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C-3-MODIFIED ANALOGUES OF 1D-*MYO*-INOSITOL AS INHIBITORS OF PHOSPHATIDYLINOSITOL 3-KINASE

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Tzenge-Lien Shih

May, 1992

DEDICATION

This thesis is dedicated to my parents and brother

for their spiritual support and endless love.

ACKNOWLEDGMENTS

I am deeply grateful to my advisor, Professor David C. Baker, for his advice, encouragement, and support during my graduate study. I also thank Dr. Laurens Anderson for kindly supplying L-(−)-quebrachitol, which served as the starting material for most of the syntheses described herein. I deeply appreciate Dr. Thomas L. Benjamin and his associates, J. Dahl and K. Dower, for the biological testing on the *myo*-inositol analogues. My thanks also go to D. J. A. Schedler, S. C. Johnson, Dr. Kuai-Lin Sun, Dr. Tsu-Hsing Lin, Qi Chao, and Shi-Jia Yan for their friendship and assistance in the laboratory.

ABSTRACT

The phosphoinositol pathway involves the synthesis of phosphatidylinositol (PtdIns) from inositol and cytidine diphosphate diglyceride (PtdCMP) via the action of phosphatidylinositol synthetase (EC 2.7.8.11). Phosphorylation of the resulting phosphatidylinositol derivative occurs first at C-4, and then at C-5, via specific enzymes, 1-phosphatidylinositol kinase (EC 2.7.1.67) and 1-phosphatidylinositol 4-phosphate kinase (EC 2.7.1.68), to give phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2]. Signal transduction is mediated by the calcium (Ca^{2+}) mobilization, which is also affected by the hydrolysis of PtdIns(4,5)P_2 by phospholipase C (EC 3.1.4.3) to yield inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] and 1,2-di-*O*-acylglycerol (Ac_2Gro). The Ins(1,4,5)P_3 can be further phosphorylated by phosphatidylinositol 3-kinase in certain cell systems. Studies carried out on oncogenic systems, however, suggest somewhat different modes of action. It seems that phosphatidylinositol is first phosphorylated to phosphatidyl-inositol 3-phosphate via the action of phosphatidylinositol 3-kinase. The inositol 1,3,4,5-tetrakisphosphate has been shown to be associated with net Ca^{2+} transport across membranes and has some influence on pathophysiological processes, including oncogenesis.

In order to inhibit the activity of PtdIns 3-kinase, the strategy is that of modifying *myo*-inositol at the 3-position so that the compounds would retain respectable substrate active with phosphatidyl synthetase, then hope that the resulting phosphatidyl analogues would block the action of this enzyme. *myo*-Inositol is a meso compound. The advantage in synthesizing optically active D-*myo*-inositol analogues from an optically active compound, L-quebrachitol [1L-(-)-2-*O*-methyl-*chiro*-inositol], instead of *myo*-inositol itself, is that optical resolution methods are not required to isolate pure enantiomeric products.

The analogues synthesized in this thesis include 1D-3-deoxy-*myo*-inositol (**4**), 1D-3-deoxy-4-*O*-methyl-*myo*-inositol (**5**), 1D-4-*O*-methyl-*myo*-inositol (**10**), 1D-3-chloro-3-deoxy-*myo*-inositol (**14**), and 1D-3-deoxy-3-mercapto-*myo*-inositol (**17**).

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LIST OF ABBREVIATIONS

Phosphatidylinositol 4,5-bisphosphate	PtdIns(4,5)P_2
Inositol 1,4,5-trisphosphate	Ins(1,4,5)P_3
Diacylglycerol	Ac_2Gro
Endoplasmic reticulum	ER
Protein kinase C	PKC
Arachidonic acid	AA
Platelet-derived growth factor	PDGF
Phospholipase C	PLC
Phosphatidylinositol	PtdIns
Phosphatidylinositol 4-phosphate	PtdIns(4)P_1
Cytidine monophosphate	CMP
Cytidine diphosphodiacylglycerol	PtdCMP
Inositol 1,3,4,5-tetrakisphosphate	Ins(1,3,4,5)P_4
Inositol 1,3,4,5,6-pentakisphosphate	Ins(1,3,4,5,6)P_5
Tributyltin hydride	Bu_3SnH
α,α'-Azobis-(2-methylpropionitrile)	AIBN
Diethyl azodicarboxylate	DEAD

Triphenylphosphine . . . . . TPP

9-(4-Methoxyphenyl)xanthenethiol . . . . . AXT

2,2-Dimethyl-2-silapentane-5-sulfonate . . . . . DSS

Tetramethylsilane . . . . . TMS

N,*N*-Dimethylformamide . . . . . DMF

p-Toluenesulfonic acid . . . . . *p*-TSA

Potassium thioacetate . . . . . KSAc

p-Nitrobenzoic acid . . . . . *p*-NBA

I. INTRODUCTION

A. *myo*-Inositol: History and Biological Role

Cyclohexanehexols, also known as inositols (or cyclitols), are comprised of nine stereoisomers, seven of which are meso compounds (see Figure 1).[1] These stereoisomers are *scyllo*- (no axial OH), *myo*- (1 axial OH), *neo*- and *epi*- (2 axial OHs), *cis*-, *muco*-, and *allo*-inositol (3 axial OHs). The remaining two are chiral compounds: D-*chiro*- and L-*chiro*-inositol (2 axial OHs).

The most important and abundant inositol in nature is *myo*-inositol. The phosphorylation of *myo*-inositol plays a pivotal role in hormone reactions and in neural transmitter-related transducing systems, which result in the mobilization of calcium within cells. The phosphoinositides, which are membrane-related lipids, are the precursors of intracellular second messengers that stimulate a variety of biochemical functions, including metabolism, cell proliferation, secretion, and protein synthesis.[2,3,4]

Signal transduction is caused by calcium (Ca^{2+}) mobilization, which is also affected by the hydrolysis of phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2]. The receptors of the hormones and hormone-like signals are activated by a membrane-bound G protein (GTP-binding protein).[4] The G protein activates phospholipase C to hydrolyze

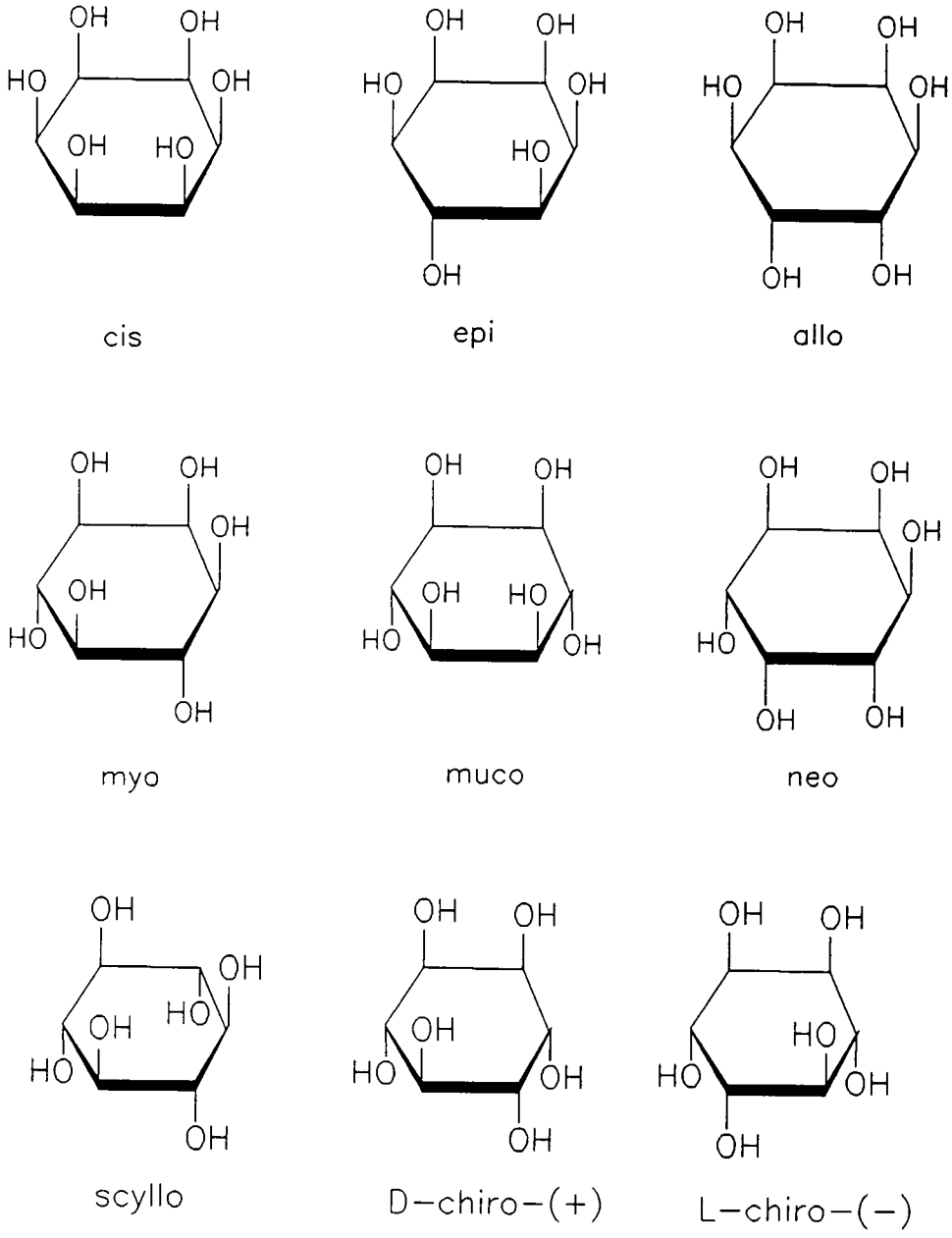
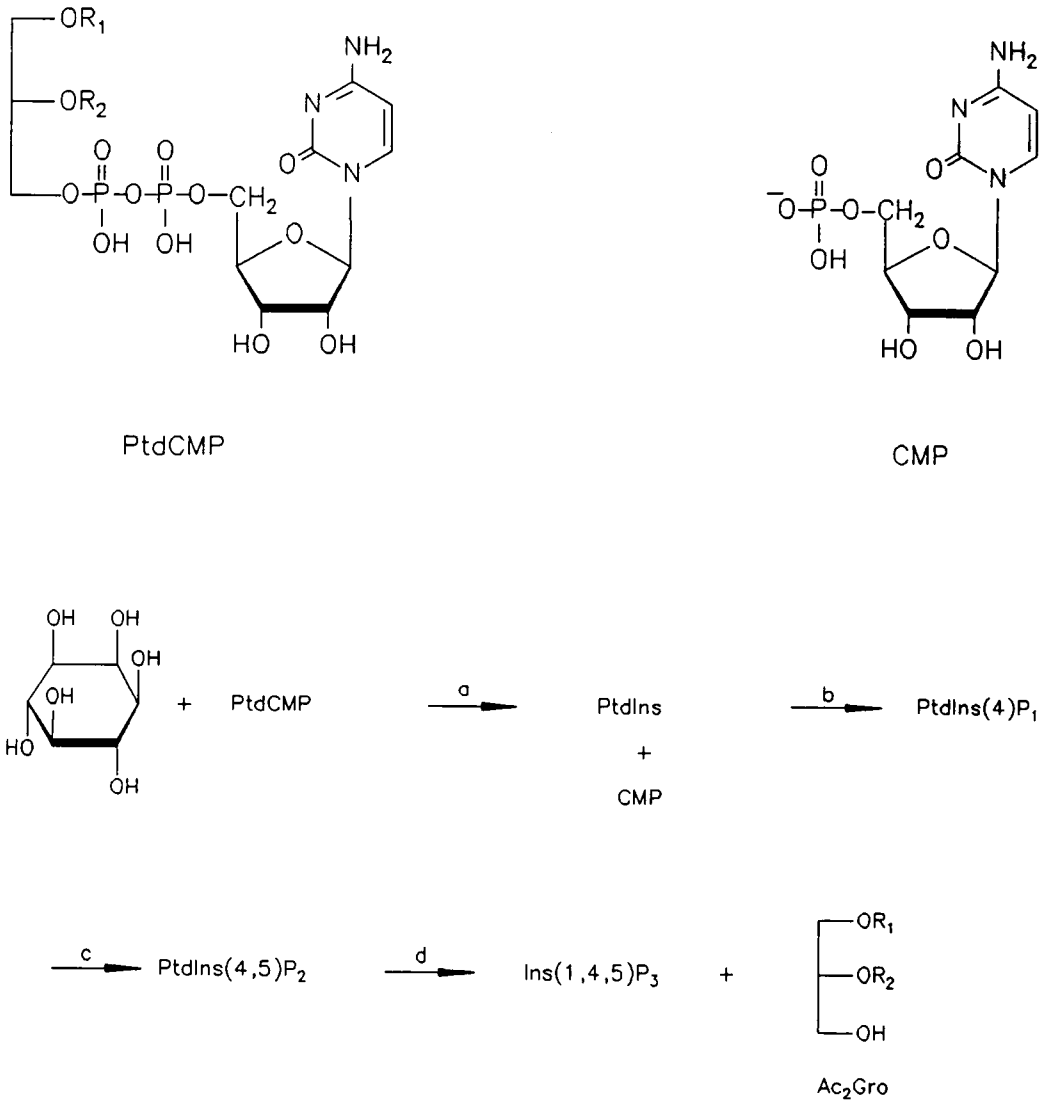


Figure 1. Stereoisomers of Inositols

the PtdIns(4,5)P_2 to form inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] and diacylglycerol (Ac_2Gro). The two products from this reaction, Ins(1,4,5)P_3 and Ac_2Gro, play very important signal-transducing roles in the cell. The Ins(1,4,5)P_3 anchors onto the surface of the endoplasmic reticulum (ER), which releases into the cytoplasm the Ca^{2+} that is stored inside the ER.[4,5] The Ac_2Gro can activate an enzyme called protein kinase C (PKC),[5] which stimulates the phosphorylation of some intraceullar proteins, such as vinculin, microtubule-associated protein, and ribosome S6 protein.[7] The PKC is a Ca^{2+} and a phospholipid-related enzyme that has been confirmed to be linked to signal transduction. The fatty acids in the first and second position of Ac_2Gro are enriched in stearic acid (a saturated acid) and arachidonic acid (AA), an unsaturated acid, respectively. These acids comprise 80% of the total Ac_2Gro species in phosphatidylinositol (PtdIns), phosphatidylinositol 4-phosphate [PtdIns(4)P], and phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2].[6] AA also serves as a precursor of prostanoids, which are a vast family of physiologically active lipids. Therefore, AA is a primary starting material for prostanoid biosynthesis, which depends in part on Ac_2Gro as a carbon source. Kinetic studies show that a small amount of Ac_2Gro can tremendously promote the affinity of PKC for Ca^{2+}, but triacylglycerol, monoacylglycerol, and free fatty acids are totally inactive.[7] As the level of Ac_2Gro increases, the cytoplasmic pH also increases, which can stimulate the blood platelet-derived growth factor (PDGF), epidermal growth, and production of bombesin and vasopressin in Swiss 3T3 cells.[2,7,8] The PKC-binding interaction of with Ac_2Gro serves as the target of phorbol esters which are known to promote tumors.[8]

B. Phospholipid Metabolism (PtdIns Cycle)

The phosphorylation of *myo*-inositol is a very important step in physiological processes which are related to the signal transduction arising from the mobilization of Ca^{2+}. By far, the most abundant inositol lipid in nature is phosphatidylinositol (PtdIns), which comprises about 5% of the membrane phospholipid of cells.[5,6] The formation of PtdIns results from PtdCMP forming a phosphodiester bond to the 1D OH group of *myo*-inositol (see Figure 2). This step is catalyzed by phosphatidylinositol synthetase (EC 2.7.8.11). The other two inositol lipids are created by phosphorylation of the 4- and 5-OH groups of PtdIns in sequence to form PtdIns(4)P_1 and PtdIns(4,5)P_2, respectively (see Figure 3). The former is phosphorylated via ATP and 1-phosphatidylinositol kinase (PtdIns 4-kinase, also known as PtdIns kinase II, EC 2.7.1.67), and the latter was phosphorylated in turn via 1-phosphatidylinositol-4-kinase (PtdIns-4-P 5-kinase, EC 2.7.1.68)[2,6] (see Figure 2). By using intact human neutrophils and labeling with ^{32}P, which was incorporated into the phosphate groups of polyphosphoinositide, it was found that the radioactivity at C-5 was higher than that at C-4; in turn, the radioactivity at C-4 was higher than that at C-1.[11] These experimental data support the fact that PtdIns(4,5)P_2 is synthesized using PtdIns(4)P_1, which, in sequence, began with the phosphorylation of PtdIns. Furthermore, PtdIns(4,5)P_2 is hydrolyzed to PtdIns(4)P_1 and to PtdIns by the phosphatases, PtdIns(4,5)P_2 phosphomonesterase and PtdIns(4)P_1 phosphomonoesterase.[12]



R_1, R_2 = long-chain fatty acid.

a: Phosphatidylinositol synthetase (PtdIns synthetase)
b: Phosphatidylinositol kinase (PtdIns kinase)
c: Phosphatidylinositol 4-phosphate kinase (PtdIns-4-P kinase)
d: Phospholipase C

