SYNTHETIC STUDIES TOWARD BORON-CONTAINING NUCLEOSIDES

By

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SYNTHETIC STUDIES TOWARD
BORON-CONTAINING NUCLEOSIDES

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PREFACE

The purpose of this study is the investigation of synthetic routes toward boron-containing thymidine and uridine analogues that have potential for acting as substrates for DNA polymerases to be incorporated into replicating DNA.

Three methods of investigation were utilized in the search for boron-containing thymidine and uridine analogues. The first investigation involved the coupling and cyclization of a boronate and a urea. Model studies involving benzylurea were used to investigate reaction conditions for the coupling but led to the decomposition and rearrangement of the boronate. The second investigation entailed using thiophene as a synthetic backbone in the preparation of precursors to thymidine analogues. The trigonal boron atom in 2-benzyl-1,3-dihydro-1-hydroxythieno[3,2-*c*][1,5,2]diazaborin-3(2*H*)-one, the thiophene-based precursor, was shown to be stable in a variety of solvent environments. The third investigation employed asymmetric synthesis of boronic esters in the preparation of boron-containing acyclic precursors to uracil. This method continues to be investigated for its applications in the synthesis of boron-containing nucleoside analogues.

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to Dr. Steven M. Graham for his advice and encouragement during my years at Oklahoma State University. Without his support and never-ending ideas this research could not have been possible.

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Additional thanks need to be extended to Dr. Donald S. Matteson, along with the faculty and staff of Washington State University's chemistry department, for the opportunity to continue research as part of the degree process.

Special thanks go to my family and friends for their determination and strength which have become so infectious over the past few years, especially, to my parents who have walked every step of the way on this project. Also, thanks to Heather, Eric and Craig for being my never-ending sources of encouragement.

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CHAPTER ONE

INTRODUCTION

Design and Significance of Boron-Containing Nucleoside Analogues

The projects presented within this document were pursued at Oklahoma State University and Washington State University under the direction of Steven Graham and Donald Matteson. Though geographically separated, the visions of the advisors for the projects were quite similar. The initial goal was to synthesize boron-containing nucleoside analogues which have unexplored potential as inhibitors of HIV, as anticancer agents, and as tools to study the basic mechanisms of DNA (deoxyribonucleic acid) replication, alkylation and repair.

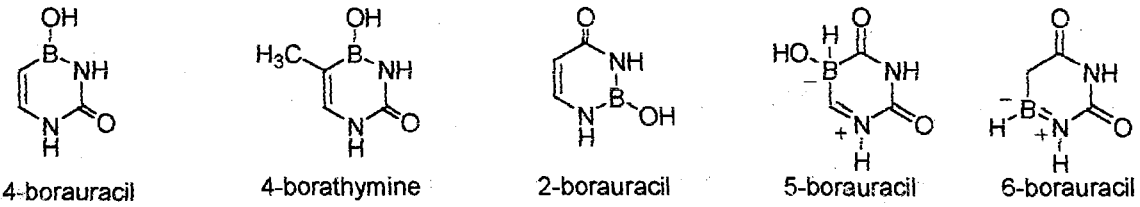
Because there are differing beliefs about the properties of these molecules, this initial discussion will present these beliefs with the understanding that they are merely speculative. In addition, the two projects were first presented with different ideas as to the eventual use of the boron-containing nucleoside analogues. Therefore, the potential uses of boron-containing nucleoside analogues will also be discussed in some detail.

Experimental Background

Over 40 years ago, Dewar established a class of compounds, designated as borazaro, which includes 4-borauracil and 4-borathymine.[1,2] These boron analogues of uracil and thymine (Figure 1) are the only analogues expected to have some stability toward water. The 2-isomer has two hydrolytically unstable B-N bonds[3] making it useless for the

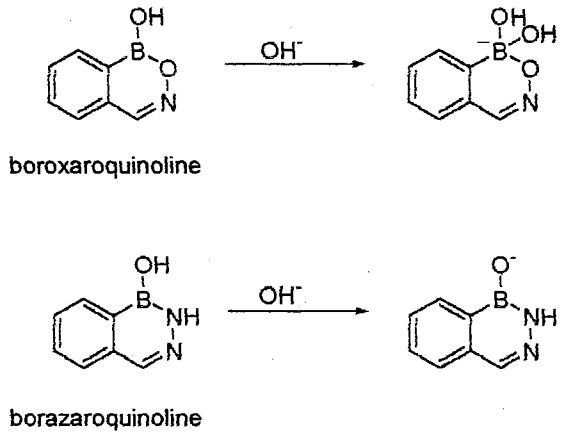
applications proposed for boron-containing nucleoside analogues. The 5- and 6-isomers are not allowed due to valence rules.

Figure 1. Boron Analogues of Uracil and Thymine.



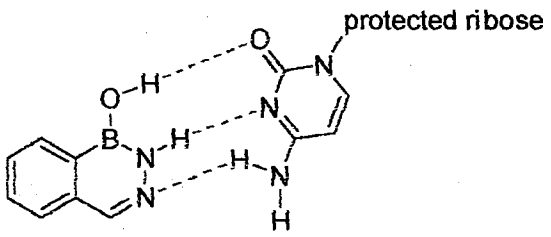
With the predicted stability of 4-borauracil and the similar compound 4-borathymine, these two compounds became the targets of the syntheses in the following chapters. In spite of the predicted stability of 4-borauracil, several attempts to synthesize this compound by different research groups have failed over the last 35 years.[4,5]

Figure 2. Reactions of Boron Heterocycles with Base.



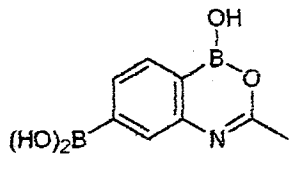
Though attempts to prepare 4-borauracil have been unsuccessful, there is some precedent for the synthesis of boron-containing heterocycles. Dewar and coworkers prepared boroxaroquinoline and borazaroquinoline (Figure 2).[2] These two compounds react very differently when exposed to hydroxide ion, as evidenced by the ^{11}B NMR. Boroxaroquinoline reacts with the hydroxide ion at the boron to form the tetracoordinate borate but the more aromatic borazaroquinoline loses a proton from the hydroxyl making it analogous to phenol (Figure 2).[6,7] Groziak *et al.* later proved that these two analogues maintain planar, aromatic structures in aqueous solution using NMR. They were also able to demonstrate that hydrogen bonding occurs between borazaroquinoline and a cytidine derivative (Figure 3).[8]

Figure 3. Hydrogen Bonding Pattern of Borazaroquinoline and a Cytidine Derivative.

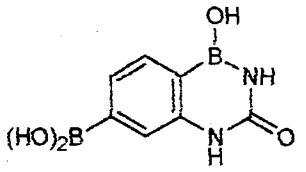


Groziak and coworkers also synthesized other benzo-fused heterocycles and found that these compounds tended to hydrate in water.[9] Soloway also recently achieved the synthesis of benzoborauracil and showed that it is stable in water. To date, benzo-fused heterocycles of varying stability (Figure 4)[9-11] have been prepared, but the search continues for a way to synthesize borauracil or borathymine without the added aromatic support. The work presented in the following chapters is simply a continuation of this 35-year old search.

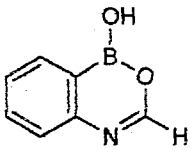
Figure 4. Structures of Known Benzo-fused Boron Heterocycles.

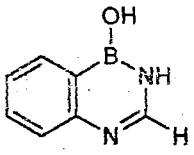


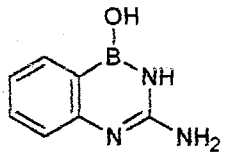
Stable to boiling 15% KOH or conc. HCl >2 hrs



Recrystallized from water







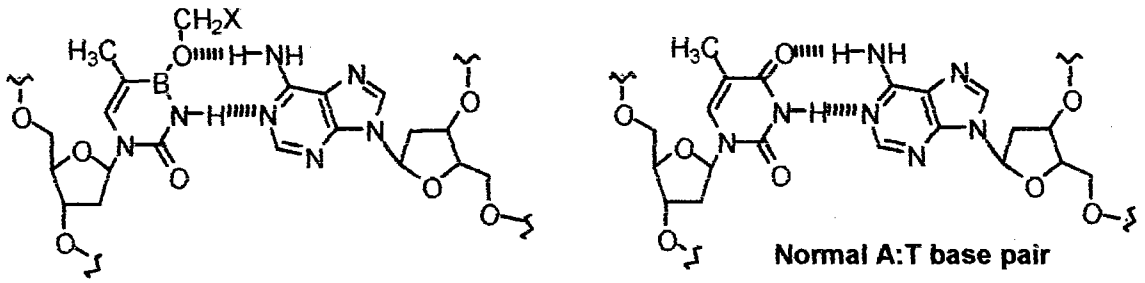
Desired Properties of Boron-Containing Nucleoside Analogues

Once the boron analogues of uridine and thymidine are synthesized, the potential for these compounds to substantially mimic the natural molecules must be explored. The first step would be to study whether or not the nucleosides could become phosphorylated to their corresponding nucleotides. The simplest method for predicting phosphorylation is to use affinity chromatography, where the interaction of immobilized nucleosides with kinases is explored. Even though this technique may be a useful indicator as to whether or not the reaction takes place, based on the accessibility and binding of the nucleosides to the active sites of the kinases, it may not be a realistic representation of the rate of the reaction.[12]

The second step would be to investigate hydrogen bonding possibilities with natural nucleobases using nuclear magnetic resonance. If hydrogen bonding could be shown to occur with one of the natural nucleobases in the normal Watson-Crick hydrogen bonding pattern, there would be a greater potential for these compounds to be incorporated into

replicating DNA. This property is subject to debate and may be based on the boron substitution. Because of the odd valence of boron, borauracil without other substituents is not expected to maintain the Watson-Crick type hydrogen bonding. However, it is believed that the substitution of an alkyl or electrophilic alkyl group on the borinate may maintain the original hydrogen bonding pattern of the normal base (Figure 5). Any of these molecules that could be prepared would have potential as a drug for antiviral or anticancer treatment or as a chemical probe for studying the mechanisms associated with DNA.

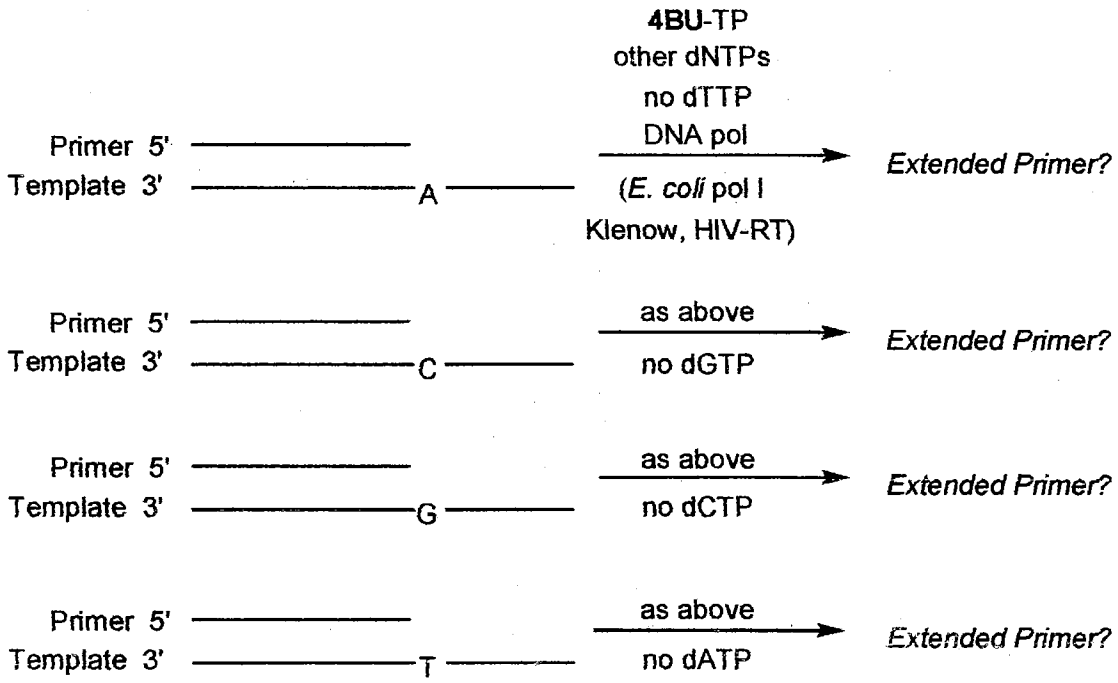
Figure 5. Proposed Hydrogen Bonding Pattern for the Alkyl Form of Borauridine.



For some of the potential uses of boron-containing nucleoside analogues, it would be beneficial for the triphosphates of the boron-containing nucleoside analogues to be used by DNA polymerases (*E. Coli* pol I, Klenow, bacteriophage T4, HIV reverse transcriptase) during replication. Preliminary studies to determine whether these compounds act as substrates would make use of oligomeric DNA templates annealed to short radiolabeled complementary primers.[13] Incubation of the template-primer pair would then be carried out in the presence of three of the four natural deoxynucleotides and the analogue (Figure 6). Repeating this experiment with all four combinations would provide information as to the extent of incorporation into DNA or disruption of DNA synthesis in the presence of the

analogue. Extension of the growing DNA strand up to and including the missing natural nucleotide implies that the analogue is able to mimic the missing nucleotide and establishes which base is complementary to the analogue. If extension continues beyond the unique nucleotide, it is implied that the analogue does not block DNA synthesis.[14,15]

Figure 6. Primer Extension Studies for Analogues with DNA Polymersases.

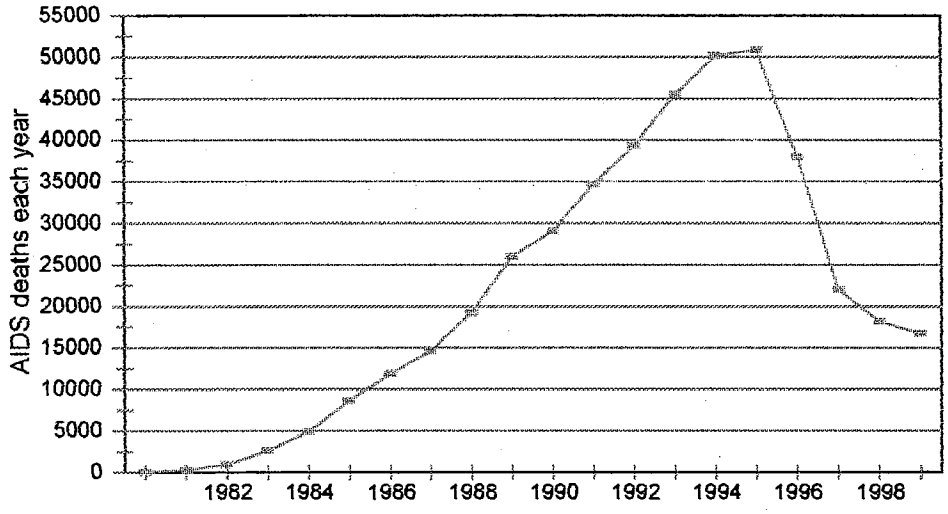


HIV History

When the original proposals for the research presented here were submitted, astonishing statistics had just been released by the Center for Disease Control, causing a stir in the research community. In 1995, the announcement that HIV (Human Immunodeficiency Virus) was no longer just another epidemic but now the leading cause of death for those aged 25-44 in the United States came as a shock. We were unable to

comprehend how such a disease could rise from non-existence to major killer within a fifteen-year span (Figure 7).[16] Around that same time, continuing research with HIV showed

Figure 7. AIDS Deaths from 1980 to 1999 in the United States.



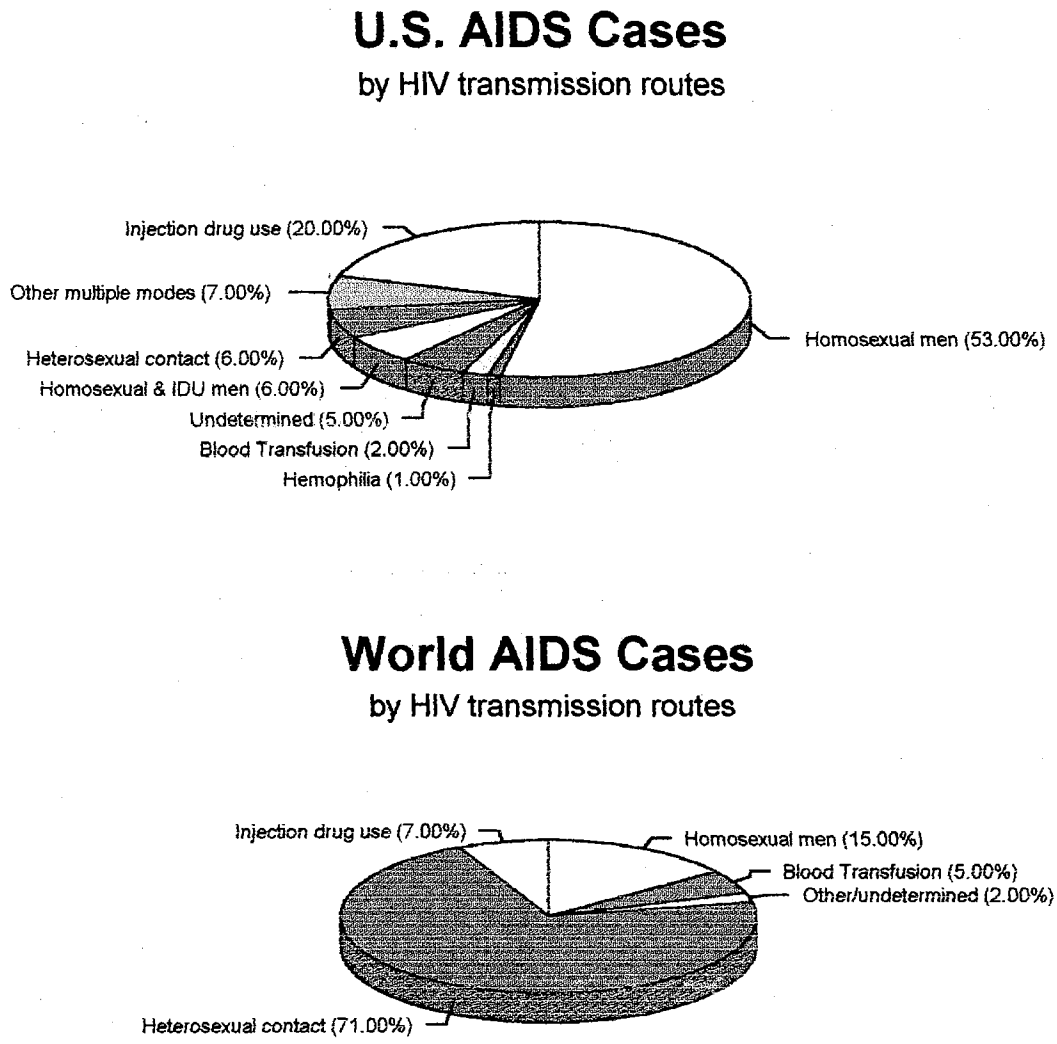
that the virus does not lie dormant during the apparent latent phase but is continually replicating at a rate of more than one billion times per day.[17] These two statements, made to the public, renewed the push to find a cure for the newest plague of our society. Educators and health care professionals took on the challenge of informing the public as to how to help control the transmission of the disease. Scientists began searching for new and improved ways to treat and conquer the virus that was plaguing the United States.

The first cases of AIDS (Acquired Immune Deficiency Syndrome) in the United States appeared in five young, previously healthy, homosexually active men in Los Angeles in the spring of 1981. These men were first diagnosed with a rare upper respiratory infection, *Pneumocystis carinii* pneumonia, that later became linked to a reduced ability to

fight off even the simplest infections, such as the common cold.[18-20] Within one month, 10 other cases of the same rare infection were identified in addition to 26 cases of Kaposi's sarcoma, a rare cancer of the connective and vascular tissue.[21] By December of 1981, there had been 257 cases of AIDS identified and reported to the Center for Disease Control.[19,22] This initial growth statistic seems overwhelming until we look more carefully at the rates of infection, progression to AIDS, and death within the United States. Only then is the full power of the disease unveiled.

Since the appearance of HIV in the United States, there has been an ongoing search to identify the initial mode of infection and rapid transmission of the virus. With the initial cases being identified in homosexual males, focus was first placed on this particular lifestyle. We now know that the main mode of transmission is through sexual contact, whether homosexual or heterosexual. In fact, depending on the part of the world being considered, these two modes of transmission may be interchanged as the most prevalent route of infection (Figure 8).[23]

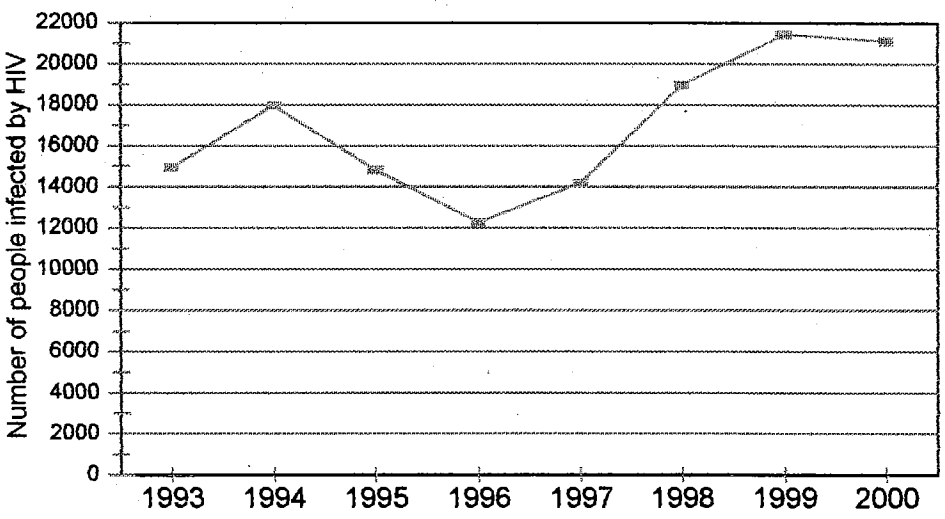
Figure 8. Comparison of HIV Transmission Routes for the United States and the World.



In 1995, a new case of HIV was reported every fifteen seconds, more than 5000 per day in the world.[24] As if this weren't shocking enough, someone dies from AIDS every 30 minutes. At that time, an estimated 216,000 people were living with AIDS in the United States.[25] Though it was thought that the rate of infection would dramatically decrease as a result of the educational programs (Figure 9), this trend was not seen in the next five years. Due, in part, to a push for people in high risk categories to be tested for HIV, an unexpected

up-turn in infection was seen rather than a decline during this period. As a result of continued infection, at the end of 2000 there were an estimated 313,000 people living with AIDS in this country alone.[26] Obviously, the epidemic is as real today as it was in the 1980s when scientists and physicians were first figuring out how to identify HIV/AIDS as anything other than the opportunistic diseases that afflict people infected with HIV.

Figure 9. Annual HIV Infections Reported within the United States from 1993 to 2000.



In addition to the fear of becoming infected with HIV, with 313,000 people living with HIV/AIDS in the United States, the public health system will soon be stressed beyond its available resources at the expense of others in the community. Unfortunately, with the cost of medical treatment, infected people will not be able to pay for care out of their own resources. The estimate for necessary medical care to protect against opportunistic diseases from HIV infection to death is anywhere from $119,000 to 147,000 for one infected person.[27,28] Of this, half of the expenses ($62,000 to 73,000) are associated with treatment

from the onset of AIDS to death,[27,28] a 12- to 15-month span in most cases. The hope of finding a cure is not only for those suffering from HIV/AIDS but for the rest of society.

Viral Cycle of HIV

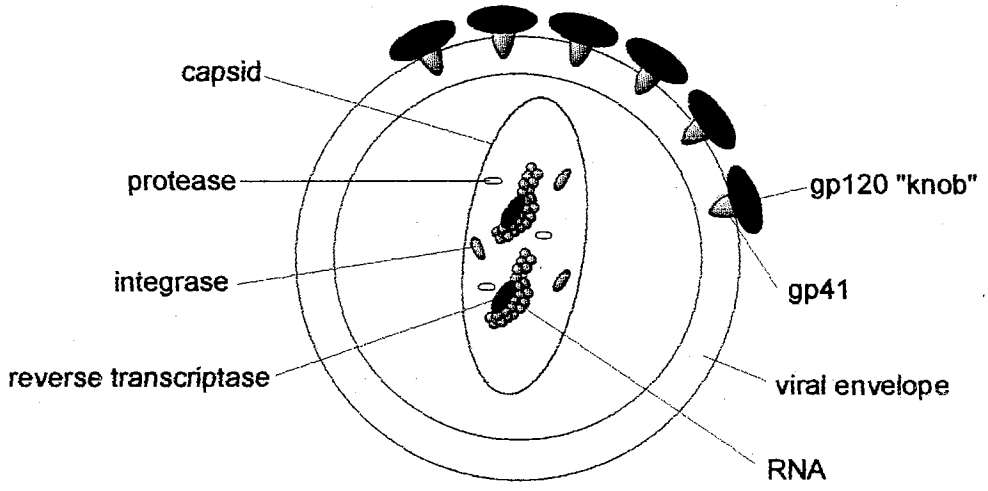
HIV belongs to a group of retroviruses known more specifically as cytopathic lentoviruses. Lentoviruses are able to integrate their viral genetic material into a host cell's genetic material through reverse transcription. The use of reverse transcription is the one feature that distinguished retroviruses from other viruses. Though there are several retroviruses known to man, focus has been placed on two main types in recent years. The first, Human T-cell Lymphotropic Virus type-I (HTLV-I) and HLTV-II, causes unrestrained growth and proliferation of white blood cells, which eventually leads to the onset of leukemia. The second, HIV-1 and HIV-2, causes a profound deterioration of the immune system by the loss and dysfunction of T-helper lymphocytes.[23] Since these two types of viruses share T-helper cells as a common pathogenic target, research leading to the suppression of one may be beneficial for the treatment of the other. For this reason, little emphasis will be placed on HTLV itself within this document.

HIV targets the human body at cells of the central nervous system and the immune system giving rise to weakened immune defenses. Though lay people in the discussion of the plague of our lifetime frequently interchange HIV and AIDS, these two acronyms apply to very different stages of the viral cycle. In fact, AIDS may never develop as a result of HIV infection. Long-term nonprogressors, people who survive with HIV for more than 10 to 15 years and show no signs of HIV disease, remain a mystery to scientists. Speculations as to what protects these individuals range from extraordinary killer T-cells effective against

the virus to infection by a weak strain of the virus and even genetics. In terms of genetics, the focus becomes the presence of certain receptors. Binding of HIV involves the CD_4 molecule and CCR-2, CXCR-4 or CCR-5 coreceptors. Evidence indicates that people who inherit two mutated genes for the CCR-5 receptor may be highly resistant to HIV infection while those who inherit only one mutated copy tend to be long-term nonprogressors.[29]

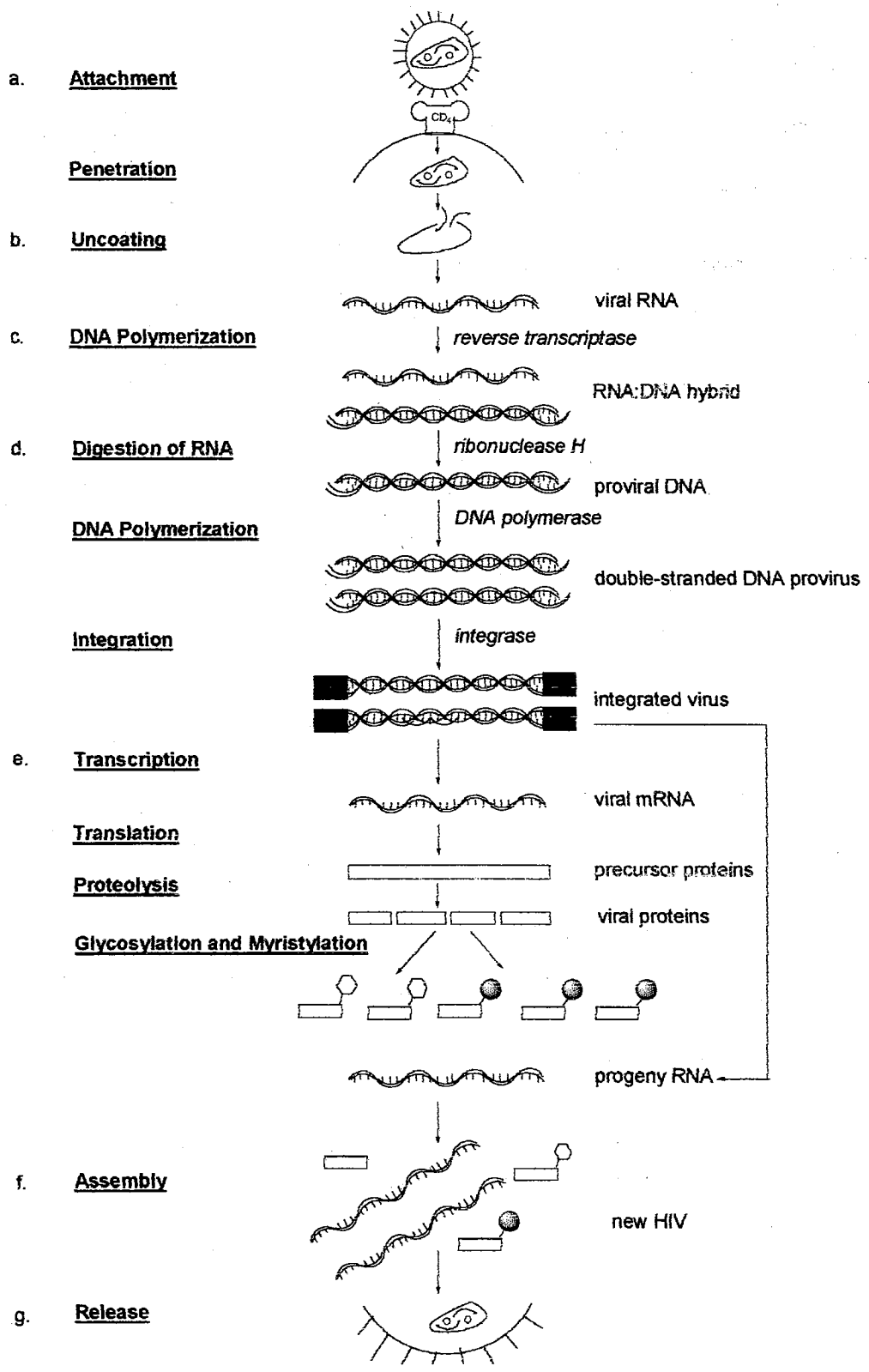
Upon infection, HIV seeks to attach itself to T-helper lymphocytes. The virions, which look like small balls covered with suction cups (Figure 10),[29] use their envelope

Figure 10. Schematic of a Mature HIV Virion.



surface protein, gp120, to attach to the outside of the membrane encasing the T-helper cells at the CD_4 (cluster determinant 4) receptor (Figure 11a). The CD_4 receptor is the gate for all infectious agents[30] and must be present for the infection of the T-helper cell by HIV to

Figure 11. Replication Cycle of HIV from Infection to the Formation of New Virions.



occur. Once the virus has entered the host cell, the envelope protein is stripped from the virion (Figure 11b) and the viral RNA is transcribed into DNA by reverse transcriptase (Figure 11c), an enzyme specifically designed to copy RNA into DNA. The new retroviral DNA is then integrated into the genetic material of the host cell forming proviral DNA (Figure 11d).[30,31] With this insertion, each time the host cell transcribes its DNA into messenger-RNA a new retrovirus (viral mRNA) is also made.[23,32]

The newly-transcribed viral mRNA produces the structural proteins, envelope proteins, regulatory proteins and RNA necessary for the production of HIV (Figure 11e).[19,33] The new viral RNA is then encapsulated as the core of the newly produced proteins in the assembly of new HIV (Figure 11f).[34,35] Once the assembly of the virion is complete, the replicated virus particles bud and are released from the host cell, killing the host cell in the process (Figure 11g).[23,32]

There are many phases that occur with the infection of T-helper cells by HIV. The first period is very brief but intense in terms of the amount of viral replication that occurs.[36] During this early period, HIV multiplies and disseminates rapidly causing massive quantities of virus (viremia) to be present throughout the body. This phase of mass production is followed by a latent phase. It was originally thought that HIV particles were actually dormant during this time. In 1995, research by Coffin *et al.* indicated that the virus was not hibernating during this time but hiding within the T-helper cells, inserting its genetic material into the host cell's DNA, and constantly

replicating.[17] The "silent" infection that takes place during this time is not synonymous with asymptomatic periods that occur throughout the progression of HIV infection.[37-39] The difference between these two states is the detection of the virus by the body's immune

system. During the "silent" infection, the body is unable to identify and fight the hidden virus, while during asymptomatic periods the body is able to fight the virus just enough for the person to seem healthy.

The dormant HIV particles that have been hiding in the T-cells are triggered by other opportunistic viruses, such as herpes simplex, hepatitis B, HTLV, or Epstein-Barr.[34,40] The attack of these viruses seems to weaken the immune system further and cause the synthesis of proteins and assembly of new HIV to be initiated.[20,30] Once the T-helper cell has been overwhelmed by the growing virus, the new virus particles bud from the host cell, destroying a small piece of the immune system in the process.[39,41] The destruction that occurs can happen on many levels. The first is the straightforward destruction of the host cell by breaking open the outer membrane of the cell. The second occurs when the virus sheds fragments of envelope protein gp120, which then adhere to CD_4 surface molecules of uninfected, neighboring T-helper cells. This attachment of gp120 to CD_4 receptors causes the formation of profoundly damaged cells (syncytia), in this case a multinucleated clump of functionally incapacitated cells,[30,39,42] and the subsequent immune response to destroy these otherwise healthy cells.[43] The third occurs when unintegrated HIV DNA interferes with the fundamental function of T-helper cells.[34] In addition to these specific methods, several other means by which HIV destroys cells have been well established.[43]

Disrupting Replication

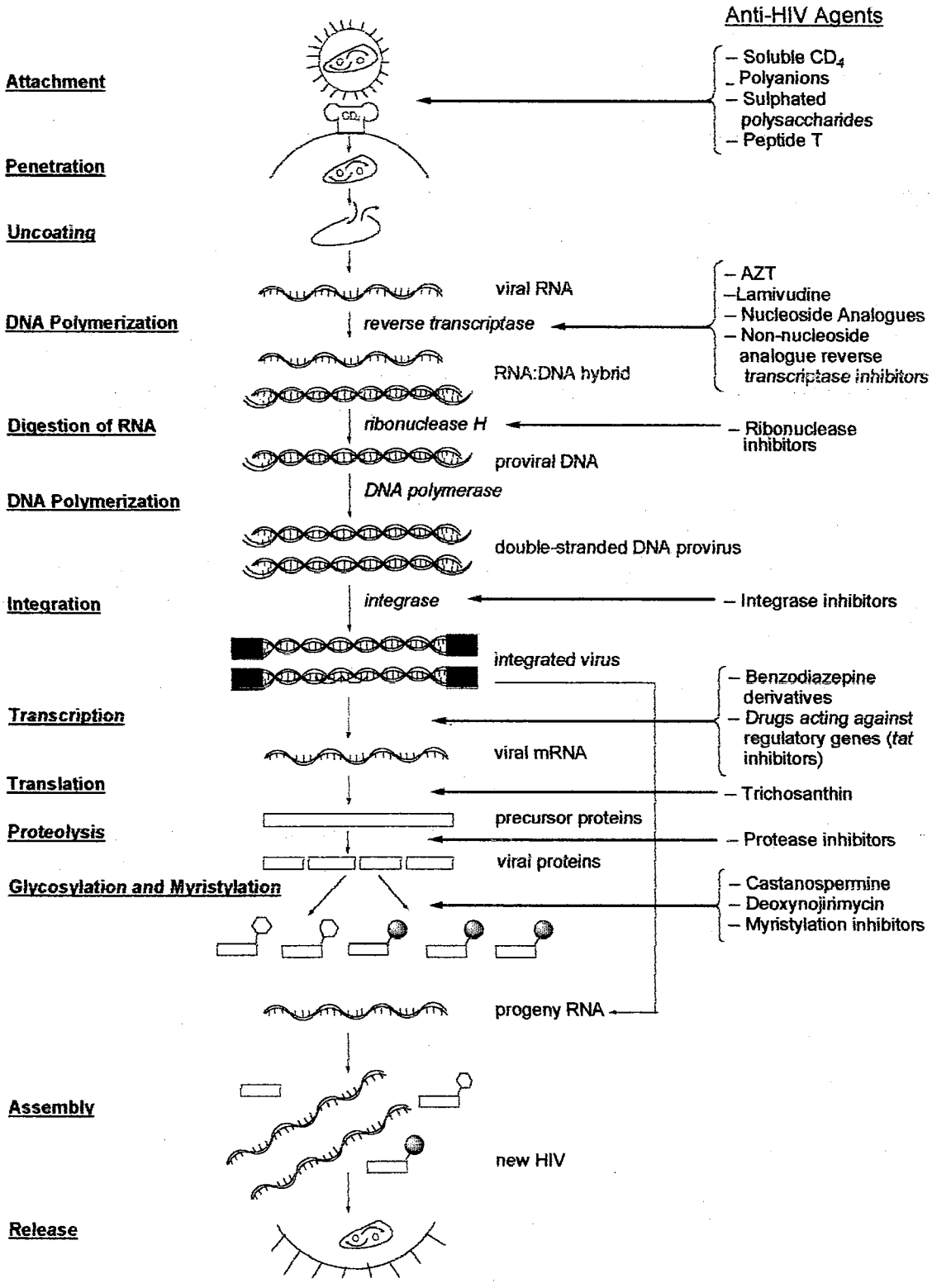
When a virus is defined, care must be taken to distinguish the status of the virus inside a cell from that of the virus outside a cell. Inside the cell, the virus is actively

multiplying while on the outside it appears to be merely another chemical. Therefore, research targets processes within the cell for the development of antiviral agents.

As seen previously, there are many steps involved in the replication of HIV. Each step along the path could be targeted by antiviral drugs (Figure 12)[32] designed to interfere with the replication cycle. For example, amantadine, a drug used to treat an influenza A infection, prevents the virus from ever entering the host cell.[32] Many antiviral drugs, including the herpes drug, acyclovir, interfere with the replication of the nucleic acid.[32] The other main target for antiviral agents occurs later in the cycle when genetic material of the progeny are assembled and released. Interferon, an agent produced by the body, as well as synthetically, affects many viruses at this later stage.[32]

Theoretically, numerous targets exist for the development of drugs that could be selectively active against HIV. Since the hope would be to target a step in the replication process that will distinguish the virus from the host cell, reverse transcription has become the target of most drug development. Reverse transcription is the ideal process because this step does not occur in the host cells while retroviruses rely on this process for multiplication. Therefore, targeting reverse transcription, more specifically the enzyme reverse transcriptase, should have little toxicity to host cells. Unfortunately, the drugs currently in use all have significant toxicity so the search continues for non-toxic agents.

Figure 12. Anti-viral Drugs Tested Against HIV.



DNA Polymerase Inhibition

One strategy for disrupting replication is the use of a boron-containing nucleoside analogue to inhibit DNA polymerases, including HIV reverse transcriptase. This property may be independent of the inclusion of boron-containing nucleoside analogues into replicating DNA but is tested in a manner similar to that previously described. All four natural deoxynucleotides and the analogue are incubated with the template-primer pair in the presence of a DNA polymerase. If the DNA polymerase preferentially incorporates the natural deoxynucleotides, incomplete or decelerated extension of the primer implies that the boron-containing nucleoside analogue acts as an inhibitor of the polymerase.[44] Of course, these studies could easily be repeated to determine the kinetic and equilibrium parameters, K_m and k_{cat}.

Alkylation of DNA

Alkylation-induced damage of DNA is a common strategy for disrupting replication in the treatment of cancer and may be applicable to the treatment of viruses.[45-48] Unfortunately the alkylating drugs (mitomycin C, nitrogen mustards, nitrosoureas) suffer from the inability to distinguish replicating from non-replicating DNA and deactivation of the drugs by reaction with non-DNA cellular nucleophiles.

The damage caused by the inability of the drugs to distinguish a diseased cell from a healthy cell has been implicated in mutagenesis within the healthy cell and its progeny.[48-51] The changes in DNA, as a result of alkylation, are manifested as either alteration of the Watson-Crick hydrogen bonding pattern or mutations within the genetic material of the cell.

The observed mutation is often the result of continued replication in which a mismatched base is incorporated opposite the alkylated base.[50,51]

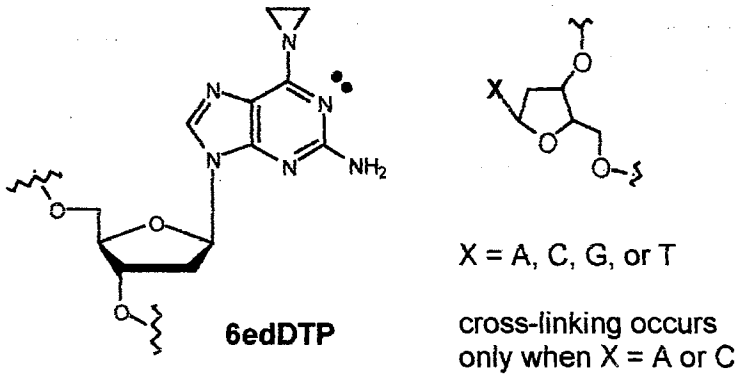
Mismatched-induced mutagenesis can potentially be avoided in two ways. First, a double alkylation may be introduced to form an interstrand cross-link that would hinder mutations by suppressing the separation of DNA necessary for subsequent replication. A cross-link of this sort also makes DNA resistant to denaturation and *in vivo* repair. Second, alkylations that result in the maintenance of the natural hydrogen bonding patterns will be less mutagenic if the alkylation does not lead to an interstrand cross-link.[48,52]

DNA Cross-linking

Highly reactive DNA cross-linking agents can be controlled by combining the alkylating agent with an oligonucleotide in order to create a site-specific cross-linking agent.[14,47,53-59] If the alkylating functionality can be properly placed so as to have a complimentary base trigger cross-linking upon hybridization of duplex DNA, reactivity may also be modulated.[14,56,57] While these two approaches have been studied and were successful in forming site-specific interstrand cross-links, very few investigations have been carried out using replicating DNA, implying that little is known about the association of the agents with DNA polymerase and the involved kinases.

A study carried out by Cowart and Benkovic,[14] in which the nucleoside analogue, 6edDTP (Figure 13), was synthesized and then subjected to various tests, demonstrated the potential use of nucleoside analogues in alkylation and cross-linking of DNA. Though the synthesis of the aziridine - containing nucleoside triphosphate analogue based on

Figure 13. 6edDTP was Used to Demonstrate the Potential for Analogues to Alkylate and Cross-link DNA.

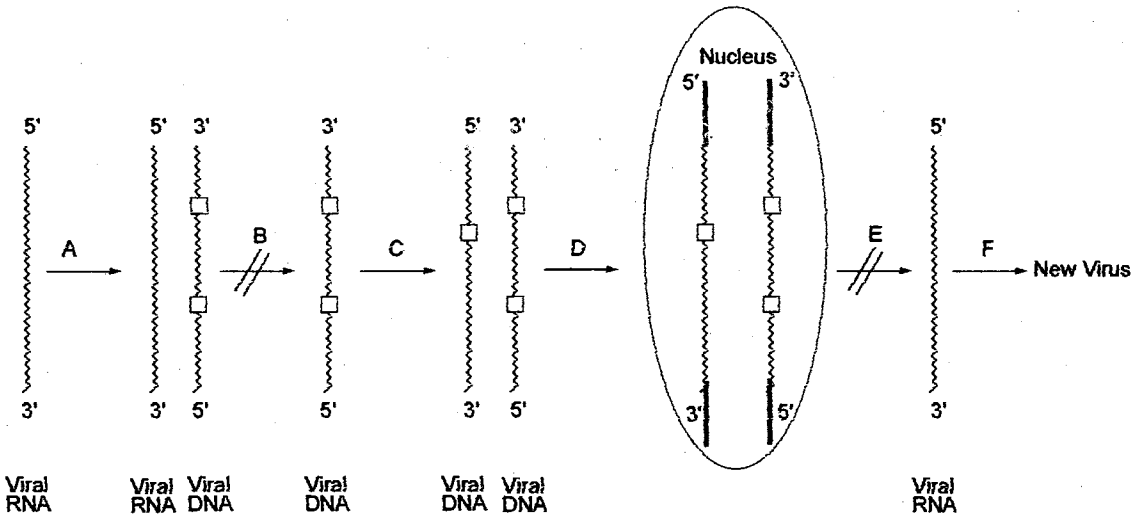


deoxyguanosine triphosphate was not promising, with a less than 1% yield, and unnatural hydrogen bonding was seen, the fact that several DNA polymerases used the analogue as a substrate made the compound feasible for use in further investigations. DNA pol I (Klenow fragment) showed little selectivity in placing 6edDTP opposite complimentary bases. However, cross-linking occurred only when the analogue was placed opposite the highly nucleophilic deoxycytosine or deoxyadenosine. The other important finding was polymerization continued beyond the cross-link, indicating that little perturbation had occurred within the DNA duplex structure.

The introduction of cross-linking agents during viral replication (Figure 14) could suppress replication by covalently linking genetic material and inhibiting the digestion of viral RNA and transcription of new viral RNA (Figure 11d). In order for this to occur, a therapeutic cross-linking agent should possess these four characteristics: (1) it should be a nucleoside analogue that is a substrate for DNA polymerases and kinases; (2) the alkylating functionality should be small and should not noticeably perturb the DNA duplex, so as to avoid repair; (3) the alkylating group should be placed in close proximity to a nucleophile

on the complementary strand of DNA; and (4) the analogue should fully retain the Watson-Crick hydrogen bonding pattern of the parent nucleoside to avoid mismatched-induced mutation and ensure its use by DNA polymerases.

Figure 14. Proposed Insertion of Cross-linking Analogues into Replicating HIV.



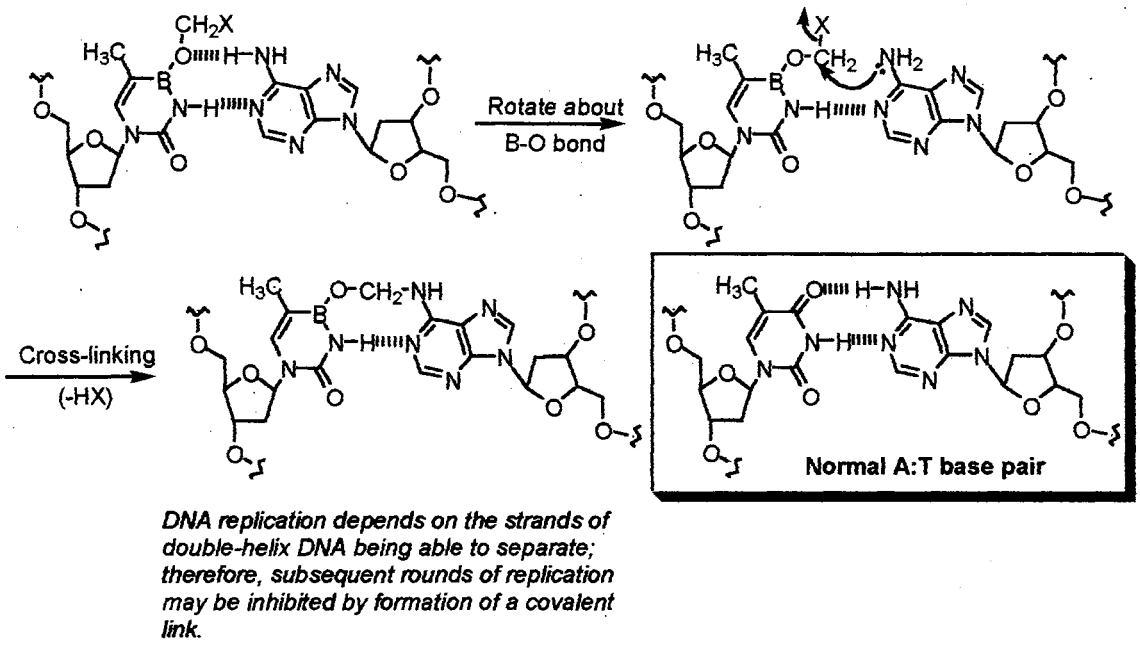
A = Reverse Transcriptase synthesis of the RNA-DNA hybrid
B = Ribonuclease H digestion of the viral RNA
C = DNA Polymerase synthesis of the DNA-DNA duplex
D = Integration into the host genome
E = Transcription into new viral mRNA
F = Translation and assembly of new virus particles

Steps B and/or E could be inhibited by cross-linking

The proposed boron-containing nucleoside analogues could potentially satisfy all four of these requirements (Figure 15). It is believed that the triphosphate of 4-borathymidine could maintain Watson-Crick hydrogen bonding, as previously seen (Figure 5), when paired with adenosine. With the insertion of the boron-containing nucleoside analogue into replicating DNA, the alkylating/cross-linking functionality should be within close proximity of the adenosine on the opposite strand. A rotation around the B-O bond

places the alkylating group in a favorable position for nucleophilic attack. If the alkylating group is kept small (-OCH_2X) there should be little disturbance of the DNA duplex structure. Once incorporated into the replicating DNA, the analogues could also be used in another form of cancer treatment, boron neutron capture therapy.

Figure 15. Proposed Hydrogen Bonding and Cross-linking of Boron Analogues within Replicating DNA.



DNA replication depends on the strands of double-helix DNA being able to separate; therefore, subsequent rounds of replication may be inhibited by formation of a covalent link.

Boron Neutron Capture Therapy

Boron neutron capture therapy (BNCT) has developed as a specified branch of radiation therapy in which radiation is generated from the capture reaction of thermal (slow) neutrons by nonradioactive ^{10}B compounds. BNCT has been proposed as part of a binary system of cancer treatment in which surgery is the first means of defense and BNCT is used to seek out residual cells in the area where a tumor has been removed. Such binary systems

currently exist in cancer therapy: chemotherapy and radiotherapy, surgery and chemotherapy, surgery and radiotherapy. Although current methods have proven to be quite effective on several types of tumors throughout the body, malignant tumors within the brain provide additional challenges. Unlike most malignant tumors, those of the brain possess little propensity to metastasize to other organs but are highly infiltrative of the brain itself.[61] Surgery alone cannot meet the therapeutic objective requiring a combination of surgery with conventional radiotherapy or chemotherapy. These types of therapy have major limitations where the brain tissue is concerned. Conventional radiotherapy must be administered at a dose high enough to destroy cancer cells, the magnitude of which is enough to seriously compromise normal brain cells.[62] In addition, the radiation is unable to locate and destroy tumor cells whose precise location is not known. The most obvious limitation of chemotherapy is that drugs administered to destroy tumor cells are not normally confined to the target cells but can also adversely affect normal brain cells. Additionally, reversing the toxic effects of administered radiation and/or chemotherapy is not possible. Researchers interested in BNCT have had these limitations in mind while working to develop a better form of therapy.

