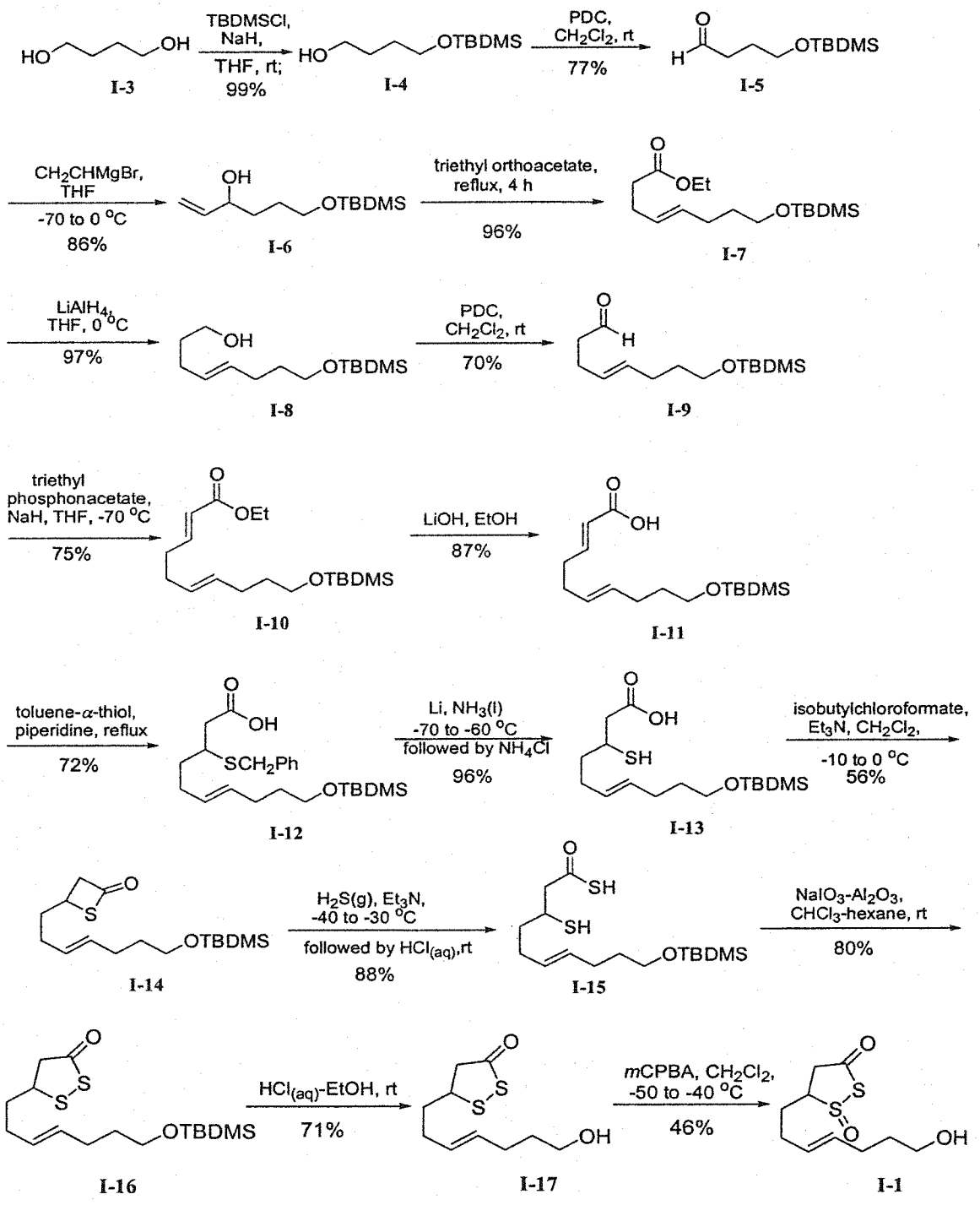
Leinamycin, isolated from *Stretomyces sp.* in 1989, is the only antibiotic possessing 1,2-dithiolan-3-one 1-oxide moiety which is fused in a spiro fashion to an 18-membered lactam.[6,7] This natural product displays potent antitumor properties and has been examined clinically as an anticancer drug candidate.[24] Unlike any previous discovered DNA-cleaving reagents and microbial metabolites, leinamycin possesses a novel structural feature that acts as its major DNA-cleaving functionality.[8-10]

Owing to the lack of decisive experimental evidence, the precise DNA cleaving mechanisms of leinamycin are currently still in dispute.[8,9] In fact, both Gates' radical and Asai's alkylation mechanisms were derived from the study of calf thymus DNA that contains thousands of base pairs. Due to the complex conformational features and sequence diversity of calf thymus DNA, chemical reactions between such a macromolecular nucleic acid and an antibiotic can hardly be characterized. The significance of this project is threefold. Firstly, leinamycin is the only antibiotic discovered to date that possesses the novel and major DNA-cleaving functionality 1,3-dioxo-1,2-dithiolane incorporated into 18-membered lactam ring.[4-6] Characterizing the precise mechanism of DNA-cleavage of this antibiotic is crucial for further exploration to this gift from the nature and its utilities. Secondly, since leinamycin is capable of cleaving DNA and possessing anticancer activities,[4,5] the knowledge of mechanism of action of leinamycin at the molecular level is important for determining the suitability of leinamycin as an anticancer drug candidate at the later stages of drug development.

Thirdly, our designed mimics of leinamycin's core functionality are structurally much simpler than their parent compound. Thus, the mimics will be accessed rapidly and efficiently, and the biological evaluation of them will therefore provide valuable information for selecting advanced and alternative antitumor drug candidates on the molecular basis.[25]

We have recently completed the synthesis of Molecules **I-1** and **I-2**, the core functionalities of leinamycin, via a 15 steps synthetic pathway. The detailed synthesis of leinamycin's cores **I-1** and **I-2**, as well as their derivatives and those entities' biological activities will be presented accordingly.[26,27] Compound **I-1** was selected as one of our synthetic targets for cytotoxic examination and thiol-mediated DNA-cleavage because its structure not only resembles the 1,3-dioxo-1,2-dithiolane moiety of leinamycin but also contains an appropriately positioned olefinic double bond mimicking that found at the gamma position of lactam ring of the antibiotic molecule. The route was shown in Scheme 1.7, which was envisioned with well-established methodologies.

Our synthesis began with commercially available 1,4-butanediol **I-3**. Protection of one of the hydroxyl group of 1,4-butanediol was achieved by using 1.1 molar equivalent of sodium hydride as a base, in THF as a solvent. The monosodium alkoxide salt was silylated with 1.1 equivalent of *tert*-butyldimethylsilyl chloride (TBDMSCl). Monosilylated product **I-4** then was rewarded with excellent yield. We employed TBDMS to protect the hydroxyl group because TBDMS ether is one of the most popular silyl protective groups used in organic synthesis. It is easily introduced with a wide variety of reagents, has the virtue of being quite stable to a variety of reaction conditions



Scheme 1.7 Synthetic route to afford compound **I-1**

and is readily removed under media that do not attack other functionalities. It is approximately 10^4 times more stable to basic hydrolysis than trimethylsilyl group (TMS).[28] Though, it has excellent stability toward base, it is sensitive to acid. The ease of introduction and removal of the TBDMS ether is influenced by steric factors.[28a] The subsequent aldehyde **I-5** was prepared in 77% yield by reacting alcohol **I-4** with pydridinium dichromate (PDC) in distilled CH_2Cl_2. This methodology allows the efficient oxidation of a wide range of alcohols to carbonyl compounds in CH_2Cl_2 with only a modest excess of oxidant. Actually, PDC has emerged as a very useful and versatile oxidant which is clearly deserving of widespread application.[29] Neither the reagent nor its application to the oxidation of alcohol is new. In fact, this species is probably present in the Sarett[30] and Cornforth[31] oxidizing mixtures. During the course of the synthesis of **I-5**, we found that we benefited from a more complete oxidation and a higher yield of the desired product by employing the molecular sieve modification of PDC oxidation.

In general, vinylmagnesium bromide reacts with various electrophiles including aldehydes, ketones, ester, lactones, anhydrides, acid chlorides, carboxylic acid, nitriles, amides, epoxides and allyl halides.[32,33] We prepared the vinylmagnesium bromide *in situ* from 1 M vinyl bromide –THF solution (obtained from Fluka) and magnesium tunings in distilled THF. This Grigard reagent was freshly prepared and transferred by means of hypodermic syringe or cannula under nitrogen atmosphere as the reagent might deteriorate with time and ignite in contact with moisture and air. Unreacted Mg metal and insoluble components in the Griganrd reagent were filtered by wrapping a piece of filter paper at the tip of the cannula and transfered it under N_2. To this stirring Grignard

reagent was added a solution of aldehyde **I-5** in distilled THF through a dropping funnel under N_2 at –70 °C. The stirring was continued overnight under N_2 and the temperature was allowed to raise to ambient. Allylic alcohol **I-6** was afforded with 86% yield. Ethyl ester **I-7** was attained by reacting **I-6** with triethyl orthoacetate in the presence of catalytic amount of propanoic acid, the unreacted orthoacetate was recovered by distillation.

We have tried to prepare aldehyde **I-9** from ethyl ester **I-7** directly with dissobutylaluminium hydride (DIBAL),[34] but it failed. So an alternative route was tried. Reducing **I-7** to **I-8** with lithium aluminium hydride (LAH) in THF was proved to be a successful way. **I-8** was then re-oxidized with PDC in CH_2Cl_2 to afford aldehyde **I-9** in fairly good yield. And the work-up process was simply filtering the dark brown slurry reaction mixture through a pad of silica gel in sintered glass filtering funnel and the filter-cake was washed with diethyl ether, followed by EtOAc. After silica gel column chromatography, an essentially pure **I-9** was obtained as colorless oil.

The reaction of aldehyde **I-9** with phosphonate carbanions containing electron withdrawing groups in an aprotic polar solvent constituted a useful olefination. The Horner-Emmons reactions (phosphonate anions)[35] have a number of advantages over the triarylphosphoranes or Wittig reagents.[36] They are in most cases much less expensive and they react with a wider variety of ketones and aldehydes, usually under milder reaction conditions. A convenient method for preparing phosphonate anions consists of adding the phosphonate at room temperature to slurry of sodium hydride in THF. Elevated temperature is optional due to the facile self-condensation of the anion. Indeed,

some alkyl phosphate anions which do not incorporate with activating group can be decomposed at the elevated temperature. The solution of aldehyde **I-9** was then added dropwise to the anion solution with hypodermic syringe under N_2. The solution was then heated to reflux for 1 h, sodium diethyl phosphate formed then increased the viscosity of the mixture. The product **I-10** was isolated in 75% by purification with flash chromatography over silica gel.

Hydrolysis of ester **I-10** to carboxylic acid **I-11** posed another challenge in our synthesis because **I-10** behaved unexpectedly stable toward most common hydrolysis reagents including sodium hydroxide and potassium hydroxide/aqueous-ethanol. More drastic condition, i.e. KOH/aqueous-ethanol reflux gave the deprotection of the hydroxyl group. Gratifyingly, we completed the hydrolysis by using *tert*-butanol/ potassium *tert*-butoxide and optimized the yield to 87% by employing LiOH/ EtOH.[37] Production of benzyl thioether **I-12** was accomplished by reacting prop-2-enoic acid **I-11** with toluene-α-thiol in piperidine.[38] Thiols added to olefins by electrophilic, nucleophilic, or free-radical mechanisms. In the absence of initiators the addition to simple olefins is by an electrophilic mechanism, and Markovnikov's rule is followed. However, this step proceeds incredibly slow and often cannot be completed or requires severe conditions unless a proton or Lewis acid catalyst is added. In the presence of free-radical initators, thiols are added to double bond by free-radical mechanism and the product follows anti-Markovnikov's rule.[39] When thiols are added to substrates susceptible to nucleophilic attack, bases catalyze the reaction and the mechanism is nucleophilic. These substrates maybe of the Michael type or maybe polyhalo olefins.

As the mercapto acid **I-13** is not stable in various purification procedures, so we took efforts to search for a method to debenzylated **I-12** in a clean and effective transformation. It is certainly that the use of Li/ liquid ammonia to prepare mercapto acid **I-13** could guarantee the success of preparation.[40] In general, debenzylations are achieved by reacting the substrates with sodium in liquid ammonia. We report here the use of lithium instead of sodium afforded considerably better yield and shorter reaction time in our works. The superiority of employing lithium as a reducing metal lies in a nearly ideal combination of its properties: (i) higher reduction potential, ie. –2.99 V in liquid ammonia compared to those of sodium (-2.59 V) and potassium (-2.73 V).[41] (ii) high solubility and low atomic weight, making possible high concentrations and high actual reduction potentials, and (iii) slow rate of reaction with alcohols. The deprotection was performed in a three-necked flask equipped with a stirrer, calcium chloride drying tube, nitrogen inlet, dry ice condenser and ammonia inlet. Cosolvent was needed in the hope of obtaining a homogenous reaction mixture because of poor solubility of **I-12** in liquid ammonia. THF was introduced to the mixture, which was found to be better than diethyl ether and ethylene glycol dimethyl ether because of its good solubility to the starting material and ease to be dried by distillation with Na/benzophenone. The typical reaction mixture consisted of a deep blue liquid phase covered by a layer of lithium-ammonia "bronze". Quantity of Li metal added depending on the color of liquid phase, the criteria was to keep the deep blue color throughout the reaction time by adding Li wire in small pieces over the period. After quenching with solid NH_4Cl cautiously and routine work-up, the low melting product was afforded in 96% yield.

Cyclization of the resulting mercapto acid **I-13** in a solution of isobutyl chlormate and triethylamine gave rise to the thiolactone **I-14**.[22] Subsequent ring opening of thiolactone **I-14** to the corresponding mercapto thioic acid **I-15** was done by treating the solution of **I-14** in $CaCl_2$ dried carbon tetrachloride saturated with dry hydrogen sulfide gas dropwise with freshly distilled triethylamine. Thiolactone underwent bases catalyzed ring opening with the hydrogen sulfide attacking on the electrophilic carbonyl carbon, followed with sulfur-carbonyl carbon bond rupture to afford **I-15** in 88% yield.[16,19,22] The H_2S gas we used was ordered in package of lecture bottles assayed of 99.5%+ purity. We had generated the gas from iron (II) sulfide and dilute hydrochloric acid (1:3) in a Kipp's apparatus;[42] it was then washed with water to remove acid spray and was passed through $CaCl_2$ for drying. Unfortunately, the reaction did not lead to the deserved product, impurities and moisture were blamed for the failure.

The isolated mercapto thiolic acid **I-15** further proceed to oxidative ring closure to generate dithiolane **I-16** by exposure to sodium iodate supported on neutral alumina.[43] The usage of iron (III) chloride as an oxidizing agent[44] posed a problem in our route because its acidity led to deprotection of TBDMS group. Organic chemists have long been benefited from the use of solid-supported reagents or reactants in synthetic operations.[23] It is understood that solid supports, whether they are inorganic salts or organic polymers, are capable of facilitating organic reactions by four factors: (i) an increase in the effective surface area for reaction to take place, (ii) the juxtaposition of reagent and substrate at close proximity to one another, (iii) the presence of pores which entrap both reagent and substrate so as to lower the entropy of activation for reaction, and (iv) in the cases of inorganic supports such as silica gel and alumina, the possibility

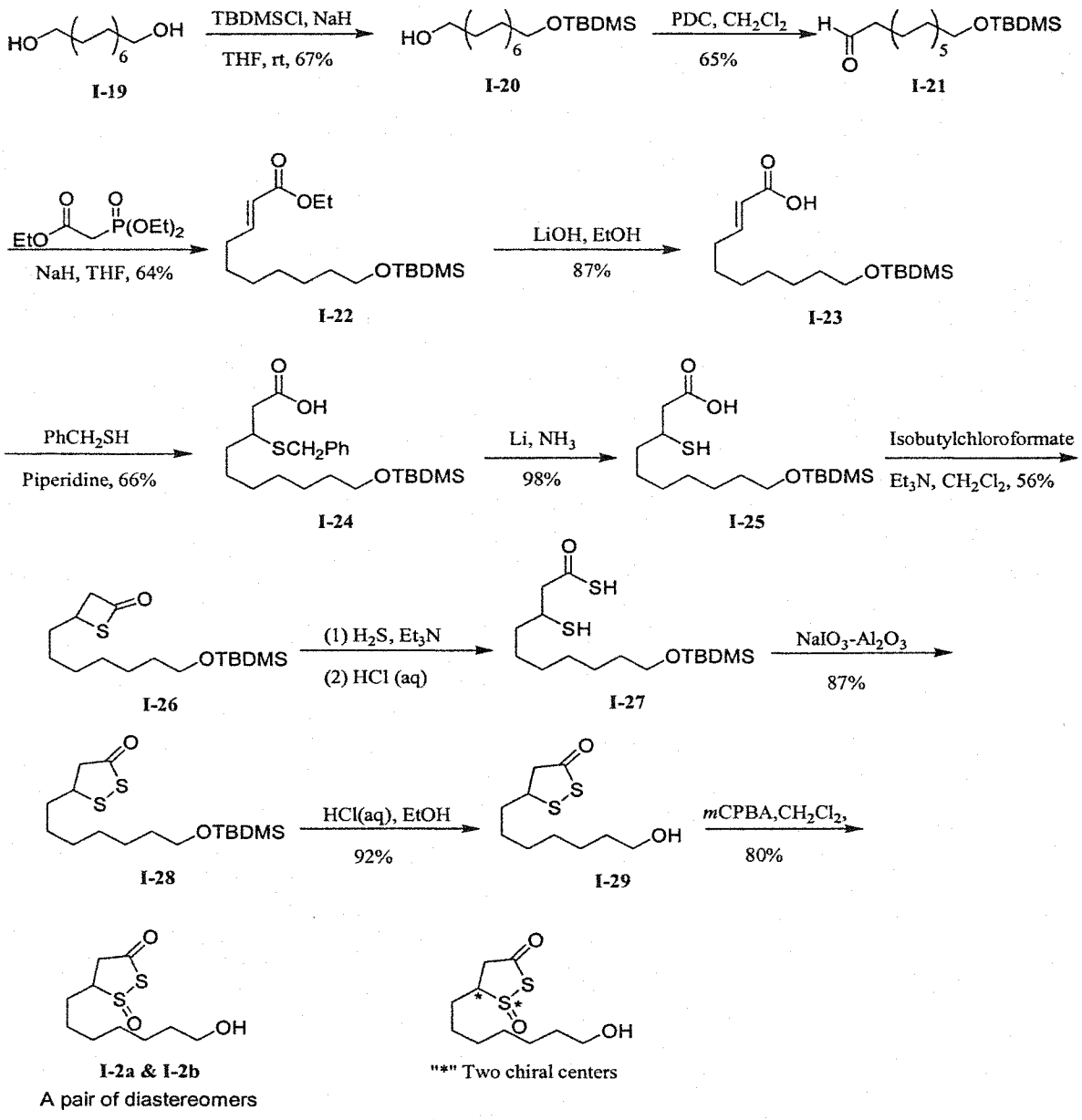
of catalytic effects of surface species such as H^+, OH^-, Al^{3+} etc. In addition to these attributes is the fact that in many cases the supported reagents are insoluble in organic solvents. The reactant and the product transformed in the course of a reaction are tenaciously adsorbed on the surface of the inorganic support or remain intercalated in it so as to allow removal of contaminants prior to dislodging of product by elution with the proper solvent. The attractiveness of such work-up procedures in what amounts to crude chromatography by simple filtration is a special feature in reactions employing solid-supported reagents. Encouraged by the success of previous works and Hirano's example,[43] the oxidative coupling of **I-15** with sodium iodate supported on alumina under biphases condition was attempted, dithiolane **I-16** was obtained in 80% yield after subjecting to simple work-up and purification.

Desilylation[28b] of **I-16** in hydrochloric acid/ ethanol was the most convenient method to produce alcohol **I-17** in good yield. We employed *m*CPBA to accomplished the *S*-oxidation of 1,2-dithiolan-3-one moiety to afford 1,2-dithiolan-3-one 1-oxide **I-1** in 46% yield. The reaction mixture was stirred for 30 min at –40 to –50 °C. Upon warming to 0 °C, saturated aqueous sodium sulfite solution was added to quench the oxidation. Stirring at the low temperature was believed to be the key of success of this step as it diminished the risk of epoxidation of the olefin and delivered a better *S*-oxo control.[16,19,22]

Once the compound **I-1** was prepared, the synthesis of its analog **I-2** with the saturation of olefinic double bond was started. Scheme 1.8 depicts our route, starting with protection of one of the hydroxyl group of 1,8-octanediol **I-19** using stoichiometric

equivalent of sodium hydride as base and THF as solvent. Silylation of the monosodium alkoxide salt with 1.1 equiv of *tert*-butyldimethylsilyl chloride gave the monosilylated product **I-20** in fairly good yield.[28] The monoprotected alcohol was oxidized with PDC in CH_2Cl_2,[29] and was monitored by TLC; the reaction was terminated by diluting the reaction mixture with diethyl ether. After usual work-up, pure **I-21** (65%) was afforded as colorless syrup after evaporating off the solvent. Similar to the previous case, the olefination of **I-21** was achieved by reacting with phosphonate anion in a Horner-Witting type reaction.[35] LiOH/EtOH was utilized to carry out the hydrolysis of **I-22**, yield of which was 64%. Since the mercapto acid **I-25** was not available in various purification processes due to its chemical lability, Li/liquid ammonia was utilized as a tool to achieve debenzylation of **I-24**. Thiolactone **I-26** was then obtained via cyclization of the corresponding mercapto acid **I-25** in a mixture of isobutyl chloroformate and triethylamine. The mercapto thiolic acid **I-27** underwent oxidative cyclization to give **I-28** in 92% yield by reacting it with sodium iodiate supported on neutral alumina in a biphases circumstance.[16,19,22,43] Deprotection of TBDMS group was routinely attained by stirring the substrate in hydrochloric acid- ethanol mixture, desilylated product was isolated from the extracts with 80% yield. Finally, *m*CPBA was used to achieve the oxo-selective *S*-oxidation of 1,2-dithiolan-3-one, yielding **I-2a** and **I-2b** entities. A pair of diastereomers was found, their R_f are only slightly different, but readily separated by column chromatography over silica gel with gradient elution. This pair of diastereomers bears the structural features showed in Scheme 1.8 below. Formation of sulfoxide in the *S*-oxidation introduced the second chiral centers into the pair of enantiomers. This event brought about four isomers which were divided into two pairs of diastereomers. Proton

NMR and 2D COSY spectra provided some hints to confirm the existence of diastereomers.



Scheme 1.8 Synthetic route to afford compound **I-2**

Alternative preparation of moiety **I-24** started with carboxylic acid **I-12**, one of the precursors of 1,2-dithiolan-3-one 1-oxide **I-1** (See Scheme 1.9). Hydrogenation of the olefinic double bond was accomplished with PtO_2 as the catalyst and relative high pressure of hydrogen (1000 psi.) was required to saturate the double bond completely. Pd/C was not an option of catalyst because debenzylation[45] happened during the course of hydrogenation and the completion of olefin saturation was hampered (thiols may poison the Pd/C catalyst). Although the above mentioned route is an alternative pathway to obtained **I-2**, we found that it is not efficient enough when compared to the previous one described in Scheme 1.8. The reasons behind are manifold: (i) the intermediates obtained from the adopted pathway were available in fairly good yield overall; (ii) it is not necessary to use expensive catalyst and relative high pressure of hydrogen to saturate the olefinic double bond which was prepared through a hardship; (iii) the route detailed in Scheme 1.8 did not involve the use of high pressure reaction condition, this can be an advantage if the preparation is scaled up.

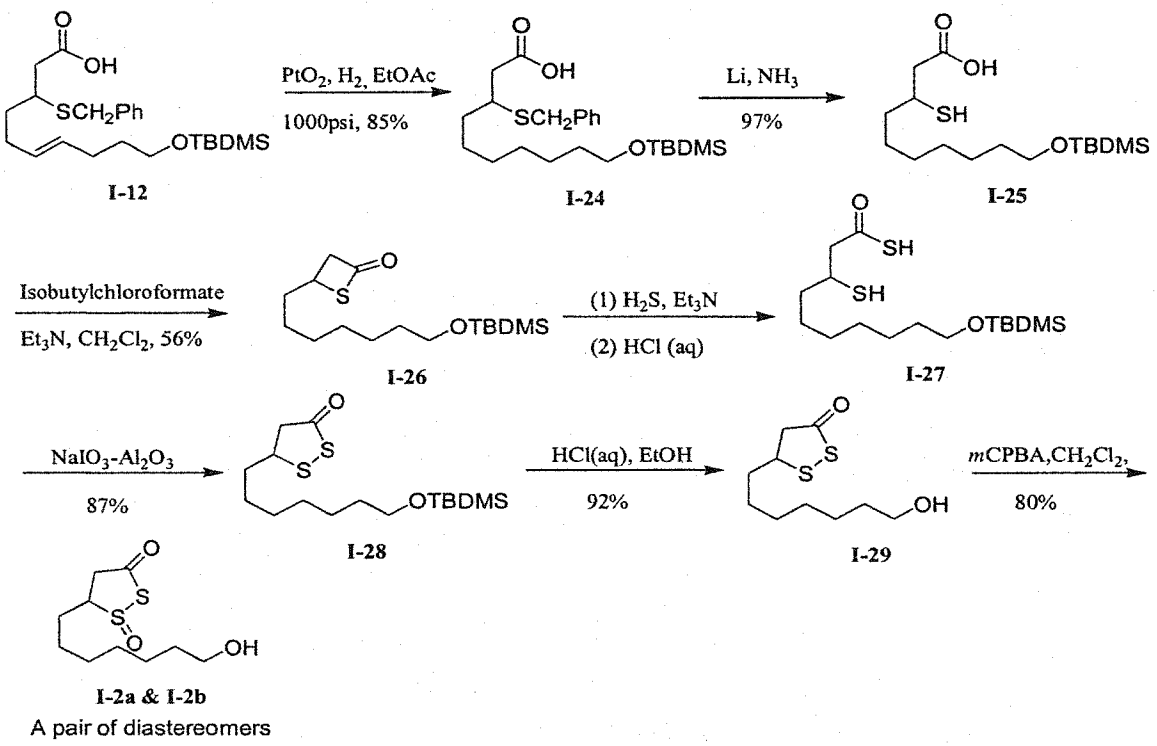
I.2.2 Cytotoxicities of Leinamycin's Mimics

In order to compare our mimics's antitumor and cytotoxic activities with leinamycin, the cytotoxic tests (IC_{50}) for molecules **I-1** and **I-2**[8,10] synthesized in our laboratory were carried out. One of the points we want to illustrate in our project is to elucidate the mimics' cytotoxicities, DNA-damaging properties come from the *S*-heterocycle core functionality.[4-7] The role of C6-C7 double bond incorporated into the mimic's scaffold would be studied too. The cytotoxic properties of the two mimics **I-1** and **I-2** were evaluated against six different human cancer cell lines, were expressed as

the concentration of these compounds that inhibited 50% of cell proliferation (IC_{50}). The clinically used anticancer drug doxorubicin was also tested parallelly for its cytotoxic activity on the same set of cell lines as a control. As shown in Table 1.1, the IC_{50} values of the two mimics against these six cancer cell lines are all at the micromolar levels. From the result, we found that both mimics sharing similar cytotoxicities, and the slightly higher cytotoxicity of **I-2** (molecule without C6-C7 olefinic double bond) against the MDA231, UMUC3, FDIGROV and MCF-7 cell lines may not account for the difference of *in vitro* cytotoxic properties of them. On the other hand, we discovered **I-1** (molecule possessing C6-C7 double bond) has a shorter half life when compared to its single-bonded counterpart. This discrepancy is aroused from the robust reactivity of co-existing *S*-heterocycle and double bond in proximity. The high reactivity and short half life of our two synthetic mimics limited their usage, and required fresh preparations before tests.

Table 1.1 Cytotoxic properties of **I-1** and **I-2** against six human cancer cell lines.

Cell type	Cell line	IC_{50} (μM) **I-1**	IC_{50} (μM) **I-2**	IC_{50} doxorubicin (nM)
human colon cancer	HT-29	>3.0	>3.0	59.80
human prostate cancer	PC-3	1.99	>3.0	14.98
human breast cancer	MDA231	>3.0	1.70	169.46
human bladder cancer	UMUC3	4.49	1.02	3.16
human ovarian carcinoma	FDIGROV	1.44	0.71	40.48
human breast carcinoma	MCF-7	2.46	1.51	8.89

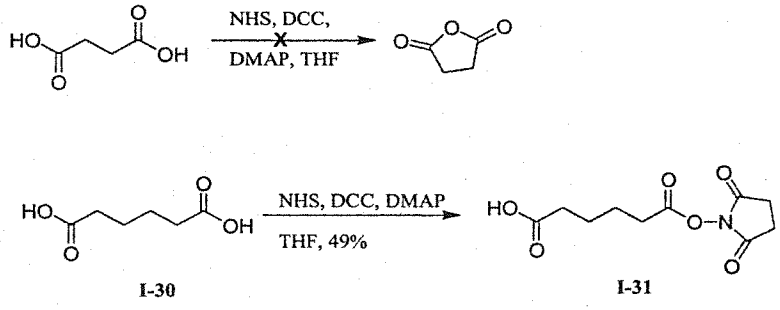


Scheme 1.9 Alternative pathway lead to compound **I-2**

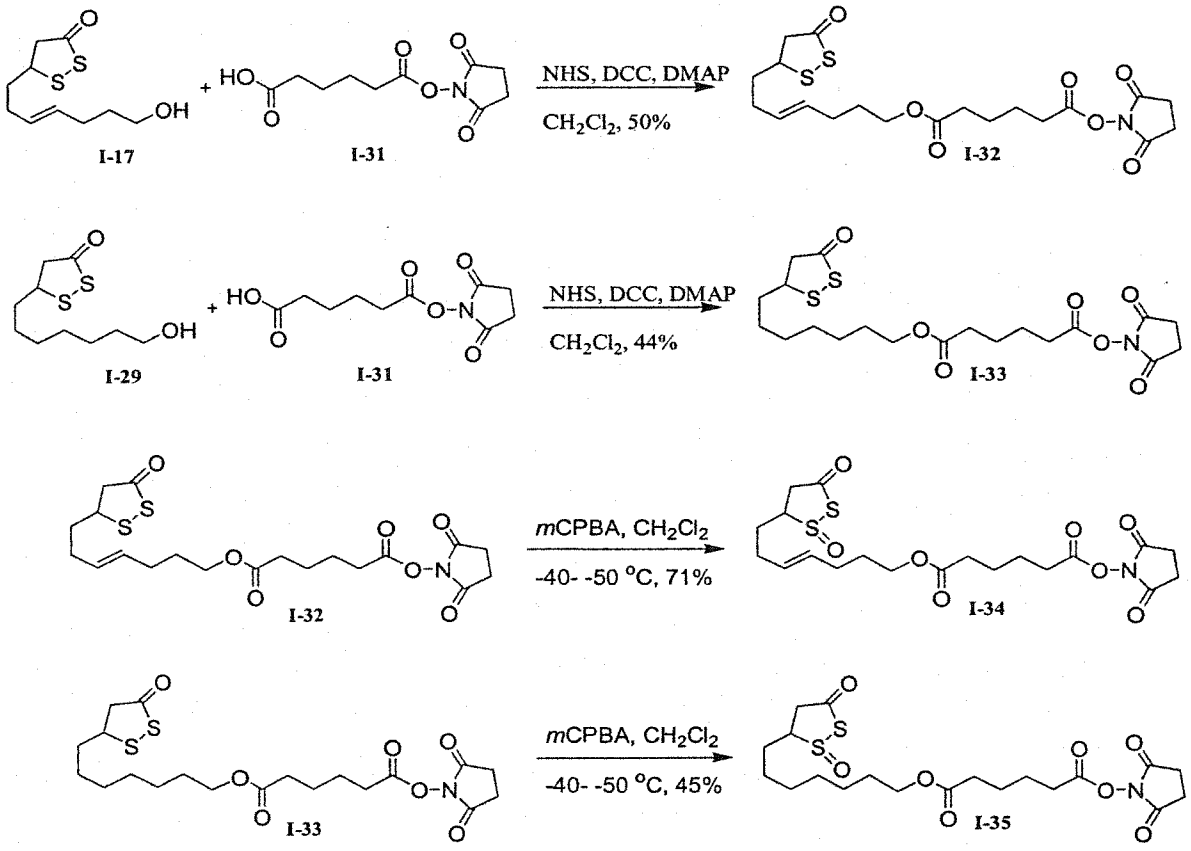
Form the literature, we find that previous studies on the leinamycin's mechanisms have not been conclusive. The radical mechanism proposed by Gates's group demonstrated that the five membered ring heterocycle alone suffices for DNA-damaging activity and the reactions were inhibited by radical scavengers.[8] On the other hand, Asai *et al* emphasized that the core functionality of leinamycin's DNA cleaving includes the C6-C7 olefinic double bond and the 1,2-dithiolan-3-one 1-oxide moiety. Inspired by Gates's study and work from Hara *et al.*[4-7] We worked out the reactions of leinamycin's mimics with pBR322 DNA using acetonitrile as a cosolvent for studying the mimics DNA-cleaving properties and approached to address and clarify the issue of DNA-cleaving mechanisms.[46,50]

In order to explore the methodology for studying the DNA-cleaving mechanisms of leinamycin and its mimics on the molecular level, and prepare for the future research of binding core functionalities of leinamycin covalently to antisense oligonucleotides,[11,12] we have built the linker in the following way as shown in Schemes 1.10 and 1.11.

The linker **I-31** was afforded in 49% yield by mixing the solution of adipic acid and *N*-hydroxysuccinimide in distilled THF with DCC and catalytic amount of DMAP under N_2 at 0 °C.[47] Attempts were tried to prepare our linker from succinic acid in similar pathway, but succinic anhydride was isolated as a major product. This served as a proof once again; the five member ring is energetically favored to form instead of seven member ring. Condensation of 1,2-dithiolan-3-ones **I-17** and **I-29** with linker **I-31** gave **I-32** and **I-33** respectively. **I-32** and **I-33** were then subjected to *S*-oxidation with *m*CPBA to give our targets molecules **I-34** and **I-35** in 71 and 45% yields.[26,27]



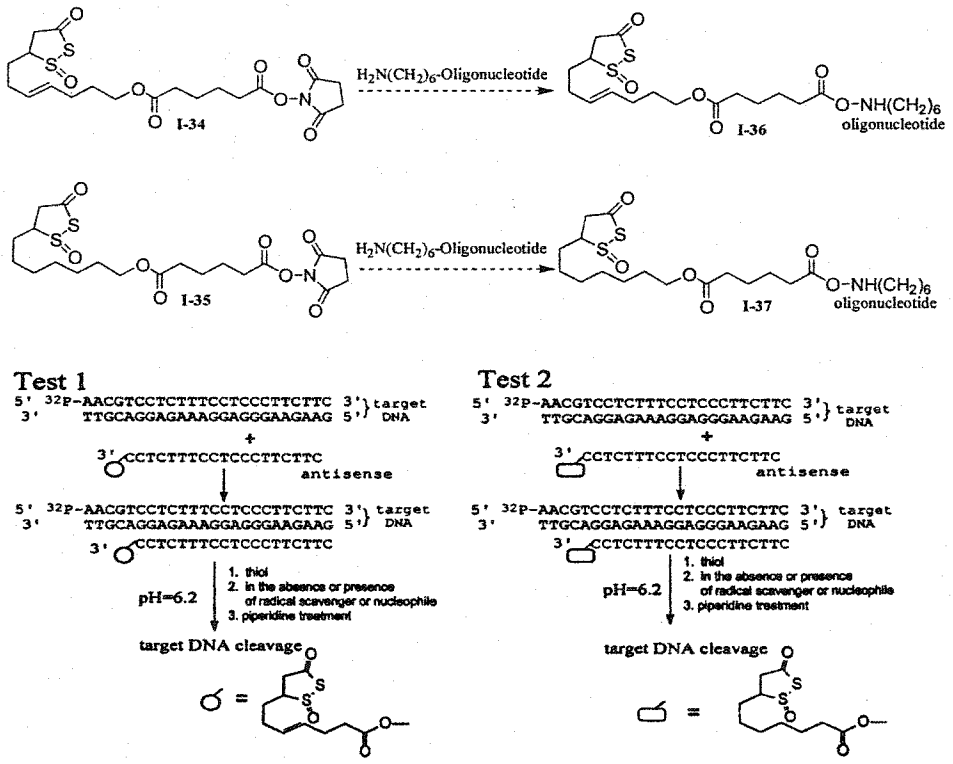
Scheme 1.10 Preparation of linker **I-31**



Scheme 1.11 Preparation of leinamycin's cores-activated ester conjugates **I-34** and **I-35**

With the candidates **I-34** and **I-35** in hand, we were able to link them to the selected antisense oligonucleotides with $H_2NC_6H_{12}$-modification at its 3' end. The coupling reactions between the selected oligonucleotides and compounds **I-36, I-37** will be carried out as described in Scheme 1.12. With the aid of polyacrylamide gel electrophoresis and phosphorimager analysis, the DNA cleaving mechanisms of compounds **I-36** and **I-37** will be characterized. Total synthesis of linker-incorporating compounds **I-34** and **I-35** introduced an opening for the plausible future works on the

more sophisticated and elaborated study on leinamycin/ leinamycin's mimics' mechanisms of DNA-cleavage.[46] Moreover, it acts as a starting point of one of the newly developed research strategies of our team. In such a way, the mimics will be not only brought closely to target DNA but also confined to specific region within the target DNA duplex. As the leinamcycin interacts with duplex DNA *in vivo*, double stranded oligoxynucleotides should be selected as its DNA target in order to mimic the natural condition to this end, as shown in the following Scheme 1.12. The formations of triple helices of **I-36** and **I-37** with duplex are designed to meet such requirement.[11-13] We deem this modification will be an vital tool to overcome the research obstacles on leinamycin DNA-cleavage mechanism caused by the poor affinity of leinamycin to its DNA targets. A part of the related studies is currently undertaking in our laboratory.



Scheme 1.12 Formation of antisense oligonucleotides and their expected interaction with duplex DNA

I.2.3 DNA-Cleaving Assays of Leinamycin's Mimics

Both leinamycin and its simple, synthetically prepared dithiolan-3-one 1-oxide mimics appear to represent a new class of DNA-damaging agents with a unique thiol-mediated mechanism.[8] As mentioned before, the DNA-cleaving properties of the two synthetic leinamycin mimics compounds **I-1** and **I-2** prepared in our laboratory recently were investigated. In order to gain insight into the DNA-cleaving properties of our selected candidates, the investigation on the following profiles of their abilities to cleave supercoiled plasmid DNA pBR322 was launched: (i) in different concentrations of DNA-damaging agents with fixed concentrations of used thiols; (ii) in various concentrations of thiols with the agents concentrations kept constant; (iii) in varying pH values; (iv) in the presence of various radical scavengers to probe their DNA-cleavage mechanisms. Different kinds of radical scavengers and addictives were mixed with the reaction mixtures in microtubes and incubated at 37 °C for stated period of time. 0.8% agarose gel electrophoresis was employed to assay the cleavage of DNA by our two candidates under physiologically relevant conditions. Thiol-mediated single-strand (Form II) and sometimes even double-strand (Form III) scissions of supercoiled pBR322 DNA by leinamycin's mimics were observed.

The DNA-cleavage activity was determined with pBR322 DNA obtained from BioLabs Ins. and GIBCO BRL. The reaction mixtures included freshly prepared solutions of DNA-cleaving agents in acetonitrile and the final reaction mixtures in microtubes containing 10% v/v of acetonitrile in 50 mM sodium phosphate buffer. As DMSO is known to be an effective free radical scavenger, we did not use it as a co-

solvent for all the thiol-dependent DNA cleavage attempts. A typical procedure for thiol-dependent plasmid pBR322 DNA cleavage is as follows: Given concentration of DNA-cleaving agents in acetonitrile (2 μL) was added to a solution containing 50 mM sodium phosphate with given pH values, 0.5 μg of pBR322 and water. Followed immediately by the addition of given concentration of 2-mercaptoethanol (2 μL) and/or addictives to make up a total volume of 20 μL in a microtube. The mixture was agitated with a vortex mixer, spun for 3 s in a tabletop microcentrifuge, and incubated in dark at 37 °C for 12-24 h.

Agarose gel electrophoresis of pBR322 DNA was started after quenching the DNA cleavage with 3 μL of loading buffer containing 0.25% bromophenol blue, 0.25% xylene cyanol FF and 40% (w/v) sucrose in water.[46,48] The resulting mixtures were loaded into the wells of the submerged 0.8% agarose gel using a disposable micropipette. The gel was electrophoresed at 100 V for 1 h (The gel was run until the bromophenol blue and xylene cyanol FF had migrated the appropriate distances through the gel) in 1 X TAE buffer and then stained in an aqueous ethidium bromide solution (0.2 μg/mL) for 2-6 h. The DNA was then visualized by UV transillumination and the gel was photographed using a Roche Lumilmager 4.

Caution: Ethidium bromide is a powerful mutagen and is moderately toxic. Gloves should be worn when handling the gels and solutions that contain this dye. After use, these materials were decontaminated by soaking with bleaching water or 0.5 M $KMnO_4$-2.5 N HCl aqueous solution overnight.[49]

The DNA cleaving activities of compounds **I-1** and **I-2** were determined by monitoring their effectiveness in converting supercoiled DNA (form I) to nicked DNA (form II). In some cases, more extensive DNA cleavage leads to the appearance of linearized (Form III) plasmid migrating just below the nicked form in the electrophoresed agarose gels shown. Ten pieces of gels were selected and discussed herein. Various concentrations of compounds **I-1** or **I-2** were incubated at 37 °C in the mixtures containing 2-mercaptoethanol with concentrations from one to several times that of compounds **I-1** or **I-2** and pBR322 DNA in 50 mM of sodium phosphate buffer with varying pH values under aerobic conditions in dark for indicated period of time usually for hours.

From Figures 1.1 and 1.2, we found that the percentage of DNA cleaved by both **I-1** and **I-2** increased with increasing concentrations of added 2-mercaptoethanol from 0.4 molar equivalent to 20 molar equivalent (with respected to the concentrations of **I-1** and **I-2** in the reaction mixtures); (lanes 2 to 7). Increasing the thiol concentration to 14.3 mM led to little diminished DNA cleavage (lane 8). This result is in-line with those found by Gates,[8b] we proposed that is caused by the self-quenching mechanism by excess thiol in the system. In fact, thiols can act as radical scavengers in high concentration was documented in literature;[50] similar trend was found in neocarzinostatin previously.[51] Without added thiol, no significant DNA cleavage recorded (lane 1). Thiols are capable of causing DNA cleavage through reduction of molecular oxygen to superoxide radical.[52] Figure 1.3 recorded the comparison of DNA strand scission by **I-1** and **I-2** in pH 6.5. From that gel we found the **I-2** (100 μM) gave approximately 50% of nicked plasmid after 25 hours incubation with 10 molar

equivalence of thiol. And the plasmid DNA (Form I) was completely converted to nicked form (Form II) when the amount of 2-mercaptoethanol increased to 50 molar equivalent. Excess of thiol added (lanes 4 and 7) resulted in diminished DNA strand scission just like those observed in Figures 1.1 and 1.2. Form III DNA cleavage happened when 100 μM of **I-1** was incubated with supercoiled circular plasmid DNA in 10 and 50 molar equivalents of thiols for a day; the compound **I-2**, however, did not give the same result under the same condition. Moreover, **I-1** cleaved plasmid DNA more effective than **I-2** in the condition corresponding to Figure 1.3. These observations were also recorded in the other gels and were attributed to the difference of **I-1** and **I-2** molecular functionalities and DNA cleavage mechanisms that they possess.

Meanwhile, we attempted to confirm that DNA cleaving activities of **I-1** and **I-2** could be triggered by thiols other than 2-mercaptoethanol. In Figure 1.4, the abilities of 2-mercaptoethanol and glutathione to mediate the DNA strand scissions by both agents were compared. Lanes 2, 5 and 4, 7 show the two thiols functioned with approximately equal efficacy as the cofactors of the DNA cleavage mediated by our two agents. This implies the thiol functionality (SH) alone is sufficient to facilitate the strand scission process.[8] Apart from catalase,[8,10,53] radical scavengers such as methanol, ethanol (results not shown) and mannitol[53,54] were tested for their quenching properties in the thiol-dependent DNA cleavage triggered by our agents. 10 mM of mannitol was found to be sufficient to inhibit DNA strand scissions by **I-1** and **I-2** (lanes 3 and 6). Methanol, ethanol and mannitol are known to be the guaranteed hydroxyl radical ($HO^{\cdot}$) scavengers. Thus, the inhibition outcomes further proved the DNA cleavage mediated by our two

agents was via radical mechanism.[54] Of course, we cannot rule out the DNA cleavage by **I-1** undergoes an alkylation pathway at the same time. Detailed mechanistic studies were continued in the experiments represented by Figures 1.7 and 1.8, also supplementary information from other experiments involving the comparison of DNA cleaving activities of **I-1** and **I-2** served as the proof consistent to our standpoint upheld.

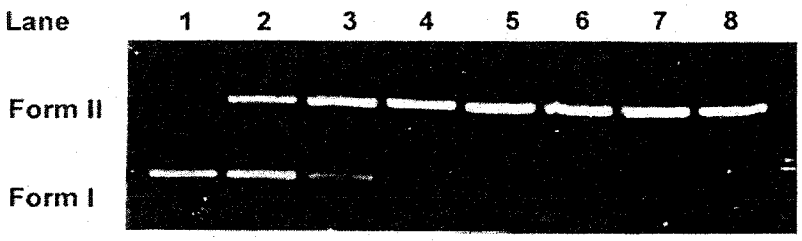


Figure 1.1 Thiol-dependent cleavage of supercoiled pBR322 DNA by 250 μM **I-1** in various concentrations of 2-mercaptoethanol. Reactions were incubated at 37 °C for 38 h in 50 mM sodium phosphate, pH 7.0, containing 10% v/v acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, pBR322 alone; lane 2, 250 μM **I-1** + 100 μM 2-mercaptoethanol; lane 3, 250 μM **I-1** + 250 μM 2-mercaptoethanol; lane 4, 250 μM **I-1** + 500 μM 2-mercaptoethanol; lane 5, 250 μM **I-1** + 1.25 mM 2-mercaptoethanol; lane 6, 250 μM **I-1** + 2.5 mM 2-mercaptoethanol; lane 7, 250 μM **I-1** + 5 mM 2-mercaptoethanol; lane 8, 250 μM **I-1** + 14.3 mM 2-mercaptoethanol.

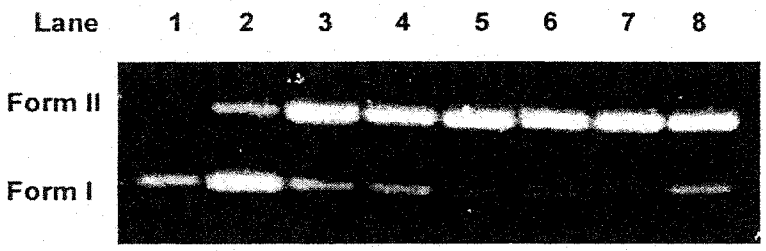


Figure 1.2 Thiol-dependent cleavage of supercoiled pBR322 DNA by 250 μM **I-2** in various concentrations of 2-mercaptoethanol. Reactions were incubated at 37 °C for 30 h in 50 mM sodium phosphate, pH 7.0, containing 10% v/v acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, pBR322 alone; lane 2, 250 μM **I-2** + 100 μM 2-mercaptoethanol; lane 3, 250 μM **I-2** + 250 μM 2-mercaptoethanol; lane 4, 250 μM **I-2** + 500 μM 2-mercaptoethanol; lane 5, 250 μM **I-2** + 1.25 mM 2-mercaptoethanol; lane 6, 250 μM **I-2** + 2.5 mM 2-mercaptoethanol; lane 7, 250 μM **I-2** + 5 mM 2-mercaptoethanol; lane 8, 250 μM **I-2** + 14.3 mM 2-mercaptoethanol.

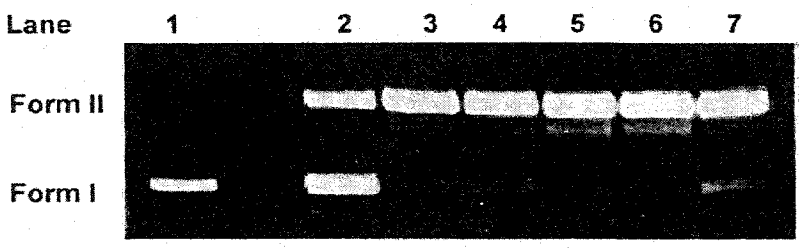


Figure 1.3 Thiol-dependent cleavage of supercoiled pBR322 DNA by 100 μM **I-2** and **I-1** in various concentrations of 2-mercaptoethanol. Reactions were incubated at 37 °C for 25 h in 50 mM sodium phosphate, pH 6.5, containing 10% v/v acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, pBR322 alone; lane 2, 100 μM **I-2** + 1 mM 2-mercaptoethanol; lane 3, 100 μM **I-2** + 5 mM 2-mercaptoethanol; lane 4, 100 μM **I-2** + 14.3 mM 2-mercaptoethanol; lane 5, 100 μM **I-1** + 1 mM 2-mercaptoethanol; lane 6, 100 μM **I-1** + 5 mM 2-mercaptoethanol; lane 7, 100 μM **I-1** + 14.3 mM 2-mercaptoethanol.

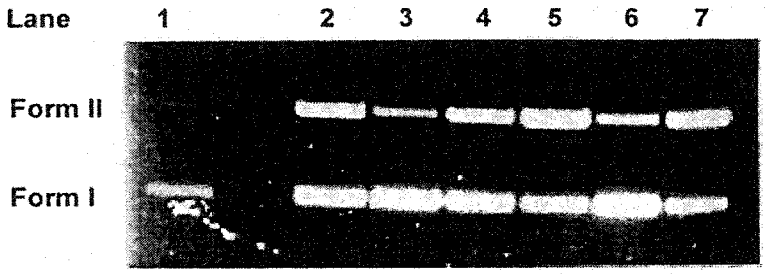


Figure 1.4 Thiol-dependent cleavage of supercoiled pBR322 DNA by 100 μM **I-1** and **I-2**. Reactions were incubated at 37 °C for 21 h in 50 mM sodium phosphate, pH 7.0, containing 10% v/v acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, pBR322 alone; lane 2, 100 μM **I-1** + 1.8 mM glutathione; lane 3, 100 μM **I-1** + 1.8 mM glutathione + 10 mM mannitol; lane 4, 100 μM **I-1** + 250 μM 2-mercaptoethanol; lane 5, 100 μM **I-2** + 1.8 mM glutathione; lane 6, 100 μM **I-2**+ 1.8 mM glutathione + 10 mM mannitol; lane 7, 100 μM **I-2** + 250 μM 2-mercaptoethanol.

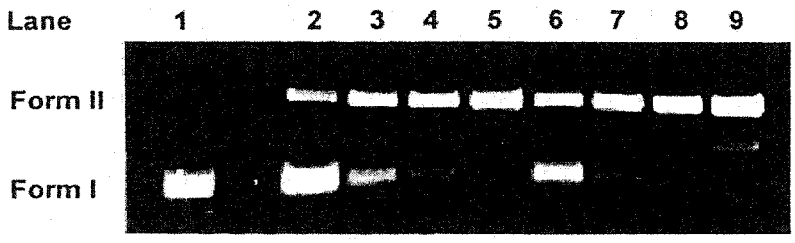


Figure 1.5 Time-dependent cleavage of supercoiled pBR322 DNA by 100 μM **I-2** and **I-1** in 500 μM 2-mercaptoethanol. Reactions were incubated at 37 °C in varying time in 50 mM sodium phosphate, pH 6.0, containing 10% v/v acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, pBR322 alone; lane 2, 100 μM **I-2** + 500 μM 2-mercaptoethanol (4 h); lane 3, 100 μM **I-2** + 500 μM 2-mercaptoethanol (14 h); lane 4, 100 μM **I-2** + 500 μM 2-mercaptoethanol (24 h); lane 5, 100 μM **I-2** + 500 μM 2-mercaptoethanol (38 h); lane 6, 100 μM **I-1** + 500 μM 2-mercaptoethanol (4 h); lane 7, 100 μM **I-1** + 500 μM 2-mercaptoethanol (14 h); lane 8, 100 μM **I-1** + 500 μM 2-mercaptoethanol (24 h); lane 9, 100 μM **I-1** + 500 μM 2-mercaptoethanol (38 h).

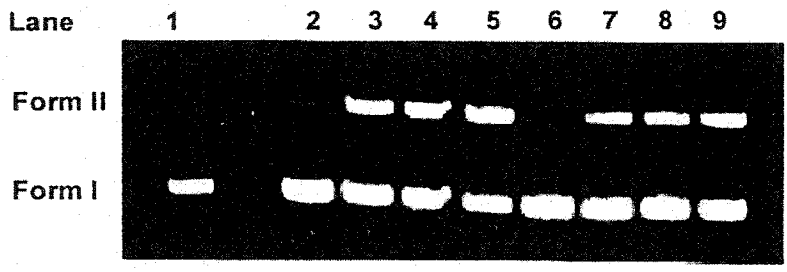


Figure 1.6 Time-dependent cleavage of supercoiled pBR322 DNA by 100 μM **I-2** and **I-1** in 500 μM 2-mercaptoethanol . Reactions were incubated at 37 °C in varying time in 50 mM sodium phosphate, pH 7.0, containing 10% v/v acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, pBR322 alone; lane 2, 100 μM **I-2** + 500 μM 2-mercaptoethanol (4 h); lane 3, 100 μM **I-2** + 500 μM 2-mercaptoethanol (21 h); lane 4, 100 μM **I-2** + 500 μM 2-mercaptoethanol (28 h); lane 5, 100 μM **I-2** + 500 μM 2-mercaptoethanol (40 h); lane 6, 100 μM **I-1** + 500 μM 2-mercaptoethanol (4 h); lane 7, 100 μM **I-1** + 500 μM 2-mercaptoethanol (21 h); lane 8, 100 μM **I-1** + 500 μM 2-mercaptoethanol (28 h); lane 9, 100 μM **I-1** + 500 μM 2-mercaptoethanol (40 h).

Figures 1.5 and 1.6 showing the time dependence of thiol-mediated DNA cleavage by both compounds **I-1** and **I-2**. The DNA-cleavage reactions by both compounds at pH 7.0 (Figure 1.6) were quite slow, we found no observable cleavage after incubation for 4 hours (lanes 2 and 6). But the rates of both candidates were greatly increase at pH 6.0 (Figure 1.5), nearly 100% Form I plasmid pBR322 DNA converted to the nicked form after 24 hour incubation in dark (lane 4 and 8). Also the Form III cleavage of DNA was observed in lane 9 on Figure 1.5. (pH factor of thiol-dependent DNA cleavage will be discussed in Figures 1.9 and 1.10.) And the rate of cleavage by 100 μM concentration of **I-1** at 37 ^{o}C, pH 6.0 and 7.0 was slightly faster than the same concentration of **I-2**. We assumed that **I-1** cleaved DNA with dual mechanisms: radical and alkylation pathways, so it might turn out faster in DNA damaging compared to **I-2** which only cleaved DNA in radical pathway (Figure 1.8 has a detail description on the mechanistic studies of **I-1** and **I-2**).

The metal chelator, diethylenetriaminepentaacetic acid (DETAPAC) is known to sequester adventitious traces of transition metals in a form that inhibits the trace metals from catalyzing the conversion of peroxides to DNA-cleaving hydroxyl radical in Fenton reaction.[8,10,55] Thus, the finding on Figure 1.7, addition of DETAPAC to our reaction mixtures caused significant inhibition of thiol-mediated DNA cleavage by both candidates **I-1** and **I-2**. This assay illustrates that the DNA-cleaving mechanism of the two compounds involves the radical mechanism, and serves as additional information implicating the role of oxygen radicals played in the DNA cleavage. The effective quenching concentration of DETAPAC started at 1 mM, and increasing the concentration to 10 mM and even to 100 mM could enhance the quenching effectiveness

(lane 3 to lane 5) and (lane 7 to lane 9). However, the higher concentration of DETAPAC is likely to be limited by its solubility in aqueous buffer. **I-1** gave the higher DNA cleaving rate than **I-2** when comparing the lanes 3 to 5 with lanes 7 to 9 might acted as the evidence for their difference of mechanistic pathway. **I-2** cleaved DNA via radical route and it should be quenched a little bit stronger than that of **I-1** which is capable to cause DNA damage by both alkylation and radical mechanisms.

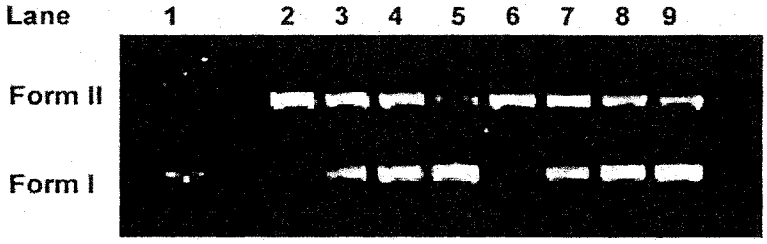
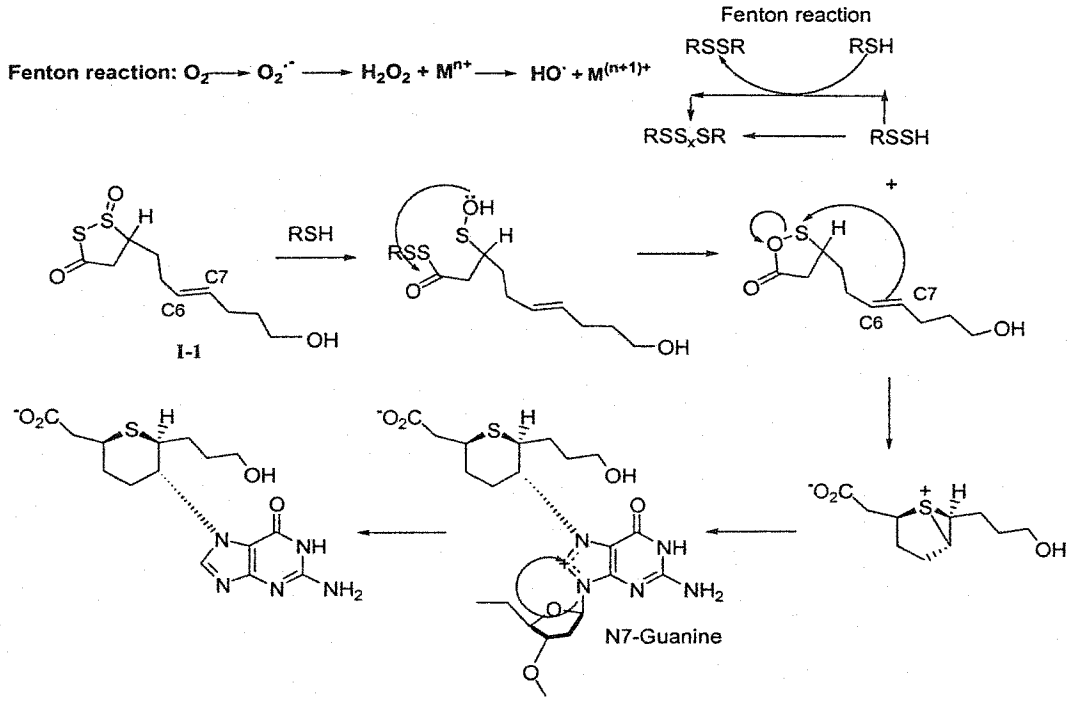


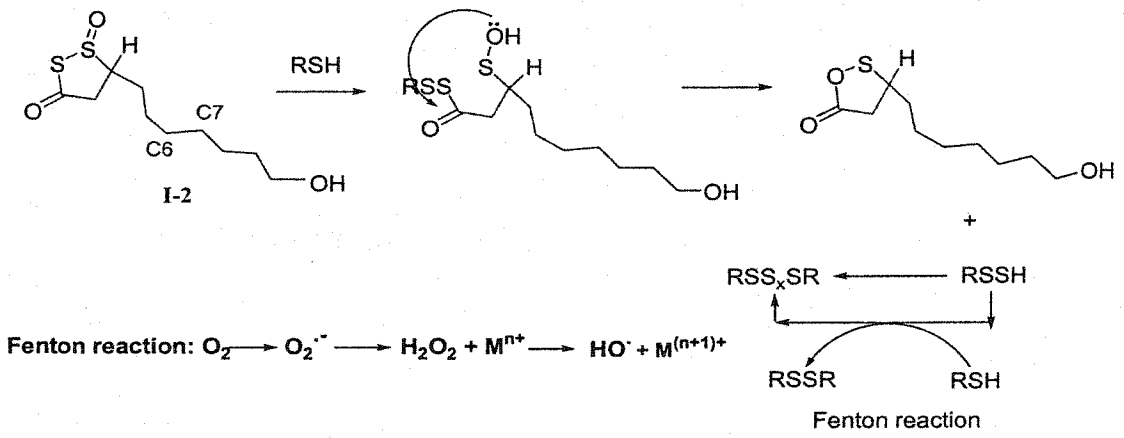
Figure 1.7 Thiol-dependent cleavage of supercoiled pBR322 DNA by 250 μM **I-1** and **I-2** in 1.25 mM 2-mercaptoethanol with varying concentration of DETAPAC. Reactions were incubated at 37 °C for 23 h in 50 mM sodium phosphate, pH 7.0, containing 10% v/v acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, pBR322 alone; lane 2, 250 μM **I-1** + 1.25 mM 2-mercaptoethanol; lane 3, 250 μM **I-1** + 1.25 mM 2-mercaptoethanol +1 mM DETAPAC; lane 4, 250 μM **I-1** + 1.25 mM 2-mercaptoethanol +10 mM DETAPAC; lane 5, 250 μM **I-2** + 1.25 mM 2-mercaptoethanol +100 mM DETAPAC; lane 6, 250 μM **I-2** + 1.25 mM 2-mercaptoethanol; lane 7, 250 μM **I-2** + 1.25 mM 2-mercaptoethanol +1 mM DETAPAC; lane 8, 250 μM **I-2** + 1.25 mM 2-mercaptoethanol +10 mM DETAPAC; lane 9, 250 μM **I-2** + 1.25 mM 2-mercaptoethanol +100 mM DETAPAC.

The mechanistic study was also done by evaluating the inhibitory effects of catalase and imidazole as radical scavenger and nucleophile[8,10,53-55] on the DNA cleavage process. The substantial quenching of thiol-mediated DNA cleavage reactions by catalase,[53] the hydrogen peroxide destroying enzyme, suggested that in combination with thiols, molecular oxygen was converted to hydrogen peroxide, which decomposed to hydroxyl radical via Fenton reaction, and cleaved the DNA via the radical mechanism.[8,10] On the other hand, the quenching of thiol-mediated DNA cleavage by imidazole[56] served as nucleophile and competed with those bases such as guanine residue to react with episulfonium ion generated in the course, implying the existence of alkylation mechanism. Figure 1.8 showed explicitly that the DNA cleaving activity of **I-1** decreased as imidazole added (lane 3) while its activity was only slightly affected by catalase (lane 4). Compound **I-2**, however, showed the reversed results that the DNA cleaving activity was only slightly affected by adding imidazole (lane 6) but quenched significantly by catalase (lane 7). Those observations are the evidence that compound **I-1** cleaves DNA in both radical and alkylation mechanisms and **I-2** cleaves DNA in radical mechanism solely. Our proposed mechanisms, both radical and alkylation, are figured out as follows (Schemes 1.13 and 1.14). Compound **I-1** with C6-C7 olefinic double bond and 1,2-dithiolan-3-one 1-oxide functionalities, cleaves DNA via an alkylation pathway. The initial attack of thiols on the heterocycle moiety leads to the formation of sulfenic acid that cyclizes to the 1,2-oxanthiolan-5-one and releases a hydrodisulfides (RSSH). The electrophilic oxanthiolan-5-one undergoes intramolecular reaction with C6-C7 olefin to generate an alkylating agent-episulfonium ion. The episulfonium ion efficiently alkylates double stranded DNA, most probably, forming a covalent bond at the N7 position of deoxyguanosine residues. Meanwhile, production of

hydrodisulfides (RSSH) causes the DNA damage in the radical pathway via Fenton reaction.[57]



Scheme 1.13 Proposed dual radical and alkylation mechanisms of DNA cleavage by compound **I-1**



Scheme 1.14 Proposed radical mechanism of DNA cleavage by compound **I-2**

Thiol-dependent DNA cleavage by our candidate compound **I-2** is thought to proceed through the free radical pathway. It initiates with oxygen-mediated oxidation of the unstable hydrodisulfide intermediate (RSSH), which produces in the reaction of 1,2-dithiolan-3-one 1-oxide moiety with thiols. The resulting polysulfides (RSS_xSR) then cause further thiol-dependent oxidative DNA damage via the Fenton reaction with excess thiols that regenerate RSSH intermediates which are readily oxidized. (Scheme 1.14) The facile oxidation of hydrodisulfides (RSSH) is resulted from the fact that this moiety (pKa ~ 6.2) exists predominantly in the deprotonated form (RSS^-) at physiological pH.[8d] The anion (RSS^-) is thermodynamically favourable in single electron oxidation when compared with its thiolate (RS^-) counterpart, and it is the active substrate for the Fenton reaction (trace-metal, oxygen-mediated oxidation) that yields hydroxyl radical ($HO^{\cdot}$) as the culprit of DNA cleavage. And this is the reason why catalase substantially quenched thiol-mediated DNA cleavage reactions of compound **I-2**.

In fact, hydrodisulfide intermediate and polysulfides generated from the reaction of thiols with our DNA-cleaving candidates **I-1** and **I-2** are essential in the initiation of the activated oxygen species formation. To understand the Fenton reaction that takes place in DNA cleavage process, the role of oxygen plays should be realized. The electron structure of oxygen makes it susceptible to form the free radical. It possesses two unpaired electrons in two π-orbitals. In the presence of catalytic metals such as copper, iron, zinc and manganese; electron donors; or free radical initiator, the oxygen molecule is then activated. Depending on the change of electronic configuration, oxygen has various reactive forms. They are free radicals, superoxide ($O_2^{\cdot-}$) and the hydroxyl

radical (HO˙); excited form but not the free radicals, singlet oxygen ($^1O_2$) and peroxide (O_2^{2-}) or hydrogen peroxide (H_2O_2).[57a] The electronic configurations of ground state and some reactive forms of oxygen are shown below.

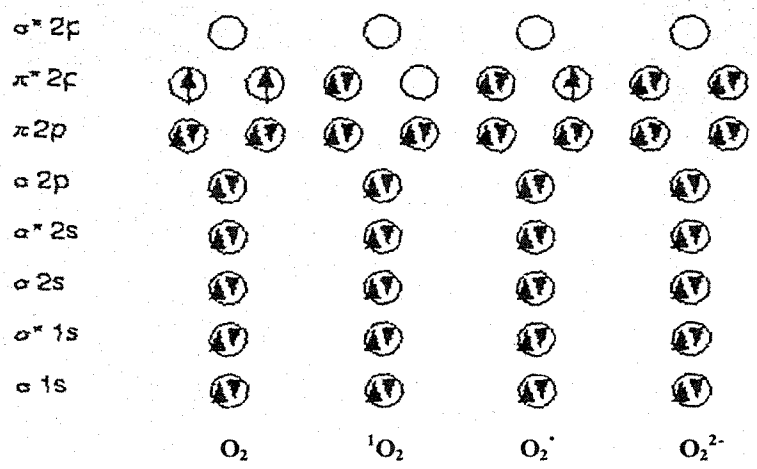


Chart 1.2 Electronic configurations of ground state oxygen molecule and some reactive forms of oxygen[57a]

Singlet oxygen has its implication in photodynamic therapy. The treatment of psoriasis with psoralens or treatment of pulmonary carcinoma with hermatoporphyrin results in the formation of singlet oxygen which is reactive enough to damage tissue.[57,58] Its life is very short but still long enough to cause damage. Superoxide radical is the first reactive oxygen species formed during the univalent reduction of oxygen. It forms when an oxygen molecule capture an electron and localizes on the anti-bonding orbital. Thus, superoxide is an anionic radical and exhibits paramagnetic property.[59] Superoxide can be produced *in vivo* during exposure of hydrogen peroxide to UV. Dismutation reaction of superoxides is comprised by single electron transfer from one superoxide radical to another. This ends up with the production of hydrogenperoxide and oxygen. Superoxide dismutase (SOD) is the enzyme using trace amount of metals mentioned before to

catalyze dismutation. The equations below summarize the reactions of superoxide dismutation.[53-57,60]

$$O_2^{\cdot} + O_2^{\cdot} + 2H^+ \rightarrow H_2O_2 + O_2$$

$$O_2^{\cdot} + M^{n+} \rightarrow M^{(n-1)+} + O_2$$

$$O_2^{\cdot} + M^{(n-1)+} \rightarrow M^{n+} + H_2O_2$$

Superoxide radical involves in Harber-Weiss reaction[8,53-58] and a mixture of oxygen, hydroxyl radical is formed as shown below:

$$O_2^{\cdot} + H_2O_2 \rightarrow O_2 + HO^- + HO^{\cdot}$$

The biological significant Fenton reaction (equation below), a variant of Harber-Weiss reaction takes place, consists in the formation of hydroxyl radical (HO$^{\cdot}$) which is the most powerful among the reactive oxygen species. This radical is capable of destroying any organic molecule and living systems. It is believed that the hydroxyl radical is generated by our thiol-mediated DNA-cleaving agents **I-1** and **I-2** via Fenton reaction.[8,10,53-58]

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^- + HO^{\cdot}$$

Hydrogen peroxide forms mainly in cellular electron transport in mitochondria.[57a,61] Owing to its oxidative properties, cells have evolved a mechanism to get rid of its harm. Fenton reaction and iron-containing enzymes are responsible to the decomposition of hydrogen peroxide. The equation below summarizes the catalysis carried out by catalase.[8,10,53]

$$H_2O_2 + H_2O_2\text{-catalase-}Fe^{3+} \rightarrow O_2 + H_2O$$

And this equation is the basis of our quenching study when using catalase to probe the hydroxyl radical present in our DNA-cleaving procedure. The existence of hydroxyl radicals in our reaction system and other living systems react rapidly with any organic compound (RH), including DNA molecules, proteins, carbohydrates, lipids. Then the carbon center radicals or other kinds of radical arise.[53,55-57,62]

$$HO^{\cdot} + RH \rightarrow R^{\cdot} + H_2O$$

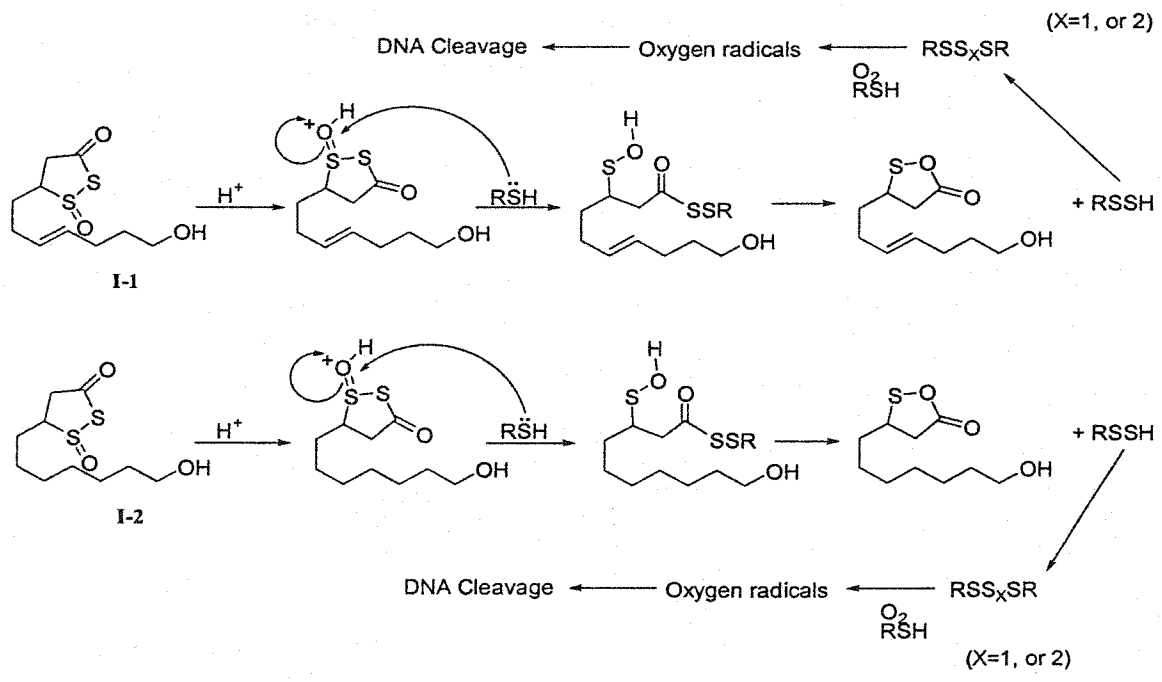
The reactivity of hydroxyl radical exceeds superoxide radical and is among the highest in all chemistry. Formation of hydroxyl radical in close proximity (approx 200 nm) to biological molecules, such as DNA or regulating enzymes, is likely to cause oxidative damage. The reaction of $HO^{\cdot}$ with organic molecules leads to formation of organic peroxides ($RO_2^{\cdot}$), this peroxidation once began, the free radical initiation and propagation will be followed.[57,63]

$$ROO^{\cdot} + RH \rightarrow R^{\cdot} + ROOH$$

$$ROO^{\cdot} + RSH \rightarrow RS^{\cdot} + ROOH$$

Figures 1.9 and 1.10 show the pH-dependent DNA cleavage of **I-1** and **I-2**, thiol-mediated DNA-cleavage by the agents reached their maximum at pH 5.5 (lane 3) then diminished slowly to their minimum at pH 8.0 (lane 11). Since thiol has pKa's of *ca.* 8, we would not expect to see a pH-dependent DNA-cleaving behavior caused by thiols.[10,52] We assured that the profile of pH dependence of the agent's DNA-strand

cleavage was related to the acid-catalysed ring opening of the 1,2-dithiolan-3-one 1-oxide entity as if our proposed mechanism (Scheme 1.15). The proton present in relatively acidic physiological relevant condition, i.e. pH 5.5 protonates 1,2-dithiolan-3-one 1-oxide heterocycle at the 1-oxide position. The resulting entity undergoes nucleophilic attack by thiol on the sulfenyl sulfur more readily than the neutral form. We assumed this simply change of ionic properties of the molecules could facilitate the complicated and consequent succession of DNA strands damaging. On the other hand, the degree of DNA cleavage by both our candidates in pH 7 and pH 8 conditions did not change a lot. This may suggest that presence of OH^- has little interference on the system.



Scheme 1.15 Plausible mechanism for pH-dependent thiol-mediated DNA damaging by **I-1** and **I-2**

In addition, we have attempted to remove the dissolved atmospheric gases from the samples by "freeze-pump-thaw" technique which is a routine practice in synthetic organic chemistry for solvent degassing.[64] We found that, however, there was no significant diminishing of DNA cleavage. This result is different from Gates's observation,[8b] and we are quite sure that the forementioned method may not be adequate for removal of dissolved oxygen in the only 20 μL reaction mixture, especially when the solvent is an aqueous buffer which have the high polarity and that gases are tightly trapped in liquid and solid phases. (Water's physical property is featured by the increase of total volume in solid phase, so the dissolved gases won't be unleashed during freezing. It is different from those cases of organic solvents that subjected to "freeze-pump-thaw" technique for degassing in routine organic synthesis.)

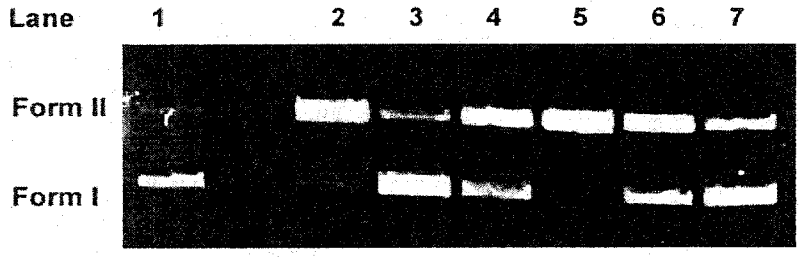


Figure 1.8 Thiol-dependent cleavage of supercoiled pBR322 DNA by 100 μM **I-1** and **I-2** in 500 μM 2-mercaptoethanol. Reactions were incubated at 37 °C for 18 h in 50 mM sodium phosphate, pH 6.0, containing 10% v/v acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, pBR322 DNA alone, lane 2, 100 μM **I-1** + 500 μM 2-mercaptoethanol; lane 3, 100 μM **I-1** + 500 μM 2-mercaptoethanol + 10 mM imidazole; lane 4, 100 μM **I-1** + 500 μM 2-mercaptoethanol + 26.6 μg/mL catalase; lane 5, 100 μM **I-2** + 500 μM 2-mercaptoethanol; lane 6, 100 μM **I-2** + 500 μM 2-mercaptoethanol + 10 mM imidazole; lane 7, 100 μM **I-2** + 500 μM 2-mercaptoethanol + 26.6 μg/mL catalase.

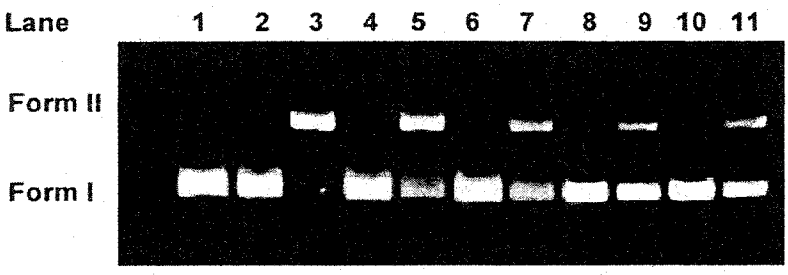


Figure 1.9 pH-dependent cleavage of supercoiled pBR322 DNA by 60 μM **I-1** in 300 μM 2-mercaptoethanol at various pH values. Reactions were incubated at 37 °C for 5 h in 50 mM sodium phosphate, containing 10% v/v acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, pBR322 alone; lane 2, 300 μM 2-mercaptoethanol at pH 5.5; lane 3, 60 μM **I-1** + 300 μM 2-mercaptoethanol at pH 5.5; lane 4, 300 μM 2-mercaptoethanol at pH 6.0; lane 5, 60 μM **I-1** + 300 μM 2-mercaptoethanol at pH 6.0; lane 6, 300 μM 2-mercaptoethanol at pH 6.5; lane 7, 60 μM **I-1** + 300 μM 2-mercaptoethanol at pH 6.5; lane 8, 300 μM 2-mercaptoethanol at pH 7.0; lane 9, 60 μM **I-1** + 300 μM 2-mercaptoethanol at pH 7.0; lane 10, 300 μM 2-mercaptoethanol at pH 8.0; lane 11, 60 μM **I-1** + 300 μM 2- mercaptoethanol at pH 8.0.

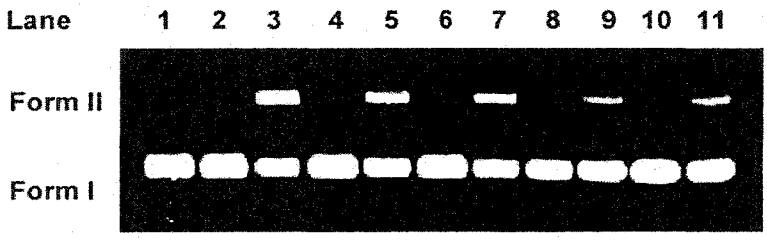


Figure 1.10 pH-dependent cleavage of supercoiled pBR322 DNA by 100 μM **I-2** in 500 μM 2-mercaptoethanol at various pH values. Reactions were incubated at 37 °C for 7 h in 50 mM sodium phosphate, containing 10% v/v acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, pBR322 alone; lane 2, 500 μM 2-mercaptoethanol at pH 5.5; lane 3, 100 μM **I-2** + 500 μM 2-mercaptoethanol at pH 5.5; lane 4, 500 μM 2-mercaptoethanol at pH 6.0; lane 5, 100 μM **I-2** + 500 μM 2-mercaptoethanol at pH 6.0; lane 6, 500 μM 2-mercaptoethanol at pH 6.5; lane 7, 100 μM **I-2** + 500 μM 2-mercaptoethanol at pH 6.5; lane 8, 500 μM 2-mercaptoethanol at pH 7.0; lane 9, 100 μM **I-2** + 500 μM 2-mercaptoethanol at pH 7.0; lane 10, 500 μM 2-mercaptoethanol at pH 8.0; lane 11, 100 μM **I-2** + 500 μM 2-mercaptoethanol at pH 8.0.

I.3 Conclusion

In summary, these studies show the novel synthesized molecules **I-1** and **I-2** can function as the thiol-mediated DNA cleaving agents. It is evident that the mimics of leinamycin provide us a new insight into the evaluation of leinamycin DNA cleaving mechanisms: **I-1** acts as leinamycin mimic with 1,2-dithiolan-3-ones 1-oxide embedding C6-C7 olefinic double bond, cleaving the DNA in both radical and alkylation mechanisms; **I-2** acts as the mimic with the heterocyclic moiety cleaving DNA in radical mechanism.[26,27] Moreover, the pH dependence of the agents suggests that leinamycin DNA cleaving activities involving the ring opening of 1,2-dithiolan-3-ones 1-oxide. Thus, **I-1**, **I-2** and their structural derivatives can be used for the design of new DNA-cleaving agents with innumberable potential uses in biochemical and chemotherapeutic applications.[65] Our approach advanced the mechanistic and DNA cleavage activities studies of the novel antitumor antibiotic leinamycin by rivaling the complexity and diversity of natural product with challenging development of rapid and effective synthetic pathway of DNA cleavers mimicking this antibiotic. With the intention of covalently links the core functionality of leinamycin to certain DNA-binding agents for the future uses, **I-34** and **I-35** were also synthesized. The activated ester linker **I-31** was prepared from the reaction of adipic acid with *N*-hydroxysuccinimide, and this was condensed with compounds **I-17** and **I-29** to yield **I-34** and **I-35** after subsequent *S*-oxidation by *m*CPBA.[26,27] The continuous studies about linking the DNA cleavers to the selected antisense oligonucleotides with $H_2NC_6H_{12}$-modification at its 3' end are now under investigation in our laboratory. In such a way, the mimics are not only brought closely to target DNA but also confined to specific region within the target DNA duplex.

I. 4 Experimental

I.4.1 Organic Synthesis of Leinamycin's Mimics

General Information

^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker Advance DPX 400 FT-NMR Spectrometer or Varian Unity INOVA 500 FT-NMR Spectrometer as solutions in $CDCl_3$ unless otherwise indicated, and the chemical shifts are reported in parts per million (ppm, δ). Coupling constants are reported in hertz (Hz). Low resolution mass spectra were obtained on Fisons VG Platform Mass Spectrometer, or Hewlett Packard G1800C GCD Series II GCMS. High resolution mass spectra (HRMS) were measured by Finnigan MAT95 and Thermo Finnigan MAT95XL Mass Spectrometers. IR spectra were recorded on Nicolet Avatar 360 FT-IR Spectrometer as a thin film on the polished NaCl plates and the characteristic bands are reported in reciprocal centimeters (cm^{-1}). All reactions were monitored by thin layer chromatography (TLC) performed on Merck precoated silica gel 60 F_{254} plates. Flash chromatography was carried out on columns of NA or Merck silica gel 60 (230-400 mesh). THF was distilled from Na/benzophenone and dichloromethane was distilled from CaH_2 respectively prior to use. Unless otherwise noted, materials and solvents were obtained from commercial suppliers and used without further purification.

4-*tert*-Butyldimethylsiloxy-*n*-butan-1-ol (I-4). A solution of **I-3** (23.0 mL, 0.25 mol) in THF (40 mL) was added to a suspension of sodium hydride (11.20 g, 0.28 mol) in THF (600 mL), and the mixture was stirred at room temperature for 2.5 h. *tert*-

Butyldimethylsilyl chloride (42.2 g, 0.28 mol) in THF (60 mL) was added and the mixture was stirred at room temperature overnight followed by the addition of saturated aqueous K_2CO_3 (300 mL). The mixture was extracted with diethyl ether (3× 250 mL) and the combined organic extracts were washed with brine (150 mL), dried (Na_2SO_4) and concentrated in *vacuo* to yield the known compound,[24] **I-4** (57.42 g, 99%) as a colorless syrup: IR (film) 3682, 3611, 3339, 3017, 2930, 2858, 1424, 1265, 1219, 1091, 1025, 784, 666 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 3.65 (m, 4H), 1.64 (m, 4H), 0.90 (s, 9H), 0.07 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 63.3, 62.8, 30.2, 29.9, 25.9, 18.3, -5.5; ESIMS *m/z* (%): 205 ($[M+1]^+$, 100).

Preparation of pyridinium dichromate (PDC). Pyridine (80.6 mL, 1.0 mol) was gradually added to a cooled solution of (100 g, 1.0 mol) of CrO_3 in 100 mL of water at < 30 °C. The solution was stirred for 1 h, and followed by adding acetone (400 mL). The solution was then cooled to -18°C until the PDC was fully crystallized. The orange crystals were collected by suction filtration, washed with cold acetone (30 mL) and dried in *vacuo* to yield an essentially pure PDC (127.5 g, 68%)[29] as a bright orange crystal.

4-*tert*-Butyldimethylsiloxy-1-butyraldehyde (I-5). A solution of **I-4** (31.5 g, 0.15 mol) in distilled CH_2Cl_2 (80 mL) was added to a mixture of PDC (84.6 g, 0.23 mol) and activated molecular sieves (type 5A powder, 25 g) in distilled CH_2Cl_2 (200 mL), and the mixture was stirred for 8 h. Diethyl ether (50 mL) was added, and the ethereal mixture was suction-filtered and the filter-cake was washed with diethyl ether and EtOAc. The organics were concentrated in *vacuo* and the residue was purified by flash

chromatography (hexane: EtOAc 20:1) to afford **I-5**[25] (29.6 g, 77%) as a colorless oil: IR (film) 3058, 2991, 2955, 2924, 2853, 1716, 1424, 1271, 1096, 892, 840, 743 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 9.79 (s, 1H), 3.65 (t, 2H, J=6.0 Hz), 2.50 (td, 2H, J=7.0, 1.5 Hz), 1.86 (quintet, 2H, J=6.5 Hz), 0.88 (s, 9H), 0.04 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 202.6, 62.0, 40.7, 25.8, 25.4, 18.2, -5.5; ESIMS *m/z* (%): 203 ($[M+1]^+$, 8), 187 (100); HREIMS calcd for $C_{10}H_{21}O_2Si$ $[M-H]^+$ 201.1305; found: 201.1305.

6-*tert*-Butyldimethylsiloxy-hex-1-en-3-ol (I-6). A few drops of iodomethane were added to a mixture of magnesium turnings (2.8 g, 0.106 mol) and distilled THF (10 mL) followed by the addition of 1 M of vinyl bromide in THF (92.0 mL). After reflux for 1 h, the resultant Grignard reagent was cooled to room temperature, filtered and transferred to a different flask by cannula. A solution of **I-5** (15.6 g, 76.7 mmol) in distilled THF (35.0 mL) at –70 °C was added to this filtered Grignard reagent, and the mixture was stirred at the same temperature for 1 h. The mixture was further stirred overnight while the solution was allowed to warm up to room temperature. Saturated aqueous NH_4Cl (50 mL) was added into the white turbid solution chilled in an ice bath. The aqueous phase was extracted with EtOAc (3× 100 mL) and the combined organic extracts were washed with water (50 mL) and brine (2× 50 mL), dried (Na_2SO_4) and concentrated in *vacuo* to give a yellow syrup. Purification with flash column chromatography (hexane: EtOAc 15:1) to afford **I-6** (15.3 g, 86%) as a slightly yellow oil: IR (film) 3442, 3345, 3017, 2960, 2925, 2847, 1465, 1255, 1209, 1096, 927, 835, 753, 661 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.87 (m, 1H), 5.23 (d, 1H, J=17.5 Hz), 5.09 (d, 1H, J=10.5 Hz), 4.12 (m, 1H), 3.66 (t, 2H, J=6.0 Hz), 1.63 (m, 4H), 0.90 (s,

9H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 141.2, 114.3, 72.6, 63.4, 34.4, 28.7, 25.9, 18.3, -5.4; ESIMS *m/z* (%): 231 ($[M+1]^+$, 100), 213 (24), 205 (31), 195 (20), 187 (12); HRESIMS calcd for $C_{12}H_{27}O_2Si$ $[M+H]^+$ 231.1773; found: 231.1775.

8-*tert*-Butyldimethylsiloxy-oct-4-enoic acid ethyl ester (I-7). A mixture of **I-6** (12.7 g, 55.1 mmol), triethyl orthoacetate (80 mL, 0.496 mol) and catalytic amount of propanoic acid (0.41 mL, 5.50 mmol) was refluxed for 4 h followed by the removal of triethyl orthoacetate. The residues were dissolved in EtOAc (80 mL), and the mixture was extracted with brine (2× 30 mL), dried (Na_2SO_4) and concentrated in *vacuo*. The residue was purified by flash column chromatography (hexane: EtOAc 25:1) to afford **I-7** (15.9 g, 96%) as a yellow syrup: IR (film) 3012, 2955, 2925, 2848, 1721, 1521, 1470, 1255, 1214, 1086, 1040, 835, 748, 661cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.43 (m, 2H), 4.12 (q, 2H, *J*=7.0 Hz), 3.58 (t, 2H, *J*=6.4 Hz), 2.32 (m, 4H), 2.02 (q, 2H, *J*=6.3 Hz), 1.55 (quintet, 2H, *J*=6.6 Hz), 1.26 (t, 3H, *J*=11.0 Hz), 0.88 (s, 9H), 0.03 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.3, 131.1, 128.3, 62.5, 60.2, 34.4, 32.5, 28.7, 27.9, 25.9, 18.3, 14.2, -5.3; ESIMS *m/z* (%): 301 ($[M+1]^+$, 100), 169 (25); HRCIMS calcd for $C_{16}H_{31}O_3Si$ $[M-H]^-$ 299.2034; found: 299.2048.

8-*tert*-Butyldimethylsiloxy-oct-4-en-1-ol (I-8). $LiAlH_4$ (2.34 g, 58.8 mmol) was added to a solution of **I-7** (16.08 g, 53.5 mmol) in distilled THF (150 mL) at 0 °C, and the solution was stirred at room temperature for 1.5 h. EtOAc (20 mL) and 10% aqueous HCl were added and the mixture was extracted with EtOAc (3× 90 mL). The combined extracts were washed with water (40 mL) and brine (40 mL), dried (Na_2SO_4) and

concentrated in *vacuo*. The residue was purified by flash column chromatography (hexane: EtOAc 4:1) to give **I-8** (13.41 g, 97%) as a colorless oil: IR (film) 3621, 3452, 3350, 3017, 2935, 2848, 1255, 1209, 1091, 835, 739, 661 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.44 (m, 2H), 3.65 (t, 2H, J=6.5 Hz), 3.60 (t, 2H, J=6.5 Hz), 2.05 (m, 4H), 1.63 (quintet, 2H, J=6.5 Hz), 1.55 (quintet, 2H, J=6.5 Hz), 0.89 (s, 9H), 0.04 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 130.6, 129.8, 62.6, 32.6, 32.4, 28.9, 28.8, 25.9, 18.3, -5.3; ESIMS *m/z* (%): 258 (M^+, 18), 209 (14), 187 (29), 177 (11), 145 (59), 127 (100); HRFABMS calcd for $C_{14}H_{31}O_2Si$ $[M+H]^+$ 259.2088; found: 259.2086.

8-*tert*-Butyldimethysiloxy-oct-4-en-1-al (I-9). A solution of **I-8** (8.99 g, 34.80 mmol) in distilled CH_2Cl_2 (120 mL) was added to a mixture of PDC (19.64 g, 52.20 mmol) and activated molecular sieves (type 5A powder, 7.2 g) in distilled CH_2Cl_2 (250 mL) at room temperature, and the mixture was stirred at the same temperature for 3 h. Diethyl ether (90 mL) was added, and the ethereal phase was suction-filtered. The combined organics were concentrated in *vacuo* and the residue was purified by flash column chromatography (hexane: EtOAc 20:1) to yield **I-9** (6.25 g, 70%) as a colorless oil: IR (film) 3360, 3022, 2929, 2853, 1726, 1470, 1260, 1209, 1096, 835, 758, 666 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 9.76 (s, 1H), 5.44 (m, 2H), 3.58 (t, 2H, J=6.5 Hz), 2.49 (td, 2H, J=7.0, 1.5 Hz), 2.32 (q, 2H, J=7.0 Hz), 2.03 (q, 2H, J=7.5 Hz), 1.55 (quintet, 2H, J=7.5 Hz), 0.88 (s, 9H), 0.04 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 202.4, 131.4, 127.9, 62.5, 43.5, 43.4, 32.4, 28.7, 25.9, 25.2, 18.3, -5.3; ESIMS *m/z* (%): 257 ($[M+1]^+$, 100), 228 (20), 125 (12); HRESIMS calcd for $C_{14}H_{29}O_2Si$ $[M+H]^+$ 257.1929; found: 257.1917.

Ethyl (*E*)-10-*tert*-butyldimethylsiloxy-dec-2-enoate (I-10). Triethyl phosphonoacetate (7.2 mL, 36.32 mmol) was added to NaH (2.61 g, 32.40 mmol) in THF (100 mL), and the solution was stirred at room temperature for 1 h followed by the addition of **I-9** (7.76 g, 30.27 mmol) in THF (60 mL). The mixture was heated at reflux for 1 h and allowed to cool down to room temperature. After removal of the solvents, the residue was dissolved in CH_2Cl_2, and the mixture was washed with water (30 mL) and brine (40 mL), dried (Na_2SO_4) and concentrated in *vacuo*. The residue was purified by flash column chromatography (hexane: EtOAc 60:1) to afford **I-10** (7.41 g, 75%) as a slightly yellow syrup: IR (film) 3017, 2930, 2853, 1711, 1655, 1470, 1368, 1250, 1209, 1096, 1035, 840, 743, 661 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.95 (s, 1H), 5.81 (dd, 1H, J=15.5, 1.5 Hz), 5.42 (m, 2H), 4.18 (q, 2H, J=7.0 Hz), 3.59 (t, 2H, J=7.0 Hz), 2.25 (q, 2H, J=7.0 Hz), 2.14 (q, 2H, J=6.0 Hz), 2.03 (q, 2H, J=6.5 Hz), 1.56 (quintet, 2H, J=7.0 Hz), 1.28 (t, 3H, J=6.5 Hz), 0.89 (s, 9H), 0.04 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.7, 148.6, 131.1, 128.7, 121.5, 62.5, 60.1, 32.5, 32.2, 32.2, 30.9, 28.7, 25.9, 18.3, 18.3, 14.3, -5.3; ESIMS *m/z* (%): 327 ($[M+1]^+$, 13), 277 (10), 255 (12), 235 (13), 214 (9), 213 (100), 167 (22); HRCIMS calcd for $C_{18}H_{33}O_3Si$ $[M-H]^-$ 325.2204; found: 325.2204.

(*E*)-10-*tert*-Butyldimethylsiloxy-dec-2-enoic acid (I-11). LiOH (25.52 g, 0.340 mol) was added to a solution of **I-10** (18.64 g, 56.74 mmol) in ethanol (120 mL) and water (5 mL), and the mixture was stirred at room temperature for 22 h. The mixture was filtered and the filter-cake was washed with EtOAc. The combined organics were concentrated in *vacuo* and the residue was dissolved in water (30 mL). The aqueous solution was acidified to pH 4-5 by adding 10% aqueous HCl. The mixture was extracted with

CH_2Cl_2 (3× 90 mL) and brine (30 mL), dried (Na_2SO_4) and concentrated in *vacuo*. The crude product was purified by flash column chromatography (hexane: EtOAc 10:1) to afford **I-11** (14.83 g, 87%) as a white solid: mp 35.0-36.8 °C; IR (film) 3611, 3442, 3349, 3017, 2925, 2848, 1695, 1655, 1429, 1214, 1096, 1045, 835, 753, 656 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.06 (m, 1H), 5.83 (dt, 1H, J=15.5, 2.0 Hz), 5.43 (m, 2H), 3.60 (t, 2H, J=6.5 Hz), 2.28 (q, 2H, J=6.5 Hz), 2.16 (q, 2H, J=7.0 Hz), 2.04 (q, 2H, J=7.0 Hz), 1.56 (quintet, 2H, J=6.5 Hz), 0.85 (s, 9H), 0.04 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.7, 151.6, 131.3, 128.5, 120.9, 62.6, 32.5, 32.3, 30.8, 28.7, 25.9, 18.3, -5.3; ESIMS *m/z* (%): 298 ($[M+1]^+$, 24), 227 (11), 213 (31), 186 (8), 185 (100), 167 (40); HRESIMS calcd for $C_{16}H_{30}O_3Si$ $[M+H]^+$ 298.1956; found: 298.1951.

3-Benzylsulfanyl-10-*tert*-butyldimethylsiloxy-dec-6-enoic acid (I-12). A mixture of **I-11** (12.12 g, 40.33 mmol), toluene-α-thiol (5.51 mL, 44.36 mmol) and freshly distilled piperidine (30 mL) was refluxed under nitrogen overnight. The mixture was chilled in an ice bath followed by the addition of 10% aqueous HCl to allow the pH of the aqueous solution to reach 2 to 3. The suspension was extracted with diethyl ether (3× 100 mL) and the combined extracts were washed with brine (3× 40 mL), dried (Na_2SO_4) and concentrated in *vacuo*. The residue was purified by column chromatography (hexane: EtOAc 10:1 initially and 3:1 subsequently) to afford **I-12** (12.21 g, 72%) as a yellow oil: IR (film) 3683, 3339, 3017, 2935, 2848, 1706, 1521, 1419, 1209, 1096, 968, 922, 748, 661 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.31 (m, 4H), 7.22 (m, 1H), 5.33 (m, 2H), 3.75 (s, 2H), 3.59 (t, 2H, J=6.5 Hz), 2.97 (quintet, 1H, J=6.0 Hz), 2.60 (dd, 2H, J=7.0, 2.5 Hz), 2.05 (m, 4H), 1.60 (m, 4H), 0.90 (s, 9H), 0.05 (s, 6H); ^{13}C NMR ($CDCl_3$, 100

MHz) δ 176.7, 138.5, 130.8, 130.5, 129.6, 129.1, 128.9, 128.5, 127.0, 62.6, 40.6. 35.5, 34.6, 32.5, 29.6, 28.8, 25.9, 25.6, 18.3, -5.3; ESIMS *m/z* (%): 423 ($[M+1]^+$,29), 422 (M^+, 29), 377 (12), 376 (80), 310 (14), 309 (100), 252 (83), 227 (30), 219 (40), 213 (39), 190 (44), 167 (14); HRCIMS calcd for $C_{23}H_{37}O_3SSi$ $[M-H]^-$ 421.2238; found: 421.2246.

10-*tert*-Butyldimethylsiloxy-3-mercapto-dec-6-enoic acid (I-13). A flask equipped with a stirrer, calcium chloride drying tube, nitrogen inlet, dry ice condenser and ammonia inlet was flushed thoroughly with a stream of nitrogen. A solution of **I-12** (0.791 g, 1.878 mmol) in distilled THF (8 mL) was added to the flask chilled at –78 °C. Gaseous ammonia (ca 25 mL) was passed through and condensed into the flask at the same temperature. Finely divided metal lithium was added and a deep blue color of the solution persisted. The mixture was stirred at –78 °C for 30 min followed by the addition of solid NH_4Cl. After removal of remaining ammonia by a stream of nitrogen, the residue was dissolved in water (20 mL) and the mixture was acidified by the addition of 15 % aqueous HCl to allow the pH of the aqueous solution to reach 2 to 3. The mixture was extracted with CH_2Cl_2 (3× 50 mL) and brine (25 mL), dried (Na_2SO_4) and concentrated in *vacuo* to afford **I-13** (0.596 g, 96%) as a colorless syrup: IR (film) 3395, 3012, 2924, 2585, 1711, 1516, 1465, 1409, 1209, 1045, 840, 758, 666 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.42 (m, 2H), 3.60 (t, 2H, J=6.5 Hz), 3.18 (m, 1H), 2.73 (dd, 1H, *J*=16.5, 5.5 Hz), 2.57 (dd, 1H, *J*=16, 8.5 Hz), 2.11 (m, 4H), 1.70 (d, 1H, *J*=8.0 Hz), 1.57 (m, 4H), 0.89 (s, 9H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 176.8, 131.2, 128.7, 62.6, 44.0, 37.9, 35.5, 32.5, 29.9, 28.8, 25.9, 25.6, 18.3, -5.3; ESIMS *m/z* (%): 332 (M^+,

12), 330 (22), 286 (8), 285 (14), 284 (78), 227 (100), 217 (41), 146 (20), 139 (18), 129 (8); HRESIMS calcd for $C_{16}H_{34}O_3SSi$ $[M+2]^+$ 334.1989; found: 334.2010.

4-(7-*tert*-Butyldimethysiloxyhept-3-enyl)thietan-2-one (I-14). Freshly distilled isobutyl chloroformate (1.40 mL, 10.76 mmol) was added to a solution of **I-13** (3.23 g, 9.786 mmol) and Et_3N (1.27 mL, 11.74 mmol) in CH_2Cl_2 (25 mL) at –10 °C. The mixture was stirred for 15 min while its temperature was allowed to warm up to 0 °C. The mixture was diluted with CH_2Cl_2 (25 mL) and washed with cold 15 % aqueous HCl (3× 15 mL), water (20 mL) and brine (20 mL). The organic phase was dried (Na_2SO_4) and concentrated in *vacuo*. The resultant residue was purified by flash column chromatography (hexane: EtOAc 15:1) to yield **I-14** (1.378 g, 56%) as a slightly yellow oil: IR (film) 3053, 2980, 2925, 2853, 1747, 1655, 1419, 1265, 1096, 979, 886, 840, 753, 702 cm^{-1}; ^1H NMR (C_6D_6, 500 MHz) δ 5.28 (m, 1H), 5.17 (m, 1H), 3.54 (t, 2H, J=6.5 Hz), 3.24 (q, 1H, J=7.0 Hz), 2.81 (q, 1H, J=4 Hz), 2.77 (m, 1H), 2.06 (q, 2H, J=7.0 Hz), 1.70 (m, 2H), 1.56 (quintet, 2H, J=6.5 Hz), 1.32 (q, 2H, J=8.0 Hz), 0.99 (s, 9H), 0.07 (s, 6H); ^1H NMR ($CDCl_3$, 500 MHz) δ 5.46 (m, 1H), 5.39 (m, 1H), 4.01 (q, 1H, J=10 Hz), 3.60 (t, 2H, J=6.5 Hz), 3.52 (m, 2H), 2.06 (m, 5H), 1.90 (m, 1H), 1.57 (quintet, 2H, J=6.5 Hz), 0.89 (s, 9H), 0.04 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 190.9, 131.8, 128.1, 62.5, 60.6, 37.6, 32.7, 32.6, 32.5, 31.7, 28.8, 25.9, 18.3, -5.3; ESIMS *m/z* (%): 315 ($[M+1]^+$, 100), 273 (71), 257 (89), 183 (46), 149 (14), 141 (39); HRCIMS calcd for $C_{16}H_{29}O_2SSi$ $[M-H]^-$ 313.1663; found: 313.1657.

10-*tert*-Butyldimethylsiloxy-3-mercapto-dec-6-enethioic acid (I-15). A stirred solution of the thiolactone **I-14** (1.021 g, 3.247 mmol) in CCl_4 (20 mL) at ca –35 °C was saturated with hydrogen sulfide gas, and the mixture was stirred at the same temperature for 20 min. Freshly distilled Et_3N (0.55 mL, 4.871 mmol) was added to the reaction mixture through a hypodermic syringe. The solution was further saturated with H_2S gas for 8 h at the same temperature. The resultant mixture was diluted with $CHCl_3$ (20 mL) and washed with 10% aqueous HCl (2× 10 mL), water (10 mL) and brine (10 mL), dried (Na_2SO_4) and concentrated in *vacuo* to give **I-15** (0.996 g, 88%) as a yellow oil which was subjected to the oxidative reaction in the next step without further purification.

Preparation of sodium iodate supported on neutral alumina. Sodium iodate (0.4 g, 2mmol) was added to 5 mL of distilled water in a 50 mL round-bottom flask, and the mixture was warmed up to allow the solution to become transparent. 1.0 g of alumina was added to the mixture, and the mixture was shaken for 30 min followed by the removal of solvent in *vacuo*.

5-(7-*tert*-Butyldimethysiloxyhept-3-enyl)-1,2-dithiolan-3-one (I-16). Excess $NaIO_3/Al_2O_3$ - $NaIO_3$ (8.1 g) was added to **I-15** (0.996 g, 2.854 mmol) in a mixture of hexane and $CHCl_3$ (16 mL: 2 mL), and the mixture was stirred at room temperature for 40 min followed by suction filtration. The filter-cake was washed with CH_2Cl_2 (50 mL). The combined organics were concentrated in *vacuo*, and the residue was purified by flash column chromatography (hexane: EtOAc 15:1) to afford **I-16** (0.791 g, 80%) as a slightly yellow oil: IR (film) 3012, 2935, 2858, 1705, 1470, 1429, 1388, 1255, 1214,

1096, 968, 840, 763, 661, 615 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.50 (m, 1H), 5.39 (m, 1H), 3.70 (quintet d, 1H, *J*=7.3, 2.5 Hz), 3.60 (t, 2H, *J*=6.5 Hz), 3.00 (dd, 1H, *J*= 16, 5.5 Hz), 2.67 (dd, 1H, *J*=16.5, 8.5 Hz), 2.17 (quintet, 2H, *J*= 6.5 Hz), 2.03 (q, 2H, *J*=6.5 Hz), 1.82 (m, 2H), 1.57 (quintet, 2H, *J*=7.0 Hz), 0.89 (s, 9H), 0.04 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 206.5, 132.1, 127.9, 62.5, 49.2, 48.3, 32.5, 32.4, 30.6, 28.8, 25.9, 19.1, 18.3, -5.3; ESIMS *m/z* (%): 350 (20), 347 ($[M+1]^+$, 14), 339 (38), 307 (68), 275 (84), 257 (100), 233 (54), 199 (63), 193 (40), 161 (49); HRCIMS calcd for $C_{16}H_{30}O_2S_2Si$ $[M]^-$ 346.1462; found: 346.1460.

5-(7-Hydroxyhept-3-enyl)-1,2-dithiolan-3-one (I-17). 15% Aqueous HCl was added to a solution of **I-16** (0.439 g, 1.267 mmol) in absolute EtOH (15 mL), and the mixture was stirred at room temperature for 30 min followed by the addition of Et_3N (0.5 mL). The neutralized solution was extracted with CH_2Cl_2 (20 mL) and washed with water (10 mL) and brine (10 mL). The extracts were dried (Na_2SO_4) and concentrated in *vacuo*. The residue was purified by flash chromatography (hexane: EtOAc 5:1) to afford **I-17** (0.207 g, 71%) as a yellow syrup: IR (film) 3688, 3606, 3447, 3345, 3022, 2919, 2858, 1721, 1404, 1214, 1091, 1045, 835, 758, 677 cm^{-1}; ^1H NMR (C_6D_6, 500 MHz) δ 5.26 (m, 1H), 5.10 (m, 1H), 3.38 (t, 2H, *J*=6.0 Hz), 2.92 (m, 1H), 2.28 (dd, 1H, *J*=15.5, 5.0 Hz), 1.97 (m, 3H), 1.73 (m, 2H), 1.44 (quintet, 2H, *J*=7.0 Hz), 1.33 (m, 1H), 1.12 (m, 1H); ^{13}C NMR (C_6D_6, 100 MHz) δ 204.5, 131.6, 128.5, 61.9, 48.6, 48.2, 32.6, 32.3, 30.6, 29.1; ESIMS *m/z* (%): 234 ($[M+2]^+$, 31), 232 (M^+, 43), 223 (23), 217 (12), 203 (25), 201 (43), 199 (100), 185 (22), 171 (47); HRESIMS calcd for $C_{10}H_{16}O_2S_2$ $[M]^+$ 232.0683; found: 232.0687.

5-(7-Hydroxyhept-3-enyl)-1,2-dithiolan-3-one 1-oxide (I-1). A solution of 70-75% *m*-CPBA (0.480 g, 1.902 mmol) in distilled CH_2Cl_2 (6 mL) was added to a solution of 1,2-dithiolan-3-one **I-17** (0.178 g, 0.761 mmol) in distilled CH_2Cl_2 (10 mL), and the mixture was stirred at –40 to –50 °C for 30 min and warmed up to 0 °C. Saturated sodium sulfite solution was added, and the aqueous suspension was extracted with CH_2Cl_2 (3× 20 mL). The combined organic extracts were washed with saturated sodium sulfite solution (20 mL), water (20 mL) and brine (20 mL), dried (Na_2SO_4) and concentrated in *vacuo*. The resultant residue was purified by flash column chromatography (hexane: EtOAc 3:1 initially and 2:1 subsequently) to afford **I-1** (0.087 g, 46%) as a slightly yellow oil: IR (film) 3339, 3012, 2925, 2853, 1721, 1440, 1209, 1040, 758, 661 cm^{-1}; ^1H NMR (C_6D_6, 500 MHz) δ 5.25 (m, 1H), 5.06 (m, 1H), 3.32 (t, 2H, J=6.5 Hz), 2.69 (dd, 1H, J=17.0, 12.5 Hz), 2.33 (m, 1H), 2.16 (dd, 1H, J=17.0, 5.5 Hz), 1.95 (q, 2H, J=7.0 Hz), 1.69 (m, 2H), 1.53 (m, 1H), 1.42 (q, 2H, J=6.5 Hz), 1.21 (m, 1H); ^{13}C NMR (C_6D_6, 100 MHz) δ 200.1, 132.2, 63.6, 61.8, 41.7, 32.4, 29.6, 29.1, 27.7; ESIMS *m/z* (%): 249 ($[M+1]^+$,100), 217 (10); HRCIMS calcd for $C_{10}H_{16}O_3S_2$ $[M]^-$ 248.0546; found 248.0539.

8-*tert*-Butyldimethylsiloxy-*n*-octan-1-ol (I-20). 60% w/w Sodium hydride (9.0 g, 0.226 mol) was suspended in distilled THF (400 mL) followed by the addition of octane-1,8-diol **I-19** (29.70 g, 0.204 mol) in THF (150 mL). The mixture was stirred at room temperature for 2 h, large amount of opaque white precipitates were formed. After the addition of *tert*-butyldimethylsilyl chloride (34.0 g, 0.226 mol) in THF (50 mL), the mixture was stirred vigorously at room temperature overnight. The reaction was quenched by the addition of *ca* 300 mL of saturated aqueous K_2CO_3 and extracted with

diethyl ether (2× 250 mL). The combined organic extracts were washed with brine (150 mL), dried over Na_2SO_4 and concentrated in *vacuo* to afford **I-20** (35.60 g, 67%) as colorless syrup: ^{1}H NMR δ ($CDCl_3$) 0.04 (s, 6H), 0.89 (s, 9H), 1.34 (m, 6H), 1.54 (m, 6H), 3.59 (t, 2H, *J*=6.5 Hz), 3.64 (t, 2H, *J*=6.5 Hz); ^{13}C NMR δ ($CDCl_3$) -5.3, 18.4, 25.6, 25.7, 25.7, 25.9, 29.4, 32.8, 32.8, 63.1, 63.3; ESIMS *m/z* (%) 261 [$(M+1)^+$, 8], 257 (12), 169 (6), 147 (100).

8-*tert*-Butyldimethylsiloxy-*n*-octan-1-al (I-21). To a slurry mixture of PDC (61.0 g, 0.161 mol) and activated molecular sieves (type 5A powder, 20 g) in distilled CH_2Cl_2 (150 mL) was added a solution of **I-20** (28.5 g, 0.108 mol) in distilled CH_2Cl_2 (100 mL). The mixture was stirred vigorously at room temperature for 4 h. After the addition of diethyl ether (100 mL), the ethereal mixture was suction-filtered and the filter-cake was washed with diethyl ether and EtOAc. After evaporation under vacuum, the residue was purified by flash column chromatography with hexane: EtOAc (15:1, v/v) as eluent to afford **I-21** (18.16 g, 65%) as a colorless syrup: ^{1}H NMR δ ($CDCl_3$) 0.04 (s, 6H), 0.89 (s, 9H), 1.31 (bs, 6H), 1.50 (bt, 2H, *J*=6.5 Hz), 1.63 (bt, 2H, *J*=7.0 Hz), 2.42 (td, 2H, *J*=7.5, 2.0 Hz), 3.59 (t, 2H, *J*=6.5 Hz), 9.76 (t, 1H, *J*=2.0 Hz); ^{13}C NMR δ ($CDCl_3$) -5.3, 18.29, 21.9, 25.6, 25.9, 29.1, 32.7, 43.8, 63.1, 202.7; ESIMS *m/z* (%) 259 [$(M+1)^+$ 100], 257 (31); HRESIMS found 259.2225, calculated for $C_{14}H_{31}O_2Si$ 259.2257.

10-*tert*-Butyldimethylsiloxy-dec-2-enoic acid ethyl ester (I-22). Triethyl phosphonoacetate (13.0 mL, 65.8 mmol) was added dropwise to 60% w/w NaH (5.26 g, 65.8 mmol) in distilled THF (250 mL) at room temperature. The mixture was stirred at

room temperature for 2 h at that time certain heat evolutions were observable. After the addition of **I-21** (17.0 g, 65.8 mmol) in distilled THF (150 mL), the mixture was heated at reflux for 1.5 h. After cooling the mixture to room temperature, solvent was removed under reduced pressure to yield a yellow syrup. The residue was dissolved in CH_2Cl_2, washed with water (30 mL), brine (40 mL), and dried over Na_2SO_4. After evaporation under vacuum, the crude product was purified by flash column chromatography with hexane: EtOAc (30:1, v/v) as eluent to afford **I-22** (13.8 g, 64%) as a slightly yellow oil: ^{1}H NMR δ ($CDCl_3$) 0.04 (s, 6H), 0.89 (s, 9H), 1.28 (t, 3H, J=7.0 Hz), 1.30 (bs, 4H), 1.47 (m, 6H), 2.18 (m, 2H), 3.58 (t, 2H, *J*=7.0 Hz), 4.17 (q, 2H, *J*=7.5 Hz), 5.80 (dt, 1H, *J*=15.5, 1.5 Hz), 6.95 (m, 1H); ^{13}C NMR δ ($CDCl_3$) -5.3, 14.3, 18.4, 25.7, 26.0 27.9, 29.1, 29.2, 32.2, 32.8, 60.1, 63.3, 121.3, 149.5, 166.8; ESIMS *m/z* (%) 329 [$(M+1)^+$, 100]; HRESIMS found 329.2418, calculated for $C_{18}H_{37}O_3Si$ 329.2384.

10-*tert*-Butyldimethylsiloxy-dec-2-enoic acid (I-23). To a solution of **I-22** (12.67 g, 38.56 mmol) in ethanol (70 mL) and water (2 mL) was added 56% w/w LiOH (9.70 g, 231.0 mmol) at room temperature. The mixture was stirred vigorously at the same temperature for 20 h followed by filtration of the mixture through a filter paper. The filter-cake was washed with an appropriate volume of EtOAc. After evaporation under vacuum, the residue was dissolved in water (30 mL) which was acidified to pH 4-5 by adding 10% aqueous HCl. The aqueous mixture was extracted with CH_2Cl_2 (3× 90 mL). The organic phase was washed with brine (30 mL) and dried over Na_2SO_4. After evaporation under vacuum, the crude product was purified by flash column chromatography using hexane: EtOAc (10:1→ 5:1, v/v) as eluent to afford **I-23** (10.08 g,

87%) as a colorless oil which was readily formed a white crystal at room temperature: ^{1}H NMR δ ($CDCl_3$) 0.05 (s, 6H), 0.89 (s, 9H), 1.31 (bs, 5H), 1.48 (m, 5H), 2.22 (q, 2H, J=7.0 Hz), 3.60 (t, 2H, J=7.0 Hz), 5.83 (d, 1H, J=15.5 Hz), 7.08 (quintet, 1H, J=7.0 Hz); ^{13}C NMR δ ($CDCl_3$) -5.3, 18.4, 25.7, 25.9, 27.8, 29.1, 29.1, 32.3, 32.8, 63.2, 120.6, 152.4, 171.8; ESIMS *m/z* (%) 301 [$(M+1)^+$, 100], 275 (5), 187 (7), 169 (7); HRESIMS found 300.2122, calculated for $C_{16}H_{32}O_3Si$ 300.2124.

3-Benzylsulfanyl-10-(*tert*-butyldimethysiloxy)decanoic acid (I-24). A mixture of **I-23** (8.15 g, 27.1 mmol), toluene-α-thiol (3.20 mL, 27.3 mmol) and piperidine (15 mL) was heated at reflux overnight. After cooling the mixture in ice bath, 10% aqueous HCl was added to allow the pH of the solution to reach 2-3. The resultant white suspension was extracted with diethyl ether (3× 100 mL) and the combined extracts were washed with brine (3× 40 mL) and dried over Na_2SO_4. After evaporation under vacuum, the crude product was purified by flash column chromatography with hexane: EtOAc (10:1→ 3:1, v/v) as eluent to afford pure **I-24** (7.57 g , 66%) as a colorless oil: ^{1}H NMR δ ($CDCl_3$) 0.05 (s, 6H), 0.90 (s, 9H), 1.26 (m, 7H), 1.38 (m, 1H), 1.51 (m, 4H), 2.60 (m, 2H), 2.96 (quintet, 1H, J=6.0 Hz), 3.59 (t, 2H J=6.5 Hz), 3.76 (s, 2H), 7.23 (dt, 1H, J=7.0 Hz), 7.32 (m, 4H); ^{13}C NMR δ ($CDCl_3$) -5.3, 18.4, 25.3, 25.6, 25.7, 25.9, 26.5, 29.0, 29.2, 29.2, 29.7, 32.5, 32.8, 34.6, 34.8, 35.5, 40.6, 41.1, 63.3, 127.0, 128.5, 128.9, 138.2, 176.9; ESIMS *m/z* (%) 424 [$(M+1)^+$, 31], 394 (16), 379 (11), 378 (68), 327, (28), 311 (28), 256 (12), 228 (19), 227 (100); HRESIMS found 424.2430, calculated for $C_{23}H_{40}O_3SSi$ 424.2457.

10-(*tert*-Butyldimethysiloxy)-3-mercaptodecanoic acid (I-25). A flask was equipped with a stirrer, calcium chloride drying tube, nitrogen inlet, dry ice condenser and ammonia inlet. After the flask was flushed thoroughly with a stream of nitrogen, the ammonia was passed through and condensed into the flask followed by the addition of **I-24** (6.80 g, 16.0 mmol) in distilled THF (50 mL) at –78 °C. Finely divided metal lithium was added to allow the deep blue color of the solution to persist. The mixture was stirred at –60 to –78 °C for 1.5 h followed by the addition of adequate amount of solid NH_4Cl to quench the reaction. After passing N_2 through the white slurry mixture to remove the remaining ammonia at room temperature, the solid residue was dissolved in water (50 mL) which was acidified with 15 % v/v aqueous HCl to allow its pH to reach 2-3. The aqueous mixture was extracted with CH_2Cl_2 (3× 100 mL). The combined organics was washed with water (30 mL), brine (3× 25 mL), and dried over Na_2SO_4. Evaporation of solvent under vacuum afforded **I-25** (5.26 g, 98%) as a white turbid syrup with foul smell. This compound was subjected to the next step reaction without purification (this mercaptan is readily oxidized by oxygen in air): ^{1}H NMR δ ($CDCl_3$) 0.05 (s, 6H), 0.89 (s, 9H), 1.31 (bs, 6H), 1.51 (m, 5H), 1.66 (m, 1H), 1.72 (d, 1H, J=7.5 Hz), 2.57 (dd, 1H, J=16.0, 8.5 Hz), 2.74 (dd, 1H, J=16.0, 5.0 Hz), 3.19 (m, 1H), 3.60 (t, 2H, J=6.5 Hz); ^{13}C NMR δ ($CDCl_3$); -5.3, 18.4, 25.6, 25.7, 25.9, 26.9, 29.0, 29.1, 29.2, 32.8, 36.1, 38.1, 44.1, 63.3, 176.8; ESIMS *m/z* (%) 333 (M^+, 19), 327 (18), 311 (21), 275 (22), 256 (61), 228 (49), 227 (32), 211 (31), 183 (52), 143 (63), 136 (100).

4-(7-*tert*-Butyldimethysiloxyheptyl)thietan-2-one (I-26). To a solution of **I-25** (5.26 g, 15.7 mmol) and freshly distilled Et_3N (2.20 mL, 20.4 mmol) in CH_2Cl_2 (40 mL) was

added isobutyl chloroformate (2.30 mL, 17.3 mmol) at –10 °C. The mixture was stirred for 30 min at which time its temperature was allowed to warm up to reach 0 °C. It was diluted with CH_2Cl_2 (25 mL) and washed successively with cold 15 % aqueous HCl (3× 15 mL), diluted Na_2CO_3 aqueous solution (20 mL) and brine (20 mL). The combined organics were dried over Na_2SO_4. After evaporation under vacuum the residue was purified by flash column chromatography with hexane: EtOAc (20:1, v/v) as eluent to yield **I-26** (2.74 g, 56%) as a colorless syrup: ^{1}H NMR δ (C_6D_6) 0.07 (s, 6H), 0.98 (s, 9H), 1.03 (m, 2H), 1.05 (m, 2H), 1.12 (m, 2H), 1.28 (m, 4H), 1.49 (quintet, 2H, J=6.5 Hz), 2.77 (m, 1H), 2.87 (dd, 1H, J=17.5, 3.5 Hz), 3.31 (dd, 1H, J=17.0, 7.0 Hz), 3.56 (t, 2H, J=6.0 Hz); ^{13}C NMR δ (C_6D_6) -4.4, 19.2, 26.9, 29.5, 30.0, 30.2, 33.7, 33.9, 38.5, 61.6, 63.9, 189.6; ESIMS *m/z* (%) 318 [$(M+1)^+$, 16], 317 (M^+,100), 301 (8); HRESIMS found 316.1756, calculated for $C_{16}H_{32}O_2SSi$ 316.1742.

10-(*tert*-Butyldimethysiloxyheptyl)-3-mercaptodecanethioic acid (I-27). A solution of **I-6** (2.08 g, 6.58 mmol) in CCl_4 (12 mL) chilled at –30 to –40 °C was saturated with hydrogen sulfide gas. The mixture was stirred at the same temperature for 20 min followed by the addition of freshly distilled Et_3N (1.07 mL, 9.87 mmol). The resultant solution was saturated with H_2S gas for 7 h followed by the introduction of N_2 to expel the remaining H_2S gas at 0 °C. The mixture was diluted with $CHCl_3$ (20 mL) and washed with water (2× 10 mL). The aqueous mixture was acidified with 10% aqueous HCl to allow its pH to reach 1-2. The organic phase was washed with brine (2× 10 mL) and dried over Na_2SO_4. Evaporation of solvent under reduced pressure to afford **I-27** as a yellow oil with foul odor which was subjected to the oxidative coupling reaction in the

next step without further purification (this thioic acid is not stable enough for further characterization): ^{1}H NMR δ ($CDCl_3$) 0.05 (s, 6H), 0.91 (s, 9H), 0.98 (bs, 2H), 1.40 (m, 4H), 1.43 (m, 6H), 2.67 (dd, 1H, *J*=16.0, 9.0 Hz), 3.00 (dd, 1H, *J*=16.0, 5.5 Hz), 3.60 (t, 2H, *J*=14.5 Hz), 3.65 (m, 1H), 3.70 (m, 1H).

5-(7-*tert*-Butyldimethysiloxyheptyl)-1,2-dithiolan-3-one (I-28). To a solution of **I-27** (2.33 g, 6.58 mmol) in the mixture of hexane: $CHCl_3$ (24 mL: 2 mL) was added $NaIO_3/Al_2O_3$ - $NaIO_3$ (14.2 g). The mixture was stirred at room temperature for 40 min followed by filtration. The filter-cake was washed with appropriate volume of CH_2Cl_2. The filtrate was concentrated in *vacuo*. The resultant residue was purified by flash column chromatography with hexane: EtOAc (15:1, v/v) as eluent to afford **I-28** (2.01 g, 87%) as a slightly yellow oil: ^{1}H NMR δ ($CDCl_3$) 0.04 (s, 6H), 0.89 (s, 9H), 1.32 (bs, 6H), 1.49 (m, 4H), 1.78 (m, 2H), 2.67 (dd, 1H, *J*=16.0, 8.5 Hz), 3.00 (dd, 1H, *J*=16.5, 5.5 Hz), 3.60 (t, 2H, *J*=7.0 Hz), 3.70 (m, 1H); ^{13}C NMR δ ($CDCl_3$) -5.3, 18.3, 25.7, 25.9, 27.77, 29.1, 32.7, 32.9, 49.2, 49.3, 63.2, 206.5; ESIMS *m/z* (%) 349 [$(M+1)^+$, 19], 317 (33), 301 (7), 256 (11), 236 (11), 235 (100), 217 (22); HRESIMS found 348.1588, calculated for $C_{16}H_{32}O_2S_2Si$ 348.1605.

5-(7-Hydroxyheptyl)-1,2-dithiolan-3-one (I-29). To a solution of **I-28** (1.462 g, 4.201 mmol) in EtOH (20 mL) was added aqueous HCl (15%, 0.9 mL). The mixture was stirred at room temperature for 40 min followed by the addition of Et_3N (0.5 mL). The organic phase was concentrated in *vacuo*. The residue was dissolved in CH_2Cl_2 (30 mL), washed with water (20 mL), brine (20 mL) and dried over Na_2SO_4. After evaporation

under vacuum, the crude product was purified by flash column chromatography with hexane: EtOAc (5:1, v/v) to yield **I-29** (0.906 g, 92%) as a yellow syrup: ^{1}H NMR δ (C_6D_6) 0.92 (m, 3H), 1.01 (m, 4H), 1.18 (m, 3H), 1.35 (quintet, 2H, J= 7.5 Hz), 1.97 (dd, 2H, J=16.5, 8.5 Hz), 2.25 (dd, 1H, J=16.5, 5.5 Hz), 2.83 (m, 1H), 3.35 (t, 2H, J=7.0 Hz); ^{13}C NMR δ (C_6D_6) 25.98, 27.83, 29.29, 29.40, 32.72, 33.01, 48.80, 49.02, 62.56, 205.44; ESIMS *m/z* (%) 234. (M^+, 100), 217 (24); HRESIMS found 234.0747, calculated for $C_{10}H_{18}O_2S_2$ 234.0746.

5-(7-Hydroxyheptyl)-1,2-dithiolan-3-one 1-oxide (I-2). To a solution of **I-29** (0.521 g, 2.22 mmol) in distilled CH_2Cl_2 (15 mL) was added a solution of 70-75% *m*-CPBA (1.410 g, 5.55 mmol) in distilled CH_2Cl_2 (10 mL). The mixture was stirred at –40 to –50 °C for 30 min. After warming up the mixture to 0 °C, saturated sodium sulfite solution was added followed by the extraction of the aqueous suspension with CH_2Cl_2 (3× 20 mL). The combined organic extracts were washed with saturated sodium sulfite aqueous solution (20 mL), water (20 mL), brine (20 mL) and dried over Na_2SO_4. After evaporation under vacuum, the crude product was purified by flash column chromatography with hexane: EtOAc (5:1→ 1:2, v/v) as eluent to afford two diastereomers **I-2a** and **I-2b**, **I-2a** (0.220 g, 39%, a colorless oil): ^{1}H NMR δ (C_6D_6) 0.827 (m, 1H), 1.01 (m, 2H), 1.11 (m, 4H), 1.21 (quintet, 2H, J=7.0 Hz), 1.43 (m, 3H), 2.18 (dd, 1H, J=20.0, 5.0 Hz), 2.24 (m, 1H), 2.72 (dd, 1H, J=16.5, 12.0 Hz), 3.40 (t, 2H, J=6.2 Hz); ^{13}C NMR δ (C_6D_6) 25.9, 26.6, 28.0, 29.2, 29.4, 32.9, 41.8, 62.5, 64.4, 200.3; ESIMS *m/z* (%) 251 [$(M+1)^+$, 100], 233 (38), 65 (35); HRESIMS found 251.0959, calculated for $C_{10}H_{19}O_3S_2$ 251.0944. **I-2b** (0.231 g, 41%, a colorless oil): ^{1}H NMR δ

(C_6D_6) 0.64 (m, 1H), 0.77 (m, 4H), 0.98 (m, 3H), 1.17 (m, 2H), 1.39 (quintet, 2H, *J*=5.0 Hz), 2.19 (dd, 1H, *J*=17.0, 1.0 Hz), 2.69 (q, 1H, *J*=6.0 Hz), 3.20 (dd, 1H, *J*=17.5, 6.5 Hz), 3.40 (t, 2H, *J*=6.0 Hz); ^{13}C NMR δ (C_6D_6) 25.9, 27.2, 28.8, 29.2, 32.9, 42.3, 62.5, 66.8, 201.4; ESIMS m/z (%) 251 [$(M+1)^+$, 100], 233 (41), 65 (55); HRESIMS found 251.0986, calculated for $C_{10}H_{19}O_3S_2$ 251.0944.