Figure 2. Phosphatidylinositol Cycle

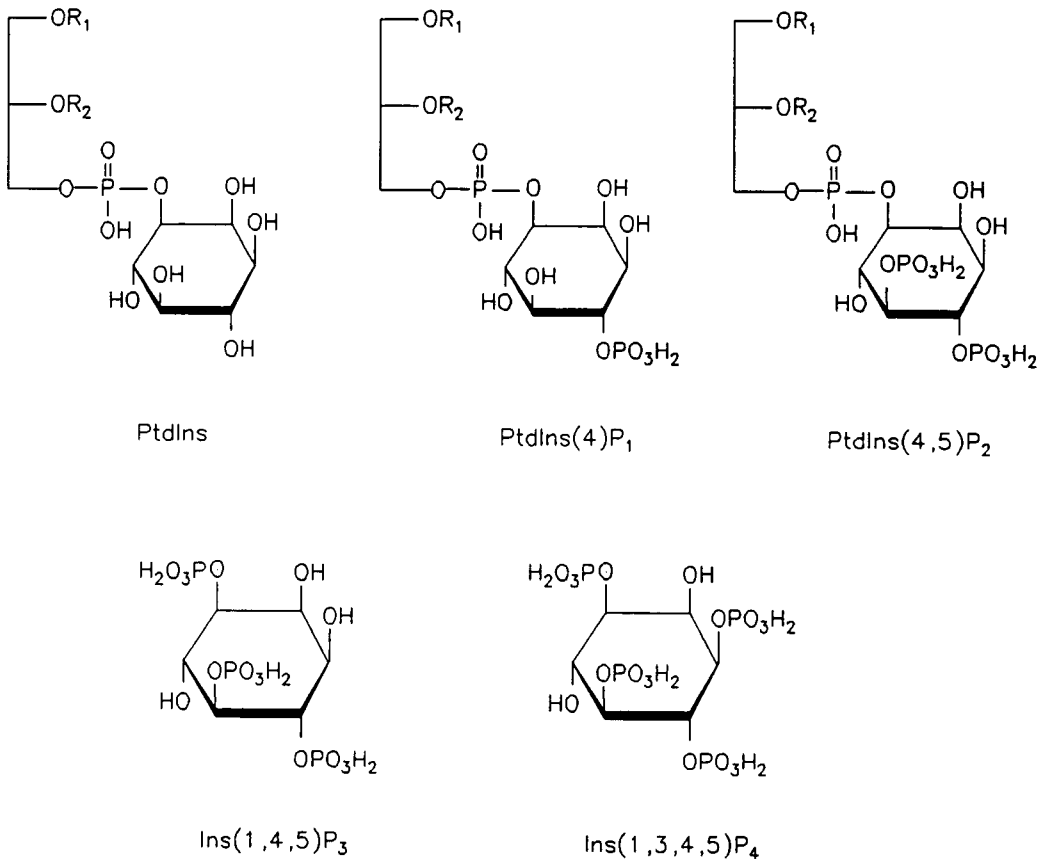


Figure 3. Inositol Phosphate Derivatives

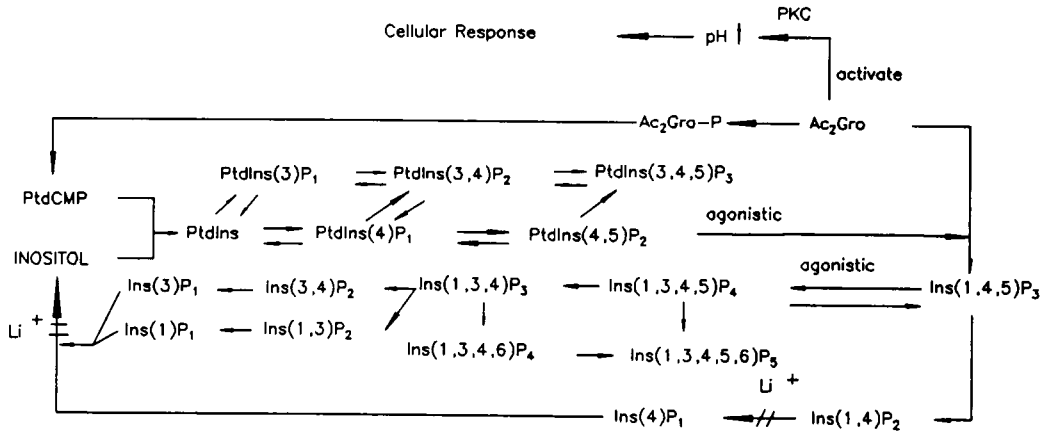


Figure 4. Phosphatidylinositol Metabolism.[2,5,7,9,10,11,14]

The generation of agonistic activity within the cell depends on the hydrolysis of PtdIns(4,5)P_2 by phospholipase C (PLC) to yield two products, Ac_2Gro and Ins(1,4,5)P_3, which are related to the production of intracellular signals. These interrelationships are illustrated in Figure 4. These two products [Ac_2Gro and Ins(1,4,5)P_3] are, for the most part, directed respectively into a lipid cycle and an inositol phosphate cycle, which later can once again form PtdIns.

There are two known fates of Ins(1,4,5)P_3. Studies of red blood cells have provided evidence that Ins(1,4)P_2 is derived from the dephosphorylation of Ins(1,4,5)P_3 by an enzyme located at the plasma membrane.[2,26] Ins(1,4)P_2 can be degraded to Ins(4)P_1, then to inositol that combines with PtdCMP to form PtdIns to regenerate the cycle. The other fate is the phosphorylation of Ins(1,4,5)P_3 by D-*myo*-inositol trisphosphate 3-kinase to form Ins(1,3,4,5)P_4. There is evidence that the Ins(1,3,4,5)P_4 also serves as another second messenger.[11,13] Ins(1,3,4,5)P_4 either modulates intracellular Ca^{2+} mobilization from inside the cell or reenters the cell via the extracellular membrane.

In addition, Ins(1,3,4,5)P_4 provides the other branch pathway of the PtdIns cycle, which produces additional inositol phosphates, such as Ins(1,3,4,6)P_4 and Ins(1,3,4,5,6)P_5.[13,14]

A phosphatidylinositol 3-kinase (PtdIns 3-kinase, also known as PtdIns kinase I) has been found and is linked to the action of growth factors and oncogene products.[17] However, the PtdIns 3-kinase reaction seems to lack specificity. The enzyme can phosphorylate PtdIns, PtdIns(4)P, and PtdIns(4,5)P_2 to yield PtdIns(3)P, PtdIns(3,4)P_2, and PtdIns(3,4,5)P_3, respectively. These phospholipids collectively account for less 2% of total inositol phospholipids in human platelets; however, their role in oncogenesis may be profound.[5,18]

Recently, it has been found from PDGF-stimulated fibroblast and smooth muscle cell experiments that the products of PtdIns 3-kinase phosphorylation at the D-3 position of *myo*-inositol are poor substrates for both brain and liver phospholipase C.[15,19] The pathway for PtdIns phosphorylation at the D-3 position is different from the pathway of PtdIns conversion to PtdIns(4,5)P_2, diacylglycerol, and Ins(1,4,5)P_3. This new pathway has revealed new PtdIns metabolism and intracellular signaling activity.[16]

An experiment using middle T antigen-transformed cell activation of PtdIns 3-kinase has shown that Ins(3,4)P_2 is derived from the Ins(1,3,4)P_3 intermediate.[14] Ins(3,4)P_2 and Ins(1,3,4)P_3 arise from consecutive dephosphorylation of Ins(1,3,4,5)P_4. The concentrations of Ins(1,3,4)P_3 and Ins(1,3,4,5)P_4 increase after PDGF treatment of a quiescent cell culture. One might guess that those two inositol phosphitides were involved in the hydrolysis of PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 by the catalysis of phospholipase.[14] Alternatively, the PtdIns 5-kinase has been found to incorporate ^{32}P into

PtdIns(3,4)P_2 to yield PtdIns(3,4,5)P_3 in human platelets.[18] These data indicate that PtdIns(3,4,5)P_3 did not result from phosphorylation of PtdIns(4,5)P_2, but of PtdIns(3,4)P_2. This result has thus defined the pathway for the biosynthesis of these lipids in the order of PtdIns → PtdIns(3)P → PtdIns(3,4)P_2 → PtdIns(3,4,5)P_3. Finally, these inositol phosphatides are converted into *myo*-inositol by successive dephosphorylation. The PtdIns cycle once again begins with *myo*-inositol reacting with PtdCMP making PtdIns plus CMP.

Lithium has been known to be an inhibitor of some of the enzymes that hydrolyse inositol phosphates to regenerate *myo*-inositol (see Figure 4).[6,12,20] Because lithium reduces inositol concentration, signal transduction is indirectly slowed by an insufficient supply of Ins(1,4,5)P_3 and Ac_2Gro. The scenario is as follows: Ins(1,4,5)P_3 is released in the hydrolysis of PtdIns(4,5)P_2 by phospholipase C. Finally, Ins(1,4,5)P_3 is degraded to the monophosphate and then to free inositol. Free inositol is not formed in the presence of lithium (Li^+) where inositol monophosphates accumulate in proportion to the stimulated breakdown of PtdIns(4,5)P_2. Because Li^+ blocks the production of free inositol, PtdCMP accumulates. Furthermore, the blocking result by lithium can indicate phosphoinositide turnover during embryogenesis[12] as determined by measuring the concentration of PtdCMP.

C. A Synthetic Approach to D-myo-Inositol Analogues from Optically Active Precursors

A number of procedures to prepare Ins(1,4,5)P_3 from *myo*-inositol have been developed.[22,25] In biological systems, a transformation through the action of Ins(1,4,5)P_3 to yield Ins(1,3,4,5)P_4 by Ins 3-kinase has been demonstrated as a means to regenerate intracellular pools of the tetrakisphosphate. The action of Ins 3-kinase on Ins(1,4,5)P_3 to generate Ins(1,3,4,5)P_4 as a secondary messenger is not really clear,[23] but agents that affect the metabolism of Ins 3-kinase should be considered as potential probes of inositol phosphate function. Because *myo*-inositol is a meso compound, however, synthetic intermediates are a racemic mixture. Optical resolution methods must be applied to isolate pure enantiomers. An alternative method is to use an optically active starting material to synthesize the desired D-*myo*-inositol analogues without using a chemical resolution method.

Inositols in plants exist as a number of methyl ethers[21] that can serve as candidates to create the optically active *myo*-inositol derivatives. The most abundant monomethyl ether inositol is L-quebrachitol [1L-(-)-2-*O*-methyl-*chiro*-inositol], which comes from the extract of the serum of the rubber tree (*Hevea brasilliensis*).[21,23] The L-quebrachitol constitutes from 1 to 1.5% (12 to 18% of the solids) of the aqueous phase (serum) of the latex exudate.[21] The author's purpose is to utilize L-quebrachitol as a useful starting material for the synthesis of 3-substituted 1D-*myo*-inositol derivatives in optically active form.

II. STATEMENT OF THE PROBLEM

A. Design and Synthesis of the *myo*-Inositol Analogues

My research goal is to design and synthesize 3-modified *myo*-inositol analogues that would remain as good substrates for phosphatidylinositol synthetase, the first enzyme encountered in the phosphatidylinositol pathway. C-3-modified lipids so obtained would then serve as potential inhibitors of either phosphatidylinositol 3-kinase or inositol 3-kinase. L-Quebrachitol, a gift from Professor L. Anderson (University of Winsconsin, Madison), was chosen as the starting material.

The selective protection of hydroxy groups on L-quebrachitol is very important in modifying the first position (L-*chiro*-inositol numbering) to convert it into 3-modified *myo*-inositols. To prepare optically active 3-modified *myo*-inositol analogues (including 3-deoxy, 3-halo, 3-mercapto, and 3-hydroxyl derivatives), the position at which the additional phosphate groups were added to yield PtdIns(3)P_1 or PtdIns(3,4)P_2 would be inhibited. The chemical methods I apply here include the stereospecific inversion of the 1-OH group (L-*chiro* numbering), followed by the demethylation of the 2-OCH_3 group.

B. Evaluation of D-*myo*-Inositol Analogues with PtdIns Synthetase

Two methods are employed to investigate the inhibition of *myo*-inositol analogues with PtdIns synthetase, the enzyme which catalyzes the coupling of *myo*-inositol with PtdCMP: (1) Inhibition of *myo*-inositol uptake and (2) CMP formation from PtdCMP. The research was carried out by our collaborators, J. D. K. Dawer and T. L. Benjamin of the Department of Pathology at Harvard University.

III. RESULTS AND DISCUSSION

A. Design and Synthetic Approach to *myo*-Inositol Analogues

The numbering system throughout this paper is based on that used for *myo*-inositol (Figure 1) and D,L-*chiro*-inositol. By convention, if the numbering of carbons in inositol is viewed clockwise from the top of the ring, it indicates the L configuration, and if viewed counterclockwise, it indicates the D configuration.[21,27]

My research goal was to modify the 3-position of D-*myo*-inositol and yet maintain respectable substrate activity with the first enzyme of the phosphatidylinositol pathway, phosphatidylinositol synthetase. Lipids modified at the 3-position should then be potential inhibitors of phosphatidylinositol 3-kinase, which phosphorylates PtdIns to make PtdIns(3)P_1 and D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] to produce D-*myo*-inositol 1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)P_4]. The PtdIns(3)P_1 or D-*myo*-inositol-1,3,4,5-tetrakisphosphate are therefore considered to be the second messenger that stimulates the Ca^{2+} to enter the cell from an extracellular environment.

The starting material we chose was the naturally abundant optically active inositol, L-quebrachitol, the monomethyl ether of L-*chiro*-inositol (Figure 5). By selective protection (Scheme I) of the four hydroxyl groups (at C-3, C-4, C-5, and C-6 by *chiro*

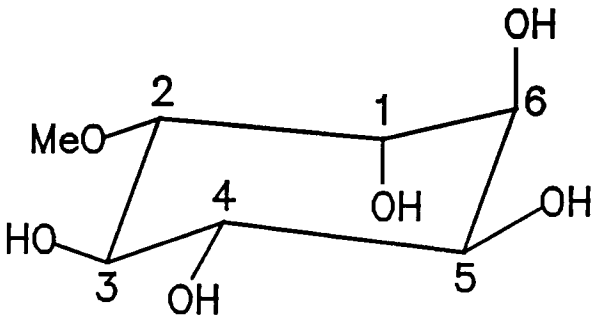
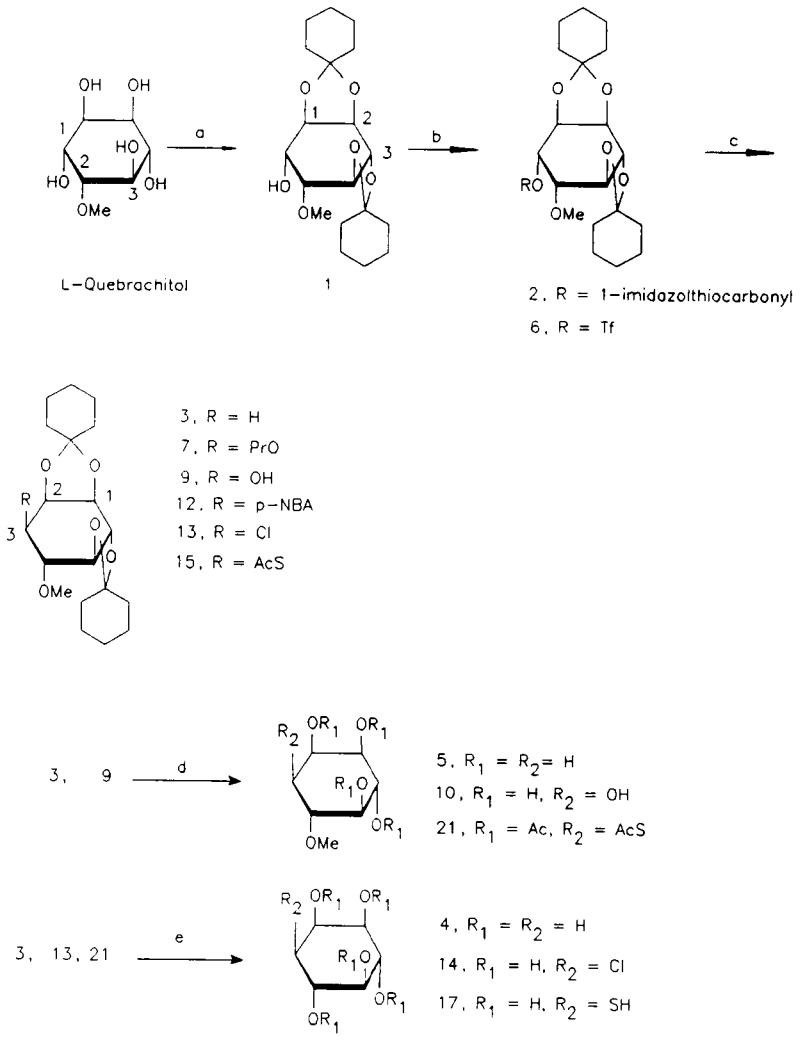


Figure 5. L-Quebrachitol [1L-(-)-2-*O*-methyl-*chiro*-inositol]

SCHEME I



a. 1—Ethoxycyclohexene; b. c. Various reagents; d. CF_3COOH solution;

e. BBr_3—CH_2Cl_2

numbering. The numbering system will be changed to C-4, C-3, C-2, and C-1 after protection), we can modify (deoxygenate, invert, or substitute) the 6-position (3-position in D-*myo*-inositol), followed by deacetalation with an aqueous CF_3COOH solution and demethylation with 1.0 M BBr_3—dichloromethane to obtain the *myo*-inositol analogues.

An elimination product **8** was inevitably formed when steps were taken to invert the configuration at the C-3 position either by reaction of cesium propionate (Scheme III, p. 25) or lithium chloride (Scheme IV, p. 33), or potassium thioacetate (Scheme V, p. 37) with the triflate product **6**. The trans relationship between the triflate and the hydrogen at C-5 (*chiro* numbering) favors the formation of the product **8**.