During the development of neutron capture therapy over the past 60 years, several radioactive and nonradioactive nuclides were targeted for investigation as a result of their ability to absorb thermal or slow neutrons.[63] In the long-term investigation of these nuclides, three attributes were established as guidelines for considering new nuclides. The first of these was a search for high capture cross sections for thermal neutrons. The values shown in Table 1[64] are large by nuclear standards, many are more than 2 orders of magnitude greater than observed for normal tissue composition (Table 2).[64]

Table 1. Capture Cross Section Values of Various Nuclides for Thermal Neutrons

nuclide	cross section capture value[a]	nuclide	cross section capture value[a]
^{6}Li	942	^{151}Eu	5800
^{10}B	3838	^{155}Gd	61000
$^{22}Na^{b}$	32000	^{157}Gd	255000
$^{58}Co^{b}$	1900	^{164}Dy	1800
^{113}Co	19800	^{184}Os	3000
$^{126}I^{b}$	6000	^{199}Hg	2000
$^{135}Xe^{b}$	2600000	$^{230}Pa^{b}$	1500
$^{148m}Pm^{b}$	10600	$^{235}U^{b}$	580
^{149}Sm	42000	$^{241}Pu^{b}$	1010

[a] Cross section capture values in barns (1 barn = 10^{-24} cm^2)
[b] Radioactive

Table 2. Thermal Neutron Capture Cross Section Values of Tissue Elements and Their Percentages

nuclide	weight (% in tissue)	cross section capture value[a]	nuclide	weight (% in tissue)	cross section capture value[a]
H	10.00	0.332	P	1.16	0.18
C	18.0	0.0034	S	0.20	0.53
N	3.0	1.82	Cl	0.16	32.68
O	65.0	1.8×10^{-4}	K	0.20	2.1
Na	0.11	0.43	Ca	2.01	0.4
Mg	0.04	0.053	Fe	0.01	2.57

[a] Cross section capture values in barns (1 barn = 10^{-24} cm^2)

Second, the nature of the fission products needed to be considered. Identity of the fission products helped determine whether these components could be confined to the cell of origin or whether they produce radiation which might leave the cell and cause damage to the surrounding tissue. Third, radioactive nuclides were quickly removed from the studies due to an inability to confine these nuclides to malignant cells, causing exposure of normal structures to undesired radiation.

From the nuclides that have been studied, a brief history of neutron capture therapy

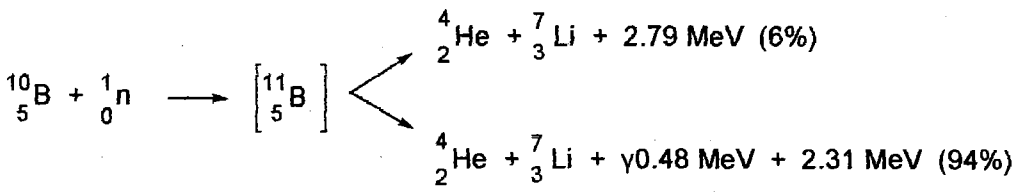
can be constructed to show how the three attributes mentioned were established. Early in the development of NCT, ^{235}U was investigated as a potential nuclide.[65,66] The fission particles from the capture of thermal neutrons by radioactive ^{235}U are very large and of relatively low energy. Because of these two properties the destructive radiation would be confined to the cell in which capture takes place. Unfortunately, most uranium compounds are toxic, and though biologically stable ligands of uranium have been synthesized, they are of no use when toxicity is considered. Nonradioactive ^{6}Li was considered at the same time as ^{235}U.[67,68] Due to the size and energy of the particles released during fission (Figure 16), ^{6}Li appeared to be a promising nuclide. The large, high linear energy transfer (LET) particles could be confined to a radius of 9-10 μm, approximately the dimensions of a single

Figure 16. Neutron Capture by ^{6}Li

$$^{6}_{3}Li + ^{1}_{0}n \longrightarrow \left[^{7}_{3}Li\right] \longrightarrow ^{4}_{2}He + ^{3}_{1}H + 4.3\ MeV$$

cell. The only limitation is that ^{6}Li is an alkali metal and under biological conditions ^{6}Li compounds are broken down to lithium cations. Nonradioactive boron-10 soon came into the picture as a potential nuclide for several reasons. Among the top reasons were natural abundance (20% of natural boron), fission particles (Figure 17) similar to ^{6}Li,[69-72] and small atomic size that would permit its replacement of carbon in many organic structures.

Figure 17. Neutron Capture by ^{10}B.



The ^{10}B nuclide was eventually chosen as the focus of synthetic studies due to the stability of boron-containing compounds with structures and chemical and physical properties analogous to their carbon counterparts. Hydrolytically and metabolically stable compounds containing covalent bonds with various heteroatoms and boron clusters (polyhedral borane anions and carboranes) have already been synthesized and studied. However, the search for possible nuclides continued with more recent investigation of ^{157}Gd.[73-75] Though ^{157}Gd possesses a capture cross section 66 times greater than boron-10, the radiation emitted counteracts this benefit. The fission products from this nuclide are gamma-rays and Auger and Coster-Kronig electrons.[76] Gamma-rays are not confined to the cell where capture occurs. Auger and Coster-Kronig electrons have such a low energy that the nuclide must be closely associated with the tumor cell DNA to have any effect.

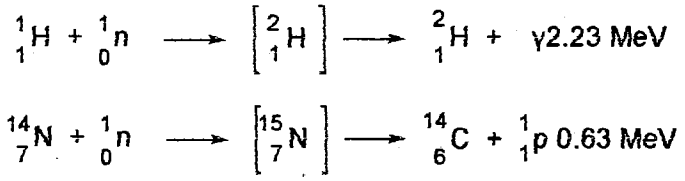
The objective of BNCT is to destroy tumor cells and processes without compromising nearby or contiguous normal tissue. Unlike conventional radiation, neutron capture therapy promises to be able to eradicate tumor cells whose precise location may not be fully known so residual cancer cells do not become foci for recurrences. However, the current limitations of the therapy include a lack of information regarding the permeability differences between tumor and contiguous normal tissues depending on the biochemical differences between the cell types. The assumption has been made that malignant cells

would have a greater uptake rate and radiation sensitivity than normal tissues.

The question remains, in the minds of researchers, as to whether or not compounds containing thermal neutron absorbing nuclides can be designed and synthesized that are capable of selectively targeting malignant cells and at a concentration level sufficient to deliver an effective radiation dosage. As a means of focusing on this question, research has been divided into three areas: design, synthesis, and evaluation of more selective tumor targeting agents; optimizing delivery; and improving neutron beam characteristics from reactors and accelerators.

Design and synthesis of selective tumor targeting agents is aimed at realizing four important parameters: (1) achieving tumor concentrations in the range of 20-35 μg ^{10}B/g; (2) a tumor:normal tissue differential greater than 1; (3) sufficiently low toxicity so that the dose administered would be well tolerated; and (4) a concentration differential would persist throughout the neutron irradiation period.[77] A concentration of 20-35 μg ^{10}B/g or 10^9 ^{10}B atoms/cell must be achieved to minimize the contribution of radiation from hydrogen and nitrogen in the tissue exposed to the neutron beam (Figure 18). At this concentration, 85% of the radiation dose comes from the ^{10}B capture reaction. The nonradioactive nuclide exists as 20% of natural boron which can be activated by 0.025 eV neutrons to produce high LET particles capable of eradicating boron-containing tumor cells without causing damage to surrounding tissues by being confined to a radius of 9-10 μm. In addition, neutrons at an energy of 0.025 eV are below the threshold energy required to ionize tissue components. The use of such a low-energy source would allow irradiation of boron-containing cells without disturbing other tissue components.

Figure 18. Neutron Capture by ^{1}H and ^{14}N in Normal Tissue.



Compound design continues to focus on analogs of cellular building blocks in which carbon has been replaced by boron, generating isosteres that offer the potential for biologically simulating the parent compounds. The knowledge that tumor cells are in various stages of rapid cell division and may be in search of the constituents needed for replication provides the basis for this particular focus. These boron-containing isosteres could have the capacity to compete with the natural substrates. Such competition would allow for incorporation of the fraudulent building blocks and preparation of the tumor cell for BNCT. As is always the case, toxicity of such compounds is of great importance. Since the synthetic analogues are meant to very closely mimic the natural components, there is potential for the molecules to trigger or suppress biochemical systems in a toxic manner.

Of all the possible cellular building block analogues, potential precursors of nucleic acids continue to be the main thrust of chemical efforts. With the main target for the high LET radiation generated by the boron neutron capture reaction being the nucleus (genetic material) of the tumor cell, it is only rational to search for analogues of the purines and pyrimidines which comprise DNA and RNA.

There are many approaches to obtaining structural analogues of purines and pyrimidines. Boron may be inserted into the ring structure in place of carbon.[78-85] When

boron is flanked by two nitrogen atoms, the heterocycle may be easily formed but this linkage is very hydrolytically unstable. Such molecules have been synthesized with the addition of bulky or aromatic groups for stability but were found to no longer biochemically emulate the normal substrates. The boron moiety may also be attached to a pyrimidine nucleus.

The optimization of delivery of neutron absorbers is most pertinent with the potential treatment of brain tumors. The search for molecules that can cross the blood- brain barrier continues, as other methods of introduction are considered highly invasive. Among the current methods being used are intratumoral injection, implantation of sustained release polymers,[86] convection-enhanced delivery, blood-brain barrier disruption, and transport through cationization.[77]

Current neutron beams contain a broad spectrum of energy: thermal, epithermal, and fast neutron, in addition to gamma rays. Thermal neutrons have an energy below the threshold value for scattering interaction between neutrons and hydrogen. Energies of greater than 10 keV cause scattering that produces protons capable of ionizing tissue components. Epithermal neutron beams consisting of 0.5 to 10keV[87] are being developed, for the treatment of more deep-seated tumors of the brain, to counteract the dissipation of thermal neutrons. As the epithermal beams traverse the tissue, they become "thermalized" by losing energy to the surrounding tissue. The last two types of radiation in current neutron beams contribute radiation in a nonselective manner. The presence of these intense types

of radiation gives rise to the need for narrowed neutron sources from reactors and accelerators.

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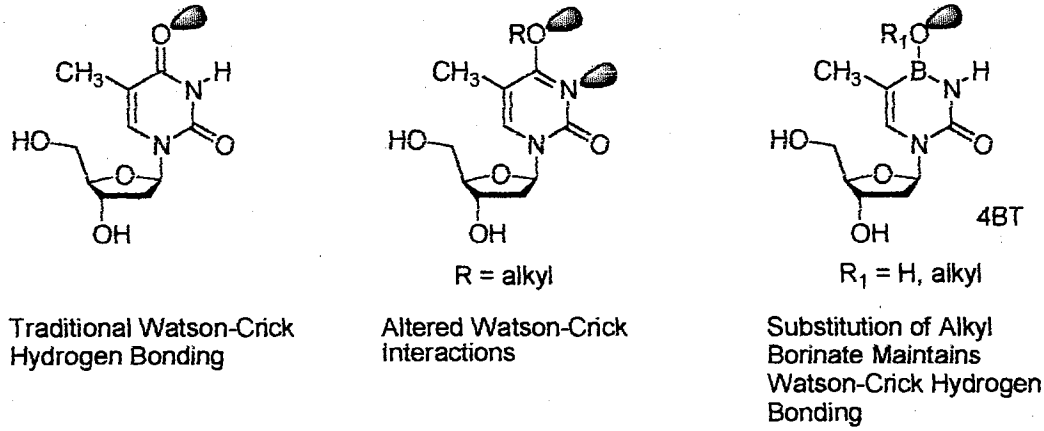
CHAPTER TWO

STUDIES TOWARD THE SYNTHESIS OF 4-BORATHYMIDINE

Oklahoma State University

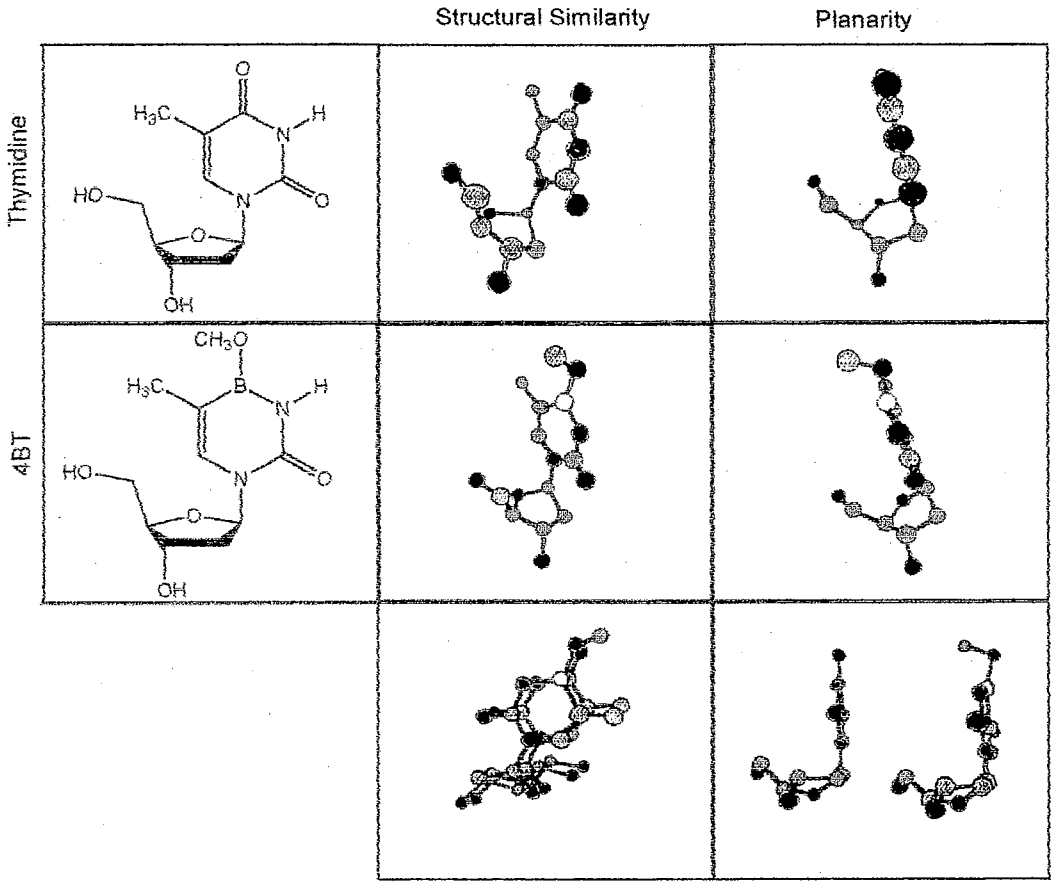
The boron-containing thymidine analogue 4-borathymidine (4BT, Figure 19) has been designed for investigation of DNA replication, alkylation, and repair by introducing a functionality that allows for attachment of a potential reporter group or reactive moiety to the exocyclic oxygen at the 4-position. The key design feature of 4BT is the replacement of the thymidine *C*4 carbonyl group (C=O) by an alkyl borinate (B-O-R). The borinate may allow traditional Watson-Crick hydrogen bonding interactions in duplex DNA to be maintained, whereas attachment of a reporter group or reactive center to *O*4 of thymidine disrupts normal Watson-Crick hydrogen bonding due to loss of the donor hydrogen at *N*3 (Figure 19).

Figure 19. Comparison of Watson-Crick Hydrogen Bonding Patterns.



Synthesis of an analogue possessing an electrophilic alkyl borinate (B-O-RX, X = Cl, Br, I) functionality introduces the possibility of alkylation of the complementary amino group, thereby forming a covalent interstrand cross-link between two nucleic acid strands. For this to be accomplished, 4BT must be incorporated into DNA as an analogue of thymidine without disrupting the inherent helical structure. Therefore, planarity of the boron-containing base is essential. A crude examination of the structural similarities, specifically molecular shape and planarity, to thymidine (Figure 20) make 4BT attractive as a potential antiviral/anticancer therapeutic.

Figure 20. Three-dimensional Study Comparison of Thymidine to 4-Borathymidine (4BT)



It has been established that at least for some anticancer therapeutics, alkylation and cross-linking are the mechanisms by which DNA replication and transcription is inhibited.[1-5] The main drawback of the commonly used alkylating drugs is their inability to selectively target DNA in diseased cells (therapeutic cytotoxicity) as opposed to DNA in normal healthy cells.[4,6-8] Since DNA alkylation has also been implicated in mutagenesis, it is clear that any clinically useful alkylating/cross-linking agent must strike a delicate balance between toxicity and mutagenicity.

Another promising anticancer strategy besides DNA alkylation is boron neutron capture therapy,[9,10] currently in clinical trials. In this therapy, boron-rich tumor cells are selectively destroyed by the high-energy decay products $^{4}He^{2+}$ and $^{7}Li^{+}$ emitted when ^{10}B captures a thermal (slow) neutron. A nucleoside analogue containing boron that can act as a substrate for DNA polymerases may allow tumor cells to accumulate boron in sufficiently high quantities as to make this a potentially useful alternative to the current boron-based drugs.[9-13]

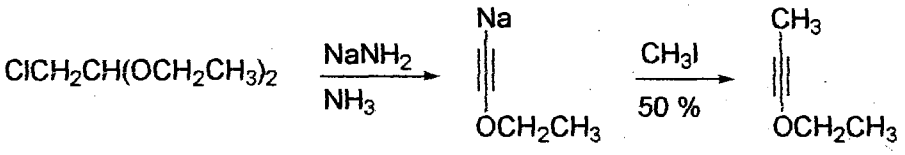
Results and Discussion

The three major issues considered in the synthesis of 4BT were (1) how to prepare the required boron intermediate(s), (2) how to prepare the boron-containing heterocyclic base, and (3) how to install the ribose moiety.

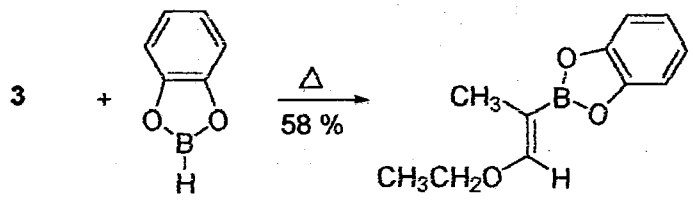
The synthesis of the key boron reactant **5** is shown in Scheme 1. Reaction of 1-ethoxy-1-propyne (**3**)[14,15] (derived from chloroacetaldehyde diethyl acetal, **1**) with catechol borane (**4**), gave *Z*-(1-ethoxy-1-propen-2-yl)-1,3,2-benzodioxaborole (**5**).[16] The hydroboration reagent added across the triple bond in a *syn* orientation[17,18] with the boron

attached exclusively to *C*2 of the propene.

Scheme 1. Preparation of *Z*-(1-Ethoxy-1-propen-2-yl)-1,3,2-benzodioxaborole



1 **2** **3**

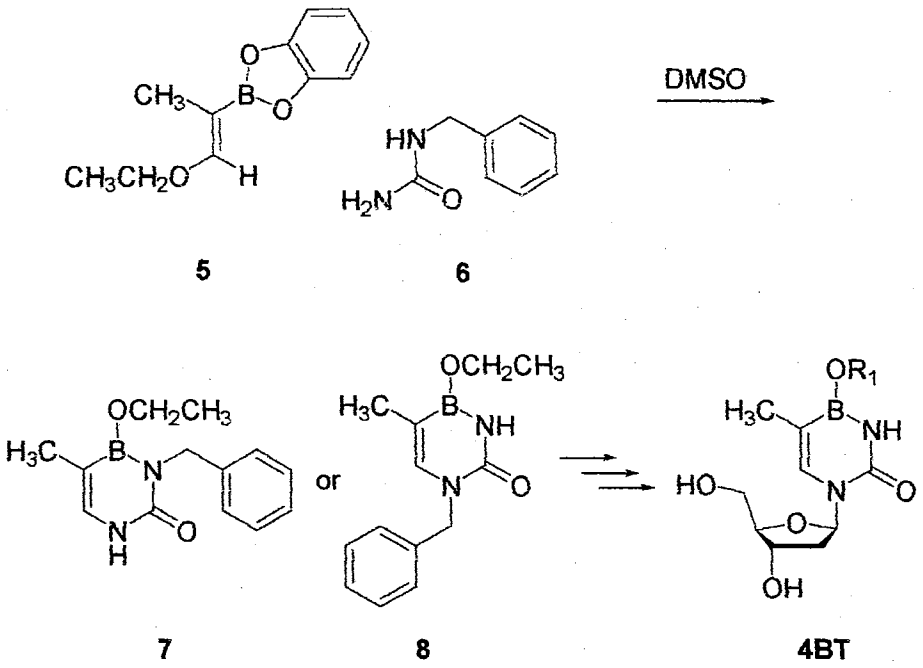


4 **5**

To test the feasibility of preparing the desired boron heterocycle, compound **5** was reacted with *N*-benzylurea (**6**) as depicted in Scheme 2. Trials using various solvents (ethanol, methanol, acetonitrile, ethyl acetate, *N*,*N*-dimethylformamide, acetone, ether, tetrahydrofuran, and dimethyl sulfoxide) with an acid catalyst (sulfuric, acetic, phosphoric acids), a base catalyst (anhydrous ammonia, ammonium hydroxide, sodium ethoxide), or no catalyst were performed. Of all the combinations, the reaction carried out in DMSO, upon being chromatographed on silica gel, gave a product whose ^{1}H NMR, ^{13}C NMR, and FAB MS data were consistent with that expected for *N*3-benzyl-O^4-ethyl-5-methyl-4-borapyrimidin-2-one (**7**) or the corresponding *N*1-benzyl compound **8**. After demonstrating the validity of the method, **7** (and **8**, after removal of the benzyl group) can potentially be

glycosylated,[19-24] 2'-deoxygenated,[19,25] and deprotected[26] according to literature procedures to provide 4BT.

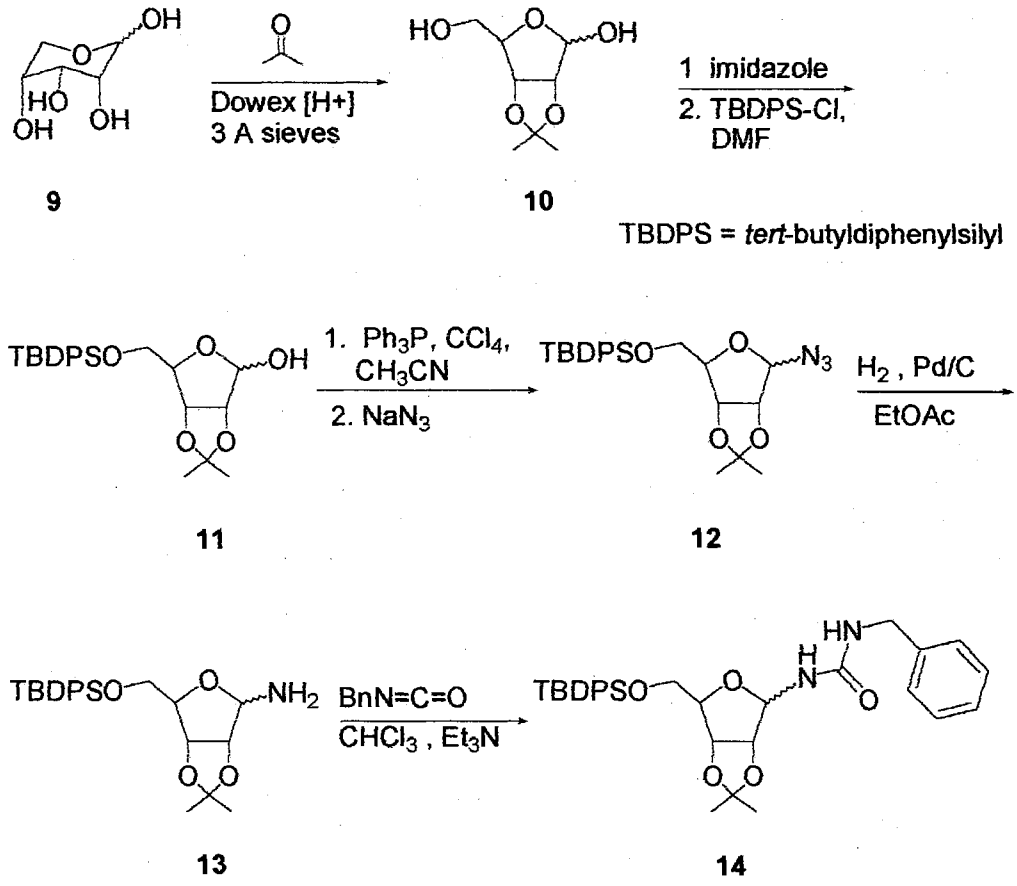
Scheme 2. Attempted Synthesis of *N*3-Benzyl-*O*^{4}-ethyl-5-methyl-4-borapyrimidin-2-one (*N*3-Benzyl-*O*^{4}-ethyl-4-borathymine)



During further characterization of **7** and **8**, an alternative route to 4BT was pursued. This alternative route (Scheme 3) required the synthesis of a fully protected ribofuranosyl urea **14** for cyclization with **5**. D-Ribose (**9**) was protected as its 2,3-*O*-isopropylidene acetal **10** using acid catalysis.[27,28] Subsequent protection of the 5-hydroxyl as its *tert*-butyldiphenylsilyl (TBDPS) ether[29] gave the protected ribofuranose **11**, which was treated with triphenylphosphine and carbon tetrachloride to form a ribosyl chloride[30] that was immediately reacted with sodium azide to provide 5-*O*-*tert*-butyldiphenylsilyl-2,3-*O*-isopropylidene ribofuranosyl azide (**12**).[31-33] The azide **12** was then reduced under standard conditions[31,34] and reacted with benzyl isocyanate to provide the fully protected ribofuranosyl

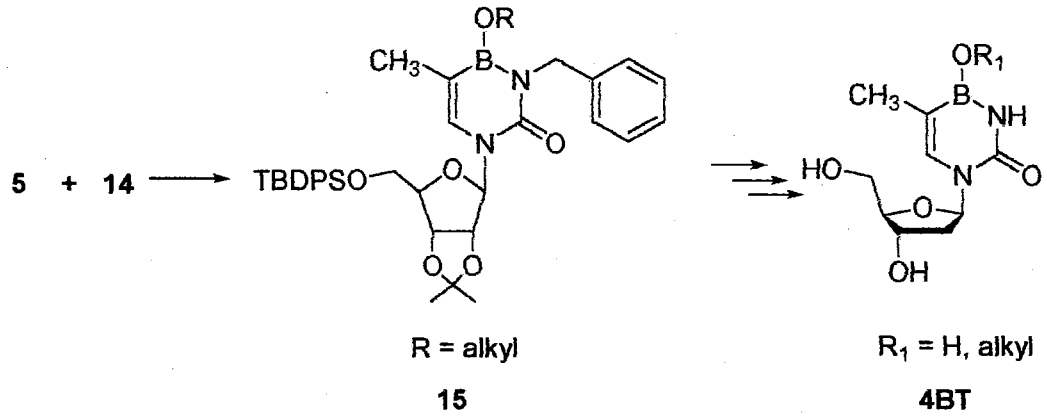
urea **14**[31,35] in 19% yield for five steps.

Scheme 3. Synthesis of *N*3-Benzyl-5-*O*-*tert*-butyldiphenylsilyl-2,3-*O*-isopropylidene Ribofuranosyl Urea



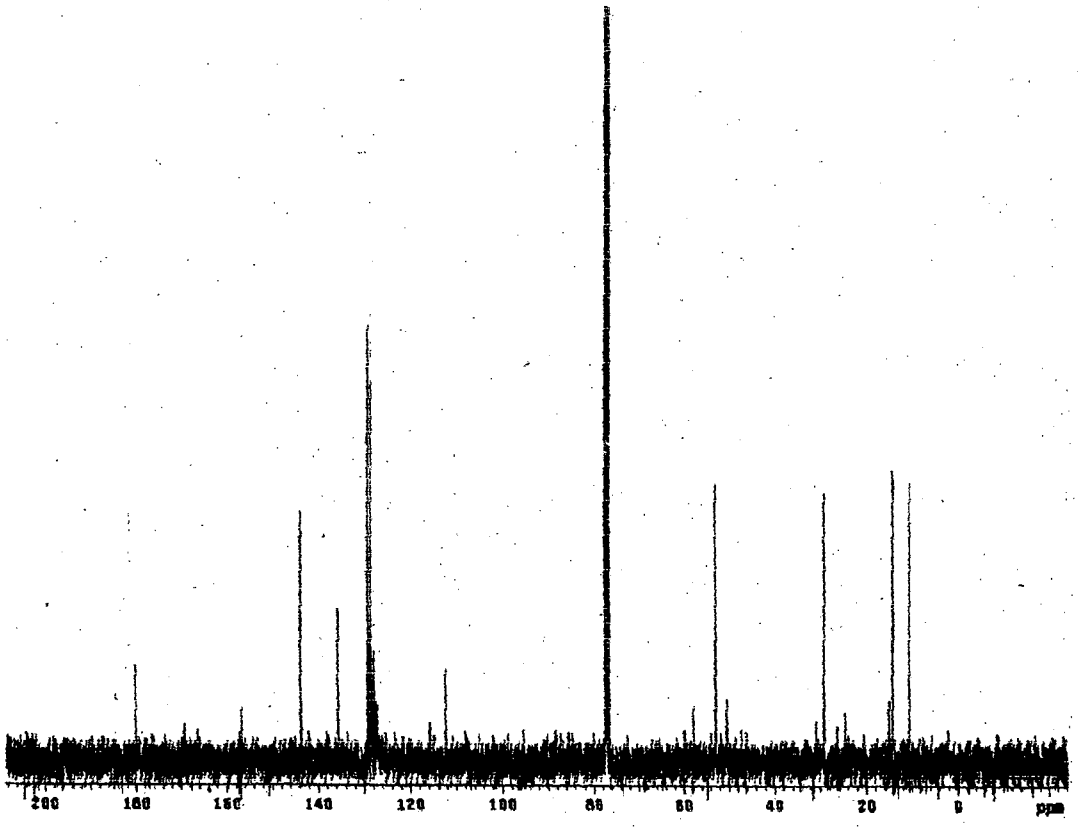
At this point, based on the reaction of **5** with **6**, the reaction of **5** with **14** in the presence of DMSO (Scheme 4) should have given the fully protected ribofuranose form of analogue **15**; this was not observed.

Scheme 4. Proposed Synthesis of 4-Borathymidine



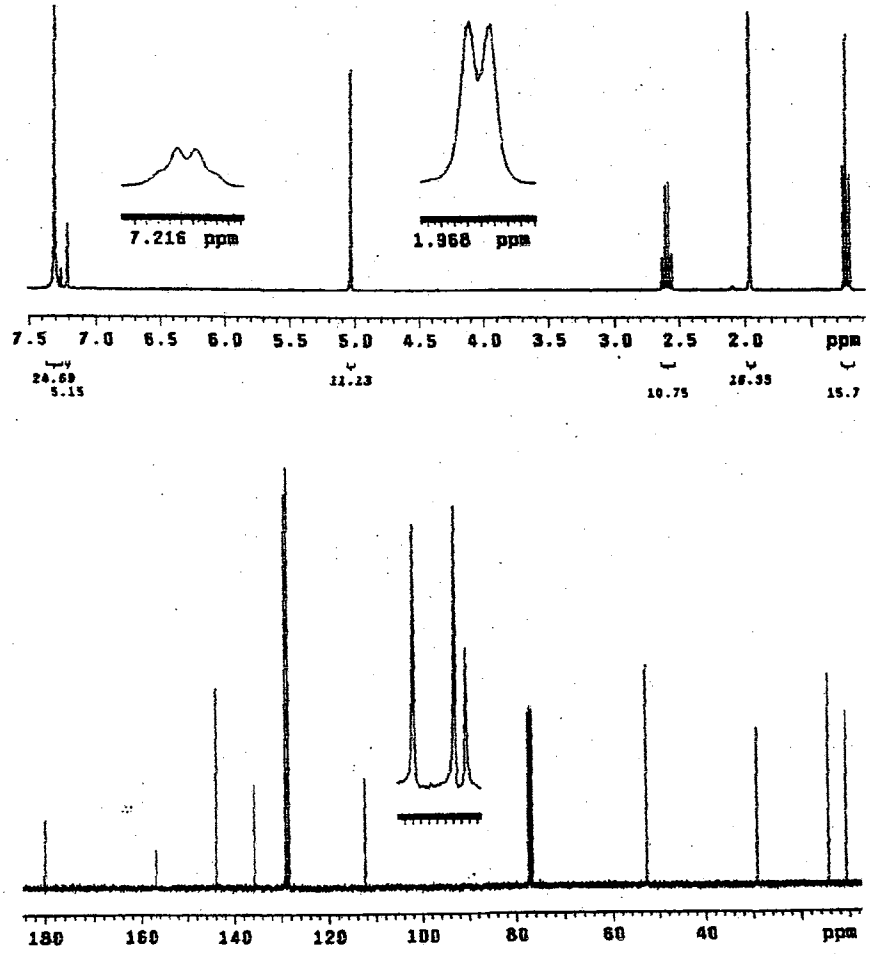
The additional investigation to verify the identity of compound 7 involved elemental analysis in which boron was assayed in addition to carbon, nitrogen, and hydrogen. Unexpectedly, the elemental analysis that was returned indicated no boron was present in the sample. A curious result for what appeared to be a straightforward interpretation of the initial data. Initially, LR-FAB MS supported the calculated formula mass of 244, seen as MH^+ equal to 245, ^1H NMR splitting patterns and integration were consistent with the proposed structure, and ^{13}C NMR showed 11 signals with the carbon next to the boron not being observed (Figure 21). This is a common phenomenon caused by the quadrupolar moment of boron broadening the signal of the neighboring carbon.

Figure 21. Initial ^{13}C NMR of Compound **7**



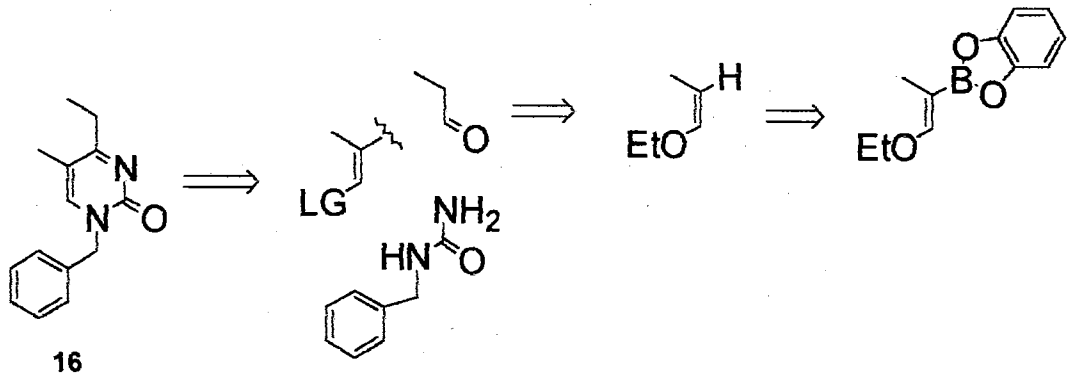
Repeated investigation of **7** led to data that was intriguing. LR-FAB MS now indicated a formula mass of 228 and ^{13}C NMR showed 12 signals (Figure 22). This new data paved the way for further investigation into what was actually occurring. First, a structure needed to be proposed from the new data. Compound **16** matched the data provided by elemental analysis, LR-FAB MS, ^{1}H NMR and ^{13}C NMR so an investigation into the origin of this product began.

Figure 22. Spectra Obtained During Further Investigation of Compound 7



Retrosynthetically, the possible involvement of propionaldehyde in the reaction was suggested (Figure 23). In order to examine this possibility, the reaction of **5** with **6** in the presence of DMSO was repeated with the addition of propionaldehyde and with the addition of butyraldehyde to observe any changes that may occur in the outcome of the reaction. In the propionaldehyde case only 6% of the desired product was observed, and with butyraldehyde, the product of interest was not observed indicating that the reaction did not proceed as proposed.

Figure 23. Retrosynthetic Analysis for Compound **16**



Conclusions

The work presented in the preceding pages shows the complexity of building an acyclic precursor for the purpose of incorporating boron into a heterocyclic compound. The method of introducing boron must be carefully chosen so as not to destroy the acyclic precursor before the boron is securely in place. Among the many considerations to be taken into account is the bond strength between boron and other atoms, such as carbon, nitrogen, oxygen, and the halogens. The boron-nitrogen bond is rather labile compared to bonds with carbon and oxygen and, therefore, posed a problem in the desired synthesis.

The observation to be made from this work is that, when attempting to form a boron-nitrogen bond, abundant spectral data must be taken to ensure that the desired product has indeed been achieved. As a result of this observation, future work includes ^{11}B NMR data to support the assumption that boron is present in the molecule.

Experimental

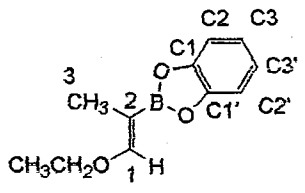
General Synthetic Procedures. NMR spectra were obtained on Gemini 300 or Unity Inova 400 or 600 MHz spectrometers from Varian. Unless otherwise stated, the ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ and referenced to either internal TMS or to the central line of the residual solvent multiplet. Infrared spectra were obtained using KBr on a System 2000 FT-IR instrument from Perkin-Elmer. Gas chromatography-mass spectrometry (electron ionization) was performed on a Hewlett-Packard G1800A GCD system. Low resolution fast atom bombardment (LR-FAB) mass spectra were obtained on a model ZAB-2SE VG Analytical Limited spectrometer. The high resolution fast atom bombardment (HR-FAB) mass spectra were performed by the Washington University Resource for Biomedical and Bio-organic Mass Spectrometry, St. Louis, MO. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. Melting points were determined on a Thomas Hoover apparatus and are uncorrected. All reactions were run under an inert atmosphere of nitrogen using glassware dried at 100°C. D-Ribose was dried under vacuum with P_2O_5. When used as a reaction solvent, chloroform was ethanol-free. Other solvents were dried using established procedures and appropriate drying agents: dimethylsulfoxide (DMSO) and dimethylforamide (DMF) over 3Å sieves; ethanol, ethyl acetate, and methanol over 4Å sieves; acetone distilled from $CaSO_4$; triethylamine (Et_3N) distilled from KOH; acetonitrile distilled from CaH_2; carbon tetrachloride distilled from P_2O_5. Unless otherwise noted, reactions were dried over Na_2SO_4 followed by filtration through glass wool. Solutions were evaporated *in vacuo* using a rotary evaporator at 30°C and 20 mm Hg (water aspirator). A cylinder of Drierite ™ (W.A. Hammond) was placed in the vacuum line between the aspirator and rotary evaporator to protect the contents from

moisture. Drying *in vacuo* refers to removal of residual solvent using an oil pump (<0.1 mm Hg) at room temperature. Thin-layer chromatography was accomplished using Machery-Nagel Polygram Sil G/UV™ plates with detection using either ultraviolet light (254 nm) or *p*-dimethylaminobenzaldehyde stain[36]. Purification was accomplished by column chromatography[37] on silica using combinations of chloroform (C), ethanol (E), and hexanes (H) as the main solvents. Chemicals were purchased from Aldrich and solvents from Fisher Scientific unless otherwise stated.

1-Ethoxy-1-propyne (3). A 1-L, 3-necked round-bottomed flask equipped with a dry ice condenser, a mechanical stirrer, and a gas inlet tube was placed in a dry ice-acetone bath. After flushing with nitrogen, anhydrous ammonia was introduced until 600 mL of liquid ammonia had collected. The gas inlet was replaced by a septum and the cooling bath was lowered. To the ammonia was added iron(III) nitrate nonahydrate (0.59 g, 1.5 mmol) followed by a small piece of freshly cut sodium. Once hydrogen evolution ceased, small pieces of sodium were added over a 20 min period until 35.8 g (1.56 mol) had been introduced. The reaction was allowed to stir until the dark blue color dissapeared to give a dark gray suspension. To the reaction was added chloroacetaldehyde diethyl acetal (76.0 mL, 77.4 g, 0.51 mol) at a rate of 1 mL/min by syringe pump. [Note: Excessive foaming was controlled by stopping the addition or immersing the flask in the cooling bath.] After 30-60 min, the mixture became light gray and methyl iodide (69.0 mL, 157 g, 1.1 mol) was added slowly (0.3-1.6 mL/min) by syringe pump. The mixture was stirred vigorously for 2.5 h, after which the septum was replaced by a pressure-equalizing addition funnel. Cooled, saturated ammonium chloride (30 mL) was cautiously added, followed by pentane (120 mL)

and additional cooled ammonium chloride (370 mL). The contents of the flask were transferred to a 2-L separatory funnel, the layers separated, and the aqueous layer extracted with pentane (2 x 75 mL). The combined organic layers were filtered through glass wool to remove emulsions, dried with $MgSO_4$, and filtered. Distillation of the pentane solution gave 26.3 mL (22.0 g, 261 mmol, 50%) of 1-ethoxy-1-propyne as a colorless liquid: bp 88-90 °C. ^{1}H NMR δ 4.01 (q, 2 H, J = 7.1 Hz, CH_2), 1.75 (s, 3 H, ≡CCH_3), 1.35 (t, 3 H, J = 7.1 Hz, -CH_2CH_3). ^{13}C NMR δ 88.03, 73.62, 32.43, 14.25, 1.47.

***Z*-(1-Ethoxy-1-propen-2-yl)-1,3,2-benzodioxaborole (5).** Catecholborane (1,3,2-benzodioxaborole, 1.09 g, 9.1 mmol) was added to 1-ethoxy-1-propyne (**3**, 0.82 g, 9.7 mmol) under nitrogen at 0 °C. The reaction mixture was stirred at 70 °C for 5.5 h. Distillation (0.4 mm Hg) of the reaction mixture gave 1.09 g (5.3 mmol, 58%) of *Z*-(1-ethoxy-1-propen-2-yl)-1,3,2-benzodioxaborole as a colorless liquid, bp 74-77 °C. [Note: *Z*-(1-ethoxy-1-propen-2-yl)-1,3,2-benzodioxaborole is air and moisture sensitive. A deep red color appears upon contact with air but the compound is stable when stored under N_2 at 5 °C.] ^{1}H NMR δ 7.08 (q, 1 H, J = 1.5 Hz, H1), 7.19-7.00 (m, 4 H, J = 7.1 Hz, ArH), 4.05 (q, 2 H, J = 7.1 Hz, CH_2), 1.80 (d, 3 H, J = 1.5 Hz, =CCH_3), 1.35 (t, 3 H, -CH_2CH_3). ^{13}C NMR δ 159.85, 149.05, 122.75, 112.49, 69.50, 15.97, 10.06. The alkene carbon (*C*2) attached to boron was not observed due to strong quadrupolar line-broadening.[38]



***N*3-Benzyl-*O*^{4}-ethyl-5-methyl-4-borapyrimidin-2-one (*N*3-Benzyl-*O*^{4}-ethyl-4-borathymine, 7) or *N*1-Benzyl-*O*^{4}-ethyl-5-methyl-4-borapyrimidin-2-one (*N*1-Benzyl-*O*^{4}-ethyl-4-borathymine, 8).** A solution of benzylurea (81 mg, 0.59 mmol) and *Z*-(1-ethoxy-1-propen-2-yl)-1,3,2-benzodioxaborole (**5**, 100 µL, 104 mg, 0.51 mmol) in DMSO (1 mL) was stirred at room temperature for 25 h then heated to 65 °C for an additional 28 h. The solvent was evaporated under vacuum on a rotary evaporator and the resulting syrup chromatographed (100% C→100% E) to yield 42.0 mg (172 mmol, 30%) of **7** or **8**, R_f 0.55 (90:10 C:E). Initial Data: MS (LR-FAB) *m/z* 245 (MH^+). ^{1}H NMR δ 7.36-7.28 (m, 6 H, Ar and NH), 6.93 (q, 1 H, J = 3.7 Hz, H6), 5.05 (s, 2 H, -CH_2-), 2.61 (q, 2 H, J = 7.3 Hz, -CH_2CH_3), 1.97 (d, 3 H, J = 3.3 Hz, =C-CH_3), 1.24 (t, 3 H, J = 7.3 Hz, -CH_3). ^{13}C NMR δ 180.03, 143.82, 135.61, 129.10, 128.57, 128.46, 127.90, 112.39, 52.89, 29.18, 14.31, 10.65.

Compound **16** based on revised data from **7**: MS (LR-FAB) *m/z* 228 (MH^+). ^{1}H NMR δ 7.36-7.28 (m, 6 H, Ar and NH), 6.93 (q, 1 H, J = 3.7 Hz, H6), 5.05 (s, 2 H, -CH_2-), 2.61 (q, 2 H, J = 7.3 Hz, -CH_2CH_3), 1.97 (d, 3 H, J = 3.3 Hz, =C-CH_3), 1.24 (t, 3 H, J = 7.3 Hz, -CH_3). ^{13}C NMR δ 179.92, 156.67, 143.74, 135.64, 128.99, 128.48, 128.34, 112.24, 52.75, 29.12, 14.23, 10.56.

2,3-*O*-Isopropylidene Ribofuranose (10). Acetone (50.0 mL, 681 mmol), 3 Å sieves (1.86 g), and Dowex 50W-X8[H^+] ion exchange resin (1.77 g) were combined. D-Ribose (3.07 g, 20.0 mmol) was added and the mixture was stirred at room temperature for 10.5 h. The mixture was filtered, solvent evaporated, and the resultant thick syrup was chromatographed (80:20→90:10 EtOAc:petroleum ether) to yield 2.57 g (13.5 mmol, 68%)

of 2,3-*O*-isopropylidene ribofuranose, R_f 0.61 (EtOAc). MS (LR-FAB) *m/z* 173 (MH^+-H_2O). ^{1}H NMR δ (β anomer) 5.42 (d, 1 H, *J* = 6.0 Hz, H1), 4.85 (d, 1 H, *J* = 6.0 Hz, H2), 4.59 (d, 1 H, *J* = 12.0 Hz, H3), 4.42 (t, 1 H, *J* = 2.4 Hz, H4), 3.80-3.40 (m, 4 H, H5',5", CH_2OH, *OH*), 1.49 (s, 3 H, CH_3), 1.33 (s, 3 H, CH_3). ^{13}C NMR δ 112.34, 103.24, 87.09, 87.94, 88.15, 63.91, 26.71, 24.82.

5-*O*-*tert*-Butyldiphenylsilyl-2,3-*O*-isopropylidene Ribofuranose (11). A solution of *tert*-butyldiphenylsilyl chloride (2.1 mL, 2.2 g, 8.1 mmol) in DMF (12.5 mL) was slowly added, via 250 μm capillary over 4 h, to a stirred mixture of 2,3-*O*-isopropylidene ribofuranose (**10**, 1.07 g, 5.6 mmol) and imidazole (1.20 g, 17.7 mmol). After 4.5 h, the reaction mixture was diluted with ether (20 mL), washed with H_2O (2 x 10 mL), NaCl (10 mL), dried and evaporated. The crude clear syrup was chromatographed (95:5:0.5 C:H:Et_3N→99:1:0.5 C:E:Et_3N) to give 2.34 g (5.5 mmol, 97%) of 5-*O*-*tert*-butyldiphenylsilyl-2,3-*O*-isopropylidene ribofuranose, R_f 0.33 (99:1 C:E). ^{1}H NMR δ (major anomer) 7.72-7.37 (m, 10 H, Ar), 5.35 (d,1 H, *J* = 10.4 Hz, H1), 4.72 (d, 1 H, *J* = 6.0 Hz, H2), 4.61 (d, 1 H, *J* = 6.0 Hz, H3), 4.53 (d, 1 H, *J* = 10.4 Hz, H4), 4.28 (s, 1 H, OH), 3.82 (dd, 1 H, *J* = 11.3, 2.8 Hz, H5' or H5"), 3.66 (dd, 2 H, *J* = 11.3, 2.4 Hz, H5" or H5'), 1.48 (s, 3 H, CH_3), 1.32 (s, 3 H, CH_3), 1.06-1.09 (s, 9 H, *t*-Bu). ^{13}C NMR δ 135.94, 135.76, 135.01, 131.81, 131.71, 130.64, 130.44, 128.32, 128.18, 128.11, 127.92, 112.33, 103.63, 103.60, 87.56, 87.28, 65.70, 27.10, 27.06, 26.77, 26.68, 25.17, 19.34.

5-*O*-*tert*-Butyldiphenylsilyl-2,3-*O*-isopropylidene Ribofuranosyl Azide (12). A solution of 5-*O*-*tert*-butyldiphenylsilyl-2,3-*O*-isopropylidene ribofuranose (**11**, 5.19 g, 12.1

mmol), carbon tetrachloride (3.4 mL, 5.4 g, 35 mmol), and triphenylphosphine (3.89 g, 14.8 mmol) in DMF (9.2 mL) was stirred at room temperature for 4.5 h. The reaction mixture was poured into a stirred mixture of 20 mL of ether, 20 mL of petroleum ether, and 40 mL of ice water. Celite was added and the mixture filtered through a Celite pad. The aqueous layer was separated and washed with 1:1 petroleum ether:ether. The combined organic layers were dried, evaporated to 1/2 volume, filtered through silica, and evaporated to give a crude syrup of 5-*O*-*tert*-butyldiphenylsilyl-2,3-*O*-isopropylidene ribofuranosyl chloride which was used directly, R_f 0.80 (99:1:0.5 C:E:Et_3N). ^1H NMR δ (major anomer) 7.69-7.36 (m, 10 H, Ar), 6.09 (s, 1 H, H1), 4.90 (d, 1 H, J = 5.9 Hz, H2), 4.83-4.81 (m, 1 H, H3), 4.43-4.41 (m, 1 H, H4), 3.87-3.82 (m, 2 H, H5',5"), 1.56 (s, 3 H, CH_3), 1.40 (s, 3 H, CH_3), 1.05-1.14 (9 H, *t*-Bu). ^{13}C NMR δ 135.86, 135.81, 135.00, 133.35, 133.20, 130.07, 129.87, 127.99, 113.43, 98.75, 90.27, 89.67, 81.66, 63.50, 27.07, 27.01, 26.78, 26.71, 25.53, 19.45. Acetonitrile (69.0 mL) and sodium azide (6.59 g, 101 mmol) were added to the crude syrup and the resultant suspension was stirred at reflux (80-85 °C) for 7 h, then at room temperature for 7 h. After filtration and evaporation, the resulting syrup was chromatographed (40:60:0.5→80:20:0.5 C:H:Et_3N) yielding 2.26 g (5.0 mmol, 41%) of 5-*O*-*tert*-butyldiphenylsilyl-2,3-*O*-isopropylidene ribofuranosyl azide, R_f 0.22 (45:55:0.5 C:H:Et_3N). FT-IR (KBr) ν 2124 (N_3). MS (LR-FAB) *m/z* 453 (MH^+). ^1H NMR δ (major anomer) 7.70-7.35 (m, 10 H, Ar), 5.18 (d, 1 H, J = 4.0 Hz, H1), 4.82 (dd, 1 H, J = 6.4, 1.1 Hz, H3), 4.77 (dd, 1 H, J = 6.4, 4.0 Hz, H2), 4.27 (broad t, 1 H, H4), 3.86 (dd, 1 H, J = 11.4, 2.9 Hz, H5' or H5"), 3.69 (dd, 1 H, J = 11.4, 2.6 Hz, H5" or H5'), 1.59 (s, 3 H, CH_3), 1.38 (s, 3 H, CH_3), 1.03-1.10 (9 H, *t*-Bu).

