The Enantioselective Synthesis of C_{18}-Sphingosines

by

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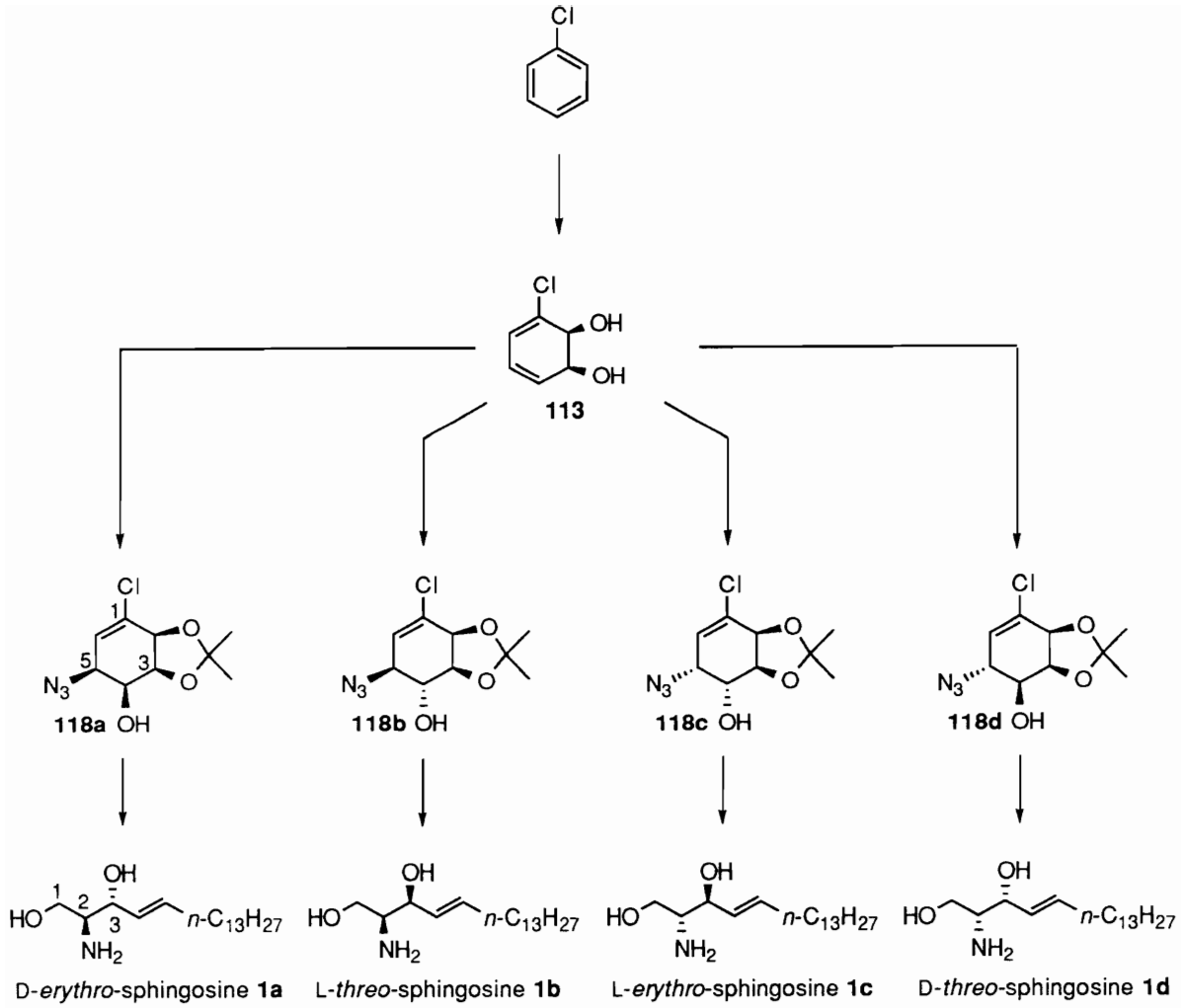
(Abstract)

Biocatalytic conversion of chlorobenzene to the corresponding homochiral cyclohexadiene *cis*-diol (**113**) allows, through careful symmetry-based planning, the stereodivergent synthesis of all sphingosine stereoisomers. This was achieved *via* the selective preparation of the appropiate diastereomer of azidoalcohol (**118**), were C-4 and C-5 correspond to C-3 and C-2 of the sphingosine skeleton, respectively (Scheme 1).


Cl
OH
OH
113
118a OH
118b OH
118c OH
118d OH
D-erythro-sphingosine 1a
L-threo-sphingosine 1b
L-erythro-sphingosine 1c
D-threo-sphingosine 1d

Scheme 1

ACKNOWLEDGMENTS

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my father and mother - Thomas and Susan Nugent - thanks for everything

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I. INTRODUCTION

In 1876 Thudichum, a London surgeon-chemist, described the chemical composition of the brain and alluded to the presence of cerebroside (or cerebral galactoside) and its exact chemical composition, including a unique aliphatic alkaloid called "sphingosine." [1] Sphingosines constitute a group of related long-chain aliphatic 2-amino-1,3-diols, of which 2-amino-D-*erythro*-4E-octadecene-1,3-diol (**1**) occurs most frequently in animal glycosphingolipids,[2] the glycosides of N-acylsphingosines or ceramides. The structural variation inherent in fatty acids, sphingosines, and carbohydrates results in a great number of chemically distinct glycosphingolipids,[2] which are of intense interest[3] because of their diverse biological roles.

The intention of the synthetic project devised in this thesis is the enantioselective synthesis of all four stereoisomers of sphingosine from one chiral synthon. This goal was achieved using the versatile homochiral chlorocyclohexadiene-*cis*-diol synthon. The general utility of this synthon is apparent when one looks at the number of total syntheses of natural products that use this compound.[4]

Chlorocyclohexadiene-*cis*-diol is one of many enantiomerically pure diols of type **113**, accessible *via* the biocatalystic oxidation of aromatic compounds. Recently, it has been suggested that biotransformations could be used to remove toxic aromatic waste from the environment. It has been known for years that the soil bacterium *Pseudomonas putida* oxidatively degrades aromatic compounds to catechols and ultimately to muconates. In 1970 Gibson isolated a mutant strain of bacteria (*Pseudomonas putida* 39D) that arrested the oxidation of aromatics to catechols at the stage of the substituted cyclohexadiene-*cis*-diol. These bacterial metabolites are >95% enantiomerically pure. Industrially, aromatic waste such as benzene and chlorobenzene (over 3 million tons of each produced annually) could be converted into chiral synthons of type **113**.

It is this marriage of chemistry and microbiology that has allowed the field of biotransformations to grow so rapidly recently. Biotransformations or biocatalysis are chemical transformations mediated by either purified enzymes or by whole cell organisms (e.g. bacteria, fungi, plant or mammalian tissue culture). Included under this broad title are steroselective hydrolysis, esterifications, oxidations, and reductions catalyzed by enzymes.

Organic synthesis is rich with examples of total syntheses of challenging and complex natural products, but upon examination of the aliphatic alkaloids called "sphingosines" one might be less excited. The molecule appears very simple, and it is. So the question arises, "Why are people still trying to make sphingosine?"

Since the first total synthesis in 1954, there have been at least another thirty syntheses. Over the years, overall yield has dramatically increased along with efficiency, to a point now of great desparity from the original synthesis. This has been accomplished mostly through great insight and to a lesser degree through the use of new reagents. The continued interest has been fueled by two main factors. One is the fact that only D-*erythro*-sphingosine exists in any significant amount in nature. Second, occasionally one of the other three isomers gives more encouraging results in biological assays. So, low natural abundance, lack of the other three isomers and diverse biological activities create a demand and keep the synthetic mind open to its synthesis. It is in this context that we became involved in the total synthesis of sphingosines, supported by interest from Genencor International, a biocatalysis-oriented company. What ensues is our story of the synthesis of sphingosines.

1. Sphingosines

1.1 Sphingolipid and Lysosphingolipid Structure

This thesis is concerned with the synthesis of sphingosines and necessitates a definition of their structures. Essential for the discussion of synthetic targets and bioactivity is a familiarity with the structures of sphingolipids and lysosphingolipids, which are themselves part of a larger category of biomolecules, namely the lipids.

Broadly defined, a lipid is any molecule of molecular weight between 100 and 5000 which has a substantial portion of its constitution as either aliphatic or aromatic in nature. Included are the hydrocarbons, steroids, soaps, detergents, and more complex molecules i.e. triacylglycerols, phospholipids, gangliosides, and lipopolysaccharides. The physical behavior of such chemically divergent molecules will be equally diverse. Indeed, one of the most interesting characteristics of lipids is their varied behavior in aqueous systems, ranging from almost total insolubility (e.g. paraffin oil, sterol esters) to nearly complete solubility (e.g. soaps, detergents, bile salts, and gangliosides). This particular aspect of lipids is important biologically because all cells exist in an aqueous milieu.[5]

The simplest sphingolipid is ceramide (Figure 1). All other sphingolipids have head groups attached at the C-1 carbon. Ceramides comprise sphingoid bases with an amide-linked fatty acyl chain, examples of which are stearoyl, oleoyl, palmitoyl, or linoleoyl residues. The stearoyl residue is the most commonly occurring fatty acyl chain. With the exception of sphingomyelin, which has a phosphoryl choline head group at C-1 linked through a phosphodiester bond, sphingolipids have a glycosidic bond at C-1. These sugar head groups can vary in complexity from a single glucose or galactose, as in cerebrosides, to more complex structures, such as lactosylceramide with two sugars, trihexosides with three, and higher order carbohydrate moieties. Certain subclasses are characterized by additional components, such as sulfatides, which contain sulfate, and gangliosides, which carry sialic acid residues. Lysosphingolipids are based on sphingosine in a manner analogous to the way sphingolipids are based on ceramide (Figure 1). For each parental sphingolipid there is a corresponding lysosphingolipid that has an identical head group at C-1, but that lacks the amide-linked fatty acyl group at C-2. All lysosphingolipids share two important structural features with sphingosine *i.e.* a charged amine at C-2 and a hydrophobic hydrocarbon tail. Note that sphingosine itself is a lysosphingolipid and not a sphingolipid.

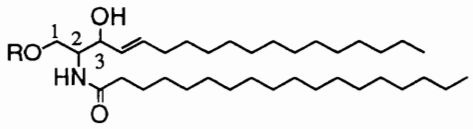
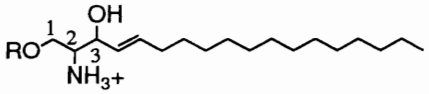
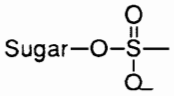
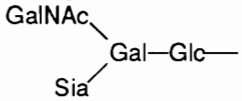
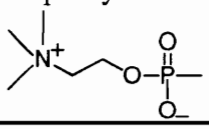
HEAD GROUP	SPHINGOLIPID	LYSOSPHINGOLIPID
		
R = H	Ceramide	Sphingosine
R = Galactose	Galactocerebroside	Psychosine (Galactosylsphingosine)
R = Sulfogalactose 	Sulfatide	Lysosulfatide (Sulfogalactosylsphingosine)
R = Ganglioside, *e.g.* 	GM_2	Lyso GM_2
R = Phosphorylcholine 	Sphingomyelin	Lysosphingomyelin

Figure 1

Although sphingosine is the predominant long-chain base in many sphingolipids and lysosphingolipids, other sphingoids may be present (Figure 2).[6]

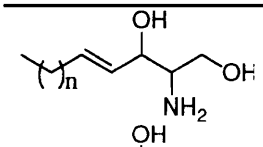
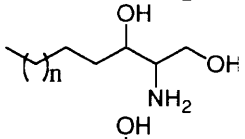
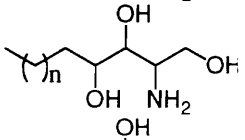
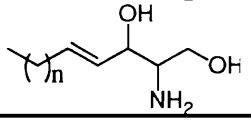
STRUCTURE	n	NAME	OTHER NAMES
	12	sphingosine	sphing-4-ene
	12	sphinganine	dihydroxysphinganine
	12	phytosphingosine	4-D-hydroxysphinganine
	14	eicosasphingenine	D-*erythro*-2-amino-4-*trans*-eicosene-1,3-diol

Figure 2

1. Sphingosines

1.2 Isolation and Structure Elucidation

Nerve tissue contains a high concentration of extremely complex lipid material. The three primary lipid constituents are cerebrosides,[7] sphingomyelins,[7] and gangliosides.[8] The only practical source of the sphingolipids is the brain or spinal cord, although they occur in blood, liver, kidney, spleen, and other organs in small amounts. Brain is reported to contain 2.5 to 3.0 per cent of cerebroside[9] and 1.0 to 3.0 per cent of sphingomyelin by weight.[10] A variety of methods serve to isolate sphingolipids from nerve tissue. Practically all involve (1) dehydration of the fresh tissue, (2) extraction of the glycerophosphatides, and (3) extraction of the cerebrosides and sphingomyelins.[11]

Thus Carter *et al.*[11] described the process using 100 pounds of freshly ground beef brain or spinal cord. The yield of crude sphingolipid from 45.4 kilos (100 pounds) of spinal cord ranged from 2.2 g to 2.5 g, compared to 1.3 g to 1.6 g when brain was used. The sphingolipids isolated from the spinal cord were of higher purity. For a brief procedural summary see reference 12.

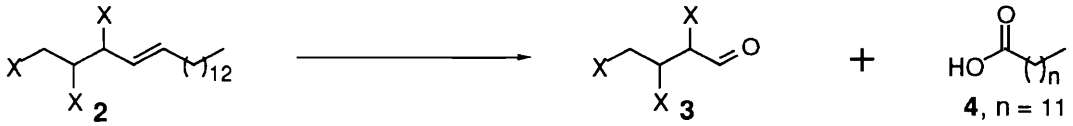
The study of the chemical composition of the brain dates to the end of the eighteenth century. Foucroy made this task possible when he introduced organic solvents

(e.g. ethanol and ether) and mineral acids (e.g. H_2SO_4 and HNO_3). In 1793 he was able to isolate, among other things, a substance with properties like cholesterol[13] from brain matter. It was not until 1812 that Vauquelin, Fourcroy's pupil, showed that phosphorus[14] was inherent in brain matter. In 1834 Couerbe isolated pure cholesterol *via* exhaustive extraction of brain matter.[15] He also isolated a mixture of lipids, which was later referred to as protagon. In 1865 Liebreich hypothesized[16] that the brain consisted of a single chemically distinct compound which he named protagon (Gr. *protos*, first; *agonistes*, a combatant.[17]). He described it as a white powdery brain substance which had been freed from fat and cholesterol by extraction with ethanol and ether. Nineteen years later Thudichum, now known as the chemist of the brain, published his work concerning protagon.[18] His work showed that protagon was a mixture of phosphatides and cerebrosides and stated further that there were at least 14 adducts, which he isolated and analyzed. His contemporaries denounced his views, as Mathews[19] points out, "Our knowledge of the chemical constitution of the brain is owing largely to Thudichum, a man of extraordinary care, accuracy, insight and industry, whose abilities were much underrated during his life. For, owing partly to an unusually combative nature, he alienated many of his colleagues and his work was neglected. There is now, however, no question that he was far in advance of all others in this difficult field and his 1901 book, entitled *Die chemische Konstitution des Gehirns des Menschen und der Tiere, nach eigenen Forschungen bearbeitet,* is a monument to his ability and insight."[20]

It was through extensive experimentation on the hydrolysis of phrenosine (a phosphorus-free entity of the brain), by Thudichum, that a parent base of "alkaloidal nature" was isolated. Of which he said, "in commemoration of the many enigmas which it presented to the inquirer" he gave the name sphingosine (Gr. *sphingein*, to bind tightly).[21]

Thudichum suggested the first formulation for sphingosine, $C_{17}H_{35}NO_2$. In 1906 Thierfelder[22] proved the presence of a double bond by adding Br_2. In 1912 Levene[23] showed that sphingosine could be reduced to dihydosphingosine, that it forms a dimethyl ether, and that all of its nitrogen is present as a primary amine. In the same year both Levene[23] and Theirfelder[24] independently made the triacetate and concluded that sphingosine was an unsaturated dihydroxy amine. One year later Levene's oxidative cleavage of the double bond yielded the aminotetrose **3**[25] and the fatty acid **5** (upon further oxidation). This established the position of the double bond on the aliphatic chain. Unfortunately the oxidized fatty acid portion was misidentified as tridecanoic acid

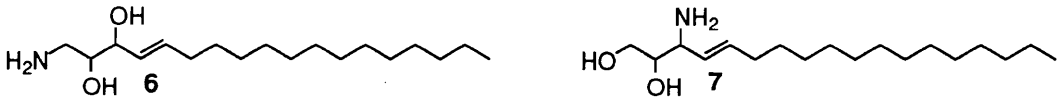
4, when in actuality it was tetradecanoic acid **5** (Scheme 1). It was not until 1929 that Klenk[26] reinvestigated the chromic acid oxidation and established that the fatty acid was indeed tetradecanoic acid **5**. He also provided the correct empirical formula, $C_{18}H_{37}NO_2$, for sphingosine.



Where X represents two alcohols and one primary amine of unknown position.

Scheme 1

Several structural questions still remained. What was the relative order of the hetereo atoms on C-1 through C-3? What was their relative stereochemistry, and finally, what was the geometry of the olefin? The assignment of the relative position of the hetereo atoms was not a trivial one, because the first two assignments were incorrect (Scheme 2). In 1913 Levene[27] made the first misassignment represented by structure **6**. The second misassignment was made by Klenk[28] when he suggested structure **7**, in 1931.

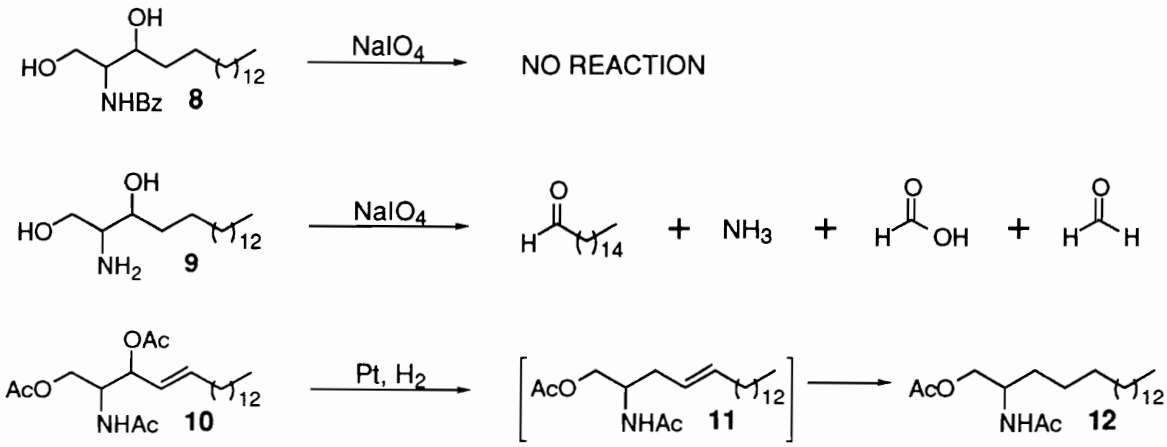


Scheme 2

It was not until 1941 that Seydel[29] proposed, in his dissertation, that the amino functionality resided on C-2. He came to this conclusion after periodate failed to cleave the assumed vicinal diol of the N-acyldihydrosphingosine derivative of **7**. One year later Carter[30] studied the same problem with N-benzoyldihydrosphingosine and found, as Seydel did, that periodate did not attack this molecule. Their independent conclusions were the same, namely sphingosine was a 2-amino-1,3-dihydroxy-4-octadecene.

In addition to the periodate reaction a few more reactions were performed that buttressed their structure proof (Scheme 3). Periodate cleavage of dihydrosphingosine **9** showed that all three hetero atoms were indeed on the first three carbons. In addition Carter found that the triacetate of sphingosine consumed 2.0 equivalents of hydrogen, implying that an allylic acetate was present. Hydrogenolysis of the allylic acetate formed olefin **11**, which was further reduced to the fully saturated compound **12**. Structure **12** was not identified until 1951 when Carter[30c] confirmed the D-configuration of C-2 using

a chemical correlation study. He accomplished this feat by further derivatizing diacetate **12** to the known D-α-benzamidostearic acid. Two years later Carter[31] published several papers concerning the synthesis of D-*erythro*-dihydrosphingosine and showed that the synthetic and natural dihydrosphingosine were identical in every respect.



Scheme 3

In 1947 Ohno provided a chemical correlation study which confirmed the *trans* geometry of the olefin in sphingosine.[32] Degrading sphingosine to hexadecenal, he was then able to oxidize it to the known *trans*-hexadecenoic acid. This result was supported by Mislow[33] who observed a strong infrared absorption at 10.3 μ, indicative of a trans double bond. A very informative and more detailed account of this structural proof can be found in *Chemistry of Sphingolipids* by David Shapiro.[21]

1. Sphingosines
1.3 Bioactivity

At least 300 different sphingolipids are synthesized in various mammalian cell types. This diversity has intrigued investigators for many years. Thudichum, noted for his discovery of sphingosines,[1] published his first report on the chemical constitution of the brain[34] over 120 years ago (1874). In his last publication (1901), entitled "*Die chemische Konstitution des Gehirns des Menschen und der Tiere*," Thudichum summarizes his life-work on cerebrosides, sphingomyelins, and sphingosine.[35,36]

In the latter half of the 1980's it was discovered that lysosphingolipids, the breakdown products of cellular sphingolipids, are biologically active. This bioactivity generated a new interest in the role of these molecules in cell physiology and pathology. Sphingolipid breakdown products, sphingosine and lysospingolipids, inhibit protein kinase C, a pivotal enzyme in cell regulation and signal transduction. These compounds also affect significant cellular responses and exhibit anti tumor promoter activities in various mammalian cells. These molecules may play a role as endogenous modulators of cell function and possibly as second messengers.[37]

Until recently scientists were only able to allude to the important cellular functions of the vast array of complex cellular lipids. A recurring question was why these molecules had survived eons of evolution when a simple phospholipid would suffice in bilayer formation to delimit cells and to divide the cytoplasm into its organelles and compartments.[37] The answer, while incomplete, has more to do with the breakdown products and metabolites of membrane lipids than with the lipids themselves. Many of these breakdown products and metabolites function predominantly in signal transduction as agonists or as second messengers. They include diacylglycerol,[38] platelet activating factor,[39] phosphatidic acid,[40] arachidonic acid[41] (4), prostaglandins,[41] leukotrienes,[41,42] (4,5) eicosanoids,[41] thromboxanes,[41] lipoxins,[42] inositol phosphates,[43] and inositol glycans.[44] Although some of these, such as diacylglycerol and phosphatidic acid, are present constitutively in cells under resting conditions, most of the others are generated when cells are activated.[44]

A recent addition to this growing family of biologically and physiologically active lipids emerged from the investigation of sphingolipid breakdown products. When added to cells, sphingolipid-derived molecules, sphingosine and lysosphingolipids, elicit various pharmacological responses. Some of these responses are inhibition of platelet and neutrophil activation,[45] inhibition of growth factor action,[46] modulation of receptor function,[47] and inhibition of phorbol ester-induced responses.[48]

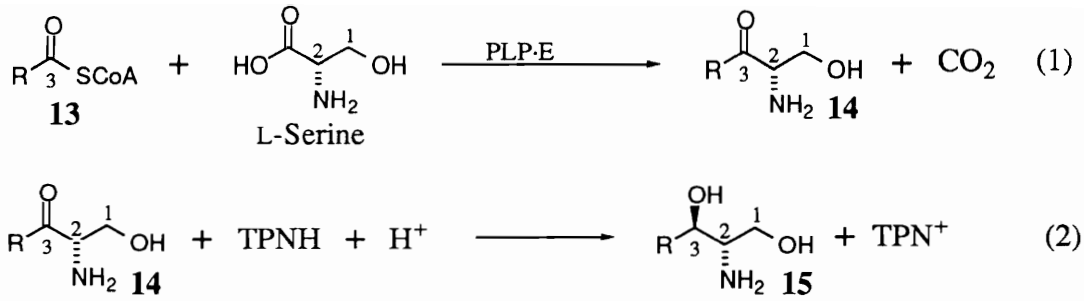
A number of biological and pathological functions are attributed to different sphingolipids, they are summarized in Table 1 of the appendix.[49]

1. Sphingosine
1.4 Biosynthesis

Many nutritional experiments in animals have indicated that vitamin B_6 plays an undefined role in lipid metabolism, and particularly in the formation of polyunsaturated fatty acids.[50] In an attempt to further assess these claims, Haskell and Snell[51] conducted a careful comparison of the lipids of a yeast, *Hanseniaspora valbyensis*, grown with excess vitamins or deficient in vitamin B_6, pantothenic acid, or biotin. They found that the palmitoleic acid content of lipids from vitamin B6-deficient cells was greatly reduced relative to that found in normal control cells, and that this effect was specifically related to the deficiency in vitamin B_6. These analyses also showed that the sphingolipid base content was substantially reduced as a result of the vitamin deficiency. The latter finding caught the attention of researchers interested in the metabolism of the sphingolipid bases.[52]

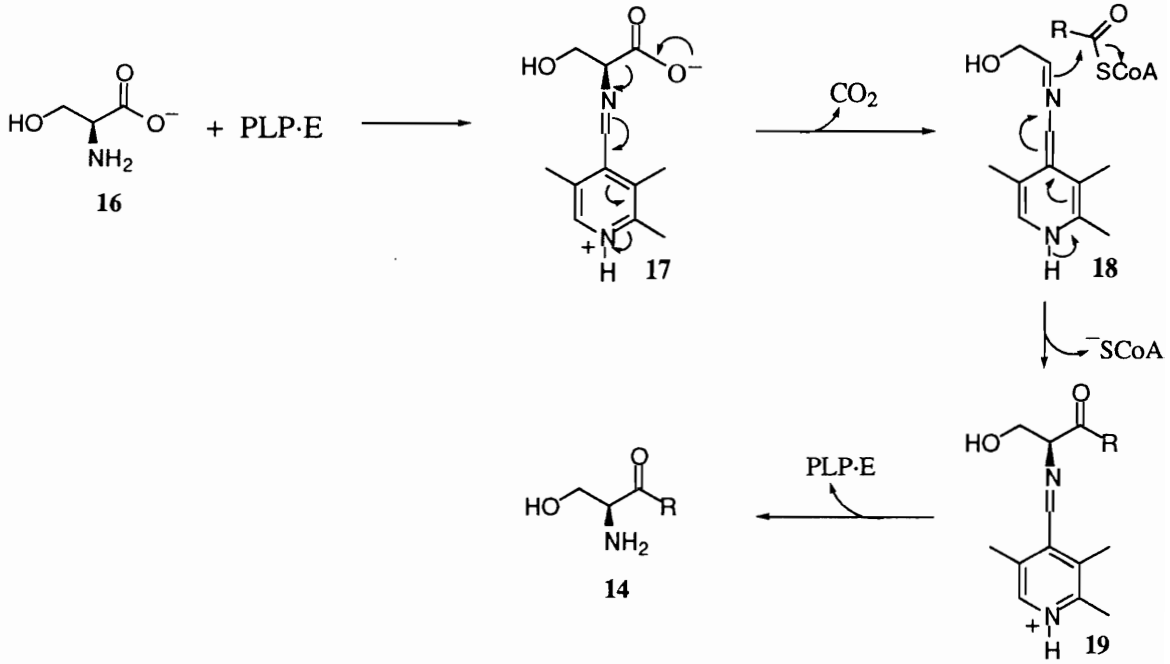
The literature revealed an excellent rationale for the participation of pyridoxal-phosphate (pyridoxal-P) in formation of the sphingolipids.[52] Labeling experiments in animals had shown that sphingolipid bases arose from palmitate and serine, with loss of the carboxyl group of serine, a reaction reminiscent of the pyridoxal-P-dependent enzymatic decarboxylation of amino acids. One postulated course for this reaction was via a 3-keto intermediate, formed by replacing the carboxyl group of serine with the palmityl group. Such a reaction would be closely analogous to the formation of δ-aminolevulinic acid from succinyl CoA and glycine, a reaction known to require pyridoxal-P.[52] In addition, Brady *et al.*[53] had already achieved cell-free (*in vitro*) synthesis of dihydrosphingosine in brain microsomes from palmitaldehyde or palmityl CoA and serine, and had shown that this reaction was largely inhibited by dialysis of their preparations against cysteine (a good complexing agent for pyridoxal-P).[53]

When it was found that a strain of *Hansenula ciferri*, a yeast, excretes relatively large quantities of dihydrosphingosine and phytosphingosine in the form of their acylated derivatives, a practical attempt at the biosynthesis problem could be started.[54] These products arose from serine and palmitic acid.[55] Using the knowledge gained from previous studies, Snell *et al.*[52] proposed the biosynthesis of dihydrosphingosine followed the reaction sequence shown by equation 1 and 2 (Scheme 4).



Scheme 4

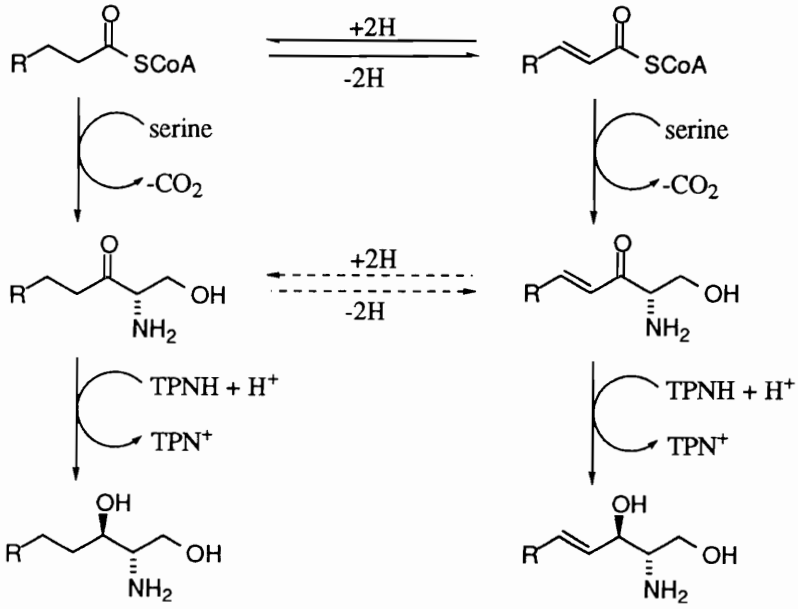
The key features of this proposal are the previously unknown 3-keto intermediate **14**, and the implicit assumption that two distinct enzymatic reactions are involved. From equations (1) and (2) it is evident that the proposed ketonic intermediate **14** should accumulate in the absence of TPNH (NADPH). Indeed, in the absence of TPNH the 3-keto intermediate **14** was formed. Separate experiments showed it to be inhibited by cysteine, i.e. reaction (1) appears to be pyridoxyl-P-dependent [PLP·E (pyridoxal-P enzyme)]. Thus Snell proposed a likely mechanism of formation (Scheme 5).



Scheme 5

A question still remained: when was the unsaturation introduced at C-4 and C-5? To test their hypothesis, that the unsaturation was incorporated after formation of ketone

14, synthetic 3-keto-dihydrosphingosine was treated TPNH (*i.e.* same reaction conditions, but without palmityl CoA and serine). The only observed product was dihydrosphingosine, *via* reduction of the ketone. No sphingosine was found. Since these enzyme preparations form sphingosine in addition to dihydrosphingosine (sphinganine) when palmityl CoA and serine are the substrates, one can tentatively conclude that desaturation during the synthesis of sphingosine must occur at the fatty acyl CoA level,[52] and not at the ketonic level (Scheme 6).[56]



Scheme 6: Probable Course of Biosynthesis for Sphingosine and Dihydrosphingosine.

2. Syntheses

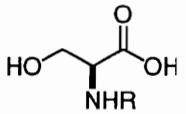
2.1 L-Serine Approaches

Many syntheses of optically pure sphingosines have relied on the use of L-serine as a chiral building block. The first application of L-serine to sphingosine synthesis was reported by Newman[57] in 1973. Since then, exhaustive work using serine derivatives has culminated in highly efficient and diastereoselective synthesis of D-*erythro*- and L-*threo*-sphingosine. The starting materials for the syntheses to follow are shown in Table 1 and are commercially available.

Table 1

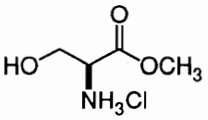
L-serine when R = H

N-(*t*-butoxycarbonyl)-L-serine (N-Boc-L-serine) when R = CO_2t-Bu

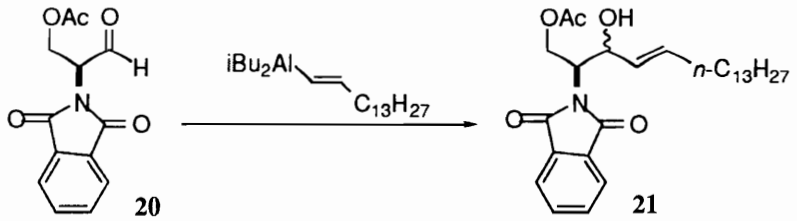


N-(benzyloxycarbonyl)-L-serine (N-Cbz-L-serine) when R = $CO_2CH_2C_6H_5$

L-serine methyl ester hydrochloride

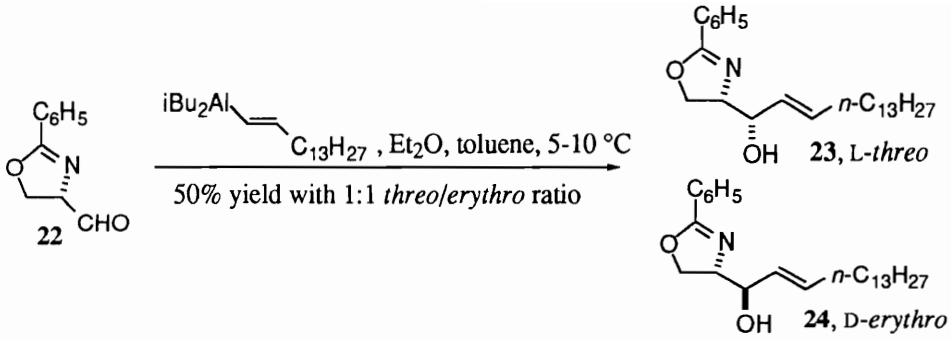


Newman[57] published the first synthesis of sphingosine originating from L-serine. N-phthaloylation of L-serine followed by treatment with acetic anhydride gave a primary acetate. Treatment with thionyl chloride provided the acid chloride which was subsequently reduced to aldehyde **20** (Scheme 7). Treatment of aldehyde **20** with *trans*-pentadecenyldiisobutylalane (*trans*-vinyl-alane) gave the protected sphingosine **21** in predominantly the *erythro* form.



Scheme 7

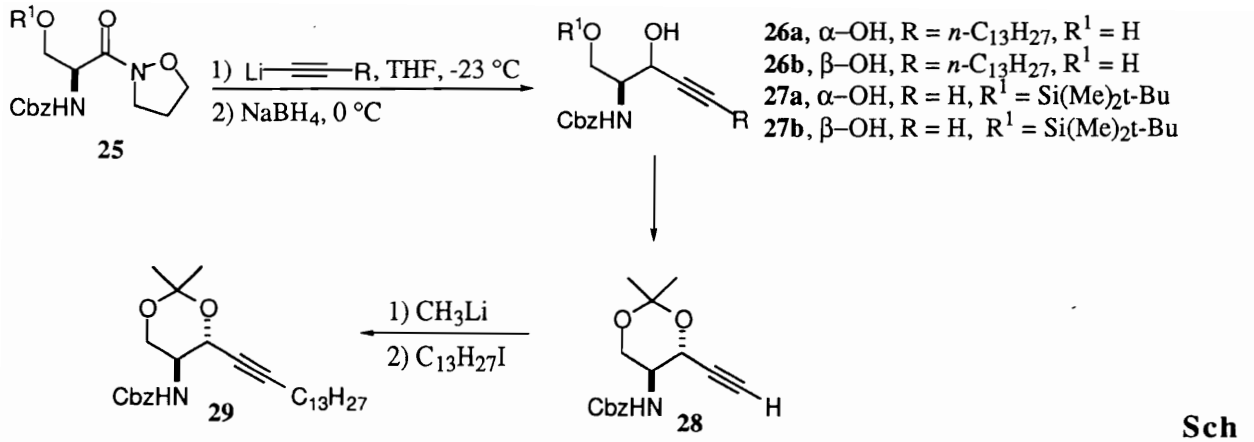
Thornton *et al.*[58] made *erythro*- and *threo*-N-oleoyl-D-sphingosines (ceramides) and galactosylceramides (cerebrosides) in 1981. Starting from L-serine the oxazoline aldehyde **22** was made in three steps and in 65% overall yield. The key step involved the addition of *trans*-vinylalane to **22**, yielding a 1:1 *erythro/threo* ratio of protected sphingosines **23** and **24** in a combined yield of 50% (Scheme 8). In a one pot sequence from **23** or **24** the corresponding ceramides were made in 13% overall yield.



Scheme 8

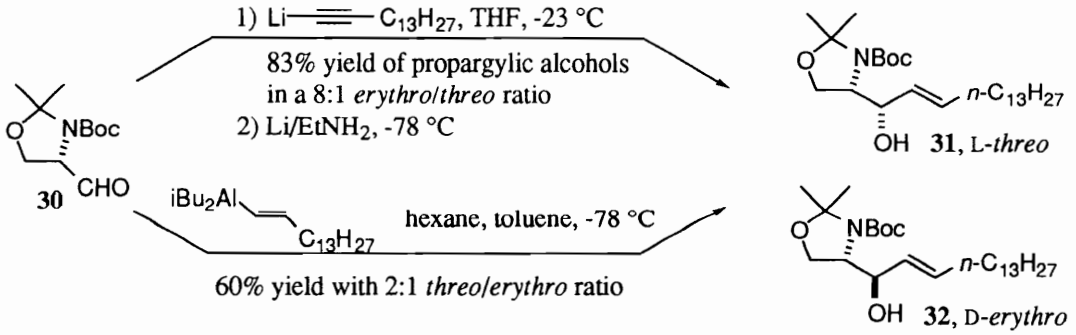
In 1986 Rapoport *et al.*[59] were the first to employ the lithium pentadecyne approach. The synthesis began with N-Cbz-L-serine which was converted to the protected L-serine isoxazolidide **25** in two steps and with 76% yield (Scheme 9). Lithium pentadecyne was added to isoxazolidide **25** (R^1= H) to give an ynone in 90% yield. Reduction of the ynone yielded propargylic alcohols **26** as a mixture of diastereomers. In an attempt to improve the diastereoselectivity of the reduction, a large variety of reducing reagents were investigated. Unfortunately no reducing agent gave high diastereoselectivities and high yields of propargylic alcohols **26**. The best results provided a 5:1 ratio of diastereomers *erythro* **26a** and *threo* **26b** in a combined yield of 63% (53% erythro).

In an attempting to improve the diastereoselectivity and yield, an alternate approach was investigated. Addition of lithium ethyne to **25** (R^1= Si(Me)$_2$*t*-Bu) afforded the corresponding ynone (racemized on chromatography with Florisil) in 60 - 80 % yield. Reduction of the ynone using $NaBH_4$ and $CeCl_3$ provided an 85% yield of **13** (Scheme 9) in a 6:4 ratio of diastereomers (accurate assignment as **27a** *vs* **27b** was not made). Acetonide formation revealed the major diastereomer to be **28**. Terminal alkyne **28** was deprotonated and alkylated to give **29** in 35% yield from **28**. Low yields and or low diastereoselectivity hampered this total synthesis. D-*erythro*-sphingosine was made in 22% overall yield in five steps. Note that **26a** and **26b** were only separable when silica gel treated with sodium borate was used (Boric acid impregnated silica gel has been used to differentiate diastereomeric 1,2 diols by TLC).[60]



Scheme 9

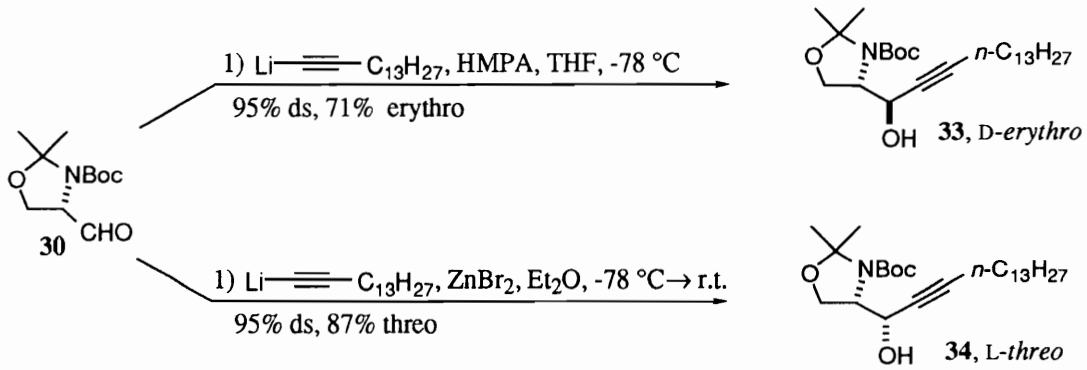
In 1988, Garner *et al.*[61] used N-Boc-L-serine to make D-*erythro*-sphingosine triacetate in 39% overall yield. This was accomplished in eight steps with 8:1 *erythro/threo* diastereoselectivity. The first three steps were used to form the acetonide of N-Boc-L-serinal **30**. No further chromatographic purifications were neccessary for the remainder of the synthesis. The key step, addition of lithium pentadecyne, yielding propargylic alcohols which were then reduced to sphingosine derivatives **31** and **32** (Scheme 10). This method afforded D-*erythro*-sphingosine in 39% overall yield. Garner also investigated addition of Newman's[62] *trans*-vinylalane to N-Boc-serinal acetonide **30** and found a 2:1 *threo* /erythro diastereoselectivity.



Scheme 10

In 1988 Herold[63] used N-Boc-L-serine to make D-*erythro*- and L-*threo*-sphingosine triacetate, and their *cis* geometric isomers. In addition he made N-octadecanoyl-D-*erythro*-sphingosine, which is a ceramide or N-acylated sphingosine. The approach resembles that of Garner's in that both used lithium pentadecyne as the carbon nucleophile in the additions to N-Boc-serinal acetonide **30**. By employing chelation control through judicious choice of Lewis acids, solvent, and temperature, Herold was able to add lithium pentadecyne with 95% ds to give either *erythro* or *threo* propargylic alcohols **33** or **34** selectively (Scheme 11). The triacetate of D-

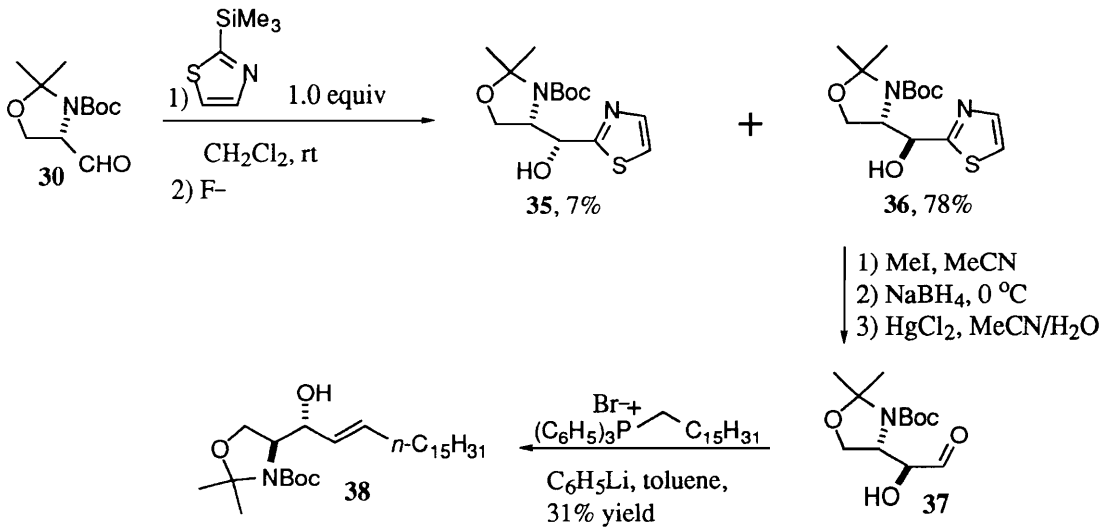
erythro-sphingosine or L-*threo*-sphingosine was obtained in eight steps with 18% and 21% yields, respectively. The alkynes were reduced to the *trans* olefins using sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al) in Et_2O at 0 °C to room tempertaure in 65% yield.



Scheme 11

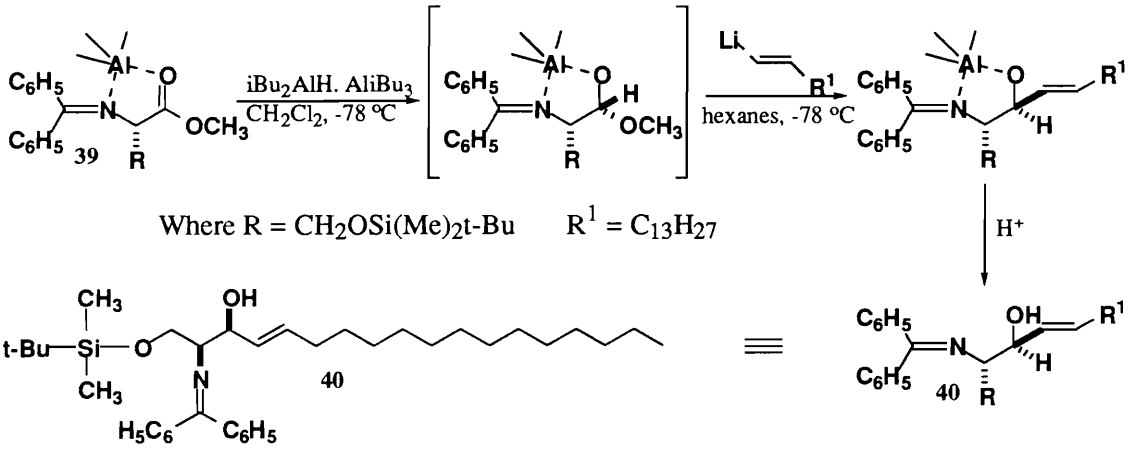
In 1988 a third independent synthesis of D-*erythro*- and L-*threo*-sphingosine was published, by Liotta *et al.*[64] These chemists also took advantage of the N-Boc-L-serinal acetonide **30** and lithium pentadecyne as Garner and Herold did. Using lithium pentadecyne in THF at -78 °C a 9:1 *erythro/threo* ratio of propargylic alcohols **33** and **34** was observed. Alcohols **33** and **34** gave a combined yield of 90% (81% *erythro*). Unique to this approach was the conversion of propargylic alcohol **33** to **34** using standard Mitsunobu conditions in 70% yield. Reduction of alkynes **33** or **34** with $Na°/NH_3$ proceeded in 90% yield when performed on a small scale (< 100 mg). When larger scale reductions were desired lithium aluminum hydride in refluxing 1,2 dimethoxyethane had to be employed. These large scale reductions proceeded in 70% yield. In six steps D-*erythro*-sphingosine was made in 28% yield and L-*threo*-sphingosine in eight steps and in 20% yield.

In 1990 Dondoni *et al.*[65] used N-Boc-L-serine to make the D-*erythro*-C_{20}-sphingosine triacetate. This synthesis was accomplished in ten steps (six purifications) and with 12:1 *erythro/threo* diastereoselectivity, in an overall yield of 15%. In addition phytosphingosine was synthesized. These researches took advantage of a stable thiazole masked aldehyde, which could be deprotected at the appropriate time using mild conditions.[66] The subsequent aldehyde would then be subjected to Wittig olefination. Addition of 2-(trimethyl-silyl)thiazole to N-Boc-L-serinal acetonide **30** afforded the one carbon homologues, i.e. the masked aldehydes **35** and **36**, with 85-90% anti-diastereoselectivity at the new asymmetric center (Scheme 12). Deprotection of thiazole **36** and Wittig olefination of the resulting aldehyde **37** gave the protected D-*erythro*-C_{20}-sphingosine **38**, which was deprotected and then converted to its triacetate for the purposed of identification.



Scheme 12

L-Serine methylester hydrochloride was used by Polt *et al.*[67] to prepare L-*threo*-sphingosine triacetate in 1992. The synthesis was accomplished in four steps, with > 95% diastereoselectivity and in an overall yield of 59%. Diastreoselective syntheses of sphingosine and C1-deoxygenated sphingosines, derived from L-alanine, of varying chain lengths were achieved. The key step involved the reaction of α-imino esters (O'Donnell's Schiff bases) with aluminum hydrides to produce acetal-like intermediates which underwent subsequent reaction with carbon nucleophiles. For the synthesis of L-*threo*-sphingosine (Scheme 13) the Schiff base methyl *O*-(tert-butyldimethylsilyl) -N-(diphenyl-methylene)-L-serinate (**39**), shown pre-cordinated as an aluminum acetal, was converted to the protected L-*threo*-sphingosine (**40**) in > 20:1 *threo/erythro* selectivity in one pot and with 76% combined yield (72% *threo*).

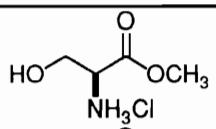
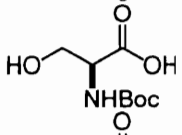
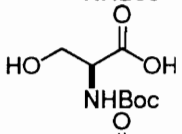
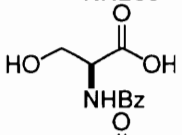
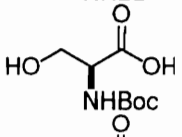
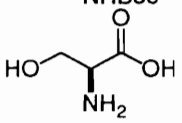
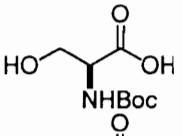
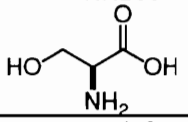


Scheme 13

This synthesis proceeded without epimerization, confirmed *via* use of Mosher's amide, and with a high degree of *threo* selectivity. Although some Boc-protected amino aldehyde derivatives show resistance to racemization,[68] facile loss of optical activity in α-amino aldehyde derivatives is a general problem.[69] Sphingosine derivative **40** was fully deprotected to L-*threo*-sphingosine using HCl in dioxane at 100 °C by Polt.[67] The overall yield of L-*threo*-sphingosine was 59% from L-serine methylester hydrochloride.

The novelty and efficiency of sphingosine synthesis from serine has progressed by leaps and bounds since the first synthesis by Newman (Table 2). The syntheses have been arranged according to their overall percent yields.

Table 2. Syntheses of Sphingosine From Serine

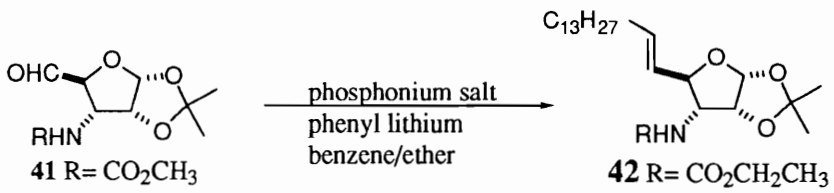
Author	Year	Starting Material	Product	Steps	Overall % Yield	Ref
Polt	1992		D-*threo*-sphingosine	5	59	67
Garner	1988		D-*erythro*-sphingosine	6	39	61
			D-*threo*-sphingosine	6	14	
Liotta	1988		D-*erythro*-sphingosine	6	28	64
			D-*threo*-sphingosine	8	20	
Rapoport	1986		D-*erythro*-sphingosine	5	22	59
Herold	1988		D-*erythro*-sphingosine	8	18	63
			D-*threo*-sphingosine	8	21	
Thornton	1981		1-benzoyl-D-*erythro*-sphingosine	5	16	58
			1-benzoyl-D-*threo*-sphingosine	5	16	
Dondoni	1990		D-*erythro*-sphingosine	10	15	65
Newman	1973		D-*erythro*-sphingosine	6	*	57

All yields were calculated based on information obtained from the experimental sections of the cited papers and references therein, when available. * Many reactions were reported without a percent yield.

2.2 Sugar Approaches

A crucial problem with the L-serine approach is the introduction of the second asymmetric center. One way to avoid unwanted mixtures of diastereomers is to begin the synthetic sequence with the stereocenters already established. The chiral pool of sugars become attractive building blocks for such an endevor.

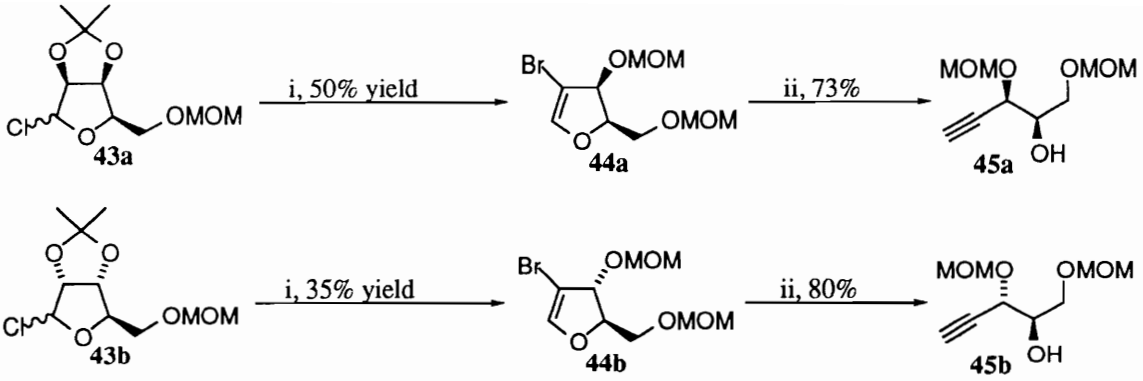
The first sphingosine synthesis from sugars was accomplished by Reist *et al.*[70] in 1970. Starting with 3-amino-3-deoxy-1,2:5,6-di-isopropylidene-α-D-allofuranose[71] the synthesis of D-*erythro*-sphingosine was accomplished in 8 steps with a 5% overall yield. The key step was the Wittig olefination of aldehyde **41** which provided the coupled *trans* product **42** in 60% yield (Scheme 14). The strong band at 970 cm^{-1} in the infrared spectrum was used to assign the geometry of the olefin. Upon examination of the spectral data for olefin **42** it was found that the methyl carbamate had been converted to the ethyl carbamate. The authors reasoned that phenyl lithium had attacked the solvent (ether) to produce ethylene and an ethoxy anion, which then proceeded to transesterify the methyl carbamate. Reist previously published a synthesis of D-dihydrosphingosine,[72] from the same starting material.



41 R= CO_2CH_3 **42** R= $CO_2CH_2CH_3$

Scheme 14

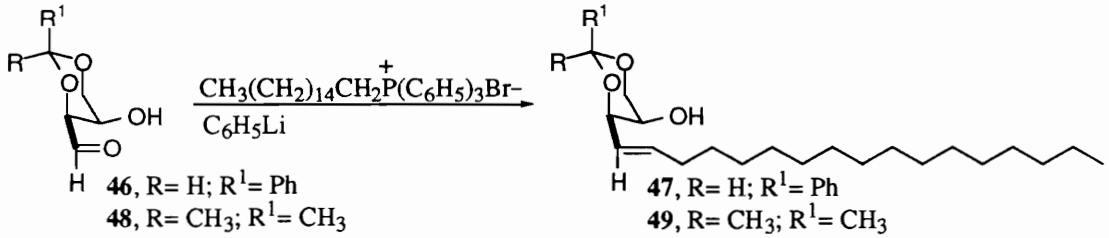
It was not until 1985 that sphingosines were again synthesized *via* a sugar route. Schlosser *et al.*[73] were able to convert D(+)-mannose and D(+)-ribono-1,4-lactone into D-*erythro*- and L-*threo*-sphingosine respectively. Schlosser states that the key intermediates were the two epimers of 4-bromo-3-methoxymethyl-2-methoxymethoxymethyl-2,3-dihydrofurans **44a** and **44b** (Scheme 15). Treatment of **44** with *n*-butyllithium gave the alkynes **45** which were subsequently alkylated to give the sphingosine skeleton. In twenty four steps and in 1.3% overall yield D-*erythro*-sphingosine was made, while the preparation of L-*threo*-sphingosine took twenty steps and proceeded in 1.6% overall yield.



Reagents and conditions: (i) (1) Li, NH_3, (2) $ClCH_2OCH_3$, $EtN(iPr)_2$, (3) Br_2, CCl_4, (4) DBU, THF; (ii) *n*-BuLi, THF.

Scheme 15

Three efficient syntheses of D-*erythro*-sphingosine were published in the first six months of 1986. Independently, Schimdt, Kiso, and Ogawa employed very similar approaches. Schmidt and Zimmermann[74] published a total synthesis of D-*erythro*-sphingosine from D-galactose in seven steps with an overall yield of 7%. The crucial step was the Wittig olefination of 2,4-O-benzylidene-D-threose **46** (Scheme 16) to yield the *trans*-olefin **47** in 68% yield. Conversion of the free alcohol in*trans*-olefin **47** to an azide (*via* the triflate) with inverse stereochemistry, followed by deprotection provided azido D-*erythro*-sphingosine. Despite the low overall yield the synthesis is an efficient one. If the first step is eliminated (formation of 4,6-O-benzylidene-D-galactose from D-galactose, 26% yield) the overall yield increases to 28%. In 1988 a detailed paper with full experimental procedures was published.[75] In this publication Schmidt reports that 2,4-O-benzyliden-D-threose **46** can be made from D-galactose and D-arabinose, and further shows that 2,4-O-isopropylidene-D-threose **48** can be made from D-xylose and D-glucose.

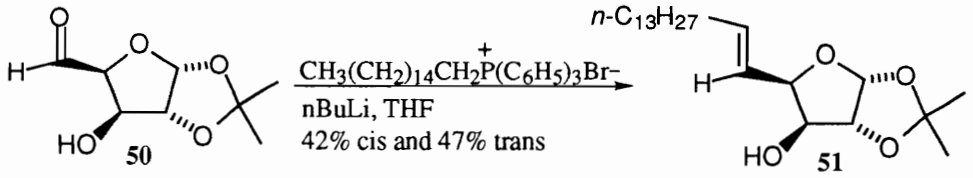


Scheme 16

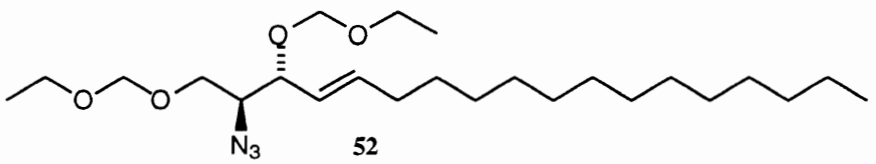
Kiso *et al.*[76] employed the same synthetic strategy as Schmidt, the only differences being the initial isopropylidene protecting group *vs* Schimdt's benzylidene protecting group and the Wittig condensation. Using Schlosser Wittig betaine-ylid conditions, 2,4-O-isopropylidene-D-threose **48**, available from 3,5-O-isopropylidene-D-xylofuranose[77] or 4,6-O-isopropylidene-D-galactopyranose[78] was converted to **49** in 40% yield (35% *cis*-olefin isolated, Scheme 16). Thus from D-xylose, D-*erythro*-sphingosine was made in seven steps and in 6% overall yield. Unlike

many researchers Kiso did not photoisomerize the *cis*-olefin **49** (these photoisomerizations[79] generally proceed in high yield) to the *trans*-olefin **49**, lowering his overall yield. As with Schmidt's synthesis the lowest yielding step is the first one, namely the formation of the 3,5-O-isopropylidene-xylofuranose from D-xylose. Kiso also converted his sphingosine product into several ceramides.

Ogawa *et al.*[80] used D-glucose to arrive at aldehyde **50** in three steps. Use of standard Wittig condensation procedures provided a 42% of the *trans* olefin **51**, and 47% of the *cis* olefin (Scheme 17). The *cis* geometric isomer was photoisomerized to the *trans* olefin **51**, so that an 87% yield of **51** could be realized from aldehyde **50**. Ogawa was interested in the synthesis of ceramides, thus these researchers never made azido D-*erythro*-sphingosine or D-*erythro*-sphingosine, but instead a related compound **52** was made (Scheme 18). Thus, from D-glucose, protected azido D-*erythro*-sphingosine **52** was made in eleven steps and in 14% overall yield. Noteworthy was the reduction of azide **52**, accomplished in 90% yield using sodium borohydride in refluxing isopropanol.

