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Synthesis of Bipyridine Carbohydrate Receptors

Maxwell Addo

THE DESIGN AND SYNTHESIS OF PYRIDINE-BASED LECTIN MIMICS (CARBOHYDRATE RECEPTORS)

A Thesis

by

MAXWELL ADDO

Submitted to Texas A&M International University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

May 2017

Major Subject: Biology

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ABSTRACT

Design and Synthesis of Pyridine-based Lectin Mimics (Carbohydrate Receptors). (May 2017)

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Chair of Committee: Dr. Daniel Mott

Macrocyclic molecules consisting of aromatic groups with amino side chains are known to bind selectively to carbohydrates, mainly monosaccharides and oligosaccharides. Some of these macrocyclic molecules, for example, Pyridine-based and Naphthyridine-based macrocyclic structures, behave like carbohydrate receptors and bind selectively to carbohydrates in aqueous solutions through hydrogen bonding, hydrophobic and electrostatic interactions. Bipyridine receptors mimic natural lectins. A bipyridine receptor was designed and synthesized from Tris(2-aminoethyl)amine and 2,2-bipyridine-4,4-dicarboxyaldehyde. The receptor was purified and characterized using extraction, HPLC (High Performance Liquid Chromatography), NMR (Nuclear Magnetic Resonance) spectroscopy and ESI-MS (Electrospray Ionization Mass Spectrometry). Masses and peaks obtained matched the expected structure which was synthesized. The binding constants between the bipyridine receptor and six selected sugars in water were determined by UV-Vis (Ultraviolet Visible) spectroscopy titration. Studies confirmed that the bipyridine receptor was able to bind to three of the sugars. The observed dissociation constant values ranged from 0.16mM to 0.37mM. These values compared favorably with the reported binding constants between a 1,8-naphthyridine receptor and monosaccharide complexes, as well reported lectin/monosaccharide complexes in nature. The divalent version

of the bipyridine receptor, which was expected to have better binding properties towards sugar substrates, was also prepared using solid phase synthesis. Two of the sugars that were able to bind to the bipyridine receptor occurred in high concentration in certain cancer tumor tissues. It is expected that the bipyridine receptor and the findings in this research can serve as source of information for further research work into early detection and treatment of cancer or other cellular communications in which glycoconjugates are important.

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INTRODUCTION

Cell surfaces are covered by carbohydrates. Current research works have shown that these carbohydrates are essential in cell-cell communication. The communication occurs by cell-cell contact or by other particles attaching themselves to the cell-surface carbohydrates. These particles that attach themselves to the cell-surface carbohydrates are highly specific to the type of carbohydrate to which they attach. Effective communication will also depend on the effectiveness of the binding between the cell-surface carbohydrates and their specific receptors (Brandley et al., 1986; Lepenies et al., 2010; Reenberg et al., 2007).

Carbohydrates are considered one of the most important biomolecules in living organisms. They are the most abundant group of biomolecules and make up a higher percentage than any of the other groups of biomolecules in nature (Boyer, 2006). Chemically, carbohydrates are an important family of organic compounds with their own unique functional group and set of physical and chemical properties (Brown et al., 2006). In human nutrition, carbohydrates constitute one of the main classes of nutrients (Dryden, 2008). Basically, carbohydrates are known as reactants for energy production in living cells; for example, glucose, a monosaccharide, reacts with oxygen to generate energy (Boyer, 2006). These basic roles of carbohydrates have been known to scientists for a long time. Current research works, however, have unearthed other important roles played by carbohydrates in living systems. One of such roles is the function of carbohydrates in the area of biological recognition processes; a role previously known to be associated with proteins and nucleic acids. Carbohydrates are now viewed as playing prominent roles in areas of control and dissemination of information in living

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This thesis follows the model of *Current Organic Chemistry Journal.*

cells (Boyer, 2006; Catterall et al., 1997; Davis et al., 1999; Sharon et al., 1987).

There are some important cellular processes that are known to be controlled fully or partly by signals from cell-cell contact. Complex cell-surface codes may control intercellular interactions including binding of certain agents to cells via cell-surface carbohydrates and carbohydrate receptor interactions. For example, many immunoglobulins (antibodies) and peptide hormones contain glycoprotein sequences, a sequence composed of a peptide chain linked to a carbohydrate. Circulating enzymes cleave these carbohydrates and the liver determines their degradation by recognizing differences in length of the carbohydrate portion of the glycoprotein (Boyer, 2006; Devlin, 2011). This and other biological processes, show that cell-surface carbohydrates play a role in cell-cell recognition. Carbohydrates are also essential for other processes like immune response, pathogenesis, cell differentiation, metastasis, cell colonization in tumor formation, and even in fertilization process between the sperm and ovum (Catterall et al., 1997; Sharon et al., 1987; Striegler, 2003).