Hexanedioic acid mono-(2,5-dioxo-pyrrolidin-1-yl)ester (I-31). To a solution of adipic acid **I-30** (5.02 g, 34.1 mmol) and *N*-hydroxysuccinimide (4.05 g, 40.9 mmol) in distilled THF (60 mL) at 0 °C was added *N,N'*-dicyclohexylcarbodiimide (7.22 g, 34.9 mmol) in distilled THF (20 mL). The mixture was stirred at the same temperature for 15 min followed by the addition of catalytic amount of DMAP (~30 mg) and further stirred overnight during which time period the mixture was allowed to warm up to room temperature. The resultant DHU was filtered off and the filtrate was concentrated in *vacuo*. The resultant residue was redissolved in CH_2Cl_2 (50 mL). The organics were washed with water (2× 20 mL) and brine (20), dried (Na_2SO_4) and concentrated in *vacuo*. The resultant residue was purified by flash column chromatography (hexane: EtOAc: CH_2Cl_2 10:1:1) to afford **I-31** (3.83 g, 49%) as white crystals: IR (film) 3017, 1742, 1521, 1424, 1214, 1060, 758, 677 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.84 (brd, 4H, *J*=4.5 Hz), 2.65 (t, 2H, *J*=7.0 Hz), 2.42 (t, 2H, *J*=7.0 Hz), 1.79 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 179.2, 169.5, 168.5, 33.6, 30.8, 25.8, 24.1, 23.9; ESIMS *m/z* (%): 244 ($[M+1]^+$, 38), 226 (11), 225 (100), 111 (9), 91 (14); HRESIMS calcd for $C_{10}H_{13}NO_6$ $[M]^+$ 243.1029; found: 243.1018.

Hexanedioic acid 2,5-dioxo-pyrrolidin-1-yl ester 7-hept-3-enyl-[5-(1,2-dithiolan-3-one)] ester (I-32). *N,N'*-dicyclohexylcarbodiimide (0.110 g, 0.506 mmol) in distilled CH_2Cl_2 (2 mL) was added to a solution of **I-17** (0.108 g, 0.456 mmol) and **I-31** (0.125 g, 0.506 mmol) in CH_2Cl_2 (4 mL) at 0 °C, and the mixture was stirred at the same temperature for 5 min. A catalytic amount of DMAP (~10 mg) was added, and the mixture was stirred at room temperature for 3 h. The resultant DHU was filtered off and the filter-cake was washed with CH_2Cl_2. The combined organics were concentrated in *vacuo* and the resultant residue was purified by flash column chromatography with hexane: EtOAc (5:1, v/v) to afford **I-32** (0.104 g, 50%) as a white semi solid: IR (film) 3048, 2981, 1819, 1783, 1741, 1419, 1363, 1260, 1204, 1060, 892, 733, 702 cm^{-1}; ^1H NMR (C_6D_6, 500 MHz) δ 5.19 (m, 1H), 5.09 (m, 1H), 3.99 (t, 2H, J=6.5 Hz), 2.95 (m, 1H), 2.30 (dd, 1H, J=13.5, 8.0 Hz), 2.07 (t, 2H, J=6.5 Hz), 1.99 (dd overlap with t, 3H, J=17.0, 7.0 Hz), 1.89 (q, 2H, J=7.5 Hz), 1.78 (m, 1H), 1.70 (m, 3H), 1.50 (m, 4H), 1.42 (m, 5H), 1.16 (m, 1H); ^{13}C NMR (C_6D_6, 100 MHz) δ 205.0, 172.3, 168.7, 168.7, 13.1, 129.1, 63.4, 48.6, 48.1, 33.5, 32.2, 30.6, 30.5, 28.9, 28.5, 25.1, 24.1, 24.0; ESIMS *m/z* (%): 457 (M^+, 33), 344 (13), 343 (100), 317 (49), 225 (21), 217 (30), 215 (38), 212 (24), 149 (21), 135 (35), 91 (52); HRCIMS calcd for $C_{20}H_{27}NO_7S_2$ $[M]^-$ 457.1234; found: 457.1225.

Hexanedioic acid 2,5-dioxo-pyrrolidin-1-yl ester 7-hept-3-enyl-[5-(1,2-dithiolan-3-one 1-oxo)] ester (I-34). A solution of 70-75% *m*-CPBA (0.110 g, 0.434 mmol) in CH_2Cl_2 (3 mL) was added to a solution of 1,2-dithiolan-3-one **I-32** (0.080 g, 0.170 mmol) in CH_2Cl_2 (10 mL), and the mixture was stirred at –40 to –50 °C for 30 min.

After warming the mixture up to 0 °C, saturated sodium sulfite aqueous solution was added, followed by the extraction of the aqueous suspension with CH_2Cl_2 (2× 15 mL). The combined organic extracts were washed with saturated sodium sulfite aqueous solution (3× 10 mL), water (20 mL) and brine (20 mL), dried (Na_2SO_4) and concentrated in *vacuo*. The residue was purified by flash column chromatography (hexane/EtOAc 8:1 initially and 3:1 subsequently) to afford **I-34** (0.058 g, 71%) as a colorless oil consisting of two inseparable diastereomers* due to the chiral centers at 1' and 3 positions of the molecule: IR (film) 3017, 2955, 1818, 1782, 1736, 1516, 1419, 1214, 1060, 922, 748, 666 cm^{-1}; ^1H NMR (C_6D_6, 500 MHz) δ 5.13 (m, 1H), 4.96 (m, 1H), 3.98 (m, 2H), 3.24 (dd, 0.5H, J=17.0, 6.0 Hz)*, 2.84 (q, 0.5H, J=6.5 Hz)*, 2.74 (dd, 0.5H, J=17.0, 13.0)*, 2.44 (m, 0.5H)*, 2.25 (m, 1H), 2.08 (t, 2H, J=7.0 Hz), 1.99 (m, 2H), 1.88 (quintet, 2H, J=7.0 Hz), 1.69 (m, 3H), 1.49 (m, 9H), 1.30 (m, 1H), 1.12 (m, 1H); ^{13}C NMR (C_6D_6, 100 MHz) δ 172.4, 168.8, 168.7, 131.4, 131.15, 128.9, 128.4, 65.9, 63.6, 63.3, 42.2, 41.7, 33.5, 30.5, 29.8, 29.5, 28.9, 28.9, 28.5, 28.4, 28.2, 27.7, 25.2, 24.1, 24.0; ESIMS *m/z* (%): 474 ($[M+1]^+$, 22), 360 (8), 359 (51), 317 (23), 231 (16), 225 (100), 213 (23), 135 (21), 91 (26); HRCIMS calcd for $C_{20}H_{27}NO_8S_2[M]^-$ 473.1184; found: 473.1191.

Hexanedioic acid 2,5-dioxo-pyrrolidin-1-yl ester 7-heptyl-[5-(1,2-dithiolan-3-one)] ester (I-33). To a solution of **I-29** (0.301 g, 1.239 mmol) and **I-31** (0.264 g, 1.130 mmol) in distilled CH_2Cl_2 (6 mL) was added *N,N'*-dicyclohexylcarbodiimide (0.260 g, 1.241 mmol) in distilled CH_2Cl_2 (2 mL) at 0 °C. The mixture was stirred at the same temperature for 10 min followed by the addition of a catalytic amount of DMAP (~15 mg). After 3 h at room temperature, the resultant DHU was filtered off. The filter-cake

was washed with CH_2Cl_2. The filtrate was concentrated in *vacuo*. The residue was purified by flash column chromatography with hexane: EtOAc (5:1, v/v) as eluent to afford **I-33** (0.223 g, 44%) as a white semi solid: ^{1}H NMR δ (C_6D_6) 0.91 (m, 3H), 1.03 (m, 2H), 1.13 (m, 2H), 1.24 (m, 1H), 1.43 (m, 6H), 1.56 (bs, 2H), 1.71 (bs, 2H), 2.00 (t, 2H, *J*=8.5 Hz), 2.04 (dd, 1H, *J*=16.0, 6.5 Hz), 2.08 (t, 2H, *J*=7.0 Hz), 2.31 (dd, 1H, *J*=16.0, 5.5 Hz), 2.89 (m, 1H), 4.00 (t, 2H, *J*=7.0 Hz); ^{13}C NMR δ (C_6D_6) 24.0, 24.1, 25.2, 25.9, 27.7, 28.8, 29.1, 30.5, 32.6, 33.6, 48.7, 48.9, 64.1, 168.7, 169.8, 172.4, 205.2; ESIMS *m/z* (%) 460 [$(M+1)^+$, 24], 449 (44), 349 (9), 345 (21), 225 (100); HRESIMS found 459.1861, calculated for $C_{20}H_{29}NO_7S_2$ 459.1857.

Hexanedioic acid 2,5-dioxo-pyrrolidin-1-yl ester 7-heptyl-[5-(1,2-dithiolan-3-one 1-oxo)] ester (I-35). To a solution of **I-33** (0.188 g, 0.409 mmol) in distilled CH_2Cl_2 (20 mL) was added 70-75% *m*-CPBA (0.250 g, 1.024 mmol) in distilled CH_2Cl_2 (4 mL). The mixture was stirred at –40 to –50 °C for 45 min. Upon raising temperature to 0 °C, saturated sodium sulfite aqueous solution was added followed by the extraction of the aqueous suspension with CH_2Cl_2 (3× 15 mL). The combined organic extracts were washed with saturated sodium sulfite aqueous solution (3× 10 mL), water (20 mL), brine (20 mL) and dried over Na_2SO_4. After evaporation under vacuum, the crude product was purified by flash column chromatography with hexane: EtOAc (5:1→ 1:1, v/v) as eluent to afford **I-35** (two inseparable diastereomers* in 1:1 ratio, 0.090 g, 45%) as a colorless oil: ^{1}H NMR δ (C_6D_6) 0.83 (m, 2H), 0.96 (m, 2H), 1.04 (m, 3H), 1.15 (m, 3H), 1.44 (m, 6H), 1.55 (bs, 2H), 1.69 (bs, 2H), 2.01 (t, 2H, *J*=7.0), 2.07 (t, 2H, *J*=7.0 Hz), 2.22 (dd, 1H, *J*=19.5, 2.5 Hz), 2.31 (m, 0.5 H)*, 2.75 (dd, 1H, *J*=16.5, 12.5 Hz), 3.20 (m, 0.5 H)*,

4.01 (t, 2H, J=7.0 Hz); ^{13}C NMR δ (C_6D_6) 24.1, 24.2, 25.3, 26.00, 26.7, 27.2, 28.1, 28.8, 28.9, 29.00, 29.1, 29.3, 30.6, 33.7, 41.9, 42.3, 64.2, 64.3, 66.89, 168.8, 168.9, 172.5, 200.3; ESIMS m/z (%) 476 (M^+, 29), 450 (9), 449 (35), 361 (21), 226 (12), 225 (100), 65 (21); HRESIMS found 476.1444, calculated for $C_{20}H_{29}NO_8S_2$ 476.1475.

I.4.2 DNA Cleavage Assays of Leinamycin's Mimics

Materials and Methods for pBR322 DNA Cleavage Assays

Materials. Supercoiled pBR322 plasmid DNA was purchased from GIBCO BRL and BioLabs Inc. Buffers, chelators, radical scavengers, addicitves and thiols were purchased from GIBCO BRL, Aldrich Chemical Co. and Sigma Chemical Co. Catalase was purchased from Calbiochem and superoxide dismutase was purchased from Sigma Chemical Co. HPLC grade acetonitrile was purchased from Tedia company Inc. Water was distilled, deionized, and glass-redistilled. High purity agarose gel was purchased from GIBCO BRL. Ethidium bromide pellets were from Acros Chemicals.

Preparation of the buffers. Sodium phosphate buffer (50 mM, various pH): 3.34 g of sodium phosphate $Na_2HPO_4{\cdot}7H_2O$ was dissolved in deionized water and phosphoric acid 85% H_3PO_4 was added to adjust the pH value to the desired one (pH 5.5, pH 6.0, pH 6.5, pH 7.0 or pH 8.0). The volume was brought to 30 mL finally to give 250 mM sodium phosphate buffer. TAE running buffer: 100 mL of 10X TAE buffer obtained from Life Technologies™ was diluted to 1X TAE by bring the final volume to 1000 mL with adding of deionized water.

Preparation o f the gels. 0.8% Agarose gel: 0.40 g of powdered agarose obtained from GIBCO BRL was dissolved in 50.0 mL 1X TAE buffer, the slurry was heated in a microwave oven until the agarose well dissolved. When the temperature of the solution was cooled down to ca 60 °C, it was poured to an assembled mold and waited for gel was completely set (45-60 min at room temperature). The gel was put in a vat and 1X TAE running buffer was poured into the vat until the gel was submerged under ca 1 mm of the buffer and the comb was removed from the gel plate.

DNA Cleavage Reactions. The leinamycin analogs **I-1** and **I-2** were used as freshly prepared solutions of acetonitrile and the final reaction mixtures in microtubes contained 10% v/v of acetonitrile in 50 mM sodium phosphate buffer. (*see Results and Discussion Section*)

Gel Electrophoresis. *For information on the general aspect, please refer to the Results and Discussion Section of this part.*

I.5 References

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PART II

Exploring the Antibiotics Varacin, Varacin B, Varacin C and the Structural Related Entities for Anti-Cancer Drug Candidates

II. 1. Introduction

Organic polysulfides have aroused considerable interests among organic chemists in the past few decades, for their synthetic utilities and potent biological activities.[1,2,3] In 1991, the unprecedented natural polysulfides varacin **II-1** was isolated from ascidians belonging to the genus *Lissoclinum* (Didemnidae), which exhibited potent antibiotic, cytotoxic and antiviral properties.[1,2,4,5] In the course of Makarieva *et al* work on biologically active marine natural products, they have isolated varacin **II-1** and three new related polysulfides **II-2, II-3, II-4** in 1995, from the far-eastern ascidian *Polycitor* sp.[5] The colonial ascidian *Polycitor* sp. (phylum Chordata, subphylum Urochordata, class Ascidiaceae, suborder Aplousobranchia, family Polycitoridae) was collected by dredging from a depth of 90 m in the Sea of Japan (43°04'N, 134°30'E). Searle and Molinski have isolated a group of five new alkaloids from the tropical ascidian, *Lissoclinum* sp. Two of these, the dibenzotetrathiocin lissoclinotoxin D **(I)** as well as lissoclintoxin A **(II)** were found to possess antifungal activity.[2,6] Faulkner, Carté and co-workers have described the isolation of some structurally related benzopentathiepind from two *Lissoclinum* species. These include the 5-methylsulfanyl varacins **(III, IV)**, two very similar benzotrithiole analogues, and 3,4-desmethylvaracin **(V)** from a *Eudistoma* species.[7] (See Chart 2.1 for their molecular structures)

As a member of unusual dopamine-derived cyclic polysulfides, varacin bears the first pentathiepin ring found in nature.[1] It was reported to has antifungal

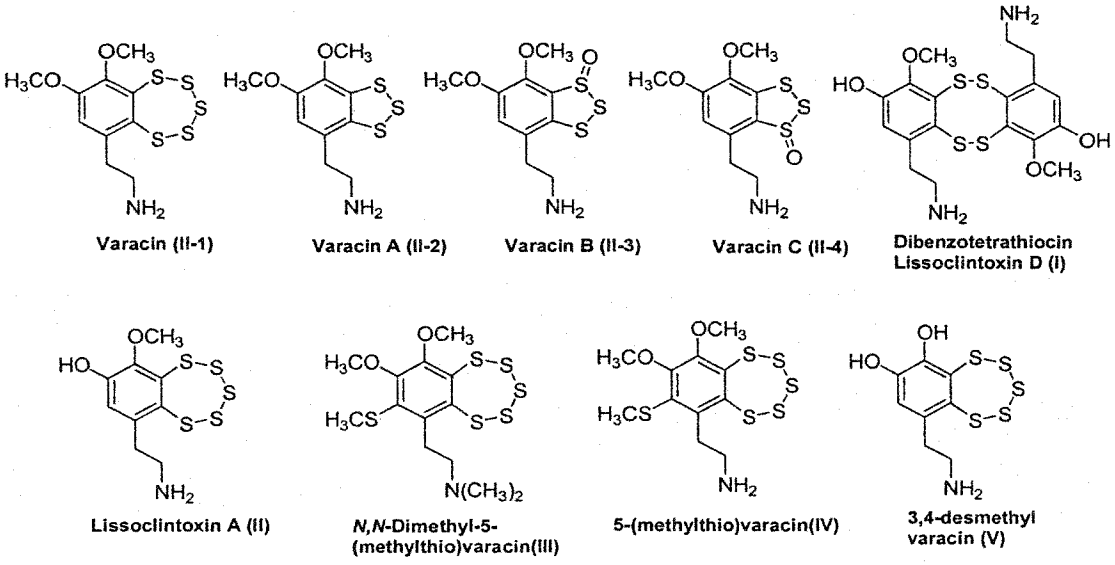
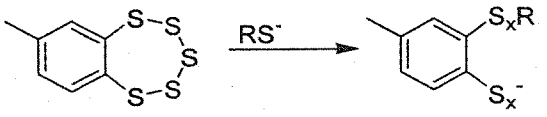


Chart 2.1 Structures of selected polysulfide antibiotics discovered by various groups worldwide

cytotoxic activities, and shown cytotoxicity toward the CHO cell lines EM9 (chlorodeoxyuridine sensitive) and BR1 (BCNU resistant), implying varacin's mechanism of action may involve the formation of single stranded DNA breaks.[1,8] Results of further experiments indicated that the simple structural analog of varacin, 7-methylbenzopentathiepin **(VI)**, in conjunction with thiols, mediated the conversion of molecular oxygen to DNA-cleaving oxygen radicals. Chatterji and Gates[5-8] suggested that thiol-dependent DNA cleavage by the simple analog involved conversion of molecular oxygen, probably via superoxide radical, to hydrogen peroxide, followed by a trace-metal-dependent Fenton reaction to generate the DNA-cleaving agent hydroxyl radical. These findings may pertain to the biological properties of naturally occurring varacin and other benzopentathiepin antibiotics, as it is well known that the production

of reactive oxygen species including superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (HO$^{\bullet}$) by xenobiotics can be cytotoxic. Chart 2.2 describes the reactive hydropolysulfide anions generated in the reaction of thiols with (**VI**) are the key intermediates in the production of DNA-cleaving oxygen radical.



VI (X=2 to 4)

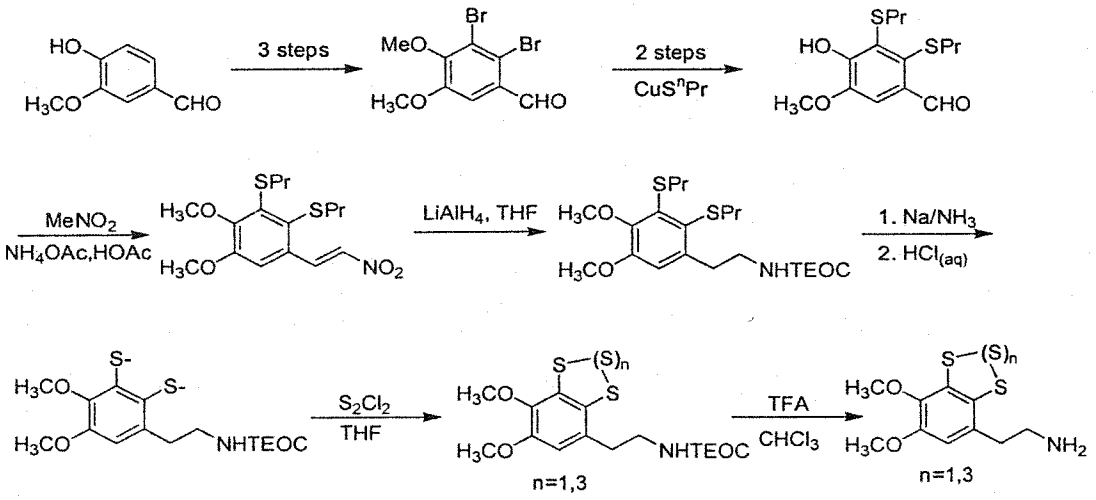
Chart 2.2 Generation of hydropolysulfide anions by (**VI**) during thiol-mediated DNA cleavage reaction via free radical pathway

In addition, varacins B and C isolated by Makarieva *et al* are the first antibiotic discovered to date that possess the unique structural feature of trithiole 1-oxide as their core functionality.[5] Subsequent physiological examinations on these two natural products revealed that both of them exhibit potent antifungal and antimicrobial activities against *Candida albicans* and *Bacillus subtilus*.[5] However, the modes of action of varacins B and C at the molecular level have not been identified up until now.

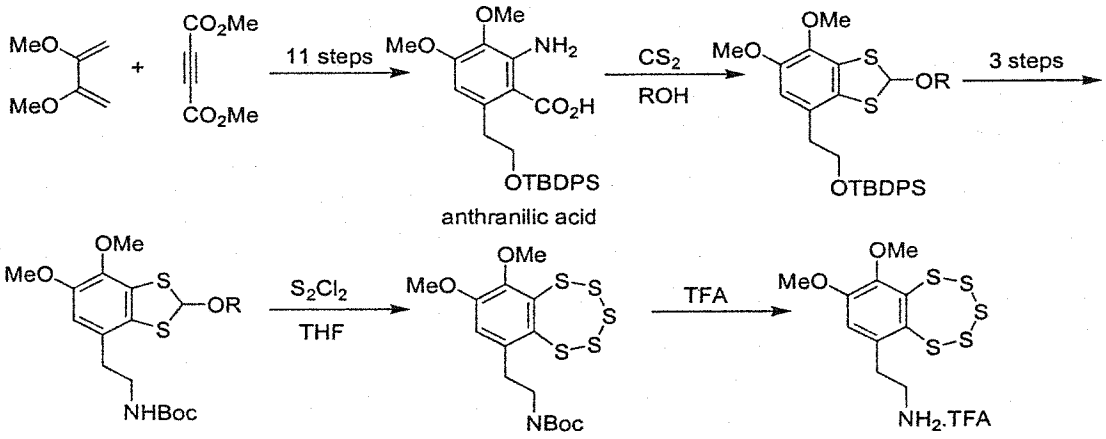
Preparations of pentathiepins with several strategies have been published in literature.[9] Davidson has accomplished the total synthesis of varacin **II-1** in 10 steps from vanillin, providing unambiguous confirmation of the unique dopamine-related benzopentathiepin structure. His general approach involved the formation of the pentathiepin ring by addition of sulfur monochloride (S_2Cl_2) to an appropriate dithiol or

dithiolate precursor. The sulfur atoms were introduced onto the aromatic ring as sulfides by the nucleophilic substitution of an aromatic dibromoarene with cuprous *n*-propyl mercaptide.[9b] In the same year, Danishefsky reported the alternative pathway to prepare the novel antibiotic varacin.[9a] Their synthetic effort began with a Diels-Alder reaction of commercially available 2,3-dimethoxy-1,3-butadiene and dimethylacetylene dicarboxylate (DMAD). After the 11-step synthetic work and functionality transformations, the key intermediate anthranilic acid was afforded. Treating the acid with isoamyl nitrite in the presence of carbon disulfide, isoamyl alcohol was then thermal decomposed. Following the installation of pentathiepin moiety with removal of the ortho ester effected by S_2Cl_2, the final product was yielded as its trifluoroacetate salt. Still *et al* published an new route to the synthesis of varacin two years later.[9d] They used readily available inexpensive 3,4-dimethoxyphenethylamine as the starting material. After protecting the amino group quantitatively, they introduced a thiocyanate group regioselectively by an electrophilic thiocyanation with *N*-thiocyanatosuccinimide (NTS). Conversion of the thiocyanate to its *tert*-butyl sulfide was achieved by an organometallic reaction with the cuprate $^{t}Bu_2Cu(CN)Li_2$. Regioselective lithiation with di-*tert*-butyl disulfide, the desired bissulfide was afforded in good yield. Constructing the pentathiepin ring with $S_2Cl_2/BaCO_3$ system gave an improved reaction yield compared to the previous procedures. As a whole, their synthesis of varacin was achieved with shorter pathway and 18% overall yield. The following Scheme 2.1 serves as a graphical summary of previous preparations for this novel pentathiepin antibiotic varacin.

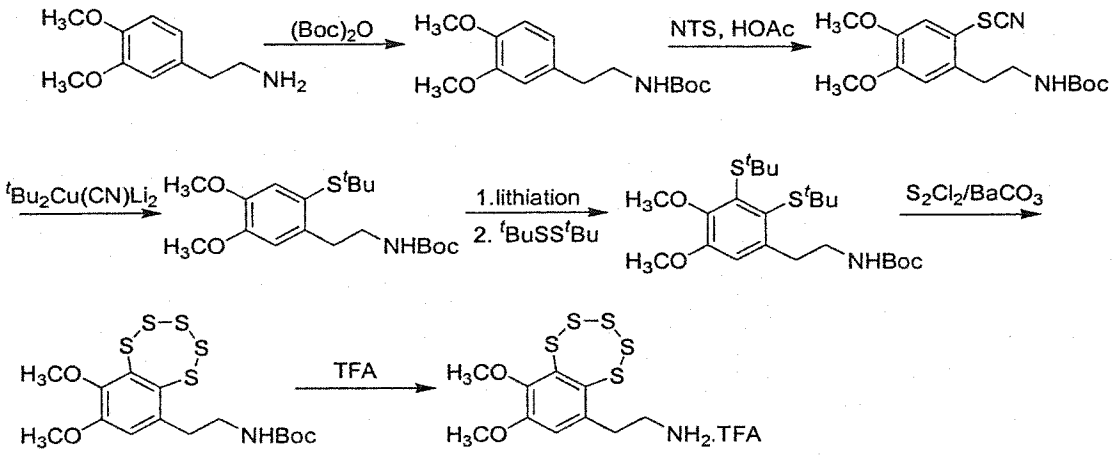
Davidson's synthetic pathway for varacin:



Danishefsky's method of varacin preparation:



Still's work of varacin synthesis:



Scheme 2.1 Summary of previous procedures for novel pentathiepin antibiotic varacin synthesis

In contrast, conduction of the total synthesis of varacins B **II-3** and C **II-4** have not yet been reported. With the aim of clarifying the mode of action of these two antibiotics at the molecular level, benzotrithole oxide analogs of varacins B and C were designed and prepared in our lab.[10] Furthermore, we have demonstrated for the first time that these synthetic benzotrithiole oxides are the potent thiol-dependent DNA-cleaving agents under the physiological condition.[10] Developing from these promising preliminary results, total synthesis of varacins B and C, studies of their modes of DNA cleavage and illustrations of their cytotoxicity with IC_{50} versus several cancer cell lines were carried out.[11] On the other hand, the total synthesis of varacin was achieved by modifying the Davidson's methodology.[9b,12] Our attempts to study varacin's mechanism of plasmid DNA cleavage, is the first approach to use varacin to cleave DNA.[12]

Precedent study was carried out by using simple benzopentathiepins rather than the natural product.[8]

The objectives of our project are (1) to study the modes of action of varacin, varacins B and C at the molecular level, (2) to evaluate the suitability of varacin and its structure analogs as the anticancer drug candidates or lead structures. In order to achieve our goal, we attempted to synthesize varacin by modifying previous strategy.[9] To the best of our knowledge, our laboratory is the first to report the total synthesis of varacins B and C.[11] By utilizing these synthetic products, we can address the following unanswered questions: (a) Whether varacin, varacins B and C are capable of cleaving DNA as benzopentathiepinan[8] and benzotrithiole oxide[10,13] did in thiol mediated physiological condition? (b) If they indeed exhibit DNA-cleaving properties, what are the corresponding mechanisms these antibiotics undergo? Are they via free radical,[14] alkylation[15] or both pathways?[14,16] (c) What are the IC_{50} values of our synthetic products against various cancer cell lines and their potent cytotoxicity?

The significance of our stand is fourfold. Firstly, varacin, varacins B and C are the only antibiotics discovered to date that possess benzopentathiepin and benzotrithiole 1-oxide as a major functionality respectively.[1,5] Identification of the biological targets that varacin, varacins B and C act on could provide chemists with a new insight into the chemistry that has been long utilized in nature. Secondly, as varacin and its structure similar polysulfides including varacins B and C exhibit potent antimicrobial and antifungal activities against *Candida albicans* and *Bacillus subtilus*,[1-5] the information on the modes of action of these antibiotics at the molecular level is essential for

determining the possibility of them to serve as anticancer drug candidates. Thirdly, exploration of some short and molecular effective synthetic routes for varacins B and C provide us significant amount of antibiotics for further studies instead of extracting them from natural source with meager yields and via tedious processes. Fourthly, we can deal with several interesting problems with the proposed synthetic strategy. The chemistry of benzotrithiole 1-oxide has not been fully elucidated, incorporation of such a trithiole 1-oxide into a pentasubstituted aromatic ring added to the complexity of the goal. It is difficult to deduce the number of sulfur atoms in the polysulfide ring during the varacin preparation, because pentathiepin rings readily lose S_2 in mass spectrum and base catalyzed condition.[9,17] And only limited information is available from ^{1}H NMR and ^{13}C NMR about the substitution pattern of the aromatic ring, which hampers the characterizations of the intermediates and products

II. 2. Results and Discussion

II.2.1 Synthesis of Antibiotics Varacin, Varacins B, C and Their Analogs

Varacin **II-1** and lissoclinotoxin **(I)**, **(II)** isolated from ascidians belonging to the genus *Lissoclinum* (Didemnidae), are the two members of the cyclic polysulfide antibiotics[1] exhibiting potent antibiotic, cytotoxic, and antiviral activities.[2,3] In the course of Makarieva's continuing studies on biologically active marine natural products,[5] they had isolated varacin and three new related polysulfides from the ascidian *Polycitor sp.* (see Chart 2.3 for our synthetic antibiotics and analogs)

To our knowledge, the reported methodologies for the synthesis of benzopentathiepins and varacin related compounds[1,9] didn't include the varacins B **II-3** and C **II-4**. Subsequently biological examinations on varacins A **II-2**, B **II-3** and C **II-4** demonstrated that, similar to varacin, those three antibiotics exhibit potent antifungal and antimicrobial activities against *Candida allbicans and Bacillus subtilus*.[5]

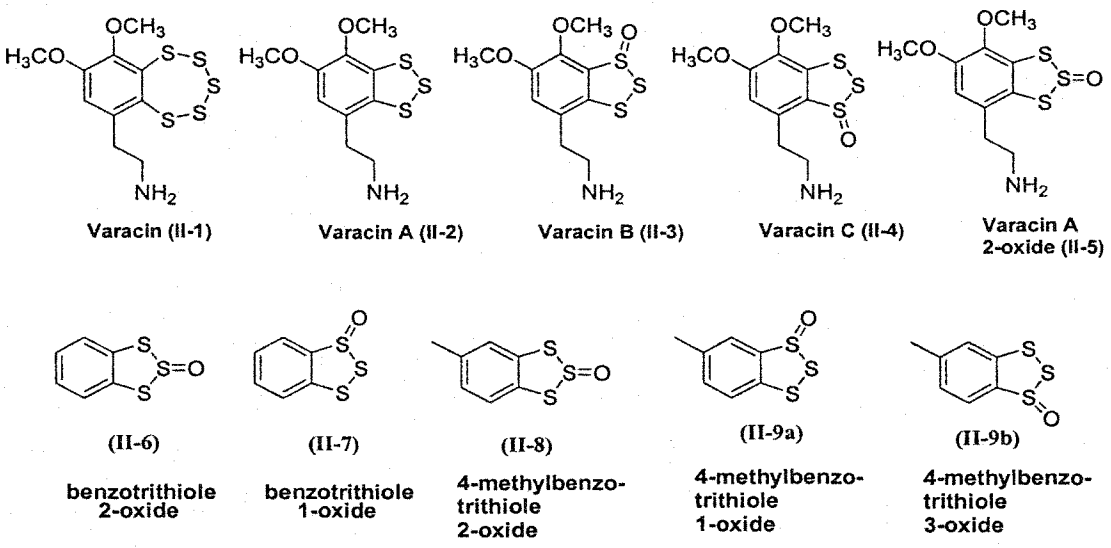


Chart 2.3 Structures of varacin and its sulfur-containing cyclic analogs we prepared in our lab.

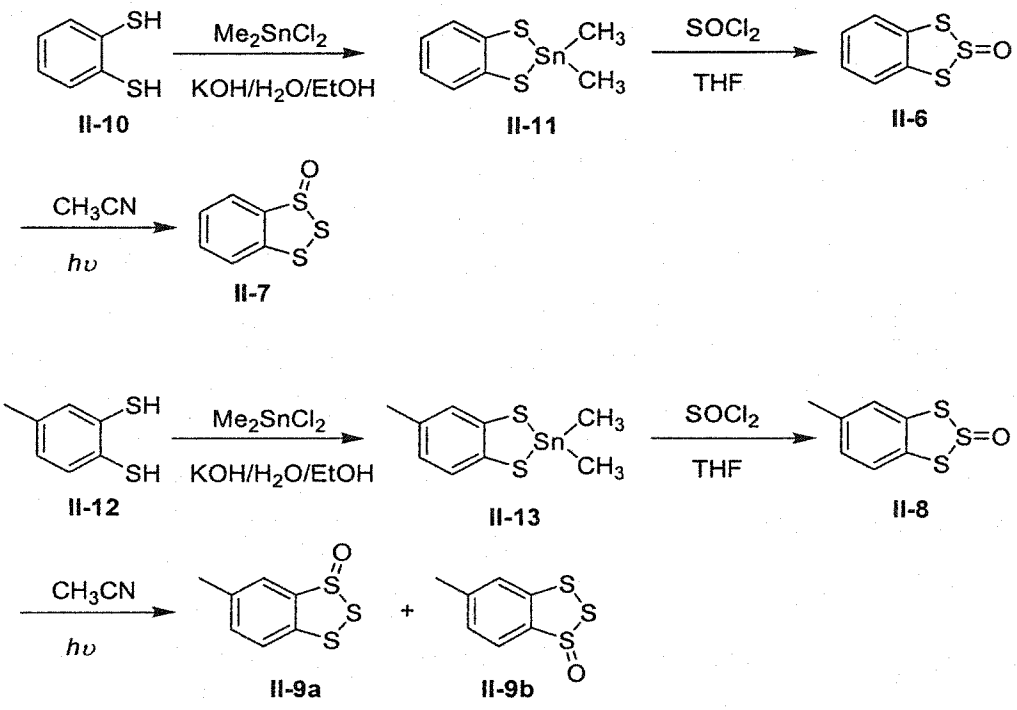
Unlike varacin and any other previously discovered microbial metabolites in structures,[1,3,18] varacins B and C possess the unique trithiole 1-oxide scaffold as their reactive functionality.[5] Although previous studies illustrated their biological activities,[5] the mechanisms of action of varacins B and C at the molecular level and the corresponding total synthesis have not been brought to light until now. In fact only a

single article was found by search for the word "varacin B" or "varacin C" through Scifinder.

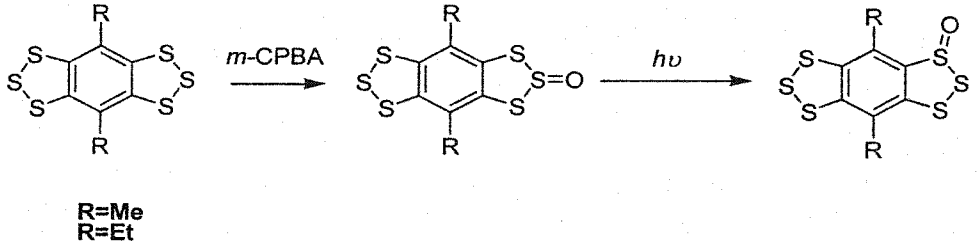
The opportunities for carrying the first total synthesis of varacins B and C, studying their modes and origins of biological activities, and the challenging structural features of these natural products, have prompted us to launch extensive investigations into the mentioned entities.

The simpler structural analog of varacins B and C, benzotrithiole 1-oxide **II-7** and its precursor benzotrithiole 2-oxide **II-6** (see Chart 2.3) were designed and successfully synthesized in our laboratory.[10] The synthetic routes toward these structural analogs are illustrated in the following schemes.[19,20] We started our synthesis with commercially available 1,2-benzenedithiols **II-10**. Treatment of dithiols with dimethyltin dichloride in KOH-ethanolic solution gave 2,2-dimethyl-1,3,2-benzodithiastannoles **II-11** in moderate yield. To a solution of stannole in THF was added 1.5 equiv. of thionyl chloride at –60 °C under Ar. The reaction mixture was stirred for 2 h with the temperature raised to 0 °C at the end. After usual work-up the crude product was purified with flash chromatography to afford benzotrithiole 2-oxide in 87% yield. Photolysis of benzotrithiole 2-oxide was achieved by irradiating the trithiole 2-oxide in acetonitrile upon a Pyrex filtered 100 W high-pressure mercury lamp under argon. The corresponding 1-oxide was obtained in 80% yield. On the other hand, we have prepared the inseparable mixture of 4-methyl benzotrithiole 1-oxide **II-9a** and the 3-oxide **II-9b** from the 2-oxide **II-8** in a similar manner starting with 4-methyl 1,2-benzenedithiols **II-12** (Scheme 2.2). This kind of intramolecular photochemical oxygen

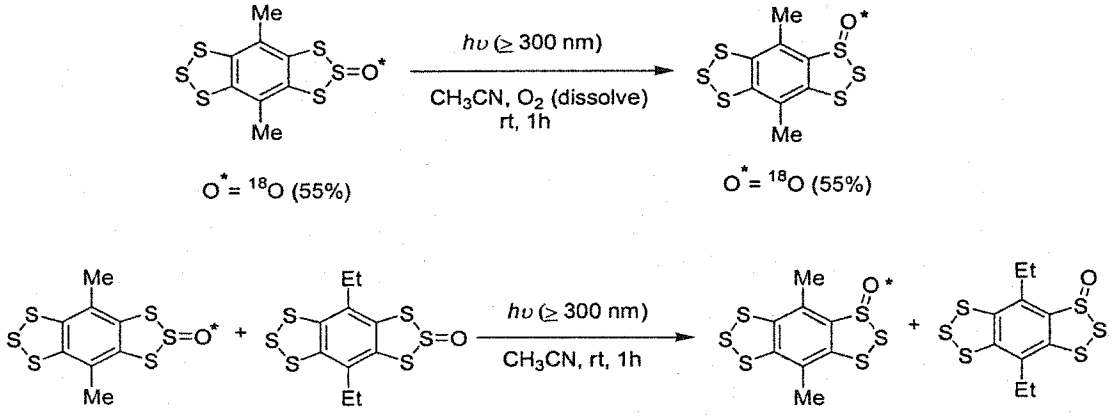
migration was firstly reported by Sato *et al.*[19] During their studies on the oxidation of the trithioles, they found that 4,8-dialkylbenzo[1,2-d;4,5-d']bis[1,2,3] trithiole 2-oxides which obtained from the oxidation of 4,8-dialkylbenzo[1,2-d;4,5-d']bis[1,2,3]trithioles with *m*-chloroperbenzoic acid were slowly converted to its 1-oxides upon exposure to diffuse light (Scheme 2.3). They also reported that these migrations did not take place thermally, and they were not affected by the addition of a few drops of acids such as trifluoroacetic acid. Moreover, the aromatic ring fused to the trithiole 2-oxide ring was believed to play an important role for the oxygen migrations. The reactions were not quenched even upon irradiation in the presence of some triplet quenchers such as oxygen, 1,3-cyclohexadiene and 1,3-pentadiene. Also, the oxygen migrations were proved to proceed intramolecularly, not intermolecularly. Scheme 2.4 below summarizes Sato *et al* ^{18}O-labelled and cross-over experiments that demonstrated the photochemical intramolecular oxygen migration.[21,22] Their results suggested that the oxygen migration proceeded via an excited singlet state. Trithiole 2-oxides in CH_3CN showed a strong UV absorption band in the range from 245-285 nm which could be assigned as a π-π^* transition of the aromatic ring fused to the trithiole 2-oxide ring.[20a] Those our synthetic compounds have been subjected to DNA-cleavage tests and cytotoxicity examinations.[10]



Scheme 2.2 Synthetic routes to benzotrithiole 1-oxide, 2-oxide and 4-methyl-benzotrithiole 1-oxide, 2-oxide and 3-oxide



Scheme 2.3 First example of intramolecular photochemical oxygen migration in benzotrithiole 2-oxide



Scheme 2.4 ^{18}O-labeled cross-over photolysis experiments on trithiole oxides

Based on the findings of many sulfur-containing antibiotics such as leinamycin and thiarubrine C which are the thiol-dependent DNA-cleaving agents,[14,15,23] we examined the DNA-cleaving properties of benzotrithiole 2-oxide **II-6** and 1-oxide **II-7** respectively. Through our pBR322 plasmid-based DNA cleavage assays,[24] we discovered for the first time that benzotrithiole 2-oxide **II-6** and 1-oxide **II-7** efficiently converted supercoiled (Form I) DNA to the nicked form (Form II) at 500 nM of these synthetic compound with the presence of thiol. (Figures 2.1 and 2.2) In addition, as shown in these Figures, addition of thiol was essential for the DNA-cleavage by the two entities. This thiol-dependence of the DNA-cleavage implies that the active DNA-cleaving species must be generated from the reactions between trithiole 1-oxide **II-7** and corresponding 2-oxide **II-6** with thiols. The thiol-dependent DNA-cleavage examinations were repeated for 4-methyl benzotrithiole 2-oxide **II-8** and the mixture of 4-methyl benzotrithiole 1-oxide **II-9a** and the 3-oxide **II-9b**. The results showed that they shared similar behavior toward DNA-cleavage with benzotrithiole 1-oxide **II-7** and

2-oxide **II-6**. It was anticipated that these preliminary results from our studies on the synthetic benzotrithiole oxides and derivatives could pertain to biological properties of the antibiotics varacins B **II-3** and C **II-4** since they own the common constitutively active functionality.

Our strategy for the total synthesis of varacins B **II-3** and C **II-4** was benefited from the Davidson's methodology for the benzopentathiepins varacin **(I)** and isoliccoclintoxin A **(II)** preparations.[1,9] Starting with 4-hydroxy-3-methoxybenzaldehyde (vanillin), 5,6-dibromovanillin **II-14** was prepared in a single step by the iron tunings catalysed bromination in glacial acetic acid (see Scheme 2.5).[25] To a well-stirred solution of quinoline/pyridine were added dibromovanillin **II-15** and freshly prepared cuprous *n*-butylmercaptide,[26] the resulting white slurry was heated to 160 °C and stirred for 2 h under argon.[27] The crude product was purified by flash column chromatography using hexane/EtOAc eluent to afford the pure benzaldehyde **II-16**. Methylation of the hydroxyl group in **II-16** by iodomethane in THF-water biphases system with phase transfer catalyst tetrabutyl ammonium iodide, the dimethoxybenzaldehyde **II-17** was afforded in quantitative yield.[9b,28] A mixture of **II-17**, nitromethane, glacial acetic acid and ammonium acetate was reacted in an ultrasonic bath for 8 h and gave the *β*-nitrostyrene **II-18** in 78% yield.[29] It is worth to note that using ultrasonic energy is a better choice alternative to heating because of the higher yield and fewer byproducts. In our first attempt to prepare *β*-nitrostyrene **II-18** with heating, the yield was 32% instead of 79% reported by Davidson *et al*, and the product accompanied with a lot of uncharacterized impurities. Fortunately, those problems encompassed this step were

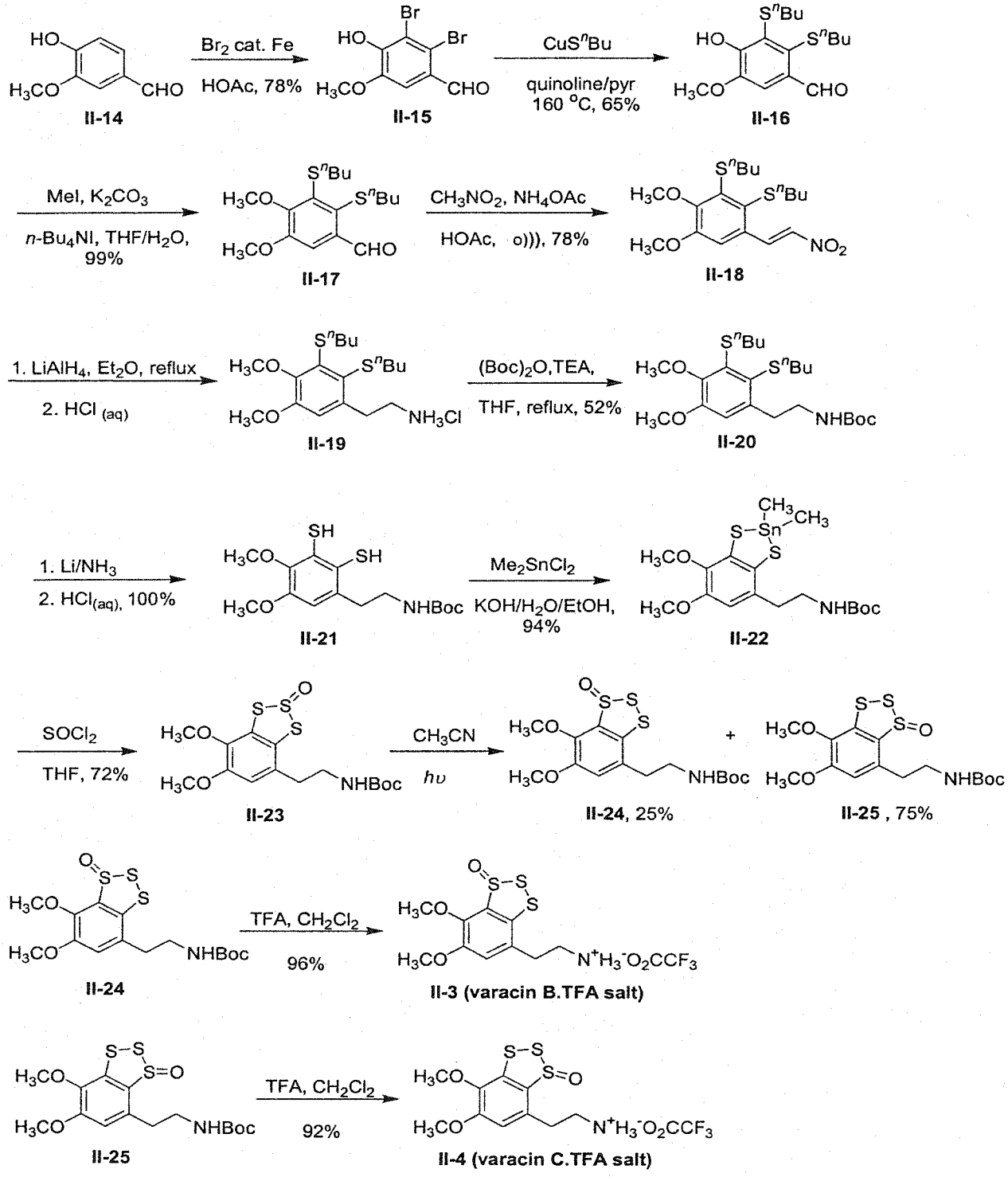
solved by promoting the reaction with ultrasonic energy.[11] Reduction of **II-18** with $LiAlH_4$ completed the installation of the phenethylamine side chain, affording the primary amine chloride salt **II-19**, which was subjected to next step without purification within few hours after preparation.[30]

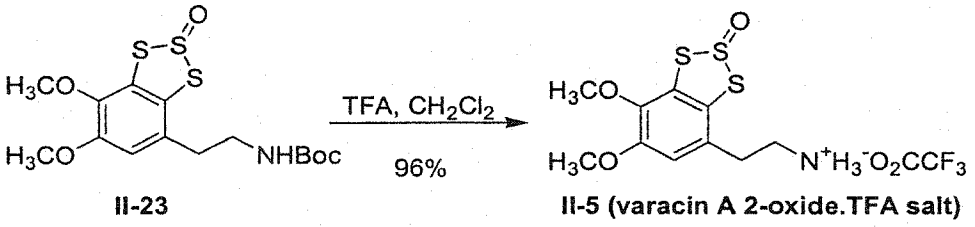
After protecting the primary amine group with *t*-butyl carbamate (Boc), the crude product was capable for purification by flash column chromatography over silica gel to afford the pure Boc-protected amine **II-20**.[31] The reason for using Boc group rather than other protecting groups was its extensive usage in peptide synthesis for amine protections. It is available at low price and wide sources, not ready to hydrolyse under basic conditions and inert to many other nucleophilic reagents. Treatment of **II-20** with Li/NH_3 resulted in the removal of *n*-butyl groups from the disulfide to give the dithiolate anion. Ammonia was allowed to evaporate under a stream of N_2 and the residue was acidified with 15% v/v aqueous HCl. After usual work-up, dithiol **II-21** was isolated in quantitative yield.[27,32]

To a stirring solution of the dithiols in KOH-ethanol was added dimethyltin dichloride. After stirring for 1.5 h, CH_2Cl_2 was added into the mixture. The organic extracts were washed with water, brine, dried over Na_2SO_4 and concentrated to yield the essentially pure benzodithiastannoles **II-22** quantitatively.[19] Addition of 1.5 equiv. of thionyl chloride to the stannole in THF at –60 °C under Ar providing the benzotrithiole 2-oxide **II-23** in 72%. Photolysis of the benzotrithiole 2-oxide was achieved by irradiating the trithiole 2-oxide in acetonitrile through a Pyrex filtered 100 W high-pressure mercury lamp under argon.[20] When the reaction was completed, as monitored

by TLC, the solvent was evaporated to give the crude product as a yellow paste which consisted of two isomers with very close R_f. The **II-24** (24%) and **II-25** (75%)[5] were afforded as yellow solid and light yellow prism solid respectively after flash column chromatography. Removal of the Boc group was routinely achieved with trifluoroacetic acid to yield varacinium B trifluoroacetate **II-3** and varacinium C trifluoroacetate **II-4** respectively (Scheme 2.5).[9,31]

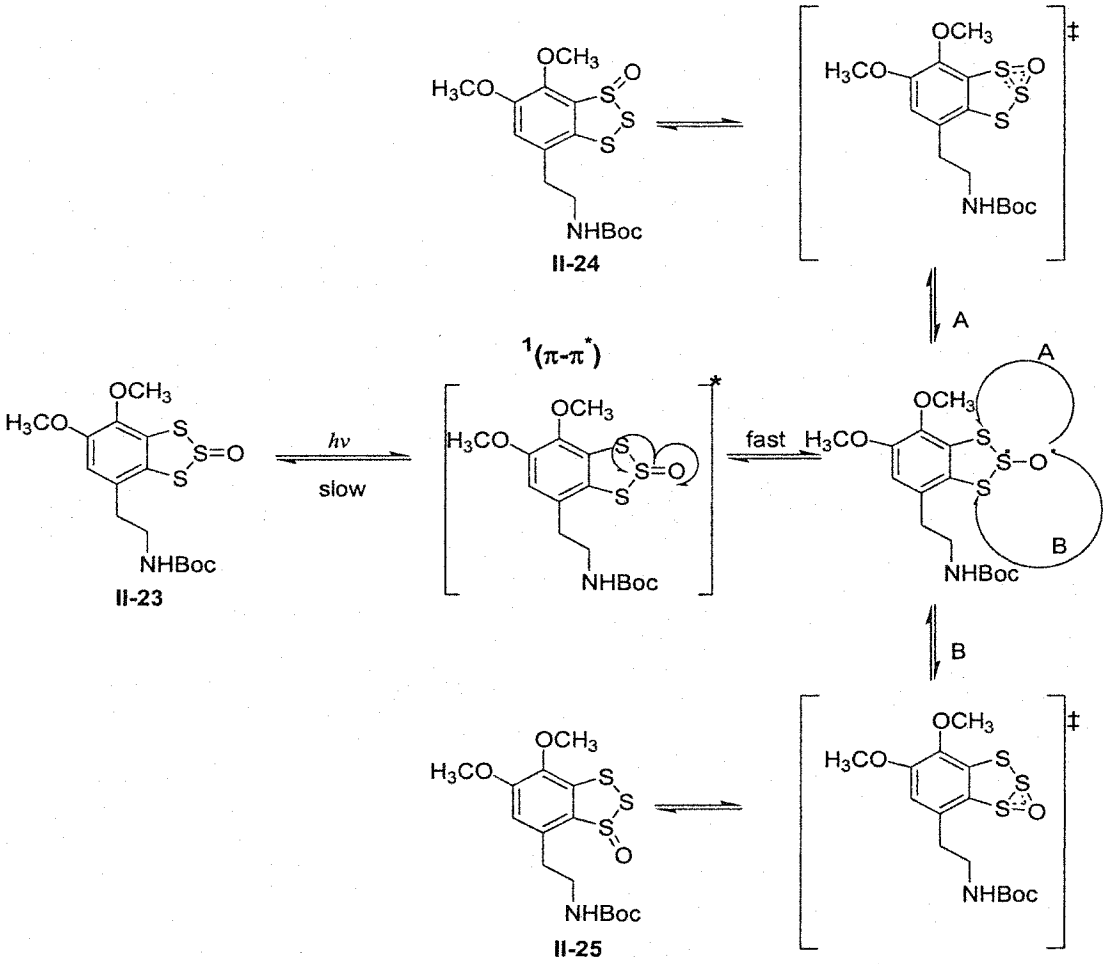
We have prepared varacinium B / C trifluoroacetates derivative **II-5** – varacin A 2-oxide trifluoroacetate by Boc deprotection from **II-23**. This is a synthetic compound structurally derived from varacins B and C. As it has not been reported before, we are curious about whether it is a real precursor of the two antibiotics in nature, like the case existed in our synthetic pathway. It is a fundamental question to the Mother Nature about the order of existence. Apart from asking the question, we were interested about the biological activities of this derivative. Thus we examined the thiol-dependent DNA-cleaving properties of varacins B **II-3**, C **II-4** as well as their structural derivative varacin A 2-oxide **II-5** on pBR322 plasmid DNA. Detection of any circular nicked form (Form II) or linearized form (Form III) plasmid DNA from incubation reactions of the synthetic compounds with supercoiled form (Form I) DNA would be the decisive evidence for DNA-cleavage caused by the candidates. Moreover, the cytotoxicity (IC_{50}) on the chosen cancer cell lines was analyzed and reported in the following Tables 2.1 and 2.2.


II-14
Br2 cat. Fe
HOAc, 78%
II-15
CuSnBu
quinoline/pyr
160 °C, 65%
II-16
MeI, K2CO3
n-Bu4NI, THF/H2O,
99%
II-17
CH3NO2, NH4OAc
HOAc, o))), 78%
II-18
1. LiAlH4, Et2O, reflux
2. HCl (aq)
II-19
(Boc)2O,TEA,
THF, reflux, 52%
II-20
1. Li/NH3
2. HCl(aq), 100%
II-21
Me2SnCl2
KOH/H2O/EtOH,
94%
II-22
SOCl2
THF, 72%
II-23
CH3CN
hυ
II-24, 25%
II-25 , 75%
TFA, CH2Cl2
96%
II-3 (varacin B.TFA salt)
TFA, CH2Cl2
92%
II-4 (varacin C.TFA salt)



II-23 II-5 (varacin A 2-oxide.TFA salt)

Scheme 2.5 Preparation of varacins B, C and varacin A 2-oxide

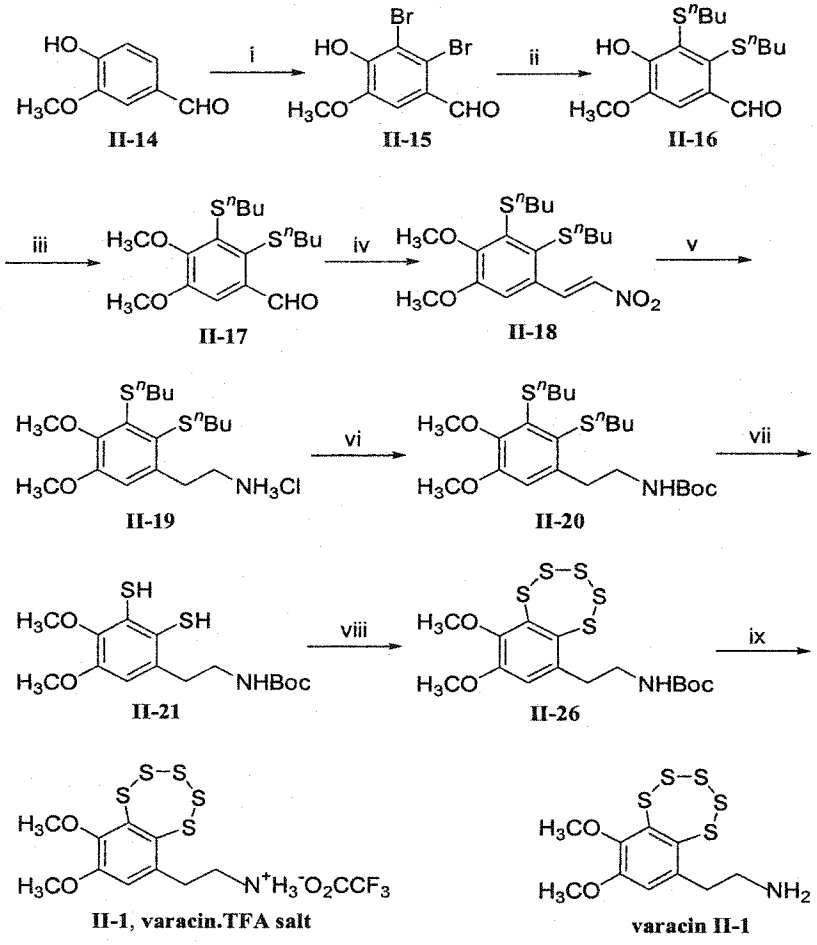


Scheme 2.6 Plausible photolysis mechanism of conversion **II-23** to **II-24** and **II-25**

Scheme 2.6 above is the plausible mechanism of photolytic intramolecular oxygen migration from **II-23** to **II-24** and **II-25**. We excited **II-23** photochemically to its π-π^* energy level, followed by the intramolecular migration of oxygen to the neighboring sulfur atoms via radical transfer and tricyclic strained species formation. **II-24** and **II-25**, the *N*-Boc-varacins B and C were yielded respectively.

Preparation of varacin **II-1** is one of our aims in our research. We obtained it easily from the branch of our synthetic pathway. The syntheses of varacin was not for developing a new synthetic methodology as there are some very effective pathways have been reported by Davidson, Toste, and Danishefsky.[9] Our goal was to prepare the varacin for studying the thiol-dependent DNA-cleavage based on plasmid pBR322. We were surprised by the absence of examples using varacin as a DNA-cleaving agent in literature. The most related publication of this was reported by Chatterji *et al.* who investigated the DNA-cleaving ability of a simple varacin analog, 7-methylbenzapentathiepin,[8,17] and provided the first direct evidence on DNA-cleavage ability of pentathiepins under physiologically relevant conditions. Thanks to the inspiration from their works, we proceed further within the topic by employing varacin instead of its analog. As pointed out by the authors of the article, all naturally occurred benzopentathiepins bear an aminoethyl functionality. This group probably exists in the charged form under physiological conditions and is likely to influence pharmacokinetics of these agents.[8,33] Furthermore, the positively charged amino moiety may confer DNA affinity on the antibiotics due to the negatively charged phosphate on the nucleotides.

Under some conditions, the aminoethyl substituent may catalyse the reaction of thiols with cyclic polysulfide of the natural products.[8,9,34] Therefore, the amino bearing antibiotics and their metabolites may be somewhat more efficient thiol-mediated DNA-cleaving agents than simple analogs.[8,33]



Scheme 2.7 Preparation of varacin. *Reagents and conditions*: i, Br_2, cat Fe, HOAc, reflux, 78%; ii, CuS^nBu, quinoline-pyridine (4:1), 160 °C, 65%; iii, MeI, K_2CO_3, $^nBu_4NI$, THF/H_2O, 99%; iv, $MeNO_2$, NH_4OAc, HOAc, o))), 78%; v, $LiAlH_4$, Et_2O, reflux then HCl(aq) 95%; vi, $(Boc)_2O$, TEA, THF, reflux, 52%; vii, Li-NH_3(l), HCl(aq), 100%; viii, S_2Cl_2-$BaCO_3$, THF, 35%; ix, TFA, CH_2Cl_2, 92%.

Scheme 2.7 illustrates how the preparation of varacin **II-1** was secured in the pathway. *N*-(Boc)-2',3'-dimercapto-4',5'-dimethoxyphenethylamine **II-21** served as the branch point for the synthesis. To a solution of dithiol **II-21** in distilled THF containing barium carbonate solid at 0 °C was slowly added a solution of disulfide dichloride in distilled CH_2Cl_2.[9,35] The resulting tan yellow suspension was stirred at room temperature for 2 h. When the reaction was completed, as monitored by TLC, the dark brown reaction mixture was filtered. Upon flash column chromatography, *N*-(Boc)-varacin **II-26**[9d] was afforded in 35% yield. Boc group deprotection was routinely achieved with trifluoroacetic acid to yield the varacinium salt quantitatively. The product was subjected to the DNA-cleavage tests.

II.2.2 First X-Ray Crystallographic Details of Antibiotics Varacin and Varacin C

Other than total synthesis work, DNA-cleavage examination, and test of cytotoxicity on selected cancer cell lines, the X-ray crystallographic analysis for the crystal structures of *N*-Boc-varacin C **II-25** and the novel antibiotic varacin **II-1** was a topic of our research.[36] With the aid of X-ray diffraction, we were able to confirm that the varacin C's structure was featured with benzotrithiole 3-oxide (unique structural properties) and proved this functionality was stable in the course of synthetic pathway (chemical activities). **II-25** was a chiral molecule as its S(1) atom was bonded to O(1), torsion angle [O(1)-S(1)-S(2)-S(3)] is –77.1(2)°. The two sulfur atoms [S(1) and S(3)] were nearly coplanar with the aromatic ring, while S(2) lied out of the plane as indicated by the torsion angles [C(1)-S(1)-S(2)-S(3), S(1)-S(2)-S(3)-C(2)] were 30.50(18)° and –

28.99(18)°. The sulfur-oxygen bond length was 1.468(5) Å, sulfur-sulfur bond lengths [2.067(2), 2.074(2 Å] were similar to S_8, sulfur-carbon (sp^2) [C(1)-S(1), C(2)-S(3)] bond lengths were 1.776(5) ,1.751(5) Å, closed to those found in benzopentathiepin (1.770, 1.768 Å).[9b,37,38] The hydrogen bonds existed in the crystal, N(1)-H(1B)...O(4). Bond distances of D-H...A are 0.86, 1.99 and 2.810 (5) Å, angle between (DHA) is 158.8°.

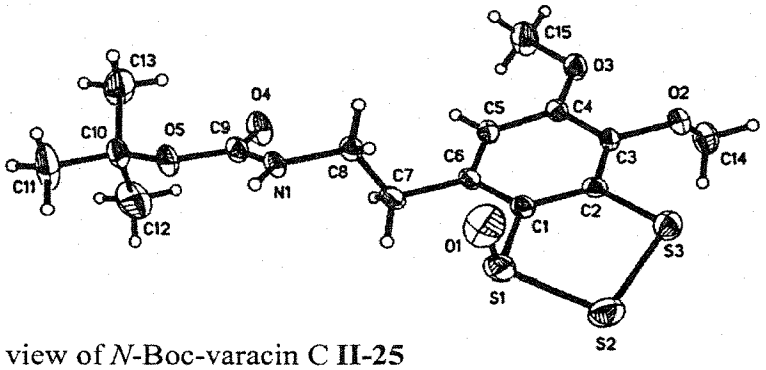


Chart 2.4 ORTEP view of *N*-Boc-varacin C **II-25**

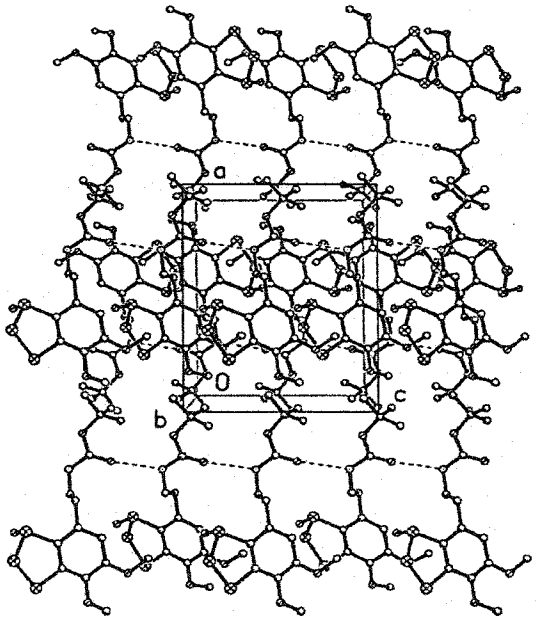
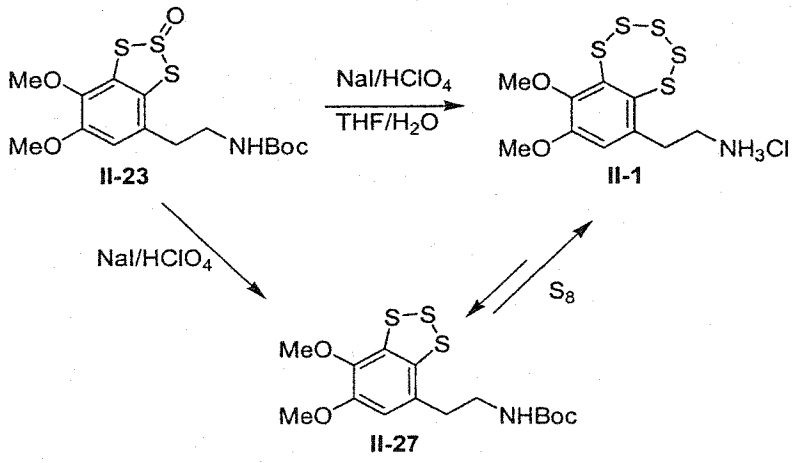


Chart 2.5 Crystal packing of *N*-Boc-varacin C **II-25**

X-Ray analysis of the crystal of **II-25** was shown in Charts 2.4 and 2.5. Crystal data: Monoclinic symmetry, and accurate lattice constants of a=12.546(3) Å, b=15.353(3) Å, c=9.957(2) Å were determined and refined from a full-matrix least-squares on F^2 (Goodness-of-fit on F^2 = 1.020). The empirical formula was $C_{15}H_{21}NO_5S_3$, the formula weight was 391.51 amu and crystal density, 1.356 Mg/m^3, indicated that four molecules made up the unit cell (volume, z: 1917.7(7) $Å^3$, 4). F(000) was 824. Systematic extinctions were consistent with the $P2_1/c$ space group. All data were collected using Mo $K\alpha$ radiation (λ=0.71073 Å). Number of reflections collected was 12749, and the number of independent reflections was 4401 (R_{int}=0.0925), 223 parameters. Completeness to (θ = 27.62°) was 98.8%. Final R indices [I>2σ(I)], R1 = 0.0776, wR2 = 0.1754, R indice (all data) R1 = 0.2441, wR2 = 0.2149. The Largest diff. Peak and hole were 0.742 and –0.294 e $Å^{-3}$.

The novel varacin **II-1** is the first known benzopentathiepin antibiotic,[1-5] it was prepared before as in Scheme 2.7 pathway and now accidentally from reducing the benzotrithiole 2-oxide **II-23** with sodium iodide and perchloric acid,[39] see Scheme 2.8.

In the course of our work toward the synthesis of antibiotic varacin A **II-2**, the title compound varacin, was obtained as an unexpected product in 90% yield. After careful examination of the X-ray crystallographic data and other resources,[36] we believed that we have obtained the novel antibiotic varacin **II-1** fortuitously with a new methodology. We proposed that the varacin A formed in the reaction reacted with S_8 generated from the quenching agent $Na_2S_2O_7$, and subsequently afforded varacinium



Scheme 2.8 Accidental preparation of varacin **II-1** from the benzotrithiole 2-oxide **II-23**

chloride. In fact, a similar equilibrium between varacin and varacin A + S_8 was first reported by Makarieva *et al.* in 1995.[5] The benzopentathiepin ring exists in the expected chiral conformation similar to previous reports for pentathiepin (Chenard *et al.*, 1987)[38] and varacin (Ford *et al.*, 1994).[9b] The ring formed a chair conformation with torsion angles [C(1)-S(1)-S(2)-S(3), S(3)-S(4)-S(5)-C(2)] were –93.78(17)° and 93.11(19)° and the [S(1)-S(2)-S(3)-S(4), S(2)-S(3)-S(4)-S(5)] torsion angles were 73.00(11)° and –75.16(11)°, The angle of S(1)-S(2)-C(1)-C(2) was 83.4(4)° . Interestingly, the sulfur-sulfur bond lengths [S(1)-S(2) 2.036(2) Å], [S(2)-S(3) 2.051(2) Å], [S(3)-S(4) 2.054(2) Å], [S(4)-S(5) 2.043(3) Å], were similar to the sulfur-sulfur bond lengths in S_8.[19b] The sulfur-carbon (S-Csp^2) [S(1)-C(1) 1.777(4) Å], [S(5)-C(2) 1.773(5) Å] bond lengths were marginally longer than those of the general sulfur-carbon (sp^2) single bond lengths (1.75 Å), but comparable to those (1.774, 1.777 Å) found in the benzopentathiepin (Fehér *et al.*, 1979).[37] From the packing diagram (Chart 2.7), we

observed dipolar interactions between ionic chlorine with methoxy oxygen and sulfur S(2) atoms, and the molecules were linked by Cl^- ions through N-H···Cl hydrogen bonds to form a network structure.[36]

X-Ray analysis of the crystal of varacinium hydrogen chloride salt **II-1** was shown in Charts 2.6 and 2.7. Corresponding crystal data for varacin **II-1**: Monoclinic symmetry, and accurate lattice constants of a=16.891(3) Å, b=10.6971(16) Å, c=9.1959(13) Å were determined and refined from a full-matrix least-squares on F^2 (Goodness-of-fit on F^2 = 0.905). The empirical formula was $(C_{10}H_{14}NO_2S_5)^+Cl^-$, the formula weight was 375.97 amu and crystal density, 1.535 Mg/m^3, indicated that four molecules made up the unit cell (volume, z: 1627.2(4) $Å^3$, 4). F(000) is 784. Systematic extinctions were consistent with the $P2_1/c$ space group. All data were collected using Mo $K\alpha$ radiation (λ=0.71073 Å).

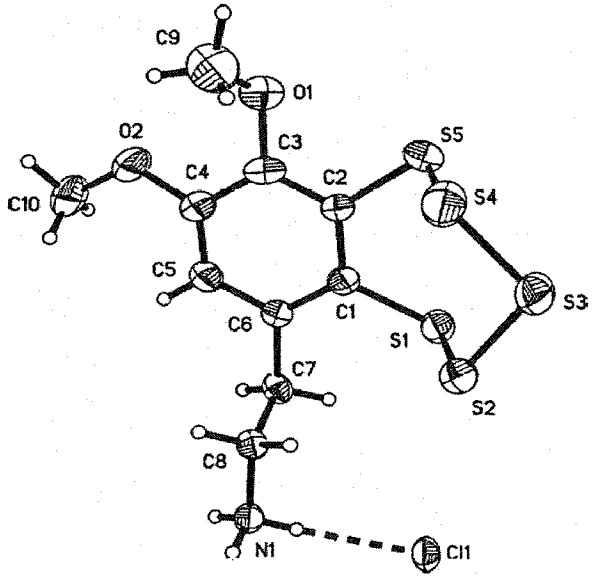


Chart 2.6 ORTEP view of varacin **II-1**

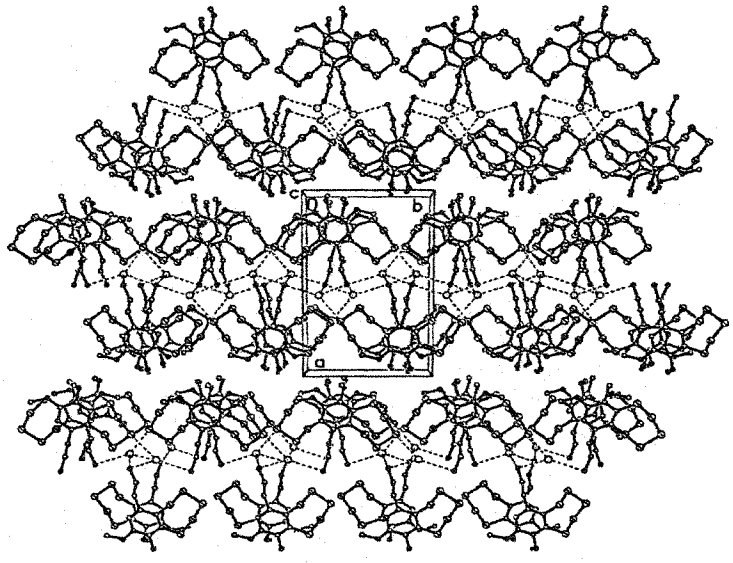


Chart 2.7 Unit cell of varacin **II-1** crystal packing

II.2.3 DNA-Cleaving Assays of Varacin, Varacins B, C and Their Analogs

We have investigated the variations of DNA-cleaving properties of nine benzopentathiepins and benzotrithiole oxides (Chart 2.3, except varacin A **II-2**) with the given concentrations of 2-mercaptoethanol used to trigger the cleavage. DNA cleavage was monitored by observing the conversion of supercoiled (Form I) plasmid DNA to the circular nicked form (Form II) that occurred upon single-strand scission. In some cases, more extensive DNA cleavage led to the appearance of linearized form (Form III) migrating below the nicked plasmid in 0.9% agarose gels. We found that in the presence of 2-mercaptoethanol, one of the commonly used thiols to trigger the DNA cleavage, nanomolar to micromolar concentrations of the DNA-cleaving agents efficiently carried out single-strand cleavage of plasmid DNA. At the concentrations used in our studies,

those agents did not cleave DNA efficiently in the absence of added thiol. This finding indicated that the compounds we synthesized are potent thiol-mediated DNA-cleaving agents. Our controls showed that under physiologically relevant conditions we prepared in experiments, 2-mercaptoethanol alone did not significantly cleave plasmid DNA though some studies suggested that thiols are capable of causing DNA cleavage through reduction of molecular oxygen to superoxide radical.[40] The typical DNA-cleavage reactions were handled as delineation in the experimental part.

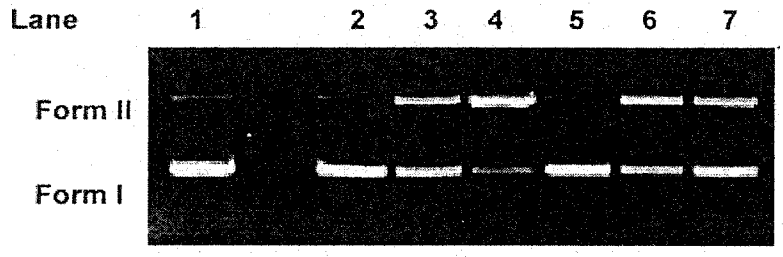


Figure 2.1 2-Mercaptoethanol-dependent cleavage of supercoiled pBR322 DNA by **II-6** and **II-7**. Reactions were incubated at 37 °C for 16 h in 50mM sodium phosphate, pH 7.0, containing 10% v/v acetonitrile and supercoiled pBR322 DNA (38 μM/bp). Lane 1, pBR322 DNA alone; lane 2, 5 μM **II-6** + 100 μM 2-mercaptoethanol; lane 3, 10 μM **II-6** + 200 μM 2-mercaptoethanol; lane 4, 25 μM **II-6** + 500 μM 2-mercaptoethanol; lane 5, 5 μM **II-7** + 100 μM 2-mercaptoethanol; lane 6, 10 μM **II-7** + 200 μM 2-mercaptoethanol; lane 7, 25 μM **II-7** + 500 μM 2-mercaptoethanol.

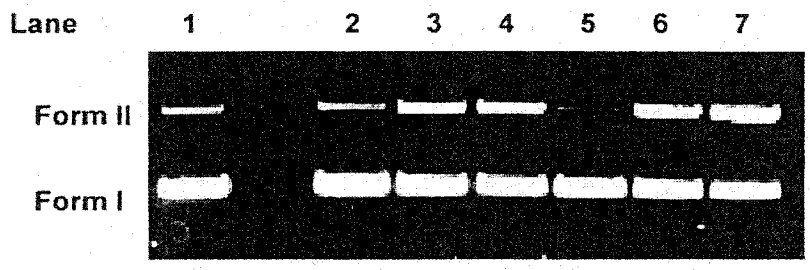
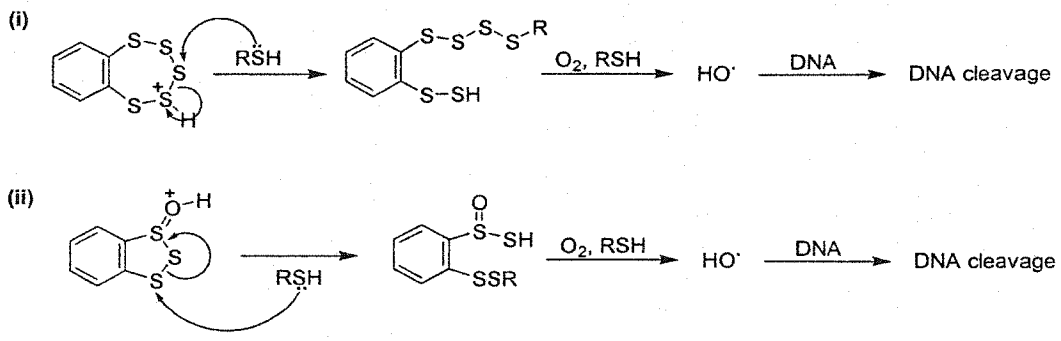


Figure 2.2 2-Mercaptoethanol-dependent cleavage of supercoiled pBR322 DNA by **II-8** and **II-9ab**. Reactions were incubated at 37 °C for 16 h in 50 mM sodium phosphate, pH 7.0, containing 10% v/v acetonitrile and supercoiled pBR322 DNA (38 μM/bp). Lane 1, pBR322 DNA alone; lane 2, 5 μM **II-8** + 100 μM 2-mercaptoethanol; lane 3, 10 μM **II-8** + 200 μM 2-mercaptoethanol; lane 4, 25 μM **II-8** + 500 μM 2-mercaptoethanol; lane 5, 5 μM **II-9ab** + 100 μM 2-mercaptoethanol; lane 6, 10 μM **II-9ab** + 200 μM 2-mercaptoethanol; lane 7, 25 μM **II-9ab** + 500 μM 2-mercaptoethanol.

In addition to the above assays, we attempted to show the variation of pH values in the medium resulted on the effects of DNA-cleavage. The encouraging turns out illustrated that the changes of pH in the physicological conditions in our experiments have a tremendous effect on supercoiled plasmid DNA cleavage by **II-1**, **II-3-6**. We have prepared sodium phosphate buffers with pH values ranged from pH 5.0 to pH 8.0. Our assays indicated that the rates of thiol-mediated DNA-cleavage by the agents **II-1**, **II-3-6** reached their maximum at pH 5.5 from pH 5.0, and then decayed slowly to their minimum at pH 8.0. To the best of our knowledge, the relationship between pH values and the rates of thiol-dependent DNA cleavage is rarely reported. We would like to propose that these observations might come from a couple of reasons listed below:

(1) at low pH value, amino functionality of varacin **II-1**, varacin B **II-3**, C **II-4** and their structural analog **II-5** were protonated to certain extents and the binding affinity to negatively charged phosphate backbones was largely increased.[8,34] However, the negative charges of phosphate backbones of DNA were neutralized at such a low pH 5.0 which might consequently decreased the binding affinity of our positively charged DNA cleaving agents to target DNA;

(2) the proposed mechanisms of acid-promoted thiol-dependent DNA cleavage by (i) varacin involved protonation of sulfur atom in the polysulfide cycle to increase the electrophilicity and lead to higher susceptibility to thiol attack; (ii) varacin B and varacin C involved protonation of sulfoxide entity at the oxygen atom, then triggered the nucleophilic attack from thiol and cleaved the DNA via a chain reaction of Fenton type (Scheme 2.9).[41]



Scheme 2.9 Plausible mechanisms of acid-promoted DNA cleavage potentiated by varacin **II-1**, varacins B **II-3**, and C **II-4**.

This unpublished discovery demonstrates that the authentic molecular structures of varacin and its family are capable of causing highly efficient DNA cleavage promoted by their acidic surroundings. It is of interest in both medicinal chemistry and biological science, because the extracellular pH in most tumor cells is lower than that in normal tissue (i.e. around pH 6.5 or below depends on the locations of tumor, *in vivo* circumstances and the methods of pH-determination, diagnostic sampling) and the antibiotics possessing acid-triggered functionalities are rare in nature.[42] The activating mode of varacinium antibiotics described in this report could consequently offer helpful hints to chemists with respect to design the acid-activated architectures of molecules as the candidates of tumor cell-activated prodrugs.

In our previous studies on leinamycin's analogs, the varacinium compounds and the work published by Gates *et al*,[8,14] added thiol was shown to be absolutely required for DNA-cleavage by certain types of agents including our molecules. Our research then expanded to the studies on the variations of added thiol concentrations to the degree of

thiol-dependence DNA cleavage reactions by **II-1**, **II-3-6**. Results showed that the maxima were displayed at approximately 2000 to 4000 equivalents of thiol added (depended on the identity of agents), and the lowest concentration of added thiol needed to trigger the reaction was in common to our compounds and equaled to 4 molar equivalent. Such thiol dependence is expected in a system where thiols both stimulates the formation of DNA-cleaving reactive intermediates and can also quench those reactive species as the thiols are the radical scavengers.[43] A similar trend in the dependence of DNA-cleavage efficiency on thiol concentration is observed for enediyne antibiotics where the formation of thiol-quenchable reactive intermediates is triggered by thiols.[44]

Besides of the above experiments, we have tried other organic compounds to trigger the DNA cleavage. The 2-mercaptoethanol is not the unique trigger in the reactions, glutathione, cysteine, thiophenol share its ability to potentiate DNA cleavage by the entities **II-1**, **II-3-6**. In the following Figures 2.7, 2.11, 2.16, 2.21 and 2.27, entries of 4-ethylphenol and 4-methylaniline gave no observable DNA-cleavage promotion. Interestingly, we found that cysteine (see the corresponding concentrations used) cut the supercoiled plasmid DNA in controls. This is the only one exception so far we have discovered that the co-reagent itself is capable of causing DNA cleavage. Cysteine may reduce molecular oxygen effectively to superoxide radical and cut the DNA through radical mechanism.[40] Glutathione was found to be competent to drive DNA cleavage in our experiments just like those recorded by Chatterji *et al.*[8b] Glutathione is a biological reducing agent in thiol-dependent enzyme reactions, and is recently reported to have anticancer activity.[45] Glutathione levels in antigen-presenting

cells modulate Th1 versus Th2 response patterns.[46] Thiolphenol delivered weaker potentiation of DNA-cleavage comparing to other thiols employed in our works.

One of our aims in this research is to find out the DNA-cleaving mechanisms by our candidates of DNA-cleaving agents. Although previous reports indicated that simple analogs of benzopentathiepins gave radical single-strand DNA-cleavage,[8,9] few of the elaborated studies on varacin and its other families' DNA-damaging mechanisms were done. Strands scission on DNA molecules can be resulted from (i) alkyation mechanism by some electrophiles,[15] and (ii) radical mechanism by oxygen radicals, carbon radicals.[14,23] Our attempts to prove the kind of mechanism responsible for thiol-mediated DNA cleavage by varacin and its derivatives were described in the following. We have employed different additives in our trials, including radical scavengers- methanol, ethanol and mannitol. Mannitol is reported to be an effective scavenger of hydroxyl radical but not alkoxyl radicals.[47] Methanol and ethanol are known to react rapidly with hydroxyl radical but much slower with alkoxyl radicals. The studies indicated that those methanol, ethanol and mannitol were capable of quenching DNA-cleavage by **II-1**, **II-3-6**. The quenching behavior of these alkanols is consistent with the proposal that oxygen radicals are generated in the course. However, we could not conclude the co-existence of alkoxide radical in the presence of hydroxyl radical.[8,14b]

The significant quenching of our thiol-mediated DNA cleavage reactions by catalase- the hydrogen peroxide destroying enzyme, suggested that in combination with thiols, molecular oxygen was converted to hydrogen peroxide, which decomposed to hydroxyl radical via Fenton reaction, and cleaved the DNA under our experimental

conditions.[14b,48] Hydrogen peroxide might be formed directly or from spontaneous dismutation of superoxide radical. Catalase activates the decomposition of hydrogen peroxide, a reactive oxygen species, into water and oxygen. It functions as a natural antioxidant, protecting cells against oxidative damage to proteins, lipids and nucleic acids.[49] Catalase has also been used to study the role of reactive oxygen species play in gene expression and apoptosis.[50]

Addition of superoxide dismutase (SOD), a superoxide destroying enzyme to our reaction mixtures did not quench the DNA-cleavage by our agents.[41] Instead, it enhanced the thiol-mediated DNA-cleavage in certain extends. As mentioned before, hydrogen peroxide might be formed directly or from spontaneous dismutation of superoxide radical.[14b] Participation of superoxide radical in some DNA cleavage reactions has been indicated by diminished DNA cleavage upon addition of superoxide dismutase to the reaction mixtures. SOD catalyses the conversion of two molecules of superoxide radical to one molecule of molecular oxygen and one molecule of hydrogen peroxide. The product of the superoxide dismutase reaction, hydrogen peroxide causes DNA cleavage by producing hydroxyl radical via Fenton reaction.[8,14] In cases, where superoxide mediated DNA-cleavage is inhibited by SOD which converts the superoxide that may serve as a necessary reducing agent to drive the Fenton chemistry to hydrogen peroxide in enzymatic catalysis. However, the slight enhancement of DNA-cleavage in our experiments might be explained by the hydrogen peroxide which produced in SOD-mediated reaction, while the presence of superoxide radical in these reactions has not yet been unambiguously confirmed.[14b]

When comparing the results with Gates's works, we were only capable to show minor quenching effects of added DETAPAC on thiol-mediated DNA cleavage by our compounds. The addition of metal chelators such as deferral and diethylenetriaminepentaacetic acid (DETAPAC) are known to sequester adventitious traces of transition metals in a form that inhibits these metals from catalyzing the conversion of species such as peroxides to DNA-cleaving oxygen radicals such as hydroxyl radical in Fenton pathway.[14, 51] This part of our results arbitrarily served as an additional evidence implicating oxygen radicals in the DNA cleavage.

2,2,6,6-Tetramethylpiperidine 1-oxyl (TEMPO) was used as one of the additives in our assays. It is a stable free radical used for radical trapping in mechanistic studies. TEMPO reacts preferentially with carbon-centered radicals.[52] It is also used as a catalyst in the oxidation of primary alcohols to aldehydes with NaOCl.[53] Our results proposed that the carbon-centered radicals existed in our thiol-mediated DNA-cleavage by candidates **II-1**, **II-3-6**. We are curious about the origin of carbon-centered radicals in our physicological conditions of experiments. Whether those radicals are really presence as the carbon radical of nucleotide backbones yielded from the hydroxyl radical attack or the TEMPO participated in some kind of unknown or unrealized routes, which resulted in the influence of DNA-cleavage are opened for further discussion. Imidazole, pyridine and 4-methyl aniline were added to our reaction mixtures too. They took up the roles of nucleophiles to testify the effect of nucleophiles on thiol-mediated DNA-cleavage by the compounds. They showed neither enhancement, nor inhibition in the cleavage, implying the DNA cleavage is not following an alkylation pathway and there is no electrophile involvement. Also, potassium iodide (KI) that decreases the concentration of hydrogen

peroxide (H_2O_2), Tiron and dithiothreitol (DTT), the superoxide radical ($O_2^{\cdot-}$) scavengers, were used to illustrate the presence of the radical species in the reaction mixtures.[54] Besides the above discussions, the following sections present as the brief reports and observations on the DNA-cleaving assays mediated by our titled compounds one-by-one.

II.2.3.1 DNA Cleavage by Benzotrithiole 2-Oxide II-6

It was initially reported by Rasheed *et al.* in 1980 that benzotrithiole 2-oxide **II-6** was a biologically useful molecule that acted as herbicides, fungicides, acaricides, nematocides and insecticides.[13] This sulfur-containing compound used in our DNA-cleaving studies was synthesized according to the procedures reported in literature[19,20] with certain modifications and its DNA-cleaving ability was tested by monitoring the conversion of circular supercoiled DNA (form I) to circular relaxed (form II) DNA. Plasmid pBR322 was accordingly incubated with **II-6** in different buffer solutions with pH values ranged from 5.0 to 7.5.[10] As shown in Figure 2.3, benzotrithiole 2-oxide **II-6** caused single stranded DNA cleavage effectively in the presence of 2-mercaptoethanol at pH 5.0 (81%, Lane 2), 5.5 (89%, Lane 4) and 6.0 (76%, Lane 6) while such DNA-cleaving activity decreased slightly as the pH of the corresponding buffer solutions increased to 6.5 (55%, Lane 8), 7.0 (44%, Lane 10) and 7.5 (31%, Lane 12). In addition, benzotrithiole 2-oxide generated detectable formation of form II DNA at a concentration of 250 μM at pH 5.5 (Lane 2, Figure 2.4) and in a concentration-dependent fashion. It should be pointed out that the efficiency of this DNA-cleaving process decreased with

increasing the concentration of benzotrithiole 2-oxide from 25 to 250 μM (Lanes 4, 3 and 2). One of the possible causes of this observation could be benzotrithiole 2-oxide **II-6** was capable of acting as an inefficient oxygen radical scavenger, the effect of which manifested itself only when its concentration increased. It is certainly that alternative causes of this observation are also possible. In addition, the amount of DNA cleavage by benzotrithiole 2-oxide increased with increasing the concentration of added 2-mercaptoethanol (Figure 2.5) while such DNA-cleaving activity was not observable in the absence of added thiol (Lane 1).

The inhibitory effects of certain free radical scavengers and nucleophile on the DNA-cleaving process by **II-6** were investigated.[14] As shown in Figure 2.6, addition of 4-methylaniline to the corresponding mixtures did not slow down the rate of the reactions (Lanes 10), which could be the sign of the absence of active electrophilic species involved in the DNA-cleaving process. Conversely, the known superoxide radical ($O_2^{\bullet -}$) scavengers Tiron (Lane 3) and DTT[54] (Lane 4) inhibited this DNA-cleaving reaction efficiently. In addition, the rate of this DNA-cleaving course was reduced by catalase[14,48] (Lane 5) and potassium iodide[54] (Lane 6) that decrease the concentration of hydrogen peroxide (H_2O_2) in solution. Moreover, hydroxyl radical ($OH^{\bullet}$) scavengers- ethanol (Lane 7) and mannitol (Lane 8) exhibited effective inhibitory effect on the DNA-cleaving process.[14]

Interestingly, addition of diethylenetriamine pentaacetic acid (DETAPAC) also slowed down the rate of this DNA-cleaving process by benzotrithiole 2-oxide. Based on these observations, it can be assumed that hydrogen peroxide was generated from the reaction of benzotrithiole 2-oxide **II-6** with molecular oxygen activated by the addition of thiols through the intermediate superoxide radical.[14-19] It is noted that addition of superoxide-decomposing superoxide dismutase (SOD) to our reaction mixture did not slow down the rate of the DNA-cleavage reaction (Lane 11, Figure 2.6).[48]

This happened most likely because the hydrogen peroxide produced by SOD from superoxide could itself lead to DNA cleavage through a Fenton reaction[14,48] as mentioned before. Also, Figure 2.7 shows that thiol 2-mercaptoethanol is not unique in its ability to potentiate plasmid DNA cleavage by benzotrithiole 2-oxide **II-6**. Thiophenol gave only little effect to trigger DNA cleavage, 4-ethylphenol has no effect at all. Glutathione reduced, 2-mercaptoethanol and cysteine illustrated the strong effect on the potentiation of DNA cleavage by our agent. Cysteine has some capability of causing DNA cleavage by itself,[14b,40] and the degree of DNA strand scission was greatly enhanced after combining with benzotrithiole 2-oxide (lanes 9 and 10).

Benzotrithiole 2-oxide II-6

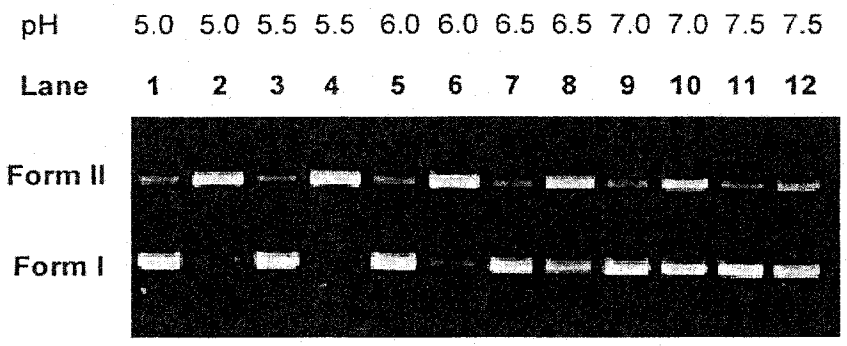


Figure 2.3 DNA cleavage by benzotrithiole 2-oxide **II-6** in buffer solutions of different pH values. Reaction mixtures were incubated at 37 °C for 4 h in 50 mM sodium phosphate buffer solution containing 25 μM **II-6**, 10% v/v acetonitrile and 38 μM (bp) supercoiled pBR322 DNA in the presence or absence of 1 mM glutathione. Lane 1, pH 5.0; lane 2, pH 5.0 in the presence of glutathione; lane 3, pH 5.5; lane 4, pH 5.5 in the presence of glutathione; lane 5, pH 6.0; lane 6, pH 6.0 in the presence of glutathione; lane 7, pH 6.5; lane 8, pH 6.5 in the presence of glutathione; lane 9, pH 7.0; lane 10, pH 7.0 in the presence of glutathione; lane 11, pH 7.5; lane 12, pH 7.5 with glutathione.

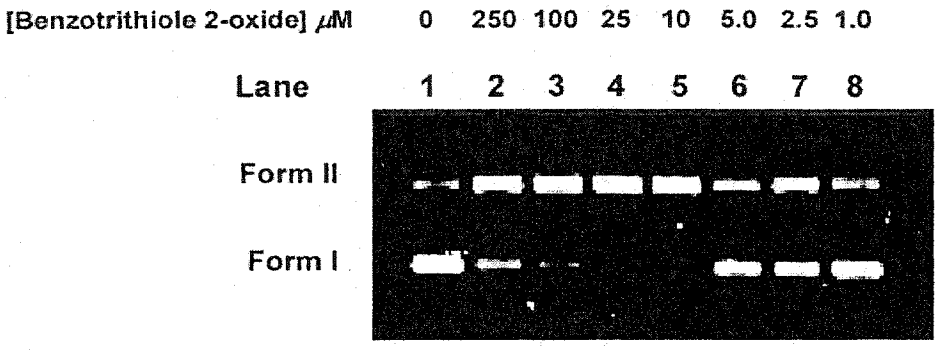


Figure 2.4 DNA-cleavage by various concentrations of benzotrithiole 2-oxide **II-6**. Reaction mixtures were incubated at 37 °C for 4 h in 50 mM sodium phosphate buffer solution (pH 5.5) containing **II-6**, 10% v/v acetonitrile, 38 μM (bp) supercoiled pBR322 DNA and 1 mM glutathione. Lane 1, pBR322 alone; lane 2, 250 μM **II-6**; lane 3, 100 μM **II-6**; lane 4, 25 μM **II-6**; lane 5, 10 μM **II-6**; lane 6, 5 μM **II-6**; lane 7, 2.5 μM **II-6**; lane 8, 1 μM **II-6**.

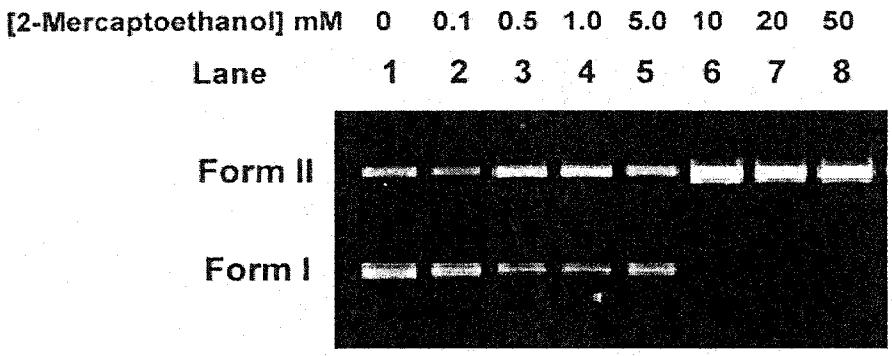


Figure 2.5 Thiol-dependent cleavage of supercoiled pBR322 DNA by benzotrithiole 2-oxide **II-6** in various concentrations of 2-mercaptoethanol. Reaction mixtures were incubated at 37 °C for 12 h in 50 mM sodium phosphate, pH 5.5, containing 10% v/v acetonitrile, 38 μM (bp) supercoiled pBR322 DNA and 25 μM **II-6** in the presence of 2-mercaptoethanol. Lane 1, pBR322 alone; lane 2, 100 μM 2-mercaptoethanol; lane 3, 500 μM 2-mercaptoethanol; lane 4, 1 mM 2-mercaptoethanol; lane 5, 5 mM 2-mercaptoethanol; lane 6, 10 mM 2-mercaptoethanol; lane 7, 20 mM 2-mercaptoethanol; lane 8, 50 mM 2-mercaptoethanol.

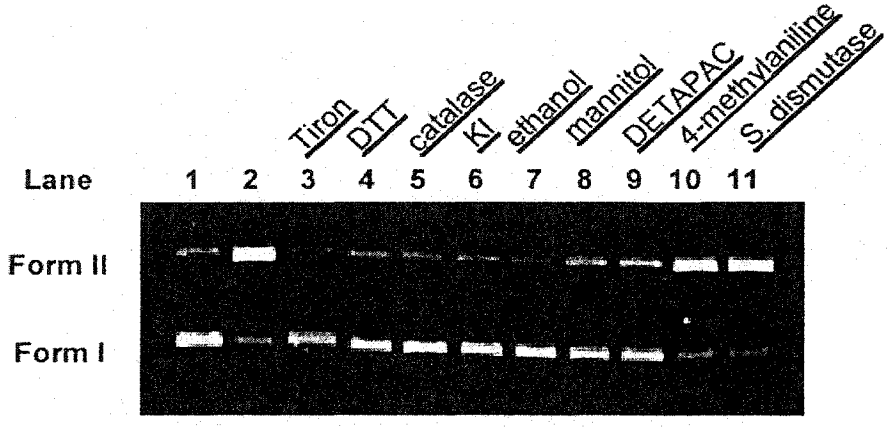


Figure 2.6 DNA-cleavage by benzotrithiole 2-oxide **II-6** in the presence of radical scavengers or nucleophiles. Reaction mixtures were incubated at 37 °C for 7 h in 50 mM sodium phosphate buffer solution (pH 5.5) containing 25 μM **II-6,** 10% v/v acetonitrile, 38 μM (bp) supercoiled pBR322 DNA and 2.5 mM 2-mercaptoethanol. Lane 1, absent of benzotrithiole 2-oxide; lane 2, standard reaction in the absence of added radical scavengers or nucleophile; lane 3, 200 mM Tiron; lane 4, 80 mM DTT; lane 5, 53.2 μg/mL catalase; lane 6, 100 mM KI; lane 7, 1 M ethanol; lane 8, 100 mM mannitol; lane 9, 2.5 mM DETAPAC; lane 10, 10 mM 4-methylaniline; lane 11, 80 μg/mL superoxide dismutase.

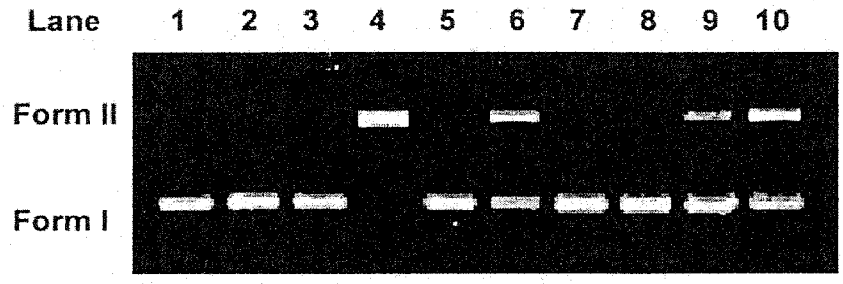


Figure 2.7 Thiol-dependent cleavage of supercoiled pBR322 DNA by 25 μM **II-6** in various thiols 1 mM. Reactions were incubated at 37 °C for 4 h in 50 mM sodium phosphate, pH 5.5, containing 10% v/v acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, 1 mM thiophenol; lane 2, 25 μM **II-6** + 1 mM thiophenol; lane 3, 1 mM glutathione reduced; lane 4, 25 μM **II-6** + 1 mM glutathione reduced; lane 5, 1 mM 2-mercaptoethanol; lane 6, 25 μM **II-6** + 1 mM 2-mercaptoethanol; lane 7, 1 mM 4-ethylphenol; lane 8, 25 μM **II-6** + 1 mM 4-ethylphenol; lane 9, 1 mM cysteine; lane 10, 25 μM **II-6** +1 mM cysteine.

II.2.3.2 DNA Cleavage by Varacin C II-4

Varacin C **II-4** alone did not cleave DNA significantly under our experimental conditions (In Figure 2.9, Lane 1). DNA cleavage by our agent increased with increasing amounts of added thiol from 20 to 2000 equivalence. No diminished DNA cleavage even at very high concentration of thiols. This observation was different from what we recorded in the case of leinamycin analogs in Part I. Plasmid pBR322 was accordingly incubated with varacin C in different buffer solutions (pH values 5.0-7.5). As shown in Figure 2.10, varacin C **II-4** caused single stranded DNA cleavage effectively at pH 5.0 (41%, Lane 2), 5.5 (47%, Lane 4) and 6.0 (39%, Lane 6), but not at higher pH: [pH 7.0 (23%, Lane 10) and pH 7.5 (12%, Lane 12)]. Moreover, even at a low concentration, varacin C (50 nM) could generate a detectable amount of form II DNA at pH 5.5 (Lane 4, Figure 2.8).

The activating course of the DNA cleavage by varacin C **II-4** was next examined by adding different types of thiols to the corresponding reaction mixtures. As shown in Figure 2.11, both glutathione[8,14] and cysteine[40] were capable of triggering varacin C-mediated DNA-cleavage as effectively as 2-mercaptoethanol (Lanes 4 and 6). However, when 2-mercaptoethanol was replaced with phenol or aniline (Lanes 8 and 10), DNA cleavage did not occur.

Varacin C II-4

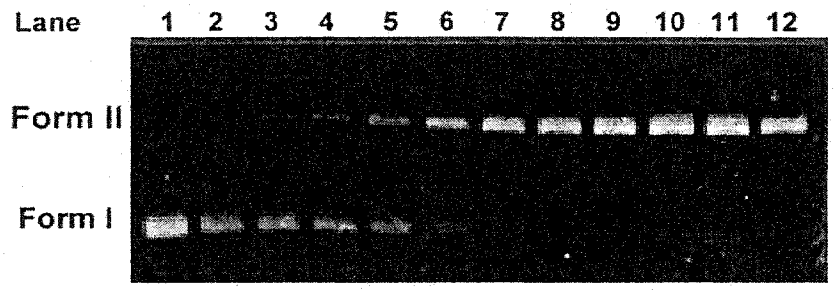


Figure 2.8 Thiol-dependent cleavage of supercoiled pBR322 DNA by various concentrations of varacin C **II-4**. Reactions were incubated at 37 °C for 12 h in 50 mM sodium phosphate, pH 5.5, containing 10% v/v acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, DNA alone; lane 2, 10 nM **II-4** +1 mM 2-mercaptoethanol; lane 3, 25 nM **II-4** +1 mM 2-mercaptoethanol; lane 4, 50 nM **II-4** +1 mM 2-mercaptoethanol; lane 5, 100 nM **II-4** +1 mM 2-mercaptoethanol; lane 6, 250 nM **II-4** +1 mM 2-mercaptoethanol; lane 7, 500 nM **II-4** +1 mM 2-mercaptoethanol; lane 8, 1 μM **II-4** + 1 mM 2-mercaptoethanol; lane 9, 2.5 μM **II-4** + 1 mM 2-mercaptoethanol; lane 10, 5 μM **II-4** + 1 mM 2-mercaptoethanol; lane 11, 10 μM **II-4** + 1 mM 2-mercaptoethanol; lane 12, 25 μM **II-4** + 1 mM 2-mercaptoethanol.

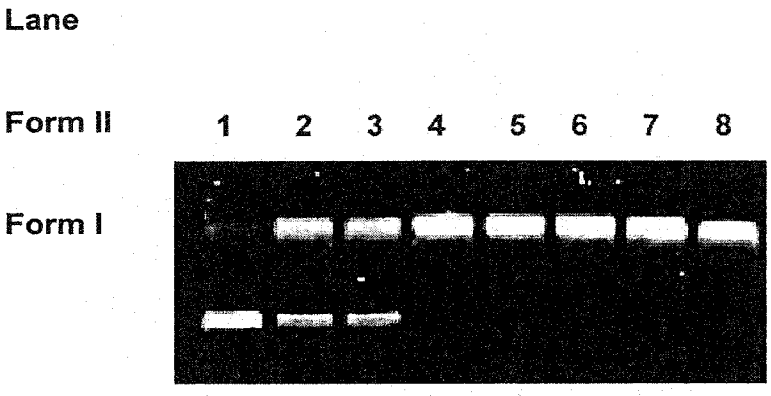


Figure 2.9 Thiol-dependent cleavage of supercoiled pBR322 DNA by 250 nM varacin C **II-4** in various concentrations of 2-mercaptoethanol. Reactions were incubated at 37 °C for 16 h in 50 mM sodium phosphate, pH 5.5, containing 10% v/v acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, 250 nM **II-4** alone; lane 2, 250 nM **II-4** + 5 μM 2-mercaptoethanol; lane 3, 250 nM **II-4** + 10 μM 2-mercaptoethanol; lane 4, 250 nM **II-4** + 25 μM 2-mercaptoethanol; lane 5, 250 nM **II-4** + 50 μM 2-mercaptoethanol; lane 6, 250 nM **II-4** + 100 μM 2-mercaptoethanol; lane 7, 250 nM **II-4** + 250 μM 2-mercaptoethanol; lane 8, 250 nM **II-4** + 500 μM 2-mercaptoethanol.

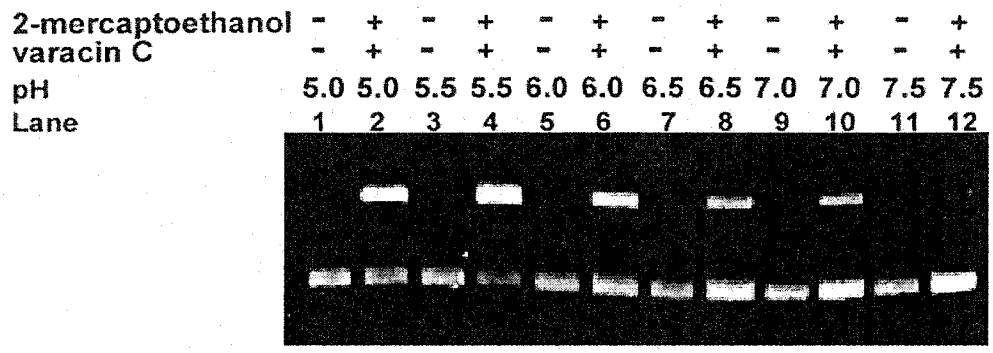


Figure 2.10 Thiol-dependent cleavage of supercoiled pBR322 DNA by 5 μM varacin C **II-4** in 500 μM 2-mercaptoethanol at various pH values. Reactions were incubated at 37 ^{o}C for 2 h in 50 mM sodium phosphate, containing 10% v/v acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, DNA alone at pH 5.0; lane 2, 5 μM **II-4** + 500 μM 2-mercaptoethanol at pH 5.0; lane 3, DNA alone at pH 5.0; lane 4, 5 μM **II-4** + 500 μM 2-mercaptoethanol at pH 5.5; lane 5, DNA alone at pH 6.0; lane 6, 5 μM **II-4** + 500 μM 2-mercaptoethanol at pH 6.0; lane 7, DNA alone at pH 6.5; lane 8, 5 μM **II-4** + 500 μM 2-mercaptoethanol at pH 6.5; lane 9, DNA alone at pH 7.0; lane 10, 5 μM **II-4** + 500 μM 2-mercaptoethanol at pH 7.0; lane 11, DNA alone at pH 7.5; lane 12, 5 μM **II-4** + 500 μM 2-mercaptoethanol at pH 7.5.

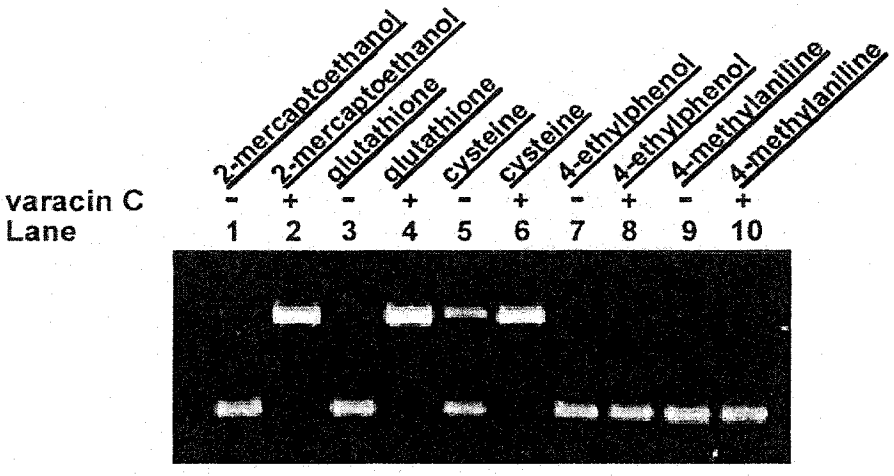


Figure 2.11 Thiol-dependent cleavage of supercoiled pBR322 DNA by 5 μM varacin C **II-4** in various thiols 1 mM. Reactions were incubated at 37 °C for 6 h in 50 mM sodium phosphate, pH 5.5, containing 10% v/v acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, 1 mM 2-mercaptoethanol; lane 2, 5 μM **II-4** + 1 mM 2-mercaptoethanol; lane 3, 1 mM glutathione reduced; lane 4, 5 μM **II-4** + 1 mM glutathione reduced; lane 5, 1 mM cysteine; lane 6, 5 μM **II-4** + 1 mM cysteine; lane 7, 1 mM 4-ethylphenol; lane 8, 5 μM **II-4** + 1 mM 4-ethylphenol; lane 9, 1 mM 4-methylaniline; lane 10, 5 μM **II-4** + 1 mM 4-methylaniline.

Investigating the inhibitions of DNA cleavage mediated by varacin C **II-4**, Figure 2.12 shows that addition of pyridine or 4-methylaniline to the reaction mixture did not slow down the rate of the reactions (Lanes 4 and 5), a result which could be indicative of the absence of an active electrophilic species produced in the DNA-cleaving process. However, the rate of this DNA-cleaving reaction was reduced drastically by the addition of the known superoxide radical ($O_2^{\bullet-}$) scavenger dithiothreitol (DTT) (lane 8) and Tiron (lane 12).[54] In addition, catalase (an enzyme that reduces the concentration of hydrogen peroxide in solution)[14,48] (Lane 3) exhibited significant inhibitory effects on the DNA-cleavage reaction. Moreover, this DNA-cleaving process was inhibited by the efficient hydroxyl radical ($OH^{\bullet}$) scavengers methanol (lane 6), ethanol (lane 7) and mannitol (lane 9).[14,48] Besides these oxygen radical scavengers, the metal chelator diethylenetriaminepentaacetic acid (DETAPAC)[51] also inhibited the varacin C-promoted DNA-cleavage reaction (Lane 10).

Based on these observations, we suggested tentatively that varacin C in concert with a thiol led to the conversion of molecular oxygen to hydrogen peroxide, which was then further converted to the DNA-cleaving hydroxyl radical by a trace-metal-dependent Fenton reaction (Scheme 2.10).[55] This proposed mechanism is analogous to the mode of action of antibiotic leinamycin[14-16,24] established by Gates and associates earlier.

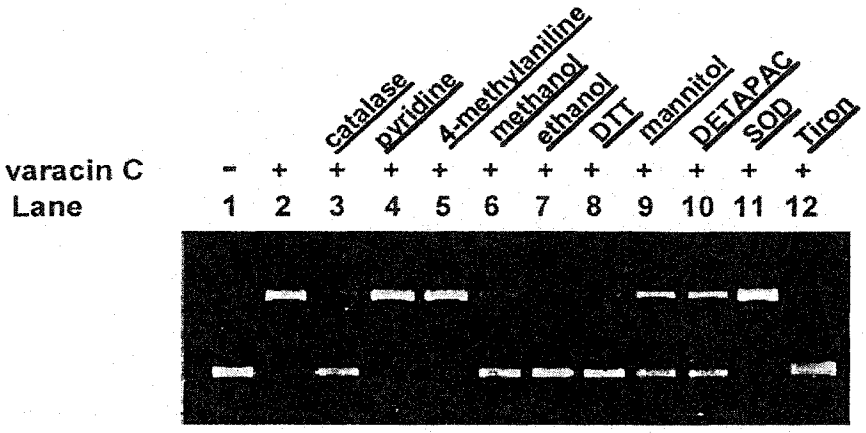
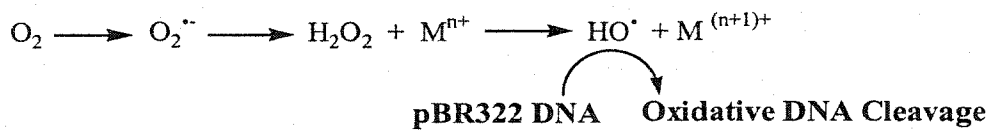


Figure 2.12 Thiol-dependent cleavage of supercoiled pBR322 DNA by 5 μM varacin C **II-4** in 500 μM 2-mercaptoethanol. Reactions were incubated at 37 °C for 3 h in 50 mM sodium phosphate, pH 5.5, containing 10% v/v acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, DNA alone; lane 2, 5 μM **II-4** + 500 μM 2-mercaptoethanol; lane 3, 5 μM **II-4** + 500 μM 2-mercaptoethanol + 53.2 μg/mL catalase; lane 4, 5 μM **II-4** + 500 μM 2-mercaptoethanol + 10 mM pyridine; lane 5, 5 μM **II-4** + 500 μM 2-mercaptoethanol + 10 mM 4-methylaniline; lane 6, 5 μM **II-4** + 500 μM 2-mercaptoethanol + 1M methanol; lane 7, 5 μM **II-4** + 500 μM 2-mercaptoethanol + 1 M ethanol; lane 8, 5 μM **II-4** + 500 μM 2-mercaptoethanol + 80 mM DTT; lane 9, 5 μM **II-4** + 500 μM 2-mercaptoethanol + 100 mM mannitol; lane 10, 5 μM **II-4** + 500 μM 2-mercaptoethanol + 2.5 mM DETAPAC; lane 11, 5 μM **II-4** + 500 μM 2-mercaptoethanol + 80 μg/mL SOD; lane 12, 5 μM **II-4** + 500 μM 2-mercaptoethanol + 200 mM Tiron.



Scheme 2.10 Mechanism of varacinium compounds mediated DNA cleavage

Addition of superoxide dismutase (SOD) (an enzyme which decomposes superoxide)[14b,41a] to the reaction mixture did not slow down the rate of the DNA-cleavage reaction (lane 11, Figure 2.12). This observation was same to those recorded before. A phenomenon observed in the DNA-cleaving reaction by leinamacin.[14,56]

II.2.3.3 DNA Cleavage by Varacin B II-3

In parallel with varacin C **II-4**, varacin B **II-3** has comparable DNA cleaving activities with its cousin. It could lead to detectable DNA cleavage even at very low concentration (500 nM) with added thiol (Figure 2.13, Lane 6). The activating course of the DNA cleavage by varacin B **II-3** was tested by adding various concentrations of 2-mercaptoethanol and adding different types of thiols. The amounts of DNA cleavage by varacin B **II-3** was thiol concentrations dependent, and 20 equivalence (Figure 2.14, Lane 3) of 2-mercaptoethanol was enough to trigger the reaction. We recorded the Form III DNA cleavage as well (Lane 7, with 400 equivalent of thiol added). Those 2-mercaptoethanol (Figure 2.16, Lane 2) glutathione reduced (Lane 4) and thiophenol

(Lane 6) were capable to trigger DNA cleavage potientated by varacin B **II-3**, but not for the same concentrations of phenol and aniline added (Lanes 8 and 10).

The pH of the reaction buffer plays a role on the DNA cleavage potentiated by varacin B **II-3**, Figure 2.15 shows the acid-promoted DNA-cleavage activities of varacin B. Radical scavengers methanol, ethanol, mannitol [14,41] and TEMPO[53] (Figure 2.17, Lanes 6, 7, 8 and 9) significantly quenched DNA cleavage. Addition of hydrogen peroxide destroying enzyme catalase remarkably inhibited the DNA strands scission (Lane 3). Incubating with superoxide-destroying enzyme superoxide dismutase (SOD)[41,48] gave no inhibition but slightly enhanced our DNA cleavage reaction by varacin B **II-3** with the presence of thiol (Lane 4). With our experimental concentration, metal chelator DETAPAC[51] merely demonstrated an insignificant quenching on DNA cleavage (Lane 10). The thiol-mediated DNA cleavage efficiency of varacin B was certainly unaffected by the added nucleophiles imidazole[16] (Lane 5), pyridine (Lane 11) and methylaniline (Lane 12). We found that it might effect the thiol-dependent DNA cleavage in the similar mechanism or manner of what the other agents we have studied.

Varacin B II-3

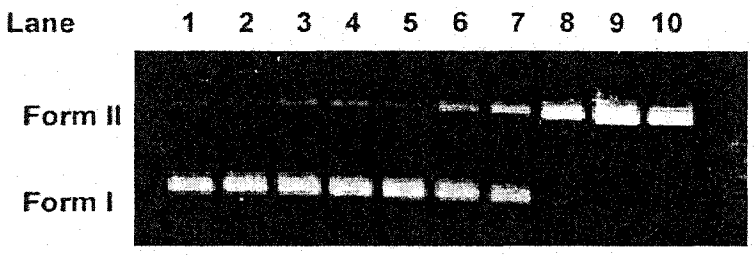


Figure 2.13 Thiol-dependent cleavage of supercoiled pBR322 DNA by various concentrations of varacin B **II-3**. Reactions were incubated at 37 °C for 12 h in 50 mM sodium phosphate, pH 5.5, containing 10% v/v acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, pBR322 alone; lane 2, 10 nM **II-3** +1 mM 2-mercaptoethanol; lane 3, 25 nM **II-3** +1 mM 2-mercaptoethanol; lane 4, 50 nM **II-3** +1 mM 2-mercaptoethanol; lane 5, 100 nM **II-3** +1 mM 2-mercaptoethanol; lane 6, 500 nM **II-3** +1 mM 2-mercaptoethanol; lane 7, 1 μM **II-3** +1 mM 2-mercaptoethanol; lane 8, 5 μM **II-3** + 1 mM 2-mercaptoethanol; lane 9, 10 μM **II-3** + 1 mM 2-mercaptoethanol; lane 10, 25 μM **II-3** + 1 mM 2-mercaptoethanol.

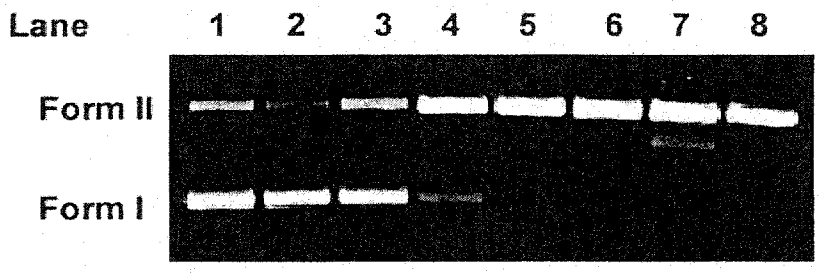


Figure 2.14 Thiol-dependent cleavage of supercoiled pBR322 DNA by 250 nM of varacin B **II-3** in various concentrations of 2-mercaptoethanol. Reactions were incubated at 37 °C for 16 h in 50 mM sodium phosphate, pH 5.5, containing 10% v/v acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, pBR322 alone; lane 2, 250 nM **II-3**; lane 3, 250 nM **II-3** + 5 μM 2-mercaptoethanol; lane 4, 250 nM **II-3** + 10 μM 2-mercaptoethanol; lane 5, 250 nM **II-3** + 25 μM 2-mercaptoethanol; lane 6, 250 nM **II-3** + 50 μM 2-mercaptoethanol; lane 7, 250 nM **II-3** + 100 μM 2-mercaptoethanol; lane 8, 250 nM **II-3** + 250 μM 2-mercaptoethanol.

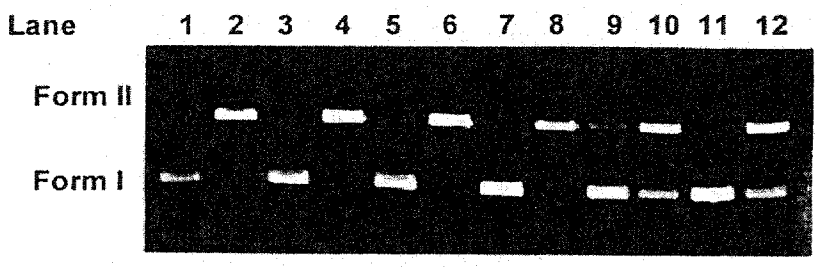


Figure 2.15 Thiol-dependent cleavage of supercoiled pBR322 DNA by 5 μM of varacin B **II-3** in 500 μM 2-mercaptoethanol at various pH values. Reactions were incubated at 37 °C for 3 h in 50 mM sodium phosphate, containing 10% v/v acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, 5 μM **II-3** at pH 5.0; lane 2, 5 μM **II-3** + 500 μM 2-mercaptoethanol at pH 5.0; lane 3, 5 μM **II-3** at pH 5.0; lane 4, 5 μM **II-3** + 500 μM 2-mercaptoethanol at pH 5.5; lane 5, 5 μM **II-3** at pH 6.0; lane 6, 5 μM **II-3** + 500 μM 2-mercaptoethanol at pH 6.0; lane 7, 5 μM **II-3** at pH 6.5; lane 8, 5 μM **II-3** + 500 μM 2-mercaptoethanol at pH 6.5; lane 9, 5 μM **II-3** at pH 7.0; lane 10, 5 μM **II-3** + 500 μM 2-mercaptoethanol at pH 7.0; lane 11, 5 μM **II-3** at pH 7.5; lane 12, 5 μM **II-3** + 500 μM 2-mercaptoethanol at pH 7.5.

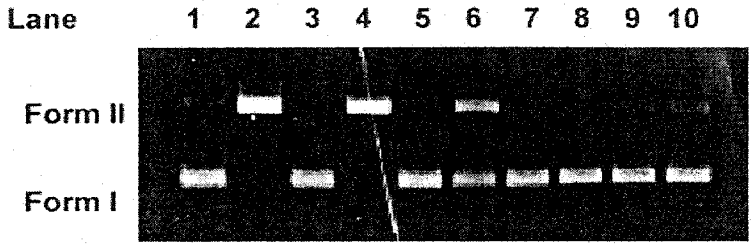


Figure 2.16 Thiol-dependent cleavage of supercoiled pBR322 DNA by 10 μM of varacin B **II-3** in various thiols 1 mM. Reactions were incubated at 37 °C for 6 h in 50 mM sodium phosphate, pH 5.5, containing 10% v/v acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, 5 μM **II-3**; lane 2, 5 μM **II-3** + 1 mM 2-mercaptoethanol; lane 3, 5 μM **II-3**; lane 4, 5 μM **II-3** + 1 mM glutathione reduced; lane 5, 5 μM **II-3**; lane 6, 5 μM **II-3** + 1 mM thiophenol; lane 7, 5 μM **II-3**; lane 8, 5 μM **II-3** + 1 mM 4-ethylphenol; lane 9, 5 μM **II-3**; lane 10, 5 μM **II-3** + 1 mM 4-methylaniline.

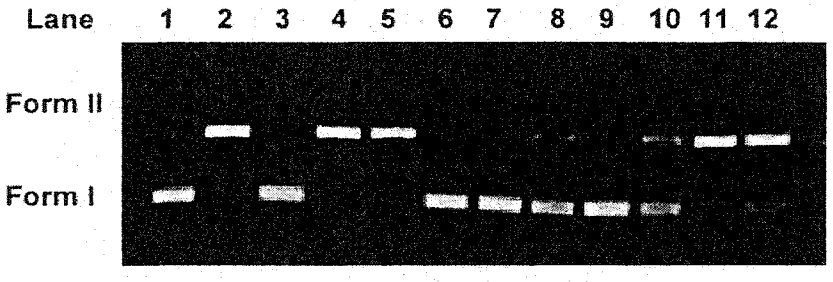


Figure 2.17 Thiol-dependent cleavage of supercoiled pBR322 DNA by 5 μM of varacin B **II-3** in 500 μM 2-mercaptoethanol. Reactions were incubated at 37 °C for 5 h in 50 mM sodium phosphate, pH 5.5, containing 10% v/v acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, 5 μM **II-3**; lane 2, 5 μM **II-3** + 500 μM 2-mercaptoethanol; lane 3, 5 μM **II-3** + 500 μM 2-mercaptoethanol + 53.2 μg/mL catalase; lane 4, 5 μM **II-3** + 500 μM 2-mercaptoethanol + 80 μg/mL SOD; lane 5, 5 μM **II-3** + 500 μM 2-mercaptoethanol + 10 mM imidazole; lane 6, 5 μM **II-3** + 500 μM 2-mercaptoethanol + 1M methanol ; lane 7, 5 μM **II-3** + 500 μM 2-mercaptoethanol + 1 M ethanol; lane 8, 5 μM **II-3** + 500 μM 2-mercaptoethanol + 100 mM mannitol; lane 9, 5 μM **II-3** + 500 μM 2-mercaptoethanol + 5 mM TEMPO; lane 10, 5 μM **II-3** + 500 μM 2-mercaptoethanol + 1 mM DETAPAC; lane 11, 5 μM **II-3** + 500 μM 2-mercaptoethanol + 10 mM pyridine; lane 12, 5 μM **II-3** + 500 μM 2-mercaptoethanol + 10 mM 4-methylaniline.

