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Synthesis of Novel Antimycobacterials and a Fluorescent Sensor for Simple Carbohydrates

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Synthesis of Novel Antimycobacterials and a Fluorescent Sensor for Simple Carbohydrates

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By

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SLeX	Sialyl Lewis X
CRD	Carbohydrate Recognition Domain
PSGL-1	P-Selectin Lectin
IC_{50}	Inhibitory Concentration at 50%
NeuNAc	Sialic Acid
Gal	Galactose
KLH	Keyhole Lymphet Hemocyanin
LPG	Lipophosphoglycan
LAM	Lipoarabinomannan
MTB	Mycobacterium Tuberculosis
PET	Photoinduced Electron Transfer
OFPBA	2-*o*-Formylphenylboronic Acid
HIV	Human Immunodeficiency Virus
IFN	Interferon
TNF	Tumor Necrosis Factor
IL	Interlukin
RNA	Ribodeoxynucleic Acid
DNA	Deoxyribonucleic Acid
PhCHO	Benzaldehyde
TLC	Thin Layer Chromatography
TFA	Trifluoroacetic Acid
DIBAH	Diisobutyl Aluminum Hydride

TfOH	Triflic Acid
DEAD	Diethylazodicarboxylate
DMF	Dimethylformamide
DCMME	Dichloromethyl Methoxy Ether
DMAP	4-(*N*,*N*)-Dimethylaminopyridine
TACO	Tryptophane Aspartate Containing Coat Protein
AG	Arabinogalactan
^{1}H NMR	Hydrogen Nuclear Magnetic Resonance
^{13}C NMR	Carbon Nuclear Magnetic Resonance
LAH	Lithium Aluminum Hydride
THF	Tetrahydrofuran
MHz	Megahertz
TMS	Trimethylsilane
FT-IR	Fourier-Transform Infrared Spectroscopy
UV	Ultraviolet Spectroscopy
ESI MS	Electrospray Ionization Mass Spectrometry
s	singlet
d	doublet
t	triplet
q	quartet
R_f	Retention Factor

Abstract

SYNTHESIS OF NOVEL ANTIMYCOBACTERIALS AND A FLUORESCENT SENSOR FOR SIMPLE CARBOHYDRATES

By Brian T. Walker

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University

Virginia Commonwealth University, 2006.

Director: Todd A. Houston, Assistant Professor of Chemistry.

Cell surface carbohydrates play an important role in a wide variety of biological processes such as inflammation, tumor metastasis, and viral and bacterial infection. The goal of our research has been two-fold. The first objective was the synthesis of antimycobacterial compounds. A mannose containing tetrasaccharide from the mannan core of lipoarabinomannan (LAM) of *Mycobacterium tuberculosis* has been synthesized using α-D-methylmannopyranoside as starting material and Koeings-Knorr reactions to couple saccharides. The synthesis was completed in nine steps and in 14% total yield. This compound should be useful in competitive inhibition studies with macrophages or as an immunological marker. We have successfully synthesized nonsulfated mimics of the aminosterol antibiotic from 5α-cholestan-3-one in two steps in 40-70% total yield. The critical step in this synthesis is the addition of the boronic acid functional group using 2-*o*-formylphenylboronic acid. It is hypothesized that the addition of boronic acids will

improve the antibacterial and anti-angiogenic activity of these compounds. The second objective was the synthesis of a simple fluorescent receptor for simple carbohydrates. A receptor using anthracene as the fluorophore has been completed demonstrating an improved yield over previous methods. This receptor is the first to show selectivity for *myo*-inositol over other saccharides.

Chapter 1

Synthesis of a Tetrasaccharide Fragment from the Mannan Core of Lipoarabinomannan of *Mycobacterium tuberculosis.*

Introduction

Mycobacterium tuberculosis (MTB), the cause of tuberculosis, is responsible for approximately three million deaths per year worldwide. It is estimated that one-third of the world's population is infected with MTB.[i] MTB was under control in the United States for most of the 20th century with a mortality rate of <5/100,000. However, from 1985 to 1992, the number of cases increased by an average of 20%; this occurred primarily in urban areas where drug abuse and HIV infection are most common. The problem is more rampant in developing countries where MTB is the leading cause of death. In 1993, the World Health Organization (WHO) declared MTB a global emergency. The highest incidence of tuberculosis infections is in sub-Saharan Africa and south Asian nations. The WHO estimates that two million people die annually from tuberculosis and that two billion people are infected with MTB.[2] The onset of active tuberculosis, among those infected, is one in ten. Global tuberculosis incidence is growing at 1% per year and is estimated to kill 35 million over the next twenty years.

Mycobacterium tuberculosis (MTB) is a slow-growing aerobic bacterium that divides every 16 to 20 hours.[3] This is extremely slow compared to other bacteria, which tend to have division times measured in minutes. Among the fastest growing bacteria is a strain of *E. coli* that can double every 20 minutes. In contrast, *Mycobacterium leprae* divides every 20 days. MTB is not classified as either Gram-positive or Gram-negative

because it does not have the chemical characteristics of either, although it contains peptidoglycan in the cell wall, which means it is related to the Gram-positive organisms. If a Gram stain is performed, it stains very weakly Gram-positive or not at all (ghost cells). MTB is a small rod-like bacillus which can withstand weak disinfectants and can survive in a dry state for weeks and only grows within a host organism. Initially, *in vitro* culture of *M. tuberculosis* took a long time to be achieved, but is now a routine laboratory procedure. MTB is identified microscopically by its staining characteristics: it retains certain stains after being treated with acidic solution, and so it is classified as an "acid-fast bacillus" or "AFB". In the most common staining technique, the Ziehl-Neelsen stain, AFB are stained a bright red which stands out clearly against a blue background. Acid-fast bacilli can also be visualized by fluorescent microscopy, and by an auramine-rhodamine stain.[3]

The *M. tuberculosis* complex includes three other mycobacteria which can cause tuberculosis: *M. bovis, M. africanum,* and *M. microti*. The first two are very rare as a cause of disease and the latter does not cause human disease. Nontuberculous mycobacteria (NTM) are other mycobacteria which may result in pulmonary disease resembling TB, lymphadenitis, skin disease, or disseminated disease.[3]

Studies indicate that the cell surface carbohydrate lipoarabinomannan (LAM) (**1**) (Figure 1) plays a central role in infection and mycobacterial survival within the host organism. LAM was first isolated in the 1960's and was described as a mannophospho-

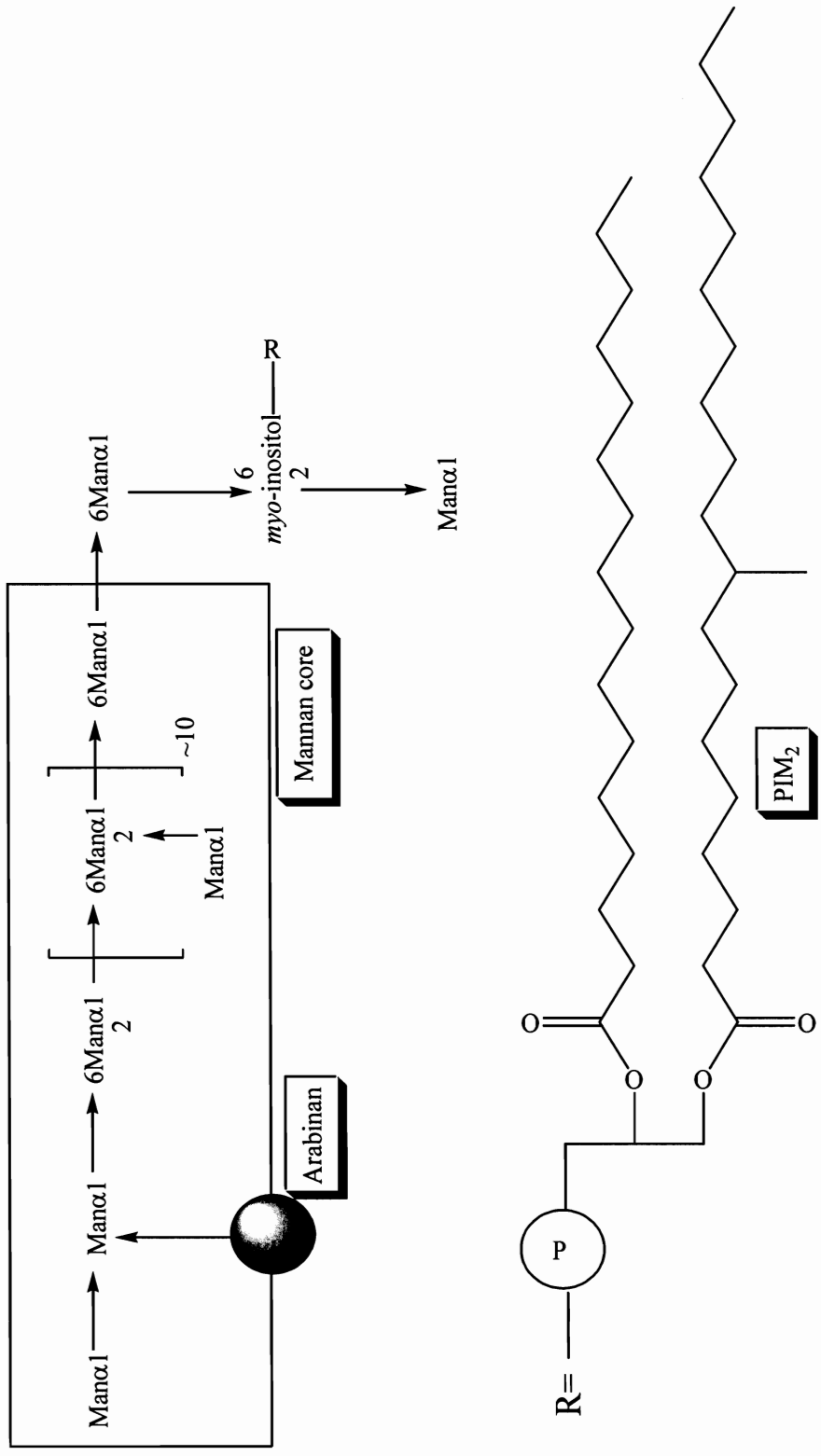

Figure 1. Structure of Lipoarabinomannan (LAM) (**1**)[4].

inositide.[4] LAM is an oligosaccharide that consists of a phosphatidylinositol unit at the non-reducing end, which anchors it to the cell membrane of the pathogen. The phosphatidylinositol is linked to the mannan core, which is a decamer of repeating Man2-Manα1 units. The mannan core is linked to oligosaccharides known as the arabinan, which consists of four to six arabinofuranosyl residues, and then terminated with an identical Man2-Manα1 linkage present in the mannan core which can have some variation.[5] A more recent discovery has been that LAMs from a number of *M. tuberculosis* strains contain a 5-deoxy-5-methylthio-pentose residue. To date, this substituent has been identified in both laboratory strains (H37Rv and H37Ra), as well as clinical isolates (CSU20 and MT1032) of *M. tuberculosis*.[6] Lowary has determined that the 5-deoxy-5-methylthio-pentose residue is typically linked in a to the 4-position of a mannopyranose residue within the arabinan.[7]

LAM is responsible for mediating binding to the host organism via phagocytosis by mannose receptors. Virulent strains (H37Rv) are phagocytized by mannose receptors, while non-virulent strains are not.[8] MTB infection is initiated by ingesting, inhaling, or being inoculated with virulent bacteria. The mouth and upper respiratory tract are resistant to infection. However, the alveolar surface of the lungs is the tissue most susceptible to infection. Alveolar macrophages may kill the bacteria or the macrophage may be deactivated. In the latter case, the cycle of infection and replication of the mycobacteia begins with multiplication of the mycobacteria within the macrophage, which causes the cell to rupture, releasing mycobacteria which are phagocytized by other macrophages.[9] Successive occurrences of multiplication and lysis of infected

macrophages lead to the development of lesions over the course of a few weeks from the initial infection.[10]

Macrophages play dual roles during tuberculosis (TB) infection.[11] In one role, they serve as the preferred host for MTB, the intracellular pathogen that causes TB. Despite this, they also help to alert the immune system to the presence of MTB, and, if activated, can eliminate it directly. Activation depends on the presentation of antigenic peptide–MHC class II (pMHC) complexes on the macrophage surface that can bind T cell receptors (TCRs) on cognate $CD4^+$ T helper cells. pMHC-TCR binding induces $CD4^+$ T helper cells to secrete IFN-γ, which stimulates macrophages to produce molecules capable of killing MTB such as nitric oxide.[12] This process constitutes an important arm of cell-mediated immunity and may determine the infection outcome.[13]

It is well established that MTB inhibits antigen presentation in macrophages.[14] Initial studies showed that fewer macrophages infected with mycobacteria express detectable levels of antigen on their surface compared with uninfected macrophages.[15] It was later confirmed that infected macrophages are unable to signal $CD4^+$ T helper cells by measuring T cell response. The magnitude of T cell response is proportional to pMHC levels.[16] Further studies provided more evidence that an inverse relationship exists between MTB infectious dose and T cell response.[17]

LAM inhibits or induces the production of various cytokines, which are soluble proteins that modulate the activities of cells and tissues.[18] LAM is a major virulence factor and is considered a modulin because of its capacity to manipulate the host immune system. LAM has been implicated in triggering multiple signaling pathways that modulate the apoptosis and IL-12 production of macrophages and dendritic cells. LAM

isolated from *M. tuberculosis* does not induce IL-12 and apoptosis and may actually inhibit the induction of these two pathways via agonists such as lipopolysaccharide (LPS) or bacterial infection.[19] LAM blocks macrophage activation by interferon (IFN)-γ and TNFα, allowing the bacterium to survive within the macrophage. LAM and MTB induce transcription of mRNA for several cytokines, including TNF, Interlukin(IL)-1α, 1β, IL-6, IL-8, and IL-10. It is believed that these cytokines mediate clinical manifestations of MTB such as fever, weight loss, and tissue necrosis.[20,21]

The development of a novel line of antimycobacterial drugs has become urgently needed as a consequence of developing drug resistance, opportunistic infections due to HIV, and difficulty in monitoring arduous drug regimens in third world areas most affected by MTB. Bacillus of Calmette and Guérin (BCG) is a vaccine against tuberculosis that is prepared from a strain of the attenuated (weakened) live bovine tuberculosis bacillus, *Mycobacterium bovis* that has lost its virulence in humans by specially culturing in artificial medium for years. The bacilli have retained enough strong antigenicity to become an effective vaccine for the prevention of human tuberculosis. The vaccine was developed for human use in 1921. It has an efficacy of between 50 and 80 percent and is not currently recommended in developed countries as routine childhood vaccination because the incidence of tuberculosis is much lower in these areas.[22]

Isoniazid (**3**, Figure 2), the most widely used chemotherapeutic agent, is a prodrug which is processed by mycobacterial catalase-peroxidase, releasing the active form of the drug. The active form inhibits enoyl-ACP reductase, which is necessary for the synthesis of the bacterial cell wall. Pyrazinamide (**4,** Figure 2) acts through involvement with pyridine nucleotide biosynthesis. The well-known antibiotic streptomycin acts by

inhibiting the translation of mRNA.[23] Ethambutol (**2,** Figure 2) prevents arabinan biosynthesis by its actions as an arabinosyltransferase inhibitor.[24] Through mass spectrometry data, it has been determined that ethambutol inhibits arabinan biosynthesis, but does not interfere with the biosynthesis of the mannan core, which contains the Manα1-Man2 motif recognized for phagocytosis by the human macrophage mannose receptor.[25] Kanamycin is an aminoglycoside antibiotic which works by affecting translocation, which causes the mRNA to be misread by the ribosome, resulting in cell death.[21]

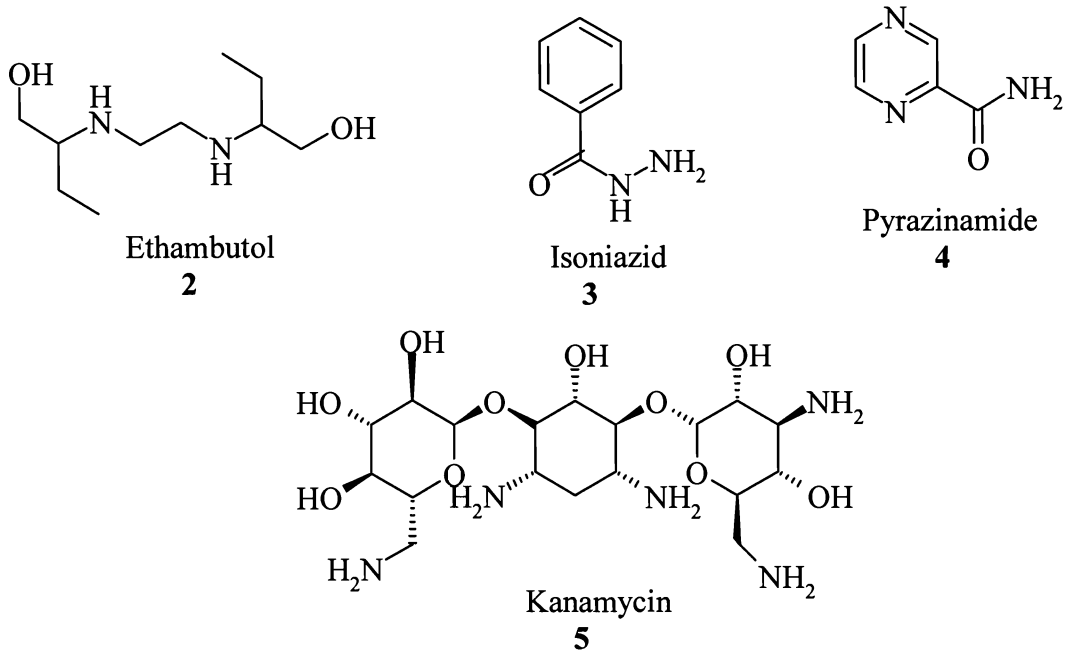

Figure 2. Structures of currently used antimycobacterial drugs.

History

Mycobacterium tuberculosis first appeared in humans 8000 years ago and has been present in every great civilization from that time to the present.[263] It has been hypothesized that MTB shares a common ancestor with *Mycobacterium bovis* due to their shared DNA homology. The immunological properties of MTB suggest that the two are subspecies and MTB evolved through genetic mutations from *M. bovis* in humans after the domestication of cattle.[27] In 1882, Robert Koch was the first to isolate the tubercle bacillus. He demonstrated that the isolated bacillus induced the disease in inoculated animals and that the organism could be recovered from those tissues and the process repeated in another animal. He believed, incorrectly, that MTB and *M. bovis* were the same bacterium. Theobold Smith demonstrated that the two were different. The mode of transmission by droplet nuclei was first elucidated in the 1930s by Wells and further explained by Richard Riley.[28]

Anti-mycobacterial Drugs and Drug Resistance

The era of MTB chemotherapy began in 1944 with the use of streptomycin. Drug resistance appeared quickly, but the development of isoniazid and p-aminosalicylic acid (PAS) led to effective drug cocktail therapies.[29] MTB does not acquire drug resistance through the exchange of plasmids, but from random mutations in DNA at normal replication rates in the absence of antibiotic exposure. Cavities in the lesions caused by MTB contain 10^7 to 10^9 bacteria, thus mutants typically develop within a lesion (Table 1).[30] The result of treating a patient with a single drug is the suppression of susceptible

bacteria and the growth of a drug-resistant strain. Chemotherapy involves combinations of the aforementioned drugs to lower the possibility of developing resistant strains. However, in third world countries where the disease is most prevalent, it is difficult to monitor patients to ensure that they take the complete therapeutic regimen. Many patients either stop taking the medication when they feel better, but before the bacteria have been completely eliminated or are prescribed a single drug and resistance to these drugs in the host occurs.

Table 1. Spontaneous mutation rates in mycobacteria.[31]

Antimycobacterial Agent	**Mutation Rate**
Isoniazid	10^{-8}-10^{-9}
Streptomycin	10^{-8}-10^{-9}
P-Aminosalicylic Acid	10^{-8}-10^{-9}
Ethambutol	10^{-7}
Rifampin	10^{-10}

Research Aim

The aim for this research was to synthesize a mannose-containing tetra-saccharide from the mannan core of lipoarabinomannan (LAM) of *Mycobacterium tuberculosis*, starting from methyl-α-D-mannopyranoside (**6**) using the Koenigs-Knorr glycosylation strategy.[32] Disaccharide **7** would be formed in three steps, providing the key intermediate which would be manipulated to form a separate glycosyl donor, **9**, and glycosyl acceptor, **8**. Koenigs-Knorr coupling followed by deprotection would yield the desired tetrasaccharide, **10**.

Previous Work

To date, LAM is commercially available only in limited supplies of microgram quantities. Other carbohydrate moieties within LAM have been synthesized. Lowary has successfully synthesized one known arabinan from LAM[33], while Kim has synthesized another.[34] Lowary has also synthesized a Manα6-Manα1 disaccharide substituted with an amine and a fluoride at the terminal 2-hydroxyl position. This work has shown the enzymes sinvolved in LAM biosynthesis will tolerate substituents that are of equal or lesser size than a hydroxyl substituent.[35] Fraser-Reid synthesized the dimannoinositol bridgehead of LAM.[36] There have been no reports on the synthesis of any fragment of the mannan core of LAM. Medicines based on carbohydrate structures are typically poor drug candidates because they are orally inactive and susceptible to hydrolysis in the stomach, and so require intravenous delivery. These compounds are sensitive to

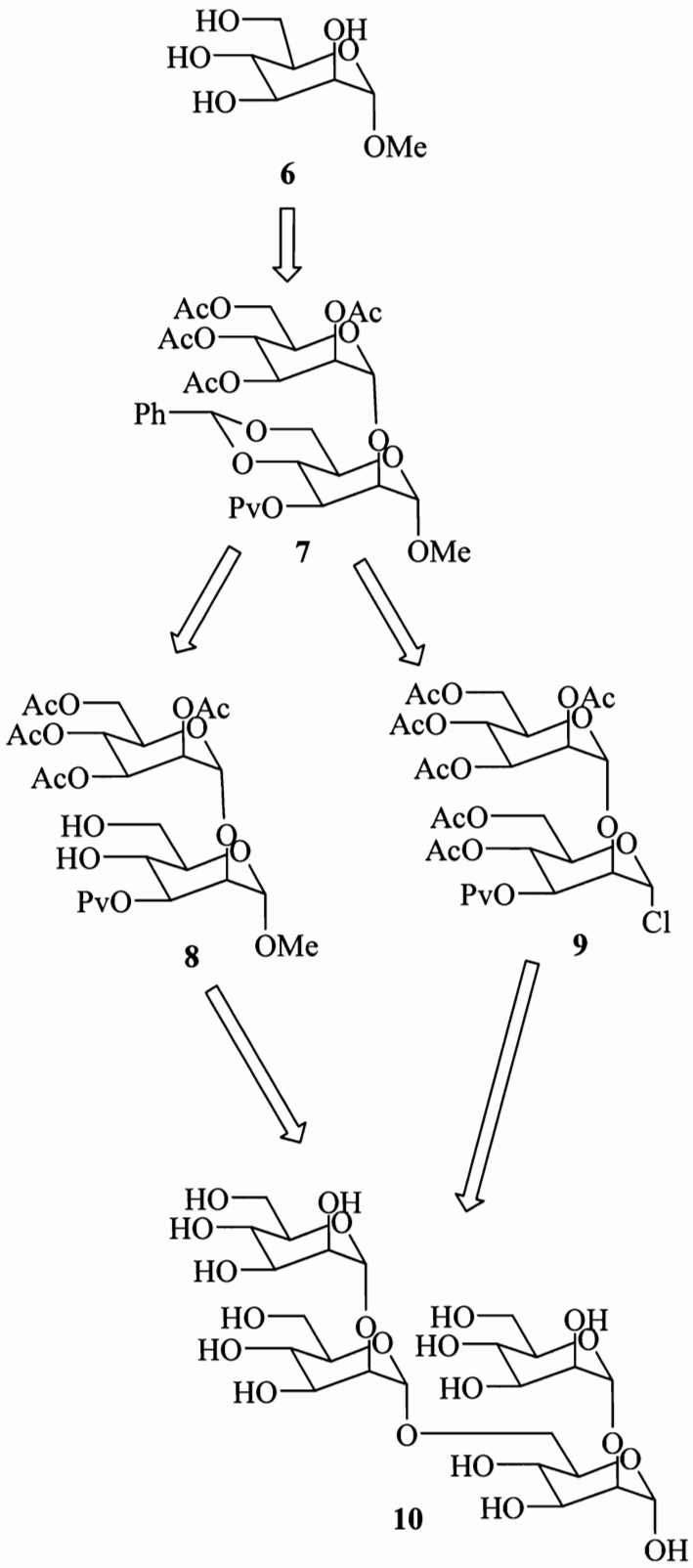

Figure 3. Strategy for tetrasaccharide sythesis from a common dissacharide intermediate.

glycosidases *in vivo* and have low affinity for protein receptors. In addition, carbohydrates are too small to provoke an immune response on their own.[37] A potential utility for carbohydrates as drugs is in the form of glycoconjugates, which are carbohydrate moieties attached to carrier proteins. While carbohydrates are unable to provoke an immune response on their own, proteins are large enough to elicit a desired immune response. When attached to a carrier protein, a carbohydrate is established as a fragment of the foreign agent and can provoke an immune response when subsequently introduced into the host. Danishefsky's work on tumor antigens is a current example of this strategy at work. Danishefsky has synthesized a methyl glycoside of GM1[38] and Globo-H[39],which are known tumor antigens, for the purpose of conjugating them to keyhole limphet hemocyanin (KLH), a common carrier protein for potential immunotherapeutics. These conjugated tumor antigens are administered in concert with an additional immunoadjuvant for the purpose of obtaining a consistent, sustained immune response to specific tumors. His group has also synthesized a glycopeptide containing multiple tumor antigens with the intent of provoking an immune response to multiple tumor antigens with one glycoconjugate.[40] A mannan core fragment may serve as a precursor of a possible MTB drug or antigen for a vaccine using the same glycoconjugate strategy. An additional use of mannan core oligosaccharides may be to determine the binding affinity for mannose receptors found on the alveolar surface of the lung. Another direction could be the use of LAM fragments to study the carbohydrate-binding of drugs and their potential use against MTB.

Results and Discussion

Synthesis by Benzylidene Opening

An initial strategy used for the oligosaccharide synthesis (**10**) is illustrated in Figure 3 which involved opening of benzylidene rings. The initial mannose species with substituents at the 1,2, and 6 positions, used required multiple protecting groups. Methyl-α-D-mannopyranoside (**6**) is a readily available and inexpensive starting material. In addition, the methoxy group at the anomeric carbon not only serves as a protecting group, but locks the saccharide into the desired alpha-pyranose conformation. The original strategy was to use $ZnCl_2$ and benzaldehyde to protect the remaining hydroxyl groups on the sugar (Figure 4), rather than attempt several different protection reactions. The cis conformation of C2 and C3 allowed for the synthesis of a dibenzylidene compound.[41] Selective opening of the benzylidene protecting groups would yield 3,4-di-*O*-benzylmethyl-α-D-mannopyranoside (**8**)[42]; then, the 6-hydroxyl could be selectively protected with TBSCl to give **9**.[43] This 1,3,4,6-*O*-protected compound would serve as a glycosyl acceptor in a coupling to create a disaccharide. A suitable disaccharide would be deprotected either at the 1 or 6-hydroxyl to serve as synthons for further oligosaccharide scaffolding.

The synthesis began with the reaction of methyl-α-D-mannopyranoside (**6**) to $ZnCl_2$ and PhCHO to yield 4:6, 2:3-di-*O*-benzylidene-α-D-methylmannopyranoside (**11**). This procedure was performed multiple times with greater than a 90% yield on every occasion.[38] The next procedure proved to be far more difficult. The theory behind the benzylidene opening reactions is that a Lewis acid would coordinate to one of the

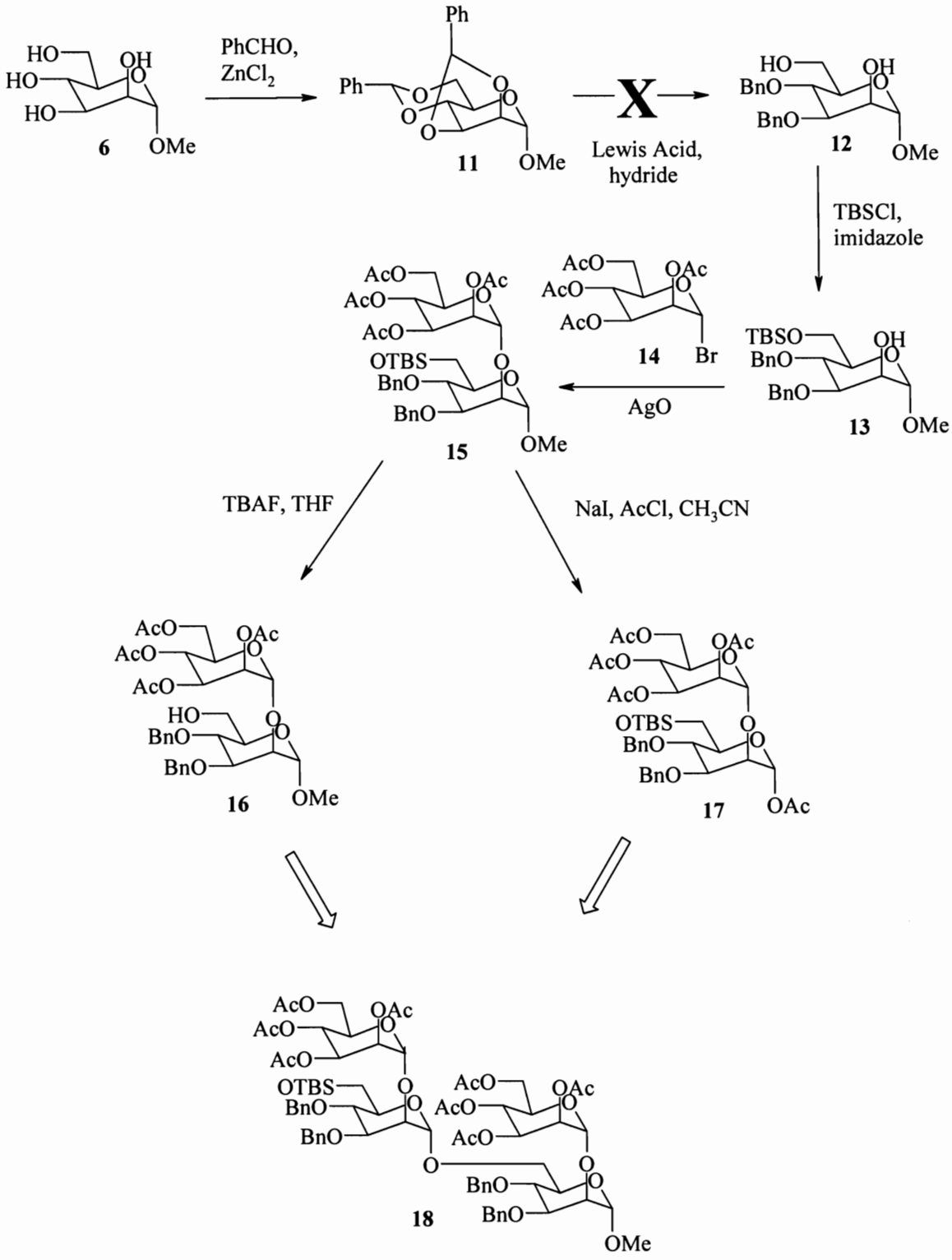

Figure 4. Initial strategy for LAM oligosaccharide synthesis.

oxygens in the 1,3-dioxolane system (Figure 5). A hydride ion could then attack the benzylic carbon, resulting in the donation of the electrons in the C-O bond from the dioxolane ring to the electron deficient oxygen. Another possibility is that the bond scission between the benzylic carbon and the coordinated oxygen could occur before the actual attack of the hydride. A Lewis acid (i.e., $AlCl_3$) would be required to generate a 4-*O*-benzyl compound from a benzylidene-protected sugar because the steric bulk of the acid would be too large to coordinate to the 4-position, requiring it to coordinate to the 6-position. The resulting reductive cleavage would then produce the 4-*O*-benzyl compound (**12**).[41]

Numerous procedures for benzylidene openings were attempted without success (Table 2). The first procedure attempted was aluminum trichloride as the acid catalyst and Me_3NBH_3 as the hydride donor in toluene.[44] Several attempts of this reaction under conditions of increasing time and temperature yielded no product. Reaction progress was followed by TLC and ^1H NMR through the disappearance of the benzylidene proton peak at 5.5 ppm and the appearance of a peak for two benzylic protons at 4.5 ppm. Only the presence of unreacted starting material **11** (Figure 4) was observed.

Numerous other hydride reagents were used in the attempt to complete this conversion (Table 2). None of them worked despite increases in reagent strength, time, and temperatures of the reaction conditions. Use of Lewis acid was necessary to complex to the less sterically hindered 6-hydroxyl. $AlCl_3$ was the most common large Lewis acid used in the literature to provide the 4-*O*-benzyl compound.[44,45,46] Use of a proton acid, such as HCl or TFA would result in the 6-O benzyl compound, which was not desired.

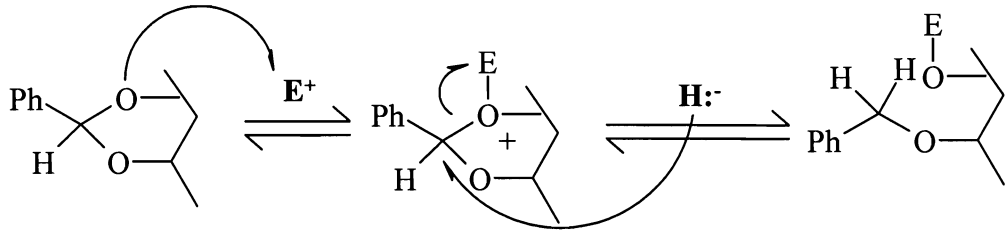

Figure 5. Mechanism for benzylidene ring opening reaction.[42]

Table 2. Summary of conditions for attempted benzylidene-opening reactions (Figure 4).

Acid	**Hydride Source**	**Solvent**
$AlCl_3$	Me_3NBH_3	Toluene[43]
$AlCl_3$	Me_3NBH_3	Et_2O/CH_2Cl_2[44]
$AlCl_3$	BH_3:THF	THF
$AlCl_3$	$NaCNBH_3$	CH_2Cl_2
$AlCl_3$	$LiAlH_4$	Et_2O/CH_2Cl_2[45]
TfOH	BH_3:THF	THF
None	DIBAH	CH_2Cl_2

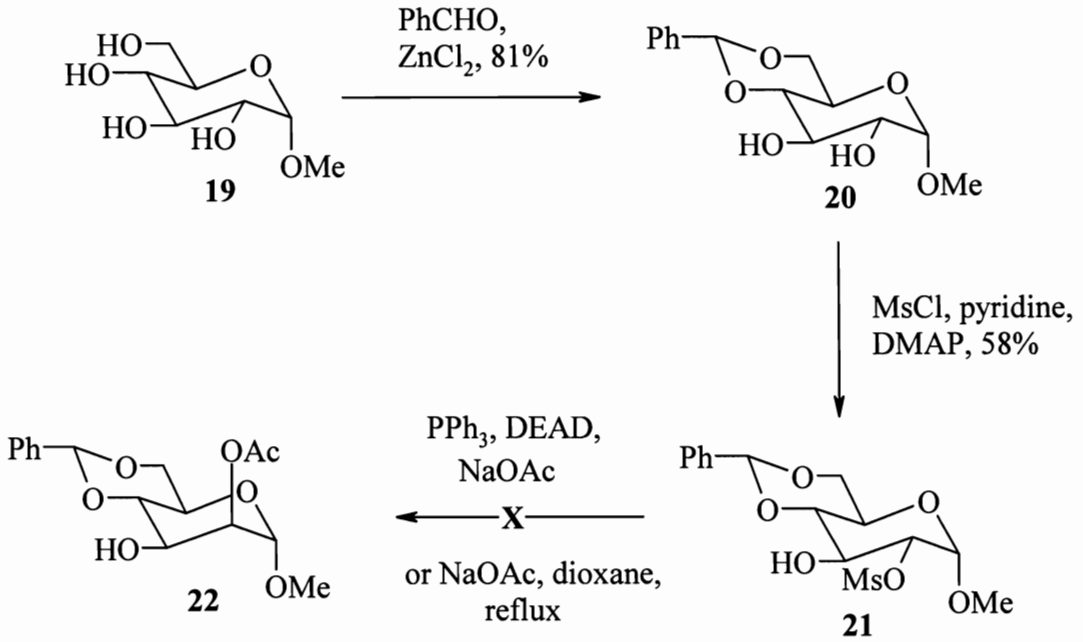

Figure 6. Attempt at oligosaccharide synthesis using stereochemical inversion

The inability to open the benzylidene groups without cleaving them required a search for alternative methods of synthesis. Glucose differs from mannose only by inversion at C2 and contains cis-diols only in the alpha configuration. A strategy was devised to protect a glucopyranoside and invert the C2 stereochemistry to yield a protected mannose (Figure 6). The reaction of methyl-α-D-glucopyranoside (**19**) with zinc chloride and benzaldehyde, as previously stated,[38] provided 4,6-*O*-benzylidene-α-D-glucopryanoside (**20**) in 81% yield (Figure 6). The conversion to 2-*O*-mesylate (**21**) was achieved with one equivalent of mesyl chloride in pyridine in 58% yield after purification by column chromatography using a 1:1 mixture of hexanes and ethyl acetate as eluent.

A Mitsunobu reaction was attempted using PPh_3, diethylazodicarboxylate (DEAD), and sodium acetate to provide **22**.[47] The Mitsunobu reaction (Figure 7) is used to convert an alcohol into a variety of functional groups, such as esters using triphenylphosphine and DEAD with an inversion of stereochemistry, which in this case would provide a protected mannose compound from the glucose precursor.[48] Temperatures were varied from room temperature to refluxing in THF, but a successful conversion was not achieved. While acetic acid would have deprotected the benzylidene moiety, sodium acetate did not possess an acidic proton for scavenging, which was the likely reason the stereochemical inversion strategy was unsuccessful. An alternative set of conditions using sodium acetate in refluxing dioxane was attempted, however, all of these reactions failed to provide the desired mannose derivative. After being unsuccessful with benzylidene opening strategies and stereocenter inversion using glucose, a decision was made to synthesize a glycosyl acceptor with only one benzylidene group protecting the 4 and 6 positions.

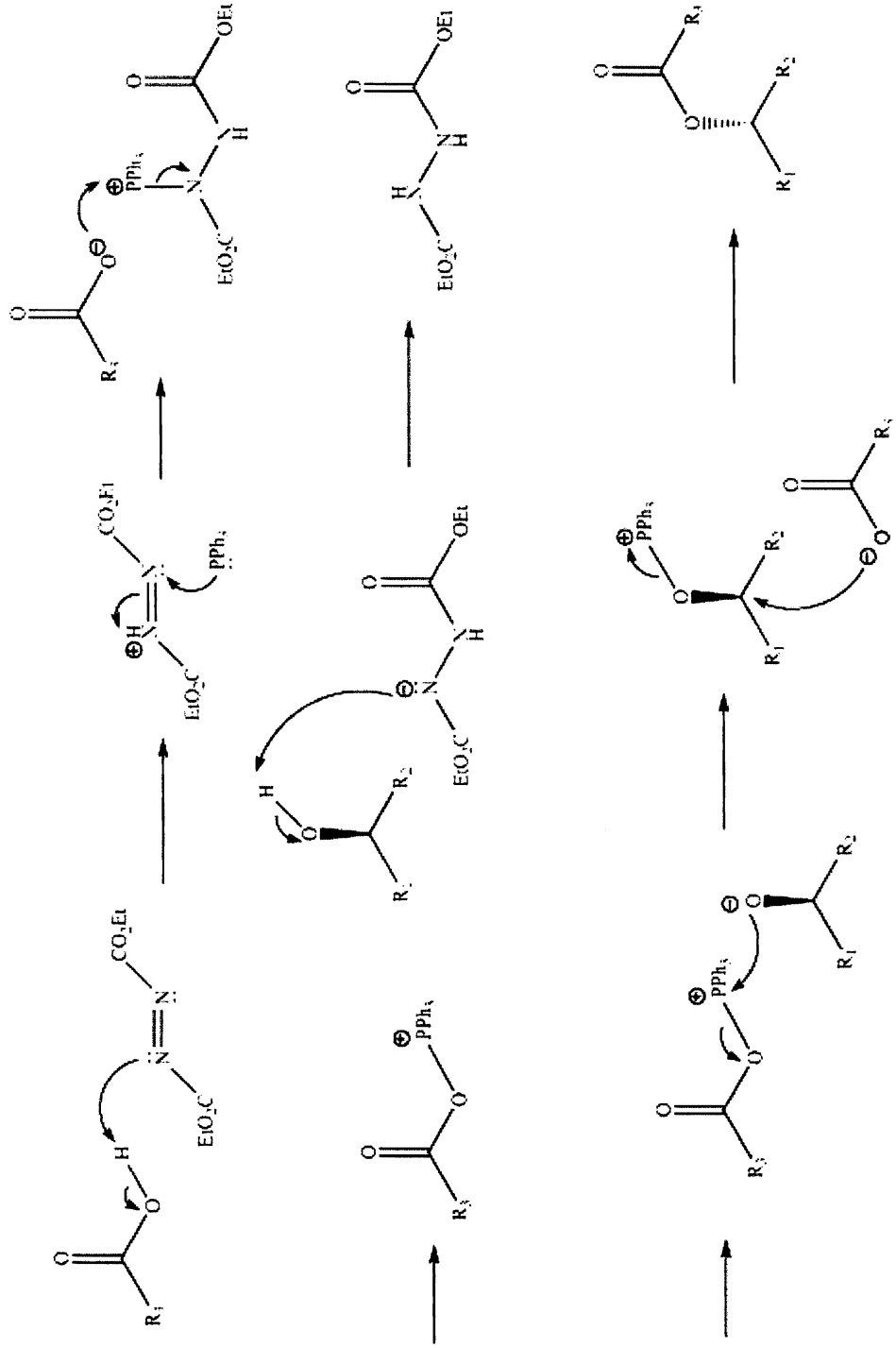

Figure 7. Mechanism of the Mitsunobu reaction.[43]

Using a 4,6-O-protected mannose derivative, the 3-hydroxyl could be selectively protected at this time, leaving only the 2-OH position free for glycosylation (Figure 9). Following the synthesis of compound **27**, the strategy planned is depicted in Figure 3, which involves the conversion of a single disaccharide to yield a glycosyl donor and acceptor. Methyl-α-D-mannopyranoside was reacted with formic acid and benzaldehyde to provide 4,6-O-benzylidene-*O*-methyl-α-D-mannopyranoside, **25**, in 50% yield. This was double the yield of Winnik's original procedure.[49] Another procedure using 2-methoxypropene, tosic acid, and DMF was attempted to obtain compound **25** in higher yield, but provided only **11**.[50] Two different procedures were attempted to obtain a compound protected at O-3 (**26**). In the first reaction, two to three equivalents of trimethylacetyl chloride in pyridine was used.[51] The procedure was successful in 60% yield after column chromatography. Addition of the protecting group was verified in the proton NMR by the observed doublet of doublets integrating to one proton at 5.3 ppm for H3 on the carbohydrate ring, along with the appearance of a nine hydrogen singlet at 1.2 ppm for the trimethyl acetyl group. Unfortunately, only freshly distilled pyridine achieved results and sufficiently anhydrous conditions were difficult to maintain. As a result, an alternative method was attempted.

An alternative method for the selective protection of the equatorial hydroxyl groups in carbohydrates is the use of stannylenes.[52] Stannylenes are formed by reacting dibutyltin oxide (DBTO) with a diol (Figure 8). Treatment with a benzyl or acetyl halide provides a monoprotected diol. In the case of pyranose rings containing 1,2-cis-diols, regioselectivity for an equatorial oxygen is obtained.[53]

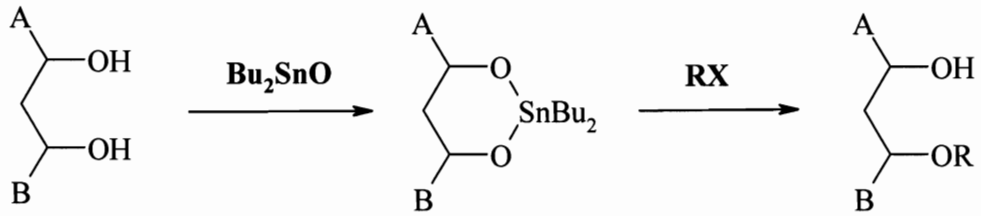

Figure 8. Diagram of stannylene mediated protection.

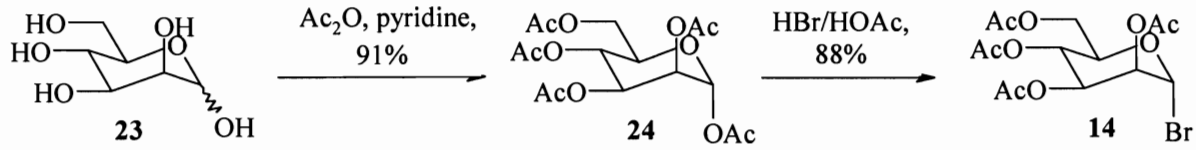

Figure 9. Synthesis of glycosyl bromide

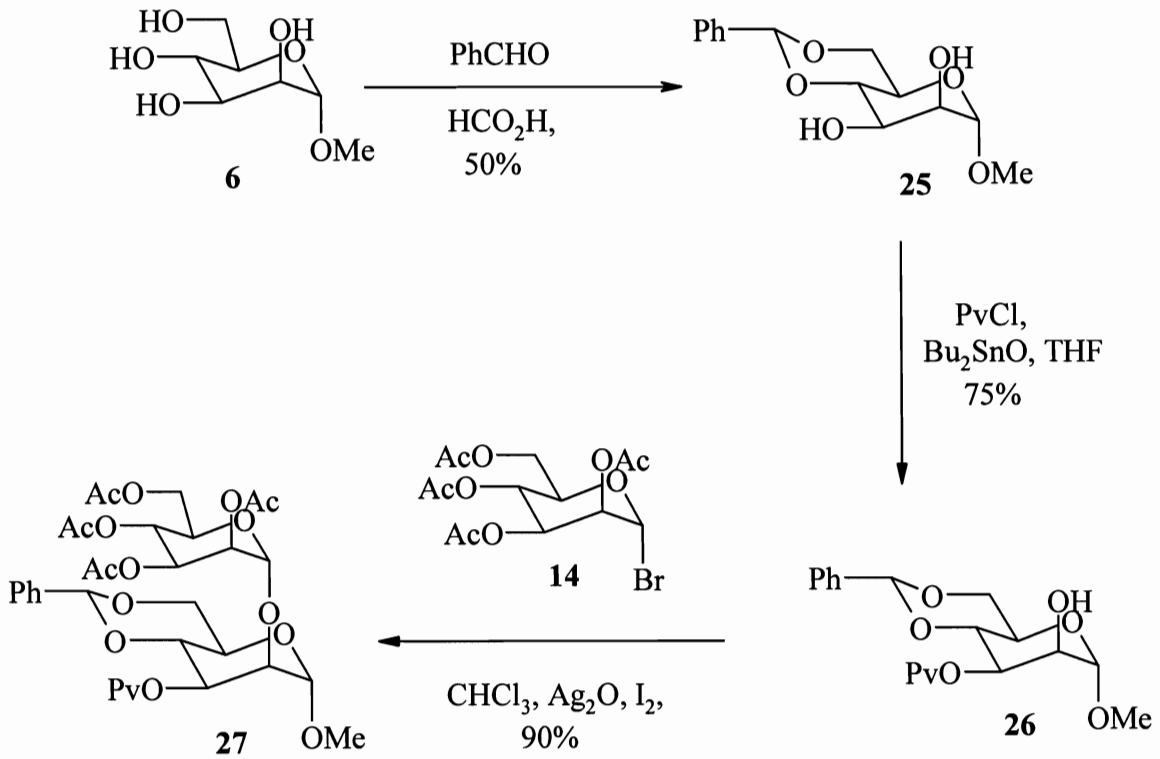

Figure 10. Synthesis of mannose disaccharide precursor.

Reacting **25** with dibutyltin oxide in refluxing MeOH for 4 hours provided the intermediate stannylene species (Figure 8). This intermediate was treated with pivaoyl chloride and heated at reflux in THF overnight to yield **26** in 75% yield.[54] The glycosyl donor, 2,3,4,6-*O*-acetyl-α-D-mannopyranosyl bromide (**14**), was synthesized in two steps from D-mannose (Figure 9).[55] D-mannose was reacted with acetic anhydride and pyridine to provide the α-pentaacetate **24** in 91% yield. The pentaacetate was reacted with 30% HBr in acetic acid to provide the glycosyl bromide **14** in 88% yield. Conversion to the bromide was determined by ^{1}H NMR (Appendix 9) and verified by the downfield shift of the H1 of the non-reducing sugar from 5.97 to 6.29 ppm. An additional sign of conversion to the glycosyl bromide was the appearance of a doublet of doublets for H2 at 5.71 ppm.

Koenigs-Knorr methodology was chosen to couple **26** and **14**, to provide dissacharide **27**.[56] The Koenigs-Knorr reaction (Figure 11) is one of the oldest methods for the preparation of 1,2-*trans*-glycosides involving per-*O*-acetylated glycopyranosyl halides as donors and silver salts as promoters.[32] In relation to the anomeric stereochemistry of the glycosylation reaction, three significant methods have been used; a) the neighboring group assisted method for the construction of 1,2-trans-glycosides such as β-gluco or α-manno type glycoside, b) the in situ anomerization method[57] for the synthesis of β-gluco or α-manno type glycoside, and c) the heterogenic catalyst method[58] for the preparation of β-mannoglycoside were developed in this area.[59] In the Koenigs-Knorr method, heavy metal salts (mainly silver and mercury salts) are used as activating reagents.[61] The order of reactivity of some representative catalysts was generally confirmed.[62]

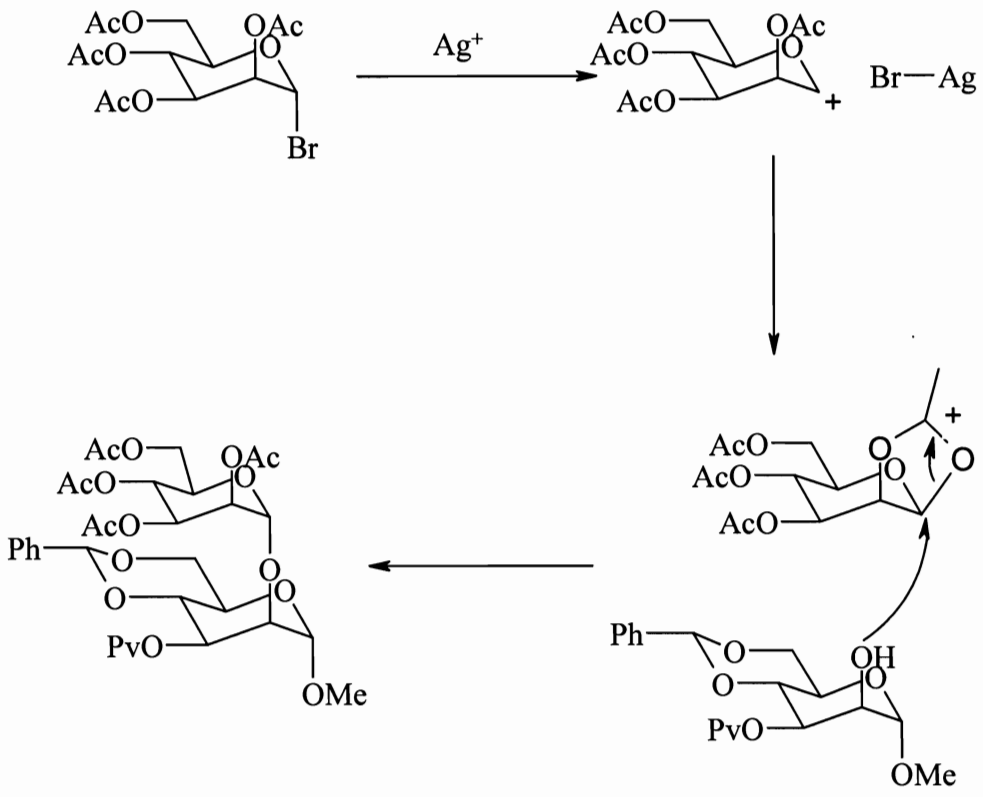

Figure 11. Mechanism for Koenigs-Knorr reaction for coupling of mannosides.[60]

One equivalent of Ag_2O and **26** and with anhydrous $CaSO_4$ in CH_2Cl_2 was stirred for one hour under nitrogen in a covered reaction vessel, as silver salts are light sensitive. A solution of CH_2Cl_2 containing **14** and a catalytic amount of I_2 crystals was added slowly over 10 minutes, to form a more reactive glycosyl iodide *in situ*. After stirring under nitrogen overnight, the disaccharide **27** was obtained in 90% yield (Figure 10). The disaccharide **27** provided the material for conversion to the disaccharide glycosyl donor and acceptor (Figures 13 and 14). Synthesis of a glycosyl donor directly from **27** using DCMME was attempted (Figure 12); however, the zinc chloride cleaved the benzylidene to yield **28**, requiring another approach.[63] In an alternative method[64] (Figure 13), compound **27** was dissolved in a 1:1 mixture of TFA/H_2O to remove the benzylidene protecting group from the 4 and 6-hydroxyl positions of the multiprotected mannose residue (Figure 13).[65] After 10 minutes, the reaction was complete according to TLC and provided **29** in 90% yield. Compound **29** was treated with NaOAc/Ac_2O to yield hexaacetate **30** in 85% yield.[66] Compound **30** was treated with 1.5 equivalents of DCMME and catalytic $ZnCl_2$ in $CHCl_3$ to yield glycosyl donor **31** in 74% yield, as shown in Figure 14. Compounds **31** and **29** were combined using Koenigs-Knorr reaction conditions (Figure 14), similar to the previous glycosylation using Ag_2O as the Lewis acid and catalytic I_2 to provide **32** in 90% yield. Conversion was evident based on the ^1H NMR spectra (Appendix 22) from the change of the anomeric proton peak from 6.19 ppm to 5.80 ppm. Total deprotection was accomplished in two steps using NaOMe to cleave the ester groups and BBr_3 to cleave the ethers and ketals in 82% yield for the two steps.[66, 67] The desired tetrasaccharide, **10**, was synthesized in nine steps in 14% total yield. At this time, no biological testing has been performed on this compound. Samples will be

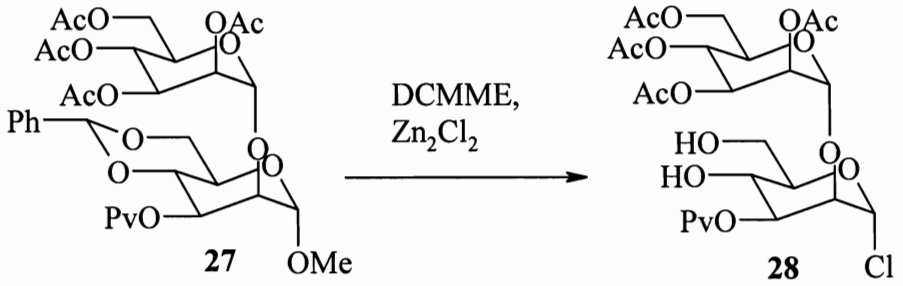

Figure 12. Attempted one step conversion of **27**to a glycosyl donor.