5-*O*-*tert*-Butyldiphenylsilyl-2,3-*O*-isopropylidene Ribofuranosyl Amine (13). The reduction of 5-*O*-*tert*-butyldiphenylsilyl-2,3-*O*-isopropylidene ribofuranosyl azide (**12**) was carried out by balloon hydrogenation. A solution of 5-*O*-*tert*-butyldiphenylsilyl-2,3-*O*-isopropylidene ribofuranosyl azide (**12**, 205 mg, 0.45 mmol) in ethyl acetate (6.0 mL), and 10% Pd/C (25 mg), were stirred under H_2 for 2.5 h, filtered through $MgSO_4$, and evaporated. Drying under vacuum gave 188 mg (0.44 mmol, 98%) of 5-*O*-*tert*-butyldiphenylsilyl-2,3-*O*-isopropylidene ribofuranosyl amine as a clear syrup, R_f 0.16 (99:1:0.5 C:E:Et_3N). MS (LR-FAB) *m/z* 428 (MH^+). ^1H NMR δ 7.70-7.30 (m, 10 H, Ar), 5.14 (d, 1 H, *J* = 4.0 Hz, H1), 4.75 (dd, 1 H, *J* = 6.0 Hz, H2), 4.62 (dd, 1 H, *J* = 6.0, 3.7 Hz, H3), 4.04 (broad t, 1 H, *J* = 3.1 Hz, H4), 3.78 (dd, 1 H, *J* = 11.0, 3.7 Hz, H5' or H5"), 3.66 (dd, 1 H, *J* = 11.0, 3.1 Hz, H5" or H5'), 2.14 (broad s, 2 H, NH_2), 1.53 (s, 3 H, CH_3), 1.37 (s, 3 H, CH_3), 1.08-1.02 (9 H, *t*-Bu).

***N*3-Benzyl-5-*O*-*tert*-butyldiphenylsilyl-2,3-*O*-isopropylidene Ribofuranosyl Urea (14).** To a stirred solution of 5-*O*-*tert*-butyldiphenylsilyl-2,3-*O*-isopropylidene ribofuranosyl amine (**13**, 42 mg, 0.10 mmol), benzyl isocyanate (40 µL, 43 mg, 0.32 mmol), and $CHCl_3$ (0.75 mL) was added a solution of triethylamine (20 µL, 15 mg, 0.15 mmol) in $CHCl_3$ (0.25 mL) via a 250 µm capillary fiber. The reaction was stirred at room temperature for 5 h then washed with 2 *M* HCl (1 mL), H_2O (1 mL), $NaHCO_3$ (1 mL), and H_2O (3 x 1 mL). The organic layer was dried and evaporated. The crude product was chromatographed (75:25:0.5 C:H:Et_3N→100 C) to yield 41 mg (7.3 µmol, 73%) of *N*3-benzyl-5-*O*-*tert*-butyldiphenylsilyl-2,3-*O*-isopropylidene ribofuranosyl urea as a dark orange solid, R_f 0.14 (99:1:0.5 C:E:Et_3N). MS (LR-FAB) *m/z* 561 (MH^+). ^1H NMR δ 7.66-7.27 (m, 15 H, Ar), 5.85 (dd, 1 H, *J* = 8.8, 4.4 Hz, H1), 5.51 (d, 1 H, *J* = 9.2 Hz, NH), 4.91 (broad t, 1 H, *NH*-CH_2), 4.80 (d, 1 H, *J* =

6.2 Hz, H3), 4.60 (dd, 1 H, J = 6.2, 4.4 Hz, H2), 4.47 (dd, 1 H, J = 14.7, 5.5 Hz, PhCH_2), 4.41 (dd, 1 H, J = 14.7, 5.9 Hz, PhCH_2), 4.10 (t, 1 H, H4), 3.79 (dd, 1 H, J = 11.0, 3.7 Hz, H5' or H5"), 3.67 (dd, 1 H, J = 11.0, 2.7 Hz, H5" or H5'), 1.50 (s, 3 H, CH_3), 1.36 (s, 3 H, CH_3), 1.26-1.06 (9 H, *t*-Bu).

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CHAPTER THREE

2-BENZYL-1,4-DIHYDRO-1-HYDROXYTHIENO[3,2-*c*][1,5,2]DIAZABORIN-3(2*H*)-ONE

Synthesis of a Novel Heterocycle

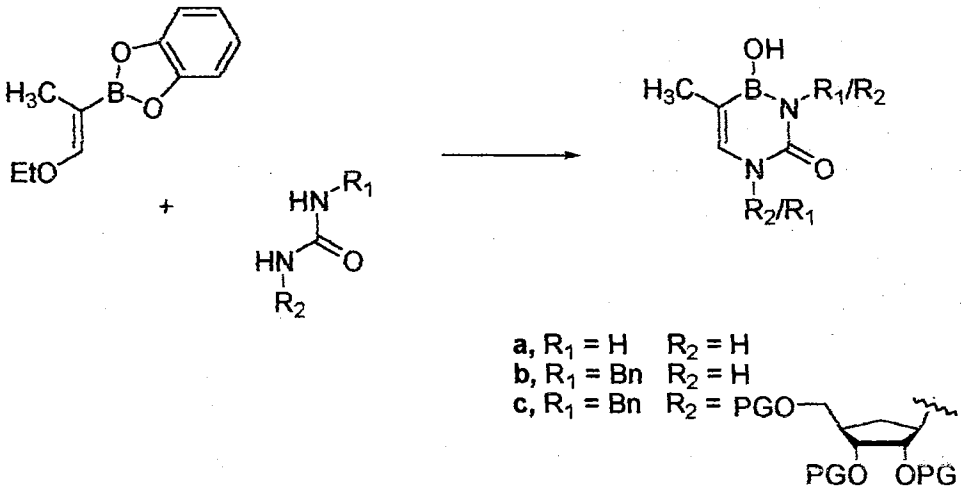
For quite some time, boron has been recognized as a valuable component of synthetic reagents. Only recently has boron emerged on the biological scene with great potential for biologically active compounds containing boron.[1] As the scientific community continues to search for innovative and effective therapeutic agents, we were motivated to design and synthesize nucleoside analogues in which the carbon atom of a pyrimidine or purine is replaced by boron. The ability of boron to maintain a trigonal planar conformation similar to that of a carbonyl, brought about a curiosity focused on the preservation of the Watson-Crick hydrogen bonding patterns. Preserving the bond donor and acceptor patterns found in parent nucleosides lends to a greater possibility for the designed endocyclic boron-containing nucleosides (BCNs) to serve as DNA structural probes and as potential inhibitors of DNA replication.

The boron-for-carbon replacement has been successfully used to prepare analogues of amino acids[2] and peptides;[3] boron-containing amino acids with pendant boron groups;[1b,4] as well as exocyclic boron-containing nucleosides with the boron found as a nucleobase,[5,6] sugar,[1b] or phosphate[7] substituent are also known. Known nucleobase analogues with endocyclic boron[1e,8-20] provide the most relevant structural information for the work described herein. To our knowledge, the task of incorporating such endocyclic boron

nucleobases into a nucleoside has not yet been accomplished.[21]

Our first attempt at synthesizing a borauracil analogue has previously been described and is shown here in Scheme 5. After being unsuccessful at preparing the desired analogue by methods adapted from work by Shaw[22] and others,[23] another approach was investigated. Gronowitz has prepared 1,2,3-diazaborine and 1,2-azaborine heterocycles *via* nickel desulfurization of the corresponding thieno-fused diaza- and azaborines.

Scheme 5. Attempted Synthesis of a Borathymidine Analogue



The advantage of the Gronowitz thieno-fused pathway is that the boron heterocycle can be constructed using a stable starting template, like the aromatic thiophene ring, instead of vinyl ureas or alkenyl boronates. Though Gronowitz has reported the synthesis of thieno-fused diaza- and azaborines, there are no reports describing the preparation of thieno-fused 1,5,2-diazaborin-3(2*H*)-ones (thieno-fused borauracils) using this, or any other, method prior to 1998. The few cases in which the 1,5,2-diazaborin-3(2*H*)-one ring system has been

reported depict this system stabilized as the benzo-fused 4-borauracil derivative. In the reported cases, the 1,5,2-diazaborin-3(2*H*)-one ring system was prepared by reaction of (2-aminophenyl)boronic acids with isocyanates. Unfortunately, direct extension of this approach in an attempt to obtain thieno-fused 4-borauracils would require the synthesis of (2-amino-3-thienyl)boronic acid. This route would be likely to suffer from the instability of 2-aminothiophenes lacking strong electron withdrawing groups.

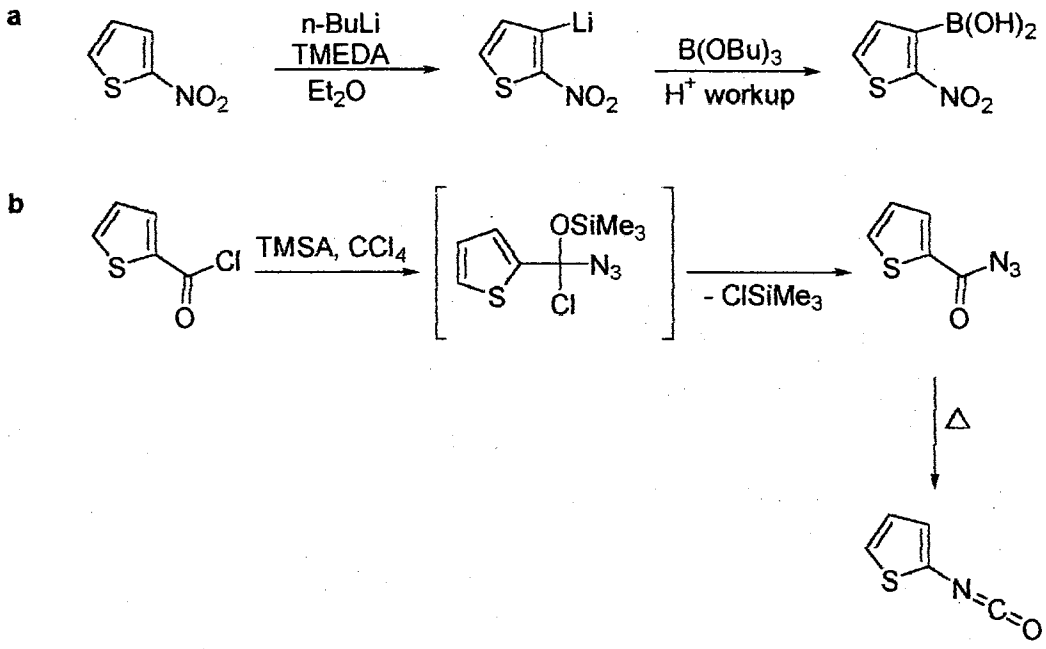
An alternative route to the boron heterocycle was investigated to avoid encountering the unstable 2-aminothiophene considered in the previous route. The alternative, a less common procedure involves synthesis of an acyclic precursor of the desired boron heterocycle followed by treatment with an appropriate boron reagent which introduces the boron atom and allows cyclization. Upon analysis of the desired thieno-fused 4-borauracil structure, 2-thienylurea was chosen as the target acyclic precursor for our synthetic route. Preparation of the urea can be considered from two obvious directions, via 2-aminothiophene or 2-thienylisocyanate. Approach via 2-aminothiophene still posed complications so 2-thienylisocyanate became the target intermediate for the preparation of 2-thienylurea.

Results and Discussion

Initially, attempts were made to convert 2-thienylnitrate to (2-thienylnitro)boronic acid by methods described by McMurry[24] and Gronowitz[25] (Scheme 6a). After several attempts at introducing the boron to the system in this manner, the synthesis of 2-thienylurea was approached. First, by the possibility of converting 2-thienophenecarbonyl chloride to

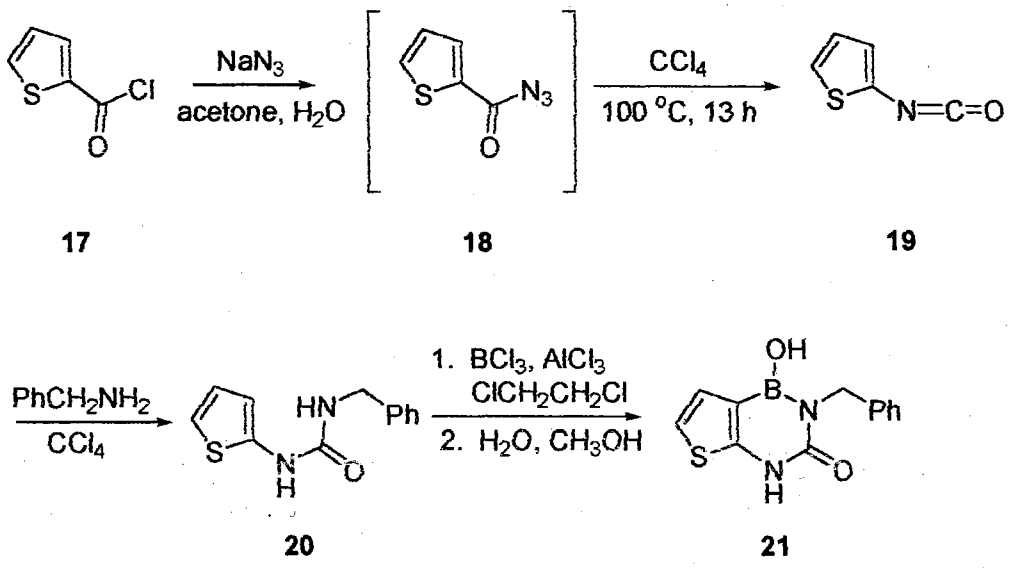
2-thienylisocyanate by the method of Toselli and Zanirato[26] (Scheme 6b). As this method proved unsuccessful, further investigation led to the formation of 2-thienophenecarbonyl

Scheme 6. Synthetic Studies Toward a Stable Template for Preparation of 2-Benzyl-1,4-dihydro-1-hydroxythieno[3,2-*c*][1,5,2]diazoborin-3(2*H*)-one



azide by conversion of 2-thienophenecarbonyl chloride using sodium azide in aqueous acetone (Scheme 7). Azide **18** was then converted to 2-thienylisocyanate (**19**) in 37% yield via Curtius rearrangement which required heating a carbon tetrachloride solution of **18** at 100 °C for 13 h. Examination of the ^{1}H NMR spectrum of the crude **19** after evaporation of the carbon tetrachloride showed that the 2-thienylisocyanate produced was sufficiently

Scheme 7. Preparation of *N*-Benzyl-*N'*-(2-thienyl)urea



17 **18** **19**

20 **21**

pure to proceed, although purification could be accomplished by vacuum distillation. The addition of benzylamine to a solution of isocyanate **19** in carbon tetrachloride precipitated N-benzyl-N'-2-thienylurea (**20**) as a white solid in 94% yield. Finally, 2-benzyl-1,4-dihydro-1-hydroxythieno[3,2-*c*][1,5,2]diazaborin-3(2*H*)-one (**21**) was produced by heating a solution of the urea **20** in 1,2-dichloroethane containing 1.3 equivalents of boron trichloride and catalytic aluminum chloride. The desired heterocycle **21** was purified by preparative HPLC (C_{18} column, 65:35 water:acetonitrile) and characterized by ^{1}H, ^{13}C, and ^{11}B NMR spectroscopy in addition to elemental analysis and high resolution fast atom bombardment mass spectrometry.

NMR Studies of

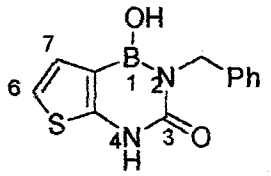
2-Benzyl-1,4-dihydro-1-hydroxythieno[3,2-*c*][1,5,2]diazaborin-3(2*H*)-one

^{1}H, ^{13}C, and ^{11}B NMR data for compound **21** were recorded in a variety of solvents to confirm the proposed structure. ^{1}H NMR data (Table 3) is in agreement with the proposed structure, as indicated by the observance of a single broad, exchangeable signal (the proton on N4), a single sharper, exchangeable signal (the proton on O1), two doublets (J = 5.4 Hz) for the thiophene protons (H6 and H7), and a singlet for the benzyl methylene group. The ^{13}C NMR data provided additional evidence for the structure of **21**. Compound **21** gave only nine observed carbon signals while urea **20** clearly showed ten carbon signals. This piece of data was expected due to the strong quadrupolar line broadening of boron[8,9,15] which generally precludes observation of the carbon signal for the carbon directly attached to the boron. Finally, the ability of the boron within compound **21** to remain trigonal planar when exposed to various solvents was investigated using ^{11}B NMR.

Boron-11 NMR spectroscopy has been used as a very sensitive probe of boron hybridization[26] to study the relative tendency of boron heterocycles to undergo a change from trigonal to tetrahedral due to solvent addition.[8-10,20] The chemical shifts (Table 3) of approximately 33 ppm using the boron trifluoride·etherate scale are indicative of a trigonal boron atom. We see no evidence for tetrahedral boron in the ^{11}B NMR spectra (no signal approximately 20-30 ppm upfield from the observed signal) in any of the solvents used, including methanol-d_4. However, previously reported data for the benzo-fused 1,5,2-diazaborin-3(2*H*)-one ring system indicated partial[10,20] or complete[20] conversion to the tetrahedral adduct in the presence of methanol-d_4. Though less sensitive as indicators of a change from trigonal to tetrahedral hybridization, the ^{1}H and ^{13}C NMR spectra also show no

evidence for tetrahedral boron. To date, there is no explanation for the apparent resistance of **21** versus the benzo-fused ring system towards tetrahedral adduct formation.

Table 3. ^{1}H and ^{11}B NMR Data for 2-Benzyl-1,4-dihydro-1-hydroxythieno[3,2-*c*][1,5,2]diazaborin-3(2*H*)-one (**21**)



	NH	OH	H6	H7	Ph	CH_2	B1 [d]
acetone -d_6 [a]	10.31	8.42	7.22	6.85	7.41 (app dm, 7.6 Hz)	4.87	33.5
	(brs)	(s)	(d, 5.4 Hz)	(d, 5.4 Hz)	7.26 (app tm, 7.6 Hz) 7.17 (app tm, 7.5 Hz)	(s)	
deuteriochloroform [b]	8.79	4.79	6.99	6.77	7.44 (app d, 6.9 Hz)	4.91	
	(brs)	(s)	(d, 5.4 Hz)	(d, 5.4 Hz)	7.34-7.22 (m)	(s)	
acetonitrile-d_3 [b]	9.46	7.01	7.15	6.81	7.32-7.19	4.80	33.7
	(brs)	(s)	(d, 5.4 Hz)	(d, 5.4 Hz)	(m)	(s)	
methanol-d_4 [c]	exch.	exch.	7.36	6.92	7.35-7.14	4.81	32.3
			(d, 5.5 Hz)	(d, 5.4 Hz)	(m)	(s)	

[a] At 600 MHz
[b] At 300 MHz
[c] At 400 MHz
[d] All ^{11}B NMR spectra were recorded at 128 MHz

Conclusions

The successful synthesis of 2-benzyl-1,4-dihydro-1-hydroxythieno[3,2-*c*][1,5,2]diazaborin-3(2*H*)-one (**21**) has renewed interest in obtaining other boron-containing analogues of nucleobases prepared by methods similar to those described above. To date, 2-benzyl-1,4-dihydro-1-hydroxythieno[3,2-*c*][1,5,2]diazaborin-3(2*H*)-one has not been

assayed to determine its susceptibility to glycosylation and phosphorylation or its usefulness as a therapeutic agent. However, with the data obtained through ^{11}B NMR, 2-benzyl-1,4-dihydro-1-hydroxythieno[3,2-*c*][1,5,2]diazaborin-3(2*H*)-one appears to remain unchanged in a variety of solvents. This stability was observed as the absence of a tetrahedral boron signal, indicating a lack of coordination between the solvent and boron atom. The maintenance of the trigonal boron suggests that if 2-benzyl-1,4-dihydro-1-hydroxythieno[3,2-*c*][1,5,2]diazaborin-3(2*H*)-one could be glycosylated and phosphorylated, there would be great expectations for the heterocyclic base to be able to mimic natural nucleotides during DNA and RNA replication.

Experimental

General synthetic procedures. NMR spectra were obtained on Gemini 300 or Unity Inova 400 or 600 MHz spectrometers from Varian. Unless otherwised stated, the ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ and referenced to either internal TMS or the central line of the residual solvent multiplet. The ^{11}B NMR spectra are reported on the boron trifluoride·etherate scale ($\delta = 0$ ppm); the spectra were referenced by setting the signal of an external trimethyl borate standard to 18.3 ppm. Infrared spectra were obtained in carbon tetrachloride solution on a System 2000 FT-IR instrument from Perkin-Elmer; ultraviolet spectra were obtained on a Hewlett-Packard 843 Diode Array Spectrophotometer; gas chromatography-mass spectrometry (electron ionization) was performed on a Hewlett-Packard G1800A GCD system; low resolution fast atom bombardment (LR-FAB) mass spectra were obtained on a model ZAB-2SE VG Analytical Limited spectrometer; the high resolution fast atom bombardment (HR-FAB) mass spectra were performed by the

Washington University Resource of Biomedical and Bio-organic Mass Spectrometry, St. Louis, Mo. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. The HPLC system used was a Waters 600E pump/controller outfitted with a 996 photodiode array detector. Analytical HPLC runs used a 4.6 x 260 mm (5 micron) MicrosorbMV™ C_{18} reversed-phase column (1 mL/min) from Rainin; preparative runs used a Dynamax™ 21.4 x 250 mm (8 micron) column (12 mL/min) also from Rainin. Melting points were determined on a Thomas Hoover apparatus and are uncorrected. All reactions were run under an inert atmosphere of nitrogen using glassware dried at 100°C. The carbon tetrachloride was dried by distillation from P_2O_5; all other chemicals and solvents were used as received. Unless otherwise noted, reactions were dried over Na_2SO_4 followed by filtration through glass wool. Solutions were evaporated *in vacuo* using a rotary evaporator at 30°C and 20 mm Hg (water aspirator). A cylinder of Drierite™ (W. Hammond) was placed in the vacuum line between the aspirator and rotary evaporator to protect the contents from moisture. Drying *in vacuo* refers to removal of residual solvent using an oil pump (<0.1 mm Hg) at room temperature. Thin-layer chromatography was accomplished using Machery-Nagel Polygram Sil G/UV™ plates with detection using either ultraviolet light (254 nm) or *p*-dimethylaminobenzaldehyde stain[28]. Chemicals were purchased from Aldrich and solvents from Fisher Scientific unless otherwise stated.

2-Thienylisocyanate (19). A solution of 2-thiophenecarbonyl chloride (4.0 mL, 5.6 g, 38 mmol) in acetone (20 mL) was cooled to 0 °C and treated by dropwise addition of a solution of sodium azide (2.87 g, 44.1 mmol) in water (24 mL). The cooled solution was stirred for 3.5 h until analysis by GC-MS indicated complete conversion of **17** to 2-

thiophenecarbonyl azide (**18**). FT-IR ν 2154, 2129 (N_3), 1687 (C=O). Carbon tetrachloride (20 mL) and saturated sodium bicarbonate (10 mL) were added, the layers separated, and the organic layer was dried and evaporated. Curtius rearrangement was performed by dissolving the above crude azide **18** in carbon tetrachloride (25 mL) and heating in a heavy-walled glass pressure vessel sealed with a threaded Teflon plug at 100°C for 13 h. **Caution: This step should be conducted behind a blast shield.** After cooling to room temperature, the solvent was removed *in vacuo* with protection from moisture and the residue distilled to give 1.72 g (37%) of **19**,[26] bp 71-74 °C (1.2 mm Hg). FT-IR ν 2277 (N=C=O). ^{1}H NMR (300 MHz) δ 6.92 (dd, 1 H, J = 5.6, 1.4 Hz, H5), 6.81 (dd, 1 H, J = 5.6, 3.7 Hz, H4), 6.68 (dd, 1 H, J = 3.7, 1.4 Hz, H3). ^{13}C NMR (75 MHz) δ 133.5, 125.8, 120.6, 120.4.

N-Benzyl-N'-2-(thienyl)urea (20). Benzylamine (550 μL, 0.54 g, 5.1 mmol) was added dropwise to a stirred solution of 2 *M* 2-thienylisocyanate (**19** in CCl_4, 2.5 mL, 0.63 g, 5.0 mmol) in carbon tetrachloride (37 mL) forming a white precipitate on contact. The mixture was stirred for 15 min then filtered and washed with carbon tetrachloride to isolate N-benzyl-N'-2-(thienyl)urea (1.10 g, 94%), mp 166-167 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35-7.23 (m, 5 H, Ar), 7.00 (dd, 1 H, J = 5.6, 1.4 Hz, H5), 6.86 (dd, 1 H, J = 5.6, 3.7 Hz, H4), 6.69 (dt, 1 H, J = 3.7, 1.4 Hz, H3), 6.49 (broad s, 1 H, NH), 5.19 (broad t, 1 H, *NH*-CH_2), 4.43 (d, 2 H, J = 5.9 Hz, CH_2); (300 MHz, acetone-d_6) δ 8.88 (broad s, 1 H, NH), 7.35-7.13 (m, 5 H, Ar), 6.75 (m, 2 H, H5 and H4), 6.45 (dd, 1 H, J = 3.2, 1.9 Hz, H3), 6.35 (broad s, 1 H, *NH*-CH_2), 4.42 (d, 2 H, J = 6.0 Hz, CH_2); (300 MHz, DMSO-d_6) δ 9.52 (broad s, 1 H, NH), 7.34-7.22 (m, 5 H, Ar), 6.75-6.67 (m, 3 H, H4, H5, and *NH*-CH_2), 6.14 (dd, 1 H, J = 3.2,1.9 Hz, H3), 4.41 (d, 2 H, J = 6.0 Hz, CH_2). ^{13}C NMR (75 MHz, acetone-d_6) δ 155.55,

143.33, 141.36, 129.32, 128.29, 127.82, 122.75, 116.42, 109.76, 44.34; (75 MHz, DMSO-d_6) δ 154.53, 142.21, 140.42, 128.43, 127.20, 126.87, 124.11, 115.21, 108.25, 42.95.

2-Benzyl-1,4-dihydro-1-hydroxythieno[3,2-*c*][1,5,2]diazaborin-3(2*H*)-one (21). To a stirred solution of 1.0 *M* BCl_3 in xylene (1.20 mL, 0.141 g, 1.20 mmol) in 1,2-dichloroethane (10 mL) was added via cannula over 25 minutes a solution of *N*-benzyl-*N'*-(2-thienyl)urea (**20**, 220 mg, 0.95 mmol) in 1,2-dichloroethane (50 mL). Aluminum chloride (10.0 mg, 0.08 mmol) was then added and the reaction refluxed under nitrogen. Reaction aliquots (100 µL) were removed, quenched with a mixture of water (500 µL), 200 m*M* sodium phosphate buffer (5 µL, *p*H 7.5 buffer), and methanol (1 mL) and analyzed by HPLC (C_{18} column, 4.6 x 250 mm, 1 mL/minute, 100:0 water:acetonitrile to 65:35 water:acetonitrile over 35 min). After 14 d, the reaction was quenched with a mixture of water (20 mL) and 200 m*M* sodium phosphate buffer (6 mL, *p*H 7.5 buffer). The organic layer was separated, dried, filtered, and evaporated. The crude product was purified by preparative HPLC (C_{18} column, 21.4 x 250 mm, 12 mL/min, 65:35 water:acetonitrile). Evaporation of solvent and drying *in vacuo* afforded 30 mg (13%) of the desired product, mp 109-112 °C. UV (CH_3CN) λ max 214 nm (ε 34,500), λ max 268 nm (ε 6,500); (CH_3OH) λ max 253 nm (ε 17,300), λ max 265 nm (ε 19,700). MS (LR-FAB, 3-nitrobenzyl alcohol matrix) *m/z* 259 (MH^+), 394 (MH^+ + matrix - H_2O); (LR-FAB, glycerol matrix) *m/z* 259 (MH^+), 333 (MH^+ + matrix - H_2O); (HR-FAB) *m/z* 259.0714 (MH^+) $C_{12}H_{11}BN_2O_2S$ requires 259.07136. ^1H NMR (600 MHz, acetone-d_6) δ 10.31 (broad s, 1 H, NH), 8.42 (s, 1 H, OH), 7.40 (apparent dm, 2 H, *J* = 7.6 Hz, Ar H2, H2'), 7.26 (apparent tm, 2 H, *J* = 7.6

Hz, Ar H3, H3'), 7.22 (d, 1 H, J = 5.4 Hz, H6), 7.17 (apparent tm, 1 H, J = 7.5 Hz, Ar H4), 6.85 (d, 1 H, J = 5.4 Hz, H7), 4.87 (s, 2 H, CH_2); (300 MHz, $CDCl_3$) δ 8.79 (broad s, 1 H, NH), 7.44 (apparent d, 2 H, J = 6.9 Hz, Ar H2, H2'), 7.34-7.22 (m, 3 H, Ar H3, H3', H4), 6.99 (d,1 H, J =5.4 Hz, H6), 6.77 (d,1 H, J =5.4 Hz, H7), 4.91 (s, 2 H, CH_2), 4.79 (s, 1 H, OH); (300 MHz, acetonitrile-d_3) δ 9.46 (broad s, 1 H, NH), 7.32-7.19 (m, 5 H, Ar), 7.15 (d, 1 H, J =5.5 Hz, H6), 7.01 (s, 1 H, OH), 6.81 (d, 1 H, J =5.4 Hz, H7), 4.80 (s, 2 H, CH_2); (400 MHz, methanol-d_4) δ 7.36 (d, 1 H, J =5.4 Hz, H6), 7.35-7.14 (m, 5 H, Ar), 6.92 (d, 1 H, J =5.4 Hz, H7), 4.81 (s, 2 H, CH_2); ^{13}C NMR (75 MHz, acetone-d_6) δ 156.4, 156.1, 141.5, 129.0, 128.9, 128.2, 127.5, 115.5, 44.8; (75 MHz, acetonitrile-d_3) δ 156.5, 156.2, 141.3, 129.4, 128.4, 128.3, 127.8, 116.2, 45.0; ^{11}B NMR (128 MHz, acetone-d_6) δ 33.5 ($\nu_{1/2}$ = 238 Hz); (128 MHz, acetonitrile-d_3) δ 33.7 ($\nu_{1/2}$ = 275 Hz); (128 MHz, methanol-d_4) δ 32.3. *Anal.* Calcd. for $C_{12}H_{11}BN_2O_2S$: C, 55.84; H, 4.30; N, 10.85. Found: C, 56.12; H, 4.63; N, 10.66.

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CHAPTER 4

STUDIES TOWARD 4-BORAURACIL

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With the prospect of treating cancer or HIV using boron neutron capture therapy, the search for stable boron-containing compounds continues. Focus has been placed on biological building blocks with a great deal of consideration being given to the components of DNA and RNA. The work presented within this chapter describes ongoing studies toward the synthesis of 4-borauracil, one of the many avenues for investigating potential boron neutron capture agents.

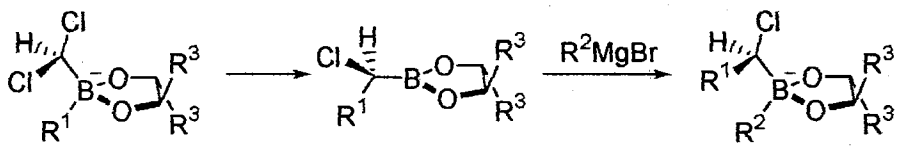
The explored avenue incorporates the concept of asymmetric synthesis using boronic esters to direct the insertion of a CHCl group into a boron-carbon bond. This type of synthesis has been under investigation since before 1980[1,2] and continues to be a promising route to many boron-containing molecules. During these investigations, the methods for inserting the α-halo group have relied on the use of organolithium reagents along with zinc chloride as a catalyst and chloride ion scavenger.[3] *In situ* generation of the common organolithium reagent, (dichloromethyl)lithium from dichloromethane and butyllithium at -100 °C or lithium diisopropylamine at temperatures up to -20 °C.[4]

This process of "homologation" or "chain extension" has been analyzed and a mechanism of action proposed to explain the migration of the alkyl group from the boron and the catalytic involvement of the zinc chloride. The mechanism does not involve any sort of enantioface selection, as is the case in most asymmetric syntheses. The initial

establishment of the asymmetric center involves selective displacement of one of the two diastereotopic halides during rearrangement.[3]

The sequential double diastereoselection seen with boronic esters of C_2-symmetry depends in the inversion of the carbon atom when the alkyl group migrates to displace one chloride ion from the dimethylborate complex **22** to form the (α-chloroalkyl)boronic ester **23**. Inversion places the remaining chloride of **23** in the vulnerable position for displacement in the subsequent borate complex **24**. The C_2-symmetry makes both faces of the boron atom equivalent in bornic ester intermediates such as **23**. This is not the case with pinanediol esters which show a nonequivalence at the boron in such intermediates.

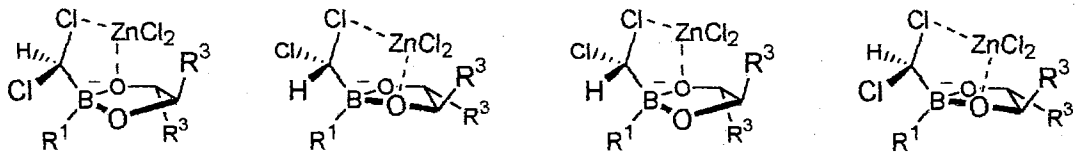
Figure 24. Alkyl Group Migration in the Dimethylborate Complex **22**.



The strong effect of zinc chloride in promoting and directing the migration of R^1 in anion **22** suggests that the zinc cation might complex with one of the boronic ester oxygens while assisting the chloride ion to depart. A comparison of two drawings of the transition state, **26** and **28**, was presented by Matteson to explain the observed products.[5] These tentative illustrations of the transition state led Corey and coworkers to write a more reasonable structure, **25**, based on some analogies and qualitative arguments.[6] Independently, Midland carried out a set of Gaussian calculations in which he discovered

that Corey's proposed structure gave a lower transition state energy than the three other possible structures.[7]

Figure 25. Effect on Zinc Chloride on Migration of the Alkyl Group (R^1).



25 **26** **27** **28**

These drawings may exaggerate the extent to which the five-membered 1,3,2-dioxaborolane (diol boronic ester) ring flexes so that the R^3 groups are pseudoaxial (**25**, **27**) or pseudoequatorial (**26**, **28**), but in any event the ring is not planar and eclipsing strain between R^3 and H on the adjacent carbon is avoided. 1,3,2-Dioxaborolanes containing trigonal boron (e.g. **23**) do have very nearly planar rings in spite of the eclipsing strain, presumably as a result of strong B-O π-bonding,[8] but even weak coordination of a fourth ligand to the boron atom is enough to make the ring nonplanar.[9]

Insertion of α-halo groups in these boronic esters allows for the introduction of other functional groups by substitution of the halide using standard methods. The functional groups that have been successfully incorporated include azido, alkoxy,[10] acyloxy,[11] alkyl,[10] bis(trimethylsilyl)amino,[12] α-tributylstannyl ethers,[13] and thiols.[3] In the instances examined to date, the boronic esters are stable under the variety of conditions necessary for these substitutions to occur. The chain extension and substitution work best with nonpolar substituents. Much effort has been expended in attempts to incorporate common functional substituents into substrates for the boronic ester chain extension. The pattern that has

emerged is that substituents of relatively low polarity work best. After hydrocarbons, ethers are the most compatible substrate. Highly polar functions such as *N*, *N*-dialkylamides appear to be incompatible with the chain extension reaction.[3]

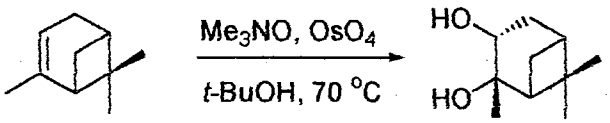
Results and Discussion

Chiral Directors

Three very different types of boronic esters were used in the work presented here. Initially, the idea of using asymmetric synthesis to form the desired basic ring structure of the nucleobase was investigated using pinanediol and (*S*, *S*)-1,2-dicyclohexyl-1,2-ethanediol ("*S*,*S*-DICHED") to protect the boronic acid and to direct the stereochemistry within the growing chain. The use of these chiral directors proved to be expensive and unnecessary due to the racemization of the α-carbon during acetoxy substitution. As a result, the boronic esters were prepared with less expensive pinacol. However, the preparation and stereo-directing properties of pinanediol and DICHED are worth noting.

Asymmetric (*S*)-pinanediol (Scheme 8, **29**) prepared from (+)-α-pinene by osmium tetraoxide catalyzed oxidation with trimethylamine *N*-oxide was introduced by Matteson and Ray in 1980 as an alternative to the published preparation using *N*-methylmorpholine *N*-oxide from 1976.[5] Pinanediol still remains a useful chiral director, even though its stereocontrol is not as precise as that of DICHED or other diols of C_2-symmetry. Once formed, pinanediol boronic esters are extremely resistant to hydrolysis allowing reactions to take place along the growing chain without loss of the ester. When treated with hydrogen peroxide, peroxidic deboronation occurs leaving the boronic ester completely intact and oxidizing the α-carbon in the process.[3]

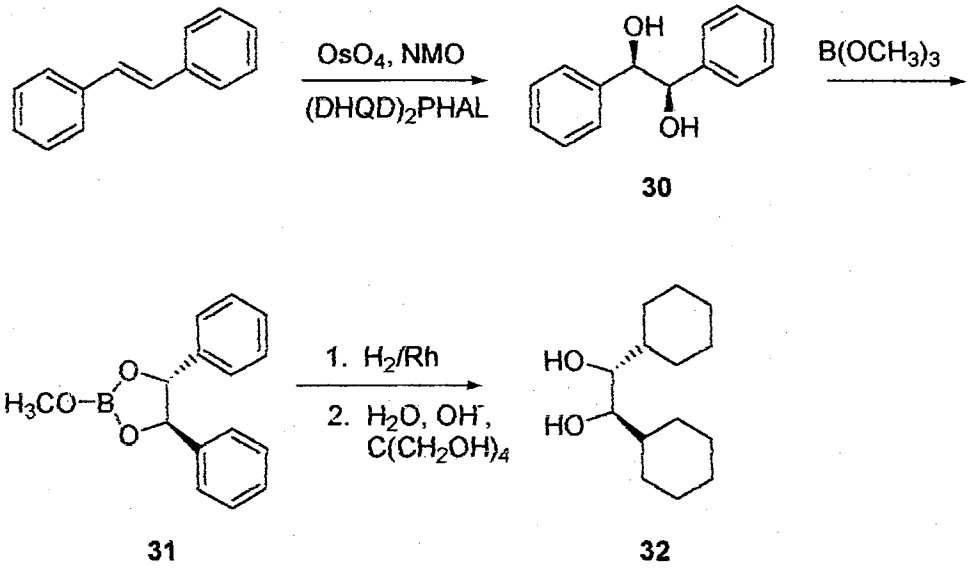
Scheme 8. Preparation of Pinanediol from (+)-α-Pinene.



29

DICHED (Scheme 9, **32**), introduced by Hoffmann and coworkers,[14] is a chiral director of C_2-symmetry. It can be obtained in large quantities by a four-step synthesis starting from *trans*-stilbene. Sharpless dihydroxylation using osmium tetraoxide in the presence of NMO and $(DHQD)_2PHAL$ gives (*R*)- or (*S*)-(*R**,*R**)-1,2-diphenylethane-1,2-diol (**30**).[15] Diol **30** is then converted to a borate ester **31** before hydrogenation over rhodium trichloride and alumina, generating a highly active catalyst *in situ* when hydrogen is introduced. Rhodium can then be recycled as the trichloride. The borate ester is finally hydrolyzed to DICHED (**32**).[16]

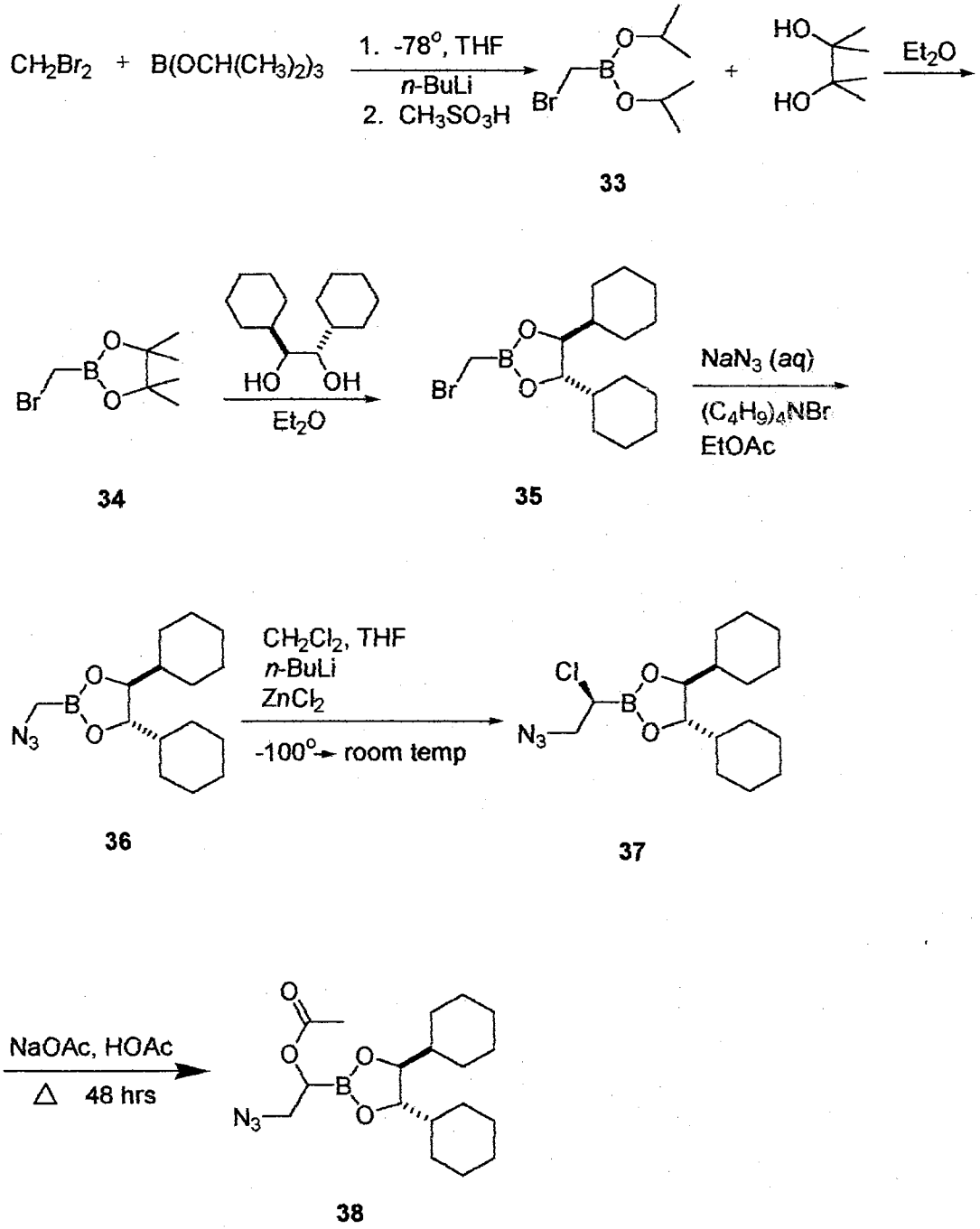
Scheme 9. Preparation of DICHED from *trans*-Stilbene via Sharpless Oxidation.



Synthetic Investigations

Investigations into the preparation of an acyclic precursor to the desired nucleobase began with the preparation of diisopropyl bromomethyl boronate (**33**) from triisopropyl borate (Scheme 10). Once the initial boronic ester **33** was prepared, the ester protecting group could be exchanged to provide better stereocontrol. In the case of the (*S*,*S*)-DICHED ester, **33** must first be converted to the pinacol ester **34**. This conversion allows for a rapid exchange with DICHED resulting in the formation of a more stable ester of C_2-symmetry **35**. In addition to added stability, exchanging the pinacol group with DICHED increases the molecular mass of the ester. Stable, high molecular weight compounds are desired when working with azido compounds to reduce the likelihood of creating explosive materials.

Scheme 10. Synthetic Studies Involving DICHED Boronates.

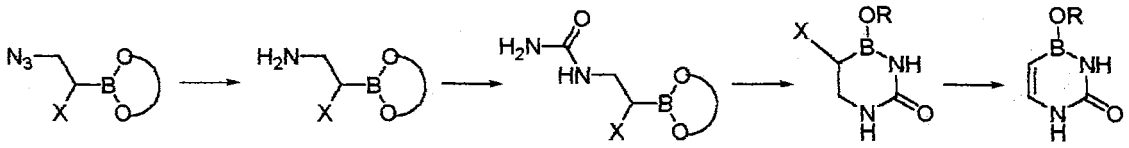


Synthesis of the azidomethyl boronic ester **36** was accomplished by a phase transfer reaction with sodium azide prior to homologation to insert a CHCl group next to the boron

atom.[5] This insertion is highly stereospecific resulting in the isolation of almost entirely the *S*-enantiomer of **37**.

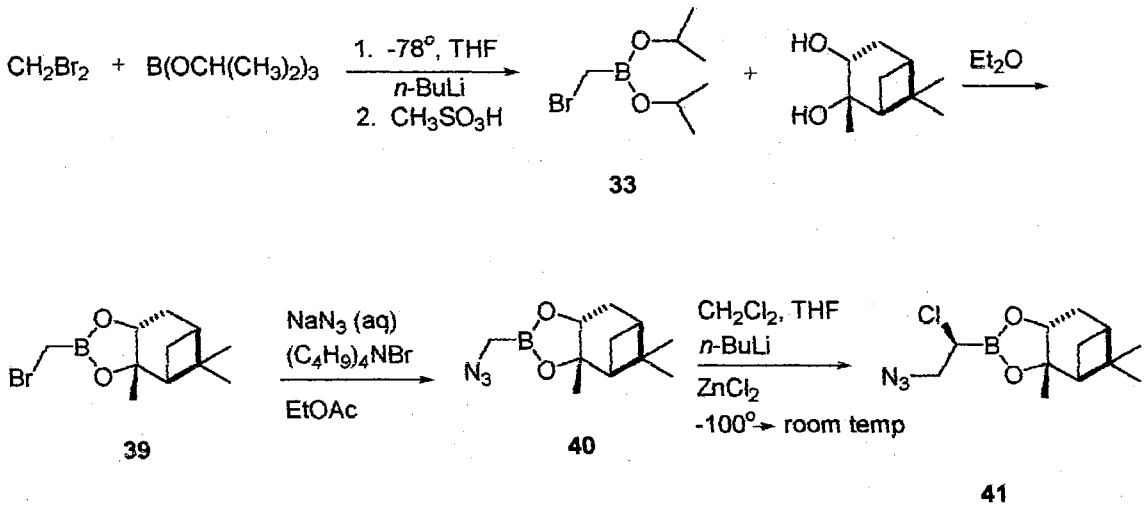
Substitution of the newly introduced Cl using sodium acetate in acetic acid results in the racemization of the α-carbon (**38**) indicating that DICHED is not a reasonable choice for this synthetic method. However, the desired product does not necessarily need to be enantiomerically pure, as the final step in the proposed synthesis is the elimination of the acetoxy group to form a double bond within the ring of the nucleobase (Figure 26).

Figure 26. Proposed Synthetic Pathway to 4-Borauracil.



Upon realizing that the use of DICHED would not result in selective substitution, investigations were continued using S-pinanediol as the stereodirector with the idea that the asymmetric nature of the pinanediol might promote formation of one enantiomer over the other in the acetoxy substitution. Preparation of **41** (Scheme 11) is analogous to the previously described synthesis of **37**. However, the synthesis of (*S*)-pinanediol 1-acetoxy-2-azidoethyl boronate was not carried out due to the lack of stereoselectivity in the formation of α-chloroboronic ester **41**. At this point, it was decided that asymmetric synthesis was neither necessary nor advantageous for the pursuit of the desired nucleobase.

Scheme 11. Synthetic Studies Involving Pinanediol Boronates.

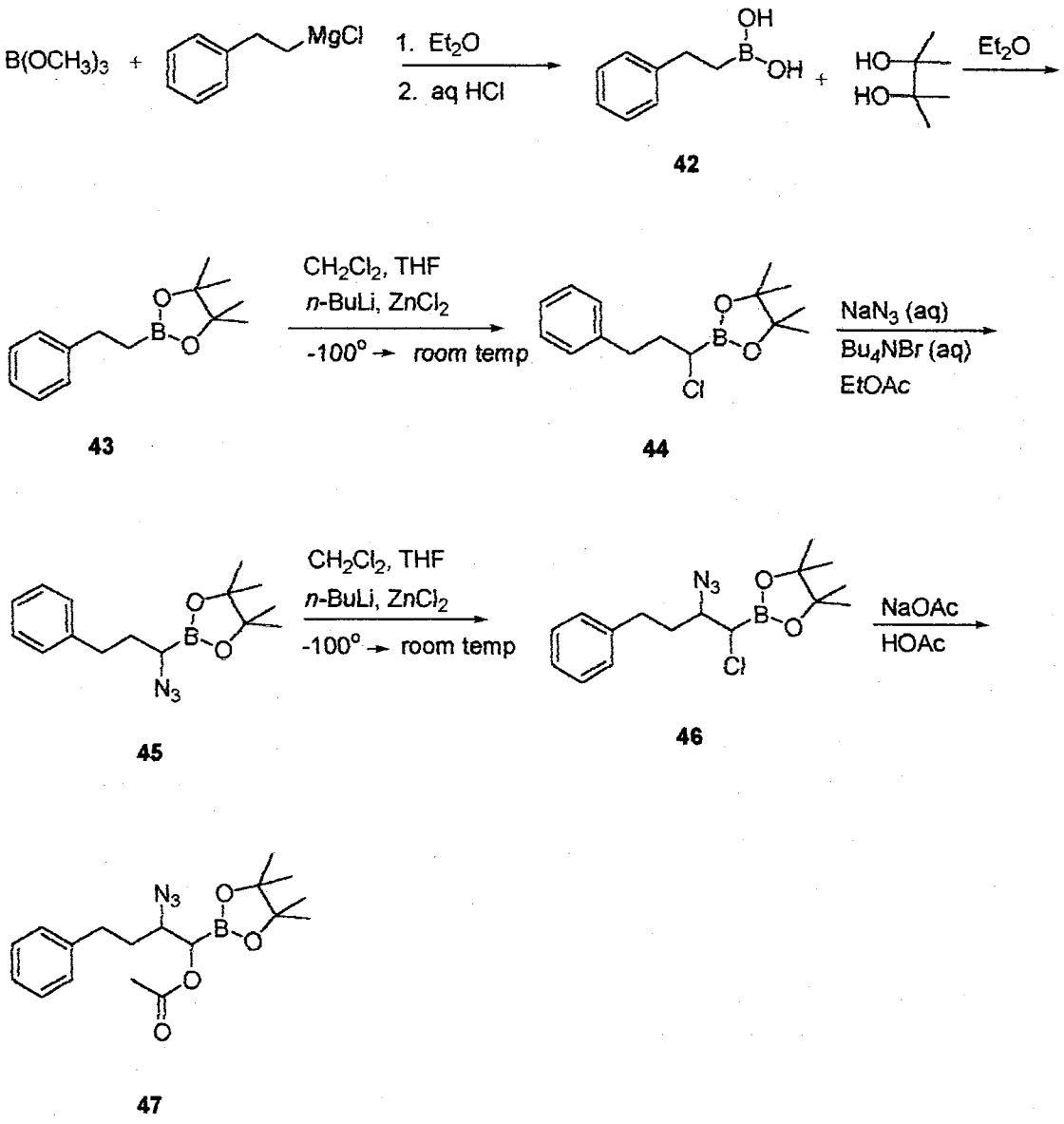


In addition, synthetic complications arose during attempts to reduce the azido group to the corresponding amine. With the azido group in either the α- or β-position, the typically uncomplicated reduction causes the decomposition of the α- and β-azido boronic esters. In the case of the 1-acetoxy-2-azidoboronic esters **37** and **41**, the decomposition appears to *proceed* by loss of the acetoxy group in addition to disruption of the ester.

