THE DESIGN, DEVELOPMENT AND SYNTHESIS OF SECONDARY BINDING ELEMENTS FOR THE CONSTRUCTION OF POLYAZAMACROCYCLIC INOSITOL PHOSPHATE RECEPTORS FOR USE AS BIOLOGICAL PROBES OF PHOSPHATIDYLINOSITOL METABOLISM AND THERAPEUTIC AGENTS FOR THE TREATMENT OF BIPOLAR DISORDER

by

ANTHONY WAYNE WARREN

(Under the Direction of Thomas E. Johnson)

ABSTRACT

Significant progress has been made in the design, development and synthesis of compounds able to provide secondary binding elements in the construction of a new class of polyazamacrocyclic inositol phosphate receptors. The secondary binding elements explored were based on a *myo*-inositol core structure, and were functionalized at the C1 and C4 positions with ether linked extended masked aldehyde groups as cyclization handles. Installation of the extended aldehyde on the inositol ring system was accomplished using the Williamson method for ether synthesis and provided consistent yields of about 80% across the gamut of latent aldehydes tested. Several molecules bearing differentially protected aldehyde moieties, for the purpose of regioselective macrocyclization into a compound designed to host Ins(1,4)$_{P2}$ molecules, were synthesized. The three specific molecules holding the most promise for future efforts in this area were (±)1,4-*O*-Bis(2-[1,3]Dioxanylethoxy)-2,3:5,6-Di-O-cyclohexylidene-*myo*-inositol, (±)1,4-Di-*O*-allyl-2,3:5,6-Di-*O*-cyclohexylidene-*myo*-inositol and (±)1,4-Di-*O*-propargyl-2,3:5,6-Di-*O*-cyclohexylidene-*myo*-inositol. The latent aldehyde alkynyl function present in the last compound was successfully unmasked via hydroboration-oxidation chemistry and was readily isolated as a near crystalline compound, following size exclusion chromatography using SX-8 mesh BioBeads.

INDEX WORDS: polyazamacrocycles, phosphatidylinositol metabolism, *myo*-inositol, host-guest chemistry, second messengers, bipolar disorder, lithium treatment, manic depression, phospholipids, phosphatidylinositol cycle, G-protein coupled receptors, receptor-ligand complexes, signal transduction, inositol phosphate receptors, anion receptors, molecular recognition

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DEDICATION

I owe this work, and any opportunities that may come from it, to the one person who has never faltered in her belief in me—my wife Maria. Nine years ago, she rescued me from what I believed to be a lifelong date with an uncertain destiny in exchange for a lifelong love affair with the woman of my dreams. Her love and commitment to me, and our marriage, transformed my existence and breathed new life into a tired soul. Her confidence in me grew stronger as the years ensued and she provided a bedrock solid support system to counter my manic tendencies. This milestone of achievement in my life could not have been reached without her constant compassion, understanding and commitment to my efforts, both physically and emotionally. She has unselfishly taken on the care of both myself, and our beautiful daughter Hannah, during these past five months while I've been chained to this laptop and locked away in my office. I am also grateful to all the time she has managed to find during this hectic period to help me with the spectra found throughout this work. Thank you so much Maria, I love you with all my heart.

I must also dedicate part of this work to my daughter, Hannah Jade, for her patience and understanding as she has been somewhat fatherless over the past few months while daddy "conshentwates" and makes late night runs to "chemistreet." Thank you also for the rocks, Buga, I'll cherish them like Zuzu's petals.

Finally, I dedicate an equal portion of this manuscript to my parents Peggy and Alonzo Warren for their many many years of tireless emotional and financial support, even when it seemed certain to me that I'd never make it—they always knew better.

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Dr. Robert Phillips simply knows everything about everything and it's scary to think just how much more he probably used to know. Like Detective Robert Goran on *Law and Order: Criminal Intent*, his insight always provided just the right amount of enlightenment to push you further into the dark.

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And then there's George…oh, look! I'm out of space! Thanks George, really.

TABLE OF CONTENTS

Page

CHAPTER 1

LIGAND-RECEPTOR MEDIATED SIGNAL TRANSDUCTION: A HISTORICAL PERSPECTIVE OF CELLULAR COMMUNICATION

1.1 Life, Communication and Cellular Signaling

One of the defining characteristics of life is the ability to interact with the surrounding environment. The ability of a living creature to communicate with, respond to, and perhaps even transform its external environment is a central theme across all levels of biological organization and complexity. From whole organisms to individual cells, the living entity must constantly monitor and regulate its immediate environment in order to survive in a land of limited resources.

For the simplest unicellular organisms the molecular events of life's critical processes are carried out within a single non-compartmentalized cell, and it's pretty much every cell for itself in the survival game. Even as the complexity of life increases and highly developed tissue, organ, and nervous systems evolve into the levels of cooperation seen in multi-cellular organisms, life still turns on cellular level molecular events. At this level communication with the external environment is critical to life sustaining processes such as locomotion, metabolism, immunity, and growth and development. Only by adhering to a strict regimen of environmental homeostasis will the organism thrive under ever changing external conditions.

Since its inception, a central goal of cell biology has been to fully understand the molecular basis of important cellular events such as communication with the environment. Furthering our understanding of cellular communication requires the ability to manipulate events on the molecular level. By better understanding such occurrences it may be possible to learn the molecular basis of human diseases and disorders, thus enabling courses of treatment to be developed. Great advances have been made toward this goal using the analysis tools of molecular cell biology. Specific cellular constituents can now be isolated, identified, and altered, either genetically or synthetically. For example, the amounts of a particular component can be manipulated by gene expression levels, or by microinjection of proteins and small molecules. The molecular structures of proteins can be modified by site directed mutagenesis of DNA or by covalent modifications of amino acid residues to probe the molecular basis of cellular events.[1]

Through such efforts we have learned that complex multi-cellular eukaryotic organisms have met the challenge of communicating with their environment by developing ligand-receptor signal transduction networks. The ligand portion of these networks is the first messenger in the communication event because it is the primary source of signal initiation. The receptor portion acts as a conduit between the two environments by coupling to proteins inside the cell. Signal transduction across the membrane is usually completed by small molecule second messengers inside the cell charged with relaying extracellular communicae to the intracellular machinery of life.

Many of the previously mentioned life sustaining processes, for which extracellular communication is crucial, are mirrored in ligand-receptor mediated behaviors such as cell growth, secretion, contraction, motility, and adhesion.

1.2 First Messengers in Ligand-Receptor Mediated Signal Transduction: Hormones, Neurotransmitters, and Local Mediators

An exhaustive coverage of ligand-initiated signal transduction is beyond the scope of this dissertation, however a sufficient amount of background knowledge in this area is needed to appreciate the relevance of my research. A historical treatment of the field is presented, followed by recent advances, as well as the rationale behind our research.

The aforementioned first messengers, or primary sources of signal initiation, can be divided into three families based on their modes of migration and communication between the source of the ligand, and the target cell receptor.[2]

The most well known family of first messengers is the hormones of the endocrine system. Hormones are secreted from specialized cells in endocrine glands and are transported through the bloodstream to another area of the body where they bind to target cells and initiate a signal response **(Table 1.1)**.[2]

Although hormones are said to initiate the signaling response, the hypothalamus acts as an important interface between the nervous system and the endocrine system. Nervous impulses originating in the brain lead to the generation of releasing hormones and inhibitors from the hypothalamus. These hypothalamus hormones in turn effect the release of hormones from the two lobes of the pituitary gland.[2]

Table 1.1 Major endocrine glands/organs and the hormones they produce.[2]

Endocrine Gland	Hormones Produced
Hypothalamus	Releasing factors/inhibitors
Pituitary, anterior	Trophic hormones, prolactin
Pituitary, posterior	Oxytocin, vasopressin
Parathyroid	Parathyroid hormone
Thyroid	Thyroid hormones
Heart atrium	Atrial natriuretic peptides
Liver	Insulin-like growth factors
Pancreas	Insulin/glucagon
Gut	Gastrin, secretin, cholecystekinin
Adrenal cortex	Corticosteroids
Adrenal medulla	Epinephrine
Ovaries	Estrogen, progesterone
Testes	Testosterone
Placenta	Choriogonadotrophin, placental lactogen

The anterior portion of the pituitary gland releases trophic hormones, which stimulate hormone release and growth in other endocrine glands. The posterior pituitary gland secretes two major hormones, vasopressin and oxytocin, which regulate smooth muscle contraction and therefore regulate blood pressure and events associated with childbirth, respectively.[2]

Some other important endocrine first messengers include thyroid hormones which regulate gene expression, insulin and glucagon released by the pancreas to regulate blood sugar levels, and the adrenal gland hormones.[2]

Nerve cells directly innervate the adrenal medulla or the adrenal cortex and are responsible for triggering hormone release from these glands. Epinephrine, or adrenaline, the "fight or flight" hormone, is a well-known first messenger released by the adrenal

medulla. The adrenal cortex is specialized for the synthesis of steroid hormones that regulate gene expression.[2]

Specialized cells in the testes, ovaries, and placenta (during pregnancy) are responsible for the secretion of the sex hormones, and those associated with gestation and childbirth.[2]

When considering endocrine factors as a family of first messengers it is clear that the nervous system plays an important role in the release of hormones. From this realization comes a second family of first messengers, namely the neurotransmitters. The mode of communication utilized by neurotransmitters differs greatly from that seen in hormones. Neurotransmitters are released at the terminal ends of nerve cells, and unlike the relatively long distances traveled by hormones to effect communication, diffuse only across a small cleft or gap to their target cells.[2] Due to their short migration requirements neurotransmitters are often transient compounds having a much shorter lifespan than their hormone first messenger counterparts.

A third family of first messengers can be loosely described as local mediators and are considered to be part of either the paracrine, autocrine, or juxtacrine systems. Local mediators fall between hormones and neurotransmitters in their mode of communication because they are released by many non-specialized cell types and travel through extracellular fluid to target cells within the same local area.[2]

An adopted family of first messengers, falling outside the scope of hormones, neurotransmitters, and local mediators is populated by signal initiators that originate outside, rather than within, the organism. Some external factors capable of initiating a

signal and cellular response are light, odorants, and taste substances found in food as well as nutrients and other chemicals in the case of less complex forms of life.[3]

Many first messengers are derived from amino acids and other small water-soluble metabolites. Some of these messengers include acetylcholine, the purines (such as the adenosine nucleotides; adenosine monophosphate (AMP), adenosine diphosphate (ADP), and adenosine triphosphate (ATP)), catecholamines (dopamine, epinephrine, norepinephrine), and the thyroid hormones. Other first messengers such as eicosanoids, prostaglandins, and steroids are synthesized from lipids. Another structural class of first messengers is peptides. Insulin, vasopressin, and oxytocin are all peptide messengers.[2]

1.3 Second Messengers in Ligand-Receptor Mediated Signal Transduction

The interaction between a ligand and its target receptor on the cell surface is usually highly specific and binding can occur even at extremely low concentrations in the nanomolar or even picomolar range.[2] However, the mere binding of a ligand to a cell surface receptor cannot in and of itself generate a well-defined response. In order for a response or behavior to be realized, the receptor must somehow convey to the cell interior the information that a binding event has occurred.

Prior to the 1950s it was speculated that the physical movement of the ligand-receptor complex from the cell surface into the cytoplasm might be one means of signal transduction. In this scenario, either the internalized complex is able to perform a specific function, or the ligand is released by the receptor to elicit some sort of intracellular response.[2]

This idea was refuted by work done by Sutherland in the 1950s which gave rise to the concept of a second messenger system as a model for signal transduction.[4,5] A second messenger could relay the extracellular signal across the cell membrane obviating the need for ligand-receptor complex endocytosis.[2]

In Sutherland's time, it was known that adrenaline caused the breakdown of glycogen in the liver and that an enzyme called phosphorylase catalyzed a key step in the catabolic pathway, but the connection between all the players was not clear. Using a cell-free system, he was able to demonstrate that upon interaction with a cellular fraction containing particulate matter, adrenaline led to the production of a compound that activated phosphorylase in the soluble fraction.[2]

It was also discovered that ATP was required for the whole process to work, and further investigation led to the conclusion that the unusual nucleotide adenosine 3',5'-cyclic monophosphate (cyclic AMP or cAMP) was the compound responsible for phosphorylase activation. Cyclic AMP was duly christened as a second messenger in signal transduction due to its connection with the primary signal initiators, or first messengers.[2]

Since Sutherland's discoveries much evidence has been gathered indicating that a large number of first messengers exert their influence via the second messenger cAMP **(Table 1.2)**.

The regulation or modulation of biological events within the cell would not be complete without the means to both up-regulate and down-regulate biochemical pathways. The first messengers shown in Table 1.2 all function to increase intracellular levels of cAMP by up-regulating the enzyme responsible for its production, namely

adenylate cyclase. Not surprisingly, many first messengers are known to decrease intracellular concentrations of cAMP by down-regulating adenylate cyclase **(Table 1.3)**.

Table 1.2 Some first messengers whose effects are mediated by cyclic AMP.[2]

Messenger	Target Cell	Physiological Effects
Glucagon	Liver	Glycogen breakdown Gluconeogenesis
Adrenaline	Adipose	Lipolysis Inhibition of fatty acid synthesis
Adrenaline	Heart	Increased heart rate Increased contractile force
Vasopressin	Kidney	Na^+/water reabsorption
Lutropin	Ovary	Progesterone synthesis
ACTH	Adrenal cortex	Glucocorticoid synthesis
Thyrotropin	Thyroid gland	Thyroid hormone synthesis
Parathormone	Bone	Bone resorption

Table 1.3 Some first messengers that inhibit adenylate cyclase.[2]

Messenger	Target Cell	Physiological Effects
Adrenaline	Vascular Smooth Muscle	Contraction
Adenosine	Adipose	Inhibits lipolysis
Acetylcholine	Heart	Relaxation
Enkefalin	Neurones	Behavioral effects
Somatostatin	Anterior pituitary	Inhibits ACTH release

1.4 The Phosphatidylinositol Response and Inositol Triphosphate as a Second Messenger

A flurry of work spurred by the newly discovered second messenger cAMP ensued in the scientific community and the entire field of ligand-receptor interactions, first and second messengers, and signal transduction attracted a substantial following of researchers leading to several key discoveries.

In 1953, Hokin and Hokin reported that acetylcholine stimulated the incorporation of radioactive phosphate into the minor phospholipid phosphatidylinositol, but not into the other major phospholipids present in the membrane of pancreatic cells.[6] Subsequently, other first messengers were found to produce this phosphatidyl-inositol response **(Table 1.4)**; however the significance of this observed increase in the turnover of phosphatidylinositol upon agonist stimulation was not understood for a long time.

Table 1.4 Some first messengers that activate phosphoinositide breakdown.[2]

Messenger	Target Cell	Physiological Effects
Adrenaline	Liver	Glycogen breakdown
Vasopressin	Liver	Glycogen breakdown
ATP	Liver	Glycogen breakdown
Acetylcholine	Exocrine pancreas	Secretion
Acetylcholine	Smooth muscle	Contraction
Thrombin	Platelets	Aggregation
PDGF	Fibroblasts	Cell Proliferation

In 1967, Michael Berridge discovered that 5-hydroxytryptamine (5-HT) stimulated the release of fluid from the salivary gland of the blowfly. He later observed

that the same stimulatory effect could be achieved by adding cAMP even in the absence of 5-HT hinting that cAMP may be acting as a second messenger for 5-HT. Further investigation indicated that 5-HT was actually operating through two second messenger pathways. One signaling pathway was using cAMP to control potassium pumps and a second employed calcium to regulate the parallel flow of chloride ions.[7]

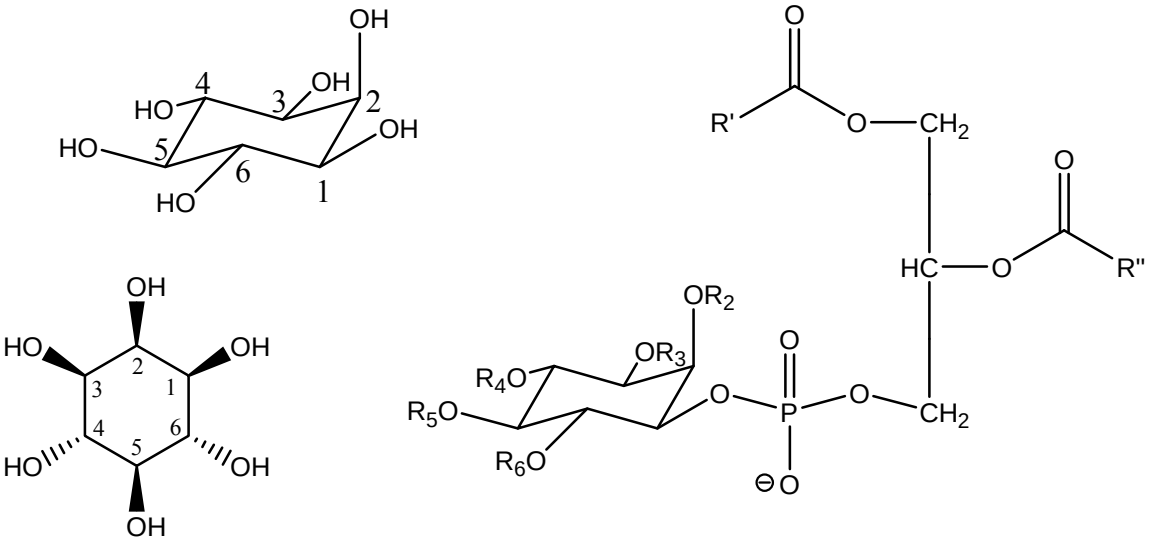
The potential role of calcium as a second messenger was largely obscured at the time by the exciting work being done with cAMP, and may have been easily dismissed, however Berridge posed the question "how does an agonist acting on cell surface receptors trigger the rapid mobilization of internal stores of calcium?"

Inspired by Bob Michell's discovery in 1975 that changes in the membrane levels of the inositol phospholipid, phosphatidylinositol-4,5-bisphosphate ($PtdIns(4,5)P_2$) **(Figure 1.1)** were accompanied by fluxes in intracellular calcium ion levels,[8] and Hokin and Hokin's earlier work on agonist stimulated phosphatidylinositol turnover,[6] Berridge began to connect the dots and draw some signal transduction conclusions around inositol phospholipids.

Berridge first showed that 5-HT caused a dose-dependent increase in the efflux of inositol, a small molecule component of phosphatidylinositol lipids. Then by using 5-HT stimulation of inositol production in the salivary glands, and frequent washing away of the inositol to prevent its reincorporation into the membrane, he was able to gradually deplete the gland of its inositol phospholipids.[7]

Measurements of calcium ion fluxes revealed that calcium signaling declined in parallel with the loss of the inositol phospholipids, but was readily restored when cells

were allowed to reconstitute their inositol phospholipid pool by being provided with free inositol.[7]



***myo*-inositol** **Phosphatidylinositol (PtdIns)**

R' = saturated alkyl chain
R" = unsaturated alkyl chain
$R_{2\text{-}6}$ = H except where noted

R_4 = PO_3^{2-} = Phosphatidylinositol-4-phosphate (PtdIns(4)P)

$R_{4,5}$ = PO_3^{2-} = Phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2)

Figure 1.1 Chair conformation and wedge and dash bond structures for *myo*-inositol and structures for the membrane lipid phosphatidylinositol.

The fact that the calcium signaling could be manipulated in accordance with the inositol phospholipid content provided the first direct evidence that phosphatidylinositol lipids were crucial in transducing the signal of 5-HT across the plasma membrane.[7]

The question of how the turnover of the membrane-bound inositol phospholipid resulted in the mobilization of internal calcium stores remained unanswered.

In the early 1980's, while using lithium to suppress the monophosphatase known to dephosphorylate *myo*-inositol-1-phosphate (Ins(1)P), Berridge observed the accumulation of Ins(1)P as expected, but he also observed the unexpected presence of the inositol phosphates *myo*-inositol-1,4-bisphosphate ($Ins(1,4)P_2$) and *myo*-inositol-1,4,5-trisphosphate (IP_3).[9] Later he was able to show that 5-HT stimulated the hydrolysis of membrane bound $PtdIns(4,5)P_2$ to form the IP_3 observed during the lithium experiments.[7]

Berridge's work culminated in 1983 when he and his co-workers in Cambridge, England and at the Max-Planck Institute of Biophysics in Frankfurt, Germany, were able to show that IP_3 caused the release of calcium from a non-mitochondrial calcium pool,[10] and *myo*-inositol 1,4,5-trisphosphate was declared to be the second messenger responsible for the observed calcium fluxes seen upon 5-HT stimulation.[7]

Many subsequent studies have supported Berridge's conclusion and it is now widely accepted that IP_3 is indeed a second messenger capable of coupling agonist stimulation outside the cell to intracellular calcium mobilization.

1.5 Ion Channels, Tyrosine Kinases, and G-Protein linked Receptor Types in Ligand-Receptor Mediated Signal Transduction

Building on the pioneering discoveries of Hokin and Hokin, Sutherland, Michell, and Berridge, and countless others who followed, our current knowledge of signal transduction has greatly expanded. Our understanding in one area of this diverse field indicates that there are three primary types of receptors through which ligands can relay

extracellular signals to the cytosol. The receptors can function as ion channels, they can perform as enzymes, or they can generate intracellular second messengers either directly (cAMP, cyclic guanosine monophosphate (cGMP)) or through coupling to guanosine triphosphate binding proteins (G-proteins) **(Figure 1.2)**.[2]

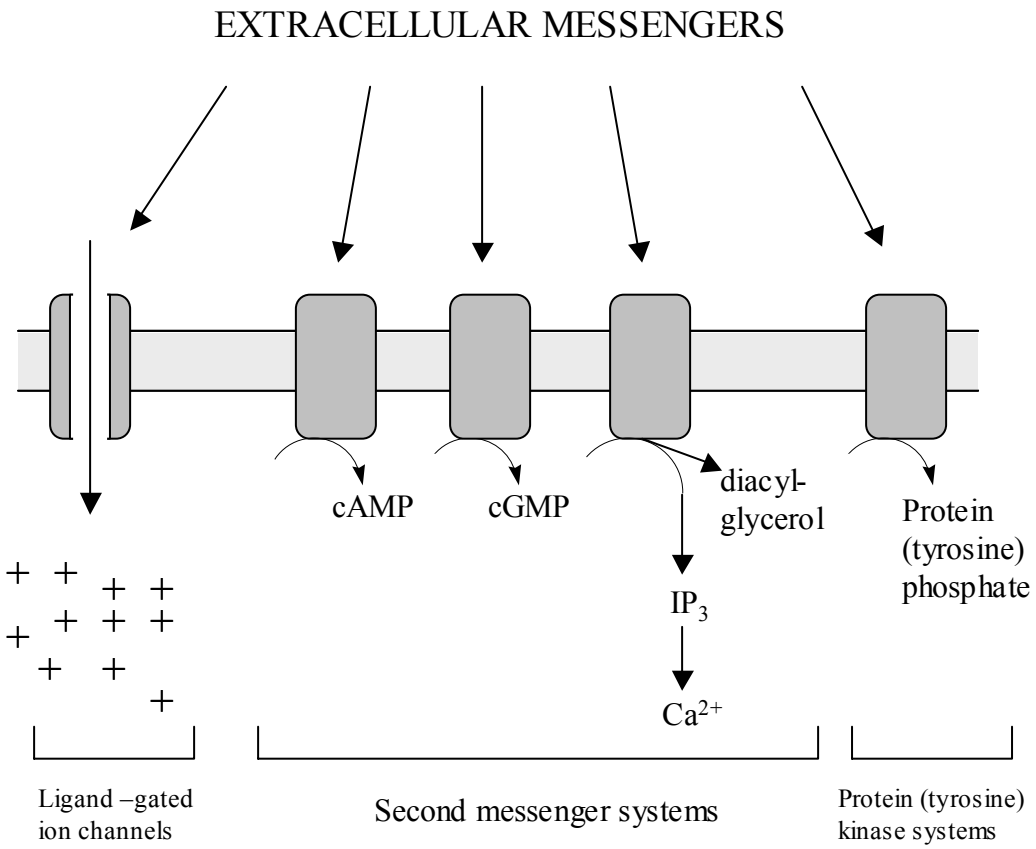


Figure 1.2 Types of signal transduction mechanisms associated with cell surface receptors.

Receptors that function as ion channels typically have neurotransmitters for ligands and control rapid responses like muscle contraction by regulating the flux of ions such as K^+ and Na^+ across the cell membrane.[1]

Receptors that are also enzymatic, often become activated via ligand induced self-phosphorylation, and act as tyrosine kinases toward other cellular enzymes producing long-term behavioral responses to cell surface binding events.[1] This tyrosine kinase activity is in striking contrast to other cellular kinases, which are predominantly serine/threonine kinases.

These receptors/tyrosine kinases have been classified into three families, all of which are composed of growth factor receptors: the epidermal growth factor (EGF) receptor family, the platelet-derived growth factor (PDGF) receptor family, and the insulin receptor family.[1]

Based on the sheer number of biological processes associated with G-protein linked receptors, they are perhaps the most important mechanism by which signals are transduced into the cell. The long-touted first second messenger cAMP, stemming from Sutherland's work, has been shown to be modulated by G-protein linked receptors. As we have seen **(Tables 1.2, 1.3)**, receptor binding by any number of ligands may either activate the enzyme adenylate cyclase leading to the production of the second messenger cAMP, or deactivate the enzyme leading to a decrease in intracellular stores of cAMP. These two competing processes are used in conjunction to modulate the cell's response to bound ligands.[1]

A primary target of cAMP is protein kinase A (pKA, or the cAMP- dependent protein kinase),[11] which upon binding, leads to phosphorylation of serine and threonine residues on protein substrates, thus modulating their activity.[1]

Branching out of Berridge's work came a more relevant example of G-protein linked signal transduction as it pertained to my research project. In this example of second messenger release ligand-bound receptors activate the enzyme known as phospholipase C (PLC) leading to the production of IP_3 and 1,2-diacylglycerol (DAG) as second messengers from PtdIns(4,5)P_2.[12]

As Berridge showed, IP_3 diffuses into the cytoplasm where it causes the release of calcium ions from various internal stores **(Figure 1.3)**.[13] There appear to be two distinct areas within the endoplasmic reticulum that are used for calcium storage, one is an IP_3-sensitive pool, making up between 30 and 50% of the total releasable Ca^{2+}, and the other is an IP_3-insensitive pool.[2]

The IP_3 receptor on the endoplasmic reticulum is an ion channel containing a large cytoplasmic region that houses the IP_3 binding site and the native receptor is believed to be tetramer requiring three subunits to be bound to IP_3 before the channel opens.[2]

Calcium released from the endoplasmic reticulum upon IP_3 binding appears to be a second messenger in its own right, and performs as a ubiquitous regulator of a diverse range of cellular function by mediating its target protein, calmodulin.[14]

Calcium-calmodulin complexes modulate the activity of many intracellular enzymes, including various protein kinases as well as glycogen synthase and microtubule-associated proteins.

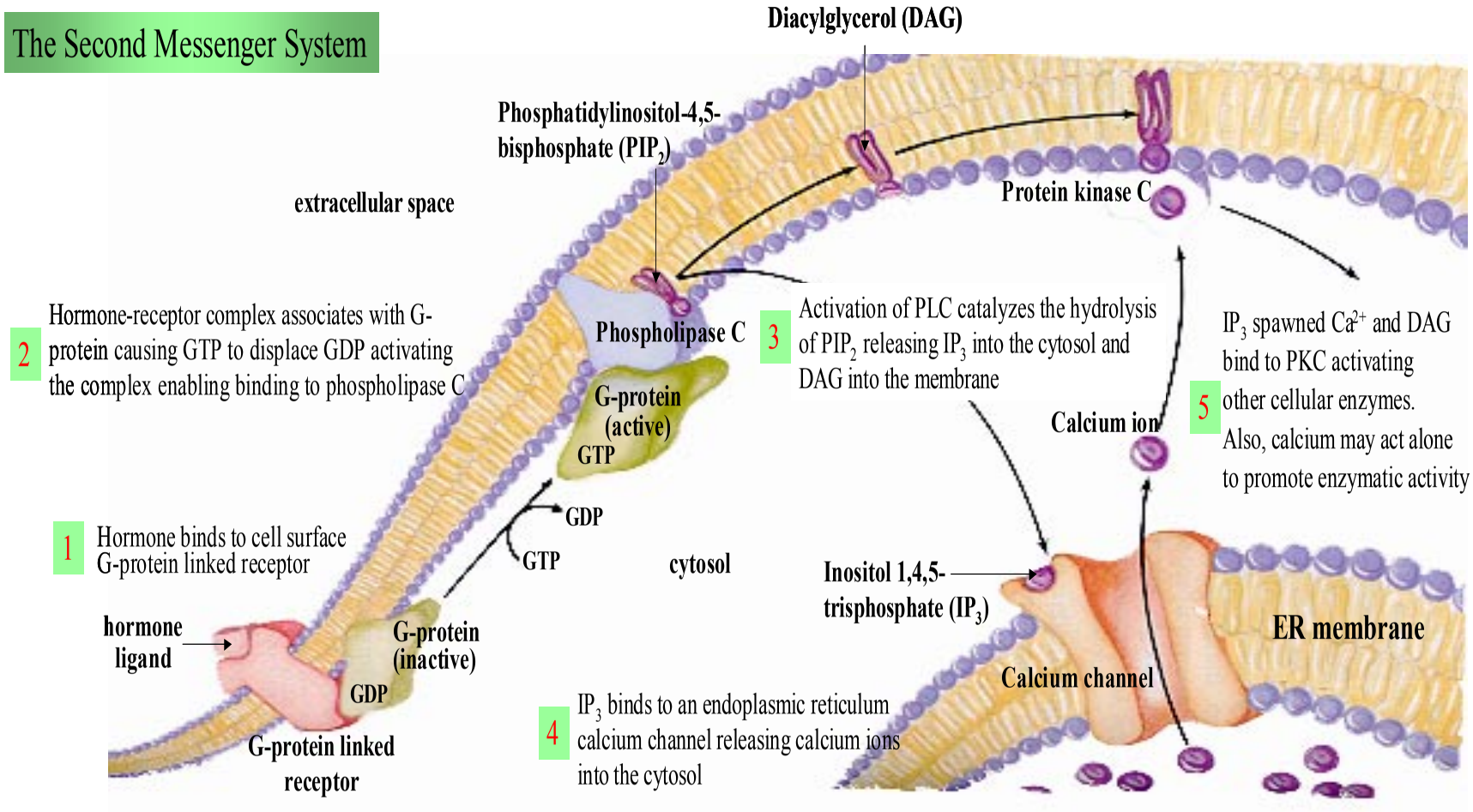


Figure 1.3 The IP_3 Second Messenger System.
Adapted from Lehninger, *Principles of Biochemistry.*

Diacylglycerol (DAG), the other product of PLC induced PIP_2 hydrolysis, diffuses within the membrane and activates the membrane associated enzyme known as protein kinase C (pKC), which much like the cAMP mediated pKA, phosphorylates serine and threonine residues on target proteins to alter their activity.[2] Known phosphorylation targets of PKC include the insulin receptor, β-adrenergic receptor, cytochrome P-450, and tyrosine hydroxylase.[15]

In an evil twist of attenuation hell, pKC has among its substrates a variety of cell surface receptors such as epidermal growth factor, insulin, transferrin, interleukin-2, and β-adrenergic receptors.[16] Membrane transport proteins for glucose, Na^+ and H^+ and cytoplasmic proteins involved in cellular migration and adhesion may also be under the regulation of pKC and thus the phosphatidylinositol second messenger system.[1]

More recently another second messenger system has been discovered which is the cGMP system, where the intracellular concentration of cGMP can be increased by activation of the enzyme guanylate cyclase in a similar fashion to that seen with adenylate cyclase,[17] or conversely decreased by the activation of the enzyme cGMP-specific phosphodiesterase.[18] Not unexpectedly, the protein target of cGMP is an enzyme known as pKG, whose substrates are not well-characterized.[1]

1.6 Guanosine Triphosphate-Protein Linked Receptors in Ligand-Receptor Mediated Signal Transduction

The role of G-proteins in signal transduction is to act as intermediates between the signal initiating ligand-receptor complex and the resultant second messengers. The pivotal part played by G-proteins in human health is underscored by the observation that

drugs targeting G-protein-mediated pathways make up the largest class of drugs.[3] The importance of this mechanism of signal transduction is also illustrated by the fact that 80% of the known hormones and neurotransmitters utilize G-protein coupled receptors as their mode of action.[1]

Because of its importance in human disorders and diseases much effort has been directed toward deciphering the physiological details behind G-protein linked receptors and signal transduction. Molecular and cell biologists have determined that the inactive form of the G-protein consists of α–, β–, and γ-subunits with a molecule of guanosine diphosphate (GDP) bound to the α-subunit. Upon interaction of the G-protein with the ligand-bound receptor complex, GDP is released from the α-subunit and is replaced with guanosine triphosphate (GTP) at the same site. Swapping of GDP for GTP results in the separation of the α-subunit from the β– and γ-subunits (which remain together). Separation activates the α-GTP subunit which in turn can modulate the activity of effectors (catalytic proteins, enzymes or ion channels, capable of changing levels of second messengers)—the ones directly involved in the signaling response.[1]

As of 1990, in a paper by Birnbaumer[19] there were approximately 80 identified receptors (currently in the 100s believed to be upwards of 1000) that interact with 12 known G-proteins, and G-proteins are now known to influence the production of at least six second messengers: cAMP, cGMP, IP_3, DAG, Ca^{2+}, and arachidonic acid.[2]

Structural and functional diversity within the G-protein family in a given eukaryotic organism is conferred by expression of multiple α–, β–, and γ-subunits that form the functional heterotrimers. As of 1977, Gudermann, e*t al*, had identified 23 distinct G_α proteins in mammals.[3] There is much less diversity seen in the number of

genes coding for the G_β and G_γ subunits such that there are only a few different G_β and maybe a dozen G_γ proteins.[3]

A single ligand-bound receptor may be capable of coupling to more than one kind of G-protein, and one G-protein may be capable of regulating more than one kind of effector protein. In addition, a ligand-bound receptor may also be able to couple to several G-proteins of the same type before dissociating, and in turn, each of the active α-GTP complexes generated may be able to modulate several effectors. It has been estimated that this multiplicity or amplification effect can turn the binding of one ligand to one receptor into the generation of 100-1000 IP_3 molecules leading to the release of 10,000 calcium ions in less than one minute.[7]

In terms of the change in basal levels of IP_3 required to elicit a response it is very minimal. In cells considered at rest the basal level of IP_3 is between 0.1 and 0.2 μM. Upon stimulation initiated by an extracellular agonist, this concentration rises to less than 1 μM. There are some exceptions to this as seen in AR 42J pancreatoma and SH-SY5Y human neuroblastoma cell lines whose basal levels are from 1 to 4 μM which upon stimulation spike to 25 μM. The only explanation offered for this difference is that perhaps IP_3 is somehow sequestered in the cell and not really free to interact with it's receptor.[7]

For a G-protein coupled receptor, ligand binding often leads to the simultaneous release of several second messengers into the cytoplasm. The existence of multiple second messengers working to accomplish the cellular response to a ligand, is now seen to be a common theme in signal transduction.[1]

The three basic receptor types discussed in this section (ion channels, tyrosine kinases, and G-protein linked receptors) give rise to a surprisingly huge variety of responses in their target cells. However, with a few exceptions, a common element in all these responses is that they are mediated by modulation of kinase and phosphatase activity—enzymes that phosphorylate and dephosphorylate serine, threonine, or tyrosine residues on target proteins.[2]

A sampling of the responses include: stimulation and contraction of both smooth and striated muscle, glycogen breakdown, protein synthesis, boosting of carbohydrate oxidation in the Kreb's cycle, glycolysis and gluconeogenesis, lipolysis, amino acid breakdown, heart muscle contraction, and gene expression.[2]

Not all cells in the body contain receptors for all the possible signal molecules they are likely to encounter. The types of receptors presented by a given cell type are tailored for the specific function of the cell or tissue where the cell resides. For example photoreceptor cells in the retina detect light, and may have very few, if any, receptors for the detection of other environmental signals.[3]

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CHAPTER 2

EXPLORATION OF THE PHOSPHATIDYLINOSITOL CYCLE: THE NEED FOR MORE INVESTIGATIVE TOOLS

2.1 Introduction

During the course of Berridge's studies it became evident that there was an inositol recycling system in place allowing for the reincorporation of inositol into the membrane as phosphatidylinositol after it had finished its messenger role. Evidence at the time pointed to a simple phosphatidylinositol recycling pathway where a series of three phosphatases were believed to sequentially remove phosphate groups from IP_3 until only inositol remained, which could be re-introduced into membrane phospholipids.[1]

In the mid 1980s the discovery of the previously unknown *myo*-inositol 1,3,4-trisphosphate ($Ins(1,3,4)P_3$) and *myo*-inositol 1,3,4,5-tetrakisphosphate ($Ins(1,3,4,5)P_4$) hinted that the phosphatidylinositol recycling pathway might be more complex than previously envisaged by Berridge making it an area ripe for further investigation.[1]

2.2 The Phosphatidylinositol Cycle and a Multitude of Inositol Phosphates

Nearly 20 years and countless studies later, the life of IP_3 from its cell surface mediated birth to its reincarnation as a cell membrane phospholipid has indeed proven to be a complex cycle inundated with a series of metamorphic events **(Figure 2.1)**.

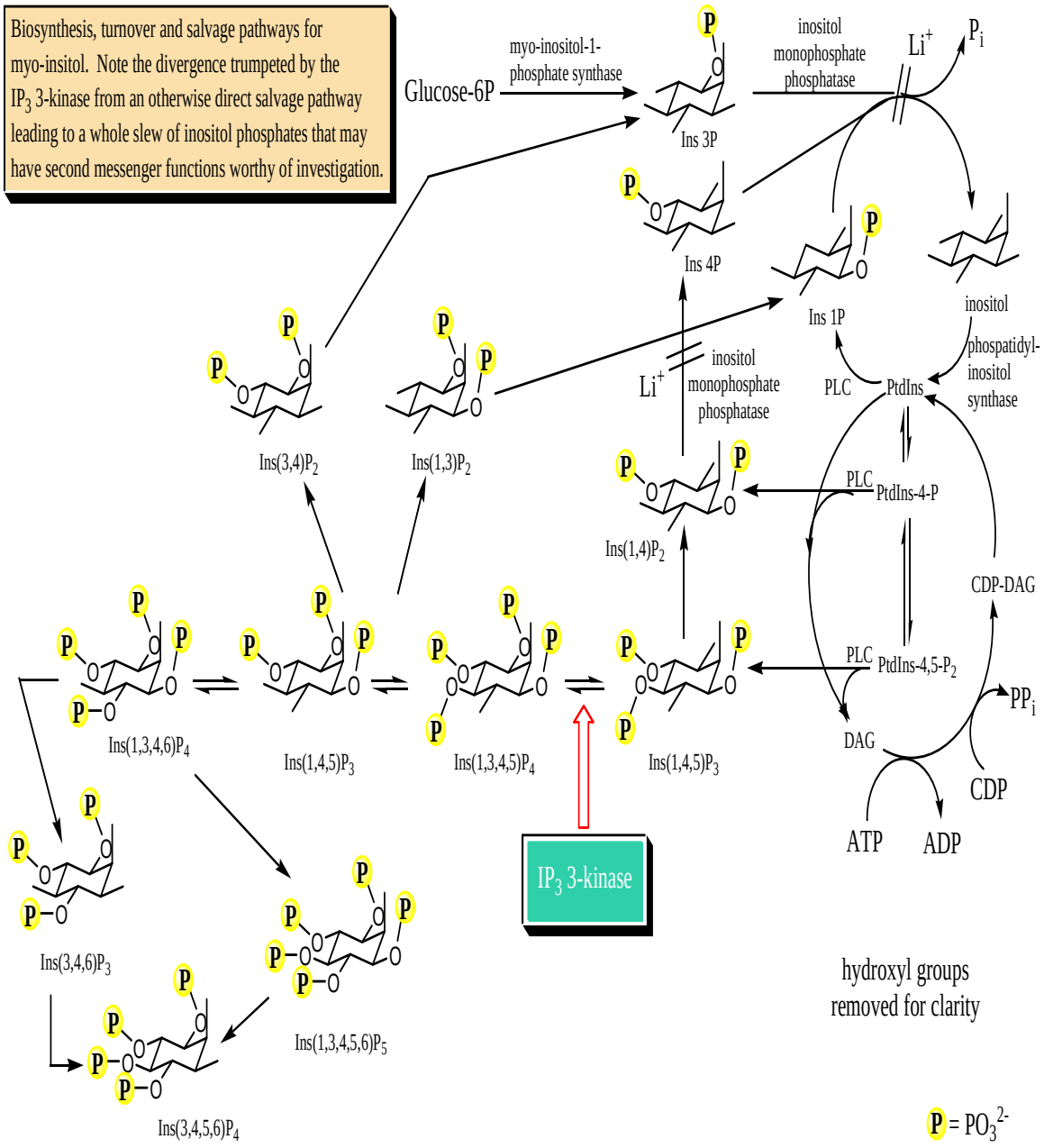

Figure 2.1 The Inositol Phosphate Cycle.

Examination of the elucidated phosphatidylinositol cycle reveals that two potential fates await IP_3 following completion of its duty as a second messenger. In what would be considered a direct pathway, IP_3 5-phosphatase deactivates the second messenger by removing a phosphate group to generate *myo*-inositol 1,4-bisphosphate which continues to undergo step-wise dephosphorylation in two additional reactions. A rather puzzling branch, in what would be an otherwise straightforward inositol recycling pathway, emerges in the second component of the bifurcating pathway where IP_3 3-kinase phosphorylates IP_3 to give the tetraphosphate $Ins(1,3,4,5)P_4$. While it is true that this route also serves to deactivate IP_3, the divergence from the 5-phosphatase route gives rise to many different inositol phosphates whose significance are not fully understood.[2] In fact, because of this detour, more than 20 different inositol phosphates, differing in their phosphorylation pattern, have been discovered in the cells of eukaryotic organisms.[3]

At first glance, all of these inositol phosphates simply appear to be intermediates in the PIP_2 regeneration pathway, as they eventually converge to inositol; however because natural systems are generally models of efficiency, a complex anabolic pathway strewn with a disparate degree of intermediates, seems to be a recycling bypass burdened with litter. Our faith in nature's sense of purpose leads us to believe that there must be ulterior motives for such a meandering return to the phospholipid fold and that these variants may have second messenger properties like IP_3 before them, and do not represent evolutionary baggage. We believe that their presence in the cell is indeed functional, and not merely the by-product of a scenic metabolic joyride.

2.3 Inositol Phosphates, a Multitude of Second Messengers?

So the big question that comes to mind about this inositol joy ride revolves around the IP_3 recycling pathway, and whether or not other inositol phosphates have roles beyond being intermediates in a convoluted metabolic pathway.

Others have provided evidence that Ins(1,3,4,5)P_4, product of the rebel IP_3 3-kinase, may also be a second messenger that influences the influx of calcium ions from outside the cell, or that it may have a hand in replenishing depleted endoplasmic reticulum stores.[4]

Another explanation for the bifurcating pathway involves the multi-specificity toward inositol phosphate substrates a handful of inositol phosphomonoesterase enzymes exhibit. In other words, some of these enzymes are capable of removing phosphate groups from a variety of inositol phosphate molecules. This ability may be more efficient in terms of enzyme conservation, but it would give rise to the observed seemingly inefficient proliferation of many different inositol phosphate intermediates.[2]

However, neither of these explanations address the presence of other kinases responsible for generating even more highly phosphorylated inositol derivatives and it may actually be that these pathways are merely evolutionary baggage that organisms have retained for no apparent reason.[2]

Exploring the possibility that various inositol phosphates may serve other cellular roles besides being intermediates in the phosphatidyl inositol recycling pathway certainly seems warranted.

2.4 Efforts Toward Assigning Second Messenger Roles to Inositol Phosphates.

In the area of non-IP_3 inositol phosphate second messengers, Hermosura *et al*.[5] have presented some functional studies on Ins(1,3,4,5)P_4—the alternative product of the bifurcating pathway emanating from IP_3 3-kinase. One known function of Ins(1,3,4,5)P_4 is to protect IP_3 from hydrolytic degradation at the hands of inositol phosphate 5-phosphatase. Inositol phosphate 5-phosphatase is a multisubstrate enzyme capable of removing the number five phosphate group from several different inositol phosphates including Ins(1,3,4,5)P_4 and IP_3. The tetraphosphate acts as a sacrificial lamb of sorts by virtue of its 10-fold higher affinity for the enzyme than IP_3 meaning that Ins(1,3,4,5)P_4 is dephosphorylated at a more rapid clip, thus preserving IP_3.

Only but a moment of reflection should suffice to realize that none of that makes much sense since IP_3 is consumed when generating its supposed protector.[6] So how does the production of Ins(1,3,4,5)P_4 from IP_3 result in a net gain of IP_3? Inositol phosphate 5-phosphatase has a one hundred-fold lower V_{max} for Ins(1,3,4,5)P_4 as compared with IP_3 which means it has a longer residence time in the cell after stimulation has ceased.[7] Hermosura report data that indicates this residual population of Ins(1,3,4,5)P_4 can serve to protect any new *myo*-inositol 1,4,5-trisphosphate that is released by subsequent stimulation. In this manner Ins(1,3,4,5)$_4$ acts as a short-term memory that the cell has recently had a cell-surface stimulatory event.[6]

The investigators also reported another interesting function for Ins(1,3,4,5)P_4—IP_3 receptor antagonist. This ability to compete with IP_3 for the endoplasmic reticulum bound receptor becomes physiologically significant when the Ins(1,3,4,5)P_4:IP_3 ratio is greater than about 10:1, however the relevance of this function is not clear. Speculation

is that it may be a means of tightening intracellular calcium oscillations in the convoluted world of regulatory mechanisms only a cell's mother could love. IP_3 induced Ca^{2+} release activates the IP_3 3-kinase boosting Ins(1,3,4,5)P_4 levels possibly to the 10:1 ratio point of IP_3 receptor antagonism, thereby slowing calcium release and allowing stores to build up faster for the next uptick.[6]

The hydrolysis of Ins(1,3,4,5)P_4 via IP_3 3-kinase activity to produce Ins(1,3,4)P_3 has some additional consequences. First, Ins(1,3,4)P_3 is the main regulator of inositol 3,4,5,6-tetrakisphosphate levels, itself a second messenger that controls chloride efflux.[8] Second, IP_3 3-kinase may be the first step in a receptor-regulated synthesis pathway leading to the 'higher 'inositol phosphates, such as IP_5, IP_6 and —perhaps the most surprising inositol phosphate 'proliferation ' of all —IP_7 and IP_8![6,8]

Our research in this area is based on the theory that these pathways are not some sort of vestigial aberration of evolution gone awry, but rather that the intermediate inositol phosphates seen in the phosphatidylinositol cycle do have important second messenger roles in the cell that have yet to be determined. There is considerable precedence for this, and proponents of this idea conjecture that because of the strong presence of the phosphatidylinositol system in the brain, and the diverse effects of calcium, it is likely that inositol polyphosphates have second messenger duties in neurons, however due to the lack of pharmacological tools, much still remains unknown about these roles.[1]

Experiments using lithium, which effectively depletes neurons of inositol, thus reducing their ability to generate IP_3 and DAG, have suggested an important role for

phosphatidyl-inositol turnover in the brain, however use of lithium does not allow for the uncoupling of inositol phosphate functions from those of DAG.[1]

Drugs designed to specifically block the Ca^{2+} mobilization branch of the phosphatidylinositol cycle are needed to test for the role of inositol phosphates in neuronal cells independently of DAG. The obvious target for such compounds is the endoplasmic reticulum bound IP_3 receptor and drugs such as decavanadate, heparin, and neomycin are known to inhibit IP_3 binding to the receptor, however these drugs are not highly specific.[1]

Clearly, there is a demonstrated need for better methods of perturbing intracellular inositol phosphate levels for potentially elucidating the second messenger roles of these compounds. In addition to perturbation, methods to detect the concentration of inositol phosphates in both resting cells and in agonist stimulated cells are needed. The development of improved perturbation and detection methods could allow for comparative studies to be done on various ligand-receptor complexes to probe for a multitude of physiological responses.

The standard approach to these problems has been to use steady-state radioactive labeling of the phosphate groups in inositol and phosphatidylinositol by introducing [^{3}H]*myo*-inositol to the cells, but this doesn't necessarily approach the biological reality. It may also take several days for the system to reach isotopic equilibrium and certain inositol phosphates take longer than others to reach steady-state.[1]

A frequent goal of such studies is to measure the sum concentration of downstream metabolites of the competing pathways i.e. [Ins1P+Ins(1,4)P_2] vs. [Ins(1,3,4,5)P_4+Ins(1,3,4)P_3+Ins(3,4)P_2+Ins(1,3)P_2+Ins3P+Ins1P]. Problems occur

because the fluxes in the concentrations of these species are very rapid making it hard to prevent IP_3 and Ins(1,3,4,5)P_4 from being completely dephosphorylated to inositol before any useful information can be obtained.

Again, lithium is the tool of choice typically used to fight exhaustive dephosphorylation by suppressing Ins(1,3,4)P_3/Ins(1,4)P_2 1-phosphatases and inositol monophosphatase, however it cannot completely block those pathways and it can actually activate 5-phosphatase, thus skewing the analysis away from the more complex branch of the bifurcating pathway that leads to higher-order inositol polyphosphates.[1]

Methods have been developed to specifically measure the amounts of IP_3 and IP_4 in crude cell extracts,[9,10] however they require a complex separation of all the inositol phosphates via HPLC prior to being assayed, so they have not gained widespread use.[1]

Recently its been speculated that Ins(1,3,4,5,6)P_5 and IP_6 (phytic acid) may act as extracellular messengers much like ATP. They have been shown to reduce blood pressure and heart rate. There is also evidence they may have neurotransmitter-like roles. Phytic acid causes the influx of calcium into the cells of certain tissues, and this increase in calcium uptake correlated with an increase in neurotransmitter release.[1]

Many of the higher inositol polyphosphates are known to chelate divalent cations to form both soluble and insoluble complexes suggesting they may activate neurons via depletion of Ca^{2+}.[1]

2.5 The Inositol Phosphate Receptor Research Project

Much of the research in the area of assigning second messenger status to inositol phosphates other than IP_3 has focused on the synthesis of inositol polyphosphate analogues designed to inhibit enzymes in an effort to affect a physiological response.

Our efforts will focus on developing receptors that intercept specific *myo*-inositol phosphate in hopes of affecting a physiological response and assigning second messenger roles to the wide variety of inositol phosphates found in the cell. This can be accomplished through the synthesis of a series of abiotic organic macrocyclic and macropolycyclic receptor molecules, which are designed to specifically bind to the many *myo*-inositol phosphate secondary messengers found throughout the cell. More importantly, the metabolic consequences and physiological responses associated with such binding events can be explored in an effort to develop therapeutic agents for a range of diseases linked to phosphatidyl inositol metabolism. These inositol phosphate receptors would also add to the collection of molecular biology tools available for the study of diverse cellular processes. Because they have the potential to act as probes for numerous signal transduction events they would be useful in efforts to understand the molecular basis of many human diseases and disorders.

One major medical problem, in which our research may have direct bearing upon, is in the treatment of bipolar disease. Bipolar affective disorder, also termed manic depressive illness, is a disorder of mood characterized by recurrent periods of mania and major depression. During periods of mania, patients exhibit expansiveness, elation, agitation, hyperexcitability, hyperactivity, and apparent increased speed of thought and speech. Depressive episodes are characterized by feelings of sadness, despair and

discouragement, and are often associated with feelings of low self-esteem, guilt and self-reproach, withdrawal from inter-personal contact and somatic symptoms such as eating and sleep disturbances.[2]

Currently the FDA has approved the use of two drugs—lithium and valproic acid—for the treatment of bipolar disorder. The putative target of lithium is inositol monophosphatase, an enzyme involved in the recycling of IP_3 into PIP_2. The lithium induced inhibition of inositol monophosphatase results in the intracellular accumulation of inositol monophosphate, inositol bisphosphates, and IP_3.[11]

Bipolar disorder is one malady known to be directly influenced by phosphatidyl-inositol metabolism, however isolation and cloning of G-protein linked receptor subtypes along with mutation studies have implicated the receptors in a wide variety of human disorders such as retinitis pigmentosa and other visual disorders, nephrogenic diabetes insipidus, development, malignant transformation, autoimmune disease, drug addiction and alcoholism, therefore these diseases may also have ties to the IP_3 second messenger system and phosphatidylinositol cycle.[12]

The cloning and mutation studies have also provided structural information useful in designing therapeutic agents geared toward treating G-protein maladies. Synthetic organic chemistry and pharmacology have been used to modify small molecule ligands such as norepinephrine and histamine to provide new selective agonists and antagonists with restricted therapeutic activity and limited side effects.[12]

With the advancement of molecular biology techniques has also come the ability to manipulate larger, more complex peptide and protein ligands for the treatment of

diseases. The fields of antisense and gene therapy are also sure to play a role in our understanding and treatment of G-protein based disorders.[12]

We also believe that our inositol phosphate receptors can play an important diagnostic role in the area of G-protein linked receptor maladies.

The following chapter will embark on a review of the recent literature concerning, the current state of anion receptors and the concept of building abiotic inositol phosphate receptors as probes of biological functions, second messenger roles of non-IP_3 inositol phosphates, and the current state of inositol phosphate synthesis. It will become evident in later chapters that all these areas are relevant to the design and synthesis of our inositol phosphate receptor molecules.

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CHAPTER 3

A REVIEW OF THE CURRENT LITERATURE ON SYNTHETIC NITROGEN CONTAINING ANION RECEPTORS AND INOSITOL PHOSPHATE BOUND HOST-GUEST COMPLEXES

3.1 Host-Guest Chemistry, A Brief History

In natural systems, host-guest interactions have long been known to trigger many biologically important events. These highly specific molecular recognition events include: substrate binding to receptor proteins; enzymatic reactions; assembling of protein-protein complexes; immunological antigen-antibody association; intermolecular reading; translation and transcription of the genetic code; signal induction by neuro-transmitters; cellular recognition, etc.[1] It is not surprising that the discovery of such interactions in living systems inspired synthetic organic chemists to mimic these events using artificial, or abiotic host-guest systems.

Pioneers in the field of abiotic host-guest chemistry include Nobel Laureates Charles Pedersen,[2,3] Jean-Marie Lehn[1,4-6] and Donald Cram.[7,8] Thanks to the early and continued (for the living) efforts of these individuals and the hundreds of followers they motivated, the field was well-established and well-populated with researchers by the late 1960s.

Some of earliest examples exhibiting the field defining interactions were the "metal deactivators" developed by Pedersen at DuPont.[9,10] The first of these was *N,N'*-

bis(*o*-hydroxybenzylidene)-1,2-propanediamine (**Figure 3.1**) an excellent deactivator for copper which has been used industrially for many years.

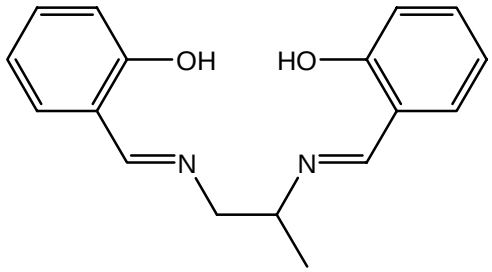


Figure 3.1 Pedersen's copper chelating *N*,*N*'-bis(*o*-hydroxybenzylidene)-1,2-propane-diamine.

Pedersen's crowning achievement in the area of host-guest chemistry was the discovery of polyether macrocycles called crown ethers (**Figure 3.2**). Crown ethers entered the host-guest fray with the ability to bind cationic metal centers such as sodium and potassium, as well as the ammonium ion, with some degree of specificity.

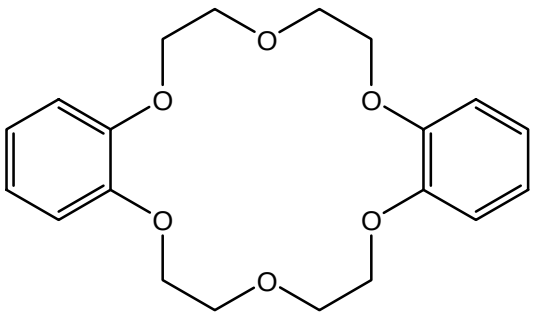


Figure 3.2 Pedersen's dibenzo[18]crown-6.

Lehn's contributions to the field began with his interest in binding cations (K^+ and Na^+) associated with nerve cell function in an effort to study excitation and ion distributions across biological membranes.[6,11] With this interest came the synthesis of cryptands (**Figure 3.3**) which were able to form complexes with the aforementioned cations. Lehn further went on found an entirely new field of chemistry known as supramolecular chemistry.[1]

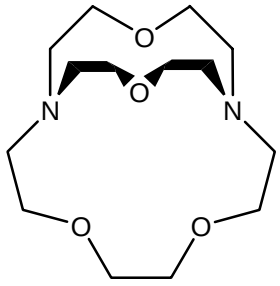


Figure 3.3 Lehn's cryptand.

Donald Cram was recognized for his contribution to the field of host-guest chemistry with compounds he christened spherands.[12,13] Cram recognized that crystal structures of Pedersen's crown ethers and Lehn's cryptand's did not show evidence of highly preorganized cavities or convergent binding elements. Spherands were designed as completely preorganized macrocycles, consisting of extremely rigid phenyl groups, bearing convergent binding elements (**Figure 3.4**).

A major conceptual leap in the field came with the idea of molecular recognition, introduced by Lehn in 1973. It was a unifying concept tying together decades of

observations stemming from Emil Fischer's 1894 "lock and key" theory of enzyme-substrate recognition.[14,15]

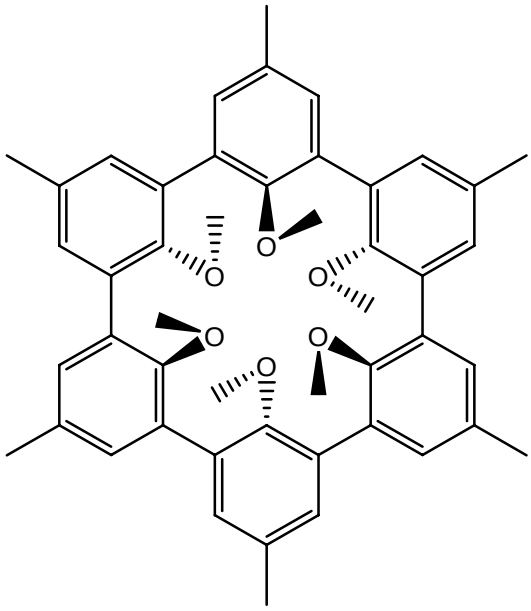


Figure 3.4 Cram's spherand.

A key component of molecular recognition is that recognition by a host molecule is not simply the binding of a guest compound. The condition of true recognition is met only when the binding of one substrate to a receptor occurs preferentially to the binding of others in a competitive mixture of many possible substrates.[1] Furthermore, the association must be stronger than nonspecific collisions or proximity induced casual molecular interactions. Lehn postulated that the preferential binding of a single substrate to a receptor is the result of large differences between the binding free energies of competing guest molecules. This idea will be revisited throughout this chapter.

Molecular recognition is a primary concern in the building of artificial receptors designed to manipulate cellular function. Selectivity in the binding event is of tantamount importance. Significant binding of molecules in the cell not specifically targeted by an artificial receptor could have disastrous cellular consequences, which may resonate throughout the entire organism.

With the concept of molecular recognition firmly in mind, Lehn built upon Pedersen's crown ether theme by adding a new structural dimension resulting in molecules called cryptands. Cryptands can be viewed as macropolycyclic compounds, whereas crown ethers are best seen as macrocyclic polyethers, or more generally as macrocycles.

By introducing the polycyclic dimension, Lehn successfully added structural rigidity, a larger contact area, a greater number of binding sites, and a solvent-shielding cavity to Pedersen's theme. These additions resulted in better ligand binding leading to more specific interactions.

Lehn was further able to elaborate on the cryptand theme by introducing non-oxygen heteroatoms (namely sulfur and nitrogen) at receptor binding sites. This progression into heteroatom binding sites vastly opened up the array of guest molecules for which receptors might be built.

Of particular relevance to this research project was the use of nitrogen heteroatoms in the ring giving rise to molecules known as polyamino- or polyazamacro-cycles. This was a major step in the history of artificial receptors since prior to the introduction of basic sites capable of proton abstraction, receptors were only able to bind cationic guests.

Jean-Marie Lehn had some great insights into the enormous potential of macrocycles during his early work in the field of host-guest chemistry. He was one of the first to recognized that manipulation of linkers between the amino binding sites of the receptor could be used for the linear recognition of molecular length. The idea that heteroatomic binding sites coupled to linear recognition of molecular length might be useful in the design of receptor molecules capable of binding not merely ions, but also charged molecular species, was born.

The number of disciplines influenced by Lehn and his Nobel cohorts, loosely falling under the umbrella of host-guest chemistry, is enormous. Other monikers one could image are ligand-receptor interactions, anion receptors, cation receptors, molecular recognition, molecular receptors, chelation, coordination chemistry, metal-ligand complexation, chemosensors, and the beat goes on. As a broad indoctrination, some major milestones achieved in artificial receptor design and synthesis grounded in lock and key inspired chemistry include cryptand-cation, polyaza-anion, cyclophane-aromatic, boronic acid-sugar, and guanidinium-carboxylate partnerships,[16] and there are several new milestones on the horizon.

In order to provide a literature review both meaningful to the focus of this project and digestible to the reader, the receptor portion will be limited to nitrogen-centric macrocycles. Literature on macrocycles containing other heteroatoms such as sulfur and oxygen is not particularly relevant since these binding motifs are geared toward cation complexation, and thus will not be considered. The coverage of acyclic molecules will be limited as well, unless they bear special interest. Also, in keeping with relevance to my research project, the bulk of the nitrogen containing macrocycles covered will be of

the amino variety as opposed to amide, urea, guanidinium, or other assorted nitrogen containing compounds.

3.2 Design Aspects Important For Molecular Recognition

The defining feature of host-guest chemistry is the interaction or binding event responsible for bringing the two partners together. When considering these binding interactions, the key indicator describing the strength of the attraction between the two partners is the binding or stability constant (K_S). The condensed phase binding constant measures the difference in free energies between the host-guest complex and the separated fully solvated partners and thus does not reflect the total intrinsic free energy of association. Because of this it is conceivable that structural modifications at sites remote from the actual binding sites may affect the binding strength of the bimolecular association. The process of bimolecular binding requires that two highly solvated charged molecules both shed their solvent cages in favor of mutual interactions.[17]

One of those charged molecules is the hosting species. Having an understanding or definition of what it takes to be a host molecule will aid in the design and construction of molecular receptors. There is no universal consensus on the criteria to be met by molecular hosts, but a number of features[17] seem to be essential.

One historic criterion that does not apply to modern experimental observations stems from the lock-and-key metaphor used by Emil Fischer to describe enzyme-substrate interactions.[14,15] Visualization of host-guest interactions *vis-à-vis* a lock-and-key analogy has the guest penetrating and being wrapped by the host structure. Although

this encapsulation type of host-guest interaction has advantages it is not required to have host-guest complexation.[17]

Another consideration is the types of chemical interactions typically at work in host-guest complexes. While the majority of these interactions have been non-covalent, there are successful and universally accepted systems where the host binds its guest covalently.[17]

One criterion that appears to be mandatory in defining a host is that the host-guest binding equilibrium be established rapidly—at least within the usual time frame of kinetic experiments involving physical measurements, which generally means on the ^{1}H NMR time-scale This time criterion can be roughly translated into an upper limit for the free energy of binding in the complex. A crude estimate for a 1:1 stoichiometric association gives a log K_S of about 13 (ΔG = 18kcal/mol). (The rate of bimolecular association is taken at the diffusion limit of 10^9 M^{-1} s^{-1} and the half-time for the dissociation complex at $T_{1/2}$ = 3 hr).[17] A survey of the experimental data shows that in practice, the theoretical upper limit is not reached. The range of Log K_S values observed typically ranges from 2 to 11.[18] However, supramolecular complexes of higher stability are well known in biology and show greater binding constants (log $K_S \approx 15$) than the theoretical upper limit suggested by diffusion kinetics in artificial systems.[17]

Host-guest binding is considered to be occurring on an appreciable level with log K_S values between about 3 and 4, good binding is reached between 4 and maybe 5.5, and the exceptional threshold appears to be crossed around log K_S = 6. Anything over 8 is truly a worthy achievement, but few receptors appear to reach that pinnacle. Absolute

success cannot be measured with log K_S values alone, as this data must be tempered by the envisioned application for the host system.

A final criterion is that the host must show selectivity for a given guest.[17] This can be presented as a ratio of association constants for potential guest species under a given set of experimental conditions. For most applications a selectivity factor of 1000-fold for the targeted guest over all others appears to be the gold standard in measuring successful molecular recognition in receptor molecules. Selectivity arises from the molecular interactions between the binding partners and by understanding these interactions it may be possible to optimize complexation through host engineering.

Before the engineering process begins it is important to ask some fundamental questions about the host-guest relationship. According to Schmidtchen and Berger,[17] three particular questions are of interest: What is the chemical nature of the host and guest? In what type of environment will the binding take place (solvent/solution)? What purpose must the complexation serve?

The Chemical Nature of the Host and Guest.

The chemical nature of the guest is the design element commanding the most attention by molecular architects of anion receptors. Items to consider are the attractive and repulsive forces present between the two partners along with complementary shape and topology as well as the geometric preorganization of host molecules.[17]

The Chemical Environment Where Binding Occurs.

Complexation of the host and guest molecules invariably alters the solvent organization about the binding partners. The net free energy of this process can either favor or hamper the association between the binding partners. Gelb and Zomba[19,20] concluded from binding studies with various halide- and oxyanions that host-guest interactions in water are driven by an entropy effect. The mutual interaction of oppositely charged species coming together results in an enthalpic effect as well, but it is believed that the gain in enthalpy achieved by this association is largely offset by the energy required to the release the molecules solvating the partners. The entropy effect comes into play when solvent molecules are freed by the association of binding partners and overcomes the negative entropy of combining two partners into one complex. This provides evidence that solvent reorganization is a major contributor to complex stability, and may play a more important role than direct electrostatic interaction between the host and the guest.

Fully understanding the solvation energies involved in molecular recognition is not a trivial matter and results in this aspect of receptor engineering are scarce at the present time.[17]

The Purpose Served by the Binding Interaction.

It is important to know what purpose the host-guest complexation will serve. Is the aim of the interaction to maximize binding or is it to serve a more functional purpose such as membrane transport or catalytic activity.[17]

With these three broad questions in mind, one step toward the rational design of receptors can be taken to help optimize the binding interaction responsible for complexation.

Because most types of binding interactions are cumulative, it is expected that the total interaction energy will increase as the number of independently recognized substructures (epitopes) in the guest increases. For this reason, the larger and more diversely structured the guest molecule is, and the better its functional surface, size, and topography are matched to the host, the stronger its binding constant will be. Cram postulated that a rigid host having all its anchor groups preorganized to compliment the guest should show the strongest binding.[7,8]

Implementing this idea of preorganized binding sites complementary to guest molecules represents a difficult synthetic challenge, especially in the case of larger multifunctional guests. Placing binding sites in fixed spatial topology and orientation often requires a macropolycyclic framework representing one dimension beyond macrocyclic compounds. Besides being synthetically challenging, macropolycyclic structures do not readily lend themselves to easy modification in case some tweaking of the binding states is required to optimize binding. There is also the risk of slow guest exchange kinetics with these encapsulation interactions that may preclude the system from potential applications even if selective binding is achieved.[17]

It is interesting to note that the inherent complexity of macrocyclic receptors might not be a necessary design element if one looks to nature for guidance. In natural systems, biological receptors often do not adhere to this idea of a rigid, preorganized framework, rather they follow a modular construction principle in which binding domains

are connected in linear or branched fashion. The correct alignment of binding domains in terms of topology and orientation is achieved in a free energy intensive folding process. Because of this, a foldable host having the same number and type of binding sites as a rigid host will exhibit less selective binding due to the entropic cost of organizing the flexible chain into a well-defined three dimensional structure. Also solid-state structural evidence determined by X-ray crystallography may not be readily translated into solution phase structures for flexible hosts versus rigid hosts whose X-ray data can be assumed to be valid in solution. Flexible hosts do however offer some advantages over rigid hosts in terms of ease of synthesis and ability to modify. Flexible hosts would also probably not suffer from slow guest exchange rates seen with encapsulation motifs.[17]

The previous paragraph illustrates the importance of understanding the purpose of the host-guest complexation the matchmaker had in mind. If selectivity is not an issue, then acylic hosts may suffice for the application at hand. This may be the case in a system designed for reaction catalysis where the feedstock chemicals are well known and delivery is easily controlled. In other applications, such as those where the host must function in a cellular environment, selectivity may be more important, thus more complex macrocyclic or macropolycyclic hosts might work best.

Whether the application calls for hosts to be acyclic or cyclic will also depend a great deal on the guest. Designing molecules capable of housing a guest in a comfortable environment that enhances binding, requires molecular architects to fully understand both the chemical and physiochemical properties of the substrate.[21] For the purpose of this review, only anion guests will be considered, although many of these ideas would be applicable to the design of cation receptors as well.

The salient feature of anions is their negative charge which makes electrostatic binding to cationic hosts the most obvious choice for anion receptor design. Another consideration, especially important in the design of receptors for monoatomic anions, is that they are relatively large in comparison to cations: F- (1.36Å), Cl- (1.81 Å), Br- (1.95 Å), and I-(2.16 Å).[21] The smallest of this series, F-, is nearly the size of the largest monatomic cation, K+ (1.38Å).[21]

Anions also have higher Gibbs free energies of solvation (ΔG) relative to cations, meaning they are more strongly hydrated in aqueous medium, therefore anion hosts must compete more effectively with the solvent for their guests.[22] In the case of ion pairing host-guest interaction—the electrostatic attraction of oppositely charged species—this solvation issue is nearly a non-event. In fact, it requires extremely dilute conditions ($<10^{-4}$ M) of ions in a highly solvating solution (water) to prevent ion pairing from occurring.[21]

It may also be of little concern in some organic systems because removal of an anion from an aqueous phase to a non-hydrogen bonding phase greatly increases its nucleophilicity, and thus possibly its attraction to hosts, as the ion is stripped of its waters of hydration.[21]

Another consideration is that a large variety of atomic and molecular geometries may be encountered, spherical (F^-, Cl^-, Br^-, I^-), linear (N_3^-, CN^-, SCN^-, etc.), planar (NO_3^-, CO_3^{2-}, R-CO_2^-, etc.), tetrahedral (PO_4^{2-}, SO_4^{2-}, ClO_4^-, etc.), octahedral ($Fe(CN)_6^{4-}$, $Co(CN)_6^{3-}$, etc.),[21] and many others given the possibilities of more complex molecular structures.

In addition to being negatively charged most anionic species exhibit Lewis basicity and this feature is certainly exploitable in the design of receptors. Lewis basicity

adds directionality to the guest system rendering it sensitive to the spatial arrangement and orientation of binding groups. This is an indispensable screen to differentiate between anions of very similar size and charge as seen in the biological distinction of phosphate and sulfate.[23,24]

Finally, when considering anion properties it is important to understand that the anion state may only exist in a limited pH range, i.e. above pH 5-6 for the carboxylates.[21]

Because the size and geometry of the anion dictate the size and shape of the receptor, and given the range of potential anionic guest species, the designer is faced with many decisions on whether to utilize acyclic, macrocyclic, macrobicyclic, or macropolycyclic topologies to house the binding elements and accommodate a given guest.[21]

By fully understanding the chemical and physiochemical aspects of the guest, the process of rational receptor design can begin by identifying the recognition elements needed in the host to selectively bind the guest. The ammonium and guanidinium sites have been the most widely utilized, but other positively charged groups such as sulfonium and phosphonium have also been considered. Other functional groups of interest for anion binding sites are the physiologically electroneutral amide, urea and thiourea groups. Even Lewis acid functions possessing electron deficient centers (tin, mercury, boron, silicon) have been studied.[21]

A final consideration for binding specific anions is the identification of appropriate linkers to piece together the recognition elements and place them in space such that maximum complimentarity with anion binding epitopes is achieved.[21]

3.3 Nitrogen Centric Anion Binding Motifs in Abiotic Receptors

Nitrogen-based anion receptors can be broadly classified into positively charged or electroneutral species.[17] Cationic receptors capable of forming ion pairs with anions in solution are most easily prepared by protonation of suitable basic compounds. Because many anions, are also basic, host-guest binding often depends on relative proton affinities in interconnected multiple equilibria. In water, proton equlibria are easily established as are the corresponding pK_a values, so it is no surprise that water was the solvent of choice in which to study anion binding to a great number of polyaza compounds.[17]

Perhaps the most widely studied of these are those of the polyammonium variety. Polyammonium-, or polyazamacrocycles, are the simplest form of nitrogen containing cyclic compounds used to bind anionic substrates and their use will be extensively reviewed in the following sections.[21]

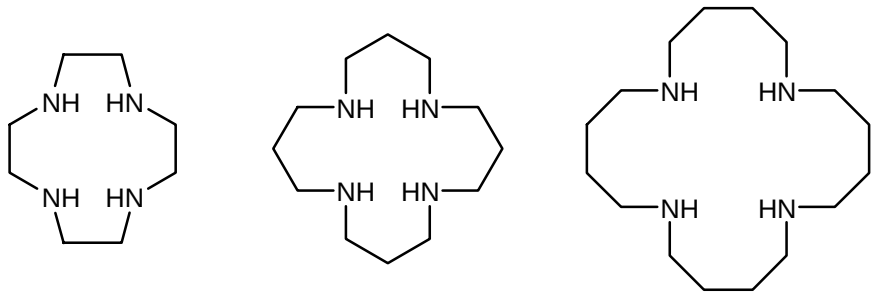
Polyazamacrocycles

The impetus driving the synthesis of the first polyazamacrocycles was the desire to build molecular hosts capable of binding to biological phosphates, especially the adenosine nucleotides, due to their energy currency role in cells.[22] Nucleotides in general make up the bulk of the phosphates seen in living systems primarily due to their presence in DNA and RNA.