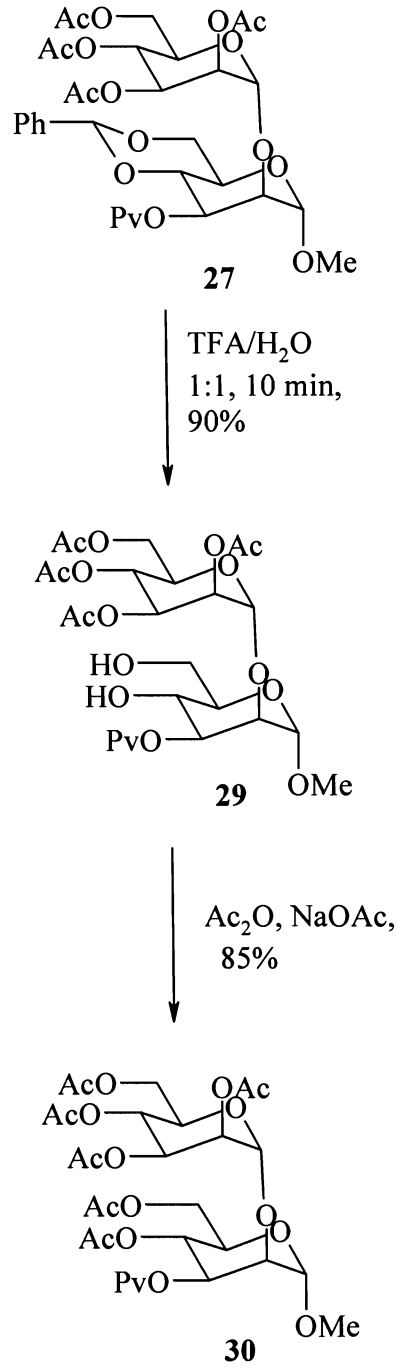

Figure 13. Synthesis of glycosyl donor (**28**) and precursor for glycosyl acceptor.

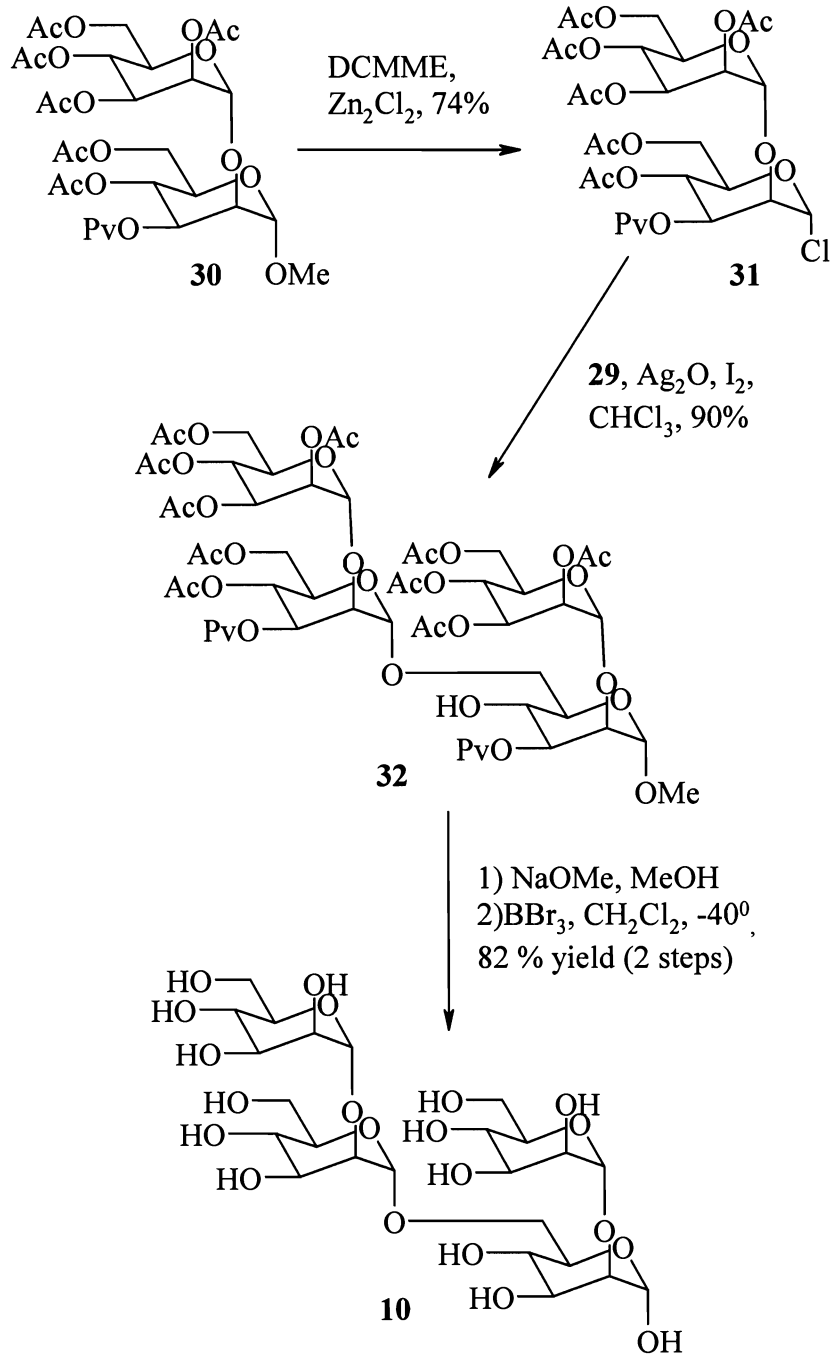

Figure 14. Synthesis of tetrasaccharide target **10**.

will be submitted for direct antimycobacterial activity. This compound will also be conjugated to a carrier protein to test for specific immune response.

This is the first synthesis of a tetrasaccharide present in the LAM repeating unit. Construction from the non-reducing end minimizes deprotection and reprotection steps during synthesis and increasing throughput. In addition, larger oligosaccharides could be made using **27** as a building block which could be selectively deprotected or converted to a glycosyl halide. This could be highly useful as LAM and its fragments in tuberculosis infection are investigated further to meet the increasing need for synthetic analogs of these carbohydrates.

Chapter 2

Synthesis of Squalamine Derivatives as Antimycobacterials

Introduction

The need for new antimycobacterial compounds has been previously discussed in Chapter 1. Squalamine (**33**) (Figure 15) was the first aminosterol that has been isolated from the dogfish shark *Squalas acanthias.*[68] It was found to have potent antimicrobial activity against Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeroginosa*), Gram positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*) and fungi (*Candida albicans*, *Paramecium caudatum*).[69] Squalamine also inhibits angiogenesis and tumor growth in several animal models. This biological activity led to the development of squalamine into an anticancer chemotherapeutic[70] and is currently in phase II clinical trial for treatment of advanced nonsmall cell lung cancer.[71] Squalamine is obtained in small amounts from the liver and gall bladder tissue of the dogfish shark (0.001–0.002 wt %). Squalamine also occurs in the spleen, stomach, intestines, gills, and testes. Recently, attempts to obtain large amounts of squalamine from the liver of the dogfish shark resulted in the discovery, isolation and characterization of seven new aminosterols related to squalamine and showed anti-bacterial and anti-fungal properties comparable to squalamine. [72] These aminosterols have a relatively invariant steroid skeleton with a *trans* A/B ring junction and a spermidine or spermine introduced at the equatorial C3 position. Aminosterols from *Squalas acanthias*, other than squalamine, have been also isolated in small quantities (0.00005–0.00025%).[73]

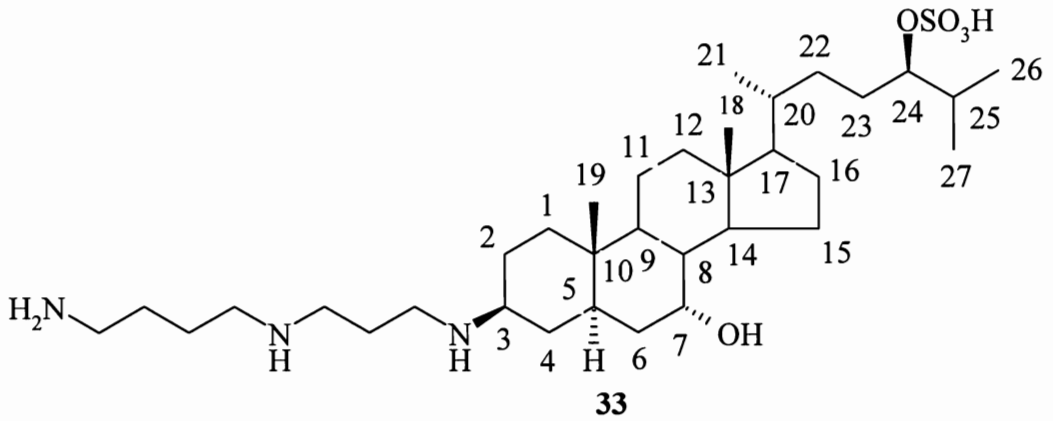

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Figure 15. Structure of squalamine with steroid numbering.

Several syntheses of squalamine have been published.[74,75,76,77,78] However, these methods afford mixtures of epimers at C3 due to the introduction of the polyamine side chain via a reductive amination. Typical syntheses were fourteen to seventeen steps.[77,78] Low isolated yields and long synthetic schemes with expensive starting material illustrate the need for squalamine analogues with similar biological acitivity and shorter synthetic routes. The synthesis of several squalamine analogues has also been reported.[79,80,81] Biological studies of squalamine and analogues led to the following conclusions: the sulfate could be removed from the sterol side chain and the structure and position of polyamine side chain on the steroid could be varied.[82] Since large amounts of squalamine analogues are necessary for chemotherapeutic application, and these compounds cannot be obtained by isolation or multistep synthetic methods, the design of simplified squalamine is analogues essential.

Research Aim

The goal of this work was to synthesize boronic acid derivatives of squalamine from commercially available cholesterol. These compounds would serve as potential antimycobacterial and antibiotic compounds.

Previous Work

The concept that squalamine derivatives would work as antituberculosis agents is based on four facts: a) the presence of mycolic acids on the mycobacterial surface, b) the affinity of mycobacteria for cholesterol, c) the activity of antibiotics against MTB, and d) the carbohydrate binding potential of boronic acids. One reason squalamine derivatives could be effective as antimycobacterial drugs is the presence of mycolic acids and other long chain fatty acids on the cell wall surface of MTB. These fatty acids are directly related to cell wall fluidity and permeability.[83] Highly polar drugs may not be able to permeate the lipid bilayer of the cell membrane. However, steroidal compounds may be able to permeate the fatty acid layers of the cell membrane because of its non-polar carbon based skeleton. In addition, lipophilic fatty acid derivatives have been shown to be active against several strains of mycobacteria, including multidrug resistant MTB.[84]

Contained within MTB is a tryptophane aspartate-containing protein (TACO).[85] Mycobacteria actively recruit and retain TACO to prevent fusion with cellular lysosomes. Phagosomes are cells that sequester foreign material to be degraded. After phagocytosis, these cells fuse with lysosomes, which degrade consumed material with acid hydrolases. Mycobacteria retain TACO at the phagosome, which prevent fusion with lysosomes.[86]

This absence of lysosomes helps ensure the survival of MTB within the macrophage. TACO associates with the phagosome in a cholesterol dependent manner. Mycobacteria display a high binding capacity for cholesterol. Cholesterol inhibition reduces macrophage uptake of *M. bovis* by 85-90% and MTB by 85%.[86] The glycolipid-rich cell wall may contain compounds involved in cholesterol mediated entry and the affinity of mycobacteria for cholesterol could be taken advantage of by designing drugs that have structural similarities to cholesterol as a result.

Several antibiotics are currently used in anti-tuberculosis chemotherapies. Isoniazid, the most widely used chemotheraputic agent, is processed by mycobacterial catalase-peroxidase, releasing the active form of the drug. The active form inhibits enoyl-ACP reductase, which is necessary for the synthesis of the bacterial cell wall. Rifampin is an antibiotic that targets mycobacterial RNA polymerase. Pyrazinamide acts through involvement with pyridine nucleotide biosynthesis. The well-known antibiotic streptomycin acts by inhibiting the translation of mRNA.[23]

The mechanism for the antimicrobial activity of squalamine is under investigation. Initially, it was believed that squalamine acted as a proton ionophore. The spermidine side chain (Figure 15) could interact with the 24-sulfate, forming a polar face that would line the aqueous interior of a trans-membrane pore. The formation of this stable pore would compromise the integrity of the organism, resulting in cell-death.[87] Selinsky has synthesized analogs of squalamine with shorter amine chains or hydroxyl groups at the 3 position which are incapable of forming a membrane pore and possess antimicrobial activity. Structure-activity relationships have shown that different analogs of squalamine have different potencies against different organisms.[81] The fluctuations in

antimicrobial activity suggest that these agents are acting upon sites specific for each organism. Another study performed by Selinksy showed squalamine is not a proton ionophore.[88] As a result, the antimicrobial activity of these compounds is unrelated to whether they can act as proton ionophores or not. This information leads to the belief that the 24-sulfate may not be necessary for antimicrobial activity.[82] Work on the interactions of squalamine with vascular endothelial cells indicate that it binds with cell membranes, inhibits the membrane Na^+/H^+ exchanger and may further function as a calmodulin chaperone. These primary actions appear to promote inhibition of several vital steps in angiogenesis, such as the blockade of mitogen induced actin polymerization, cell–cell adhesion and cell migration, leading to suppression of endothelial cell proliferation.[89]

El Kihel has synthesized squalamine analogues with a spermidine side chain at the C7 of the steroid, replacing the hydroxyl group. These compounds were made using cholesterol and cholestanol as starting material and has shown activity against *S. aureus* with minimum inhibitory concentrations in the low micromolar range.[90]

Angiogenesis is the process whereby new blood vessels are formed from preexisting vascalature. This takes place in wound repair and reproduction. It also plays a role in other degenerative conditions. In arthritis, new capillary blood vessels invade the joint and destroy cartilage. In diabetes, new capillaries in the retina invade the vitreous, bleed, and cause blindness. Tumor growth and metastasis are angiogenesis-dependent. A tumor must continuously stimulate the growth of new capillary blood vessels for the tumor itself to grow. Furthermore, the new blood vessels embedded in a tumor provide a gateway for tumor cells to enter the circulation and to metastasize to

other sites, such as the liver, lung, and bone.[91] Squalamine has been shown to improve antitumor activity when co-administered with carboplatin or cis-platin.[92] Glucosidase inhibitors such as castanospermine and N-methyldeoxynojirimycin have demonstrated anti-angiogenic properties. It appears that certain tumor cell surface oligosaccharides were required for angiogenesis to occur.[93]

Cell surface carbohydrates are known to play an important role in numerous biological processes such as inflammation,[94] tumor metastasis,[95] and viral and bacterial infection.[96,97,98,99] The incorporation of boronic acids into receptor sites has provided a means to form covalent bonds to a target saccharide. Boronic acids form boronate esters with contiguous hydroxyl groups of sugars. Boeseken first studied the interaction between boronic acids and polyols.[100] Torssell published extensively on aromatic boronic acids and their interactions with polyols and their effects on biological systems.[101] Lorand and Edwards were first to report the selectivity and stability trends of phenylboronic acid binding to vicinal diols. It was confirmed that the rigid cyclic structures of saccharide complexes have larger association constants than the corresponding acyclic diols by an order of magnitude (Figure 16). This applies to nonaromatic boronic acid systems as well.[102] The formation of boronate esters is favored at alkaline pH. Carbohydrates are soluble only in polar protic solvents which are competitive with the guest as in a hydrogen bonding receptor. As a result, the covalent interaction of a boronic acid binding to a sugar is far stronger than the hydrogen bonding interactions of receptors based on electrostatic and hydrogen bonding interactions.

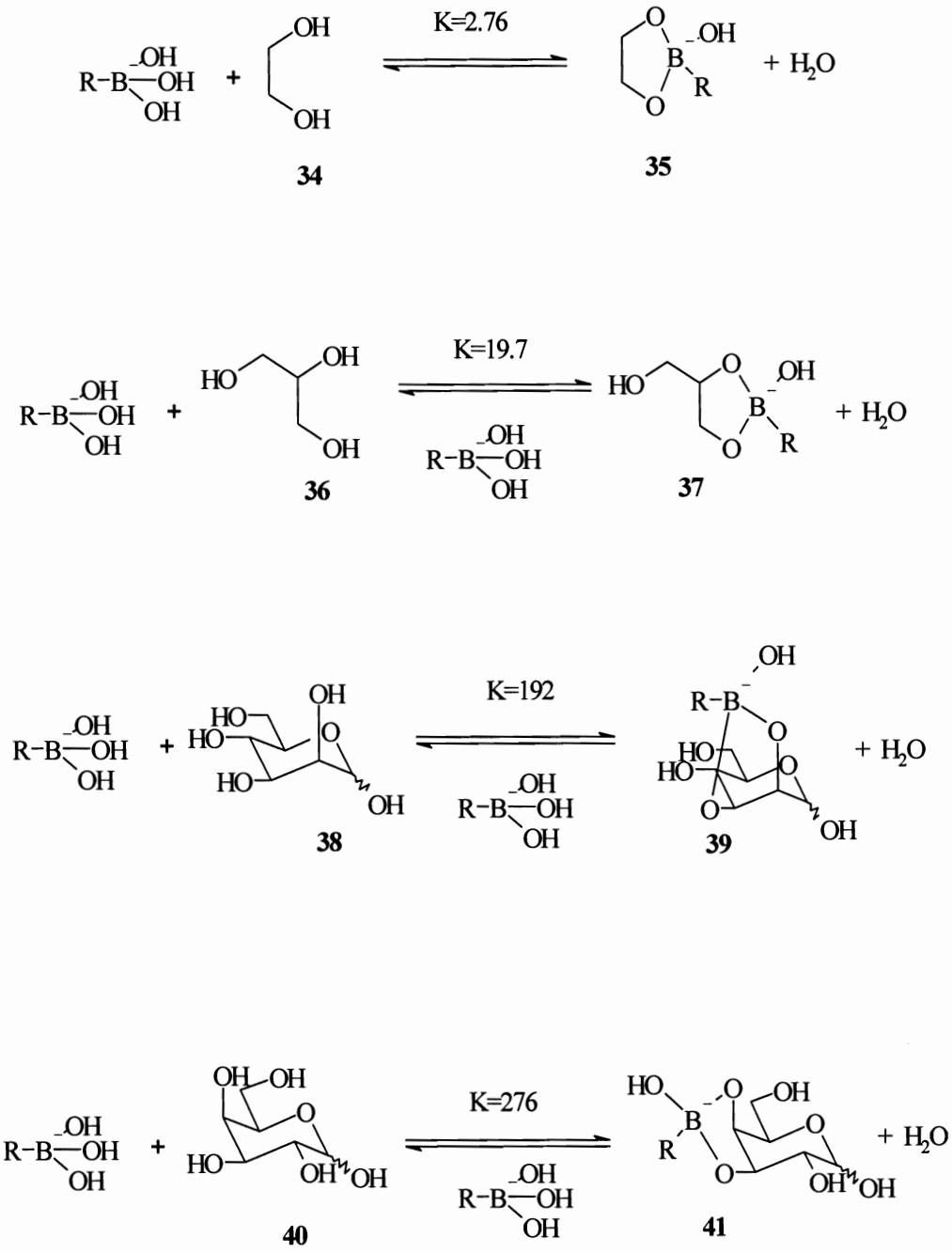

Figure 16. Binding constants of diol-boronic acid interactions.[102]

Boronic acids can be added to the terminal amine of squalamine via reductive amination. Carbohydrate moieties on the mycobacterial surface include LAM and arabinogalactan (AG) are structures that contain ligands capable of binding boronates.

Other microorganisms that contain cell-surface carbohydrates may also be bound by boronate containing compounds. The carbohydrate 6-phospho-(1-manno-pyranosyl)-galactose (**42**) (Figure 17) is one of these carbohydrates; it is an oligomeric component

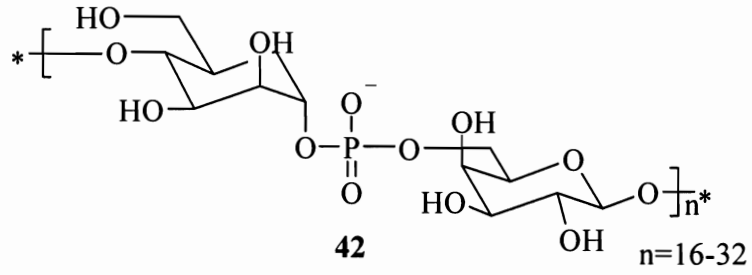

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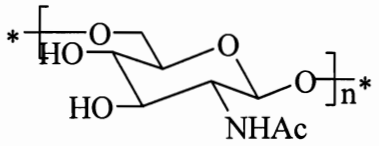
Figure 17. Structure of lipophosphoglycan (LPG) of *Leishmania* protozoa.[97]

of the lipophosphoglycan (LPG) that is found on the cell-surface of *Leishmania* protozoa. LPG is implicated in numerous functions contributing to the parasite's pathogenesis. These functions include host-cell recognition and deactivation of host macrophages through inhibition of β-galactosidase. LPG is the only known example of a 4-O substituted mannose linkage in eukaryotic systems.[103] It consists of a neutral galactose

cap; the repeating LPG unit and a hexasaccharide core are linked to the cell membrane through a lipid anchor. Although *Leishmania* is typically restricted to tropical regions, there are some cases found in the United States.[104]

Staphylococcus aureus is a bacterial pathogen frequently acquired during extended hospital stays[105] and is a significant pathogen in dairy cows.[106] Resistance to first-line antibiotics has resulted in problems treating methicillin-resistant *S. aureus* strains which are increasingly common. *S. aureus* contains a repeating chitin-like β-N-acetylglucosamine polymer on its surface with a molecular weight of approximately three million (Figure 18).[107]



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MW=3,000,000

Figure 18. Structure of cell-surface polysaccharide of *Staphylococcus aureus.*[107]

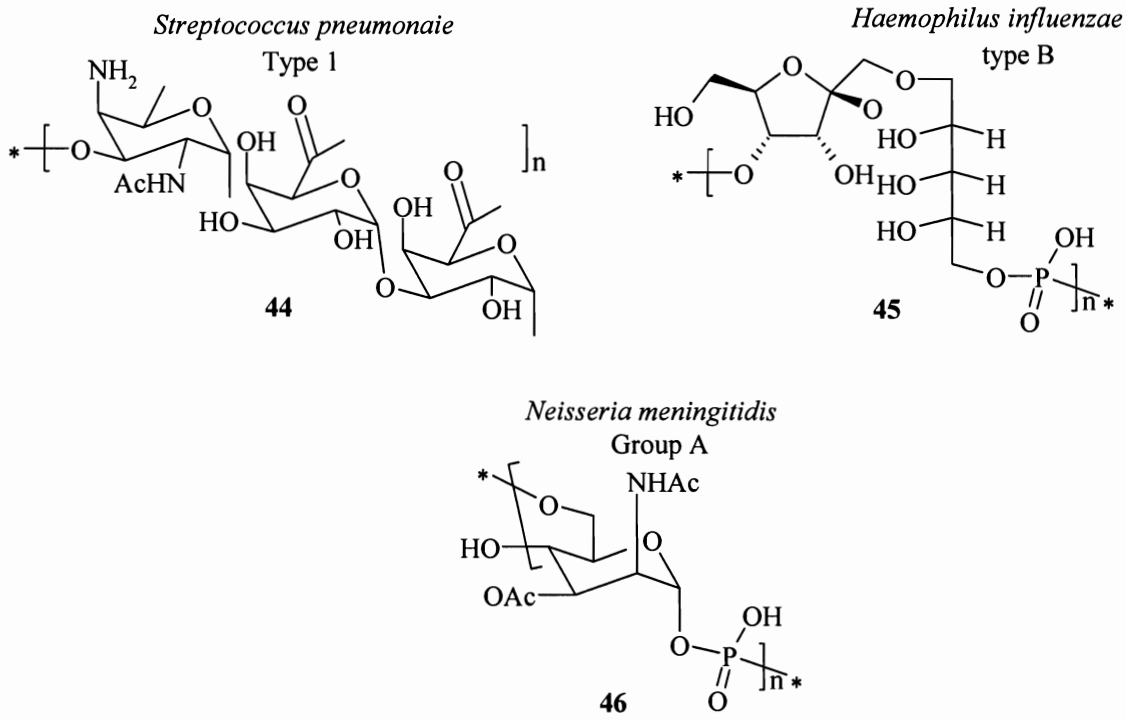

Figure 19. Structures of additional bacterial capsular polysaccharides.

Other examples of capsular polysaccharides involved in diseases (Figure 19) include *Streptococcus pneumonaie* which is the main cause of middle ear infections in children and lower respiratory tract infection in adults.[108] *Haemophilus influenzae* is a Gram-negative organism that causes meningitis, a serious infection among infants that causes severe, permanent neurological damage.[109] *Neisseria meningitides* is an organism that causes meningitis in children and adults and possesses a high mortality rate in the absence of antibiotics.[99] A larger concern is the emergence of strains with reduced susceptibility to vancomycin, which is the antibiotic of last resort. The appearance of these vancomycin-resistant strains raises the possibility of untreatable staphylococcal infections; therefore, a need for alternative therapies exists.[110] The addition of boronic acids to squalamine may serve as an additional delivery system for the drug to the mycobacterial surface that may enhance its antimicrobial and its anti-angiogenic activity.

Results and Discussion

Initially, cholesterol was chosen as the starting material for the squalamine derivatives. The synthetic plan was to oxidize the hydroxyl group in the 3 position, then perform reductive aminations with a series of amines. Reductive amination of the resulting compounds at the terminal amine with OFPBA would add the boronic acid to the terminal amine. [111]

A Swern oxidation is a low-temperature reaction of dimethyl sulfoxide (DMSO), with oxalyl chloride to provide a ketone from a secondary alcohol (Figure 20).[112] A Swern oxidation (Figure 21) successfully converted the hydroxyl group of cholesterol (**47**) to a ketone (**48**), but the double bond shifted to the A ring of the steroid, according to the ^{1}H NMR spectra, which has an sp^2 proton at 5.5 ppm. Another strategy involved conversion of the 3-hydroxyl of **47** to the 3-bromide and subsequent nucleophilic substitution. Reaction of the 3-OH with PBr_3 yielded the 3-bromide **49** in 71% yield, as shown in Figure 21. The 3-H next to the bromine on the A ring of **47** was shifted slightly downfield in the NMR spectrum from 3.17 ppm to 3.92 ppm, indicating that a positive reaction had occured. Nucleophilic substitution was attempted using excess ethylenediamine to increase the likelihood of reaction of only one of the two primary amines. Reactions were attempted in refluxing chloroform, and then in neat refluxing ethylenediamine, however, both failed. The bromide proved to be unreactive under these conditions. The next strategy involved hydrogenation of the double bond, as shown in Figure 22. Cholesterol was protected with MeI forming the methoxy ether **51**.[113]

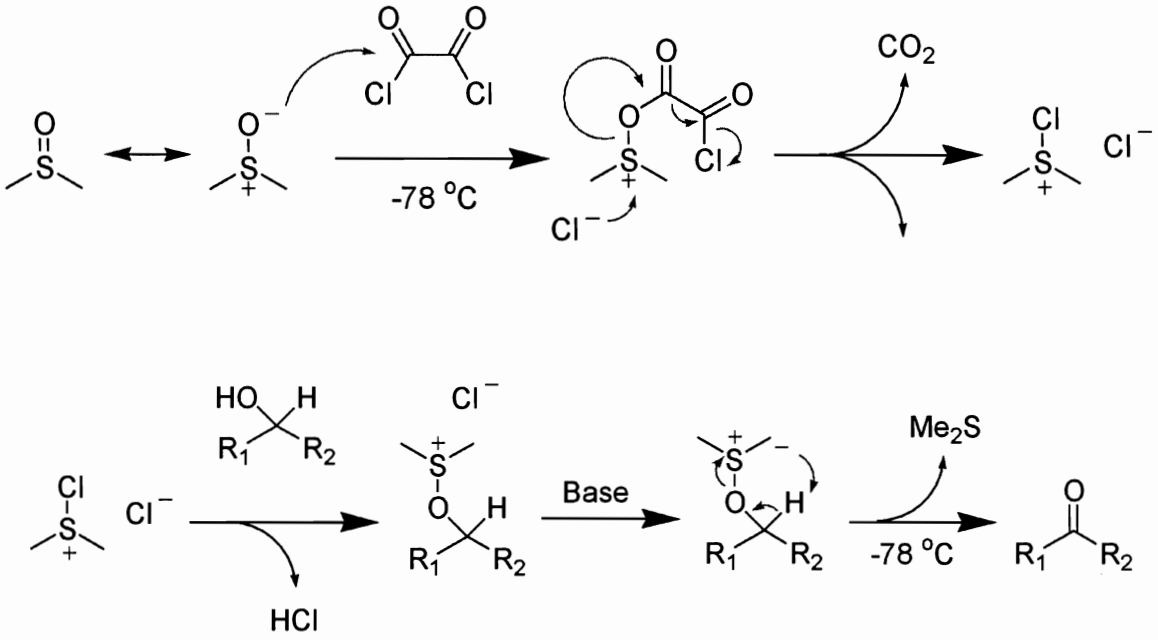

Figure 20. Mechanism of the Swern oxidation.[112]

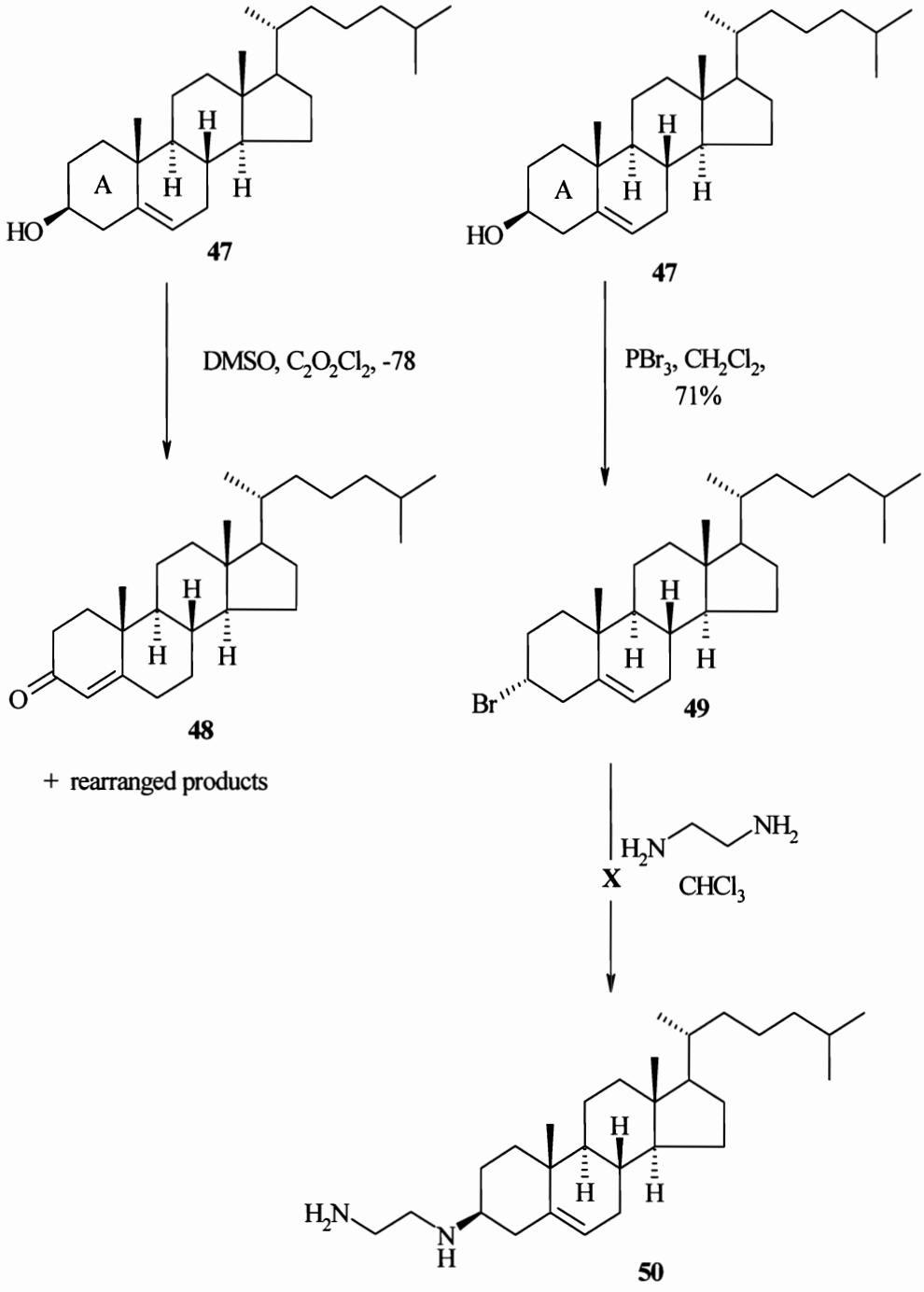

Figure 21. Oxidation of cholesterol and substitution as potential routes to aminosterols.

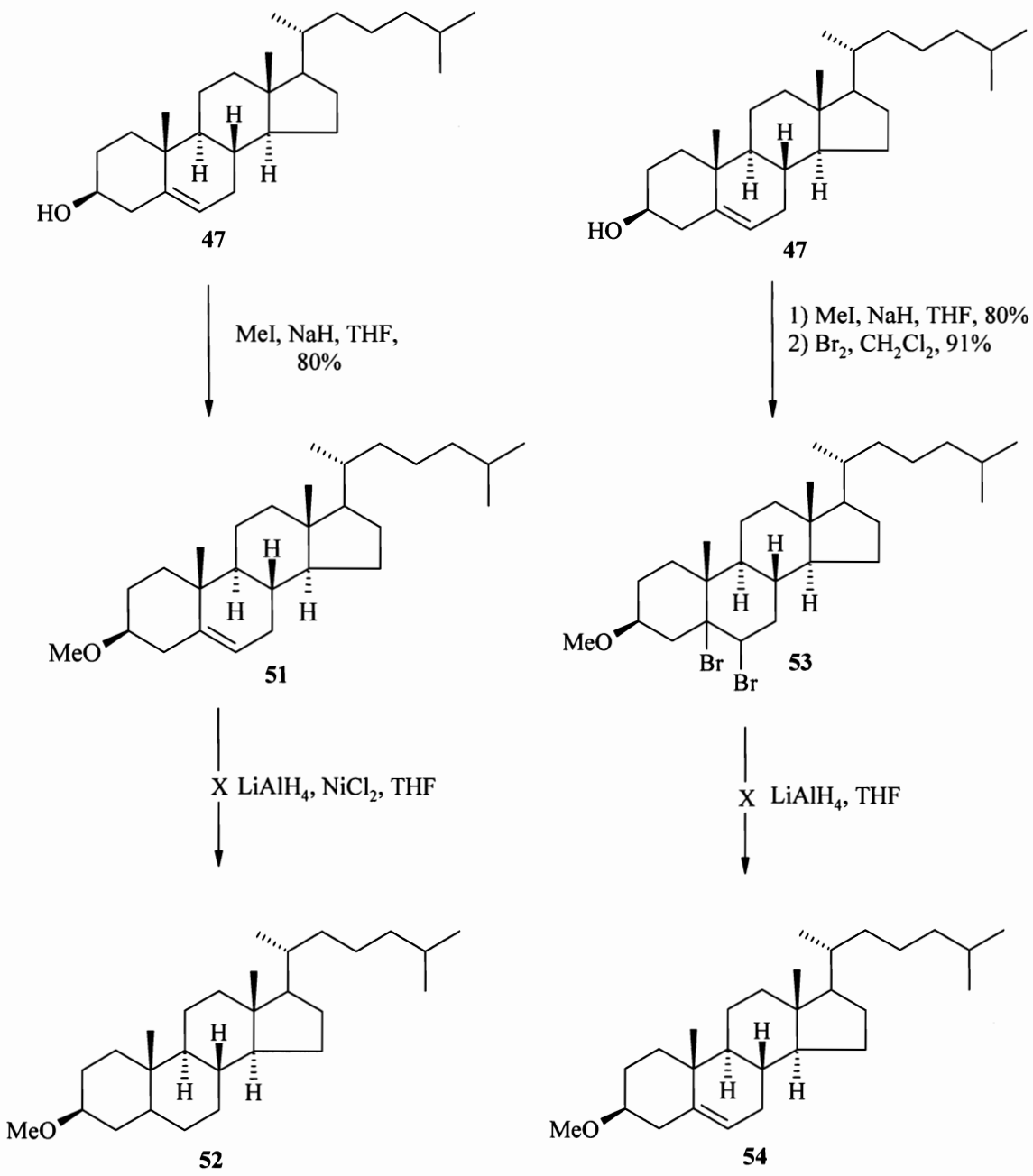

Figure 22. Hydrogenations as failed routes to saturated steroids.

Reduction of **51** with LAH and $NiCl_2$ in THF at –40° C was attempted; however, after several hours, only starting material was recovered. The next approach to eliminate the double bond was to brominate **47** and react the dibromide with a metal to form the unsaturated compound. Protection with MeI was followed by Br_2 addition which proceeded in 90% yield to give **53** (Figure 22). Reaction with LAH in THF was expected to yield unsaturated product through an elimination pathway[114]; however, the bromines were cleaved and the double bond was restored.

After attempting to convert cholesterol (**47**) to obtain suitable precursors that would not be prone to rearrangements, 5α-cholestan-3-one (**64**) was chosen as the starting material. The synthetic plan was to reductively aminate **64** with a series of amines, then reductively aminate **65-69** with OFPBA by adding the boronic acid to the terminal amine, as shown in Figure 24.[106] Reductive amination is a type of amination reaction which involves the conversion of a carbonyl group to an amine. In this reaction the amine was first reacted with the carbonyl group, to form the imine accompanied by the loss of one molecule of water. The imine was then reduced to the amine with a suitable reducing agent, such as $NaBH_4$ or $NaCNBH_3$. In this lab the utility of reductive aminations using *o*-formylphenylboronic acid (OFPBA) (**55**) and amines for the synthesis of δ-aminoboronic acids was demonstrated.[106] Reactions with simple amines were performed (Figure 23) using one equivalent of **55** and one equivalent of amine in methanol. OFPBA and the amine were stirred at room temperature for 3-4 hours to provide the corresponding δ-aminoboronic acid in >90% yield without further purification. The first step, using 5α-cholestan-3-one, was carried out as a one pot synthesis using **64**, an excess of diamine to avoid addition to both primary amines, and

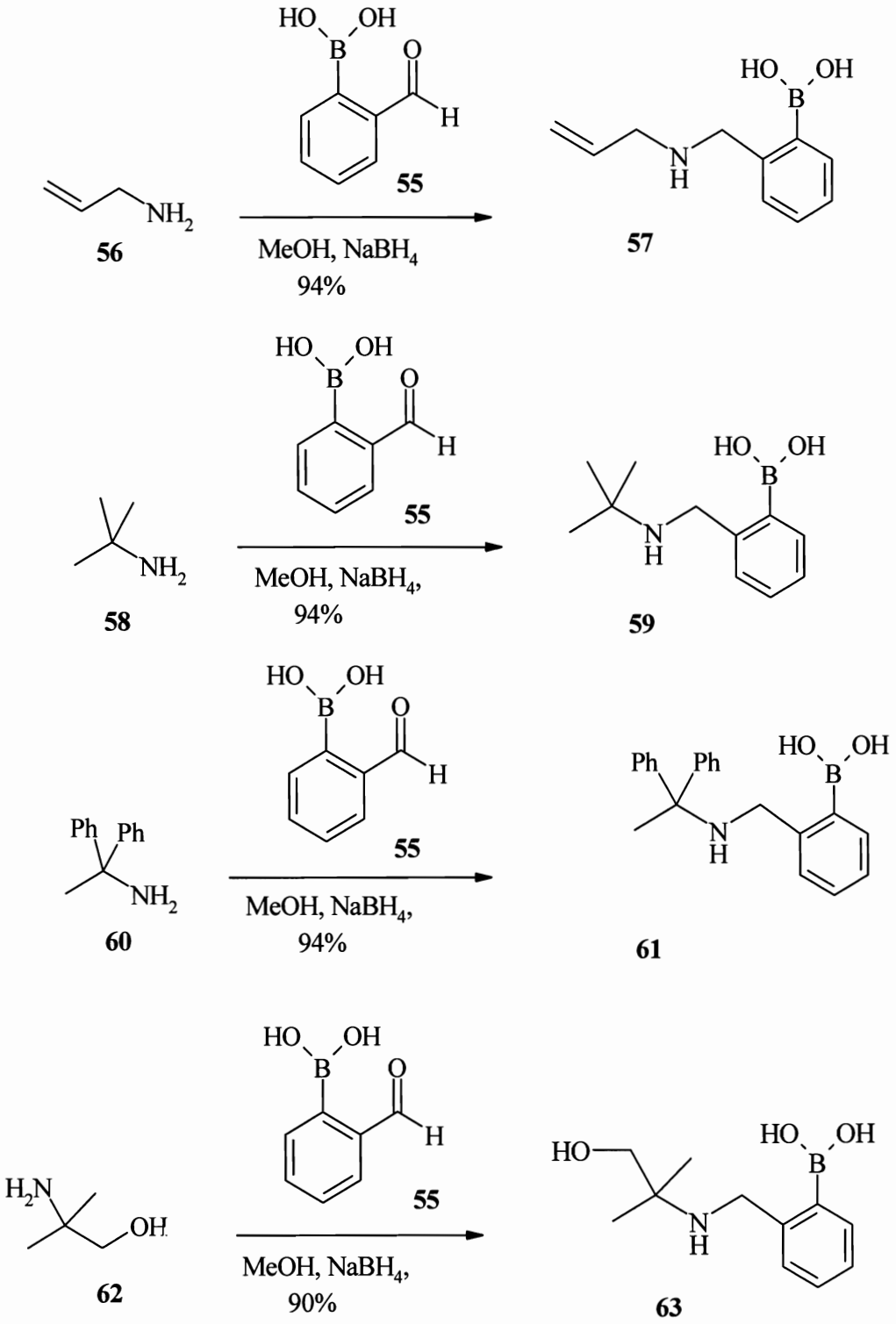

Figure 23. Reaction of various amines with OFPBA (**55**).[106]

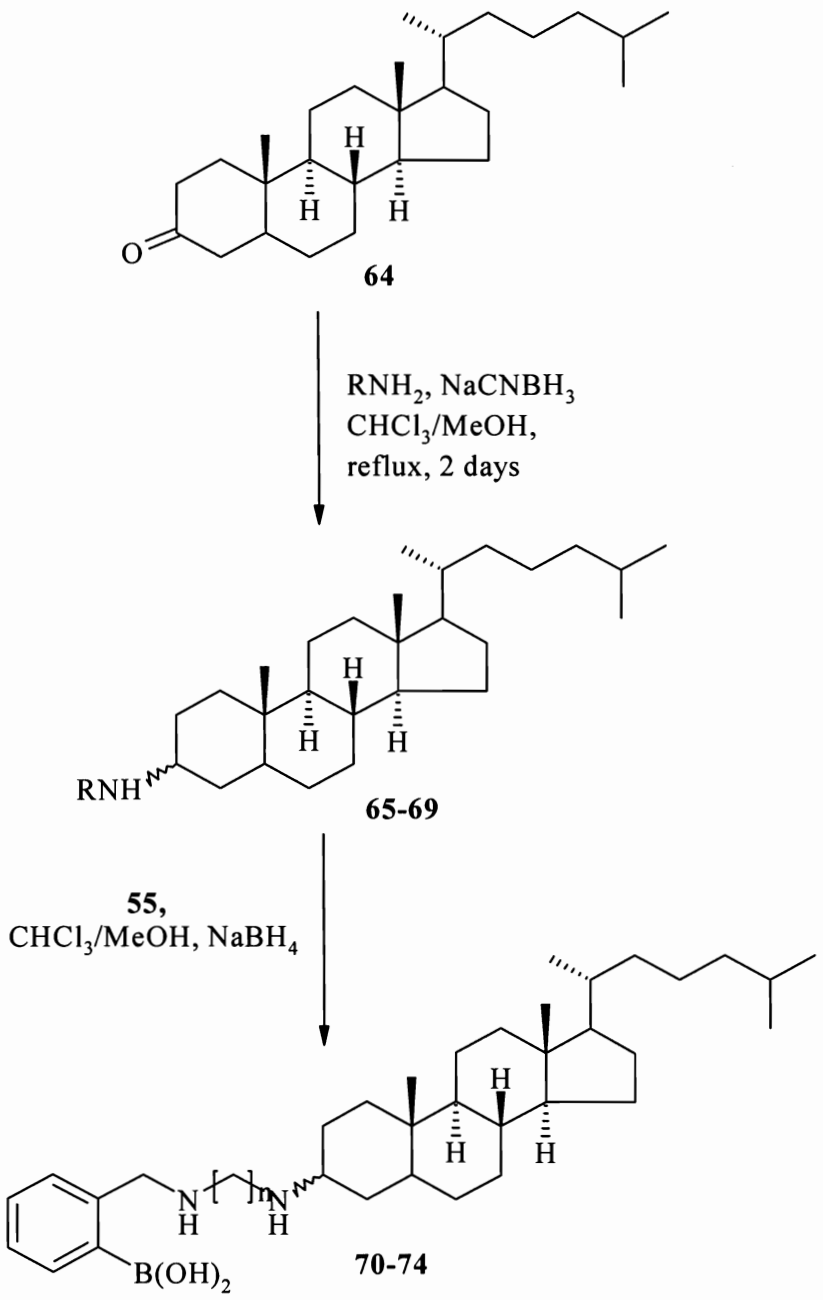

Figure 24. Reductive amination of 5α-cholestan-3-one.

$NaCNBH_3$ (Figure 24). Cyanoborohydride reduces imines, not ketones or aldehydes.[115] Conversion to the amine was established by ^{13}C NMR spectroscopy (Appendices 36, 38, 41, 44, 46), where the disappearance of the carbonyl carbon at 210 ppm demonstrated conversion from the ketone to the amine. The reaction was heated for 48 h at reflux for complete conversion, generating compounds **65-68** in fairly high yield (>85%) and **69** obtained in 52% yield (Table 3). The triethylenetetraamine was available only in a 60% technical grade and was not purified, explaining the lower yield. The harsh conditions relative to the procedures in Figure 23 were necessary due to the higher difficulty of amine condensation with a ketone relative to that of an aldehyde. The next step was to reductively aminate the terminal amine of compounds **65-69** with OFPBA, as shown in Figure 24. The imine was generated in 3-4 hours, then borohydride was added and the solution was allowed to stir overnight. Purification by column chromatography yielded the final boronic acid products **70-74** in good yield (76-87%) (Table 4).

These compounds may have some utility as general antibiotics or antitumor compounds. As previously discussed, the boronic acid could act as a "warhead" to deliver the drug directly to the cell surface of a tumor or bacterium by carbohydrate binding where the biological activity of the aminosterol could fight bacterial infection or tumor metastasis. The cholestane should be able to penetrate the lipid-rich membrane of MTB and be actively transported by cholesterol binding proteins. Biological testing will determine whether the presence of boronates on the amines enhances its cell-surface affinity further. At this time, no biological testing has been performed on this compound.

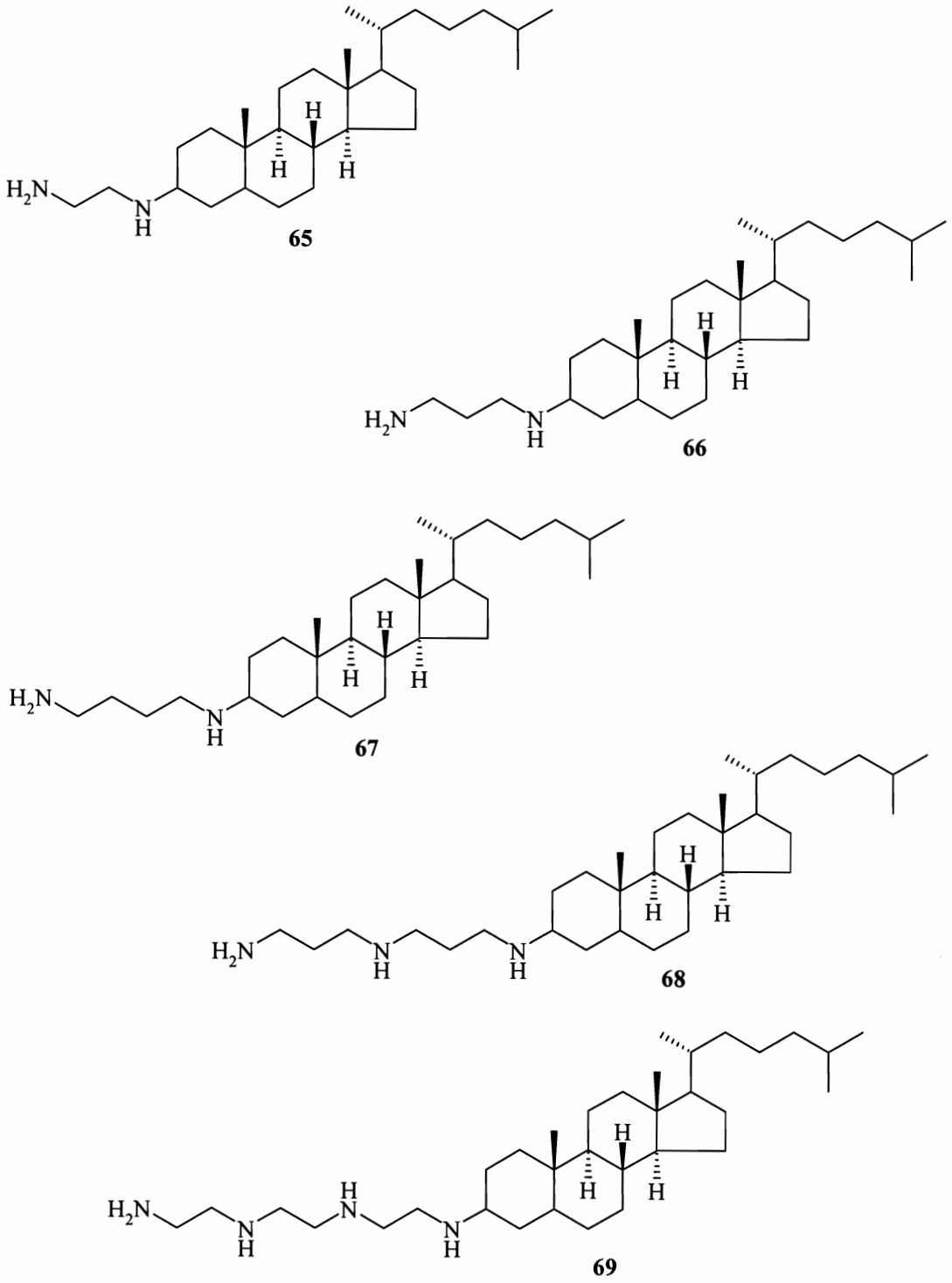

Figure 25. Products of reductive amination with 5α-cholestan-3-one (**64**).