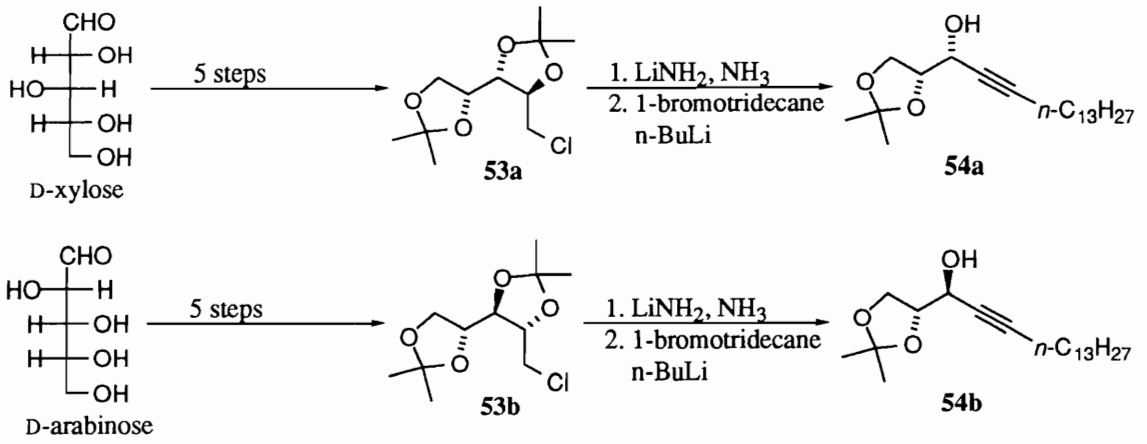


Scheme 17



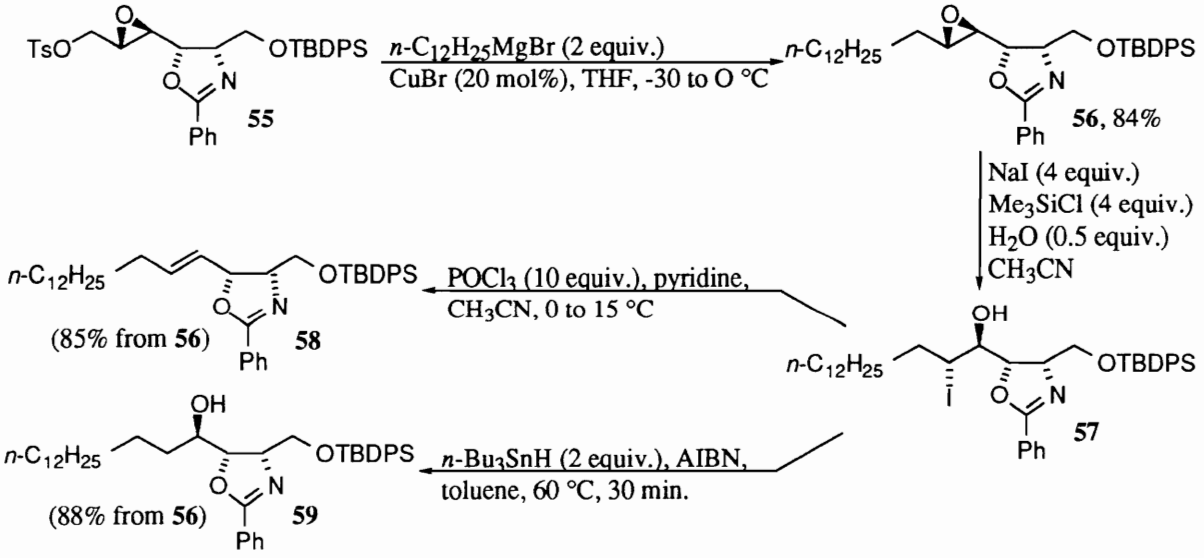
Scheme 18

In 1993 Yadav *et al.*[81] synthesized D-*erythro*- and L-*threo*-sphingosine from D-xylose and D-arabinose respectively. The key reaction was a base-induced double elimination of chloride **53** followed by alkylation of the terminal alkyne to give high yields
(**53a** → **54a** in 78% yield) of the corresponding propargylic alcohols **54** (Scheme 19). While the β-alkoxy chloride elimination to give propargylic alcohols **54** is interesting[82] this synthesis offered no improvement over past syntheses. For both series fourteen steps were needed and an estimated overall yield of < 10% and > 5% was assumed based on the available data, as many yields were not reported in the paper.[81]



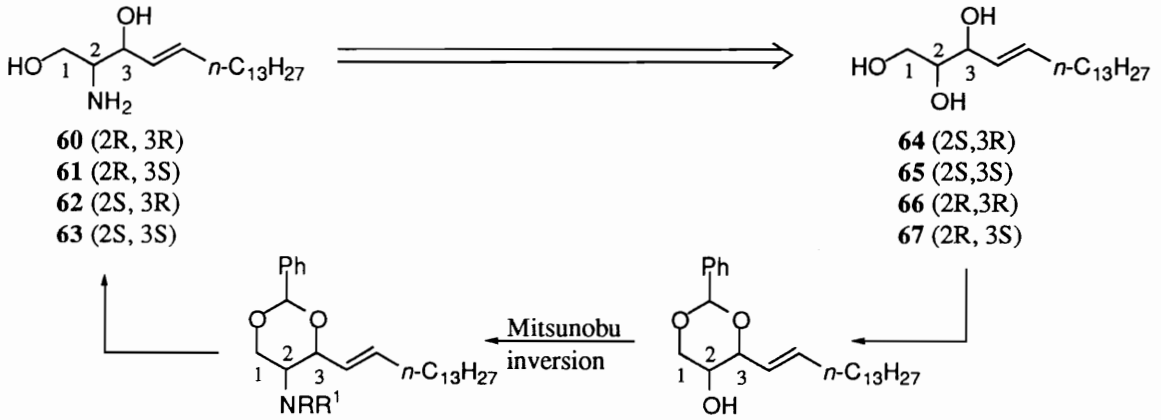
Scheme 19

In 1994 additional syntheses of sphingosines were published. First Murakami *et al.*[83] made D-*erythro*-sphingosine and phytosphingosine from D-glucosamine hydrochloride. After nine steps epoxytosylate **55** was made and then coupled with a dodecyl cuprate to give epoxide **56** (Scheme 20). The epoxide was then opened with NaI to give the corresponding iodohydrin **57**, from which either D-*erythro*-sphingosine or phytosphingosine was made. This synthesis in 15 steps provides D-*erythro*-sphingosine in 19% overall from the hydrochloride of D-glucosamine.[84]



Scheme 20

Late in 1994 Wu *et al.*[85] published a formal synthesis of D-*erythro*-**62** and L-*threo*-sphingosine **63**. The strategy was to make the four stereoisomers of 1,2,3-trihydroxy-4E-octadecene **64-67** and then to protect them as their 1,3-O-benzylidene-4-octadecene-1,2,3 triols, subject them to Mitsunobu conditions and then liberate the desired sphingosine **60-63** (Scheme 21). To this end they investigated the syntheses of **64** - **67** from sugars (Table 3).



Scheme 21

Table 3

Triol	Starting material	Overall % yield, # of steps
64	D-mannose	39%, 6
65	L-tartaric acid	13%, 8
65	D-xylose	20%†, 7
66	D-xylose	9%, 5
66	D-glucose	27%, 5
66	D-galactose	10%, 5
67	D-glucose	35%, 4

† No % yield given for one step, so it was arbitrarily assigned 50%.

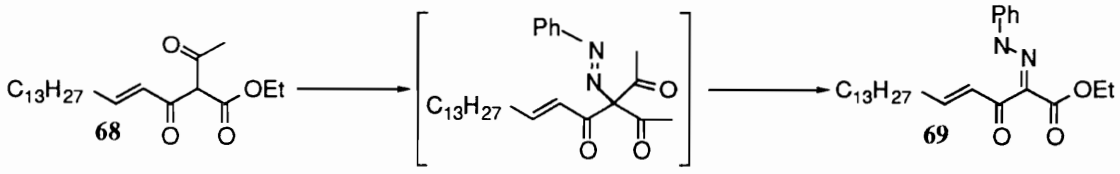
While the syntheses from sugars do not suffer from mixtures of diastereomers, they generally lack the higher overall yields associated with the L-serine approaches. Many times the initial protection of the sugar is the lowest yielding step. If this fact is taken into account, then many of these syntheses are highly efficient and noteworthy. The sugar-based syntheses have been organized in tabular form with overall percent yield as the criterion for their relative order (Table 4). Note that several (1,2,3)-trihydroxy-4E-octadecene have been included in the table, some representing formal syntheses.

Table 4. Syntheses of Sphingosine From Sugars

Author	Year	Starting Material	Product	Steps	%	Ref
Wu	1994	D-glucose	(1,2R,3R)-trihydroxy-4E-octadecene	5	27	85
		D-glucose	(1,2R,3S)-trihydroxy-4E-octadecene	4	35	85
		D-mannose	(1,2S,3R)-trihydroxy-4E-octadecene	6	39	85
		D-xylose	(1,2S,3S)-trihydroxy-4E-octadecene	7	20	85
Murakami	1994	D-glucosamine	D-*erythro*-sphingosine	13	19	83
Ogawa	1986	D-glucose	1,3-diethoxymethoxy-D-*erythro*-sphingosine	11	14	80
Kiso	1986	D-galactose	D-*erythro*-sphingosine	7	10	76
Schmidt	1986	D-galactose, D-xylose, D-arabinose, or D-glucose	D-*erythro*-sphingosine	7	7	74 75
Reist	1970	D-glucosamine	D-*erythro*-sphingosine	7	6	70
Yadav	1993	D-xylose	D-*erythro*-sphingosine	14	<10	81
		D-arabinose	D-*threo*-sphingosine	14	<10	81
Schlosser	1985	D-mannose	D-*erythro*-sphingosine	24	1	73
		D-ribono-1,4-lactone	D-*threo*-sphingosine	20	1	73

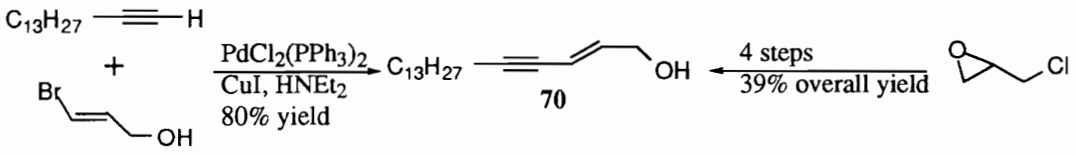
2.3 First Total Synthesis and Miscellaneous Approaches

Seventy-two years after the initial isolation of sphingosine by Thudichum, the first total synthesis of the alkaloid sphingosine appeared and confirmed the assigned structure. Shapiro *et al.*[86] began their synthesis with a Knoevenagel-Doebner condensation of myristryl aldehyde (tetradecyl aldehyde) with malonic acid. The crucial reaction took place four steps later when ester **68** was treated with benzenediazonium chloride in the presence of sodium acetate and ammonium chloride to give hydrazone **69** (Scheme 22). This was the first time the Japp-Klingemann reaction[87] was used on such a substrate. At the time the coupling of a diazonium salt with akyl substituted aceto-acetic esters was known, but had not been carried out with α,α-diacyl ester in which one of the acyl groups is α,β-unsaturated. This synthesis can be performed on tens of grams and proceeded in 3% overall yield to give DL-*erythro*-sphingosine in nine steps.



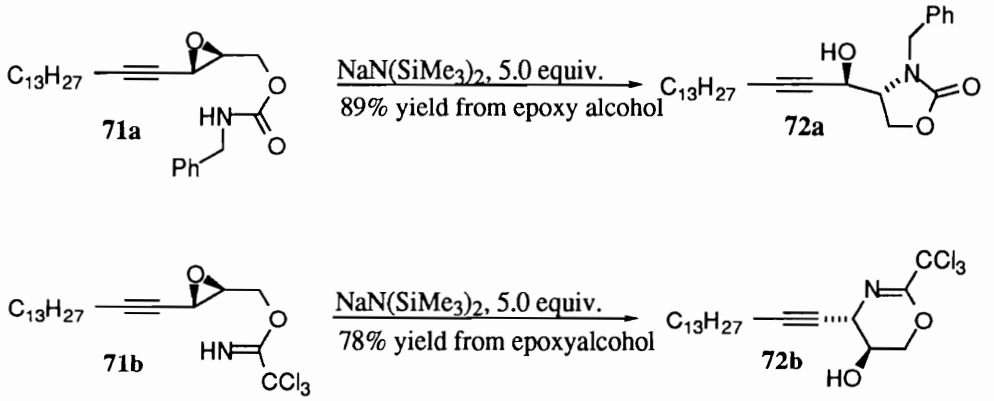
Scheme 22

In 1983, Vasella *et al.*[88] published a synthesis of D-*erythro*-sphingosine. Of the several key reactions, the first was the generation of enynol **70** (Scheme 23), *via* a Pd coupling reaction. Sharpless epoxidation of **70** proceeded in high yield and enantiomeric purity. This epoxy alcohol was then converted to carbamate **71a** or imidate **71b** (Scheme 24). Intramolecular cyclization of carbamate **71a** gave the desired regiochemical opening of the epoxide to afford oxazolidinone **72a**. This oxazolidinone was then converted to D-*erythro*-sphingosine. The trichloroimidate **71b** preferred to cyclize with the opposite regiochemical opening and was used to synthesize the 3-amino-2-hydroxy-L-*erythro*-isomer of sphingosine.



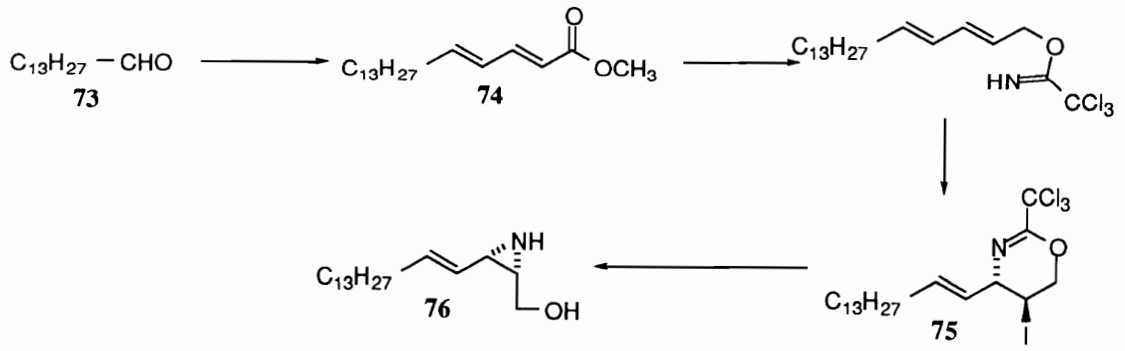
Scheme 23

In 1986 Vasella published a second generation synthesis of D-*erythro*-sphingosine.[88b] The synthesis of enynol **70** was improved so that the palladium coupling was no longer needed (Scheme 23). In addition the percent yield of both the Sharpless epoxidation and the reduction of the alkyne to the trans alkene were increased. D-*erythro*-Sphingosine was synthesized in 50% overall yield and in six steps.



Scheme 24

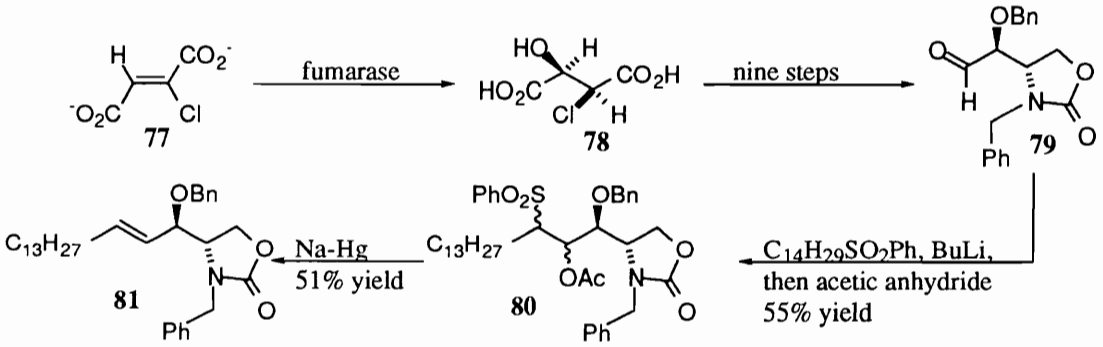
In 1986 a (±)-*erythro*-sphingosine synthesis was reported by Cardillo et al.[89] A Wittig-Horner olefination of tetradecylaldehyde **73** yielded the diene ester **74**. Lithium aluminum hydride reduction of diene ester **74**, formation of the trichloroimidate and treatment of the diene trichloroimidate with N-iodo-succinimide gave the corresponding 4,5-dihydro-1,3-oxazine **75** (Scheme 25). These researchers noted the same problem that



Scheme 25

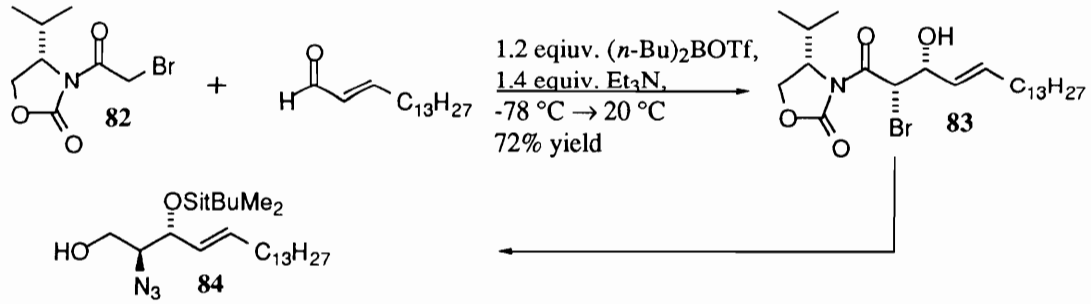
Vasella *et al.* observed with imidate **71b** (Scheme 24). Acidic hydrolysis of **75** afforded the ammonium salt, which upon treatment with base formed aziridine **76**. This aziridine opened exclusively at the allylic sight with Amberlyst A 26 (in the acetate form) to give racemic acetamido-sphingosine in 32% yield and in seven steps.

In 1987 George Whitesides *et al.*[90] was investigating the breadth of synthetically useful substrates accepted by fumarase. Thus chlorofumaric acid **77** was stereospecifically hydrated to give L-*threo*-chloromalic acid **78**, in ≥ 99.5 % ee (Scheme 26). L-*threo*-chloromalic acid **78** was converted in nine steps to give oxazolidinone **79**. Julia coupling[91] yielded the desired sulfone acetate **80**, which gives olefin **81**. From acid **78**, D-*erythro*-sphingosine was made in thirteen steps and in 3% overall yield.



Scheme 26

Another synthesis of sphingosine was published in 1988 by Nicolaou *et al.*[92] Starting with the chiral oxazolidinone **82**, the corresponding boron enolate was condensed with an α,β-unsaturated aldehyde to afford derivative **83** in 72% yield. Nucleophilic displacement of bromine with an azide group, silyl protection of the allylic alcohol, followed by reduction with $LiBH_4$, gives the very important sphingosine derivative **84** in 52% yield from **82**. From oxazolidinone **82** D-*erythro*-sphingosine was made in 42% yield and in six steps (Scheme 27).

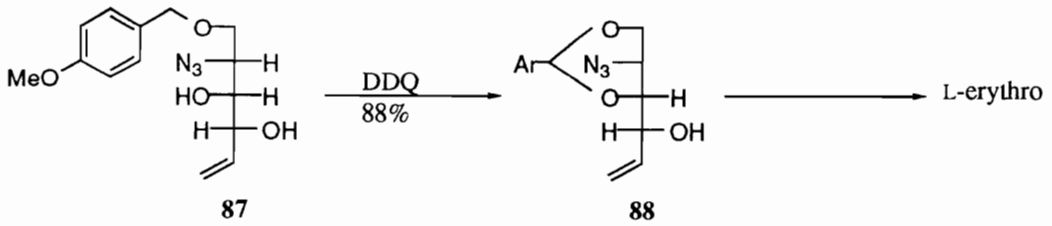


Scheme 27

(R,R) **85** **86** meso

Scheme 28

Takano *et al.*[93] took advantage of a Katsuki-Sharpless epoxidation of (R,R) and *meso*-1,2-divinylethylene glycols to give epoxides **85** and **86** (Scheme 28). After four steps epoxide **85** was converted to diol **87**. Oxidative cyclization of **87** using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in the presence of 4 Å molecular sieves gave 1,3-bezylidene **88** (Scheme 29). Mesylation of the free alcohol and treatment with lauryl magnesium bromide, CuI gives the 1,3-benzylidene-azido-sphingosine. Thus epoxide **85** is converted to L-*threo*-sphingosine in 11% overall yield and in 10 steps. In a similar manner epoxide **86** was converted to D-*threo*-sphingosine in 16% overall yield in 10 steps.

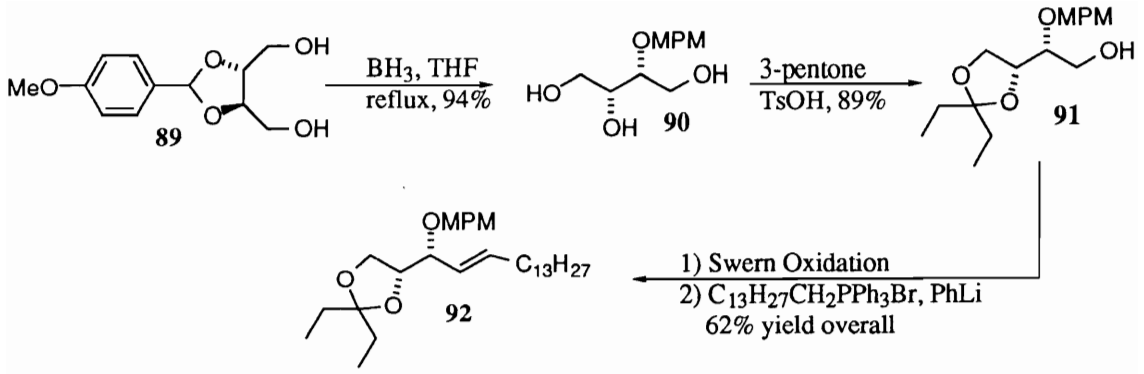


87 **88**

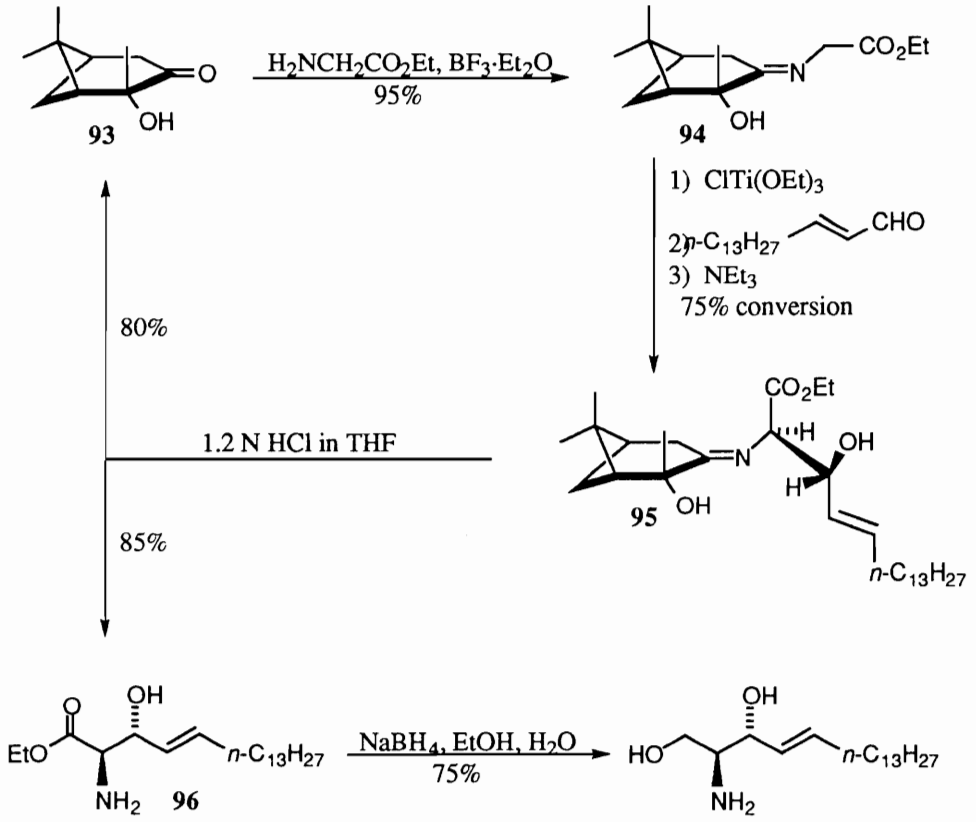
Scheme 29

Somfai and Olsson[94] in 1993 made a derivative similar to Nicolaou's azide **84** (Scheme 27) although in much lower yield and in twice the number of steps. Starting with diethyl-D-tartrate diol, diol **89** was made in two steps and in quantitative yield (Scheme 30). Treatment with diborane yields the unsymmetrical triol **90** and protection of the 1,2 vicinal diol affords **91**. The key step is the oxidation of alcohol **91** to the aldehyde which is not purified further, but subjected to Wittig conditions using freshly prepared phenyl lithium to ensure trans-selectivity.[149b]

In 1994 Solladie-Cavallo and Koessler[95] published a four-step diasteroe- and enantioselective synthesis of D-*erythro*-sphingosine. Utilizing (+)-(R,R,R)-hydroxypinanone **93** as a chiral template, iminoglycinate **94** was synthesized (Scheme 31). Formation of the titanium enolate of **94**, followed by addition of E-hexadecenal afforded imino ester **95** in 60% yield. Note: 20% of both the iminoglycinate **94** and E-hexadecenal were recovered. Hydrolysis of imino ester **95** afforeded compound **96** in 85% yield. The chiral auxillary (+)-(R,R,R)-hydroxypinanone **93** was also recovered, in 80% yield. Reduction of the ester moiety in **96** afforded D-*erythro*-sphingosine in 35 - 40% overall yield, based on recovered starting materials.



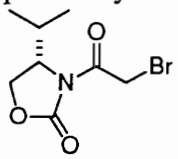
Scheme 30



Scheme 31

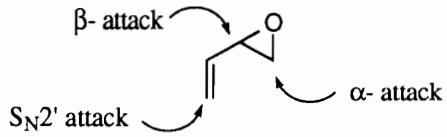
All the miscellaneous syntheses have been arranged according to their overall percent yield in Table 5.

Table 5. Miscellaneous Syntheses of Sphingosine

Author	Year	Starting Material	Product	Steps	Overall Yield	Ref
Vasella	1983	pentadecyne	D-*threo*-sphingosine	6	50%	88
Nicolaou	1988		D-*erythro*-sphingosine	6	42%	92
Solladie	1994	(+)-(R,R,R)-hydroxypinanone	D-*erythro*-sphingosine	4	35%	95
Cardillo	1986	tetradecyl-aldehyde	(±)-sphingosine	7	32%	89
Somfai	1993	diethyl-D-tartrate	D-*erythro*-sphingosine	13	31%	94
Takano	1991	meso-1,2-divinylethylene-glycol	L-*threo*-sphingosine D-*threo*-sphingosine	10 10	11% 16%	93
Whitesides	1987	chlorofumaric acid	D-*erythro*-sphingosine	13	3	90
Shapiro	1954	tetradecyl-aldehyde	(±)-sphingosine	9	3	86

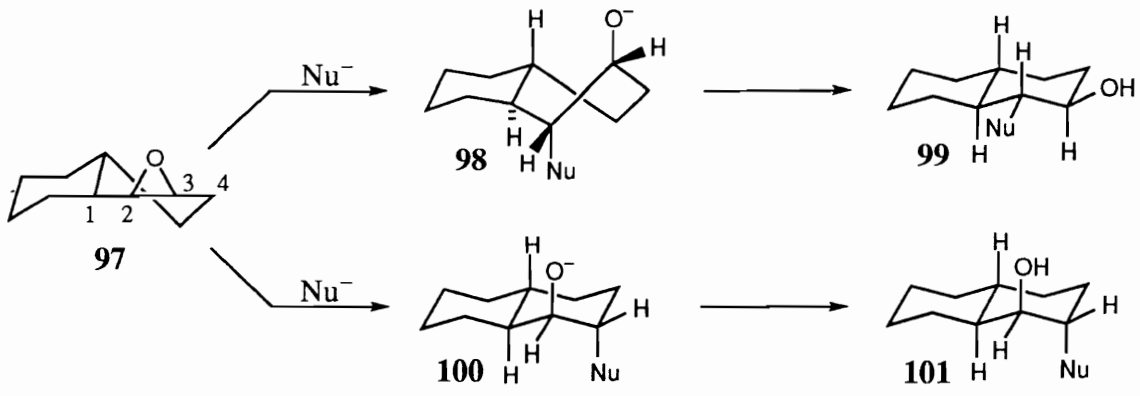
3.0 Vinylic oxirane openings with carbon and heteroatom nucleophiles.

Unlike isolated oxiranes which have two possible sites for nucleophilic attack vinyl oxiranes exhibit three (Scheme 32). Vinyl oxiranes, endowed with all of the reactivities of the former, also lend themselves to a whole new class of reactions, namely palladium catalyzed nucleophilic additions. A brief overview of the regio- and stereospecificity of these reactions ensues.



Scheme 32

Nucleophilic S_N2 opening of epoxides affords trans products. When unsymmetrical epoxides are ring opened with nuclephiles, a mixture of regioisomeric products generally ensues. Unsymmetrical cyclic epoxides, on the other hand, give products resulting from only one of two possible trans-diaxial openings.[96] The exclusive formation of one product is the result of the stereoelectronic demands inherent in the transition states. For example, the conformationally rigid cyclic epoxide **97** (Scheme 33), gives only one product **101**.[96] The transition state in the S_N2 reaction requires that the reaction at C-2 must give the twist-boat intermediate **98** which would led to the diequatorial product **99**. When attack is at C-3 the chair intermediate **100** is required and the diaxial product **101** ensues. This is a classic example of a kineticly controlled reaction product. Intermediate **100** is lower in energy than **98** and thus the higher energy diaxial product **101** is formed preferentially over the lower energy diequatorial product **99**. In general, when the energy gap between the two possible transition states (e.g. **98** & **100**) is large, exclusive formation of one product is found. As the energy gap between the two transitions states decreases, formation of the other regioisomer becomes possible. The same principles apply to vinyl oxiranes, as will be seen in the following paragraphs.

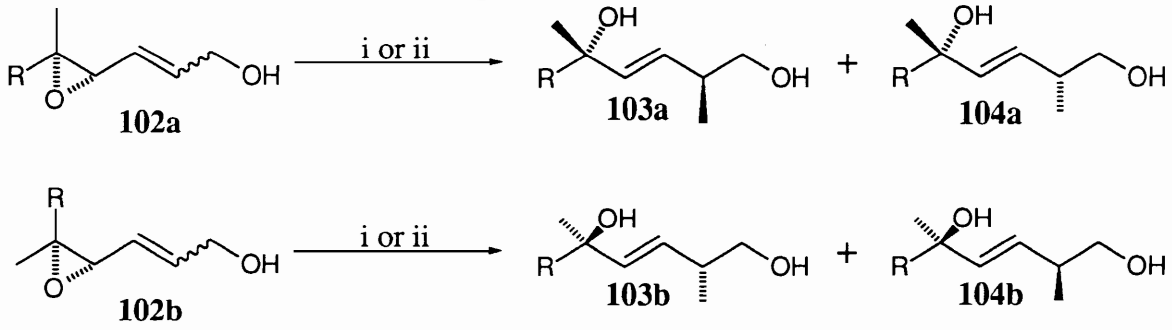


Scheme 33

The addition of carbon nucleophiles to vinyl oxiranes has been studied extensively. Organocuprate and Grignard reagents add stereoselectively in a S_N2' manner (vinylogous attack) to yield allylic alcohols.[97] Table 6 demonstrates the stereoselective *anti* addition of organocuprates and the preponderance for retention of the initial olefinic geometry.[97b] Organocuprate addition to cyclic vinyl oxiranes proceeds in an analogous *anti* S_N2' mode. β-Attack has been observed but usually is a secondary product to vinylogous attack and can generally be suppressed by the use of cyanocuprate reagents (Scheme 34). These streoelectronic effects cease to dominate when stericly demanding reactive centers are involved.[97a,98] Extensive work has been published concerning organocuprate additions to 1,3-cyclopentadiene, 1,3-cyclohexadiene, and 1,3-cycloheptadiene monoepoxides.[97a,98,99]

Vinyl and phenyl organostannanes couple with vinyl oxiranes in good yield to give allylic alcohols.[100] Other organostannanes either fail to react (allyl, benzyl, and alkyl) or do so by other pathways. The most common mode of addition is *anti* S_N2', although β-addition sometimes occurs. The reactions are palladium catalyzed and are believed to proceed as indicated in Scheme 35.

Table 6. S_N2' Cuprate Addition to Vinyl Oxiranes

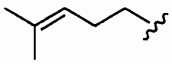


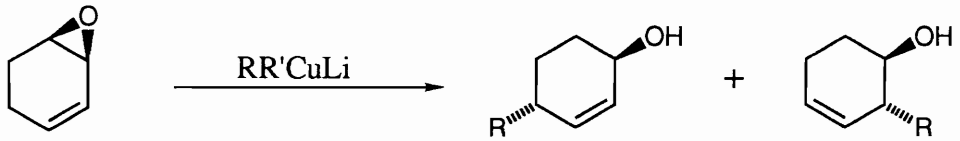
102a **103a** **104a**

102b **103b** **104b**

vinyl oxirane	ratio of **103**:**104**	yield (%)
trans **102a**	84:16	81
cis **102a**	1:99	88
trans **102b**	14:86	95
cis **102b**	97:3	95

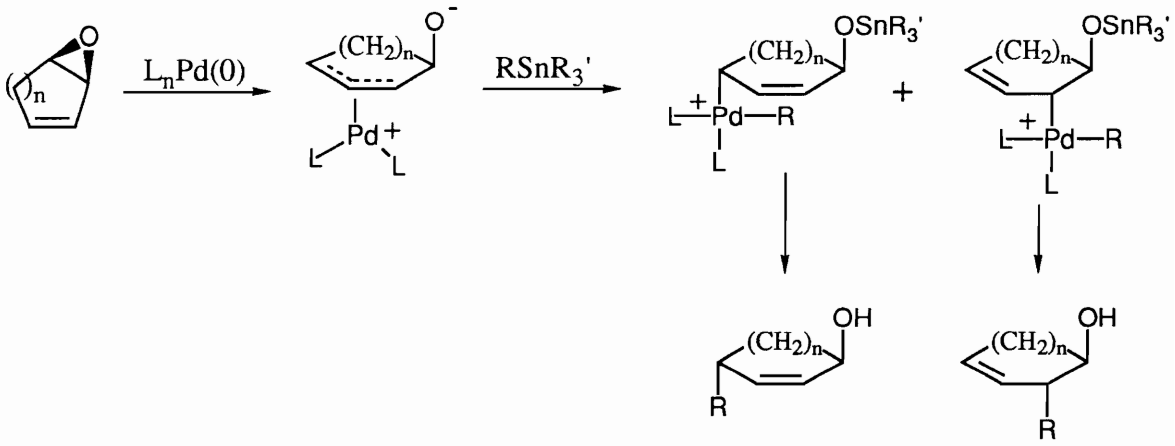
i. $LiMe_2Cu$, THF/Et_2O (4:1), 0 °C; ii. LiMeCuCN, Et_2O, 0 °C

R = H, $SiMe_2tBu$, 



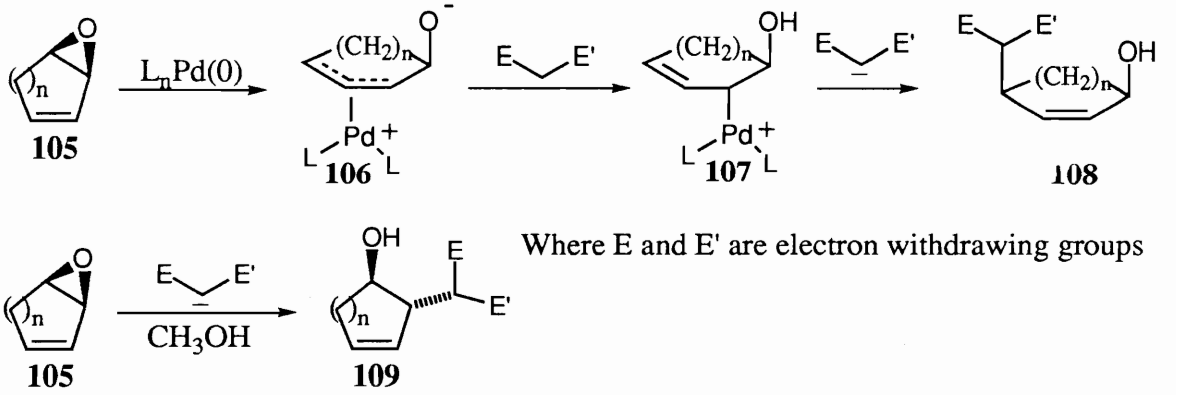
1,3-cyclohexadiene-monoepoxide

Scheme 34



Scheme 35

In contrast to the organometallic carbanions mentioned so far the addition of stabilized carbanions proceeds under neutral conditions. Malonate-type additions to cyclic and acyclic vinyl oxiranes, using palladium catalysis affords *syn* S_N2' products (Scheme 36).[101] The *syn* addition is explained by initial coordination of Pd (0) , followed by deprotonation of the malonate species by the alkoxyanion **106** of the former epoxide **105**. Attack by the malonate anion then occurs at the least sterically hindered face of the π-allylpalladium complex. When the Pd catalyst is absent products arising from *anti* β-attack are observed (Scheme 36).

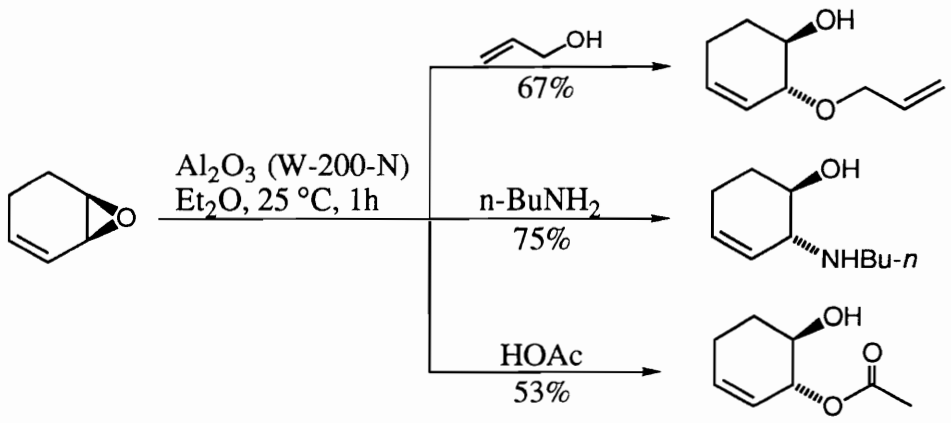


Where E and E' are electron withdrawing groups

Scheme 36

The heteroatomic opening of vinyl oxiranes has been examined extensively.[99] This brief discussion will be limited to alcohols, acids, anhydrides, esters, thiols, amines, and azide nucleophiles. Treatment of 1,3-cyclohexadiene monoepoxide with alcohols or amines in the presence of alumina affords products of β-addition (Scheme 37).[102] When

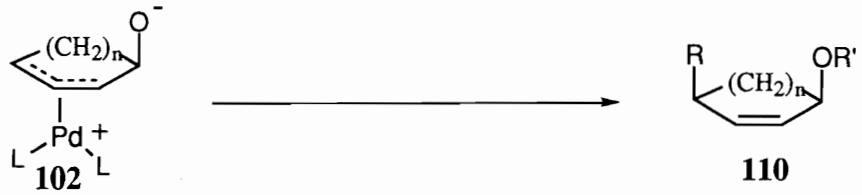
acidic reaction conditions are employed mixtures of β-addition (S_N2 and S_N2') products are observed.

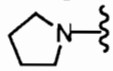
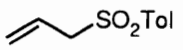
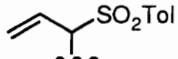
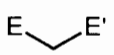
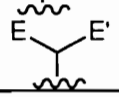


Scheme 37

Under Pd(0) catalysis (*e.g.* tetrakis(triphenylphosphine)palladium) cyclic and acyclic vinyl oxiranes can afford a diverse number of mono- and diprotected cis-2-(cyclo)alkene-1,4-diols (Table 7).[101] The chemistry is closely related to the malonate "type" addition found in Scheme 36, were the zwitterion **102** is the same. In fact the premise for the addition is the same. The oxygen in **102** can function as an alkoxide base while the electron deficient allylic system reacts with nucleophiles in a manner analogous to normal π-allylpalladium complexes.[101a] Thus any nucleophile containing a proton of lower acidity than an alcohol should in theory add to **102**, Table 6, in a *syn* S_N2' fashion. The nucleophilicity of the alkoxide anion in **102** has also been exploited, entries 3- 5.[101a] Tenaglia[103] notes that direct amination, with primary amines, of complexes of type **102** do not occur, "because of the occurence of stable unreactive intermediate palladium complexes." The use of secondary amines however does not have this short coming, entry 7 produced **110** in 92% yield.[104] Note that the *syn* S_N2' additions in Table 7 do not exclude acyclic cases, the transformation of **102** to **110** is solely for illustrative purposes.

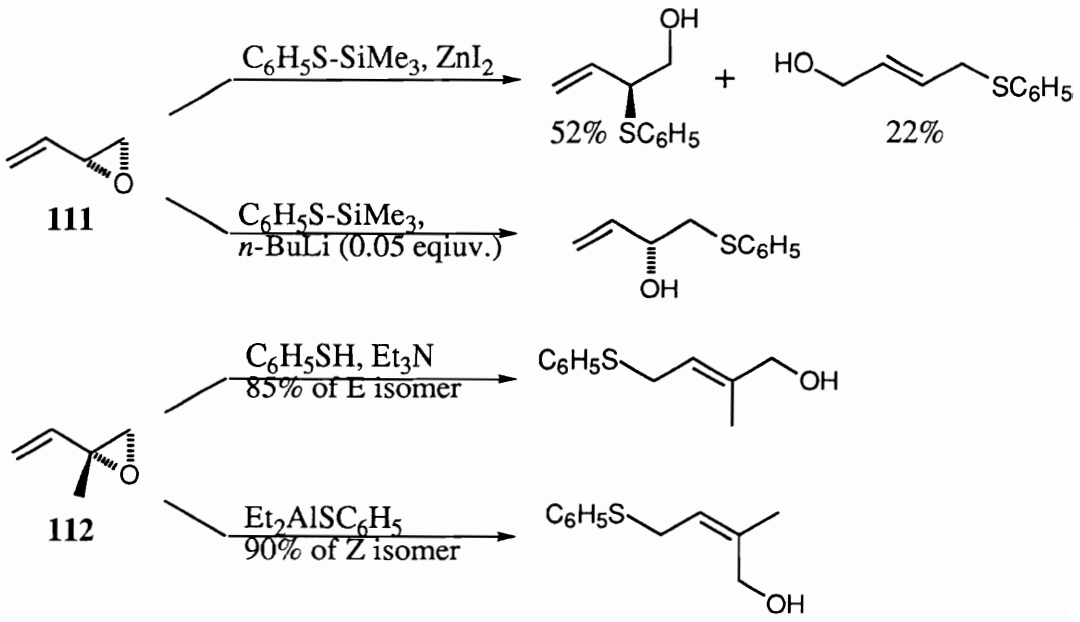
Table 7. Palladium Catalyzed Heteroatomic Addition to Vinyl Oxiranes



Entry number	Nucleophile	R	R'	Reference
1	$R''CO_2H$	$R''CO_2$	H	fa)
2	ArOH	ArO	H	fa)
3	AcOAr	OAr	Ac	fa)
4	$(R''CO)_2O$	$R''CO_2$	R''CO	fa)
5	$R_3''SiOCOR'''$	R3''SiO	R'''CO	fb)
6	NaN_3	N_3	H*	g
7			H	h
8			H	h
9			H	e,h

* Note that H_2O, in the reaction mixture, served as the proton source.

The reaction of vinyl oxiranes with sulfur nucleophiles is unique in that α-, β-, and S_N2' addition can be controlled by selecting the appropriate conditions.[99] For example vinyl epoxide **111** under Lewis acid conditions afforded the β-addition product in 52% yield. Note that upon treatment of the same epoxide with a catalytic amount of *n*-BuLi α addition dominates. Interestingly S_N2' addition of thiol phenol to vinyl oxirane **112** can be controlled to give exclusively *cis* or *trans* olefins (Scheme 38).

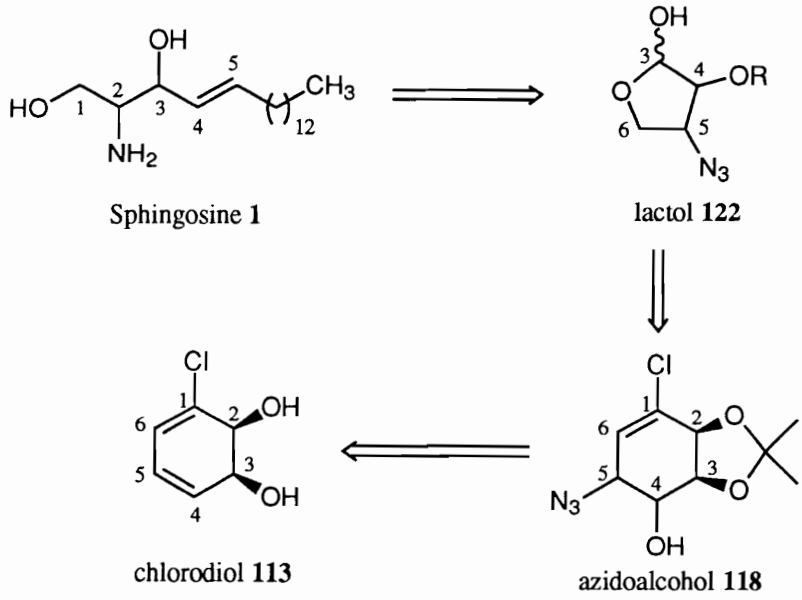

C6H5S-SiMe3, ZnI2
OH
52% SC6H5
+
HO
SC6H5
22%
111
C6H5S-SiMe3,
n-BuLi (0.05 eqiuv.)
SC6H5
OH
C6H5SH, Et3N
85% of E isomer
C6H5S
OH
112
Et2AlSC6H5
90% of Z isomer
C6H5S
OH

Scheme 38

III. DISCUSSION

1. Introduction - Strategy

The objective of this investigation was the synthesis of all four stereoisomers of sphingosine **1**. We reasoned that this could be accomplished from one common starting material, namely (2R,3S)-2,3-dihydroxy-1-chlorocyclohexa-4,6-diene[105] (in future text referred to as chlorodiol) **113** (Scheme 39). Although several possibilities exist, our retrosynthetic analysis was biased by a working knowledge of the reactivity of chlorodiol **113** and similar diols.[106] The synthesis presented here is the culmination of the chemical knowledge gained during the investigation of the original proposal.



Scheme 39

Crucial to our success would be the synthesis of all four C4 and C5 stereoisomers of azidoalcohol **118** (Scheme 39). Earlier work on chlorodiol **113** suggested the use of an acetonide protecting group.[107] This would combine a high degree of steric hindrance on the β-face with an unsymmetrical diene unit in the cyclohexene ring (Scheme 40). Any subsequent chemical transformations performed on the diene would then be biased toward the α-face.[107] Because of the stereoelectronic features of the diene, the C4–C5 double bond is more electron rich, and thus reacts with electrophiles preferentially over the C1–C6 double bond. Thus, exploitation of acetonide **114**, should allow for the high degree of regio- and stereocontrol needed to synthesize all four diastereomers of sphingosine in an enantiomerically pure form.

The stereocenters of sphingosine would be established at the cyclic stage, were the high degree of stereocontrol needed is more easily achieved. This could be accomplished by

synthesizing the four diastereomers of azidoalcohol **118** (Scheme 39). Azidoalcohols **118** could then be exhaustively cleaved to provide lactols **122** (Note: The carbon atoms of chlorodiol **113** have retained the same numbers in compounds **118** and **122** of Scheme 39). A Wittig reaction of lactol **122** with the ylide of tetradecyltriphenylphosphonium bromide could then provide an azidosphingosine product. Simple reduction of the azide moiety would then provide the natural product, sphingosine.

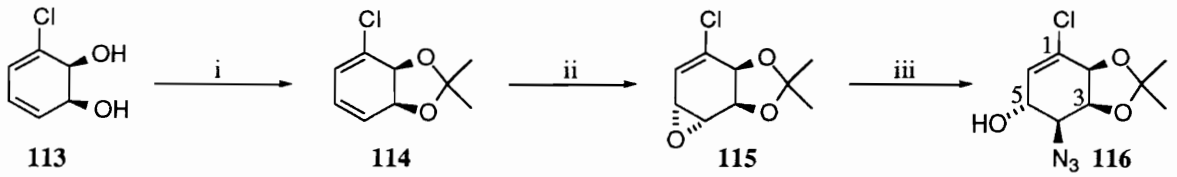
Carbons C1 through C4 of sphingosine **1** would originate from the chlorodiol **113** (Scheme 39). Treatment of the vinyl chloride moiety, in **118,** with ozone, followed by reductive work-up should provide the primary alcohol of sphingosine. Carbons C2 and C3 of sphingosine correspond to C5 and C4 of azidoalcohol **118**, respectively. Oxidative cleavage of C2–C3 of azidoalcohol **118** should provide an aldehyde at C3 which corresponds to C4 of sphingosine. Wittig olefination will provide the rest of the aliphatic skeleton of sphingosine.

2. Results

2.1 A Misassigned Structure

Based on the literature data,[108] an assumption was made that treatment of epoxide **115**[109] with NaN_3 would result in a homo allylic opening of the epoxide to provide azidoalcohol **116** (Scheme 40). Our original goal was to synthesize the four C4 and C5 stereoisomers of type **116**, a regioisomer of azidoalcohol **118** (Scheme 39).

Facile protection of chlorodiol **113**, followed by epoxidation (*m*-CPBA) afforded epoxy acetonide **115**[109] in a regio- and stereospecific manner. It was reported[108] that epoxy acetonide **115** upon treatment with NaN_3 and NH_4Cl in the polar solvent mixture of 1,2-dimethoxyethane, ethanol, and water, provided azidoalcohol **116** (Scheme 40). Using this reaction protocol, an azidoalcohol believed to have structure **116** was obtained.

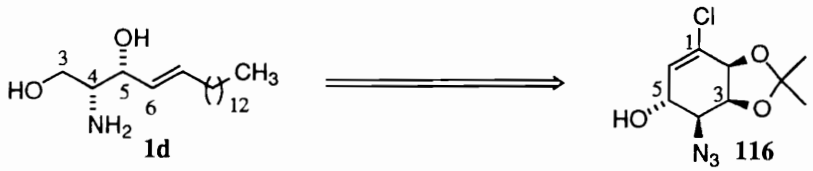


113 114 115

Scheme 40

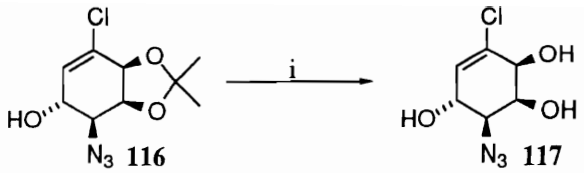
Reagents and Conditions: (i) 2,2-dimethoxypropane , cat. *p*-TsOH, CH_2Cl_2; (ii) *m*-CPBA, CH_2Cl_2, 0 °C; (iii) NaN_3, $NH_4^+Cl^-$, 1,2-dimethoxyethane, EtOH, H_2O, 70 °C.

The relevance of azidoalcohol **116** is apparent in light of its retrosynthetic analysis (Scheme 41). Although azidoalcohol **116** could be dismantled in several different ways, we decided to cleave the C2–C3 bond first. Removal of the acetonide, in **116**, would afford a vicinal diol **117** (Scheme 42), which could then be oxidatively cleaved, *i.e.* with $NaIO_4$. To this end, azidoalcohol **116** was deprotected to afford a vicinal diol, believed to have structure **117**.



1d

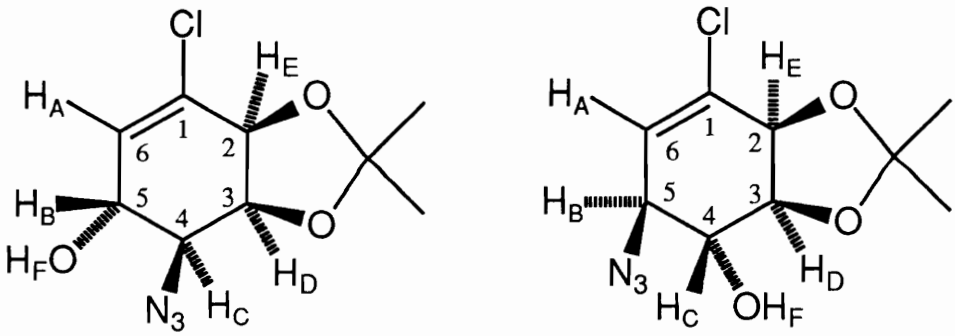
Scheme 41



Scheme 42

Reagents and Conditions: (i) Amberlyst 15 (wet) ion-exchange resin-strongly acidic, Aldrich Chemical Co.

Careful scrutiny of the spectral data, obtained for this novel compound **117**, revealed inconsistencies. The proton spectrum of the presumed triol **117** was recorded in DMSO-d_6 (Figure 4). Addition of D_2O eliminated the exchangeable protons, largely simplifying the resonance patterns (Figure 5). The connectivity assignment began with the readily identifiable olefinic proton H_A (δ 5.72 d, J= 2.6 Hz, 1H). The only other proton resonance showing the same coupling constant (J= 2.6 Hz) was at 3.93 ppm (J= 2.6, 7.8 Hz, 1H) and therefore must be H_B, residing on carbon C5. This was supported by selective irradiation experiments. Surprisingly, the resonance pattern of this proton (H_B) appeared to be unchanged upon addition of D_2O (see Figures 4 and 5), indicating that no alcohol functionality was present on carbon C5. Since an azide functionality was clearly present, as indicated by the IR spectrum, it seemed logical that the azide resided on carbon C5. Further scrutiny of the spectrums confirmed the observations discussed and left us with only one conclusion, *i.e.* azidoalcohol **116** is NOT formed under the reaction conditions found in the literature[108] (Scheme 40). Instead its regioisomer **118b** was the main product (Figure 3).



azidoalcohol **116** azidoalcohol **118b**

Figure 3. Regioisomeric Azidoalcohols **116** and **118b**

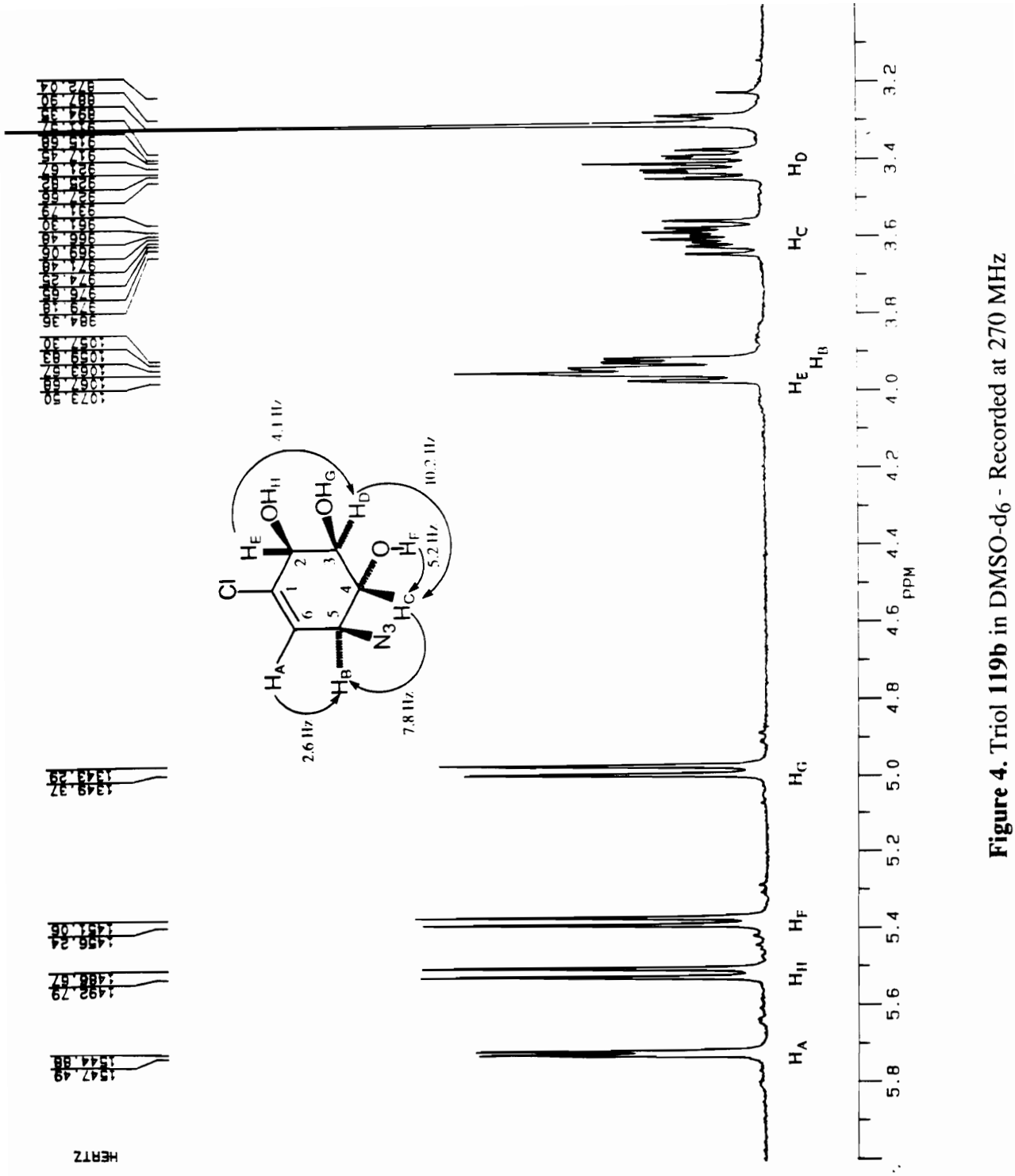


Figure 4. Triol **119b** in DMSO-d_6 - Recorded at 270 MHz

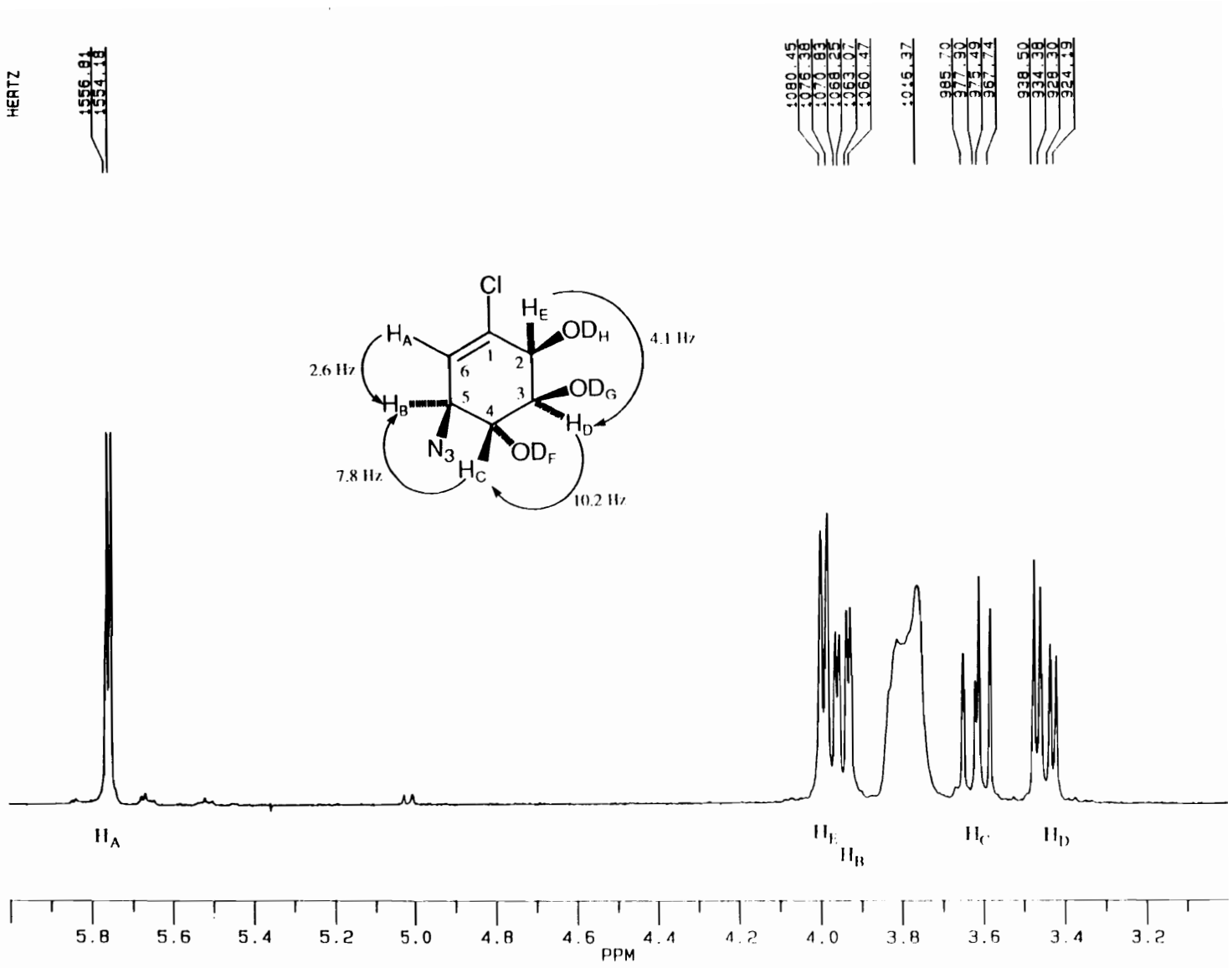
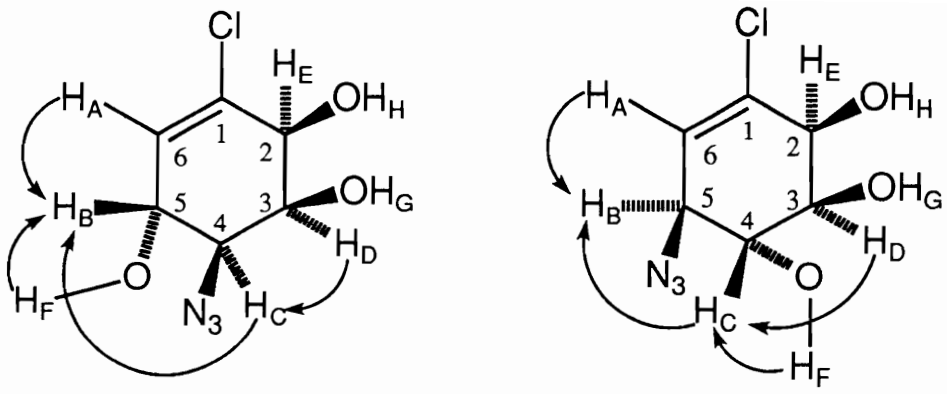


Figure 5. Triol **119b** in DMSO-d_6 With a Drop of D_2O - Recorded at 270 MHz

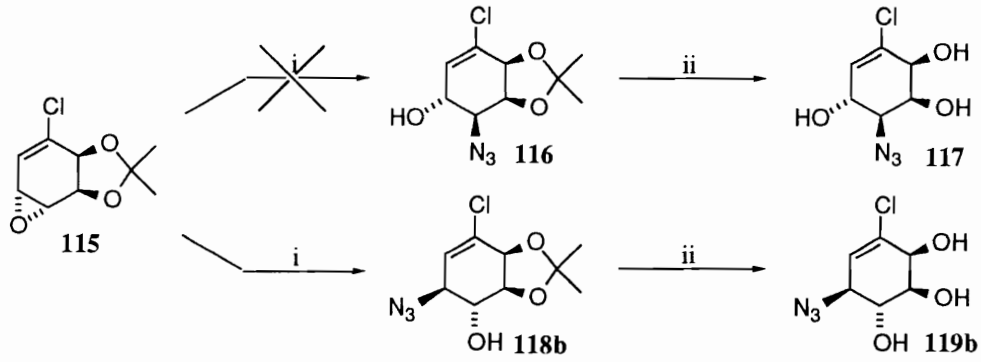


triol **117** triol **119b**

Figure 6. The expected Triol **117** and the Actual Triol formed **119b**

The article,[108] which contains the misassigned structure, describes a large variety of nucleophilic openings of epoxide **115**. The authors state that all nucleophiles examined, except azide, opened epoxide **115** at the allylic site. After examining all of our data, we now believed that azide had also attacked at the allylic site, as all the other nucleophiles had. Recognizing this fact we were able to explain all of the inconsistencies we were finding with the spectral data we presumed was for triol **117** (Figure 6).

