SYNTHESIS OF CARBORANE DERIVATIVES OF CHOLESTEROL FOR INCORPORATION INTO UNILAMELLAR LIPOSOMES AND EVALUATION AS POTENTIAL AGENTS FOR BORON NEUTRON CAPTURE THERAPY

THESIS

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For the Degree

Master of Science

By

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DEDICATION

This is for my parents, Rodney and Patricia Tate, for their love and support throughout the years. Without them, I never would have been the person that I am. I would also like to thank my mentor, Dr. Debra A. Feakes, for all of her love and support in my career as a chemist. I count myself extremely lucky to have had a mentor such as her. Without her, I would not have been able to succeed in my professional career. I would also like to thank my committee members Dr. Michael Blanda and Dr. Chad Booth for their help and support. Lastly, I would like to thank my wonderful wife, Sara. When the road became difficult, she was always there to help me make it up the path. I would also like to dedicate this work to Banjo the Great, Remington the Quick and Mariah the Gentle.

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1.0 ABSTRACT

Boron neutron capture therapy, or BNCT, is a binary cancer therapy based on the ability of the boron-10 isotope to capture thermal neutrons. The neutron capture reaction is the formation of a highly energetic boron-11 atom. The boron-11 atom fissions, producing both a lithium atom and an alpha particle. Along with the two products mentioned, a significant amount of energy is produced. The energy is dissipated within a distance of approximately 10 μm in tissue or roughly the size of a single eukaryotic cell. Hence, if a large enough concentration of boron-10 atoms can be selectively localized in the tumor cell, irradiation of the cell would result in the destruction of the tumor cell while leaving surrounding, normal tissue unaffected.

The major limitation of BNCT is the production of boron-containing compounds that will selectively localize in the tumor cell with large enough concentrations to be effective. In 1999, a series of carborane-containing derivatives of cholesterol, containing a six carbon-atom tether between the carborane and the cholesterol moieties, was prepared. Although the attempt was made to incorporate some of the compounds into the hydrophobic bilayer of unilamellar liposomes, the compounds that were incorporated did not contain sufficient boron for the biodistribution experiments. Additionally, the synthetic schemes were somewhat cumbersome, yields were relatively low, and problems with the reported syntheses were noted.

Therefore, a shorter synthetic route would enhance the ability to prepare the compounds in a given period of time and also increase the overall yield. Additionally, the length of the alkyl chain tether should affect the ability of the carborane derivatives to pack into the bilayer of the liposomes. Therefore, the goal of this research project is the

design of an improved synthetic scheme and the preparation of new carborane derivatives of cholesterol, with varying tether lengths, for incorporation into unilamellar liposomes. New synthetic routes have been developed for the cholesterol derivatives and eight new compounds have been prepared.

2.0 INTRODUCTION

2.1 Boron Neutron Capture Therapy

Boron neutron capture therapy (BNCT) is a technique that is used to treat certain types of brain tumors and metastatic melanoma. The technique is based on the reaction that occurs when a stable isotope of the element boron, boron-10, captures a low energy (0.025 eV), or thermal, neutron. The capture yields a high linear energy transfer (LET) alpha particle and a recoiling lithium-7 nuclei as shown in to Figure 1.

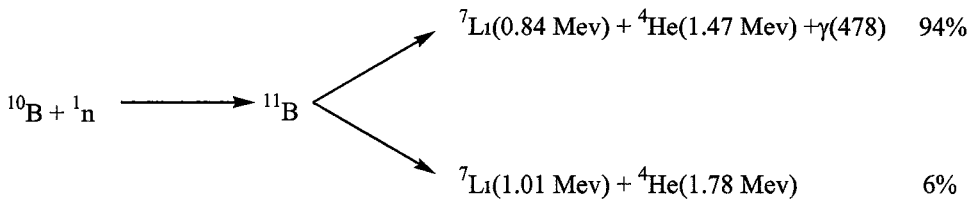


Figure 1: Reaction scheme for α decay of boron-11 atoms after irradiation of boron-10 atoms by a thermal neutron beam.[1]

The thermal neutron cross section for boron-10 is relatively high (σ = 3838 barns) compared to other low atomic weight elements. In tissue, the products of the neutron capture reaction travel approximately 8-10 μm, dissipating the associated energy within that distance. The distance corresponds roughly to the diameter of one eukaryotic cell. If sufficiently high concentrations of boron-10 can be accumulated, in the cells, theoretically, the neutron capture reaction will destroy only the tumor cells.[2] Even though the investigation of BNCT for medicinal application has been ongoing since 1936, when Locher first proposed the idea, there have been serious limitations. The

major inadequacy of the current therapy is that large concentrations of boron are required, in each tumor cell. The estimated theoretical concentration required for successful therapy is calculated to be approximately 15 μg of boron per gram of tissue.[3] In these calculations, the location of the boron target nuclei within the tumor cell is critical. As the distance from the target nuclei to the critical nuclear constituentsdecreases, the calculated concentration of boron required for cell destruction decreases. Consequently, the concentration of the target nuclei can be much less if the target nuclei localize within the cell as opposed to the exterior cell wall. Additionally, the neighboring tissues, including blood, must have a minimal concentration of compound to reduce the lethal affects to normal tissue.

Theoretically, BNCT is an ideal binary therapy in which each component is non-lethal. As attractive as BNCT is, significant limitations have been encountered in the application of BNCT. BNCT trials were performed in the 1950's and 1960's at both Brookhaven National Laboratories (BNL) and the Massachusetts Institute of Technology (MIT) utilizing malignant gliomas. The compounds, $Na_2[B_4O_7]$ and $Na_2[B_{10}H_{10}]$, were injected and irradiated with thermal neutrons.[4] Both trials yielded poor results and the treatment modality was given a large amount of negative press. These results have been attributed to insufficient penetration by the thermal neutron beam, as well as poor tumor specificity of the compounds used. The penetration issue was resolved by partial resection of the scalp and skull, the use of an epithermal neutron source, and the development of better beam designs.[5] The poor tumor specificity associated with the compounds is still an issue with the application of BNCT.

Since the original clinical trials in the 1950's and 60's, some encouraging results have been obtained using BNCT for the treatment of malignant brain tumors and metastatic melanoma. The Hatanaka group in Japan use boron-10 enriched borocaptate sodium ($Na_2B_{12}H_{11}SH$, BSH) (Figure 2), and clinical trials in the United States have been using *p*-boronophenylalanine (*p*-BPA) (Figure 3). The two compounds have been shown to accumulate in a number of small animal glioma models.[6]

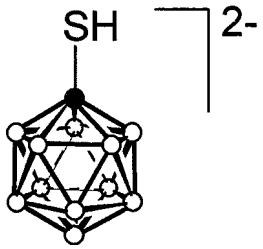


Figure 2: Structure of $Na_2B_{12}H_{11}SH$ (BSH).

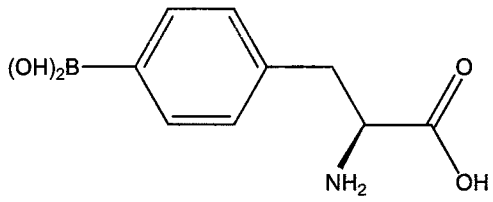


Figure 3: Structure of *p*-BPA.

Even though BSH and *p*-BPA are currently being used for BNCT, the compounds lack the desired retention properties and localization within the tumor to be a truly viable cancer agent. Therefore, the design and preparation of boron-containing compounds is currently the basis for extensive research. At present, there are two general methods being utilized in the development of compounds for evaluation as BNCT agents. One

method is based on the synthesis of boron-containing derivatives of a species known to gather in the tumor or have the potential for *in vivo* metabolization. This category includes boron derivatives of amino acids,[7] nucleosides and nucleotides,[8] monoclonal antibodies,[9] and porphyrins.[10] The second method is based on the design of a tumor selective delivery system that deposits a boron-containing compound which possesses no inherent tumor specificity. While low-density lipoproteins incorporating lipophilic boron-containing compounds have been studied,[11] the primary system used in this category is the utilization of unilamellar liposomes as delivery vehicles.[12]

For a number of years, unilamellar liposomes, such as the one depicted in Figure 4, have been studied as medicinal delivery agents.[13] The liposomes that have been

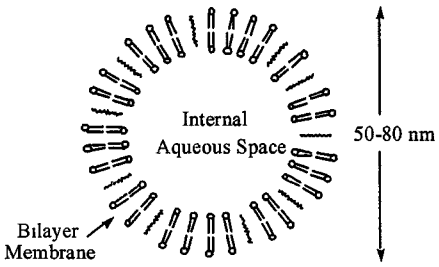
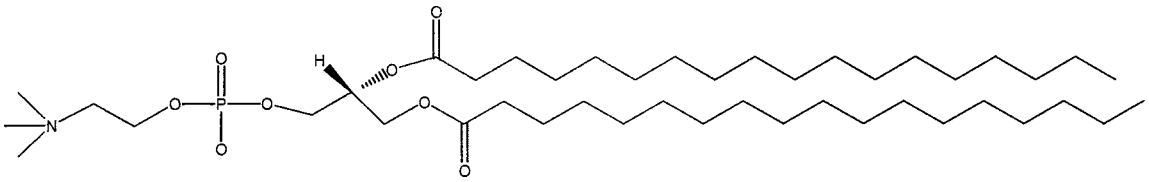
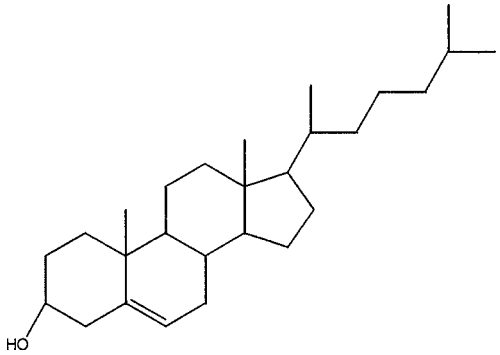


Figure 4: Cross section of a small unilamellar liposome

studied for application in BNCT, and used for this study, are of a specific composition and size. The bilayer is composed of an equimolar ratio of cholesterol and 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) (Figure 5). DSPC is purchased as a pure synthetic product and yields greater *in vivo* stability when compared to natural phospholipids.



1,2-Distearoyl-sn-Glycero-3-Phosphocholine (DSPC)



Cholesterol

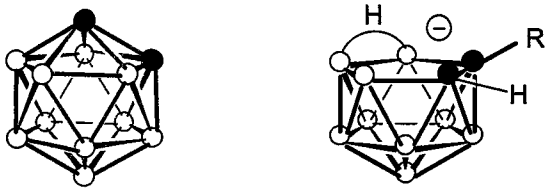
Figure 5: The structures of cholesterol and DSPC which make up the bilayer of unilamellar liposomes

The proposed mechanism of delivery, for liposomes of 80 nm or less, is based on the hypothesized ability of the liposome to seep through the immature vascular tissue that is characteristic of rapidly growing tumor systems. Upon delivery to the tumor, the liposomes are endocytosed by a coated pit-coated vesicle mechanism.[14] After the liposome is coated, the vesicle ruptures and the contents are discharged into the cytoplasm of the cell.[14] The retention of the liposomal compounds is dependent on the compounds size, charge, and the ability of the compound to interact with the intracellular protein moieties.

The reason that unilamellar liposomes are appealing for application as delivery agents in BNCT is due to their potential to deliver a wide variety of boron-containing compounds to the interior of tumor cells. Hydrophilic boron-containing compounds can

be encapsulated in the aqueous interior of the liposomes while hydrophobic boron-containing compounds can be incorporated into the lipophilic bilayer of the liposomes. Previous research has concentrated on the delivery of water-soluble polyhedral borane anions.[15] Although water-soluble polyhedral borane anions can be incorporated into unilamellar liposomes and lead to a high degree of tumor uptake, at low injected doses, relative to other BNCT studies, the boron concentration required for application in BNCT has been attained by encapsulation of hypertonic solutions of the polyhedral borane anions ranging from 750-900 mOsM. Due to detrimental affects to the stability of the liposome formulation, higher concentrations of the water soluble polyhedral borane anion cannot be encapsulated. Also, due to the low incorporation efficiencies ($\approx$ 3%) obtained in the production of unilamellar liposomes by sonication, large quantities of boron-containing compounds must be prepared.[16] This observation is of particular importance when the preparation of boron-10 enriched compounds is ultimately required.

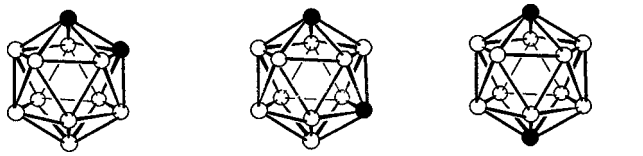
In order to increase the amount of boron delivered by the liposome, lipophilic boron-containing compounds have been prepared for incorporation into the bilayer of liposomes which also encapsulate the water-soluble polyhedral borane anions.[17] The boron-containing compounds contain the lipophilic carborane substituent. Carboranes are defined as a boron cluster that have one or more carbon atoms within the borane cluster framework.[18] Two of the most common carborane structures (Figure 6) are called the *closo*-carborane, which has a closed cage geometry, and the *nido*-carborane, which has an open cage geometry and an associated negative one charge.



closo-carborane *nido*-carborane

Figure 6: Structures of *closo*-carborane and *nido*-carborane

Nido-carboranes are traditionally prepared by the degradation of the *closo*-carborane using potassium hydroxide in ethanol.[18] Three isomers of *closo*-carborane are known: *ortho*-carborane, *meta*-carborane, and *para*-carborane (Figure 7).



ortho-carborane *meta*-carborane *para*-carborane

Figure 7: Structures of *ortho*-, *meta*-, and *para*-carborane

The isomeric assignment is based on the location of the carbon atoms relative to each other. *Ortho*-carborane is prepared from the reaction of decaborane and an appropriate alkyne.[18] The alkyne carbons are incorporated into the structure of the cage. *Meta*-carborane and *para*-carborane are prepared from the *ortho*-carborane, consecutively, by heating at high temperatures. *Ortho*-carborane is the easiest of the three isomers to convert into the *nido*-carborane derivatives. Based on the direct synthesis of the *closo*-carborane derivatives from the appropriate alkyne and the relative ease in conversion to

the *nido*-carborane derivatives, *ortho*-carboranes have been investigated for application in BNCT to a far greater extent than either *meta*-carborane or *para*-carborane.

The potassium salt of [*nido*-7-$CH_3(CH_2)_{15}$-7,8-$C_2B_9H_{11}$] (Figure 8), a *nido*-carborane compound characterized by a polar *nido*-carborane head group and a long lipophilic alkyl chain, has been successfully incorporated into the bilayer of the liposomes for investigation as a potential agent in BNCT.[19]

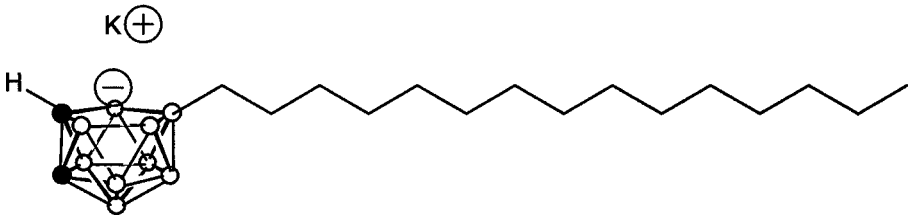
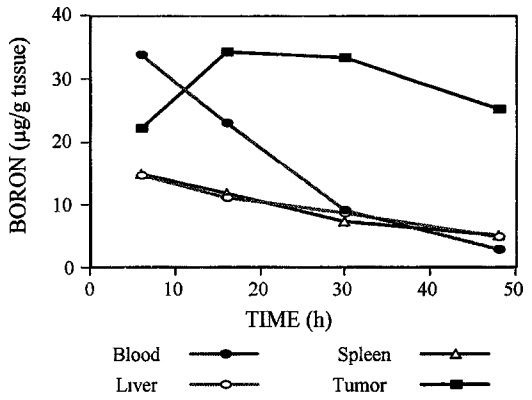
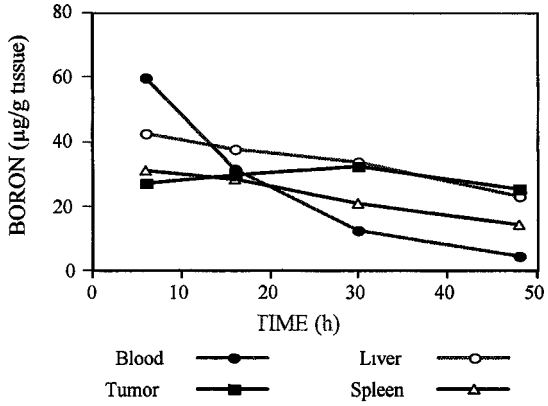


Figure 8: Structure of K[*nido*-7-$CH_3(CH_2)_{15}$-7,8-$C_2B_9H_{11}$

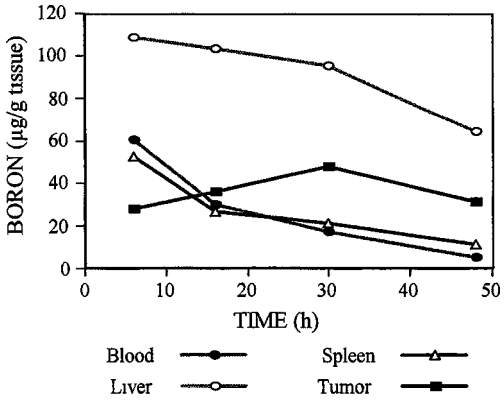
Biodistributions are performed using BALB/c mice bearing EMT6 mammary adenocarcinomas implanted in the left flank of the mouse. Five mice are sacrificed at each time increment and the points on the graph represent the average of the five boron concentrations, measured by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) at the Idaho National Engineering and Environmental Laboratories (INEEL). The biodistribution of the *nido*-carborane, incorporated in unilamellar liposomes containing boron-free buffer displayed an enhanced tumor selectivity (Figure 9a). This characteristic is consistent with liposomes with a negatively charged bilayer. Incorporation of the *nido*-carborane into unilamellar liposomes encapsulating the polyhedral borane anion, $Na_3[B_{20}H_{17}NH_3]$, exhibite an increase in concentration of boron in the tumor relatively to unilamellar liposomes which only encapsulated the polyhedral borane anion (Figure 9b and 9c). Therefore, incorporation of the lipophilic boron-



a) Lipophilic $K[CH_3(CH_2)_{15}C_2B_9H_{11}]$ in BALB/c Mice Bearing EMT6 Tumors



b) Hydrophilic $Na_3[ae\text{-}B_{20}H_{17}NH_3]$ in BALB/c Mice Bearing EMT6 Tumors



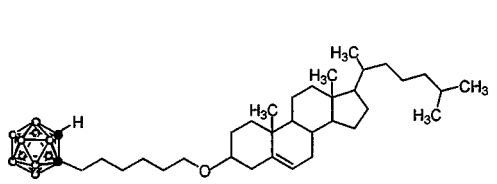
c) Combination of Lipophilic and Hydrophilic Species in BALB/c Mice Bearing EMT6 Tumors

Figure 9: Biodistribution studies for a) Lipophilic $K[CH_3(CH_2)_{15}C_2B_9H_{11}]$, b) Hydrophilic $Na_3[ae\text{-}B_{20}H_{17}NH_3]$ and c) Combination of lipophilic and hydrophilic species in BALB/c mice bearing EMT6 tumors

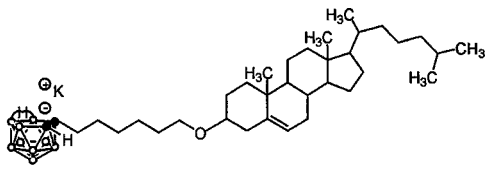
containing compound into the bilayer of liposomes which also contain an encapsulated polyhedral borane anion enables an increase in boron dosage and a higher boron concentration in the tumor.

2.2 Synthesis of Lipophilic Boron-Containing Compounds

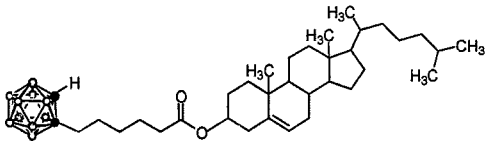
In 1999, four carborane containing derivatives of cholesterol were developed and reported (Figure 10).[21]



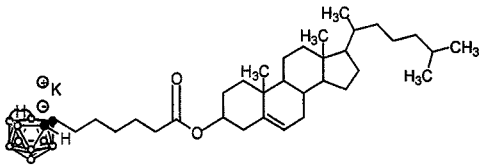
Closo-carborane with an ether linkage



Nido-carborane with an ether linkage



Closo-carborane with an ester linkage



Nido-carborane with an ester linkage

Figure 10: Developed carborane derivative of cholesterol

The four compounds developed contained a six carbon atom tether between the cholesterol and the carborane sustituent. In the synthetic scheme reported, the synthesis required six steps to prepare the *ortho*-carborane derivatives with the ester linkage (Figure 11) and five steps to prepare the *ortho*-carborane derivatives with the ether linkage (Figure 12).

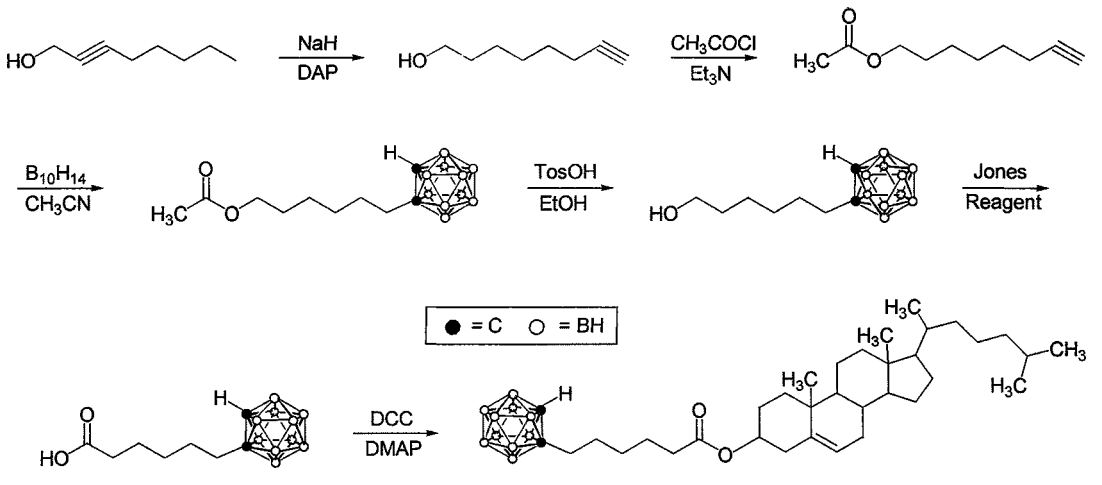


Figure 11: Original synthetic scheme for the ester-linked derivatives

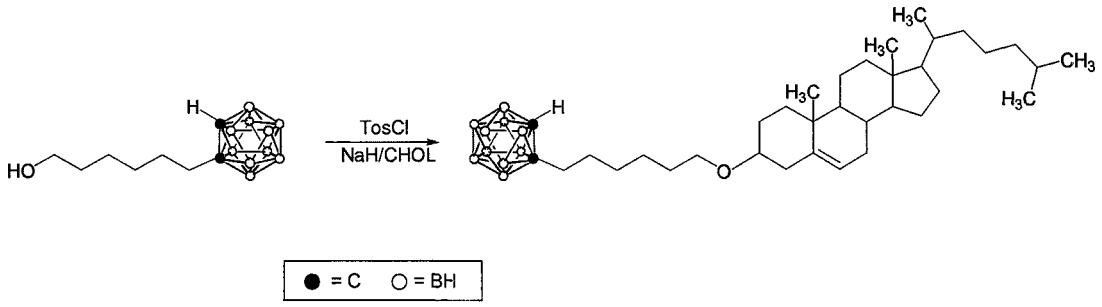


Figure 12: Original synthetic scheme for the ether-linked compounds

To prepare the *nido*-carborane derivatives, an additional step was required. Therefore, the preparation of the *nido*-carborane derivative with the ester link (Figure 13) required a total of seven steps while the *nido*-carborane derivative with the ether linkage (Figure 13) required a total of six steps.