Table 3. Yields of initial reductive aminations with 5α-cholestan-3-one (**64**) (Figure 25).

Amine	Yield
Ethylenediamine (**65**)	92%
Propylenediamine (**66**)	89%
1,4-Diaminobutane (**67**)	90%
N-(3-aminopropyl)-1,3-propanediamine (**68**)	86%
Triethylenetetraamine (**69**)	52%

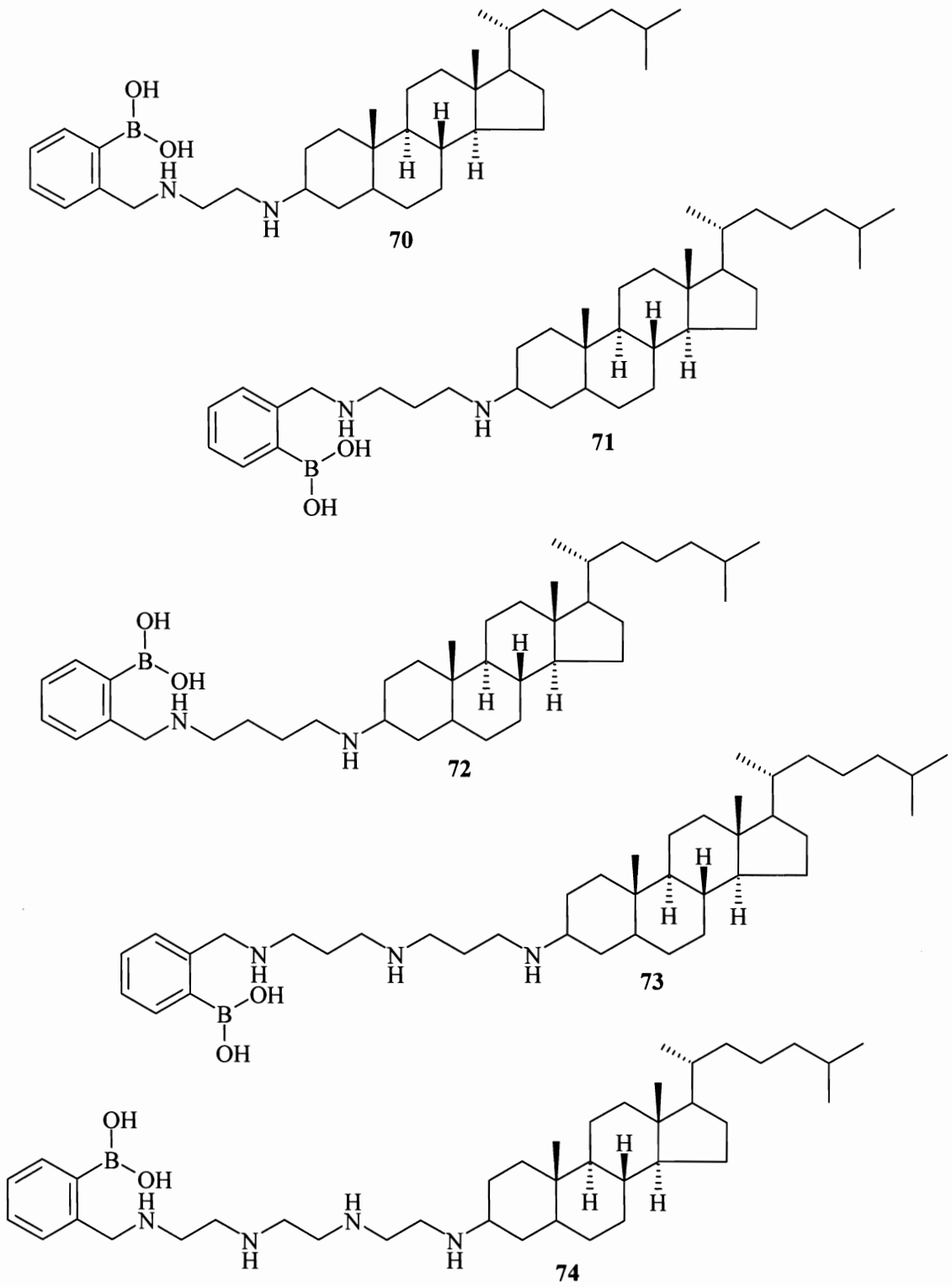

Figure 26. Products of reductive amination of aminosterols **65-69** with **55**.

Table 4. Yields of reductive aminations of aminosterols **65-69** with **55** (Figure 26).

Amine on Steroid	Reactant	Yield
Ethylenediamine **(70)**	**65**	76%
Propylenediamine **(71)**	**66**	87%
1,4-Diaminobutane **(72)**	**67**	81%
N-(3-aminopropyl)-1,3-propanediamine **(73)**	**68**	78%
Triethylenetetraamine **(74)**	**69**	79%

Chapter 3

Design of a Fluorescent Receptor for Simple Carbohydrates

Introduction

Cell surface carbohydrates play an important role in numerous biological processes such as inflammation, tumor metastasis, and viral and bacterial infection.[88-93] A great deal of interest has surfaced in designing probes which can detect and/or quantify the presence of simple saccharides within a given system. Photoinduced electron transfer (PET) has emerged as a strategy for fluorescent sensor design for several analytes.[116] The distance between the binding site and the PET system offers a way to design molecular sensory systems with a high degree of sensitivity and precision. The binding interactions are not disturbed by the PET system. The modification of the fluorescence intensity, accomplished by the remote suppression of the PET quenching, preserves the properties of the fluorophore while providing recognition.

The incorporation of boronic acids into receptors has provided a means to form covalent bonds to a target saccharide. Boronic acids form boronate esters with contiguous hydroxyl groups of sugars. The rigid cyclic structures of saccharide-boronate complexes are stabiliized by the conformational restriction and lower entropy of ring formation over acyclic systems (Figure 16). This construct provides greater association constants than the corresponding acyclic diols by an order of magnitude.[96] The formation of boronate esters is favored at alkaline pH. Carbohydrates are soluble only in polar protic solvents which compete with the guest hydrogen bonding receptor. As a result, the

covalent interaction of a boronic acid binding to a sugar is far stronger than the hydrogen bonding interactions of receptors based on electrostatic and hydrogen bonding interactions. This is important because interactions of an analyte with the solvent must be overcome for sensing applications.

Photoinduced electron transfer involves quenching fluorescence by the transfer of an electron lone pair into an aromatic ring system.[117] Bonding the lone pair electrons of an amine prevents fluorescence quenching via photoinduced electron transfer (PET). Thus a Lewis acid-base complex, between the boron and nitrogen, is a tunable method of occupying the amine lone electron pair. Tethering a boronic acid to a fluorophore is one design to form a fluorescent receptor. A carbohydrate-boronic acid construct provides a better Lewis acid, thus creating a stronger boron-nitrogen interaction. This stronger interaction prevents PET from occurring, causing an increase in the fluorescence signal observed (Figure 27).

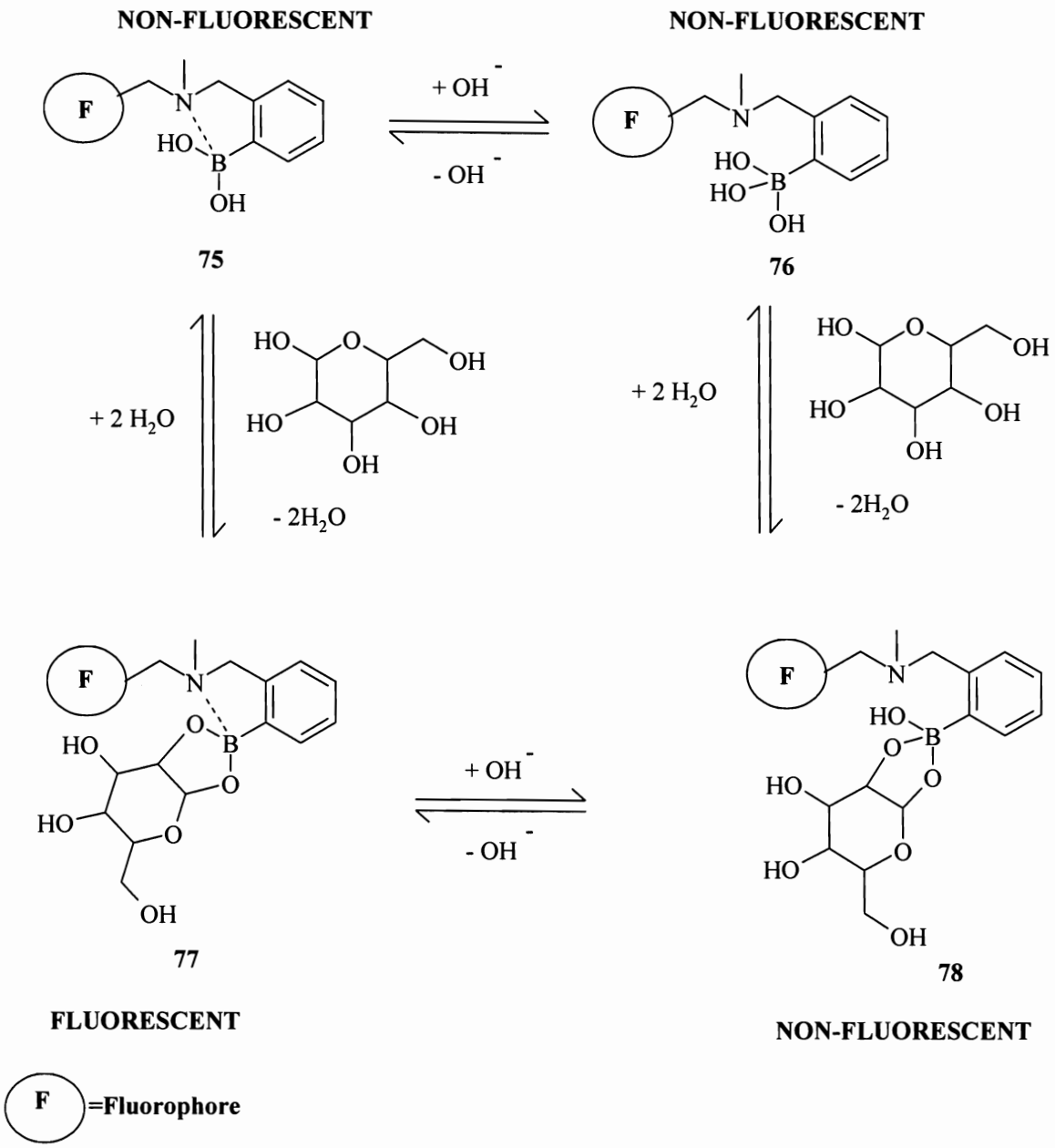

Figure 27. Demonstration of PET for detection of carbohydrates.

Research Aim

The first objective of this research was to synthesize a fluorescent receptor for simple carbohydrates. The starting material was 9-anthraldehyde (**93**) and the boronic acid functional group was attached by amination with 2-formylphenylboronic acid (**55**). The long term goal was to synthesize fluorescent receptors that would bind preferentially to specific oligosaccharide motifs found on pathogenic cell surfaces, such as LAM (**1**).

Previous Work

A wide range of research has been performed with respect to boronic acid-based receptors for carbohydrates. Shinkai and Sandanayake reported the first known synthetic molecular color sensor for saccharides (**79**).[118] The color change was caused by a decrease in the pK_a of the neighboring amine after complexation which caused electronic changes in the receptor chromophore. Shinkai's group designed a receptor that discriminated between D and L sugars using chiral 1,1-binapthyl as the chromophore of choice (**80**).[119] Subsequently, James synthesized a receptor with a crown-ether (**81**) that discriminated between cationic D-glucosamine hydrochloride and D-glucose.[120] The Shinkai reaction scheme (Figure 29) for a basic fluorescent sensor involved converting 2,4,6-o-tolylboroxin (**84**) to 2,2-dimethylpropane-1,3-diyl[o-(bromomethyl)phenyl]-boronate (**86**) (84%). The 9-[(methylamino)methyl]anthracene (**87**) moiety was then added to the bromide (**88**) to yield the fluorescent receptor (**79**). [121] More recently, Shinkai has coupled a synthetic receptor to the sugar binding pocket of a Concanavalin

A, a natural lectin[122], and synthesized a diboronic acid using 1,10-phenanthroline as a platform.[123] James has more recently experimented with multiple fluorescent boronic acids connected by a flexible aliphatic linker.[124]

Some receptors have been designed with the goal of colorimetric response to different sugars. A receptor (**82**) for glucose-6-phosphate using a hexasubstituted benzene as the platform and fluorophore was synthesized by Anslyn (Figure 28).[125] Strongin developed a resorcinarene-based boronic acid receptor (**83**) that causes color changes in solution according to the type of monosaccharide that is bound.[126] Other receptors have been designed with binding of specific carbohydrates in mind. Ansyln has also designed a colorimetric boronic acid receptor for carboxy and phospho sugars[127] and a separate receptor for heparin.[128] Smith designed an anionic receptor using anthracence as the fluorophore and attached it to a cationic polyallylamine. The latter compound has modest success in binding to ribonucleoside phosphates. Experiments that failed to bind ribonucleosides with an electronic interaction alone[129] demonstrated the superiority of boronic acids to bind carbohydrate structures relative to electrostatic interactions.

Wang has also synthesized boronic acid containing compounds for carbohydrate binding.[130] 5-Quinolineboronic acid, 8-quinolineboronic acid, and dibenzofuran-4-boronic acid are compounds designed as fluorescent water-soluble boronic acids. Dibenzofuran-4-boronic acid shows increased fluorescence upon carbohydrate binding at multiple wavelengths. 5-Quinolineboronic acid demonstrates a unique pKa switching mechanism that provides maximum fluorescence through a zwitterionic species that does not contain a boron-nitrogen bond as the fluorescence quenching species.[131]

Smith demonstrated that boronic acids facilitate transport of monosaccharides across a lipid membrane (Figure 30).[132] Monosaccharides and boronic acids are water soluble species. When bound in its neutral form, the monosaccharide-boronic acid complexes are organic soluble. Smith has used crown ether boronic acids to transport catecholamines across lipid membranes.[133] A potential application of this is the transport of hydrophilic drugs across cell membranes. This may be particularly pertinent for the design of drugs for MTB, which contains a lipid rich cell membrane. Smith's group has also used polymeric boronic acids to bind sialic acid in the presence of glucose, demonstrating selectivity between sugars.[134]

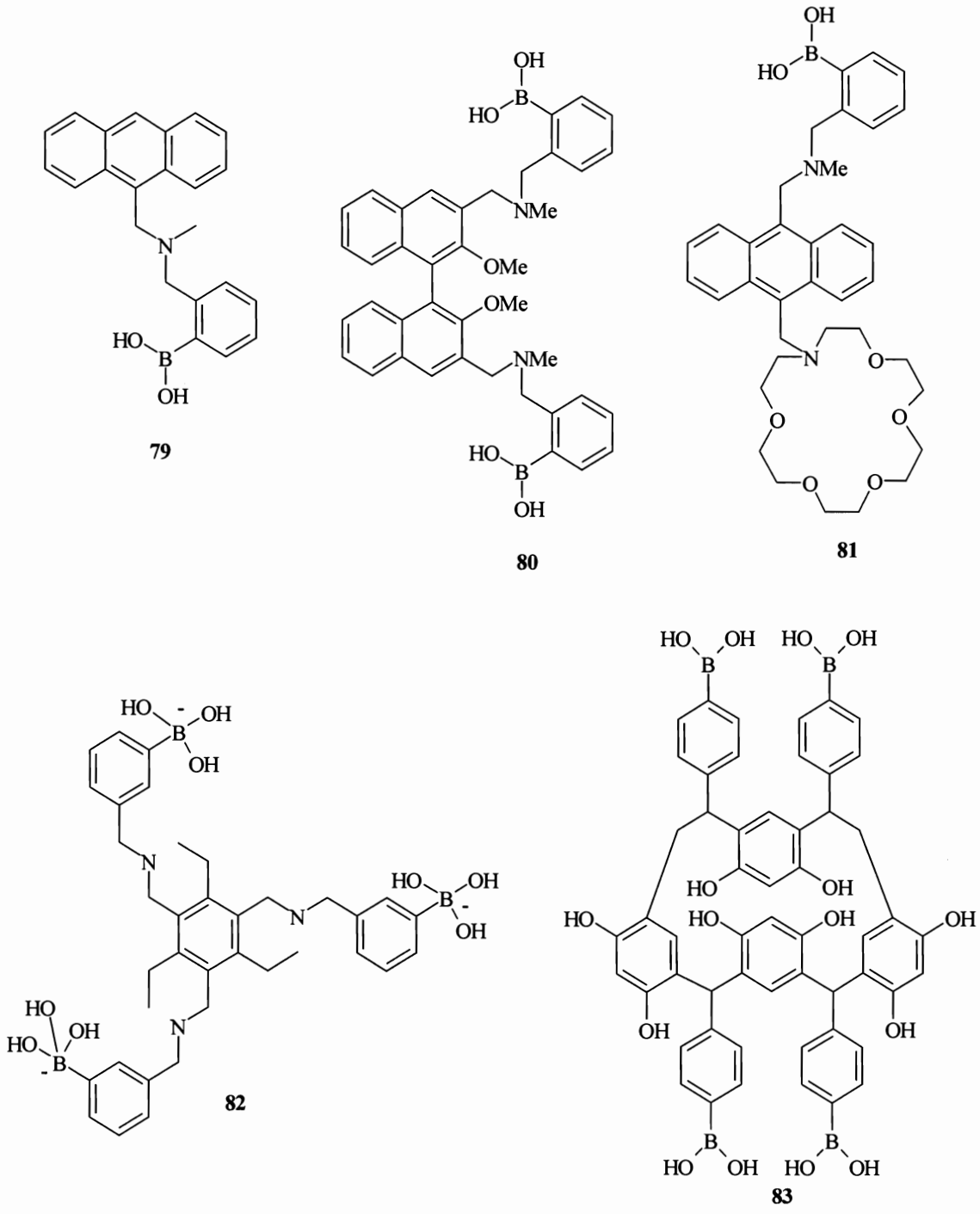

Figure 28. Examples of existing fluorescent sensors for carbohydrates.[118-121, 126]

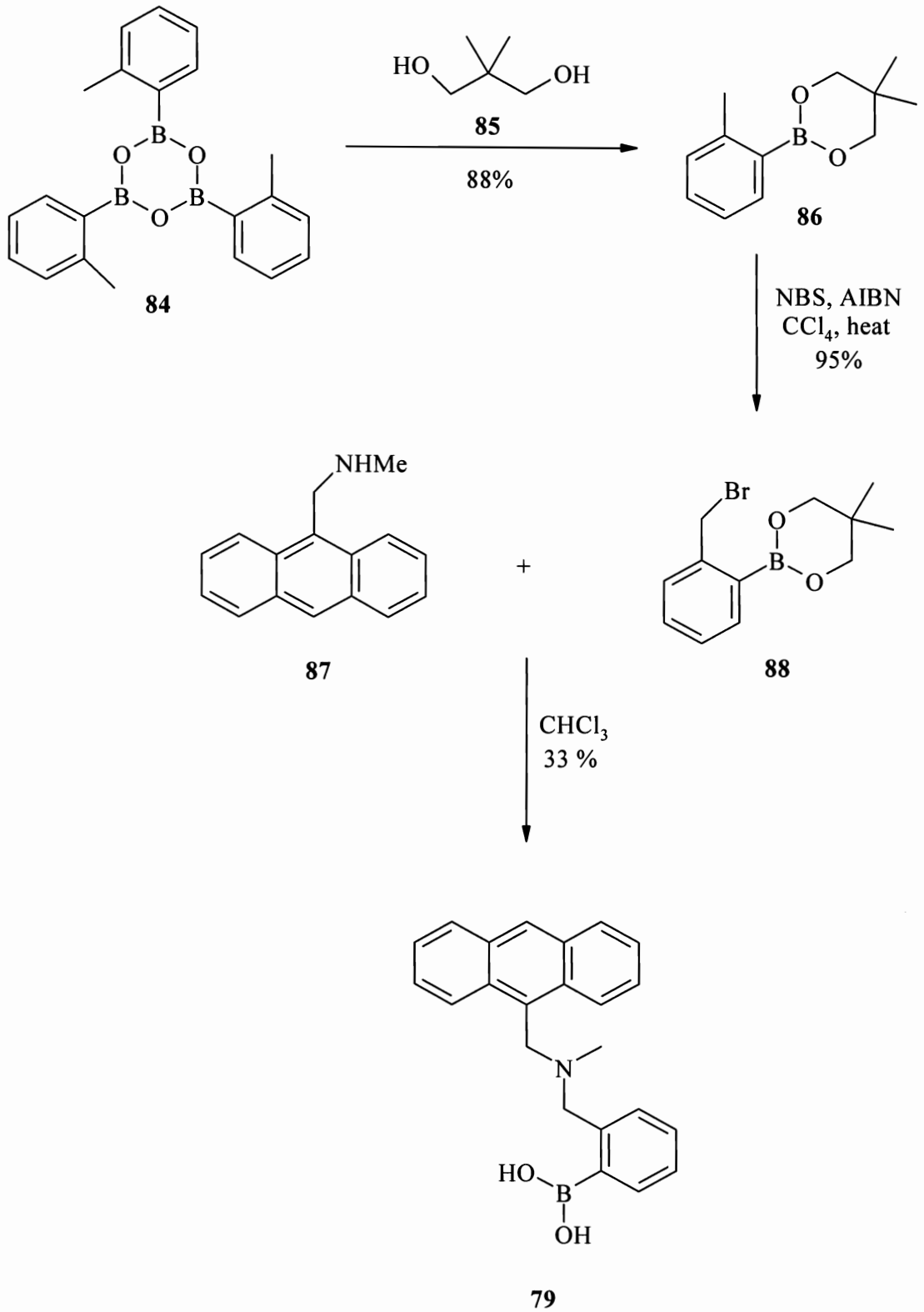

Figure 29. Shinkai synthesis of fluorescent receptor.[121]

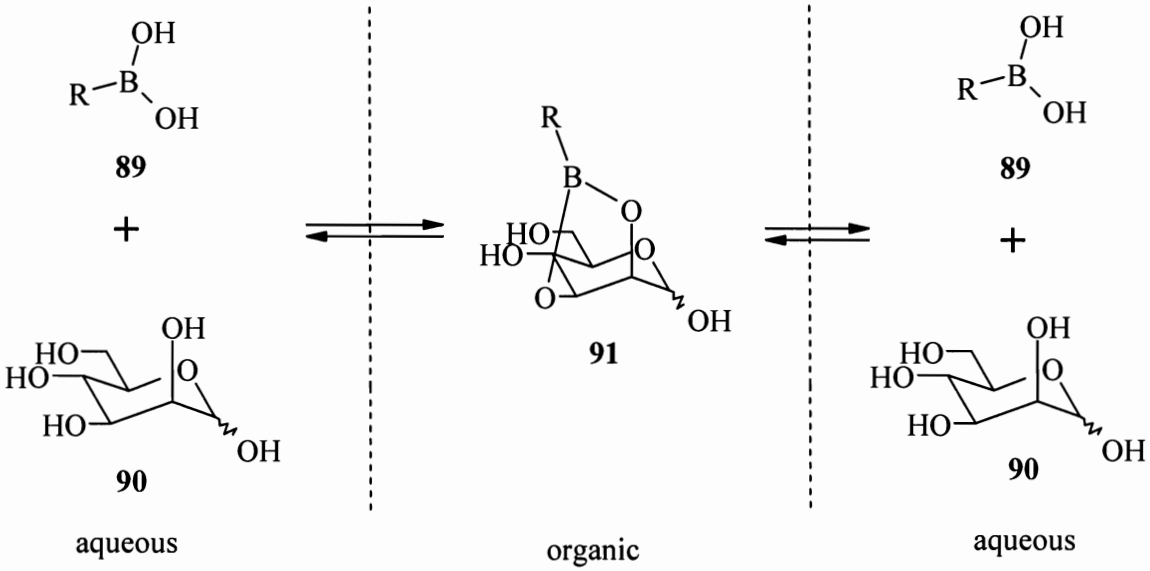

Figure 30. Demonstration of saccharide transport across a lipid membrane.[132]

Results and Discussion

A more direct synthetic route to anthracene-based sensors was designed for this study. The utility of reductive aminations, using *o*-formylphenylboronic acid (OFPBA) (**55**) and amines for the synthesis of δ-aminoboronic acids, was developed in this laboratory.[111] To determine the scope of this method, reactions with simple amines were performed as shown in Figure 23 (Chapter 2). For these reactions, one equivalent of **55** and one equivalent of amine in methanol were reacted at room temperature. Excess sodium borohydride was used to reduce the corresponding δ-aminoboronic acid in >90% yield. To form a fluorescent sensor, 9-anthraldehyde (**92**) was aminated with benzylamine (**93**) (Figure 31) forming **94** in 90% yield. Compound **94** was reductively aminated with OFPBA (**55**), to provide 9-[[N-Benzyl-N-(o-boronbenzyl)amino]methyl]-anthracene (**95**) in 79% yield. This was a much better yield than that obtained previously for the addition of the boronic acid functional group. The overall yield was 72% in two steps, which was also a significant improvement over the 28% total yield in three steps for the Shinkai procedure (Figure 31).[121]

The equilibrium equation for the reaction between a fluorescent sensor and a carbohydrate shown in Figure 32 and equation **1** was used for determining the equilibrium constant:

K= [bound receptor-sugar complex]/[unbound receptor][unbound sugar] **(1)**

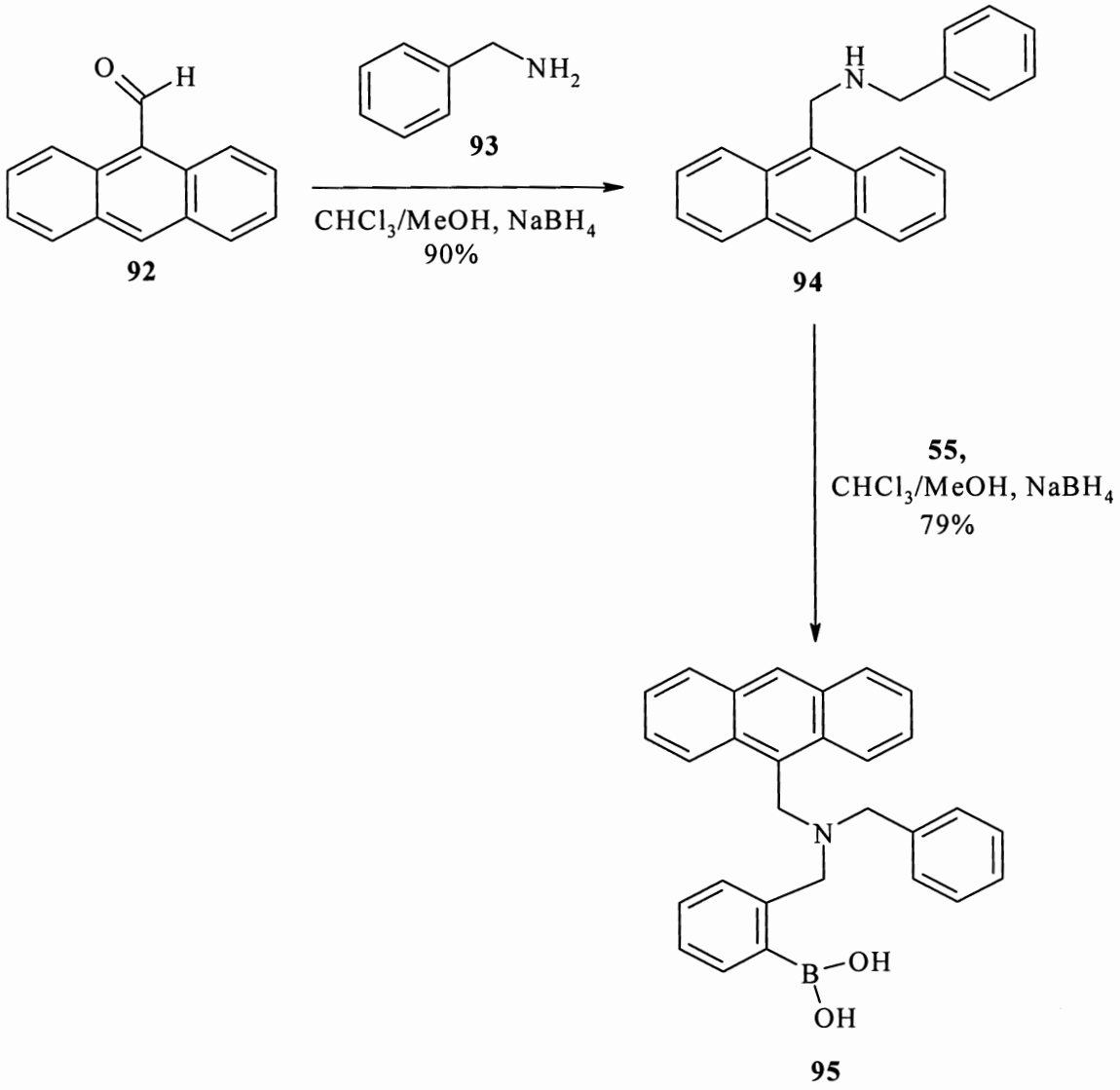

Figure 31. Synthesis of fluorescent sensor for binding simple carbohydrates.

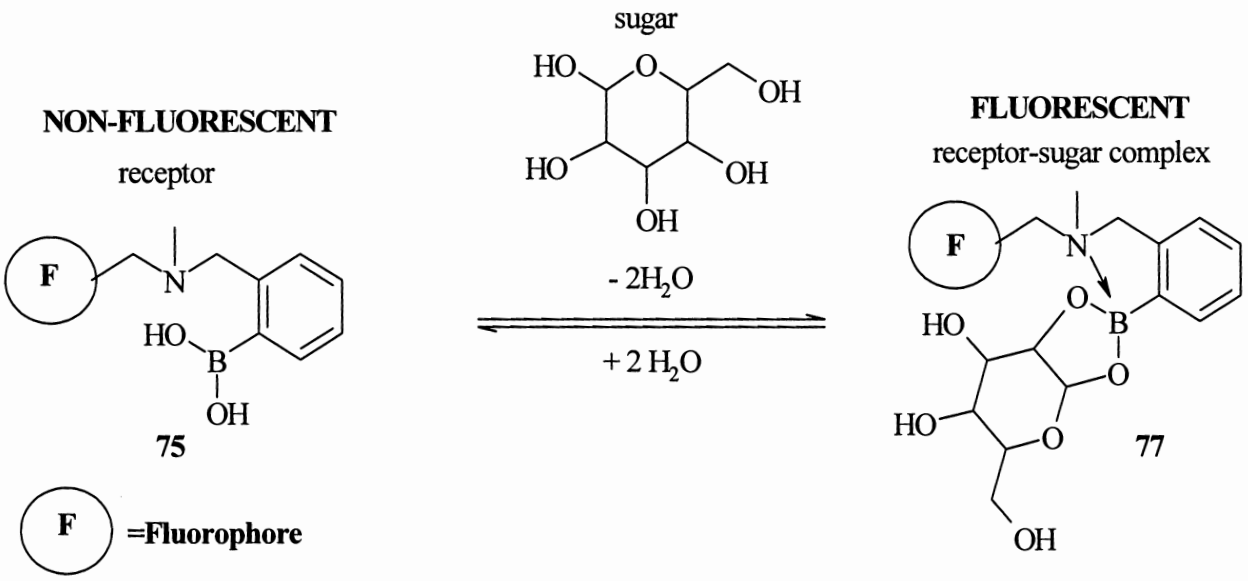

Figure 32. Binding interaction between a simple carbohydrate and a fluorescent sensor.

The addition of carbohydrate to the reaction mixture maximized fluorescence. Unbound receptor (**95**) would quench fluorescence and lower the measured intensity (I/I_o). A maximum signal indicated that all of the boronic acid receptor was bound to saccharide in the reaction mixture, preventing PET from quenching the signal. Reactions were performed in 99:1 10 mM phosphate buffer (pH=7.8):methanol. This mixture contains more water than previous experiments, which used 90:10 10 mM phosphate buffer (pH=7.8):methanol.[121] The higher water content provided a more biologically relevant environment. In one experimental method, a known concentration of fluorescent receptor was added to the stock solution and the binding constants calculated were reproducible at a boronic acid concentration of 10^{-5} M. The carbohydrate of interest was added directly to the 10^{-5} M boronic acid solution and fluorescence intensity was measured. When the signal no longer increased with additional sugar, the receptor was 100% bound to carbohydrate in solution. A second method was developed to validate the data obtained by the first method. A stock solution with fixed boronic acid concentration (10^{-5} M) and a fixed carbohydrate concentration (1.0 M) was prepared. Fluorescence intensity was measured at an excitation frequency of 387 nm as the 1.0 M carbohydrate solution was added in small portions to a stock solution with the same fixed boronic acid concentration, but no carbohydrate. Based on calculations from the previous method, a solution with 10^{-5} M boronic acid and 1.0 M carbohydrate produces a maximum fluorescence signal. Adding this solution to a solution containing boronic acid alone in small portions verified binding constants from the previous method. A plot of the fluorescence intensity versus carbohydrate concentration is shown in Figure 34. The

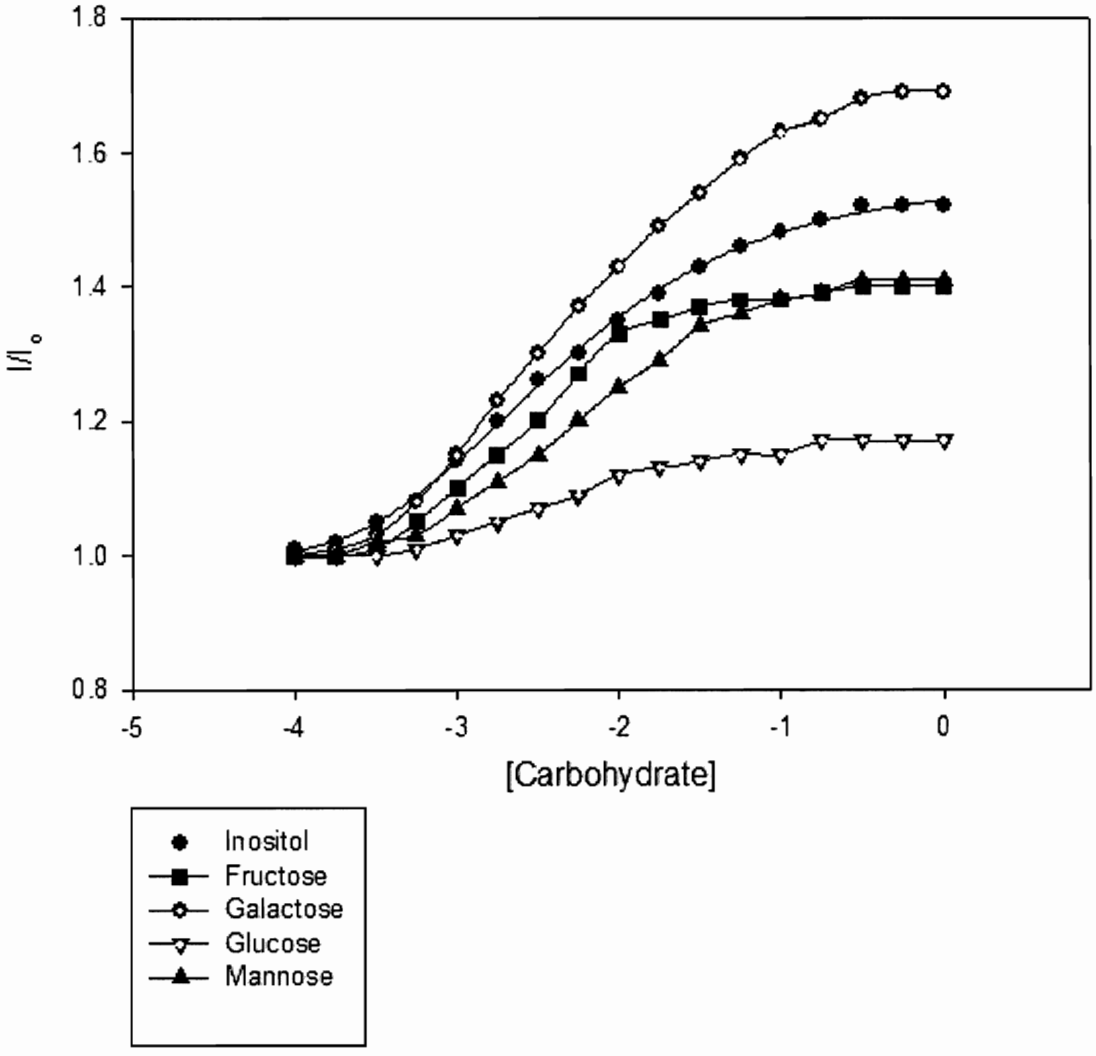

Figure 33. Plot of fluorescence intensity versus sugar concentration for binding with **95**.

Table 5. Calculated binding constants for the fluorescent receptor (**95**).

Carbohydrate	Binding Constant	I/I_o
Inositol	354	1.52
Fructose	316	1.40
Galactose	233	1.69
Glucose	200	1.17
Mannose	178	1.41

binding constants were calculated based on the concentration of each species when the carbohydrate concentration was 50% of the concentration used to obtain a maximum signal. At one-half of the intensity change, it was assumed that the receptor is 50% bound. These values were used in the equilibrium constant equation (**1**) to generate a binding constant for the saccharide-boronic acid interactions.

The K values for the binding constants are similar to those from previous experiments (Table 5). The constants obtained in this study for glucose and mannose are an improvement over the constants obtained by Shinkai for his receptor.[121] The method described above provided the first reported binding constant for *myo*-inositol.[135] The K value obtained for galactose and is less than the value calculated for phenylboronic acid by Lorand and Edwards.[102] The K value for glucose is considerably higher, with the binding to mannose being approximately equal. The fluorescence intensity of the bound complex versus the unbound receptor (I/I_o) were within the reported range,[121] with glucose being the lowest in these experiments.

Recently, James has produced a pyren-1-yl, diboronic acid structure with a significant improvement in the binding constants reported previously.[119,121] This work has promise for the development of diboronic acids for sensing more complex saccharide structures, which remains the long-term goal of this laboratory.[136] The solubility of **95** in the 99:1 H_2O/MeOH system was surprising because of the presence of the anthracene ring system. The solubility provided the opportunity to work in a system closer to the *in vivo* environment of biological systems where these receptors would be utilized. Overall, the new receptor gave binding data similar to previous carbohydrate binding

molecules.[126] The 2-formylphenylboronic acid were incorporated with boronic acids to form fluorescent sensors in greater yield than previously reported methods.[119, 120, 121] The synthesis and utilization of 9-[[N-benzyl-N-(o-boronbenzyl)amino]methyl]-anthracene (**95**) as a receptor for simple carbohydrates in this study provides an improved method for further fluorescence quenching studies.

Summary

A tetrasaccharide fragment of LAM of *M. tuberculosis*, **10**, has been synthesized in nine steps and 14 % total yield. While the bridgehead[33] and various arabinans[36] have been previously synthesized, this is the first synthesis of any fragment of the mannan core of LAM. The synthetic strategy used here is amenable to the construction of larger oligosaccharides through the use of a common intermediate **27** as a precursor to glycosyl acceptors and donors for further synthesis. This molecule is a fragment of a longer repeating oligosaccharide unit which is recognized by the human macrophage mannose receptor in the process of phagocytosis of MTB. This carbohydrate moiety may have some utility in blocking the macrophage or as a tool in stimulating the immune system when conjugated to a carrier protein. These carbohydrate fragments or analogues thereof may possess some additional utility in investigating the roles of LAM in mycobacterial infection and chemotheraputic strategies against MTB.

A set of squalamine derivatives has been synthesized in varying yields from 5α-cholestan-3-one in two steps. The initial step was reductive amination of the ketone with an excess of an oligomeric amine. The final step was a second reductive amination of the terminal primary amine with OFPBA (**55**). These aminosterols may be able to permeate the fatty acid layers of the mycobacterial cell membrane. It is known that the presence of cholesterol is required for phagocytosis of MTB. The boronic acids may serve as a delivery system for the drug to the mycobacterial surface that may enhance its biological activity. These compounds may also serve as part of a larger database of compounds from which more detailed structure-activity relationships may be determined.

A fluorescent receptor for carbohydrates, 9-[[N-benzyl-N-(o-boronbenzyl)amino]methyl]anthracene (**96**) has been successfully synthesized in two steps and 72% total yield. This is an improvement on the synthesis of a similar receptor, which was synthesized in three steps and 28% total yield. Reductive amination with OFPBA was demonstrated as a single-step, high-yield method to these receptor through the addition of arylboronic acids to amines.[111] Our receptor was shown to have binding constants similar to existing fluorescent probes in solvent systems with 90% lower methanol content than previous experiments. Future directions would include receptors with multiple boronic acids for detection of carbohydrates which play specific roles in disease, such as tumor antigens[39,40],or bacterial determinants such as LAM[4].

Experimental Section

General Methods. ^{1}H NMR and ^{13}C NMR spectra were recorded at ambient temperature on a 300 MHz Varian spectrometer. Chemical shifts were internally referenced to the residual proton resonance in $CDCl_3$ (δ=7.24) or with tetramethylsilane (TMS) as an internal standard. Coupling constants (*J*) were reported in Hertz (Hz). All melting points were taken on a Mel-Temp II melting point apparatus. IR spectra were recorded on an Avatar 320 FT-IR. Mass spectra were obtained on a Micromass Quatro 2 time-of-flight mass spectrometer by Dr. Lambert Ngoka at VCU. Dichloromethane from CaH_2, THF from Na/Benzophenone, and pyridine from KOH were distilled prior to use. All reactions were run under atmospheric conditions unless otherwise noted. All reactions were monitored by thin-layer chromatography (TLC) using Whatman 20 x 20 cm silica gel 1B-F plates. TLC results were viewed by UV light and $KMnO_4$ stain. All solvents were obtained from Fisher Scientific and used without purification unless otherwise noted.

2:3, 4:6-Di-*O*-benzylidene-α-D-methylmannopyranoside, 11. α-D-Methyl-mannopyranoside (**6**) (3.5 g, 19.4 mmoles, Aldrich) was added to PhCHO (30 mL, Fisher) and $ZnCl_2$ (4.0 g, 29.6 mmoles, Fisher). The reaction mixture was allowed to stir for 20 h. Water was added and the biphasic mixture was cooled to 0^{o} for 30 min. The reaction mixture solidified and was filtered and rinsed with water (2 x 30 mL) and hexanes (2 x 30 mL) and was recrystallized from chloroform. The overall yield was 6.2 g (93%) of a white solid, **11**. mp 93-94 ° C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.49-7.15

(10 H, m, Ar-H), 5.51 (2 H, s, two Ph-CH-), 4.94 (1 H, s, H1), 4.28 (1 H, dd, *J*=5.4 Hz, H-5), δ=4.03 (2 H, m, H-3, H-4), δ=3.86 (3 H, m, H-2), δ=3.39 (3 H, s, -OCH_3); IR (cm^1): 3032 (C-H), 2940 (C-H), 2862 (C-H), 1190 (C-O), 1026 (C-O).

Attempted syntheses of 3,4-Di-*O*-benzyl-α-D-methylmannopyranoside, 12.

A) $AlCl_3$, Me_3NBH_3, Toluene: 4:6, 2:3-Di-*O*-benzylidene-α-D-methyl-mannopyranoside (**11**) (185 mg, 0.5 mmoles) was dissolved in dry toluene (15 mL). $AlCl_3$ (790 mg, 6.0 mmoles, 12 eq, Lancaster) and Me_3NBH_3 (420 mg, 6.0 mmoles, 12 eq, Aldrich) were added and the solution was allowed to stir at room temperature overnight under N_2. Thin-layer chromatography showed no reaction progress. The mixture was heated at reflux for 5 days with no reaction progress based on TLC. An aliquot was removed and concentrated for ^{1}H NMR analysis. No benzyl peaks could be detected by ^{1}H NMR at 4.5 ppm; the benzylidene peaks were present in the proper integration for the dibenzylidene starting material.

B) $AlCl_3$,Me_3NBH_3,Et_2O/CH_2Cl_2: 4:6,2:3-Di-*O*-benzylidene-α-D-methylmannopy-ranoside (**11**) (185 mg, 0.5 mmoles) was dissolved in 1:1 Et_2O/CH_2Cl_2 (20 mL) $AlCl_3$ (792 mg, 6.0 mmoles, 12 eq, Lancaster) and Me_3NBH_3 (420 mg, 6.0 mmoles, 12 eq, Aldrich) were added and the solution was allowed to stir at room temperature overnight under N_2. Thin-layer chromatography showed no reaction progress. The mixture was heated at reflux for 3 days with no reaction progress demonstrated by TLC. An aliquot was removed and concentrated for ^{1}H NMR analysis. No benzyl peaks were detected by

^{1}H NMR at 4.5 ppm; the benzylidene peaks were present in the proper integration for the dibenzylidene starting material.

C) $AlCl_3$, BH_3/THF : 4:6, 2:3-Di-*O*-benzylidene-α-D-methyl-mannopyranoside (**11**) (185 mg, 0.5 mmoles) was dissolved in THF (20 mL). $AlCl_3$ (792 mg, 6.0 mmoles, 12 eq, Lancaster) and BH_3:THF (5.0 mL, 5.0 mmoles, 10 eq, Aldrich) were added and allowed to stir at room temperature for 3 days under N_2 with periodic determinations of reaction progress by TLC. After no progress was shown by TLC, an aliquot was removed and concentrated for ^{1}H NMR analysis. No benzyl peaks were detected by ^{1}H NMR at 4.5 ppm.

D) $AlCl_3$, $NaCNBH_3$, CH_2Cl_2 : 4:6, 2:3-Di-*O*-benzylidene-α-D-methyl-mannopyranoside (**11**) (185 mg, 0.5 mmoles) was dissolved in CH_2Cl_2 (20 mL) $AlCl_3$ (792 mg, 6.0 mmoles, 12 eq, Aldrich) and $NaCNBH_3$ (370 mg, 6.1 mmoles, 12 eq, Aldrich) were added and the solution was allowed to stir at room temperature overnight under N_2. Thin-layer chromatography showed no reaction progress. The mixture was heated at reflux for 2 days with no reaction progress demonstrated by TLC. An aliquot was removed and concentrated for ^{1}H NMR analysis. No benzyl peaks were detected by ^{1}H NMR at 4.5 ppm. The benzylidene peaks were present in the proper integration for the dibenzylidene starting material.

E) $AlCl_3$, $LiAlH_4$, Et_2O/CH_2Cl_2 : 4:6, 2:3-Di-*O*-benzylidene-α-D-methylmannopyranoside (**11**) (185 mg, 0.5 mmoles) was dissolved in 1:1 Et_2O/CH_2Cl_2

(20 mL) $AlCl_3$ (792 mg, 6.0 mmoles, 12 eq, Aldrich) and 1.0 M solution of $LiAlH_4$ in THF (6.0 mL, 6.0 mmoles, 12 eq, Aldrich) were added and the solution was allowed to stir at room temperature overnight under N_2. Thin-layer chromatography showed no reaction progress. The mixture was heated at reflux for 40 h with no reaction progress demonstrated by TLC. An aliquot was removed and concentrated for ^1H NMR analysis. No benzyl peaks were detected by ^1H NMR at 4.5 ppm. The benzylidene peaks were present in the proper integration for the dibenzylidene starting material.

F) TfOH, BH_3:THF : 4:6, 2:3-Di-*O*-benzylidene-α-D-methyl-mannopyranoside (**11**) (185 mg, 0.5 mmoles) was dissolved in THF (20 mL) . BH_3:THF (5.0 mL, 5.0 mmoles, 10 eq, Aldrich) and TfOH (0.44 mL, 750 mg, 5.0 mmoles, 10 eq, Aldrich) were added and the solution was allowed to stir at room temperature overnight under N_2. Thin-layer chromatography showed no reaction progress. The mixture was heated at reflux for 3 days with no reaction progress demonstrated by TLC. An aliquot was removed and concentrated for ^1H NMR analysis. No benzyl peaks were detected by ^1H NMR at 4.5 ppm. The benzylidene peaks were present in the proper integration for the dibenzylidene starting material.

G) DIBAH, CH_2Cl_2: 4:6, 2:3-Di-*O*-benzylidene-α-D-methyl-mannopyranoside (**11**) (185 mg, 0.5 mmoles) was dissolved in CH_2Cl_2 (20 mL) Diisobutylaluminum hydride (1.0 M solution in CH_2Cl_2, 5.0 mL, 5.0 mmoles, 10 eq) was added and solution was stirred under N_2 with occasional monitoring by TLC. After 6 h, a lower spot on TLC was detected. The reaction was quenched with 10 mL water. Upon quenching, a white solid

formed which was filtered off. The reaction mixture was extracted with water (20 mL), saturated bicarbonate (20 mL), and water (20 mL). Organic extracts were dried with sodium sulfate and concentrated by rotary evaporation. ^{1}H NMR analysis showed a compound different from starting material, but the absence of benzylic peaks at δ=4.5 ppm indicated the lack of expected product.

4,6-*O*-Benzylidene-α-D-methylglucopyranoside, 20. α-D-Methylglucopyranoside (**19**) (11.4 g, 60 mmoles, Aldrich) was added to a stirring mixture of PhCHO (50 mL, Fisher) and $ZnCl_2$ (9.6 g, 71.1 mmoles, Fisher). The reaction mixture was allowed to stir for 20 h. Water was added and the biphasic mixture was cooled to 0° for 30 minutes. The reaction mixture solidified. The solid was filtered and rinsed with water (2 x 30 mL) and hexanes (2 x 30 mL) and was recrystallized from ethanol. The overall yield was 14.0 g (81%) of a white solid, **20;** mp 151-153 °C; ^{1}H NMR, (300 MHz, $CDCl_3$): δ=7.48 (2H, m, ArH), 7.37 (3 H, m, ArH), 5.53 (1H, s, Ph-CH-), 4.76 (1 H, d, *J*=2.0 Hz, H-1), 4.38 (1 H, d, *J*=2.4 Hz, H-2), 4.20 (1 H, dd, *J*=8.6 Hz, 1.8 Hz, H-3), 3.73 (3 H, m, H-6, H-6', H-4), 3.36 (3 H, s, -OCH_3), 3.02 (1H, m, H-5),.

2-*O*-Mesyl-4,6-*O*-benzylidene-α-D-methylglucopyranoside, 21. 4,6-*O*-Benzylidene-α-D-methylglucopyranoside (**20**) (2.83 g, 10.0 mmoles) was dissolved in freshly distilled pyridine (30 mL) and cooled to 0° under N_2. A solution of mesyl chloride (1.0 g, 12.5 mmoles, 1.25 eq, Aldrich) and DMAP (80 mg, catalytic, Aldrich) in freshly distilled pyridine (5.0 mL) was added. The reaction mixture was allowed to stir overnight under N_2. The mixture was concentrated by rotary evaporation and then diluted with

chloroform (100 mL). The diluted mixture was extracted with 1.0 M HCl (2 x 50 mL) and brine (50 mL). The organic extracts were dried with sodium sulfate, and concentrated by rotary evaporation. Purification by column chromatography with 2:3 hexanes/ethyl acetate (R_f=0.78) yielded 2.10 g (58%) of yellow-tinted syrup, **21**; ^{1}H NMR, (300 MHz, $CDCl_3$): 7.46 (2H, m, ArH), 7.31 (3 H, m, ArH), 5.53 (1H, s, Ph-CH-), 4.79 (1 H, d, *J*=2.0 Hz, H-1), 4.48 (1 H, d, *J*=2.4 Hz, H-2), 3.98-3.64 (4 H, m, H-4, H-5, H-6, H-6'), 3.36 (3 H, s, -OCH_3), 2.10 (3H, s, -OSO_2CH_3).

Attempted synthesis of 2-*O*-Acetyl-4,6-*O*-benzylidene-α-D-methylmannopyranoside, 22:

A) Mitsunobu reaction: 2-*O*-Mesyl-4,6-*O*-benzylidene-α-D-methylglucopyranoside (**21**) (180 mg, 0.5 mmoles) was dissolved in THF (15 mL). Sodium acetate (120 mg, 1.5 mmoles, 3 eq, Aldrich), Ph_3P (170 mg, 0.65 mmoles, 1.3 eq, Aldrich), and DEAD (0.1 mL, 113 mg, 0.65 mmoles, 1.3 eq, Aldrich) were added to the reaction mixture and was allowed to stir at room temperature overnight under N_2. Thin-layer chromatography showed a small new spot which was less polar (1:1 hexanes/ethyl acetate), but no product was isolated.

