Summer 2014

Synthesis of Hybrid Inositol Glycan Analogues

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SYNTHESIS OF HYBRID INOSITOL GLYCAN ANALOGUES

A Thesis

Presented to

The Faculty of the Department of Chemistry

San José State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Sudi Sabet

August 2014

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The Designated Thesis Committee Approves the Thesis Titled

SYNTHESIS OF HYBRID INOSITOL GLYCAN ANALOGUES

by

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August 2014

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ABSTRACT

SYNTHESIS OF HYBRID INOSITOL GLYCAN ANALOGUES

by Sudi Sabet

A 2010 study by Centers for Disease Control and Prevention has projected that one of three US adults could have diabetes by the year 2050. More than 90% of the diagnosed cases are Type II diabetes, a condition characterized by insulin resistance. In search of new antidiabetic drugs, a class of natural, phosphorylated, inositol-containing pseudosaccharides called inositol glycans (IGs) has been studied for years. These compounds have insulin-mimetic activity in insulin-sensitive cells and can stimulate processes such as lipogenesis and glucose transport. Due to the heterogeneity of IGs, it is very difficult to isolate them from their natural sources, and the potent synthetic ones are complex structures demanding lengthy and laborious chemistry.

It has been observed that a hybrid IG, an inositol-containing disaccharide conjugated with a non-carbohydrate fluorophore, exhibits relatively high insulin-mimetic activity. We propose the generation of a library of similar hybrid IGs (HIGAs), via a relatively short synthetic pathway, in which each HIGA is prepared from the attachment of the phosphorylated inositol core to a readily available non-carbohydrate piece. The ability of each HIGA to activate insulin-sensitive cells will be assayed to identify the ones with maximum insulin-like response. The active HIGAs would be further studied in future research as potential pharmaceutical agents for treatment of diabetes.

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LIST OF ABBREVIATIONS

AChE	- Acetylcholinesterase
ACL	- ATP Citrate Lyase
Akt	- Same as PKB
AP	- Alkaline Phosphatase
ATP	- Adenosine Tri-Phosphate
DAG	- Diacylglycerides
DCM	- Dichloromethane, Methylene Chloride
DEAD	- Diethyl Azodicarboxylate
DIAD	- Diisopropyl Azodicarboxylate
DMAP	- 4-Dimethylaminopyridine
DMP	- Dess–Martin Periodinane
DRM	- Detergent Resistant Membranes
FDA	- U.S. Food and Drug Administration
Glut4	- Glucose Transporter 4
GPI	- Glycosylphosphatidylinositol
GS	- Glycogen Synthase
IAEDANS	- 5-((((2-Iodoacetyl)amino)ethyl)amino)Naphthalene-1-Sulfonic Acid)
IG	- Inositol Glycan
IKK	- I κ B Kinase
IPG	- Inositol Phosphate Glycan
IR	- Insulin Receptor

IRS-1	- Insulin Receptor Substrate 1
IRS-2	- Insulin Receptor Substrate 2
JNK	- c-Jun N-terminal Kinase
MHC	- Major Histocompatibility Complex
MIR	- Maximal Insulin Response
PDK	- Phosphoinositide-Dependent Kinase
PDK1	- Phosphoinositide-Dependent Kinase 1
PI-PLC	- Phosphatidylinositol Phospholipase C
PI3K	- Phosphatidylinositol 3 Kinase
PKA	- Protein Kinase A
PKB	- Protein Kinase B
PKC	- Protein Kinase C
PNB	- para-Nitrobenzoic Acid
PPh₃	- Triphenylphosphine
TBAI	- Tetrabutylammonium Iodide
TEA	- Triethylamine
THF	- Tetrahydrofuran
TLC	- Thin Layer Chromatography
TLR	- Toll-Like Receptor
TLR4	- Toll-Like Receptor 4
VSG	- Variant Surface Glycoprotein

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CHAPTER 1

INTRODUCTION

1.1 Diabetes Overview

1.1.1 Diabetes

The most recent data, from the diabetes fact sheet of 2011, show that 25.8 million Americans (which is 8.3% of the population in the United States) have diabetes.¹ In 2010 alone, 1.9 million new cases were added to this pool. The Diabetes Report Card 2012 indicates that, if the current trend continues, by 2050 one in every three American adults could have diabetes.²

Diabetes is diagnosed when the concentration of glucose in blood, or glycemia, reaches 120 mg/dL or higher. Normally, blood glucose level is controlled by the opposing action of two pancreatic hormones, insulin and glucagon. Between meals or during physical activity, the glucose level in blood decreases, triggering the pancreas to release glucagon. Although many different cell types in the body are responsive to this hormone, it mainly stimulates the liver to increase the blood concentration of glucose by releasing its stored glucose into the blood. On the other hand, after having a meal by which the glucose level in blood is elevated, the pancreas releases insulin into the circulatory system. Again, many tissues respond to this hormone, but mainly insulin affects the muscle, liver, and fat cells and instigates the uptake of glucose, which in turn lowers the blood glucose concentration.

The balancing effect of glucagon and insulin maintains the glycemia in the narrow range of 4–7 mmol/L (70-100 mg/dL). Various circumstances can cause this system to collapse and allow blood glucose to attain much higher levels. This is the main symptom of the diabetic condition.

1.1.2 Diabetes Type I

There are two major forms of this disease: Type I or insulin-dependent diabetes and Type II or insulin independent diabetes. Type I diabetes is fundamentally an autoimmune disease in which the pancreatic β cells that are responsible for production and secretion of insulin are attacked by the patient's own immune system. This autoimmune response is induced in susceptible individuals, usually children, by a foreign antigen, such as a virus, that immunologically mimics the β cells. Certain alleles of Class II major histocompatibility complex (MHC) proteins, which are the normal components of the immune system, are prevalent among Type I diabetic patients. When these genetically variant proteins are bound to the β cell-resembling antigen, they trigger such a strong and prolonged immunological response that eventually, in addition to the foreign antigen, the β cells of the patient's pancreas are eliminated.³ When this loss reaches 80%, the patient suffers from acute insulin deficiency which leads to toxic levels of elevated blood glucose concentration, or hyperglycemia.

From the etiology, it is evident why Type I diabetes is also referred to as insulin-dependent diabetes. In fact, until the isolation of insulin in 1921 (Figure 1), this disease was fatal within days or weeks of the onset.⁴ However, since then, millions of children

and young adults are saved by the administration of exogenous insulin. Although it remains a life-changing illness, the majority of patients can maintain a comparatively normal life with a robust diet, regular exercise, and routine monitoring of their blood glucose level.

(a)

(b)

Figure 1. First isolation of insulin. (a) Frederick Banting (right) and Charles Best purified insulin in summer of 1921 at the University of Toronto. (b) “Insulin: Toronto’s Gift to the World” Exhibit went on display in 2011 in Toronto, to celebrate the 90th birthday of insulin.⁴

1.1.3 Diabetes Type II

In Type II diabetes the pathology lies on the receiving end of the insulin signal. Contrary to Type I, at the early stages of the disease, the β cells of the pancreas are healthy and able to produce and secrete insulin. In fact, at this stage the patients are simultaneously hyperglycemic and hyperinsulinemic (having elevated amounts of insulin in the blood). However, the cells that normally respond to insulin, such as hepatocytes (liver cells), adipocytes (fat cells), and myocytes (muscle cells) fail to do so. It seems that there is a defect in the insulin signal transduction pathway, a disconnect between

receiving the extracellular insulin and generating an intracellular response. In other words, insulin-sensitive cells have become insulin resistant.³

1.1.4 Insulin Signal Transduction

The signaling pathway starts when insulin binds to the alpha subunit of insulin receptors (IRs) in the membrane of insulin-sensitive cells. These receptors are transmembrane heterotetrameric tyrosine kinases that become activated upon binding insulin.⁵ This results in autophosphorylation of the receptor itself, and tyrosine phosphorylation of insulin receptor substrate-1 and substrate-2 (IRS-1 and IRS-2). The now activated IRS-1 interacts with the regulatory subunit (p85) of phosphatidylinositol 3-kinase (PI3K),⁶ which activates p110, the catalytic subunit of the same kinase.⁷ PI3K then phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-trisphosphate (PIP3). This molecule then binds to phosphoinositide-dependent kinase 1 (PDK1) which participates in the activation of Akt, also referred to as protein kinase B (PKB). Akt/PKB is a critical node in the insulin phosphorylation cascade. It phosphorylates a specific site of ATP citrate lyase (ACL), leading to lipid biosynthesis, or lipogenesis. It also increases the rate of glycogen synthesis by preventing the inactivation of the glycogen synthase (GS). Glycogen synthesis is directly up-regulated when glycogen-associated protein phosphatase 1 (PP1), which is activated downstream of PI3K, dephosphorylates and hence activates the GS.⁸⁻⁹

Perhaps the most well known physiological response associated with insulin is the uptake of glucose from the blood and the resulting decrease in blood glucose level. This

happens when Akt, either directly or through the activation of other kinases, causes the translocation of glucose transporters from the intracellular vesicles to the cell membrane. There is also a second phosphorylation cascade that leads to the same event and that starts when insulin-bound IR phosphorylates APS protein.⁹ Glucose transporters exist in several isoforms including glucose transporter 4 (Glut4) that is responsible for insulin-induced glucose transport. When Glut4 is present on the cell membrane, glucose uptake occurs by facilitated diffusion. Figure 2 summarizes these major events of the insulin signal transduction pathway.⁸⁻⁹

Figure 2. Schematic summary of the major events of the insulin signal transduction pathway. —○ denotes catalysis, —→ denotes activation, and - - -→ denotes indirect activation.

1.1.5 Diabetes Type II Etiology

A multitude of factors can render cells resistant to insulin. This makes Type II diabetes a complex and etiologically heterogeneous disease. The most common risk factor is obesity. A recent study lists three ways by which overeating provokes insulin resistance: increased inflammation, lipid metabolism alterations, and gastrointestinal changes in the microbiota.¹⁰

It has been observed that the number of proinflammatory macrophages in adipose and hepatic tissues of obese people is substantially higher than in non-obese people. Genetic loss- and gain-of-function techniques have shown a direct correlation between macrophage-mediated inflammation and insulin resistance. For example, in adipose tissue, macrophages release a particular cytokine that activates c-Jun N-terminal kinase (JNK) and I κ B kinase (IKK) that deactivate IRS-1 by serine phosphorylation.¹⁰ Unfortunately, anti-inflammatory drug development against diabetes has not been successful so far.

Alteration in the metabolism of lipids in obese people is also observed. Such changes can result in the lingering of high levels of fatty acids in the blood stream, which trigger cellular signaling networks that negatively interact with the insulin pathway. For example, circulating saturated fatty acids stimulate a member of the toll-like receptor (TLR) family, toll-like receptor 4 (TLR4), which is a cell surface, transmembrane protein associated with the innate immune system.¹¹ TLR4 in turn activates JNK and IKK, linking lipotoxicity and proinflammatory pathways and eventually promoting insulin resistance by serine phosphorylation of IRS-1. Secondly, certain byproducts of fat

metabolism cause insulin resistance. For instance diacylglycerides (DAGs), produced by fatty acids metabolism, activate protein kinase C (PKC), which deactivates IRS-1, again by serine phosphorylation. Ceramides, another byproduct of lipid metabolism, prevent Akt activity and thus block the insulin signal propagation.¹⁰

It is still not very clear if the change in the gastrointestinal microbiome is the cause or effect of obesity and insulin resistance. Also, most studies in this area have been conducted on mice models, and there are questions on how well they can be translated to humans. Nonetheless, these studies show that increased amount of food-sourced energy in obesity causes dysbiosis, an imbalance in the otherwise stable intestinal microbiota of the individual. Leakage of the bacterial products or bacteria themselves into the circulatory system causes proinflammatory responses with adverse effects in insulin responsive cells.¹⁰

1.1.6 Complications and Treatment

More than 90% of all cases of diabetes are diabetes Type II. If left untreated, diabetes causes complications such as cardiovascular disease, which accounts for 50% of all fatalities related to this disease. Diabetes is also the main cause of end stage renal disease in which patients need dialysis or kidney transplantation. Damage to the nerves, especially in the toes, feet, and lower limb, causes loss of feeling which consequently allows injuries to worsen unnoticed. Eventually, severe infections or gangrenes might leave the patient with no other option than amputation. Damage to the retina of the eye and loss of vision are also common in diabetic patients.

Currently, there is no cure available. When diagnosed, the patients are advised to make major lifestyle changes involving diet and exercise. Usually, after some time, prescription of antidiabetic drugs are necessary. None of these drugs is perfect and scientists are still in search of better ones. In fact, the FDA has recently approved a medication by Johnson and Johnson called Invokana® that forces the kidneys to dispose of the excess glucose in the urine.¹² On the other hand, older medications, like Januvia® by Merck, are under FDA scrutiny due to new data indicating that they may cause cancer.¹³ So, the quest for better treatment options is by no means over. Although the multifactorial nature of Type II diabetes is overwhelming, it does offer multiple pharmacological entry points.

1.2 The Second Messenger

The signaling network that was explained in section 1.1.4 does not have any provision to allow one biological event to happen independent of another. As mentioned above, glycogen synthesis and glucose transport are prominent physiological responses triggered by insulin. Although in most cases these events happen together, it has been observed that, in certain tissues and under specific conditions, they are activated exclusive of each other. For example, when rat heart was treated with insulin, glucose transport was observed but not glycogen synthesis. However, in rat diaphragm, insulin activated glycogen synthase, but glucose transport stayed dormant.¹⁴ In order to explain these observations Lerner, in 1972, for the first time, hypothesized the presence of a “new intermediate”, a second messenger in the insulin signaling pathway.¹⁵ Until then, the

propagation of insulin stimulation was explained by a phosphorylation network initiated with the auto-phosphorylation of the insulin receptor tyrosine kinase upon binding to insulin. But that model failed to explain the disconnect between insulin-associated responses.

The hypothesis gained strength when in 1974 Larner isolated one or more compounds from the extracts of insulin treated murine muscle and liver cells that inhibited protein kinase A (PKA) and activated glycogen synthase phosphatase.¹⁴ As described before, these activities are both associated with insulin. A few years later, Jarett and Seal demonstrated that the same compound could activate mitochondrial pyruvate dehydrogenase, another insulin-linked event.¹⁶ So by 1979, the compound was referred to as the “insulin mediator”.¹⁷

Identifying the chemical structure of the insulin mediator proved to be an elusive goal. It was suspected at first that it is a peptide.¹⁷ Later, carbohydrates were found in the purified compound.¹⁸ In 1986, Saltiel made the important discovery that tritiated inositol and glucosamine are incorporated in the structure of the mediator and that phosphatidyl-inositol phospholipase C was able to release the mediator from the hepatic cell membranes.¹⁹ Also the polar nature of the compound implied the presence of phosphate groups. The exact chemical structure of the insulin mediator was not known yet, but through ion exchange chromatography, molecular sizing, and phase partitioning purification methods, it was determined that it is negatively charged, highly polar, and thus water soluble, and weighs between 1000-1500 dalton. All this information

suggested that the mediator is an inositol phosphate glycan (IPG), sometimes known more simply as an inositol glycan (IG).²⁰

On the basis of these findings, the biological activity of several already well established natural inositol phosphate compounds were auditioned. Neither the chemical properties nor the biological effects of the known compounds matched those of the insulin mediator. But then a new structure was found, a complex compound that anchored proteins in the cell membrane, a glycosylphosphatidylinositol or GPI.