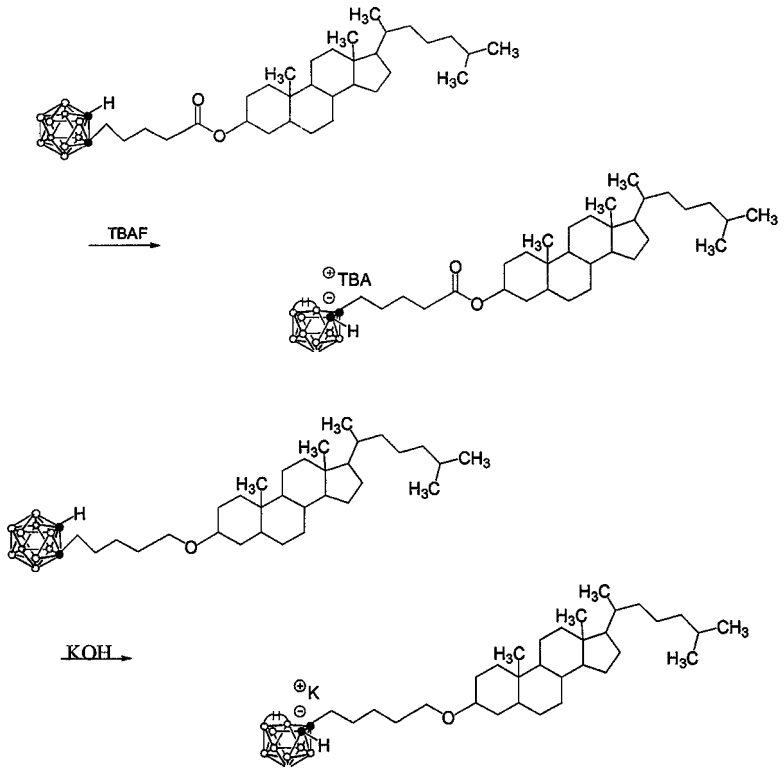


Figure 13: Original synthetic scheme for the conversion of the *closo*-carborane derivatives to form the *nido*-carborane derivatives

The synthesis of the ester-linked, *closo*-carborane derivative was initiated by the formation of a terminal alkyne from an internal alkyne through base catalyzed isomerization.[22] The alcohol was protected using an acetate moiety before the formation of the *ortho*-carborane. This was necessary to prevent an intramolecular alkoxide ion mediated cage degradation.[23] In the first step of the *closo*-carborane formation, decaborane is allowed to react with acetonitrile in toluene to activate the decaborane. The protected terminal alkyne is then added to the activated reaction mixture to produce the protected *closo*-carborane. The acetate moiety is removed to yield the free alcohol which in turn is oxidized using the Jones' reagent to form the *closo*-carboranyl acid. The acid was coupled to cholesterol using the standard N,N'-dicyclohexylcarbodiimide (DCC) as the coupling reagent and dimethylaminopyridine (DMAP) as a neucleophillic

catalyst. Since the ester linkage is sensitive to base cleavage, the standard KOH/ethanol degradation of the *closo*-carborane substituent to form the *nido*-carborane moiety could not be used. To circumvent this problem, anhydrous tetrabutylammonium fluoride in tetrahydrofuran (THF) was used for the conversion.[24] This route produced the tetrabutylammonium salt of the *nido*-carborane, ester linked derivative with a six carbon atom tether.

For the synthesis of the ether linked *closo*-carborane derivatives of cholesterol (Figure 14), the tosylate of the *closo*-carborane alcohol was produced via the reaction of the substituted *closo*-carborane with *p*-toluenesulfonyl chloride in pyridine. The tosylated *closo*-carborane was then coupled to the cholesteryl anion, which had been prepared by allowing cholesterol to react with sodium hydride.

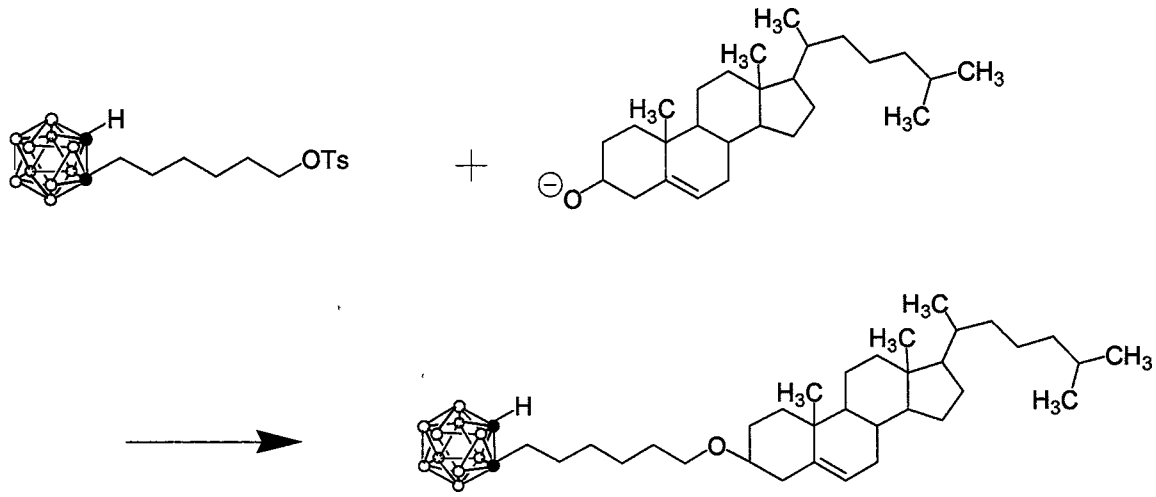


Figure 14: Williamson ether synthesis for the ether linked *closo*-carborane derivatives of cholesterol

The degradation of the ether linked, *closo*-carborane to form the *nido*-carborane

derivative follows much the same procedure as the degradation of the ester linked, *closo*-carborane. The standard KOH/EtOH degradation pathway could be used in the presence of the stable ether linkage, but the milder tetrabutylammonium salt was used to produce the *nido*-carborane product (Figure 15).

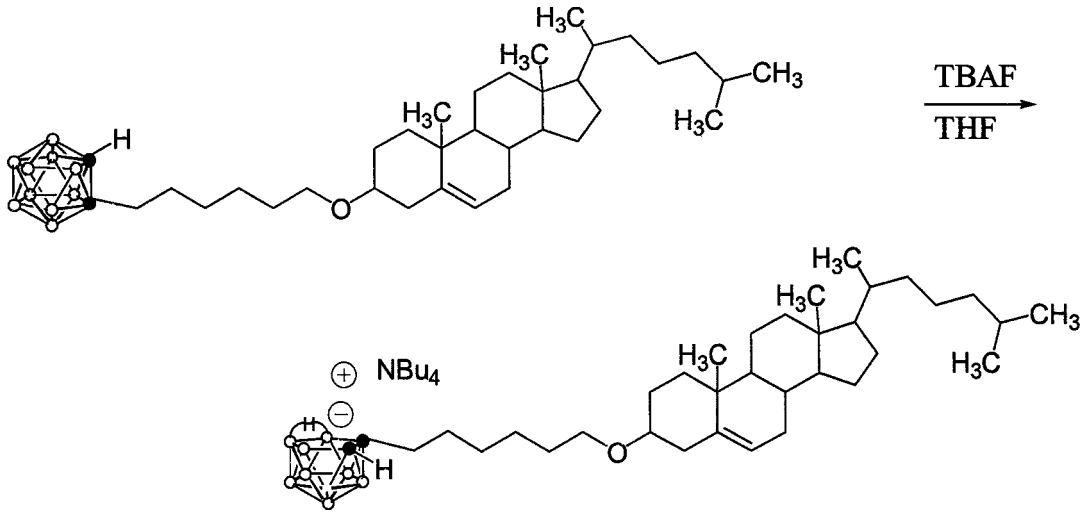


Figure 15: Synthetic scheme for the degradation of the *closo*-carborane to the *nido*-carborane

While these compounds provided an opportunity for evaluation for use in BNCT, problems with the reported synthesis were noted. First, the reaction schemes were relatively long and the yields that were obtained in several steps were relatively low. This in turn caused the overall yields to be very low, sometimes causing problems with the evaluation of the compounds. Also, difficulties were observed during the scale-up process. Due to problems in yield and scale-up of the reactions, only two compounds, the *closo*-carborane derivatives, were obtained in amounts suitable for the liposomal incorporation. Also, the *closo*-carborane derivatives were not incorporated into the bilayer of the liposomes in suitable amounts for the murine biodistribution experiments. Another problem with the reported reaction scheme was that the decaborane was added in

the third step of the reaction sequence. Since decaborane is such a valuable chemical, it would be better to add it closer to the end of the reaction sequence to avoid loss due to subsequent reactions.

Therefore, a shorter synthetic route would enhance the ability to prepare the compounds in a given period of time and to a greater overall yield. Additionally, the length of the alkyl chain tether should affect the ability of the carborane derivatives to pack into the bilayer of the liposomes. The design of a new synthetic scheme and a series of derivatives, with different tether lengths of three and nine carbon atoms have been proposed.

The specific goals for the research project are:

1. Design a new synthetic route for the formation of carborane derivatives of cholesterol which contain an ester linkage and use the scheme to:
   - Prepare *closo*-carborane derivatives of cholesterol which are linked through a tether containing three carbon atoms or nine carbon atoms.
   - Prepare *nido*-carborane derivatives of cholesterol which are linked through a tether containing three carbon atoms or nine carbon atoms.
2. Design a new synthetic route for the formation of carborane derivatives of cholesterol which contain an ether linkage and use the scheme to:
   - Prepare *closo*-carborane derivatives of cholesterol which are linked through a tether containing three carbon atoms or nine carbon atoms.
   - Prepare *nido*-carborane derivatives of cholesterol which are linked through a tether containing three carbon atoms or nine carbon atoms.

3.0 EXPERIMENTAL

3.1 Materials and Methods

Decaborane, $B_{10}H_{14}$, was obtained from either Alfa Aesar (Ward Hill, MA) or KatChem (Czech Republic). Decaborane from Alfa Aesar was sublimed under vacuum before use; decaborane from KatChem was used as received. **Caution: Decaborane is a highly toxic, impact sensitive compound that forms explosive mixtures, especially with halogenated materials. A careful examination of the MSDS is recommended before use.** Other starting materials, 10-undecynoic acid and 4-pentynoic acid were obtained from Lancaster (Windham, NH) and used without furtherpurification. The DSPC was acquired from Avanti Polar Lipids, Inc (Alabaster, AL). All other reagents were obtained from the Sigma-Aldrich Chemical Company (Milwaukee, WI) and were used without further purification. All of the solvents that were used were reagent grade and were distilled in the presence of the appropriate drying agents under dry argon atmosphere prior to use. The reactions were performed under argon using anhydrous conditions.

The melting points were measured using a Mel-Temp II apparatus and are uncorrected. The ^{1}H, ^{13}C, and ^{11}B Fourier transform nuclear magnetic resonance (NMR) spectra were obtained with a Varian INOVA spectrometer operating at 400 MHz, 100 MHz and 128 MHz, respectively. Proton and carbon chemical shifts were referenced either to the internal standard, tetramethylsilane (TMS), or the residual solvent protons. Proton integrations in the 0.50-2.50 ppm region of the spectrum are estimated in the boron-containing precursor compounds based on theoretical values or, in the case of the

cholesterol derivatives, are not provided for multiplets in the region due to the complexity of the cholesterol spectrum in combination with the underlying boron-hydrogen baseline signal. Boron chemical shifts were externally referenced to $BF_3 \cdot Et_2O$ in C_6D_6; peaks upfield from the reference are designated as negative. All boron spectra were obtained in the proton-decoupled mode. The Fourier transform infrared (IR) spectra were obtained using either a Perkin-Elmer 1600 or a Perkin-Elmer Spectrum One instrument. Boron analyses were performed by the Idaho National Engineering and Environmental Laboratories (INEEL, Idaho Falls, ID). Elemental analysis (C, H, N) were performed by Quantitative Technologies, Inc. (Whitehouse, NJ). Biodistribution studies are performed by Dr. Patrick Gavin at Washington State University (Pullman, WA).

3.2 Synthesis

3.2.0 Coupling of Cholesterol and 4-Pentynoic Acid

Into a 500-mL round-bottom flask equipped with a stir bar and an argon inlet, 4-pentynoic acid (4.0 g, 41 mmol), cholesterol (17.344 g, 44.853 mmol), dicyclohexylcarbodiimide (DCC, 9.2544 g, 44.852 mmol), and 4-dimethylaminopyridine (DMAP, 0.6476 g, 5.301 mmol) were added. The solid mixture was dried under vacuum for two hours to ensure that the reactants were free of water. Freshly distilled diethyl ether (350 mL) was added to the reaction vessel via cannulation. Upon addition of ether, a white precipitate of dicyclohexylurea instantly formed. The identity of the dicyclohexylurea was verified by ^{1}H and ^{13}C NMR spectroscopy. The reaction was allowed to stir at room temperature overnight. The precipitate was filtered from the

solution and the remaining solution was extracted with hydrochloric acid (5%, 3 x 200 mL), saturated sodium bicarbonate (3 x 200 mL), and brine (1 x 200 mL). The ether solution was collected and dried over anhydrous magnesium sulfate, filtered, and the solvent was removed under reduced pressure by rotary evaporation and placed under vacuum to dry. The product was isolated in 58 % yield from the white powder by recrystallization from absolute ethanol and water. MP = 130-135 °C; ^{1}H NMR ($CDCl_3$) δ 5.365 (s, 1H), 4.641 (s, 1H), 2.501 (s, 2H), 2.316 (s, 1H), 1.963 (s, 1H), 1.862-1.834 (d, 2H), 1.567-1.533 (d, 2H), 1.483-1.115 (m), 1.008 (s, 3H), 0.940-0.905 (d,3H), 0.854 (d, 6H), 0.664 (s, 3H); ^{13}C ($CDCl_3$) δ 172.214,140.635,123.787, 83.635, 74.435, ,69.996, 57.752, 57.206, 51.085, 43.377, 40.798, 40.586, 39.152, 38.029, 37.658, 37.256, 36.861, 34.730, 32.970, 32.924, 29.291, 29.078, 28.836, 25.346, 24.898, 23.882, 23.624, 22.691, 22.099, 20.817, 20.377, 19.786, 18.686, 15.500, 12.921, 4.978; IR (nujol) 3054.41, 2725.99, 1722.32, 1464.79 cm^{-1}.

3.2.1 Coupling of Cholesterol and 10-Undecynoic Acid

Into a 500-mL round-bottom flask equipped with a stir bar and an argon inlet, 10-undecynoic acid (5.0 g, 27 mmol), cholesterol (11.6695 g, 30.1771 mmol), dicyclohexylcarbodiimide (DCC, 6.2265 g, 30.177 mmol), and 4-dimethylaminopyridine (DMAP, 0.4355 g, 3.565 mmol) were added. The solid mixture was dried under vacuum for two hours to ensure that the reactants were free of water. Freshly distilled diethyl ether (350 mL) was added to the reaction vessel. Upon addition of the ether, a white precipitate of dicyclohexylurea instantly formed. The identity of the dicyclohexylurea was verified using ^{1}H and ^{13}C NMR spectroscopy. The reaction was allowed to stir at

room temperature overnight. The precipitate was filtered from the solution and the remaining solution was extracted with hydrochloric acid (5%, 3 x 200 mL), saturated sodium bicarbonate (3 x 200 mL), and brine (1 x 200 mL). The ether solution was collected and dried over anhydrous magnesium sulfate, filtered, and the solvent was removed under reduced pressure by rotary evaporation and placed under vacuum to dry. The product was isolated in 49 % yield from the white powder by recrystallization using absolute ethanol and water. MP = 117-124°C; ^{1}H NMR ($CDCl_3$) δ 5.2310-5.304 (d, 1H), 4.561 (s, 1H), 2.282-2.116 (m, 3H), 1.994-1.827(m), 1.819-0.985 (m), 0.997(s, 3H), 0.956-0.866(d, 3H), 0.850 (d, 6H), 0.836-0.669(m), 0.652 (s,3H) ; ^{13}C ($CDCl_3$) δ 173.230, 139.549, 122.526, 73.659, 68.083, 56.697, 56.621, 56.113, 50.067, 42.193, 39.728, 39.455, 38.089, 37.217, 36.929, 36.489, 36.413, 35.738, 34.592, 31.831, 31.528, 30.822, 29.039, 28.842, 28.599, 28.175, 27.932, 27.742, 26.278, 24.662, 23.805, 22.766, 22.516, 21.021, 19.330, 19.246, 18.662, 11.789; IR (nujol) 3036.76, 2925.87, 1737.32, 1464.81,1057.11 cm^{-1}.

3.2.2 Preparation of closo-Carborane Derivative of 3.2.0

Into a 500-mL 3-necked round-bottom flask equipped with stir bar, argon inlet, pressure equalized dropping funnel (PED), and water condenser, 200-mL of freshly distilled toluene was added. Natural abundance decaborane ($B_{10}H_{14}$, 1.81 g, 14.8 mmol) and freshly distilled acetonitrile (7.2 mL, 1.4 x 10^2 mmol) were added also. The solution was allowed to stir overnight at reflux temperature. A solution of 3.2.0 (6.91 g, 14.8 mmol) in freshly distilled toluene was added dropwise via the PED to the yellowish solution and allowed to reflux overnight. The reaction mixture was cooled to room

temperature and transferred to a 500-mL separatory funnel. The mixture was extracted with 1M NaOH (4 x 100 mL) to remove most of the boron by-products. The organic layer was collected and dried over anhydrous magnesium sulfate. The solution was filtered and the solvent was removed under reduced pressure by rotary evaporation. The resulting orange-red viscous residue was extracted with 100-mL increments of boiling heptane repeatedly until no residue was extracted into the heptane. The decanted heptane solutions were combined and the heptane was removed under reduced pressure by rotary evaporation. The remaining residue was passed through a silica gel plug, using chloroform as the solvent, to remove the darker colored contaminant. The chloroform was collected and removed under reduced pressure by rotary evaporation and the product was isolated in 40% yield by recrystallization from ethanol and water. MP = 180-186°C; ^{1}H NMR($CDCl_3$) δ 5.378 (m, 1H), 4.611-4.585 (m, 1H), 3.735 (bs, 1H), 2.559-2.500 (s), 2.483 (s), 2.353 (s), 2.290 (s), 2.01-1946 (m), 1.883-1.813 (m), 1.630-0.859(m), 0.669 (s, 3H); ^{13}C ($CDCl_3$) δ 170.851, 139.272, 123.008, 118.964, 94.394, 75.050, 61.480, 56.883, 50.602, 42.310, 40.338, 37.986, 36.181, 35.779, 33.769, 32.896, 31.887, 30.681, 29.551, 28.208, 27.670, 25.386, 24.271, 23.437, 22.807, 21.025, 20.767, 19.470, 18.711, 17.474, 12.400, 11.853; ^{11}B ($CDCl_3$) δ -2.603, -6.089, -9.955, -12.056, -13.304; IR (nujol).2924.18, 2853.70, 2589.29, 17.22.47, 1463.86, 1376.96, 1263.55, 1192.17, 1003.70, 722.82 cm^{-1}.

3.2.3 Preparation of Closo-Carborane Derivative of 3.2.1

Into a 500-mL 3-necked round-bottom flask equipped with stir bar, argon inlet, pressure equalized dropping funnel (PED), and water condenser, 300-mL of freshly distilled toluene was added. Natural abundance decaborane ($B_{10}H_{14}$, 2.218 g, 18.10

mmol) and freshly distilled acetonitrile (9.00 mL, 173 mmol) were added. The solution was allowed to stir overnight at reflux temperature. A solution of 3.2.1 (10.0 g, 18.2 mmol) in freshly distilled toluene was added dropwise via the PED to the yellowish solution and allowed to reflux overnight. The reaction mixture was cooled to room temperature and transferred to a 500-mL separatory funnel. The mixture was extracted with 1M NaOH (4 x 100 mL) to remove any of the boron by-products. The organic layer was collected and dried over anhydrous magnesium sulfate. The solution was filtered and the solvent was removed under reduced pressure by rotary evaporation. The resulting orange-red viscous residue was extracted with 100-mL increments of boiling heptane repeatedly until no residue was extracted into the heptane. The decanted heptane solutions were combined and the heptane was removed under reduced pressure by rotary evaporation. The resulting viscous yellow liquid was dissolved in chloroform and eluted through a silica gel plug to remove the darker colored contaminant. The chloroform was collected and removed under reduced pressure by rotary evaporation and the product was isolated in 54% yield by recrystallization from ethanol and water. MP = 120-130°C; ^{1}H NMR ($CDCl_3$) δ 5.365-5.354 (s, 1H), 4.610-4.547 (m, 1H), 3.547 (bs, 1H), 2.301-2.231 (m), 2.190-1.981 (m), 1.937-1.796 (m), 1.604-1.586 (m), 1.554 (s), 1.522-1.030 (m), 1.007 (s, 3H), 0.992-0.894 (d, 3H), 0.863-0.846 (m, 6H), 0.665 (s, 3H); ^{13}C ($CDCl_3$) δ 173.188, 139.666, 122.636, 73.746, 56.678, 56.124, 50.017, 42.310, 39.724, 39.511, 38.168, 38.077, 36.985, 36.598, 36.173, 35.787, 34.603, 31.895, 31.857, 29.134, 28.967, 28.906, 28.876, 28.830, 28.216, 28.004, 27.814, 24.909, 24.279, 23.816, 22.807, 22.557, 21.025, 19.318, 18.711, 11.853; ^{11}B ($CDCl_3$) δ -5.067, -9.876, -12.013, -14.220; IR (nujol) 2928.6, 2592.40, 1715.64, 1464.24, 1377.91, 1261.24, 1020.24, 722.24 cm^{-1}.

3.2.4 Reduction of 3.2.2 Using Sodium Borohydride

To a 100-mL round-bottom flask that had been flushed with argon, freshly distilled tetrahydrofuran (26.00 mL) and boron trifluoride diethyl etherate (BF_3•Et_2O, 13.70 g, 108.1 mmol) were added. To the tan colored solution, 3.2.2 (2.0 g, 3.5 mmol) was added and allowed to dissolve. To the above solution, sodium borohydride (0.6003 g, 15.87 mmol) was added and a condenser was connected. Gas evolution was observed instantly. The solution was cooled to room temperature and allowed to stir for two hours then at then at reflux temperature for two hours. The reaction was monitored by FT-IR spectroscopy. After two hours at reflux, the reaction was not completed and was, therefore, cooled to room temperature and allowed to stir overnight. The cloudy white solution was quenched with saturated sodium carbonate and extracted into diethyl ether (2 x 75 mL). The diethyl ether solution was washed with saturated sodium bicarbonate (2 x 75 mL) and brine (2 x 75 mL). The ether extract was dried over anhydrous magnesium sulfate, filtered, and the ether removed under reduced pressure by rotary evaporation. The resulting white viscous liquid was recrystallized from ether to remove the alcohol by-product. The product was then separated via column chromatography using silica gel as the stationary phase and a mixture of 50% acetonitrile and 50% hexane as the mobile phase in 54% yield. MP = 162-168 °C; ^{1}H NMR ($CDCl_3$) δ 3.597 (bs, 1H), 3.585 (t, 2H), 3.401 (m, 1H), 3.387-0.713 (m), 0.627 (s, 3H); ^{13}C ($CDCl_3$) δ 79.014, 70.052, 67.547, 61.783, 60.048, 57.358, 51.945, 49.352, 46.549, 44.758, 41.435, 40.284, 39.018, 37.552, 36.837, 35.216, 33.521, 32.437, 31.125, 29.585, 27.211, 26.627, 25.432, 24.052, 23.548, 22.548, 20.446, 19.356, 16.543, 14.915, 14.007, 12.248; ^{11}B ($CDCl_3$) δ -5.000, -8.478, -11.956, -14.171, -15.693; IR (nujol) 2954.09, 2924.35, 2854.23, 2596.03, 1463.37,

1377.19, 1060.60, 1019.37, 722.12 cm^{-1}.