Attempted synthesis of 22 by sodium acetate/dioxane displacement: 2-*O*-Mesyl-4,6-*O*-Benzylidene-α-D-methylglucopyranoside (**21**) (180 mg, 0.5 mmoles) was dissolved in 95% dioxane (20 mL, Fisher). NaOAc (240 mg, 3.0 mmoles, Fisher) was added and the solution was heated at reflux. Thin-layer chromatography showed a small percentage of

conversion to product after 5 days reflux. The reaction was not worked up due to poor yield.

4,6-*O*-Benzylidene-α-D-methylmannopyranoside, 25. α-D-Methylmannopyranoside (**6**) (3.5 g, 19.4 mmoles, Aldrich) was dissolved in formic acid (25 mL, Aldrich). Immediately upon dissolution, PhCHO (25 mL, Fisher) was added and the mixture was allowed to stir for 15 min. The benzaldehyde solution was added dropwise to a rapidly stirring mixture of hexanes (150 mL, Fisher) and $NaHCO_3$ (60 g, Fisher) in water (150 mL). The solution was allowed to stir for 40 minutes and then filtered. The resulting white solid was washed with EtOAc and the filtrate was concentrated. The resulting solid was washed with hexanes and filtered, leaving a white solid, **25** (2.55 g, 50%). R_f=0.68 (1:1 hexanes/ethyl acetate). mp 148-149 °C; ^{1}H NMR, (300 MHz, $CDCl_3$): δ=7.48 (2H, d, *J*=3 Hz, ArH), 7.37 (3 H, m, ArH), 5.57 (1 H, s, Ph-CH-), 4.76 (1 H, d, H-1), 4.28 (1 H, dd, *J*=5.4 Hz, H-2), 4.03 (2 H, m, H-3, H-4), 3.86 (3 H, m, H-5, H-6, H-6'), 3.39 (3 H, s, -OCH_3); ^{13}C NMR, (300 MHz, $CDCl_3$): δ=137.4, 129.5, 129.4, 128.6, 126.5, 126.2, 102.5, 101.6, 79.1, 71.1, 69.1, 68.9, 63.3, 55.4; IR (KBr, cm^{-1}): 3520 (O-H), 3053 (C-H), 2952 (C-H), 1432 (C-H), 1105 (C-O); ESI MS (m/z) $(M + Na)^+$ calcd.306.30, found 306.362.

3-O-Pivaloyl-4,6-*O*-benzylidene-α-D-methylmannopyranoside, 26. 4,6-*O*-Benzylidene-α-D-methylmannopyranoside (**25**) (1.1 g, 4.0 mmoles) was dissolved in CH_3OH (20 mL). Bu_2SnO (1.0 g, 4.0 mmoles, Aldrich) was added and the solution was heated to reflux for 4 h. The reaction mixture was concentrated by rotary evaporation. Freshly

distilled THF (20 mL) and pivaoyl chloride (0.56 mL, 540 mg, 4.5 mmoles) were added and solution was heated at reflux for 18 h. The reaction mixture was concentrated by rotary evaporation and chromatographed on silica gel using 1:1 hexanes/ethyl acetate, R_f= 0.75. This provided 1.1g (75%) of an amorphous solid, **26**. mp 98-100 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.42 (2 H, d, *J*=3Hz, phenyl protons), 7.33 (3 H, m, ArH), 5.57 (1 H, s, Ph-CH-), 5.22 (1H, dd, *J*=3.0 Hz, 10.2 Hz, H-3), 4.75 (1 H, s, H-1), 4.30 (1 H, d, *J*=6.0 Hz, H-2), 4.10 (2 H, dd, *J*=9.0 Hz, 2.8 Hz, H-4, H-5), 3.89 (2 H, m, H-6, H-6'), 3.40 (3 H, s, $-OCH_3$), 1.22 (9 H, s, $-C(CH_3)_3$); ^{13}C NMR, (300 MHz, $CDCl_3$): δ=177.6, 137.5, 129.0, 128.5, 128.3, 126.5, 126.1, 101.8, 101.6, 76.5, 70.0, 69.1, 64.0, 55.3, 39.4, 27.5; IR (KBr, cm^{-1}): 3352 (O-H), 3057 (C-H), 2940 (C-H), 1664 (C=O), 1085 (C-O); ESI MS (m/z) $(M + 2\ Na)^+$ calcd. 406.41, found 406.478.

2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl bromide, 14. 1,2,3,4,6-Penta-O-acetyl-α-D-mannopyranose (**24**) (3.0 g, 16.7 mmoles, Aldrich) was dissolved in CH_2Cl_2 (20 mL) HBr in HOAc (30 %, 4.0 mL) was added through a septum and the reaction mixture was allowed to stir for 4 h. The reaction mixture was extracted with water, saturated sodium bicarbonate, and water. Organic phase was dried with sodium sulfate (Fisher) and concentrated by rotary evaporation, yielding 2.88 g (88 %) as a golden syrup **14**. ^{1}H NMR (300 MHz, $CDCl_3$): δ=6.24 (1H, d, *J*=1.2 Hz, H-1), δ=5.70 (1 H, dd, *J*=3.3 Hz, 10.2 Hz, H-2), 5.38 (2 H, m, H-3, H-4), 4.32 (1 H, dd, *J*=4.5 Hz, 12.5 Hz, H-5), 4.25 (1 H, m, H-6), 4.10 (1 H, m, H-6), 2.14 (3 H, s, $-COCH_3$), 2.08, (3 H, s, $-COCH_3$), 2.06 (3H, s, $-COCH_3$), 2.02 (3 H, s, $-COCH_3$); ^{13}C NMR, (300 MHz, $CDCl_3$): δ=170.6, 169.8, 169.6, 169.5, 83.4, 73.0, 72.3, 68.1, 65.4, 61.7, 21.1, 20.97, 20.95, 20.87; IR (KBr, cm^{-1}):

2966 (C-H), 1720 (C=O), 1385 (C-O); ESI MS (m/z) $(M + Na - Ac)^+$ calcd.406.17, found 406.94.

2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosy(l → 2)-4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-methylmannopyranoside, 27. 3-O-Pivaloyl-4,6-*O*-benzylidene-α-D-methylmannopyranoside (**26**) (1.1 g, 3.0 mmoles) was dissolved in methylene chloride (15 mL) and covered with aluminum foil. Silver (I) oxide (800 mg, 6.5 mmoles, Aldrich) and anhydrous calcium sulfate (1.0 g, Commercial Drierite) were added and solution was stirred under N_2 for 45 min. After this time, 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl bromide (1.25 g, 3.0 mmoles) (**14**) in methylene chloride (15 mL) and a catalytic amount of iodine were added. The solution was allowed to stir under nitrogen at room temperature for 20 h. After this time, the reaction mixture was filtered through a pad of Celite and concentrated by rotary evaporation. Purification by column chromatography (1:1 hexanes/ethyl acetate, Rf=0.78) yielded 1.88 g (90 %) as a brown syrup, **27**. ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.48 (2 H, d, *J*=3 Hz, ArH), 7.37 (3 H, m, ArH), 5.97 (1 H, s, H-1), 5.58 (1 H, dd, *J*=3 Hz, 10.5 Hz, -CH-Ph), 5.39-5.21 (4 H, m), 4.72 (1 H, s), 4.31-4.19 (4 H, m), 4.13-4.00 (4 H, m), 3.88-3.83 (1 H, m), 3.36 (3 H, s), 2.20, 2.16, 2.08, 2.05 (3 H each, s, $-C(O)OCH_3$), 1.22 (9 H, s, $-C(O)C(CH_3)_3$); ^{13}C NMR, (300 MHz, $CDCl_3$): 177.6, 171.0, 170.4, 170.2, 169.9, 137.5, 129.0, 128.5, 128.3, 126.5, 126.1, 101.8, 101.6, 98.8, 70.0, 69.1, 68.6, 66.5, 66.4, 65.8, 64.0, 62.9, 62.8, 55.3, 39.4, 27.5; IR (KBr, cm^{-1}): 3016 (C-H), 2942 (C-H), 1735 (C=O), 1120 (C-O); ESI MS (m/z) $(M + Na - \text{terminal carbohydrate})^+$ calcd.372.42, found 372.379.

2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosy(l →2)-3-*O*-pivaoyl-α-D-methylmanno-pyranoside, 29. A 1:1 solution (10 mL) of TFA (Aldrich)/water was added to **27** (1.05 g ,1.5 mmoles) and stirred for 10 min. The reaction mixture was concentrated by rotary evaporation. Column chromatography (1:1 hexanes/ethyl acetate, Rf=0.58) of the residue provided 820 mg (90%) **29** as a light brown syrup. ^{1}H NMR (300 MHz, $CDCl_3$): δ=6.09 (1 H, d, *J*=2.1Hz, H-1)), 5.41 (1H, dd, *J*=3.3 Hz, 10.2 Hz, H-1'), 5.35-5.25 (4 H, m), 4.72 (1 H, s), 4.31-4.22 (4 H, m), 4.18-4.09 (4 H, m), 3.69-3.67 (1 H, m), 3.40 (3 H, s, $-OCH_3$), 2.17, 2.12, 2.09, 2.02 (3H each, s, $-C(O)CH_3$), 1.22 (9 H, s, $-C(O)C(CH_3)_3$); ^{13}C NMR, (300 MHz, $CDCl_3$): δ=171.0, 170.4, 170.2, 169.9, 98.8, 92.4, 70.4, 69.8, 69.3, 69.1, 68.6, 66.5, 66.4, 65.8, 62.9, 62.8, 55.6, 27.5, 21.3, 21.1; IR (KBr, cm^{-1}): 3426 (O-H), 2953 (C-H), 1742 (C=O), 1405 (C-H), 1229 (C-O); ESI MS (m/z) (M + Na - terminal carbohydrate)$^{+}$ calcd.372.42, found 372.612.

2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl(l → 2)- 4,6-di-*O*-acetyl-3-*O*-pivaoyl-α-D-methylmannopyranoside, 30. Compound **29** (290 mg, 0.42 mmoles) was dissolved in Ac_2O (10 mL). NaOAc (300 mg, 3.7 mmoles, Fisher) was added and the solution was heated at reflux for 1h. The reaction mixture was poured into water (50 mL) and extracted with CH_2Cl_2 (2 x 20 mL), dried with Na_2SO_4 (Fisher) and concentrated. Purification of the residue by column chromatography (1:1 hexanes/ethyl acetate, Rf=0.71) yielded 260 mg (85%) as a light brown syrup, **30**. ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.90 (1 H, s, H-1), 5.50-5.15 (6 H, m), 4.85 (1 H, m, H-1'), 4.40-3.95 (6 H, m), 3.40 (3 H, s, $-OCH_3$), 2.19, 2,17, 2.10, 2.08, 2.05, 2.01 (3 H ea., s, $-C(O)CH_3$), 1.2 (9 H, s, $-C(O)C(CH_3)_3$); ^{13}C NMR, (300 MHz, $CDCl_3$): δ=170.2, 169.9, 169.8, 169.3,

168.0, 166.4, 100.5, 98.7, 73.1, 70.6, 69.5, 69.1, 68.7, 68.4, 68.2, 65.3, 55.6, 27.3, 26.8, 22.0, 20.82, 20.80, 20.77, 20.71, 20.67; IR (KBr, cm^{-1}): 2988 (C-H), 1754 (C=O), 1396 (C-H), 1054 (C-O); ESI MS (m/z) $(M + Na)^+$ calcd.716.65, found 716.167.

2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl(1 ⟶ 2)- 4,6-di-*O*-acetyl-3-*O*-pivaoyl-1-chloro-α-D-mannopyranose, 31. Compound **30** (280 mg, 0.39 mmoles) was dissolved in chloroform (20 mL). Freshly fused $ZnCl_2$ (Fisher) and DCMME (0.2 mL, Aldrich) were added to the mixture. The reaction mixture was heated at reflux for 70 min, until TLC indicated completion by the presence of a higher running spot (1:1 hexanes/ethyl acetate, Rf=0.88). The reaction mixture was extracted with water (20 mL) and saturated bicarbonate (20 mL). Organics were dried with sodium sulfate and concentrated by rotary evaporation, yielding 210 mg (74%) as a yellow syrup, **31**. ^1H NMR (300 MHz, $CDCl_3$): δ=6.19 (1 H, s, H-1), 5.48-5.12 (6 H, m), 4.83 (1 H, m, H-1'), 4.42-3.98 (6 H, m), 2.18, 2,17, 2.11, 2.07, 2.05, 2.00 (3 H ea., s, $-C(O)CH_3$), 1.2 (9 H, s, $-C(O)C(CH_3)_3$); ^{13}C NMR, (300 MHz, $CDCl_3$): δ=170.5, 169.9, 169.7, 169.5, 168.1, 166.5, 98.5, 90.5, 73.1, 70.7, 69.5, 69.2, 68.9, 68.4, 68.3, 66.2, 65.5, 27.1, 26.9, 22.2, 20.9, 20.82, 20.77, 20.73, 20.69. IR (KBr, cm^{-1}): 2974 (C-H), 1745 (C=O), 1391 (C-H), 1075 (C-O); ESI MS (m/z) (M + Na - terminal carbohydrate)$^+$ calcd.414.80, found 414.263.

2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosy(1 ⟶ 2)-4,6-O-diacetyl-3-O-pivaoyl-α-D-mannopyranosyl(1⟶ 6)-2-O-(2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl)-3-O-pivaoyl-α-D-methylmannopyranoside, 32. In a 100 mL round-bottomed flask,

compound **28** (175 mg, 0.26 mmoles) was dissolved in chloroform (20 mL) and covered with aluminum foil. Silver (I) oxide (320 mg, 2.6 mmoles) and anhydrous calcium sulfate (commercial Drierite) were added to the flask and the reaction was allowed to stir for 45 min. Compound **31** (210 mg, 0.31 mmoles) and a catalytic amount of iodine in methylene chloride (20 mL) were added to the reaction flask and the solution was allowed to stir for 20 h. After this time, the reaction mixture was filtered through a pad of Celite and concentrated by rotary evaporation. Purification by column chromatography (1:1 hexanes/ethyl acetate, Rf=0.73) yielded 260 mg (90 %) as a brown syrup, **32**. ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.92 (1 H, s), 5.88 (1 H, s), 5.52-5.09 (12 H, m), 4.89 (1 H, s), 4.84 (1 H, s), 4.45-3.92 (12 H, m), 3.42 (3 H,s, $-OCH_3$), 2.19, 2.17, 2.16, 2.15, 2.13, 2.11, 2.10, 2.09, 2.06, 2.04 (3 H, ea., s, $-C(O)CH_3$), 1.2 (9H, s, $-C(O)C(CH_3)_3$); ^{13}C NMR, (300 MHz, $CDCl_3$): δ=170.5, 169.9, 169.7, 169.5, 168.6, 168.1, 167.5, 166.8, 166.5, 100.9, 100.2, 98.8, 97.3, 73.1, 70.7, 70.1, 69.8, 69.6, 69.5, 69.4, 69.2, 68.9, 68.7, 68.4, 68.3, 67.9, 67.7, 67.3, 66.2, 66.0, 65.8, 65.5, 55.3, 27.1, 26.9, 22.2, 21.0, 20.92, 20.88, 20.86, 20.82, 20.77, 20.75, 20.73, 20.71, 20.69. IR (KBr, cm^{-1}): 3315 (O-H), 3046 (C-H), 2940 (C-H), 1762 (C-H), 1422 (C-H), 1095 (C-O).

α-D-Mannopyranosyl(1 → 2)-α-D-mannopyranosyl(1 → 6)-2-O-(α-D-mannopyranosyl)-α-D-mannopyranose, 10. In a 50 mL round bottomed flask, compound **32** (260 mg, 0.2 mmoles) was dissolved in methylene chloride (20 mL) and cooled to –40 C^{o} in a dry ice/acetone bath. BBr_3 (0.04 mL, 102 mg, 0.4 mmoles, 1.86 eq, Aldrich) was added and allowed to equilibrate to room temperature over 2 h under N_2. The reaction mixture was extracted with water (50 mL) and the with saturated

bicarbonate (50 mL). Organics were dried with sodium sulfate (Fisher), filtered, and concentrated by rotary evaporation. The crude product was dissolved in 1:10 21% sodium ethoxide/ethanol (10 mL) and stirred until reactant disappeared on TLC. Dowex 50W-X8 (Mallinkrodt) ion exchange resin was added until the mixture was neutralized. The reaction mixture was filtered and concentrated by rotary evaporation. Purification by flash column (10% CH_3OH/methylene chloride) yielded 110 mg (82% over two steps) as a clear syrup, **10**. ^1H NMR (300 MHz, D_2O): δ=5.29 (d, J = 1.2 Hz, 1H), 5.09 (d, J = 1.2 Hz, 1H), 5.01 (d, J = 1.5 Hz, 1H), 4.41 (d, J = 7.9 Hz, 1H), 4.11 (dd, J = 1.8, 3.1 Hz, 1H), 4.05-4.04 (m, 1H), 4.00-3.50 (m, 24H), 2.99-2.95 (m, 2H), 1.65-1.60 (m, 5H), 1.38 (br s, 4H); ^{13}C NMR, (300 MHz, $CDCl_3$): δ=103.7, 102.8, 101.2, 98.4, 79.3, 79.1, 77.3, 75.9, 73.8, 73.1, 72.0, 71.6, 70.9, 70.5, 70.5, 69.6, 69.2, 68.6, 67.7, 67.5, 61.8, 61.8, 61.7, 60.9. IR (KBr, cm^{-1}): 3523 (O-H), 2968 (C-H), 1443 (C-H); ESI MS (m/z) (M + Na - terminal dissacharide)$^+$ calcd.406.34, found 406.088.

Cholesteryl bromide, 49: In a 50 mL round bottomed flask, cholesterol (**47**) (380 mg, 1.0 mmoles, Aldrich) was dissolved in CH_2Cl_2 (10 mL) and cooled to 0 °C. Phosphorous tribromide (270 mg, 0.10 mL, 1.0 mmoles, Aldrich) was added and stirred at 0 °C under N_2 overnight. The reaction mixture was slowly poured into ice water; the water was decanted and the organic portion was extracted with bicarbonate and dried with Na_2SO_4. Rotary evaporation provided 268 mg (71%) of an off-white solid **49**. ^1H NMR, (300 MHz, $CDCl_3$): δ=5.34 (1 H, t, J=4.0 Hz), 3.92 (1 H, m), 2.05-0.60 (43 H, m).

Attempted substitution reaction: In a 50 mL round bottomed flask, compound **49** (220 mg, 0.50 mmoles, synthetic) was dissolved in $CHCl_3$. Ethylene diamine (300 mg, 0.34 mL, 5.0 mmoles, Aldrich) was added and the solution was heated at reflux for 72 h. The reaction mixture was extracted with 1 M HCl and water. The organics were dried and concentrated by rotary evaporation. ^1H NMR showed only starting material. The reaction was repeated with the exception that ethylenediamine was used as the solvent (10 mL). The reaction mixture was heated at reflux for 72 hours. The reaction mixture was partitioned between $CHCl_3$ and 1 M HCl and extracted. The organics were dried and concentrated. ^1H NMR again indicated only starting material was in the spectrum.

3-Methoxycholesterol, 51: In a 50 mL round bottomed flask, cholesterol (**47**) (385 mg, 1.0 mmole, Aldrich) was dissolved in freshly distilled THF (10 mL). NaH was added in small amounts until the mixture no longer emitted bubbles upon addition. The reaction mixture was stirred at room temperature under N_2 for 10 min, then MeI (280 mg, 0.12 mL, 2.0 mmoles, Aldrich) was added through a septum and was allowed to stir overnight under N_2 . The reaction was quenched with water and extracted with CH_2Cl_2 (10 mL). The organic extracts were dried with Na_2SO_4, filtered, and concentrated by rotary evaporation providing 320 mg (80%) **51** as a white powder. mp 250-253 °C; ^1H NMR, (300 MHz, $CDCl_3$): δ=5.36 (1 H, t, *J*=3.8 Hz), 3.38 (3 H, s), 3.08 (1 H, m), 2.21-1.01 (45 H, m).

Attempted catalytic hydrogenation of 51: In a 50 mL round bottomed flask, compound **51** (100 mg, 0.25 mmoles, synthetic) was dissolved in freshly distilled THF (10 mL) under N_2. $NiCl_2$ (32 mg, 0.25 mmoles, Lancaster) was added and the reaction mixture was cooled to –70 °C with a dry ice/acetone bath. After cooling for 30 min, 1.0 M LAH/ CH_2Cl_2 (0.25 mL) was added. The reaction mixture-stirred overnight under N_2 and was allowed to warm to room temperature. The reaction was quenched with ice water and extracted with CH_2Cl_2 (2 x 10 mL). Organics were dried and concentrated by rotary evaporation. ^{1}H NMR indicated only starting material in the spectrum.

3-Methoxy-5,6-dibromocholesterol, 53: In a 50 mL round bottomed flask, compound **47** (320 mg, 0.80 mmoles, synthetic) was dissolved in CH_2Cl_2 (10 mL). Br_2 (30%) in CH_2Cl_2 was added until the solution remained orange. The reaction mixture was allowed to stir for 1 h and the flask was cooled with an ice bath to precipitate product. The product was precipitated from the mixture and recrystallized from CH_2Cl_2 to provide 510 mg (91%) **53** as white crystals, mp 165-168 °C. ^{1}H NMR, (300 MHz, $CDCl_3$): δ=3.78 (1H, m), 3.24 (3H, s), 2.12-0.76 (44 H, m).

Attempted reduction of 53: In a 50 mL round bottomed flask, compound **53** (275 mg, 0.50 mmoles, synthetic) was dissolved in freshly distilled THF (15 mL) and cooled to –70 °C with a dry ice/acetone bath under N_2. After cooling for 30 minutes, 1.0 M LAH/ CH_2Cl_2 (1.0 mL) was added and the reaction mixture was allowed to stir under under N_2 for 24 h and warm to room temperature over that time. The reaction was quenched with ice water and extracted with CH_2Cl_2. Organics were dried and concentrated. ^{1}H NMR

indicated that the expected product was not present; compound **51** was present in the sample (the original alkene).

3-*N*-(*o*-Boronbenzyl)methylamino-1-propene, 57: In a 100 mL round bottomed flask, OFPBA (**55**) (150 mg, 1.0 mmol, Aldrich) was dissolved in MeOH (15 mL) and allylamine was (**56**) (107 mg, 1.0 mmol, 0.11 mL, Aldrich) added to the reaction mixture. After 3 h, $NaBH_4$ (57 mg, 1.5 mmoles, Aldrich) was added to the reaction mixture and allowed to stir overnight. The mixture was concentrated by rotary evaporation. CH_2Cl_2 (10 mL) was added to the flask and stirred for 5 min. The solution was filtered, and the filtrate was concentrated to yield 180 mg (94%) of a clear oil, **57**. ^{1}H NMR (300 MHz, CD_3OD): δ=7.43 (1 H, t, *J*=4.0 Hz), 7.17 (3 H, m), 6.06 (1 H, m), 5.38 (2 H, m), 3.96 (2 H, s), 3.45 (2 H, d); ^{13}C NMR (300 MHz, $CDCl_3$): δ=132.3, 130.5, 127.5, 126.8, 122.7, 119.9, 53.1, 50.2.

2-*N*-((*o*-Boronbenzyl)methylamino)-2-methylpropane, 59: In a 100 mL round bottomed flask, OFPBA (**55**) (150 mg, 1.0 mmol, Aldrich) was dissolved in MeOH (15 mL) and *t*-butylamine (**58**) (73 mg, 1.0 mmol, 0.11 mL, Aldrich) was added. After 3 h, $NaBH_4$ (57 mg, 1.5 mmoles, Aldrich) was added to the reaction mixture and allowed to stir overnight. The mixture was concentrated by rotary evaporation. CH_2Cl_2 (10 mL) was added to the flask and stirred for 5 min. The solution was filtered and the filtrate was concentrated by rotary evaporation to yield 154 mg (94%) of a clear oil, **59**. ^{1}H NMR (300 MHz, CD_3OD): δ= 7.78 (1 H, d, *J*=6.9 Hz), 7.42 (3 H, m), 3.36 (2 H, s), 1.32 (9H, s); ^{13}C NMR (300 MHz, CD_3OD): δ=134.8, 129.9, 127.2, 126.0, 54.9, 48.0, 25.4.

***N*-((*o*-Boronbenzyl)methylamino)diphenylmethane, 61**: In a 100 mL round bottomed flask, OFPBA (**55**) (150 mg, 1.0 mmol, Aldrich) was dissolved in MeOH (15 mL) and diphenylaminomethane (**60**) (186 mg, 1.0 mmol, 0.17 mL, Aldrich) was added. After 3 h, $NaBH_4$ (57 mg, 1.5 mmoles, Aldrich) was added to the reaction mixture and allowed to stir overnight. The mixture was concentrated by rotary evaporation. Methylene chloride (10 mL) was added to the flask and stirred for 5 min. The solution was filtered and the filtrate was concentrated by rotary evaporation to yield 185 mg (91%) of an opaque solid, **61**. mp 80-82 °C; ^{1}H NMR (300 MHz, CD_3OD): δ=7.48-7.18 (14 H, m), 5.13 (2 H, s), 3.67 (3 H, s); ^{13}C NMR (300 MHz, CD_3OD): δ=145.6, 142.7, 141.5, 134.9, 129.4, 128.3, 128.2, 128.0, 127.0, 126.9, 126.8, 126.0, 65.4, 53.8.

***N*-((*o*-Boronbenzyl)methylamino)-2-methyl-1-propanol, 63:** In a 100 mL round bottomed flask, OFPBA (**55**) (150 mg, 1.0 mmol, Aldrich) was dissolved in MeOH (15 mL) and 2-methyl-2-amino-1-propanol (**62**) (89 mg, 0.09 mL, 1.0 mmol, Aldrich) was added. After 3 h, $NaBH_4$ (57 mg, 1.5 mmoles, Aldrich) was added to the reaction mixture and allowed to stir overnight. The mixture was concentrated by rotary evaporation. CH_2Cl_2 (10 mL) was added to the flask and stirred for 5 min. The solution was filtered and the filtrate was concentrated by rotary evaporation to yield 162 mg (90%) of a golden oil, **63**. ^{1}H NMR (300 MHz, CD_3OD): δ= 7.36 (3 H, m), 7.15 (1 H, d, *J*=6.0 Hz), 4.11 (2 H, s), 3.63 (2H, d, *J*=9.3 Hz), 1.43 (3 H, s), 1.12 (3 H, s).

Ethylene diamine conjugate of 5α-cholestan-3-one, 65: In a 50 mL round bottomed flask, 5α-cholestan-3-one (**64**) (190 mg, 0.5 mmoles, Aldrich) was dissolved in $CHCl_3$ (5 mL). Ethylenediamine (180 mg, 3.0 mmoles, 0.20 mL, Aldrich) and $NaCNBH_3$ (150 mg, 2.5 mmoles, Aldrich) in MeOH (5 mL) were added and the mixture was heated at reflux for 48 h. After this time, the reaction mixture was diluted with $CHCl_3$ (20 mL) and extracted with water, dilute HCl, and water. The organic extracts were dried with sodium sulfate (Fisher). Concentration of the filtrate yielded **65** as a white powder (185 mg, 92%); mp 124-126 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ= 2.82 (4 H, t, *J*=6.0 Hz, NH_2-C**H$_2$**-); 2.00-0.58 (47 H, m). ^{13}C NMR (300 MHz, $CDCl_3$): δ=56.8, 56.6, 54.8, 49.1, 49.0, 48.3, 46.4, 45.7, 40.9, 40.4, 39.8, 36.1, 35.8, 34.2, 34.1, 31.10, 31.08, 28.6, 28.4, 24.6, 24.2, 23.4, 23.2, 22.9, 19.0, 12.5; IR (KBr, cm^{-1}): 3473 (N-H), 3118 (O-H), 2942 (C-H), 1522 (N-H), 1460 (C-H).

1,3-Diaminopropane conjugate of 5α-cholestan-3-one, 66: In a 50 mL round bottomed flask, 5α-cholestan-3-one (**64**) (190 mg, 0.5 mmoles, Aldrich) was dissolved in $CHCl_3$ (5 mL). 1,3-Diaminopropane (222 mg, 3.0 mmoles, 0.24 mL, Aldrich) and $NaCNBH_3$ (150 mg, 2.5 mmoles, Aldrich) in MeOH (5 mL) was added and the mixture was heated at reflux for 48 h. After this time, the reaction mixture was diluted with $CHCl_3$ (20 mL) and extracted with water, dilute HCl, and water. The organic extracts were dried with sodium sulfate (Fisher). Rotary evaporation of the solvent yielded 188 mg **66** (89%) as an off-white solid, mp 130-132 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.73 (4 H, t, *J*=3.5 Hz, NH_2-C**H$_2$**-), 1.90 (2 H, q, *J*=2.9 Hz, NH_2-CH_2-C**H$_2$**-), 2.10-0.70 (47 H,

m); ^{13}C NMR (300 MHz, $CDCl_3$): δ=58.3, 56.7, 52.9, 43.1, 40.8, 40.6, 40.5, 40.3, 37.8, 35.5, 35.7, 36.0, 33.7, 33.5, 33.1, 32.8, 29.6, 29.4, 29.3, 28.5, 26.2, 24.3, 23.9, 22.7, 22.5, 20.9, 19.0, 12.7, 12.6, 12.4; IR (KBr, cm^{-1}): 3445 (N-H), 3148 (O-H), 2954 (C-H), 1496 (N-H), 1352 (C-H); ESI MS (m/z) $(M + Na - C_3H_8N)^+$ cacl.424.73, found 423.449.

1,4-Diaminobutane conjugate of 5α-cholestan-3-one, 67: In a 50 mL round bottomed flask, 5α-cholestan-3-one (**64**) (190 mg, 0.5 mmoles, Aldrich) was dissolved in $CHCl_3$ (5 mL) 1,4-Diaminobutane (264 mg, 0.30 mL, 3.0 mmoles, Aldrich) and $NaCNBH_3$ (150 mg, 2.5 mmoles, Aldrich) in MeOH (5 mL) was added and the mixture was heated at reflux for 48 h. After this time, the reaction mixture was diluted with $CHCl_3$ (20 mL) and extracted with water, dilute HCl, and water. The organic extracts were dried with sodium sulfate (Fisher). Rotary evaporation of the solvent yielded 220 mg **67** (90%) as an off-white solid; mp 141-143 °C; ^1H NMR (300 MHz, $CDCl_3$): δ=2.82 (m, 4 H, NH_2-CH_2-), 1.95 (dd, 4 H, *J*=10.3 Hz, 1.9 Hz, NH_2-CH_2-CH_2-), 2.05-0.58 (47 H, m); ^{13}C NMR (300 MHz, $CDCl_3$): δ=54.8, 54.2, 52.1, 49.6, 47.5, 47.2, 45.6, 41.3, 38.5, 38.3, 38.2, 37.3, 36.8, 36.1, 35.9, 33.9, 33.3, 32.7, 27.8, 23.9, 23.8, 22.3, 22.2, 21.6, 21.5, 19.6, 19.1, 17.5, 12.2; IR (KBr, cm^{-1}): 3375 (N-H), 3159 (O-H), 2932 (C-H), 1503 (N-H), 1392 (C-H); ESI MS (m/z) $(M + Na)^+$ cacl.488.82, found 488.766.

N-(3-aminopropyl)-1,3-propanediamine conjugate of 5α-cholestan-3-one, 68: In a 50 mL round bottomed flask, 5α-cholestan-3-one (**64**) (190 mg, 0.5 mmoles, Aldrich)

was dissolved in $CHCl_3$ (10 mL). N-(3-aminopropyl)-1,3-propanediamine (390 mg, 3.0 mmoles, 0.40 mL, Aldrich) and $NaCNBH_3$ (150 mg, 2.5 mmoles, Aldrich) in MeOH (5 mL) was added and the mixture was heated at reflux for 48 h. After this time, the reaction mixture was diluted with $CHCl_3$ (20 mL) and extracted with water, dilute HCl, and water. The organic extracts were dried with sodium sulfate (Fisher). Rotary evaporation of the solvent yielded 216 mg **68** (86%) as a golden-brown powder, mp 116-117 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.9 (4H, m, -NH-CH_2-), 2.70 (4H, t, NH_2-CH_2-), 1.95 (4 H, dd, *J*=9.8 Hz, 2.1 Hz, -CH_2-C**H$_2$**-CH_2-), 2.00-0.61 (47 H, m); ^{13}C NMR (300 MHz, $CDCl_3$): δ= 62.3, 58.7, 56.3, 56.2, 54.7, 54.5, 49.7, 49.4, 45.8, 45.7, 45.6, 42.9, 40.3, 39.9, 37.5, 35.8, 35.6, 35.4, 34.7, 33.7, 33.5, 32.5, 28.5, 283, 28.1, 26.1, 26.0, 24.7, 24.6, 21.9, 21.7, 19.5, 12.2; IR (KBr, cm^{-1}): 3470 (N-H), 3295 (O-H), 2991 (C-H), 1569 (N-H), 1412 (C-H); ESI MS (m/z) $(M + Na - C_2H_6N)^+$ cacl.503.87, found 503.791.

Triethylene tetraamine conjugate of 5α-cholestan-3-one, 69: In a 50 mL round bottomed flask, 5α-cholestan-3-one (**64**) (190 mg, 0.5 mmoles, Aldrich) was dissolved in $CHCl_3$ (10 mL). Triethylenetetraamine (730 mg , 5.0 mmoles, 0.75 mL, 60% technical grade Aldrich) and $NaCNBH_3$ (150 mg, 2.5 mmoles, Aldrich) in MeOH (5 mL) was added and the mixture was heated at reflux for 48 h. After this time, the reaction mixture was diluted with $CHCl_3$ (20 mL) and extracted with water, dilute HCl, and water. The organic extracts were dried with sodium sulfate (Fisher). Rotary evaporation of the solvent yielded 130 mg **69** as a brown syrup (52%), ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.81 (2H, t, -NH_2-C**H$_2$**-CH_2-NH-), 2.77 (2H, t, -NH_2-CH_2-C**H$_2$**-NH-), 2.67 (8H, m), 2.08-0.66

(47 H, m); ^{13}C NMR (300 MHz, $CDCl_3$): δ=56.8, 56.5, 54.7, 48.8, 46.2, 45.6, 44.5, 42.8, 40.3, 39.8, 36.3, 36.1, 35.8, 35.6, 32.4, 31.1, 31.0, 30.2, 29.2, 28.5, 28.3, 27.7, 24.5, 24.1, 22.3, 23.1, 22.9, 21.4, 19.0, 12.7, 12.4; IR (KBr, cm^{-1}): 3502 (N-H), 3318 (O-H), 2983 (C-H), 1544 (N-H), 1376 (C-H); ESI MS (m/z) $(M + Na - C_2H_6N)^+$ cacl.503.84, found 503.713.

2-*N*-(*o*-Boronbenzyl)methylethylenediamine conjugate of 5α-cholestan-3-one, 70: In a 50 mL round bottomed flask, compound **65** (100 mg, 0.23 mmoles) was dissolved in 1:1 $CHCl_3$:MeOH (10 mL). OFPBA (**55**) (35 mg, 0.25 mmoles, Aldrich) was added and the solution was allowed to stir overnight. $NaBH_4$ (50 mg, 1.33 mmoles) was added and the solution was allowed to stir for 4h. The mixture was concentrated by rotary evaporation. CH_2Cl_2 (10 mL) was added to the flask, stirred for 5 min and filtered. The filtrate was removed by rotary evaporation to yield 98 mg (76%) **70** as a light brown solid; mp 159-164 °C; ^1H NMR (300 MHz, $CDCl_3$): 7.40-7.04 (4H, m, ArH)), 3.90 (2H, s, Ar-CH_2-NH-), 3.20-2.85 (4H, m, -NH-CH_2-), 2.12-0.56 (47 H, m); ^{13}C NMR, (300 MHz, $CDCl_3$): δ=128.7, 124.5, 121.0, 118.0, 56.8, 56.6, 54.8, 49.1, 49.0, 48.3, 46.4, 45.7, 40.9, 40.4, 39.8, 36.5, 36.1, 35.8, 34.2, 34.1, 31.1, 28.6, 28.4, 24.6, 24.2, 23.4, 23.2, 22.9, 19.0, 12.5; IR (KBr, cm^{-1}): 3542 (N-H), 3386 (O-H), 2942 (C-H), 1522 (N-H), 1372 (C-H); ESI MS (m/z) $(M + Na - B(OH)_2)^+$ calcd. 543.89, found 540.53.

3-*N*-(*o*-Boronbenzyl)methylpropylenediamine conjugate of 5α-cholestan-3-one, 71: In a 50 mL round bottomed flask, compound **66** (100 mg, 0.23 mmoles) was dissolved in 1:1 $CHCl_3$:MeOH (10 mL). OFPBA (**55**) (35 mg, 0.25 mmoles, Aldrich) was added and

the solution was allowed to stir overnight. $NaBH_4$ (50 mg, 1.33 mmoles) was added and the solution was allowed to stir for 4h. The mixture was concentrated by rotary evaporation. CH_2Cl_2 (10 mL) was added to the flask, stirred for 10 min and filtered. The solvent was removed by rotary evaporation to yield 115 mg (87%) **71** as an off-white powder; mp 168-173 °C; ^{1}H NMR (300 MHz, $CDCl_3$): 7.23 (1 H, m, ArH)), 7.18 (1 H, m, ArH), 7.05 (2 H, m, ArH)) 3.35 (2 H, s, Ar-CH_2-NH-), 2.75 (4 H, m, -NH-CH_2-), 2.10-.055 (47 H, m) ; ^{13}C NMR, (300 MHz, $CDCl_3$): δ=135.2, 130.5, 126.5, 122.7, 58.3, 54.5, 52.6, 43.7, 40.8, 40.7, 40.1, 39.9, 39.7, 37.4, 36.3, 36.0, 35.8, 33.8, 33.4, 33.0, 33.5, 28.9, 28.6, 28.1, 26.4, 24.1, 23.8, 22.5, 22.1, 21.6, 21.4, 19.4, 12.3, 12.2, 11.9; IR (KBr, cm^{-1}): 3468 (N-H), 3320 (O-H), 2960 (C-H), 1547 (N-H), 1394 (C-H) ; ESI MS (m/z) $(M + Na - B(OH)_2)^+$ calcd.540.74, found 540.69.

4-*N*-(*o*-Boronbenzyl)methyl-1,4-diaminobutane conjugate of 5α-cholestan-3-one, 72: In a 50 mL round bottomed flask, compound **67** (100 mg, 0.22 mmoles) was dissolved in 1:1 $CHCl_3$:MeOH (10 mL). OFPBA (**55**) (35 mg, 0.25 mmoles, Aldrich) was added and the solution was allowed to stir overnight. $NaBH_4$ (50 mg, 1.33 mmoles) was added and the solution was allowed to stir for 4h. The mixture was concentrated by rotary evaporation. CH_2Cl_2 (15 mL) was added to flask, stirred for 10 min, and filtered. The solvent was removed from the filtrate by rotary evaporation to yield 105 mg (81%) **72** as a light brown powder ; mp 164-167 °C; ^{1}H NMR (300 MHz, $CDCl_3$): 7.36-7.01 (4H, m, ArH), 3.93 (2H, s, Ar-CH_2-NH-), 2.82 (4 H, m), 2.65 (4 H, m), 2.00-0.58 (47 H, m); ^{13}C NMR, (300 MHz, $CDCl_3$): δ=136.0, 132.3, 128.6, 123.8, 57.3, 56.9, 56.8, 53.8, 51.9, 49.8, 48.7, 46.3, 41.1, 38.6, 38.5, 38.4, 38.2, 37.3, 36.9, 36.3, 36.0, 34.1, 33.3, 32.7, 31.7,

31.6, 27.8, 23.9, 23.7, 2.3, 20.9, 20.7, 19.2, 18.8, 17.5, 12.3; IR (KBr, cm^{-1}): 3466 (N-H), 3275 (O-H), 2912 (C-H), 1645(N-H), 1403 (C-H); ESI MS (m/z) $(M + Na - B(OH)_2)^+$ calcd. 576.95, found 576.347.

3-*N*-(*o*-Boronbenzyl)methyldipropylenetriamine conjugate of 5α-cholestan-3-one, 73: In a 50 mL round bottomed flask, compound **68** (100 mg, 0.20 mmoles) was dissolved in 1:1 $CHCl_3$:MeOH (10 mL). OFPBA (**55**) (35 mg, 0.25 mmoles, Aldrich) was added and the solution was allowed to stir overnight. $NaBH_4$ (50 mg, 1.33 mmoles) was added and the solution was allowed to stir for 6 h. The mixture was concentrated by rotary evaporation. CH_2Cl_2 (15 mL) was added to flask, stirred for 10 min and filtered. The solvent was removed from the filtrate by rotary evaporation to yield 100 mg (78%) **73** as a brown solid; mp 156-159°C; ^1H NMR (300 MHz, $CDCl_3$): 7.22 (1 H, m, ArH), 7.10-7.02 (3 H, m, ArH), 3.95 (2H, s, Ar-CH_2-NH-), 2.82 (8H, m), 2.02-0.61 (47 H, m); ^{13}C NMR, (300 MHz, $CDCl_3$): δ=136.3, 131.8, 127.9, 123.2, 61.8, 59.4, 56.3, 56.2, 54.0, 53.8, 51.7, 51.4, 44.8, 44.7, 44.6, 43.0, 39.2, 39.0, 37.5, 35.8, 35.5, 35.2, 34.4, 31.4, 31.1, 30.5, 28.7, 28.2, 28.1, 27.2, 24.2, 24.0, 22.7, 22.5, 21.0, 20.9, 19.3, 12.0; IR (KBr, cm^{-1}): 3502 (N-H), 3348 (O-H), 2957 (C-H), 1542 (N-H), , 1361 (C-H); ESI MS (m/z) $(M + Na - OH)^+$ calcd.645.85, found 645.946.

***N*-(*o*-Boronbenzyl)methyltriethylenetetramine conjugate of 5α-cholestan-3-one, 74**: In a 50 mL round bottomed flask, compound **74** (100 mg, 0.19 mmoles) was dissolved in 1:1 $CHCl_3$:MeOH (15 mL). OFPBA (**55**) (35 mg, 0.25 mmoles, Aldrich) was added and the solution was allowed to stir overnight. $NaBH_4$ (50 mg, 1.33 mmoles) was added and

the solution was allowed to stir for 6 h. The mixture was concentrated by rotary evaporation. CH_2Cl_2 (15 mL) was added to flask, stirred for 5 min, and filtered. The solvent was removed from the filtrate by rotary evaporation to yield 98 mg (79%) **74** as a dark syrup; ^{1}H NMR (300 MHz, $CDCl_3$): 7.49-6.87 (4H, m, ArH), 3.98 (2H,s, Ar-CH_2-NH-), 3.60-3.42 (6H, m), 2.63-2.45 (6H, m), 2.11-0.56 (47 H, ,m); ^{13}C NMR, (300 MHz, $CDCl_3$): δ=138.6, 125.5, 124.3, 122.7, 54.0, 53.6, 53.4, 49.2, 46.2, 45.7, 44.3, 38.3, 37.9, 37.1, 36.4, 36.2, 35.6, 34.9, 34.1, 33.4, 32.3, 32.1, 28.5, 22.2, 21.5, 21.3, 20.9, 20.6, 19.2, 18.6, 18.2, 17.4, 12.2; IR (KBr, cm^{-1}): 3552 (N-H), 3386 (O-H), 2901 (C-H), 1586 (N-H), 1408 (C-H); ESI MS (m/z) $(M + Na - 2\ OH)^+$ cacl. 645.85, found 646.41.

9-[(*N*-Benzylamino)methyl]anthracene, 94. In a 100 mL round bottomed flask, 9-anthraldehyde (**92**) (515 mg, 2.5 mmoles, Aldrich) was dissolved in 1:1 CH_2Cl_2/CH_3OH (20 mL). Benzylamine (**93**) (0.27 mL, 270 mg, 2.5 mmoles, Fisher) was added and the solution was allowed to stir overnight. $NaBH_4$ (200 mg, 5.26 mmoles, Aldrich) was added and the mixture was allowed to stir for 5 h. The reaction mixture was concentrated by rotary evaporation. CH_2Cl_2 (20 mL) was added and the solution was stirred for 5 min, then filtered. The filtrate was purified by column chromatography using 2:1 hexanes/ethyl acetate, R_f=0.84. The yield was 695 mg (91%) as a light brown powder, **94**. mp. 148-151 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.40 (1 H, s, ArH), 8.22 (2 H, dd, *J*=9.0 Hz, 1.8 Hz, ArH), 8.08 (m, 2 H, ArH), 7.25-7.55 (m, 9 H, ArH), 4.70 (2 H, d, *J*=10 Hz, NH-CH_2-), 4.05 (2 H, d, *J*=10 Hz, NH-CH_2-). ^{13}C NMR (300 MHz, $CDCl_3$): δ=136.4, 131.9, 131.8, 131.2, 130.5, 130.4, 128.6, 128.5, 128.2, 128.1, 127.9, 127.1,

126.1, 126.0, 125.3, 125.2, 125.1, 125.0, 124.6, 54.3, 50.9. IR (KBr, cm^{-1}): 3057 (N-H), 2962 (C-H), , 1526 (C-H).

9-[[*N*-benzyl-*N*-(*o*-boronbenzyl)amino]methyl]anthracene, 95 In a 100 mL round bottomed flask, 9-[(benzylamino)methyl]anthracene (**94**) (300 mg, 1.0 mmole) was dissolved in 1:1 CH_2Cl_2/MeOH (20 mL). OFPBA (**55**) (150 mg, 1.0 mmole, Aldrich) was added and the solution was allowed to stir overnight under N_2. $NaBH_4$ (100 mg, 2.63 mmoles, Aldrich) was added and the mixture was allowed to stir for 5 h. The reaction mixture was concentrated by rotary evaporation. CH_2Cl_2 (20 mL) was added and the solution was stirred for 5 minutes and filtered. The filtrate was purified by column chromatography using 2:1 Hexanes/EtOAc, R_f=0.15. Yield was 320 mg (79%) of orange syrup **95** ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.40 (1 H, s), 8.22 (2 H, dd, *J*=9.0 Hz, 1.8 Hz), 8.08 (m, 2H), 7.25-7.55 (m, 11 H), 7.15 (1 H, m), 7.05 (1 H, m), 4.80 (2 H, s, NH-CH_2-), 4.70 (2 H, s, NH-CH_2-), 4.05 (2 H, s, NH-CH_2-); ^{13}C NMR (300 MHz, $CDCl_3$): δ=139.5, 131.6, 130.7, 130.3, 129.6, 128.9, 128.6, 128.4, 128.2, 127.2, 125.9, 125.7, 125.0, 124.8, 123.9, 119.9, 69.3, 53.3, 43.6; IR (KBr, cm^{-1}): 3352 (O-H), 3057 (C-H), 2940 (C-H), 1564 (C-H), 1385 (C-H).

Experimental Procedures for Fluorescent Sensor Testing. Method one: a known concentration of fluorescent receptor (10^{-5} M) was added to the 99:1 10 mM phosphate buffer (pH=7.8):methanol solution. Small increments of carbohydrate substrate were added directly to the boronic acid containing solution and dissolved. Fluorescence readings were taken with each addition. When the signal no longer increased with

additional sugar, the concentration and binding constants were tabulated. Method Two: A solution of fixed boronic acid concentration (10^{-5} M) in 99:1 10 mM phosphate buffer (pH=7.8):methanol and fixed carbohydrate concentration (1 M) was added to a solution with the same fixed boronic acid concentration with no carbohydrate. The sugar concentration in the standard solution was higher than necessary to maximize fluorescence intensity. This standard solution was added until the fluorescence intensity of the two solutions was equal. For both methods, samples were irradiated at an excitation frequency of 387 nm. Slit widths were 10 nm and 1.5 nm for excitation and emission respectively. Photomultiplier tube voltage was set at 700 volts for each experiment.