1.3 GPI Anchors

1.3.1 Phosphatidylinositol Phospholipase C

Gangrene, a possible complication of diabetes, is a serious condition caused by a class of bacteria called clostridia that produce the most diverse array of toxins among all bacteria. Ironically these dangerous organisms have also endowed us with a valuable biological tool, phospholipase C. Although not categorized as phospholipase C at the time, this protein was first discovered in 1941 as the toxin of bacterium *Clostridium perfringens*.²¹⁻²² It turned out that phospholipase C is actually a group of enzymes, and, since some of the members of this group are strong toxins, they have been extensively studied. In 1976, scientists succeeded in purifying a type of phospholipase C from bacterium *Bacillus cereus* that specifically acted on phosphatidylinositols and produced diacylglycerols and inositolmono -1- and -1-2 cyclicmonophosphates. This enzyme is referred to as phosphatidylinositol phospholipase C or PI-PLC.^{21, 23}

The specificity of PI-PLCs has been exploited in phospholipid composition studies of membranes.²⁴ It was found that this enzyme released certain proteins such as alkaline phosphatase (AP), acetylcholinesterase (AChE), and 5'-nucleotidase that were known to reside on the surface of the cell. Prior to this unanticipated observation, membrane proteins were believed to be associated with the lipid bilayer in two ways: through a hydrophobic polypeptide sequence inserted in the membrane or through a lipophilic tail that was attached to either the free amine or the thiol function of the proteins and secured them into the membrane. PI-PLC experiments suggested a third method: anchorage in the lipid bilayer by attachment to a phosphatidylinositol-derived structure.²³

1.3.2 GPI Anchor Structure

In pursuit of the structural elucidation of the novel membrane protein anchors, Tse and his coworkers,²⁵ and Ferguson and his collaborators²⁶ made significant contributions. Thy-1 glycoprotein, the antigen of rodent thymocytes and neurons, was the first eukaryotic membrane protein for which a non-protein tail was suggested as the membrane anchor. Tse *et al.*, in 1985, demonstrated that the anchor is in fact a glycopospholipid structure. Using gas chromatography and mass spectrometry, they established that the structure contained of ethanolamine, *myo*-inositol, stearic acid, phosphate, and glycerol.²⁵

Trypanosoma brucei is a protozoan that, like other African trypanosomes, confuses the mammalian immune system by expressing the genes encoding variant

surface glycoproteins (VSGs), which form a dense coat covering the cell surface. Ferguson *et al.*, also in 1985, established that the VSGs are covalently linked to a phosphatidylinositol-containing glycolipid that includes a non-N-acetylated glucosamine, which is glycosidically attached to a *myo*-inositol monophosphate. These two important studies, along with others, established a class of compounds generally referred to as glycosylphosphatidylinositols or GPIs.

Since then, an abundance of membrane proteins that use GPI as their anchoring system has been discovered. Across the eukaryotic domain, plants, protozoa, fungi, yeast, mollusks, insects, and vertebrates benefit from GPI as anchors. Interestingly, none has been found in bacteria. In protozoa, free GPIs are also observed on the cell surface. These structures are not bound to any proteins.

Ferguson *et al.* discovered the missing pieces of the structural puzzle of *T. brucei* VSG anchor in 1988, making it the first GPI to be completely elucidated.²⁷ Since then, the structural details of many GPI anchors have been revealed. GPIs are heterogeneous in composition, but a conserved core is shared among all. As depicted in Figure 3 the core structure of the GPI consists of 6-O-aminoethylphosphoryl-Man(α 1-2)-Man(α 1-6)-Man(α 1-4)-GlcNH₂((α 1-6)-*myo*-inositol-1-O-phospholipid).²⁸ The posttranslational addition of the GPI to the nascent protein, the protein destined to mature to a GPI-anchored membrane protein, is done by a transamidase enzyme in the endoplasmic reticulum. The point of the addition, also referred to as the ω site, is usually one of the smaller amino acids such as Gly, Ala, Ser, Asn, Asp, and Cys and lies upstream of the 15-30 residue-long, C-terminus signal transamidase sequence of the pro-protein. The

transamidase concomitantly adds the GPI to the ω site and cleaves the signal sequence. The lipid tail of the GPI then gets incorporated into the plasma membrane and anchors the mature protein in the outer leaflet.^{29 - 30}

Figure 3. The conserved core structure of GPI anchors.

The aforementioned heterogeneity arises when species- or tissue-specific modifications occur at several branching sites of the GPI core. The more common changes include the presence of additional mannose residues or phosphate groups and branching at the C3 or C4 of the mannose attached to the glucosamine unit (Man I). The hydrocarbon chain of the GPI phospholipid can vary in length and degrees of unsaturation as seen in diacylglycerol, alkylacylglycerol, and ceramide type constituents. It has also been observed that some GPIs are not sensitive to PI-PLC due to acylation at the C2 position of the *myo*-inositol with extra fatty acids (Figure 4). Instead, glycosylphosphatidylinositol-specific phospholipase D (GPI-PLD), isolated from human

serum, hydrolyzes this subfamily of GPIs to phosphatidic acid and an inositolglycan piece. In contrast with cleaving product of PI-PLC enzyme, here, the phosphate group remains with the lipid moiety. This difference is depicted in Figure 4.^{23, 31, 32}

Figure 4. Cleavage sites of PI-PLC and GPI-PLD on GPI anchor and free GPI. GPIs' structures are simplified to their bare skeleton.

1.3.3 GPI Anchor Function

The structural complexity of GPI anchors, juxtaposed with the simplicity of lipids or peptides that could serve the same purpose, has left the scientific community wondering about their evolutionary advantage. Despite considerable efforts to pinpoint GPIs' functions, their only confirmed biological role is anchoring of membrane proteins. There are some other functionalities attributed to GPI anchors, such as altering the structure of their associated protein, involvement in signal transduction, facilitating cellular communication, and providing a regulation mode for their associated protein via phospholipases, but these remain controversial.²⁷

Among the possible functions, involvement in signal transduction is the most relevant to the subject at hand. One reason that GPIs are associated with signal transduction is that the GPI-anchored proteins are localized in lipid rafts, and, since a lot of signaling proteins are also gathered in this region, it is hypothesized that GPI anchors may also have a role in signal transduction.²⁷ Lipid rafts are microdomains of the cell membrane that are packed with sphingolipids, cholesterol, and transmembrane and lipidated proteins. They are characterized by insolubility in nonionic detergents such as Triton X-100 at 4 °C. In vitro, lipid rafts are referred to as detergent resistant membranes or DRMs. GPI-anchored proteins are also detergent insoluble because of their association with lipid rafts. Due to the highly organized structure of the lipid rafts and tight packing of their sphingolipids, GPI-anchors are closely packed in this region and are suggested to be involved in signal transduction like a lot of other signaling agents in the lipid rafts.^{27, 33} It has also been observed that the product of PI-PLC digestion of the GPIs has insulin-like effects.³⁴

1.4 Synthetic IGs

Around the same time that the putative insulin mediators were classified as IPGs, GPI anchors were discovered and the chemical structure of the VSG anchor was completely solved. As mentioned earlier, several known inositol phosphate-containing compounds were surveyed for insulin-like behavior, however the chromatographic and electrophoretic properties of these compounds did not match those of the insulin

mediator, and none exhibit insulin-like behavior. The novel GPI anchors, being inositol phosphate glycans, were appropriately appointed as the next candidate.

To investigate whether GPIs function as precursors to the insulin mediator IPG, and to generate large quantities of IPGs for structure-function relationship studies, Misek and Saltiel chose to work with the structurally defined *Trypanosome brucei* VSG1180 GPI anchor, in 1992.³⁴ In addition to having a defined structure, this GPI was abundantly available from the organism. Each *T. brucei* owned 10^7 identical copies of the molecule. It was observed that *T. brucei* treatment with PI-PLC and Pronase produced an insulin mediator-like substance in the medium, which was chromatographically and electrophoretically equivalent to the insulin mediators and thus assumed to be an inositol phosphate glycan structure. The obtained IPG, which was known to contain *myo*-inositol 1,2-cyclic monophosphate,²⁶ inhibited lipolysis in rat adipocyte and stimulated gluconeogenesis in hepatocyte. In order to establish whether the cyclicity of the phosphate group on the inositol motif was required for the insulin mimicry, the obtained IPG was exposed to acid hydrolysis by treatment with 100 mM HCl for 15 min. The product was the more negatively charged acyclic inositol monophosphate glycan that had lost its insulinomimetic behavior. Due to these observations, it was suggested that insulin triggers a special phospholipase that in turn hydrolyzes the GPI to afford the insulin mediator IPG as a 1,2-cyclic monophosphate. In fact, it has been realized that the formation of cyclic phosphate on the inositol is ensured by the mechanism through which PI-PLC hydrolyzes the phospholipid bond of the GPI.³⁵ The free 2-OH of the inositol nucleophilically attacks the 1-phosphate, while the lipid leaves as a fatty acid. For the

IPG to act as a mediator, it needs to become deactivated after its signaling function is accomplished. The interconversion between the cyclic and acyclic phosphate can serve as a switch, turning the IPG mediator “on” or “off”.

By this time it was confidently believed that IPGs are the elusive second messengers of the insulin pathway.³⁶ It had been observed that, through regulating metabolic enzymes such as glycogen synthase, low- K_m cAMP phosphodiesterase, adenylate cyclase, pyruvate dehydrogenase, phospholipid methyl transferase, protein kinase A, and pyruvate dehydrogenase phosphatase, IPGs evoke insulin-related physiological responses, most importantly lipogenesis. But their structure still was not unequivocally resolved. It was known that IPGs contain a free aminohexose that bridged a glycan chain and a phosphorylated inositol that was attached through a glycosidic bond. However, the stereochemistry and substitution was uncertain. Chiro and myo inositols, various degrees of phosphorylation, and different number and types of sugars in the glycan chain have all been detected. The heterogeneity and natural scarcity of the IPGs made it hard to obtain pure material, a prerequisite in interpreting the data for biological studies. It appeared that chemical synthesis was the only way to have in hand a defined structure to be used in uncovering the role of IPGs in insulin signal transduction pathway.

So in 1992, Plourde and d'Alarcao exploited the similarity between the structurally defined GPI, used by Misek and Saltiel, and the partially known structure of IPGs and designed a short synthetic scheme towards disaccharide **1** and its derivative, acyclic phosphate **2** (Figure 5).³⁶ Derivative **2** was not biologically active. However, 40 μ M of disaccharide **1** effected the incorporation of 14 C-glucose into lipids in intact rat

epididymal adipocytes. At that concentration, **1** was 30-40% as effective as insulin at stimulating lipogenesis. This successful outcome shed the spotlight on IPGs and inspired many scientists to consider them for research.

Figure 5. Disaccharide **1** is the first biologically active synthetic IPG. Acyclic phosphate **2**, which only differs from **1** in having an acyclic vs. cyclic phosphate group, did not show any biological activity.

In an extensive structure-function study in 1998, Müller and collaborators made forty-six IPG variants based on the structure of the GPI anchor of the Gce1p membrane protein of the yeast *Saccharomyces cerevisiae*.³⁷ These synthetic IPGs were divided into four classes, A, B, C, and D, according to their overall insulin-mimetic activity.

Compounds **3** and **4** (Figure 6) were in the most active groups, C and D respectively.

These compounds stimulated lipogenesis, one of the several insulin-related responses assayed in this experiment, up to 90% of the maximal insulin response (MIR) at 20

μM. Compound **5** of class B was much less potent with 20% of MIR at 100 mM and

compound **6** representing class A, was almost inactive. Structure-activity analysis

revealed that the complete conserved core glycan of the GPI, including the three mannose residues, the glucosamine, the mannose side chain in correct glycosidic linkage, and the

inositol phosphate moiety were all required for maximal insulin-like activity. The phosphate and sulfate anionic substituents had a distinct but unfortunately unpredictable influence on the insulin activity of the molecule. By way of example, exchanging the terminal sulfate of **3** to phosphate caused considerable decrease in insulin-mimetic activity of this compound, though interestingly, deviation from the naturally occurring α -glycosidic linkage between glucosamine and inositol to β -linkage improved the insulin-like activity of this species.³⁷

Figure 6. Structures **3** and **4** represent the most potent synthetic IPGs ever made with lipogenesis activity up to 90% and 80% of MIR. The potency of compound **5** is 20% of MIR and compound **6** is almost inactive.

By 2005, despite great efforts devoted to understanding the molecular basis of insulin-mimicry of IPGs, the findings remained incomplete and were conflicting at times. For example, Alvarez and coworkers³⁸ showed that a natural IPG had to be in the cytosol in order to elicit metabolic responses, but Müller and coworkers³⁹ showed that a synthetic IPG and a yeast-originated IPG only needed to bind a specific protein on the surface of the cell to instigate insulin-like metabolic responses. In order to help explain if one or both of these events are necessary to activate the cell, d'Alarcao and coworkers⁴⁰ prepared a fluorescently labeled pseudodisaccharide, a hybrid between the very first biologically active synthetic IG, disaccharide **1**, and a commercially available non-carbohydrate fluorophore, to be able to use optical techniques in tracing the molecule in the cellular environment (Figure 7). In order to do so, they replaced the sole primary hydroxyl group in **1** with a thiol function so that it could easily react with an iodoacetamide derivative of the Lucifer yellow fluorescent tag. The hybrid **7** (Figure 7) was added to the growing media of the rat epididymal adipocytes. After an hour of incubation the cells were viewed by fluorescence microscopy and analyzed by flow cytometry. They discovered that, although no cellular internalization of the fluorescent hybrid occurred, it still stimulated lipogenesis with a maximal activity of 47% of MIR. The conclusion was that, at least for this hybrid IPG, internalization to insulin-sensitive cells is not a prerequisite for insulin-like activity.⁴⁰

This finding was important, but what was even more intriguing in this experiment was the fact that, contrary to the scientists' expectation, the fluorescently conjugated IPG had more insulin-like activity than the parent unadulterated carbohydrate.⁴⁰ They

postulated that the presence of anionic sulfate groups in Lucifer yellow and their position relative to inositol are the reasons behind the improved activity. This was a fortunate coincidence. Lucifer yellow had been chosen as the fluorescent tag because it would become dianionic in physiological conditions. This would prevent nonspecific association of the conjugate to the cell surface, which could result in a false positive for cell internalization. In addition, it was hypothesized that the locality of these ionic substituents on Lucifer yellow may match the IPG's biologically active sites. A synthetically significant bonus was that they were added to the molecule via commercially available precursors. A logical question followed: are there other commercially available non-carbohydrate moieties with opportunistic positioning of the ionic groups that would render the simple disaccharide a strong insulin-mimetic pharmacophore? Answering this question is the goal of the current research.

Figure 7. The structure of fluorescent IPG derived from disaccharide **1**. The sulfate and the amine groups are ionic under physiological conditions.