II.2.3.4 DNA Cleavage by Varacin A 2-oxide II-5

Our work demonstrated **II-5** again, was an effective DNA-cleaving agent in the presence of 2-mercaptoethanol. It cleaved the plasmid DNA with the concentration as low as 1.0 μM (Figure 2.18, Lane 8). We discerned this effectiveness in DNA lesion is the result of high affinity of **II-5**'s positively charged amino group to negatively charged phosphate backbone of DNA. As shown in Figure 2.19, the amount of DNA cleavage by **II-5** increased with increasing the concentration of added 2-mercaptoethanol from 4 equivalence (Lane 2) to 2000 equivalence (Lane 10) of **II-5**, while no DNA-cleavage was found in the absence of added thiols (result not shown).[14b,40] Also, plasmid pBR322 was accordingly incubated with the agent in phosphate buffer solutions with pH values varying from 5.0 to 7.5. As shown in Figure 2.20, **II-5** caused single stranded DNA cleavage effectively at pH 5.0 (97%, Lane 2), 5.5 (86%, Lane 4) and 6.0 (72%, Lane 6) and 6.5 (41%, Lane 8) while such a DNA-cleaving activity drastically decreased as the pH of the corresponding buffer solutions increased to 7.0 (19%, Lane 10) and 7.5 (20%, Lane 12). We found that these acid-promoted DNA-cleaving properties are shared by the polysulfide antibiotics and their structural analogs we have studied.[10-12,16] Given that the extracellular pH in most tumor cells is lower than that in normal tissue[42,57] and the antibiotic possessing acid-activated functionalities is rare in nature, we hope this finding may serve a new insight into tumor chemotherapy.[58]

Varacin A 2-oxide

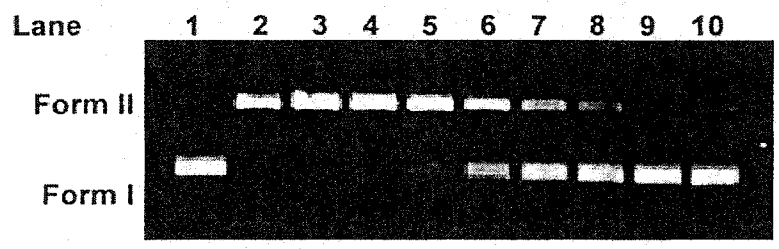


Figure 2.18 Thiol-dependent cleavage of supercoiled pBR322 DNA by various concentrations of varacin A 2-oxide **II-5**. Reactions were incubated at 37 °C for 4 h in 50 mM sodium phosphate, pH 5.5, containing 10% v/v acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, pBR322 alone; lane 2, 100 μM **II-5** + 500μM 2-mercaptoethanol; lane 3, 50 μM **II-5** + 500 μM 2-mercaptoethanol; lane 4, 25 μM **II-5** + 500 μM 2-mercaptoethanol; lane 5, 10 μM **II-5** + 500 μM 2-mercaptoethanol; lane 6, 5 μM **II-5** + 500 μM 2-mercaptoethanol; lane 7, 2.5 μM **II-5** + 500 μM 2-mercaptoethanol; lane 8, 1 μM **II-5** + 500 μM 2-mercaptoethanol; lane 9, 500 nM **II-5** + 500 μM 2-mercaptoethanol; lane 10, 100 nM **II-5** + 500 μM 2-mercaptoethanol.

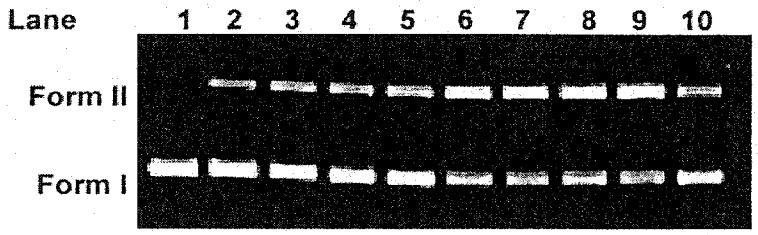


Figure 2.19 Thiol-dependent cleavage of supercoiled pBR322 DNA by 5 μM of varacin A 2-oxide **II-5** in various concentrations of 2-mercaptoethanol. Reactions were incubated at 37 °C for 4 h in 50 mM sodium phosphate, pH 5.5, containing 10% v/v acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, pBR322 alone; lane 2, 5 μM **II-5** + 20 μM 2-mercaptoethanol (1: 4); lane 3, 5 μM **II-5** + 50 μM 2-mercaptoethanol (1: 10); lane 4, 5 μM **II-5** + 100 μM 2-mercaptoethanol (1: 20); lane 5, 5 μM **II-5** + 200 μM 2-mercaptoethanol (1: 40); lane 6, 5 μM **II-5** + 500 μM 2-mercaptoethanol (1: 100); lane 7, 5 μM **II-5** + 1 mM 2-mercaptoethanol (1: 200); lane 8, 5 μM **II-5** + 2.5 mM 2-mercaptoethanol (1:500); lane 9, 5 μM **II-5** + 5 mM 2-mercaptoethanol (1:1000); lane 10, 5 μM **II-5** + 10 mM 2-mercaptoethanol (1:2000).

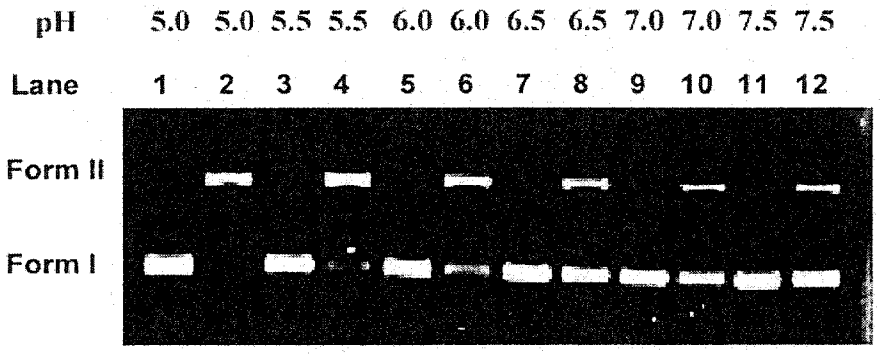


Figure 2.20 Thiol-dependent cleavage of supercoiled pBR322 DNA by 5 μM of varacin A 2-oxide **II-5** in 500 μM 2-mercaptoethanol at various pH values. Reactions were incubated at 37 °C for 6 h in 50 mM sodium phosphate, containing 10% v/v acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, 5 μM **II-5** at pH 5.0; lane 2, 5 μM **II-5** + 500 μM 2-mercaptoethanol at pH 5.0; lane 3, 5 μM **II-5** at pH 5.0; lane 4, 5 μM **II-5** + 500 μM 2-mercaptoethanol at pH 5.5; lane 5, 5 μM **II-5** at pH 6.0; lane 6, 5 μM **II-5** + 500 μM 2-mercaptoethanol at pH 6.0; lane 7, 5 μM **II-5** at pH 6.5; lane 8, 5 μM **II-5** + 500 μM 2-mercaptoethanol at pH 6.5; lane 9, 5 μM **II-5** at pH 7.0; lane 10, 5 μM **II-5** + 500 μM 2-mercaptoethanol at pH 7.0; lane 11, 5 μM **II-5** at pH 7.5; lane 12, 5 μM **II-5** + 500 μM 2-mercaptoethanol at pH 7.5.

Meanwhile, we have repeated the effects of thiols on the DNA cleavage potientated by our agent varacin A 2-oxide **II-5**. Once again, we found this cascade of activation can be triggered by glutathione and thiophenol instead of mercaptoethanol,[11,14b] but nucleophiles included 4-methylaniline and 4-methylphenol gave no effect on *in situ* **II-5** activation (Figure 2.21). The inhibitory effects of certain free radical scavengers on the DNA-cleaving process were also investigated. As shown in Figure 2.22, the DNA cleavage caused by **II-5** was inhibited effectively by the addition of catalase, (Lane 3), mannitol (Lane 7), ethanol (Lane 8)[41,48] and carbon radical scavenger TEMPO (Lane 6).[53] However, diethylenetriamine pentaacetic acid (DETAPAC)[51] had a minor effect on the quenching of DNA-cleaving process (lane 5). Same as the previous studies, addition of superoxide dismutase (SOD)[48] (Lane 4), an enzyme destroying superoxide in solution, to our reaction mixture did not slow down the DNA-cleaving reaction but speeded up the reaction a little bit. Addition of nucleophiles pyridine and 4-methylaniline showed neither observable inhibition nor catalysis on the DNA cleavage (Lanes 9 and 10). Based on this preliminary data, it is expected that **II-5** cleaves DNA in a similar manner as the antibiotics we reported before.[14-16,23,56]

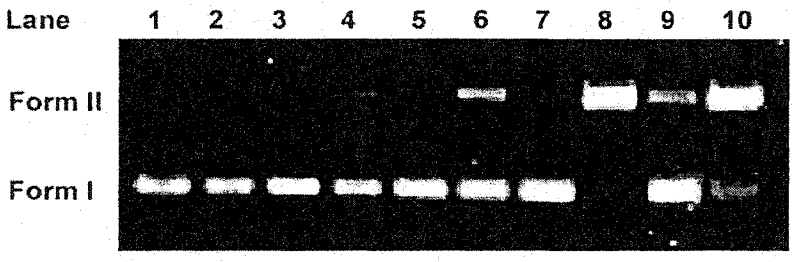


Figure 2.21 Thiol-dependent cleavage of supercoiled pBR322 DNA by 5 μM of varacin A 2-oxide **II-5** in various thiols 1 mM. Reactions were incubated at 37 °C for 5 h in 50 mM sodium phosphate, pH 5.5, containing 10% v/v acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, 5 μM **II-5**; lane 2, 5 μM **II-5** + 1 mM 4-ethylphenol; lane 3, 5 μM **II-5**; lane 4, 5 μM **II-5** + 1 mM 4-methylaniline; lane 5, 5 μM **II-5**; lane 6, 5 μM **II-5** + 1 mM thiophenol; lane 7, 5 μM **II-5**; lane 8, 5 μM **II-5** + 1 mM glutathione reduced; lane 9, 5 μM **II-5**; lane 10, 5 μM **II-5** + 1 mM 2-mercaptoethanol.

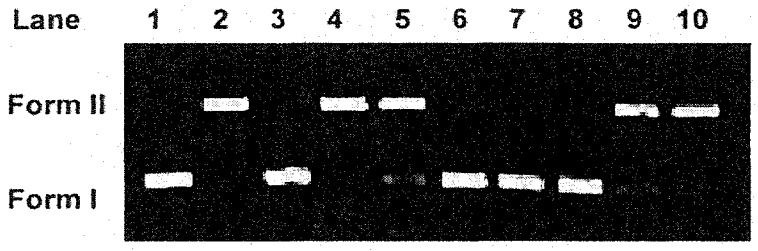


Figure 2.22 Thiol-dependent cleavage of supercoiled pBR322 DNA by 10 μM of varacin A 2-oxide **II-5** in 1 mM 2-mercaptoethanol. Reactions were incubated at 37 °C for 4 h in 50 mM sodium phosphate, pH 5.5, containing 10% v/v acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, 10 μM **II-5**; lane 2, 10 μM **II-5** + 1 mM 2-mercaptoethanol; lane 3, 10 μM **II-5** + 1 mM 2-mercaptoethanol + 53.2 μg/mL catalase; lane 4, 10 μM **II-5** + 1 mM 2-mercaptoethanol + 80 μg/mL SOD; lane 5, 10 μM **II-5** + 1 mM 2-mercaptoethanol + 500 μM DETAPAC; lane 6, 10 μM **II-5** + 1 mM 2-mercaptoethanol + 5 mM TEMPO; lane 7, 10 μM **II-5** + 1 mM 2-mercaptoethanol + 100 mM mannitol; lane 8, 10 μM **II-5** + 1 mM 2-mercaptoethanol + 1 M ethanol; lane 9, 10 μM **II-5** + 1 mM 2-mercaptoethanol + 10 mM pyridine; lane 10, 10 μM **II-5** + 1 mM 2-mercaptoethanol + 10 mM 4-methylaniline.

II.2.3.5 DNA Cleavage by Varacin II-1

As shown in Figure 2.23, varacin **II-1** caused single stranded DNA cleavage effectively at pH 5.0 (66%, Lane 2), 5.5 (81%, Lane 4) and 6.0 (54%, Lane 6) while such DNA-cleaving activity decreased slightly as the pH of the corresponding buffer solutions increased to 7.0 (34%, Lane 10) and 7.5 (24%, Lane 12). In addition, varacin **II-1** produced detectable formation of form II DNA at a concentration as low as 500 nM at pH 5.5 (Lane 2) and in a concentration-dependent fashion (Figure 2.24).[12] Moreover, the DNA-cleaving caused by varacin was a thiol-dependent process. As shown in Figure 2.25, the amount of DNA cleavage by varacin **II-1** increased with increasing the concentration of added 2-mercaptoethanol while there was no DNA-cleavage in the absence of added thiols.

As shown in Figure 2.26, the DNA cleavage caused by varacin was inhibited effectively by the addition of catalase, an enzyme that reduces the concentration of hydrogen peroxide (H_2O_2) in solution (Lane 3).[14b,41,48] In addition, radical scavengers such as mannitol (Lane 8), methanol (Lane 6) and ethanol (Lane 7) also effectively inhibited the DNA cleavage by varacin.[41,48] However, diethylenetriamine pentaacetic acid (DETAPAC)[14,51] had little effect on the DNA-cleaving process by varacin (lane 5). Similarly, addition of superoxide dismutase, an enzyme destroying superoxide in solution, to our reaction mixture did not slow down the DNA-cleaving reaction by varacin. The activating course of the DNA cleavage by varacin **II-1** was also examined

by adding different types of thiols to the corresponding reaction mixtures. As shown in Figure 2.27, both 2-mercaptoethanol and glutathione were capable of triggering varacin mediated DNA-cleavage effectively (Lanes 2 and 4). However, when 2-mercaptoethanol was replaced with 4-ethylphenol or 4-methylaniline (Lanes 8 and 10), DNA cleavage did not occur. Furthermore, the DNA-cleavage by varacin did not take place when thiol was omitted from the reaction (Lanes 1, 3) and 1 mM of thiolphenol was merely able to trigger a little bit of DNA cleavage with our used concentration of varacin (Lane 6).

Varacin II-1

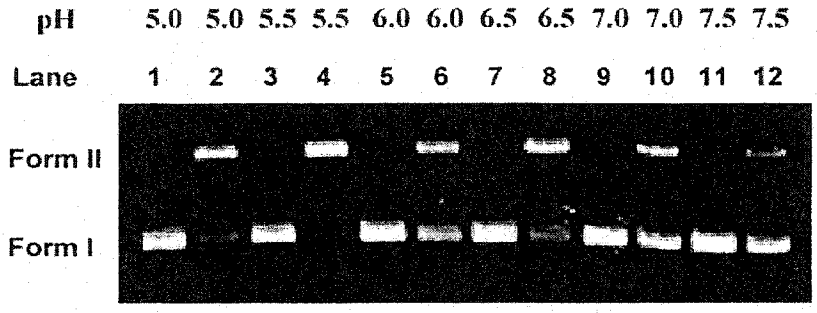


Figure 2.23 DNA cleavage by varacin **II-1** in phosphate buffer solutions at different pH values. Reactions were incubated at 37 °C for 3 h in 50 mM sodium phosphate containing 10% v/v acetonitrile, 38 μM/bp of supercoiled pBR322 DNA, 10 μM varacin and in the presence or absence of added thiols. Lane 1, pH 5.0, absent of added thiol; lane 2, pH 5.0, 1 mM 2-mercaptoethanol; lane 3, pH 5.5, absent of added thiol; lane 4, pH 5.5, 1 mM 2-mercaptoethanol; lane 5, pH 6.0, absent of added thiol; lane 6, pH 6.0, 1 mM 2-mercaptoethanol; lane 7, pH 6.5, absent of added thiol; lane 8, pH 6.5, 1 mM 2-mercaptoethanol; lane 9, pH 7.0, absent of added thiol; lane 10, pH 7.0, 1 mM 2-mercaptoethanol; lane 11, pH 7.5, absent of added thiol; lane 12, pH 7.5, 1 mM 2-mercaptoethanol.

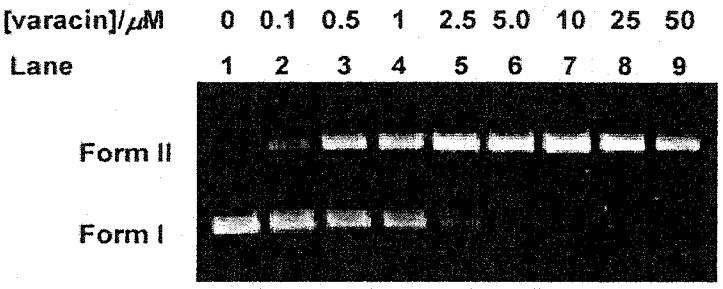


Figure 2.24 DNA cleavage by various concentrations of varacin **II-1**. Reactions were incubated at 37 °C for 18 h in 50 mM sodium phosphate, pH 5.5 containing 10% v/v acetonitrile, 38 μM/bp of supercoiled pBR322 DNA, varacin and 1 mM 2-mercaptoethanol. Lane 1, pBR322 alone; lane 2, 100 nM varacin + 1 mM 2-mercaptoethanol; lane 3, 500 nM varacin + 1 mM 2-mercaptoethanol; lane 4, 1 μM varacin + 1 mM 2-mercaptoethanol; lane 5, 2.5 μM varacin + 1 mM 2-mercaptoethanol; lane 6, 5.0 μM varacin + 1 mM 2-mercaptoethanol; lane 7, 10 μM varacin + 1 mM 2-mercaptoethanol; lane 8, 25 μM varacin + 1 mM 2-mercaptoethanol; lane 9, 50 μM varacin + 1 mM 2-mercaptoethanol.

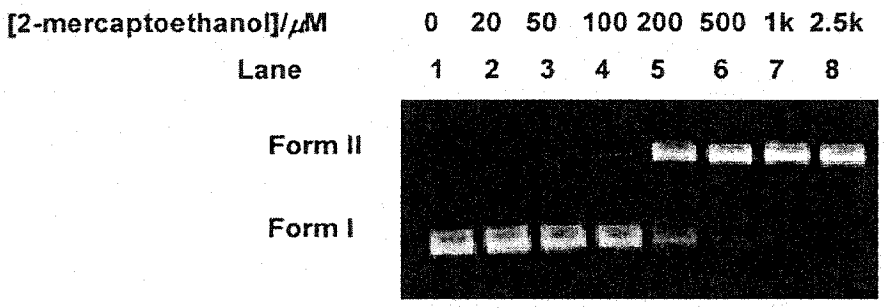


Figure 2.25 DNA cleavage by varacin **II-1** in the presence of varying concentrations of 2-mercaptoethanol. Reactions were incubated at 37 °C for 18 h in 50 mM sodium phosphate (pH 5.5) containing 10% v/v acetonitrile, 38 μM/bp of supercoiled pBR322 DNA, 5 μM varacin and 2-mercaptoethanol. Lane 1, 5 μM varacin and absent of added thiol; lane 2, 5 μM varacin + 20 μM 2-mercaptoethanol; lane 3, 5 μM varacin + 50 μM 2-mercaptoethanol; lane 4, 5 μM varacin + 100 μM 2-mercaptoethanol; lane 5, 5 μM varacin + 200 μM 2-mercaptoethanol; lane 6, 5 μM varacin + 500 μM 2-mercaptoethanol; lane 7, 5 μM varacin + 1 mM 2-mercaptoethanol; lane 8, 5 μM varacin + 2.5 mM 2-mercaptoethanol.

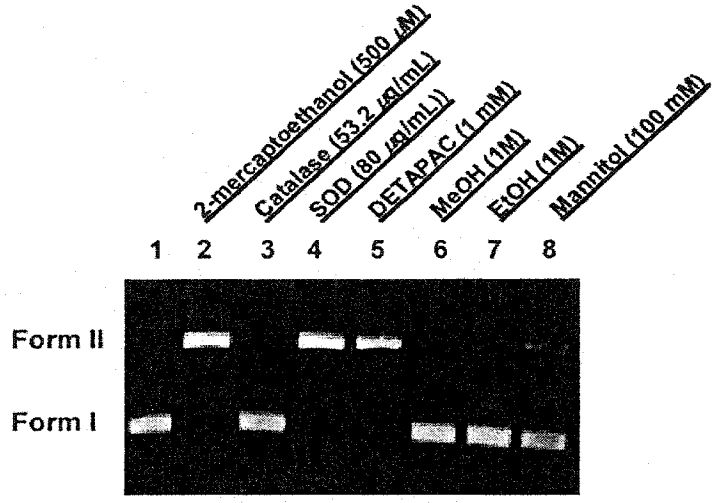


Figure 2.26 DNA cleavage by varacin **II-1** inhibited by the addition of radical scavengers and nucleophiles. Reactions were incubated at 37 °C for 4 h in 50 mM sodium phosphate (pH 5.5) containing 10% v/v acetonitrile, 38 μM/bp of supercoiled pBR322 DNA, 5 μM varacin, 500 μM 2-mercaptoethanol and additives. Lane 1, DNA alone; lane 2, 5 μM varacin + 500 μM 2-mercaptoethanol reaction absent of additive; lane 3, 5 μM varacin + 500 μM 2-mercaptoethanol + 53.2 μg/mL catalase; lane 4, 5 μM varacin + 500 μM 2-mercaptoethanol + 80 μg/mL SOD; lane 5, 5 μM varacin + 500 μM 2-mercaptoethanol + 1 mM DETAPAC; lane 6, 5 μM varacin + 500 μM 2-mercaptoethanol + 1 M methanol; lane 7, 5 μM varacin + 500 μM 2-mercaptoethanol + 1 M ethanol; lane 8, 5 μM varacin + 500 μM 2-mercaptoethanol + 100 mM mannitol.

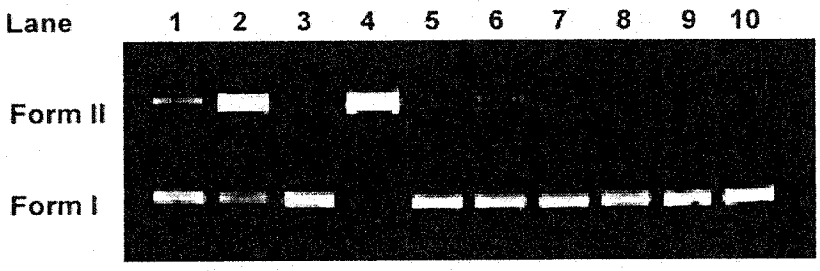


Figure 2.27 Thiol-dependent cleavage of supercoiled pBR322 DNA by 5 μM varacin **II-1** in various thiols at 1 mM concentration. Reactions were incubated at 37 °C for 6 h in 50 mM sodium phosphate, pH 5.5, containing 10% v/v acetonitrile and 38 μM (bp) of supercoiled pBR322 DNA. Lane 1, 5 μM **II-1**; lane 2, 5 μM **II-1** + 1 mM glutathione reduced; lane 3, 5 μM **II-1**; lane 4, 5 μM **II-1** + 1 mM 2-mercaptoethanol; lane 5, 5 μM **II-1**; lane 6, 5 μM **II-1** + 1 mM thiophenol; lane 7, 5 μM **II-1**; lane 8, 5 μM **II-1** + 1 mM 4-ethylphenol; lane 9, 5 μM **II-1**; lane 10, 5 μM **II-1** + 1 mM 4-methylaniline.

II.2.3.6 Cytotoxic Assays on the Titled Compounds

The cytotoxic properties of benzotrithiole 2-oxide **II-6**, varacin A 2-oxide **II-5** and varacin **II-1** were evaluated on eight different human cancer cell lines, which are expressed as the concentration of this compound that inhibits 50% of cell proliferation (IC_{50}). As a positive control, the clinically used anticancer drug doxorubicin was also tested in parallel for its cytotoxic activity against the same set of cell lines. As shown in Table 2.1, the IC_{50} values of those three tested compounds against these eight cancer cell lines were all on nanomolar scales. **II-5** was more potent than the clinically used antitumor agent doxorubicin in the cell lines of colon cancer, prostate cancer and human pancreatic carcinoma, exhibiting their IC_{50} values against these cell lines over the range from 0.93 nM to 853.6 nM. On the other hand, **II-1** was more potent than doxorubicin in the cell lines of colon, prostate cancer, pancreatic and kidney carcinoma. The IC_{50} values of **II-1** against the chosen cell lines fell in the range of 0.6 nM to 48.4 nM.

The cytotoxic activities (IC_{50}) of varacin C **II-4** and varacin **II-3** toward seven different human cancer cell lines were evaluated. Comparing these values with data obtained for doxorubicin in the same cell lines, the result was shown in Table 2.2. Varacin C and varacin B were more potent than the clinically used antitumor agent doxorubicin against the cell lines of colon cancer, prostate cancer, human breast cancer, human pancreatic carcinoma and human kidney carcinoma, exhibiting IC_{50} values against these cell lines in the range 2.4 to 48.2 nM and 2.34 to 78.99 nM respectively.[11]

Table 2.1 Cytotoxic property of benzotrithiole 2-oxide **II-6**, varacin A 2-oxide **II-5** and varacin **II-1** against eight selected cancer cell lines.

Cell type	Cell line	IC_{50} (nM) **II-6**	IC_{50} (nM) **II-5**	IC_{50} (nM) **II-1**	IC_{50} (nM) doxorubicin
colon cancer	HT-29	78.9	1.17	3.18	61.8
prostate cancer	PC-3	62.9	0.93	0.60	49.8
breast cancer	MDA231	627.0	122.73	9.52	1.95
bladder cancer	UMUC3	365.6	14.35	11.41	9.2
human pancreatic carcinoma	PACA2	981.9	5.36	1.8	8.7
lung cancer	A549	151.2	32.93	12.29	2.7
human kidney carcinoma	A4982LM	849.9	853.6	48.40	52.8
human breast carcinoma	MCF-7	209.2	1.75	1.69	1.2

Table 2.2 Cytotoxic properties of varacin C **II-4** and varacin B **II-3** toward seven cancer cell lines.

Cell type	Cell line	IC_{50} (nM) **II-4**	IC_{50} (nM) **II-3**	IC_{50} (nM) doxorubicin
colon cancer	HT-29	11.9	12.02	41.4
prostate cancer	PC-3	2.4	5.68	41.8
breast cancer	MDA231	2.9	2.34	3.5
bladder cancer	UMUC3	8.4	15.90	2.2
human pancreatic carcinoma	PACA2	7.2	16.67	17.1
lung cancer	A549	48.2	78.99	2.4
human kidney carcinoma	A4982LM	24.9	9.60	46.0

II.3 Conclusion

In conclusion, we have completed the total syntheses of antibiotics varacins B **II-3**, C **II-4** and A 2-oxide **II-5** and reported the biological studies. In the route of their first total syntheses, we were able to grow the single crystals of varacin **II-1** and *N*-Boc-varacin C **II**-25 and studied the detailed X-ray crystallographic structures of them. Then, we have demonstrated by arrays of experiments for the first time that varacin **II-1**, varacins B **II-3** and C **II-4**, varacin A 2-oxide **II-5** and benzotrithiole oxides **II-(6-9ab)** are capable of causing DNA cleavage effectively.[10-12] This may explain the observed potent cytotoxic activities of these antibiotics and structural analogs of the antibiotics, and their antifungal and antimicrobial activities against *Candida albicans* and *Bacillus subtilus*.[1-6] Most remarkably, our current studies reveal the rates of the DNA-cleaving courses by those tested candidates can be promoted by an acidic environment. Tumor cells possess a lower extracellular pH than normal cells,[42] which is an intrinsic feature of the tumor phenotype and caused by alterations either in acid export or in clearance of extracellular acid.[57] Pharmaceutical agents maybe particularly desirable in cancer chemotherapy if they undergo selective activation by the acidic microenvironments around tumor tissue.[58] Accordingly, we hope that the modes of action of these antibiotics described in this part of our work may stimulate the design of a new generation of acid-activated, tumor-activated prodrugs.[59]

We believe the antibiotics' DNA cleaving activities are related to the free radical mechanisms via Fenton reaction in the presence of thiols,[8,14,23,56] analogous to the

mode of action of antibiotic leinamycin described in Part I of this thesis. Our studies provide a classical mechanistic view to help to resolve the fundamental questions about the origin of these sulfur-containing antibiotics biological properties.[1-6] We anticipate the findings will be useful in the manipulation of their biochemical activities.

II. 4. Experimental

II.4.1 Organic Synthesis of Varacin, Varacins B, C and Their Analogs

General Information

Melting points were determined on a Reichert Microscopic apparatus or Electrothermal recorder 9100, and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker Advance DPX 400 FT-NMR Spectrometer or Varian Unity INOVA 500 FT-NMR Spectrometer as solutions in $CDCl_3$ (99.8% atom D) unless otherwise indicated, and the chemical shifts are reported in parts per million (ppm, δ). Coupling constants are reported in hertz (Hz). Low resolution mass spectra were obtained on Fisons VG Platform Mass Spectrometer, or Hewelett Packard G1800C GCD Series II GCMS. High resolution mass spectra (HRMS) were measured by Finnigan MAT95 and Thermo Finnigan MAT95XL Mass Spectrometers. X-Ray crystallographic analysis was carried out by Bruker Smart 1000 CCD area-detector diffractometer.

All reactions were monitored by thin layer chromatography (TLC) performed on Merck precoated silica gel 60 F_{254} plates, and compounds were visualized under ultraviolet light (254 or 365 nm) or with spray of 5% w/v dodecamolybdophosphoric acid in 95% ethanol, or 5% w/v potassium permanganate in aqueous solution of sulphuric acid (5%) and subsequent heating. TLC plates were also developed by exposing to iodine vapor and allowing the iodine to evaporate. Flash chromatography was carried out on columns of NA or Merck Keisel silica gel 60 (230-400 mesh).

2,2-Dimethyl-1,3,2-benzodithiastannoles (II-11). To an ethanolic solutions (5 mL) of 1,2-benzenedithiol **II-10** (0.260 g, 1.830 mmol) was treated with an ethanolic solution of

KOH (15 mL, 0.1 M). After stirring for 15 min, an aqueous solution (25 mL) of dimethyltin dichloride (0.402 g, 1.830 mmol) was added dropwise, a white precipitate of complexes formed immediately. After stirring for 50 min the white solid was filtered off and washed with water (20 mL) and ethanol (20 mL). The slightly yellow solid was dissolved in CH_2Cl_2 (25 mL). The solution was then washed with brine (2 × 20 mL), dried over Na_2SO_4 and concentrated to furnish essentially pure **II-11** (0.472 g, 89%) as a slightly yellow solid: ^{1}H NMR δ ($CDCl_3$) 7.45 (m, 2H), 6.90 (m, 2H), 1.01 (s, 6H); ^{13}C NMR δ ($CDCl_3$) 137.94, 128.88, 124.33, 2.53; ESIMS *m/z* (%) 290 [$(M+1)^+$, 38], 275 (42), 273 (36), 217 (40), 185 (79), 165 (100), 164 (92), 163 (53).

1,2,3-Benzotrithiole 2-oxide (II-6). To a suspension of benzodithiastannole **II-11** (0.473 g, 0.819 mmol) in THF (20 mL) at –60 °C was added $SOCl_2$ (0.100 mL, 1.230 mmol) under Ar. The reaction mixture was stirred for 2 h with the temperature raised to 0 °C at the end. The reaction was quenched with ice cold water (5 mL) and the resulting aqueous layer was extracted with CH_2Cl_2 (3× 15 mL). The combined extracts were washed with brine (2× 10 mL), dried over Na_2SO_4 and rotavaporated to give an orange syrup. Flash chromatography, eluting with hexane: CH_2Cl_2 (2:1, v/v), afforded **II-6** (0.120 g, 87%) as a slightly yellow powder: m.p. 76-77 °C (lit.,[19,61] 77 °C); ^{1}H NMR δ ($CDCl_3$) 7.62 (m, 2H), 7.37 (m, 2H); ^{13}C NMR δ ($CDCl_3$) 135.64, 127.33, 124.59; ESIMS m/z (%) 189 [$(M+1)^+$, 100].

1,2,3-Benzotrithiole 1-oxide (II-7). A solution of trithiole 2-oxide **II-6** (0.117 g, 0.621 mmol) in CH_3CN (40 mL) under Ar was irradiated through a Pyrex filter with a 100 W

high-pressure mercury lamp for 36 h. After evaporation of the solvent, chromatography of the residue on silica gel with hexane: CH_2Cl_2 (2:1, v/v) as eluent yielded **II-7** (0.094 g, 80%) as a slightly yellow solid after removal of solvent: m.p. 71-73 °C (lit.,[19,20,61] 73-74 °C); ^{1}H NMR δ ($CDCl_3$) 7.99 (d, 1H, J=9.0 Hz), 7.74 (d, 1H, J=8.0 Hz), 7.51 (t, 1H, J=7.0 Hz), 7.42 (t, 1H, J=7.5 Hz); ^{13}C NMR δ ($CDCl_3$) 150.11, 145.06, 131.33, 126.60, 126.50, 123.72; ESIMS *m/z* (%) 189 [$(M+1)^+$, 100].

5-Methyl 2,2-dimethyl-1,3,2-benzodithiastannoles (II-13). To an ethanolic solutions (8 mL) of 4-methyl 1,2-benzenedithiol **II-12** (0.466 g, 3.000 mmol) was treated with an ethanolic solution of KOH (20 mL, 0.1 M). After stirring for 15 min, an aqueous solution (25 mL) of dimethyltin dichloride (0.660 g, 3.000 mmol) was added dropwise, a white precipitate of complexes formed immediately. After stirring for 45 min the white solid was filtered off and washed with water (30 mL) and ethanol (20 mL). The slightly yellow solid was dissolved in CH_2Cl_2 (30 mL) then washed with brine (2 × 20 mL), dried over Na_2SO_4 and concentrated to furnish essentially pure **II-13** (0.853 g, 94%) as a slightly yellow glassy solid: ^{1}H NMR δ ($CDCl_3$) 7.33 (d, 1H, J=8.0 Hz), 7.28 (s, 1H), 6.72 (d, 1H, J=8.0 Hz), 2.23 (s, 3H), 0.99 (s, 6H); ^{13}C NMR δ ($CDCl_3$) 137.68, 134.17, 130.29, 129.53, 125.44, 20.47, 2.48; ESIMS *m/z* (%) 305 [$(M+1)^+$, 100], 303 (M^+, 64), 289 (54), 287 (43), 187 (26), 185 (70), 183 (50), 181 (40), 149 (23).

5-Methyl 1,2,3-benzotrithiole 2-oxide (II-8). To a suspension of benzodithiastannole **II-13** (0.833 g, 2.750 mmol) in THF (20 mL) at –60 °C was added $SOCl_2$ (0.350 mL, 4.220 mmol) under Ar. The reaction mixture was stirred for 3 h with the temperature

raised to 0 °C at the end. The reaction was quenched with ice cold water (8 mL) and the resulting aqueous layer was extracted with CH_2Cl_2 (3× 25 mL). The combined extracts were washed with brine (2×10 mL), dried over Na_2SO_4 and rotavaporated to yield a yellow solid. Flash chromatography, eluting with hexane: CH_2Cl_2 (2:1, v/v), afforded **II-8** (0.439 g, 79%) as a slightly yellow powder: m.p. 96-97 °C (lit.,[19,20] 97.3-97.8 °C); ^{1}H NMR δ ($CDCl_3$) 7.47 (d, 1H, *J*=8.5 Hz), 7.41 (s, 1H), 7.16 (dt, 1H, 8.0, 0.5 Hz), 2.40 (s, 3H); ^{13}C NMR δ ($CDCl_3$) 137.76, 135.74, 132.33, 128.38, 124.83, 124.21, 20.89; ESIMS *m/z* (%) 202.9 (M^+, 100).

5-Methyl 1,2,3-benzotrithiole 1-oxide (II-9a) and 6-methyl 1,2,3-benzotrithiole 1-oxide (II-9b). A solution of trithiole 2-oxide **II-8** (0.298 g, 1.470 mmol) in CH_3CN (50 mL) under Ar was irradiated through a Pyrex filter with a 100 W high-pressure mercury lamp for 48 h. After evaporation of the solvent, chromatography of the residue on silica gel with hexane: CH_2Cl_2 (1:1, v/v) as eluent yielded an inseparable mixture of **II-9a** and **II-9b** [19,20,61] (0.207 g, 70%) as a yellow syrup: ^{1}H NMR δ ($CDCl_3$) 7.86 (d, 1H, *J*=8.0 Hz), 7.79 (s, 1H), 7.61 (d, 1H, *J*=8.0 Hz), 7.53 (s, 1H), 7.32 (d, 1H, *J*=9.5 Hz), 7.21 (d, 1H, *J*= 8.0 Hz), 2.45 (s, 3H), 2.44 (s, 3H); ^{13}C NMR δ ($CDCl_3$) 150.30, 147.51, 145.45, 142.47, 141.59, 137.04, 132.31, 127.44, 126.65, 126.16, 123.71, 123.24, 21.17, 20.72; ESIMS *m/z* (%) 203 [$(M+1)^+$, 100].

5,6-Dibromo-4-hydroxy-3-methoxybenzaldehyde (II-15). To a solution of vanillin **II-14** (34.81 g, 229 mmol) in glacial acetic acid (130 mL) was added bromine (11.9 mL, 229 mmol) dropwise through a Hershberg funnel. The solution was stirred at room

temperature for 1 h followed by addition of iron filings (300 mg, 5.37 mmol) and Br_2 (25.1 mL, 490 mmol). After heating at reflux overnight, the resultant brown mixture was cooled to room temperature, filtered and washed with water. The organic phase was dissolved in acetone (400 mL) followed by removal of iron filings by filtration. Evaporation of excess solvent and recrystallization of the crude product from methanol (500 mL) afforded **II-15** (56.1 g, 78%) as a yellow solid: m.p. 223-226 °C; ^{1}H NMR δ (DMSO) 11.25 (s, 1H), 10.07 (s, 1H), 7.39 (s, 1H), 3.91 (s, 3H); ^{13}C NMR δ (DMSO) 190.85, 151.27, 147.45, 126.28, 122.21, 113.87, 110.38, 56.41; GC/MS Ins *m/z* (%) 310 (M^+, 100).

2,3-Bis(*n*-butylthio)-4-hydroxy-5-methoxybenzaldehyde (II-16). To a solution of quinoline/pyridine (250 mL, 4:1, v/v) were added **II-15** (9.611 g, 31.3 mmol) and freshly prepared cuprous *n*-butylmercaptide[5] (17.07 g, 112 mmol). The resultant white slurry was heated to 160 °C for 2 h under argon. The mixture was cooled to room temperature followed by addition of 37% (v/v) aqueous HCl (100 mL). After extraction of the mixture with EtOAc (3× 150 mL), the combined organic extracts were washed with 10% (v/v) aqueous HCl (2× 50 mL), water (2× 50 mL) and brine (40 mL). The crude product was purified by flash chromatography over silica gel using hexane: EtOAc (8:1, v/v) as eluent to afford **II-16** (6.62 g, 65%) as yellow needles: ^{1}H NMR δ ($CDCl_3$) 10.68 (s, 1H), 7.58 (s, 1H), 7.48 (s, 1H), 3.96 (s, 3H), 2.86 (t, 2H, J=7.5 Hz), 2.84 (t, 2H, J=7.5 Hz), 1.51 (m, 4H), 1.39 (m, 4H), 0.89 (t, 3H, J=7.5 Hz), 0.87 (t, 3H, J=7.5 Hz); ^{13}C NMR δ ($CDCl_3$) 192.27, 192.25, 152.75, 147.83, 137.89, 132.42, 126.14,

110.66, 56.19, 38.73, 36.28, 31.63, 31.40, 21.93, 21.89, 13.58; HRESIMS found 328.1161, calculated for $C_{16}H_{24}O_3S_2$ 328.1167.

2,3-Bis(*n*-butylthio)-4,5-dimethoxybenzaldehyde (II-17). To a solution of **II-16** (9.468 g, 29.0 mmol), K_2CO_3 (3.970 g, 29.0 mmol), tetra-*n*-butylammonium iodide (12.860 g, 34.8 mmol) in THF (80 mL) and water (80 mL) was added MeI (5.5 mL, 86.5 mmol) at room temperature. The resultant mixture was refluxed overnight followed by removal of solvent under reduced pressure. The residue was dissolved in CH_2Cl_2 (220 mL), washed with water (2× 50 mL) and brine (60 mL) and dried over Na_2SO_4. The crude product was purified by flash chromatography over silica gel with hexane: EtOAc (6:1, v/v) as eluent to afford **II-17** (9.64 g, 99%) as an orange syrup: ^{1}H NMR δ ($CDCl_3$) 10.75 (s, 1H), 7.43 (s, 1H), 3.94 (s, 3H), 3.93 (s, 3H), 2.99 (t, 2H, *J*=7.5 Hz), 2.83 (t, 2H, *J*=7.5 Hz), 1.52 (m, 4H), 1.39 (m, 4H), 0.88 (t, 3H, *J*=7.5 Hz), 0.87 (t, 3H, *J*=7.5 Hz); ^{13}C NMR δ ($CDCl_3$) 192.91, 155.25, 153.48, 137.24, 135.48, 110.35, 60.39, 55.94, 38.25, 35.31, 31.84, 31.39, 21.94, 13.62, 13.58; ESIMS *m/z* (%) 343 (M^+, 100), 311 (6), 221 (5), 87 (3).

3,4-Bis(*n*-butylthio)-1,2-dimethoxy-5-(2-nitroethenyl)benzene (II-18). A mixture of 2,3-bis(*n*-butylthio)-4,5-dimethoxybenzaldehyde **II-17** (7.430 g, 22.0 mmol), CH_3NO_2 (50 mL), glacial acetic acid (3.5 mL, 55.2 mmol) and NH_4OAc (3.392 g, 45.3 mmol) was sonicated in an ultrasonic bath at room temperature for 8 h followed by the addition of water (15 mL) to quench the reaction. After extraction of the aqueous mixture with diethyl ether (3× 65 mL), the combined organic phase was washed with water (30 mL)

and brine (40 mL) and dried over Na_2SO_4. After removal of solvent under reduced pressure, the crude product was purified by flash chromatography on silica gel eluting with hexane: EtOAc (5:1, v/v) to afford **II-18** (6.62 g, 78%) as an orange oil: ^{1}H NMR δ ($CDCl_3$) 8.91 (d, 1H, J=14.0 Hz), 7.47 (d, 1H, J=13.5 Hz), 6.99 (s, 1H), 3.92 (s, 3H), 3.92 (s, 3H), 3.00 (t, 2H, J=7.5 Hz), 2.79 (t, 2H, J=7.5 Hz), 1.50 (m, 4H), 1.39 (m, 4H), 0.88 (t, 3H, J=12.5 Hz), 0.87 (t, 3H, J=12.5 Hz); ^{13}C NMR δ ($CDCl_3$) 153.33, 139.07, 137.92, 137.55, 134.17, 131.20, 112.22, 109.65, 60.35, 55.96, 37.76, 35.33, 31.82, 31.34, 21.92, 21.89, 13.60, 13.56; HRESIMS found 385.1404, calculated for $C_{18}H_{27}NO_4S_2$ 385.1379.

2',3'-Bis(*n*-butylsulfanyl)-4',5'-dimethoxyphenethylamine salt (II-19). To slurry of LAH (0.402 g, 10.8 mmol) in distilled diethyl ether (30 mL) was added a solution of **II-18** (1.102 g, 3.06 mmol) in diethyl ether (30 mL). The mixture was heated at reflux for 1.5 h followed by cooling the reaction to 4 ^{0}C in an ice-water bath. After the addition of 15% v/v aqueous HCl (25 mL) cautiously, the aqueous mixture was extracted with EtOAc (2× 60 mL). The combined organic extracts were washed with water (30 mL) and brine (30 mL) and dried over Na_2SO_4. Removal of solvent under reduced pressure afforded the crude amine salt (1.094 g) as a yellow syrup, which was used without further purification.

***N*-(Boc)-2',3'-bis(*n*-butylsulfanyl)-4',5'-dimethoxyphenethylamine (II-20).** To a solution of **II-19** (1.094 g, 3.06 mmol) in distilled THF (30 mL) were added distilled Et_3N (1.0 mL, 17.32 mmol) and $(Boc)_2O$ (0.920 g, 4.22 mmol). The mixture was

refluxed for 2 h followed by removal of solvent under reduced pressure. The crude product was purified by flash chromatography on silica gel eluting with hexane: EtOAc (8:1, v/v) as eluent to afford **II-20** (0.73 g, 52%) as a yellow/orange syrup: ^{1}H NMR δ ($CDCl_3$) 6.74 (s, 1H), 4.65 (br s, 1H), 3.86 (s, 3H), 3.84 (s, 3H), 3.34 (br d, 2H, *J*=6.0 Hz), 3.31 (t, 2H, *J*=6.5 Hz), 3.01 (t, 2H, *J*=7.5 Hz), 2.77 (t, 2H, *J*=8.0 Hz), 1.51 (m, 4H), 1.44 (s, 9H), 1.38 (m, 4H), 0.88 (t, 3H, *J*=7.5 Hz), 0.87 (t, 3H, *J*=7.5 Hz); ^{13}C NMR δ ($CDCl_3$) 155.92, 153.03, 149.05, 140.17, 129.27, 112.90, 112.26, 59.97, 55.77, 41.86, 37.24, 36.19, 35.39, 31.92, 31.49, 28.41, 22.17, 22.04, 13.70, 13.68, 0.99; ESIMS *m/z* (%) 458 (M^+, 42), 403 (23), 402 (100), 358 (25), 356 (35), 314 (57), 317 (8); HRESIMS found 457.2351, calculated for $C_{23}H_{39}NO_4S_2$ 457.2320.

***N*-(Boc)-2',3'-dimercapto-4',5'-dimethoxyphenethylamine (II-21).** Liquid ammonia (25 mL) was condensed by means of a dry-ice condenser into a 50 mL two-necked round bottom flask containing a solution of **II-20** (2.40 g, 5.24 mmol) in distilled THF (8 mL) at –78 to –60 °C. Small pieces of lithium metal were added until a deep blue solution persisted. After stirring for 1 h, the reaction was quenched by the addition of solid NH_4Cl. The excess ammonia was allowed to evaporate under a stream of N_2. The resultant white residue was dissolved in water (5 mL) and acidified with 15 % (v/v) aqueous HCl. After extraction of the aqueous solution with CH_2Cl_2 (3 × 30 mL), the combined organic extracts were washed with water (2× 20 mL) and brine (25 mL) and dried over Na_2SO_4. Removal of solvent under reduced pressure afforded **II-21** (1.80 g, 100%) as an orange paste: ^{1}H NMR δ ($CDCl_3$) 6.57 (s, 1H), 4.77 (s, 1H), 3.83 (s, 3H), 3.82 (s, 3H), 3.35 (br t, 2H, *J*=6.5 Hz), 3.24 (s, 1H), 2.96 (t, 2H, *J*=7.5), 1.43 (s, 9H); ^{13}C

NMR δ ($CDCl_3$) 155.88, 151.64, 142.69, 137.06, 132.54, 117.12, 110.75, 59.65, 55.84, 40.57, 35.96, 28.37; ESIMS *m/z* (%) 346 [$(M+1)^+$, 21], 317 (22), 292 (11), 291 (14), 290 (100), 282 (29), 246 (38), 229 (30), 65 (10).

***N*-(Boc)-4-ethylamino-6,7-dimethoxy-2,2-dimethyl-1,3,2-benzodithiastannole (II-22).** To an ethanolic solution (45 mL) of **II-21** (0.580 g, 1.689 mmol) was added an ethanolic solution of KOH (18 mL, 0.2 M). The mixture was stirred at room temperature for 15 min followed by the addition of an aqueous solution (30 mL) of dimethyltin dichloride (0.810 g, 3.68 mmol). After stirring for 1.5 h, CH_2Cl_2 (60 mL) was added. The resultant organic phase was washed with water (2 × 30 mL) and brine (20 mL) and dried over Na_2SO_4. Removal of solvents under reduced pressure afforded **II-22** (0.79 g, 94%) as a white glassy solid: ^{1}H NMR δ ($CDCl_3$) 6.51 (s, 1H), 4.70 (br s, 1H), 3.83 (s, 3H), 3.79 (s, 3H), 3.42 (m, 2H), 2.96 (br s, 2H, *J*=6.0 Hz), 1.42 (s, 9H), 0.97 (s, 6H); ^{13}C NMR δ ($CDCl_3$) 155.88, 149.86, 146.38, 135.01, 133.53, 130.03, 111.23, 78.99, 59.57, 56.12, 39.91, 37.55, 28.39, 2.76; ESIMS *m/z* (%) 494 [$(M+1)^+$, 55], 438 (100), 436 (75), 394 (83), 392(61), 391 (35), 317 (86), 263 (50), 261 (40), 185 (66), 181 (73), 151 (67), 149 (56), 147 (29); HRFABMS found 493.0387, calculated for $C_{17}H_{27}NO_4S_2Sn$ 493.0397.

***N*-(Boc)-4-ethylamino-6,7-dimethoxy-1,2,3-benzotrithiole 2-oxide (II-23).** To a suspension of **II-22** (1.868 g, 3.79 mmol) in THF (30 mL) at –60 °C was added $SOCl_2$ (0.40 mL, 5.48 mmol) under argon. The reaction mixture was allowed to warm to -10 °C followed by the addition of ice cold water (15 mL) to quench the reaction. After

extraction of the aqueous solution with CH_2Cl_2 (3× 30 mL), the combined extracts were washed with brine (2 × 20 mL) and dried over Na_2SO_4. After removal of the solvent under reduced pressure, the crude product was purified by flash chromatography on silica gel eluting with CH_2Cl_2: EtOAc (10:1, v/v) to afford **II-23** (1.06 g, 72%) as a yellow solid: ^{1}H NMR δ($CDCl_3$) 6.82 (s, 1H), 4.63 (br s, 1H), 3.93 (s, 3H), 3.89 (s, 3H), 3.39 (br s, 2H), 2.88 (t, 2H, *J*=6.5 Hz), 1.44 (s, 9H); ^{13}C NMR δ ($CDCl_3$) 155.74, 152.33, 143.15, 131.15, 130.85, 127.35, 114.28, 60.63, 56.56, 40.21, 37.48, 28.36, 14.16; ESIMS *m/z* (%) 392 [$(M+1)^+$, 100], 336 (91), 292 (54); HRESIMS found 391.0562, calculated for $C_{15}H_{21}NO_5S_3$ 391.0582.

4-Ethylamino-6,7-dimethoxy-1,2,3-benzotrithiole 2-oxide trifluoroacetate (II-5). To a solution of *N*-(Boc)-amine **II-23** (38.3 mg, 0.098 mmol) in CH_2Cl_2 (5 mL) was added trifloroacetic acid (0.2 mL, 2.50 mmol) at 0 °C. The reaction was stirred at room temperature for 1 h. At the end of reaction, the solvent and excess TFA were removed by *vacuo*, yielding the corresponding TFA salt (40.6 mg, 99%): ^{1}H NMR δ($CDCl_3$) 7.85 (br s, 3H), 6.87 (s, 1H), 3.91 (s, 3H), 3.86 (s, 3H), 3.28 (br s, 2H), 3.02 (br s, 2H); ^{13}C NMR δ ($CDCl_3$) 152.70, 143.77, 132.19, 127.47, 126.62, 114.59, 60.74, 56.41, 39.28, 34.20; HRESIMS found 404.9960, calculated for $C_{12}H_{14}NO_5S_3F_3$ 404.9986.

***N*-(Boc)-4-ethylamino-6,7-dimethoxy-1,2,3-benzotrithiole 1-oxide (II-24).** A solution of trithiole 2-oxide **II-23** (0.692 g, 1.775 mmol) in CH_3CN (45 mL) under Ar was irradiated through a Pyrex filter with a 100 W high-pressure mercury lamp for 18 h. When the reaction was completed, as monitored by TLC, the solvent was evaporated to

give the crude product as a yellow paste which consisted of two isomers with very close R_f. Flash chromatography of the residue on silica gel with CH_2Cl_2: EtOAc (50:1, v/v) as eluent, then slowly increased the polarity of eluent to CH_2Cl_2: EtOAc (10:1, v/v) until the end. **II-24** (0.166 g, 24%) was afforded as a yellow solid or light yellow prisms **II-24**: ^{1}H NMR δ ($CDCl_3$) 6.94 (s, 1H), 4.69 (br s, 1H), 4.03 (s, 3H), 3.90 (s, 3H), 3.44 (br s, 2H), 2.94 (m, 2H), 1.44 (s, 9H); ^{13}C NMR δ ($CDCl_3$) 155.81, 151.24, 146.37, 145.24, 136.34, 129.95, 117.92, 79.58, 62.33, 56.87, 40.00, 37.59, 28.39; HRFABMS found 391.0581, calculated for $C_{15}H_{21}NO_5S_3$ 391.0582.

4-Ethylamino-6,7-dimethoxy-1,2,3-benzotrithiole 1-oxide trifluoroacetate (II-3). To a solution of *N*-(Boc)-amine **II-24** (45.1 mg, 0.116 mmol) in distilled CH_2Cl_2 (5 mL) was added slowly trifluoroacetic acid (0.25 mL, 2.90 mmol) at 0 °C. The reaction was stirred at that temperature for 2 h, the solvent and unreacted trifluroracetic acid were removed under reduced pressure to afford essentially pure **II-3** (45.2 mg, 96%) as a yellow glassy solid: ^{1}H NMR δ ($CDCl_3$) 7.74 (br s, 3 H), 7.04 (s, 1H), 4.01 (s, 3H), 3.87 (s, 3H), 3.29 (br s, 2H), 3.15 (br s, 1H), 3.09 (br s, 1H); ^{13}C NMR δ ($CDCl_3$) 151.67, 145.59, 135.27, 127.12, 118.57, 62.30, 56.61, 39.28, 34.41; HRESIMS found 404.9981, calculated for $C_{12}H_{14}NO_5F_3S_3$ 404.9986.

***N*-(Boc)-4-ethylamino-6,7-dimethoxy-1,2,3-benzotrithiole 3-oxide (II-25).** A solution of **II-23** (0.692 g, 1.78 mmol) in CH_3CN (45 mL) was irradiated upon a 100 W high-pressure mercury lamp through a Pyrex filter for 18 h. After removal of solvents by evaporation, the crude product was purified by flash chromatography on silica gel

eluting initially with CH_2Cl_2: EtOAc (50:1, v/v) and then with CH_2Cl_2: EtOAc (10:1, v/v) to afford **II-25** (0.52 g, 75%)[1] as light yellow prisms. **II-25**: ^{1}H NMR δ ($CDCl_3$) 6.77 (s, 1H), 4.81 (br s, 1H), 3,94 (s, 3H), 3.92 (s, 3H), 3.44 (m, 2H), 3.25 (m, 1H), 3.13 (m, 1H), 1.42 (s, 9H); ^{13}C NMR δ ($CDCl_3$) 155.76, 154.61, 143.13, 142.31, 141.01, 133.76, 112.84, 79.42, 60.66, 56.54, 41.41, 36.04, 28.31; ESIMS *m/z* (%) 392 [$(M+1)^+$, 100), 336 (73), 292 (43), 282 (20); HRESIMS found 391.0549, calculated for $C_{15}H_{21}NO_5S_3$ 391.0582.

4-Ethylamino-6,7-dimethoxy-1,2,3-benzotrithiole 3-oxide trifluoroacetate (II-4). To a solution of **II-25** (50.0 mg, 0.128 mmol) in distilled CH_2Cl_2 (5 mL) was added trifluoroacetic acid (0.25 mL, 2.90 mmol) at 0 °C. The mixture was stirred for 2 h. Removal of solvents and excess TFA under reduced pressure afforded **II-4** (47.5 mg, 92%) as a brown glassy solid: ^{1}H NMR δ ($CDCl_3$) 8.03 (br s, 3H), 6.87 (s, 1H), 3.94 (s, 3H), 3.90 (s, 3H), 3.33 (br s, 4H); ^{13}C NMR δ ($CDCl_3$) 155.15, 142.80, 142.08, 141.05, 131.38, 113.44, 60.73, 56.57, 40.85, 33.27; ^{1}H NMR δ (CD_3OD) 7.08 (s, 1H), 3.96 (s, 3H), 3.93 (s, 3H), 3.35 (m, 2H) 3.29 (m, 2H); ^{13}C NMR δ (CD_3OD) 156.45, 144.08, 143.66, 142.26, 132.80, 114.86, 61.19, 57.35, 41.45, 34.15; ESIMS *m/z* (%) 292 [$(M-CF_3CO_2)^+$, 100], 275 (11); HRESIMS found 404.9960, calculated for $C_{12}H_{14}NO_5F_3S_3$ 404.9986.

X-ray analysis of *N*-(Boc)-4-ethylamino-6,7-dimethoxy-1,2,3-benzotrithiole 3-oxide (II-25). The compound was recrystallized from EtOAc/hexane as yellow prisms. A crystal of **II-25** measuring approximately 0.30×0.24×0.10 mm^3 was selected and aligned

on a Bruker Smart 1000 CCD area-detector diffractometer. Preliminary X-ray diffraction patterns indicated monoclinic symmetry, and accurate lattice constants of a=12.546(3) Å, b=15.353(3) Å, c=9.957(2) Å were determined and refined from a full-matrix least-squares on F^2 (Goodness-of-fit on F^2 = 1.020). The empirical formula was $C_{15}H_{21}NO_5S_3$, T=294(2) K, the formula weight was 391.51 amu and crystal density, 1.356 Mg/m^3, indicated that four molecules made up the unit cell (volume, z: 1917.7(7) $Å^3$, 4). F(000) is 824. Systematic extinctions were consistent with the $P2_1/c$ space group. All data were collected using Mo $K\alpha$ radiation (λ=0.71073 Å). Number of reflections collected was 12749, independent reflections was 4401 (R_{int}=0.0925), 223 parameters. Completeness to (θ = 27.62°) was 98.8 %. Final R indices [I>2σ(I)], R1 = 0.0776, wR2 = 0.1754, R indice (all data) R1 = 0.2441, wR2 = 0.2149. The Largest diff. Peak and hole were 0.742 and –0.294 e $Å^{-3}$.

Data collection: *SMART* (Siemens 1995); cell refinement: *SMART*; data reduction: *SHELXTL-NT* (Siemens, 1995); program(s) used to solve structure: *SHELXS*97 (Sheldrick, 1990); program(s) used to refine structure: *SHELXL*97 (Sheldrick, 1997): molecular graphics: *SHELXTL-NT.*

6,7-Dimethoxy-9-[[(*t*-butyl carbamate)amino]ethyl]benzopentathiepin (II-26). To a solution of dithiol **II-21** (0.206 g, 0.599mmol) in distilled THF (30 mL) containing barium carbonate solid (0.580 g, 6.02 mmol) at 0 °C was slowly added a solution of disulfide dichloride (0.12 mL, 1.490 mmol) in distilled CH_2Cl_2 (4 mL). The resulting tan yellow suspension was stirred at room temperature for 2 h. When the reaction was completed, as monitored by TLC, the dark brown reaction mixture was filtered under

gravity and the residue was washed with CH_2Cl_2 (50 mL). Then the filtrate was rotavaporated to give an orange brown oil. Flash chromatography with hexane: EtoAc: CH_2Cl_2 (40:2:1v/v/v) as eluent, increasing the polarity of eluent slowly to hexane: EtOAc (4:1, v/v). The collected portions were concentrated under reduced pressure to afford *N*-(BOC)-varacin **II-26** [1,2] (97.2 mg, 35%) as a slightly yellow syrup: ^{1}H NMR δ ($CDCl_3$) 6.78 (s, 1H), 4.70 (br s, 1H), 3.89 (s, 3H), 3.84 (s, 3H), 3.33 (m, 2H), 3.12 (m, 2.H), 1.43 (s, 9H); ^{13}C NMR δ ($CDCl_3$) 155.81, 154.69, 149.45, 141.21, 140.47, 134.95, 115.56, 61.87, 56.17, 41.82, 36.94, 28.39, 28.16; ESIMS *m/z* (%) 440 [$(M+1)^+$, 100], 422 (62), 384 [$(M-Boc)^+$, 5], 356 (65).

6,7-Dimethoxy-9-[(amino)ethyl]benzopentathiepin trifluoroacetate (II-1). To a solution of *N*-(Boc)-amine **II-26** (25.8 mg, 0.059 mmol) in distilled CH_2Cl_2 (5 mL) was added slowly trifluoroacetic acid (0.20 mL, 2.60 mmol) at 0 °C. The reaction was stirred at that temperature for 2 h, the solvent and excess TFA were removed under reduced pressure to yield essentially pure **II-1** [1,2,7] (25.5 mg, 92%) as a slightly yellow glassy solid: ^{1}H NMR δ (CD_3OD) 7.08 (s, 1H), 3.95 (s, 3H), 3.82 (s, 3H), 3.33 (m, 1H), 3.24 (m, 1H), 3.14 (m, 2H); ^{13}C NMR δ (CD_3OD) 156.69, 151.26, 142.00, 140.29, 136.06, 117.18, 62.18, 56.90, 41.63, 35.13; ESIMS *m/z* (%) 340 [$(M-CF_3CO_2)^+$, 74], 330 (46), 314 (100), 312 (83), 295 (34).

6,7-Dimethoxy-9-[(amino)ethyl]benzopentathiepin hydrogen chloride/ varacin-ium chloride (II-1). To a solution of *N*-Boc-4-ethylamino-6,7-dimethoxy-1,2,3-bezotrithiole 2-oxide **II-23** (42.0 mg, 0.108 mmol) in THF (5 mL) was added NaI (oven dried, 81.5

mg, 0.540 mmol) in water (3 mL) and 20% v/v $HClO_4$ (2.0 mL) at room temperature. The reaction mixture was refluxed overnight and cooled to room temperature when the reaction was completed. After quenching with saturated aqueous $Na_2S_2O_7$, the aqueous mixture was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic extracts were washed with water (20 mL) and brine (20 mL) and dried over Na_2SO_4 to give the title compound **II-1** (24.1 mg, 90%) as a yellow film after rotary evaporation: ^{1}H NMR δ (CD_3OD) 7.14 (s, 1H), 3.97 (s, 3H), 3.82 (s, 3H), 3.35 (m, 1H), 3.25 (m, 1H), 3.16 (m, 2H); ^{13}C NMR δ (CD_3OD) 156.67, 151.24, 141.94, 140.29, 136.31, 62.18, 57.03, 41.67, 35.07; ESIMS *m/z* (%) 381 (5), 343 (3), 342 (23), 340 [$(M-Cl)^+$, 100], 323 (54), 276 (20), 259 (34), 226 (17). The title compound was then recrystallized from a CH_2Cl_2-hexane (8:1) solvent system.

X-ray analysis of varacinium chloride (II-1). A slightly yellow crystal of **II-1** measuring approximately 0.28×0.10×0.08 mm^3 was selected and aligned on a Bruker Smart 1000 CCD area-detector diffractometer. Preliminary X-ray diffraction pattern indicated monoclinic symmetry, and accurate lattice constants of a=16.891(3) Å, b=10.6971(16) Å, c=9.1959(13) Å were determined and refined from a full-matrix least-squares on F^2 (Goodness-of-fit on F^2 = 0.905). The empirical formula was $(C_{10}H_{14}Cl_2NO_2S_3)^+Cl^-$, T=294(2) K, the formula weight was 382.75 amu and crystal density, 1.562 Mg/m^3, indicated that four molecules made up the unit cell (volume, z: 1627.2(4) Å^3, 4). F(000) is 784. Systematic extinctions were consistent with the $P2_1/c$ space group. All data were collected using Mo $K\alpha$ radiation (λ=0.71073 Å). Number of reflections collected was 10836, independent reflections was 3720 (R_{int}=0.0830), 172

parameters. Completeness to (θ = 27.56°) was 99.2%. Final R indices [I>2σ(I)], R1 = 0.0590, wR2 = 0.1277, R indice (all data) R1 = 0.1774, wR2 = 0.1497. The Largest diff. Peak and hole were 0.424 and –0.426 e $Å^{-3}$.

Data collection: *SMART* (Siemens 1995); cell refinement: *SMART*; data reduction: *SHELXTL-NT* (Siemens, 1995); program(s) used to solve structure: *SHELXS*97 (Sheldrick, 1990); program(s) used to refine structure: *SHELXL*97 (Sheldrick, 1997); molecular graphics: *SHELXTL-NT.*

II.4.2 DNA Cleavage Assays of Leinamycin's Mimics

Materials and Methods for DNA Cleavage Assays

Materials. *See Experimental I.4.2 of Part I.*

DNA Cleavage Reactions. Varacinium trifluoroacetate **II-1**, varacinium B **II-3**, C triflurooacetates **II-4**, 4-Ethylamino-6,7-dimethoxy-1,2,3-benzotrithiole 2-oxide tri-fluoroacetate **II-5** and the benzotrithiole oxides **II-6-9** were used as freshly prepared solutions in acetonitrile and the final reaction mixtures in microtubes contained 10% v/v of acetonitrile in 50 mM sodium phosphate buffer. A typical procedure for thiol-dependent plasmid pBR322 DNA cleavage is as follows: Given concentration of DNA-cleaving agents in acetonitrile (1 μL) was added to a solution containing 50 mM sodium phosphate with given pH values, 0.5 μg of pBR322, water. Followed immediately by given concentration of 2-mercaptoethanol (1 μL) and/or addictives to make up a total

volume equal to 10 μL in a microtube. The mixture was agitated on a vortex mixer, spun for 3 s in a tabletop microcentrifuge, and incubated in dark at 37 °C for 6-24 h.

Gel Electrophoresis. *Referring to Experimental **I.4.2** of Part I.*

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PART III

Varacins B, C and Benzotrithiole Oxides Structural Analogs as A Novel Family of Photoinducible DNA-Cleaving Agents

III. 1 Introduction

In the past decade, the design and investigation of artificial photoinducible DNA-cleaving agents which cleave DNA strands by irritating at a specific wavelength under mild conditions without additives such as metals and thiols mediation has received the great attention owing to their significance in the fields of molecular biology and medicinal chemistry.[1,2] The applications of these photonucleases include photo-footprinting of RNA or DNA,[3] determination of secondary and higher structures of nucleic acid as well as their functions as photochemotherapeutic drugs.[2,4,5] The photonucleases advance non-photochemical DNA-cleaving agents, i.e. natural antitumor antibiotics work upon the presence of co-reagents forming the conditions which are hardly compatible with *in vivo* utilities. In addition, the photonucleases are not cytotoxic in dark, their activities can be controlled by the proper choice of light sources and irritation methods.[6]

Artificial photonucleases can be categorized by a range of functions and mechanisms:[1,2] (i) Those involving the generation of active oxygen species such as singlet oxygen, hydroxyl radicals, hydrogen peroxide etc.[7,8] (ii) Photoreactive metal center,[9] i.e. Co(III)-Belomycin and its analogues.[10] (iii) Triggering an electron transfer from DNA nucleobases.[11] (iv) Proton abstraction from DNA backbone by radical formation.[12] (v) Photoinduced alkylation pathway.[13] The light sources generally used for irradiation fall in UVA region (315-400 nm), UVB (280-315 nm), UVC (200-280 nm) and visible light spectrum (Chart 3.1). For the phototherapy

purpose, the use of red-absorbing region (~600 nm) for penetration of light within blood and body tissues is preferred, and the UVC is avoided because of it high cytotoxicity and potential to induce thymine bases dimer formation.[14]

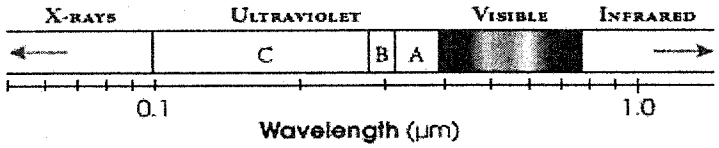


Chart 3.1 EM radiation spectrum from X-ray to Infrared region

The following paragraphs are the brief prologue of the photonuclease categories and corresponding representative examples reported in literature.[1,2,15]

(i) Photonuclease as a cancer chemotherapeutic agent.[7,16] The *in vivo* photosensitivity associated with porphyrins was reported by Meyer-Bentz as early as in 1913 and the concept of cancer photodynamic therapy (PDT) was established since then.[17] The modern therapeutic technique involves red laser irritation of a tumor following the administration of light absorbing agents: a complex mixture of haematoporphyrin and porphyrin related compounds. (See Charts 3.2 and 3.3) Excitation of porphyrins (P) and the structural analogues with red light leads to the production of the first excited singlet state (^{1}P), then it may has a fluorescence back to ground state, P, or intersystem crossing to yield the triplet state (^{3}P).[18] The red fluorescence emission is of interest as a means of a malignant tumor marker, where the relatively long-lived triplet state undergoes exchange electron energy transfer with oxygen to yield singlet oxygen. And this active form of oxygen initiates DNA

radical cleavage through a cascade of reactions and formation of oxygen radicals. The overall process is summarized as follows (Chart 3.4):

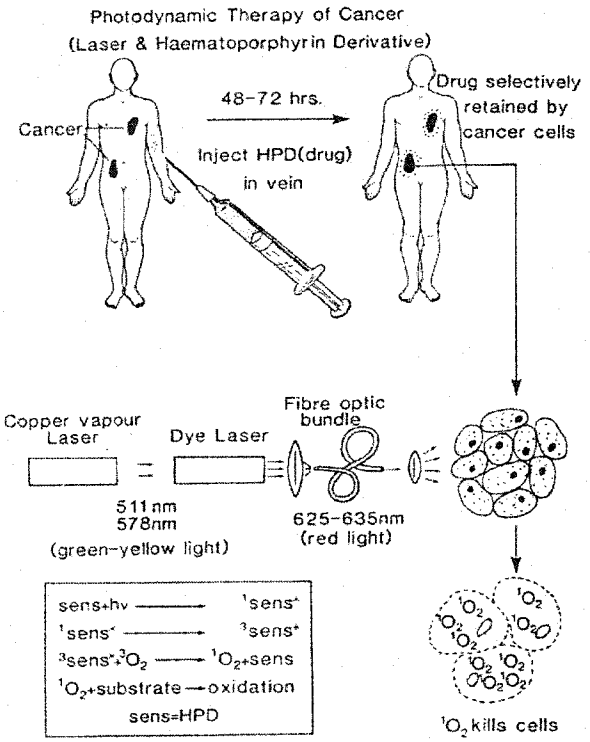


Chart 3.2 Principle of the photodynamic therapy of cancer (Illustration from Ref.[16-18])

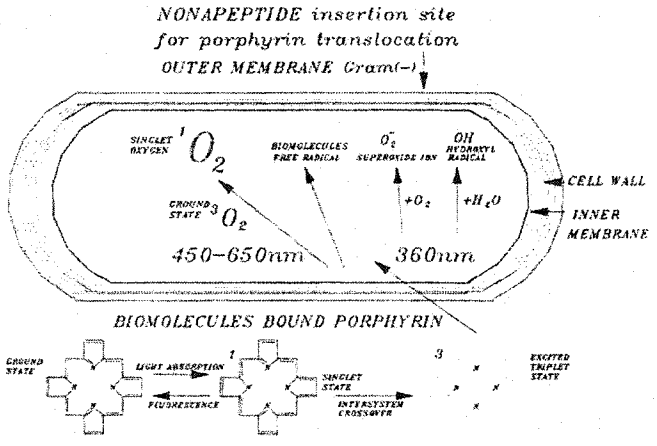


Chart 3.3 Biological interactions of porphyrin with the membrane of gram-negative bacteria and the generation of activated species in photosensitization (Illustration from Ref.[16-18])

$$P + h\nu \rightarrow {}^1P$$

$${}^1P \rightarrow P + \text{fluorescence}$$

$${}^1P \rightarrow {}^3P$$

$${}^3P + O_2 \rightarrow O_2\,({}^1\Delta_g)$$

Chart 3.4 Change of the energy states of porphyrin in phtoosensitization

(ii) Photoinduced DNA cleavage by the metal chelate of the natural products:[9,10] Co(III)-Belomycin is used as an example in Chart 3.5. The family of antibiotic bleomycin requires a metal ion Fe^{2+} and molecular oxygen for their DNA-cleaving action. Co(III)-bleomycin inflicts strand cleavage of DNA upon illumination and this kind of photocleavage does not require an aerobic condition. The light induced DNA strand damage is neither a consequence of reduction of Co(III) to Co(II) nor the generation of singlet oxygen.

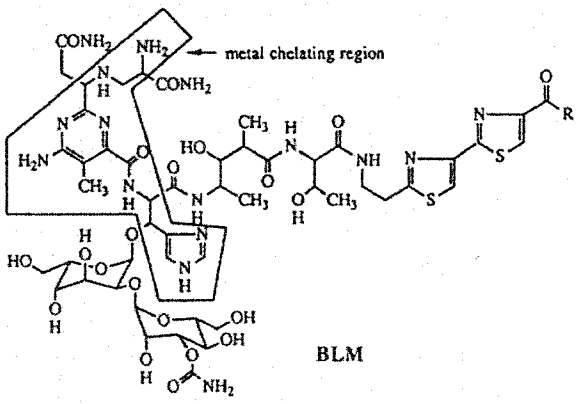


Chart 3.5 Molecular structure of Belomycin

Mascharak *et al.* described the mechanism of DNA cleavage originated from UV irradiation of the candidates. The entities generated C-N based radical of the ligand which reacted with water rapidly to yield ·OH radical.[19] The positively charged

complex bound DNA rather strongly due to dipolar interaction with the negatively charged phosphate backbone, and UV irradiation ended up with a rapid production of ·OH radical in a close proximity of the DNA helix with concomitant strand scissions.

The second example of this type of DNA-cleaving agents (Chart 3.6) is the tetraanionic diplatinum complex, $Pt_2(pop)_4^{4-}$ (pop=pyrophosphito). Thorp *et al.* found that this metal complex could serve as a sequence-neutral photocleavage agent.[1,2,9,20] The complex can also be used as a catalytic DNA cleaving agent because of its ability to oxidize organic substrates catalytically by photoinduced hydrogen atom abstraction. The DNA fragments that arise from this DNA-cleaving possess 3'-phosphate and 3'-phosphoglycolate as well as 5'-phosphate and 5'-aldehyde termini. The two sets of termini are consistent with H-abstraction from both C-4' and C-5' respectively. H-abstraction occurs upon collision between an excited-state $Pt_2(pop)_4^{4-}$ complex and DNA, and is strongly enhanced by inclusion of Mg^{2+} in the reaction buffer. This phenomenon was generated presumably because Mg^{2+} associates with DNA phosphates and/or the platinum complex effectively, thus screening the electrostatic repulsion between the reactants and increasing the DNA cleavage effectiveness.[9,20]

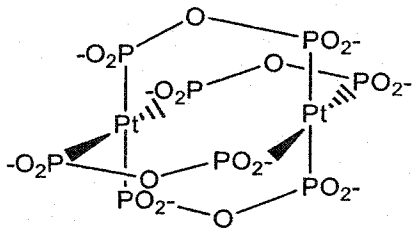
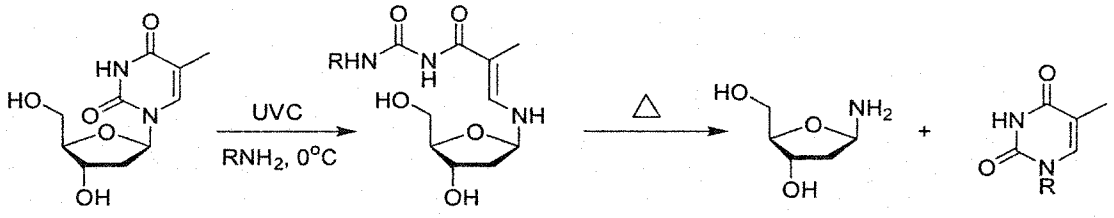


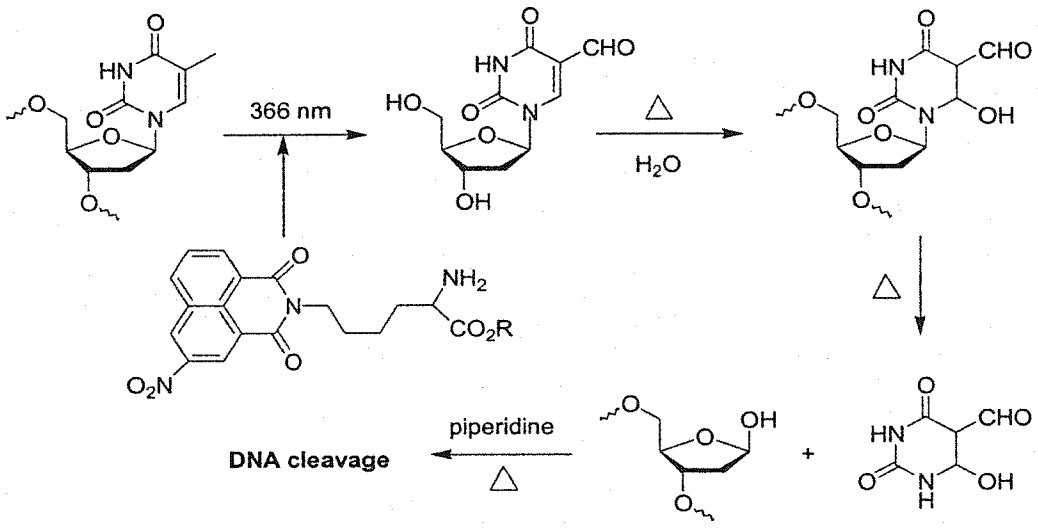
Chart 3.6 Structure of $Pt_2(pop)_4^{4-}$ complex

(iii) Activation of electron transfer from DNA nucleobases through the photochemical pathway is best illustrated by the following example.[1,11] A decade ago, Saito *et al* published a novel photochemical ring-opening of thymidine in the presence of primary amines upon UVC illumination (Scheme 3.1). This access has led to a T-specific cleavage of DNA fragments, and has been used for determining T residues in DNA sequencing of the standard Maxam-Gilbert C+T reaction with an independent confirmatory reading (Scheme 3.2).[1,11,21]



Scheme 3.1 Photochemical ring-opening of thymidine by Saito *et al.*

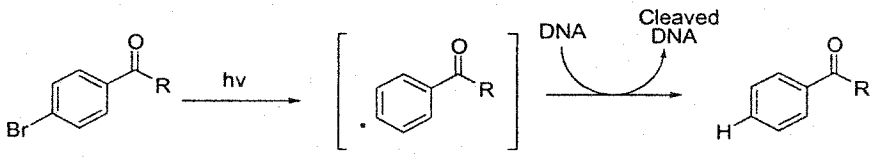
In effort to devise practically useful DNA-cleaving amino acids, they also designed and synthesized a nitro-substituted naphthalimide. Then illuminated it under 366 nm radiation in the presence of ^{32}P-end labeled double stranded DNA fragments, followed by piperidine treatment. T-specific DNA cleavage was accordingly observed. And the initial step of this T-specific cleavage of DNA was an oxidative transformation of the T-methyl group into a formyl group initiated with hydrogen abstraction from T-methyl group by the photoexcited nitro compound.[11,21]



Scheme 3.2 T-specific DNA cleavage illustrated by Saito's group

(iv) Abstraction of proton from DNA backbones by free radicals. Proton abstraction from DNA backbone by radical formation is one of the most common categories of photoinduced DNA damaging mechanism.[12] Paul A. Wender at Standford University reported the utility of bromoacetophenone-pyrrolecarboxamide conjugates as the photonucleases which are illustrated in Scheme 3.3. 4'-Bromoacetophenone derivatives upon illumination generated monophenyl radicals which were capable of abstracting hydrogen atom. Pyrrolecarboxamide (DNA minor groove binder)-conjugated 4'-bromoacetophenones were therefore cleaving DNA at the sites targeted by the recognition subunit (Chart 3.7).[22,23] The excitation of a haloarene led to homolytic cleavage of a carbon-halogen bond. This phenyl radical reacted with DNA surrogate- d_8-THF to give a monodeuterated acetophenone as a product.[12h,24] Additional example was published by Theodorakis *et al.*[12f,25] Their studies were the development of new approaches to DNA cleavage by

photoactivation of *N*-aroyloxy-2-thiopyridone derivatives. Upon illumination (>350 nm), the molecules generated aroyloxy radicals and thiopyrido radicals without the addition of metal or external oxidants, and then nicked single strand in DNA duplex form at micromolar concentrations (Scheme 3.4). Despite the linking of photocleaving ligand to 9-aminoacridine via a polymethylene chain enhanced the agent affinity for DNA duplex by intercalation, the compound (Chart 3.8) cleaved DNA single strand without base sequence selective at all.



Scheme 3.3 Bromoacetophenone as an example of photonucleases

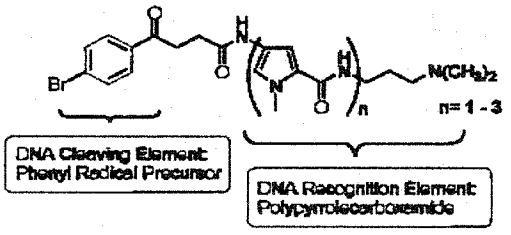
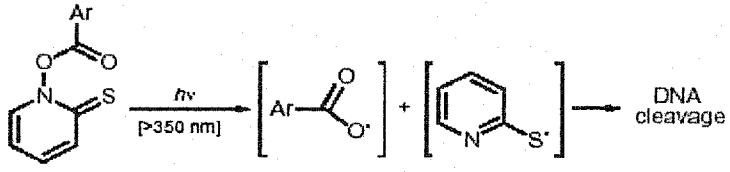


Chart 3.7 Molecular design of Wender's photonucleases



Scheme 3.4 Photoactivation of *N*-aroyloxy-2-thiopyridone derivatives

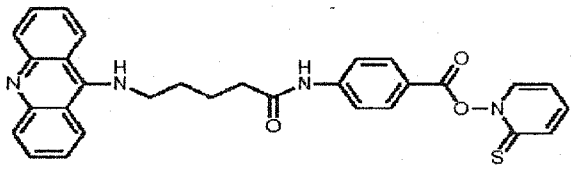


Chart 3.8 *N*-aroyloxy-2-thiopyridone- 9-aminoacridine conjugate

(v) DNA cleavage that undergoes photoinduced alkylation pathway.[1,2,13] Although photoinduction of super electrophiles under mild conditions is particular interesting, few reports have described such a type of DNA alkylation. It is partially attributed to the difficulty of distinguishing the selective guanine cleavage by alkylation after hot piperidine treatment from the G cleavage by singlet oxygen. One of the reported DNA alkylation reagents is shown in Chart 3.9. This design utilized Wolff rearrangement of dibenzoyldiazomethane (DBDM) and its derivatives thus, affording a highly electrophilic benzoylketene after irradiation. The proposed mechanism is illustrated in Scheme 3.5. DBDM was accordingly illuminated at 366 nm in aqueous acetonitrile to afford benzyl phenyl ketone. The formation of this ketone was the result of addition of water to electrophilic ketene followed by decarboxylation of the resulting β-keto acid. Photoirritation of DBDM in the presence of two molar equivalents of glycine ethyl ester (Gly·OEt) gave the amide in 77% yield and benzyl phenyl ketone in 12%. On the other hand, photoirritation of DBDM with N^{α}-Boc-*L*-Lys-OMe gave the corresponding amide in 55% yield. The elaborated development of DBDM included introduction of derivatives DBDM-OSu and

DBDM-oligonucleotide conjugate as a class of photonucleases which cleaved DNA selectively at guanine bases (Chart 3.9).[13]

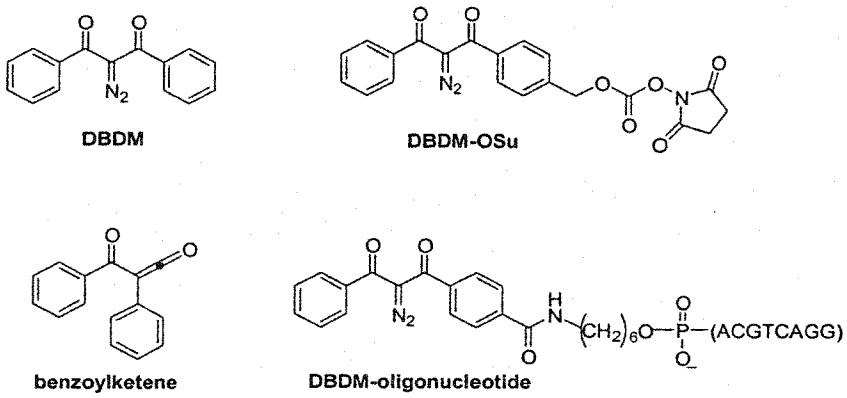
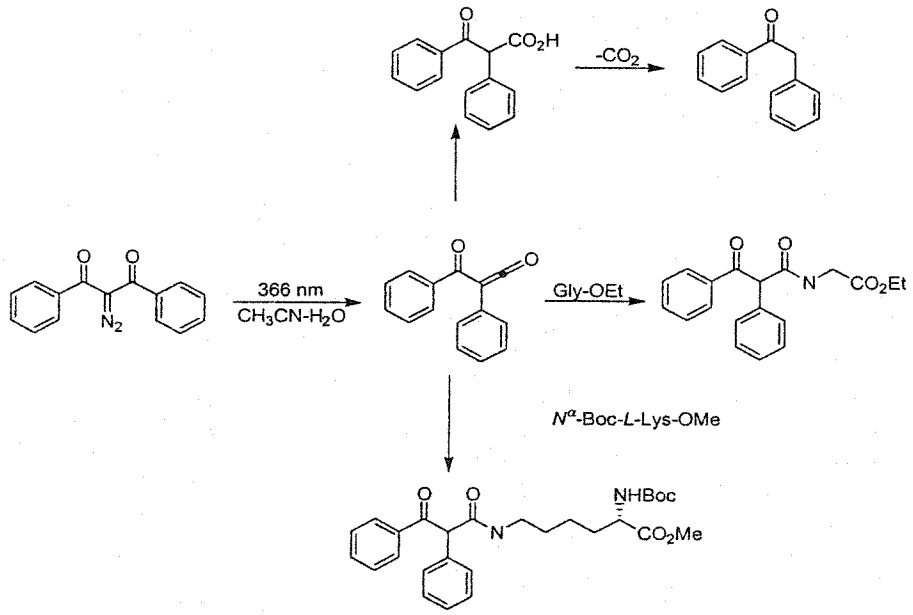


Chart 3.9 DBDM and its derivatives



Scheme 3.5 Photoirritation of DBDM

The third part of our research on the development and application of photonucleases was motivated by the usefulness and challenge of exploring a series

of DNA cleavers. They are stable in dark but highly activated upon irritation with a range of radiation. Their DNA cleaving activities can be triggered simply by application of light. The use of co-reagents such as thiols and metal ions are accordingly omitted since the utilities of co-reagents will lead to the situation hardly compatible to *in vivo* conditions.[1,26] It is believed that the photochemical approach can aid our realization and offers a new insight of the photochemotherpay into the healing of tumors and virus photoinactivation of blood products.

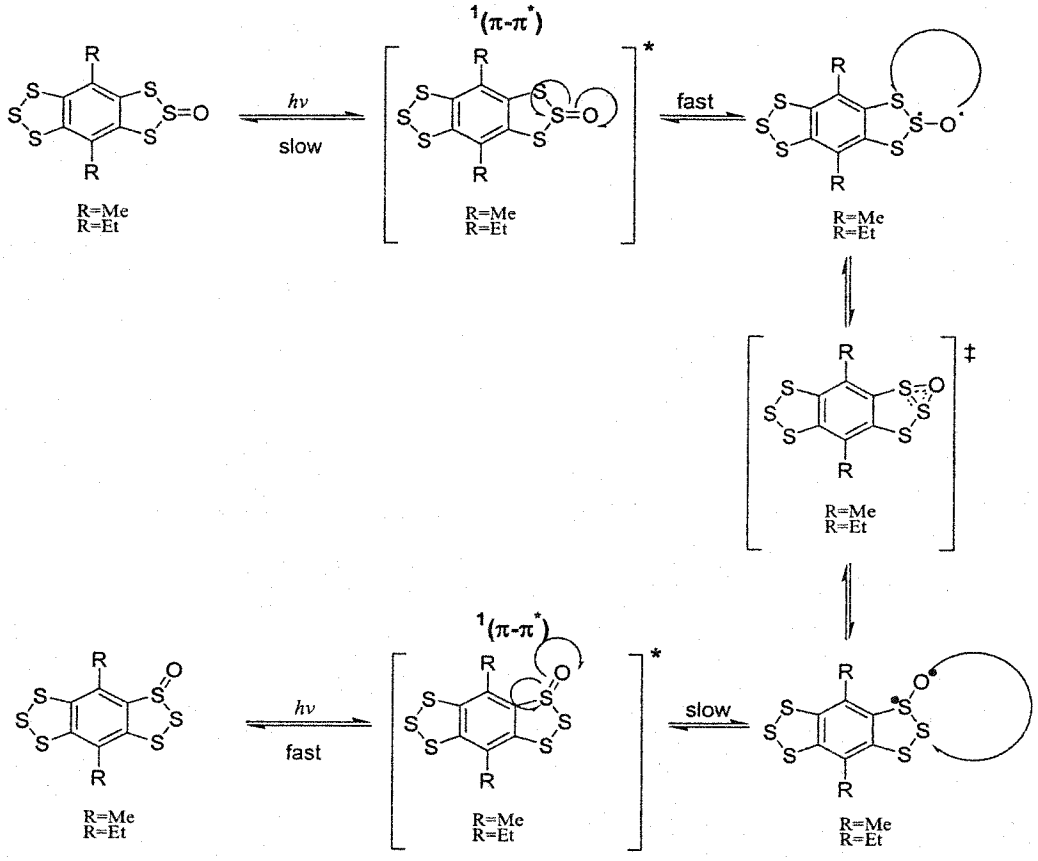
During the research on the antibiotics varacin B, varacin C and the structural related moieties serving as the thiol-mediated DNA-cleaving candidates, we have serendipitously uncovered the photoinduced DNA-cleaving properties of the benzotrithiole oxide, varacin B and varacin C which incorporating benzotrithiole oxide moiety in molecular scaffolds.[27] This idea was originated from the previous synthetic studies on photolysis of benzotrithiole 2-oxide which gave rise to benzotrithiole 1-oxide. The proposed mechanism of this intramolecular photochemical oxygen migrations by Sato *et al* involves the excitation of benzotrithiole 2-oxide upon illumination to attain singlet excited state. The singlet stated intermediate is rapidly transformed into the strained form (see Schemes 2.4 and 3.6).[28] The strained intermediate converts itself to benzotrithiole 1-oxide via intramolecular oxygen migration, or returns to the starting state. Although benzotrithiole 1-oxide can be excited to the singlet state upon irradiation, the rate of quenching (i.e. fluorescence, intersystem crossing and internal conversion of this moiety) may be much faster than its intramolecular oxygen migration to yield the

corresponding 2-oxide.[29] As a whole, the conversion is concluded as a one-way photochemical rearrangement of intramolecular oxygen, the isotopic labeling study showed that there is absent of oxygen exchange between the benzotrithiole oxide moiety with the surroundings (Scheme 2.4).[29a,b,30]

Moreover, several articles in literature indicated that this kind of photochemical reaction pre-required the aromatic ring fused to the core functionality-trithiole 2-oxide ring.[29,31] This migration does not take place thermally or triggered by the addition of acid in dark. The end point of the photochemical change is the oxygen atoms migrated to the terminal sulfur atoms in the trithiole rings which are more electron rich than the middle sulfur atoms. Based on the proposed mechanism and extended studies by several Japanese organic chemists such as Ogawa, Sato *et al*,[28-30] we are inspired that whether photochemical reaction of benzotrithiole oxides are capable to cause DNA strand cleavage through generation of radicals upon illumination.

Preliminary result from our mechanical studies and DNA cleavage assays implied that benzotrithiole oxides were capable of causing the photochemical cleavage of DNA. And this DNA cleavage was originated from the photolysis of benzotrithiole oxides to yield a singlet excited state, which was rapidly transformed to the strained intermediate and further returned to ground state with intramolecular oxygen migrations. Meanwhile, the intersystem crossing $D^* + A \rightarrow D + A^*$ (one of

the quenching mechanisms) generated hydroxyl radicals in aqueous medium and cleaved the DNA strands in the system.[29,32]



Scheme 3.6 Proposed mechanism of the photolysis of benzotrithiole oxides

III. 2 Results and Discussion

The third part of our research work has harnessed a series of benzotrithiole oxides with the photoinduced DNA cleaving properties. Chart 3.10 shows the

structures of these molecules. Their synthetic methods have been discussed in this dissertation. Preliminary results showed that both the natural products and artifically designed DNA cleavers gave the positive results in DNA cleavage upon irradiation at 254, 300 and 365 nm respectively (UVC, B and A regions).

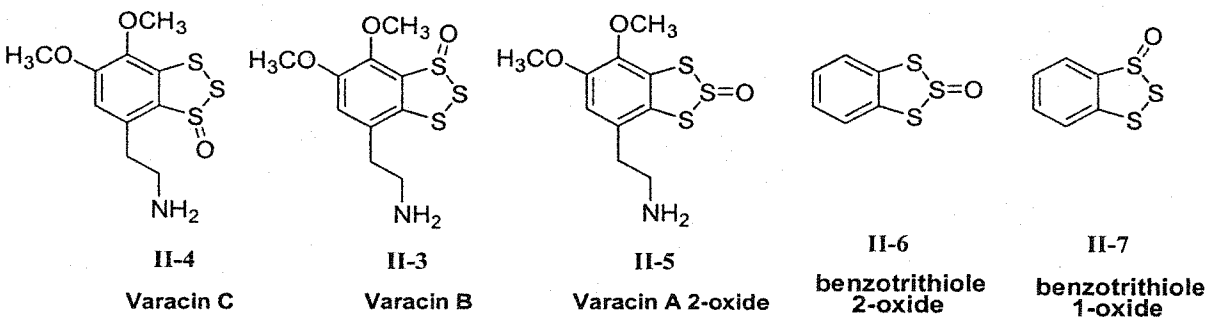


Chart 3.10 Benzotrithiole oxides employed in this part of research works

Ultraviolet radiation, particularly of the short wavelengths such as UVC or UVB is highly mutagenic.[33] DNA and proteins absorb the radiation at 254 nm to 290 nm wavelengths having the higher possibility of generating pyrimidine base dimers and other side products than that of the longer wavelengths. Such a short wavelengths of ultraviolet radiation therefore induces direct damage of DNA, RNA, proteins and lipids accordingly. We did not employ the UVC as the light source though the trithiole 2-oxides in acetonitrile were likely to show the maximum absorption in the range of 245-285 nm ($\varepsilon \sim 8000$). The excitation was assigned as a π–π^* transition of the fusion of aromatic system with trithiole oxide core.[29] In an effort to choose the proper light source for the study and design to harness the tested

candidates as potent prodrugs of anticancer therapy or as applicants for DNA cleavage in future, we have to know the biological impacts of different wavelengths of ultraviolet radiation clearly.

Yet UV radiation at different wavelengths varies in its biological impacts, and we have to cope with the dilemma (intrinsic harmful effects as well as the helpful one).[33,34] Radiation at the longer UV wavelengths of 320-400 nm, designated as UVA, plays a helpful and essential role in formation of Vitamin D by the epidermis cells, and a harmful role as well owing to it ability to cause sunburn on human skin and cataracts in our eyes. Radiation at shorter wavelengths of 290-320 nm, designated as UVB, <290 nm designated as UVC, both cause damage at the molecular level to DNA. The reason behind is that DNA readily absorbs UVB, C radiation, which commonly change the shapes of the molecules in one of the several ways.[34,35] The illustration (Chart 3.11) below reveals one of such changes of DNA due to its exposure to UVB radiation. Changes in the DNA molecules often mean that protein-building enzymes are unable to "read" the DNA codes properly at that point of the molecules or motifs. As a result, mutated proteins will be made, or cells undergo apoptosis.

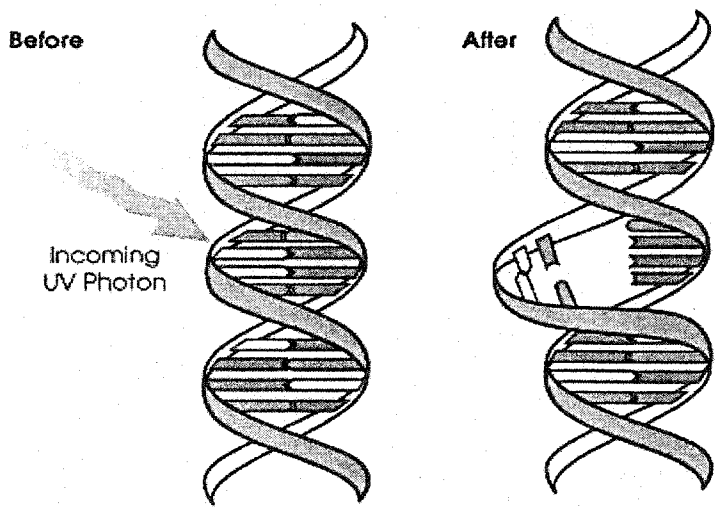


Chart 3.11 Ultraviolet (UV) photons harm the DNA molecules of living organisms in different ways. In one common damage event, adjacent bases bond with each other, instead of across the "ladder." This makes a bulge, and the distorted DNA molecule does not function properly. (Illustration by David Herring at http://earthobservatory.nasa.gov/Library/UVB/)

In fact, living cells are "smart." Over millions of years of evolution in the presence of ultraviolet radiation in our planet, cells have adapted to develop the ability to repair the cellular DNA. Some special enzymes *in vivo* can arrive at the damage site, remove the damaged section of DNA, and replace it with the proper components (based on information elsewhere on the strands of DNA molecules).[36]

III.2.1 Photoinducible DNA Cleavage by the Simple Molecule of Benzotrithiole 2-Oxide

In our investigations, we employed UVA at 365 nm as a light source owing to the reasons mentioned above.[35] Actually, this wavelength is widely accepted by other researchers worldwide as a light source for DNA photonucleases studies. The Spectroline Model ENF260CF in CM10 cabinet 6W unit was accordingly used as the UVA radiation source in our experiments. The typical peak intensity of this wavelength is 350μW/cm^2 at 150 mm. From the standpoint of application and convenience, we inclined to employ the weaker light source rather the hundred watts medium or high pressure Hg arc lamps. Our tested candidates all contain benzotrithiole oxide rings in their molecular structures, including antibiotics varacins B, C, the structural analogue-varacin A 2-oxide and benzotrithiole 1-oxide. Their DNA cleaving properties were investigated and monitored by observing the conversion of supercoiled (Form I) of plasmid DNA pBR322 or supercoiled circular ΦX174 RF DNA to the single-strand cleavage product nicked form (Form II).[37] In our reaction conditions and reagent concentrations, no double-strand scission linearized form of DNA (Form III) was observed.

At the beginning of our studies, the simple analog of varacins B, C antibiotics benzotrithiole 2-oxide **II-6** was tested as a plausible candidate of photonucleases. Then we wondered that whether the photoinitiated DNA cleavage mediated by benzotrithiole 2-oxides could be a milestone or exemplar of harnessing other

structural similar entities as the photonucleases.[27a,38] The DNA-cleaving activity of benzotrithiole 1-oxide **II-7** on plasmid pBR322 DNA was examined upon irradiation at 365 nm. The preliminary result encouraging the ambitious extended studies, we were surprised that such a simple molecule was capable to cause the DNA lesions (Figure 3.1). Only insignificant lesion of pBR322 DNA was recorded in the control (lane 1), the DNA cleavage was confirmed as the photoactivation of benzotrithiole 2-oxide **II-6** (lanes 3, 4). Owing to the benzotrithiole 2-oxide **II-6** structural similarity, we elaborated our tests to various varacinium antibiotics, they incorporate the similar functionality into the molecules.[39]

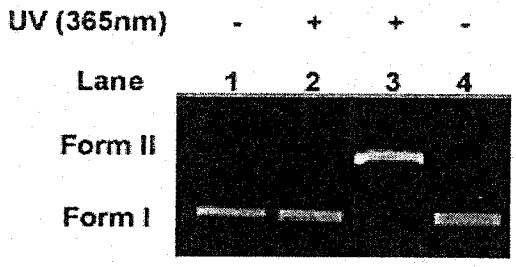


Figure 3.1 The photoinduced cleavage of the plasmid pBR322 DNA by benzotrithiole 2-oxide **II-6**. DNA cleavage reactions were irradiated with UV lamp (365 nm, 6W) from 10 cm distance for 1 h at 25 °C in sodium phosphate buffer at pH 7.0, then incubated at 37 °C in dark for 1 h. The product was analysed by gel electrophoresis (0.8% agarose gel, ethidium bromide stain). Lane 1, pBR322 DNA (38 μM/bp) in dark; lane 2, DNA (365 nm); lane 3, DNA + 250μM **II-6** (365 nm); lane 4, DNA + 250 μM **II-6** in dark.

III.2.2 DNA-Cleaving Agents' Concentration Dependence of the Photoinducible DNA-Cleaving Processes

The abilities of the antibiotics varacins B **II-3** and C **II-4** to cleave DNA under irradiation condition were determined in our studies. Their effectiveness in converting circular supercoiled ΦX174 RF DNA (Form I) and plasmid pBR322 DNA (Form I) to nicked DNA (Form II) were recorded by the 0.8% agarose gel electrophoresis.[37] For this purpose, the varacins B and C were irradiated at various concentrations in the presence of supercoiled ΦX174 RF DNA (50 μM/bp) in 1:9 acetonitrile: sodium phosphate buffer (50 mM, pH 7.0) for 1 h at 365 nm at room temperature, then incubated at 37 °C in dark for another hour. The result of this photocleavage of DNA by varacin B **II-3** at various concentrations is shown in Figure 3.2. The lowest concentration that led to observable single strand DNA cleavage was around 10 nM (lane 7). The highest concentration of varacin B used to test its DNA cleaving properties is 50 μM (lane 3). The control confirmed that the present of varacin B in dark did not lead to the desired DNA damage (lane 2). Lane 1 was the control of DNA alone under ultraviolet radiation at 365 nm, no significant conversion of supercoiled DNA (Form I) to nicked form was detected from this reaction. On the other hand, as shown in Figure 3.3, varacin C **II-4** was capable of causing the single strand cleavage (lane 6) at concentration as low as 100 nM. At higher concentrations, the extent of DNA cleavage increased and the complete disappearance of intact DNA was found at the concentration of 25 μM or above (lane 3). The antibiotic candidates capable of cleaving DNA single strand at much lower concentrations (100-2500 nM)

than the simple benzotrithiole oxides is one of the striking accomplishments we made. This happening was most likely occurred because the amino groups of varacins B and C are positively charged and holding a proximity with slightly negatively charged phosphate groups on the DNA strands with dipolar interactions as discussed in Part II.[40,41]

Comparsion between the varacins B **II-3** and C **II-4** s' photocleavage DNA properties at a range of concentrations was illustrated in Figure 3.4. It is found that with the same or comparable concentrations, the varacin C cleaved supercoiled ΦX174 RF DNA more rapidly than varacin B. This phenomenon is ground on the observation and reason underneath is open to be explored.

To elaborate the work, the benzotrithiole 2-oxide incorporated natural product, varacins B and C structural analog: 4-ethylamino-6,7-dimethoxy-1,2,3-benzotrithiole 2-oxide **II-5** trifluoroacetate was tested too. The photoinduced DNA cleavage activities of **II-5** were assayed using the plasmid pBR322 DNA and covalently closed supercoiled circular ΦX174 RF DNA. As noted in Figure 3.5, **II-5** cleaved the plasmid DNA at 25, 10 and 2.5 μM concentrations upon irradiation at 365 nm for 1 h and incubated at 37 °C for another 1 h. Only single strand breaks of DNA were recorded, leading to the nicked, circular DNA (Form II). While the DNA controls showed no significant cleavage in the identical condition upon illumination. In addition, it was found that benzotrithiole 2-oxide **II-6** at concentrations below 100

μM could not effectively cleave the plasmid DNA. So we believed that the lowest effective concentration of the photoinducible benzotrithiole 2-oxide **II-6** was optimized to 250 μM (data not showed).

III.2.3 Effect of pH on the Photoinducible DNA-Cleaving Processes by Varacins B, C as well as their Structural Analogs

Furthermore, the effect of buffer's pH on the photoinduced cleavage of plasmid DNA by benzotrithiole 2-oxide **II-6** was examined. As shown in Figure 3.6, the DNA-cleaving efficiency did not vary with the change of pH values of the corresponding buffer solutions from pH 5.5, 7.0 and 8.0 of sodium phosphate buffers. This range of pH values represents the slightly acidic, neutral and alkaline aqueous media of the artifical physiological conditions for DNA cleavage.[27a,b,41a] Different from the previous investigations on thiol-mediated DNA cleavage by the benzotrithiole oxides, this photoinducible DNA-cleaving process does not depend on the pH values of buffer solutions which indicates that the mechanisms of the DNA single strand scissions of the two pathways are simply unlikely.

Lack of pH dependence was evident from the DNA cleavage by varacins B **II-3**, C **II-4** (data not shown) and A 2-oxide **II-5** (Figures 3.7 & 3.8), just like those observed in benzotrithiole 2-oxide case. However, the identity of the buffer has a

prominent effect on the efficiency of the photoinduced supercoiled ΦX174 RF DNA cleavage that mediated by varacins B and C. The single strand lesion was significantly more ready in 50 mM sodium phosphate buffer than in 50 mM or 250 mM Tris·HCl buffer. We also observed that those the results obtained from Tris·HCl buffered reactions were far less consistent than the phosphate buffered counterpart (data not shown). The observed buffer dependence is believed to be the consequence of Tris·HCl buffer's ability to react with the radicals which responsible to the DNA cleavage. Several publications have reported the HCl or chloride ion is capable to scavenge hydroxyl radical.[42] Following Figure 3.7 is the data of varacin B **II-3**. Varacin C **II-4** pH independence (data not shown) paralleled the varacin B's result. The pH independence of photoinduced cleavage of pBR322 DNA by varacin A 2-oxide **II-5** was illustrated too. Within the range of buffers pH 6.5, 7.0, 7.5 and 8.5, no observable dependence was found to the DNA cleavage (Figure 3.8).

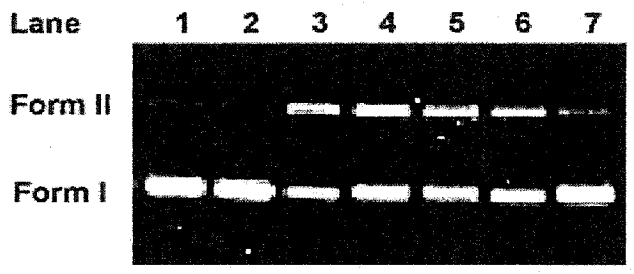


Figure 3.2 Varacin B **II-3** concentration dependence of the photoinducible ΦX174RF DNA-cleaving process. DNA cleavage reactions were irradiated with UV lamp (365 nm, 6W) from 10 cm distance for 1 h at 25 °C in 50 mM sodium phosphate buffer at pH 7.0, then incubated at 37 °C in dark for 1 h. The product was analysed by gel electrophoresis (0.8% agarose gel, ethidium bromide stain). Lane 1, ΦX174RF DNA (50 μM/bp) in dark; lane 2, DNA + 10 μM **II-3** in dark; lane 3, DNA + 50μM **II-3**; lane 4, DNA + 10 μM **II-3**; lane 5, DNA + 1.0 μM **II-3**; lane 6, DNA + 0.1 μM **II-3**; lane 7, DNA + 0.01 μM **II-3**.

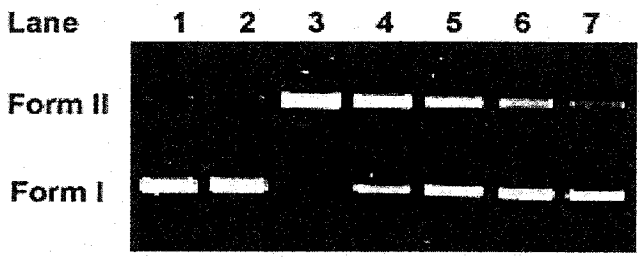


Figure 3.3 Varacin C **II-4** concentration dependence of the photoinducible ΦX174RF DNA-cleaving process. DNA cleavage reactions were irradiated with UV lamp (365 nm, 6W) from 10 cm distance for 1 h at 25 °C in 50 mM sodium phosphate buffer at pH 7.0, then incubated at 37 °C in dark for 1 h. The product was analysed by gel electrophoresis (0.8% agarose gel, ethidium bromide stain). Lane 1, ΦX174RF DNA (50 μM/bp) in dark; lane 2, DNA + 25 μM **II-4** in dark; lane 3, DNA + 25μM **II-4**; lane 4, DNA + 10 μM **II-4**; lane 5, DNA + 5 μM **II-4**; lane 6, DNA + 1 μM **II-4**; lane 7, DNA + 0.1 μM **II-4**.

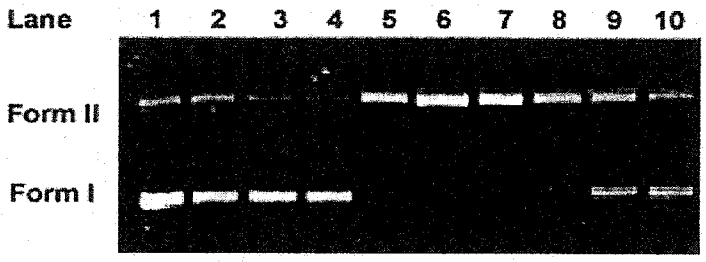


Figure 3.4 Varacins B **II-3** and C **II-4** concentration dependence of the photoinducible ΦX174RF DNA-cleaving process. DNA cleavage reactions were irradiated with UV lamp (365 nm, 6W) from 10 cm distance for 1 h at 25 °C in 50 mM sodium phosphate buffer at pH 7.0, then incubated at 37 °C in dark for 1 h. The product was analysed by gel electrophoresis (0.8% agarose gel, ethidium bromide stain). Lane 1, ΦX174RF DNA (50 μM/bp) in dark; lane 2, DNA (*hv*); lane 3, DNA + 25 μM **II-4** in dark; lane 4, DNA + 50 μM **II-3** in dark; lane 5, DNA + 25 μM **II-4**; lane 6, DNA + 10 μM **II-4**; lane 7, DNA + 5 μM **II-4**; lane 8, DNA + 50 μM **II-3**; lane 9, DNA + 10 μM **II-3**; lane 10, DNA + 1 μM **II-3**.

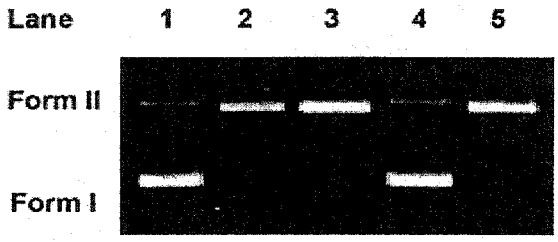


Figure 3.5 Varacin A 2-oxide **II-5** concentration dependence of the photoinducible plasmid pBR322 DNA-cleaving process. DNA cleavage reactions were irradiated with UV lamp (365 nm, 6W) from 10 cm distance for 1 h at 25 °C in 50 mM sodium phosphate buffer at pH 7.0, then incubated at 37 °C in dark for 1 h. The product was analysed by gel electrophoresis (0.8% agarose gel, ethidium bromide stain). Lane 1, pBR322 DNA (38 μM/bp) in dark; lane 2, DNA + 2.5μM **II-5**; lane 3, DNA + 10μM **II-5**; lane 4, DNA + 25 μM **II-5** in dark; lane 5, DNA + 25 μM **II-5**.

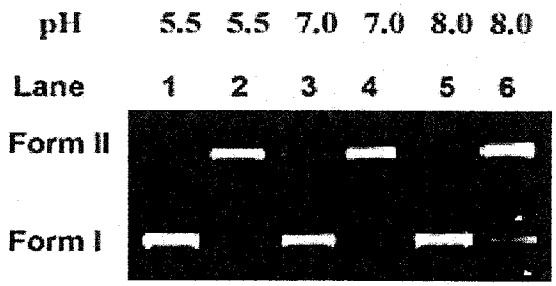


Figure 3.6 Effect of pH on photoinducible plasmid pBR322 DNA-cleaving process by benzotrithiole 2-oxide **II-6**. DNA cleavage reactions were irradiated with UV lamp (365 nm, 6W) from 10 cm distance for 1 h at 25 °C, then incubated at 37 °C in dark for 1 h. The product was analysed by gel electrophoresis (0.8% agarose gel, ethidium bromide stain). Lane 1, pBR322 DNA (38 μM/bp) at pH 5.5; lane 2, DNA + 250 μM **II-6** at pH 5.5; lane 3, DNA at pH 7.0; lane 4, DNA + 250 μM **II-6** at pH 7.0; lane 5, DNA at pH 8.0; lane 6, DNA + 250 μM **II-6** at pH 8.0.

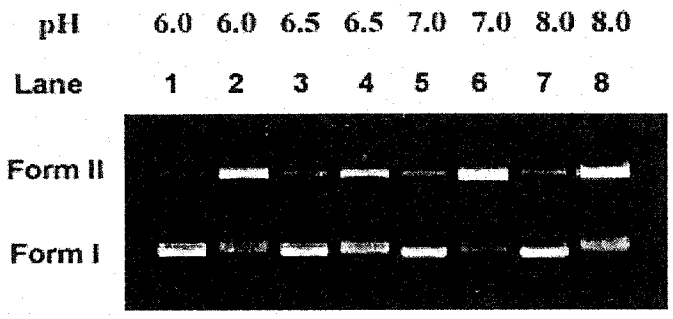


Figure 3.7 Effect of pH on photoinducible ΦX174RF DNA-cleaving process by varacin B **II-3**. DNA cleavage reactions were irradiated with UV lamp (365 nm, 6W) from 10 cm distance for 1 h at 25 °C, then incubated at 37 °C in dark for 1 h. The product was analysed by gel electrophoresis (0.8% agarose gel, ethidium bromide stain). Lane 1, ΦX174RF DNA (50 μM/bp) at pH 6.0; lane 2, DNA + 10 μM **II-3** at pH 6.0; lane 3, DNA at pH 6.5; lane 4, DNA + 10 μM **II-3** at pH 6.5; lane 5, DNA at pH 7.0; lane 6, DNA + 10 μM **II-3** at pH 7.0; lane 7, DNA at pH 8.0; lane 8, DNA + 10 μM **II-3** at pH 8.0.

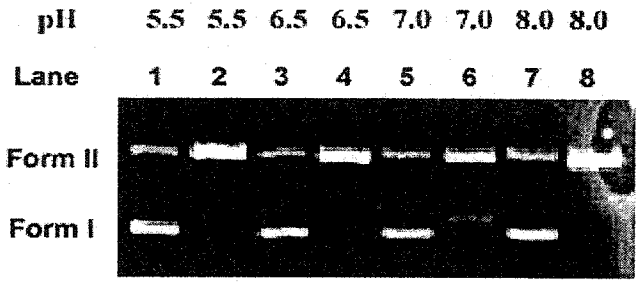


Figure 3.8 Effect of pH on photoinducible plasmid pBR322 DNA-cleaving process by varacin A 2-oxide **II-5**. DNA cleavage reactions were irradiated with UV lamp (365 nm, 6W) from 10 cm distance for 1 h at 25 °C, then incubated at 37 °C in dark for 1 h. The product was analysed by gel electrophoresis (0.8% agarose gel, ethidium bromide stain). Lane 1, pBR322 DNA (38 μM/bp) at pH 5.5; lane 2, DNA + 10 μM **II-5** at pH 5.5; lane 3, DNA at pH 6.5; lane 4, DNA + 10 μM **II-5** at pH 6.5; lane 5, DNA at pH 7.0; lane 6, DNA + 10 μM **II-5** at pH 7.0; lane 7, DNA at pH 8.0; lane 8, DNA + 10 μM **II-5** at pH 8.0.

III.2.4 Wavelength Dependence of the Photoinducible DNA-Cleaving Processes by Varacins B, C as well as their Structural Analogs

Besides of using the above DNA cleaving assays to investigate the photochemical properties of benzotrithiole 2-oxide **II-6**, varacin B **II-3**, varacin C **II-4** and varacin A 2-oxide **II-5**, comparisons of two ultraviolet light sources employed to induce the plasmid DNA cleavage by them were studied too. Figures 3.9 and 3.10 illustrate the conversion of supercoiled plasmid DNA (Form I) to circular relaxed DNA (Form II) by the varacinium agents upon illumination with UVC (254 nm) and UVA (365 nm) respectively. The UVC was extraordinary effective to promote DNA cleavage, the reactions took 20 min or shorter time to complete the conversion of pBR322 from Form I to Form II. Its effectiveness, however, cannot advance the UVC as a light source for photonucleases to UVA. The reason behind has been mentioned before in this chapter.

The success of harnessing UVA as a radiation to excite benzotrithiole oxide moieties is the trophy from Sato *et al* achievements in arena of the photochemistry of using radiation ≥300 nm though the moiety π-π^* energy gap fall in 245-285 nm UV absorption band.[28,29] In our case, we called on 6W UVA (365 nm) lamp as a high pressure and medium pressure mercury arc lamps surrogate, because the too much heat mercury lamps delivered to the specimens might cause unknown damages, serious side effects and difficulties in temperature control on the experiments.

Furthermore, the medium pressure mercury arc lamps operate at internal pressures of from 1 to 10 atm and emit radiation over region 200-1400 nm, with particularly intense emission at 313, 366, 436 and 546 nm; high pressure mercury arc lamps operate at pressure from 100 to several hundred atm and emit radiation over the region 200-1400 nm with visible light rich radiant energy. Those the light sources are the combinations of multiple wavelengths radiation, and pose an obstacle to specify the useful wavelength for photonucleases activation.[43] We envisioned that 6W UVA lamp maybe a promising candidate with single wavelength which is capable to excite the benzotrithiole oxides, and this light source is also available in our laboratory.

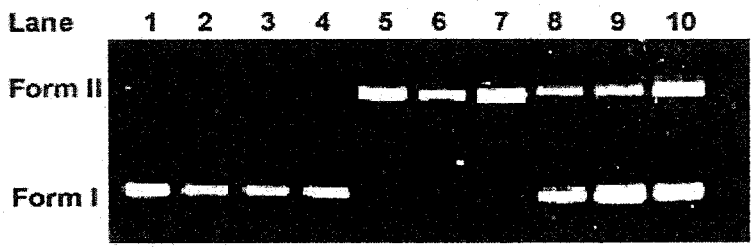


Figure 3.9 Wavelength dependence of the photoinducible plasmid pBR322 DNA-cleaving process by benzotrithiole 2-oxide **II-6**, varacin C **II-4** and varacin A 2-oxide **II-5**. DNA cleavage reactions were kept in dark, irradiated with UV lamp (254 nm, 6W or 365 nm, 6W) from 10 cm distance for 30 min at 25 °C, and further incubated at 37 °C in the absence of light for 1 h. The product was analysed by gel electrophoresis (0.8% agarose gel, ethidium bromide stain). Lane 1, pBR322 DNA (38 μM/bp) in dark; lane 2, DNA + 250 μM **II-6** in dark; lane 3, DNA + 10 μM **II-4** in dark; lane 4, DNA + 10 μM **II-5** in dark; lane 5, DNA + 250 μM **II-6** (254 nm); lane 6, DNA + 10 μM **II-4** (254 nm); lane 7, DNA + 10 μM **II-5** (254 nm); lane 8, DNA + 250 μM **II-6** (365 nm); lane 9, DNA + 10 μM **II-4** (365 nm); lane 10, DNA + 10 μM **II-5** (365 nm).

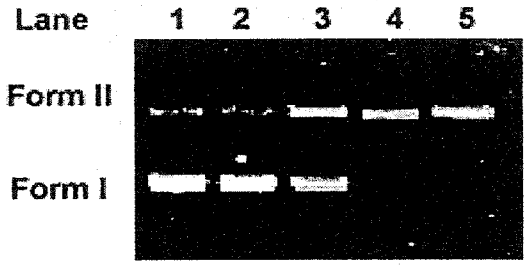


Figure 3.10 Wavelength dependence of photoinducible plasmid pBR322 DNA-cleaving by varacin B **II-3**. DNA cleavage reactions were kept in dark, irradiated with UV lamp (254 nm, 6W or 365 nm, 6W) from 10 cm distance for 30 min at 25 °C, then further incubated at 37 °C in dark for 1 h. The product was analysed by gel electrophoresis (0.8% agarose gel, ethidium bromide stain). Lane 1, pBR322 DNA (38 μM/bp) in dark; lane 2, DNA + 250 μM **II-3** in dark; lane 3, DNA + 10 μM **II-3** (365 nm); lane 4, DNA + 10 μM **II-3** (254 nm); lane 5, DNA + 10 μM **II-3** (both 254 and 365 nm).

III.2.5 Irradiation Time Dependence of the Photoinducible DNA-Cleaving Processes by Varacins B, C and their Structural Analogs

In order to probe the mechanisms of this DNA-cleaving process, the effect of irradiation time was examined. As shown in following, varacin B **II-3** demonstrated the nearly complete conversion of supercoiled (Form I) ΦX174 RF DNA (50μM/bp) to its nicked form (Form II) by 105 min (lane 10) in Figure 3.11. On the time scale, it was recorded the gradually change of Form I DNA to Form II. With the time of irradiation and incubation increased, the amount of damage DNA increased linearly. The cleavage of DNA caused by varacin B **II-3** photoinduction occurred as early as the first 15 min (lane 4). However, we found no double-strand cleavage product (Form III) DNA on that time scale, even beyond the 100 min of irradiation. It seem that the photoactivitated DNA cleavage by all of our used candidates caused the single strand DNA damage only, no matter the change of concentration, time of reaction and pH of buffer. Figure 3.12 shows the time dependence photoinducible DNA cleavage by varacin C **II-4**. As mentioned before, it is a far more powerful DNA cleaver than its varacin B counterpart. At 10 μM concentration (same to varacin B), the extent of DNA lesions increased gradually as the amount of intact DNA decreased, with complete vanishing of Form I at 45 min (lane 5). Varacin A 2-oxide **II-5** (Figure 3.13) demonstrated the fully complete conversion of supercoiled ΦX174 RF DNA (50 μM/bp) to its nicked form by means of single strand lesions of DNA by 30 min (lane 4). Those time dependence studies of the two antibiotics and a structural analog were worked out in the same reaction condition and the results were repeated with consistence.

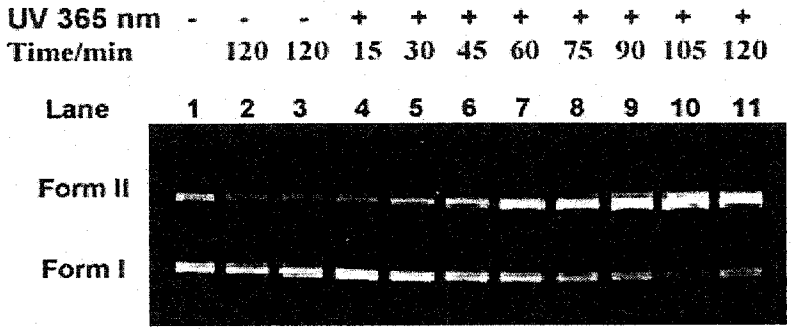


Figure 3.11 Irradiation time dependence of photoinduced supercoiled ΦX174 RF DNA (38 μM/bp) DNA-cleaving process by varacin B **II-3**. Unless otherwise indicated, DNA cleavage reactions were irradiated with UV lamp (365 nm, 6W) from 10 cm distance for various time intervals at 25 °C, then incubated at 37 °C in dark for 1 h. The product was analysed by gel electrophoresis (0.8% agarose gel, ethidium bromide stain). Lane 1, control supercoiled ΦX174 RF DNA (50 μM/bp); lane 2, DNA (no *hv*) 120 min; lane 3, DNA + 10 μM **II-3** (no *hv*) for 120 min; lane 4, DNA + 10 μM **II-3** for 15 min; lane 5, DNA + 10 μM **II-3** for 30 min; lane 6, DNA + 10 μM **II-3** for 45 min; lane 7, DNA + 10 μM **II-3** for 60 min; lane 8, DNA + 10 μM **II-3** for 75 min; lane 9, DNA + 10 μM **II-3** for 90 min; lane 10, DNA + 10 μM **II-3** for 105 min; lane 11, DNA + 10 μM **II-3** for 120 min.

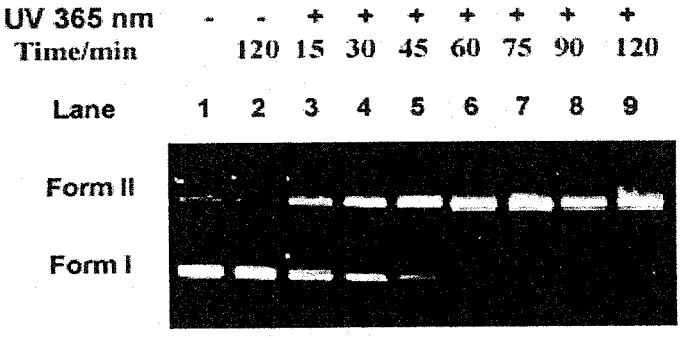


Figure 3.12 Irradiation time dependence of photoinducible supercoiled ΦX174 RF DNA (38 μM/bp) DNA-cleaving process by varacin C **II-4**. Unless otherwise indicated, DNA cleavage reactions were irradiated with UV lamp (365 nm, 6W) from 10 cm distance for various time intervals at 25 °C, then incubated at 37 °C in dark. The product was analysed by gel electrophoresis (0.8% agarose gel, ethidium bromide stain). Lane 1, control supercoiled ΦX174 RF DNA (50 μM/bp); lane 2, DNA + 10 μM **II-4** (no *hv*) for 120 min; lane 3, DNA + 10 μM **II-4** for 15 min; lane 4, DNA + 10 μM **II-4** for 30 min; lane 5, DNA + 10 μM **II-4** for 45 min; lane 6, DNA + 10 μM **II-4** for 60 min; lane 7, DNA + 10 μM **II-4** for 75 min; lane 8, DNA + 10 μM **II-4** for 90 min; lane 9, DNA + 10 μM **II-4** for 120 min.

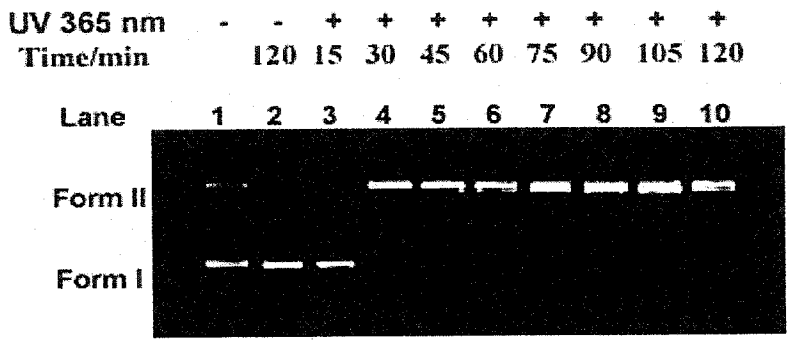


Figure 3.13 Irradiation time dependence of photoinducible supercoiled ΦX174 RF DNA (38 μM/bp) DNA-cleaving process by varacin A 2-oxide **II-5**. Unless otherwise indicated, DNA cleavage reactions were irradiated with UV lamp (365 nm, 6W) from 10 cm distance for various time intervals at 25 °C, then incubated at 37 °C in dark. The product was analysed by gel electrophoresis (0.8% agarose gel, ethidium bromide stain). Lane 1, control supercoiled ΦX174 RF DNA (50 μM/bp); lane 2, DNA + 10 μM **II-5** (no *hν*) for 120 min; lane 3, DNA + 10 μM **II-5** for 15 min; lane 4, DNA + 10 μM **II-5** for 30 min; lane 5, DNA + 10 μM **II-5** for 45 min; lane 6, DNA + 10 μM **II-5** for 60 min; lane 7, DNA + 10 μM **II-5** for 75 min; lane 8, DNA + 10 μM **II-5** for 90 min; lane 9, DNA + 10 μM **II-5** for 105 min; lane 10, DNA + 10 μM **II-5** for 120 min.

III.2.6 Determination of Reaction Mechanisms of the Photoinducible DNA-Cleaving Processes by Varacins B, C and their Structural Analogs

Investigation of the photoinducible DNA cleavage mechanism by varacins B, C and the two analogs were examined during our investigation. In general, DNA strands damage is resulted from the reaction of DNA with a variety of species including electrophiles, oxygen radicals and carbon-centered radicals.[40,41,44] Thus, certain experiments were undertaken in order to identify the nature of the reactive species responsible for the photoinduced DNA single-strand cleavage by the antibiotics and their structural analogs. Following Figures recorded the DNA-cleaving assays of benzotrithiole 2-oxide **II-6**, varacin B **II-3** and varacin C **II-4** and varacin A 2-oxide **II-5** at concentrations described in the legends. They carried out single-strand cleavage of plasmid DNA or ϕX174RF DNA upon irradiation at 365 nm ultraviolet radiation without addition of co-reagent, such as thiols and transition metal ions. The DNA cleavages mediated by the entitled agents were successfully quenched by catalase 53.2 μg/mL, catalase is a hydrogen peroxide-destroying enzyme.[41,45] Addition of the carbon radical scavenger 2,2,6,6-Tetramethylpiperidinooxy (TEMPO)[46] at 5 mM to the reaction mixtures did not inhibit the DNA cleavage by benxotrithiole 2-oxide **II-6**, varacin C **II-4** and varacin A 2-oxide **II-5** but not for varacin B **II-3**. Superoxide-destroying enzyme superoxide dismutase (SOD)[47] at concentration 80 μg/mL also gave no observable quenching effects on varacin C **II-4** and varacin A 2-oxide **II-5** mediated DNA cleavage in our reaction conditions.

Hydroxyl radical scavengers methanol, mannitol significantly inhibited DNA cleavage by all parties.[41,45] Therefore, inhibition of DNA cleavage by the two alcohols is the notion that hydroxyl radical are likely involved in the DNA cleavage by our agents.