Some of the questions that initiated research into cell-cell communication were: in which group of cell-surface molecules is a particular cell-surface code embedded; and, which types of receptors detect the cell-surface code? Although research effort in this area of science has intensified in recent times, very little is known about some of these molecules; their biochemical mechanisms and how the signals obtained affect changes in cell and tissue behaviors in living systems. Data from previous research have advanced the hypothesis that cell-surface carbohydrates can act as cell-cell recognition molecules. For example, surface carbohydrates on one cell can bind to receptors like lectins, and this can initiate a specific interaction resulting in changes in cell behavior (Brandley et al., 1986).

What has become more challenging in this area of research are the characteristics of cell-surface carbohydrates and the nature of carbohydrate receptors involved in the biological recognition processes; as well the type of interactions in which they engage. Biological recognition between cell-surface carbohydrates and their receptors can be very complex. Receptors and other agents are highly specific as to the type of carbohydrates to which they link. The structural diversity of carbohydrates in terms of linkages, ring size and functional groups also add extra complexity to the study of these interactions. Two identical monosaccharide units have the option of forming a maximum of eleven different disaccharides. Natural carbohydrate-receptor recognitions may therefore involve complex and elaborate processes. The processes may involve lots of different varieties of carbohydrate molecules. They may also involve a lot different receptors which are highly specific for the type of carbohydrates they can bond to. Current research works are also looking into the effectiveness of the binding affinities between surface cell carbohydrates and their specific carbohydrate receptors and processes of enhancing the effectiveness of these bindings for medicinal and other uses (Brandley et al., 1986; Lepenies et al., 2010; Reenberg et al., 2007).

Research works into the use of synthetic carbohydrates for biological recognition and drug delivery processes in the human body are ongoing. Research works into the use of synthetic carbohydrate receptors for diagnostic study and drug delivery processes are also ongoing. The process of designing a carbohydrate receptor that binds to carbohydrates in an aqueous medium is a relatively new area of research. Carbohydrate receptors that bind to carbohydrates in organic media have already been made (Mazik et al., 2006)**.** However, cellular

fluids are aqueous solutions; it will be of interest to make receptors which bind to carbohydrates in aqueous media.

The proposed carbohydrate receptor will have to overcome obstacles like the structural complexity of carbohydrates and the weak binding interactions between the receptor and the carbohydrate. A macrocyclic receptor molecule, if properly designed, can have characteristics to overcome these obstacles. It can be made water-soluble, possesses the structures suitable for stronger binding between the receptor molecule and the substrate, etc. (Lee et al., 2011; Brandley et al., 1986). The structure of a macrocyclic bipyridine molecule shows that it possesses the characteristic parts needed to bind to carbohydrates through hydrogen bonding and other interactions. This research work is about designing and synthesizing a macrocyclic bipyridine carbohydrate receptor which can recognize and bind to carbohydrate substrates in aqueous media (Boyer, 2006; Catterall et al., 1997; Davis et al., 1999; Holme & Peck, 1998; Sharon et al., 1987).

REVIEW OF LITERATURE

2.1 Cell-surface Carbohydrates

The name carbohydrates simply mean “hydrates of carbon”. Carbohydrates constitute a family of organic compounds primarily made up of carbon, hydrogen and oxygen. They are represented by the general formula $C_x(H_2O)_y$, with the ratio of H to O being equal to 2:1. In addition to these three elements, some carbohydrates and their derivatives also contain sulfur, nitrogen or phosphorus. A carbohydrate usually has a reactive ketone or aldehyde functional group and one or more hydroxyl groups, so, carbohydrates can be viewed as polyhydroxy aldehydes and ketones (Boyer, 2006; Brown et al., 2006).