It has long been known that the naturally occurring polyamines spermidine and spermine bind with phosphates in aqueous solution.[25] For example log K_S values, at pH 7.5, for 1:1 complexes of spermine with AMP, ADP and ATP are 2.6, 3.1 and 4.0, respectively.[26]

Early work in the field of building polyazamacrocycles focused on the azacrown ethers being pioneered by Jean-Marie Lehn. Polyprotonated azacrown ethers provided a high charge density about relatively rigid binding sites to which anions were electrostatically attracted. With the goal of intensifying coulombic attraction forces between host and guest species, it was only natural to want to place as many ammonium groups as possible in close vicinity.[17] However, limitations arise when the distance between cationic centers becomes to small.

Lehn's group addressed those limitations with some interesting studies done on a series of three tetraamine macrocycles whose structures vary by the number of methylene groups found between the nitrogens (**Figure 3.5**). The four compounds are [12]-, [16]-, and [20]azacrown-4 (**1**, **2**, **3)**, with 2, 3, and 4 methylene groups between amino nitrogens, respectively. The studies concerned the degree of protonation the polyamines can accommodate at neutral pH.



1 **2** **3**

Figure 3.5 Polyazamacrocycles [12]azacrown-4, [16]azacrown-4, [20]azacrown-4.

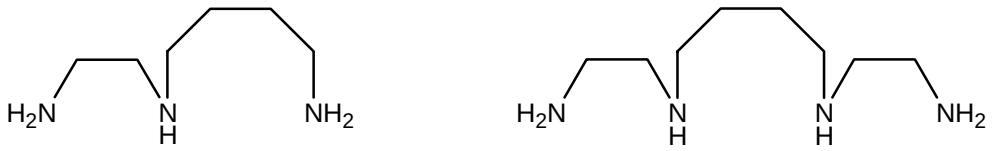
The experiments revealed that at neutral pH, based on the protonation constants for successive protonations, **1** would be diprotonated in a trans-like fashion and **3** would be tetraprotonated, and **2** could slip in and out of di- and tri- protonated states with slight fluctuations in the pH between 6.9 and 7.0.[21]

The proximity of the nitrogen atoms within the ring system can be used to explain the results of the protonation studies. The semi-rigid configuration, and often symmetrical distribution of nitrogen about the ring confers upon the macrocycle some unusual properties versus their acyclic counterparts.

The nitrogen lone pairs of the macrocycle may overlap to bring about higher electron densities within the compound's cavity making the initial protonation much easier (pKa's > 10) than seen with isolated linear amines. This stronger than normal basicity tapers off dramatically in the two smaller macrocycles **1** and **2** after a second proton is added to the cavity making further protonation very difficult.

This lack of desire to pickup more than two protons is likely due to the unfavorable electrostatic conditions established by having two positive charges in a small and relatively rigid cavity.[27] By expanding the cavity and increasing the interatomic distances between neighboring nitrogens (through the addition of extra methylene groups) these unfavorable electrostatic interactions can be overcome allowing for full protonation of amine nitrogens under neutral conditions.

These proton affinity observations are not surprising in light of spermidine and spermine **(Figure 3.6)** which have both three and four carbon atoms between two successive nitrogens and are fully protonated at physiological pH (≈ 7.2) allowing them to interact strongly with polyanionic nucleic acids.[21]



Spermidine **Spermine**

Figure 3.6 Naturally occurring acyclic polyamines spermidine and spermine.

These results have serious implications in the design of polyazamacrocyclic receptors designated for the binding of biological anions under physiological pH conditions. It is clear that ethylene spacers cannot be used to separate nitrogen groups in the macrocycle's framework if one desires a high degree of protonation to occur at near neutral pH. Conversely, it may be desirable to use ethylene spacing to control the degree of protonation while still maintaining a polyazamacrocycle foundation. An example of this can be seen with hexacyclen **(Figure 3.7)**.

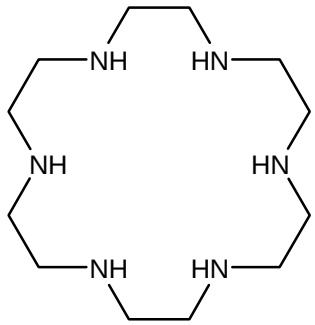


Figure 3.7 Hexacyclen ([18]azacrown-6).

Hexacyclen has been shown to complex a large variety of anions in its tri- and tetra- protonated forms, but it cannot accept more than four protons as the result of its ethylene spacers.

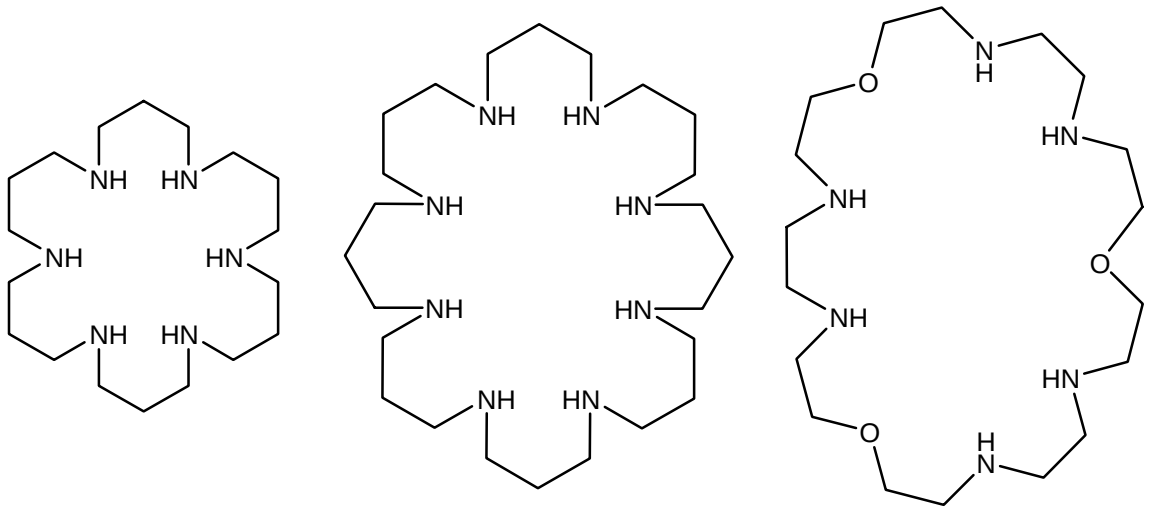
Triprotonated hexacyclen complexes polycarboxylate anions (citrate^{3-}, succinate^{2-}, malonate^{2}-, etc.), while the tetraprotonated macrocycle complexes inorganic anions such as Cl^-, Br^-, NO_3^-, ClO_4^-, and IO_3^-.[21] Tetraprotonated hexacyclen has also been observed to complex with sulfate ions (SO_4^{2-}) and even with two sulfate ions at once.[21]

It almost seems wasteful to have nitrogen atoms around that cannot be protonated as with hexacyclen. So to answer the problem of increasing charge density, the size of the macrocycle has been expanded by simply adding more amine sites. This also meshes nicely with the fact that anions are often larger than their corresponding cations, thus larger receptors are already needed to accommodate their size.

Highly chargeable macrocycles **4-6 (Figure 3.8)** of six or eight nitogens, where the amine functions are separated by 3 to 5 atoms of either carbon or oxygen, are representative examples of these larger ring systems synthesized by Lehn et al.[28] They are among the first polyazamacrocycles designed for the purpose of binding biological phosphates.[22]

Polyazamacrocycles **4-6** were tested with a variety of anions in receptor validation studies (**Table 3.1**). Hexaprotonated receptors **4** and **6** form highly stable 1:1 complexes with dianions such as sulfate, oxalate, malonate, succinate, tartrate, and AMP, (log K_S 2.2-4.7), trianions such as citrate, 1,3,5-benzenetricarboxylate, and ADP, (log K_S 3.3-7.7), and tetraanions like iron hexacyanide and ATP (log K_S 6.3-9.1).[21] Octaprotona-

ted **5** complexes 1:1 with the same anions, but with stronger binding constants. Larger anions are also very strongly complexed by **4**.



4 **5** **6**

Figure 3.8 A series of ring expanded polyazamacrocycles.

Table 3.1 Selected stability constants (log KS (± 0.2)) for Anion Binding by polyazamacrocycles 4-6 in Water.[29]

Anionic Species	**4•6H$^+$**	**5•8H$^+$**	**6•6H$^+$**
Oxalate^{2-}	3.8	3.7	4.7
Sulfate^{2-}	4.0	4.0	4.5
Fumurate^{2-}	2.2	2.9	2.6
Squarate^{2-}	3.2	3.6	3.4
Citrate^{3-}	4.7	7.6	5.8
1,3,5-benzene tricarboxlyate^{3-}	3.5	6.1	3.8
$Co(CN)_6^{3-}$	3.9	6.0	3.3
Adenosine monophosphate (AMP^{2-})	3.4	4.1	4.7
Adenosine diphosphate (ADP^{3-})	6.5	7.5	7.7
Adenosine triphosphate (ATP^{4-})	8.9	8.5	9.1

The binding constants seen with the hexaprotonated receptors **4** and **6** indicate electrostatic forces dominate the host-guest binding interactions as reflected in the increase in stability observed with increasingly negative anions. This is also seen in most cases in the higher binding constants observed with the more highly protonated compound **5** versus the less protonated **4** and **6**.

The importance of electrostatics is solidified with the binding constants for **4**•$6H^+$ with AMP, ADP and ATP (log K_S = 3.4, 6.5 and 8.9, respectively) whose core structures are very similar, but charges differ. Because of these charge differences the highly charged ATP binds more strongly than its lesser charged self and with an unheard of >300K-fold selectivity over AMP!

Structural differences, primarily governed by size, in receptors carrying the same amount of charge (**4** and **6**) provide only slight differences in binding stability when binding the same anion. The largest difference with respect to this element, is seen in the binding constants of adenosine monophosphate with macrocycles **4** and **6** which translates into a 20-fold stronger binding interaction between AMP and **6** versus AMP and **4**.

In a parallel manner, only a moderate dependence of the binding stabilities on anion structures bearing the same charge can be noted. The greatest difference in the stability of any two guests of like charge amounts to a factor of only about 60. This data implies that these simple polyazamacrocycles are incapable of significant guest discrimination.

These early examples of polyazamacrocycles being used to bind anions were expanded upon in innumerable ways with a better understanding of the protonation states

and the factors controlling them. Understanding the bonding interaction would also help in the design of future receptors.

Further expansion of the polyazamacrocycle theme has been seen with the construction of tethered macrocycles[30] where two polyazamacrocycles are connected to each other through some sort of polyether or polymethylene bridge **(Figure 3.9)**

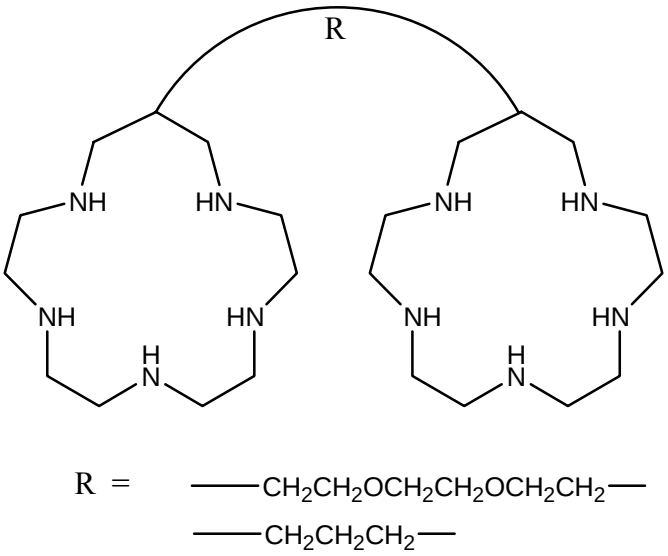


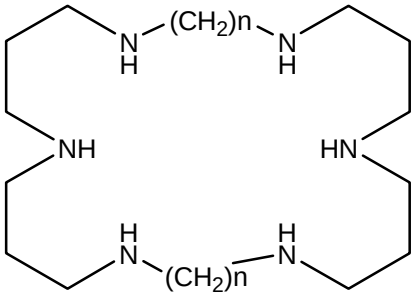
Figure 3.9 Tethered polyazamacrocycle lariat or corrand compound.

These compounds began to incorporate Lehn's concept of linear recognition of molecular length by separating two distinct anion binding domains using a neutral spacer.

Tethered bis-macrocycles like these were able to form complexes with a variety of multiply charged species (citrate^{3-}, AMP^{2-}, ATP^{4-}, HPO_4^{2-}, $Fe(CN)_6^{4-}$, $Fe(CN)_6^{3-}$),[21] however little or no improvement in binding stability was observed over their isolated

macrocycle components, indicating the one-armed receptors cannot muster the preorganization necessary for massive guest selectivity.[17]

Hosseini and Lehn designed the ditopic macrocycles **7** and **8 (Figure 3.10)** to recognize dicarboxylic acids based on both ion pairing interactions and linear recognition of molecular length.[31,32] In neutral aqueous solution these polyazamacrocycles exist in their hexaprotonated cationic forms and can bind to dicarboxylate molecules of the type $^{-}O_2C\text{-}(CH_2)_n\text{-}CO_2^{-}$.[17]



7 **n = 7**
8 **n = 10**

Figure 3.10 Ditopic polyazamacrocycles designed to recognize linear diacids.

Analysis of the stability constants of a series of diacids showed binding was strongest with the intermediate length anions (log $K_S \approx 4.5$) and tapered off with both shorter and longer chain compounds (log K_S 3-4) for a given receptor.[17]

Although achieving maximum binding enhancements of only about 30-fold, these simple macrocycles illustrated that the concept of linear recognition of molecular length was valid in that spacing of the binding sites complementary to the guest topology did

improve complex stability. It is interesting to note that an equivalent size restriction barrier is absent in flexible linear hosts which rely only upon coulombic attraction.[17]

An interesting combination of the polyazamacrocycle core and ferrocene moieties can be seen in receptors **9-12 (Figure 3.11)**.[33] These particular molecules detect the presence of analytes through changes in metal redox potentials upon complexation and find employment in ion-selective electrodes and other electrochemical applications.

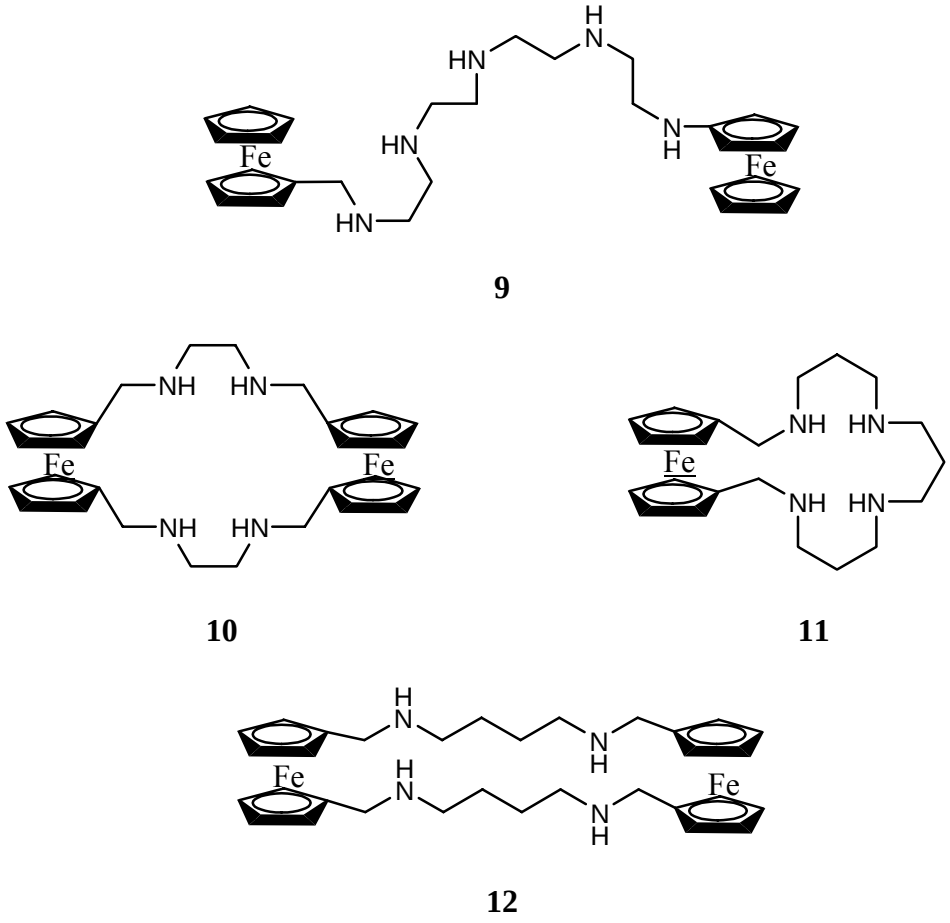
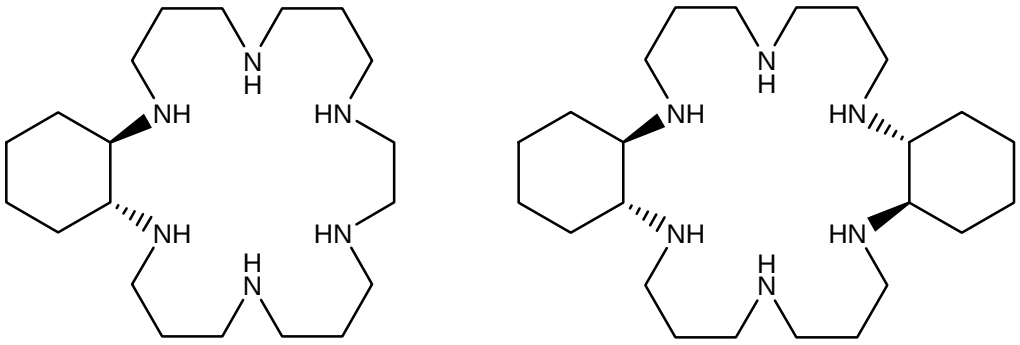


Figure 3.11 A sampling of polyazamacrocycles utilizing ferrocene moeities.

The initial interaction in these receptors comes about through ion pairing and hydrogen bonding between protonated amines and the anion. Electrostatic interaction with the iron function follows oxidation of ferrocene units to ferrocenium to provide additional binding stability.

Receptor **9** selectively binds ATP in the presence of phosphate and nitrate at pH 4.9. ATP is also selected over ADP and AMP in receptor **11** near neutral pH as it carries a higher negative charge.[34] Receptors **10**, **11**, and **12** were also seen to bind sulfate and phosphate and with adjustment of pH 7 could selectively sense phosphate anions in the presence of nitrate, acetate, and chloride.[34]

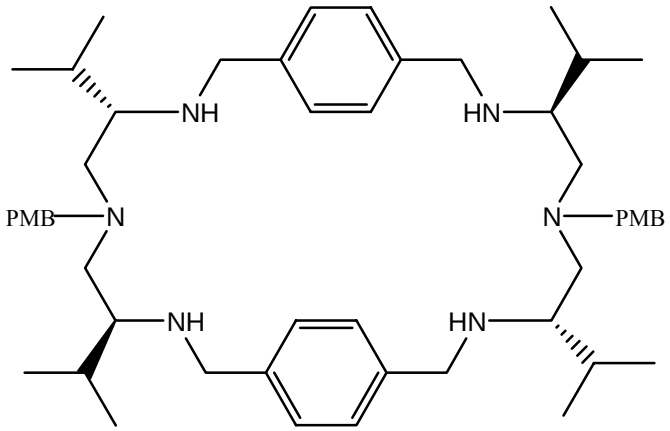
An extension of the polyazamacrocycle theme, with implications in the recognition of biological anions, can be seen with the introduction of stereogenic centers. Optically active hexaazamacrocycles **13** and **14 (Figure 3.12)** were synthesized from an enzymatically prepared starting material and upon protonation bind to chiral anions in aqueous solution.[35] Macrocycle **13** shows moderate D-preference, while **14** binds to N-Ac-D-aspartate more strongly than the corresponding L-isomer.



13 **14**

Figure 3.12 Optically active hexa-azamacrocycles with C2 and D2 symmetry, respectively synthesized from enzymatically prepared starting materials.[22]

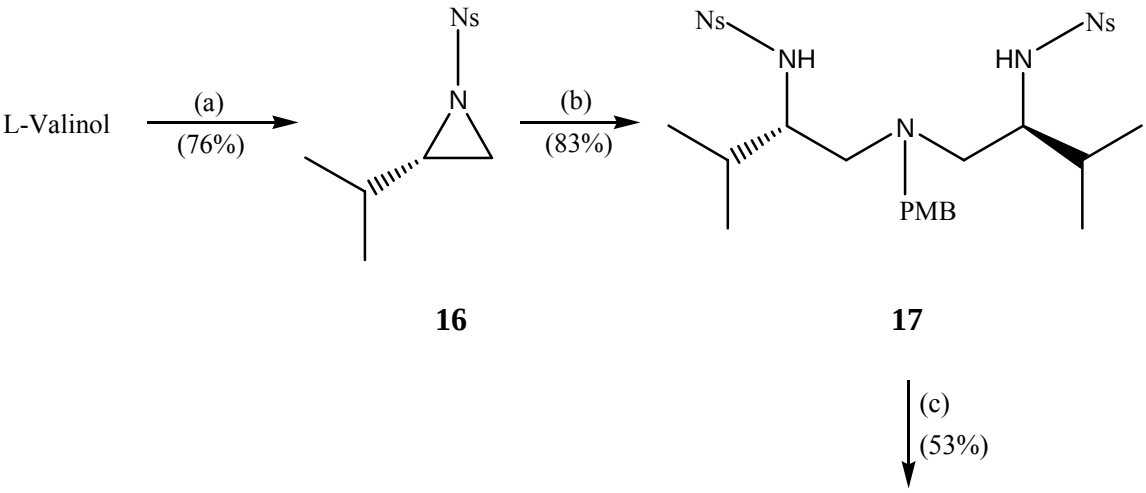
Polyazamacrocycle **15 (Figure 3.13)** containing four chiral carbons was showcased by Kim et al.[36] in a paper describing the development of an efficient, modular approach to such optically active receptors.



15

Figure 3.13 Polyazamacrocycle containing four stereogenic centers.

The receptor was constructed in a three step synthesis beginning with the chiral amino acid L-Valinol **(Scheme 3.1)**. Reaction of the starting amino acid with *p*-nitro-benzenesulfonylchloride in acetonitrile and TEA **(a)** gave nosyl-protected chiral aziridine **16** in 76% yield. Opening the aziridine ring with 0.5 equivalents of *p*-methoxybenzyl-amine **(b)** gave triamine **17** in excellent yield. Cyclization in a 2 + 2 Richman—Atkins-type coupling[37] with α,α'-dibromo-*p*-xylene in dilute acetonitrile followed by protecting group removal using *n*-propanethiol **(c)** generated the final receptor **15** in about 25% overall yield.[36] No anion binding studies information on the chiral polyazamacrocycle was presented.[36]



16 **17**

15

Scheme 3.1

In summary, while there are a few examples of very impressive selectivities with polyazamacrocycles, in general guest discrimination in these class of compounds is quite mediocre. This may be due to the multitude of host-guest binding modes available in these multiply charged, nearly symmetrical receptors with each mode having similar energies. The inherent flexibility of the polyazamacrocycles, allowing them to adapt to guest geometries with little energy cost may also contribute to the lack of selectivity.[17]

Polyazamacrobicycles

In 1968, less than a year after Pedersen's landmark work on cation binding dibenzo[18]crown-6, Park and Simmons really unlocked the door to the idea of encapsulating anions with the synthesis of the first class of purely organic ligands.[38] These hosts, which predated Lehn's work on cation receptors, were capable of binding one or two halide ions **(Figure 3.14)**. [21]

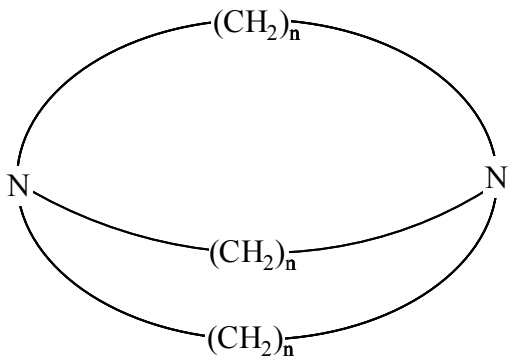


Figure 3.14 Macrobicycles by Park and Simmons, recognized as the first class of purely organic ligands.

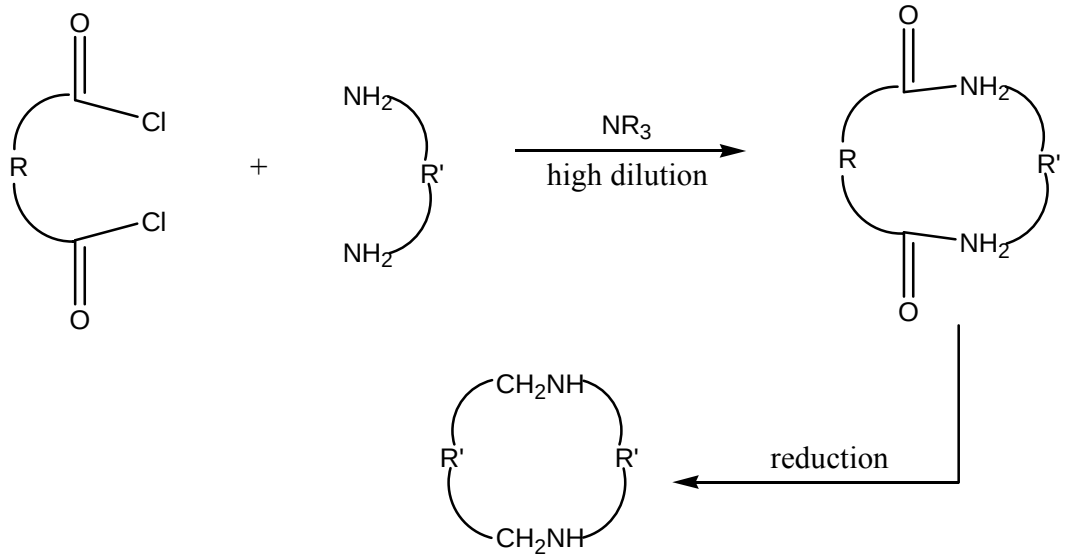
The convenient preparation of these compounds followed the work of Stetter[39,40] who showed that azamacrocycles could be synthesized by condensing an open chain diamine with an open chain diacid chloride under dilute conditions **(Scheme 3.2)**.

The dilute conditions were required to favor the formation of the cyclized product over a distribution of polymers. The initial amide formed in the reaction could be readily reduced to the amine using either lithium aluminum hydride ($LiAlH_4$), or borane/THF.

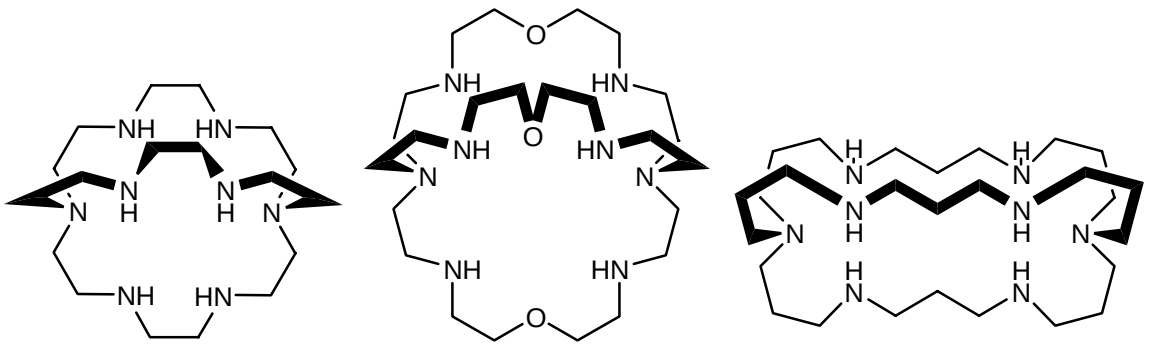
Shortly after the publication of Park and Simmons' pioneering organic ligand, Lehn's people churned out some work on the synthesis of cryptands **(Figure 3.15)**.[41-44]

Initially cryptands were designed to complex cations, but they were readily adapted to host anions. They showed better binding of anions over their macrocyclic counterparts due to the extra dimensional caging effect which allowed for a greater

degree of protonation. This added dimension to the polyazamacrocycle genre christened a new class of compounds known as polyazamacrobicycles.



Scheme 3.2



18 **19** **20**

Figure 3.15 A representative sampling of Lehn's cryptands.

Compound **18** binds fluoride with remarkable strength (log K_S = 11.2) and selectivity (ca, 10^8) over chloride ions. X-ray analysis of the hexafluoride salt confirmed a 1:1 complexation stoichiometry with fluoride encapsulated inside the cryptand.[22]

Compounds **19** and **20** were found to give reasonably stable complexes with a variety of well solvated anions in somewhat acidic aqueous solution **(Table 3.2)**.[45-48]

Table 3.2 Host-Guest Association Constants (log KS) of Anions with Bicyclic Polyazamacrocycles 19 and 20.[17]

Anionic Species	**19•6H$^+$**	**20•6H$^+$**
F^-	4.10	
Cl^-	3.00	1.70
Br^-	2.60	2.20
I^-	2.15	2.40
N_3^-	4.30	
Sulfate^{2-}	4.90	4.20
Oxalate^{2-}	4.95	4.50
Malonate^{2-}	3.10	2.85
Adenosine monophosphate (AMP^{2-})	3.85	
Adenosine triphosphate (ATP^{4-})	8.00	

A comparison of the binding constants of the oxalate and malonate anions, which differ in structure by only one carbon, indicates that strong binding is based on an inclusion process. Inclusion requires that an appropriately sized guest slip into the molecular cavity of a host structure expanded by electrostatic repulsion of the positively charged nitrogens.[17]

X-ray crystal structures confirmed that the guest is fixed by an oriented set of hydrogen bonds and the ellipsoid shape of the binding cavity seemingly compliments the

linear shape and charge distribution of the azide anion over the spherical shaped halides as reflected in their respective binding constants.[17]

Polyazamacrotricycles

Lehn's progress toward stronger binding anion receptors based on protonable nitrogens in a macropolycyclic system culminated in the aesthetically pleasing macrotricyclic azacrown ether **(Figure 3.16)**.[49,50]

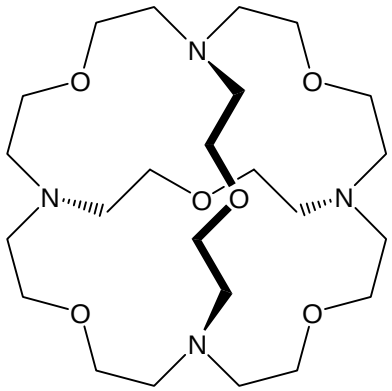


Figure 3.16 Jean-Marie Lehn's macrotricyclic azacrown ether.

The compound can be described as two interwoven polyhedra: a tetrahedron of four nitrogen atoms placed concentrially to an octahedron of oxygen sites.[17] A maximum of four protonated ammonium sites are possible and in the presence of chloride ions an array of four hydrogen bonds converges on the central cavity to hold the anion in place.

Fluoride and bromide, but not iodide, nor any of the polyatomic anions can be encapsulated by the receptor.

Notably, it was also found to bind the tetrahedral ammonium cation (NH_4^+) in its unprotonated state, and water, making it capable of binding with anionic, cationic and neutral species. The combination of a unique geometry and orientation of binding sites leads to high association constants and excellent selectivity, especially the 1000-fold preference of chloride over bromide ions.[17]

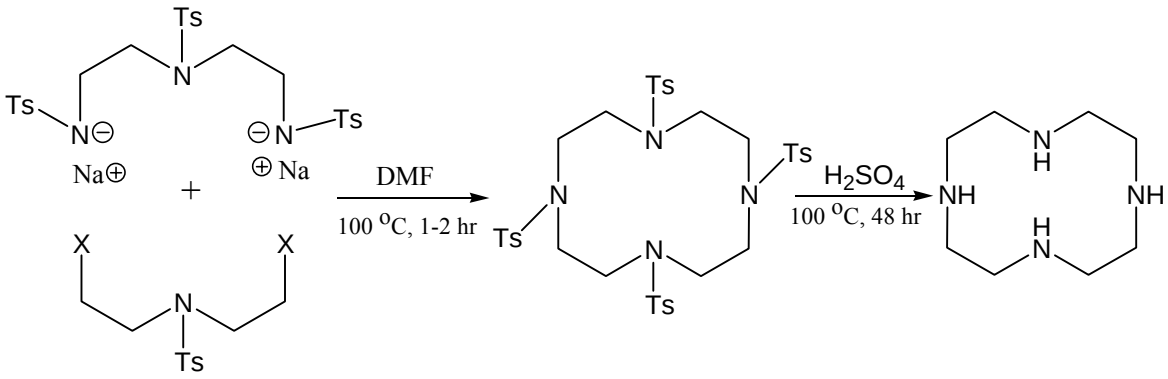
Molecular mechanics and molecular dynamics calculations[51,52] revealed a potential applications shortcoming with anion receptors modeled after the Lehn design. When chloride or bromide anions are brought from infinity toward the protonated receptor they reach a local energy minimum at a configuration placing the ion just on the outside of one tetrahedral face of the receptor. Crossing from the outside of the receptor to the interior cavity, where a lower energy minimum was found required overcoming a substantial barrier of 31.8 and 49.8 kJ/mol, respectively. In applications, such as catalysis, where the dynamic exchange of guests is desired such an energy barrier may be detrimental to receptor function.[17]

The focus of this review on polyazamacrocycles has been on their use in binding anionic substrates containing upwards of six identical anionic functional groups, however it should be mentioned that some receptors have been designed for binding di-functional anions (such as a carboxylate and a phosphate group at opposite ends of the molecule), or zwitterionic compounds possessing anionic and cationic groups (such as amino acids).[17]

Polyazamacrocycle Synthesis

The reaction of amines or sulfonamides with strong electrophiles such as halogeno compounds, acid chlorides or tosylates has been the mainstay of polyazamacro-

cycle formation chemistry.[37] Following cyclization, further manipulation such as detosylation or reduction of the appropriate bond (C=O, or N—S) maybe required to give the receptor in its final form **(Scheme 3.3)**.[37]



X = OTs, OMs, Cl, Br, I

Scheme 3.3

When a spacer linking a set of binding elements together is needed, dipode capping is used rather than the simple dipode coupling shown in Scheme 3.3 **(Figure 3.17)**.[30,53] In an interesting synthesis of a lariat type polyazamacrocycle, Bradshaw *et al*.[54,55] condensed both ends of a bis-α-chloroamide with a primary amine, followed by reduction of the carbonyls using either BH_3/THF **(Scheme 3.4)**.

These coupling and capping strategies also can be applied to macrobicycles. Macrobicyclic octaazacryptand **18 (Scheme 3.5)**[56] was made via tripod capping bicyclization using paraformaldehyde, followed by sodium borohydride reduction.

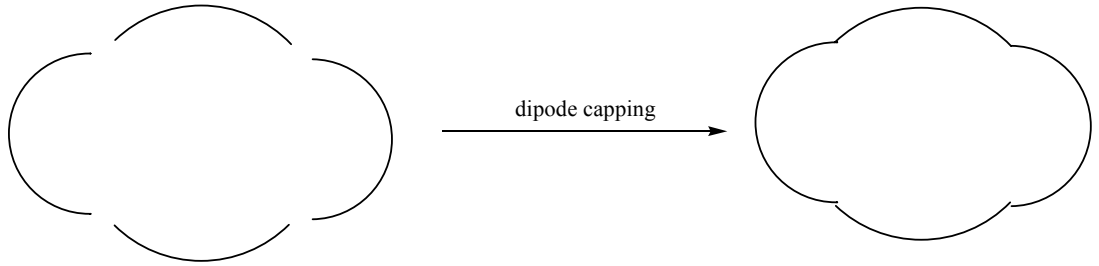
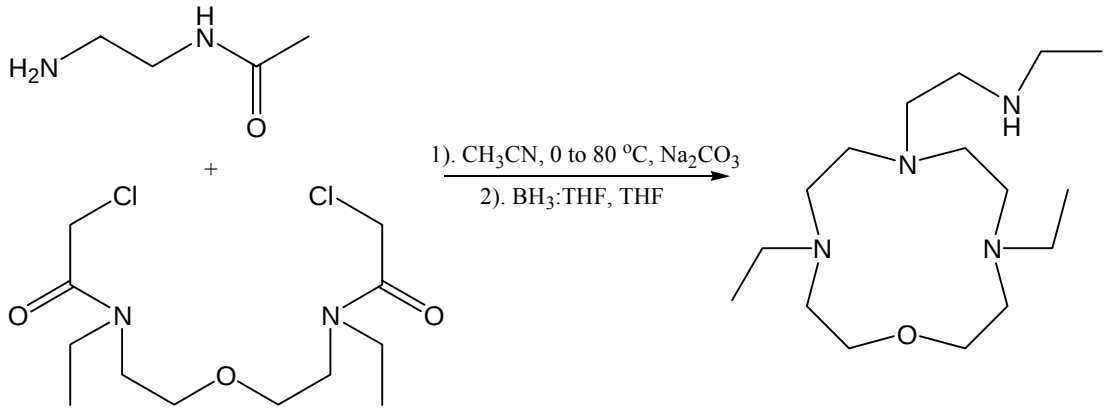
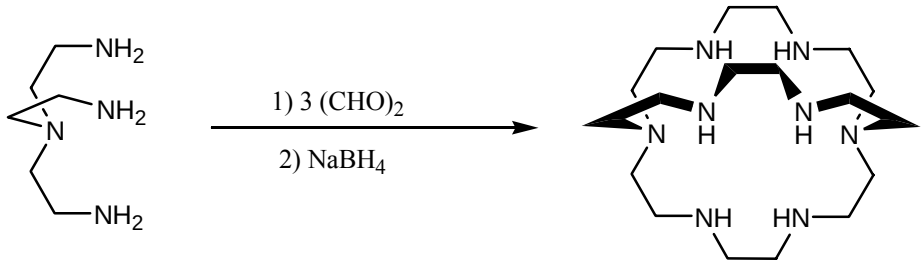


Figure 3.17 Schematic of dipode capping leading to spacer separated binding sites.



Scheme 3.4



Scheme 3.5 **18**

As simple as these reactions seem on paper, they are usually more difficult to carry out than it appears. The presence of multiple compounds containing reactive end groups is a recipe for polymerization, which competes with the cyclization process. This can be especially problematic in the case of macrobicylizations where the reaction time requirements are greater leaving more opportunity for polycondensations.[22] Another issue that may arise in the formation of especially complex or chiral receptors is the possibility of multiple up-down type isomers, or stereoisomers in the product mixture.

There are a number of techniques available to combat these problems such as high dilution, the use of efficient protecting and leaving groups, the use of compounds that can act as templates and the use of suitable reaction solvents.[22]

The idea of using high dilution reaction conditions was first applied by Ruggli[57-59] to the formation of polyamides in 1912. The basis of this technique lies in the kinetics of the two competing reactions—cyclization versus polymerization. The cyclization process follows first order kinetics giving a rate scheme where the reaction rate is directly proportional to the concentration. Because polymerization is a bimolecular reaction it is a second order process and the rate is proportional to the concentrations squared. Given that the rate constant for both processes is the same, concentrations >1 M will favor polymerization, while concentrations <1 M will favor cyclization (squaring decimals decreases the concentration factor).

Converting amino groups to their toluenesulfonamide derivatives has become the preferred method of enhancing the nucleophilicity of terminal amines and protecting against multiple alkylations.[22] In addition to this duality, many times the tosylamides transform the parent amine from liquids into solids thereby making purification by

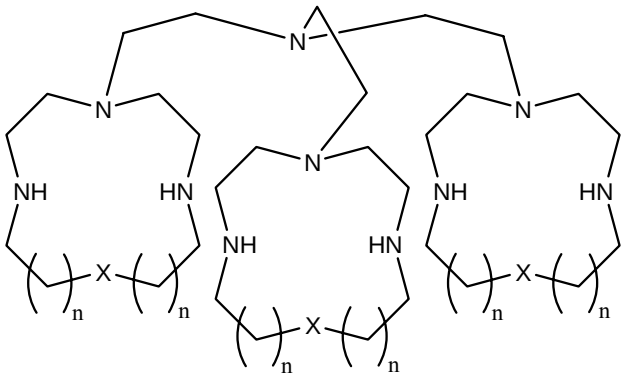
recrystallization a nice option. A popular method of deprotection requires refluxing in concentrated acid, frequently HBr/HOAc, in the presence of phenol.[60] Recently a new activating/protecting group has been used, diethoxyphosphoryl, which can removed under milder conditions—stirring in HCl(g) saturated 1,4-dioxane at room temperature for 12-24 hours.[61]

Halides provide the most obvious and ubiquitous leaving groups for polyazamacrocycle synthesis, however tosylates have become the most popular due to their high reactivity and ease of making.[62] Mesylates are also an option, and although they have often turned out to be too hot of a leaving group, they do find work in macrobicyclic construction. Again, another advantage of tosylates over halides is that they are often easier to purify solid compounds.

The most popular solvents used in polyazamacrocycle synthesis are DMF and acetonitrile which offer high polarity and reasonable boiling points helpful in product isolation.[22]

A series of three new tren-based (tren = tris(aminoethyl)-amine) tris macrocycles were reported by Bazzicalupi[63] and co-workers in a late 2002 JOC Note. The tritopic dendrimer-like macrocycles **21**, **22** and **23 (Figure 3.18)** were designed to carry multiple positive charges at physiological pH and provide a symmetrical bowl- shaped cavity in hopes of binding large inorganic substrates or anionic assemblies inside.[63]

The receptors were put together using the Richman-Atkins[37] approach from precursor **25** obtained as the hexasodium salt of N-tosylated aziridine/tren reaction product **24 (Scheme 3.7)**.



21 (n = 1, X = N) **22** (n = 2, X = O) **23** (n = 3, X = N)

Figure 3.18 A series of three tren-based tris lariat-type macrocycles.

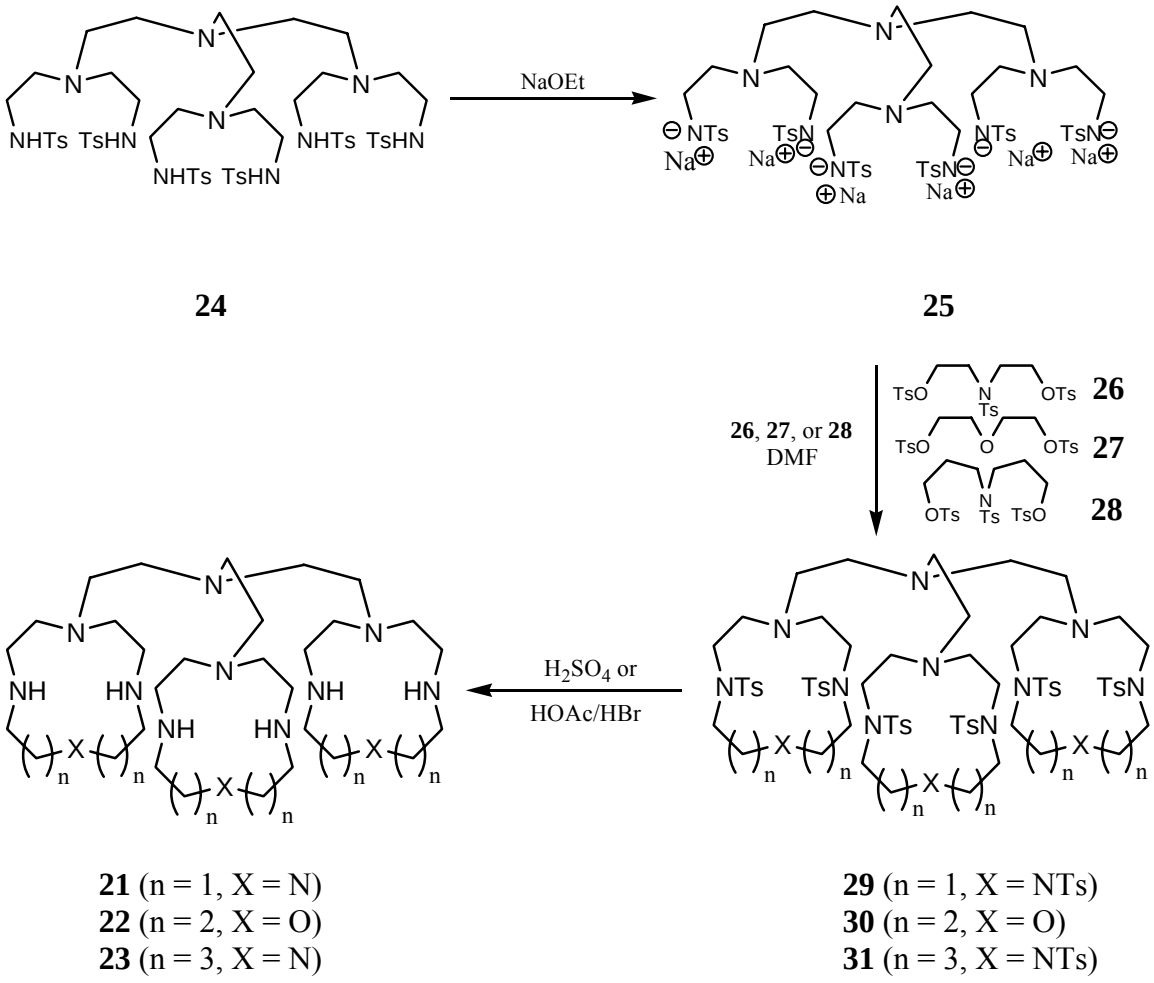
In the Richman-Atkins macrocyclization, nucleophilically enhanced tosylamides **25** reacted with tosylated diethanolamine **26**, diethylene glycol **27**, or dipropanolamine **28** in a 1:3 molar ratio in 110 ^{o}C DMF to afford tosylated macrocycles **29**, **30** and **31**. Acid removal of tosyl groups gave the receptors **21**, **22** and **23** in 15-20% yields.

The hydrochloride salt of receptor **21** was seen to form a complex crystal structure in the presence of NaClO4, namely $[(Na(ClO_4)_6) \cap L1_2H_{13}]Na_6Cl_2(ClO_4)_{12}$ where two receptors form a cage around the anion cluster $(Na(ClO_4)_6)^{5-}$ encapsulating it in a network of electrostatic and hydrogen bonds.[63]

Polyazacyclophanes

Frequently in natural systems, the guests for which receptors are designed, consist of a hydrophilic anionic region connected to a hydrophobic aromatic region, as is the case with nucleotides. In order to strongly and selectively bind these amphipathic-type

biological molecules, building a receptor able to cater to both of those features is required. For these guests, protonated cyclophanes have proven to be the host of choice.



Scheme 3.7

The first of these compounds was described by Koga[64] **(Figure 3.19)** in 1980. Koga's cyclophane formed well defined inclusion complexes with a number of aromatic guests in aqueous solution.

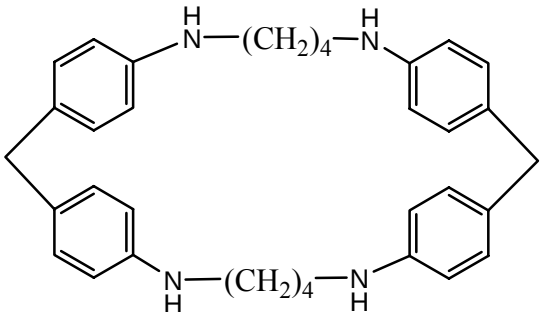
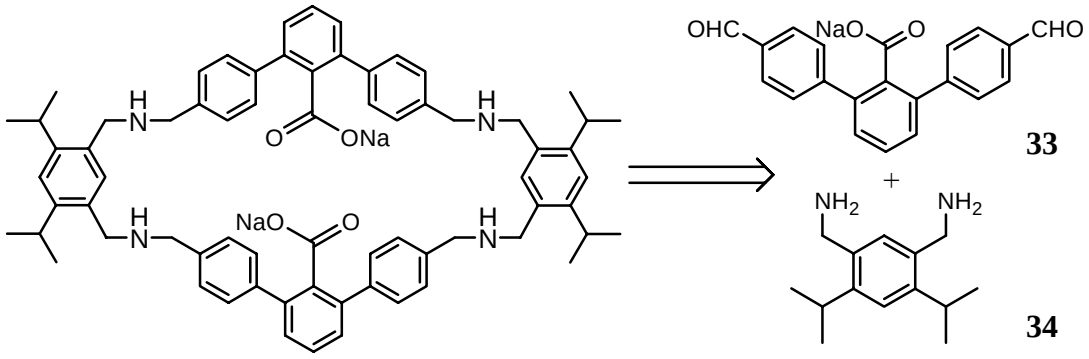


Figure 3.19 Koga's cyclophane, c. 1980.

Cyclophane chemistry was pushed into the limelight following these pioneering works and the literature is filled with descendents from Koga's line. Intercalation driven by hydrophobic interactions between aromatic hosts and guests seem to dominate the binding event of these systems with the electrostatic forces taking a back seat. Even guests lacking a charge, but possessing the same aromatic traits as their charged brethren (such as the nucleosides) could be complexed by cyclophanes, thus reducing binding selectivity. In many cases the presence of an anionic moiety only served to provide water solubility to the guest. As expected, when the anionic moiety was attached to an aliphatic residue rather than an aromatic one, weaker complexes were observed with cyclophane receptors.[65]

A cyclophane tetraazamacrocycle **32 (Figure 3.20)** containing two carboxylic acid groups oriented *endo* within the cavity was recently reported.[66] Retrosynthetic analysis of compound **32** indicated it could be assembled using Schiff base forming chemistry in a reductive amination process between dialdehyde **33** and diamine **34**.



32

Figure 3.20 Retrosynthetic analysis of carboxylic acid containing cyclophane 32.

This synthesis technique has been employed by several research groups and has often been shown to give better yields of more easily isolated product than other macrocyclization methods.[67-69] By using slow addition (10 mL/hr) of both the diamine and dialdehyde via syringe pump under dilute conditions, yields of 70% were achieved. Conversion of the imines to amines is a straightforward reduction process using $NaBH(OAc)_3$ and product isolation required nothing more than filtration.[70]

The authors envisioned macrocycle **32** to be hosts for transition metal ions, and although not specifically mentioned in the paper, receptors such as this containing both carboxylate and ammonium binding elements at physiological pH may be useful in hosting zwitterions given complementary topologies.

Cyclophane chemists, following the polyazamacrobicycle lead, began adding another ring system to give bicyclic polyazacyclophanes. Bridging 1,3,5-trisubstituted benzenes with amine containing carbon chains is an example of this extension. Hexaazacyclophane **(Figure 3.21)** was designed for complexing inorganic anions like

nitrate, chloride, or sulfate.[71,72] Strong binding of these substrates was observed with log K_S values ranging from 2.5 (monovalent) to 6.0 (divalent).

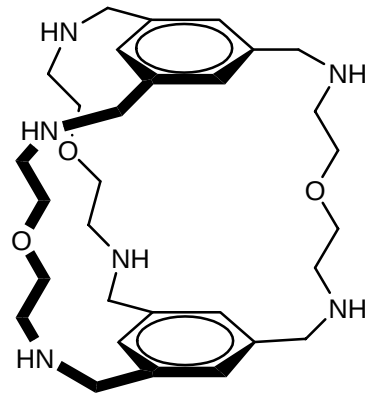
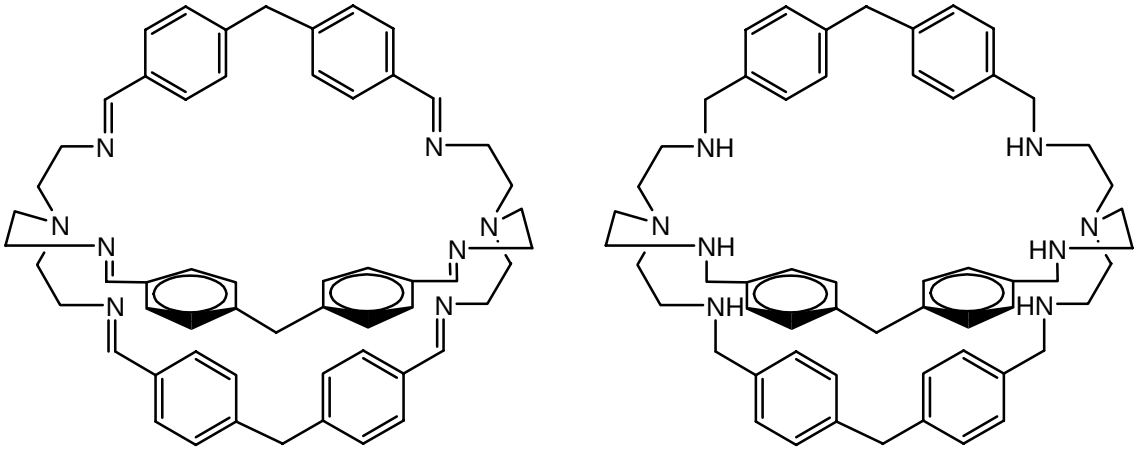


Figure 3.21 Macrobicyclic hexaazacyclophane.

Polyazacyclophane macrobicyclic compounds are also readily synthesized from aromatic aldehydes and aliphatic amines **(Figure 3.22)**. Reduction of the intermediate imine **35** gives the final macrocycle **36**.[73-78] The hexaprotonated species of **36** binds many dicarboxylates, such as the terephthalate anion, with log K_S from 3.15-4.4.[79-82] Compounds of this type also show strong, but size dependent complex formation with oxyanions and with nucleotides (log K_S 4-5).

The number of structural variants built upon the polyazamacrocycle theme seems unlimited when considering the topography options provided by macrocyclic, macrobicylcic and macrotricyclic frameworks in combination with spacer options such as the cyclophanes.

A class of compounds known as azaparacyclophanes **(Figure 3.23)** rounds out this review of receptors bearing cationic ammonium binding motifs. These rather esoteric molecules have been seen to complex anionic fluorescent probes [83,84]



35 **36**

Figure 3.22 Polyazacyclophane macrobicyclic imine **35** and corresponding amine **36.**

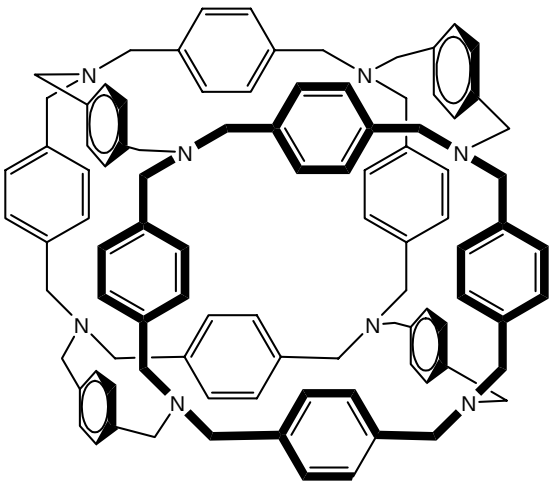


Figure 3.23 An azaparacyclophane having an interesting cubic shape.

A great number of open-chain cyclophane analogues (*seco*-cyclophanes) have also been synthesized, but in general their binding affinities fall short of their cyclic congeners in the binding of aromatic anions.[85-87]

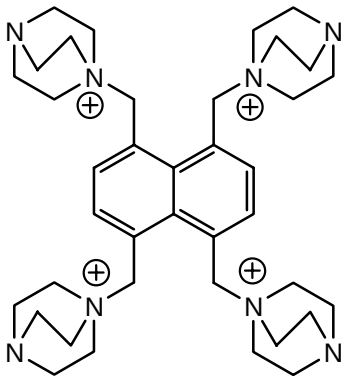
The Quaternized Nitrogen

Achieving a highly protonated state on polyazamacrocycles in aqueous solution requires careful consideration of the spacing between binding elements adding an extra level of complexity to their design. In addition, polyazamacrocycles often require quite acidic conditions to reach their fully protonated state. Such a requirement could undermine the study of any relatively basic anions, since under acidic conditions they would revert to neutrality and lose the fundamental characteristic of being negatively charged. Switching away from an aqueous solvent could be an issue as the pK_a values of the receptor may shift, thus potentially effecting protonation patterns, total charge, and the ability to complex anions.[17]

As a possible solution to these issues, the quaternization of nitrogen atoms, resulting in proton free positive centers still capable of ion pairing interactions with guest species, has been incorporated into receptor design. One unknown factor concerning this solution was whether or not ion pairing would suffice in the absence of hydrogen bonding as seen in polyazamacrocycles.

Naphthalene cores derivatized with four diazabicyclooctane (DABCO) units make up a set of acyclic anion receptors bearing the quaternized nitrogen motif **(Figure 3.24)**.[88] DABCO is often used as a membrane transfer agent and such receptors may find work in

membrane transport. Receptor **38** was engineered to contain four additional methyl groups versus receptor **37** in a steric hinderance study.



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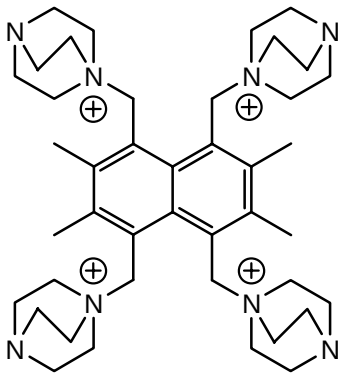
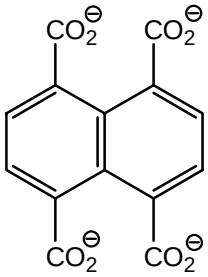


38

Figure 3.24 Acyclic receptors containing quaternized nitrogen diazabicyclooctane (DABCO) binding elements attached to a naphthalene core.

Both receptors were tested against the aromatic tetraanionic carboxylate guests **39** and **40 (Figure 3.25)**. By using naphthalene-1,4,5,8-tetracarboxylate **39** as a potential guest, we see an interesting test case of self-recognition in that the receptors are tested against the anion upon which their core structures are based. These receptors exemplify a rare breed of host molecules that incorporate part of the guest they are designed to bind in their own molecular architecture. Both **37** and **38** bound the naphthalene tetracarboxylate with log K_S = 2.59 and 2.14, respectively. When tested on the benzene tetracarboxylate **40** receptors **38** and **39** bound with log K_S = 2.79 and 2.66, respectively.



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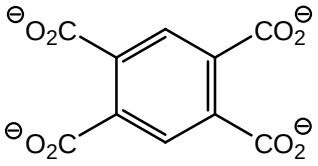


40

Figure 3.25 Tetracarboxylate guests (naphthalene-1,4,5,8-tetracarboxylate **39** and benzene-1,2,4,6-tetracarboxylate **40**) for quaternized nitrogen receptors **37** and **38**.

Two things can be observed from these numbers. One is that the tetramethylated naphthalene core of **38** may interfere with the receptor's binding elements making it bind less strongly to both cations tested. Another observation, even though cation **39** seems to be a better match for the receptors constructed on its platform, the benzene tetracation **40** actually complexed more strongly with both **37** and **38**, although at most only by a factor of ≈ 3.3. The authors speculate the reason for the better binding of **40** over **39** may be that **39** must align perfectly to fit the receptor whereas the smaller benzene tetracation has more binding flexibility in that respect.[88]

An early example of a macrocyclic anion receptor utilizing the quaternized nitrogen binding motif is seen in the polyazacyclophane synthesized by Tabushi **(Figure 3.26)**. This receptor was found to have a catalytic effect on the hydrolysis of suitable ester substrates.[89,90]

The quaternized nitrogen binding element arrangement seen in DABCO also found its way into a highly charged macrocyclic receptor **(Figure 3.27)** with the hope it could strongly bind to ATP.[91-93]

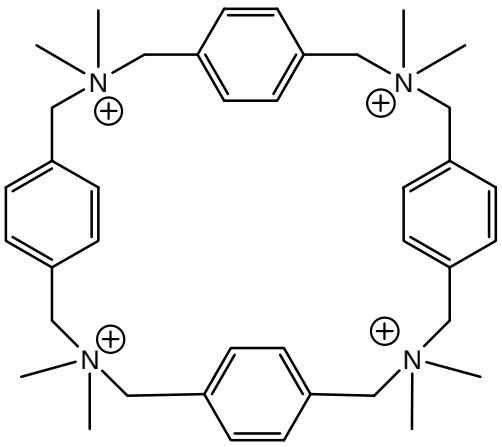


Figure 3.26 An early example of a macrocycle receptor having quaternized nitrogen binding elements.

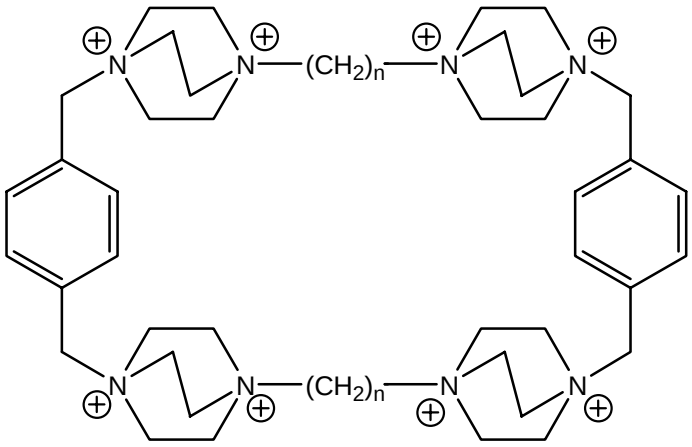
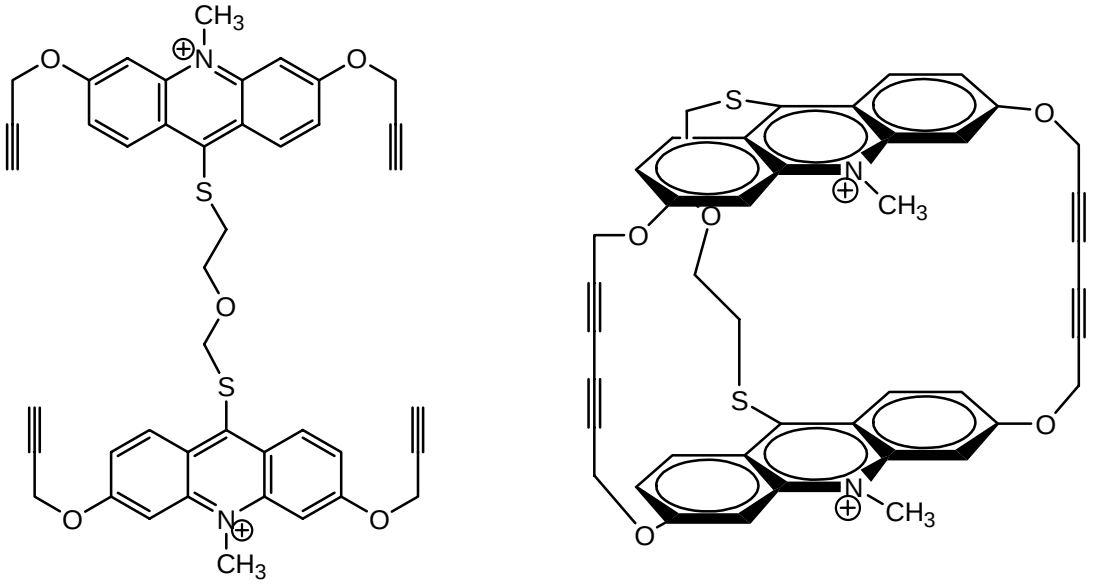


Figure 3.27 Highly charged macrocycle containing quaternized DABCO nitrogens.

Many such highly charged receptors were found to bind their guests not inside their cavities as planned, but rather outside the encapsulation space. This serves to illustrate that the simple accumulation of positive charges about a well-defined cavity is not sufficient to bring about strong associations. Often the attractive electrostatic forces

offered by encapsulation fail to match the energetic costs of desolvation required to get into the capsule.[17]

A shining example of quaternization in a bicyclic system was accomplished with the intramolecular Glaser coupling of **41** to give the acridinium cyclophane **42 (Figure 3.28)** which complexed various planar aromatic carboxylates and nucleotides in water with log K_S ranging from 4 to 7.[94-96]



41 **42**

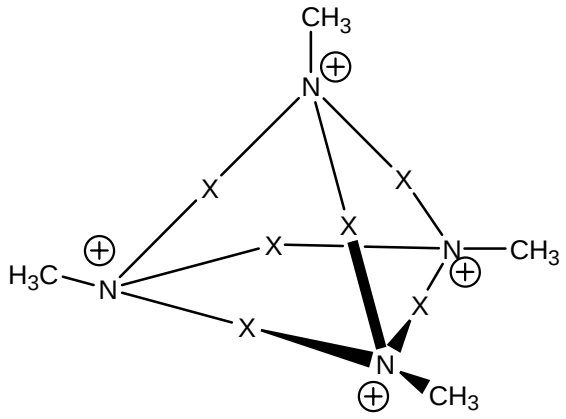
Figure 3.28 Glaser coupling precursor 41 and macrobicyclic acridinium cyclophane receptor 42 utilizing quaternized nitrogen binding elements.

Surprisingly, flexible compound **41** was found to be a better nucleotide host than the more rigid cyclophane **42**. This suggests that the substrates don't quite fit the

preorganized acridinium cyclophane and its lack of flexibility doesn't allow for optimum aromatic interactions.

Not surprisingly, many of the quaternized nitrogen compounds were synthesized effectively by alkylating the amine groups found on known polyazamacrocycles.

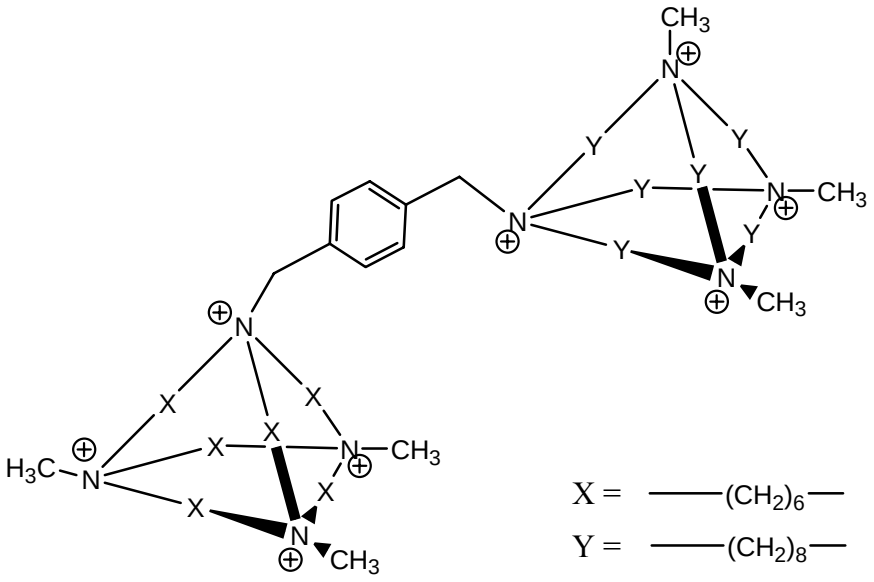
An example of this is seen in the quaternized macrotricyclic ammonium compounds **43** and **44**[97-99] **(Figure 3.29)** based on Lehn's *coup de grâce* **(Figure 3.16)**, which proved to be hosts for a broad variety of anions in water. X-ray crystal structures of **43** with iodide show that binding occurred via inclusion complexation, and the size of the cavities successfully excluded anions too large to fit inside.



43 X = $-(CH_2)_6-$
44 X = $-(CH_2)_8-$

Figure 3.29 Macrotricyclic receptors incorporating quaternized nitrogens.

Revisiting the tethering theme the ditopic macrotricyclic **45 (Figure 3.30)** was found to complex a series of dianionic dimensional probes, with a 3-fold increase in the binding stability relative to its isolated macrotricyclic components.[100,101]



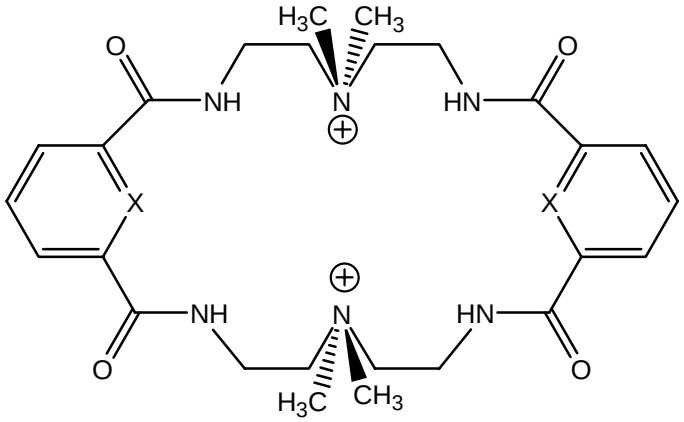
45

Figure 3.30 Tethered ditopic macrotricycle with quaternized nitrogens.

An interesting set of quaternized macrocycles coupled with amide linkages were synthesized by Hossain and co-workers earlier this year.[102] These receptors **46** and **47** **(Figure 3.31)** stand out because they combine two previously uncombined binding motifs—a positively charged quaternized amine and a group of neutral amides (a motif discussed later in the chapter)—and thus may qualify as a new class of macrocyclic anion receptors.[102] According to the authors, although a few such mixed amide/quaternary amine systems have been reported, they have not been explored as anion receptors.

X-ray crystal structure analysis of the dichloride salt of the phenyl containing **46** revealed an elongated elliptical macrocycle with quaternized amines at the far ends (unlike the structure as drawn in **(Figure 3.31)**), 12.744 Å apart from each other to minimize electrostatic repulsion. The aromatic spacers are seen in a stacking

conformation approximately 3.2 Å apart. Amide carbonyls are oriented toward the central cavity with amide protons aligned exterior to the cavity hydrogen bonded to chloride counterions located exogenous to the ring.[102]



46 X = CH
47 X = N

Figure 3.31 Hybrid macrocycles incorporating quaternized nitrogen and hydrogen-bond donating amide binding elements.

Replacement of position X with a nitrogen atom to give the pyridine spacer seen with **47** has a drastic effect on the receptors conformation. The X-ray crystal structure data show that the aromatic pyridines are stacked as the benzenes were about the same distance apart, however the amide protons point toward the pyridine nitrogens causing the macrocycle to fold into a U or V shaped molecule. It is postulated the macrocycle gains stability by amide hydrogen interaction with pyridine lone pair electrons. The two iodine counterions, unlike the chloride ions in **46**, are bound within a cleft formed by two

amides and the pyridine nitrogen. This chelate effect may be responsible for the increased affinity seen with **47** over **46** for the same series of anions **(Table 3.3)**.[102]

Table 3.3 Host-guest association constants (log K_S) of anions with mixed amide-quaternized amine receptors 47 and 48.[102]

Anionic Species	**47**	**48**
F^-	2.68	2.04
Cl^-	3.23	4.75
Br^-	2.14	4.38
I^-	2.00	2.21
$H_2PO_4^-$	4.06	5.32
HSO_4^-	n/a	3.90
NO_3^-	1.65	2.32
ClO_4^-	1.60	2.40

Oligopyrrolic Porphyrins and Sapphyrins.

There are some porphyrin containing compounds such as a porphyrin trimer (**Figure 3.32**) that have been shown to bind certain anions and even transport them across membranes.[21]

The ability of metalloporphyrins to bind anions is totally dependent on the presence of open sites available for ligation on the metal ion complexed by pyrrolic nitrogens in the central cavity. Non-metallated porphyrins do not have anion binding power due to the small size of their cavity and the inability of the two cramped N—H moieties to form stable hydrogen bonds with potential guests.

A reasonable remedy to this issue of cavity size is to expand it by incorporating more pyrrolic or other spacer units into the molecular framework. Compounds called sapphyrins seemed to fit the bill, and although known for more than 35 years, it took

considerable work to make the synthesis of these compounds accessible and to provide enough material to conduct binding studies.[21]

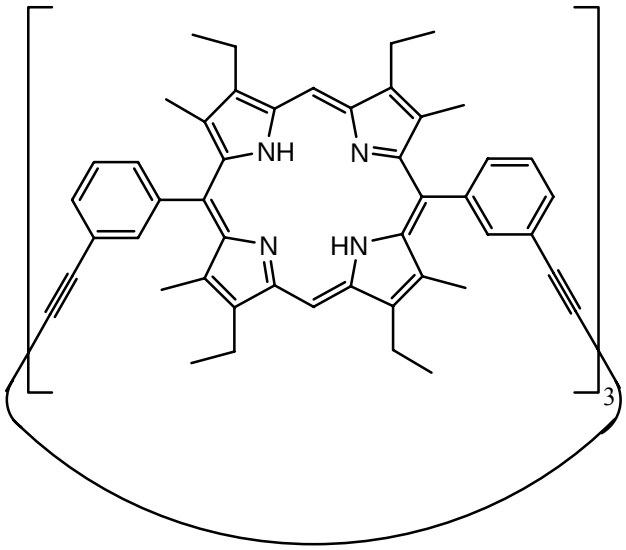


Figure 3.32 Porphyrin trimer connected by phenyl alkyne bridges.

Sessler *et al.* did just that when they prepared a large number of expanded porphyrins **(Figure 3.33)**.[103-106] The salient feature of sapphyrins is their planar pentapyrrolic skeleton of aromatic character that orientates three N—H bonds, with their positive ends, toward the center of a 5.5 Å diameter cavity. Another two protons can be added (pK_{a1} = 3.5, pK_{a2} = 9.5) allowing all the N—H loci to provide hydrogen binding and present an ideal environment for anion binding.

Sessler found that the diprotonated sapphyrin shown formed a very stable complex with fluoride ions in methanol (K_S = 5). In addition to the strong binding of

fluoride, a selectivity factor of more than 100-fold was observed over the heavier halides chloride and bromide.[107]

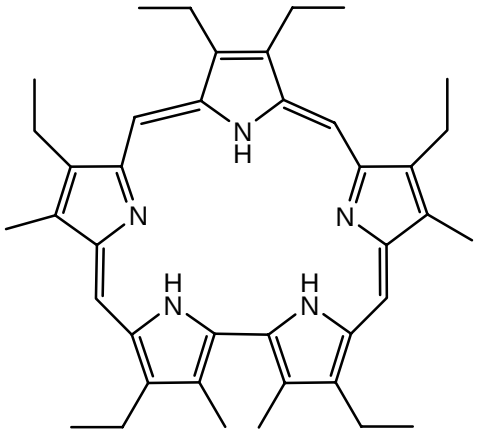


Figure 3.33 An expanded porphyrin belonging to a class of pentapyrrolic compounds called sapphyrins.