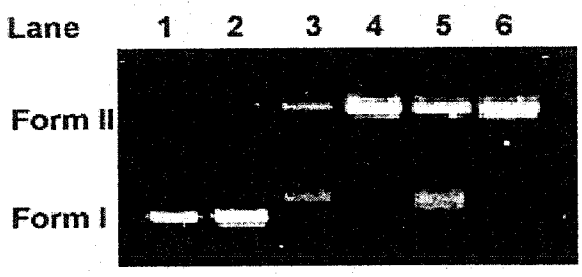


Figure 3.14 Inhibitory effect of radical scavengers on the photoinducible plasmid pBR322 DNA-cleaving process by benzotrithiole 2-oxide **II-6**. Unless otherwise indicated, all DNA cleavage reactions were irradiated with UV lamp (365 nm, 6W) from 10 cm distance for 1 h at 25 °C in sodium phosphate buffer at pH 7.0, then incubated at 37 °C in dark for 1 h. The product was analysed by gel electrophoresis (0.8% agarose gel, ethidium bromide stain). Lane 1, control pBR322 DNA (38 μM/bp); lane 2, DNA + 250 μM **II-6** (no *hv*); lane 3, DNA + 250 μM **II-6** +100 mM mannitol; lane 4, DNA + 250 μM **II-6** + 5 mM TEMPO; lane 5, DNA + 250 μM **II-6** + 53.2 μg/mL catalase; lane 6, DNA + 250 μM **II-6**.

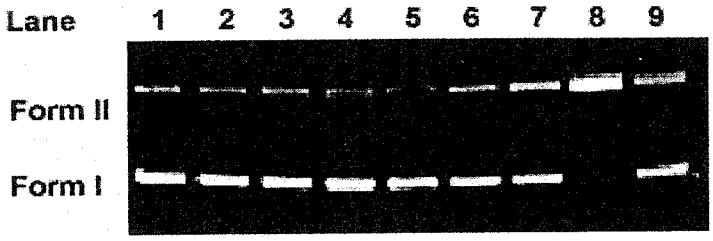


Figure 3.15 Inhibitory effect of radical scavengers on the photoinducible ΦX174RF DNA-cleaving process by varacin B **II-3**. Unless otherwise indicated, all DNA cleavage reactions were irradiated with UV lamp (365 nm, 6W) from 10 cm distance for 1 h at 25 °C in sodium phosphate buffer at pH 7.0, then incubated at 37 °C in dark for 1 h. The product was analysed by gel electrophoresis (0.8% agarose gel, ethidium bromide stain). Lane 1, control ΦX174RF DNA (50 μM/bp) ; lane 2, DNA + 10 μM **II-3** (no *hv*); lane 3, DNA + 10 μM **II-3** +1 M methanol; lane 4, DNA + 10 μM **II-3** + 100 mM mannitol; lane 5, DNA + 10 μM **II-3** + 50 mM NaN_3; lane 6, DNA + 10 μM **II-3** + 53.2 μg/mL catalase; lane 7, DNA + 10 μM **II-3** + 80 μg/mL SOD; lane 8, DNA + 10 μM **II-3**; lane 9, DNA + 10 μM **II-3** + 5mM TEMPO.

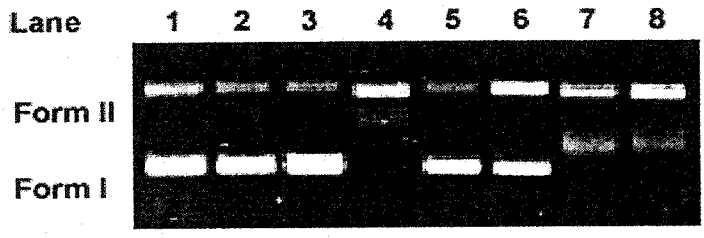


Figure 3.16 Inhibitory effect of radical scavengers on the photoinducible plasmid pBR322 DNA-cleaving process of the by varacin C **II-4**. Unless otherwise indicated, all DNA cleavage reactions were irradiated with UV lamp (365 nm, 6W) from 10 cm distance for 1 h at 25 °C in sodium phosphate buffer at pH 7.0, then incubated at 37 °C in dark for 1 h. The product was analysed by gel electrophoresis (0.8% agarose gel, ethidium bromide stain). Lane 1, control pBR322 DNA (38 μM/bp) ; lane 2, DNA + 10 μM **II-4** (no *hv*); lane 3, DNA + 10 μM **II-4** +1 M methanol; lane 4, DNA + 10 μM **II-4** + 5 mM TEMPO; lane 5, DNA + 10 μM **II-4** + 53.2 μg/mL catalase; lane 6, DNA + 10 μM **II-4** + 50 mM NaN_3; lane 7, DNA + 10 μM **II-4** 80 μg/mL SOD; lane 8, DNA + 10 μM **II-4**.

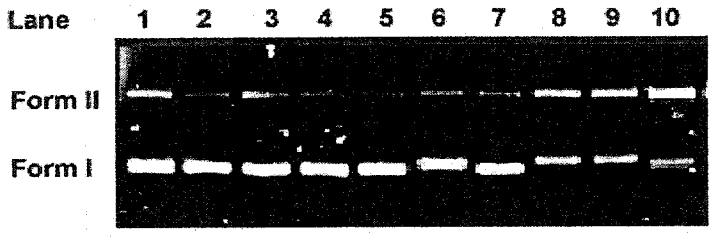


Figure 3.17 Inhibitory effect of radical scavengers on the photoinducible ΦX174RF DNA-cleaving process by varacin A 2-oxide **II-5**. Unless otherwise indicated, all DNA cleavage reactions were irradiated with UV lamp (365 nm, 6W) from 10 cm distance for 1 h at 25 °C in sodium phosphate buffer at pH 7.0, then incubated at 37 °C in dark for 1 h. The product was analysed by gel electrophoresis (0.8% agarose gel, ethidium bromide stain). Lane 1, control ΦX174RF DNA (50 μM/bp) (*hv*) ; lane 2, DNA (no *hv*); lane 3, DNA + 10 μM **II-5** (no *hv*); lane 4, DNA + 10 μM **II-5** +1 M methanol; lane 5, DNA + 10 μM **II-5** + 100 mM mannitol; lane 6, DNA + 10 μM **II-5** + 50 mM NaN_3; lane 7, DNA + 10 μM **II-5** + 53.2 μg/mL catalase; lane 8, DNA + 10 μM **II-5** + 80 μg/mL SOD; lane 9, DNA + 10 μM **II-5** + 5mM TEMPO; lane 10, DNA + 10 μM **II-5**.

The mere inhibition of the reaction by sodium azide has been frequently produced, acting as a proof that singlet oxygen alone is the mediator of phototoxicity.[48] When azide protected against killing by hematoporphorin, neutral red, methylene blue, rose bengal, and fluorescein, it was taken as evidence of singlet oxygen involvement. Thus, the finding that presence of 50 mM sodium azide caused markly quenching of DNA cleavage by varacins B **II-3**, C **II-4** and A 2-oxide **II-5**, providing the evidence that the possibility of singlet oxygen involvement in the reaction. We have added the enzymes catalase and superoxide dismutase to our reaction mixtures to monitor changes of our photonucleases' behaviors in DNA cleavage. The catalase belongs to the family of enzymes which contains the hydroperoxidases and peroxidases. Catalase catalyzes the reaction:

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

whereas peroxidases catalyze the reaction:

$$AH_2 + H_2O_2 \rightarrow A + 2\ H_2O$$

The addition of this hydrogen peroxide destroying enzyme to our reaction mixtures nearly stopped the DNA cleavage completely. This led to the suggestion of DNA damage was caused by hydroxyl radical that arose from hydrogen peroxide decomposition process-Fenton reaction. The reduction of hydrogen peroxide by iron chelates was observed by Fenton in 1894.[18,41,45,47] This process can be summarized in the equation below.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot}$$

Hydrogen peroxide may be formed from the molecular oxygen conversion, or from the spontaneous dismutation of superoxide radical. Superoxide dismutase (SOD) catalyzes the reaction between two molecules of superoxide to form hydrogen peroxide and molecular oxygen.

Superoxide dismutase is ubiquitous in aerobic organisms. Eucaryotes contain, typically, both a copper and zinc-containing superoxide dismutase (CuZnSOD) and another SOD that contains manganese (MnSOD); the two species are unrelated, and the latter is compartmentalized in the mitochondria, while the former is in the cytoplasm. Inhibition of the DNA cleavage by SOD is considered as an implication of $O_2^{\cdot -}$ in the reaction. Moreover, the product of SOD reaction, hydrogen peroxide can cause DNA cleavage through Fenton reaction. In cases of DNA cleavage quenching with SOD resulted from the SOD catalyses the formation of hydrogen peroxide, the enzyme which may serve as a necessary reducing agent to drive the Fenton reaction. However, the origin of superoxide radical in the studied reactions has not been unambiguously found because the possibility of photolysis of hydrogen peroxide in aqueous solution gives birth of $OH^{\cdot}$. It reacts with the hydrogen peroxide to yield $HO_2^{\cdot}$/ $O_2^{\cdot -}$ system.[18,47] The reaction equations are shown below.

$$H_2O_2 \rightarrow 2\ OH^{\cdot}\ (h\nu)$$

$$OH^{\cdot} + H_2O_2 \rightarrow HO_2^{\cdot} + H_2O$$

$$HO_2^{\cdot} \rightleftarrows O_2^{\cdot -} + H^+$$

Besides of studying the mechanism of photoinduced DNA cleavage by the chosen molecules with benzotrithiole oxide core with gel electrophoresis, the molecular studies were undertaken too. Attempts to isolate the products or intermediates of our photonucleases were tried. The molecular studies involved the monitoring of the organic reactions of benzotrithiole 1-oxide **II-7** and benzotrithiole 2-oxide **II-6** with hydroxyl radical quenchers including methanol, mannitol, DMSO, THF and benzophenone,[41,45,47,49a-d] was summarized in Table 3.1.

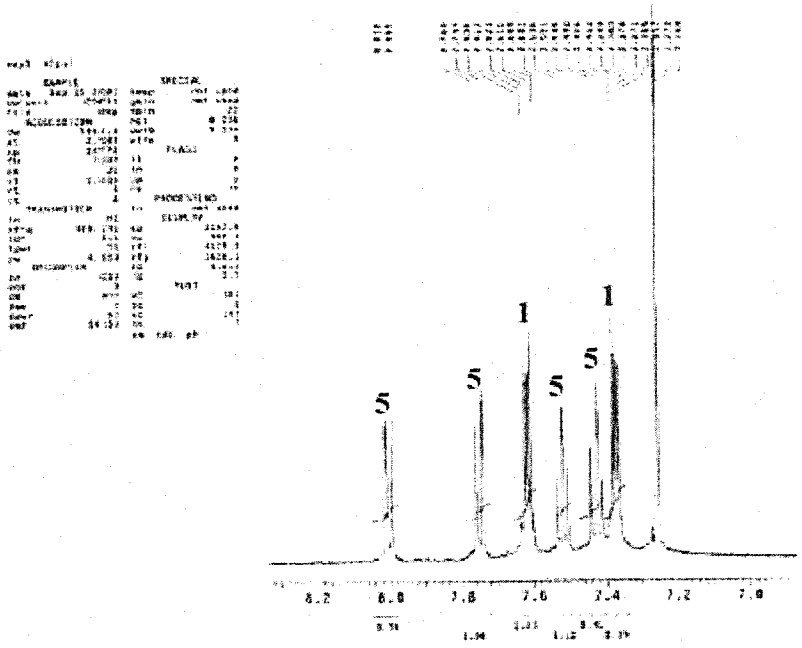
The benzotrithole 2-oxide **II-6** was allowed to react with various quenchers upon UVA (365 nm) or mercury lamp irradiation and the reaction mixture was analyzed with TLC and ^{1}H NMR. It was found that ~50% of the starting material converted to the benzotrithiole 1-oxide **II-7** via the intramolecular photochemical oxygen migration after 24 hour of illumination. As shown in Figure 3.18, pairs of doublets (δ 7.74, 7.99 ppm) and triplets (δ 7.42, 7.51 ppm) corresponding to benzotrithiole 1-oxide **II-7** were emerged in the reaction mixture. However, no change of quenchers was observed except DMSO-d_6. It is possible for us to characterize the products from the reaction between DMSO-d_6 with hydroxyl radical by magnifying the ^{1}H NMR spectra recorded in the same condition as Figure 3.18. The characterized singlets of methane sulfinic acid (MSA), dimethyl sulfone ($DMSO_2$) and the unreacted DMSO were found in Figure 3.19. The presence of hydroxyl radical as a crucial reactive intermediate has been established by the method

based on the formation of methanesulfinic acid from DMSO (see Scheme 3.7). The process of DMSO oxidation is essentially a chain reaction initiated by the OH radical.[49]

The benzotrithiole 1-oxide **II-7** underwent the similar intramolecular photochemical oxygen migration to afford benzotrithiole 2-oxide upon UVA (365 nm) or mercury pressure lamp irradiation. In our experiments, no any observed ring opening product was found. Those added hydroxyl radical quenchers gave no detectable change (using ^{1}H NMR technique) except DMSO. Addition of DMSO-d_6 to the photoactivated system yielded the reaction products identical to the benzotrithiole 2-oxide case. Figures 3.20 and 3.21 are the ^{1}H NMR spectra of the reaction mixtures recorded at 3 h and 24 h during irradiation. Proton integrations of the two fingerprint multiplets from benzotrithiole 2-oxide **II-6** at δ 7.62 and 7.37 ppm increased from 0.15 H (~6%) at 3h to 1.8 H (~50%) at 24 h. To our surprise, this finding of backward intramolecular migration of oxygen atom from benzotrithiole 1-oxide to yield benzotrithiole 2-oxide is violating the proposal settled on by Sato *et al.* They concluded that the products of regioselective oxidation of benzotrithiole analogs had the terminal sulfur atoms in the benzotrithiole rings which were more electron rich than that of the middle sulfur atoms. Consequently, the reaction was characterized as a one-way photochemical rearrangement.

Table 3.1 Results of reactions between **II-6** and **II-7** with various hydroxyl radical scavengers.

OH˙ scavenger	Methanol	Mannitol	DMSO-d_6	THF-d_8	Benzophenone
Observations of reactions with **II-6**	X	X	O	X	X
Observations of reactions with **II-7**	X	X	O	X	X



Photolysis reaction of **II-6** for 24h:

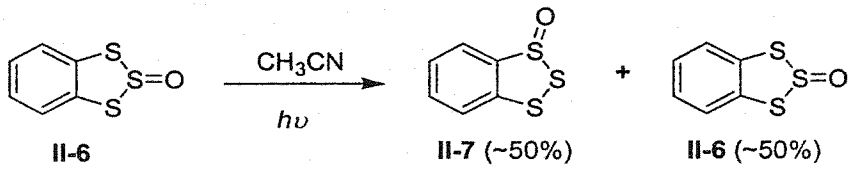


Figure 3.18 ^{1}H NMR spectrum of benzotrithiole 2-oxide after 24 h irradiation

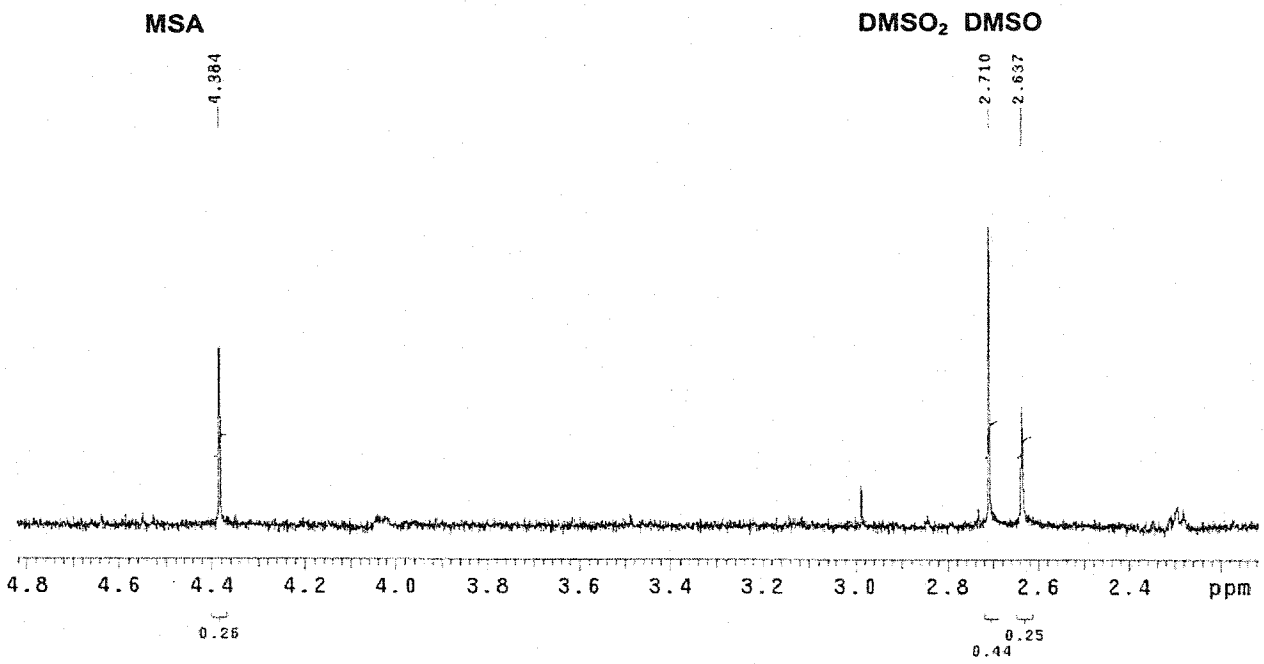
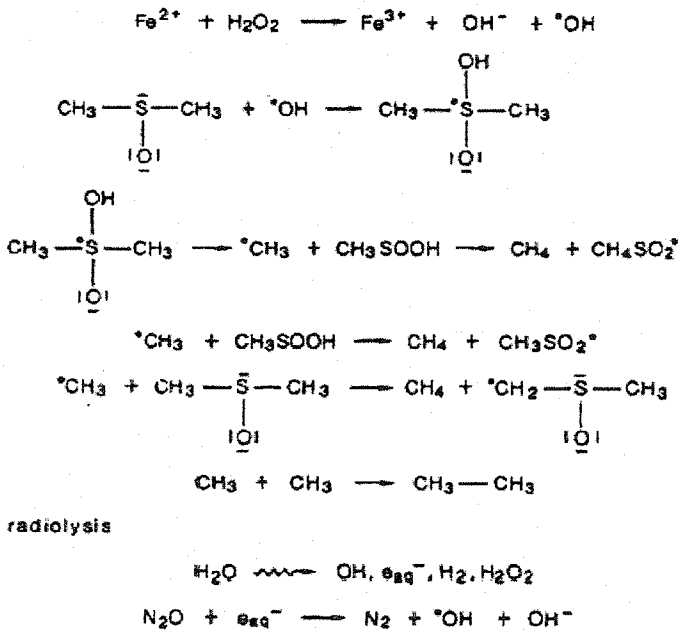
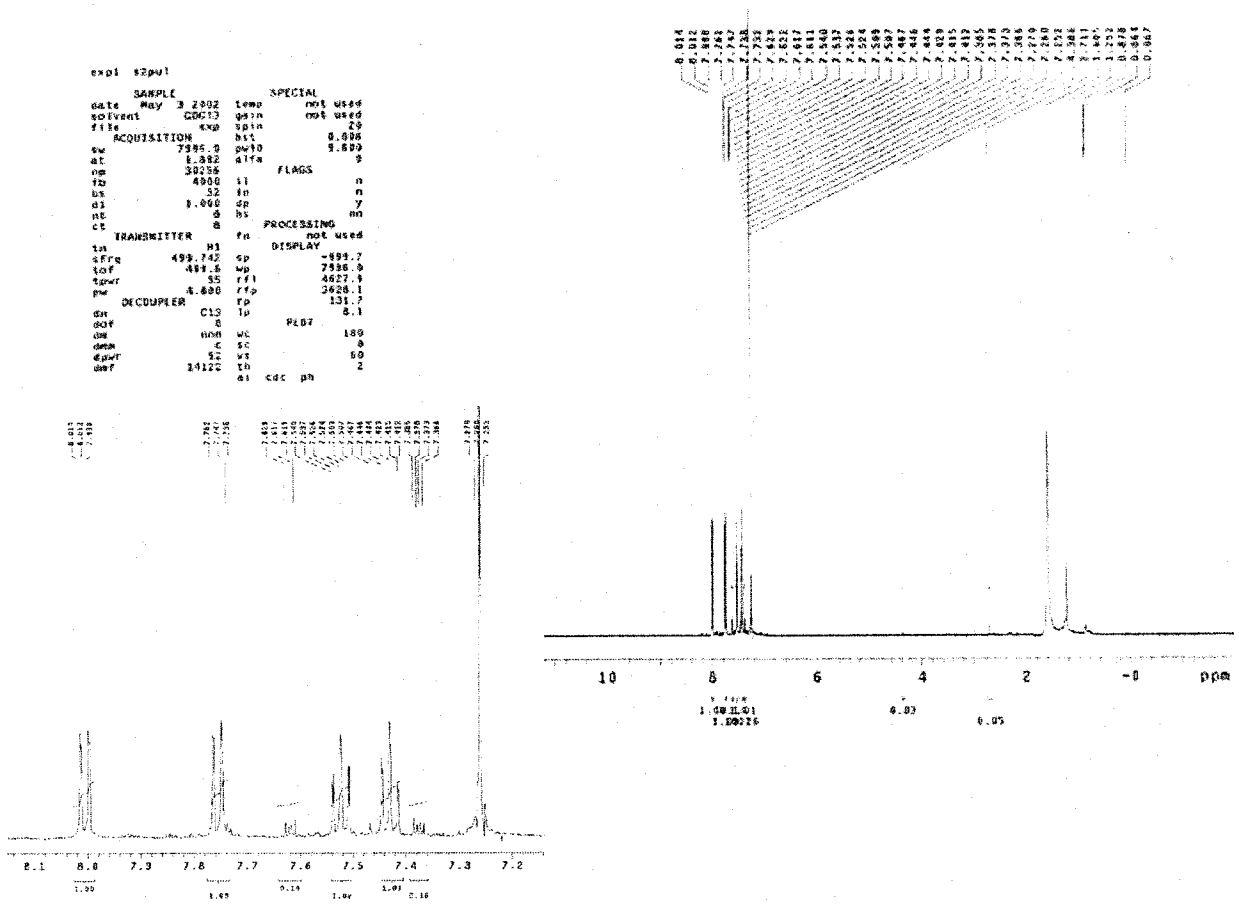


Figure 3.19 ^{1}H NMR spectrum of the reaction mixture of DMSO-d_6 with HO$^{\cdot}$



Scheme 3.7 Quenching mechanism of DMSO to hydroxyl radical (HO$^{\cdot}$)



Photolysis reaction of **II-7** for 3 h:

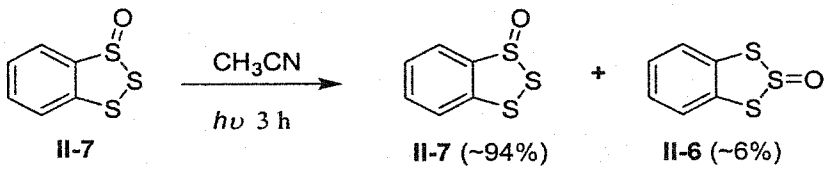
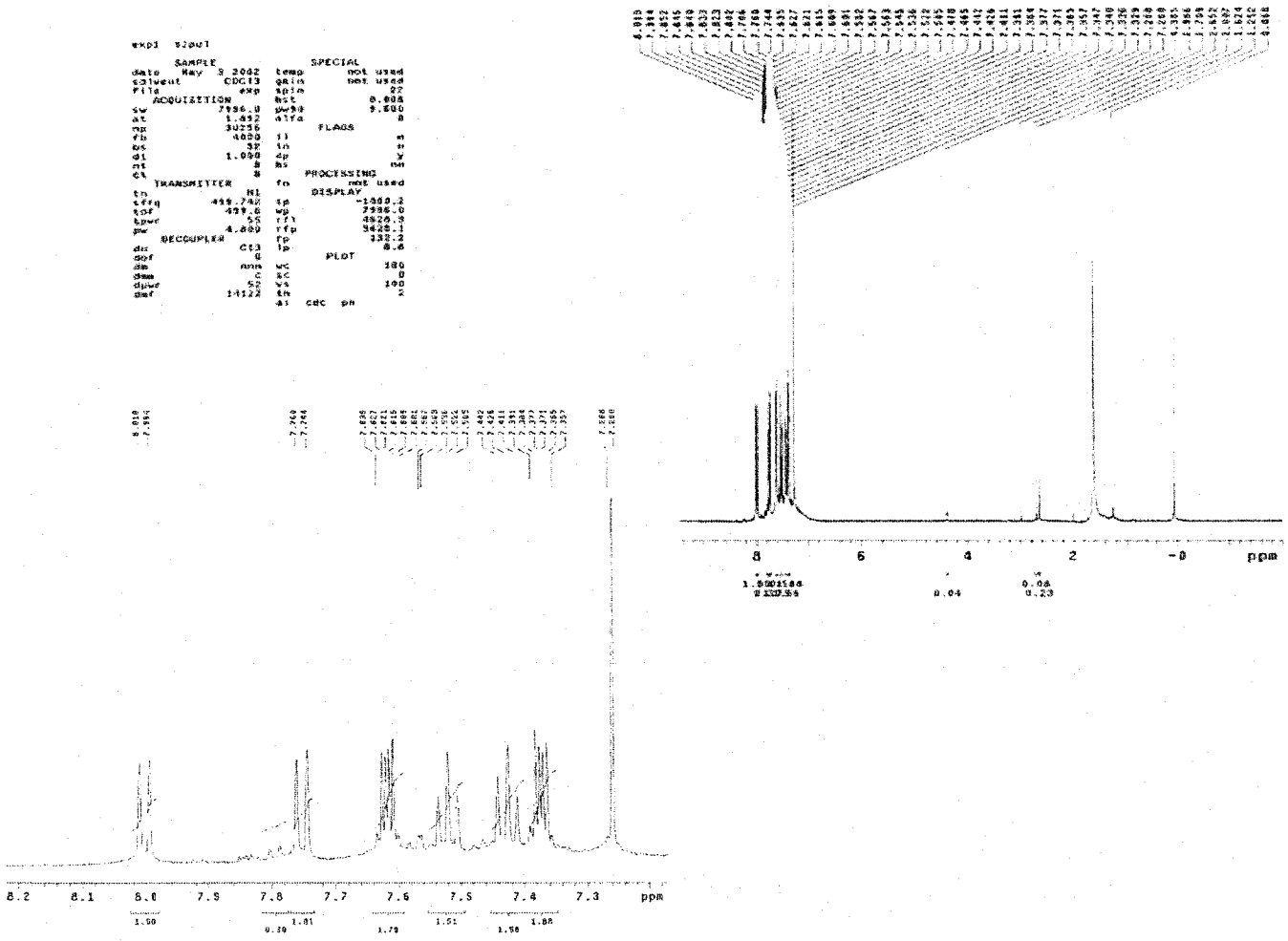


Figure 3.20 ^{1}H NMR spectra of benzothrithiole 1-oxide **II-7** under irradiation for 3 h.



Photolysis reaction of **II-7** for 24h:

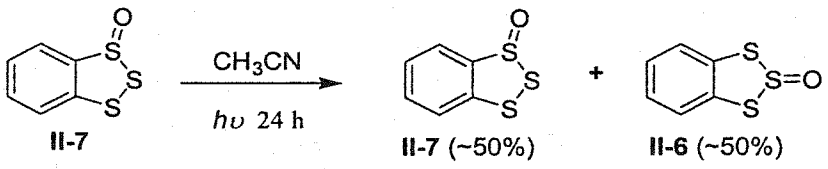


Figure 3.21 ^{1}H NMR spectra of benzothrithiole 1-oxide **II-7** under irradiation for 24 h.

The above Scheme 3.7 shows the quenching mechanism of DMSO to hydroxyl radical which is generated from Fenton reaction of trace transition metal ions with hydrogen peroxide. It is believed that the observation of DMSO quenching to the benzotrithiole oxides photochemical changes might arise from this mechanism.[50]

Although the DNA cleaving assays and molecular studies proved the presence of hydroxyl radical, possibly accompanied with singlet oxygen and superoxide, it is not likely to demonstrate the exact pathway of benzotrithiole oxides photoinduced DNA lesion unambiguously. This is a point of befuddlement in this part of research work; what it might be drawn is to narrow the mechanism to the two plasuible pathways of the photosensitizer reactions. In the attempted experiments of photonucleases, the candidates-benzotrithiole oxides in acetonitrile: sodium phosphate buffer, 1:9 v/v aqueous solution absorbed UVA at 365 nm which then excited to the singlet state π-π^* , the transition of the aromatic ring fused to the trithiole oxide ring. The singlet excited state was rapidly transformed into the strained intermediate, just like those discussed before in Scheme 3.6. One of the quenching effects- Intersystem crossing (ISC) occurred at different rates to convert singlet excited molecules into the different sublevel triplet states. ISC populates preferentially one of the triplet sublevels, which are not degenerate as they become separated in the magnet field. This preferential population gives a spin polarized triplet. Reaction of this polarized triplet with a quencher produces a pair of spin-polarized radicals as the equation and figure shown below, where $^3M^*$ is the triplet

excited molecule, Q is the quencher and R is the radical and cause DNA damage in an oxygen-independent oxidation or energy transfer reaction.[16,18,29-32] Furthermore, it may react with an oxidizable substrate, gaining an electron. Subsequently, the semi-reduced excited molecule reacts with dioxygen to yield O^{2-}, as the following shown.

$$^3M^* + Q \rightarrow R^{1\cdot}(\uparrow) + R^{2\cdot}(\uparrow)$$

$$^3M^* + AH2 \rightarrow M^{\cdot-} + AH^{\cdot} + H^+$$

$$M^{\cdot-} + O_2 \rightarrow M + O_2^{\cdot-}$$

$$^3M^* + O_2 \rightarrow M + {}^1O_2$$

Chart 3.12 Reaction of a polarized triplet sub-level with a quencher leading to spin polarized radicals and a chain of reactions with dioxygen.

Alternatively, the triplet state photosensitizer may be quenched directly by oxygen, yielding either singlet oxygen which secondarily damages the target. For most photosensitizer triplets, even in the absence of a reaction with oxidizable substrate, a small but significant percentage of interactions between the photosensitizer and molecular oxygen resulted in electron transfer, which produces $O_2^{\cdot-}$. Either of the two dioxygen radicals- the singlet oxygen or $O_2^{\cdot-}$ anion, may react with DNA target.

The second plausible pathway for photonucleases differs from the previous proposed one by the species that launch the DNA lesion. This pathway suggests the execution is done by hydroxyl radical. $OH^{\cdot}$ is a very reactive oxidizing species, capable of reacting with most organic compounds, typically by hydrogen abstraction. Superoxide, as previously illustrated, may dismute to form H_2O_2 and ultimately give rise to $OH^{\cdot}$ that reacts with DNA spontaneously; $OH^{\cdot}$ is also likely to react with H_2O_2 then produces the $HO^{2\cdot}/O_2^{\cdot-}$ system with rate constant $k = 1.7 \times 10^7$ $dm^3mol^{-1}s^{-1}$. Those species exert deleterious effects in biological systems.[18,50]

III.3 Conclusion

The synthesis, motivations and experimental studies to demonstrate the benzotrithiole 1-oxide, benzotrithiole 2-oxide, structural analogs and trithiole ring embellished natural products varacins B and C as a family of photonucleases were described. These compounds do not facilitate DNA cleavage alone, but launch the single strand lesions of DNA upon irradiation with UVA at 365 nm. The above studies showed that antibiotics varacins B and C at very low concentrations cleaved DNA much more efficient than that of the simple benzotrithiole oxides. This devastating property is the outcome of the natural products bearing aminoethyl substitutent which exists almost exclusively in the positively charged form under physiological condition.[51] There is a strong affinity among negatively charged phosphate groups in DNA strands and the positively charged amino groups in the natural molecules. Moreover, the photoactivation of the candidates exhibited a lack of

pH dependence. The mechanistic evaluations revealed that the light inducible DNA cleaving agents generated singlet excited state upon illumination, then decayed to triplet state via ISC. During ISC process, energy transfers from this polarized triplet moiety to molecular oxygen (Type II) yielded singlet oxygen to attack the DNA target directly. The formation of singlet oxygen indeed started a cascade of radical propagations. The descendant hydroxyl radical also played a crucial part in DNA cleavage.[16,18,29,32-37] This activation pathway was most likely accountable for the pH independence of our DNA-cleaving processes.

The study of singlet oxygen chemistry antedates the discovery of oxygen radicals and their effects on biological systems. Lethality due to oxygen radicals is an interesting phenomenon, the production of these oxygen radicals including $OH^{\cdot}$ by the photodynamic effect is the fancy topic in molecular clonging.

The reported investigation of photonucleases is intended to provide theoretical and experimental basis for designing potential antitumor drugs, artificial DNA cleaving agent in DNA footprinting, as well as the gene-selective drugs developments.[1-3] The advances of the described photonucleases are their inertness in dark and their usage in a wide range of physiological pH environments, discarding the companion with co-reagents which are hardly compatible with clinical applications, and their DNA cleaving activity are resurged simply upon UVA irradiation.[2-5] Those factors contribute to the great potential of the chemotherapeutic

drugs which deliver *in vivo* easily, stably and with controllable cytotoxicity in term of photoactivation.[1,2] The major cellular target attacked by these compounds is likely to be DNA.

III.4. Experimental

III.4.1 Photolysis Experiments

Photolysis of Benzotrithiole 1-Oxide II-7, 2-Oxide II-6 with DMSO-d_6. Benzotrithiole 1- or 2- oxides (70 mg, 0.37 mmol) were dissolved in 5 mL acetonitrile separately, and DMSO-d_6 (50 mg, 0.64 mmol) was added into the solutions. The reaction mixtures were illuminated with ultraviolet at 365 nm [Spectroline Model ENF260CF in CM10 cabinet 6W unit as the UV radiation source. The typical peak intensity of this wavelength is 350μW/cm^2 at 150 mm], or with mercury arc lamp, and the progresses were monitored with TLC (Hexane: CH_2Cl_2, 1:2). Upon recorded period of irradiation, the solvent was rotary evaporated and the residues were vacuum dried. Without further purification, the reaction products were analysed with ^{1}H NMR.

Photolysis of Benzotrithiole 1- Oxide II-7, 2-Oxide II-6 with quenchers (THF-d_8, methanol, mannitol, benzophenone). *The reaction procedure was followed the above steps.*

Photochemical production of hydroxyl radical OH$^{\cdot}$ and quenching with DMSO-d_6. DMSO-d_6 (20 μL) was dissolved in 35% wt hydrogen peroxide in water (200 μL) and irradiated at 254 nm (UVC) [Spectroline Model ENF260CF in CM10 cabinet 6W unit as the UVC radiation source. The typical peak intensity of this wavelength is 390μW/cm^2 at 150 mm.] for 2 days. The reaction mixture was analysed by ^{1}H NMR and shown the similar result of Figure 3.19, MSA and $DMSO_2$ were afforded.

Materials. 50 μM/bp of supercoiled ΦX174 RF DNA was purchased from GIBCO BRL. *See Experimental Sections of Parts I and II.*

III.4.2 DNA Cleavage Assays. The varacinium A 2-oxide **II-5**, B **II-3**, C **II-4** trifluoroacetates, the benzotrithiole oxides were used as freshly prepared solutions of acetonitrile and the final reaction mixtures in microtubes contained 10% v/v of acetonitrile in 50 mM sodium phosphate buffer. Given concentrations of DNA-cleaving agents in acetonitrile (1 or 2 μL) were added to solutions containing 50 mM sodium phosphate with given pH values, 38 μM/bp of supercoiled plasmid pBR322 DNA or 50 μM/bp of supercoiled ΦX174 RF DNA, deionized water. Followed immediately by given concentrations of addictives to make up a total volume equal to 10 μL in a microtube. The mixtures were agitated on a vortex mixer, spun for 3 s in a tabletop microcentrifuge. Upon ultraviolet irradiated at 365 nm [Spectroline Model ENF260CF in CM10 cabinet 6W unit as the UVA radiation source. The typical peak intensity of this wavelength is 350μW/cm^2 at 150 mm.] 10 cm distance from the light source at 25 °C for 1 h and incubated at 37 °C another hour, the reactions were quenched with 3 μL of loading buffer containing 0.25% bromophenol blue, 0.25% xylene cyanol FF and 40% (w/v) sucrose in water.[52] The following steps were referred to previous procedure of DNA-cleavage assays in Parts I and II.

Preparation of the buffers. *See Experimental Sections of Parts I and II.*

III.5 References

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APPENDIX

^{1}H and ^{13}C NMR Spectra

List of ^{1}H and ^{13}C NMR Spectra of PART I

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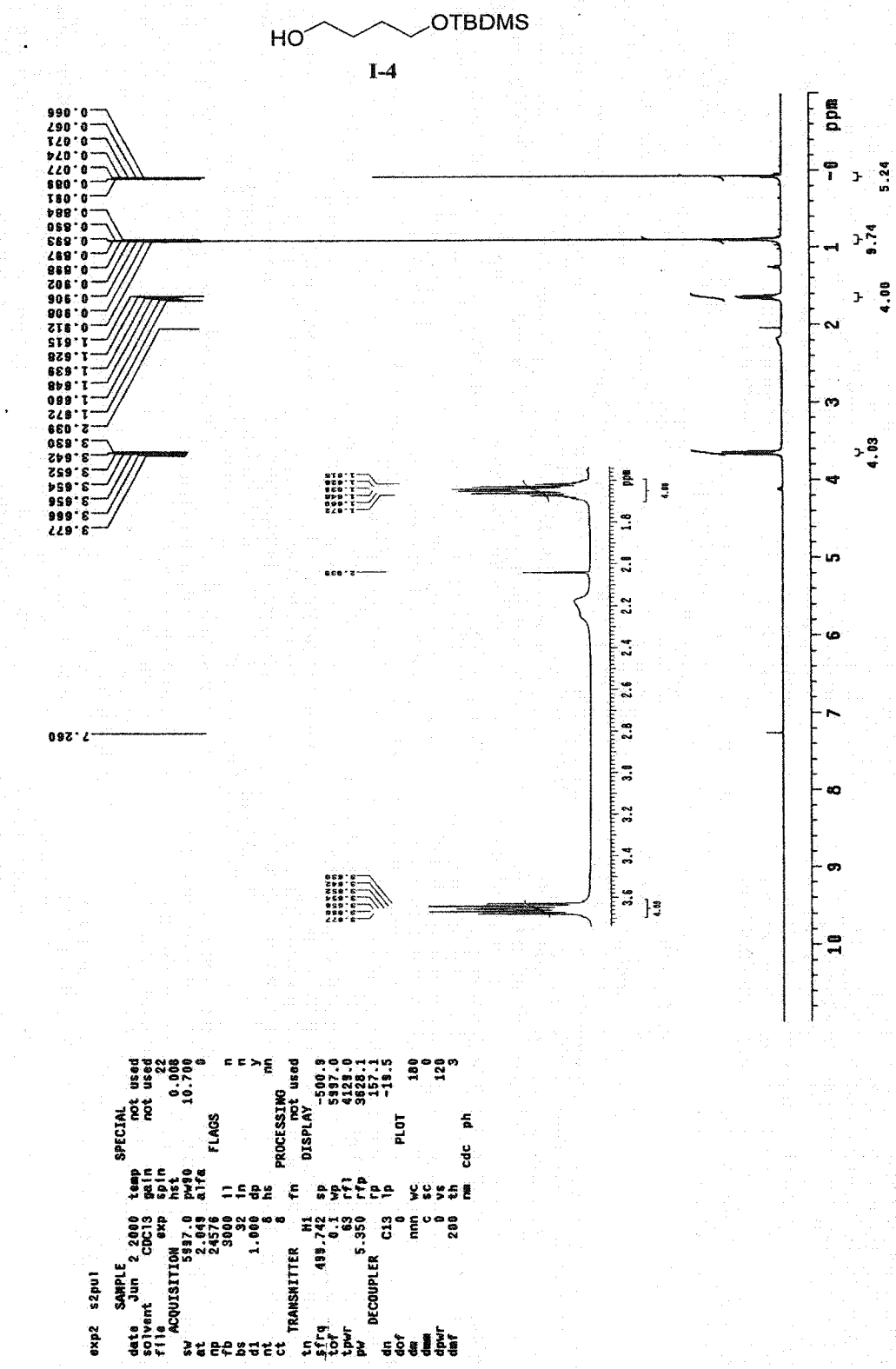
Hexanedioic acid 2,5-dioxo-pyrrolidin-1-yl ester 7-hept-3-enyl-[5-(1,2-dithiolan-3-one 1-oxo)] ester (I-34)..... 355

Hexanedioic acid 2,5-dioxo-pyrrolidin-1-yl ester 7-heptyl-[5-(1,2-dithiolan-3-one)] ester (I-33)..... 359

Hexanedioic acid 2,5-dioxo-pyrrolidin-1-yl ester 7-heptyl-[5-(1,2-dithiolan-3-one 1-oxo)] ester (I-35)..... 363



I-4

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PROCNO 1
F2 - Acquisition Parameters
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TD 65536
SOLVENT CDCl3
NS 64
DS 0
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FIDRES 0.312041 Hz
AQ 1.6024052 sec
RG 6502
DW 24.450 usec
DE 4.50 usec
TE 300.0 K
D1 1.00000000 sec
d11 0.03000000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 5.80 usec
PL1 -6.00 dB
SF01 100.6227710 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 71.00 usec
PL2 120.00 dB
PL12 17.00 dB
SF02 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127715 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 200.000 ppm
F1 20122.55 Hz
F2P -10.000 ppm
F2 -1006.13 Hz
PPMCM 10.50000 ppm/cm
HZCM 1056.43408 Hz/cm
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30.2426
62.7609
63.3409
76.6831
77.0005
77.3179
ppm
0
25
50
75
100
125
150
175

exp2 s2pul

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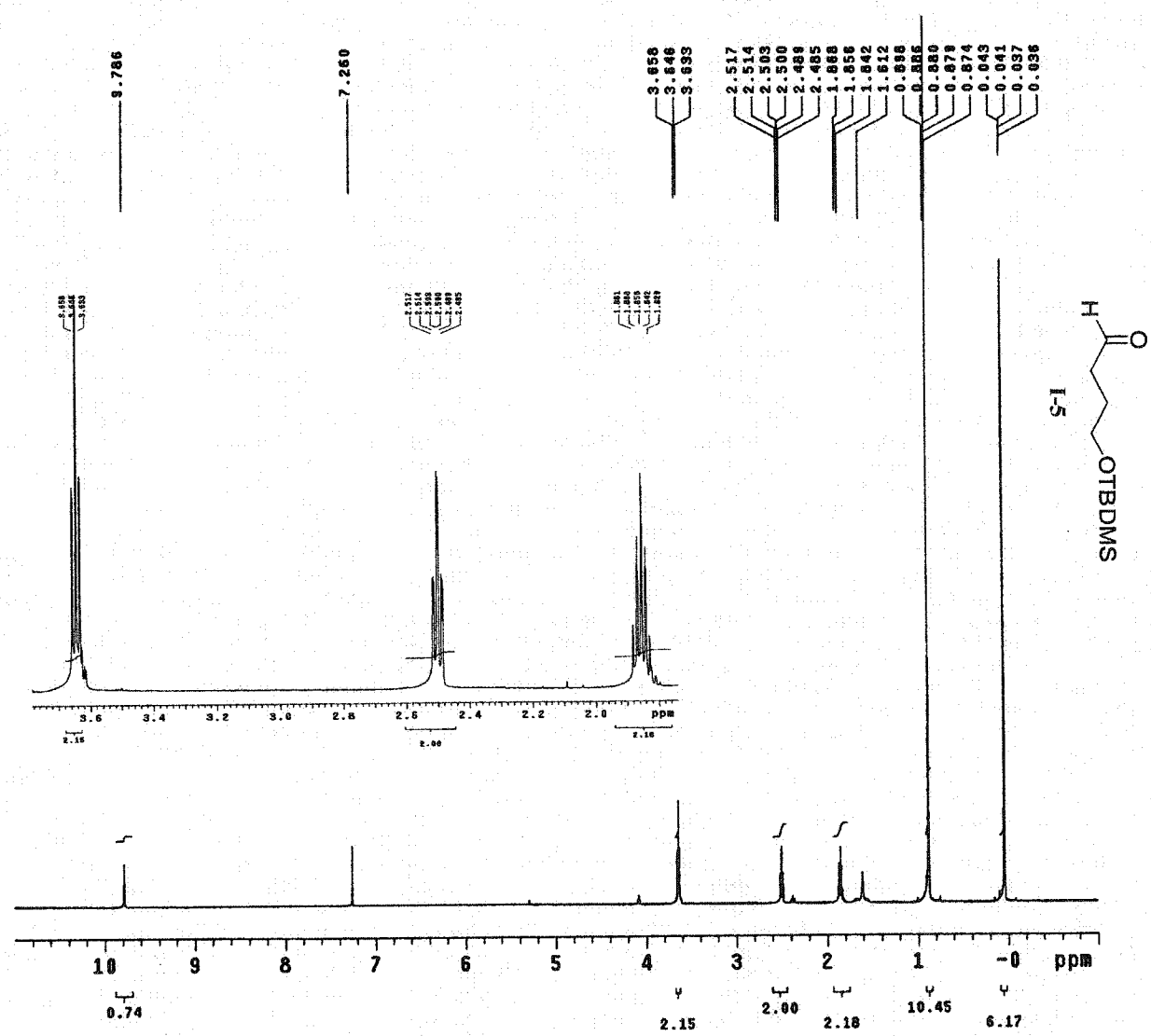
at	2.049	alfa	0
np	24576	FLAGS	
fb	3000	il	n
bs	32	in	n
d1	1.000	dp	y
nt	8	hs	nn
ct	8	PROCESSING	
TRANSMITTER		fn	not used
tn	H1	DISPLAY	
sfrq	499.742	sp	-500.9
tof	0.1	wp	5997.0
tpwr	57	rfl	4129.0
pw	4.900	rfp	3628.1
DECOUPLER		rp	-5.7
dn	C13	lp	-2.1
dof	0	PLOT	
dm	nnn	wc	180
dmm	c	sc	0
dpwr	51	vs	144
dmf	17094	th	4
		nm	cdc ph

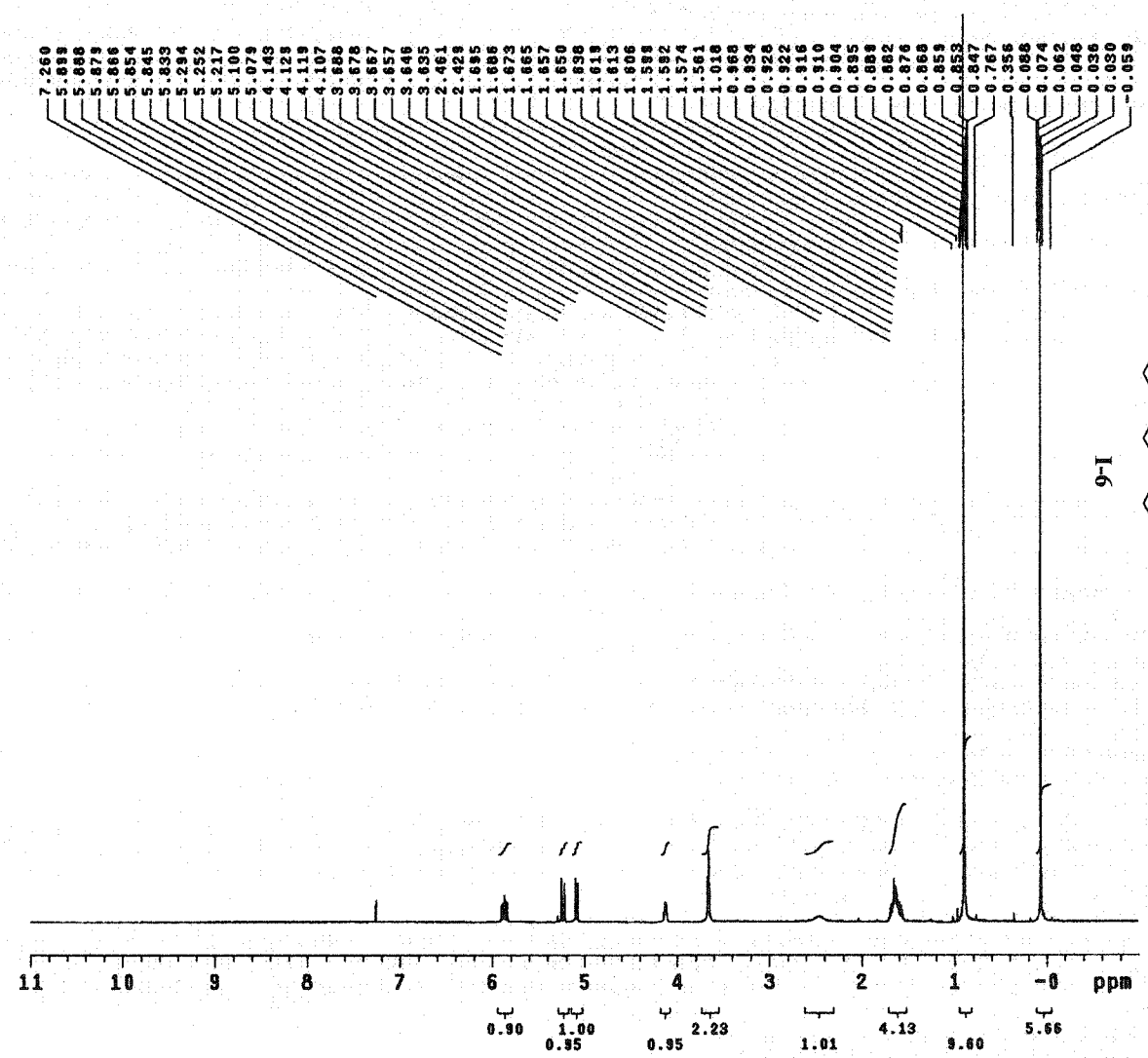


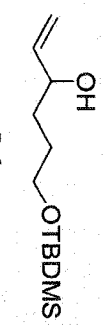
I-5

Current Data Parameters
NAME 072c13
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20000605
Time 18.49
INSTRUM dpx400
PROBHD 5 mm QNP 1H
PULPROG zgdc30
TD 65536
SOLVENT CDCl3
NS 64
DS 0
SWH 25125.629 Hz
FIDRES 0.383387 Hz
AQ 1.3042164 sec
RG 6502
DW 19.900 usec
DE 4.50 usec
TE 300.0 K
D1 1.00000000 sec
d11 0.03000000 sec
========== CHANNEL f1 ==========
NUC1 13C
P1 5.80 usec
PL1 -6.00 dB
SFO1 100.6227710 MHz
========== CHANNEL f2 ==========
CPDPRG2 waltz16
NUC2 1H
PCPD2 71.00 usec
PL2 120.00 dB
PL12 17.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127749 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
EX 20.00 cm
F1P 220.000 ppm
F1 22134.81 Hz
F2P -10.000 ppm
F2 -1006.13 Hz
PPMCM 11.50000 ppm/cm
HZCM 1157.04688 Hz/cm
ppm

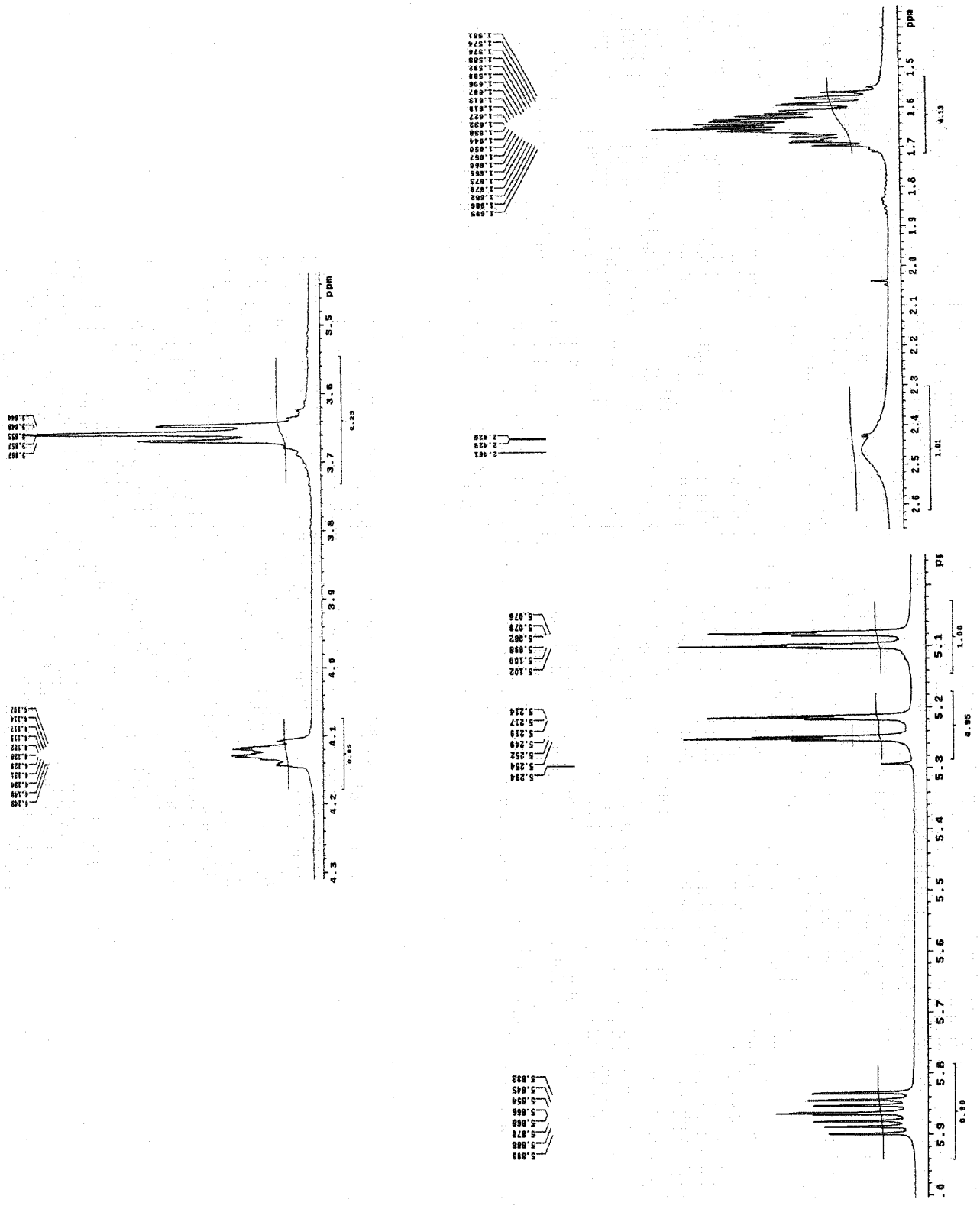
exp2 s2pul

SAMPLE		SPECIAL	
date	Jun 8 2000	temp	not used
solvent	CDCl3	gain	not used
file	exp	spin	22
ACQUISITION		hst	0.008
sw	5997.0	pw90	9.800
at	2.049	alfa	0
np	24576	FLAGS	
fb	3000	il	n
bs	32	in	n
d1	1.000	dp	y
nt	8	hs	nn
ct	8	PROCESSING	
TRANSMITTER		fn	not used
tn	H1	DISPLAY	
sfrq	499.742	sp	-499.5
tof	0.1	wp	5997.0
tpwr	57	rfl	4127.6
pw	4.900	rfp	3628.1
DECOUPLER		rp	-16.3
dn	C13	lp	0.8
dof	0	PLOT	
dm	nnn	wc	180
dmm	c	sc	0
dpwr	51	vs	155
dmf	17094	th	1
		nm	cdc ph

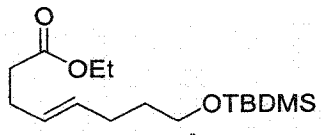




I-6



Current Data Parameters
NAME 04L-C13
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20000608
Time 22.31
INSTRUM dpx400
PROBHD 5 mm QNP 1H
PULPROG zgdc30
TD 65536
SOLVENT CDCl3
NS 240
DS 0
SWH 25125.629 Hz
FIDRES 0.383387 Hz
AQ 1.3042164 sec
RG 6502
DW 19.900 usec
DE 4.50 usec
TE 300.0 K
D1 1.00000000 sec
d11 0.03000000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 5.80 usec
PL1 -6.00 dB
SF01 100.6227710 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 71.00 usec
PL2 120.00 dB
PL12 17.00 dB
SF02 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127718 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 220.000 ppm
F1 22134.81 Hz
F2P -10.000 ppm
F2 -1006.13 Hz
PPMCM 11.50000 ppm/cm
HZCM 1157.04688 Hz/cm
ppm
0
25
50
75
100
125
150
175
200


O
OEt
OTBDMS

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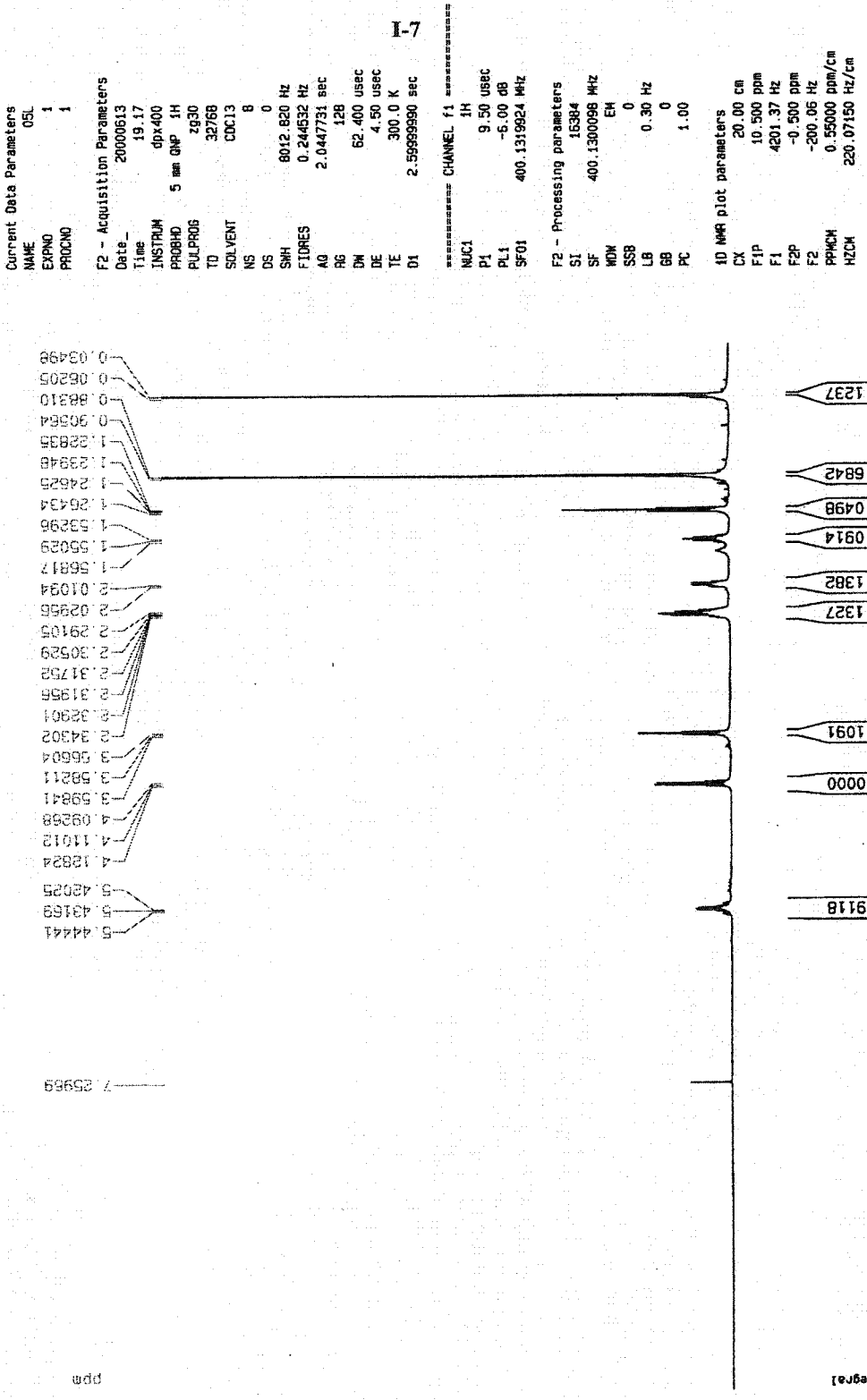
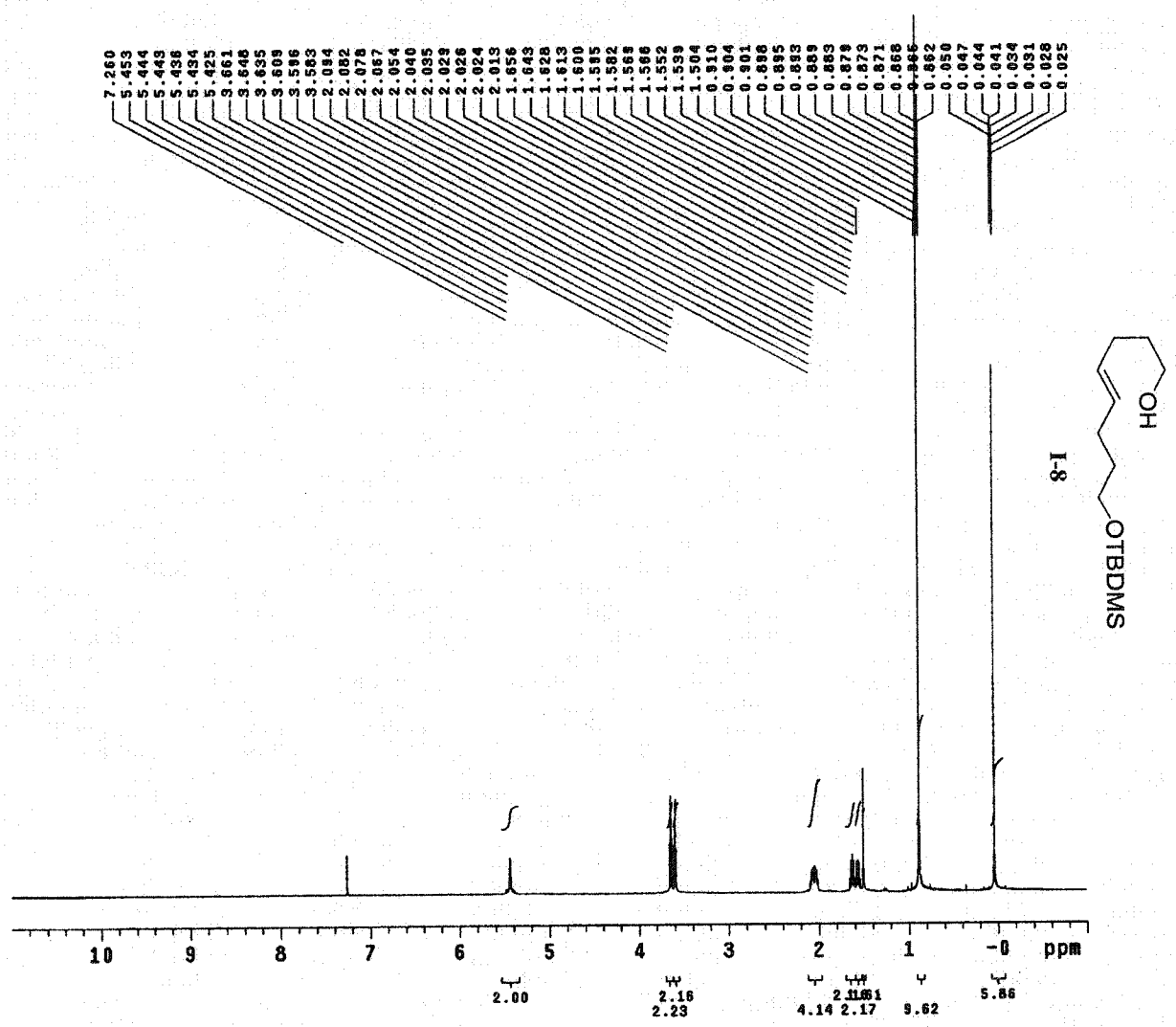
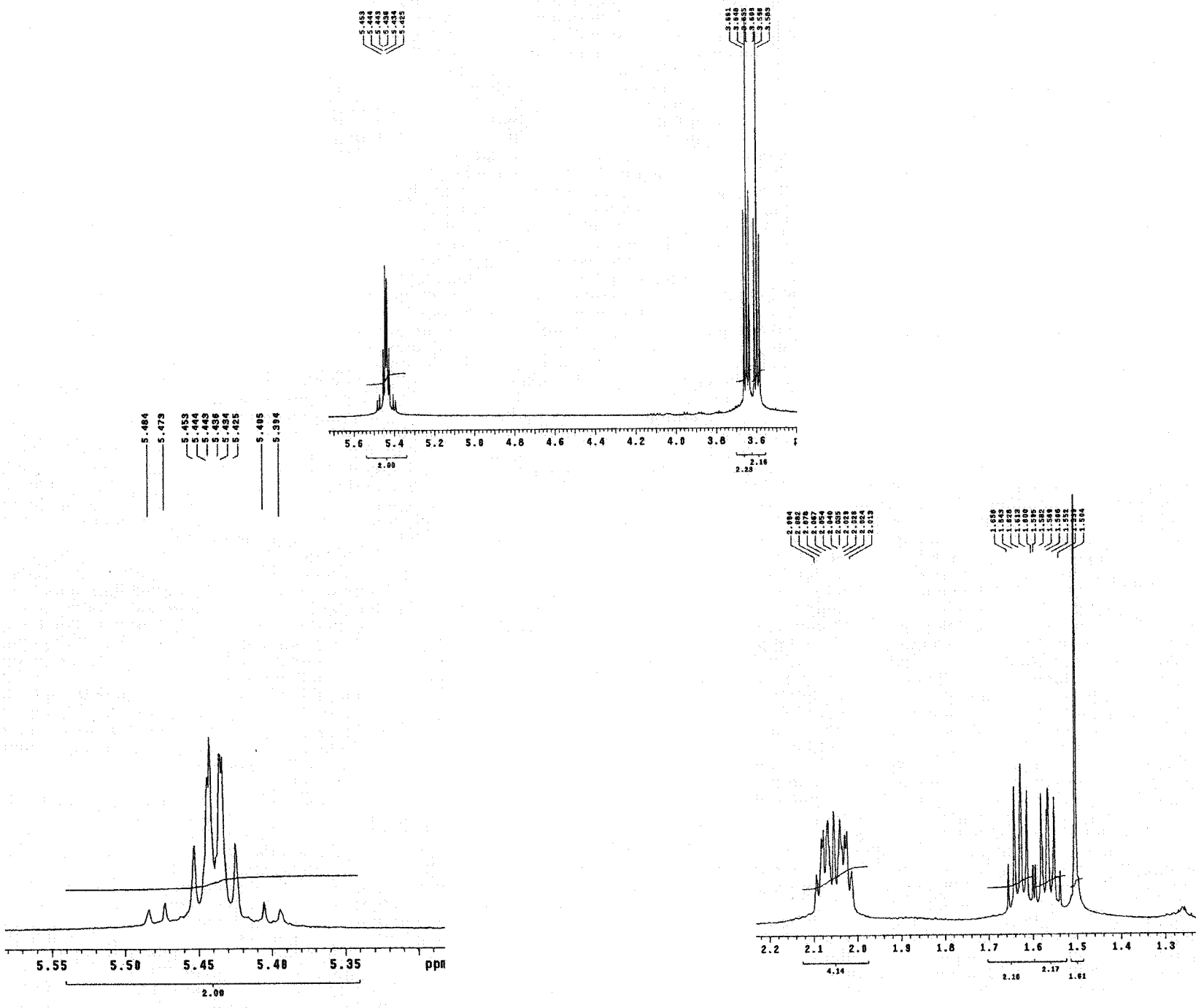

Current Data Parameters
NAME 05L
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20000613
Time 19.17
INSTRUM dpx400
PROBHD 5 mm GNP 1H
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 8
DS 0
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 2.0447731 sec
RG 128
DW 62.400 usec
DE 4.50 usec
TE 300.0 K
D1 2.59999990 sec
======== CHANNEL f1 ========
NUC1 1H
P1 9.50 usec
PL1 -6.00 dB
SFO1 400.1319924 MHz
F2 - Processing parameters
SI 16384
SF 400.1300098 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
1D NMR plot parameters
CX 20.00 cm
F1P 10.500 ppm
F1 4201.37 Hz
F2P -0.500 ppm
F2 -200.06 Hz
PPMCM 0.55000 ppm/cm
HZCM 220.07150 Hz/cm
6.1237
9.6842
3.0498
2.0914
2.1382
4.1327
2.1091
2.0000
1.9118
Integral
ppm

Current Data Parameters
NAME 05L-C13
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20000612
Time 22.43
INSTRUM dpx400
PROBHD 5 mm QNP 1H
PULPROG zgdc30
TD 65536
SOLVENT CDCl3
NS 240
DS 0
SWH 25125.629 Hz
FIDRES 0.383387 Hz
AQ 1.3042164 sec
RG 6502
DW 19.900 usec
DE 4.50 usec
TE 300.0 K
D1 1.00000000 sec
d11 0.03000000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 5.80 usec
PL1 -6.00 dB
SFO1 100.6227710 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 71.00 usec
PL2 120.00 dB
PL12 17.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127718 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 220.000 ppm
F1 22134.81 Hz
F2P -10.000 ppm
F2 -1006.13 Hz
PPMCM 11.50000 ppm/cm
HZCM 1157.04688 Hz/cm
ppm



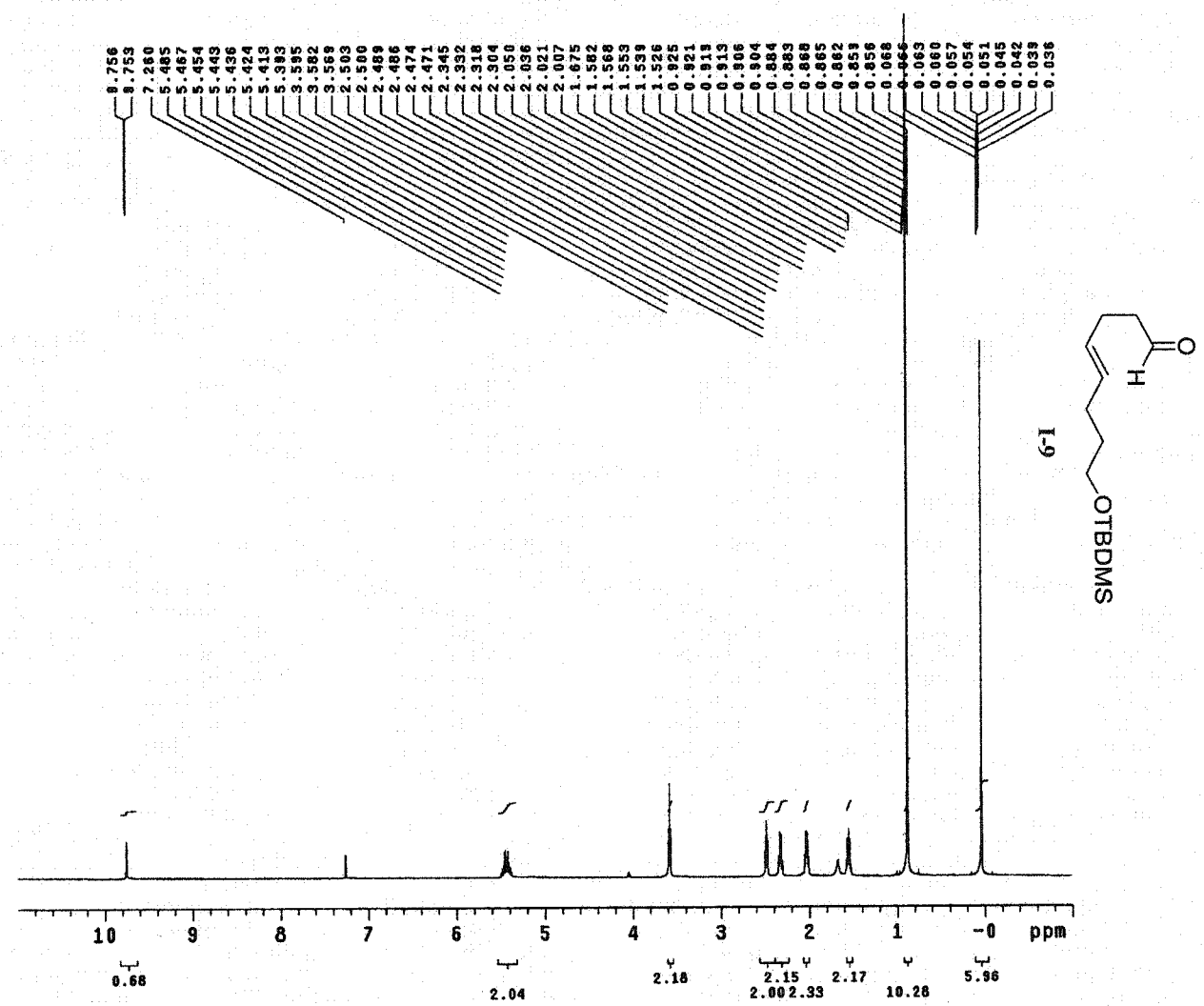

```
exp1  s2pul

      SAMPLE                SPECIAL
date   Mar 23 2001   temp       not used
solvent       CDCl3   gain       not used
file            exp   spin             22
   ACQUISITION        hst           0.008
sw           5997.0   pw90          9.800
at            2.049   alfa              0
np            24576         FLAGS
fb             3000   il                n
bs               32   in                n
d1            1.000   dp                y
nt                8   hs               nn
ct                8       PROCESSING
   TRANSMITTER        fn         not used
tn               H1        DISPLAY
sfrq        499.742   sp           -500.6
tof             0.1   wp           5997.0
tpwr             57   rfl          4128.7
pw            4.900   rfp          3628.1
    DECOUPLER         rp             19.5
dn              C13   lp             11.0
dof               0         PLOT
dm              nnn   wc              180
dmm               c   sc                0
dpwr             51   vs              140
dmf           17094   th                2
                      nm  cdc  ph
```


5.484
5.479
5.453
5.444
5.443
5.436
5.434
5.425
5.405
5.394
5.55
5.50
5.45
5.40
5.35
ppm
2.00
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
2.00
2.16
2.23
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
4.14
2.16
2.17
1.01

Current Data Parameters
NAME 06L-C13
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20000615
Time 17.33
INSTRUM dpx400
PROBHD 5 mm QNP 1H
PULPROG zgdc30
TD 65536
SOLVENT CDCl3
NS 520
DS 0
SWH 25125.629 Hz
FIDRES 0.383387 Hz
AQ 1.3042164 sec
RG 6502
DW 19.900 usec
DE 4.50 usec
TE 300.0 K
D1 1.00000000 sec
d11 0.03000000 sec
========== CHANNEL f1 ==========
NUC1 13C
P1 5.80 usec
PL1 -6.00 dB
SFO1 100.6227710 MHz
========== CHANNEL f2 ==========
CPDPRG2 waltz16
NUC2 1H
PCPD2 71.00 usec
PL2 120.00 dB
PL12 17.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127718 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 200.000 ppm
F1 20122.55 Hz
F2P -10.000 ppm
F2 -1006.13 Hz
PPMCM 10.50000 ppm/cm
HZCM 1056.43408 Hz/cm
ppm
0
25
50
75
100
125
150
175

exp2 s2pul

SAMPLE		SPECIAL	
date	Jun 20 2000	temp	not used
solvent	CDCl3	gain	not used
file	exp	spin	22
ACQUISITION		hst	0.008
sw	5997.0	pw90	10.700
at	2.049	alfa	0
np	24576	FLAGS	
fb	3000	il	n
bs	32	in	n
d1	1.000	dp	y
nt	8	hs	nn
ct	8	PROCESSING	
TRANSMITTER		fn	not used
tn	H1	DISPLAY	
sfrq	499.742	sp	-500.9
tof	0.1	wp	5997.0
tpwr	63	rfl	4129.0
pw	5.350	rfp	3628.1
DECOUPLER		rp	183.3
dn	C13	lp	-13.1
dof	0	PLOT	
dm	nnn	wc	180
dmm	c	sc	0
dpwr	0	vs	144
dmf	200	th	1
		nm	cdc ph



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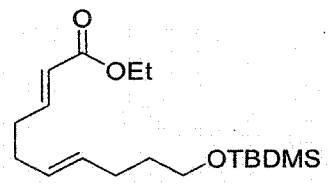
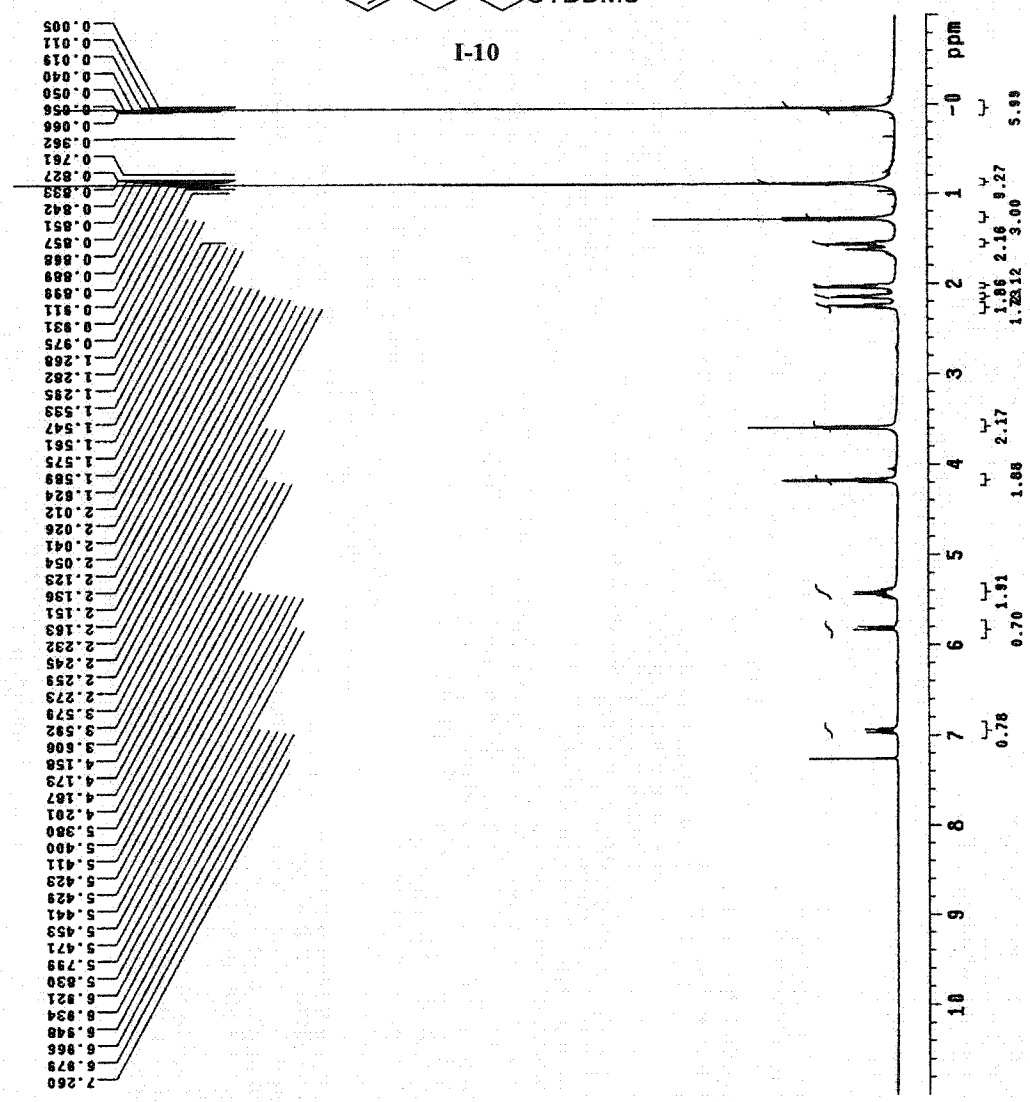
Current Data Parameters
NAME 07L-C13
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20000620
Time 20.15
INSTRUM dpx400
PROBHD 5 mm QNP 1H
PULPROG zgdc30
TD 65536
SOLVENT CDCl3
NS 520
DS 0
SWH 25125.629 Hz
FIDRES 0.383387 Hz
AQ 1.3042164 sec
RG 6502
DW 19.900 usec
DE 4.50 usec
TE 300.0 K
D1 1.00000000 sec
d11 0.03000000 sec
========== CHANNEL f1 ==========
NUC1 13C
P1 5.80 usec
PL1 -6.00 dB
SFO1 100.6227710 MHz
========== CHANNEL f2 ==========
CPDPRG2 waltz16
NUC2 1H
PCPD2 71.00 usec
PL2 120.00 dB
PL12 17.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127711 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 220.000 ppm
F1 22134.81 Hz
F2P -10.000 ppm
F2 -1006.13 Hz
PPMCM 11.50000 ppm/cm
HZCM 1157.04688 Hz/cm
-6.301
19.332
26.146
26.939
28.718
32.438
43.419
43.489
62.483
76.611
76.882
76.929
77.000
77.247
77.318
127.998
131.437
202.368
ppm
0
25
50
75
100
125
150
175
200
ppm

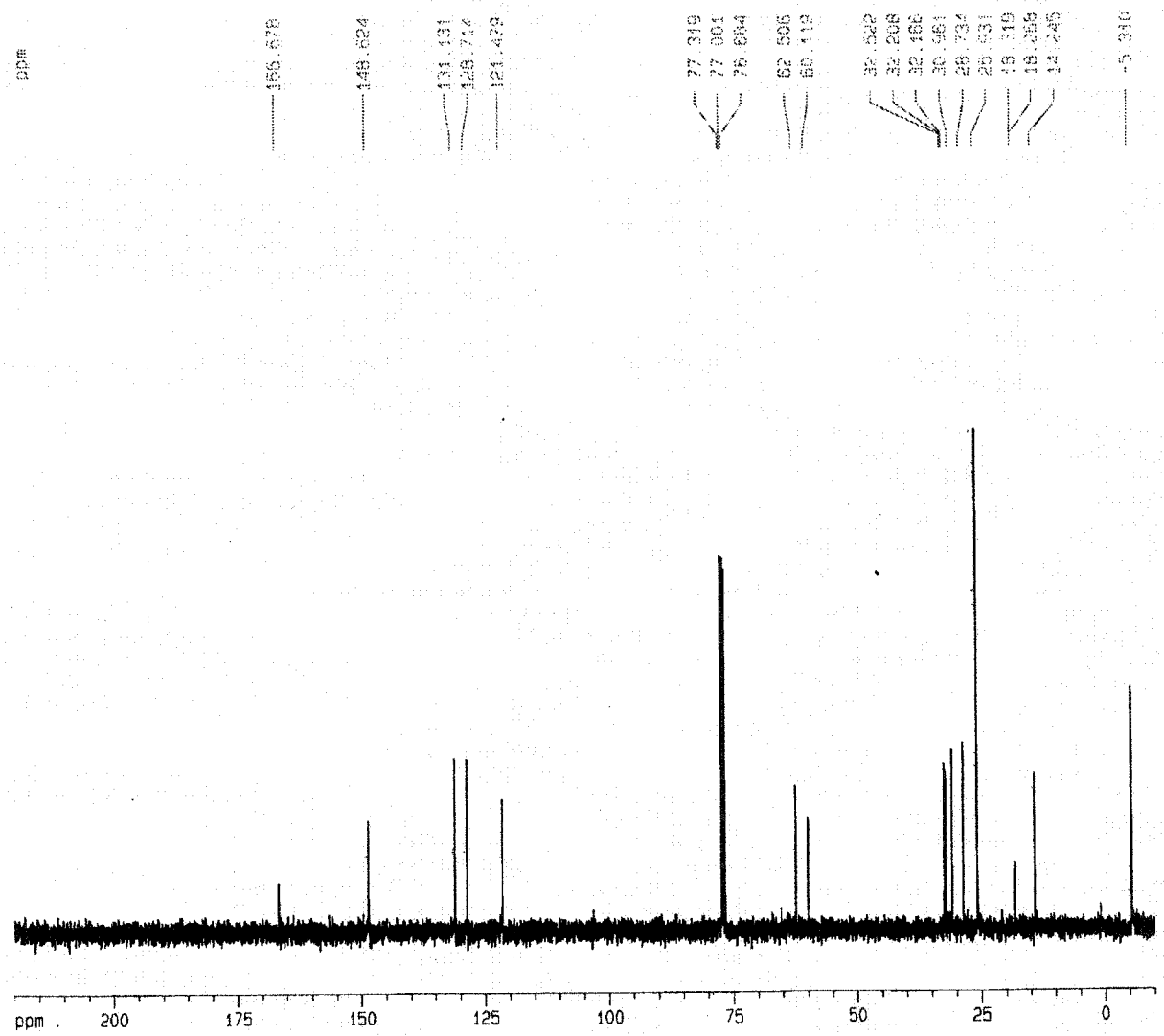


I-10




```
exp2  s2pul

        SAMPLE                          SPECIAL
date   Jun 22 2000   temp        not used
solvent      CDCl3   gain        not used
file           exp   spin              22
     ACQUISITION     hst           0.008
sw          5997.0   pw90         10.700
at          2.049   alfa               0
np          24576                FLAGS
fb           3000   il                 n
bs             32   in                 n
d1          1.000   dp                 y
nt              8   hs                nn
ct              8   PROCESSING
     TRANSMITTER    fn        not used
tn             H1   DISPLAY
sfrq       499.742   sp          -501.3
tof            0.1   wp          5997.0
tpwr            63   rfl         4129.4
pw          5.350   rfp         3628.1
     DECOUPLER       rp          185.7
dn             C13   lp           -1.6
dof              0   PLOT
dm             nnn   wc            180
dmm              c   sc              0
dpwr             0   vs            220
dmf            200   th              2
                     nm   cdc   ph
```



Current Data Parameters

NAME	06L-C13
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20000622
Time	21.46
INSTRUM	dpx400
PROBHD	5 mm QNP 1H
PULPROG	zgdc30
TD	65536
SOLVENT	CDCl3
NS	271
DS	0
SWH	25125.629 Hz
FIDRES	0.383387 Hz
AQ	1.3042164 sec
RG	6502
DW	19.900 usec
DE	4.50 usec
TE	300.0 K
D1	1.00000000 sec
d11	0.03000000 sec

========== CHANNEL f1 ==========

NUC1	13C
P1	5.80 usec
PL1	-6.00 dB
SFO1	100.6227710 MHz

========== CHANNEL f2 ==========

CPDPRG2	waltz16
NUC2	1H
PCPD2	71.00 usec
PL2	120.00 dB
PL12	17.00 dB
SFO2	400.1316005 MHz

F2 - Processing parameters

SI	32768
SF	100.6127718 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

1D NMR plot parameters

CX	20.00 cm
F1P	220.000 ppm
F1	22134.81 Hz
F2P	-10.000 ppm
F2	-1006.13 Hz
PPMCM	11.50000 ppm/cm
HZCM	1157.04688 Hz/cm

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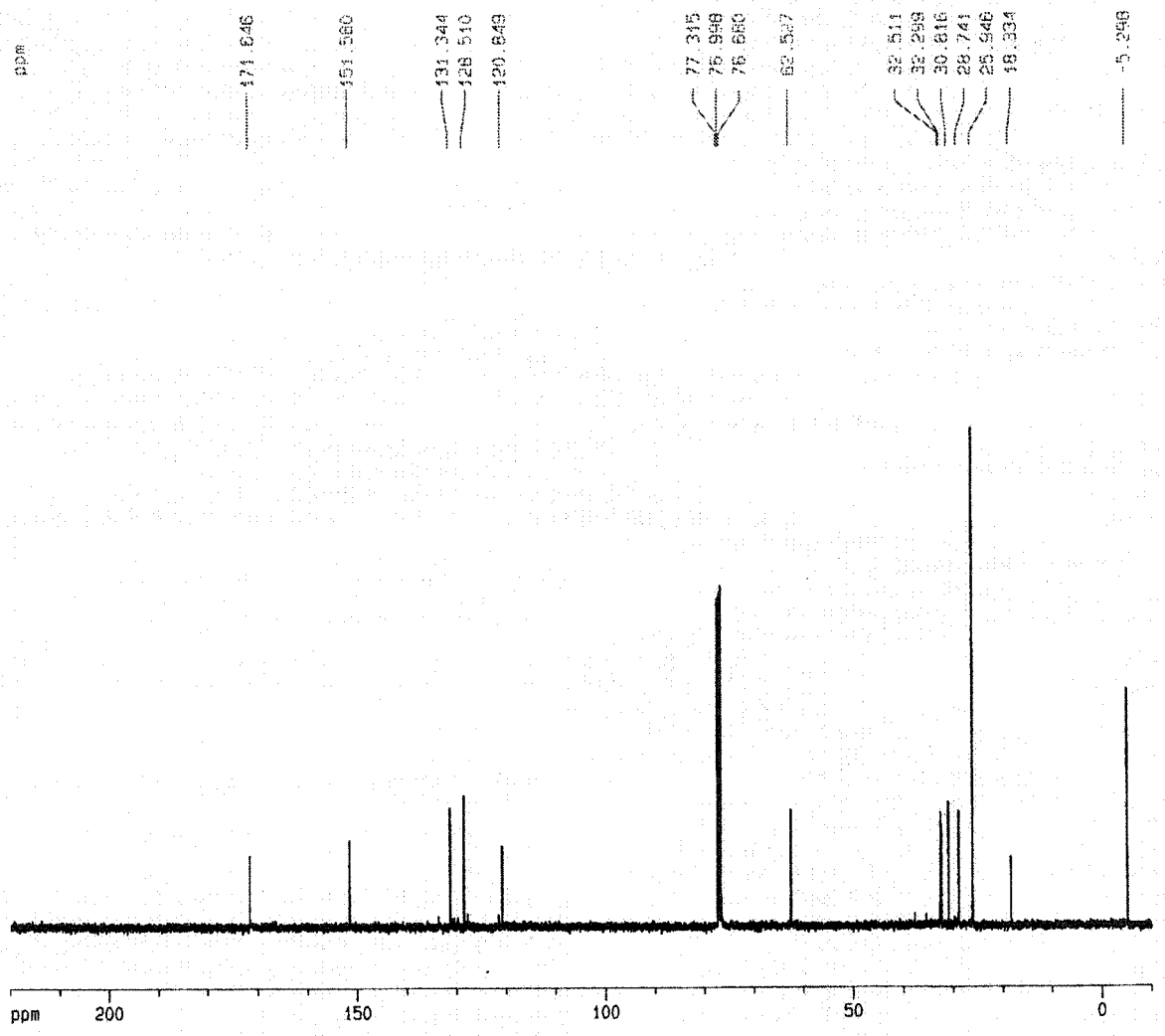


Current Data Parameters
NAME 07L-C13
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20000710
Time 17.57
INSTRUM dpx400
PROBHD 5 mm QNP 1H
PULPROG zgdc30
TD 65536
SOLVENT CDCl3
NS 1200
DS 0
SWH 25125.629 Hz
FIDRES 0.383387 Hz
AQ 1.3042164 sec
RG 6502
DW 19.900 usec
DE 4.50 usec
TE 300.0 K
D1 1.00000000 sec
d11 0.03000000 sec

========== CHANNEL f1 ==========
NUC1 13C
P1 5.80 usec
PL1 -6.00 dB
SF01 100.6227710 MHz

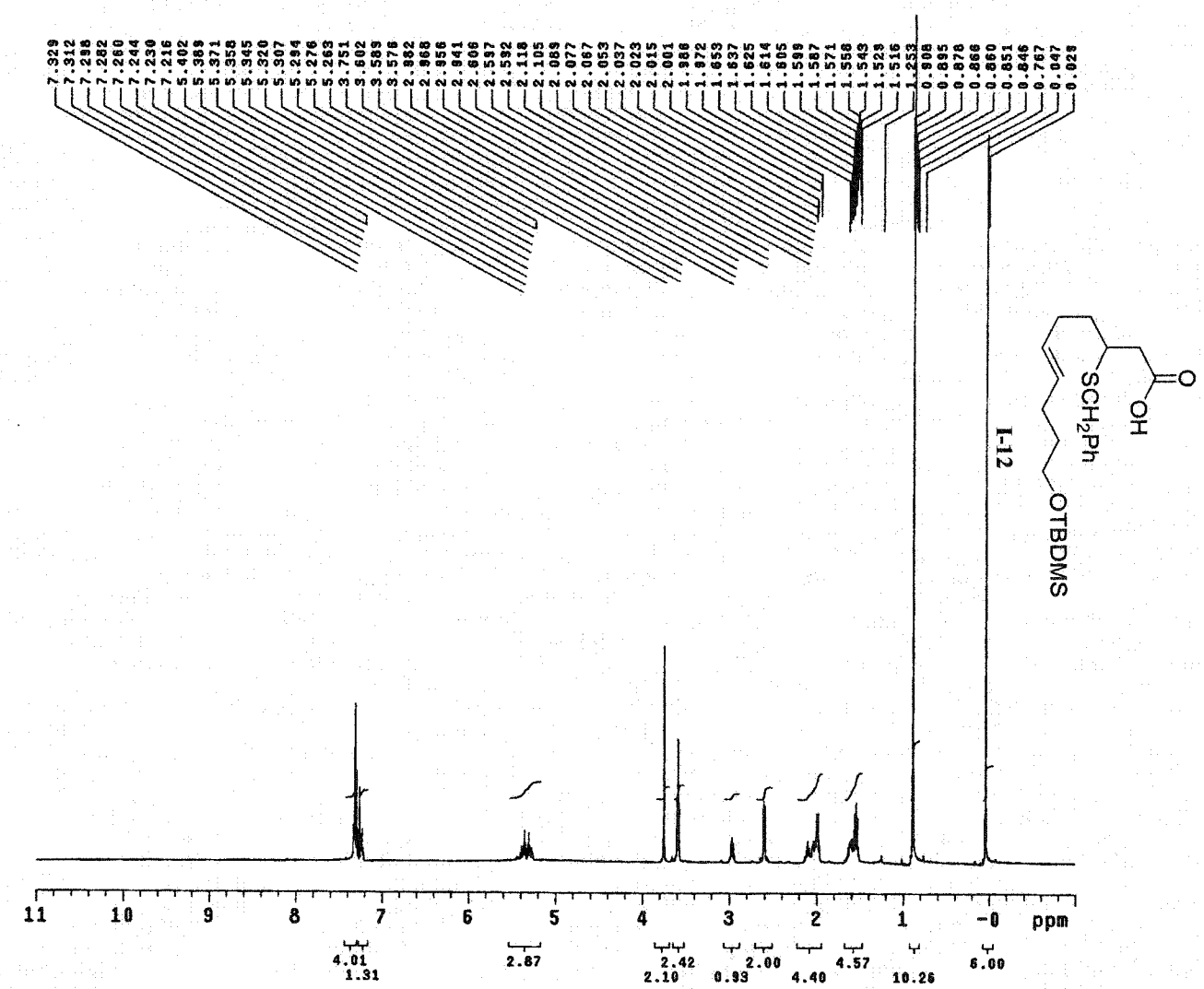
========== CHANNEL f2 ==========
CPDPRG2 waltz16
NUC2 1H
PCPD2 71.00 usec
PL2 120.00 dB
PL12 17.00 dB
SF02 400.1316005 MHz

F2 - Processing parameters
SI 32768
SF 100.6127711 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

1D NMR plot parameters
CX 20.00 cm
F1P 220.000 ppm
F1 22134.81 Hz
F2P -10.000 ppm
F2 -1006.13 Hz
PPMCM 11.50000 ppm/cm
HZCM 1157.04688 Hz/cm

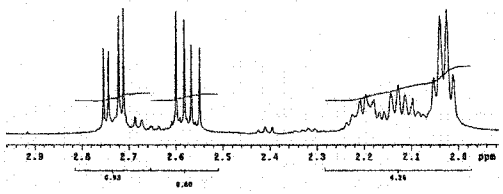
exp2 s2pul

SAMPLE		SPECIAL	
date	Jul 15 2000	temp	not used
solvent	CDCl3	gain	not used
file	exp	spin	22
ACQUISITION		hst	0.008
sw	5997.0	pw90	9.800
at	2.049	alfa	0
np	24576	FLAGS	
fb	3000	il	n
bs	32	in	n
d1	1.000	dp	y
nt	8	hs	nn
ct	8	PROCESSING	
TRANSMITTER		fn	not used
tn	H1	DISPLAY	
sfrq	499.742	sp	-500.2
tof	0.1	wp	5997.0
tpwr	57	rfl	4128.3
pw	4.900	rfp	3628.1
DECOUPLER		rp	-9.0
dn	C13	lp	18.5
dof	0	PLOT	
dm	nnn	wc	180
dmm	c	sc	0
dpwr	51	vs	180
dmf	17094	th	1
		nm	cdc ph



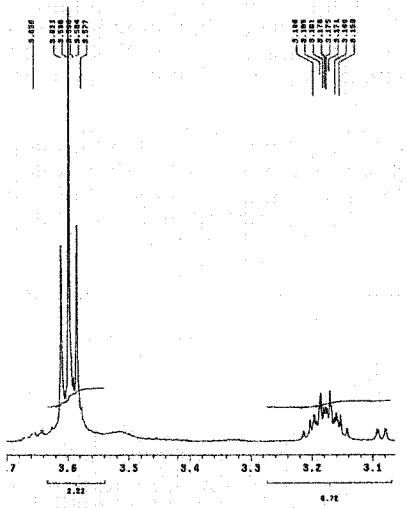
I-12

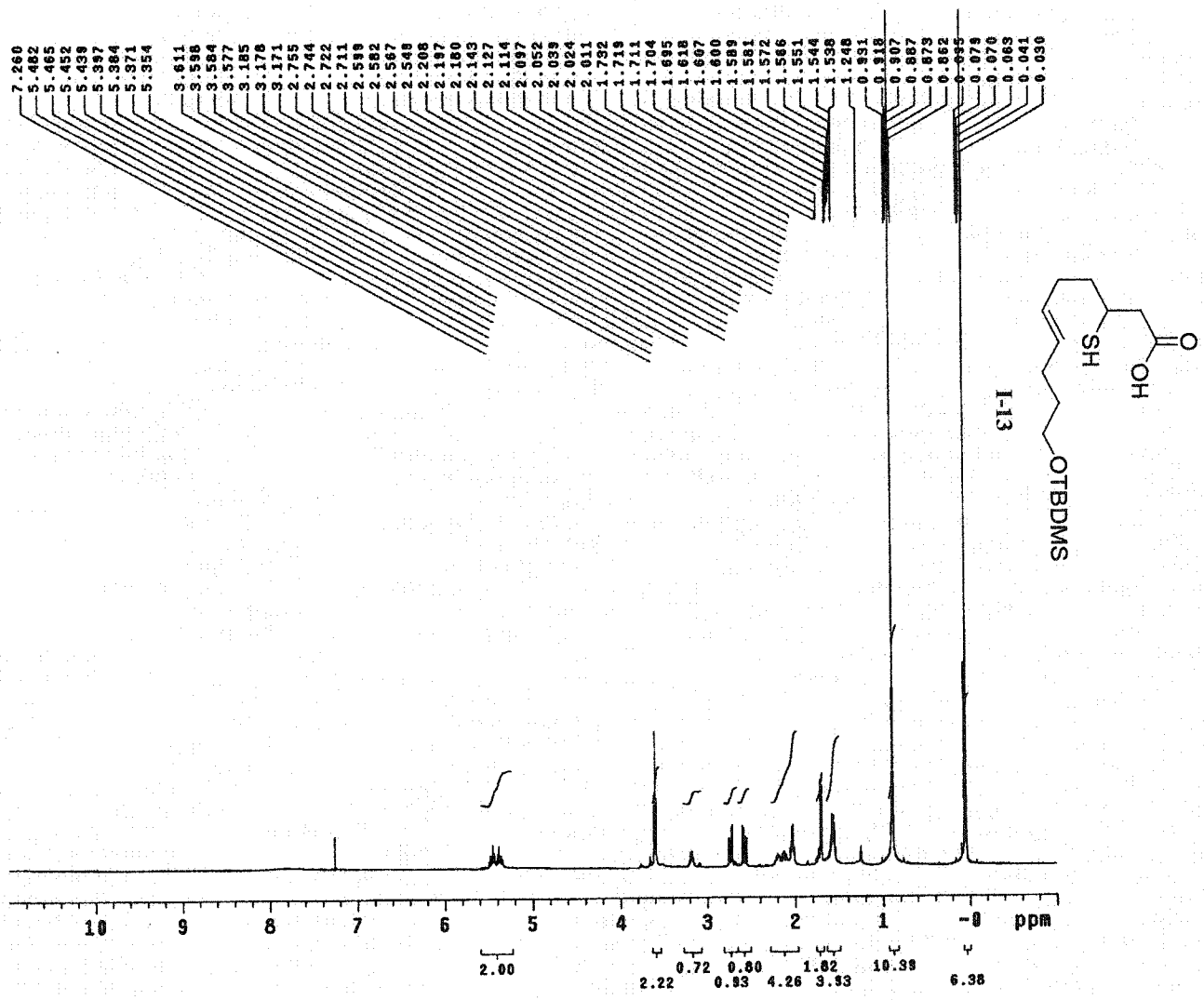
Current Data Parameters
NAME 09L-C13
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20000717
Time 23.08
INSTRUM dpx400
PROBHD 5 mm QNP 1H
PULPROG zgpc30
TD 65536
SOLVENT CDCl3
NS 237
DS 0
SWH 25125.629 Hz
FIDRES 0.383387 Hz
AQ 1.3042164 sec
RG 6502
DW 19.900 usec
DE 4.50 usec
TE 300.0 K
D1 1.00000000 sec
d11 0.03000000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 5.80 usec
PL1 -6.00 dB
SFO1 100.6227710 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 71.00 usec
PL2 120.00 dB
PL12 17.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127726 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 200.000 ppm
F1 20122.55 Hz
F2P -10.000 ppm
F2 -1006.13 Hz
PPMCM 10.50000 ppm/cm
HZCM 1056.43408 Hz/cm
-5.276
18.335
25.605
25.953
28.756
29.585
32.541
34.630
35.451
40.589
62.629
76.885
77.001
77.318
127.032
128.474
128.913
129.086
129.630
130.498
130.835
136.151
176.736
ppm
0
25
50
75
100
125
150
175
ppm



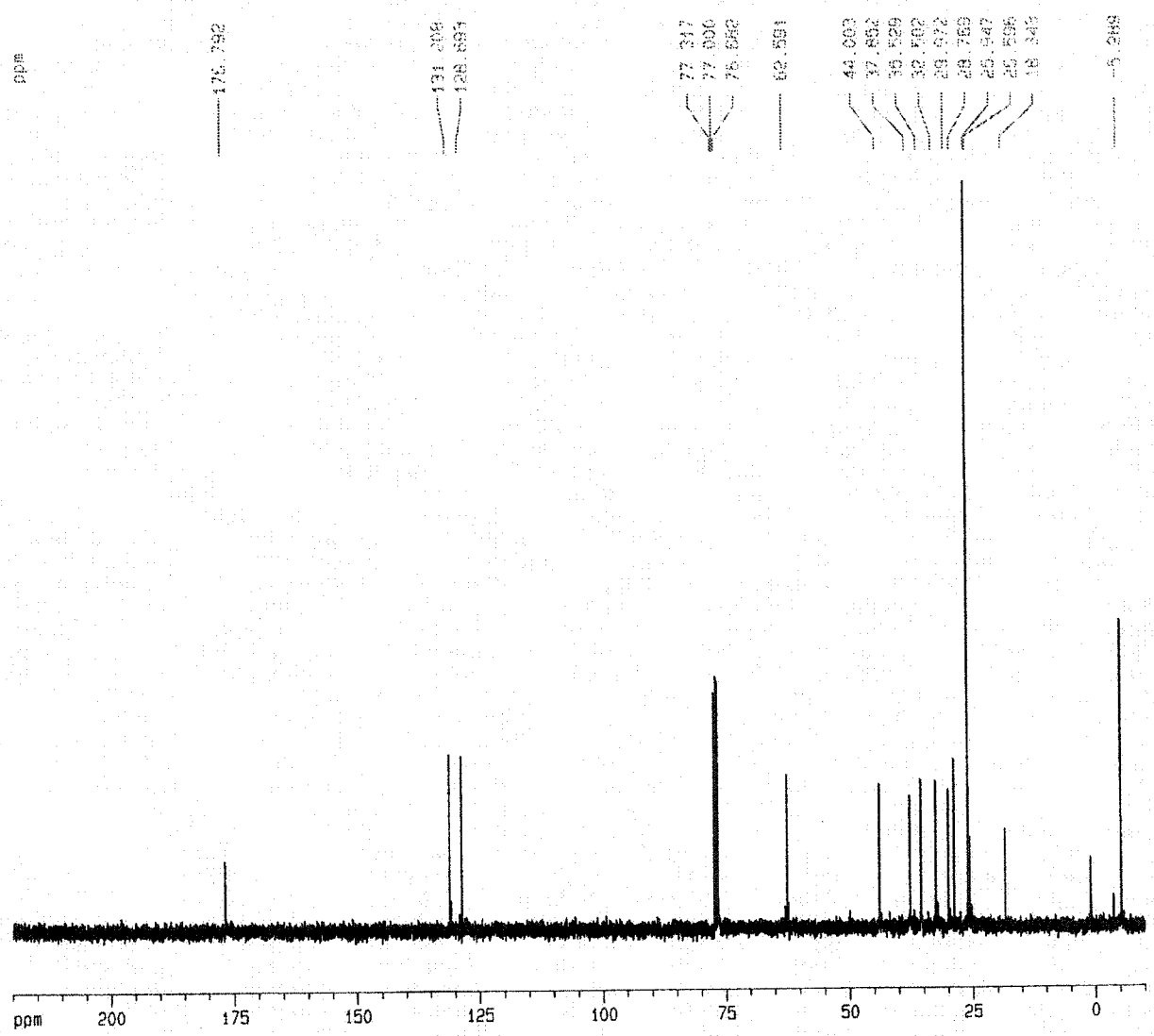
exp1 s2pul

SAMPLE		SPECIAL	
date	Aug 2 2000	temp	not used
solvent	CDCl3	gain	not used
file	exp	spin	22
ACQUISITION		hst	0.008
sw	5997.0	pw90	9.800
at	2.049	alfa	0
np	24576	**FLAGS**	
fb	3000	il	n
bs	32	in	n
d1	1.000	dp	y
nt	8	hs	nn
ct	8	**PROCESSING**	
TRANSMITTER		fn	not used
tn	H1	**DISPLAY**	
sfrq	499.742	sp	-500.9
tof	0.1	wp	5997.0
tpwr	57	rf1	4129.0
pw	4.900	rfp	3628.1
DECOUPLER		rp	-8.3
dn	C13	lp	-29.9
dof	0	**PLOT**	
dm	nnn	wc	180
dmm	c	sc	0
dpwr	51	vs	240
dmf	17094	th	2
		nm	cdc ph





I-13



Current Data Parameters

NAME	11L-C13
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20000803
Time	14.33
INSTRUM	dpx400
PROBHD	5 mm QNP 1H
PULPROG	zgdc30
TD	65536
SOLVENT	CDCl3
NS	526
DS	0
SWH	25125.629 Hz
FIDRES	0.383387 Hz
AQ	1.3042164 sec
RG	6502
DW	19.900 usec
DE	4.50 usec
TE	300.0 K
D1	1.00000000 sec
d11	0.03000000 sec

========== CHANNEL f1 ==========

NUC1	13C
P1	5.80 usec
PL1	-6.00 dB
SFO1	100.6227710 MHz

========== CHANNEL f2 ==========

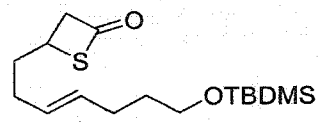
CPDPRG2	waltz16
NUC2	1H
PCPD2	71.00 usec
PL2	120.00 dB
PL12	17.00 dB
SFO2	400.1316005 MHz

F2 - Processing parameters

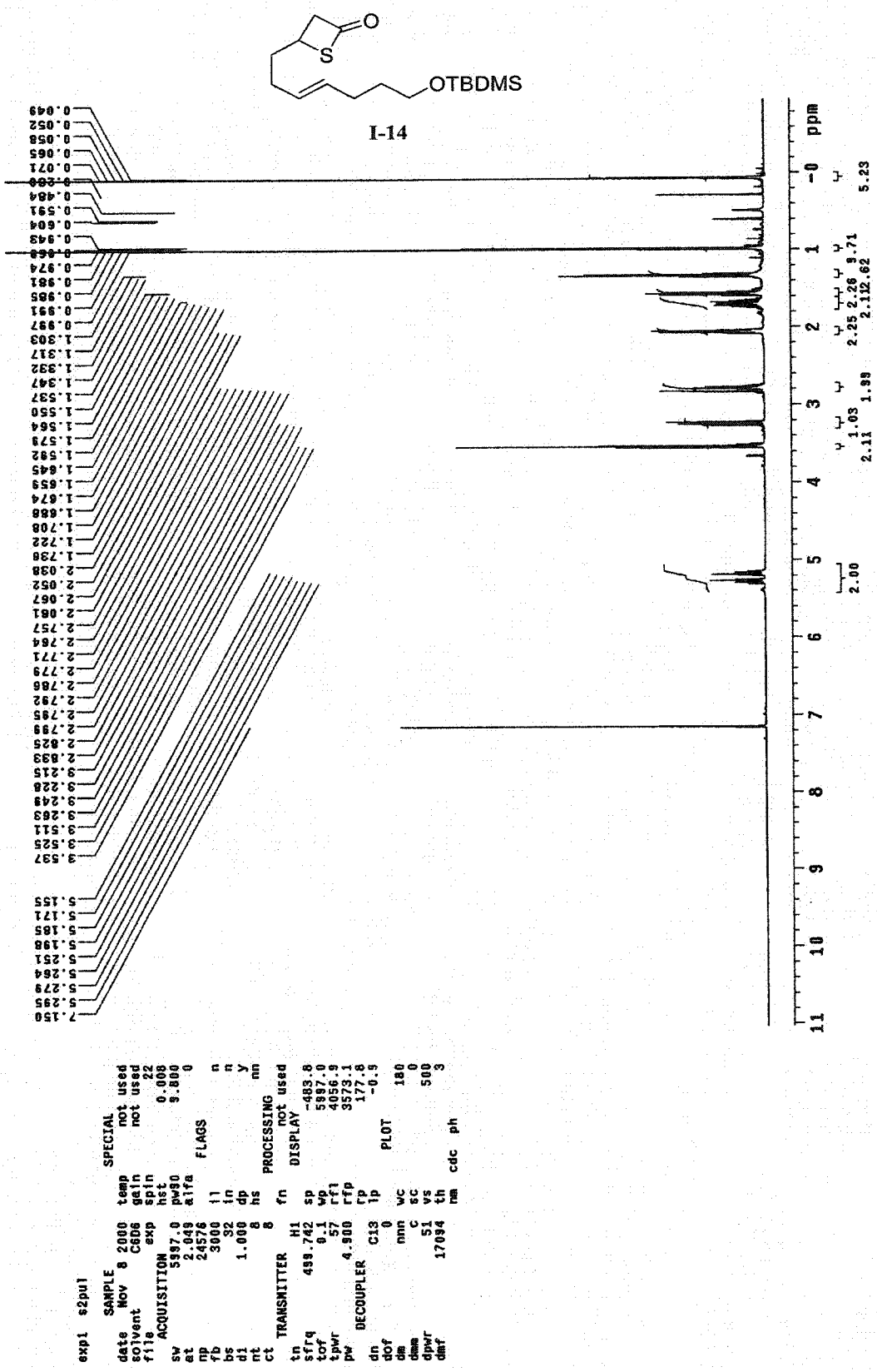
SI	32768
SF	100.6127718 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

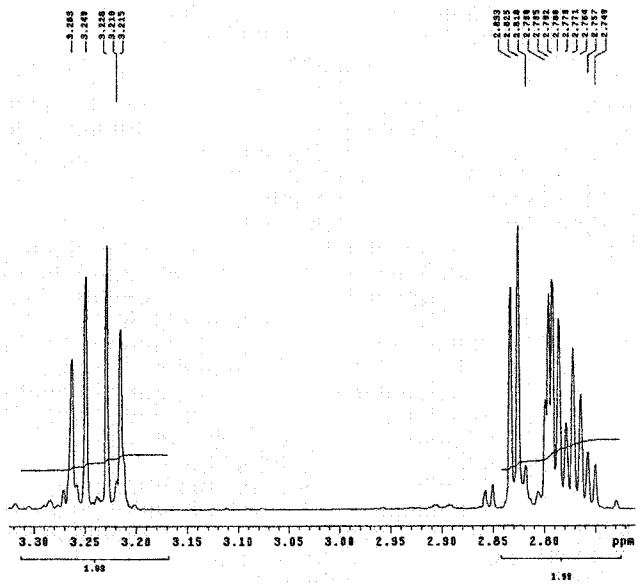
1D NMR plot parameters

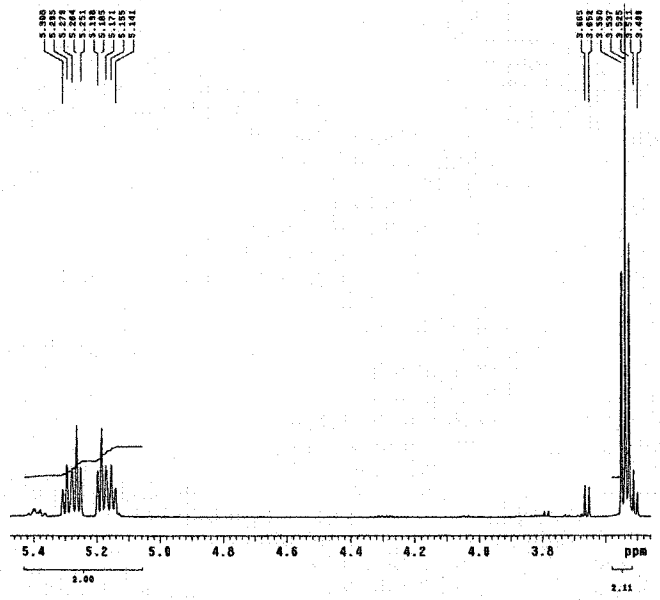
CX	20.00 cm
F1P	220.000 ppm
F1	22134.81 Hz
F2P	-10.000 ppm
F2	-1006.13 Hz
PPMCM	11.50000 ppm/cm
HZCM	1157.04688 Hz/cm

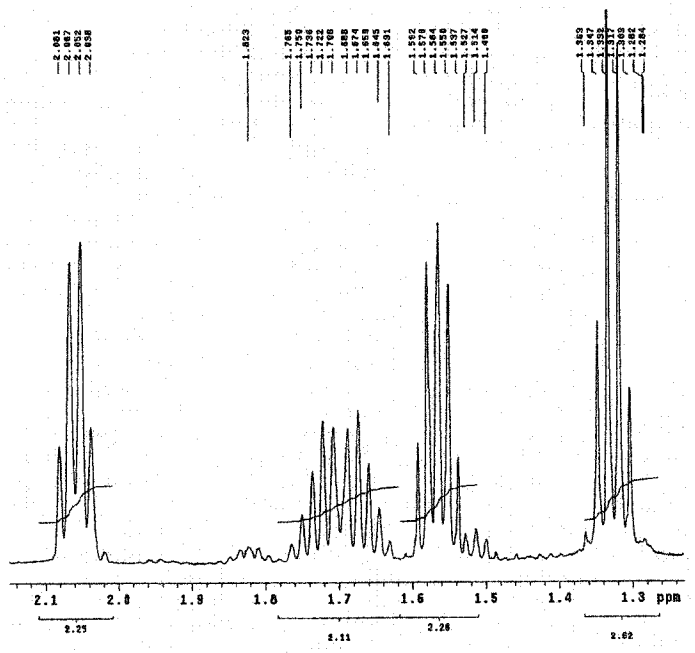

O
S
OTBDMS

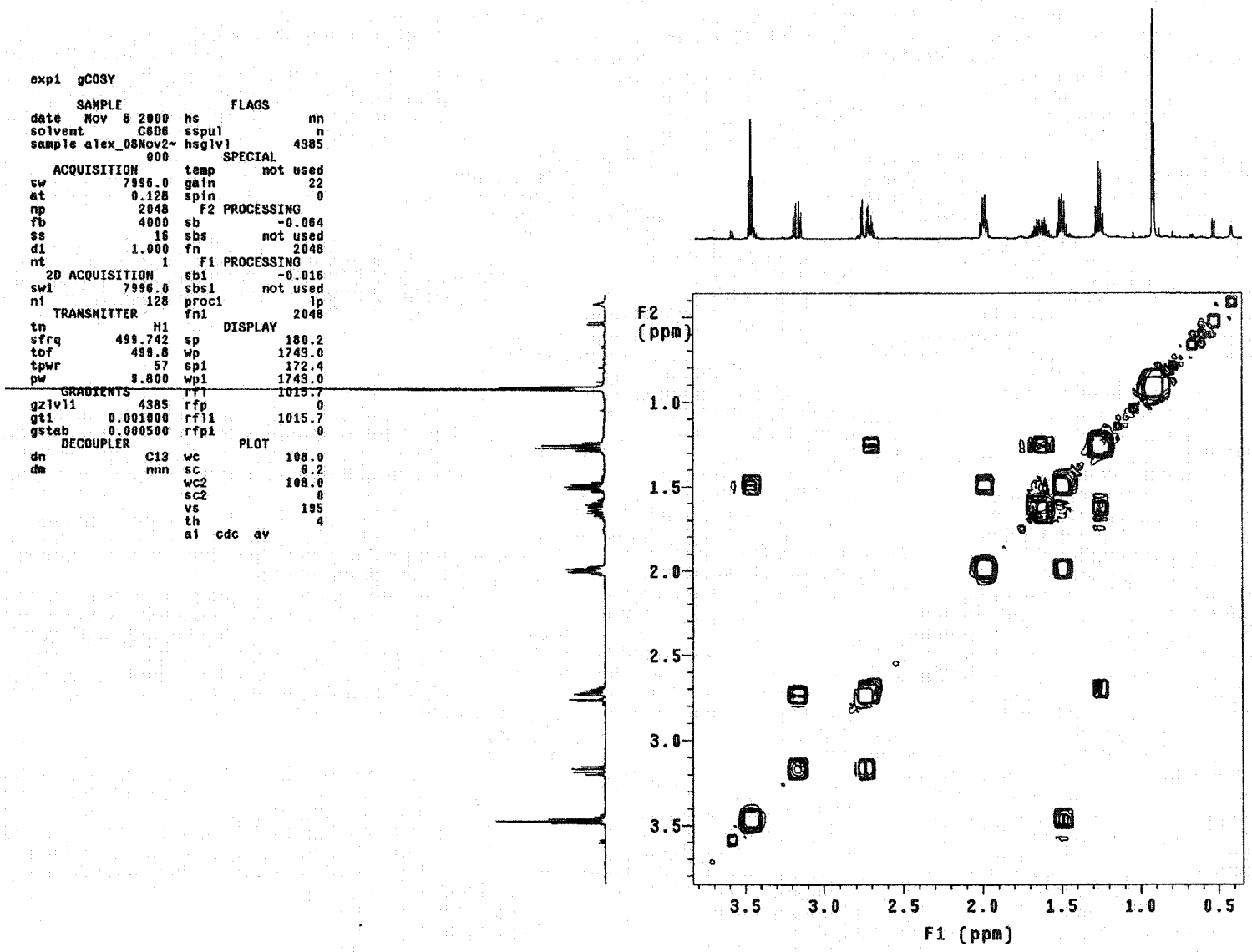
I-14

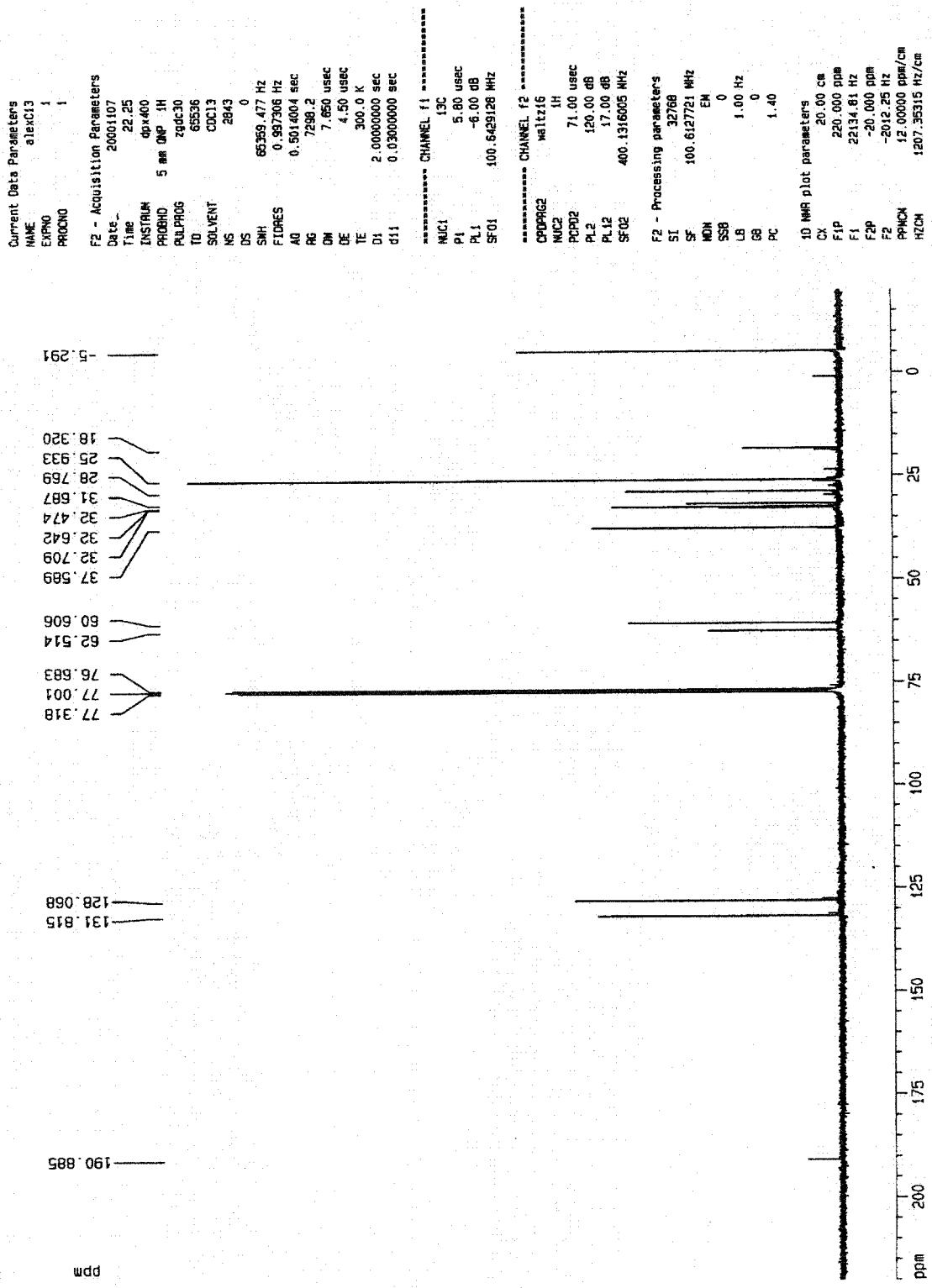

ppm
-0
1
2
3
4
5
6
7
8
9
10
11
5.23
9.71
2.26
2.25
2.11
2.62
1.99
1.03
2.11
2.00
0.049
0.052
0.058
0.065
0.071
0.080
0.484
0.591
0.604
0.943
0.968
0.974
0.981
0.985
0.991
0.997
1.303
1.317
1.332
1.347
1.537
1.550
1.564
1.579
1.592
1.645
1.659
1.674
1.688
1.708
1.722
1.736
2.038
2.052
2.067
2.081
2.757
2.764
2.771
2.779
2.786
2.792
2.795
2.799
2.825
2.833
3.215
3.228
3.249
3.263
3.511
3.525
3.537
5.155
5.171
5.185
5.198
5.251
5.264
5.279
5.295
7.150
exp1 s2pul
SAMPLE
date Nov 8 2000
solvent C6D6
file exp
ACQUISITION
sw 5997.0
at 2.049
np 24576
fb 3000
bs 32
d1 1.000
nt 8
ct 8
TRANSMITTER
tn H1
sfrq 499.742
tof 0.1
tpwr 57
pw 4.900
DECOUPLER
dn C13
dof 0
dm nnn
dmm c
dpwr 51
dmf 17094
SPECIAL
temp not used
gain not used
spin 22
hst 0.008
pw90 9.800
alfa 0
FLAGS
il n
in n
dp y
hs nn
PROCESSING
fn not used
DISPLAY
sp -483.8
wp 5997.0
rfl 4056.9
rfp 3573.1
rp 177.8
lp -0.9
PLOT
wc 180
sc 0
vs 500
th 3
nm cdc ph

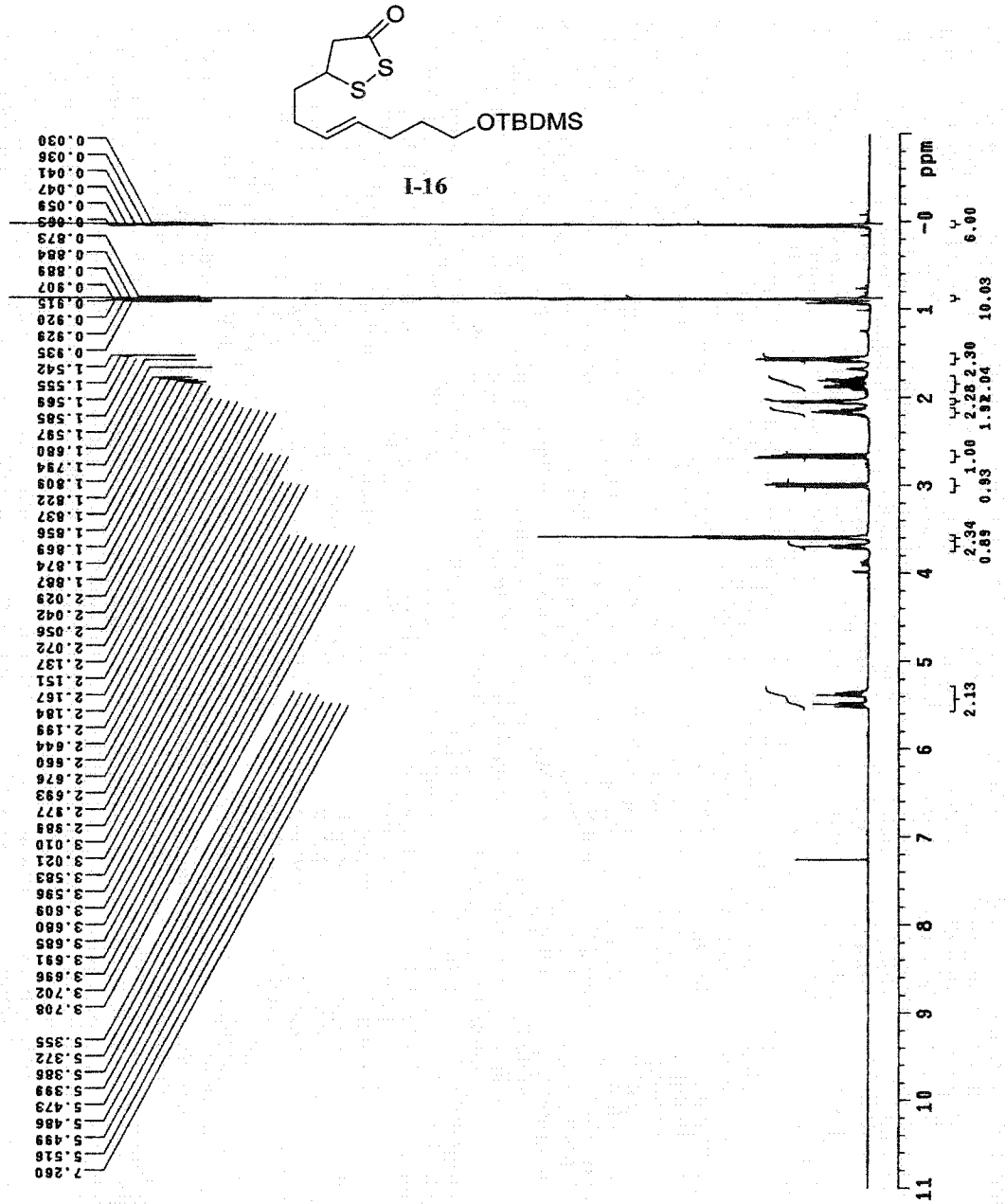

3.30 3.25 3.20 3.15 3.10 3.05 3.00 2.95 2.90 2.85 2.80 ppm
1.00
1.99


5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 ppm
3.00
2.11


2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4 1.3 ppm
2.25
2.11
2.28
2.62