3.2.5 Reduction of 3.2.3 using Sodium Borohydride

To a 100-mL round-bottom flask that had been flushed with argon, freshly distilled tetrahydrofuran (20.00 mL) and boron trifluoride diethyl etherate (BF_3•Et_2O, 11.74 g, 92.66 mmol) was added. To the tan colored solution, 3.2.3 (2.0 g, 3.0 mmol) was added and allowed to dissolve along with sodium borohydride (0.3405 g, 9.243 mmol). Gas evolution was observed instantly. The solution was allowed to stir at room temperature for two hours and then at reflux temperature for two hours. The reaction was monitored by FT-IR spectroscopy. After two hours at reflux temperature, the reaction was not complete and was, therefore, cooled to room temperature and allowed to stir overnight. The white cloudy solution was quenched with saturated sodium carbonate and was extracted into diethyl ether (2 x 75 mL). The diethyl ether solution was washed with saturated sodium bicarbonate (2 x 75 mL) and brine (2 x 75 mL). The ether solution was dried over anhydrous magnesium sulfate, filtered, and dried under reduced pressure by rotary evaporation. The resulting yellow viscous liquid was then recrystallized from ether to remove the alcohol by-product. After the alcohol by-product was removed, the product was eluted through a silica gel plug using a mobile phase composed of 30% hexane and 70% methylene chloride. The product was recovered in 45 % yield. MP = 110-115°C; ^{1}H NMR ($CDCl_3$) δ 3.448 (bs, 2H), 2.379-1.349 (m), 1.283 (m), 1.140-0.932 (m, 6H), 0.898-0.882 (m), 0.855-0.105 (m, 6H), 0.096 (s, 3H); ^{13}C ($CDCl_3$) δ 75.437, 67.996, 64.044, 62.951, 60.911, 55.343, 54.470, 51.686, 50.708, 47.029, 44.935, 42.614, 40.080, 38.100, 37.076, 35.521, 34.216, 33.837, 32.153, 31.387, 30.211, 29.301, 28.261,

27.336, 26.168, 25.432, 24.226, 23.854, 21.245, 20.284, 19.394, 183263, 16.860, 14.122, 13.507, 12.309; ^{11}B ($CDCl_3$) δ -0.095, -12.028, -14.053, -14.920, -15.834; IR (nujol) 2876.42, 2863.54, 2523.57, 1468.62, 1038.14 cm^{-1}.

3.2.6 Cage Degradation of 3.2.2 Using Cesium Fluoride

Into a 250-mL round-bottom flask, 3.2.2 (1.0 g, 1.7 mmol), a solution of cesium fluoride (CsF) in absolute ethanol (0.6584 M, 5.1 mmol, 8.0 mL), and a stir bar were added. An additional 100 mL absolute alcohol was added. A condenser was connected and the solution was allowed to reflux overnight. The clear solution was cooled to room temperature and the solvent was removed under reduced pressure by rotary evaporation and placed under vacuum to dry. The white crystals were washed with acetone to remove any borate by-products that were formed during the reaction. The crystalline white solid was isolated by filtration. The product was isolated in a 98% yield by recrystallization from ethanol and water. MP = 169-180°C; ^{1}H NMR ($CDCl_3$) δ 4.675 (s, 1H), 3.867 (s, 1H), 1.662-1.624 (m), 1.329 (m), 1.303-0.426 (m), , 0.237-0.226 (m), 0.175 (s), 0.161(s,6H), 0.0841 (s, 3H); ^{13}C ($CDCl_3$) δ 173.188, 139.666, 122.636, 73.746, 56.678, 56.124, 50.017, 42.310, 39.724, 39.511, 38.168, 38.077, 36.985, 36.598, 36.173, 35.787, 34.603, 31.895, 31.857, 29.134, 28.967, 28.830, 27.814, 24.909, 24.279, 23.816, 22.807, 22.557, 21.025, 19.318, 18.711, 11.853; ^{11}B ($CDCl_3$) δ -11.538, -14.460, -17.550, -18.928, -22.474, -33.821, -37.703; IR (nujol) 2924.66, 2852.99, 2529.79, 1736.27 1464.17, 1376.75, 11.74.22, 722.91 cm^{-1}.

3.2.7 Cage Degradation of 3.2.3 Using Cesium Fluoride

Into a 250-mL round-bottom flask, 3.2.3 (1.0g, 1.4 mmol), a solution of CsF in absolute ethanol (0.6584M, 4.4 mmol, 6.8 mL), and a stir bar were added. An additional 100 mL of absolute ethanol was added. A condenser was connected and the solution was allowed to reflux overnight. The clear solution was cooled to room temperature and the solvent was removed under reduced pressure by rotary evaporation and placed under vacuum to dry. The white crystals were washed with acetone to remove any borate by-products that were formed during the reaction. The crystalline white solid was isolated by filtration. The product was isolated in 90% yield by recrystallization from absolute alcohol and water. MP = 148-150°C; ^{1}H NMR ($CDCl_3$) δ 5.354 (m, 1H), 4.572 (m, 1H), 2.293-2.262(m, 8H), 2.243-2.131 (m), 2.002-1.817 (m), 1.565-0.0785 (m, 12H), 0.653 (s, 3H); ^{13}C ($CDCl_3$) δ 169.690, 139.621, 122.545, 121.665, 114.102, 84.639, 73.662, 71.758, 68.079, 62.640, 56.632, 49.964, 47.787, 42.257, 40.285, 39.466, 38.108, 36.135, 35.749, 31.804, 29.058, 28.186, 27.958, 25.060, 24.233, 23.786, 22.785, 20.987, 19.272, 18.673, 18.332, 11.808; ^{11}B ($CDCl_3$) δ -11.275, -13.748, -17.683, -21.823, -33.422, -37.296; IR (neat) 2927.17, 2852.82, 2513.43, 1732.79, 1714.94, 1466.34, 734.18 cm^{-1}.

3.2.8 Cage Degradation of 3.2.4 Using Cesium Fluoride

Into a 250-mL round-bottom flask, 3.2.4 (0.6 g, 1 mmol), a solution of CsF in absolute ethanol (0.6584M, 3.15 mmol, 4.78 mL), and a stir bar were added. An additional 100 mL of absolute alcohol was added. A condenser was connected and the solution was allowed to reflux overnight. The clear solution was cooled to room temperature and the solvent was removed under reduced pressure by rotary evaporation

and placed under vacuum to dry. The dried residue was dissolved in acetone and the insoluble borate by-products were removed by filtration. The acetone was removed under reduced pressure by rotary evaporation and placed under vacuum to dry. The product was separated from other impurities by recrystallization from chloroform. The product was filtered then dissolved in acetone. The acetone was then removed by rotary evaporation and placed under vacuum to dry. The product was isolated in 78% yield. MP = 158-162 °C; ^{1}H NMR ($CDCl_3$) δ 3.678 (s, 2H), 2.501-2.224 (m), 2.1045-1.635 (m) 1.625-0.648 (m), 0.601 (s, 3H); ^{13}C ($CDCl_3$) δ 169.690.139.621, 122.545, 121.665, 114.102, 84.639, 73.662, 71.758, 68.079, 62.640, 56.632, 49.964, 47.787, 42.257, 40.285, 39.466, 38.108, 36.135, 35.749, 31.804, 29.058, 28.186, 27.958, 25.060, 24.233, 23.786, 22.785, 20.987, 19.272, 18.673, 18.332, 11.808; ^{11}B ($CDCl_3$) δ -10.354, -13.261, -16.526, -17.561, -21.488, -32.645, -36.534; IR (nujol) 2964.19, 2936.25, 2852.93, 2527.83, 1467.12, 1378.09, 1066.40, 1020.84, 722.12 cm^{-1}.

3.2.9 Cage Degradation of 3.2.5 Using Cesium Fluoride

Into a 250-mL round-bottom flask, 3.2.5 (1.0 g, 1.5 x 10^{-3} mol), a solution of CsF in absolute ethanol (0.6584M, 5.1 x 10^{-3} mol, 8.0 mL), and a stir bar were added. An additional 100 mL absolute alcohol was added. A condenser was connected and the solution was allowed to reflux overnight. The clear solution was cooled to room temperature and the solvent was removed under reduced pressure by rotary evaporation and placed under vacuum to dry. The yellow viscous liquid was dissolved in acetone and the insoluble borate by-products were removed by filtration. The acetone was removed under reduced pressure by rotary evaporation and placed under vacuum to dry. The

product was isolated by recrystallization from chloroform in 97% yield. MP = 100-110 °C; ^{1}H NMR ($CDCl_3$) δ 3.589 (t,8H), 2.431-2.351 (m), 2.174 (m), 1.98-0.957 (m), 0.861 (d, 6H), 0.657 (s, 3H); ^{13}C ($CDCl_3$) δ 56.266, 55.302, 50.660, 42.316, 42.134, 40.017, 39.843, 39.494, 38.985, 37.886, 36.429, 36.156, 35.762, 35.542, 34.169, 31.809, 31.347, 29.678, 28.221, 27.979, 27.303, 27.023, 25.407, 24.368, 24.170, 23.814, 22.957, 22.787, 22.532, 22.054, 21.569, 20.249; ^{11}B ($CDCl_3$) δ -12.892, -15.838, -19.925, -24.148, -35.222, -39.103; IR (nujol) 2867.42, 2851.04, 2525.87, 1466.20, 1378.27, 1038.14, 736.27 cm^{-1}.

3.3 Liposome Preparation

3.3.0 Preparation of the Buffer Solution

Into a 1000-mL beaker HEPES (2.383 g) and NaCl (9.0 g) was added. Approximately 800 mL of deionized water was added to the solids. The pH was increased, using 1M sodium hydroxide, to approximately 7.36, the pH of biological systems. The solution was then transferred to a 1000-mL volumetric flask and diluted to the mark with deionized water. The buffer solution was filtered through a Corning 0.22-μm PES sterile filter system and stored in the refrigerator. The resulting buffer solution had an osmolarity approximately equal to that of serum and is designated as 1X. For hypertonic buffer solutions, twice the amount of NaCl was added, designated as 2X, or three times the amount of NaCl was added, designated as 3X.

3.3.1 Preparation of the Lipid Mixture

Into a 10-mL test tube a mixture of DSPC, cholesterol, and 3.2.4 was added. The amounts of each of the constituents were added in an amount that ensures a 1:1 molar ratio between the DSPC and the cholesterol compounds. The molar ratio of the cholesterol to boron-containing derivatives of cholesterol is varied to give the desired boron concentration. To this mixture, a minimal amount (approximately 1 mL) of chloroform was added to dissolve the solid samples. The solution was then vortexed until all of the solids were dissolved. The test tube was placed under flowing argon until dry. After all of the chloroform had been evaporated, the test tube was placed into a vacuum desiccator and left overnight to ensure that all of the chloroform had been removed. The uniform white solid was broken apart and left under vacuum for an additional 24 hours.

3.3.2 Preparation of the Liposomes

Upon removal from the vacuum desiccator, 6.0 mL of 2X buffer was added to the test tube. The solid was insoluble in the buffer solution and floated to the top. The test tube was put into a 250-mL beaker full of warm (60-65 °C) water and placed on the microtip of a Sonic & Materials, Inc. Vibracell sonicator and sonicated for 35 minutes. The mixture, upon sonication, converted from the clear liquid with solid floating on the top to an opalescent mixture with no solid. Once the liposomes were formed, the liposomes were separated by size-exclusion chromatography using three Pharmacia Biotech PD-10 columns which had been prepared by eluting approximately 35 mL of the prepared buffer (1X) through each column. Approximately 2 mL of the liposome mixture was placed on each column. The first fraction collected off of each column was

approximately 2.5 mL in volume and contained the buffer solution. The next fraction collected off of each column was approximately 2.6 mL in volume and contained the liposomes. The liposome fractions were combined and diluted to a total volume of 12 mL using the 1X buffer. The liposome mixture was filtered, consecutively, through a 0.45 μm and a 0.22 μm syringe filter, the final filtration going directly into a sterile vial. The liposome formulations were stored in the refrigerator and observed to determine if any precipitate formed. Mixtures which formed no precipitate were sent to the INEEL for boron analysis.

4.0 RESULTS and DISCUSSION

4.1 Results

The coupling reaction of 4-pentynoic acid to cholesterol was achieved using a standard dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP) procedure.

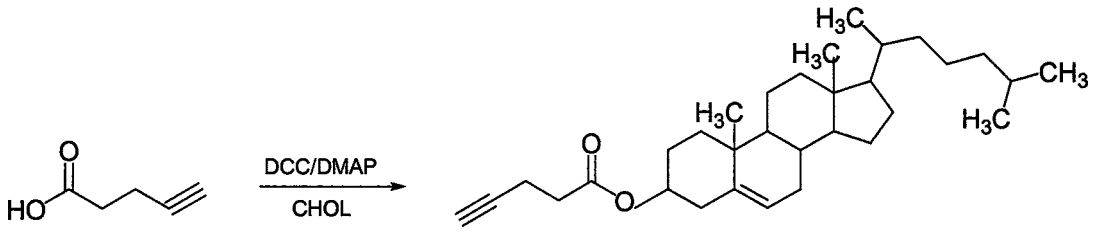


Figure 16: Synthetic scheme for the coupling of 4-pentynoic acid and cholesterol

The reaction was monitored by the shift of the signal in the ^{1}H NMR spectrum centered at 3.25 ppm, corresponding to the proton on the carbon atom bound to the alcohol substituent in cholesterol, to 4.59 ppm. When the cholesterol has been coupled to the 4-pentynoic acid the tertiary hydrogen shifts downfield due to the carbonyl electron withdrawing group. In the ^{1}H NMR the signal at 5.36 ppm corresponds to the hydrogen of the double bond in cholesterol. The remaining peaks are assigned to the cholesterol moiety and were not used in the structural assignment of the coupled product. In the ^{13}C NMR spectrum the identity of the product was confirmed by the existence of the carbonyl peak at 172.21 ppm along with the peaks at 83.635 ppm and 75.43 ppm which correspond

to the terminal alkyne carbons. The FT-IR spectrum showed that the coupling reaction went to completion by the loss of the OH peak and the shift of the carbonyl peak.

The reaction of the decaborane and the alkyne on the cholesterol derivative substituent was initiated by reacting decaborane with acetonitrile to produce the activated decaborane-acetonitrile complex. The acetonitrile substituted decaborane was allowed to react with the terminal alkyne derivative of cholesterol at reflux temperature overnight.

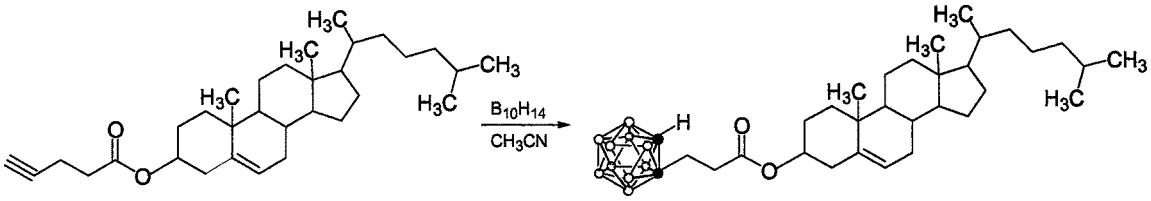


Figure 17: Synthetic scheme for the preparation of the borane derivative

The ^{1}H NMR spectrum of the product indicated the presence of the carborane substituent by the addition of the proton signal at 3.73 ppm, which corresponds to the hydrogen attached to the carbon in the carborane lattice. A rolling baseline that starts around 3.0 ppm and continues upfield to around 0 ppm can be observed. The rolling baseline is also consistent with the compounds containing the carborane lattice. The rolling baseline produced by the carborane hydrogen atoms prevents accurate integration of the ^{1}H NMR spectrum. The ^{13}C NMR spectrum confirmed the identity of the desired product by the addition of the carborane carbon signals at 68.92 ppm and 61.47 ppm. The FT-IR spectrum is consistent with the NMR spectra by the existence of an absorption at 2524 cm^{-1}, corresponding to the hydrogen-boron bond found in the carborane lattice structure.

Traditionally, the degradation of an *ortho*-carborane substituent to the *nido*-carborane substituent is achieved using potassium hydroxide in ethanol; however, the susceptibility of the ester connection to base hydrolysis prevents the use of these reagents. Therefore, degradation of the ester-linked, *closo*-carborane derivative of cholesterol to the charged ester-linked, *nido*-carborane derivative of cholesterol was achieved by allowing the *closo*-carborane derivative to react to react with cesium fluoride in absolute ethanol.

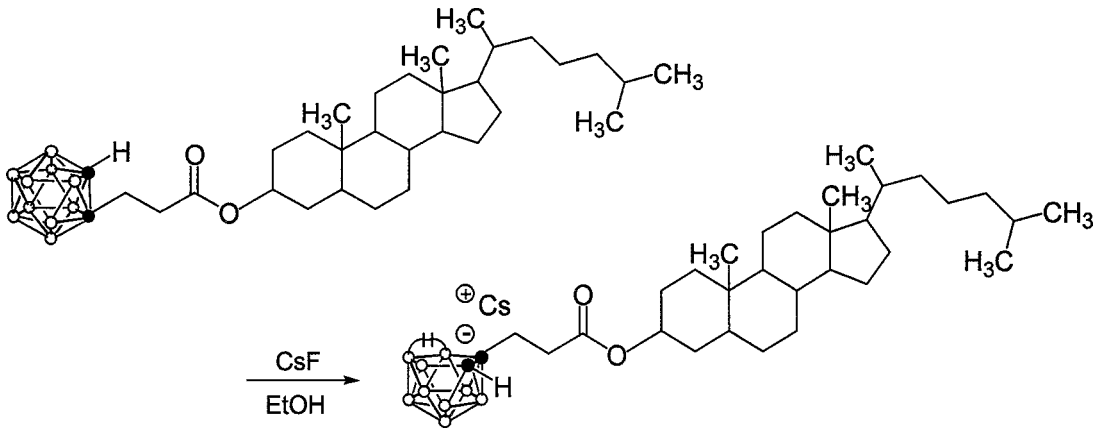


Figure 18: Synthetic scheme for the degradation of the *closo*-carborane into the *nido*-carborane

The reaction was monitored by ^{11}B NMR. The ^{11}B NMR spectrum exhibits a shift in all of the peaks upfield and the presence of two new peaks at the extreme upfield portion of the spectrum, at -33.4 ppm and -37.2 ppm, respectively. These correspond to the unique boron atom in the open face of the cage and the boron atom connected to both of the carbon atoms. In the ^{1}H NMR, spectrum, the peak at 3.25 ppm, corresponding to the hydrogen attached to the carbon atom in the carborane lattice, disappears, consistent with

known *nido*-carborane derivatives. As anticipated, no significant shift in the boron-hydrogen absorption was observed in the FT-IR spectrum.

The reduction of the ester-linked, *closo*-carborane derivative of cholesterol to form the ether-linked, *closo*-carborane derivative of cholesterol was achieved by allowing the ester-linked derivative to react with boron trifluoride-diethyl etherate and sodium borohydride. The reaction was monitored by the disappearance of the 1600 cm^{-1} peak

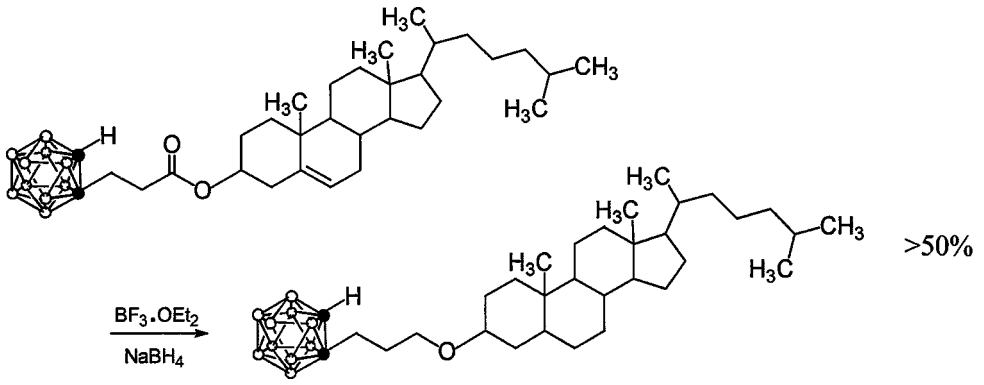


Figure 19: Synthetic scheme for the reduction of the ester into the ether

in the FT-IR spectrum, corresponding to the carbonyl functionality. The ^{1}H NMR spectrum of the product displays a shift of the proton connected to the carborane atom of the cholesterol which is involved in the link, from 4.59 ppm to 3.60 ppm. The triplet in the ^{1}H NMR spectrum of the product corresponds to the hydrogen atoms on the carbon adjacent to the carbonyl functionality. In the ester compounds, the triplet is underneath the cholesterol signals and a shift is observed as the ether is formed. Also, the disappearance of the proton peak at 5.36 ppm corresponds to the loss of the alkene bond within the cholesterol moiety. In the ^{13}C NMR spectrum, the loss of the peaks at 170.836, 139.234, and 122.985 ppm, are consistent with the structure of the product.

These peaks correspond to the carbonyl carbon atom, and the alkene bond in the cholesterol moiety, respectively. The reduction of the cholesterol double bond is not anticipated to hinder the biological distribution of the product.

The ether linkage, unlike the ester linkage, is not susceptible to base hydrolysis; however, for consistency, the degradation of the ether-linked, *closo*-carborane derivative of cholesterol to the charged ether-linked, *nido*-carborane derivative of cholesterol was also achieved by allowing the *closo*-carborane derivative to react with cesium fluoride in absolute ethanol.

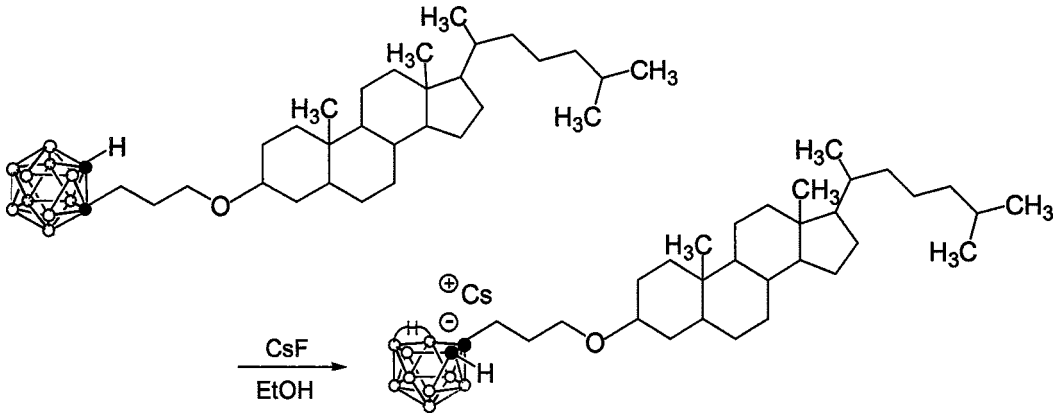


Figure 20: Synthetic scheme for the degradation of the *closo*-carborane to form the *nido*-carborane

The reaction was monitored by ^{11}B NMR spectroscopy. The ^{11}B NMR spectrum exhibits a shift in all of the peaks upfield and the presence of two peaks at the extreme upfield portion of the spectrum, at -32.6 ppm and -36.5 ppm which correspond to the unique boron atom in the open face of the cage and the boron atom connected to both of the carbon atoms. In the ^{1}H NMR spectrum, the peak at 3.5 ppm, corresponding to the hydrogen atom attached to the carbon atom in the carborane lattice, disappears, consistent

with known *nido*-carborane derivatives. As anticipated, there was no significant shift in the boron-hydrogen absorption in the FT-IR spectrum

The coupling reaction of 4-pentynoic acid to cholesterol was achieved using the standard dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP) coupling pathway.

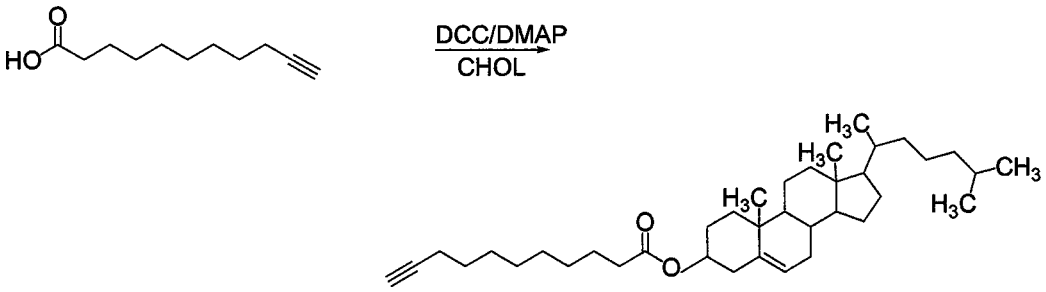


Figure 21: Synthetic scheme for the coupling of 10-undecynoic acid and cholesterol

The reaction was monitored by the shift of the signal in the ^{1}H NMR spectrum centered at 3.46 ppm, corresponding to the proton attached to the carbon atom bound to the alcohol substituent in the cholesterol, to 4.57 ppm. When the cholesterol is coupled to the 10-undecynoic acid, the proton signal shifts downfield as a result of the carbonyl electron withdrawing group. The ^{1}H NMR spectrum, the signal at 5.29 ppm corresponds to the proton of the double bond in cholesterol. The remaining peaks are assigned to the cholesterol moiety and were not used in the structural assignment of the coupled product. In the ^{13}C NMR spectrum, the identity of the product was confirmed by the existence of the carbonyl peak at 174.37 ppm along with the peaks at 82.35 ppm and 71.55 ppm which correspond to the terminal alkyne carbons. The FT-IR spectrum was used to

confirm that the coupling reaction went to completion by the loss of the OH peak and the shift of the carbonyl peak.