CHAPTER 2

RESEARCH GOAL AND SYNTHETIC PLAN

2.1 Research Goal

A great body of evidence has suggested that IPGs may act as second messengers of insulin action. Due to the scarcity and heterogeneity of these compounds, it is difficult to isolate them from their natural sources in sufficient purity and quantity for biological studies. To compensate for these difficulties, scientists have resorted to organic synthesis to generate structurally defined IPGs in workable quantities.

The most potent IPG synthesized to date is hexasaccharide **3** (Figure 6), which at 20 μ M induces insulin-mimetic activity equivalent to 90% of MIR. However, synthesizing such a complex molecule requires lengthy and arduous carbohydrate chemistry. Disaccharide **1** (Figure 5), on the other hand, is much simpler to make, but its insulin-mimetic activity, at comparable concentrations, is rather modest (less than 30% of MIR). Serendipitously, it was also observed that conjugation of **1** to a fluorescent non-carbohydrate moiety increased the MIR to 47% (cf. **7**, Figure 7).

The enhanced biological activity of hybrid **7** was credited to a correct spatial distribution of its charged substituents. Therefore, it was hypothesized that there must exist other commercially available non-carbohydrate moieties with ionizable groups positioned as such as to produce even more active hybrids. The current research is designed to evaluate this hypothesis. A relatively short synthetic pathway is proposed to quickly populate an analogue library of these hybrid inositol glycans to be examined for

insulinomimetic biological responses such as glucose uptake, glycogen synthesis, and lipogenesis. The structure of the first entry of this library, the first hybrid inositol glycan analogue (HIGA), is depicted in Figure 8.

Figure 8. The structure of the first synthesized HIGA with its three parts color-coded: phosphorylated inositol core in blue, non-carbohydrate fluorophore in green and a thiol linker in red.

The synthesis of this compound has previously been attempted.⁴¹ However, only small amounts of the product, enough to be identified by mass spectrometry, were obtained. The goal of the current work is to improve the reaction schemes and conditions, and consequently, the yields, so that sufficient amounts of the HIGAs would be synthesized for thorough chemical and biological analysis.

2.2 Synthetic Strategy

One characteristic that active IPGs, such as **1**, **3**, and **4**, have in common is the proximity of an amine to the cyclic phosphate on C1 and C2 of the inositol. In the above

examples, the amine is part of the glucosamine that is attached to inositol via an $\alpha(1-6)$ linkage. In order to shorten the synthetic route to HIGAs, this glucosamine was eliminated. To set the stage for a new protonatable nitrogen close to the cyclic phosphate, and for a reactive center to attach the linker, the inositol core was derivatized with an azide function at C6. Azide is a stellar functional group for ligation purposes. There are at least two ligation methods that involve the azide function: Staudinger ligation and Huisgen cycloaddition. In both methods, after the ligation has happened the azide converts into a protonatable-nitrogen containing system.

The structure of a HIGA (cf. **8** and **9**, Figures 8 and 9) consists of three parts, a phosphorylated inositol core (blue), a non-carbohydrate fluorophore (green), and a linker (red) that attaches the other two pieces together. The initial strategy employed to synthesize the first HIGA (cf. **9**, Figure 9) involved traceless Staudinger ligation to connect a cysteine-based linker to the azide derivative of the inositol core. Cysteine was desirable since it provided a thiol group as a “hook” for the iodoacetamide non-carbohydrate fluorophore, and provided an additional amine group, that would neighbor the cyclic phosphate. However, this strategy had to be abandoned because it still entailed too many synthetic steps and involved an intermediate product that failed to form.⁴¹

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Figure 9. The structure of the lead HIGA using traceless Staudinger ligation. The three parts are: phosphorylated inositol core in blue, non-carbohydrate fluorophore in green, and a cysteine-based linker in red.

The second strategy was to employ copper (I) catalyzed regioselective ligation of azides and terminal alkynes to fuse a thiol linker to the inositol core. This reaction was developed by Barry Sharpless in 2002⁴² and was a new contribution to the family of click reactions. It is basically a variant of Huisgen 1,3-dipolar cycloaddition. Contrary to the parent reaction, which usually requires elevated temperatures and results in a mixture of 1,4- and 1,5-disubstituted products, this transformation is simple to run and results in 1,4-disubstituted 1,2,3-triazole products only. By this reaction, the alkyne side of a thiol linker is coupled to the azidoinositol core. The thiol side acts again as a “hook” for the iodoacetamide non-carbohydrate moiety, leading to HIGA **8** in 11 fewer steps. Thus the second strategy was followed in the previous⁴¹ and current work toward the synthesis of HIGAs.

The retrosynthetic analysis of HIGA **8** is shown in Schemes 1 and 2. Compound **8** can be obtained by attaching the non-carbohydrate fluorophore, IAEDANS (**24**), to thiol **23** by an S_N2 reaction. Compound **23** is reached by the exhaustive deprotection of

22, converting the benzyl ethers and the benzyl thioether to the corresponding unprotected species, the alcohols and the thiol respectively. Compound **22** is the result of the phosphorylation of diol **21** with methylpyridinium dichlorophosphate, forming a cyclic phosphate that incorporates the vicinal hydroxyl groups at C1 and C2 of the inositol. Compound **21** is produced by the “click” chemistry between azide **19b** and thiol-yne **20**. The benzyl-protected thiol-yne **20** is produced by a substitution reaction between commercially available α -toluene thiol and propargyl bromide. Compound **19b** comes about by osmium tetroxide aided syn dihydroxylation of azide **18**, which in turn, is produced by mesylation and subsequent azide displacement of conduritol **17b**. This compound has to be purified from an epimeric mixture with **17a** (Scheme 2).

Conduritols **17a** and **17b** are, respectively, referred to as syn and anti conduritol, and their synthesis is well established in this laboratory.⁴³ These epimers are the products of the ring closing metathesis on a mixture of the 1,6-dienes **16b** and **16a**, catalyzed by the first generation Grubbs catalyst. The dienes are produced from Grignard reaction between aldehyde **15** and vinyl bromide. Aldehyde **15** comes from the Swern oxidation of alcohol **14**, which is the consequence of the addition of a vinyl group to the reducing sugar **13** by the Wittig reaction. Compound **13** is made by hydrolysis of the benzylated methylglycoside **12**. Protection of the non-anomeric hydroxyl groups of **11** by conversion to benzyl ethers gives fully protected **12**. In turn, **11** is synthesized by Fischer glycosidation of commercially available D-xylose, **10**.

Scheme 1. Retrosynthetic analysis of the first HIGA, part 1

Scheme 2. Retrosynthetic analysis of the first HIGA, part 2

As is evident from this retrosynthetic survey, the non-anomeric hydroxyl groups of D-xylose have the stereochemistry of the hydroxyl groups of *myo*-inositol at positions 3, 4, and 5, and they stay protected as benzyl ethers until the last step of the synthesis. The anomeric position that was protected as a glycoside during the benzylation is subsequently revealed and utilized as a reactive center to manipulate the molecule toward the final product. Additionally, D-xylose is a rather abundant and inexpensive compound, which makes it an even more attractive starting material.

2.3 Two Strategies Suggested to Selectively Access **17a** or **17b**

It has been known to this research group that the chromatographic separation of syn and anti conduritols, **17a** and **17b**, is quite inefficient. However, both epimers are valuable intermediates in this laboratory. In order to avoid the purification process and still have exclusive access to both conduritols, the following strategy was suggested: oxidation of the mixture of **17a** and **17b** to enone **25** and selective reduction of **25** to either conduritol (Scheme 3).

Scheme 3. Selective access to **17a** or **17b** through enone **25**

A preliminary literature investigation recommended the use of Dess-Martin periodinane for the oxidation step⁴⁴ and Luche condition,⁴⁴ the use of DIBAL,⁴⁵ or bulky reducing agents such as LS-Selectride⁴⁶ (Figure 10) for the reduction step. The Luche condition was expected to give the syn conduritol **17a**, and LS-Selectride seemed

especially promising in producing the other epimer. It was hoped that the steric demand of this bulky reducing agent would encourage equatorial hydride attack and improve the selectivity toward the axial alcohol, the anti conduritol **17b**. That was the first strategy to replace the chromatographic separation of syn and anti conduritol and still preserve access to both epimers.

Figure 10. Structure of LS-Selectride, lithium tris(3-methylbutyl)borohydride, a bulky reducing agent.

The second strategy was to employ the Mitsunobu reaction. This reaction is a popular method for the inversion of primary and secondary alcohols in organic and medicinal chemistry⁴⁷ and has been used in this laboratory before to interconvert chromatographically separated syn and anti conduritol to each other.⁴³ In case the equatorial hydride attack of enone **25** did not work as had hoped, the syn conduritol obtained from the Luche reduction would be inverted to anti epimer by the Mitsunobu reaction (Scheme 4).

Scheme 4. Selective access to **17b** through Mitsunobu inversion of **17a**

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Synthesis of Syn and Anti Conduritols, **17a** and **17b**

The synthetic scheme and reaction conditions that were followed toward the synthesis of the HIGA are summarized in Scheme 5 and Scheme 11. The current research started with compound **13**, which had already been synthesized in large scale following previously verified methods in our lab.⁴⁸ However, for the sake of completeness and clarity, the three steps leading to **13** from D-xylose are also included in Scheme 5. Under glycosidation conditions, D-xylose was refluxed in methanol in the presence of acidic dowex for 48 h, affording methyl xyloside **11**. Benzylation of **11** resulted in the differentially protected **12**. To deprotect the anomeric hydroxyl group, **12** was hydrolyzed by refluxing under acidic conditions in dioxane for 12 h to afford reducing sugar **13** in 59% overall yield over the three steps.

Toward the synthesis of the conduritols **17a** and **17b**, Wittig reagent, methylene-triphenylphosphorane, was first prepared from a suspension of methyltriphenylphosphonium bromide in THF and dropwise addition of butyllithium under strictly anhydrous conditions. After the orange-red color of the phosphorus ylide endured for 2 h, the solution of **13** in THF was added gradually with stirring. The 5-en-1-ol **14** was obtained after a quick filtration of the crude through silica plug. Swern oxidation of **14** gave 5-en-1-one **15**. Grignard reaction of **15** with freshly prepared vinylmagnesium bromide resulted in expected (but inseparable) epimers of 1,7-dien-3-ol, **16a** and **16b**. This

mixture was subjected to ring closing metathesis with ruthenium-based first generation Grubbs' catalyst which converted **16a** and **16b** to conduritols **17a** and **17b**, respectively.

Scheme 5. Synthetic scheme toward first HIGA, part 1, from D-xylose to conduritols **17a** and **17b**⁴³

3.2 Selective Synthesis of **17a** and **17b**

As mentioned in Section 2.3, since the separation of epimers **17a** and **17b** using silica gel column chromatography required successive purifications and proved to be extremely inefficient, an alternative route was devised: oxidation of the mixture of the two epimers to an enone and stereoselective reduction of the enone to either epimer. To this end, using Dess-Martin periodinane, the mixture of **17a** and **17b** was converted to enone **25**.⁴⁴ Reduction under Luche conditions using NaBH₄ and CeCl₃·7H₂O was attempted in methanol at 25, 0, -20, and -78 °C and in tetrahydrofuran (THF) at 0 and -78 °C. Although a mixture of **17a** and **17b** was observed under all conditions, the selectivity toward **17a** was greatly improved when the reduction was carried out at the initial temperature of -78 °C in methanol.⁴⁴ Analysis by NMR (page 72) indicated that, under these conditions, the ratio of **17a** to **17b** was 96 to 4 (Scheme 6).

Scheme 6. Oxidation of **17a** and **17b** to enone **25** and selective reduction of **25** to **17a**

Next, we explored the applicability of DIBAL and LS-Selectride as reducing agents toward the selective synthesis of **17b** from the enone **25**. Two equivalents of DIBAL were added to a solution of **25** in dichloromethane at room temperature. TLC

indicated the presence of both epimers. The experiment was repeated at -78 °C, however the lower temperature did not improve the selectivity of the reaction toward **17b**. The reduction was also tried with LS-Selectride, the bulkiest of the reducing agents tried. Based on analytical TLC, two major unknown products were formed in addition to a slight amount of both **17a** and **17b**. It seemed that the axial attack of the enone by reducing agents is not favored enough to allow the stereoselective production of **17b**. This approach was therefore dismissed at this point.

It was decided to produce **17a** exclusively by Luche reduction of the enone **25** and then epimerize **17a** to **17b** by the Mitsunobu reaction.⁴³ Traditionally, this reaction is performed using diethyl azodicarboxylate (DEAD), but due to security measures, it was difficult to obtain this reagent commercially. Moreover, this exact transformation had been successfully achieved in d'Alarcao's group in the past, by a variation of the Mitsunobu method.⁴³ Instead of DEAD, equimolar amounts of diisopropyl azodicarboxylate (DIAD) was used with equimolar amounts of p-nitrobenzoic acid (PNB) and triphenylphosphine (PPh₃).⁴³

It turned out that the Mitsunobu reaction was extremely water sensitive, and the reaction would not simply move forward if there were any trace of water in the solvent or the solid reagents. Ultimately, the use of commercial anhydrous ether, drying the solid reagents under vacuum in a drying pistol, and increasing the amount of DIAD, PPh₃, and PNB from one to two molar equivalents of the starting material proved to be effective in completely converting **17a** to a p-nitrobenzoyl derivative. However, TLC data showed the presence of a minor product, that eluted closely with the desired compound. Using

preparative TLC, the two products were isolated, and the ^1H NMR spectrum of each (pages 75 and 77) was compared to the references.⁴³ It was confirmed that the two products were nitrobenzoate epimers **26a** and **26b** (7).

Scheme 7. Mitsunobu conversion of **17a** to **26a** and **26b**

The reagents for the reaction are triphenylphosphine (PPh_3), p-nitrobenzoic acid (PNB), and diisopropyl azodicarboxylate (DIAD).

Nitrobenzoate **26a**, the major and the desired product, is produced as the result of $\text{S}_{\text{N}}2$ reaction between the nucleophilic nitrobenzoate anion (the conjugate base of nitrobenzoic acid) and the activated alcohol from **17a**. Considering the allylic position of the alcohol, the formation of epimer **26b** raised a concern over the accessibility of $\text{S}_{\text{N}}2'$ mechanistic pathway. Contrary to $\text{S}_{\text{N}}2$, in $\text{S}_{\text{N}}2'$ both faces of the molecule are open to nucleophilic attack, and depending on which face the nucleophile approaches, in addition to **26b**, the enantiomer of **26a** could also form. As illustrated in Scheme 8, that could compromise the optical purity that has been maintained so far in the synthetic scheme.

Scheme 8. Possible products of S_N2' reaction

In S_N2' pathway, front side attack of the nucleophile results in **26c**. Turning this molecule 120° clockwise makes it visually clear that it is the enantiomer of the desired product **26a**. Back side attack of the nucleophile results in **26d**. Again, 120° clockwise rotation of this structure makes it visually clear that it is the enantiomer of the structure **26b**, the minor product obtained in the Mitsunobu reaction, which could be the result of S_N1 or a double S_N2.