The article stated, that a homo allylic opening with azide (top pathway Scheme 43) had occurred, but we now knew that an allylic opening had occurred to give azidoalcohol **118b** which we converted to triol **119b**. The ^1H NMR analysis revealed the regiochemistry of the product, but its absolute stereochemistry was still debatable.



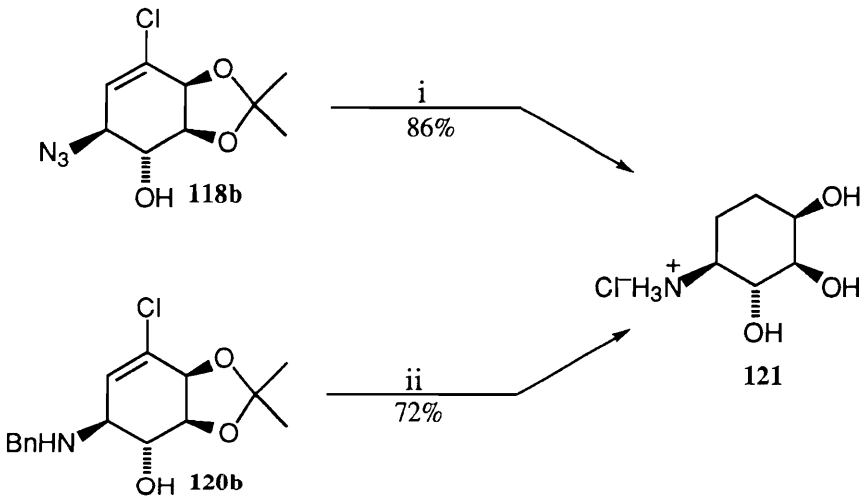
Scheme 43

Reagents and Conditions: (i) NaN_3, NH_4Cl, DME/ethanol/water (3:3:2), 65 °C; (ii) Amberlyst 15 (wet) ion-exchange resin-strongly acidic, Aldrich Chemical Co.

While it was assumed that the epoxide **115** was opened in a S_N2 'like' fashion by attack of azide, further proof was needed. Attempts to define the relative stereochemistry of the

azidoalcohol **119b** by interpretation of the ^1H NMR coupling constants of H_B and H_C (Figure 4 and 5), suggested a *trans* relationship between H_B and H_C (J= 7.8 Hz) and between H_C and H_D (J= 10.2 Hz). These data support structure **119b**.

In order to unequivocally prove the regio- and stereochemistry of azidoalcohol **118b**, a chemical correlation study was undertaken. Since benzylaminoalcohol **120b** (Scheme 44) was a known compound,[108] it was our intention to degrade both **120b** and azidoalcohol **118b** to trihydroxyamine hydrochloride **121**. Hydrogenolysis of azidoalcohol **118b** using Adams' catalyst afforded the ammonium salt **121** in 86% yield (Scheme 44). When benzylamino alcohol **120b** was subjected to the same reaction conditions a mixture of products corresponding to different levels of reduction was obtained. Pearlman's catalyst (20% $Pd(OH)_2$) gave similar results. In contrast, catalytic transfer hydrogenolysis provided ammonium salt **121** in 72% yield. The two products were indistinguishable by ^1H NMR, ^{13}C NMR, and $[\alpha]_D$ comparison. This confirmed the structure of azidoalcohol **118b**.



Scheme 44

Reagents and conditions: (i) 80 psi H_2, PtO_2, MeOH; (ii) ammonium formate, 10% Pd–C, CH_3OH reflux.

While the chemical correlation study was ongoing we were able to grow a suitable crystal of azidoalcohol **118b**, for x-ray crystallographic analysis. The data obtained confirmed all of our previous findings (Figure 7).

It should be noted, that a large number of reaction conditions were examined in the hopes of synthesizing azidoalcohol **116** from epoxide **115**, but all attempts failed. Azidoalcohol **116** was later synthesized in our group using an alternative route.[106]

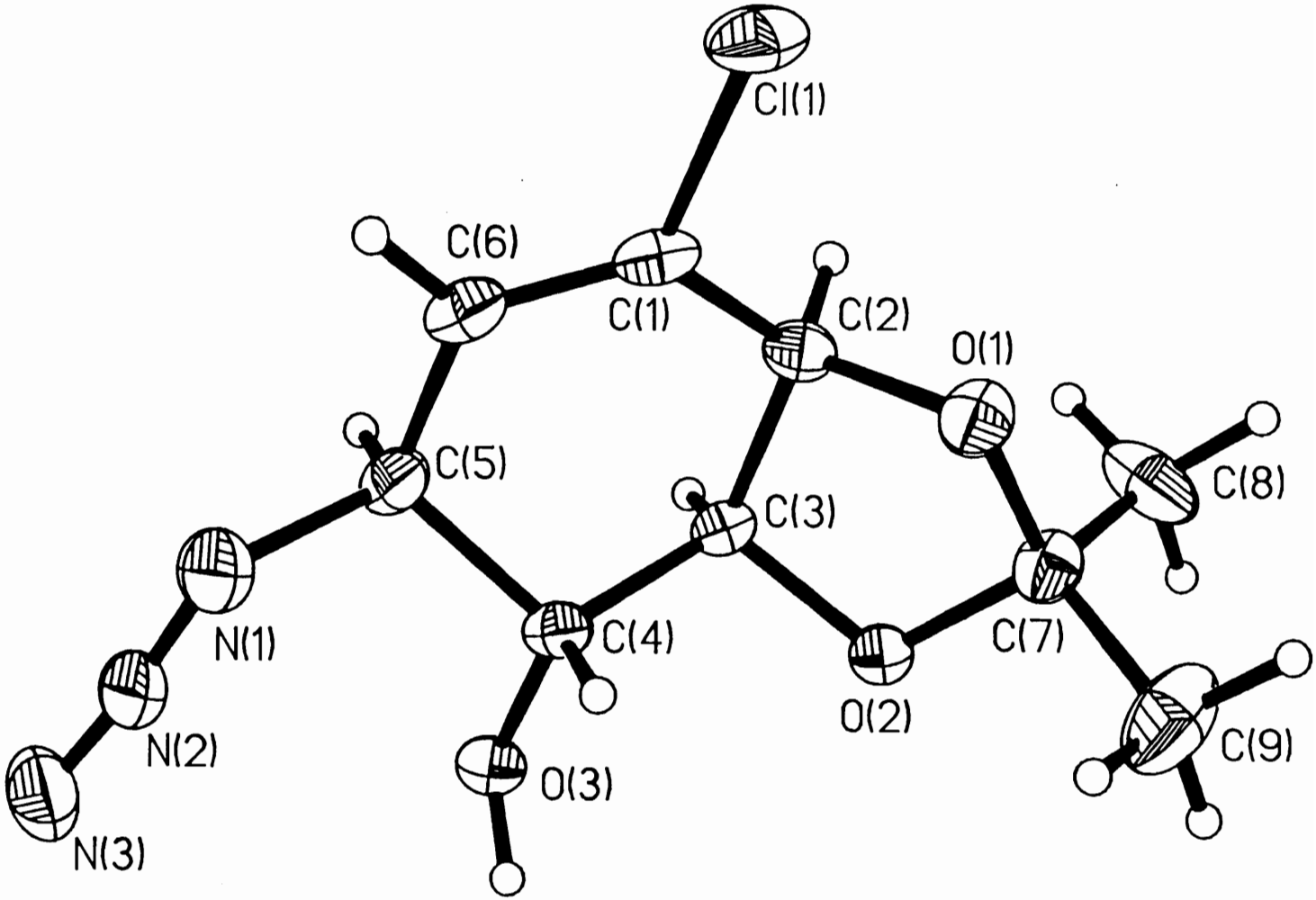
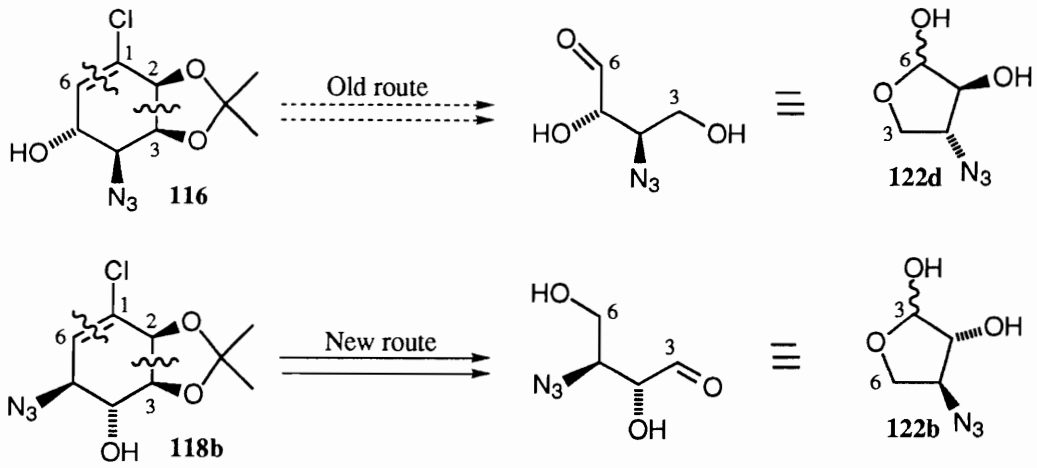


Figure 7. X-ray Structure of Azidoalcohol **118b**

2. Results

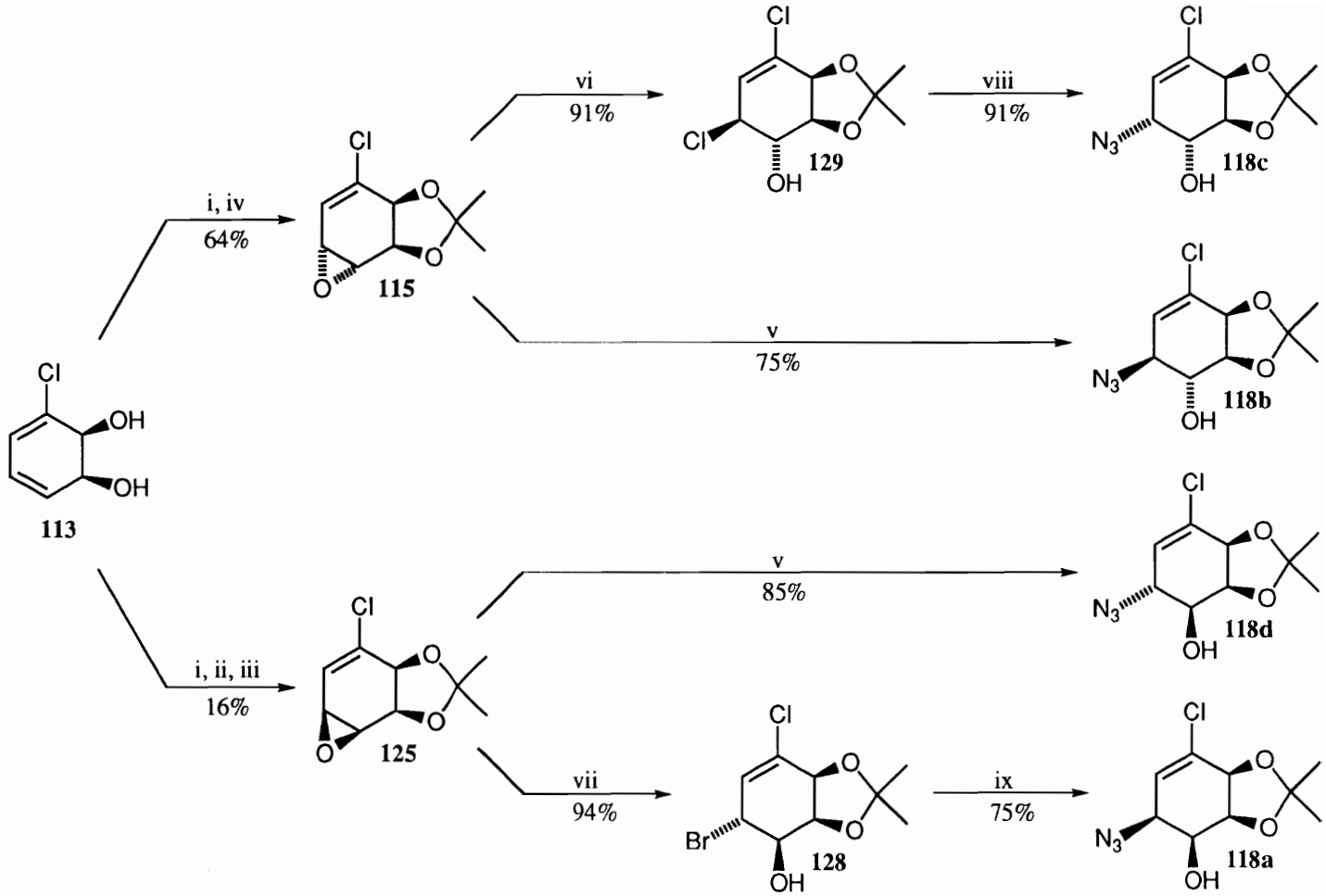
2.2 The Synthesis of Four Azidoalcohol Stereoisomers.

Having the structure of azidoalcohol **118b** firmly established, we needed to modify our proposed synthesis which was no longer valid. The original synthetic proposal called for oxidative cleavage (treatment with O_3, followed by dimethylsulfide) of the C1–C6 double bond, of **116**, and a reductive cleavage (treatment with $NaIO_4$, followed by $NaBH_4$) of the diol-moiety, C2-C3 (Scheme 45). The new strategy would require the reductive cleavage (treatment with O_3, then $NaBH_4$) of the C1-C6 double bond, in **118b**, and oxidative cleavage ($NaIO_4$) of the diol-moiety (C2-C3) to afford the enantiomeric hemiacetal **122b** (Scheme 45). Depending on the diastereomer of azidoalcohol **118** chosen, all four diastereomers of lactol **122** should be accessible.



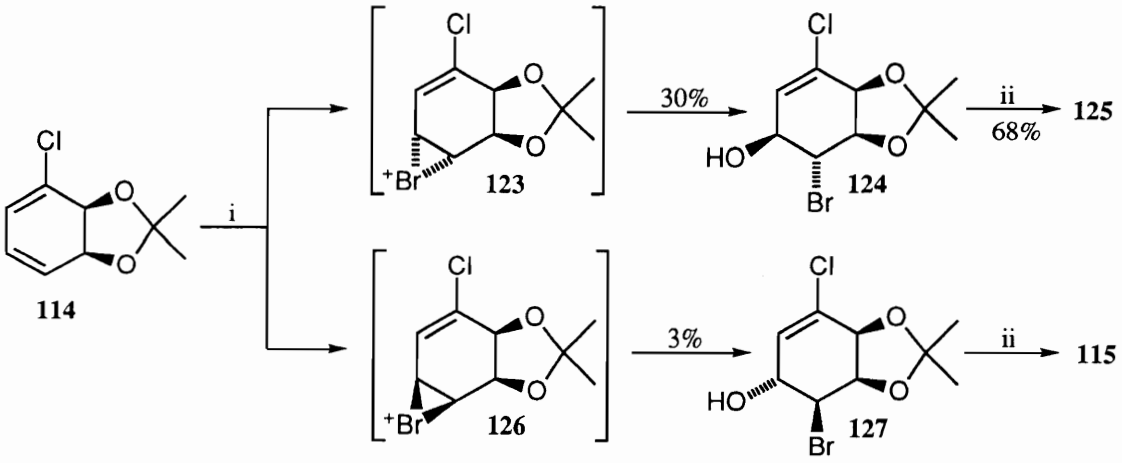
Scheme 45

With the new synthetic strategy in hand, our goal became the synthesis of two isomeric epoxides, **115** and **125**. Their nucleophilic opening and subsequent manipulation could open access to all four stereoisomers of azidoalcohol **118**. Thus, methods were needed to synthesize both α- and β-epoxides **115** and **125** respectively (Scheme 46). Procedures leading to both epoxides **115**[109] and **125**[110] had already been developed in our group. Full characterization of epoxide **125** and compounds leading to it were left until this project.



Scheme 46. Reagents and conditions: (i) 2,2-dimethoxypropane, catalytic *p*-TsOH, CH_2Cl_2; (ii) NBS, DME/H_20 (3:2), 0 °C; (iii) NaOH (1.1 equiv), Bu_4NHSO_4 (1.0 equiv), CH_2Cl_2 reflux; (iv) *m*-CPBA, CH_2Cl_2; (v) NaN_3, $NH_4^+Cl^-$, DME/EtOH/H_2O (3:3:2), 65 °C; (vi) LiCl (5.0 equiv), ethyl acetoacetate (3.0 equiv), THF, 45 °C; (vii) LiBr (1.1 equiv), ethyl acetoacetate (2.0 equiv), THF, 35 °C; (viii) NaN_3 (3.0 equiv), DMF; (ix) NaN_3 (15 equiv), DMSO.

Exposure of acetonide **114** to N-bromosuccinimide (NBS), in the presence of H_2O, led predominately, but not exclusively to bromohydrin **124** in 30% yield (Scheme 47). The minor isomer **127** was produced in 3% yield and was shown to cyclize to the known epoxide **115**, to aid in its identification. The electrophilic bromine species attacks the more electron rich olefin, predominately from the sterically less hindered α-face. Subsequent nucleophilic attack by H_2O at the allylic site yields the corresponding *trans* bromohydrins **124** and **127**. Their exposure to base furnished epoxides **125** and **115** respectively.

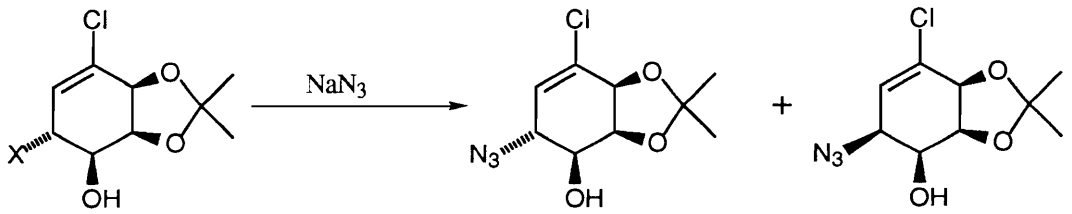


Scheme 47

Reagents and Conditions: (i) NBS, catalytic *p*-TsOH, 1,2-dimethoxyethane, H_2O; (ii) NaOH, Bu_4NHSO_4, CH_2Cl_2 reflux.

Azidoalcohols **118b** and **118d** should be directly accessible from epoxides **115** and **125** respectively (Scheme 46). Indeed, azidoalcohols **118b** and **118d** were obtained in high yield upon exposure of the epoxides (**115** and **125**) to NaN_3. The synthesis of the two remaining azidoalcohols **118a** and **118c** presented a greater challenge. Inversion of stereochemistry at the allylic site of halohydrins **128** and **129** would be necessary to form the *cis* azidoalcohols **118a** and **118c**.

When bromohydrin **128** was treated with NaN_3 (Table 8) an intra- and intermolecular competition of epoxide formation *vs* azide introduction ensued. This competition could be controlled by varying the concentration of NaN_3 present. Surprisingly, depending on the excess/concentration of sodium azide used, azidoalcohol **118a** or **118d** could be obtained as the major product. When the concentration of azide did not greatly exceed that of the bromohydrin **128**, intramolecular closure prevailed and epoxide **125** was formed. Subsequent opening of the epoxide, with azide, yields the *trans* azidoalcohol **118d** (Scheme 48). On the other hand a large excess of NaN_3, compared to the halohydrin, leads preferentially to an intermolecular displacement of the halogen by azide and afforded the *cis* azidoalcohol **118a** as the major product (Table 8).



128, X = Br **118d** **118a**

Scheme 48

Table 8. Reactivity of Halohydrins to Sodium Azide

Halohydrin, where X equals	Molarity	Molarity of NaN_3	Equiv. ratio NaN_3 / SM	Solvent	Final temp.	% of **118a**	% of **118d**
Chlorine	0.18	0.70	3.89	DMF	85 °C	12.7	77.3
Bromine	0.20	0.80	4.00	DMF	90 °C	14.4	75.6
Chlorine	0.19	5.7	30.0	DMSO	40 °C†	0	0
Bromine	0.61	9.7	16.0	DMSO	40 °C†	74.8	21.8

SM starting material (halohydrin); † An ultrasound sonicator was used to maintain homogeneity. The temperature of the bath was 40 °C.

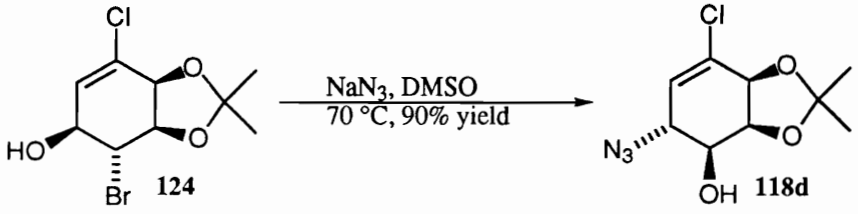
Before the optimum conditions (last row of Table 8) to synthesize **118a** were found, a variety of different approaches were examined. Thus, attempts at oxidizing azidoalcohol **118b** (Scheme 46) to the corresponding ketone and then reducing it with a hydride reagent preferentially from the least hindered, α-face, failed at the ketone stage. The alcohol of **118b** turned out to be highly resistant to oxidation, Swern conditions and tetra-*n*-propylammonium perruthenate (TPAP)[111] failed to produce the ketone. In still another attempt to exploit **118b**, the tosylate was formed. Treatment of the tosylate with a nucleophilic source of oxygen (NaOH and KNO_2[112]) also failed to produce azidoalcohol **118a**.

Attempts to protect the alcohol moiety of **128** (Scheme 48), prior to bromide displacement by azide, also failed. The acetyl, trimethylsilyl and benzyl protecting groups were investigated. Formation of the acetate proceeded in high yield, but attack by azide proved troublesome. Slow reaction times and a complex mixture of products forced us to abandon this work. Treatment of the trimethylsilyl ether of bromohydrin **128** with azide resulted in deprotection of the silyl group. Attempts to form the benzyl ether of **128** were unsuccessful, only the β-epoxide **125** was formed.

In yet another attempt to synthesize azidoalcohol **118a**, β-epoxide **125** was treated with iodine, and triphenylphosphine in CH_2Cl_2 to give the corresponding iodohydrin.[113] The iodohydrin showed visible signs of decomposition after 20 minutes at room temperature. However if immediately treated with sodium azide in DMSO, the desired azidoalcohol **118a** was

exclusively formed. Even though this sequence was fruitful, the reaction conditions shown in Table 8 were more reliable.

The synthesis of the last azidoalcohol **118d** went effortlessly, in 85%, *via* the direct opening of epoxide **125** with NaN_3 (Scheme 46). Interestingly, **118d** could also be accessed directly from bromohydrin **124**[114] (Scheme 49). The spectral data for the two independent synthesizes of azido alcohol **118d** were indistinguishable. When **118d** is synthesized from bromohydrin **124**, the overall yield from chlorodiol **113** is 27%. Using the route shown in Scheme 46 the overall yield from chlorodiol **113** is 13%.

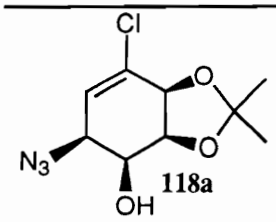
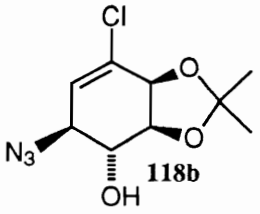
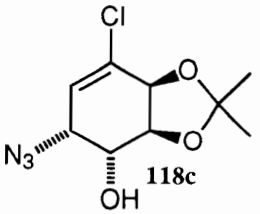
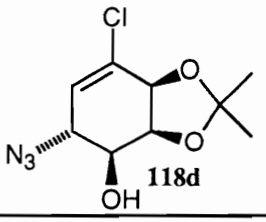


Scheme 49

In an attempt to take advantage of this unique reaction (Scheme 49) bromohydrin **124** was treated with NaBr in DMSO in the hopes of forming bromohydrin **128** (Scheme 46). Unfortunately none of the desired bromohydrin **128** was observed. Similar results were observed when NaCl was used.

That all four azidoalcohols **118a-d** were accesible from chlorodiol **113**, was now firmly established. The efficiency of their synthesis is shown in Table 9. It was now our intent to synthesize a stereoisomer of sphingosine. To this end we chose **118b** to be carried through to L-*threo*-sphingosine.

Table 9. Efficiency of Azidoalcohol Synthesis from Chlorodiol **113**

Compound	Number of steps from chlorodiol **113**	Overall % yield from chlorodiol **113**
 **118a**	five steps	11%
 **118b**	three steps	48%
 **118c**	four steps	53%
 **118d**	three steps	28%

2. Results

2.3 Exhaustive Cleavage of Azidoalcohols 7a & 7b - Synthesis of D-*erythro*-C_{18}- and L-*threo*-C_{18}-Sphingosine.

Since azido alcohol **118b** could be synthesized in high yield it was chosen for our initial investigation, which would ultimately lead to the unnatural L-*threo*-sphingosine. Content that L-*threo*-sphingosine could be synthesized from an aldehyde of type **D** (Figure 8), several possibilities for its synthesis were considered. Ozonolysis of the C1-C6 double bond in compound **A** would lead to compounds of type **B** and subsequent periodate cleavage (C2-C3), after deprotection, would yield aldehyde **D**. Alternatively, periodate cleavage of **118b** would form compounds of type **C,** ozonolysis of which should yield aldehyde **D**. Compound **D** the open from of lactol **122** would then be subjected to Wittig olefination, providing the skeleton of sphingosine. (Note: *For the synthesis of a particular stereoisomer of sphingosine, all compounds leading to its synthesis will carry the same letter designation. If another stereoisomer of sphingosine is synthesized using stereoisomeric compounds they will have the same numbers, but a different letter. For example all compounds which could potentially led to the synthesis of* D-*erythro-sphingosine* ***1a*** *will bear the letter "a".*)

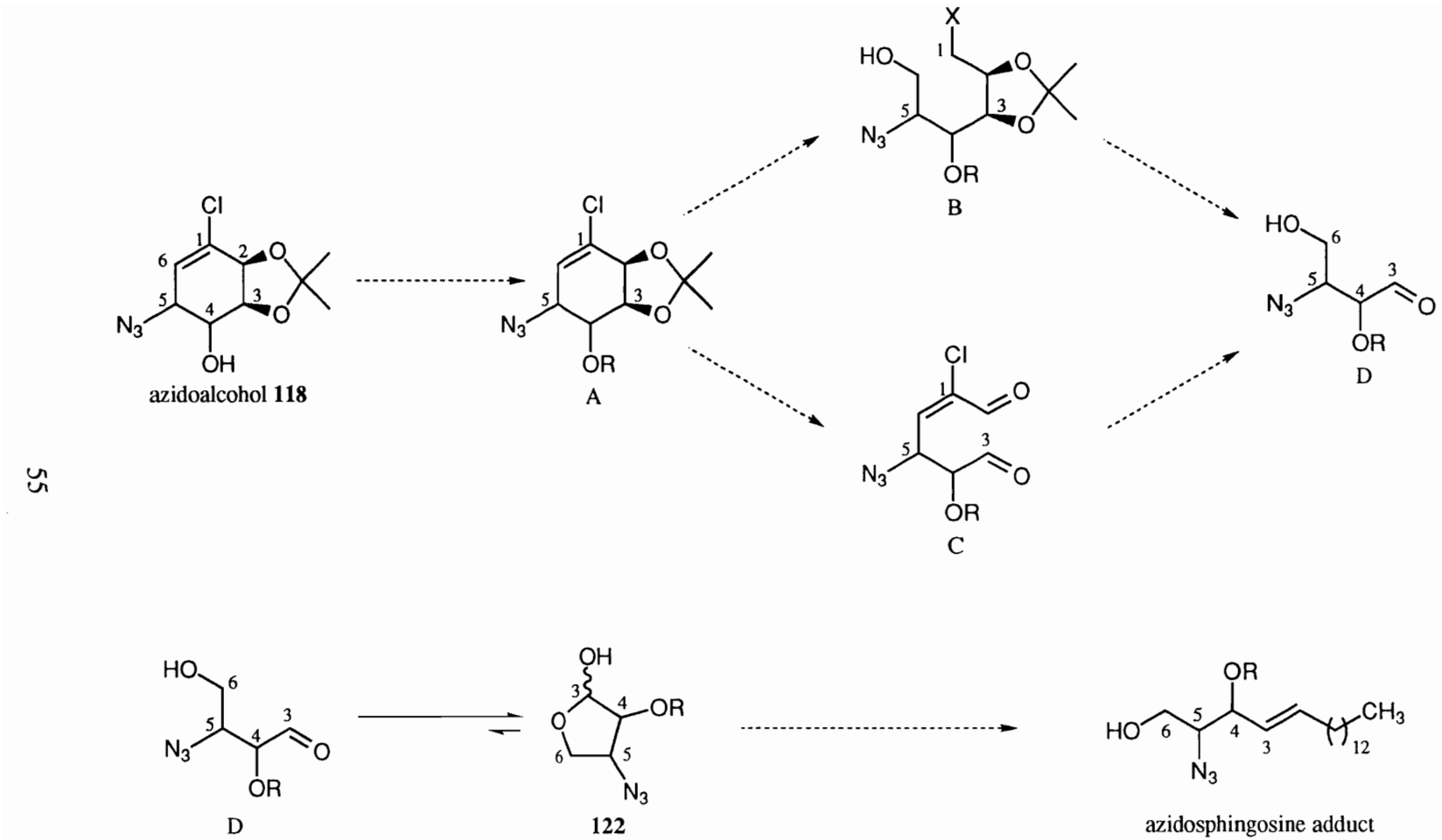
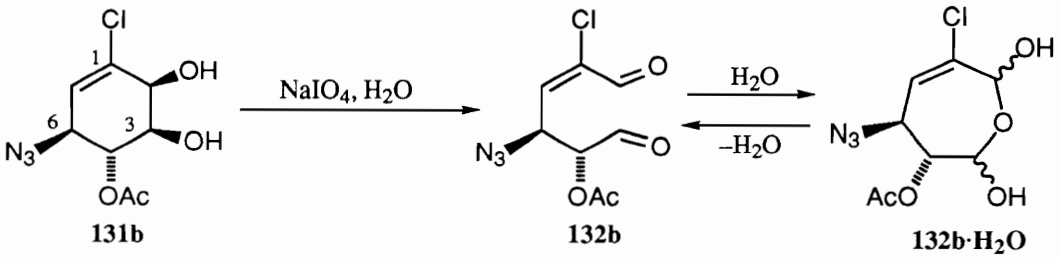


Figure 8. Proposed Route to Sphingosine.

To this end, alcohol **118b** was protected as an acetate **130b** to provide the first substrate for the ring cleavage experiments. Scheme 50 summarizes the cleavage chemistry performed. Acetonide **130b** was deprotected to afford diol **131b**, which upon treatment with $NaIO_4$ in H_2O gave at least four compounds as evidenced by the complicated ^1H NMR spectrum of the crude product mixture. Readily identifiable aldehyde and lactol resonances suggested the presence of dialdehyde **132b**, along with a cyclic dihemiacetal (presumably *via* the addition of one equivalent of water, Scheme 51). An attempt to simplify the reaction product mixture via reduction ($NaBH_4$, both normal and Luche's conditions[115]) proved fruitless and the route was abandoned.

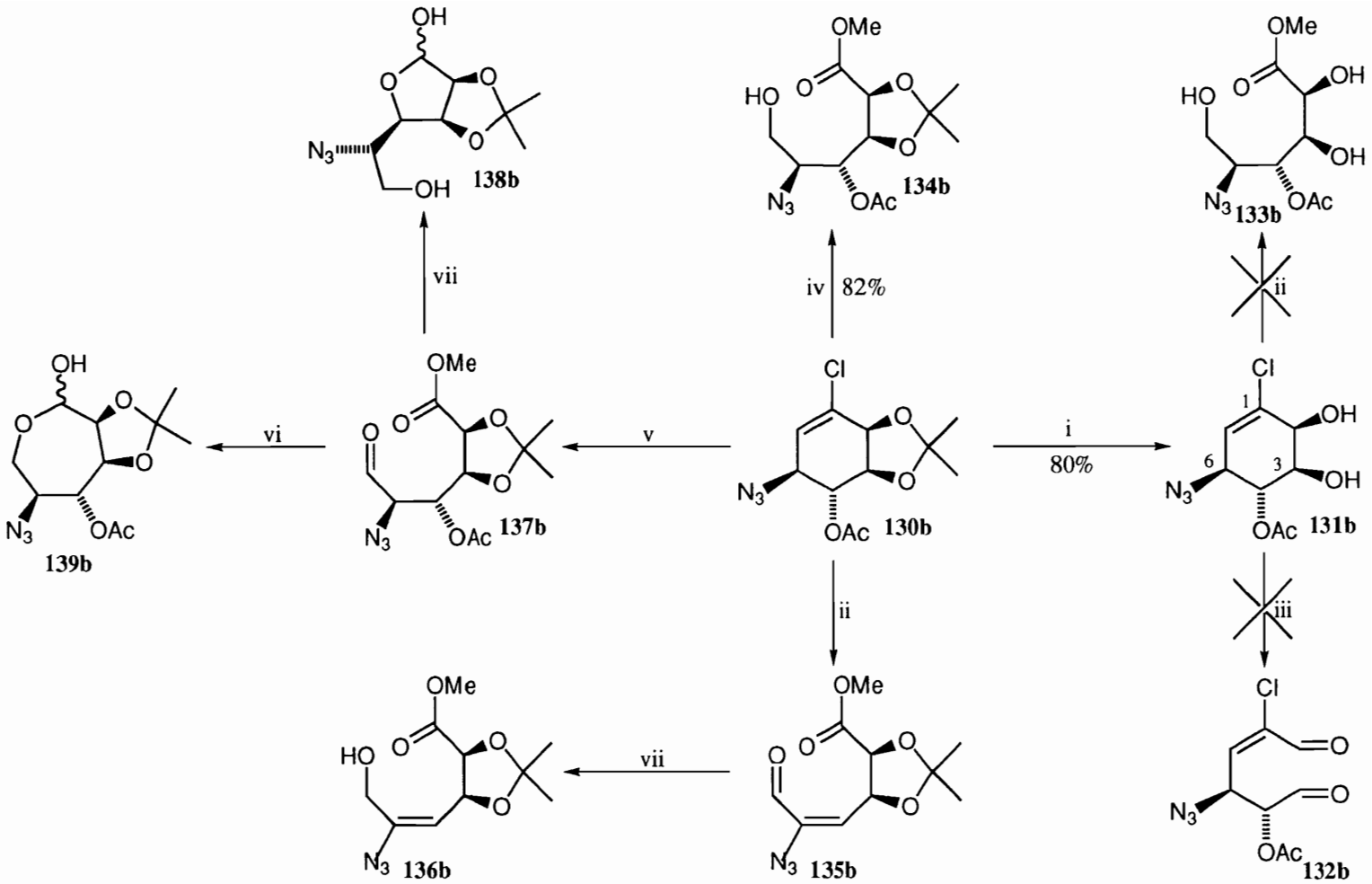


131b **132b** **132b·H_2O**

Scheme 51

Disappointed by the outcome of the periodate cleavage reaction, we focused our attention on the cleavage of the vinyl chloride moiety in **131b**. Ozonolysis in MeOH, followed by reductive work-up with $NaBH_4$, did not yield methyl ester **133b** (Scheme 50). The large number of products formed (as evidenced by the TLC) made this particular route unattractive for our goal.

Since we were unable to synthesize any compounds of interest from **131b**, we decided to examine the cleavage of the vinyl chloride moiety in **130b**. Ozonolysis (methanol, 5.0 equivalents of sodium bicarbonate), followed by $NaBH_4$ work-up, surprisingly gave vinyl azide **135b** (Scheme 50). Evidently an elimination occurred, in which acetate served as the leaving group.

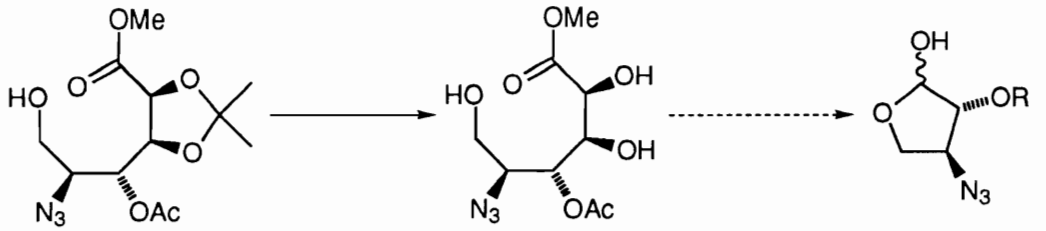


Scheme 50. Reagents and Conditions: (i) Amberlyst 15 (wet) ion-exchange resin-strongly acidic, Aldrich Chemical Co. (ii) (1) O_3 (excess), $NaHCO_3$ (5.5 equiv), MeOH, (2) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, -20 °C; (iii) (1) $NaIO_4$, H_2O; (iv) (1) O_3 (excess), MeOH, (2) $NaBH_3CN$, 0 °C; (v) (1) O_3 (excess), MeOH, (2) $NaBH_4$, -30 °C or rt; (vi) $NaBH_4$, MeOH, 0 °C; (vii) $NaBH_4$, MeOH, rt.

The stage at which this elimination occurred was not clear. Ozonolysis of compounds containing a vinyl chloride moiety (*e.g.* **130b**) generate HCl when the latent alkanoyl chloride is attacked by methanol, the solvent. To maintain a pH close to neutral, sodium bicarbonate is sometimes added. In this particular case, after $NaBH_4$ work-up of the ozonide at -20 °C, sodium bicarbonate or sodium carbonate could have easily deprotonated the acidic α-proton of aldehyde **137b** (Scheme 50). Elimination of acetate *via* the enolate of **137b** might explain the formation of the α,β-unsaturated aldehyde **135b**.

This hypothesis is strengthened by the observation that in the absence of sodium bicarbonate aldehyde **137b** is the main product (none of the elimination product **135b** was found). Interestingly even under the forceful conditions of the excess $NaBH_4$ the ozonide reduction, of **130b**, always provided the aldehyde **137b** and none of the corresponding alcohol. However, if the crude aldehyde **137b** was isolated (after acidic work-up - pH= 3.5) and treated with $NaBH_4$ at 0 °C, a seven-membered lactol **139b** ensued. If the reduction was carried out at room temperature a five-membered lactol **138b** was formed.

The cleavage experiments performed on acetate **130b** (Scheme 50) provided us with further important information. When sodium cyanoborohydride was used to reduce the ozonide of **130b**, methyl ester **134b** was isolated in 82% yield. We expected the corresponding vicinal diol **133b**, obtained from acetonide hydrolysis of **134b**, to provide lactol **122b** when treated with $NaIO_4$ (Scheme 52). Unfortunately, after the acetonide protecting group was removed, a migration of the acetyl group occurred. Several different reaction conditions were examined, but no improvements were observed. The propensity of this acetate to migrate made isolation of triol **133b** impossible.



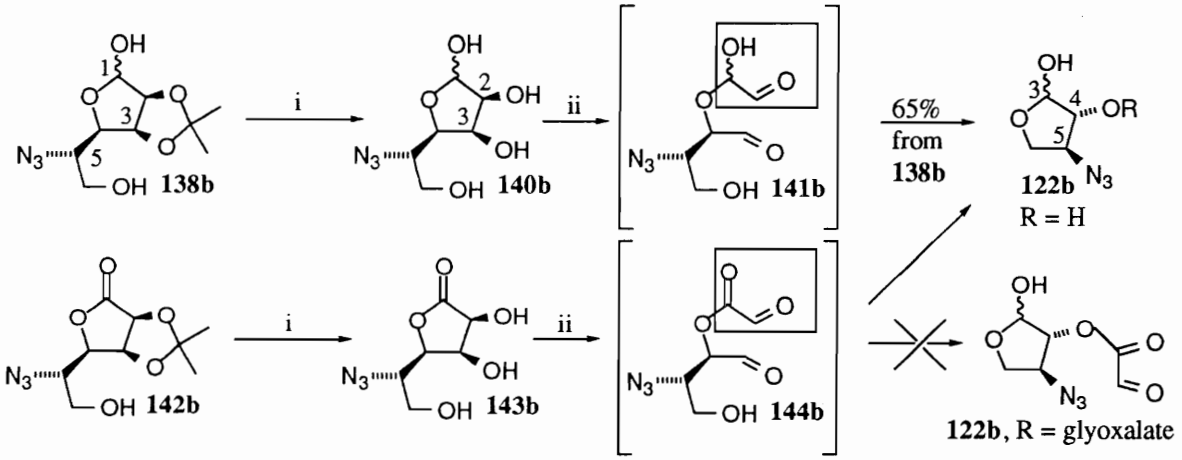
134b **133b** **122b R = acetyl**

Scheme 52

At this point it became clear that a different protecting group had to be utilized. When the ozonide of **130b** was reductively worked-up in the absence of sodium bicarbonate, lactol **138b** was obtained (Scheme 50). Obviously the acetate protecting group of **130b** was also cleaved under the reaction conditions, and the resulting free alcohol participated in an intramolecular

lactolization. The possibility of an "intramolecular protection" of this alcohol was extremely appealing and thus considered further.

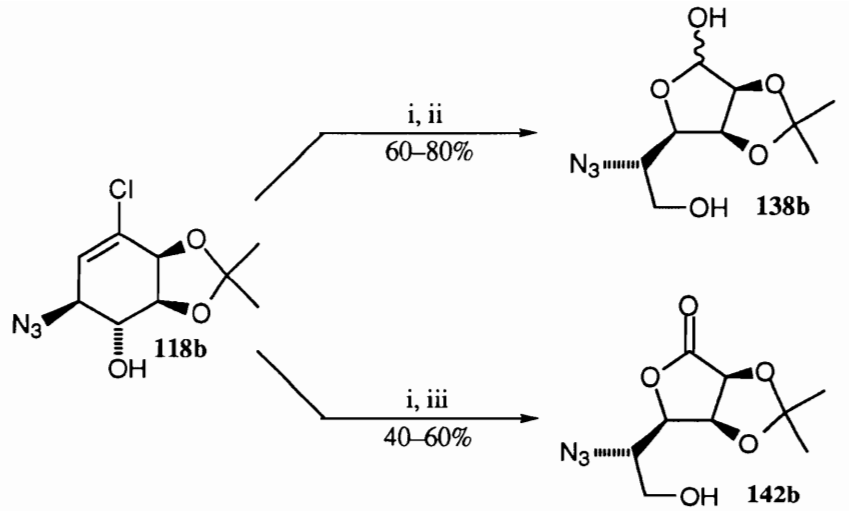
Thorough examination of this "protecting group" revealed an obvious problem. At the stage of compound **141b** (Scheme 53) the "protecting group" would be connected to the alcohol *via* a hemiacetal bond. Thus, once the vicinal diol of **140b** was cleaved, the protecting group would be readily hydrolyzed under the reaction conditions. In an attempt to overcome this lability problem, compound **144b** was considered. At the oxidation level of an ester, the gyloxalate moiety (in square) might be stable under the reaction conditions and yield lactol **122b**, where R = glyoxalate. Investigation of the synthesis of **142b** therefore appeared worthwhile.



Scheme 53

Reagents and conditions: (i) Amberlyst 15 (wet) ion-exchange resin-strongly acidic, Aldrich Chemical Co.; (ii) $NaIO_4$, H_2O.

Since the C-4 acetate was cleaved during the $NaBH_4$ work-up of the ozonide of **130b**, we expected direct ozonolysis of alcohol **118b** to provide lactol **138b** in an analogous fashion. Indeed, under these conditions ozonolysis of alcohol **118b** afforded lactol **138b** in yields varying from 60 - 80%. To obtain lactone **142b**, the ozonide of **118b** was reduced with sodium cyanoborohydride[116] (Scheme 54). Acid catalyzed hydrolysis of the acetonide in **142b** followed by periodate cleavage failed to give **122b** (R = glyoxalate, Scheme 53), but instead yielded **122b** (R = H). Even though our initial strategy warranted protection of the hydroxy group in lactol **122b**, where R = H, we decided to evaluate its potential as a Wittig substrate.



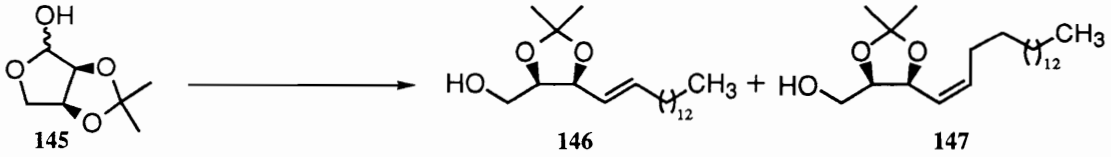
Scheme 54

Reagents and conditions: (i) O_3 (excess), MeOH, -78 °C; (ii) $NaBH_4$, MeOH, -55 °C to rt; (iii) $NaBH_3CN$, MeOH, pH ≅ 3.0, 0 °C.

Wittig olefination, completing the carbon skeleton of sphingosines, was examined in great detail. Even though a control reaction, using known lactol **145** (synthesized from the chlorodiol **113**,[117] Table 10) gave olefins **146** and **147** in almost quantitative yield, with substrate **122b** (R = H) the overall yield of azidosphingosine was never greater than 30% (Table 11). In theory, a minimum of three equivalents of base and tetradecyltriphenylphosphonium bromide is required for the reaction. The results suggest that a slight excess of the phosphonium salt is required or the reaction yield will be seriously compromised.

Azidoalcohol **118a** (precursor to naturally occurring D-*erythro*-sphingosine) was exhaustively cleaved, in the same manner as **118b**, to give the diastereomeric lactol **122a**. Thus **118a** was converted to lactol **138a**, which was then transformed into lactol **122a** (see top pathway, Scheme 53, for reaction type). When lactol **122a** (Table 11) was treated under the same optimized Wittig olefination conditions as **122b** similar yields of azidosphingosines **148a** and **149a** were observed.

Table 10. Wittig Olefination of Lactol **48**

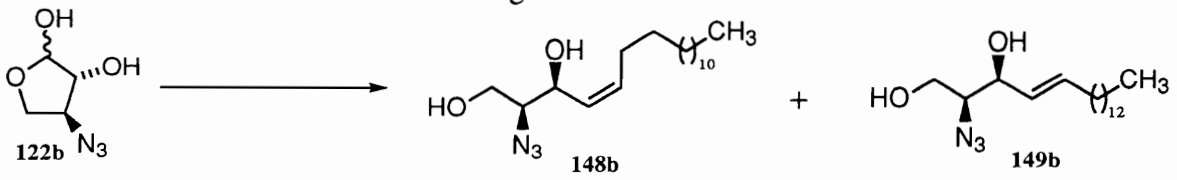


145 146 147

Equiv of salt§	Equiv of base	Temperature	Solvent	% Yield Z/E
2.2	2.25 of *n*-BuLi	room temperature	THF	80 / 15
2.2	4.12 PhenylLi	-35 °C	THF	6 / 61

§ Equiv of salt, refers to equivalents of tetradecyltriphenylphosphonium bromide.

Table 11. Wittig Olefination of Lactol **122**



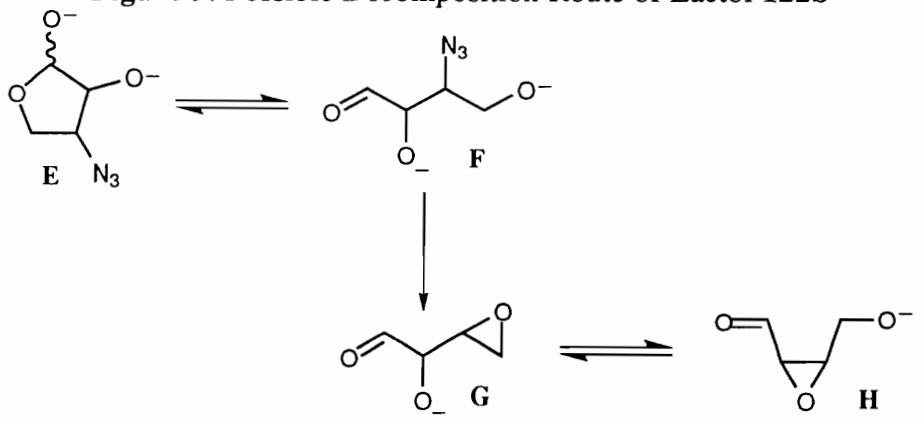
122b **148b** **149b**

Lactol	Ref.	Equiv of salt§	Equiv of base	Temperature	Solvent	% Yield Z/E
122b	117a	4.0	4.0 *n*-BuLi	room temperature	THF	Δ
122b	118†	1.15	4.6 PhenylLi	-35 °C	THF/toluene	0.9 / 1.8
122b	†	1.20	5.5 PhenylLi	-78 °C	THF/toluene	Δ
122b	119*	4.0	8.0 Dimsyl anion	room temperature	DMSO	Δ
122b	120	2.0	2.0 Na amylate	room temperature	THF/toluene	10 / 7
122b		3.2	2.7 *n*-BuLi	room temperature	THF	24 / 6‡
122a		3.2	2.7 *n*-BuLi	room temperature	THF	14 / 4

§ Equiv of salt, refers to equivalents of tetradecyltriphenylphosphonium bromide. † Denotes modified Schlosser-Wittig conditions. * The dimsyl anion was generated by the addition of 8.0 equiv of NaH to DMSO (the reaction solvent). ‡ Note this yield was not repeatable, subsequent reactions gave yields very similar to those of lactol **122a**, *i.e.* combined yields (Z and E) of 20%. Δ Neither *cis*- or *trans*-azidosphingosine were observed.

It is known that aldehydes with α-hydroxy groups generally give poor yields in Wittig reactions.[121] This alone could account for our poor yields, but we also questioned the inherent stability of these lactols under the basic conditions of a Wittig reaction. Although many Wittig reactions have been performed on lactols,[119] none could be located in the literature with a disposition of functional groups found in our lactols (Figure 9). Under the reaction conditions it was assumed that both acidic protons were removed. Once the lactol was opened, structure **F**, an intramolecular nucleophilic displacement of azide could ensue, forming epoxides **G** and **H**. Due to the insolubility of LiN_3 or NaN_3 in the reaction medium, azide would effectively be removed from the reaction. Further experiments and data collection would be required to support the suppositions of Figure 9. Possibly, the phosphonium ylide itself may attack the azide, complicating the picture further. Generally, these Wittig reactions did not show a satisfactory mass recovery.

Figure 9. Possible Decomposition Route of Lactol **122b**

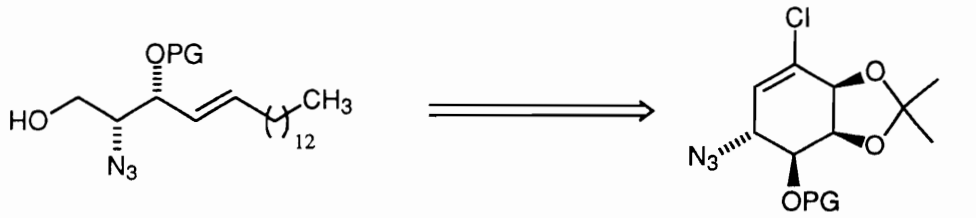


Sphingosine contains a *trans* olefin, yet the Wittig reactions we performed gave predominantly the *cis*-azidosphingosine product. Therefore *cis*-azidosphingosines **148a** (D-*erythro*-series) and **148b** (L-*threo*-series) were photoisomerized to *trans*-azidosphingosines **149a** and **149b** by means of a Hanovia 450 W lamp, Pyrex filter, and diphenyl disulfide.[79] Reduction of *trans*-azidosphingosine **149b** with hydrogensulfide gave L-*threo*-sphingosine **1b**, which was not isolated, but instead treated with acetic anhydride and pyridine to provide its triacetate, indistinguishable from an authentic sample. *trans*-Azidosphingosine **149a**, a known compound, displayed ^1H NMR and $[\alpha]_D^{24} = -34.2°$ (c 1.58, $CHCl_3$) [lit.[122] $[\alpha]_D^{20} = -32.9°$ (c 4.0, $CHCl_3$)] in agreement with the literature values. The synthesis of D-*erythro*-azidosphingosine **149a** constitutes a formal synthesis of D-*erythro*-sphingosine.

2. Results

2.4 Exhaustive Cleavage of Azidoalcohol 118d - Synthesis of D-*threo*-C_{18}-Sphingosine

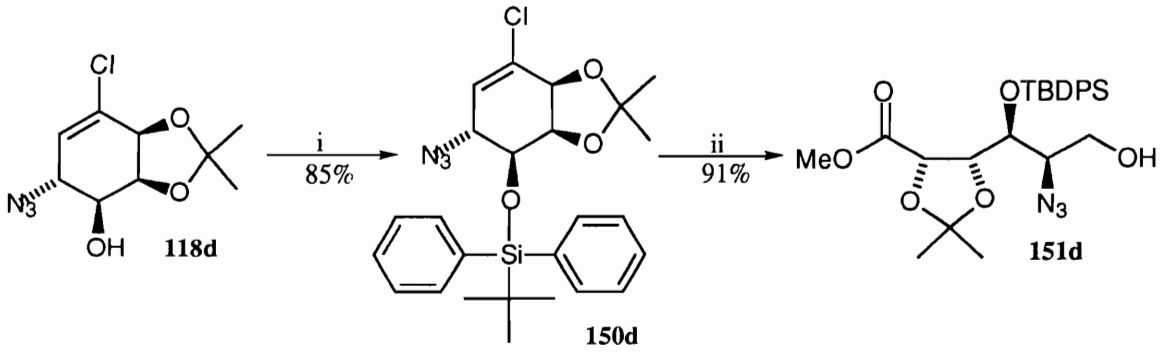
To meet our goal of synthesizing all four stereoisomers of sphingosine, D-*threo*- and L-*erythro*-sphingosine needed to be synthesized. This goal could have been met by repeating the protocols developed for the synthesis of the first two sphingosines. Instead we embarked on a second generation synthesis of sphingosine. Our original goal of synthesizing a lactol of type **122** with the alcohol protected had never been met. With this objective in mind we set our sights on such a synthesis, in the hopes of improving the Wittig olefination reaction. The protecting group choosen would have to be stable under the acetonide deprotection conditions, as well as the basic conditions of a Wittig reaction, and finally be easily removed at the azidosphingosine stage (Scheme 55).



protected azidosphingosine — protected azidoalcohol **118d**

Scheme 55

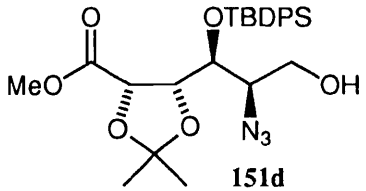
It was decided that a *tert*-butyldiphenylsilyl (TBDPS) ether protecting group would fulfill the requirements necessary.[123] To this end, alcohol **118d** was protected as its *tert*-butyldiphenylsilyl ether (TBDPS ether) **150d** and subsequently treated with ozone, to provide methyl ester **151d** (Scheme 56).



Scheme 56

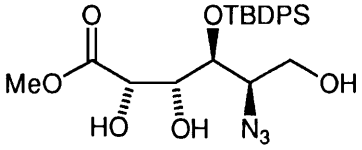
Reagents and conditions: (i) *tert*-butyldiphenylsilyl chloride (1.97 equiv), imidazole (2.39 equiv), THF; (ii) (1) O_3 (excess), MeOH, -78 °C, (2) $NaBH_4$ (excess) , MeOH, 0 °C to room temperature.

Acetonide **151d,** upon treatment with Amberlyst 15 (wet) strongly acidic ion-exchange resin, provided the desired vicinal diol **152d**, although in poor yield (44%). The remaining mass-balance consisted of three new compounds (**153d** - **155d**, Scheme 57). Full characterization of these new compounds showed that deprotection of the acetonide was not a problem. Instead it suggested that the product, **152d**, was inherently unstable under the acidic reaction conditions. The product distribution was essentially unchanged when $THF/H_2O/CH_3CO_2H$, $THF/H_2O/CF_3CO_2H$ (see Scheme 58 for an example found in the literature[124]); and/or $CH_3OH/H_2O/HCl$ systems were examined. This result was unexpected, especially in light of the result shown in Scheme 58.[124] In any event the products **152d** through **155d** are readily explained. It seems almost without question that deprotection of the acetonide to form vicinal diol **152d** occurs first. The TBDPS group then migrates to the adjacent alcohol. This migration provides what we believe is triol **153d**, based on the ^1H NMR spectrum. If triol **42d** is stored neat or exposed to silica gel (short columns of silica gel and fast elution are advised for purification) lactones **154d** and **154d** are formed. Full characterization of diol **153d** was not attempted because of its unstable nature. It is interesting to note that when pure lactone **154d** or **155d** was placed in DMSO-d_6, a 1:1 ratio of the two lactones (**154d** and **155d**) immediately ensued, as evidenced by ^1H NMR, and that this ratio does not change over a period of a couple of days. Yet when pure lactone **154d** or **155d** is placed in $CDCl_3$ no sign of the other lactone is evident by ^1H NMR.

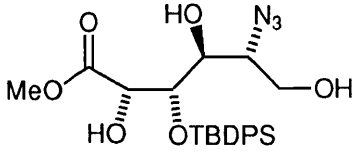


151d

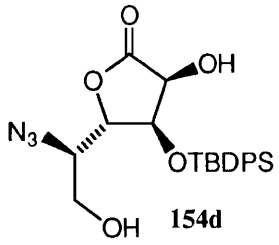
Acidic reaction conditions



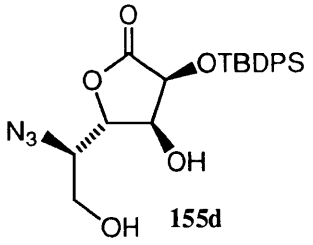
152d



153d



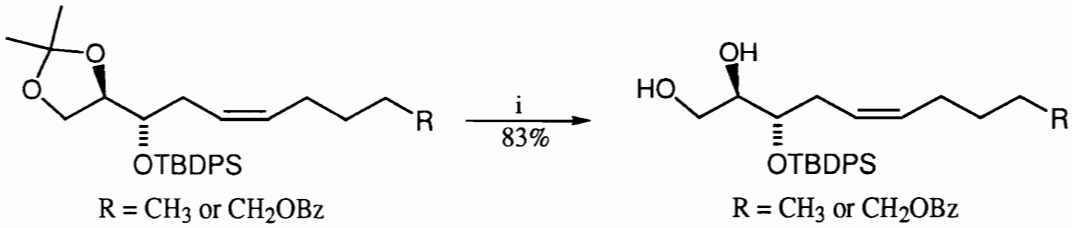
154d



155d

Scheme 57

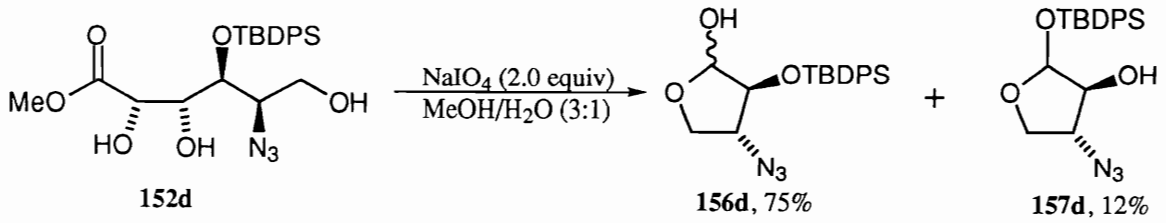
In an attempt to thwart the silyl migration of diol **152d**, some nontraditional reagents were examined. Thus when **151d** was treated with dimethylaluminum chloride[125] the results were very encouraging (TLC), but upon work-up, the four products were found as usual. Finally, when 1% I_2 in MeOH (w/v) was employed[126] an 80% yield of **152d** was obtained. No further optimization of this reaction was carried out.



Scheme 58

Reagents and conditions: (i) $THF/H_2O/CF_3CO_2H$ (20:5:1), 0 °C → rt.

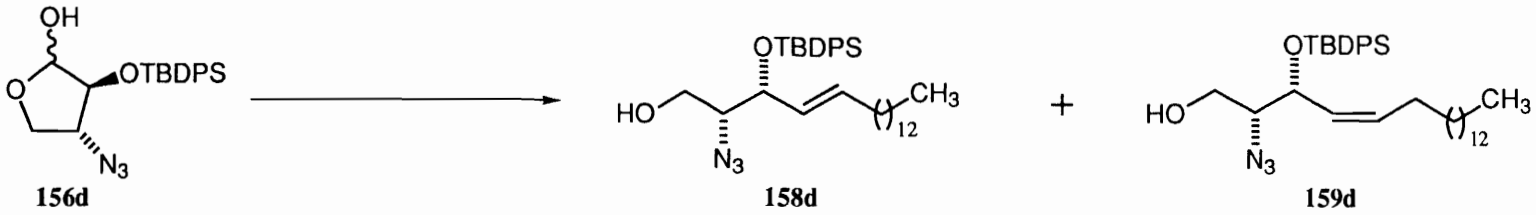
Proceeding with the synthesis, vicinal diol **152d** was treated with $NaIO_4$ to provide lactol **156d** in 75% yield (Scheme 59). The silyl migration product **157d** was also observed.