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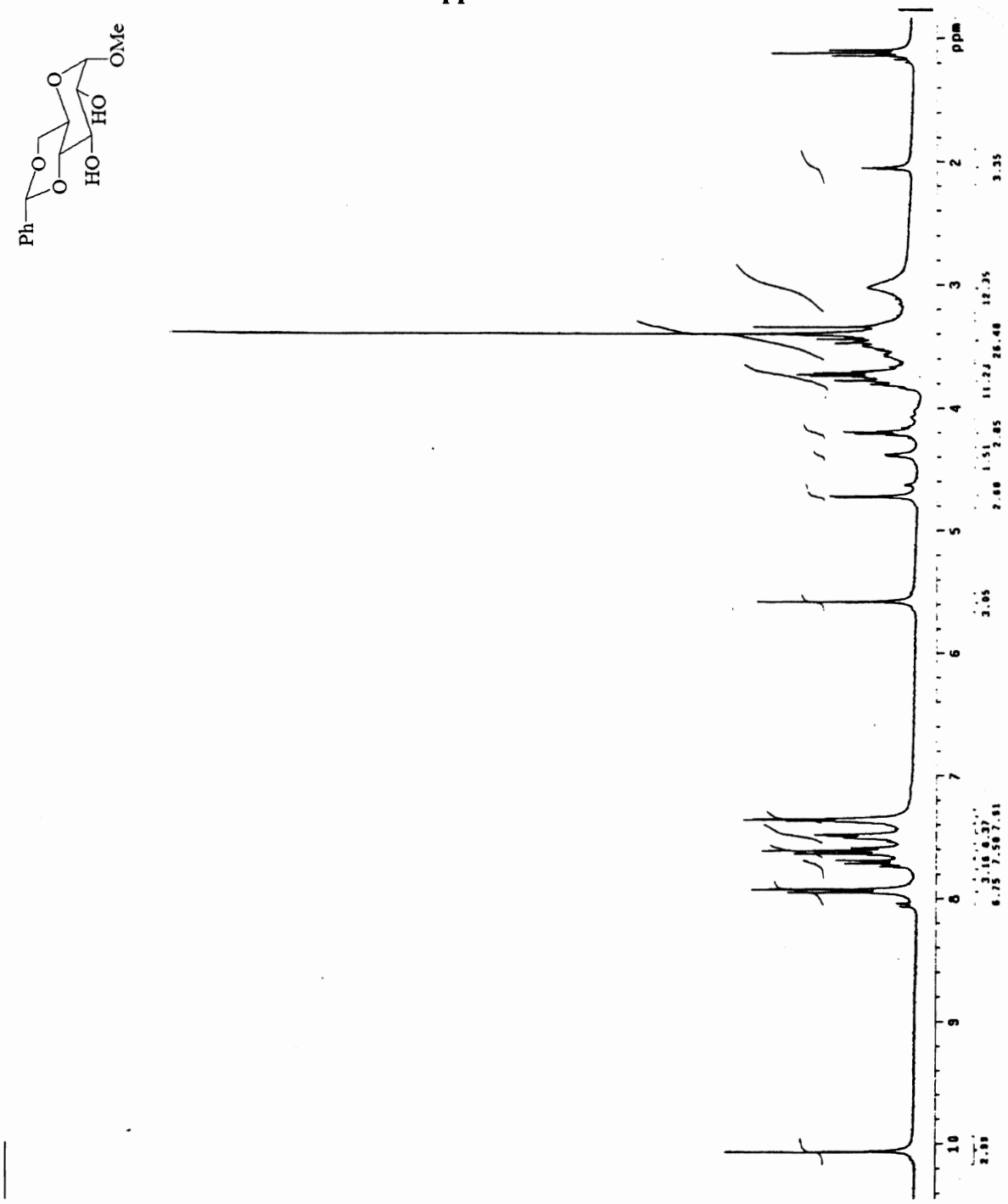
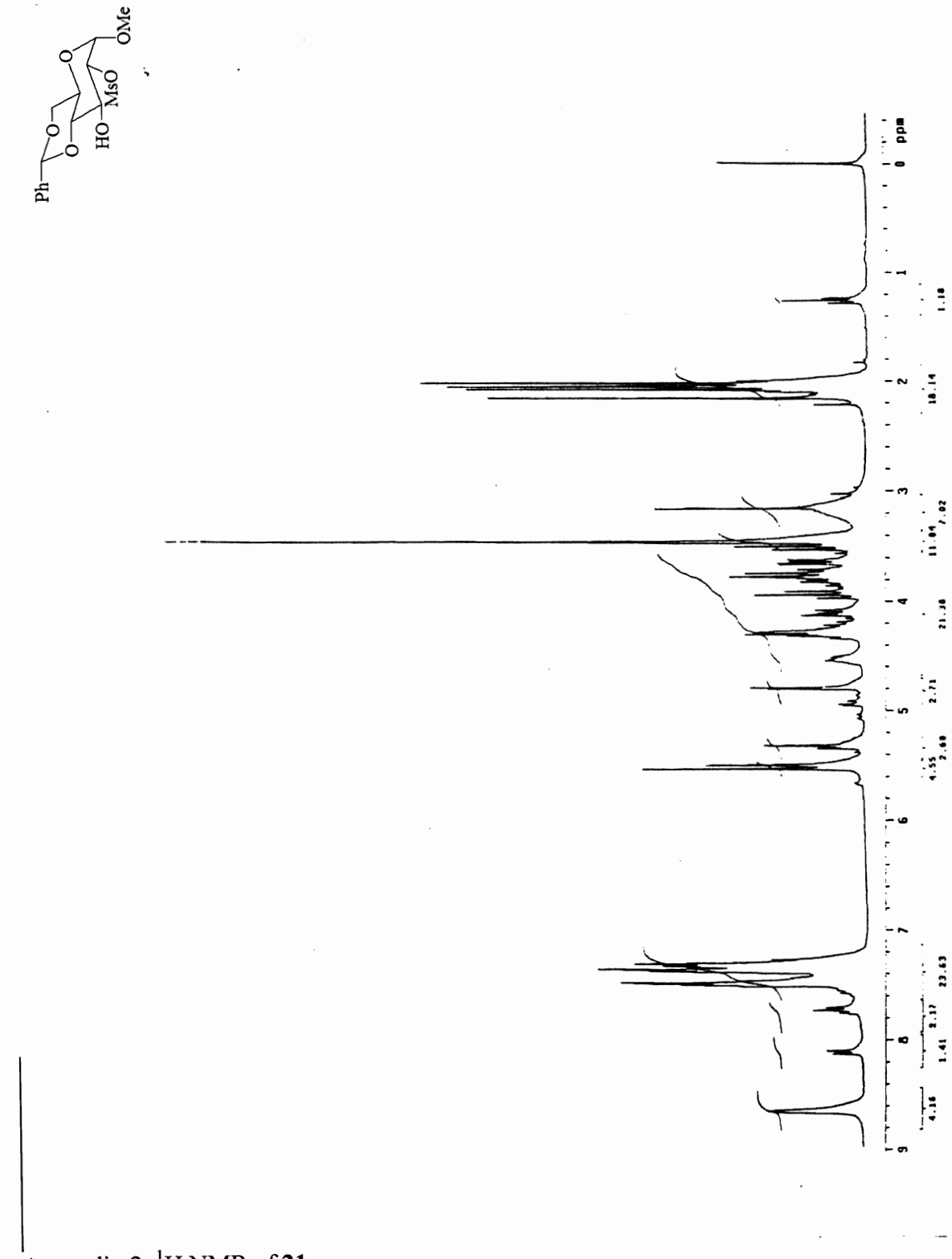
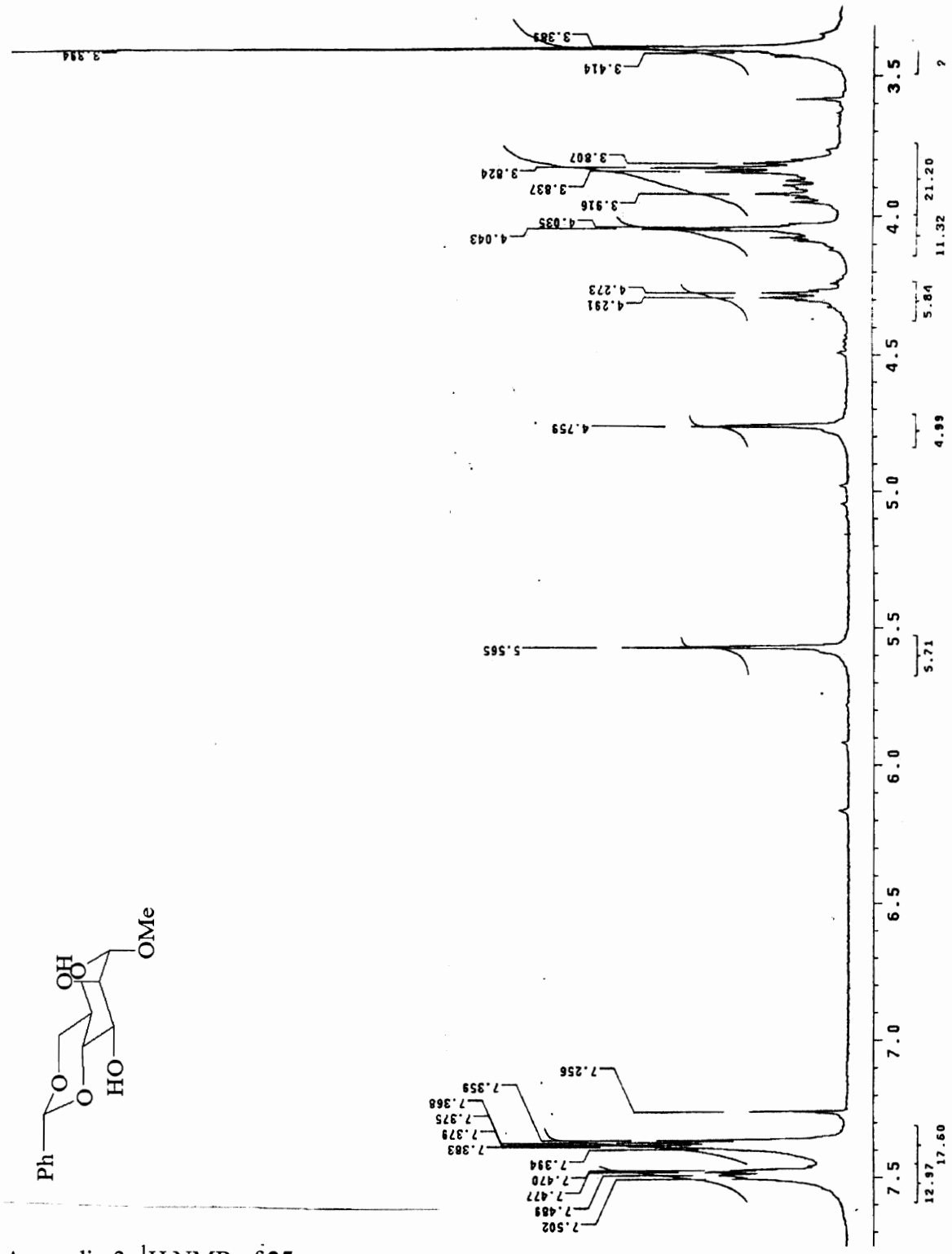
Appendices



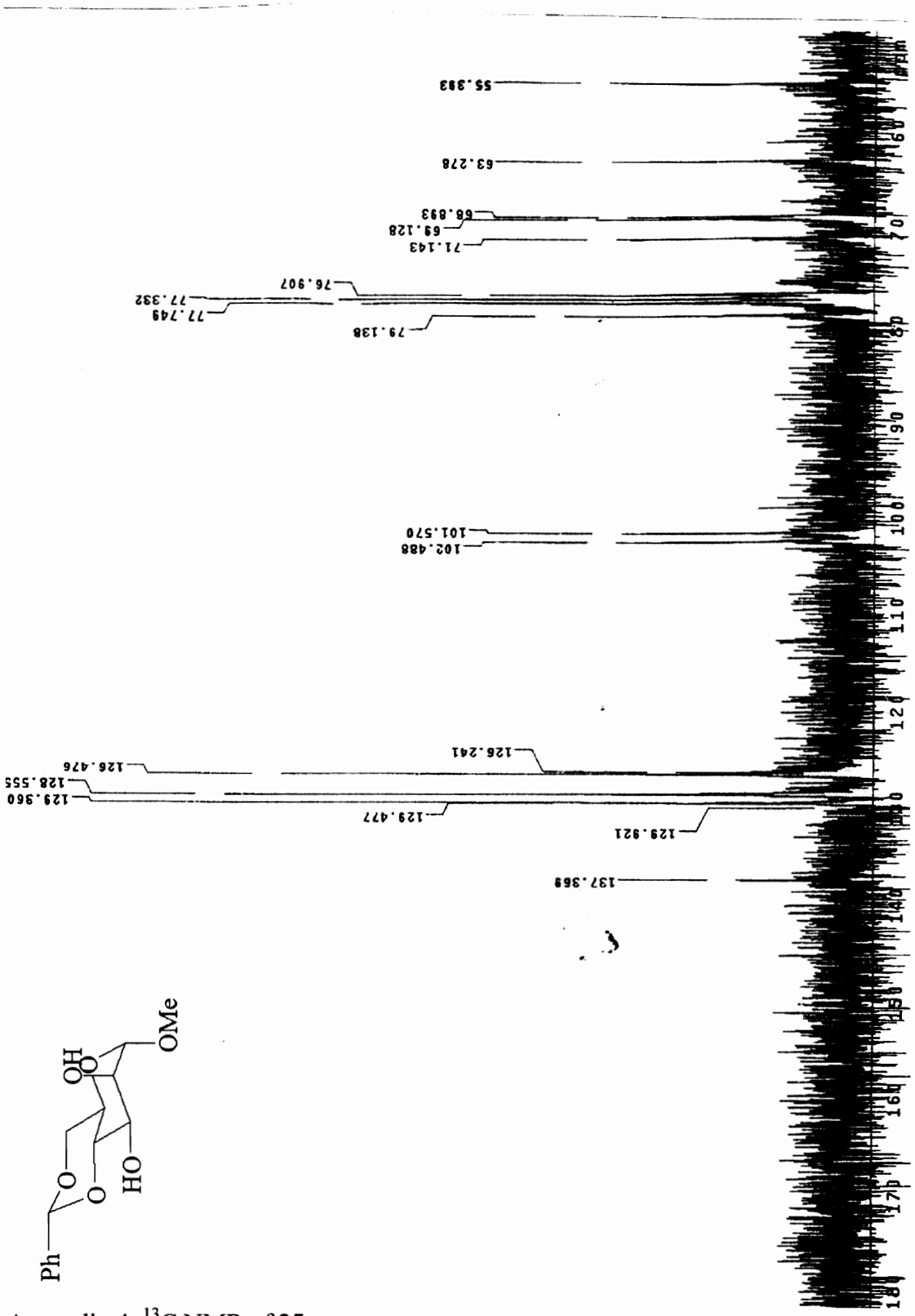
Appendix 1. ^{1}H NMR of **20**.



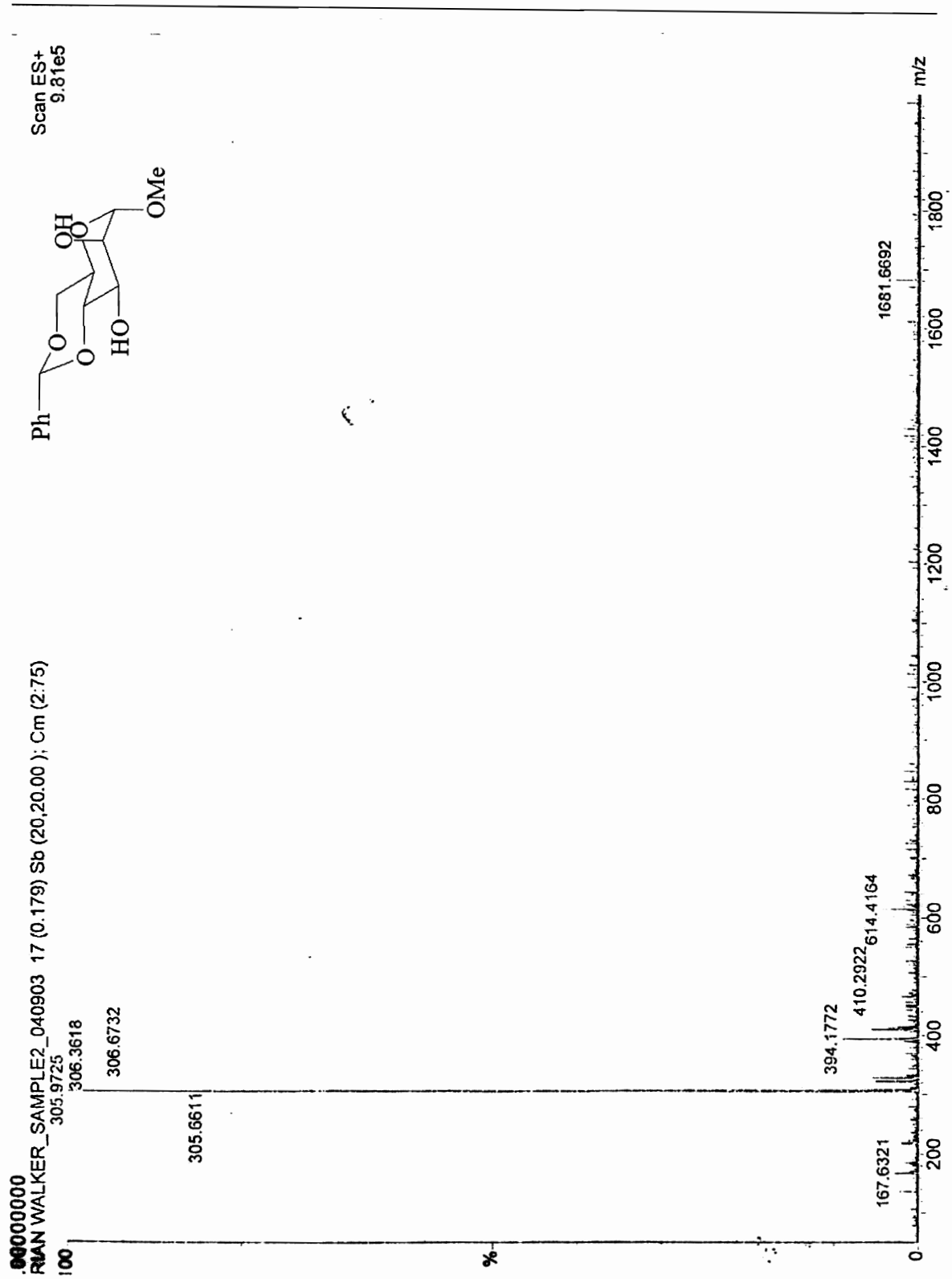
Appendix 2. ^{1}H NMR of **21**.



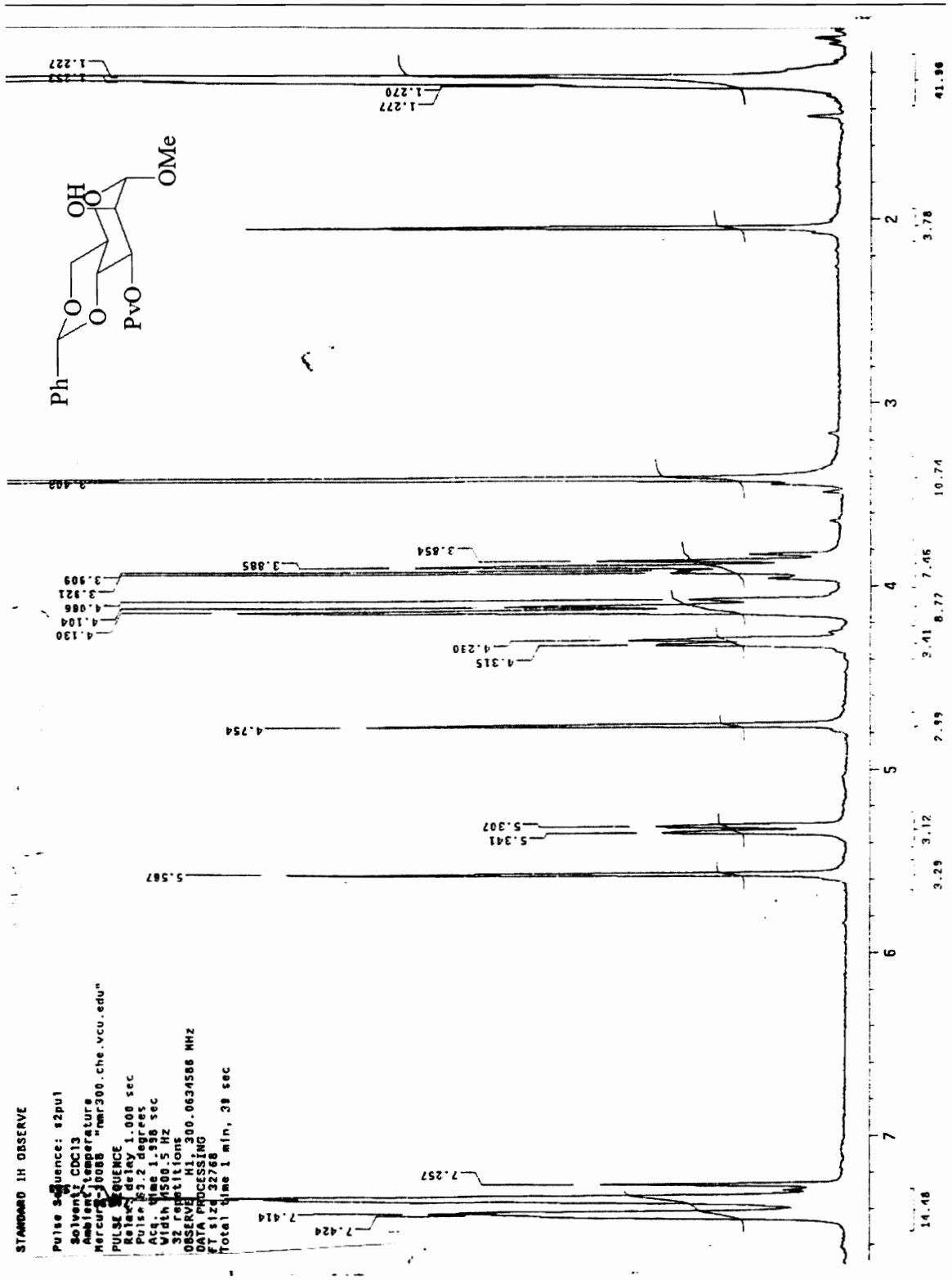
Appendix 3. ^{1}H NMR of **25.**



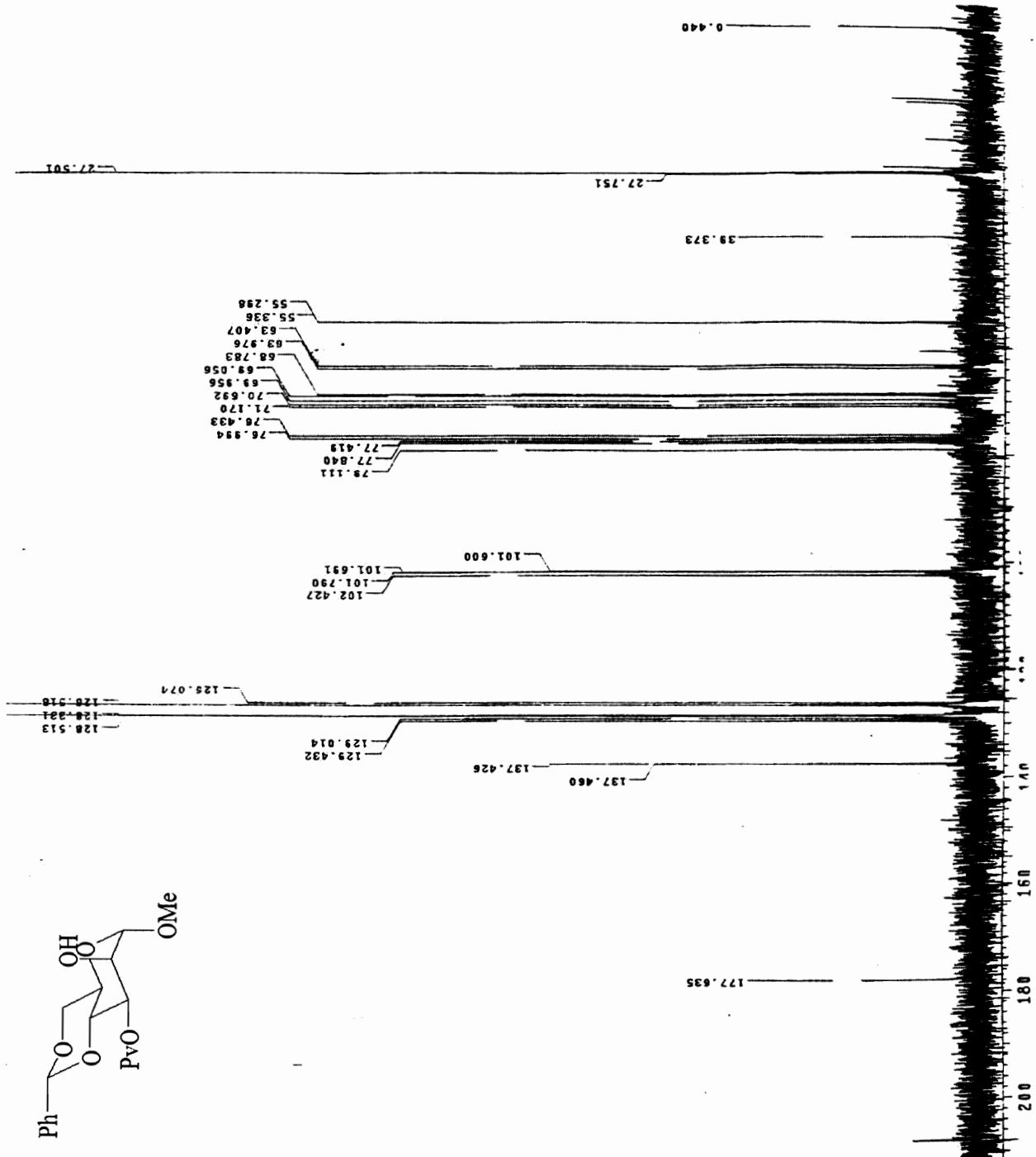
Appendix 4. ^{13}C NMR of **25**.



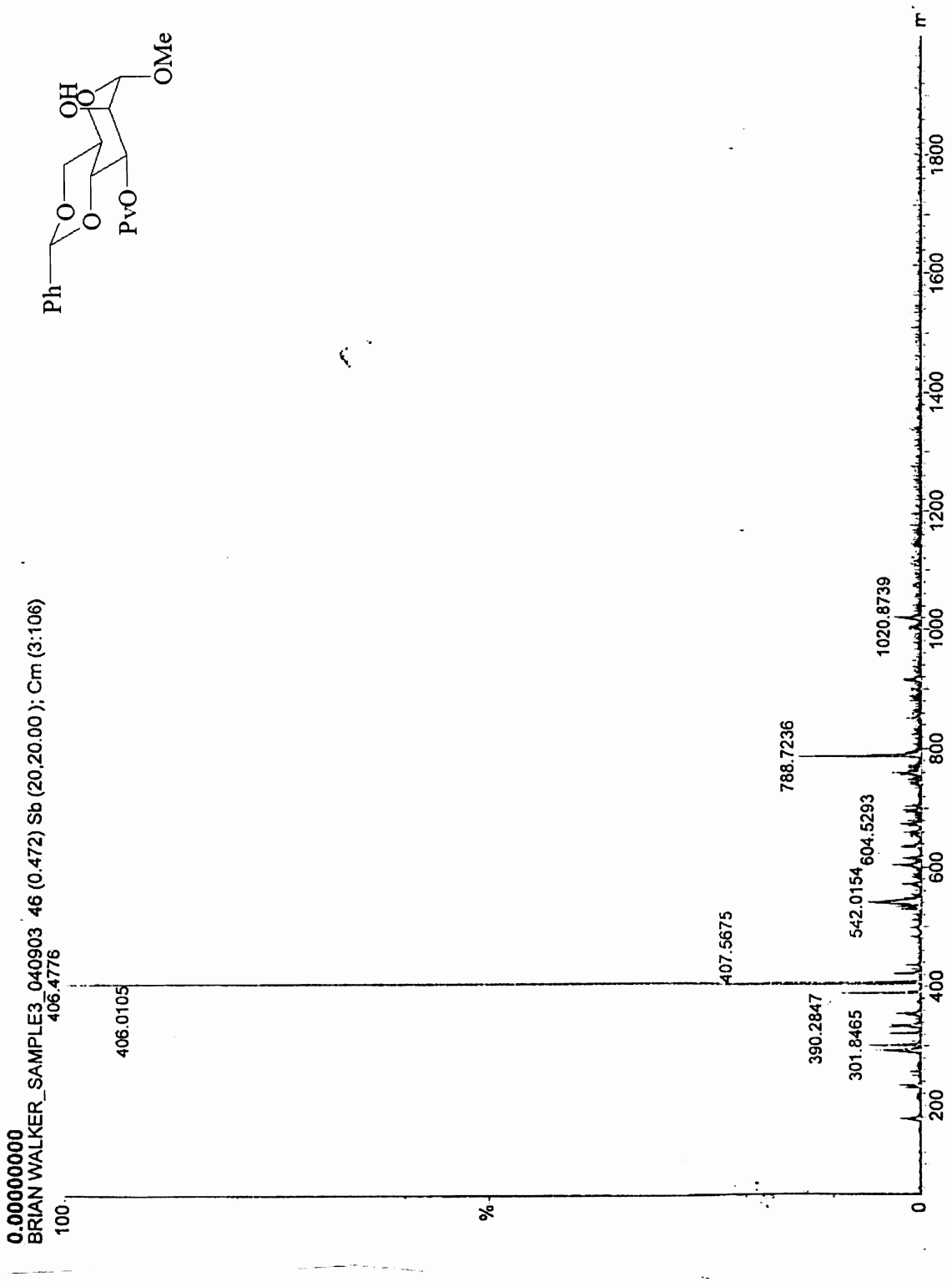
Appendix 5. Mass Specturm of **25**.



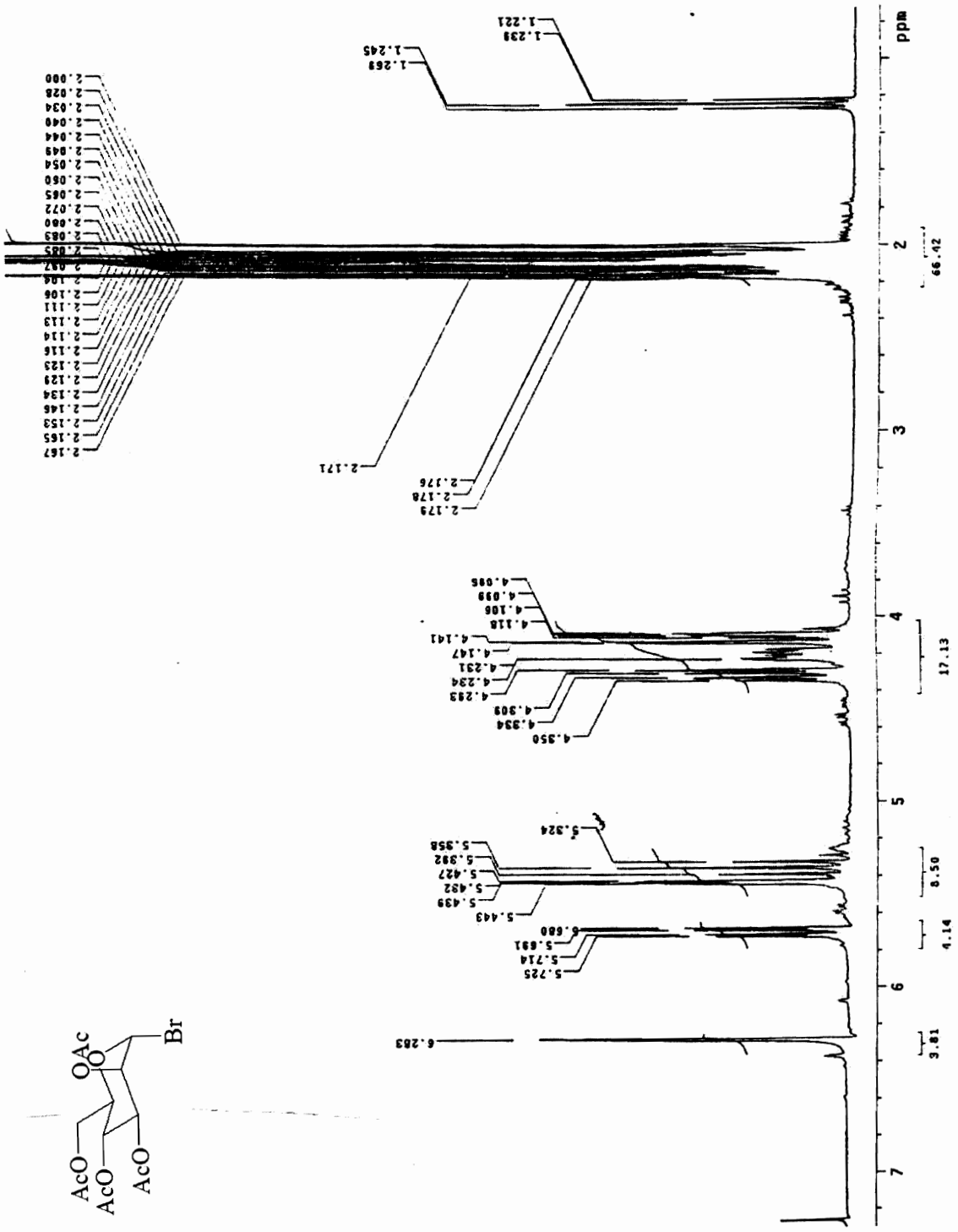
Appendix 6. ^{1}H NMR of **26**.



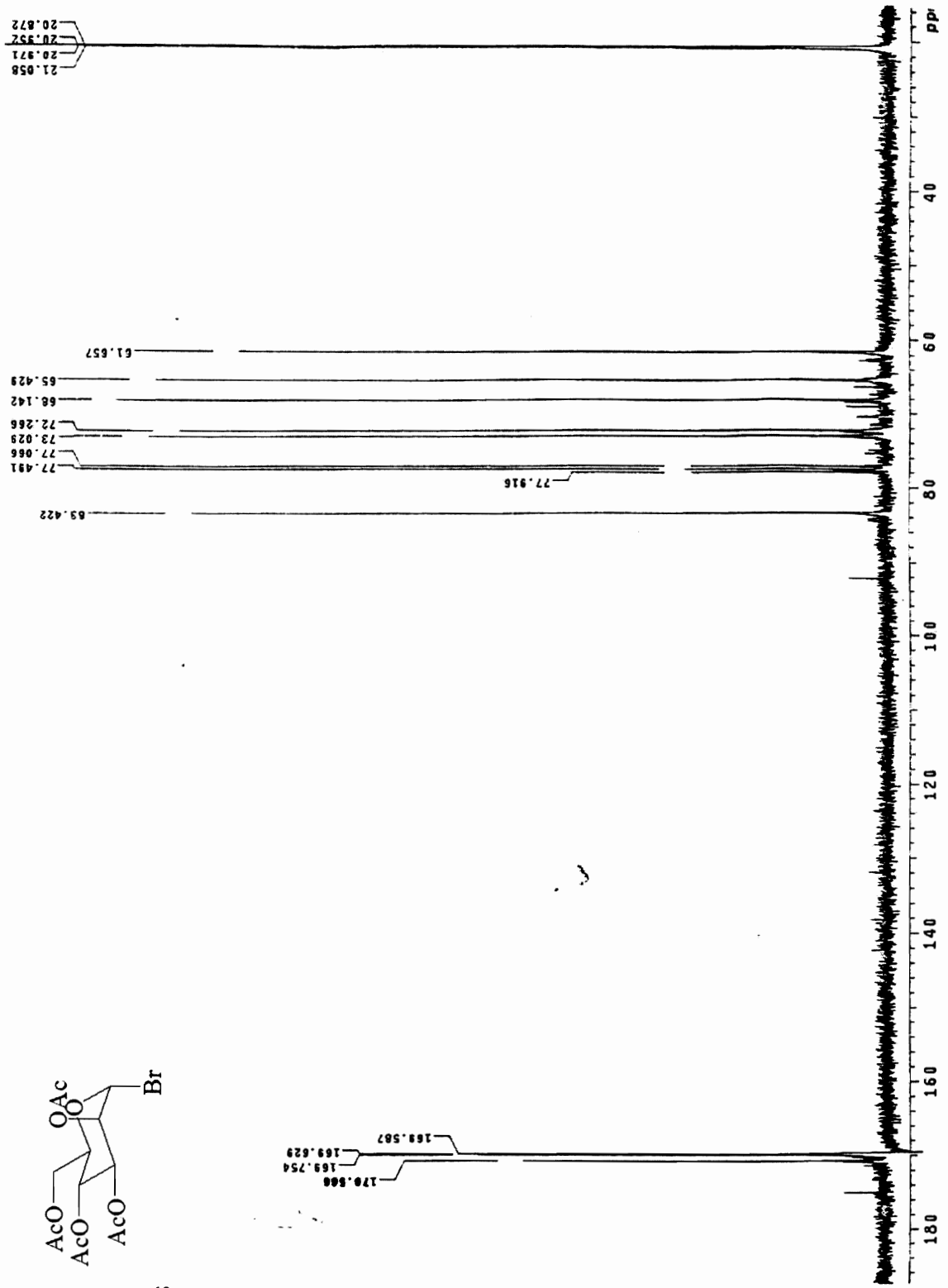
Appendix 7. ^{13}C NMR of **26.**



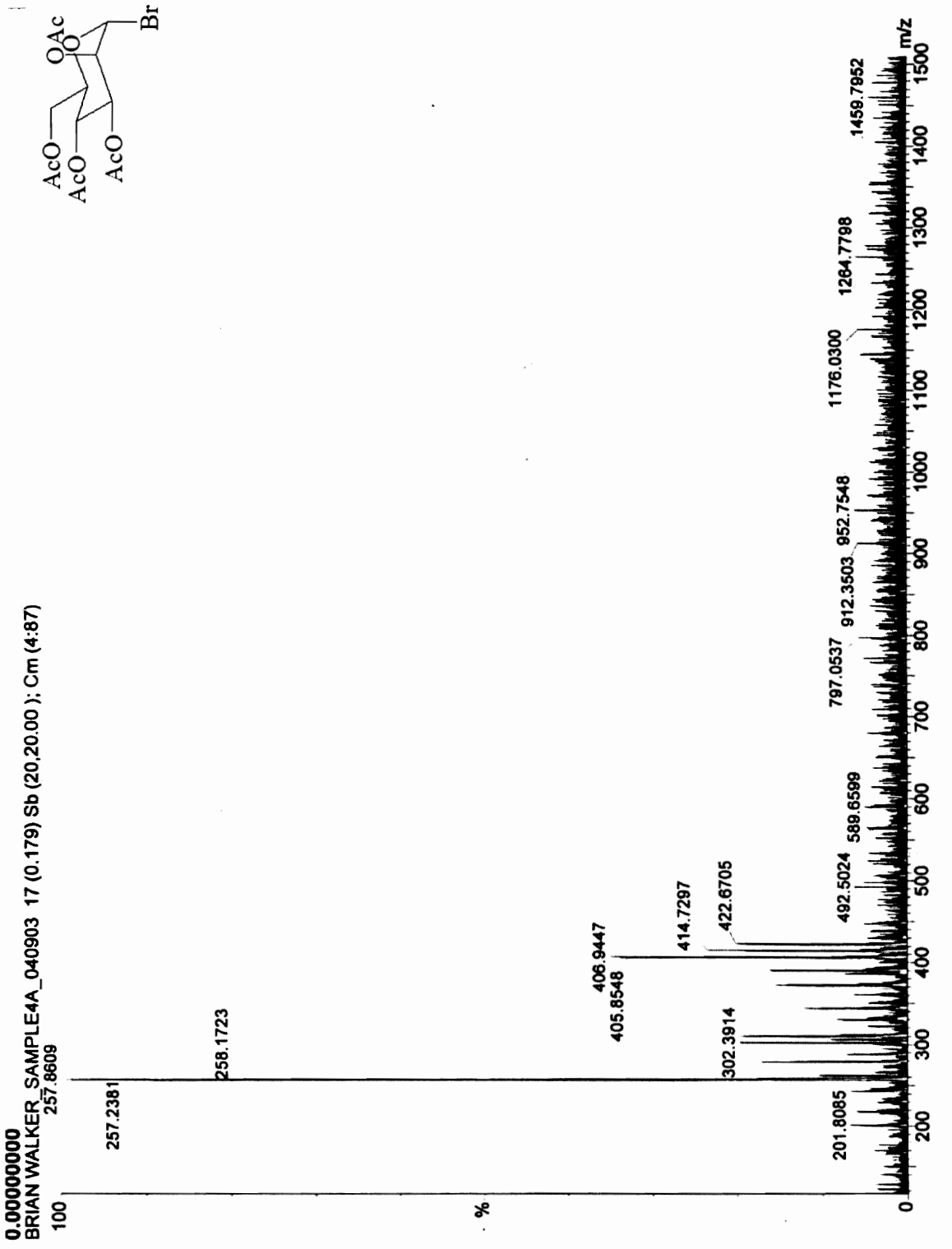
Appendix 8. Mass spectrum of **26.**



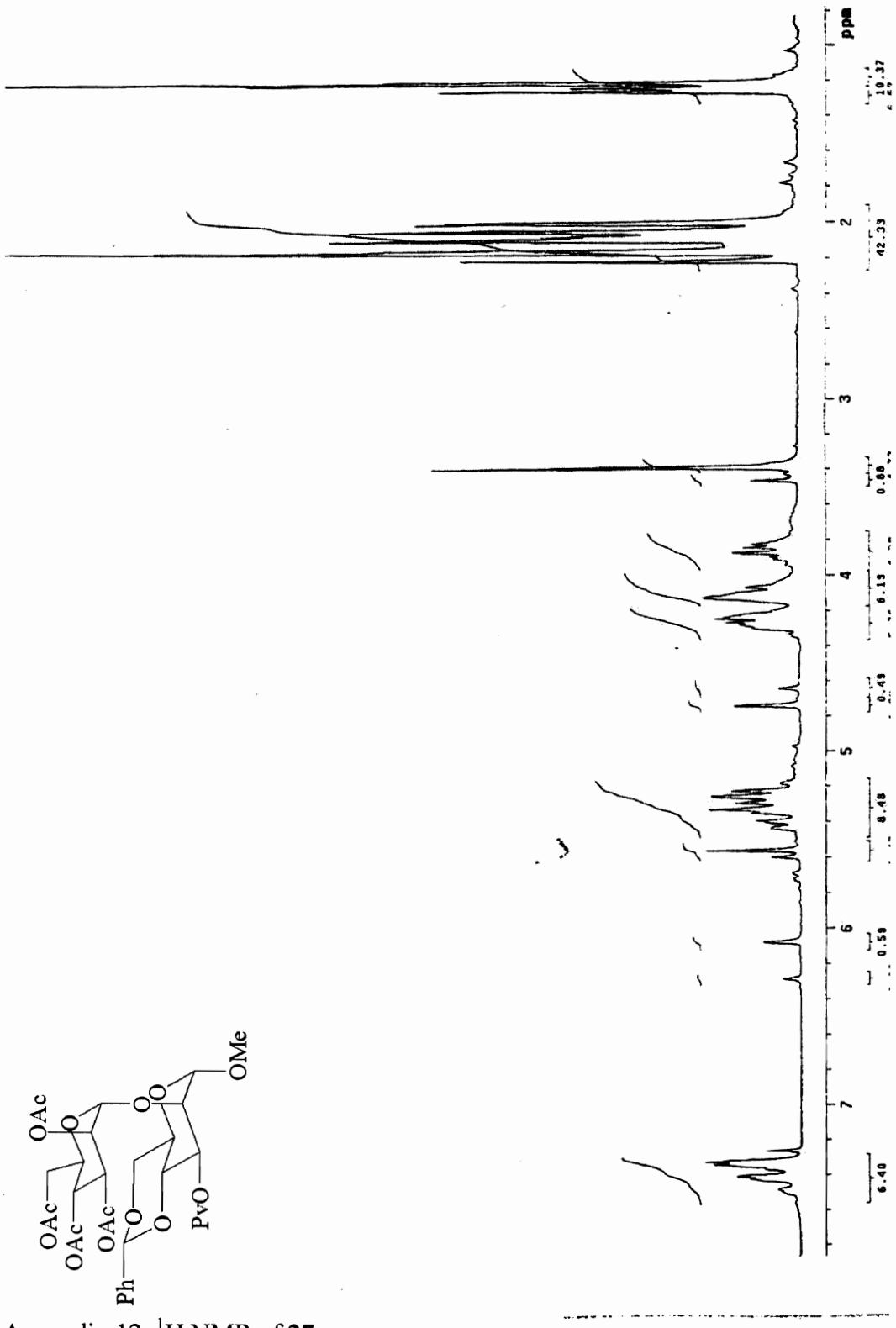
Appendix 9. ^{1}H NMR of **14.**



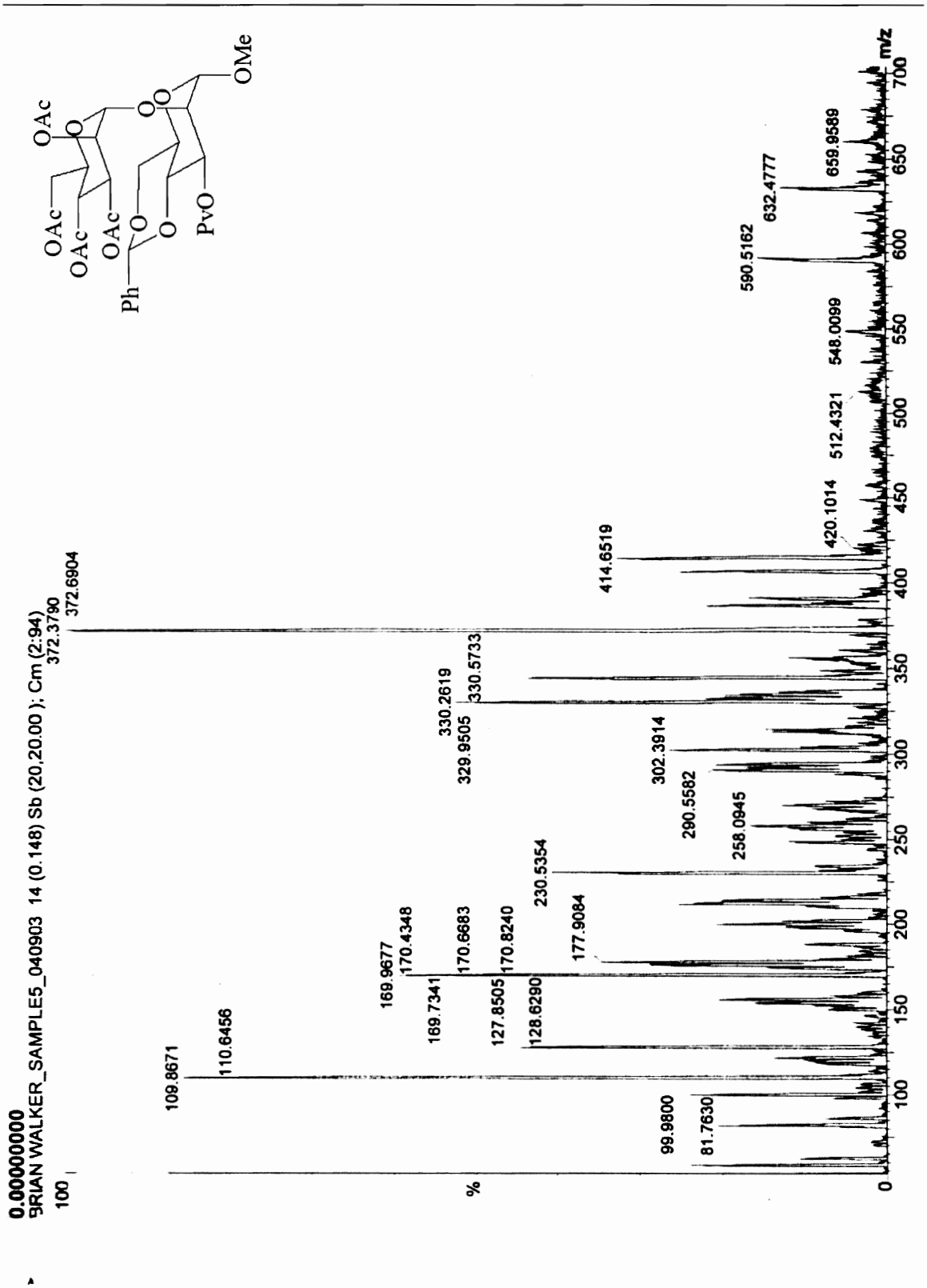
Appendix 10. ^{13}C NMR of **14.**



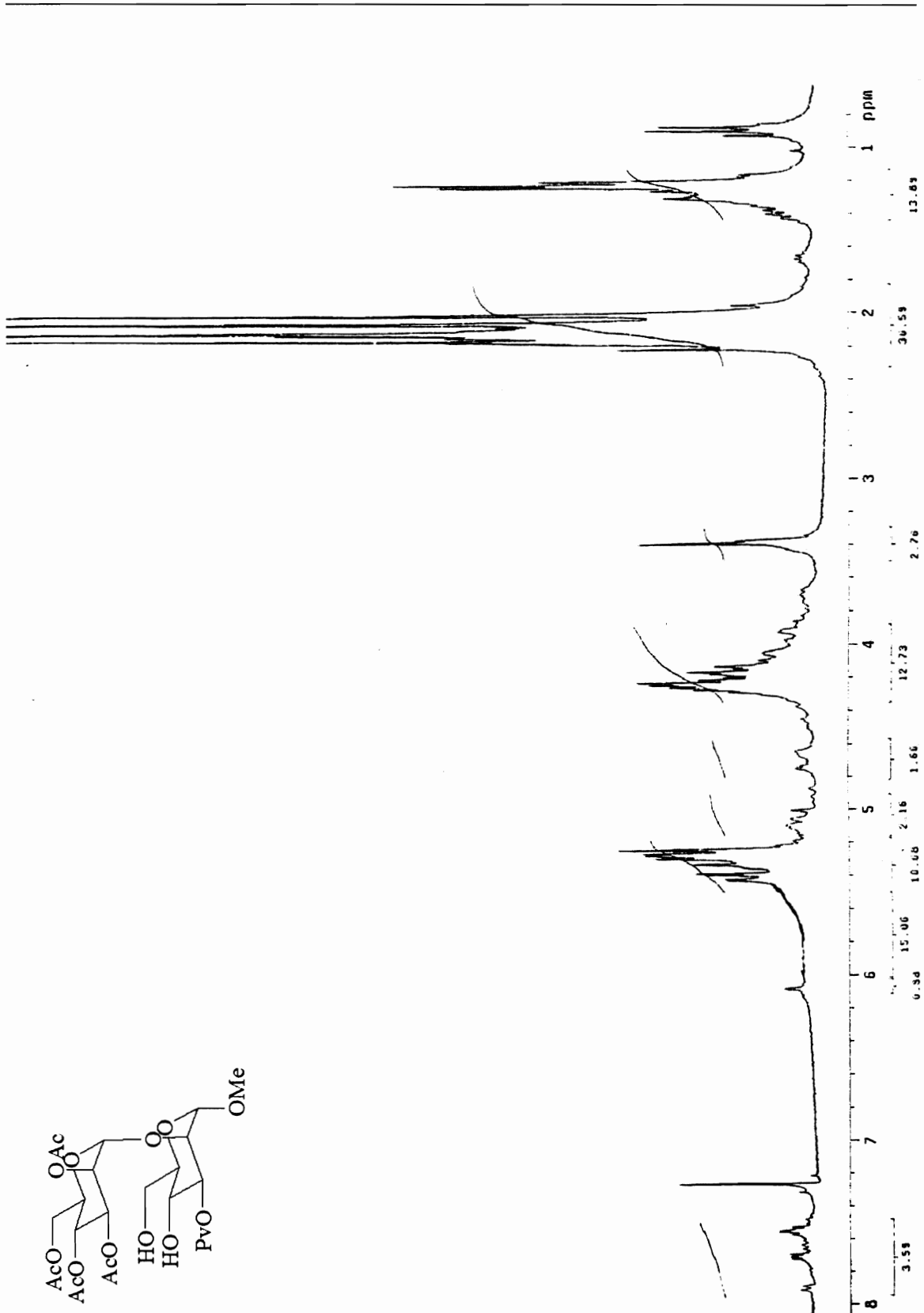
Appendix 11. Mass spectrum of **14.**



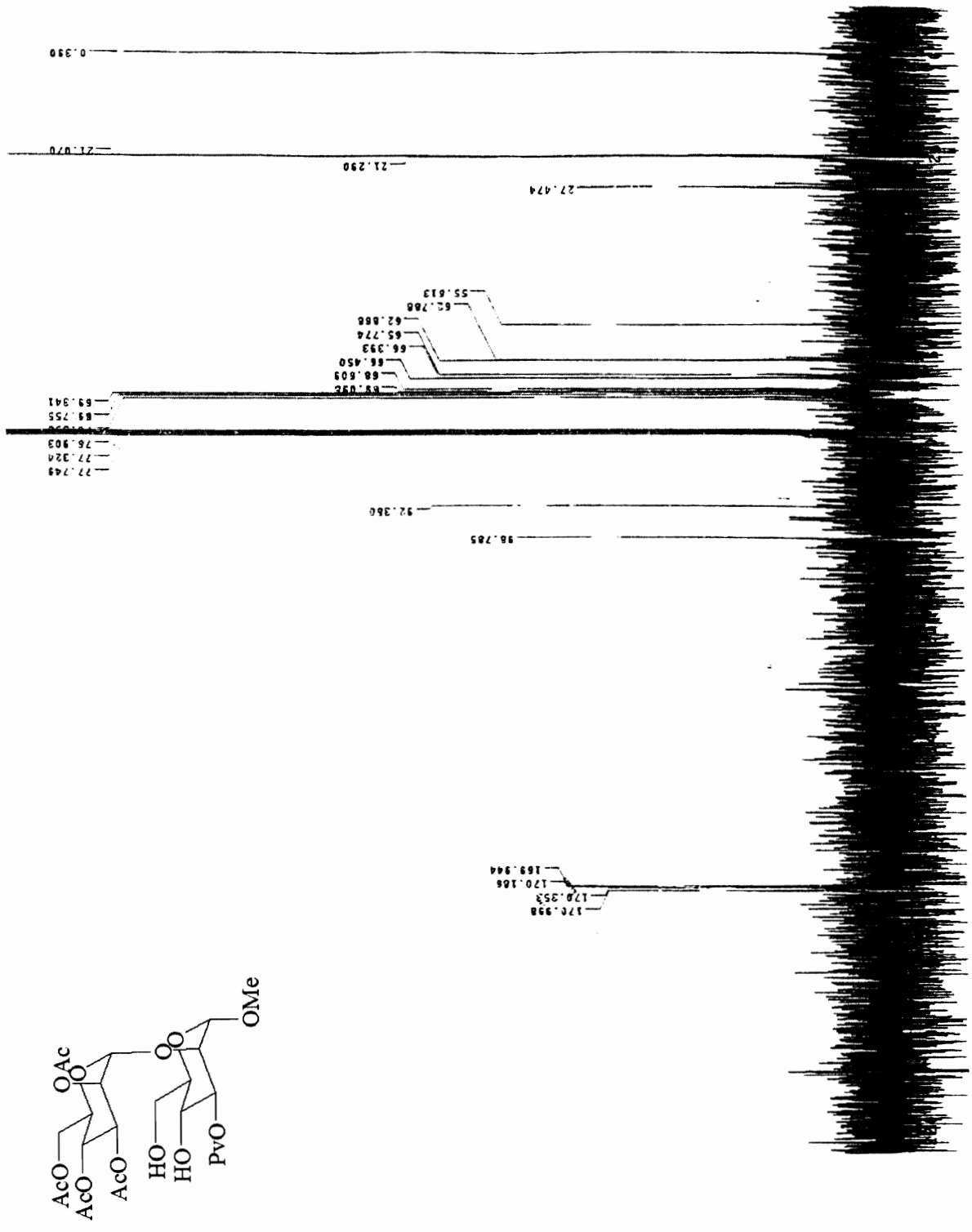
Appendix 12. ^{1}H NMR of **27.**



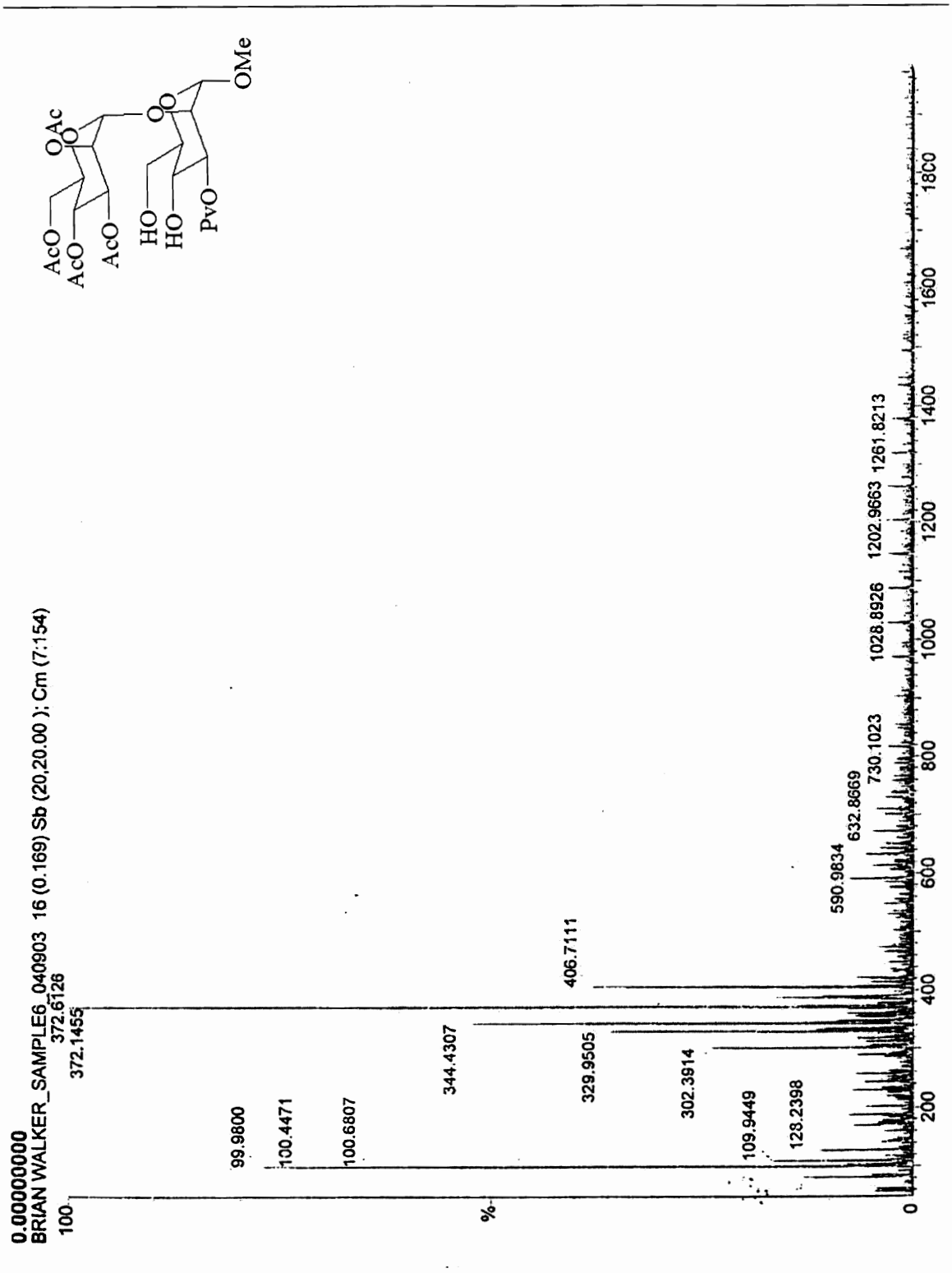
Appendix 13. Mass spectrum of **27**.