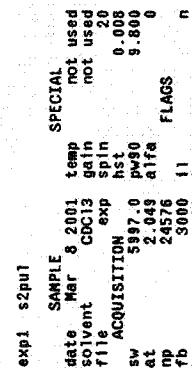

exp1 gCOSY
SAMPLE
date Nov 8 2000
solvent C6D6
sample alex_08Nov2~
000
ACQUISITION
sw 7996.0
at 0.128
np 2048
fb 4000
ss 16
d1 1.000
nt 1
2D ACQUISITION
sw1 7996.0
ni 128
TRANSMITTER
tn H1
sfrq 499.742
tof 499.8
tpwr 57
pw 8.800
GRADIENTS
gzlvl1 4385
gt1 0.001000
gstab 0.000500
DECOUPLER
dn C13
dm nnn
FLAGS
hs nn
sspul n
hsglvl 4385
SPECIAL
temp not used
gain 22
spin 0
F2 PROCESSING
sb -0.064
sbs not used
fn 2048
F1 PROCESSING
sb1 -0.016
sbs1 not used
proc1 lp
fn1 2048
DISPLAY
sp 180.2
wp 1743.0
sp1 172.4
wp1 1743.0
rf1 1015.7
rfp 0
rf11 1015.7
rfp1 0
PLOT
wc 108.0
sc 6.2
wc2 108.0
sc2 0
vs 195
th 4
ai cdc av
F2 (ppm)
1.0
1.5
2.0
2.5
3.0
3.5
3.5
3.0
2.5
2.0
1.5
1.0
0.5
F1 (ppm)

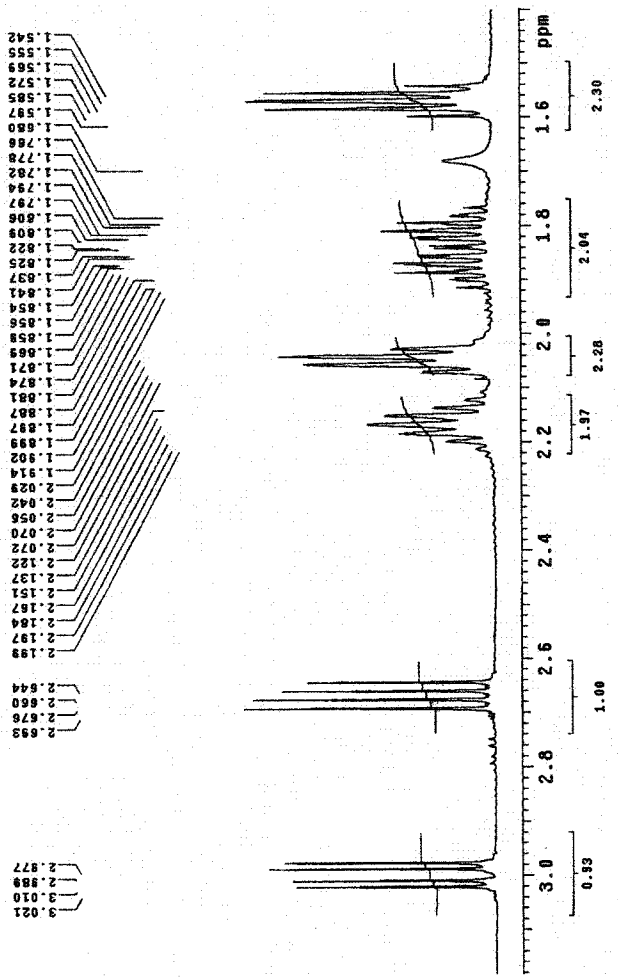

Current Data Parameters
NAME alexC13
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20001107
Time 22.25
INSTRUM dpx400
PROBHD 5 mm QNP 1H
PULPROG zgdc30
TD 65536
SOLVENT CDCl3
NS 2843
DS 0
SWH 65359.477 Hz
FIDRES 0.997306 Hz
AQ 0.5014004 sec
RG 7298.2
DW 7.650 usec
DE 4.50 usec
TE 300.0 K
D1 2.00000000 sec
d11 0.03000000 sec
========== CHANNEL f1 ==========
NUC1 13C
P1 5.80 usec
PL1 -6.00 dB
SF01 100.6429128 MHz
========== CHANNEL f2 ==========
CPDPRG2 waltz16
NUC2 1H
PCPD2 71.00 usec
PL2 120.00 dB
PL12 17.00 dB
SF02 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127721 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 220.000 ppm
F1 22134.81 Hz
F2P -20.000 ppm
F2 -2012.25 Hz
PPMCM 12.00000 ppm/cm
HZCM 1207.35315 Hz/cm
-5.291
18.320
25.933
28.769
31.687
32.474
32.642
32.709
37.589
60.606
62.514
76.683
77.001
77.318
128.068
131.815
190.885
ppm
0
25
50
75
100
125
150
175
200
ppm

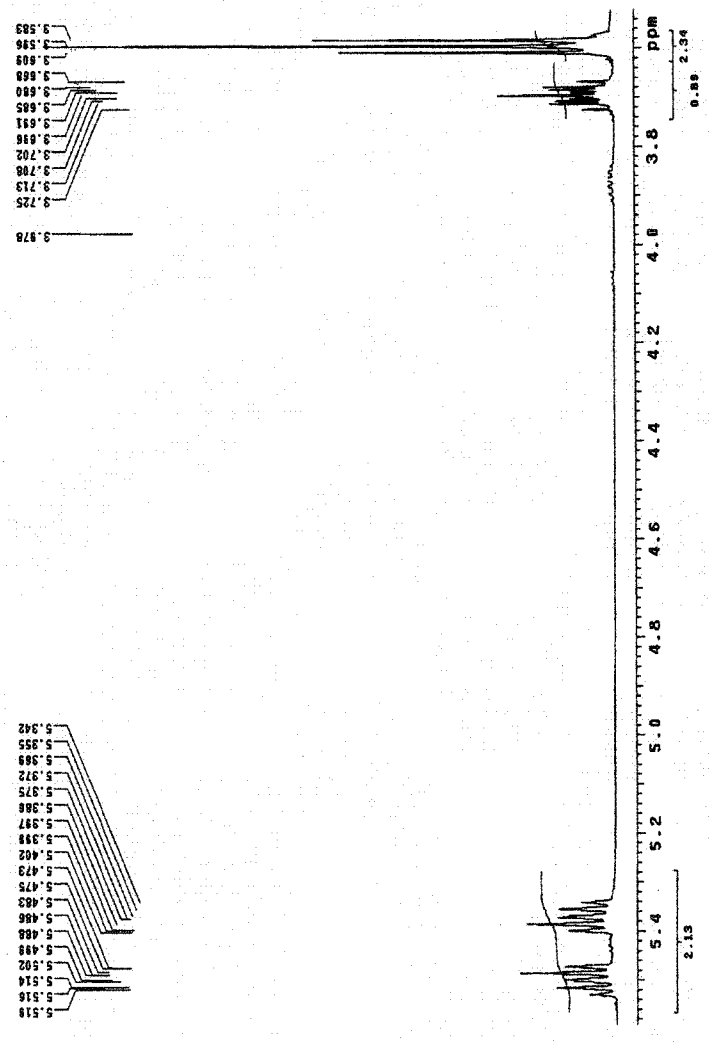

O
S
S
OTBDMS

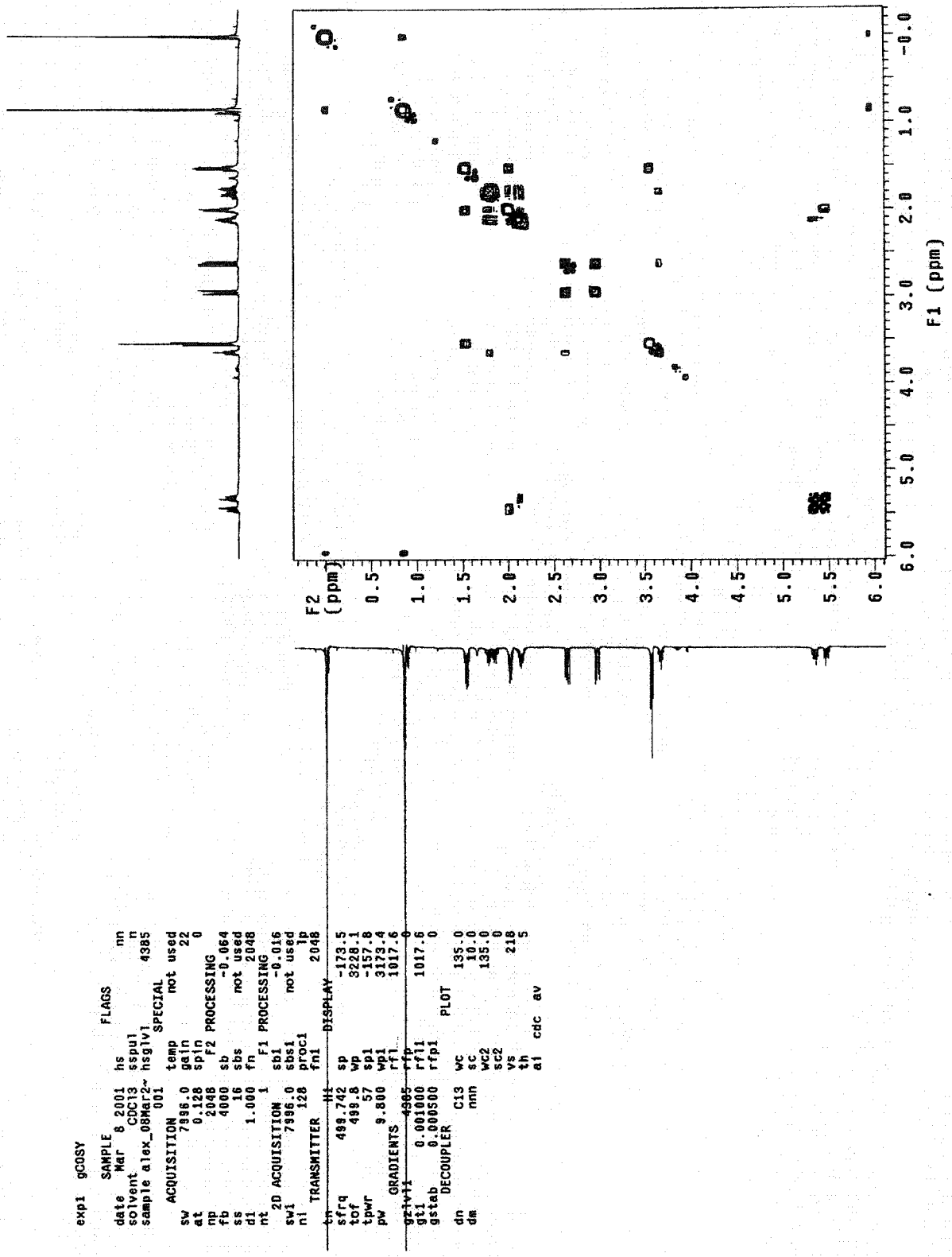
I-16

ppm
2.34
0.89
1.00
0.93
2.28 2.30
1.92.04
10.03
2.13
6.00

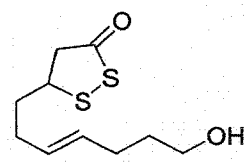

exp1 s2pul
SAMPLE
date Mar 8 2001
solvent CDCl3
file exp
ACQUISITION
sw 5997.0
at 2.049
np 24576
fb 3000
bs 32
d1 1.000
nt 8
ct 8
TRANSMITTER
tn H1
sfrq 499.742
tof 8.1
tpwr 57
pw 4.900
DECOUPLER
dn C13
dof 0
dm nnn
dmm c
dpwr 51
dmf 17094
SPECIAL not used
temp not used
gain 20
spin
hst 0.008
pw90 9.800
alfa 0
FLAGS
il n
in n
dp y
hs nn
PROCESSING not used
fn
DISPLAY
sp -500.2
wp 5997.0
rf1 4128.3
rfp 3628.1
rp -98.2
lp -7.6
PLOT
wc 180
sc 0
vs 500
th 3
nm cdc ph






exp1 gCOSY
SAMPLE
date Mar 8 2001
solvent CDCl3
sample alex_08Mar2~
ACQUISITION
sw 7996.0
at 0.128
np 2048
fb 4000
ss 16
d1 1.000
nt 1
2D ACQUISITION
sw1 7996.0
ni 128
TRANSMITTER
tn H1
sfrq 499.742
tof 499.8
tpwr 57
pw 9.800
GRADIENTS
gt1 0.001000
gstab 0.000500
DECOUPLER
dn C13
dm nnn
FLAGS
hs
sspul
hsglv1 4385
SPECIAL
temp not used
gain 22
spin 0
F2 PROCESSING
sb -0.064
sbs not used
fn 2048
F1 PROCESSING
sb1 -0.016
sbs1 not used
proc1 lp
fn1 2048
DISPLAY
sp -173.5
wp 3228.1
sp1 -157.8
wp1 3173.4
rfl 1017.6
rfp 0
rfl1 1017.6
rfp1 0
PLOT
wc 135.0
sc 10.0
wc2 135.0
sc2 0
vs 218
th 5
al cdc av
F2 (ppm)
F1 (ppm)

Current Data Parameters
NAME C13bsct2.ONP
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20010112
Time 22.38
INSTRUM dpx400
PROBHD 5 mm QNP 1H
PULPROG zgdc30
TD 65536
SOLVENT DMSO
NS 3681
DS 0
SWH 65359.477 Hz
FIDRES 0.997306 Hz
AQ 0.5014004 sec
RG 7298.2
DW 7.650 usec
DE 4.50 usec
TE 300.0 K
D1 1.00000000 sec
D11 0.03000000 sec
========== CHANNEL f1 ==========
NUC1 13C
P1 5.80 usec
PL1 -6.00 dB
SFO1 100.6429128 MHz
========== CHANNEL f2 ==========
CPDPRG2 waltz16
NUC2 1H
PCPD2 71.00 usec
PL2 120.00 dB
PL12 17.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127741 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 220.000 ppm
F1 22134.81 Hz
F2P -20.000 ppm
F2 -2012.25 Hz
PPMCM 12.00000 ppm/cm
HZCM 1207.35327 Hz/cm
-5.293
18.324
19.073
25.933
28.784
30.573
32.437
32.531
48.294
49.186
62.491
76.678
76.996
77.313
127.926
132.113
206.519
ppm
0
25
50
75
100
125
150
175
200
ppm



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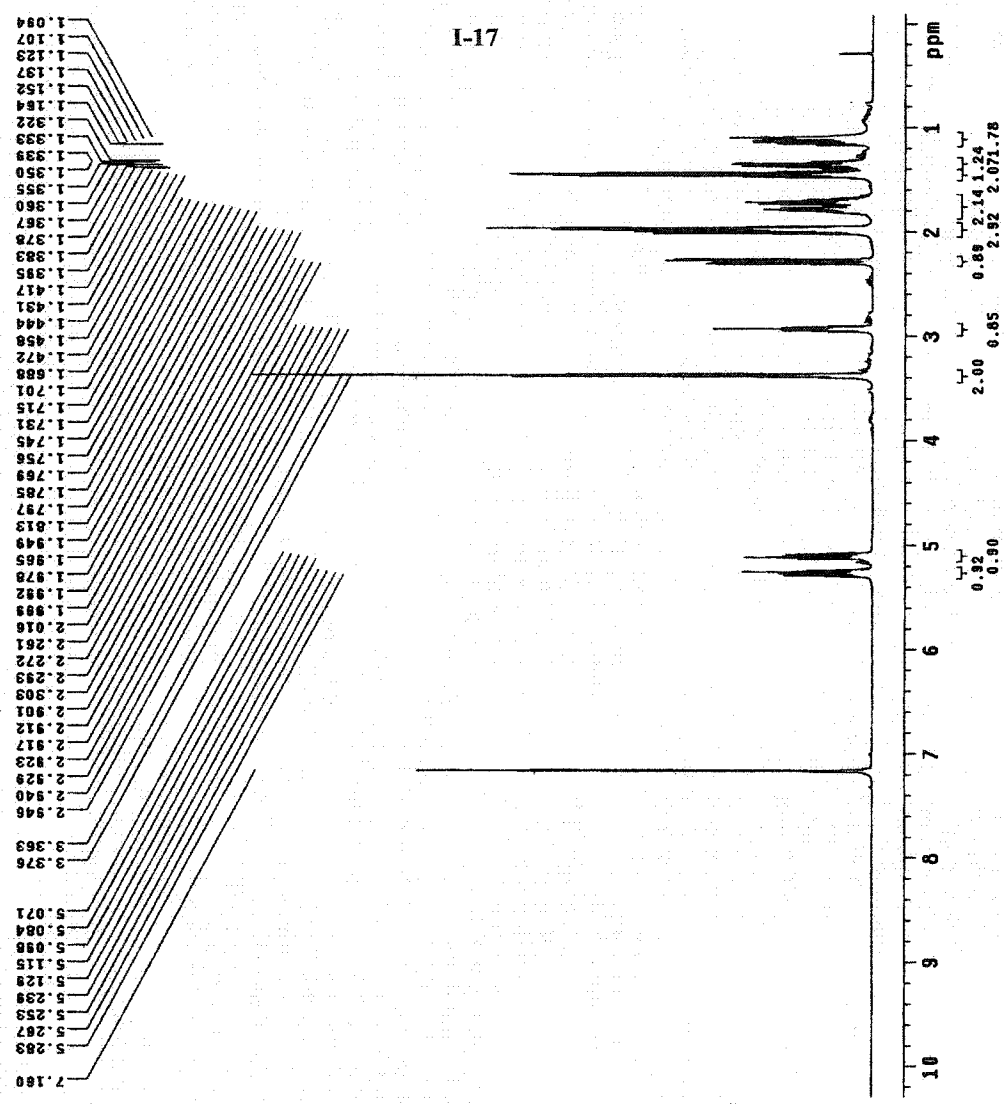
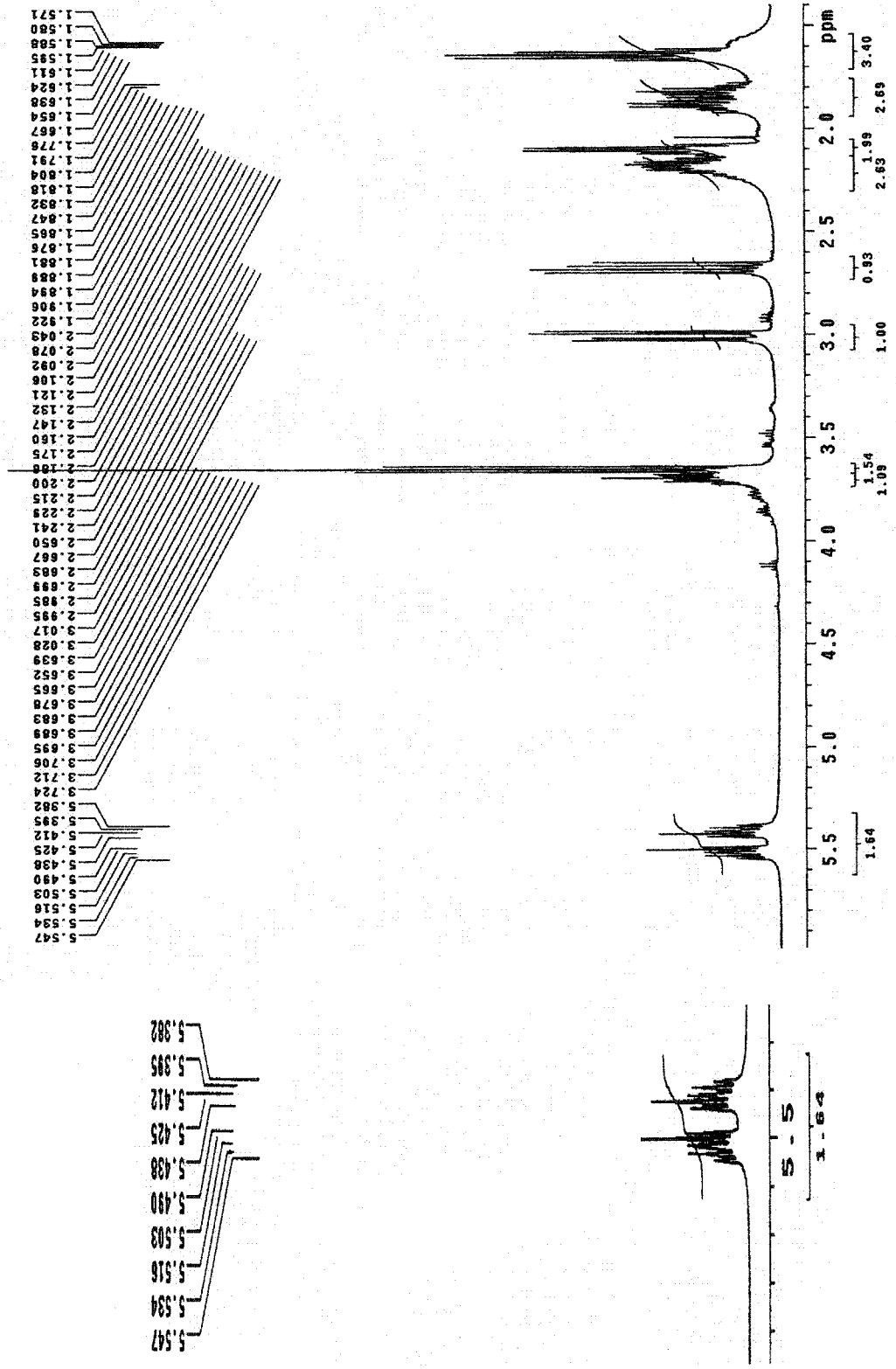
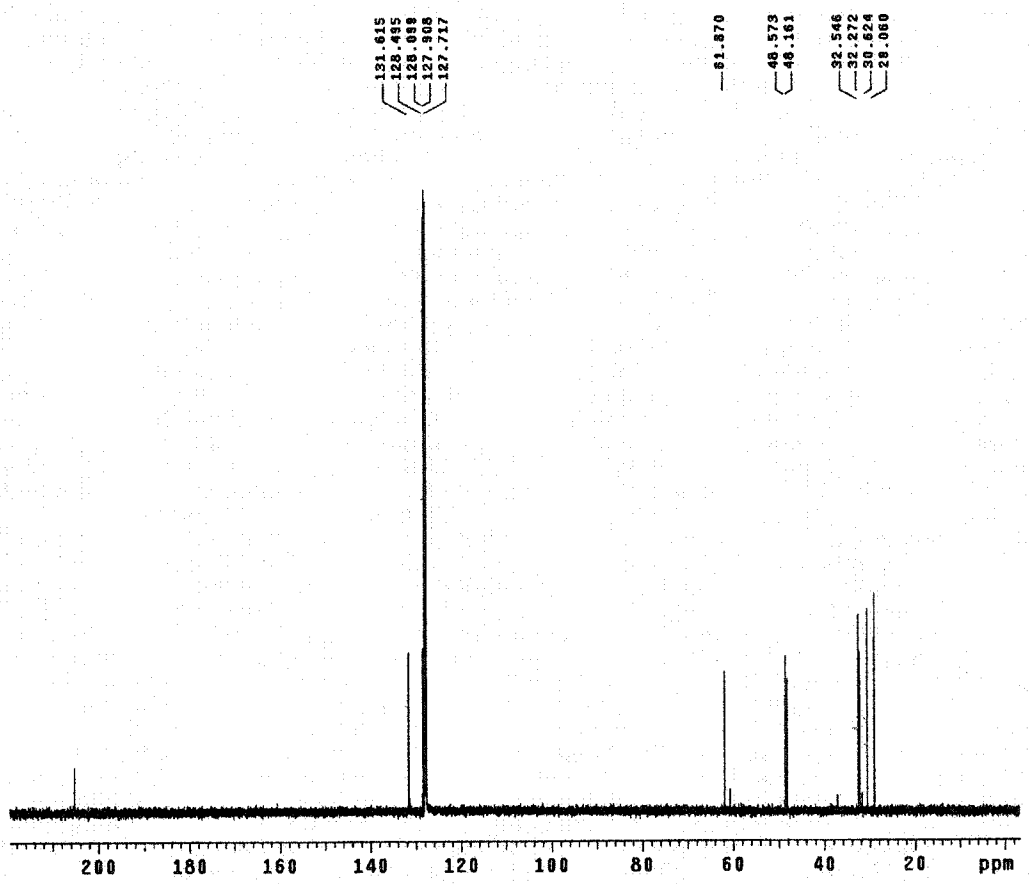


```
exp1  s2pul
        SAMPLE                          SPECIAL
date   Feb 28 2001  temp      not used
solvent      C6D6   gain      not used
file /export/home/~ spin            22
chenj/vnmrsys/data~ hst          0.008
/cj-02-02-28-1_28F~ pw90         9.800
  eb2001/PROTON.fid alfa            0
    ACQUISITION                 FLAGS
sw          7996.0  il            n
at          2.049   in            n
np          32768   dp            y
fb          4000    hs            nn
bs            32          PROCESSING
di          1.000   fn      not used
nt            8           DISPLAY
ct            8     sp          -47.7
    TRANSMITTER     wp         5190.6
tn            H1    rfl        4557.1
sfrq      499.742   rfp        3578.1
tof         499.8   rp         -117.7
tpwr         57     lp          -6.9
pw          4.900         PLOT
    DECOUPLER       wc          180
dn            C13   sc            0
dof             0   vs          111
dm            nnn   th            6
dmm             c   al   cdc  ph
dpwr           51
dmf         17094
```



exp1 s2pul

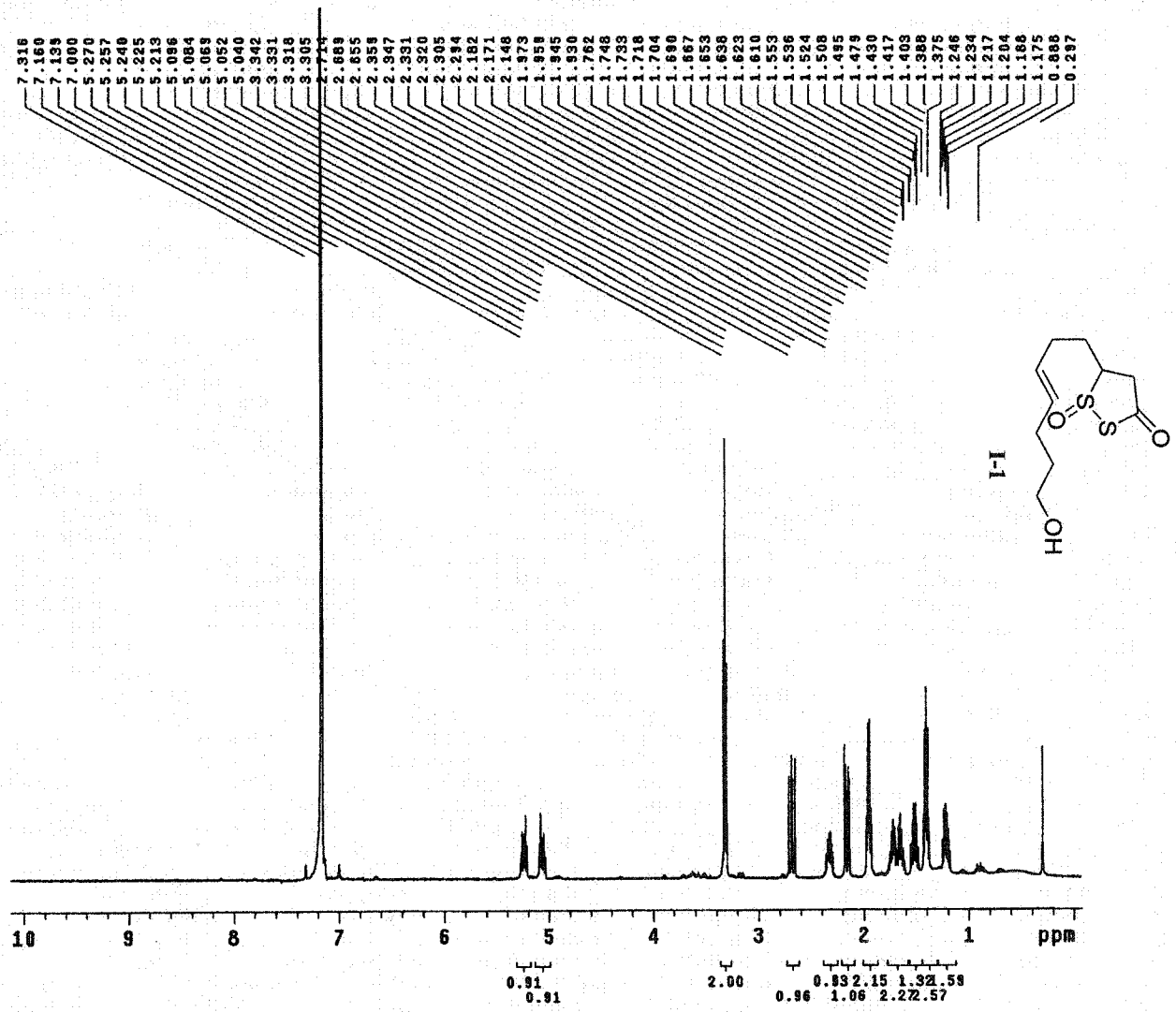
SAMPLE		SPECIAL	
date	Feb 28 2001	temp	not used
solvent	C6D6	gain	not used
file	exp	spin	16
ACQUISITION		hst	0.008
sw	31409.5	pw90	22.300
at	1.043	alfa	10.000
np	65536	FLAGS	
fb	17000	fl	n
bs	64	in	n
d1	1.000	dp	y
nt	1000	hs	nn
ct	1000	PROCESSING	
TRANSMITTER		lb	0.50
tn	C13	fn	not used
sfrq	125.673	DISPLAY	
tof	1883.5	sp	-416.5
tpwr	63	wp	27990.3
pw	11.150	rfl	1882.2
DECOUPLER		rfp	0
dn	H1	rp	-60.1
dof	0	lp	-244.2
dm	yyy	PLOT	
dmm	w	wc	180
dpwr	42	sc	0
dmf	11696	vs	387
		th	8
		al	cdc ph



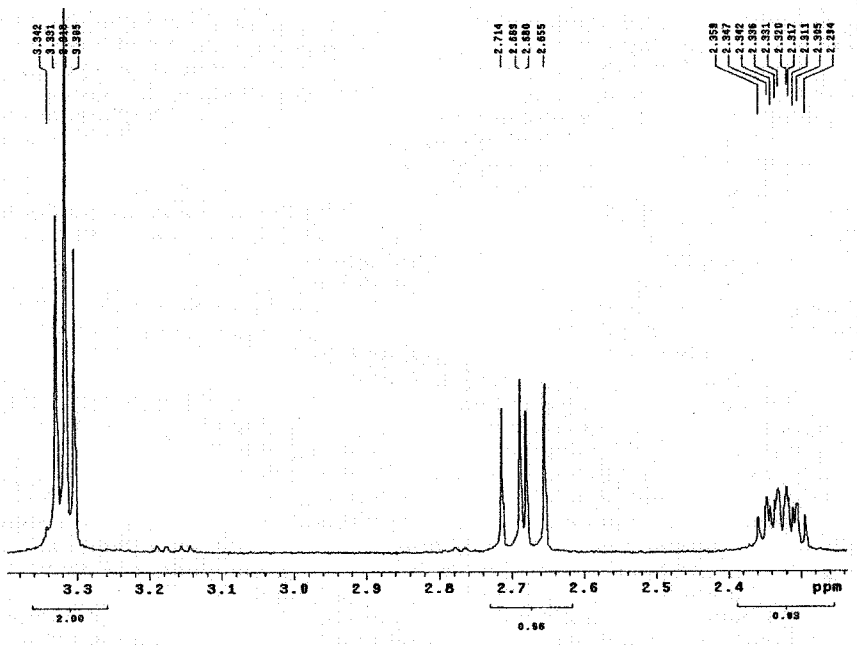
Target cpd

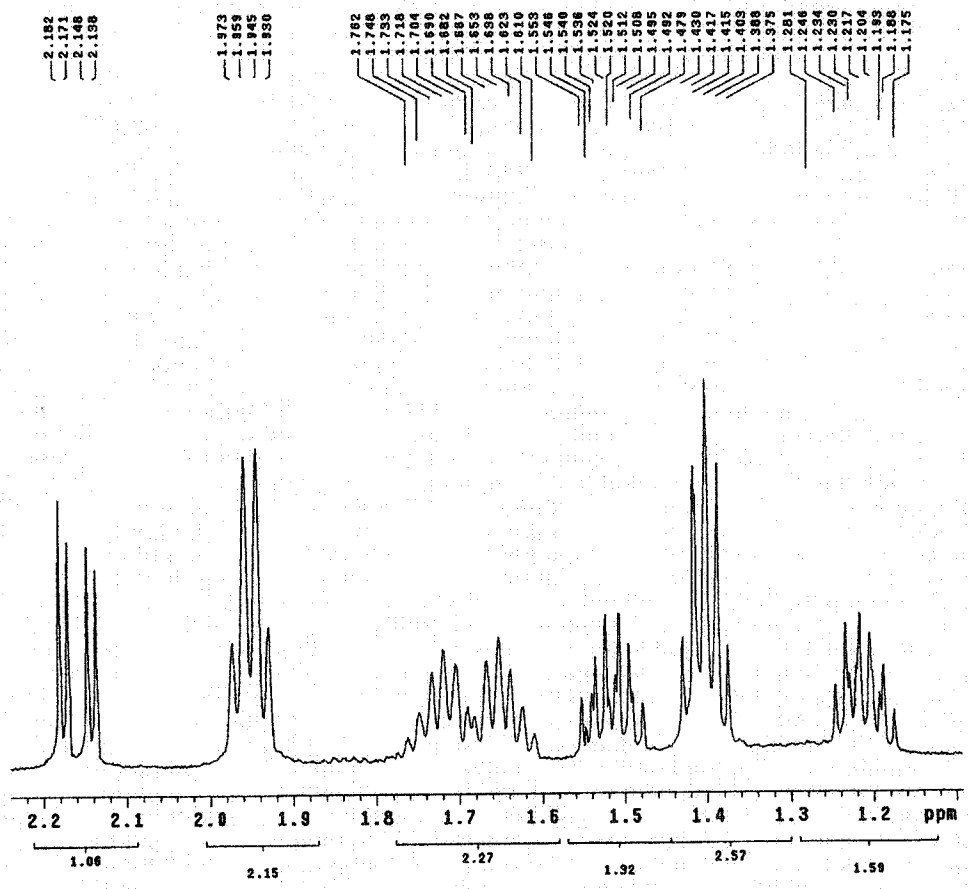
expl s2pul

SAMPLE		SPECIAL	
date	Feb 9 2001	temp	not used
solvent	C6D6	gain	not used
file	/export/home/~	spin	22
chenj/vnmrsys/data~		hst	0.008
/cj-02-02-09-1_09f~		pw90	9.800
eb2001/PROTON.fid		alfa	0
ACQUISITION		**FLAGS**	
sw	7996.0	il	n
at	2.048	in	n
np	32768	dp	y
fb	4000	hs	nn
bs	32	**PROCESSING**	
d1	1.000	fn	not used
nt	8	**DISPLAY**	
ct	8	sp	-37.5
TRANSMITTER		wp	5098.3
tn	H1	rfl	4557.1
sfrq	499.742	rfp	3578.1
tof	499.8	rp	-42.9
tpwr	57	lp	6.9
pw	4.900	**PLOT**	
DECOUPLER		wc	180
dn	C13	sc	0
dof	0	vs	101
dm	nnn	th	2
dmm	c	al	cdc ph
dpwr	51		
dmf	17094		



I-1

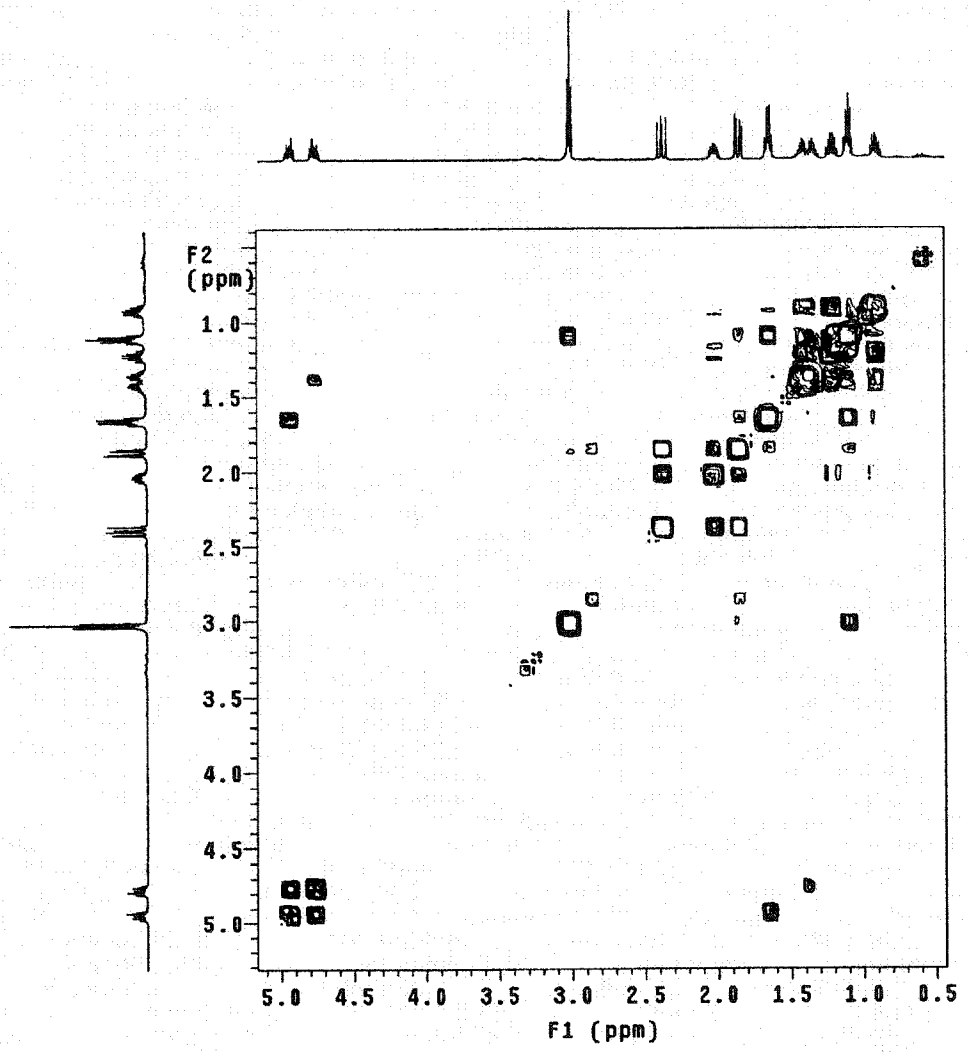

3.342
3.331
3.320
3.309
2.714
2.693
2.680
2.655
2.358
2.347
2.342
2.336
2.331
2.320
2.317
2.311
2.305
2.294
3.3
3.2
3.1
3.0
2.9
2.8
2.7
2.6
2.5
2.4
ppm
2.00
0.96
0.93


2.182
2.171
2.148
2.138
1.973
1.959
1.945
1.930
1.782
1.748
1.733
1.718
1.704
1.690
1.682
1.667
1.653
1.638
1.623
1.610
1.553
1.546
1.540
1.538
1.524
1.520
1.512
1.508
1.485
1.482
1.476
1.430
1.417
1.415
1.403
1.388
1.375
1.281
1.246
1.234
1.230
1.217
1.204
1.183
1.188
1.175
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
ppm
1.06
2.15
2.27
1.92
2.57
1.59

Target cpd

exp1 gCOSY

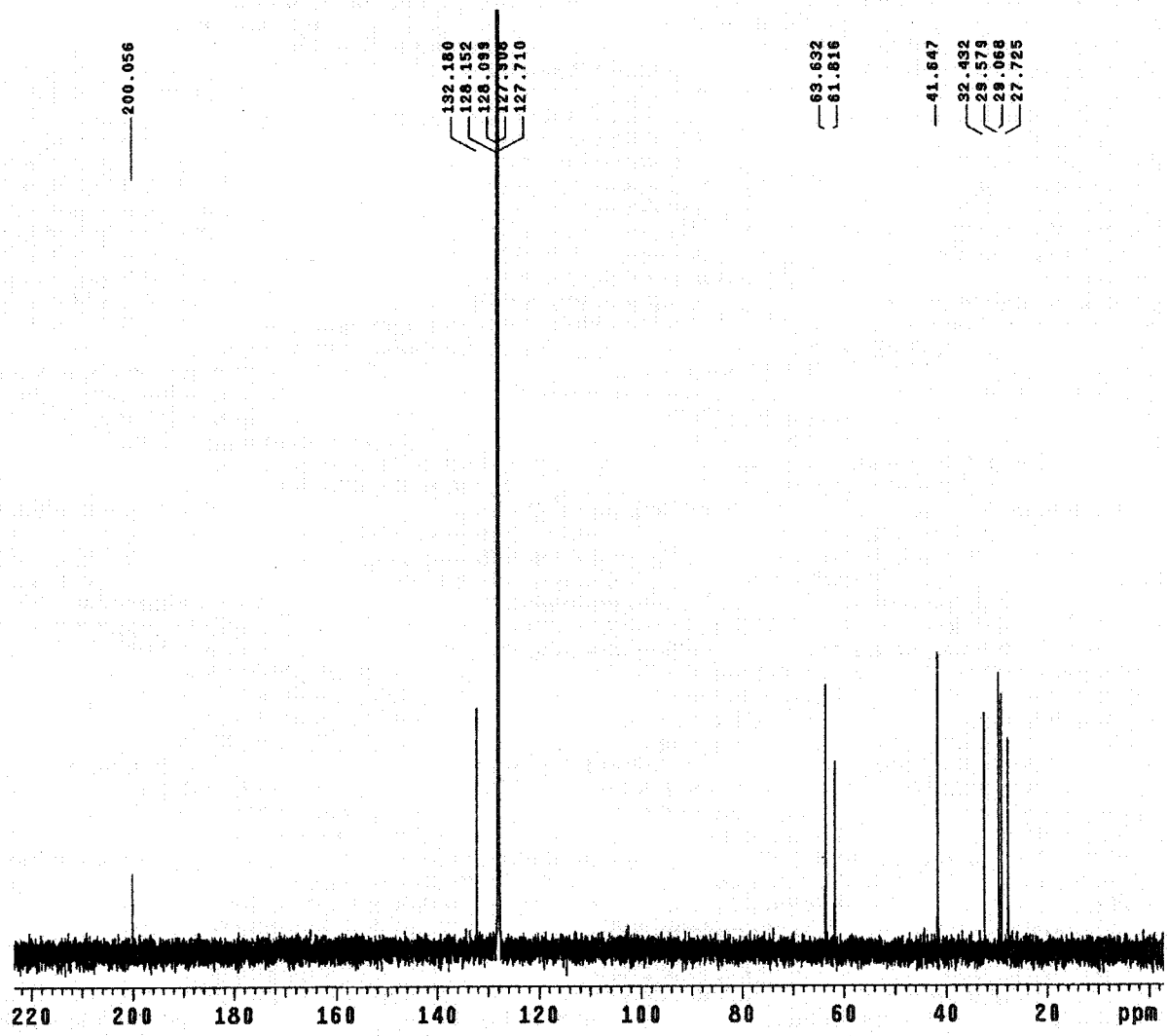
SAMPLE		FLAGS	
date	Feb 9 2001	hs	nn
solvent	C6D6	sspul	n
sample	cj-02-02-09~	hsglvl	4385
	-2_09Feb2001	SPECIAL	
ACQUISITION		temp	not used
sw	7996.0	gain	34
at	0.128	spin	0
np	2048	F2 PROCESSING	
fb	4000	sb	-0.064
ss	16	sbs	not used
d1	1.000	fn	2048
nt	1	F1 PROCESSING	
2D ACQUISITION		sb1	-0.016
swi	7996.0	sbs1	not used
ni	128	proc1	lp
TRANSMITTER		fn1	2048
tn	H1	DISPLAY	
sfrq	499.742	sp	193.5
tof	499.8	wp	2462.1
tpwr	57	sp1	217.0
pw	9.800	wp1	2360.5
GRADIENTS		rf1	1127.4
gzlvl1	4385	rfp	0
gt1	0.001000	rf11	1127.4
gstab	0.000500	rfp1	0
DECOUPLER		PLOT	
dn	C13	wc	108.0
dm	nnn	sc	6.2
		wc2	108.0
		sc2	0
		vs	214
		th	4
		ai cdc av	

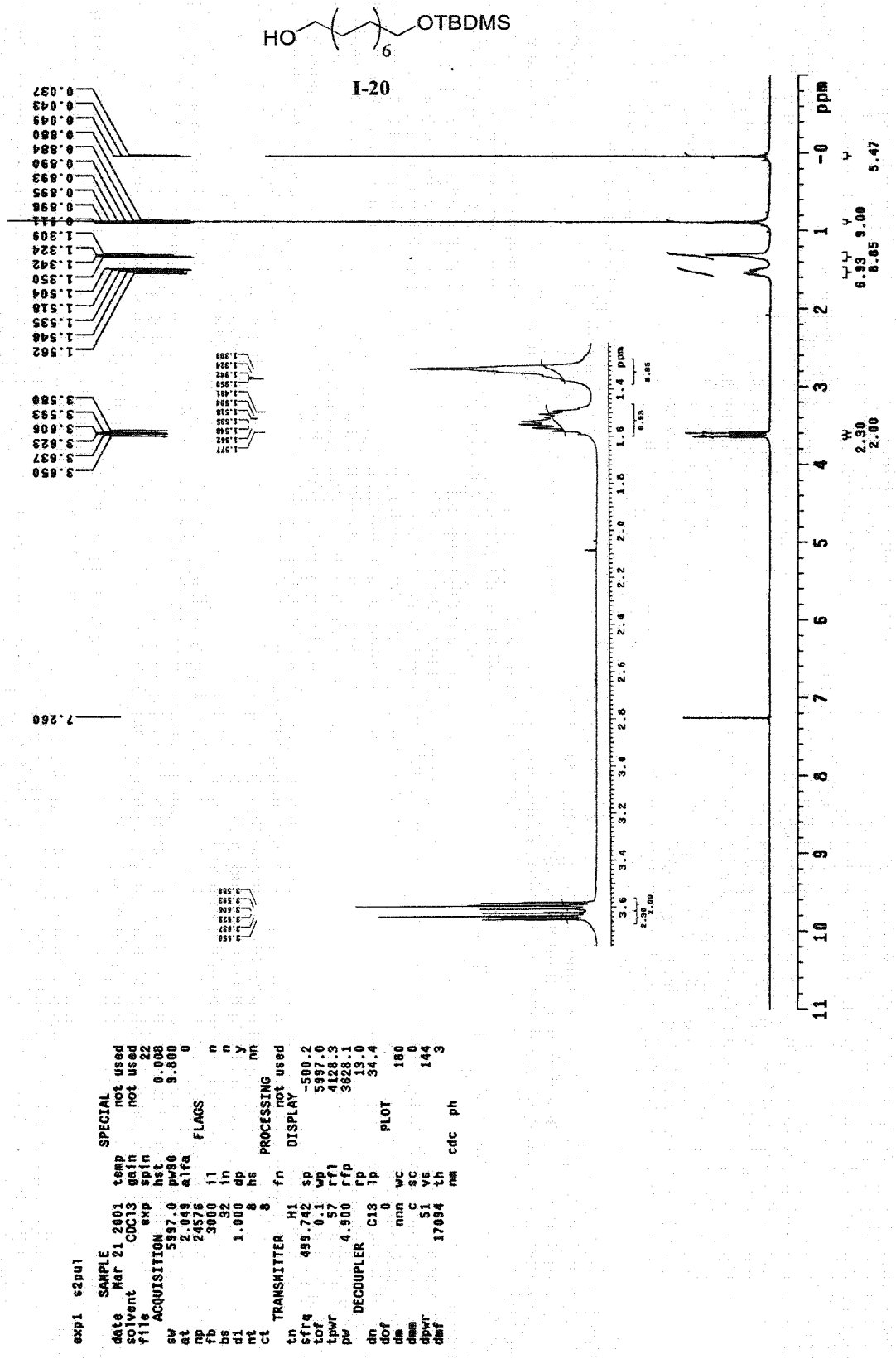


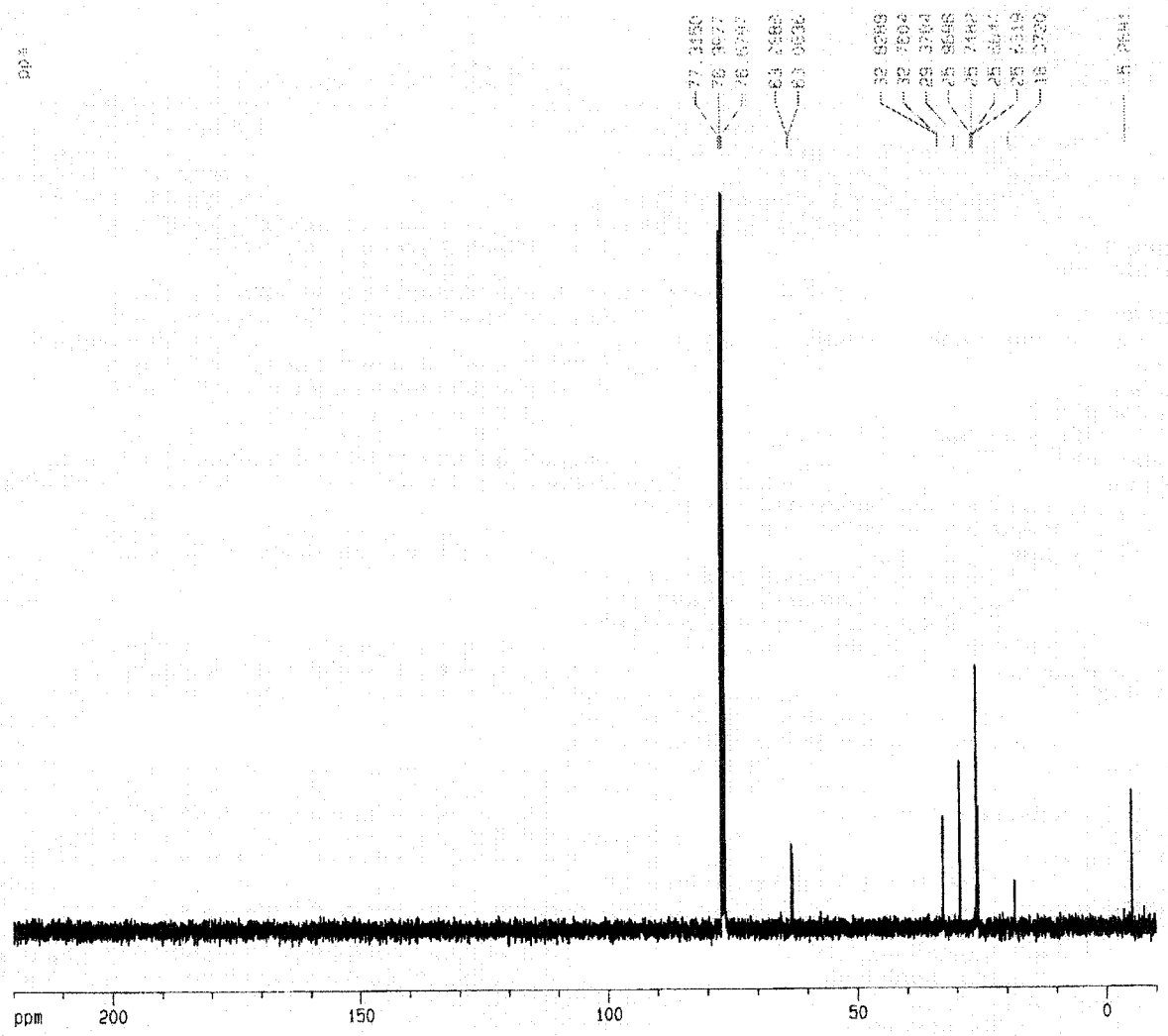
target cpd

expl s2pul

SAMPLE		SPECIAL	
date	Mar 14 2001	temp	not used
solvent	C6D6	gain	not used
file	exp	spin	16
ACQUISITION		hst	0.008
sw	31409.5	pw90	22.300
at	1.043	alfa	10.000
np	65536	FLAGS	
fb	17000	il	n
bs	64	in	n
d1	1.000	dp	y
nt	1000	hs	nn
ct	1000	PROCESSING	
TRANSMITTER		lb	0.50
tn	C13	fn	not used
sfrq	125.673	DISPLAY	
tof	1883.5	sp	-394.4
tpwr	63	wp	28473.4
pw	11.150	rfl	1882.2
DECOUPLER		rfp	0
dn	H1	rp	-108.5
dof	0	lp	-226.1
dm	yyy	PLOT	
dmm	w	wc	180
dpwr	42	sc	0
dmf	11696	vs	1168
		th	7
		al	cdc ph




HO
OTBDMS
6
I-20
ppm



Current Data Parameters
NAME 07L-C13
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20010323
Time 0.33
INSTRUM dpx400
PROBHD 5 mm QNP 1H
PULPROG zgdc30
TD 65536
SOLVENT CDCl3
NS 702
DS 0
SWH 25125.628 Hz
FIDRES 0.383387 Hz
AQ 1.3042164 sec
RG 6502
DW 19.900 usec
DE 4.50 usec
TE 300.0 K
D1 1.00000000 sec
d11 0.03000000 sec

========== CHANNEL f1 ==========
NUC1 13C
P1 5.80 usec
PL1 -6.00 dB
SFO1 100.6227710 MHz

========== CHANNEL f2 ==========
CPDPRG2 waltz16
NUC2 1H
PCPD2 71.00 usec
PL2 120.00 dB
PL12 17.00 dB
SFO2 400.1316005 MHz

F2 - Processing parameters
SI 32768
SF 100.6127711 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

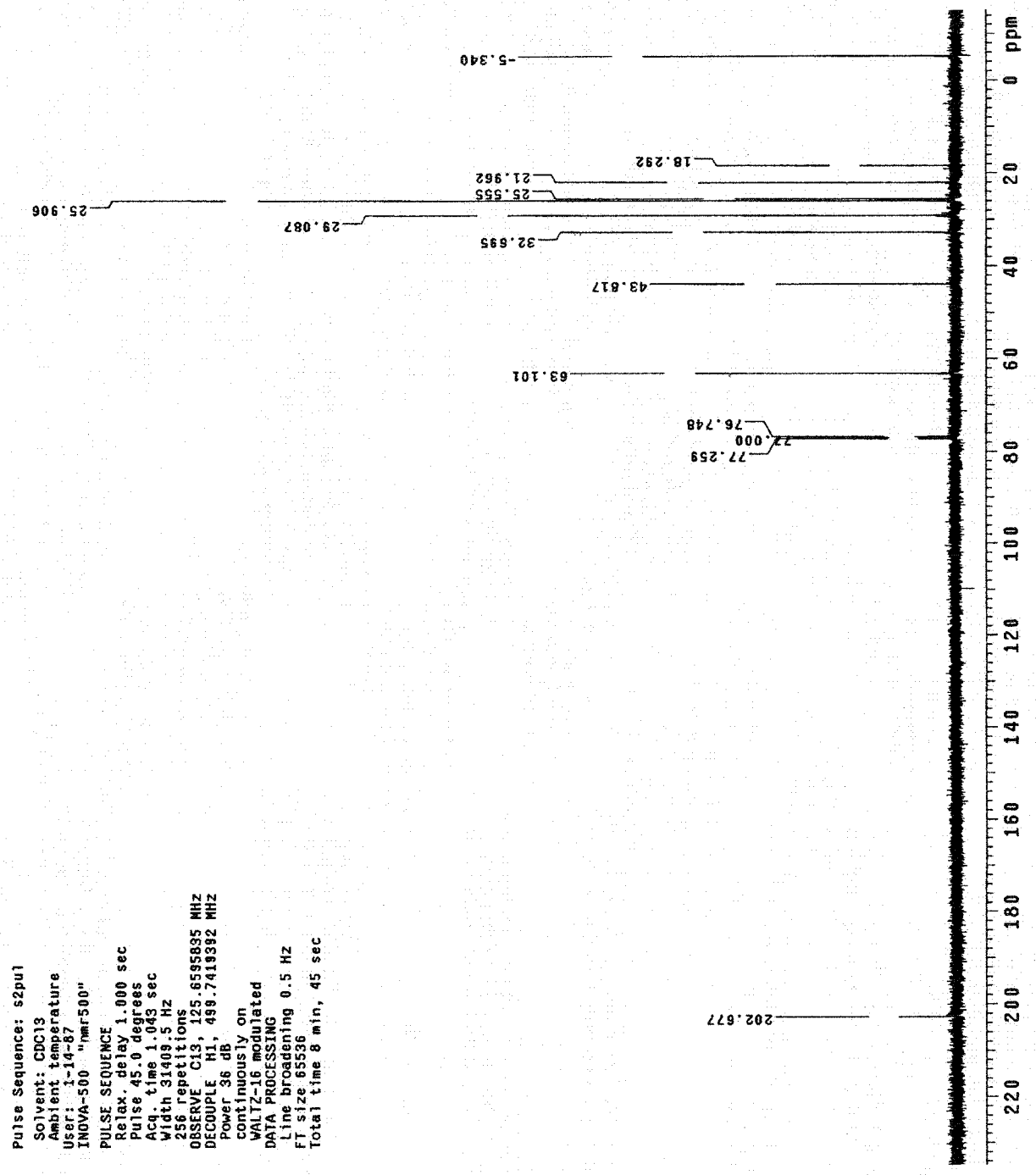
1D NMR plot parameters
CX 20.00 cm
F1P 220.000 ppm
F1 22134.81 Hz
F2P -10.000 ppm
F2 -1006.13 Hz
PPMCM 11.50000 ppm/cm
HZCM 1157.04688 Hz/cm

H
O
5
OTBDMS

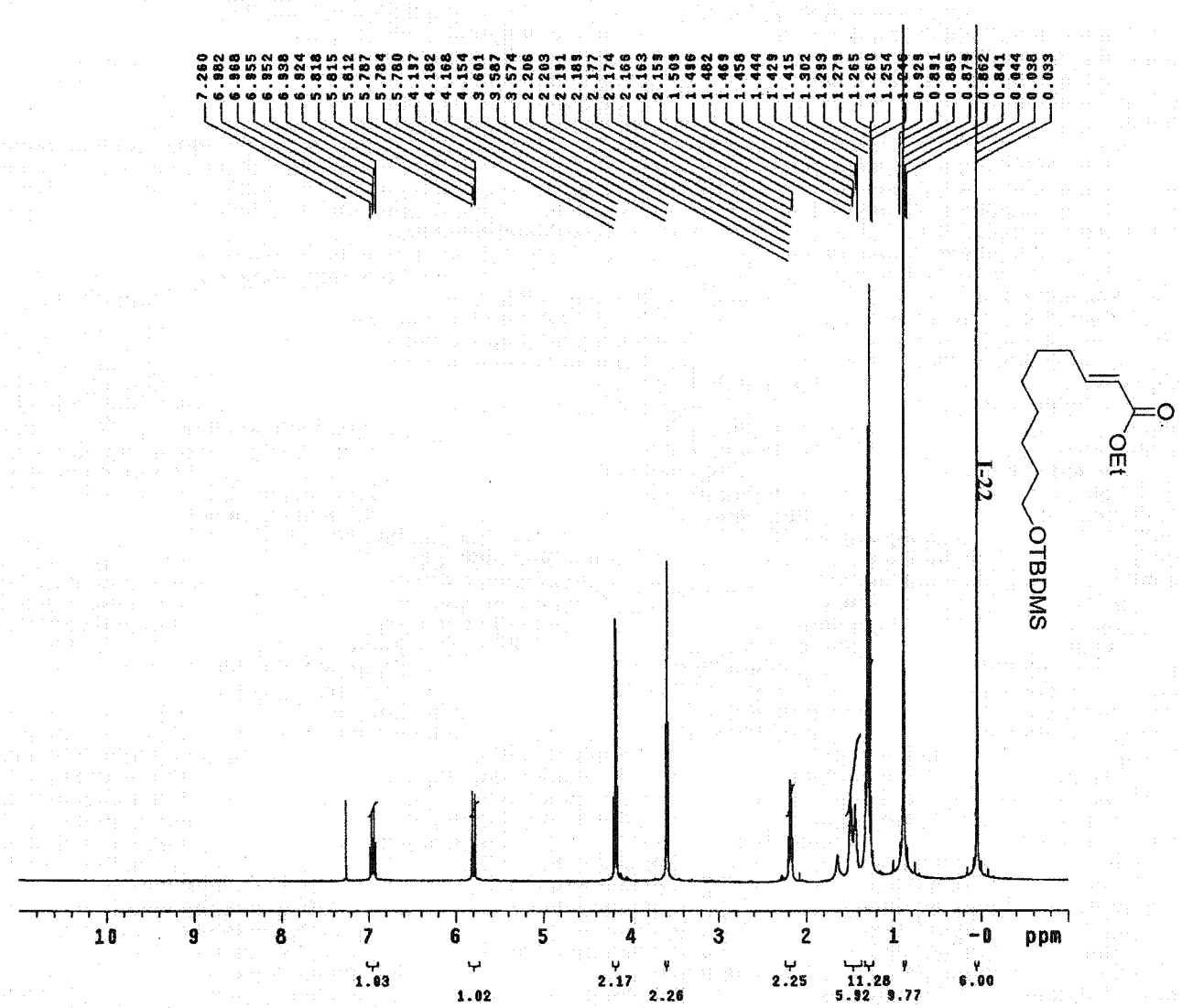
I-21

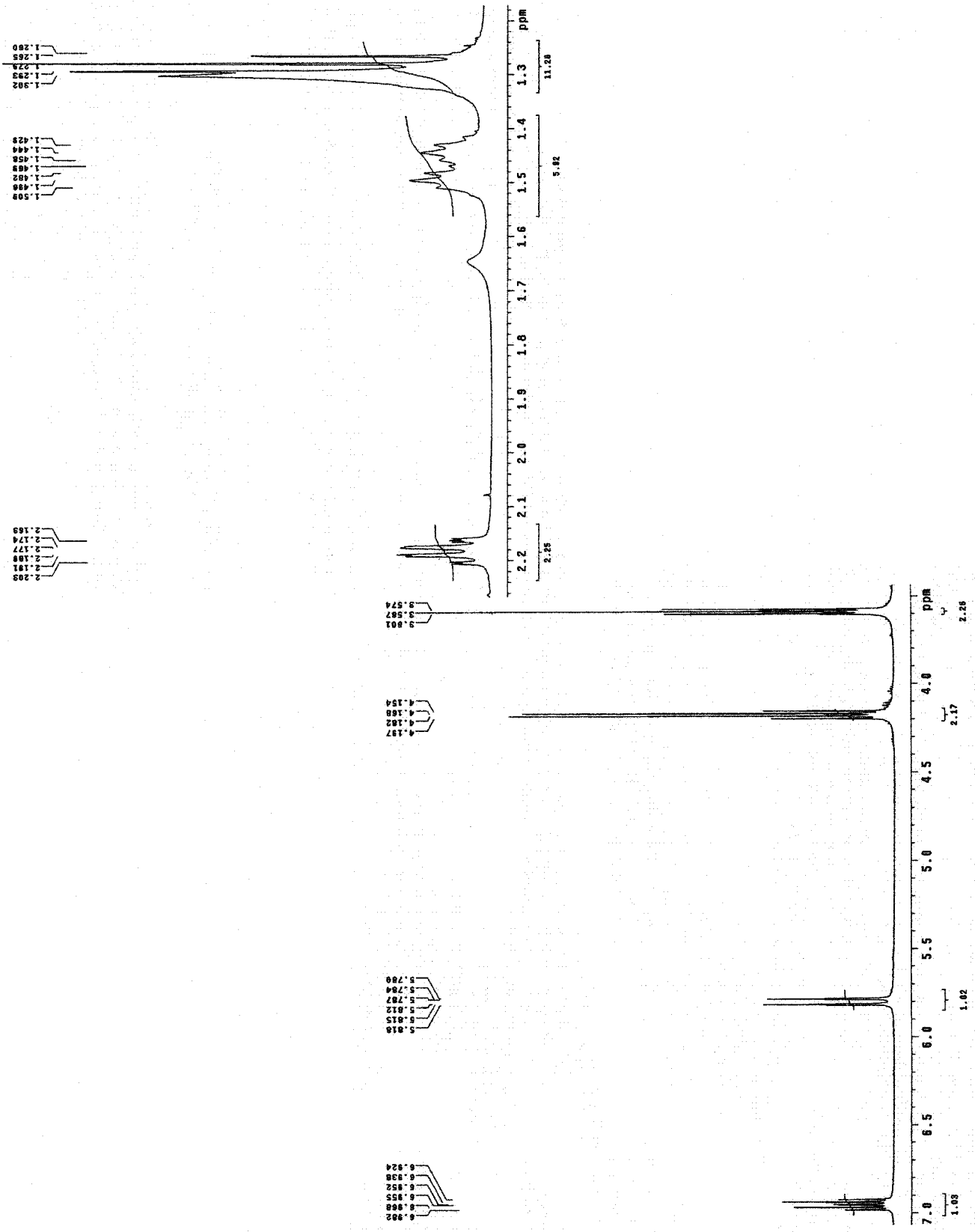
0.033
0.040
0.044
0.046
0.054
0.881
0.887
1.315
1.328
1.487
1.500
1.513
1.610
1.625
1.639
2.400
2.404
2.415
2.418
2.429
2.439
3.577
3.590
3.603
7.260
9.753
9.759
9.763
ppm
0.63
2.05
1.87
2.38
2.22
10.65
6.00

exp1 s2pul
SAMPLE
date Apr 6 2001
solvent CDCl3
file exp
ACQUISITION
sw 5997.0
at 2.049
np 24576
fb 3000
bs 32
d1 1.000
nt 8
ct 8
TRANSMITTER H1
tn
sfrq 499.742
tof 0.1
tpwr 55
pw 4.800
DECOUPLER C13
dn
dof 0
dm nnn
dmm c
dpwr 52
dmf 14122
SPECIAL not used
temp not used
gain
spin 22
hst 0.008
pw90 9.600
alfa 0
FLAGS
fl n
in n
dp y
hs nn
PROCESSING not used
fn DISPLAY
sp -500.2
wp 5997.0
rf1 4128.3
rfp 3628.1
rp -111.1
lp 0.3
PLOT
wc 180
sc 0
vs 150
th 2
nm cdc ph

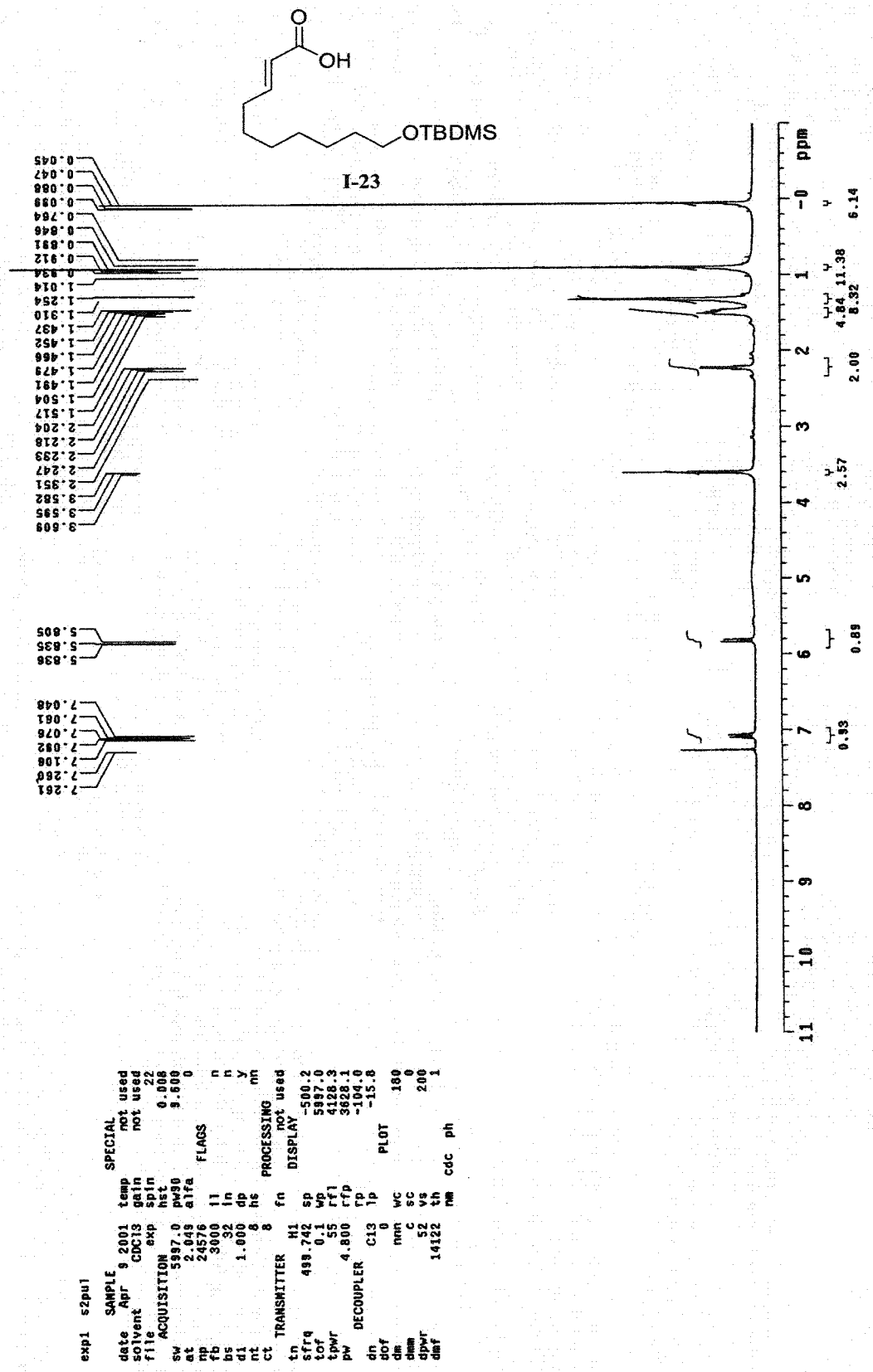

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
User: 1-14-87
INOVA-500 "nmr500"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.043 sec
Width 31409.5 Hz
256 repetitions
OBSERVE C13, 125.6585835 MHz
DECOUPLE H1, 499.7419392 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 8 min, 45 sec
202.677
77.259
77.000
76.748
63.101
43.617
32.695
29.087
25.906
25.555
21.962
18.292
-5.340
220
200
180
160
140
120
100
80
60
40
20
0
ppm

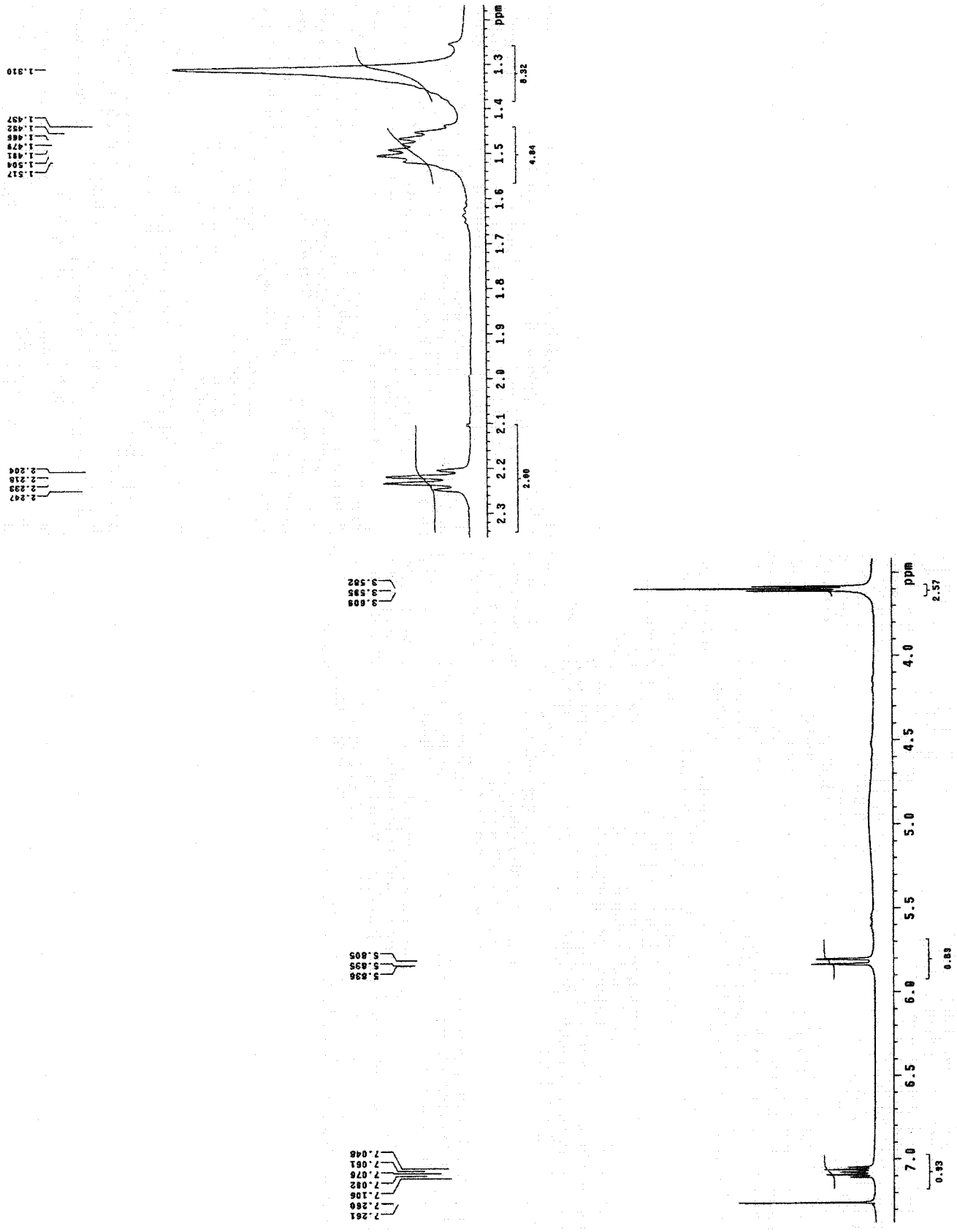
```
exp1  s2pul

      SAMPLE                  SPECIAL
date   Mar 21 2001  temp        not used
solvent       CDCl3  gain        not used
file            exp  spin              22
   ACQUISITION      hst            0.008
sw           5997.0  pw90           9.800
at            2.049  alfa               0
np            24576        FLAGS
fb             3000  il                 n
bs               32  in                 n
d1            1.000  dp                 y
nt                8  hs                nn
ct                8      PROCESSING
   TRANSMITTER      fn          not used
tn               H1       DISPLAY
sfrq        499.742  sp            -500.6
tof             0.1  wp            5997.0
tpwr             57  rfl           4128.7
pw            4.900  rfp           3628.1
    DECOUPLER        rp             23.5
dn              C13  lp             -5.7
dof               0        PLOT
dm              nnn  wc              180
dmm               c  sc                0
dpwr             51  vs              500
dmf           17094  th                4
                     nm  cdc  ph
```

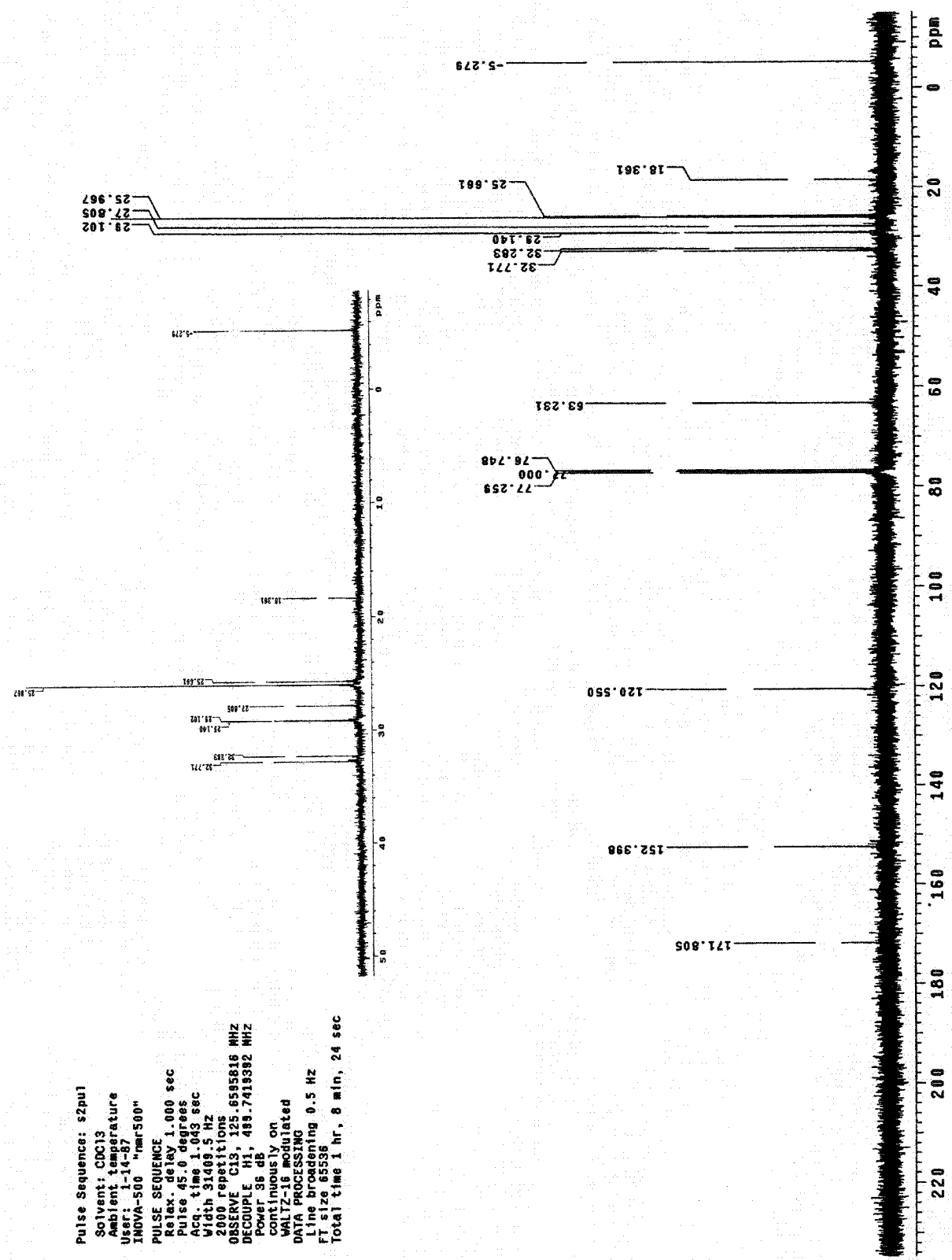


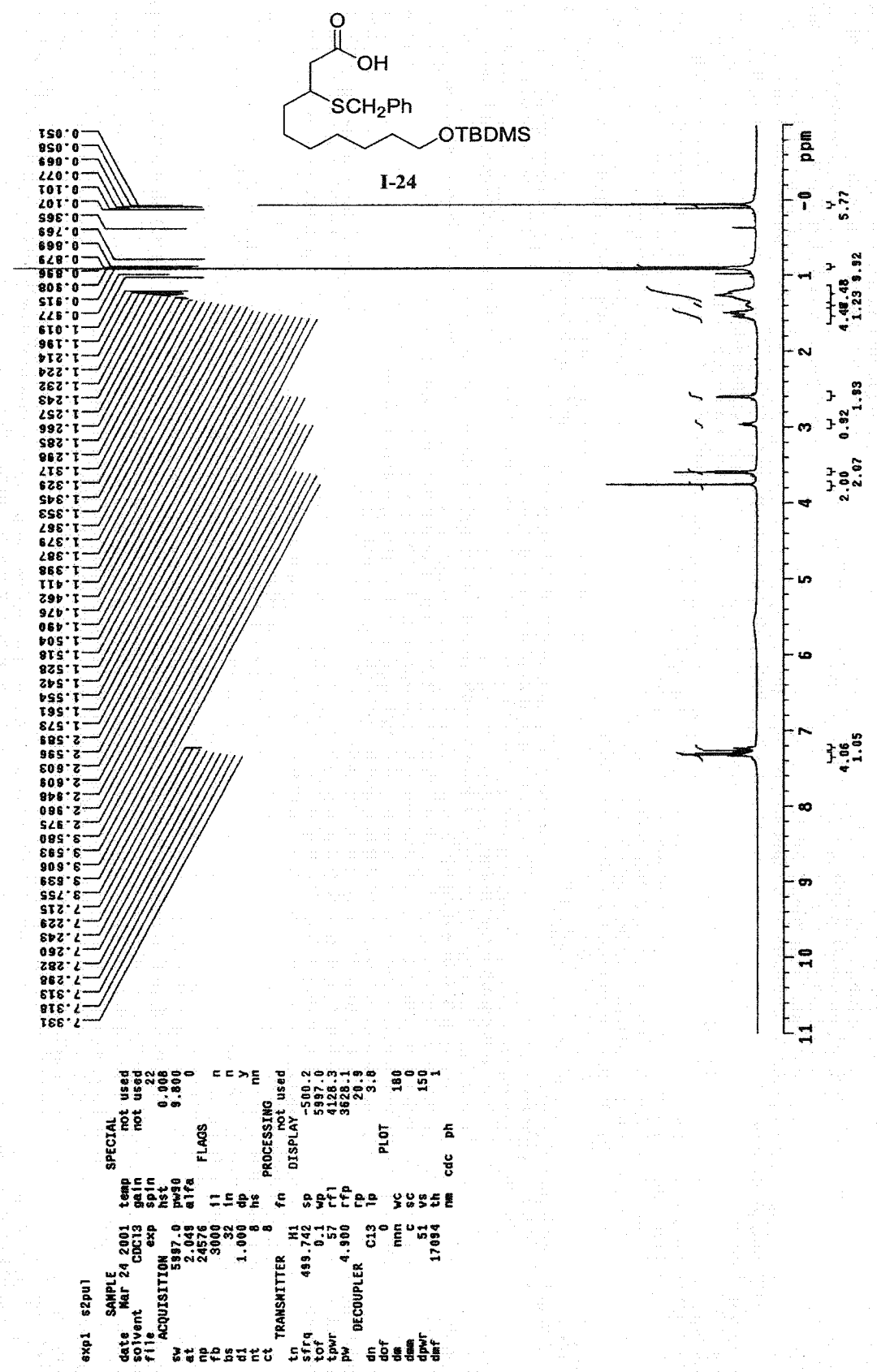


Current Data Parameters
NAME 05L-C13
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20010322
Time 21.03
INSTRUM dpx400
PROBHD 5 mm QNP 1H
PULPROG zgdc30
TD 65536
SOLVENT CDCl3
NS 760
DS 0
SWH 25125.629 Hz
FIDRES 0.383387 Hz
AQ 1.3042164 sec
RG 6502
DW 19.900 usec
DE 4.50 usec
TE 300.0 K
D1 1.00000000 sec
d11 0.03000000 sec
========== CHANNEL f1 ==========
NUC1 13C
P1 5.80 usec
PL1 -6.00 dB
SFO1 100.6227710 MHz
========== CHANNEL f2 ==========
CPDPRG2 waltz16
NUC2 1H
PCPD2 71.00 usec
PL2 120.00 dB
PL12 17.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127680 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 200.000 ppm
F1 20122.55 Hz
F2P -10.000 ppm
F2 -1006.13 Hz
PPMCM 10.50000 ppm/cm
HZCM 1056.43408 Hz/cm
ppm
0
25
50
75
100
125
150
175


O
OH
OTBDMS
I-23
ppm

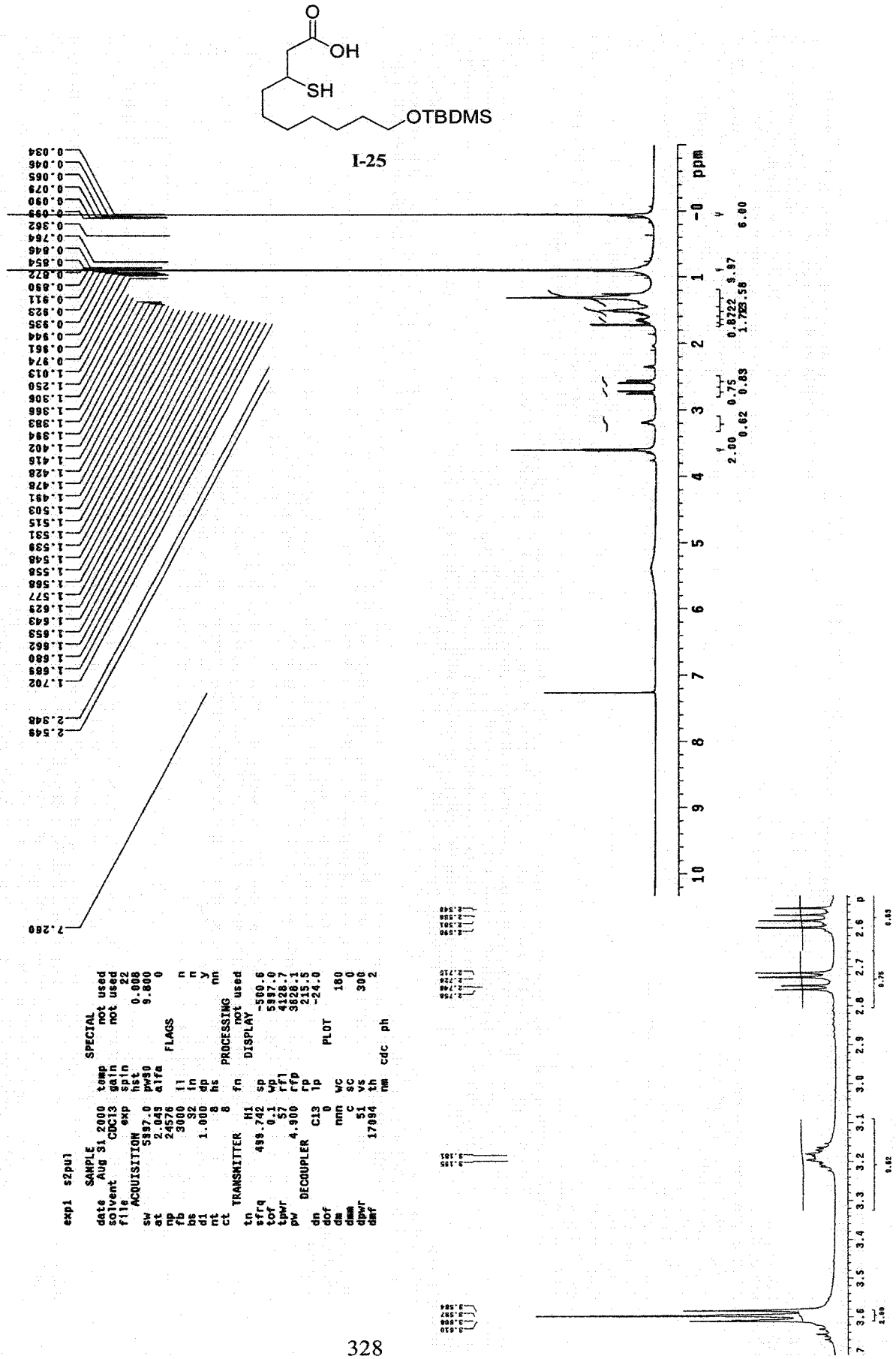

1.310
1.457
1.462
1.466
1.479
1.491
1.504
1.517
2.047
2.203
2.216
2.233
2.264
3.582
3.595
3.609
5.805
5.695
5.836
7.046
7.061
7.076
7.082
7.106
7.260
7.261
ppm
2.57
0.83
0.83


Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
User: 1-14-87
INOVA-500 "mar500"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.043 sec
Width 31409.5 Hz
2000 repetitions
OBSERVE C13, 125.6595816 MHz
DECOUPLE H1, 499.7418392 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 1 hr, 8 min, 24 sec
-5.279
18.361
25.661
25.967
27.805
28.102
29.140
32.283
32.771
63.281
76.748
77.000
77.259
120.550
152.398
171.805
ppm

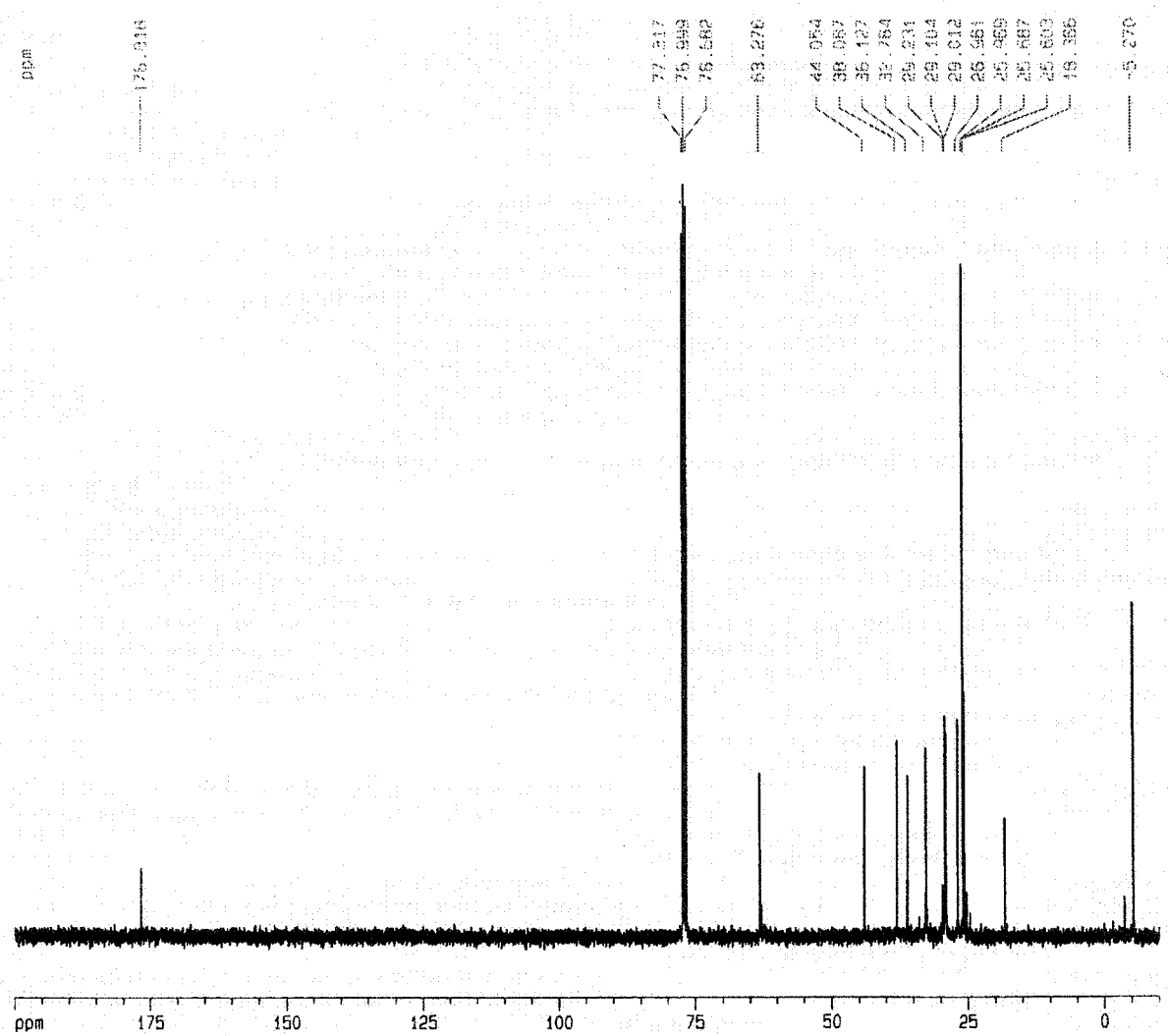

O
OH
SCH2Ph
OTBDMS
I-24
ppm

7.374
7.364
7.331
7.318
7.313
7.296
7.282
7.269
7.261
7.260
7.243
7.229
7.215
7.4
7.3
7.2
4.06
1.05
9.755
9.606
9.593
9.580
3.85
3.80
3.75
3.70
3.65
3.60
3.55
2.00
2.07
2.984
2.975
2.960
2.948
2.934
2.641
2.627
2.619
2.609
2.603
2.586
2.568
2.571
2.557
3.0
2.9
2.8
2.7
2.6
2.5
0.92
1.93

Current Data Parameters
NAME 13b-C13
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20000830
Time 12.30
INSTRUM dpx400
PROBHD 5 mm QNP 1H
PULPROG zgdc30
TD 65536
SOLVENT CDCl3
NS 400
DS 0
SWH 25125.629 Hz
FIDRES 0.383387 Hz
AQ 1.3042164 sec
RG 6502
DW 19.900 usec
DE 4.50 usec
TE 300.0 K
D1 1.00000000 sec
d11 0.03000000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 5.80 usec
PL1 -6.00 dB
SF01 100.6227710 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 71.00 usec
PL2 120.00 dB
PL12 17.00 dB
SF02 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127726 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 200.000 ppm
F1 20122.55 Hz
F2P -10.000 ppm
F2 -1006.13 Hz
PPMCM 10.50000 ppm/cm
HZCM 1056.43408 Hz/cm
ppm
0
25
50
75
100
125
150
175



I-25



Current Data Parameters
NAME 09L-C13
EXPNO 1
PROCNO 1

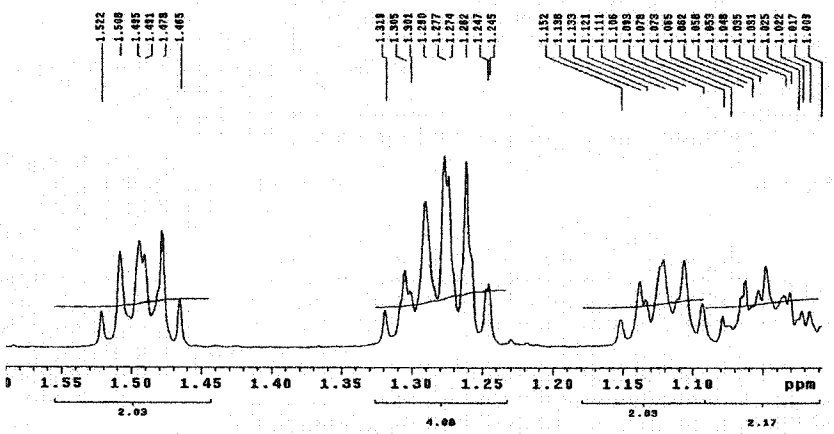
F2 - Acquisition Parameters
Date_ 20000901
Time 16.29
INSTRUM dpx400
PROBHD 5 mm QNP 1H
PULPROG zgdc30
TD 65536
SOLVENT CDCl3
NS 562
DS 0
SWH 25125.629 Hz
FIDRES 0.383387 Hz
AQ 1.3042164 sec
RG 6502
DW 19.900 usec
DE 4.50 usec
TE 300.0 K
D1 1.00000000 sec
d11 0.03000000 sec

========== CHANNEL f1 ==========
NUC1 13C
P1 5.80 usec
PL1 -6.00 dB
SFO1 100.6227710 MHz

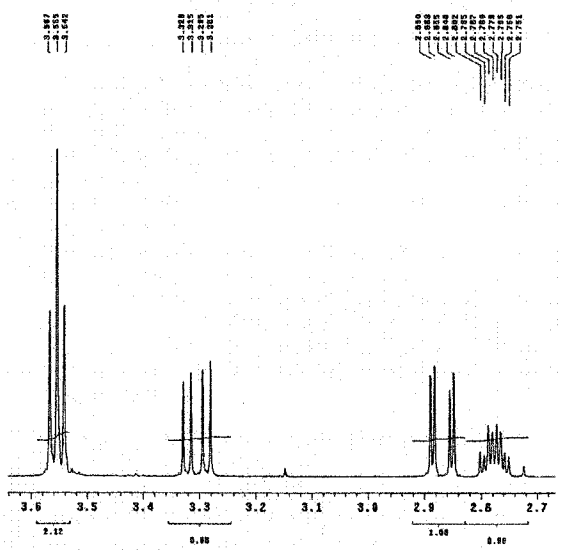
========== CHANNEL f2 ==========
CPDPRG2 waltz16
NUC2 1H
PCPD2 71.00 usec
PL2 120.00 dB
PL12 17.00 dB
SFO2 400.1316005 MHz

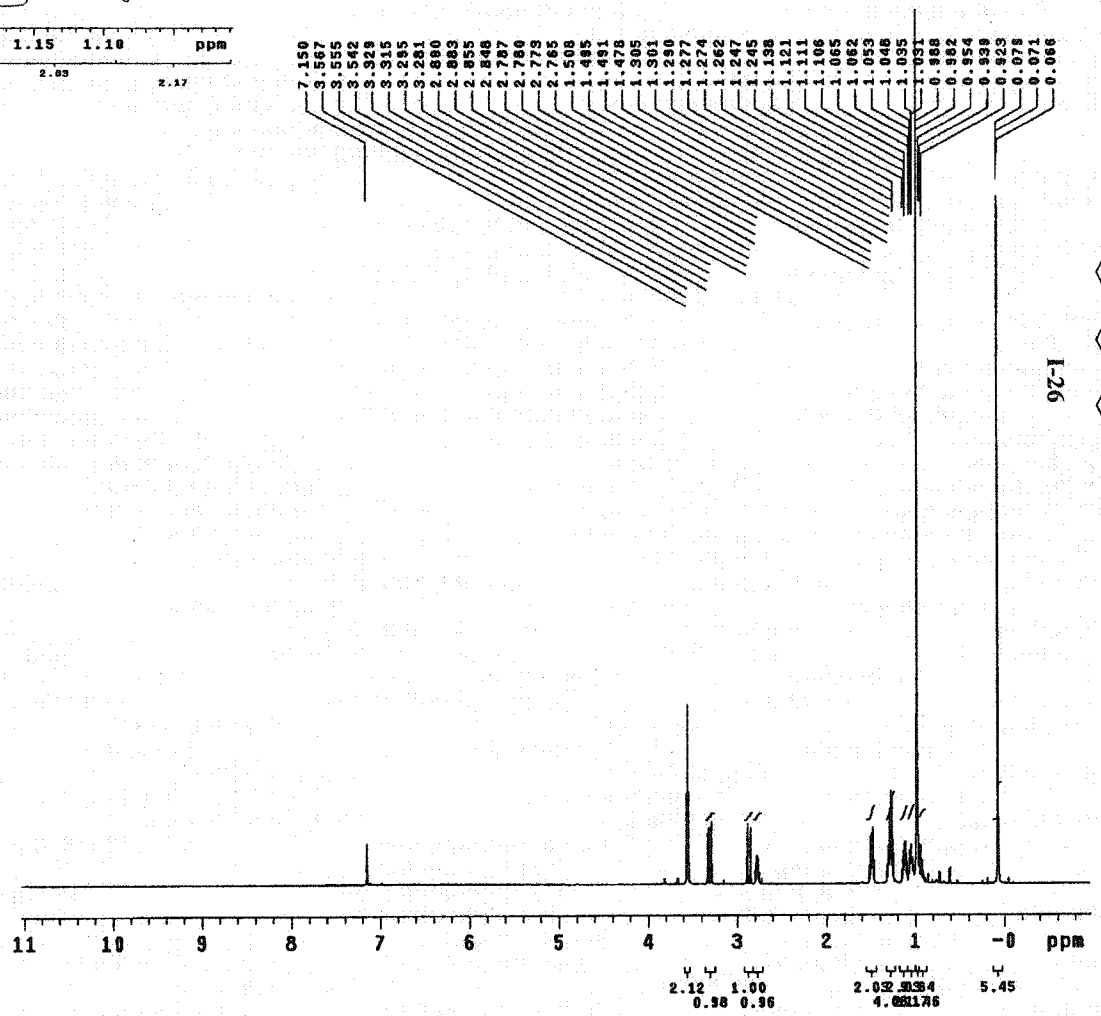
F2 - Processing parameters
SI 32768
SF 100.6127718 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

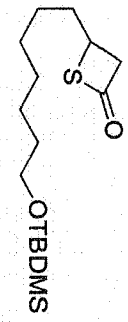
1D NMR plot parameters
CX 20.00 cm
F1P 200.000 ppm
F1 20122.55 Hz
F2P -10.000 ppm
F2 -1006.13 Hz
PPMCM 10.50000 ppm/cm
HZCM 1056.43408 Hz/cm



SAMPLE		SPECIAL	
date	Mar 28 2001	temp	not used
solvent	C6D6	gain	not used
file	exp	spin	22
ACQUISITION		hst	0.008
sw	5997.0	pw90	9.600
at	2.049	alfa	0
np	24576	FLAGS	
fb	3000	il	n
bs	32	in	n
di	1.000	dp	y
nt	8	hs	nn
ct	8	PROCESSING	
TRANSMITTER		fn	not used
tn	H1	DISPLAY	
sfrq	499.742	sp	-483.8
tof	0.1	wp	5997.0
tpwr	55	rfl	4056.8
pw	4.800	rfp	3573.1
DECOUPLER		rp	-142.9
dn	C13	lp	-0.0
dof	0	PLOT	
dm	nnn	wc	180
dmm	c	sc	0
dpwr	52	vs	150
dmf	14122	th	4
		nm	cdc ph





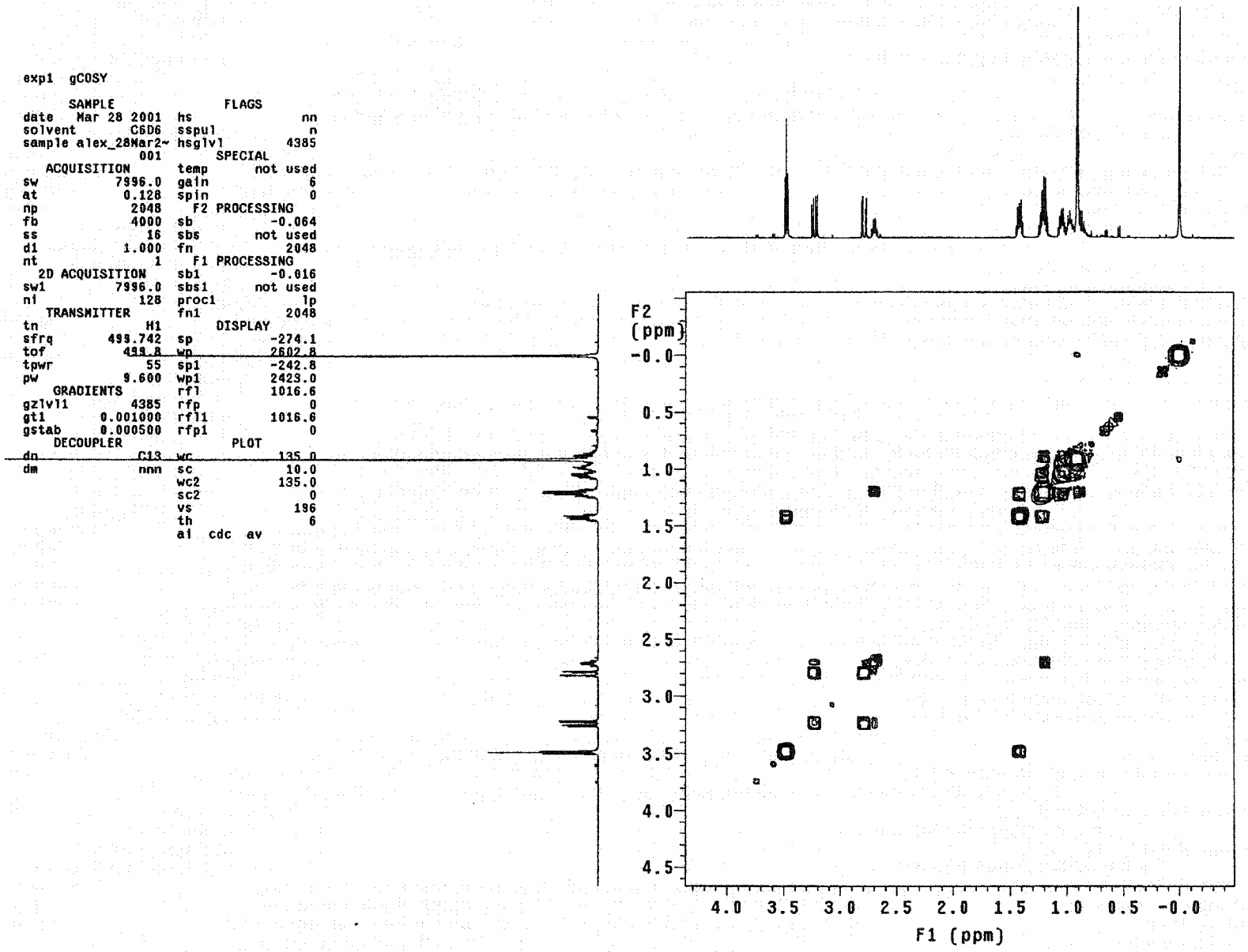


330

I-26

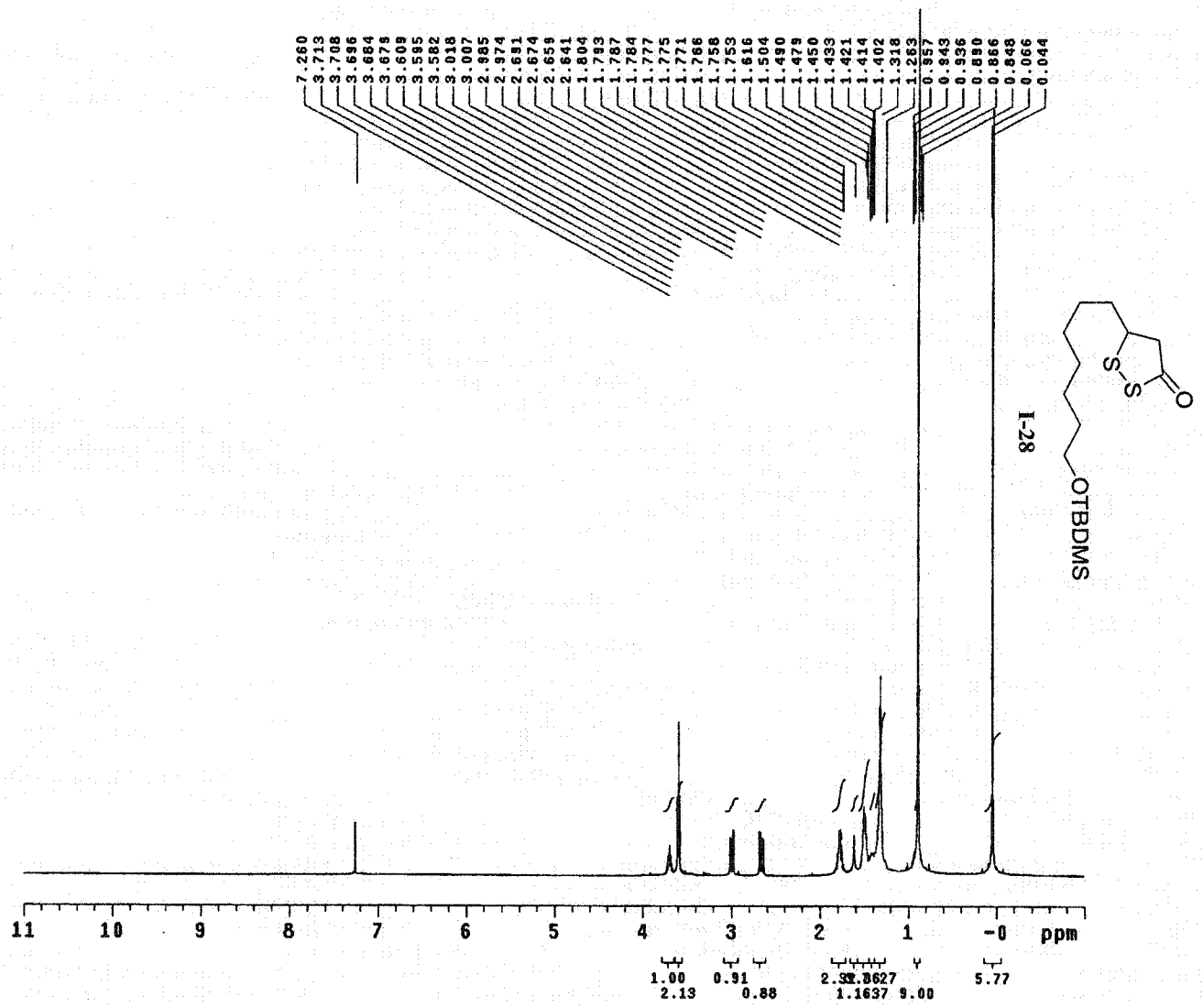
exp1 gCOSY

SAMPLE		FLAGS	
date	Mar 28 2001	hs	nn
solvent	C6D6	sspul	n
sample	alex_28Mar2~001	hsglvl	4385
ACQUISITION		SPECIAL	
sw	7996.0	temp	not used
at	0.128	gain	6
np	2048	spin	0
fb	4000	F2 PROCESSING	
ss	16	sb	-0.064
d1	1.000	sbs	not used
nt	1	fn	2048
2D ACQUISITION		F1 PROCESSING	
sw1	7996.0	sb1	-0.016
ni	128	sbs1	not used
TRANSMITTER		proc1	lp
tn	H1	fn1	2048
sfrq	499.742	DISPLAY	
tof	499.8	sp	-274.1
tpwr	55	wp	2602.8
pw	9.600	sp1	-242.8
GRADIENTS		wp1	2423.0
gzlvl1	4385	rfl	1016.6
gt1	0.001000	rfp	0
gstab	0.000500	rfl1	1016.6
DECOUPLER		rfp1	0
dn	C13	PLOT	
dm	nnn	wc	135.0
		sc	10.0
		wc2	135.0
		sc2	0
		vs	196
		th	6
		al cdc av	

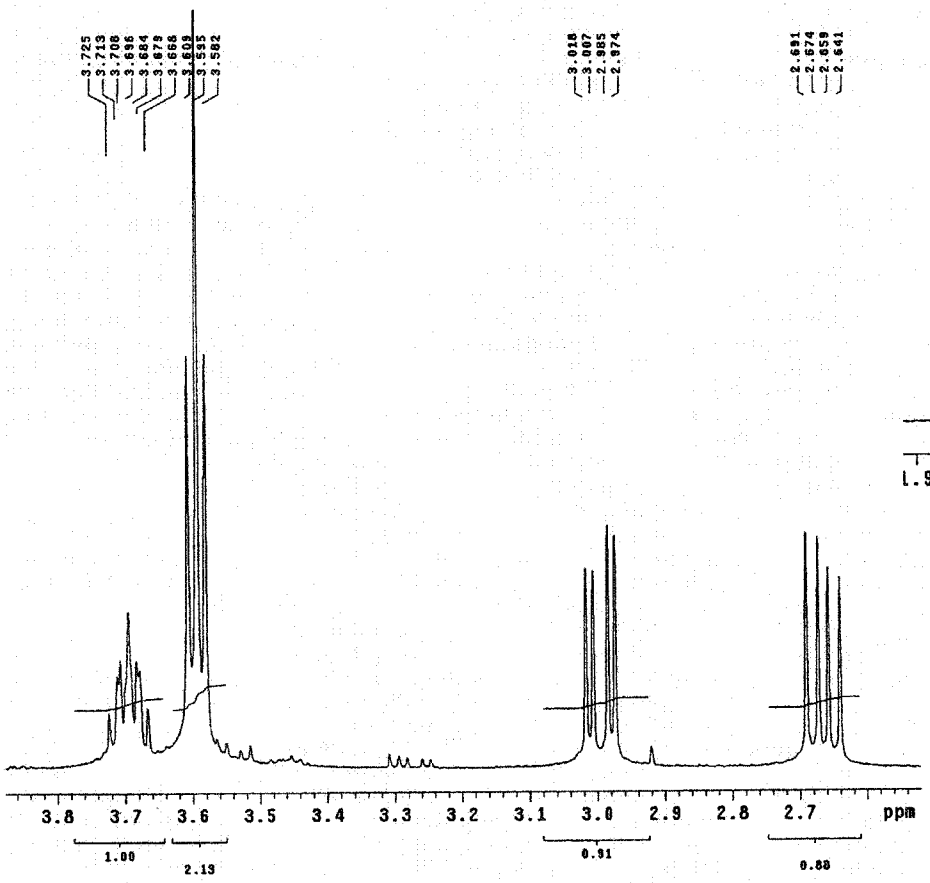


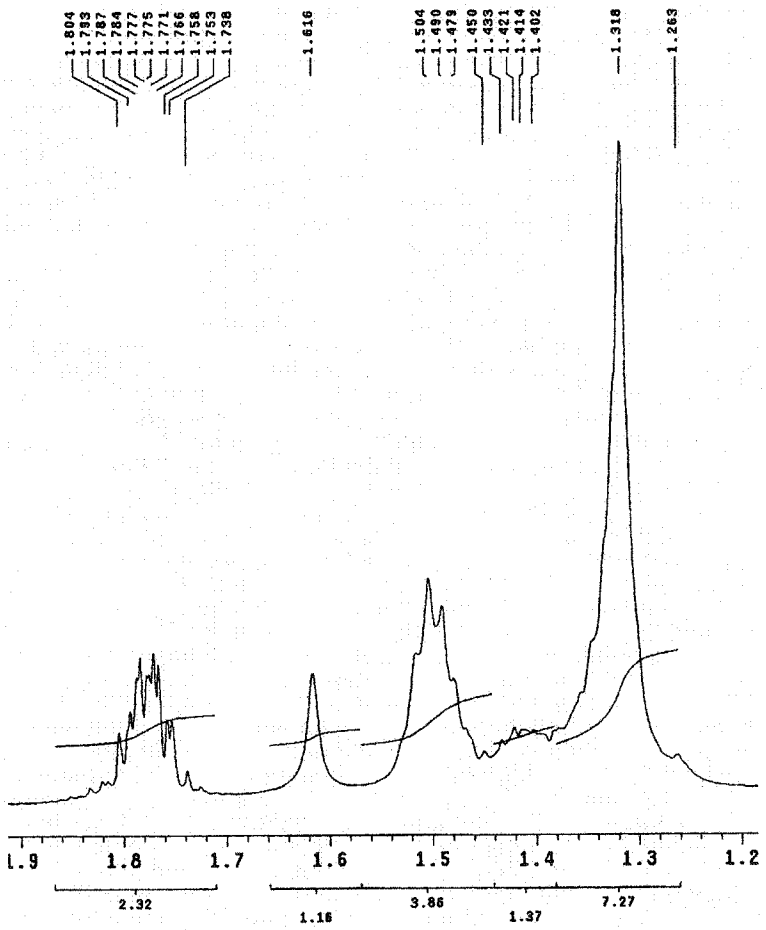
Current Data Parameters
NAME C13bact2.QNP
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20010327
Time 21.31
INSTRUM dpx400
PROBHD 5 mm QNP 1H
PULPROG zgdc30
TD 65536
SOLVENT C6D6
NS 402
DS 0
SWH 65359.477 Hz
FIDRES 0.997306 Hz
AQ 0.5014004 sec
RG 5792.6
DW 7.650 usec
DE 4.50 usec
TE 300.0 K
D1 1.00000000 sec
d11 0.03000000 sec
========== CHANNEL f1 ==========
NUC1 13C
P1 5.80 usec
PL1 -6.00 dB
SFO1 100.6429128 MHz
========== CHANNEL f2 ==========
CPDPRG2 waltz16
NUC2 1H
PCPD2 71.00 usec
PL2 120.00 dB
PL12 17.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6126787 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 220.000 ppm
F1 22134.79 Hz
F2P -20.000 ppm
F2 -2012.25 Hz
PPMCM 12.00000 ppm/cm
HZCM 1207.35205 Hz/cm
-4.444
19.204
26.853
29.494
29.988
30.190
33.574
33.875
38.533
61.610
63.893
128.455
128.694
128.932
189.579
ppm
ppm

```
expl  s2pul

      SAMPLE                  SPECIAL
date   Mar 30 2001   temp        not used
solvent       CDCl3  gain        not used
file            exp  spin              22
   ACQUISITION       hst            0.008
sw           5997.0  pw90           9.600
at            2.049  alfa               0
np            24576        FLAGS
fb             3000  il                 n
bs               32  in                 n
d1            1.000  dp                 y
nt                8  hs                nn
ct                8       PROCESSING
   TRANSMITTER       fn          not used
tn               H1        DISPLAY
sfrq        499.742  sp            -500.2
tof             0.1  wp            5997.0
tpwr             55  rfl           4128.3
pw            4.800  rfp           3628.1
    DECOUPLER        rp            -110.7
dn              C13  lp               2.0
dof               0         PLOT
dm              nnn  wc               180
dmm               c  sc                 0
dpwr             52  vs               200
dmf           14122  th                 2
                     nm  cdc  ph
```



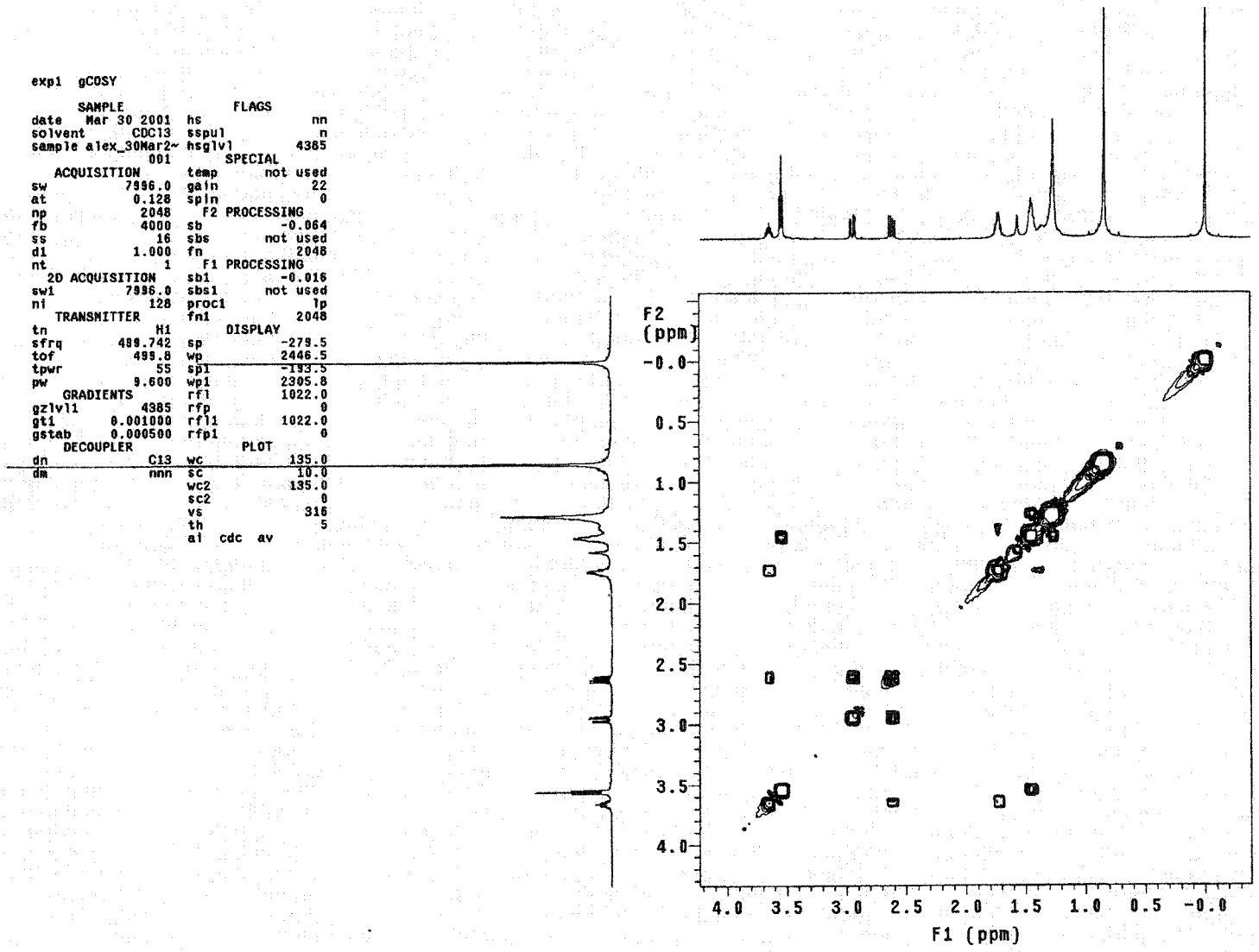
I-28

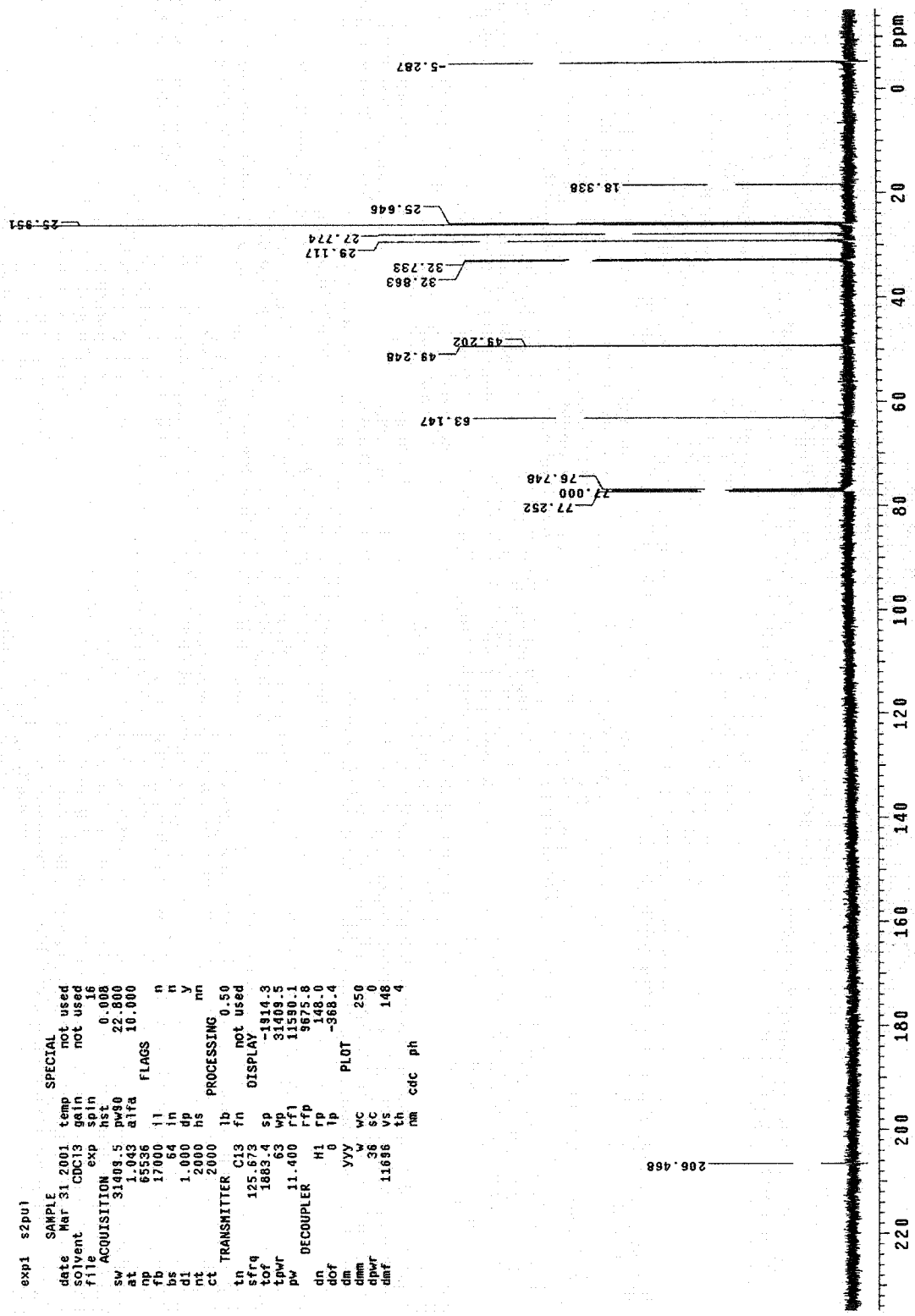

3.725
3.713
3.708
3.696
3.684
3.679
3.668
3.603
3.595
3.582
3.018
3.007
2.985
2.974
2.691
2.674
2.659
2.641
3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 ppm
1.00
2.13
0.91
0.88


1.804
1.793
1.787
1.784
1.777
1.775
1.771
1.766
1.758
1.753
1.738
1.616
1.504
1.490
1.479
1.450
1.433
1.421
1.414
1.402
1.318
1.263
1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2
2.32
1.16
3.86
1.37
7.27

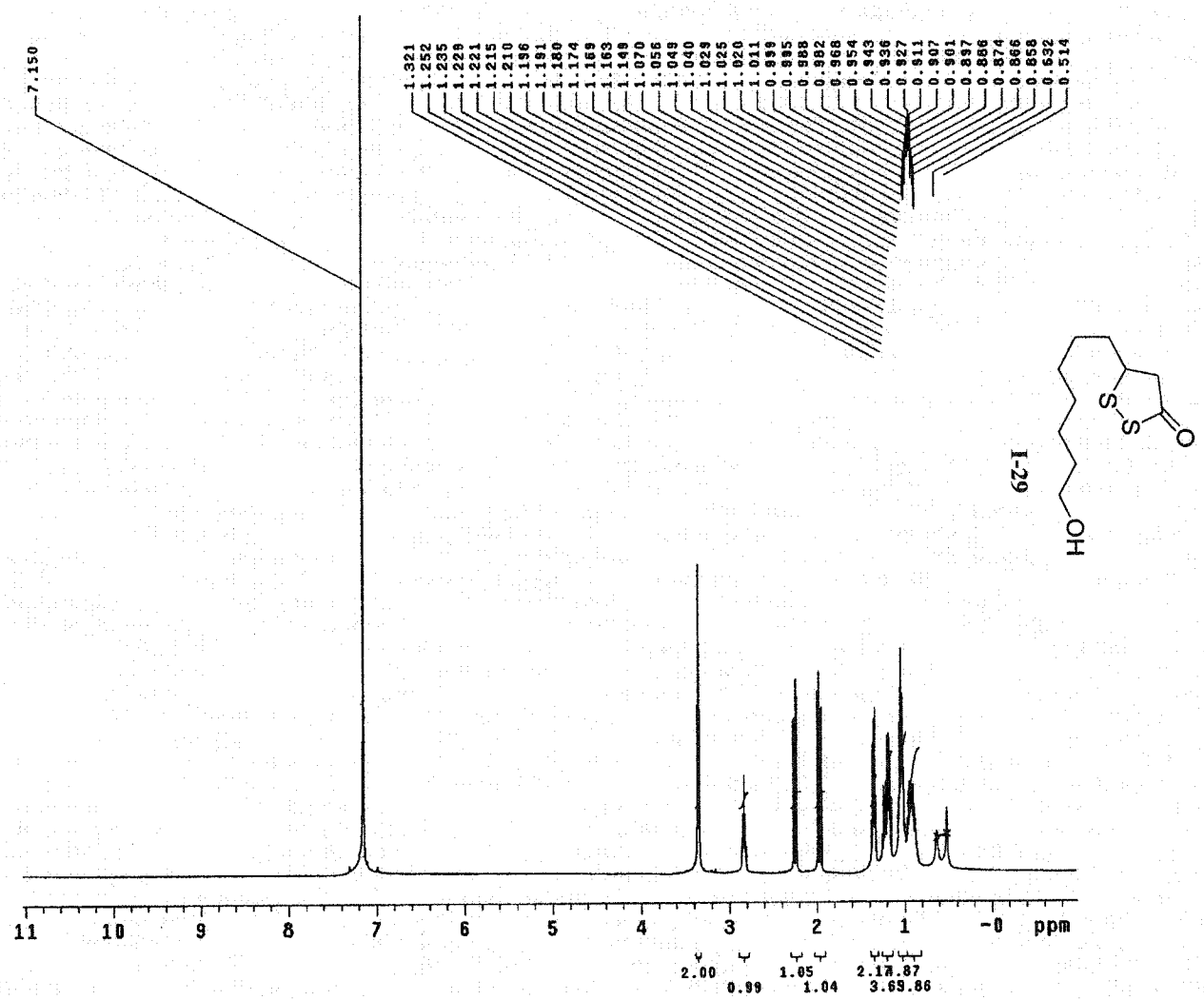
exp1 gCOSY

SAMPLE		FLAGS	
date	Mar 30 2001	hs	nn
solvent	CDCl3	sspul	n
sample	alex_30Mar2~001	hsglvl	4365
ACQUISITION		SPECIAL	
sw	7996.0	temp	not used
at	0.128	gain	22
np	2048	spin	0
fb	4000	F2 PROCESSING	
ss	16	sb	-0.064
d1	1.000	sbs	not used
nt	1	fn	2048
2D ACQUISITION		F1 PROCESSING	
sw1	7996.0	sb1	-0.016
ni	128	sbs1	not used
TRANSMITTER		proc1	lp
tn	H1	fn1	2048
sfrq	499.742	DISPLAY	
tof	499.8	sp	-279.5
tpwr	55	wp	2446.5
pw	9.600	sp1	-193.5
GRADIENTS		wp1	2305.8
gzlvl1	4385	rf1	1022.0
gt1	0.001000	rfp	0
gstab	0.000500	rf11	1022.0
DECOUPLER		rfp1	0
dn	C13	PLOT	
dm	nnn	wc	135.0
		sc	10.0
		wc2	135.0
		sc2	0
		vs	316
		th	5
		al cdc av	