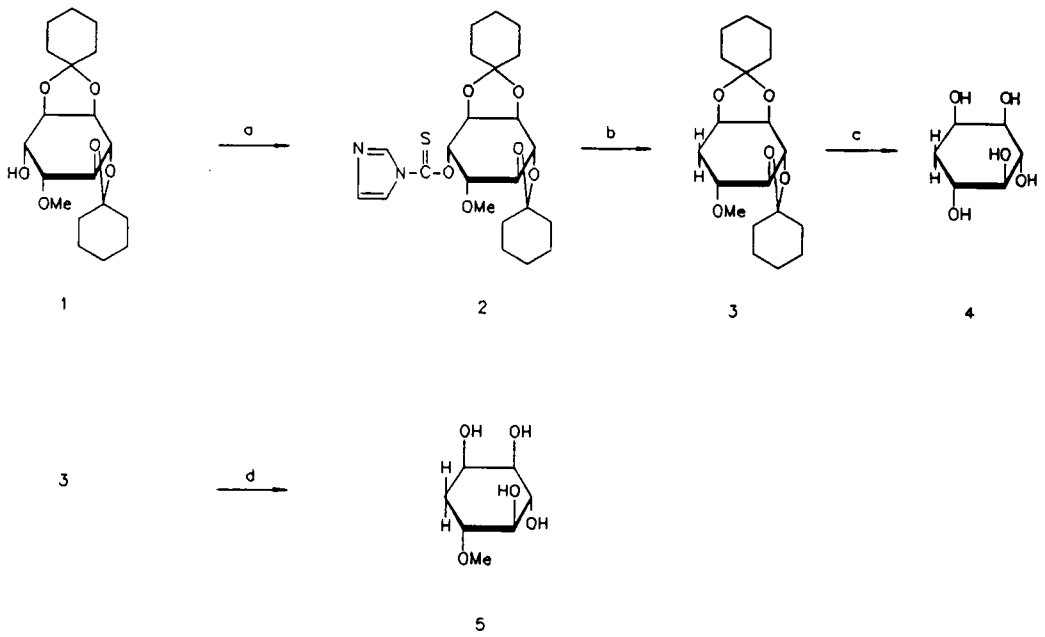
1. Improved preparation of di-*O*-cyclohexylidene derivatives of L-quebrachitol [1L-(-)-2-*O*-methyl-*chiro*-inositol]. When we used 1,1'-diethoxycyclohexane as the protecting group reagent, the yield of **1** was very low and the monoprotected compound was predominant, even though the reaction mixture was refluxed under vigorous conditions (120 °C) for a few days. The alternative method using 1-ethoxycyclohexene followed known procedures.[28] If we treated L-quebrachitol with relatively pure 1-ethoxycyclohexene (usually 4 equiv, >75% pure) with a catalytic amount of *p*-TSA in DMF solution, the yield of the di-*O*-cyclohexylidene derivative **1** was increased tremendously (up to 85%). The crude mixture of mono- and di-*O*-cyclohexylidene derivatives could be separated by a fractional crystallization from 95% EtOH. The di-*O*-cyclohexylidene derivative crystallized first as colorless rectangular crystals (mp 117—118 °C). The proton assignments for **1** in the ^1H NMR spectrum are very ambiguous because of the two broad multiple resonances at δ 3.58—3.75 and δ 4.18—4.44, each of which

is equal to three hydrogen atoms. The structure was confirmed by integration at the region of δ 1.23—1.80 (20 H, 2 cyclohexylidenes) and δ 2.79 (dd, 1 H, OH).

2. 3-Deoxy-*myo*-inositol. Compound **4**, 1L-1-deoxy-*chiro*-inositol, i.e., the 1- or 3-deoxy-*myo*-inositol, is also named (-)-viburnitol (Scheme II). Existing synthetic routes[34,35,36] to the optically active isomers **4** are generally of low yield and are lengthy. Racemic compound **4** was synthesized by C. Jiang et al.[30] in high yield (88%). In order to have the optically active compound **4**, we chose the optically active starting material, L-quebrachitol, instead of *myo*-inositol. Demethylation of **3** was achieved by adding 1 M BBr_3 in CH_2Cl_2, and removal of the acetal functions was carried out by adding MeOH, which generates HBr to cleave the cyclohexylidene groups forming **4** in 63% yield.

1D-3-Deoxy-*myo*-inositol (4). A direct route to this compound is shown in Scheme II. The deoxygenation step used the method of Barton and McCombie,[29] in which **1** is treated with 1,1'-thiocarbonyldiimidazole in to give the 6-*O*-(1-imidazolthiocarbonyl) derivative in *N*,*N*-dimethylformamide (DMF). This thioester derivative was obtained in 94% yield after column chromatography and was treated with tributyltin hydride (Bu_3SnH) and α,α'-azobis-(2-methylpropionitrile) (AIBN) to give the deoxygenated compound **3** in 74% yield. The yields of the preceding reactions were found to match the results reported in a previous publication.[30] Evidence for the deoxygenation can be supported by the two high-field multiplicities (δ 2.37, with the remaining one almost overlapping with two cyclohexylidene resonances at the region δ 1.21—1.78), which are typical of geminal protons coupling with themselves and the adjacent two protons. Demethylation of **3** was accomplished by adding 1 M BBr_3 in dichloromethane solution,

SCHEME II



a. 1,1'-Thiocarbonyldiimidazole—DMF; b. Bu_3SnH—AIBN—Toluene;

c. BBr_3—CH_2Cl_2; d. 3:1 CF_3COOH—H_2O

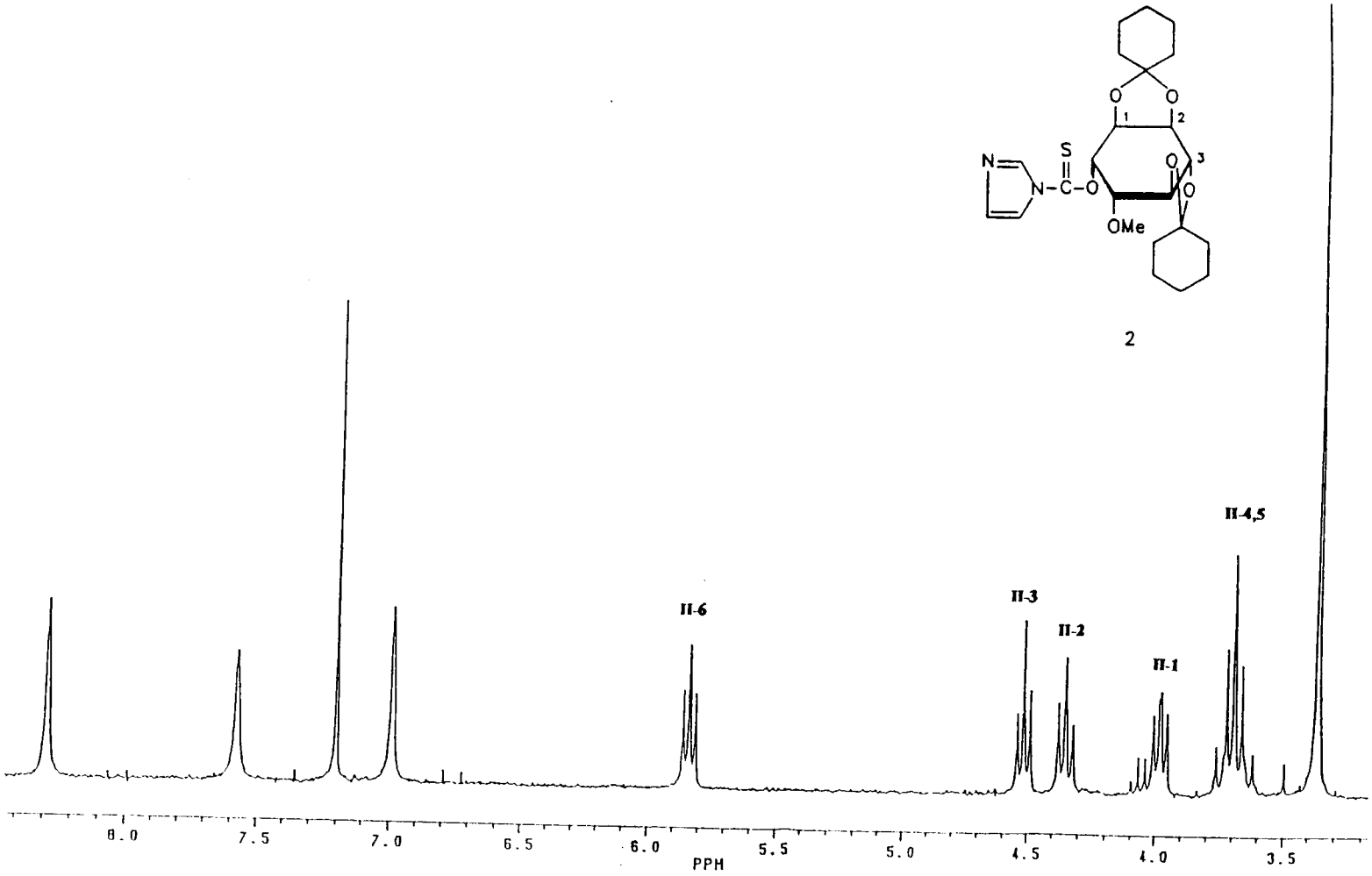
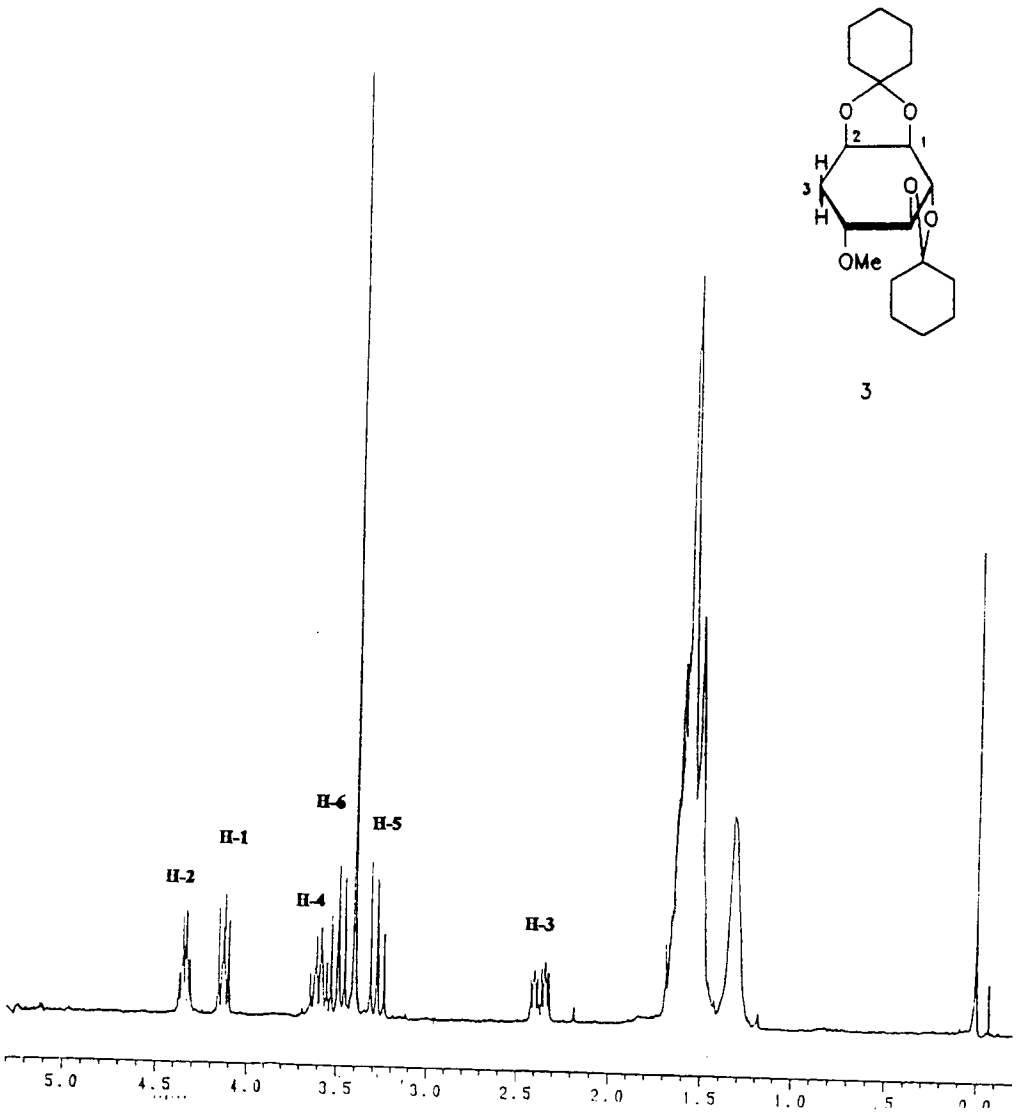


Figure 6. Partial ^{1}H NMR spectrum for **2**.



3

Figure 7. Partial ^{1}H NMR spectrum for **3**.

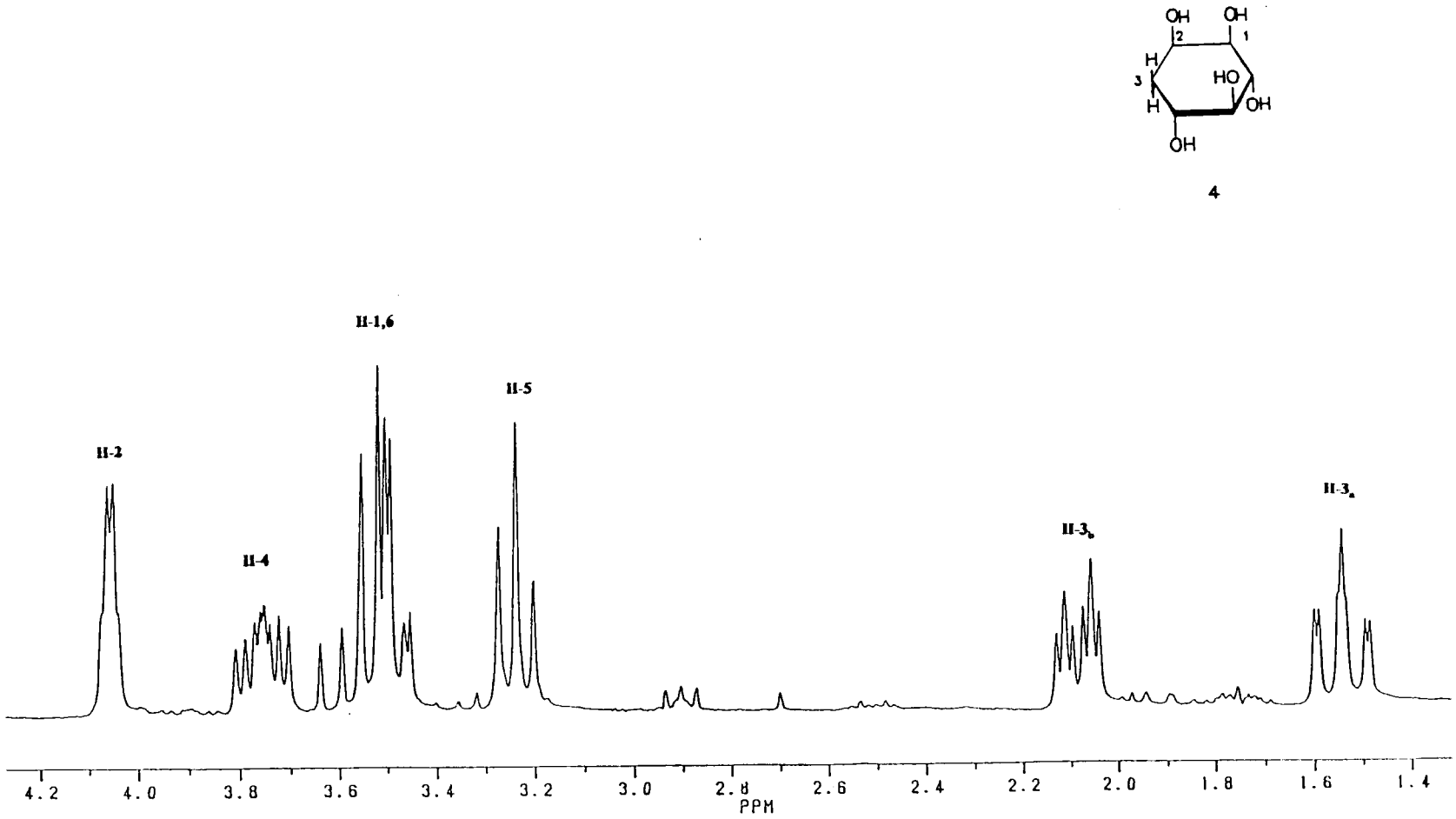


Figure 8. Partial ^{1}H NMR spectrum for **4**.

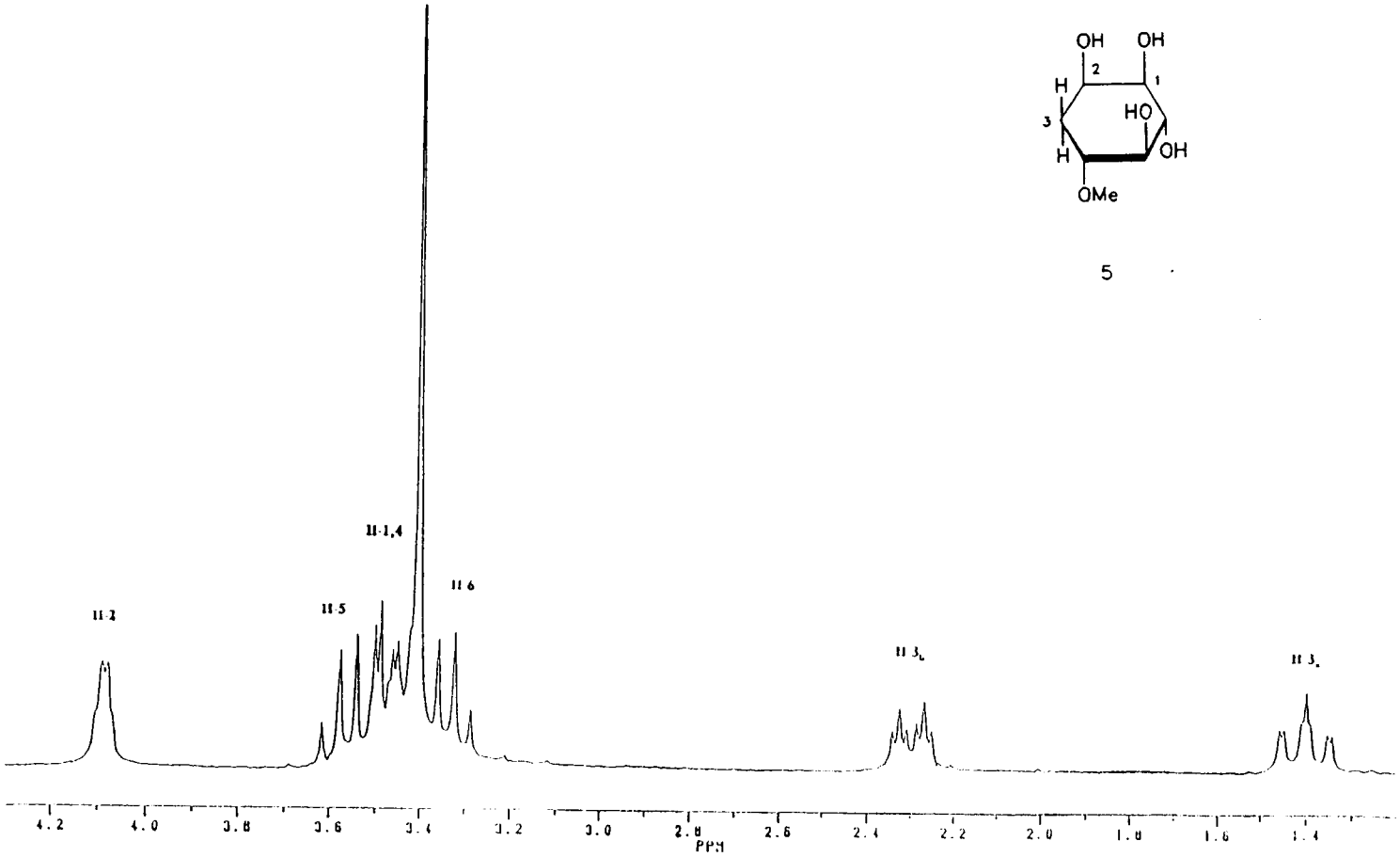


Figure 9. Partial ^{1}H NMR spectrum for **5**.