Due to the results of the attempted reductions, the question arose as to whether the azide could possibly be reduced in the presence of a boronic ester. An investigation into the effect of the azide position on the likelihood of the reduction being successful began with the preparation of phenethylboronic acid (**42**) as a means of introducing an internal azide rather than a terminal azide as seen before (Scheme 12). The other reason for incorporating the longer chain was to aid in the identification and stabilization of decomposition products if reduction of the azide were to cause the dissociation of the azido-portion of the molecule

from the boronic ester. In the earlier cases, the azide portion of the molecule was never isolated or identified.

Scheme 12. Synthetic Studies Based on Pinacol Boronates.



Protection of the boronic acid was achieved using pinacol as opposed to the more expensive chiral directors, (*S*,*S*)-DICHED and (*S*)-pinanediol, for the purpose of

investigating the azide reduction. The pinacol ester **43** was then subjected to homologation resulting in formation of the α-chloroboronic ester **44**. Azide substitution followed by a second homologation gave the β-azido-α-chloroboronic ester **46**. For the investigation to be complete, **46** had to be converted to the α-acetoxy compound **47**. Preparation of this series of compounds allowed additional studies on α- and β-azido reduction. Once again, reduction using catalytic hydrogenation, sodium borohydride, or sodium cyanoborohydride gave decomposition of the pinacol boronic esters (**45**, **46**, and **47**).

Boron-11 NMR Studies of Compounds 42-47

Boron-11 NMR data for the pinacol boronates (**42** - **47**) were recorded in a variety of solvents to confirm the proposed structures. Specifically of interest was the ability of the boron to remain trigonal planar when exposed to various conditions. Boron-11 NMR spectroscopy has been used as a sensitive probe of hybridization. The chemical shifts of approximately 33 ppm using the boron trifluoride·etherate scale are indicative of a trigonal boron atom.

Only one set of conditions shows evidence for tetrahedral boron in the ^{11}B NMR spectra (a signal of approximately 20-30 ppm upfield). As expected, reaction of phenethylboronic acid (**42**) with NaOH led to the observation of a signal at 4.3 ppm. The fact that no other tetrahedral signal was observed is encouraging for the future of these synthetic methods, in that the acetoxy, azido, and chloro substituents appear to have little or no effect on the stability of the boron atom.

Table 4. Chemical Shifts and Peak Widths of ^{11}B Signals for Compounds **42-47**

		^{11}B Chemical Shift		*tri- or*
Compound	**Solvent**	**(ppm)**	**$v_{1/2}$ (Hz)**	**tetracoordinate**
42	chloroform-*d*	31.80	139.4	Tricoordinate
	chloroform-*d* + NaOH	31.60	301.5	Tricoordinate
		4.31	545.2	Tetracoordinate
	acetone-d_6	32.46	149.9	Tricoordinate
	acetonitrile-d_3	32.35	113.9	Tricoordinate
	methanol-d_4	31.57	97.2	Tricoordinate
	deuterium oxide	32.16	339.4	Tricoordinate
43	chloroform-*d*	33.14	237.3	Tricoordinate
	chloroform-*d* + NaOH	33.09	237.2	Tricoordinate
	acetone-d_6	33.97	117.5	Tricoordinate
	acetonitrile-d_3	32.35	116.8	Tricoordinate
	methanol-d_4	33.82	163.1	Tricoordinate
44	chloroform-*d*	30.66	260.9	Tricoordinate
	chloroform-*d* + NaOH	29.76		Tricoordinate
	acetone-d_6	31.31	136.8	Tricoordinate
	acetonitrile-d_3	31.15	150.2	Tricoordinate
	methanol-d_4	31.21	181.0	Tricoordinate
45	chloroform-*d*	31.37	380.8	Tricoordinate
	chloroform-*d* + NaOH	31.44		Tricoordinate
	acetone-d_6	32.03	156.6	Tricoordinate
	acetonitrile-d_3	31.86	117.3	Tricoordinate
	methanol-d_4	31.80	220.9	Tricoordinate
46	chloroform-*d*	31.11	335.0	Tricoordinate
	chloroform-*d* + NaOH	30.53	348.5	Tricoordinate
	acetone-d_6	30.25	273.6	Tricoordinate
	acetonitrile-d_3	30.97	314.4	Tricoordinate
	methanol-d_4	31.17		Tricoordinate
47	chloroform-*d*	33.02	238.0	Tricoordinate
	acetone-d_6	34.00	141.8	Tricoordinate
	acetonitrile-d_3	33.64	154.3	Tricoordinate
	methanol-d_4	33.72	183.4	Tricoordinate

Conclusions

Though obstacles were encountered in the reduction of the β-azido boronic ester, research continues to probe the use of asymmetric synthesis as a means of constructing acyclic precursors to boron-containing heterocycles. Current investigations are exploring other methods of introducing the desired β-amino group in addition to searching for conditions to accomplish the azide reduction attempted in these studies.

Fortunately, the ^{11}B NMR research gave one piece of valuable information about the stability of these compounds. Once synthesized, the boronic esters are stable in the presence of strong aqueous bases. This knowledge reduces the concern of possible side reactions resulting in the loss of the boron entity and allows consideration of other reactions along the growing chain.

Experimental

General Synthetic Procedures. NMR spectra were obtained on Mercury 300 or Unity Inova 400 or 600 MHz spectrometers from Varian. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, unless otherwise stated, and referenced to either internal TMS or to the central line of the residual solvent multiplet. Infrared spectra were obtained using a System 2000 FT-IR instrument from Perkin-Elmer. Low and high resolution electron-impact (LR-EI and HR-EI) mass spectra were performed by the Washington State University Mass Spectrometry Lab. Elemental analyses were performed by Desert Analytics, Tucson, AZ. Melting points were determined on a MelTemp apparatus and are uncorrected. All reactions were run under an inert atmosphere of argon or nitrogen using glassware dried at 100°C. Tetrahydrofuran was dried over benzophenone ketyl. Unless otherwise noted, reactions are

dried over Na_2SO_4 followed by filtration through glass wool. Solutions were evaporated *in vacuo* using a rotary evaporator at 30°C and 20 mm Hg (water aspirator). Drying *in vacuo* refers to removal of residual solvent using an oil pump (<0.1 mm Hg) at room temperature. Thin-layer chromatography was accomplished using Whatman™ Flexible-Backed adsorption plates with aluminum backing with detection using either ultraviolet light (254 nm) or ceric ammonium sulfate stain.[17] Purification was accomplished by column chromatography[18] on silica using combinations of pentane, ether, and hexanes as the main solvents. Chemicals were purchased from Aldrich and solvents from Fisher Scientific unless otherwise stated.

Diisopropyl Bromomethyl Boronate (33). A 1-L 3-necked round-bottomed flask equipped with a stirring mechanism (stir bar for small scale, mechanical stirrer for large scale), an Ar line and a septum was placed in an ambient temperature acetone or ethanol bath. THF (375 mL), triisopropyl borate (58 mL, 47 g, 251 mmol), and dibromomethane (23 mL, 57 g, 303 mmol) were added to the flask and the bath cooled to –78 °C. The septum was then replaced by an addition funnel. *n*-Butyllithium (1.6 *M* in hexanes, 138 mL, 301 mmol) was added slowly over 2.5 h via the addition funnel. After the addition, the reaction was allowed to stir for 10 min then methanesulfonic acid (17 mL, 25 g, 262 mmol) was added over 10 min. The reaction mixture was allowed to warm slowly to room temperature overnight. The THF was distilled off at atmospheric pressure using an oil bath (small scale) or steam bath (large scale). After the THF was removed, the flask was cooled, the mechanical stirrer was replaced by a stopper, and vacuum distillation of the mixture continued at room temperature to remove any remaining traces of solvent. The receiving flask was cooled to –78 °C and the distillation temperature raised to 90-100 °C until

distillation stopped. Additional purification was accomplished using fractional vacuum distillation to obtain 26.8 g (48%) of diisopropyl bromomethyl boronate as a clear, colorless liquid, bp 51 °C at 8 mm Hg. **Caution: Diisopropyl bromomethyl boronate is a lachrymator.** MS (HR-EI) *m/z* 272.0580 (M^{+}) $C_{11}H_{18}BBrO_2$ requires 272.0583. ^{1}H NMR δ 4.45 (septet, 2 H, J = 6.1 Hz, CH), 2.54 (s, 2 H, CH_2Br), 1.20 (d, 12 H, J = 6.1 Hz, CH_3). ^{13}C NMR δ 66.5, 24.68. The carbon attached to the boron was not observed.

Pinacol Bromomethyl Boronate (34). A solution of pinacol (55 g, 47 mmol) in ether (50 mL) was added via cannula to a round-bottomed flask containing diisopropyl bromomethyl boronate (**33**, 103 g, 46.2 mmol) and the reaction was stirred at room temperature for 24 h. Evaporation of the solvent yielded a crude mixture of products which was distilled to yield 91.8 g (90%) of diisopropyl bromomethyl boronate, bp 35 °C at 0.3 mm Hg. ^{1}H NMR δ 2.59 (s, 2 H, $BrCH_2$), 1.29 (s, 12 H, CH_3). ^{13}C NMR δ 84.72, 24.88.

(*S*,*S*)-1,2-Dicyclohexyl-1,2-ethanediol Bromomethyl Boronate (35). (*S*,*S*)-DICHED (9.20 g, 40.6 mmol) was added to a round-bottomed flask containing pinacol bromomethyl boronate (**34**, 9.13 g, 41.3 mmol) in ether (75 mL) and the reaction was stirred at room temperature for 24 h. The resulting organic phase was extracted with H_2O (2 x 20 mL), dried, and filtered. Evaporation of the solvent yielded a crude mixture of products which was distilled at less than 1 mm Hg. All impurities were removed below 100°C. Distillation afforded 9.24 g (68%) of pure (*S*,*S*)-DICHED bromomethyl boronate. ^{1}H NMR δ 3.96 (apparent d, 2 H, O-CH), 2.62 (s, 2 H, CH_2Br), 1.83-0.90 (m, 20 H, ring H's). ^{13}C NMR δ 84.55, 43.17, 33.69, 28.57, 27.59, 26.75, 26.62, 26.52, 26.34, 26.19.

(*S*,*S*)-1,2-Dicyclohexyl-1,2-ethanediol Azidomethyl Boronate (36). (*S*,*S*)-DICHED bromomethyl boronate (**35**, 9.23 g, 28.0 mmol) was dissolved in ethyl acetate (10 mL). Aqueous sodium azide (24.9 g, 383 mmol) in 50 mL H_2O and tetrabutylammonium bromide (1.87 g, 5.80 mmol) in 10 mL H_2O were then added to the stirred boronic ester solution and the reaction stirred under Ar for 24 h. The layers were then separated and the aqueous layer extracted with ether (2 x 20 mL). The combined organic layers were evaporated to yield 8.3 g (102%) of (*S*,*S*)-1,2-dicyclohexyl-1,2-ethanediol azidomethyl boronate as a pale yellow syrup.

(*S*, *S*)-1,2-Dicyclohexyl-1,2-ethanediol 2-Azido-1-chloroethyl Boronate (37). To a 1-L, 3-necked round-bottomed flask equipped with a mechanical stirrer, a septum, and an Ar inlet was added THF (170 mL) and CH_2Cl_2 (6.8 mL, 9.0 g, 106 mmol). The flask was cooled to -100 °C using ethanol, dry ice, and liquid N_2. To the cooled THF/CH_2Cl_2 solution was added 1.6 *M n*-butyllithium in hexanes (35 mL, 3.6 g, 56 mmol) slowly down the walls of the flask. The flask was rinsed with THF (5 mL) and allowed to stir at -100 °C for 30 min. [Note: If the solution turns black during this 30 min period, do not proceed. The resulting solution should be colorless to pale yellow at the end of the 30 min.] A solution of (*S*,*S*)-1,2-dicyclohexyl-1,2-ethanediol azidomethyl boronate (**36**, 8.15 g, 28.0 mmol) in THF (15 mL) was then added via syringe directly to the stirred solution while avoiding the walls of the flask. The mixture was stirred for an additional 30 min at -100 °C. A 1.0 *M* solution of $ZnCl_2$ in ether (65 mL, 8.9 g, 65 mmol) was then added over 10 min using a dropping funnel and the mixture was allowed to warm slowly overnight to room temperature. Ether (100 mL) was added to the flask and the solution washed with 50%

aqueous NH_4Cl (2 x 35 mL). The organic layer was separated, dried, and evaporated to yield 9.09 g (96%) of (*S,S*)-1,2-dicyclohexyl-1,2-ethanediol 2-azido-1-chloroethyl boronate as a pale yellow syrup. ^1H NMR δ 3.98 (d, 2 H, O-CH), 3.72-3.55 (m, 3 H, CH_2-N_3 and CH-Cl), 1.90-0.80 (m, 20 H, cyclohexyl). ^{13}C NMR δ 84.5, 54.6, 42.8, 28.2, 27.2, 26.4, 26.0, 25.9.

(*S,S*)-1,2-Dicyclohexyl-1,2-ethanediol 1-Acetoxy-2-azidoethyl Boronate (38). *S,S*-DICHED 1-chloro-2-azidoethyl boronate (**37**, 1.00 g, 2.94 mmol), sodium acetate (242 mg, 2.94 mmol) and acetic acid (2.8 mL) were combined in a reflux apparatus and heated at 75°C for 48 h. The acetic acid was evaporated and the resulting syrup purified by silica gel chromatography (pentane→ether gradient)to yield 228 mg (22%) of (*S,S*)-1,2-dicyclohexyl-1,2-ethanediol 1-acetoxy-2-azidoethyl boronate. FT-IR ν 2099 (N_3), 1734 (C=O). ^1H NMR δ 4.34 (ddd, 1 H, CH-OAc), 3.89 (apparent. t, 2 H, O-CH), 3.65-3.48 (m, 2 H, CH_2N_3), 2.13 (s, 3 H, CH_3), 1.83-0.85 (m, 20 H, cyclochexyl). ^{13}C NMR δ 172.7, 84.5, 52.1, 43.0, 28.8, 27.8, 26.8, 26.3, 26.2, 20.6.

(*S*)-Pinanediol Bromomethyl Boronate (39). A solution of (*S*)-pinanediol (4.08 g, 24.0 mmol) in ether (25 mL) was added via cannula to a round-bottomed flask containing diisopropyl bromomethyl boronate (**33**, 5.26 g, 23.6 mmol) and the reaction was stirred at room temperature for 2 h. Evaporation of the solvent yielded a crude mixture of products which was chromatographed on silica gel (30:1 pentane:ether). Pure (*S*)-pinanediol bromomethyl boronate was recovered in 89% yield as a clear syrup, R_f 0.20 (30:1 pentane:ether). MS (HR-EI) *m/z* 272.0580 (M^+) $C_{11}H_{18}BBrO_2$ requires 272.0583. ^1H NMR

δ 4.38 (dd, 1 H, J = 8.8, 1.9 Hz, OCH), 2.64 (s, 2 H, CH_2Br), 2.40-2.22 (m, 2 H, ring H), 2.09 (apparent t, 1 H, ring H), 1.96-1.87 (m, 2 H, ring H), 1.42 (s, 3 H, CH_3), 1.30 (s, 3 H, CH_3), 1.20 (d, 1 H, J = 11.2 Hz, CH), 0.85 (s, 3 H, CH_3).

(*S*)-Pinanediol Azidomethyl Boronate (40). (*S*)-Pinanediol bromomethyl boronate (**39**, 2.99 g, 11.0 mmol) was dissolved in ethyl acetate (40 mL). Aqueous sodium azide (25 g, 385 mmol) in 85 mL H_2O and tetrabutylammonium bromide (1.77 g, 5.49 mmol) in 25 mL H_2O were then added to the stirred boronic ester solution and the reaction was stirred under Ar for 5 d. The layers were then separated and the aqueous layer extracted with ether (2 x 20 mL). The combined organic layers were evaporated to yield 2.2 g (84%) of (*S*)-pinanediol azidomethyl boronate as a pale yellow syrup. ^{1}H NMR δ 4.36 (dd, 1 H, J = 8.8, 1.9 Hz, OCH), 3.10 (s, 2 H, CH_2N_3), 2.39-2.22 (m, 2 H, ring H), 2.08 (dt, 1 H, J = 4.5, 3.0 Hz, ring H), 1.96-1.92 (m, 1 H, ring H), 1.88 (dd, 1 H, J = 3.3, 1.9 Hz, CH), 1.43 (s, 3 H, CH_3), 1.30 (s, 3 H, CH_3), 1.13 (d, 1 H, J = 11.2 Hz, CH), 0.85 (s, 3 H, CH_3). ^{13}C NMR δ 87.0, 78.7, 51.3, 39.7, 38.4, 35.4, 28.8, 27.3, 26.8, 24.3.

(*S*)-Pinanediol 2-Azido-1-chloroethyl Boronate (41). To a 1-L, 3-necked round-bottomed flask equipped with a mechanical stirrer, a septum, and an Ar inlet was added THF (60 mL) and CH_2Cl_2 (2.5 mL, 3.3 g, 39 mmol). The flask was cooled to -100 °C using ethanol, dry ice, and liquid N_2. To the cooled THF/CH_2Cl_2 solution was added 1.6 *M n*-butyllithium in hexanes (13.3 mL, 1.36 g, 21.3 mmol) slowly down the walls of the flask. The flask was rinsed with THF (5 mL) and allowed to stir at -100°C for 30 min. [Note: If the solution turns black during this 30 min period, do not proceed. The resulting solution

should be colorless to pale yellow at the end of the 30 min.] A solution of (*S*)-pinanediol azidomethyl boronate (**40**, 2.5 g, 10.6 mmol) in THF (8 mL) was then added via syringe directly to the stirred solution while avoiding the walls of the flask. The mixture was stirred for an additional 30 min at -100 °C. A 1.0 *M* solution of $ZnCl_2$ in ether (21.2 mL, 2.98 g, 21.2 mmol) was then added over 10 min using a dropping funnel and the mixture was allowed to warm slowly overnight to room temperature. Ether (40 mL) was added to the flask and the solution washed with 50% aqueous NH_4Cl (2 x 20 mL). The organic layer was separated, dried, and evaporated to yield 3.9 g (130%) of (*S*)-pinanediol 2-azido-1-chloroethyl boronate.

Phenethylboronic Acid (42). To a 1-L, 3-necked round-bottomed flask equipped with a mechanical stirrer, an Ar inlet, and a septum was added ether (300 mL). The flask was cooled to –78 °C before trimethyl borate (35 mL, 32 g, 308 mmol) was added via syringe. The mixture was stirred and the septum replaced by an addition funnel. A 1.0 *M* solution of phenethylmagnesium chloride in THF (300 mL, 300 mmol) was introduced via syringe into the addition funnel and slowly added to the reaction flask over 45 min. The reaction was allowed to stir for 30 min then warmed to room temperature. The reaction mixture was poured over 300 mL of ice and acidified with 2 *M* HCl (200 mL) then poured back into the reaction vessel to dissolve the solid which had clumped in the flask. The biphasic solution was separated and the aqueous layer extracted with ether (2 x 200 mL). The combined ether extracts were washed with water (100 mL) and saturated NH_4Cl (100 mL) then dried and evaporated. The crude product was used directly in the synthesis of **74**.

^{1}H NMR δ 7.35-7.15 (m, 5 H, Ph), 5.75 (br s, 2 H, OH), 2.75 (t, 2 H, J = 8.2 Hz, CH_2-Ph), 1.15(t, 2 H, J = 8.2 Hz, CH_2-B). ^{13}C NMR δ 144.1, 128.2, 127.7, 125.5, 30.23.

Pinacol 2-Phenethyl Boronate (43). A solution of pinacol (36.4 g, 308 mmol) in ether (125 mL) was added to a solution of phenethylboronic acid (**42**, 45.0 g, 300 mmol) in ether (125 mL) and the reaction stirred at room temperature for 30 min. An additional portion of ether (100 mL) was added and the solution washed with water (2 x 50 mL) followed by saturated NH_4Cl (50 mL). The ether layer was dried and evaporated. Simple distillation of the crude product served to purify 51.5 g (71.4% based on phenethylmagnesium chloride) of pinacol 2-phenethyl boronate, bp 110 °C at <1 mm Hg. ^{1}H NMR δ 7.30-7.12 (m, 5 H, Ar), 2.75 (t, 2 H, J = 8.2 Hz, CH_2Ph), 1.22 (s, 12 H, CH_3), 1.15 (t, 2 H, J = 8.2 Hz, CH_2B). ^{13}C NMR δ 144.2, 128.1, 127.9, 125.4, 83.1, 30.0, 24.9.

Pinacol 1-Chloro-3-Phenylpropyl Boronate (44). To a 1-L, 3-necked round-bottomed flask equipped with a mechanical stirrer, a septum, and an Ar inlet was added THF (500 mL) and CH_2Cl_2 (29 mL, 38 g, 452 mmol). The flask was cooled to -100 °C using ethanol, dry ice, and liquid N_2. To the cooled THF/CH_2Cl_2 solution was added 1.6 M *n*-butyllithium in hexanes (88 mL, 9.0 g, 141 mmol) slowly down the walls of the flask. The flask was rinsed with THF (5 mL) and allowed to stir at -100 °C for 30 min. [Note: If the solution turns black during this 30 min period, do not proceed. The resulting solution should be colorless to pale yellow at the end of the 30 min.] A solution of pinacol 2-phenethyl boronate (**43**, 27.04 g, 116.5 mmol) in THF (5 mL) was then added via syringe directly to the stirred solution while avoiding the walls of the flask. The mixture was stirred for an

additional 30 min at -100 °C. A 1.0 *M* solution of $ZnCl_2$ in ether (235 mL, 32.0 g, 235 mmol) was then added over 10 min using a dropping funnel and the mixture was allowed to warm slowly overnight to room temperature. Ether (150 mL) was added to the flask and the solution washed with 50% aqueous NH_4Cl (150 mL). The organic layer was separated, dried, and evaporated to yield 38.6 g (118%) of 1-chloro-3-phenylpropyl pinacol boronate as an orange-brown syrup. ^1H NMR δ 7.33-7.18 (m, 5 H, Ar), 3.43 (t, 1 H, J = 7.4 Hz, CH_2Cl), 2.82 (ddt, 2 H, J = 7.4, 7.1, 6.2 Hz, CH_2Ph), 2.13 (dt, 2 H, J = 7.4, 7.1 Hz, CH_2), 1.30 (s, 12 H, CH_3). ^{13}C NMR δ 140.9, 128.5, 128.3, 125.9, 84.4, 35.7, 33.3, 24.8.

Pinacol 1-Azido-3-phenylpropyl Boronate (45). To a stirred solution of pinacol 1-chloro-3-phenylpropyl boronate (**44**, 38.57 g, 137.5 mmol) in EtOAc (30 mL) was added aqueous sodium azide (36.17 g, 556.3 mmol) in 90 mL H_2O and aqueous tetrabutylammonium bromide (7.02 g, 21.8 mmol) in 10 mL H_2O. The reaction mixture was heated at 75°C for 18 h protected by Ar and a reflux condenser. The reaction was cooled to room temperature and the phases separated. The aqueous phase was washed with ether (15 mL). The combined organic phases were then washed with saturated NH_4Cl (25 mL), separated, dried, and evaporated. The crude syrup was purified using silica gel chromatography (pentane→ether gradient) to give 19.7 g (59%) of pinacol 1-azido-3-phenylpropyl boronate from pinacol phenethyl boronate. FT-IR ν 2095 (N_3). ^1H NMR δ 7.32-7.17 (m, 5 H, Ar), 3.11 (t, 1 H, J = 7.2 Hz, CH_2N_3), 2.82-2.71 (m, 2 H, CH_2Ph), 1.97 (dt, 2 H, J = 7.7, 7.2 Hz, CH_2), 1.28 (s, 12 H, CH_3). ^{13}C NMR δ 141.1, 128.5, 128.4, 128.3, 125.9, 84.5, 33.4, 32.5, 24.9, 24.8.

Pinacol 2-Azido-1-chloro-4-phenylbutyl Boronate (46). To a 1-L, 3-necked round-bottomed flask equipped with a mechanical stirrer, a septum, and an Ar inlet was added THF (500 mL) and CH_2Cl_2 (20 mL, 26 g, 312 mmol). The flask was cooled to -100 °C using ethanol, dry ice, and liquid N_2. To the cooled THF/CH_2Cl_2 solution was added 1.6 *M n*-butyllithium in hexanes (60 mL, 6.1 g, 96 mmol) slowly down the walls of the flask. The flask was rinsed with THF (5 mL) and allowed to stir at -100 °C for 30 min. [Note: If the solution turns black during this 30 min period, do not proceed. The resulting solution should be colorless to pale yellow at the end of the 30 min.] A solution of pinacol 1-azido-3-phenylpropyl boronate (**45**, 23.18 g, 80.72 mmol) in THF (5 mL) was then added via syringe directly to the stirred solution while avoiding the walls of the flask. The mixture was stirred for an additional 30 min at -100°C. A 1.0 *M* solution of $ZnCl_2$ in ether (165 mL, 22.5 g, 165 mmol) was then added over 10 min using a dropping funnel and the mixture was allowed to warm slowly overnight to room temperature. Ether (150 mL) was added to the flask and the solution washed with 50% aqueous NH_4Cl (150 mL). The organic layer was separated, dried, and evaporated to obtain 34.8 g (129%) of pinacol 2-azido-1-chloro-4-phenylbutyl boronate. FT-IR ν 2102 (N_3). ^{1}H NMR δ 7.4-7.2 (m, 5 H, Ph), 5.3 (s, 2 H, O-CH), 3.7-3.6 (m, 1 H, CH-Cl), 3.5 (dd, 2 H, CH_2-Ph), 2.9-2.6 (m, 2 H, CH_2-N_3), 2.0 (s, 2 H, CH_2), 1.1 (s, 12 H, CH_3).

Pinacol 1-Acetoxy-2-azido-4-phenylbutyl Boronate (47). A mixture of pinacol 2-azido-1-chloro-4-phenylbutyl boronate (**46**, 10.03 g, 29.9 mmol), sodium acetate (2.48 g, 30.3 mmol), and acetic acid (30 mL) was heated at 75 °C for 63 h. The excess acetic acid was removed by simple vacuum distillation. The crude mixture was dissolved in ether and

washed with water to remove any excess salts and neutralized with NaOH. After separation of the layers and evaporation of the ether the product was chromatographed (pentane → ether gradient) to yield 1.9 g (18%) of pinacol 1-acetoxy-2-azido-4-phenylbutyl boronate. FT-IR ν 2101 (N_3), 1730 (C=O). MS (HR-EI) *m/z* 359.2016 (MH^+) $C_{18}H_{26}BN_3O_4$ requires 360.2054.

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Plate I: ^{1}H NMR of Compound **3** in chloroform-*d*