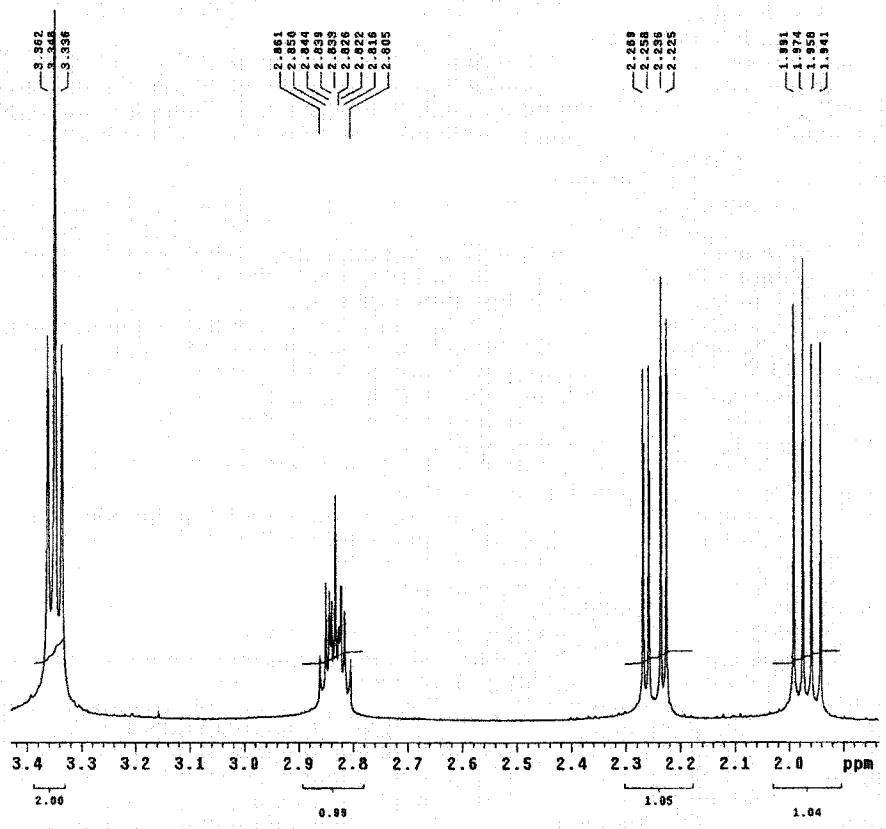


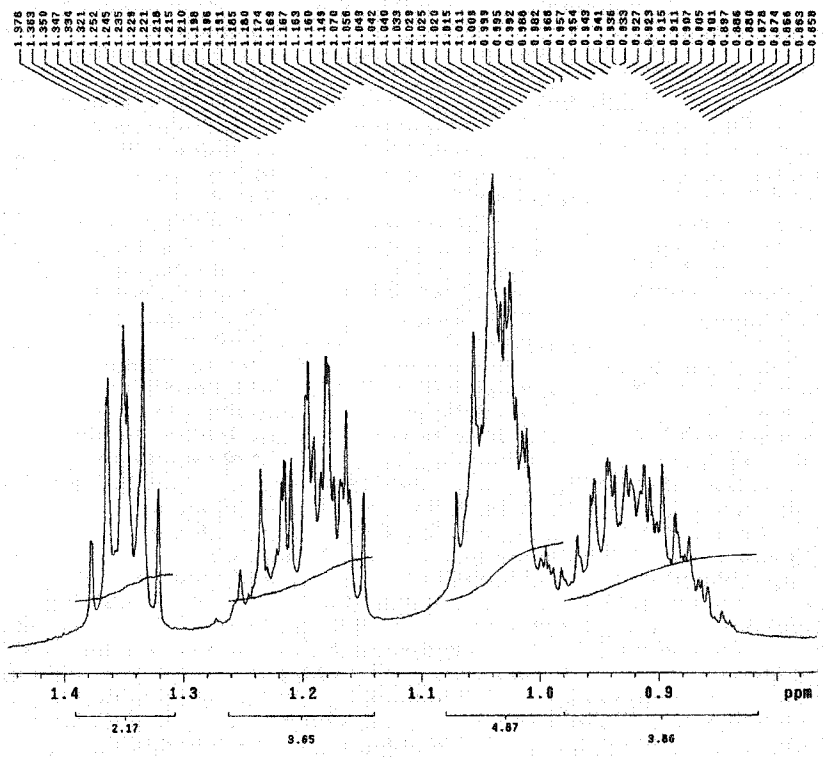

exp1 s2pul
SAMPLE
date Mar 31 2001
solvent CDCl3
file
ACQUISITION
sw 31409.5
at 1.043
np 65536
fb 17000
bs 64
d1 1.000
nt 2000
ct 2000
TRANSMITTER
tn C13
sfrq 125.673
tof 1883.4
tpwr 63
pw 11.400
DECOUPLER
dn H1
dof 0
dm yyy
dmm w
dpwr 36
dmf 11696
temp
gain
spin
hst
pw90
atfa
FLAGS
il
in
dp
hs
PROCESSING
lb
fn
sp
wp
rfl
rfp
rp
lp
PLOT
wc
sc
vs
th
nm
SPECIAL
not used
not used
16
0.008
22.800
10.000
n
n
y
nn
0.50
not used
DISPLAY
-1914.3
31409.5
11590.1
9675.8
148.0
-368.4
250
0
148
4
cdc ph
206.468
77.252
77.000
76.748
63.147
49.202
49.248
32.863
32.733
29.117
27.774
25.646
25.351
18.338
-5.287
220 200 180 160 140 120 100 80 60 40 20 0 ppm

```
exp1  s2pul

      SAMPLE                SPECIAL
date   Apr  2 2001   temp       not used
solvent        C6D6  gain       not used
file            exp  spin             22
   ACQUISITION       hst           0.008
sw           5997.0  pw90          9.600
at            2.049  alfa              0
np            24576        FLAGS
fb             3000  il                n
bs               32  in                n
d1            1.000  dp                y
nt                8  hs               nn
ct                8      PROCESSING
   TRANSMITTER       fn         not used
tn               H1       DISPLAY
sfrq        499.742  sp          -483.8
tof             0.1  wp          5997.0
tpwr             55  rfl         4056.9
pw            4.800  rfp         3573.1
    DECOUPLER        rp          -156.9
dn              C13  lp             2.0
dof               0         PLOT
dm              nnn  wc             180
dmm               c  sc               0
dpwr             52  vs             144
dmf           14122  th               4
                     nm  cdc  ph
```



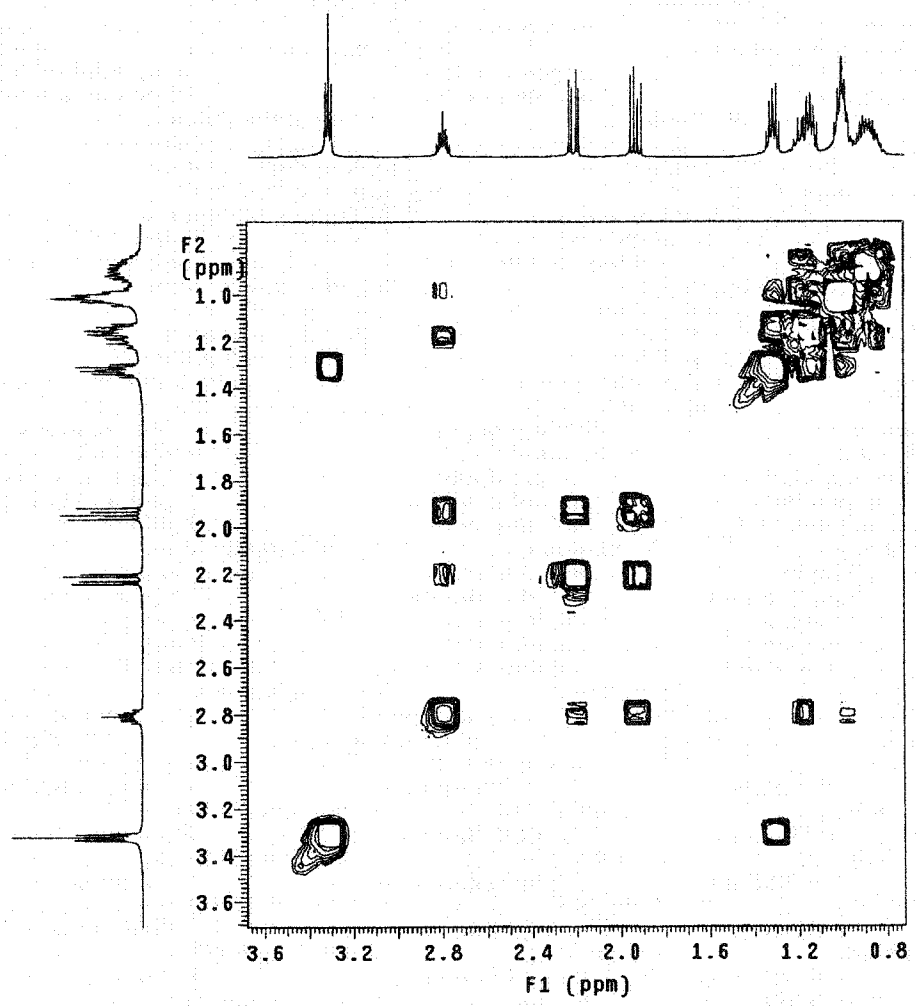
I-29

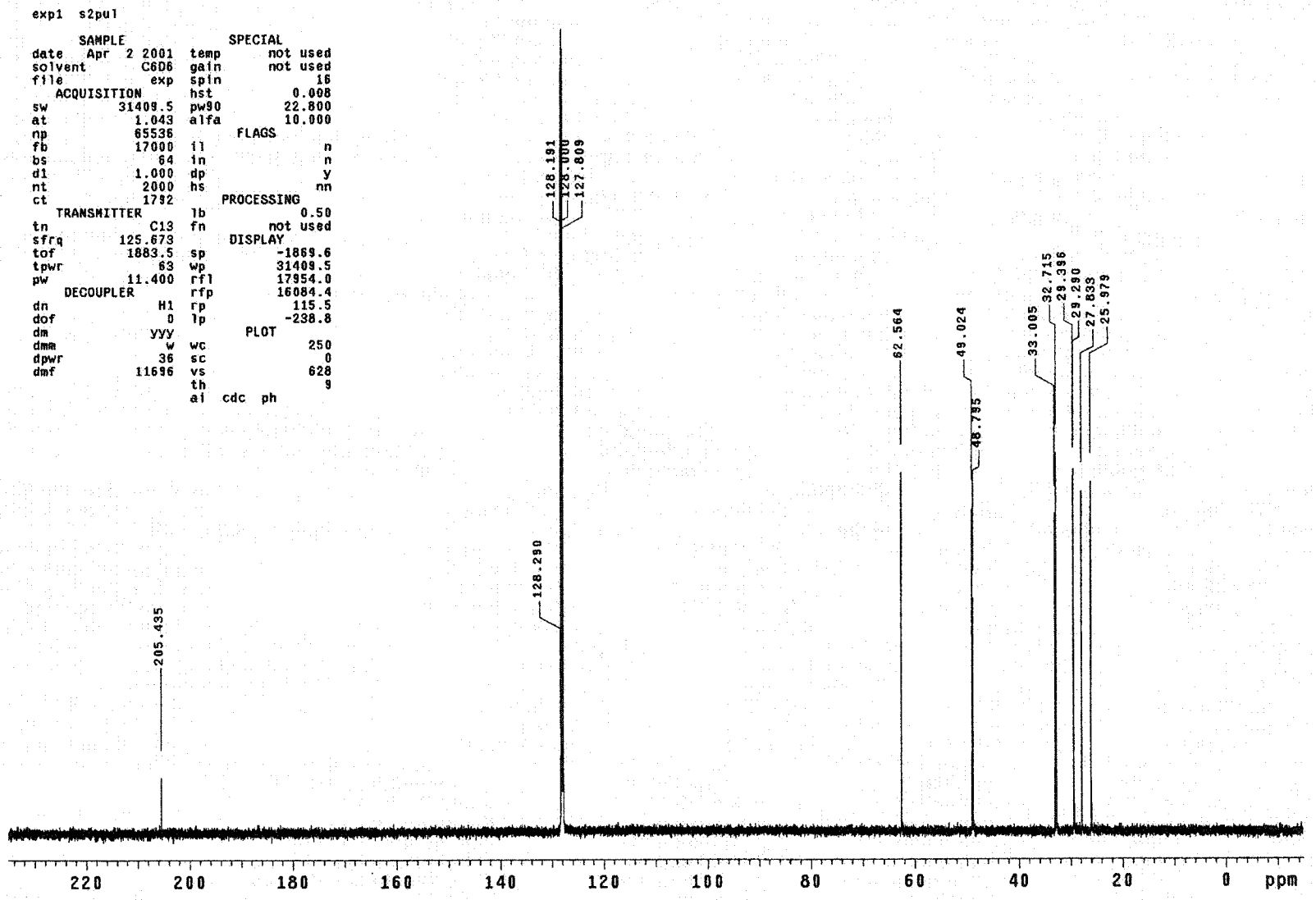

3.362
3.348
3.336
2.861
2.850
2.844
2.839
2.833
2.826
2.822
2.816
2.805
2.269
2.258
2.236
2.225
1.991
1.974
1.958
1.941
3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1 2.0 ppm
2.00
0.88
1.05
1.04

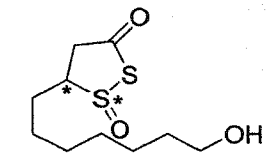

1.376
1.363
1.350
1.347
1.334
1.321
1.252
1.245
1.235
1.229
1.221
1.218
1.215
1.210
1.198
1.188
1.181
1.165
1.160
1.174
1.168
1.167
1.163
1.160
1.149
1.070
1.056
1.049
1.042
1.040
1.033
1.029
1.025
1.020
1.015
1.011
1.001
0.999
0.995
0.992
0.986
0.982
0.968
0.957
0.954
0.949
0.941
0.938
0.933
0.927
0.923
0.915
0.911
0.907
0.905
0.901
0.897
0.886
0.880
0.878
0.874
0.866
0.863
0.858
1.4 1.3 1.2 1.1 1.0 0.9 ppm
2.17
3.65
4.87
3.86

exp1 gCOSY

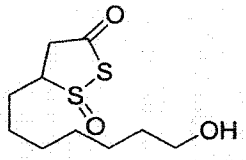
SAMPLE		FLAGS	
date	Apr 2 2001	hs	nn
solvent	C6D6	sspul	n
sample	alex_02Apr2~ 001	hsglvl	4385
ACQUISITION		SPECIAL	
sw	7996.0	temp	not used
at	0.128	gain	30
np	2048	spin	0
fb	4000	F2 PROCESSING	
ss	16	sb	-0.064
d1	1.000	sbs	not used
nt	1	fn	2048
2D ACQUISITION		F1 PROCESSING	
sw1	7996.0	sb1	-0.016
ni	128	sbs1	not used
TRANSMITTER		proc1	lp
tn	H1	fn1	2048
sfrq	499.742	DISPLAY	
tof	499.8	sp	344.8
tpwr	55	wp	1508.5
pw	9.600	sp1	360.5
GRADIENTS		wp1	1477.3
gzlvl1	4385	rf1	988.6
gt1	0.001000	rfp	0
gstab	0.000500	rfl1	989.6
DECOUPLER		rfp1	0
dn	C13	PLOT	
dm	nnn	wc	135.0
		sc	10.0
		wc2	135.0
		sc2	0
		vs	222
		th	6
		ai cdc av	



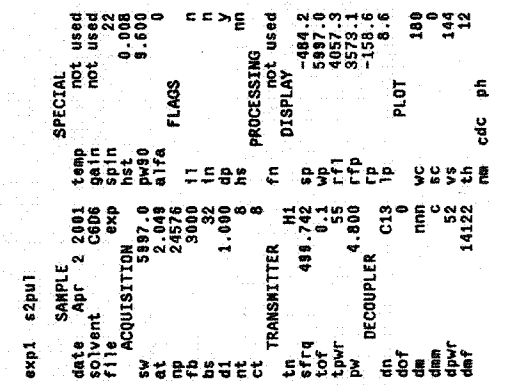

exp1 s2pul
SAMPLE
date Apr 2 2001
solvent C6D6
file exp
ACQUISITION
sw 31409.5
at 1.043
np 65536
fb 17000
bs 64
d1 1.000
nt 2000
ct 1792
TRANSMITTER
tn C13
sfrq 125.673
tof 1883.5
tpwr 63
pw 11.400
DECOUPLER
dn H1
dof 0
dm yyy
dmm w
dpwr 36
dmf 11696
SPECIAL
temp not used
gain not used
spin 16
hst 0.008
pw90 22.800
alfa 10.000
FLAGS
il n
in n
dp y
hs nn
PROCESSING
lb 0.50
fn not used
DISPLAY
sp -1869.6
wp 31409.5
rfl 17954.0
rfp 16084.4
rp 115.5
lp -238.8
PLOT
wc 250
sc 0
vs 628
th 9
ai cdc ph
205.435
128.290
128.191
128.000
127.809
62.564
49.024
48.795
33.005
32.715
29.396
29.290
27.833
25.979
220
200
180
160
140
120
100
80
60
40
20
0
ppm

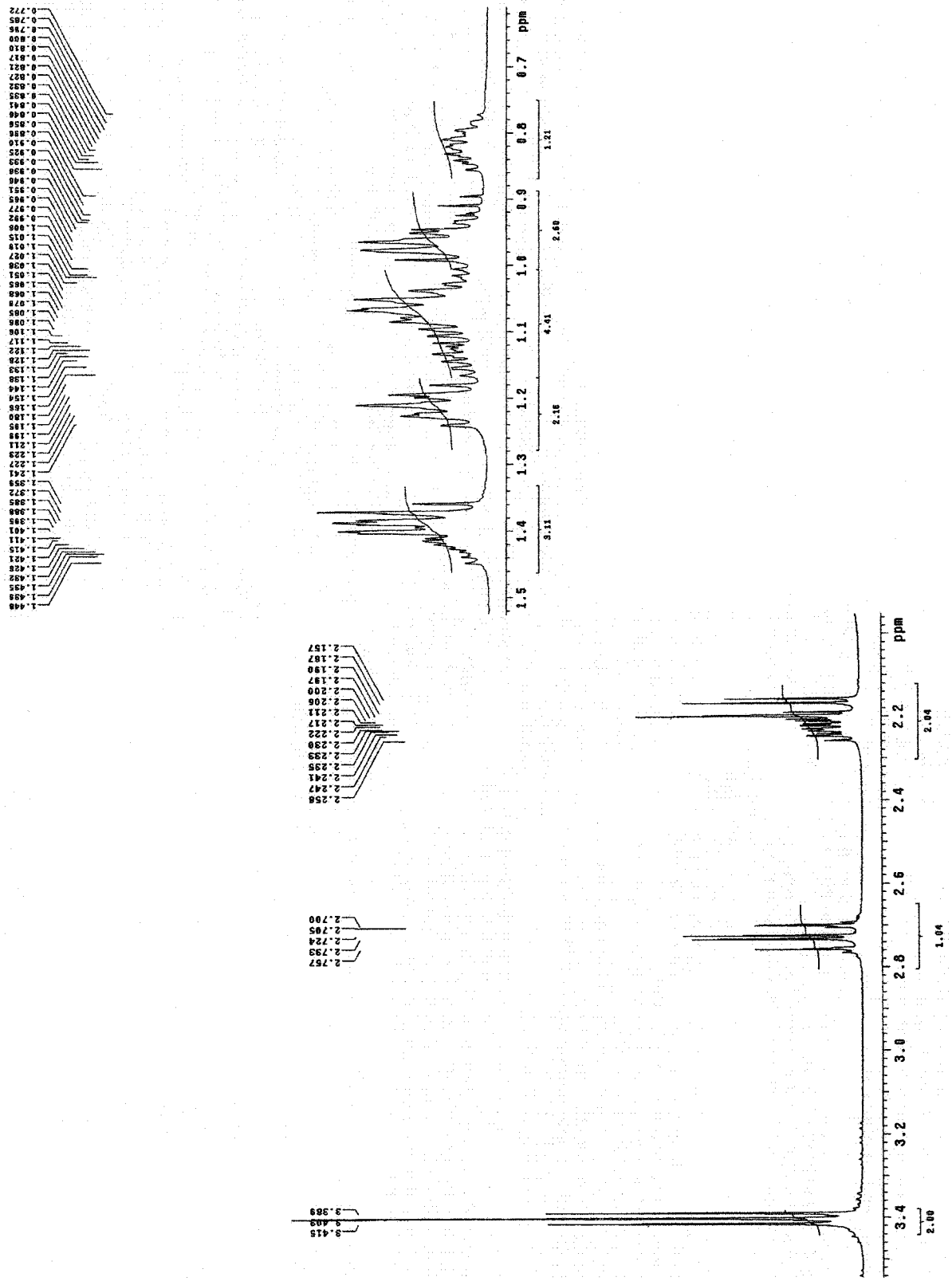


"*" Two chiral centers



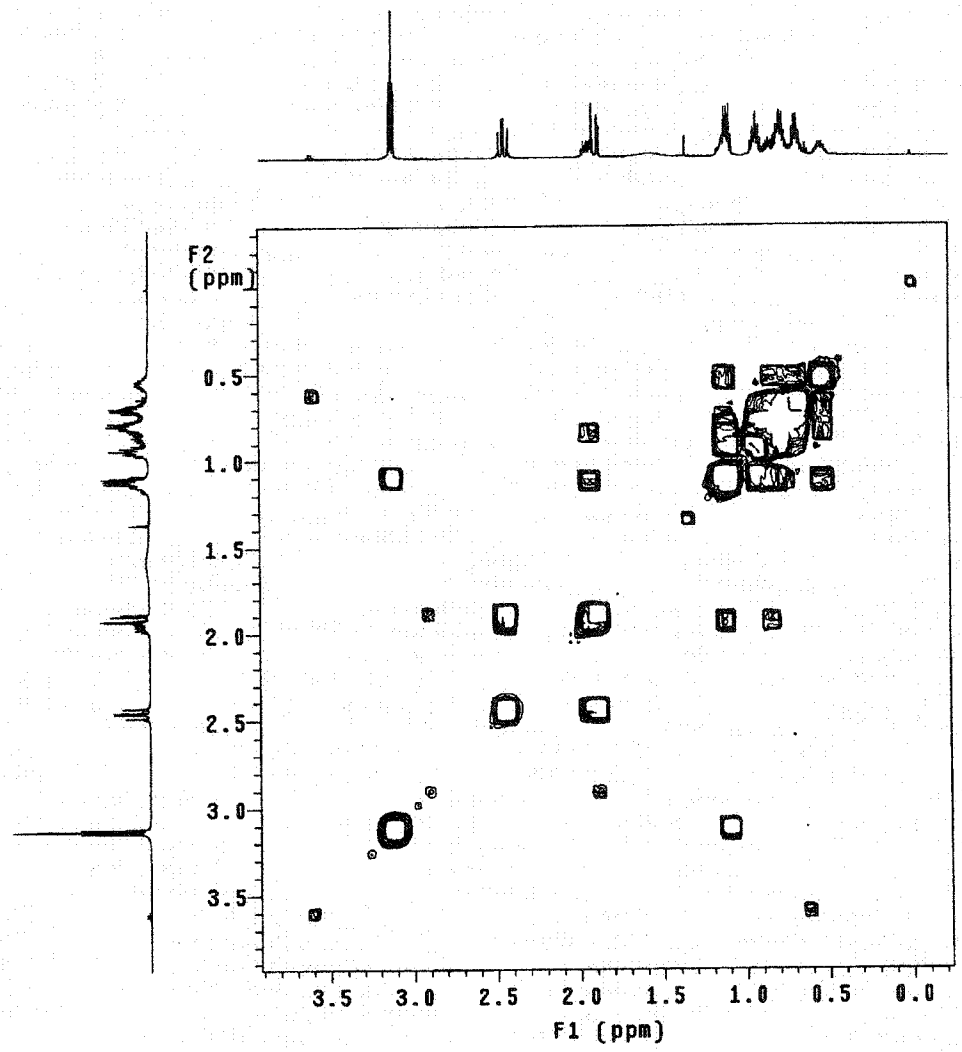
I-2a

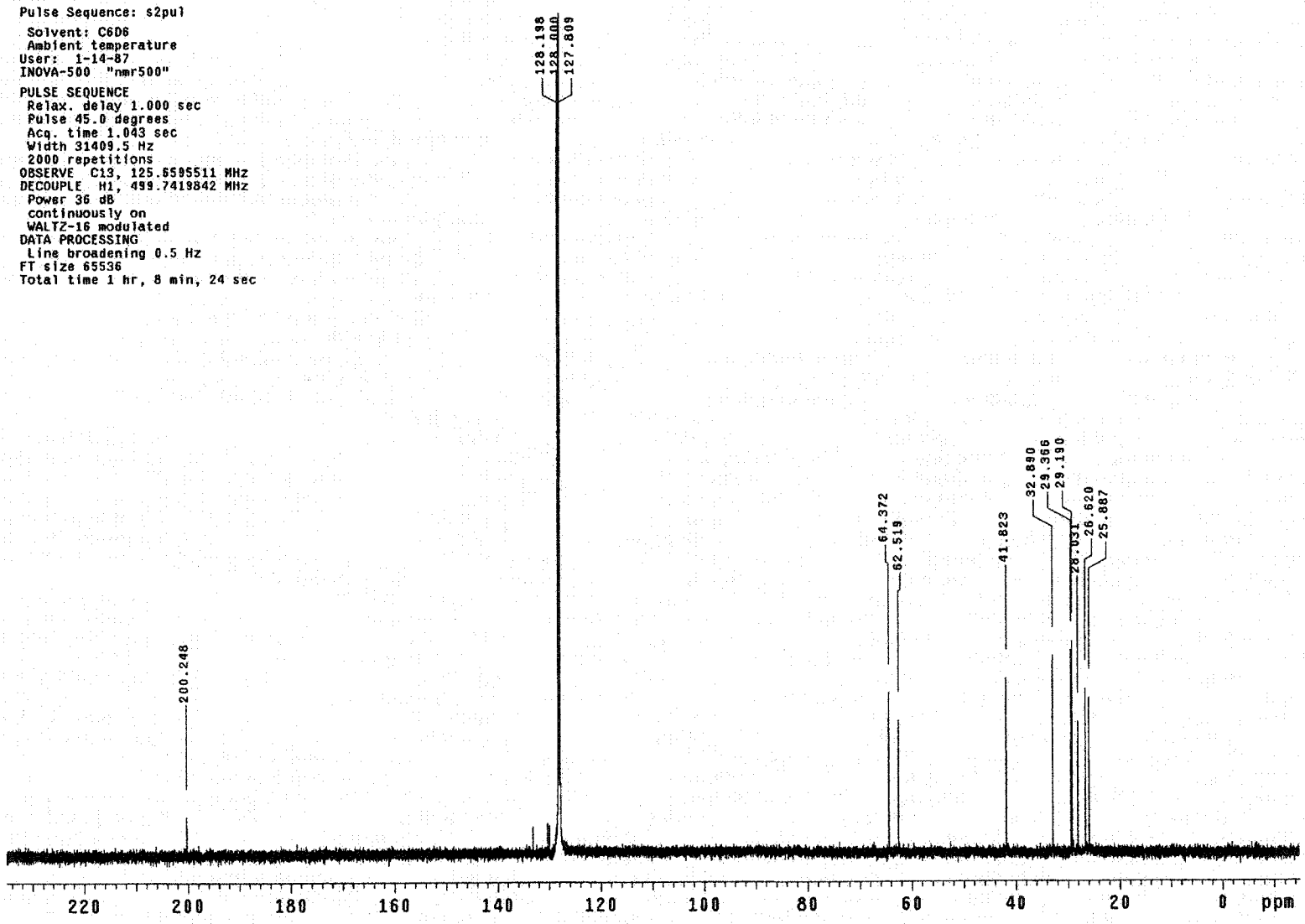


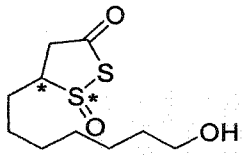


exp1 gCOSY

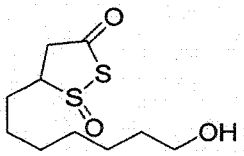
SAMPLE		FLAGS	
date	Apr 2 2001	hs	nn
solvent	C6D6	sspul	n
sample	alex_02Apr2~	hsglvl	4385
	001-18:25:17	SPECIAL	
ACQUISITION		temp	not used
sw	7996.0	gain	28
at	0.128	spin	0
np	2048	F2 PROCESSING	
fb	4000	sb	-0.064
ss	16	sbs	not used
d1	1.000	fn	2048
nt	1	F1 PROCESSING	
2D ACQUISITION		sb1	-0.016
swi	7996.0	sbs1	not used
ni	128	proc1	lp
TRANSMITTER		fn1	2048
tn	H1	DISPLAY	
sfrq	499.742	sp	-170.4
tof	499.8	wp	2133.8
tpwr	55	sp1	-115.7
pw	9.600	wp1	2071.3
GRADIENTS		rf1	1124.0
gzlvl1	4385	rfp	0
gt1	0.001000	rf11	1124.0
gstab	0.000500	rfp1	0
DECOUPLER		PLOT	
dn	C13	wc	108.0
dm	nnn	sc	6.2
		wc2	108.0
		sc2	0
		vs	116
		th	6
		al cdc av	



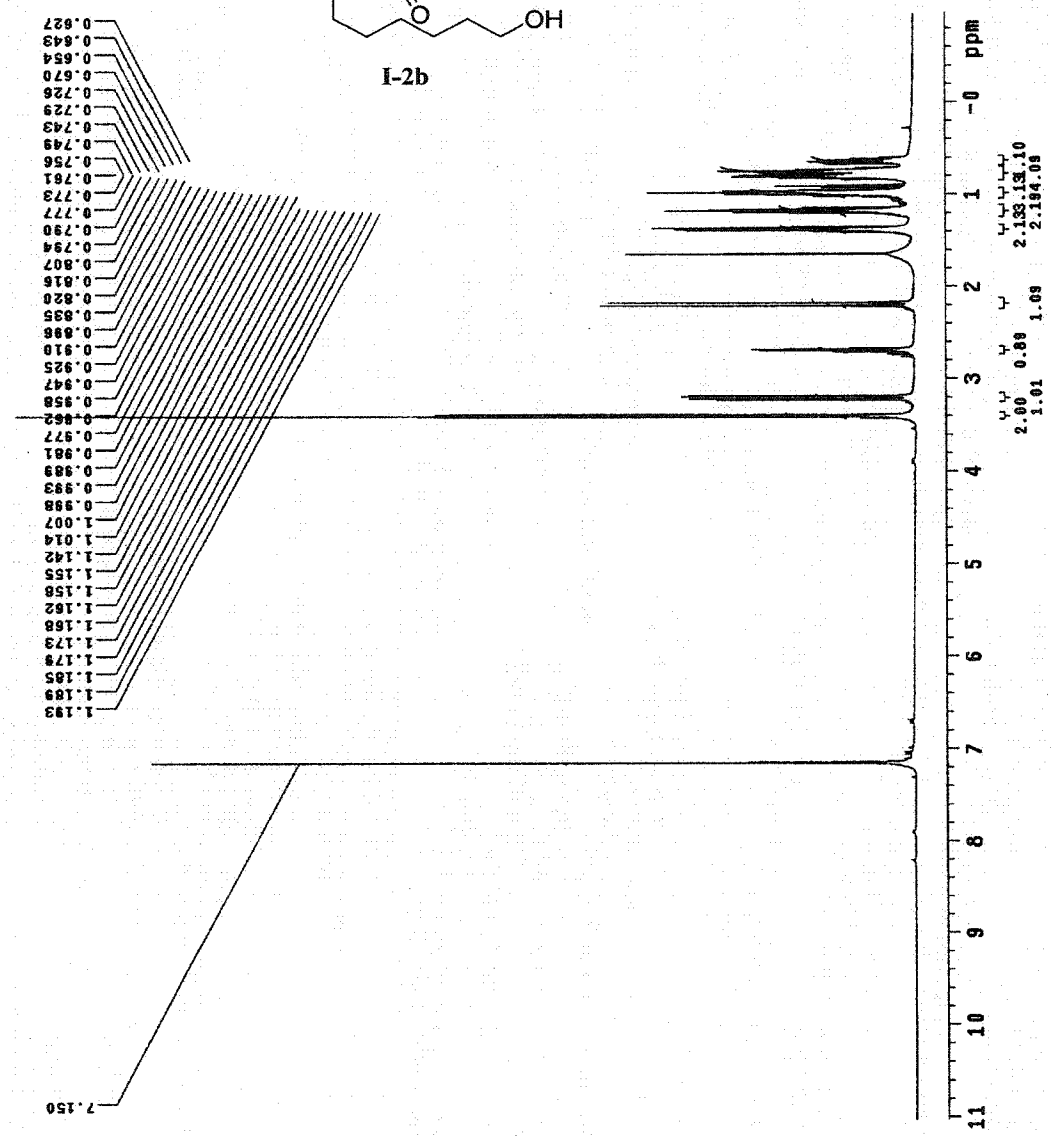

Pulse Sequence: s2pul
Solvent: C6D6
Ambient temperature
User: 1-14-87
INOVA-500 "nmr500"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.043 sec
Width 31409.5 Hz
2000 repetitions
OBSERVE C13, 125.8595511 MHz
DECOUPLE H1, 499.7419842 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 1 hr, 8 min, 24 sec
128.198
128.000
127.809
200.248
64.372
62.519
41.823
32.880
29.366
29.190
28.031
26.620
25.887
220
200
180
160
140
120
100
80
60
40
20
0
ppm



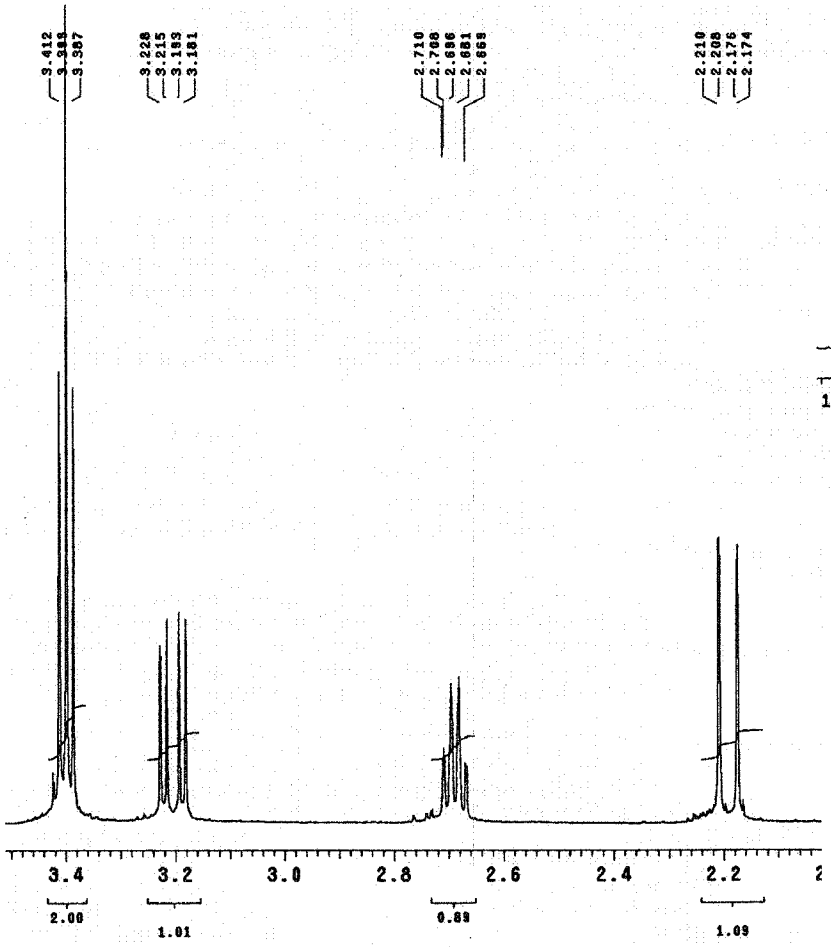
"*" Two chiral centers

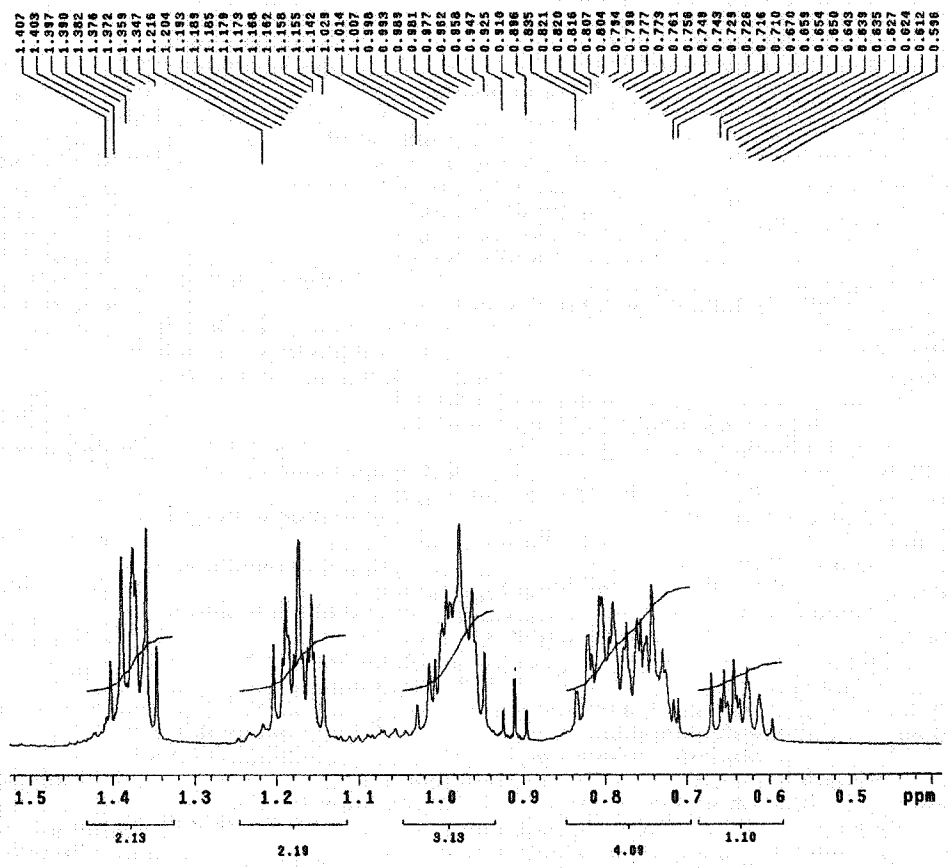


I-2b



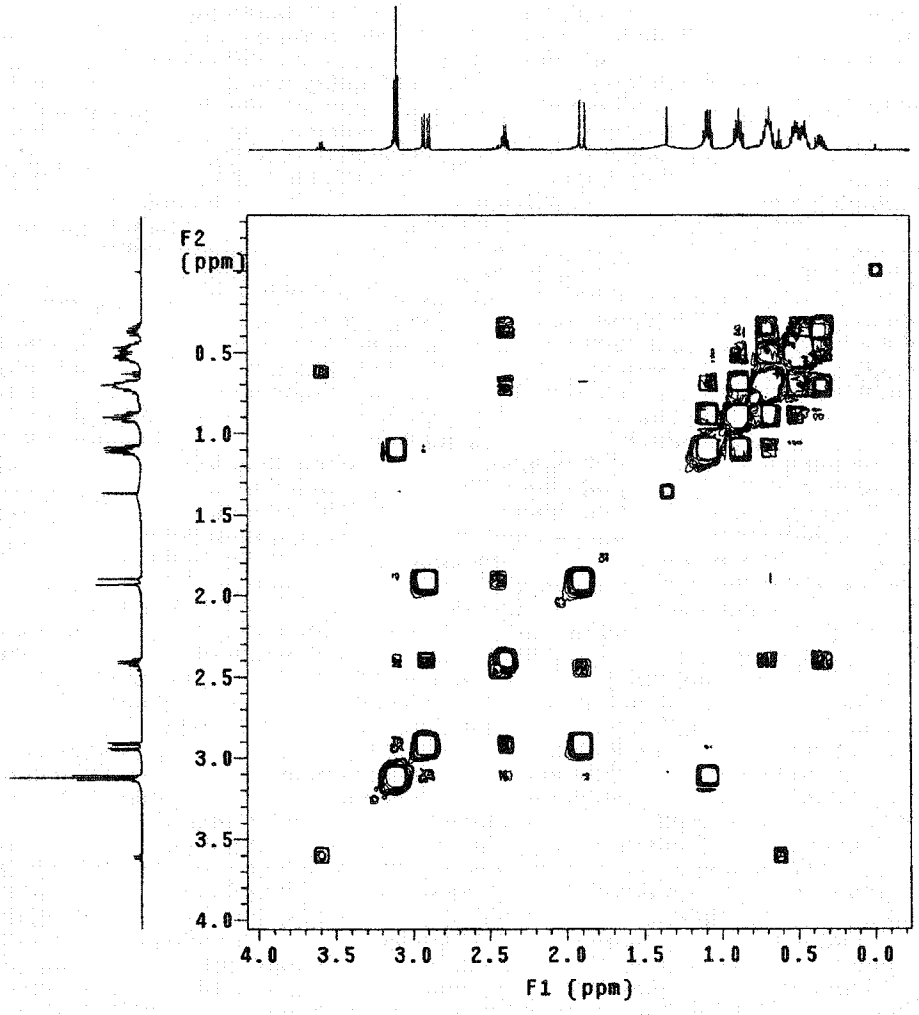

```
exp1  s2pul

          SAMPLE                          SPECIAL
date   Apr  2 2001   temp      not used
solvent       C6D6   gain      not used
file          exp    spin            22
     ACQUISITION     hst          0.008
sw           5997.0  pw90         9.600
at           2.049   alfa             0
np           24576          FLAGS
fb           3000   il            n
bs             32   in            n
d1           1.000  dp            y
nt             8    hs           nn
ct             8          PROCESSING
          TRANSMITTER   fn      not used
tn             H1          DISPLAY
sfrq       499.742   sp        -484.2
tof            0.1   wp         5997.0
tpwr            55   rf1        4057.3
pw           4.800   rfp        3573.1
     DECOUPLER       rp        -152.3
dn             C13   lp            0.8
dof             0          PLOT
dm           nnn   wc           180
dmm            c   sc             0
dpwr          52   vs           144
dmf        14122   th             9
                   nm   cdc  ph
```


3.412
3.398
3.387
3.228
3.215
3.193
3.181
2.710
2.708
2.686
2.681
2.668
2.210
2.208
2.176
2.174
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.00
1.01
0.89
1.09


1.407
1.403
1.397
1.390
1.382
1.376
1.372
1.359
1.347
1.216
1.204
1.193
1.189
1.185
1.179
1.173
1.168
1.162
1.156
1.155
1.142
1.029
1.014
1.007
0.998
0.993
0.985
0.981
0.977
0.962
0.958
0.947
0.925
0.916
0.896
0.895
0.821
0.820
0.816
0.807
0.804
0.794
0.788
0.777
0.773
0.761
0.756
0.749
0.743
0.729
0.726
0.716
0.710
0.670
0.655
0.654
0.650
0.648
0.639
0.635
0.627
0.624
0.612
0.596
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
ppm
2.13
2.19
3.13
4.09
1.10

exp1 gCOSY

SAMPLE		FLAGS	
date	Apr 2 2001	hs	nn
solvent	C6D6	sspul	n
sample	alex_02Apr2~	hsglvl	4385
	001-18:54:01	SPECIAL	
ACQUISITION		temp	not used
sw	7996.0	gain	24
at	0.128	spin	0
np	2048	F2 PROCESSING	
fb	4000	sb	-0.064
ss	16	sbs	not used
d1	1.000	fn	2048
nt	1	F1 PROCESSING	
2D ACQUISITION		sb1	-0.016
sw1	7996.0	sbs1	not used
ni	128	proc1	lp
TRANSMITTER		fn1	2048
tn	H1	DISPLAY	
sfrq	499.742	sp	-169.5
tof	499.8	wp	2196.4
tpwr	55	sp1	-114.7
pw	9.600	wp1	2149.5
GRADIENTS		rf1	1123.0
gzlvl1	4385	rfp	0
gt1	0.001000	rf11	1123.0
gstab	0.000500	rfp1	0
DECOUPLER		PLOT	
dn	C13	wc	135.0
dm	nnn	sc	10.0
		wc2	135.0
		sc2	0
		vs	136
		th	7
		ai cdc av	

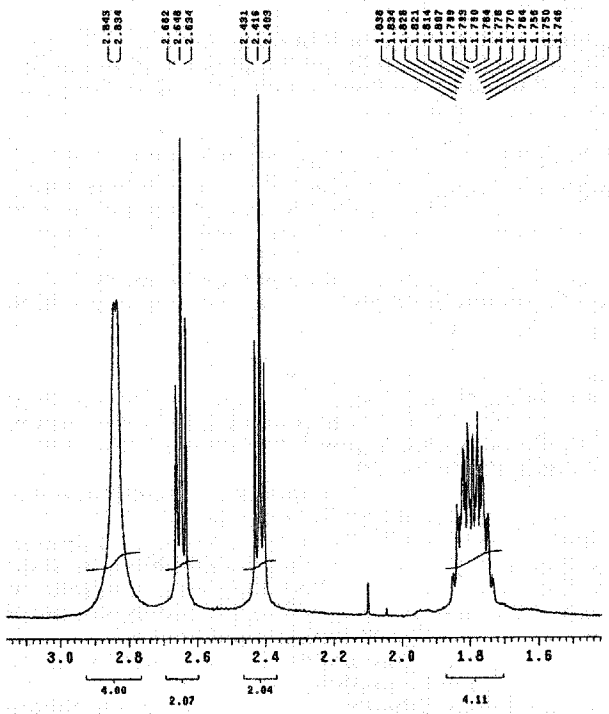


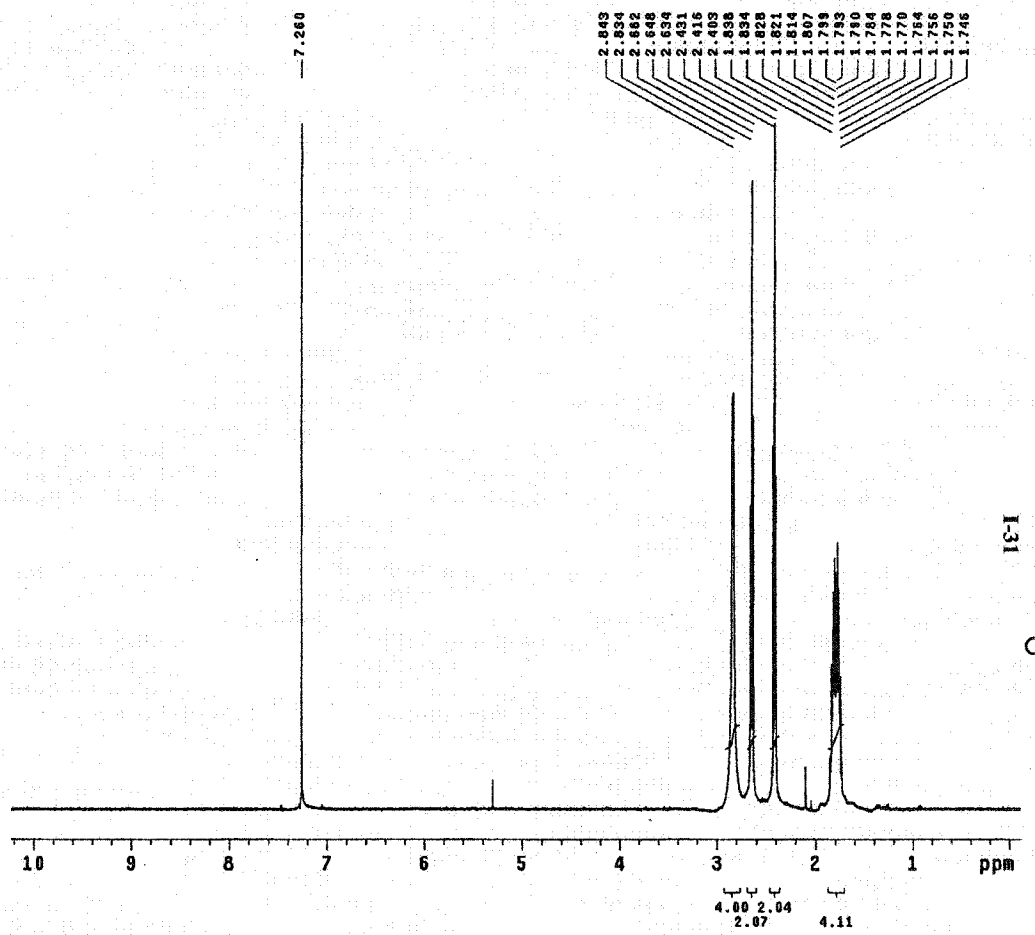
Pulse Sequence: s2pul
Solvent: C6D6
Ambient temperature
User: 1-14-87
INOVA-500 "nmr500"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.043 sec
Width 31409.5 Hz
5000 repetitions
OBSERVE C13, 125.6585511 MHz
DECOUPLE H1, 499.7419842 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 2 hr, 51 min, 0 sec
128.191
128.000
127.802
128.290
201.438
66.882
62.503
42.273
32.867
29.183
28.809
27.161
25.895
220
200
180
160
140
120
100
80
60
40
20
0
ppm

Active ester

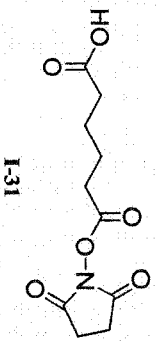
exp1 s2pul

SAMPLE		SPECIAL	
date	Feb 27 2001	temp	not used
solvent	CDCl3	gain	not used
file	/export/home/~	spin	22
	chenj/vnmrsys/data~	hst	0.008
	/cj-02-02-27-1_27F~	pw90	9.800
	eb2001/PROTON.fid	alfa	0
ACQUISITION		FLAGS	
sw	7998.0	il	n
at	2.049	in	n
np	32768	dp	y
fb	4000	hs	nn
bs	32	PROCESSING	
d1	1.000	fn	not used
nt	8	DISPLAY	
ct	8	sp	-58.0
TRANSMITTER		wp	5170.1
tn	H1	rfl	999.5
sfrq	499.742	rfp	0
tof	499.8	rp	-97.7
tpwr	57	lp	-31.3
pw	4.900	PLOT	
DECOUPLER		wc	180
dn	C13	sc	0
dof	0	vs	96
dm	nnn	th	11
dmm	c	al	cdc ph
dpwr	51		
dmf	17094		



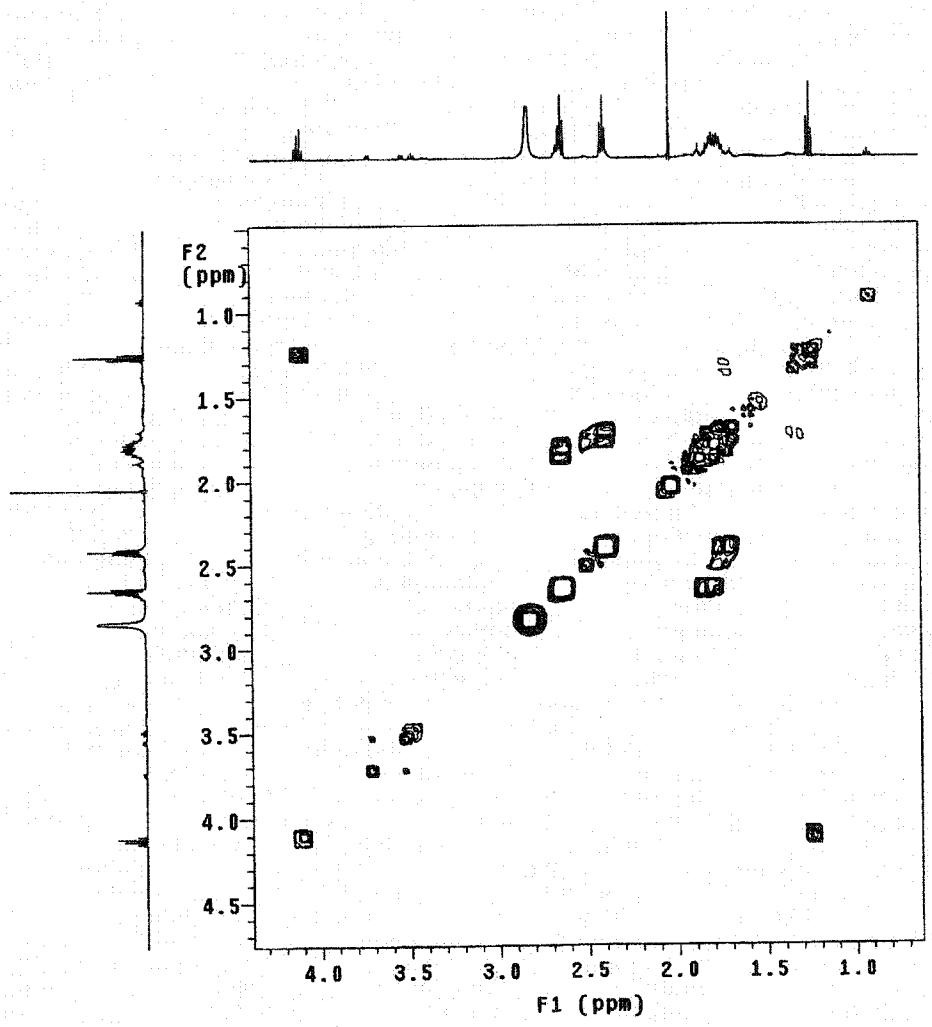


I-31



exp1 gCOSY

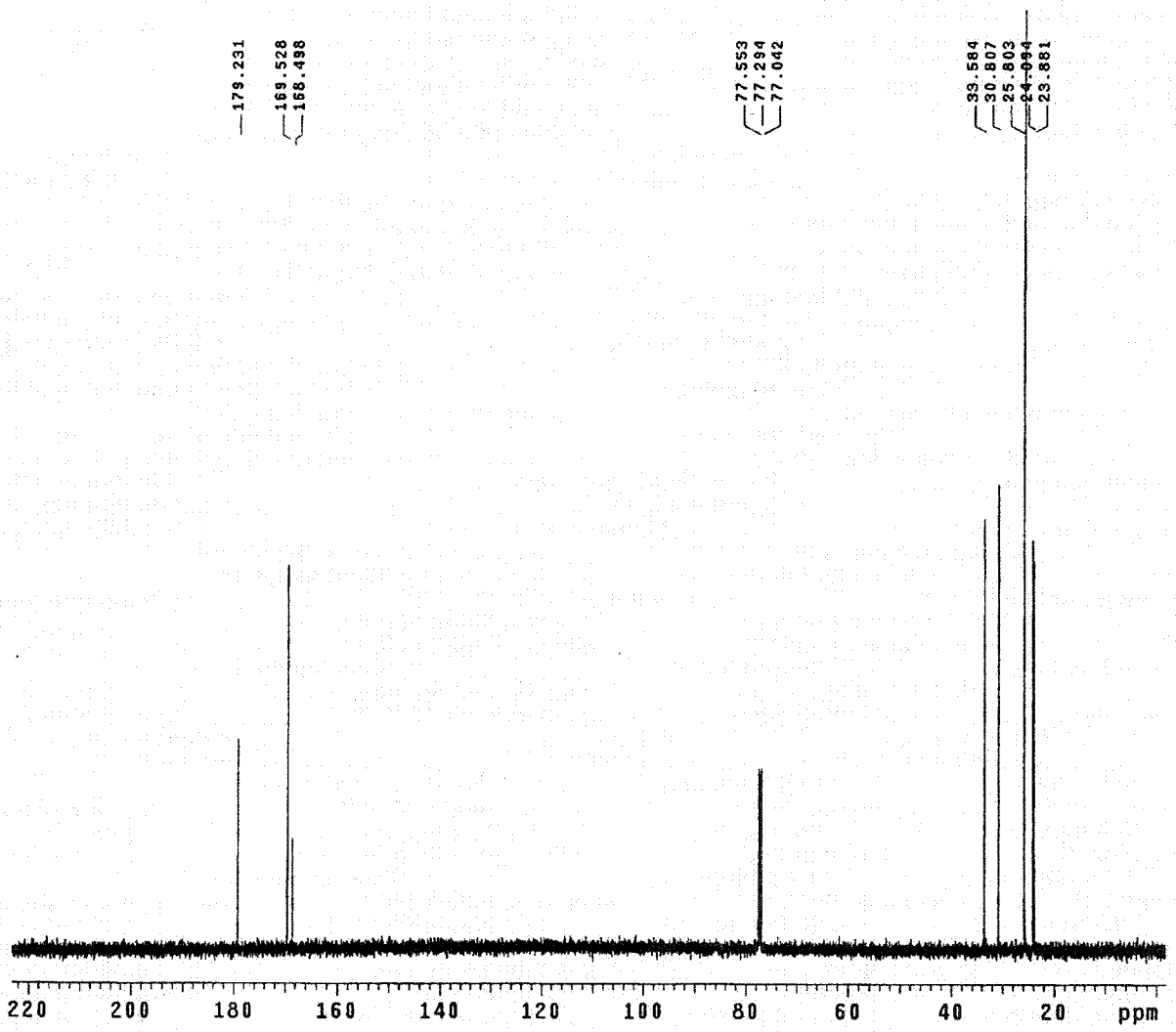
SAMPLE		FLAGS	
date	Apr 11 2001	hs	nn
solvent	CDCl3	sspul	n
sample	alex_11Apr2~001	hsglvl	4385
ACQUISITION		**SPECIAL**	
sw	7996.0	temp	not used
at	0.128	gain	28
np	2048	spin	0
fb	4000	**F2 PROCESSING**	
ss	16	sb	-0.064
di	1.000	sbs	not used
nt	1	fn	2048
2D ACQUISITION		**F1 PROCESSING**	
sw1	7996.0	sb1	-0.016
ni	128	sbs1	not used
TRANSMITTER		proc1	lp
tn	H1	fn1	2048
sfrq	499.742	**DISPLAY**	
tof	499.8	sp	243.3
tpwr	55	wp	2133.8
pw	8.600	sp1	313.6
GRADIENTS		wp1	1875.9
gzlvl1	4385	rf1	999.5
gt1	0.001000	rfp	0
gstab	0.000500	rf11	999.5
DECOUPLER		rfp1	0
dn	C13	**PLOT**	
dm	nnn	wc	135.0
		sc	10.0
		wc2	135.0
		sc2	0
		vs	60
		th	8
		ai cdc av	



active ester

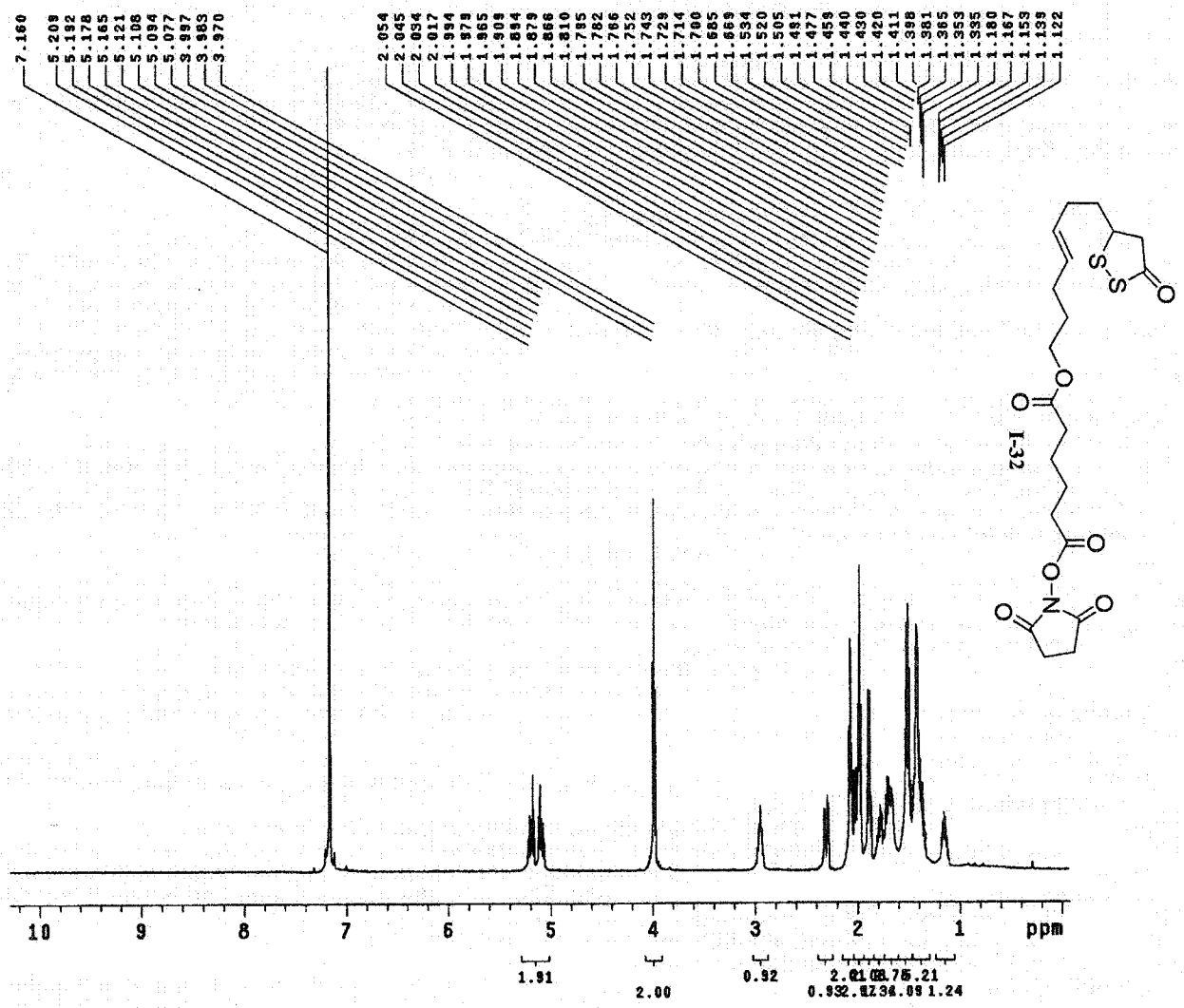
exp1 s2pul

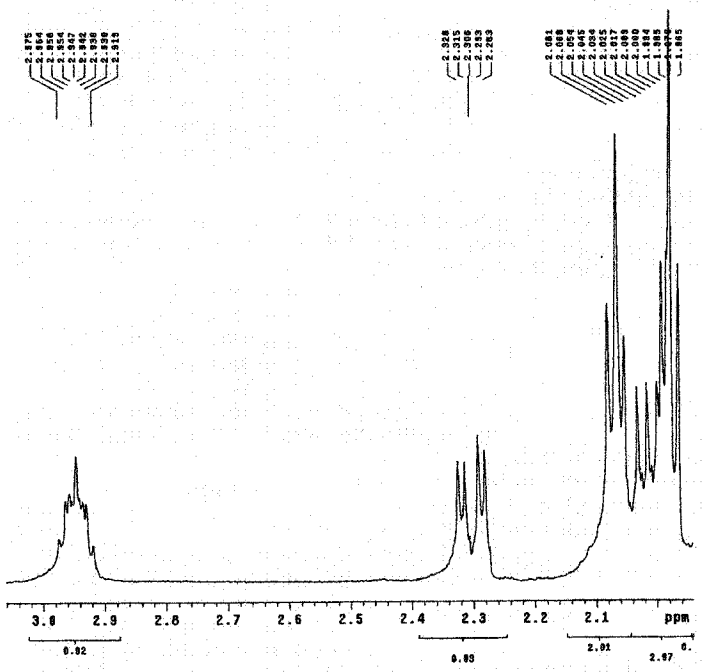
SAMPLE		SPECIAL	
date	Feb 27 2001	temp	not used
solvent	CDCl3	gain	not used
file	exp	spin	16
ACQUISITION		hst	0.008
sw	31409.5	pw90	22.300
at	1.043	alfa	10.000
np	65536	FLAGS	
fb	17000	il	n
bs	64	in	n
d1	1.000	dp	y
nt	2000	hs	nn
ct	1684	PROCESSING	
TRANSMITTER		lb	0.50
tn	C13	fn	not used
sfrq	125.673	DISPLAY	
tof	1883.4	sp	-233.4
tpwr	63	wp	28272.1
pw	11.150	rfl	1882.1
DECOUPLER		rfp	0
dn	H1	rp	-62.7
dof	0	lp	-234.1
dm	yyy	PLOT	
dmm	w	wc	180
dpwr	42	sc	0
dmf	11696	vs	683
		th	6
		ai	cdc ph

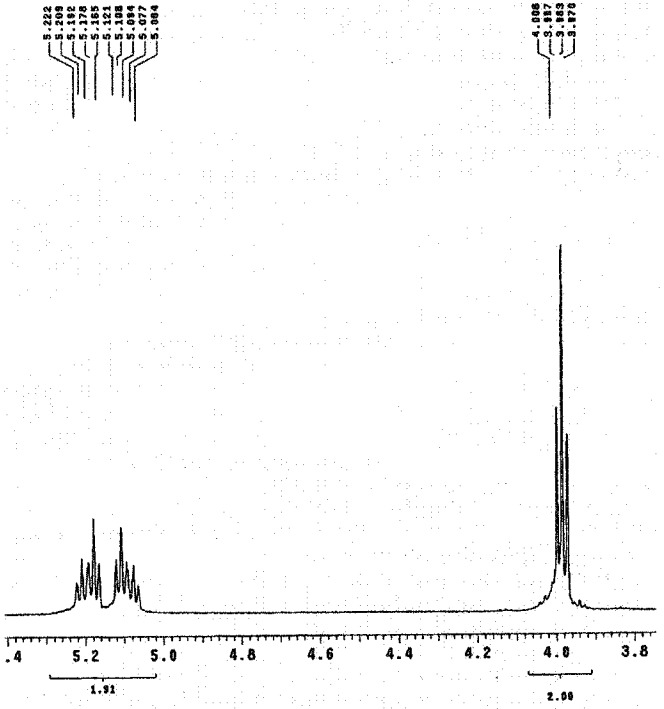


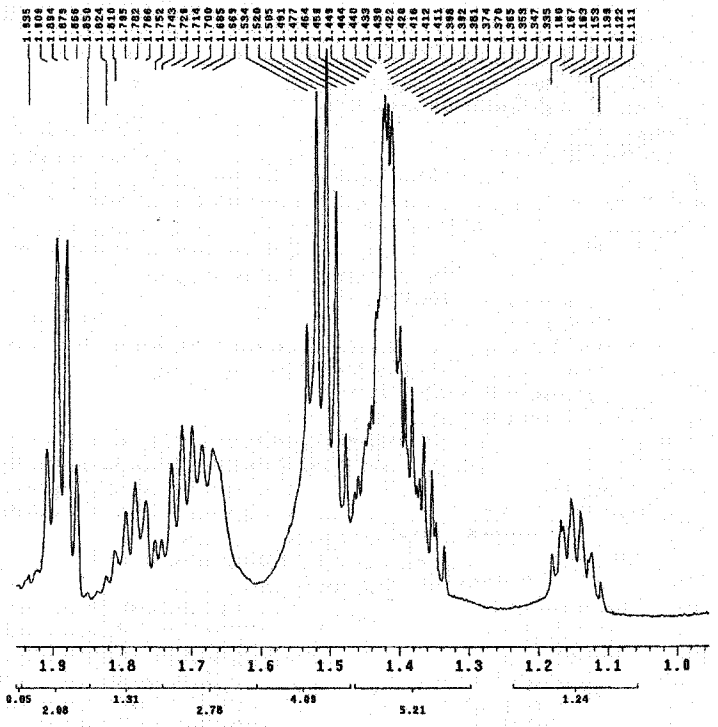
exp1 s2pul

SAMPLE		SPECIAL	
date	Mar 22 2001	temp	not used
solvent	C6D6	gain	not used
file	exp	spin	22
ACQUISITION		hst	0.008
sw	7986.0	pw90	9.800
at	2.049	alfa	0
np	32768	**FLAGS**	
fb	4000	il	n
bs	32	in	n
d1	1.000	dp	y
nt	8	hs	nn
ct	8	**PROCESSING**	
TRANSMITTER		fn	not used
tn	H1	**DISPLAY**	
sfrq	499.742	sp	-37.0
tof	499.8	wp	5180.3
tpwr	57	rfl	4556.6
pw	4.900	rfp	3578.1
DECOUPLER		rp	20.9
dn	C13	lp	-8.8
dof	0	**PLOT**	
dm	nnn	wc	180
dmm	c	sc	0
dpwr	51	vs	126
dmf	17094	th	5
		ai cdc ph	



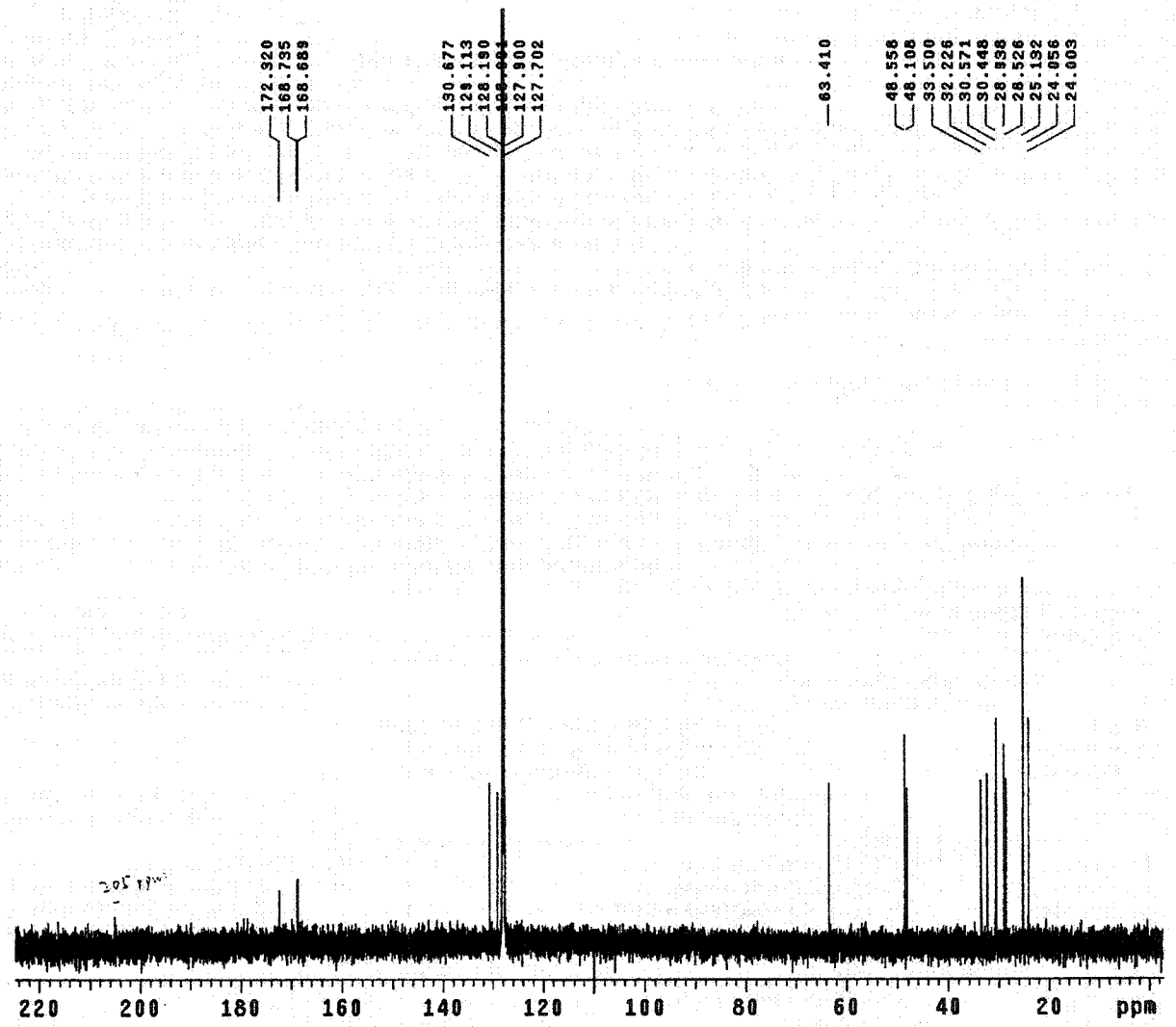

3.8
2.9
2.8
2.7
2.6
2.5
2.4
2.3
2.2
2.1
ppm

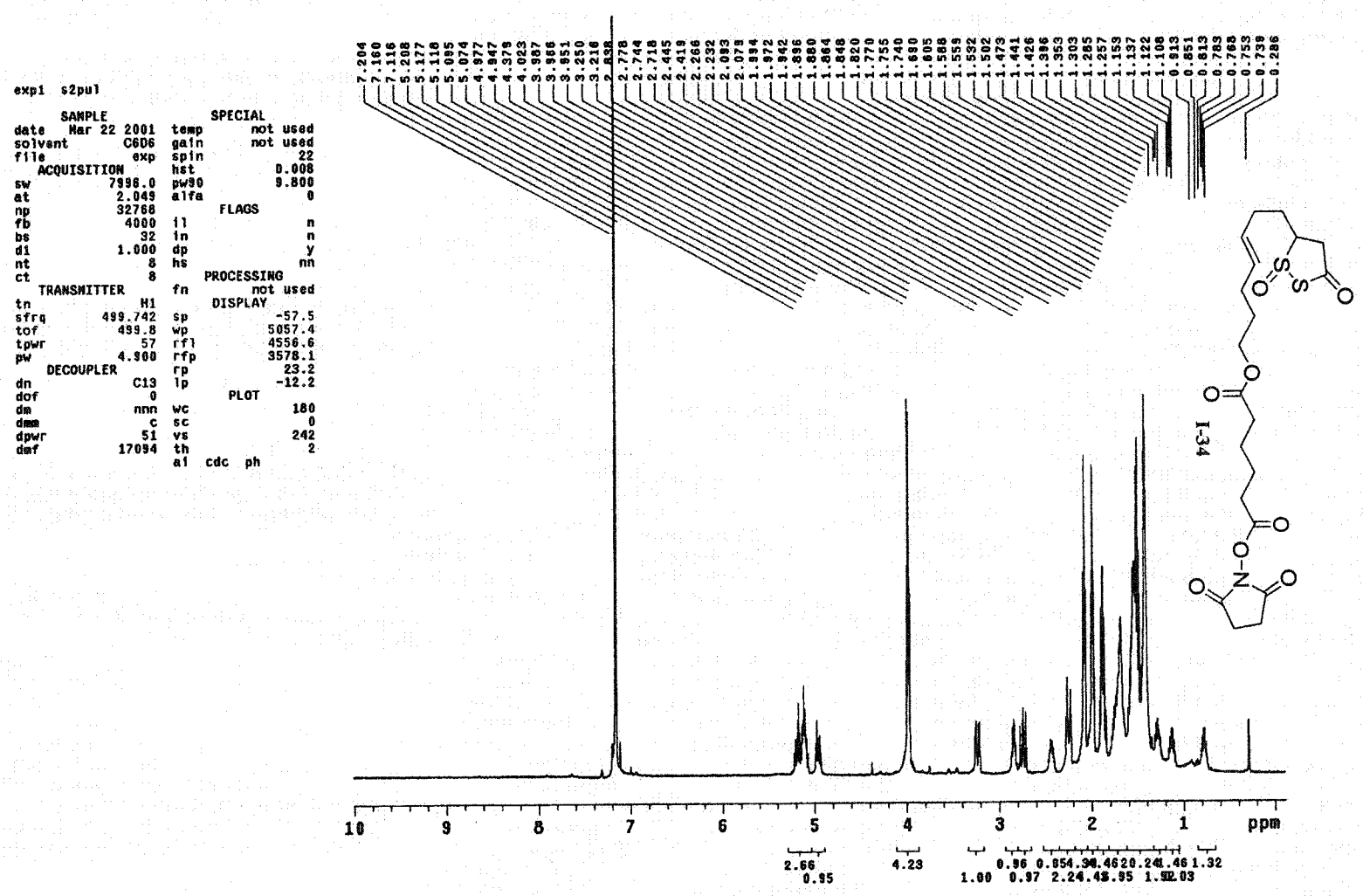

5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8

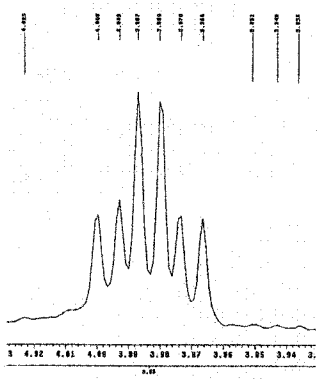

1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0

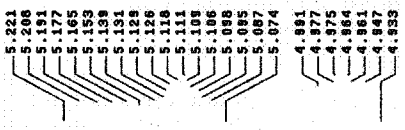
exp1 s2pul

SAMPLE		SPECIAL	
date	Mar 22 2001	temp	not used
solvent	C6D6	gain	not used
file	exp	spin	16
ACQUISITION		hst	0.008
sw	31409.5	pw90	22.300
at	1.043	alfa	10.000
np	65536	**FLAGS**	
fb	17000	il	n
bs	64	in	n
d1	1.000	dp	y
nt	2000	hs	nn
ct	2000	**PROCESSING**	
TRANSMITTER		lb	0.50
tn	C13	fn	not used
sfrq	125.673	**DISPLAY**	
tof	1883.5	sp	-354.2
tpwr	63	wp	28594.2
pw	11.150	rfl	1882.2
DECOUPLER		rfp	0
dn	H1	rp	-109.8
dof	0	lp	-226.1
dm	yyy	**PLOT**	
dmm	w	wc	180
dpwr	42	sc	0
dmf	11696	vs	1901
		th	3
		ai	cdc ph

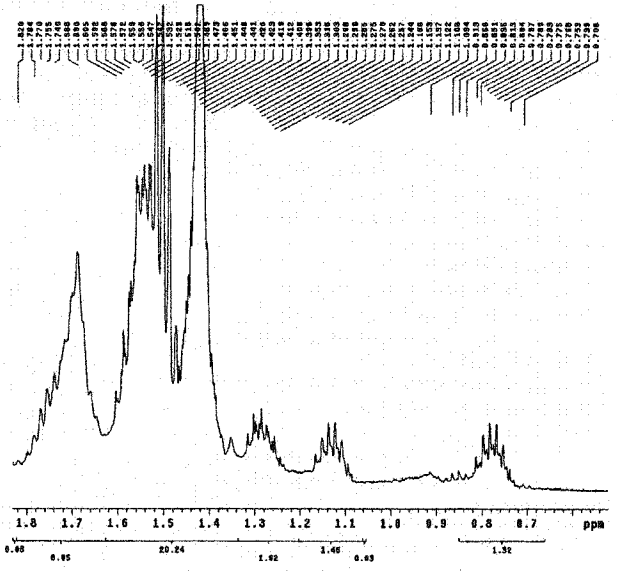


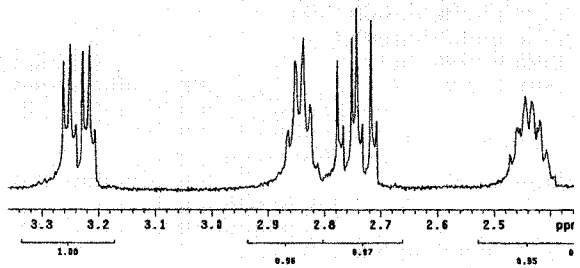

exp1 s2pul
SAMPLE
date Mar 22 2001
solvent C6D6
file exp
ACQUISITION
sw 7996.0
at 2.049
np 32768
fb 4000
bs 32
d1 1.000
nt 8
ct 8
TRANSMITTER
tn H1
sfrq 499.742
tof 499.8
tpwr 57
pw 4.900
DECOUPLER
dn C13
dof 0
dm nnn
dmm c
dpwr 51
dmf 17094
SPECIAL
temp not used
gain not used
spin 22
hst 0.008
pw90 9.800
alfa 0
FLAGS
il n
in n
dp y
hs nn
PROCESSING
fn not used
DISPLAY
sp -57.5
wp 5057.4
rfl 4556.6
rfp 3578.1
rp 23.2
lp -12.2
PLOT
wc 180
sc 0
vs 242
th 2
ai cdc ph
ppm
I-34

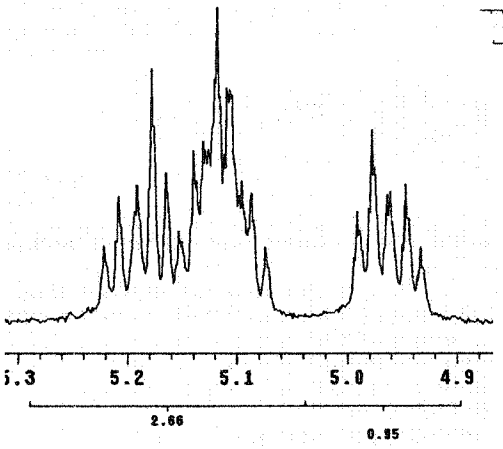


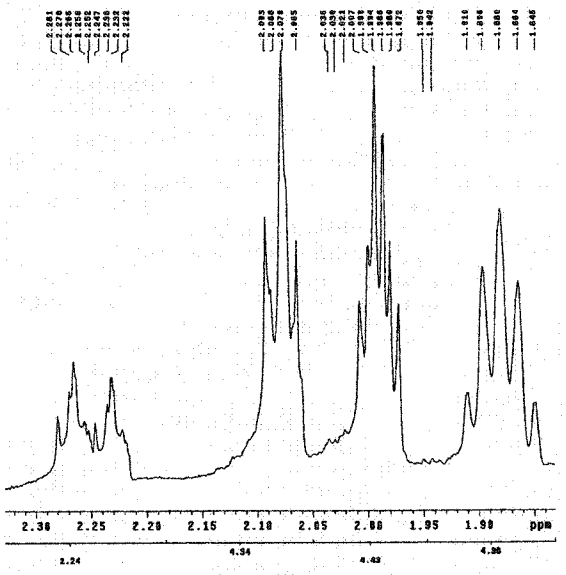








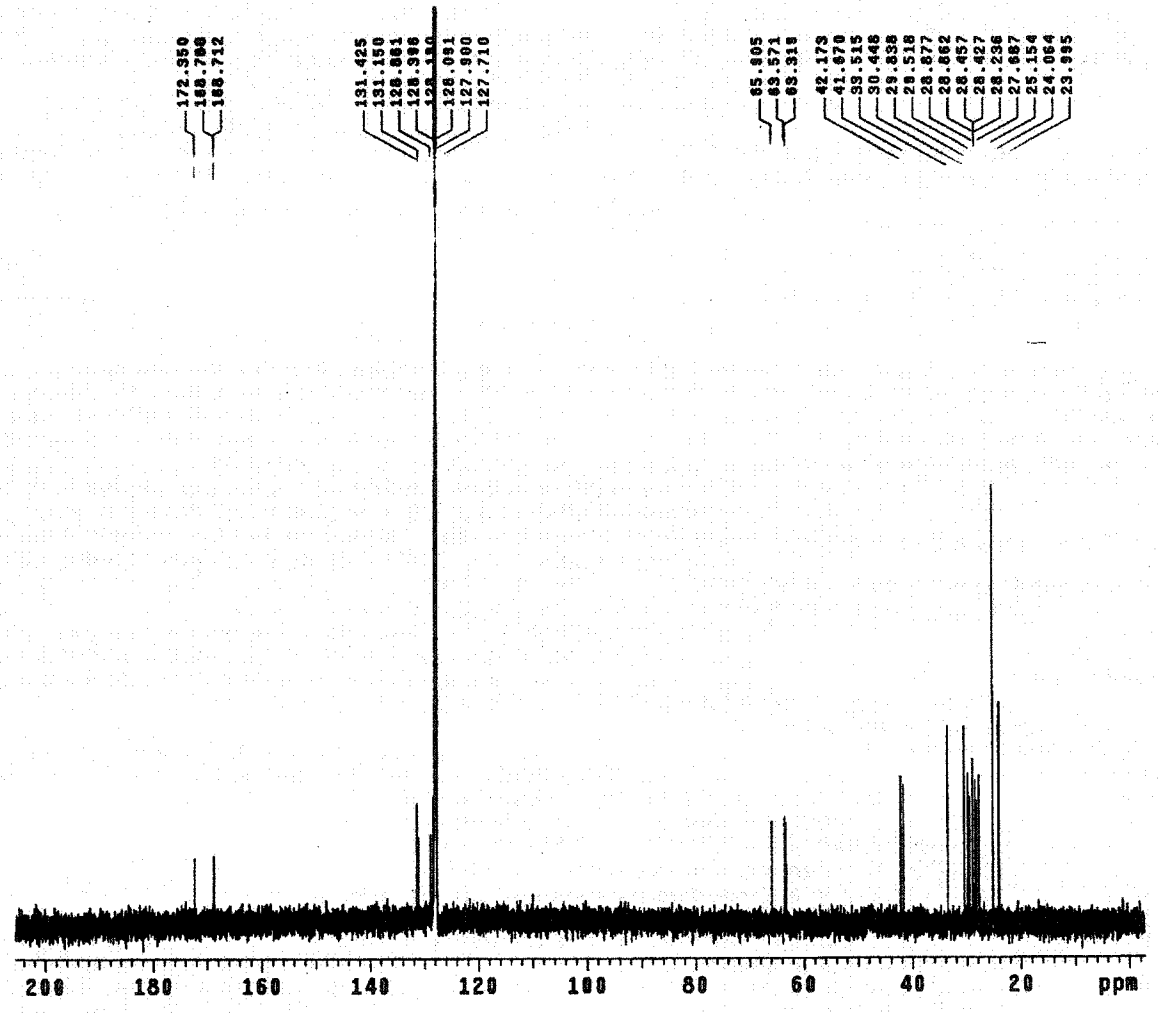


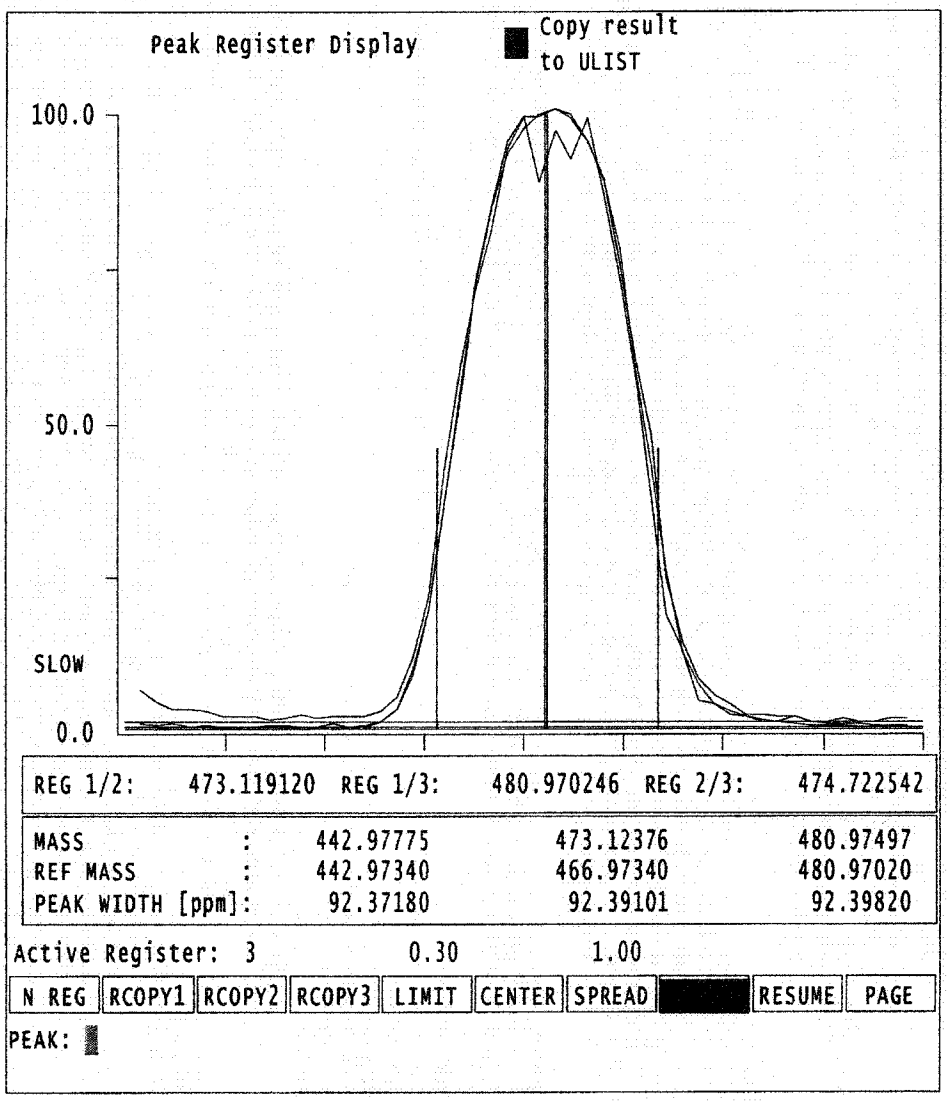


Final target cpd

expl s2pul

SAMPLE		SPECIAL	
date	Mar 22 2001	temp	not used
solvent	C6D6	gain	not used
file	exp	spin	16
ACQUISITION		hst	0.008
sw	31408.5	pw90	22.300
at	1.043	alfa	10.000
np	65536	FLAGS	
fb	17000	il	n
bs	64	in	n
d1	1.000	dp	y
nt	5000	hs	nn
ct	5000	PROCESSING	
TRANSMITTER		lb	0.50
tn	C13	fn	not used
sfrq	125.673	DISPLAY	
tof	1883.5	sp	-354.2
tpwr	63	wp	26180.5
pw	11.150	rfl	1882.2
DECOUPLER		rfp	0
dn	H1	rp	-104.5
dof	0	lp	-232.5
dm	yyy	PLOT	
dmm	w	wc	180
dpwr	42	sc	0
dmf	11696	vs	2777
		th	5
		ai cdc ph	

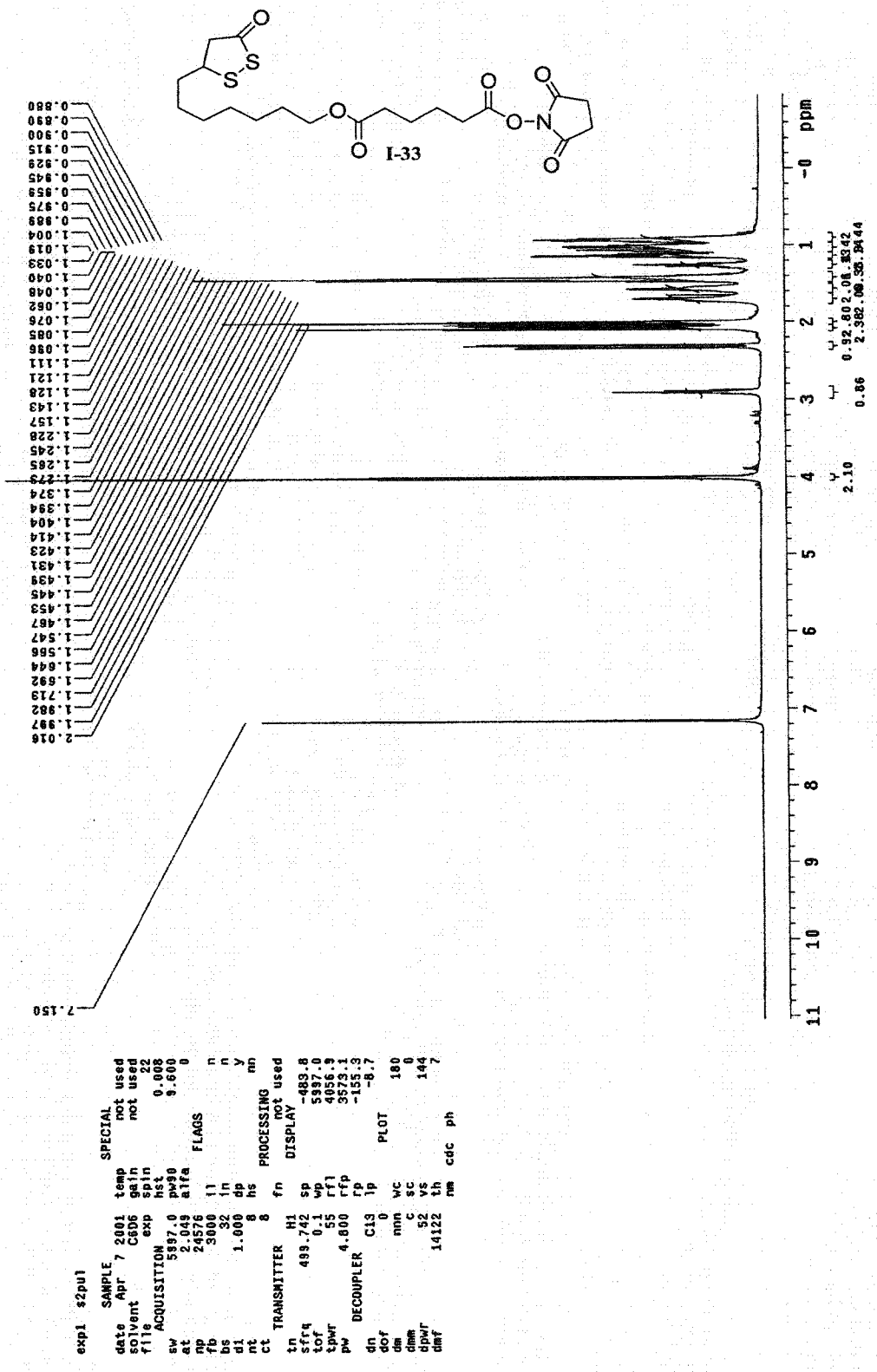


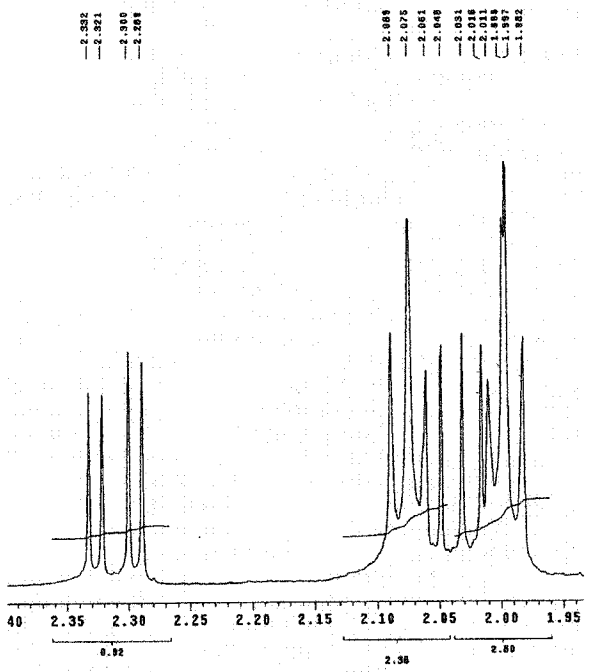


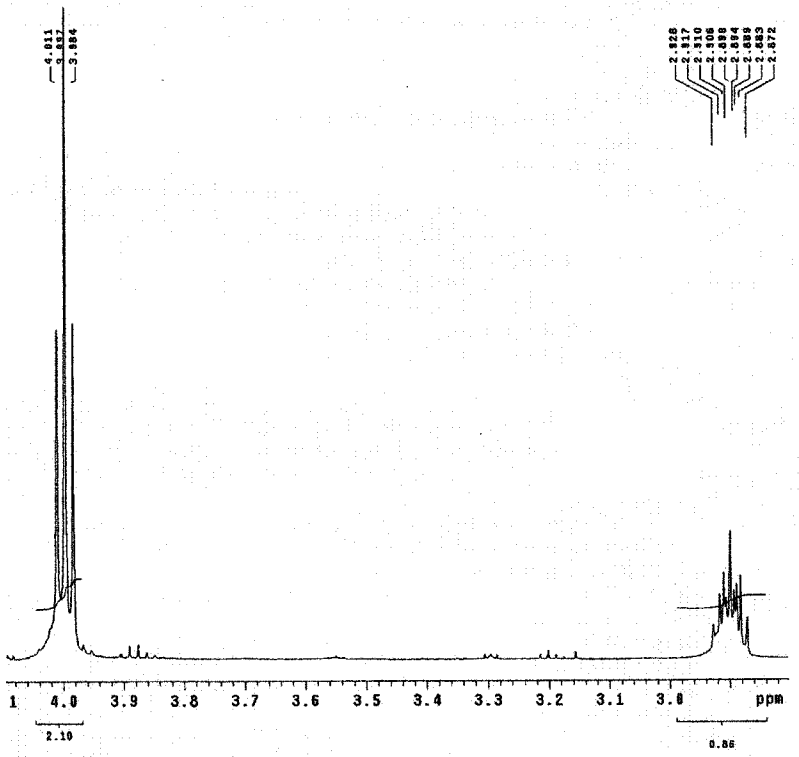
Mf. $C_{20}H_{27}N_1O_8S_2$

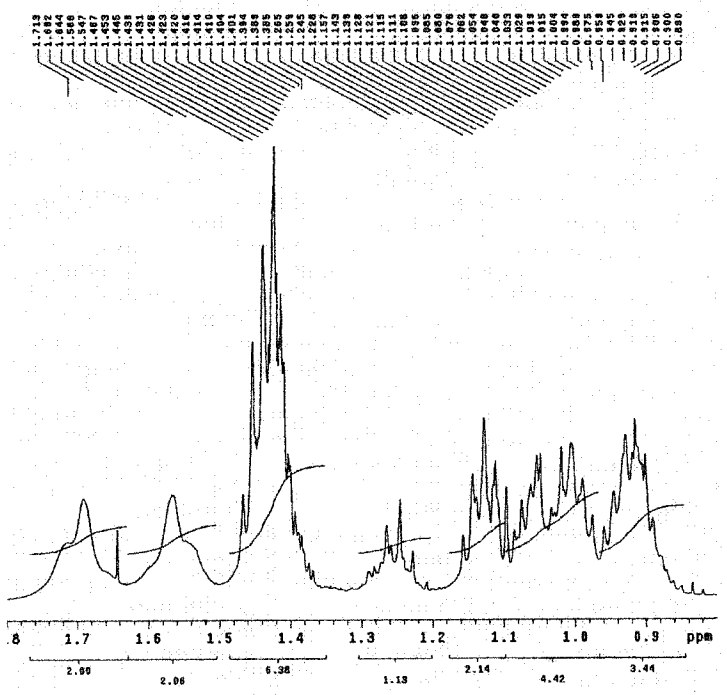
~~$(M+H)^+$ (theoretical) =~~

$(M)^-$ (theoretical) = 473.1184


I-33
ppm


2.332
2.321
2.300
2.289
2.089
2.075
2.061
2.048
2.031
2.016
2.011
1.997
1.982
40 2.35 2.30 2.25 2.20 2.15 2.10 2.05 2.00 1.95
0.92
2.36
2.80


4.011
3.987
3.984
2.928
2.917
2.910
2.906
2.898
2.894
2.889
2.883
2.872
1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 ppm
2.10
0.86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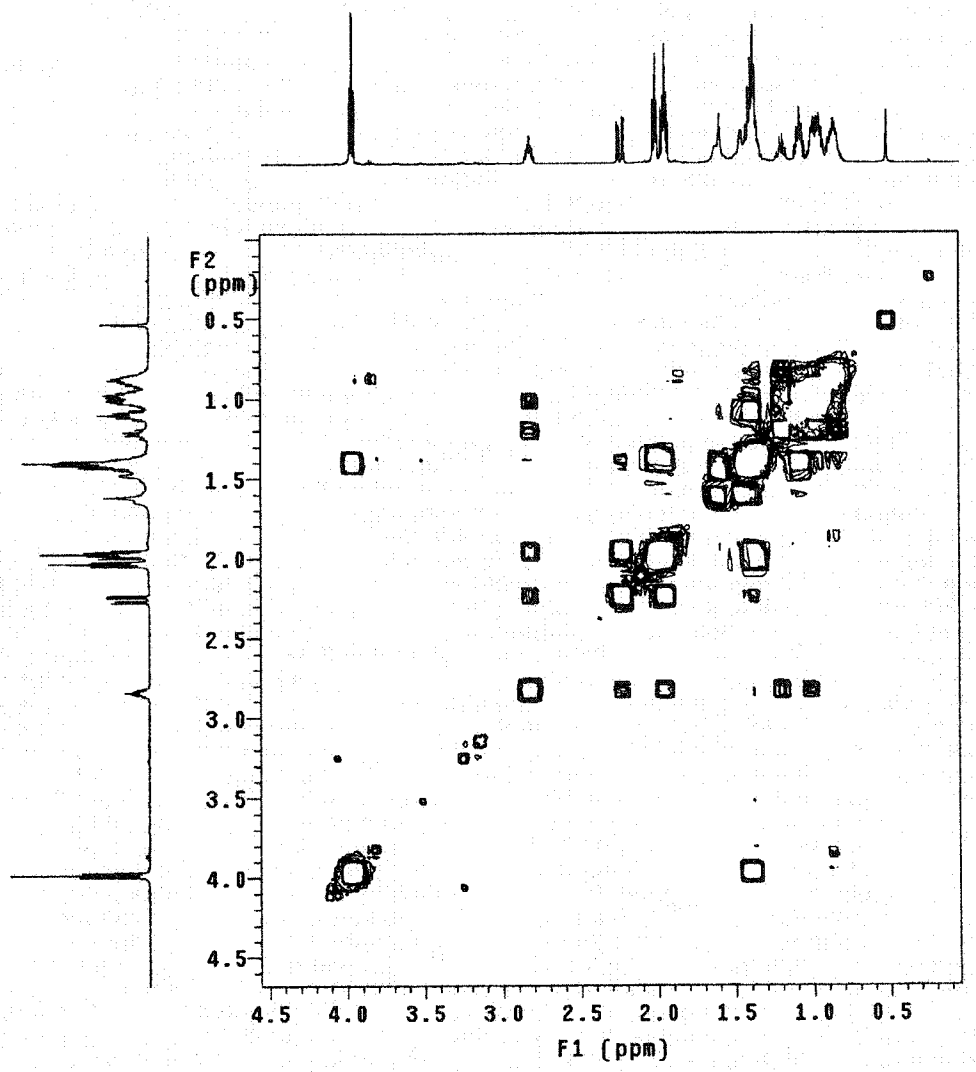
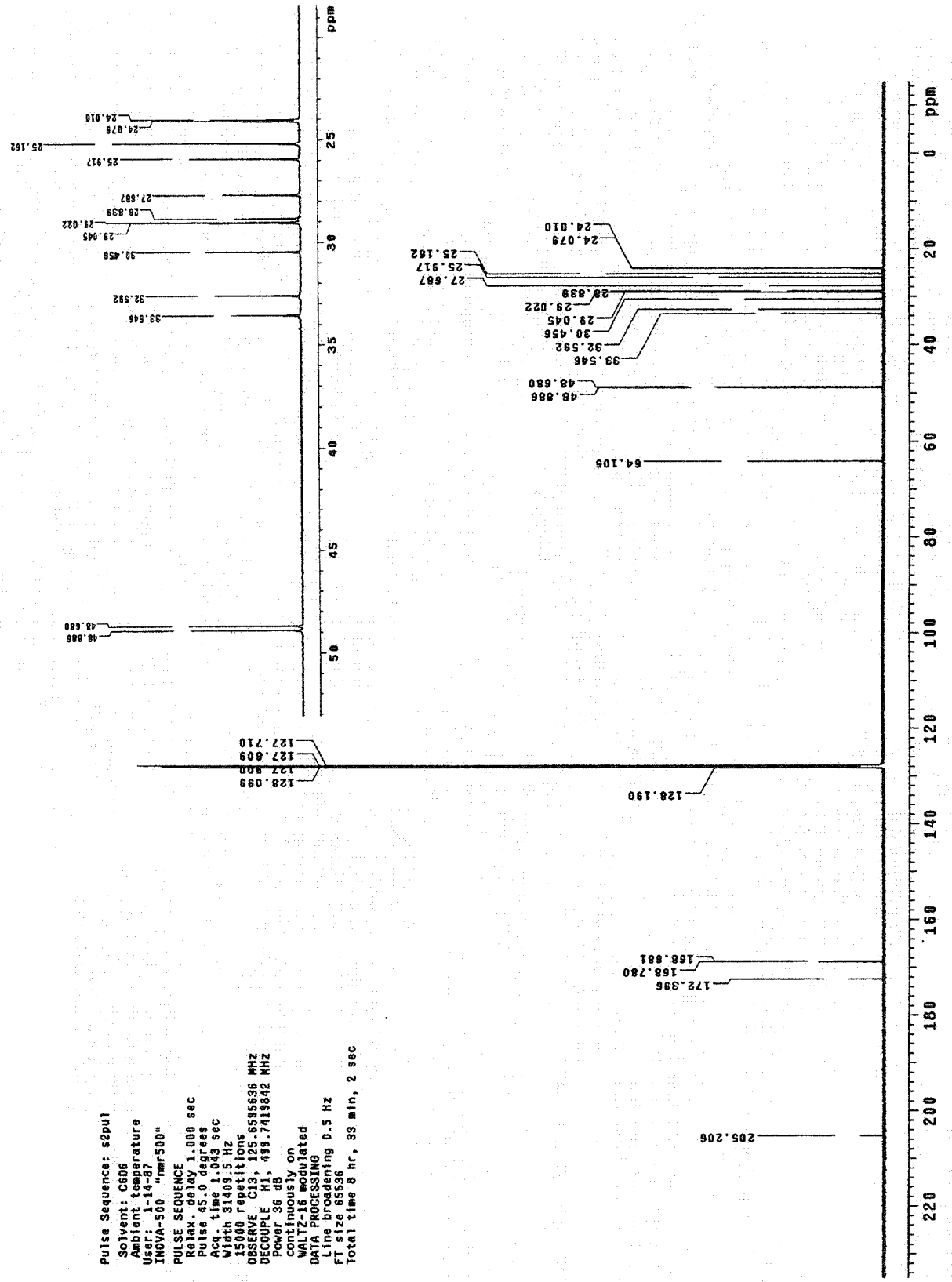
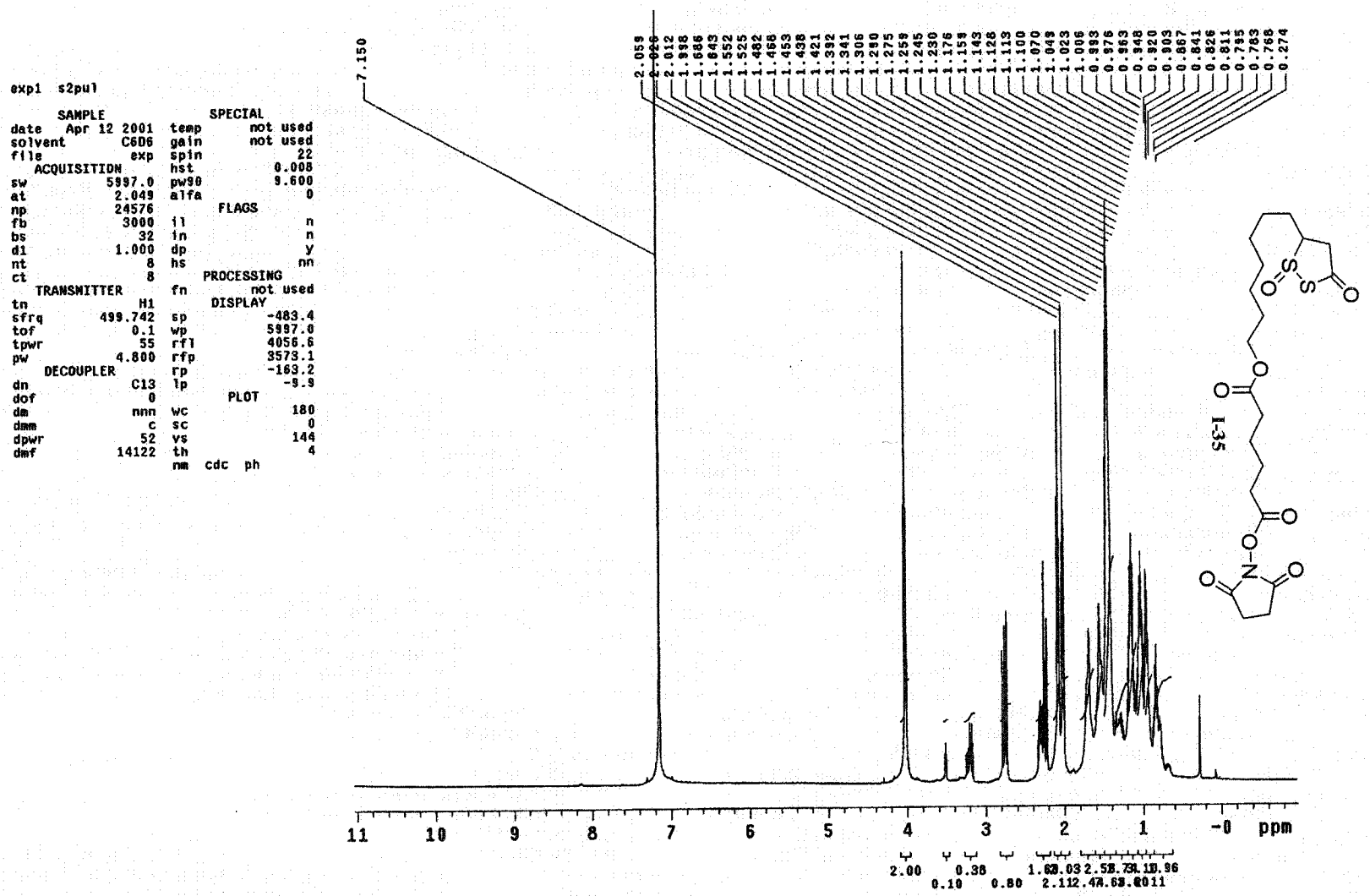
1.682
1.644
1.580
1.547
1.467
1.453
1.446
1.438
1.431
1.426
1.423
1.420
1.416
1.414
1.410
1.404
1.401
1.394
1.388
1.385
1.265
1.259
1.245
1.228
1.157
1.143
1.139
1.128
1.121
1.115
1.111
1.108
1.096
1.085
1.080
1.078
1.062
1.054
1.048
1.040
1.033
1.029
1.019
1.015
1.004
0.994
0.989
0.975
0.959
0.945
0.929
0.918
0.915
0.906
0.900
0.880
.8 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 0.9 ppm
2.00
2.06
6.38
1.13
2.14
4.42
3.44

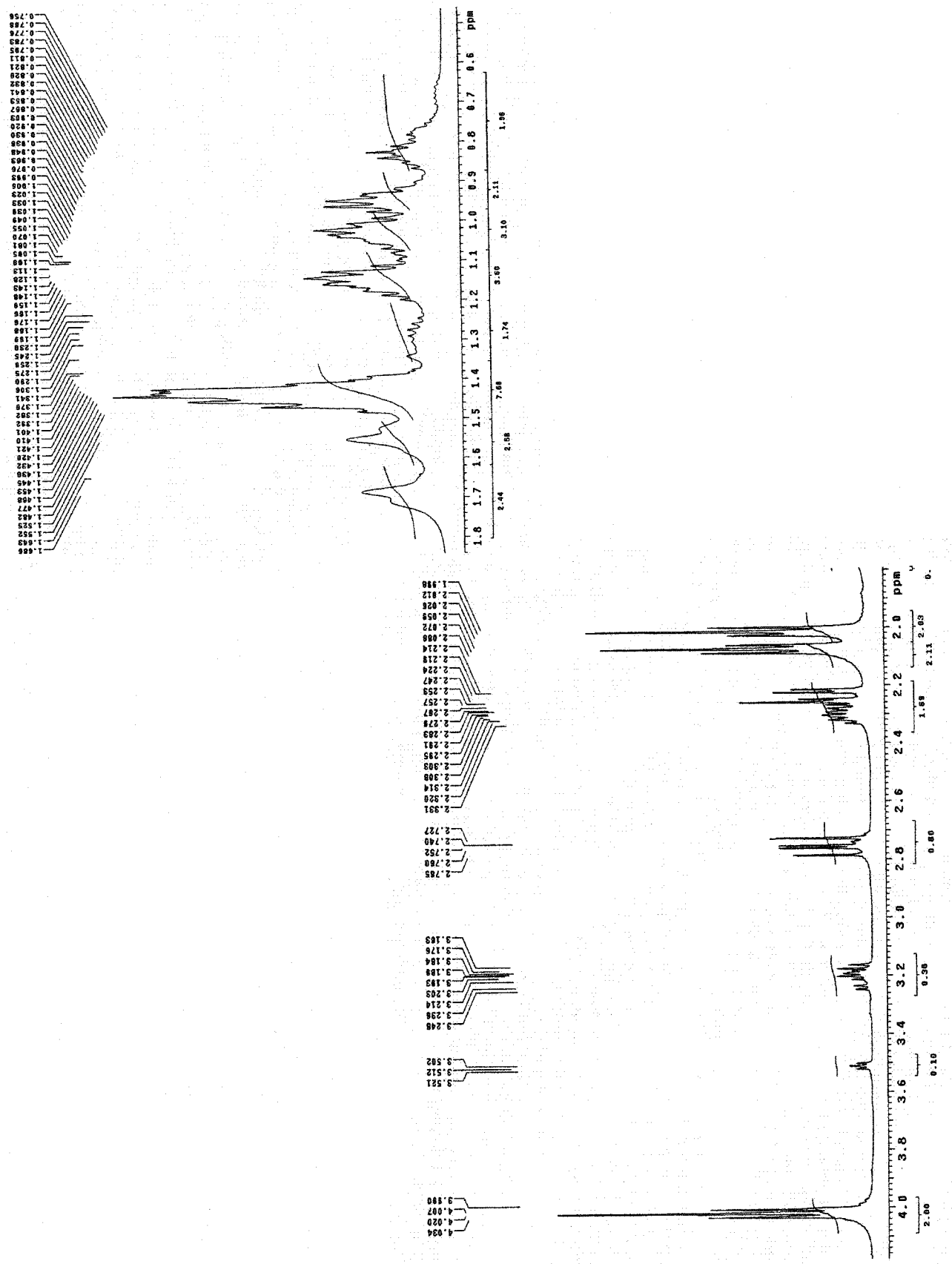
exp1 gCOSY

SAMPLE		FLAGS	
date	Apr 6 2001	hs	nn
solvent	C6D6	sspul	n
sample	alex_06Apr2~	hsglvl	4385
	001-22:59:56	SPECIAL	
ACQUISITION		temp	not used
sw	7996.0	gain	28
at	0.128	spin	0
np	2048	F2 PROCESSING	
fb	4000	sb	-0.064
ss	16	sbs	not used
d1	1.000	fn	2048
nt	1	F1 PROCESSING	
2D ACQUISITION		sb1	-0.016
sw1	7996.0	sbs1	not used
ni	128	proc1	lp
TRANSMITTER		fn1	2048
tn	H1	DISPLAY	
sfrq	499.742	sp	-14.7
tof	499.8	wp	2352.7
tpwr	55	sp1	24.4
pw	9.600	wp1	2251.1
GRADIENTS		rf1	999.6
gzlvl1	4385	rfp	0
gt1	0.001000	rf11	999.6
gstab	0.000500	rfp1	0
DECOUPLER		PLOT	
dn	C13	wc	135.0
dm	nnn	sc	10.0
		wc2	135.0
		sc2	0
		vs	232
		th	6
		ai cdc av	



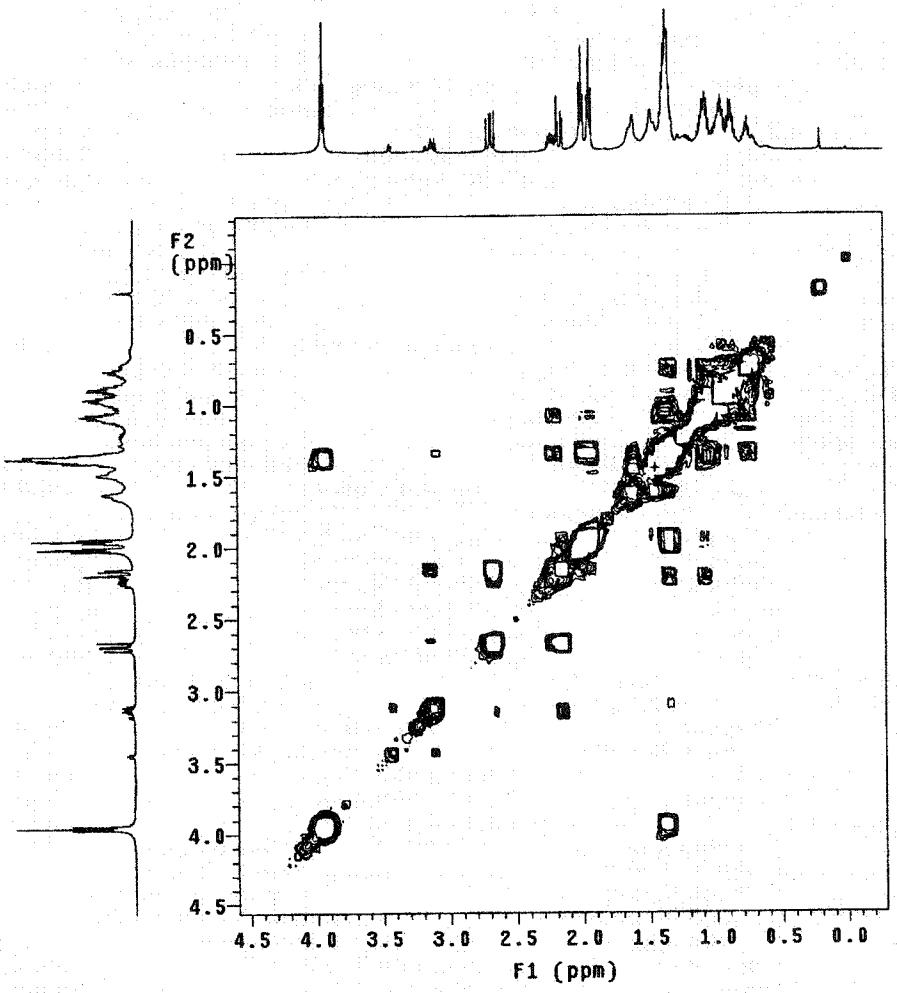

Pulse Sequence: s2pul
Solvent: C6D6
Ambient temperature
User: 1-14-87
INOVA-500 "nmr500"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.043 sec
Width 31409.5 Hz
15000 repetitions
OBSERVE C13, 125.6585636 MHz
DECOUPLE H1, 499.7419842 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 8 hr, 33 min, 2 sec
ppm
205.206
172.396
168.780
168.681
128.190
128.099
127.809
127.710
64.105
48.886
48.680
33.546
32.592
30.456
29.045
29.022
28.839
27.687
25.917
25.162
24.079
24.010

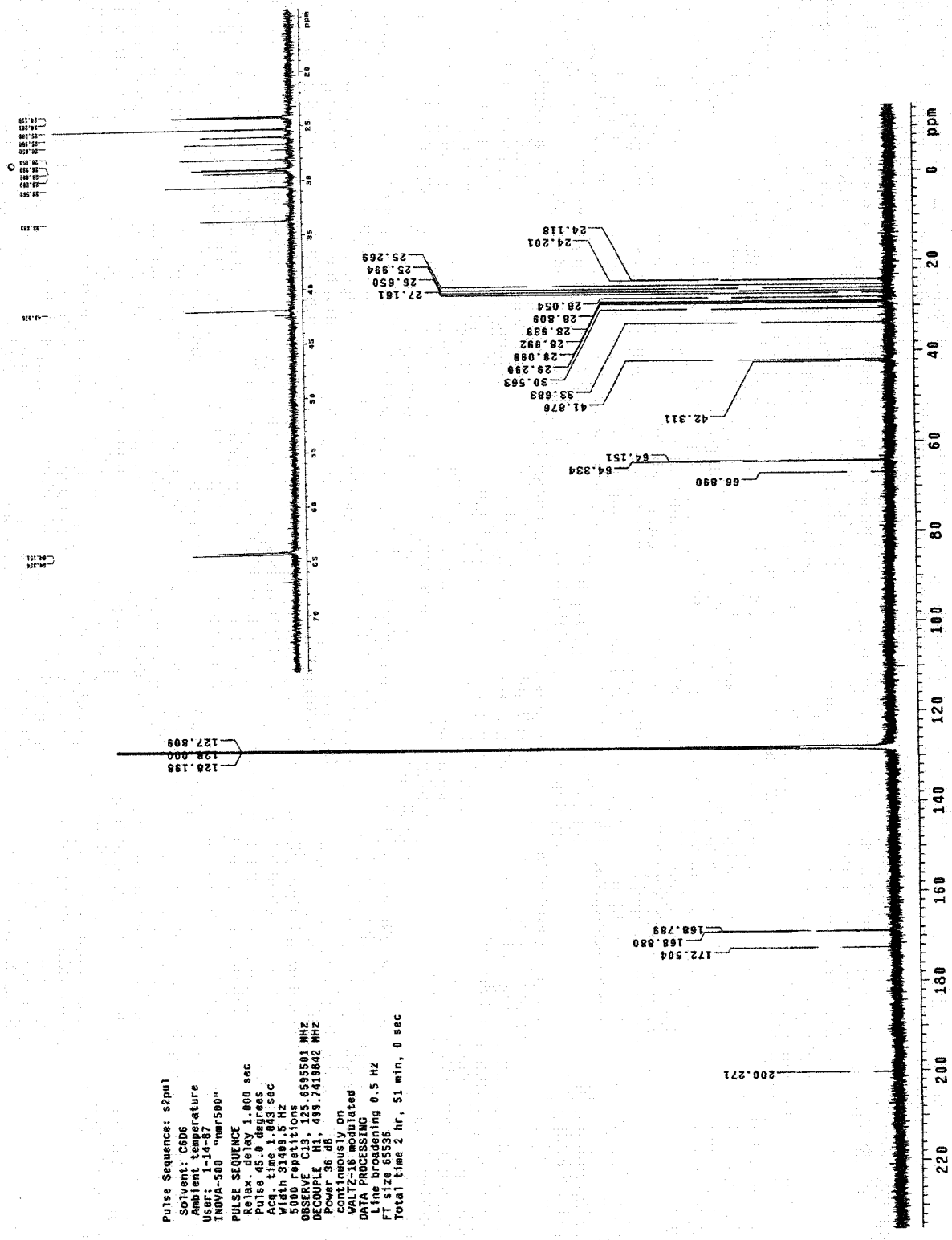

exp1 s2pul
SAMPLE
date Apr 12 2001
solvent C6D6
file exp
ACQUISITION
sw 5997.0
at 2.049
np 24576
fb 3000
bs 32
d1 1.000
nt 8
ct 8
TRANSMITTER
tn H1
sfrq 499.742
tof 0.1
tpwr 55
pw 4.800
DECOUPLER
dn C13
dof 0
dm nnn
dmm c
dpwr 52
dmf 14122
SPECIAL
temp not used
gain not used
spin 22
hst 0.008
pw90 9.600
alfa 0
FLAGS
il n
in n
dp y
hs nn
PROCESSING
fn not used
DISPLAY
sp -483.4
wp 5997.0
rfl 4056.6
rfp 3573.1
rp -163.2
lp -9.9
PLOT
wc 180
sc 0
vs 144
th 4
nm cdc ph
7.150
2.059
2.026
2.012
1.998
1.686
1.643
1.552
1.525
1.482
1.466
1.453
1.438
1.421
1.392
1.341
1.306
1.280
1.275
1.259
1.245
1.230
1.176
1.159
1.143
1.128
1.113
1.100
1.070
1.049
1.023
1.006
0.993
0.976
0.963
0.948
0.920
0.903
0.867
0.841
0.826
0.811
0.795
0.783
0.768
0.274
I-35
11
10
9
8
7
6
5
4
3
2
1
-0
ppm
2.00
0.38
1.62
3.03
2.58
3.73
4.10
3.96
0.10
0.80
2.11
2.47
4.63
3.80
11



exp1 gCOSY

SAMPLE		FLAGS	
date	Apr 12 2001	hs	nn
solvent	C6D6	sspul	n
sample	alex_12Apr2~001	hsglvl	4385
ACQUISITION		SPECIAL	
sw	7996.0	temp	not used
at	0.128	gain	22
np	2048	spin	0
fb	4000	F2 PROCESSING	
ss	16	sb	-0.064
d1	1.000	sbs	not used
nt	1	fn	2048
2D ACQUISITION		F1 PROCESSING	
sw1	7996.0	sb1	-0.016
ni	128	sbs1	not used
TRANSMITTER		proc1	lp
tn	H1	fn1	2048
sfrq	499.742	DISPLAY	
tof	499.8	sp	-159.8
tpwr	55	wp	2438.7
pw	9.600	sp1	-144.2
GRADIENTS		wp1	2438.7
gzlvl1	4385	rf1	1019.6
gt1	0.001000	rfp	0
gstab	0.000500	rfl1	1019.6
DECOUPLER		rfp1	0
dn	C13	PLOT	
dm	nnn	wc	135.0
		sc	10.0
		wc2	135.0
		sc2	0
		vs	417
		th	7
		ai cdc av	




Pulse Sequence: s2pul
Solvent: CDD6
Ambient temperature
User: I-14-87
INOVA-500 "nmr500"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.043 sec
Width 31408.5 Hz
5000 repetitions
OBSERVE C13, 125.6585501 MHz
DECOUPLE H1, 499.7419842 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 2 hr, 51 min, 0 sec
200.271
172.504
168.880
168.769
128.186
128.000
127.809
66.890
64.334
64.151
42.311
41.876
33.683
30.563
29.290
29.099
28.892
28.839
28.809
28.054
27.161
26.650
25.994
25.269
24.201
24.118
ppm

APPENDIX

^{1}H and ^{13}C NMR Spectra

List of ^{1}H and ^{13}C NMR Spectra of PART II

2,2-Dimethyl-1,3,2-benzodithiastannoles (II-11)..... 369

1,2,3-Benzotrithiole 2-oxide (II-6)..... 371

1,2,3-Benzotrithiole 1-oxide (II-7)..... 373

5-Methyl 2,2-dimethyl-1,3,2-benzodithiastannoles (II-13)..... 375

5-Methyl 1,2,3-benzotrithiole 2-oxide (II-8)..... 377

5-Methyl 1,2,3-benzotrithiole 1-oxide (II-9a) and 6-methyl 1,2,3-benzotrithiole 1-oxide (II-9b)..... 379

5,6-Dibromo-4-hydroxy-3-methoxybenzaldehyde (II-15)..... 382

2,3-Bis(*n*-butylthio)-4-hydroxy-5-methoxybenzaldehyde (II-16)..... 384

2,3-Bis(*n*-butylthio)-4,5-dimethoxybenzaldehyde (II-17)..... 387

3,4-Bis(*n*-butylthio)-1,2-dimethoxy-5-(2-nitroethenyl)benzene (II-18)..... 389

N-(Boc)-2',3'-bis(*n*-butylsulfanyl)-4',5'-dimethoxyphenethylamine (II-20).... 391

N-(Boc)-2',3'-dimercapto-4',5'-dimethoxyphenethylamine (II-21)..... 394

N-(Boc)-4-ethylamino-6,7-dimethoxy-2,2-dimethyl-1,3,2-benzodithiastannole (II-22)..... 397

N-(Boc)-4-ethylamino-6,7-dimethoxy-1,2,3-benzotrithiole 2-oxide (II-23)..... 399

4-Ethylamino-6,7-dimethoxy-1,2,3-benzotrithiole 2-oxide trifluoroacetate (II-5)..... 401

N-(Boc)-4-ethylamino-6,7-dimethoxy-1,2,3-benzotrithiole 1-oxide (II-24)..... 404

4-Ethylamino-6,7-dimethoxy-1,2,3-benzotrithiole 1-oxide trifluoroacetate (II-3)..... 407