The reaction of the decaborane and the alkyne on the cholesterol derivative to form the *ortho*-carborane substituent was initiated by reacting decaborane with acetonitrile to produce the activated decaborane-acetonitrile complex. The acetonitrile substituted decaborane was allowed to reflux overnight to react with the terminal alkyne derivative of cholesterol.

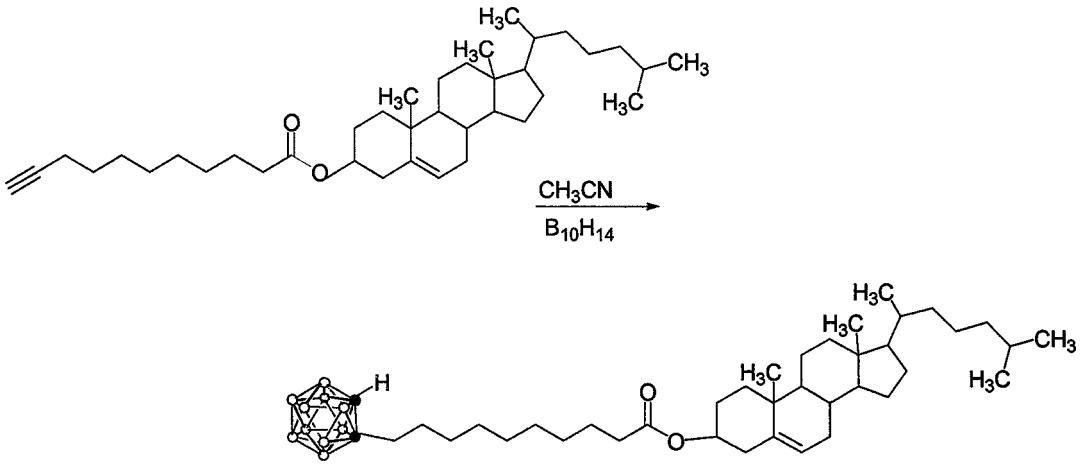


Figure 22: Synthetic scheme for the preparation of the borane derivatives

The ^{1}H NMR spectrum indicated the presence of the carborane substituent by the addition of the proton signal at 3.54 ppm which corresponds to the hydrogen attached to the carbon in the carborane lattice. A rolling baseline that starts around 3.0 ppm and continues upfield to around 0 ppm is observed. The rolling baseline is also consistent with compounds containing the carborane lattice. The rolling baseline produced by the carborane hydrogen atoms prevents accurate integration of the ^{1}H NMR spectrum. The ^{13}C NMR spectrum of the product confirmed the identity of the desired product by the

addition of the carborane peaks at 69.49 ppm and 59.15 ppm. The FT-IR spectrum is consistent with the NMR spectrum as inscated by the existence of an absorption at 2524 cm^{-1}. This signal corresponds to the hydrogen-boron bond found in the carborane lattice structure.

The degradation of the ester-linked, *closo*-carborane derivative of cholesterol to the charged ester-linked, *nido*-carborane derivative of cholesterol was also achieved by allowing the *closo*-carborane derivative to react with cesium fluoride in absolute ethanol.

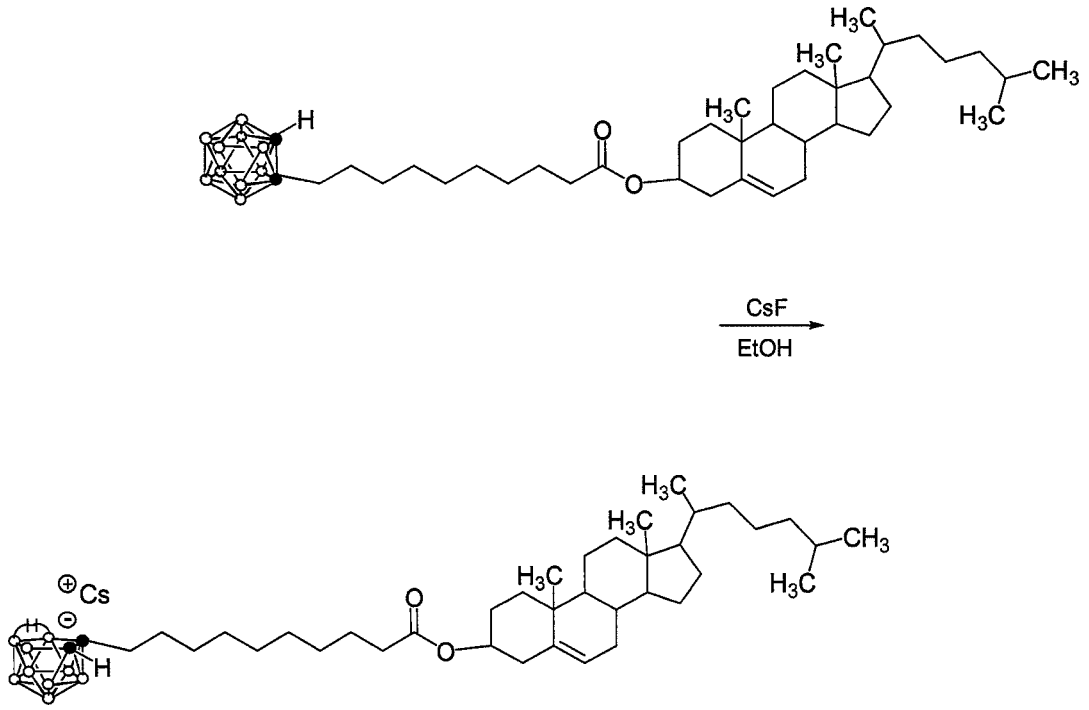


Figure 23: Synthetic scheme for the degradation of the *closo*-carborane to form the *nido*-carborane

The reaction was monitored by ^{11}B NMR spectroscopy. The ^{11}B NMR spectrum exhibits a shift in all of the peaks upfield and the presence of two peaks at the extreme upfield portion of the spectrum, at -33.4 ppm and -37.2 ppm, respectively. These signals

correspond to the unique boron atom in the open face of the cage and the boron atom connected to both of the carbon atoms. In the ^{1}H NMR spectrum, the peak at 3.55 ppm, corresponding to the hydrogen atom attached to the carbon atom in the carborane lattice, disappears, which is consistent with known *nido*-carborane derivatives. No significant shift in the boron-hydrogen absorption in the FT-IR spectrum was observed.

The reduction of the ester-linked, *closo*-carborane derivative of cholesterol to form the ether-linked, *closo*-carborane derivative of cholesterol was achieved by allowing the ester-linked derivative to react with boron trifluoride-diethyl etherate and sodium borohydride.

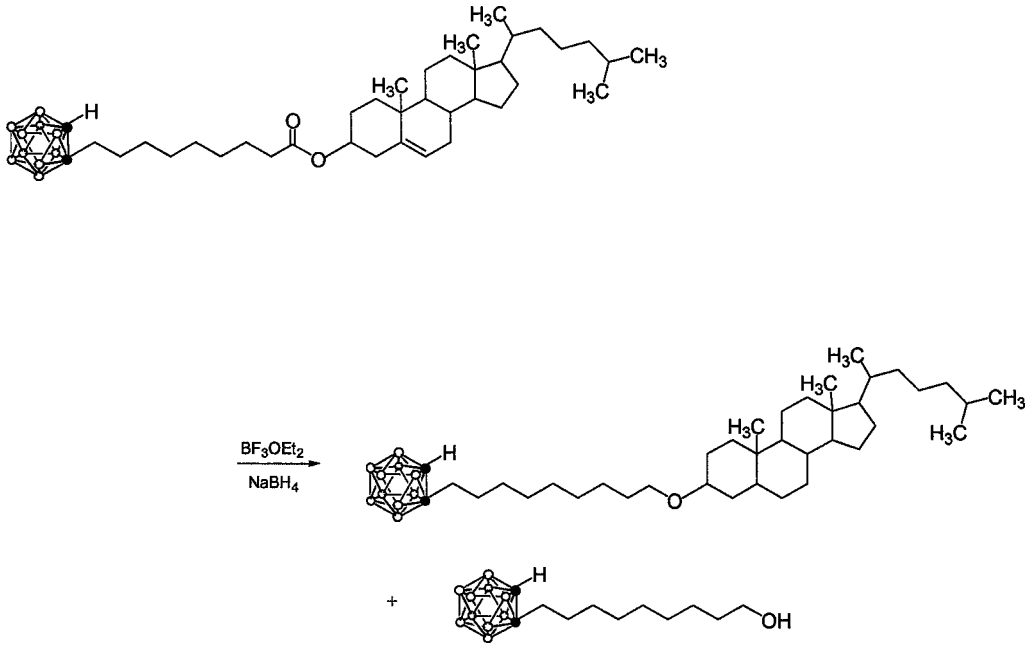


Figure 24: Synthetic scheme for the reduction of the ester to form the ether

The reaction was monitored by the disappearance of the 1600 cm^{-1} absorption in the FT-IR spectrum, corresponding to the carbonyl functionality. The ^{1}H NMR spectrum

displays a shift of the proton connected to the carbon atom of the cholesterol, which is involved with the link, from 4.59 ppm to 3.45 ppm. The triplet in the ^{1}H NMR spectrum of the product corresponds to the hydrogen atoms on the carbon adjacent to the carbonyl functionality. Also, the disappearance of the proton peak at 5.35 ppm corresponds to the loss of the alkene bond within the cholesterol moiety. In the ^{13}C NMR spectrum, gives a loss of the peaks at 173.188, 139.666, and 122.636 which is consistent with the structure of the product. These peaks correspond to the carbonyl linker and the alkene bond in the cholesterol moiety, respectively. It is not thought that the reduction of the cholesterol double bond will hinder the biological distribution of the product.

The degradation of the ester-linked, *closo*-carborane derivative of cholesterol to the charged ester-linked, *nido*-carborane derivative of cholesterol was achieved by allowing the *closo*-carborane derivative to react with cesium fluoride in absolute ethanol.

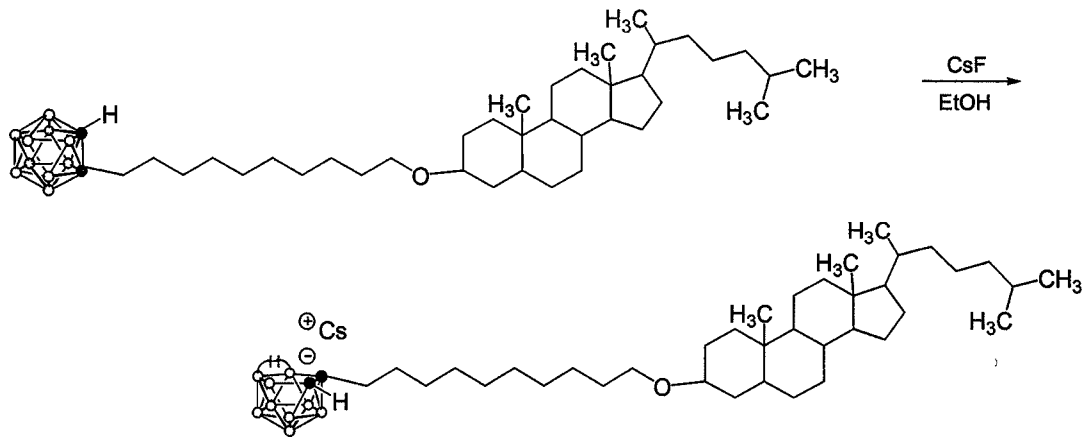


Figure 25: Synthetic scheme for the degradation of the *closo*-carborane to form the *nido*-carborane

The reaction was monitored by ^{11}B NMR spectroscopy. The ^{11}B NMR spectrum exhibits

a shift in all of the peaks upfield as well as the presence of two peaks at the extreme upfield portion of the spectrum at -35.2 ppm and -39.1 ppm , respectively These signals correspond to the unique boron atom in the open face of the cage and the boron atom connected to both of the carbon atoms. The ^{1}H NMR spectrum, the peak at 3.45 ppm, corresponding to the hydrogen atom attached to the carbon atom in the carborane lattice, disappears, which is consistent with known *nido*-carborane derivatives. No significant shift in the boron-hydrogen absorption in the FT-IR spectrum was observed, as anticipated.

4.2 Discussion

A series of eight boron-containing derivatives of cholesterol that vary in the length of the carbon-atom tether, identity of the connecting group, and charge on the carborane moiety have been synthesized. These compounds are suitable for incorporation into unilamellar liposomes and evaluation as potential agents for BNCT. The synthesis of the *closo*-carborane derivatives linked by an ester functionality were accomplished in two steps, as shown for the three carbon atom linker in Figure 26.

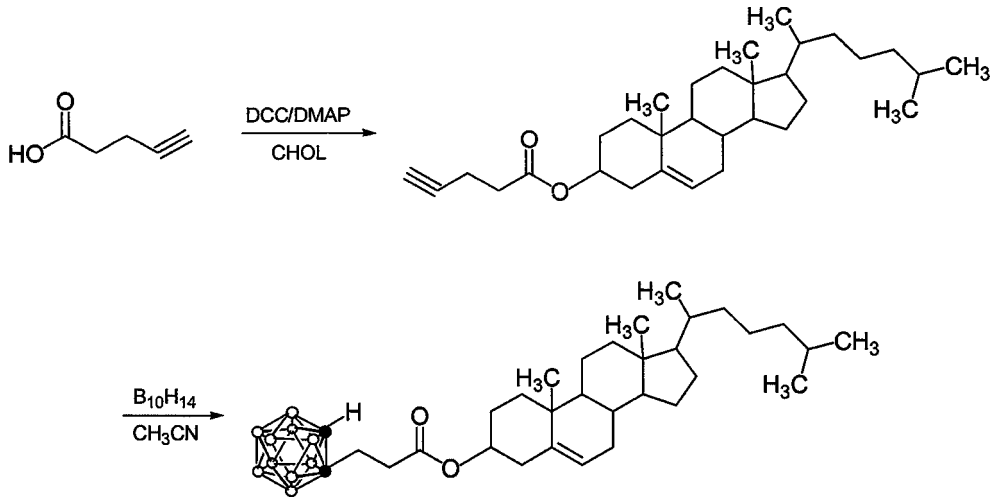


Figure 26: New synthetic scheme for the formation of the *closo*-carborane

The new route significantly shortens the original synthesis which took six steps. The decaborane is not added until the second step, significantly reducing the waste of the relatively precious compound. For the ether synthesis, a much more efficient reaction scheme, shown for the three carbon atom linker in Figure 27, was used to replace the original low yield Williamson ether synthesis.

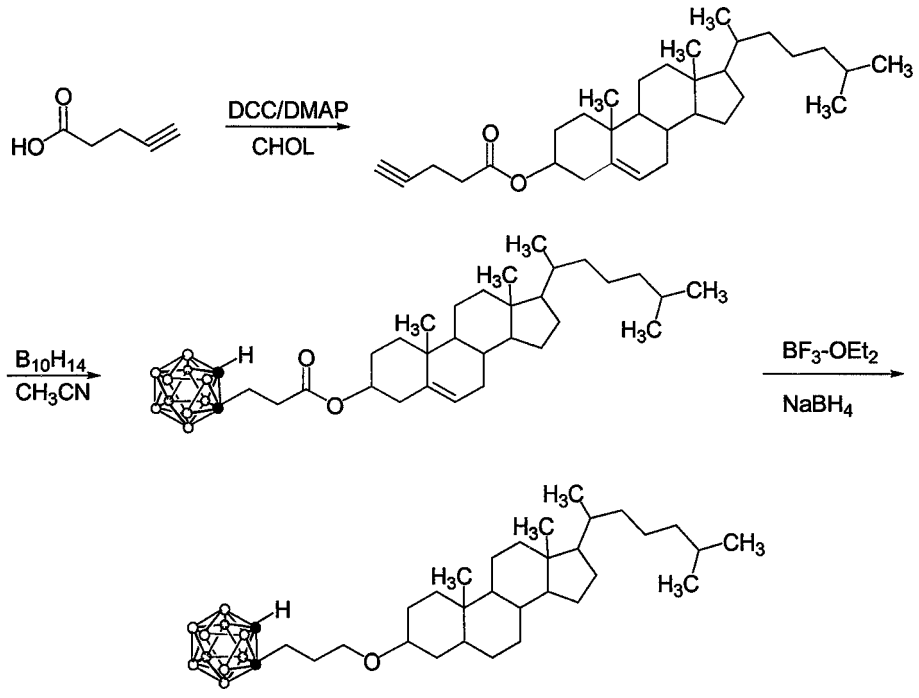


Figure 27: New synthetic scheme for the formation of the ether-linked, *closo*-carborane

Another advantage of the new synthetic scheme for the formation of the ether-linked, *closo*-carborane derivative is that the byproduct, the carboranyl alcohol, can be used to form more product using the original synthetic scheme. Degradation of the *closo*-carborane derivatives to form the *nido*-carborane derivatives has been accomplished using cesium fluoride. The new synthetic route, shown for the three carbon atom linker in Figure 28, enables the production of the *nido*-carborane with the cesium counterion as opposed to the more biologically toxic tetrabutylammonium counterion which had been used in the original synthesis.

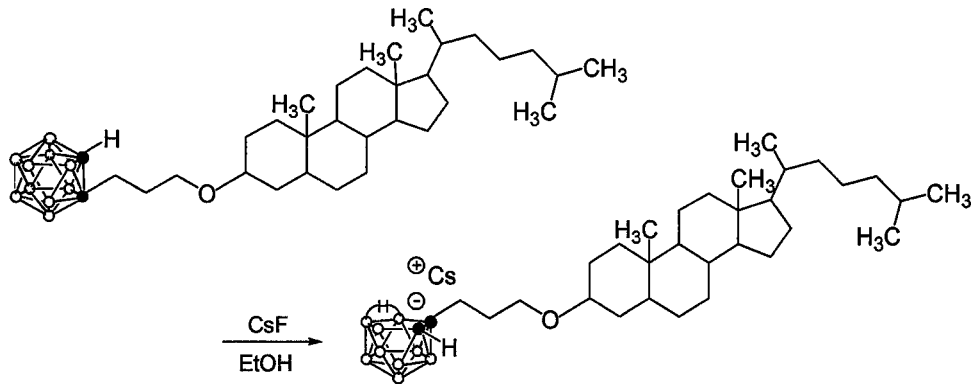


Figure 28: New synthetic scheme for the degradation of the *closo*-carborane derivative to form the *nido*-carborane derivative

Therefore, the specific goals for the project have all been achieved.

5.0 SUGGESTIONS FOR FUTURE RESEARCH

Although the specific goals of this research project were achieved, the new compounds have not been incorporated into the unilamellar liposomes for biological testing. Therefore, the primary emphasis for future research should be the incorporation of the prepared compounds into unilamellar liposomes. The design of the preparation should include varying the amounts of each of the compounds in order to maximize the boron concentration within the liposomes. It is anticipated that other compounds which also contain the *nido*-carborane head group should pack in the bilayer to a greater extent and also exhibit enhanced tumor uptake and retention. The packing should be enhanced due to the consistent bond angle throughout the linker and the uptake and retention should also be enhanced due to the negative charge imparted by the *nido*-carborane head group. Once the liposomes are prepared, the size of the liposomes should be determined and the concentration of boron established. The biodistributions can be completed by Dr. Patrick Gavin at Washington State University in Pullman, Washington.

There is some speculation in the literature that the hydroxyl substituent on the cholesterol is necessary for maximum packing of the bilayer constituents, and, therefore, maximum stability of the bilayer. If this is the case, then substitution at the hydroxyl group of cholesterol may not be optimum. Derivatives of cholesterol can be purchased which enable the modification of the side chain rather than the hydroxyl group. These compounds may be more suitable for liposomal incorporation because they would retain the hydroxyl group and thereby enhance packing. They could also be modified to include the *ortho*-carborane or *nido*-carborane substituent.

Finally, there is increased interest in modifying compounds which are known to specifically localize within the brain. One class of these compounds would include neurotransmitters, such as epinephrine, dopamine, or norepinephrine. Modification of these compounds to include the *ortho*-carborane or *nido*-carborane substituent may provide a means of directly accessing the primary target tumor for application in BNCT, *glioblastoma multiforme*.

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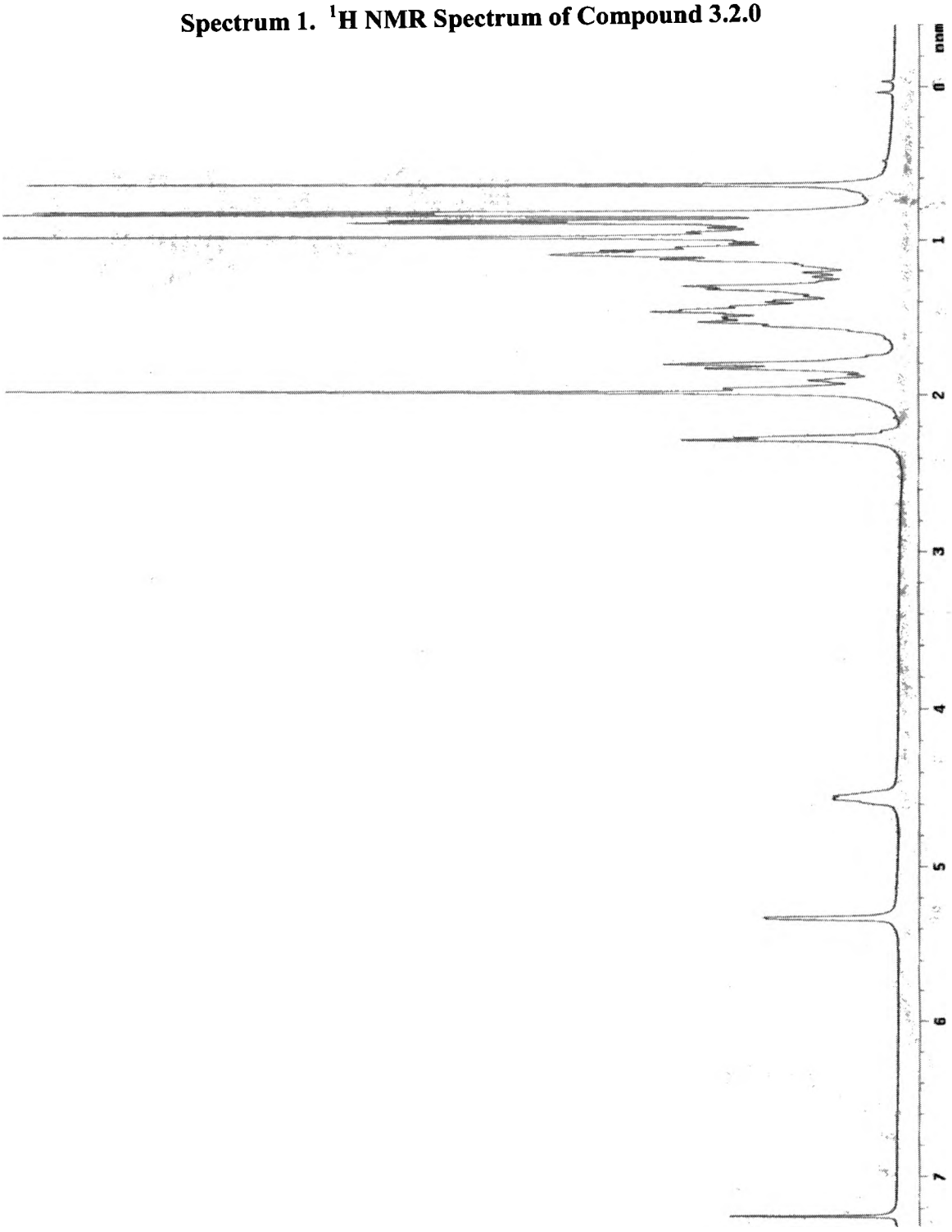
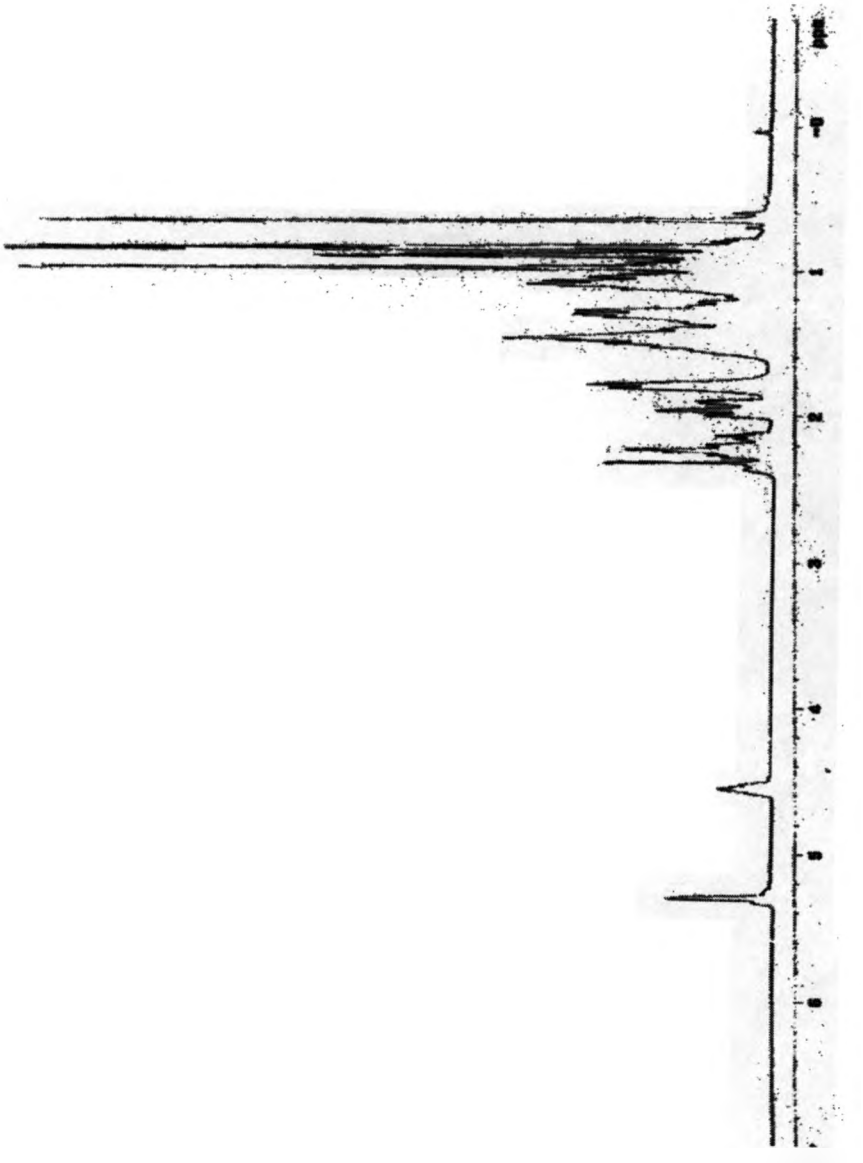
APPENDIX: SPECTRA