Sapphyrins have found use as carrier molecules, affinity chromatography resins, and binders of single- or double-stranded DNA.[17]

Porphyrin's have been linked to other metallo-anion binding motifs such as cobaltocenium **(Figure 3.34,** fourth *meso*-position amide linkage left out for clarity**)**. This compound provides an example of one of the more unusual anion receptors built using the porphyrin ring system as a scaffold.[108] It was constructed through amide bond formation between the all-cis atropisomer of tetrakis(*o*-aminophenyl)porphyrin and cobaltocenium moieties. The resulting tetracation was observed to complex with a variety of anions in acetonitrile, however with log K_S values of less than three and a selectivity factor of about four.

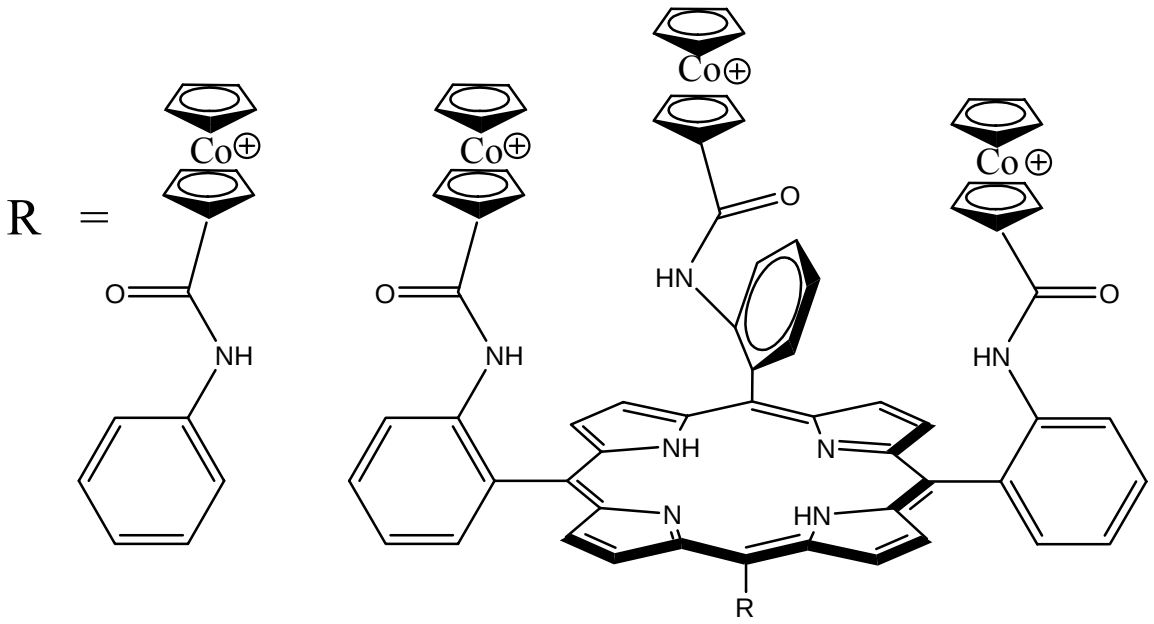


Figure 3.34 Cobaltocenium binding moieties amide linked to a porphyrin scaffold.

Guanidinium.

Another nitrogen containing group useful in the construction of positively charged anion receptors is the guanidinium ion **(Figure 3.35)**. The core structure of the guanidinium/guanine moeity is an sp^2 hybridized carbon center surrounded by three amino groups. It is a feature prevalent in the biochemical building blocks of life. The guanidinium group is found in the amino acid arginine, and therefore makes its way into proteins, enzymes, and other cellular polypeptides. Guanine is a purine base found in the nucleic acids DNA and RNA, and in the nucleotides GMP, GDP, and GTP (of G-protein linked receptor fame).

Being so highly represented in biological systems, it is only natural to extend the guanidinium group to the field of molecular recognition and host-guest chemistry,

especially for the hosting of cellular guests. Indeed, this has been the case as guanidinium-based receptors appear to be more geared toward biological substrates when compared to the polyazamacrocycles.

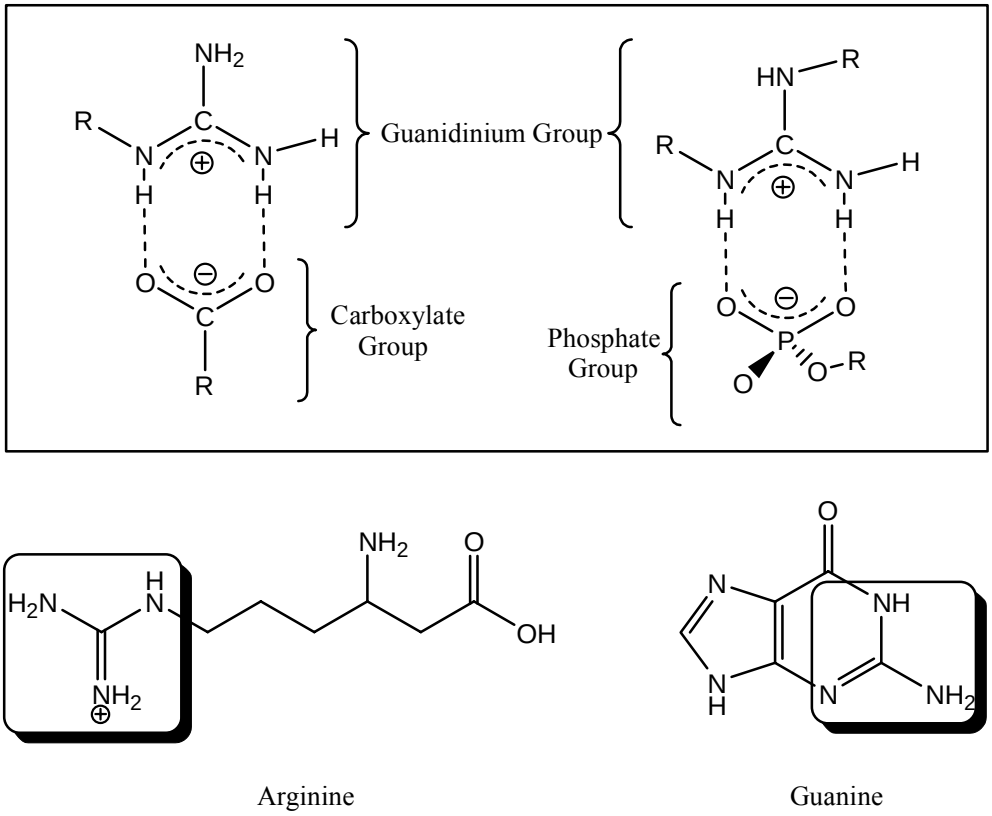


Figure 3.35 Binding pattern of guanidinium groups with oxyanions and the guanidinium group in naturally occurring amino acid arginine and nucleic acid guanine.

Several interesting features of the group are notable. The guanidinium group has a high protonation constant ($pK_a \approx 13.5$) making it protonated across virtually the entire pH spectrum, unlike the polyaza compounds. This could be an advantage in the design of anion receptors where a high degree of protonation is required for binding.[21]

The guanidinium binding motif's strong binding potential is anchored by its positive electrostatic center flanked by two parallel hydrogen bonds donors complementary to the charge distribution of oxyanions like carboxylates and phosphates.

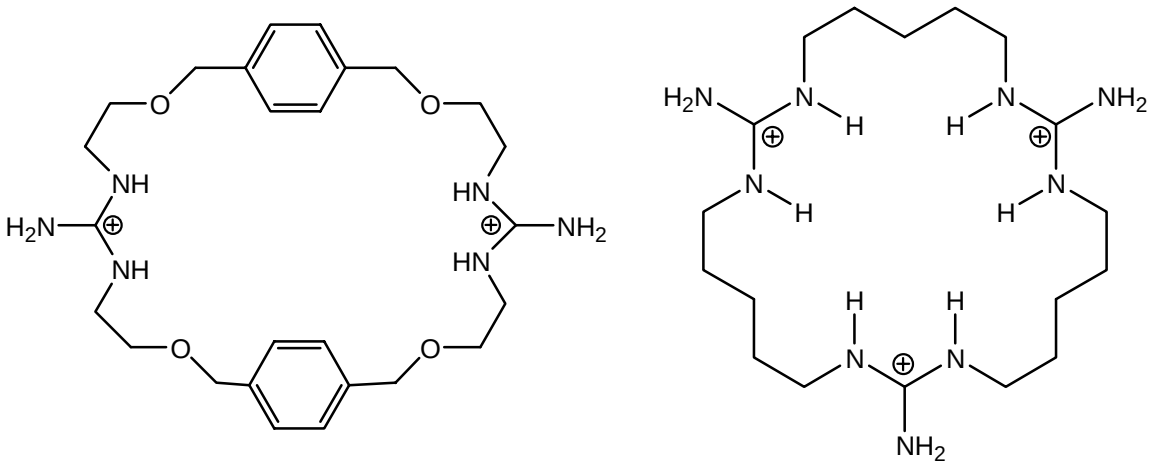
Some disadvantages of the guanidinium group are that it is very strongly solvated in water, and it has a lower charge density than the ammonium based receptors leading to weaker electrostatic interactions.[17]

Lehn, *et al*. were the first to report macrocyclic guanidinium based receptors **(Figure 3.36)**.[109] Triguanidinium macrocycles **48** and **49** showed only weak complexation of PO_4^{3-} (log K_S 1.7 and 2.4, respectively) in methanol/water and the interaction was shown to be largely driven by ion pairing, and therefore charge dependent.

The low binding affinities may be due to the limited number of binding sites available to the anion, or more probably a receptor conformation leading to poorly aligned guanidinium moeities unable to form strong pairs of hydrogen bonds with the substrate.[21] In either case, the poor binding properties and difficulties associated with the synthesis of these compounds dissuaded further research in the area and the focus was shifted to acyclic guanidinium based receptors.[21] Several such receptors have been made over the years and have been shown to form relatively stable complexes with many phosphate and carboxylate anions.[21]

A simple acyclic bisguanidinium compound **(Figure 3.37)** was synthesized by Hamilton's group as an artificial enzyme that bound strongly to phosphodiesters (log K_S 4.7) and increased transesterifications by a factor of 300.[110] The equivalent monoguanidinium receptor was seen to increase the reaction by only 2.5-fold, indicating

an important cooperative binding role for the cleft like arrangement of guanidinium groups in a preorganized manner.



48 **49**

Figure 3.36 Macrocyclic receptors incorporating the guanidinium binding element.

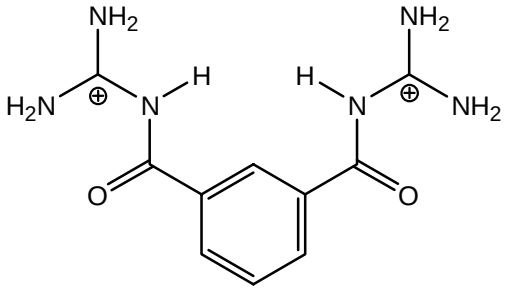


Figure 3.37 Simple acyclic receptors bearing guanidinium binding elements.

A receptor bearing three aminoimidazolinium binding motifs was designed to complex the tricarboxylic acid citrate and was selective over mono- and dicarboxylates, phosphates, sugars and simple salts in water **(Figure 3.38)**.[111] This receptor was used in

conjunction with a fluorescent dye to determine the citrate concentrations in commercial beverages.

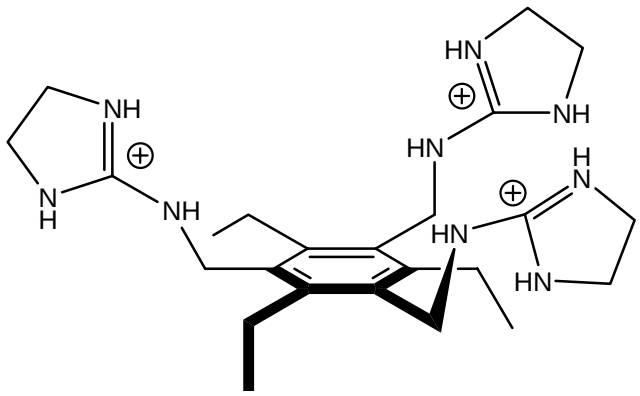
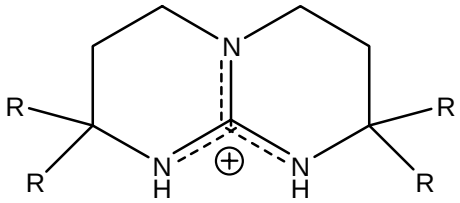


Figure 3.38 Guanidinium-based receptor using aminoimidazolium binding motifs.

In addition to steering away from macrocyclic guanidinium based receptors, research efforts have been geared toward the synthesis of a more rigid framework to house the business ends of the receptors. This has come in the form of sequestering the guanidinium group in a bicyclic system with the idea of restricting rotational movement to maintain a more favorable orientation for anion binding.

The idea of more rigid, preorganized binding sites caught on, and in a 1980 paper Schmidtchen showcased guanidinium binding motifs embedded in a bicyclic framework that helped advance the field (**Figure 3.39**).[112] Even simplest acyclic derivatives based on this re-engineered motif were seen to strongly complex p-nitrobenzoate in chloroform ($\log K_S = 5.15$).[113] Crystal structures of acetate ion binding in these receptors show that, as expected, the anion is bound via two hydrogen bonds to the guanidinium unit.[21]



R = CH_3, $CH_2CH{=}CH_2$, or $CH_2CH_2CH_2OH$

Figure 3.39 Sequestering the guanidinium group in a rigid bicyclic ring system.

Chiral analogues of the bicyclic embedded guanidinium-based receptors were developed along with improved methods of synthesis enabling these structures to be extensively investigating for use in enantioselective recognition, catalysis, and transport of specific substrates across membranes.[17]

One such elaboration is seen in a receptor designed **(Figure 3.40)** to bind with the zwitterionic substrate, dioctanoyl-L-α-phosphatidylcholine.[114] The chiral guanidinium unit, targeting the substrate's phosphoester linkage, is linked to a calix[6]arene pocket targeting the tetraalkylammonium function. Binding of the membrane lipid was relatively strong weighing in with a log K_S of 4.86.

In an effort to bind nucleotides, a pincer-like receptor molecule was built as reported in a 1992 paper by de Mendoza *et al* **(Figure 3.41)** and was able to complex with AMP via multiple modes of binding.[115] NMR data led to the deduction of a structure for the complex where ion pairing, π stacking, and hydrogen bonding intertwine to bring about bimolecular association **(Figure 3.42)**.

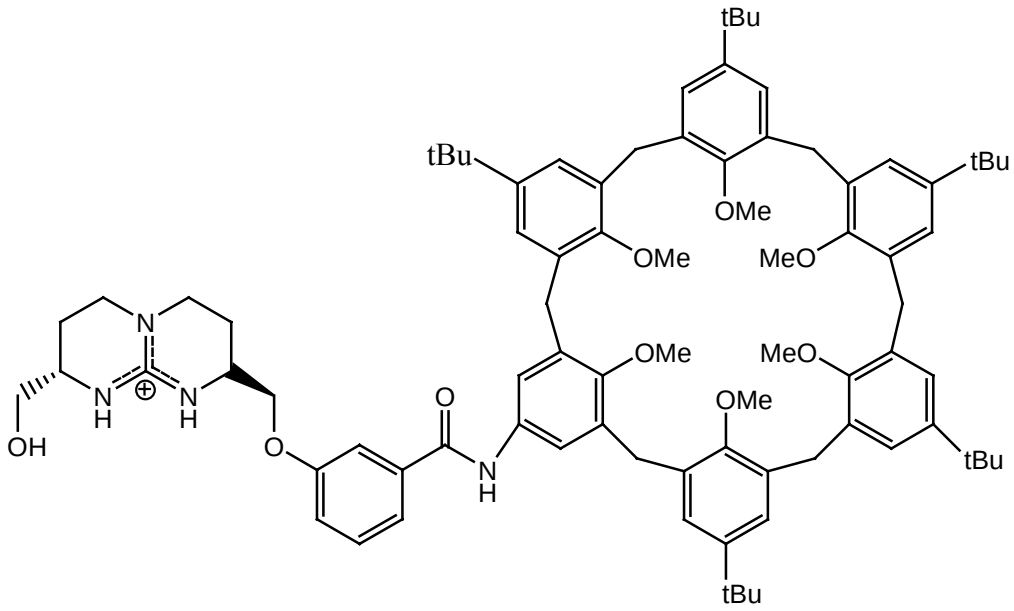


Figure 3.40 Zwitteranionic targeting chiral guanidinium-based receptor linked to a calixarene moiety.

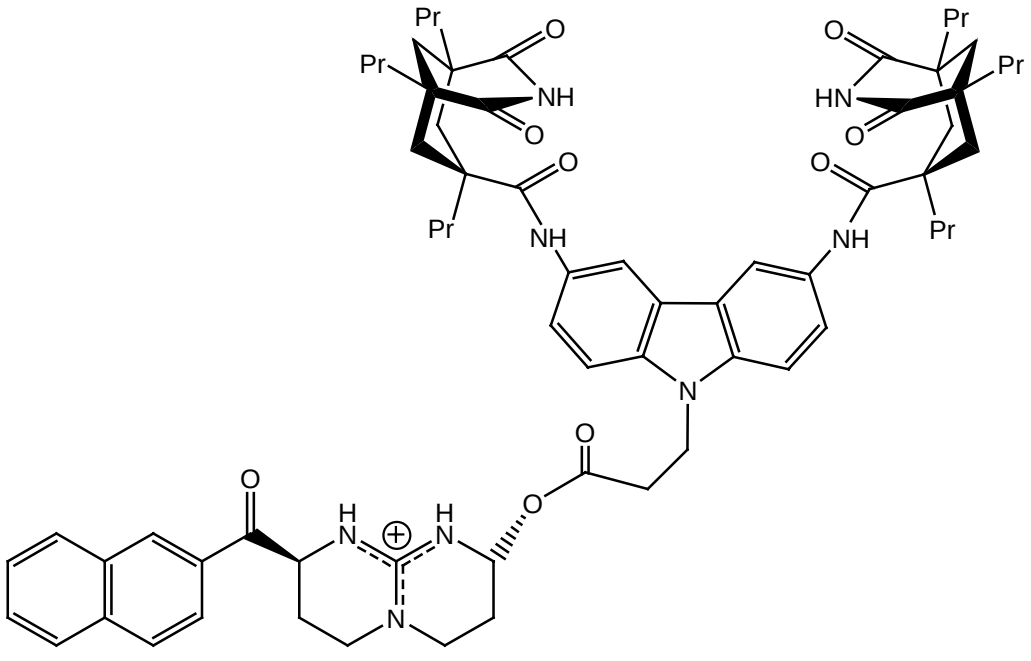


Figure 3.41 Pincer-like chiral guanidinium-based receptor designed to bind to adenosine monophosphate.

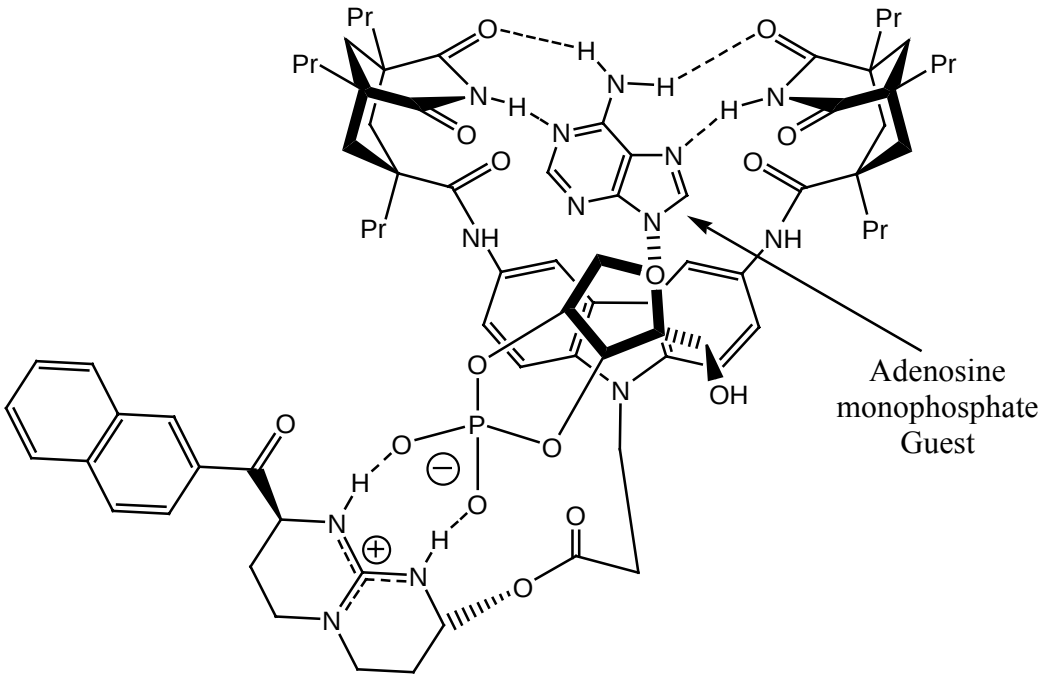


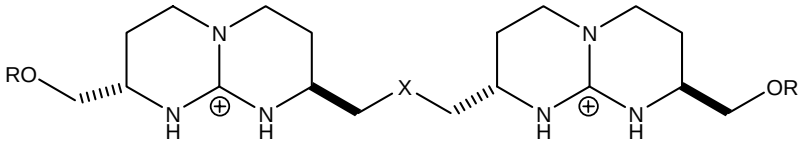
Figure 3.42 NMR deduced structure of complex formation showing hydrogen-bonding interactions, ion-pairing and aromatic stacking between AMP and de Mendoza's receptor.

Despite the successful association, the receptor exhibited only moderate selectivity for cyclic adenosine monophosphates over the corresponding guanosine analogues. Replacement of the naphthoyl group with another adenine recognition site gave a compound having high affinity for an adenosine dinucleotide.[116]

Tetrahedral oxyanions, such as phosphates and sulfates, are important functional groups in biological systems and thus represent intriguing targets for receptor engineers. The theme of bicyclic embedded guanidinium binding sites was expanded into polytopic receptors in hopes of achieving better binding to such oxyanions.

The idea of linking two bicyclic guanidinium groups with a linear spacer has been realized in compounds **(Figure 3.43)**.[117-121] Designers of such receptors envisioned that

binding to a suitable tetrahedral anionic guest would initiate a folding of the molecule, placing the two binding motifs perpendicular to each other and in perfect orientation for complexing the oxyanion **(Figure 3.44)**. All of these compounds did exhibit successful binding to a variety of biological oxyanions in water.



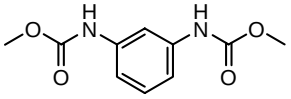
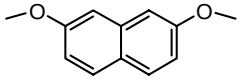
	B	X
50	—$SiPh_2{}^tBu$	
51	H	
52	—$SiPh_2{}^tBu$	
53	H	

Figure 3.43 Linearly linked bis-bicyclic guanidinium binding elements.[17]

One of the critical design elements built into the ditopic guanidinium-based receptors were spacer segments flexible enough to allow for the observed orthogonal orientation of the primary binding sites. To examine the influence of spacer flexibility on binding, a series of increasingly rigid mannitol based linker molecules **(Figure 3.45)** were designed and incorporated into **50-53**. The resulting receptors exhibited binding constants with no discernible trends indicating that spacer flexibility did not play a major role in guest affinity.

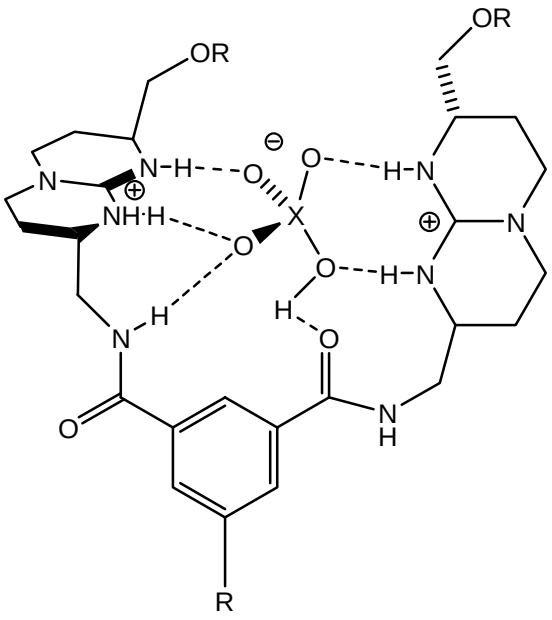


Figure 3.44 Presumed binding geometry in the interaction of ditopic guanidinium receptors with tetrahedral oxyanions.[17]

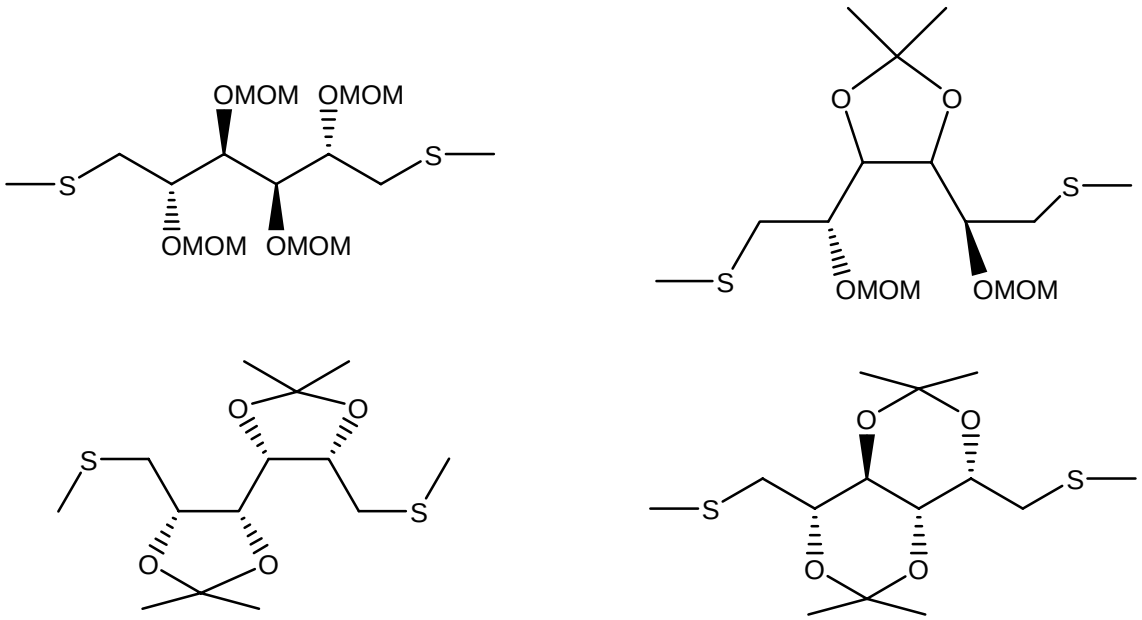


Figure 3.45 From top left to right, increasingly more rigid linkers incorporated into receptors **50**-**53**.

As with the polyazamacrocycles there are many more variations on the guanidinium theme including the incorporation of aromatic functions.[122-124]

Metallo-complexed nitrogens

Observations that phosphates can act as substrates for, or inhibitors of zinc enzymes, through coordination with Zn^{2+}, have led to the use of zinc (II) complexes of cyclens as models for zinc enzymes such as carbonic anhydrase and alkaline phosphatase A.[18] Kimura et al. have developed a series of Zn^{2+}-complexed cyclen-type receptors for the binding of organic phosphates **(Figure 3.46)**. The base unit is represented in receptor **54**, receptor **55** adds another cyclen unit and **56** adds a third cyclen. Binding with a variety of organic phosphates in aqueous media was tested and it was found that in all cases where data was available the tricyclen receptor **56** had 100 to 300 times greater affinity for the substrate than did cyclen **54** as would be expected in an electrostatic interaction.[18]

An interesting application of hydrophobic tail appended Zn^{2+}-complexed receptor **54** was the selective transfer of AZT from an aqueous layer into an organic layer with >90% efficiency.[18] Binding experiments of Zn^{2+}-complexed receptors similar to **54** and **55** to various nucleotides, including AZT, were carried out and showed log K_S values ranging from ≈ 3.3 for the monocyclen receptor to ≈ 5.5 for dicyclen receptors configured *meta* on the benzene core, to ≈ 6.0 for dicyclen receptors configured *para* to each other.[18]

The zinc(II)-macrocyclic polyamine complexes are stronger acids than their polyazamacrocycle precursors. Kimura claims that his receptors bearing zinc(II)-phosphate interactions are generally more kinetically and thermodynamically stable in

aqueous media than those utilizing protonated polyamine-phosphate interactions. This may make such receptors more useful in biological systems.[18]

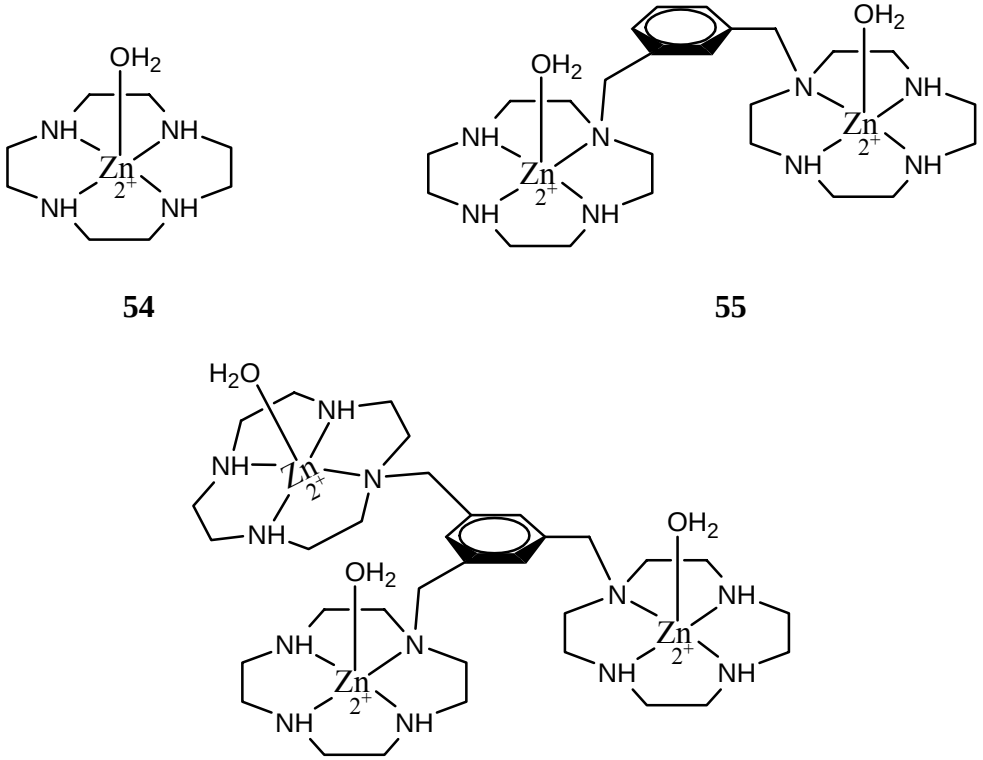


Figure 3.46 Zinc II complexes of cyclens with increasing "topic" complexity.

Another receptor of this type is the biscopper(II) complex of **19 (Figure 3.47)** which at pH 8 forms an extremely strong complex with the biologically important inorganic molecule pyrophosphate (log K_S = 10.5).[125]

A similar compound containing the fluorescent 9,10-anthracenyl reporter group has been used to sense linear anions in aqueous solution **(Figure 3.48)**.[126] The presence of the reporter group enables binding to be detected as anion complexation quenches the

fluorescence of the fluorophore. These type of receptors are often referred to as chemosensors. Halides and diffuse anions, such as NO_3^-, HCO_3^-, and SO_4^{2-} are not well complexed in comparison to the linear N_3^- or NCO^-, and the azide was favored over NCO^-.[34]

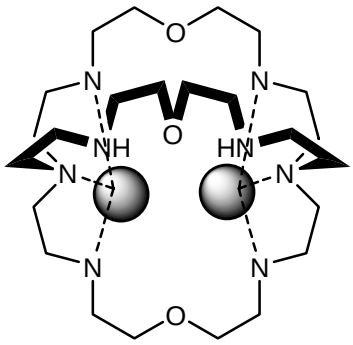


Figure 3.47 Biscopper complex of compound 19 strongly binds pyrophosphate.

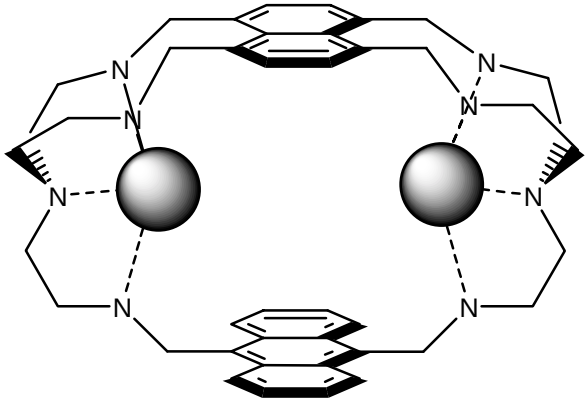


Figure 3.48 Dizinc cage chemosensor bearing anthracenyl reporter group.

The utilization of metallocenes **(Figure 3.49)** was propagated by Beer and Takahashi.[127-130] Some important virtues of these compounds are their ready accessibility, reversible redox responsiveness, chemical stability, potentially cationic, modifiability ease and building block modularity. The common theme among many of the successful metallocenes is the incorporation of cobaltocenium moieties into the macrocyclic structure. It was determined that with these compounds the positive charge born on cobaltocenium moieties is not sufficient to bind anions, rather hydrogen bonding and other interactions form the brunt of the complexing interactions.[17]

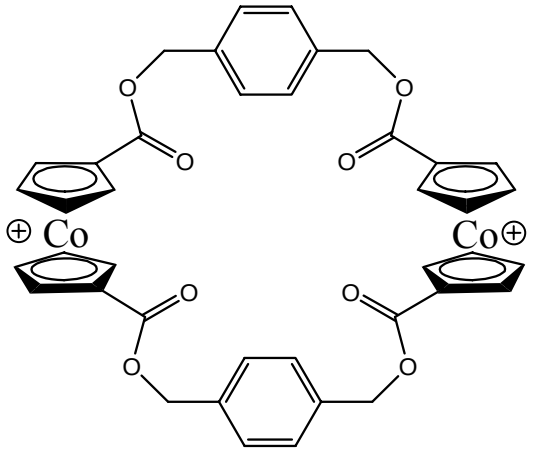


Figure 3.49 Macrocyclic cobaltocenium metallocene.

Poly Lewis Acids.

This class of compounds actually falls outside the boundaries of nitrogen-based anion binding motifs, however it is included here as an introduction to electroneutral receptors.

Lewis acids are defined as chemical entities capable of accepting a pair of electrons in a bonding event, whereas Lewis bases are electron pair donors. Some of the earliest host molecules known, Pedersen's crown ethers, were essentially preorganized arrangements of Lewis basic oxygen atoms connected in an organic framework. Such compounds were able to bind even the weakest coordinating cations. In a reversal of this idea it seems plausible that by substituting Lewis basic moieties with Lewis acid ones, receptors could be made to bind anionic substrates. This reciprocal arrangement has been coined anti- or inversecrown chemistry and several receptors based on this idea have been produced.[17]

Host electroneutrality is one of the commanding features of these compounds responsible for a series of other properties that sets them apart from the receptors discussed thus far. In a host molecule lacking charged groups there are no counter-anion interactions to interfere or compete with guest binding. These type of hosts are expected to be more selective in their binding resulting in an interaction with the guest that is covalent. With pure coulombic forces responsible for partner association, only parameters such as size, charge density and distance control the host-guest interaction, however with Lewis acid—Lewis base interaction the parameters of stereoelectronics, molecular orbital symmetry, softness, back bonding ability, etc., provide more subtle means to differentiate binding partners.[17]

Covalent binding of the substrate may be a major disadvantage in the application of Lewis acid based receptors. Another issue that complicates the usability of these receptors is that, although there are no counter ions, virtually every solvent will compete with the guests for the receptor because with the exception of hydrocarbons, all other

organic solvents exhibit Lewis basicity as well and would be present in many fold excess to the desired substrate.[17]

As mentioned, Lewis acid-based receptors are not nitrogen-based and are geared more toward applications such as potentiometric inorganic anion sensing by ion selective electrodes, therefore this class of receptors will not be explored any further.

Ion-Dipole Binding Motifs.

Ion-dipole bonding based receptors offer a non-covalent binding electroneutral alternative to Lewis acid containing receptors. One area of concern is that although driven by the same electrostatic forces that dominate ion pairing interactions between full charge bearing partners, ion dipole interactions are much weaker and fall off with distance more steeply.[17]

Another possible disadvantage of this motif, because of the strong hydrogen bonding network seen in water, is that host-guest interactions established in organic solvents, often vanish when moving to an aqueous environment[17].

One advantage of this motif, aside from electroneutrality, is the directionality of the interaction which is most often manifested as hydrogen bonding. The average hydrogen bond can add 2 to 5 kcal/mol of energy to the binding interaction of two partners—roughly the 1/10 the energy of a carbon-carbon covalent bond.[131]

Receptors incorporating this function are also able to simultaneously strike up several binding relations and the chemistry available for embedding suitable hydrogen bonding motifs into the receptor framework is rich and well established. Even the most primitive hydrogen bond donor hosts can bind anions as long as competing hydrogen

bond acceptors can be kept at bay—a major obstacle in experimental design. The host is faced with standing up against competing solvation of both binding partners.

Natural systems provide precedence that anion binding in aqueous solution can be achieved via hydrogen bonding alone. An example of this is a sulfate binding protein that is a highly efficient carrier able to sequester sulfate with a log $K_S = 6$ and shows about a hundred-fold discrimination against HPO_4^{2-}, an anion of the same charge and about the same size.[132]

The selectivity can be explained by the X-ray crystallography data of the complex which shows the anion to be deeply embedded in the protein and held in place by seven hydrogen bonds. There are no functional groups present in the sulfate binding domain capable of acting as a hydrogen bond acceptor as would be required to bind hydrogen phosphate.[17]

Some general conclusions about ion-dipole host design have been reached. The accumulation of hydrogen bond donor sites in close proximity to one another enables maximum hydrogen bonding to guest species and provides suboptimal conditions for solvation of binding sites both of which lead to stronger host-guest binding.[133-135] What follows is a review of specific receptor types utilizing this binding motif.

Polylactams and Polyureas

The amide, like guanine, is another functional group strongly represented in biology, and is capable of ion-dipole interactions *vis-a-vis* hydrogen bonding. Several X-ray structures of anion binding proteins have revealed that many amide protons are involved in the binding of inorganic oxyanions like sulfate and phosphate.[136] As far back

as the early 1980s Kimura mentioned that in a cyclam-type compound **(Figure 3.50)**, modified to include two amide groups, both the ammonium and amide sites were involved in the binding event.[21] However, reports on this type of receptor are scarce.

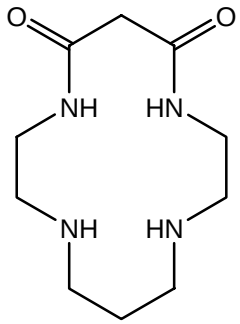


Figure 3.50 Cyclam compound in which amides and protonated amines were found to be involved in anion binding.

The urea and thiourea arrangement of two nitrogens sharing a carbonyl or sulfonyl have also been used as a binding motif in the construction of anion receptors.

The major drawback of the amide unit, and the closely related urea and thiourea motifs, is the difficulty in handling this binding site in the framework of macrocyclic and macropolycyclic systems because of its rigid directionality.[21] This problem also plagues the bicyclic embedded guanidinium unit which is why many of these type of receptors are acyclic.

A representative acyclic amide—a derivative of a cyclohexane tricarboxylic acid **(Figure 3.51)** was made by Moran *et al*. and complexed phenyl phosphate dianion with log K_S = 4.18 in dimethyl sulfoxide.[137] This compound failed to work in chloroform due to dimerization of the receptor.

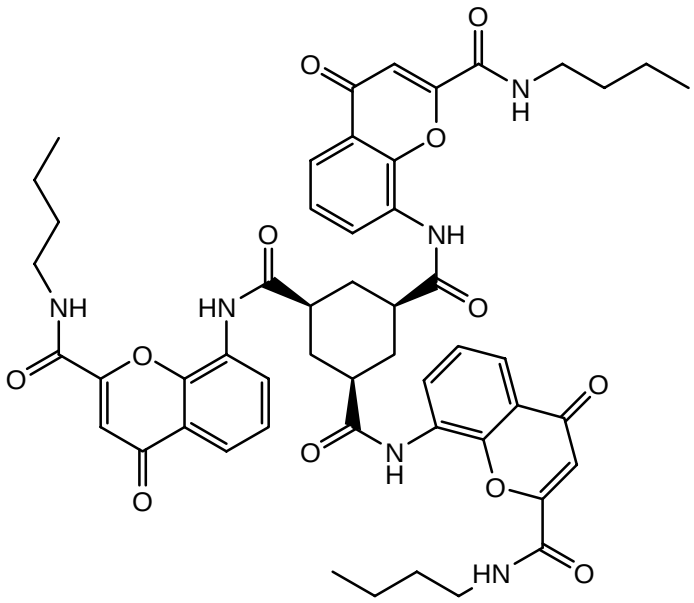


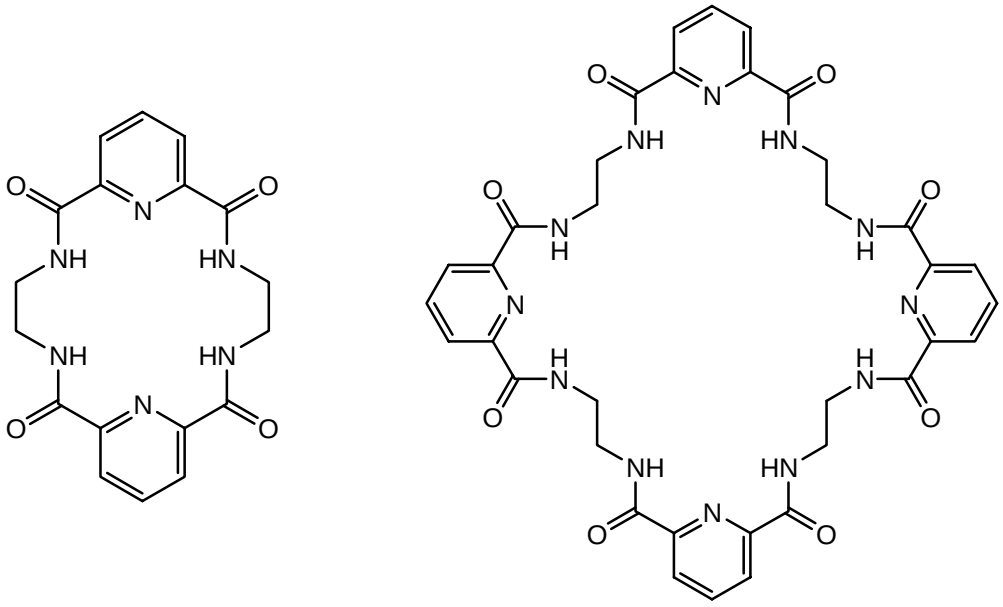
Figure 3.51 Acyclic polylactam receptor geminating from cyclohexane core.

In spite of the potential downfalls associated with incorporating the amide motif in a macrocyclic framework, stubborn organic chemists have been able to synthesize polylactams and test them for anion binding ability **(Figure 3.52)**.[138]

Polylactam **57** was tested with chloride, fluoride, dihydrogen phosphate, acetate, and the *p*-nitrophenoxide anions. Stability constants were determined in ^{1}H NMR titration experiments in [D_6]DMSO at room temperature. Overall, the strongest binding was observed with the bidentate-type oxyanions acetate and dihydrogen phosphate (log K_S = 3.42 and 3.23, respectively).

Of the three oxyanions tested, *p*-nitrophenoxide is the strongest base, yet showed much weaker complexation (log K_S = 1.83) and the weakest base (acetate) showed the strongest binding (log K_S = 3.42). With the two spherical anions, **57** was seen to bind F^-

much better than Cl^- (log K_S = 2.92 vs. 1.81, respectively) giving a selectivity factor of ≈10-fold (within experimental error).



57 **58**

Figure 3.52 Macrocyclic receptors incorporating the polylacatam binding motif.

The fluoride anion is known to be a better hydrogen bond acceptor than the chloride anion which could explain the selectivity and binding strength numbers observed. However, X-ray crystal structure determination shows that Cl^- is too bulky to be included inside the cavity and resides 1.9 Å above the median plane of the receptor, but F^- fits quite well and only floats 0.9 Å above the ring, enabling stronger complexation.

Compound **58** was actually a by-product of the reaction that afforded **57** and was shown by X-ray structure analysis to be suitable to complex a planar $(H2O\text{-}Cl^-)_2$ assembly.[138]

The authors of this paper, Jurczak and Szumna, claim to be the first to use electrospray ionization mass spectrometry (ESI MS) to analyze the binding stoichiometries of hydrogen-bonded anion complexes of neutral receptors. Negative ion mode ESI MS was used to examine **57** complexed to fluoride, chloride and acetate anions to show *m/z* signals corresponding to 1:1 complexation with the all three anions and a small amount of 2:1 (receptor:acetate) complexation with acetate, all of which corroborated X-ray and ^{1}H NMR studies.[138]

One of the many challenges present in field of anion receptor construction is to design receptors that bind their substrates strongly and selectively. The challenge of developing receptors capable of strongly binding many anions has mostly been met, however it is the area of selectivity that currently presents the most difficulty. According to Hamilton and Choi,[139] molecular architects have made extensive use of hydrogen bonding in receptor design, but there are few notable examples where the binding groups have been carefully arranged in a convergent and manner.[140,141]

A polylactam representing their efforts toward receptors combining the features of rigidity, convergent hydrogen bonding sites and geometrically optimized binding groups was synthesized **(Figure 3.53)**. The receptor incorporates three amide groups projecting into the center of the cavity providing a convergence of hydrogen bonding sites for anion complexation. Monte Carlo conformational iterations using the MM2 force field showed the cavity of the lowest energy conformer to be ≈5 Å in diameter and lined only with hydrogens—three from the amides and three from the aryl groups.

Binding of the anions selected for the complexation studies was fairly strong across the board and log K_S values ranged from 3.94 to 5.66. Selectivity by a factor of

nearly 15 was seen for iodide over chloride. The two molecular anions tested were NO_3^- and the p-tosylate anion ($pTsO^-$). The p-tosylate anion is a tetrahedral oxyanion and falls into the same geometry class as phosphate and sulfate ions. The nitrate anion is planar.

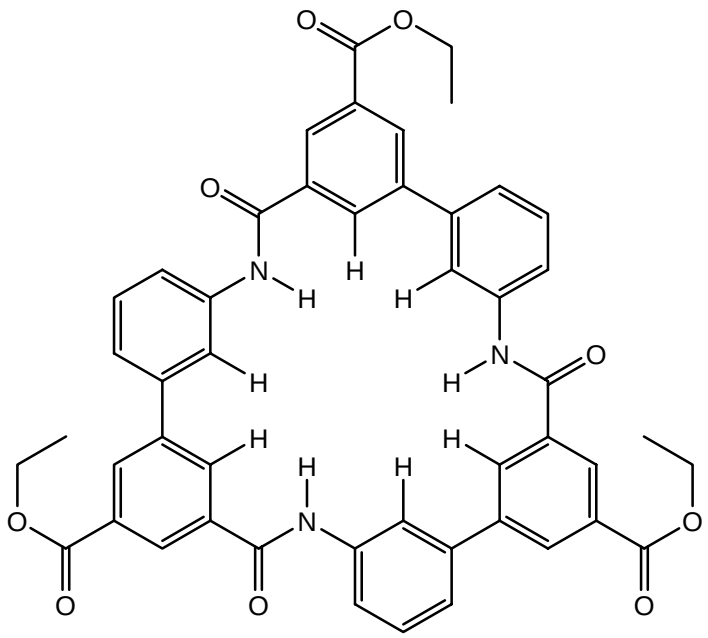
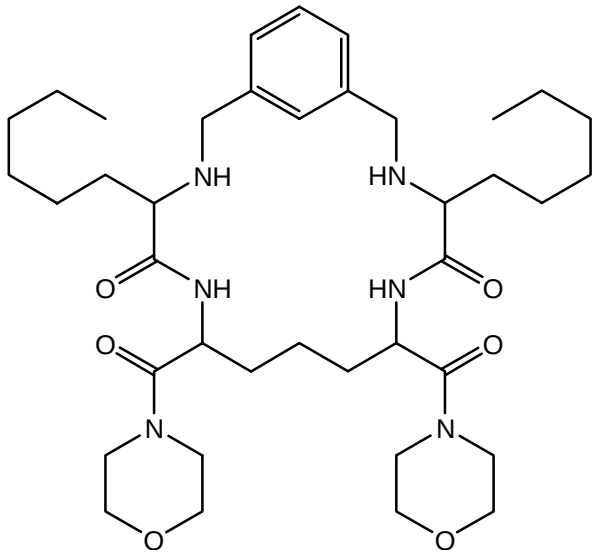


Figure 3.53 Rigid polylactam macrocycle containing convergent hydrogen bonding sites and geometrically optimized binding elements.

The receptor formed strong complexes with both of these geometrically disparate anions nearly equally well (log K_S = 5.41 and 5.66, for tosylate and nitrate, repectively), with a narrow ≈1.8 to 1 selectivity for the planar species. The macrocycle was found to bind much better (≈1500-fold) to the anions than its open-chain triamide penultimate

synthetic precursor[139] and several of the anions formed 1:2 sandwich complexes with the macrocycle.

Zhu et al. developed an unusual synthesis for a unique mixed amide/amine macrocycle **(Figure 3.54)**.[142] Macrocycle **59** was made in a one-pot four-component synthesis **(Scheme 3.8)**.

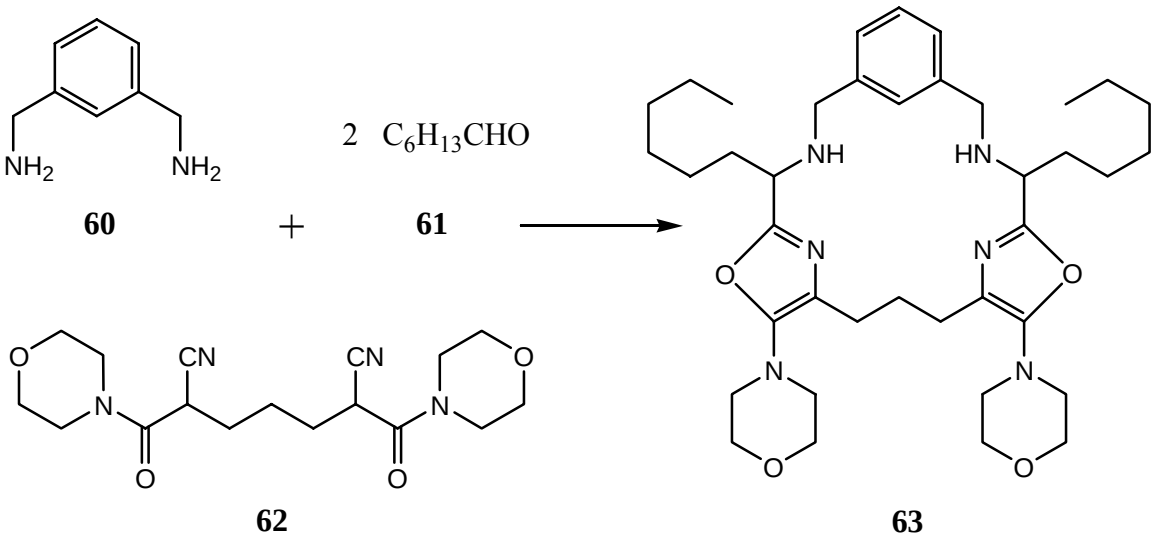


59

Figure 3.54 Polylactam/polyamine hybride macrocycle generated from an unusual oxazole precursor.

The synthesis was carried out by refluxing *m*-xylylenediamine **60** with 2 equivalents of *n*-heptanal **61** in methanol for 30 minutes. Addition of 2,6-diisocyano-heptanedioic acid bismorpholinylamide **62** and reacting an additional four hours gave the interesting amine/oxazole macrocycle **63** in about 50% yield. The final step involved opening up the oxazole rings via hydrolysis using an 8:2:1 solution of THF:H_2O:TFA to

give **59** in over 85% yield. All six diastereomers were readily separated and identified by LC/MS.[142]



Scheme 3.8

A series of three complex optically active polylactam macrobicycles, too hard to draw much less synthesize, were produced in low yields and questionable purity by Diedrich and Welti as putative carbohydrate receptors.[143]

Computer modeling using MacroModel 6.0 with the OPLS* force field and 4000-step *pseudo*-Monte Carlo multiple minimum (MCMM) conformational searches indicated that the cyclophane amides harbored an open cavity ripe for carbohydrate inclusion.[143]

Three alkylated optically active variations of glucose (Octyl β-D-glucoside, Octyl α-D-glucoside, Octyl α-L-glucoside) were tested against the three receptors and the best binding any of them could muster was log $K_S = 2.43$ with most log $K_S < 2$.

Enantiomeric selectivity was also nearly non-existent sporting at best a factor of ≈1.5-fold. The authors speculate, in spite of computational predictions to the contrary, that the receptors do not have a well-defined preorganized binding cavity and that the binding they did measure was not via inclusion, but rather exterior to the host.[143]

Taking a cue from nature, Ishida *et al*.[144] synthesized an optically active cyclic peptide **(Figure 3.55)** which provides a rigid structure orienting hydrogen bond donors toward the macrocyclic center. Binding of p-nitrophenyl phosphate in DMSO was exceptional with log K_S = 6.1.

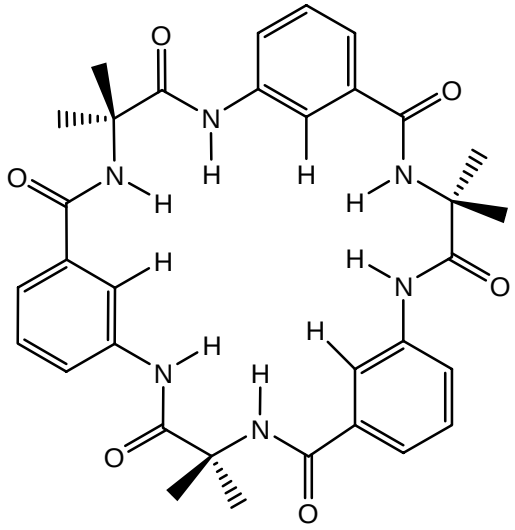


Figure 3.55 Optically active cyclic peptide incorporating rigid covergent hydrogen bonding sites.

A peptidic bicyclic cyclophane **(Figure 3.56)** has been successfully used as a receptor for anions and shows an affinity for acetate and nitrate over cyanide.[140]

Encapsulation was observed in the X-ray data as was ligand binding by six hydrogen bonds.

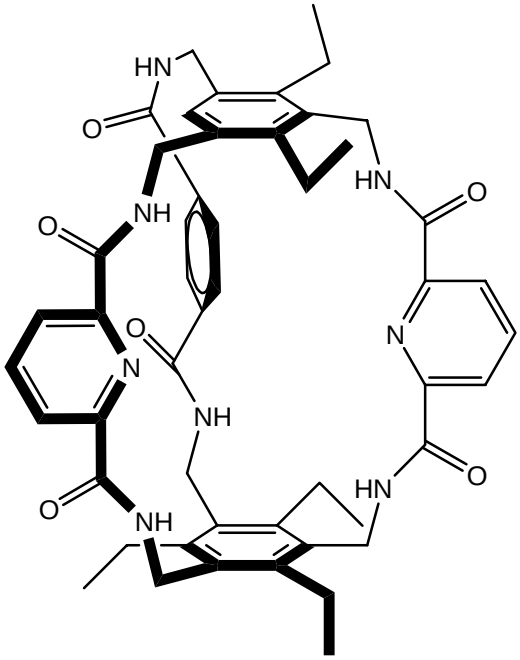


Figure 3.56 An amide linked bicyclic cyclophane.

Two relatively simple acyclic urea-based hosts capable of complexing oxyanions were synthesized in the early 1990s **(Figure 3.57)**.[145] The investigators, Kelly and Kim, determined that greater Brönsted basicity and higher charge in the guest led to stronger binding with ion-dipole hosts. As a corollary, higher acidity in the host is also expected to increase binding strength. However, in each of these cases the interdependence is rather flat. The dependence on solvent is more pronounced with K_S decreasing as solvent polarity increases.[17] It was also found that structural elements in the receptor influencing the hydrogen bond donating ability of the urea moiety greatly altered binding constants.[146,147]

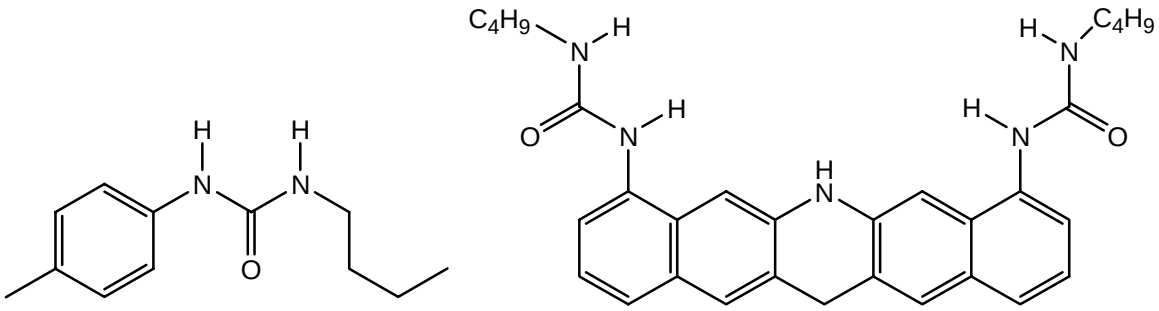


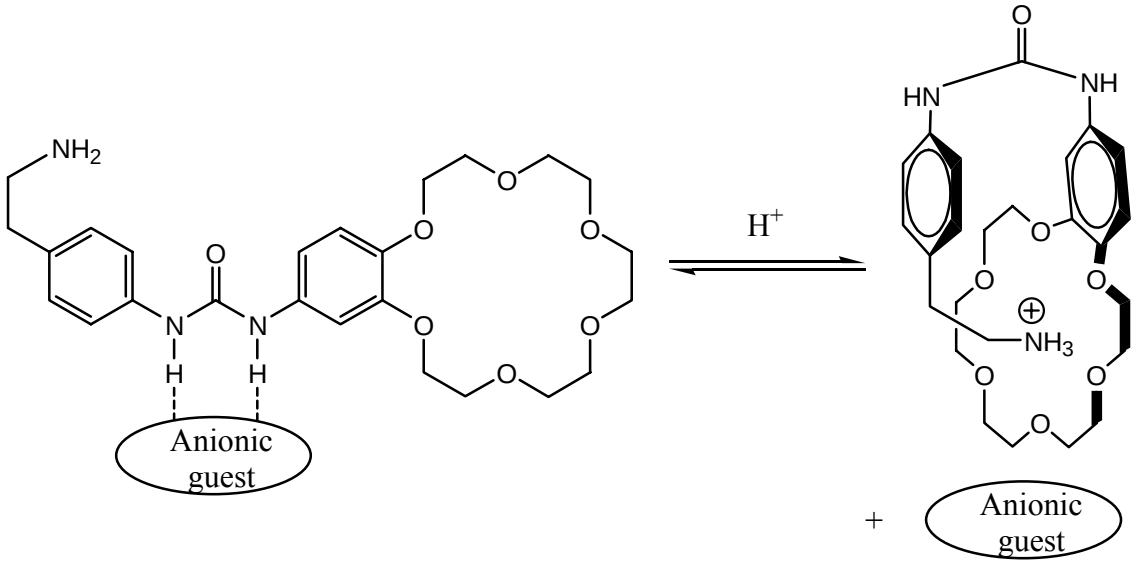
Figure 3.57 Simple acyclic urea-based anionic host molecules.

Since most organic functional groups acting as hydrogen bond donors also contain corresponding hydrogen bond acceptors, there is the danger of intramolecular self-interaction if given enough receptor flexibility. This property could also result in supramolecular self-assembly instigated by intermolecular interactions between receptors. Obviously these type of events would seriously impair, if not prevent, host-guest complexation, so designing receptors with restricted flexibility is expected to suppress self-anihilation interactions and lead to stronger substrate binding.

An elegant twist of negative allostery capitalizing on self-anihilation, serves as an example of more complex polyurea binding motifs. Branda and Al-Sayah[148] developed a receptor which upon protonation of a single amino group caused intramolecular blocking of binding elements distal from the site of protonation **(Scheme 3.9)**.

The allosteric elements flanking the binding site consist of an aliphatic amine and a crown ether appendage. Under pH conditions leaving the amine group unprotonated the urea-based binding elements remain exposed and capable of complexing the acetate anion. Decreasing the pH protonates the amine which binds to the crown ether moiety

throwing the urea binding site out of a conformation suitable for substrate binding. The conformational change induced by the intramolecular allosteric interaction causes the acetate ion to be released. The whole process reverses by increasing the pH which frees the binding site for anion complexation.



Scheme 3.9

A urea linked macrocycle sporting the guanidinium binding motif **(Figure 3.58)** provides a preorganized set of two cooperative binding motifs housed in a chiral framework capable of six hydrogen bonds oriented toward the central cavity.[149] Phosphates are bound tightly, however they do not enter the cavity. The chloride complex was found to have perfect C_2 symmetry at any temperature indicating encapsulation of the anion

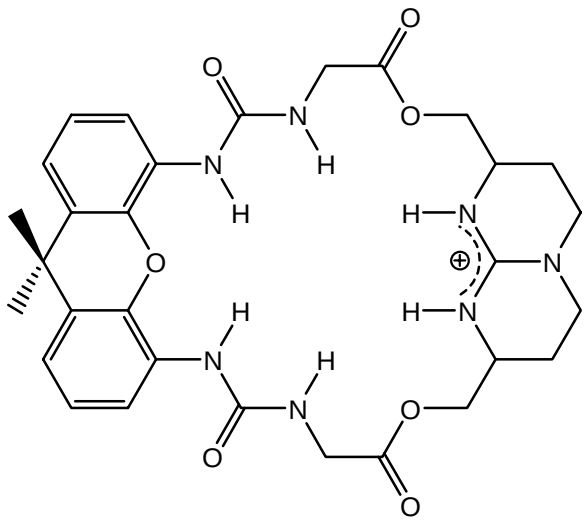


Figure 3.58 Urea linked hybrid macrocycle with a guanidinium binding element.

Calixarenes

Calixarenes represent another relevant group of compounds containing electro-neutral ion-dipole binding motifs which operate through hydrogen bonding. Strictly speaking calixarenes do not directly harbor anion binding motifs (the sister class calixpyrroles are an exception), but they do provide motif anchor points. Connecting sulfonamide functions to the upper rim of calix[4]arene gave a receptor **(Figure 3.59)** able to distinguish between hydrogen sulfate and chloride or nitrate.[150]

Dangling urea or thiourea functions from calix[4]- or calix[6]arene, respectively gave anion receptors designed to complex phosphates **(Figure 3.60)**.[151,152] The investigators were surprised that phosphates did not bind, but rather chlorides took up residence in a 1:1 fashion as unexpected guests. Compound **65** also showed strong binding to 1,3,5-benzenetricarboxylate (log K_S = 5.3) in $CDCl_3$. Isomeric benzene tricarboxylates were bound 10-100 times less strongly.

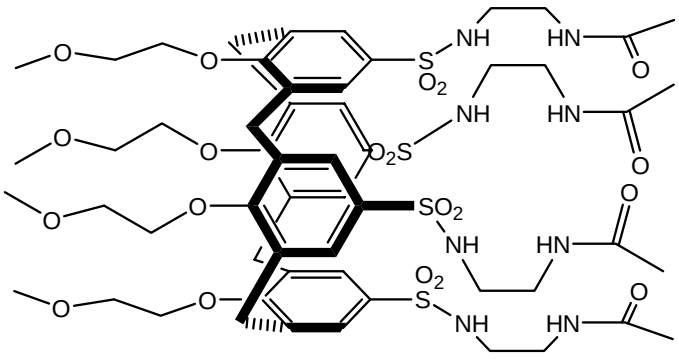
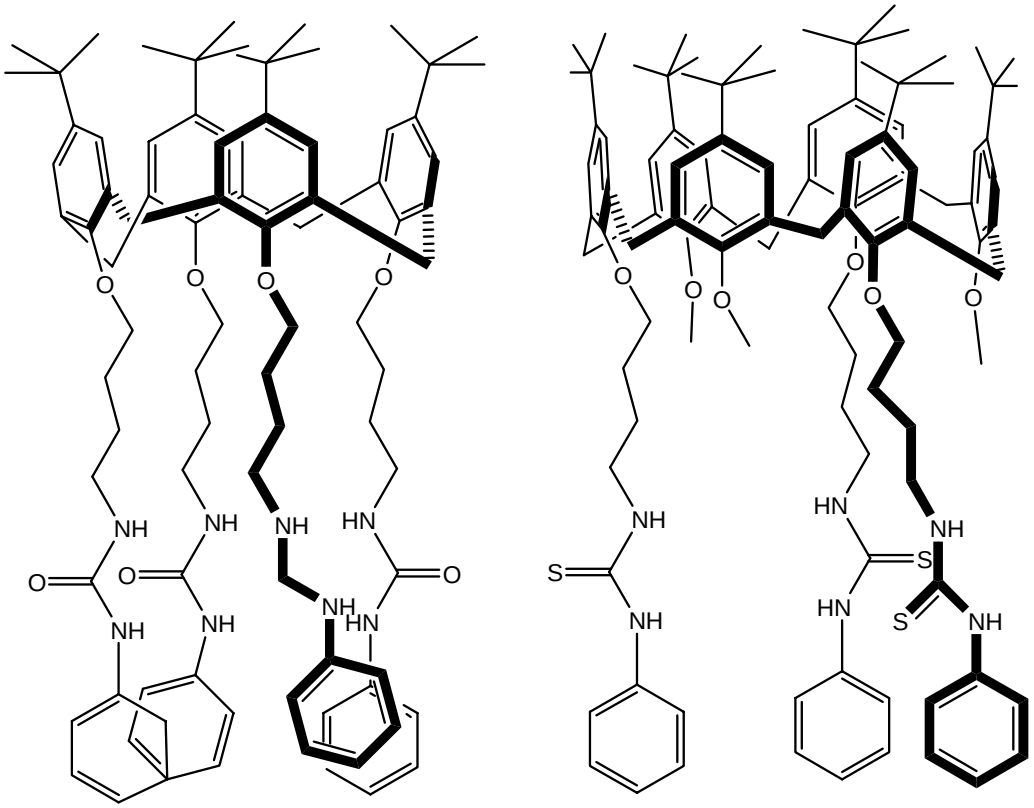


Figure 3.59 Calix[4]arene host molecule (shown on its "side") with sulfonamide linked amide and amine binding motifs eminating from the upper rim.



64 **65**

Figure 3.60 Calix[4]- and calix[6]arenes (**64**,**65** respectively) tethered to urea or thiourea binding motifs (three thiourea chains left off **65** for clarity).

Calixpyrrole **(Figure 3.61)** known as acetone-pyrrole, has been well-known for over a century[153,154] and is easily synthesized in a one step condensation of its namesake components. It has recently been characterized as an electroneutral ion-dipole host capable of binding fluoride in dichloromethane (log K_S = 4.23) with a strong preference over both chloride and dihydrogen phosphate (log K_S = 2.54 and 1.99, respectively).[141]

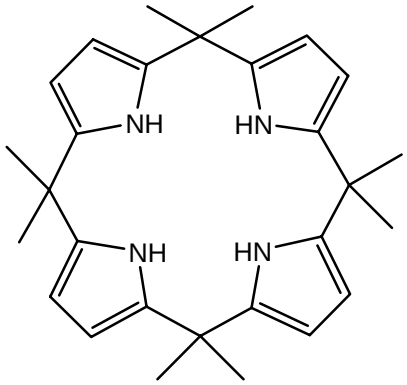


Figure 3.61 A calix[4]pyrrole more commonly known as acetone-pyrrole.

X-ray crystal structures indicate a conformational change occurs in the pyrrolic macrocycle upon complexing fluoride such that a cone shaped receptor results with all four N—H groups pointing toward the anion. The anion, in spite of its diminutive size, must still perch above the even smaller cavity of the macrocycle.

In a recent Organic Letters paper,[155] the first synthesis of novel calix[4]arene-porphyrin conjugates connected via urea functions was reported. Several variations of this "kitchen sink" receptor **(Figure 3.62)** were assembled with porphyrin-capped urea anion-binding elements extending from the top rim of the cone-shaped calixarene.

Acetate groups dangling from the lower rim of the calixarene created cation binding sites. It was envisioned that changes in the UV-visible spectrum of the appended porphyrin groups upon anion complexation would provide a reporter mechanism. One to one complexes of the receptors with a variety of anions were determined using Job plots constructed from both UV/vis and ^{1}H NMR titration data. Binding constants with the halide series chloride, bromide, iodide showed that binding affinity decreased with increased anion diameter.[155]

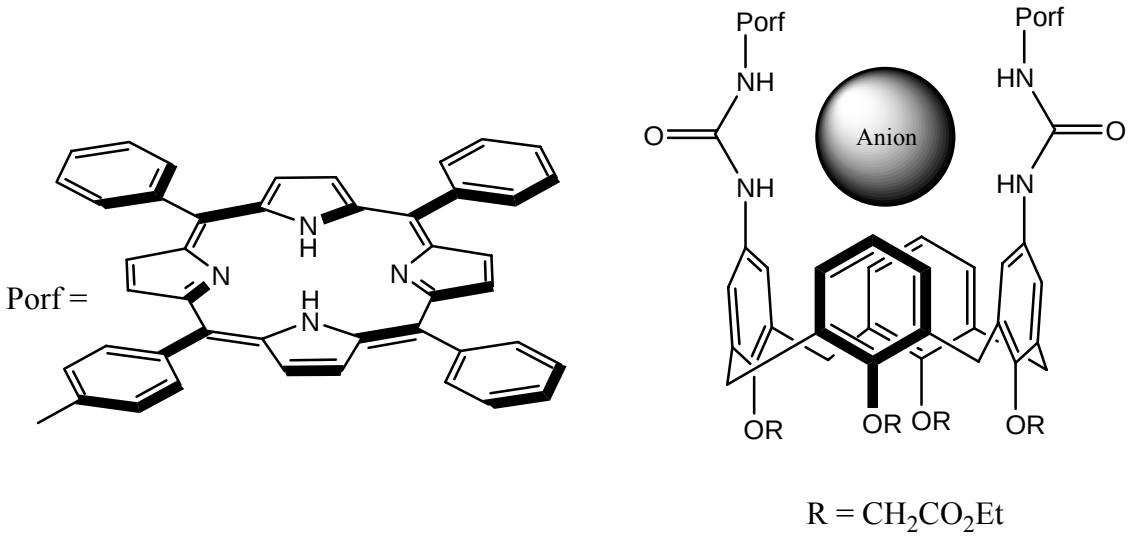


Figure 3.62 "Kitchen sink"-receptor containing everything but, including urea binding elements tethered to tetraphenylporphyrin moieties along with acetates dangling from the bottom rim.

Molecularly Imprinted Polymers

An entirely new class of chemical entities with great potential in the field of molecular recognition has carved out an interesting niche in the world of organic

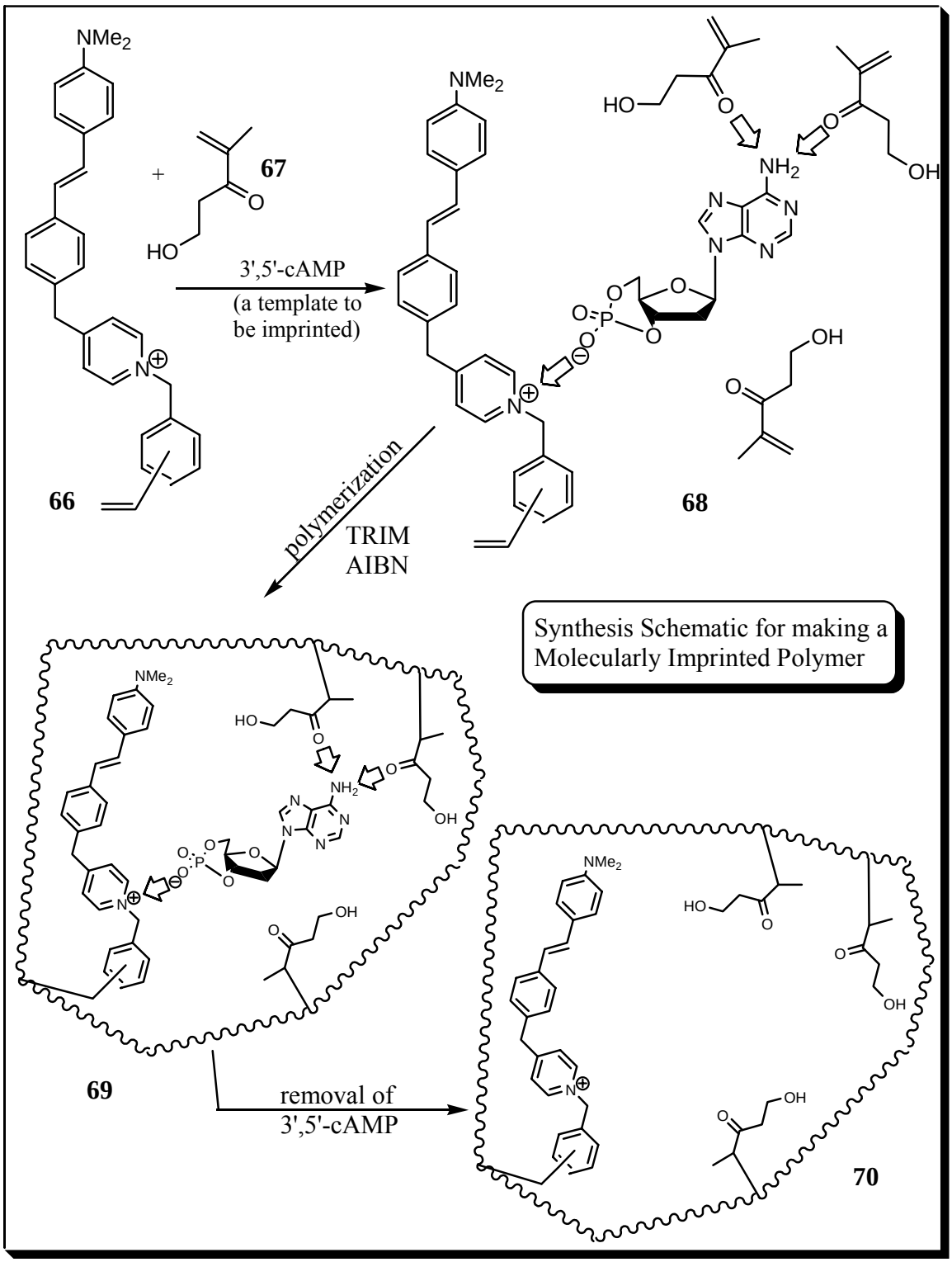
chemistry. These compounds are known as molecularly imprinted polymers (MIPs) and they rely heavily on the idea of templating.