N-(Boc)-4-ethylamino-6,7-dimethoxy-1,2,3-benzotrithiole 3-oxide (II-25)..... 410

4-Ethylamino-6,7-dimethoxy-1,2,3-benzotrithiole 3-oxide trifluoroacetate (II-4)..... 413

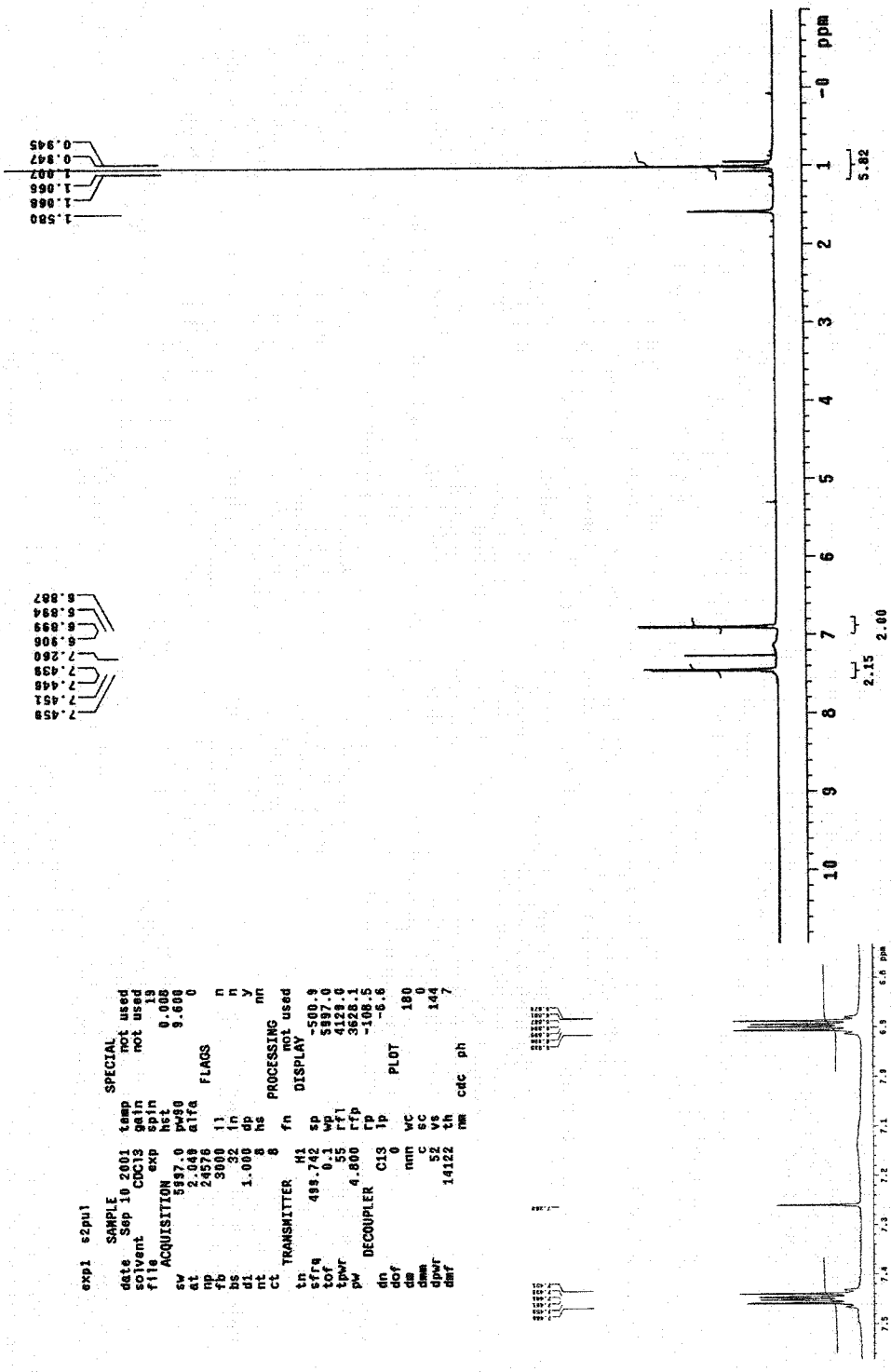
6,7-Dimethoxy-9-[[(*t*-butyl carbamate)amino]ethyl]benzopentathiepin (II-26)..... 417

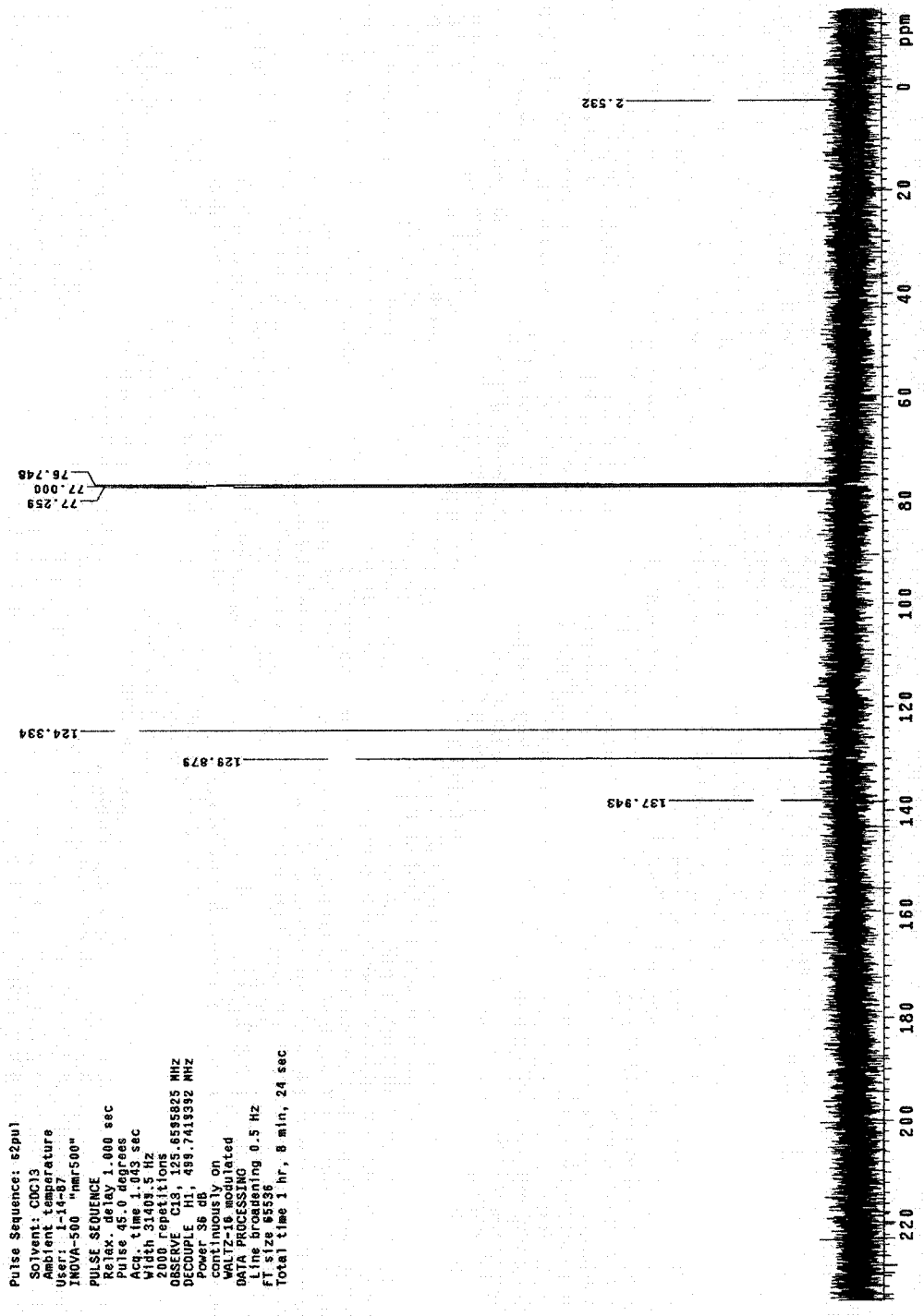
6,7-Dimethoxy-9-[(amino)ethyl]benzopentathiepin trifluoroacetate (II-1)..... 419

6,7-Dimethoxy-9-[(amino)ethyl]benzopentathiepin hydrogen chloride/ varacinium chloride (II-1)..... 421



II-11

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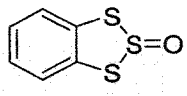
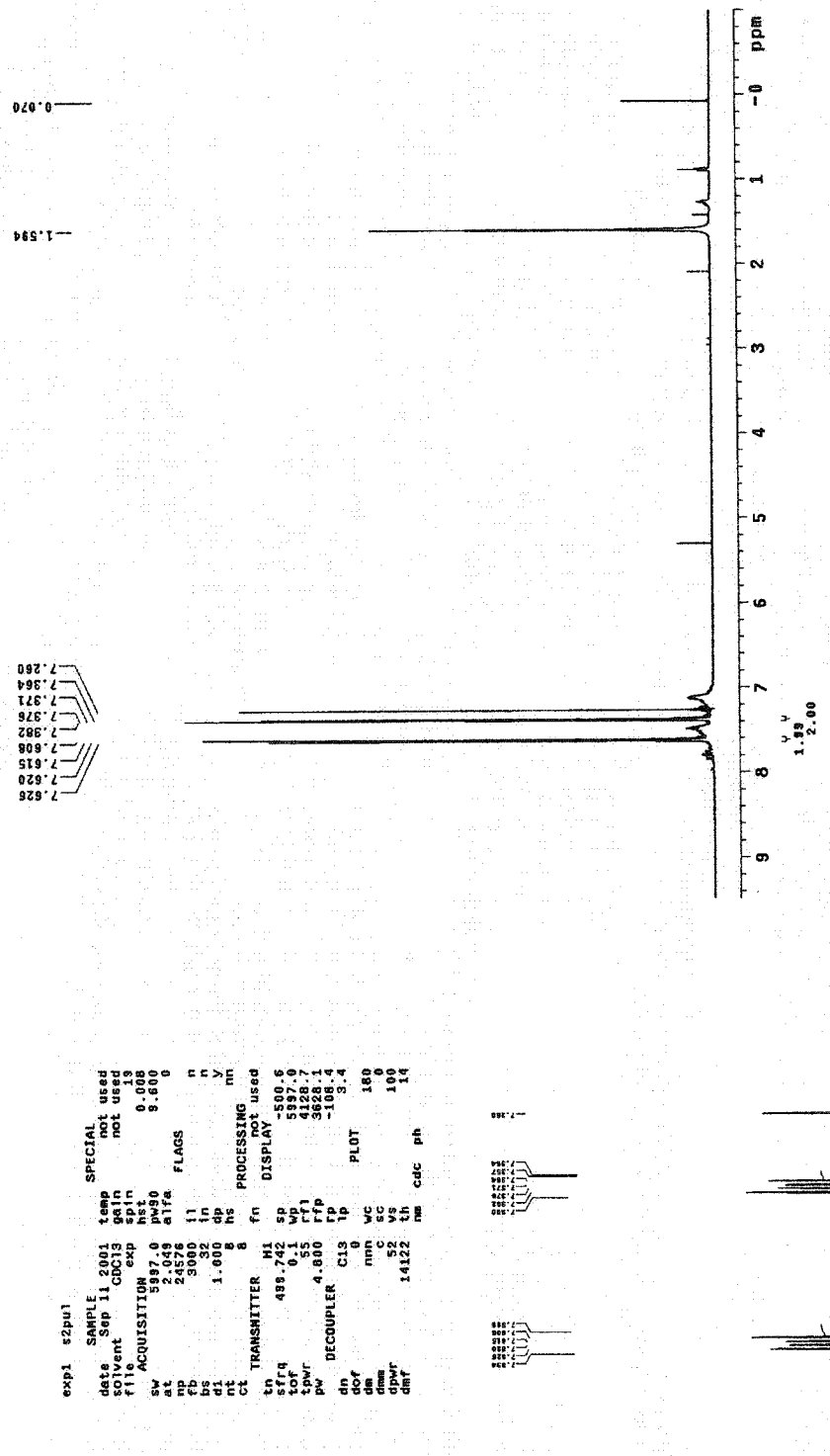
Solvent: CDCl3
Ambient temperature
User: I-14-87
INOVA-500 "nmr500"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.043 sec
Width 31409.5 Hz
2000 repetitions
OBSERVE C13, 125.6595825 MHz
DECOUPLE H1, 499.7419392 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 1 hr, 8 min, 24 sec
137.943
129.879
124.334
77.259
77.000
76.748
2.532
220
200
180
160
140
120
100
80
60
40
20
0
ppm



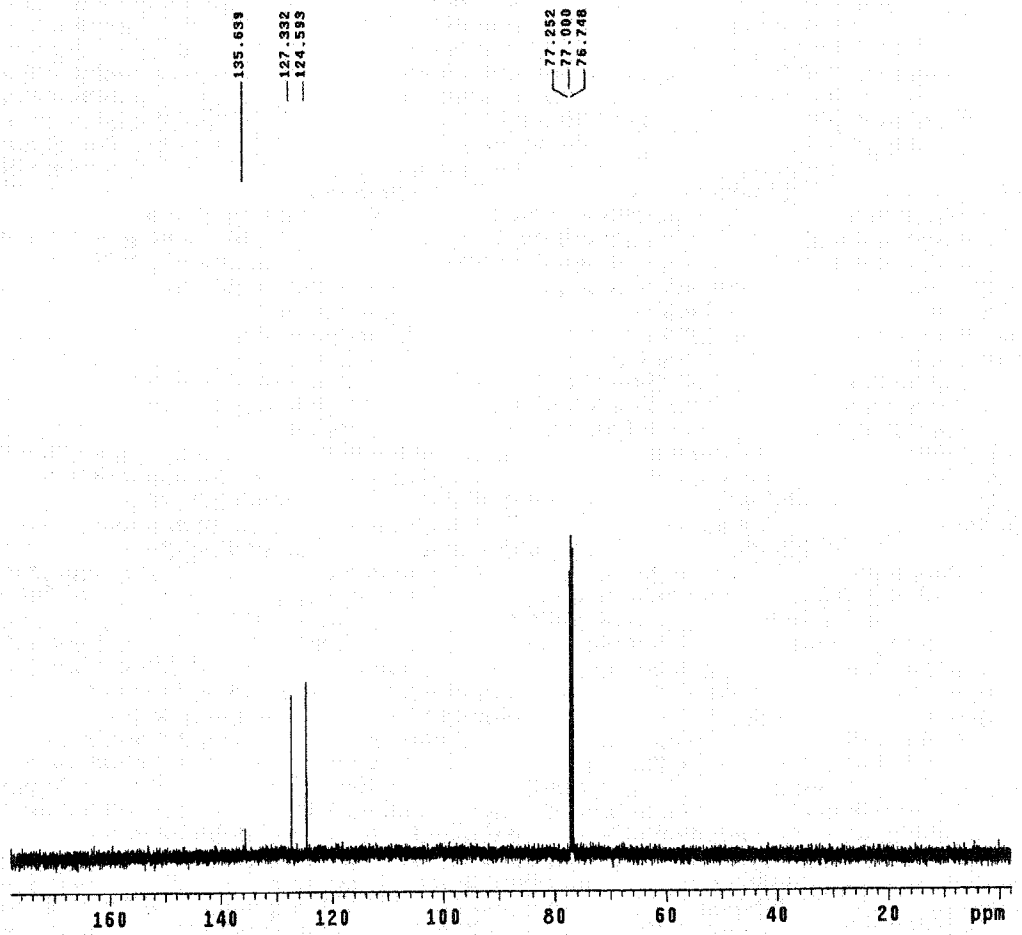
II-6



Test-Alex

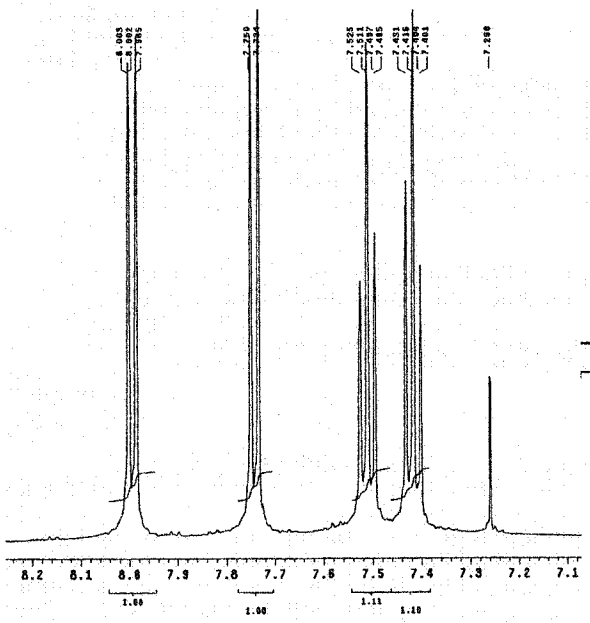
exp2 s2pul

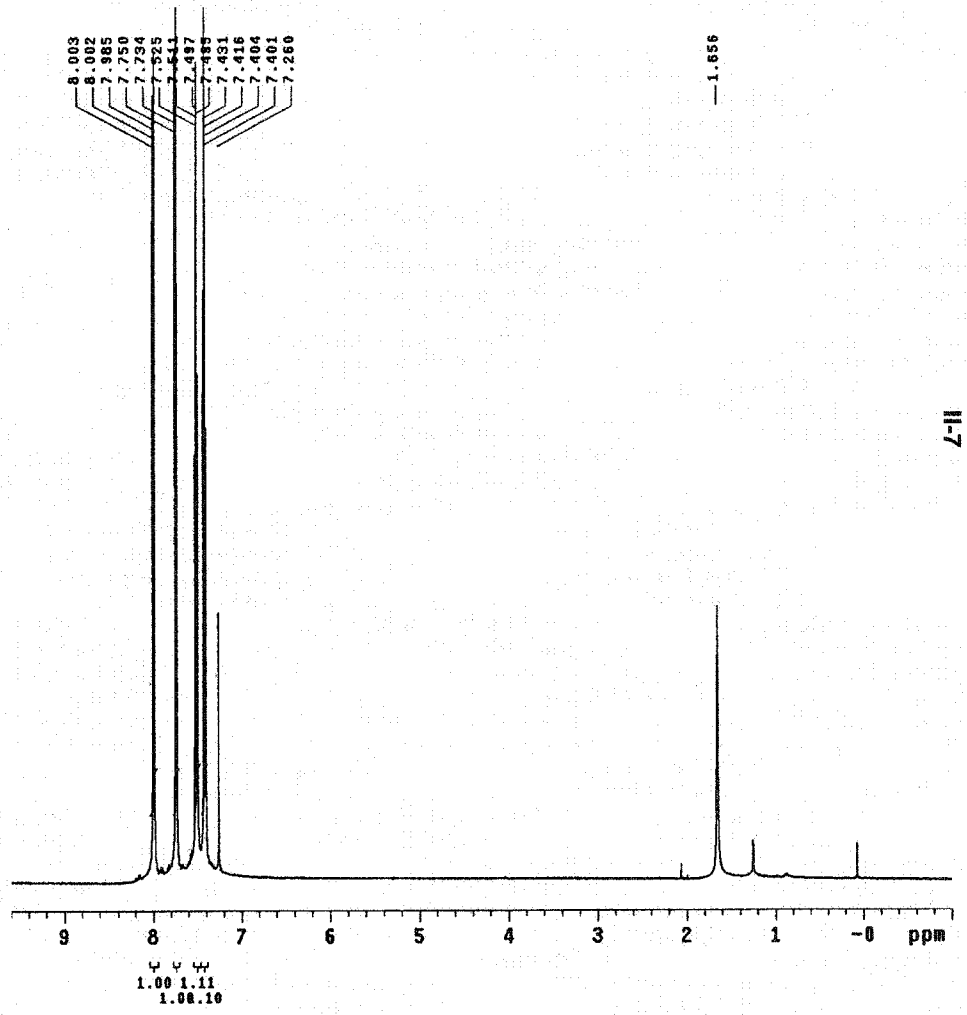
SAMPLE		SPECIAL	
date	Sep 11 2001	temp	not used
solvent	CDCl3	gain	not used
file	/export/home/~	spin	20
guorw/vnmrsys/data~		hst	0.008
/test-Alex_11Sep20~		pw90	22.800
01/CARBON.fid		alfa	10.000
ACQUISITION		**FLAGS**	
sw	31409.5	il	n
at	1.043	in	n
np	65536	dp	y
fb	17000	hs	nn
bs	64	**PROCESSING**	
d1	1.000	lb	0.50
nt	5000	fn	not used
ct	5000	**DISPLAY**	
TRANSMITTER		sp	-268.4
tn	C13	wp	22618.4
sfrq	125.673	rf1	11588.2
tof	1883.4	rfp	9675.8
tpwr	63	rp	12.6
pw	11.400	lp	-234.8
DECOUPLER		**PLOT**	
dn	H1	wc	180
dof	0	sc	0
dm	yyy	vs	1386
dmm	w	th	4
dpwr	35	ai	cdc ph
dmf	11696		



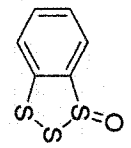
exp1 s2pul

SAMPLE		SPECIAL	
date	Sep 15 2001	temp	not used
solvent	CDCl3	gain	not used
file	exp	spin	22
ACQUISITION		hst	0.008
sw	5987.0	pw90	9.600
at	2.049	alfa	0
np	24576	**FLAGS**	
fb	3000	il	n
bs	32	in	n
d1	1.000	dp	y
nt	8	hs	nn
ct	8	**PROCESSING**	
TRANSMITTER		fn	not used
tn	H1	**DISPLAY**	
sfrq	499.742	sp	-500.9
tof	0.1	wp	5997.0
tpwr	55	rfl	4129.0
pw	4.800	rfp	3628.1
DECOUPLER		rp	-116.0
dn	C13	lp	10.1
dof	0	**PLOT**	
dm	nnn	wc	180
dmm	c	sc	0
dpwr	52	vs	144
dmf	14122	th	31
		nm	cdc ph

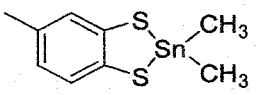




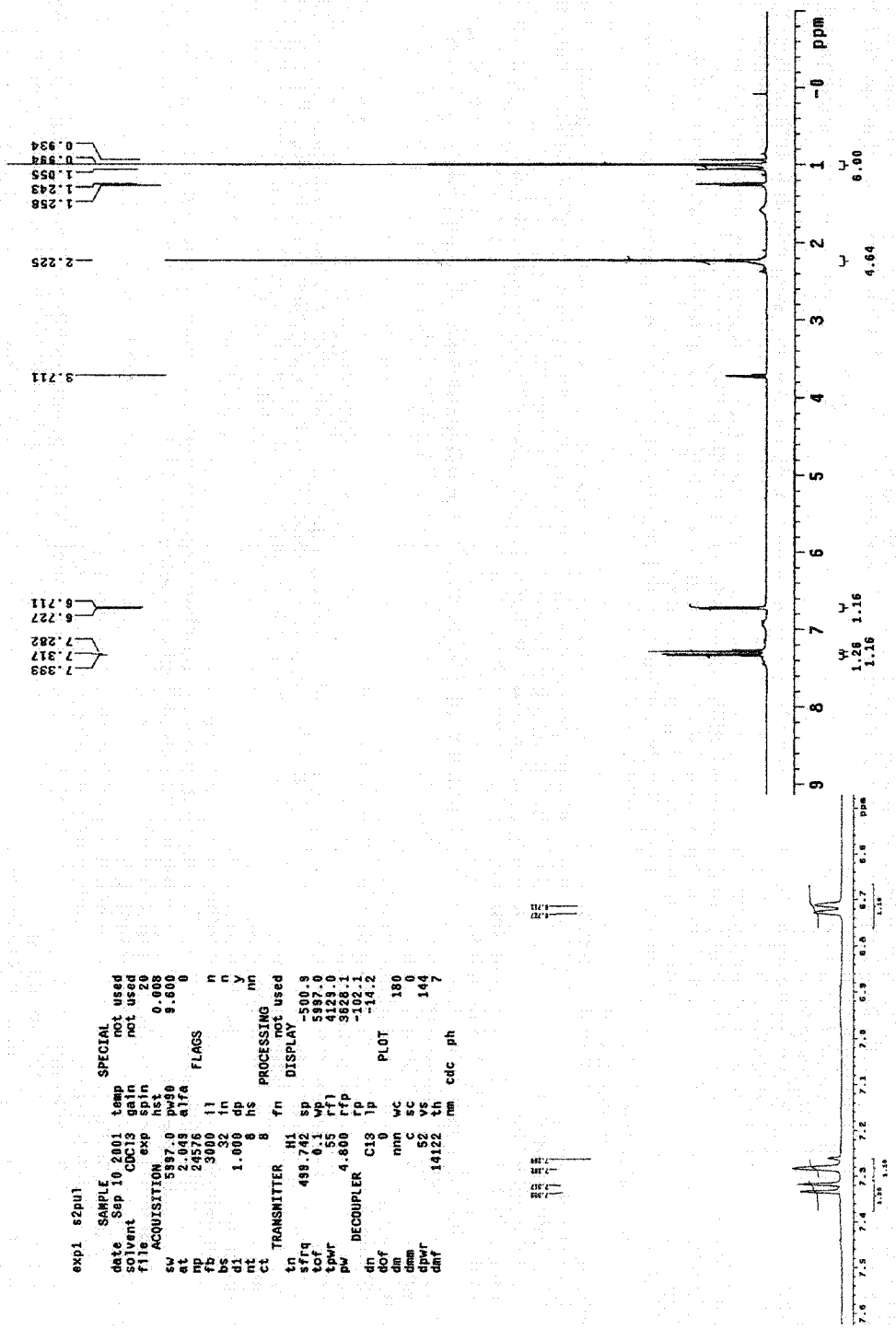
III-7

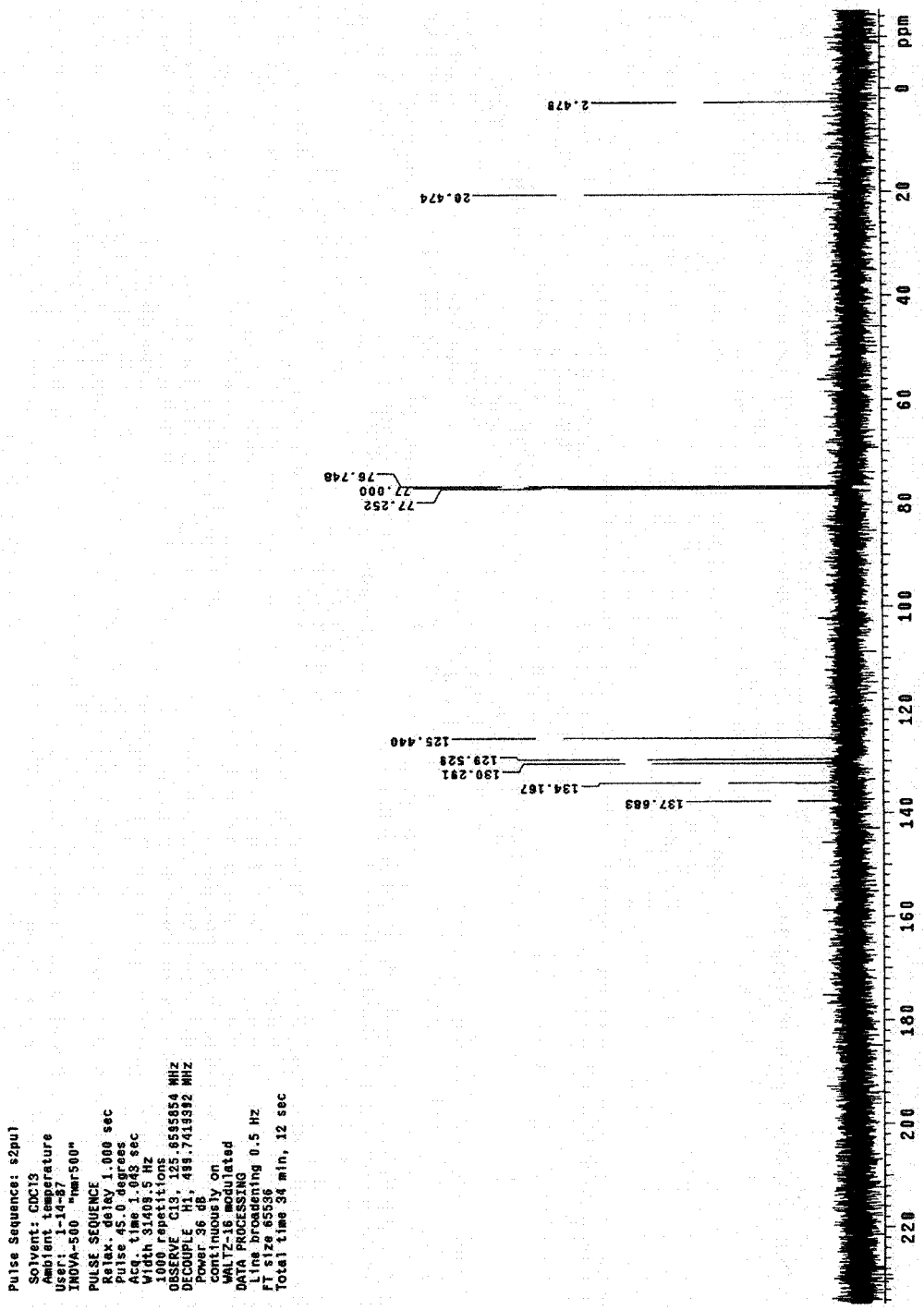


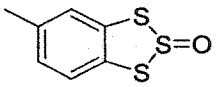
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
User: 1-14-87
INOVA-500 "nmr500"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.043 sec
Width 31408.5 Hz
2000 repetitions
OBSERVE C13, 125.6595873 MHz
DECOUPLE H1, 499.7419392 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 1 hr, 8 min, 24 sec
150.110
145.060
131.329
126.599
126.500
123.723
77.259
77.000
76.748
ppm



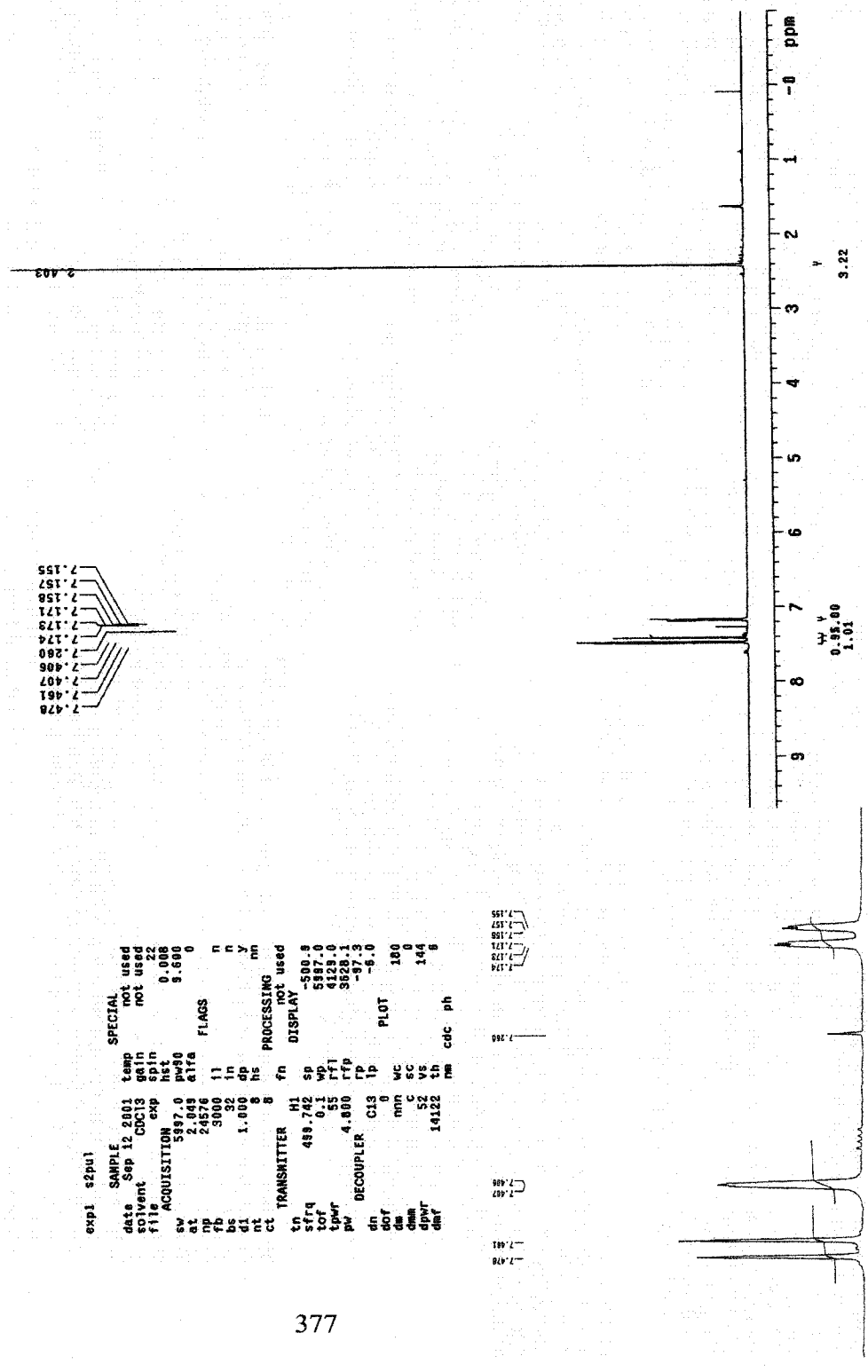
II-13


0.934
0.994
1.055
1.243
1.258
2.225
3.711
6.711
6.727
7.282
7.317
7.393
ppm
6.00
4.64
1.16
1.26
1.16

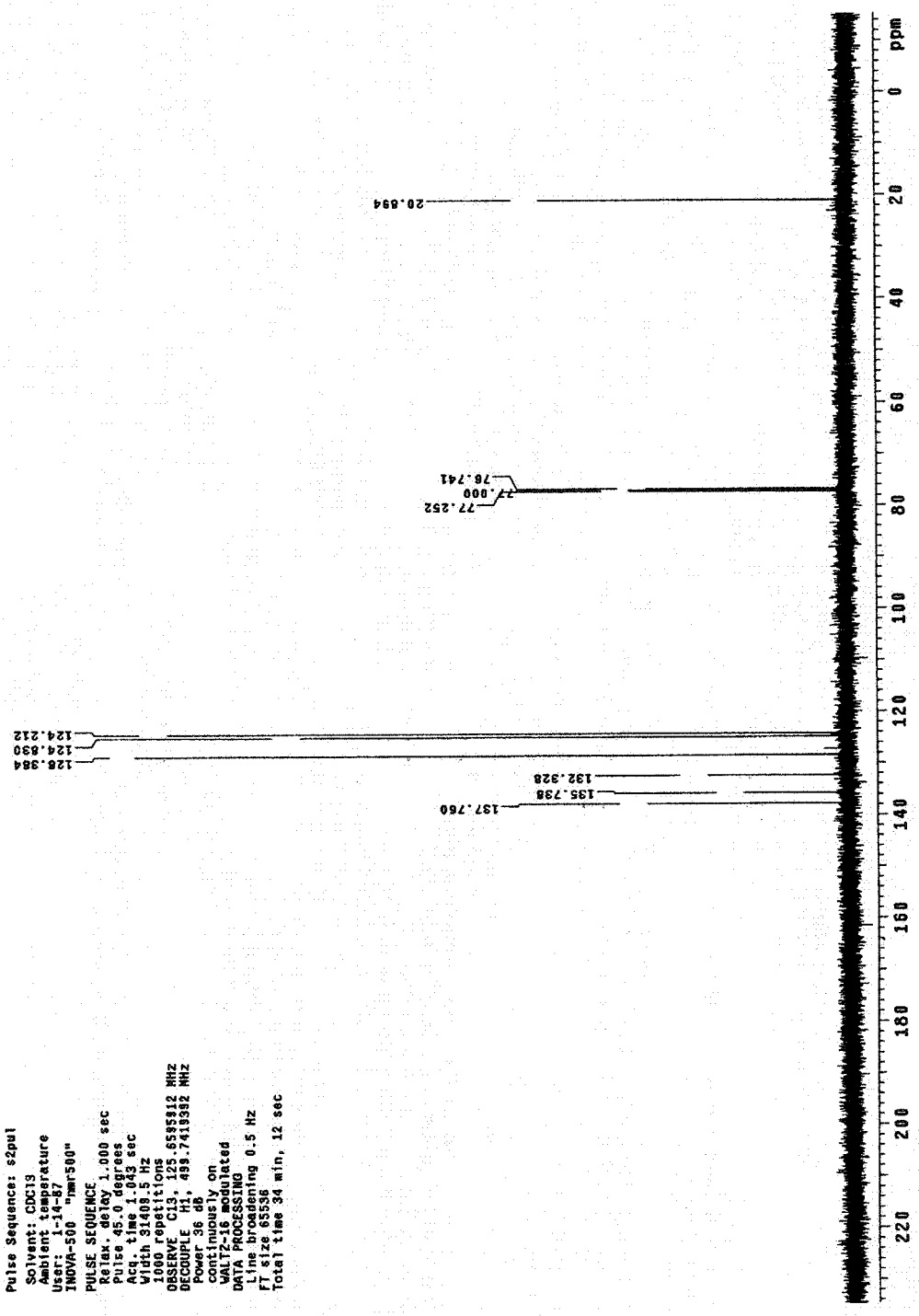

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
User: 1-14-87
INOVA-500 "mar500"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.048 sec
Width 31403.5 Hz
1000 repetitions
OBSERVE C13, 125.6595854 MHz
DECOUPLE H1, 499.7419392 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 34 min, 12 sec
137.683
134.167
130.291
129.528
125.440
77.252
77.000
76.748
20.474
2.478
ppm

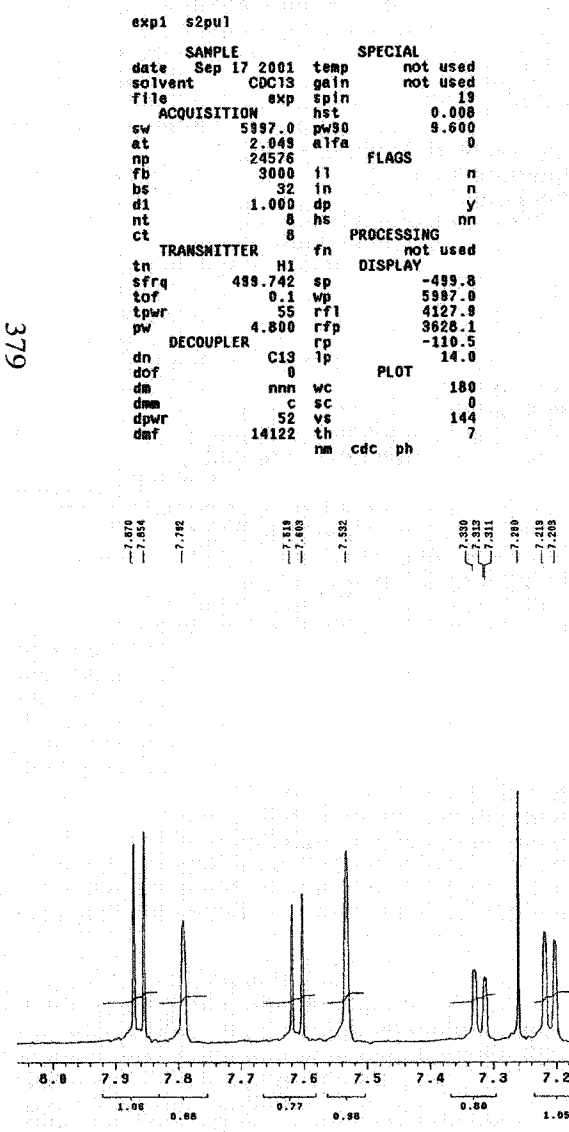


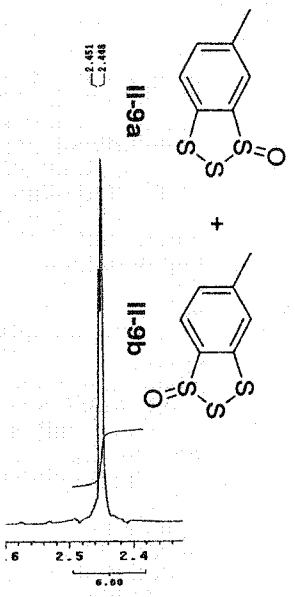
II-8



7.155
7.157
7.158
7.171
7.173
7.174
7.260
7.406
7.407
7.461
7.478
2.403
ppm
3.22
0.95.00
1.01

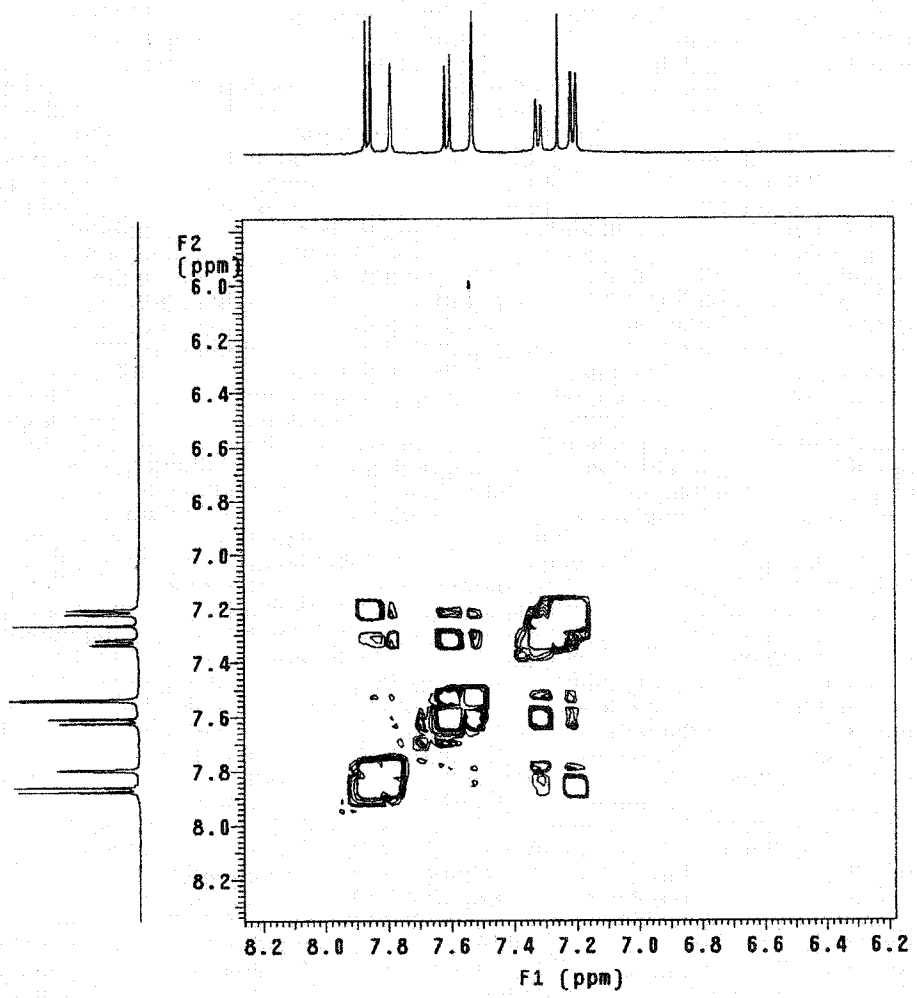

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
User: 1-14-07
INOVA-500 "nmr500"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.043 sec
Width 31408.5 Hz
1000 repetitions
OBSERVE C13, 125.6585812 MHz
DECOUPLE H1, 499.7419382 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 34 min, 12 sec
128.384
124.830
124.212
137.760
135.738
132.328
77.252
77.000
76.741
20.894
220
200
180
160
140
120
100
80
60
40
20
0
ppm

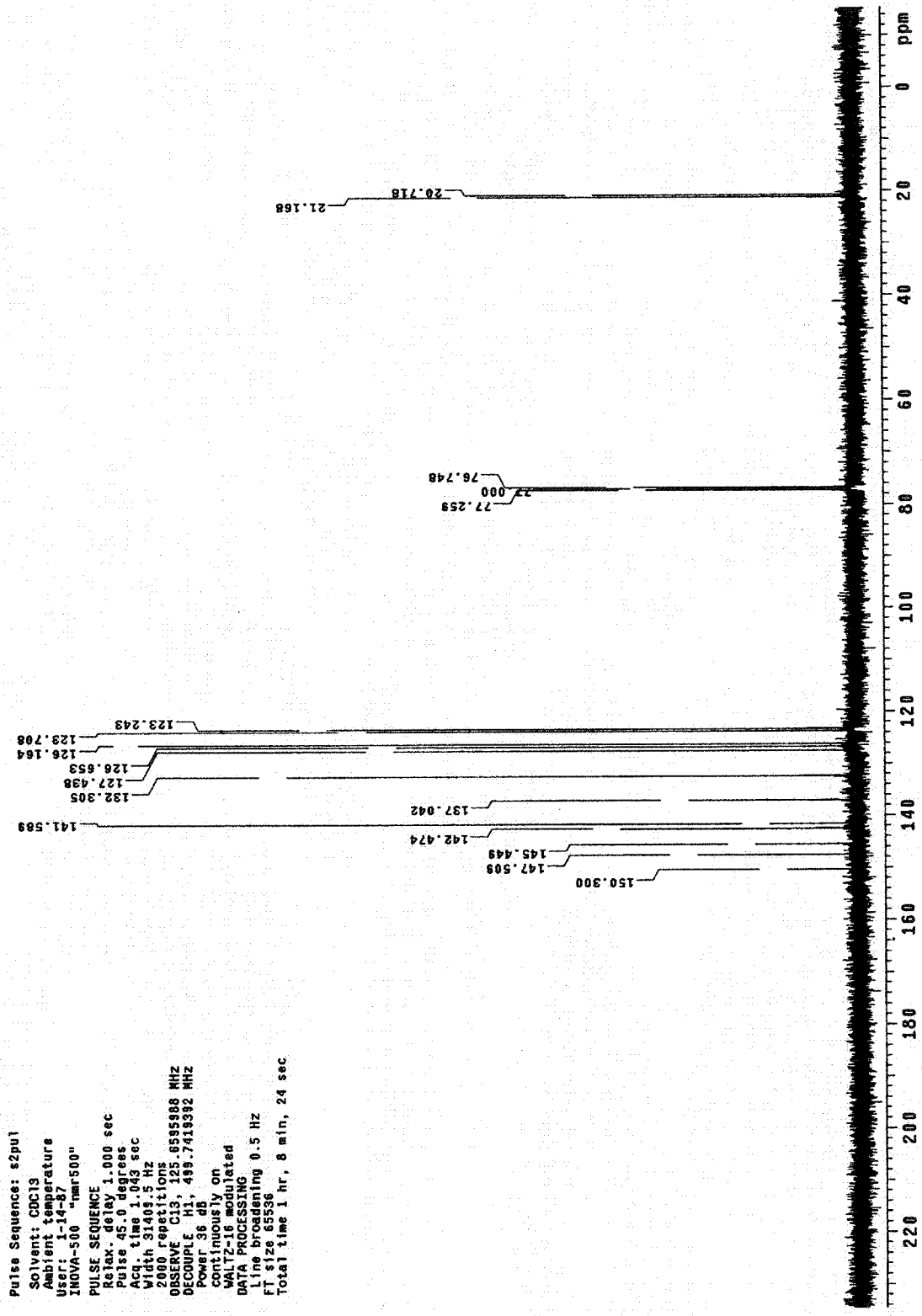

exp1 s2pul
SAMPLE
date Sep 17 2001
solvent CDCl3
file exp
ACQUISITION
sw 5987.0
at 2.048
np 24576
fb 3000
bs 32
d1 1.000
nt 8
ct 8
TRANSMITTER
tn H1
sfrq 499.742
tof 0.1
tpwr 55
pw 4.800
DECOUPLER
dn C13
dof 0
dm nnn
dmm c
dpwr 52
dmf 14122
SPECIAL
temp not used
gain not used
spin 19
hst 0.008
pw90 9.600
alfa 0
FLAGS
il n
in n
dp y
hs nn
PROCESSING
fn not used
DISPLAY
sp -499.8
wp 5987.0
rfl 4127.9
rfp 3628.1
rp -110.5
lp 14.0
PLOT
wc 180
sc 0
vs 144
th 7
nm cdc ph
7.870
7.854
7.792
7.619
7.603
7.532
7.330
7.313
7.311
7.260
7.219
7.203
2.451
2.448
1.628
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
1.08
0.66
0.77
0.98
0.80
1.05
9
8
7
6
5
4
3
2
1
-0
ppm
6.00

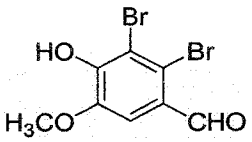

II-9a
+
II-9b

exp1 gCOSY

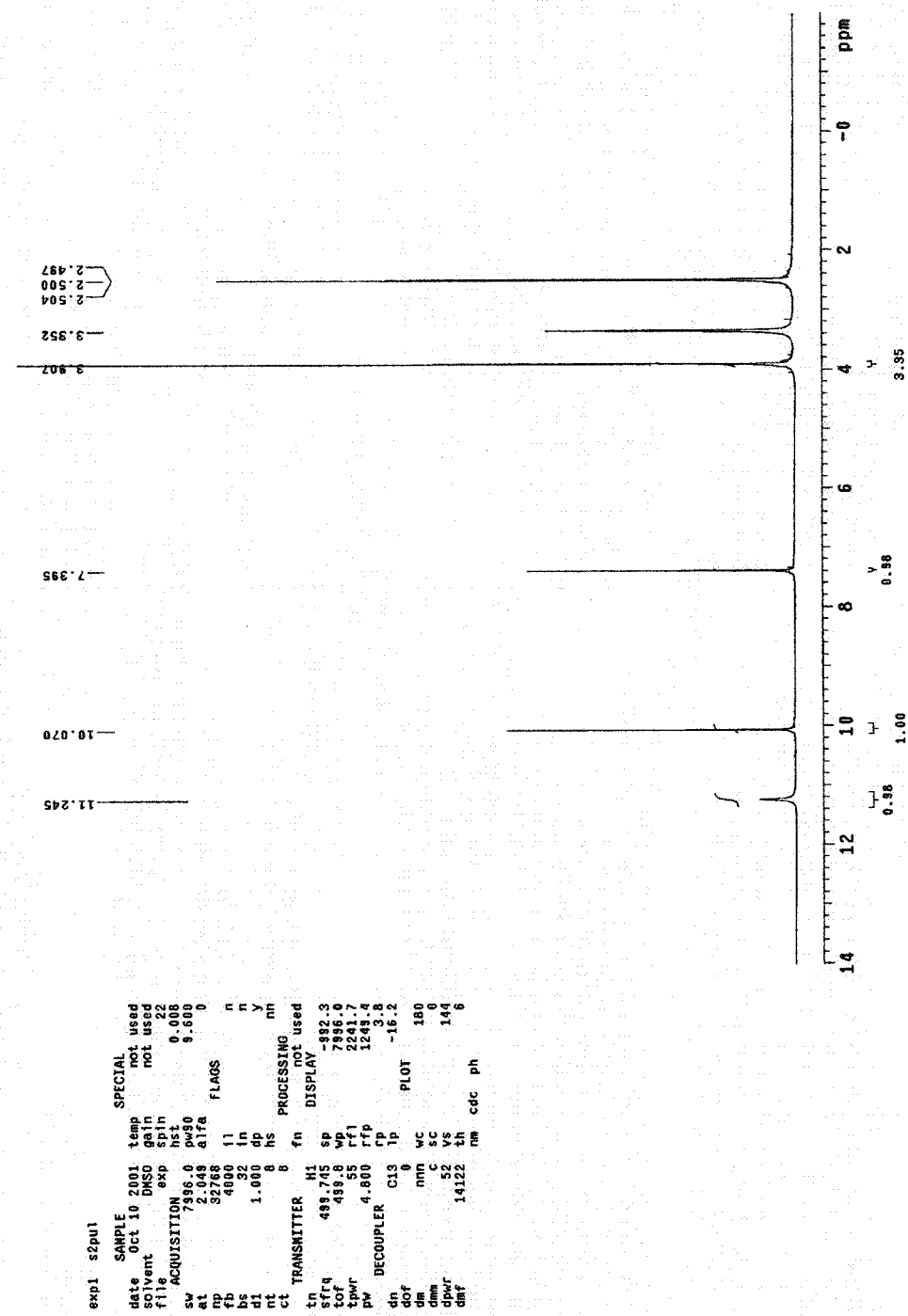
SAMPLE		FLAGS	
date	Sep 17 2001	hs	nn
solvent	CDCl3	sspul	n
sample	alex_17Sep2~001	hsglvl	4385
ACQUISITION		SPECIAL	
sw	7996.0	temp	not used
at	0.128	gain	36
np	2048	spin	0
fb	4000	**F2 PROCESSING**	
ss	16	sb	-0.064
d1	1.000	sbs	not used
nt	1	fn	2048
2D ACQUISITION		**F1 PROCESSING**	
sw1	7996.0	sb1	-0.016
ni	128	sbs1	not used
TRANSMITTER		proc1	lp
tn	H1	fn1	2048
sfrq	499.742	DISPLAY	
tof	499.8	sp	2877.4
tpwr	55	wp	7287.5
pw	9.600	sp1	3088.4
GRADIENTS		wp1	1039.6
gzlvl1	4385	rf1	999.5
gt1	0.001000	rfp	0
gstab	0.000500	rfl1	999.5
DECOUPLER		rfp1	0
dn	C13	PLOT	
dm	nnn	wc	135.0
		sc	10.0
		wc2	135.0
		sc2	0
		vs	54
		th	4
		ai cdc av	



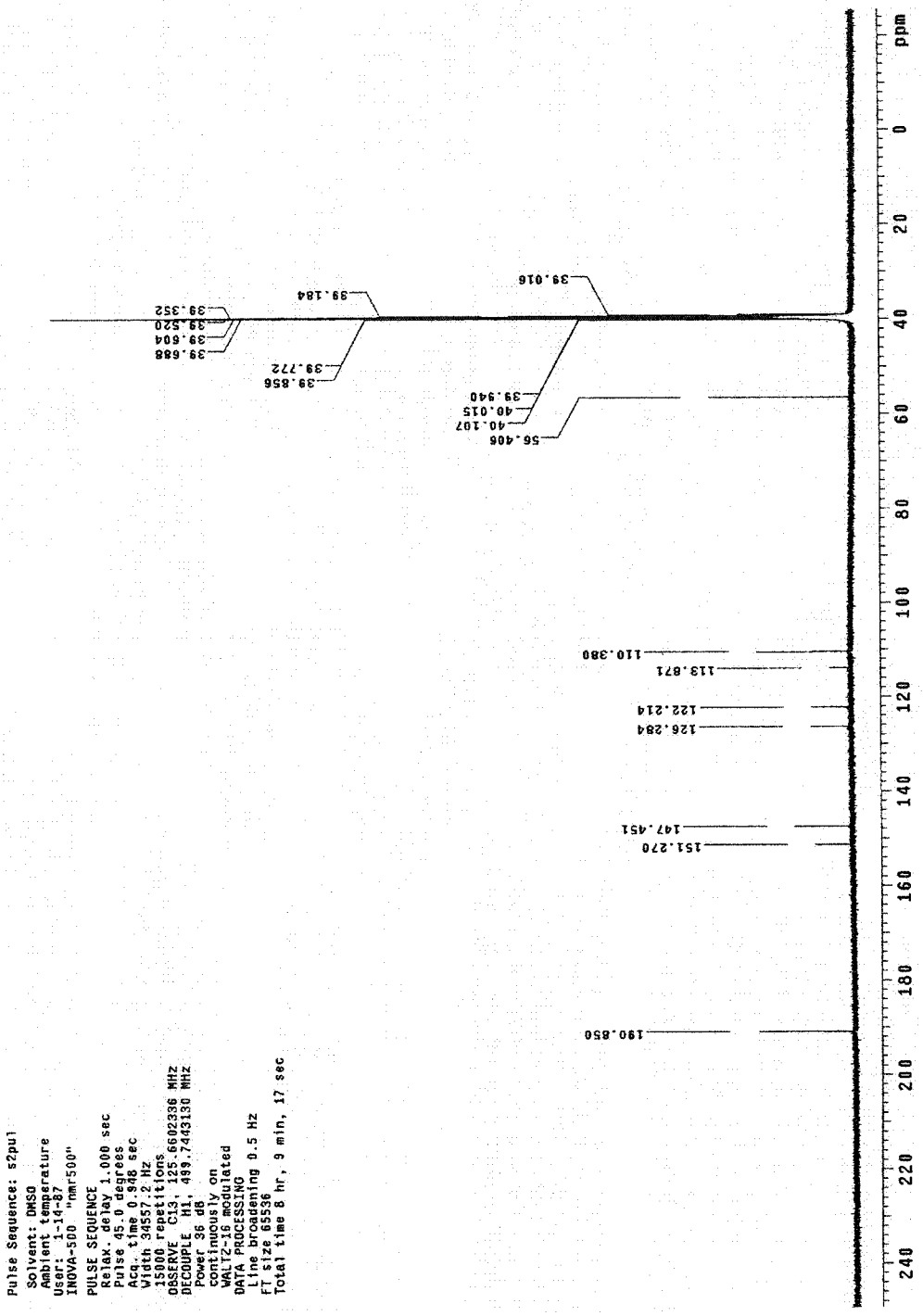

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
User: 1-14-87
INOVA-500 "nmr500"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.043 sec
Width 31405.5 Hz
2000 repetitions
OBSERVE C13, 125.6595888 MHz
DECOUPLE H1, 499.7419392 MHz
Power 38 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 1 hr, 8 min, 24 sec
21.168
20.718
77.000
76.748
77.259
123.243
123.708
126.164
126.653
127.438
132.305
137.042
141.589
142.474
145.449
147.509
150.300
ppm



II-15



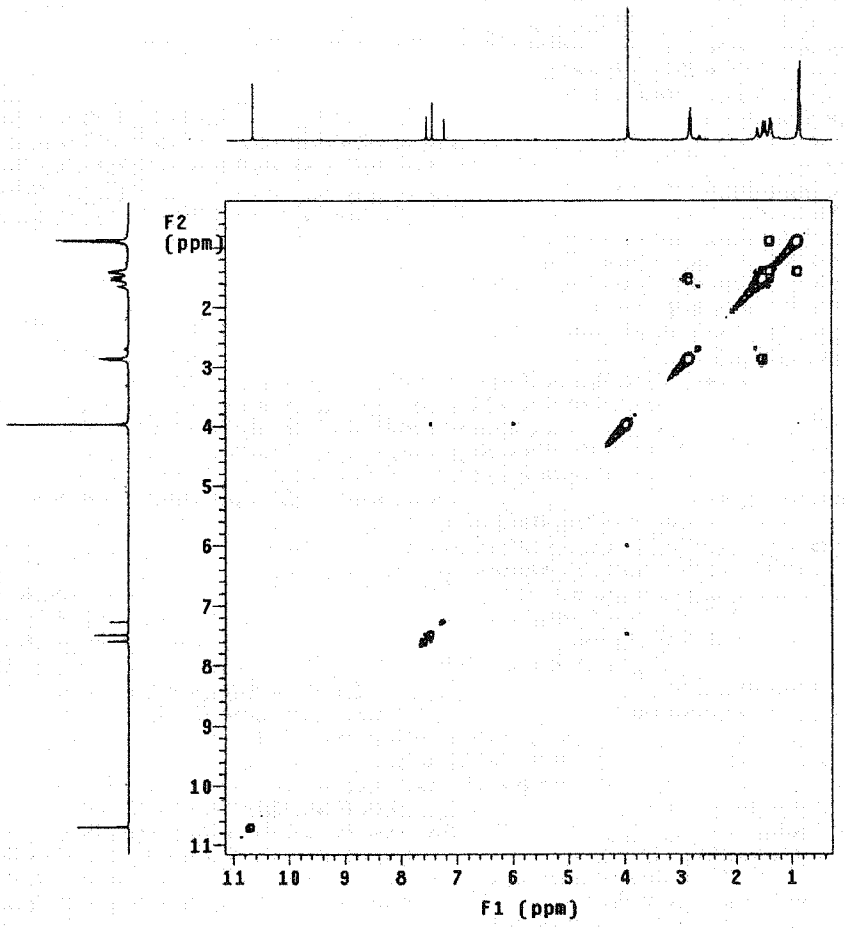

```
exp1  s2pul

          SAMPLE                          SPECIAL
date   Oct 10 2001  temp      not used
solvent        DMSO  gain      not used
file            exp  spin            22
      ACQUISITION    hst        0.008
sw          7996.0  pw90       9.600
at          2.049  alfa           0
np          32768
fb          4000  FLAGS
bs             32  il          n
d1          1.000  dp          y
nt              8  hs          nn
ct              8  PROCESSING
      TRANSMITTER    fn     not used
tn             H1  DISPLAY
sfrq        499.745  sp       -992.3
tof         499.8  wp        7996.0
tpwr           55  rf1       2241.7
pw          4.800  rfp       1249.4
      DECOUPLER      rp        3.8
dn             C13  lp       -16.2
dof              0  PLOT
dm             nnn  wc         180
dmm              c  sc           0
dpwr            52  vs         144
dmf          14122  th           6
                    nm  cdc  ph
```

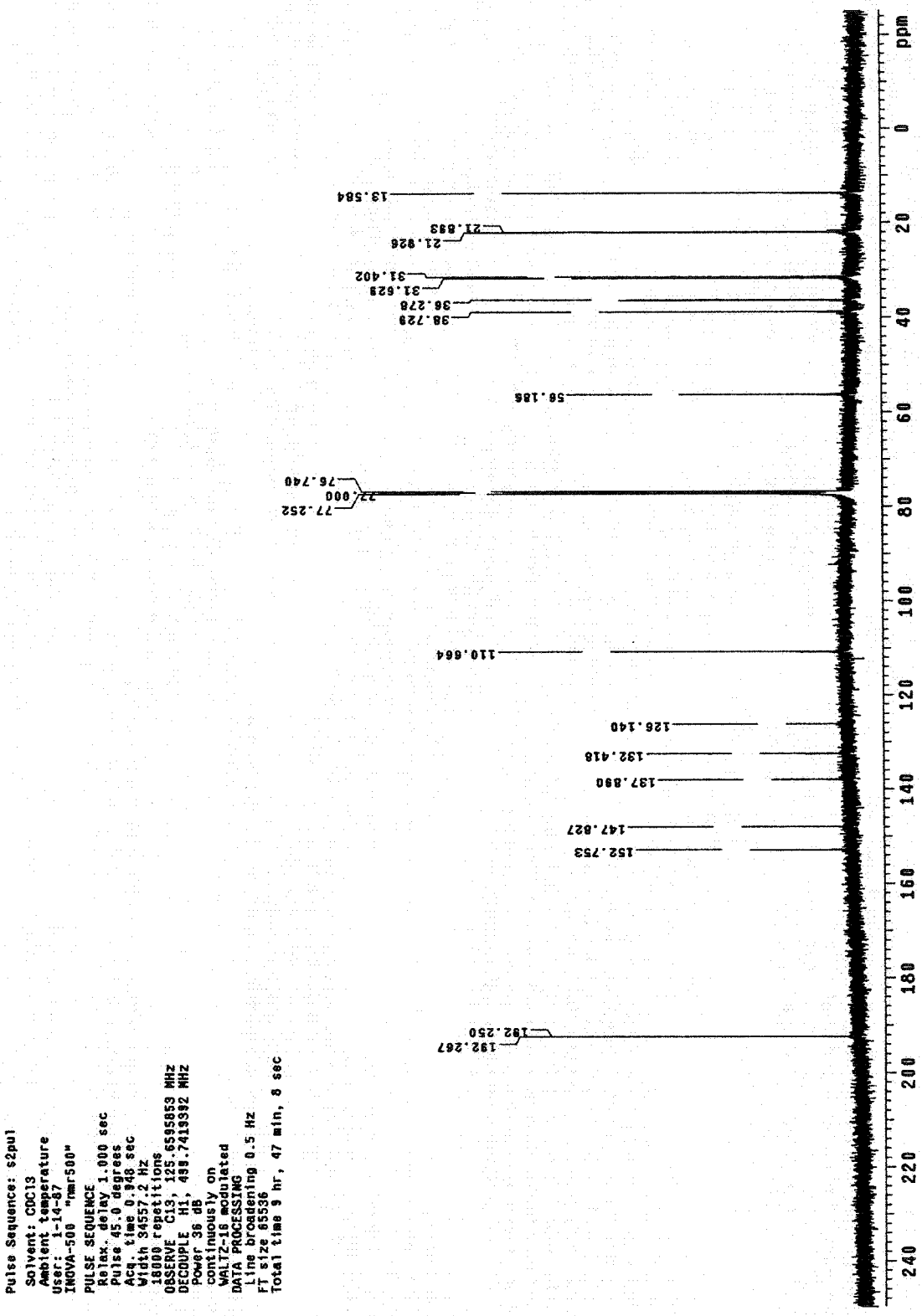

Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
User: 1-14-07
INOVA-500 "nmr500"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 0.908 sec
Width 34557.2 Hz
15000 repetitions
OBSERVE C13, 125.6602336 MHz
DECOUPLE H1, 499.7443130 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 8 hr, 9 min, 17 sec
190.850
151.270
147.451
126.284
122.214
118.871
110.380
56.406
40.107
40.015
39.940
39.772
39.856
39.688
39.604
39.520
39.352
39.184
39.016
ppm
240
220
200
180
160
140
120
100
80
60
40
20
0

S^{n}Bu
HO
S^{n}Bu
H$_3$CO
CHO
II-16

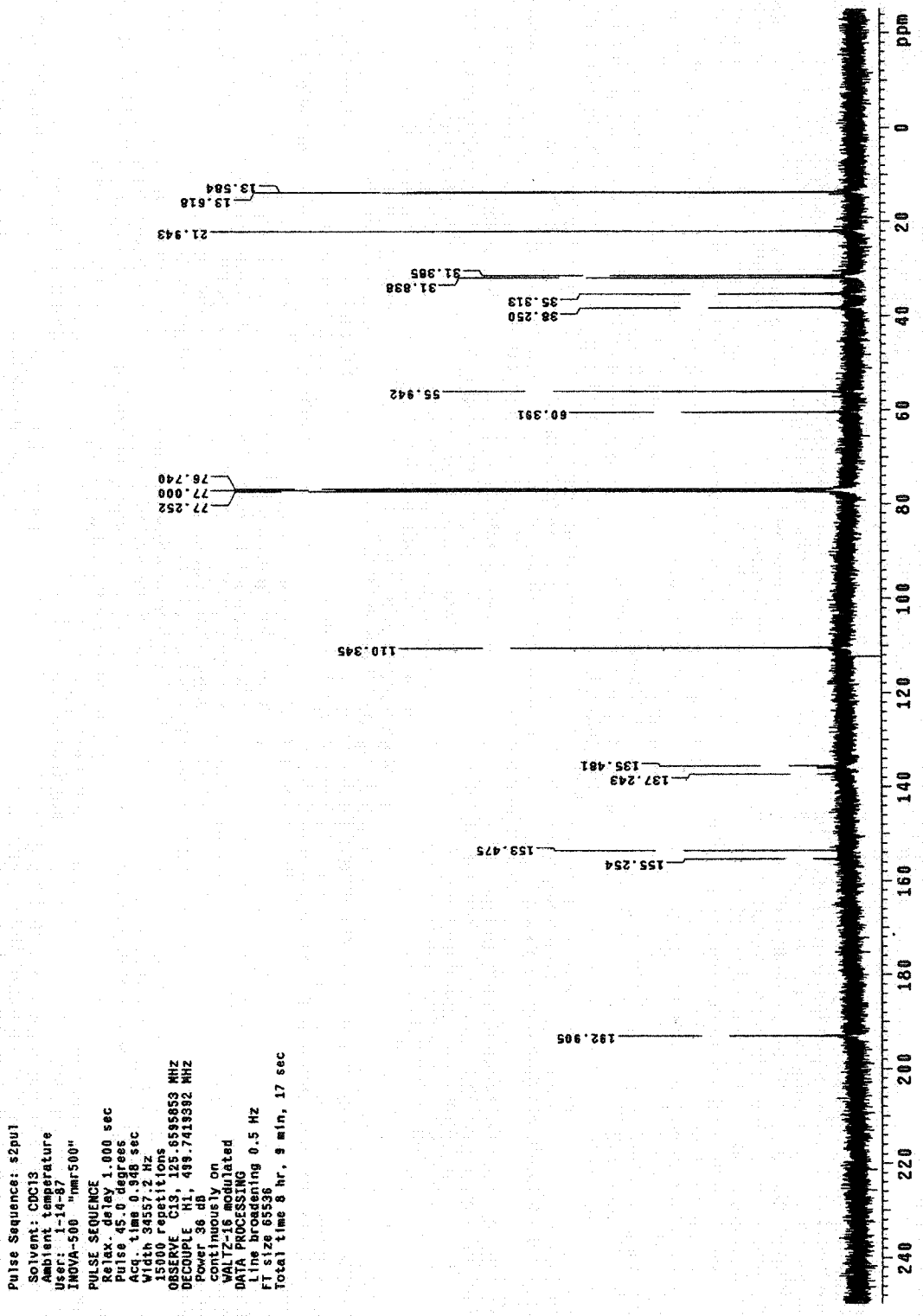
exp1 gCOSY

SAMPLE		FLAGS	
date	Oct 12 2001	hs	nn
solvent	CDCl3	sspul	n
sample	alex_12Oct2~001	hsglvl	4385
ACQUISITION		SPECIAL	
sw	7986.0	temp	not used
at	0.128	gain	34
np	2048	spin	0
fb	4000	F2 PROCESSING	
ss	16	sb	-0.064
di	1.000	sbs	not used
nt	1	fn	2048
2D ACQUISITION		F1 PROCESSING	
sw1	7986.0	sb1	-0.016
ni	128	sbs1	not used
TRANSMITTER		proc1	lp
tn	H1	fn1	2048
sfrq	499.742	DISPLAY	
tof	499.8	sp	110.4
tpwr	55	wp	5463.5
pw	9.600	sp1	133.9
GRADIENTS		wp1	5432.3
gzlvl1	4385	rf1	999.5
gt1	0.001000	rfp	0
gstab	0.000500	rf11	999.5
DECOUPLER		rfp1	0
dn	C13	PLOT	
dm	nnn	wc	135.0
		sc	10.0
		wc2	135.0
		sc2	0
		vs	70
		th	6
		al cdc av	



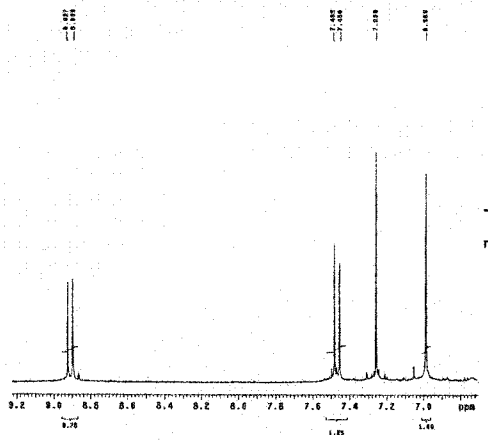

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
User: 1-14-87
INOVA-500 "mar500"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 0.948 sec
Width 34557.2 Hz
18000 repetitions
OBSERVE C13, 125.6595853 MHz
DECOUPLE H1, 499.7419392 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 9 hr, 47 min, 8 sec
192.267
192.250
152.753
147.827
137.890
132.418
126.140
110.664
77.252
77.000
76.740
56.186
38.729
38.278
31.629
31.402
21.826
21.693
13.584
ppm

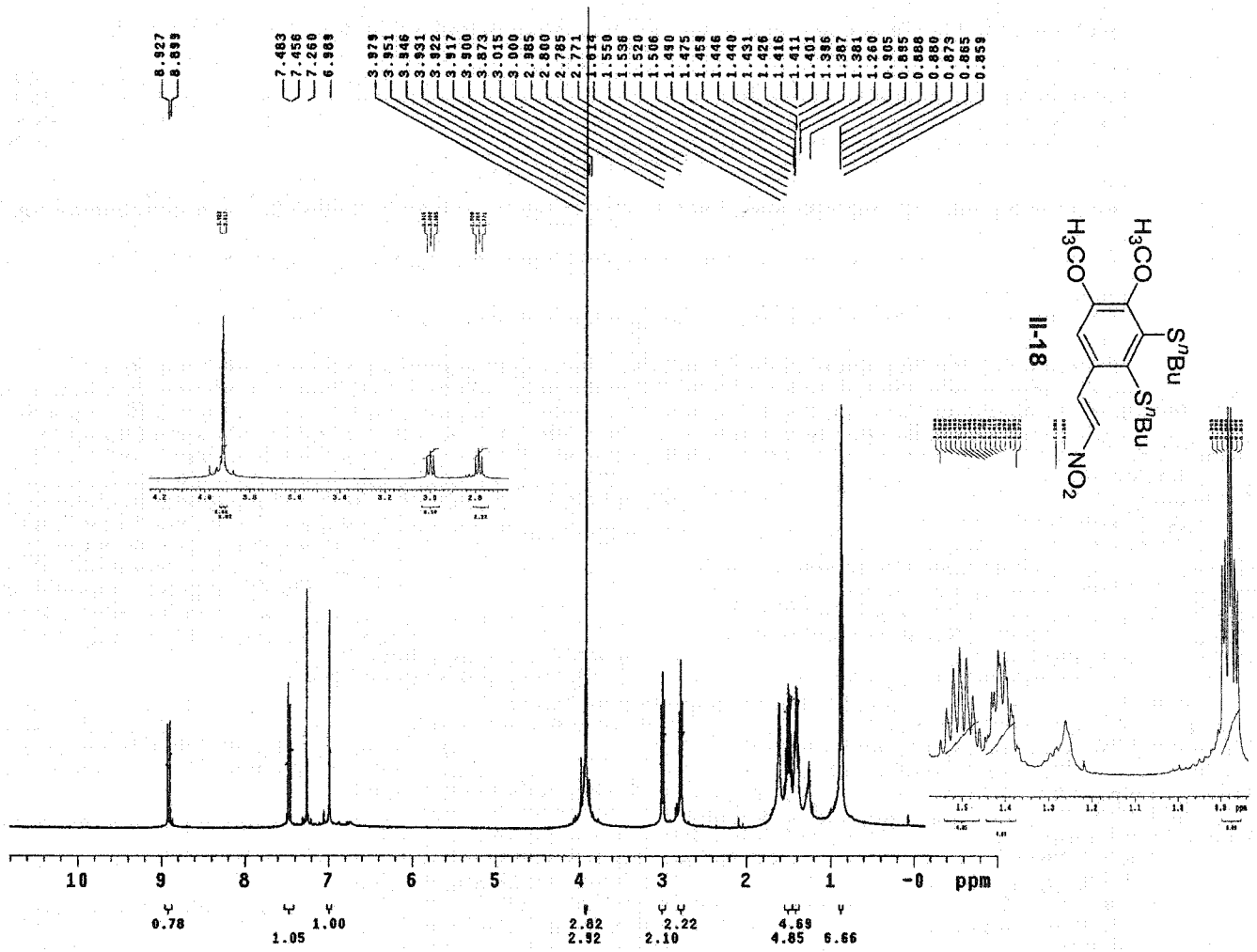
II-17


Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
User: 1-14-97
INOVA-500 "nmr500"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 0.948 sec
Width 34557.2 Hz
15000 repetitions
OBSERVE C13, 125.6595853 MHz
DECOUPLE H1, 499.7413392 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 8 hr, 9 min, 17 sec
182.905
155.254
153.475
137.243
135.481
110.345
77.252
77.000
76.740
60.391
55.942
38.250
35.313
31.858
31.385
21.943
13.618
13.564
ppm

exp1 s2pul

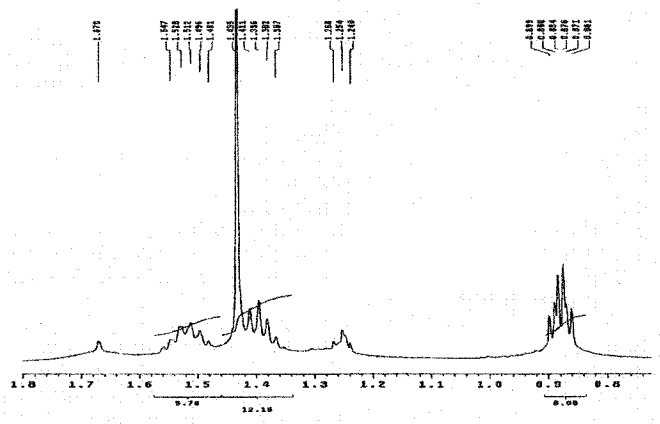
SAMPLE		SPECIAL	
date	Oct 15 2001	temp	20.0
solvent	CDCl3	gain	not used
file	exp	spin	22
ACQUISITION		hst	0.008
sw	5997.0	pw90	9.600
at	2.049	alfa	0
np	24576	FLAGS	
fb	3000	fl	n
bs	32	in	n
di	1.000	dp	y
nt	8	hs	nn
ct	8	PROCESSING	
TRANSMITTER		fn	not used
tn	H1	DISPLAY	
sfrq	499.742	sp	-500.9
tof	0.1	wp	5997.0
tpwr	55	rfl	4129.0
pw	4.800	rfp	3628.1
DECOUPLER		rp	-113.0
dn	C13	lp	-0.4
dof	0	PLOT	
dm	nnn	wc	180
dmm	c	sc	0
dpwr	52	vs	144
dmf	14122	th	7
		nm	cdc ph





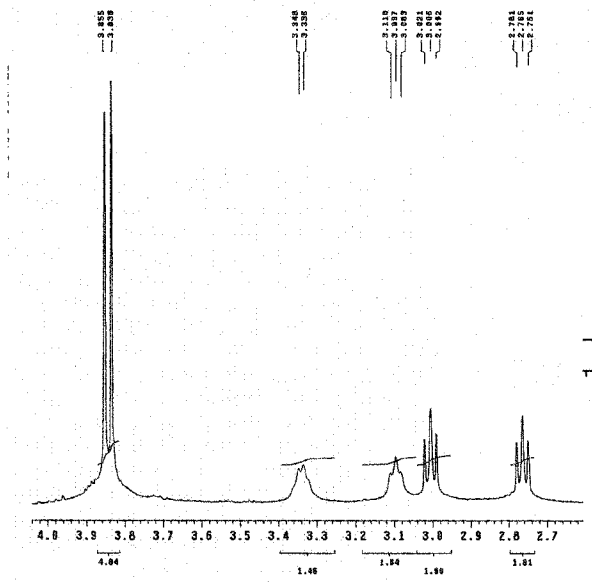
II-18

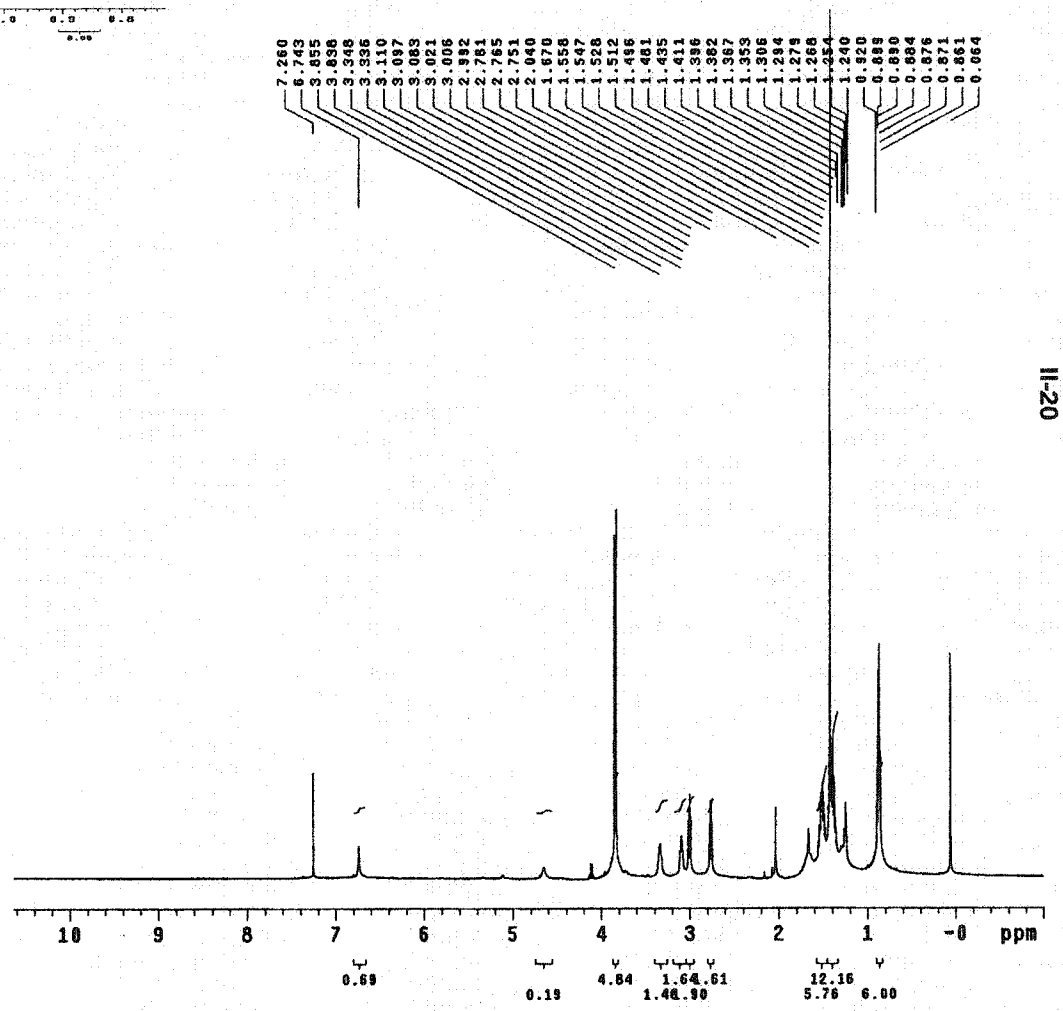
Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 20.0 C / 293.1 K
User: lj14-87
INOVA-500 "nmr500"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 0.348 sec
Width 34557.2 Hz
2000 repetitions
OBSERVE C13, 125.6595874 MHz
DECOUPLE H1, 499.7419392 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 1 hr, 5 min, 14 sec
13.559
13.601
21.885
21.918
31.335
31.822
35.330
37.764
55.959
60.349
76.740
77.000
77.252
109.648
112.216
131.201
134.172
137.546
137.915
139.065
153.382
ppm

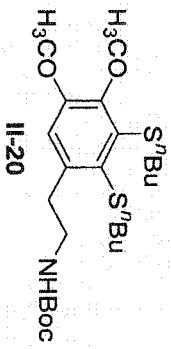


expi s2pul

SAMPLE		SPECIAL	
date	Oct 16 2001	temp	20.0
solvent	CDCl3	gain	not used
file	exp	spin	22
ACQUISITION		hst	0.008
sw	5997.0	pw90	9.600
at	2.049	alfa	0
np	24576	**FLAGS**	
fb	3000	il	n
bs	32	in	n
di	1.000	dp	y
nt	8	hs	nn
ct	8	**PROCESSING**	
TRANSMITTER		fn	not used
tn	H1	**DISPLAY**	
sfrq	499.742	sp	-501.3
tof	0.1	wp	5997.0
tpwr	55	rfl	4128.4
pw	4.800	rfp	3628.1
DECOUPLER		rp	-115.6
dn	C13	lp	2.8
dof	0	**PLOT**	
dm	nnn	wc	180
dmm	c	sc	0
dpwr	52	vs	144
dmf	14122	th	4
		nm	cdc ph



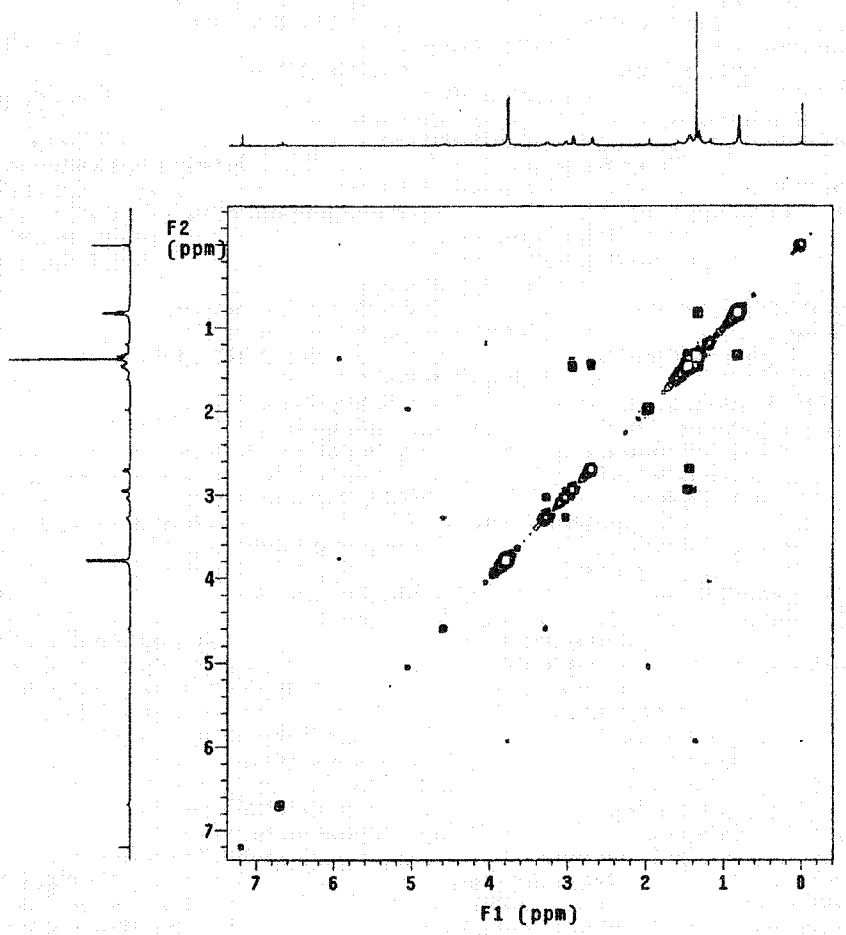


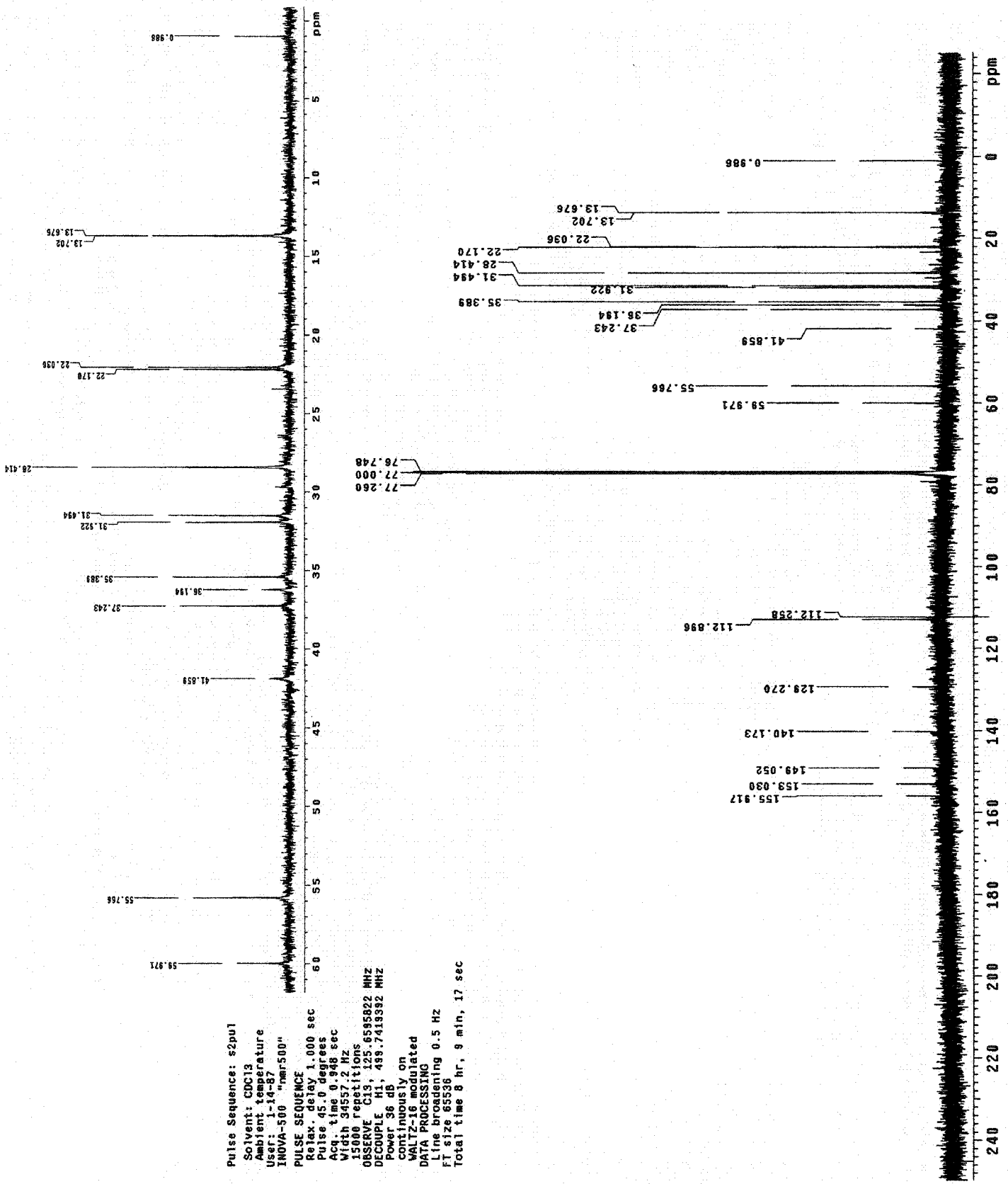


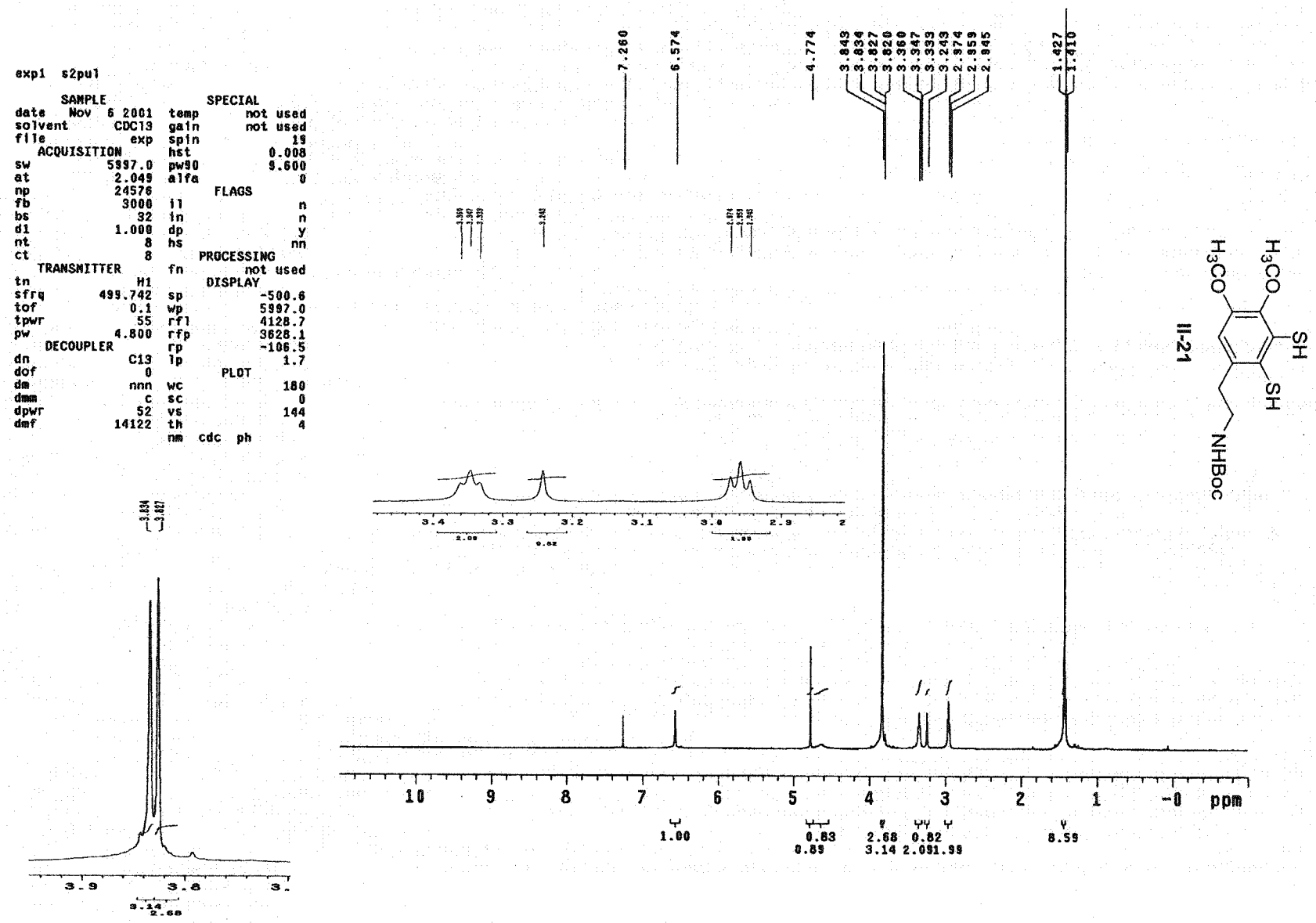
II-20

exp1 gCOSY

SAMPLE		FLAGS	
date	Oct 17 2001	hs	nn
solvent	CDCl3	sspul	n
sample	alex_17Oct2~	hsglvl	4385
	001	SPECIAL	
ACQUISITION		temp	not used
sw	7996.0	gain	28
at	0.128	spin	0
np	2048	F2 PROCESSING	
fb	4000	sb	-0.064
ss	16	sbs	not used
d1	1.000	fn	2048
nt	1	F1 PROCESSING	
2D ACQUISITION		sb1	-0.016
sw1	7996.0	sbs1	not used
ni	128	proc1	lp
TRANSMITTER		fn1	2048
tn	H1	DISPLAY	
sfrq	498.742	sp	-226.2
tof	499.8	wp	3900.3
tpwr	55	sp1	-202.8
pw	9.600	wp1	3884.7
GRADIENTS		rf1	1031.3
gzlvl1	4385	rfp	0
gt1	0.001000	rfl1	1031.3
gstab	0.000500	rfp1	0
DECOUPLER		PLOT	
dn	C13	wc	135.0
dm	nnn	sc	10.0
		wc2	135.0
		sc2	0
		vs	58
		th	5
		ai cdc av	




0.986
13.676
13.702
22.036
22.170
28.414
31.494
31.922
35.389
36.194
37.243
41.859
55.766
59.971
76.748
77.000
77.260
112.258
112.896
129.270
140.173
149.052
153.080
155.917
ppm
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
User: 1-14-87
INOVA-500 "nmr500"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 0.948 sec
Width 34557.2 Hz
15000 repetitions
OBSERVE C13, 125.6595822 MHz
DECOUPLE H1, 499.7419392 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 8 hr, 9 min, 17 sec

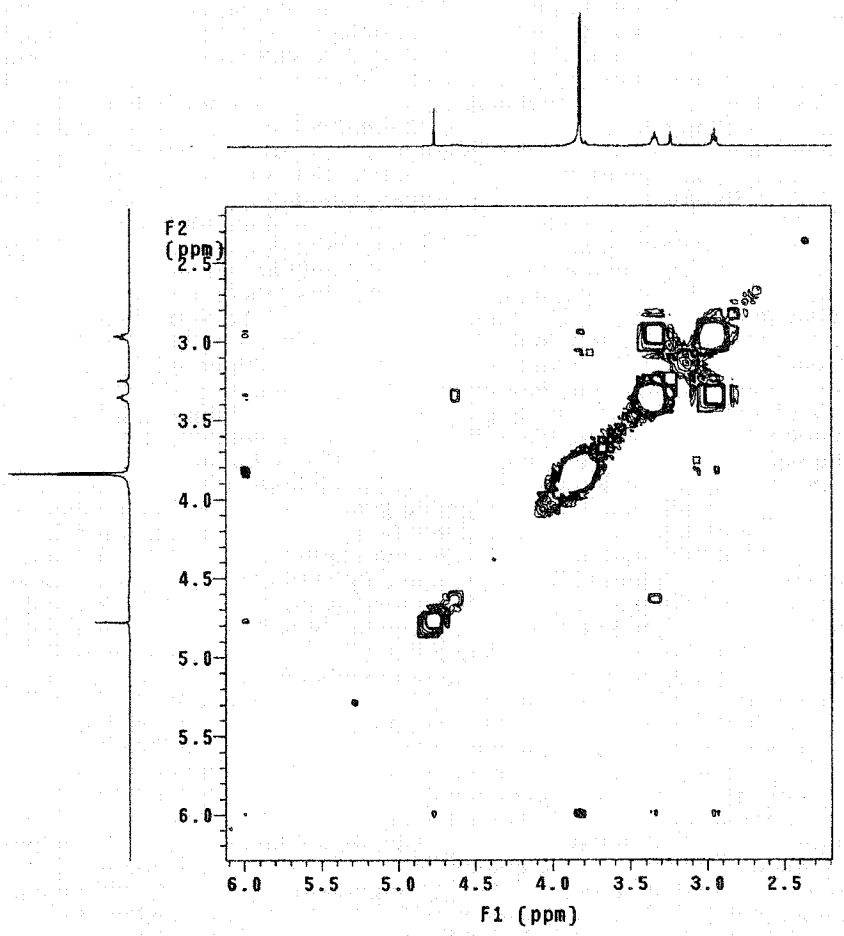


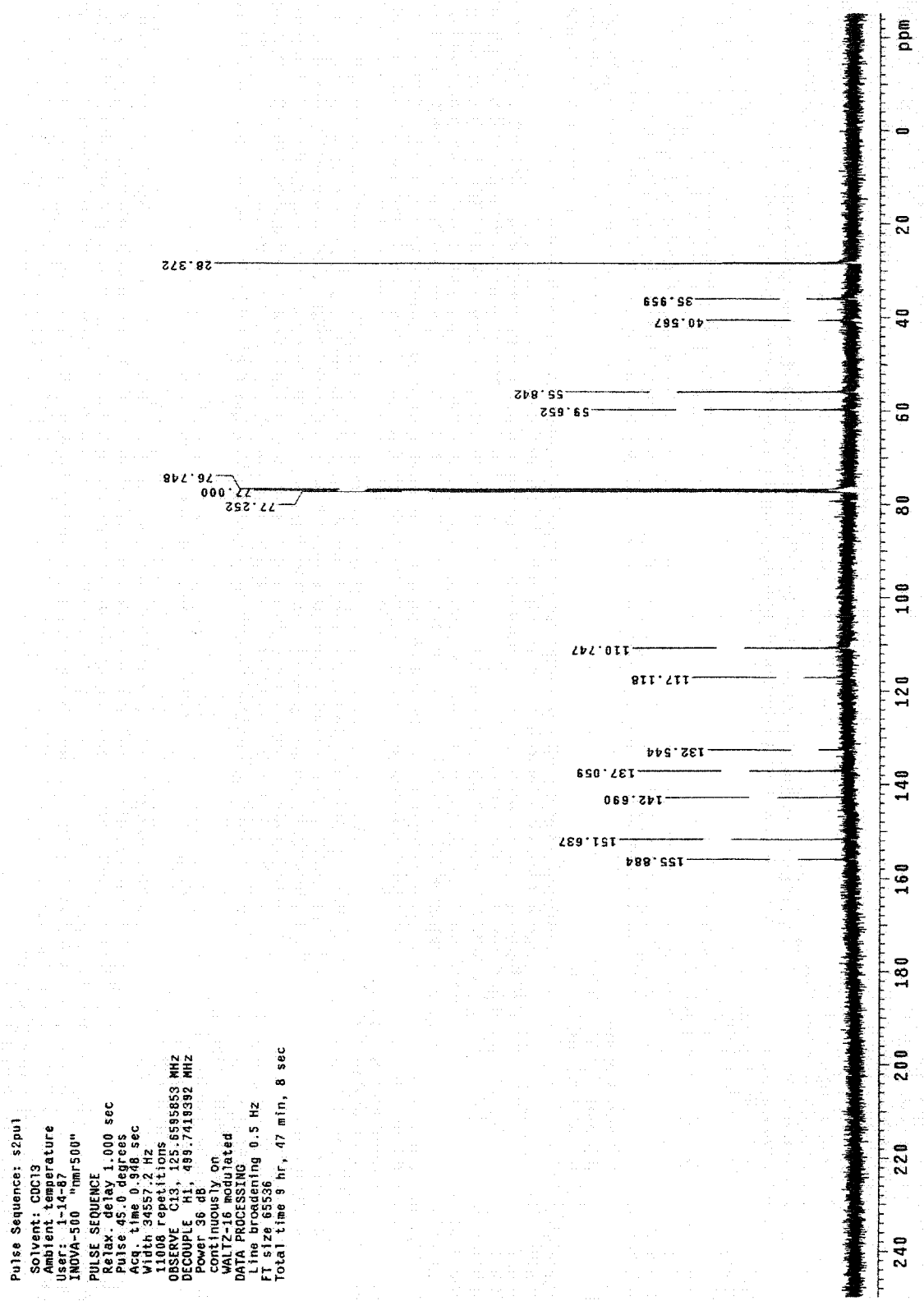
exp1 s2pul
SAMPLE
date Nov 6 2001
solvent CDCl3
file exp
ACQUISITION
sw 5997.0
at 2.049
np 24576
fb 3000
bs 32
d1 1.000
nt 8
ct 8
TRANSMITTER
tn H1
sfrq 499.742
tof 0.1
tpwr 55
pw 4.800
DECOUPLER
dn C13
dof 0
dm nnn
dmm c
dpwr 52
dmf 14122
SPECIAL
temp not used
gain not used
spin 19
hst 0.008
pw90 9.600
alfa 0
FLAGS
il n
in n
dp y
hs nn
PROCESSING
fn not used
DISPLAY
sp -500.6
wp 5997.0
rfl 4128.7
rfp 3628.1
rp -106.5
lp 1.7
PLOT
wc 180
sc 0
vs 144
th 4
nm cdc ph
7.260
6.574
4.774
3.843
3.834
3.827
3.820
3.360
3.347
3.333
3.243
2.974
2.959
2.945
1.427
1.410
3.834
3.827
3.9
3.8
3.14
2.68
3.4
3.3
3.2
3.1
3.0
2.9
2
10
9
8
7
6
5
4
3
2
1
-0
ppm
1.00
0.83
0.89
2.68
3.14
0.82
2.09
1.99
8.59
H3CO
H3CO
SH
SH
NHBoc

II-21

exp1 gCOSY

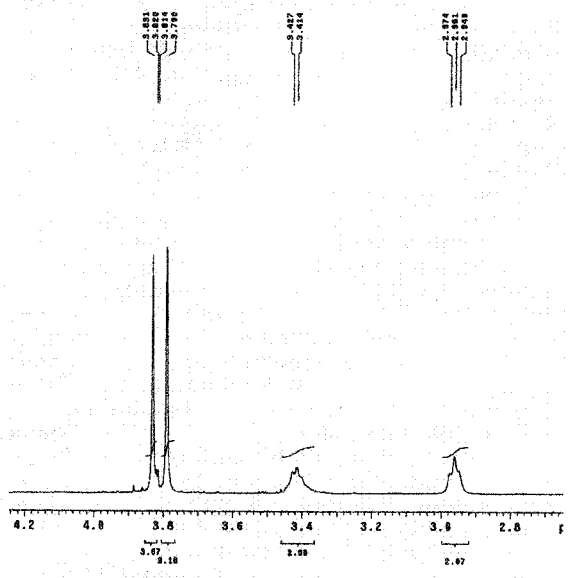
SAMPLE		FLAGS	
date	Nov 6 2001	hs	nn
solvent	CDCl3	sspul	n
sample	alex_06Nov2~001	hsglvl	4385
ACQUISITION		SPECIAL	
sw	7996.0	temp	not used
at	0.128	gain	28
np	2048	spin	0
fb	4000	F2 PROCESSING	
ss	16	sb	-0.064
d1	1.000	sbs	not used
nt	1	fn	2048
2D ACQUISITION		F1 PROCESSING	
sw1	7996.0	sb1	-0.016
ni	128	sbs1	not used
TRANSMITTER		proc1	lp
tn	H1	fn1	2048
sfrq	499.742	DISPLAY	
tof	499.8	sp	1071.8
tpwr	55	wp	2071.3
pw	9.600	sp1	1095.3
GRADIENTS		wp1	1861.9
gzlvl1	4385	rfl	999.5
gt1	0.001000	rfp	0
gstab	0.000500	rfl1	999.5
DECOUPLER		rfp1	0
dn	C13	PLOT	
dm	nnn	wc	135.0
		sc	10.0
		wc2	135.0
		sc2	0
		vs	26
		th	2
		al cdc av	

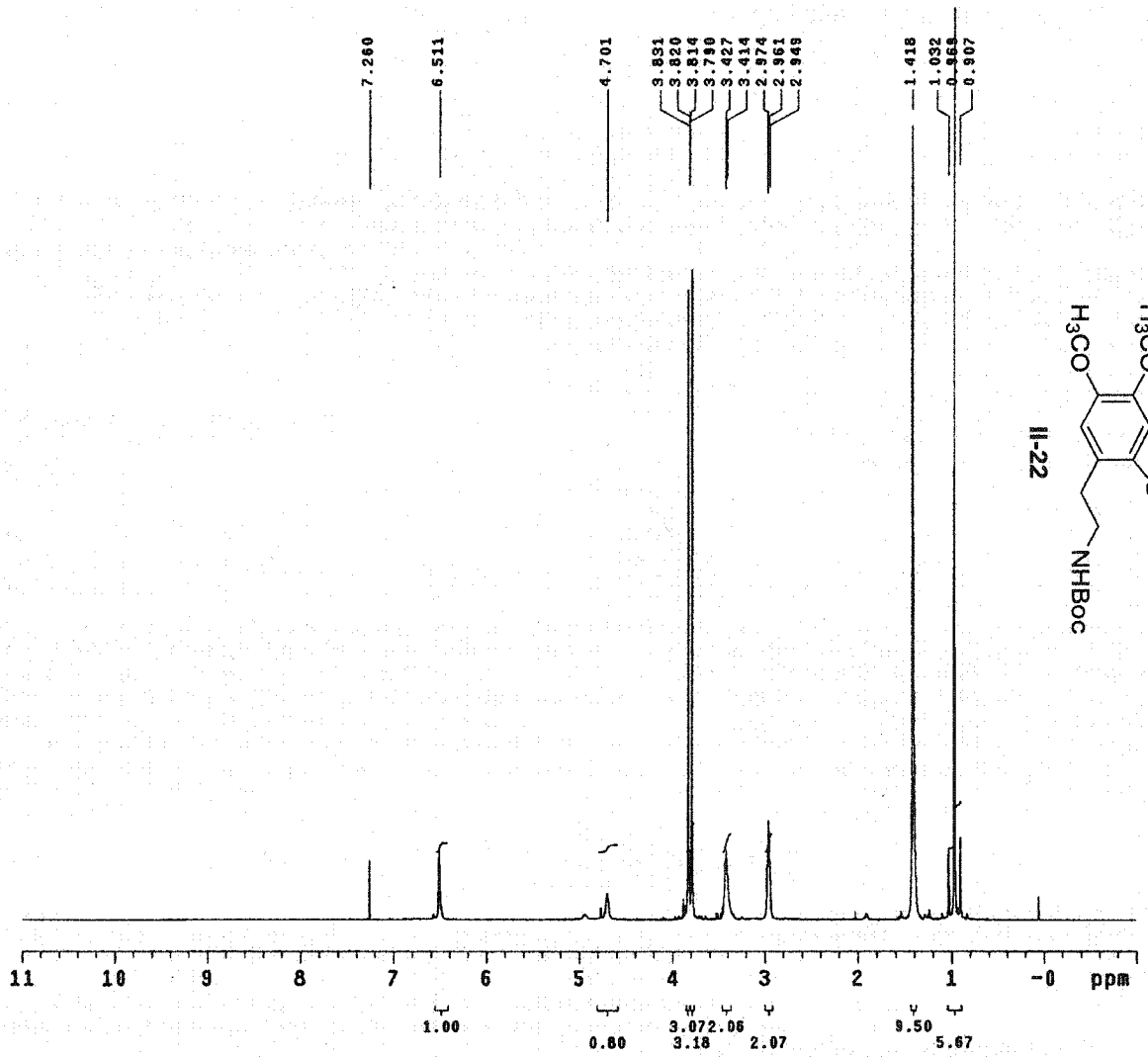


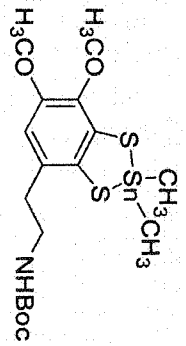

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
User: 1-14-87
INOVA-500 "nmr500"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 0.948 sec
Width 34557.2 Hz
11008 repetitions
OBSERVE C13, 125.6595853 MHz
DECOUPLE H1, 499.7418392 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 9 hr, 47 min, 8 sec
28.372
35.959
40.567
55.842
59.652
76.748
77.000
77.252
110.747
117.118
132.544
137.059
142.690
151.637
155.884
ppm
0
20
40
60
80
100
120
140
160
180
200
220
240

exp1 s2pul

SAMPLE		SPECIAL	
date	Nov 6 2001	temp	not used
solvent	CDCl3	gain	not used
file	exp	spin	20
ACQUISITION		hst	0.008
sw	5997.0	pw90	9.600
at	2.049	alfa	0
np	24576	FLAGS	
fb	3000	il	n
bs	32	in	n
d1	1.000	dp	y
nt	8	hs	nn
ct	8	PROCESSING	
TRANSMITTER		fn	not used
tn	H1	DISPLAY	
sfrq	499.742	sp	-500.2
tof	0.1	wp	5997.0
tpwr	55	rfl	4128.3
pw	4.800	rfp	3628.1
DECOUPLER		rp	-98.3
dn	C13	lp	3.5
dof	0	PLOT	
dm	nnn	wc	180
dmm	c	sc	0
dpwr	52	vs	144
dmf	14122	th	4
		nm cdc ph	







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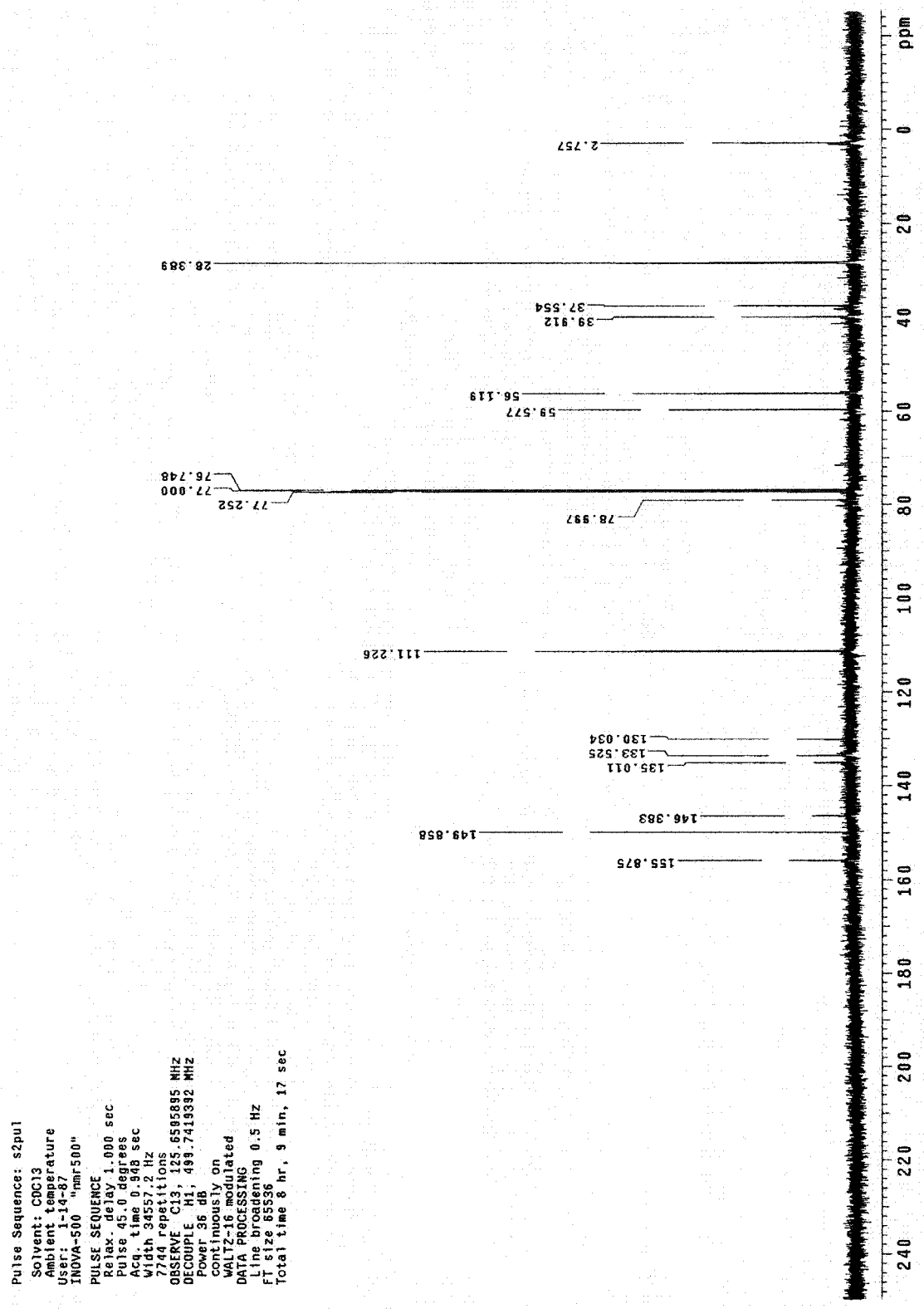
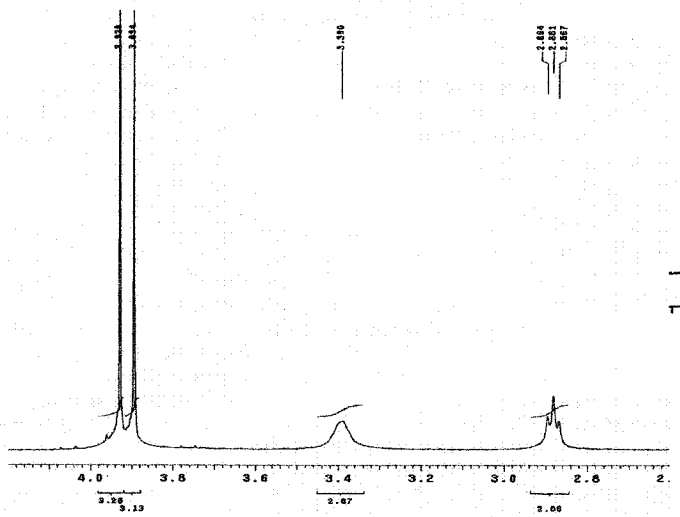
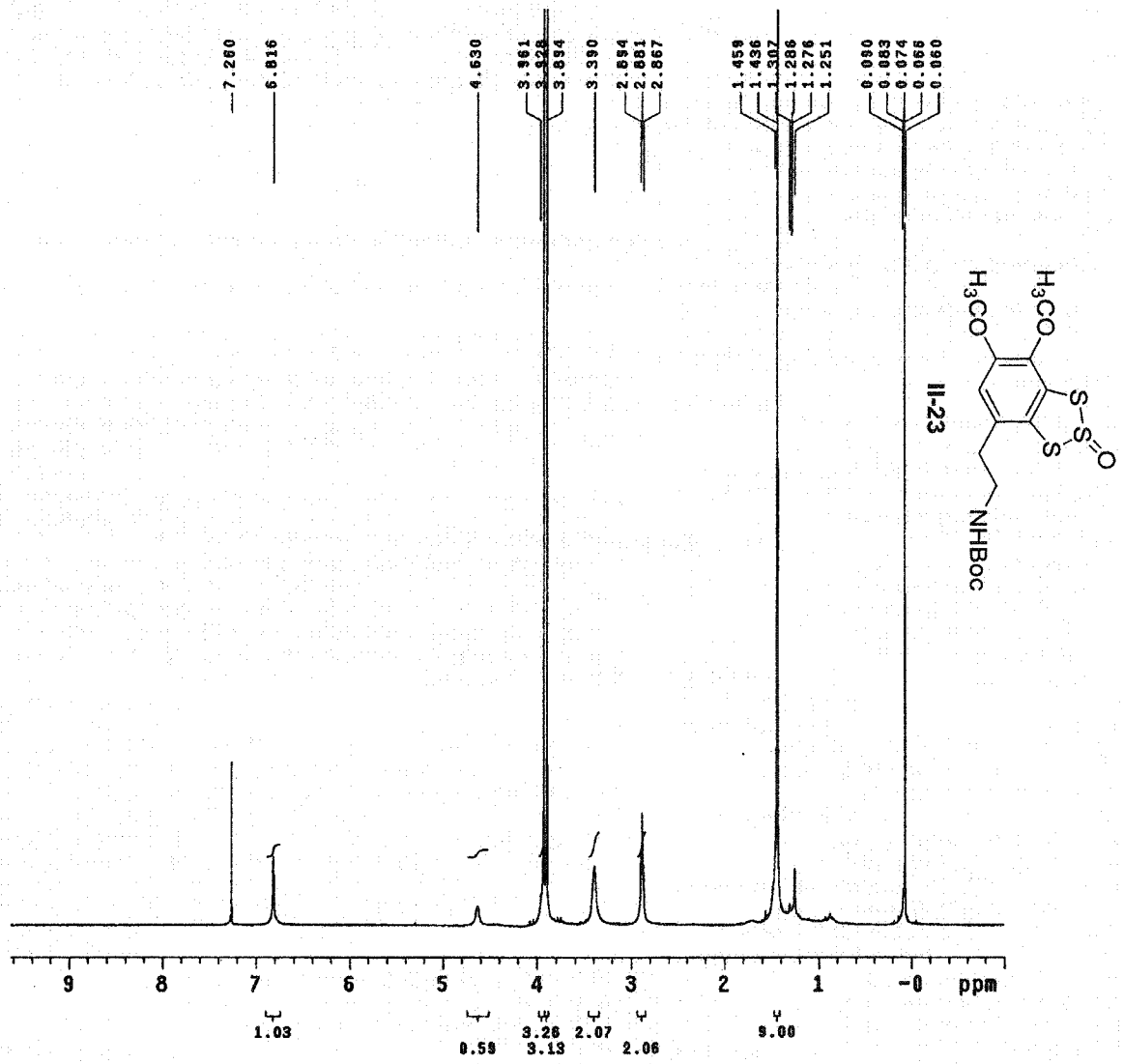
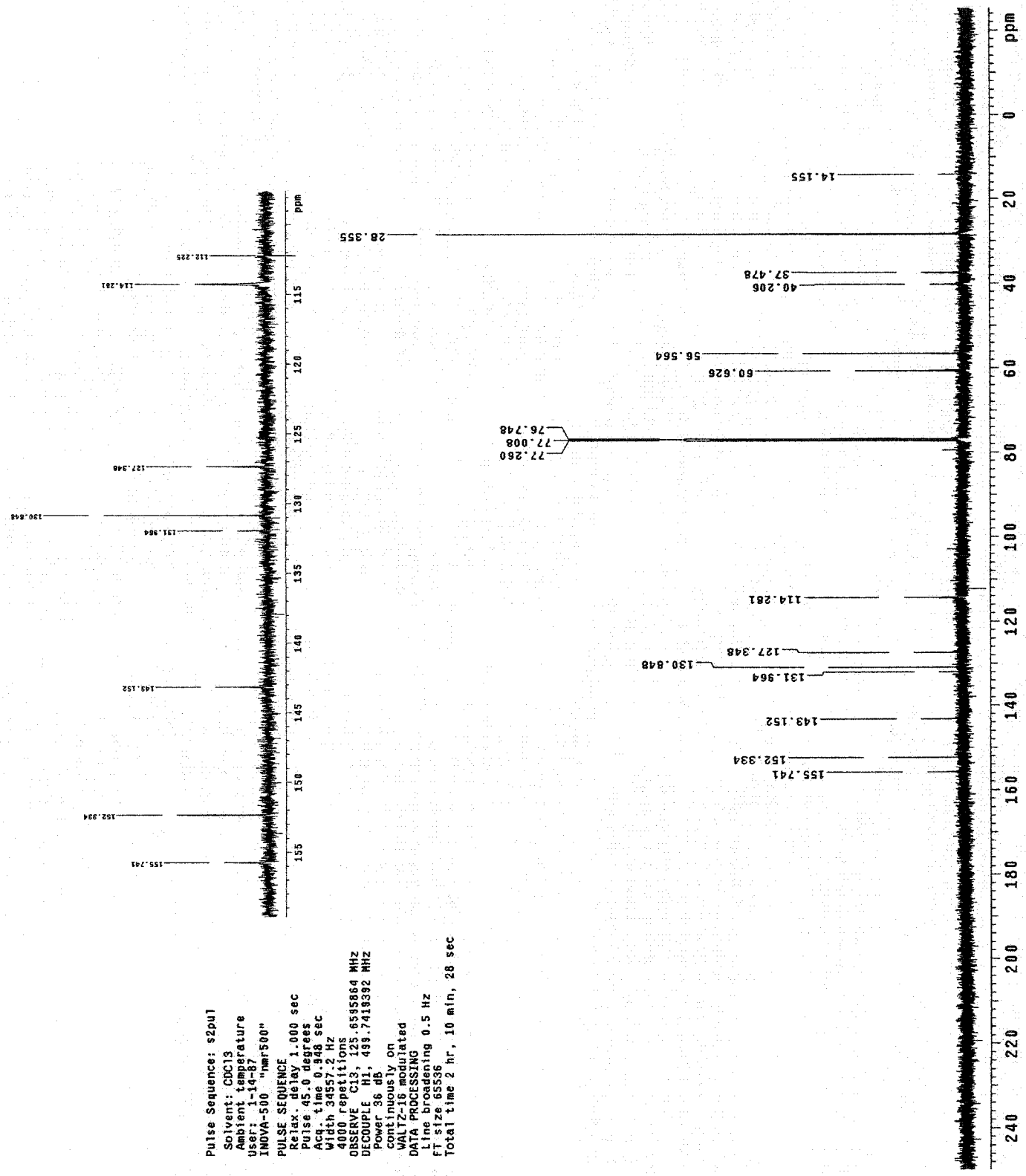

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
User: 1-14-87
INOVA-500 "mr500"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 0.948 sec
Width 34557.2 Hz
7744 repetitions
OBSERVE C13, 125.6585895 MHz
DECOUPLE H1, 499.7419392 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 8 hr, 9 min, 17 sec
2.757
28.389
37.554
39.912
56.119
59.577
76.748
77.000
77.252
78.997
111.226
130.034
133.525
135.011
146.383
149.858
155.875
ppm
0
20
40
60
80
100
120
140
160
180
200
220
240

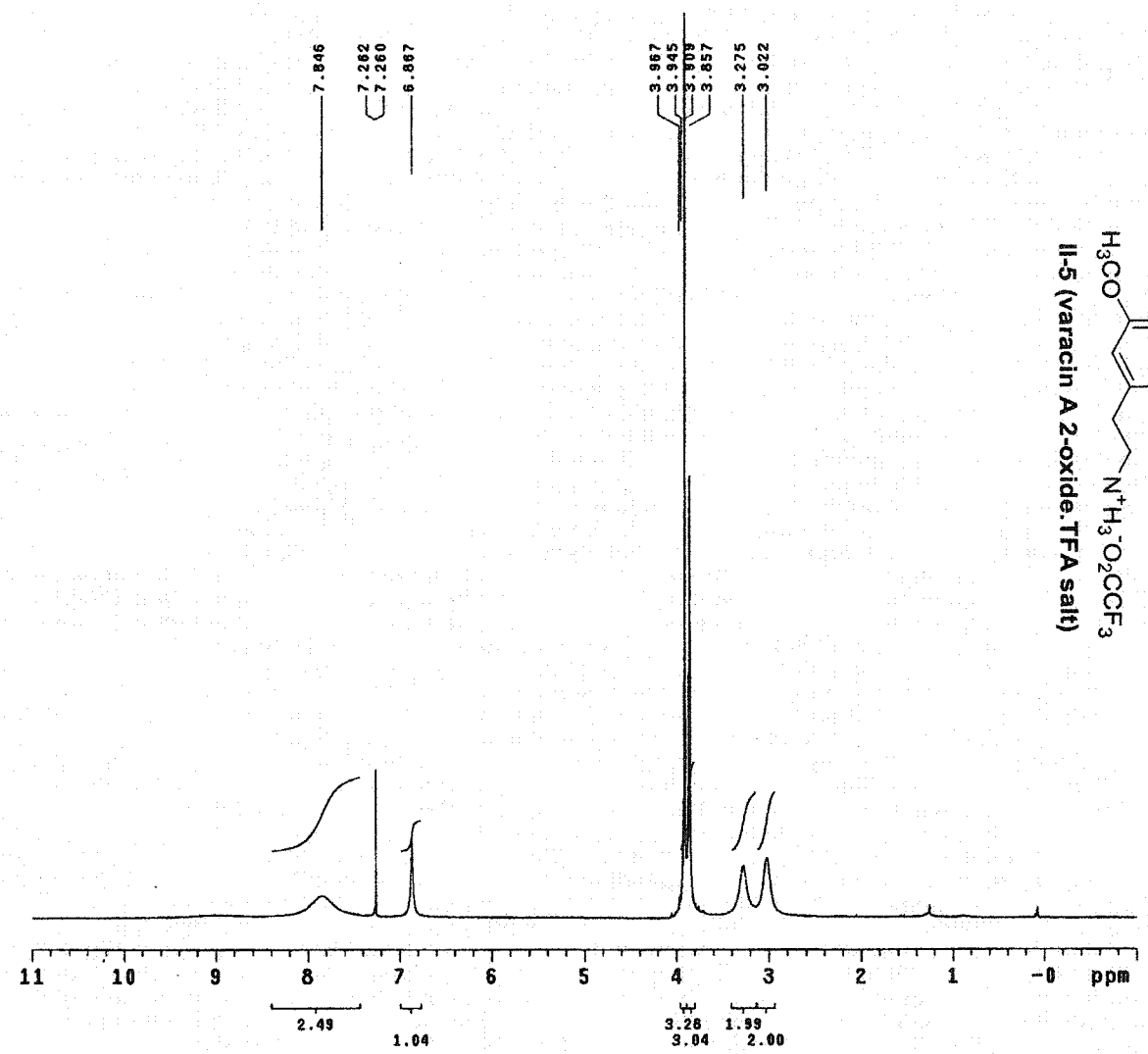
exp1 s2pul

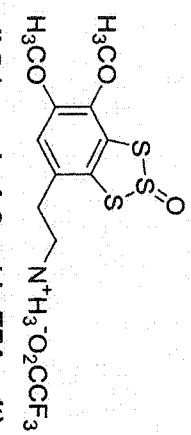
SAMPLE		SPECIAL	
date	Oct 22 2001	temp	not used
solvent	CDCl3	gain	not used
file	exp	spin	20
ACQUISITION		hst	0.008
sw	5997.0	pw90	9.600
at	2.048	alfa	0
np	24576	FLAGS	
fb	3000	il	n
bs	32	in	n
d1	1.000	dp	y
nt	8	hs	nn
ct	8	PROCESSING	
TRANSMITTER		fn	not used
tn	H1	DISPLAY	
sfrq	499.742	sp	-500.6
tof	0.1	wp	5997.0
tpwr	55	rfl	4128.7
pw	4.800	rfp	3628.1
DECOUPLER		rp	-104.2
dn	C13	lp	-2.9
dof	0	PLOT	
dm	nnn	wc	180
dmm	c	sc	0
dpwr	52	vs	300
dmf	14122	th	2
		nm	cdc ph






Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
user: 1-14-87
INOVA-500 "nmr500"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 0.848 sec
Width 34557.2 Hz
4000 repetitions
OBSERVE C13, 125.6595864 MHz
DECOUPLE H1, 499.7418392 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 2 hr, 10 min, 28 sec
ppm
14.155
28.355
37.478
40.206
56.564
60.626
76.748
77.008
77.260
114.281
127.348
130.848
131.964
143.152
152.334
155.741