Scheme 59

From azidoalcohol **118d**, TBDPS protected lactol **156d** was synthesized in 46% overall yield. For comparision lactol **122b**, R = H, was synthesized from azidoalcohol **118b** in the same overall yield, 46%. Disappointed with the results of earlier Wittig olefination reactions, we looked forward to the Wittig olefination of lactol **156d** with great anticipation. Confident that success was at hand, modified Schlosser-Wittig conditions[118b] (2.2 equiv of phosphonium salt, 4.40 equiv of phenyllithium) were examined first (Table 12). These conditions destroyed the starting lactol and none of the desired product was observed. Amazed by the reactivity of this lactol, the previous conditions (Table 11) using sodium amylate and *n*-BuLi were examined. Thus the best conditions afforded a combined yield of *cis*-and *trans*-azidosphingosine **148d** and **149d** in 13% after Wittig olefination and *tert*-butyldiphenylsilyl ether cleavage. Photoisomerization of *cis*-azidosphingosine **148d** provided *trans*-azidosphingosine **149d** and was found to have ^1H NMR and $[\alpha]_D^{25} = -2.4°$ (c 0.27, $CHCl_3$) in agreement with the literature values.[93]

Table 12. Wittig Olefination Conditions Performed on Lactol **156d** (R = TBDPS)

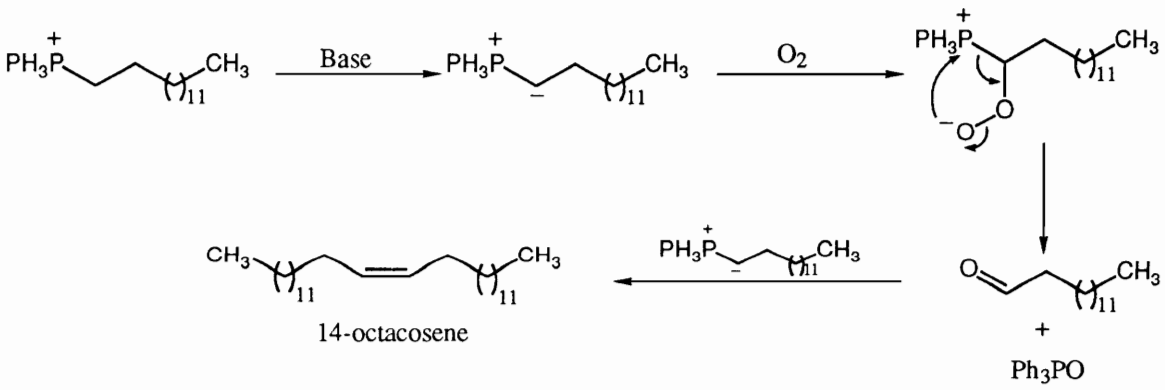


Equiv of salt	Equiv of base	Temperature	Solvent	**158d & 159d**	Dimer †	Silanol◊	*trans*-2-hexadecene	$P\emptyset_3$
2.20	4.40 PhenylLi	-50 to 0 °C	THF	0%	§	57%	not present	§
1.77	1.77 Na amylate	rt	THF	9.0%	§	43%	§	§
1.77	1.77 Na amylate	0 °C to rt	THF	3.7%‡	20.0%	47%	§	§
3.50	3.50 Na amylate	0 °C to rt	THF	13%‡	19.1%	≈19%	12%	11.5%
2.20	2.0 of *n*BuLi	rt	THF	0%	§	66%	not present	§
3.00	2.7 of *n*BuLi	rt	THF*	≈5%	≈5%	≈40%	not present	≈5%
3.50	3.2 of *n*BuLi	-78 °C to rt	Toluene	0%Δ	20.0%	§	not present	§
4.00	3.6 of *n*BuLi	rt	THF	0%ΔΔ	§	§	not present	§

† The dimer refers to 14-octacosene ($C_{28}H_{56}$). ◊ The silanol is *tert*-butyldiphenylsilanol. § Was not looking for or did not isolate. ‡ Percent yield determined by treating the crude product with F^- and isolating the corresponding azidosphingosine. * Deoxygenated the solvent. Δ 36% of the silylmigrated product **157d** and 35% of starting lactol isolated. ΔΔ TLC shows silylmigrated product **157d**, within minutes, after 15 hrs neither starting lactol or **157d** remains.

A common observation while performing these Wittig reactions was that of silyl migration. After five minutes of reaction time compound **157d** (Scheme 59) was always present in the reaction mixture. The work-up of these reactions was not a trival task. Simple addition of water caused silyl ether cleavage. In an effort to suppress this unwanted silyl cleavage subsequent reactions were chilled to -50 °C before quenching with saturated NH_4Cl thus suppressing the silyl ether cleavage.

Finally the formation of a dimer was noted, namely 14-octacosene ($C_{28}H_{56}$). Closer scrutiny of the reaction conditions revealed, that it was forming during the phosphonium ylide formation, *i.e.* before the lactol was added. The normal protocol for Wittig formation was as follows. To tetradecyltriphenylphosphonium bromide, under argon, freshly distilled solvent was added. After cooling this solution in an ice-bath, base was added. Regardless of the base used a color change ensued (clear to a dark orange or yellow). The phosphonium ylide was then stirred for 30 minutes at 0 °C, at which time the ice-bath was removed. Once at room temperature the reaction was stirred for an additional 30 minutes. The temperature of the ylide solution was then adjusted to the desired reaction temperature. It has been suggested that perhaps dissolved oxygen is the culprit of this dimer formation (Scheme 60). Deoxygenation of the solvent diminished the dimer formation (Table 12, entry 6). Further experimentation would be needed to draw more accurate conclusions.



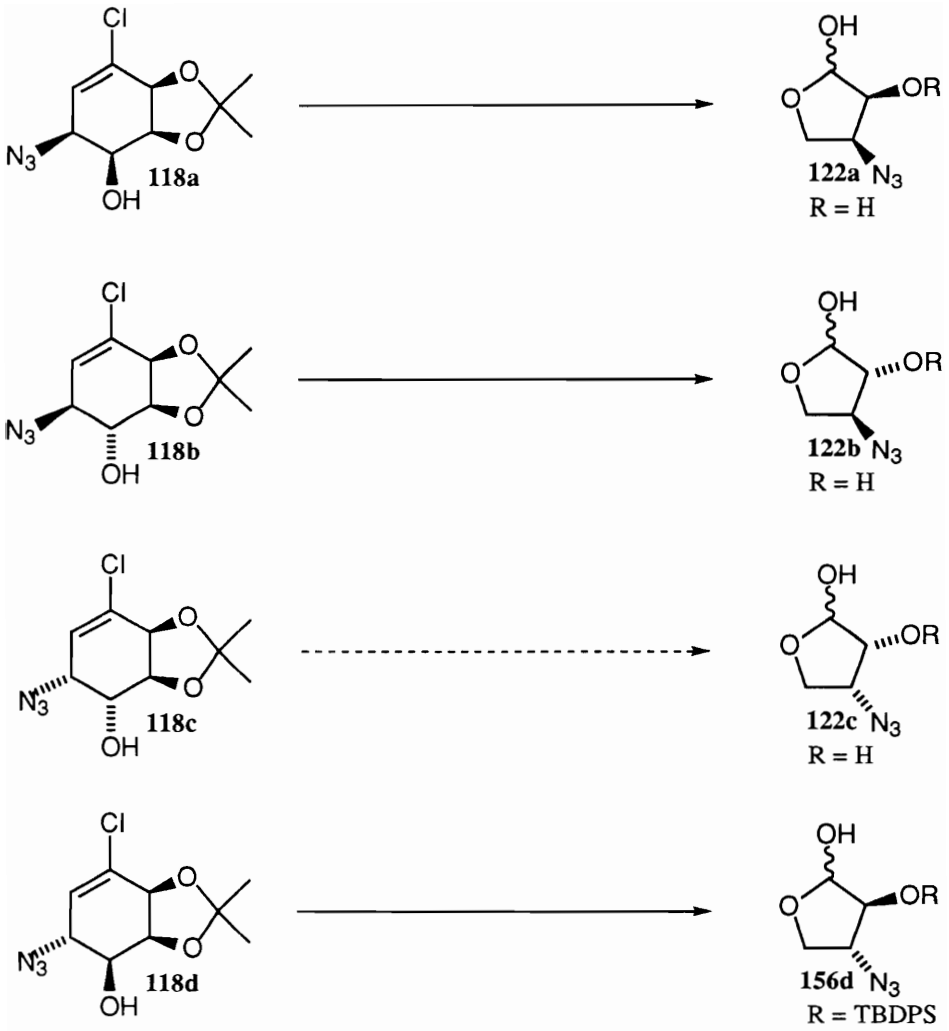
Scheme 60

IV. SUMMARY, CONCLUSION and OUTLOOK

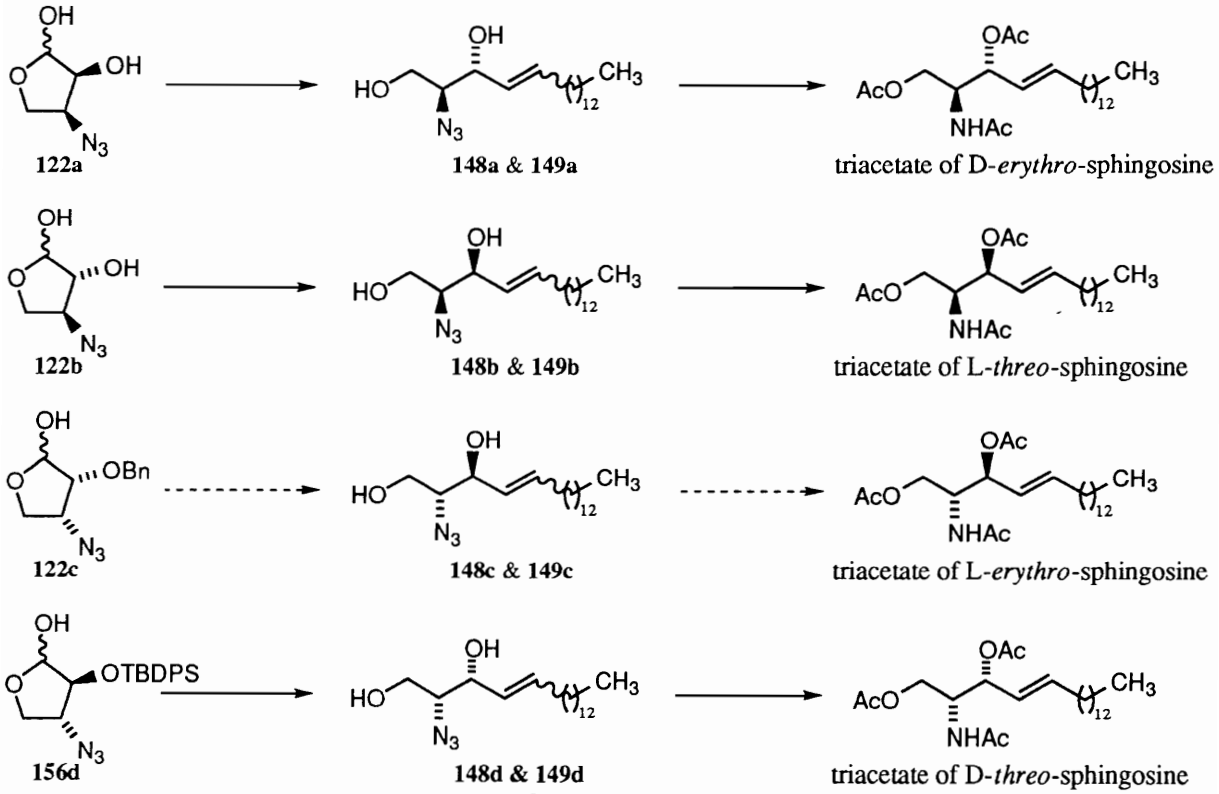
The utility of chlorodiol **113** as a general synthon for sphingosine synthesis has been unequivocally shown. The synthesis of three out of the four possible stereoisomers of sphingosine from one starting material is a feat in its own, and bodes well for the enantiodivergent accessibility of the fourth isomer from chlorodiol **113**.

At present the Achilles' tendon of this total synthesis is the Wittig olefination of lactols **122a**, **122b**, and **156d**. To date the olefination has been plagued by a myriad of set backs. Further work after this dissertation will try to address some of the following questions. Are lactols of type **122** and **156** inherently unstable to basic reaction conditions? Is the azide attacked by the phosphonium ylides? Can a stable protecting group be found and still be easily removed at the end of the synthesis? If an appropriate protecting group is found will it increase the Wittig olefination yields? These are few of the questions which need to be addressed.

The fourth isomer will be synthesized after the this dissertation is submitted. The strategy will entail protection of azidoalcohol **118c** as a benzyl ether. Using the same set of reactions used on the previous isomers, lactol **122c**, where R = benzyl, will be subjected to the Wittig olefination. If no improvement in yield is apparent, the azide (of lactol **122c**) will be reduced and protected and this new lactol will then be subjected to Wittig olefination. Depending on the outcome, most of the above questions will be answered. Below are Schemes 61 and 62 which summarize what has been accomplished during this investigation.


Cl
O
O
N3
OH
118a
OH
OR
O
122a N3
R = H
Cl
O
O
N3
OH
118b
OH
OR
O
122b N3
R = H
Cl
O
O
N3
OH
118c
OH
OR
O
122c N3
R = H
Cl
O
O
N3
OH
118d
OH
OR
O
156d N3
R = TBDPS

Scheme 61


OH
OH
N3
122a
HO
OH
CH3
12
N3
148a & 149a
AcO
OAc
NHAc
triacetate of D-erythro-sphingosine
122b
148b & 149b
triacetate of L-threo-sphingosine
OBn
122c
148c & 149c
triacetate of L-erythro-sphingosine
OTBDPS
156d
148d & 149d
triacetate of D-threo-sphingosine

Scheme 62

V. EXPERIMENTAL

General: All reactions were carried out in an argon atmosphere with standard techniques for the exclusion of air and moisture. Glassware used for moisture sensitive reactions were flame dried under vacuum. All solvents were reagent grade. Anhydrous solvents were dried immediately before use. THF and toluene were distilled from sodium benzophenone ketyl. A one-piece reflux apparatus was used when reflux conditions were needed (it was custom-made in a glass shop and consists of a jointless connection of a round bottom flask and a reflux condenser), it can be substituted for by using a condenser and a round bottom flask.

Dry oxygen containing about 2.5% ozone was introduced at a rate of 4L/min into the solution of a substrate.

TLC plates were visualized by immersion in a vanillim stain, followed by warming on a hot plate. Flash chromatography was carried out on Merck Kieselgel 60 silica gel (230 - 400 mesh). The impure products were impregnated on silica gel and then loaded onto the column.

^{1}H NMR and APT ^{13}C NMR were recorded at 270 MHz and 68 MHz. Proton and carbon chemical shifts are reported in parts per million (ppm) relative to $CDCl_3$ (^{1}H NMR, 7.24 and 13C NMR, 77.0 ppm - middle peak of the triplet). Elemental analyses were performed by Atlantic Microlabs, Inc.

(3S,4S,5S,6S)-3-azido-1-chloro-5,6-O-isopropylidene-1-cyclohexene-4,5,6-triol (118a). To a 50 mL Erlenmeyer flask was added bromohydrin **128** (6.039 g, 21.30 mmol), NaN_3 (22.00 g, 338.4 mmol, 15.9 eq) and then DMSO (35.0 mL). The mixture solidified immediately, so it was broken into much smaller pieces (CAUTION: A metal spatula can initiate an explosion with dry NaN_3 and possibly with these thick pastes of NaN_3). The ensuing thick paste was sonicated for 1.5 hrs and then more DMSO (15.0 mL) was added. The reaction vessel was sonicated for another 2.5 hrs, at which point no bromohydrin **128** was apparent by TLC. Distilled H_2O was added until all the NaN_3 dissolved and then the solution was extracted with EtOAc (X5). The combined organics were dried with $MgSO_4$, filtered, and volatiles removed. Column chromatography using gradient elution (hexanes/EtOAc, 8.5:1.5 and ending with 7:3) gave azidoalcohol **118a** (3.915 g, 15.94 mmol) as a clear oil in 74.8% yield and an epimeric azidoalcohol [(3R,4S,5S,6S)-3-azido-1-chloro-5,6-O-isopropylidene-1-cyclohexene-4,5,6-triol (**118d**)] (1.143 g, 4.652 mmol) as a brown oil (pure by ^{1}H NMR) in 21.8% yield. $\mathbf{R_f}$= .35 (hexanes/EtOAc, 2:1); $[\alpha]_D^{23}$= + 288.8 (c 1.06, $CHCl_3$); **IR** (neat) υ 3420, 3060, 2995, 2910, 2090, 1635 cm^{-1}; **^{1}H NMR** ($CDCl_3$) δ 6.00 (d, J = 5.0 Hz, 1H), 4.49 (m, 2H), 4.02 (m, 2H), 2.77 (br.s, 1H), 1.51 (s, 3H), 1.39 (s, 3H); **^{13}C NMR** ($CDCl_3$) δ 137.4 (C), 111.6 (C), 121.7 (CH), 75.9 (CH), 75.4 (CH), 67.0 (CH), 58.1 (CH), 27.4 (CH_3), 25.7 (CH_3); **MS** (CI, 70 eV) *m/z* (rel. intensity) 246 (M^++1, 3.0), 232 (19), 230 (53), 220 (42), 218 (92),

205 (23), 203 (60), 182 (39), 59 (100); **Anal. calcd for** $C_9H_{12}ClN_3O_3$: C, 44.00; H, 4.92; N, 17.1. **Found**: C, 43.91; H, 4.93; N, 16.93.

(3R,4S,5S,6S)-3-azido-1-chloro-5,6-O-isopropylidene-1-cyclohexene-4,5,6-triol (118d). Epoxide **125** (250 mg, 1.23 mmol), 1,2-dimethoxyethane (13.0 mL), ethanol (10.0 mL), H_2O (8.0 mL), NaN_3 (320 mg, 4.92 mmol, 4.00 equiv), and NH_4Cl (264 mg, 4.94 mmol, 4.00 mmol) were added to a rb flask in the order stated. The rb flask was equipped with a reflux condenser and the apparatus placed in an oil bath at 55 °C. After 60 min the reaction was allowed to cool to rt and then had H_2O (40.0 mL) added to it. This was extracted with EtOAc (X3), the organic extracts were combined, dried with $MgSO_4$, filtered, and volatile organics removed. Column chromatography of this crude product using gradient elution (Hex/EtOAc 3:1 → 2:1) afforded azido alcohol (**118d**) (262.1 mg, 1.07 mmol) in 87% yield as a clear oil . This clear oil turns to a dark brown oil within 5 hrs at rt, yet no decomposition is noticable by ^1H NMR. An analytical sample was obtained using column chromatography with an acetone/CH_2Cl_2 solvent system (1% acetone in CH_2Cl_2 → 2% acetone in CH_2Cl_2). $\mathbf{R_f}$= .45 (hexanes/EtOAc, 2:1); $[\alpha]_D^{22}$= -120.1 (c 1.00, $CHCl_3$); **IR** (neat) υ 3430, 2995, 2930, 2895, 2100, 1645, 1225, 1040 cm^{-1}; **^1H NMR** ($CDCl_3$) δ 5.81 (d, J = 2.0 Hz, 1H), 4.57 (m, 2H), 4.27 (td, J= 1.8, 8.6 Hz, 1H), 3.81 (dt, J= 2.1, 8.1 Hz, 1H), 2.54 (d, J= 7.5 Hz, -OH), 1.44 (s, 3H), 1.43 (s, 3H); **^{13}C NMR** ($CDCl_3$) δ 134.2 (C), 111.1 (C), 123.9 (CH), 76.7 (CH), 76.3 (CH), 72.0 (CH), 60.9 (CH), 27.3 (CH_3), 26.3 (CH_3); **MS** (CI, 70 eV) *m/z* (rel. intensity) 246 (M^++1, 3.0), 160 (3.0), 145 (20), 133 (3.0), 117 (3.0), 114 (4.0), 101 (15.0), 96 (15.0), 59 (100); **Anal. calcd for** $C_9H_{12}ClN_3O_3$: C, 44.00; H, 4.92; N, 17.11. **Found**: C, 44.34; H, 4.96; N, 16.96.

(1R, 2S, 3S, 6S)-6-Azido-4-chloro-cyclohex-4-ene-1,2,3-triol (119b). To a solution of azidoalcohol (**118b**) (513 mg, 2.088 mmol) in CH_3OH (25 mL) was added 1.6 grams of Amberlyst 15(wet) ion-exchange resin. After 8 days no more azidoalcohol remained by TLC. The solution was filtered through a pad of celite and the solvent was evaporated to give 453.9 mg of crude product. Purification by column chromatography (hexane/ethylacetate, 1:4) gave 11mg of starting material and 405.7 mg (1.973 mmol, 97%) of azidotriol as a white solid. For data collection a portion was recrystallized from ethylacetate. $\mathbf{R_f}$= 0.47 (Ethylacetate); **mp** 137-138 °C; $[\alpha]_D^{20}$= - 11.0 (c 1.1, CH_3OH); **IR** (KBr) υ 3290, 3190, 2900, 2840, 2060, 1640, 1430, 1240 cm^{-1}; **^1H NMR** (DMSO) δ 5.72 (1H, d, J 2.6), 5.51 (1H, d, J 6.1), 5.38 (1H, d, J 5.2), 4.98 (1H, d, J 6.1), 3.98 (1H, m , J 4.1, 6.1), 3.93 (1H, m , J 2.6, 7.8), 3.62 (1H, ddd, J 5.2, 7.8, 10.2), 3.45 (1H, ddd, J 4.1, 6.1, 10.2); **^{13}C NMR** (DMSO) δ 135.4 (C), 125.0 (CH), 71.4 (CH), 71.0 (CH), 70.0 (CH), 63.6 (CH); **MS** (CI 70 eV) *m/z* (rel. intensity) 237 (5), 212

(5), 182 (8), 180 (26), 178 (24), 160 (30), 145 (80), 116 (80), 114(100); **Anal. calcd for** $C_6H_8N_3O_3Cl$: C, 35.05; H, 3.92; **Found**: C, 35.19; H, 3.87.

1R, 2R, 3R - Cyclohexanetriol-4S-amine: Method #1: To a steel vessel was added azido alcohol (**118b**) (396mg, 1.612 mmol), CH_3OH (14mL), and PtO_2 (45mg, 0.1982 mmol). The vessel was charged with hydrogen and purged, charged again and maintained at 80 psi. After 6h no starting material remained by TLC, the solution was filtered through Celite and the solvent evaporated to give a light brown oil. To the oil was added aqueous HCl (1 mL, 2.42 N), followed by 4 mL of CH_3OH with stirring (5 min). The solution was evaporated to dryness and $CHCl_3$ (2 x 15mL) was added and subsequently evaporated. The crude product was then dissolved in CH_3OH (1-2 mL) and the product was precipitated from solution by adding hexane, then ether dropwise. The lightly pink precipicate was precipitated again to give the salt as a white precipitate (255.9 mg, 86%).
Method #2: To a one-piece reflux apparatus was added benzylamino alcohol (**120**) (249.0 mg, 0.804 mmol), followed by CH_3OH (4 mL), 10% Pd/C (180 mg) and ammonium formate (250.0 mg, 3.965 mmol). The reaction mixture was brought to reflux and after 10 min. no starting material remained by TLC. The solution was filtered through Celite and evaporated to give a brown oil. The crude oil was worked-up as described in method #1 using 1 mL of 1.21 N HCl, a white precipitate ensued (105.8 mg, 72%). $\mathbf{R_f}$= 0.0 (ethyl acetate); **mp** > 200 °C; $[\alpha]_D^{24}$= -26.24 (c 1.0, H_2O); **IR** (KBr) υ 3410, 3320, 3050, 2940, 1660, 1595, 1515, 1075 cm^{-1}; **^{1}H NMR** (DMSO) δ 7.96 (3H, bs), 5.36 (1H, bs), 4.84 (1H, bs), 4.60 (1H, bs), 3.77 (1H, s), 3.52 (1H, t, J 9.8), 3.16 (1H, d, J 8.4), 2.74 (1H, bs), 1.67 (3H, m), 1.41 (1H, m); **^{13}C NMR** (DMSO) δ 74.5 (CH), 70.5 (CH), 68.0 (CH), 53.5 (CH), 27.4 (CH_2), 22.4 (CH_2); **MS** (CI 70 eV) *m/z* (rel. intensity) 148 (100), 112 (30), 85 (11), 84(9); **Anal. calcd for** $C_6H_{14}ClNO_3$: C, 39.24; H, 7.69; **Found**: C 39.24; H 7.70

General procedure for the formation of lactols 122a and 122b. A 0.1 M soln of lactol **138** in distilled H_2O had amberlyst 15(wet) ion-exchange resin (4.0 weight eq. based on **138**) added. The temp was raised to 65 °C and after 5h the rxn was finished by TLC. The solution containing crude product **140b** was passed through a fritted glass filter and the pH adjusted to 7.0 (+ or - 0.5) with sat. $NaCO_3H$. This solution was diluted with H_2O until the molarity was lowered to 0.05 M. $NaIO_4$ (1.0 eq., based on **11**) was added and the rxn flask was totally enclosed with aluminum foil, to exclude all light. Rxn time varies for epimers, **122a** needs 0.5

hrs, **122b** needs > 2 hrs. The reaction soln was saturated with NaCl and the product was extracted with EtOAc/isopropanol (1:1) until no more product could be detected in the aqueous layer by TLC. The combined organic extracts had NaCl added to them, were then decanted, dried with $MgSO_4$, filtered and solvent removed. The crude product was chromatographed using hexanes/EtOAc 1:4, producing **122** as a clear oil.

(2R)-hydroxy-(3S)-azido-γ-butyrolactol (122b). This compound was obtained in 64% yield from **138b** following the general procedure. R_f= 0.35 (CH_2Cl_2/acetone, 3:1); $[\alpha]_D^{23}$= + 39.58 (c 1.2, acetone); **IR** (film) υ 3400, 2105, 1660, 1640 cm^{-1}; **^{1}H NMR** (DMSO) δ 6.38 (m, 2H), 5.60 (d, J = 4.39 Hz, 1H), 5.30 (d, J = 6.83 Hz, 1H) 5.06 (t, J = 5.42, 4.52 Hz, 1H), 5.00 (dd, J = 4.6, 1.3 Hz, 1H), 4.02 (m, 3H), 3.80 (m, 2H), 3.70 (m, 1H), 3.40 (m, 2H); **^{13}C NMR** (DMSO) δ 102.5 (CH), 95.7 (CH'), 80.4 (CH), 75.8 (CH'), 65.3 (CH), 64.7 (CH'), 68.3 (CH_2), 66.7 (CH_2'); **MS** (CI 70 eV) *m/z* (rel. intensity) 128 (15), 118 (8), 103 (6), 88 (93), 85 (54), 73 (30), 60 (100); **Anal. calcd for** $C_4H_7N_3O_3$: C, 33.11; H, 4.86; **Found**: C, 33.37; H, 4.98.

(2S)-hydroxy-(3S)-azido-γ-butyrolactol (122a). This compound was obtained in 68% yield from **138a** following the general procedure. R_f= 0.29 (CH_2Cl_2/acetone, 3:1); $[\alpha]_D^{25}$= + 5.47 (c 1.17, acetone); **IR** (film) υ 3400, 2950, 2900, 2500, 2105, 1725, 1650 cm^{-1}; **^{1}H NMR** (DMSO) δ 6.39 (d, J = 5.2 Hz, -OH), 5.70 (d, J = 5.1 Hz, -OH), 5.00 (dd, J = 5.2, 2.3 Hz, 1H), 3.95 (m, 3H), 3.63 (dd, J = 9.0, 4.0 Hz, 1H); **^{13}C NMR** (DMSO) δ 101.8 (CH), 77.2 (CH), 67.5 (CH_2), 61.1 (CH); **MS** (CI 70 eV) *m/z* (rel. intensity) 128 (45), 118 (8), 103 (12), 100 (13), 85 (68), 72 (46), 60 (100); **Anal. calcd for** $C_4H_7N_3O_3$: C, 33.11; H, 4.86.

(3S,4R,5R,6S)-4-bromo-1-chloro-5,6-O-isopropylidene-1-cyclohexene-3,5,6-triol (124). To chlorocyclohexadienediol **113** (5.00g, 34.11 mmol) in acetone (20 mL, HPLC grade) was added 2,2-dimethoxypropane (30 mL) and cat. p-toluenesulfonic acid monohydrate (175 mg). After 20 min. at RT, no more diol **113** remained by TLC. Separatory funnel washing with sat. $NaHCO_3$ was followed by CH_2Cl_2 extraction of the aqueous layer. The combined organic extracts were dried with $MgSO_4$, and solvent removed. The crude acetonide product was placed under high vacuum for 15 min. and then had 1,2-dimethoxyethane (140 mL) and distilled

H_2O (35 mL) added to it. This soln. was cooled in an ice/NaCl-bath and then NBS (1.6 eq., based on **113**), 54.58 mmoles, 9.715 g) was added. The reaction was then maintained at 0 °C for 10 hrs, at which point the rxn was finished by TLC (note this rxn has not been optimized). To the soln. was added brine (100 mL) and EtOAc. The EtOAc extracts were combined and dried wtih $MgSO_4$. Removal of volatiles gave 4.20 g of crude product, this residue was disolved in hot hexanes and decanted to another flask. Upon cooling a white precipitates forms [this is an undesired diepimeric bromohydrin (270 mg, 0.952 mmoles, 2.8% yield from diol **113**) which is difficult to remove by chromatography, $\mathbf{R_f}$= 0.30 (hexanes/EtOAc, 4:1)], the soln. is once again decanted hexanes removed to give an oilly residue. Column chromatogaphy of the residue using acetone/CH_2Cl_2 (1:50) gives the desired bromohydrin **124** (2.968 g, 10.47 mmoles, 30.7 % from diol **7**) as a clear oil. $\mathbf{R_f}$= .35 (hexanes/EtOAc, 4:1); **mp**= 43-47 °C; $[\alpha]_D^{23}$= + 17.24 (c 1.16, $CHCl_3$); **IR** (neat) υ 3485, 3000, 2940, 1650, 1380, 1220, 1060 cm^{-1}; **^{1}H NMR** ($CDCl_3$) δ 6.10 (d, J = 4.4 Hz, 1H), 4.61 (dm, J = 2.27 Hz, 2H), 4.35 (dddm, J = 9.1, 4.4, 4.2 Hz, 1H), 4.27 (m, 1H), 2.88 (d, J = 9.1 Hz, 1H), 1.51 (s, 3H), 1.41 (s, 3H); **^{13}C NMR** ($CDCl_3$) δ 133.1 (C), 112.2 (C), 126.9 (CH), 78.1 (CH), 75.3 (CH), 70.2 (CH), 48.6 (CH), 27.9 (CH_3), 26.4 (CH_3); **MS** (CI, 70 eV) *m/z* (rel. intensity) 285 (0.25), 283 (M^+, 0.40), 281 (0.27), 269 (40.0), 267 (38), 211 (20), 209 (65), 207 (50), 181 (23), 145 (23), 128 (21), 59 (100); **Anal. calcd for** $C_9H_{12}BrClO_3$: C, 38.12; H, 4.266. **Found**: C, 38.00; H, 4.26.

(1S,4S,5S,6S)-3-chloro-4,5-O-isopropylidene-7-oxa-bicyclo[4.1.0]hept-2-ene-4,5-diol (125). To an argon flooded one-piece reflux condenser was added bromohydrin **124** (1.650 g, 5.829 mmol) and CH_2Cl_2 (25 mL), followed by n-tetrabutylammoniumhydrogensulfate (195.2 mg, 0.575 mmol) and NaOH (300.0 mg, 5.749 mmol, note NaOH pellets were crushed in a crucible, weighed and immediately added). This refluxed for 1h and then stirred at rt for another 12h at which point the reaction was finished by TLC. The reaction was quenched with saturated NH_4Cl and the aqueous layer was extracted with CH_2Cl_2 (X2). The combined CH_2Cl_2 extracts were dried with $MgSO_4$, filtered, and solvent removed. Column chromatography using gradient elusion (hexanes/EtAOc, 9:1 and ending with 7:3) gave epoxide **125** (805.7 mg, 3.98 mmol) as a white solid in 68.3% yield. Recrystallization from hexanes gave an analytical sample. $\mathbf{R_f}$= .46 (hexanes/EtOAc, 2:1); **mp**= 89-90 °C; $[\alpha]_D^{23}$= - 111.9 (c 1.05, $CHCl_3$); **IR** (neat) υ 3070, 2990, 2910, 2875, 1650, 1375, 1255, 1210, 1060, 1040 cm^{-1}; **^{1}H NMR** ($CDCl_3$) δ 6.40 (d, J = 4.3 Hz, 1H), 4.59 (dd, J = 6.7, 1.8 Hz, 1H), 4.52 (dd, J = 6.8, 2.6 Hz, 1H), 3.61 (ddd, 4.2, 2.6, 1.7 Hz, 1H), 3.48 (t, J = 4.2 Hz, 1H), 1.52 (s, 3H), 1.40 (s, 3H); **^{13}C NMR** ($CDCl_3$) δ 135.7 (C), 109.1 (C), 125.6 (CH), 75.5 (CH), 74.0 (CH), 54.5 (CH), 49.6 (CH), 27.1 (CH_3), 25.3 (CH_3); **MS** (CI, 70 eV) *m/z* (rel. intensity) 203 (M^++1, 1.0), 187 (80.0), 145 (60), 117

(70), 109 (75), 59 (100); **Anal. calcd for** $C_9H_{11}ClO_3$: C, 53.34; H, 5.47. **Found**: C, 53.44; H, 5.55.

(3R,4R,5S,6S)-3-bromo-1-chloro-5,6-O-isopropylidene-1-cyclohexene-4,5,6-triol (128). To an argon flooded one-piece reflux condenser was added syn-epoxyacetonide **125** (5.000 g, 24.67 mmol) and distilled THF (80.0 mL), followed by lithium bromide (2.570 g, 29.60 mmol, 1.2 eq) and ethylacetoacetate (6.24 mL, 49.34 mmol, 2.0 eq). After 4 hrs at 30 °C, no epoxide remained by TLC (do not use hexane/EtOAc mixtures to monitor rxn, epoxide and bromohydrin cospot, use acetone/CH_2Cl_2). The rxn soln was washed with saturated NH_4Cl (X3), then dried with $MgSO_4$, filtered, and volatiles removed, the viscous residue was then left under high vacuum at rt for 48 hrs (to remove most of the ethylacetoacetate). Column chromatography using gradient elution (acetone/CH_2Cl_2, 1:99 and ending with 3:97 or hexanes/ EtOAc, 9:1 and ending with 7:3) gave bromohydrin **10b** (6.535 g, 23.22 mmol) as a white solid in 94.1% yield. Recrystallization from hexanes gave an analytical sample. $\mathbf{R_f}$= 0.40 (hexanes/EtOAc, 2:1); **mp**= 66-67 °C; $[\alpha]_D^{23}$= - 89.3 (c 1.00, $CHCl_3$); **IR** (KBr) υ 3420, 3070, 2995, 2935, 2905, 1630, 1370, 1220 cm^{-1}; **^{1}H NMR** ($CDCl_3$) δ 6.06 (d, J = 2.7 Hz, 1H), 4.75 (ddd, J = 7.7, 2.6, 1.8 Hz, 1H), 4.64 (dd, J = 5.4, 1.7 Hz, 1H), 4.60 (dd, J = 5.4, 2.4 Hz, 1H), 4.06 (dd, J = 7.7, 2.4 Hz, 1H), 1.43 (s, 3H), 1.41 (s, 3H); **^{13}C NMR** ($CDCl_3$) δ 133.7 (C), 111.2 (C), 126.4 (CH), 76.1 (CH), 75.8 (CH), 72.7 (CH), 48.8 (CH), 27.2 (CH_3), 26.1 (CH_3); **MS** (CI, 70 eV) *m/z* (rel. intensity) 287 (7.5), 285 (22), 283 (M^+,19), 269 (14), 267 (11), 209 (16), 203 (25), 147 (32.4), 145 (90), 59 (100); **Anal. calcd for** $C_9H_{12}BrClO_3$: C, 38.12; H, 4.27. **Found**: C, 38.29; H, 4.23.

General procedure for the formation of lactols 138a and 138b. A 0.4 M soln of azido alcohol **118** in methanol was cooled in a dry-ice/acetone bath and an excess of O_3/O_2 was bubbled through the solution until a persistent blue color was apparent, indicating the presense of O_3 and the fact that it is no longer being consumed. Nitrogen was then bubbled through the solution for half an hour at -78 °C. The reaction temperature was then raised to -55 °C and 3.0 eq. of $NaBH_4$ were added slowly so that the rxn temperature never rose above -30 °C (note the reduction is routinely done with the round bottom open to the atmosphere). This solution is allowed to come to rt over a 6h period slowly. If the reduction is not complete by TLC analysis, more $NaBH_4$ should be added at rt very slowly; use 0.3 eq. of $NaBH_4$, stir for 0.5 hrs, check by TLC. If not complete repeat this routine until the reduction is complete, note over reduction can occur. The reaction was acidified using aqueous HCl (1.0 M) until pH = 4.5 (+ or - 0.5). Extract with EtOAc (X4), wash combined organics with brine, dry with $MgSO_4$, remove volatiles. Column chromatography using gradient hexanes/EtOAc (6.5:3.5 ending with 1:1) gives a clear oil in 70 - 80% yield.

(2S,3S,4R)-2,3-O-Isopropylidene-4-((1'S)-1'-azido-2'-hydroxyethyl)-γ-butyrolactol (138b). Note the equilibrium between the two possible anomeric lactols is evident in the ^{1}H NMR and ^{13}C NMR, but one anomeric form dominates in an approximate ratio of 9 to 1 (conc. of 10 - 20 mg in 0.5 mL of $CDCl_3$). **R_f**= .29 (hexanes/EtOAc, 1:1); $[\alpha]_D^{21}$= + 31.03 (c 0.97, $CHCl_3$); **IR** (neat) υ 3400, 2995, 2940, 2115, 2095, 1755, 1725 cm^{-1}; **^{1}H NMR** ($CDCl_3$) δ 5.40 (s, 1H), 4.73 (dd, J = 5.9, 3.6 Hz, 1H), 4.61 (d, J = 5.9 Hz, 1H), 4.24 (dd, J = 8.8, 3.4 Hz, 1H), 4.11 (br.d, J = 2.0 Hz, 1H), 3.82 (m, 2H), 3.71 (m, 1H), 2.92 (br.t, J = 5.5 Hz, 1H), 1.44 (s, 3H), 1.29 (s, 3H); **^{13}C NMR** ($CDCl_3$) δ 112.9 (C), 100.9 (CH), 85.9 (CH), 79.9 (CH), 79.5 (CH), 63.5 (CH), 62.1 (CH_2), 25.9 (CH_3), 24.8 (CH_3); **MS** (CI, 70 eV) *m/z* (rel. intensity) 246 (M^++1, 0.5), 230 (12), 218 (2), 202 (2.5), 159 (20), 73 (25), 59 (100); **HRMS calcd for** $C_xH_yO_z$:....; **Found**: .....

(2S,3S,4S)-2,3-O-Isopropylidene-4-((1'S)-1'-azido-2'-hydroxyethyl)-γ-butyrolactol. (138a). Note the equilibrium between the two possible anomeric lactols is evident in the ^{1}H NMR and ^{13}C NMR. Also the number of protons listed for the splitting patterns of the ^{1}H NMR will not always be integers because the pair of anomeric lactols are present in an approximate ratio of 2 to 1 (when approximately 20 mg of compound are in 0.5 mL of $CDCl_3$). If the conc. is decreased (approximately 3 mg of compound in 0.5 mL of $CDCl_3$) the anomeric ratio changes to approximately 3 to 1. **R_f**= .26 (hexanes/EtOAc, 1:1); $[\alpha]_D^{23}$= + 11.7 (c 0.94, $CHCl_3$); **IR** (neat) υ 3430, 2995, 2950, 2100, cm^{-1}; **^{1}H NMR** ($CDCl_3$) δ 5.44 (s, 1H), 5.38 (d, J = 3.83 Hz, 0.5H), 4.83 (dd, J = 6.0, 1.1 Hz, 1H), 4.72 (dd, J = 6.8, 2.8 Hz, 0.5H), 4.67 (dd, J = 6.9, 3.9 Hz, 0.5H), 4.61 (d, J = 5.92 Hz, 1H), 4.1 (dd, J = 5.5, 2.8 Hz, 0.5H), 4.06 (dd, J = 9.36, 1.22 Hz, 1H), 3.97 (dd, J =11.8, 4.0 Hz, 1H), 3.81 (m, 2.5 H), 3.62 (m, 1.5H), 1.55 (s, 1.5H), 1.46 (s, 3H), 1.38 (s, 1.5H), 1.31 (s, 3H); **^{13}C NMR** ($CDCl_3$) δ 115.2 (C), 112.9 (C), 103.2 (CH), 96.5 (CH), 86.0 (CH), 85.7 (CH), 82.3 (CH), 80.9 (CH), 80.7 (CH), 79.5 (CH), 64.6 (CH), 64.2 (CH), 62.9 (CH_2), 62.4 (CH_2) 26.5 (CH_3), 26.2 (CH_3), 24.9 (CH_3); **MS** (CI, 70 eV) *m/z* (rel. intensity) 246 (M^++1, 4), 218 (23), 202 (25), 200 (25), 188 (41), 159 (64), 142 (61), 69 (69), 59 (100).

General procedure for the formation of 148a,b and 149a,b. To a flame dried round bottom under Ar was added n-tetradecyltriphenylphosphonium bromide (3.20 eq.). After 5 min. under vacuum the RB was flooded with Ar. THF was added until a 1.0 M soln was obtained. This soln was cooled with an ice bath and *n*-BuLi (2.75 eq., 2.0 M in hexanes) was added giving a brownish/yellow color. After stirring for 5 min the ice-bath was removed and the soln was allowed to come to rt. After stirring at rt for 15 min the lactol (1.0 eq.) in THF (0.7 M) was added. The round bottom originally containing the lactol was rinsed with additional THF and this was immediately transferred to the reaction flask. The rxn continued to stir at rt and was monitored by

TLC. TLC's at 1, 2, 3, and 5 hrs revealed no apparent change in product or starting material (a small amount remains) intensity, thus after 5 hrs the rxn was quenched with saturated NH_4Cl soln. Extraction with EtOAc (X3), combination of the organic extracts, drying with $MgSO_4$, filtration, and removal of the volatile solvents gave a viscous residue. Gradient elution using Hexane/EtOAc (beginning with 15% EtOAc and ending with 30% EtOAc) gave cis and trans azidosphingosines in approximately a 4 to 1 ratio respectively, by ^1H NMR.

(2S,3S,4E)-2-azido-4-octadecen-1,3-diol (149b). A 6.1% yield of the trans product was observed. **R_f**= 0.45 (hexane/EtOAc, 2:1); $[\alpha]_D^{24}$= + 3.11 (c 0.53, $CHCl_3$); **IR** (neat) υ 3360, 2920, 2855, 2095, 1520 cm^{-1}; **^1H NMR** ($CDCl_3$) δ 5.78 (dtd, J = 15.4, 6.7, 0.7 Hz, 1H), 5.50 (ddt, J = 15.4, 7.1, 1.4 Hz, 1H), 4.19 (t, J = 6.5 Hz, 1H), 3.80 (dd, J = 11.5, 4.3 Hz, 1H), 3.68 (dd, J = 11.5, 6.3 Hz, 1H), 3.45 (dt, J = 6.0, 4.3 Hz, 1H), 2.04 (q, J = 6.7 Hz, 2H), 1.63 (br.s, >2H), 1.22 (m, >22H), 0.86 (t, J = 6.6 Hz, 3H); **^{13}C NMR** ($CDCl_3$) δ 135.5 (CH), 128.4 (CH), 73.6 (CH), 67.8 (CH), 63.0 (CH_2), 32.3 (CH_2), 31.9 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.0 (CH_2), 22.7 (CH_2), 14.0 (CH_3); **MS** ; **Anal. calcd for** $C_{18}H_{35}N_3O_2$: C, 66.42; H, 10.84; N, 12.91; **Found**: C, 66.87; H, 10.70; N, 12.41.

(2S,3S,4Z)-2-azido-4-octadecen-1,3-diol (148b). The cis isomer was produced in 24.4 % yield. **R_f**= 0.52 (hexane/EtOAc, 2:1); **^1H NMR** ($CDCl_3$) δ 5.64 (dt, J = 11.1, 7.5 Hz, 1H), 5.44 (tt, J = 9.9, 1.5 Hz, 1H), 4.53 (m, 1H), 3.79 (m, 1H), 3.65 (m, 1H), 3.44 (dt, J = 6.3, 4.1 Hz, 1H), 2.06 (m, 4H), 1.23 (m, >22H), 0.86 (t, J = 6.6 Hz, 3H); **^{13}C NMR** ($CDCl_3$) δ 135.7 (CH), 127.7 (CH), 68.4 (CH), 68.1 (CH), 62.8 (CH_2), 31.9 (CH_2), 29.64 (CH_2), 29.56 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 28.0 (CH_2), 22.7 (CH_2), 14.0 (CH_3);

(2S,3R,4E)-2-azido-4-octadecen-1,3-diol (149a). A 3.8 % yield of the *trans* product was observed. **R_f**= .38 (hexane/EtOAc, 2:1); $[\alpha]_D^{24}$ = - 34.1 (c 1.58, $CHCl_3$); **^1H NMR** ($CDCl_3$) δ 5.80 (dtd, J = 15.4, 6.7, 0.7 Hz, 1H), 5.51 (ddt, J = 15.4, 7.3, 1.3 Hz, 1H), 4.23 (t, J = 6.4 Hz, 1H), 3.77 (m, 2H), 3.45 (q, J = 5.3 Hz, 1H), 2.05 (q, J = 7.0 Hz, 2H), 1.94 (br.s, 2H), 1.23 (m, >22H), 0.86 (t, J = 6.8 Hz, 3H); **^{13}C NMR** ($CDCl_3$) δ

(2S,3R,4Z)-2-Azido-4-octadecen-1,3-diol (148a). A 14.0% yield of the *cis* isomer was observed. **R_f**= .43 (hexanes/EtOAc, 2:1); **^1H NMR** ($CDCl_3$) δ 5.67 (dtd, J = 11.0, 7.5, 0.8 Hz, 1H), 5.45 (tt, J = 10.9, 1.5 Hz, 1H), 4.58 (ddd, J = 8.8, 5.8, 0.9 Hz, 1H), 3.77 (m, 2H), 3.49 (q, J = 5.4 Hz, 1H), 2.19 (br.s, 2H), 2.08 (m, 2H), 1.23 (m, >22H), 0.85 (t, J = 0.85, 3H)

(3R,4S,5S,6S)-3-Azido-4-*t*butyldiphenylsilyl-1-chloro-5,6-O-isopropylidenecyclohex-1-ene-4,5,6-triol (150d). To a one-piece reflux apparatus was added azido alcohol **118d** (2.302 g, 9.37 mmol) as a solution in THF (10.0 mL), an additional 4.0 mL of THF was used to rinse the solution rb and this was placed in the reflux apparatus. *t*Butyldiphenylsilylchloride (5.074 g, 18.46 mmol, 1.97 equiv) and imidazole (1.524 g, 22.37

mmol, 2.387 equiv.) were then added. The apparatus was then placed in a 75 °C oil bath with stirring. After 4 h of heating the reaction was allowed to cool to rt, and then stirred for another 12 h. Note: as the reaction proceeds a white precipitate accumulates. Work-up: The solution was filtered through celite and the reaction rb and filter are rinsed with methylene chloride, the filtrate is washed with sat. ammonium chloride, and then with brine. The combined organic layers were then dried with $MgSO_4$, filtered, and evaporated to give 7.214 g of a viscous brown oil. Purification by flash chromatography using gradient elution (100% hexanes → 3% ethylacetate in hexanes) provided 3.846 g (7.945 mmol, 85%) of the silyl ether as a very viscous clear oil. **R_f**= 0.45 (hexanes/EtOAc, 9:1); **$[\alpha]_D^{24}$**= -62.5 (c 0.95, $CHCl_3$); **IR** (neat) υ 3075, 3050, 2985, 2930, 2895, 2860, 2105, 1430, 1380, 1225 cm^{-1}; **^{1}H NMR** ($CDCl_3$) δ 7.78 (m, 4H), 7.40 (m, 6H), 5.73 (m, J = 1.7 Hz, 1H), 4.37 (dm, J= 8.7 Hz, 1H), 4.10 (m, 2H), 3.68 (dm, J = 8.8 Hz, 1H), 1.42 (s, 3H), 1.27 (s, 3H), 1.09 (s, 9H); **^{13}C NMR** (CCl_3D) δ 133.9 (C), 133.8 (C), 132.2 (C), 110.6 (C), 19.4 (C), 136.0 (CH), 135.9 (CH), 130.2 (CH), 129.9 (CH), 128.0 (CH), 127.6 (CH), 76.6 (CH), 76.4 (CH), 72.8 (CH), 61.7 (CH), 27.5 (CH_3), 26.8 (CH_3), 26.2 (CH_3); **MS** CI *m/z* (rel. intensity) 484 (M^+, 0.52), 426 (17), 400 (15), 399 (11), 398 (38), 386 (10), 385 (36), 384 (27), 383 (100), 378 (34), 368 (29), 348 (21), 340 (24), 320 (27), 305 (37); **Anal. calcd for** $C_{25}H_{30}ClN_3O_3Si$: C, 62.03; H, 6.25; N, 8.68. **Found**: C, 62.41; H, 6.52, 8.35.

(2S,3R,4S,5R)-5-Azido-4-*t*butyldiphenylsilyloxy-1,2-O-isopropylidenehexanoicmethylester-2,3,4-triol (151d). To a rb was added vinyl chloride (**150d**) (3.740 g, 7.73 mmol) followed by MeOH (51 mL). This solution was heated gently to completely dissolve the vinyl chloride. The solution was then cooled in a dryice/acetone bath and ozone was bubbled through the solution. After 20 min the solution was saturated with O_3, as indicated by the characteristic blue color. TLC indicated no starting material remained. The generation of ozone was terminated and the reaction rb had Ar bubbled through it for 20 min at -78 °C. The reaction flask was then placed in an ice-bath. With the rb open to the atmosphere several portions of $NaBH_4$ (Note: use lumps of $NaBH_4$ *vs* powder when possible. If powder is used, add slowly *i.e.* over ≅ 3 - 5 min) were added. 580 mg (15.33 mmol, 1.98 equiv) of $NaBH_4$ were added first, ≅ 20 - 30 min transpired and then more $NaBH_4$ (220 mg) was added. 25 min passed and another portion of $NaBH_4$ (240 mg) was added, an additional 25 min transpired. At this point the ice-bath was removed, and an another 20 min passed and then two more portions of $NaBH_4$ (340 mg, wait 20 min, then 107 mg) were added. Note: it is <u>crucial</u> to monitor this reaction by TLC after each addition of $NaBH_4$; depending on the reaction more or less $NaBH_4$ may be needed. Work-up: Add distilled H_2O (150 mL) and then acidify with 1.2 N HCl acid, to a pH of 3.5 ± 0.5. Immediately extract the aqueous solution with EtOAc (X4), wash the combined EtOAc

extracts with brine (X2), dry with $MgSO_4$, filter, and evaporate the EtOAc. This procedure provided an oil which was purified by flash chromatography, using gradient elution (15% EtOAc in hexanes → 40% EtOAc in hexanes), and provided 3.592 g (6.99 mmol, 90%) of the methyl ester **151d** as a viscous clear oil. $\mathbf{R_f}$= 0.42 (hexanes/EtOAc, 2:1); $[\alpha]_D^{23}$= +29.6° (c 1.56, $CHCl_3$); **IR** (neat) υ 3500, 3070, 3050, 2980, 2950, 2930, 2890, 2850, 2100, 1755, 1590 cm^{-1}; **^{1}H NMR** ($CDCl_3$) δ 7.72 (m, 4H), 7.41 (m, 6H), 4.40 (ddm, J= 3.9, 6.9 Hz, 1H), 4.22 (m, 2H), 3.52 (m, 6H), 1.57 (s, 3H), 1.31 (s, 3H), 1.05 (s, 9H); **^{13}C NMR** (CCl_3D) δ 170.3 (C), 133.3 (C), 133.0 (C), 110.6 (C), 19.6 (C), 136.0 (CH), 135.9 (CH), 130.0 (CH), 127.8 (CH), 127.7 (CH), 80.1 (CH), 75.4 (CH), 70.4 (CH), 64.6 (CH), 51.8 (CH), 62.3 (CH_2), 27.0 (CH_3), 26.4 (CH_3), 25.1 (CH_3); **MS** CI *m/z* (rel. intensity) 514 (M^++1, 2), 487 (33), 486 (100), 429 (17), 428 (73.6), 408 (60), 379 (12), 378 (73), 358 (31), 350 (25), 300 (22), 291 (45), 240 (25), 220 (49), 199 (21), 144 (21), 135 (21), 59 (25); **Anal. calcd for** $C_{26}H_{35}N_3O_6Si$: C, 60.80; H, 6.87; N, 8.18. **Found**: C, 61.16; H, 7.15; N 7.87.

(2S,3R,4S,5R)-5-Azido-4-*t*butyldiphenylsilyloxy-hexanoicmethylester-2,3,4-triol (152d). To a rb was added acetonide (**151d**) (1.730 g, 3.367 mmol) followed by 30 mL of 1% I_2 in MeOH (*i.e.* 1.0 g of I_2 per 100 mL of MeOH). This solution was placed in an oil bath at 45 °C with stirring. After 46 h no more acetonide remained by TLC and the reaction was quenched with 80 mL of saturated sodium thiosulfate ($Na_2S_2O_3$). This solution was extracted with EtOAc (X3), the organic extracts were combined, dried with $MgSO_4$, filtered, and the volatile organics removed under vacuum. This procedure provided the crude product as a viscous oil which was purified using gradient elution (25% EtOAc in Hexanes → 65% EtOAc in hexanes) chromatography. (Note: Four inches of silica gel are sufficient and necessary. This product is prone to silyl migration and lactonization on prolonged exposure to silica gel.) This provided 1.278 g (2.699 mmol, 80% yield) of the desired vicinal diol (**152d**). Note: If the reaction is performed at higher temperatures two knew products (lactones **154d** and **155d**) appear. The physical data for the lactones follows this experimental. $\mathbf{R_f}$= 0.19 (hexanes/EtOAc, 1:1); $[\alpha]_D^{23}$= +13.4° (c 1.00, $CHCl_3$); **mp**= 78 - 82 °C; **IR** (KBr) υ 3470, 3430, 3350, 3075, 3050, 3020, 3000, 2950, 2930, 2890, 2855, 2095, 1755, 1590, 1425, 1100, 1080, 810 cm^{-1}; **^{1}H NMR** (DMSO-d_6) δ 7.69 (m, 4H), 7.42 (m, 6H), 5.62 (d, J= 5.9 Hz, -OH), 5.31 (d, J= 5.8 Hz, -OH), 4.91 (t, J= 5.2 Hz, -OH), 4.27 (t, J= 6.1 Hz, 1H), 3.83 (t, J= 4.3 Hz, 1H), 3.76 (q, J= 5.4 Hz, 1H), 3.66 (q, J= 5.7 Hz, 1H), 3.52 (s, 3H), 3.37 (t, J= 5.6 Hz, 2H), 0.98 (s, 9H); **^{13}C NMR** (CCl_3D) δ 172.9 (C), 132.8 (C), 19.5 (C), 136.0 (CH), 130.2 (CH), 127.9 (CH), 75.4 (CH), 71.8 (CH), 71.6 (CH), 64.5 (CH), 52.5 (CH), 61.4 (CH_2), 27.0 (CH_3); **MS** CI *m/z* (rel. intensity) 514 (M^++1, 2); **Anal. calcd for** $C_{23}H_{31}N_3O_6Si$: C, 58.33; H, 6.59; N, 8.87. **Found**: C, 64.47; H, 7.49; N 9.30.

(2S,3S,4S)-2-hydroxy-3-(*tert*-Butyldipenylsilyl)oxy-4-((1R')-1'-azido-2'-hydroxyethyl)-γ-butyrolactone (154d). The procedure used to make **(2S,3R,4S,5R)-5-Azido-4-*t*butyldiphenylsilyloxy-hexanoicmethylester-2,3,4-triol (152d)** also provided this lactone. $\mathbf{R_f}$= 0.45 (hexanes/EtOAc, 1:1); $[a]_D^{24}$= -45.3° (c 1.03, $CHCl_3$); **mp=** 104-105 °C; **IR** (KBr) u 3350, 3070, 3045, 2940, 2890, 2850, 2105, 1790, 1775, 700 cm^{-1}; **^{1}H NMR** ($CDCl_3$) δ 7.66 (m, 4H), 7.46 (m, 6H), 4.57 (m, 1H, note with a drop of D_2O present the multiplet is simplified to a d, J= 6.0 Hz, 1H), 4.34 (d, J= 5.9 Hz, 1H), 3.99 (d, J= 2.1 Hz, 1H), 3.61 (dd, J= 7.7, 11.2 Hz, 1H), 3.44 (dd, 5.4, 11.2 Hz, 1H), 2.92 (br s, 1H, -OH), 2.69 (m, 1H, note with a drop of D_2O present the multiplet is simplified to a heptet, J= 2.2, 5.4, 7.6 Hz, 1H), 1.64 (br s, 1H, -OH), 1.09 (s, 9H), (Note: If the ^{1}H NMR solvent is DMSO-d_6 or acetone-d_6 the lactone (**155d**) immediately appears.); **^{13}C NMR** (CCl_3D) d 174.8 (C), 132.5 (C), 131.4 (C), 19.2 (C), 135.7 (CH), 130.8 (CH), 130.6 (CH), 128.3 (CH), 83.2 (CH), 71.7 (CH), 68.2 (CH), 62.15 (CH), 62.24 (CH_2), 26.9 (CH_3); **MS** CI *m/z* (rel. intensity) 514 (M^++1, 2); **Anal. calcd for** $C_{22}H_{27}N_3O_5Si$: C, 59.84; H, 6.16; N, 9.52. **Found**: C, 59.46; H, 6.15; N 9.34.

(2S,3S,4S)-2-(*tert*-Butyldipenylsilyl)oxy-3-hydroxy-4-((1R')-1'-azido-2'-hydroxyethyl)-γ-butyrolactone(155d) second lactone. The procedure used to make **(2S,3R,4S,5R)-5-Azido-4-*t*butyldiphenylsilyloxy-hexanoicmethylester-2,3,4-triol (152d)** also provided this lactone. $\mathbf{R_f}$= 0.28 (hexanes/EtOAc, 1:1); $[\alpha]_D^{24}$= -77.2° (c 1.00, $CHCl_3$); **mp=** 121-123 °C; **IR** (KBr) υ 3350, 3070, 3045, 2960, 2935, 2890, 2860, 2105, 1785, 700 cm^{-1}; **^{1}H NMR** ($CDCl_3$) δ 7.82 (m, 2H), 7.69 (m, 2H), 7.47 (m, 6H), 4.65 (d, J= 5.5 Hz,1H), 4.44 (d, J= 2.4 Hz, 1H), 3.81 (d, J= 6.4 Hz, 1H), 3.78 (dd, J= 0.8, 5.5 Hz, 1H), 3.61 (ddd, J= 2.5, 6.4 Hz, 1H), 2.98 (br s, -OH), 2.05 (br s, -OH), 1.16 (s, 9H), (Note: If the ^{1}H NMR solvent is DMSO-d_6 the lactone (**154d**) immediately appears.); **^{13}C NMR** (CCl_3D) δ 173.3 (C), 132.2 (C), 131.0 (C), 19.3 (C), 135.9 (CH), 135.5 (CH), 130.6 (CH), 130.5 (CH), 128.1 (CH), 128.0 (CH), 82.1 (CH), 70.2 (CH), 69.5 (CH), 62.3 (CH), 62.8 (CH_2), 26.8 (CH_3); **MS** CI *m/z* (rel. intensity) 442 (M^++1, 2.5), 385 (11), 384 (43), 365 (22), 364 (100), 328 (26), 310 (11), 309 (18), 308 (83), 290 (26), 263 (17), 249 (20), 222 (17), 221 (24), 202 (25), 200 (21), 199 (34), 163 (17), 60 (52); **Anal. calcd for** $C_{22}H_{27}N_3O_5Si$: C, 59.84; H, 6.16; N, 9.52. **Found**: C, 59.83; H, 6.23; N 9.42.