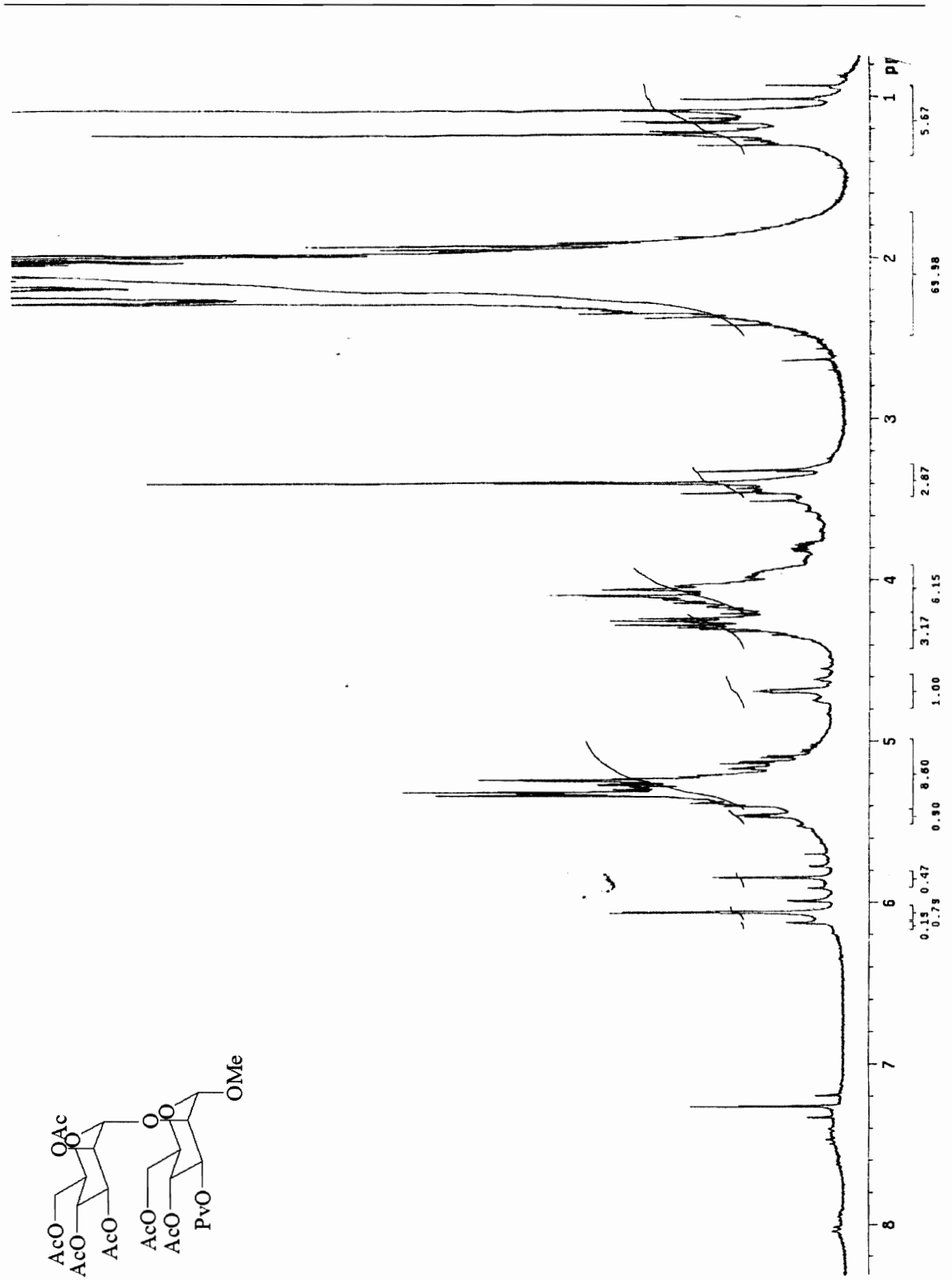
Appendix 14. ^{1}H NMR of **29**.



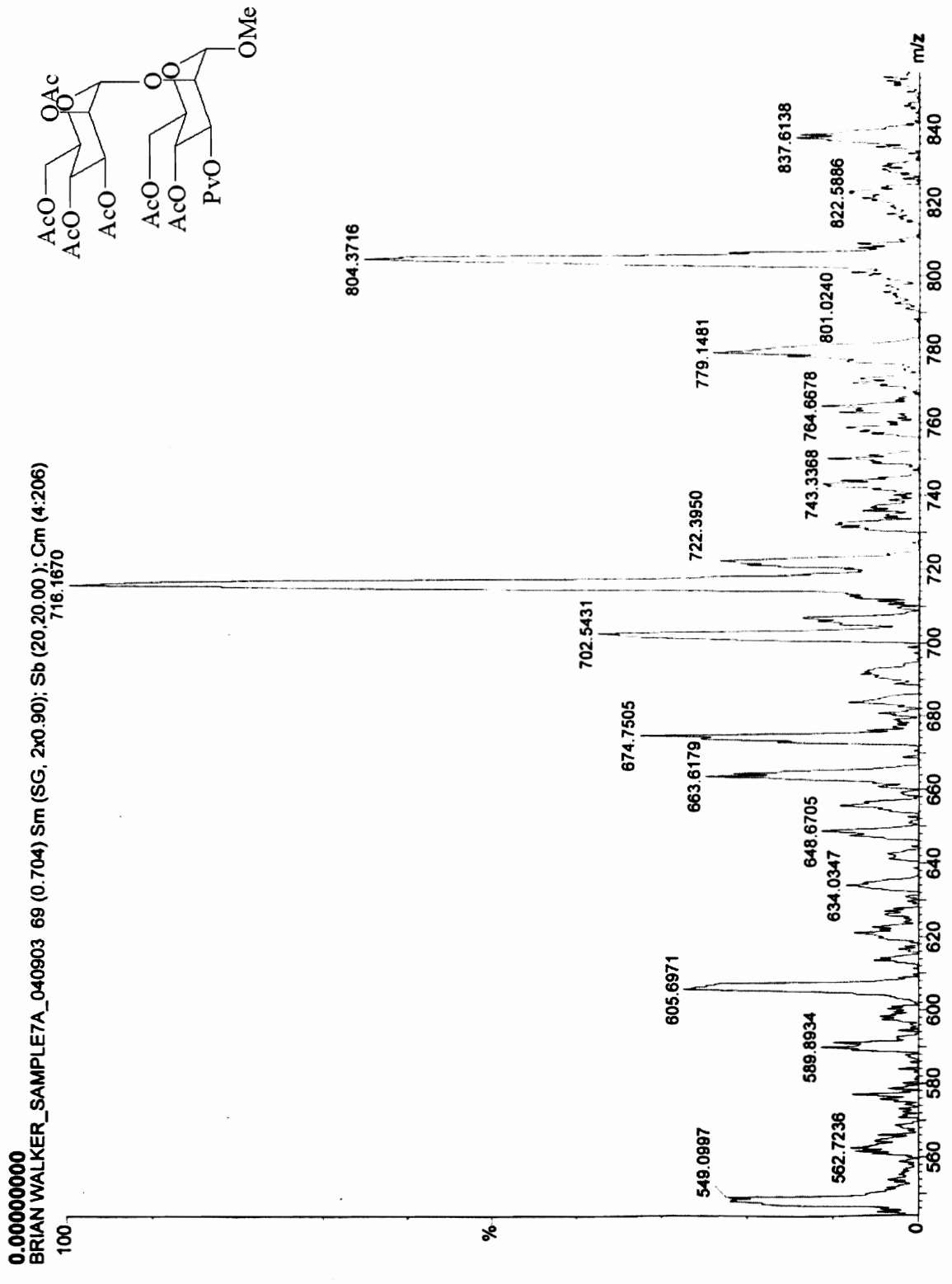
Appendix 15. ^{13}C NMR of **29.**



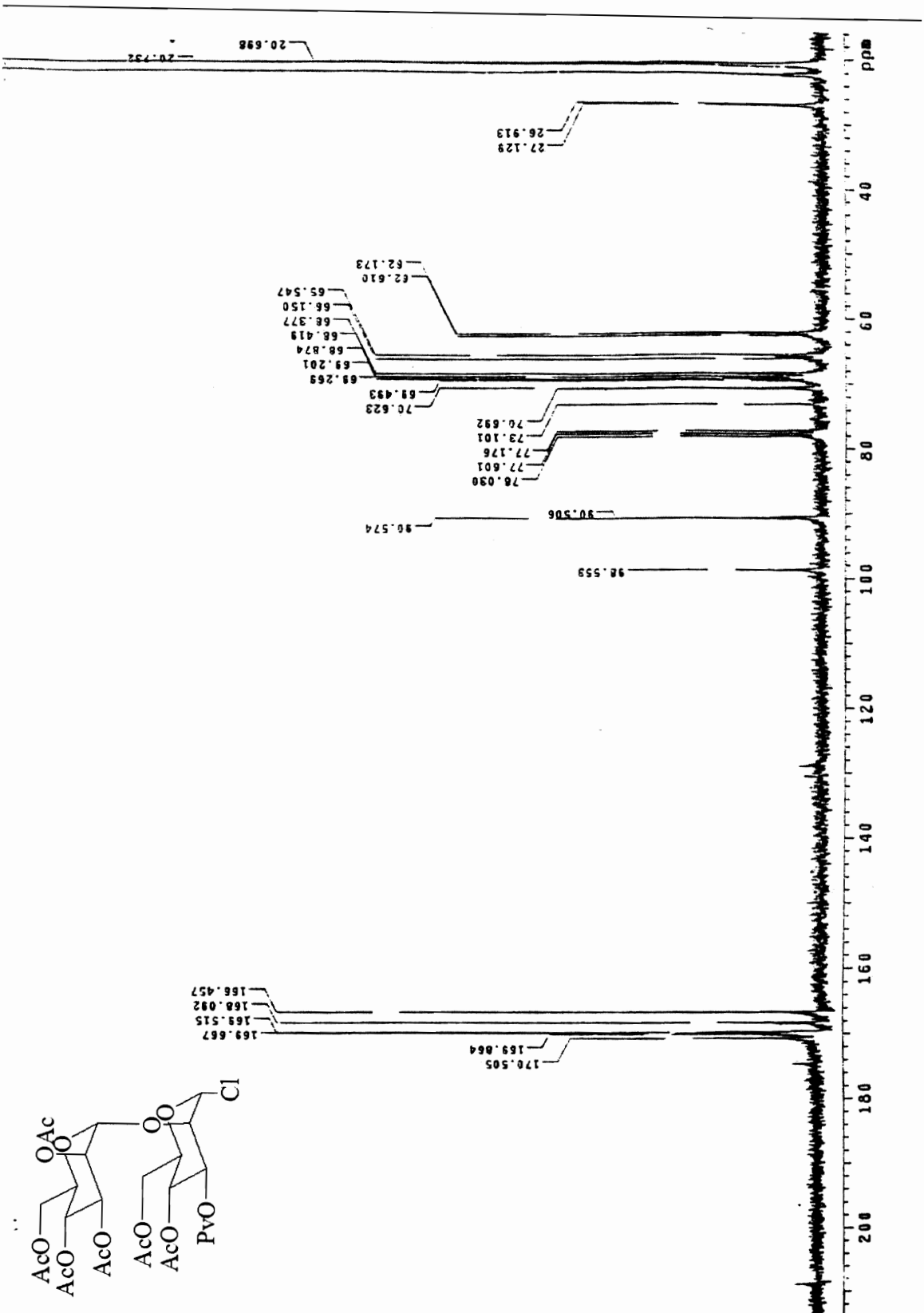
Appendix 16. Mass spectrum of **29.**



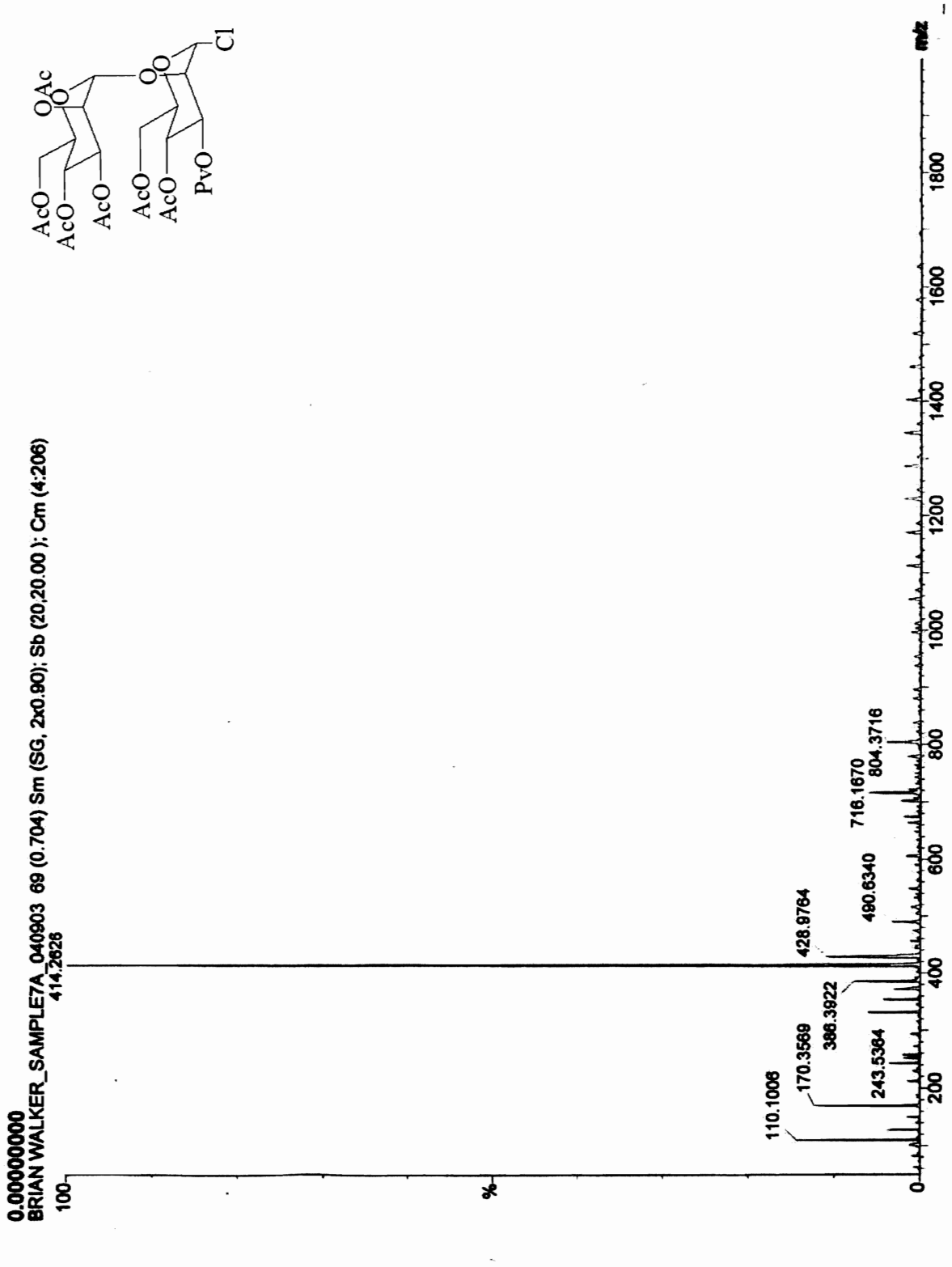
Appendix 17. ^{1}H NMR of **30**.



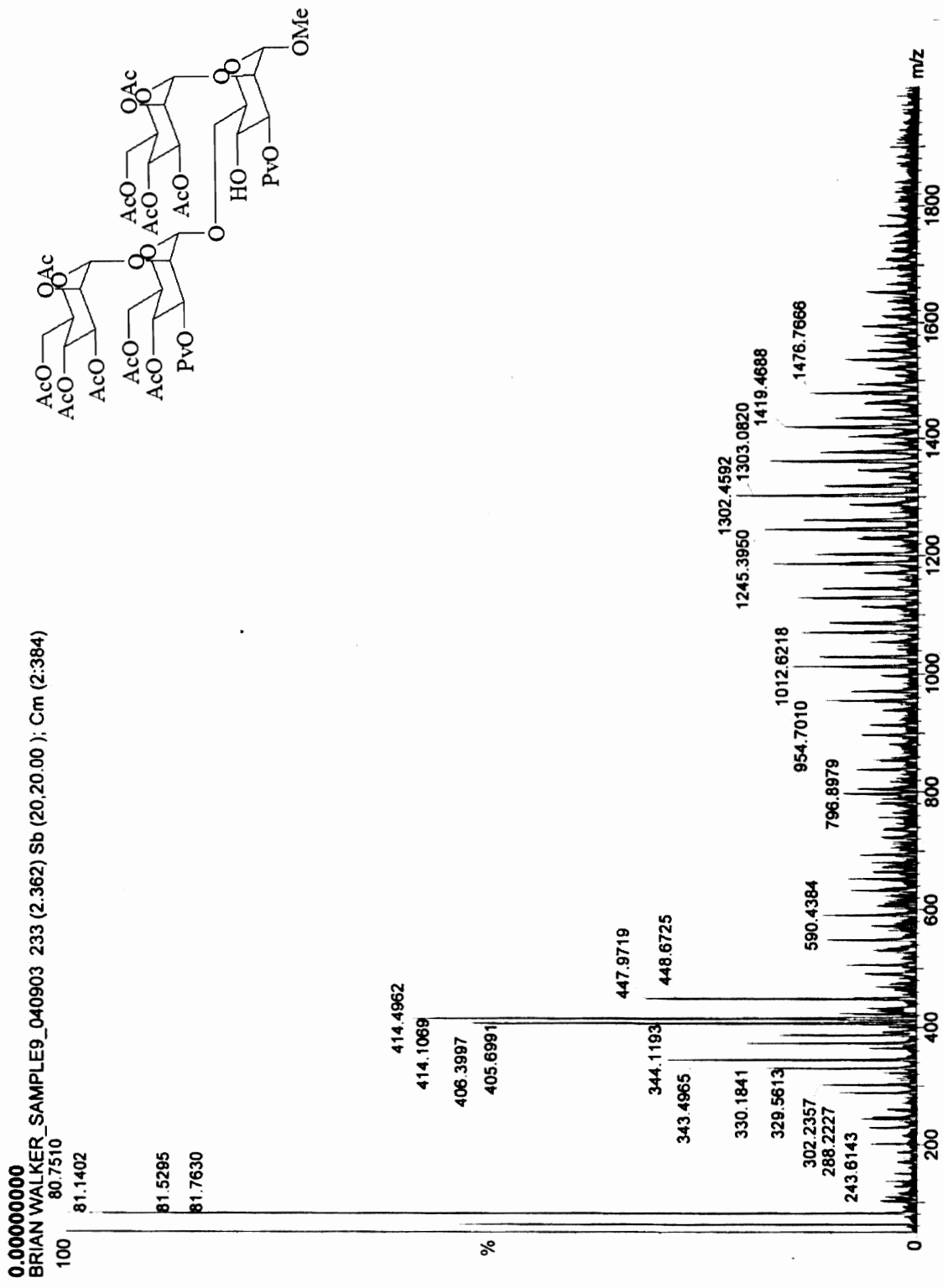
Appendix 18. Mass spectrum of **30.**



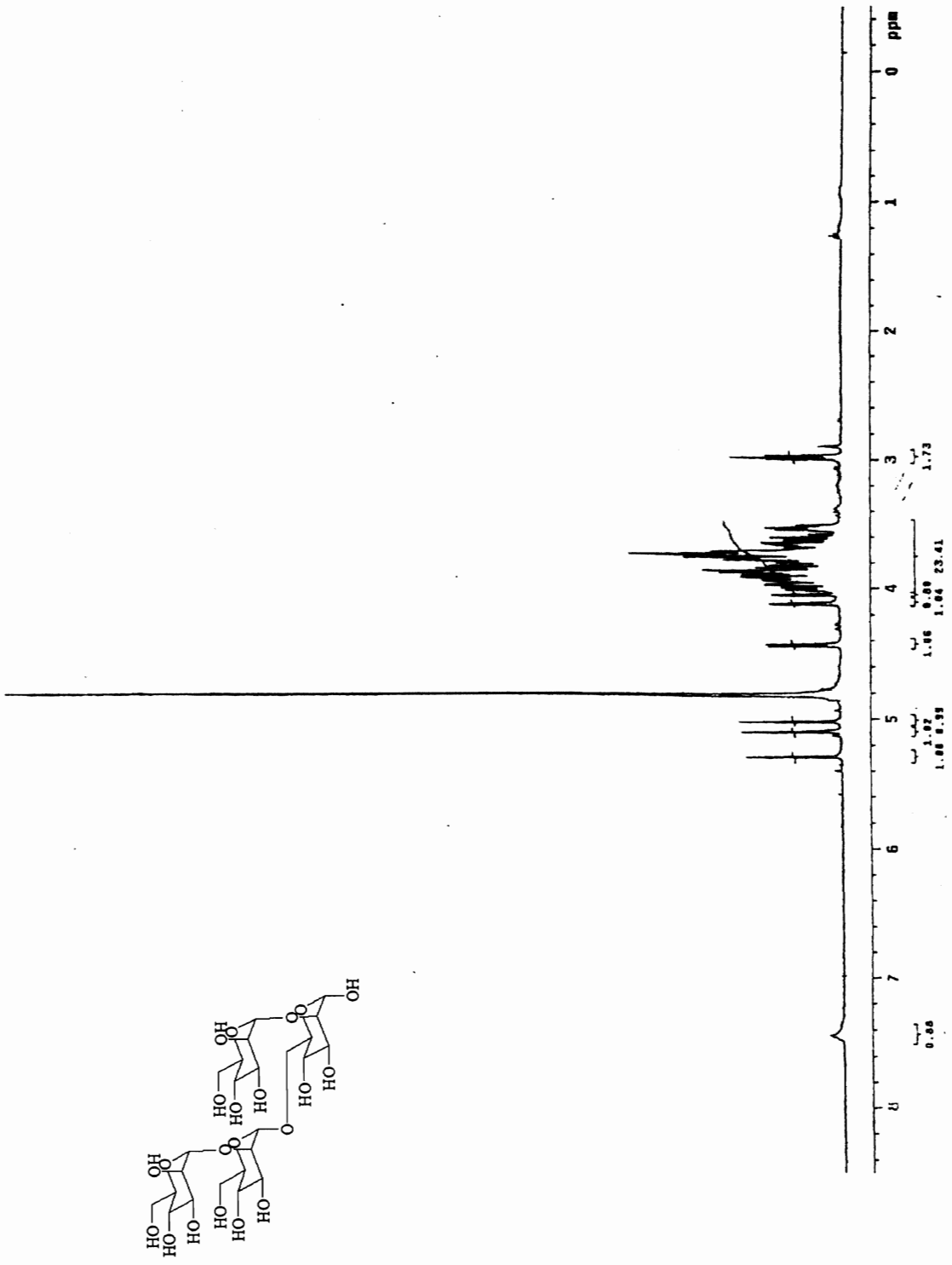
Appendix 19. ^{13}C NMR of **31.**



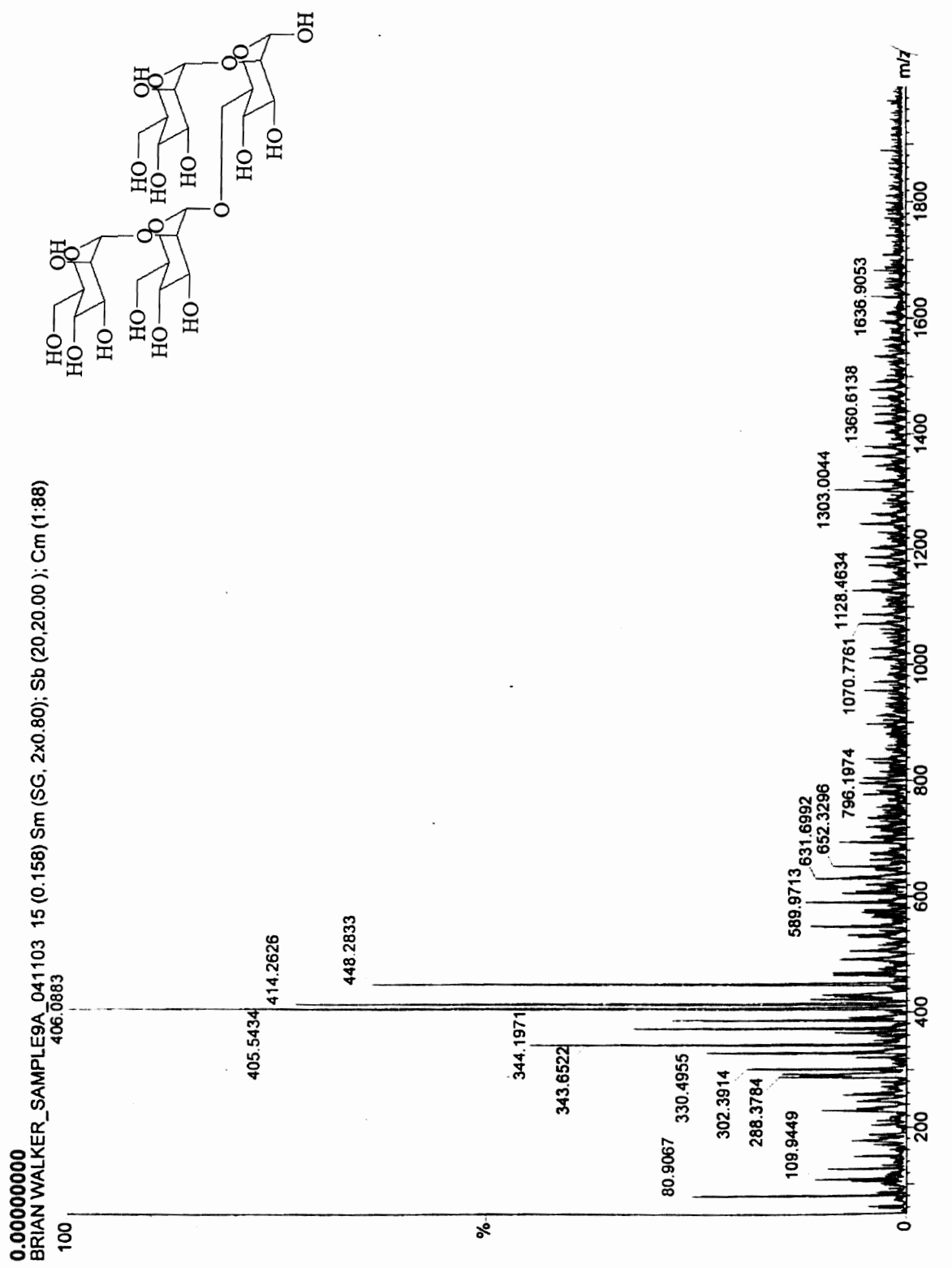
Appendix 20. Mass spectrum of **31**.



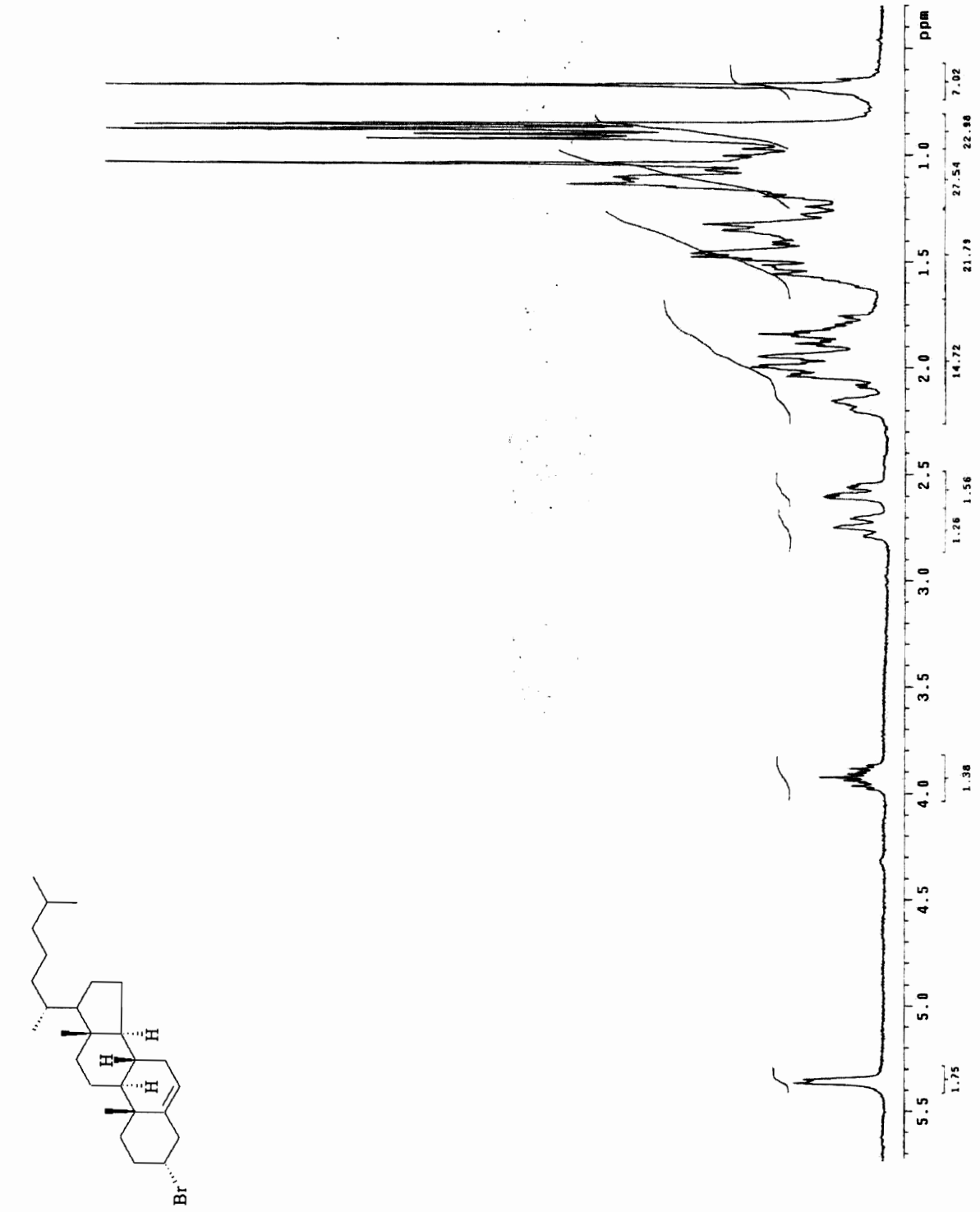
Appendix 21. Mass spectrum of **32**.



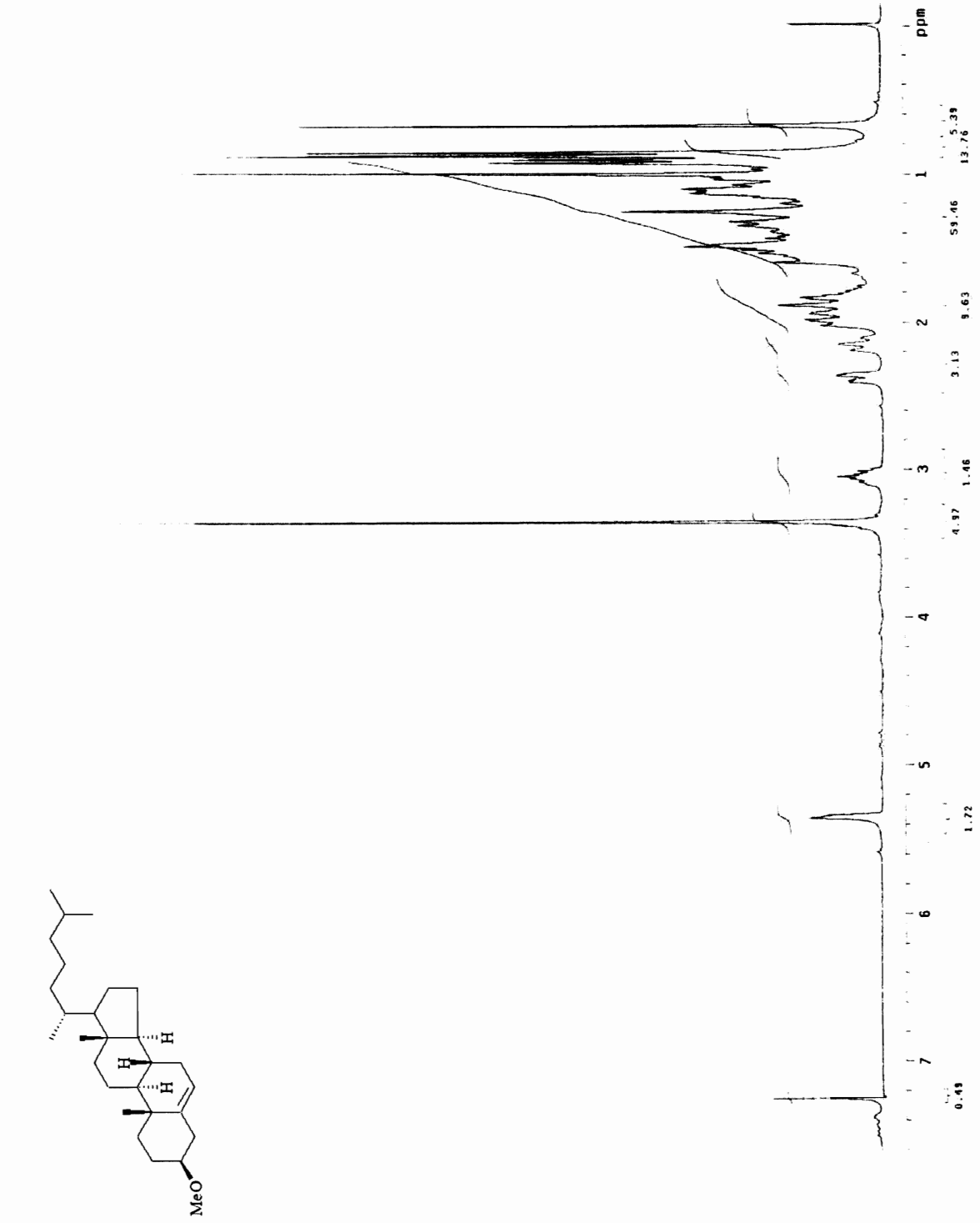
Appendix 22. ^{1}H NMR of **10**.



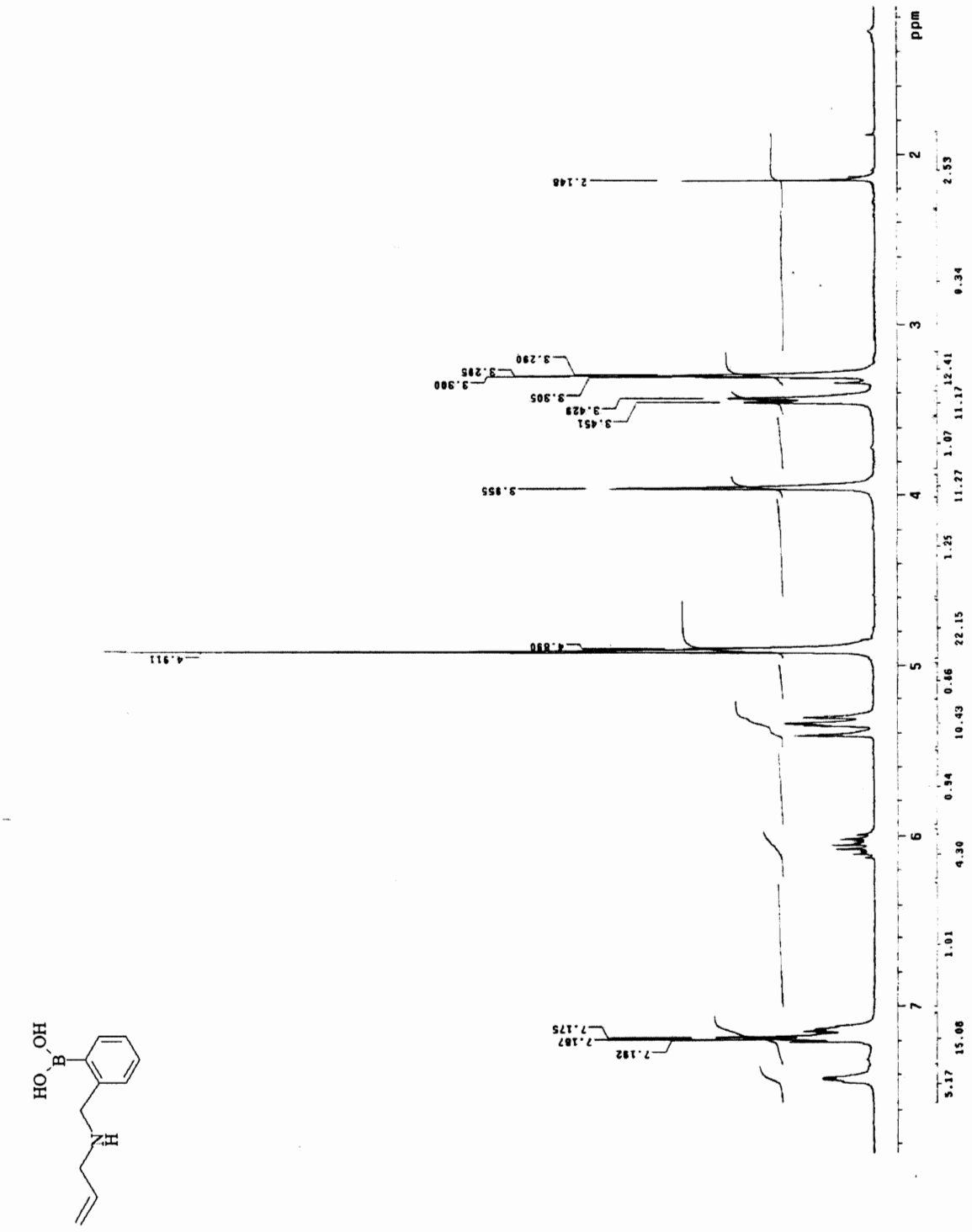
Appendix 23. Mass spectrum of **10**.



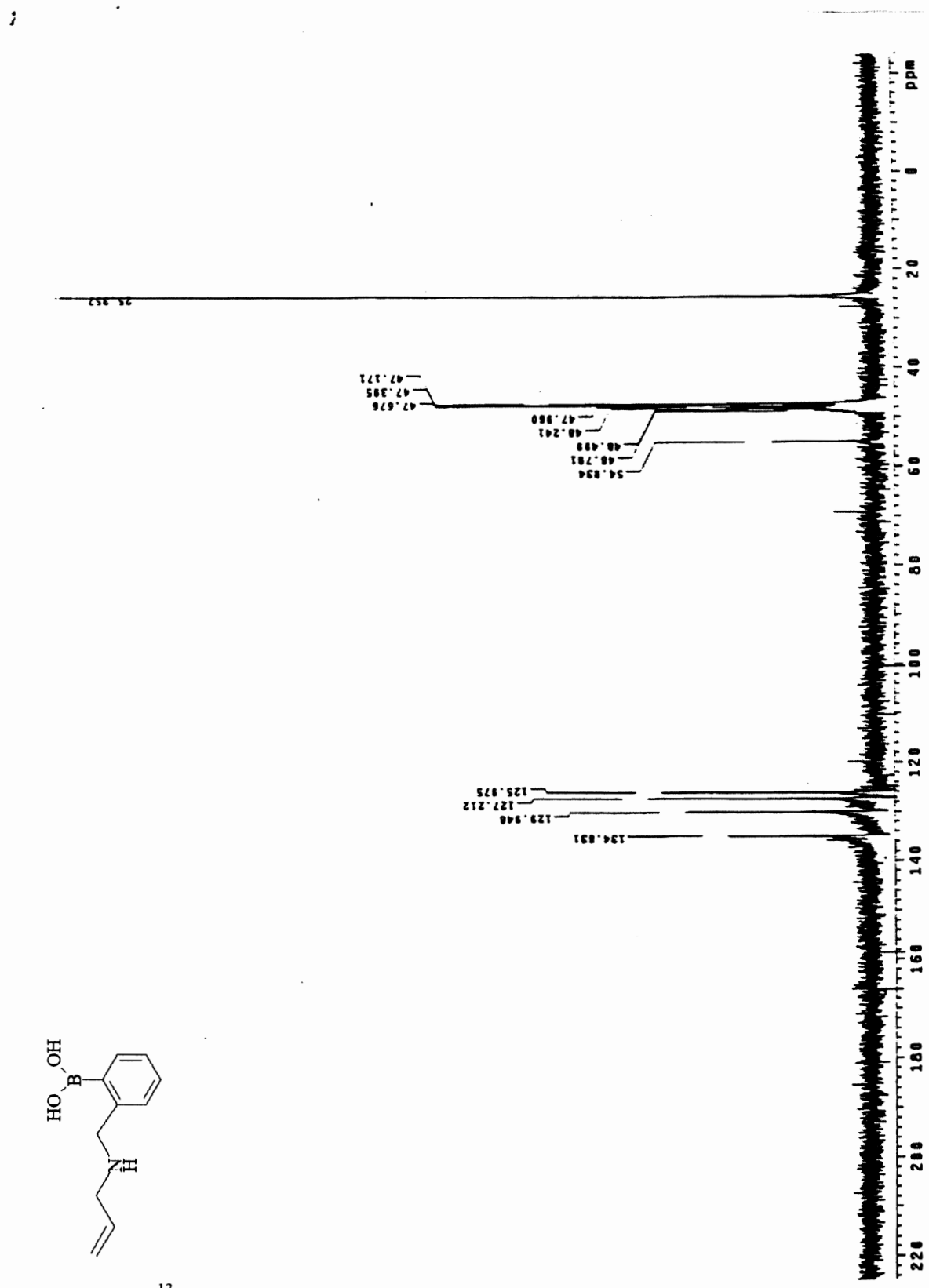
Appendix 24. ^{1}H NMR of **49**.



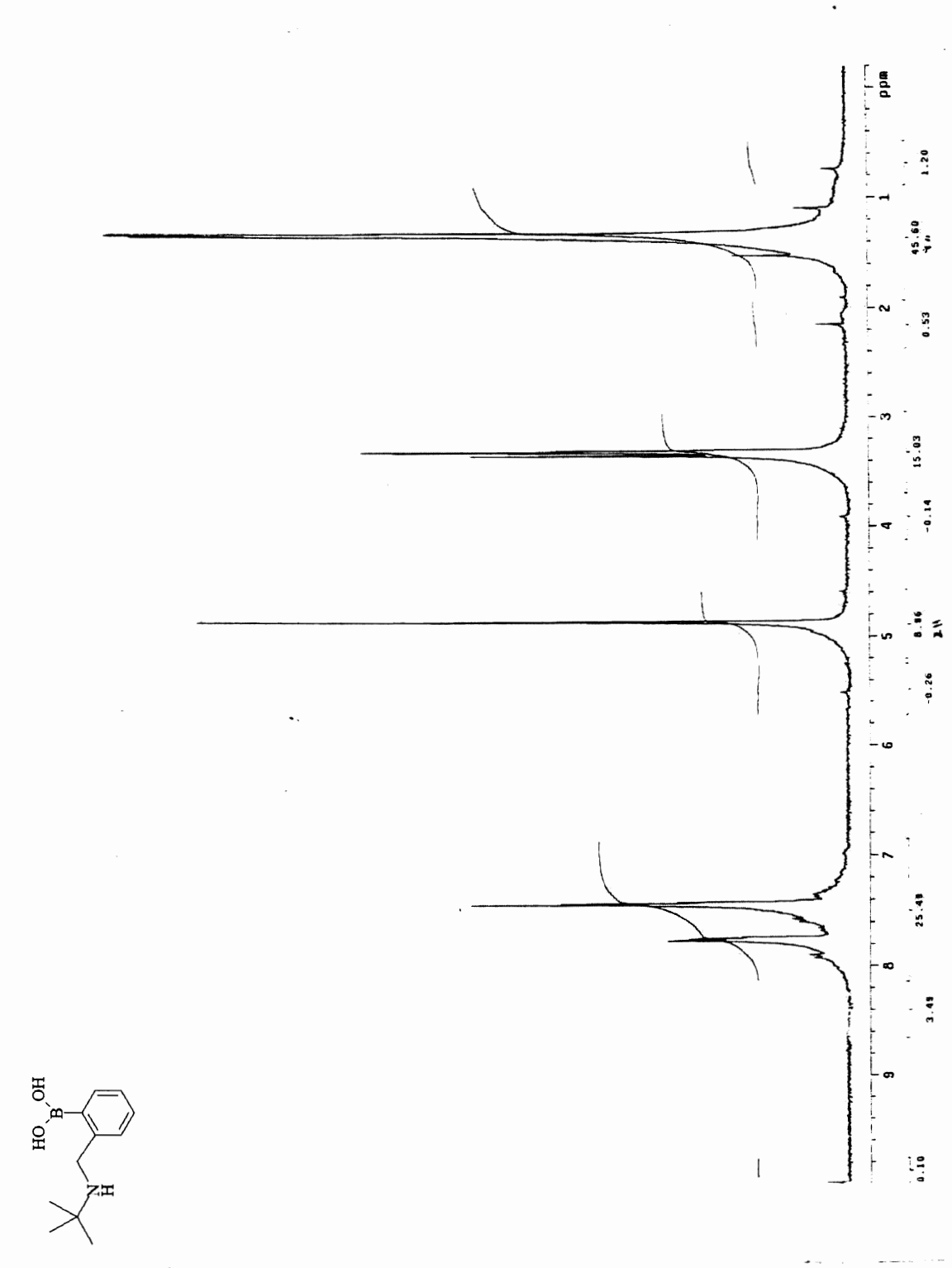
Appendix 25. ^{1}H NMR of **51**.



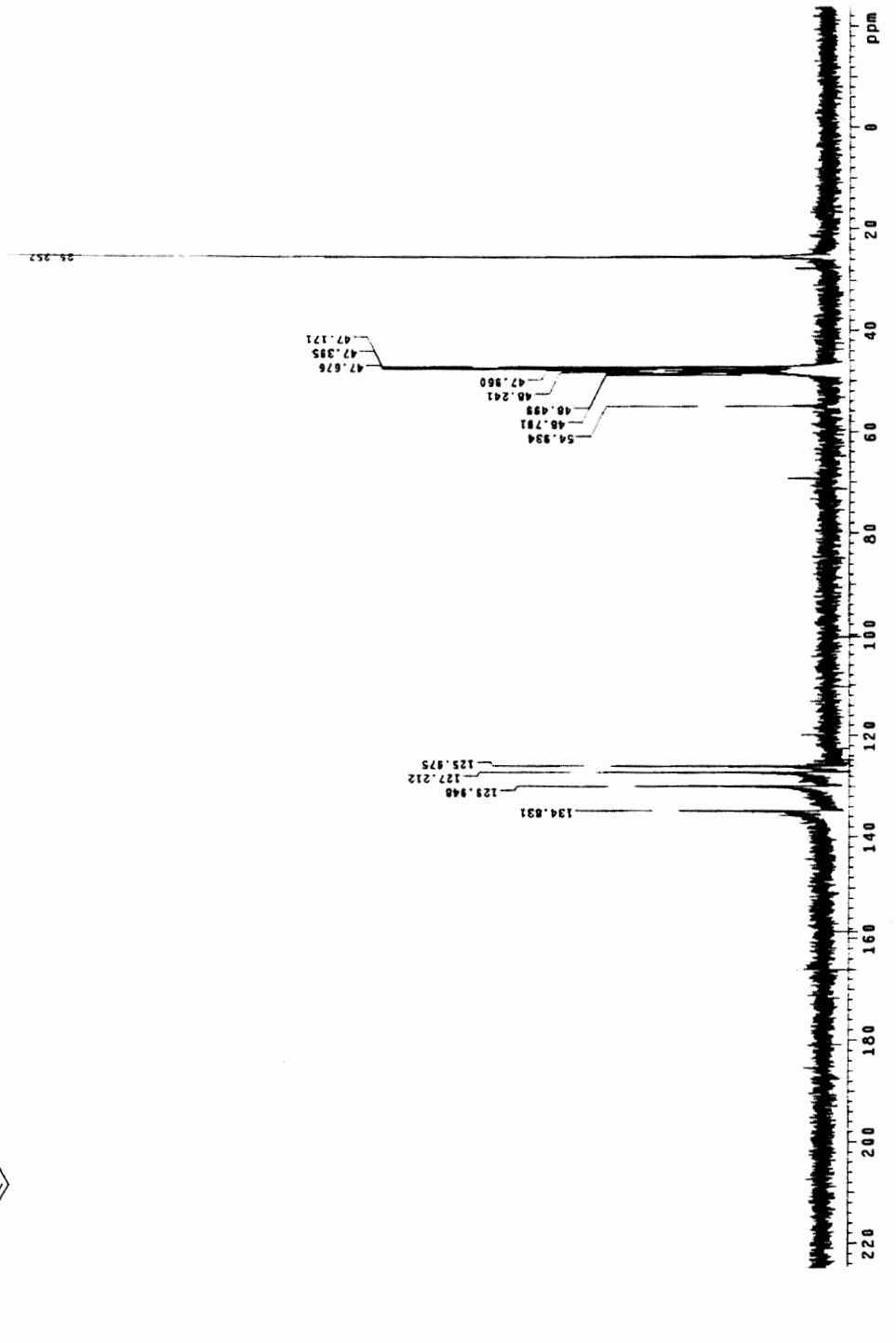
Appendix 26. ^{1}H NMR of **57**.

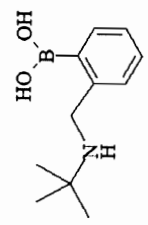


Appendix 27. ^{13}C NMR of **57**.