Carbohydrates are widely distributed in both plant and animal tissues; together with other organic macromolecules like proteins, lipids, and nucleic acids, they serve various functions in living things. Some monosaccharides like glucose and fructose are used in energy generation in living organisms; others like ribose and deoxyribose sugars are one of the main components of nucleic acids. Some polysaccharides, like glycogen and starch, serve as chemical stores for energy production. Some carbohydrates and their derivatives also serve as structural components in living things; for example, the rigid cell wall structures in bacterial and plant cells, cartilage in animals, and the exoskeleton of arthropods all consist of carbohydrates and carbohydrate derivatives. Carbohydrates are also important raw materials for many biochemical reactions in living cells; they are also used as industrial raw materials for fermentation reaction for production of alcohol (Boyer, 2006; Solomons & Fryhle, 2004).

Until recently, the knowledge and importance of carbohydrates has been limited to these uses and functions already mentioned above. Over the past fifty years, research works into other uses of carbohydrates have been growing steadily. One of such areas is the use of carbohydrates in the control and dissemination of information in living cells. Numerous biological particles, including antibodies, enzymes, hormones, glycoproteins, glycolipids, etc. are known to bind to cell-surface carbohydrates, where they engage in this control and dissemination of information in living cells (Bucior & Burger, 2004).

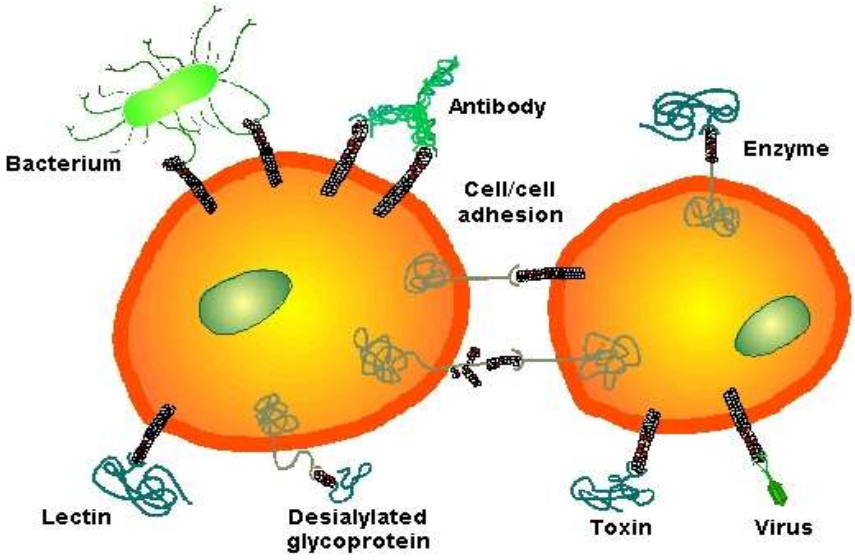

Figure 1. A diagram showing some of the chemicals and structures that can bind to cell-surface carbohydrates (Magnani, 2009).

The biological process of cell-cell recognition enables carbohydrates to play significant roles in embryogenesis, spermatogenesis, implantation, host-pathogen interaction, immune response, cell differentiation, tumor cell colonization and metastasis. (Boyer, 2006; Brandley et al., 1986; Brandley et al., 1987; Bucior & Burger, 2004; Lee et al., 2011; Lepenies et al., 2010).

The various roles that cell-surface carbohydrates play in animals cannot be underestimated. Cell-surface carbohydrates are known to play a unique role in the spread of cancer from the disease origin to other parts of the body. Carbohydrates are essential in aiding cancer cells to break away from their disease origin and are carried away to other areas of the body to enhance the spreading of the disease. This is due to the phenomenon that when cells are diseased or become cancerous, the carbohydrate on the cell surfaces changes. These changes in cell-surface carbohydrates enable cancer cells to escape immune-surveillance and other means by which they can be destroyed (Ghazarian et al., 2010).

Sialic acid, a type of cell-surface carbohydrate, occurs on cell surfaces in humans and thus has exposed locations; coupled with the negative charge on them, they engage in cell-cell recognition by either masking recognition sites or controlling the recognition. This phenomenon is important in nervous system embryogenesis, inflammatory and immune response processes (Buschiazzo & Alzari, 2008; Kwak et al., 2012; Rutishauser 2008). Most pathogens use the production of sialic acids to evade the immune system of their hosts; others which cannot produce sialic acid use the enzymes they produce to control or moderate the immune system of their host (Devlin 2011; Severi et al., 2007). A strain of *Salmonella enterica*, *S. taphi*, uses sialic acid on the surface of cells to fix itself to the cell surface during infection (Sakarya et al., 2010). Some types of viruses, specifically certain types of Adenovirus and avian infectious bronchitis virus have been known to use sialic acid molecules as cellular receptors (Arnberg et al. 2002; Winter et al., 2006). The human influenza virus uses glycoproteins it produces to attach to sialic acid in human cells (Thompson et al., 2006). Anti-influenza drugs are therefore designed to be just like sialic acid derivatives; an example is Zanamivir, a drug that inhibits the