Series III compound leading to D-*threo*-sphingosine

(3R)-Azido-(2S)-*tert*-butyldiphenylsilyloxy-γ-butyrolactol (156d). To a rb flask was added vicinal diol (**152d**) (1.278 g, 2.699 mmol), 80 mL of a 3:1 mixture of MeOH/H_2O, and $NaIO_4$ (1.154 g, 5.397 mmol, 2.00 equiv). The reaction was excluded from light and stirred. The

starting material was consumed after 3 h. Note: A white precipate accumulates as the reaction proceeds, it is not the product. Work-up: CH_2Cl_2 (80 mL) was added to the reaction and the resulting solution was passed through a plug of silica gel over celite. To the filtrate was added H_2O (80 mL). This solution was placed in a separatory funnel and the organic layer removed. The aqueous layer was extracted with CH_2Cl_2 (X3), the organic extracts were combined, dried with $MgSO_4$, filtered, and volatiles removed to provide an oil. Gradient elution (25% EtOAc in hexanes → 50% EtOAc/hexanes) chromatography provided 778.1 mg (2.029 mmol, 75%) of lactol (**156d**) as a viscous clear oil. In addition 134.4 mg (0.350 mmol, 13%) of the silyl migrated product (**157d**) was isolated. Note the two possible anomeric lactols are evident in the ^1H NMR and ^{13}C NMR, complicating the spectrum. The two anomers exist in an approximate ratio of 1 to 1.2 (conc. of 10 - 20 mg in 0.5 mL of $CDCl_3$). H and H' are used to indicate the separate lactol resonances, as established by TOCSY NMR. **R_f**= 0.28 (hexanes/EtOAc, 4:1); $[\alpha]_D^{25}$= -2.22° (c 1.35, $CHCl_3$); **IR** (neat) υ 3435, 3080, 3055, 2980, 2950, 2895, 2860, 2100, 1590, 1425, 1110, 695 cm^{-1}; **^1H NMR** ($CDCl_3$) δ 7.66 (m, 4H), 7.45 (m, 6H), 5.28 (dd, J = 4.0, 9.8 Hz, 1H'), 5.25 (d, J= 7.9 Hz, 1H), 4.28 (dd, J= 5.2, 9.8 Hz, 1H), 4.18 (dd, J= 4.6, 9.8 Hz, 1H' & d, J= 1.5 Hz, 1H), 4.12 (dd, J= 2.2, 4.0 Hz, 1H'), 4.01 (dd, J= 2.3, 9.8 Hz, 1H), 3.96 (d, J= 9.8 Hz, -OH'), 3.76 (dt, J= 1.7, 5.0 Hz, 1H), 3.66 (ddd, J= 0.5, 2.1, 9.8 Hz, 1H'), 3.62 (p, J= 2.1, 4.6 Hz, 1H'), 2.63 (d, J= 7.9 Hz, -OH), 1.13 (s, 9H), 1.09 (s, 9H); **^{13}C NMR** (CCl_3D) δ 132.7 (C), 132.4 (C), 132.0 (C), 131.4 (C), 19.1 (C), 19.0 (C), 135.6 (CH), 135.58 (CH), 130.6 (CH), 130.5 (CH), 130.24 (CH), 130.19 (CH), 128.2 (CH), 128.1 (CH), 128.0 (CH), 127.9 (CH), 103.5 (CH), 97.0 (CH), 81.1 (CH), 76.5 (CH), 66.2 (CH), 65.9 (CH), 71.2 (CH_2), 68.2 (CH_2), 26.9 (CH_3), 26.8 (CH_3).

(3R)-Azido-1-*tert*-butyldiphenylsilyloxy-(2S)-hydroxy-γ-butyrolactol (157d). The procedure used to make **(3R)-Azido-(2S)-2-*tert*-butyldiphenylsilyloxy-γ-butyrolactol (156d)** also provided 134.4 mg (0.350 mmol, 13%) of this anomeric silyloxy protected lactol (**157d**). **R_f**= 0.45 (hexanes/EtOAc, 4:1); $[\alpha]_D^{25}$= -86.2° (c 1.30, $CHCl_3$); **IR** (neat) υ 3505, 3070, 3050, 2955, 2930, 2895, 2855, 2100, 1590, 1425, 1110, 695 cm^{-1}; **^1H NMR** ($CDCl_3$) δ 7.67 (m, 4H), 7.43 (m, 6H), 5.41 (d, J = 3.7 Hz, 1H), 4.20 (dd, J= 5.7, 9.7 Hz, 1H), 4.13 (m, 2H), 3.66 (dd, J= 4.4, 9.0 Hz, 1H), 2.91 (d, J= 7.5 Hz, -OH), 1.12 (s, 9H); **^{13}C NMR** (CCl_3D) δ 132.5 (C), 132.4 (C), 19.2 (C), 135.6 (CH), 135.5 (CH), 130.2 (CH), 130.1 (CH), 127.9 (CH), 127.8 (CH), 97.2 (CH), 77.2 (CH), 66.1 (CH), 68.6 (CH_2), 26.8 (CH_3);

Series III compound leading to D-*threo*-sphingosine

(2R,3R,4E)-2-Azido-4-octadecen-1,3-diol (148d/149d). To a flame dried rb flask under Ar was added *n*-tetradecyltriphenylphosphonium bromide (358.8 mg, 0.665 mmol, 3.50 equiv). The phosphonium salt was put under vacuum (0.075 ± 0.025 mm Hg) for 1 h and subsequently flooded with Ar. THF (2.50 mL) was added* and the solution was placed in an ice-bath. After stirring for 15 min, sodium amylate† (0.27 M, 2.5 mL, 3.5 equiv) was added§ dropwise over 15 sec. The solution immediately became an orange color. This solution stirred for 15 min and then the ice-bath was removed. After 30 min at rt the ylide solution was cooled to 0 °C again. The lactol (72.9 mg, 0.190 mmol, 1.0 equiv) in THF (1.5 mL) was added dropwise over 1 min. After 0.5 h the ice-bath was removed. After 1.5 h†† of total reaction time the reaction was cooled to -55 °C and then quenched with saturated NH_4Cl (10 mL). This solution was extracted with EtOAc (X3), the organic extracts were combined, dried with $MgSO_4$, filtered, and the volatile organics removed to yield the crude product as a viscous dark oil. Gradient elution chromatography (100% hexanes → hexanes/EtOAc, 7:3) provided 32 mg of impure sphingosine adduct (##). Further attempts at purification were futile. This crude product had THF (2.0 mL) added to it followed by *n*-BuNF (40 mg). After 5 min the reaction was filtered and the THF evaporated to provide an oil. Column chromatography of this oil, using gradient elution (100% hexanes → hexanes/EtOAc, 7:3), provided 8.3 mg (0.025 mmol, 13% yield) of azidosphingosine (##).

* Note: The phosphonium salt completely dissolves in the THF, but the solution is not transparent.

† For preparation of sodium amylate see: R.P. Short, B.C. Ranu, J.M. Revol and T. Hudlicky, General Method of Synthesis of Cyclopentanoid Terpenic Acids. Stereocontrolled Total Syntheses of (±)-Isocomenic Acid and (±)-Epiisocomenic Acid, *J. Org. Chem.*, **48**, 4453-4461 (1983).

§ You must heat the sodium amylate to ≅ 65-70 °C, then remove it *via* syringe, and add it quickly to the cooled solution of the phosphonium salt. If the syringe cools to rt the amylate will crash out of solution and jamn the syringe.

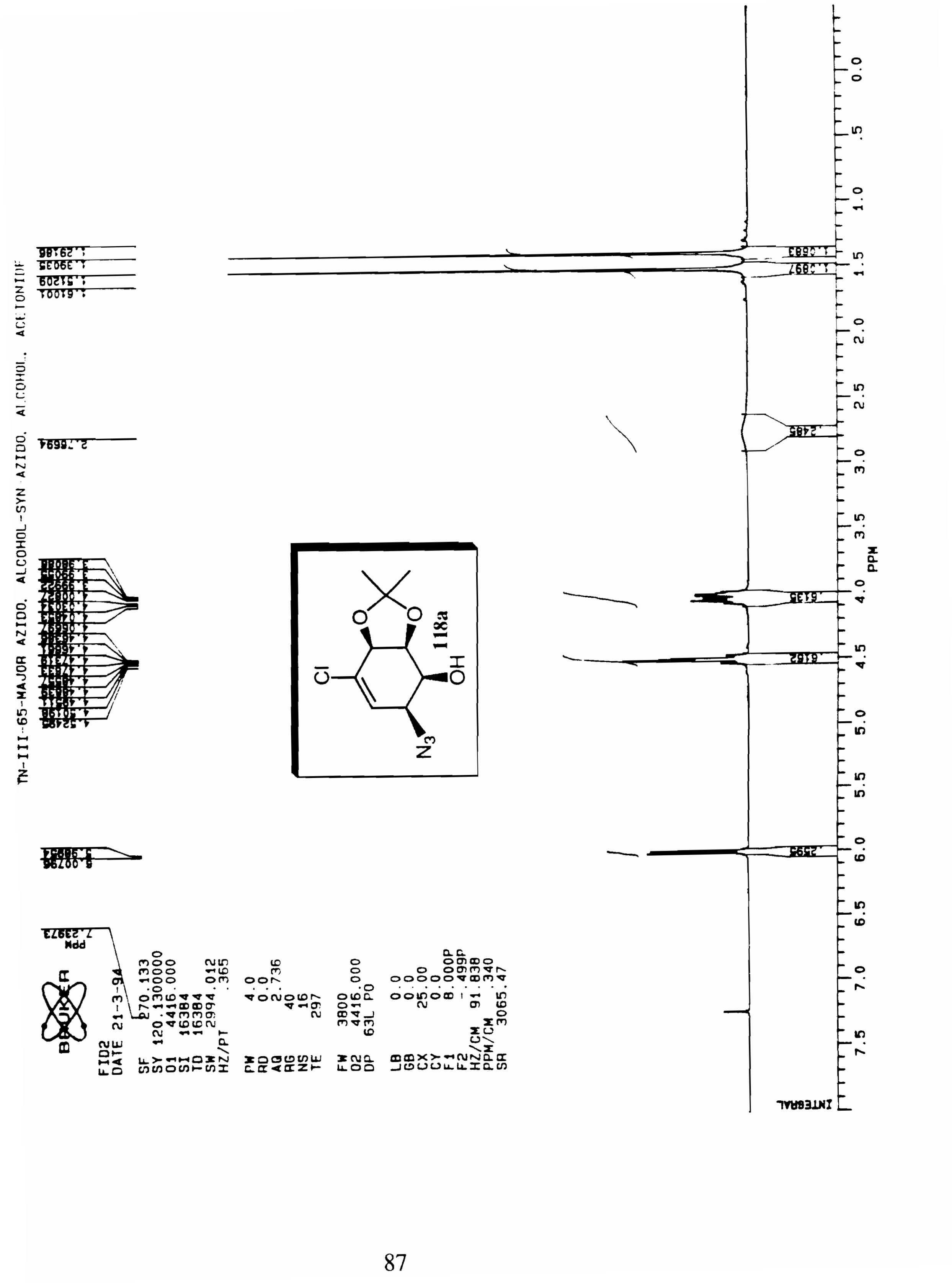
†† Note: After 15 min (45 min total reaction time) at rt the solution turned a non translucent brown color.

(2R,3R,4Z & 4E)-2-Azido-3-*tert*-butyldiphenylsilyloxy-4-octadecen-1,3-diol (159d). The ^1H NMR of the impure silyloxy azidosphingosine indicates a 9:1 *cis/trans* ratio of geometric isomers. Note: When the reaction is run at rt a 6:4 *cis/trans* ratio is observed. One proton will be defined as the sum of equivalent *cis* and *trans* proton integration areas. The major impurity is *tert*-butyldiphenylsilanol. **^1H NMR** ($CDCl_3$) δ 7.67 (m, 4H),7.42 (m, 6H), 5.72 (m, 0.1H,*trans*), 5.55 (dt, J= 7.3, 11.0 Hz, 0.9H,*cis*), 5.38 (m, 1H), 4.43 (pentet, J= 4.8 ave., 0.9H, *cis*), 4.13 (q, J= 5.6 Hz, 0.1H, *trans*), 3.8 (m, 4H), 3.42 (m, 2H), 1.99 (m, 2H), 1.26 (s, 22H), 0.89 (t, J= 6.9 Hz, 3H).

(2R,3R,4Z)-2-Azido-4-octadecen-1,3-diol (148d). The cis isomer was produced in 12.0 % yield. **R_f**= 0; **^{1}H NMR** ($CDCl_3$) δ 5.66 (dtm, J= 7.7, 11.0 Hz, 1H), 5.46 (ddt, J= 1.5, 10.8, 10.8 Hz, 1H), 4.52 (m, 1H), 3.81 (m, 1H), 3.67 (m, 1H), 3.46 (ddd, J= 4.1, 6.4, 6.4 Hz, 1H), 2.08 (m, 2H), 1.90 (m, 2H), 1.24 (s, >20H), 0.90 (t, J= 7.0 Hz, 1H).

VI. SELECTED SECTRA

On the following pages selected spectra are provided.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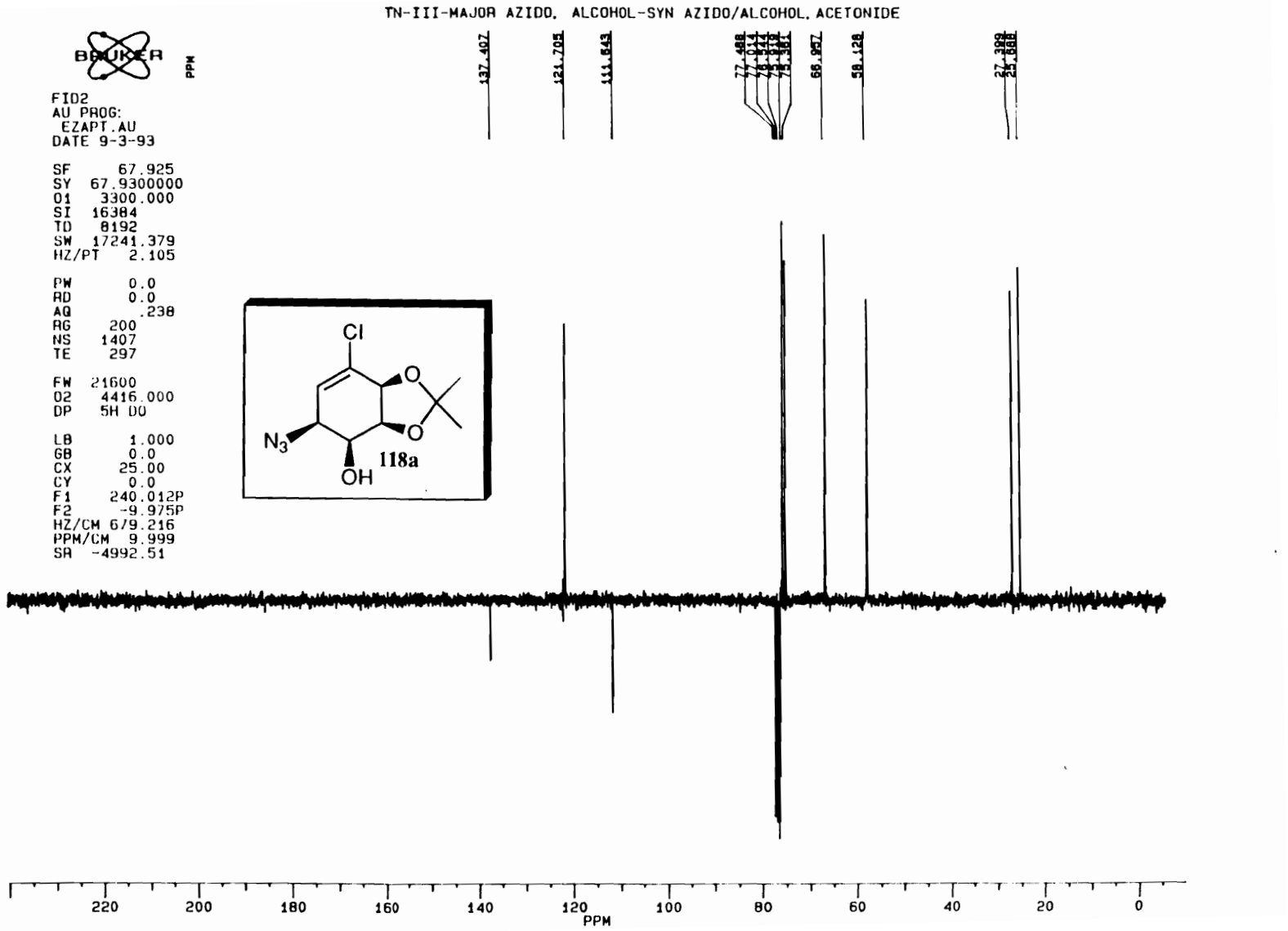
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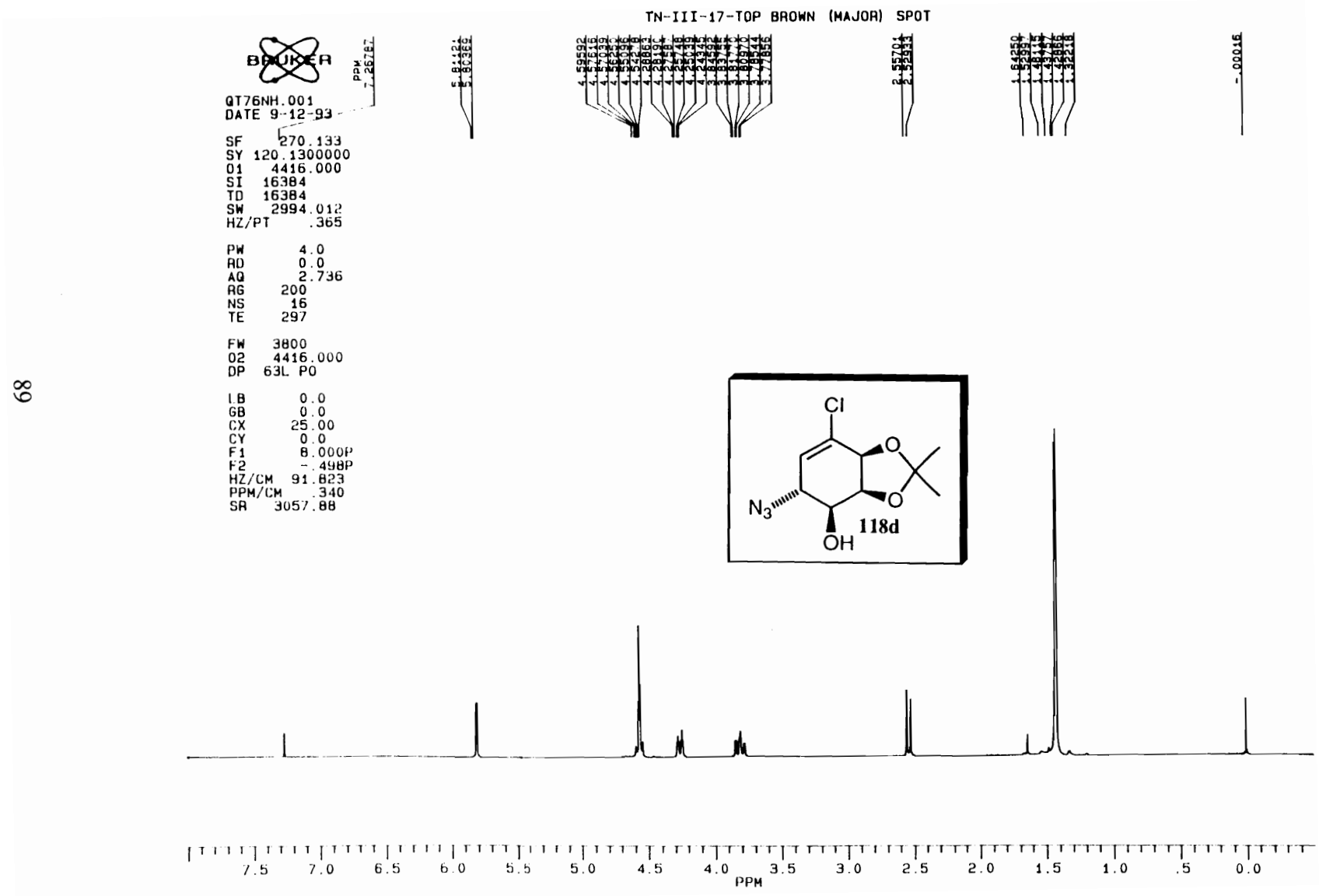
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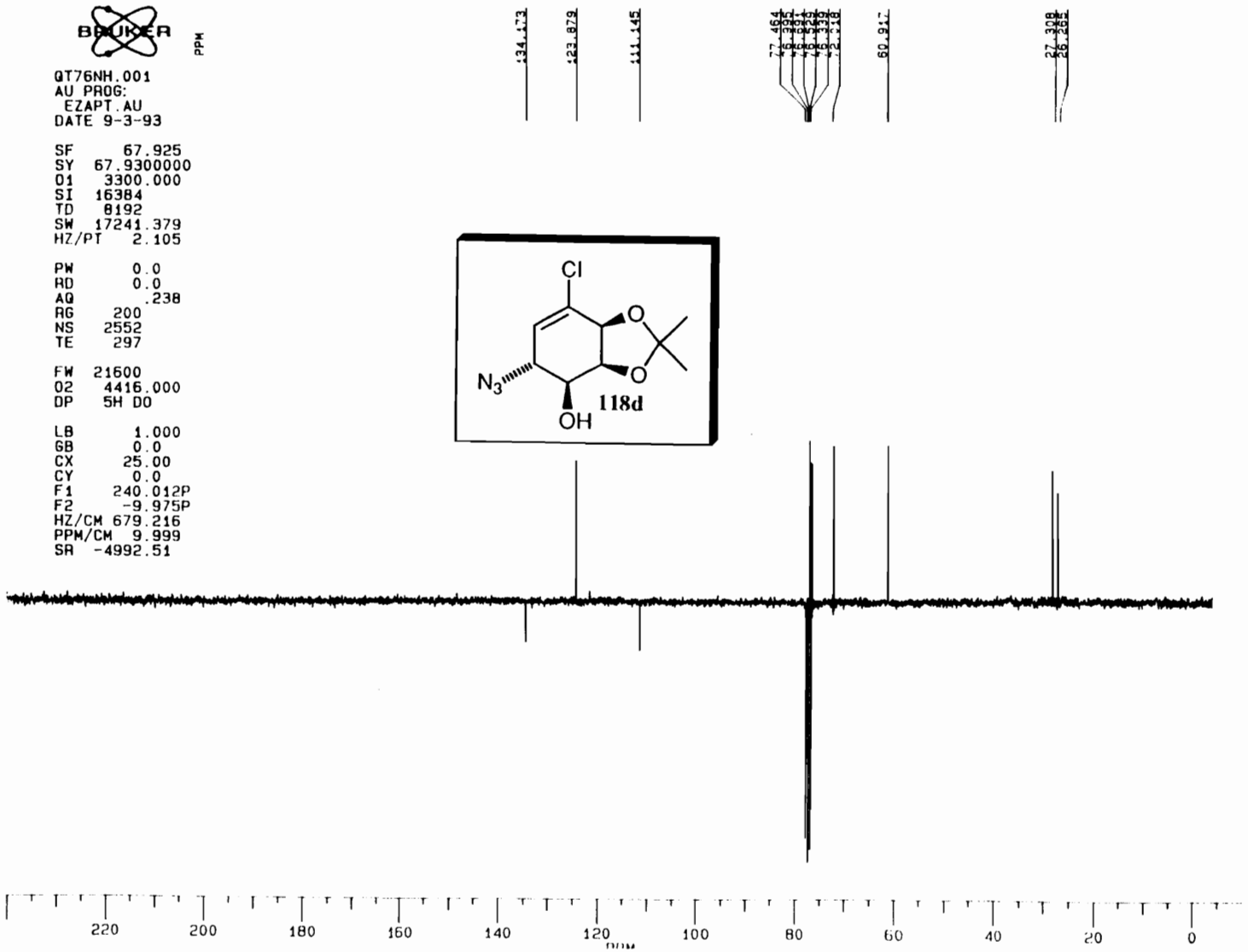

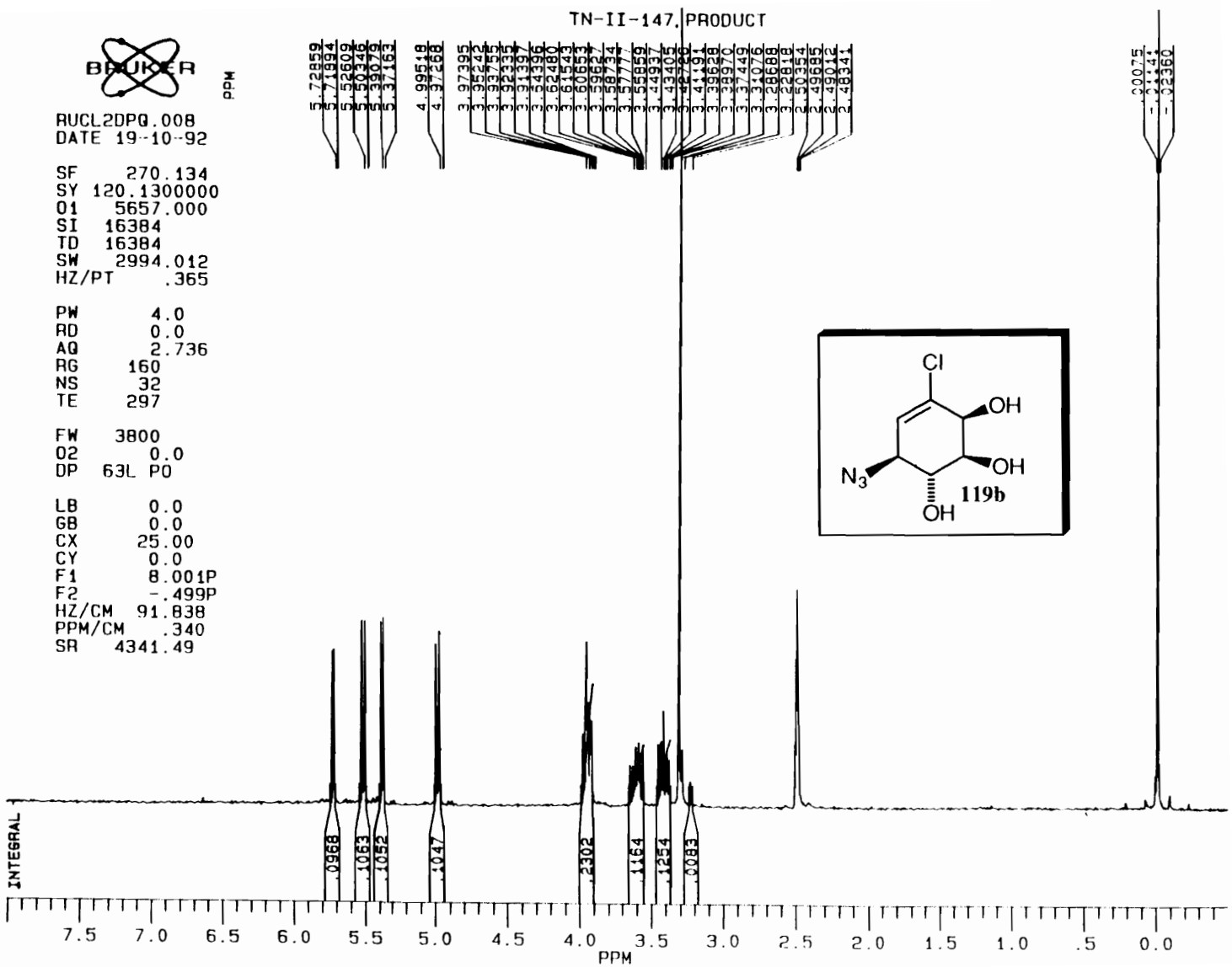
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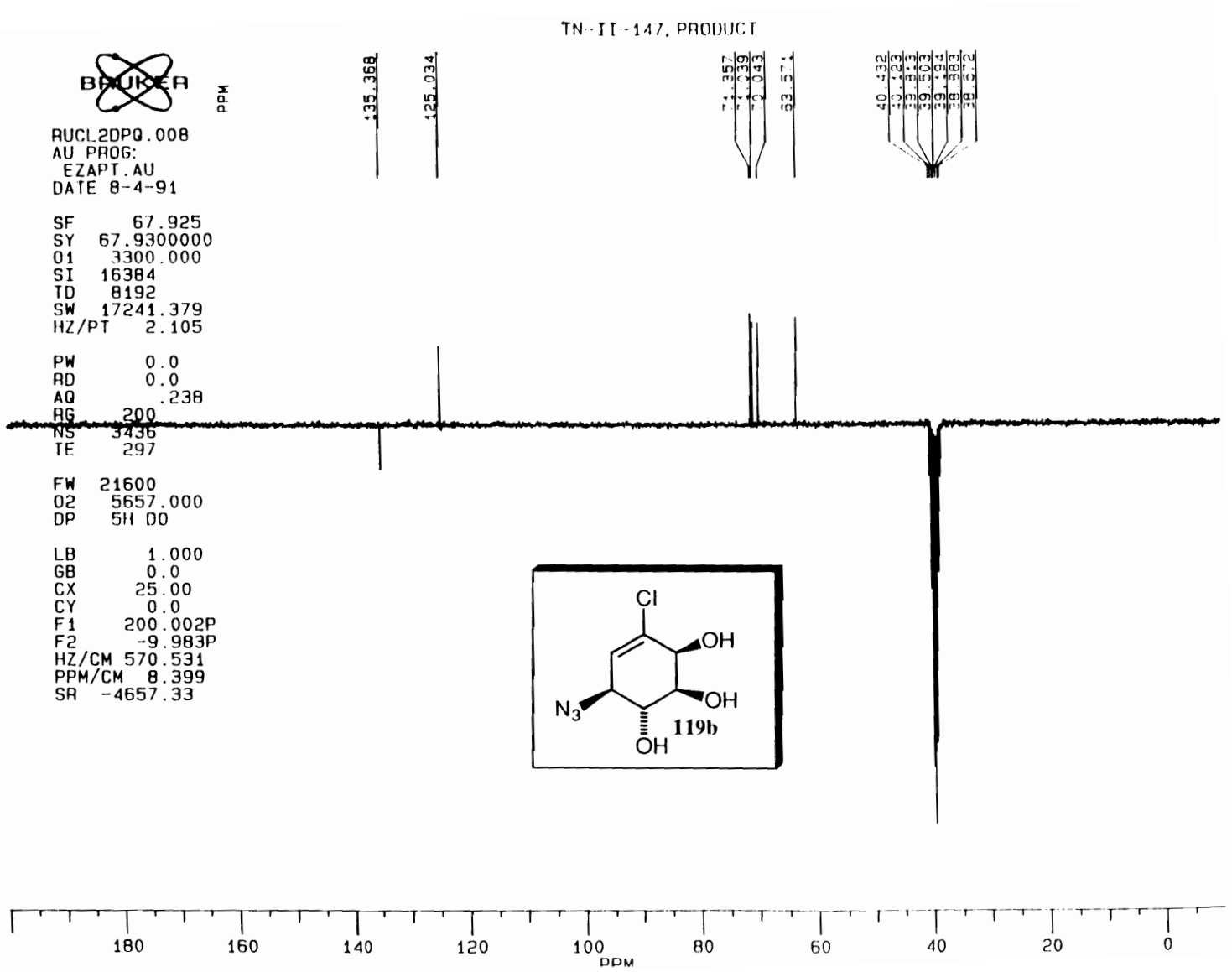
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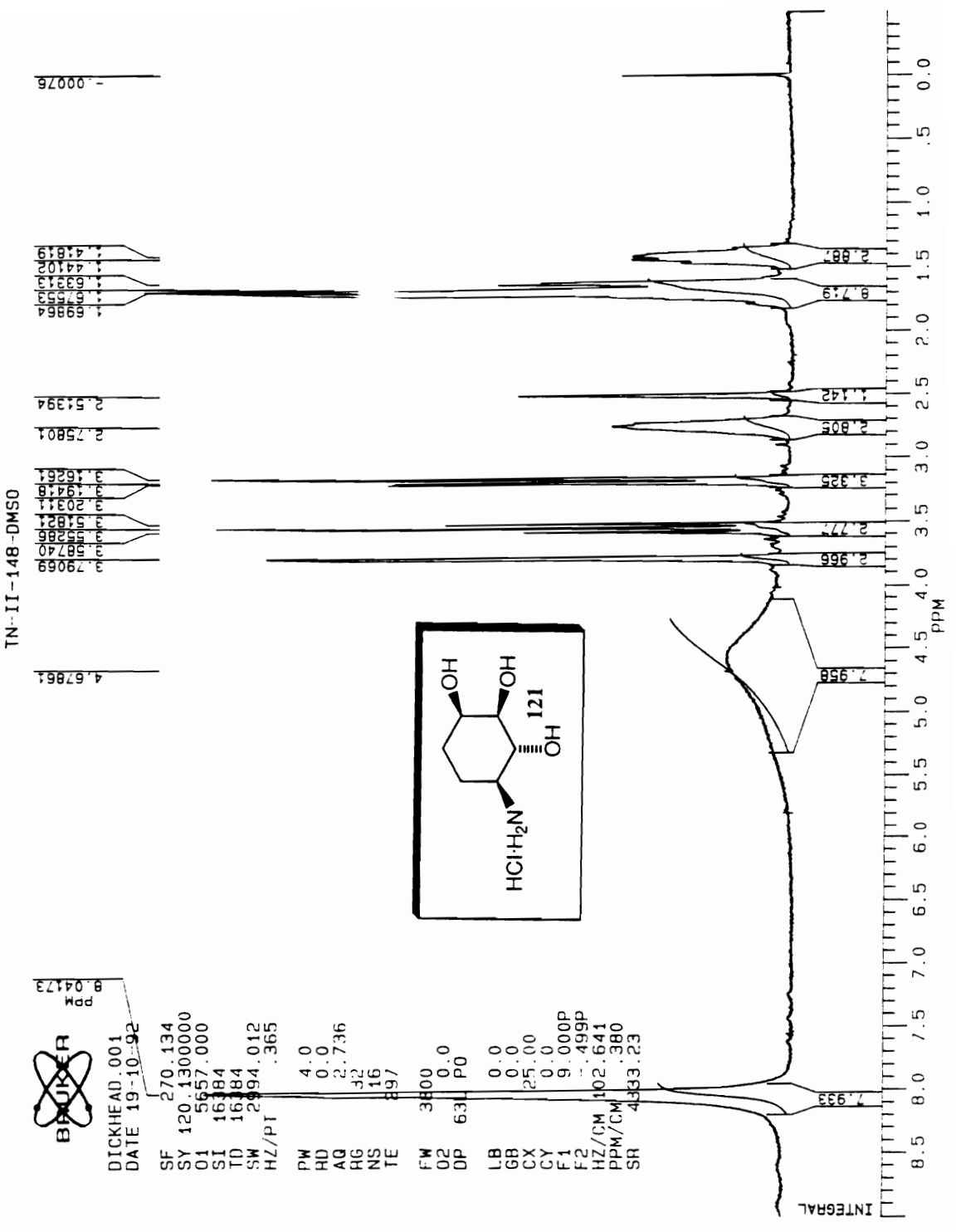
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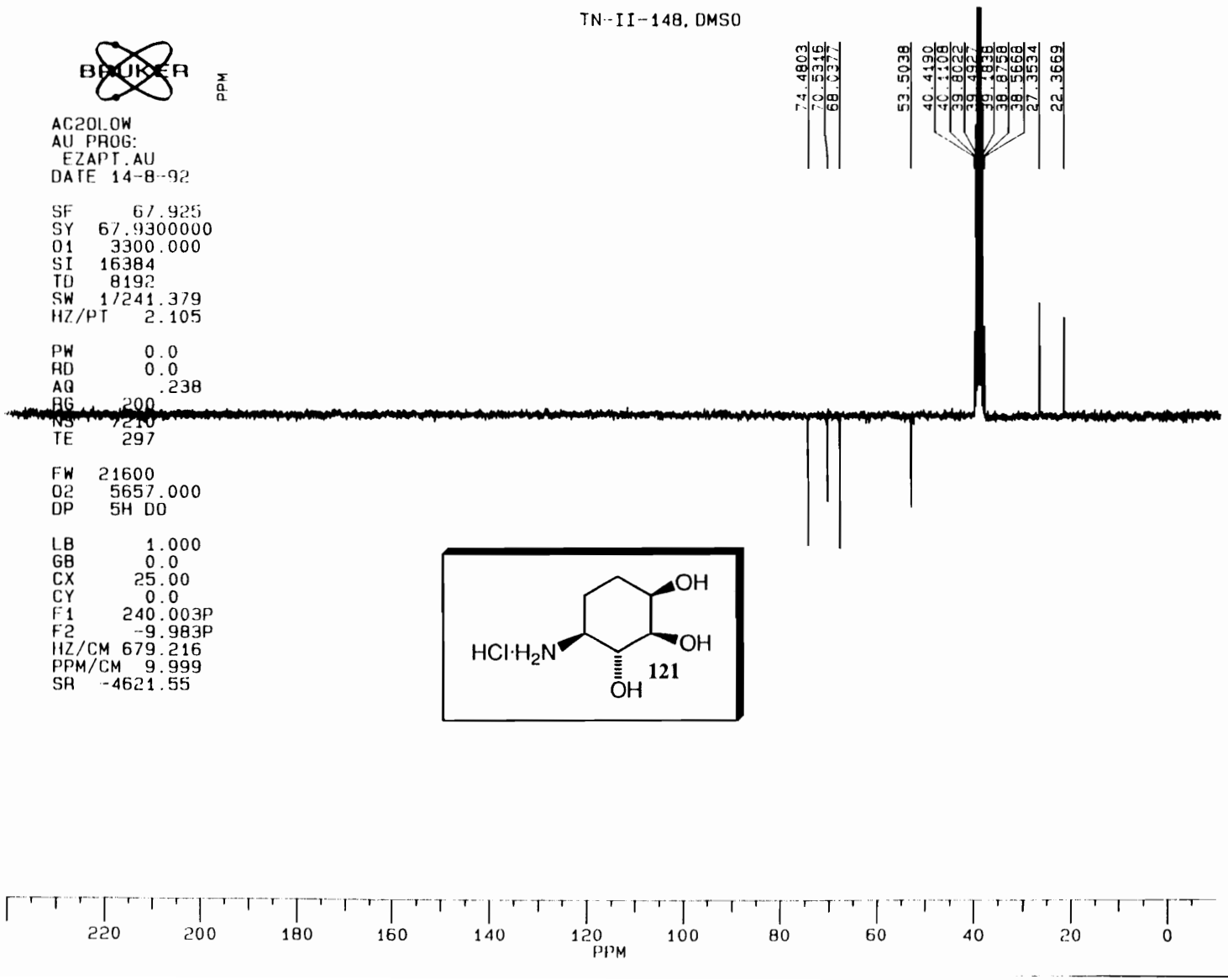
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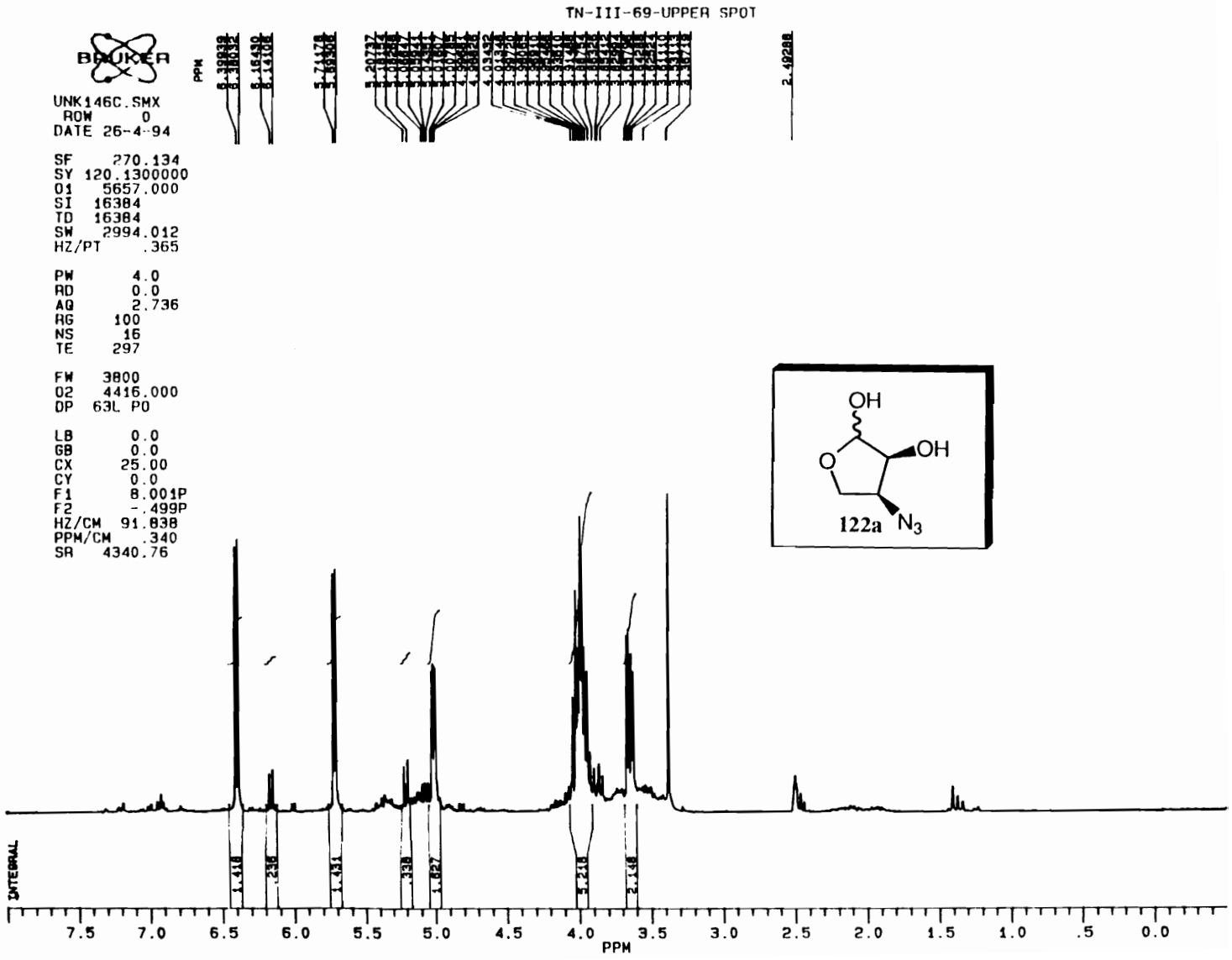
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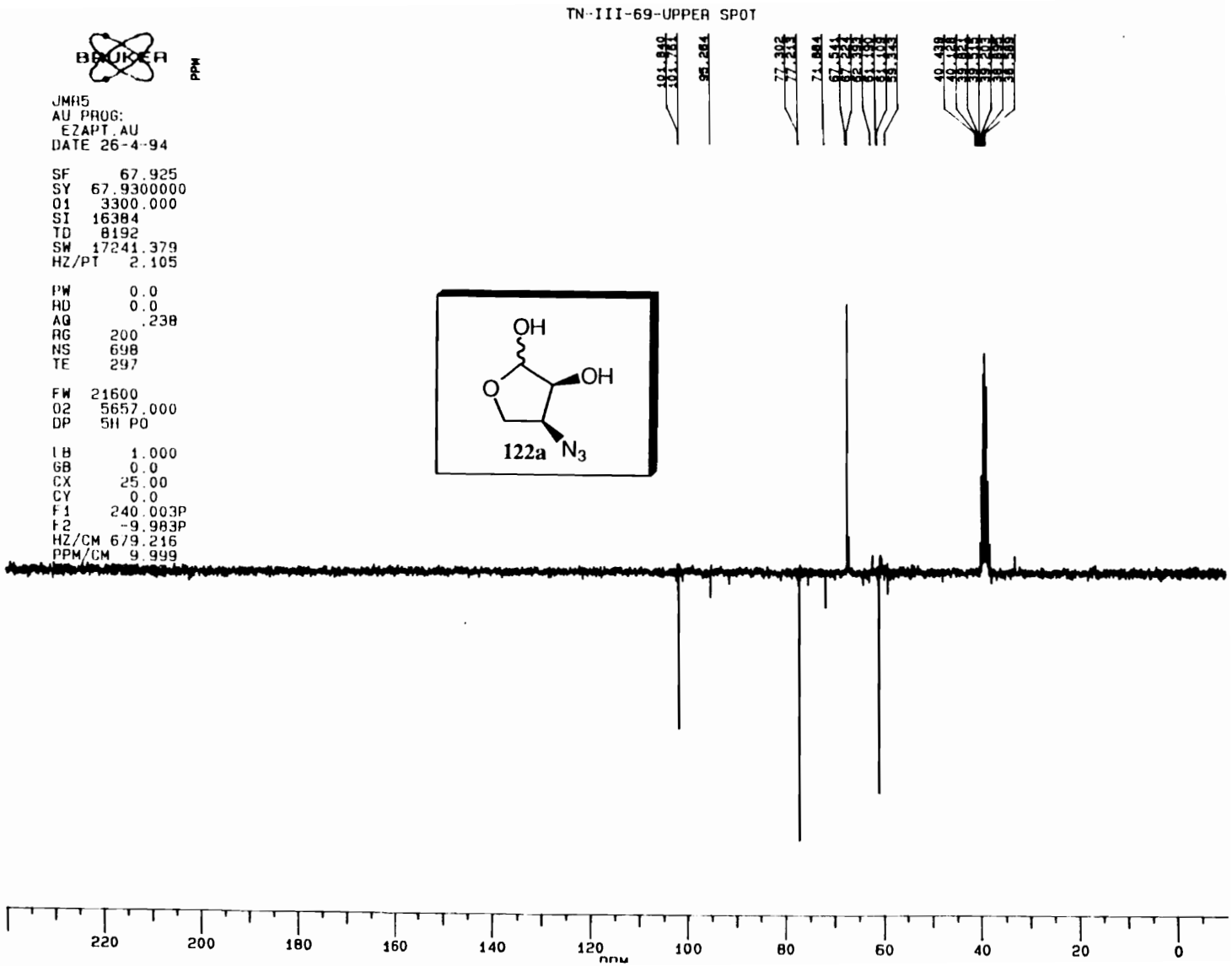
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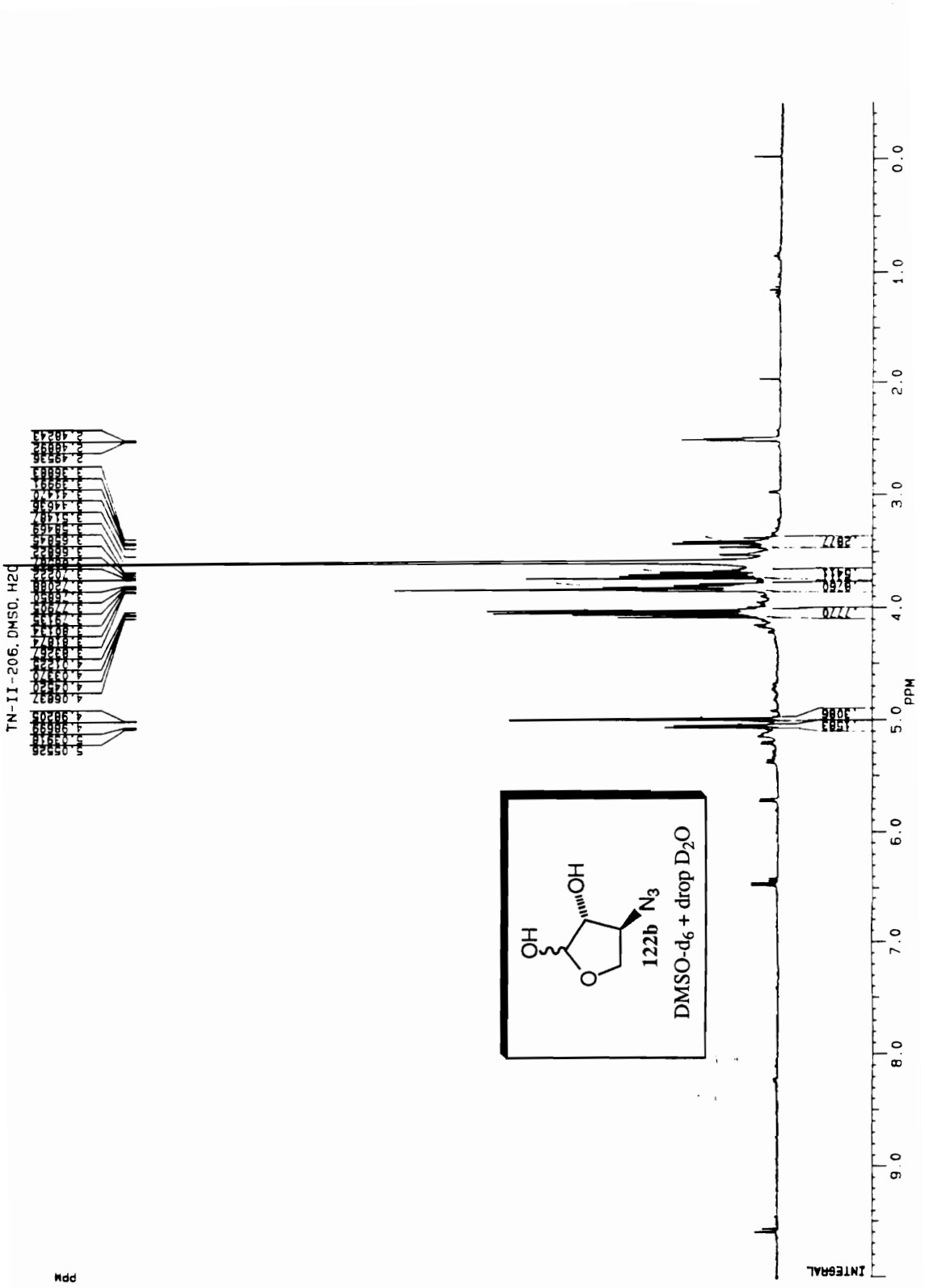
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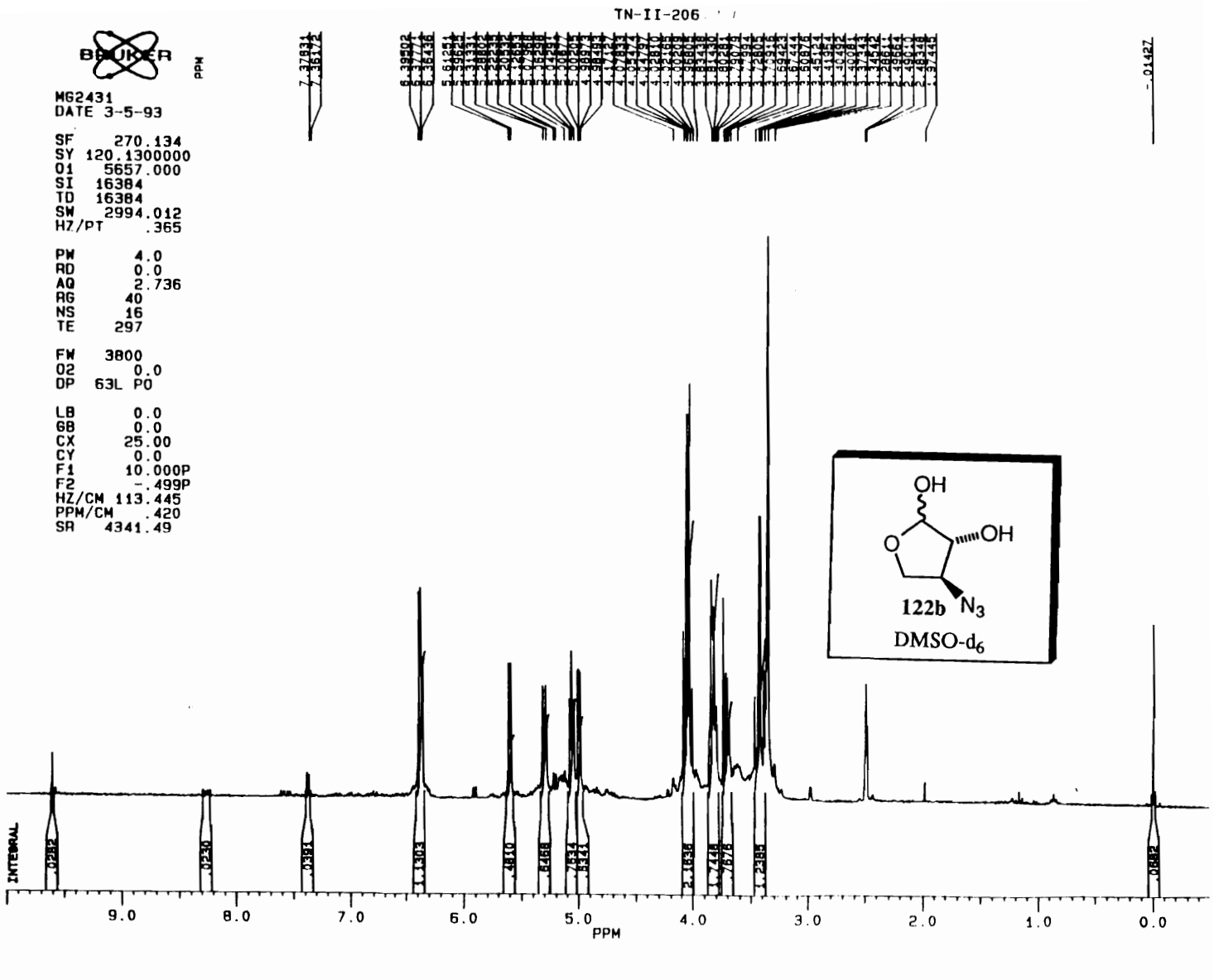
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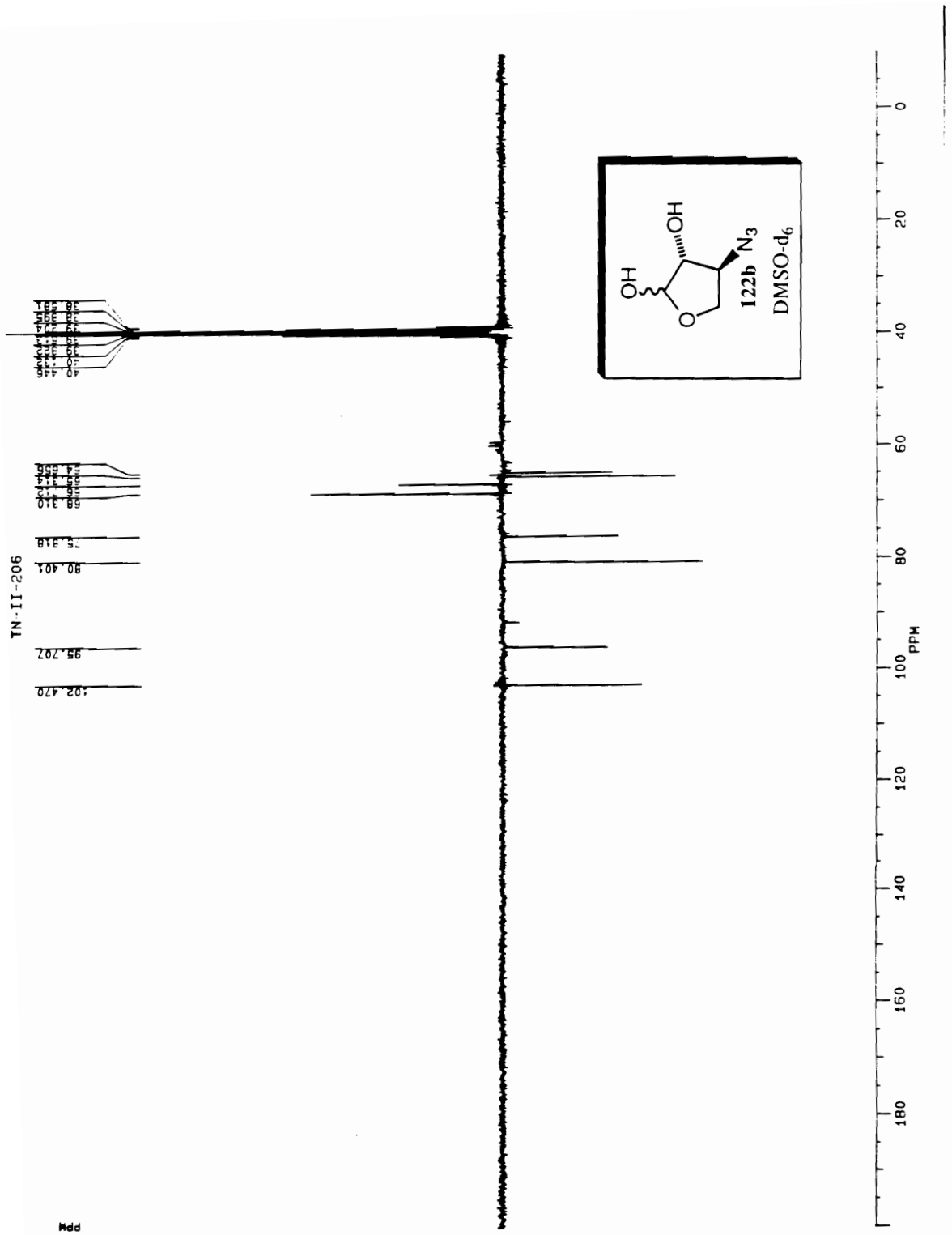
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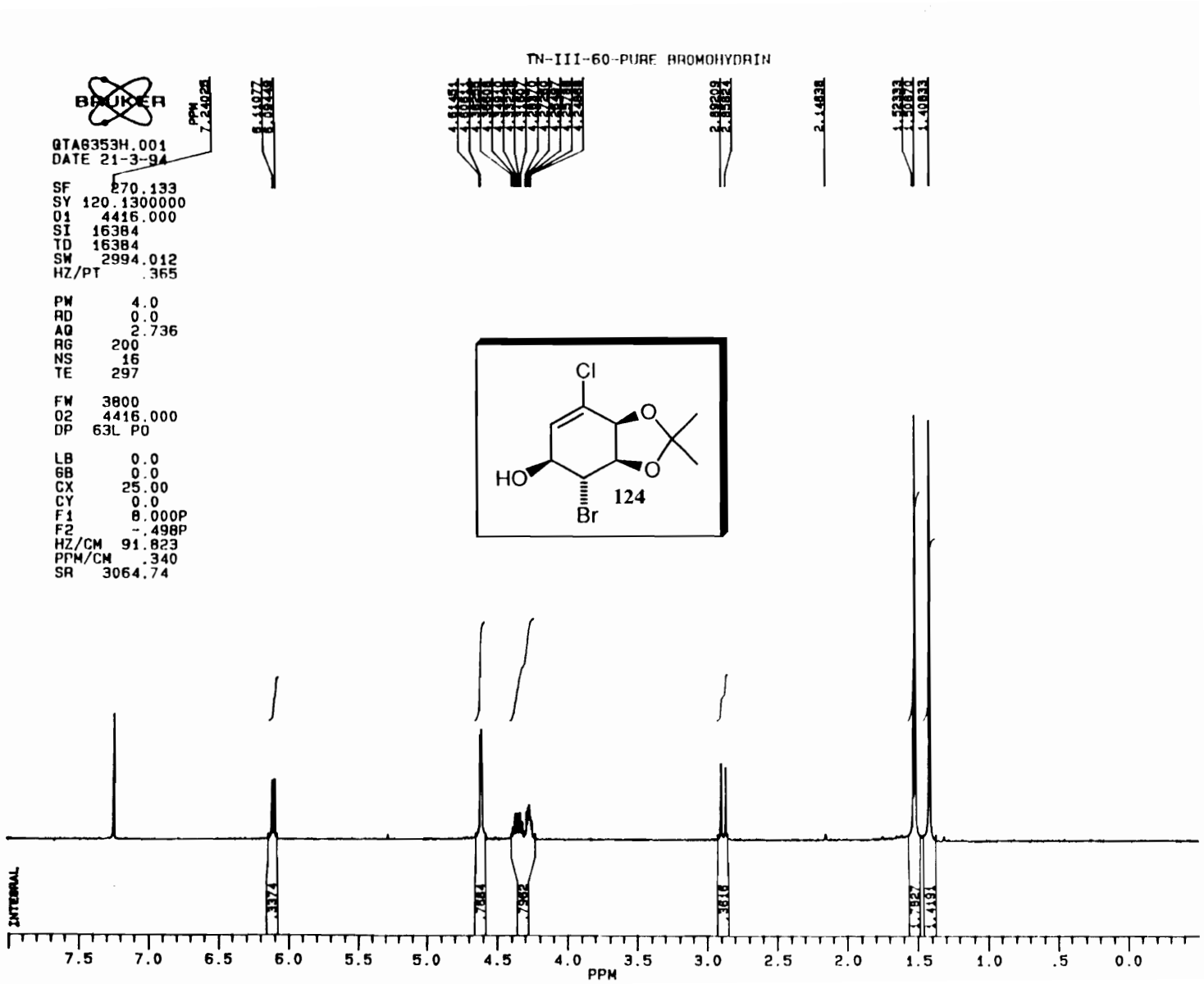
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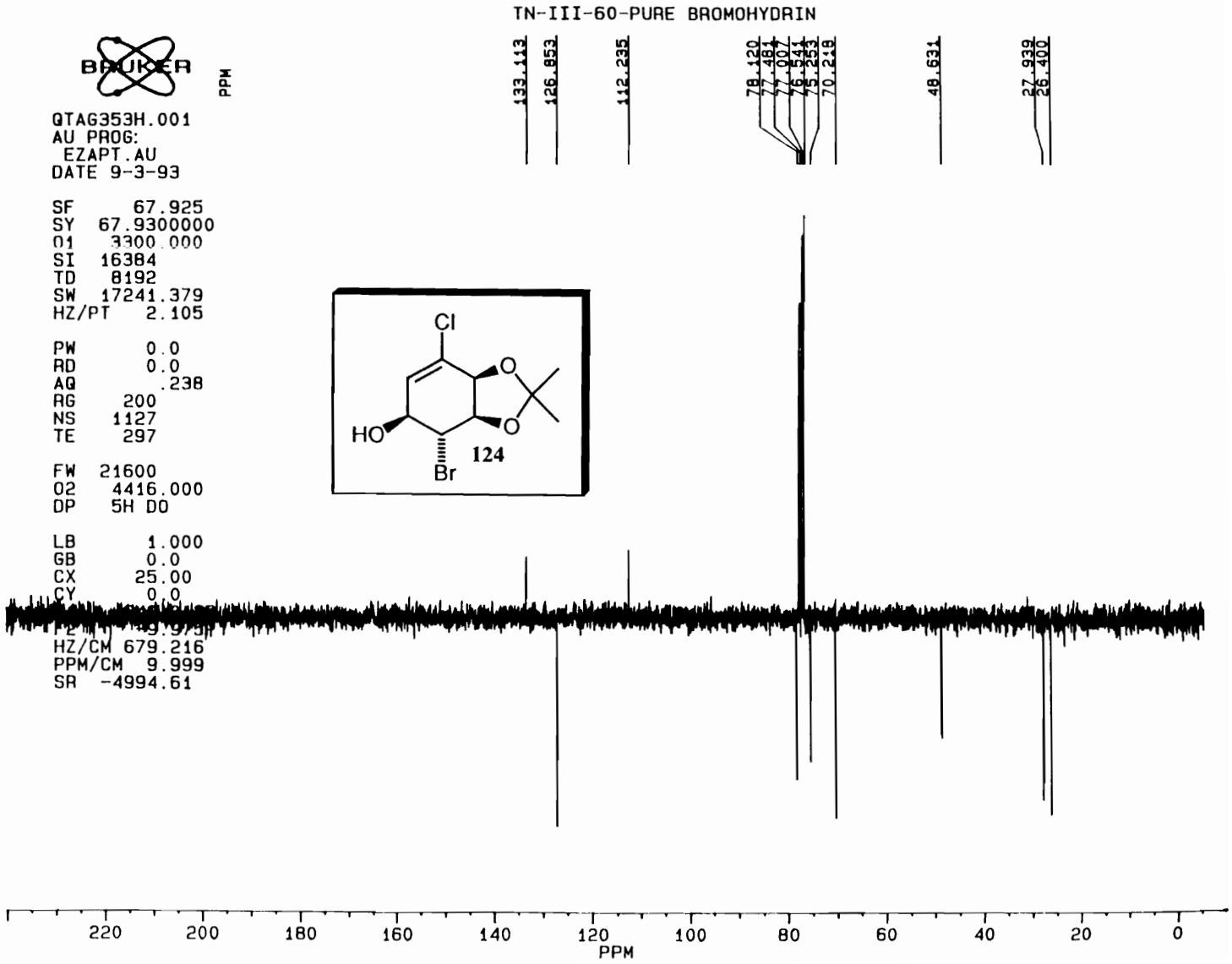
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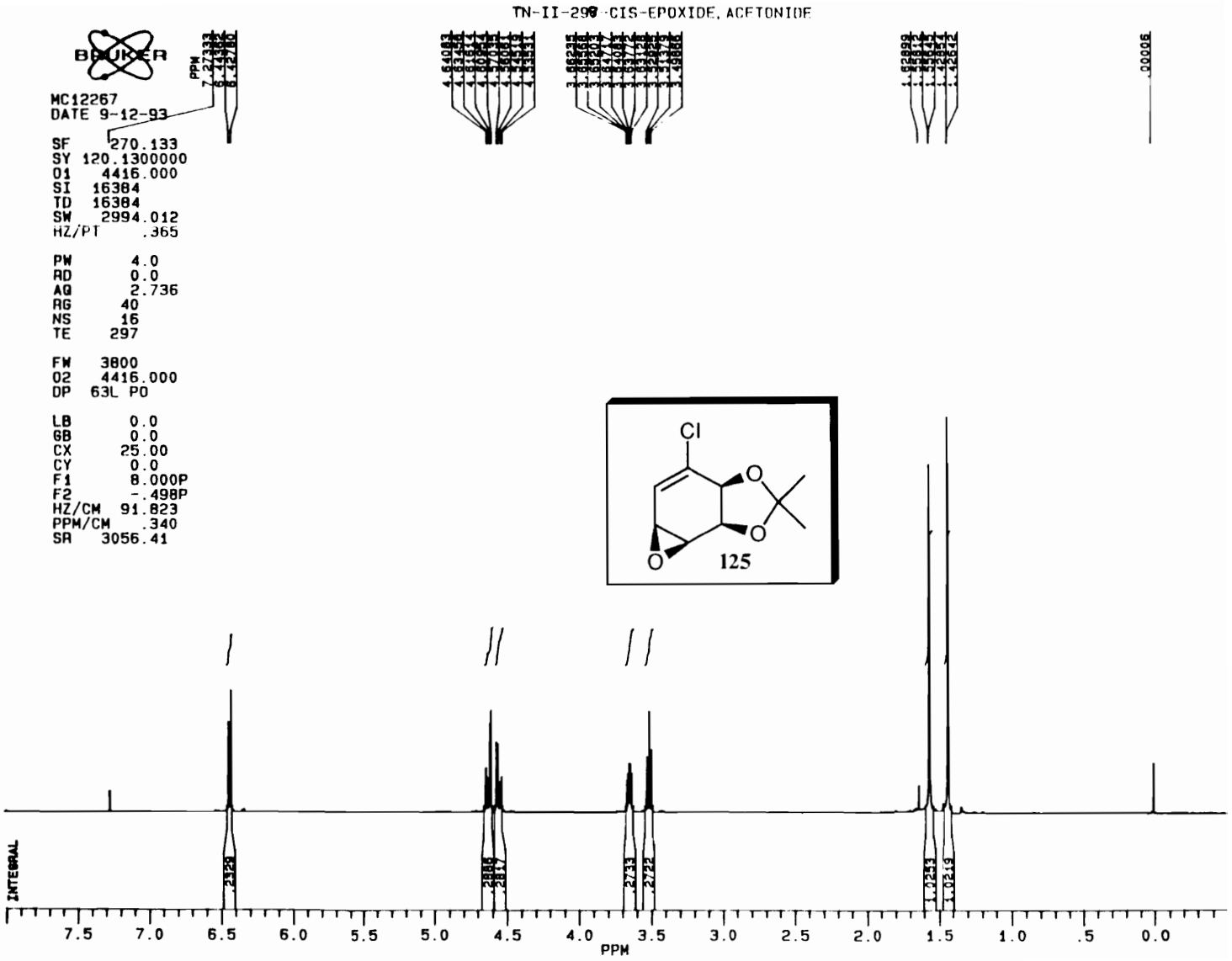
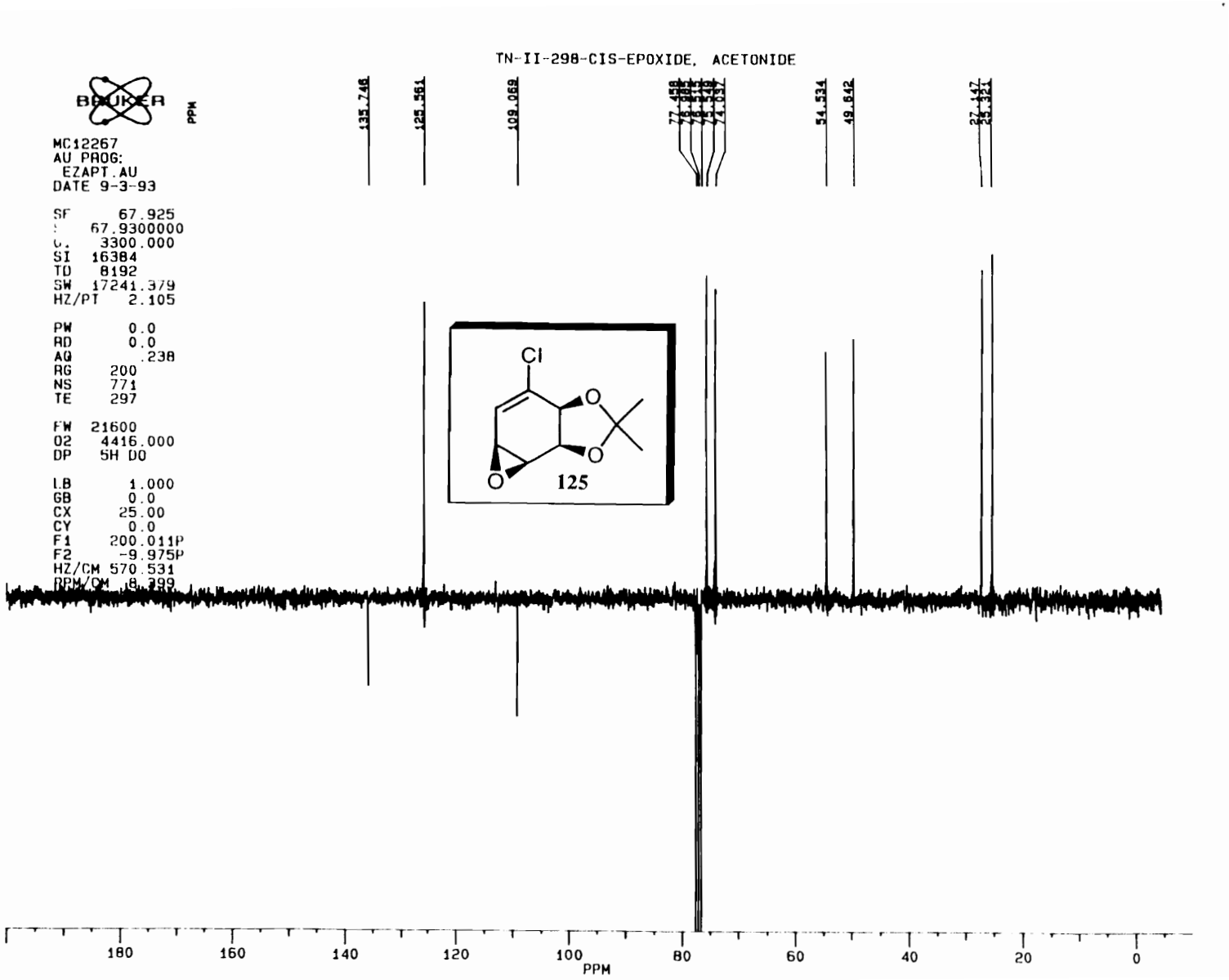
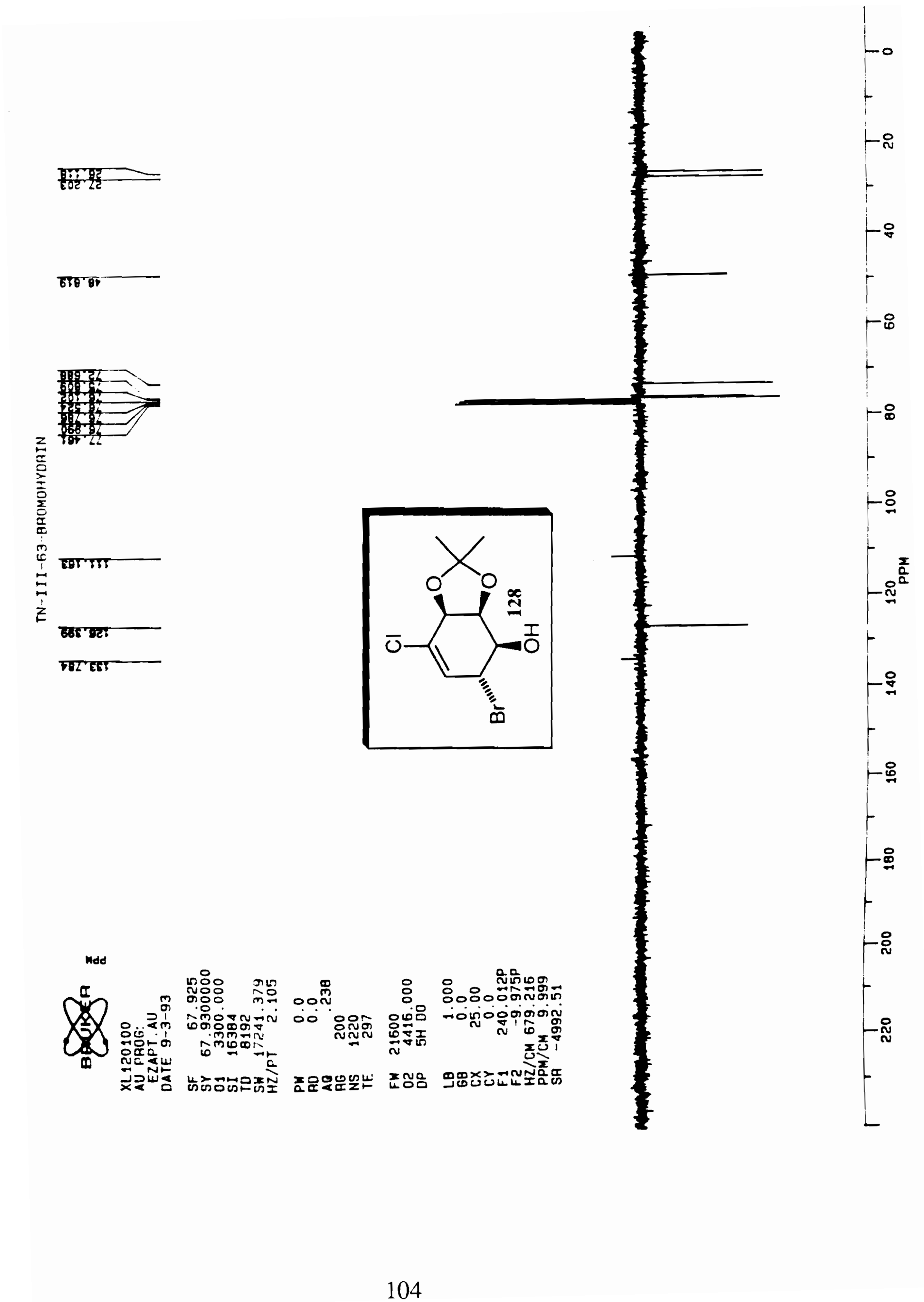
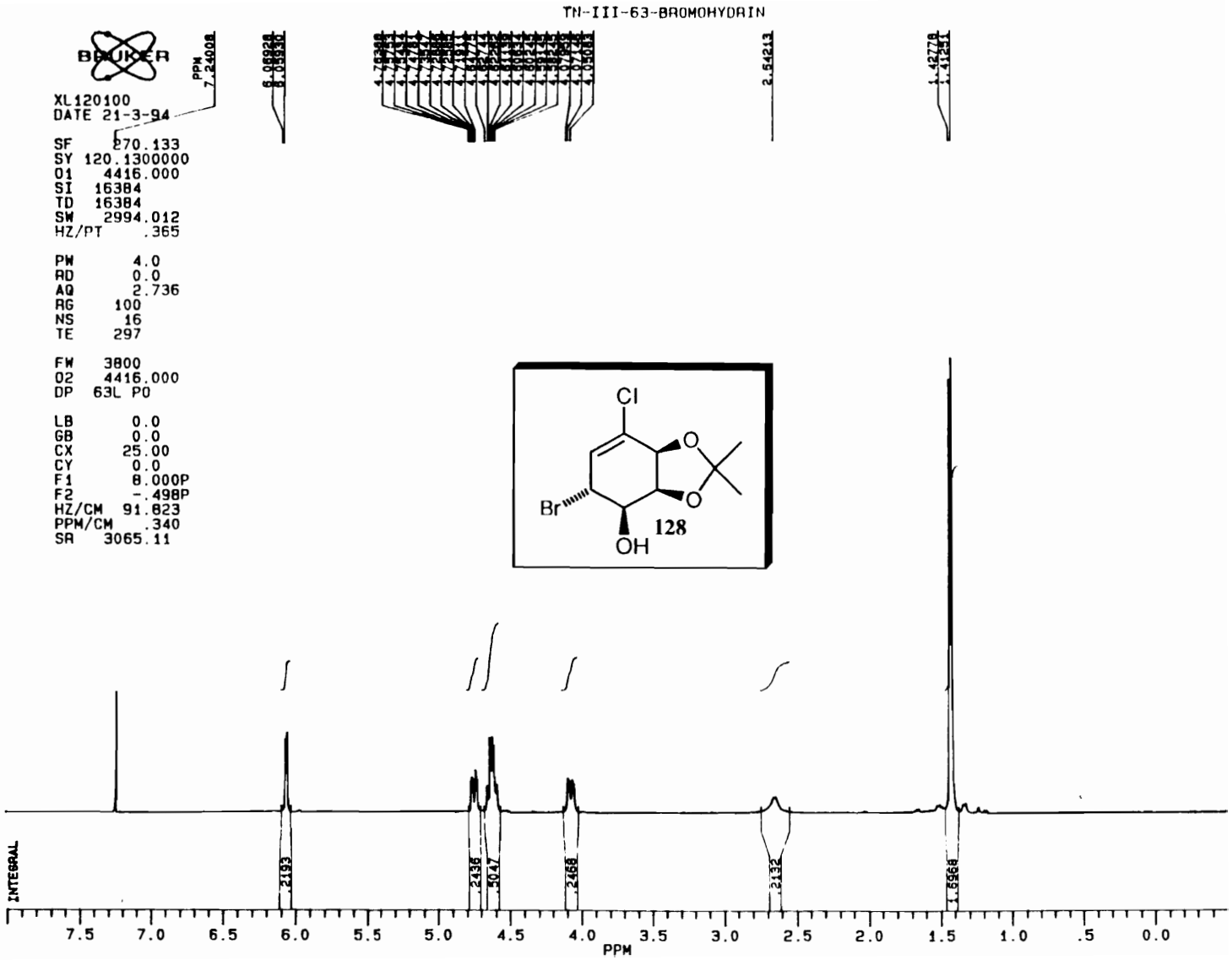
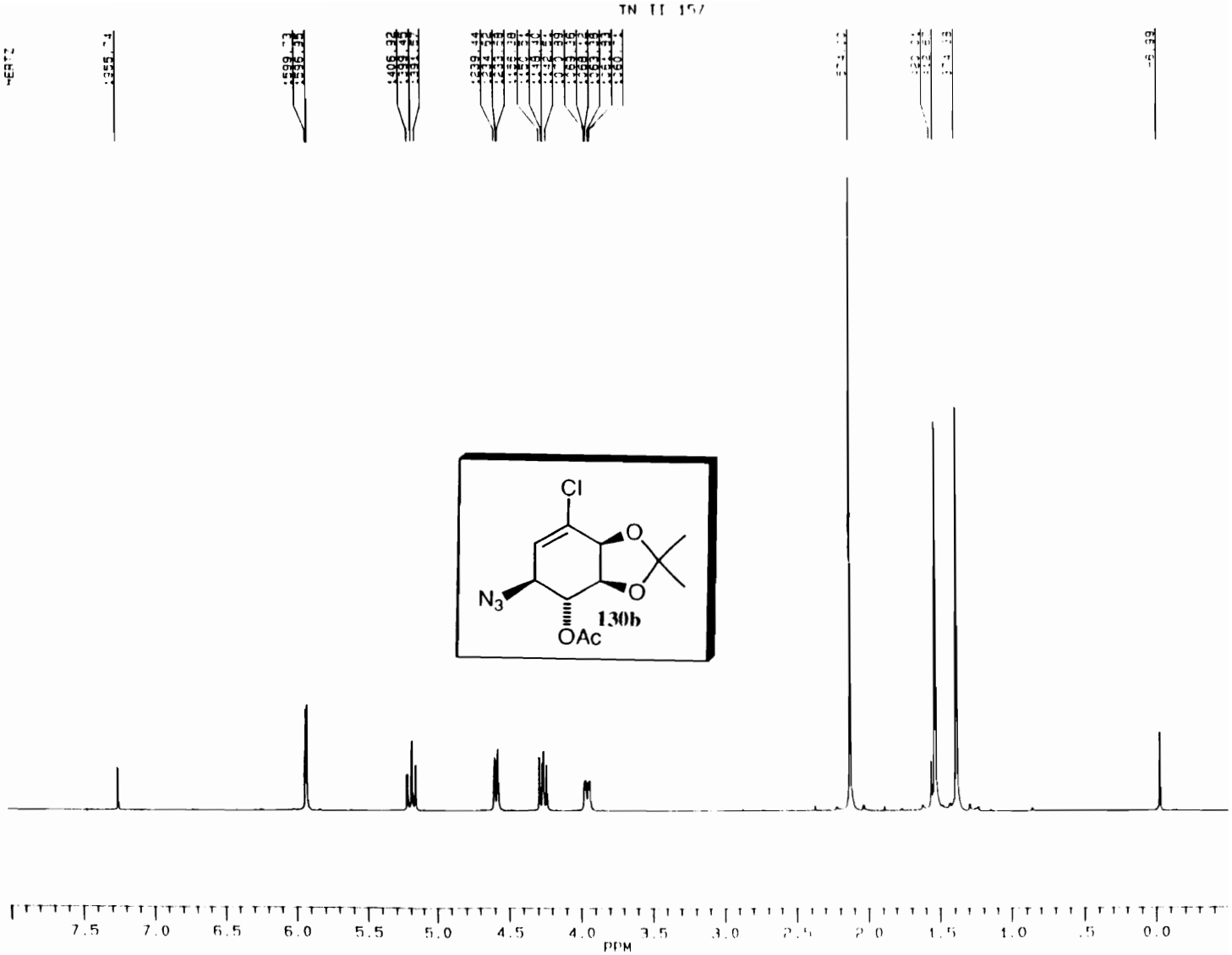
PPM