followed by repeatedly adding several portions of dry MeOH so that coevaporation would remove the excess boron as $B(OMe)_3$. The structure of **4** was confirmed from its ^1H NMR spectrum that had previously been reported for d,l-viburnitol.[30] The geminal H's at C-3 are located at δ 2.09 and 1.56, respectively, the latter of which can be assigned the axial hydrogen because of the large axial—axial coupling with H-4. The equatorial H at C-2 is at low field at δ 4.06, and its splitting with H-1 is small. One cannot determine the $J_{1,2}$ value because of the splitting of its shoulder edges of its doublet of doublets, but its value is reflected in the H-1 resonance at δ 3.48, which is about 2.2 Hz. H-4 is a typical multiplet (ddd) with the center at δ 3.75. The optical rotation of **4** is approximately that reported.[21]

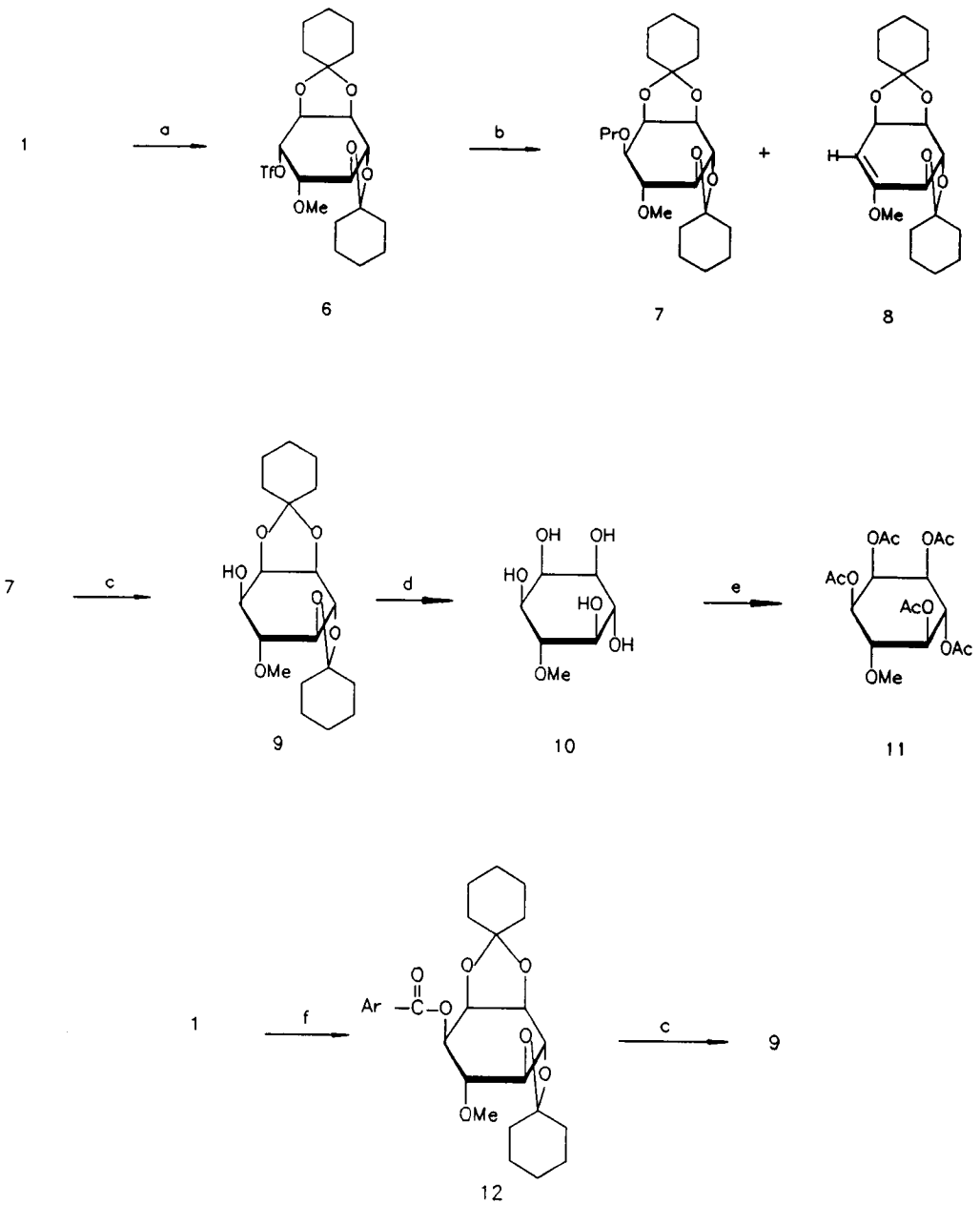
1D-3-Deoxy-4-*O*-methyl-*myo*-inositol (5). Deprotection of **3** was carried out in trifluoroacetic acid solution for 10 min at room temperature, followed by addition of abs. EtOH to remove the excess acid by coevaporation, to give **5**. The splitting patterns of the geminal H's at C-3 were the same as those for **4**, but were shifted to δ 2.29 and 1.40, respectively. The two pseudotriplet's resonance center at δ 3.57 and 3.32 could be assigned H-5 and H-6, respectively, according to decoupling experiments. H-1 and H-4 are very hard to distinguish because of their overlapping at the region δ 3.37—3.51 with part of the methoxy group resonance.

3. The 4-monomethyl ether of *myo*-inositol. Compound **10**, known also as D-(+)-ononitol, is D-*myo*-inositol methylated at C-4. D-Ononitol is present in the leaf tissues of some trees formed in tropical regions.[37] D-Ononitol was proved to be the biochemical precursor[38] of D-pinitol (1D-3-*O*-methyl-*chiro*-inositol) in the Jojoba tree.

D-Ononitol and D-pinitol are epimers. By using a $^{14}CO_2$ pulse-chase experiment[39] on young, expanding leaves,[38,39] the inositol was shown to be methylated and readily converted into D-ononitol and D-pinitol in young leaf tissue, but synthesis terminated when the plants were mature. Previous synthetic routes[40,41,42] to D-ononitol have been reported in low yield. Some synthetic methods[43,44,45] have been reported to convert *myo*-inositol into either (+)-ononitol or (±)-ononitol in moderate yield.

1D-(+)-Ononitol (1D-4-*O*-methyl-*myo*-inositol, 10). A direct route to this compound is shown in Scheme III. In order to invert the C-3 center, we treated **1** with trifluoromethanesulfonic anhydride to give triflyl intermediate **6**, which was not purified because of its instability. Reaction of **6** with cesium propionate gave **7** in 29% yield, accompanied by elimination product **8** in 32% yield. Deprotection of **9** was carried out in aqueous CF_3COOH solution to give **10** in 75% yield. The COSY technique indicated a doublet of doublets centered at δ 3.47 that could be assigned as either H-1 or H-3. One of the signals was overlapped with the other hydrogen and methoxy group in the region δ 3.56—3.66. The most downfield and small pseudotriplet splitting pattern, which indicates the equatorial—axial hydrogen coupling can be assigned as H-2. The proton assignments cannot be determined as H-2 by homodecoupling because of the overlapping at the region δ 3.3—3.4 and 3.56—3.66. The coupling constant problem can be resolved by peracetylating **10** to afford **11** and observing the ^1H NMR spectrum of the peracetate. The pseudotriplet in the most upfield region can be assigned as H-4, the carbon where the methoxy group is attached. The coupling constant of $H_{4,5}$, $H_{5,6}$, and $H_{6,1}$ are almost

SCHEME III



Tf— = CF_3SO_2— Pr— = CH_3CH_2CO— Ar— = *p*-NO_2-C_6H_5—

a. $(CF_3SO_2)_2O$—CH_2Cl_2—Pyridine; b. $CsOCOCH_2CH_3$—DMF; c. NaOMe—MeOH

d. 3:1 CF_3COOH—H_2O; e. Ac_2O—Pyridine; f. DEAD—TPP—Benzene—*p*-NBA

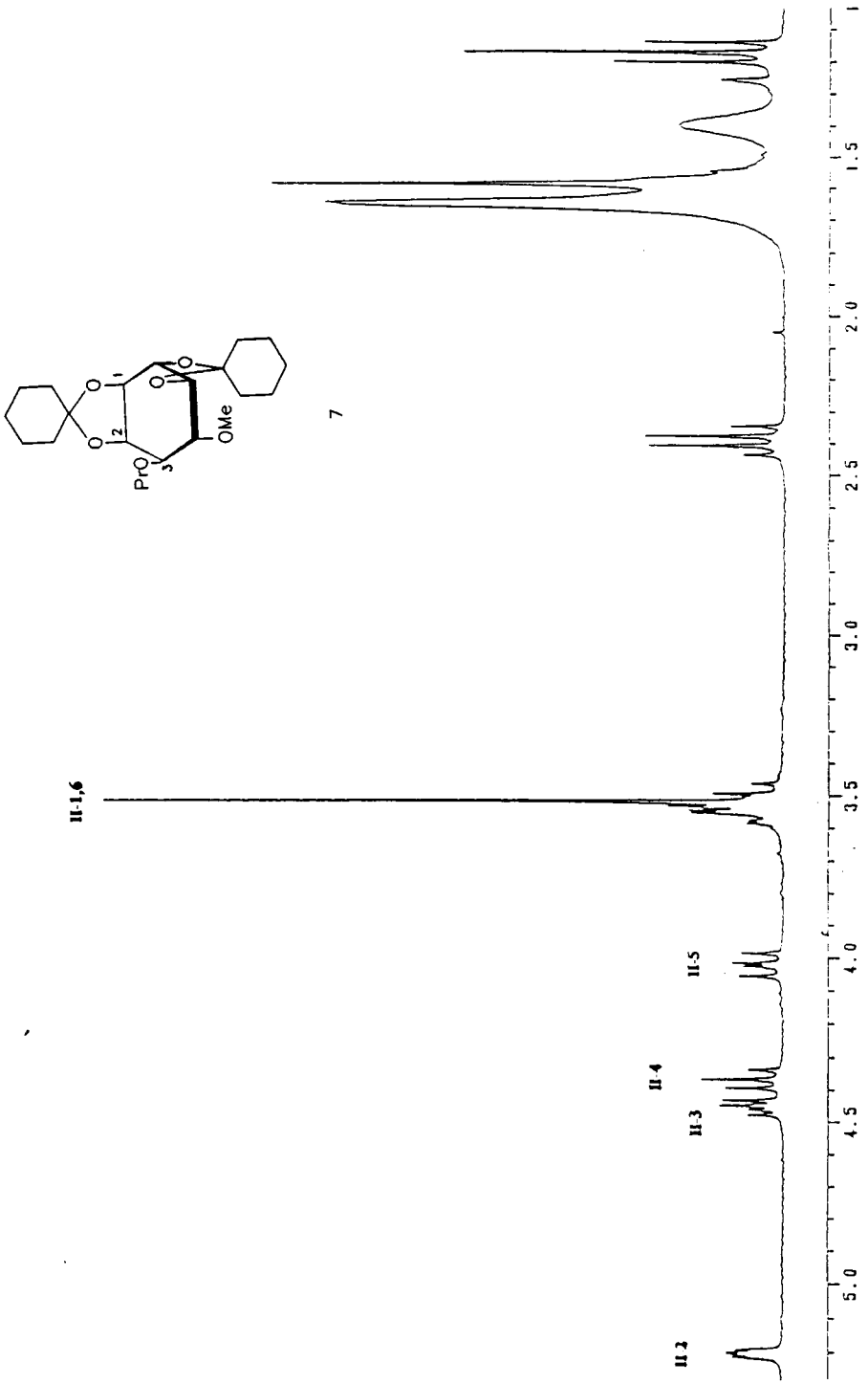


Figure 10. Partial ^{1}H NMR spectrum for **7**.

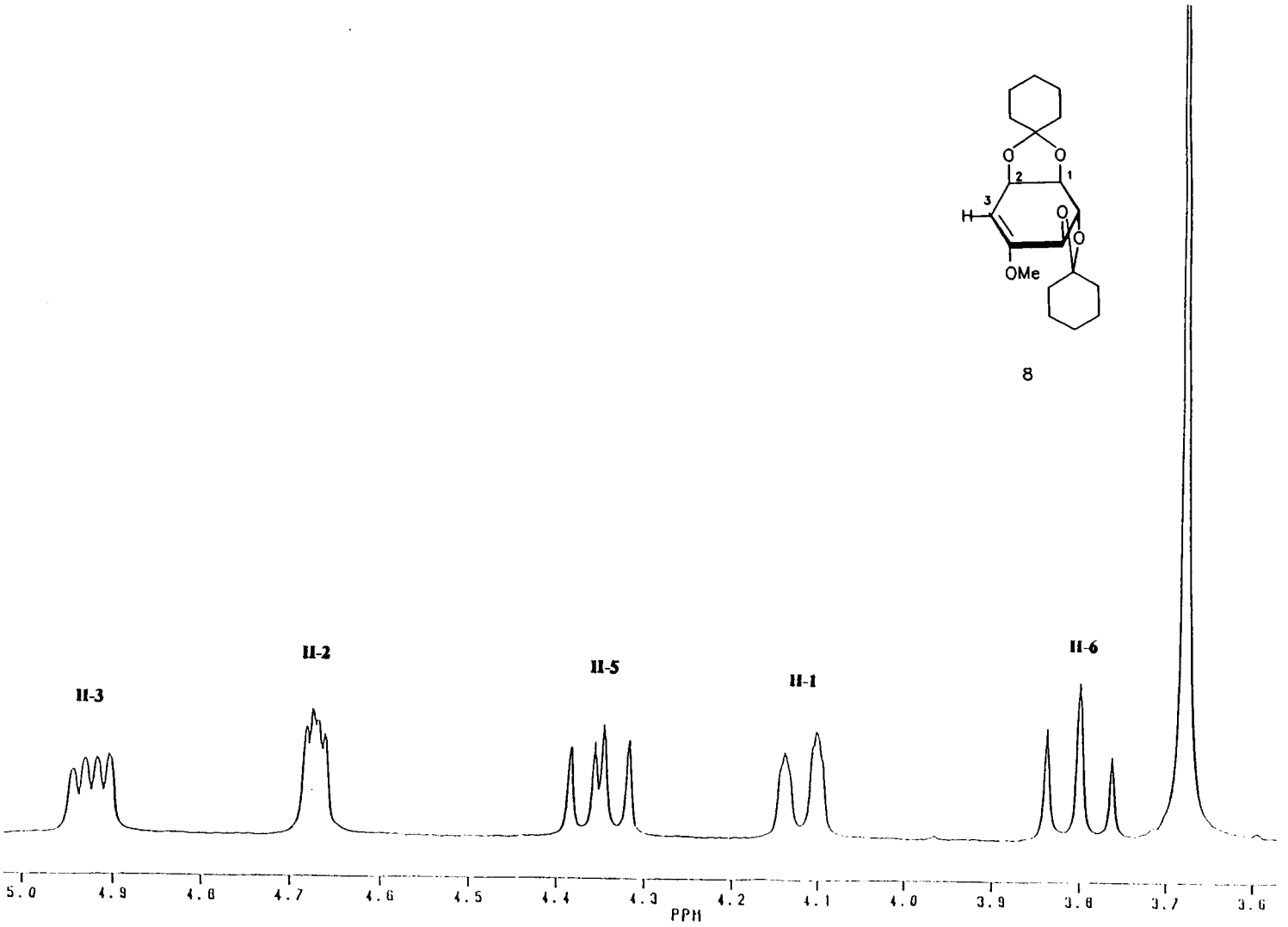


Figure 11. Partial ^{1}H NMR spectrum for **8**.