function of the viral neuraminidase enzyme and thus prevents the reproduction of the virus. (Cyranoski, 2005). Aged glycoproteins lose some of the sialic acid component. This makes it possible for liver cells to easily detect and degrade these aged glycoproteins by using proteolytic enzymes (Marshall et al., 1974). More than half of the sialic acid in the human body is found in gangliosides in the human central nervous system. Gangliosides bind to toxins and provide specific recognition determinants on cell surfaces. Certain polymers of sialic acid control neural cell adhesion during embryonic development. Expression of sialic acids on cell surfaces has been linked to metastasis of several human tumors; this means the behavior of these cell-surface sialic acids can be studied and applied in treatment of tumors (Tang et al., 1997). Sialic acid analysis in cell membranes is used to study not only malignant cancer growth, but also, diabetes mellitus. Overexpression of sialic acid may be an indication of certain malignant cancer growth, whereas under-expression may be an indication of diabetes mellitus (Kwak et al., 2012).

Bacteria as well as their toxins attach themselves to cell-surface carbohydrates. Symptoms linked to most bacterial diseases are caused by these toxins being attached to cell-surface carbohydrates and hence altering the normal functions of the host cell (De Greve et al., 2007; Nizet & Esko, 2009). Lectins also link to cells via cell-surface carbohydrates like sialic acids. Lectins are known to react this way with cells which have undergone malignant transformation. This phenomenon is employed in bone marrow transplants in children and for leukemia treatment (Sharon & Lis, 2002). Cell-surface carbohydrates are, therefore, important when studying most biological processes including infections by bacteria and viruses, interactions between the cells of the human body and cells of lower eukaryotes, specific

binding of sperm to egg during fertilization in human reproduction and recirculation of lymphocytes to diseased regions in the body (Catterall & Turner, 1997; De Greve *et al*. 2007; Sharon and Lis, 2002).

As stated above, an important feature of cell-surface carbohydrates that research has shown is the idea that cell-surface carbohydrates change with the onset of certain diseases. It is known, for example, that cell-surface carbohydrates change with the onset of cancer and inflammation (Couldrey & Green, 2000). The change can be in a form of the loss of the normal or original carbohydrate and subsequent replacement with another; the overexpression of the normal carbohydrate (Rodgers et al., 2000); the persistence of incomplete or truncated structure of the normal carbohydrate; the accumulation of precursors of the original carbohydrate; or the expression of certain new carbohydrates that do not normally occur on healthy cells (Gorelik et al., 2001; Kannagi et al., 2004; Xu et al., 2005). This information may be linked to the behavior of certain diseased cells as the disease progresses. The information obtained from studying this may be used for diagnostic purposes and selective treatment for those diseased cells. This phenomenon has led to research which divert attention to the synthesis of chemically defined cell-surface carbohydrate analogs, as well as mimics of certain structures or chemicals that interact with these cell-surface carbohydrates, to reveal the mechanics of a potential cellular language involved in intercellular interactions, which then can be used for disease detection and treatment.

Carbohydrates are among the most complex and varied natural products; sometimes, even considered more complex and varied than proteins or nucleic acids. Their complexity and variability originates from the fact that a single monosaccharide unit can form variety of bonds

at multiple positions in its skeletal structure. Isomerism ensures that even the α- and the β-isomeric linkages produce carbohydrates with different properties. Branching in the carbon chain may also produce different carbohydrates most of which are naturally stable compounds. Carbohydrate structures can be modified by replacing the hydroxyl group with other functional groups producing different functionalized carbohydrates. Glycoproteins, glycolipids, proteoglycans, glycosaminoglycans, sialic acids, etc. are all examples of functionalized carbohydrates (Boyer, 2006; Gorelik *et al*., 2001; Solomons & Fryhle, 2004). Hence, cell surface carbohydrates can be complex and varied. The binding affinities in ordinary carbohydrates are very weak. However, when carbohydrates are converted into glycoproteins, glycolipids and proteoglycans, the binding affinities increase dramatically and that is the reason why cell-surface carbohydrates are mostly in the form of glycoproteins, glycolipids and proteoglycans (Bucior & Burger, 2004; Yoneda & Couchman, 2003).