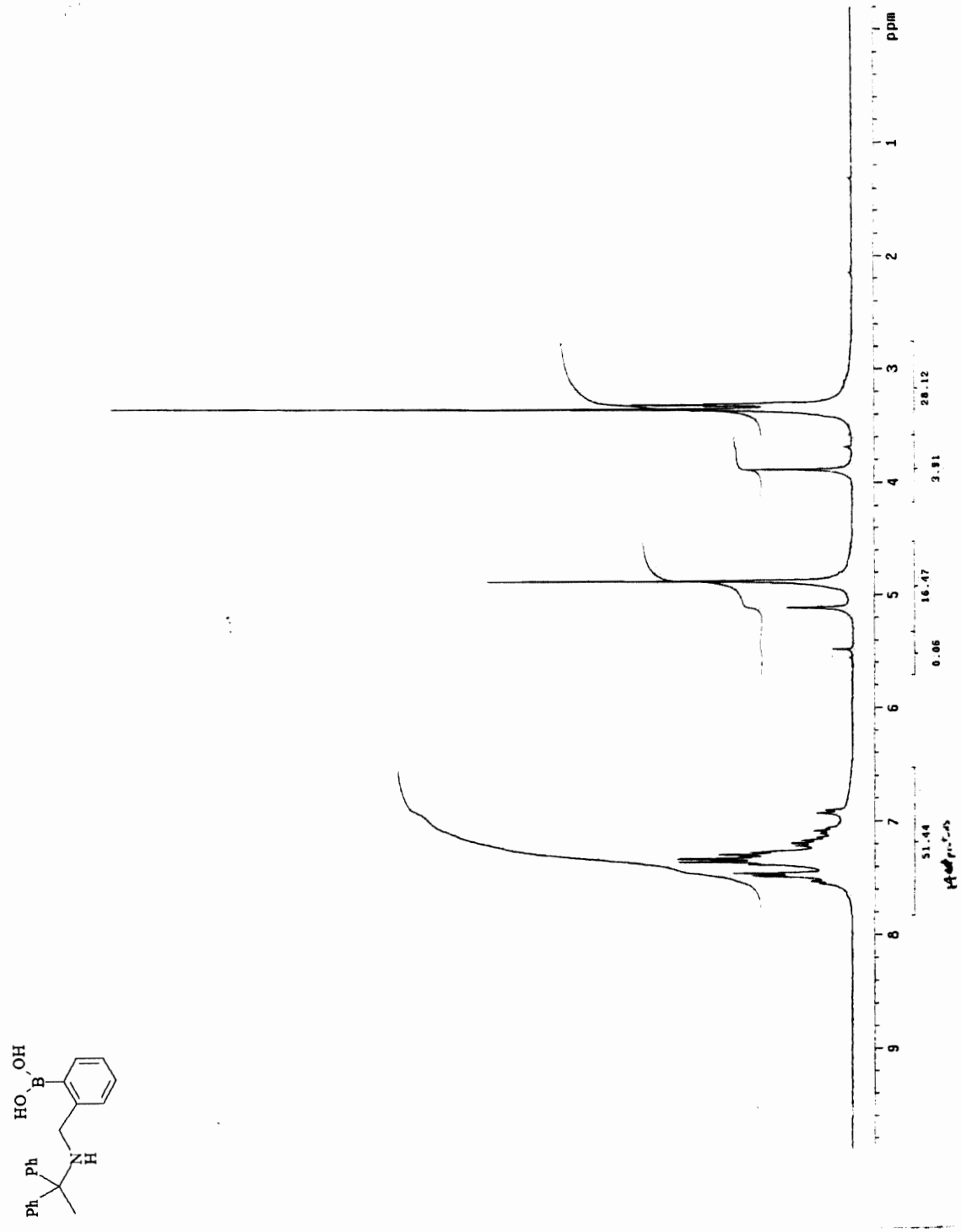


Appendix 28. ^{1}H NMR of **59**.



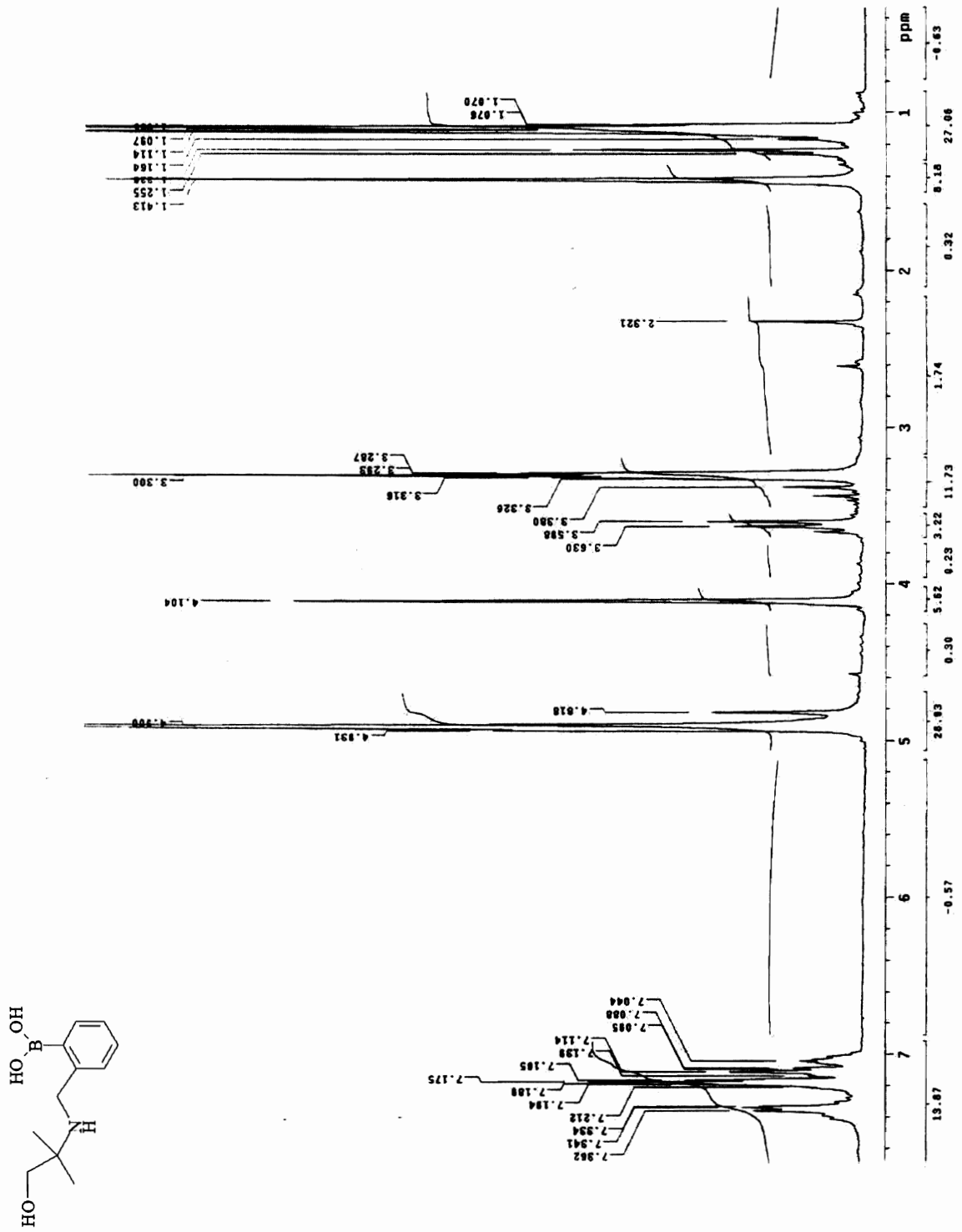


Appendix 29. ^{13}C NMR of **59**.

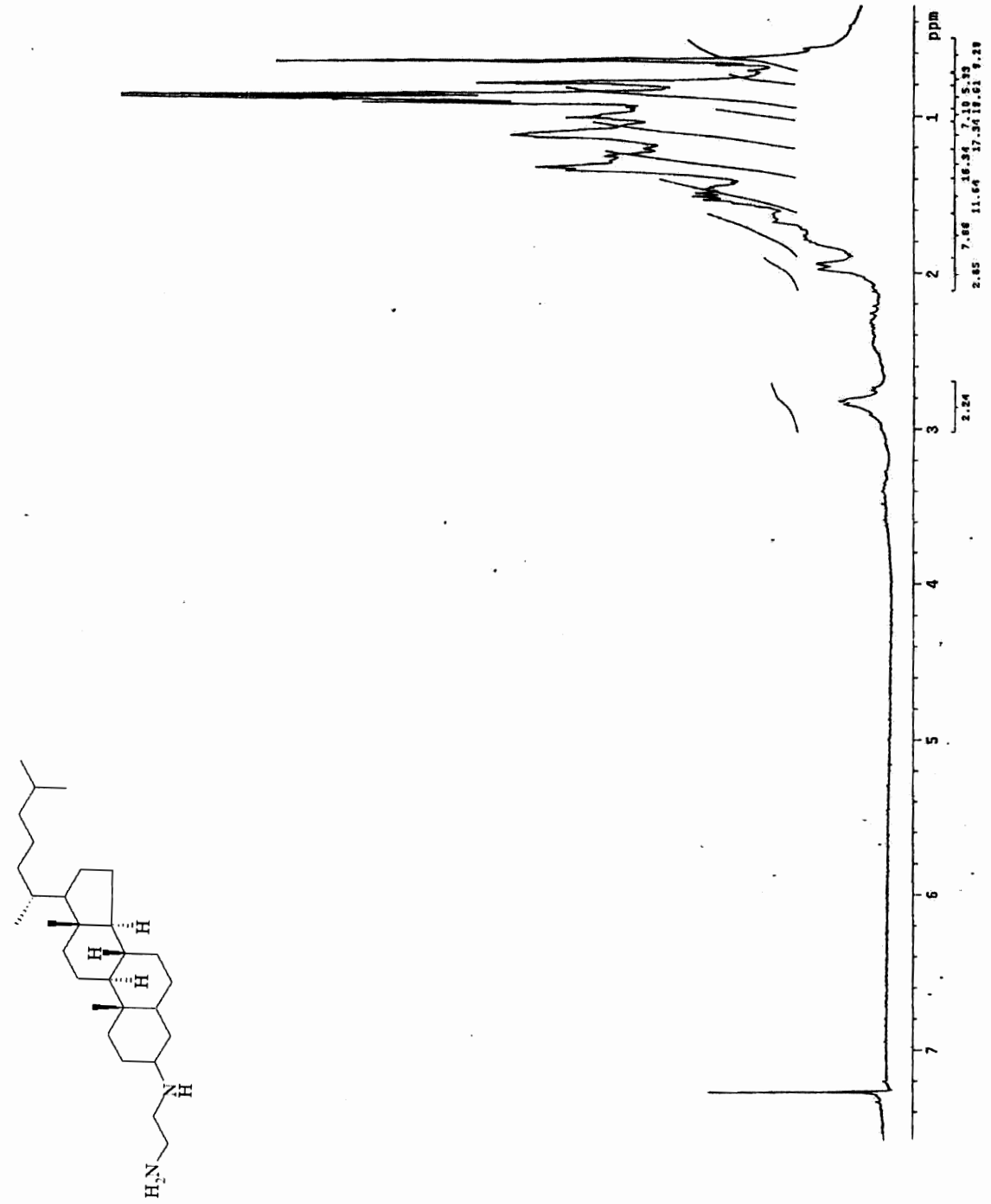


Appendix 30. ^{1}H NMR of **61**.

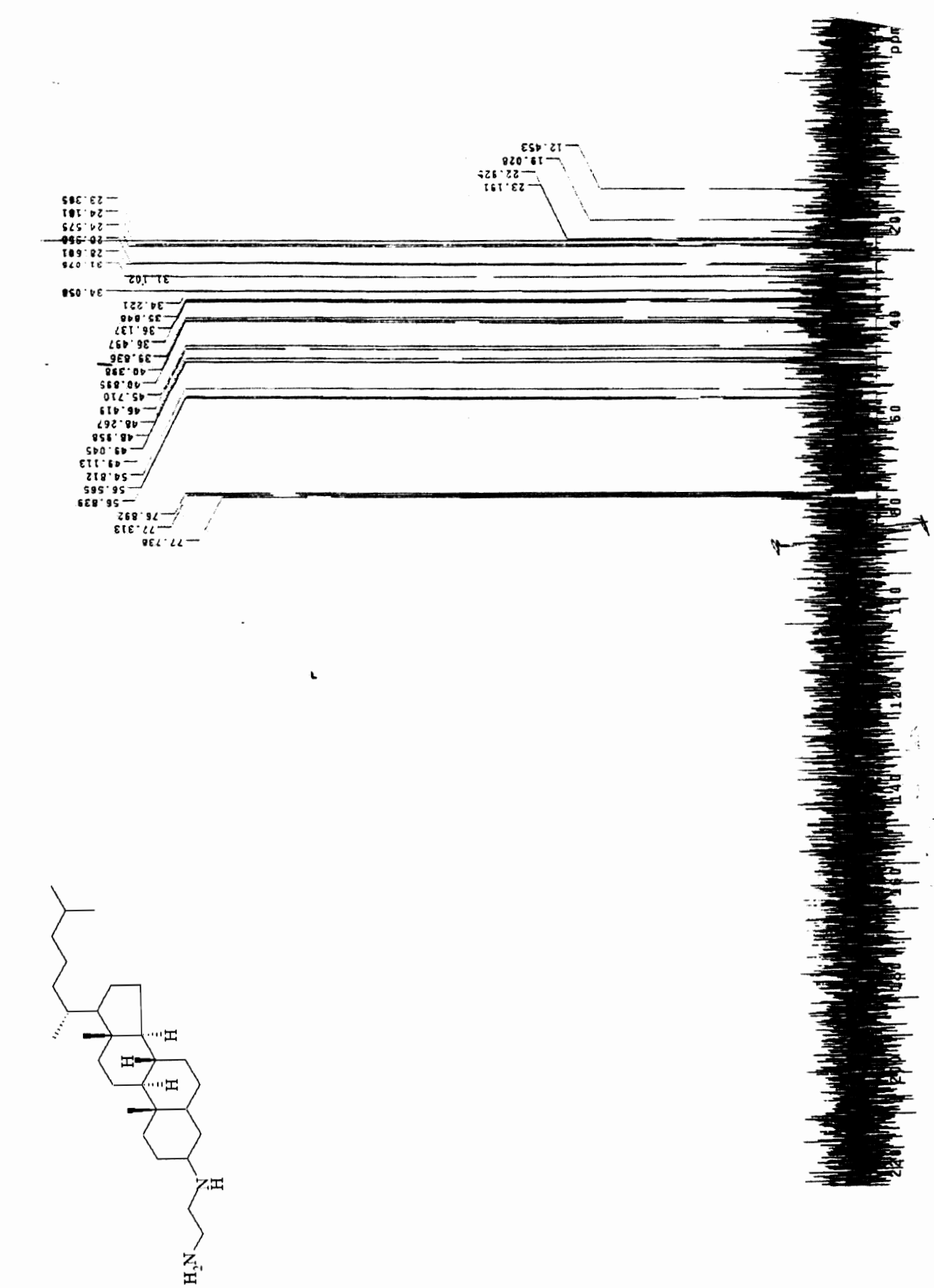
Appendix 31. ^{13}C NMR of **61**.



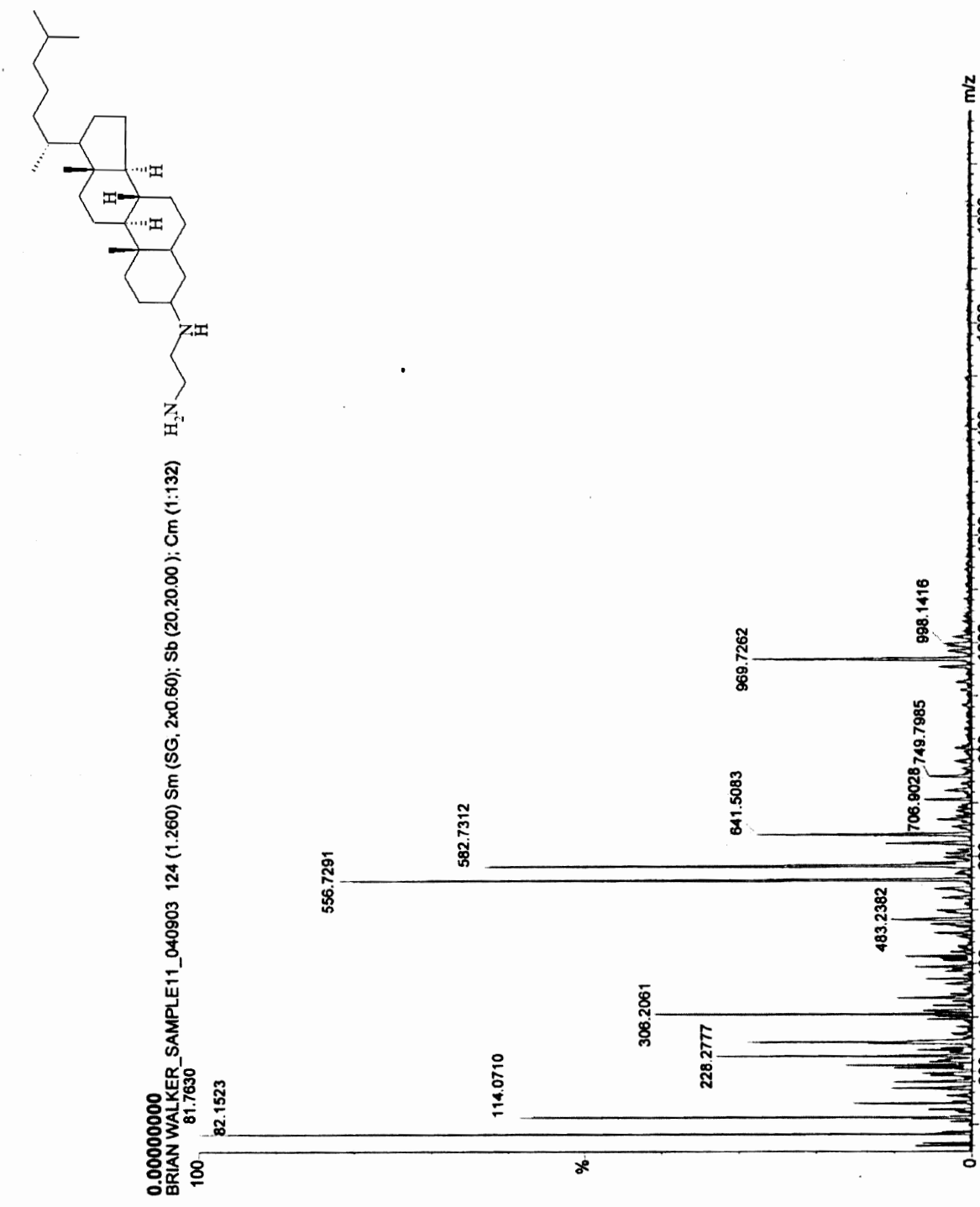
Appendix 32. ^{1}H NMR of **63**.



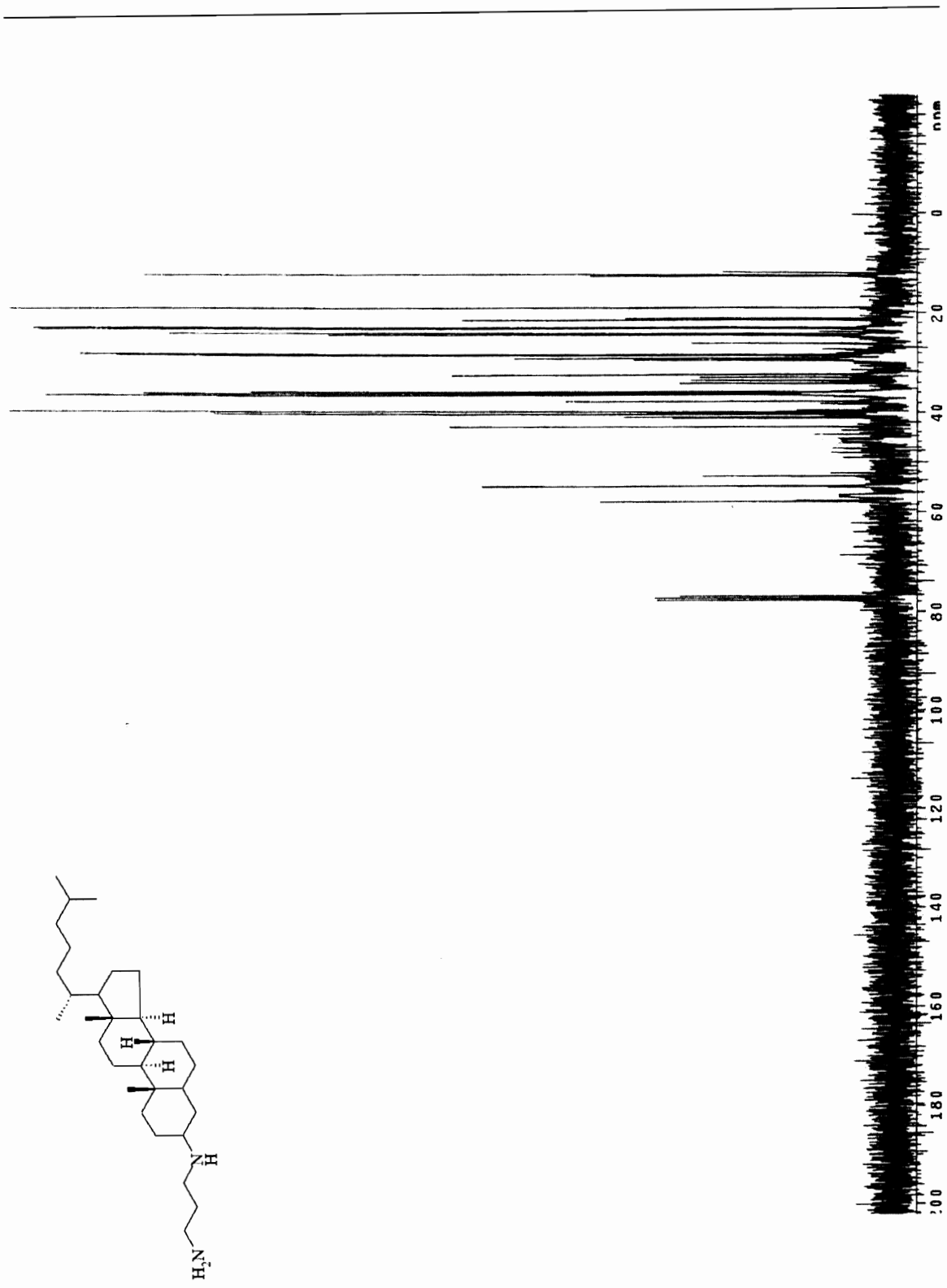
Appendix 33. ^{1}H NMR of **65**.



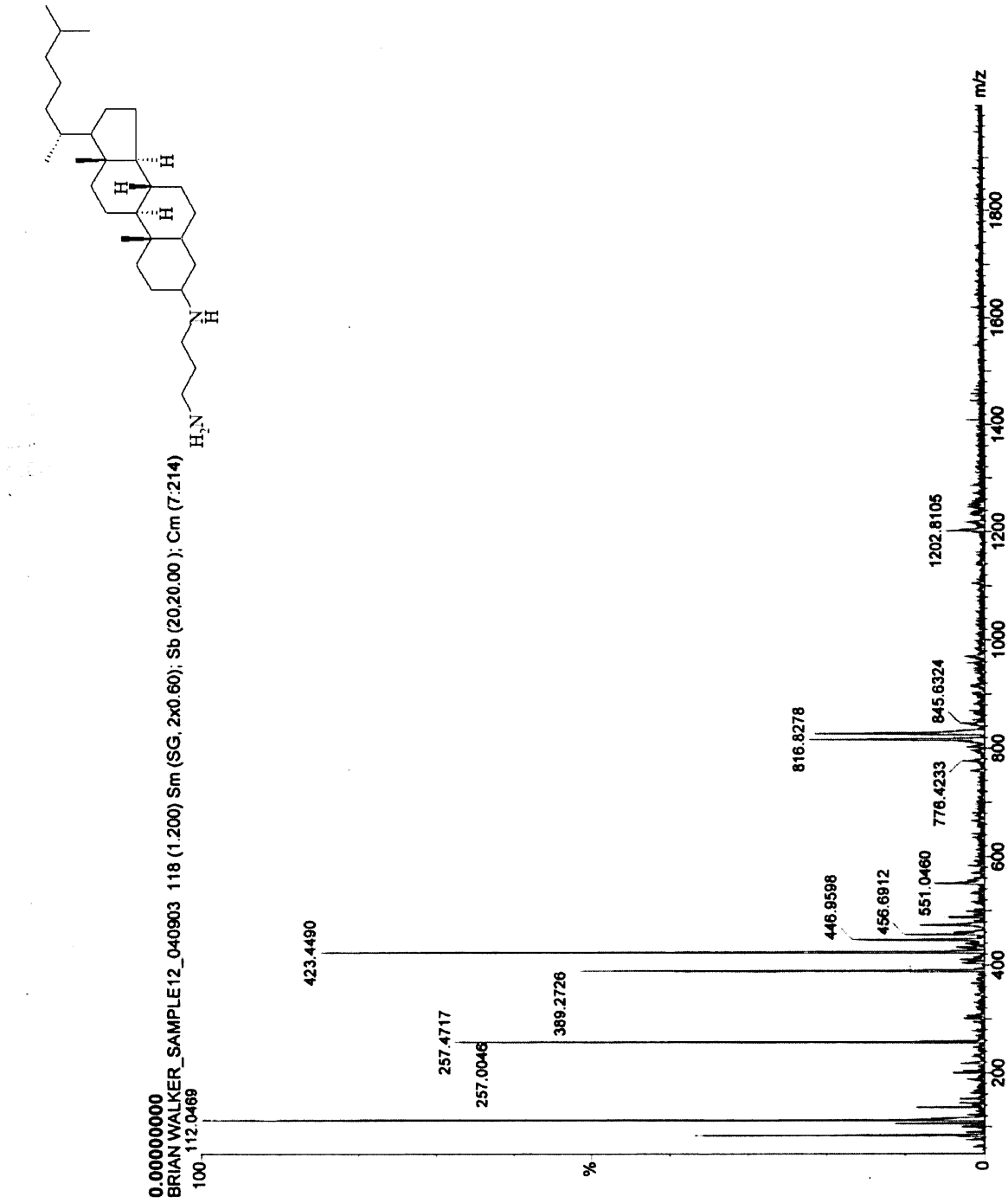
Appendix 34. ^{13}C NMR of **65**.



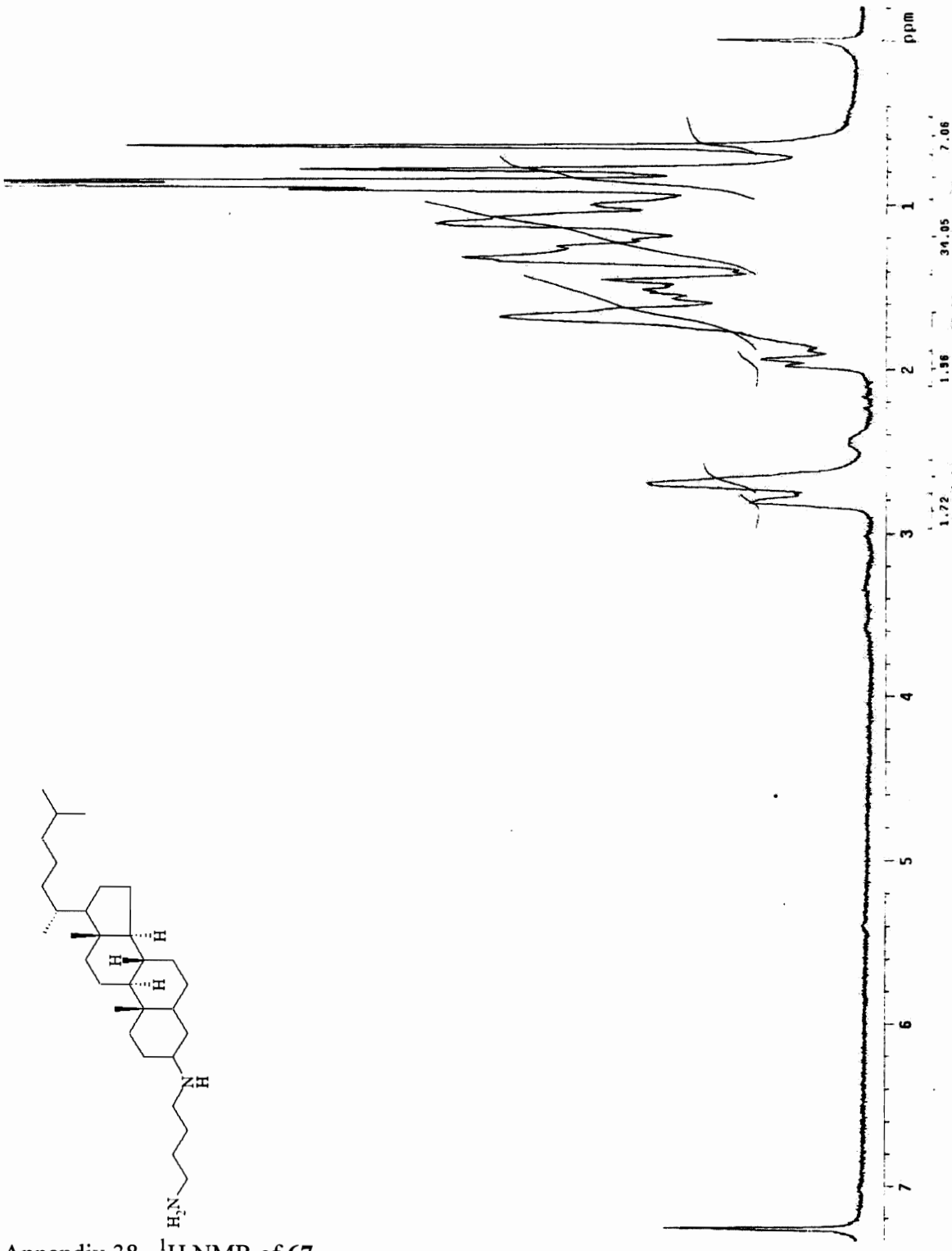
Appendix 35. Mass spectrum of **65**.



Appendix 36. ^{13}C NMR of **66**.

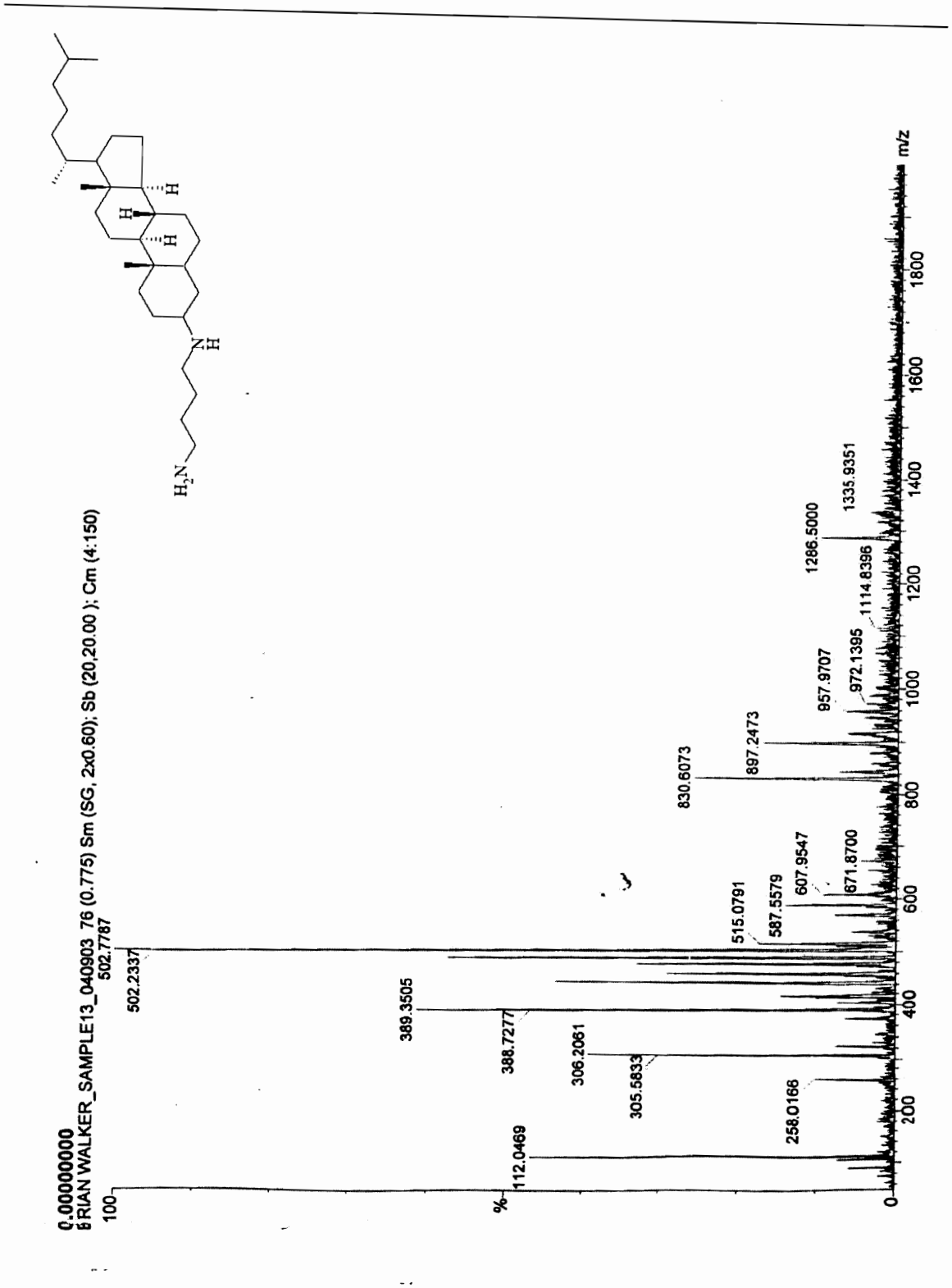


Appendix 37. Mass spectrum of **66**.

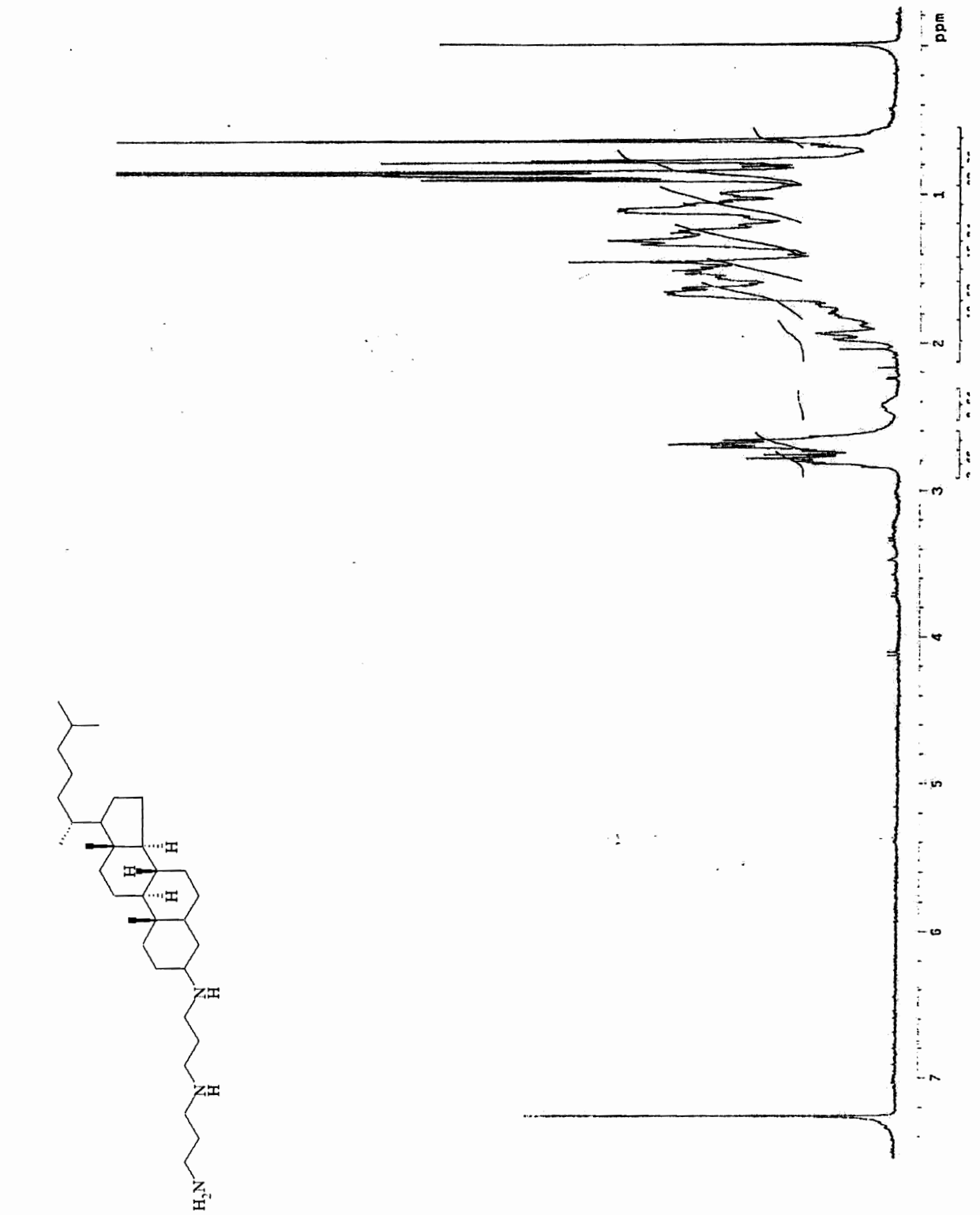


Appendix 38. ^{1}H NMR of **67**.

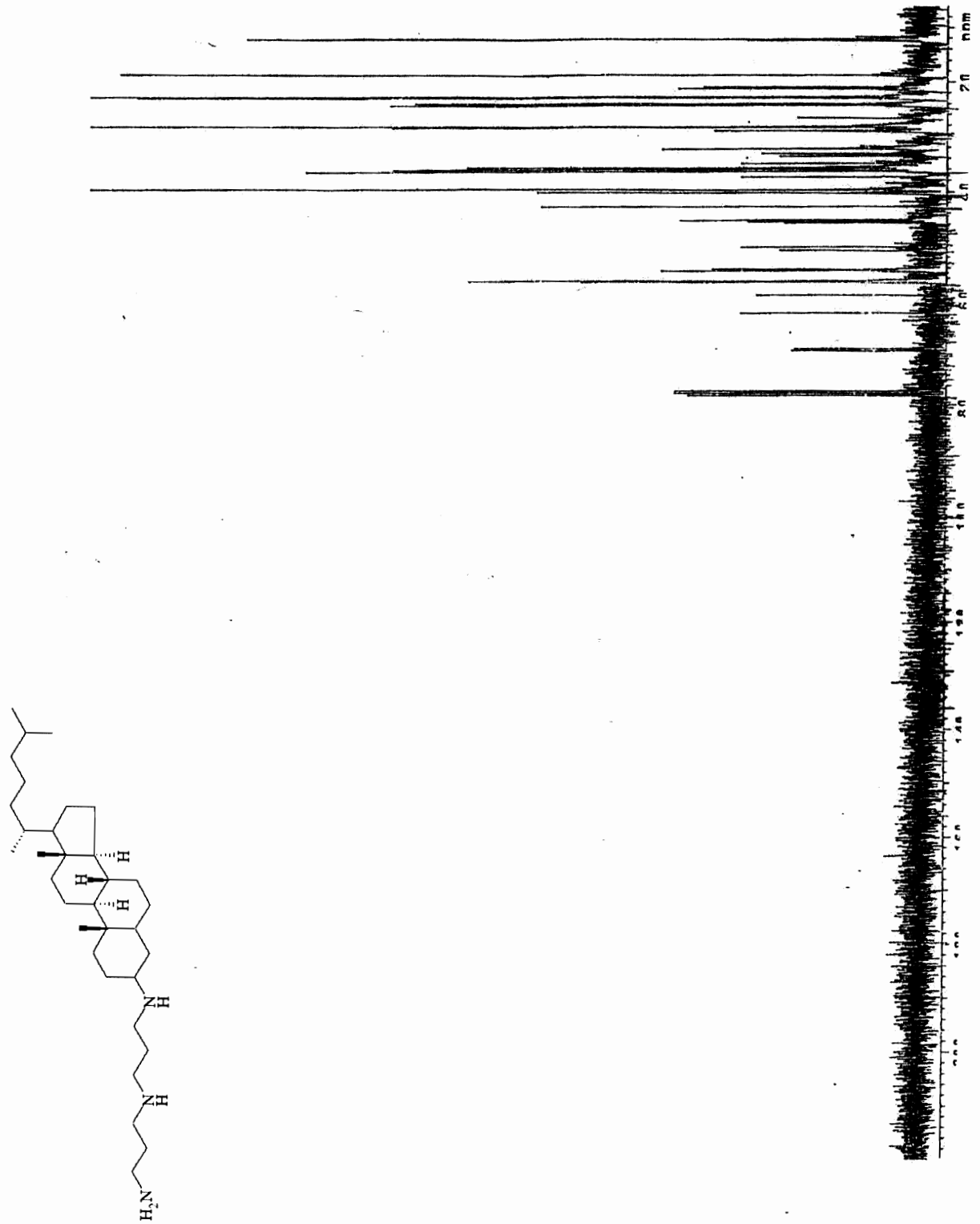
Appendix 39. ^{13}C NMR of **67**.



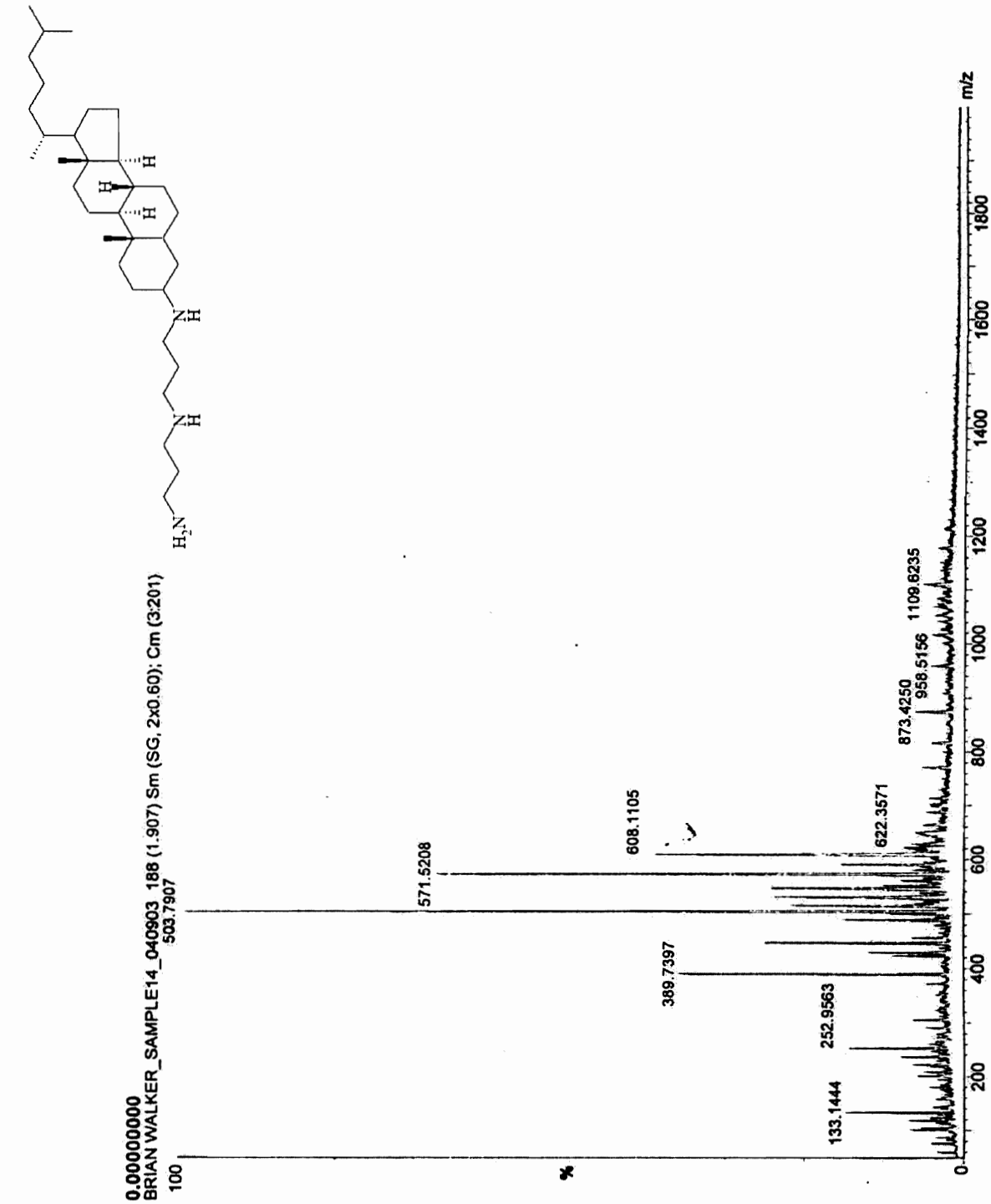
Appendix 40. Mass spectrum of **67**.



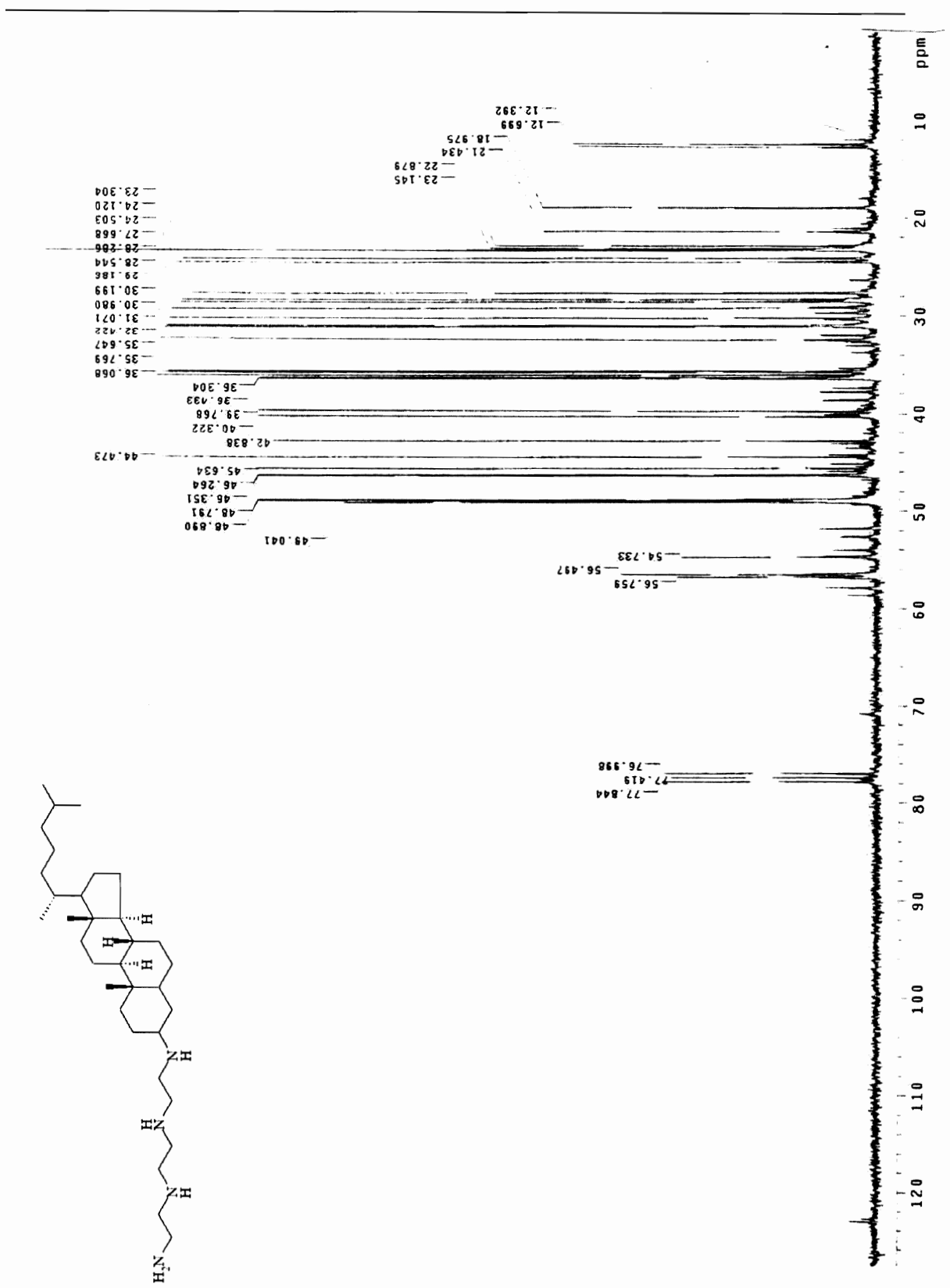
Appendix 41. ^{1}H NMR of **68**.



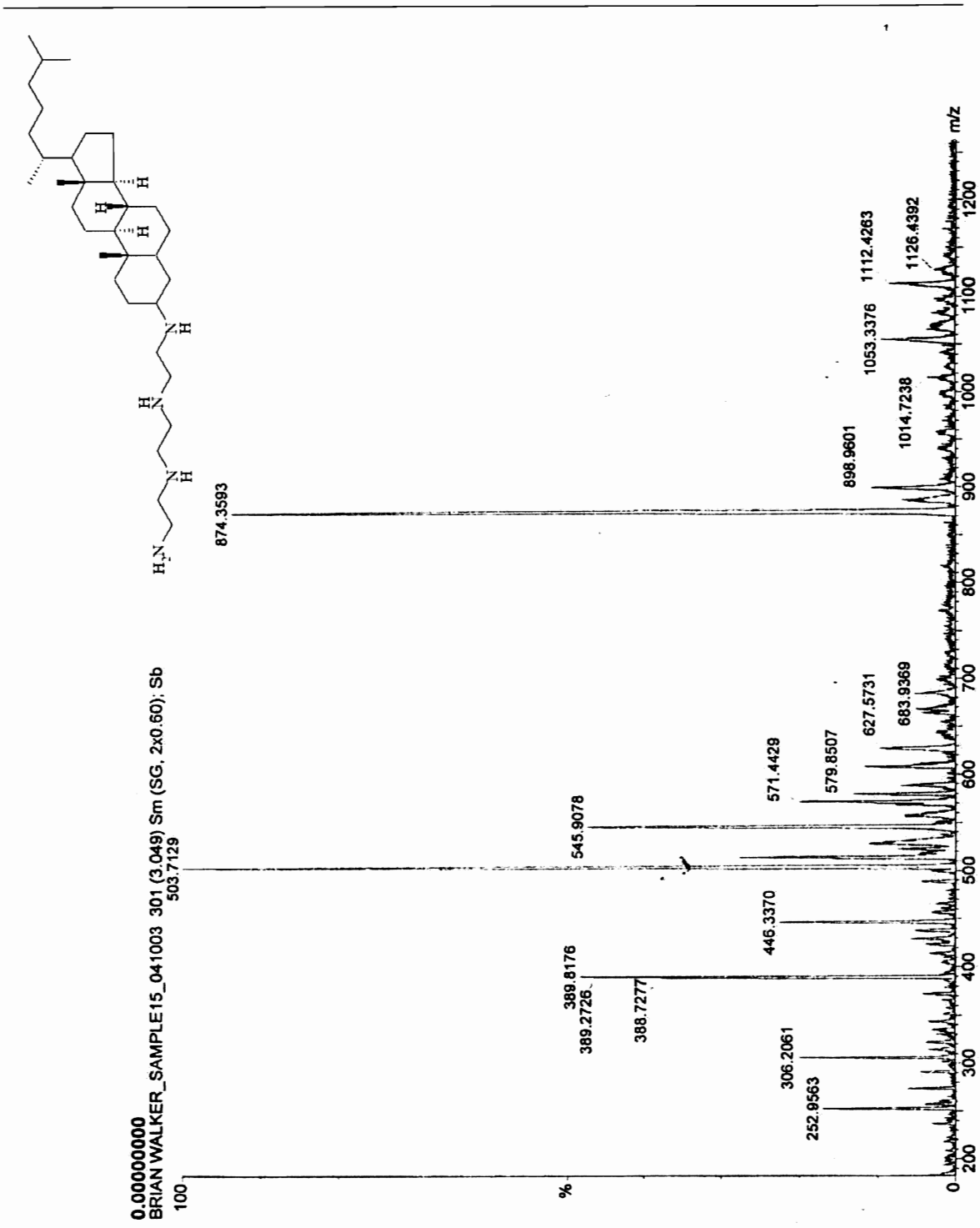
Appendix 42. ^{13}C NMR of **68**.