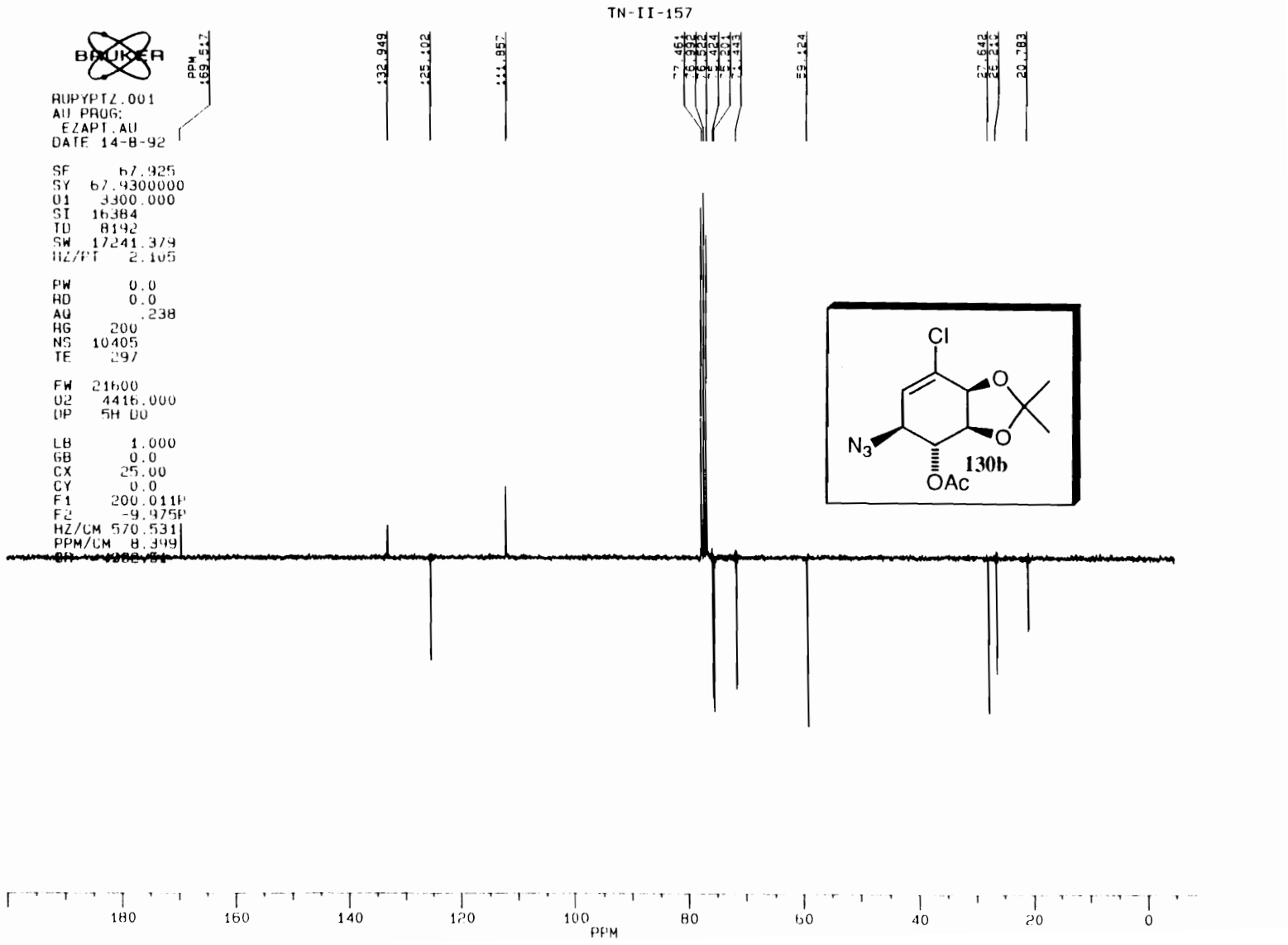

TN-II-290 -CIS-EPOXIDE, ACETONIDE
BRUKER
MC12267
DATE 9-12-93
SF 270.133
SY 120.1300000
O1 4416.000
SI 16384
TD 16384
SW 2994.012
HZ/PT .365
PW 4.0
RD 0.0
AQ 2.736
RG 40
NS 16
TE 297
FW 3800
O2 4416.000
DP 63L PO
LB 0.0
GB 0.0
CX 25.00
CY 0.0
F1 8.000P
F2 -.498P
HZ/CM 91.823
PPM/CM .340
SR 3056.41
Cl
O
O
O
125
INTEGRAL
PPM

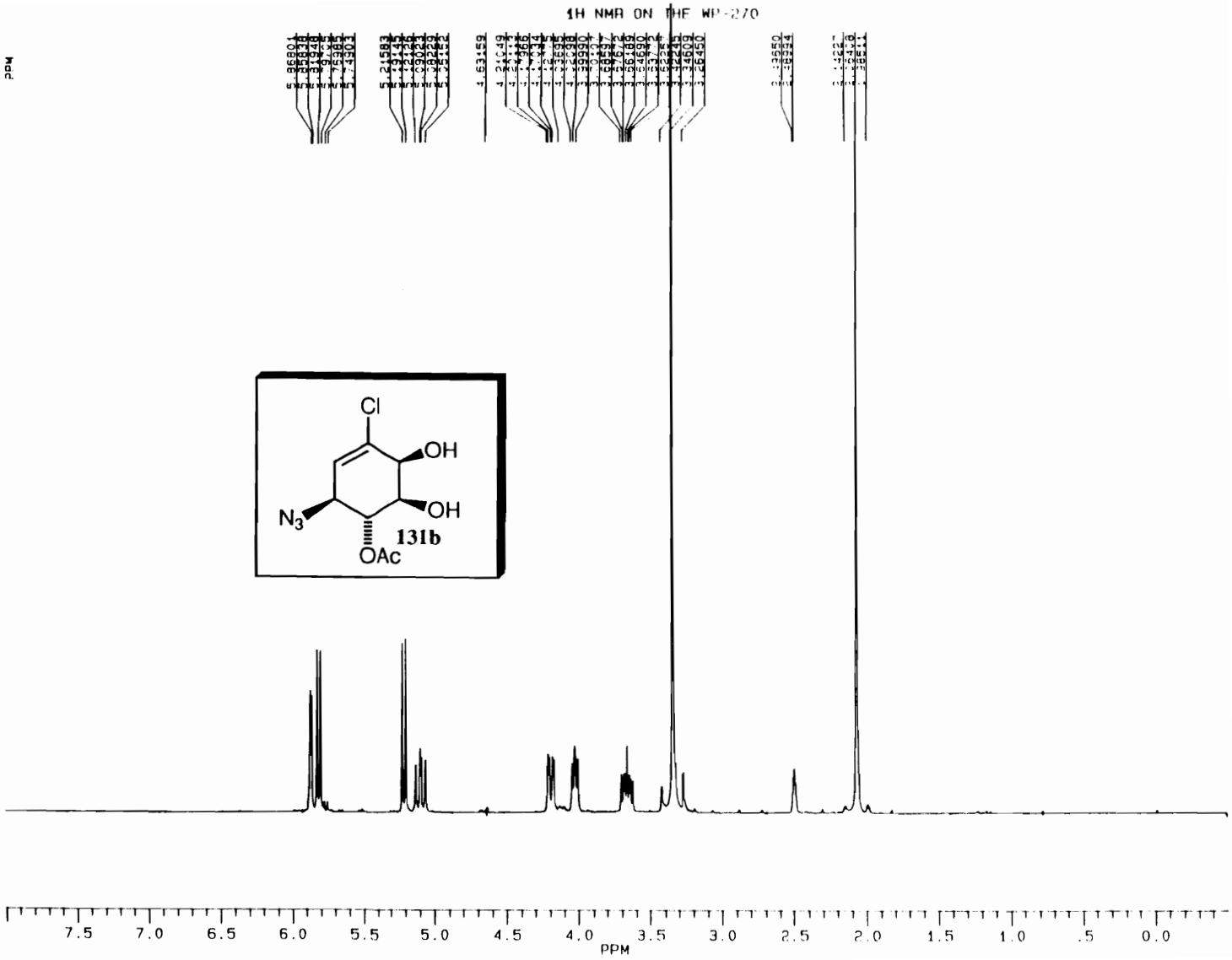

TN-II-298-CIS-EPOXIDE, ACETONIDE
BRUKER
PPM
135.746
125.561
109.069
77.458
54.534
49.642
27.147
25.321
MC12267
AU PROG:
EZAPT.AU
DATE 9-3-93
SF 67.925
67.9300000
3300.000
SI 16384
TD 8192
SW 17241.379
HZ/PT 2.105
PW 0.0
RD 0.0
AQ .238
RG 200
NS 771
TE 297
FW 21600
02 4416.000
DP 5H D0
LB 1.000
GB 0.0
CX 25.00
CY 0.0
F1 200.011P
F2 -9.975P
HZ/CM 570.531
PPM/CM 8.399
Cl
O
O
O
125
180
160
140
120
100
80
60
40
20
0
PPM

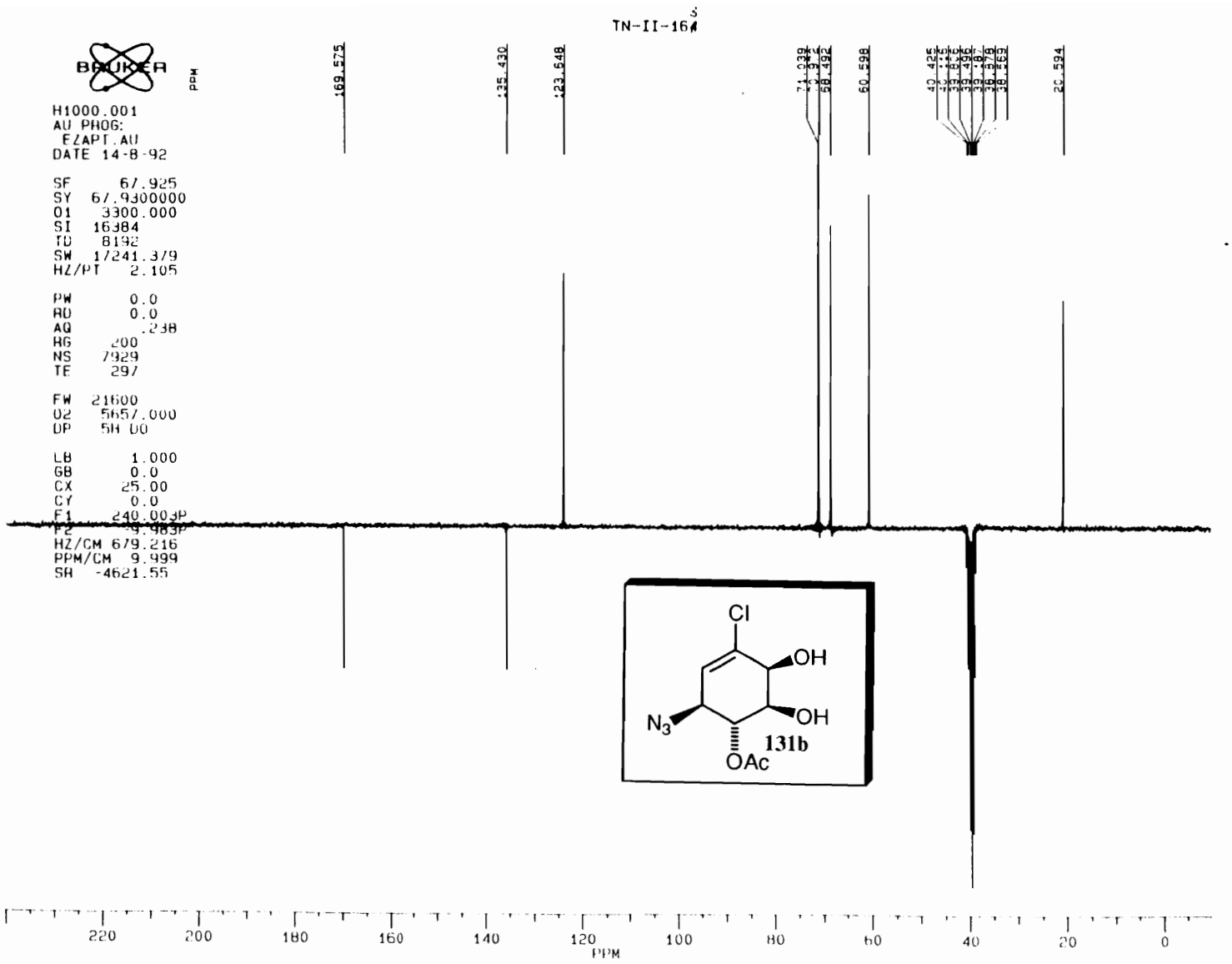

TN-III-63-BROMOHYDRIN
133.784
128.399
111.163
77.481
48.819
27.203
XL120100
AU PROG:
EZAPT.AU
DATE 9-3-93
SF 67.925
SY 67.9300000
O1 3300.000
SI 16384
TD 8192
SW 17241.379
HZ/PT 2.105
PW 0.0
RD 0.0
AQ .238
RG 200
NS 1220
TE 297
FW 21600
O2 4416.000
DP 5H DO
LB 1.000
GB 0.0
CX 25.00
CY 0.0
F1 240.012P
F2 -9.975P
HZ/CM 679.216
PPM/CM 9.999
SR -4992.51
Cl
O
O
Br
OH
128
PPM

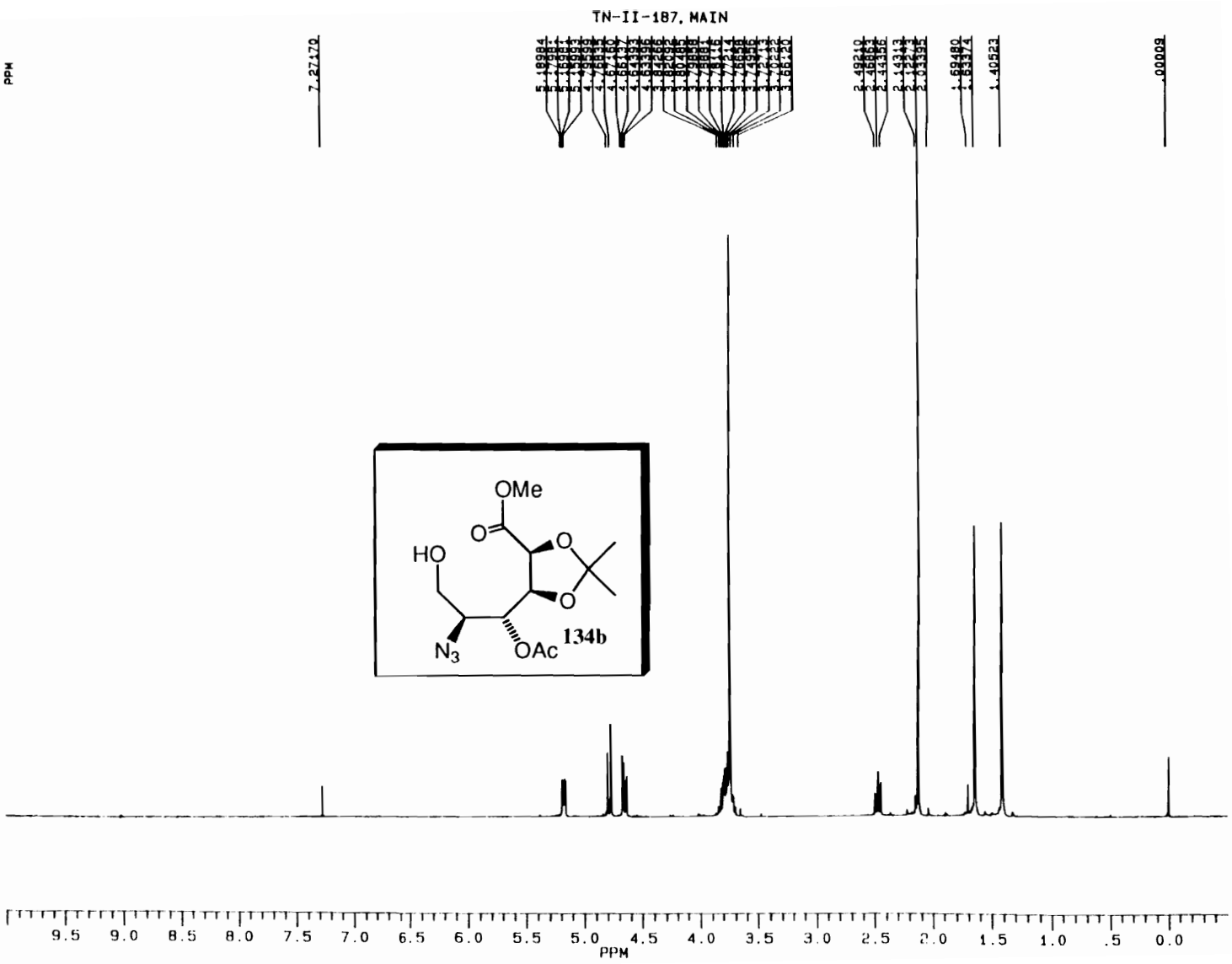

TN-III-63-BROMOHYDRIN
BRUKER
XL120100
DATE 21-3-94
SF 270.133
SY 120.1300000
O1 4416.000
SI 16384
TD 16384
SW 2994.012
HZ/PT .365
PW 4.0
RD 0.0
AQ 2.736
RG 100
NS 16
TE 297
FW 3800
O2 4416.000
DP 63L P0
LB 0.0
GB 0.0
CX 25.00
CY 0.0
F1 0.000P
F2 -.498P
HZ/CM 91.823
PPM/CM .340
SR 3065.11
Cl
O
O
Br
OH
128
INTEGRAL
2193
2436
5047
2468
2132
1.5566
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 .5 0.0
PPM

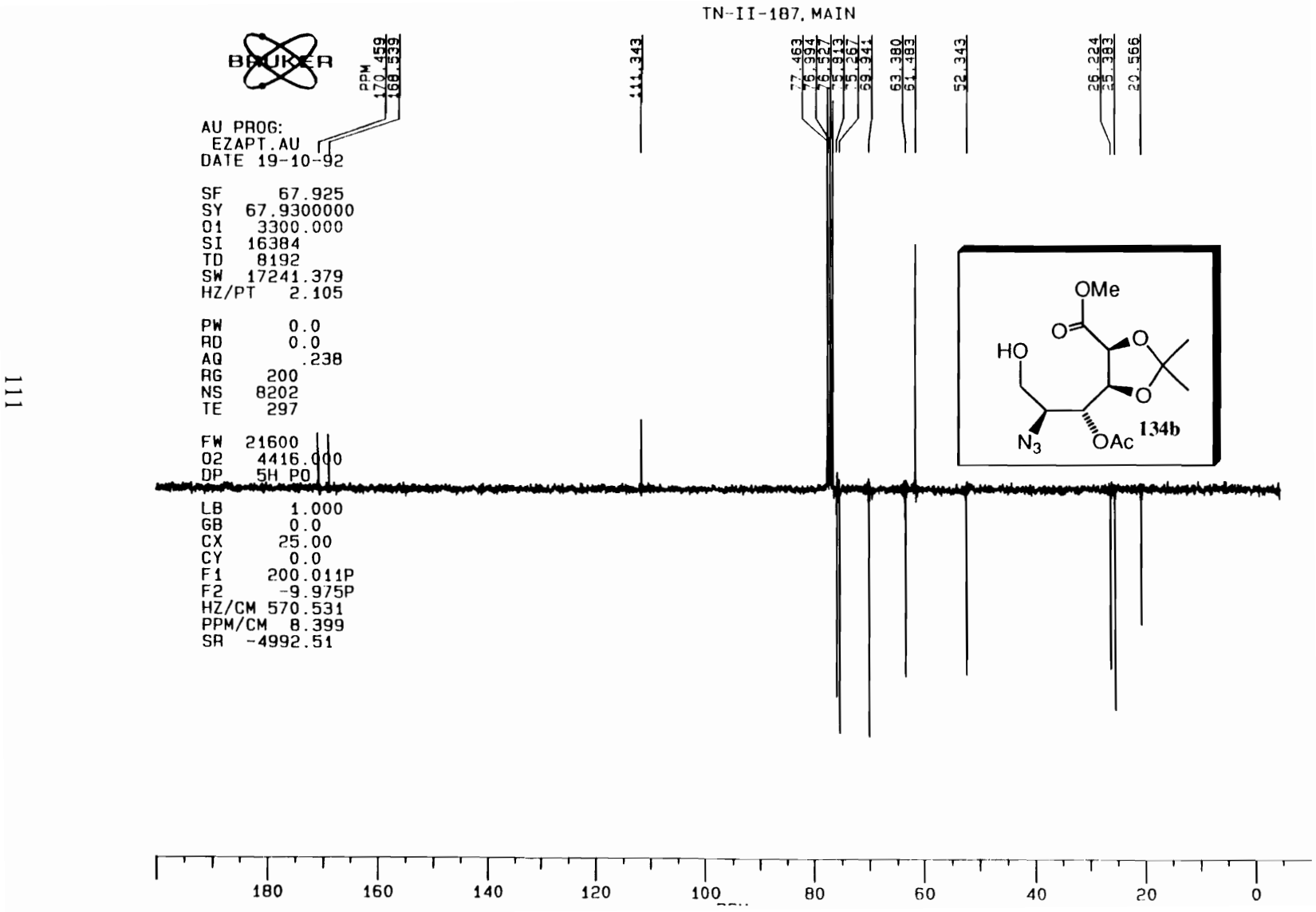

Cl
O
O
N3
OAc
130b
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
0.0
PPM

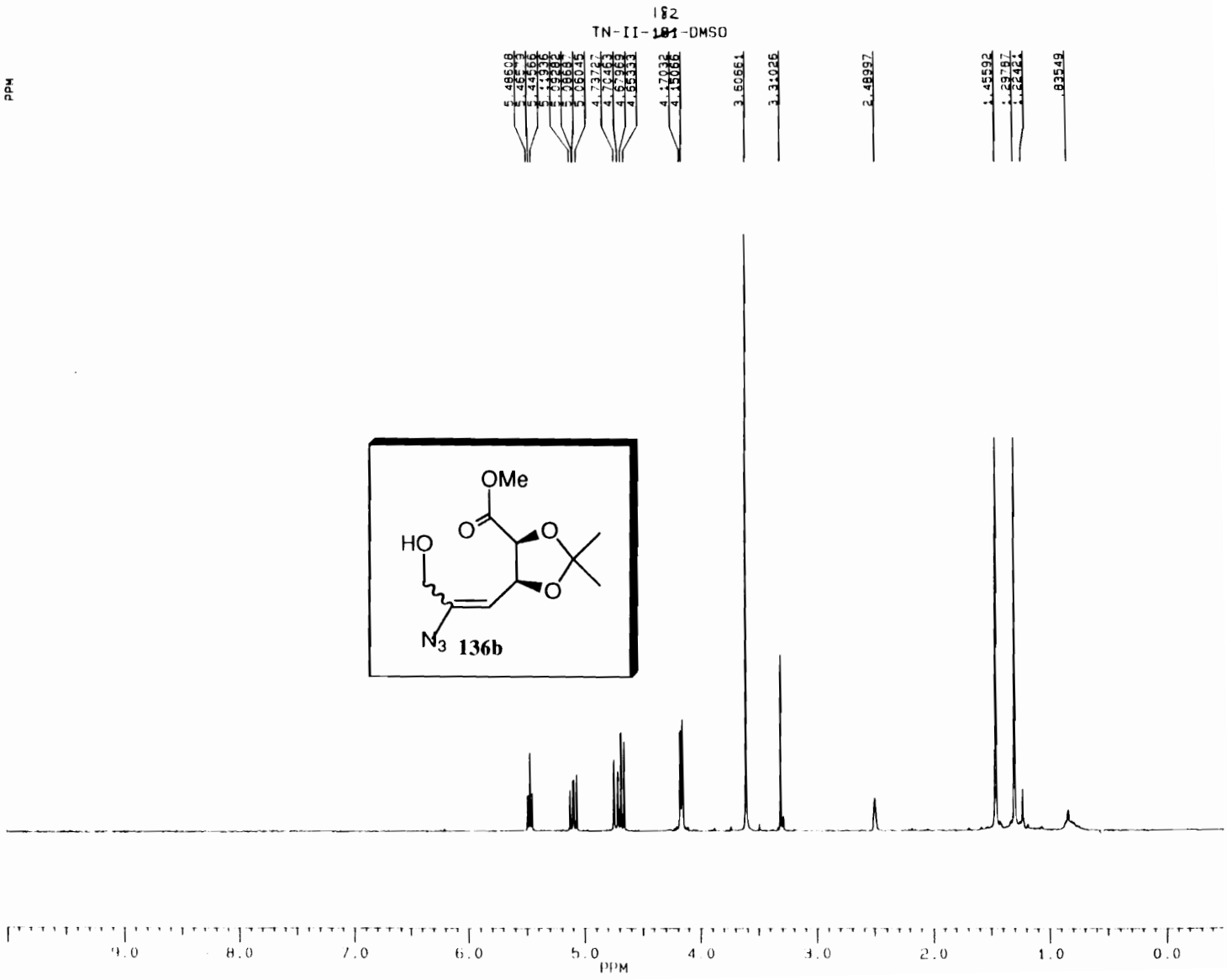

TN-II-157
BRUKER
RUPYPTZ.001
AU PROG:
EZAPT.AU
DATE 14-8-92
SF 67.925
SY 67.9300000
O1 3300.000
SI 16384
TD 8192
SW 17241.379
HZ/PT 2.105
PW 0.0
RD 0.0
AQ .238
RG 200
NS 10405
TE 297
FW 21600
O2 4416.000
DP 5H DO
LB 1.000
GB 0.0
CX 25.00
CY 0.0
F1 200.011P
F2 -9.975P
HZ/CM 570.531
PPM/CM 8.399
PPM
169.517
132.949
125.102
111.857
77.461
59.124
22.642
20.783
Cl
O
O
N3
OAc
130b
180
160
140
120
100
80
60
40
20
0
PPM

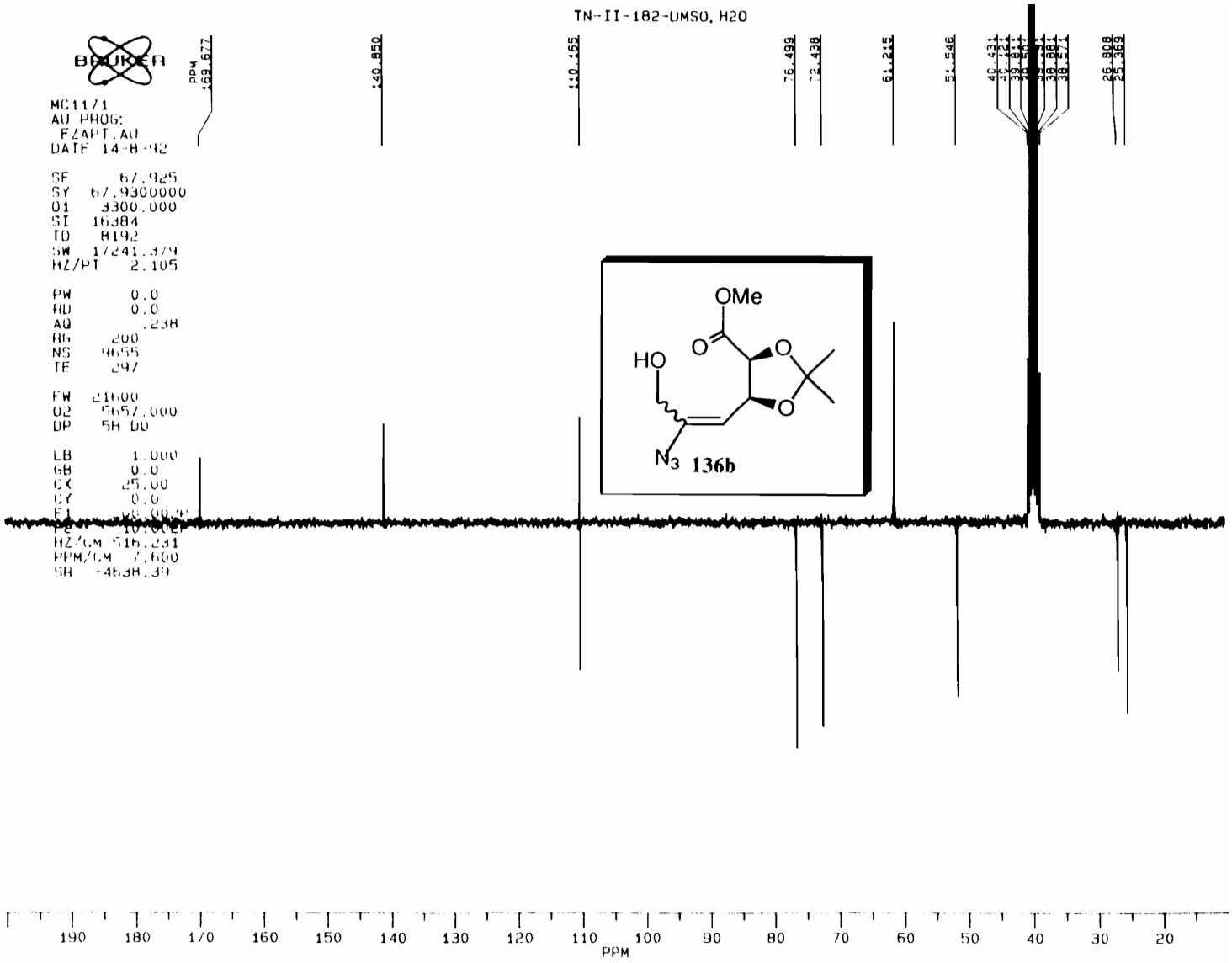

1H NMR ON THE WP-270
PPM
Cl
OH
OH
N3
OAc
131b
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
0.0
PPM

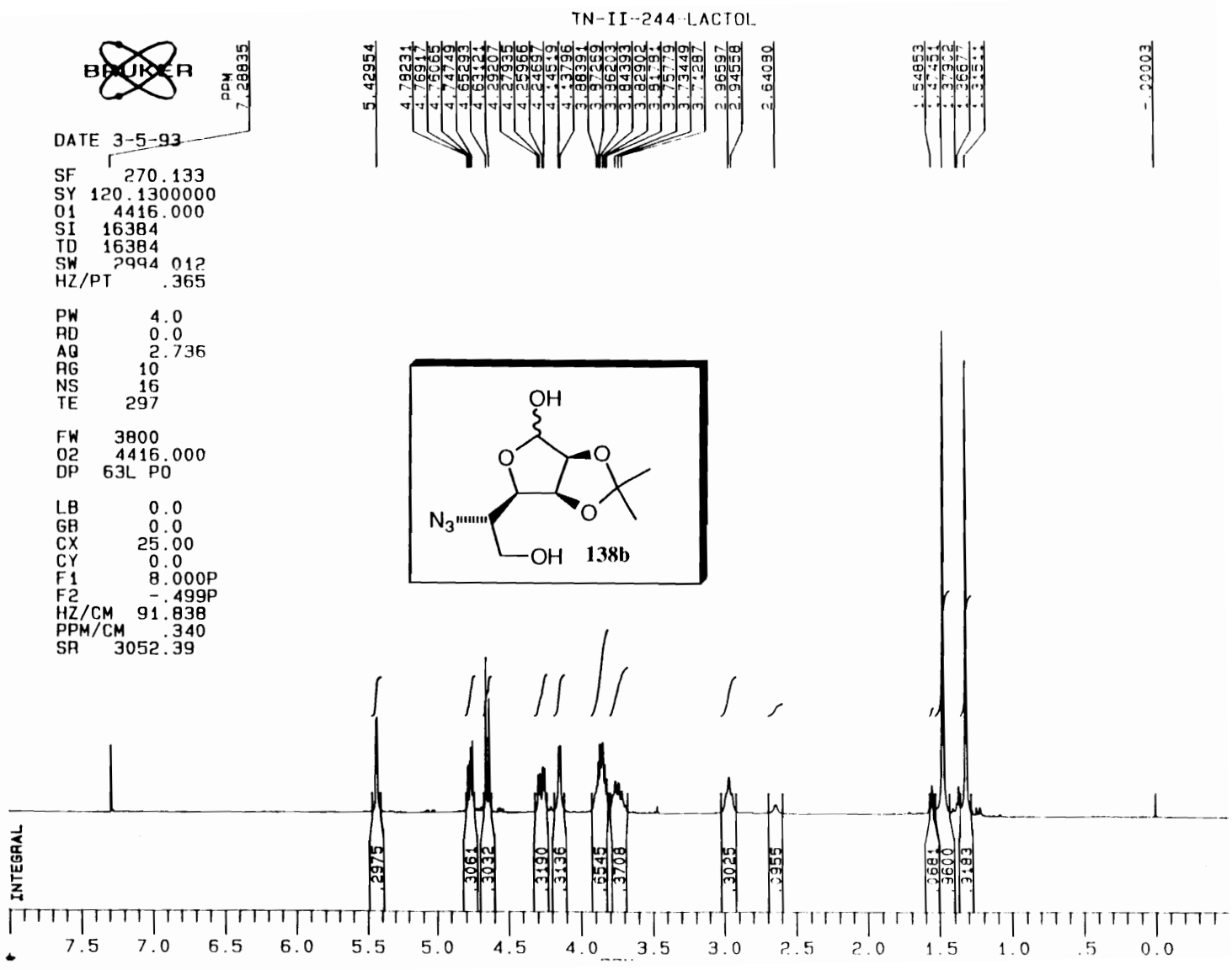

TN-II-164
BRUKER
PPM
169.575
135.430
123.648
74.039
73.844
68.492
60.598
49.425
39.495
38.169
26.594
H1000.001
AU PH06:
EZAPT.AU
DATE 14-8-92
SF 67.925
SY 67.9300000
O1 3300.000
SI 16384
TD 8192
SW 17241.379
HZ/PT 2.105
PW 0.0
RD 0.0
AQ .238
RG 200
NS 7929
TE 297
FW 21600
O2 5657.000
DP 5H D0
LB 1.000
GB 0.0
CX 25.00
CY 0.0
F1 240.003P
F2 -9.983P
HZ/CM 679.216
PPM/CM 9.999
SR -4621.55
Cl
OH
OH
N3
OAc
131b
220
200
180
160
140
120
100
80
60
40
20
0
PPM

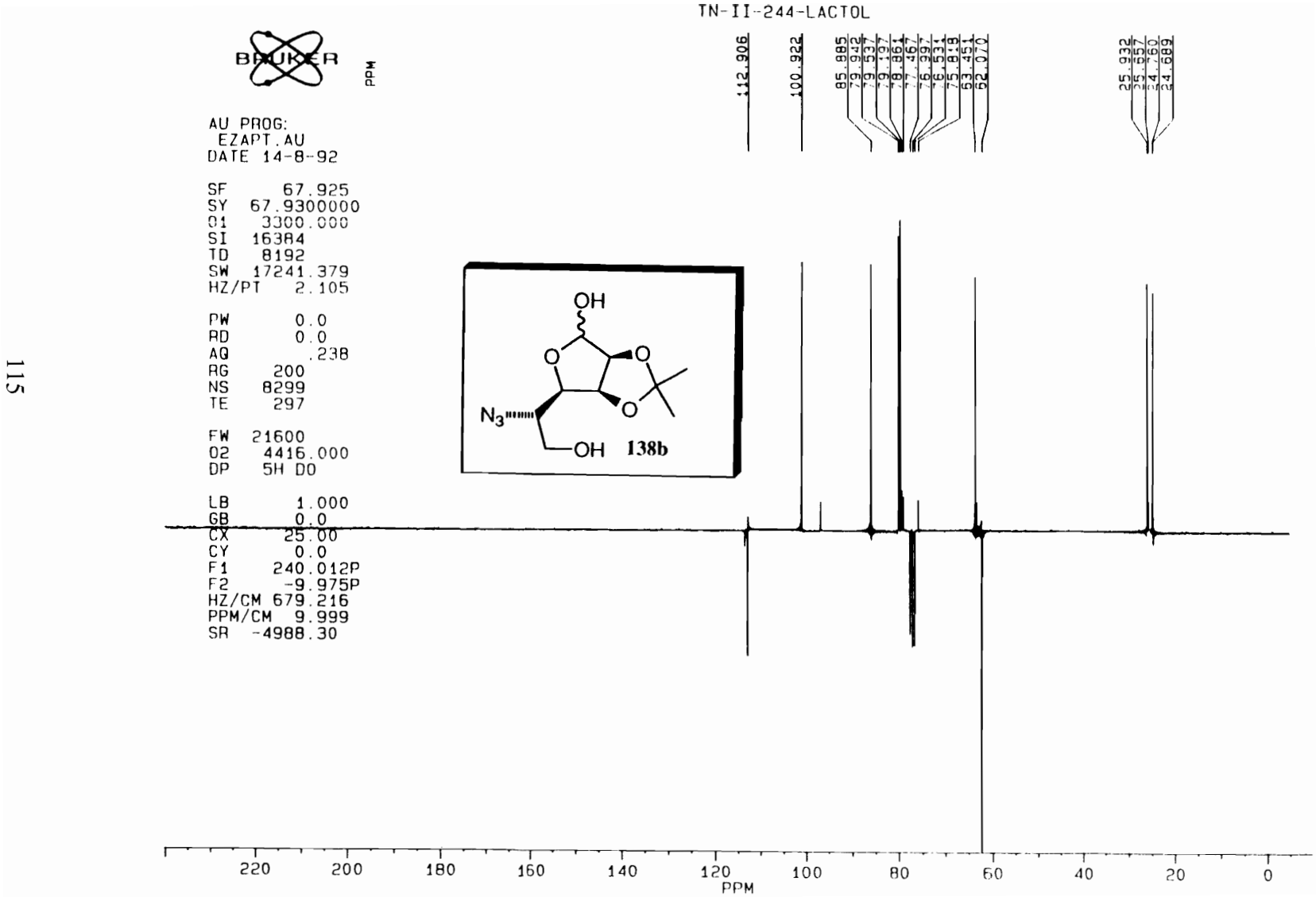

TN-II-187, MAIN
PPM
7.27170
1.40523
.00009
OMe
O
HO
O
O
N3
OAc
134b
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 .5 0.0
PPM

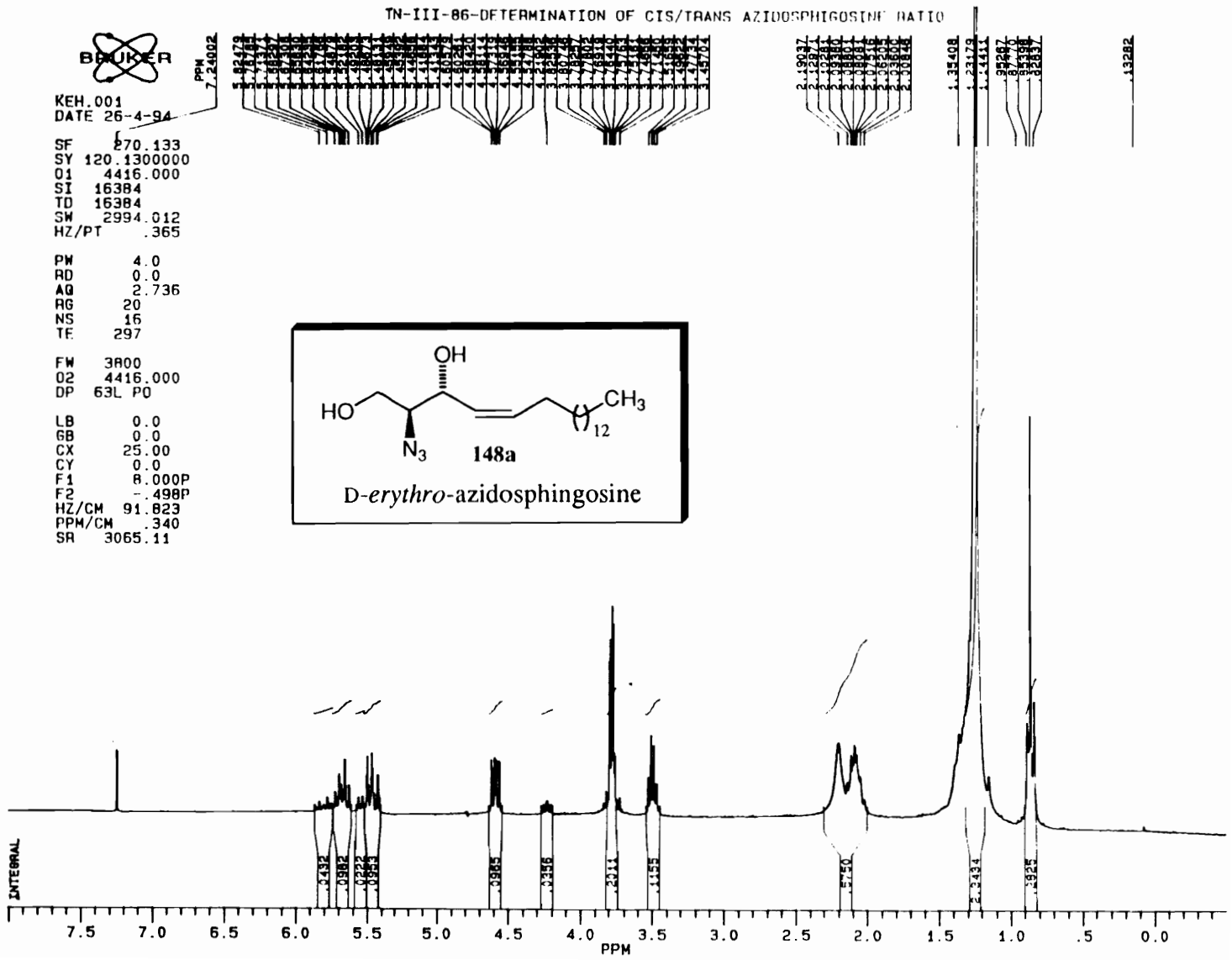

TN-II-187, MAIN
BRUKER
PPM
170.459
168.539
111.343
77.463
76.994
76.527
69.941
63.380
61.483
52.343
26.224
25.383
20.566
AU PROG:
EZAPT.AU
DATE 19-10-92
SF 67.925
SY 67.9300000
O1 3300.000
SI 16384
TD 8192
SW 17241.379
HZ/PT 2.105
PW 0.0
RD 0.0
AQ .238
RG 200
NS 8202
TE 297
FW 21600
O2 4416.000
DP 5H PO
LB 1.000
GB 0.0
CX 25.00
CY 0.0
F1 200.011P
F2 -9.975P
HZ/CM 570.531
PPM/CM 8.399
SR -4992.51
OMe
O
HO
O
O
N3
OAc
134b
180
160
140
120
100
80
60
40
20
0