2.2 Lectins

Carbohydrates on cell surfaces bind to other structures in the body. One of such structures is lectin. The word “lectin”, first used by William Boyd in 1954, comes from the latin word, “legere” which means “to select” (Lam & Ng, 2011). Lectins are carbohydrate-binding proteins that are highly specific for their carbohydrate moieties. The occurrence of lectins in nature has been known since the turn of the nineteenth century; however, research interest in lectins only intensified about twenty years ago. Formally known as hemagglutinins, it was from the seeds of *Ricinus communis* that the first lectin was isolated by Stillmark in 1888 (Gabius et al., 2011; Sharon et al., 1987; Singh et al. 2011).

Lectins are produced from non-immunogenic sources. Lectins are known to bind to specific carbohydrates on cell surfaces in a non-catalytic manner. In terms of binding, lectins can be monovalent or multivalent. The nature of the binding of lectins to carbohydrates depends on the stereochemistry of the carbohydrates. A lectin has a carbohydrate-binding site that binds to the carbohydrate ligand. The binding occurs through a type of "lock and key" system which is then stabilized by a complex network of hydrogen bonds and van der Waals forces. The binding between lectins and carbohydrates by hydrogen bonds involve the hydroxyl groups in the carbohydrates and the polar groups on the amino acid residue in lectins (Singh et al., 2011).

Lectins play many important roles in biological systems. Lectins are known to mediate symbiotic activity between leguminous plants and the nitrogen-fixing bacterium *Rhizobium*. It is also known that plant lectins play a role in protecting seedlings against fungal attack; for example, a lectin in potato is known to protect young potato plants against *Botrytis cinerea*, a phytopathogenic fungus (Sharon et al., 1990). Germination of spores of *Neurospora crassa*, *Aspergillus amstelodami*, and *Botryodiplodia theobromae* are known to be inhibited by certain lectins (Brambl et al., 1985). Lectins in marine algae are known to control certain biological activities. Alga lectins from *Eucheuma serra* and *Galaxaura marginata* have been observed to inhibit the fish pathogen Vibrio *vulnificus* (Liao et al., 2003). Lectins in alga gametes enhance recognition and adherence during sexual reproduction (Bolwell et al., 1979; Han et al., 2012). The phenomenon of yeast binding to carbohydrates via lectins is an important application in the brewery and food industry (Singh et al., 2011). Lectins are involved in assessment of protein folding in the endoplasmic reticulum (Gerlach et al., 2012). It is known that certain

parasitic amoebae, fungi, bacteria and viruses attach themselves to the host cell by binding to lectins; lectins, in this way, aid in specific recognition between the pathogens and their host cells. Lectins are suspected of being involved in the storing and moving of certain carbohydrates because of their ability to adhere to specific carbohydrates. Other important biological functions lectins engage in include removing glycoproteins from the circulatory system; recognizing and delivering hydrolytic enzymes to lysosomes; exclusively detecting carbohydrates found on the surface of cells of pathogens; and massing of leucocytes to sites of inflammation. In medicine, a lectin, PHA-L, is used to trace the path of efferent axons, while, Banlec, a lectin from banana is used to inhibit HIV-1 in-vitro. Lectins are also involved in food allergies and sensitivities, inflammation and autoimmune diseases (Balzarini, 2006; Ji et al., 2004; Singh et al., 2012).

The use of lectins in the study of carbohydrate recognition by proteins is gaining momentum because of the ease of obtaining lectins and their large variety of sugar specificity. Lectins are also being used to investigate the changes in cell-surface carbohydrates, from cell differentiation, through metastasis, to, for example, giving a clue to the health status of a cell (or tissue) and giving information on the onset of certain diseases. Future research would be targeting the use lectins as carrier systems for cell-specific (or tissue-specific) drug delivery to different diseased parts of the body (Sharon et al., 1989).

The classification of lectins is very broad due to the large number of lectins found in all living organisms. There are animal, plant and microbial sources of lectins (Sharon & Lis, 2004). Lectins have been isolated from peanuts (*Arachis hypogaea*), the European eel (*Anguilla anguilla*) and the common mushroom (*Agaricus bisporus*). Bacterial lectins are commonly

known as adhesins; certain bacteria fix themselves onto host cells using these adhesins via glycans. The influenza virus fixes itself to erythrocytes by using hemagglutinin, a lectin that recognizes sugars on the surfaces of erythtrocytes. According to the animal lectin homepage (www.imperial.ac.uk/ *research/animallectin/ctld/lectins.html*), there are 13 groups of animal lectins. Lectins can be classified based on the type of carbohydrates they recognize (Singh et al., 2012). Lectins are termed as intracellular if they engaged in protein trafficking and sorting; they can also be termed as extracellular if they engaged in signaling and pathogen recognition (Kilpatrick, 2002; imperial research website).