Spectrum 1. ^{1}H NMR Spectrum of Compound 3.2.0



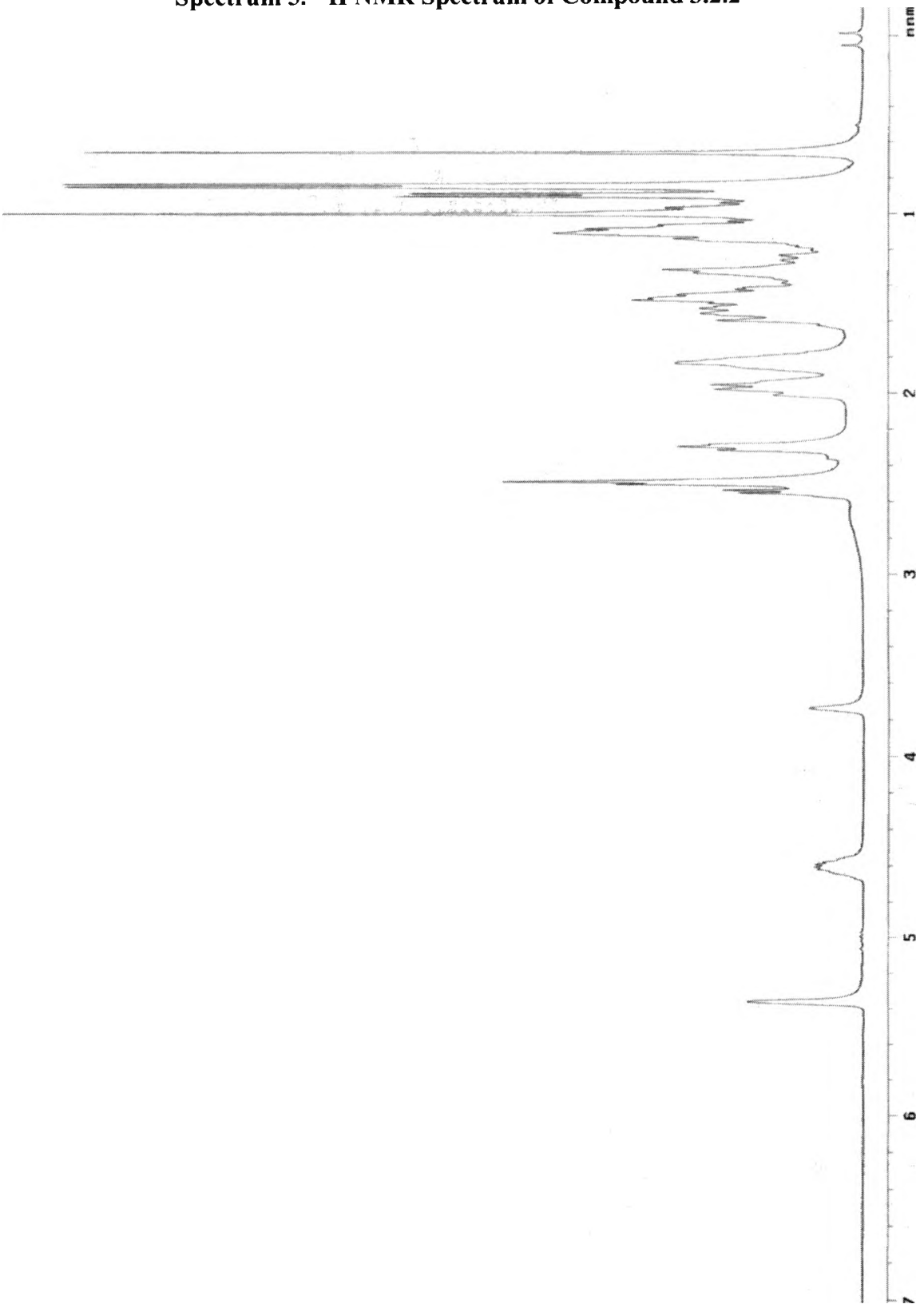
Spectrum 2. ^{13}C NMR Spectrum of Compound 3.2.0

Spectrum 3. ^{1}H NMR Spectrum of Compound 3.2.1

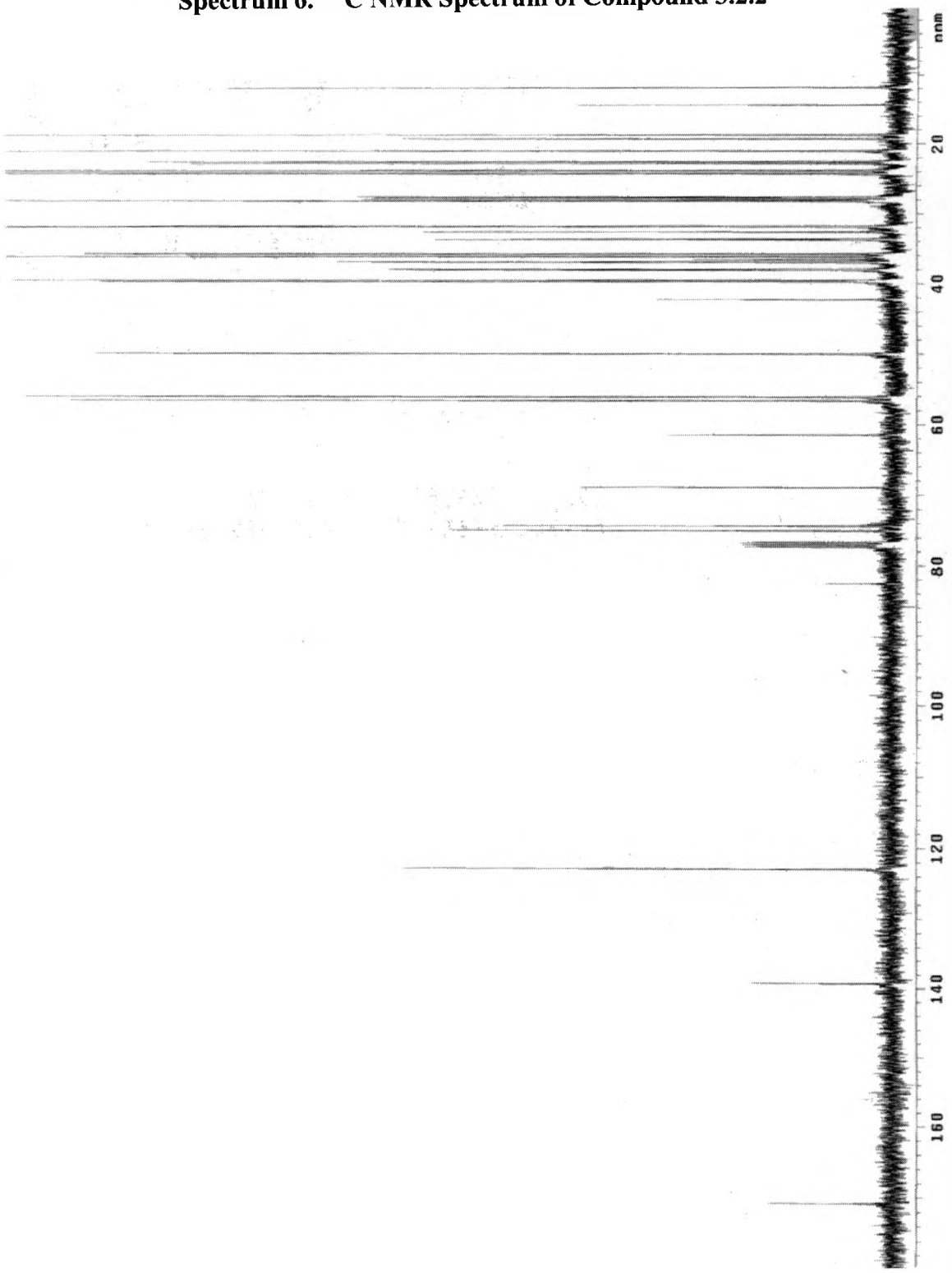


Spectrum 4. ^{13}C NMR Spectrum of Compound 3.2.1

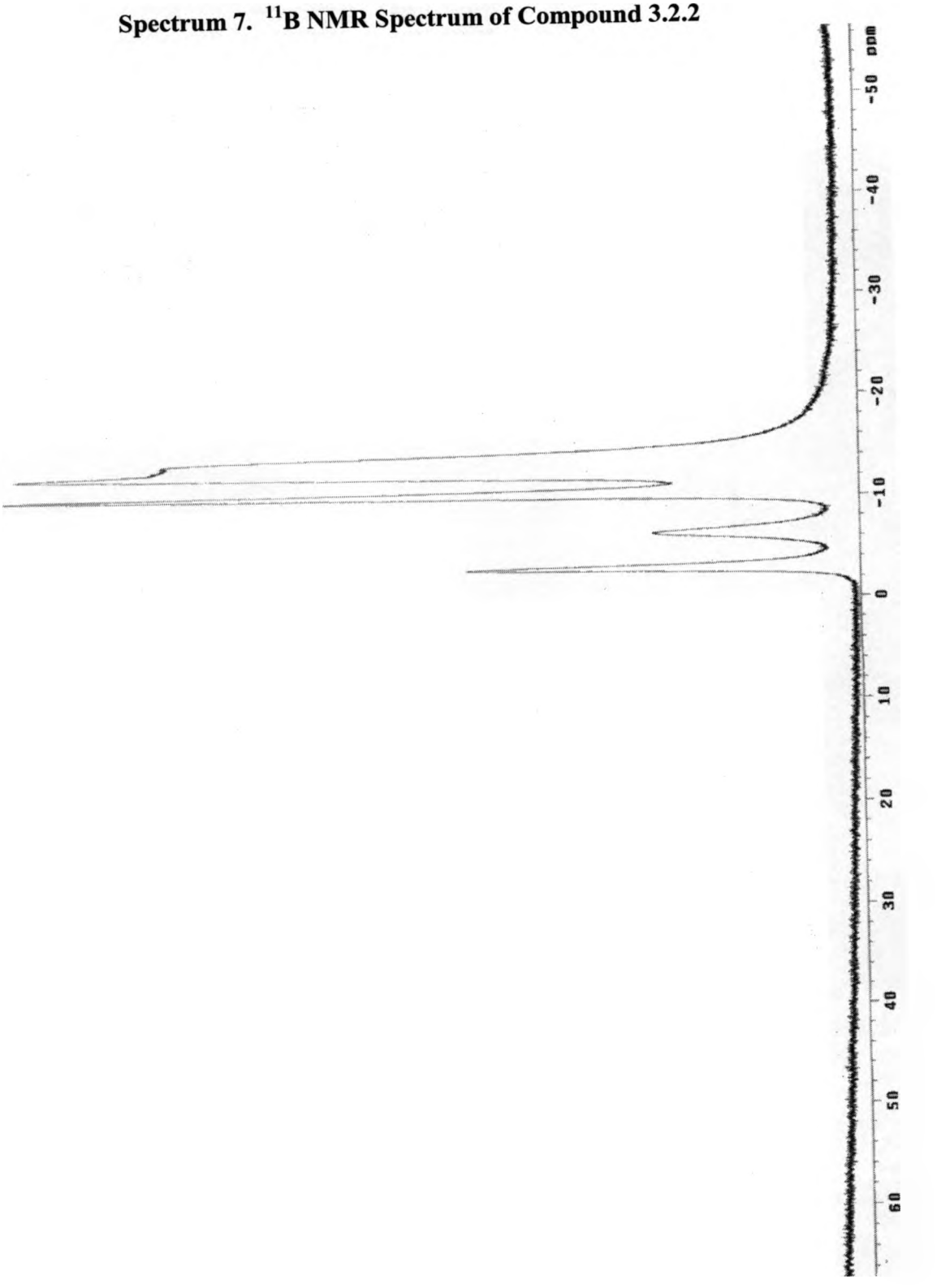
Spectrum 5. ^{1}H NMR Spectrum of Compound 3.2.2



Spectrum 6. ^{13}C NMR Spectrum of Compound 3.2.2



Spectrum 7. ^{11}B NMR Spectrum of Compound 3.2.2



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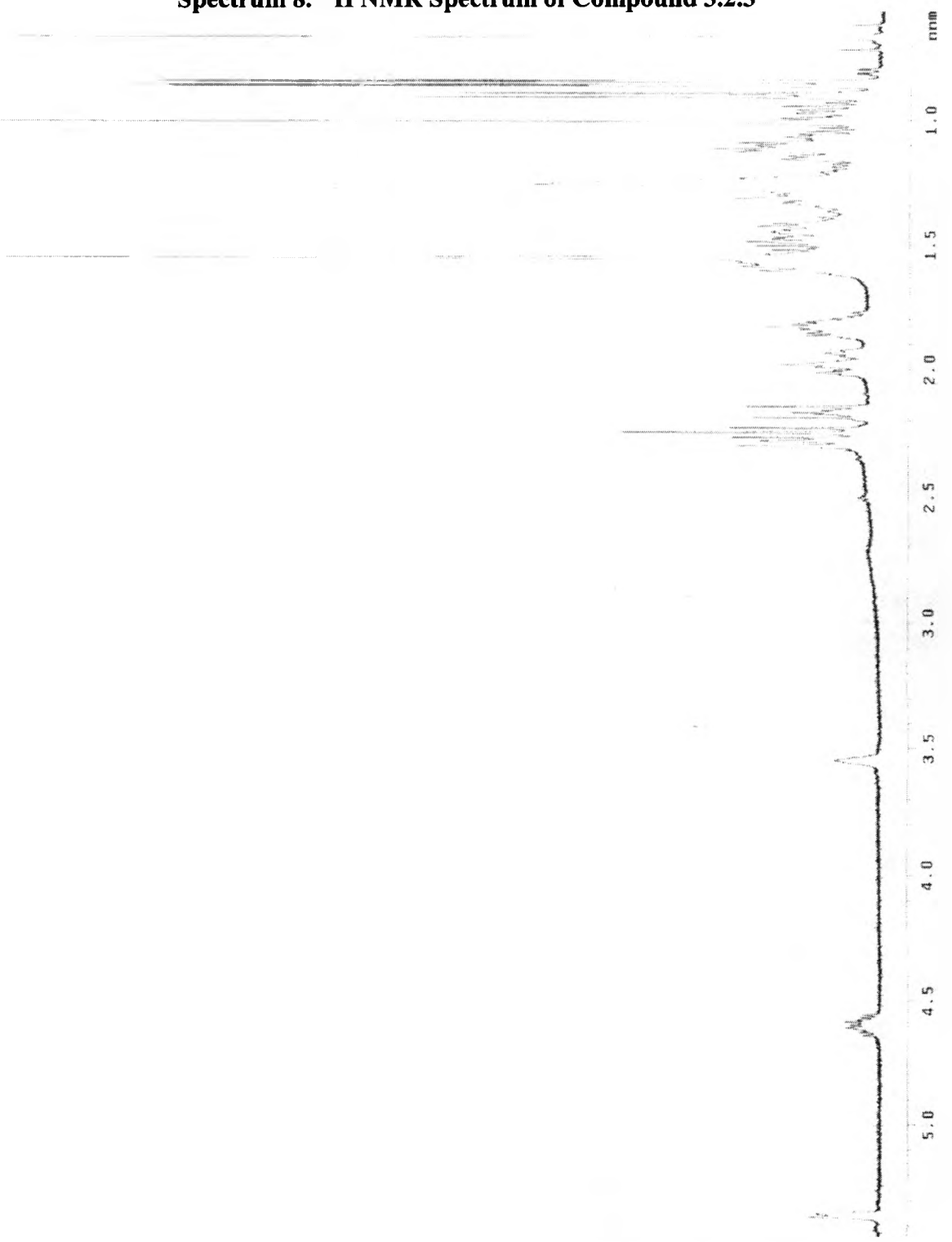
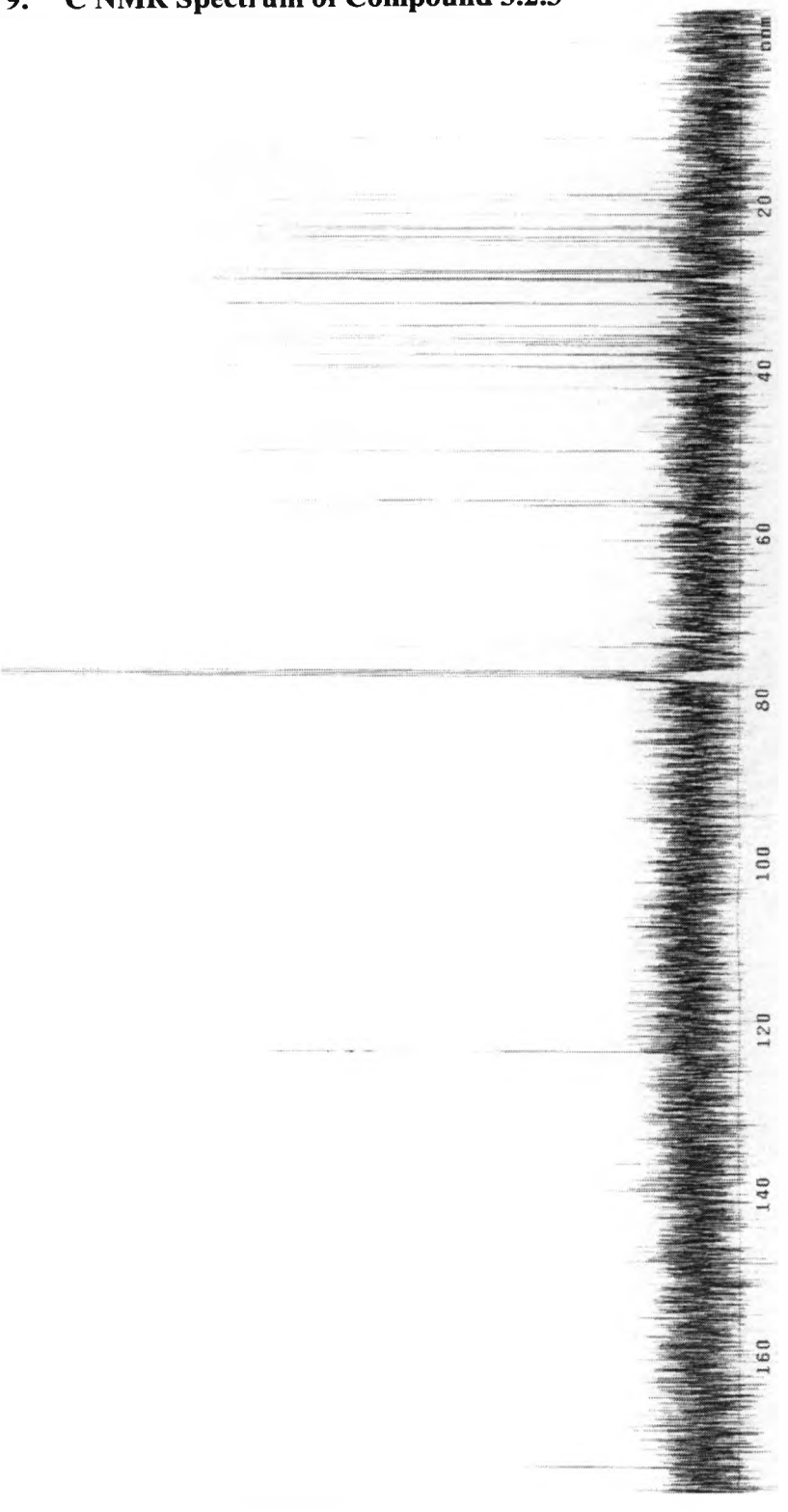
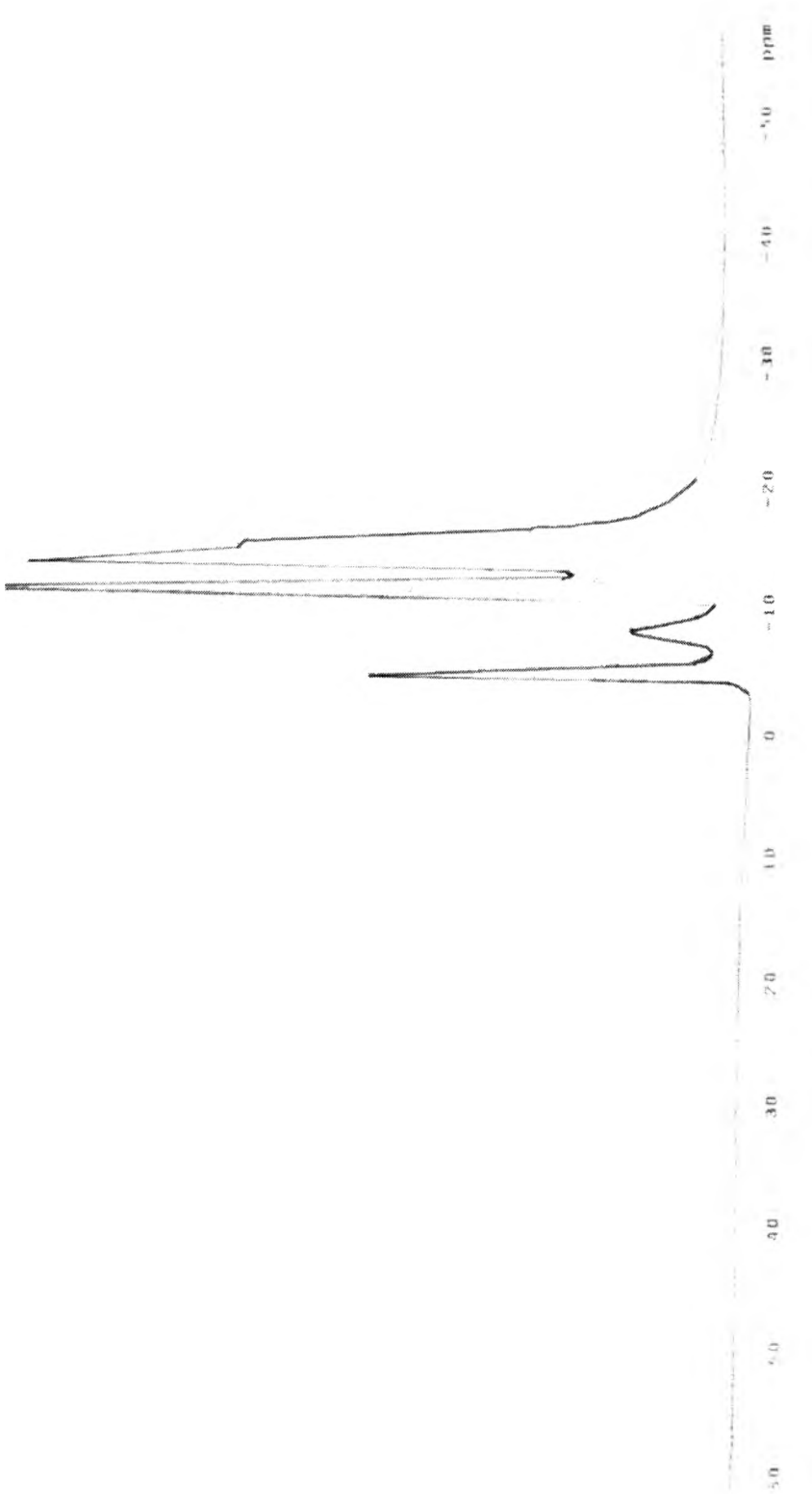