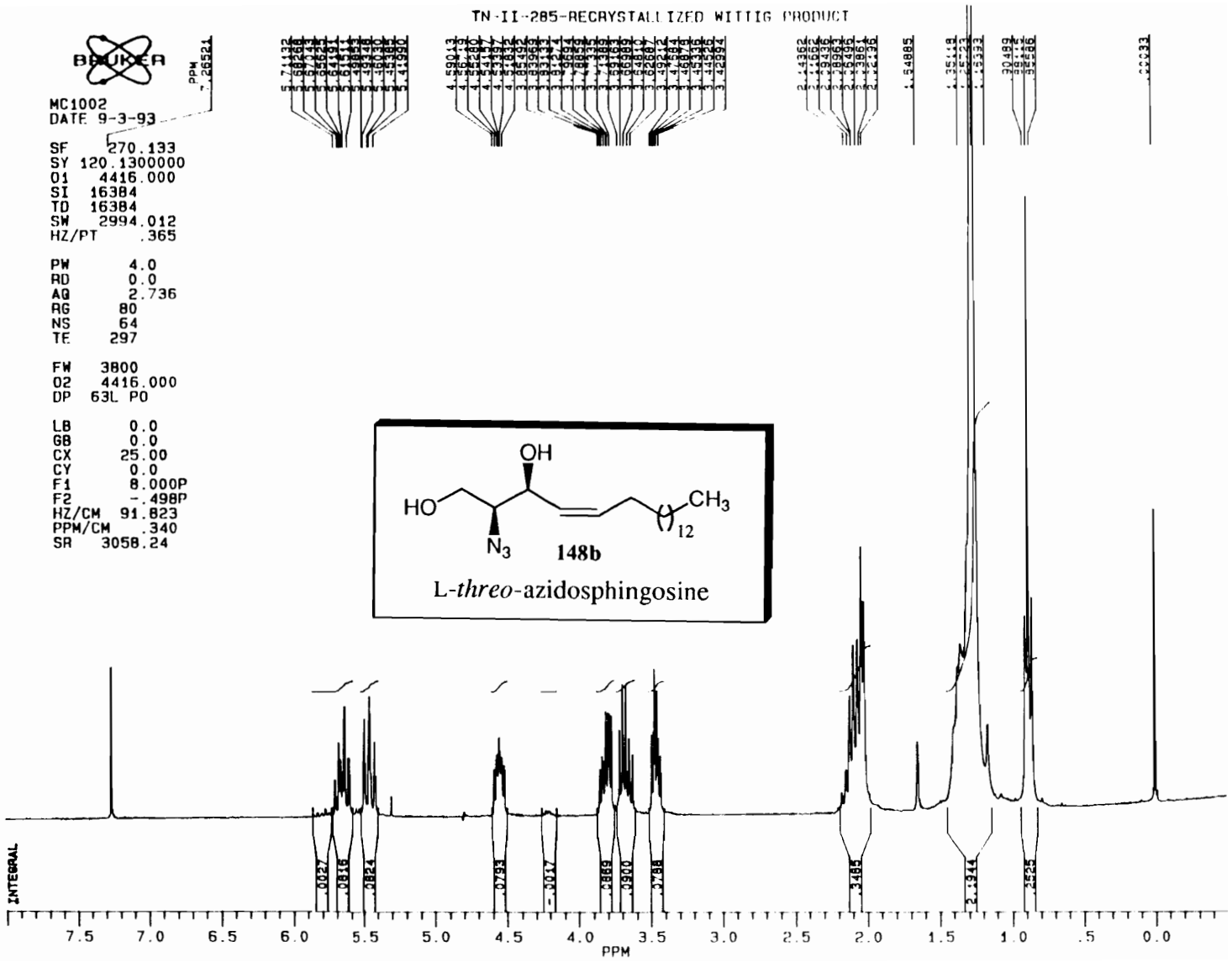

TN-II-181-DMSO
PPM
3.60661
3.31026
2.48997
1.45592
.83549
OMe
O
HO
O
O
N3
136b
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
PPM

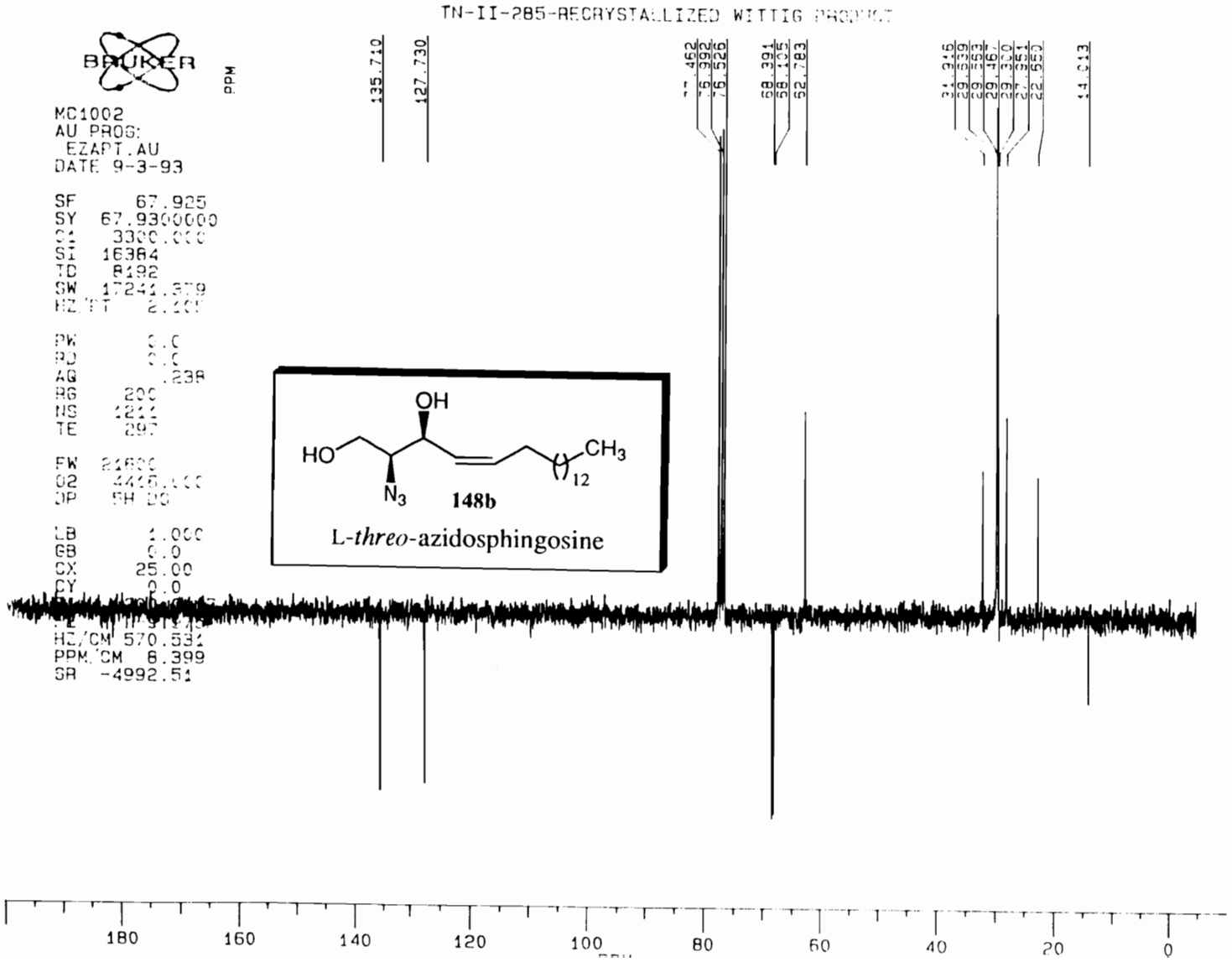

TN-II-182-DMSO, H2O
BRUKER
PPM
169.677
140.850
110.165
76.499
72.438
61.215
51.546
40.431
26.808
25.369
MC11/1
AU PROG:
EZAPT.AU
DATE 14-8-92
SF 67.925
SY 67.9300000
O1 3300.000
SI 16384
TD 8192
SW 17241.379
HZ/PT 2.105
PW 0.0
RD 0.0
AQ .238
RG 200
NS 9655
TE 297
FW 21600
O2 5657.000
DP 5H DD
LB 1.000
GB 0.0
GX 25.00
GY 0.0
HZ/CM 516.231
PPM/CM 7.600
SR -4638.39
OMe
O
O
O
HO
N3
136b
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
PPM

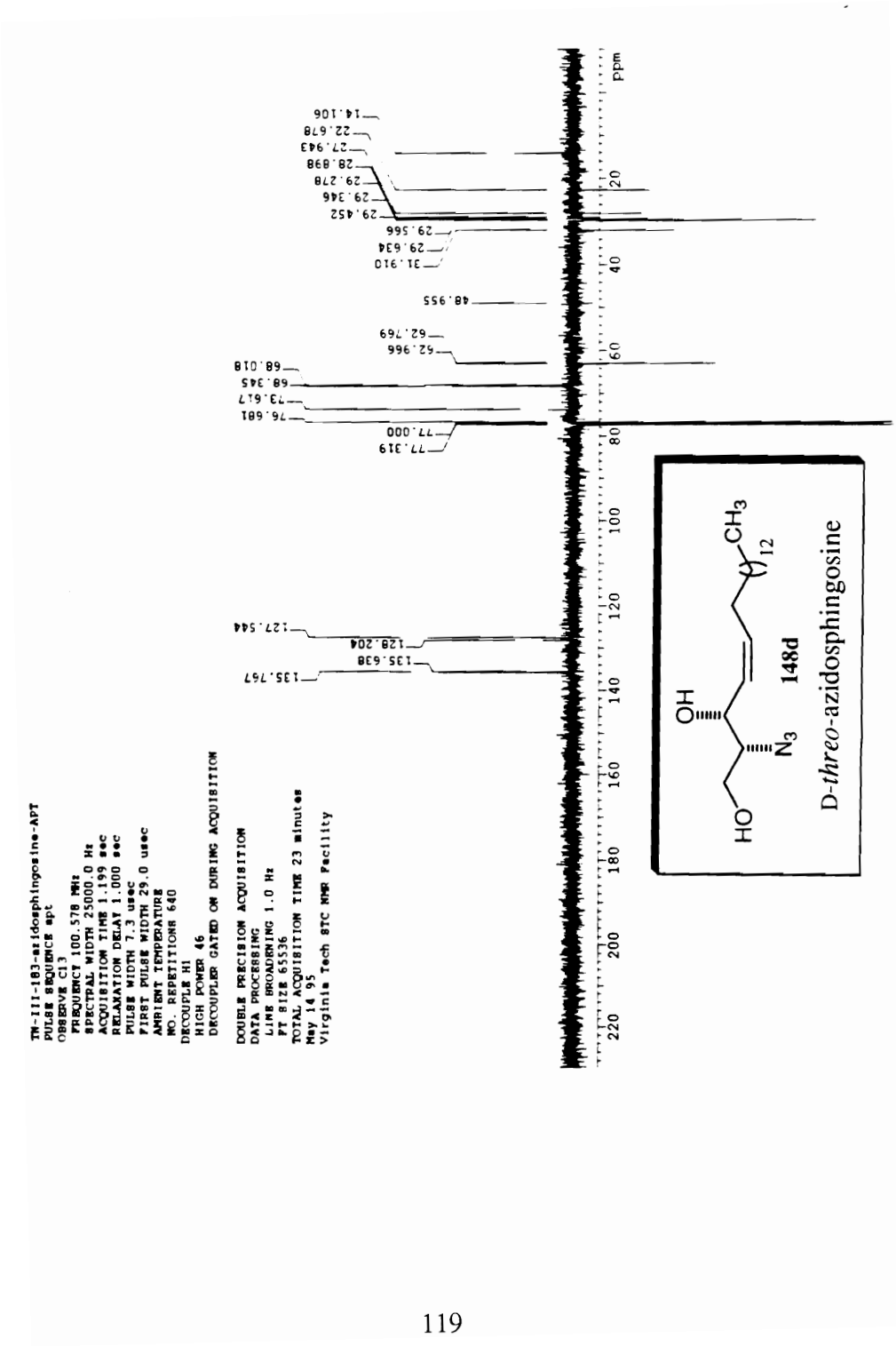

TN-II-244-LACTOL
BRUKER
DATE 3-5-93
SF 270.133
SY 120.1300000
O1 4416.000
SI 16384
TD 16384
SW 2994.012
HZ/PT .365
PW 4.0
RD 0.0
AQ 2.736
RG 10
NS 16
TE 297
FW 3800
O2 4416.000
DP 63L PO
LB 0.0
GB 0.0
CX 25.00
CY 0.0
F1 8.000P
F2 -.499P
HZ/CM 91.838
PPM/CM .340
SR 3052.39
OH
O
O
O
N3
OH
138b
INTEGRAL
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
0.0

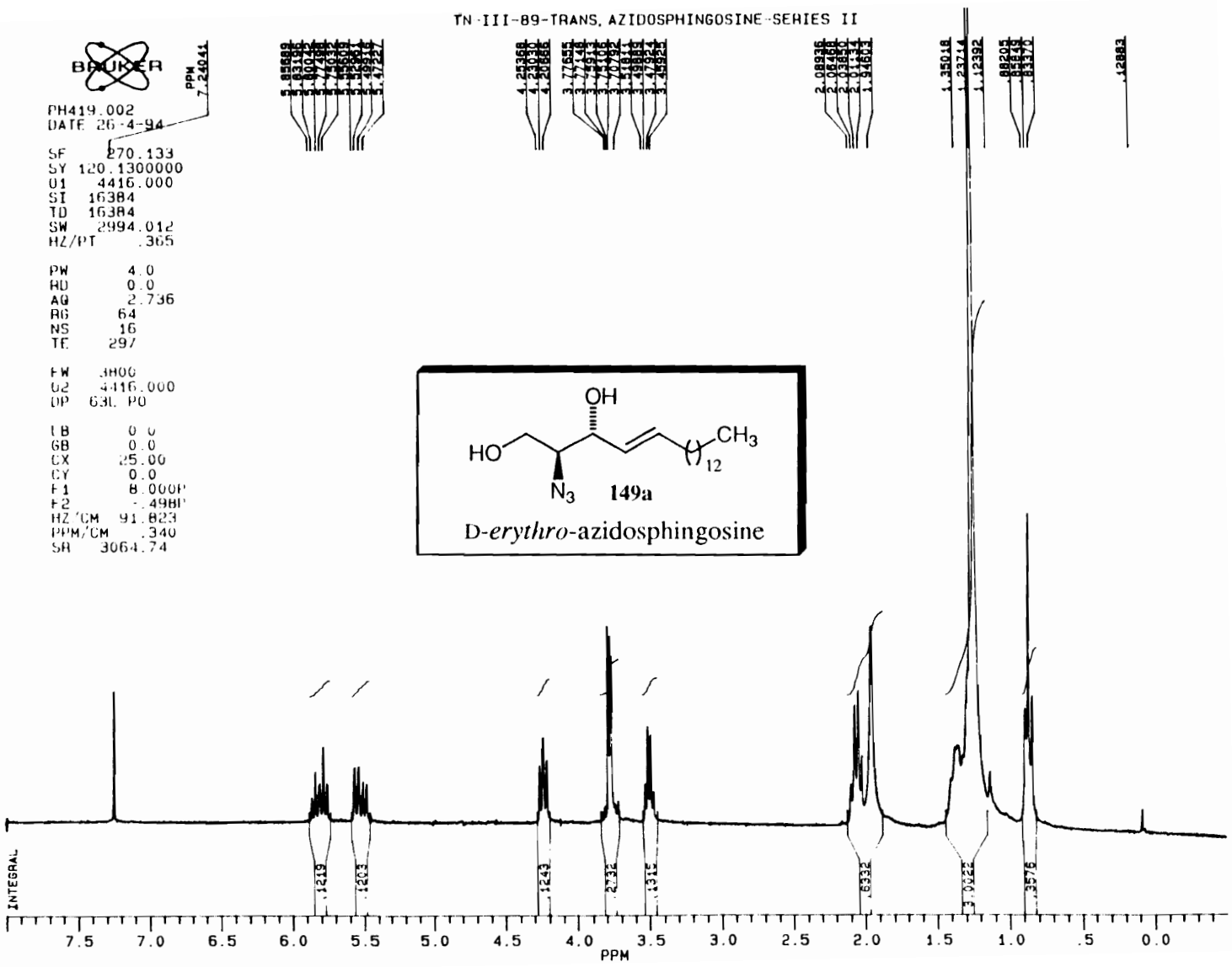

TN-II-244-LACTOL
BRUKER
PPM
AU PROG:
EZAPT.AU
DATE 14-8-92
SF 67.925
SY 67.9300000
O1 3300.000
SI 16384
TD 8192
SW 17241.379
HZ/PT 2.105
PW 0.0
RD 0.0
AQ .238
RG 200
NS 8299
TE 297
FW 21600
O2 4416.000
DP 5H D0
LB 1.000
GB 0.0
CX 25.00
CY 0.0
F1 240.012P
F2 -9.975P
HZ/CM 679.216
PPM/CM 9.999
SR -4988.30
112.906
100.922
85.985
79.942
79.537
79.197
78.861
77.467
76.997
76.531
75.818
63.451
52.070
25.932
25.557
24.760
24.689
OH
O
O
O
N3
OH
138b
220
200
180
160
140
120
100
80
60
40
20
0
PPM

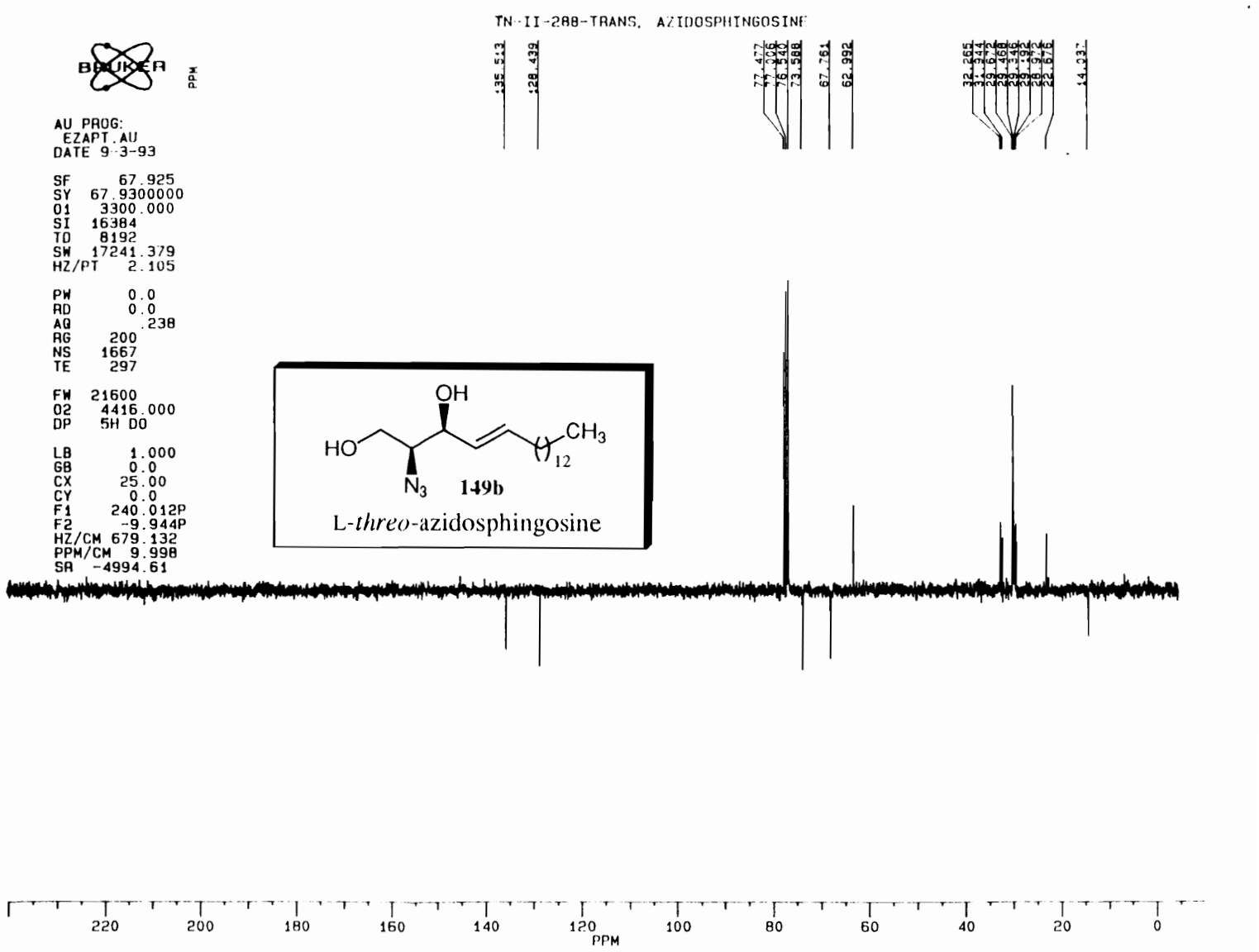

TN-III-86-DETERMINATION OF CIS/TRANS AZIDOSPHIGOSINE RATIO
BRUKER
KEH.001
DATE 26-4-94
SF 270.133
SY 120.1300000
O1 4416.000
SI 16384
TD 16384
SW 2994.012
HZ/PT .365
PW 4.0
RD 0.0
AQ 2.736
RG 20
NS 16
TE 297
FW 3800
O2 4416.000
DP 63L PO
LB 0.0
GB 0.0
CX 25.00
CY 0.0
F1 8.000P
F2 -.498P
HZ/CM 91.823
PPM/CM .340
SR 3065.11
OH
HO
CH3
12
N3
148a
D-erythro-azidosphingosine
INTEGRAL
PPM

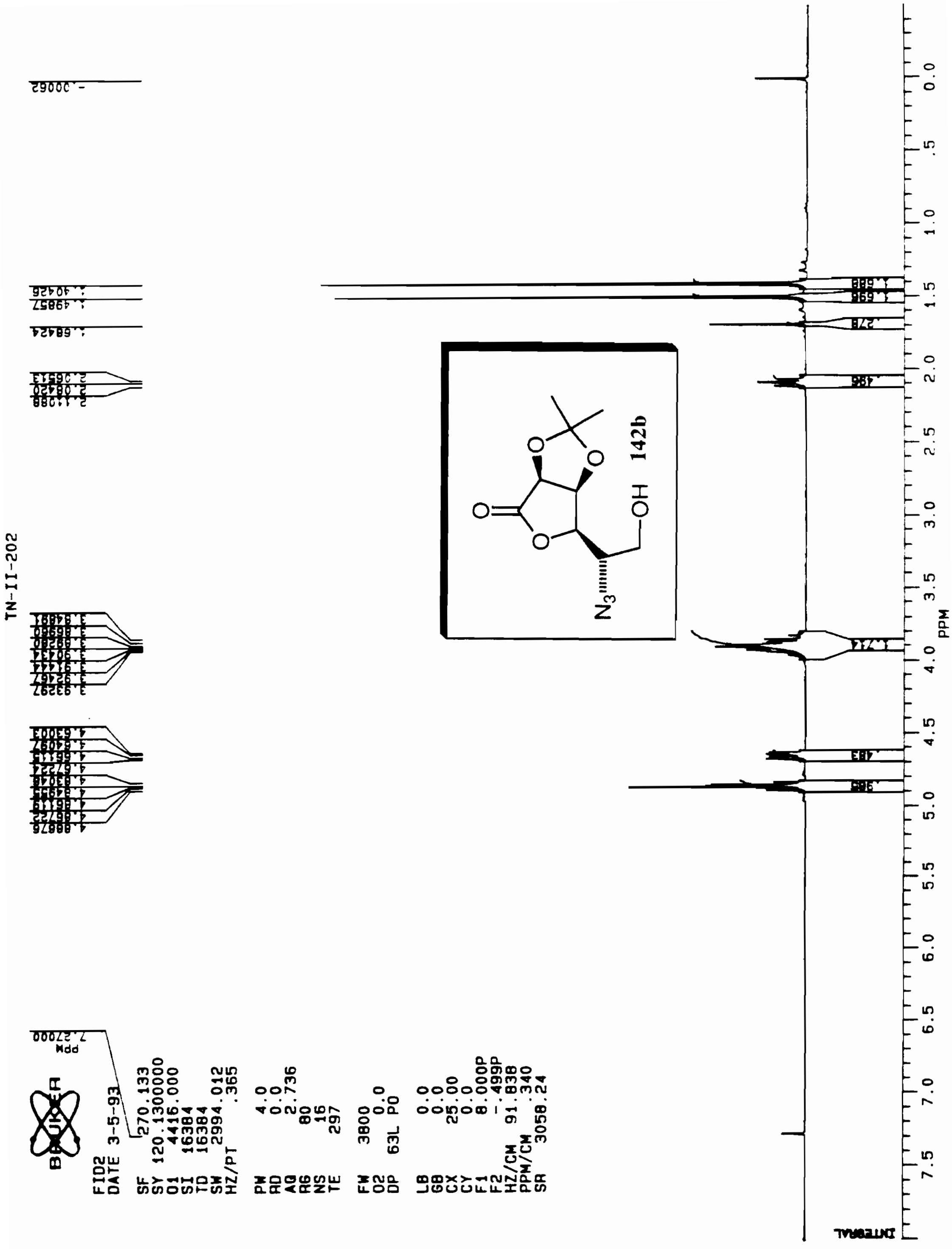

TN-II-285-RECRYSTALLIZED WITTIG PRODUCT
BRUKER
MC1002
DATE 9-3-93
SF 270.133
SY 120.1300000
O1 4416.000
SI 16384
TD 16384
SW 2994.012
HZ/PT .365
PW 4.0
RD 0.0
AQ 2.736
RG 80
NS 64
TE 297
FW 3800
O2 4416.000
DP 63L PO
LB 0.0
GB 0.0
CX 25.00
CY 0.0
F1 8.000P
F2 -.498P
HZ/CM 91.823
PPM/CM .340
SR 3058.24
OH
HO
N3
CH3
12
148b
L-threo-azidosphingosine
INTEGRAL
.0027
.0816
.0824
.0793
-.0017
.0869
.0900
.0788
.3485
2.1944
.3525
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
0.0
PPM

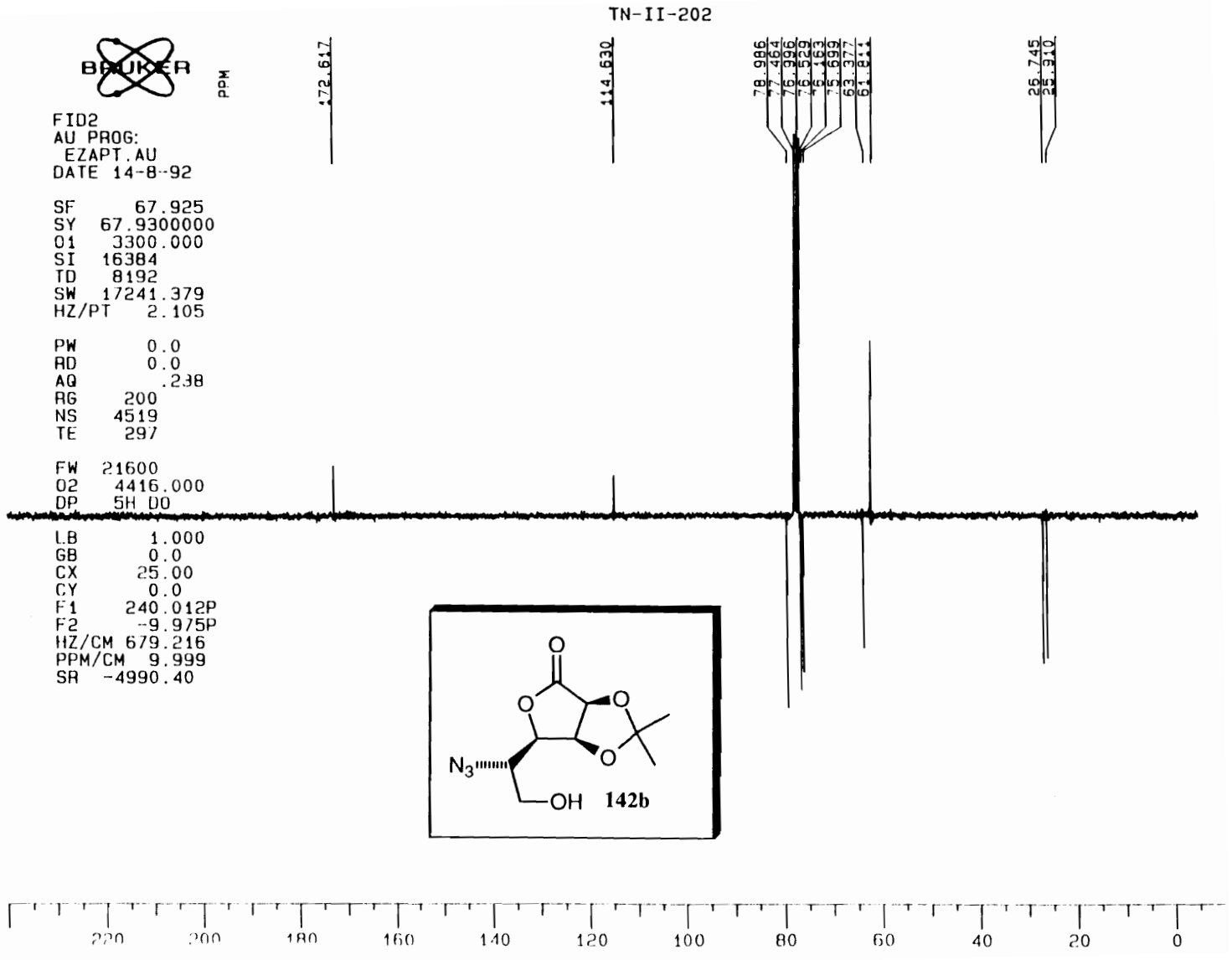

TN-II-285-RECRYSTALLIZED WITTIG PRODUCT
BRUKER
PPM
135.710
127.730
77.462
76.992
76.526
68.391
52.783
31.915
29.639
29.563
29.462
29.330
22.560
14.013
MC1002
AU PROG:
EZAPT.AU
DATE 9-3-93
SF 67.925
SY 67.9300000
SI 16384
TD 8192
SW 17241.379
PW 9.0
AQ .238
RG 200
NS 1211
TE 297
LB 1.000
GB 0.0
SX 25.00
CY 0.0
HZ/CM 570.532
PPM/CM 8.399
SR -4992.51
OH
HO
CH3
12
N3
148b
L-threo-azidosphingosine
180
160
140
120
100
80
60
40
20
0

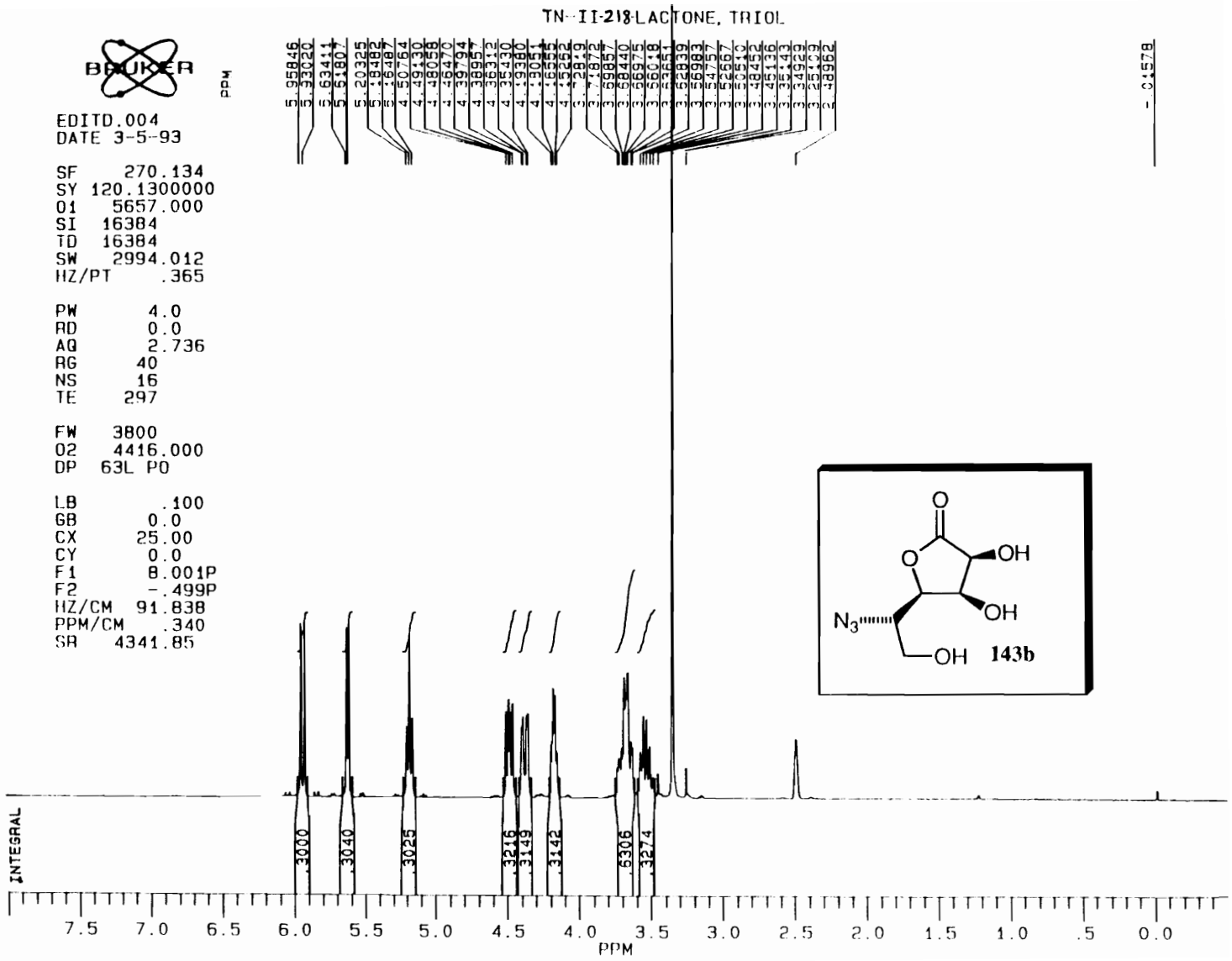

TN-III-183-azidosphingosine-APT
PULSE SEQUENCE apt
OBSERVE C13
FREQUENCY 100.578 MHz
SPECTRAL WIDTH 25000.0 Hz
ACQUISITION TIME 1.199 sec
RELAXATION DELAY 1.000 sec
PULSE WIDTH 7.3 usec
FIRST PULSE WIDTH 29.0 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 640
DECOUPLE H1
HIGH POWER 46
DECOUPLER GATED ON DURING ACQUISITION
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
LINE BROADENING 1.0 Hz
FT SIZE 65536
TOTAL ACQUISITION TIME 23 minutes
May 14 95
Virginia Tech STC NMR Facility
135.767
135.638
128.204
127.544
77.319
77.000
76.681
73.617
68.345
68.018
62.769
62.966
48.955
31.310
29.634
29.566
29.452
29.346
29.278
28.898
27.943
22.678
14.106
ppm
OH
N3
HO
CH3
12
148d
D-threo-azidosphingosine

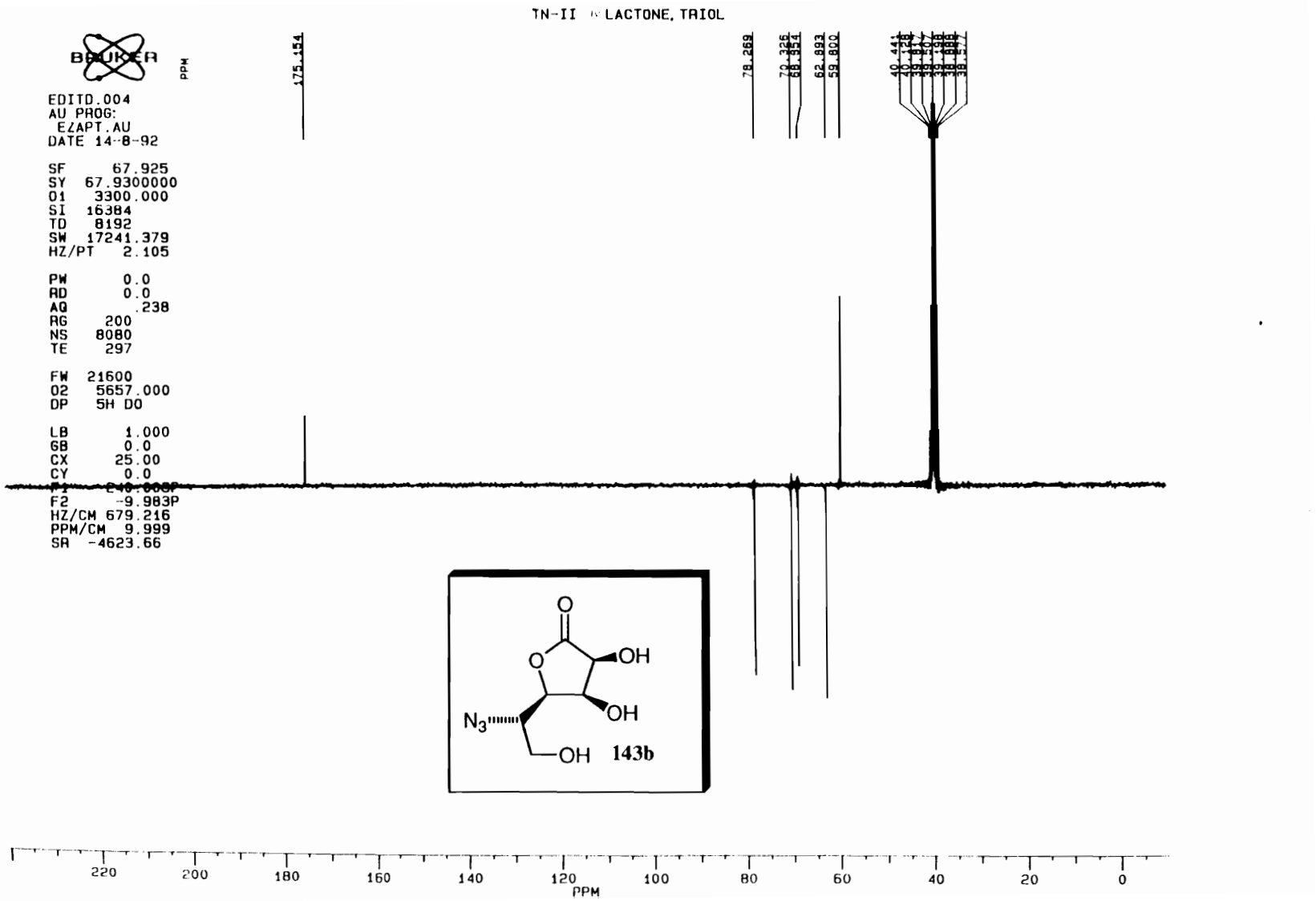

TN-III-89-TRANS, AZIDOSPHINGOSINE-SERIES II
BRUKER
PH419.002
DATE 26-4-94
SF 270.133
SY 120.1300000
O1 4416.000
SI 16384
TD 16384
SW 2994.012
HZ/PT .365
PW 4.0
RD 0.0
AQ 2.736
RG 64
NS 16
TE 297
O2 4416.000
CX 25.00
CY 0.0
HZ/CM 91.823
PPM/CM .340
SR 3064.74
OH
HO
CH3
12
N3
149a
D-erythro-azidosphingosine
INTEGRAL
PPM

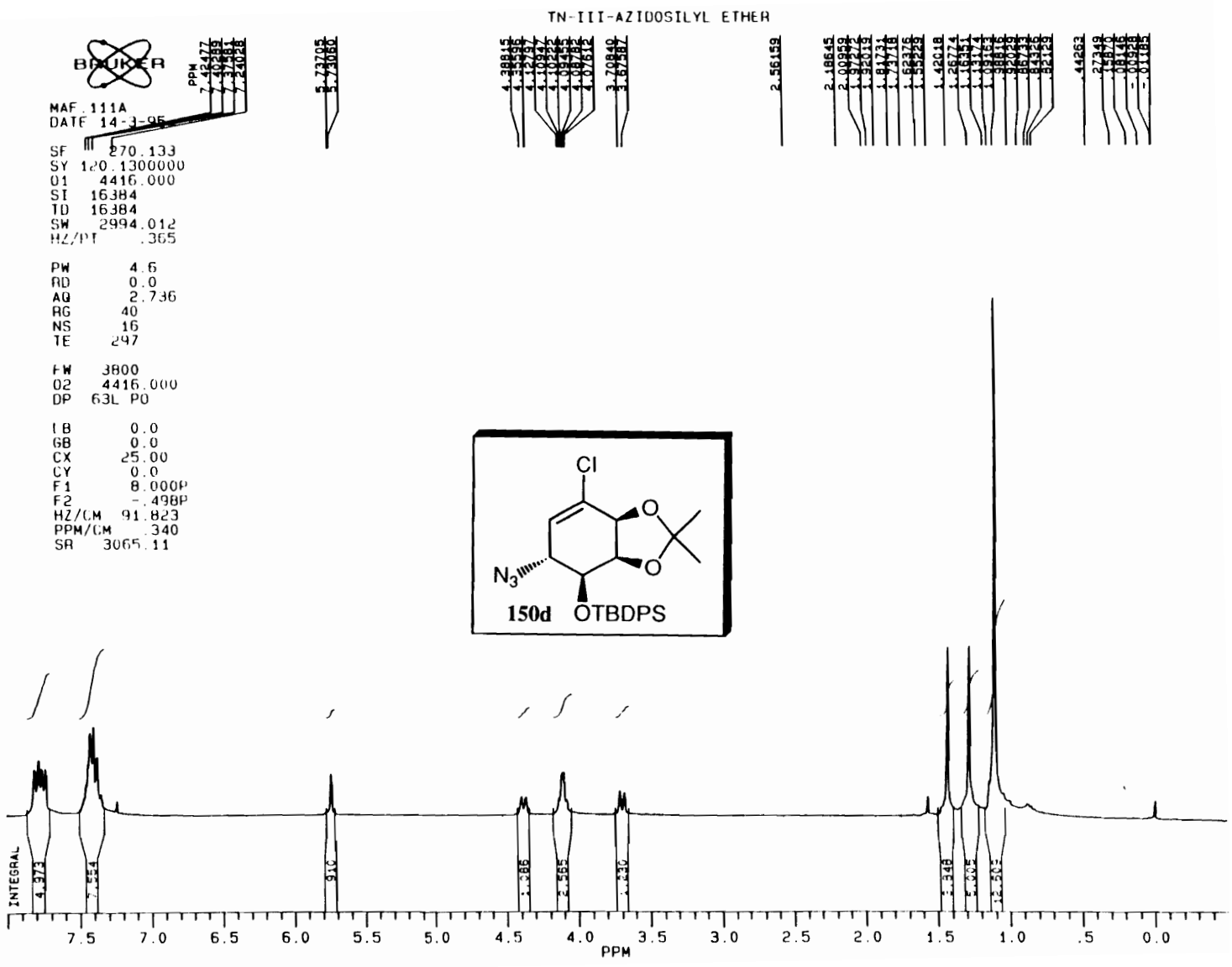

TN-II-288-TRANS. AZIDOSPHINGOSINE
BRUKER
PPM
135.513
128.439
77.477
77.056
76.540
73.588
67.761
62.992
32.265
31.944
29.675
29.468
29.346
29.192
28.972
22.676
14.037
AU PROG:
EZAPT.AU
DATE 9-3-93
SF 67.925
SY 67.9300000
O1 3300.000
SI 16384
TD 8192
SW 17241.379
HZ/PT 2.105
PW 0.0
RD 0.0
AQ .238
RG 200
NS 1667
TE 297
FW 21600
O2 4416.000
DP 5H D0
LB 1.000
GB 0.0
CX 25.00
CY 0.0
F1 240.012P
F2 -9.944P
HZ/CM 679.132
PPM/CM 9.998
SR -4994.61
OH
HO
CH3
12
N3
149b
L-threo-azidosphingosine
220
200
180
160
140
120
100
80
60
40
20
0
PPM

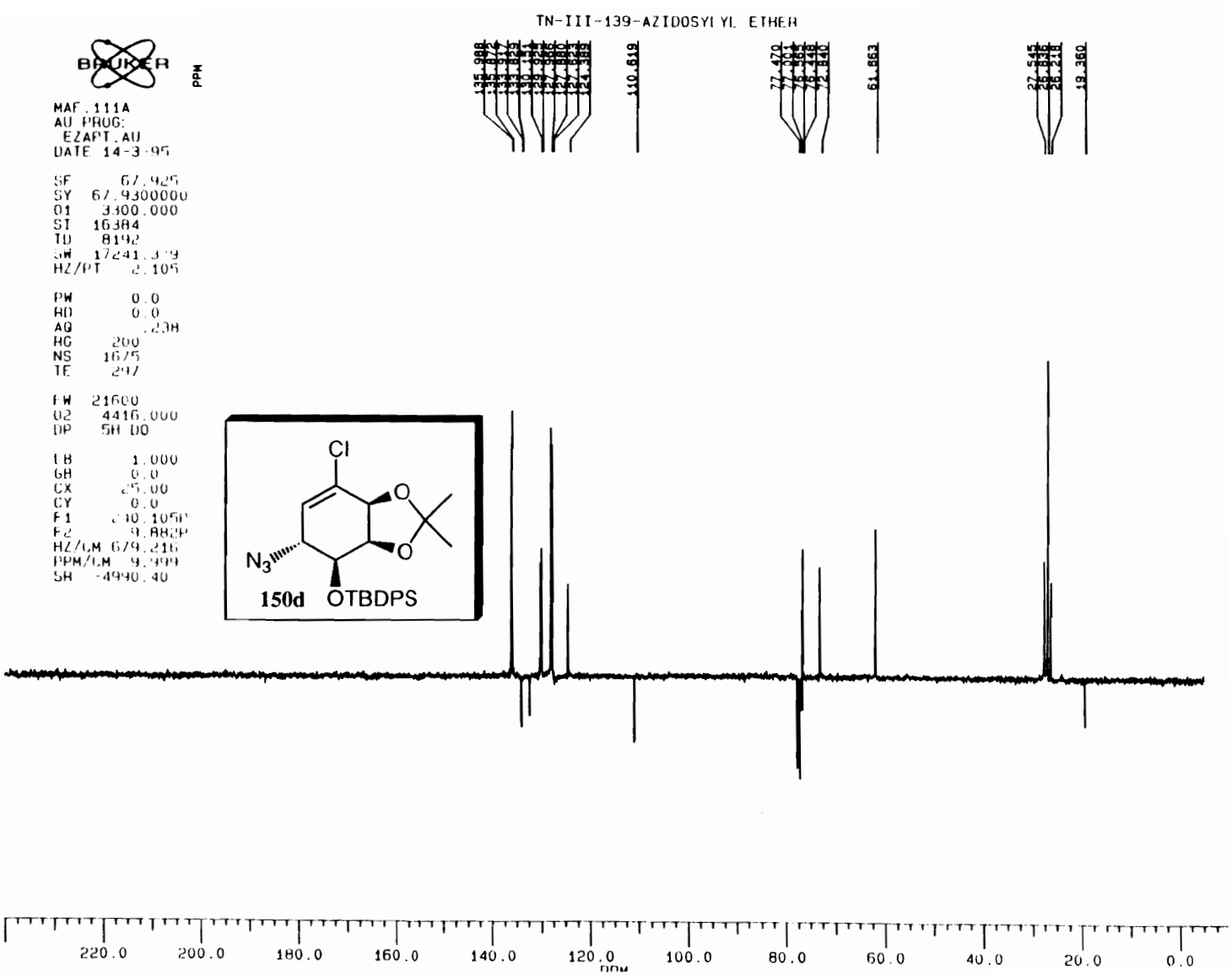

TN-II-202
142b
PPM
INTEGRAL

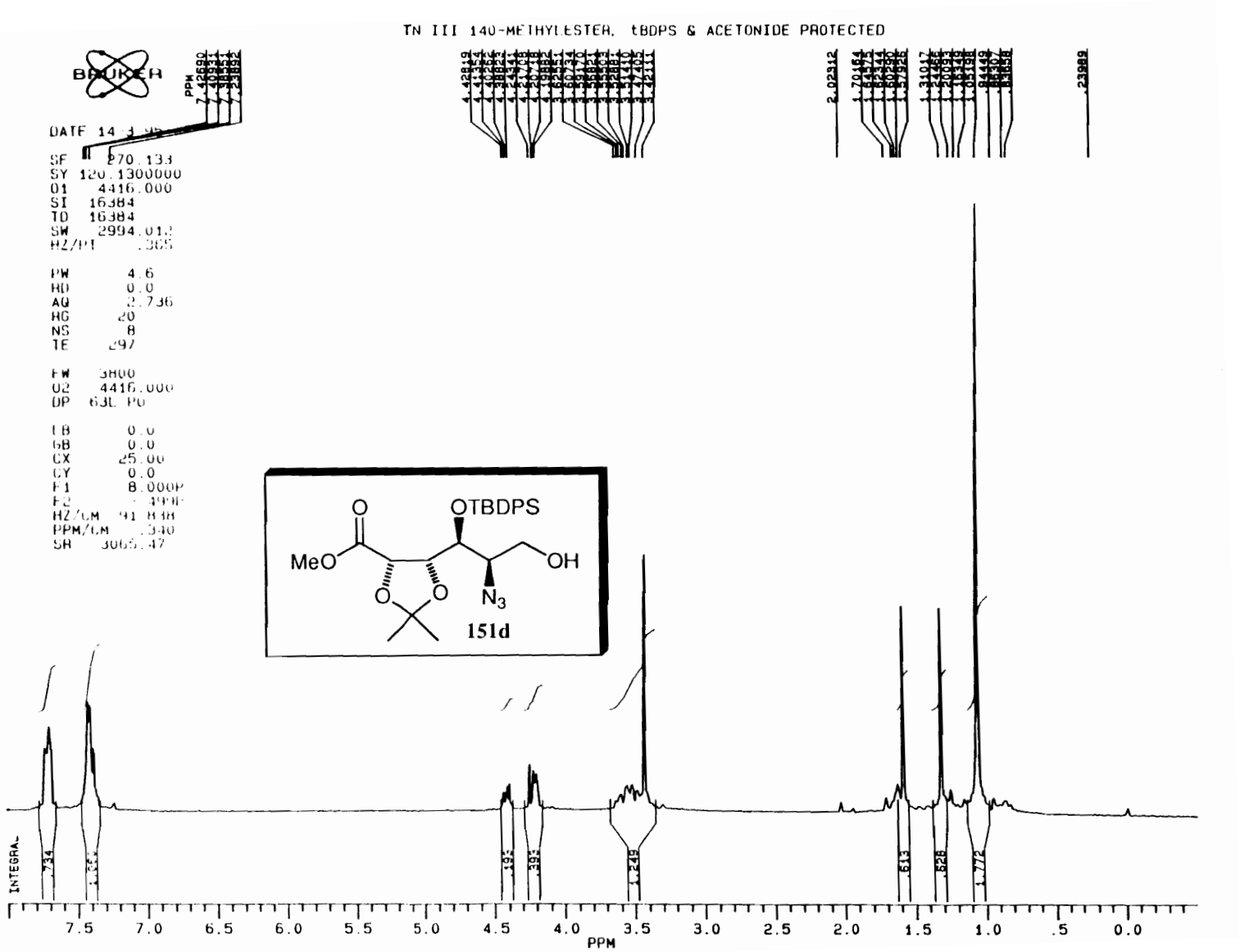

TN-II-202
BRUKER
PPM
172.617
114.630
78.986
77.464
76.996
76.529
76.163
75.699
63.377
61.844
26.745
25.910
FID2
AU PROG:
EZAPT.AU
DATE 14-8-92
SF 67.925
SY 67.9300000
O1 3300.000
SI 16384
TD 8192
SW 17241.379
HZ/PT 2.105
PW 0.0
RD 0.0
AQ .238
RG 200
NS 4519
TE 297
FW 21600
O2 4416.000
DP 5H DO
LB 1.000
GB 0.0
CX 25.00
CY 0.0
F1 240.012P
F2 -9.975P
HZ/CM 679.216
PPM/CM 9.999
SR -4990.40
O
O
O
O
N3
OH
142b
220
200
180
160
140
120
100
80
60
40
20
0

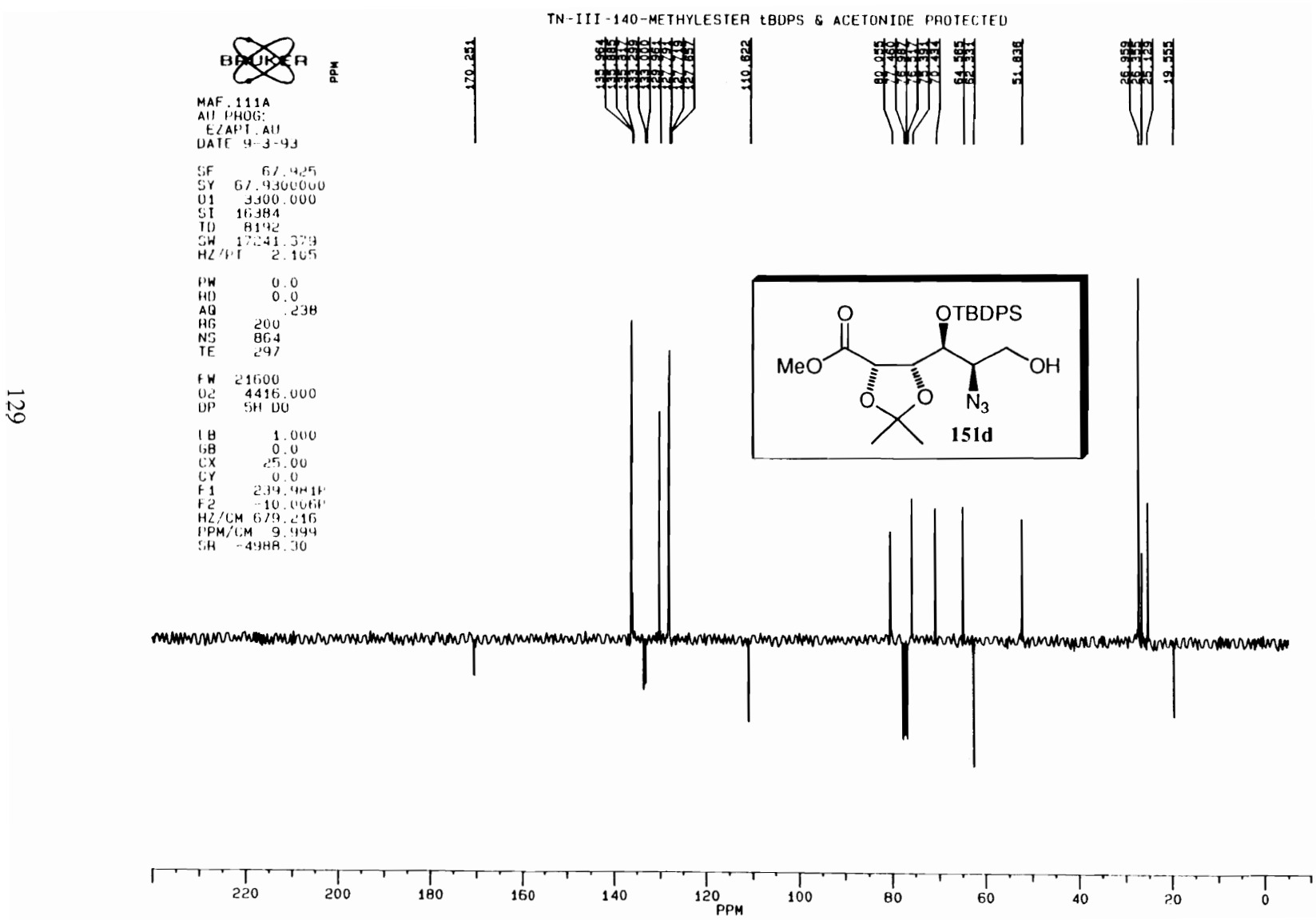

TN-II-218-LACTONE, TRIOL
EDITD.004
DATE 3-5-93
SF 270.134
SY 120.1300000
O1 5657.000
SI 16384
TD 16384
SW 2994.012
HZ/PT .365
PW 4.0
RD 0.0
AQ 2.736
RG 40
NS 16
TE 297
FW 3800
O2 4416.000
DP 63L PO
LB .100
GB 0.0
CX 25.00
CY 0.0
F1 8.001P
F2 -.499P
HZ/CM 91.838
PPM/CM .340
SR 4341.85
INTEGRAL
.3000
.3040
.3025
.3216
.3149
.3142
.5306
.3274
PPM
OH
OH
OH
143b

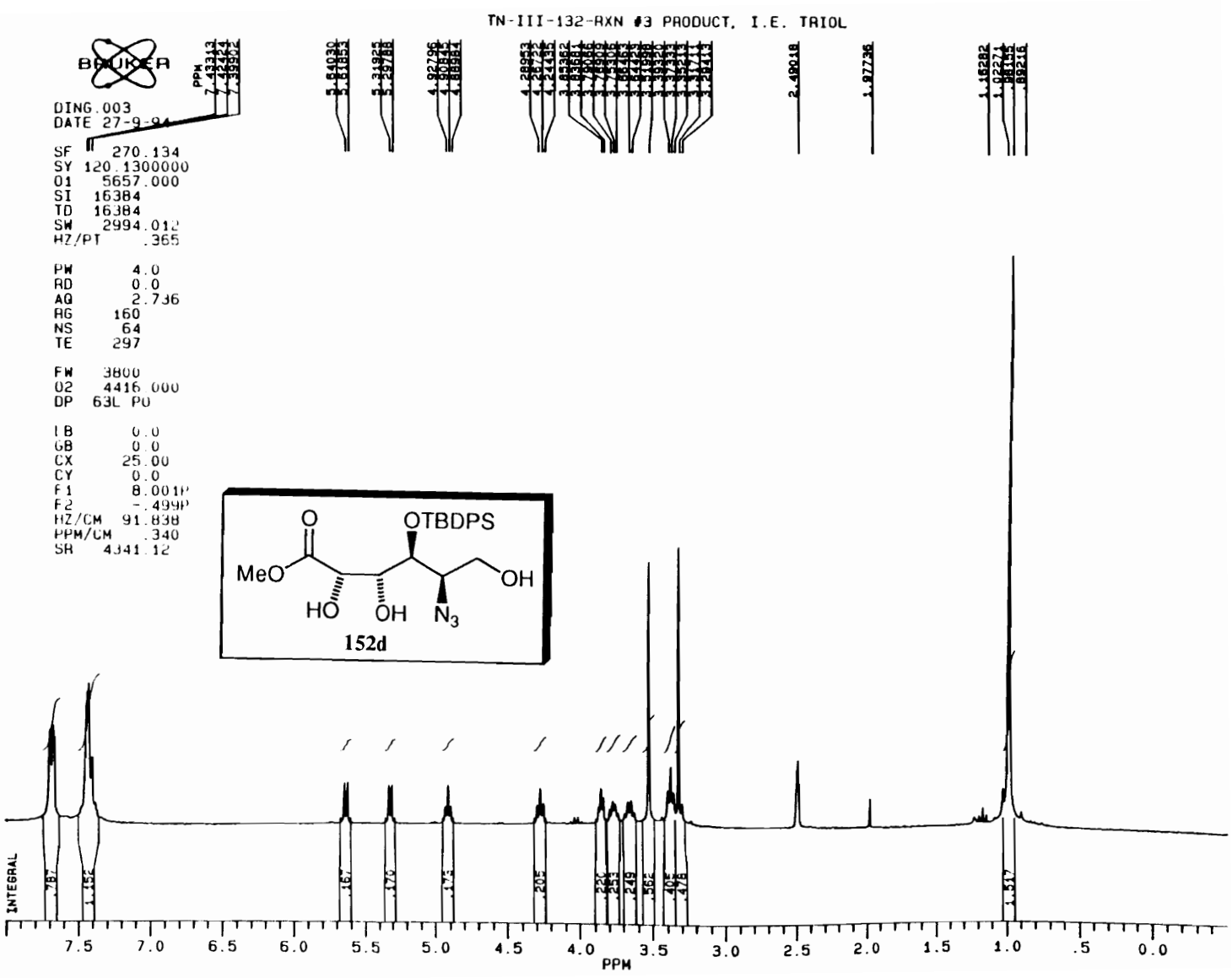

TN-II LACTONE, TRIOL
BRUKER
PPM
175.154
78.269
72.326
68.354
62.893
59.800
40.441
EDITD.004
AU PROG:
EZAPT.AU
DATE 14-8-92
SF 67.925
SY 67.9300000
O1 3300.000
SI 16384
TD 8192
SW 17241.379
HZ/PT 2.105
PW 0.0
RD 0.0
AQ .238
RG 200
NS 8080
TE 297
FW 21600
O2 5657.000
DP 5H DO
LB 1.000
GB 0.0
CX 25.00
CY 0.0
F2 -9.983P
HZ/CM 679.216
PPM/CM 9.999
SR -4623.66
O
OH
OH
N3
OH
143b
220
200
180
160
140
120
100
80
60
40
20
0
PPM