Making receptors using MIPs involves building a polymer around a molecular template which is either designed to closely mimic the compound to be bound by the receptor, or actually is the compound to be bound by the receptor. Following polymerization around the target, the templating molecule is removed leaving behind an imprint of the molecule. The MIP is a highly preorganized receptor molded precisely to the topography of a target anion and is thus expected to be very selective in its binding.

An example of such a compound, with an embedded fluorophore for binding detection, was polymerized and imprinted using a cAMP template **(Scheme 3.10)**.[156] The cationic fluorophore **66** and 2-hydroxyethyl methacrylate (HEMA) **67** were mixed with cAMP forming **68** an organized supramolecular-like matrix of binding associations. Polymerization with trimethylolpropane (TRIM) in the presence of 2,2'-azobisisobutyl-nitrile (AIBN) afforded **69**.[18]

Upon removal of cAMP, and given the polymerization is able to successfully lock a ternary complex of binding elements in place, a preorganized pocket or imprint of the substrate **70** should remain to welcome new guests as they arrive. This particular MIP showed remarkable selectivity for cAMP over cGMP,[34] and bound cAMP with a log K_S = 5.5 in aqueous solution.[18]

Kimura *et al.* have recently reported another example of an anion receptor embedded within MIPs.[18] The idea arose when they observed that the Zn^{2+}-complexed cyclen **54** (Figure 3.46) formed a 2:1 complex with 4-nitrophenylphosphate ($NPP2^-$)(2 Zn^{2+}-complexed cyclens/$NPP2^-$). Polymerization of a styrenyl derivative of the complex


NMe2
67
HO
3',5'-cAMP
(a template to
be imprinted)
NMe2
HO
NH2
OH
66
68
OH
polymerization
TRIM
AIBN
Synthesis Schematic for making a
Molecularly Imprinted Polymer
NMe2
HO
NH2
OH
OH
69
removal of
3',5'-cAMP
NMe2
HO
OH
OH
70

Scheme 3.10

with ethylene glycol dimethylacrylate (EGDMA) in the presence of AIBN, followed by removal of $NPP2^-$ gave an MIP. They were able to determine the concentration of Zn^{2+}-cyclen units present in the polymer by using a zinc selective fluorophore, however the MIP itself does not contain any binding event reporting mechanism. The group expects the MIP to selectively recognize $NPP2^-$ and other phosphate monoesters for separation, hydrolytic catalysis, and other applications, however no data has yet emerged.[18]

Chemosensors

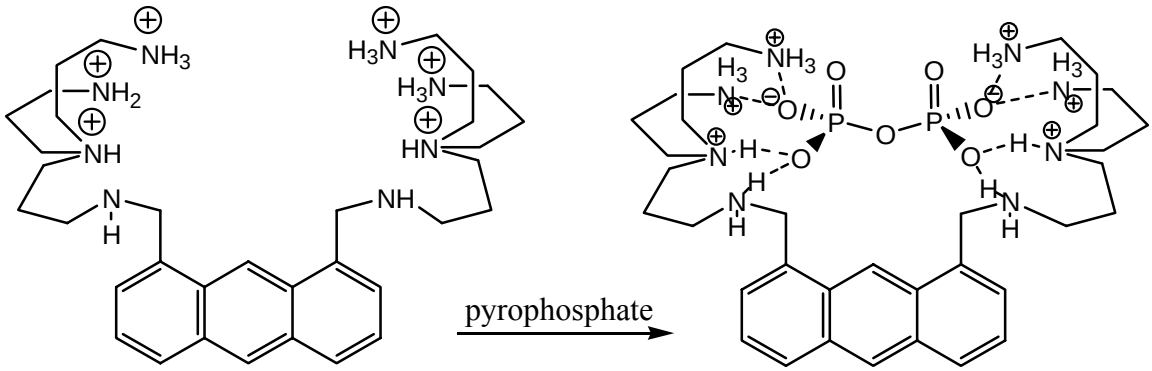
In the previous sections numerous examples have been given to illustrate the diversity of anion receptors possible using nitrogen centric binding motifs as recognition elements. In this section, one particularly interesting application of such receptors will be explored with a few examples of recent work in the area of chemosensors.

Chemosensors are receptors that not only bind an analyte or substrate, but also report that binding has occurred. Traditionally chemosensors have targeted monoatomic ions such as sodium, potassium, mercury, lead and calcium, but of late that list has expanded to include small anions such as phosphate, phosphodioesters, azide and even medium sized molecular anions like citrate.[16]

Binding event reporting is usually accomplished by fluorophores or chromophores covalently attached to the host compound. Upon substrate binding, conformational or electronic changes occur in the complex which lead to a detectable signal from the reporter.

Two mechanisms by which a binding event signal is transduced are Fluorescence resonance energy transfer (FRET) and photoinduced electron transfer (PET).[16]

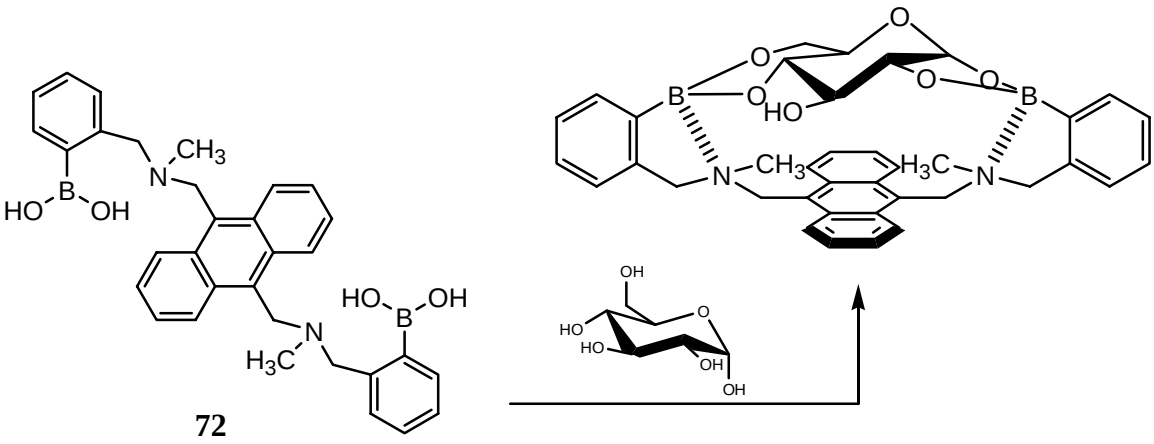
An example of the latter mode of signal detection can be seen in compound **71 (Scheme 3.11)** which displays a 1000-fold selectivity for pyrophosphate over phosphate ions.[157,158] Another interesting example can be seen in **72 (Scheme 3.12)**, a bisboronic host capable of signaling the presence of glucose bound via boronic acid-diol interaction.[159]



71

low fluorescence high fluorescence

Scheme 3.11



72

low fluorescence high fluorescence

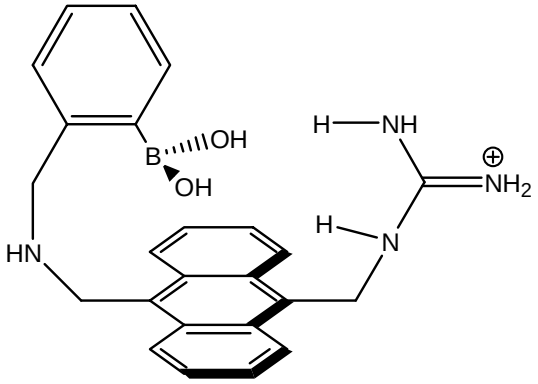
Scheme 3.12

A different approach for detecting host-guest binding events in chemosensors is to use competition assays. In a competition assay the reporter element, generally a dye compound, is not covalently attached to the receptor, but is rather loosely associated with the recognition elements via non-covalent interactions. In the presence of the anion, a competition between the reporter compound and the analyte is set up whereby the stronger binding analyte displaces the reporter. Displacement modulates the optical properties of the reporter dye molecule allowing for detection of the binding event via UV/vis or fluorescence spectroscopy.[16]

The advantage of this approach, over covalently attached reporters, is that less covalent bond architecture is required. A disadvantage is that these systems are not useful in applications requiring full imaging of a surface or volume, such as whole cell imaging, and they are single use systems.[16]

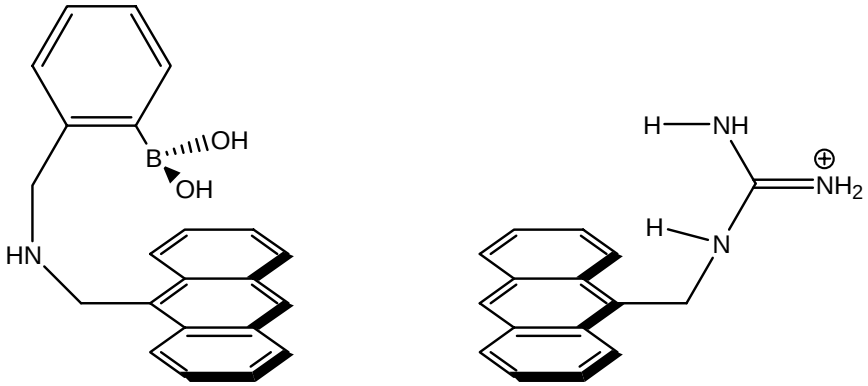
In a February 2003 communication Wang, Yang, Yan, and Fang reported the first fluorescent sensor for D-glucarate based on the cooperative action of boronic acid and guanidinium groups.[160] The sensor **73 (Figure 3.63)** consists of an anthracene backbone to which a guanidinium unit and an amine linked phenyl boronic acid are attached. The design makes use of the reversible formation of cyclic esters of boronic acid with diols in aqueous media and the well-known strong interaction between the guanidinium group and carboxylates.[160,161] Anthracene was chosen as a spacer for both its ability to report binding via fluorescence and molecular modeling analysis indicating that it could optimally orient the two binding elements.[160]

Binding studies were done with a series of structurally similar saccharides on receptor **73** and two control molecules **74** and **75 (Figure 3.64 and Table 3.3).**



73

Figure 3.63 Hybrid chemosensor incorporating the guanidinium and boronic acid binding elements.



74 **75**

Figure 3.64 Control molecules **74** *sans* guanidinium, and **75** *sans* boronic acid binding elements.

Table 3.3 Host-Guest Association Constants (log K_S) of Anionic Saccharides with Receptors **73-75** at 25 ^{o}C in 50% MeOH/0.1 M aqueous HEPES buffer at pH 7.4.[160]

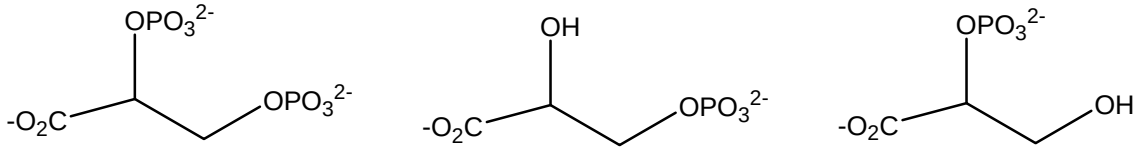
Saccharide	73	74	75
D-Glucarate	3.71	2.93	undetectable
D-Gluconate	3.16	2.83	Not tested
D-Sorbitol	3.11	3.22	Not tested
D-Glucuronic acid	1.66	1.43	Not tested
D-Glucose	1.79	1.26	Not tested

Control **74** is made from the anthracene core attached only to the boronic acid binding element. Control **75** is made from the anthracene core attached only to the guanidinium binding element. Fluorescent measurements on **75** with glucarate indicated little if any detectable association between the substrate and the isolated guanidinium group, so no further testing on **75** was warranted.

Control compound **74** showed the greatest affinity for sorbitol, as expected for a monoboronic acid, and greater than 5-fold less affinity for the target glucarate substrate than did the full-fledged receptor **73**. As expected, receptor **73** showed the highest affinity for the target substrate over the other similar saccharides with ≈ 3.5-fold selectivity over gluconate. The >5-fold (within experimental error) increase in binding stability exhibited by **73** over **74** for glucarate was attributed to carboxylate-guanidinium group interaction present in host **73**, but absent in host **74**.

This study was significant because it represented one of the few examples where fluorescent receptors containing two different binding sites to capture a single species has been reported.[162-164] Another such example has been found and is described in the following passage.

Phosphoglycerate mutase catalyzes the interconversion of 3-phosphoglycerate and 2-phosphoglycerate **(Figure 3.65)**. During the isomerization, 2,3-Bisphosphoglycerate (BPG) is formed as an intermediate. It plays and important role in the regulation of oxygen transport by binding to hemoglobin and decreasing its affinity for oxygen. Abnormal concentrations of BPG may lead to a diseased state making its detection a desirable goal for diagnostic reasons.[165]



2,3-bisphosphoglycerate (BPG) | 3-phosphoglycerate | 2-phosphoglycerate

Figure 3.65 Phosphoglycerate substrates for phosphoglycerate mutase.

An N-oxidized bis-bipyridine complexed europium binding motif tethered to a triethyl dibenzyl amine via amide linkages was synthesized to bind and detect BPG **(Figure 3.66)**.

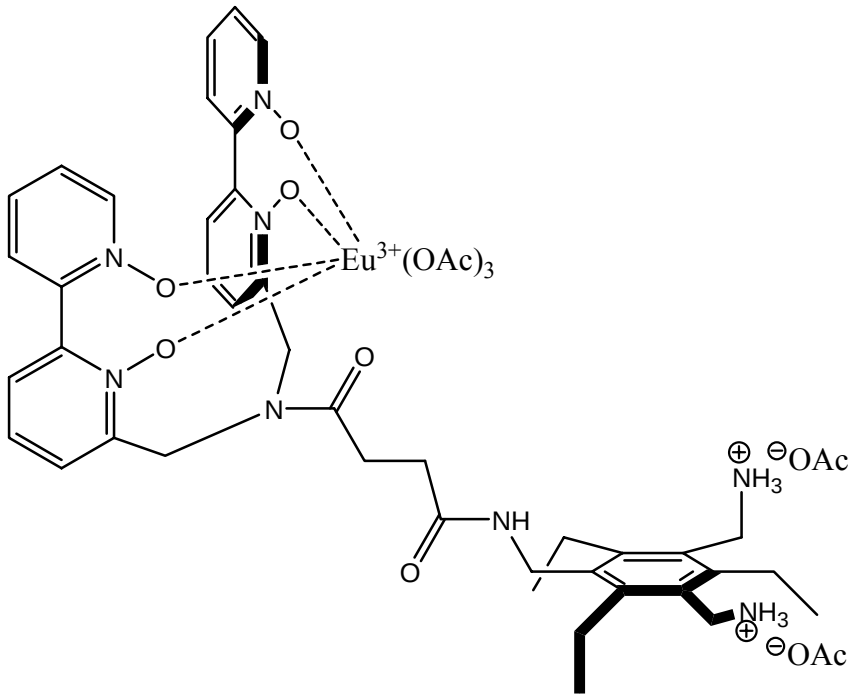


Figure 3.66 Chemosensor laced with europium binding N-oxidized bis-bipyridine units tethered to a triethyl dibenzyl amine binding element via amide linkages.

Detection of the binding event was accomplished by monitoring the fluorescence of the europium bipyridine complex as modulated by anion complexation. Upon displacement of acetate counterions, the europium moiety also served as a binding domain for the β-phosphate group of BPG.

At the benzene end of the receptor, all three amino groups of the precursor were oriented on one side of the ring due to ethyl group sterics. Following amide linkage and protonation, the two ammonium-based anion binding elements were positioned above the aromatic ring poised to synergistically complex the remaining phosphate and carboxylate groups of BPG.[165]

In 50% methanol/acetonitrile, BPG was found to strongly bind with the receptor (log K_S = 5.83) in a 1:1 stoichiometry. Related phosphoglycerates, namely 2- and 3-phosphoglycerate, lacking a second phosphate group bound in a 2:1 ratio to the receptor.

A control receptor lacking the ammonium elements bound BPG in a 2:1 ratio indicating the importance of those elements in maintaining a strict 1:1 binding stoichiometry. Phenyl phosphate, containing only one phosphate group complexed 1:1 with the receptor with a log K_S over three times less than BPG.

3.4 Abiotic Inositol Phosphate Receptors.

The receptors covered in this section serve to narrow the focus of this literature inquiry and zero in on work similar to that presented in this dissertation. The following are recent literature reports of receptors used to host *myo*-inositol 1,4,5-trisphosphate.

Of all the anion binding motifs explored, polyazamacrocycles generally have the highest affinities for anion binding, however incorporating a high degree of specificity to

these types of receptors is usually limited to altering the cavity size.[166] Using an acyclic cleft motif may have an advantage over the macrocycle because the shape is more versatile and often easier to synthesize, however few such receptors strongly bind anions in protic media.[166] In order to detect the nanomolar concentrations of IP_3 typically found in the cell strong binding is required.

Based on evidence suggesting that arginine residues in the endoplasmic reticulum bound natural IP_3, Niikura chose to design and build an IP_3 receptor around a cleft-like guanidinium anion binding motif **(Figure 3.67)**. The synthesis was reported in 1998, and when paired to an optical signaling molecule the receptor is able to quantify IP_3 at nanomolar concentrations.[166]

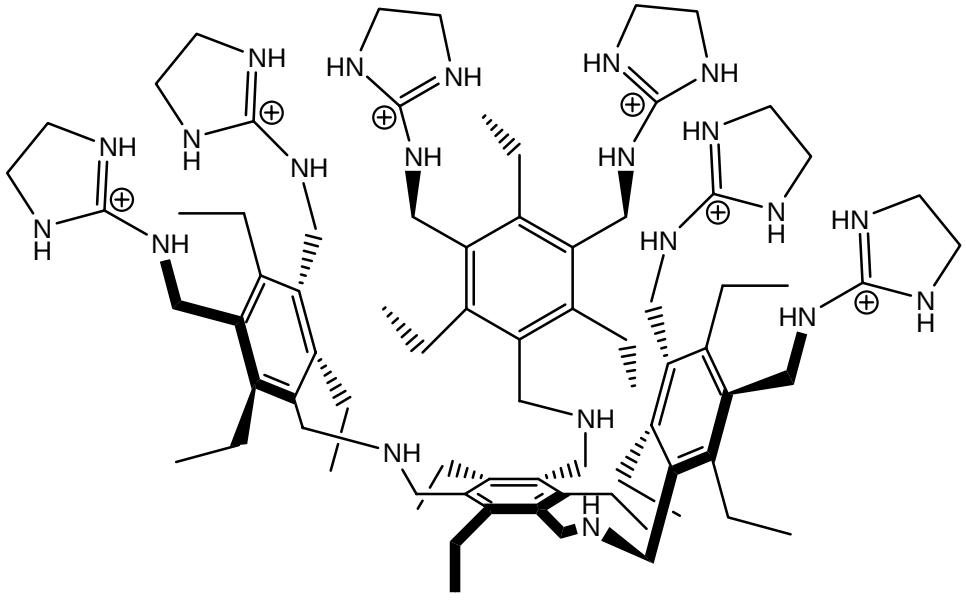


Figure 3.67 Guanidinium-based *myo*-inositol 1,4,5-trisphosphate receptor.

The receptor was synthesized by reacting 1,3,5-tris(bromomethyl)-2,4,6-triethylbenzene with 1-aminomethyl-3,5-[bis(4,5-dihydro-1H-imidazol-2-yl)aminomethyl]-2,4,6-triethylbenzene in methylene chloride. Isolation of the receptor as a white powder followed gel filtration chromatography.

The binding association constants were determined for a group of anions in a competition assay measuring the UV/vis spectroscopic blue shift resulting from anion displacement of the optical signaling molecule 5-carboxyfluorescien (**Table 3.4**).[166]

Table 3.4 Host-guest association constants of various anions with the *myo*-inositol 1,4,5-trisphosphate receptor at 20 ^{o}C, 10 mM hepes buffer (pH 7.4).[166]

Anionic Species	**log K_S**
5-carboxyfluorescien	4.34
myo-inositol 1,4,5-trisphosphate^{3-}	5.67
Benzene-1,3,5-triphosphate^{3-}	5.70
Phytic acid^{6-}	5.88
Adenosine triphosphate (ATP^{4-})	4.36
Fructose-1,6-diphosphate^{2-}	4.34
Citrate^{3-}	3.90
Ethylenediamine tetraacetate (EDTA)$^{4-}$	3.30
Inositol	<2.70

Although seen to bind IP_3 nearly 1000 times better than non-phosphorylated neutral inositol, selectivity among the other biologically relevant anions seems mediocre at best. With the exception of inositol, the best selectivity was seen between IP_3 and EDTA where IP_3 was complexed more strongly by a factor of about 230. This may not be relevant for biological applications of the receptors since EDTA is not a biological molecule. The next best degree of selectivity was seen with the biologically relevant

citrate where nearly a 60-fold binding preference for IP_3 over the tricarboxylic acid was observed.

It is also interesting to note that the receptor slightly favored binding to the fully phosphorylated inositol phosphate (phytic acid) over the triphosphate IP_3 by a factor of about 1.5. Because there is virtually no selectivity between these two IPs, such a host compound would be unable to function along the lines envisioned for our inositol phosphate receptors. The receptor did show an increased affinity for IP_3 in methanol with a log $K_S = 8$, however this too, is irrelevant in biological applications.

Another related area that has received attention lately is the delivery of inositol phosphates through the cell membrane to the cytosol. A critical aspect of this endeavor is the anionic nature of the phosphoinositides making them impermeable to lipid membranes. Both electroneutrality and lipophilicity are required to pass through biological phospholipid membranes.

Along these lines several lipid soluble analogues of inositol triphosphates ($Ins(1,3,4)P_3$, $Ins(1,4,5)P_3$ aka IP_3) and phosphoinositides ($PtdIns(3,4,5)P_3$)[167-169] and caged ($Ins(1,3,4,5)P_4$, $Ins(1,4,5)P_3$, phytic acid) have been synthesized and used in cellular studies.[170]

After crossing the membrane barrier, photoinduced deprotection or enzymatic cleavage of carrier bonds was required to release the active inositol phosphate into the cell. Controlled release of the active phosphates into the cell was often difficult because of multiple carrier bonds undergoing disproportionate cleavage.[171]

Other efforts toward monitoring changes in cellular localization of inositol phosphates are centered around the use of green fluorescent proteins (GFPs) fused to

phosphatidylinositol polyphosphate-specific pleckstrin homology binding domains (PH). The chimeras, produced within the cell, migrate to membrane sites when cells are stimulated.[171]

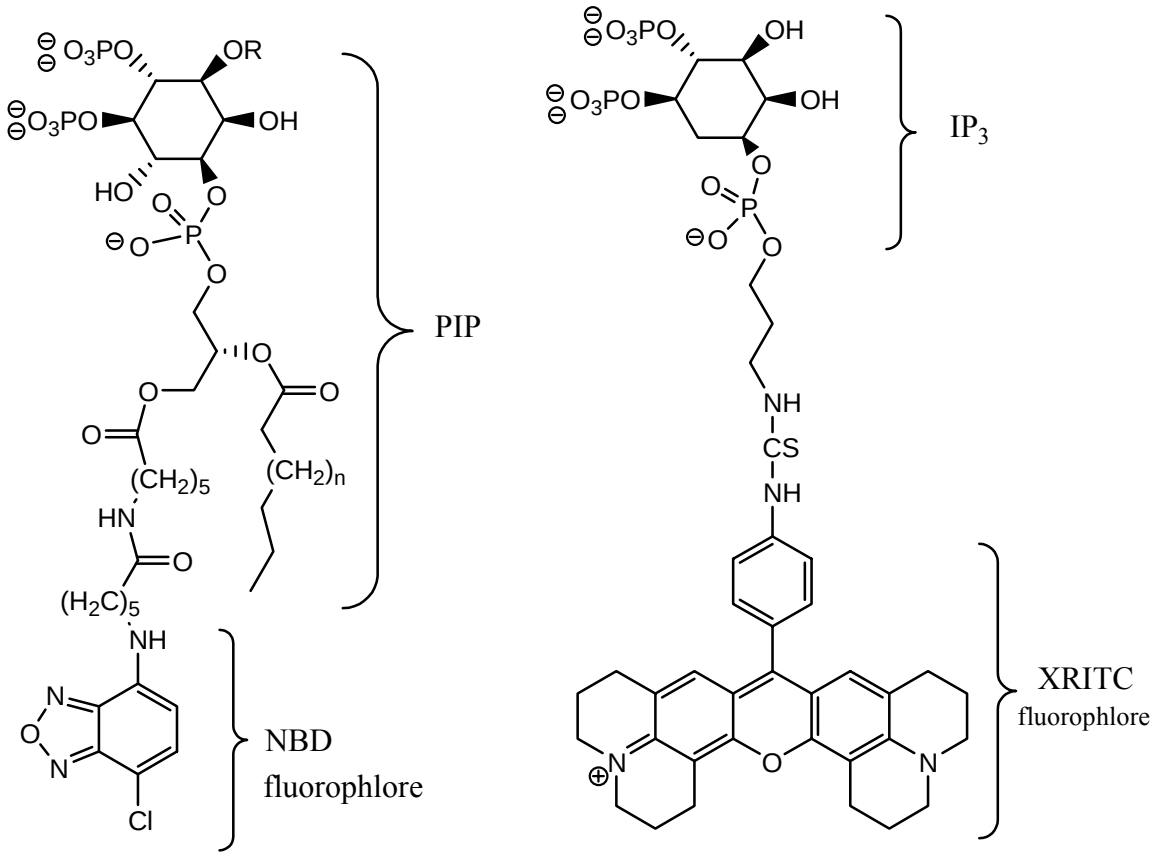
Three deficiencies are associated with the use of GFP-PH fusion proteins. First, the PH domain often binds with free cytosolic inositol phosphate progeny of the parent phosphatidylinositol phosphate, just as well as with the targeted phosphatidylinositol phosphate. Second, GFP-PH constructs have not been observed to traverse intracellular membranes such as the nuclear envelope, endoplasmic reticulum or trafficking vesicles within the cell. This is significant because of the substantial presence of nuclear phosphatidylinositol-4,5-bis phosphate detected in mammalian cells. Finally, these chimeric constructs do not offer a means to deliver exogenously applied phosphatidyl-inositol phosphates.[171]

Ozaki and co-workers developed an alternative approach to inositol phosphate monitoring and delivery from those above in an effort to address some of the limitations therein.

In their approach, fluorescently tagged anionic phosphatidylinositol phosphates (PIPs) or inositol phosphates (IPs) were complexed and neutralized with fluorescently tagged cationic polyamine carriers for rapid cellular internalization **(Figure 3.68)**.

Phosphatidylinositol phosphates PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 were labeled on their acyl tails using a nitrobenzene diazole fluorophore abbreviated as NBD. Inositol phosphate IP_3 was fluorescently labeled with a compound from Molecular Probes known as Rhodamine X isothiocyanate (XRITC). The carrier molecules (not shown) used were

aminoglycoside neomycin compounds labeled with either XRITC or the similar fluorophore known as Rhodamine B isothicyanate.[171]



PtdIns(4,5)P_2-NBD
R = H, n = 1 or 13

PtdIns(3,4,5)P_3-NBD
R = PO_3^{2-}, n = 11

Ins(1,4,5)P_3-XRITC

Figure 3.68 Fluorophlore linked phosphatidylinositol phosphates or inositol phosphates.

The results of the study showed fluorescently labeled PIPs and IPs failed to enter cell cultures in the absence of the neomycin carriers during the 30 minute experimental time frame. Addition of preformed complexes of PIPs and/or IPs with carrier aminoglycosides to cultured cells resulted in rapid cellular uptake of the complex as observed via fluorescent microscopy.[171]

The authors of the study were able to demonstrate the successful delivery of PIPs into mammalian, plant, bacteria, protozoal and yeast cells using neomycin carrier molecules and a dendritic polyamine. Polybasic protein histone carriers (Type III-S) were able to deliver *myo*-inositol 1,4,5-trisphosphate as well as a variety of PIPs into the cells. They also found that carrier delivered IP_3 was able to induce changes in cytosolic calcium concentration as expected, and that the delivery of PtdIIns$(4,5)_2$ resulted in a time delayed increase in calcium levels due to the time lag of PLC hydrolysis action on the phosphatidylinositol phosphate precursor of cellular *myo*-inositol 1,4,5-trisphosphate.[171]

In a recent chemosensor application, the stochastic sensing of nanomolar concentrations of *myo*-inositol 1,4,5-trisphosphate (IP_3) using an engineered transmembrane α-hemolysin (αHL) ion pore was reported by the Bayley group out of Texas A&M.[172]

Stochastic sensing uses single-molecule detection to both quanity and identify analytes. The presence of the analyte is detected as a function of its modulation of current flow through the transmembrane ion channel. The nature of the binding events as measured by such quantities as signal amplitude and signal duration may be used for the identification of analytes.

Proteins known to bind phosphate esters generally do so through the positively charged side-chains of arginine, lysine and histidine residues. Capitalizing on this, it was envisioned that a ring of seven arginine residues could be placed within the lumen of a heptameric α-hemolysin ion pore by replacing a single residue in each of the protein monomers with arginine.[172] Such a protein was engineered, assembled into a channel named αHL-M113R, and put through the conductivity mill.

In the absence of inorganic phosphate the channel αHL-M113R connecting an outer chamber to an inner chamber through an artificial membrane was wide open (conductance 732 ± 9 pS (+20 mV). With phosphate present in both chambers channel blockage was observed. Addition of phosphate to the inner chamber alone failed to appreciably alter membrane conductance, however addition to the outer chamber alone resulted in almost complete channel block (12 ± 3 pS (+20 mV).

Other biologically significant ions, namely nitrate, sulfate, citrate, and perchlorate did not interact with αHL-M113R when administered from either chamber.[172] Biological phosphates were also tested. Administration of cAMP or AMP from the outer chamber did not alter conductance, ADP and inositol 2-phosphate were observed to bind, but more weakly than phosphate. Phytic acid and *myo*-inositol 1,4,5-trisphosphate bound very tightly to the arginine residues within αHL-M113R. None of these molecular phosphates bound from the inner chamber.

Hill plots, Woodhull and kinetic analysis all suggested that in the cases of IP_3 and phytic acid, a single molecule was bound within the pore. Under that assumption, the binding constants (log K_S) were determined to be 7.90 and 6.65, respectively for IP_3-αHL-M113R and IP_6- αHL-M113R complexation. In fact, IP_3 exhibited the strongest

binding of all the phosphates tested and showed about a 2-fold preference over ATP and a suspiciously exorbitant eight million-fold selectivity over inorganic phosphate.[172]

While this initial approach was certainly deemed successful, lining the lumen with 14 arginine residues proved to be even more successful and enabled the detection of IP_3 at the nanomolar level as described below.[172]

Experiments like these conducted with artificial membranes using carefully controlled single analyte solutions cannot hope to mimic the realities of the cellular environment. The interior of the cell contains a veritable mess of small and large, charged and neutral, metabolites of various concentrations at different times that could interfere with stochastic sensing.

To determine if the 14-arginine residue mutant channels were capable of biosensing IP_3 in a more physiologically accurate environment the authors set up some mixing experiments in the presence of ATP and $Mg2^{+}$. These particular species were chosen because of their potential as spoilers due to high intracellular concentrations. Intracellular free magnesium ions are found in approximately 0.5 mM, and ATP ranges from 1-10 mM, with IP_3 typically less than 500 nM.[172]

Results of the experiments showed that the signatures of ATP and IP_3 could readily be distinguished in single-channel recordings of the 14-arginine mutated αHL-M113R pores, and a calibration curve constructed from a typical experiment indicated detection of IP_3 at concentrations of less than 100 nM was possible.[172]

The authors went on to suggest that incorporating engineered αHL pores into a patch electrode that could be inserted into a living cell may function as a real-time sensing device for determining intracellular IP3 and phytic acid levels. They also implied

that additional engineering of arginine, lysine, and histidine sites into the lumen may lead to more specialized pores capable of detecting other biological phosphates of interest.

3.5 Conclusion

It is clear that synthetic chemists have been inspired to mimic nature's designs when it comes to building receptor compounds able to bind anions. The simplest hosts were modeled on the natural polytopic amines spermine and spermidine, spurring a generation of polyazamacrocycles utilizing both hydrogen bonding and electrostatics to entice their guests. Lehn, Park, Simmons and others followed with more complex molecular frameworks seen in the macrobi- and macrotricycles. Aromatic moieties came along as a new recognition element in azacyclophanes designed to intercalate nucleic acids. The biologically inspired guanidinium binding motif has been exploited in both its flexible acyclic form and in a bicyclic sequestered backbone. Examples of hosts relying on electrostatics rather than hydrogen bonding for guest complexation were seen with the family of quaternized nitrogen compounds, as well as the flipside where hydrogen bonding rather than electrostatics was seen in the electroneutral amide, urea and thiourea bearing receptors. Calix-type molecules offered scaffolding upon which to hang a variety of anion recognition groups, and several examples of hybrid receptors utilizing different binding motifs in the same molecule were explored. The relatively new molecularly imprinted polymers have been used to capture anionic species and may hold a bright future in molecular selectivity. A few examples of anion receptors being designed and used in chemosensor applications were also presented.

There is no question that great strides in the field of host-guest chemistry have been made, however the vast majority of the guests being hosted are still simple monovalent inorganic anions such as the halides. A few slightly more complex inorganic anions have been targeted as guests such as the sulfate, phosphate, nitrate and perchlorate species. There have even been some examples of small multiply-charged organic compounds targeted as guest molecules which include the adenosine nucleosides, citrate, tartrate, and some dicarboxylates. Every now and then, carbohydrates pop up as molecular targets for recognition, although this field has seen a recent upsurge. However, in spite of all these advances, the field is still far from reaching any sort of selective binding pinnacle based on Lehn's idea of true molecular recognition and hasn't fully embraced the idea of molecular recognition of linear length in many receptor designs.

To achieve these lofty goals more attention needs to be given to all the design elements present in a receptor for a specific molecule. All physical components of the receptors molecular framework, including spacers/linkers and binding motifs as well as the chemical and spatial aspects of those substructures must be designed to work in concert toward guest complexation.

Our design efforts in this project have been geared to the specific requirements of our chosen class of guests—the inositol phosphates—whereas past efforts in the field seem to rely less on design and more on happenstance when it comes to binding a well-defined molecule. Many anion receptors seem to have been built around known binding motifs with the general goal of binding any ions which happen to come along, a testament to their nonspecificity. Some have been designed to specifically bind to say, carboxylates for example, but not a particular carboxylate, just ones that happen to fit. Few receptors

actually seem to have been specifically designed to bind to only one molecule, the way Lehn probably envisioned. This is where our efforts are different and the design concepts for those efforts will be formulated in the next two chapters.

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CHAPTER 4

THE DESIGN AND SYNTHESIS OF ABIOTIC HOSTS FOR INOSITOL POLYPHOSPHATE GUEST MOLECULES I

4.1 Introduction

Over time evolution is expected to rid the cell of futile metabolic pathways thus streamlining cellular function to maximize efficiency and survivability. We saw in the phosphatidylinositol cycle **(Figure 2.1)**, where IP_3 released from membrane bound phospholipids is reincorporated into the plasma membrane by a complex salvage pathway, that the interesting IP_3 3-kinase emerged to introduce a puzzling branch to an otherwise straightforward inositol recycling pathway.

This seemingly inefficient divergence has spurred us to further investigate the propagation of numerous species of inositol phosphate molecules with the belief that these variants have second messenger properties, like IP_3 before them, and do not represent evolutionary baggage. Offering support to this idea has been the discovery of more than twenty unique inositol phosphates in the cells of eukaryotic organisms, indicating that their presence in the cell is indeed functional , and not merely the by-product of a scenic metabolic joyride.

The long term focus of our research is to synthesize a family of receptor molecules capable of binding in a specific manner to each of the different inositol phosphates found within the cell. The development of such receptors would aid in

elucidating any possible secondary messenger functions of all the inositol phosphates and help other researchers fully explore the nuances of phosphatidylinositol metabolism. In addition to their potential roles as molecular probes, these receptors may also be instrumental as therapeutic agents in the fight against diseases associated with inositol phosphates such as bipolar disease.

Over the years, as clearly indicated by these project goals, this research will evolve into a true interdisciplinary project as it moves away from the bench top and into the realm of molecular biology. In the early stages, receptor design and synthesis development will comprise the bulk of work required for the project's progression.

Work reviewed in the previous chapter underpins the importance of understanding the chemical nature of the guest when it comes to receptor design. A broad range of receptor binding motifs ranging from cationic compounds to electroneutral species to polymers are also at the disposal of molecular architects. Synthetic methodologies for constructing host molecules rounds out the design aspects to be considered.

4.2 The Physiochemical Nature of Inositol Phosphates

The inositol family of isomers can be viewed as hexahydroxycyclohexanes with each of the six ring carbons bearing a single hydroxyl group **(Figure 4.1)**. Inositols have the same molecular formula and mass as hexose carbohydrates ($C_6H_{12}O_6$, m.w. = 180.16 g/mol), but are not hemiacetals.

When viewed as a Haworth projection the alcohol substituents on each of the six carbons can be oriented either above or below the cyclohexane ring. Statistically such an arrangement leads to 2^6 or 64 molecular possibilities based on the inositol constitution,

however molecular symmetry limits permutations to only nine unique isomeric inositol compounds **(Figure 4.2)**.[1]

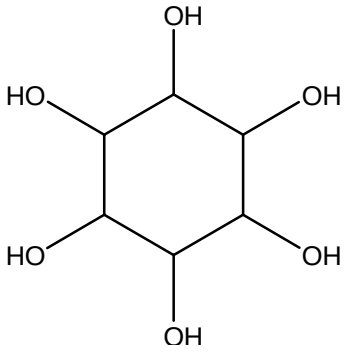


Figure 4.1 The Hexahydroxycyclohexane Structure of Inositol.

Examination of the predominant chair form (maximization of equatorial hydroxyls) of these nine stereoisomers reveals some interesting structural aspects. The only isomer without an axial hydroxyl in its preferred conformation is *scyllo*-inositol. The only inositol isomer containing a single axial hydroxyl group is the infamous *myo*-inositol. A total of four inositols model two axial hydroxyls; *neo*-inositol, *epi*-inositol D-*chiro*-inositol and L-*chiro*-inositol. The remaining three isomers—*cis*-, *muco*-, and *allo*-inositol—all present three axial hydroxyl groups in either chair conformation so they actually ring-flip quite easily.

With the exception of enantiomers D- and L-*chiro*-inositol and *allo*-inositol all of the stereoisomers posses at least one internal plane of symmetry relegating them to meso status. Interestingly the ring-flip conformer of *allo*-inositol is its enantiomer, so with rapid interconversion between conformers it exists as a racemate.[1]

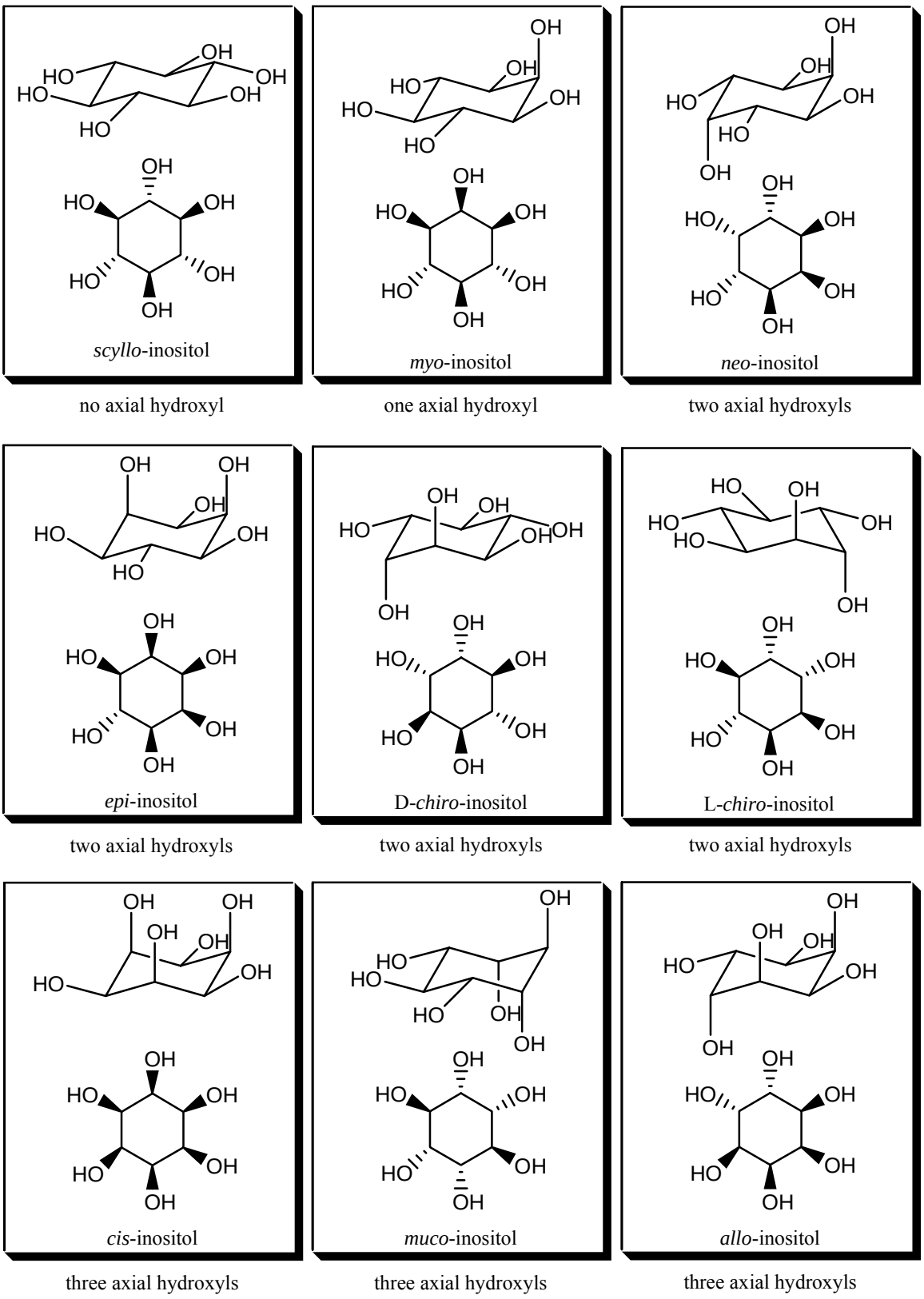
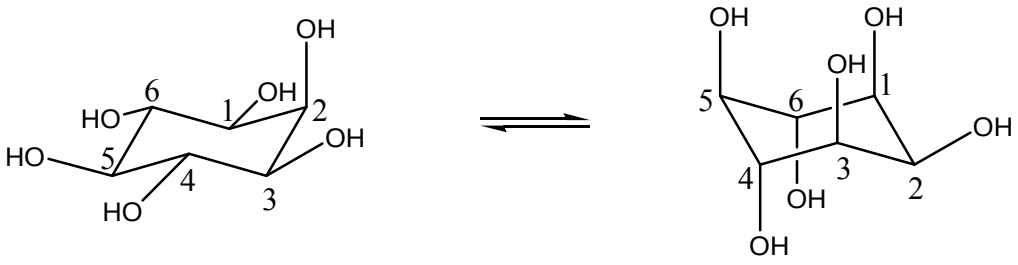


Figure 4.2 The Nine Stereoisomers of Inositol.

Although several members of the inositol family have been found in nature, *myo*-inositol is the most abundant, and is the only one of the nine inositol stereoisomers found in phospholipids. Speculation as to why this is so has to do with it being the only stereoisomer bearing a single axial hydroxyl in its preferred conformation which may act as a recognition handle for enzymes.[1]

As with most saturated six-membered ring structures, the least energetic conformation available to *myo*-inositol is the chair form. There are two readily interconvertible chair forms available to the molecule , the standard form **(Figure 4.3)** with a single axial hydroxyl, and its ring-flipped conformer which is less widely used to illustrate the compound.



standard form

ring-flip conformer of standard form

Figure 4.3 Two interconvertible conformers of *myo*-inositol.

When viewed in standard form, with the perspective of looking down on the ring such that four of the six alcohol groups are projecting above the ring, the axial hydroxyl of *myo*-inositol is attached to carbon number two (C2). The remaining ring carbons are

numbered as indicated such that numbering proceeds around the ring in a clockwise fashion. It is important to note that the clockwise numbering convention applies only to the unsubstituted *myo*-inositol molecule for reasons discussed below.

Although C1 and C2 are chemically equivalent, and C4 and C6 are chemically equivalent, each of these positions are stereogenic and any symmetry breaking substitution of the hydroxyl protons at these positions will generate non-meso optically active derivatives. Since C2 and C5 lie in the symmetry plane, substitution of the hydroxyl protons at either one or both of these positions does not alter the molecules meso status. Any type of reaction leading to substitution of the entire hydroxyl on a particular carbon could result in an inversion of configuration at that carbon, taking you out of the realm of *myo*-inositols and into one of the other eight inositols.

Faced with a bewildering array of chirality nomenclature issues, it was decided by the Nomenclature Committee of the International Union of Biochemistry[2] to retain a rigid numbering system for the carbon skeleton of *myo*-inositol upon substitution rather than using the lowest possible locant rule for new substituents. Upon substitution, the axial hydroxyl seen in the standard view remains attached to C2, however the numbering of the remaining carbons reverses direction and proceeds around the ring in a counterclockwise manner.[1]

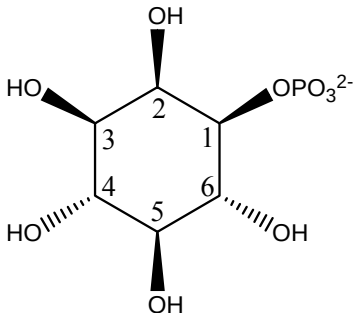
This reversal of the numbering scheme when going from *myo*-inositol to, say, a phosphate derivative, is in deference to the order of attachment to the diacylglyceride backbone enjoyed by *myo*-inositol as a cell membrane phospholipid. The phosphodiester bridge linking the *myo*-inositol moiety to the membrane anchored diacylglyceride in the phosphatidylinositols is rooted at C1 for priority sake. Carbon number one in the

membrane bound lipid corresponds to C1 in any meso-meandering *myo*-inositol derivatives.

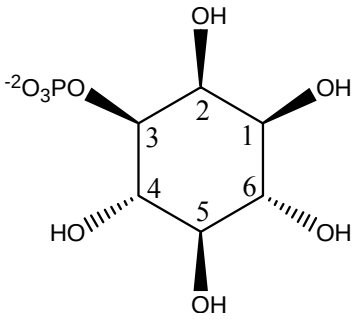
Just as there were many isomeric possibilities inherent in the inositol core structure, so too when it comes to *myo*-inositol phosphates. The total number of permutations when it comes to spreading one to six phosphate groups around the *myo*-inositol skeleton is sixty six.[1] Throw in some cyclic phosphates and pyrophosphates, and that only about two dozen different inositol phosphates have been identified in the cell, its easy to imagine many more second messengers finding employment in the cellular communication business.

With a rigid locant numbering system for also came the need to strictly define the naming of the many *myo*-inositol phosphates. The Ins(n)P_n nomenclature system was implemented and can be illustrated using some monophosphates (**Figure 4.4)**.

If the phosphate group is attached to C1, the long name of the molecule is *myo*-inositol-1-phosphate and the Ins(n)P_n name is Ins(1)P. If the phosphate group is attached to the number three carbon the short name becomes Ins(3)P.



Ins(1)P



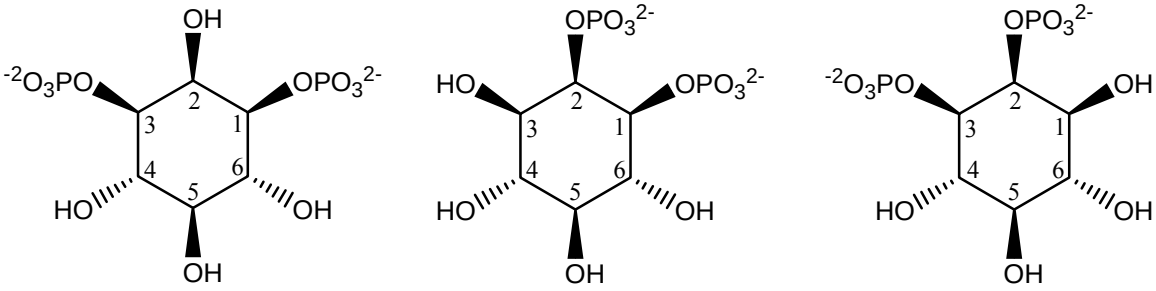
Ins(3)P

Figure 4.4 Enantiomeric *myo*-inositol monophosphates.

One of the downfalls of this nomenclature system is that it obscures important relationships between stereoisomers. For example, Ins(1)P and Ins(3)P are enantiomers—something not immediately obvious from the names.

Understanding the stereochemical relationships between the dozens of *myo*-inositol phosphates takes some practice, however a few helpful hints and examples should smooth the way. The key to this system goes back to understanding the stereogenic nature of C1, C3, C4 and C6, and the mesogenic nature of C2 and C5. Any phosphorylations at C2 or C5 do not upset the mesodom, nor do any symmetrical phosphorylations at enantio-paired carbons. C1 & C3 are enantio-paired as are C4 & C6.

A simple example using some diphosphates will serve to clarify this. Consider Ins(1,3)P_2, Ins(1,2)P_2 and Ins(2,3)P_2 **(Figure 4.5)**.



Ins(1,3)P_2 Ins(1,2)P_2 Ins(2,3)P_2

Figure 4.5 Three *myo*-inositol diphosphates.

Phosphorylation of the enantio-paired C1 and C3 hydroxyls maintains the meso character of the molecule, therefore no enantiomers exist. Phosphorylation at C1 and C2 is a different story because C1 is stereogenic and it loses functional group symmetry with

its enantio-pair C3, thus making Ins(1,2)P_2 part of a pair of enantiomers. C2 is not stereogenic, so substitution at that position is irrelevant.

Once it has been determined that a phosphorylation pattern seen on a particular inositol phosphate gives rise to enantiomers, it may be desirable to identify that compound's enantiomer. This can be done from the numbers alone, without regard to visualizing the structure by using the aforementioned enantio-pair relationships. By knowing that C1 is enantio-paired to C3, simply replacing one with three in the name Ins(1,2)P_2 and retaining the ascending order of the numbers within the parenthesis, gives the name of the enantiomer, Ins(2,3)P_2. Notice that this is the name of the third molecule used in this example and that the mesogenic C2 remains C2 in both names.

This method can be applied to the C4 and C6 enantio-pair as well or combinations of substitutions. Take Ins(1,4,5)P_3 for example, the numbers tell us we have one of a pair of enantiomers because there is either a one without its enantio-pair three, or a four without its enantio-pair six (in this case both are seen). Replacing one with three and four with six, and reordering gives Ins(3,5,6)P_3 the enantiomer of Ins(1,4,5)P_3.

Ins(1,4,5)P_3 is that most famous of all inositol phosphates, being the long confirmed second messenger released from the membrane upon cell surface stimulation, and more commonly goes by its exclusive name, IP_3.

4.3 Design Aspects of Inositol Phosphate Receptors

With twenty five or so known *myo*-inositol phosphates floating around in the cells of eukaryotic organisms and upwards of eighty total possibilities when accounting for

cyclic- and pyrophosphates, the task of designing and building receptors for all these compounds is daunting, and thus requires both a rational and modular approach.

Given the multitude of binding motifs available, as discussed in Chapter 3, many possible design strategies come to mind, however in a effort to prove the validity of our concept the well-established polyazamacrocycle binding motif was chosen for our first generation receptors.

Polyazamacrocycles have a proven history of successful complexation with phosphates and they have often done so with strong affinity resulting from both electrostatic and hydrogen bonding interactions. This is because at physiological pH phosphate groups exist as divalent oxyanions capable of strong ion-pairing interactions and hydrogen bonding, and given the opportunity can also form hydrogen bonding partnerships through lone pair electrons on the phosphonyl oxygen.

The diversity of polyazamacrocycles is also well-documented and arises from the use of macrocyclic, macrobicyclic or macrotricyclic molecular frameworks within which a specific number and variety of amine-based phosphate binding sites can be installed to optimize the complexation of specific inositol phosphate molecules. Specificity will largely come from coordinating binding element topology and the number of positive charges present in the receptors to the physiochemical nature of the target inositol phosphate.

The more preorganized macrocyclic foundation is also expected to provide better specificity than acyclic counterparts, an important consideration in light of the number of potential targets. It will, however be important to retain a certain degree of flexibility in the receptors design to accommodate the phosphate groups tetrahedral geometry.

Examination of some simple *myo*-inositol phosphates **(Figure 4.6)** reveals a three level hierarchy of phosphate patterns that can be exploited for receptor design. Level one is the type of phosphate encountered (isolated, cyclic, or pyro). Level two is the number of phosphate groups encountered and level three is the spatial arrangement of the charged phosphates. These levels are not mutually exclusive as some overlapping may occur. For example the type of phosphate determines the amount of charge carried at one loci and the spatial arrangement of those charges.

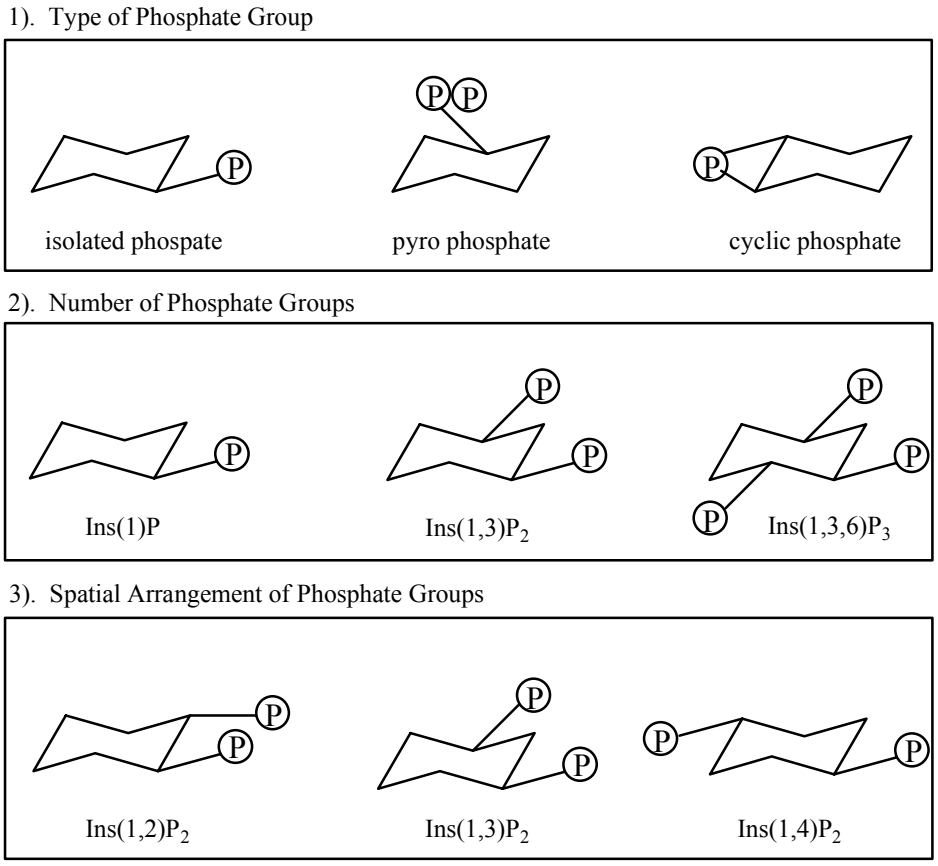


Figure 4.6 Three levels of phosphate patterns that can be exploited for receptor design.

The target compound for which our first receptor was designed is Ins(1,4)P_2. This particular bisphosphate was chosen primarily because of its simple, nearly symmetrical structure placing two phosphate groups at the far ends of the molecule. However, it was also chosen because of its known presence in the inositol recycling pathway.

Inspection of this pathway **(Figure 2.1)** reveals that it is produced by the IP_3 5-phosphatase catalyzed hydrolysis of IP_3, and that is only two metabolic steps away from the phospholipid membrane as the inositol component of phosphatidylinositol. Lithium has been shown to inhibit the inositol monophosphatase responsible for the conversion of Ins(1,4)P_2 to Ins(4)P, and although there has been no direct evidence linking bipolar disorder and the inositol cycle, Ins(1,4)P_2 may have therapeutic potential, thus an abiotic Ins(1,4)P_2 receptor would be of great interest for both mechanistic and therapeutic studies.

For the relatively simple 1,4 bisphosphate arrangement a ditopic receptor with binding elements separated by spacer groups should suffice to complex the target *myo*-inositol phosphate **(Figure 4.7)**.

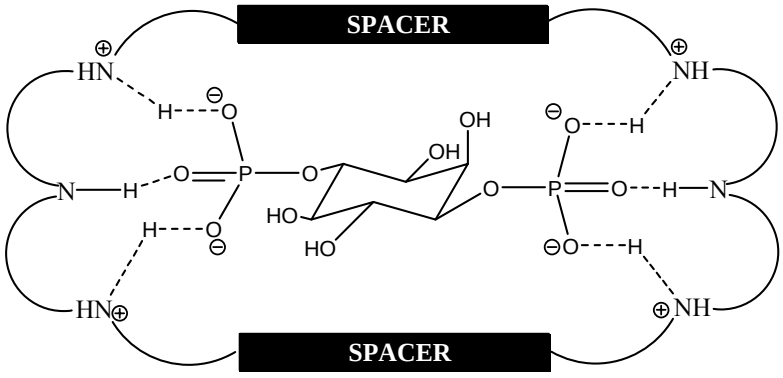


Figure 4.7 Schematic of a receptor for *myo*-inositol-1,4-bisphosphate.

Based on results reported by Dhaenens,[3] such a receptor can be constructed from the appropriate spacers and anion binding motifs, using standard 2 + 2 diamine-dialdehyde condensation chemistry, followed by imine reduction.

One of the important binding motif design factors discussed in section 3.3 was the degree of protonation within a given motif. Recall that the number of methylene groups between successive nitrogen atoms dictates the amount of proximal positive centers in a given region. When the interatomic distance between nitrogen atoms is insufficient coulombic repulsion forces arising from closely spaced like-charges prevents complete protonation. This lack of complete protonation is observed when only two methylene groups (a.k.a. an ethylene group) separate successive nitrogen atoms within the macrocycle.

A speciation graph of diethylenetriamine **(Figure 4.8)** illustrates this idea. This dialkyl triamine, separated by ethylene groups, has multiple-proton equilibria in aqueous solution as exemplified by the six possible protonation states seen in the graph.

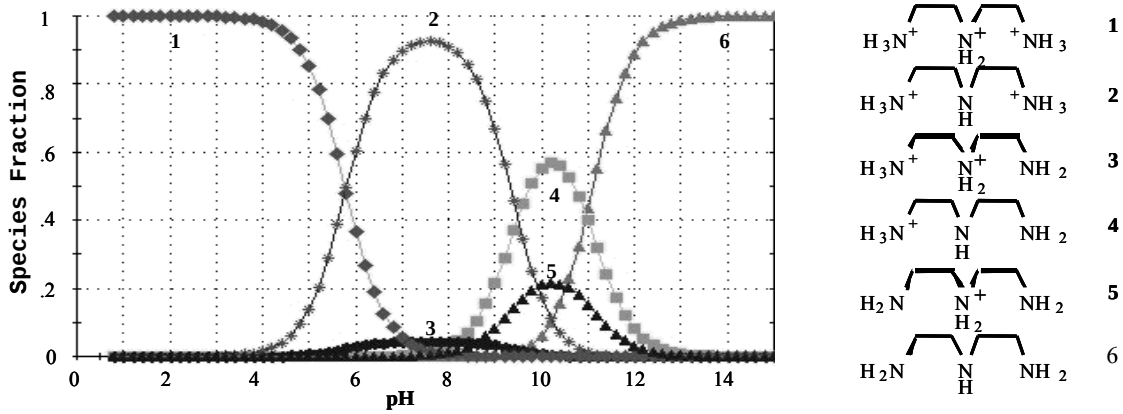


Figure 4.8 Speciation Plot of Deithylenetriamine.

Of special importance to this project is the species predominating the population under normal physiological conditions. In the pH range where our receptors are to be used (7.2-7.5), diprotonated **2**, displaying charged terminal nitrogens, makes up at least 90% of the diethylenetriamine population.

This protonation pattern bodes well in our receptor design for Ins(1,4)P_2 for a number of reasons. For instance, proper spacing of two diethylenetriamine units at opposing ends of an elongated macrocycle is expected to provide a complimentary charge density landscape favorable for Ins(1,4)P_2 binding. In addition, the tetraprotonated receptor perfectly counters the phosphates oxyanions so that upon binding a membrane friendly electroneutral species is obtained. Electroneutrality is a feature generally required of compounds wishing to effectively navigate the amphipathic plasma membrane—a journey we may want ours to take someday.

Although several features of diethylenetriamine make it appealing for use in our first generation of receptors, the commercial availability of some twenty symmetrical, unsymmetrical and chiral diamines, which may also provide suitable phosphate binding sites within the polyazamacrocyclic frame work, contributes greatly to the modular approach of our design strategy. The availability of so many diamines provides a great deal of flexibility in receptor design enabling the tweaking of binding sites for optimum host-guest interaction.

This cache of available amines also provides a means (pun intended) of altering the electrostatics of the binding site. Binding site electrostatics, as determined by the number of ammonium groups in the amine, can be tuned by controlling the methylene

group content between successive nitrogen atoms, thus modulating the net charge of the host-guest complex.

Having decided on the polyazamacrocycle binding motif exploiting the physiological protonation pattern observed in diethylenetriamine, our design attention turned, almost as an afterthought, to the spacer element.

In our original design, stemming from Dhaenens azacyclophane work using a napthalene spacer between diethylenetriamine units, a benzene ring, or phenyl spacer unit was chosen for the prototype receptor.

A few factors contributed to this choice. The first was the long well-known ease with which aromatic aldehydes react with aliphatic amines to form Schiff bases as witnessed in the Dhaenens paper. This reaction is at the core of the macrocyclization of the two receptor components bearing intermolecularly reactive functional groups at each end. However, azacyclophanes of this type with large aromatic spacers such as napthalene, tend not to be water soluble even upon protonation, so could not be used as we envisioned. The smaller phenyl spacer element is expected to allow for water solubility, while retaining the ability to readily form Schiff bases with amines.

There is also a substantial degree of modularity built into the phenyl framework as one could imagine multiple varieties of formylated benzene useful for the construction of macrocycles and macropolycycles. Additionally, it was envisioned that through the rich chemistry of aromatic substitution other groups might be put in place about the ring to better interact with guest *myo*-inositol phosphate molecules. Flourescent substituents could also be readily introduced on the phenyl spacers, thus building an important sensing function into the receptors.

Perhaps the worst factor that should have steered us from our choice was that in essence, cyclophanes are designed for the binding of like-substrates—i.e. aromatic entities. Nothing about our guest targets spoke aromatic to these class of receptors, and no matter how much you dressed them up to attract inositol phosphates, they were hydrophobes at heart seeking water loving fools.

4.4 Design and Synthesis Strategies for Azacyclophane Inositol Phosphate Receptors

While a better plan was being hatched, the design work of phenyl-based polyazamacrocycle *myo*-inositol phosphate receptors continued with the subsequent synthesis of such a receptor using the diethylenetriamine binding motif.

A general macrocyclic structure, incorporating the previously discussed features of a potential Ins(1,4)P_2 receptor, was designed to contain phenyl spacers with -$(CH_2)_m$ between the aromatic ring and the terminal nitrogens of the triamine binding moiety and -$(CH_2)_n$ between adjacent nitrogen atoms **(Figure 4.9)**.

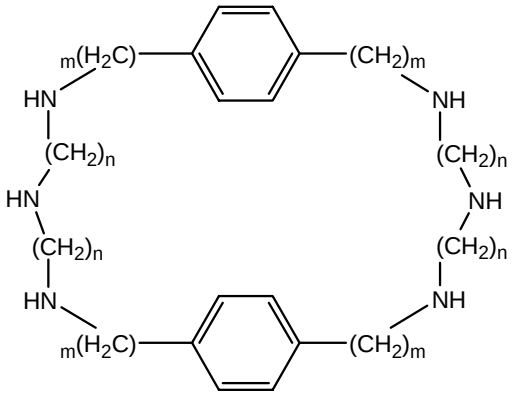
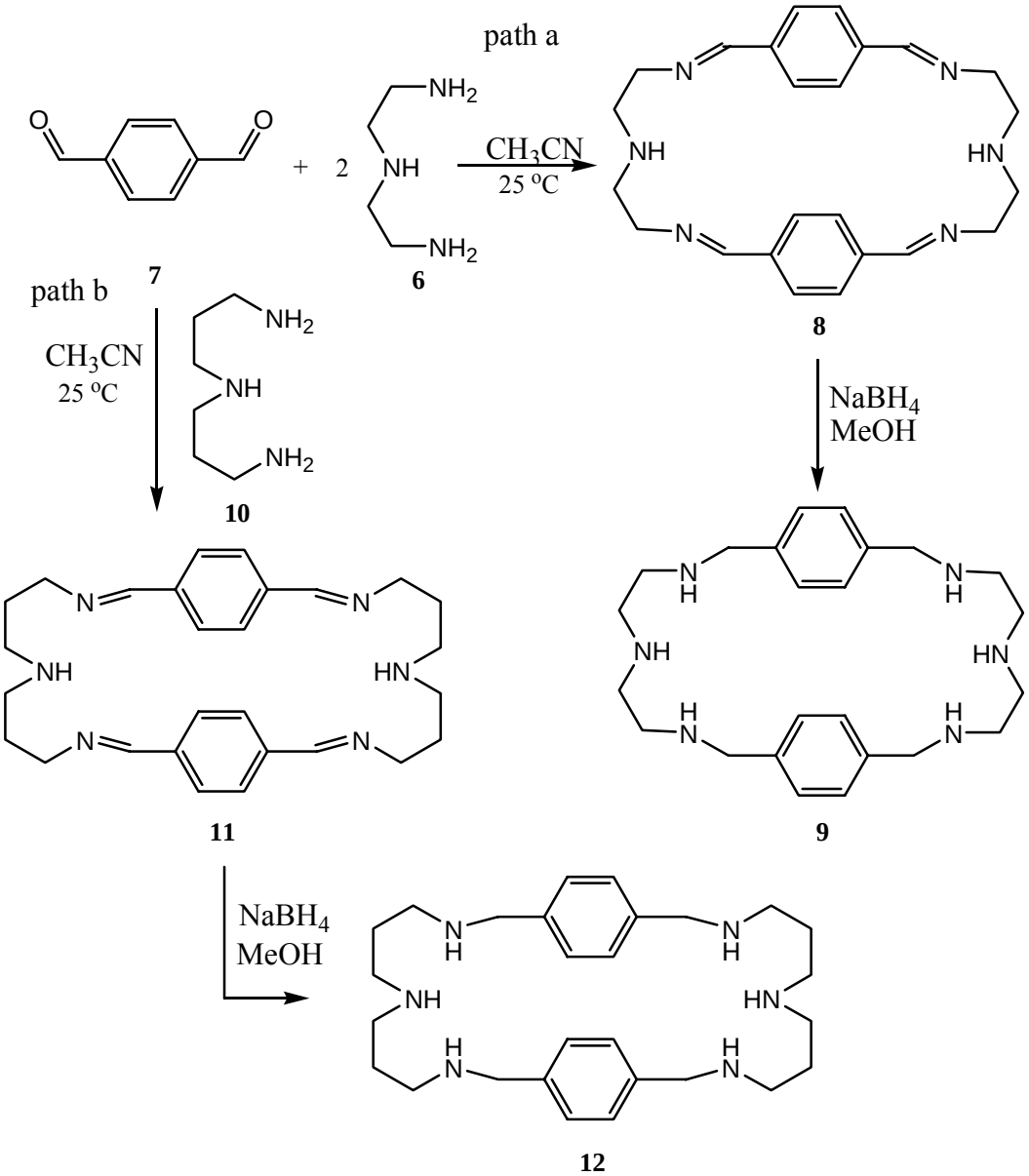


Figure 4.9 General structure for a potential inositol-1,4-bisphosphate receptor molecule.

As an example reaction for this series of -$(CH_2)_m$, -$(CH_2)_n$ receptors **(Scheme 4.1, path a)**, using terephthalaldehyde **7** and diethylenetriamine **6**, equimolar masses of each component are condensed to afford the Schiff base intermediate **8**. Reduction of the resulting tetraimine using sodium borohydride gives the m = 1, n = 2 receptor **9**.[3]

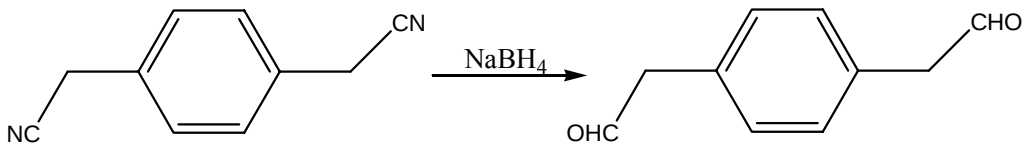


Scheme 4.1

By replacing diethylenetriamine with the commercially available *N*-(3-Aminopropyl)-1,3-propanediamine **10** (path b) an m = 1, n = 3 receptor **12** can be similarly made as well as a multitude of other compounds given the variety of amines readily at hand.

It should be noted that moving away from the m = 1 design has potential synthetic consequences, mainly loss of the use of the readily available terephthalaldehyde as a starting material. A parallel consideration is the necessary change from an aromatic to an aliphatic aldehyde which may adversely affect the Schiff base forming reaction. Nonetheless, design strategies for these type of receptors were extensively explored.

Luckily, the phenyl dialdehyde spacer **14** required as a starting material in the m = 2, n = 2 and m = 2, n = 3 receptors can be produced by reducing the commercially available dinitrile **13** with sodium borohydride or lithium tri-t-butoxy aluminum hydride **(Scheme 4.2)**. Completion of the polyazamacrocycles receptor in this case is accomplished by coupling to the appropriate amine (**6** or **10**).



13 **14**

Scheme 4.2

The synthesis of m ≥ 3, n = 2 or 3 receptors, involve more elaborate pathways. One approach envisioned still makes use of our friend terephthalaldehyde. In this

synthon strategy, the synthons required for expanding the distance between the benzene ring and the nitrogen atoms, to give the corresponding "m" values desired, are the bis-benzylic cation (played by terephthalaldehyde) and an anionic alkyl aldehyde or aldehyde equivalent **(Figure 4.10)**.

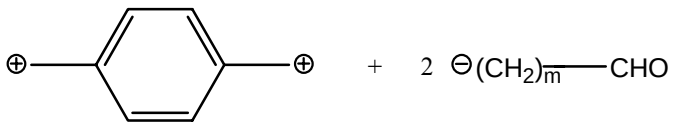


Figure 4.10 Synthons required for the synthesis of m ≥ 3 receptors.

Given the carbonyl function of terephthalaldehyde, one possible player for the other synthon is Grignard reagents. The use of aldehyde containing Grignard reagents necessitates protecting the aldehyde group from intermolecular self-anhilation. Potentially useful aldehydes masked as acetals include the Grignard reagents of 2-bromomethyl-1,3-dioxolane and 2-(2-Bromoethyl)-1,3-dioxolane, which could be used in the construction of m = 3 and m = 4 receptors, respectively. Both of the bromoalkyl dioxolane Grignard precursors are available from Aldrich Chemical Company.