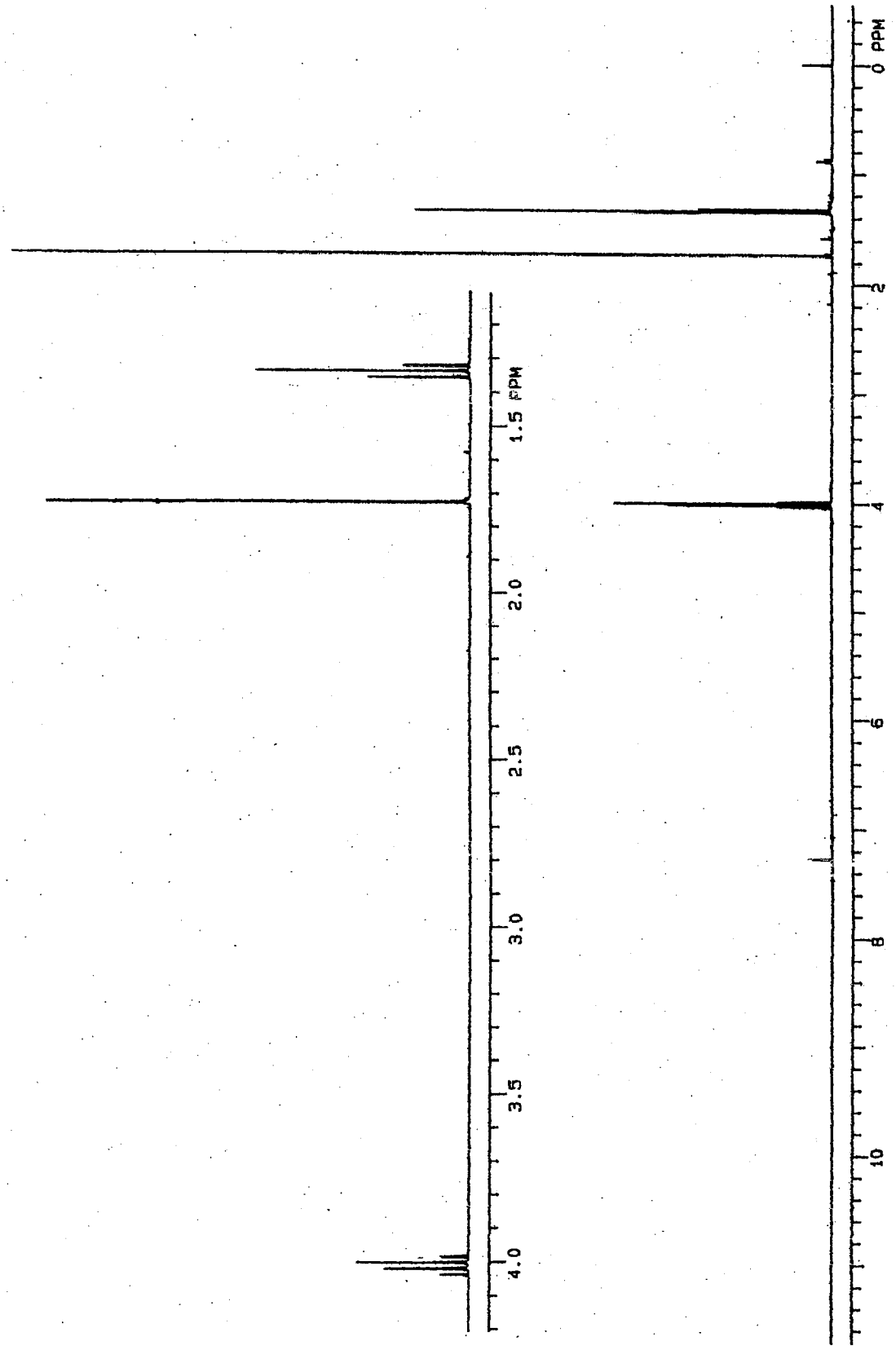


Plate II: ^{13}C NMR of Compound **3** in chloroform-*d*

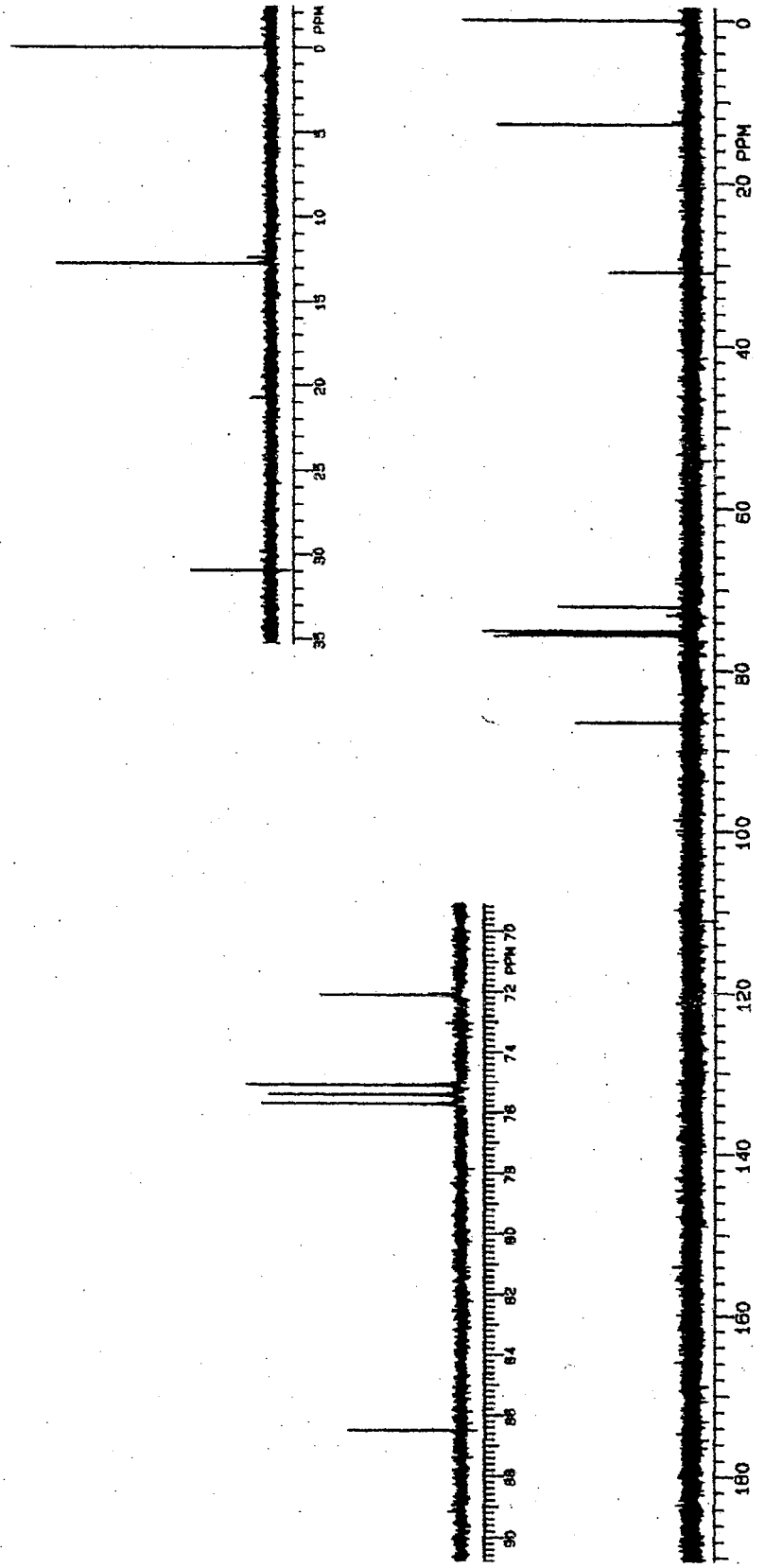


Plate III: ^{1}H NMR of Compound **5** in chloroform-*d*

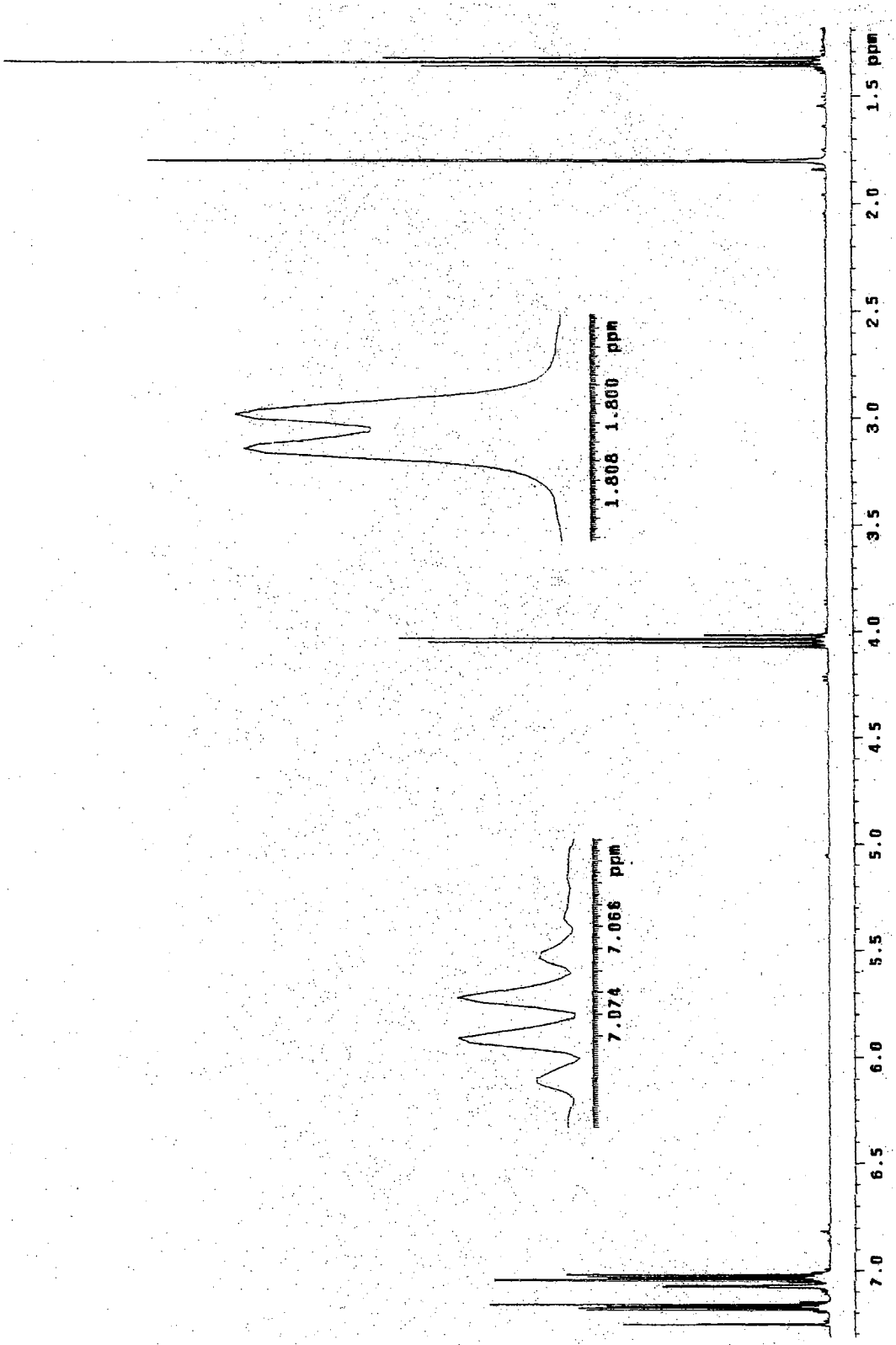


Plate IV: ^{13}C NMR of Compound **5** in chloroform-*d*

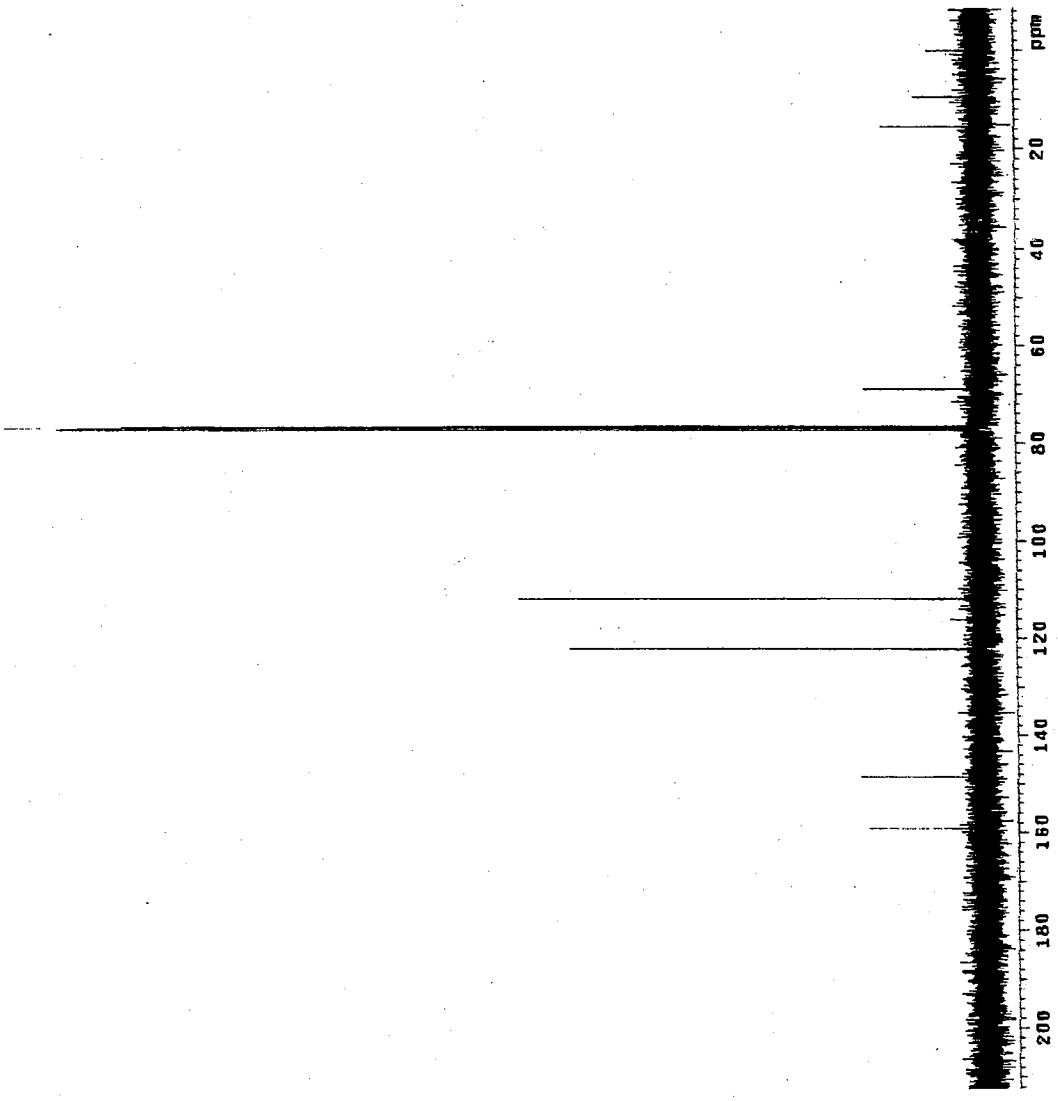


Plate V: ^{1}H NMR of Compound 7 in chloroform-*d*

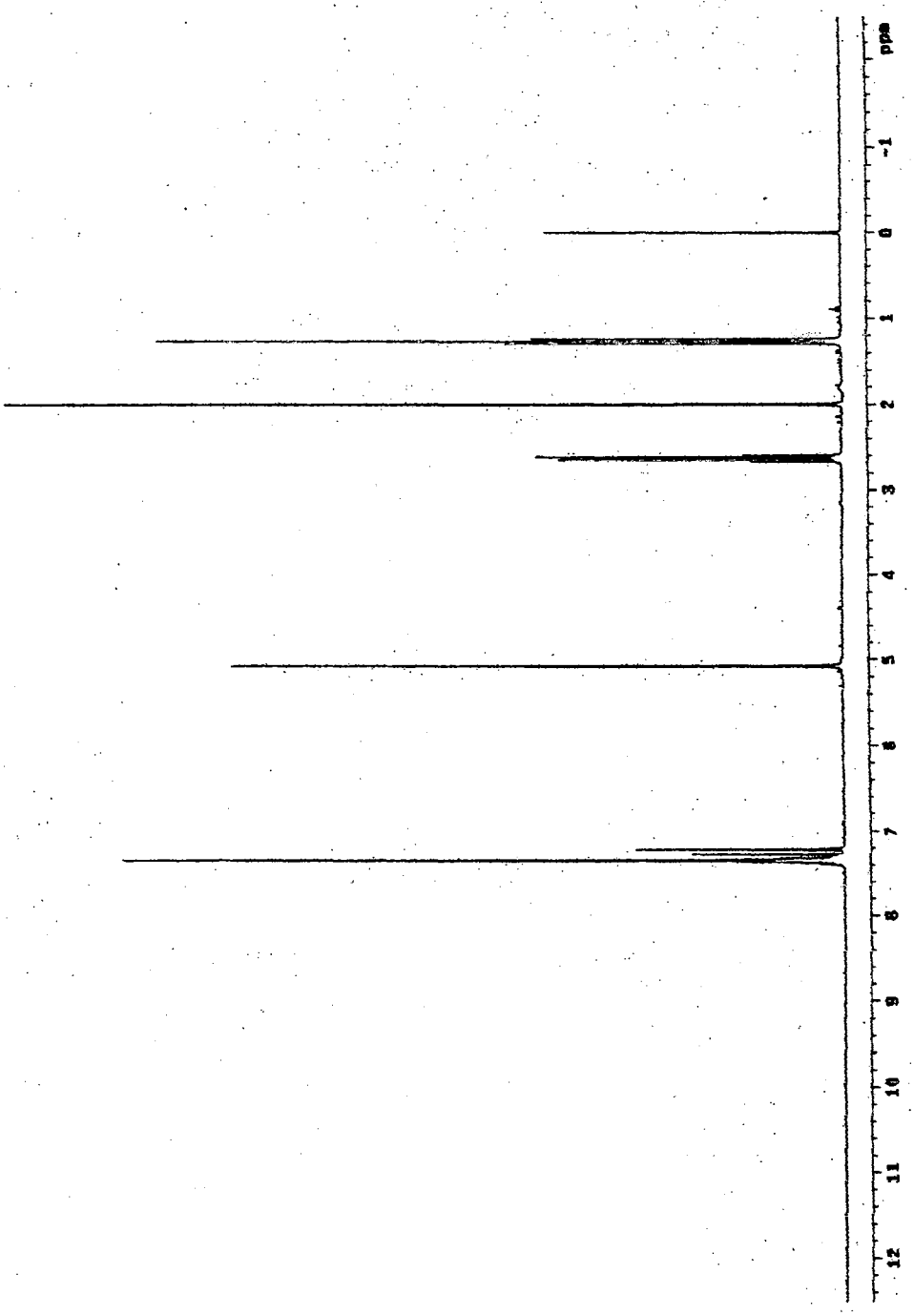


Plate VI: ^{13}C NMR of Compound **7** in chloroform-*d*

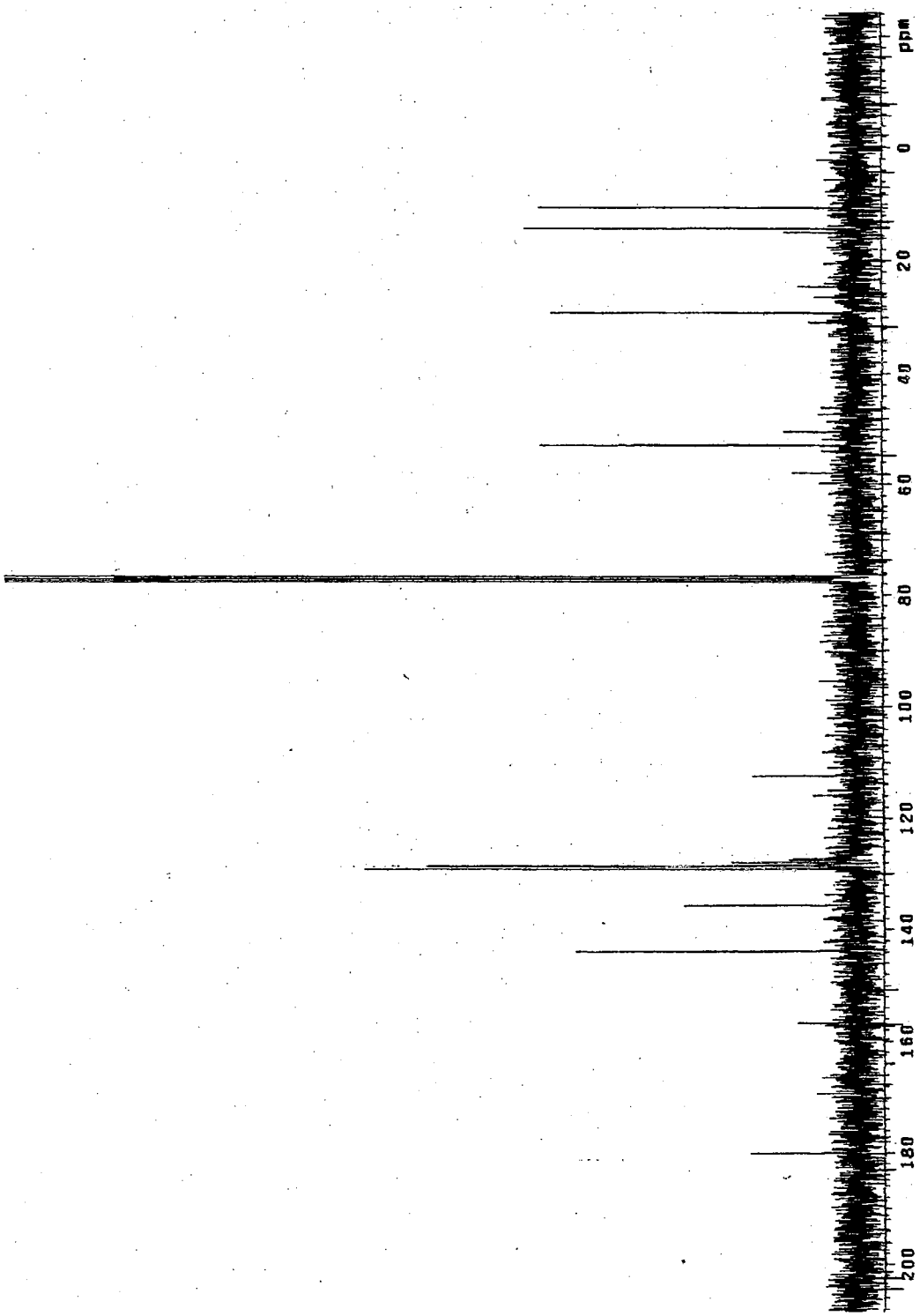


Plate VII: ^{1}H NMR of Compound **10** in chloroform-*d*

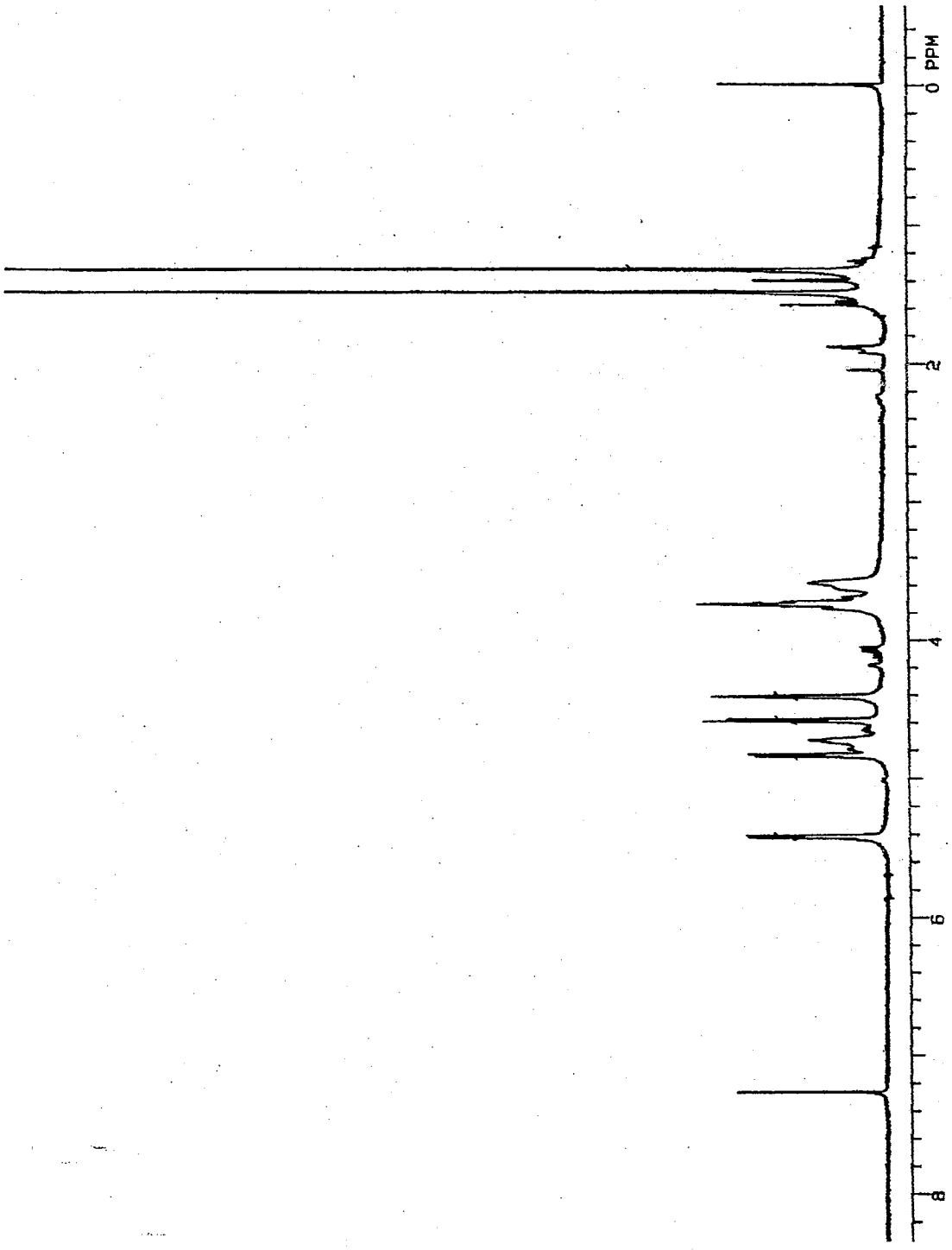


Plate VIII: ^{1}H NMR of Compound **11** in chloroform-*d*

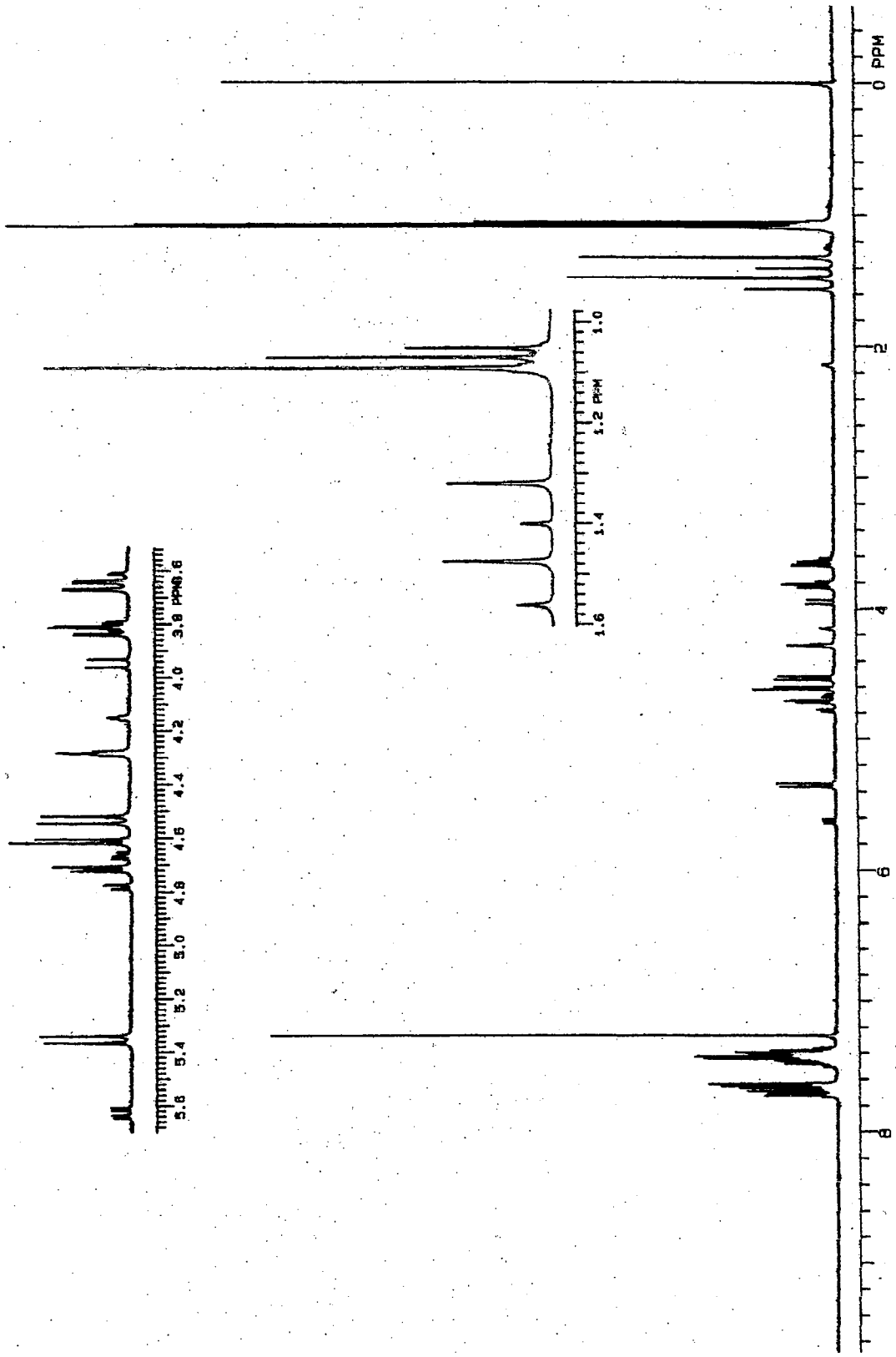


Plate IX: ^{13}C NMR of Compound **11** in chloroform-*d*

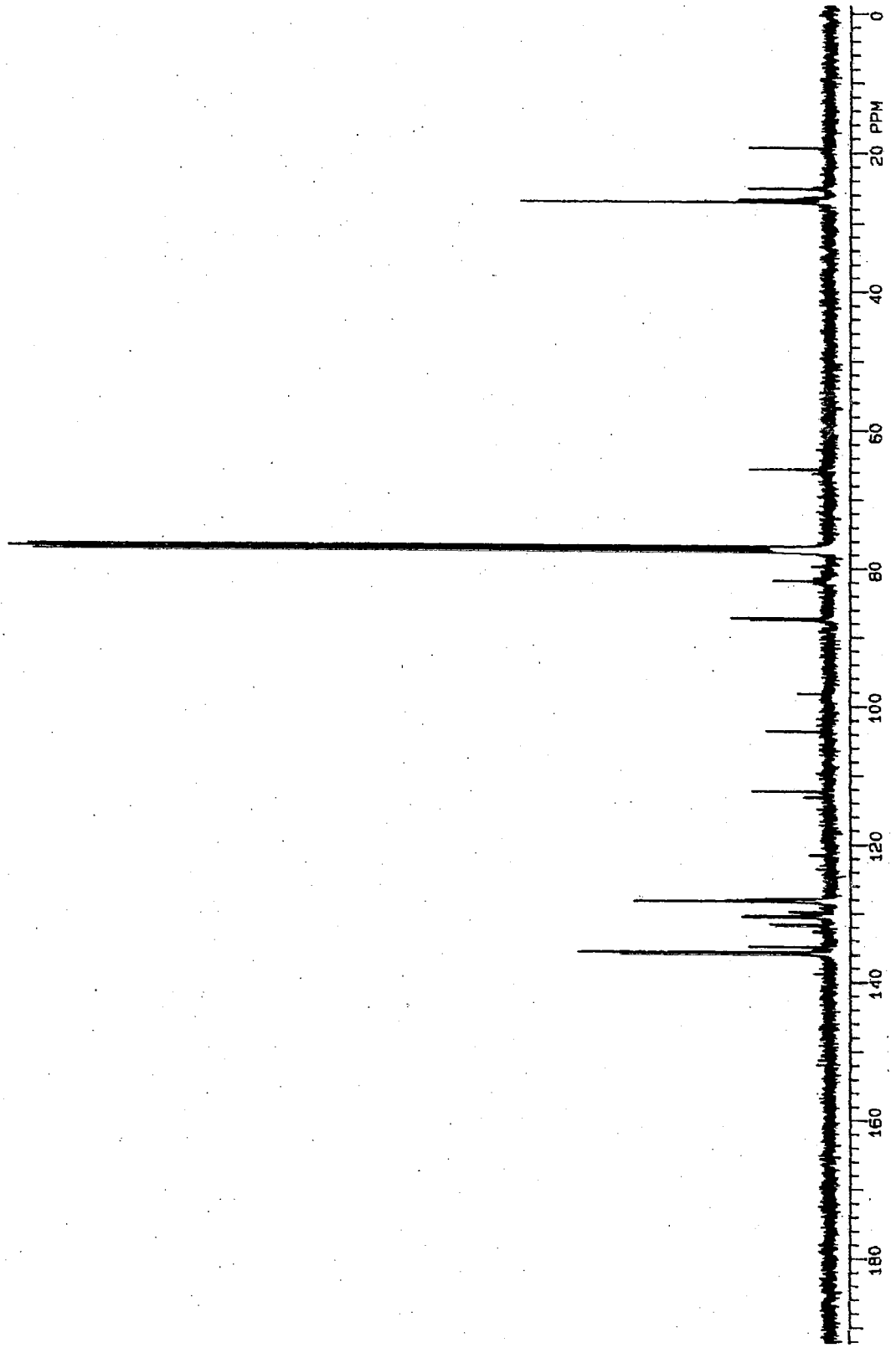


Plate X: ^{1}H NMR of Chloride Intermediate in Preparation of Compound **12** in chloroform-*d*

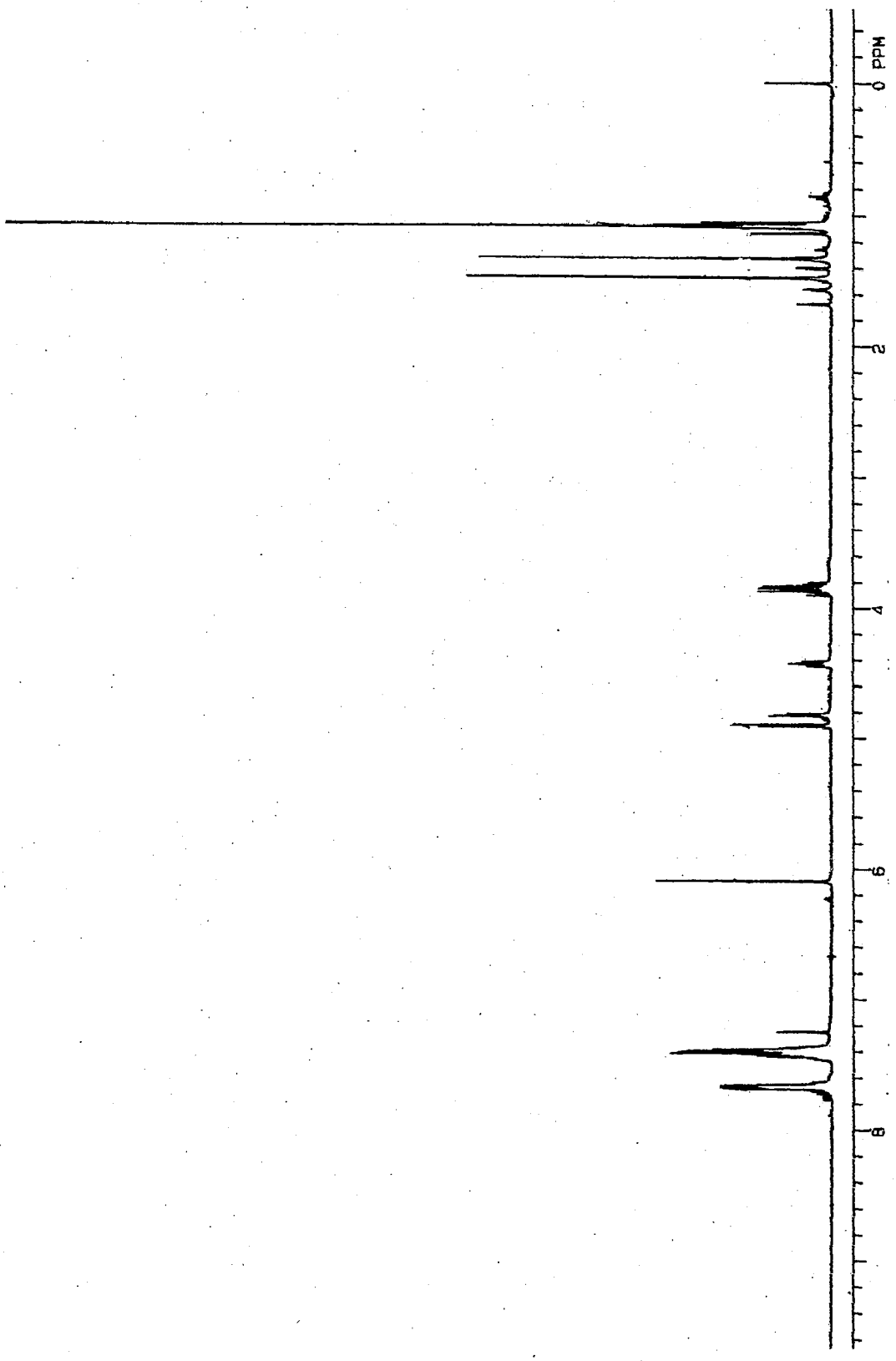


Plate XI: ^{13}C NMR of Chloride Intermediate in Preparation of Compound **12** in chloroform-*d*