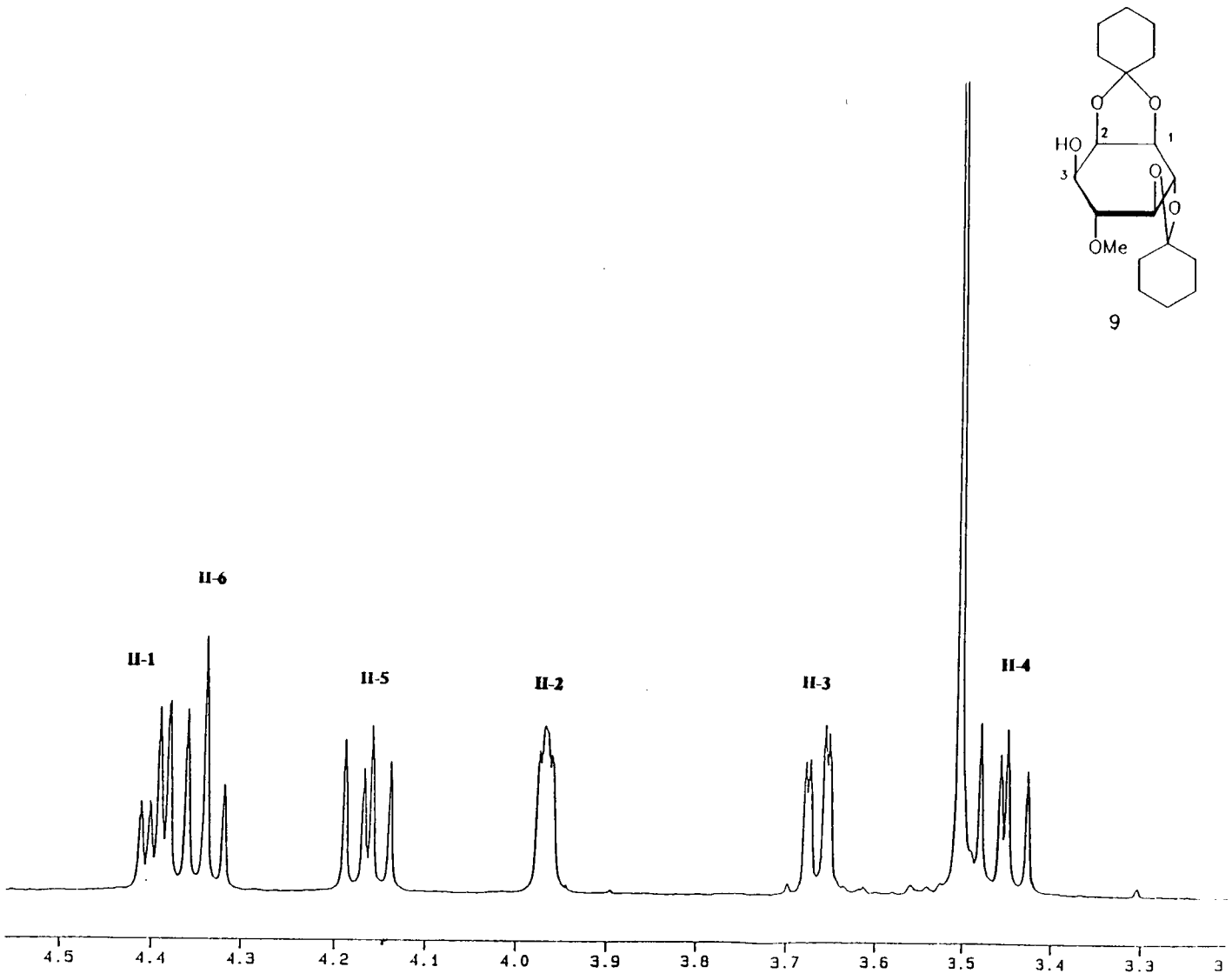


Figure 12. Partial ^{1}H NMR spectrum for **9**.

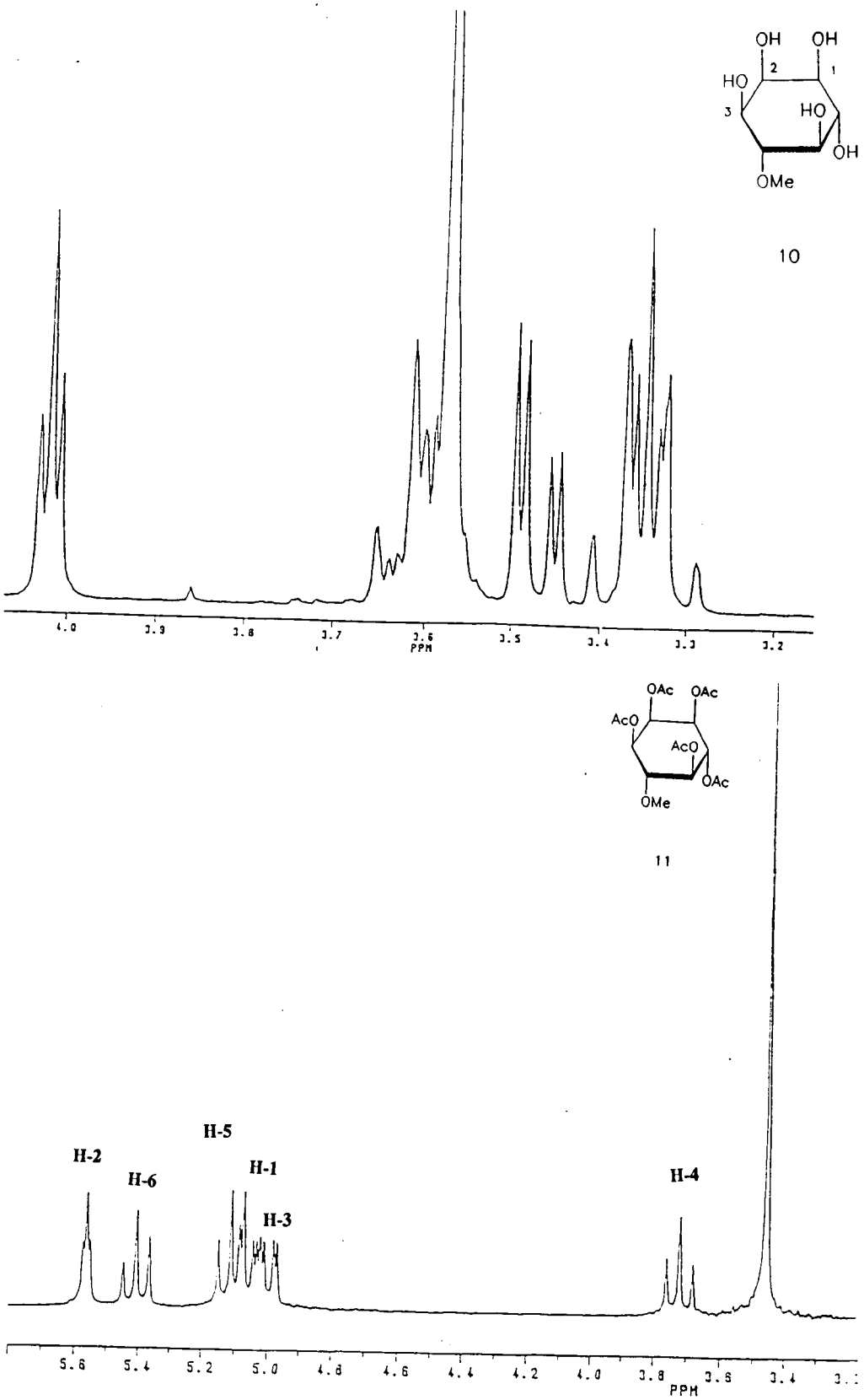


Fig 13. Partial ^{1}H NMR spectra for **10** and **11**.

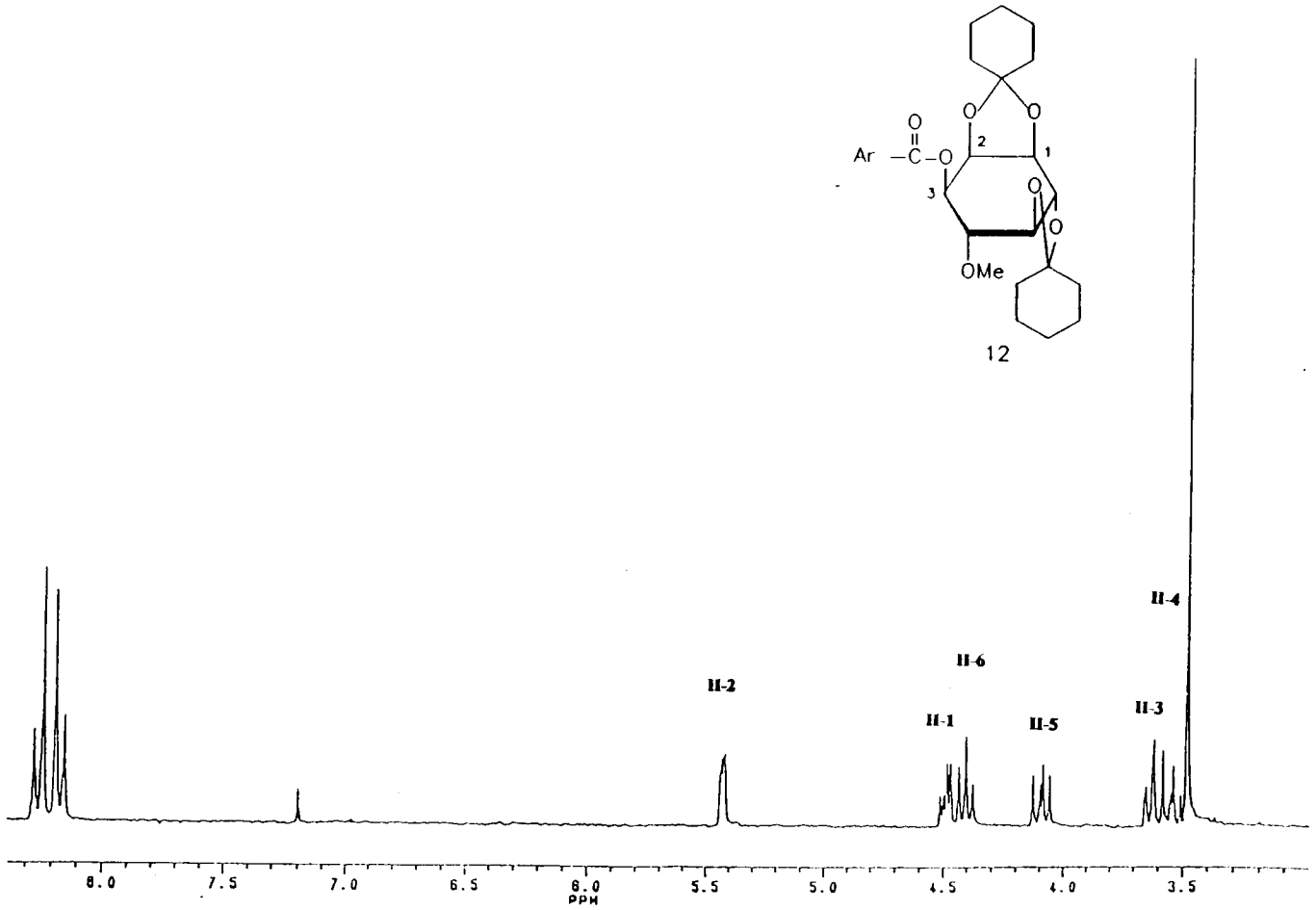


Figure 14. Partial ^{1}H NMR spectrum for **12**.

equal at about 10 Hz, giving patterns that are essentially pseudotriplets. The small—small coupling at the most downfield region is indicated to be that of H-2.

From the decoupling techniques used on **8**, the alkene H at the C-3 center had a long-range coupling with H-5 of 7.1 Hz. The coupling constant between H-3 and H-1 was very small at almost 0 Hz. Deprotection of **8** was unsucessfully attempted by treatment with an aqueous CF_3COOH solution. It was hoped that the resulting methyl enol ether would be converted into the ketone by hydrolysis and tautomerization.

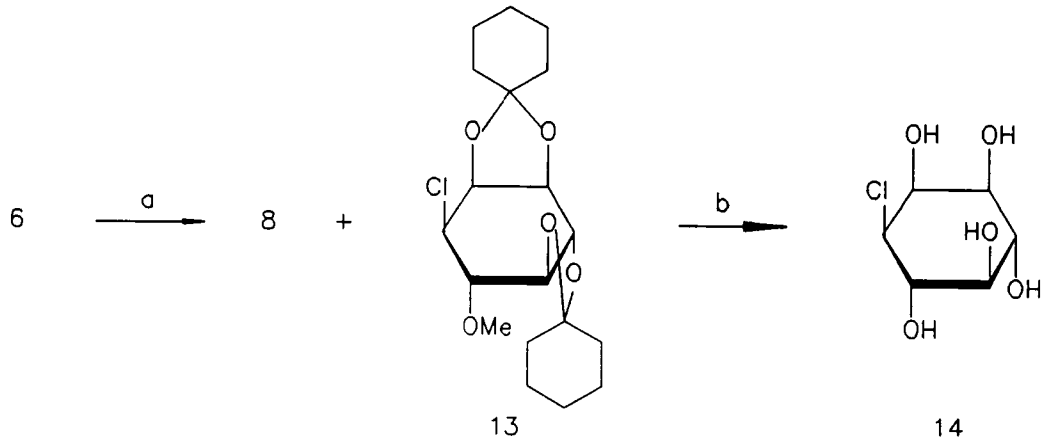
In order to prepare a more stable leaving group at C-3 on **1**, an alternative method is to treat **1** with *p*-toluenesulfonyl chloride in DMF solution. However, the replacement cannot be accomplished by cesium propionate as the resulting tosyl functional group is too unreactive, even under vigorous refluxing conditions.

An alternative method to prepare D-(+)-ononitol from intermediate **12** was employed. This method involved a modification of the Mitsunobu reaction as developed by Martin and Dodge.[46] Compound **1** was treated with *p*-nitrobenzoic acid (*p*-NBA), triphenylphosphine (TPP), and diethyl azodicarboxylate (DEAD), at room temperature in benzene, rather than in THF. After stirring for 10 h, TLC showed a minor amount of product along with a preponderant amount of starting material. This mixture was refluxed for 13 h at which time TLC indicated enhancement of product (having an R_f value similar to that of compound **8**) and residual starting material **1**. Even after the addition excess DEAD, followed by stirring at room temperature for 12 h, then refluxing (90 °C) for 38 h, some starting compound still remained. Column chromatography recovered **1** and afforded **12** in 74.1% yield (based on recovered **1**). Use of this

modified method afforded compound **12** in much greater yield when compared to the method of cesium propionate replacement of **6** (29%) and avoided the unwanted production of **8**. According to previously discussed procedures, saponification of **12** gave **9**, which when followed by deprotection, afforded 1D-(+)-ononitol **(10)**.

4. 3-Deoxyhalo-*myo*-inositol: 1D-3-chloro-3-deoxy-*myo*-inositol (14). A direct route to this compound is shown in Scheme IV. The halo-de-hydroxylation of **1** to synthesize the deoxychloro derivative could not effectively be accomplished by treating **1** with triphenylphosphine—carbon tetrachloride—imidazole[31] (in CH_2Cl_2 or CH_3CN or DMF at reflux). The yield of chloro compound was only 2—8% after column chromatography. The starting material was almost fully recovered. An alternative method for preparation of **14** was to prepare the triflate derivative **6** and displace with lithium chloride in stirring DMF—imidazole solution at room temperature. After the DMF was removed under vacuum, the resulting residue was chromatographed to yield 44.8% and 36.4% of **8** and **13**, respectively. From the decoupling of **13**, the most downfield resonance was assigned to H-2 (δ 4.48), of which the coupling with H-1 ($J_{1,2}$ = 6 Hz) was larger than the coupling with H-3 ($J_{2,3}$ = 4.5—5.1 Hz). The axial hydrogen at C-3 has a moderate coupling with both H-2 and H-4 ($J_{2,3} = J_{3,4}$ = 5.1 Hz) and thus appears as a pseudotriplet. Part of the H-6 resonance is overlapping with H-3, but the axial—axial couplings with H-1 and H-5 can be determined from the upfield resonance of the H-5 (at δ 3.38) and the downfield resonance of H-1 (at δ 4.29). The *J*-coupling is indicated, $J_{5,6}$ = 10.5 Hz and $J_{1,6}$ = 8.1 Hz, respectively. Demethylation

SCHEME IV



a. LiCl—DMF—Imidazole; b. BBr_3—CH_2Cl_2

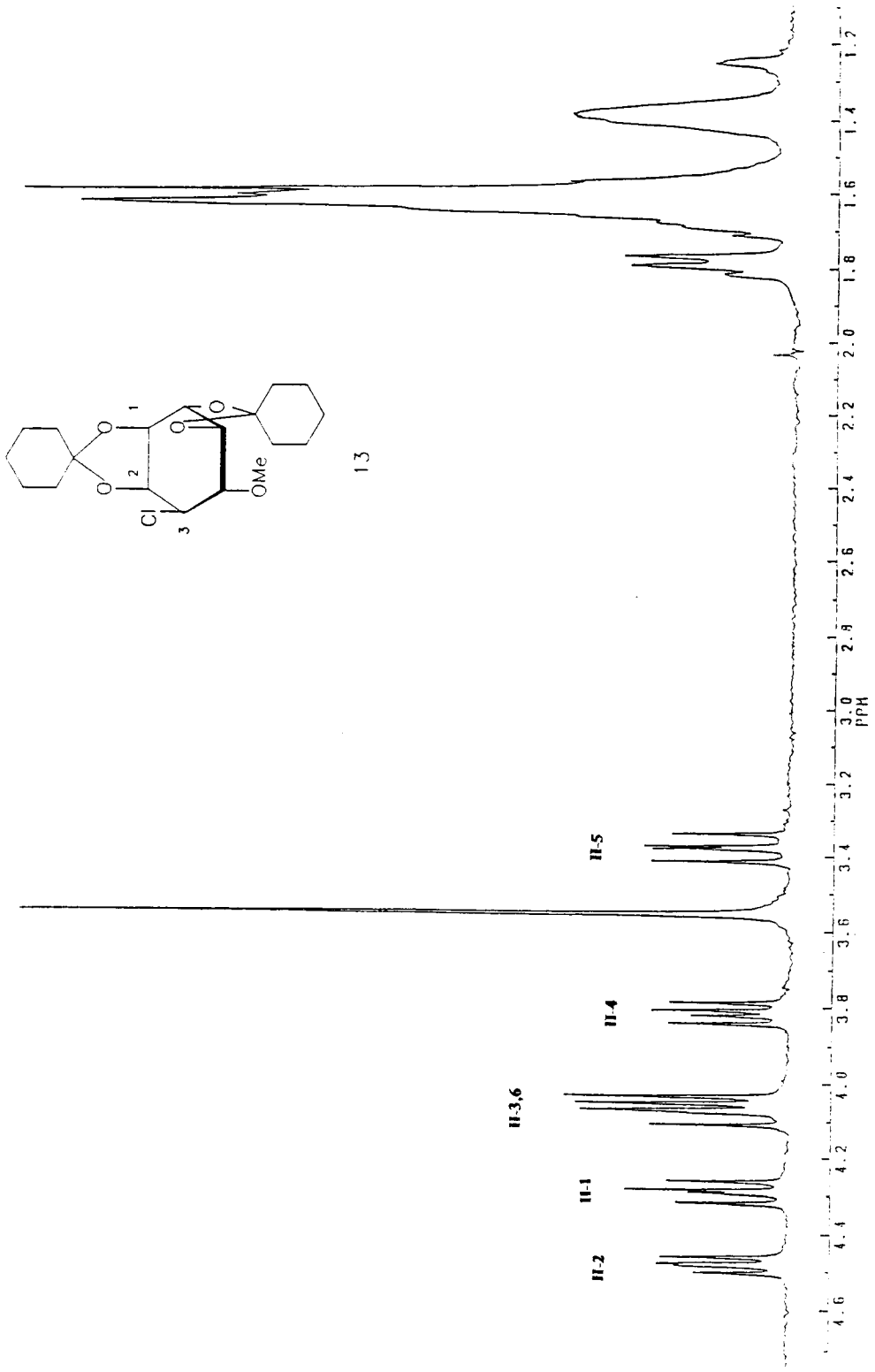


Figure 15. Partial ^{1}H NMR spectrum for **13**.