The ability of lectins to bind to carbohydrates is an important factor to consider in diseased cell recognition, drug delivery to specific sites in the human body, and the treatment of diseased cells and tissues (Castonguay et al., 2011; Lakhtin et al., 2010; Singh et al., 2012). In considering the use of lectins in the afore-mentioned areas, the binding properties and specificity of lectins need to be assessed. In assessing these characteristics, there is the need to consider a specific lectin and its characteristics. For the focus of this research, legume lectin is being considered for this assessment because of its wide use in other biomedical researches (Sharon & Lis, 2004).

2.3. Legume Lectins

There are several reasons why a discussion on legume lectins is important for this research. Legume lectins form one of the largest families of lectins. Over 100 legume lectins from different members of the family *Leguminoseae* have been isolated and characterized. Most legume lectins were obtained from the seeds of various leguminous plants (Sharon & Lis, 1990; 2002). Legume lectins are the most extensively studied of all lectin families. Legume

lectins can be easily obtained in their pure form and they easily show carbohydrate selectivity, a phenomenon key to this research. Studies in legume lectins can readily be related to other lectin families, and this is a key stepping stone into the studies of other lectins like the C-type animal lectin which is important in medicinal and pharmaceutical research (Sharon & Lis, 1990; 2002).

A legume lectin consists of two to four single polypeptide chains known as protomers. The protomers can be the same type or they can differ slightly. Each polypeptide chain usually consists of about 250 amino acids. The amino acids engage in hydrogen bonding and hydrophobic interactions with carbohydrates. Each polypeptide chain contains a site for recognizing a carbohydrate; for each specific legume lectin, the carbohydrate recognizing sites have the same carbohydrate specificity. These carbohydrate recognition sites are shallow depressions on the surface of the protein and they serve as binding pockets (Sharon & Lis, 1990; 1998; 2002).

Studies have also shown that legume lectins possess hydrophobic binding sites in addition to their carbohydrate binding sites. Most legume lectins bind strongly to carbohydrates using Ca^{2+} ion, and a transition metal ion like Mn^{2+}. These metal ions are so essential that if the lectin is treated with acid to remove the metal ion, the lectin loses its carbohydrate binding property (Sharon & Lis, 1990)**.** This means that a legume lectin requires positive electrostatic forces for it binding to carbohydrates. The positive charge on the lectin binds to the negative charge on a cell-surface carbohydrate. Another important feature of legume lectins is that they are generally glycoproteins; up to 10% of the lectin is a carbohydrate. Currently, it is known that this carbohydrate portion plays no role in the sugar

binding properties of legume lectin; when the carbohydrate is removed, the lectin still binds to specific sugars (Sharon & Lis, 1990). Legume lectins, like any typical lectin, differ highly in their carbohydrate specificity; this has been examined by analytical methods like equilibrium dialysis, spectrophotometry, fluorimetry, and nuclear magnetic resonance. The binding constants obtained from these studies show that legume lectins bind stronger to oligosaccharides than to monosaccharides (Sharon & Lis, 1990).

Four main characteristics are thus expressed by legume lectin as a typical lectin: shallow depressions serving as carbohydrate recognizing sites; electrostatic forces of attraction envisaged by the presence of positively charged ions; a carbohydrate portion attached to a polypeptide which plays no role in its sugar recognition; and finally, the polypeptide chain known to generate the hydrogen bonds between the nitrogen (N) of the lectin and the oxygen (O) of the hydroxide of the specific sugar the legume lectin is likely to bind to. These are the important characteristics found in lectins that we seek to mimic. Three of these characteristics are also very essential in lectin-carbohydrate complex formation.

2.4. Lectin mimics as artificial carbohydrate receptors

Two important ideas come to mind considering cell-surface carbohydrates and lectins: cell surface carbohydrates changes due to health status of the cell (Baba & Catoi, 2007) and lectins are specific in the type of carbohydrates they bind to (Berg et al., 2002; Sharon & Lis, 1998). These two phenomena can then be called into play in considering research work into their applications in biomedical sciences. Lectins can be used to investigate the changes in cell-surface carbohydrates which can then give a clue to the health status of a cell, tissue or organ.