In order to investigate whether the S_N2' pathway is in fact accessible, **26a** and, if present, its enantiomer, **26c**, were separated chromatographically from **26b** and its enantiomer **26d**. The assumed enantiomeric mixture of **26a** and **26c** were hydrolyzed and then derivatized with optically pure (R)-Mosher acid chloride (Scheme 9).⁴⁹ If the material were in fact an enantiomeric mixture, it was expected that the product would be

a diastereomeric mixture of Mosher esters **27a** and **27b**. However, both TLC and NMR data (page 81) confirmed that only one product, **27a**, was obtained from the Mosher ester analysis, therefore **26a** must have been optically pure. Hydrolysis of **26a** gave anti conduritol **17b**, the NMR data of which matched the literature.⁴³

Scheme 9. Hydrolysis and Mosher ester analysis of **26a** and **26c**^{43, 49}

Since it is determined that the Mitsunobu reaction does not undermine the optical purity of the desired intermediate **17b**, Scheme 10 could be adopted as an alternative “workaround” to gain exclusive access to anti conduritol, **17b**, avoiding the inefficient

isolation from mixtures with syn conduritol **17a**. The overall yield for the “workaround” is 45%.

Scheme 10. “Workaround” scheme

3.3 Mesylation and Azide Displacement

Scheme 11 shows the second half of the synthetic scheme. The isolated anti conduritol **17b** was converted to azide **18**, through the intermediacy of its mesylated derivative generated by the reaction with methanesulfonic anhydride, and then the azide displacement was achieved using sodium azide. Similar to the Mitsunobu inversion, the mesylation reaction also resulted in two epimers, **28a** (desired product) and **28b** (Scheme 12). These mesylates are reactive species, therefore they cannot be isolated and characterized though their presence, and the fact that they are epimers of each other, are confirmed since their hydrolysis generates syn and anti conduritols, whereas the reaction had started with the anti epimer only. After numerous experiments, it was discovered that the order of the addition of the reagents affects the ratio of the two products. It is important that triethylamine (TEA), which acts as both the catalyst and the base in this reaction, be added dropwise after methanesulfonic anhydride is already in the reaction mixture. After each drop of TEA, the reaction should be monitored and, once complete, stopped before the emergence of the undesired epimer **28b**, which elutes faster than **28a**

on silica plate.

Scheme 11. Synthetic scheme toward first HIGA, part 2, from **17b** to HIGA **8**

Scheme 12. Mesylation reaction resulting in two epimers, **28a** and **28b**

Desired product

It was also discovered that the use of 4-dimethylaminopyridine (DMAP) improved the efficacy of the reaction and that **28a** formed as the major product.⁵⁰ However, TLC data showed that during the workup some of the product hydrolyzed back to the starting alcohol. To avoid this problem, it was planned to proceed directly to the azide displacement reaction and skip the aqueous workup. The drawback of this approach is the possible formation of two explosive byproducts: mesyl azide and diazidomethane. If extra mesyl anhydride remained from the mesylation step, it could react with the sodium azide in the next step and form mesyl azide.⁵¹ Additionally, if after concentrating the mesylation crude some dichloromethane was left behind, it could also react with the sodium azide in the next step, producing diazidomethane. The chemical shift of the equivalent hydrogens of the diazidomethane is expected to be 4.8 ppm.⁵² NMR data of the crude product of the mesylation reaction did not have any peak with that specific chemical shift, indicating that our reaction conditions were not conducive to the formation of that particular byproduct. Nevertheless, extreme caution was taken at this step and fortunately there was no trouble. Scheme 13 shows the optimized conditions for the two-step transformation from conduritol **17b** to azide **18a**.

Scheme 13. Use of DMAP in the mesylation step to optimize the reaction conditions

3.4 Preparation and Separation of the Diols

Osmium tetroxide catalyzed syn dihydroxylation of azide **18** produced diols **19a** and **19b** (Scheme 11). The reaction took about a week to complete, and more catalyst, osmium tetroxide, and oxidant, N-methylmorpholine N-oxide were added to push the reaction to completion. It is important that both the catalyst and the oxidant be added if necessary, since it has been observed that addition of the catalyst only could result in the formation of a black solid and a dramatically low yield. Separation of **19b**, the desired diol, from **19a** proved to be a challenging endeavor, even by preparative TLC. In order to find an optimum chromatography solvent system, different solvent combinations were tested (Table 1). Ultimately, it was discovered that addition of one drop of ammonium hydroxide per 10 ml of either 1:2 solution of hexane-ether, or neat ether, greatly improves the resolution power of these solvent systems. For best results on the preparative TLC, the diol mixture was once eluted with hexane-ether-NH₄OH system and then with ether (neat)-NH₄OH solution. At this stage the *myo*-inositol core of the HIGAs is prepared.

Table 1. Solvent systems tried for isolation of **19a** and **19b**

Solvent system	Ratio of solvents	Result*
Chloroform : acetonitrile	1 : 1	Not resolved
Chloroform	Neat	No elution
Chloroform : DCM	1 : 2	No elution
Chloroform : Ether	1 : 2	No elution
DCM	Neat	No elution
DCM → Ether	Neat → Neat	Not resolved

Solvent system	Ratio of solvents	Result*
DCM : Ether	1 : 2	Not resolved
DCM : Methanol	4 : 1	Eluted w/ solvent front
DCM : Methanol	10 : 0.2	$R_f = 0.3$, not resolved
Ether	Neat	$R_f = 0.75$, resolved poorly
Ether : Hexane : acetonitrile	3 : 3: 1	$R_f = 0.5$, not resolved
Ether : NH_4OH	Neat : 1 drop	$R_f = 0.5$, $\Delta R_f = 0.1$
Hexane : Ether	1 : 2.5	$R_f = 0.5$, poor resolution
Hexane : Ethyl acetate	1 : 2	R_f too high, not resolved
Hexane : Ether : NH_4OH	1 : 2 : 1 drop	$R_f = 0.3$, $\Delta R_f = 0.1$
Toluene	Neat	No elution
Dioxane	Neat	Not resolved

* The reported R_f is for **19b**, which elutes faster than **19a** on the TLC plate.

3.5 The Click Reaction

Before phosphorylating the inositol core (Scheme 11), the thiol linker is attached via copper (I) catalyzed alkyne-azide cycloaddition, the so-called “click reaction”.⁵³ The linker was made from commercially available reagents, α -toluene thiol and propargyl bromide (Scheme 14).⁵⁴ Although it was reported previously that the product does not stain with p-anisaldehyde,⁴¹ in our hands, a white spot was observed. Depending on the solvent system, especially if methanol is present, the product may stain light green. NMR analysis revealed that the green color of the stain is indicative of the co-elution of an impurity. A solution of 6 to 1 ether-hexane proved to be a good solvent system for isolating the pure triazole **21** (Scheme 11). Uncharacteristic of the click chemistry, the

reaction between **19b** and **20** produced many side products, and the isolated yield was never more than 50%.

3.6 Phosphorylation of the Model Diol **31** and Triazole **21**

The phosphorylation reaction that converts diol **21** to phosphate **22** needs freshly prepared N-methylpyridinium dichlorophosphate which is provided from anhydrous pyridine and commercially available methyl dichlorophosphate (MeOPOCl₂) (Scheme 15).⁵⁵ It has been shown by others that it is prudent to evaluate the efficacy of this reagent in creating a cyclic phosphate on a model compound, before letting it react with the precious synthetic intermediate.⁵⁶ In order to do so, the model diol **31** (Scheme 16) was prepared from *myo*-inositol following a previously reported synthesis.⁵⁷ First, two of the three vicinal cis diols of *myo*-inositol were protected as a cyclohexylidene. Although the literature reported 93% yield for this step, in our hands, it resulted in multiple side products that adversely affected the yield (2%). The next step was the benzylation of the remaining hydroxyl groups. During the workup of this step, the presence of bromine was detected by the yellow color of the ethyl acetate solution. Therefore, 1% aqueous sodium thiosulfate was used to reduce the bromine before the workup was completed. Acidic

hydrolysis of the cyclohexylidene proceeded smoothly, and an adequately pure diol was obtained after the first crystallization.

Scheme 15. Preparation of the phosphorylating reagent, N-methylpyridinium dichlorophosphate

Scheme 16. Synthesis of the model diol **31**

During the preparation of the phosphorylating reagent (Scheme 15), it was expected that white turbidity would develop within a half hour of mixing the reactants.⁵⁸ If this was not observed, the reaction was discarded and the quality of the reactants were examined. The phosphorylation reaction was then undertaken by addition of the

phosphorylating reagent to the solution of the model diol **31** in anhydrous pyridine (Scheme 17). Since pyridine hindered TLC-monitoring, the reaction was instead monitored by mass spectrometry. When the mass of the starting material disappeared from the spectrum, the reaction was considered complete. Significantly, it was noticed that the successful formation of the cyclic phosphate, as confirmed by ^{31}P NMR, and hence the potency of the phosphorylating reagent could be judged by a mass signature: the presence of $m/z = 682.25$ and 620.24 and the absence of $m/z = 621.22$. The calculated exact mass of the phosphorylated model diol is 602.21 .

Scheme 17. Synthesis of the model phosphate **32**

Two different workup procedures were attempted for the phosphorylation reaction. In the first one,⁵⁶ established by this research group, the reaction was simultaneously quenched and neutralized by the addition of saturated sodium bicarbonate. Then the solvents were co-evaporated with heptane. The residue was dissolved in water, carefully adjusted to pH=1, and extracted with ethyl acetate. This step is time consuming, but it guarantees the protonation of the phosphate group, and thus promotes the solubility of **32** in organic solvent. The other workup method is much

simpler and faster.⁵⁸ It calls for 25 times dilution of the reaction mixture with dichloromethane and 3 times wash with brine. However, after trying both methods and realizing that silica gel chromatography of **32** is not possible because of extremely low yields, the first approach was favored since it resulted in a cleaner product. It should be noted though, that as indicated by ³¹P NMR, the cyclic phosphate group survived both workup procedures without hydrolyzing to an acyclic one.

After it was determined how to confirm the potency of the phosphorylating reagent and to monitor the reaction using mass spectrometry, the phosphorylation reaction was carried out on the triazole **21**. The phosphorylating reagent was first used on the model diol, and, after the mass signature was observed, it was added to the pyridine solution of **21**. Mass spectrometry showed the presence of the cyclic phosphate **22** (Scheme 11) and consumption of all the starting material.

3.7 Deprotection of the Model Phosphate **32**

The first time deprotection was tried on compound **22**, using dissolving-metal reduction, the reaction failed. No trace of the starting material or the product was detected even by mass spectrometry. Hydrogenolysis has been employed before in this laboratory to deprotect the benzyl groups of a disaccharide.⁵⁶ It was observed that the presence of the amine group on the benzylated disaccharide interfered with accessing the deprotected compound after hydrogenolysis, so the deprotection was achieved by dissolving-metal reduction using liquid ammonia and sodium. Nonetheless, it was planned to try hydrogenolysis on **22** hoping that the triazole would not behave like an

amine. However, literature research led us to another example in which the triazole moiety deactivated the palladium catalyst and prevented the hydrogenolysis of the benzyl groups.⁵⁹ Therefore, it was decided to stay with the dissolving-metal reduction as the deprotection method but to optimize it on the model compound **32** prior to deprotecting the synthetic intermediate.

In the literature procedure that was followed, solid ammonium chloride was added to quench the reaction, and, when the blue color of the solvated electrons disappeared, methanol was poured in. However, in our hands, the reduction was successful when the workup was changed as follows: after the addition of ammonium chloride, the reaction was let to stir overnight, allowing liquid ammonia to evaporate without adding methanol. The solids that were left behind were dissolved in methanol the next day and concentrated. The residue was desalted using Sephadex G10. ³¹P NMR and mass spectrometry confirmed the presence of salt free phosphorylated inositol **33** (Scheme 18).

Scheme 18. Deprotection of the model phosphate **32**

CHAPTER 4

CONCLUSION AND FUTURE DIRECTIONS

The challenges of each step of the synthesis, from the mixture of conduritols to the global deprotection, were successfully overcome. The inefficient isolation of the anti conduritol from its mixture with the syn epimer was addressed by designing a “workaround” scheme in which the mixture of syn and anti conduritols was oxidized to an enone. The syn conduritol was then exclusively obtained by Luche reduction of the enone, and the anti conduritol was afforded by Mitsunobu inversion of the syn epimer. Enantiomeric analysis of the Mitsunobu intermediates suggested strongly that the route to S_N2' is inaccessible and the optical purity of the product is maintained by the “workaround” scheme. The conditions of the mesylation reaction were optimized so as to obtain the desired product in considerable preponderance. A drop of ammonium hydroxide was all that was needed to separate the diols by preparative TLC, and leaving methanol out of the solvent system was found to be the key to a pure click product. An unexpected but consistently observed mass predicted the efficacy of the phosphorylating reagent in creating cyclic phosphates, and reevaluating the established workup procedure made the deprotection with dissolving-metal reduction a success.

Toward the synthesis of HIGA, the synthetic phosphate will be deprotected following the newly found method, and, at the last step, the exposed thiol function of the linker will be reacted with the non-carbohydrate fluorophore, IAEDAN, to produce the first HIGA. It might be prudent to determine if the triazole function present in the linker

survives the deprotection method, maybe by designing a model triazole. The reaction of the thiol group with IAEDAN is expected to progress smoothly according to the established protocols. In the future, the synthetic scheme could be repeated to prepare other HIGAs containing different non-carbohydrate moieties. The HIGAs will be assayed for insulinomimetic biological activity in insulin-sensitive cells by comparing the amount of glucose uptake in the presence and the absence of HIGAs.

CHAPTER 5

EXPERIMENTAL PROCEDURES

Reagents and solvents purchased from commercial sources (Fisher Scientific, Sigma Aldrich, or Life Technologies) were used without further purification with the following exceptions: methylene chloride was distilled from calcium hydride. Pyridine and triethylamine were distilled from barium oxide. Tetrahydrofuran (THF) and diethylether were distilled from sodium benzophenone ketyl. Reactions that required anhydrous conditions were performed under argon or nitrogen atmosphere with prior drying of hygroscopic solids by azeotropic co-evaporation with toluene or ethyl acetate.

The progress of the reactions was monitored by thin layer chromatography (TLC) on SiliaPlate glass backed silica gel plates (0.25 mm thickness) with a 254-nm fluorescent indicator. Visualization was achieved either by ultraviolet illumination or by staining the plates with ethanolic solution of p-anisaldehyde (2.5% p-anisaldehyde, 3.5% sulfuric acid, and 1% acetic acid) and then baking on a hot plate. Organic extracts collected during workups were dried over MgSO_4 and concentrated *in vacuo* on a rotary evaporator. Purifications were carried out by flash chromatography on SiliaFlash silica gel (40-63 μm), preparative TLC, or gel filtration using Sephadex G-10. Nuclear magnetic resonance (NMR) data were gathered using Inova-400 MHz NMR spectrometer and were reported in parts per million (δ) relative to internal standard tetramethylsilane (TMS) for ^1H , ^{13}C , or external standard H_3PO_4 (85% in D_2O) for ^{31}P . Mass spectrometry data were obtained on Agilent 6520 Quadrupole Time-of-Flight LC/MS mass spectrometer using electrospray as the ionization method (ESI-MS).