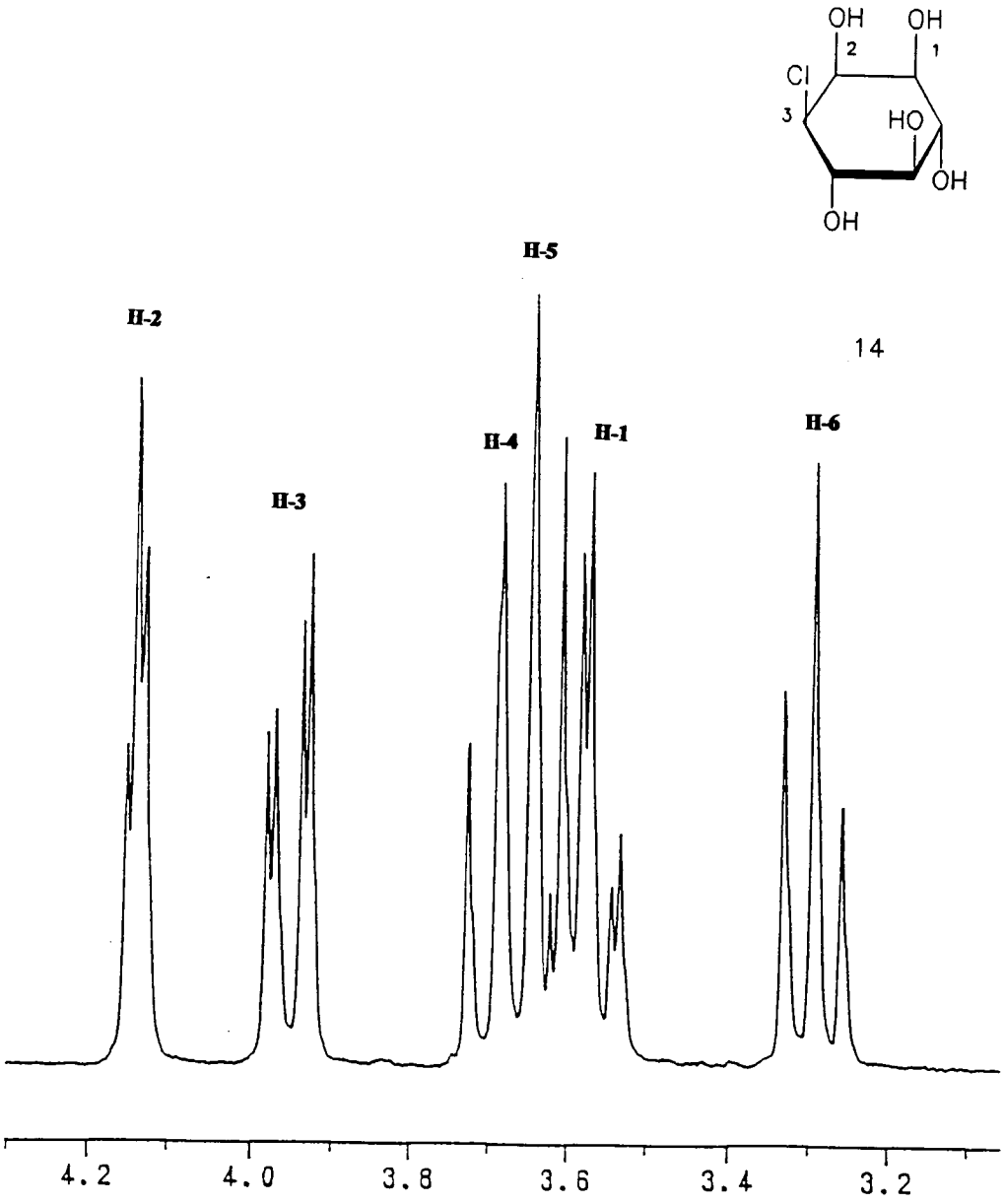
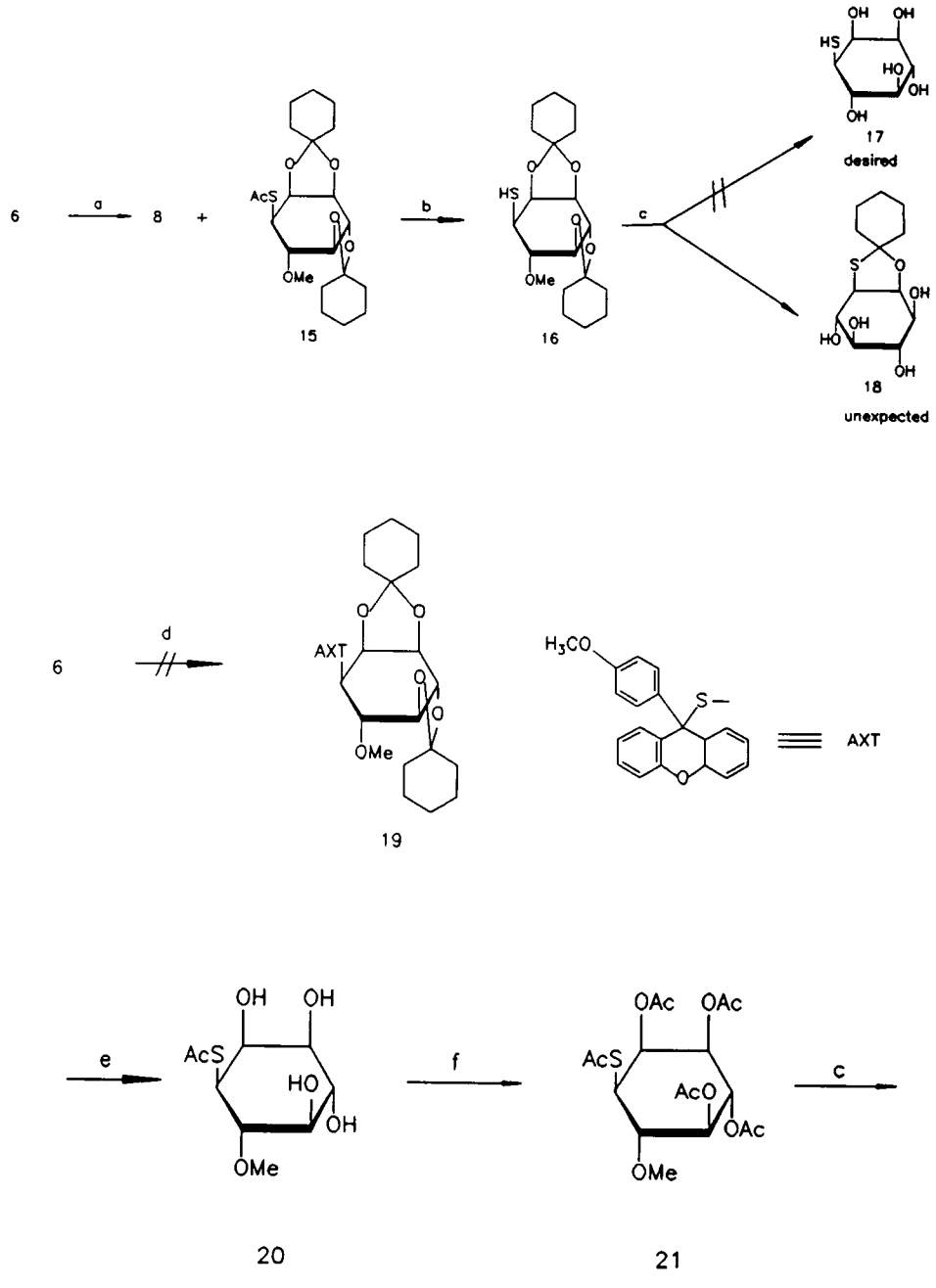


Figure 16. Partial ^{1}H NMR spectrum for **14**.

of **13** was accomplished using the same procedure as in the preparation of **4**. An alternative method was used to remove the residual boric acid by passing a solution of **14** through IRA-743 resin.[32] The small equatorial—axial coupling of H-2 with both H-1 and H-3 indicated that the most downfield resonance was H-2. The resonances of H-1, H-4, and H-5 are slightly overlapping in the region of δ 3.53—3.73. Both H-4 and H-5 are pseudotriplets, and each of the two peaks in these pseudotriplets are overlapping. H-4 is determined to be more downfield than H-5 from decoupling the most upfield resonance H-6 at δ 3.29.

5. 1D-3-Deoxy-3-mercapto-*myo*-inositol (17). (Scheme V). There are a couple of routes to consider for the preparation of **17**. As it had been determined that the Mitsunobu reaction[47,48] cannot convert **1** to **15** in THF or benzene solution by treatment of triphenylphosphine—diethyl azobicarbonate—thioacetic acid. Compound **6** was treated with potassium thioacetate in CH_3CN or DMF solution and stirred at room temperature to give **15** in 51% yield along with **8** in 8% yield. Deacetylation of **15** in sodium methoxide—MeOH solution afforded **16** in 81% yield. Demethylation—deprotection of **16** to the desired **17** was not accomplished by treating with 1 M BBr_3. An unexpected compound appears to be **18**, where one of the cyclohexylidene groups migrated from the oxygen to the sulfur to form the more stable hemithioketal. MS, ^{13}C NMR, and DEPT data for peracetylated **18** confirmed the structure. A third alternative route to the thio compound is via the use of 9-(4-methoxyphenyl)xanthenethiol (AXT).[49,50] However, the reaction fails when a stirred solution of **6** in DMF is treated with AXT (the nucleophile) and N^1,N^1,N^3,N^3-tetramethylguandine (a base) is added dropwise at room temperature.

SCHEME V



a. KSAc—CH_3CN; b. NaOMe—MeOH; c. BBr_3—CH_2Cl_2; d. AXT—DMF; e. 9:1 CF_3COOH—H_2O; f. Ac_2O—Pyridine

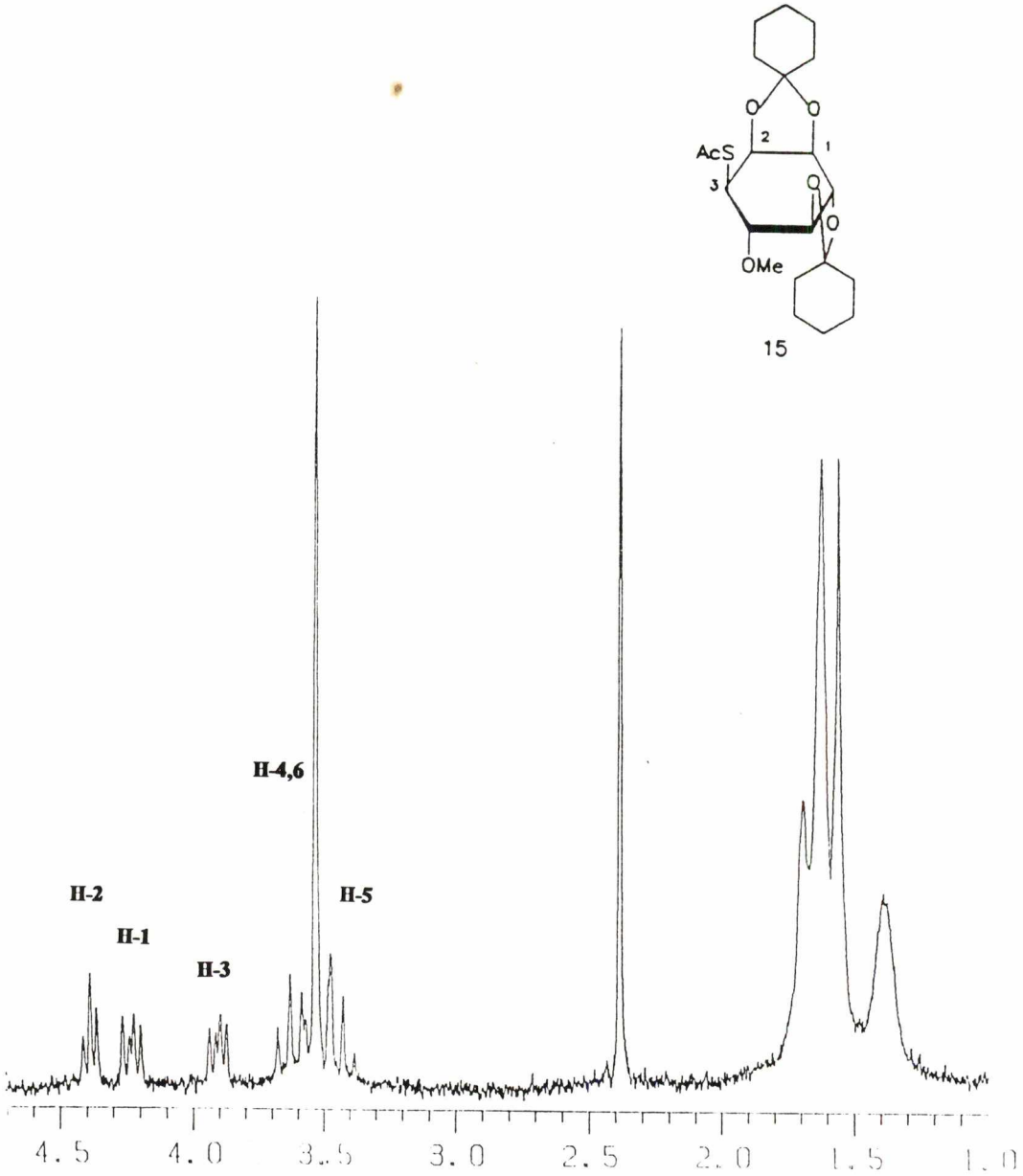


Figure 17. Partial ^{1}H NMR spectrum for **15**.

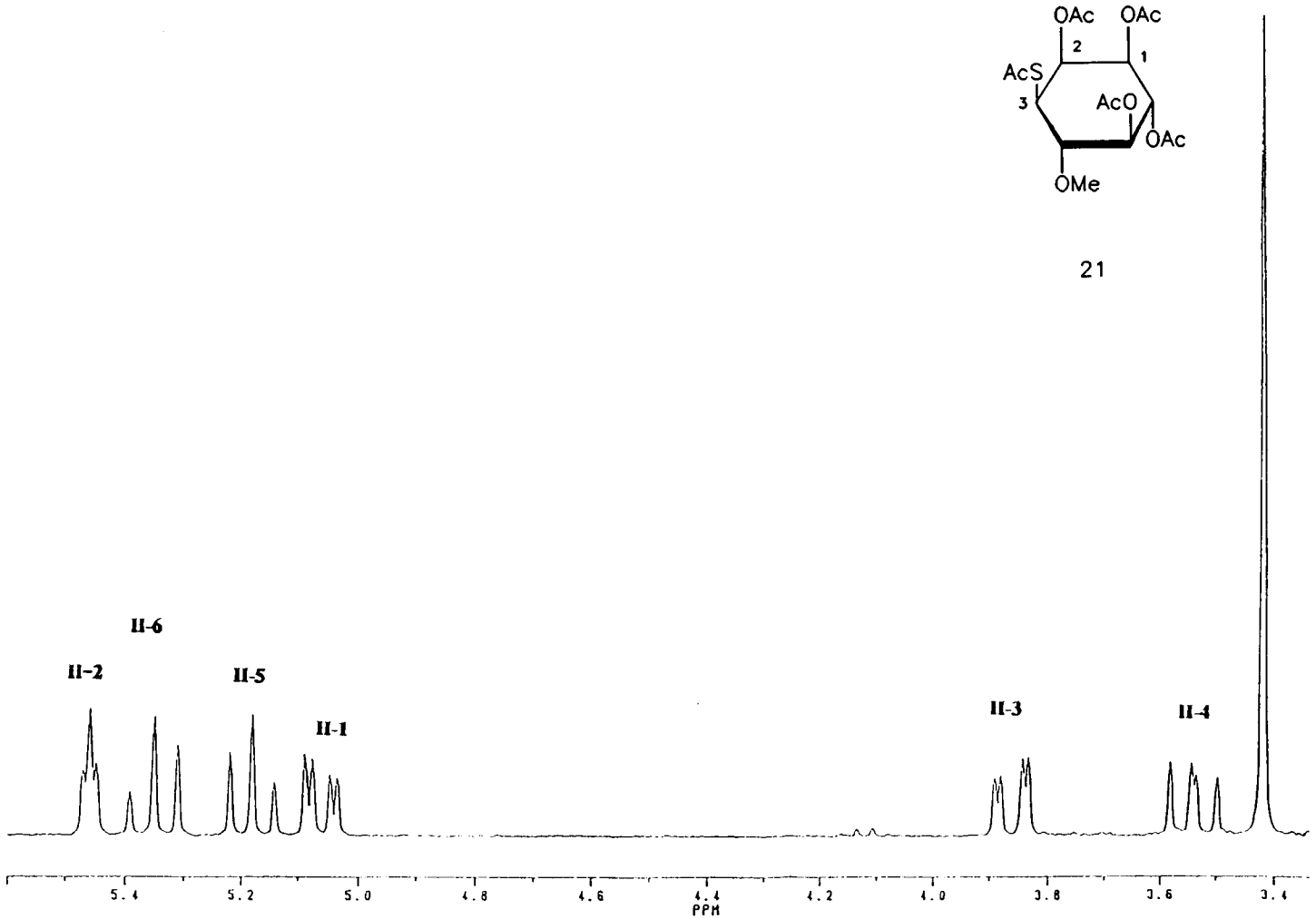
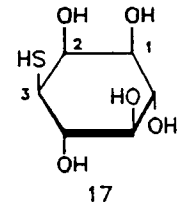


Figure 18. Partial ^{1}H NMR spectrum for **21**.