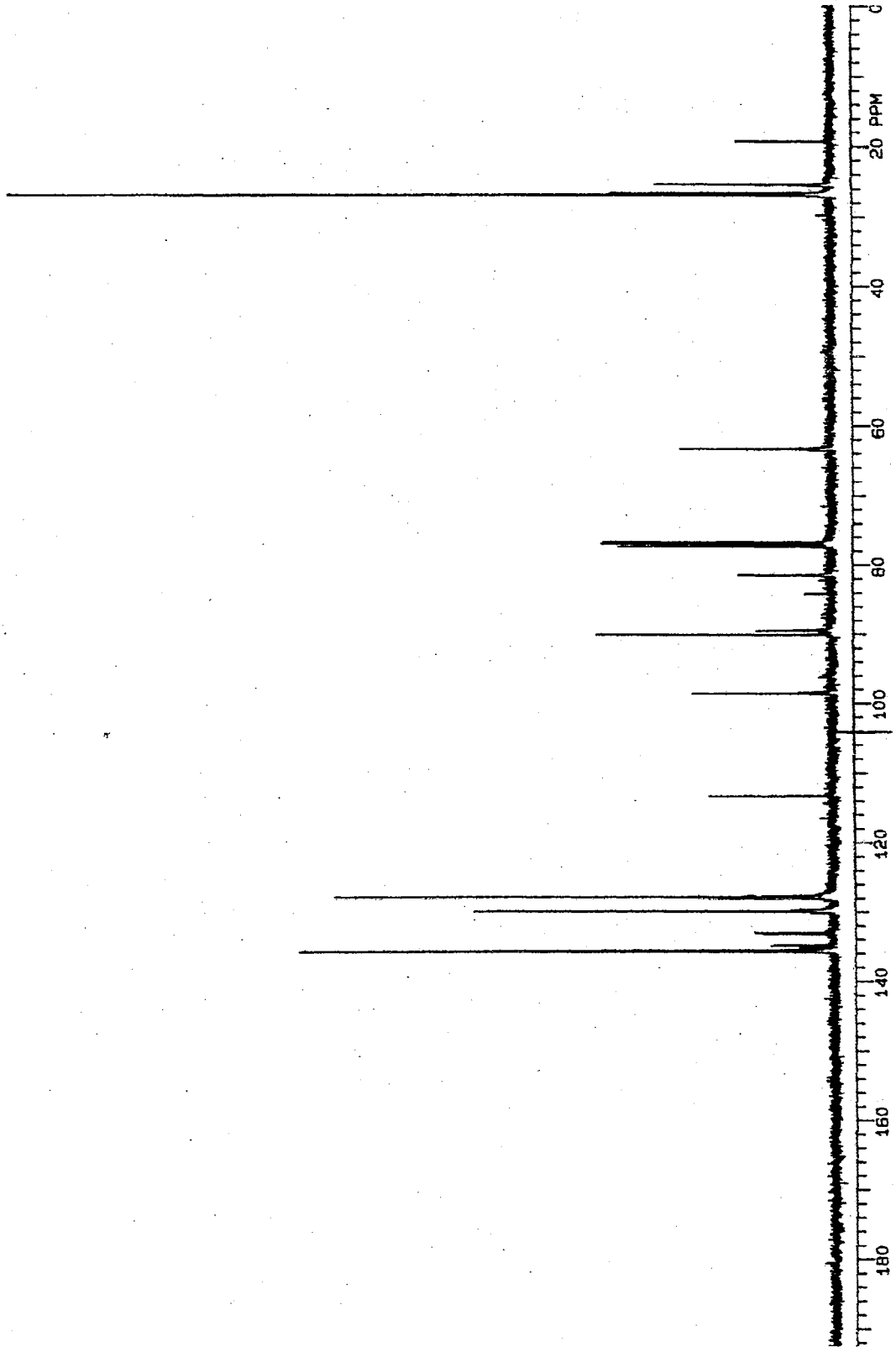


Plate XII: FT-IR of Compound **12**

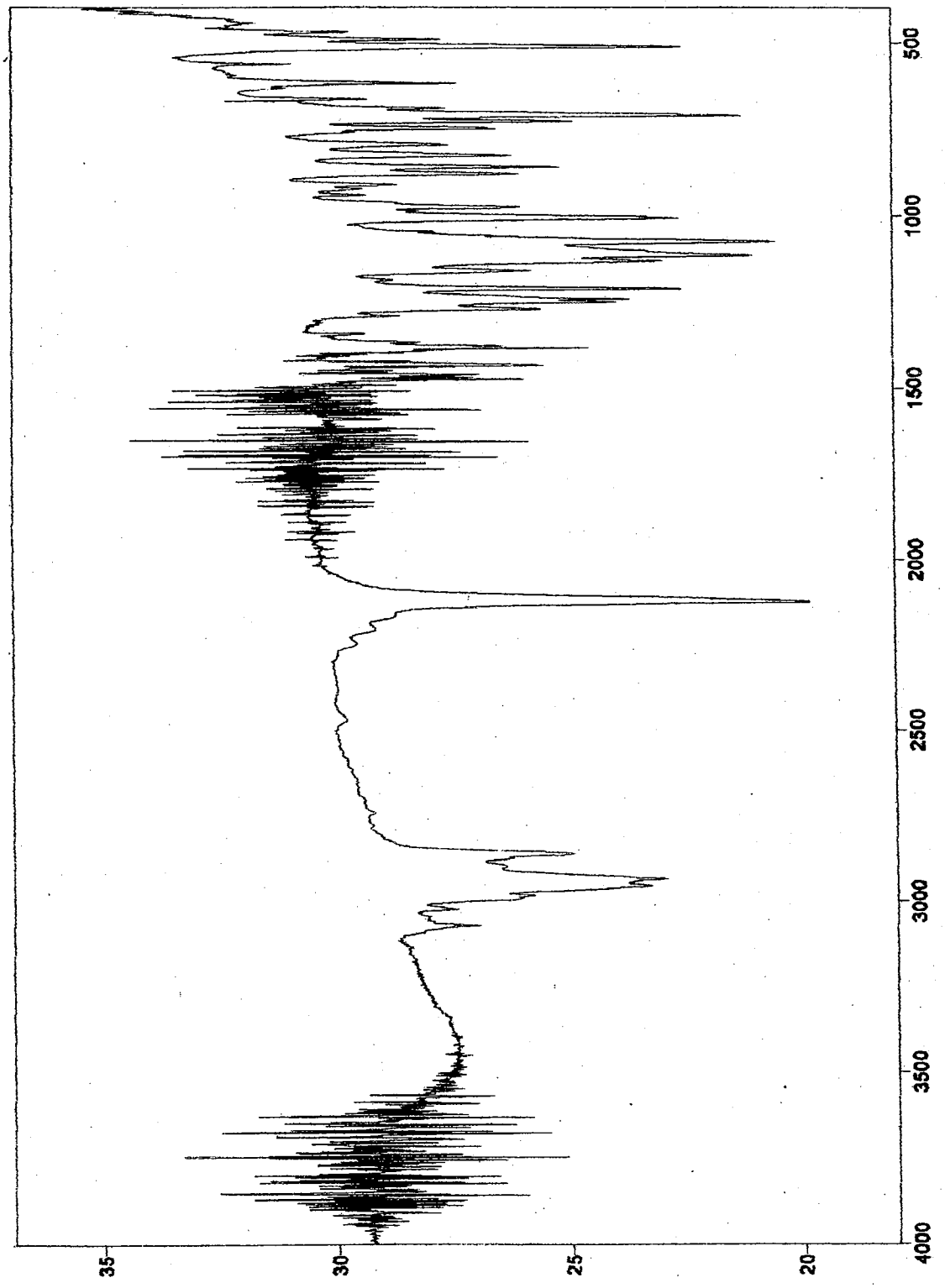


Plate XIII: ^{1}H NMR of Compound **14** in chloroform-*d*

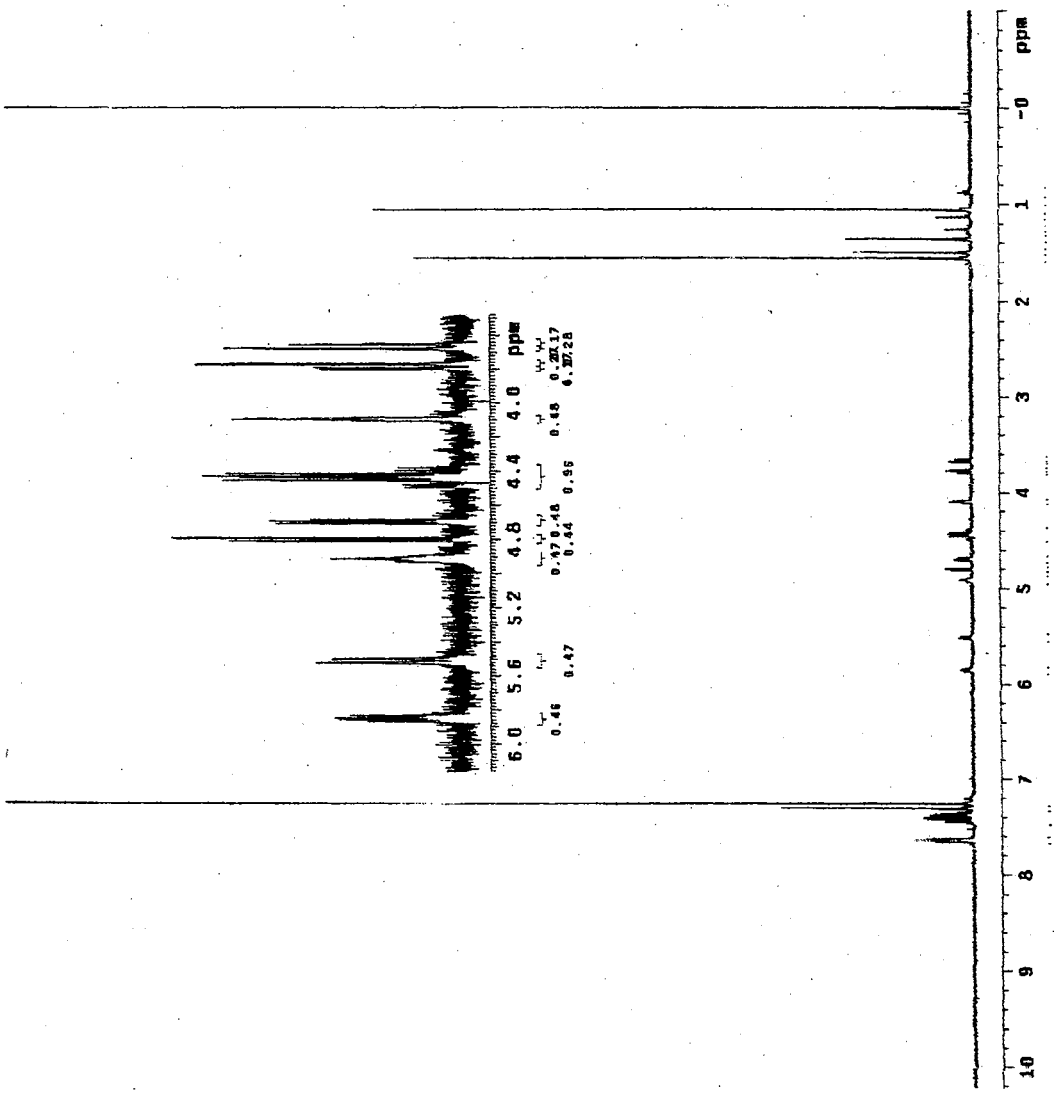


Plate XIV: COSY of Compound **14** in chloroform-*d*

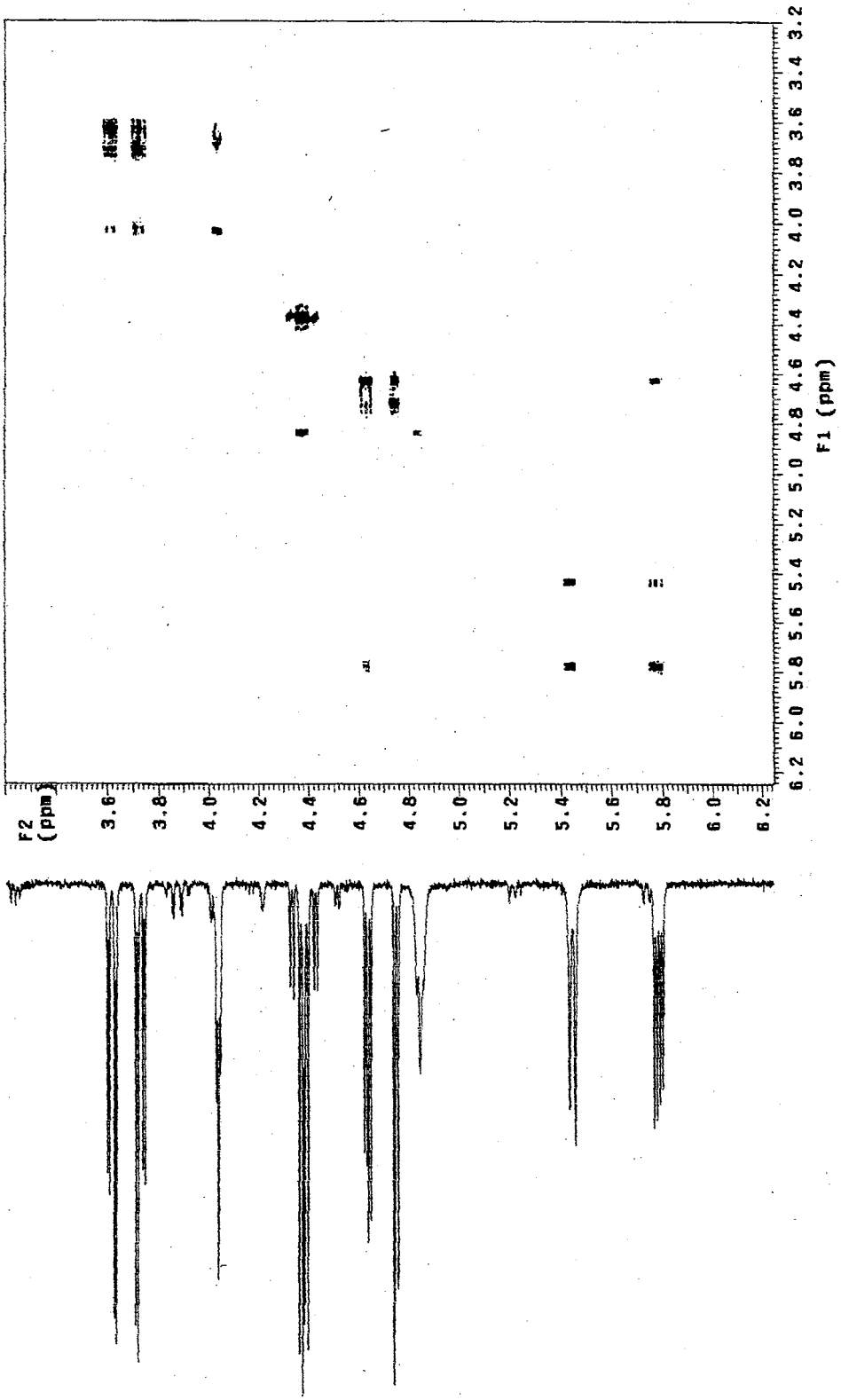


Plate XV: ^{1}H NMR of Compound **16** in chloroform-*d*

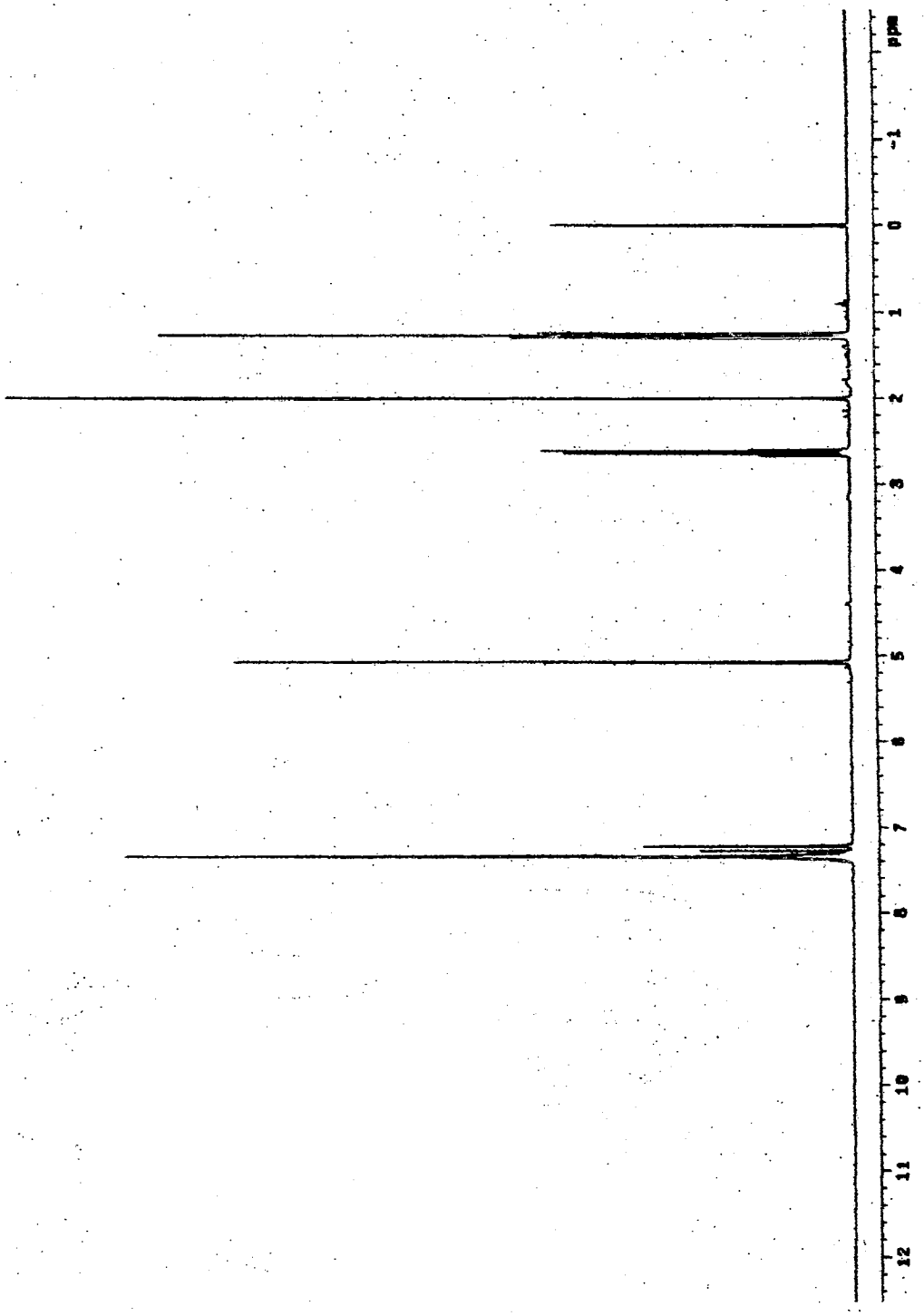


Plate XVI: FT-IR of Compound **18**

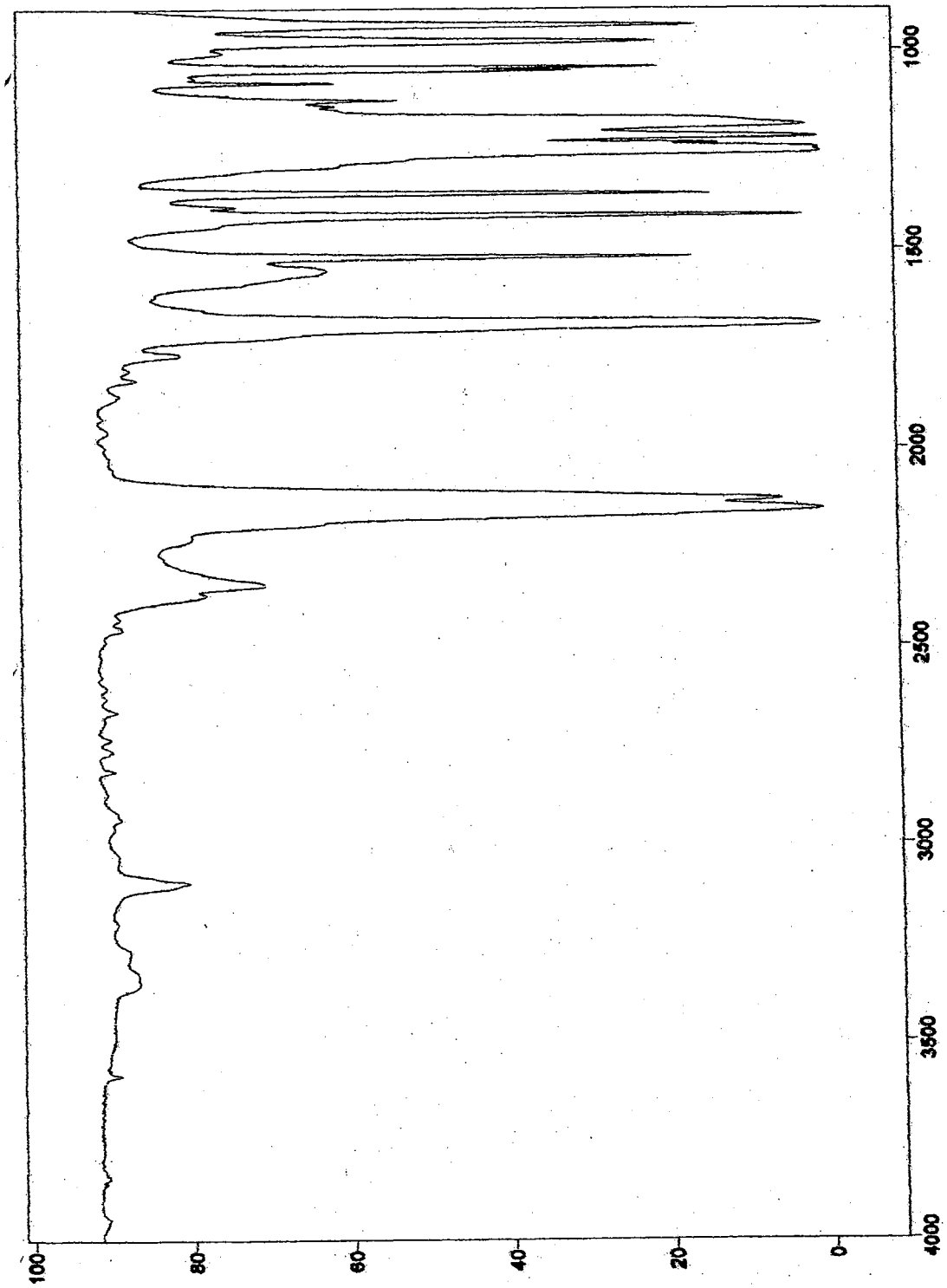


Plate XVII: FT-IR of Compound **19**

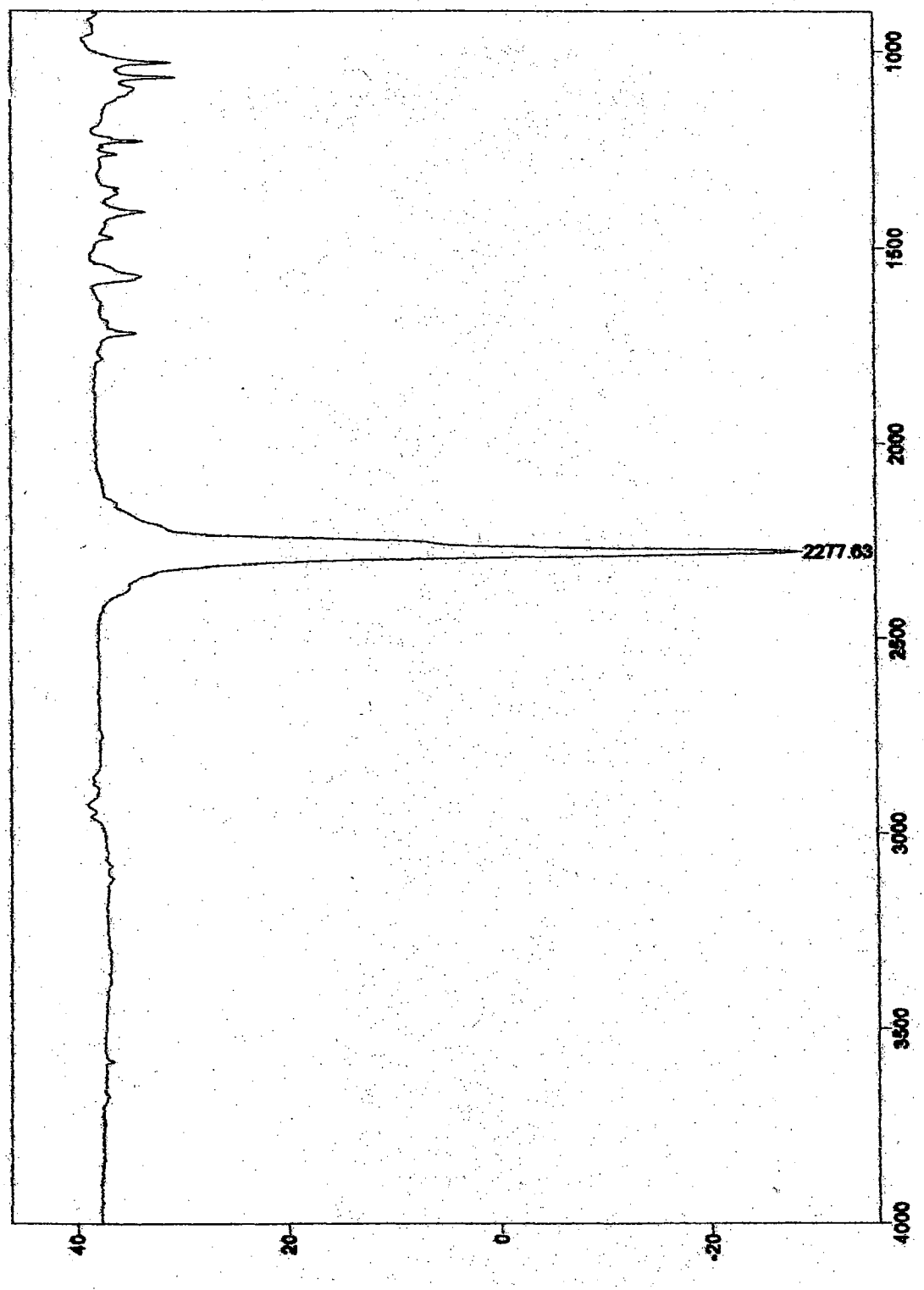


Plate XVIII: ^{1}H NMR of Compound **19** in chloroform-*d*

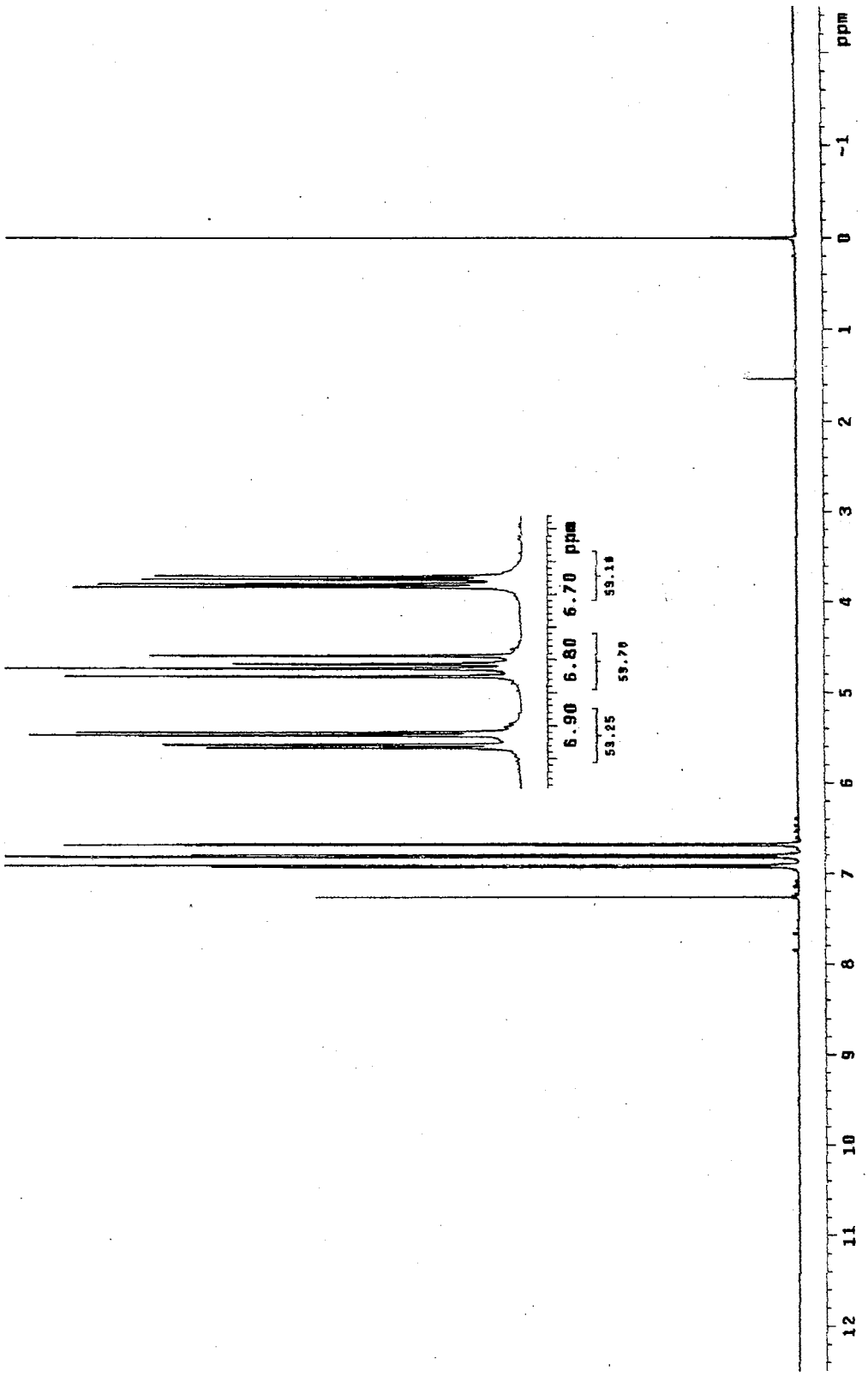


Plate XIX: ^{13}C NMR of Compound **19** in chloroform-*d*

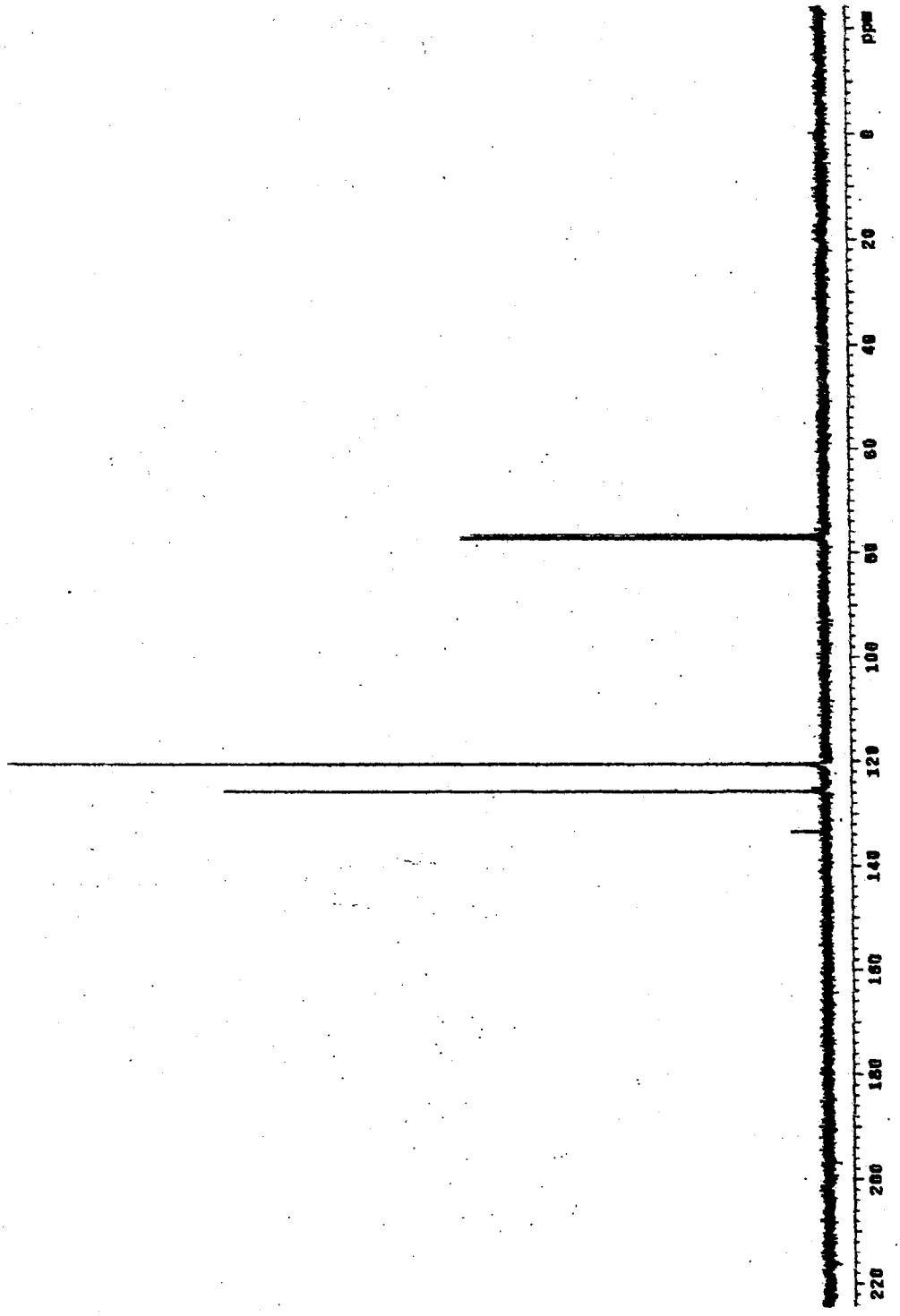


Plate XX: ^{1}H NMR of Compound **20** in chloroform-d

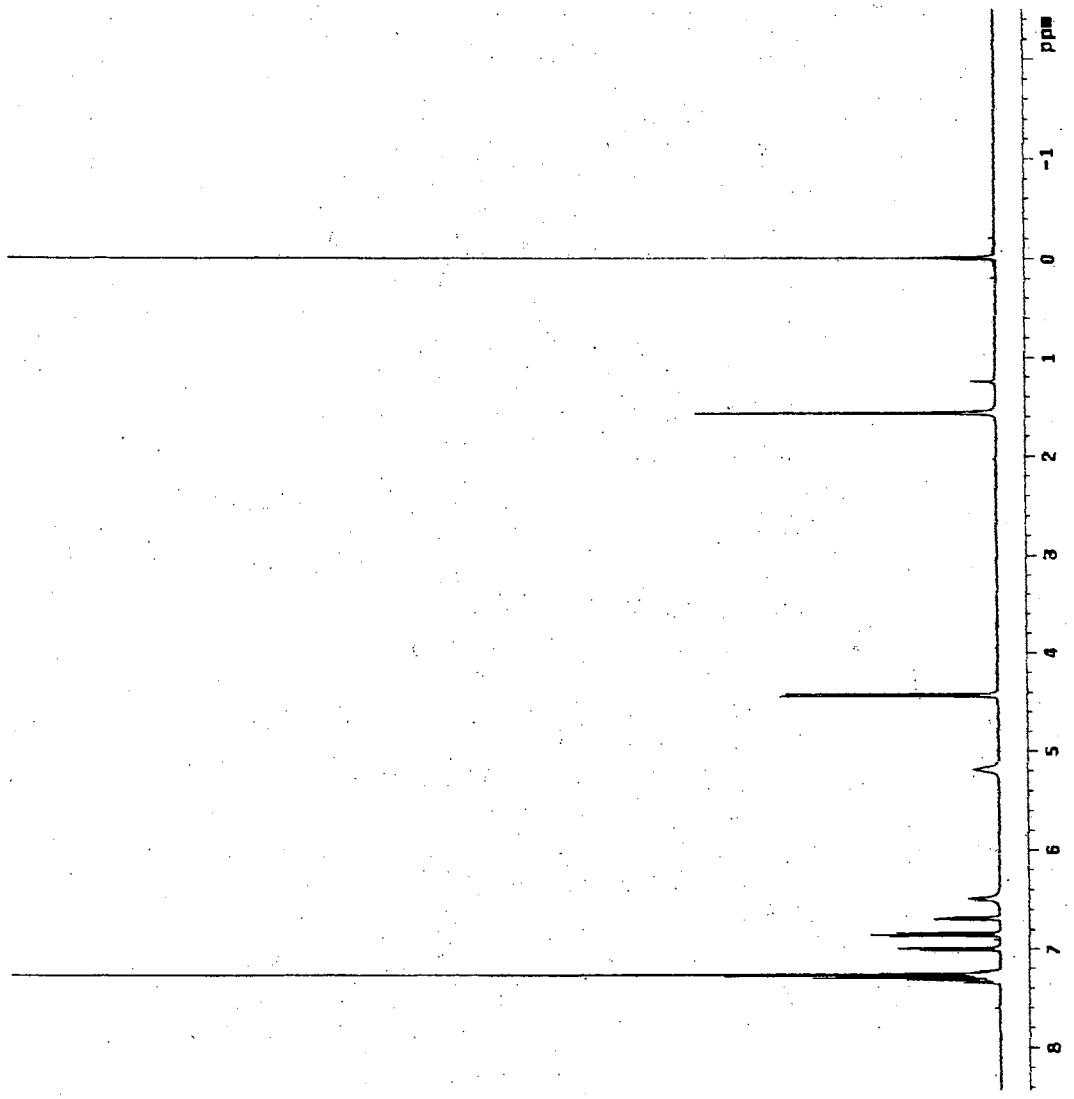


Plate XXI: ^{1}H NMR of Compound **20** in acetone-d_6

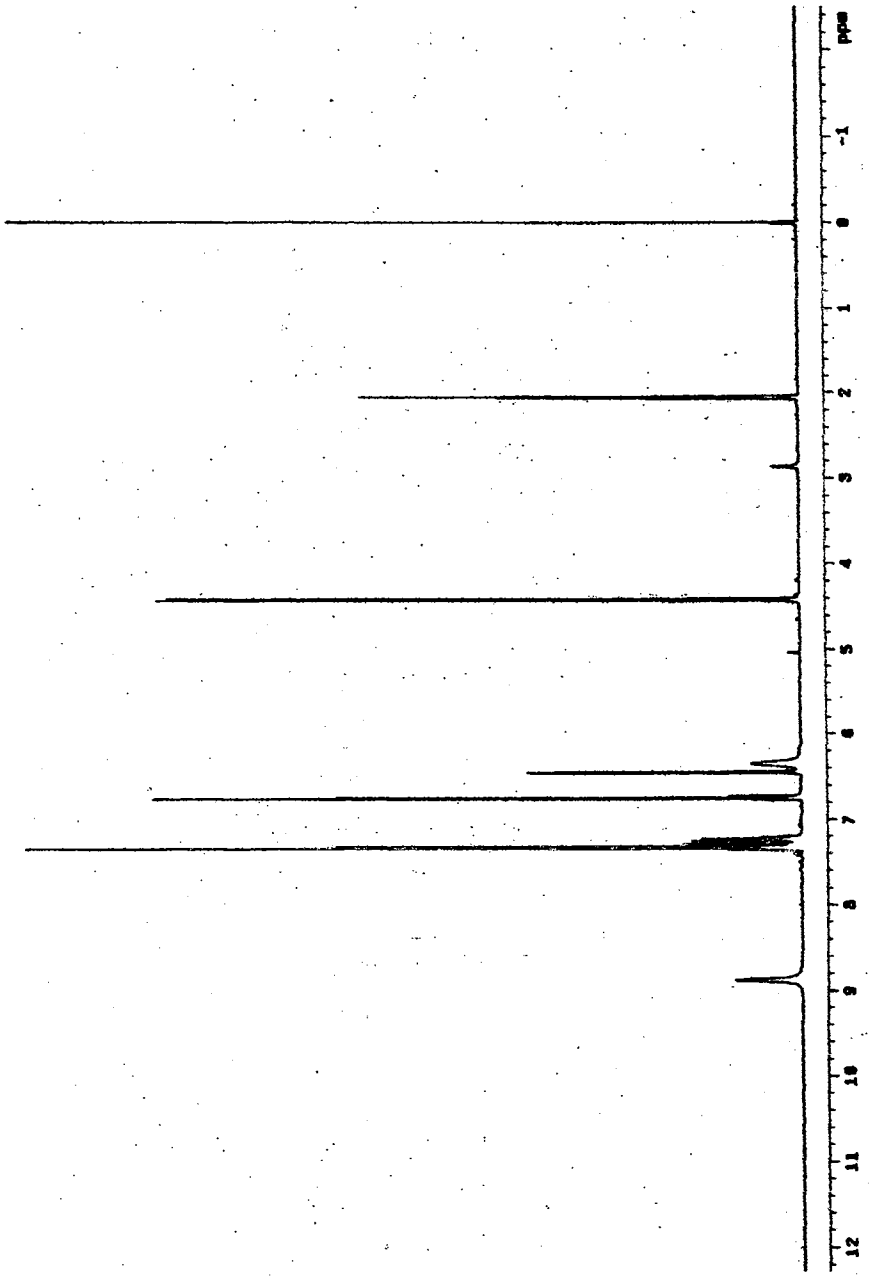


Plate XXII: ^{1}H NMR of Compound **20** in dimethyl sulfoxide-d_6

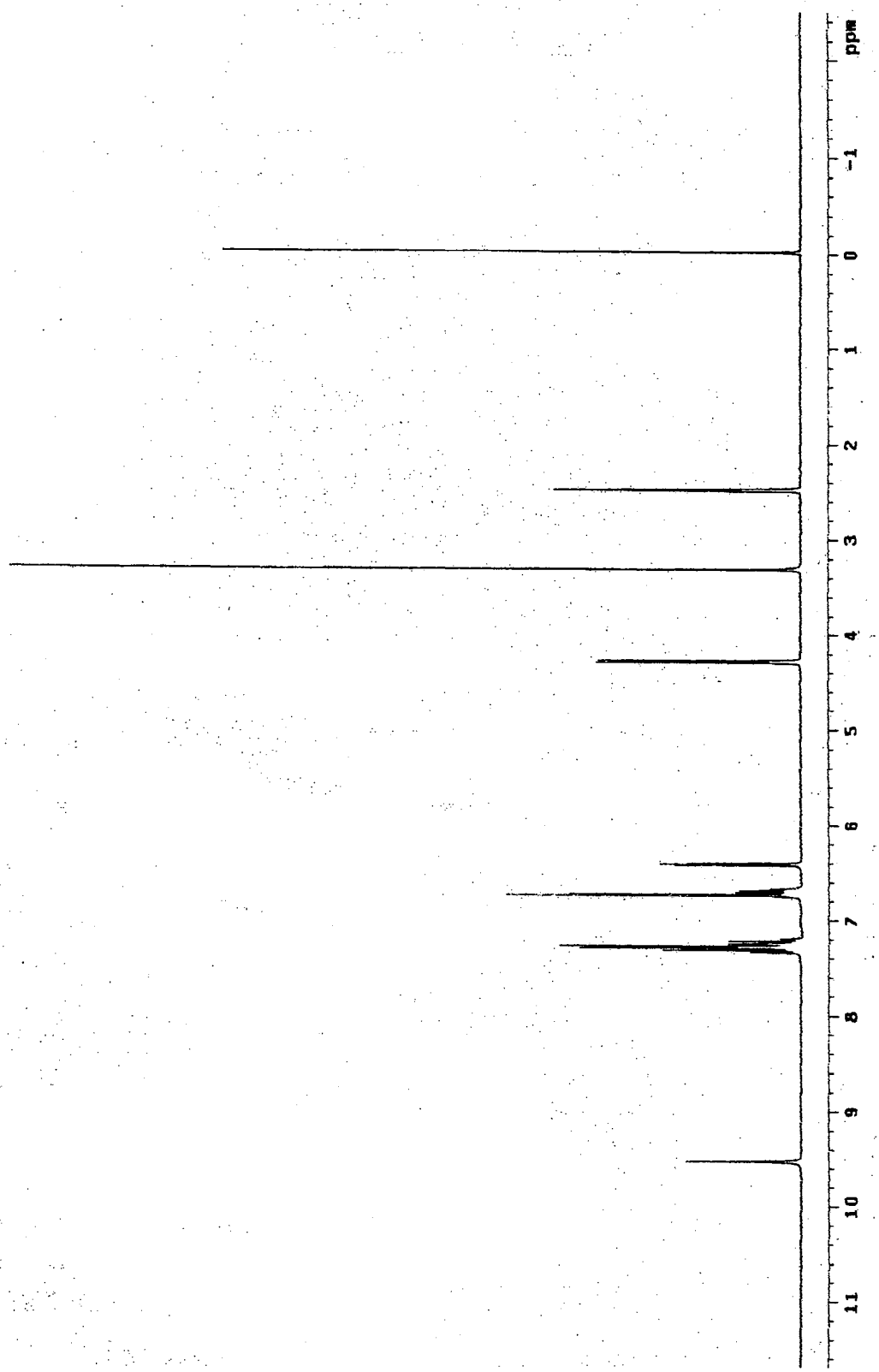


Plate XXIII: ^{13}C NMR of Compound **20** in acetone-d_6

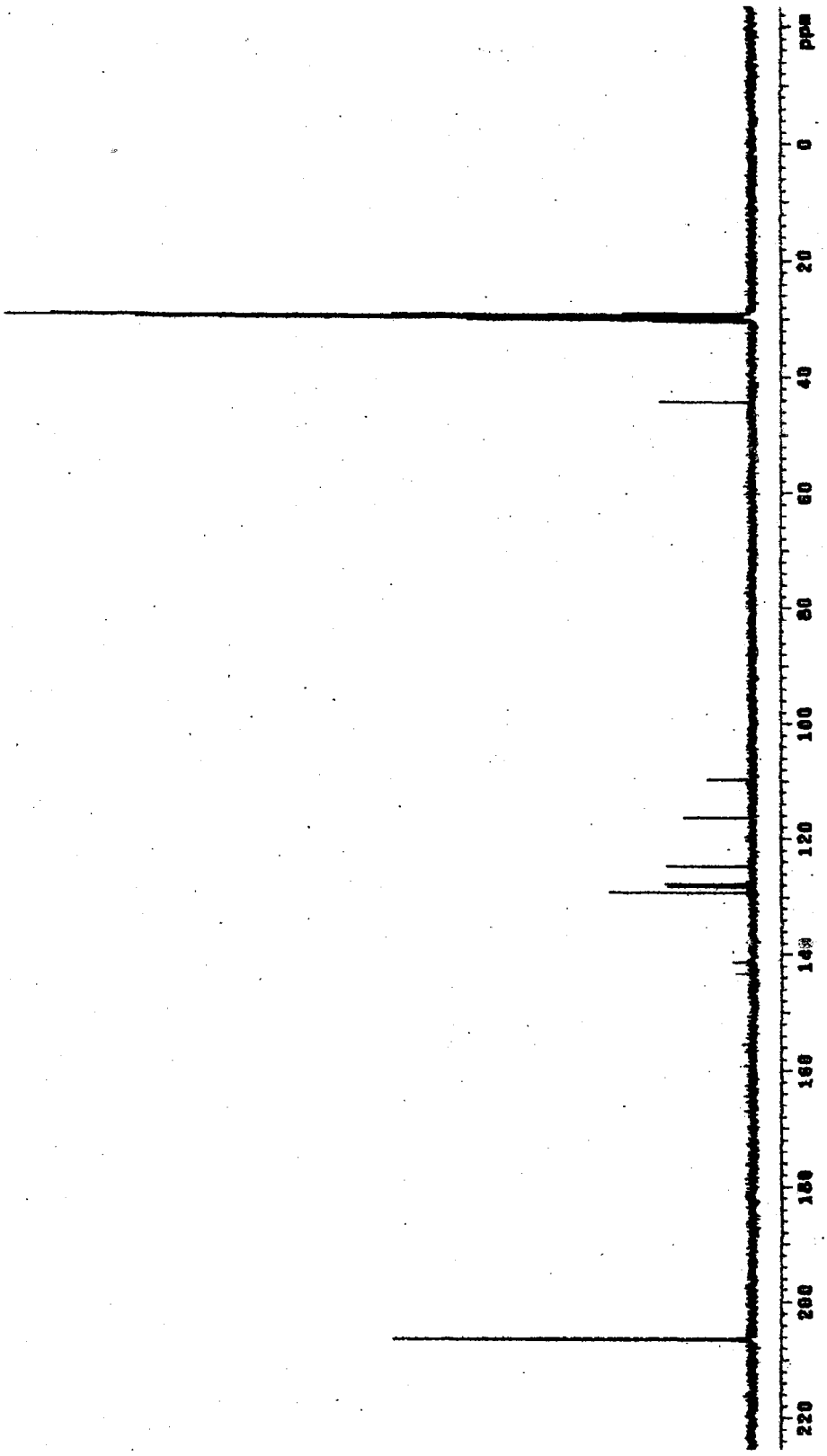


Plate XXIV: ^{13}C NMR of Compound **20** in dimethyl sulfoxide-d_6

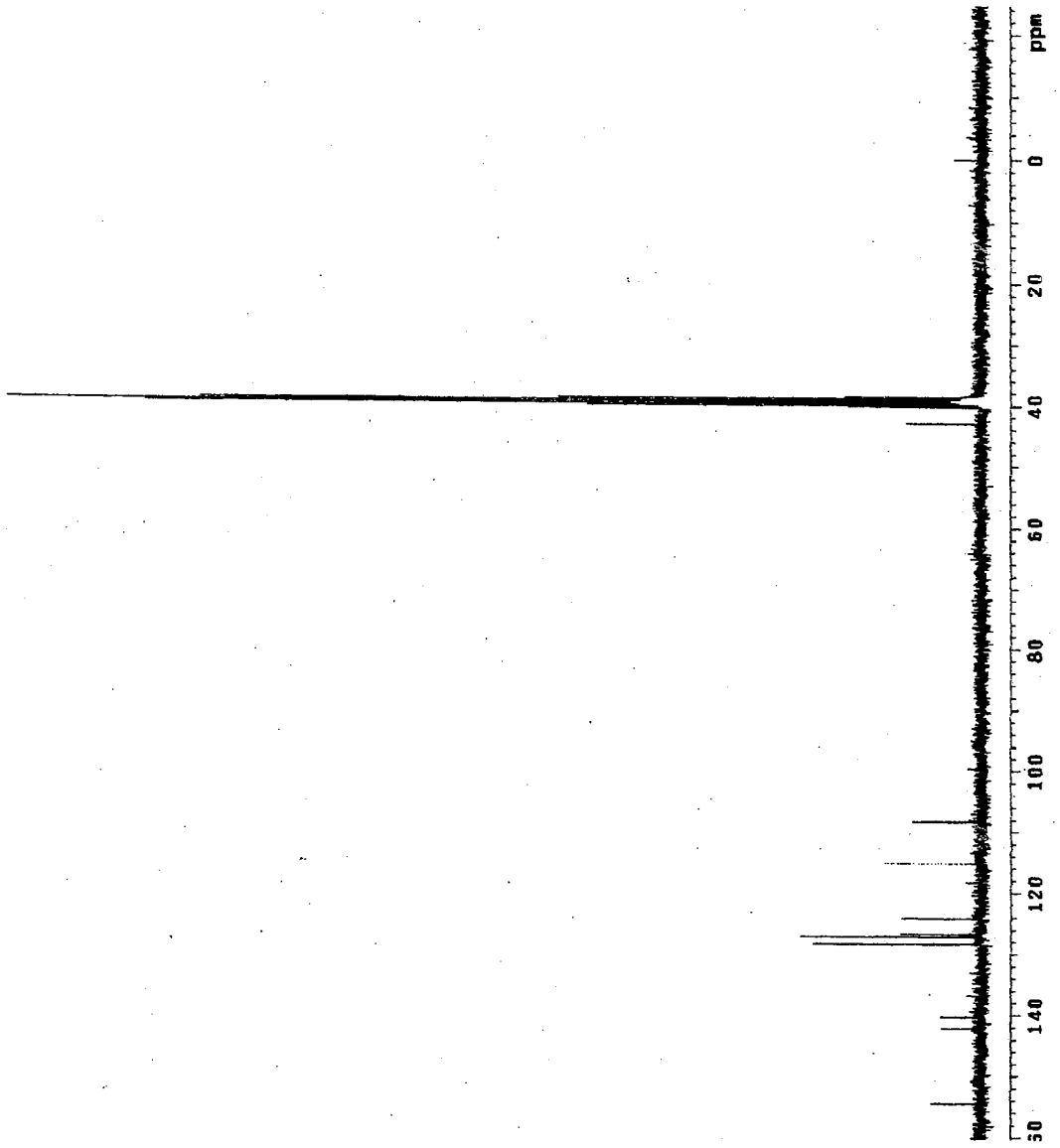


Plate XXV: ^{1}H NMR of Compound **21** in acetone-d_6

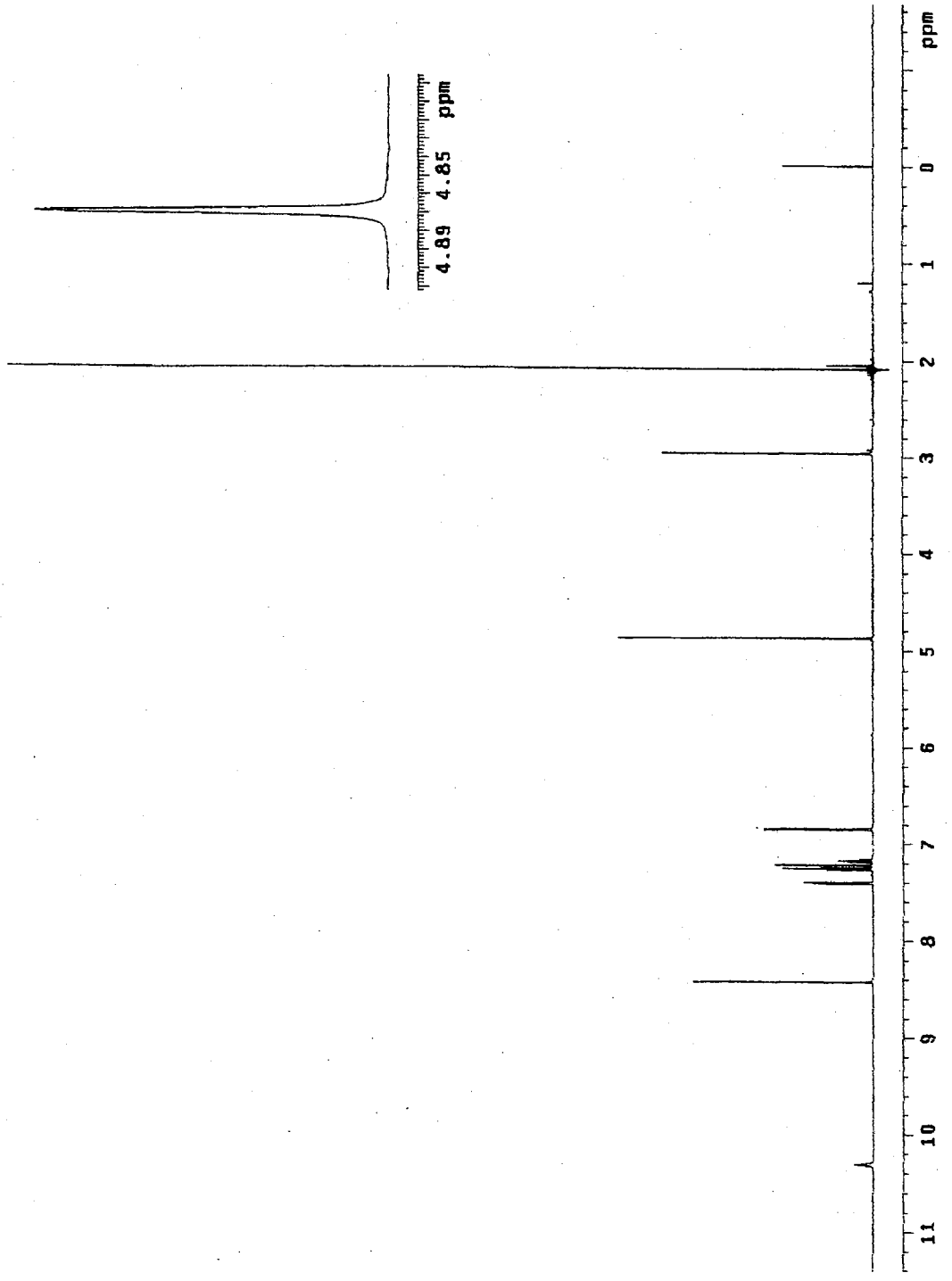


Plate XXVI: ^{1}H NMR of Compound **21** in chloroform-*d*

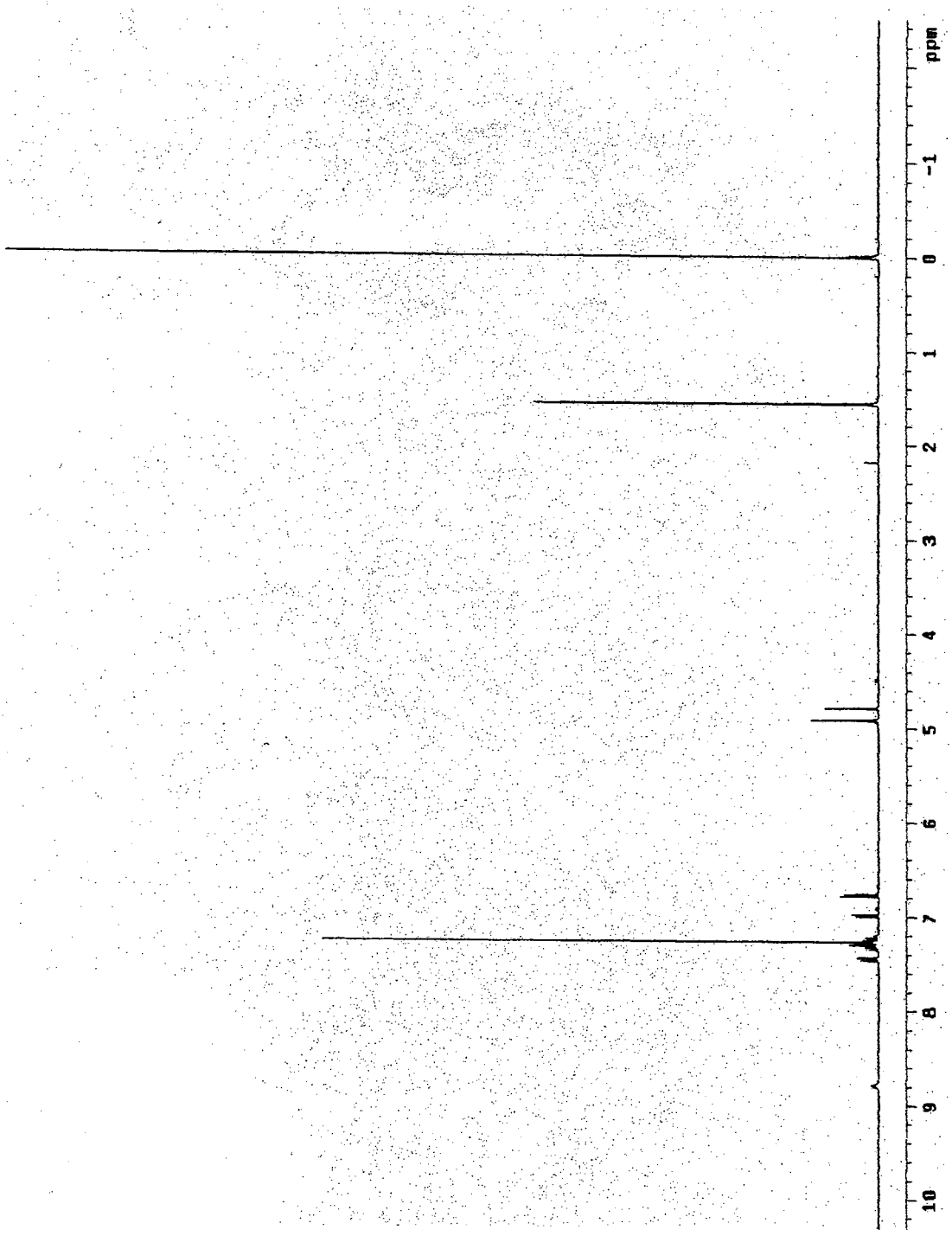


Plate XXVII: ^{1}H NMR of Compound **21** in acetonitrile-d_3

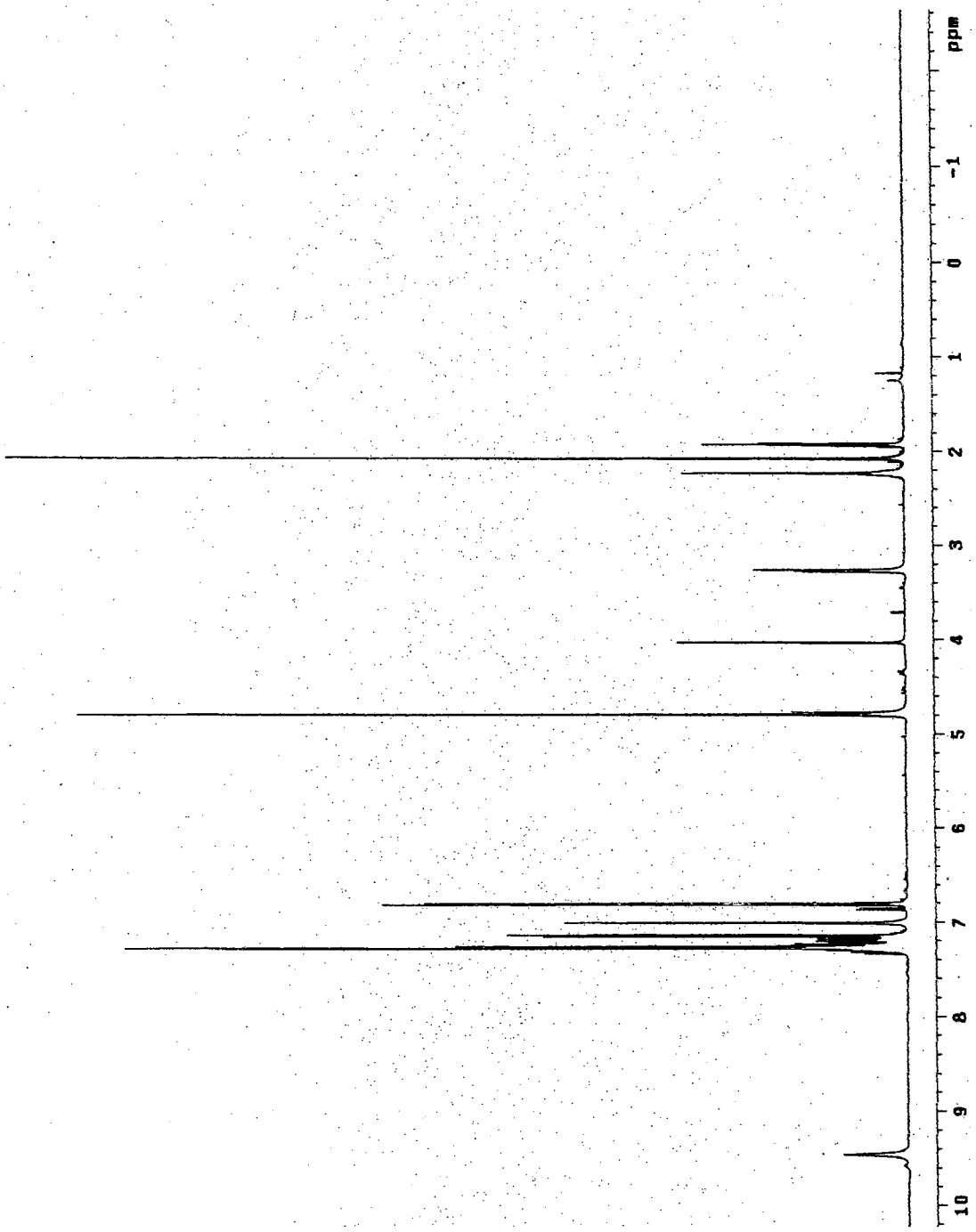


Plate XXVIII: ^{1}H NMR of Compound **21** in methanol-d_4

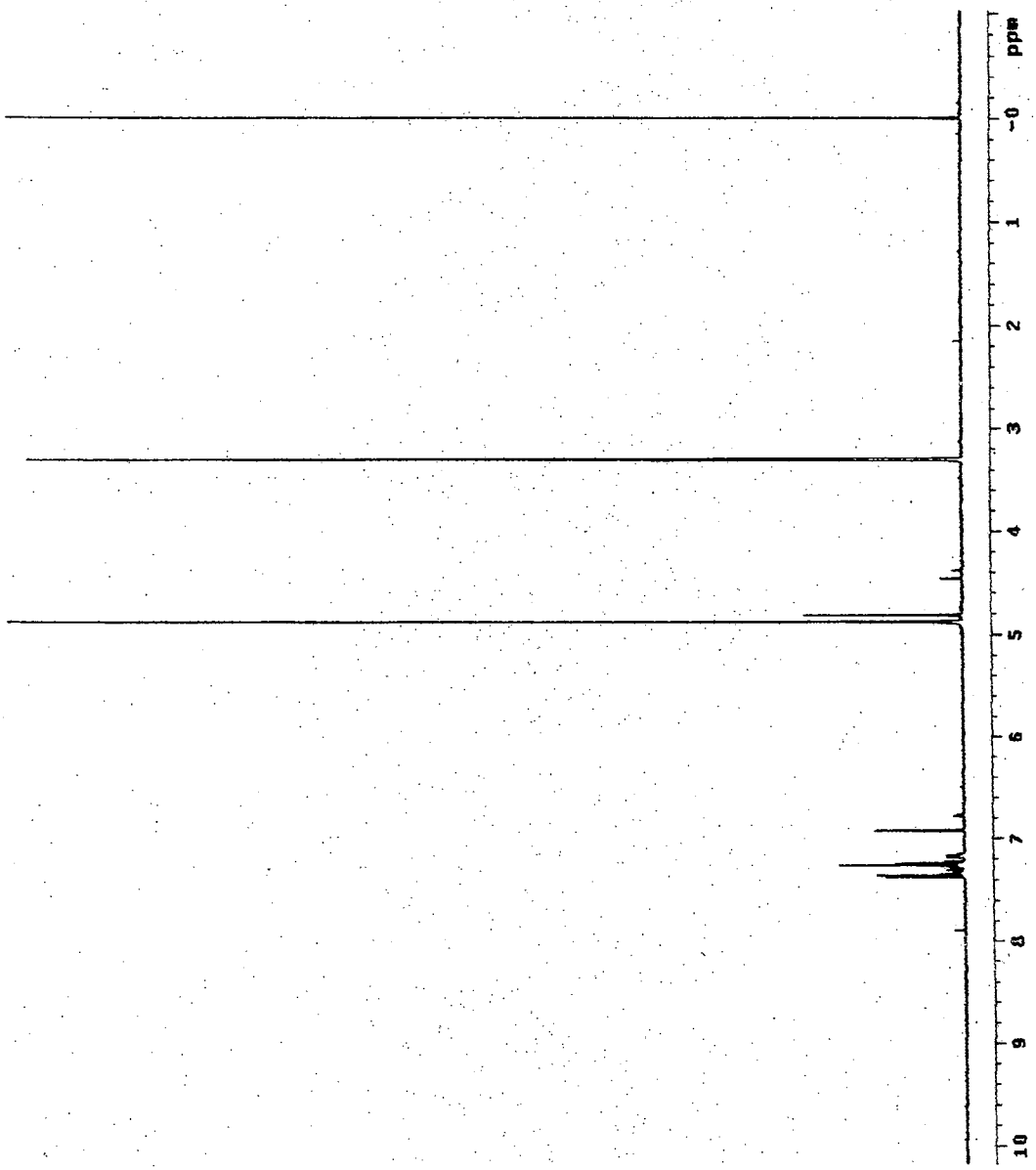


Plate XXIX: ^{13}C NMR of Compound **21** in acetone-d_6

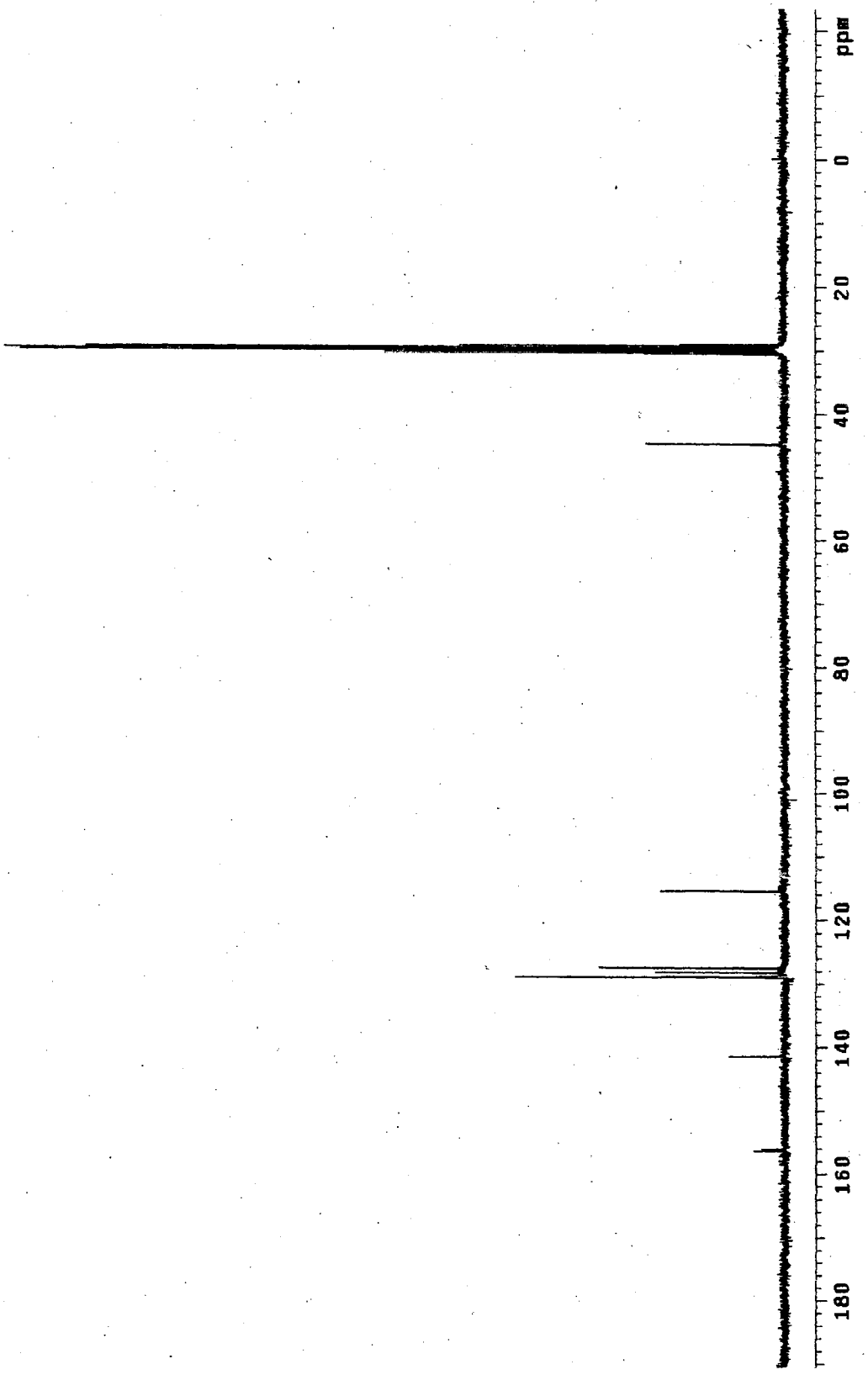


Plate XXX: ^{13}C NMR of Compound **21** in acetonitrile-d_3

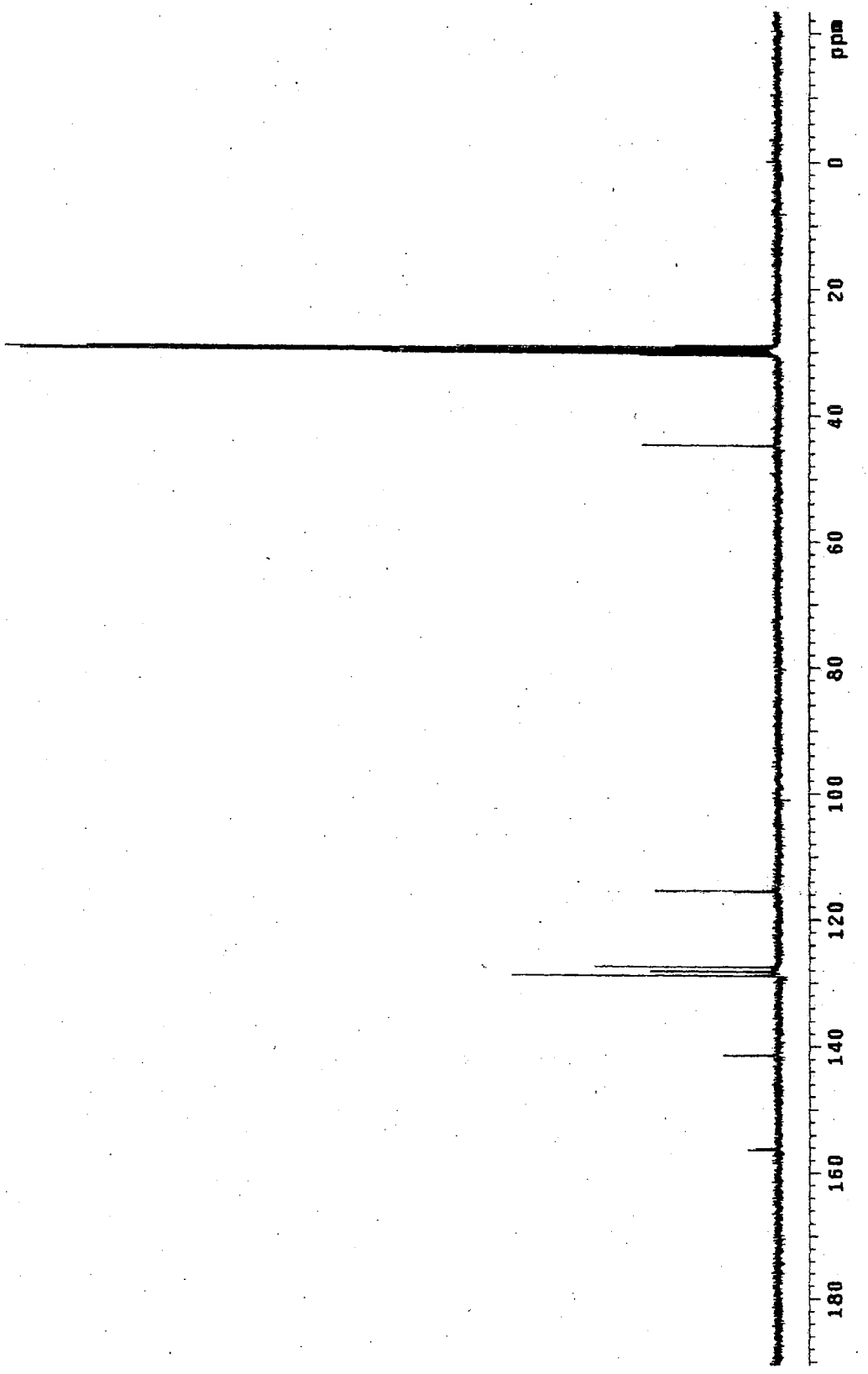


Plate XXXI: ^{11}B NMR of Compound **21** in acetone-d_6