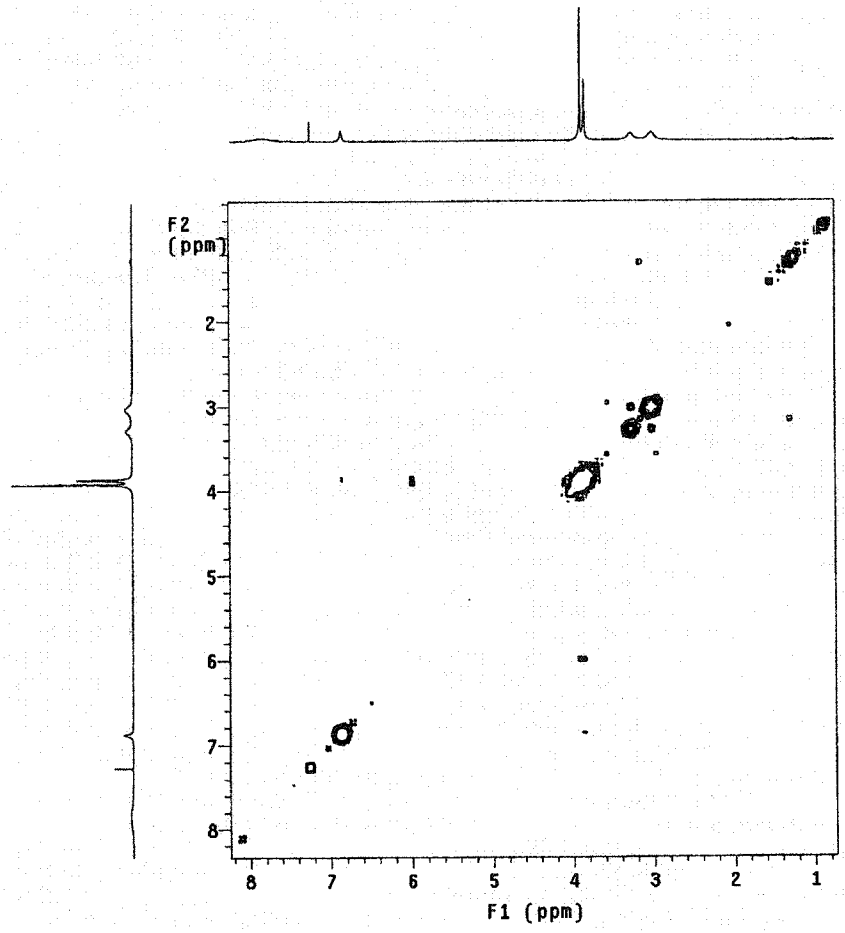


II-5 (varacin A 2-oxide.TFA salt)

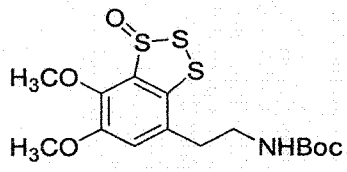
```
exp1  s2pul

      SAMPLE                SPECIAL
date   Nov  7 2001  temp        not used
solvent      CDCl3  gain        not used
file           exp  spin              22
   ACQUISITION      hst           0.008
sw          5997.0  pw90          9.600
at           2.049  alfa               0
np           24576        FLAGS
fb            3000  il                 n
bs              32  in                 n
d1           1.000  dp                 y
nt               8  hs                nn
ct               8      PROCESSING
   TRANSMITTER      fn          not used
tn              H1       DISPLAY
sfrq       499.742  sp           -500.2
tof            0.1  wp           5997.0
tpwr            55  rfl          4128.3
pw           4.800  rfp          3628.1
    DECOUPLER       rp           -101.1
dn             C13  lp              1.0
dof              0        PLOT
dm             nnn  wc              180
dmm              c  sc                0
dpwr            52  vs              144
dmf          14122  th                2
                    nm  cdc  ph
```

exp1 gCOSY

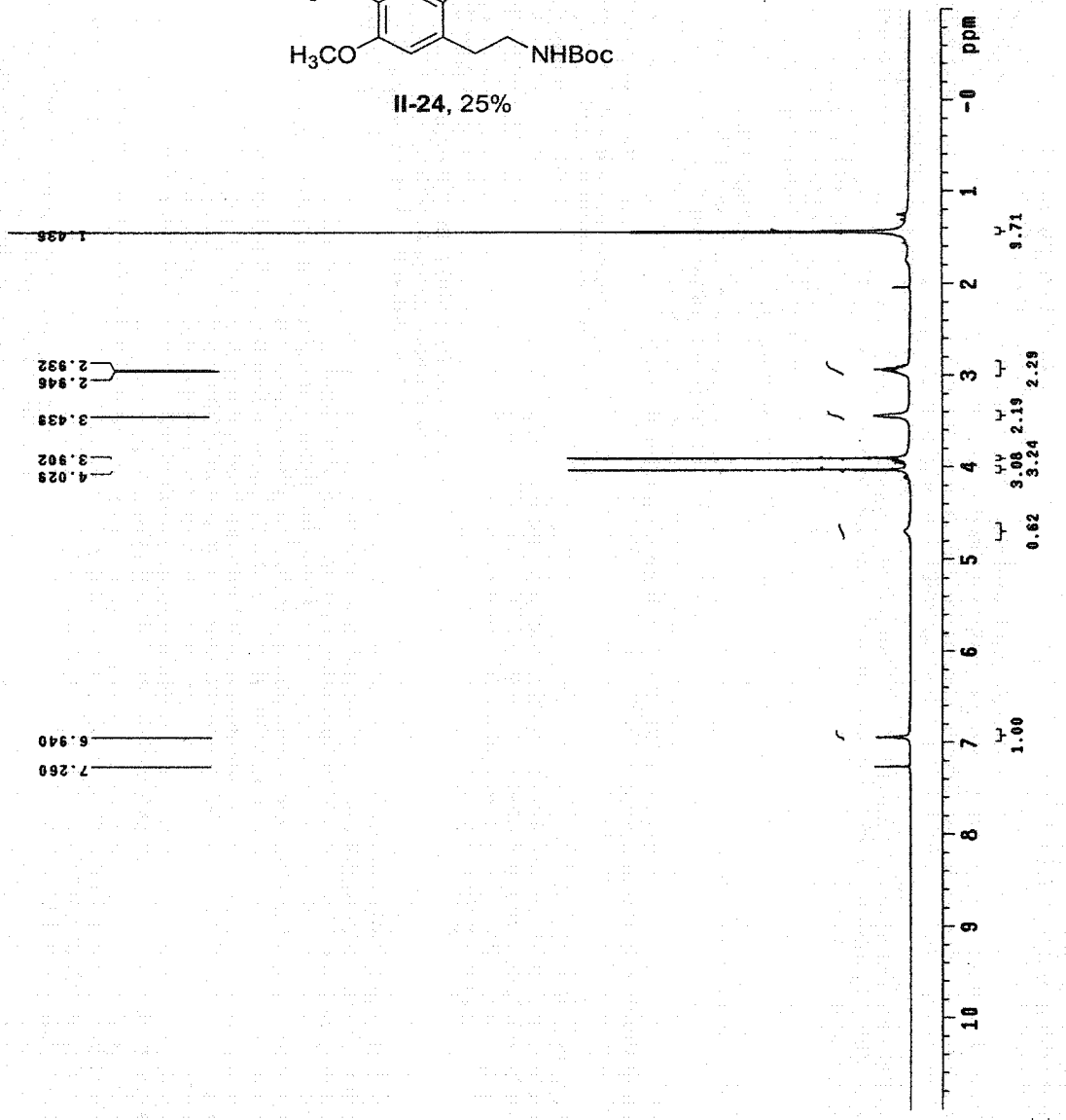
SAMPLE		FLAGS	
date	Nov 7 2001	hs	nn
solvent	CDCl3	sspul	n
sample	alex_07Nov2~001	hsglvl	4385
ACQUISITION		SPECIAL	
sw	7996.0	temp	not used
at	0.128	gain	30
np	2048	spin	0
fb	4000	F2 PROCESSING	
ss	16	sb	-0.064
d1	1.000	sbs	not used
nt	1	fn	2048
2D ACQUISITION		F1 PROCESSING	
sw1	7996.0	sb1	-0.016
ni	128	sbs1	not used
TRANSMITTER		proc1	lp
tn	H1	fn1	2048
sfrq	499.742	DISPLAY	
tof	499.8	sp	290.2
tpwr	55	wp	3876.8
pw	9.600	sp1	368.3
GRADIENTS		wp1	3751.8
gzlvl1	4385	rf1	999.5
gt1	0.001000	rfl1	999.5
gstab	0.000500	rfp1	0
DECOUPLER		PLOT	
dn	C13	wc	135.0
dm	nnn	sc	10.0
		wc2	135.0
		sc2	0
		vs	86
		th	2
		ai cdc av	



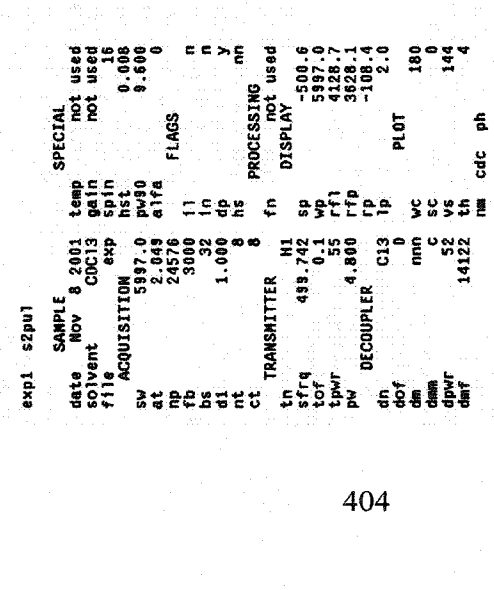
Current Data Parameters
NAME var-36C13
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20011108
Time 6.58
INSTRUM dpx400
PROBHD 5 mm QNP 1H
PULPROG zgdc30
TD 65536
SOLVENT CDCl3
NS 1843Z
DS 0
SWH 65359.477 Hz
FIDRES 0.997306 Hz
AQ 0.5014004 sec
RG 5792.6
DW 7.650 usec
DE 4.50 usec
TE 300.0 K
D1 1.00000000 sec
d11 0.03000000 sec
CHANNEL f1
NUC1 13C
P1 5.80 usec
PL1 -6.00 dB
SFO1 100.6429128 MHz
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
PCPD2 71.00 usec
PL2 120.00 dB
PL12 17.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127721 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 220.000 ppm
F1 22134.81 Hz
F2P -10.000 ppm
F2 -1006.13 Hz
PPMCM 11.50000 ppm/cm
HZCM 1157.04688 Hz/cm
34.196
39.279
56.406
60.743
76.683
77.000
77.202
77.318
114.587
126.619
127.471
132.193
143.769
152.704
ppm
ppm
200
175
150
125
100
75
50
25
0



II-24, 25%



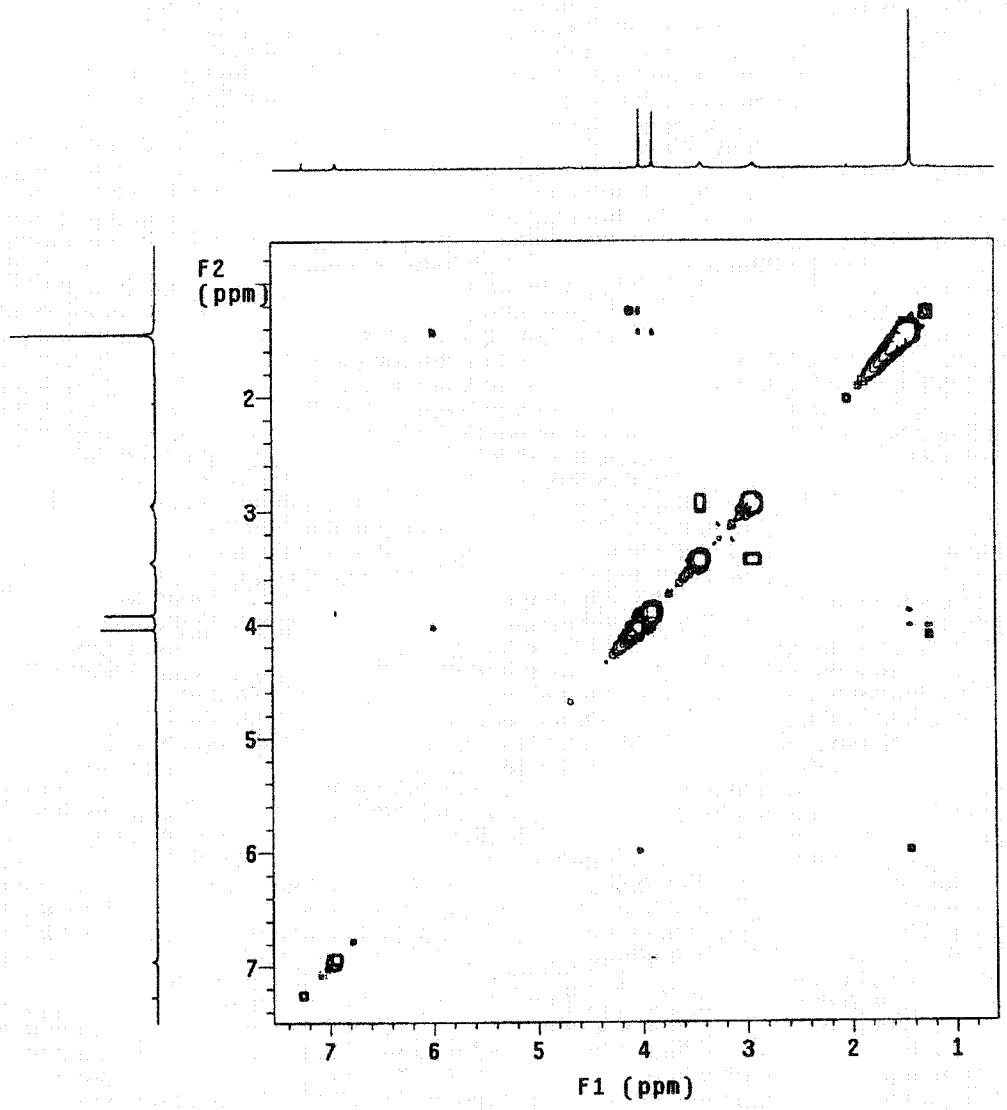
1.436
2.932
2.946
3.439
3.902
4.029
6.940
7.260
ppm
1.00
0.62
3.08
3.24
2.19
2.29
9.71



exp1 s2pul
SAMPLE
date Nov 8 2001
solvent CDCl3
file exp
ACQUISITION
sw 5997.0
at 2.049
np 24576
fb 3000
bs 32
d1 1.000
nt 8
ct 8
TRANSMITTER
tn H1
sfrq 499.742
tof 0.1
tpwr 55
pw 4.800
DECOUPLER
dn C13
dof 0
dm nnn
dmm c
dpwr 52
dmf 14122
SPECIAL
temp not used
gain not used
spin 16
hst 0.008
pw90 9.600
alfa 0
FLAGS
il n
in n
dp y
hs nn
PROCESSING
fn not used
DISPLAY
sp -500.6
wp 5997.0
rfl 4128.7
rfp 3628.1
rp -108.4
lp 2.0
PLOT
wc 180
sc 0
vs 144
th 4
nm cdc ph

exp1 gCOSY

SAMPLE		FLAGS	
date	Nov 8 2001	hs	nn
solvent	CDCl3	sspul	n
sample	alex_08Nov2~001	hsglvl	4385
ACQUISITION		SPECIAL	
sw	7996.0	temp	not used
at	0.128	gain	30
np	2048	spin	0
fb	4000	F2 PROCESSING	
ss	16	sb	-0.064
d1	1.000	sbs	not used
nt	1	fn	2048
2D ACQUISITION		F1 PROCESSING	
sw1	7996.0	sb1	-0.016
ni	128	sbs1	not used
TRANSMITTER		proc1	lp
tn	H1	fn1	2048
sfrq	499.742	DISPLAY	
tof	499.8	sp	321.4
tpwr	55	wp	3423.5
pw	9.600	sp1	305.8
GRADIENTS		wp1	3462.6
gzlvl1	4385	rfl	999.5
gt1	0.001000	rfp	0
gstab	0.000500	rfl1	999.5
DECOUPLER		rfp1	0
dn	C13	PLOT	
dm	nnn	wc	135.0
		sc	10.0
		wc2	135.0
		sc2	0
		vs	35
		th	3
		ai cdc av	

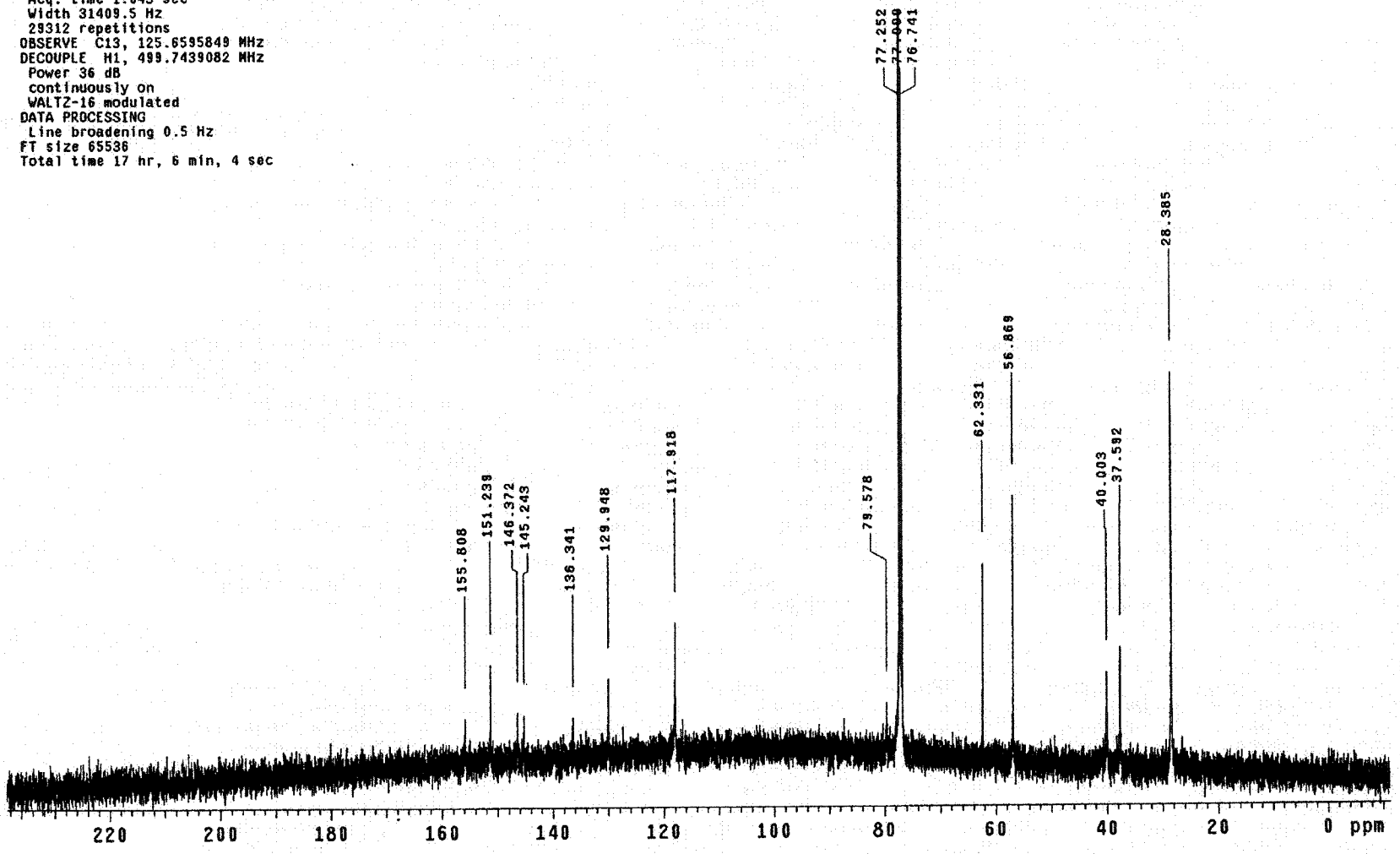


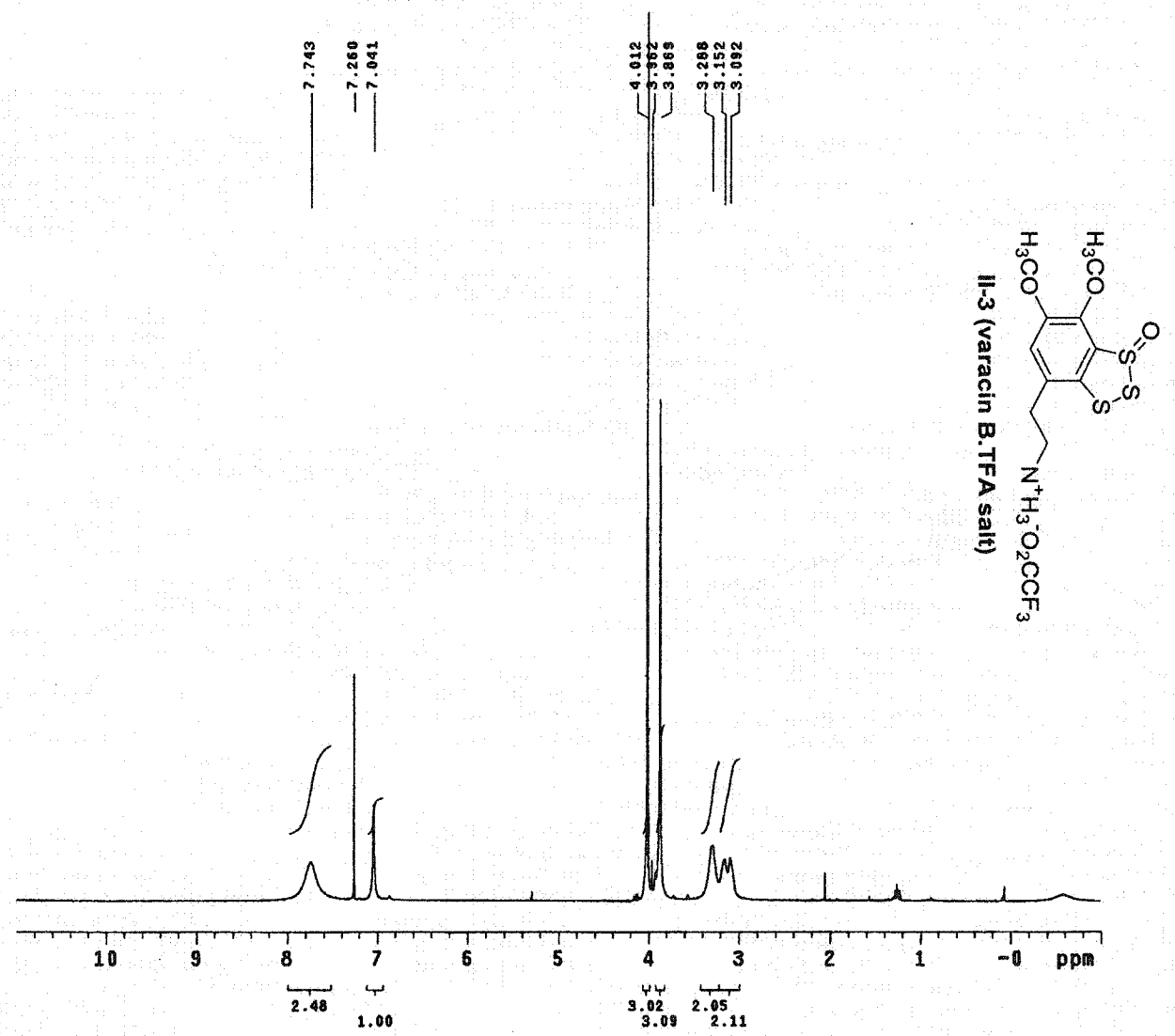
alex11

Pulse Sequence: s2pul

Solvent: CD3OD
Ambient temperature
User: 1-14-87
File: alex11
INOVA-500 "nmr500"

PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.043 sec
Width 31409.5 Hz
29312 repetitions
OBSERVE C13, 125.6595849 MHz
DECOUPLE H1, 499.7439082 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 17 hr, 6 min, 4 sec



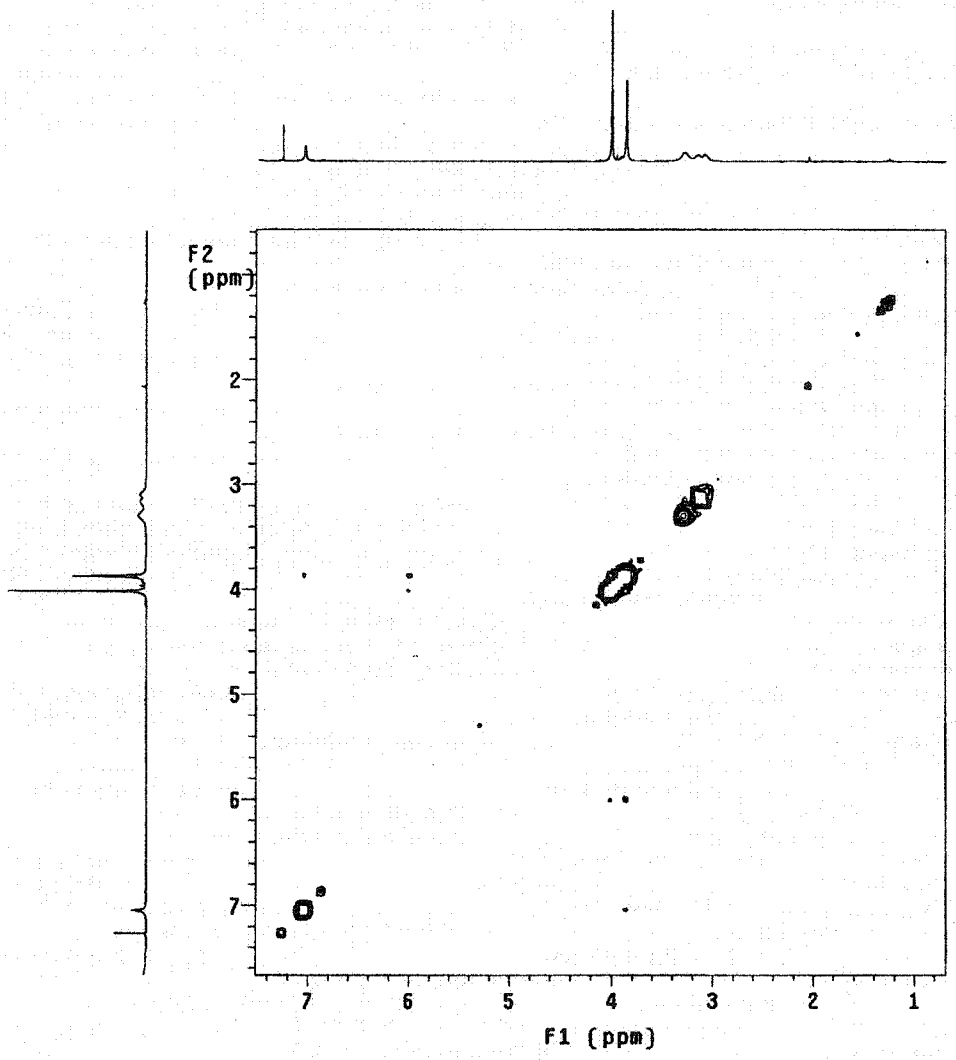


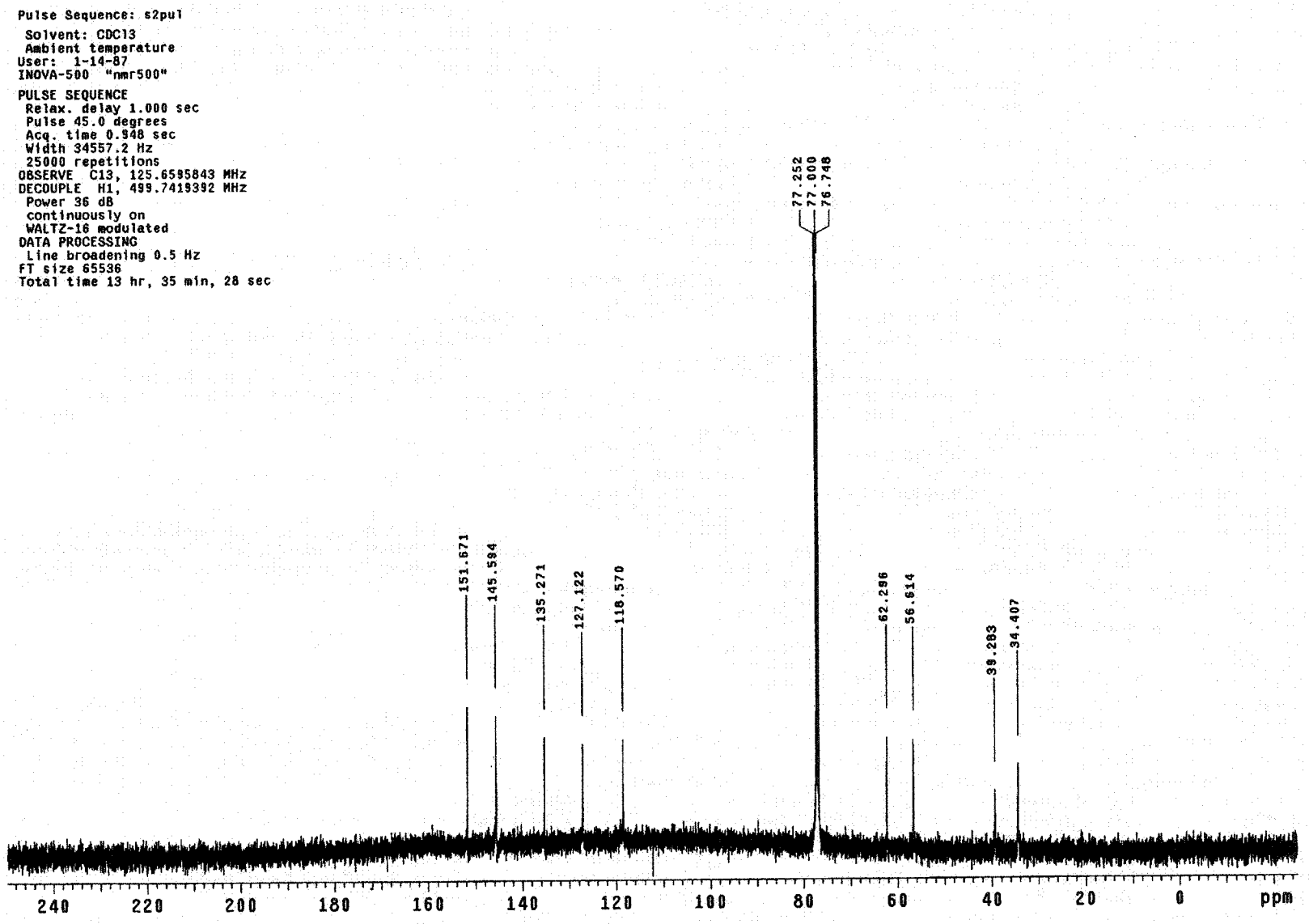
II-3 (varacin B.TFA salt)

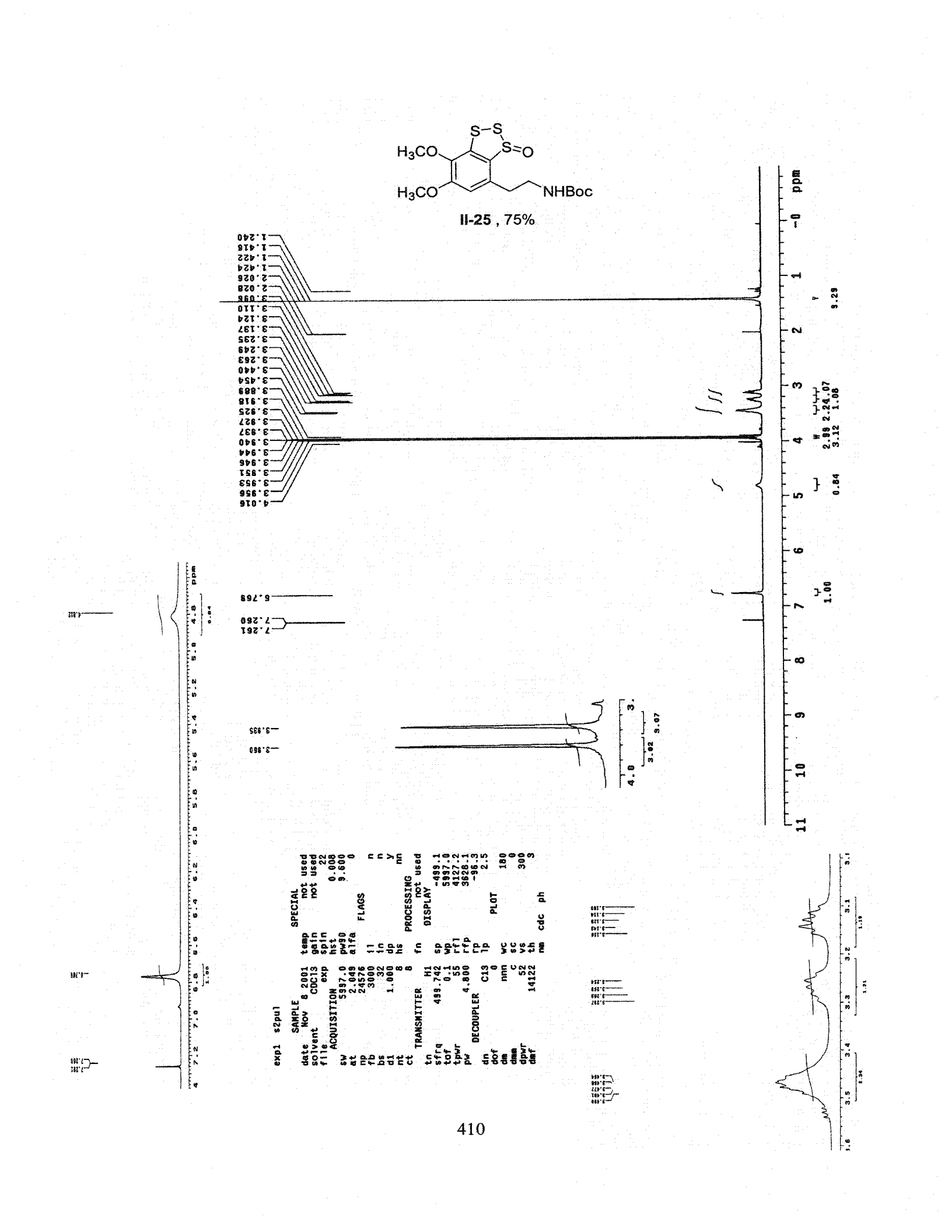
```
exp1  s2pul

      SAMPLE                SPECIAL
date    Nov  8 2001  temp       not used
solvent       CDCl3  gain       not used
file            exp  spin             20
   ACQUISITION       hst           0.008
sw           5997.0  pw90          9.600
at            2.049  alfa              0
np            24576        FLAGS
fb             3000  il                n
bs               32  in                n
d1            1.000  dp                y
nt                8  hs               nn
ct                8      PROCESSING
   TRANSMITTER      fn         not used
tn               H1       DISPLAY
sfrq        499.742  sp           -500.6
tof             0.1  wp           5997.0
tpwr             55  rfl          4128.7
pw            4.800  rfp          3628.1
    DECOUPLER        rp           -102.6
dn              C13  lp             -3.2
dof               0         PLOT
dm              nnn  wc              180
dmm               c  sc                0
dpwr             52  vs              144
dmf           14122  th                5
                     nm  cdc  ph
```

exp1 gCOSY

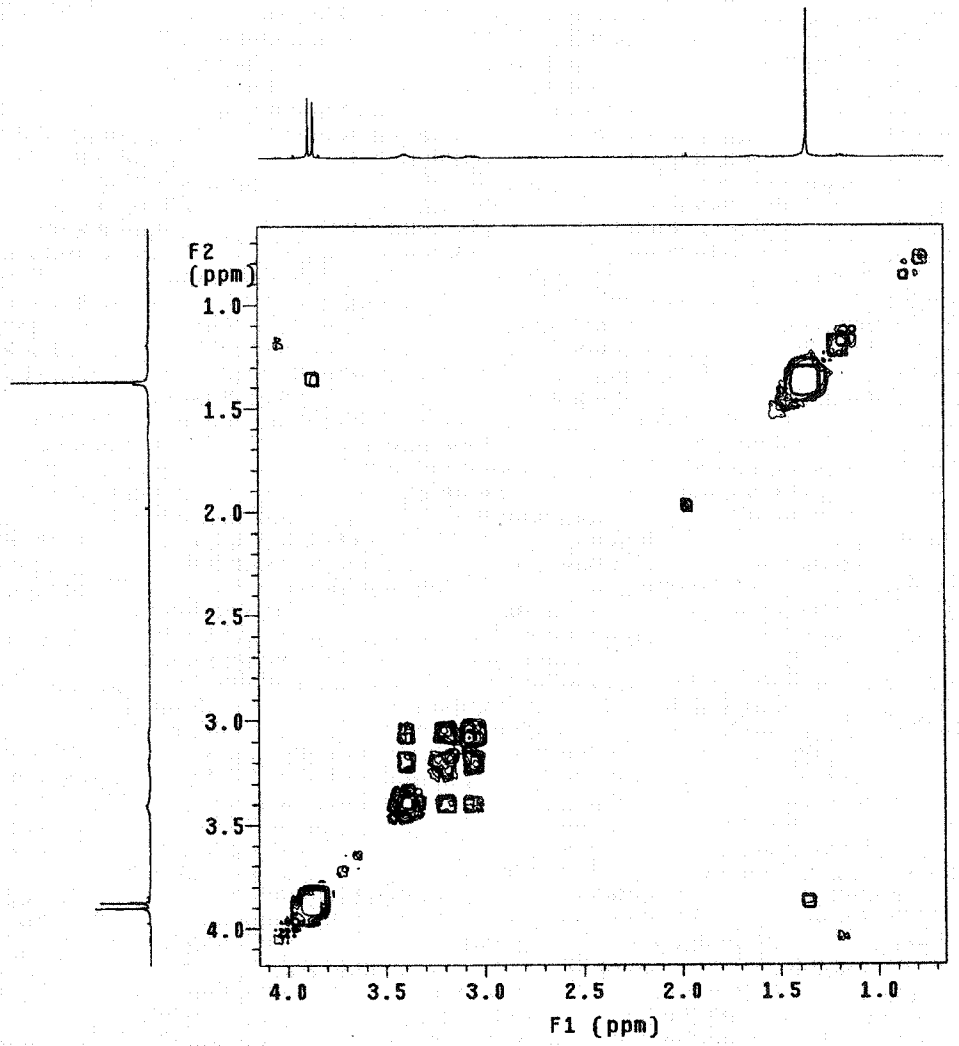
SAMPLE		FLAGS	
date	Nov 8 2001	hs	nn
solvent	CDCl3	sspul	n
sample	alex_08Nov2-001-17:31:06	hsglvl	4385
ACQUISITION		SPECIAL	
sw	7996.0	temp	not used
at	0.128	gain	30
np	2048	spin	0
fb	4000	F2 PROCESSING	
ss	16	sb	-0.064
d1	1.000	sbs	not used
nt	1	fn	2048
2D ACQUISITION		F1 PROCESSING	
sw1	7996.0	sb1	-0.016
ni	128	sbs1	not used
TRANSMITTER		proc1	lp
tn	H1	fn1	2048
sfrq	499.742	DISPLAY	
tof	499.8	sp	290.2
tpwr	55	wp	3540.8
pw	9.600	sp1	344.9
GRADIENTS		wp1	3407.9
gzlvl1	4385	rfl	999.5
gt1	0.001000	rfp	0
gstab	0.000500	rfl1	999.5
DECOUPLER		rfp1	0
dn	C13	PLOT	
dm	nnn	wc	135.0
		sc	10.0
		wc2	135.0
		sc2	0
		vs	79
		th	4
		al cdc av	



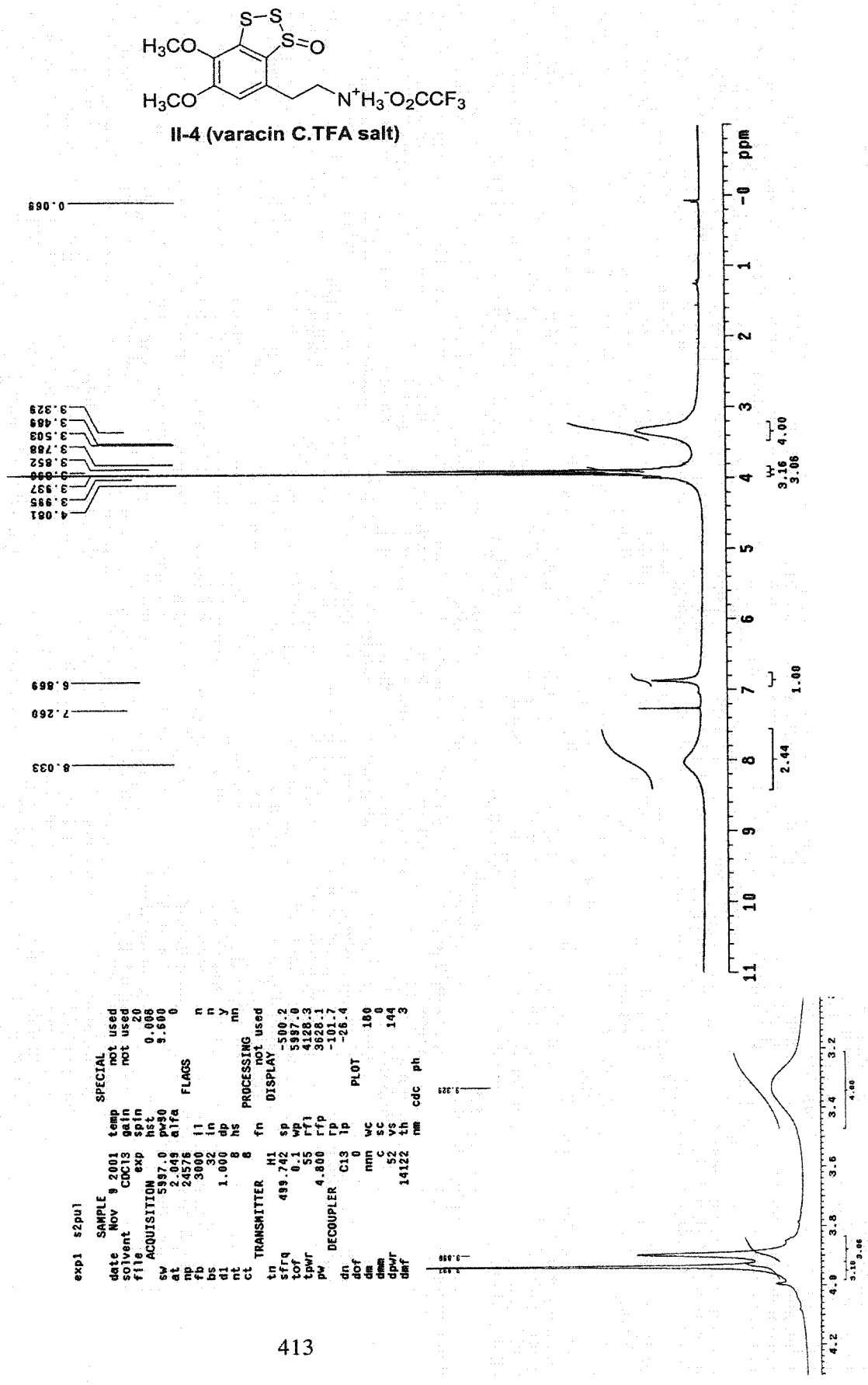

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
User: 1-14-87
INOVA-500 "nmr500"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 0.948 sec
Width 34557.2 Hz
25000 repetitions
OBSERVE C13, 125.6595843 MHz
DECOUPLE H1, 499.7419392 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 13 hr, 35 min, 28 sec
77.252
77.000
76.748
151.671
145.584
135.271
127.122
118.570
62.286
56.614
39.283
34.407
240
220
200
180
160
140
120
100
80
60
40
20
0
ppm

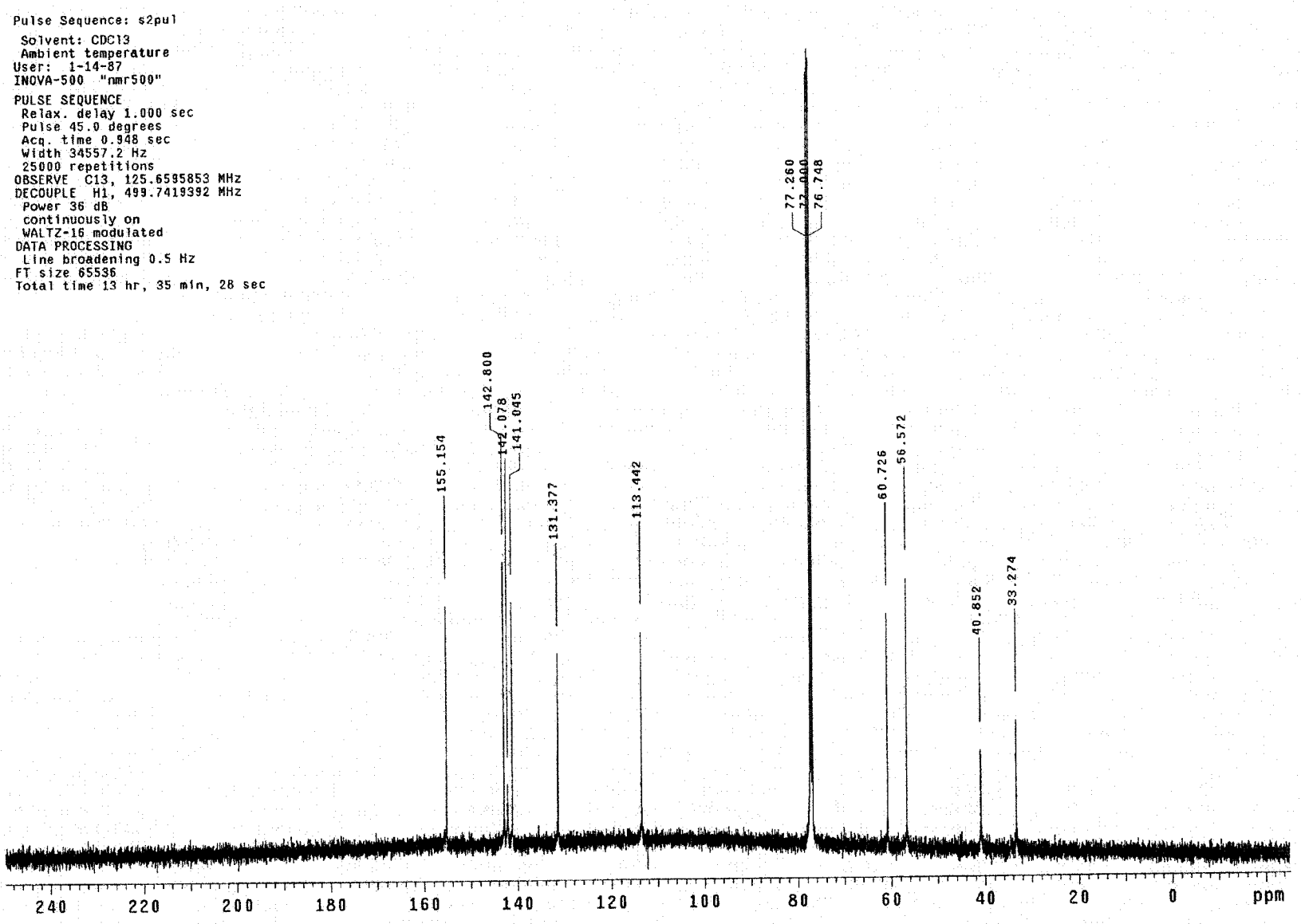

S–S
S=O
H3CO
H3CO
NHBoc
II-25 , 75%

```
exp1  gCOSY

      SAMPLE                FLAGS
date   Oct 26 2001  hs                nn
solvent       CDCl3  sspul             n
sample alex_26Oct2~ hsglvl          4385
      001-17:55:36        SPECIAL
   ACQUISITION      temp       not used
sw           7996.0  gain              34
at            0.128  spin               0
np             2048    F2 PROCESSING
fb             4000  sb           -0.064
ss               16  sbs        not used
d1            1.000  fn              2048
nt                1    F1 PROCESSING
  2D ACQUISITION    sb1          -0.016
sw1          7996.0  sbs1       not used
ni              128  proc1             lp
   TRANSMITTER      fn1             2048
tn               H1        DISPLAY
sfrq        499.742  sp             312.1
tof           499.8  wp            1774.3
tpwr             55  sp1            327.8
pw            9.600  wp1           1743.0
    GRADIENTS       rf1           1032.3
gzlvl1         4385  rfp                0
gti        0.001000  rfl1          1032.3
gstab      0.000500  rfp1               0
    DECOUPLER              PLOT
dn              C13  wc             135.0
dm              nnn  sc              10.0
                     wc2            135.0
                     sc2                0
                     vs                27
                     th                 5
                     a1  cdc  av
```



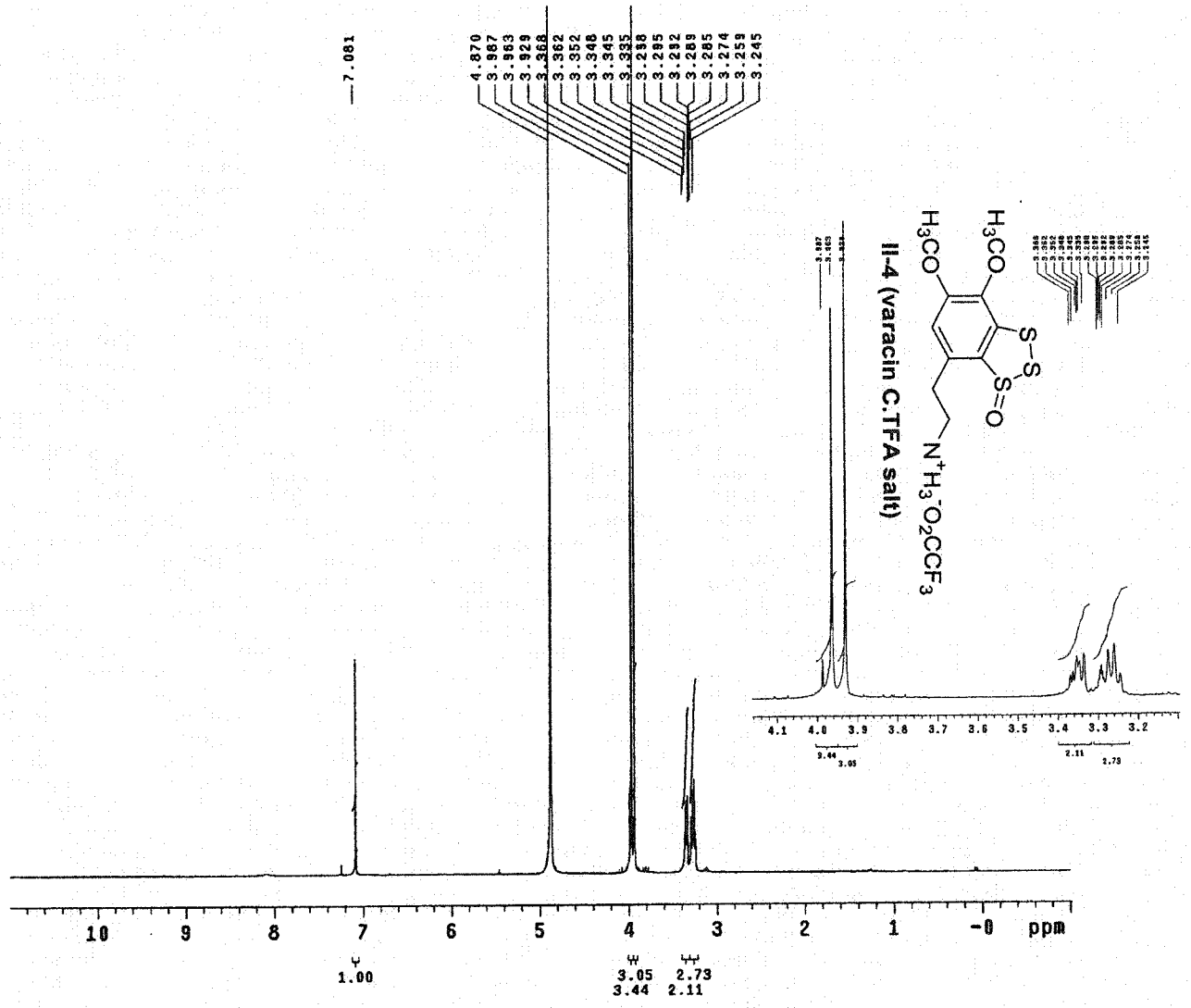
Current Data Parameters
NAME var88BoC13
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20011108
Time 23.08
INSTRUM dpx400
PROBHD 5 mm QNP 1H
PULPROG zgdc30
TD 65536
SOLVENT CDCl3
NS 1162
DS 0
SWH 65359.477 Hz
FIDRES 0.997306 Hz
AQ 0.5014004 sec
RG 5792.6
DW 7.650 usec
DE 4.50 usec
TE 300.0 K
D1 1.00000000 sec
d11 0.03000000 sec
========== CHANNEL f1 ==========
NUC1 13C
P1 5.80 usec
PL1 -6.00 dB
SFO1 100.6429128 MHz
========== CHANNEL f2 ==========
CPDPRG2 waltz16
NUC2 1H
PCPD2 71.00 usec
PL2 120.00 dB
PL12 17.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127781 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 220.000 ppm
F1 22134.81 Hz
F2P -10.000 ppm
F2 -1006.13 Hz
PPMCM 11.50000 ppm/cm
HZCM 1157.04688 Hz/cm
28.312
36.043
41.412
56.539
60.660
76.675
76.993
77.311
79.416
112.835
133.755
141.013
142.317
143.132
154.611
155.760
ppm
0
25
50
75
100
125
150
175
200
ppm

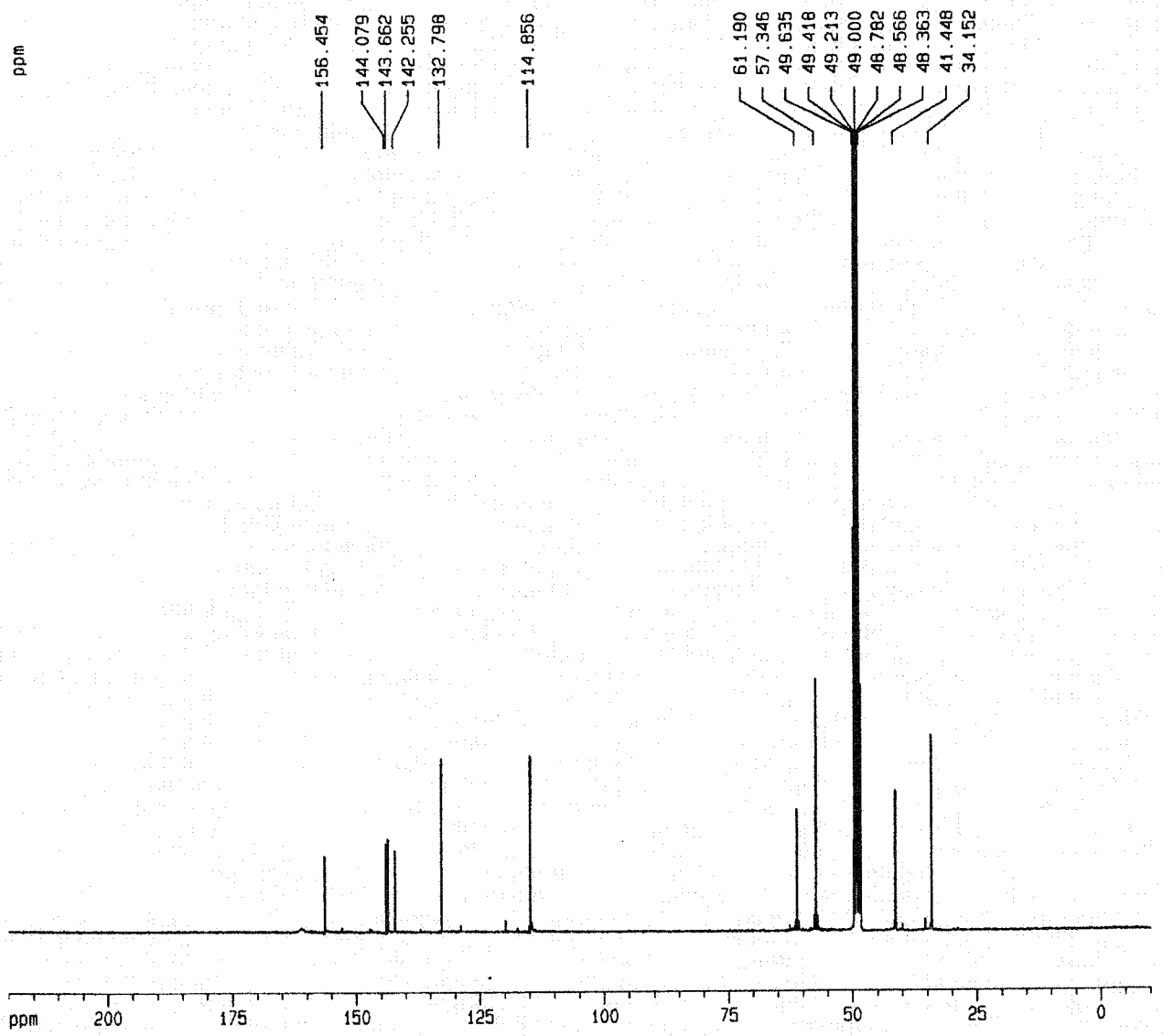

S–S
S=O
H3CO
H3CO
N+H3 -O2CCF3
II-4 (varacin C.TFA salt)
0.089
3.329
3.469
3.503
3.788
3.852
3.898
3.937
3.985
4.081
6.869
7.269
8.033
ppm
4.00
3.16
3.06
2.44
1.00
exp1 s2pul
SAMPLE
date Nov 9 2001
solvent CDC13
file exp
ACQUISITION
sw 5997.0
at 2.049
np 24576
fb 3000
bs 32
d1 1.000
nt 8
ct 8
TRANSMITTER
tn H1
sfrq 499.742
tof 0.1
tpwr 55
pw 4.800
DECOUPLER
dn C13
dof 0
dm nnn
dmm c
dpwr 52
dmf 14122
SPECIAL
temp not used
gain not used
spin 20
hst 0.008
pw90 9.600
alfa 0
FLAGS
il n
in n
dp y
hs nn
PROCESSING
fn not used
DISPLAY
sp -500.2
wp 5997.0
rfl 4128.3
rfp 3628.1
rp -101.7
lp -26.4
PLOT
wc 180
sc 0
vs 144
th 3
nm cdc ph


Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
User: 1-14-87
INOVA-500 "nmr500"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 0.948 sec
Width 34557.2 Hz
25000 repetitions
OBSERVE C13, 125.6595853 MHz
DECOUPLE H1, 499.7419392 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 13 hr, 35 min, 28 sec
155.154
142.800
142.078
141.045
131.377
113.442
77.260
77.000
76.748
60.726
56.572
40.852
33.274
240
220
200
180
160
140
120
100
80
60
40
20
0
ppm

exp1 s2pul

SAMPLE		SPECIAL	
date	Nov 10 2001	temp	not used
solvent	CD3OD	gain	not used
file	exp	spin	22
ACQUISITION		hst	0.008
sw	5997.0	pw90	9.600
at	2.048	alfa	0
np	24576	**FLAGS**	
fb	3000	il	n
bs	32	in	n
d1	1.000	dp	y
nt	8	hs	nn
ct	8	**PROCESSING**	
TRANSMITTER		fn	not used
tn	H1	**DISPLAY**	
sfrq	499.744	sp	-506.4
tof	0.1	wp	5997.0
tpwr	55	rfl	2840.1
pw	4.800	rfp	2433.7
DECOUPLER		rp	-0.5
dn	C13	lp	-0.9
dof	0	**PLOT**	
dm	nnn	wc	180
dmm	c	sc	0
dpwr	52	vs	144
dmf	14122	th	5
		nm	cdc ph





Current Data Parameters

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EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

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TD	65536
SOLVENT	MeOH
NS	18432
DS	0
SWH	65359.477 Hz
FIDRES	0.997306 Hz
AQ	0.5014004 sec
RG	5792.6
DW	7.650 usec
DE	4.50 usec
TE	300.0 K
D1	1.00000000 sec
d11	0.03000000 sec

========== CHANNEL f1 ==========

NUC1	13C
P1	5.80 usec
PL1	-6.00 dB
SFO1	100.6429128 MHz

========== CHANNEL f2 ==========

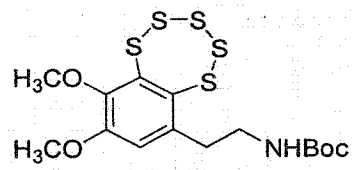
CPDPRG2	waltz16
NUC2	1H
PCPD2	71.00 usec
PL2	120.00 dB
PL12	17.00 dB
SFO2	400.1316005 MHz

F2 - Processing parameters

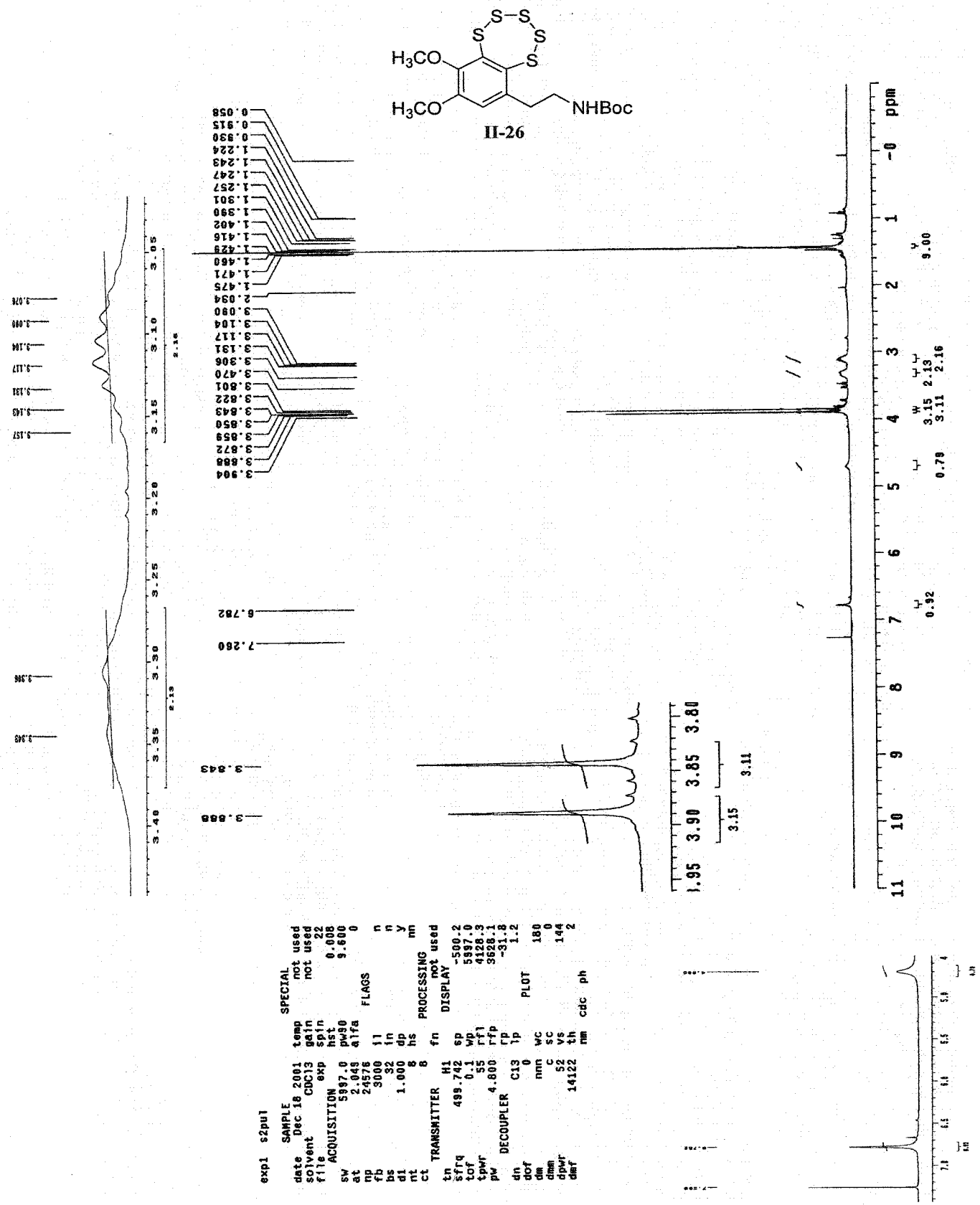
SI	32768
SF	100.6126333 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

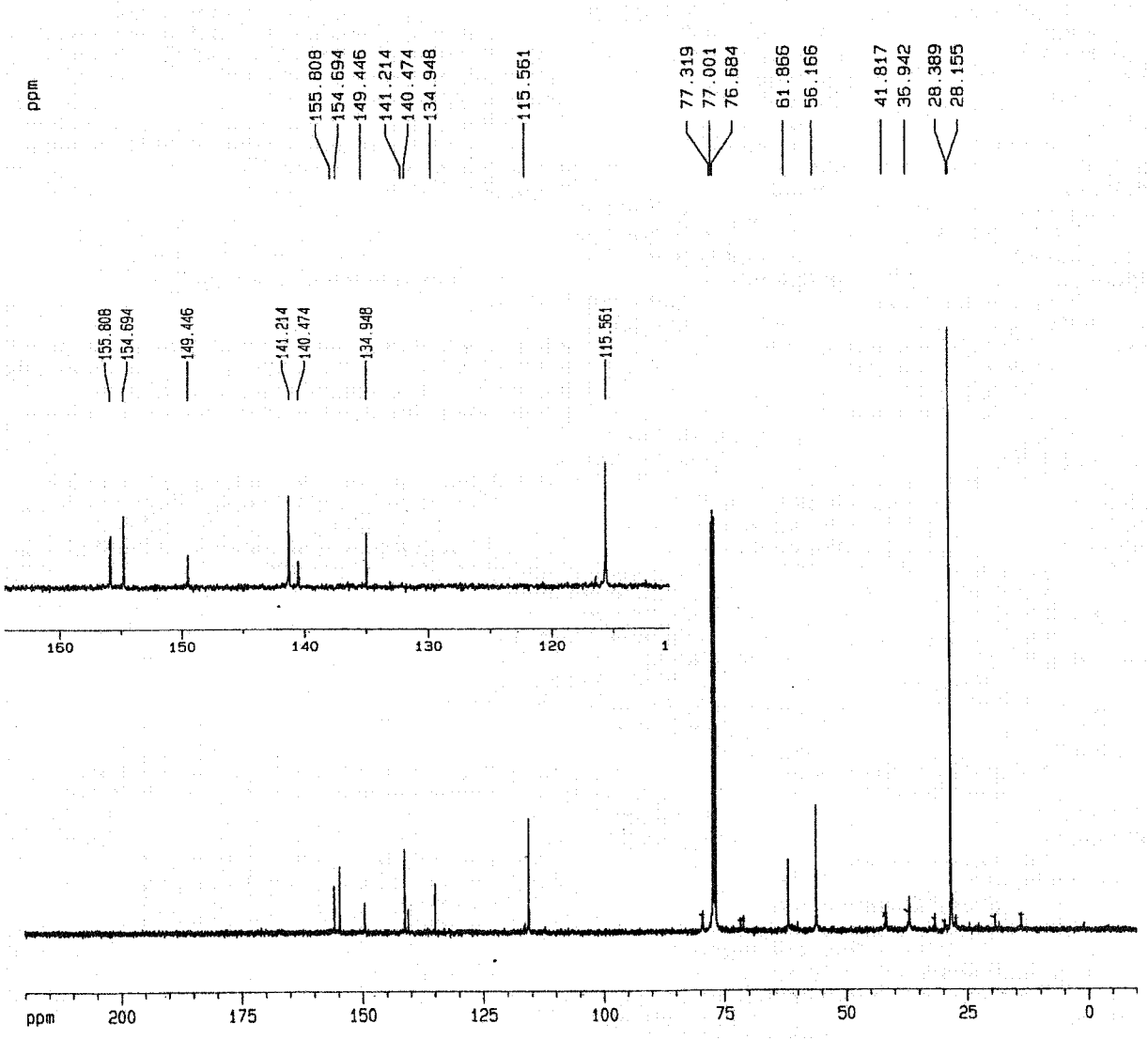
1D NMR plot parameters

CX	20.00 cm
F1P	220.000 ppm
F1	22134.78 Hz
F2P	-10.000 ppm
F2	-1006.13 Hz
PPMCM	11.50000 ppm/cm
HZCM	1157.04529 Hz/cm



II-26





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EXPNO 1
PROCNO 1

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Time 4.43
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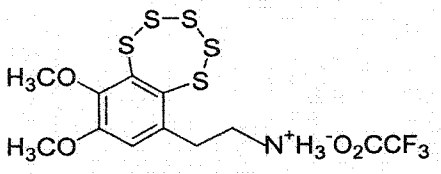
PULPROG zgdc30
TD 65536
SOLVENT CDCl3
NS 18432
DS 0
SWH 65359.477 Hz
FIDRES 0.997306 Hz
AQ 0.5014004 sec
RG 5792.6
DW 7.650 usec
DE 4.50 usec
TE 300.0 K
D1 1.00000000 sec
d11 0.03000000 sec

========== CHANNEL f1 ==========
NUC1 13C
P1 5.80 usec
PL1 -6.00 dB
SFO1 100.6429128 MHz

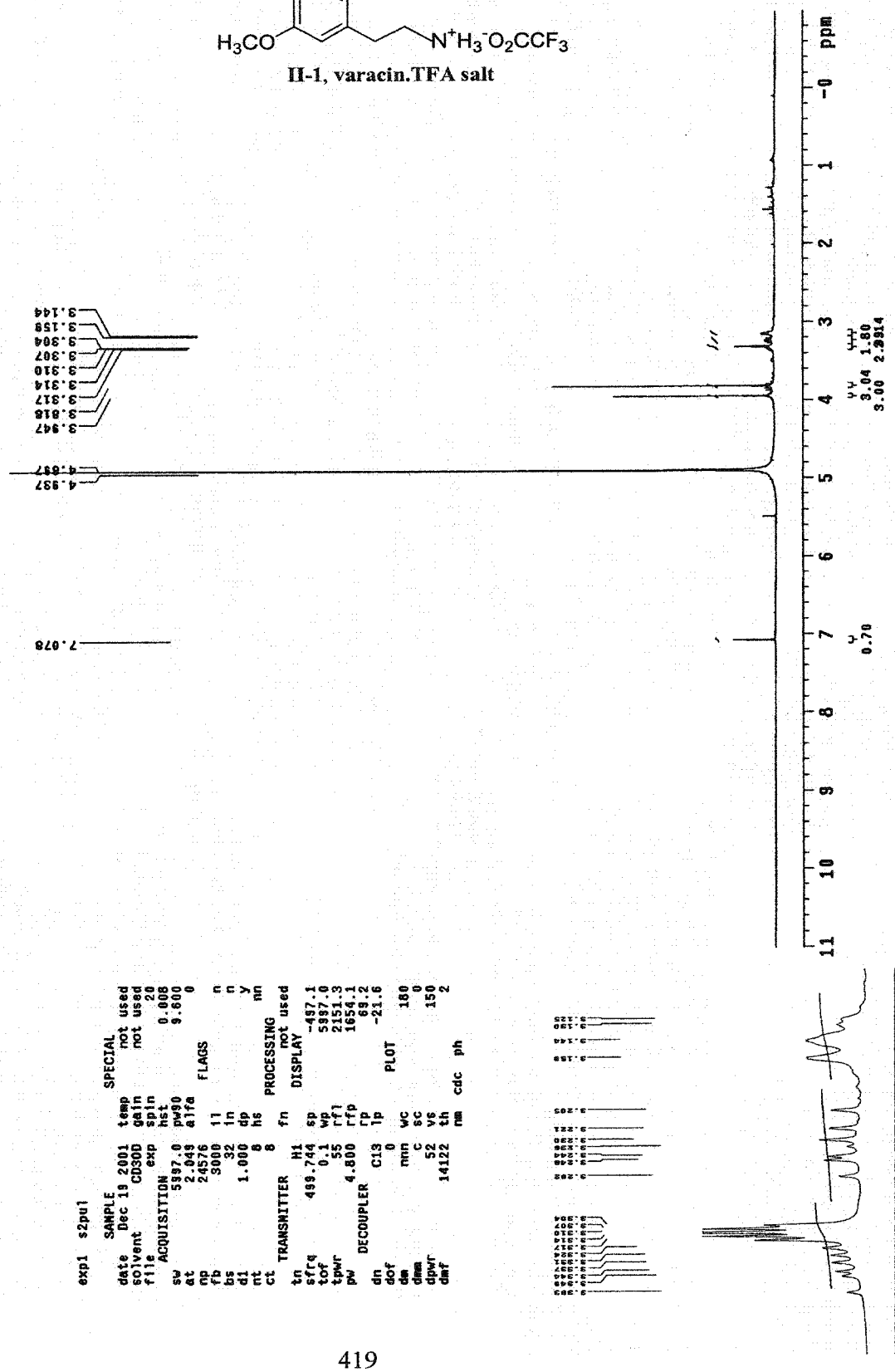
========== CHANNEL f2 ==========
CPDPRG2 waltz16
NUC2 1H
PCPD2 71.00 usec
PL2 120.00 dB
PL12 17.00 dB
SFO2 400.1316005 MHz

F2 - Processing parameters
SI 32768
SF 100.6127741 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

1D NMR plot parameters
CX 20.00 cm
F1P 220.000 ppm
F1 22134.81 Hz
F2P -10.000 ppm
F2 -1006.13 Hz
PPMCM 11.50000 ppm/cm
HZCM 1157.04688 Hz/cm



II-1, varacin.TFA salt



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PROCNO 1
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PROBHD 5 mm QNP 1H
PULPROG zgdc30
TD 65536
SOLVENT MeOH
NS 19432
DS 0
SWH 65359.477 Hz
FIDRES 0.997306 Hz
AQ 0.5014004 sec
RG 5792.6
DW 7.650 usec
DE 4.50 usec
TE 300.0 K
D1 1.00000000 sec
d11 0.03000000 sec
CHANNEL f1
NUC1 13C
P1 5.80 usec
PL1 -6.00 dB
SFO1 100.6429128 MHz
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
PCPD2 71.00 usec
PL2 120.00 dB
PL12 17.00 dB
SFO2 400.1318005 MHz
F2 - Processing parameters
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SF 100.6126313 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 200.000 ppm
F1 20122.53 Hz
F2P -10.000 ppm
F2 -1006.13 Hz
PPMCM 10.50000 ppm/cm
HZCM 1056.43262 Hz/cm
35.128
41.629
48.363
48.567
48.783
49.000
49.213
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49.635
56.895
62.181
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136.063
140.293
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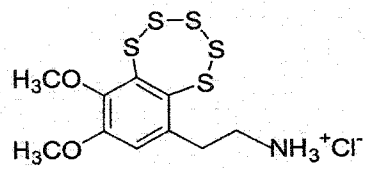
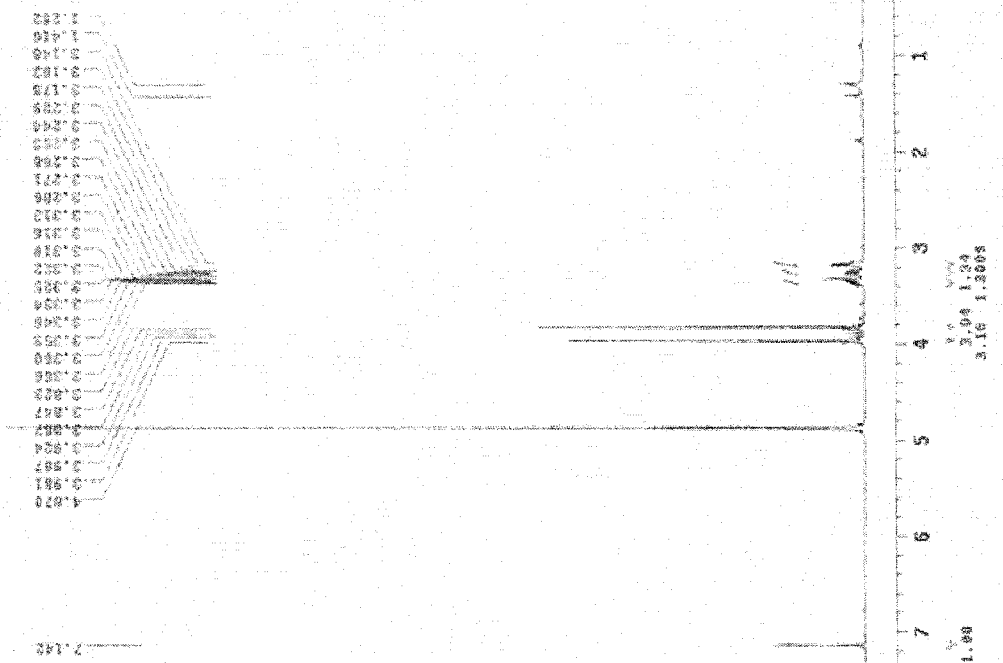
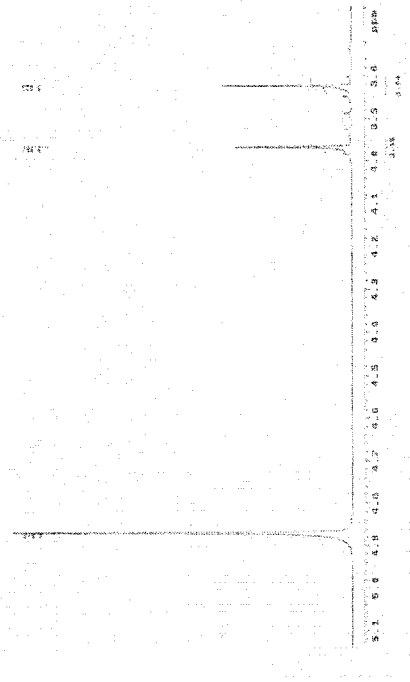
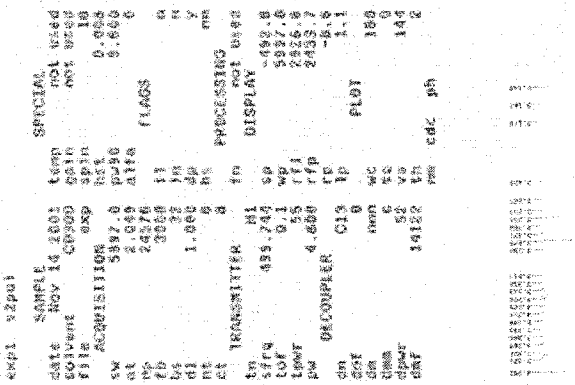
151.257
156.687
ppm

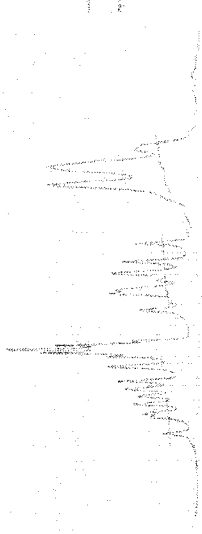


II-1, varacinium chloride









APPENDIX

X-Ray Crystallographic Structure Analysis

List of X-Ray Data of PART II

6,7-Dimethoxy-9-[(amino)ethyl]benzopentathiepin hydrogen chloride/ varacinium chloride (II-1)..... 424

***N*-(Boc)-4-ethylamino-6,7-dimethoxy-1,2,3-benzotrithiole 3-oxide (II-25)..... 433**

Table 1. Crystal data and structure refinement for lhf1.

Identification code	ABClhf1 2/12-2002	
Empirical formula	C10 H14 Cl N O2 S5	
Formula weight	375.97	
Temperature	294(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P2(1)/c	
Unit cell dimensions	a = 16.891(3) Å	α= 90°.
	b = 10.6971(16) Å	β= 101.673(4)°.
	c = 9.1959(13) Å	γ = 90°.
Volume	1627.2(4) Å3	
Z	4	
Density (calculated)	1.535 Mg/m3	
Absorption coefficient	0.872 mm-1	
F(000)	776	
Crystal size	0.28 x 0.10 x 0.08 mm3	
Theta range for data collection	3.02 to 27.56°.	
Index ranges	-16<=h<=21, -13<=k<=13, -11<=l<=11	
Reflections collected	10857	
Independent reflections	3726 [R(int) = 0.0813]	
Completeness to theta = 27.56°	99.3 %	
Absorption correction	Empirical	
Max. and min. transmission	0.9335 and 0.7924	
Refinement method	Full-matrix least-squares on F2	
Data / restraints / parameters	3726 / 0 / 174	
Goodness-of-fit on F2	0.810	
Final R indices [I>2sigma(I)]	R1 = 0.0535, wR2 = 0.1111	
R indices (all data)	R1 = 0.1683, wR2 = 0.1332	
Largest diff. peak and hole	0.402 and -0.272 e.Å-3	

Table 2. Atomic coordinates (x $10^{4)}$ and equivalent isotropic displacement parameters ($Å^2$x 10^3) for lhf1. U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U(eq)
Cl(1)	5585(1)	3852(1)	2063(1)	62(1)
S(1)	3084(1)	4802(1)	1985(2)	67(1)
S(2)	3680(1)	5644(1)	3878(2)	76(1)
S(3)	2951(1)	7126(1)	4133(2)	89(1)
S(4)	1998(1)	6258(1)	4793(2)	99(1)
S(5)	1342(1)	5453(1)	2917(2)	92(1)
O(1)	458(2)	3212(4)	3376(5)	96(1)
O(2)	989(2)	839(3)	3664(5)	94(1)
C(1)	2493(3)	3626(4)	2621(5)	54(1)
C(2)	1735(3)	3914(4)	2957(6)	62(1)
C(3)	1253(3)	2944(6)	3267(6)	74(2)
C(4)	1521(3)	1705(5)	3343(6)	69(2)
C(5)	2270(3)	1453(4)	3051(5)	61(1)
C(6)	2756(3)	2391(4)	2680(5)	49(1)
C(7)	3578(3)	2005(4)	2414(5)	50(1)
C(8)	4186(3)	1997(4)	3843(5)	48(1)
C(9)	286(4)	3095(6)	4758(7)	117(3)
C(10)	1209(4)	-435(5)	3654(7)	98(2)
N(1)	4995(2)	1573(3)	3649(4)	51(1)

Table 3. Bond lengths [Å] and angles [°] for lhf1.

S(1)-C(1)	1.777(4)
S(1)-S(2)	2.036(2)
S(2)-S(3)	2.0505(19)
S(3)-S(4)	2.054(2)
S(4)-S(5)	2.043(3)
S(5)-C(2)	1.773(5)
O(1)-C(9)	1.366(6)
O(1)-C(3)	1.396(6)
O(2)-C(4)	1.363(5)
O(2)-C(10)	1.413(5)
C(1)-C(6)	1.391(6)
C(1)-C(2)	1.411(6)
C(2)-C(3)	1.385(6)
C(3)-C(4)	1.398(6)
C(4)-C(5)	1.373(6)
C(5)-C(6)	1.382(6)
C(5)-H(5)	0.9300
C(6)-C(7)	1.515(5)
C(7)-C(8)	1.495(6)
C(7)-H(7A)	0.9700
C(7)-H(7B)	0.9700
C(8)-N(1)	1.484(5)
C(8)-H(8A)	0.9700
C(8)-H(8B)	0.9700
C(9)-H(9A)	0.9600
C(9)-H(9B)	0.9600
C(9)-H(9C)	0.9600
C(10)-H(10A)	0.9600
C(10)-H(10B)	0.9600
C(10)-H(10C)	0.9600
N(1)-H(1A)	0.8900
N(1)-H(1B)	0.8900
N(1)-H(1C)	0.8900
C(1)-S(1)-S(2)	104.17(17)
S(1)-S(2)-S(3)	103.59(9)
S(2)-S(3)-S(4)	102.05(8)
S(5)-S(4)-S(3)	105.36(9)
C(2)-S(5)-S(4)	103.66(19)
C(9)-O(1)-C(3)	115.9(5)
C(4)-O(2)-C(10)	117.9(4)
C(6)-C(1)-C(2)	119.7(4)
C(6)-C(1)-S(1)	119.3(4)
C(2)-C(1)-S(1)	120.8(4)
C(3)-C(2)-C(1)	118.6(4)
C(3)-C(2)-S(5)	117.8(4)
C(1)-C(2)-S(5)	123.5(4)
C(2)-C(3)-O(1)	118.4(5)
C(2)-C(3)-C(4)	121.5(5)
O(1)-C(3)-C(4)	119.9(5)
O(2)-C(4)-C(5)	125.7(5)
O(2)-C(4)-C(3)	115.7(5)
C(5)-C(4)-C(3)	118.6(5)

C(4)-C(5)-C(6)	121.6(5)
C(4)-C(5)-H(5)	119.2
C(6)-C(5)-H(5)	119.2
C(5)-C(6)-C(1)	119.8(4)
C(5)-C(6)-C(7)	116.9(4)
C(1)-C(6)-C(7)	123.2(4)
C(8)-C(7)-C(6)	110.4(3)
C(8)-C(7)-H(7A)	109.6
C(6)-C(7)-H(7A)	109.6
C(8)-C(7)-H(7B)	109.6
C(6)-C(7)-H(7B)	109.6
H(7A)-C(7)-H(7B)	108.1
N(1)-C(8)-C(7)	112.4(3)
N(1)-C(8)-H(8A)	109.1
C(7)-C(8)-H(8A)	109.1
N(1)-C(8)-H(8B)	109.1
C(7)-C(8)-H(8B)	109.1
H(8A)-C(8)-H(8B)	107.9
O(1)-C(9)-H(9A)	109.5
O(1)-C(9)-H(9B)	109.5
H(9A)-C(9)-H(9B)	109.5
O(1)-C(9)-H(9C)	109.5
H(9A)-C(9)-H(9C)	109.5
H(9B)-C(9)-H(9C)	109.5
O(2)-C(10)-H(10A)	109.5
O(2)-C(10)-H(10B)	109.5
H(10A)-C(10)-H(10B)	109.5
O(2)-C(10)-H(10C)	109.5
H(10A)-C(10)-H(10C)	109.5
H(10B)-C(10)-H(10C)	109.5
C(8)-N(1)-H(1A)	109.5
C(8)-N(1)-H(1B)	109.5
H(1A)-N(1)-H(1B)	109.5
C(8)-N(1)-H(1C)	109.5
H(1A)-N(1)-H(1C)	109.5
H(1B)-N(1)-H(1C)	109.5

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for lhf1. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ (ha^*)^2\ U_{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U_{12}\ ]$

	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
Cl(1)	76(1)	41(1)	66(1)	6(1)	7(1)	-5(1)
S(1)	66(1)	63(1)	80(1)	13(1)	31(1)	8(1)
S(2)	64(1)	59(1)	104(1)	2(1)	20(1)	1(1)
S(3)	89(1)	54(1)	134(1)	-3(1)	46(1)	3(1)
S(4)	110(1)	76(1)	130(2)	-2(1)	70(1)	8(1)
S(5)	61(1)	75(1)	147(2)	23(1)	38(1)	24(1)
O(1)	65(3)	109(3)	115(4)	20(3)	21(3)	9(2)
O(2)	64(3)	68(3)	154(4)	9(3)	29(3)	-17(2)
C(1)	47(3)	58(3)	59(3)	9(3)	21(3)	5(3)
C(2)	42(3)	55(3)	91(4)	11(3)	19(3)	3(3)
C(3)	42(4)	87(4)	94(4)	4(4)	19(3)	6(3)
C(4)	50(4)	63(4)	92(4)	6(3)	13(3)	-6(3)
C(5)	57(4)	51(3)	74(4)	3(3)	10(3)	7(3)
C(6)	45(3)	48(3)	56(3)	3(2)	12(3)	4(3)
C(7)	57(3)	46(3)	49(3)	2(2)	14(3)	10(3)
C(8)	52(3)	48(3)	48(3)	1(2)	22(3)	9(2)
C(9)	144(7)	135(6)	87(5)	12(4)	58(5)	36(5)
C(10)	94(5)	65(4)	136(6)	-1(4)	25(4)	-35(4)
N(1)	52(3)	46(2)	57(3)	5(2)	17(2)	2(2)

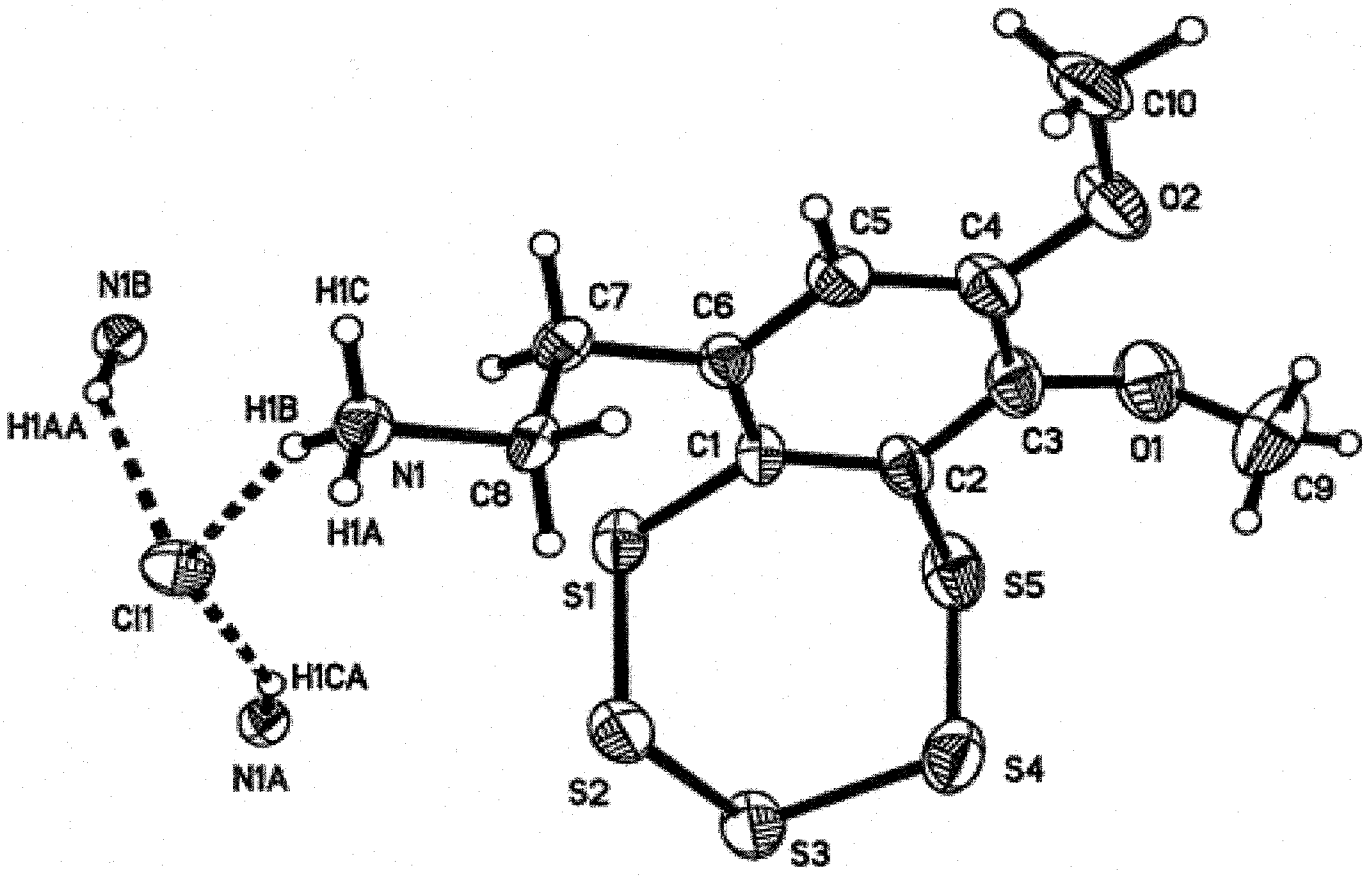
Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for lhf1.

	x	y	z	U(eq)
H(5)	2455	632	3104	73
H(7A)	3543	1177	1974	60
H(7B)	3749	2583	1725	60
H(8A)	4232	2834	4260	57
H(8B)	3999	1449	4543	57
H(9A)	-235	3454	4760	176
H(9B)	690	3523	5467	176
H(9C)	282	2226	5017	176
H(10A)	781	-943	3878	147
H(10B)	1693	-574	4387	147
H(10C)	1303	-653	2691	147
H(1A)	5337	1582	4523	76
H(1B)	5175	2084	3023	76
H(1C)	4958	800	3284	76

Table 6. Torsion angles [°] for lhf1.

C(1)-S(1)-S(2)-S(3)	-93.78(17)
S(1)-S(2)-S(3)-S(4)	73.00(11)
S(2)-S(3)-S(4)-S(5)	-75.16(11)
S(3)-S(4)-S(5)-C(2)	93.11(19)
S(2)-S(1)-C(1)-C(6)	-101.5(4)
S(2)-S(1)-C(1)-C(2)	83.4(4)
C(6)-C(1)-C(2)-C(3)	-3.1(7)
S(1)-C(1)-C(2)-C(3)	172.0(4)
C(6)-C(1)-C(2)-S(5)	180.0(4)
S(1)-C(1)-C(2)-S(5)	-4.9(6)
S(4)-S(5)-C(2)-C(3)	108.5(4)
S(4)-S(5)-C(2)-C(1)	-74.6(4)
C(1)-C(2)-C(3)-O(1)	-170.4(4)
S(5)-C(2)-C(3)-O(1)	6.6(7)
C(1)-C(2)-C(3)-C(4)	3.9(8)
S(5)-C(2)-C(3)-C(4)	-179.0(4)
C(9)-O(1)-C(3)-C(2)	-112.1(6)
C(9)-O(1)-C(3)-C(4)	73.4(7)
C(10)-O(2)-C(4)-C(5)	-2.1(8)
C(10)-O(2)-C(4)-C(3)	175.8(5)
C(2)-C(3)-C(4)-O(2)	179.7(5)
O(1)-C(3)-C(4)-O(2)	-6.0(8)
C(2)-C(3)-C(4)-C(5)	-2.2(9)
O(1)-C(3)-C(4)-C(5)	172.0(5)
O(2)-C(4)-C(5)-C(6)	177.5(5)
C(3)-C(4)-C(5)-C(6)	-0.3(8)
C(4)-C(5)-C(6)-C(1)	1.1(7)
C(4)-C(5)-C(6)-C(7)	178.7(4)
C(2)-C(1)-C(6)-C(5)	0.7(7)
S(1)-C(1)-C(6)-C(5)	-174.5(3)
C(2)-C(1)-C(6)-C(7)	-176.8(4)
S(1)-C(1)-C(6)-C(7)	8.0(6)
C(5)-C(6)-C(7)-C(8)	-87.2(5)
C(1)-C(6)-C(7)-C(8)	90.4(5)
C(6)-C(7)-C(8)-N(1)	177.6(3)

Symmetry transformations used to generate equivalent atoms: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O2
C5
C4
N1B
H1C
C7
C6
H1AA
H1B
C1
C3
O1
C9
N1
C8
C2
H1A
S1
S5
Cl1
H1CA
N1A
S2
S4
S3

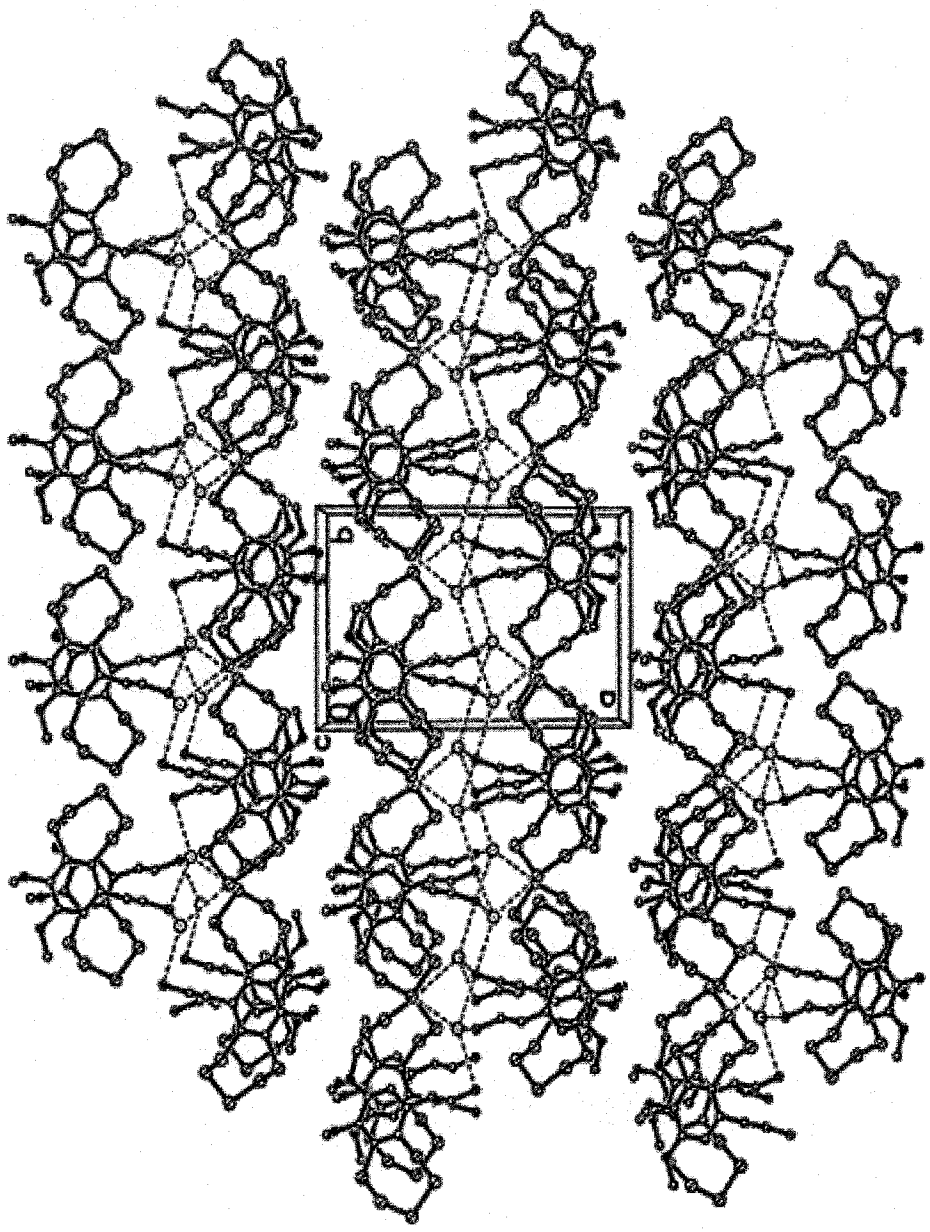


Table 1. Crystal data and structure refinement for 1hf2.

Identification code	ABC1hf2 (12/12-2001)
Empirical formula	$C_{15}H_{21}NO_5S_3$
Formula weight	391.51
Temperature	296(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1/c$
Unit cell dimensions	a = 12.546(3) Å alpha = 90° b = 15.353(3) Å beta = 90.832(5)° c = 9.957(2) Å gamma = 90°
Volume, Z	1917.7(7) $Å^3$, 4
Density (calculated)	1.356 Mg/m^3
Absorption coefficient	0.410 mm^{-1}
F(000)	824
Crystal size	0.30 x 0.24 x 0.10 mm
θ range for data collection	2.95 to 27.62°
Limiting indices	$-15 \le h \le 16$, $-20 \le k \le 19$, $-10 \le l \le 12$
Reflections collected	12749
Independent reflections	4401 (R_{int} = 0.0925)
Completeness to θ = 27.62°	98.8 %
Absorption correction	Empirical
Max. and min. transmission	0.9602 and 0.8870
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4401 / 5 / 223
Goodness-of-fit on F^2	1.020
Final R indices [I>2σ(I)]	R1 = 0.0776, wR2 = 0.1754
R indices (all data)	R1 = 0.2441, wR2 = 0.2149
Largest diff. peak and hole	0.742 and -0.294 $eÅ^{-3}$

Table 2. Atomic coordinates [x 10^4] and equivalent isotropic displacement parameters [$Å^2$ x 10^3] for lhf2. U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U(eq)
S(1)	5299(1)	466(1)	8232(2)	78(1)
S(2)	6410(2)	-490(1)	8677(2)	108(1)
S(3)	7707(1)	-19(1)	7631(2)	81(1)
O(1)	5476(4)	1263(3)	9018(5)	135(2)
O(2)	8469(2)	585(2)	5077(3)	63(1)
O(3)	7324(2)	1430(2)	3132(4)	77(1)
O(4)	2457(3)	2578(3)	4157(4)	86(1)
O(5)	1238(2)	2957(2)	5704(3)	68(1)
N(1)	2801(3)	2469(2)	6375(4)	55(1)
C(1)	5856(4)	687(3)	6639(5)	50(1)
C(2)	6929(3)	507(3)	6403(5)	49(1)
C(3)	7394(3)	756(3)	5222(5)	47(1)
C(4)	6813(4)	1195(3)	4264(5)	55(1)
C(5)	5716(4)	1339(3)	4468(5)	59(2)
C(6)	5231(4)	1093(3)	5652(5)	52(1)
C(7)	4050(3)	1298(3)	5815(5)	64(2)
C(8)	3919(3)	2236(3)	6248(5)	57(2)
C(9)	2178(4)	2651(3)	5308(5)	60(2)
C(10)	399(4)	3151(4)	4776(5)	75(2)
C(11)	-523(7)	3382(8)	5682(10)	90(3)
C(12)	39(5)	2329(5)	4050(8)	144(3)
C(13)	740(13)	3814(9)	3716(14)	162(8)
C(14)	8719(4)	-55(4)	4044(5)	86(2)
C(15)	6726(4)	1862(5)	2091(6)	87(3)

Table 3. Bond lengths [Å] and angles [°] for lhf2.

S(1)-O(1)	1.468(5)	S(1)-C(1)	1.776(5)
S(1)-S(2)	2.067(2)	S(2)-S(3)	2.074(2)
S(3)-C(2)	1.751(5)	O(2)-C(3)	1.383(5)
O(2)-C(14)	1.460(5)	O(3)-C(4)	1.353(5)
O(3)-C(15)	1.433(5)	O(4)-C(9)	1.209(5)
O(5)-C(9)	1.334(5)	O(5)-C(10)	1.422(5)
N(1)-C(9)	1.341(6)	N(1)-C(8)	1.454(5)
N(1)-H(1B)	0.8600	C(1)-C(6)	1.394(6)
C(1)-C(2)	1.397(6)	C(2)-C(3)	1.375(6)
C(3)-C(4)	1.369(6)	C(4)-C(5)	1.412(6)
C(5)-C(6)	1.387(6)	C(5)-H(5A)	0.9300
C(6)-C(7)	1.526(6)	C(7)-C(8)	1.514(6)
C(7)-H(7A)	0.9700	C(7)-H(7B)	0.9700
C(8)-H(8A)	0.9700	C(8)-H(8B)	0.9700
C(10)-C(13)	1.531(9)	C(10)-C(11)	1.519(7)
C(10)-C(12)	1.520(6)	C(11)-H(11A)	0.9600
C(11)-H(11B)	0.9600	C(11)-H(11C)	0.9600
C(12)-H(12A)	0.9600	C(12)-H(12B)	0.9600
C(12)-H(12C)	0.9600	C(13)-H(13A)	0.9600
C(13)-H(13B)	0.9600	C(13)-H(13C)	0.9600
C(14)-H(14A)	0.9600	C(14)-H(14B)	0.9600
C(14)-H(14C)	0.9600	C(15)-H(15A)	0.9600
C(15)-H(15B)	0.9600	C(15)-H(15C)	0.9600

O(1)-S(1)-C(1)	105.0(3)	O(1)-S(1)-S(2)	112.4(2)
C(1)-S(1)-S(2)	93.12(17)	S(1)-S(2)-S(3)	100.16(9)
C(2)-S(3)-S(2)	94.48(17)	C(3)-O(2)-C(14)	114.9(3)
C(4)-O(3)-C(15)	118.5(4)	C(9)-O(5)-C(10)	122.1(4)
C(9)-N(1)-C(8)	122.3(4)	C(9)-N(1)-H(1B)	118.8
C(8)-N(1)-H(1B)	118.8	C(6)-C(1)-C(2)	120.2(4)
C(6)-C(1)-S(1)	119.3(4)	C(2)-C(1)-S(1)	120.3(4)
C(3)-C(2)-C(1)	120.7(4)	C(3)-C(2)-S(3)	119.1(3)
C(1)-C(2)-S(3)	120.2(4)	C(4)-C(3)-C(2)	120.3(4)
C(4)-C(3)-O(2)	122.1(4)	C(2)-C(3)-O(2)	117.6(4)
O(3)-C(4)-C(3)	117.2(4)	O(3)-C(4)-C(5)	123.6(5)
C(3)-C(4)-C(5)	119.1(5)	C(6)-C(5)-C(4)	121.4(5)
C(6)-C(5)-H(5A)	119.3	C(4)-C(5)-H(5A)	119.3
C(5)-C(6)-C(1)	118.1(4)	C(5)-C(6)-C(7)	118.2(5)
C(1)-C(6)-C(7)	123.7(5)	C(8)-C(7)-C(6)	109.7(4)
C(8)-C(7)-H(7A)	109.7	C(6)-C(7)-H(7A)	109.7
C(8)-C(7)-H(7B)	109.7	C(6)-C(7)-H(7B)	109.7
H(7A)-C(7)-H(7B)	108.2	N(1)-C(8)-C(7)	111.5(4)
N(1)-C(8)-H(8A)	109.3	C(7)-C(8)-H(8A)	109.3
N(1)-C(8)-H(8B)	109.3	C(7)-C(8)-H(8B)	109.3
H(8A)-C(8)-H(8B)	108.0	O(4)-C(9)-O(5)	125.6(5)
O(4)-C(9)-N(1)	124.0(4)	O(5)-C(9)-N(1)	110.3(4)
O(5)-C(10)-C(13)	112.0(7)	O(5)-C(10)-C(11)	103.0(5)
C(13)-C(10)-C(11)	118.5(8)	O(5)-C(10)-C(12)	110.3(5)
C(13)-C(10)-C(12)	107.9(8)	C(11)-C(10)-C(12)	104.7(6)
C(10)-C(11)-H(11A)	109.5	C(10)-C(11)-H(11B)	109.5
H(11A)-C(11)-H(11B)	109.5	C(10)-C(11)-H(11C)	109.5
H(11A)-C(11)-H(11C)	109.5	H(11B)-C(11)-H(11C)	109.5
C(10)-C(12)-H(12A)	109.5	C(10)-C(12)-H(12B)	109.5
H(12A)-C(12)-H(12B)	109.5	C(10)-C(12)-H(12C)	109.5
H(12A)-C(12)-H(12C)	109.5	H(12B)-C(12)-H(12C)	109.5

C(10)-C(13)-H(13A)	109.4	C(10)-C(13)-H(13B)	109.5
H(13A)-C(13)-H(13B)	109.5	C(10)-C(13)-H(13C)	109.5
H(13A)-C(13)-H(13C)	109.5	H(13B)-C(13)-H(13C)	109.5
O(2)-C(14)-H(14A)	109.5	O(2)-C(14)-H(14B)	109.5
H(14A)-C(14)-H(14B)	109.5	O(2)-C(14)-H(14C)	109.5
H(14A)-C(14)-H(14C)	109.5	H(14B)-C(14)-H(14C)	109.5
O(3)-C(15)-H(15A)	109.5	O(3)-C(15)-H(15B)	109.5
H(15A)-C(15)-H(15B)	109.5	O(3)-C(15)-H(15C)	109.5
H(15A)-C(15)-H(15C)	109.5	H(15B)-C(15)-H(15C)	109.5

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters [$Å^2$ x 10^3] for 1hf2.
The anisotropic displacement factor exponent takes the form:
$-2\pi^2$ [$(ha^*)^2U_{11}$ + ... + 2hka*b*U_{12}]

	U11	U22	U33	U23	U13	U12
S(1)	84(1)	80(1)	72(1)	7(1)	30(1)	1(1)
S(2)	118(1)	98(1)	111(2)	40(1)	34(1)	20(1)
S(3)	70(1)	95(1)	79(1)	22(1)	4(1)	11(1)
O(1)	146(4)	154(4)	105(4)	9(4)	16(3)	36(4)
O(2)	38(2)	79(2)	72(3)	-3(2)	5(2)	5(2)
O(3)	57(2)	99(3)	75(3)	28(2)	23(2)	20(2)
O(4)	62(2)	126(3)	70(3)	-7(2)	19(2)	21(2)
O(5)	46(2)	110(3)	47(2)	0(2)	11(2)	22(2)
N(1)	43(2)	82(3)	41(2)	1(2)	19(2)	7(2)
C(1)	51(3)	47(3)	52(3)	-1(2)	10(3)	1(2)
C(2)	50(3)	36(3)	60(3)	-5(2)	9(3)	-7(2)
C(3)	35(3)	51(3)	54(3)	0(3)	1(2)	9(2)
C(4)	47(3)	59(3)	58(3)	2(3)	14(3)	1(3)
C(5)	46(3)	68(4)	63(4)	8(3)	3(3)	6(3)
C(6)	44(3)	46(3)	65(4)	-10(3)	14(3)	-6(2)
C(7)	48(3)	58(3)	85(4)	-4(3)	17(3)	-9(3)
C(8)	49(3)	61(4)	59(3)	-6(3)	11(3)	0(3)
C(9)	53(3)	79(4)	49(3)	4(3)	24(3)	10(3)
C(10)	47(3)	108(5)	70(4)	15(4)	7(3)	20(3)
C(11)	47(6)	131(8)	94(9)	65(8)	41(6)	70(6)
C(12)	92(5)	186(8)	152(7)	-46(6)	-12(5)	-43(5)
C(13)	131(11)	214(14)	143(12)	162(11)	69(9)	108(10)
C(14)	60(4)	107(5)	90(5)	-12(4)	15(3)	19(3)
C(15)	55(7)	134(8)	72(8)	64(7)	50(6)	44(6)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$ x 10^3) for lhf2.

	x	y	z	U(eq)
H(1B)	2531	2489	7164	66
H(5A)	5310	1605	3795	71
H(7A)	3674	1202	4970	76
H(7B)	3747	914	6483	76
H(8A)	4282	2324	7104	68
H(8B)	4247	2616	5593	68
H(11A)	-365	3915	6149	135
H(11B)	-626	2923	6321	135
H(11C)	-1160	3455	5148	135
H(12A)	605	2121	3495	215
H(12B)	-576	2455	3499	215
H(12C)	-138	1890	4697	215
H(13A)	1284	3563	3168	243
H(13B)	1014	4326	4153	243
H(13C)	136	3967	3162	243
H(14A)	9476	-140	4018	128
H(14B)	8468	152	3186	128
H(14C)	8377	-597	4247	128
H(15A)	7183	1983	1349	130
H(15B)	6445	2399	2431	130
H(15C)	6150	1495	1796	130

Table 6. Torsion angles [°] for lhf2.

O(1)-S(1)-S(2)-S(3)	-77.1(2)
C(1)-S(1)-S(2)-S(3)	30.50(18)
S(1)-S(2)-S(3)-C(2)	-28.99(18)
O(1)-S(1)-C(1)-C(6)	-87.0(4)
S(2)-S(1)-C(1)-C(6)	158.9(4)
O(1)-S(1)-C(1)-C(2)	89.0(4)
S(2)-S(1)-C(1)-C(2)	-25.1(4)
C(6)-C(1)-C(2)-C(3)	2.6(7)
S(1)-C(1)-C(2)-C(3)	-173.4(3)
C(6)-C(1)-C(2)-S(3)	-178.9(3)
S(1)-C(1)-C(2)-S(3)	5.1(5)
S(2)-S(3)-C(2)-C(3)	-163.3(3)
S(2)-S(3)-C(2)-C(1)	18.1(4)
C(1)-C(2)-C(3)-C(4)	0.8(7)
S(3)-C(2)-C(3)-C(4)	-177.7(4)
C(1)-C(2)-C(3)-O(2)	177.6(4)
S(3)-C(2)-C(3)-O(2)	-0.9(6)
C(14)-O(2)-C(3)-C(4)	-69.8(6)
C(14)-O(2)-C(3)-C(2)	113.4(5)
C(15)-O(3)-C(4)-C(3)	177.7(5)
C(15)-O(3)-C(4)-C(5)	1.1(7)
C(2)-C(3)-C(4)-O(3)	179.2(4)
O(2)-C(3)-C(4)-O(3)	2.5(7)
C(2)-C(3)-C(4)-C(5)	-4.0(7)
O(2)-C(3)-C(4)-C(5)	179.3(4)
O(3)-C(4)-C(5)-C(6)	-179.4(5)
C(3)-C(4)-C(5)-C(6)	4.0(7)
C(4)-C(5)-C(6)-C(1)	-0.7(7)
C(4)-C(5)-C(6)-C(7)	178.0(4)
C(2)-C(1)-C(6)-C(5)	-2.6(7)
S(1)-C(1)-C(6)-C(5)	173.4(4)
C(2)-C(1)-C(6)-C(7)	178.8(4)
S(1)-C(1)-C(6)-C(7)	-5.2(6)
C(5)-C(6)-C(7)-C(8)	-81.3(6)
C(1)-C(6)-C(7)-C(8)	97.4(5)
C(9)-N(1)-C(8)-C(7)	-78.4(6)
C(6)-C(7)-C(8)-N(1)	178.3(4)
C(10)-O(5)-C(9)-O(4)	6.7(8)
C(10)-O(5)-C(9)-N(1)	-176.0(4)
C(8)-N(1)-C(9)-O(4)	5.8(8)
C(8)-N(1)-C(9)-O(5)	-171.6(4)
C(9)-O(5)-C(10)-C(13)	-57.1(9)
C(9)-O(5)-C(10)-C(11)	174.4(6)
C(9)-O(5)-C(10)-C(12)	63.2(6)

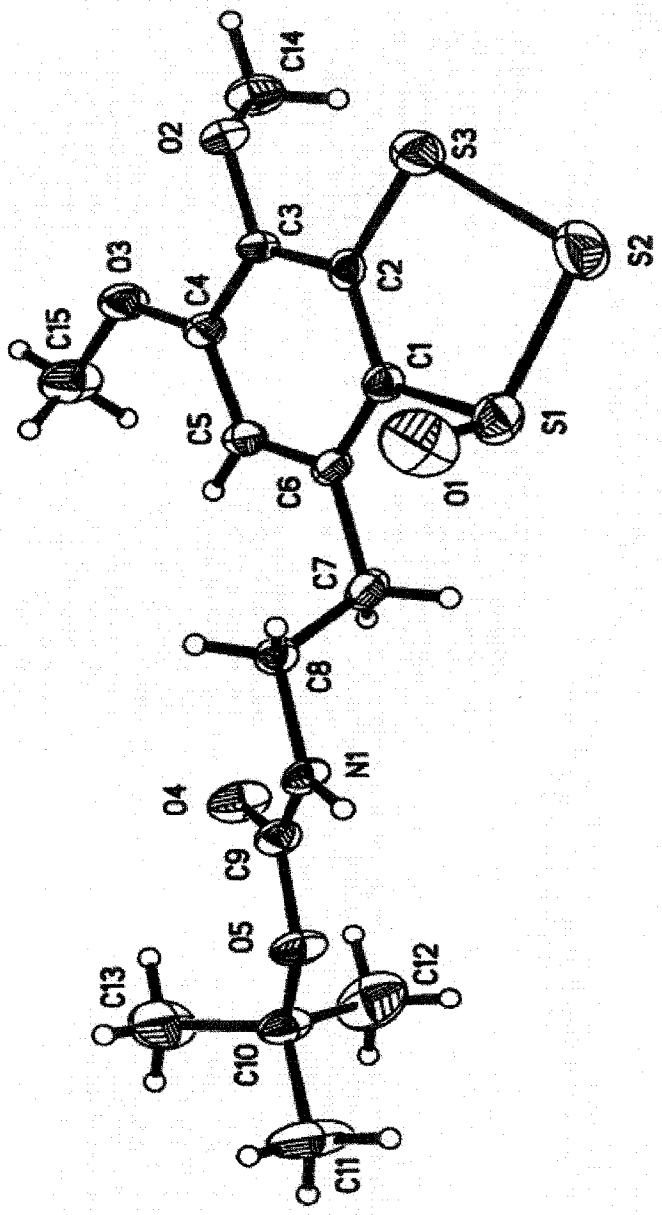
Symmetry transformations used to generate equivalent atoms:

Table 7. Hydrogen bonds for 1hf2 [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N(1)-H(1B)...O(4)#1	0.86	1.99	2.810(5)	158.8

Symmetry transformations used to generate equivalent atoms:

#1 x,-y+1/2,z+1/2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O2
S3
C3
S2
O3
C4
C2
C15
C1
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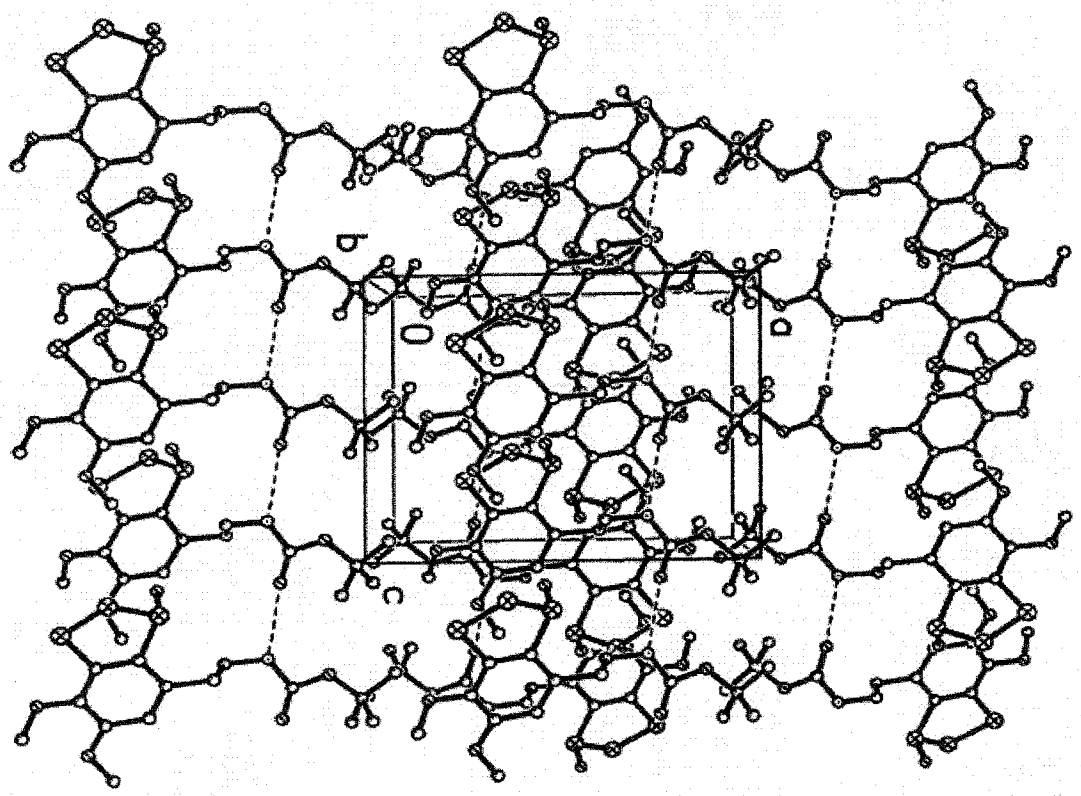
N1
O4
C9
O5
C12
C13
C10
C11


b
O
a
c

LIST of SELECTED PUBLICATIONS DURING Ph. D. STUDY (Searchable on SciFinder Scholars)

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5. Lee, Alex H. F.; Chan, Albert S. C.; Li, Tianhu. **Benzotrithiole 2-oxide: A new family of thiol-Activated DNA-Cleaving functionalities.** Bioorganic & Medicinal Chemistry Letters (2002), 12(22), 3259-3261. CODEN: BMCLE8 ISSN:0960-894X. AN 2002:808829 CAPLUS

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9. Chen Jian; Liu Dongsheng; Lee Alex H F; Qi Jianying; Chan Albert S C; Li Tianhu **G-quadruplex as a new class of structural entities for directing the formation of circular oligodeoxyribonucleotides.** Chem Commun (Camb) (2002 Nov 21), (22), 2686-7. Journal code: 9610838. ISSN:1359-7345. DN 22398946 PubMed ID 12510299 AN 2003005148 MEDLINE

10. Lee Alex H F; Chen Jian; Liu Dongsheng; Leung Thomas Y C; Chan Albert S C; Li Tianhu **Acid-promoted DNA-cleaving activities and total synthesis of varacin C.** JOURNAL OF THE AMERICAN CHEMICAL SOCIETY (2002 Nov 27), 124(47), 13972-3. Journal code: 7503056. ISSN:0002-7863. DN 22329125 PubMed ID 12440877 AN 2002681020 MEDLINE

11. Lee Alex H F; Chan Albert S C; Li Tianhu **Benzotrithiole 2-oxide: a new family of thiol-activated DNA-cleaving functionalities.** BIOORGANIC AND MEDICINAL CHEMISTRY LETTERS (2002 Nov 18), 12(22), 3259-61. Journal code: 9107377. ISSN:0960-894X. DN 22280384 PubMed ID 12392727 AN 2002634509 In-process for MEDLINE

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pBR322

NEW ENGLAND BioLabs Inc.

4,361 base pairs
GenBank Accession #: J01749
See page 132 for ordering information

pBR322 is an *E. coli* plasmid cloning vector containing the origin of replication from pMB1 (a plasmid in the ColE1 compatibility group; 1–4). The *rop* gene product, which regulates plasmid replication by stabilizing the interaction between RNAI and RNAII transcripts, maintains the copy number at about 25 per cell. However, pBR322 can be amplified with chloramphenicol or spectinomycin (4).

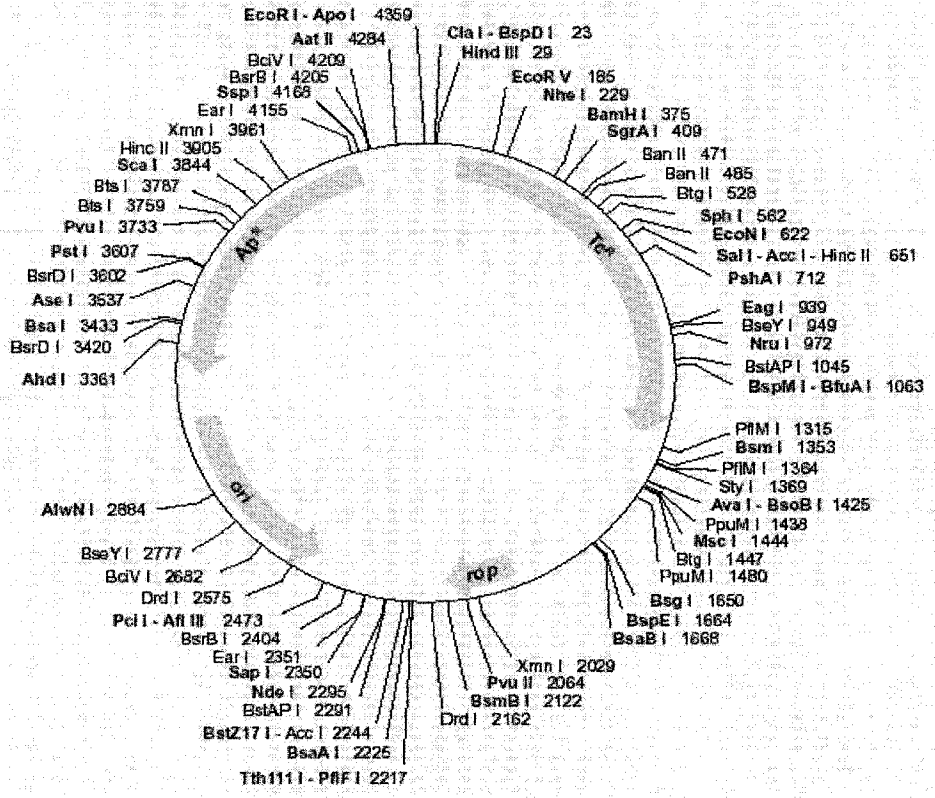
Enzymes with unique restriction sites are shown in **bold** type and enzymes with two restriction sites are shown in regular type. The accompanying table shows restriction sites of these enzymes that cut a moderate number of times. Restriction site coordinates refer to the position of the 5′-most base on the top strand in each recognition sequence.

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

Origin of replication coordinates include the region from the –35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. *bla* (Ap^R) gene coordinates include the signal sequence.

Feature	Coordinates	Source
tet (Tc^R)	86–1276	pSL101
bla (Ap^R)	4153–3296	Tn3
rop	1915–2106	pMB1
origin	3122–2534	pMB1

ori = origin of replication
Ap = ampicillin, Tc = tetracycline



References

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2. Sutcliffe, J.G. (1979) *Cold Spring Harb. Symp. Quant. Biol.* 43, 77–90.
3. Watson, N. (1988) *Gene* 70, 399–403.
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ϕX174

NEW ENGLAND BioLabs Inc.

5,386 base pairs
GenBank® Accession # NC_01422
See page 133 for ordering information

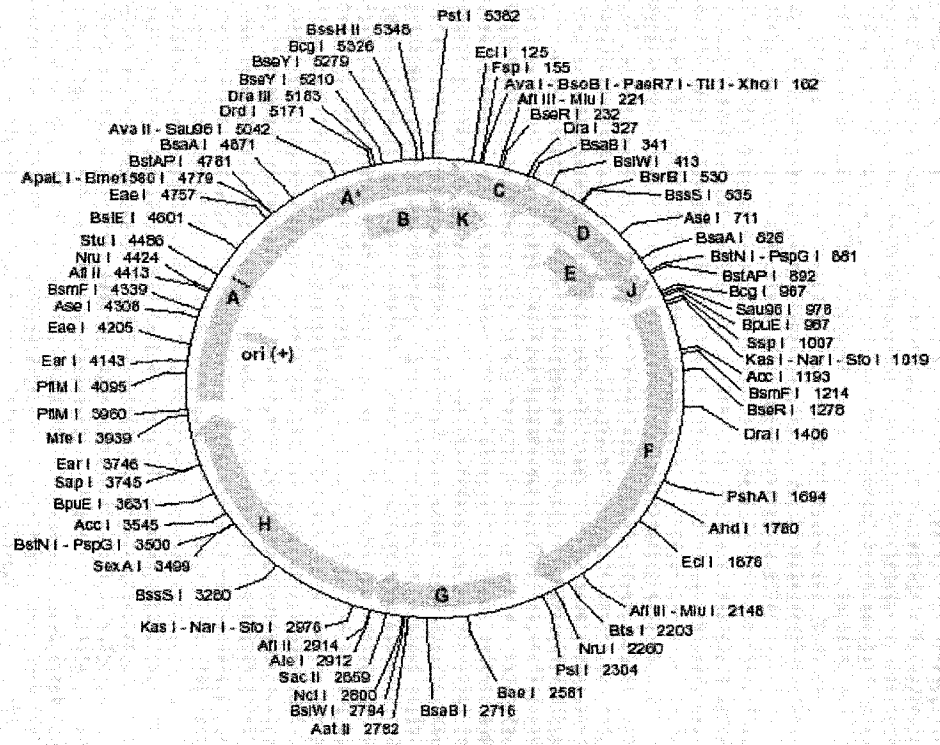
ϕX174 is a small *E. coli* bacteriophage with a circular, single-stranded DNA genome coding for 11 proteins (1,2). Double-stranded forms of the molecule (RFI and RF II) arise during DNA replication. The DNA strand in the virion, termed the plus strand, is shown below. Nucleotide numbering begins at the first G in the unique Pst I site (...CTGCAG...).

Enzymes with unique restriction sites are shown in **bold** type and enzymes with two restriction sites are shown in regular type. The accompanying table shows restriction sites of those enzymes that cut a moderate number of times. Restriction site coordinates refer to the position of the 5′-most base on the top strand in each recognition sequence.

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

Feature	Description	Coordinates
ori	origin of plus strand replication	4306
gene A	RF replication	3981-136 (cw)
gene A*	shut off host DNA synthesis	4497-136 (cw)
gene B	capsid morphogenesis	5075-51 (cw)
gene C	DNA maturation	133-358
gene D	capsid morphogenesis	359-848
gene E	cell lysis	568-843
gene F	major coat protein	1001-2284
gene G	major spike protein	2395-2922
gene H	minor spike protein, absorption	2931-3917
gene J	core protein; DNA condensation	848-1061
gene K	function unknown	51-221

(cw) = clockwise



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