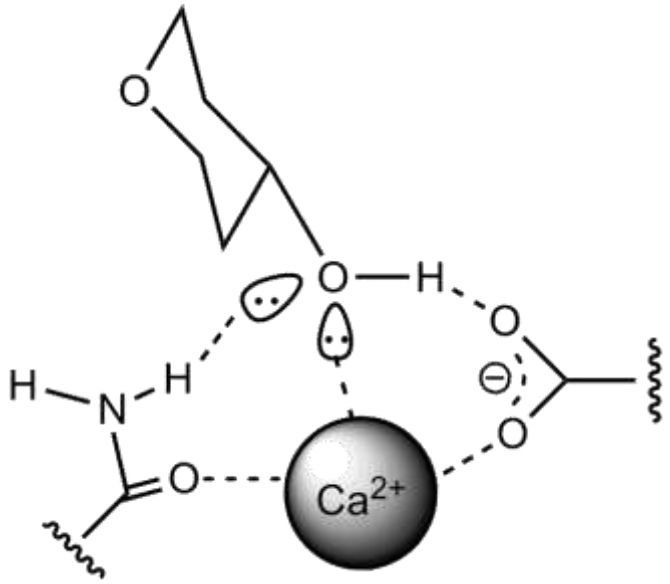

Figure 2: A diagram showing various types interactions in lectin-carbohydrate binding. There is an electrostatic force of attraction between the positively charged Ca^{2+} ion and the O in the carbohydrate or in any amino acid with an O; hydrogen bonds between the O of the OH in the carbohydrate and the H of the NH of the amino acid (Protein - Carbohydrate binding, by Goldon999 - Licensed under Creative Commons).

The information deduced from the investigation into the changes in cell-surface carbohydrates can be used to predict the onset of certain diseases like cancer and malignant tumor (Tao et al., 2008). Lectins can be employed as carrier systems for target drug delivery to different diseased tissues in the body. This is another plausible research area which is still under investigation (Gupta et al., 2009). Research works are also ongoing to produce synthetic carbohydrates to cover specific cell surfaces as a clue in studying these cells if and when they are diseased; and as carrier systems to deliver vaccines for cancer therapy (Zhu et al., 2009).

The research into the use of synthetic lectins to study diseased cells still faces some challenges. The size of lectin as a protein molecule, the nature of proteins and their stability under harsh body conditions show that using smaller molecules as lectin mimics may work

more effectively in these areas of research already mentioned above (Jin et al., 2010). Lectins, being proteins may be easily destroyed by enzymes in the body. Also, the making and storing of lectins may be expensive because proteins are easily denatured by heat. It should also be noted that introducing lectins into the body can cause immune shock (Janeway et al., 2001). Binding affinities of lectins for natural carbohydrates in biological systems are very weak, although the carbohydrate and the lectin being multivalent enhance binding (Dam et al., 2000).

2.5. Rationale behind the design of Bipyridine Carbohydrate receptor

The goal in this research is to design a receptor molecule that can recognize carbohydrates. Such a carbohydrate receptor will be acting as a lectin mimic. There are two main hurdles that need to be overcome. The first is the three dimensional complex nature of the monosaccharide molecules. This makes it difficult for effective complex formation between a carbohydrate receptor molecule and the carbohydrate. The second is the ability of the carbohydrate receptor to have high specificity in differentiating between monosaccharides that are closely related such as stereoisomers (Inouye et al., 1995).

Earlier research has shown that binding occurs between aromatic groups with amino acid side chains when they come into contact with oligosaccharides moieties (Mazik et al., 2000). This phenomenon makes macrocyclic structures important scaffolds in the design of carbohydrate receptor molecules. Macrocyclic structures which contain aromatic groups such as polypyridine units contain lipophilic cavities which serve as binding sites. Such structures can be designed into carbohydrate receptors capable of using hydrogen bonding, electrostatic, hydrophobic, pi-pi and CH-pi interactions to bind to carbohydrates. Finally, the macrocyclic structures with the polypyridine units can exhibit strong absorption and fluorescence emission

bands. Changes in their absorption due to carbohydrate-receptor complex formation can be studied and stability constants of complexes formed can be determined and used in analyzing effectiveness of complexes formed (Mazik et al., 2000, Mensah & Cudic, 2011).