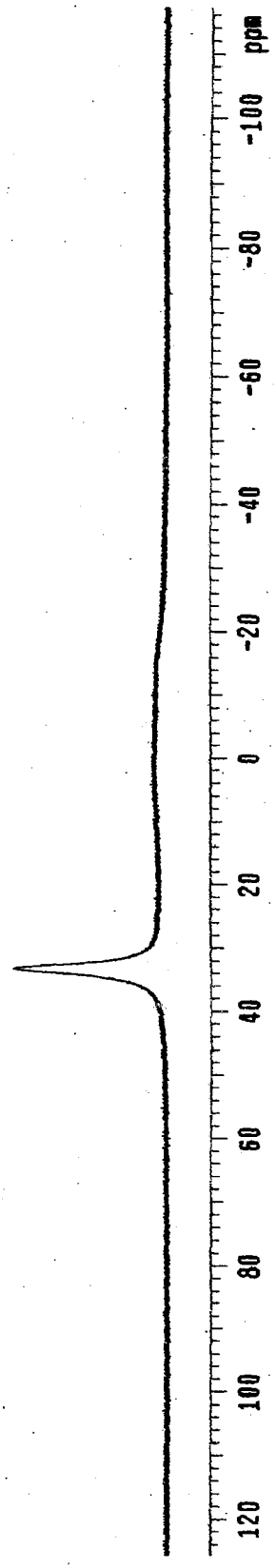


Plate XXXII: ^{11}B NMR of Compound **21** in acetonitrile-d_3

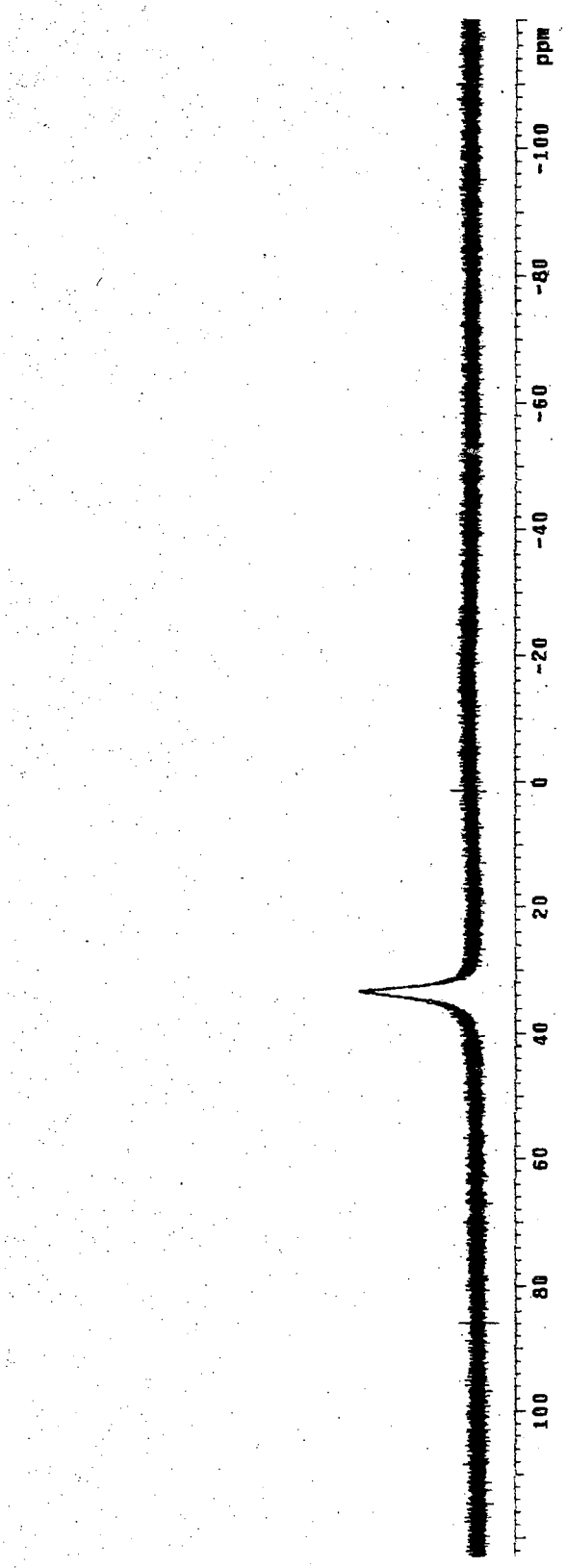


Plate XXXIII: ^{11}B NMR of Compound **21** in methanol-d_4

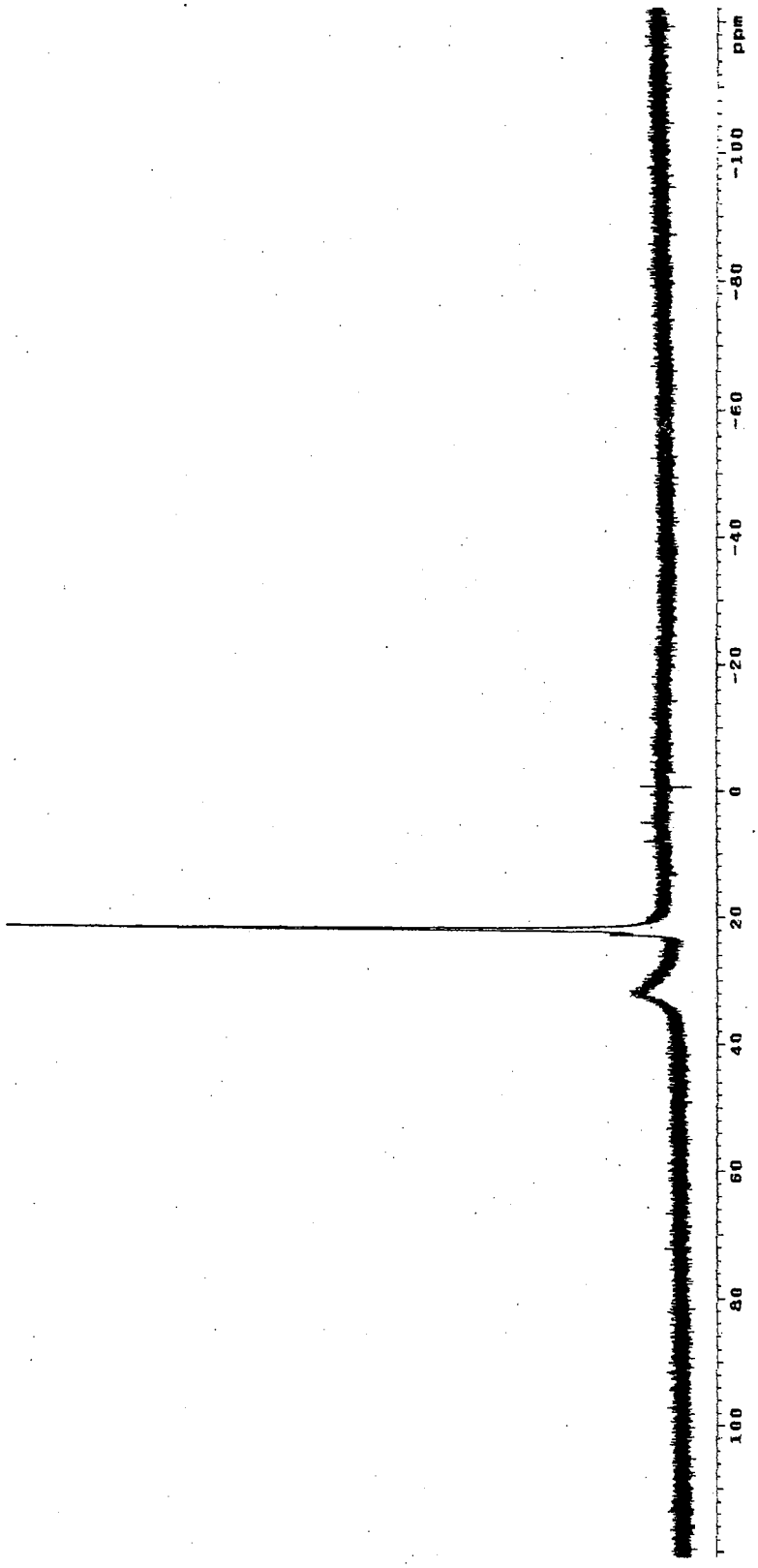


Plate XXXIV: ^{1}H NMR of Compound **33** in chloroform-*d*

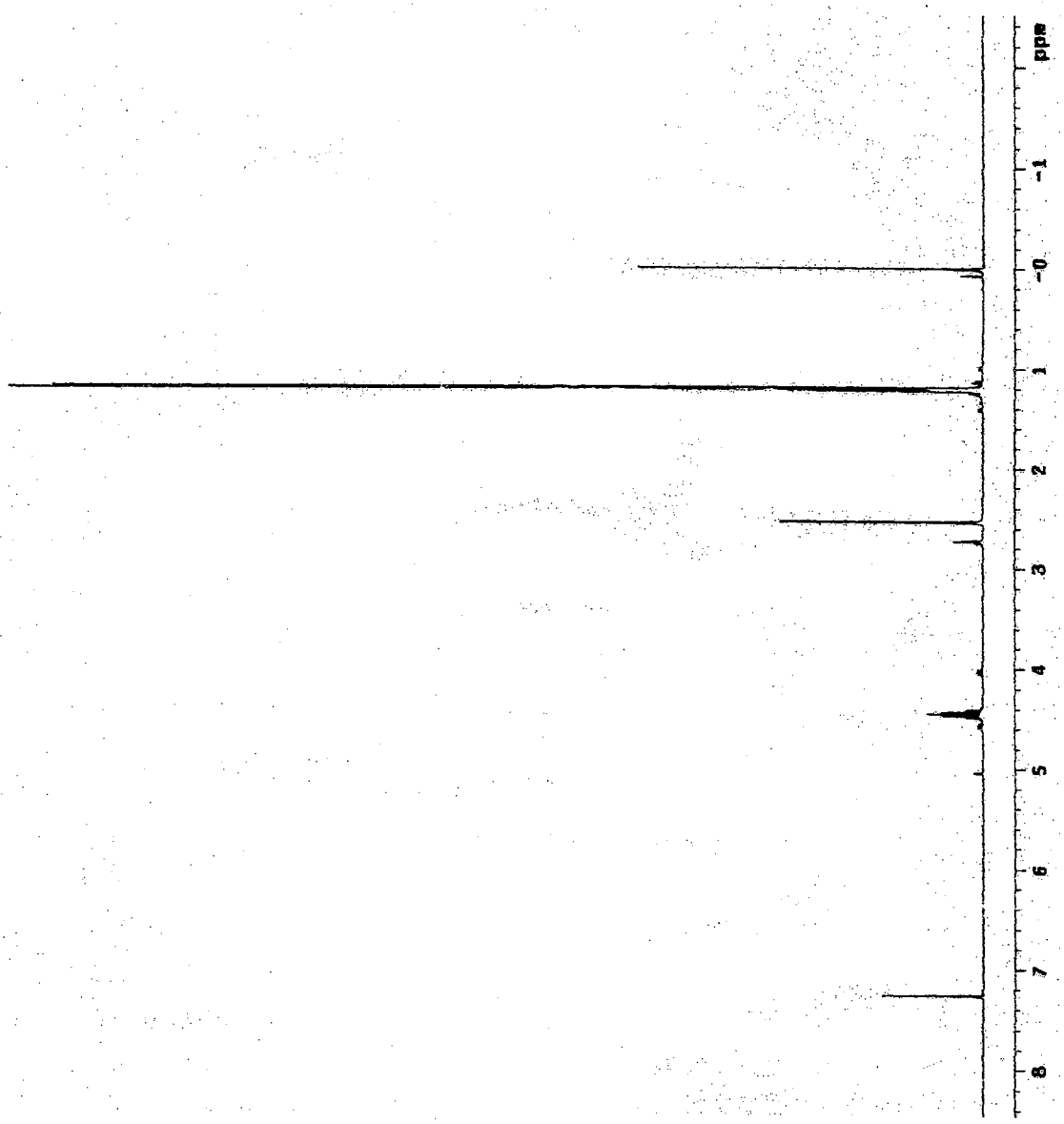


Plate XXXV: ^{13}C NMR of Compound **33** in chloroform-*d*

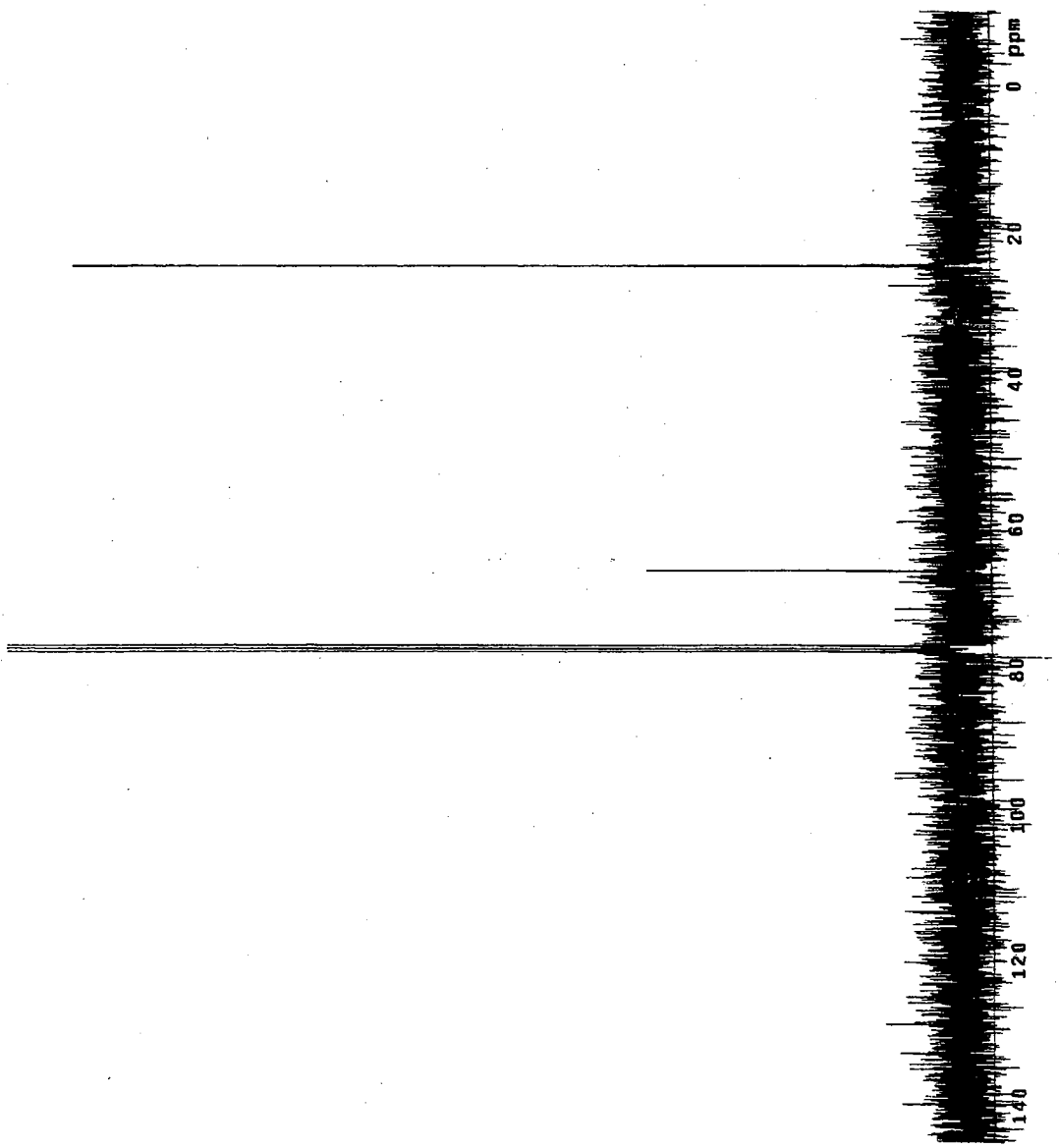


Plate XXXVI: ^{1}H NMR of Compound **34** in chloroform-*d*

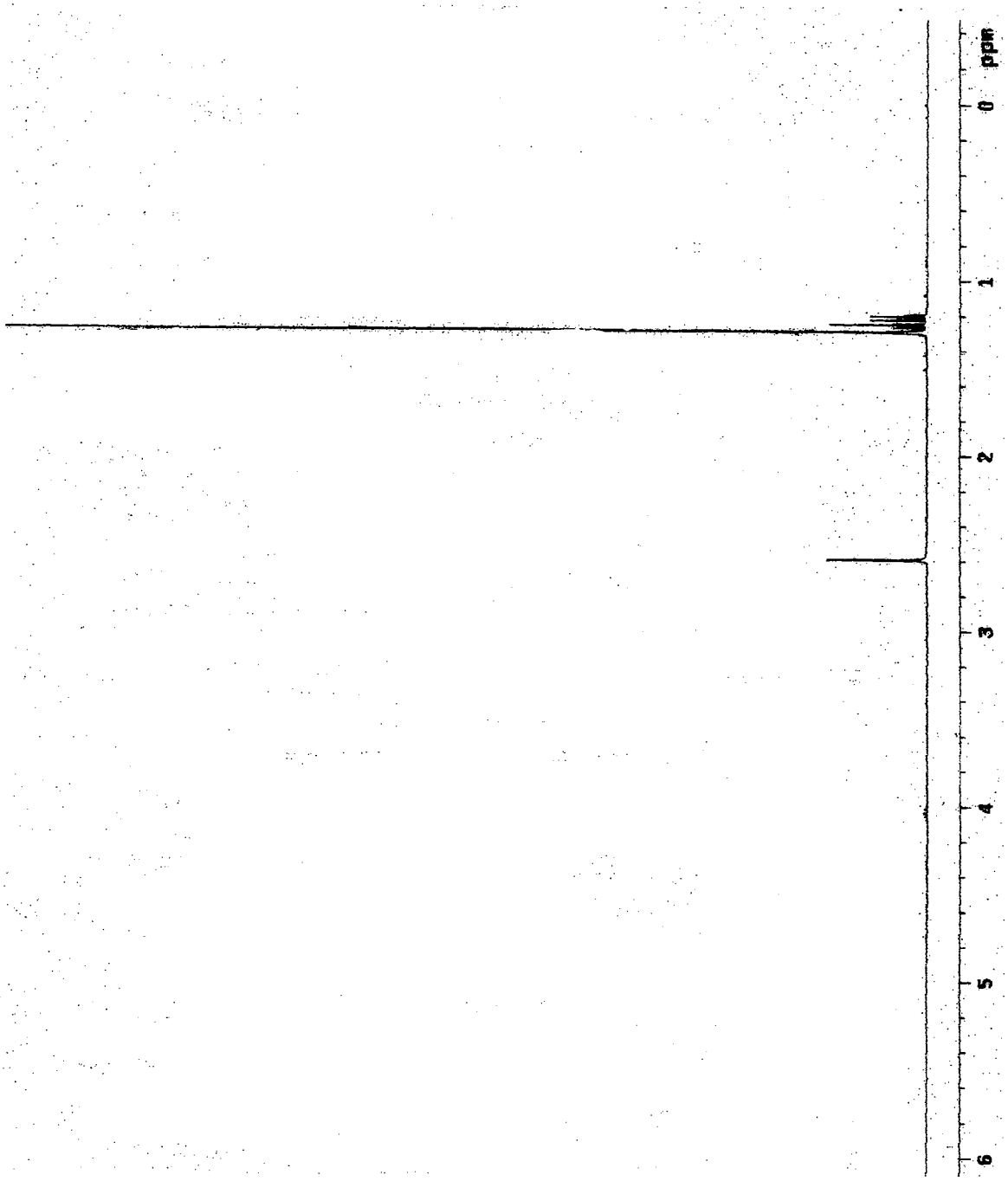


Plate XXXVII: ^{13}C NMR of Compound **34** in chloroform-*d*

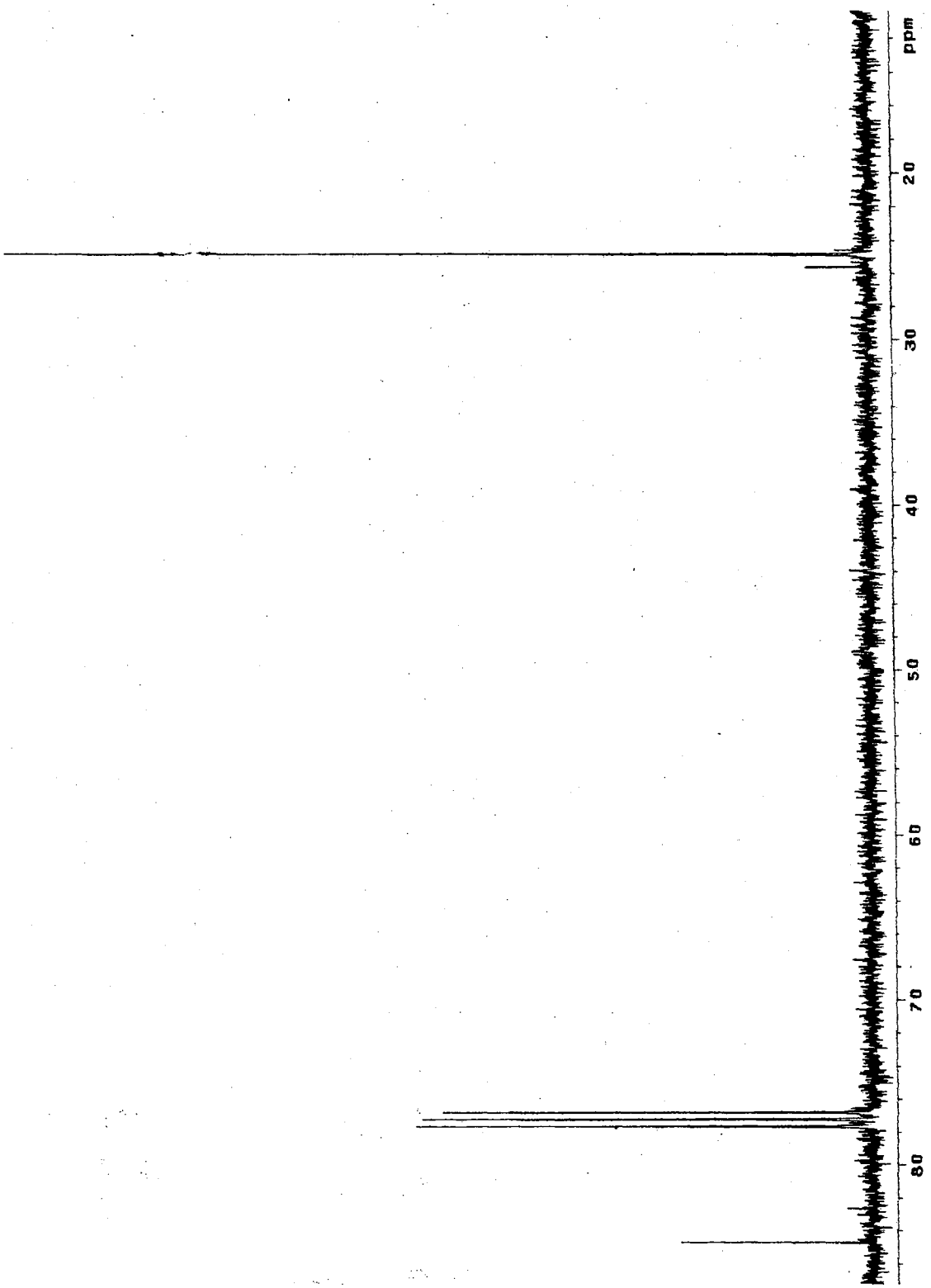


Plate XXXVIII: ^{1}H NMR of Compound **35** in chloroform-*d*

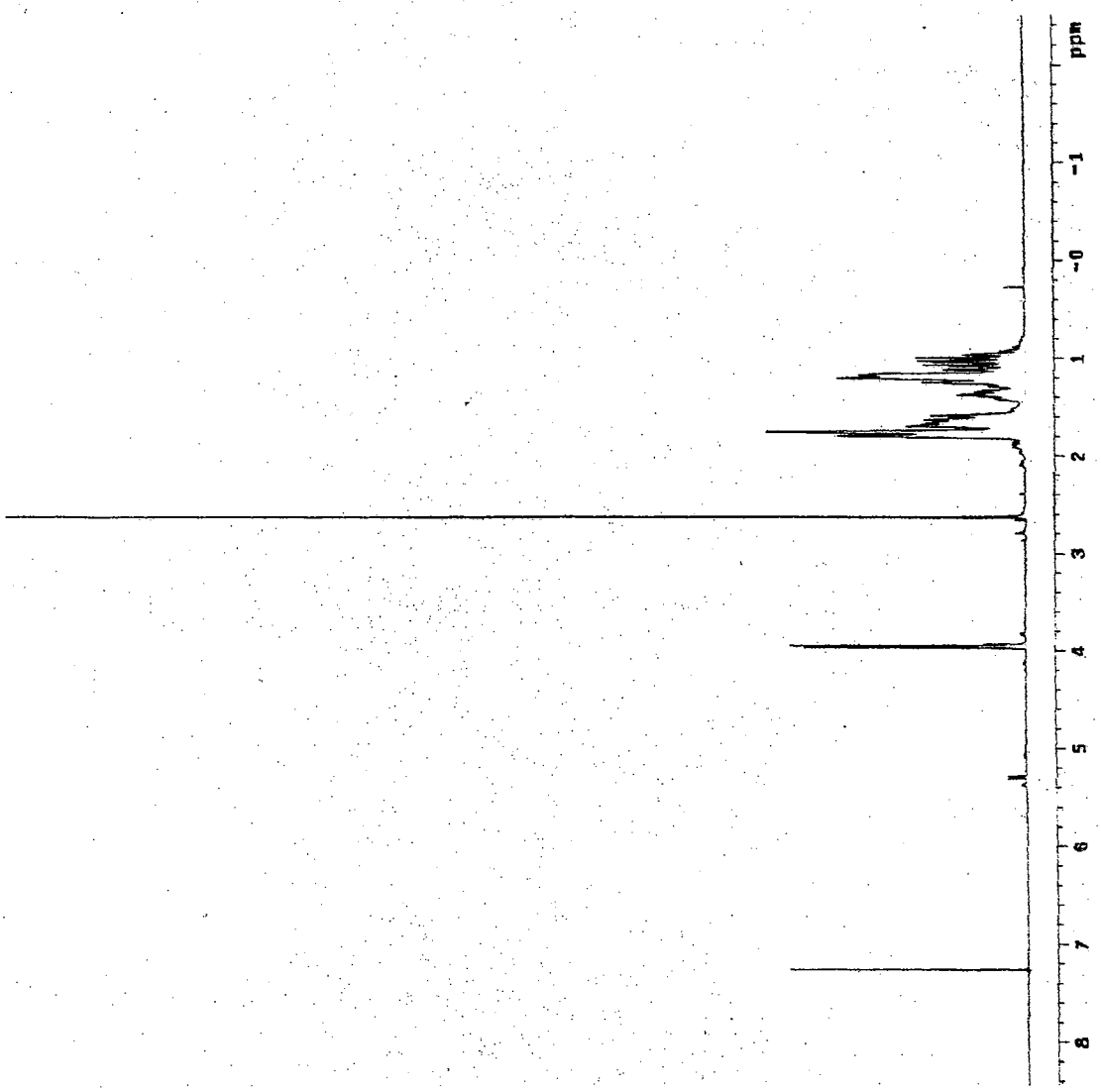


Plate IXL: ^{13}C NMR of Compound **35** in chloroform-*d*

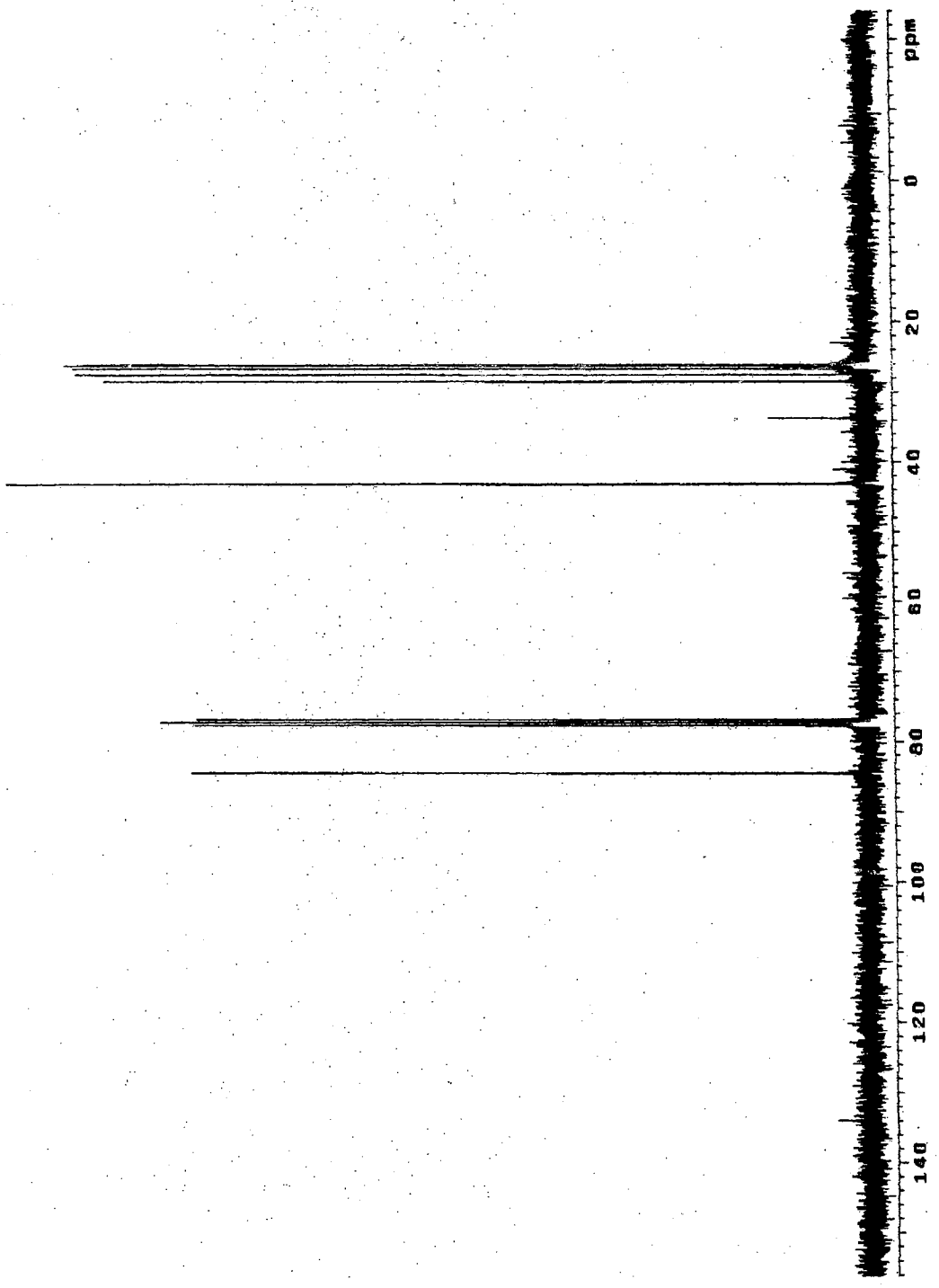


Plate XL: ^{1}H NMR of Compound **37** in chloroform-*d*

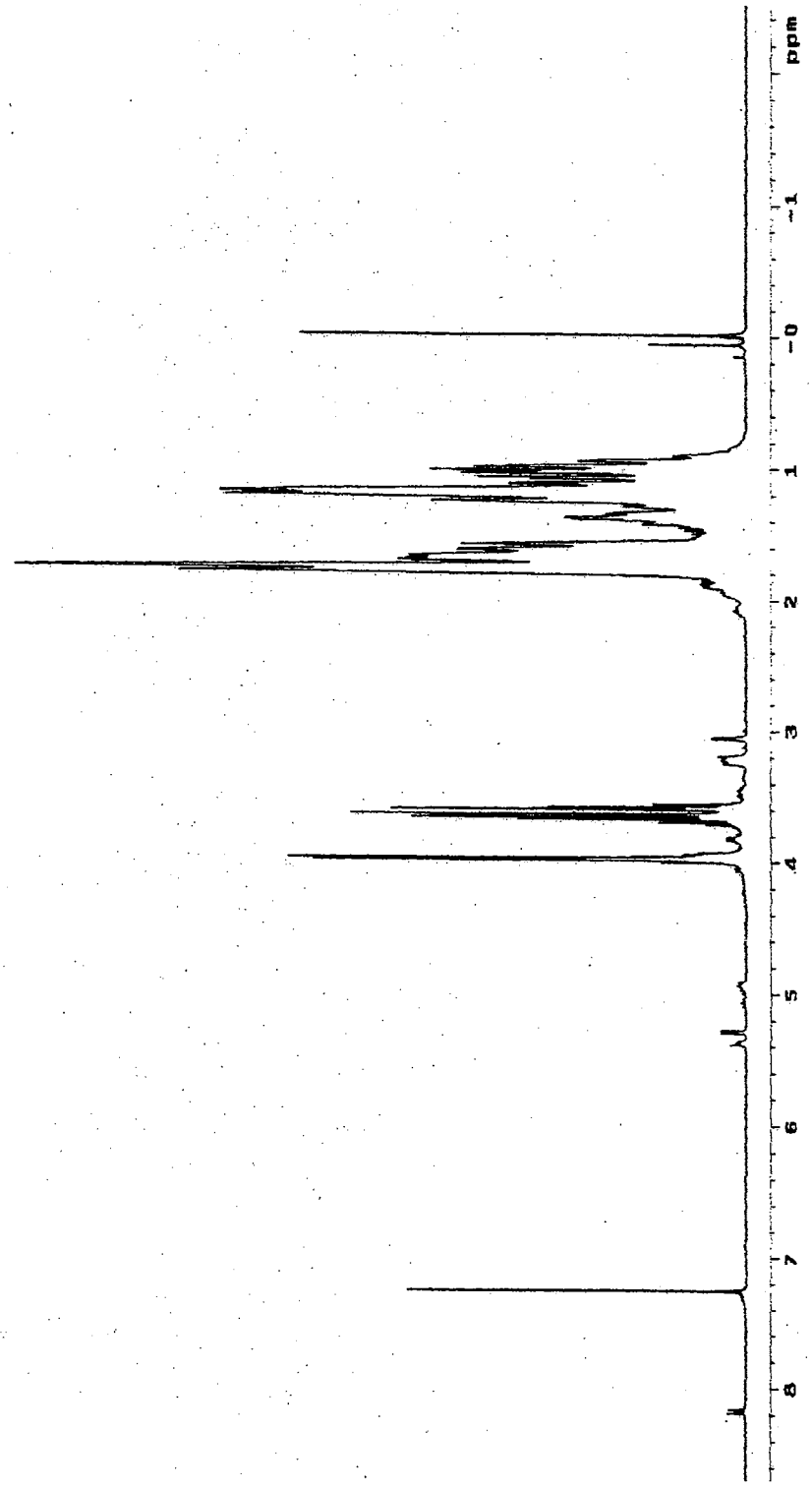


Plate XLI: ^{13}C of Compound **37** in chloroform-*d*

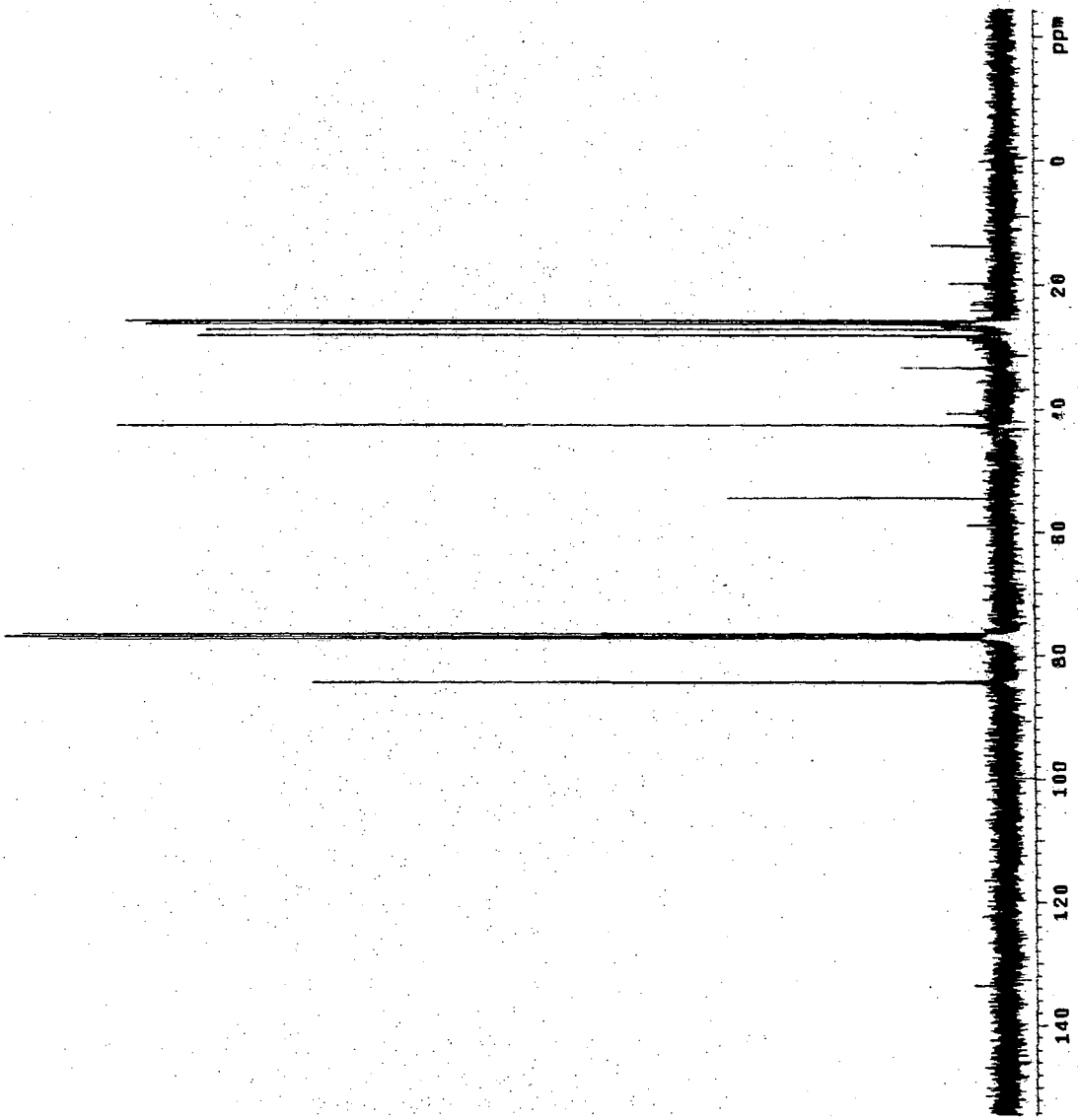


Plate XLII: FT-IR of Compound **38**

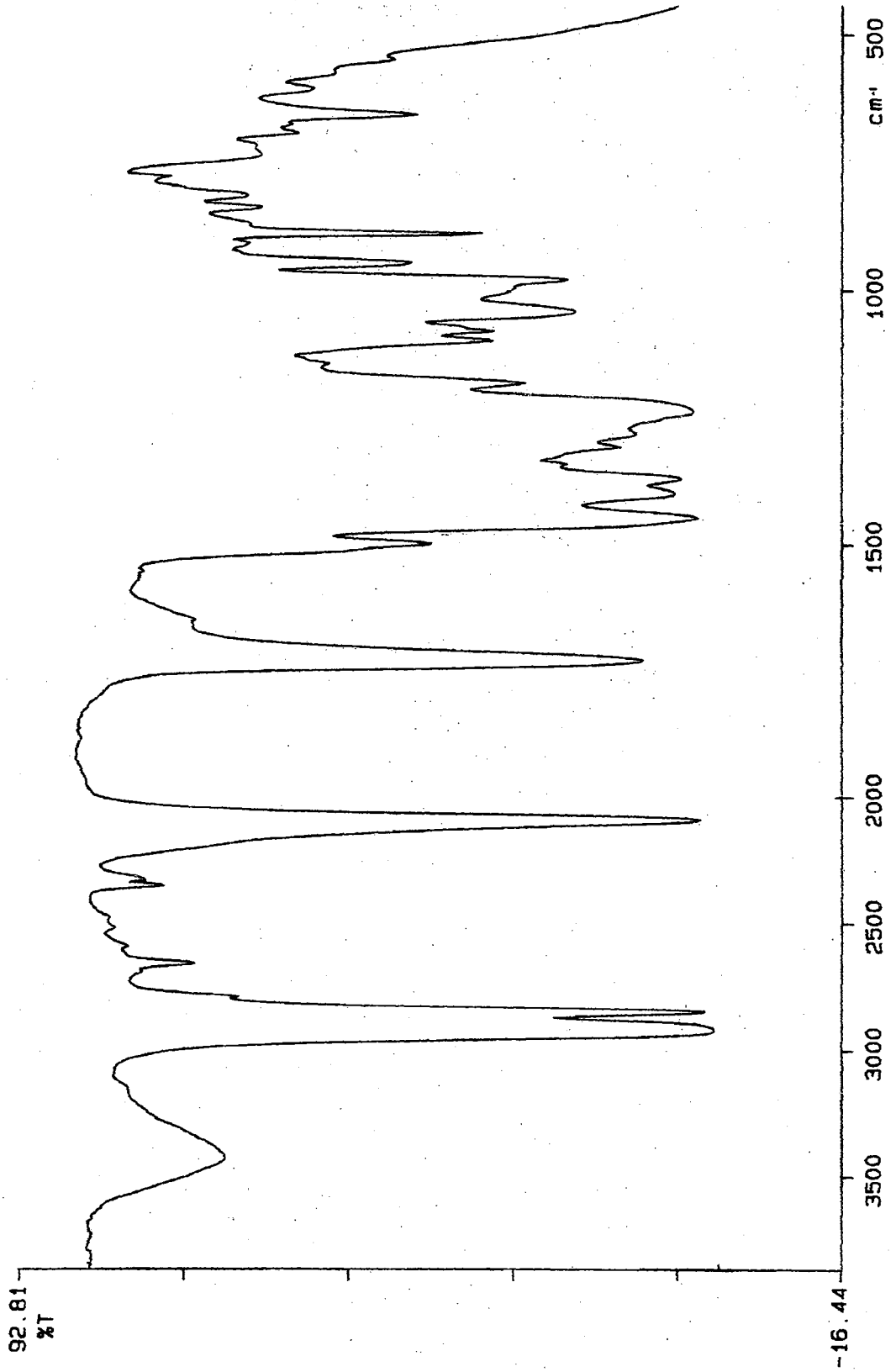


Plate XLIII: ^{1}H NMR of Compound **38** in chloroform-*d*

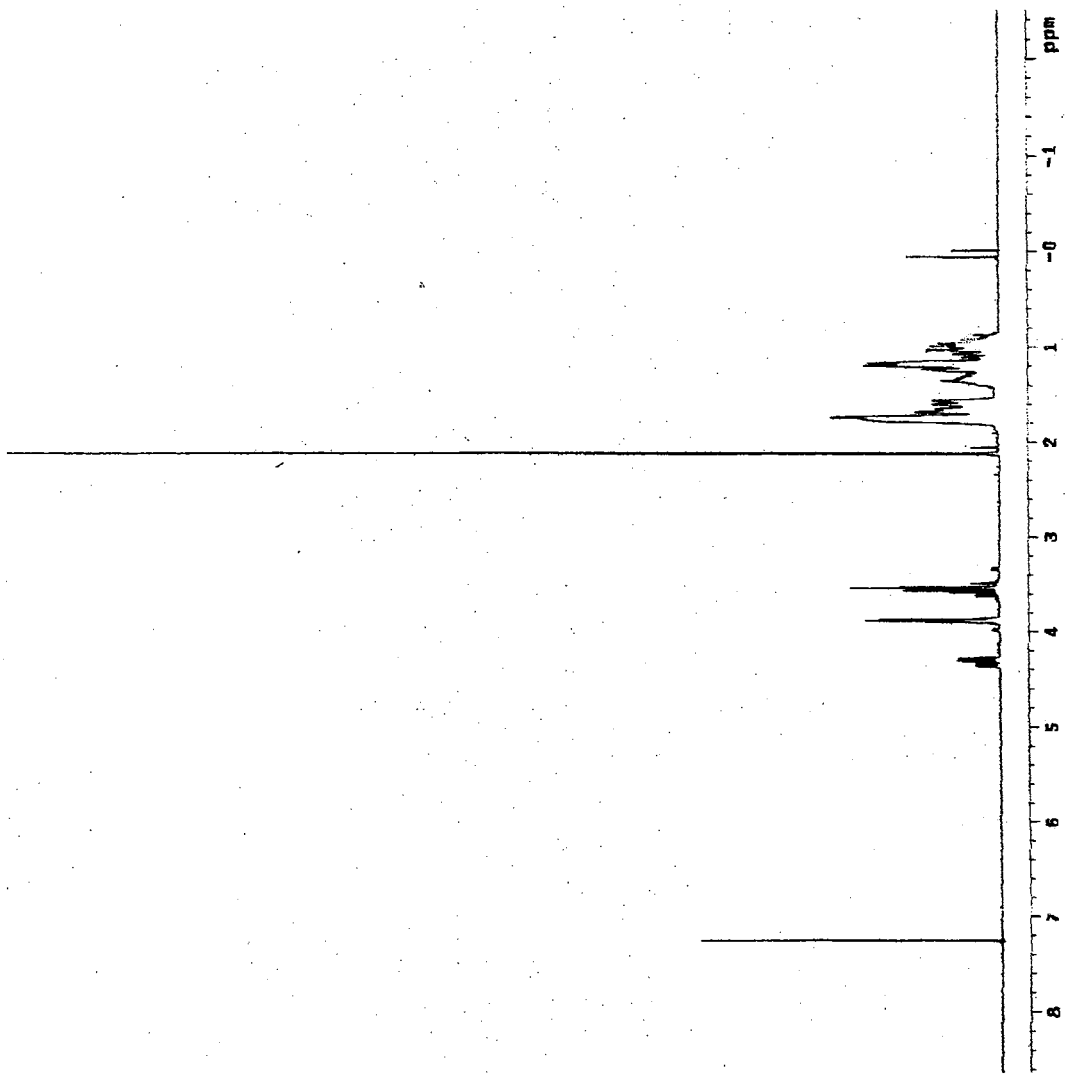


Plate XLIV: ^{13}C NMR of Compound **38** in chloroform-*d*

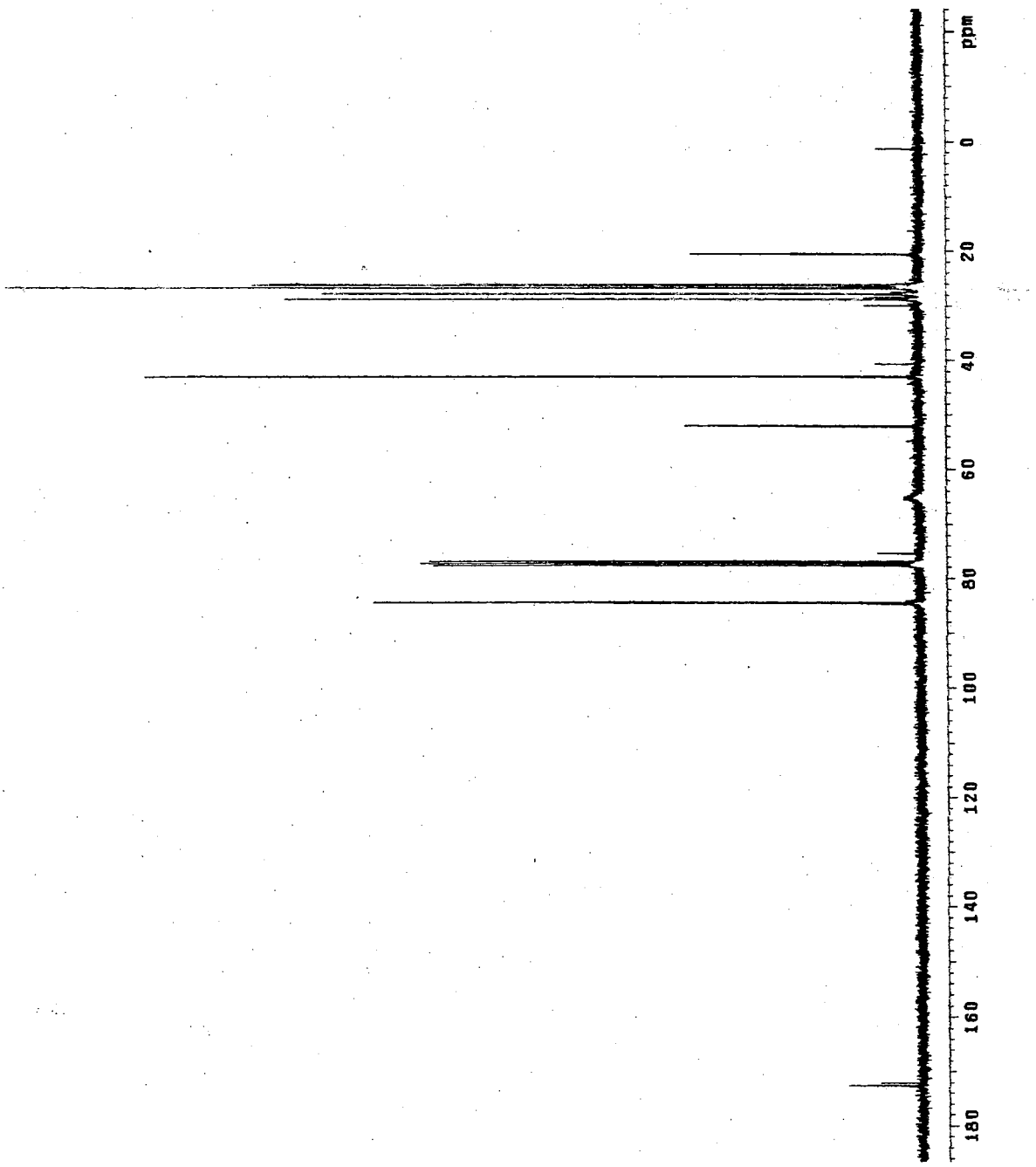


Plate XLV: ^{1}H NMR of Compound **39** in chloroform-*d*

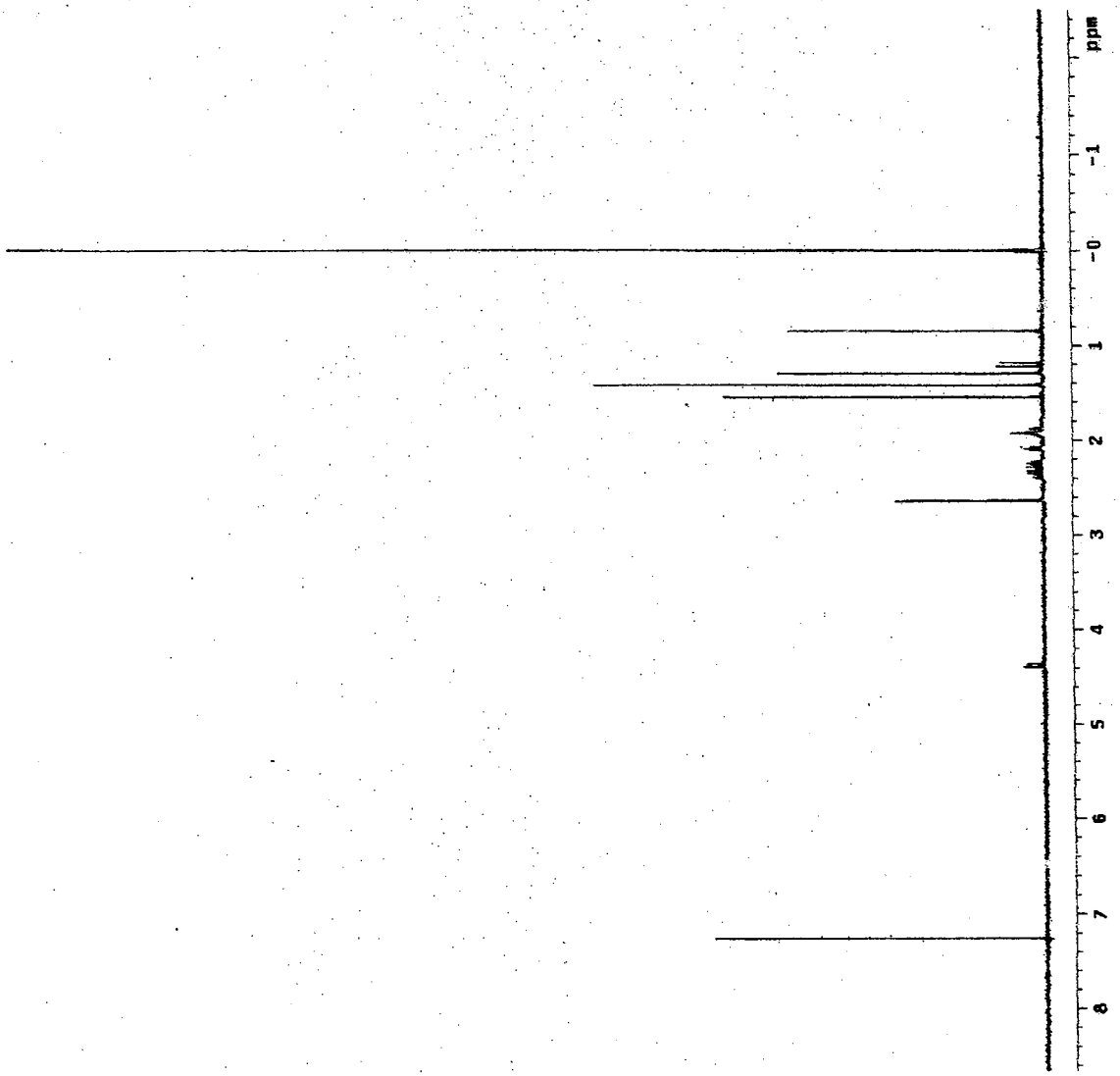


Plate XLVI: ^{1}H NMR of Compound **40** in chloroform-*d*

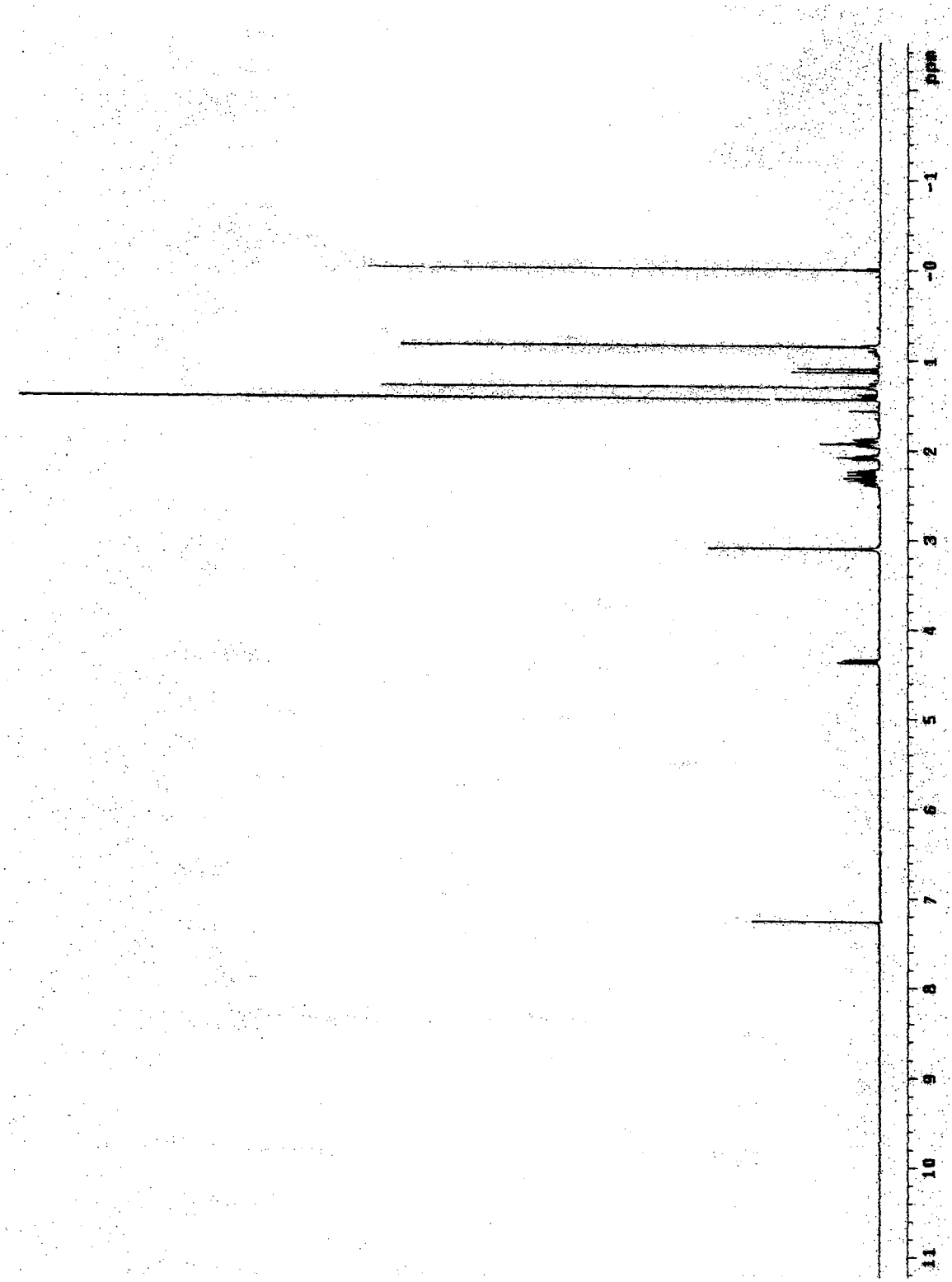


Plate XLVII: ^{13}C NMR of Compound **40** in chloroform-*d*

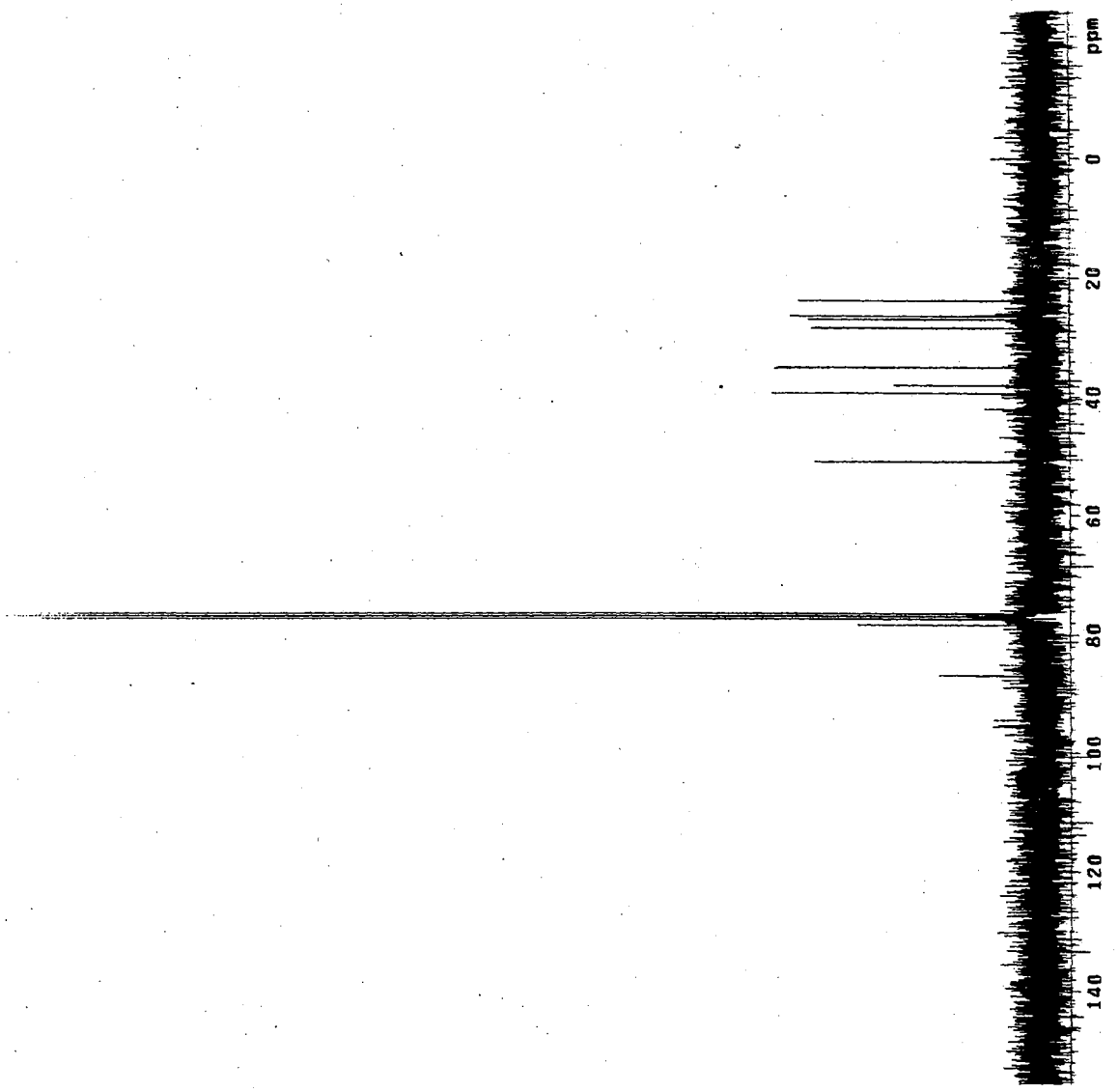


Plate XLVIII: ^{1}H NMR of Compound **42** in chloroform-*d*

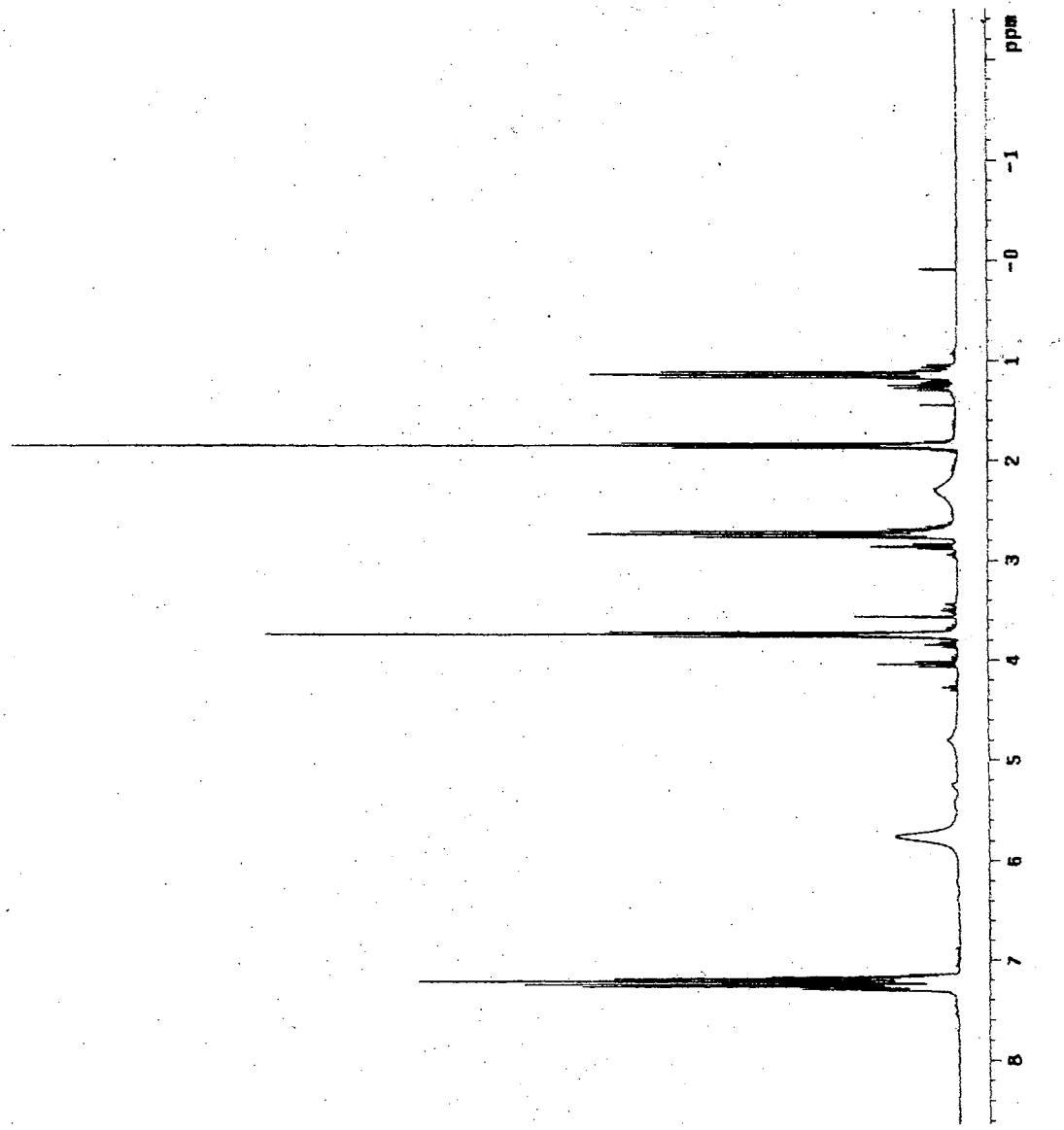


Plate IL: ^{13}C NMR of Compound **42** in chloroform-*d*

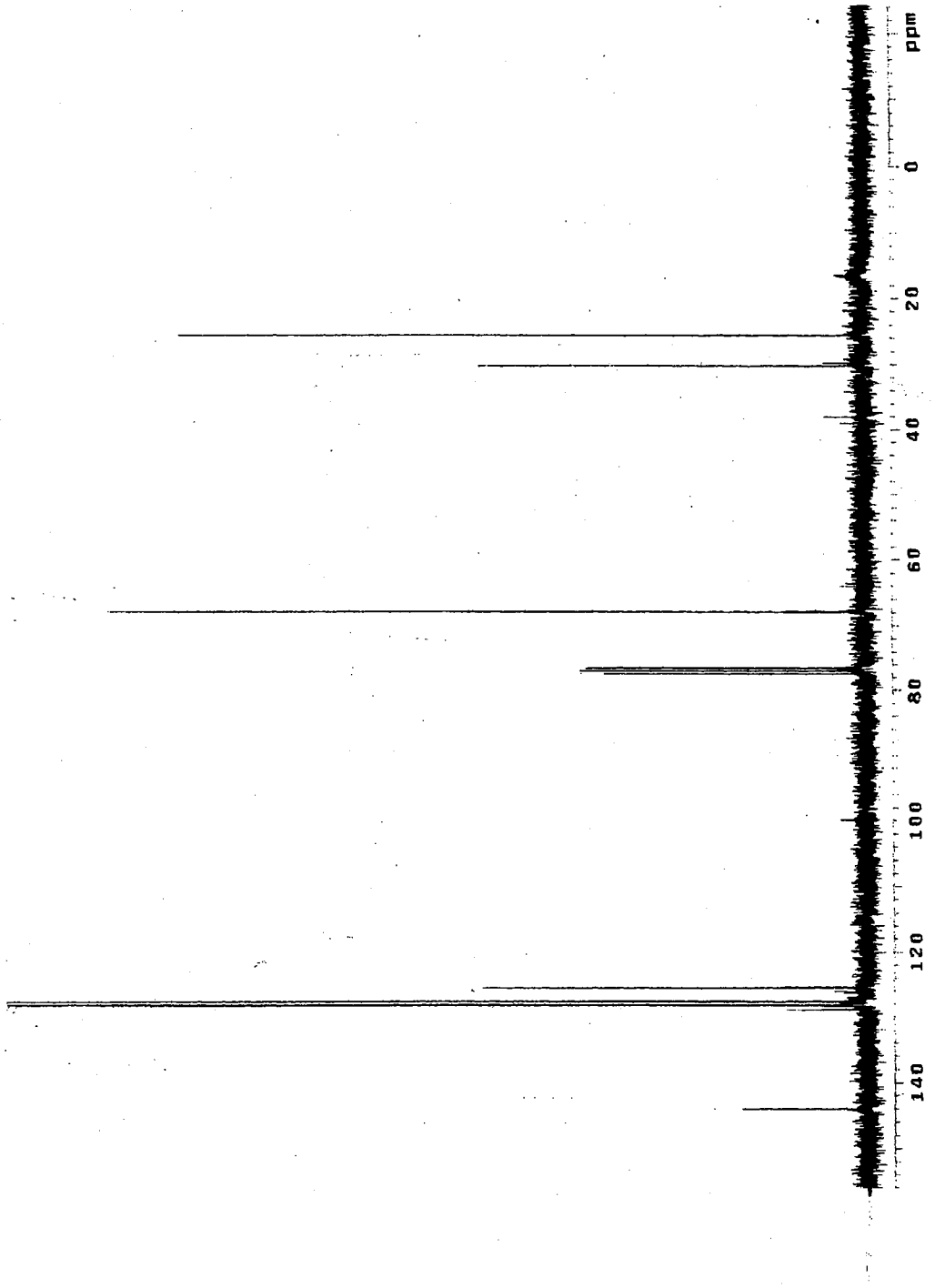


Plate L: ^{11}B NMR Comparison of Compound **42** to Compound **42** after reaction with NaOH