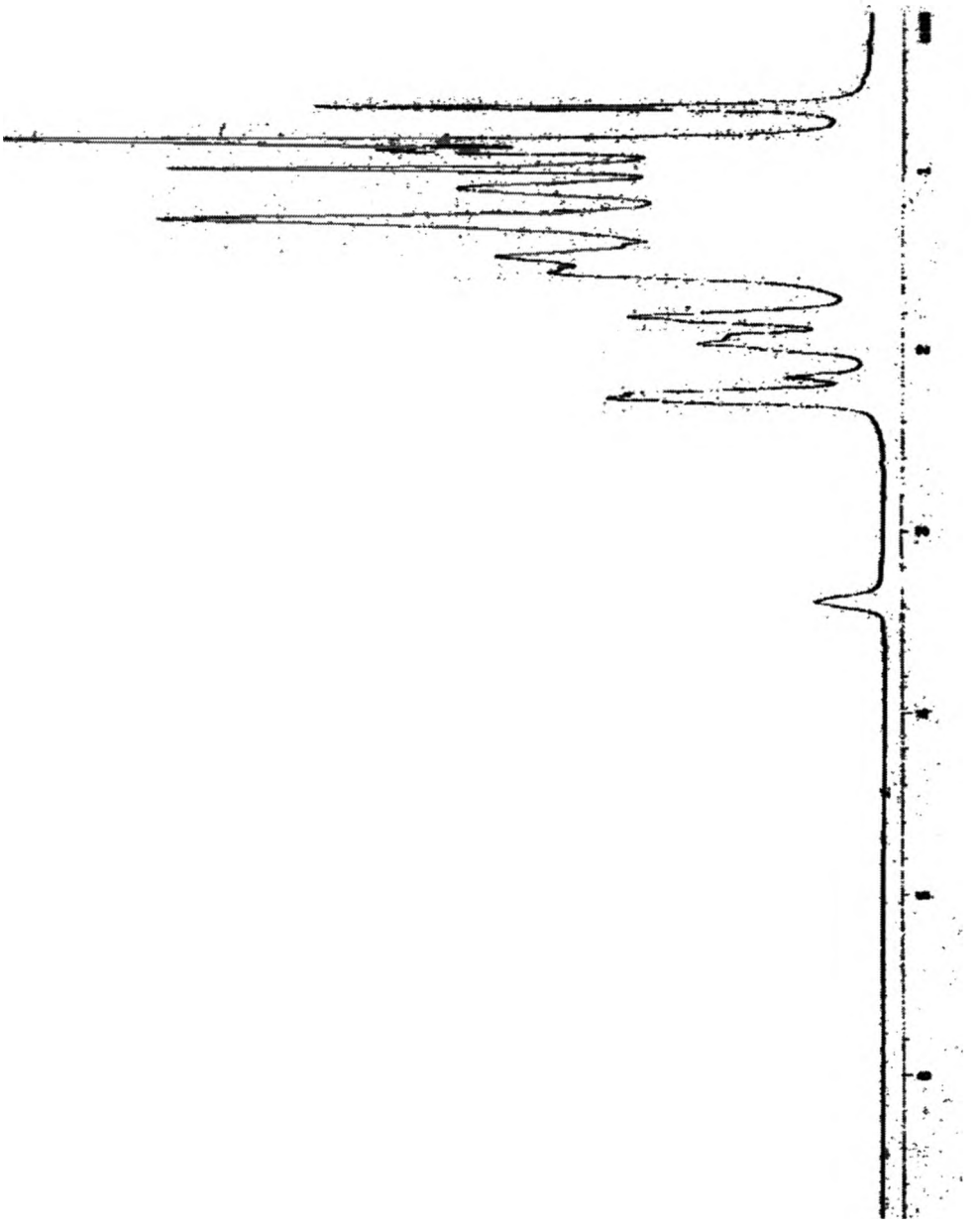
Spectrum 9. ^{13}C NMR Spectrum of Compound 3.2.3



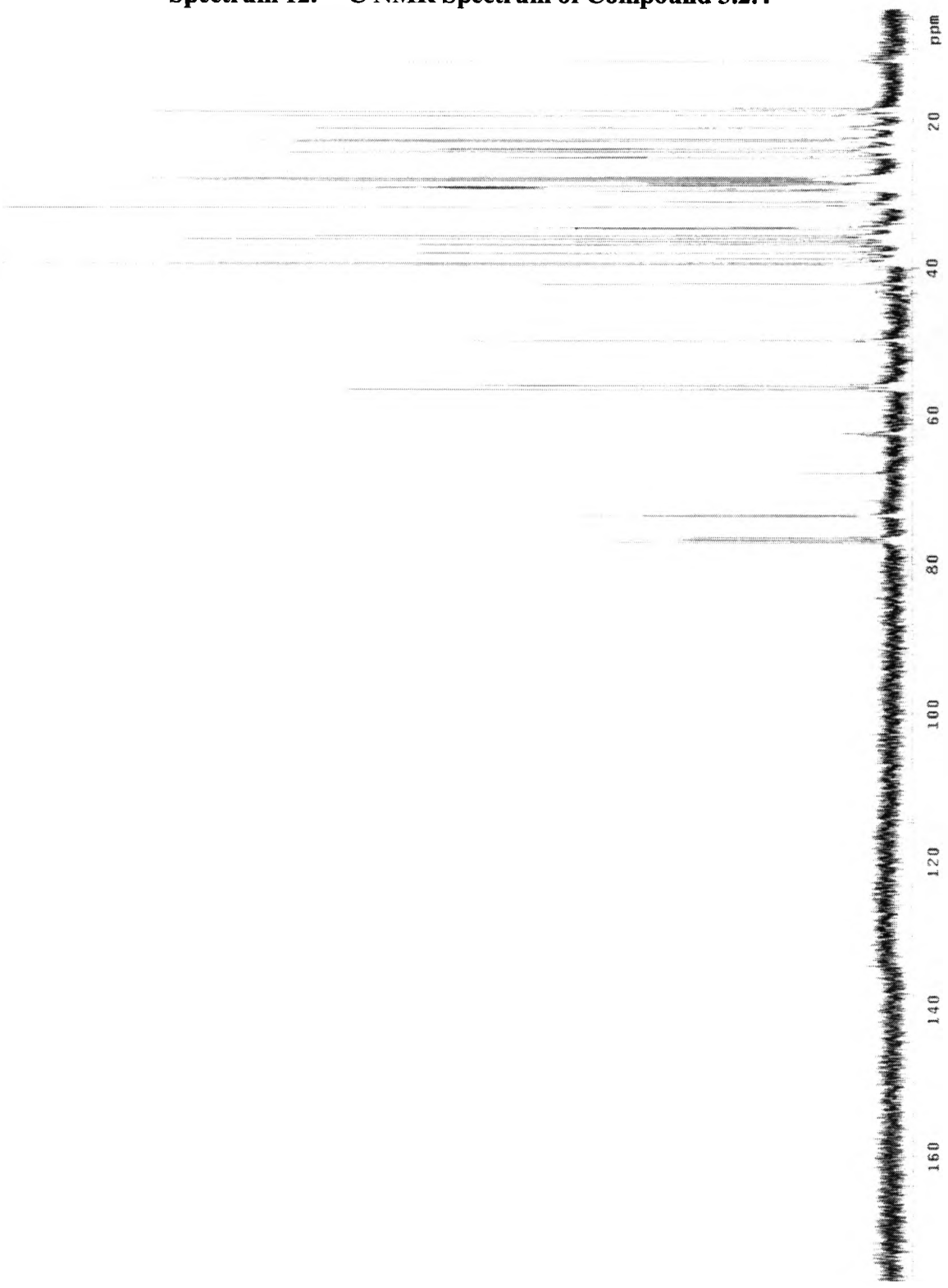
Spectrum 10. ^{11}B NMR Spectrum of Compound 3.2.8



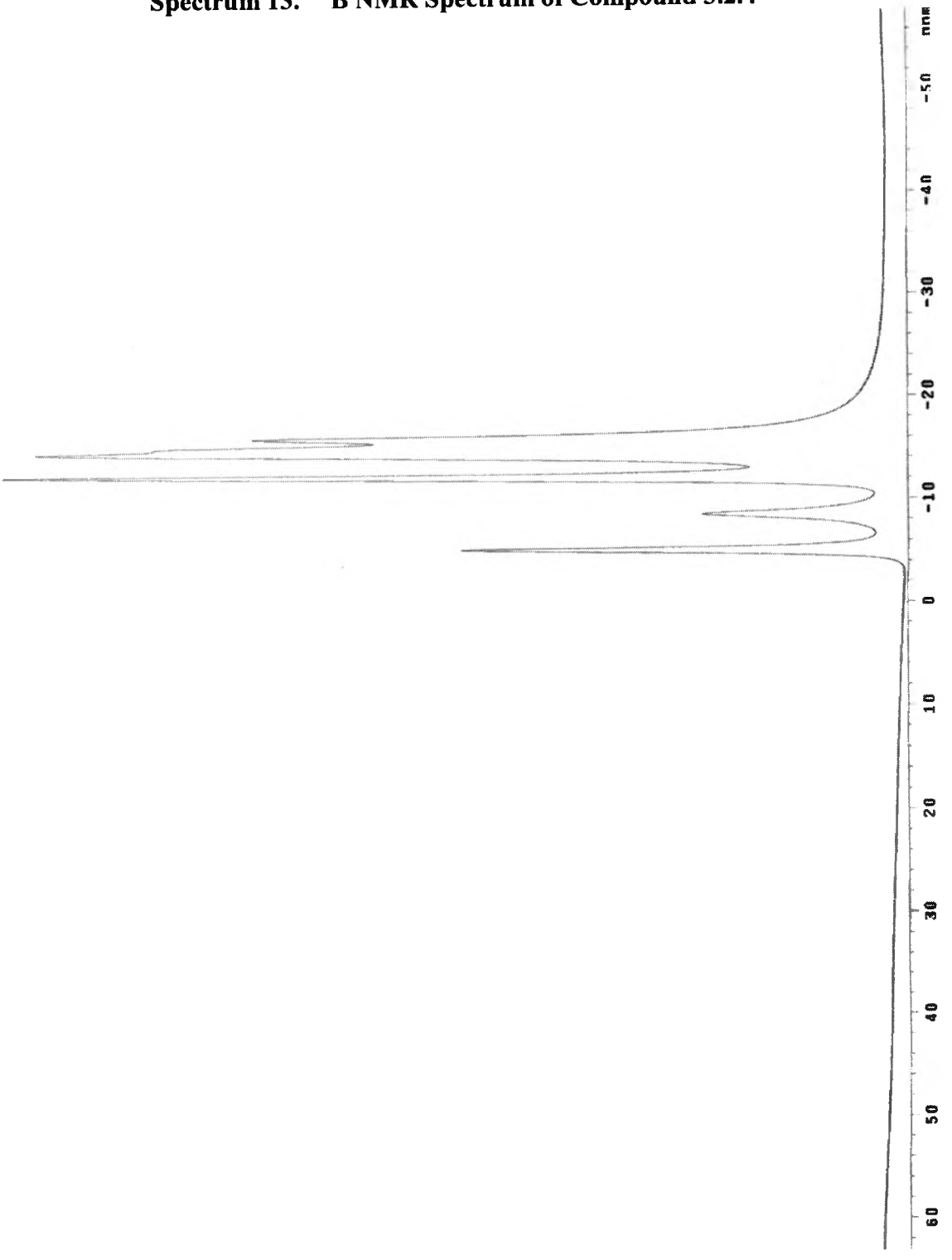
Spectrum 11. ^{1}H NMR Spectrum of Compound 3.2.4



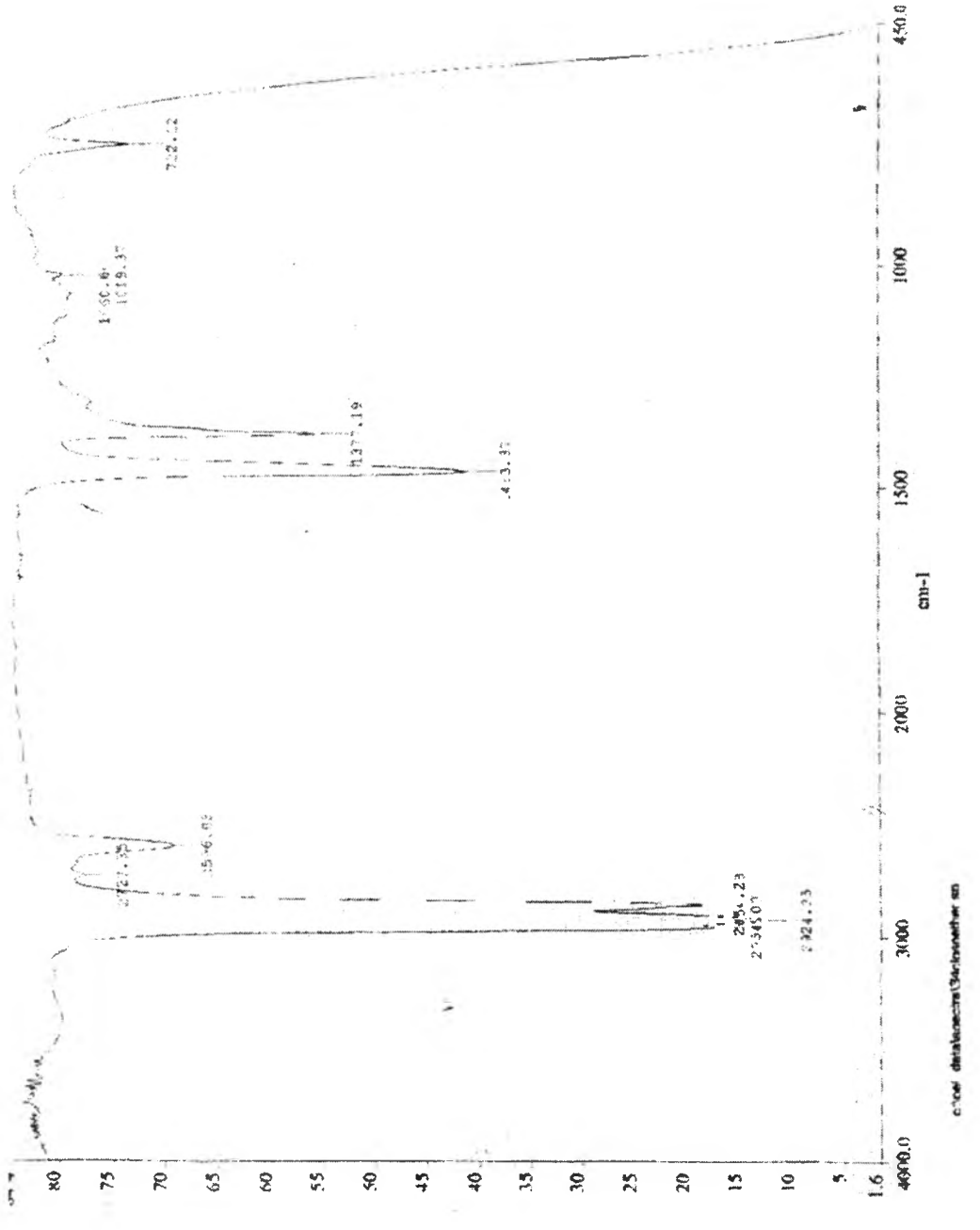
Spectrum 12. ^{13}C NMR Spectrum of Compound 3.2.4



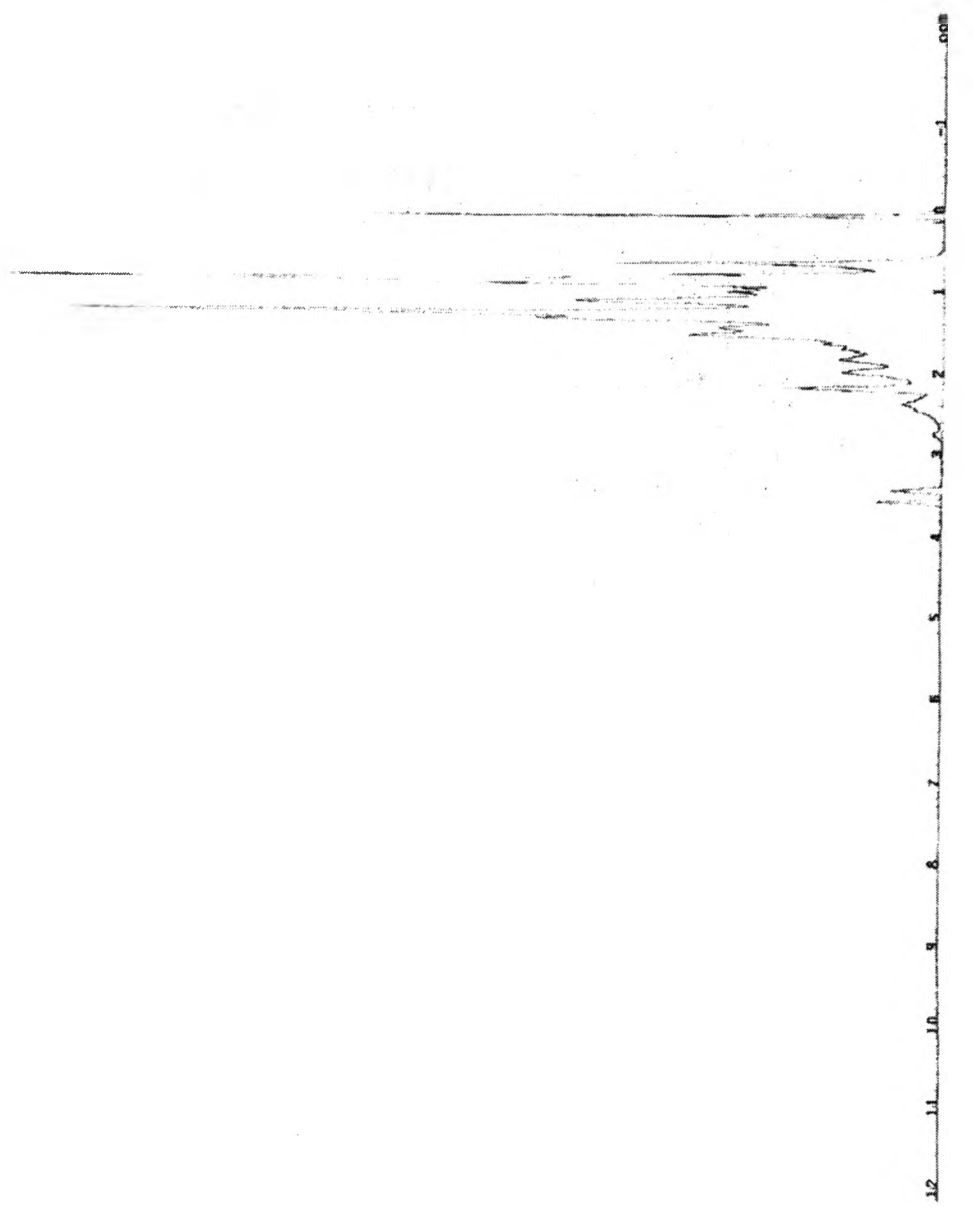
Spectrum 13. ^{11}B NMR Spectrum of Compound 3.2.4



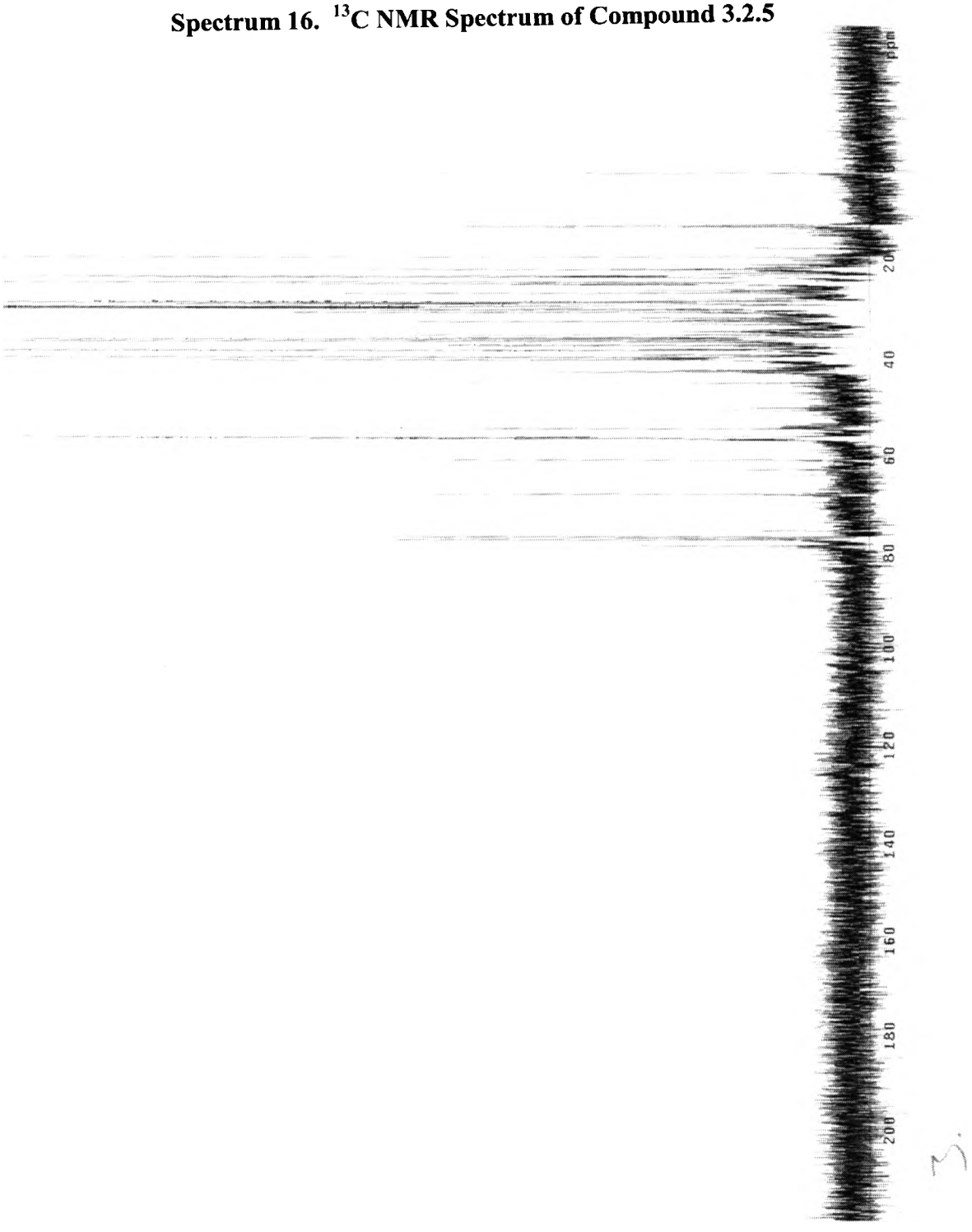
Spectrum 14. FT-IR Spectrum of Compound 3.2.4