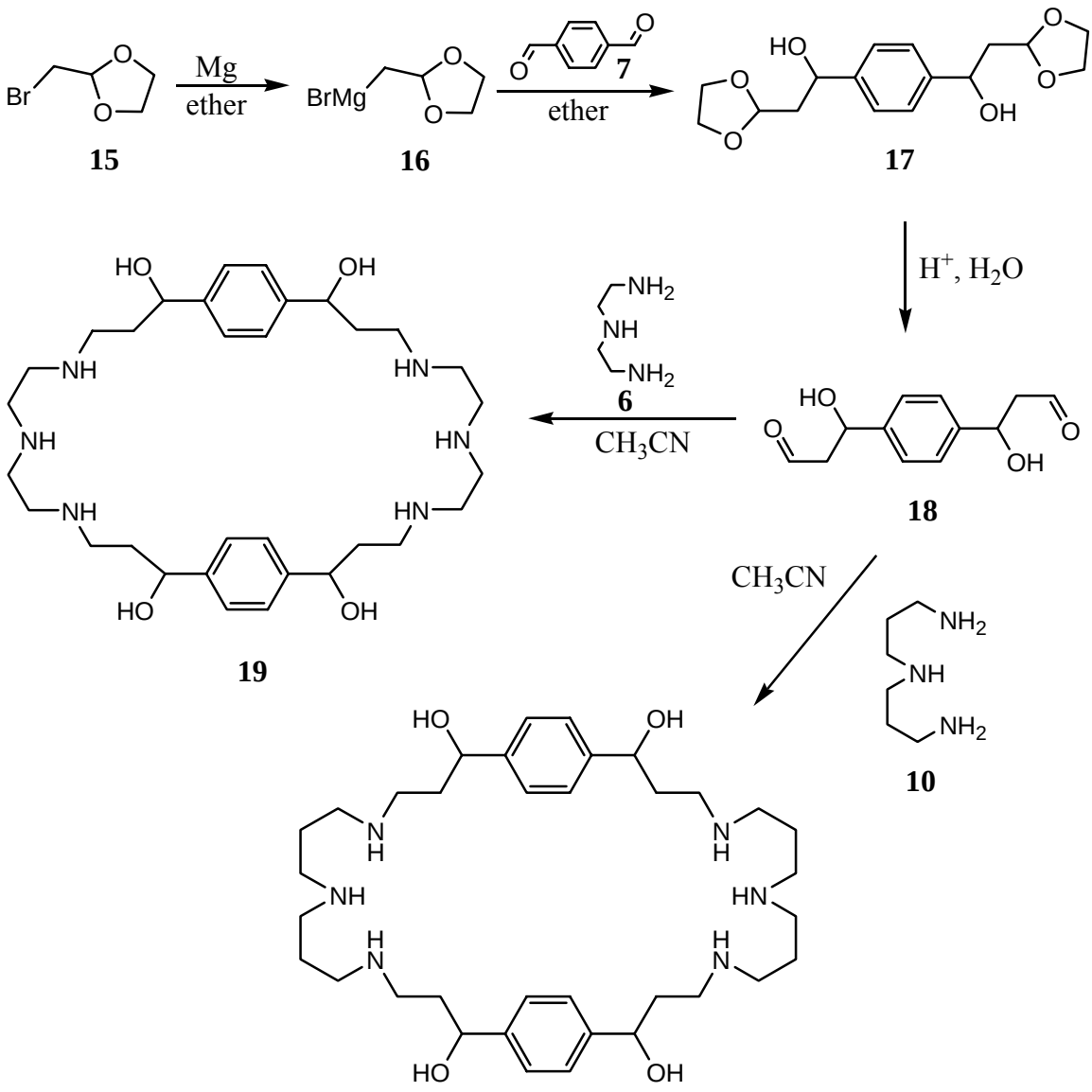
Bromoalkyl alcohols that could easily be converted to their THP-protected ethers, or purchased already protected, were also considered as potentially useful Grignard reagents. Two factors limiting their usefulness are: the possible inopportune unmasking during the acidic Grignard work-up; the nature of the unmasked functional group, being an alcohol, requires an additional oxidation step to give the target aldehyde, so they were not further explored.

The synthesis of receptors where m = 3 and n = 2 or 3 using 2-bromomethyl-1,3-dioxolane **15 (Scheme 4.3)** begins by formation of the Grignard reagent **16** using magnesium in ether or THF. Reaction with terephthalaldehyde **7**, followed by hydrolysis would give the benzylic diol diacetal **17**, which could be unmasked (if the oxo-magnesium hydrolysis hadn't already do so) to give dialdehyde **18**. Cyclization of the dialdehyde with either diethylenetriamine **6** or *N*-(3-Aminopropyl)-1,3-propanediamine **10** would give the desired receptors. A similar route to the m = 4 and n = 2 or 3 receptor could be achieved by starting with 2-(2-Bromoethyl)-(1,3) dioxolane instead of 2-bromomethyl-1,3-dioxolane.

Inspection of the schemes shows that the use of Grignard reagents presents the problem of introducing benzyl alcohols to the spacer component not designed into the original receptors. However, these polar, hydrogen bond donating groups may actually help favor host-guest complexation, be useful handles for fluorescent tagging and/or enhance the water solubility of the macrocycle. For these reasons they can either be left as is, or removed prior to cyclization by a tosylation-reduction sequence.

Straying away from terephthalaldehyde chemistry might be valuable as well. Some of the above Grignard-based homologation reactions using bromoalkyl dioxolanes could also be applied to compound **14** to afford m = 4 and m = 5 receptors.

Another option considered was the use of lithium dialkyl cuprates constructed from the bromoalkyl dioxolanes or the abandoned bromoalkyl THP ethers. The cuprates would likely be quite expensive and would require tosylated benzene diols to instigate spacer group homologation.



Scheme 4.3

4.5 Synthesis of a Prototype Azacyclophane Inositol Phosphate Receptor

As previously mentioned, the prototype receptor chosen to initiate the project, was inspired by Dhaenens and constructed from two diethylenetriamine **6** binding motifs **(Scheme 4.1, path a)** connected by terephthalaldehyde **7** linkers.

Freshly recrystallized terephthalaldehyde was dissolved in acetonitrile and the dilute solution was purged with argon while stirring. A dropping funnel containing a solution of acetonitrile and diethylenetriamine was fitted to the flask and the funnel contents were allowed to drip into the flask over one hour. After the addition, the reaction was allowed to proceed under argon for an additional 36 hours. The reaction became cloudy and light yellow and solids formed. The solids were filtered to afford 900 mg (72%) of light yellow powder as the crude product. Recrystallization was done by dissolving the crude product in dichloromethane at room temperature followed by the slow addition of hexanes to afford 675 mg (54% overall) of very light yellow crystalline tetraimine. The crystals were observed to decompose in the MelTemp® starting around 191^{o}C and continued to do so until evaporating around 250^{o}C.

The first indication of a successful synthesis **(Figure 4.11, S11)** was the loss of the aldehyde proton signal present in terephthalaldehyde and the replacement of those nuclei with less deshielded imine protons resonating as a singlet at 7.54 ppm. The eight equivalent aromatic protons come into resonance as a singlet at 8.30 ppm and the inner and outer methylene protons (8 each) showed up as a pair of triplets centered at 3.0 and 3.8 ppm, respectively. The poorly resolved hump around 2 ppm was believed to be the readily solvent exchangeable nitrogen protons. Further verification of the target imine was provided by ^{13}C NMR **(Figure 4.11, S12)**.

The final piece of evidence, provided by Dr. Dennis Phillips, was given by laser desorption ionization mass spectrometry (LDMS) **(Figure 4.12)**. The molecular formula for the tetraimine macrocycle ($C_{24}H_{30}N_6$) yields a molecular weight of 402.50 g/mol, which closely agreed with the $(M + H)^{+1}$ base peak of 403 *m/z* in the mass spectrum.

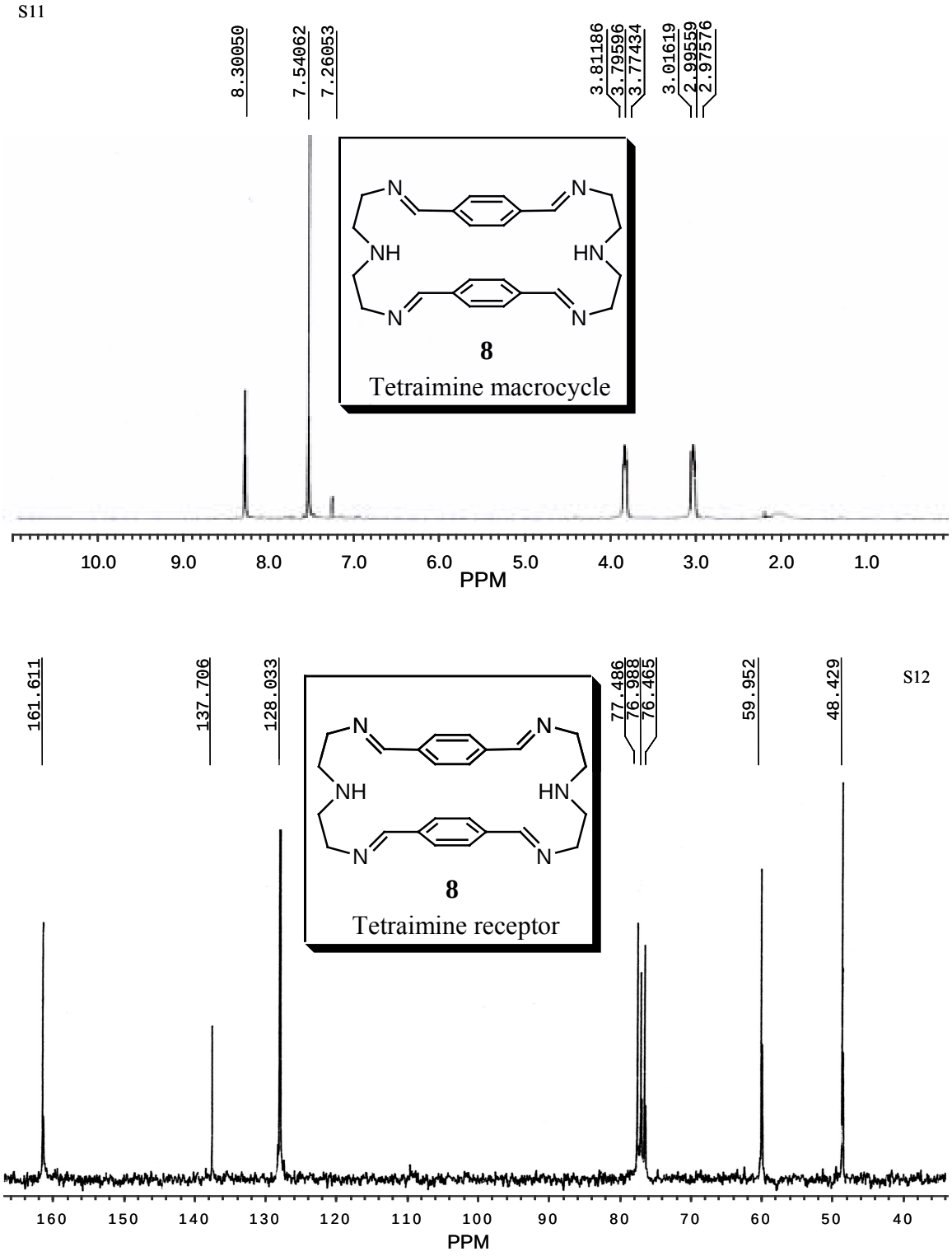


Figure 4.11 The ^{1}H NMR (S11) and ^{13}C NMR (S12) of tetraimine macrocycle **8**.

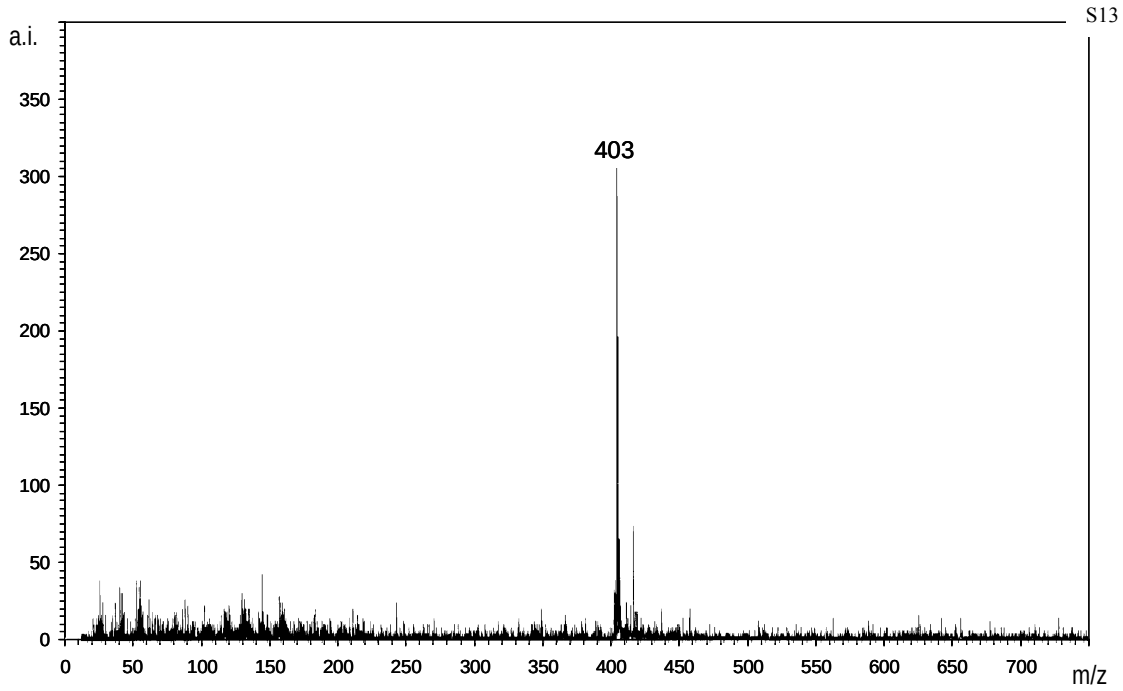


Figure 4.12 The LDMS spectrum of tetraimine **8**.

The cyclization reaction was repeated several times to accumulate sufficient material with only one change—the recrystallization method. Instead of using hexanes in room temperature dichloromethane to bring about crystal formation, the crude reaction product was dissolved in a minimal amount of boiling acetonitrile and enough methanol to complete the dissolution, then allowed to cool to room temperature and stand overnight in the freezer.

The next step required to produce the target polyazamacrocycle was the reduction of the tetraimine to afford the hexamine receptor. A procedure in accordance with the Dhaenens paper[3] using sodium borohydride was employed.

The tetraimine was dissolved in methanol and placed into a 100 mL three-neck round bottom flask along with a stir bar. The system was capped and purged with argon

while stirring on a magnetic stir plate as previously described for the imine synthesis and was maintained under inert conditions throughout the course of the reaction. A solution of sodium borohydride in methanol was dripped slowly to the tetraimine through a dropping funnel.

Following the addition, a reflux condenser was installed in place of the dropping funnel and the solution was allowed to reflux for 45 minutes. After cooling, aqueous sodium hydroxide was added to the flask. Solvent was removed from the reaction under reduced pressure to yield a white residue.

Various attempts to precipitate the hydrochloride salt of the target hexamine by dissolving the residue in hot tetrahydrofuran (THF) and a small amount of methanol followed by treatment with hydrogen chloride (g) saturated THF failed.[4] Attempts to crystallize the hexamine solids from aqueous solution via THF diffusion into the water layer also failed. Several attempts to extract the putative hexamine from an aqueous solution of the evaporated residue into dichloromethane (both in a separatory funnel and over several days in a Soxhlet extractor) and 1-butanol failed as well.

A new approach from the tetraimine to the hexamine was taken. With the aid of Phil Robinson, then a graduate student in Dr. Phil Bowen's lab, heterogeneous catalytic hydrogenation of the tetraimine was conducted.

The tetraimine was dissolved in methanol and placed into a Parr® shaker reaction vessel along with palladium on carbon catalyst. After several cycles of oxygen purging and nitrogen back flushing, pressurized hydrogen gas (pressure unknown) was introduced to the vessel and it was allowed to react for five hours.

Suction filtration through a layer of Celite in a glass scintered funnel was used to separate the catalyst from solution. The methanol filtrate was evaporated under reduced pressure on a room temperature water bath to afford nearly white solids.

A significant loss in color between the starting tetraimine and the putative hexamine, recovered from the hydrogenation reaction, was observed indicating a loss of conjugation. The solids were recrystallized from boiling acetonitrile following cooling of the solution to room temperature to give a 68% yield (≈ 40% overall from terephthal-aldehyde) of fine, short needles having a fluffy, cotton like appearance and a sharp melting point range of 143-144 ^{o}C.

The molecular formula for the hexamine macrocycle ($C_{24}H_{38}N_6$) yields a molecular weight of 410.56 g/mol, which closely agreed (0.15% difference) with the $(M + Na)^{+1}$ sodiated base peak of 432.9 *m/z* in the relatively clean mass spectrum as determined by LDMS **(Figure 4.13)**.

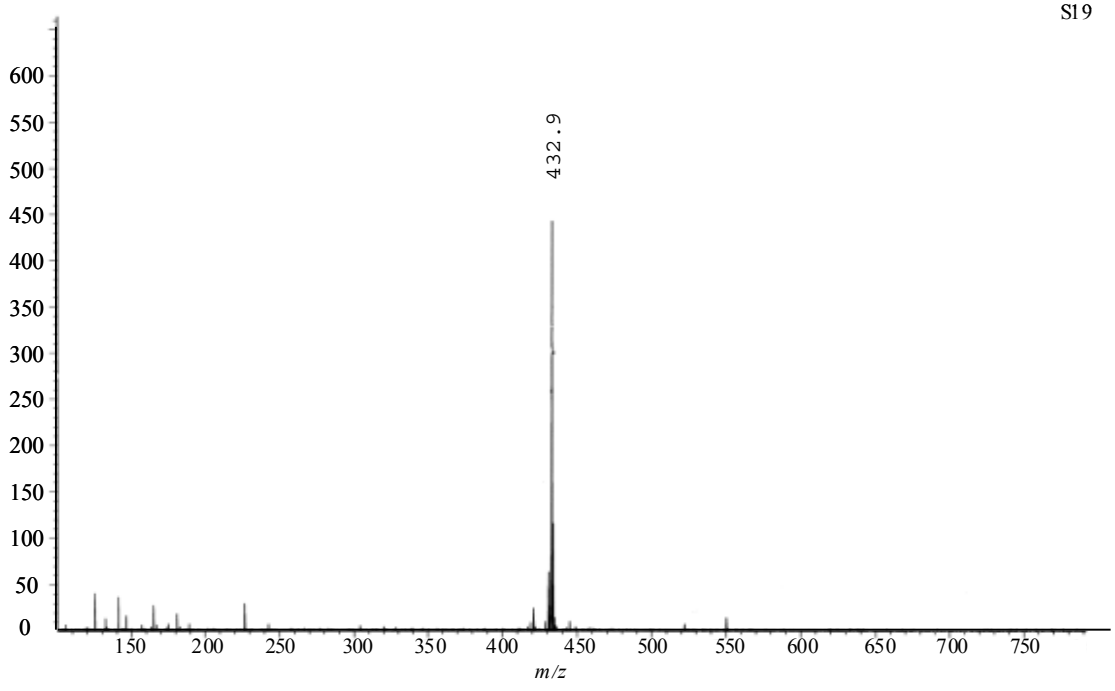


Figure 4.13 LDMS spectrum of hexamine receptor **9**.

Inspection of the ^{1}H NMR for the hexamine **(Figure 4.14)** (Varian/Unix Mercury 400 MHz NMR) was disturbing due to the apparent lack of aromatic protons. They may buried in with the $CDCl_3$ signal at 7.27 ppm. The singlet at 3.78 ppm can be assigned to the benzylic protons and the pair of leaning triplets, possibly quartets, centered at 2.78 and 2.80 ppm (see expansion) belong to the non-benzylic methylene protons. The broad peak centered at 1.6 ppm is assigned to solvent exchangeable nitrogen protons. Integration of the four signals, including the 7.27 ppm solvent/aromatic peak, corresponds well to the number and type of protons present in the hexamine structure.

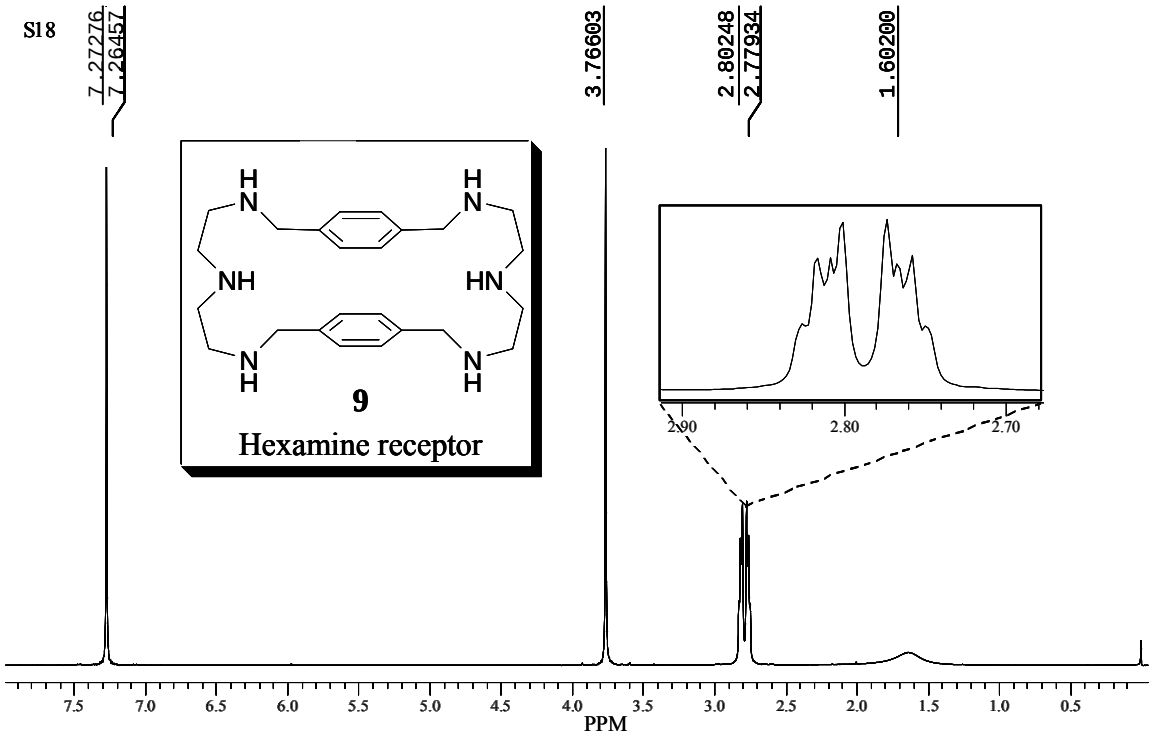
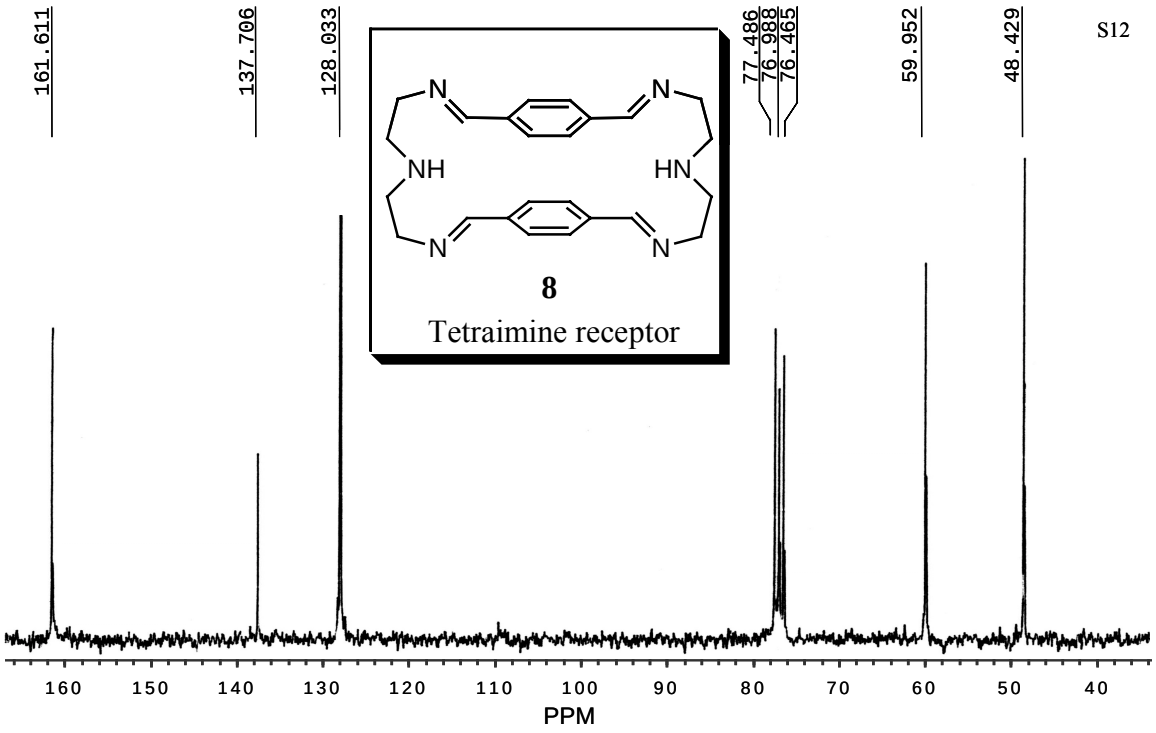


Figure 4.14 The ^{1}H NMR of hexamine receptor compound **9**.

Comparison of the ^{13}C NMR of the tetraimine to the hexamine **(Figure 4.15)** shows the upfield movement of the imine carbon from ≈ 161 ppm to ≈ 53.7 ppm, a region associated with methylene carbons.



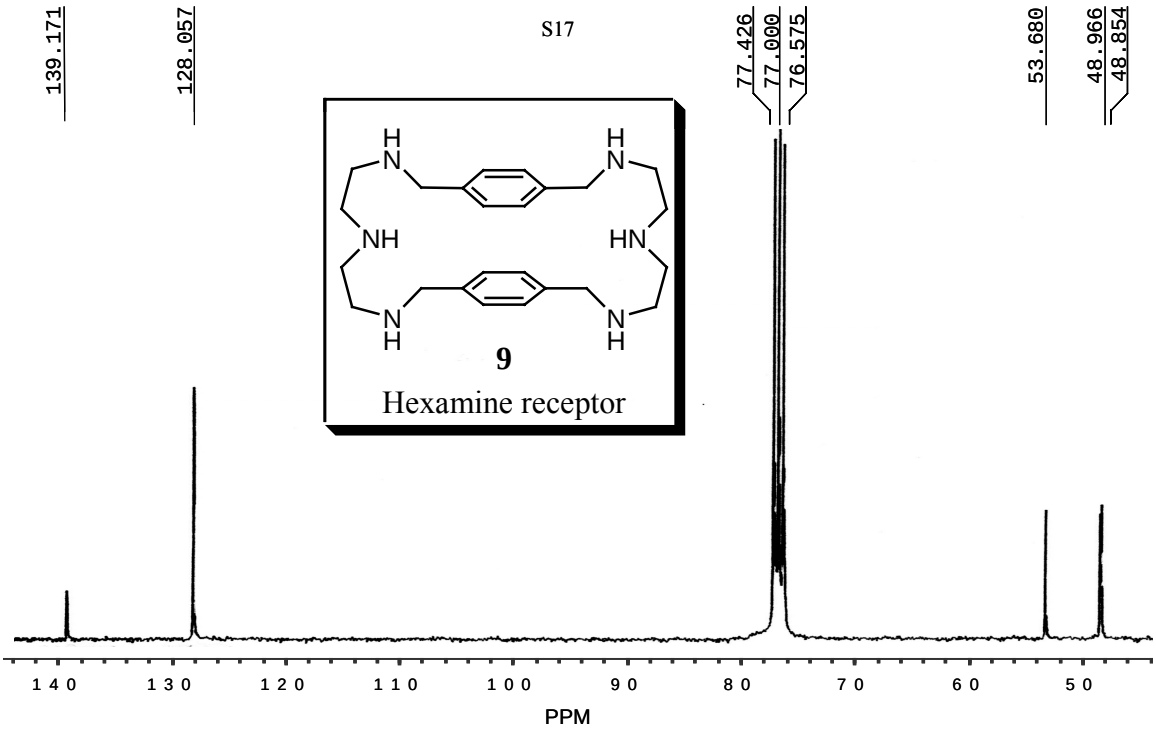


Figure 4.15 The ^{13}C NMR of tetraimine **8** (S12) and hexamine **9** (S17).

4.6 Conclusion

Although synthesis of our first generation prototype azacyclophane receptor was successful, some Corey-Pauling-Koltum (CPK) van der Waals radii modeling studies indicated the m = 1 framework was actually too small to allow entry of the Ins(1,4)P_2 guest into the macrocycles cavity. Providing complete access to the host cavity required a phenyl to external amine nitrogen distance of between 3 and 4 carbons.

The modeling studies also indicated that an n = 3, rather than an n = 2 quantity of methylenes between the terminal/external nitrogen atoms provided a more complimentary fit between phosphate oxygens and the pro-ammonium nitrogen binding elements.

Given these findings, the synthesis focus was shifted from slowly building out the m = 1, n = 2 receptor, to immediately constructing an m = 4, n = 3 supersized receptor using some of the homologation techniques discussed in section 4.4.

As the synthesis of these class of receptors, based on phenyl spacers and triamine binding elements began building steam, the whole idea of coming to the ball in search of hydrophilic aliphatic dancing partners in a hydrophobic aromatic carriage continued to eat away at us. It was becoming clear that there must be a better fit and one day, in the eleventh hour, Dr. Johnson came to me, glass slipper in hand—*myo*-inositol phosphates.

4.6 Experimental

General experimental aspects. Acetonitrile, methanol and diethylenetriamine were purchased from Aldrich Chemical Company. Acetonitrile and methanol were dried and distilled prior to use and diethylenetriamine, a nasty toxic liquid, was used from the

bottle without further purification. Terephthalaldehyde (original supplier unknown, obtained from Dr. Chu's lab) was recrystallized using 700 mL of boiling water to initially dissolve 25 g, which upon evaporating to 100 mL followed by slow cooling produced 13 g of slightly yellow needle-like crystals with a sharp melting point (116^{o}C). Carbon and ^{1}H NMR spectroscopy were performed on either a Bruker 250 or 300 MHz instrument using $CDCl_3$ (Isotech) unless otherwise noted. Industrial grade argon purchased from National Welder's Supply (Charlotte, NC) was used as indicated. Melting points were determined using a Mel-Temp II® apparatus from Laboratory Devices using a standard mercury thermometer and are uncorrected for atmospheric pressure.

Diphenyl Hexaaza Tetraimine Cyclophane (8). Into a 500 mL three-neck round bottom flask fitted with a rubber septum in one side-neck was added 250 mL of acetonitrile, 832 mg (6.2 mmol) of terephthalaldehyde and a magnetic stir bar. A glass stopper was placed in the other side-neck. Argon was allowed to flow into the flask through a needle penetrating the rubber septum while the solvent stirred on a magnetic stir plate. The center neck was connected to a vacuum-pump oil filled bubbler via glass adaptor and Tygon® tubing as an argon outlet. The system was purged with argon while a pressure equalized 500 mL dropping funnel was charged with 250 mL of acetonitrile and 640 mg (6.2 mmol) of diethylenetriamine. The argon outlet tube was removed to the top of the dropping funnel and the funnel was fitted to the center neck in its place. The system was allowed to purge with argon an additional thirty minutes before the stopcock was opened sufficiently to allow slow dripping of the funnel contents into the round bottom flask over a period of one hour. Following the complete addition of the dropping funnel solution, the reaction was allowed to proceed under argon for an additional thirty

six hours. Over the thirty six hour period the flask contents became cloudy and light yellow in color and solids formed. Water aspirated vacuum filtration yielded 900 mg (72%) of light yellow powder as the crude reaction product. Recrystallization of the crude product from 20 mL of room temperature dichloromethane, followed by the slow addition of 45 mL of hexanes provided 675 mg (54% overall) of very light yellow crystalline tetraimine. While determining their melting point, the crystals began decomposing ≈ 191°C and continued to do so until evaporating ≈ 250°C. ^{1}H NMR ($CDCl_3$) δ 8.30 (s, aromatic), 7.54 (s, imine), 3.79 (t, aliphatic), 2.99 (t, aliphatic), 2.00 (m, amine). ^{13}C NMR δ 161.61, 137.71, 128.03, 59.95, 48.43. MW for $C_{24}H_{30}N_6$ = 402.54 g/mol, exact mass = 402.25 g/mol, found $(M+H)^+$ *m/z* 403 via LDMS.

Diphenyl Hexaaza Tetraamine Cyclophane (9). Into the Parr® shaker reaction vessel was placed 942 mg (2.34 mmol) of tetraimine (**8**) dissolved in methanol (volume not recorded, but probably dictated by reaction vessel) and 503 mg palladium on carbon catalyst. After several cycles of oxygen purging and nitrogen back flushing, pressurized hydrogen gas (pressure unknown) was introduced to the vessel and it was allowed to react for five hours. Water aspirated filtration through a layer of Celite in a glass scintered funnel was used to separate the catalyst from solution. The methanol filtrate was evaporated under reduced pressure on a room temperature water bath to afford nearly white solids. The solids were recrystallized from 75 mL of boiling acetonitrile following cooling of the solution to room temperature to yield 655 mg (68% from tetraimine, ≈ 40% overall from terephthalaldehyde) of fine, short needles having a fluffy, cotton like appearance. Melting point 143-144 °C. ^{1}H NMR (Varian/Unix Mercury 400 MHz NMR) ($CDCl_3$) δ 7.27 (s, aromatic, 8 H), 3.76 (s, benzylic, 8 H), 2.80, 2.77 (t, methylene,

16 H). ^{13}C NMR (Varian/Unix Mercury 400 MHz NMR) ($CDCl_3$) δ 139.17, 128.06, 53.68, 48.97, 48.85. MW for $C_{24}H_{38}N_6$ = 410.60 g/mol, exact mass = 410.32 g/mol, found $(M+Na)^+$ *m/z* 432.9.

4.8 References

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CHAPTER 5

THE DESIGN AND SYNTHESIS OF ABIOTIC HOSTS FOR INOSITOL POLYPHOSPHATE GUEST MOLECULES II

5.1 Introduction

Like with Cinderella and her Prince Charming bonding requires strong interaction between proximal entities. Had she not been enticed to the ball, the mean stepsisters may have prevailed and forever relegated her to a life of sweeping cinders. Developing a more enticing host, with which to search the kingdom of competing guests, became a priority, with the ultimate goal of creating the perfect glass slipper for our phosphorylated guests.

During the initial receptor design phase not much thought was put into the spacer elements responsible for separating the polyaza phosphate binding elements. A cyclophane design was chosen more as a matter of convenience rather than for any compelling physiochemical attributes. The ready availability of benzene derivatives and seemingly easy ability to manipulate the constituent lengths through different homologation approaches made phenyl spacers an attractive option. The capacity to carry fluorescent tags also played into the decision, but that was about the extent of determining factors. Re-examination of the needs of the guest led to the design and development of a new class of receptors, for which there is little precedence.[1]

5.2 Design Aspects of Secondary Binding Elements

True molecular recognition, the pinnacle of host-guest chemistry, requires very specific complexation interactions well beyond the universal electrostatic force seen in general ion pairing.

Conceptually, this idea is certainly not unheard of in the world of host-guest chemistry as chemists realized it was the sum total of all types of binding interactions which led to stronger complexation and ultimately selectivity. However, examination of the major milestones achieved in artificial receptor design (cryptand-cation, polyaza-anion, cyclophane-aromatic, boronic acid-sugar, and guanidinium-carboxylate partnerships),[2] revealed that little thought had been put into optimizing spacer components for substrate interaction.

The major focus in the synthesis of these systems has been—and in many instances continues to be—the interaction between charged anion regions and receptor binding elements and has largely ignored the peripheral aspects of the guest. Receptor architects having seemingly been blinded by the drive to build better and more diverse anionic recognition sites and have lost sight of potential spacer-guest interactions as a means to attain selectivity and true molecular recognition.

Of the milestone receptors synthesized to date, only the cyclophanes ostensibly incorporated this recognition algorithm by having intercalating spacers connecting charged binding elements, however as it turned out, the spacers actually provided the bulk of the binding interaction with electrostatics engaged more in aqueous solubility.

In spite of a few efforts to the contrary, a review of the literature has shown the primary purpose of the spacer component in receptor design has historically been, well,

spacing—to achieve linear recognition of molecular length through the appropriate separation of binding elements. It is our belief that in order to obtain stronger binding and greater selectivity, the role of the spacer component must be greatly expanded and serve not only to template molecular length in the guest but also to actively engage in the physiochemical recognition of substrate attributes. This expanded role for spacer components elevates their status from linkers to secondary binding elements, stressing their importance in the selective binding process.

To be sure, this idea of secondary binding interactions with spacer elements is not completely new as indicated by the following passage in a 1997 review by Schmidtchen and Berger entitled "*Artificial Organic Host Molecules for Anions*:"

"The condensed phase binding constant measures the difference in free energies between the host-guest complex and the separated fully solvated partners and thus does not reflect the total intrinsic free energy of association. Because of this it is conceivable that structural modifications at sites remote from the actual binding sites may affect the binding strength of the bimolecular association."[3]

An extension of this idea in the same review article states: "The problematic aspect (of building spacer segregated binding sites into a receptor) is how to define an anchor (spacer) group knowing that in principle every part of the entire structure contributes to overall binding and any structural segregation is ultimately arbitrary and artificial."[3]

Since binding effectiveness and selectivity is a free energy driven function, where often the enthalpic cost of desolvation is equally balanced by the enthalpic gain of complexation, the decision on which state is more thermodynamically stable resides in

the realm of entropy. When it comes to ion pairing interactions between anionic guest molecules and oppositely charged amine-based binding elements the change in entropy associated with chaotic desolvation wins out, solvent is shed, and complexation occurs between the partners.

The chemical nature of the binding interactions between polyazamacrocycles and their anionic guests is two-fold. One can envision that electrostatic repulsion forces help in the preorganization of the binding sites by expanding them to the size needed to fit the guest. The electrostatic attractive (ion pairing) forces between oppositely charged regions on the receptor and substrate bring the two compounds within binding range. The initial "ice-breaking" is followed with multiple, cumulative, non-electrostatic interactions between all regions of the pre-organized receptor and the proximal guest providing the final push toward strong association of the two binding partners at the expense of excluded solvent molecules.

Guest attributes already considered in the design of the primary polyaza binding elements **(Section 4.3)** include the type, number and position of phosphate groups. By incorporating well-designed secondary binding elements into a receptor compound already containing well-established nitrogen-centric primary binding elements, we can tap into a previously unexplored synergism of molecular recognition.

Tapping into this idea of holistic binding synergy requires a much greater degree of receptor engineering than seen in the past. Optimizing the receptor for substrate binding requires the installation of carefully thought out secondary binding elements designed to enhance the primary electrostatic interaction. In this manner, the sum total of

molecular interactions throughout the host-guest complex may be amplified to bring about greater thermodynamic stability.

Our inositol phosphate guests provide a rich environment of non-electrostatic regions which may be exploited alongside the negatively charged phosphate groups in the design of secondary binding elements. Some of the physiochemical guest properties we can take advantage of are hydrogen bonding with free hydroxyls, hydrophobic interactions with the cyclohexane core and molecular topography including features like length, shape and most importantly, chirality—in short those attributes which aid in the recognition of self.

Having said that, a key design feature in the second generation of receptors was the incorporation of *myo*-inositol into the *myo*-inositol phosphate receptor architecture as a self-recognizing secondary binding element. This strategy provides pre-organized, chiral binding sites affording precise three-dimensional molecular cavities into which the desired substrates may penetrate. To a great extent, this approach automatically defaults to the idea of linear recognition of molecular length. Additionally, by incorporating substrate stereochemistry into the structure of its own receptor, we take advantage of crystal packing-type forces stemming from hydrogen bonding interactions between highly compatible secondary binding elements and guest molecules.

While there is no question that using inositol compounds as secondary binding elements will improve the intrinsic stability of the host-guest complex, it is expected that these contributions will be small relative to electrostatic interactions at the primary binding site. One concern is that any stability enhancements gained through secondary binding element engineering, may still be negated by desolvation enthalpy requirements.

However, it is conceivable that the boost seen with crystal packing forces, and the cumulative effect of several potential spacer-guest modes of interaction, may lead to greater obligatory shedding of solvent molecules, thus increasing entropy and favor the free energy of binding. One thing is certainly clear—spacer elements between primary binding sites should not be overlooked in receptor design.

Through interactions of the binding elements with both the inositol and the phosphate groups, our receptors should possess strong affinity and selectivity for the targeted inositol phosphates, thus distinguishing them from their isomers and from other biological phosphates. Although inherently simple, this approach combines several features that are seldom found together in previously described synthetic receptors.

5.3 Design and Synthesis Aspects of Inositol-based Inositol Phosphate Receptors

Many of the design and synthesis strategies hammered out in our first generation polyazacyclophane receptors could be readily adapted to this new generation of proposed molecular hosts. The amine-based primary binding elements remained intact and formed the electrostatic foundation on which to build secondary binding elements. The target inositol phosphate, Ins(1,4)P_2, also remained unchanged, such that the basic macrocyclic framework needed is similar to the phenyl-based receptor already synthesized.

The cyclization approach by way of imine formation between a dialdehyde and diethylenetriamine, followed by reduction, was kept as well. The only difference being the identity of the dialdehyde, where an inositol derivative substitutes for the phenyl dialdehyde. Do recall however, that macrocycle size was an issue of concern with the cyclophane receptor, and it was determined that some distance between three and four

methylene groups away from the phenyl ring was required for optimal spacing of the polyaza binding elements.

Interestingly, new modeling studies showed the same distance requirements for an inositol-based Ins(1,4)P_2 receptor which could be accomplished by using a dialdehyde derivative of Ins(1,4)P_2 **(Figure 5.1)** as a secondary binding element. The requirement of such a derivative meant returning to the drawing board and developing synthetic strategies that could be used to obtain the newly designed receptor.

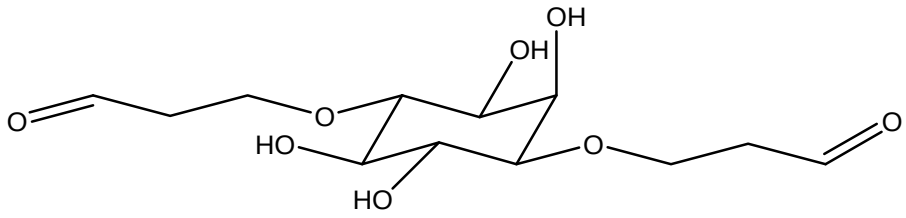


Figure 5.1 The extended dialdehyde derivative of Ins(1,4)P_2 for use as a secondary binding element in the synthesis of inositol phosphate receptors.

Perhaps the most challenging aspect of synthesizing *myo*-inositol derivatives is the specific placement of functional groups where needed either about the cyclohexane ring, or appended to hydroxyl group oxygens, in the presence of six secondary alcohols, four chiral centers and two meso positions. Any type of substitution chemistry has the potential to invert configurations leading to altogether different varieties of inositols.

As discussed in Chapter 4, *myo*-inositol also contains four stereogenic centers in the form of enantio-paired carbons C1:C3 and C4:C6, with C2 and C5 being mesogenic. Combining chirality with either S_N1 or S_N2 reactions at stereogenic centers could lead to

complex mixtures of intractable stereoisomers, especially in light of incomplete reactions at multiple carbons or oxygens.

Ironically, it is these problematic aspects of synthesis—polyhydroxylated chiral centers—that are highly desirable in their role as secondary binding elements in our receptors.

Due to these problems, we thought it best to avoid ring substitutions altogether and concentrate on ways to get the requisite *myo*-inositol dialdehyde through Williamson ether synthesis chemistry. In the Williamson method of preparing ethers the nucleophilic alkoxide of an alcohol, formed by reacting the alcohol with a suitably strong base, attacks an alkyl halide displacing the halogen in a substitution reaction to generate the ether product.

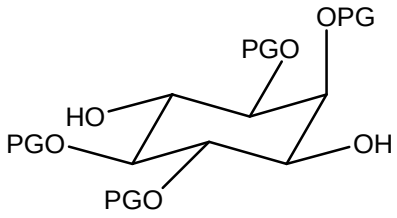
By employing this type of chemistry many, but not all, of the isomeric issues are resolved (pun 2). Inversion of configuration at chiral centers is no longer an issue because the inositol compound becomes nucleophilic rather than electrophilic. Generation of stereoisomers is still possible though when unsymmetric reactions occur at oxygens attached to the stereogenic C1:C3 or C4:C6 carbons, but not at either C2 or C5. Since our design called for extended aldehydes at C1 and C4 to complement the Ins(1,4)P_2 target molecule we would indeed have to deal with this problem.

Although the Williamson approach eliminates the problems associated with ring substitution, it presumably does nothing to diminish the problem of attaching the aldehyde moeities specifically to the C1 and C4 oxygens in the presence of all the other hydroxyl groups. There is no reason to believe any selectivity exists between the six hydroxyl groups when subjected to proton abstraction conditions of the Williamson

method. There is also no reason to think that, once stripped of their protons, the alkoxide groups would not all attack the electrophile with equal ferocity resulting in a hexa-etherated product. Consequently, there must be some strategy employed to leave free hydroxyls at C1 and C4, and protect the rest of the alcohol groups from the ravages of wild hydride.

So the Williamson method for attaching aldehyde ending ethers to the C1 and C4 positions of *myo*-inositol offered a mixed blessing. Inversion of configuration resulting from ring carbon substitution was eliminated because no such reaction was required. However, it was problematic because the ether reaction products were enantiomeric and in our receptor design a crucial element was the incorporation of the specific enantiomer Ins(1,4)P_2 as opposed to Ins(3,6)P_2 into the receptor. We were also faced with the aforementioned protecting group issues to ensure etherfication of the desired C1 and C4 positions. It struck us that both these problems were actually one in the same and ideally could be resolved by selective simultaneous protection of the C2, C3, C5, and C6 positions with the same substituent **(Figure 5.2)**.

PG = protecting group

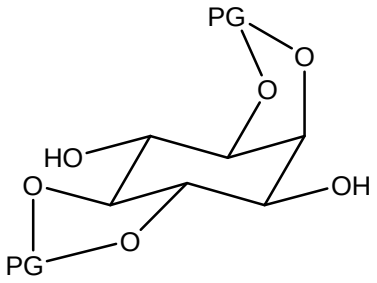


+ 179 other structures

Figure 5.2 An inositol protected at C2, C3, C5, C6 with four protecting groups leaving hydroxyl groups at C1 and C4 free.

Of course, such a substituted inositol cannot exist without first going through some sort of resolving stage due to the chemical equivalencies of C3 and C1, and of C6 and C4. Even then the likelihood of all four protecting groups going to the desired set of alcohols, given all the possible permutations (6!/(6-4)!4!) 180 unique combinations, many are enantiomers), is low. The number of possible unique compounds is drastically reduced if groups bridging adjacent hydroxyls are used to protect four hydroxyls in various patterns around the ring leaving two alcohols free for reacting **(Figure 5.3)**.

PG = protecting group



+ 8 other structures

Figure 5.3 An inositol protected at C2, C3, C5, C6 with two protecting groups each spanning two oxygens leaving hydroxyl groups at C1 and C4 free.

In this scenario there are nine unique combinations—four enantiomeric pairs and a meso compound. Again there is no guarantee as to which two will be left unprotected and enantiomers still prevail in the unresolved reaction. So the prospect of using a single reaction to exclusively free up C1 and C4 hydroxyl groups for Williamson ether chemistry wasn't very promising.

The next best thing might be to make use of resolving/protecting agent, bridging vicinal diols, that simultaneously converts the meso inositol into a pair of readily

separable diastereomers while leaving the hydroxyls we want to do chemistry on free for manipulation. This is the same strategy as the last scenario only with a chiral protecting group instead of an achiral one.

At first glance it seems like there would be the same number of unique combinations (nine) only there would be no enantiomers present in the reaction mix. Actually this turns out not to be the case due to the cyclic asymmetric nature of chiral bridging protecting groups which gives rise to two regioisomers every time it reacts with vicinal diols. Combine two regioisomers at two different sets of vicinal hydroxyls for each protection event along with eight original diastereomers and a meso just for fun and you end up with thirty three unique molecules. Again not a desirable situation.

All these combinations ultimately arise from *myo*-inositol being a meso compound and the fact that it usually goes optical upon reacting. The simultaneous protection of four hydroxyls using either chiral cyclic or achiral cyclic protecting groups also contributes to the quagmire. Using acyclic protecting groups on *myo*-inositol doesn't present a favorable option either, due to the expected lack of selectivity and multiple combinations.

A solution to this mess may be cutting down the number of hydroxyls protected at one time to a more manageable level and then using some selective protecting group options to further manipulate the molecule as desired. Protection of two vicinal hydroxyl groups with an achiral cyclic protecting group would give rise to only six unique molecules (along with a presumably small amount of tetra-protected material) existing as three sets of enantiomers **(Figure 5.4)**.

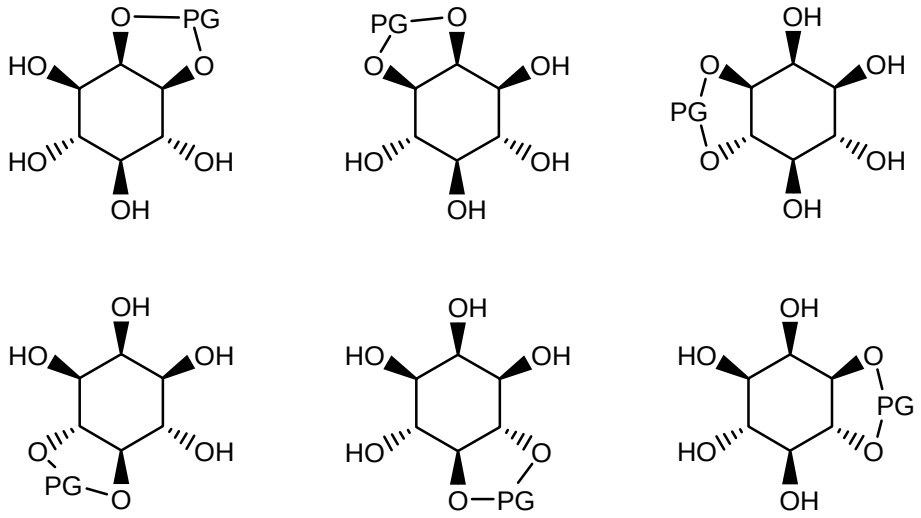


Figure 5.4 The set of six unique isomers arising from the protection of two vicinal hydroxyl groups on *myo*-inositol using an achiral cyclic protecting group.

Protection of two hydroxyls with a chiral cyclic protecting group would generate six diastereomers, each of which posses two regioisomers leading to a total of twelve unique molecules. These options seem a little less daunting, although certainly not ideal.

It should be noted that in calculating the possible combinations it was assumed that protecting group reactions would occur at each hydroxyl group with equal probability. However, it has been observed that there does exist some degree of reactivity differences between the six secondary hydroxyl groups on *myo*-inositol which can be exploited during protecting group manipulations. The installation of one or more protecting groups will also alter the reactivity of the remaining free hydroxyl groups as the molecule undergoes some conformational changes.

Racemic mixtures of multiple enantiomers and the desire to leave specific hydroxyls open for reaction are not the only considerations in the synthetic design work. Another issue arises when looking downstream to the cyclization reaction between the extended inositol dialdehyde and diethylenetriamine. Even if enantiomerically pure dialdehyde can be obtained, coupling within the framework of the macrocycle can lead to two positional isomers in the product mix because of the asymmetric nature of the inositol dialdehyde **(Figure 5.5)**.

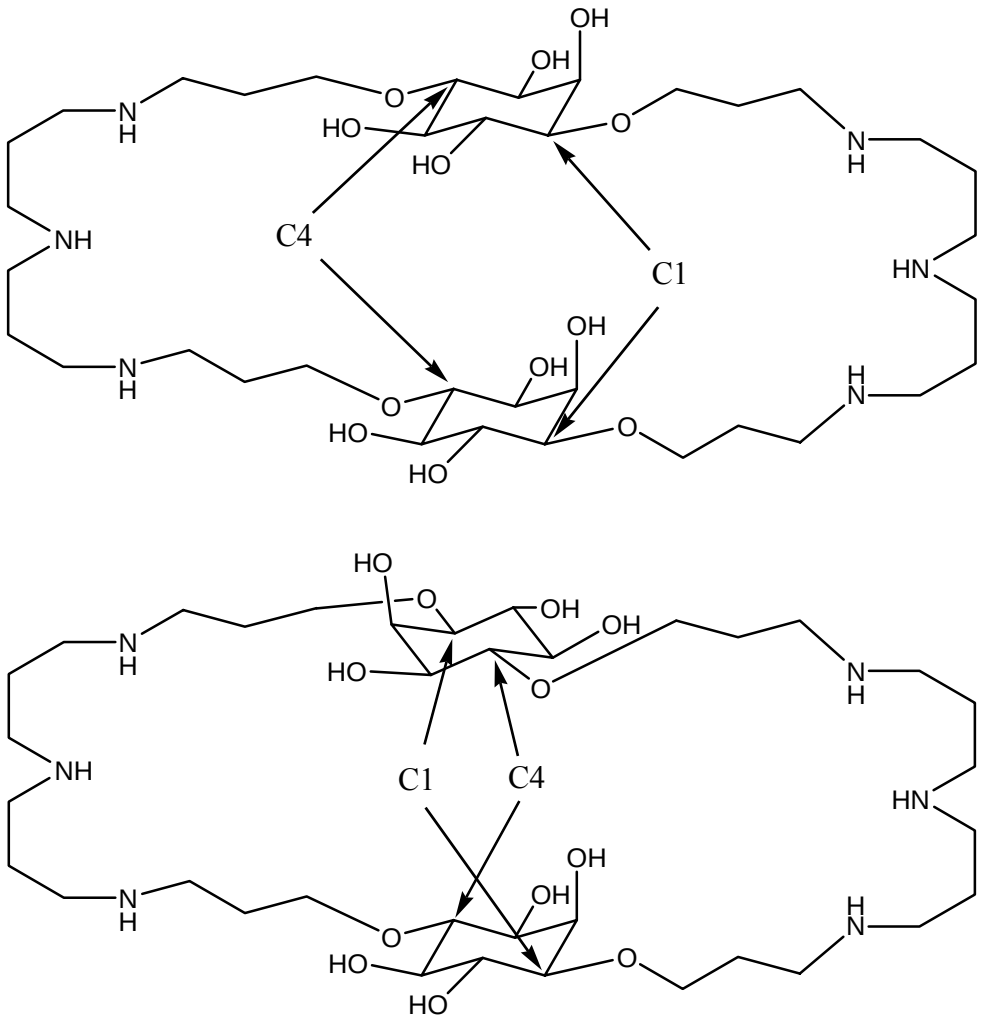


Figure 5.5 The two regioisomers arising from head-to-head or head-to-tail cyclization.

The aldehyde extending from the C1 position of one of the inositols could couple, through the diethylenetriamine primary binding domain, to the aldehyde of the second inositol at either the C1 or C4 position. Because of this it was desirable to install differentially protected latent aldehyde functions extending from the C1 and C4 positions of the inositol spacer which could be unmasked independently to control the regiochemistry of macrocyclization.

The use of latent, or protected aldehydes, would be required irregardless of the regioisomer issue due to the incompatibility of a free aldehyde with the Williamson method. In other words it would not be possible to attach the extended aldehyde group by simply reacting the alkoxide with 3-bromopropanal since enolate formation is expected. Many of the homologation design strategies previously explored while considering the cyclophane receptors led to the discovery of several latent aldehydes potentially useful in the current receptor design and they will be revisited later.

5.4 Synthesis of Inositol Phosphate Derivatives: The Camphor Years

When faced with the synthesis of inositol derivatives through extensive protecting group manipulations and resolution of the inevitable pot of enantiomers, the first place to turn is Reitz's Bible (albeit, old testament): "*Inositol Phosphates and Derivatives; Synthesis, Biochemistry, and Therapeutic Potential*."[4] Although Reitz's book specifically addresses the synthesis of *myo*-inositol phosphates, many of the strategies covered apply to the synthesis of other derivatives as well.

There are two major schools of thought on how *myo*-inositol derivatives should be synthesized.[5] One approach is to use the meso *myo*-inositol compound as a starting

material, perform non-stereoselective reactions on hydroxyl groups to generate racemic mixtures of products, selectively deprotect where needed, followed by carrying out the desired reaction.

Chiral resolution, as needed throughout the course of the reaction by way of crystallization or chromatography of the diastereomers, followed by any further deprotections, affords the pure enantiomer desired. Common protecting groups used in the field of inositol chemistry include: acetyl, benzoyl (Bz), benzyl (Bn), *p*-methoxy-benzyl (PMB), allyl (All), isopropylidene, cyclohexylidene and orthoformate. Typical resolving agents employed include: (+)menthylchloroformate, (+)camphanic acid (or its acid chloride) and D-camphor.

The other major route to *myo*-inositol derivatives makes use of some of the eight inositol isomers discussed in Chapter 4 especially the chiral starting materials D-pinitol (D-5-O-methyl-*chiro*-inositol) and L-quebrachitol (L-2-O-methyl-*chiro*-inositol), the methyl ethers of D- and L-*chiro*-inositol. Getting the *myo*-inositol compound out of the *chiro*-inositol precursors requires inversion of a hydroxyl group somewhere along the way. By starting with chiral compounds the issues of chiral resolution can be averted, however these starting materials are very expensive.

It was decided to pursue the inositol needed for our Williamson ether synthesis derived inositol dialdehyde using the more traditional approach starting with *myo*-inositol rather than delving into the *chiro*-inositols. We needed to find a relatively straight forward route to selectively protected enantiomerically pure *myo*-inositol having free hydroxyl groups only at the C1 and C4 positions in order to satisfy our receptor design requirements. Recalling the long term goals of this project, the synthesis of receptors for

all the inositol phosphates found in the cell, it was highly desirable to find a modular approach that could be used in the synthesis of a wide variety of *myo*-inositol derivatives for use in future receptor construction. This was important because we envisioned extending the macrocycle sturucture to more elaborate macrobicyclic and maybe even macrotricyclic receptors for the encapsulation of more complex cellular inositol phosphates.

Although the Reitz book provided many methods for the synthesis of enantiomerically pure *myo*-inositol phosphates, there were very few instances where hydroxyl groups were left unprotected at the positions desired and none were really suitable for the modular approach envisioned.

In a 1992 paper authored by Bruzik,[6] he showcased a systematic approach for the synthesis of many different *myo*-inositol phosphates which meant just prior to phosphorylation free hydroxyls were present in a variety of patterns.

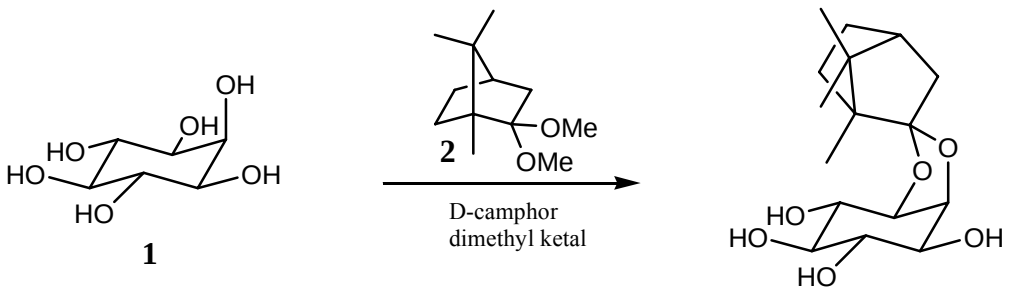
The promise of the paper was the production of *myo*-inositols having free hydroxyl groups at the following positions: (1); (1,2); (1,4); (1,6); (1,3,4); (1,4,5); (1,2,6); (1,3,4,5). More importantly those compounds were enantiomerically pure and the chiral auxillary was put in place as a useful protecting group to help guide subsequent synthetic manipulations. Our target (free hydroxyl groups at C1 and C4) compound made his laundry list of useful alcohols, so we bellied up to the bar and bought a round.

The crux of Bruzik's strategy was the immediate upstream use of D-camphor ((1R)-(+)-camphor) as a chiral auxillary protecting group which eliminated the need for optical resolution downstream. This strategy combined attributes of both major methods

used in the synthesis of inositol phosphate derivatives because meso *myo*-inositol was used as the starting material, yet it was only one step away from being chirally resolved.

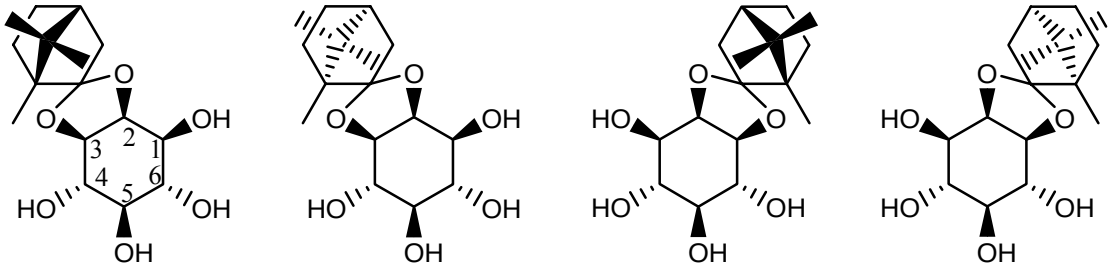
As discussed in section 5.4, the use of a chiral, cyclic protecting group to block only two adjacent hydroxyl groups was one of the better options giving rise to twelve possible molecules. This idea was corroborated by Bruzik as he observed that protection of *myo*-inositol with various ketalization reagents (e.g. cyclohexanone, 1,1-dimethoxy-cyclohexane, or 2,2-dimethoxypropane) followed by hydrolysis of *trans*-ketal groups gave 1,2-*O*-ketals in yields of up to 85%. He reasoned from this that protection of one set of *cis* vicinal diols, namely at the 1,2 positions, was the most efficient protecting group strategy known in *myo*-inositol chemistry.

The chiral auxillary used by Bruzik was the dimethyl ketal of D-camphor ((1R)-(+)-2,2-Dimethoxy-1,7,7-trimethyl-bicyclo[2.2.1]heptane) **2** which upon reacting with *myo*-inositol **1** generated a mixture of mono- and bis-ketals. Following removal of trans protecting groups via alcoholysis and crystallization from methanol the desired 2,3 protected *myo*-inositol (1D-2,3-*O*-(D-1',7',7'-Trimethyl[2.2.1]bicyclohept-2'-ylidene)-*myo*-inositol) **3a** was obtained in 31% yield **(Scheme 5.1)**.



Scheme 5.1

After alcoholysis and prior to crystallization, the bulk of the product mixture consisted of four diastereomers **(Figure 5.6)**. This is consistent with my previous analysis on using a chiral cyclic protecting group on the meso *myo*-inositol starting material. Protection is regioselecitive and occurs at either C1, C2 hydroxyls or C2, C3 hydroxyls with the bornanediyl protecting group bridge system oriented either *endo* or *exo* relative to the inositol ring.



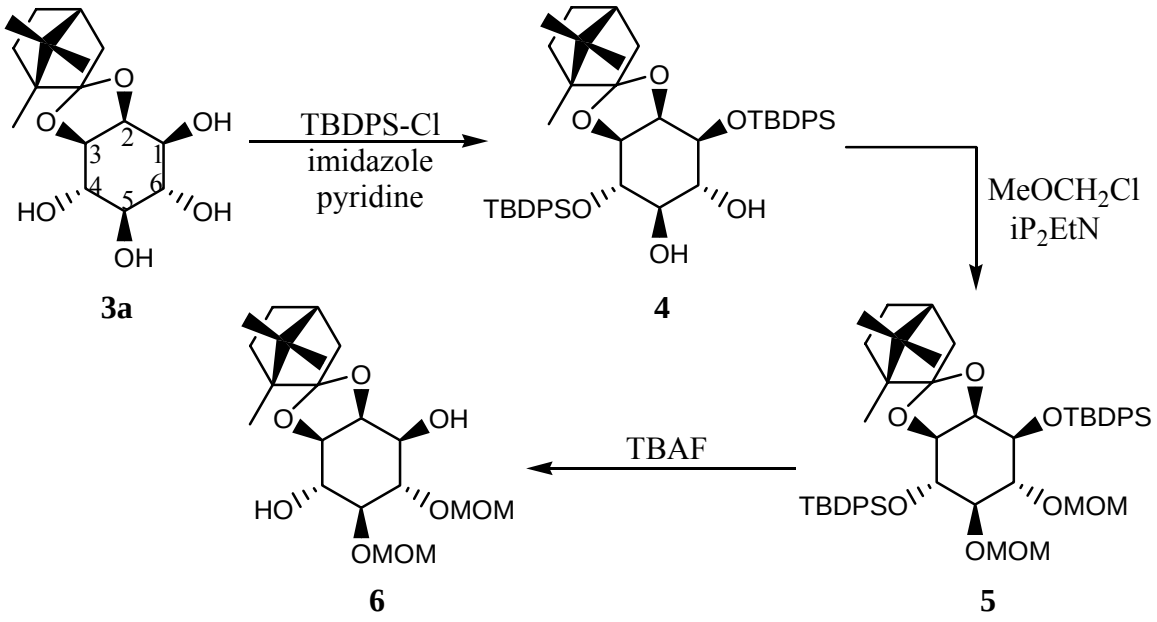
3a **3b** **3c** **3d**

Figure 5.6 The four diastereomers (**3a-3d**) generated from the protection of *myo*-inositol using the dimethylketal of D-camphor.

There is no ready explanation as to why the favored protection seems to be centered at the number two hydroxyl, presumably its because of the axial orientation which makes the OH group protrude beyond the others rendering it more reactive and the fact that it is the centerpiece of the only two pairs of *cis*-hydroxyls around.

An important aspect of Bruzik's approach wass the seeming ability to selectively crystallize out only one of the four diastereomers such that the enantiomerically pure compound **3a** could be further used in subsequent non-stereogenic reactions without any

loss of enantiopurity. Based on his strategy, a synthesis scheme was developed to give us our Williamson ether synthesis precursor **(Scheme 5.2)**.



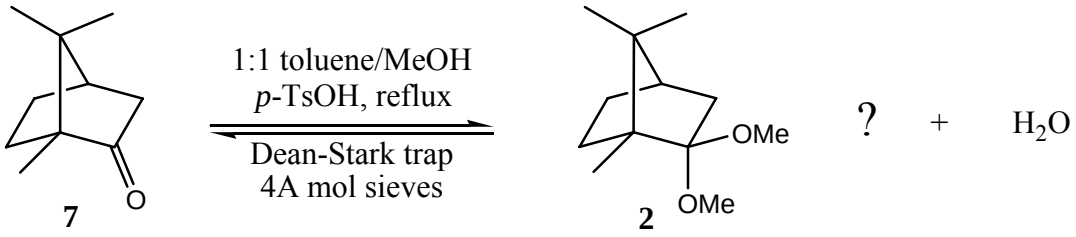
Scheme 5.2

The Bruzik route starts with the enantiomerically pure camphor dimethyl ketal protected *myo*-inositol **3a** which when subjected to protection conditions using *tert*-butyldiphenylsilyl chloride provides compound **4** selectively protected at the C1 and C4 hydroxyl groups. Protection of the remaining free alcohols is done using chloromethyl methoxy ether to provide **5** and treatment with tetrabutylammonium fluoride removes the silyl groups leaving compound **6** with free hydroxyls at C1 and C4 as desired.

Seventy seven pages of lab notebook space and nearly as many spectra were dedicated to the seemingly trivial conversion of D-camphor to its dimethyl ketal. On the surface ketalization is simply a matter of reacting the ketone with an alcohol in the

presence of acid to give the desired product. The reaction's reversibility also necessitated the removal of water, or the product formed, to drive the equilibrium toward the product.

In my first ketalization effort, D-camphor **7** was dissolved in equal volumes of toluene and methanol along with some *p*-toluenesulfonic acid **(Scheme 5.3).** A Dean-Stark trap was used to collect azeotroped water and 4Å molecular sieves were added to the reaction as an extra water trap.



7 **2**

Scheme 5.3

After several hours of refluxing the only thing that distilled, based on vapor temperature, into the Dean-Stark trap appeared to be methanol. The fundamental flaw in this set-up was using an alcohol having a much lower boiling point than the compound (toluene) with which it was intended to azeotrope.

A search on ketalization methods turned up a useful conversion that relied on water removal via trimethylorthoformate rather than azeotropic distillation.[7] In the case of ketalization via methanol, trimethylorthoformate has a dual effect on the equilibrium. It reacts with water formed in the reaction to help push ketalization and upon hydrolysis

produces methanol—the alcohol used as a reactant. The other hydrolysis product is methyl formate, which along with methanol is easily removed by rotary evaporation.

Prior to attempting any further ketalization utilizing the new orthoformate method it was deemed prudent to examine the ^{1}H and ^{13}C NMR spectra of D-camphor and to consider the changes expected in those spectra following a successful conversion to the ketal **(Figure 5.7)**.

Assignments of proton signals arising from D-camphor were done based on information found in "*Modern NMR Spectroscopy*" by Jeremy Sanders.[8] Of primary importance in the determination of ketalization would be the appearance of two new proton singlets between 3.3 and 4.0 ppm roughly equal in height to the three upfield methyl singlets arising from the two quaternary carbons present in D-camphor. These singlets correspond to the methoxy group protons of the D-camphor dimethyl ketal which are slightly deshielded by oxygen and would appear in an uncluttered region of the spectrum.

In the ^{13}C NMR spectrum of D-camphor the carbonyl carbon signal appears way downfield at 212 ppm. Upon ketalization this signal was expected to disappear and be replaced with a ketal carbon signal between 100 and 120 ppm. In addition, two new methoxy carbon signals were expected between 50 and 65 ppm.

Even with the added benefit of trimethylorthoformate, early ketalization attempts were still done by pretty much flying blind. There was no specific camphor ketalization procedure to follow so it wasn't even known if the product ketal was a liquid or a solid making work-up prospects a kind of seat-of-the-pants operation.

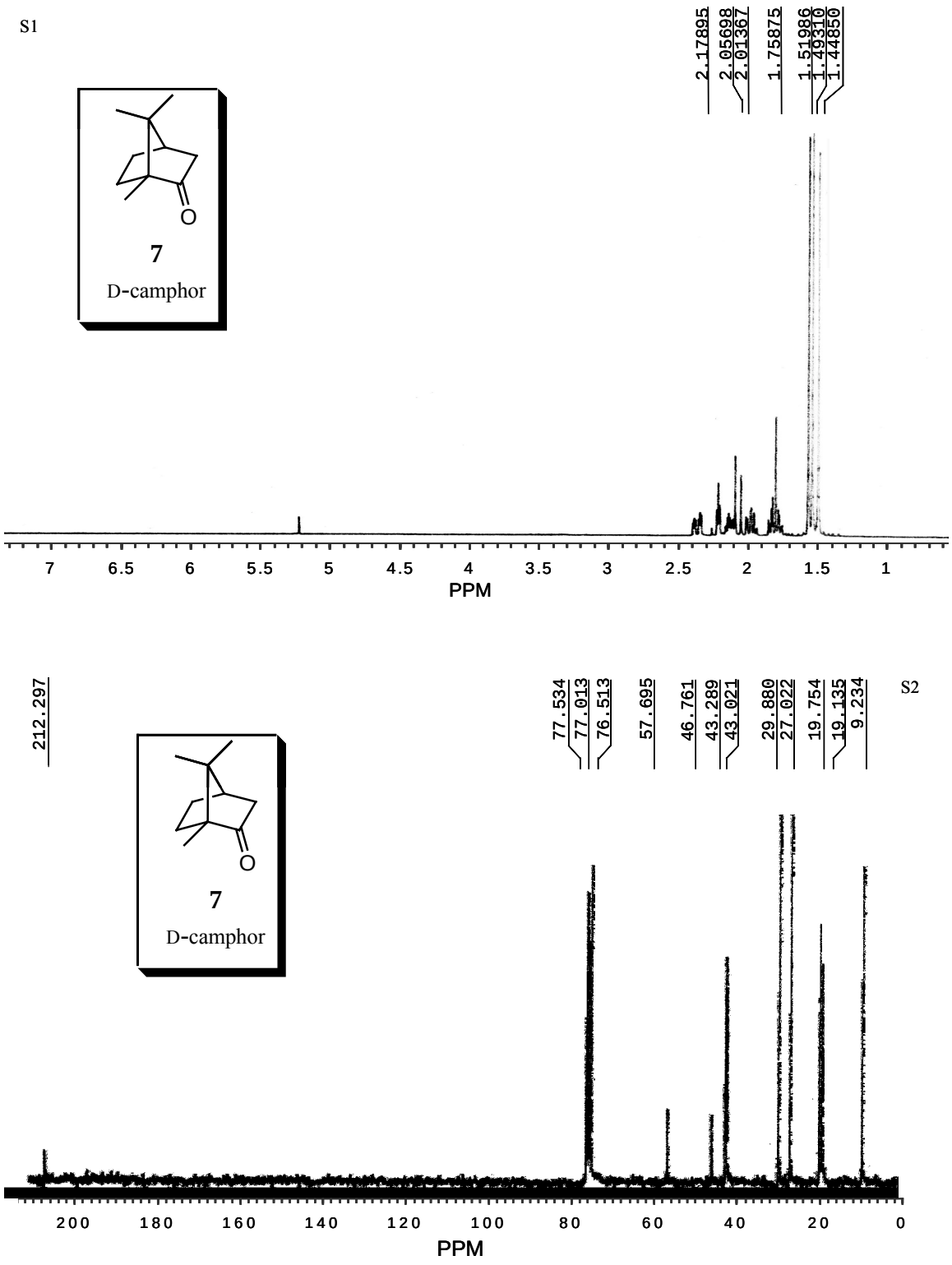
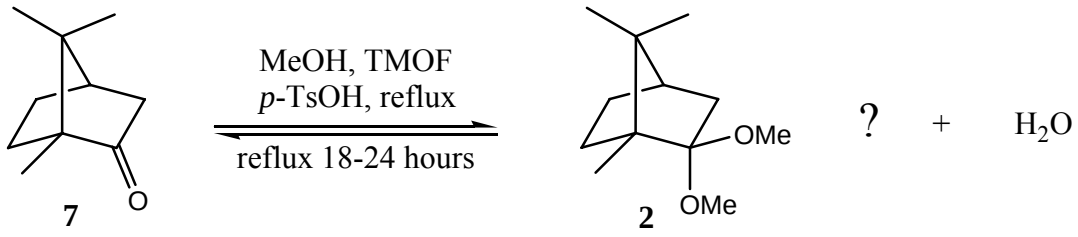


Figure 5.7 The ^{1}H NMR (S1) and ^{13}C NMR (S2) of D-camphor.

The procedure used at the time, adopted from Wenkert's generic ketalization paper, entailed combining D-camphor **7**, trimethylorthoformate, methanol and *p*-toluene-sulfonic acid into a flask and refluxing under argon for eighteen to twenty four hours **(Scheme 5.4)**.