(2*R*,3*R*,4*S*)-2,3,4-Tris(benzyloxy)hex-5-en-1-ol (14).⁴⁸

This reaction was performed under extremely anhydrous conditions. All glassware and needles were flame-dried. THF was freshly distilled from a sodium benzophenone ketyl solution. Methyltriphenylphosphonium bromide was dried in a drying pistol in the presence of phosphorus pentoxide and heated in hot water bath for 3 days. Xylose **13** was azeotroped with toluene three times. A three-neck flask was setup to accommodate a dropping funnel, an argon inlet, and a rubber septum to be used for injecting the reagents. To the suspension of dried methyltriphenylphosphonium bromide (13.13 g, 36.74 mmol) in anhydrous THF, in the three-neck flask, was added butyl lithium (21.77 mL of 1.6 M hexane solution) dropwise, at 0 °C. The color of the reaction mixture was expected to turn orange-red upon formation of phosphonium ylide. More equivalents of butyl lithium were prepared to be added in case the reaction wouldn't go to completion. After the red color was maintained for 2 h, the solution of **13** (5.3 g) in THF (42 ml) was added to the phosphonium ylide solution using the dropping funnel. The reaction was stirred over night at room temperature and refluxed for 2 h after TLC (hexanes-EtOAc, 7:3) indicated that the reaction was complete. The reaction was diluted with NH₄Cl (60 ml, 1 M) and water (100 mL) and extracted with ether twice. Combined organic layers were

washed with brine, dried over MgSO_4 , and concentrated. The crude was quickly filtered through a 4" long silica plug using 15% and then 30% ethyl acetate in hexane to afford 2.8 g of pure **14** in 54% yield. ^1H NMR (CDCl_3): δ = 7.37 – 7.24 (m, 15H), 5.88 (ddd, J = 17.7, 10.5, 7.4 Hz, 1H), 5.33 – 5.28 (m, 2H), 4.74 (s, 2H), 4.63(d, J = 12.0 Hz, 1H), 4.61 (s, 2H), 4.37 (d, J = 12.0 Hz, 1H), 4.10 (m, 1H), 3.73 – 3.48 (m, 4H), 1.97 (br s, 1 H).

The Wittig product

exp3 s2pu1

SAMPLE		SPECIAL	
date	Feb 16 2012	temp	not used
solvent	CDCl3	gain	not used
file	/export/home/~sp1	20	
ssabet/SSI-76-4.f4		0.000	
acq		14.000	
acq		6.600	
ACQUISITION		FLAGS	
sw	6395.9	at	3.744 11
np	47892	in	4.000 11
bs	16	hs	1.000 16
nt	1.000	fn	not used
ct	16	sp	367.9
tn	H1	wp	3606.9
sfrq	399.746	rf1	799.5
tof	399.5	rfp	91.0
tpwr	7.000	lp	-30.0
pc	DECOUPLER	C13	WC 200
dn	0	SC	25
dm	0	nmr	1.5
dpr	36	nm	cdc ph
dar	17054

14

exp3 s2pu1

SAMPLE	date	solvent	port	CDCl3	gain	temp	SPECIAL
1	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
2	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
3	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
4	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
5	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
6	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
7	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
8	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
9	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
10	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
11	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
12	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
13	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
14	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
15	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
16	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
17	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
18	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
19	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
20	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
21	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
22	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
23	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
24	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
25	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
26	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
27	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
28	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
29	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
30	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
31	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
32	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
33	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
34	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
35	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
36	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
37	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
38	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
39	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
40	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
41	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
42	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
43	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
44	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
45	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
46	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
47	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
48	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
49	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
50	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
51	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
52	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
53	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
54	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
55	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
56	Feb 16 2012	CH2Cl2	1	0.00	0.00	not used	
57	Feb 16 2012	CH2Cl2	1	0.00	0.00

(2*S*,3*R*,4*S*)-2,3,4-Tris(benzyloxy)hex-5-enal (15).⁴⁸

All glassware and needles were flame-dried and kept under argon till used. To a dry flask was transferred anhydrous methylene chloride (30 mL) and cooled to -78 °C. Oxalyl chloride (2.13 mL, 0.0249 mol, 98% pure) and DMSO (3.53 mL, 0.0498 mol) was syringed in, dropwise. Evolution of gases was observed and the reaction mixture turned cream in color. Stirring continued for another 15 min. Alcohol **14** (5.2 g, 0.0124 mol) dissolved in 30 mL of dry methylene chloride was added dropwise into the reaction mixture, at -78 °C, and stirred for 30 min, after which time triethylamine (13.8 mL) was added dropwise. The cloudy reaction mixture was stirred for another 5 min and then was let to warm to room temperature. After TLC (hexanes-EtOAc, 7:3) indicated the completion of the reaction, it was diluted with water and extracted with methylene chloride. The organic layer was washed with 1 M NH₄Cl, water, then brine, and dried over MgSO₄. Since TLC indicated a pure product the crude was kept frozen in benzene to be used in the next reaction without any purification. ¹H NMR spectrum was not obtained.

(3*S*,4*R*,5*R*,6*S*)-4,5,6-Tris(benzyloxy)octa-1,7-dien-3-ol (16a) and (3*R*,4*R*,5*R*,6*S*)-4,5,6-tris(benzyloxy)octa-1,7-dien-3-ol (16b).⁴⁸

To a flame-dried three-neck flask was attached a mechanical stirrer, a condenser, and a rubber septum. Finely cut magnesium ribbons (3.2 g, 0.130 mol) were dropped into the flask and freshly distilled THF (65 mL) was syringed in. The flask was lowered in an ice bath, and after 5 min, vinyl bromide (15 mL) was quickly poured in. To initiate the reaction, the flask was removed from the ice bath, but was returned to it as the progression of the reaction generated heat and gas. When no change was observed after about an hour the reaction was let to run at room temperature until a deep dark green color was observed and magnesium ribbon cuts were consumed. To the prepared 2M vinylmagnesium bromide, cooled to -78 °C, was added methylene chloride (300 mL) and then the solution of aldehyde **15** (5.4 g) in methylene chloride (15 mL). The reaction was complete, after 4 h, as indicated by TLC (hexanes-EtOAc, 8:2), quenched with methanol, and brought to room temperature. It was then washed with water, 1 M NH₄Cl, again water, and brine, dried over MgSO₄ and concentrated. The crude was purified by flash chromatography over silica gel, eluting with (hexane-EtOAc, 85:15) to afford 3.03 g of

the inseparable mixture of **16a** and **16b** as a yellowish oil (52%). ¹H NMR of the mixture was identical to what was previously reported.⁴⁸

The original product

exp3 s2pu1

SAMPLE		SPECIAL	
date	Feb 27 2012	temp	not used
solvent	CDCl3	gain	not used
file	/export/home/~spin	spin	20
ssebet/SSI-79-3.f4~	hst	0.000	
pw90	14.000		
acq	6.600		
sw	6395.9	delta	6.600
at	3.744	11	
np	47892	1n	n
fb	4000	dp	y
rs	1.000	hs	
nt	1.000	fn	not used
ct	B	sp	not used
tr	399.746	rf1	399.746
sfreq	399.746	rf1	399.746
tof	399.5	rfp	0
tpwr	58	rp	60.3
pw	7.000	lp	13.9
de	DECOUPLER	g13	Wc
dof	0	sc	200
dm	nnn	vs	25
dpr	c	th	130
ph	3.0	nm	5
der	17084	cdc	ph

(1*S*,4*S*,5*R*,6*R*)-4,5,6-Tris(benzyloxy)cyclohex-2-en-1-ol (17a) and

(1*R*,4*S*,5*R*,6*R*)-4,5,6-Tris(benzyloxy)cyclohex-2-en-1-ol (17b).⁴³

To a stirred solution of **16a** and **16b** (3.03 g, 6.82 mmol) in methylene chloride was added first generation Grubbs' catalyst (0.590 g, 7.18 mmol), at room temperature and under argon atmosphere, to produce a deep dark purple color. The reaction was complete in 24 h as indicated by TLC (hexane-EtOAc, 7:3) and concentrated. After four iterations of flash chromatography (silica gel, hexanes-ether, 1:1) **17a** was obtained as a white flaky solid (1.3 g) and **17b** was afforded as a dark, brown oil (470 mg), with total yield of 68%.

For **17a**, ¹H NMR (CDCl₃): δ = 7.25 - 7.36 (m, 15H), 5.70 (m, 2H), 5.03 (d, *J* = 11.3 Hz, 1H), 4.92 - 4.25 (m, 5H), 4.32 - 4.25 (m, 2H), 3.79 (dd, *J* = 7.4, 10.2 Hz, 1H), 3.53 (dd, *J* = 8.0, 10.1 Hz, 1H), 2.22 (d, *J* = 3.9 Hz, 1H).

For **17b** ¹H NMR (CDCl₃): δ = 7.35 - 7.25 (m, 15H), 5.88 (d, *J* = 1.9 Hz, 2H), 4.94 - 4.66 (m, 6H), 4.29 (m, 1H), 4.10 (d, *J* = 7.4 Hz, 1H), 4.50 (dd, *J* = 7.2, 9.7 Hz, 1H), 3.56 (dd, *J* = 4.1, 9.7 Hz, 1H), 2.71 (d, *J* = 2.5 Hz, 1H).

The Grubbs products: syn and anti condur
tols

exp3 s2pu1

SAMPLE		SPECIAL	
date	Jul 17 2013	temp	not used
solvent	CDCl3	gain	not used
file	/export/home/~spn	spin	20
scat	/SSII-31-5	scat	0.000
acq	12.600	pe90	12.600
acq	6.600	al1a	6.600
ACQUISITION			
sw	4796.7	al1a	6.600
at	3.744	al1a	6.600
fd	35500	al1a	6.600
bs	3000	dp	16
nt	1.000	fn	not used
PROCESSING			
ct	TRANSMITTER	sp	DISPLAY
tn	399.741	wp	3997.3
sfrq	399.741	rfl	414.0
tor	-0.3	rfa	155.0
pw	6.300	lp	-16.0
DECOUPLER			
dn	C13	mc	200
dof	0	sc	25
dms	nmr	th	8
dpr	39	at	5
def	29412	cdc	ph

The Gr

exp3 s2pu1

SAMPLE	date	July 17 2013	temp	SPECIAL
solvent	CDCl3	gain	not used	
1/expo/home		0.008	not used	
assay/3511-212	pu30	12.600		
ACQUISITION	alpha	6.600		
pw	4795.7	11	FLDS	n
pc	3591.8	11		n
pp	3000	dp		y
ps	16	fs	PROCESSING	nn
pt	1.000	fn	not used	nn
ct	8		DISPLAY	
transmitter	sp	0.0		
freq	399.741	Hz		314.0
tof	-0.3	r/p		414.0
temp	57	r/p		155.8
pw	6.300	11	PLOT	-16.9
decoupler	C13	wc		200
scf	0	sc		25
am	nm	vs		60
am	nm	vs		80
ddpr	39	at	cdc ph	5
	28412

Pure anti1 condurito1

exp3 s2pul

SAMPLE		SPECIAL	
date	Dec 7 2011	temp	not used
solvent	CDCl3	gain	not used
file	/export/Amc/	hst	0.008
ssabef/SST-65-05	hst	pead0	14.000
acq	6.000	afre	6.000
ACQUISITION			
sw	6395.9	fl	11
ns	37240	in	n
fb	47092	dp	y
bs	4000	hs	nn
dl	1.000	fn	nn
nt	32	fr	nn
ct	32	fn	nn
PROCESSING			
tn	395.746	rf1	3957.2
sfreq	395.746	rf1	799.5
tofr	395.5	rfp	81.6
pr	7.000	lp	-10.5
PLOT			
dn	200	wc	200
dsf	130	vc	130
dsm	130	vs	130
dpcr	36	th	5
daf	17094	cdc	ph

17b

Pure ent1 condurito1

exp3 s2pul

SAMPLE		SPECIAL	
date	Dec 7 2011	temp	not used
solvent	CDCl3	gain	not used
time	0.008	phase	0.008
ssbref	SS1-01	ht	14.000
acq	1d	pw90	6.600
ACQUISITION		fla	FLAUS
sw	6395.9	fl	1
at	3776.6	in	n
fb	4769.2	dp	y
bs	16	hs	mn
dl	1.000	fn	not used
nt	32	display	-0.2
ct	TRANSMITTER	32	SP
tn	H1	wp	3197.8
sfreq	395.746	rf1	799.5
tof	395.5	rfp	81.6
tdr	7.000	lp	-10.5
pw	7.000	lp	-10.5
DECOUPLER		C13	WC
dn	200	sc	25
dof	8	sc	135
dsb	nmc	th	5
dsm	36	nm	cdc
dpur	17094	ph	
dsf

Pure syn conduritol

exp3 s2pu1

date	Apr 11 2012	temp	not used
solvent	CDCl3	gain	not used
file	/export/home/~	spin	20
ssadet/SSI-81-4pur~		0.000	
ACQUISITION	14.000	14.000	
SW	6395.9	11	n
at	3.744	11	n
np	47892	11	y
bs	16	hs	nn
d1	1.000	fn	not used
nt	64	fn	not used
ct	54	sp	DISPLAY
tn	H1	wp	-0.2
sfrq	399.746	rfl	3997.2
tof	399.5	rfl	799.5
tpur	1.58	tp	68.1
pw	7.000	tp	7.2
DECOUPLER	C13	WC	200
dn	0	SC	25
dof	nm	VS	150
dm	36	nm	cdc
dpur	17094	ph	
def

Pure syn conduritol

exp3 szpul
 date Apr 11 2012 temp not used
 file report/none/~ hst
 ssabes/SSI-41-40ur/~ hst
 6.11d pw90 14.000
 6.600
 ACQUISITION
 635.9 11
 4782 1n n
 4000 dp y
 16 hs
 1.000 fn
 64
 64
 TRANSMITTER H1 wp -0.2
 399.746 3197.8
 399.746 799.5
 399.746 799.5
 58 rp 68.1
 7.000 lp 7.2
 DECOUPLER C13 wc 200
 130
 130
 36 nm cdc ph 5
 17034

(4*S*,5*R*,6*S*)-4,5,6-Tris(benzyloxy)cyclohex-2-en-1-one (25).⁴⁴

To **17a** and **17b** (42.5 mg, 0.10 mmol) dissolved in CH₂Cl₂ (0.6 mL) were added NaHCO₃ (42 mg, 0.50 mmol) and Dess-Martin periodinane (86.5 mg, 0.20 mmol) at room temperature. After 40 min. sat. aq. Na₂S₂O₃ was added and the reaction stirred vigorously until it turned clear. The reaction was partitioned between CH₂Cl₂ and sat. aq. NaHCO₃, the layers were separated, and the aqueous layer was extracted once with 20 mL CH₂Cl₂. The combined organic layers were washed with brine, dried over MgSO₄ and concentrated to give clear oil. Purification over silica gel (hexanes-EtOAc, 8:2) afforded a light yellowish viscous oil (39.2 mg, 94%). ¹H NMR (CDCl₃): δ = 7.46 - 7.30 (m, 15H), 6.82 (dd, *J* = 2.0, 10.2 Hz, 1H), 6.05 (dd, *J* = 2.4, 10.4 Hz, 1H), 5.10 (d, *J* = 11.2 Hz, 1H), 4.98 (d, *J* = 11.2 Hz, 1H), 4.79 (m, 4H), 4.37 (dd, *J* = 2.4, 8.0 Hz, 1H), 4.02 (m, 2H).