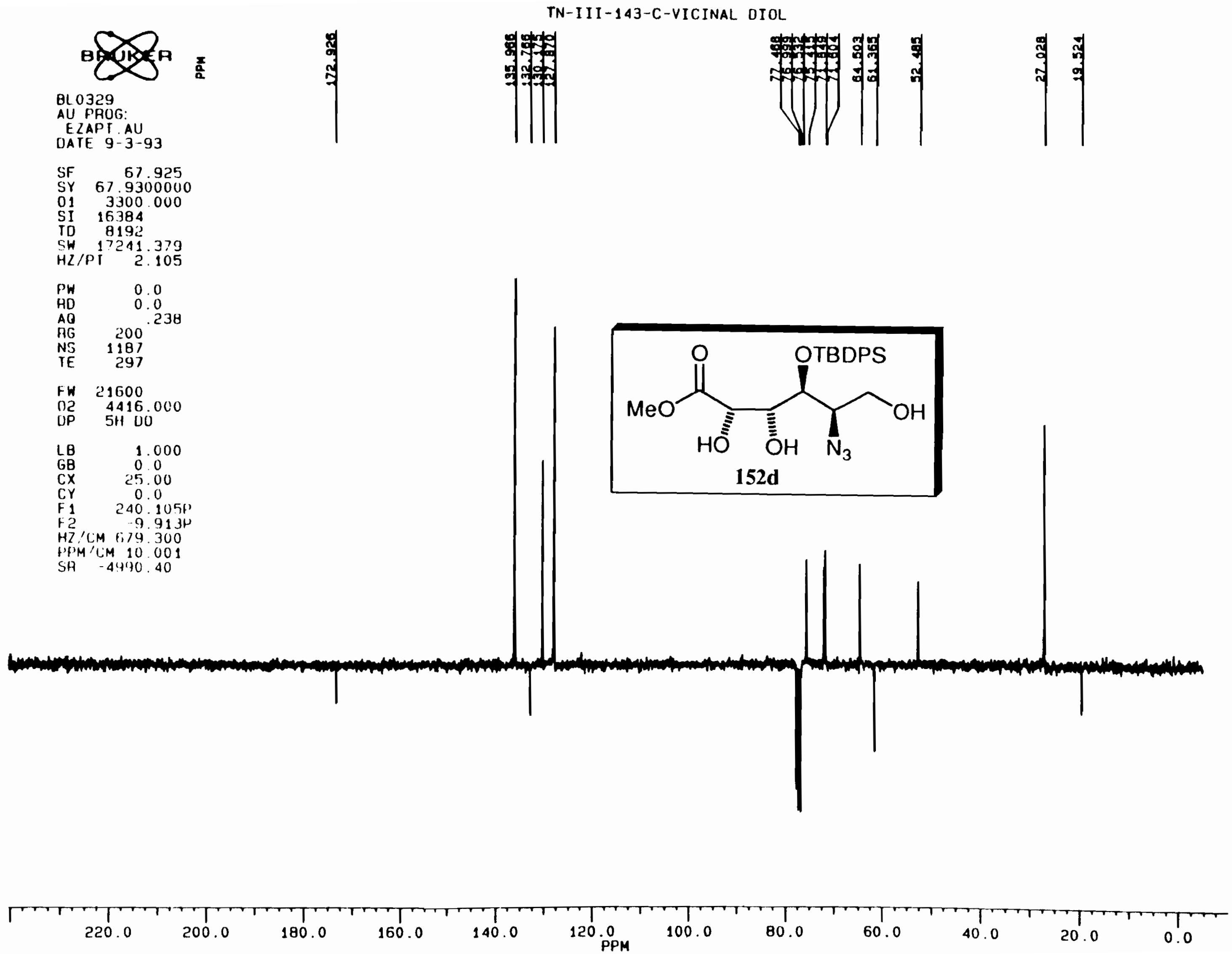

TN-III-AZIDOSILYL ETHER
BRUKER
MAF.111A
DATE 14-3-95
SF 270.133
SY 120.1300000
O1 4416.000
SI 16384
TD 16384
SW 2994.012
HZ/PT .365
PW 4.6
RD 0.0
AQ 2.736
RG 40
NS 16
TE 297
FW 3800
O2 4416.000
DP 63L PO
LB 0.0
GB 0.0
CX 25.00
CY 0.0
F1 8.000P
F2 -.498P
HZ/CM 91.823
PPM/CM .340
SR 3065.11
Cl
O
O
N3
150d OTBDPS
INTEGRAL
4.373
2.554
9.16
1.386
2.565
2.236
3.348
5.805
14.505
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
0.0
PPM

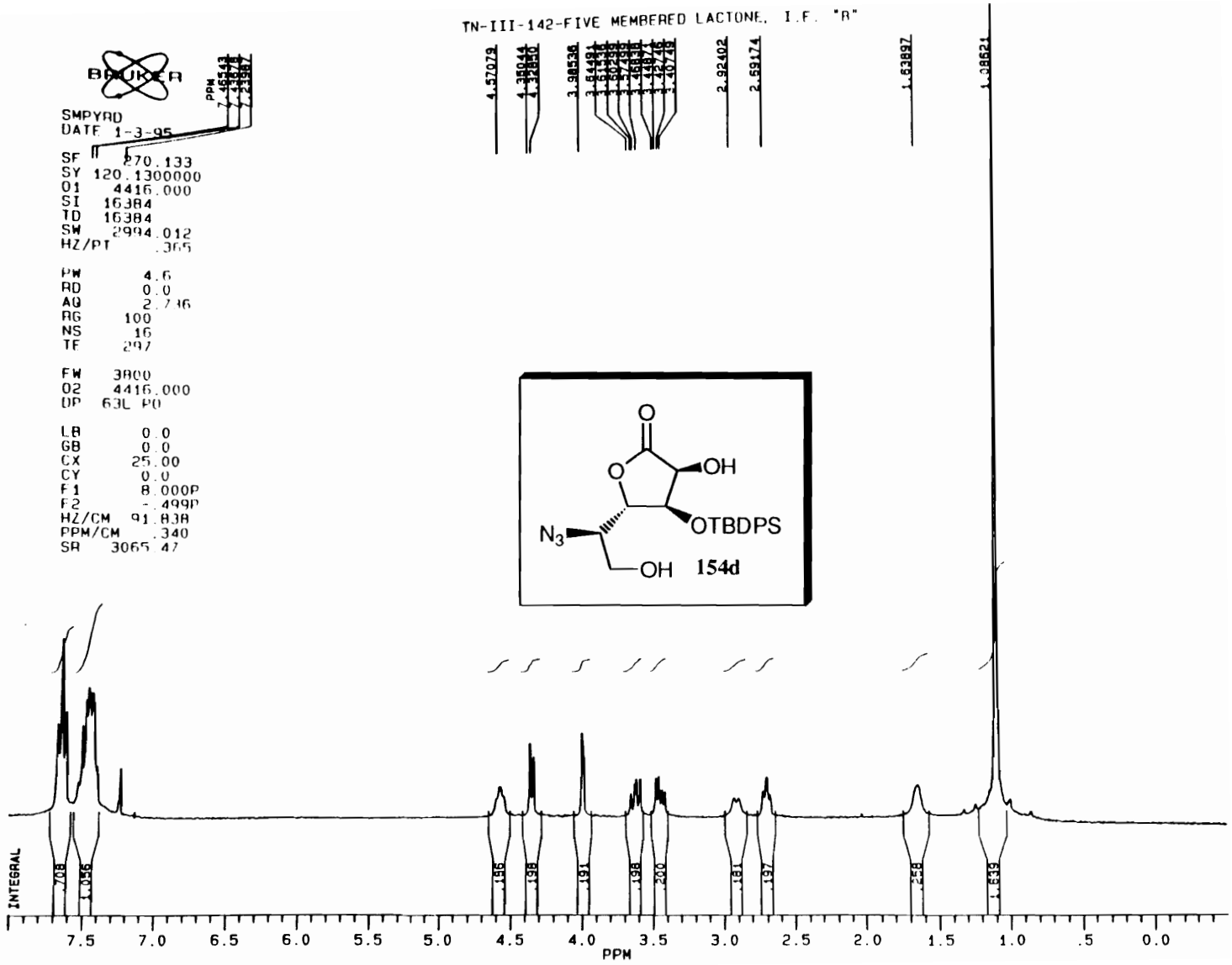

TN-III-139-AZIDOSYLYL ETHER
BRUKER
PPM
MAF.111A
AU PROG:
EZAPT.AU
DATE 14-3-95
SF 67.925
SY 67.9300000
01 3400.000
SI 16384
TD 8192
SW 17241.379
HZ/PT 2.105
PW 0.0
RD 0.0
AQ .238
RG 200
NS 1675
TE 297
FW 21600
02 4416.000
DP 5H D0
LB 1.000
GB 0.0
GX 25.00
GY 0.0
F1 -30.1050
F2 9.8820
HZ/CM 679.216
PPM/CM 9.999
SR -4990.40
Cl
O
O
N_3
150d OTBDPS
135.988
133.817
110.619
77.470
61.863
27.545
26.836
26.218
19.360
220.0
200.0
180.0
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
PPM

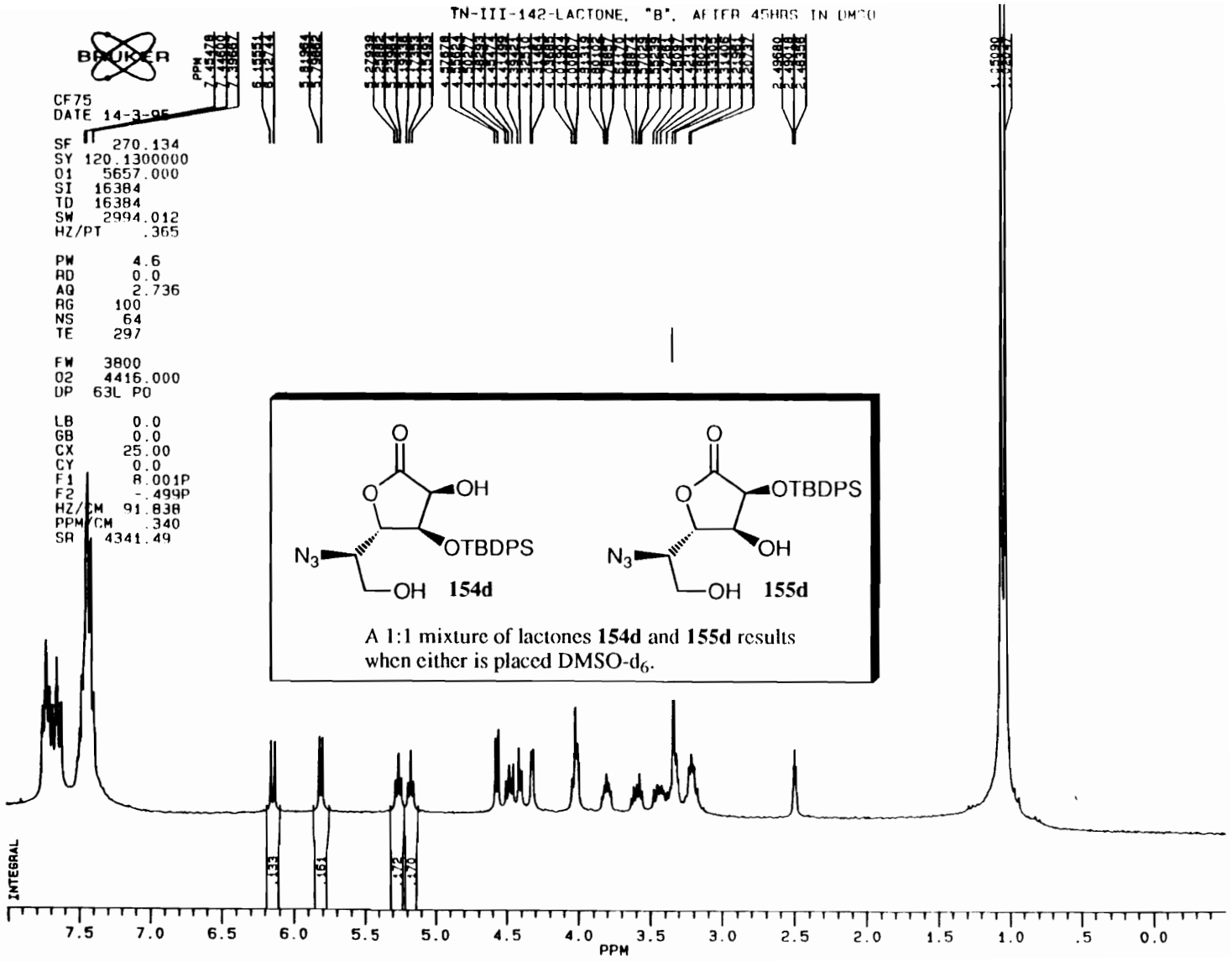

TN III 140-METHYLESTER, tBDPS & ACETONIDE PROTECTED
BRUKER
PPM
DATE 14 3 95
SF 270.133
SY 120.1300000
01 4416.000
SI 16384
TD 16384
SW 2994.013
HZ/PT .365
PW 4.6
RD 0.0
AQ 2.736
RG 20
NS 8
TE 297
FW 3800
02 4416.000
DP 63L PO
LB 0.0
GB 0.0
CX 25.00
CY 0.0
F1 8.000P
F2 .499P
HZ/CM 91.838
PPM/CM .340
SR 3065.47
MeO
O
OTBDPS
OH
O
O
N3
151d
INTEGRAL
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
0.0
PPM

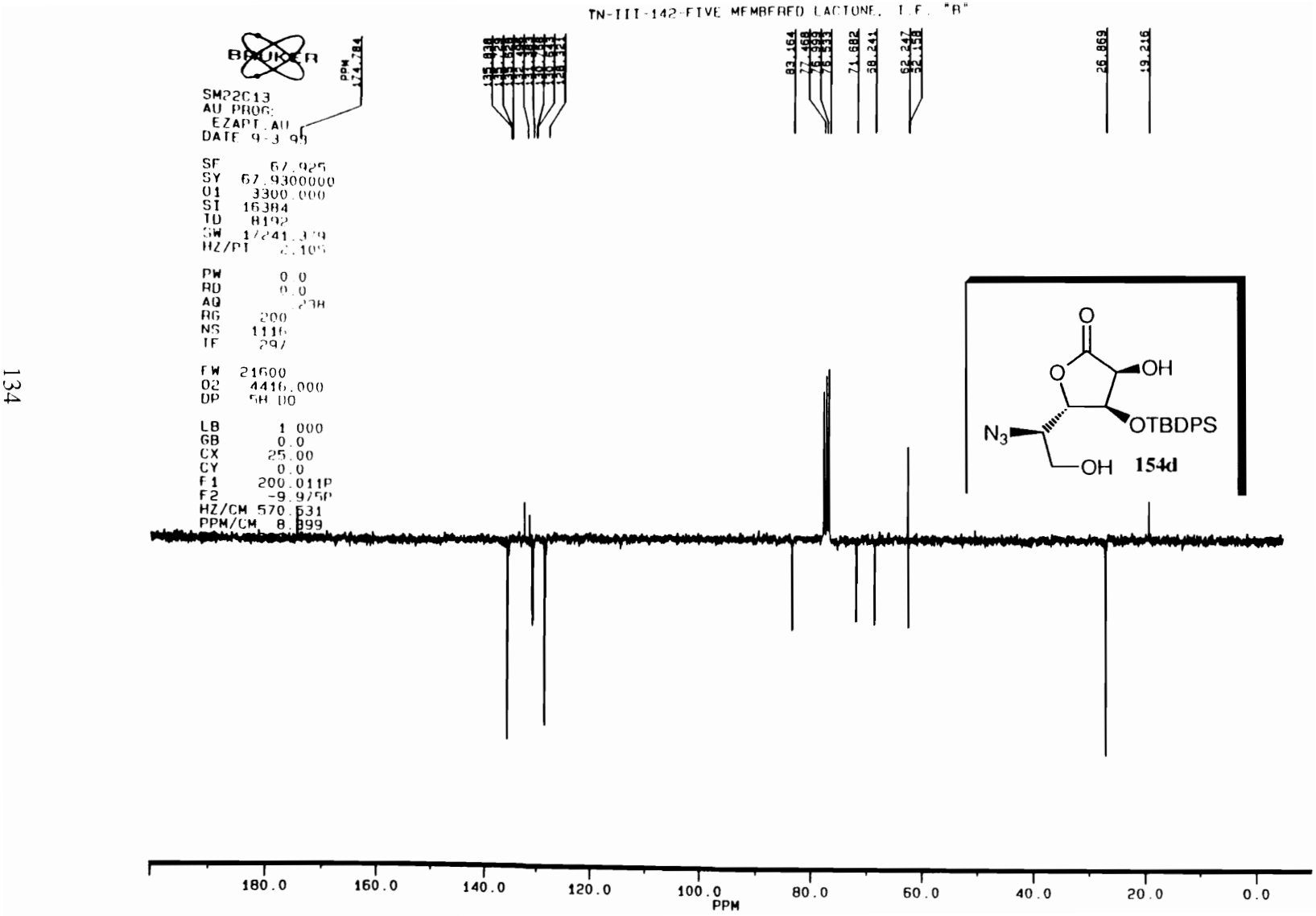

TN-III-140-METHYLESTER tBDPS & ACETONIDE PROTECTED
BRUKER
PPM
MAF.111A
AU PROG:
EZAPT.AU
DATE 9-3-93
SF 67.925
SY 67.9300000
O1 3300.000
SI 16384
TD 8192
SW 17241.379
HZ/PT 2.105
PW 0.0
RD 0.0
AQ .238
RG 200
NS 864
TE 297
FW 21600
O2 4416.000
DP 5H D0
LB 1.000
GB 0.0
CX 25.00
CY 0.0
F1 239.9818
F2 -10.0068
HZ/CM 679.216
PPM/CM 9.999
SR -4988.30
170.251
110.822
51.838
19.553
O
OTBDPS
MeO
OH
O
O
N3
151d
220
200
180
160
140
120
100
80
60
40
20
0
PPM

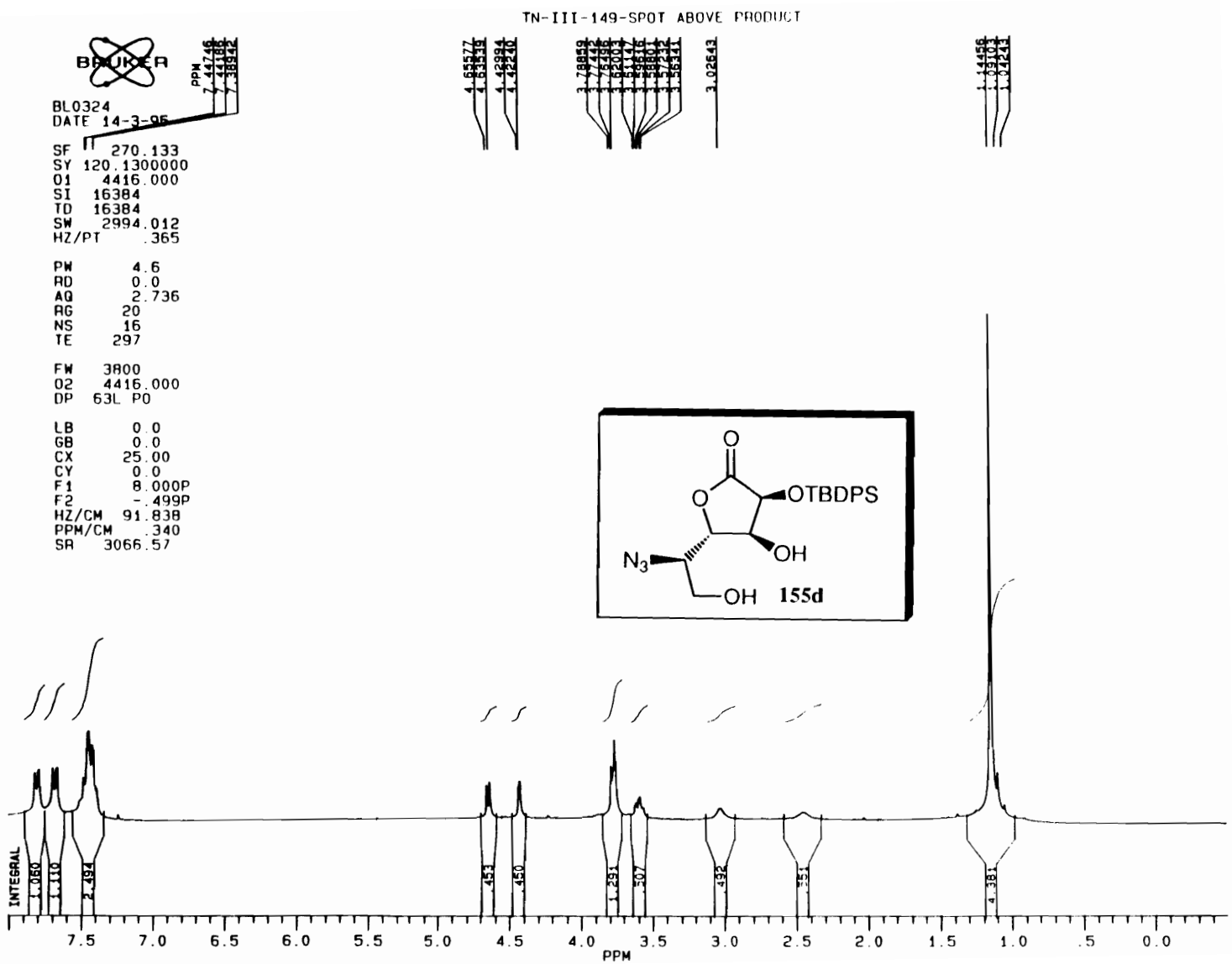

TN-III-132-AXN #3 PRODUCT, I.E. TRIOL
BRUKER
DING.003
DATE 27-9-94
SF 270.134
SY 120.1300000
O1 5657.000
SI 16384
TD 16384
SW 2994.012
HZ/PT .365
PW 4.0
RD 0.0
AQ 2.736
RG 160
NS 64
TE 297
FW 3800
O2 4416.000
DP 63L P0
LB 0.0
GB 0.0
CX 25.00
CY 0.0
F1 8.001P
F2 -.499P
HZ/CM 91.838
PPM/CM .340
SR 4341.12
MeO
O
OTBDPS
OH
HO
OH
N3
152d
INTEGRAL
PPM

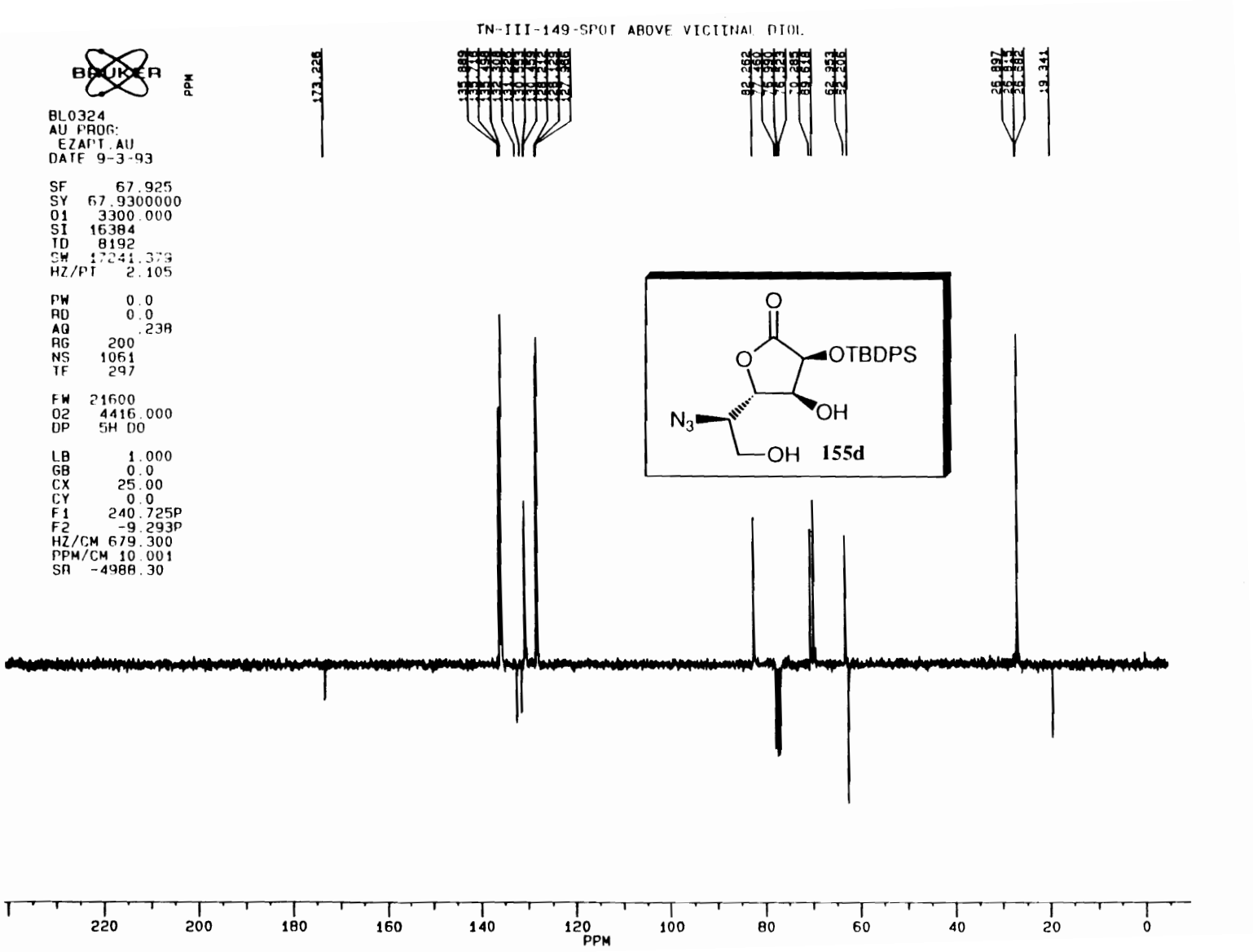

TN-III-143-C-VICINAL DIOL
BRUKER
PPM
BL0329
AU PROG:
EZAPT.AU
DATE 9-3-93
SF 67.925
SY 67.9300000
01 3300.000
SI 16384
TD 8192
SW 17241.379
HZ/PT 2.105
PW 0.0
RD 0.0
AQ .238
RG 200
NS 1187
TE 297
FW 21600
02 4416.000
DP 5H DO
LB 1.000
GB 0.0
CX 25.00
CY 0.0
F1 240.105P
F2 -9.913P
HZ/CM 679.300
PPM/CM 10.001
SR -4990.40
172.926
135.966
132.789
130.175
127.870
77.468
76.999
76.532
75.415
71.849
71.804
64.503
61.365
52.485
27.028
19.524
OTBDPS
MeO
O
OH
HO
OH
N3
152d
220.0
200.0
180.0
160.0
140.0
120.0
PPM
100.0
80.0
60.0
40.0
20.0
0.0

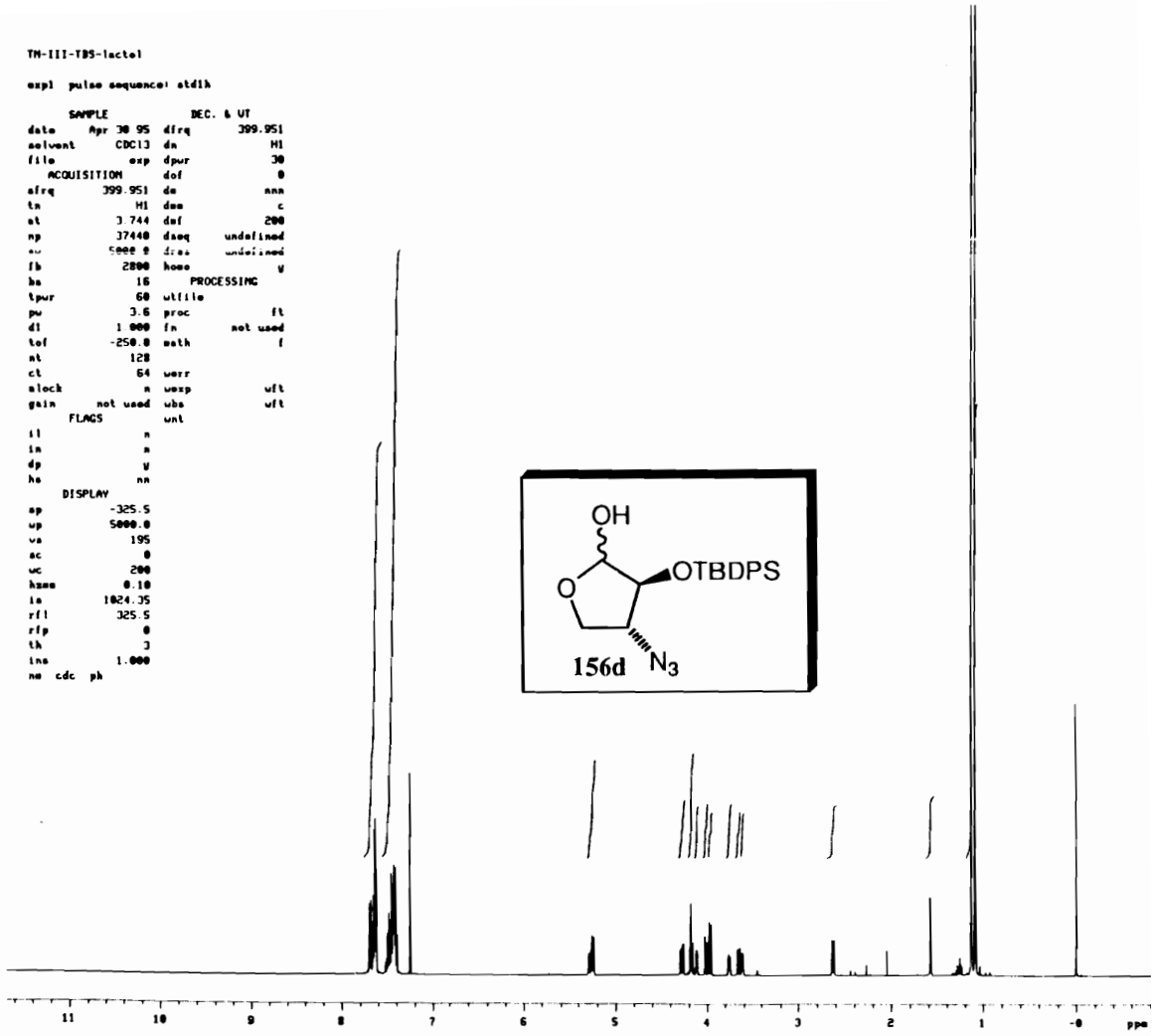

TN-III-142-FIVE MEMBERED LACTONE, I.F. "B"
SMPYRD
DATE 1-3-95
SF 270.133
SY 120.1300000
O1 4416.000
SI 16384
TD 16384
SW 2994.012
HZ/PT .365
PW 4.6
RD 0.0
AQ 2.746
RG 100
NS 16
TF 297
FW 3800
O2 4416.000
DP 63L P0
LB 0.0
GB 0.0
CX 25.00
CY 0.0
F1 8.000P
F2 -.499P
HZ/CM 91.838
PPM/CM .340
SR 3065.47
OH
OTBDPS
N3
OH
154d
INTEGRAL
PPM

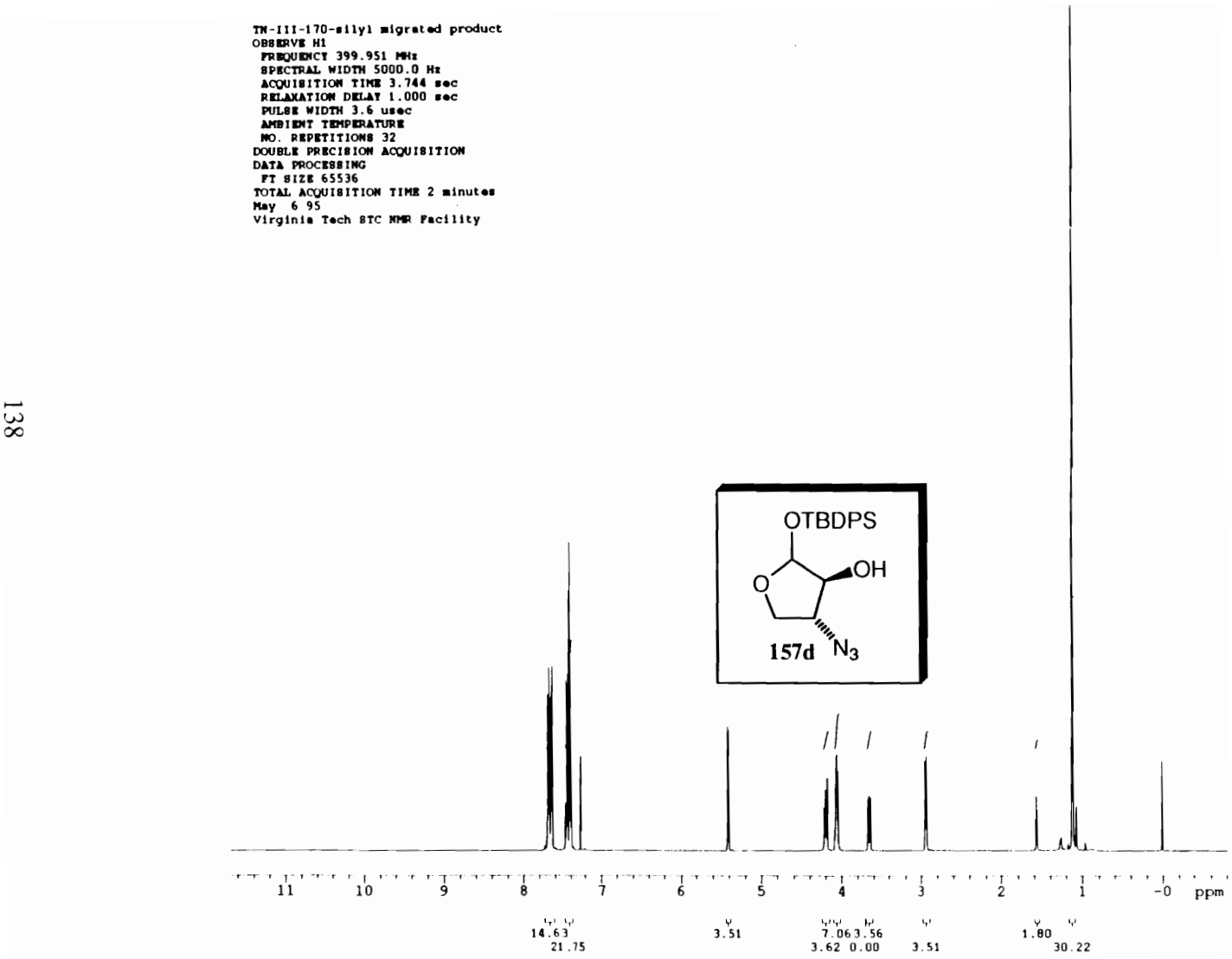

TN-III-142-LACTONE, "B", AFTER 45HRS IN DMSO
BRUKER
CF75
DATE 14-3-95
SF 270.134
SY 120.1300000
O1 5657.000
SI 16384
TD 16384
SW 2994.012
HZ/PT .365
PW 4.6
RD 0.0
AQ 2.736
RG 100
NS 64
TE 297
FW 3800
O2 4416.000
DP 63L PO
LB 0.0
GB 0.0
CX 25.00
CY 0.0
F1 8.001P
F2 -.499P
HZ/CM 91.838
PPM/CM .340
SR 4341.49
OH
OTBDPS
N3
OH
154d
OTBDPS
OH
N3
OH
155d
A 1:1 mixture of lactones 154d and 155d results when either is placed DMSO-d6.
INTEGRAL
.133
.161
.172
.176
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
0.0
PPM

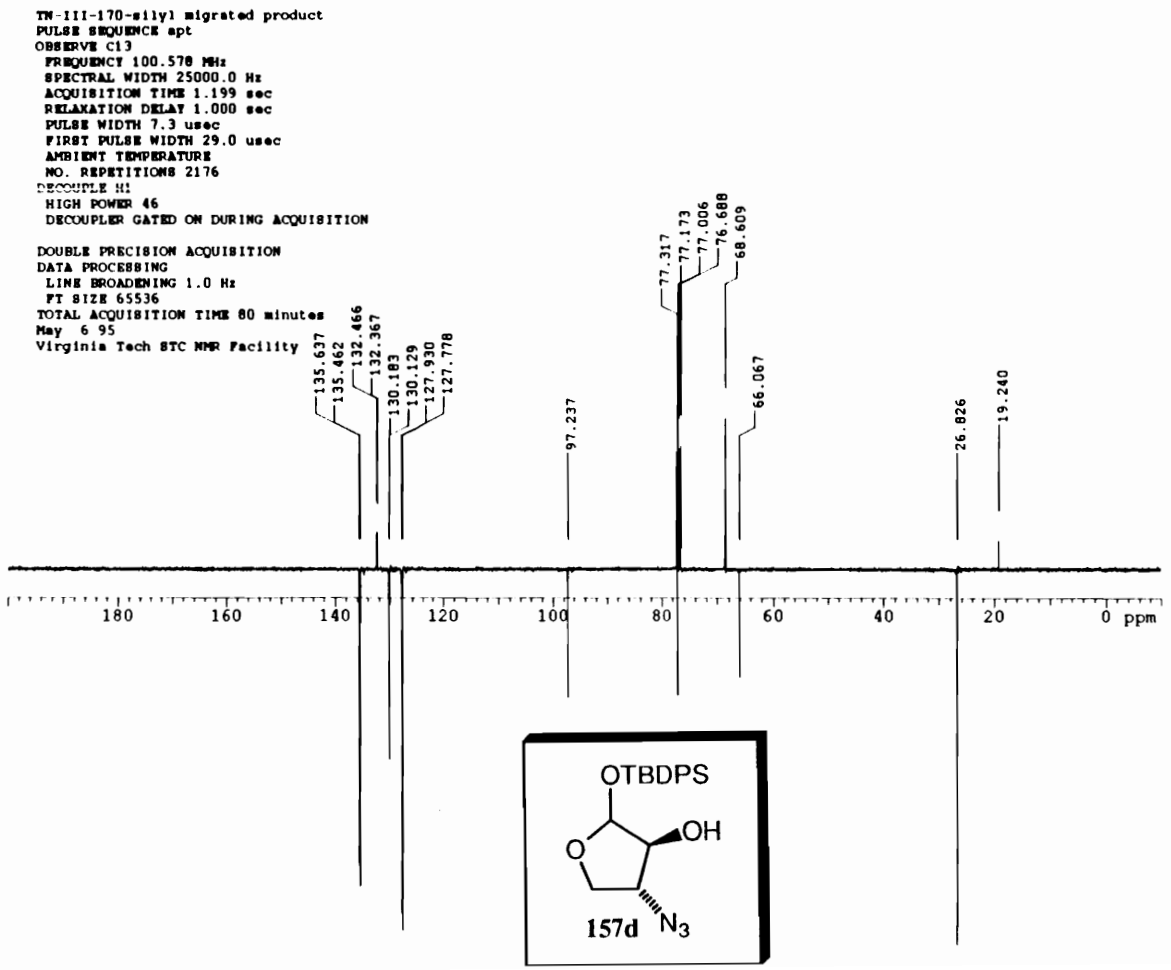

TN-III-142-FIVE MEMBERED LACTONE, I.E. "B"
BRUKER
SM22C13
AU PROG:
EZAPT.AU
DATE 9-3-99
SF 67.925
SY 67.9300000
O1 3300.000
SI 16384
TD 8192
SW 17241.379
HZ/PT 2.105
PW 0.0
RD 0.0
AQ 0.238
RG 200
NS 1116
TF 297
FW 21600
O2 4416.000
DP 58.00
LB 1.000
GB 0.0
CX 25.00
CY 0.0
F1 200.011P
F2 -9.975P
HZ/CM 570.631
PPM/CM 8.399
PPM
174.784
135.838
133.521
133.428
132.
131.
130.
128.
128.321
83.164
77.468
77.000
76.531
71.682
38.241
62.247
62.158
26.869
19.216
OH
OTBDPS
N3
OH
154d
180.0
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
PPM

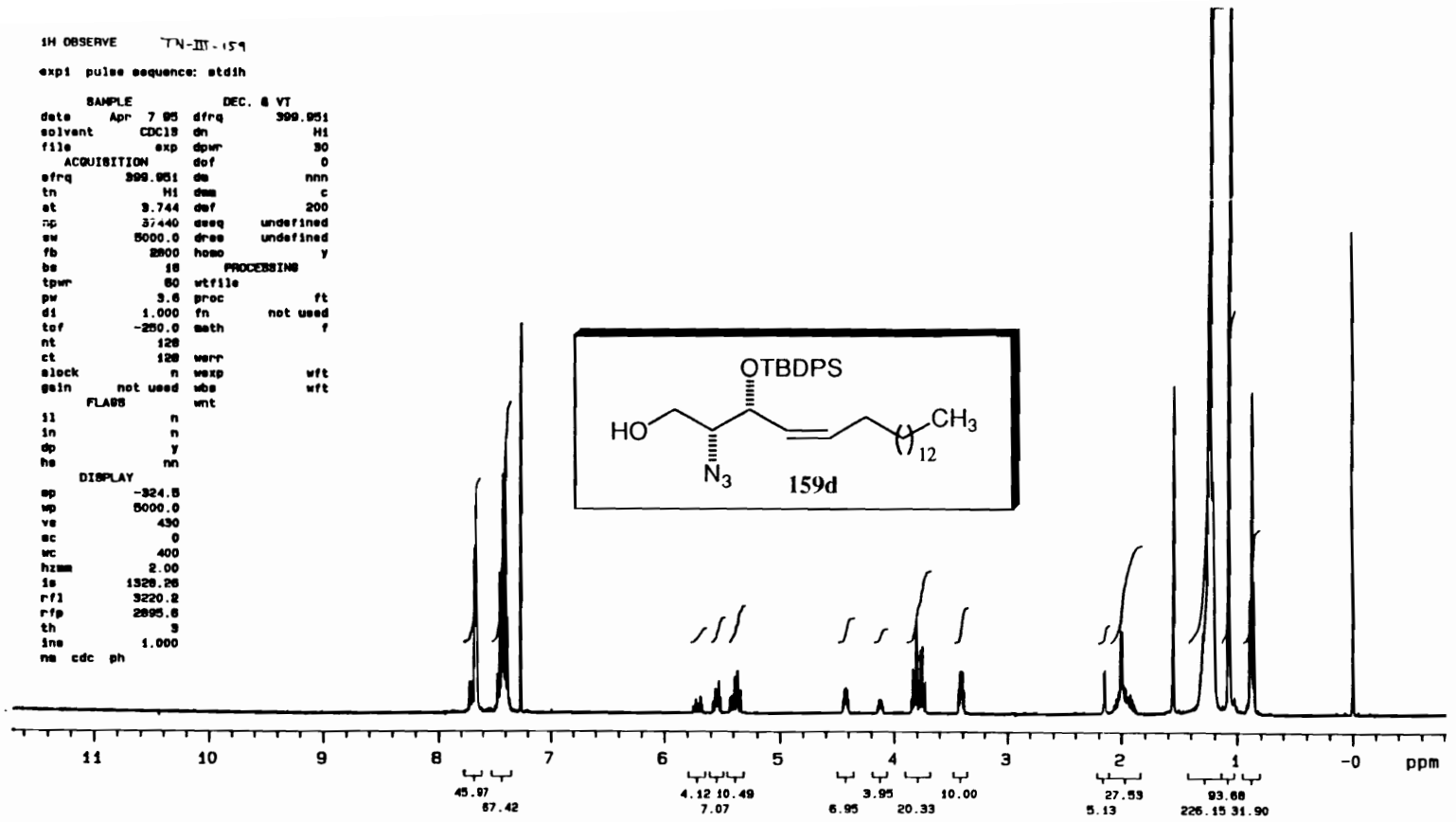

TN-III-149-SPOT ABOVE PRODUCT
BRUKER
BL0324
DATE 14-3-95
SF 270.133
SY 120.1300000
O1 4416.000
SI 16384
TD 16384
SW 2994.012
HZ/PT .365
PW 4.6
RD 0.0
AQ 2.736
RG 20
NS 16
TE 297
FW 3800
O2 4416.000
DP 63L PO
LB 0.0
GB 0.0
CX 25.00
CY 0.0
F1 8.000P
F2 -.499P
HZ/CM 91.838
PPM/CM .340
SR 3066.57
OTBDPS
OH
OH
N3
155d
INTEGRAL
1.060
1.110
2.494
.453
.450
1.291
.507
.432
.351
4.381
PPM

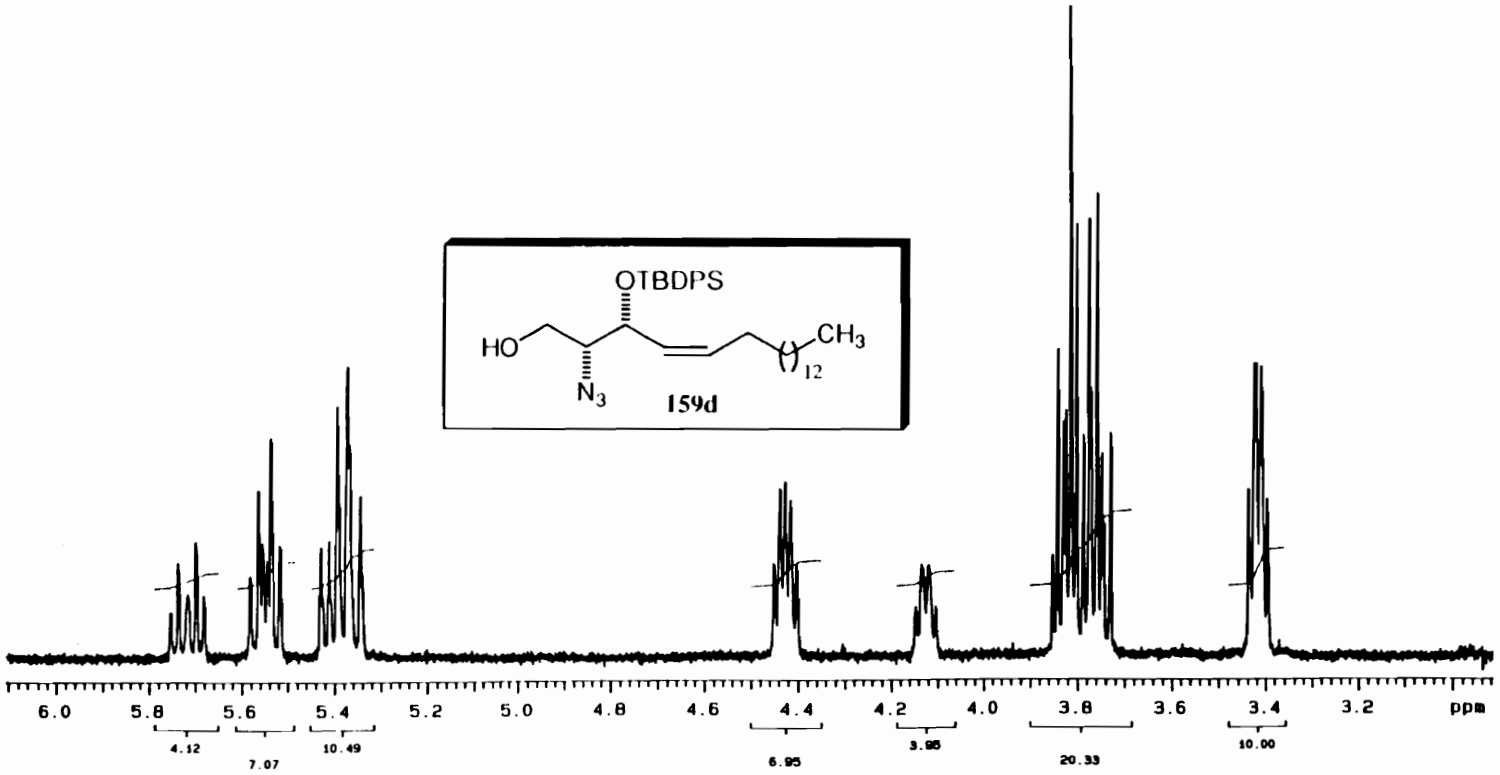

TN-III-149-SPOT ABOVE VICINAL DIOL
BRUKER
PPM
BL0324
AU PROG:
EZAPT.AU
DATE 9-3-93
SF 67.925
SY 67.9300000
O1 3300.000
SI 16384
TD 8192
SW 17241.379
HZ/PT 2.105
PW 0.0
RD 0.0
AQ .238
RG 200
NS 1061
TF 297
FW 21600
O2 4416.000
DP 5H 00
LB 1.000
GB 0.0
CX 25.00
CY 0.0
F1 240.725P
F2 -9.293P
HZ/CM 679.300
PPM/CM 10.001
SR -4988.30
OTBDPS
OH
N3
OH
155d
220
200
180
160
140
120
100
80
60
40
20
0
PPM

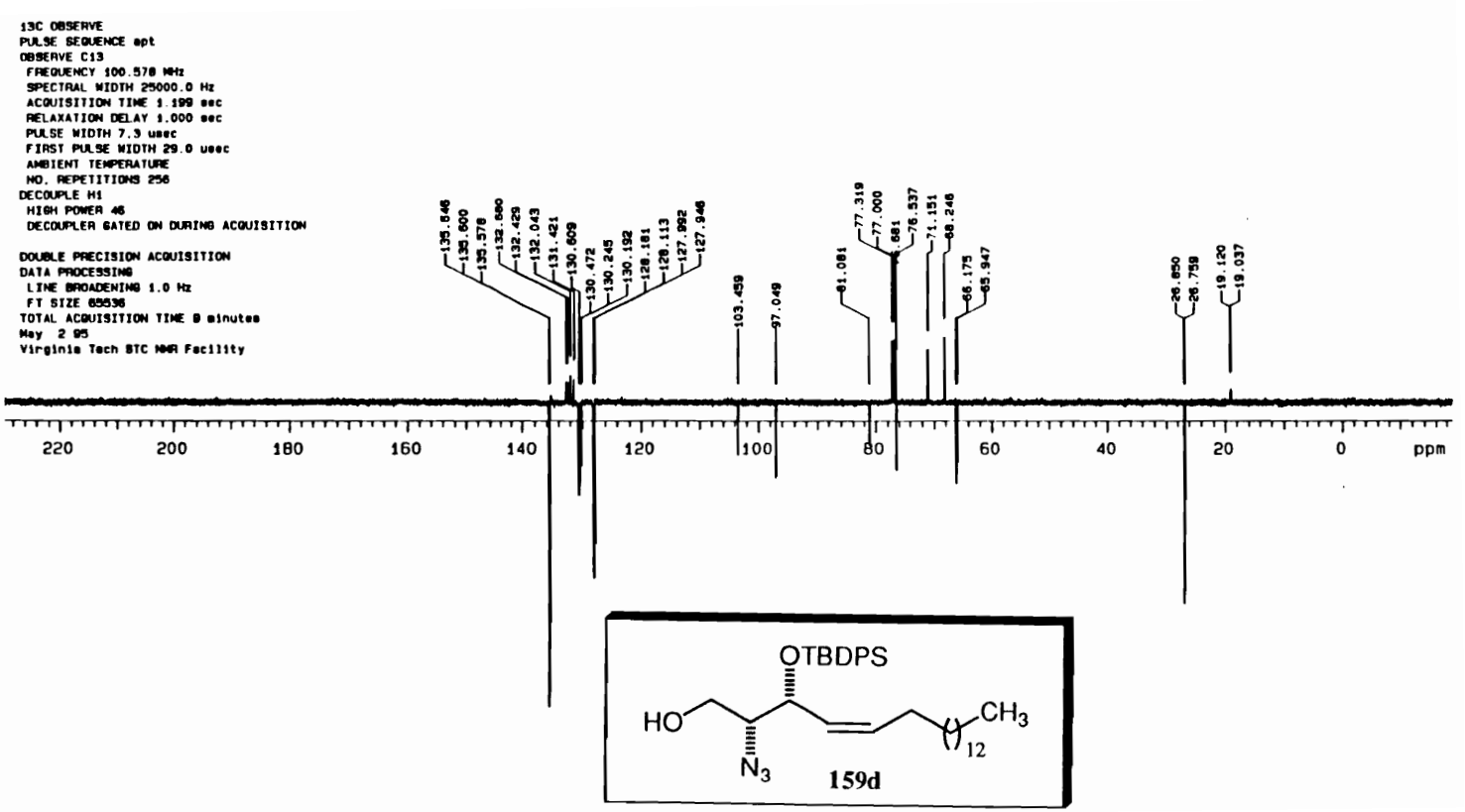

TN-III-TBS-lactol
expl pulse sequence: std1h
SAMPLE
date Apr 30 95
solvent CDCl3
file exp
ACQUISITION
sfrq 399.951
tn H1
at 3.744
np 37440
sw 5000.0
fb 2800
bs 16
tpwr 60
pw 3.6
d1 1.000
tof -250.0
nt 128
ct 64
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -325.5
wp 5000.0
vs 195
sc 0
wc 200
hzmm 0.10
is 1024.35
rfl 325.5
rfp 0
th 3
ins 1.000
ns cdc ph
DEC. & VT
dfrq 399.951
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
dseq undefined
dres undefined
homo y
PROCESSING
wtfile
proc ft
fn not used
math f
werr
wexp wft
wbs wft
wnt
OH
OTBDPS
O
156d
N3
11
10
9
8
7
6
5
4
3
2
1
-0
ppm


TN-III-170-silyl migrated product
OBSERVE H1
FREQUENCY 399.951 MHz
SPECTRAL WIDTH 5000.0 Hz
ACQUISITION TIME 3.744 sec
RELAXATION DELAY 1.000 sec
PULSE WIDTH 3.6 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 32
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
FT SIZE 65536
TOTAL ACQUISITION TIME 2 minutes
May 6 95
Virginia Tech STC NMR Facility
OTBDPS
OH
O
157d
N3
11
10
9
8
7
6
5
4
3
2
1
-0
ppm
14.63
21.75
3.51
7.06
3.56
3.62
0.00
3.51
1.80
30.22


TN-III-170-silyl migrated product
PULSE SEQUENCE apt
OBSERVE C13
FREQUENCY 100.570 MHz
SPECTRAL WIDTH 25000.0 Hz
ACQUISITION TIME 1.199 sec
RELAXATION DELAY 1.000 sec
PULSE WIDTH 7.3 usec
FIRST PULSE WIDTH 29.0 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 2176
DECOUPLE H1
HIGH POWER 46
DECOUPLER GATED ON DURING ACQUISITION
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
LINE BROADENING 1.0 Hz
FT SIZE 65536
TOTAL ACQUISITION TIME 80 minutes
May 6 95
Virginia Tech STC NMR Facility
135.637
135.462
132.466
132.367
130.183
130.129
127.930
127.778
97.237
77.317
77.173
77.006
76.688
68.609
66.067
26.826
19.240
180
160
140
120
100
80
60
40
20
0 ppm
OTBDPS
OH
O
157d N3


1H OBSERVE TN-III-159
exp1 pulse sequence: std1h
SAMPLE
date Apr 7 95
solvent CDCl3
file exp
ACQUISITION
sfrq 399.951
tn H1
at 3.744
np 37440
sw 5000.0
fb 2800
bs 16
tpwr 60
pw 3.6
d1 1.000
tof -250.0
nt 128
ct 128
alock n
gain not used
FLAGS
il n
in n
dp y
he nn
DISPLAY
sp -324.5
wp 5000.0
vs 490
sc 0
wc 400
hzmm 2.00
is 1328.26
rfl 3220.2
rfp 2895.6
th 9
ins 1.000
ns cdc ph
DEC. & VT
dfrq 399.951
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
dseq undefined
dres undefined
homo y
PROCESSING
wtfile
proc ft
fn not used
math f
werr
wexp wft
wbs wft
wnt
OTBDPS
HO
N3
CH3
12
159d
11
10
9
8
7
6
5
4
3
2
1
-0
ppm
45.97
67.42
4.12 10.49
7.07
6.95
3.95
20.33
10.00
5.13
27.59
226.15 31.90
93.68


OTBDPS
HO
N_3
CH_3
$()_{12}$
159d
6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
ppm
4.12
7.07
10.49
6.95
3.95
20.33
10.00


13C OBSERVE
PULSE SEQUENCE apt
OBSERVE C13
FREQUENCY 100.578 MHz
SPECTRAL WIDTH 25000.0 Hz
ACQUISITION TIME 1.199 sec
RELAXATION DELAY 1.000 sec
PULSE WIDTH 7.3 usec
FIRST PULSE WIDTH 29.0 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 256
DECOUPLE H1
HIGH POWER 46
DECOUPLER GATED ON DURING ACQUISITION
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
LINE BROADENING 1.0 Hz
FT SIZE 65536
TOTAL ACQUISITION TIME 8 minutes
May 2 95
Virginia Tech BTC NMR Facility
135.846
135.600
135.578
132.880
132.429
132.043
131.421
130.609
130.472
130.245
130.192
128.181
128.113
127.992
127.946
103.459
97.049
81.081
77.319
77.000
76.681
76.537
71.151
68.246
65.175
65.947
26.850
26.759
19.120
19.037
220
200
180
160
140
120
100
80
60
40
20
0
ppm
OTBDPS
HO
N_3
CH_3
12
159d

VI. APPENDIX

Table 1. Biological Acitivity Chart

Activity
Tumor antigens
GM_2 expression on human melanoma cells
GD_2 expression upon progression of melanoma
Antibodies to GD_3 have antitumor activity
TerC glycolipid marker for teratocarcinoma
Lactosylceramide marker for colon carcinoma
A sialylated ganglioside as marker for chronic myelogenous leukemia
Glycolipid alteration in *ras*-transfected NIH-3T3 cells
GD_{1a} expressed by rat hepatoma cells
Markers of cell differentiation
Increased sphingomyelin upon differentiation of hairy cell luekemia with phorbol esters
Increased sphingomyelin in 3T3-L1 cells and in polymorphonuclear leukocytes treated with dexamethasone
GM_3 elevation in differentiated HL-60 cells and U937 cells; turnover of GM_3 sialic acid residues during fibroblast growth
SSEA-1 glycolipid in embryo development
GM_1 and GD_{1a} elevation on differentiation of teratocarcinoma
GM_3 elevation in differentiating intestinal epithelium
I antigen in erythrocyte differentiation
GD_3 elevation in muscle cell differentiation
Ganglioside elevation in neuroblastoma
GM_1 elevation in lymphoid cell differentiation
Role in membrane fluidity
Gangliosides on outer leaflet of bilayer confer rigidity
Correlation of sphingomyelin content with fluidity
Modulation of cell proliferation
Nerve growth factor-like activity of GQ_{1b}
Neuritogenic and neuronotrophic activities of gangliosides
GM_1 inhibition of Swiss 3T3 cell growth

GM_3 inhibition of growth factor-induced mitogenesis
Inhibition of lymphocyte proliferation
Stimulation of astroglial and neuroblastoma proliferation by exogenous gangliosides
Modulation of protein phosphorylation
GQ_{1b}-dependent protein kinase activity
Ganglioside-inhibited protein kinase from pig brain
GM_3 inhibition of EGF-dependent tyrosine phosphorylation of EGF receptor
Ganglioside-induced phosphorylation of proteins in myelin
Inhibition of protein kinase C activity by gangliosides
GT_{1b}- and GD_{1a}-activated protein kinase
Ca^{2+}- and ganglioside-dependent protein kinase
GM_3 inhibition of tyrosine phosphorylation of the platelet-derived growth factor receptor
de-N-acetyl GM_3 stimulation of tyrosine kinase activity of EGF receptor
Cell Contact response
Inhibition of cell contact
Globoside modulation of neuromuscular junction formation
GM_2 modulation of retinal adhesion
Induction of GD_{1b} and GT_{1b} during neuroglial interaction
Gangliosides as receptors and receptor cofactors
GM_1 binds B subunit of cholera toxin and mediates its mitogenic effect
G_{1b} gangliosides as receptors for tetanotoxin
Binding of gangliosides to fibronectin
Binding of laminin, thrombospondin, and von Willebrand factor to sulfated glycolipids
Immune recognition
Blood group antigens
Autoimmune antigens
Tumor antigens
Differentation antigens
Lymphocyte markers
Miscellaneous

Embryo inversion by a complex glycolipid
Cerebrosides with antiulcerogenic activity
Stimulation of fruiting of Schizophylium commue by plant cerebrosides
Modulation of sodium transport by complex gangliosides
"Ganglioside syndrome" in rabbits intensively immunized with GM_1 and GD_{1a}
Glycolipid changes with the cell cycle

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(12) What follows, represents the experience acquired by Carter *et. al.*[G] in processing 600 pounds of brain and 2000 pounds of spinal cord: The brain or spinal cord matter is ground into a soft paste and then extracted with acetone overnight. The acetone is decanted, and more acetone is added to the paste. A total of six extractions in this manner are performed.

The residue remaining after evaporation of the combined acetone extracts gave on average 9 to 12 kilos for spinal cord and 8.5 to 12 kilos for brain, of a light brown, gummy solid. Next the glycerophosphatides were extracted using ether, in which the sphingolipids are sparing soluble. The residue left behind was air dried, giving (5.2 to 6.0 kilos for spinal cord and 6.0 to 7.5 kilos for brain) a light brown friable material, which was moderately stable on exposure to air and could be stored almost indefinitely for use in the final step. This material was extracted 3 times with boiling ethanol for 10 - 15 minutes. These ethanol extracts were combined and allowed to cool overnight in an ice box. The precipitate was filtered, washed once with acetone and air dried to give a white powdery material (2.0 g to 2.5 g for spinal cord and 1.3 to 1.6 g for brain).

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IV. VITA

Thomas Christopher Nugent was born November 04, 1967 to Mr. and Mrs. Thomas Christopher Nugent in London, England. He graduated from Tottenville High School, Staten Island, New York in the spring of 1986 and enrolled at Virginia Polytechnic Institute and State University the following fall. In December, 1990 he earned a Bachelor of Science degree in chemistry. In fall, 1991 he began his graduate career under the direction of Dr. Tomas Hudlicky. His accomplishments while at Virginia Polytechnic Institute and State University are listed below:

Publications:

1) T. Hudlicky, H. Luna, H. F. Olivo, C. Anderson, T. Nugent, J. D. Price, Biocatalysis as a Strategy of Choice in the Exhaustive Enantiocontrolled Synthesis of Conduritols, *J. Chem. Soc. Perkin 1* **1991**, 2907.

2) T. Hudlicky, M. G. Natchus, T. Nugent†, Improved Practical Synthesis of a Prostaglandin and Carbocyclic Nucleoside Synthon, *Synthetic Communications* **1992**, *22*, 151.

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† - undergraduate researcher.

Presentations:

1) "L-Threo-Sphingosine From Monohalogenated Benzenes," ACS SE Regional Meeting," Birmingham, October 1994, Thomas Nugent and Tomas Hudlicky.

2) "Sphingosines and Their Derivatives from Monohalogented Benzenes," ACS

SE Regional Meeting," Johnson City, October 1993, Thomas Nugent and Tomas Hudlicky.

3) "Synthesis and Application of Homochiral Fluoroconduritols: Fluoroaspartic Acid," ACS SE Regional Meeting, Richmond, November 1991, Thomas Nugent and Tomas Hudlicky.