7 **2**

Scheme 5.4

Dilution with ether, treatment with 5% NaOH, washing the organic layer with brine and water, followed by drying, and rotary evaporation was reported to give low molecular weight ketals **3** as an oil which could be purified via distillation.

For our work-up purposes, it was assumed the product, like D-camphor itself, was a solid and would be readily recovered from the flask upon rotary evaporation. This idea of easy recovery was quickly squelched when it was discovered that camphor, and presumably its ketal, readily sublimes which causes problems during any heated rotary evaporation or distillation operations.

Following several failed attempts to convert D-camphor to its dimethyl ketal, a paper was found in which this specific task was accomplished.[9] Thankfully, the conditions in this procedure were essentially the same as those already being employed

with the exception that they dried methanol over $Mg(OMe)_2$ and the ketal product was extracted into a dichloromethane-pentane solution rather than ether.

Another piece of very useful information was gleaned from the paper—D-camphor dimethyl ketal was a liquid, refuting our initial assumption. The authors also made a point of saying how difficult it was to ketalize camphor and that it was subject to repeated attempts, a sentiment for which I could vouch.

By following the procedure and collecting a distillate fraction beginning at 90 ^{o}C and ending when sublimed, solidified camphor began clogging the condenser, enough material was obtained to get some useful ^{1}H and ^{13}C NMR data **(Figure 5.8).**

Although neither of these spectra are pretty, some encouraging pieces of information were present. Looking at the ^{1}H NMR, methoxy proton signals can be seen at 3.39 and 3.44 ppm right where they were expected in the dimethyl ketal and indicated that ketalization had occurred. All the signals between 0.5 and 2.5 ppm arose from a mixture of D-camphor and its ketal.

The ^{13}C NMR confirmed that the sample contained a mixture of reactants and products as witnessed by all the carbon signals evident. The carbon spectra did offer additional support for the presence of the ketal by the appearance of the ≈ 108 ppm signal corresponding to the newly formed ketal carbon.

A follow-up procedure was done, and after collecting the product following distillation another set of ^{1}H NMR and ^{13}C NMR were obtained **(Figure 5.9).** Both of these spectra were quite clean relative to the previous sample and showed very little indication of a mixture. Strong methoxy proton signals arose at 3.15 and 3.22 ppm as did a strong ketal signal at 108 ppm in the carbon spectrum and there was no indication of a

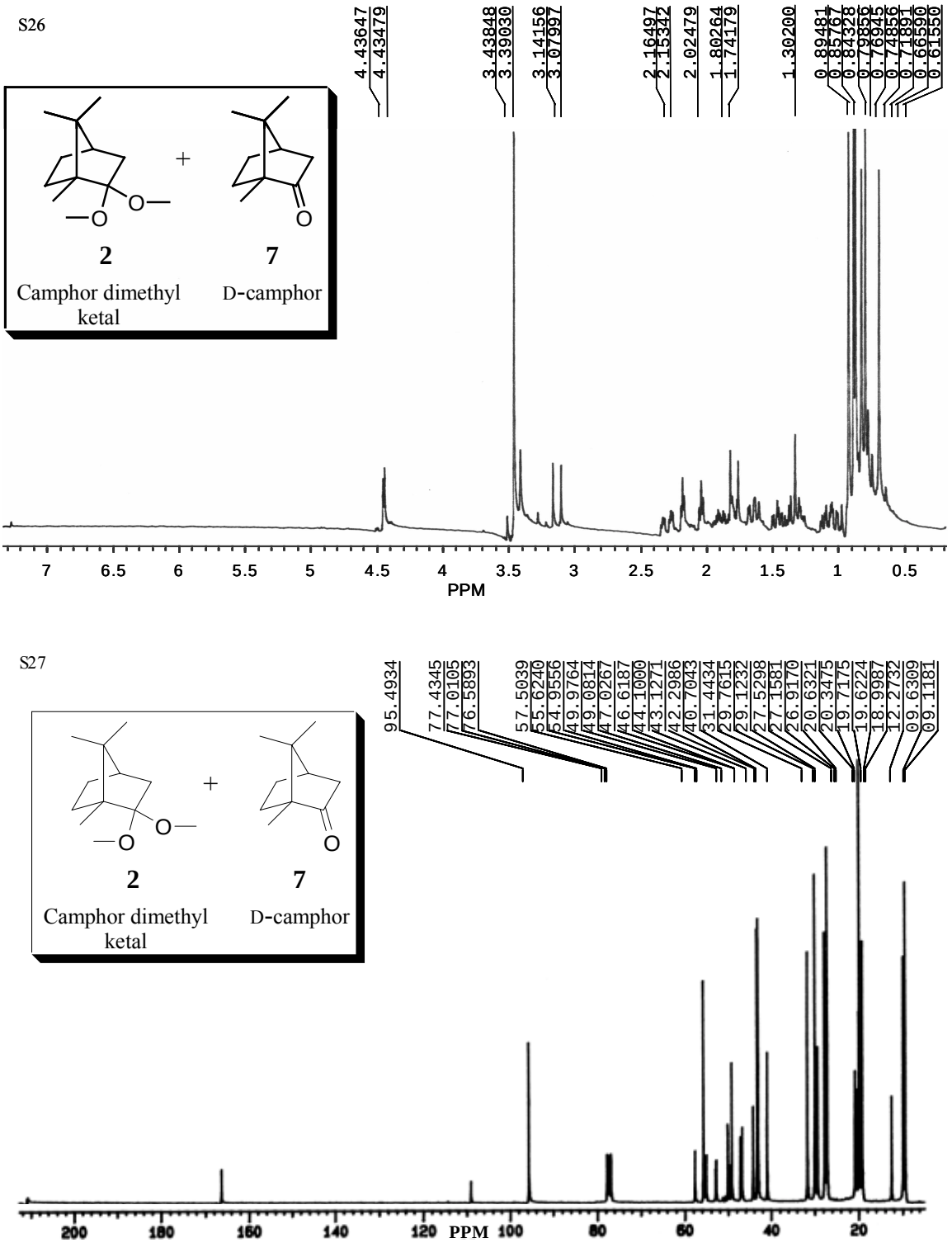


Figure 5.8 The ^{1}H NMR (S26) and ^{13}C NMR (S27) of a mixture of camphor and its dimethyl ketal.

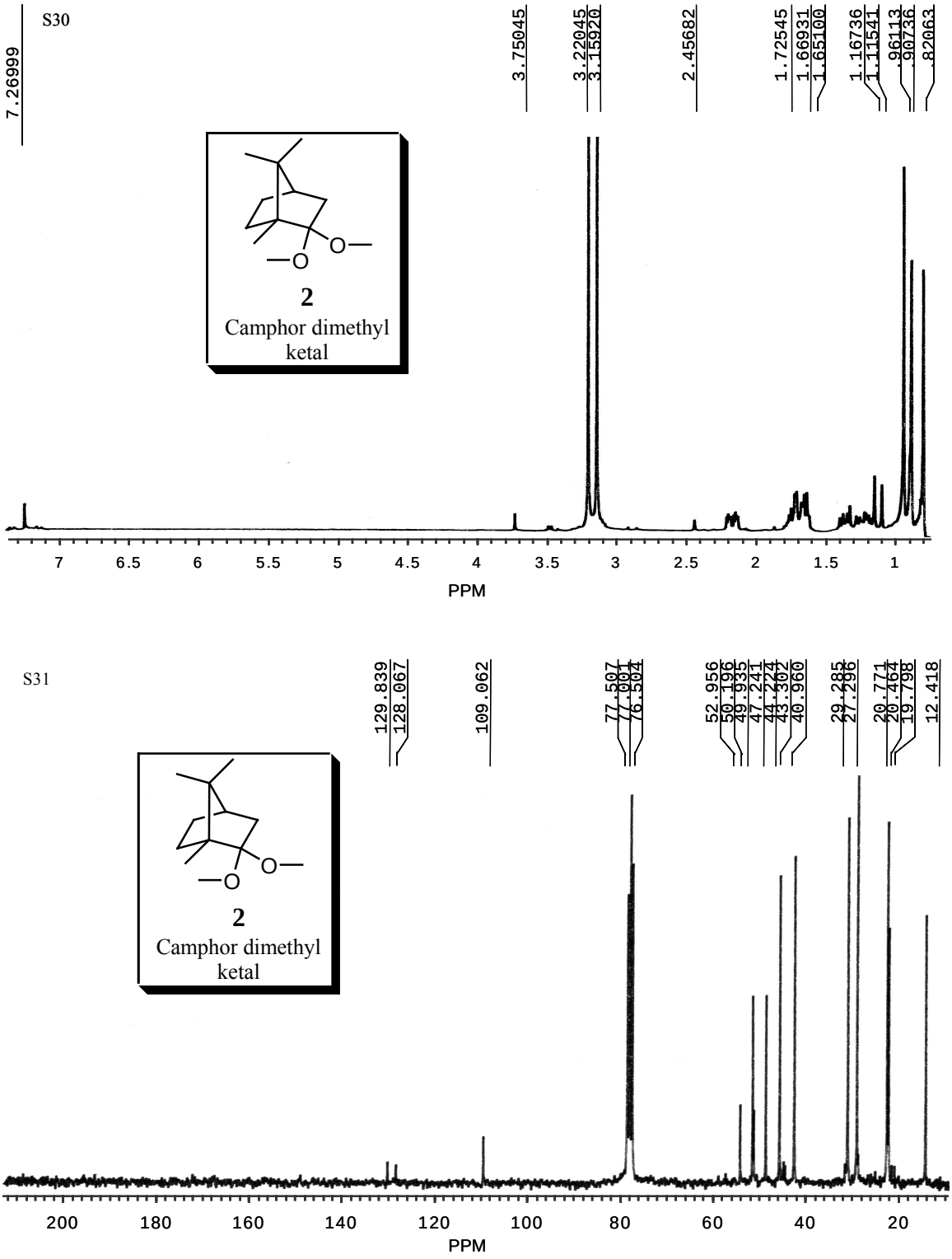


Figure 5.9 The ^{1}H NMR (S30) and ^{13}C NMR (S31) of D-camphor dimethyl ketal.

ketone carbon (no, it wasn't cut off). The remaining peaks in the ^{13}C NMR matched up well with those reported by Wiberg with the exception of two lingering camphor signals at 19.7 and 43.8 ppm and the two small mystery signals seen around 130 ppm.

The vacuum distillation worked reasonably well to separate camphor from its ketal, however it did not work as envisioned. It was expected that the ketal would distill from the camphor-ketal mixture in the pot and be collected as a distillate. Instead, the heat from the oil bath enhanced the ability of camphor to sublime causing it to collect in the condenser, leaving the ketal behind. Eventually the ketal could be made to come over, but it was decided to simply stop the distillation once a rapid temperature change from 93 ^{o}C to 97 ^{o}C was observed, indicating that camphor distillation was complete and the ketal could be collected from the distillation pot.

According to the Wiberg paper further purification of the ketal could be obtained by subjecting the camphor impurity to reduction by either diisobutylaluminum hydride (DIBAL) or liquid sodium in refluxing xylenes prior to distillation. This seemed like a worthwhile endeavor and it gave me the chance to play with some live sodium, so a crude reaction mixture following rotary evaporation was dissolved in xylenes and refluxed with freshly deoxidized sodium.[10] Distillation of the black mixture under atmospheric pressure (due to volatility and bumping tendency of xylenes) to first remove the solvent followed by collection of the 190-210 ^{o}C fraction gave a yellow distillate believed to be the ketal. Both ^{1}H and ^{13}C NMR, although not as clean as the previous batch, verified the presence of the ketal and the absence of any camphor.

A few subtle changes were made in the ketalization reaction which did seem to improve the ketal:camphor ratio, but it wasn't ever possible to completely remove camphor from the picture.

Some GC-MS work helped quantify the mixture **(Appendix A, S82-S87).** The first compound eluted from the gas chromatograph around eight minutes and showed a maximum *m/z* ratio of 166. The identity of the component was not immediately obvious, but it could have been the hemi-ketal after the loss of water. The next substantial compound to elute came off just under nine minutes and its identity was not readily obvious either, nor was any effort made to determine the molecule behind the trace. The next peak to elute from the column, which came off around 9.2 minutes, was positively identified by screening the spectrum against a database residing within the instrument's computer. That compound was identified as camphor and had a maximum *m/z* ratio of 152 matching the molecular weight perfectly. The largest GC trace came off around eleven minutes with a maximum *m/z* of 198 and matched the diketal.

A crude estimate, based on the GC peaks, indicated about 20-25% of the product mixture was not D-camphor dimethyl ketal. This crude quantification enabled us to proceed with the next steps of the Bruzik reactions, by simply adjusting the reagent measurements accordingly if desired.

As an interesting side-note, over time it became rather easy to make a qualitative judgement as to the success of a particular ketalization or purification reaction simply based on the smell of the product mixture as the two compounds had distinctly different odors to the trained nose.

The first reactions of the Bruzik procedure were actually being carried out concurrently with the ketalization efforts, in addition to some of the Grignard and Wittig homologation reactions relevant to the phenyl-based receptors which were never completely abandoned.

The Bruzik procedure for the protection of *myo*-inositol with the dimethyl ketal of chiral auxillary D-camphor was followed to the letter on several occasions with success as measured by two proton spectra (**Figure 5.10**). An effort was made to assign the proton signals reported in the Bruzik paper to the camphor dimethyl ketal protected *myo*-inositol and to corroborate those assignments with the ^{1}H NMR of the crude product **(Figure 5.11)**.

The protons on the inositol portion of the molecule were assigned using chemical shifts and coupling constants. All of the inositol protons were expected to be doublets of doublets because they are split by single protons on adjacent carbons to each side, however the proton on H-6 appeared as a triplet due to the nearly equal $J_{1\text{-}6}$ and $J_{5\text{-}6}$ values which cause two of the interior peaks to overlap. Free hydroxyl protons do not show up in the NMR due to rapid exchange with the deuterated solvent. The methyl groups on the camphor portion gave rise to the singlets as indicated in the figure and were not specifically assigned by Bruzik. The remaining protons on the camphor portion of the molecule were assigned as shown.

Although the proton signals in S94, Figure 5.10 were very weak, enough deflection from the baseline was present to be reasonably confident about the success of the reaction. The signals matched up quite well with Bruzik's data and a key indicator of success were the three singlets around 1 ppm belonging to camphor methyl groups.

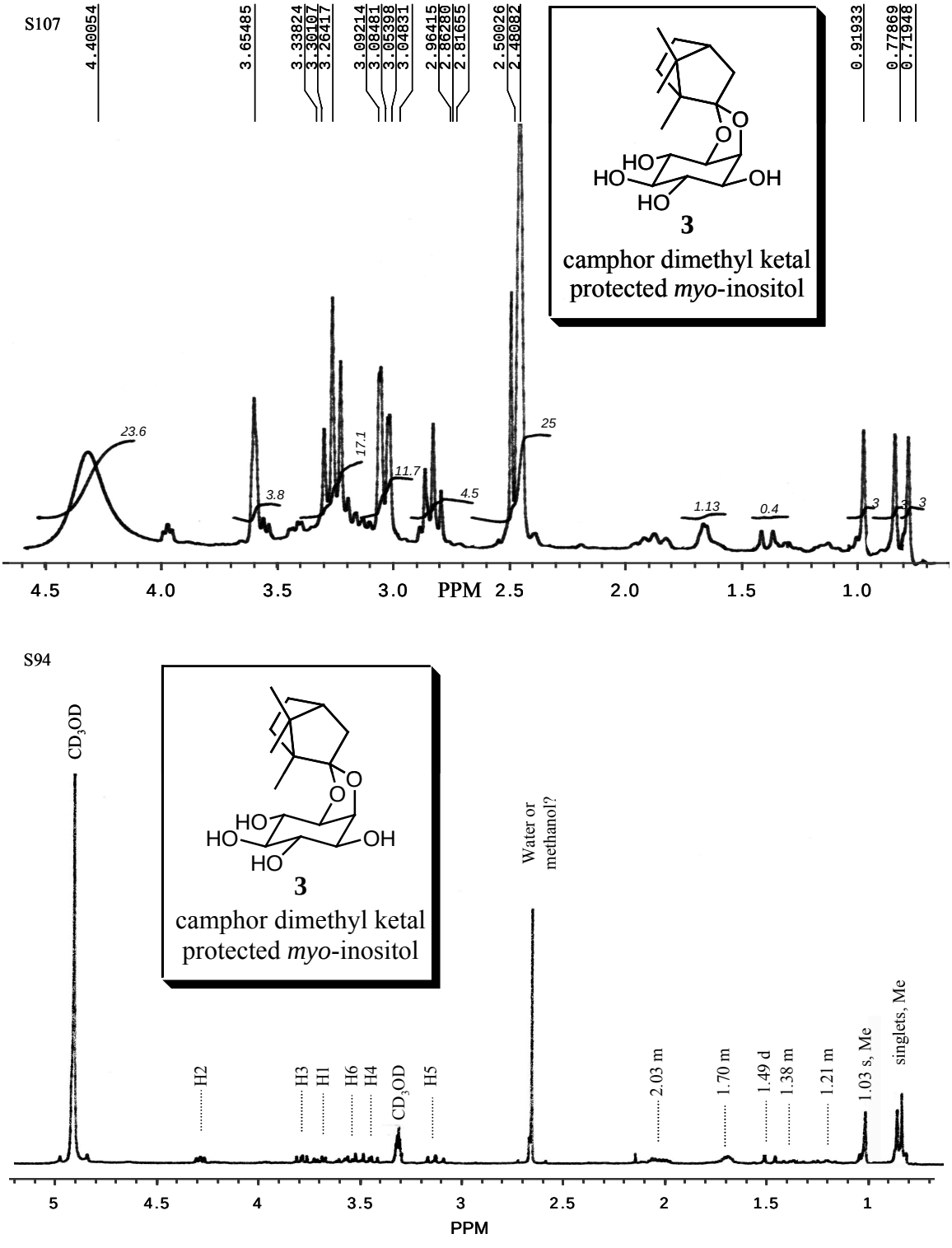
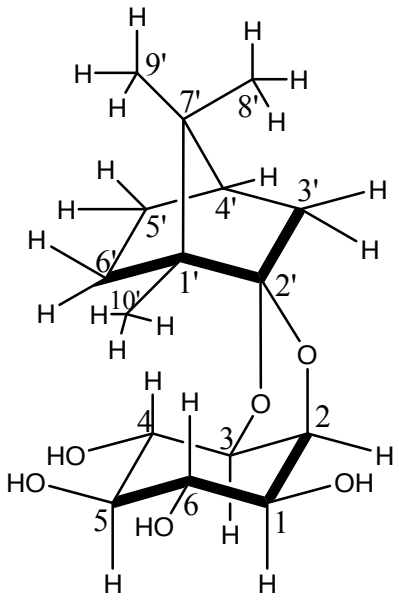


Figure 5.10 Two ^{1}H NMR (S107 and S94) of camphor dimethyl ketal protected *myo*-inositol **3**.



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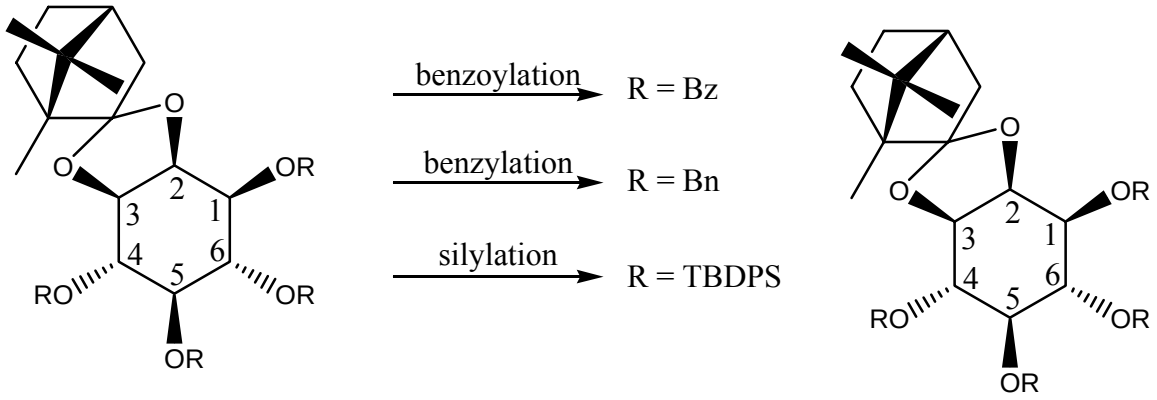
	chemical shift (ppm)	splitting pattern	proton assignment	number of protons	
inositol protons	4.27	dd	H-2	1	J_{1-2} = 4.3 Hz
	3.77	dd	H-3	1	J_{2-3} = 5.5 Hz
	3.68	dd	H-1	1	J_{3-4} = 7.2 Hz
	3.51	tr	H-6	1	J_{1-6} = 9.6 Hz
	3.44	dd	H-4	1	J_{5-6} = 9.3 Hz
	3.12	dd	H-5	1	J_{4-5} = 10.1 Hz
camphor protons	2.03	m	H-6'	2	
	1.70	m	H-5'	2	
	1.49	d	H-3'	1	
	1.38	d	H-3'	1	
	1.21	m	H-4'	1	
	1.03	s	not specifically assigned, but comes from H-8',H-9',H-10' methyl groups	3	
	0.87	s		3	
	0.85	s		3	

Figure 5.11 Analysis of the ^{1}H NMR for camphor dimethyl ketal protected *myo*-inositol.

Attempts to get the pure 2,3-*O*-bornanediyl protected compound by crystallization from methanol as described by Bruzik were unsuccessful so efforts were made to separate the diastereomers using chromatography, but these were made difficult by the inability to conveniently visualize column fractions or TLC plates.

It was decided to forge ahead with the crude diastereomeric mixture of four compounds and do some chemistry on the tetrol that might help with visualization, and thus separation of the product.

Three different reaction venues were explored; benzoylation, benzylation, and silylation of the free hydroxyl groups present on the camphor dimethyl ketal protected *myo*-inositol **(Scheme 5.5)**.



3a R = H

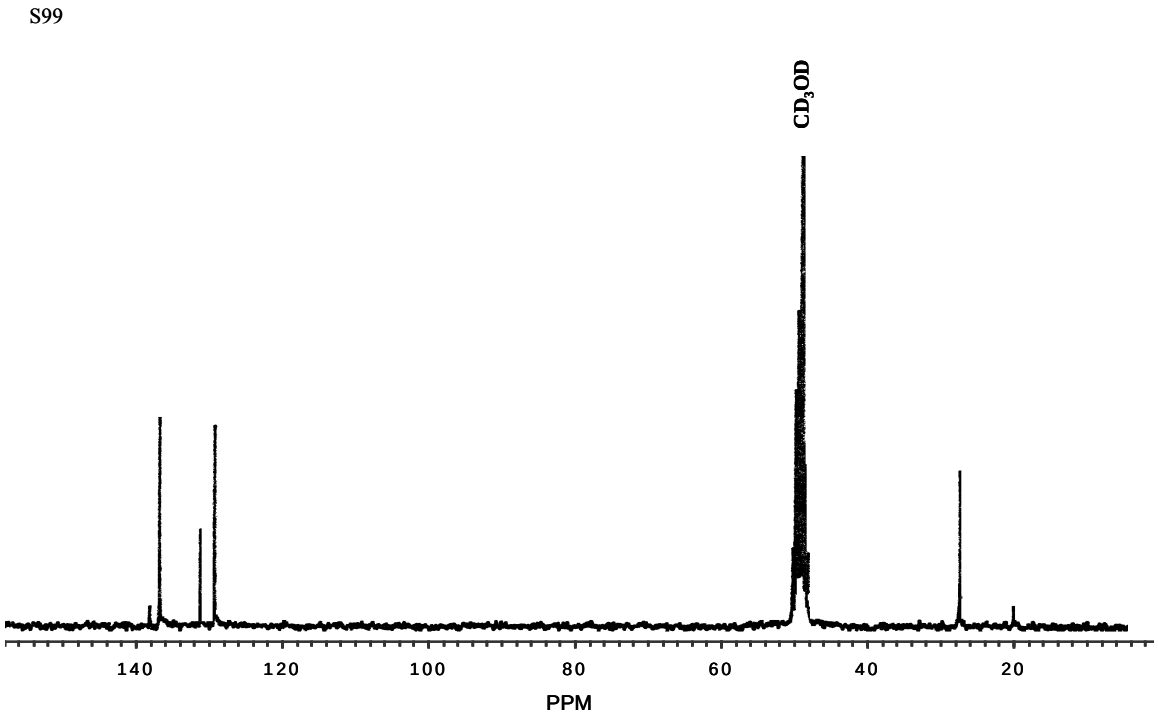
Scheme 5.5

For some inexplicable reason, the benzoylation procedure followed was not one of the ones available in the Bruzik paper which called for the straight forward removal of hydroxyl protons using potassium hydroxide followed by reaction with benzoyl chloride in dimethylaminopyridine. Instead a procedure was followed in which pyridine was use as the base.[10]

Although some evidence of a successful reaction was seen via thin-layer chromatography (observed four spots with one predominant), attempts to isolate the diastereomers via column chromatography were not successful.

A standard benzylation procedure was followed by treating the tetrol with sixteen equivalents of sodium hydride in dimethylformamide, and reacting the alkoxide with benzyl bromide. Following the work-up white crystalline solids were collected having a sharp melting point of 240 ^{o}C, however no further mention of the material could be traced in my lab notebooks.

Silylation was attempted using *tert*-butyldiphenylsilyl chloride in pyridine and imidazole and good yields (≈ 90%) of very light yellow solids were obtained on two different occasions, however the ^{1}H and ^{13}C NMR failed to support the structure of the desired compound. In fact, the spectra supported either a di-*tert*-butyldiphenyl silane or more likely a bis-*tert*-butyldiphenylsilylether compound **(Figure 5.12)**. Efforts toward the synthesis of the target inositol having free hydroxyls at the C1 and C4 positions using the methods outlined by Bruzik were not proceeding in a productive manner so alternative options were explored.



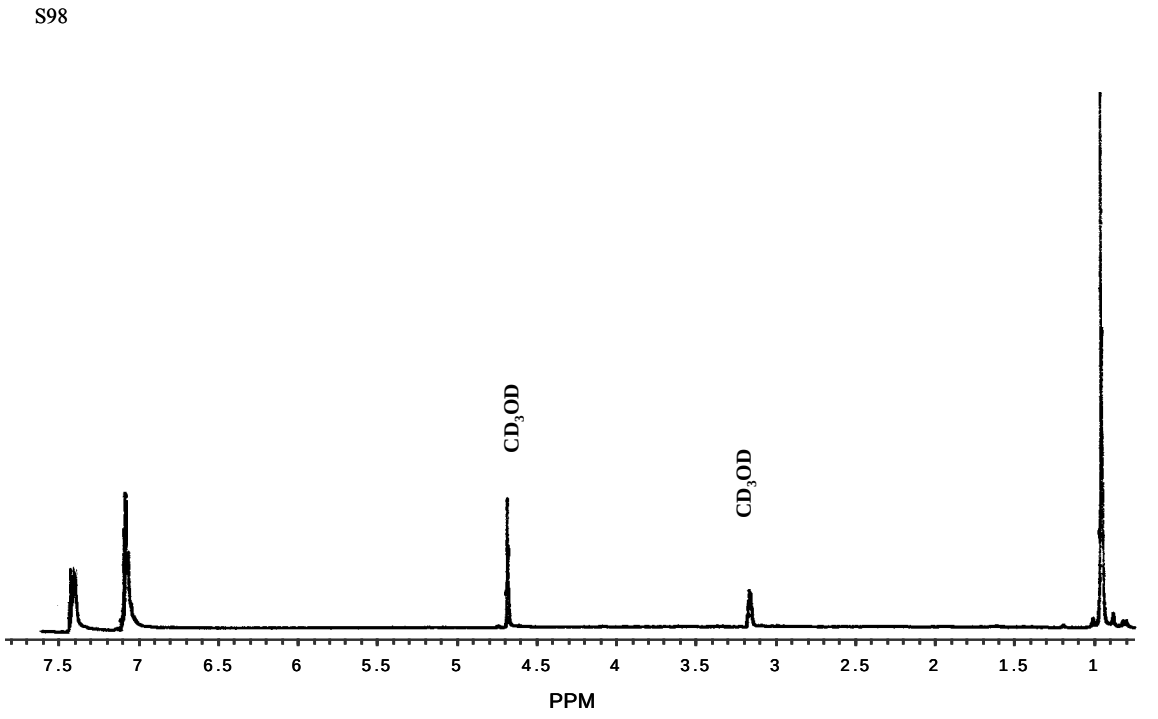


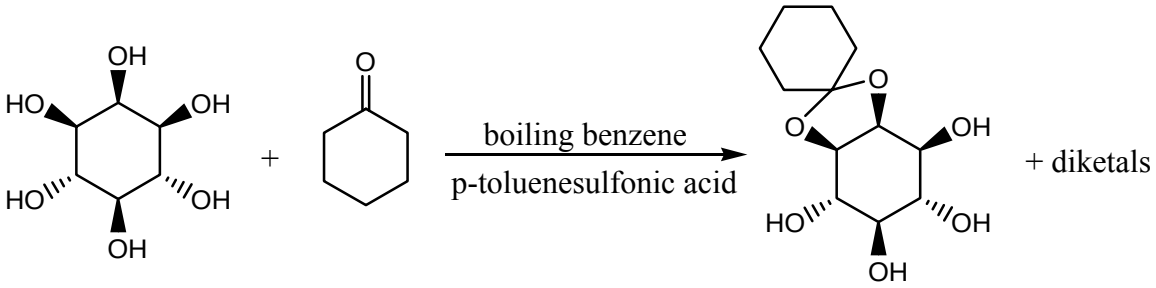
Figure 5.12 ^{1}H (S98) and ^{13}C NMR (S99) of the solids obtained following the silylation of camphor dimethyl ketal protected *myo*-inositol using *tert*-butyldiphenylsilyl chloride.

5.5 Synthesis of Inositol Phosphate Derivatives: The Cyclohexanone Years

In a seminal paper by Angyal he described the synthesis of cyclohexylidene derivatives of *myo*-inositol.[11] At that time the favored method of protecting cyclitols (as they were then called) was via acetonide formation using acetone and an acid catalyst. Angyal found that the preparation of isopropylidene *myo*-inositol ketals gave poor and inconsistent yields. This could be attributed to the equilibrium condition of the reaction requiring the removal of water to drive it toward the ketal. Acetone is not well-suited for the task of water removal via azeotropic separation, nor is it stable to strong dehydrating agents and self-condensation generates equilibrium shifting water.

Work by Micovic and Stojilkovic,[12] and Salmi,[13] who prepared cyclohexylidene derivatives of sugars and used azeotropic dehydration in the condensation of glycols with cyclohexanone inspired Angyal to test the methods on *myo*-inositol.

Under his original reaction conditions *myo*-inositol **1** eventually dissolved in a mixture of boiling benzene, cyclohexanone **8** and *p*-toluenesulfonic acid. Prolific generation of water was observed and extraction of the mixture into water afforded a monocyclohexylidene derivative **9** in only 2% yield **(Scheme 5.6)**.

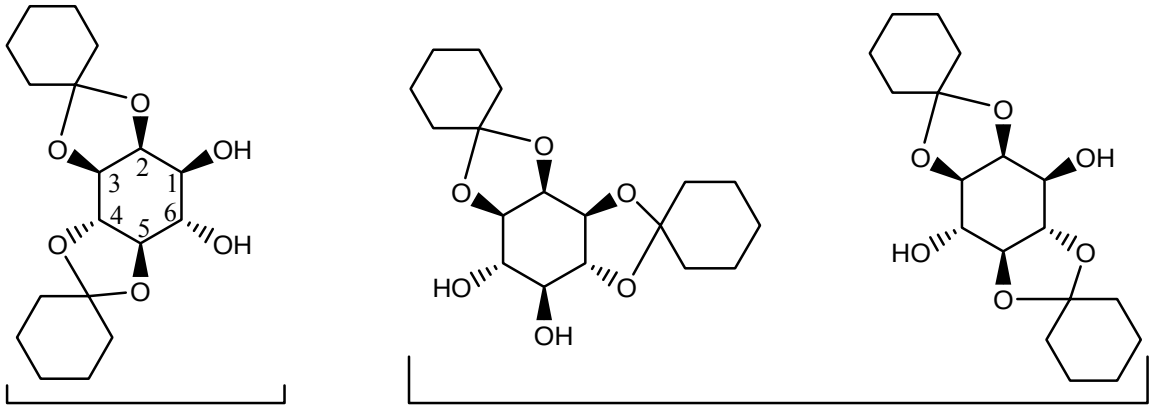


Scheme 5.6

The bulk of the starting *myo*-inositol was converted into three diketals which were painstakingly separated using alumina column chromatography and crystallization. Triketals were also expected from the reaction but none were isolated.

Through an impressive display of chemical manipulation involving acetylation, benzoylation, hydrolysis, sodium periodate oxidative cleavage and good old fashioned detective work he was able to discern the identities of the isolated compounds.

Exhaustive acetylation or benzoylation of the isolated cyclohexylidene derivatives, followed by hydrolysis of the ketals, and analysis of the alkylated inositol enabled him to determine that most of the compounds were diketals. Hydrolysis of each of the three isolated diketals under mild conditions using ethylene glycol and acid gave only 1,2-diketals indicating that all three contained one of their cyclohexylidene groups at that location **(Figure 5.13)**. In retrospect, it also indicated that the *trans*-ketals were more labile than the 1,2-*cis*-ketal.



5% yield

15-16% yield

Figure 5.13 The three diketals isolated and identified by Angyal **(Scheme 5.6)**.

In my previous analysis it was mentioned that protection of two pairs of vicinal hydroxyls around the *myo*-inositol ring by way of two non-chiral cyclic protecting groups, as is the case with cyclohexylidene, would give rise to nine distinct molecular entities—three enantiomeric pairs and a meso. That analysis was purely a statistical distribution of possible outcomes given equal reactivity of the six hydroxyl groups.

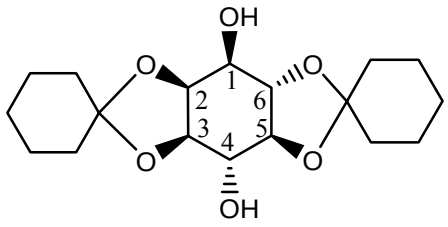
Angyal's work showed there was a strong preference for the first cyclohexanone protection to occur at the C1 and C2 positions (enantiomerically related to C2 and C3 protection, of course). This idea was certainly exploited in the Bruzik method, as well as many other future *myo*-inositol manipulations. The reason for this C1, C2 (and C2, C3) selectivity is because they are the only vicinal *cis*-hydroxyl groups on the molecule. All other vicinal hydroxyl protections using cyclic groups have to occur on trans-hydroxyl groups which are much more difficult to protect from a mechanistic viewpoint.

Conjecture holds that ketalization across the C1, C2 hydroxyls is actually required to torque the ring enough to allow for further ketalization across one of the remaining sets of vicinal *trans*-diols. Because of this C1, C2 preference only three pairs of enantiomeric diketals are expected to dominate the ketalization product mixture.

Further skewing the product mix was the observation that the (±)1,2:4,5 and (±)1,2:5,6 dicyclohexylidene protected *myo*-inositols were produced in about equal amounts (15-16% yield) and in a 3-fold greater quantity than the more sterically challenged (±)1,2:3,4 version (5% yield).

All this flashback-to-the-60s information proved to be quite useful for the synthesis of an inositol bearing free hydroxyls at C1 and C4. The protected inositol we desired to perform Williamson ether chemistry on was the (±)1,2:4,5 dicyclohexylidene

protected *myo*-inositol **(Figure 5.14)** According to Angyal's work this was one of the major compounds produced in the ketalization, albeit still only ≈ 15% and racemic to boot, but after the travails with camphor it was looking pretty good.



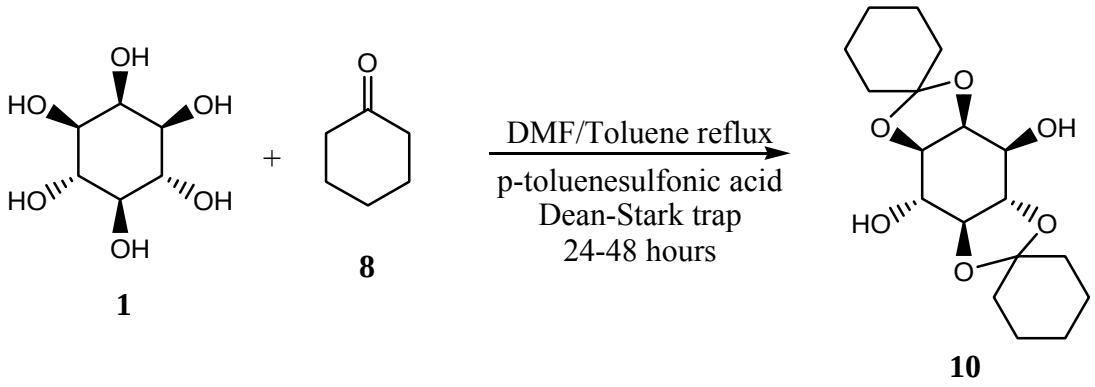
10

Figure 5.14 (±)1,2:4,5 dicyclohexylidene protected *myo*-inositol.

Thankfully, since the days of Angyal some other budding cyclitol chemists picked up the torch and were able to significantly simplify the landmark ketalization. A few particular papers were found that fit the bill quite nicely.[14-17] The methods in each of the papers were very similar, so aspects of each were combined along with my months of camphor ketalization experience and a procedure was hacked out.

The reaction involved refluxing *myo*-inositol with cyclohexanone in dimethyl formamide and toluene with *p*-toluenesulfonic acid for 24 to 48 hours **(Scheme 5.7)**. A Dean-Stark apparatus, filled with toluene, was used to trap water and return solvent back to the reaction in the azeotropic dehydration. After cooling, triethylamine was added to destroy the acid catalyst and prevent hydrolysis during the work-up. The reaction was concentrated, taken up in dichloromethane, washed with water, sodium bicarbonate

solution, water again and brine before being dried. Filtration, followed by filtrate concentration gave a golden sticky brown residue containing mostly diketals.



Scheme 5.7

Dissolution of the sticky residue in hexanes and acetone with a little heat, followed by cooling provided a white precipitate consisting of the crude (±)2,3:5,6-di-cyclohexylidene protected *myo*-inositol which upon crystallization in toluene gave nice fine white crystals with a sharp melting point and a 15% yield.

The yield and melting point coincided with Angyal's results and the ^{1}H NMR **(Figure 5.15)** matched nicely with the numbers reported by Jiang and Baker.[18] A ^{13}C NMR of the compound was also obtained **(Figure 5.15)** and based on the molecular structure was expected to have eighteen carbon signals. The spectrum did contain eighteen readily visible signals, however three of those belonged to $CDCl_3$, meaning only sixteen came from the product. Two of the 18 signals were from ketal carbons and those can be found where expected around 110 and 113 ppm. The six inositol carbon signals are mixed in with the solvent peaks between 70 and 83 ppm. That left all but the ten

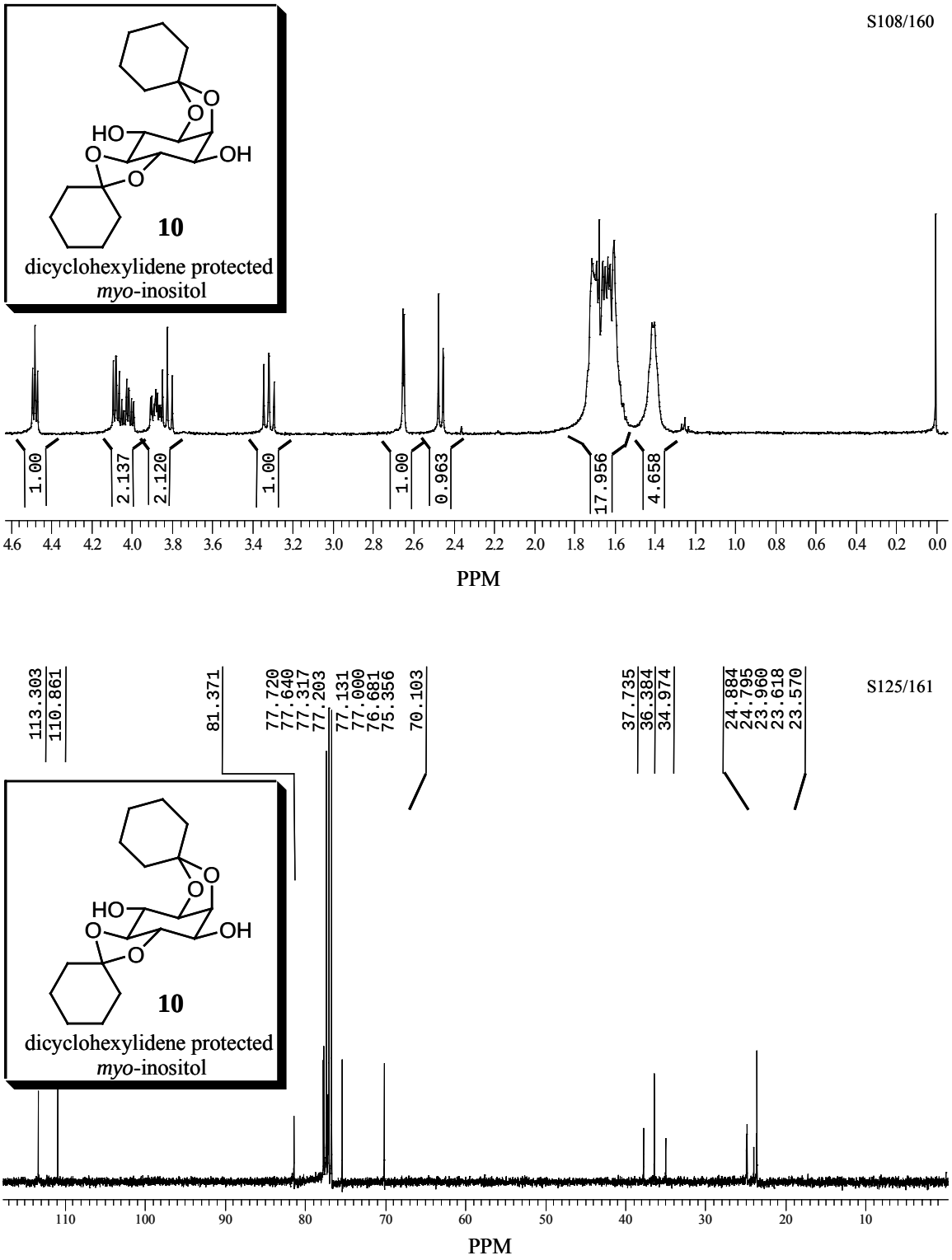


Figure 5.15 The ^{1}H NMR (S108/160) and ^{13}C NMR (S125/161) of dicyclohexylidene protected *myo*-inositol **10.**

methylene carbons of the cyclohexylidene groups accounted for in the spectrum. Seven of those ten were easily seen between 23 and 38 ppm. It may be that some of those carbons were in similar enough environments to be nearly equivalent and they overlaped beyond the instruments resolution.

Further confirmation was provided by MALDI mass spectrometry **(Figure 5.16)**. The top spectrum was taken of only the HABA (2-(4-hydroxyphenyl-azo)-benzoic acid) matrix and the bottom spectrum was from a sample containing matrix and the reaction product. The two significant differences were the peaks at *m/z* 363.41 and *m/z* 379.35 representing the sodiated and potassiated ions of the dicyclohexylidene protected *myo*-inositol (MW 340.422 g/mol).

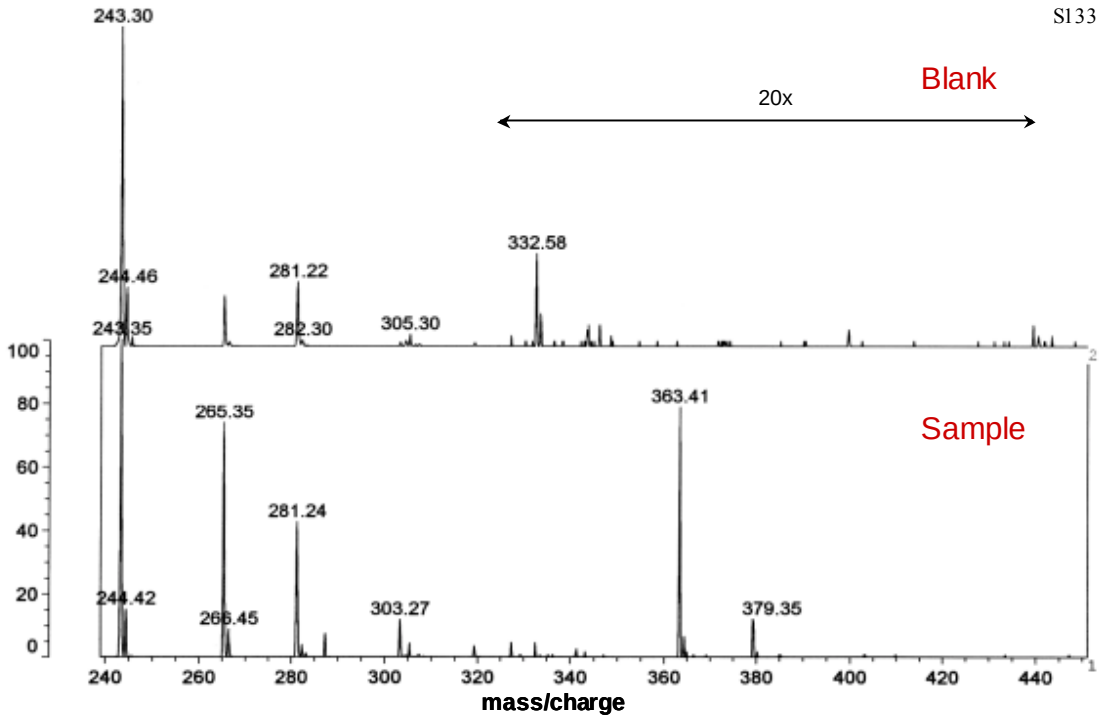


Figure 5.16 MALDI MS of the dicyclohexylidene protected *myo*-inositol **10**.

5.6 Synthesis of Inositol Phosphate Derivatives: The Williamson Years

In the early design stages of the inositol-based inositol phosphate receptors, after moving away from the highly symmetrical polyazacyclophanes, it was determined the asymmetric nature of the inositol secondary binding elements would lead to two regioisomeric macrocyclic receptors. While this may not be a major concern with the relatively simple macrocyclic designs it would become increasingly more troublesome in the synthesis of complex macrobicyclic and macrotricyclic receptors where many orders of attachment could occur. These concerns launched us into the next phase of receptor construction—the design of inositol derivatives carrying differentially protected latent aldehydes, or near aldehydes, meeting the criteria of the Williamson method.

The actual criteria were fairly simple. One end of the molecule had to have a good leaving group able to undergo displacement by the alkoxide formed from dicyclohexylidene protected *myo*-inositol. Bromides seemed to be a good choice because it was expected that many such compounds existed which could meet the remaining criteria. A second criterion was that the bromides had to have some sort of latent aldehyde or similar functional group at the other end of the molecule that was easily unmasked or converted to the free aldehyde. For the last criterion, there needed to be three methylene groups (inclusively) from the masked carbonyl carbon of the aldehyde to the carbon bearing the bromine atom. Although we did entertain the idea of longer and shorter groups at these positions.

The list of latent aldehydes can be loosely grouped into three categories **(Figure 5.17)**. With the exception of **13**, the first set of molecules **11-14** are the least removed from the aldehyde parent. Acetals **11** and **12** are 2-(2-Bromoethyl)-1,3-dioxolane, and 2-

(2-Bromoethyl)-1,3-dioxane, respectively. Compound **13** is 2-(3-Bromo-propoxy)-tetrahydropyran, the THP protected 3-bromopropanol, and thioacetal **14** is 2-(2-Bromo-ethyl)-(1,3)dithiolane.

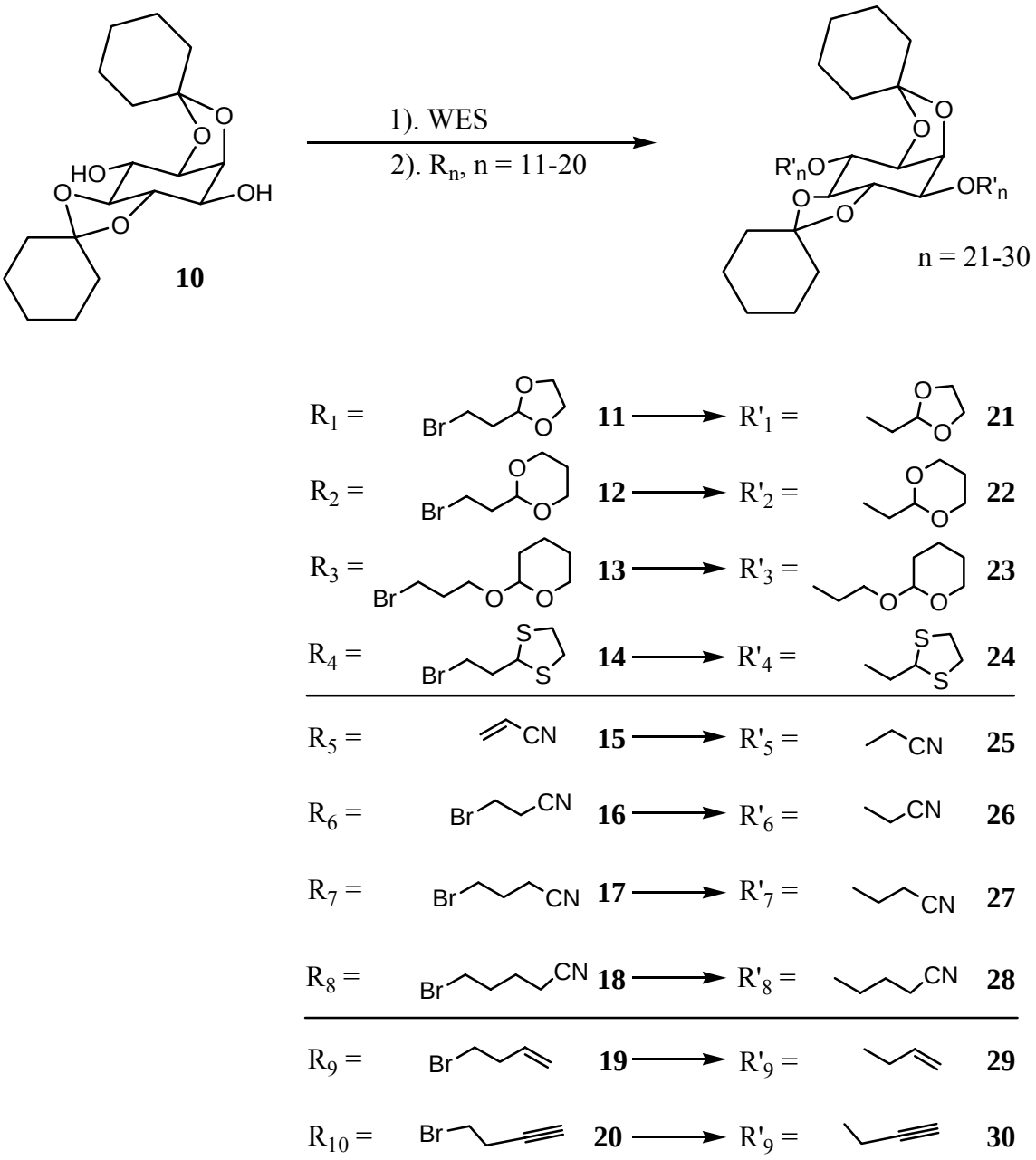


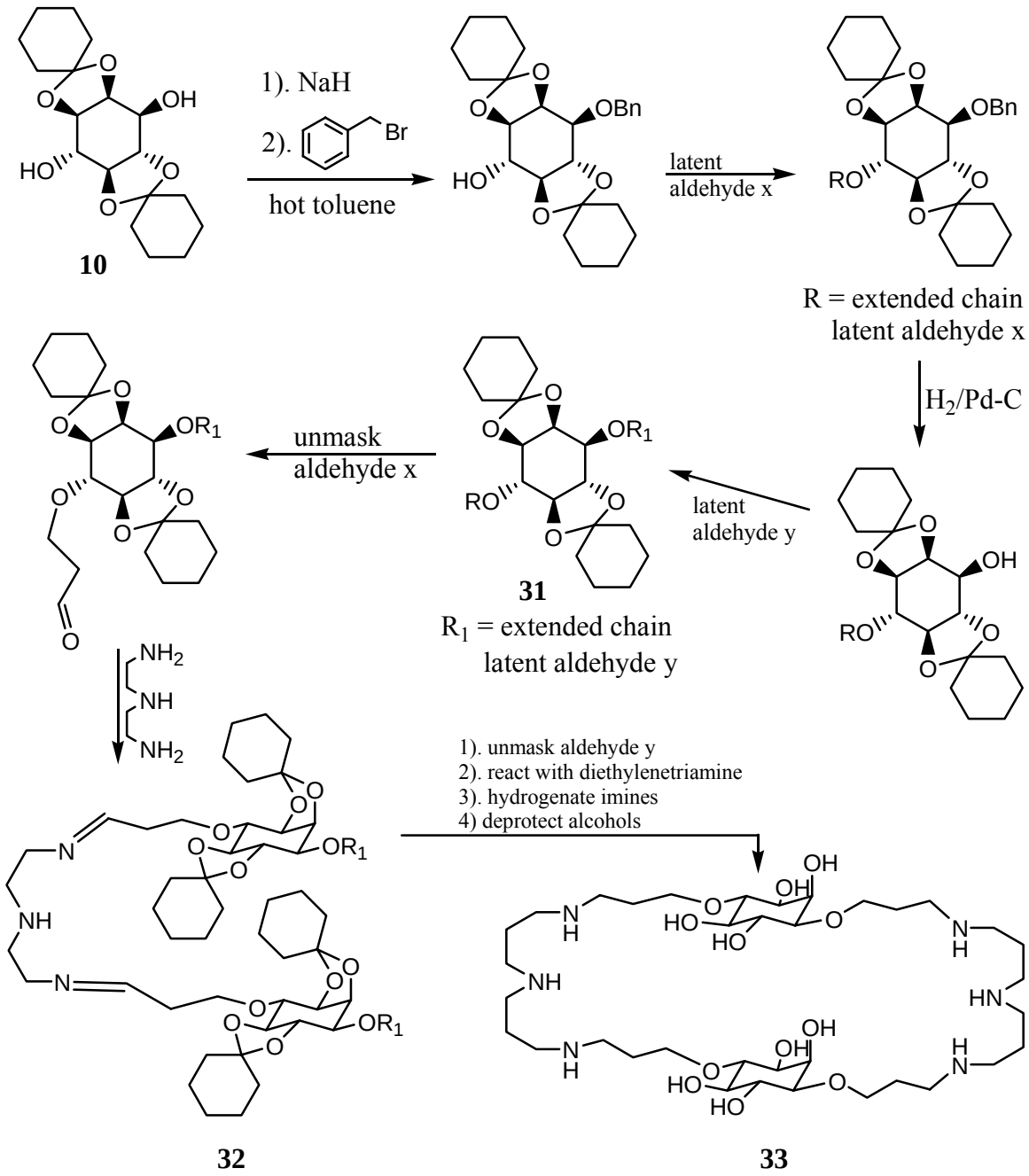
Figure 5.17 Three sets of latent aldehydes for use in the synthesis of inositol dialdehydes.

A second set of latent aldehydes **15-18** comes from the nitrile groups which can be reduced with $LiAlH(Ot\text{-}Bu)_3$ to give the aldehyde function. Acrylonitrile **15** is unique in that no halide leaving group is present. It reacts with the alkoxide through Michael addition rather than by substitution.

In the last set of latent aldehydes, allyl bromide **19** and propargylbromide **20** are terminally unsaturated alkyl halides able to undergo hydroboration-oxidation reactions to unleash their useful functionality. In the case of the allyl group (a well-known inositol protecting group) oxidation and subsequent hydrolysis of the alkyl borane formed between the dicyclohexylidene protected *myo*-inositol dialkene and 9BBN is expected to give a high proportion of the 1^o dicyclohexylidene protected *myo*-inositol diol versus the secondary diol. Like the THP protected ether **13**, the diol formed would require additional manipulation prior to cyclization. Propargyl bromide **20** makes for an interesting latent aldehyde. Upon hydroboration-oxidation with 9BBN the major initial product formed is an enol which spontaneously tautomerizes during borate ester hydrolysis to give the requisite aldehyde function.

A scheme showing how differentially protected and differentially unmasked aldehydes can be used to give a single enantiomerically and regiospecifically pure $Ins(1,4)P_2$ receptor is shown **(Scheme 5.8)**.

Benzylation of the dicyclohexylidene protected *myo*-inositol **10** is reported to be selective for the C1 hydroxyl. Following the benzylation reaction, a latent aldehyde x may be installed at the free hydroxyl group and upon hydrogenation the C1 alcohol is left free. Latent aldehyde y is installed at the newly freed C1 hydroxyl to afford compound **31** carrying differentially protected aldehydes at C1 and C4.



Scheme 5.8

Selective unmasking of the C4 aldehyde followed by reaction of two equivalents with diethylenetriamine provides imine **32**. Unmasking of the C1 aldehyde, reaction

with diethylenetriamine, hydrogenation of the imine groups and deprotection of the dicyclohexylidene protected hydroxyls yields receptor **33**.

A discerning eye will notice that chiral resolution of the racemic dicyclohexylidene protected *myo*-inositol **10** would be required before proceeding too far into this reaction sequence. A few methods of chiral resolution were explored, however it was decide to move ahead with the racemate first and see if we could knock out the target receptor and then go back and work on the resolution.

It was also decided to first establish the ability to construct secondary binding elements whose extended chain latent aldehydes were identical and to not attempt any differential dialdehyde installation during this phase of the project. As indicated in the reaction scheme, differentially protected aldehydes would best be undertaken after chiral resolution has occurred.

The dioxane and dioxolane acetals, along with the THP protected ether were throwbacks to polyazacyclophane days where they were converted into Grignard reagents for subsequent homologation reactions with terephthalaldehyde and thus provided a convenient place to start.

Dicyclohexylidene protected myo-inositol didioxane

Sodium metal in tetrahydrofuran was used to strip the alcohol protons from the dicyclohexylidene protected *myo*-inositol **10**. This was accomplished by brutally subjecting half a gram of the diol to the metal overnight. The slightly yellow solution was added via pipet to a four-fold excess of the bromoethyl dioxane **12** in a separate flask to leave the excess sodium behind. The solution was stirred and refluxed for 24 hours,

dumped into a separatory funnel and extracted with dichloromethane (and "possibly" water according to my notes). The lower layer was rotavapped via water aspiration, then using high vacuum to give a dark yellow liquid. Just to be on the safe side the upper layer in the separatory funnel was also rotavapped to give an insignificant amount of golden brown material deemed safe to dispose.

For comparison purposes a ^{1}H NMR of the starting 2-(2-Bromoethyl)-1,3-dioxane purchased from Aldrich Chemical Company was obtained **(Figure 5.18, S113)**. Analysis of the protons present in the dioxane compound leads one to believe there should be five proton signals in the spectrum, however there were upwards of seven different proton types seen in the NMR , indicating a potential purity problem.

A proton NMR was taken of the crude reaction product obtained after rotavapping the lower layer from the separatory funnel **(Figure 5.18, S114)**. Close comparison of the starting material and crude product spectra shows the small presence of additional protons in the product.

The overpowering signals seen in the product spectrum obviously arose from the dioxane starting material. Comparison with the spectrum of the dicyclohexylidene protected *myo*-inositol **(Figure 5.15 S160/S108)** showed that all the peaks present in the inositol compound could be accounted for in the spectrum of the crude product. Nearly buried in the baseline, there was the triplet around 4.5 ppm, the mess of doublets of doublets between 3.8 and 4.1 ppm (although virtually masked by the goliath dioxane signals), the triplet at 3.3 ppm, the grouping around 2.5 ppm and the mass of peaks between 1.5 and 1.7 ppm (although slightly shifted).

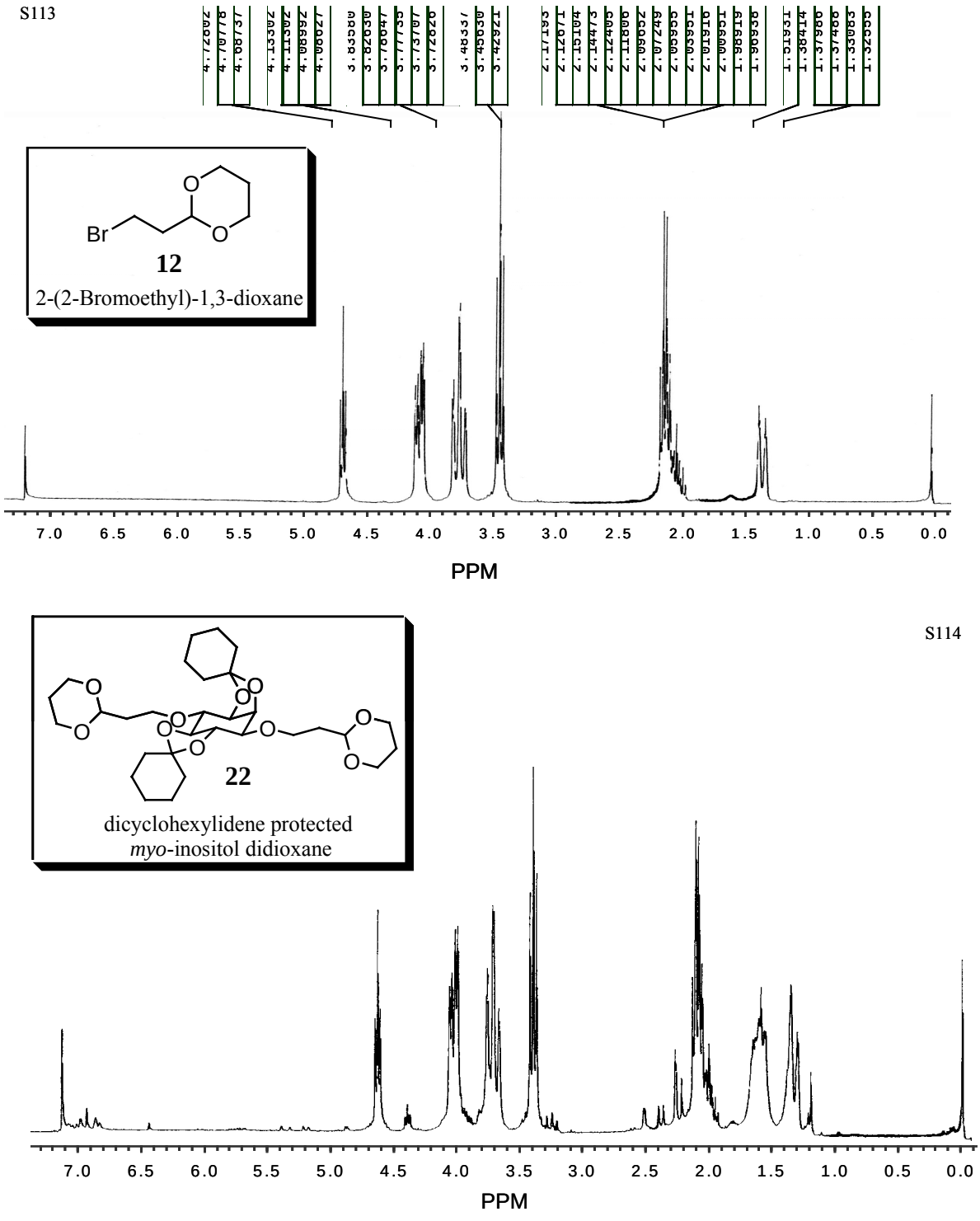
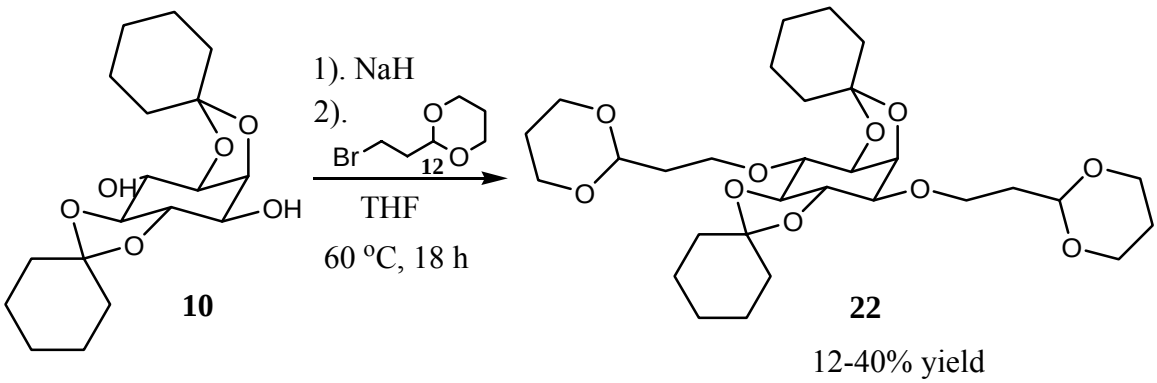


Figure 5.18 ^{1}H NMR of 2-(2-Bromoethyl)-1,3-dioxane **12** (S113) and of the material found in the dichloromethane layer **22**? following treatment of dicyclohexylidene protected *myo*-inositol with sodium metal and compound **12** (S114).

There was no doubt that both reactant components of the expected product were present in the crude product taken from the rotary evaporator, the question was did they actually react, or had I just sampled a mixture of starting materials? That would explain the apparent concentration discrepancy seen in the spectrum. An interesting development was observed with the crude reaction mixture while in its storage vial—white solids began to precipitate out of the yellow liquid.

While contemplating the identity of those solids another half gram scale reaction was set up in THF, where sodium hydride was used for proton extraction instead of sodium metal, and only fifteen minutes of contact time was allowed instead of overnight stirring **(Scheme 5.9)**.



Scheme 5.9

The bromoethyl dioxane **12** was allowed to react overnight at 60 °C with the alkoxide solution of **10** to yield a light brown mixture containing some solids that didn't appear to be sodium hydride and were believed to be sodium bromide—an early

indication of success. Following work-up and removal of extraction solvent via rotary evaporation a cloudy light yellow liquid remained.

A proton NMR of the crude material was obtained **(Figure 5.19)** and some comments recorded at the time as to the identities of the peaks can be seen. As with the spectrum taken of the crude sodium metal reaction, this spectrum too appeared to contain all of the peaks expected in the product, however there was no concentration anomaly as seen with the sodium metal reaction, making a much more convincing argument that this reaction went as planned.

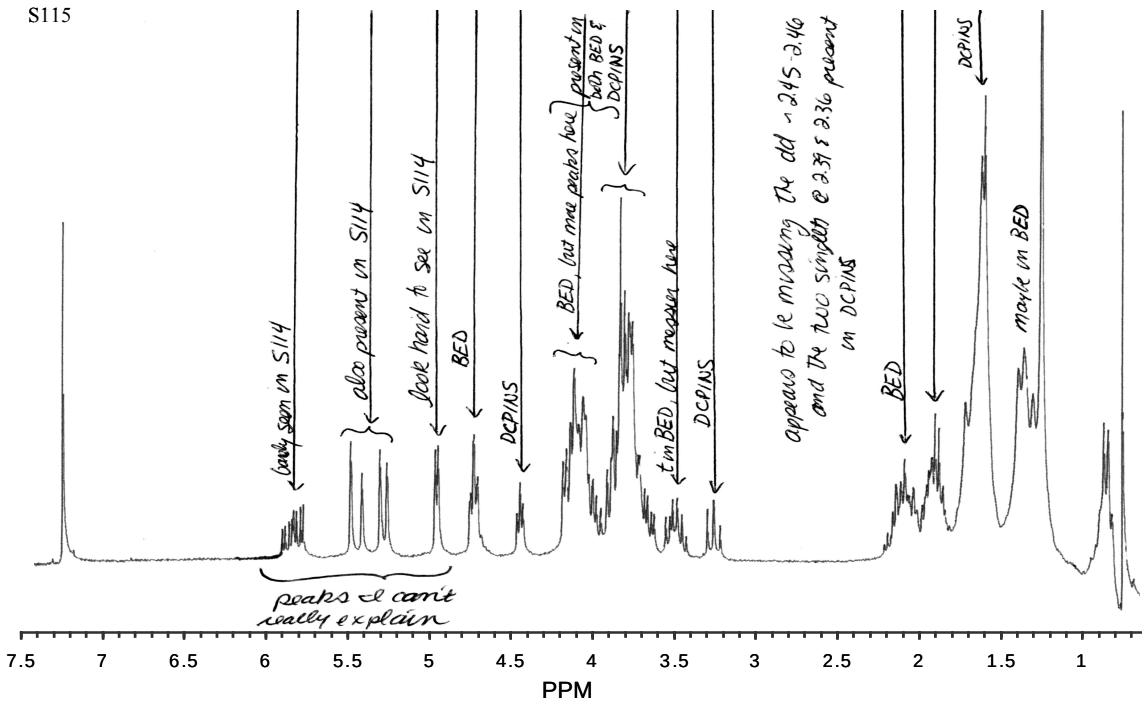


Figure 5.19 ^{1}H NMR of dicyclohexylidene protected *myo*-inositol didioxane **22**.

While the crude yellow liquid sat on the bench top over the weekend off-white semi-crystalline solids appeared as with the sodium metal reaction. Hexanes were used to transfer the colored mixture into a funnel where the solids were filtered and washed with traces of acetone (somewhat soluble) and hexanes to give white crystalline looking solids. They were recrystallized from hexanes to give 40% yield of fine white crystals having a melting point range of 125-127 ^{o}C. A ^{1}H NMR obtained matched the one of the crude with the exception of the loss of peaks seen between 5 and 6 ppm—peaks in the crude that could not be explained.

To further confirm the successful outcome of the Williamson ether synthesis reaction, Maria Warren (at the time a graduate student with Dr. Ron Orlando) did some mass spectrometry work on the recrystallized material **(Figure 5.20)**.

The first spectrum (S117), obtained from a Kratos brand MALDI mass spectrometer, was of the α-cyano 4-hydroxycinnamic acid matrix to establish the matrix ionization pattern. The second spectrum (S118) contained the sample in a 1:1 ratio with the matrix.

Three important new peaks appeared in the second spectrum that were not present in the first. They can be seen at *m/z* 568.7, *m/z* 591.9 and *m/z* 607.9. The molecular formula of the target molecule was $C_{30}H_{48}O_{10}$ which gives a molecular weight of 568.70 g/mol and an exact mass of 568.32 g/mol perfectly matching the first of the three mass to charge ratios above (technically this was off by at most 1 *m/z* unit because the ion should be protonated). The other two peaks corresponded to the sodiated and potassiated ions of the product, respectively.

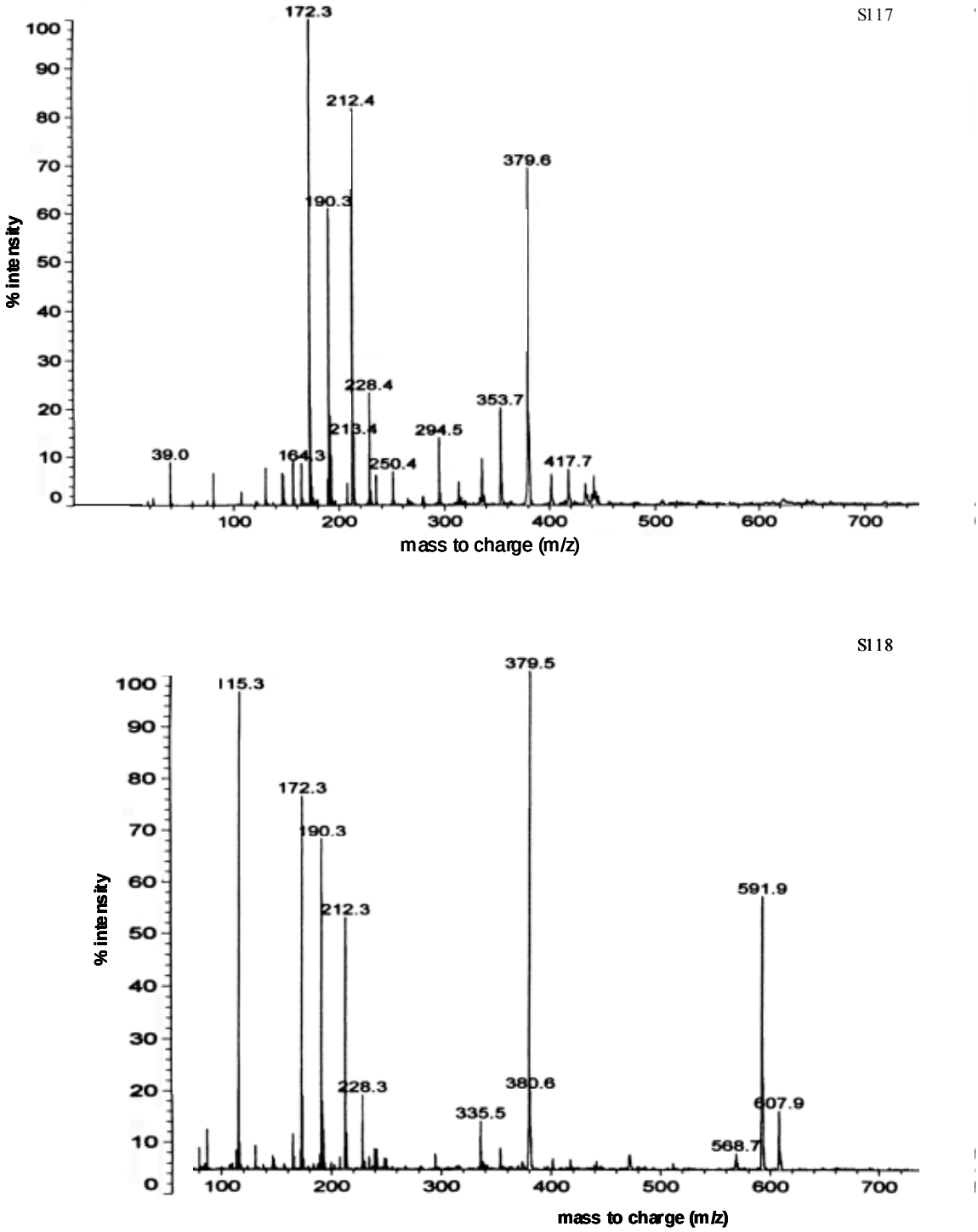
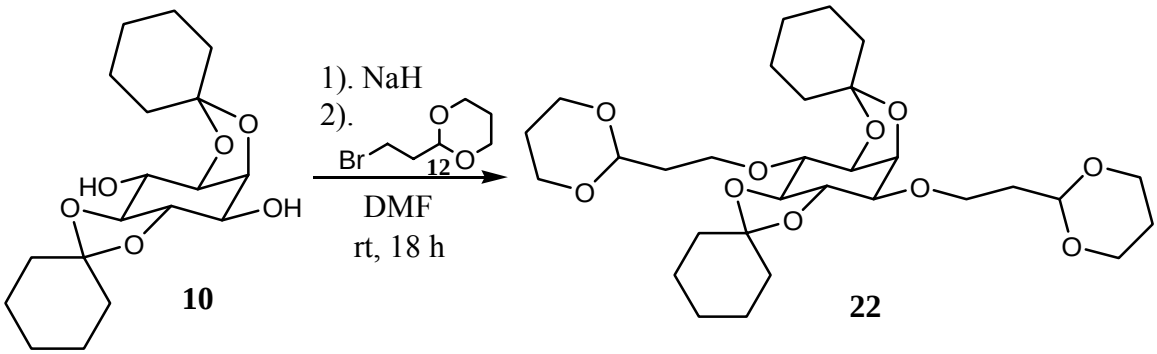


Figure 5.20 MALDI MS of dicyclohexylidene protected *myo*-inositol didioxane **22**.