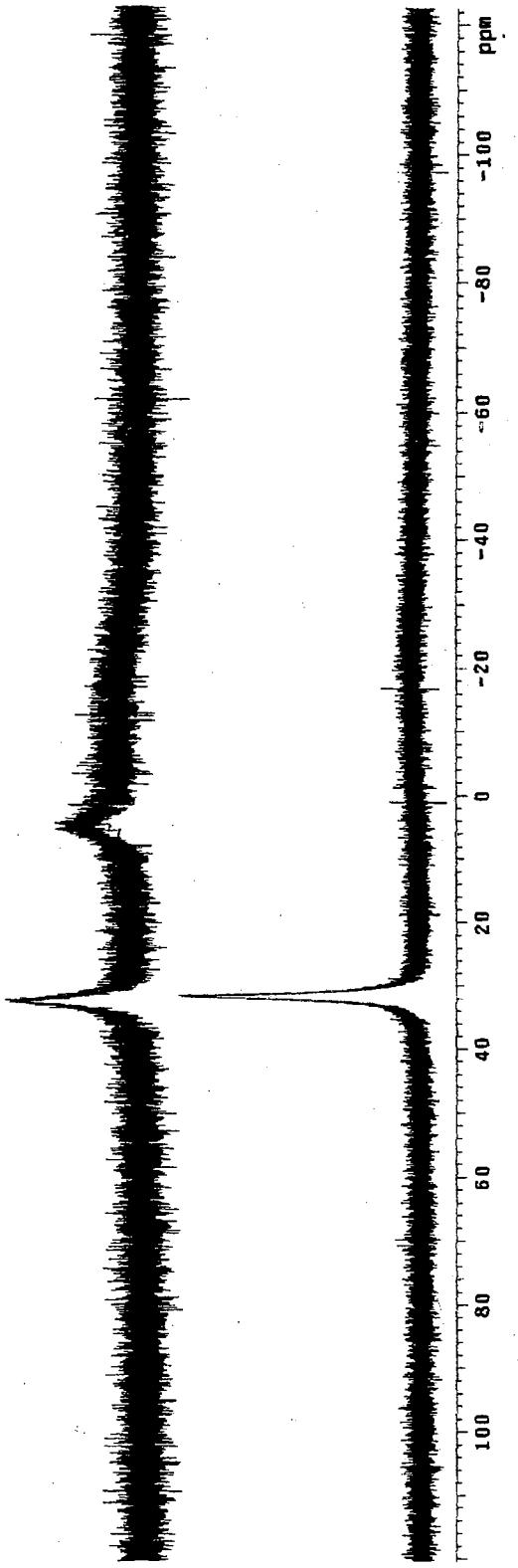


Plate LI: ^{1}H NMR of Compound **43** in chloroform-*d*

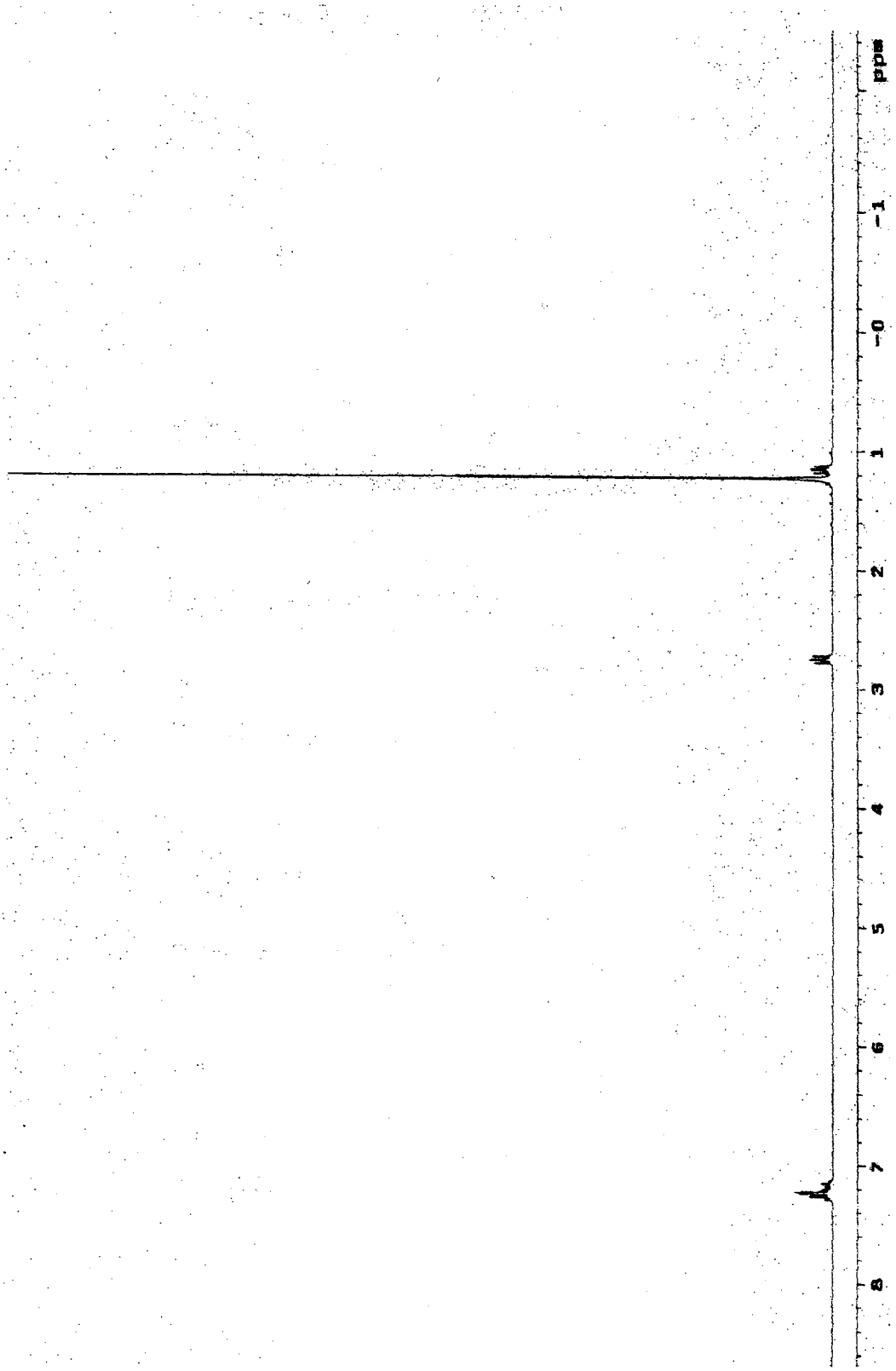


Plate LII: ^{13}C NMR of Compound **43** in chloroform-*d*

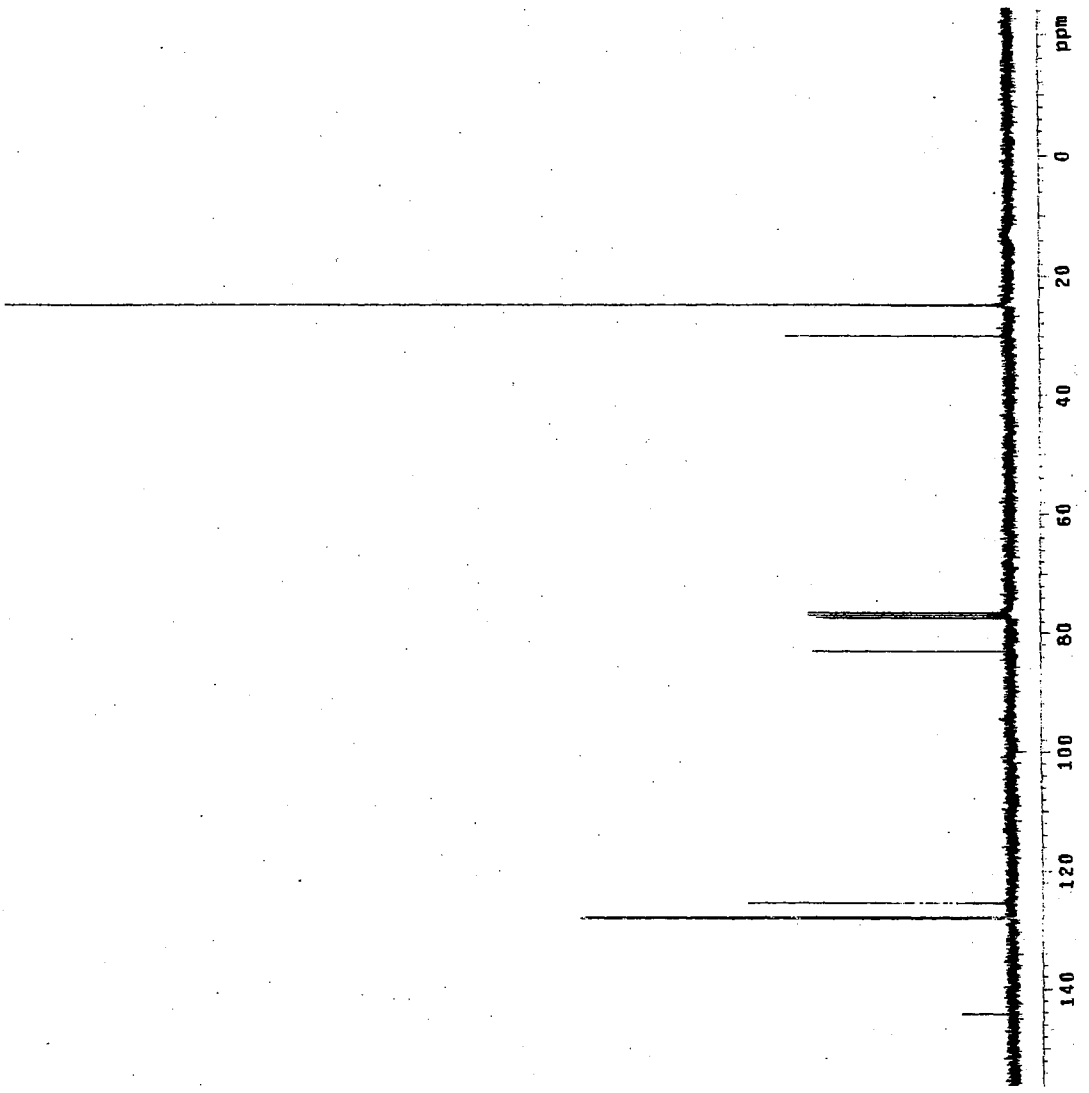


Plate LIII: ^{1}H NMR of Compound **44** in chloroform-*d*

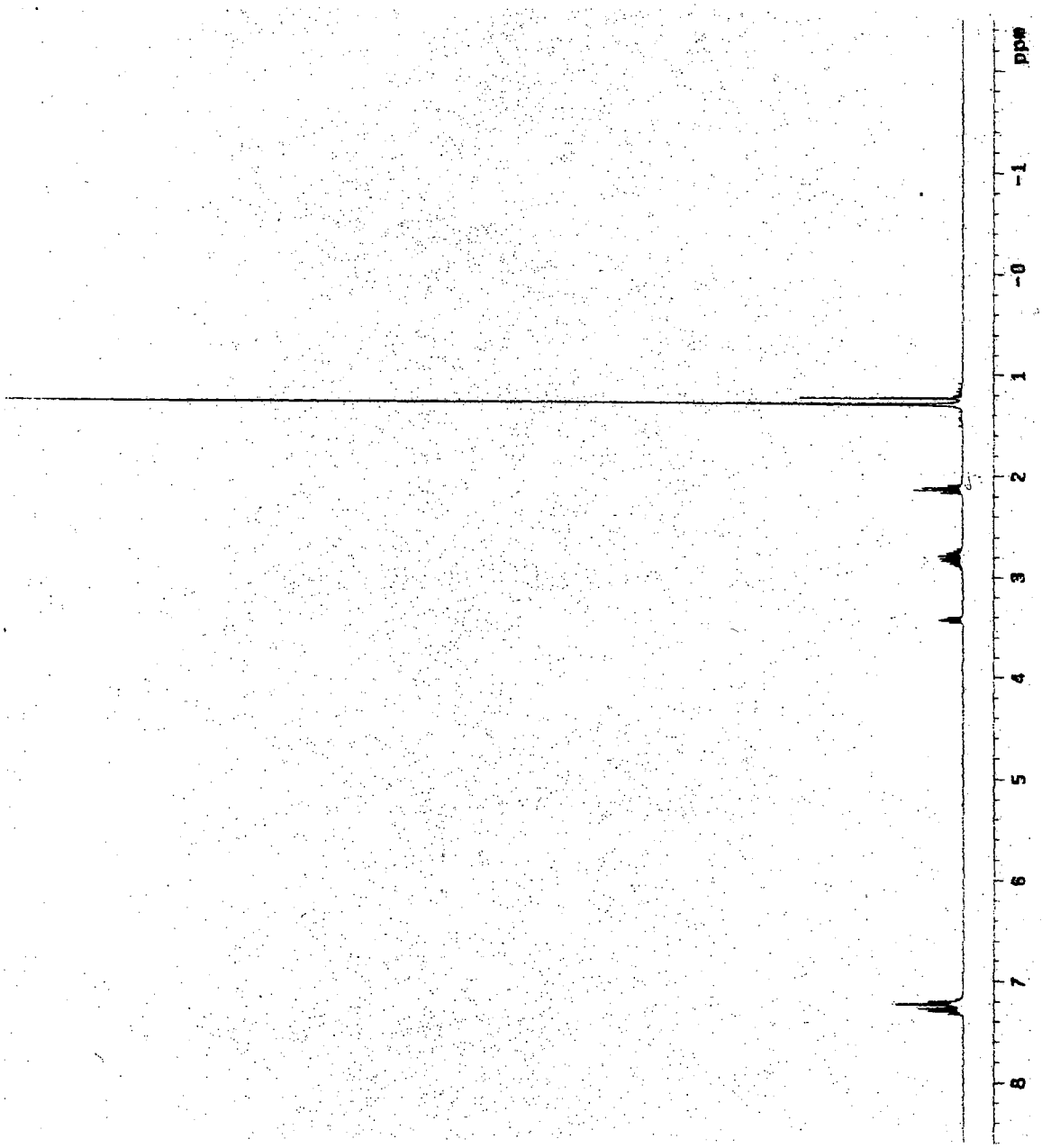


Plate LIV: ^{13}C NMR of Compound **44** in chloroform-*d*

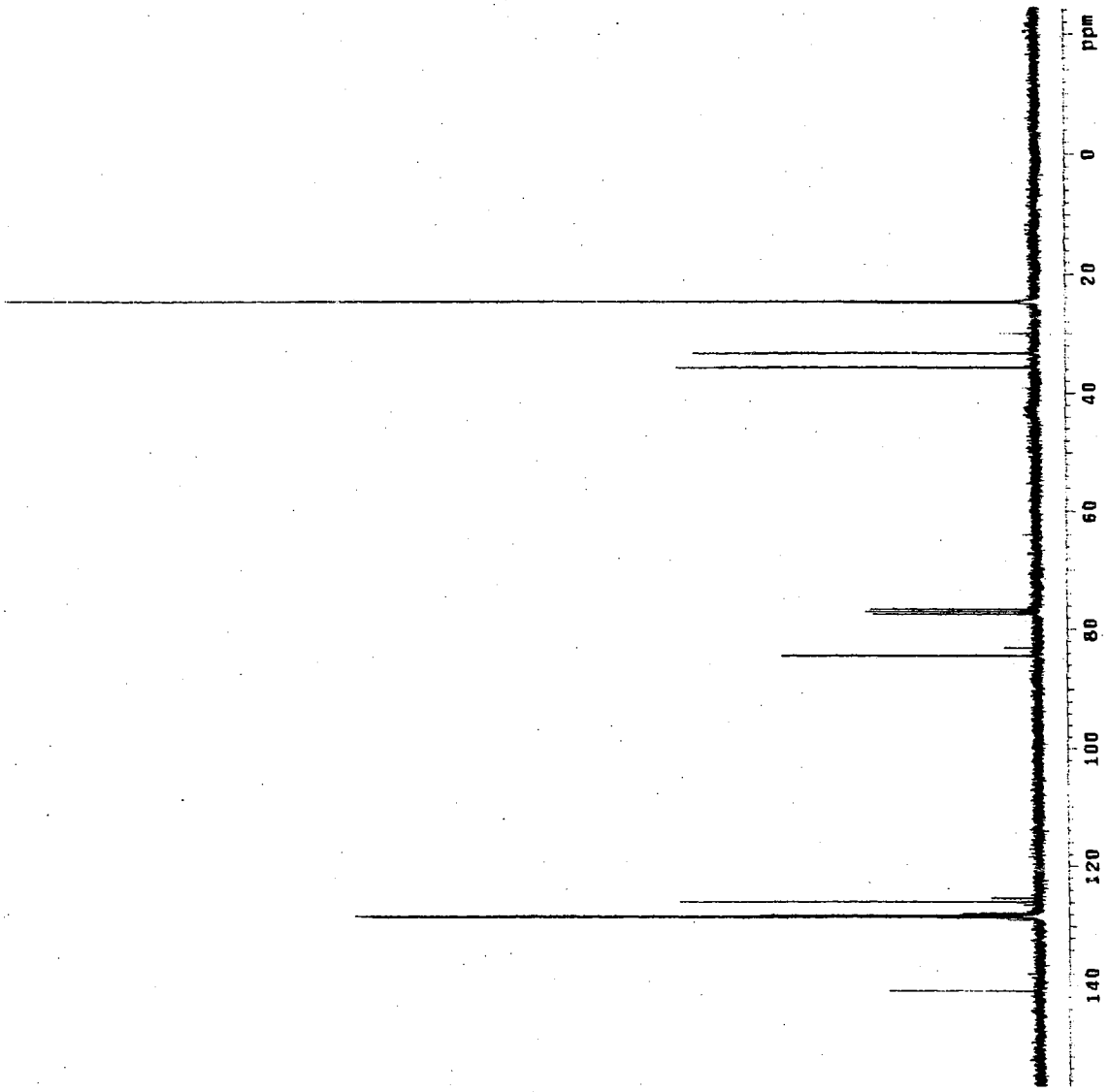


Plate LV: COSY NMR of Compound 44

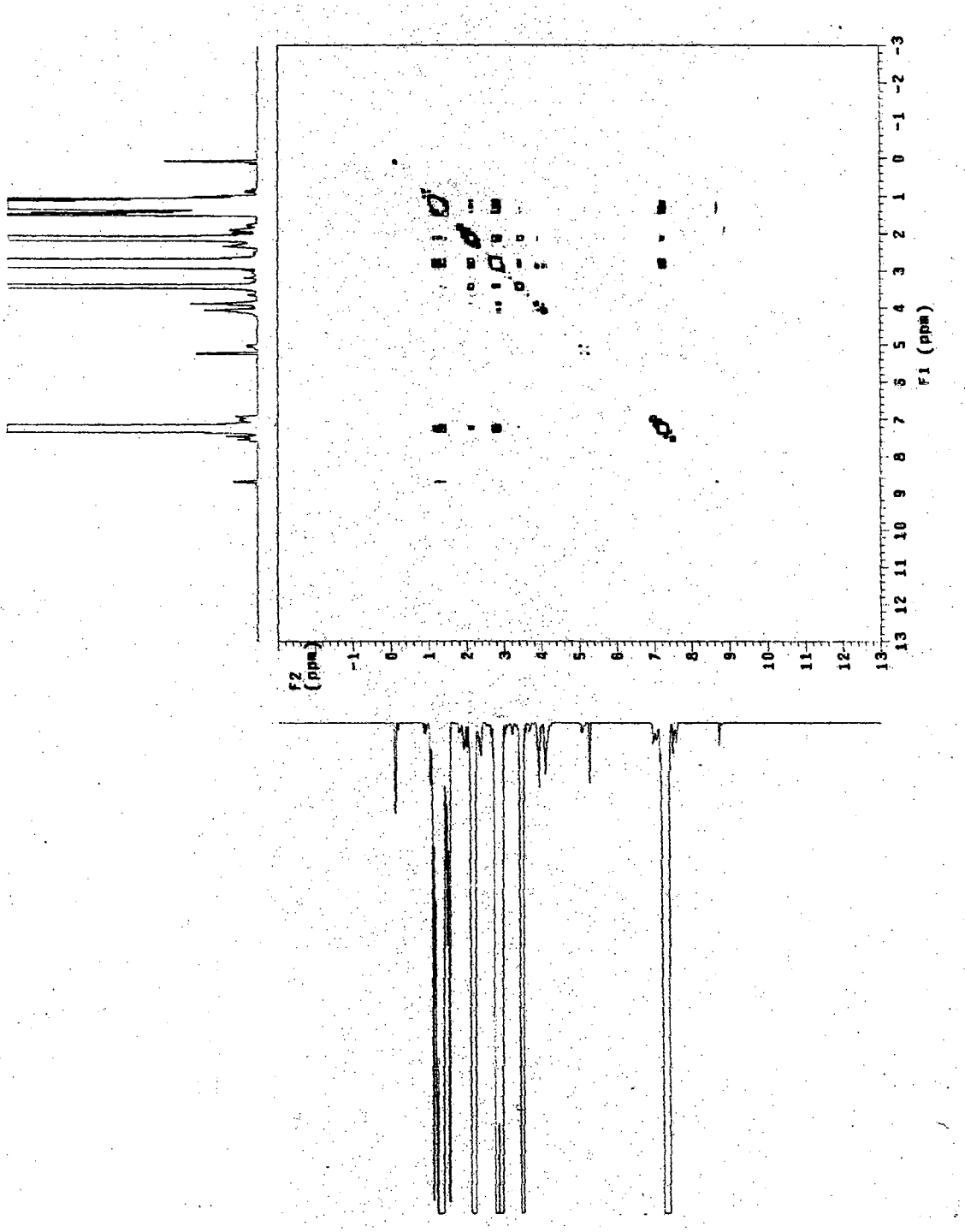


Plate LVI: FT-IR of Compound **45**

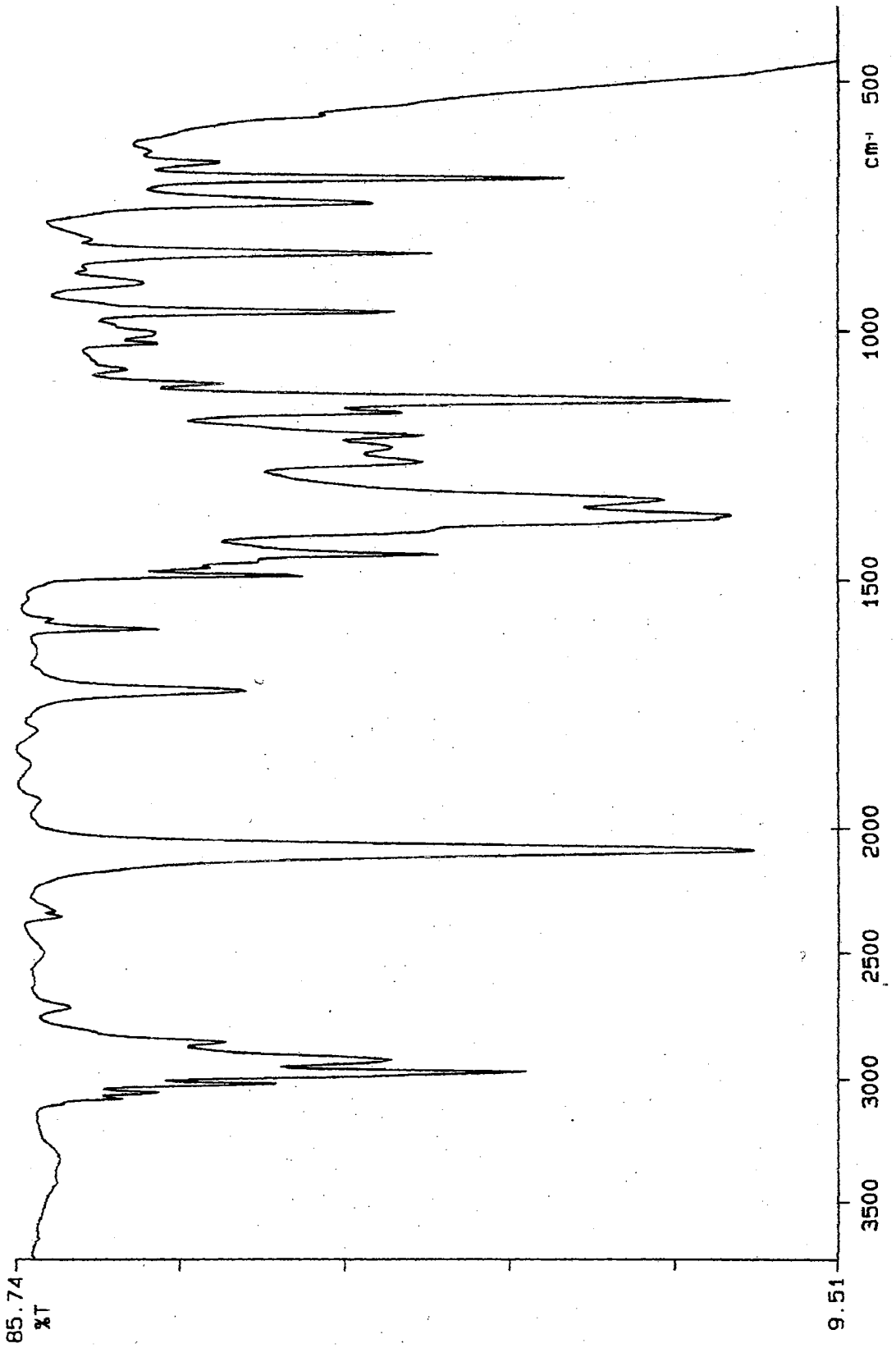


Plate LVII: ^{1}H NMR of Compound **45** in chloroform-*d*

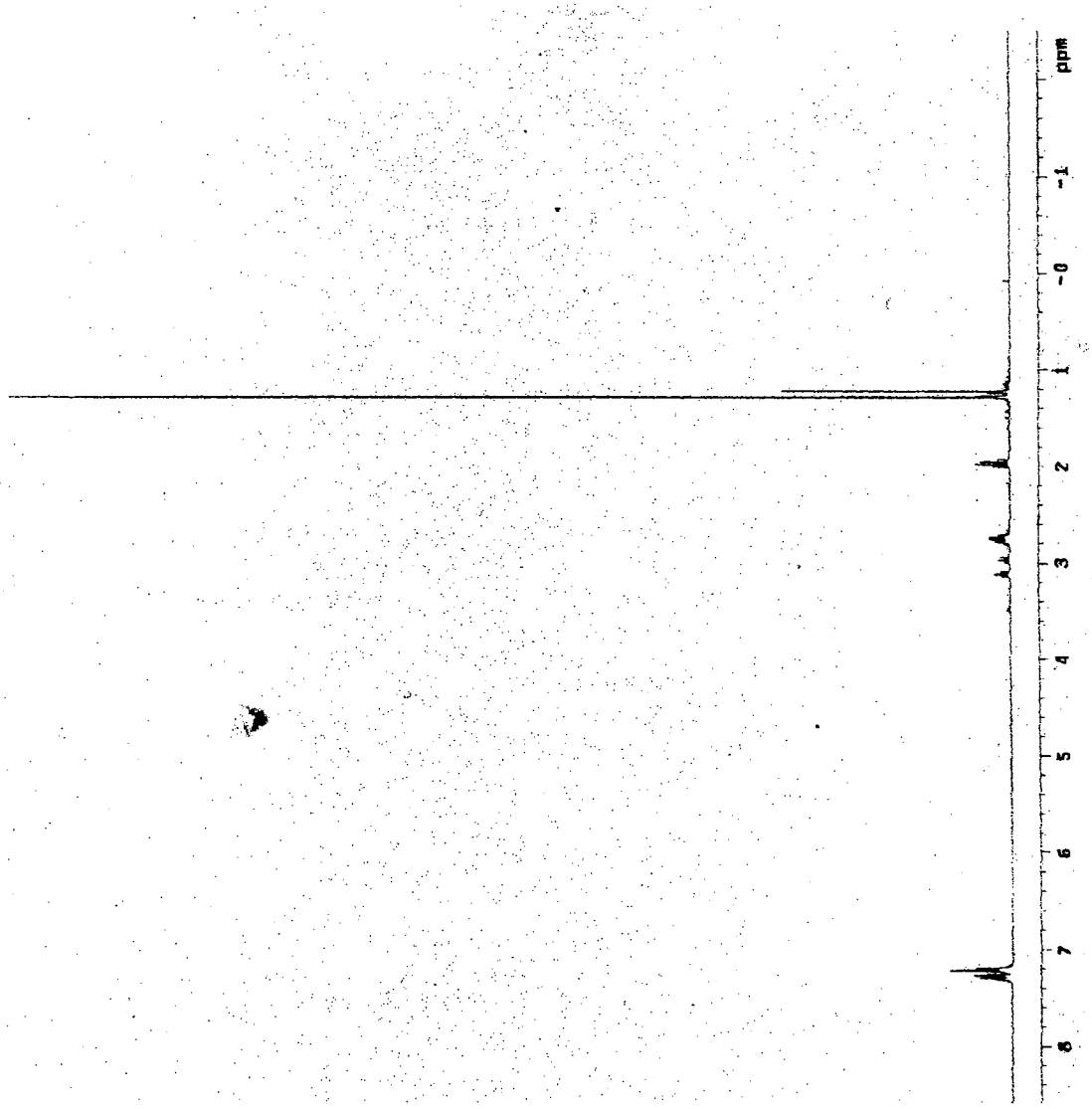


Plate LVIII: ^{13}C NMR of Compound **45** in chloroform-*d*

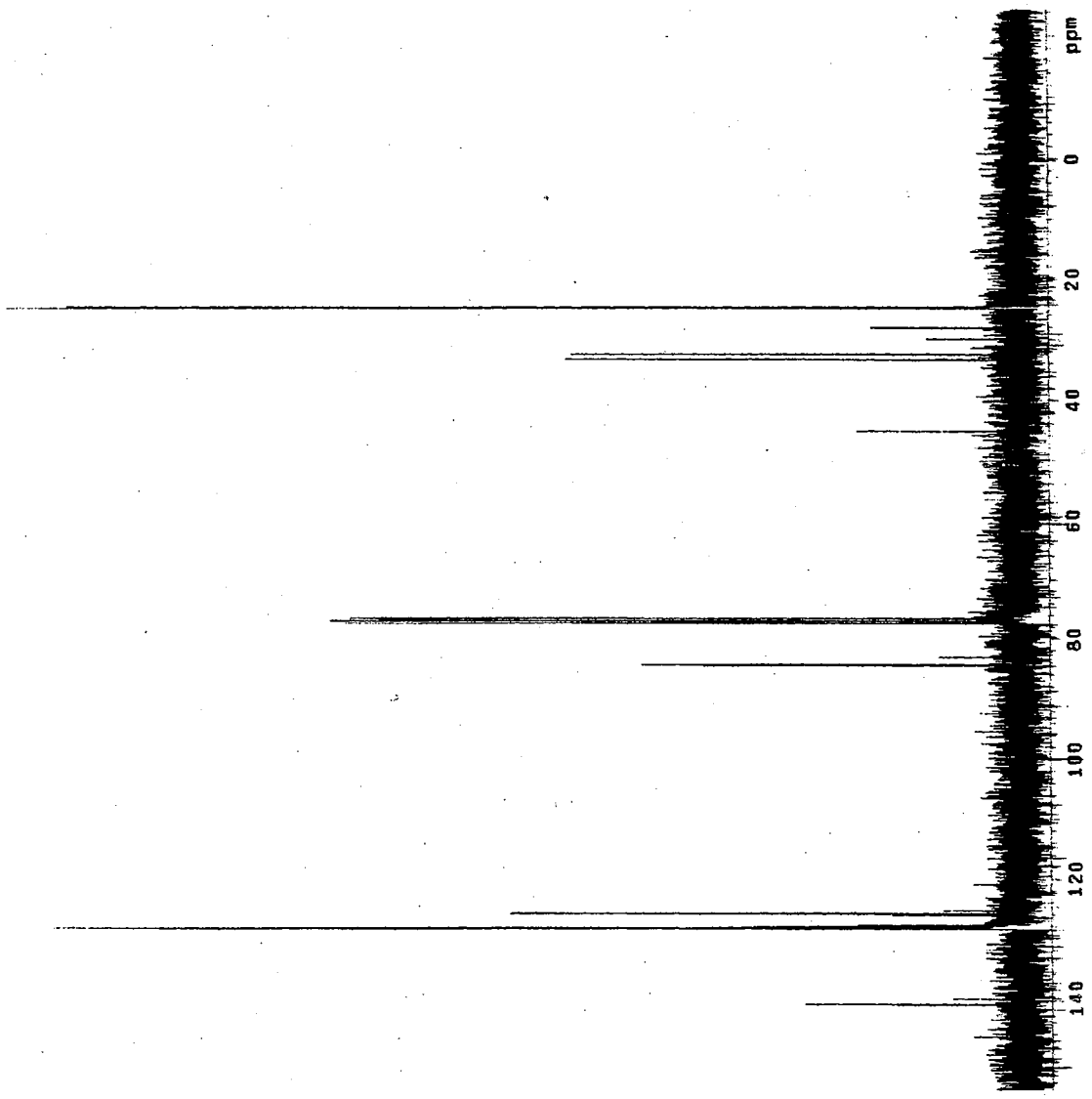


Plate LIX: FT-IR of Compound **46**

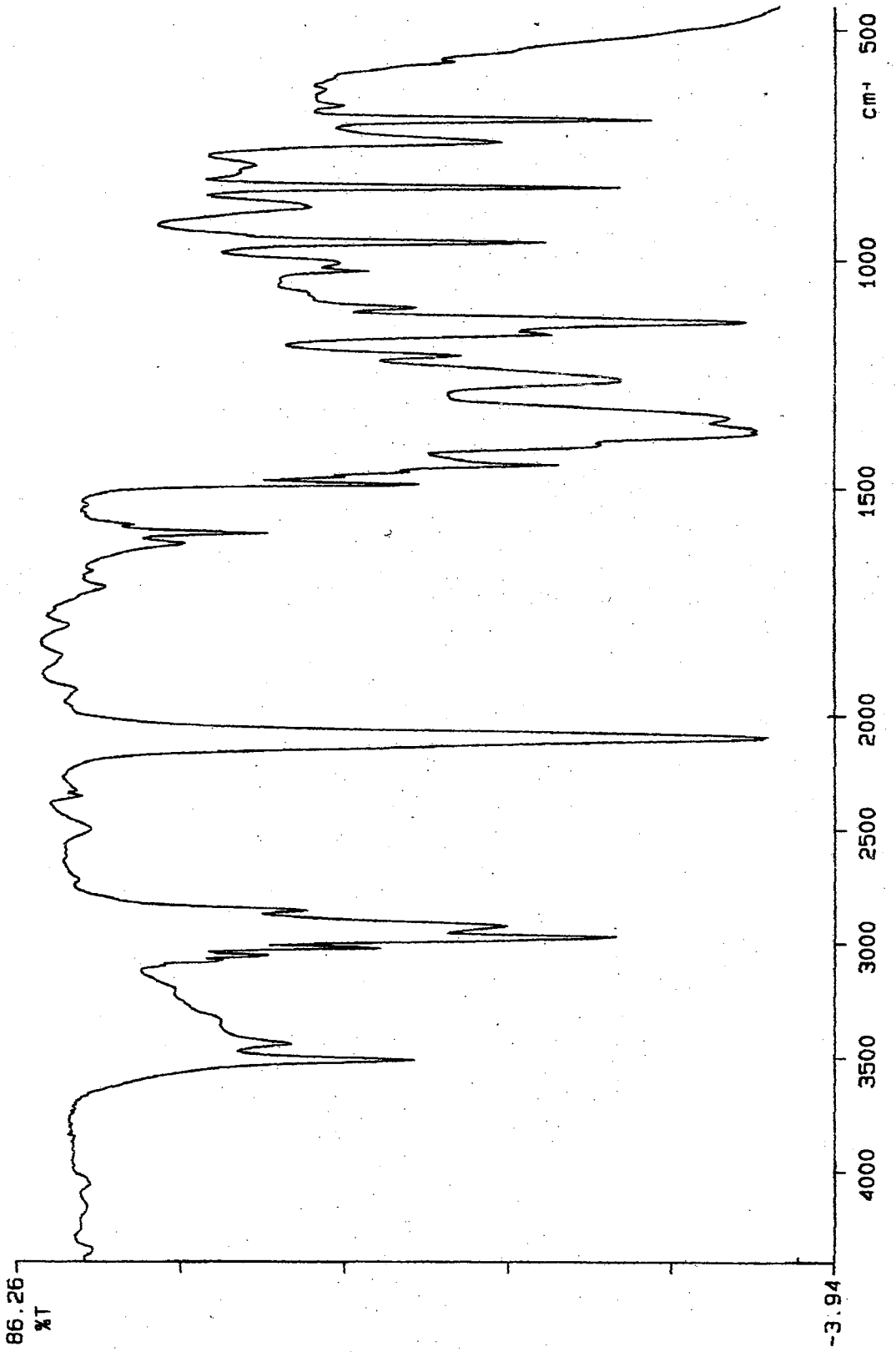


Plate LX: ^{1}H NMR of Compound **46** in chloroform-*d*

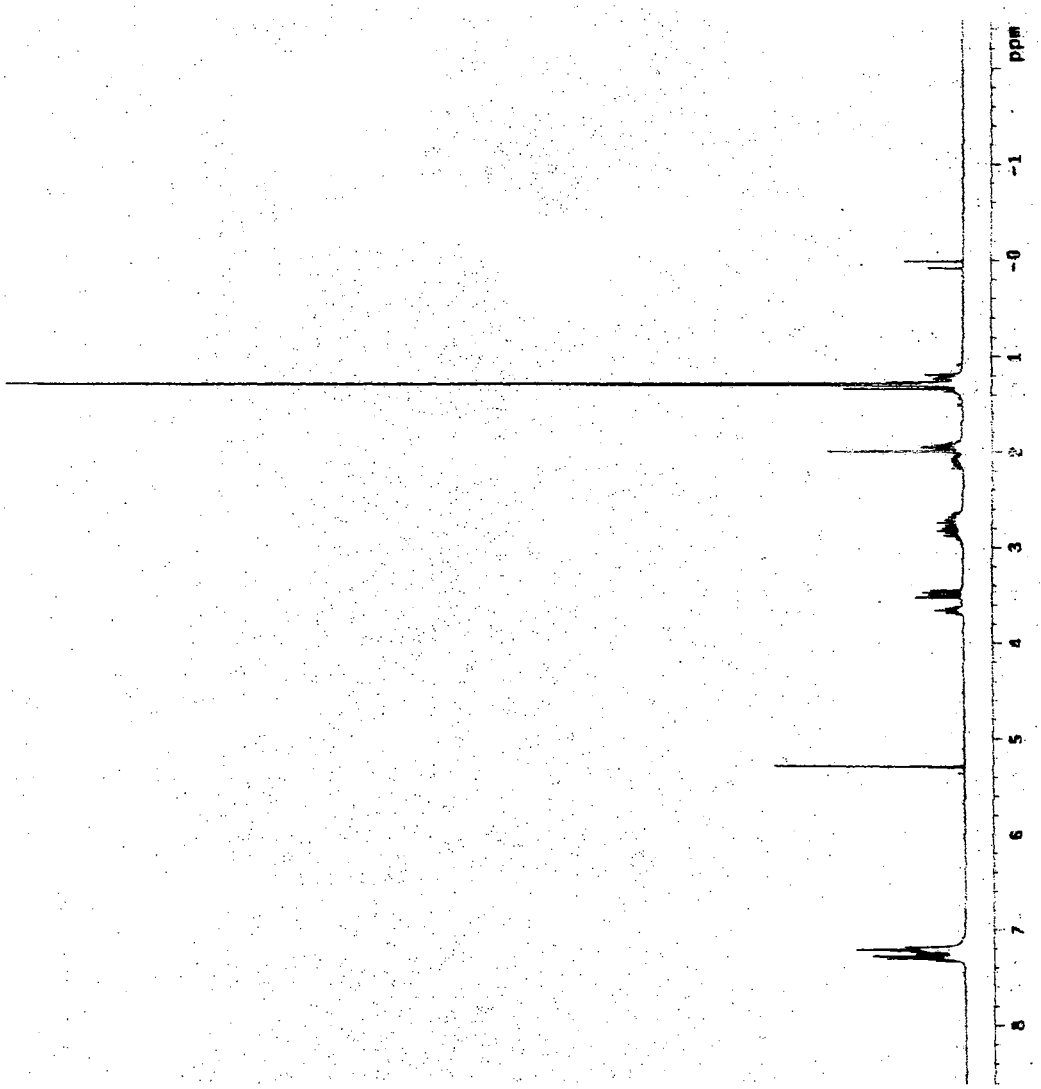
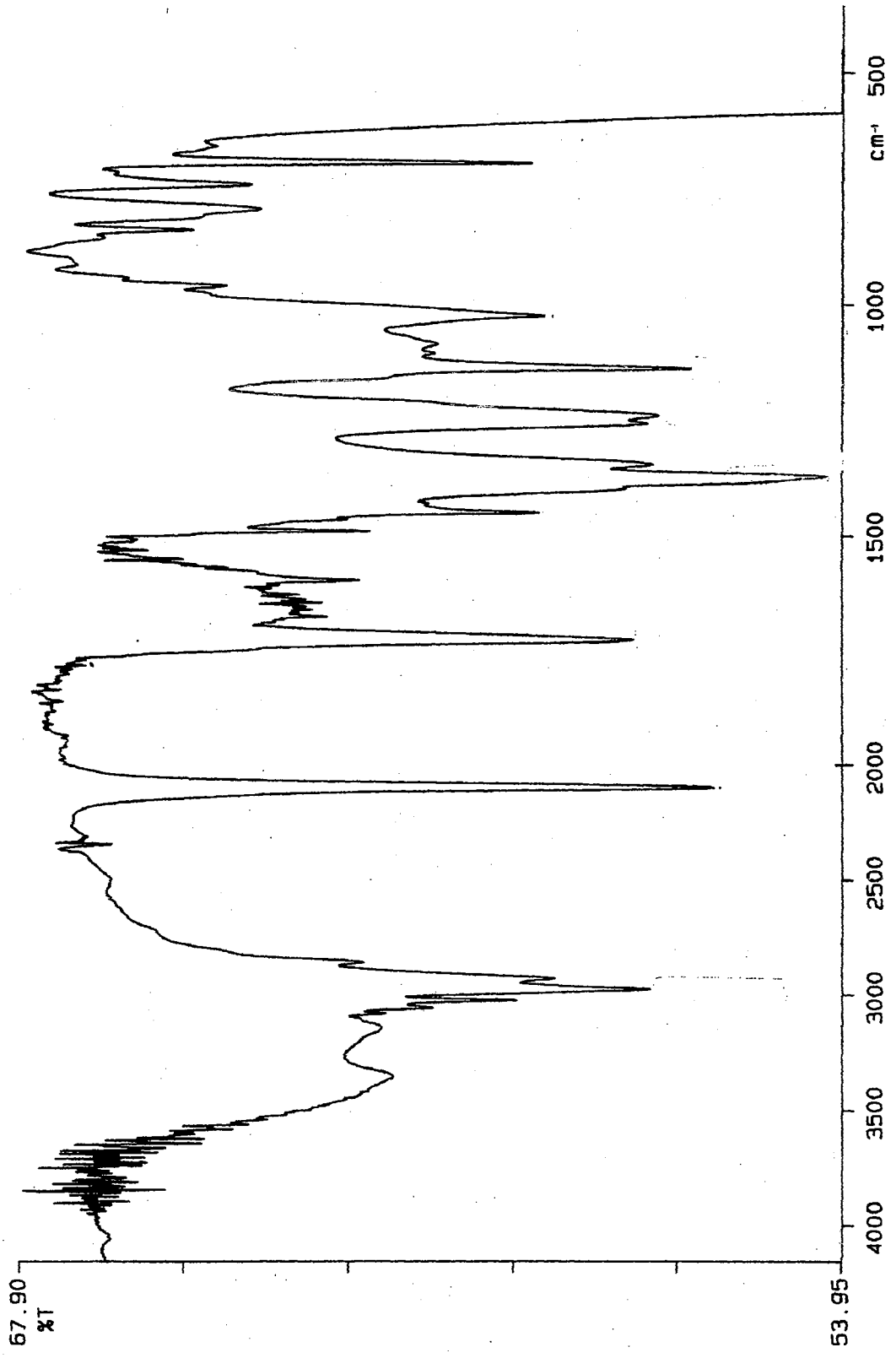


Plate LXI: FT-IR of Compound **47**



VITA 2

Loralee M. Ohrtman

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Doctor of Philosophy

Thesis: SYNTHETIC STUDIES TOWARD BORON-CONTAINING NUCLEOSIDES

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Biographical:

Personal Data: Born in Lewiston, Idaho, on June 16, 1972, the daughter of Dennis and Carol Ohrtman.

Education: Graduated from Lewiston Senior High School, Lewiston, Idaho in May 1990; received Bachelor of Arts degree in Chemistry from Augustana College, Sioux Falls, South Dakota in May 1994. Completed the requirements for the Doctor of Philosophy degree with a major in Chemistry at Oklahoma State University in August 2002.

Experience: Employed by Augustana College, Department of Chemistry as an undergraduate laboratory assistant; Washington State University, Department of Chemistry as a graduate teaching assistant and instructor; Luther College Department of Chemistry as an instructor; Oklahoma State University, Department of Chemistry as a graduate research and teaching assistant.

Professional Memberships: American Chemical Society, Phi Lambda Upsilon