17

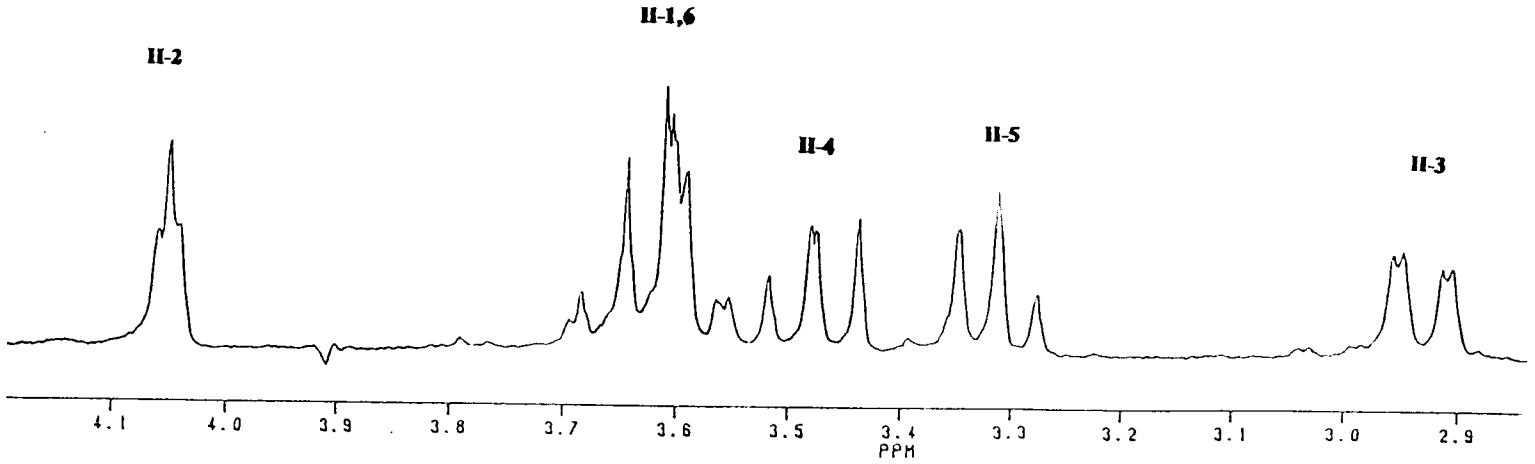


Figure 19. Partial ^{1}H NMR spectrum for **17**.

The failure may arise from the bulky structure of the AXT. In order to prevent the cyclohexylidene group from migration during treatment with BBr_3, compound **15** was treated with aqueous 9:1 CF_3COOH solution to remove these two protecting groups. After partitioning between $CHCl_3$ and H_2O, and freeze drying, **20** was peracetylated to give **21** in 82% yield. Deacetylation and demethylation of **21** were carried out by the same procedure as for **4** to afford **17** (52.6%) as a white solid after column chromatography and freeze drying.

B. Evaluation of *myo*-Inositol Analogues with PtdIns Synthetase

Two methods, (1) inhibition of *myo*-inositol uptake[51] and (2) CMP formation from the reaction of the inositol with PtdCMP,[52] were employed by Dr. T. L. Benjamin and coworkers to evaluate the *myo*-inositol analogues as inhibitors or substrates of PI synthetase. A range of inositol analogues and labelled inositol were added to measure the conversion rate of *myo*-[^{3}H]inositol to [^{3}H]-PtdIns by PI synthetase. The decrease in the rate of conversion into [^{3}H]-PtdIns indicated that these analogues either influenced or interacted with the binding site of the *myo*-inositol. These results indicate that these analogues are either competitive inhibitors or substrates of PI synthetase, competing effectively (8—40%) with [^{3}H]-*myo*-inositol for the binding site. In order to distinguish whether the analogue is either a competitor or an inhibitor, the second method, determination of CMP formation, was applied. All of the analogues except the 5-deoxy-5-fluoro compound are to be considered inhibitors rather than substrates (see Table 1).

For comparsion, data for 5-deoxy-5-fluoro-*myo*-inositol, which is considered a relatively good substrate of PI synthetase, is shown in Table 1. This activity can be explained by the fact that fluoride at C-5 is isosteric with the oxygen of *myo*-inositol. The deoxyhalo compounds **14** and **17** are effective inhibitors but poor substrates because of their larger atom size. Compounds **5** and **10** are 4-*O*-methyl-*myo*-inositol derivatives. The former is a good inhibitor but not a good substrate, while the latter is poor as both a substrate and an inhibitor of PI synthetase.

Table 1: *myo*-Inositol Analogues as Inhibitors and Substrates of Phosphatidylinositol Synthase

Analogue	% Inibition of Uptake of [^{3}H]-*myo*-inositol with PI Synthase[a]	CMP Formation[b] (% of Inositol)
5-deoxy-5-fluoro	22	28
3-deoxy (**4**)	9	6
3-deoxy-4-*O*-methyl (**5**)	28	0
3-chloro-3-deoxy (**14**)	30	1
D-(+)-ononitol (**10**)	8	0
3-mercapto (**17**)	40	1.5

[a] Assay mixtures contained 0.5 m*M* *myo*-[^{3}H]-inositol and 5 m*M* analog. The control rate was 3.6 nmol/min/mg protein. Data are averages of two or more determinations differing no more than 5%.

[b] Analogues and *myo*-inositol were tested at m*M* concentration. The control rate was 11.4 nmol/min/mg protein. Averages of two or more determinations differing no more than 5% are presented.

IV. CONCLUSIONS

The tumor-promoting actions arising from Ca^{2+} mobilization have been known for a long time. A number of modified *myo*-inositol compounds have previously been prepared.[30] Some of them are good inhibitors and substrates of PI synthetase; some of them are not. However, the other second messenger, Ins(1,3,4,5)P_4, which is caused by phosphorylation at the C-3 position of Ins(1,4,5)P_3, probably serves a signal-tranducing role even though its mode of action is not really clear. A time-saving approach to *myo*-inositol analogues from L-quebrachitol has been developed. These 3-modified *myo*-inositol analogues have been studied as substrates or inhibitors of PI synthetase. It is hoped that a powerful cell-growth regulator can be found in the future.

V. EXPERIMENTAL

General Procedures. Optical rotations were determined on a Perkin—Elmer 241 digital polarimeter in a 1-dm cell, and the concentrations were expressed in g/100mL. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Nicolet NT200, Bruker AC-250, and Bruker AM-360 instruments, at 200, 250, and 360 MHz, respectively. The chemical shifts were reported in δ-units relative to internal tetramethylsilane (TMS) in $CDCl_3$. Compounds in deuterium oxide (D_2O) were reported relative to external 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). Coupling constant are measured in Hz and multiplities are indicated as follows: d, doublet; dd, doublet of doublets; ddd, doublet of doublet of doublets; m, multiplet; q, quartet; ψt, pseudotriplet (i.e., a doublet of doublets with both *J*-values approximately equal). Mass spectra were obtained on a model VG ZABEQ instrument. Column chromatography used 230—400 mesh silica gel. Melting points were obtained from a Thomas—Hoover "Unimet" capillary melting point apparatus equipped with a Cole-Parmer model 8520-50 Digi-Sense digital thermometer/8520-55 thermocouple combination that was calibrated with known standards.

Elemental analyses were carried out by Atlantic Microlab, Inc., Norcross, Georgia. Unless otherwise noted, all the solvents and reagents were "reagent grade". Toluene was distilled from Na prior to use. *N,N*-Dimethylformamide (DMF) was distilled from

calcium hydride under reduced atmosphere and stored over 4-Å molecular sieves. Both pyridine and methylene chloride were distilled from calcium hydride and stored over 4-Å molecular sieves. Methanol was distilled from magnesium turnings and stored over 3-Å molecular sieves. The preparation of cesium propionate[33] and the protecting group, 1-ethoxycyclohexene,[28] followed known procedures.

Preparation of 1L-1,2:3,4-Di-*O*-cyclohexylidene-5-*O*-methyl-*chiro*-inositol (1). To a solution of 8.52 g (43.9 mmol) of L-quebrachitol and a catalytic amount of *p*-toluenesulfonic acid (*p*-TSA) in 300 mL of dry DMF was added 17 g (~13.5 mmol) of 1-ethoxycyclohexene at ambient temperature. The mixture was refluxed at 120 °C under a nitrogen atmosphere for 18 h, at the end of which time the mixture was concentrated under vacuum to give a yellow syrup. The residual syrup was partitioned between CH_2Cl_2 (50 mL) and H_2O (2 × 50 mL). The organic layer was separated, dried ($MgSO_4$), and concentrated under reduced pressure to afford a yellow syrup which was crystallized from 95% EtOH to give 13.1 g (85%) of **1** as colorless crystals: mp 117—118 °C [lit.[53] 117—119 °C].

1L-1,2:3,4-Di-*O*-cyclohexylidene-6-*O*-(1-imidazolthiocarbonyl)-5-*O*-methyl-*chiro*-inositol (2). To a solution of **1** (1.96 g, 5.54 mmol) in dry DMF (60 mL) was added 1,1'-thiocarbonyldiimidazole (2.2 g, 12.3 mmol). The mixture was heated under a nitrogen atmosphere at 90—100 °C for 12 h, at the end of which time concentration under high vacuum yielded a yellow syrup. The crude product was chromatographed ($CHCl_3$) to yield 2.42 g (94%) of **2** as a white glass. TLC (silica gel, anisaldehyde dip, $CHCl_3$) R_f 0.15. ^1H NMR (250 MHz, $CDCl_3$): δ 1.21—1.90 (m, 20 H, 2

cyclohexylidene), 3.36 (s, 3 H, OCH_3), 3.62—3.76 (m, 2 H, H-4; H-5), 4.04 (dd, 1 H, $J_{1,2} = J_{1,6} = 6.6$ Hz, H-1), 4.34 (dd, 1 H, $J_{1,2} = J_{2,3} = 6.6$ Hz, H-2), 4.51 (ψt, 1 H, $J_{2,3} = J_{3,4} = 6.6$ Hz, H-3), 5.83 (ψt, 1 H, $J_{1,6} = J_{5,6} = 6.6$ Hz, H-6).

1D-1,2:5,6-Di-*O*-cyclohexylidene-3-deoxy-4-*O*-methyl-*myo*-inositol (3). A solution of **2** (480 mg, 1.03 mmol) in dry toluene (10 mL) was treated with a solution of tributyltin hydride (Bu_3SnH) (0.7 mL, 2.6 mmol) and α,α'-azobisisobutyronitrile (AIBN) (0.25 g, 1.4 mmol). The mixture was refluxed under a nitrogen atmosphere for 3 h, at the end of which time the mixture was concentrated and chromatographed ($CHCl_3$) to give 257 mg (74%) of **3** as a colorless syrup. TLC (silica gel, anisaldehyde dip, $CHCl_3$) R_f 0.30. ^1H NMR (250 MHz, $CDCl_3$): δ 1.21—1.78 (m, 21 H, H_{3a}, 2 cyclohexylidene), 2.37 (ddd, 1 H, $J_{gem} = 15.2$ Hz, $J_{3b,4} = 5.7$ Hz, $J_{3b,2} = 4.3$ Hz, H-3_b), 3.28 (dd, 1 H, $J_{5,6} = 10.0$ Hz, $J_{5,4} = 8.5$ Hz, H-5), 3.49 (dd, 1 H, $J_{6,5} = 10.0$ Hz, $J_{6,1} = 8.5$ Hz, H-6), 3.59 (m, 1 H, 6 lines, H-4), 4.13 (dd, 1 H, $J_{1,2} = J_{1,6} = 8.5$ Hz, H-1), 4.34 (dd, 1 H, $J_{2,1} = 8.5$ Hz, $J_{2,3} = 4.3$ Hz, H-2). ^{13}C NMR (90 MHz, $CDCl_3$): δ 111.01, 109.08, 78.47, 78,46, 76.22, 75.93, 75.71, 72.66, 56.31, 37.07, 35.52, 35.45, 33.86, 24.04, 22.94, 22.69, 22.64, 22.57. *Anal.* Calcd for $C_{19}H_{30}O_5 \cdot 0.19$ $CHCl_3$ [FW 361.12]: C, 63.63; H, 8.72. Found C, 63.83; H, 8.43.

1D-3-Deoxy-*myo*-inositol (4). To a solution of **3** (564 mg, 1.67 mmol) in CH_2Cl_2 (50 mL) was added dropwise a solution of boron tribromide (10 mL of a 1.0 M solution in CH_2Cl_2, 1.0 mmol). The solution was stirred at 0 °C under a nitrogen atmosphere for 4 h then at ambient temperature for 8 h at which time concentration under reduced pressure gave a black residue. Methanol was sucessively added followed by

coevaporation (4 × 10 mL) to give a residue which was partitioned between H_2O (20 mL) and $CHCl_3$ (2 × 20 mL). The aqueous phase was separated, filtered through Celite, and concentrated under freeze—dry conditions to obtain 288 mg (83%) of a light yellow solid. Recrystallization from 95% EtOH gave 172 mg (63%) of a white solid: $[\alpha]_D^{25}$ = -49.77° (*c* 0.5, H_2O) [lit.[21] -50°]; ^1H NMR (250 MHz, D_2O): δ 1.54 (ddd, 1 H, J_{gem} = 14.0 Hz, $J_{3a,4}$ = 12 Hz, $J_{3a,2}$ = 3.0 Hz, H-3_a), 2.09 (dψt, 1 H, J_{gem} = 14.0 Hz, $J_{3b,2}$ = $J_{3b,4}$ = 4.2 Hz, H-3_b), 3.23 (ψt, 1 H, $J_{5,4}$ = $J_{5,6}$ = 9.0 Hz, H-5), 3.48—3.57 (m, 2 H, H-1; H-6), 3.75 (ddd, 1 H, $J_{4,3a}$ = 12 Hz, $J_{4,5}$ = 9.0 Hz, $J_{4,3b}$ = 4.2 Hz, H-4), 4.06 (dd, 1 H, $J_{2,1}$ = 2.2 Hz, $J_{2,3a}$ = 3.0 Hz, H-2). *Anal.* Calcd for $C_6H_{12}O_5 \cdot 0.3\ H_2O$ [FW 169.56]: C, 42.41; H, 7.50. Found: C, 42.44, 42.35; H, 7.23, 7.28.

1D-3-Deoxy-4-*O*-methyl-*myo*-inositol (5). To **3** (78.5 mg, 0.22 mmol) was added a solution of 3:1 CF_3COOH—H_2O (8 mL). The resulting solution was stirred at ambient temperature for 15 min. Concentration under reduced pressure, followed by coevaporation with abs. EtOH (4 × 10 mL), gave a residue which was partitioned between H_2O and $CHCl_3$. The aqueous phase was separated and concentrated under freeze-dry conditions to give 33 mg (81%) of **5** as a colorless solid: $[\alpha]_D^{25}$ = -69.07° (*c* 1.42, H_2O); ^1H NMR (250 MHz, D_2O): δ 1.40 (ddd, 1 H, J_{gem} = 14.1 Hz, $J_{3a,4}$ = 10.0 Hz, $J_{3a,2}$= 2 Hz, H-3_a), 2.29 (dψt, 1 H, J_{gem} = 14.1 Hz, $J_{3b,4}$ = $J_{3b,2}$ = 4.4 Hz, H-3_b), 3.32 (ψt, 1 H, $J_{6,1}$ = $J_{6,5}$ = 9.0 Hz, H-6), 3.40 (s, 3 H, -OCH_3), 3.42—3.49 (m, 6 lines, 2 H, H-1; H-4), 3.57 (ψt, 1 H, $J_{5,4}$ = $J_{5,6}$ = 9.0 Hz, H-5), 4.08 (dd, 1 H, $J_{2,1}$ = 2.8 Hz, $J_{2,3a}$ = 2.2 Hz, H-2). *Anal.* Calcd for $C_7H_{14}O_5 \cdot 0.074\ CHCl_3$ [FW 187.02]: C, 45.43; H, 7.58. Found: C, 45.42; H, 7.53.

1L-1,2:3,4-Di-*O*-cyclohexylidene-5-*O*-methyl-6-*O*-triflyl-*chiro*-inositol (6). To a solution of 1.25 g (3.52 mmol) of **1** and 0.18 mL (2.22 mmol) of pyridine at 0 °C in dry dichloromethane (25 mL) under a nitrogen atmosphere was added dropwise 0.36 g (2.12 mmol) of cold trifluoromethanesulfonic anhydride. The mixture was stirred at 0 °C for 2 h at which time TLC indicated one major product (R_f 0.61, $CHCl_3$). The solvent was evaporated under reduced pressure to give a yellow—red syrup. The syrup was partitioned between EtOAc (40 mL) and saturated aqueous sodium chloride (2 × 40 mL). The organic phase was dried ($MgSO_4$) and concentrated to give 1.67 g of a yellow—red syrup of **6**. The product was used in the following step without further purification.

1D-1,2:5,6-Di-*O*-cyclohexylidene-4-*O*-methyl-3-*O*-propionyl-*myo*-inositol (7). To the triflate derivative of **6** (1.04 g, 2.14 mmol) in dry DMF (40 mL) was added cesium propionate (603 mg, 2.9 mmol). This mixture was heated at 60—70 °C for 7 h. Concentration under vacuum gave a purple syrup to which CH_2Cl_2 (30 mL) was added. The resulting solution was washed with saturated aqueous sodium chloride (2 × 30 mL), and the organic phase was separated, dried ($MgSO_4$), and concentrated under reduced pressure to give a yellow syrup. Purification by column chromatography (CH_2Cl_2) afforded the elimination product **8**, which was crystallized from 95% EtOH to give 213 mg (32%) as a white solid, along with 231 mg of **7** (29%) as a colorless syrup: TLC (CH_2Cl_2) R_f 0.25, 0.47 for **7** and **8**, respectively; mp 153—155 °C (**8**). Physicochemical data for **7**: ^1H NMR (200 MHz, $CDCl_3$): δ 1.10 (t, 3 H, Pr), 1.2—1.85 (m, 20 H, 2 cyclohexylidene), 2.32 (q, 2 H, CH_2-Pr), 3.42—3.52 (m, 2 H, H-1; H-6), 3.95 (dd, 1

H, $J_{5,6}$ = 9.8 Hz, $J_{5,4}$ = 7.6 Hz, H-5), 4.33 (ψt, 1 H, $J_{4,3} = J_{4,5}$ = 7.1 Hz, H-4), 4.39 (dd, 1 H, $J_{3,4}$ = 7.1 Hz, $J_{3,2}$ = 3.6 Hz, H-3), 5.14 (dd, 1 H, $J_{2,3}$ = 3.6 Hz, $J_{2,1}$ = 2.0 Hz, H-2). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 172.9, 113.1, 111.5, 81.2, 78.0, 76.3, 73.2, 71.7, 57.7, 36.5, 36.4, 36.2, 34.0, 27.6, 25.0, 24.9, 23.7, 23.6, 23.4, 8.9. *Anal.* Calcd for **7**, $C_{22}H_{34}O_7$ [F.W. 410.29]: C, 64.37; H, 8.35. Found: C, 64.11; H, 8.41. Physicochemical data for **8**: ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.25—1.87 (m, 20 H, 2 cyclohexylidene), 3.67 (s, 3 H, -OCH_3), 3.80 (ψt, 1 H, $J_{6,1} = J_{6,5}$ = 9.2 Hz, H-6), 4.12 (dψt, 1 H, $J_{1,6}$ = 9.2 Hz, $J_{1,2}$ = 1.8 Hz, $J_{1,3}$ = 0 Hz, H-1), 4.35 (dd, 1 H, $J_{5,6}$ = 9.6 Hz, $J_{5,3}$ = 7.1 Hz, H-5), 4.67 (dd, 1 H, $J_{2,3}$ = 3.3 Hz, $J_{2,1}$ = 1.8 Hz, H-2), 4.92 (ddd, 1 H, $J_{3,5}$ = 7.1 Hz, $J_{3,2}$ = 3.3 Hz, $J_{3,1}$ = 0 Hz, H-3). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 156.1, 114.1, 110.9, 90.3, 80.6, 74.5, 74.3, 72.8, 55.4, 37.5, 36.3, 35.9, 34.5, 25.0, 24.9, 23.9, 23.6, 23.4, 23.3. *Anal.* Calcd for **8**, $C_{19}H_{28}O_5$ [FW 336.43]: C, 67.83; H, 8.39. Found: C, 67.57; H, 8.44.