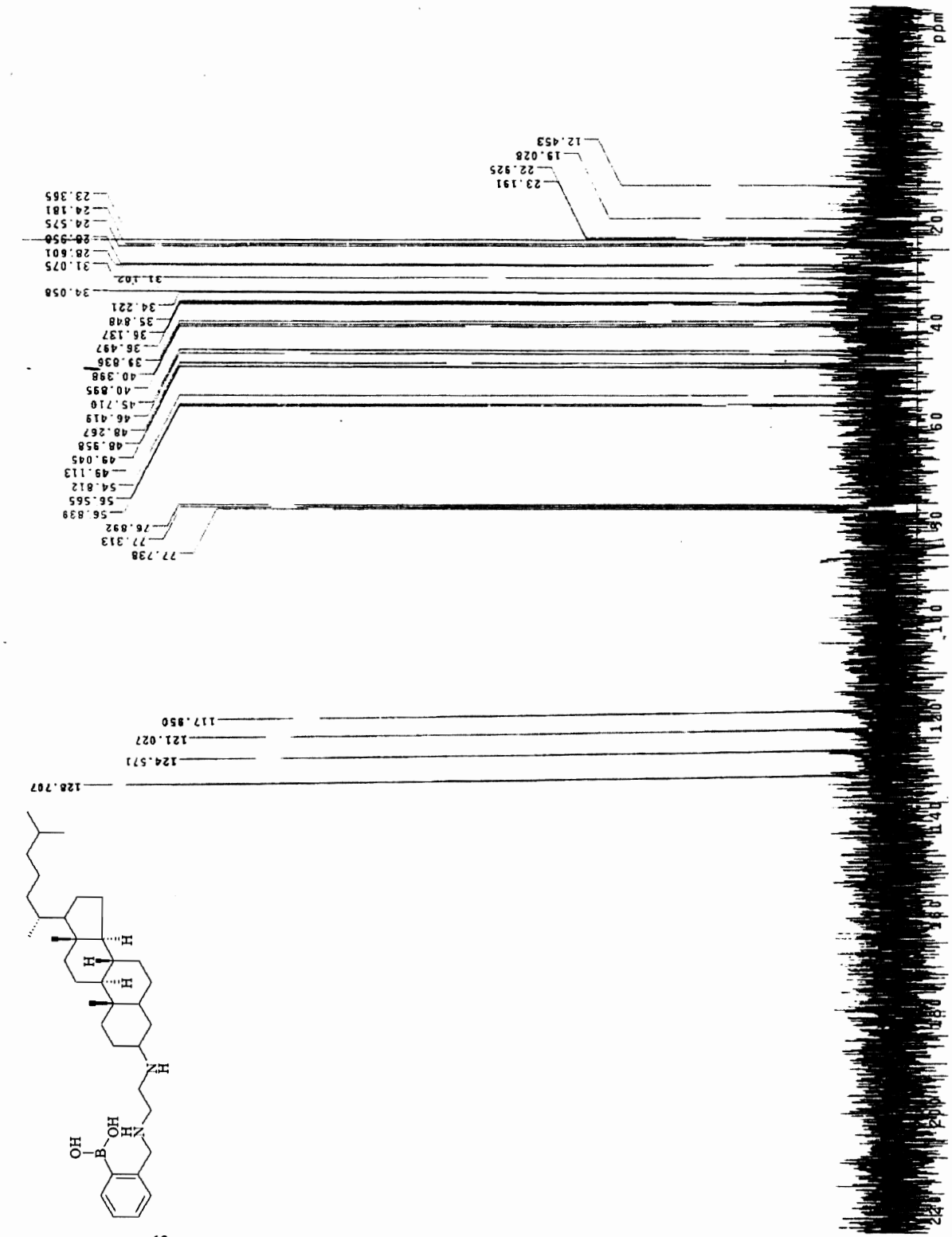
Appendix 43. Mass spectrum of **68**.



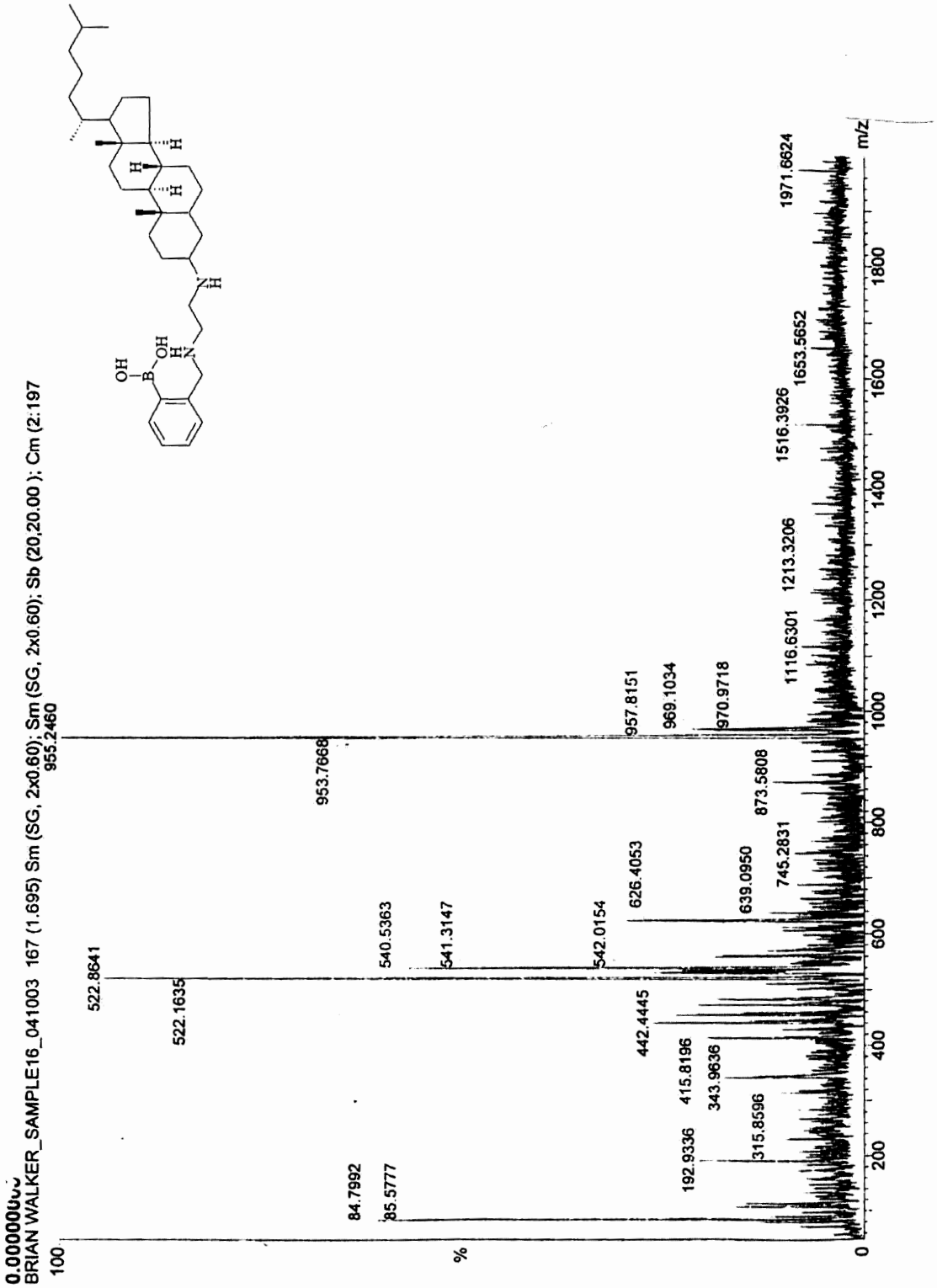
Appendix 44. ^{13}C NMR of **69**.



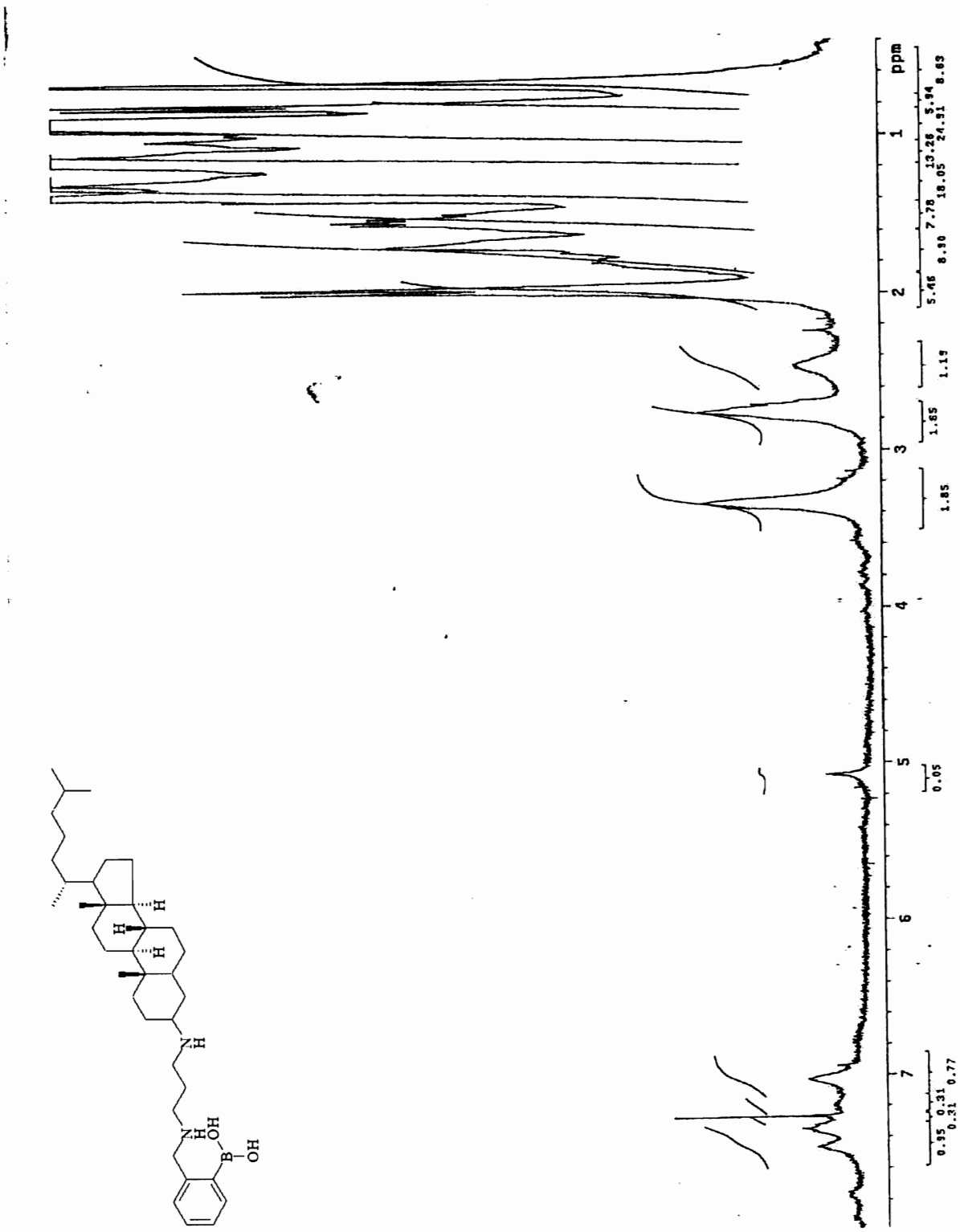
Appendix 45. Mass spectrum of **69**.



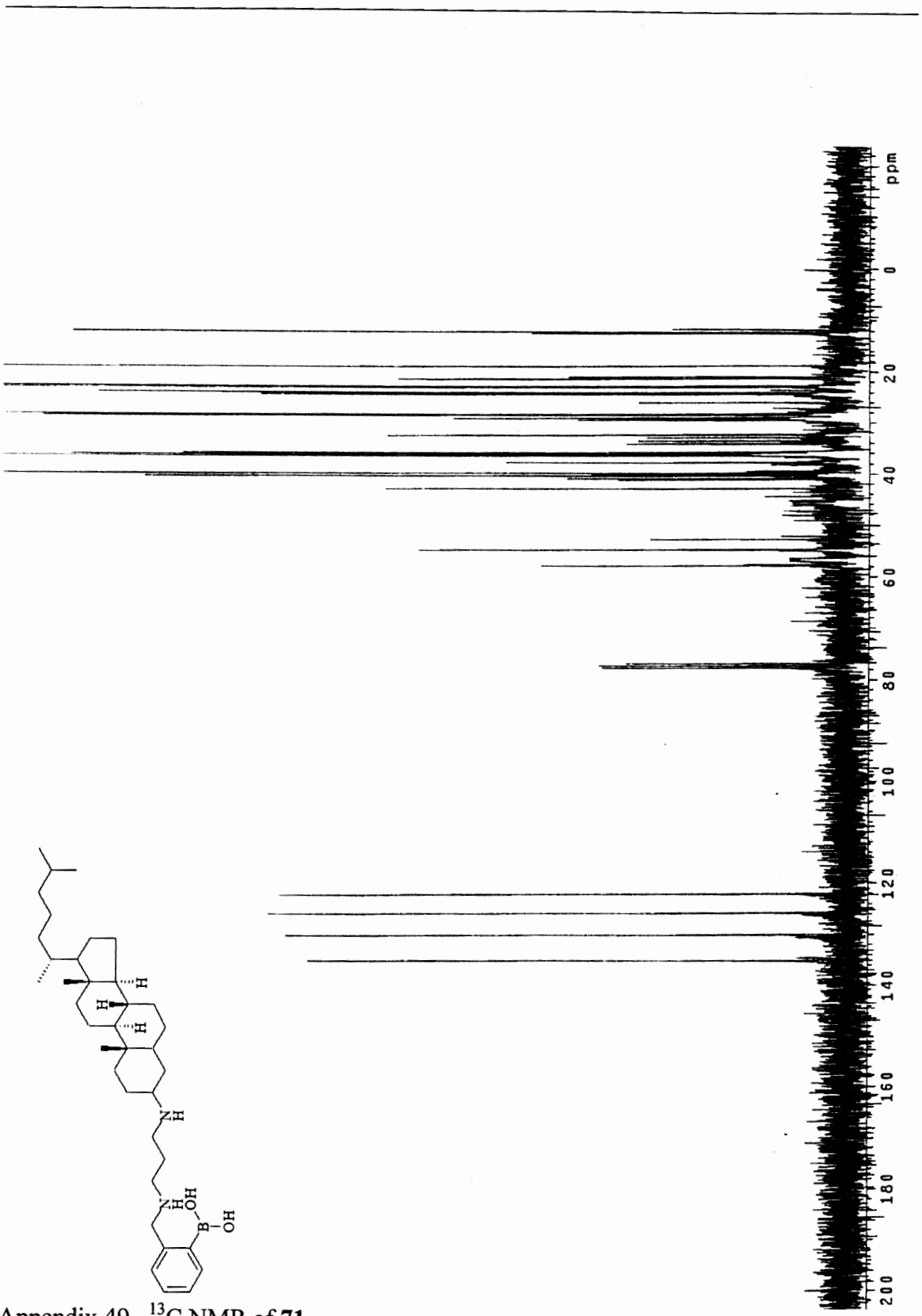
Appendix 46. ^{13}C NMR of **70**.



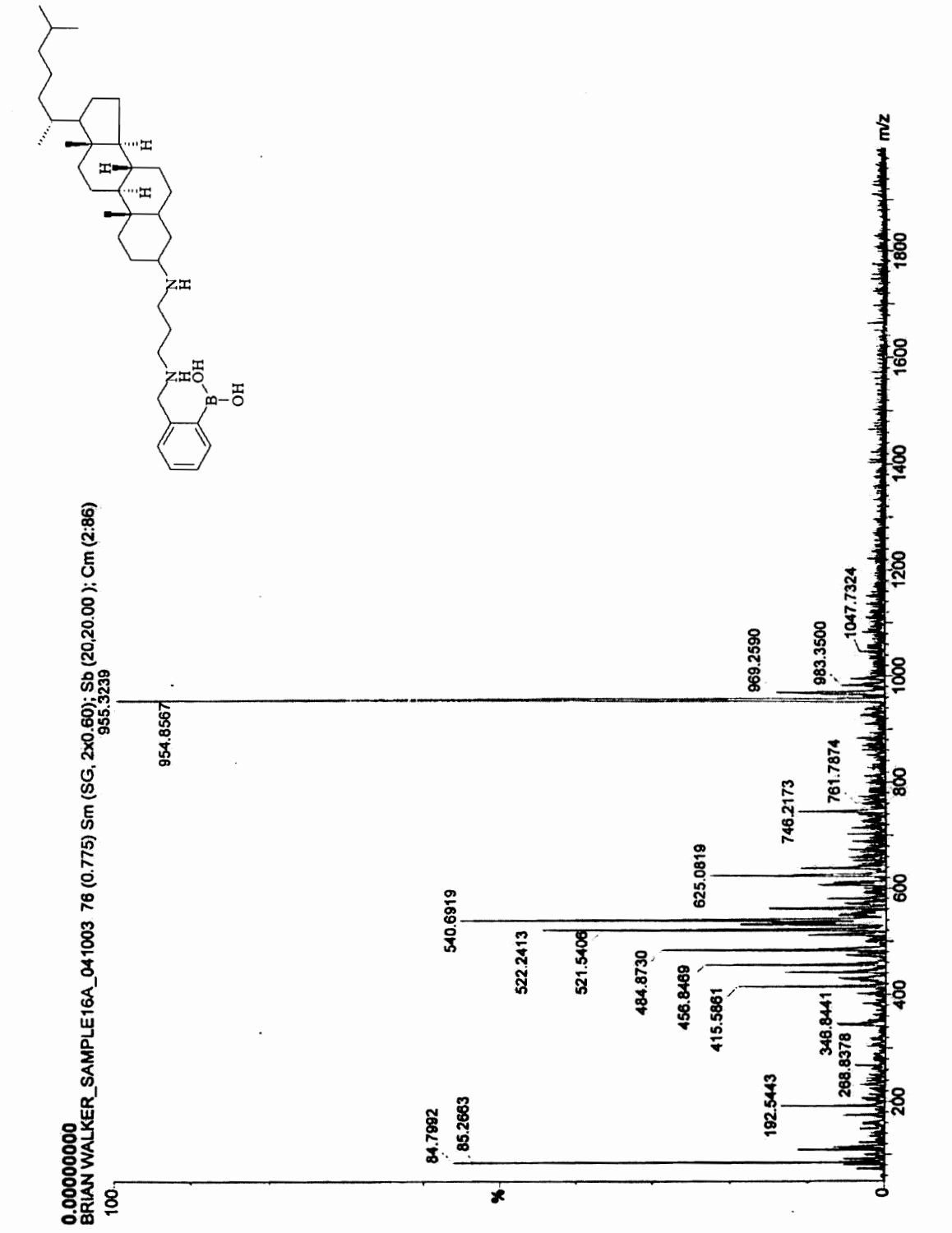
Appendix 47. Mass spectrum of **70**.



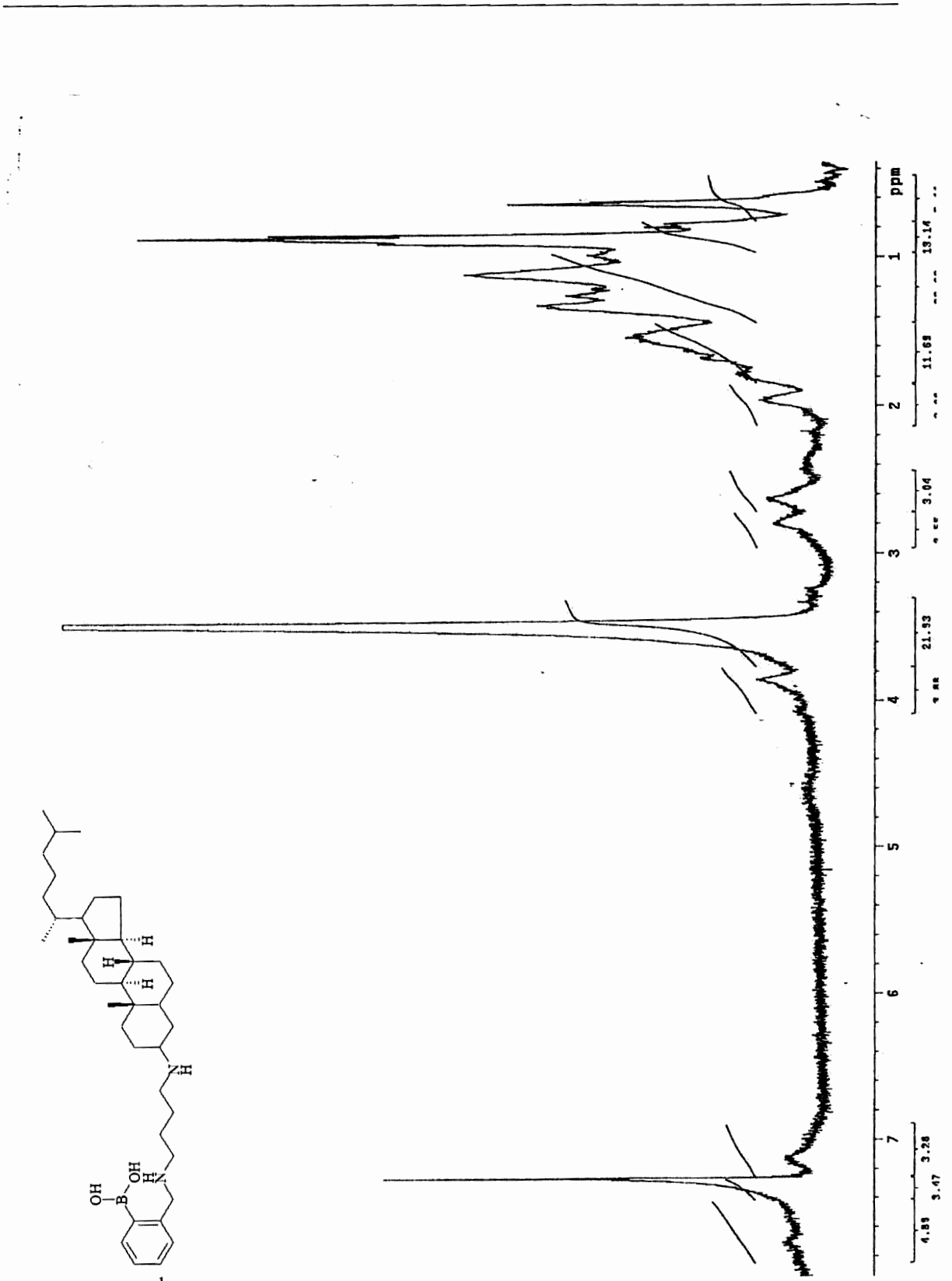
Appendix 48. ^{1}H NMR of **71**.



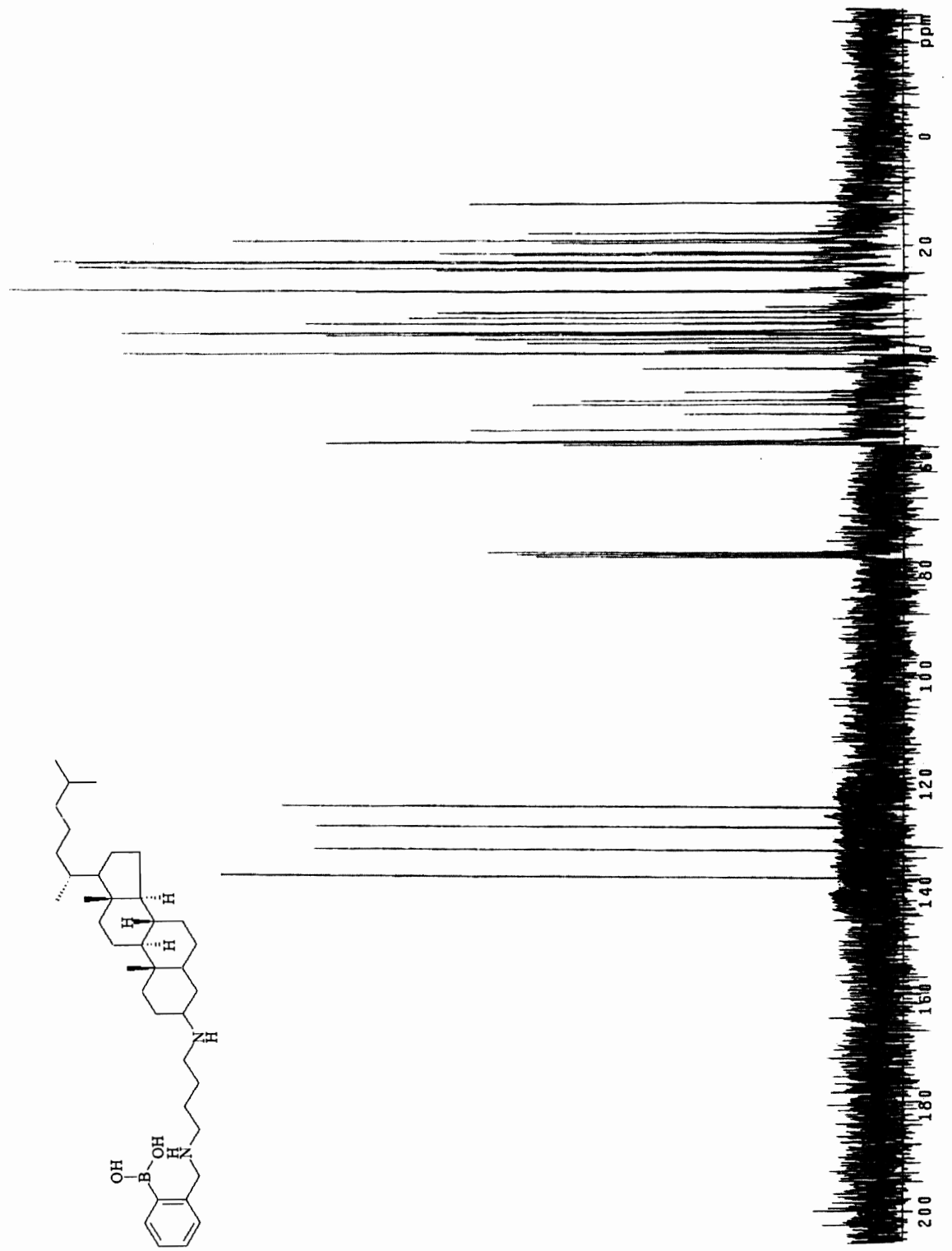
Appendix 49. ^{13}C NMR of **71**.



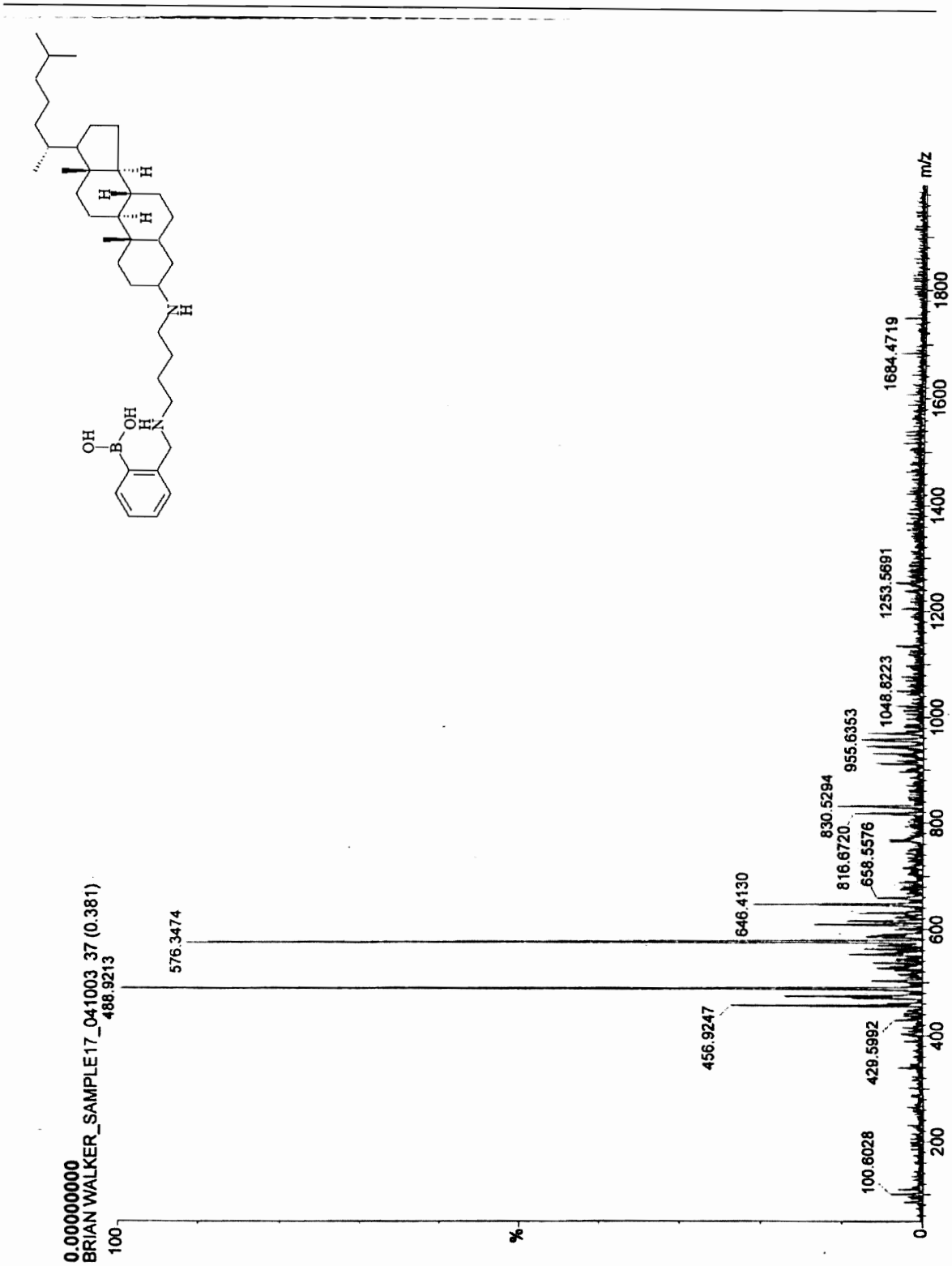
Appendix 50. Mass spectrum of **71**.



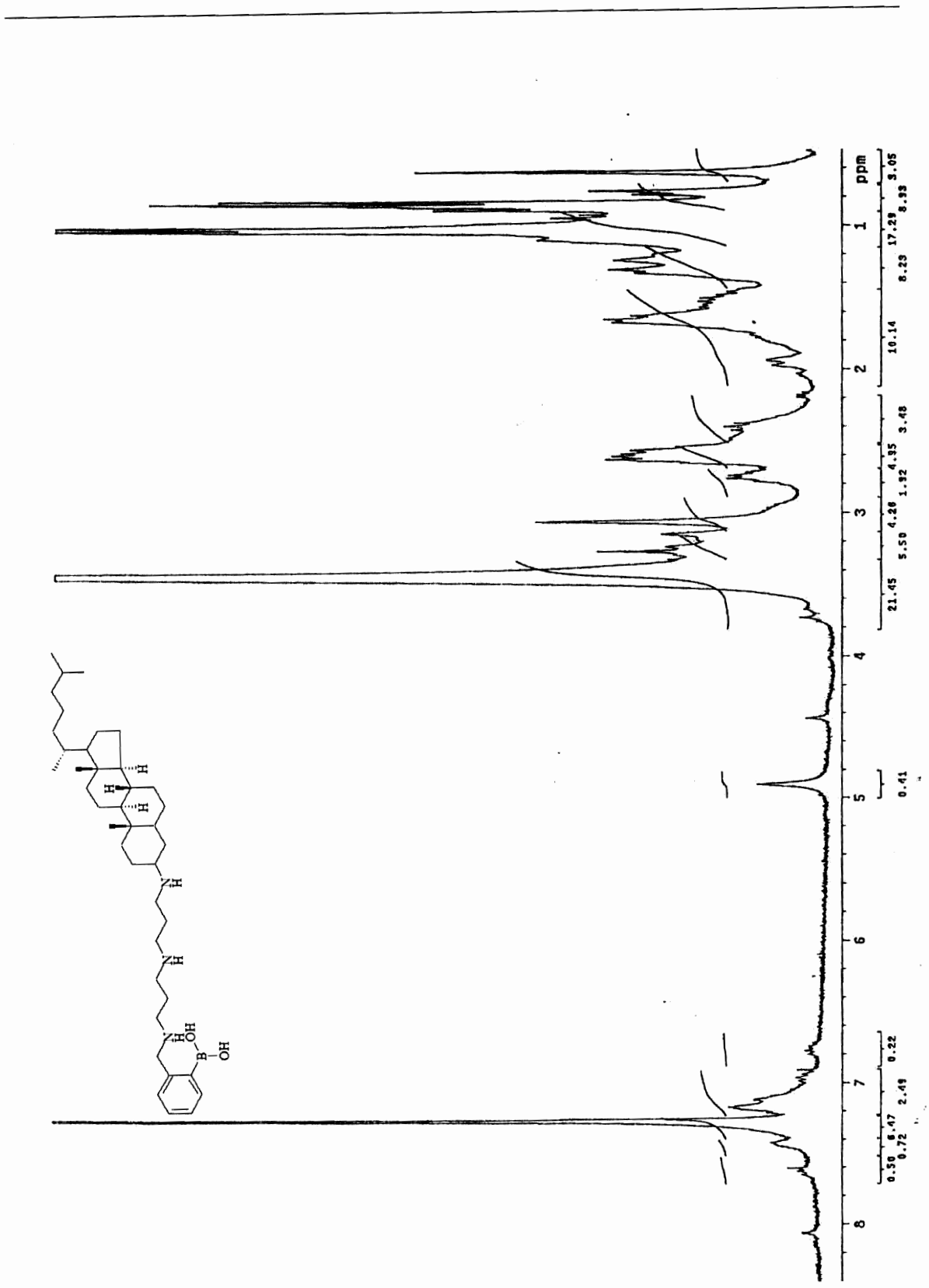
Appendix 51. ^{1}H NMR of **72**.



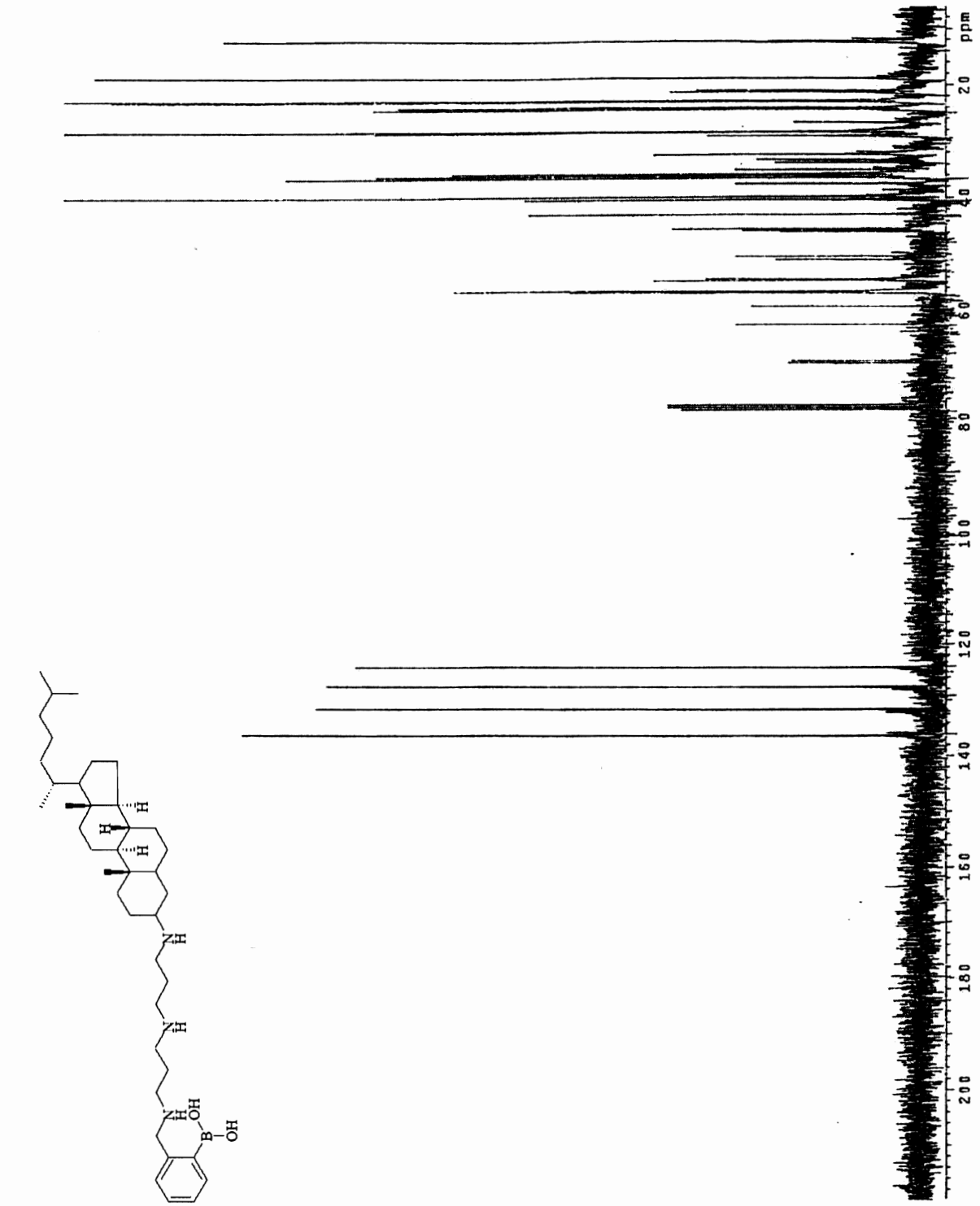
Appendix 52. ^{13}C NMR of **72.**



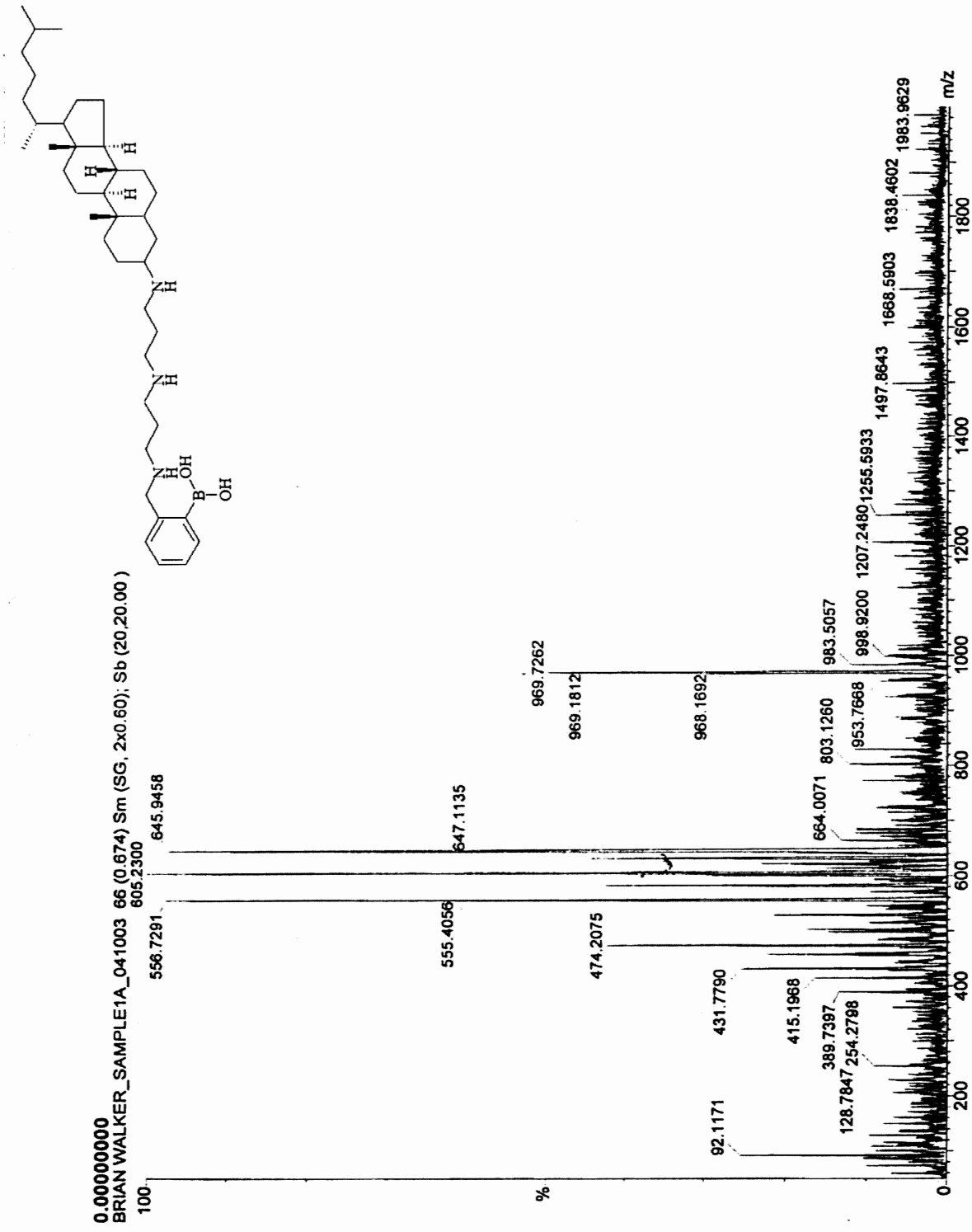
Appendix 53. Mass spectrum of **72**.



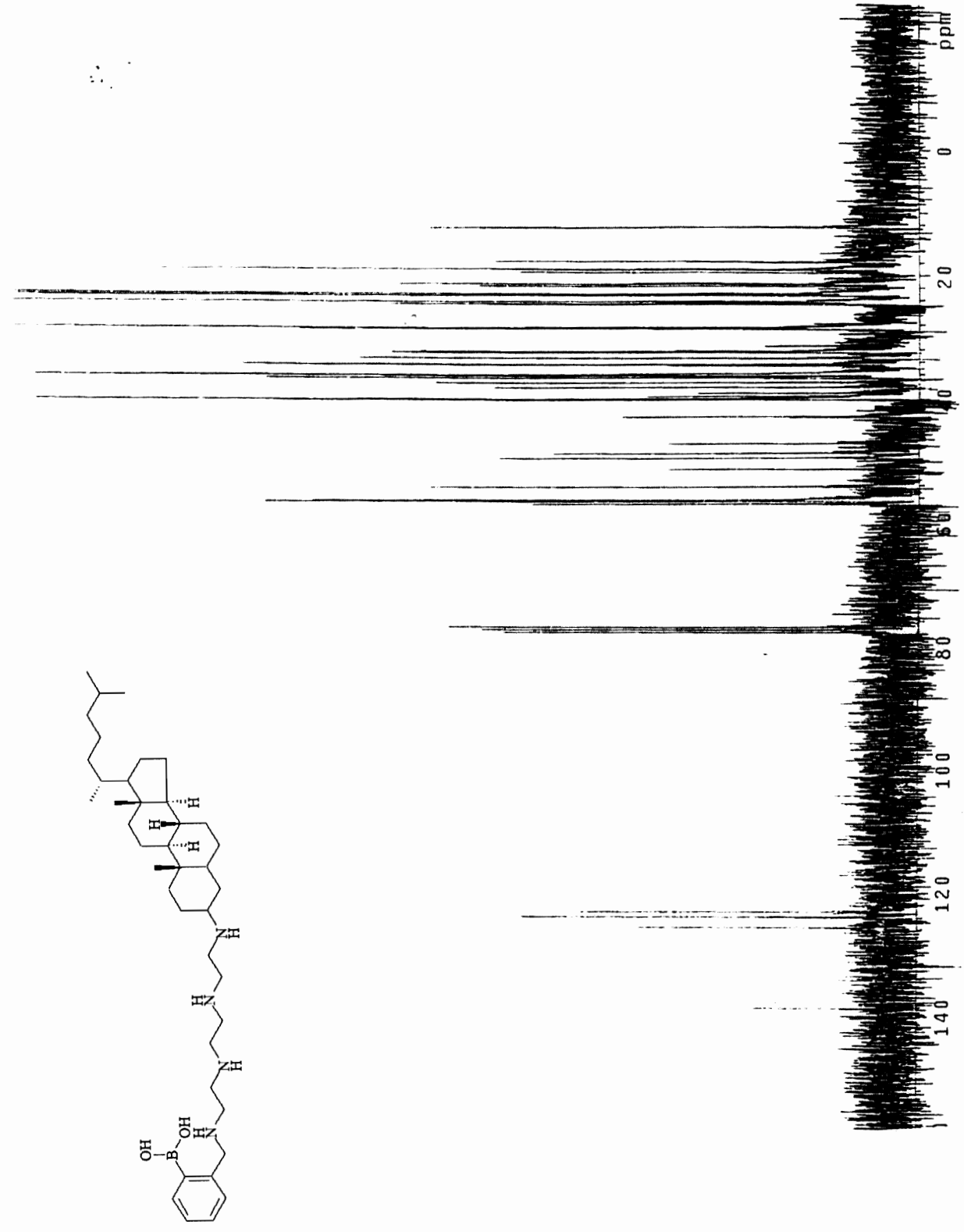
Appendix 54. ^{1}H NMR of **73**.



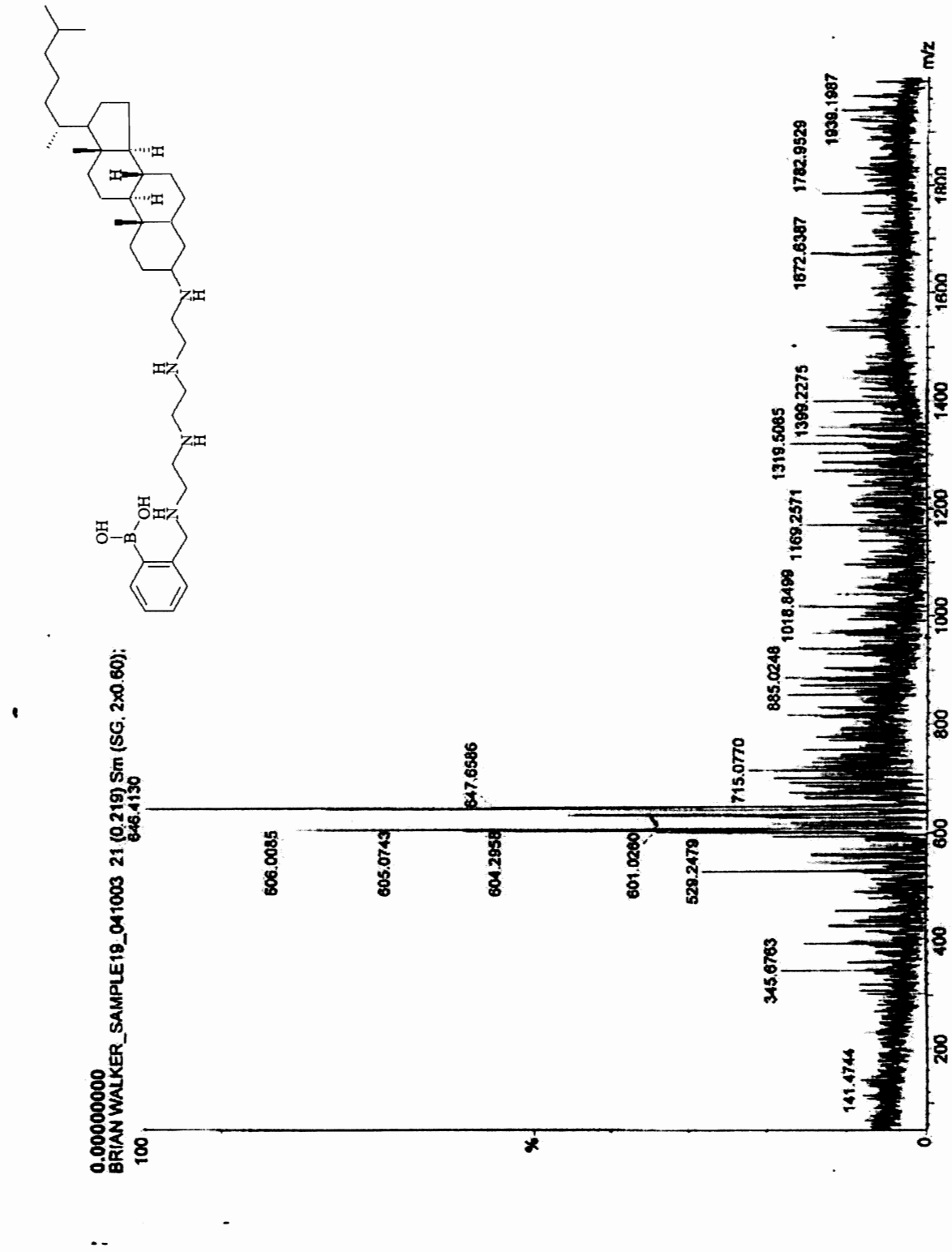
Appendix 55. ^{13}C NMR of **73**.



Appendix 56. Mass spectrum of **73**.

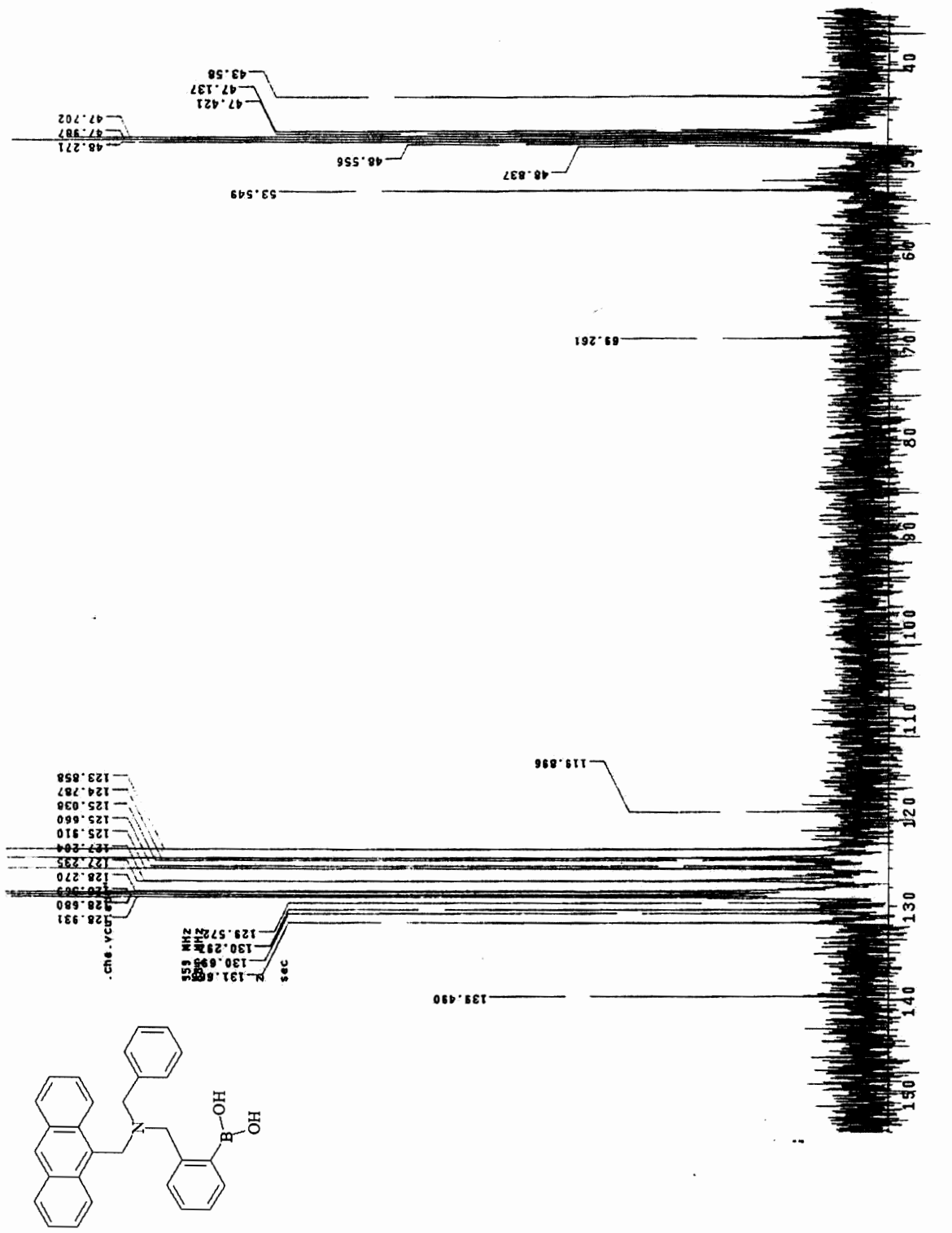


Appendix 57. ^{13}C NMR of **74.**



Appendix 58. Mass spectrum of **74**.

Appendix 59. ^{1}H NMR of **95**.



Appendix 60. ^{13}C NMR of **95**.

Vita

Brian Thomas Walker was born on December 5, 1974 in Richmond, VA. He graduated from New Kent High School in Virginia in 1992. He received his B. S. in Chemistry from the College of William and Mary in May 1996. He entered the Virginia Commonwealth University Graduate Program in August 1996. He is to receive is Doctor of Philosophy in Chemistry in August 2006. He is currently a research associate at the Hampton University School of Pharmacy.

Publications:

1. Gray, C., Walker, B., Houston, T., Foley, R. Boronate Derivatives of BioactiveAmines: Potential Neutral Receptors for Anionic Oligosaccharides. *Tetrahedron Letters*, **2003**, *44*, 3304.

2. Gray, C., Johnson, L., Walker, B., Sleevi, M., Campbell, S., Plourde, R., Houston, T. Selective Sensing Between Inositol Epimers by a Bis(boronate), *Bioorg. Med. Chem. Lett.*, **2005**, *15*, 5416.

Presentations:

1. Walker, B. T., Houston, T. A. "Toward Selective Recognition of Sialic Acid", presented at the 216th National Meeting of the American Chemical Society, Boston, MA, August 1998.

2. Walker, B. T., Houston, T. A., "Toward Selective Recognition of Sialic Acid", presented at the 15th Annual Daniel T. Watts Research Poster Symposium, VCU, Oct. 1998.

3. Walker, B. T., Kramp, K. L., Houston, T. A. "Oligomeric Boronic Acids for Targeted Binding to Cell-Surface Carbohydrate Repeats," presented at the High Technology Materials Symposium, VCU, Nov. 1998.

4. Walker, B. T., Houston, T. A. "Design of Synthetic Receptors for Unique Cell-Surface Oligosaccharides," presented at the 220th National Meeting of the American Chemical Society, Washington D.C., August 2000.