STANDARD 1H OBSERVE

exp3 s2pu1

SAMPLE		SPECIAL	
date	Sep 30 2010	temp	not used
solvent	CDCl3	spin	not used
file	/export/home/~	sp1n	20
vtarku	/SSI-002-4.~	hst	0.000
acq	14.700	pd90	14.700
sw	6395.9	af6	6.600
at	3.744	l1	
np	47692	in	n
fb	4000	dp	y
d3	1.000	hs	PROCESSING
nt	8	fn	not used
ct	8	fn	DISPLAY
tn	TRANSMITTER	sp	-0.9
fr	4000.4	sp1	4000.4
sf	399.746	rf1	739.0
tof	339.5	rfp	-107.8
tpwr	58	rfp	-31.6
pw	7.350	lp	
dn	DECOUPLER	cl3	200
dof	0	sc	25
dm	nm	vs	133
dpr	c	th	20
dev	17004	nm	cdc ph

25

(1*S*,4*S*,5*R*,6*R*)-4,5,6-Tris(benzyloxy)cyclohex-2-en-1-ol (17a).⁴⁴

Enone **25** (80 mg, 0.193 mmol) was taken up in methanol (3.21 ml) at room temperature and stirred until it was completely dissolved. The reaction flask was submerged into an acetone/dry ice bath for 10 minutes after which time $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$ (93.5 mg, 0.25 mmol) and sodium borohydride (18.1 mg, 0.48 mmol) were added. To help start the reaction, the flask was removed from the $-78\text{ }^\circ\text{C}$ bath. TLC (ethyl acetate:hexane, 4:6) indicated the completion of the reaction after 1.5 h. The reaction was terminated and worked up as follows: Separated between ethyl acetate and water, the aqueous layer was extracted with ethyl acetate, and combined organic layers were dried over MgSO_4 . After purification (silica gel, hexane-ether, 1:1) 38.6 mg of syn conduritol **17a** was obtained (48%). The ^1H NMR spectrum of the product matched what was previously reported.⁴³

exp3 s2pu1

17a

17b

(1R,4S,5R,6R)-4,5,6-Tris(benzyloxy)cyclohex-2-en-1-yl 4-nitrobenzoate (26a) and (1S,4S,5R,6R)-4,5,6-Tris(benzyloxy)cyclohex-2-en-1-yl 4-nitrobenzoate (26b).⁴³

This reaction is extremely water sensitive. Triphenylphosphane (PPh₃) and *p*-nitrobenzoic acid (PNB) were dried in drying pistol for 24 h. The starting material **17a** (9 mg, 0.0220 mmol), was azeotroped with ethyl acetate three times. PPh₃ (12 mg, 0.0440 mmol) and PNB (7.2 mg, 0.044 mmol) were added to the starting material, and the content was azeotroped one last time. Commercially obtained anhydrous ether (0.38 mL, 0.058 M) was added at room temperature. After most of the material had dissolved and no more change was observed, 2 equivalents of diisopropyl azodicarboxylate (DIAD) (8.72 μ L, 0.0440 mmol) was syringed into the mixture. The reaction occurred violently and instantaneously with disappearance of all of the starting material. The reaction mixture was concentrated under reduced pressure and the major and the minor products were isolated by preparative TLC (hexanes-ether, 8:2) with 72% yield for the major product. For **26a**: ¹H NMR (CDCl₃): δ = 8.28 (d, *J* = 8.5 Hz, 2H), 8.18 (d, *J* = 8.5, 2H), 7.36 - 7.23 (m, 15H), 6.04 - 5.87 (m, 3H), 4.99 - 4.67 (m, 6H), 4.16 - 4.04 (m, 2H), 3.73

(dd, $J = 3.0, 9.9$ Hz, 1H).

For **26b**: ^1H NMR (CDCl_3): δ = 8.24 (d, $J = 8.8$ Hz, 2H), 8.02 (d, $J = 8.8$ Hz, 2H), 7.36 - 7.10 (m, 15H), 5.83 (m, 2H), 5.64 (d, $J = 10.5$ Hz, 1H), 4.98–4.67 (m, 6H), 4.30 (dd, $J = 2.8, 4.7$ Hz, 1H), 3.93 - 3.85 (m, 2H).

The major product of the Mitsunobu reaction

exp3 52pu1

SAMPLE		SPECIAL	
date	Sep 27 2012	temp	not used
solvent	CDCl3	gain	not used
report	hst	hst	20
ssabst	viarsys/dat~	hst	0.008
a/551-95-5.fid	pw90	hst	13.700
ACQUISITION	6.600	FLAUS	
sw	6395.8	11	
sp	3161.1	11	
np	47892	1n	
bs	4800	dp	
fb	16	hs	
dl	1.000	fn	
ct	256	fn	
ct	256	fn	
TRANSMITTER	256	sp	
tn	h1	wp	
sfreq	399.741	rf1	
toar	399.56	rfp	
pw	6.850	lp	
DECOUPLER	CL3	mc	
dn	nnn	vc	
dof	nnn	vc	
dsm	c	th	
dpur	39	nm	
daf	29.412	cdc	
		ph

26a Major product

The minor product of the Mitsunobu reaction

exp3 s2pu1

SAMPLE		SPECIAL	
date	sep 27 2012	temp	not used
solvent	CDCl3	gain	not used
file	/export/home/~sp1n	sp1n	20
ssabel/vmr-sys/dat	~hst	0.008	
a/SSI-95-50.fid	pu90	13.700	
ACQUISITION	6395.9	delta	6.600
at	3.744	11	FLAGS
np	47892	1n	n
fb	4000	dp	n
ts	6	hs	y
dt	1.000	fn	not used
ct	64	fn	not used
TRANSMITTER		DISPLAY	
ts	390.741	sp	0.2
sfreq	390.741	wp	390.7
tof	395.4	rfp	789.5
tpur	56	rp	0
pw	6.850	lp	-29.2
dn	DECOUPLER	cl3	WC
dof	0	sc	200
dm	nnn	vs	25
dpr	c	th	140
drf	29412	nm	5
		cdc	ph

26b Minor product

The minor product of the Mitsunobu react

```

exp3  szpu1
=====
date  Sep 27 2012  temp  not used
file  /export/home/~ spn  not used
file  /export/home/~ spn  not used
ssabet/vnarys/dat~ hst  0.000
a/551-95-5b.fid  pedu  13.700
ACQUISITION  a1fa  6.600
=====
at  3.744  11  n
np  47892  1n  n
fb  4000  dp  y
bs  16  hs  n
nt  1.000  not used
ct  64  fm  not used
=====
TRANSMITTER  64  DISPLAY
tn  882.2
f1  399.241
f2  399.241
f3  399.241
f4  399.241
f5  399.241
f6  399.241
f7  399.241
f8  399.241
f9  399.241
f10  399.241
f11  399.241
f12  399.241
f13  399.241
f14  399.241
f15  399.241
f16  399.241
f17  399.241
f18  399.241
f19  399.241
f20  399.241
f21  399.241
f22  399.241
f23  399.241
f24  399.241
f25  399.241
f26  399.241
f27  399.241
f28  399.241
f29  399.241
f30  399.241
f31  399.241
f32  399.241
f33  399.241
f34  399.241
f35  399.241
f36  399.241
f37  399.241
f38  399.241
f39  399.241
f40  399.241
f41  399.241
f42  399.241
f43  399.241
f44  399.241
f45  399.241
f46  399.241
f47  399.241
f48  399.241
f49  399.241
f50  399.241
f51  399.241
f52  399.241
f53  399.241
f54  399.241
f55  399.241
f56  399.241
f57  399.241
f58  399.241
f59  399.241
f60  399.241
f61  399.241
f62  399.241
f63  399.241
f64  399.241
f65  399.241
f66  399.241
f67  399.241
f68  399.241
f69  399.241
f70  399.241
f71  399.241
f72  399.241
f73  399.241
f74  399.241
f75  399.241
f76  399.241
f77  399.241
f78  399.241
f79  399.241
f80  399.241
f81  399.241
f82  399.241
f83  399.241
f84  399.241
f85  399.241
f86  399.241
f87  399.241
f88  399.241
f89  399.241
f90  399.241
f91  399.241
f92  399.241
f93  399.241
f94  399.241
f95  399.241
f96  399.241
f97  399.241
f98  399.241
f99  399.241
f100  399.241
=====
DECOUPLER  C13  Wc  200
dn  0  sc  25
dof  0  vs  400
da  39  th  5
dpcr  c  th  5
dat  29412
=====

```

26b Minor product

(1*R*,4*S*,5*R*,6*R*)-4,5,6-Tris(benzyloxy)cyclohex-2-en-1-ol (17b).⁴³

To a stirred solution of **26a** (4 mg, 0.0071 mmol) in THF (0.1 mL, 0.07M), was added aq. 1 M LiOH (0.1 mL). The reaction mixture was stirred overnight at room temperature. After that time TLC (EtOAc-hexanes, 4:6) indicated the completion of the reaction. The solvent was evaporated and 2.3 mg of anti conduritol **17b** was isolated by preparative TLC (EtOAc-hexanes, 4:6). (77%) The ¹H NMR data of this product matched what was previously reported (page 63 of this document).

(S)-(1R,4S,5R,6R)-4,5,6-Tris(benzyloxy)cyclohex-2-en-1-yl 3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (27a).⁴⁹

To a solution of **17b** (2.3 mg, 0.0055 mmol, obtained from Mitsunobu conversion of 17a), Et₃N (2.3 μ L, 0.017 mmol, 3 eq), and DMAP (~ 1 mg, small crystal) in CHCl₃ was added 37 μ L of 0.178M solution of (R)-Mosher acid chloride (0.0066 mmol) in benzene. Since after 2 days the reaction did not go to completion 8 more equivalents of the Mosher acid chloride solution and 3 more equivalents of Et₃N was added to the reaction mixture. After TLC (EtOAc-hexanes, 2:8) indicated the consumption of all starting material, the solvents were evaporated under reduced pressure and the residue was let to stir with 3 drops of water over night. The reaction mixture was diluted with CHCl₃ and washed with saturated sodium bicarbonate (2x) and 0.1M HCl (2x). The organic layer was concentrated to produce **27a**. ¹H NMR (CDCl₃): δ = 7.52 (d, *J* = 8.4 Hz, 2H), 7.27 - 7.13 (m, 18H), 5.88 (dd, *J* = 2.4, 9.8 Hz, 1H), 5.83 (dd, *J* = 4.0, 4.0 Hz, 1H), 5.68 (m, 1H), 4.83 (d, *J* = 10.8 Hz, 1H), 4.73 (d, *J* = 11.2 Hz, 1H), 4.66 (d, *J* = 9.2 Hz, 1H) 4.64 (d, 9.6

Hz, 1H), 4.57 (d, $J = 11.2$ Hz, 1H), 4.49 (d, $J = 11.6$ Hz, 1H), 4.05 (d, $J = 8.0$ Hz, 1H),
3.87 (dd, $J = 8.0, 10.8$ Hz, 1H), 3.62 (dd, $J = 4, 10.8$ Hz, 1H), 3.46 (s, 3H).

^{19}F NMR (CDCl_3): $\delta = -71.0, -71.8$.

Enantioselective analysis of the anti condur
 101 obtained from Mitsunobu reaction
 exp3 s2p1

date	Oct 23 2012	temp	not used
time	06:03	spin	not used
file	/export/home/~	spin	20
ssabet/SSII-1-3HC1~	hst	0.008	
.fid	pw30	13.700	
ACQUISITION	6.000	6.000	
sw	605.9	11	FLAGS
at	3.744	11	
np	47892	in	n
fb	4000	dp	y
bs	16	hs	nn
nt	1.002	fn	not used
ct	32	fn	not used
tn	32	fn	not used
h1	399.2	wp	399.2
h2	399.2	wp	399.2
h3	399.2	wp	399.2
h4	399.2	wp	399.2
h5	399.2	wp	399.2
h6	399.2	wp	399.2
h7	399.2	wp	399.2
h8	399.2	wp	399.2
h9	399.2	wp	399.2
h10	399.2	wp	399.2
h11	399.2	wp	399.2
h12	399.2	wp	399.2
h13	399.2	wp	399.2
h14	399.2	wp	399.2
h15	399.2	wp	399.2
h16	399.2	wp	399.2
h17	399.2	wp	399.2
h18	399.2	wp	399.2
h19	399.2	wp	399.2
h20	399.2	wp	399.2
h21	399.2	wp	399.2
h22	399.2	wp	399.2
h23	399.2	wp	399.2
h24	399.2	wp	399.2
h25	399.2	wp	399.2
h26	399.2	wp	399.2
h27	399.2	wp	399.2
h28	399.2	wp	399.2
h29	399.2	wp	399.2
h30	399.2	wp	399.2
h31	399.2	wp	399.2
h32	399.2	wp	399.2
h33	399.2	wp	399.2
h34	399.2	wp	399.2
h35	399.2	wp	399.2
h36	399.2	wp	399.2
h37	399.2	wp	399.2
h38	399.2	wp	399.2
h39	399.2	wp	399.2
h40	399.2	wp	399.2
h41	399.2	wp	399.2
h42	399.2	wp	399.2
h43	399.2	wp	399.2
h44	399.2	wp	399.2
h45	399.2	wp	399.2
h46	399.2	wp	399.2
h47	399.2	wp	399.2
h48	399.2	wp	399.2
h49	399.2	wp	399.2
h50	399.2	wp	399.2
h51	399.2	wp	399.2
h52	399.2	wp	399.2
h53	399.2	wp	399.2
h54	399.2	wp	399.2
h55	399.2	wp	399.2
h56	399.2	wp	399.2
h57	399.2	wp	399.2
h58	399.2	wp	399.2
h59	399.2	wp	399.2
h60	399.2	wp	399.2
h61	399.2	wp	399.2
h62	399.2	wp	399.2
h63	399.2	wp	399.2
h64	399.2	wp	399.2
h65	399.2	wp	399.2
h66	399.2	wp	399.2
h67	399.2	wp	399.2
h68	399.2	wp	399.2
h69	399.2	wp	399.2
h70	399.2	wp	399.2
h71	399.2	wp	399.2
h72	399.2	wp	399.2
h73	399.2	wp	399.2
h74	399.2	wp	399.2
h75	399.2	wp	399.2
h76	399.2	wp	399.2
h77	399.2	wp	399.2
h78	399.2	wp	399.2
h79	399.2	wp	399.2
h80	399.2	wp	399.2
h81	399.2	wp	399.2
h82	399.2	wp	399.2
h83	399.2	wp	399.2
h84	399.2	wp	399.2
h85	399.2	wp	399.2
h86	399.2	wp	399.2
h87	399.2	wp	399.2
h88	399.2	wp	399.2
h89	399.2	wp	399.2
h90	399.2	wp	399.2
h91	399.2	wp	399.2
h92	399.2	wp	399.2
h93	399.2	wp	399.2
h94	399.2	wp	399.2
h95	399.2	wp	399.2
h96	399.2	wp	399.2
h97	399.2	wp	399.2
h98	399.2	wp	399.2
h99	399.2	wp	399.2
h100	399.2	wp	399.2