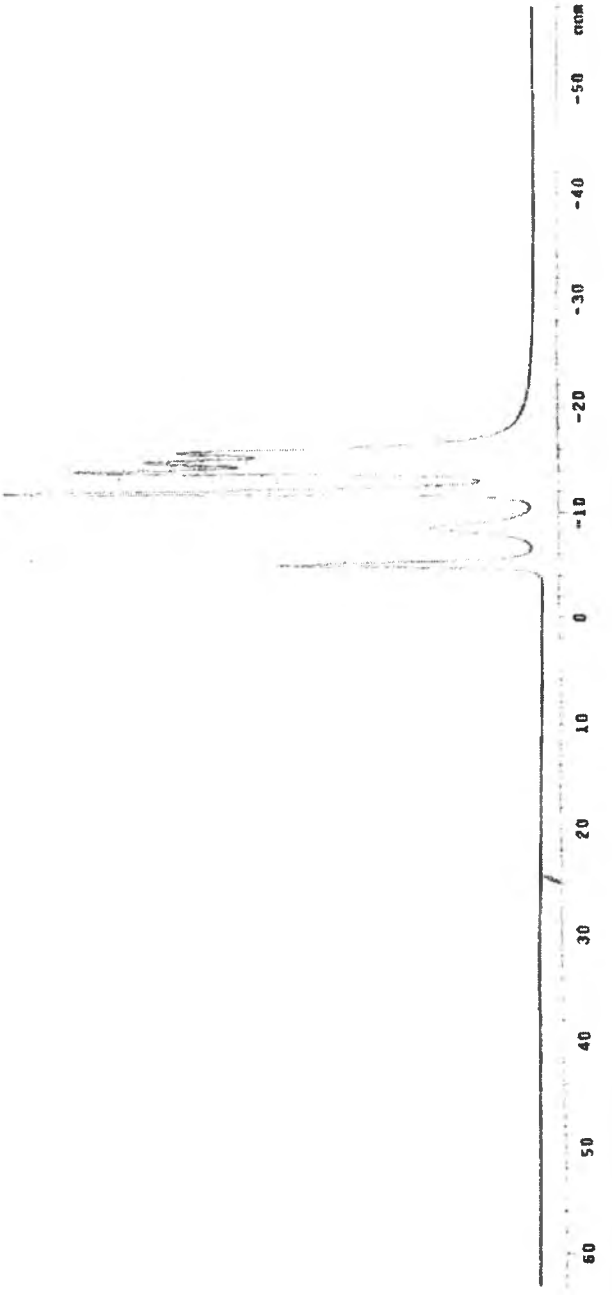
Spectrum 15. ^{1}H NMR Spectrum of Compound 3.2.5



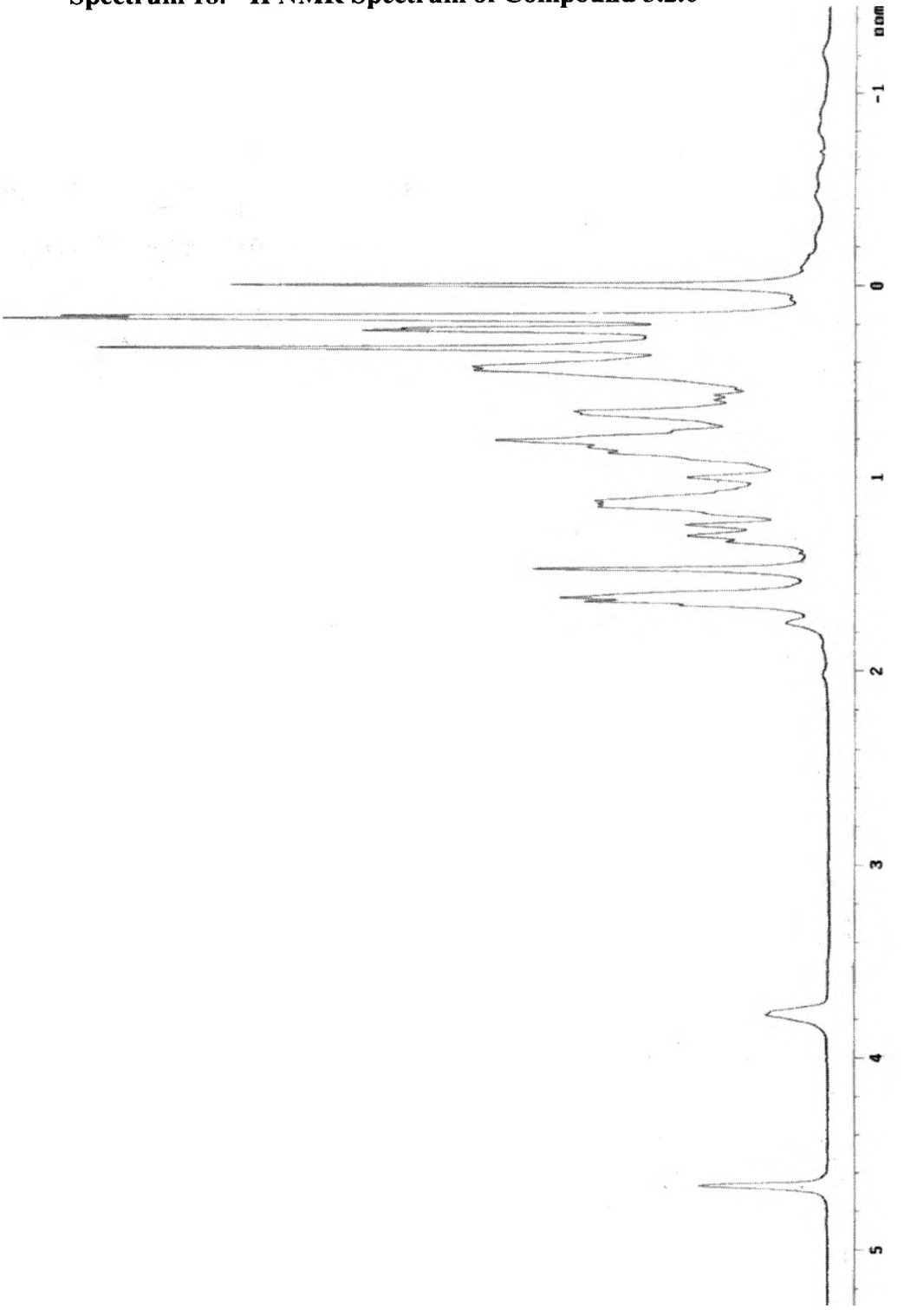
Spectrum 16. ^{13}C NMR Spectrum of Compound 3.2.5



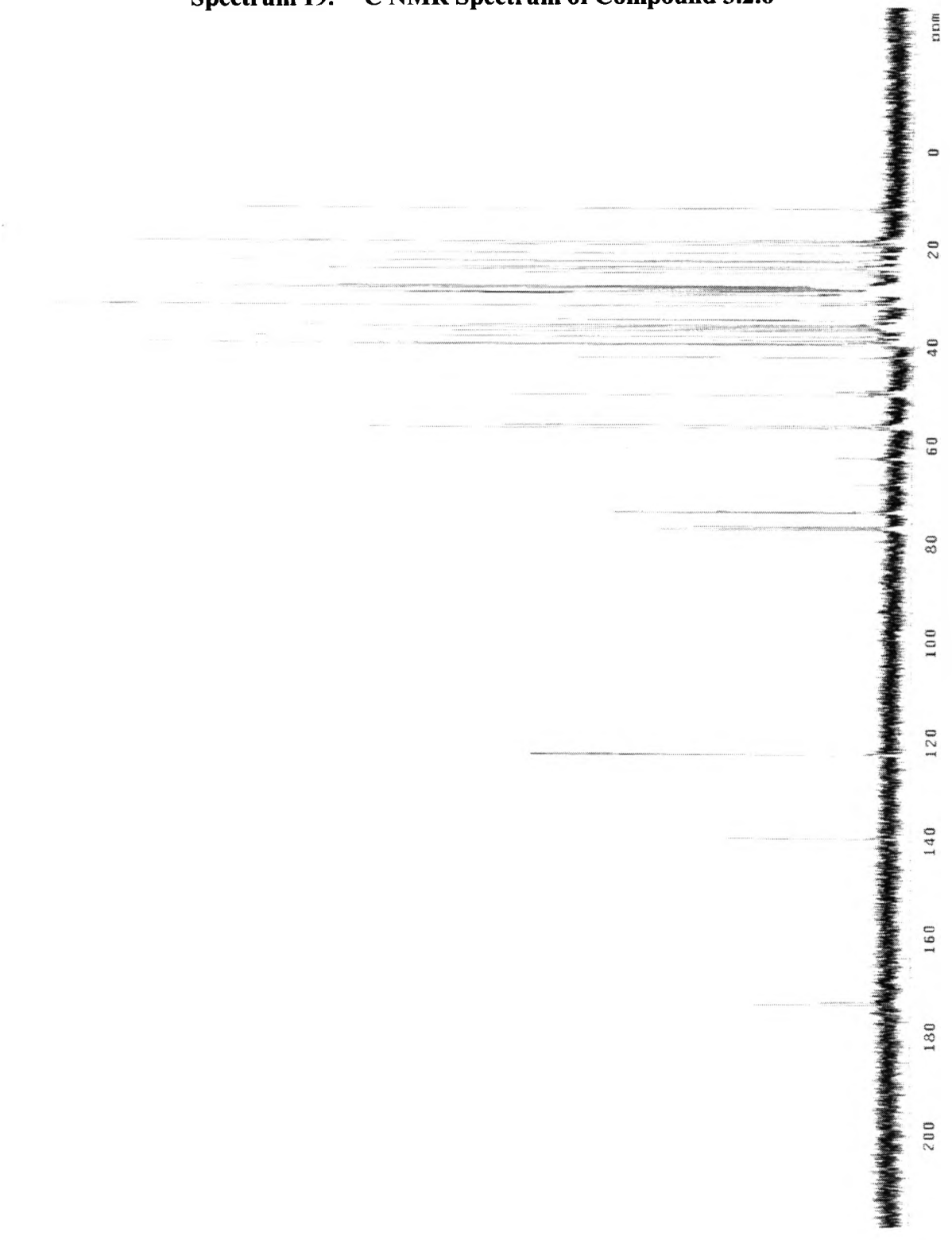
Spectrum 17. ^{11}B NMR Spectrum of Compound 3.2.5



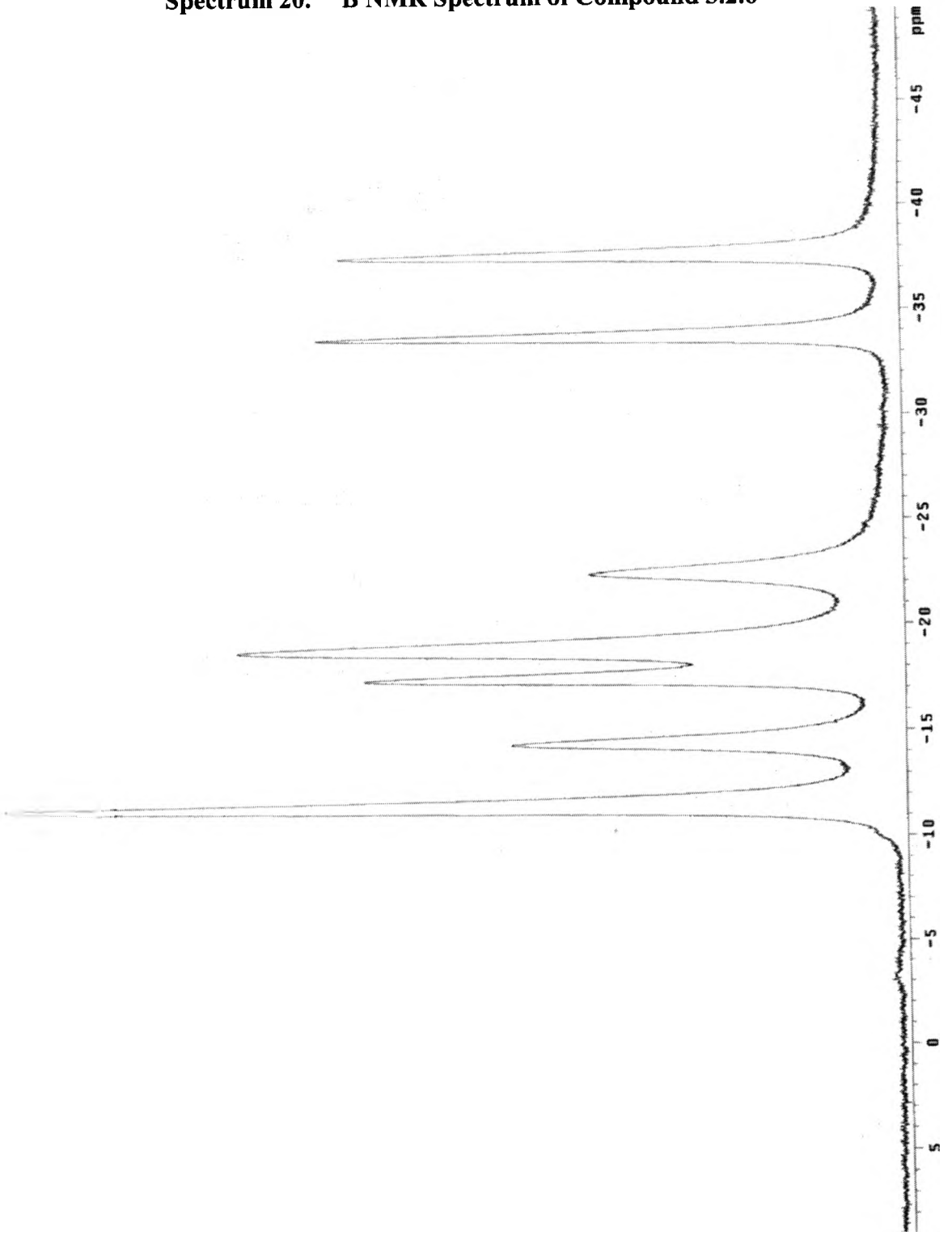
Spectrum 18. ^{1}H NMR Spectrum of Compound 3.2.6



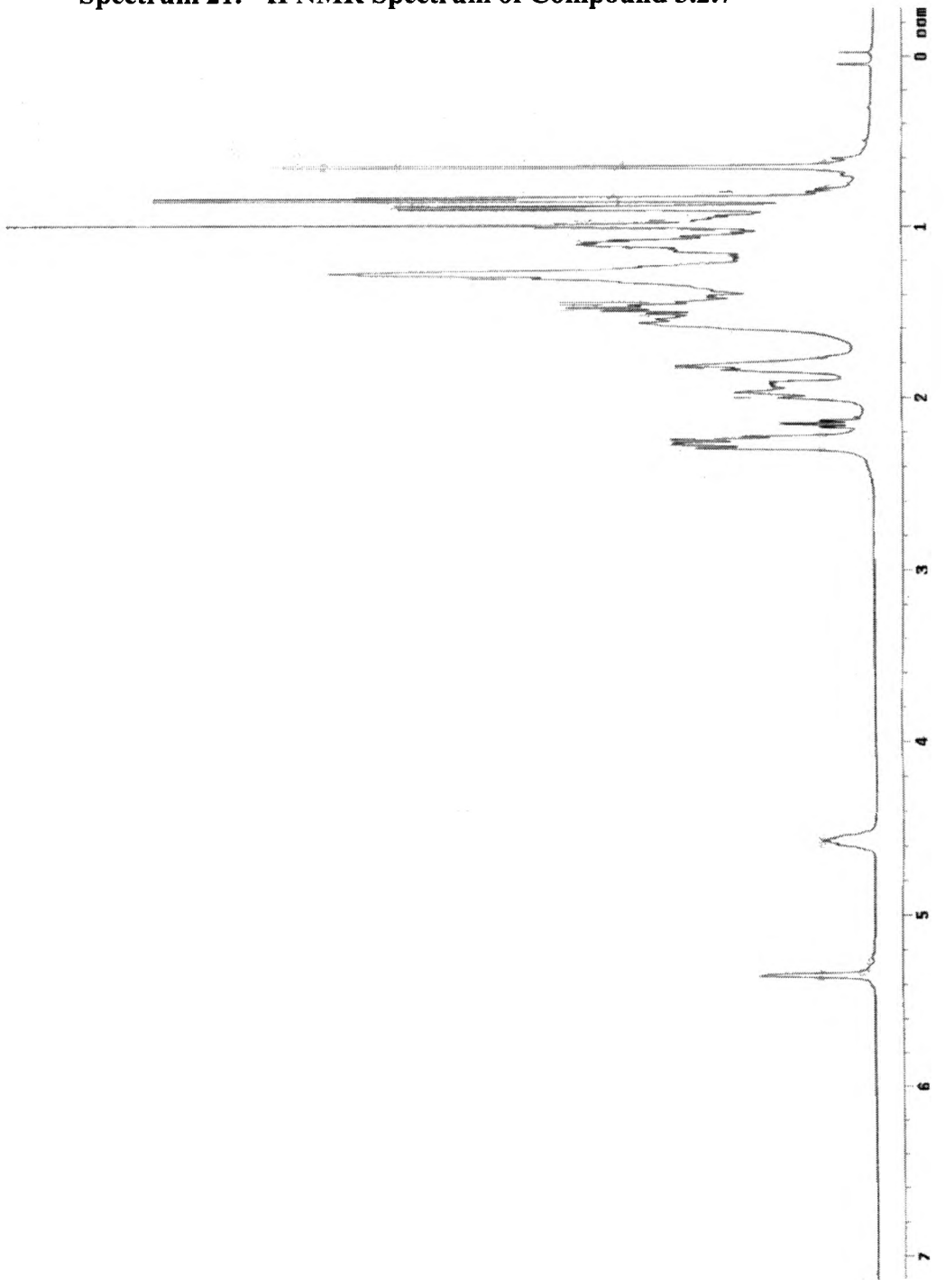
Spectrum 19. ^{13}C NMR Spectrum of Compound 3.2.6



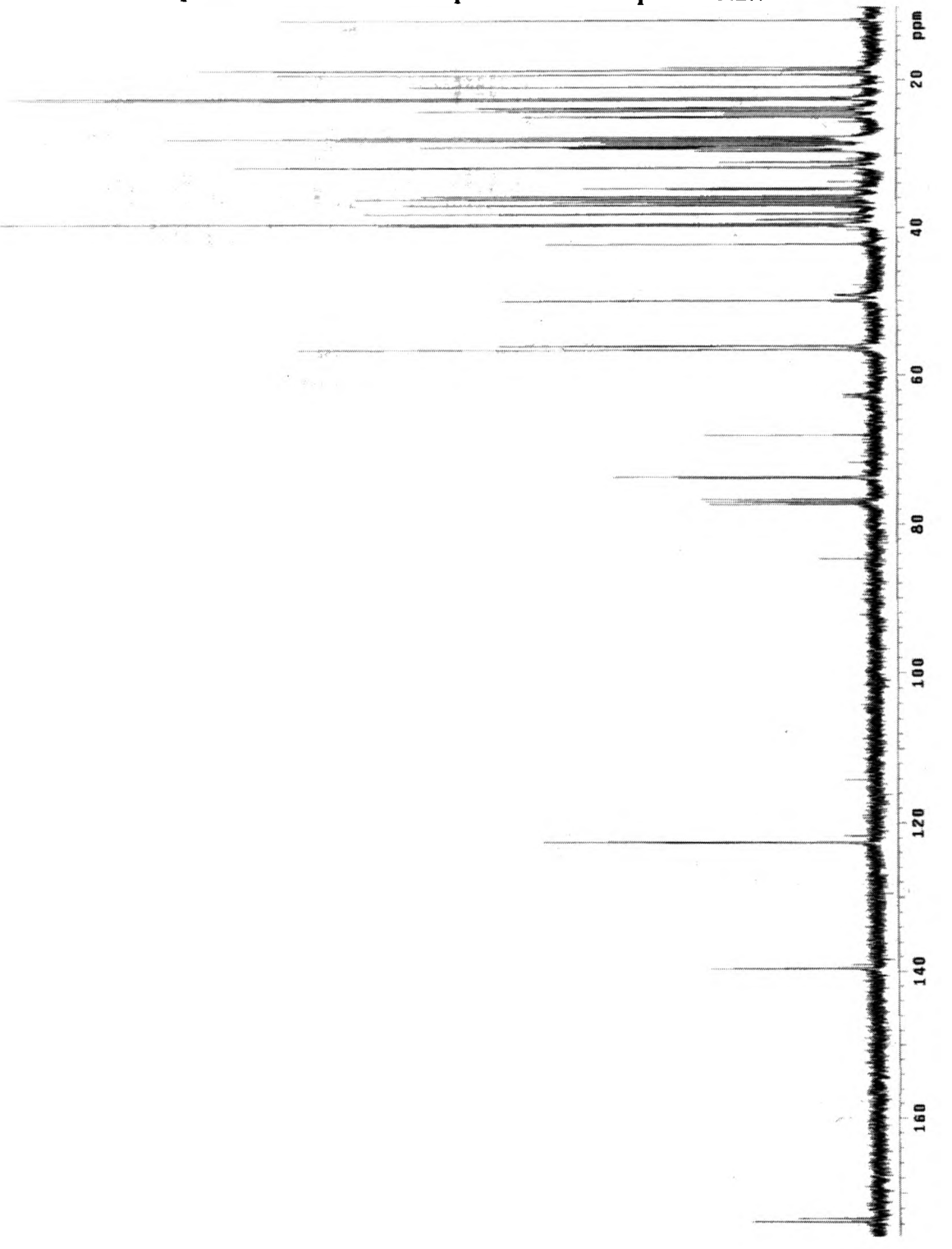
Spectrum 20. ^{11}B NMR Spectrum of Compound 3.2.6



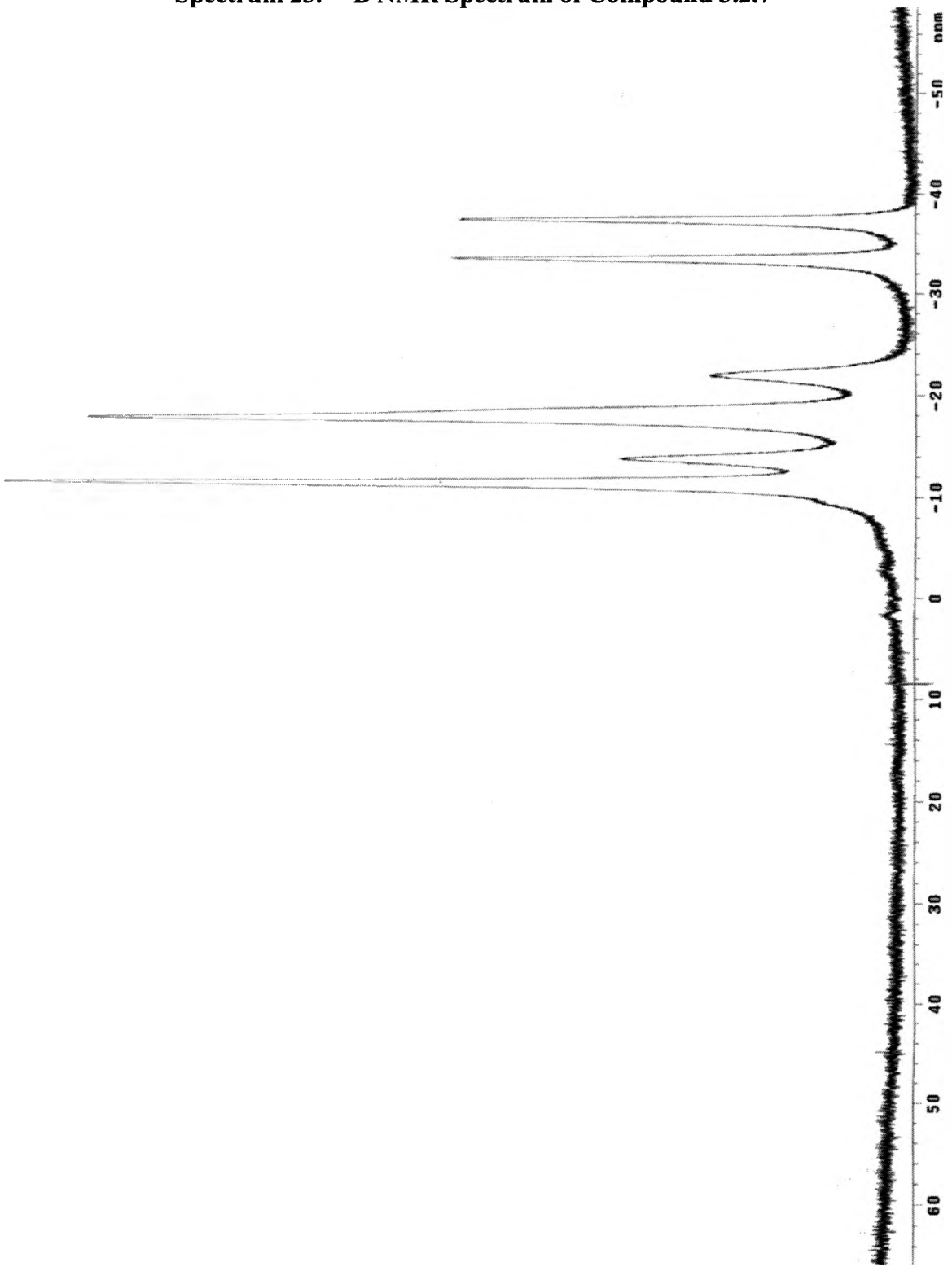
Spectrum 21. ^{1}H NMR Spectrum of Compound 3.2.7