1D-1,2:5,6-Di-*O*-cyclohexylidene-4-*O*-methyl-*myo*-inositol (9). A solution of **7** (385 mg, 0.94 mmol) in methanol (10 mL) was treated with a solution of NaOMe (1.0 mL of a 1.0 M solution, 1.0 mmol). The solution was stirred at ambient temperature for 7.5 h. The solution was concentrated under reduced pressure to give a syrup which was partitioned between H_2O (20 mL) and CH_2Cl_2 (2 × 20 mL). The organic phase was separated, dried ($MgSO_4$), and concentrated under reduced pressure. The light yellow syrup was purified by column chromatography ($CHCl_3$) to give 283 mg (90%) of **9** as a colorless syrup: TLC ($CHCl_3$) R_f 0.29; ^{1}H NMR (360 MHz, $CDCl_3$): δ 1.25—1.72 (m, 20 H, 2 cyclohexylidene), 2.62 (s, 1 H, OH), 3.45 (dd, 1 H, $J_{4,5}$ = 10.6 Hz, $J_{4,3}$ = 8.0

Hz, H-4), 3.66 (dd, 1 H, $J_{3,4}$ = 8.0 Hz, $J_{3,2}$ = 1.9 Hz, H-3), 3.98 (dd, 1 H, $J_{2,1}$ = 3.6 Hz, $J_{2,3}$ = 1.9 Hz, H-2), 4.16 (dd, 1 H, $J_{5,4}$ = 10.6 Hz, $J_{5,6}$ = 7.4 Hz, H-5), 4.34 (ψt, 1 H, $J_{6,1}$ = $J_{6,5}$ = 7.40 Hz, H-6), 4.40 (dd, 1 H, $J_{1,6}$ = 7.40 Hz, $J_{1,2}$ = 3.6 Hz, H-1). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 112.9, 111.2, 81.9, 78.5, 76.6, 75.21, 71.6, 57.5, 36.6, 36.5, 36.3, 33.4, 25.1, 25.0, 23.8, 23.7, 23.6, 23.3. *Anal.* Calcd for $C_{19}H_{30}O_6$ [FW 354.44]: C, 64.39; H, 8.53. Found: C, 64.26; H, 8.56.

1D-4-*O*-Methyl-*myo*-inositol (10). To **9** (197 mg, 0.556 mmol) was added a solution of 9:1 CF_3COOH—H_2O (7.0 mL). This solution was stirred at ambient temperature for 10 min, at which time concentration under reduced pressure, followed by coevaporations with 95% EtOH (3 × 10 mL), gave a white syrup. Recrystallization from 95% EtOH gave 80 mg (74.5%) of **10**: mp 170—172 °C [lit.[21] 172 °C]; $[\alpha]_D^{25}$ = +6.24° (*c* 0.78, H_2O) [lit.[21] +6.6°]; ^{1}H NMR (250 MHz, D_2O): δ 3.29—3.41 (m, 2 H, 7 lines, H-5, H-6 or H-4, H-5), 3.47 (dd, 1 H, H-1 or H-3), 3.57—3.68 (m, 5 H, -OCH_3, H-1, H-4 or -OCH_3, H-3, H-6), 4.02 (ψt, 1 H, $J_{2,1}$ = $J_{2,3}$ = 2.5 Hz, H-2). *Anal.* Calcd for $C_7H_{14}O_6 \cdot 0.05\ H_2O$ [FW 195.08]: C, 43.10; H, 7.28. Found: C, 42.95, 42.88; H, 7.09,7.12.

1D-1,2,3,5,6-Penta-*O*-acetyl-4-*O*-methyl-*myo*-inositol (11). To 5 mg (0.03 mmol) of **10** in 1 mL of pyridine was added 2 mL of acetic anhydride, and the mixture was stirred at ambient temperature overnight, at the end of which time the solvent was removed under vacuum, and several parts of 95% EtOH were added to remove the excess acetic anhydride to yield very light yellow syrup. ^{1}H NMR (250 MHz, $CDCl_3$): δ 2.01, 2.06, 2.09, 2.16, 2.19, (5 s, 5 × 3 H, respectively; -OAc's), 3.46 (s, 3 H, -OCH_3),

3.68 (ψt, 1 H, $J_{4,3} = J_{4,5} = 10.0$ Hz, H-4), 5.00 (dd, 1 H, $J_{3,4} = 10.0$ Hz, $J_{3,1} = 2.8$ Hz, H-3), 5.06 (dd, 1 H, $J_{1,6} = 10.0$ Hz, $J_{1,2} = 2.8$ Hz, H-1), 5.11 (ψt, 1 H, $J_{5,6} = J_{5,4} = 10.0$ Hz, H-5), 5.40 (ψt, 1 H, $J_{6,1} = J_{6,5} = 10$ Hz, H-6), 5.56 (ψt, 1 H, $J_{2,1} = J_{2,3} = 2.8$ Hz, H-2)

1D-1,2:5,6-Di-*O*-cyclohexylidene-3-*O*-*p*-nitrobenzoyl-4-*O*-methyl-*myo*-inositol (12). To a stirring solution of **1** (0.824 g, 2.32 mmol), triphenylphosphine (3.05 g, 11.6 mmol), and *p*-nitrobenzoic acid (*p*-NBA) (1.87 g, 11.2 mmol) in dry benzene (15 mL) was added diethyl azodicarboxylate (DEAD) (1.84 mL, 11.7 mmol) dropwise at room temperature. After stirring 10 h, TLC indicated mostly starting material. This mixture was refluxed at 81 °C for 13 h at which time TLC showed the presence of one major spot and a minor spot corresponding to starting material. Another portion of DEAD (0.8 mL) was added at room temperature and stirred for 12 h, then heated at 90 °C for 38 h, at the end of which time TLC showed the same results as previously described. The solvent was removed under vacuum and purified by column chromatography (14:1 hexanes—EtOAc) to recover **1** (0.315 g) and supply **12** (0.535 g, 74.1% based on recovering **1**) as a pale yellow to colorless syrup. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.13—1.68 (m, 20 H, 2 cyclohexylidene), 3.48 (s, 3 H, -OCH_3), 3.54 (dd, 1 H, $J_{4,3} = 7.8$ Hz, $J_{4,5} = 10.6$ Hz, H-4), 3.64 (dd, 1 H, $J_{3,2} = 1.6$ Hz, $J_{3,4} = 7.8$ Hz, H-3), 4.09 (dd, 1 H, $J_{5,4} = 10.6$ Hz, $J_{5,6} = 7.2$ Hz, H-5), 4.40 (ψt, 1 H, $J_{6,1} = J_{6,5} = 7.2$ Hz, H-6), 4.49 (dd, 1 H, $J_{1,2} = 3.6$ Hz, $J_{1,6} = 7.2$ Hz, H-1), 5.42 (dd, 1 H, $J_{2,1} = 3.6$ Hz, $J_{2,3} = 1.6$ Hz, H-2), 8.21 (m, 4 H, -Ar). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 162.6, 150.23, 134.5, 130.4, 123.1, 113.0, 80.6, 76.3, 76.0, 75.8, 72.8, 72.6, 57.2, 36.0,

35.5, 33.3, 31.0, 29.1, 24.4, 23.1, 22.9, 22.0, 13.5. *Anal.* Calcd for $C_{26}H_{33}NO_8 \cdot H_2O$ [FW 505.86]: C: 61.77; H, 6.98; N, 2.77. Found: C, 61.85; H, 6.64; N, 2.75.

1D-3-Chloro-1,2:5,6-di-*O*-cyclohexylidene-3-deoxy-4-*O*-methyl-*myo*-inositol (13). Following the same procedure used to prepare **6**, the triflate derivative, which was prepared from 0.148 mg (0.42 mmol) of **1**, was dissolved in 5 mL of DMF and was treated with 0.28 g (0.41 mmol) of imdiazole and 0.184 g (0.43 mmol) of LiCl. The mixture was stirred at ambient temperature under a nitrogen atmosphere for 12 h at which time TLC indicated two major spots. The mixture was concentrated under high vacuum and was purified by column chromatography (30:1 hexanes—EtOAc) to provide 56.7 mg (36.4%) of **13** as a light yellow syrup and 63 mg (44.8%) of **8**. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.19—1.72 (m, 20 H, 2 cyclohexylidene), 3.38 (dd, 1 H, $J_{5,6}$ = 10.5 Hz, $J_{5,4}$ = 8.6 Hz, H-5), 3.56 (s, 3 H, -OCH_3), 3.81 (dd, 1 H, $J_{4,5}$ = 8.6 Hz, $J_{4,3}$ = 5.1 Hz, H-4), 4.03—4.12 (m, 5 lines, 2 H, H-3; H-6), 4.29 (dd, 1 H, $J_{1,6}$ = 8.1 Hz, $J_{1,2}$= 6.0 Hz, H-1), 4.48 (dd, 1 H, $J_{2,1}$ = 6.0 Hz, $J_{2,3}$ = 4.6 Hz, H-2). MS: *m/z* 373 (M^+); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 113.03, 111.68, 83.07, 77.16, 76.34, 75.50, 75.19, 59.12, 58.59, 36.56, 36.46, 34.71, 25.00, 24.95, 23.87, 23.63, 23.50. *Anal.* Calcd for $C_{19}H_{29}O_5Cl$ [FW 372.6]: C, 61.20; H, 7.84; Cl, 9.51. Found: C, 61.27; H, 7.86; Cl, 9.52.

1D-3-Chloro-3-deoxy-*myo*-inositol (14). To a solution of **13** (114 mg, 0.3 mmol) in dry CH_2Cl_2 (5 mL) maintained at 0 °C and under an argon atmosphere was added a solution of boron tribromide (5 mL of a 1.0 M solution in CH_2Cl_2, 5.0 mmol). After stirring at 0 °C for 4 h and at ambient temperature for 8 h, any remaining volatiles were

removed under reduced pressure. The brown residue was sucessively treated with dry MeOH (5 × 20 mL), followed by evaporation under reduced pressure. The residue was dissolved in dry MeOH, and the solution was neutralized with ethylene oxide. The solvent was evaporated to give a light brown solid which was partitioned between $CHCl_3$ and H_2O. The aqueous phase was passed through IRA-743 resin to remove the boric acid and concentrated under freeze dry conditions to provide a light yellow solid. Recrystallization from abs. EtOH gave 34.5 mg (56.8%) of **14** as white solid: mp 235 °C (dec.); $[\alpha]_D^{25}$ = + 20.9° (*c* 0.54, H_2O); ^1H NMR (250 MHz, D_2O): δ 3.29 (ψt, 1 H, $J_{6,1} = J_{6,5}$ = 10.0 Hz, H-6), 3.55 (dd, 1 H, $J_{1,6}$ = 10.0 Hz, $J_{1,2}$ = 2.4 Hz, H-1), 3.64 (ψt, 1 H, $J_{5,4} = J_{5,6}$ = 10.0 Hz, H-5), 3.68 (ψt, 1 H, $J_{4,3} = J_{4,5}$ = 10.0 Hz, H-4), 3.96 (dd, 1 H, $J_{3,4}$ = 10.0 Hz, $J_{3,2}$ = 2.4 Hz, H-3), 4.14 (ψt, 1 H, $J_{2,1} = J_{2,3}$ = 2.4 Hz, H-2). *Anal.* Calcd for $C_6H_{11}O_5Cl \cdot 0.25\ H_2O$ [FW 203.11]: C, 35.48; H, 5.71. Found: C, 35.48; H, 5.46.

1D-3-Deoxy-3-*O*-acetylthio-1,2:5,6-di-*O*-cyclohexylidene-4-*O*-methyl-*myo*-inositol (15). To a stirring solution of **6** (0.662 g, 1.36 mmol) in CH_3CN (10 mL) was added potassium thioacetate (KSAc) (1.51 g, 13.2 mmol). This mixture was stirred at room temperature for 14 h at the end of which time the solvent was evaporated under vacuum and partitioned between $CHCl_3$ and H_2O. The organic layer was separated, dried ($MgSO_4$), and purified by column chromatography (15:1 hexanes—EtOAc) to afford 36.1 mg of **8** in 8.6% yield and **15** which was crystallized from hexanes to yield 261 mg (51%) as a colorless, needle crystals: mp 115—117 °C. ^1H NMR (200 MHz, $CDCl_3$): δ 1.41—1.74 (m, 20 H, 2 cyclohexylidene), 2.25—2.37 (s, 3 H, CH_3COS-), 3.43 (ψt

1 H, $J_{5,6} = J_{5,4} = 9.8$ Hz, H-5), 3.5—3.7 (m, 5 H, H-4, H-6, -OCH_3), 3.91 (dd, 1 H, $J_{3,2} = 5.0$ Hz, $J_{3,4} = 8.2$ Hz, H-3), 4.23 (dd, 1 H, $J_{1,2} = 5.0$ Hz, $J_{1,6} = 8.3$ Hz, H-1), 4.39 (ψt, 1 H, $J_{2,1} = J_{2,3} = 5$ Hz, H-2). ^{13}C NMR (62.5 Hz, $CDCl_3$): δ 194.2, 112.3, 110.5, 80.0, 79.1, 78.9, 76.4, 76.1, 58.9, 45.5, 37.7, 36.5, 36.3, 34.9, 30.3, 25.0, 23.9, 23.7, 23.5. *Anal.* Calcd for $C_{19}H_{30}O_5S$ [FW 412.54]: C, 61.14; H, 7.82; S, 7.77. Found: C, 61.21; H, 7.84; S, 7.72.

1D-1,2,3,5,6-Penta-*O*-acetyl-3-deoxy-3-mercapto-4-*O*-methyl-*myo*-inositol (21). To **15** (680 mg, 1.65 mmol) was added aqueous 9:1 CF_3COOH solution (10 mL) at room temperature. After stirring for 10 min, TLC indicated the disappearance of compound **15**. The excess CF_3COOH was removed under vacuum, followed by coevaporation with several portions of 95% EtOH, and the residue was partitioned between $CHCl_3$ and H_2O. The aqueous layer was freeze dried to afford crude **20** (410 mg) as a light yellow solid. This crude **20** was treated with acetic anhydride (4 mL) and pyridine (5 mL) and stirred at room temperature for 7 h, at which time the solvent was removed under vacuum and chromatographed (3:1 hexanes—EtOAc) to give **21** (572 mg, 1.36 mmol, 82.6% from **15**) which was crystallized as colorless crystals while standing: mp 103—105 °C. ^1H NMR (250 MHz, $CDCl_3$): δ 1.96, 2.01, 2.05, 2.08, 2.16, 2.34 (5 s, 5 × 3 H, 5-OAc), 3.42 (s, 3 H, -OCH_3), 3.54 (dd, 1 H, $J_{4,3} = 11.6$ Hz, $J_{4,5} = 10$ Hz, H-4), 3.86 (dd, 1 H, $J_{3,4} = 11.6$ Hz, $J_{3,2} = 2.6$ Hz, H-3), 5.06 (dd, 1 H, $J_{1,6} = 10$ Hz, $J_{1,2} = 2.6$ Hz, H-1), 5.18 (ψt, 1 H, $J_{5,6} = J_{5,4} = 10$ Hz, H-5), 5.35 (ψt, 1 H, $J_{6,5} = J_{6,1} = 10$ Hz, H-6), 5.46 (ψt, 1 H, $J_{2,1} = J_{2,3} = 2.6$ Hz, H-2). *Anal.* Calcd

for $C_{17}H_{24}O_{10}S$ [FW 420.43]: C, 48.57; H, 5.75; S, 7.63. Found: C, 48.51; H, 5.80; S, 7.68.

1D-3-Deoxy-3-mercapto-*myo*-inositol (17). By the same procedure used for **4**, 357 mg (0.85 mmol) of **21** was deprotected, demethylated, chromatographed (7:3 $CHCl_3$—MeOH), and freeze dried to give 91 mg (54.3%) of **17** as a white solid. MS: *m/z* 196 (M^+); $[\alpha]_D^{24}$ = + 40.87° (*c* 0.47, MeOH); ^1H NMR (250 MHz, D_2O): δ 2.93 (dd, 1 H, $J_{3,2}$ = 2.4 Hz, $J_{3,4}$ = 10.9 Hz, H-3), 3.31 (ψt, 1 H, $J_{5,4} = J_{5,6}$ = 9.0 Hz, H-5), 3.48 (dd, 1 H, $J_{4,5}$ = 9.0 Hz, $J_{4,3}$ = 10.9 Hz, H-4), 3.53—3.71 (m, 2 H, H-1;H-6), 4.05 (ψt, 1 H, $J_{2,1} = J_{2,3}$ = 2.4 Hz, H-2). *Anal.* Calcd for $C_6H_{12}O_5S \cdot 0.07\ CHCl_3$ [FW 204.58]: C, 35.64; H, 5,95; S, 15.67. Found: C, 35.60; H, 5.88; S, 15.68.

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