An attempt to scale-up the reaction by 10-fold gave a yellow crude liquid described as intractable in my lab notebook. In retrospect, the product may have been obtained by swamping the crude residue (believed to be excess, high boiling bromoethyl dioxane unable to be removed via rotavap) with hexanes, but this was apparently not attempted at the time. Scaling the reaction from the original half gram to one or two grams did give isolated product, however the yields suffered and ranged from 12-20%.

A major change was made in the procedure by substituting dimethyl formamide for tetrahydrofuran. This allowed the starting dicyclohexylidene protected *myo*-inositol **10** to be readily dissolved in the reaction solvent at room temperature and also enabled the reaction to take place without heating **(Scheme 5.10)**.



Scheme 5.10

The first efforts under the new conditions were carried out using 1 g of the dicyclohexylidene protected *myo*-inositol **10** and a yield of 87% was obtained for a

significant improvement over previous efforts. Scaling the reaction by a factor of about seven was convenient for making larger quantities of the dicyclohexylidene protected *myo*-inositol didioxane product **22** and yields (80%) suffered only mildly.

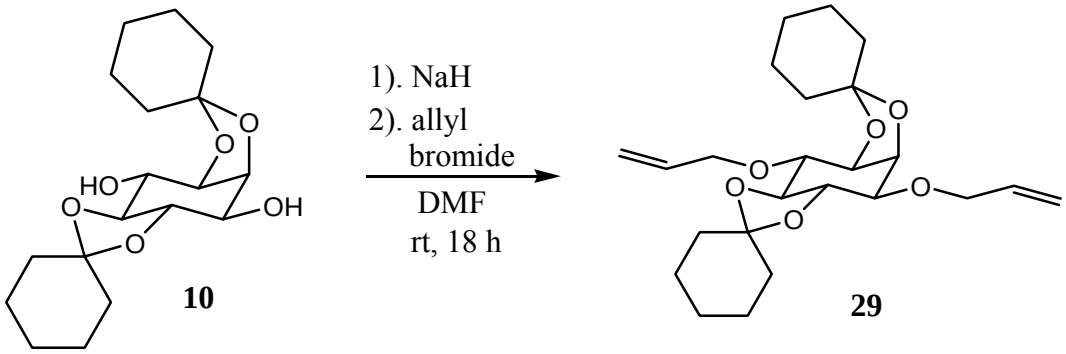
Dicyclohexylidene protected myo-inositol didioxolane

The dioxolane acetal is nearly identical to the dioxane acetal except that it has one less methylene between the two ether oxygens which makes it a five membered ring rather than the larger six membered dioxane. In terms of function, the smaller dioxolane is reportedly easier to hydrolyze than its larger six membered cousin. It is also more subject to thermal degradation and decomposes rather easily at temperatures approaching 40 ^{o}C. This made it a poor candidate for the original reaction conditions used on the dioxane employing heating in THF. Unfortunately, I was not aware of this caveat at the time and several attempts at using the dioxolane in the Williamson ether synthesis reaction using heated THF (pre-DMF discovery) were unknowingly predestined to fail, which they did. A recent inspection of my lab notebook revealed that the only attempt to make the dicyclohexylidene protected *myo*-inositol didioxolane under the milder room temperature conditions in DMF also failed.

Dicyclohexylidene protected myo-inositol dialkene

This was one of the more interesting reactions due to the way the product crystals formed from a concentrated liquid following the reaction. It was prepared by dissolving the dicyclohexylidene protected *myo*-inositol **10** in dimethyl formamide, treating with

sodium hydride, cooling the alkoxide mixture in an ice water bath and adding allyl bromide into the solution **(Scheme 5.11)**.



Scheme 5.11

Within thirty minutes the reaction was complete according to thin-layer chromatography (TLC) results, making it much more convenient than the lengthy acetal reactions. Quenching with water produced a cloudy white solution occasionally containing oily droplets that settled to the flask bottom when stirring was halted. The droplets were suspected to be excess allyl bromide. Ethyl acetate was used to extract the reaction, the extracts were dried and the presence of a non-starting material compound was observed via TLC. Following filtration and solvent removal a clear semi-viscous liquid was obtained which solidified in the flask after being out on the bench top for two days. The solids were broken up, washed and filtered to give 76% yield of product having a melting point range of 53-58 ^{o}C. In future syntheses, the liquid obtained following rotary evaporation was poured in a thin layer onto a crystallizing dish and allowed to form islands of crystals over the course of about a week.

The ^{1}H NMR **(Figure 5.21)** was compared to published data of a similar compound—the diisopropylidene version of our dicyclohexylidene protected *myo*-inositol **(Table 5.1)**.[19] When comparing the obtained spectrum to the starting material spectrum (bottom trace) three new sets of signals arise in the product. These can be seen around 4.33, 5.2-5.4 and 6 ppm. Searching Riley's table of data for these newcomers shows they match the allylic, terminal alkene and olefinic protons assigned to the diisopropylidene derivative, respectively.

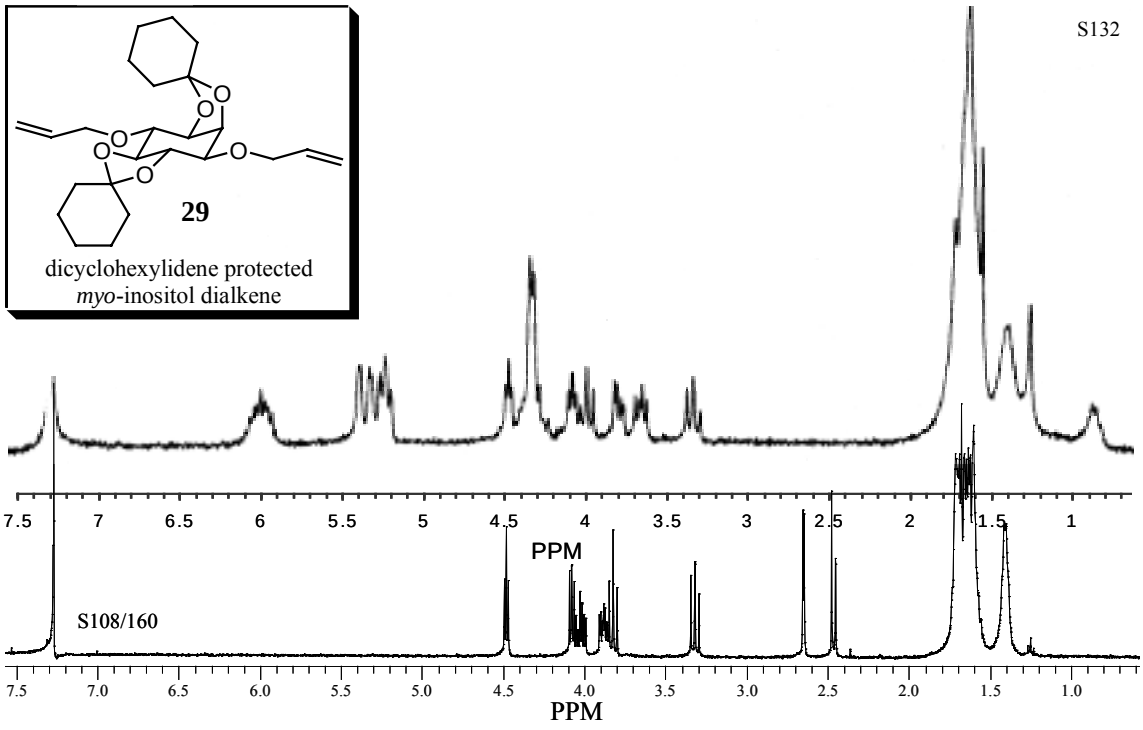


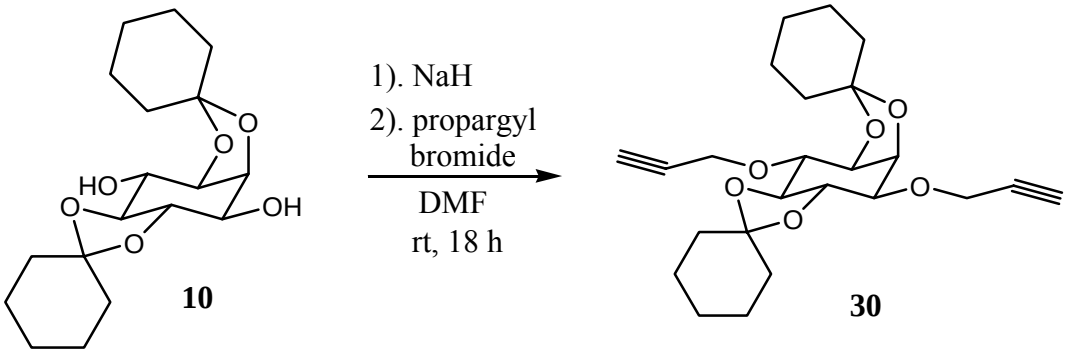
Figure 5.21 The ^{1}H NMR (S132) of dicyclohexylidene protected *myo*-inositol dialkene **29** superimposed with the ^{1}H NMR (S108/160) of dicyclohexylidene protected *myo*-inositol **10**.

Table 5.1 Selected ^{1}H NMR Data of (±)1,4-Di-*O*-allyl-2,3:5,6-Di-*O*-isopropylidene-*myo*-inositol.[19]

Proton Location	Chemical Shift (ppm)	Signal Multiplicity	Number of Protons	Coupling Constant (J)
C5-H	3.34	dd	1	10.5, 9.8
C6-H	3.66	dd	1	10.6, 6.4
C3-H	3.80	dd	1	10.3, 4.2
C4-H	3.98	dd	1	10.1, 10.1
C1-H	4.10	dd	1	6.1, 4.6
Allylic	4.21-4.37	m	4	
C2-H	4.46	dd	1	4.4, 4.4
Terminal alkene	5.17-5.35	m	4	
Olefinic	5.90-6.05	m	2	

Dicyclohexylidene protected myo-inositol dialkyne

The first of many of these reactions was done starting with five grams of dicyclohexylidene protected *myo*-inositol and gave a meager yield of 30%. The protected inositol was dissolved in dimethylformamide and while under argon sodium hydride was added to remove protons from the two free hydroxyl groups **(Scheme 5.12)**.



10 **30**

60-90% yield

Scheme 5.12

Considerable foaming was observed as the reaction was allowed to proceed for about fifteen minutes. While in an ice water bath, propargyl bromide was added changing the reaction color from hydride gray to tan. An hour later the reaction was quenched with water, diluted with ethyl acetate to dissolve, extracted into ethyl acetate, dried, filtered and rotavapped to give a yellow liquid. Pumping on the liquid overnight afforded yellow solids which were dissolved in acetone and the acetone solution was dumped into ice water to precipitate the dicyclohexylidene protected *myo*-inositol dialkyne product which was collected by filtration. Recrystallization from 95% ethanol gave fine white crystals melting between 136-137 ^{o}C. Several later reactions resulted in much improved yields (60-90%) with the ability to double the starting material for higher throughput.

The material was found to decolorize a solution of bromine in dichloromethane and formed beautiful crystals in the solution after two hours—an indication of unsaturation as would be expected with the alkyne appendages.

Infrared spectroscopy **(Figure 5.22)** was used to analyze the product and the sharp strong peak at 3300 cm^{-1} and the sharp weak peak at 2114 cm^{-1} were indicative of alkyne formation. Various spectroscopy and organic chemistry textbooks[20,21] place C≡C stretching in the IR between 2100 and 2140. The peak is described as sharp, but weak and is often lost in the baseline noise. No other functional groups, except maybe a nitrile, show up in this region. Terminal alkynes exhibit a peak ≈ 3300 cm^{-1} arising from the carbon-hydrogen bond stretching in C≡C–H.

You had to dig hard into the ^{1}H NMR **(Figure 5.23)** to find proof of structure with the strongest piece of evidence coming from the pair of triplets centered at 2.44 and

2.47 ppm. These signals were absent in the starting material and are a signature of terminal alkynes where the terminal proton is coupled to two propargylic protons to give rise to the triplets as shown.

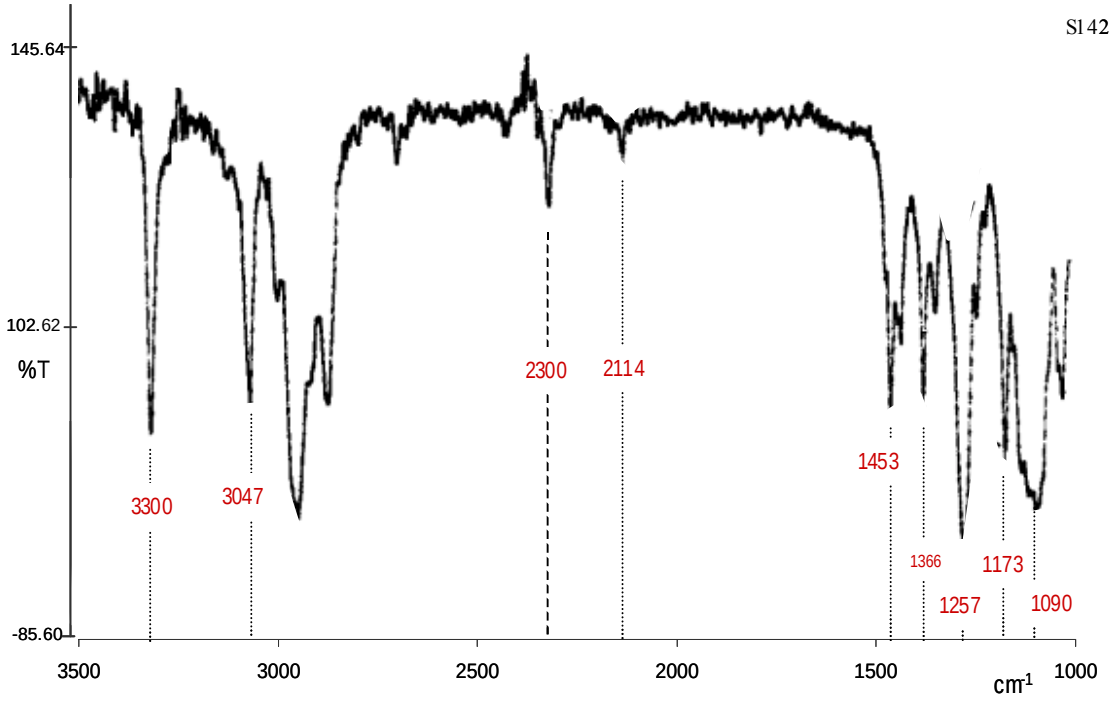


Figure 5.22 The IR spectrum of dicyclohexylidene protected *myo*-inositol dialkyne **30**.

The molecular weight of the dicyclohexylidene protected *myo*-inositol dialkyne is 416.32 g/mol with a sodiated peak expected at 439.52. MALDI mass spectrometry results **(Figure 5.24)** showed a unique non-matrix peak ≈ *m/z* 439.80 verifying the target molecule was successfully synthesized.

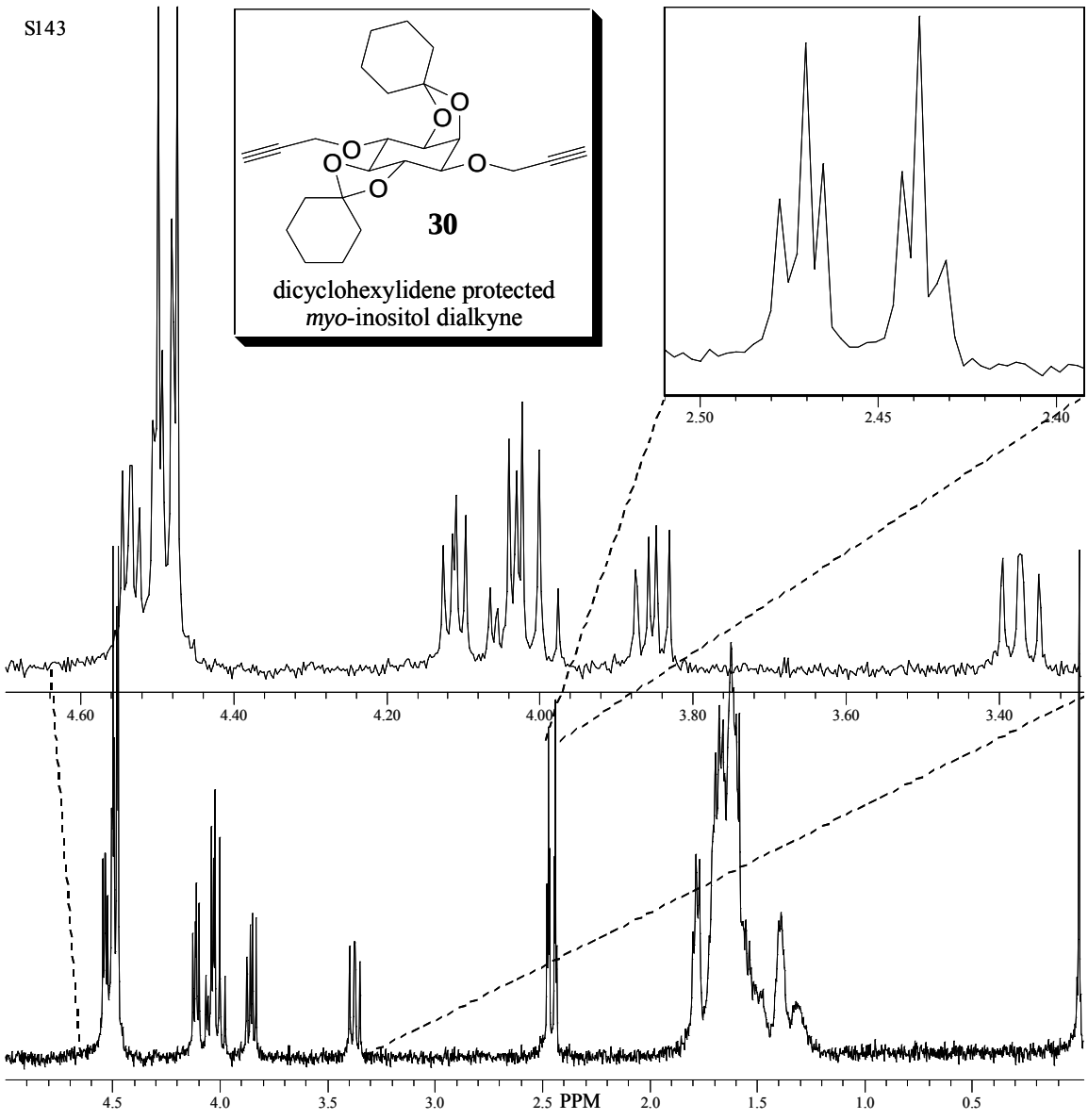


Figure 5.23 ^{1}H NMR of dicyclohexylidene protected *myo*-inositol dialkyne **30**.

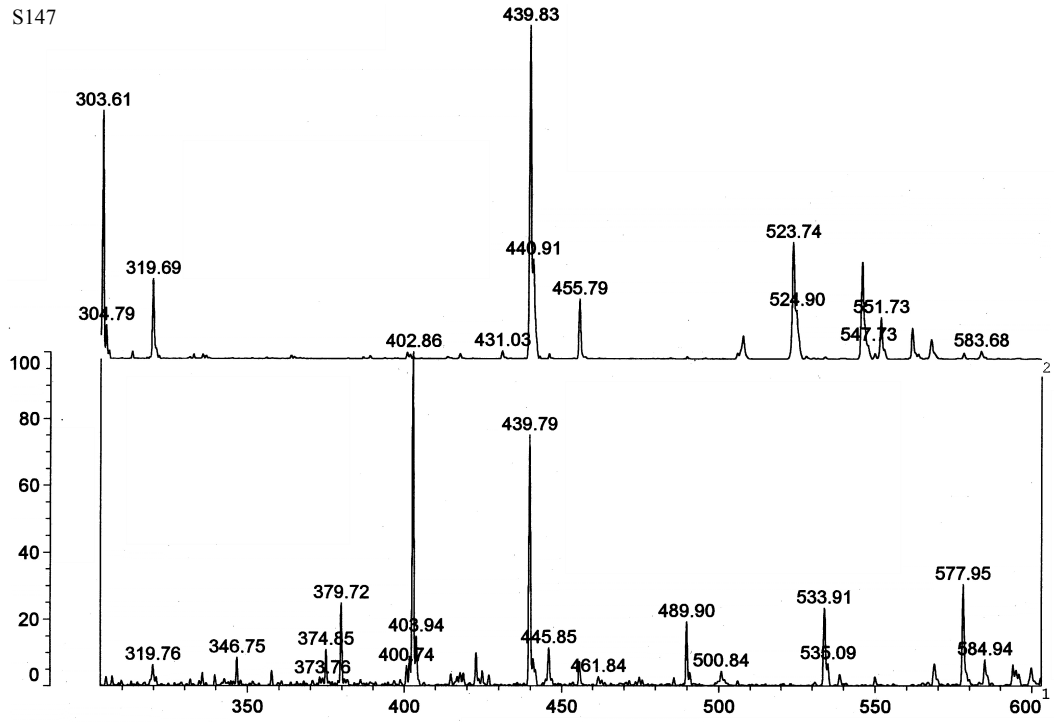


Figure 5.24 MALDI MS of the dicyclohexylidene protected *myo*-inositol dialkyne **30**.

Other latent aldehyde strategies

The THP protected ether of 3-bromopropanol was readily made from the alcohol, hydrochloric acid and dihydropyran. The target ether was verified by ^{1}H NMR, and GC-MS indicated the crude reaction mixture to be about 90% pure with 10% starting alcohol contamination (spectra not shown). An attempt to purify the ether via fractional distillation went awry and the product was destroyed.

No further effort was made to produce this pre-latent aldehyde due to questions arising about its utility. Deprotection of the THP ether would afford an alcohol rather than the aldehyde as required in the current imine cyclization approach. It was envisioned that the alcohol could either be oxidized to provide the aldehyde needed, or it

could be tosylated to be used in a Richman-Atkins[22] macrocyclization reaction with the appropriately modified diethylenetriamine.

A problem could be seen in the unmasking of the protected alcohol because the conditions of deprotection are essentially the same as those needed to deprotect the dicyclohexylidene groups which would leave six unprotected hydroxyl groups subject to either oxidation or tosylation.

The use of this group was deemed impractical and no further attempts were made to work with it. One could foresee a situation where the cyclohexylidene groups are swapped out for some non-acid labile groups making the THP ether protected alcohol potentially useful.

Although several attempts using a variety of reaction conditions were attempted on acrylonitrile **15** and bromopropionitrile **16**, none of these efforts were deemed conclusively successful in spite of trace evidence to the contrary. The crude product from some of these reactions was even taken to the reduction step without attempting to first purify or convincingly verify a successful cyanoethylation. Of course such efforts went unrewarded.

The use of thioacetal **14** was never explored as advertised and presents one of the many areas of future exploration in this synthesis saga.

5.7 Aldehydes and Alcohols: Unleashing Latent Functionality

Of all the latent aldehydes explored for use in constructing the secondary binding element of our inositol-based *myo*-inositol phosphate receptor the three most promising compounds were the (±)2,3:5,6-*O*-dicyclohexylidene protected versions of the *myo*-

inositol-1,4-*O*-didioxane **22**, the *myo*-inositol-1,4-*O*-dialkene **29**, and the *myo*-inositol-1,4-*O*-dialkyne **30 (Figure 5.25)**.

All three of these were obtained from the dicyclohexylidene protected *myo*-inositol in about the same yield (80%) using essentially the same reactions conditions, so none seemed to have an advantage over the others in that respect. In addition, all three were capable of being differentially converted into the aldehyde.

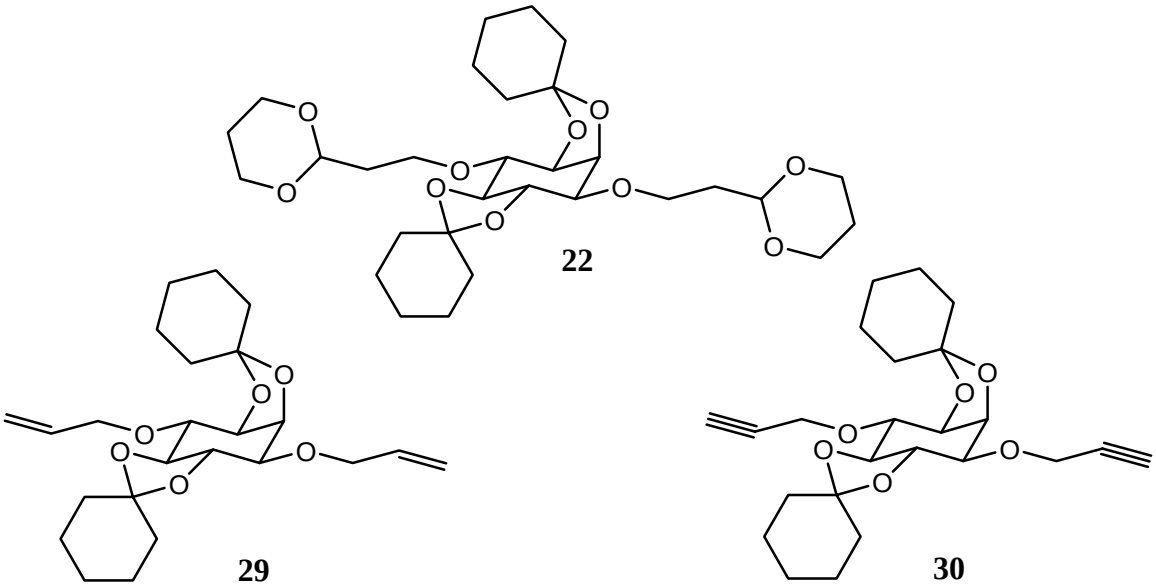


Figure 5.25 The three most promising inositol extended latent aldehyde compounds.

The most direct approach to the extended inositol dialdehyde compound was through the acid catalyzed hydrolysis of **22,** the dicyclohexylidene protected *myo*-inositol didioxane—a mixed acetal/ketal. Several experiments were conducted on this compound in an effort to generate what can be referred to as the tetrol dialdehyde, where the dioxane

acetal groups have been removed to give free aldehyde groups extending from the C1 and C4 positions, and the cyclohexylidene ketal groups have been removed to give free alcohols at C2, C3, C5 and C6.

In a paper by Stowell[23] it was reported that even boiling in acid gave only partial hydrolysis of the dioxane acetal. They were able to effectively hydrolyze the acetal using a steam distillation technique where oxalic acid was used in conjuction with azeotropic removal of product to drive the equilibrium and generate the aldehyde in 89% yield.

Their technique required that the product aldehyde be a liquid capable of forming an azeotrope with water. While the technique didn't seem applicable to the tetrol dialdehyde product **(Figure 5.1)** expected from our hydrolysis it did serve as an omen of difficulties to come in removing the stable protecting group and to illustrate the importance of providing a driving force to the equilibrium reaction.

Early attempts at removing the dioxane acetal groups were carried out in 10% sulfuric acid either at room temperature or under reflux. In neither of these cases was the aldehyde observed in the ^{1}H NMR. At the time I hadn't considered the idea that the acetals and ketals might differentially deprotect under the right conditions, so I was only looking for the formation of an aldehyde peak in the proton spectrum without any regard to the loss of ketal cyclohexylidene groups. Additionally, because I was looking for a quick answer at the instrument, none of those spectra were saved, so it is not possible to re-examine them for clues. Essentially, quick and dirty spectra were used to monitor the deprotection and if the aldehyde proton didn't jump out, it was ditched with no further consideration.

Deprotection was next tried by warming the dicyclohexylidene protected *myo*-inositol didioxane **22** in acetonitrile to form a solution and then adding a pinch of *p*-toluenesulfonic acid monohydrate. Within minutes of adding the acid solids had formed in the reaction which were collected and analyzed by TLC. Developing the silica gel plate spotted with the solid obtained from the reaction using 10% methanol in dichloromethane gave two spots. One of the spots remained on the origin and the other had an eyeballed R_f of about 0.8.

A quick and dirty ^1H NMR of the isolated solids failed to show an aldehyde peak, nor did the compound appear to lose its cyclohexylidene groups. In retrospect, I'm suspicious of the latter part of the assessment made at the time. It now seems, based on results that follow, I may have either overlooked the loss of cyclohexylidene groups in my haste to find an aldehyde peak, or possibly the *trans*-cyclohexylidene was lost leaving behind the *cis* group and its similar bunch of protons in the NMR. The two spots on the TLC plate may have been the dicyclohexylidene deprotected *myo*-inositol with the dioxane acetals still intact ($R_f \approx 0.8$) with the identity of the origin spot becoming clearer.

After doing further research on the topic it was surmised that the dioxane groups were much more stable to hydrolysis than the cyclohexylidene groups. This conclusion was based on the aforementioned observations made by Angyal,[11] Ozaki,[24] and others who determined that the *trans*-cyclohexylidene group was more labile than the *cis*-cyclohexylidene group and easily removed at room temperature via ethylene glycol in chloroform with *p*-toluenesulfonic acid. When taken together with the information presented by Stowell,[23] who said the dioxane was difficult to remove in hot acid, the

natural conclusion was that the cyclohexylidene groups were more labile than the dioxanes.

Based on these findings, it seemed reasonable that the desired tetrol dialdehyde might have to be obtained following two stage differential hydrolysis of the cyclohexylidene ketals and the dioxane acetals, respectively.

The process of testing various conditions to accomplish such a two stage deprotection started with the reaction of dicyclohexylidene protected *myo*-inositol **10** and trifluoroacetic acid (TFA) just to see how well the cyclohexylidene groups could be removed without risking any of the dioxane compound.

A small amount of the inositol derivative **10** was refluxed in 75 mL TFA with a milliliter of water for an hour. The acid was removed via rotary evaporation and the remaining brown liquid residue was diluted with water. The inositol product expected from this deprotection is fully water soluble and nearly insoluble in organics, so the aqueous solution from the rotavap was washed with dichloromethane to remove any non-hydrolyzed material, and the aqueous layer was rotavapped to give semi-white solids. The solids were found to melt between 225-228 ^{o}C, a range within which the melting point of inositol is found, so the solids were assumed to be the expected inositol product.

The tetrol dialdehyde, like inositol, was expected to be fairly soluble in water and less soluble in organic solvents due to the hydroxyl group content and the polarity of the aldehydes. This meant that the above experiment could be repeated, only using the dicyclohexylidene protected *myo*-inositol didioxane **22**, and the expected tetrol dialdehyde should be found primarily in the aqueous solution.

Following rotary evaporation of the dichloromethane washed aqueous layer, brown sugar-like solids were seen in the flask. An attempt was made obtain a ^{1}H NMR of the crude solids by first dissolving them in deuterated chloroform, however they were found to be insoluble in the solvent. Trying to dissolve the brown solids in deuterated methanol managed to dissolve the brown, but not the solids, which were actually quite white. The white solids melted between 226-230 ^{o}C, right where inositol melts. The solubility behavior seen with the NMR solvents was also consistent with inositol.

The first inclination following those events was to question whether or not the Williamson ether synthesis to produce the dicyclohexylidene protected *myo*-inositol didioxane had been successful. Once convinced of that fact, it was then surmised that cleavage of the ether linkages at the C1 and C4 positions had accompanied the TFA catalyzed hydrolysis of the two cyclohexylidene groups.

Going on the belief that TFA was perhaps too harsh of an acid, the hydrolysis experiments were repeated on the dicyclohexylidene protected *myo*-inositol didioxane **22** using 80% acetic acid.

In the first experiment the inositol derivative was refluxed for one hour in the acid solution and TLC was used to monitor the progress of the hydrolysis. Five minutes into the reaction, TLC (5% MeOH in CH_2Cl_2) showed four distinct spots (R_f = 0, 0.11, 0.45, 0.66). That was not unexpected due to the different stages of deprotection between the starting material and the target compound. After one hour two spots remained on the TLC plate, the one on the origin and the one having an R_f = 0.66.

The reaction was extracted with dichloromethane, the dichloromethane layer was neutralized, dried, filtered and rotavapped to give a few drops of liquid having an odor

not quite identifiable. The liquid was analyzed by TLC and was found to be the $R_f = 0.66$ material. It was the only spot on the plate—nothing was on the origin.

The aqueous layer was analyzed by TLC and contained only the origin spot seen on the reaction TLC plates. The two main spots seen via TLC of the reaction mixture completely partitioned themselves between the aqueous and organic layers upon workup.

Inexplicably, no effort appears to have been made to either isolate the compound present in the aqueous solution or to identify the compound in the organic layer.

With the belief that one of the two components seen in the reaction would eventually be converted into the other, another similar experiment was conducted, except that refluxing was allowed to proceed for eighteen hours. No change was observed in the distribution of compounds as seen on TLC plates.

Trying to stay on track with milder hydrolysis conditions a room temperature reaction using 80% acetic acid on the dicyclohexylidene protected *myo*-inositol **10** was set up, again just to see if those conditions could effectively remove the cyclohexylidene groups—as advertised from various sources.

After 90 minutes under those conditions the reaction was rotavapped to give white solids mixed in with an unknown liquid. Ethyl acetate was used to take up the mixture and upon filtering white crystals were obtained having a melting point of 226 ^{o}C, therefore it was assumed to be the expected hydrolysis product—inositol.

Repeating the last experiment using the dicyclohexylidene protected *myo*-inositol didioxane **22**, only over eighteen hours, resulted in crystals that could be safely washed with acetone having a melting point of 127 ^{o}C. Now that was something new, however the melting point of the starting material in that case was also right at 127 ^{o}C, so it was

looking like the room temperature acetic acid conditions capable of removing cyclohexylidene groups from inositol in the absence of any other groups was not up to the same task with the dioxane acetals waving around.

A ^{1}H NMR of the solids was obtained **(Figure 5.26)** and when compared to the proton spectrum of the dicyclohexylidene protected *myo*-inositol didioxane **22** (S115) many key differences pointed to the identity of the compound.

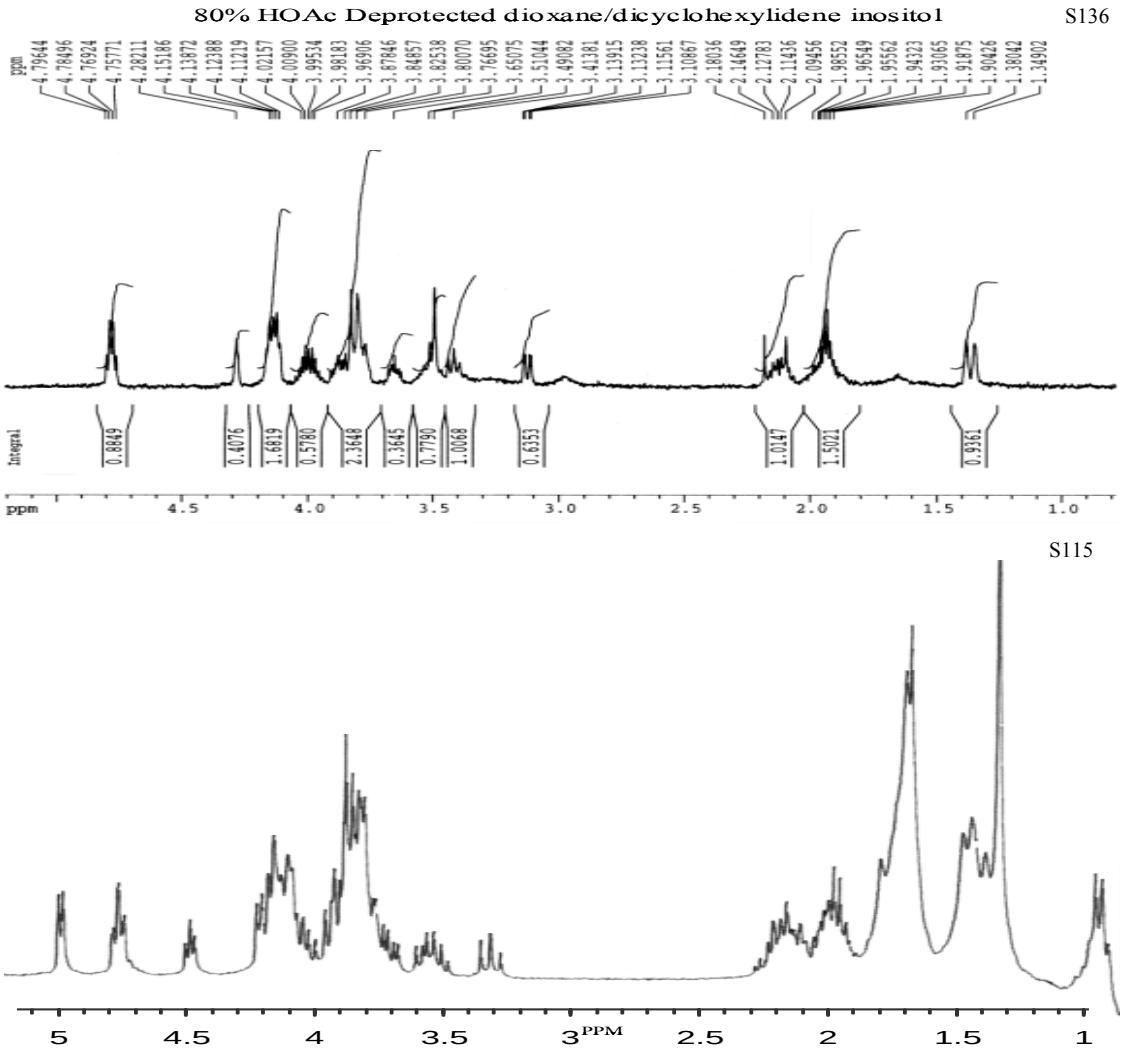


Figure 5.26 Product of the room temperature 80% acetic acid hydrolysis of dicyclohexylidene protected *myo*-inositol dialdehyde.

A major omission of significantly strong proton signals could be seen in the region between 1.4 and 1.8 ppm of the hydrolysis product versus the starting material. That is where the dicyclohexylidene protons resonate and offered good evidence the reaction conditions cleaved the ketal protecting groups from the 2,3 and 5,6 hydroxyls.

The mess of signals seen in both spectra, between 1.90 and 2.10 ppm clearly came from the dioxane protons, probably the ring protons, and indicated the ether linkage anchoring the dioxanes was still intact. Further indication that the dioxanes were still intact were the uniquely dioxane protons clustered between 4.75 and 4.80 ppm. Additionally, there was no aldehyde signal in the original S136 spectrum.

The remainder of the spectrum coincided with the inositol skeleton common to both structures. The molecular weight of the *myo*-inositol didioxane is 408.454 g/mol and MALDI mass spectrometry gave a unique non-matrix analyte peak at *m/z* 431.54 corresponding to the sodiated compound (spectrum not shown).

To round out those experiments, dicyclohexylidene protected *myo*-inositol didioxane was subjected to acid catalyzed hydrolysis by refluxing in 2 M HCl in methanol for three hours, and not unexpectedly the resulting product was inositol.

To summarize the hydrolysis events refluxing both the dicyclohexylidene protected *myo*-inositol **10** and the dicyclohexylidene protected *myo*-inositol didioxane **22** in either aqueous TFA, 2 M methanolic HCl, or as I strongly suspect even 80% acetic acid and *p*-toluenesulfonic acid in acetonitrile, gave back inositol **(Table 5.2)**. Un-fortunately, in the case of the didioxane compound, this resulted from an undesirable C1 and C4 ether cleavage catastrophe that totally undermines the receptor building efforts.

Table 5.2 A summary of all the deprotection/hydrolysis efforts made on dicyclohexylidene protected *myo*-inositol **10** and dicyclohexylidene protected *myo*-inositol dialdehyde **22.**

Starting Material	Reagents	Temperature	Time	Results
22	10% H_2SO_4	Room temp	?	No aldehyde
22	10% H_2SO_4	Reflux	?	No aldehyde
22	*p*-TsOH/MeCN	Warm	Minutes	Solids observed, no aldehyde R_f = 0.8, probably hydrolyzed dicyclohexylidene groups
22	Oxalic acid/water	Reflux	3 hours	No follow-up
22	HCl saturated THF + DMF	Reflux	3 hours	No follow-up
10	75 mL TFA + 1 mL water	Reflux	1 hour	80% recovery of white solids mp = 226 ^oC = inositol
22	10 mL TFA + 2 mL water	Reflux	1 hour	Recovered white solids from aqueous layer following dichloromethane extraction mp 226-230 ^oC = inositol
22	80% acetic acid	Reflux	1 hour	Dichloromethane layer = only 1 spot w/ R_f = 0.66. aqueous layer = only 1 spot on origin, neither was identified
10	10 mL water, oxalic acid, 80% HOAc + ethylene glycol	Room temp, but also on 60 ^oC rotavap water bath	1.5 hours	White solids, washed w/ ethyl acetate mp 226 ^oC = inositol
22	80% acetic acid	Room temp, but also on 60 ^oC rotavap water bath	12-18 hours	White crystals, washed with acetone, mp = 127 ^oC, spectra indicated deprotection of only the dicyclohexylidene groups
22	20 mL of 2M HCl in MeOH	Reflux	3 hours	White solids mp 221-228 ^oC = inositol

It did appear that the cyclohexylidene ketal groups could be independently hydrolyzed in the presence of the dioxane acetals without doing damage to the C1, C4 ether linkages if the reactions were kept at room temperature, however this observation is more of an attempt to put a positive spin on the impossibly stubborn dioxanes, than one

which may be of great utility. I suppose one could imagine a barrage of reprotection of the freed hydroxyls using groups having a different deprotection method, allowing for further attempts to free the aldehyde from the acetals, or to even cleave the C1, C4 ethers at that point and stick a new latent aldehyde out there, but that all seem rather crazy—even for an organic chemist.

Many things can be conjectured about the outcome of the preceding experiments, most of which come to mind as this is being written and didn't at that time. The reversible nature of the deprotection events should not be overlooked, especially in light of all the competing equilibria at work. The ability to remove reaction products as they are formed would do much to push the hydrolysis in the direction wanted, as was done in the aforementioned steam distillation.

This was not a viable option with a solid product, however the target molecule is not the only product produced in the hydrolysis. Deprotection of the cyclohexylidene ketals also generates cyclohexanone, while deprotection of the dioxane acetals produces 1,3-propanediol.

The boiling points of those two reaction by-products are high relative to water (155 and 214 ^{o}C, respectively) so removing them by distillation as they are formed to "pull" the equilibrium didn't seem viable because significant amounts of hydrolysis waters needed to "push" the equilibrium would be lost. If everything but the product azeotroped over, then all would be well and good provided enough water was present initially.

Often in the deprotection of acetals or ketals some sort of sacrificial molecule is introduced that is expected to react with the released protecting moiety better than it

could react with the originally protected group. For example in the case of the cyclohexylidene group, ethylene glycol is added to "soak up" or react with the cyclohexanone released so it doesn't go back and reprotect the vicinal diol. This process is known as a transketalization.

Similar strategies have also been used in the deprotection of aldehydes, where a sacrificial aldehyde or perhaps a ketone is tossed into the milieu. An interesting facet of my particular deprotection is that both an acetal and a ketal are present which sets up some interesting equilibria. Upon deprotection of the cyclohexylidene groups, cyclohexanone is released, and very possibly ethylene glycol could be used to facilitate transketalization, however if the dioxane is deprotected, it kicks out both an aldehyde capable of reacting with the added ethylene glycol and propylene glycol capable of ketalizing cyclohexanone. If ethylene glycol reacts with the freed aldehyde a dioxolane acetal would result, which may or may not be good, but the ketalization of cyclohexanone with propylene glycol would seem to work in our favor.

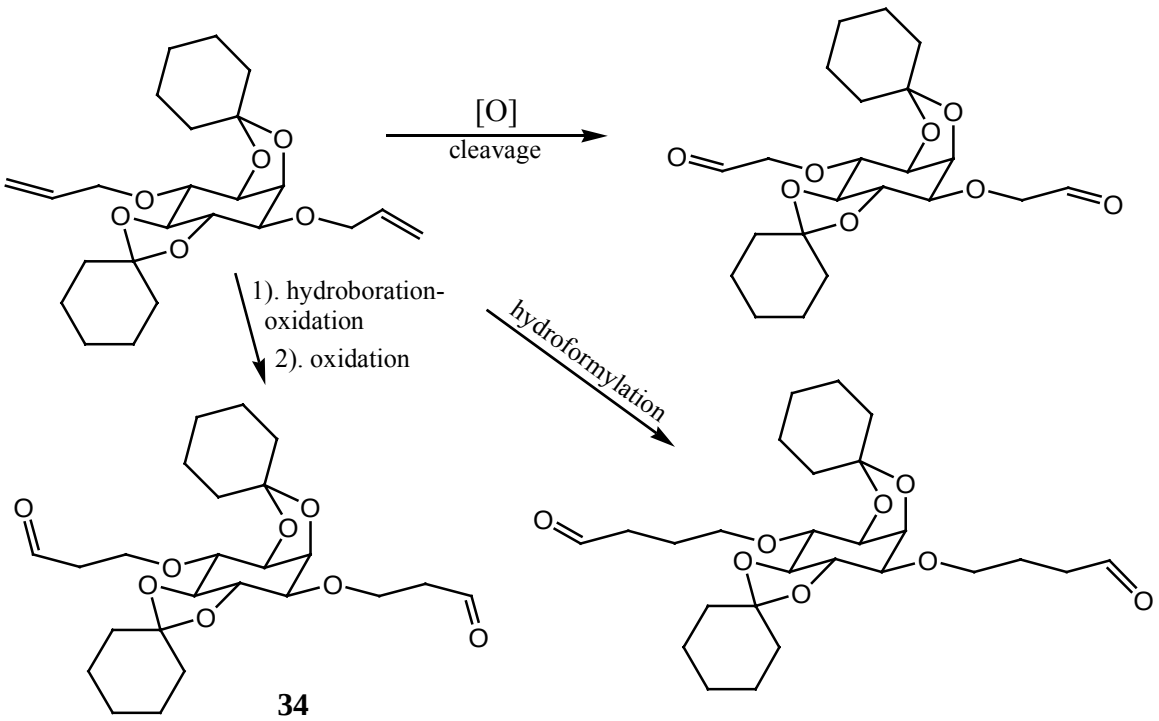
Who knows how all that might pan out in the end and what kind of product distribution could be expected, but all these competing equilibria certainly complicates things. These protecting group interactions may explain why after eighteen hours of refluxing in acetic acid no change was observed in the product distribution. Ultimately it appeared that this reaction really needed a major driving force to get it to proceed completely in the direction desired. Whether that can be accomplished, such that both the ketals and acetals are deprotected without cleaving the ether linkages to the inositol skeleton, remains to be seen.

A few questions immediately came to mind while reviewing this material. What would happen to the dicyclohexylidene protected *myo*-inositol didioxane if treated with TFA at room temperature? Refluxing takes this to inositol. What about a room temperature HCl catalyzed hydrolysis? What were the identities of the dichloromethane soluble, and the aqueous soluble materials resulting from the one hour reflux on 80% acetic acid?

There are at least five possible states of ketal/acetal deprotection available the molecule, not including the states where the C1 and/or C4 ethers have also been cleaved. How would all these states partition between two phases if a biphasic reaction were attempted? And could such a reaction be used to somehow drive the equilibrium toward the tetrol dialdehyde target compound? Since it did seem like the cyclohexylidene ketals could be removed fairly easily, perhaps the biggest question concerns whether or not the dioxane acetals can be hydrolyzed without cleaving the C1, C4 ethers.

Too many questions for what certainly appeared to be a trivial deprotection exercise. All these issues maybe should have driven us back to visit the more easily deprotected dioxolane acetal which was understandably more sensitive in the installation phase, however the research efforts were shifted full throttle in the direction of hydroboration…zoom, zoom…oxidation.

One of the appealing aspects of using the allyl functionality as a latent aldehyde was the diversity built into the group. Through additional chemical modification the allyl group as installed could be used to get three different length extended aldehydes appended to the inositol core **(Scheme 5.13)**.



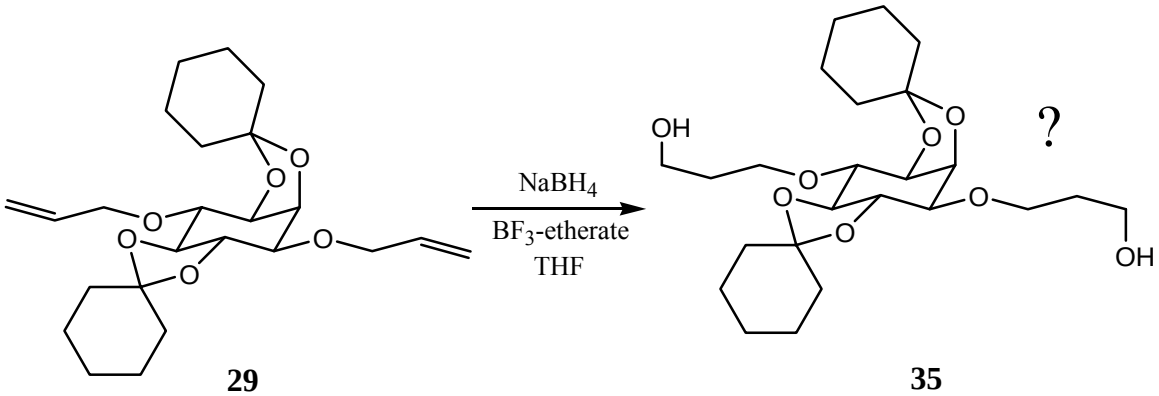
Scheme 5.13

Oxidative cleavage could be used on the three carbon allyl moiety to give a two carbon aldehyde. Hydroboration-oxidation followed by another round of oxidation would give the three carbon aldehyde **34** and hydroformylation of the allyl alkene would produce the four carbon aldehyde. These slight changes in length could be very useful in the making of macrocyclic receptors.

Revisiting Figure 5.21, S132 and Table 5.1 and Riley's data on the isopropylidene version of the inositol diallyl, three regions in the ^{1}H NMR offered guidance as to what to look for upon a successful hydroboration-oxidation reaction. Protons associated with the allylic moiety resonated around 5.98, 5.22-5.38 and 4.33 ppm and all three of those signals should relocate upfield after the oxidation. The allylic and terminal alkene

protons should take up resonance as upfield triplets between 3-4.5 ppm, putting them right in the thick of the inositol proton signals. The olefinic proton would become a pair of aliphatic protons, remaining a multiplet and should shift way upfield following hydroboration-oxidation.

A hydroboration-oxidation experiment based on information gleaned from Vogel's[25] was carried out on about half a gram of the dicyclohexylidene protected *myo*-inositol diallyl compound **29 (Scheme 5.14)**.



29 **35**

Scheme 5.14

The procedure called for the *in situ* generation of borane from sodium borohydride and boron trifluoride dietherate. Accordingly, sodium borohydride was dissolved in heated tetrahydrofuran and the inositol diallyl compound **29** was added. Boron trifluoride dietherate diluted in tetrahydrofuran was dripped into the ice water bath immersed reaction flask over about a fifteen minute period.

Within five minutes fluffy white flocculent was observed, which upon vigorous stirring broke apart to give a cloudy solution. The reaction was quenched with water

after an hour, aqueous sodium hydroxide was added, followed by aqueous hydrogen peroxide. The biphasic reaction was stirred and heated for another hour, extracted with ether, and the ether extracts were washed with water and dried. Following filtration, the ether filtrate was concentrated via rotary evaporation and the liquid residue was allowed to sit on the bench top overnight at which time it was still liquid.

A ^{1}H NMR was obtained of the crude liquid and the only significant change in the spectrum relative to the starting material was the addition of two singlets at 2.89 and 2.96 ppm **(Figure 5.27)**. There was also no loss of any of the allyl fingerprint signals between 5 and 6 ppm. Neither of those occurrences were consistent with the expected product.

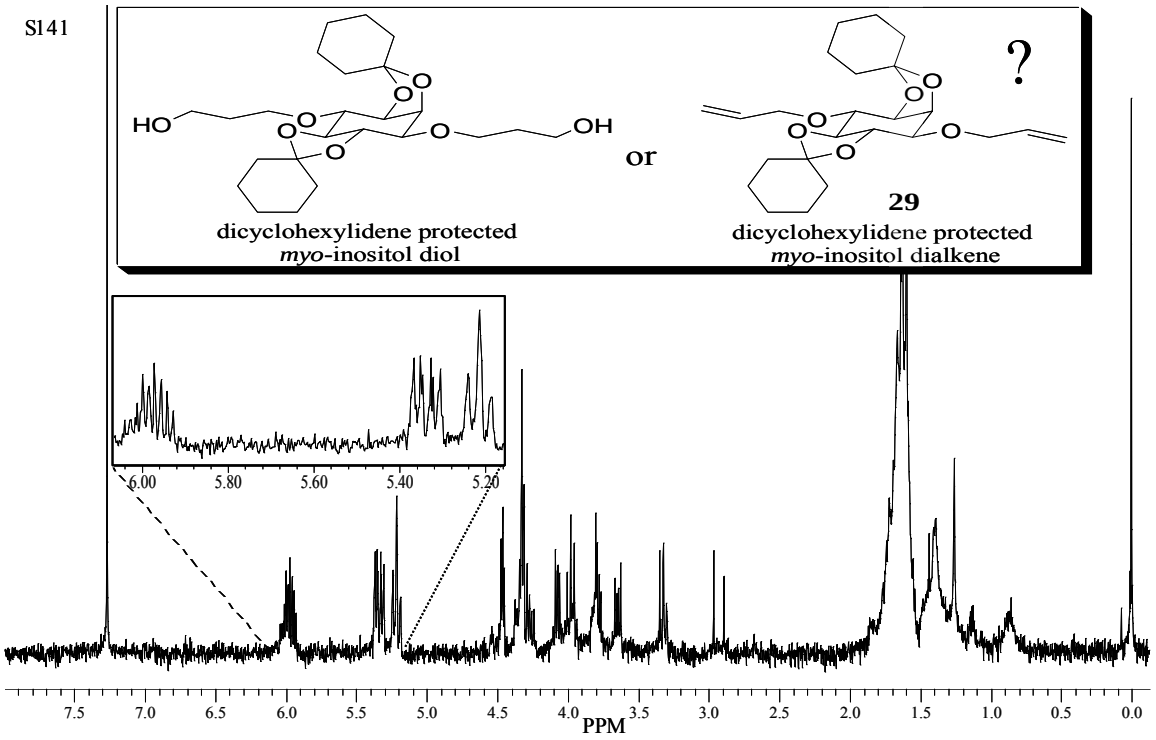


Figure 5.27 ^{1}H NMR of the liquid obtained after reacting the dicyclohexylidene protected *myo*-inositol dialkene **29** with BF_3-etherate and tetrahydrofuran .

A couple of issues were raised concerning the outcome of the hydroboration-oxidation reaction using borane. Although the reaction is known to follow Markovnikov's rule,[26] there was the possibility that some of the products formed were anti-Markovnikovian. Because the starting material was still racemic any deviations from the target molecule were not appreciated and only served to complicate an already difficult situation.

Another concern was the polymeric potential of the hydroboration half of the reaction when using the simple borane compound. This arose from the bis-functionality of the dicyclohexylidene protected *myo*-inositol dialkene combined with the non-alkylated nature of the borane molecule. Polymeric and cross-linked polymeric structures could easily be imagined. It is not clear if such a networked alkyl borane complex would be problematic in the oxidation stage or not, but it was worrisome.

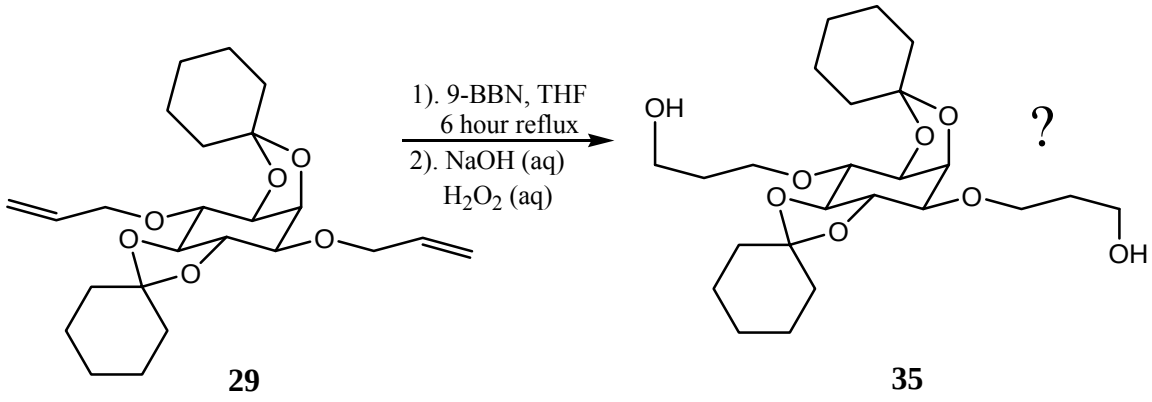
Given these concerns and the apparent failure of the previous borane reaction it was decided to pursue the hydroboration-oxidation reaction using a sterically bulky dialylated borane derivative—9-Borabicyclo[3.3.1]nonane (9-BBN).

Another change made to the procedure involved the oxidation half of the reaction. Traditionally oxidation of the boron atom is accomplished by using a basic hydrogen peroxide solution to oxidize the trialkyl borate to its borate ester, which is then hydrolyzed to give free alcohols. Further oxidation of the alcohol does not occur, however it is known that the use of pyridinium chlorochromate (PCC) in the oxidation step can oxidize the resulting alcohol directly to the aldehyde *in situ*.

Proceeding without much direction, the first efforts using 9-BBN and PCC resulted in an exothermic, multi-colored, ether buzz disaster affording little more than a headache and a sink full of cruddy glassware laced with toxic chromium by-products.

It was decided to temporarily shelve the PCC oxidation idea and to concentrate on getting a hydroboration-oxidation reaction to work. A little more direction in that area was provided in a paper by Dubber where a hydroboration-oxidation reaction was used to convert five allyl groups on a carbohydrate skeleton to their corresponding alcohols.[27]

A solution of the dicyclohexylidene protected *myo*-inositol dialkene **29** and 9-BBN in tetrahydofuran was refluxed under argon for six hours, cooled in an ice water bath, treated with aqueous sodium hydroxide and aqueous hydrogen peroxide, stirred overnight and neutralized in a separatory funnel **(Scheme 5.15)**. The organic layer was dried, filtered and the filtrate was rotavapped and allowed to stand at room temperature for two days. Ethyl acetate was added to the cloudy white residue to precipitate white solids which were collected by filtration.



29 **35**

Scheme 5.15

The solids showed some signs of melting at the same temperature as the starting material (50-60 ^{o}C) however didn't fully melt until >100 ^{o}C. A Jones reagent test for the presence of alcohols was done on the starting dialkene material alongside the isolated solids and was negative for the dialkene, but gave an instantaneous very positive test for the product. The Jones test indicated that the reaction was successful.

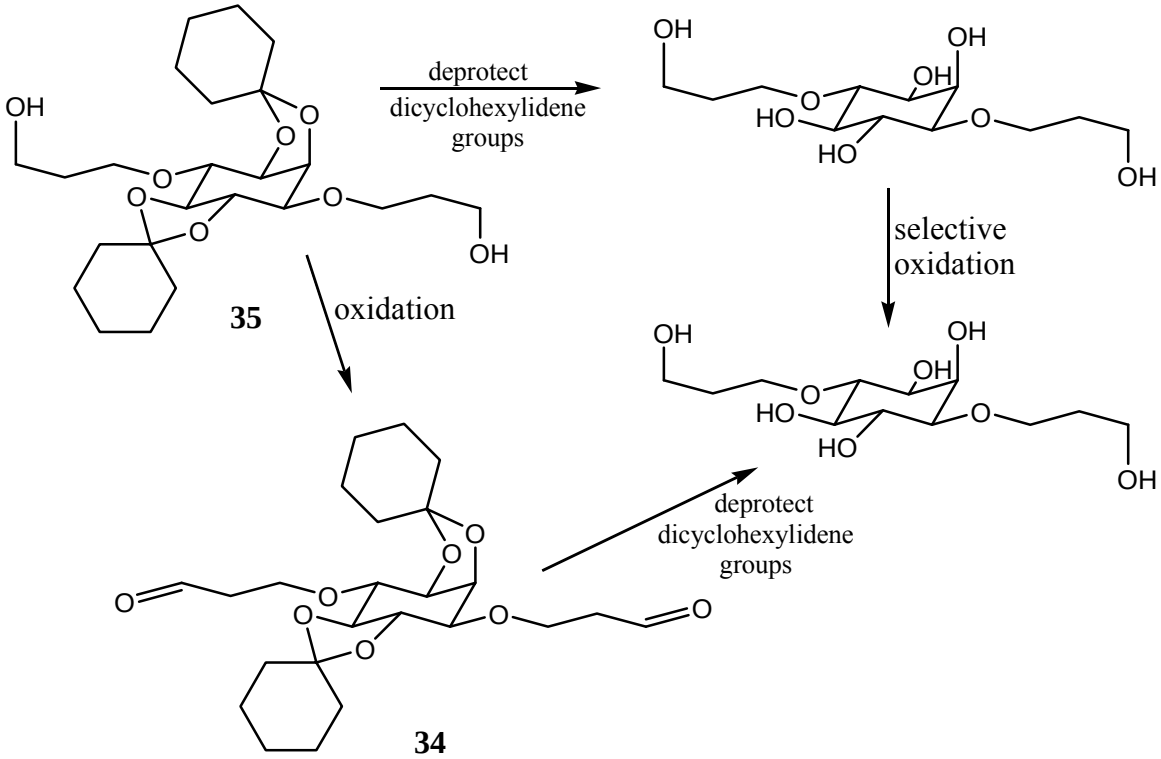
Attempts to recrystallize the product failed, as it was found to be fully soluble in water and methanol, somewhat soluble in acetone, and insoluble in boiling ethyl acetate, toluene and hexanes. No instrumental analysis of the compound appears to have been undertaken as it was used immediately in an oxidation reaction in an effort to afford the dicyclohexylidene protected *myo*-inositol dialdehyde.

In actuality, two pathways from the dicyclohexylidene protected *myo*-inositol diol **35** to the target tetrol dialdehyde can be envisioned. One would be to first remove the dicyclohexylidene groups followed by selective oxidation of the primary alcohols using for example Dess-Martin periodate chemistry, or the oxidation could be done first followed by removal of the ketals **(Scheme 5.16)**.

The latter approach seemed to be more efficient and the cyclohexylidene groups might help keep the compound organic soluble making it easier to perform chemistry on. In fact solubility issues were really beginning to creep into this whole project as it inched more and more toward alcoholic products which tend to migrate into water layers during reactions and work-up procedures making them very difficult to manipulate chemically and to isolate.

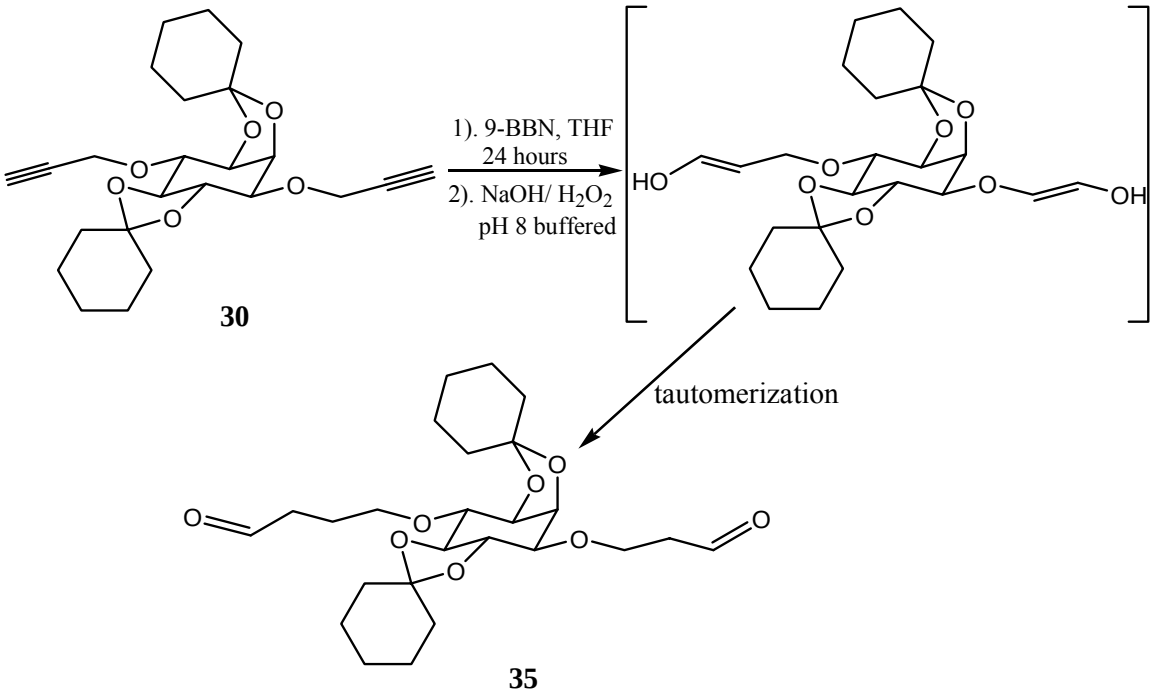
These problems apparently plagued my early attempts to oxidize the putative diol from the hydroboration-oxidation reaction. It was the combination of oxidation

difficulties, and a lot of doubt as to whether the hydroboration-oxidation reaction was actually working, that shifted the research focus to the dicyclohexylidene protected *myo*-inositol dialkyne **30**.



Scheme 5.16

After a few false starts with the dialkyne some work by the master H. C. Brown prodded me in the right direction.[28,29] A commercially purchased solution of 9-BBN in tetrahydrofuran was placed into a flask and cooled in an ice water bath. A solution of the dicyclohexylidene protected *myo*-inositol dialkyne **30** in THF was slowly added to the 9-BBN solution over thirty to forty minutes **(Scheme 5.17)**.



Scheme 5.17

After 24 hours of stirring under argon the reaction solution was added slowly to a cooled solution of basic hydrogen peroxide buffered at pH 8. A white precipitate was observed forming over time and after two hours the reaction was quenched with water and diluted with ethyl acetate to fully dissolve the viscous, oily material. Following extraction with ethyl acetate, washing the combined organic layers with brine, drying, filtering and rotavapping the filtrate, a slightly yellow sticky viscous liquid was obtained.

A small amount of the liquid residue was subjected to a 2,4-dinitrophenylhydrazine test for carbonyls and gave an instant positive result indicating the aldehyde had been successfully unleashed. The celebration lasted well into the night., but just to be certain, exploratory ^{1}H and ^{13}C NMR of the crude residue were obtained **(Figure 5.28)**.

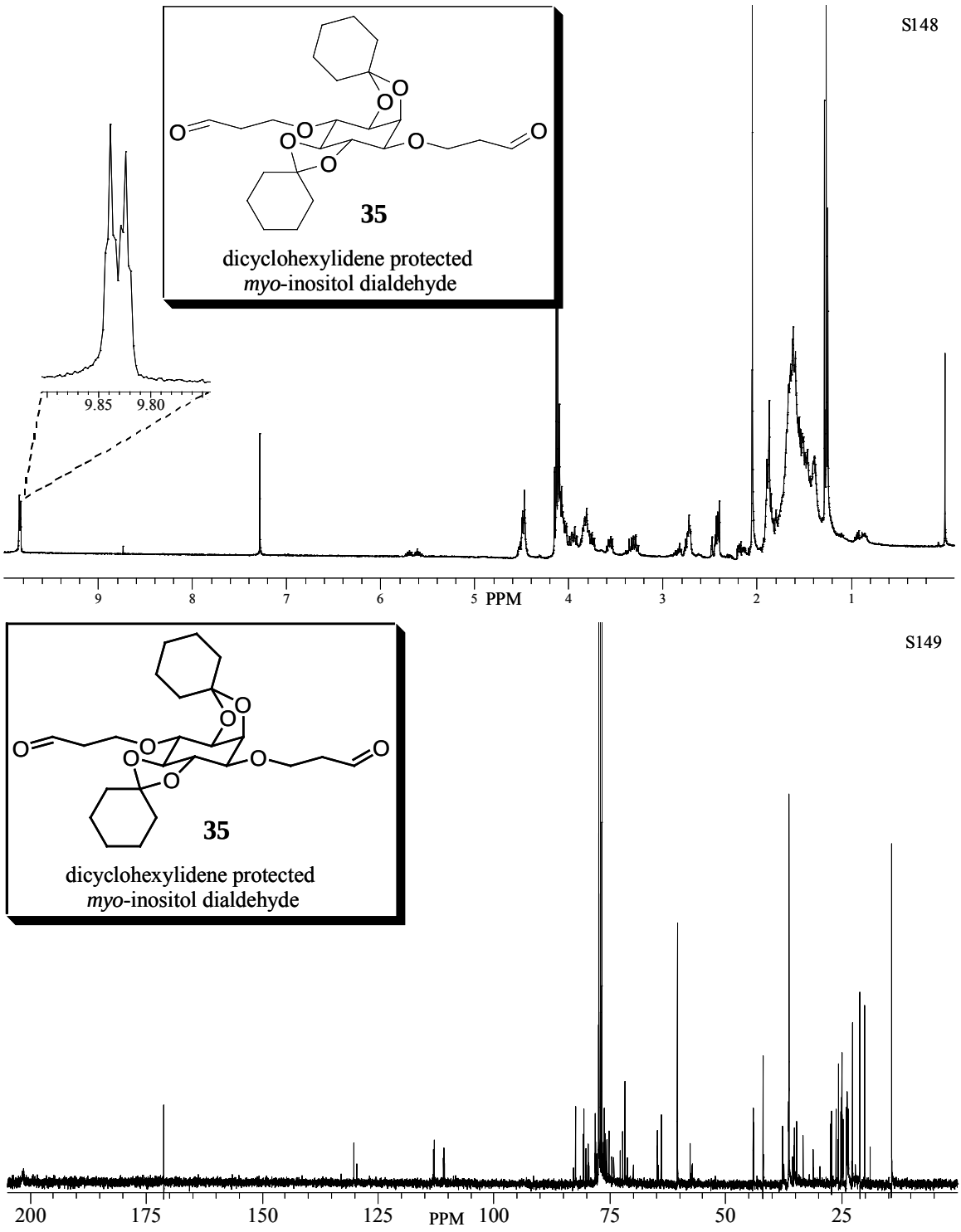


Figure 5.28 ^{1}H NMR (S148) and ^{13}C NMR (S149) of the product obtained following the 9-BBN hydroboration-oxidation of the dicyclohexylidene protected *myo*-inositol dialkyne **35**.

The main objective of those spectra was to find evidence of aldehyde groups in the product and indeed that was the case. Aldehyde peaks arising from both of the alkynes could be seen at 9.838 and 9.824 ppm where no such peaks were ever seen before in this project. The carbon spectrum also supported the presence of an aldehyde with a signal (albeit, weak) at 201.6 ppm.

A flurry of questions flooded my mind at the time of this success. With the crude dicyclohexylidene protected *myo*-inositol dialdehyde in hand what was next? Although pretty certain the target molecule was a solid, the possibility of it being liquid was not ignored, I'd been fooled before. So how should it be isolated? Should I continue rotavapping using high vacuum and risk pulling off a liquid product and loosing it, or would further removal of solvent generate nice solids to play with? Maybe purification by column chromatography was in order, but what about the fate of relatively labile cyclohexylidene groups on notoriously acidic silica gel? Should I attempt to deprotect the dicyclohexylidene groups of the hydroboration-oxidation product or perhaps try and remove those on the dicyclohexylidene protected *myo*-inositol dialkyne first and isolate the tetrol dialdehyde? Do I attempt to react the crude mixture with the diethylenetriamine in a cyclization effort and then purify? As with any good research project each milestone reached only serves to be a springboard for further investigation. Many more questions arose than had been answered, so we plodded on.

From the barrage of questions came the decision to first get the crude product in a more pure state, although I have to admit to a few unauthorized and impromptu attempts made to couple the crude dialdehyde with diethylenetriamine in hopes of miraculously producing a clean macrocycle.

One of the more important things I've learned during my tenure as a graduate student is that quick and dirty doesn't work, and this was no exception. By the way, I've also learned not to trust literature preps and that water soluble and UV transparent products suck, and the hardest part of organic chemistry is the process of product purification.

The crude reaction product from the milestone hydroboration-oxidation reaction was subjected to silica gel column chromatography. After preparing the column from a slurry of dichloromethane and silica gel and loading with sample, elution was carried out using dichloromethane and three large 500 mL fractions were collected and concentrated to about 20 mL of slightly yellow liquid.