27a

Enantiomeric analysis of the anti condur
1101 obtained from Mitsunobu reaction

exp3 52pul

SAMPLE		SPECIAL	
date	Oct 23 2012	temp	not used
solvent	CDCl3	gain	not used
file	/export/hme/~	spin	20
ssabet/SS11-1-BNC1-	htc	0.00	0.00
rfid	13.780	rfid	13.780
rfid	5.600	rfid	5.600
ACQUISITION		FLAGS	
sw	6395.9		
at	3.744	11	n
ap	4.892	1n	n
bp	0.000	1p	y
bs	1.16	hs	nn
d1	1.000	fn	not used
nt	32	fn	not used
ct	32	fn	not used
TRANSMITTER		DISPLAY	
to	32	sp	276.3
tf	399.741	rf1	2407.4
tof	399.741	rf1	814.9
tp	56	rfp	-6.0
pr	6.850	lp	-80.1
DECOUPLER		PLOT	
dn	C13	mc	200
dof	0	sc	25
da	nn	vs	230
dms	35	at	3
dmf	35	at	3

27a

19F NMR

exp3 s2pul

SAMPLE		SPECIAL	
date	Oct 23 2012	temp	not used
solvent	CDCl3	gain	not used
file	exp3	not used	
file	exp3	not used	
state	SS1-13C	hist	0.008
id	pe30	pe30	16.600
al	90.000	al	90.000
ACQUISITION			
sw	50000.0	fl	FLAGS
at	15.000	in	n
fb	28000	dp	y
bs	8	hs	nn
d1	1.500	lb	PROCESSING
nt	256	lf	0.30
ct	256	fn	not used
TRANSMITTER			
tn	F19	display	
sfrq	376.090	sp	-30393.5
tor	58	rfl	65122.8
pw	5.600	rfl	65122.8
pc	DECOUPLER	rp	-159.3
dn	H1	lp	0.0
dof	nm	wc	200
dam	c	sc	25
dpwr	38	vs	52
def	8889	th	5
		no	ph

27a

(((1*R*,2*R*,3*S*,6*S*)-6-Azidocyclohex-4-ene-1,2,3-triyl)tris(oxy))tris(methylene)tribenzen (18).⁵⁰

To triply toluene-azeotroped conduritol **17b** (32.8 mg, .0788 mmol) was added mesyl anhydride (27.5 mg, 0.1577 mmol) and DMAP (38.5 mg, 0.3152 mmol). The reaction flask was kept under argon and distilled DCM (657 mL) was syringed in. The progress of the reaction was monitored by TLC (EtOAc-hexanes, 2:3) and when no more change was observed, Et₃N was added, one drop at a time. The effect of each drop was TLC-monitored. After the completion of the reaction, sodium azide (53.8 mg, 0.8274 mmol) and DMF (400 mL) was added (Caution: mesyl azide, which is an explosive can form at this stage). The reaction was stirred behind a shield for 2 h after which time TLC (EtOAc-hexanes, 1:4) indicated the completion of the reaction. NaOH (10%) was added to the reaction which was stirred for another 15 min, and then extracted with DCM. The organic layer was washed with water, HCl, water again and then brine. The aqueous layer was extracted with DCM and the combined organic layers were dried over MgSO₄ and concentrated *in vacuo* to afford dark brown oil. The crude residue was purified by preparative TLC (EtOAc-hexanes, 1:4) to give 14.2 mg of **18** (41% yield). ¹H NMR (CDCl₃): δ = 7.35 - 7.25 (m, 15H), 5.70 (d, *J* = 10.4 Hz, 1H), 5.47 (d, *J* = 10 Hz, 1H),

4.94 - 4.66 (m, 6H), 4.11 (m, 2H), 3.70 (dd, $J = 8.0, 10.4$ Hz, 1H), 3.55 (dd, $J = 8.4, 10$ Hz, 1H).

Pure upper spotting azide

exp5 s2pu1

SAMPLE		SPECIAL	
date	01.11.2013	temp	not used
solvent	CDC13	gain	not used
file	/export/home/~sp1n	sp1n	20
ssabet/SSII-29-7pr~	hst	0.008	
cp.fid	pu90	12.600	
acq	8178	0.600	
ACQUISITION			
sw	4796.7	FLAGS	
at	3.744	11	n
np	35918	in	n
fb	3000	dp	y
ts	1.000	hs	PROCESSING
dl	32	fn	not used
nt	32	fn	not used
ct	32	fn	not used
TRANSMITTER			
tp	397.2	sp	4.8
sfreq	399.701	rf1	397.2
tof	-0.3	rfp	411.2
tpwr	57	rfp	150.7
pw	6.300	lp	-9.5
DECOUPLER			
dn	C13	wc	200
dof	0	sc	25
dm	mnn	vs	60
dma	c	th	5
dpr	31	at	cdc
dnt	29412	ph

Pure upper spotting azide

EXP5 52001

SAMPLE		SPECIAL	
date	Jul 11 2013	temp	not used
solvent	CDCl3	gain	not used
file	/export/home/~	spin	20
ssabet/SSII-29-7pr~	hst	0.008	
0490	cp.fid	12.000	
4796.7	qlf	6.000	
ACQUISITION			
at	3.744	tl	n
np	35918	in	n
bs	3000	cp	y
d1	1.000	ns	PROCESSING
nt	32	fn	not used
ct	TRANSMITTER	32	DISPLAY
tn	359.741	sp	290.2
sfreq	359.741	rf1	411.2
tof	-0.3	rff	0
tpwr	57	tp	150.7
pw	6.300	lp	-9.5
DECOUPLER			
cl3	WC	200	PLOT
dn	0	sc	25
dm	nmh	vc	80
dmr	39	at	5
dmf	29412	cdc	ph

(1*R*,2*R*,3*S*,4*R*,5*S*,6*S*)-3-Azido-4,5,6-tris(benzyloxy)cyclohexane-1,2-diol (19a).

(1*S*,2*S*,3*S*,4*R*,5*S*,6*S*)-3-Azido-4,5,6-tris(benzyloxy)cyclohexane-1,2-diol (19b).⁴³

To a solution of **18** (3.9 mg, 0.0088 mmol) in water: acetone (1:9, 440 μ L) N-methyl morpholine N-oxide (1.5 mg, 0.0132 mmol) was added followed by osmium tetroxide (22.4 mL of aqueous solution of osmium tetroxide (10 mg/mL)). The reaction mixture was stirred under argon for about a week until TLC (hexanes-ether, 1:1) indicated the completion of the reaction. Diethyl ether (4 mL) was added to the reaction mixture, and the organic layer was washed with 10% aqueous sodium thiosulfate (1.5 mL) followed by water (1.5 mL) and dried over MgSO_4 . The solvent was evaporated to give crude residue as yellow oil. ^1H NMR data shows the ratio of **19a**:**19b** to be 1:2.6. This mixture was purified by two consecutive preparative TLCs (hexanes-ether, 1:2 plus 1 drop of NH_4OH per 6 ml of organic solvents) to (neat ether plus 1 drop of NH_4OH per 6 ml of ether) with isolated yield of 31% for **19b**. ^1H NMR for **19b** (CDCl_3): δ = 7.35-7.25 (m, 15H), 5.77 (d, J = 2.32 Hz), 4.94 - 4.66 (m, 6H), 4.18 (m, 1H), 4.02 (d, J = 7.24 Hz), 3.90 (dd, J = 7.44, 7.44 Hz), 3.46 (dd, J = 4.12, 4.12 Hz), 2.59 (bs, 1H).

19a

The desired diol from the dihydroxylation

exp3 32pu1

SAMPLE		SPECIAL	
date	Jul 26 2013	temp	not used
solvent	CDCl3	gain	not used
ssref	ssref/SSII-30-4ba-hst	ht	0.008
ssref	ssref/SSII-30-4ba-hst	pe90	12.600
ssref	ssref/SSII-30-4ba-hst	ch4.fid	6.600
ACQUISITION			
sw	4795.7	fl	11
ns	3548	in	n
fb	3000	dp	y
bs	15	hs	nn
dl	1.000	fn	not used
ct	8	fn	not used
PROCESSING			
tn	399.741	wp	0.0
sfrq	399.741	rf1	3997.3
ed	-10.0	rf2	413.2
td	52	rf3	155.6
pw	6.300	lp	-14.8
PLOT			
dn	C13	wc	200
dr	nm	cc	20
dm	nm	vs	70
dpm	c	th	5
dpmr	39	a1	cdc
dpr	29412	ph

The desired diol from the dihydroxylation

exp3 s2pu1

SAMPLE		SPECIAL	
date	Jul 26 2013	temp	not used
solvent	CDCl3	gain	not used
freq	400.13	scn	0.008
ssbwt	SS11-30-4ba~	hst	12.500
sv	Acquisition	alpha	6.600
av	395.7	alpha	6.600
np	35916	in	n
fb	3000	dp	y
bs	16	hs	y
d1	1.000	fn	PROCESSING
ct	8	fn	DISPLAY
tn	8	sp	2.9
sfreq	399.741	rf1	3195.0
tpwr	-0.37	rfp	413.2
pw	6.300	tp	155.8
dn	DECODER	cl3	-14.8
dm	200	vc	200
dma	200	vc	200
dpu	200	vc	200
dmr	200	vc	200
c	th	ca	ph
29412

benzyl(prop-2-yn-1-yl)sulfane (20).⁵⁴

α -Toluene thiol (2 g, 16.1 mmol) was dissolved in degassed methanol (0.5 M, 32.2 mL) at 0 °C, and solid KOH (1.08 g, 19.2 mmol) was added to the reaction mixture. After 5 min., propargyl bromide, 80% in toluene (2.58 mL) was added, and the reaction mixture was warmed to room temperature. After 16 h, TLC (EtOAc-hexanes, 1:9) indicated minor amount of starting material remaining. The reaction was stopped by removal of the methanol under vacuum, and the residue was diluted with water (12 mL), extracted with ethyl acetate (2 x 25 mL), dried over MgSO₄ and concentrated *in vacuo*. The crude yellow oil was purified by flash chromatography (hexanes $\rightarrow$ hexanes-EtOAc, 9:1) to give **20** (1.9 g, 75% yield) as yellow oil. ¹H NMR (CDCl₃): δ = 7.38 - 7.27 (m, 5H), 3.88 (s, 2H), 3.08 (d, J = 2.8 Hz, 2H), 2.31 (t, J = 2.6 Hz, 1H).

Thiol linker

exp3 s2pu1

SAMPLE		SPECIAL	
date	Mar 1 2013	temp	not used
solvent	CDCl3	gain	not used
file	/export/home/	nt	0.008
ss	SS11-16-5.fid	nt	13.700
ss	SS11-16-5.fid	nt	6.600
ACQUISITION		FLAGS	
sw	4796.7	fl	
at	3524	tn	n
fb	3000	dp	y
bs	16	hs	
d1	1.000	fn	PROCESSING
nt	8	fn	not used
ct	TRANSMITTER	sp	6.4
tn	H1	wp	3192.5
sfrq	399.741	rfl	399.7
tof	-0.3	rfl	-23.2
tpwr	6.850	ip	-16.8
pw	DECOUPLER	ip	PLOT
dn	C13	uc	200
dof	0	sc	25
dma	mm	sc	34
dpr	39	a1	cdc
dmf	29412	ph	5

20

(1*S*,2*S*,3*S*,4*S*,5*R*,6*S*)-3,4,5-Tris(benzyloxy)-6-(4-((benzylthio)methyl)-1*H*-1,2,3-triazol-1-yl)cyclohexane-1,2-diol (21**).**⁵³

A solution of propargyl sulfide **20** (3.61 mg, .022 mmol) in THF (50 μ L) was added to **19b** (5.9 mg, 0.0124 mmol) at room temperature and the reaction mixture was let to stir. Copper sulfate pentahydrate (0.3 mg, 0.00124 mmol, 50 μ L of 6 mg/ml stock solution) followed by sodium ascorbate (0.5 mg, 0.00248 mmol) were added to the reaction mixture. TLC (neat ether) indicated complete disappearance of starting material in less than 10 min. Water (0.3 mL) was then added to the reaction mixture and the aqueous layer was extracted with ethyl acetate (2 x 0.5 mL). The organic layer was washed with water (0.5 mL) and brine (0.5 mL), dried over MgSO₄ and concentrated *in vacuo*. The crude residue was purified by preparative TLC to afford **21** (4 mg, 51% yield) as a white solid. ¹H NMR (CDCl₃): δ = 7.57 (s, 1H), 7.34 - 7.15 (m, 20H), 6.90 (dd, *J* = 3.6, 6.8 Hz, 2H), 4.94 (d, *J* = 11.2 Hz, 1H), 4.90 (d, *J* = 11.2 Hz, 1H), 4.84 (d, *J* = 3.2 Hz, 1H), 4.78 (d, *J* = 11.2 Hz, 1H), 4.59 (d, *J* = 10.4 Hz, 1H), 4.40 (d, *J* = 10.8 Hz, 1H), 4.29 (s, 1H), 4.21 (d, *J* = 10.8 Hz, 1H), 3.94 (d, *J* = 9.6 Hz, 2H), 3.68 - 3.59 (m, 5H), 2.1(bs, 2H). HRMS (ESI): *m/z* [C₃₇H₃₉N₃O₅S]⁺ calcd 638.2688, obsd 638.2684.