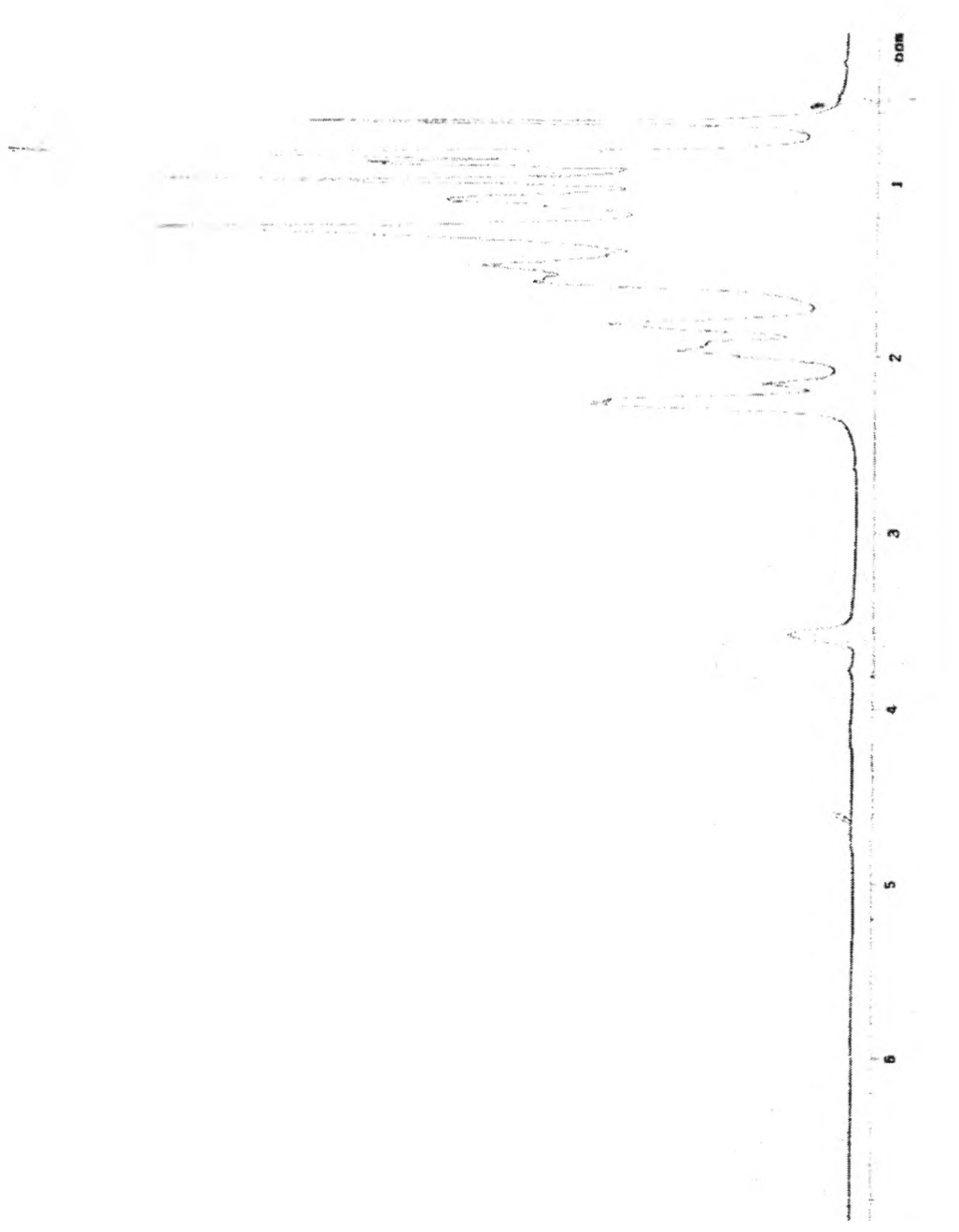
Spectrum 22. ^{13}C NMR Spectrum of Compound 3.2.7



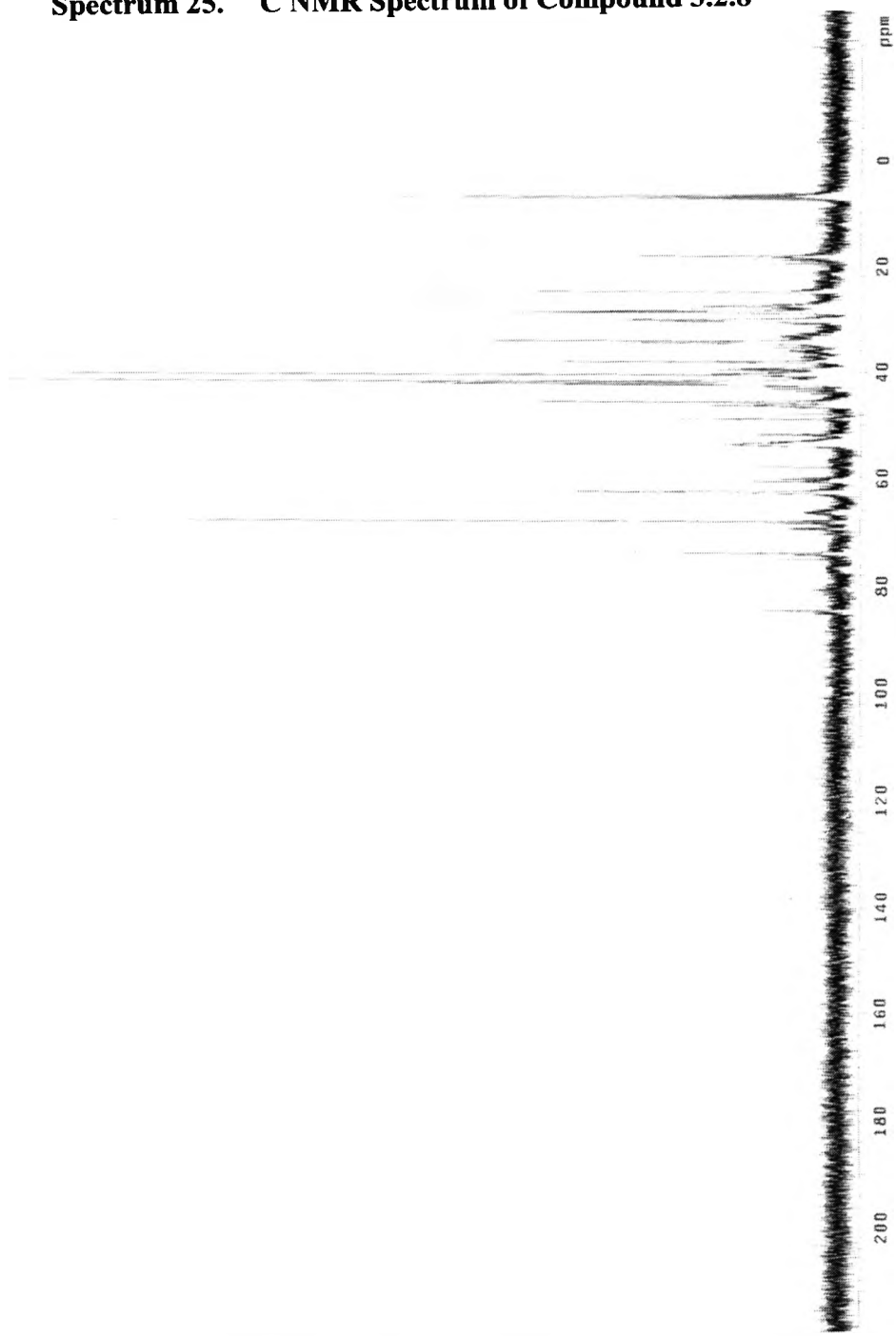
Spectrum 23. ^{11}B NMR Spectrum of Compound 3.2.7



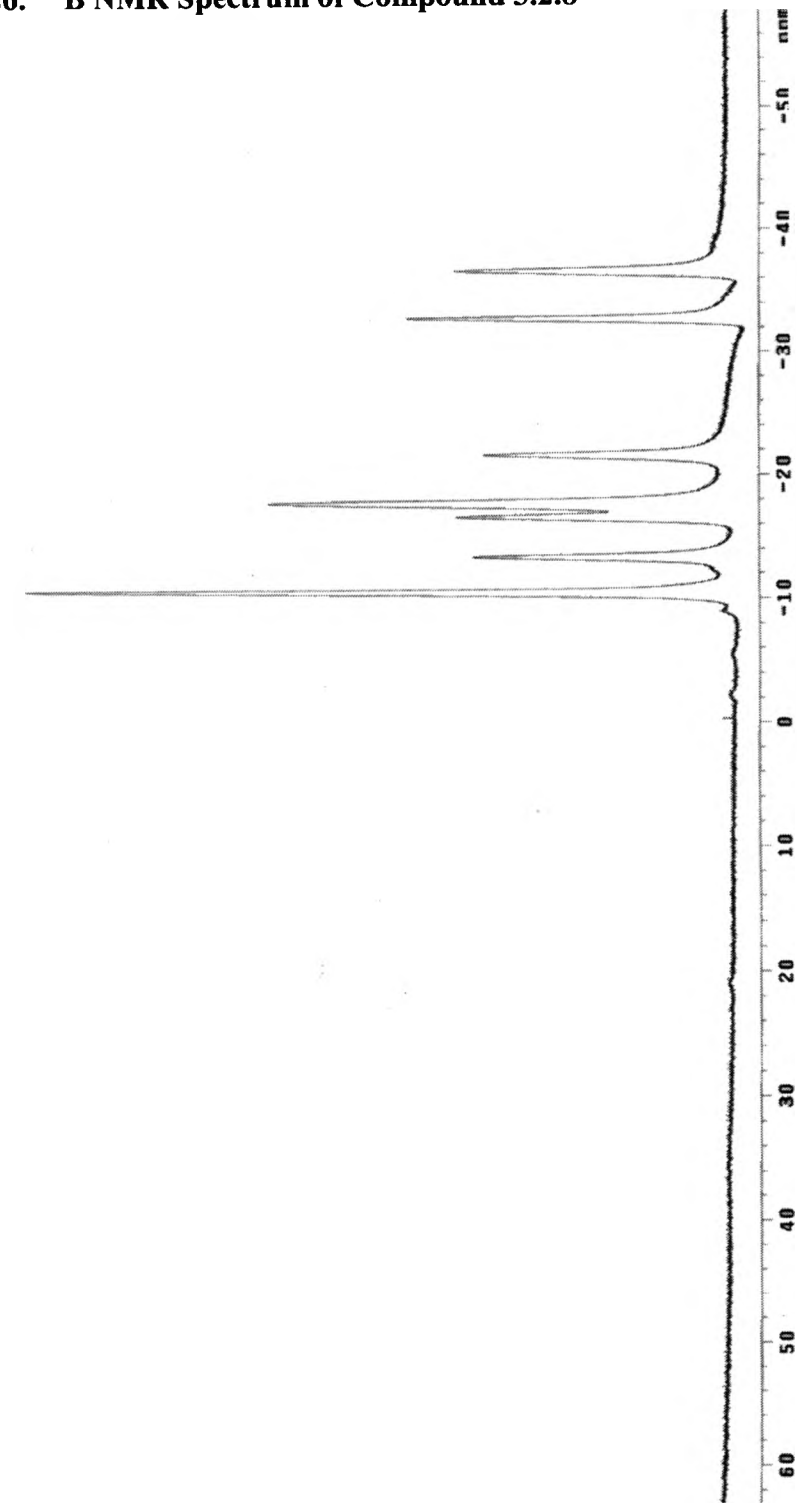
Spectrum 24. ^{1}H NMR Spectrum of Compound 3.2.8



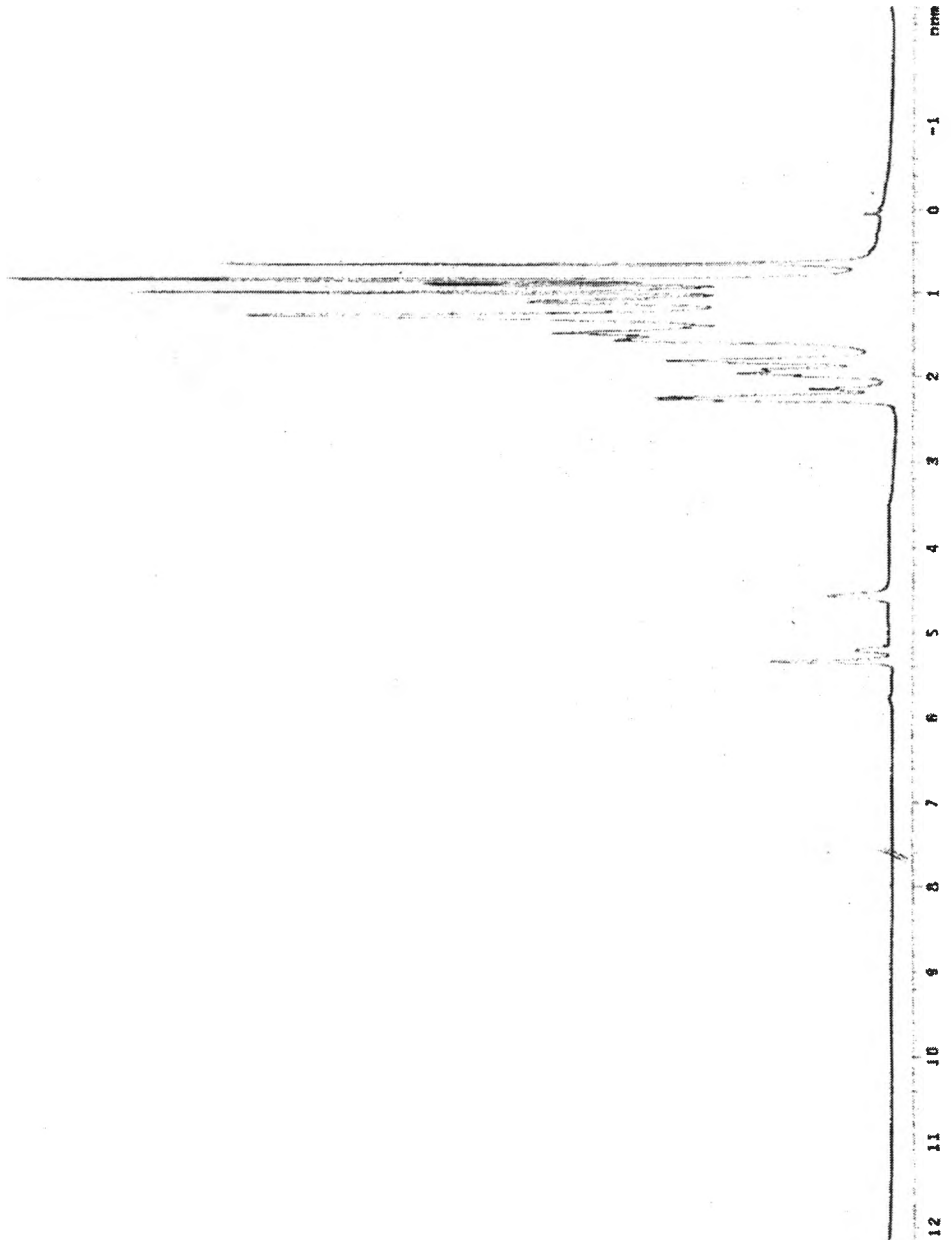
Spectrum 25. ^{13}C NMR Spectrum of Compound 3.2.8



Spectrum 26. ^{11}B NMR Spectrum of Compound 3.2.8

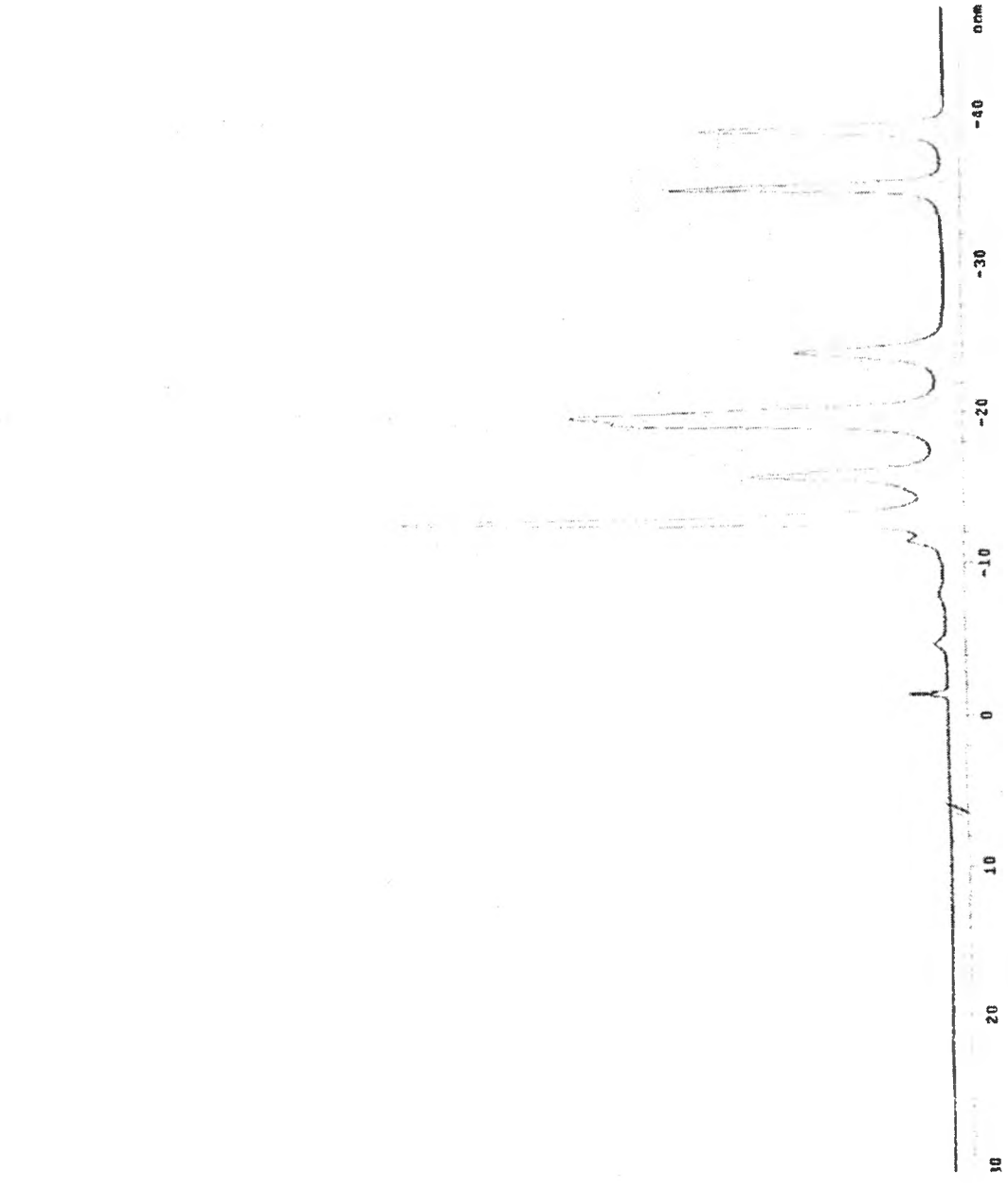


Spectrum 27. ^{1}H NMR Spectrum of Compound 3.2.9

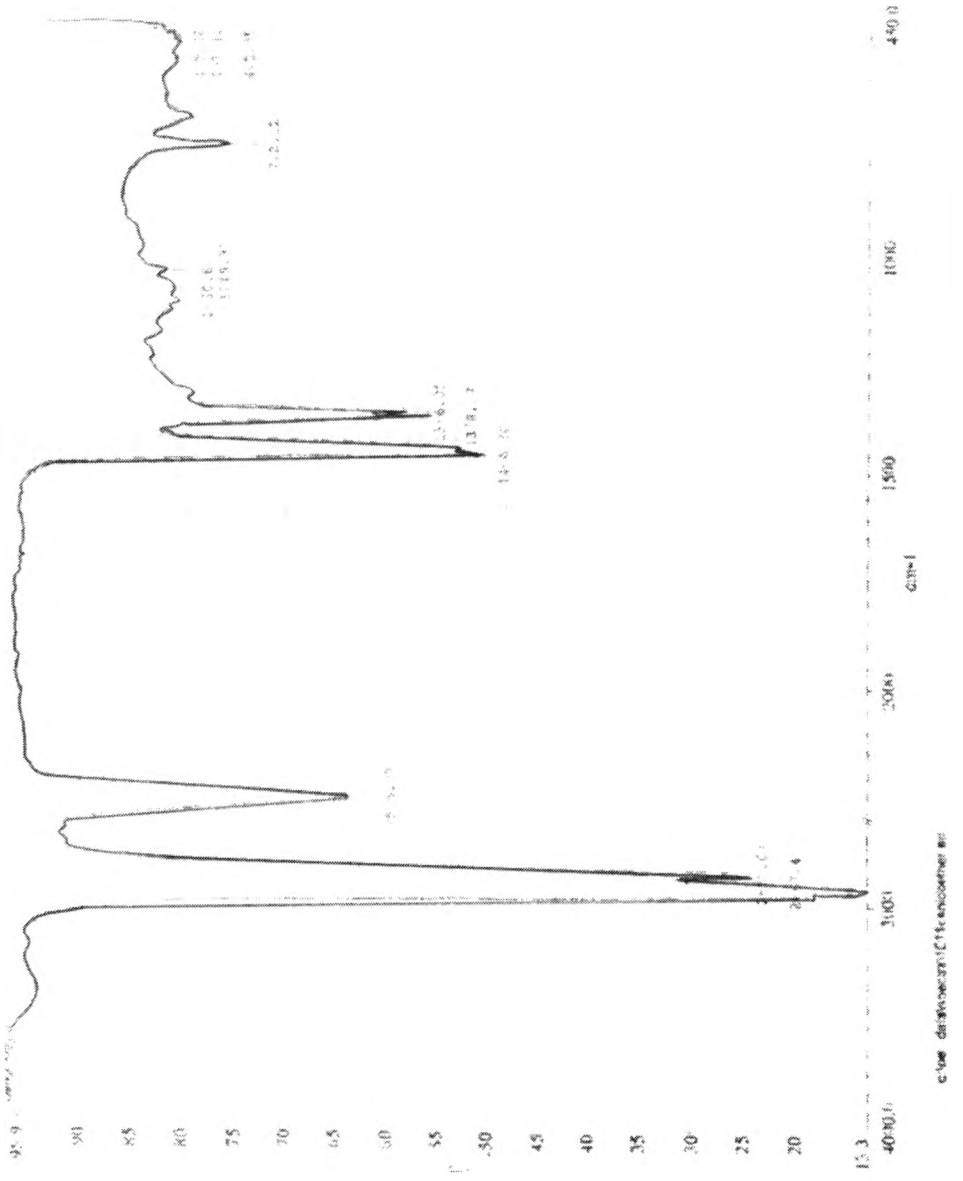


Spectrum 28. ^{13}C NMR Spectrum of Compound 3.2.9

Spectrum 29. ^{11}B NMR Spectrum of Compound 3.2.9



Spectrum 30. FT-IR Spectrum of Compound 3.2.9



VITA

Colby Clay Tate was born in Stephenville, Texas, on November 2, 1975, the son of Rodney Reeves Tate and Patricia Elaine Tate. After completing work at Brownwood High School, Brownwood, Texas, in 1994 he entered Southwest Texas State University in San Marcos, Texas. After graduation in 2000 with a Bachelor of Science in Chemistry, he entered into the Graduate School at Southwest Texas State University. After six semesters he graduated with his Master of Science and is currently attending Texas A&M, College Station, Texas, to obtain his Ph.D. in chemisty.

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This thesis was typed by Colby Clay Tate