Elution was continued by increasing the eluent polarity to a 3% methanol in dichloromethane solution as 40 x 10 mL and 40 x 20 mL fractions were collected off the column. As the methanol hit the silica, the column became warm and a blue band developed which stayed up high in the gel. A slightly yellow band was observed moving down the column which made it about two-thirds of the way down before methanol induced heat and air compromised the column and it "cracked."

About 600 mL was collected from the cracked column and in a separate fraction the yellow band was captured. Samples were spotted from the two sets of 40 fractions onto TLC paper and sprayed with 2,4-dinitrophenylhydrazine reagent to visualize the presence of aldehydes and none were seen. The two large fractions collected after the smaller ones right before the yellow band and including the yellow band both tested positive to 2,4-dinitrophenylhydrazine.

The pre-yellow band fraction was concentrated via high vacuum rotary evaporation and upon separating the flask from the bump trap a ring of clear liquid was seen in the neck of the trap which upon scraping with a spatula formed white crystalline solids. The material gave a positive 2,4-dinitrophenylhydrazine test, but there was not enough of it to get an NMR. The yellow band fraction was concentrated via high vacuum rotary evaporation and gave sticky yellow semi-solid material whose ^{1}H NMR showed aldehyde peaks at 9.82 and 9.84 ppm.

The fortuitous discovery made on the rotavap trap leading to crystalline material did show that the compound was indeed a solid and that there was hope it could be isolated in larger quantities. Several additional attempts were made to fine tune the column chromatography separation by collecting ever so smaller and smaller fractions and painstakingly analyzing the fractions using TLC and both phosphomolybdic acid and 2,4-dinitrophenylhydrazine visualization, but the dialdehyde could never be sufficiently isolated in its solid state.

It had become a fairly routine matter to generate the crude compound using the hydroboration-oxidation method. The major challenge remaining at this stage of the project was isolation of the pure dicyclohexylidene protected *myo*-inositol dialdehyde in sufficient amounts to proceed with the cyclization reaction.

During some of the experiments it was learned that water could be used to wash the sticky liquid obtained as a crude reaction product and in some cases this water wash would generate near solid-like material. Later I went to trying a boiling water wash and that also had some utility. The water was easily poured off the sticky material following

a good working and it left behind a better looking, less yellow and slightly better smelling compound. The crude dialdehyde possessed a nasty smell reminiscent of pyridine.

It was also found that the sticky smelly crude material could be handled better by freezing it for a few seconds in a dry ice-acetone solution. Upon solidification it could be broken into chunks and easily transferred from flask to flask.

Dr. Johnson thought it would be worthwhile to run the material on a column of bio-beads, which is a size exclusion chromatography resin that allows larger molecules to quickly pass through the column while smaller molecules weave in and out of the polymer matrix and are slowed down. The beads come in different sizes reflective of their pore size which in turn controls the sizes of the molecules capable of being separated by the resin.

Such a column was assembled by first soaking the beads overnight in THF and pouring the slurry into a chromatography column. The sample (150 mg) was loaded onto the column and eluted with THF. A forerun of 50 mL was collected before fraction collection commenced, and 40 fractions were then collected in one dram vials (4.2 mL). Fractions 41-63 were 1/3 of a dram. Fractions were spotted and visualized on TLC plates by spraying with PMA and heating.

Fractions 1-25 contained analyte according to PMA visualization and TLC work in either dichloromethane or 5% methanol in dichloromethane showed promising spots in fractions 20-24. Those five fractions were pooled, rotavapped and dried via vacuum pump to afford 110 mg of sticky, still semi-solid/liquid material which no longer had the nasty odor associated with the crude product loaded onto the column and tested positive with 2,4-dinitrophenylhydrazine.

Factoring in the forerun (50 mL) and 25-4.2 mL fractions, all of the semi-purified product was eluted from the column with only 155 mL of eluent which translated into ≈ 90 minutes per run.

Although the desired solid product was not obtained, the bio-bead column was deemed a useful tool because it was fairly rapid and succeeded in removing whatever the foul smelling substance was from the crude product. Also, the advantage of a bio-bead column over a silica gel column was that the bio-bead column could be used multiple times following a thorough washing between runs.

The next test of the bio-bead column was to see if 500 mg of crude material could be effectively semi-purified. The same protocol was employed to collect fractions, including a 50 mL forerun, however more extensive TLC work on the fractions was accomplished **(Table 5.3)**.

Table 5.3 Thin-layer chromatography analysis of fractions from an SX-8 bio-bead column loaded with crude dicyclohexylidene protected *myo*-inositol dialdehyde and eluted using tetrahydrofuran.

Fraction number	Number of Spots on plate	Retention Factor	2,4-DNP test results
1,2	One	0.06	Not tested
3-7	Two	0.06, 0.27	Not tested
8-17	Three	0.00, 0.06, 0.27	Not tested
18-21	Two	0.06, 0.27	Not tested
22-33	One	0.27	Positive
34-72	Two	0.06, 0.27	Negative

Based on the previous column, and the observation that fractions 22-33 contained only one spot, it was believed that the desired product resided in those fractions. They

were pooled and rotavapped to yield 265 mg of non-smelly liquid which gave a delayed positive 2,4-dinitrophenylhydrazine test. Fractions 34-72 were pooled and rotavapped and tested negative to hydrazone formation. No other fractions were tested.

The run-on seen with the R_f 0.06 and 0.27 spots may have been be due to column overloading and the continuous bleeding of material through an overtaxed resin. This idea was corroborated by the observation that analyte continued to elute even after >350 mL of eluent had been run through the column.

A new bio-bead column was assembled using the same SX-8 size exclusion resin used in previous experiments except toluene was used to both pack and elute the column rather than tetrahyrdofuran.

To avoid the overloading seen in the previous run, only 216 mg of crude dicyclohexylidene protected *myo*-inositol dialdehyde was placed on the column. Sixty fractions of 5-6 mL each were collected immediately in test tubes with no 50 mL forerun.

Spotting of the fractions onto TLC plates and spraying with 2,4-dinitrophenyl-hydrazine reagent showed the presence of aldehyde in fractions 22-46 by a bright yellow-orange spot. Of these 25 fractions, further TLC work led to the pooling of fractions 20-36 which were rotavapped to leave 123 mg of not so nasty smelling material that gave a positive 2,4-dinitrophenylhydrazine test.

Placing the material in the freezer caused it to solidify—an event that had only happened once before and certainly worth noting. Unfortunately this material fell victim to a macrocycle crazed chemist as it was wasted in a quick and dirty imine cyclization attempt—easy never works.[30]

Fractions 39-50 were pooled based on TLC plates developed in 10% ethyl acetate in dichloromethane solution and visualized by PMA showing them to contain one strong spot. The pooled fractions were washed with distilled water and the organic layer was dried, filtered and rotavapped to leave behind a yellow liquid reeking of the crude dialdehyde. The material was dissolved in 95% ethanol and tested negative to 2,4-dinitrophenylhydrazine. Again the SX-8 column demonstrated its usefulness as it was doing a decent job of cleaning up the crude sample.

One more run using the SX-8 column enabled a couple of short protocols to be developed and the material collected using these protocols was white, solid and crystalline.

In the first protocol 340 mg of crude compound was loaded onto the column and eluted with toluene. A 50 mL forerun was collected followed by 30 10 x 175 mm test tube fractions (5-6 mL). The fractions were spotted and visualized by spraying with 2,4-dinitrophenylhydrazine reagent and those testing positive were spotted on TLC plates, developed in 10% ethyl acetate in dichloromethane and visualized with PMA. Fractions up to and before the nasty smelling spot (very dark with an Rf = 0.42) were pooled and rotavapped.

The shorter protocol developed after about a dozen of these runs was to load the sample, collect a 50 mL forerun, collect a 75 mL run, collect a 100 mL post-run and rotavap down the 75 mL run to obtain reasonably pure solid dicyclohexylidene protected *myo*-inositol dialdehyde **(Figure 5.29)** without having to do any tedious TLC work.

The column was then washed with 500 mL of fresh toluene to prepare it for another run. The entire process could be completed in about 90 minutes and 4 g of crude

material from a typical hydroboration-oxidation reaction could be purified in two long days of column work.

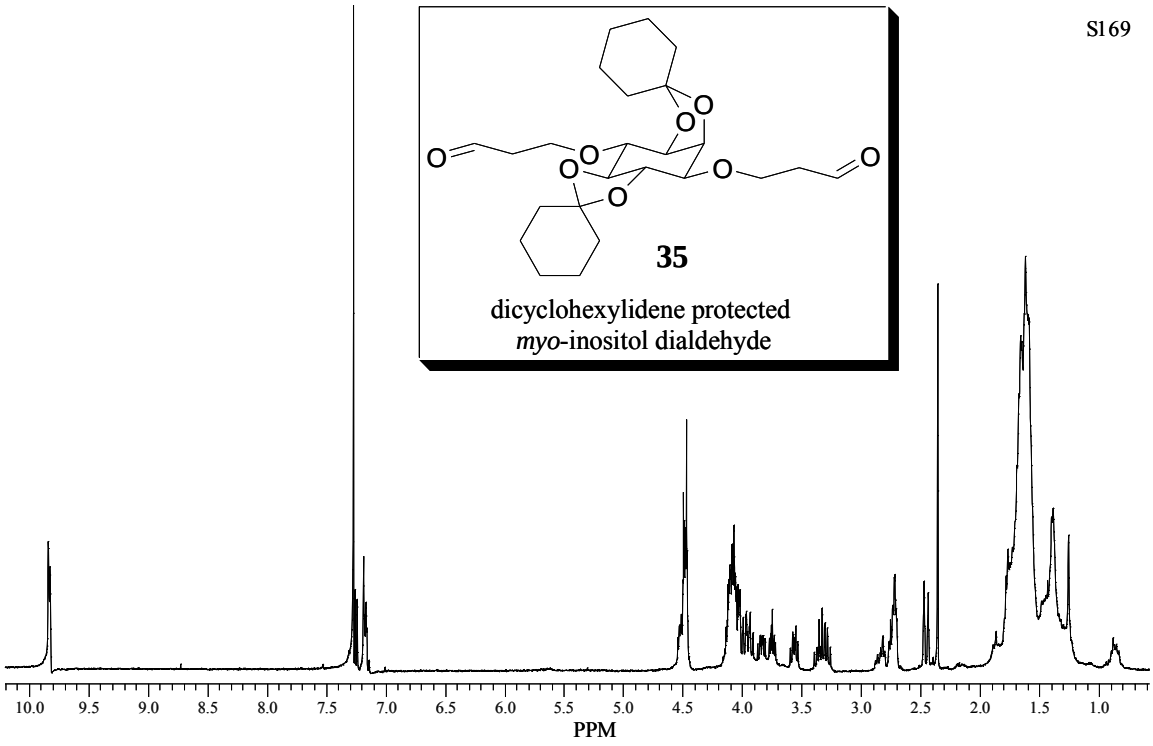
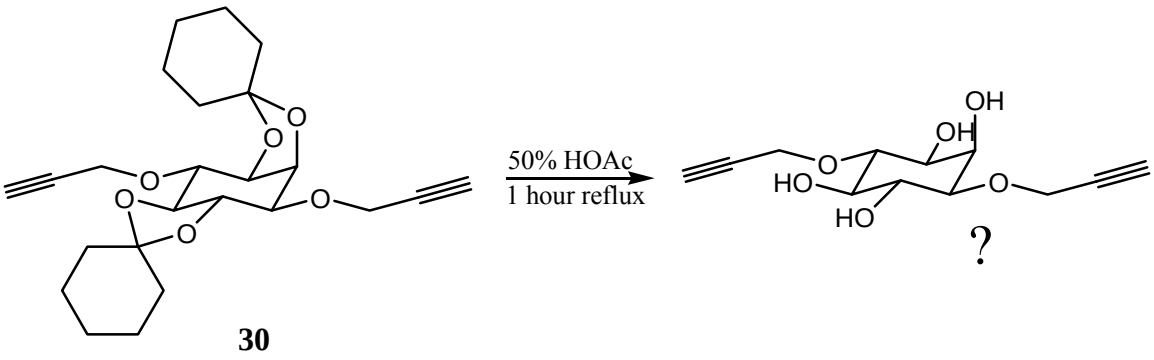


Figure 5.29 ^{1}H NMR of dicyclohexylidene protected *myo*-inositol dialkyne **35** following SX-8 size exclusion chromatography.

There was a time during the course of having to run all these TLC plates on compounds invisible under UV light, thus requiring them to be sprayed with either 2,4-dinitrophenylhydrazine reagent or phosphomolybdic acid in order to see them, when I began thinking how wonderful it would be to have groups on board easier to visualize. This prompted an effort to hydrolyze the cyclohexylidene ketals from the dicyclohexyl-

idene protected *myo*-inositol dialkyne **30 (Scheme 5.18)** so that they could be reprotected with a more UV-friendly molecule.



30

Scheme 5.18

Now, either the hydrolysis lessons of the past had been forgotten or I was feeling lucky, in either case the inositol dialkyne was refluxed in 50% acetic acid for an hour,[31] cooled and washed with dichloromethane. The dichloromethane washings were combined and solvent was removed via rotary evaporation to give a brown liquid smelling strongly of cyclohexanone—a good sign. The aqueous solution was washed with ether to remove much of the acetic acid still present and anything else that might come along. The aqueous solution was rotavapped to give off-white crystalline solids which were recrystallized from ethyl acetate to give solids having a melting point range of 157-159 ^{o}C.

Surprisingly, the melting point didn't match inositol which had been the usual result of all such hydrolysis attempts carried out in refluxing acid. Two things were

different, one was the concentration of the acid (50% versus 80% used before) and the other was the alkyne group as opposed to the dioxane.

A ^{1}H NMR was obtained of the hydrolysis product **(Figure 5.30)** and the striking absence of cyclohexylidene protons between 1.4 and 1.6 ppm offered evidence that the ketals were hydrolyzed, but were they done so without cleaving the wimpy C1, C4 ether linkages? The two triplets at 2.8 and 2.85 ppm, reminiscent of terminal alkyne protons, suggested that the ether linkages were still intact as did the signals at 4.35 and 4.49 ppm. The ^{13}C NMR spectrum (not shown) gave the number of carbon types expected in the molecule.

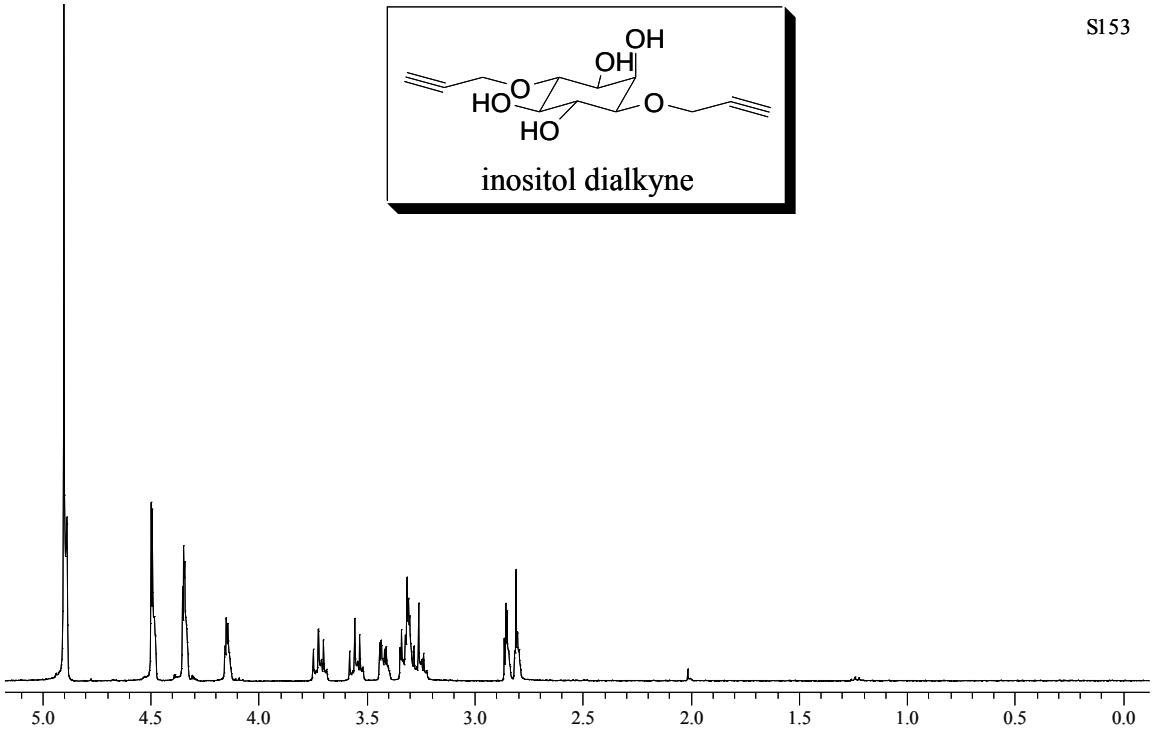


Figure 5.30 The ^{1}H NMR of the product obtained from the acetic acid hydrolysis of the dicyclohexylidene protected *myo*-inositol dialkyne **30**.

This material was further subjected to benzylidene protection[32] for better TLC visualization, however it appears that effort did not succeed.

5.8 Macrocyclization Efforts Toward an Inositol-based Inositol Phosphate Receptor

As the research efforts bore fruit closer and closer to the target macrocycle it became increasingly difficult to exercise restraint in going all out for desired receptor and several poorly conceived shots were taken at the elusive compound.

In one such effort about a gram of crude hydroboration-oxidation product dripping wet off the rotavap was slung in with some acetonitile and a little diethylene-triamine was dripped slowly into the flask. The reaction was stirred for two days and no solids were observed. This was in contrast to the Dhaenens[33] cyclophane reaction we had based our macrocyclization on, in which the solid tetraimine compound precipitated from solution. The reaction mixture was rotavapped to give only a yellow colored liquid with no evidence of the solid product expected.

The next shot at this at least came after eluting some material from a silica gel column in theory, at least, making it a little more pure than the last batch. The material was dissolved in acetonitrile in accordance with the dilute conditions designed to favor cyclization and while under argon diethylenetriamine was slowly dripped into the reaction and stirred at room temperature for two days. Cloudiness was observed in the reaction at first, but it soon turned clear and light yellow, and no solids were ever observed. Removal of the solvent via rotary evaporation gave a sticky yellow material that resembled the starting crude dicyclohexylidene protected *myo*-inositol dialdehyde and it tested positive with 2,4-dinitrophenylhydrazine reagent meaning that no reaction

had occurred. At that point I began to realize that it might be more difficult to get an aliphatic aldehyde to react with an amine than it had been to get the aromatic terephthalaldehyde to react with the same amine.

Adding to my limited knowledge on imine forming reactions I picked up some references which called for the slow addition of the aldehyde component to the amine component while cooled to well below zero.[34] Following the addition, the reaction was allowed to warm to zero degrees and then potassium hydroxide was added to push the imine formation home and generate two layers—aqueous and organic.

In those examples the amine was used neat and cyclization was not the goal, but a reaction was attempted nonetheless in dilute dichloromethane. Two layers were never observed, which was not unexpected given only a few drops of water would be released from the dilute reaction, and upon solvent removal all that was obtained was a yellow liquid testing positive to 2,4-dinitrophenylhydrazine.

A few more attempts were made. In one, the reaction was refluxed for a week only to give back starting material, another was done using counter intuitive water as the solvent[30] which did nothing but waste the first relatively pure 123 mg of material painstakingly obtained from the SX-8 column. That one was even attempted once more for good measure and I came to the conclusion that, yes, matter could be destroyed.

5.9 Experimental

(1R)-(+)-2,2-Dimethoxy-1,7,7-trimethyl-bicyclo[2.2.1]heptane (2). Into a 300 mL single neck round bottom flask were placed 30 g (197 mmol) of D-camphor, 0.1 g (0.58 mmol) of pTsOH, 60 mL of freshly distilled (over $Mg(OMe)_2$) methanol, 45 mL of

trimethylorthoformate and a magnetic stri bar. The flask was fitted with a condenser and the contents were refluxed for 48 hours while under argon. During the reflux period the reaction mixture became white wine colored and sometimes even slightly purple. The reaction was allowed to cool and 50 mL of 10% aqueous KOH solution was made. The cooled reaction was poured into the separatory funnel and 2 mL aliquots of the KOH solution were added slowly by pipet with gentle swirling until the cloudy solution separeated (22 mL of 10% KOH required). A 60:40 dichloromethane/pentane solution (500 mL) was added, shaken and allowed to separate into two layers. The lower organic layer was removed to an Erlenmeyer flask containing 15 g of anhydrous potassium carbonate. The aqueous layer was extracted 2x with 100 mL portions of dichloromethane /pentane solution and all the organic layers were combined in the drying flask. The combined organic layers were dried for thirty minutes, filtered to remove solids, and the filtrate was concentrated by rotary evaporation on a 60 ^{o}C water bath to give 32.5 g of crude cloudy yellow camphor dimethyl ketal. It was determined that 1 g of sodium metal would be required to further purify every 30 g (in camphor) scale ketalization reaction. To the crude ketal off the rotovap was added 1 g of freshly deoxidized finely divided sodium metal spheres and the mixture was vacuum distilled in a 90-125 ^{o}C oil bath to collect 16 g (41%) of clear liquid which come over between 45 and 60 ^{o}C (still head temperature). The ketal is assumed to be 80% pure and may be used as is, store in refrigerator. ^{1}H NMR ($CDCl_3$) δ 3.21 (s, methoxy methyl, 3 H), 3.14 (s, methoxy methyl, 3 H), 1.03 (s, methyl 3 H), 0.87 (s, methyl 3 H), 0.85 (s, methyl, 3 H), remaining peaks were not well resolved, but appeared to correspond with the equivalent protons in the parent camphor molecule. ^{13}C NMR ($CDCl_3$) δ 129.834, 128.087, 109.062 (ketal),

52.956, 50.196, 49.935, 47.241, 44.224, 40.960, 29.285, 27.298, 20.771, 20.484, 12.418. MW for $C_{12}H_{22}O_2$ = 198.30 g/mol, exact mass = 198.16 g/mol, found m/z 198 $(M+H)^+$.

1D-2,3-*O*-(D-1',7',7'-Trimethyl[2.2.1]bicyclohept-2'-ylidene)-*myo*-inositol (3). Into a 1L single neck round bottom flask was placed 18.1 g (100 mmol) of *myo*-inositol and 150 mL of dimethyl sulfoxide. The flask was heated in a 110 ^oC oil bath until the inositol dissolved. The solution was allowed to cool to 90 ^oC before 31.3 g (0.158 mol) of D-camphor dimethyl ketal and 0.3 mL of TMS-triflate were added. The mixture was heated at 90 ^oC for three hours and took on a peach color. Ethylene glycol (3.2 g) and 350 mL of chloroform were added and the solution was heated at 50 ^oC for two hours. Solvent was removed by rotary evaporation from a 60 ^oC water bath and the residue was re-dissolved in 1 L of a 1:2 chloroform/ether solution an transferred to a 3 L flat bottom boiling flask. The flask was placed in a 4 ^oC freezer and stirred for three days at which point it resembled a thin strawberry milk shake with some particulates present. Filtering the solution via water aspiration gave 17 g of white material after drying and had a melting point range of 205-220 ^oC (literature value 232-232 ^oC). Attempts to recrystallize from methanol met with limited success. The compound was not soluble in boiling ethanol, ethyl acetate or water (an indication it was not inositol). ^1H NMR ($CDCl_3$) δ inositol protons; 4.27 (dd, H2, 1 H), 3.77 (dd, H3 1 H), 3.68 (dd, H1 1 H), 3.51 (t, H6 1 H), 3.44 (dd, H4, 1 H), 3.12 (dd, H5 1 H); camphor protons; 2.03 (m, 2 H), 1.70 (m 2 H), 1.49 (d, 1 H), 1.38 (m, 1 H), 1.21 (m, 1 H), 1.03 (s, 3 H), 0.87 (s, 3 H), 0.85 (s, 3 H). MW for $C_{16}H_{26}O_6$ = 314.17 g/mol, exact mass = 314.37 g/mol.

(±)2,3:5,6-Di-*O*-cyclohexylidene-*myo*-inositol (10). Into a 2 L single neck round bottom flask was placed 35 g *myo*-inositol (194 mmol), 500 mL of DMF, 250 mL of

cyclohexanone, 50 mL of toluene and 4 g p-toluenesulfonic acid (23.23 mmol). A magnetic stir bar was added and the flask was fitted with a Dean-Stark trap filled with 50 mL of toluene. A condenser was put in place and the reaction was gently refluxed for 48 hours until no more water was observed coming off the reaction. Water was periodically removed from the trap over the course of the reaction. After allowing the reaction to cool to < 70 ^{o}C 3 g of triethylamine were added and the reaction was stirred for 15 minutes. Solvent was reduced by rotary evaporation in a 60 ^{o}C water bath over about one hour. Rotavapped an additional 30 minutes at 70 ^{o}C to afford a dark golden wine colored liquid as a residue. The residue was allowed to cool and was then re-dissolved in 250 mL dichloromethane and poured into a separatory funnel. It was washed 2x with 250 mL portions of water, 2x with 200 mL portions of sodium bicarbonate, 2x with with 250 mL of water and once with 250 mL brine. The organic layer was dried with anhydrous sodium sulfate overnight, filtered via water aspiration and the filtrate was concentrated using rotary evaporation on a 60 ^{o}C water bath until most of the liquid came over. Solvent removal was continue via high vacuum rotary evaporation until foaming was observed in the flask as the material began inching out. The super viscous dark golden brown liquid was taken up in a solution of 200 mL hexanes and 30 mL of acetone using gentle heating from the water bath to dissolve the material. This step is particularly critical as enough solvent must be present to prevent oiling out and subsequent solidification of the oil as the reaction is cooled in the freezer, but too much solvent will prevent the desired product from precipitating. If lucky solids will begin to precipitate immediately following dissolution of the residue and more can be obtained by placing the reaction flask in the freezer overnight. If solids fail to form upon cooling and no oil is

present it may be necessary to evaporate some solvent and try cooling again, if an oil is present in the flask after cooling more solvent must be added to dissolve the oil and the flask must be place in the freezer again. The solids were collected via water aspirated filtration and washed with 2x 50 mL portions of 3:1 hexanes/acetone to afford 11.34 g of white solids. Recrystallization from 100 mL toluene boiled down to 25 mL following an overnight stay in the freezer and filtration afforded 10.14 g (15.3%) of white crystals having a sharp melting point range of 179-180 ^{o}C (lit. 179 ^{o}C). ^{1}H NMR ($CDCl_3$) inositol protons; δ 4.48 (dd, H2, 1 H), 3.99-4.09 (2-dd, H1, H4, 2 H), 3.80-3.90 (2-dd, H3, H6, 2 H), 3.32 (dd, H5, 1 H), 2.46, 2.64 (2-d, alcohol protons, 2 H); cyclohexylidene protons; δ 1.50-1.69 (m, 20 H). ^{13}C NMR ($CDCl_3$) δ 113, 110 (ketal); 83.1, 81.4, 77.7, 76.5, 75.4, 70.14 (inositol); 56.8, 37.8, 36.4, 35.0, 24.9, 24.8, 24.0, 23.6 (cyclohexylidene). MW for $C_{18}H_{28}O_6$ = 340.19 g/mol, exact mass = 340.41 g/mol, found *m/z* 363.41 $(M+Na)^+$, *m/z* 379.35 $(M+K)^+$.

(±)1,4-*O*-Bis(2-[1,3]Dioxanylethoxy)-2,3:5,6-Di-*O*-cyclohexylidene-*myo*-inositol (22). Into an oven dried 500 mL three neck round bottom flask was placed 6.75 g (19.8 mmol) of dicyclohexylidene protected *myo*-inositol **10** and 200 mL of freshly dried and distilled (over $MgSO_4$) dimethylformamide. The solution was stirred under argon for 30 minutes before adding 10 g of 60% sodium hydride in mineral oil (250 mmol) all at once. Into a 50 mL pressure equalized dropping funnel was placed 27 mL of freshly distilled 98% 2-(2-bromoethyl)-1,3-dioxane (194 mmol). The funnel was fitted to the reaction flask and the flask was cooled in an ice water bath. The dioxane reagent was allowed to drip into the reaction over a period of one hour (cautionary note—if added too fast major foaming/expansion of reaction occurs) with continued stirring on ice and under

argon. The reaction was allowed to proceed for 18 hours as the ice melted and the system came to room temperature and the flask contents changed from hydride gray to a light orangey tan color. The mixture in the flask was transferred to a one liter single neck round bottom flask and ethyl acetate and water (100-200 mL combined total) were carefully added to destroy excess sodium hydride and fully dissolve all solids present. The solution was transferred into a one liter separatory funnel and extracted 3x with 250 mL portions of ethyl acetate. The combined extracts were dried over anhydrous sodium sulfate for one hour, vacuum filtered and the filtrate was concentrated by rotary evaporation on a 50 ^{o}C water bath. The still liquid residue was further concentrated overnight using a high vacuum pump to afford yellow solids which were washed with hexanes to give 11.40 g of slightly yellow solids as a crude product. Recrystallization of the crude product two times from 400 mL of hexanes boiled down to 275 mL afforded 9 g (80%) of off-white crystals having a sharp melting point of 126 ^{o}C. ^{1}H NMR ($CDCl_3$) δ 5.00-6.00 (various, believed to be impurities lost upon recrystallization), 4.73 (t, bromoethyl dioxane protons), 4.45 (t, dicyclohexylidene protected *myo*-inositol protons), 3.96-4.19 (various, bromoethyl dioxane and dicyclohexylidene protected *myo*-inositol protons), 3.69-3.92 (various, bromoethyl dioxane and dicyclohexylidene protected *myo*-inositol protons), 3.63 (t, various, bromoethyl dioxane protons), 3.30 (t, dicyclohexylidene protected *myo*-inositol protons), 2.10-2.18 (m, bromoethyl dioxane protons), 1.6-1.72 (m, dicyclohexylidene protected *myo*-inositol protons). MW for $C_{30}H_{48}O_{10}$ = 568.70 g/mol, exact mass = 568.32 g/mol, found *m/z* 568.7 $(M+H)^+$, *m/z* 591.9 $(M+Na)^+$ and *m/z* 607.9 $(M+K)^+$.

(±)1,4-Di-*O*-allyl-2,3:5,6-Di-*O*-cyclohexylidene-*myo*-inositol (29). Into a 250 mL single neck round bottom flask was dissolved 10 g (29.4 mmol) of dicyclohexylidene protected *myo*-inositol **10** in 125 mL dimethylformamide. The solution was stirred under argon for thirty minutes prior to adding 2.7 g of 60% sodium hydride in mineral oil (67.5 mmol). Alkoxide formation was allowed to occur for fifteen minutes, then the reaction flask was placed in an ice water bath until cooled. Allyl bromide (5.55 mL (64.5 mmol)) was added to the stirred mixture in small portions over 20 minutes as a vigorous reaction was observed. The resulting tan solution continued stirring for thirty minutes and showed no dicyclohexylidene protected *myo*-inositol present by TLC. Water was carefully added (50-75 mL) to quench the reaction by destroying excess sodium hydride and the flask contents were transferred to a 500 mL separatory funnel, extracted with 3-4 100 mL portions of ethyl acetate. The ethyl acetate layers were combined and placed back into a clean separatory funnel and allowed to more completely separate from residual water prior to drying over anhydrous potassium carbonate. The drying agent was filtered and the filtrate was concentrated via rotary evaporation in a 50-80 ^{o}C water bath to give a slightly yellow liquid. Further evaporation was accomplished using high vacuum rotary evaporation in a 60 ^{o}C water bath for thirty minutes to give a clear viscous liquid. The liquid was poured into a crystallizing dish and within two hours solids were seen forming in the dish. After leaving on the bench top over the weekend 11.40 g (86%) of white crystalline material was collected having a melting point range of 53-58 ^{o}C. ^{1}H NMR ($CDCl_3$) δ 5.98 (m, olefinic, 2 H), 5.22-5.38 (m, terminal alkene, 4 H), 4.46 (dd, inositol H2, 1 H), 4.33 (m, allylic, 4 H), 4.08 (dd, inositol H1, 1 H), 3.96 (dd, inositol

H4, 1 H), 3.81 (dd, inositol H3, 1 H), 3.64 (dd, inositol H6, 1 H), 3.33 (dd, inositol H5, 1 H), 1.39-1.63 (m, cyclohexylidene protons, 20 H). MW for $C_{24}H_{36}O_6$ = 420.54 g/mol, exact mass = 420.25 g/mol.

(±)1,4-Di-*O*-propargyl-2,3:5,6-Di-*O*-cyclohexylidene-*myo*-inositol (30). Into a 250 mL three neck round bottom flask was dissolved 5 g (14.7 mmol) of dicyclohexylidene protected *myo*-inositol **10** in 100 mL of dimethylformamide while under argon and in an ice water bath. To the flask was added 2 g of 60% NaH in mineral oil (50 mmol) and evidence of a reaction was observed. Into a dropping funnel was placed 2.9 mL of 80% propargyl bromide in toluene (32.3 mmol) which was dripped into the reaction flask over 15 minutes to give a brown colored mixture. The reaction was allowed to proceed for five hours as the ice bath melted. Excess sodium hydride was destroyed using 30 mL of water generating a white precipitate. Ethyl acetate (40-50 mL) was added and the reaction solution was transferred into a 1 L separatory funnel where it was extracted 3x with 200 mL portions of ethyl acetate. The combined ethyl acetate layers were dried over anhydrous potassium carbonate for several hours, filtered, and concentrated via rotary evaporation in a 60 ^{o}C water bath to afford a yellow solution. The solution was further concentrated using a high vacuum rotary evaporator in a 60 ^{o}C water bath for 90 minutes. Upon cooling, the residue solidified into a yellowish brown material which was broken up and place on a vacuum pump for two hours. The solids were dissolved in 250 mL of acetone and the acetone solution was poured into 750 mL of ice water to precipitate the product. The solids were collected in a medium frit scintered glass filtering funnel via vacuum filtration and recrystallized from 200 mL of 95% ethanol boiled down to 20 mL with overnight

freezing. A total of 4.32 g (71%) of slightly tan solids were obtained having a melting point range of 136-137 ^{o}C. ^{1}H NMR ($CDCl_3$) δ 4.47, 4.49 (2-d, propargylic, 2 H), 4.53 (dd, inositol H2 1 H), 4.11 (dd, inositol H1, 1 H), 3.83-4.06 (3-dd, inositol H3, H4, H6 6 H), 3.37 (dd, inositol H5, 1 H), 2.44, 2.47 (2-t, terminal alkyne 2 H), 1.40-1.80 (m, cyclohexylidene protons, 20 H). MW for $C_{24}H_{32}O_6$ = 416.51 g/mol, exact mass = 416.22 g/mol, found *m/z* 439.83 $(M+Na)^{+}$.

Hydrolysis of (±)2,3:5,6-Di-*O*-cyclohexylidene-*myo*-inositol (10) using Trifluoroacetic Acid. Into a 200 mL single neck round bottom flask was placed 333 mg (0.978 mmol) of (±)2,3:5,6-Di-*O*-cyclohexylidene-*myo*-inositol **10** and 75 mL of trifluoroacetic acid and 1 mL of water. The flask was fitted with a condenser and the reaction was refluxed with stirring for one hour. The acid was removed via rotary evaporation on a 40 ^{o}C water bath to give a brown viscous liquid which was diluted with water and washed with several portions of dichloromethane. The aqueous solution was concentrated by rotary evaporation to give semi-white solids with traces of brown. The dichloromethane layers were combined and solvent was removed via rotary evaporation to yield trace amounts of brown material to which nothing further was done. The white material weighed 0.135 g and was found to melt between 226-228 ^{o}C indicating it was the expected hydrolysis product—inositol. Assuming the material to be inositol gives a 77% yield for the deprotection reaction. MW for $C_6H_{12}O_6$ = 180.16 g/mol, exact mass = 180.06 g/mol.

Hydrolysis of (±)1,4-*O*-Bis(2-[1,3]Dioxanylethoxy)-2,3:5,6-Di-*O*-cyclohexylidene-*myo*-inositol (22) using Trifluoroacetic Acid. Into a 50 mL single neck round bottom flask was placed 100 mg (0.294 mmol) of (±)1,4-*O*-Bis(2-

[1,3]Dioxanylethoxy)-2,3:5,6-Di-*O*-cyclohexylidene-*myo*-inositol **22** and 10 mL of trifluoroacetic acid and 2 mL of water. The flask was fitted with a condenser and the reaction was refluxed with stirring for one hour. Within 15 minutes of heating the solution became a bluish/purple color and by the end of the reflux period it was chestnut brown. The acid was removed via rotary evaporation on a 40 ^{o}C water bath to give dark brown solids which were dissolved in water and washed with several portions of dichloromethane while in a separatory funnel. The aqueous solution was concentrated by high vacuum rotary evaporation on a 60 ^{o}C water bath to give 32.4 mg of brown sugar looking solids. The dichloromethane layers were combined and solvent was removed via rotary evaporation to yield a trace ring of brown material to which nothing further was done. Neither deuterated chloroform, nor deuterated methanol were able to dissolve the solids, however the methanol washed most of the brown color away and left behind white solids, which were collected by filtration. The white melted between 226-230 ^{o}C, unexpectedly indicating it was inositol. Assuming the material to be inositol gives a 61% yield for the ether ravaging deprotection reaction. MW for $C_6H_{12}O_6$ = 180.16 g/mol, exact mass = 180.06 g/mol.

Hydrolysis of (±)1,4-*O*-Bis(2-[1,3]Dioxanylethoxy)-2,3:5,6-Di-*O*-cyclohexylidene-*myo*-inositol (22) using Acetic Acid. Into a 10 mL microscale kit round bottom flask was placed 75 mg (0.132 mmol) of (±)1,4-*O*-Bis(2-[1,3]Dioxanyl-ethoxy)-2,3:5,6-Di-*O*-cyclohexylidene-*myo*-inositol **22** and 5 mL of 80% acetic acid (aq). Heat was applied to the reaction flask as an aluminum backed silica gel TLC plate was prepared containing a spotting from the reaction mixture taken after five minutes (still about room temperature) alongside a spotting of the starting material dissolved in

dichloromethane. The plate was developed in a 5% methanol in dichloromethane solution. The developed plate was visualized by spraying it with a solution of 10% phosphomolybdic acid in 95% ethanol (PMA) followed by blowing on it with a heat gun. The following results were obtained: starting material lane → one spot $R_f = 0.11$; reaction lane → four spots $R_f = 0$, 0.11, 0.45, 0.66. A similar TLC run at thirty minutes gave the following results: starting material lane → one spot $R_f = 0.11$; reaction lane → two spots $R_f = 0$, 0.66 with the 0.66 spot being much darker in the second plate versus the first. The reaction was refluxed for a total of one hour, poured into a 125 mL separatory funnel and extracted 2x with 25 mL of dichloromethane. The combined chloroform layers were washed with saturated sodium bicarbonate solution until neutral to litmus, dried over potassium carbonate, filtered and the filtrate was rotavapped on a 50 ^{o}C water bath to afford a few drops of liquid with a distinct odor which could not be pinpointed (cyclohexanone or its ketal?). The liquid was taken up in dichloromethane and a TLC of the material developed in a 5% methanol in dichloromethane solution and visualized with PMA gave a spot corresponding the previous R_f 0.66 spots. No other spots were present. A sampling of the aqueous layer remaining in the separatory funnel was analyzed via TLC developed and visualized as before and showed only one spot plastered firmly on the origin. Repeating this experiment and letting it reflux for eighteen hours gave the same results, i.e. no change in the distribution of the two products as seen by TLC was observed.

In another acetic acid hydrolysis carried out at room temperature instead of under reflux 200 mg (0.588 mmol) of (±)1,4-*O*-Bis(2-[1,3]Dioxanylethoxy)-2,3:5,6-Di-*O*-cyclohexylidene-*myo*-inositol **22** was stirred overnight in 80% acetic acid (aq). The

reaction was concentrated via rotary evaporation on a 60 ^{o}C water bath until liquid no longer came over to give a viscous liquid which was left out on the bench top overnight and formed crystals. The crystals were washed with acetone and filtered to give platelets of material having a melting point of 127 ^{o}C. ^{1}H NMR (CDCl3) δ 4.76-4.80 (m, dioxane), 4.28 (inositol), 4.11-4.15 (m, inositol), 3.98-4.00 (m, inositol), 3.77-3.87 (m, inositol), 3.76 (m, inositol), 3.41-3.65 (inositol), 3.12 (dd, inositol), 1.90-2.18 (2-m, dioxane), 1.37 (d, unknown). MW for $C_{18}H_{32}O_{10}$ = 408.44 g/mol, exact mass = 408.20 g/mol, found *m/z* 431.54 $(M+Na)^{+}$, *m/z* 447.40 $(M+K)^{+}$.

Hydrolysis of (±)2,3:5,6-Di-*O*-cyclohexylidene-*myo*-inositol (10) using Acetic Acid. Into a 50 mL single neck round bottom flask was placed 100 mg (0.294 mmol) of (±)2,3:5,6-Di-*O*-cyclohexylidene-*myo*-inositol **10**, 10 mL of water, a pinch of oxalic acid, a few drops of ethylene glycol and 10 mL of 80% acetic acid (aq). It was stirred at room temperature for 90 minutes, concentrated via rotary evaporation on a 60 ^{o}C water bath to give white solids in a small amount of liquid (cyclohexanone, ethylene glycol?). An attempt to remove the liquid via vacuum pump failed. The mixture was taken up in ethyl acetate (in which inositol is insoluble) and filtered to give white crystals having a melting point of 226 ^{o}C. The crystals are assumed to be the expected hydrolysis product—inositol. MW for $C_6H_{12}O_6$ = 180.16 g/mol, exact mass = 180.06 g/mol.

Hydrolysis of (±)1,4-*O*-Bis(2-[1,3]Dioxanylethoxy)-2,3:5,6-Di-*O*-cyclohexylidene-*myo*-inositol (22) using Hydrochloric Acid. Into a 50 mL round bottom flask was placed 400 mg (1.18 mmol) of (±)1,4-*O*-Bis(2-[1,3]Dioxanylethoxy)-2,3:5,6-Di-*O*-cyclohexylidene-*myo*-inositol **22** and 20 mL of 2M HCl in methanol. A

solution was formed within thirty minutes and the reaction was refluxed for three hours over which time it became progressively darker in color. At the end of the reflux period it was tea colored and upon cooling solids formed in the flask. It was further cooled on an ice water bath for thirty minutes and filtered to give 73 mg of white solids having a melting point range of 221-228 ^{o}C. The solids are assumed to be inositol. MW for $C_6H_{12}O_6$ = 180.16 g/mol, exact mass = 180.06 g/mol.

Hydolysis of (±)1,4-Di-*O*-propargyl-2,3:5,6-Di-*O*-cyclohexylidene-*myo*-inositol (30) using Acetic Acid. Into a 100 mL single neck round bottom flask was placed 2 g (4.80 mmol) of (±)1,4-Di-*O*-propargyl-2,3:5,6-Di-*O*-cyclohexylidene-*myo*-inositol **30**, 25 mL of glacial acetic acid, 25 mL of water and a magnetic stir bar. The mixture was refluxed for 1 hour, cooled to room temperature and transferred into a 1 L separatory funnel where it was washed with 3x 100 mL portions of chloroform and 2x 100 mL of ether. The aqueous layer was concentrated via rotary evaporation on a 60 ^{o}C water bath to afford 1.1 g of off-white crystalline solids smelling like ether. It was found to be soluble in room temperature methanol and mostly insoluble in acetone with which it was washed to give 670 mg of solids (so maybe more soluble in acetone than originally thought). The solids were recrystallized from 70-80 mL of ethyl acetate boiled down to 30 mL and cooled in the refrigerator overnight to give 589 mg (39%) of crystals (95% ethanol is suggested for future recrystallizations) having a melting point range of 157-159 ^{o}C. ^{1}H NMR (CD_3OD) δ 4.49, 4.35 (2-d, propargylic, 4 H), 4.15 (dd, H2, 1 H), 3.72 (dd, H1, 1 H), 3.55 (dd, H3, 1 H), 3.42 (dd, H6, 1 H), 3.24-3.34 (m, H5 1 from solvent, 3H), 2.86 (m, terminal alkyne, 1 H), 2.81 (m, terminal alkyne, 1 H). ^{13}C

NMR (CD_3OD) δ 82.3, 81.8, 81.1, 80.4, 76.2, 76.1, 75.5, 73.7, 73.1, 71.2, 60.9, 58.2. MW for $C_{14}H_{20}O_6$ = 284.31 g/mol, exact mass = 284.13 g/mol.

Attempted hydroboration-oxidation of (±)1,4-Di-*O*-allyl-2,3:5,6-Di-*O*-cyclohexylidene-*myo*-inositol (29). Into a three neck round bottom flask was placed 2.77 g (6.59 mmol) of (±)1,4-Di-*O*-allyl-2,3:5,6-Di-*O*-cyclohexylidene-*myo*-inositol **29**, a magnetic stir bar and enough THF to dissolve the solids. The flask was kept under argon and 224 mg (5.92 mmol) of sodium borohydride plus 1 mL boron trifluoride dietherate were added which caused significant foaming. Within five minutes a large mass of THF absorbing solids (borane-dialkene polymers?) formed leaving the flask nearly dry, so 125 mL of fresh THF were added and the solids were broken up and continued to stir for three hours at room temperature. A solution of 554 mg (13.85 mmol) of sodium hydroxide in 5-10 mL of water was prepared and 4.71 g (41.52 mmol) of 30% hydrogen peroxide was readied to add to the reaction. The sodium hydroxide solution was added followed by the hydrogen peroxide solution and the large clumps of white solids broke apart and dissolved within a few minutes. The solution was stirred for three hours, poured into a separatory funnel and extracted 2x with 100 mL portions of ethyl acetate. The ethyl acetate layers were combined, washed with brine, collected and dried over anhydrous potassium carbonate overnight. The dried ethyl acetate was filtered and the filtrate concentrated via rotary evaporation on a 40 ^oC water bath to give a clear viscous liquid that was further concentrated via high vacuum rotary evaporation on a 40 ^oC water bath to give a very sticky cloudy white substance which was soluble in ethanol and acetone, insoluble in hexanes, and gave a very exothermic positive Jones reagent test, however infrared spectroscopy gave no

indication of either an alcohol or an alkene for that matter. MW for target compound $C_{24}H_{40}O_8$ = 456.27 g/mol, exact mass = 456.57 g/mol.

(±)1,4-Di-*O*-(3-oxo-propoxy)-2,3:5,6-Di-*O*-cyclohexylidene-*myo*-inositol (34). All glassware, stoppers and stir bars were dried in a 120 ^{o}C over overnight and assembled while hot under an argon stream in the hood. Attached to the 250 mL three neck round bottom flask was a 75 mL dropping funnel and after sufficient cooling the apparatus was lowered into an ice water bath for fifteen minutes. Into the cooled flask was placed 40 mL of 0.5M 9-BBN in tetrahydrofuran (20 mmol) which was allowed to chill with stirring under argon for fifteen minutes. The solution became cloudy with the possibility of precipitate formation. The dicyclohexylidene protected *myo*-inositol dialkyne **30** (4 g (9.60 mmol)) dissolved in 40-50 mL of THF was placed in the dropping funnel. This solution was allowed to drip into the flask containing the 9-BBN solution over forty minutes with constant stirring. Following the addition the solution became clear and was allowed to react for 18 hours under argon as the ice water bath warmed to room temperature. The reaction flask contents were transferred to an Erlenmeyer flask and stoppered. The reaction flask with a magnetic stir bar was placed into an ice water bath, placed under argon and fitted with the dropping funnel. A solution of 14 mL of pH 8 buffer (potassium phosphate monobasic potassium hydroxide) topped off to the 20 mL mark in a graduated cylinder with 30% hydrogen peroxide was poured into the empty reaction flask and cooled for 15 minutes. The hydroborated alkynyl inositol from the stoppered Erlenmeyer flask was placed into the dropping funnel and added drop wise to the oxidizing solution over 40 minutes with stirring, on ice, under argon. The reaction was allowed to proceed for 2 hours, water

was added (≈ 100 mL) to quench the reaction, it was transferred into a 1 L separatory funnel and extracted with 3x 200 mL portions of ethyl acetate. The ethyl acetate extracts (upper layer) were combined and washed with 2x 200 mL portions of saturated sodium chloride solution and dried over anhydrous sodium sulfate overnight. The drying agent was filtered from the ethyl acetate and the filtrate was concentrated via rotary evaporation on a 35 ^{o}C water bath for 2 hours to yield 7.5 g of slightly yellow liquid. The residue was place on a vacuum pump for 2 days to give 6.65 g of extremely viscous, slightly yellow liquid giving a positive 2,4-dinitrophenylhydrazine reagent test indicating the presence of an aldehyde. ^{1}H NMR (CDCl3) δ 9.84, 9.82 (2 singlets), 7.17-7.26 (unknown), 4.47, 4.49 (2-d, propargylic), 4.53 (dd, inositol H2), 3.56, 3.55 (2-t, α and β aldehyde), 3.72-4.14 (multiple dd, inositol H1-H5), 2.71-2.76 (m, unknown), 2.47, 2.44 (terminal alkyne?), 2.35 (s, unknown), 1.58-1.87 (m, cyclohexylidene). MW for $C_{24}H_{36}O_8$ = 452.54 g/mol, exact mass = 452.24 g/mol.

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CHAPTER 6

CANDID OBSERVATIONS, FUTURE CONSIDERATIONS AND CONCLUSIONS ABOUT THE DESIGN AND SYNTHESIS OF INOSITOL-BASED INOSITOL PHOSPHATE RECEPTORS

6.1 Candid Observations and Future Considerations

The receptor design work undertaken in this project is a tribute to the fathers of host-guest chemistry—Lehn, Pedersen and Cram. Their ideas and contributions to the field were unabashedly borrowed and put forth in the early design stages of the polyazamacrocycle receptors. The movement from design to implementation fell squarely on the shoulders of chemistry pioneers like Williamson and Brown with some sideline assistance from Grignard. These men were deeply woven into the fabric of this receptor design and synthesis project and I am thankful for their scientific contributions.

Although the Williamson method proved to work well for the introduction of latent aldehyde functionality to the inositol skeleton, there is some concern about the stability of the ether linkage going forward. Chemical manipulations requiring hot acid may prove to be too harsh for the ether connectors. Circumvention of this issue could be accomplished using an entirely different approach to the extended aldehydes, whereby substitution chemistry on the cyclohexane skeleton of non-*myo*-inositol inositols might be considered. There seems to be enough inositol isomers available such that configuration inversions or multiple inversions of non-*myo*-inositol starting material could eventually

generate the *myo*-inositol core desired. Such an approach would necessitate a very well planned and executed set of chemistries and would certainly add more challenges to the project.

For now, the ether lability problem seems to have been averted with the use of alkynyl appendages which do not require acidic reactions to unmask latent aldehydes, nor were the ether linkages cleaved during hot acid catalyzed deprotection of the cyclohexylidene groups, however it has yet to be proven that dialdehydes from these appendages can be isolated in sufficient purity for use in macrocyclization reactions. This is where the immediate focus of the project should lie—getting a pure inositol dialdehyde derivative and successfully coupling it to diethylenetriamine in a macrocyclization reaction.

Close inspection of the dicyclohexylidene protected *myo*-inositol dialdehyde spectrum following purification using SX-8 bio-beads **(Figure 5.29)** still showed the presence of a small amount of the dialkene precursor. This may not prove to be problematic, but a cleaner conversion to the dialdehyde is always welcome and it may ultimately have to come from a different direction such as the deprotection of acetals.

Given all the difficulties experienced in the hydrolysis of acetals and ketals in the presence of one another, and the quirky sensitivity of the ether linkages, it may be worthwhile to re-examine the use of THP ether protected alcohols as potential latent pre-aldehyde moieties. Such a re-examination could bring the ever-popular Richman-Atkins[1] cyclization technique into the fold for the first time.

The few meager attempts actually made to cyclize diethylenetriamine and the dicyclohexylidene protected *myo*-inositol dialdehyde did serve to illustrate the

importance of driving a reversible reaction toward the target compound—a problem the Richman-Atkins method doesn't suffer from. However, in the case of our particular imine formation reaction it may simply be a matter of exploring different solvent systems to find one the product macrocycle crashes out of as it is being formed.

The always present purification issues of compounds becoming increasingly more water soluble is also a concern that needs to be addressed. It may be that a strategy is needed whereby certain protecting groups are removed *en route* and get replaced with different groups more suitable to the solvent environment desired. This was briefly examined with the attempt to swap out cyclohexylidene groups for benzylidene groups. These groups could also aid in the visualization of compounds not readily seen via TLC which would aid in their separation. Maintaining organic solubility and UV-visibility for as long as possible are commendable properties which ultimately ease the burden of synthesis.

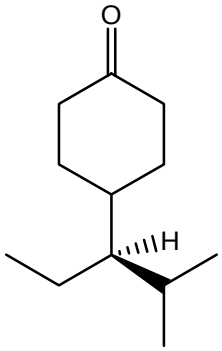
Some of the purification issues no doubt arise from the racemic nature of the dicyclohexylidene protected *myo*-inositol. Taking a racemate forward in subsequent chemical reactions, especially where two groups are being simultaneously added or manipulated, can lead to the production of many different molecules in solution, making chiral resolution early on in the reaction a top priority for future consideration. This, of course, was the big attraction for using D-camphor as a chiral auxillary in the first enantiogenic reaction on *myo*-inositol, and it is a technique worth re-exploring.

One of the drawbacks associated with the use of D-camphor, or any asymmetric cyclic resolving agent is the number of stereo- and regioisomers which arise from their

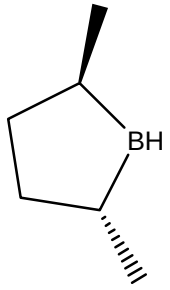
introduction. The initial reaction using D-camphor gives four major isomers due to the combination of enatiomerers and the availability of *endo/exo* positions.

One can imagine a symmetrical cyclic resolving agent such as (*R*)-4-(1-Ethyl-2-methyl-propyl)-cyclohexanone **(Figure 6.1)** having sufficient free rotation so as to eliminate positional isomeric potential, thereby eliminating half the isomers formed. You could go really crazy and replace one of the alkyl groups at the chiral carbon with a phenyl ring to impart ease of visualization when running TLC experiments.

I have also come across an old chiral dialkyl borane (JACS 1985 107 4549) **(Figure 6.1)** possibly bulky enough to act as a selective hydroborating reagent that could be useful in a later stage resolution of racemic inositol derivatives providing trialkyl borane diastereomers could be isolated.



(*R*)-4-(1-Ethyl-2-methyl-propyl)-cyclohexanone



trans-2,5-dimethylborolane

Figure 6.1 A symmetrical cyclic chiral auxillary and a chiral hydroboration reagent.

The diversity of the allyl group found in the dicyclohexylidene protected *myo*-inositol dialkene, capable of being chemically transformed into different lengths as previously discussed, warrants further investigation as a latent aldehyde.

Hydroboration-oxidation of the dialkene was rather hastily dropped after a few failed attempts in order to move onto the new and exciting inositol dialkyne derivative. New and exciting chemistry is always an enticing mistress lurking about when the old chemistry seems to be either monotonously clicking along, or worse yet, grinding to a screeching halt. It is very easy to get swept up in something new, when perhaps the best action is to stay the course and hammer things out until they work or fracture from the stress. Of course, there is no use beating a dead horse either. I guess figuring out what constitutes dead, or a horse, is the trick.

The whole idea of differential latent aldehydes is neither dead, nor a horse, and is an area of future consideration. One golden hope on this front is the orphaned thioacetal group which was never given the opportunity to shine and may just prove to hold the most luster. As of now, three differentially protected aldehydes offer the potential to provide enantiomerically and regiosterically pure macrocycles, given that a few more kinks associated with their use can be straightened out, and it is believed the thioacetal will complete the tetrahedron.

Some truly new and exciting avenues to explore in this project include a nostalgic trip back to the polyazacyclophane, with a boronic acid twist. It could be quite fruitful to use phenyl, or other aromatic aldehyde derived spacers as scaffolding for alcohol binding boronic acid moieties as previously shown in **Scheme 3.12**. Most of the *myo*-inositol

phosphates posses many hydroxyl groups which could interact with appropriately placed boronic acid secondary binding elements.

Some consideration has also been given to the exploitation of open chain dialdehydes of common hexoses such as glucose. Such compounds offer many of the same properties deemed desirable in the inositol-based secondary binding elements such as chirality, hydrogen bond donating ability through free hydroxyl groups, and size complimentarity with the target substrate.

In one hasty experiment carried out on glucose, a rather unfortunate rotavap mistake led to the overheating of the sugar. The flask was removed from the water bath and while expecting to face a charred intractable pot of carbon, I was pleasantly greeted with a whiff of the best smelling mistake to ever come out of our lab as it appeared the sugar had caramelized into a thick brown syrup ready for a stack o' hotcakes.

Another idea briefly toyed with was the use of cyclic anhydrides and cyclic imines which could be opened up by nucleophilic attack from free inositol hydroxyl groups and further manipulated to get aldehyde appendages of different lengths in place.

Given enough resources, human and financial, there is no reason why some of the other non-polyaza primary binding elements shouldn't be explored for use in the receptor design as well, especially the guanidinium group.

6.2 Conclusion

Many factors have been explored in the design and synthesis of a new class of receptor molecules which incorporate the structure of the target guest in the host architecture. Significant strides have been made toward the synthesis of a prototype

polyazamacrocycle *myo*-inositol-1,4-bisphosphate receptor designed around *myo*-inositol-1,4-bisphosphate. It has been demonstrated that the Williamson ether method can be used to successfully append latent aldehyde groups to appropriately protected inositol compounds. Strategies for the installment of differentially protected latent aldehydes were developed and explored. Much was learned about the chemistry and physical properties of a variety of inositol derivatives that will prove very useful to future research in this area. In short, a substantial amount of groundwork has been established upon which further developments toward the synthesis of these challenging abiotic receptor molecules can build. Although the pathway which lies ahead is certain to be as scenic as the inositol recycling route which spurred our research, a trail has been blazed offering friendly passage to an early milestone for those who wish to push forward in this quest.

6.3 References

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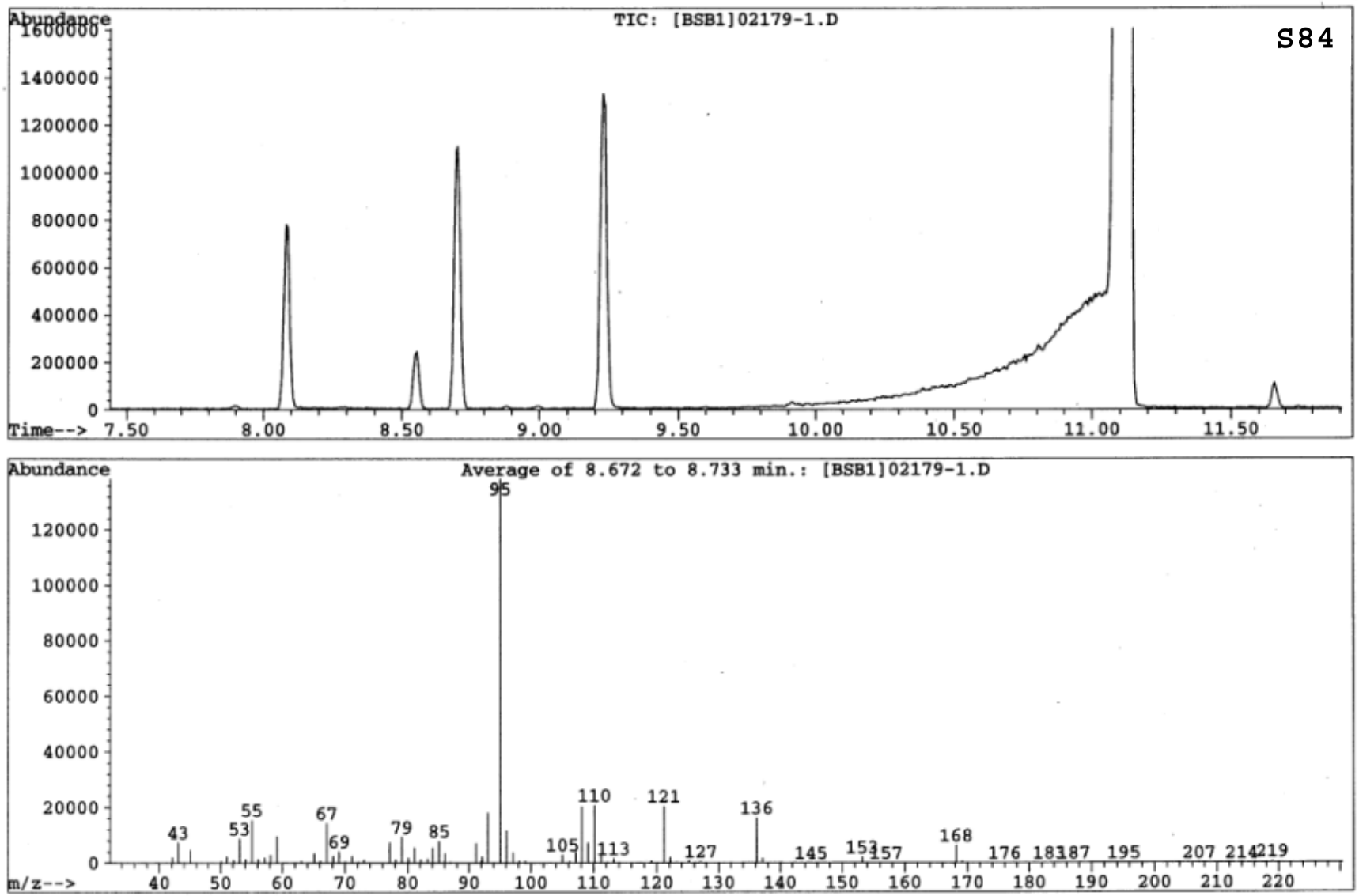
APPENDIX A

GC-MS GRAPHS ON THE KETALIZATION OF CAMPHOR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Abundance
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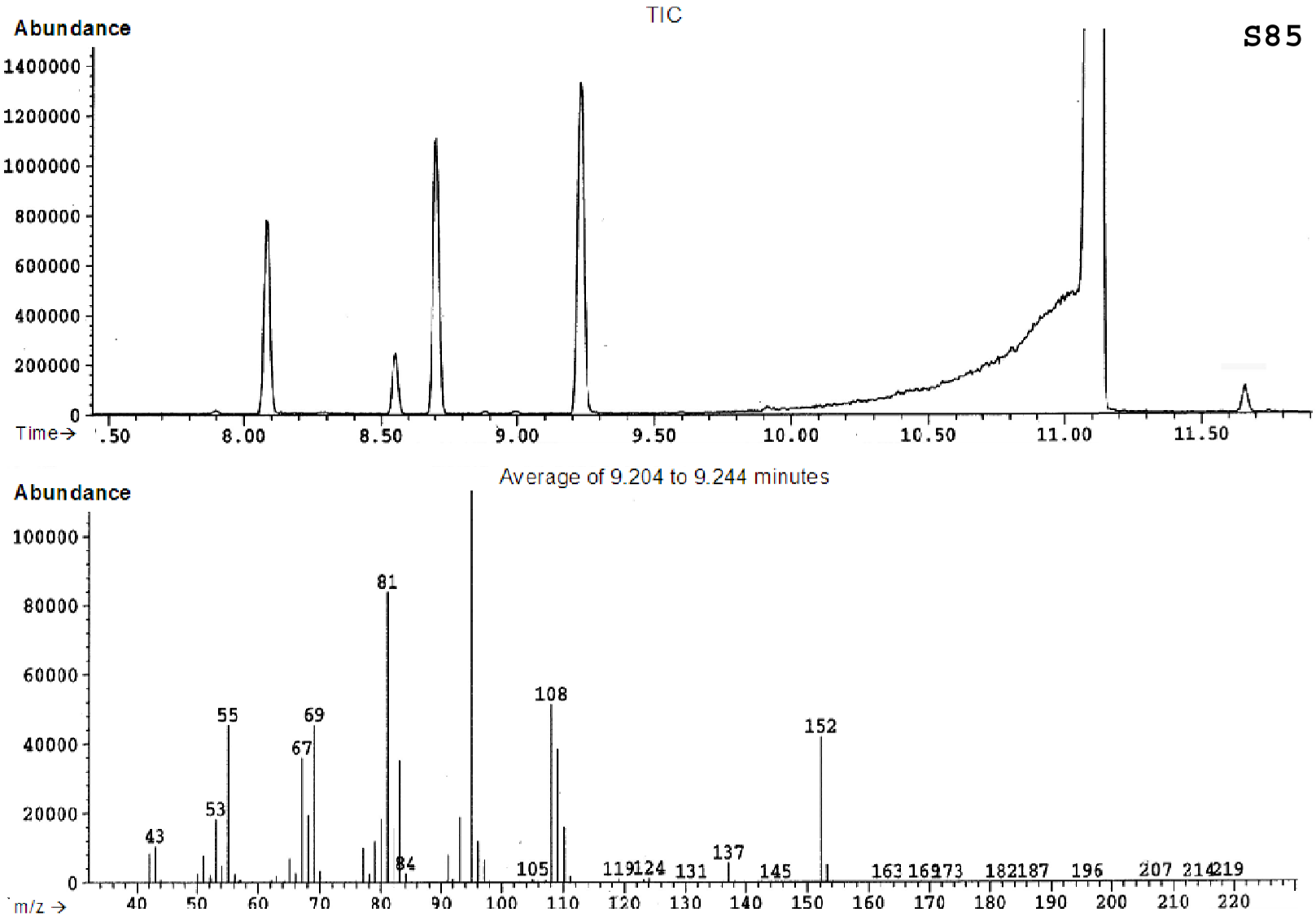
m/z-->


TIC
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Abundance
1400000
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1000000
800000
600000
400000
200000
0
Time→
.50
8.00
8.50
9.00
9.50
10.00
10.50
11.00
11.50
Average of 9.204 to 9.244 minutes
Abundance
100000
80000
60000
40000
20000
0
m/z →
43
53
55
67
69
81
84
105
108
119
124
131
137
145
152
163
169
173
182
187
196
207
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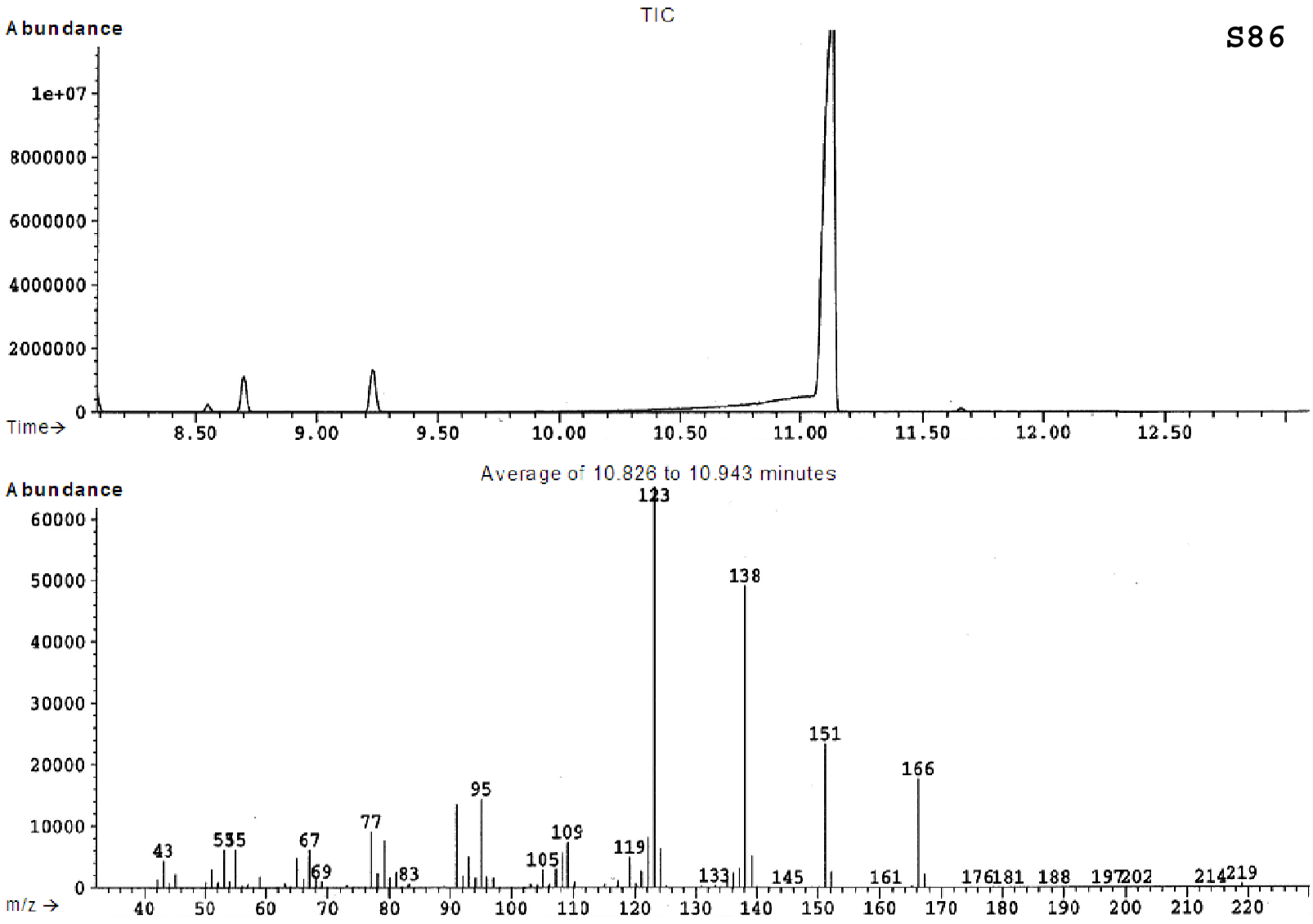
TIC
Abundance
1e+07
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6000000
4000000
2000000
0
Time→
8.50
9.00
9.50
10.00
10.50
11.00
11.50
12.00
12.50
Average of 10.826 to 10.943 minutes
Abundance
60000
50000
40000
30000
20000
10000
0
m/z →
123
138
151
166
95
77
109
119
43
53
55
67
69
83
105
133
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161
176
181
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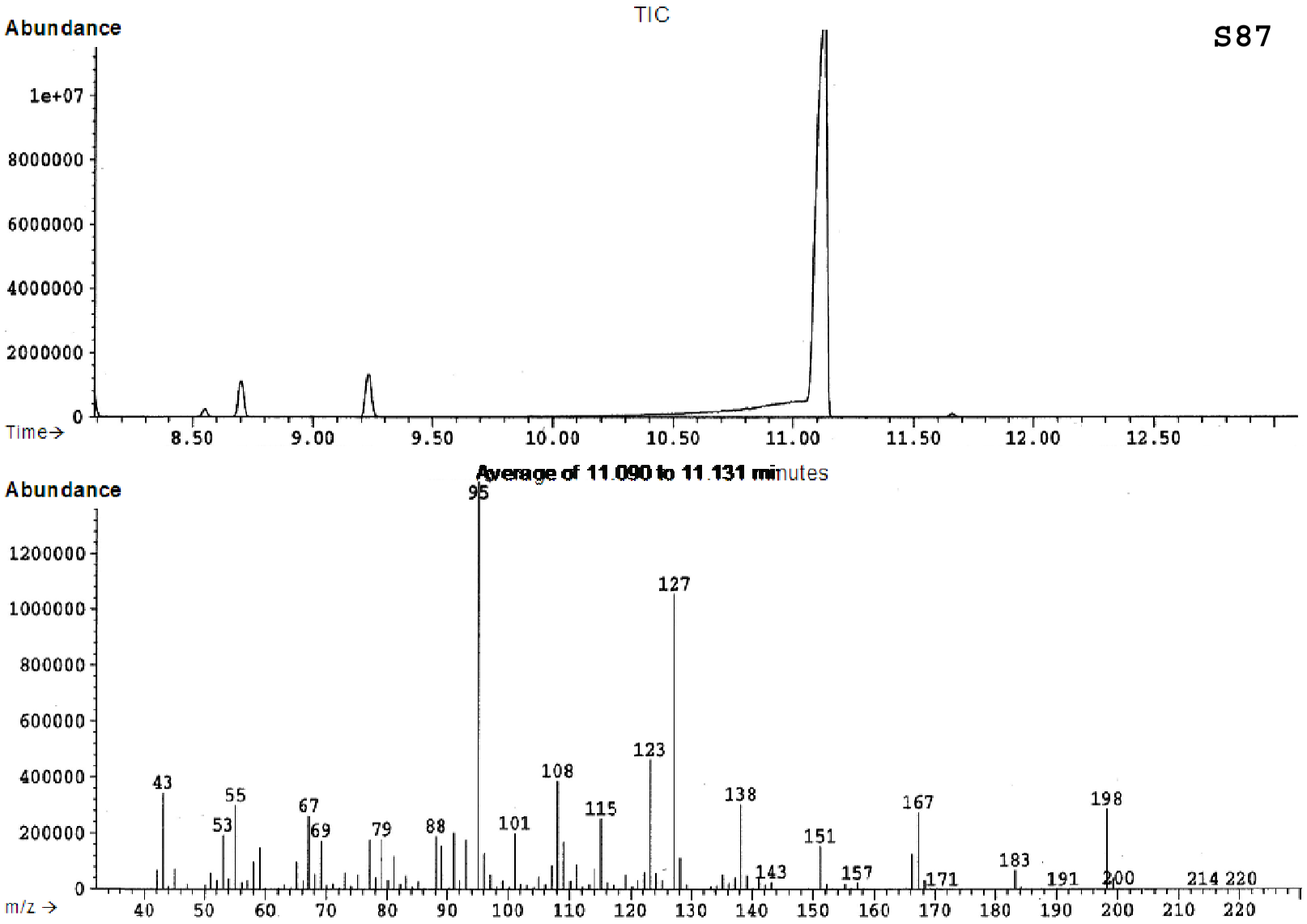
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Abundance
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9.50
10.00
10.50
11.00
11.50
12.00
12.50
Average of 11.090 to 11.131 minutes
Abundance
1200000
1000000
800000
600000
400000
200000
0
m/z →
95
127
123
108
43
55
53
67
69
79
88
101
115
138
143
151
157
167
171
183
191
198
200
214 220
40
50
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