1,2-O-cyclohexylidene-D/L-myo-inositol (**29**).⁵⁷

In a two-necked flask, *myo*-Inositol (4.707 g, 26.13 mmol) and cyclohexanone (40 mL, 385.9 mmol) were added in a 1:1 mixture of DMF (50 mL) and benzene (50 mL). The reaction mixture was heated to reflux in a Dean-Stark apparatus and *p*-toluenesulfonic acid monohydrate (*p*-TsOH.H₂O) (0.202 g, 1.06 mmol) in DMF (5 mL) was added to the reaction flask in 1.25 mL aliquots at 2 h intervals. The mixture was cloudy at first but after 10 h changed to a clear pale yellow. The reaction was heated for another 24h. The solvents were then removed by vacuum distillation to leave a viscous orange liquid that was taken up in hot EtOH (100 mL) and left to cool slowly to crystallize. The solid was filtered and re-crystallized from EtOH to give **29** as white needle-like crystals (300 mg, 2%). ¹³C NMR (DMSO): δ109.2, 79.5, 76.7, 75.7, 74.9, 73.5, 73.0, 72.6, 70.6, 38.3, 35.6, 25.3, 24.3, 24.0

(±)-3,4,5,6-Tetra-O-benzyl-1,2-O-cyclohexylidene-myoinositol (30**).⁵⁷**

Tetraol **29** (300 mg, 1.15 mmol) was transferred to a 2-necked 50 mL round bottom flask and azeotroped with toluene over night. Anhydrous THF (2.8 mL) was added to the flask and the mixture was cooled to 0 °C. To this was added NaH (276 mg, 6.90 mmol, 60% dispersion in mineral oil) and, after stirring for 30 min tetrabutylammonium iodide (TBAI) (212 mg, 0.58 mmol) was added followed by slow addition of benzyl bromide (0.84 mL, 7.04 mmol) and the reaction mixture was heated to reflux for over night. The reaction was then quenched first with MeOH and then H₂O; the mixture was washed with EtOAc and the combined organic layers washed with brine and dried over MgSO₄. The crude product was purified by column chromatography, twice, using hexanes to remove excess benzyl bromide and EtOAc-petroleum ether (1:9) to isolate **30** as an oily product (540 mg, 84%). ¹H NMR (CDCl₃): δ = 7.41-7.25 (m, 20H), 4.94 - 4.74 (m, 8H), 4.29 (t, *J* = 4 Hz, 1H), 4.1 (t, *J* = 5.2 Hz, 1H), 3.95 (t, *J* = 8.4 Hz, 1H), 3.82 (t, *J* = 9.6 Hz, 1H), 3.7 (dd, *J* = 4.0, 8.4 Hz, 1H), 3.42 (dd, *J* = 8.4, 9.6 Hz, 1H), 1.9 - 1.4 (m, 10H).

exp3 s2pu1

[illegible]

(±)-1,4,5,6-Tetra-O-benzyl-myo-inositol (_{D/L}-31**).⁵⁷**

Acetal **30** (400 mg, 0.644 mmol) was dissolved in 80% aq acetic acid (4.03 mL) and heated to a gentle reflux for 2 h. The solvent was evaporated under reduced pressure and the crude product crystallized at 0 °C from a mixture of toluene/PE (1:3) to give _{D,L}-**31** as a white solid that was re-crystallized from EtOAc/PE (1:4) (278 mg, 80%). ¹H NMR (CDCl₃): δ = 7.34 -7.26 (m, 20H), 4.94 - 4.72 (m, 8H), 4.21 (dd, *J* = 2.4, 2.7 Hz, 1H), 3.97 (dd, *J* = 9.6, 9.3 Hz, 1H), 3.84 (dd, *J* = 9.6, 9.3 Hz, 1H), 3.51 - 3.45 (m, 3H), 2.49 (s, 1H), 2.41 (d, *J* = 4.5 Hz, 1H).

After second crystallization.

exp3 s2pul

SAMPLE		SPECIAL	
date	Oct 11 2013	temp	not used
solvent	CDCl3	gain	not used
file	/export/home/~ spin		
ssubset	SS11-d1-3ar~ hst	spin	20
termpurification	hst	12.000	
temp	12.000		
fid	0178	6.600	
ACQUISITION			
sw	4796.7	11	n
at	35018	16	n
fb	3000	hs	y
bs	16		
dl	1.000	fn	not used
ct	16	sp	
tn	399.7	wp	4002.3
strq	399.741	rfl	399.7
tof	-0.3	rp	156.0
pw	8.300	lp	-28.7
DECOUPLER			
dn	C13	sc	200
dof	0	vs	25
dm	nmc	01	cdc
dpr	39	ph	5
dbr	29412

31

After second crystallization.

exp3 s2pul

SAMPLE		SPECIAL	
date	Oct 11 2013	temp	not used
solvent	CDCl3	gain	not used
exp	3	ssb	not used
ssb	SSII-41-3a	hst	0.008
ter2ndpur	ification	pw90	12.600
		alfa	6.600
ACQUISITION		FLAGS	
sw	4796.7	l1	
at	3.744	in	n
np	35916	dp	y
fb	3000	hs	
di	1.000	fn	PROCESSING
nt	16	ct	DISPLAY
TRANSMITTER		RECEIVER	
tr	16	sp	-2.4
tr	399.7	wp	3209.6
tr	399.7	ff1	399.7
tr	399.7	ff2	399.7
tr	399.7	ff3	399.7
tr	399.7	ff4	399.7
tr	399.7	ff5	399.7
tr	399.7	ff6	399.7
tr	399.7	ff7	399.7
tr	399.7	ff8	399.7
tr	399.7	ff9	399.7
tr	399.7	ff10	399.7
tr	399.7	ff11	399.7
tr	399.7	ff12	399.7
tr	399.7	ff13	399.7
tr	399.7	ff14	399.7
tr	399.7	ff15	399.7
tr	399.7	ff16	399.7
tr	399.7	ff17	399.7
tr	399.7	ff18	399.7
tr	399.7	ff19	399.7
tr	399.7	ff20	399.7
tr	399.7	ff21	399.7
tr	399.7	ff22	399.7
tr	399.7	ff23	399.7
tr	399.7	ff24	399.7
tr	399.7	ff25	399.7
tr	399.7	ff26	399.7
tr	399.7	ff27	399.7
tr	399.7	ff28	399.7
tr	399.7	ff29	399.7
tr	399.7	ff30	399.7
tr	399.7	ff31	399.7
tr	399.7	ff32	399.7
tr	399.7	ff33	399.7
tr	399.7	ff34	399.7
tr	399.7	ff35	399.7
tr	399.7	ff36	399.7
tr	399.7	ff37	399.7
tr	399.7	ff38	399.7
tr	399.7	ff39	399.7
tr	399.7	ff40	399.7
tr	399.7	ff41	399.7
tr	399.7	ff42	399.7
tr	399.7	ff43	399.7
tr	399.7	ff44	399.7
tr	399.7	ff45	399.7
tr	399.7	ff46	399.7
tr	399.7	ff47	399.7
tr	399.7	ff48	399.7
tr	399.7	ff49	399.7
tr	399.7	ff50	399.7
tr	399.7	ff51	399.7
tr	399.7	ff52	399.7
tr	399.7	ff53	399.7
tr	399.7	ff54	399.7
tr	399.7	ff55	399.7
tr	399.7	ff56	399.7
tr	399.7	ff57	399.7
tr	399.7	ff58	399.7
tr	399.7	ff59	399.7
tr	399.7	ff60	399.7
tr	399.7	ff61	399.7
tr	399.7	ff62	399.7
tr	399.7	ff63	399.7
tr	399.7	ff64	399.7
tr	399.7	ff65	399.7
tr	399.7	ff66	399.7
tr	399.7	ff67	399.7
tr	399.7	ff68	399.7
tr	399.7	ff69	399.7
tr	399.7	ff70	399.7
tr	399.7	ff71	399.7
tr	399.7	ff72	399.7
tr	399.7	ff73	399.7
tr	399.7	ff74	399.7
tr	399.7	ff75	399.7
tr	399.7	ff76	399.7
tr	399.7	ff77	399.7
tr	399.7	ff78	399.7
tr	399.7	ff79	399.7
tr	399.7	ff80	399.7
tr	399.7	ff81	399.7
tr	399.7	ff82	399.7
tr	399.7	ff83	399.7
tr	399.7	ff84	399.7
tr	399.7	ff85	399.7
tr	399.7	ff86	399.7
tr	399.7	ff87	399.7
tr	399.7	ff88	399.7
tr	399.7	ff89	399.7
tr	399.7	ff90	399.7
tr	399.7	ff91	399.7
tr	399.7	ff92	399.7
tr	399.7	ff93	399.7
tr	399.7	ff94	399.7
tr	399.7	ff95	399.7
tr	399.7	ff96	399.7
tr	399.7	ff97	399.7
tr	399.7	ff98	399.7
tr	399.7	ff99	399.7
tr	399.7	ff100	399.7

(±)-1,4,5,6-Tetra-O-benzyl-myoinositol 1,2-cyclic phosphate (32).^{56, 58}

All glassware and syringes were dried in hot oven over night. MeOP(O)Cl₂ (500 μL) was added to dry pyridine (5 mL) very slowly. The reaction was stirred at room temperature for 30 min to make a white turbid mixture. This mixture (1.3 mL, 50 eq) was added to a stirred solution of **31** (14 mg, 0.0259 mmol) in dry pyridine (0.43 mL) under argon and stirring was continued at room temperature for an hour. The completion of the reaction was determined by mass spectrometry confirming the disappearance of starting material at which time reaction was quenched by addition of saturated NaHCO₃ (10 mL) and co-evaporation with heptane (10 mL). The resulting solid was dissolved in water (0.75 mL) and adjusted to pH=1 by dropwise addition of 2 M HCl. The solution was extracted with EtOAc (5 x 0.5 mL) and the combined organic extracts were dried over MgSO₄ and concentrated *in vacuo*, to afford a crude white solid (12 mg, 78%). ¹H NMR (CDCl₃): δ = 7.35 - 7.11 (m, 20H), 4.81 - 4.43 (m, 10H), 4.17 (dd, *J* = 8, 9 Hz, 1H), 3.75 (dd, *J* = 6, 6.4 Hz, 1H), 3.635 (s, 1H), 3.35 (dd, *J* = 7.2, 8.6 Hz, 1H), 1.62 (bs, 1H). ³¹P NMR (CDCl₃): δ = 19.7)

Phosphorylated model d101.Crude

exp3 s2pu1

SAMPLE	date	Mar 21 2014	temp	SPECIAL
solvent	CDCl3	spin	not used	
1H NMR	4.000	spin	not used	
13C NMR	100.000	spin	not used	
15N NMR	125.000	spin	not used	
2D NMR	12.600	spin	not used	
3D NMR	6.600	spin	not used	
4D NMR		spin	not used	
5D NMR		spin	not used	
6D NMR		spin	not used	
7D NMR		spin	not used	
8D NMR		spin	not used	
9D NMR		spin	not used	
10D NMR		spin	not used	
11D NMR		spin	not used	
12D NMR		spin	not used	
13D NMR		spin	not used	
14D NMR		spin	not used	
15D NMR		spin	not used	
16D NMR		spin	not used	
17D NMR		spin	not used	
18D NMR		spin	not used	
19D NMR		spin	not used	
20D NMR		spin	not used	
21D NMR		spin	not used	
22D NMR		spin	not used	
23D NMR		spin	not used	
24D NMR		spin	not used	
25D NMR		spin	not used	
26D NMR		spin	not used	
27D NMR		spin	not used	
28D NMR		spin	not used	
29D NMR		spin	not used	
30D NMR		spin	not used	
31D NMR		spin	not used	
32D NMR		spin	not used	
33D NMR		spin	not used	
34D NMR		spin	not used	
35D NMR		spin	not used	
36D NMR		spin	not used	
37D NMR		spin	not used	
38D NMR		spin	not used	
39D NMR		spin	not used	
40D NMR		spin	not used	
41D NMR		spin	not used	
42D NMR		spin	not used	
43D NMR		spin	not used	
44D NMR		spin	not used	
45D NMR		spin	not used	
46D NMR		spin	not used	
47D NMR		spin	not used	
48D NMR		spin	not used	
49D NMR		spin	not used	
50D NMR		spin	not used	
51D NMR		spin	not used	
52D NMR		spin	not used	
53D NMR		spin	not used	
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55D NMR		spin	not used	
56D NMR		spin	not used	
57D NMR		spin	not used	
58D NMR		spin	not used	
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62D NMR		spin	not used	
63D NMR		spin	not used	
64D NMR		spin	not used	
65D NMR		spin	not used	
66D NMR		spin	not used	
67D NMR		spin	not used	
68D NMR		spin	not used	
69D NMR		spin	not used	
70D NMR		spin	not used	
71D NMR		spin	not used	
72D NMR		spin	not used	
73D NMR		spin	not used	
74D NMR		spin	not used	
75D NMR		spin	not used	
76D NMR		spin	not used	
77D NMR		spin	not used	
78D NMR		spin	not used	
79D NMR		spin	not used	
80D NMR		spin	not used	
81D NMR		spin	not used	
82D NMR		spin	not used	
83D NMR		spin	not used	
84D NMR		spin	not used	
85D NMR		spin	not used	
86D NMR		spin	not used	
87D NMR		spin	not used	
88D NMR		spin	not used	
89D NMR		spin	not used	
90D NMR		spin	not used	
91D NMR		spin	not used	
92D NMR		spin	not used	
93D NMR		spin	not used	
94D NMR		spin	not used	
95D NMR		spin	not used	
96D NMR		spin	not used	
97D NMR		spin	not used	
98D NMR		spin	not used	
99D NMR		spin	not used	
100D NMR		spin	not used

31P NMR

exp3 52pt

[illegible]

114

purification was not pursued). ^{31}P NMR (D_2O) δ : 17.7. HRMS (ESI): m/z $[\text{C}_6\text{H}_{12}\text{O}_8\text{P}]^+$ cald 243.0270, obsd 242.9180 ; m/z $[\text{C}_6\text{H}_{14}\text{O}_9\text{P}]^+$ cald 261.0375, obsd 260.9256; m/z $[\text{C}_6\text{H}_{11}\text{NaO}_8\text{P}]^+$ cald 265.0089, obsd 264.8451.

Deprotected model diol after desatlation
exp3 szpu1

SAMPLE		SPECIAL	
date	Apr 4 2014	temp	not used
solvent	port-ho	gain	not used
file	ssabef/ss11-57-saf~	hst	0.008
terdesatlation	fid pw90	pw90	12.600
ACQUISITION	alifa	alifa	6.600
sw	4796.7	flads	
as	35918	fl	
rp	3000	dp	
bs	1.16	hs	
dl	1.000	fn	
ct	1136	fn	
TRANSMITTER		PROCESSING	
tn	H1 wp	sp	-15.0
sfrq	399.742	rf1	4003.6
tdr	-1.2	rfp	399.7
tpwr	57	rp	73.2
pw	6.300	lp	-6.9
DECOUPLER		PLOT	
dn	C13	wc	200
ds	nnn	vs	21280
dsm	c	th	8
dper	.39	ai	
daf	29412	ph

Deprotected model d101 after desaturation

exp3 szpul

date	Apr 4 2014	temp	SPECIAL
solvent	Apr 020	gain	not used
file	/export/home/~	spin	not used
ssabet/SSII-57-54f~	hst	0.000	
term	12.600		
ACQUISITION	8176	FLAOS	
sw	4786.7	11	n
at	3.744	1n	n
np	35918	1n	y
bp	3900	dp	y
bt	1.000	ns	PROCESSING
d1	20.08	fn	not used
nt	1136	DISPLAY	
ct	TRANSMITTER	H1	SP
tn	399.742	rf1	780.8
sfrq	-0.4	rfp	1005.2
tof	57	rp	399.7
tpwr	6.300	lp	73.2
pw	DECOUPLER	C13	-6.9
dn	0	SC	200
dof	0	VS	25
da	nnn	VS	21280
dm	35	th	8
dpr	29412	ph

33

Deprotected model diol after desalination

exp3 szpul

SAMPLE		SPECIAL	
date	Apr 4 2014	temp	not used
event	szpul	spin	not used
file	export/home/	spin	not used
ssabel	SSII-57-SAF~	hist	0.008
terdesalination.fid	pw90	pw90	12.600
ACQUISITION	al1fa	al1fa	6.600
sw	4956.7	FLAGS	
np	3744	11	
bs	35018	1n	n
fb	3000	dp	y
nl	16	hs	
ct	1.000	PROCESSING	nn
	2000	not used	
	1136	fn	DISPLAY
TRANSMITTER			
tn	H1	sp	793.6
trf	399.92	wp	1601.5
trf	399.92	rf	399.92
trf	57	rfp	73.2
pw	6.300	lp	-6.9
DECOUPLER			
dc	C13	vc	290
dcf	nnn	vc	290
ds	c	th	21260
dper	39	at	ph
dpr	29412

33

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