Prior research shows that carbohydrate receptors designed from macrocyclic structures containing 1,8-naphthyridine units bind effectively to carbohydrates in aqueous media (Mensah & Cudic, 2011). The presence of 1,8-naphthyridine units in the receptor is also known to increases the compounds affinity to carbohydrates in organic media (Mazik et al., 2000).

The 2,2-bipyridine moiety is the structure being considered in the design of carbohydrate receptor in this research work.

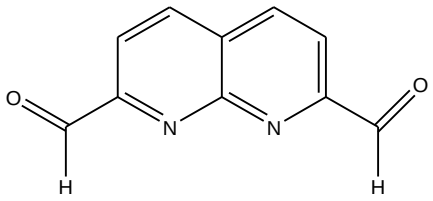

Figure 3: Naphthyridine moiety

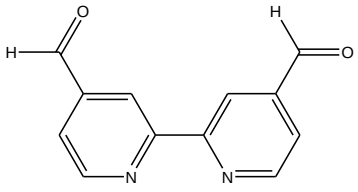

Figure 4: Bipyridine moiety

As shown in Figs. 3 and 4, the 2,2-bipyridine moiety resembles the 1,8-naphthyridine moiety and therefore it is being hypothesized that a carbohydrate receptor can be designed from 2,2-bipyridine moiety. Also, the structure of the bipyridine moiety shows that rotation of the rings is possible because the two aromatic rings are joined by a single bond, unlike in the case of the naphthyridine moiety in which the two aromatic rings are fused. The rotation in the bipyridine moiety will allow the proposed receptor to twist around and bind to sugars more strongly than the receptor produced from the naphthyridine moiety. The receptor produced from the bipyridine moiety is expected to have high entropy because of the flexibility of its building

blocks, but then, when it binds to sugars, it will lose entropy, which is expected to be gained in its enthalpy (Mensah & Cudic, 2011**).**

The purpose of this research is to design, synthesize, purify, characterize and to determine the binding constants of bipyridine-based macrocyclic carbohydrate receptors (see Figs. 5 and 6). The free amino ends of Receptor 1 however can be explored to link through coupling reactions to make a divalent receptor from Receptor 1 which will then have the ability to engage in more extensive hydrogen bonding. Receptor 2 (see Fig. 6) has no free amino groups at its ends. Dimerization of Receptor 2 to make it divalent to enhance its binding efficiency may not be possible using the coupling process that can be used to dimerize Receptor 1. The dimer of Receptor 1 (see Fig. 7) will be more multivalent and this will enhance binding and selectivity when it interacts with carbohydrates.

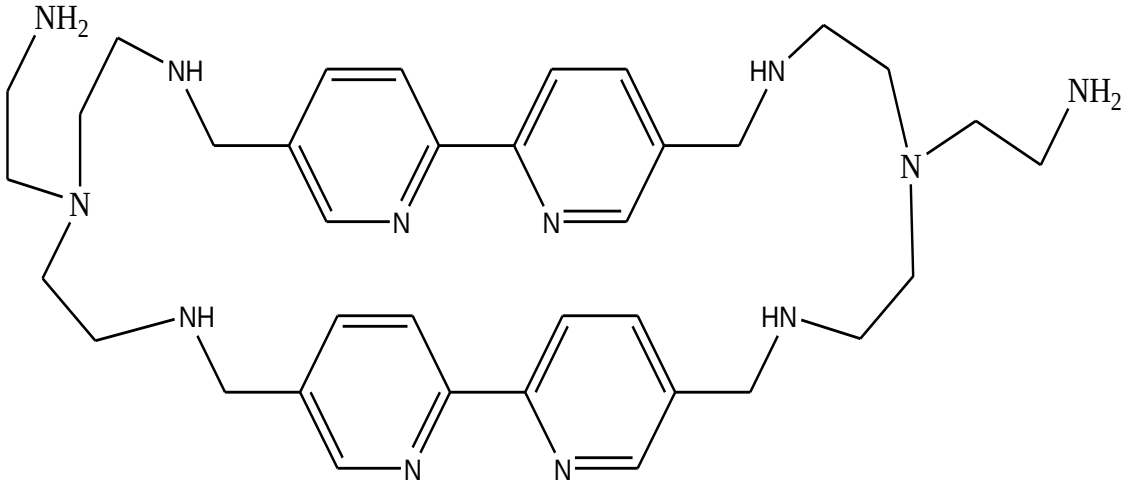

Figure 5: Receptor 1

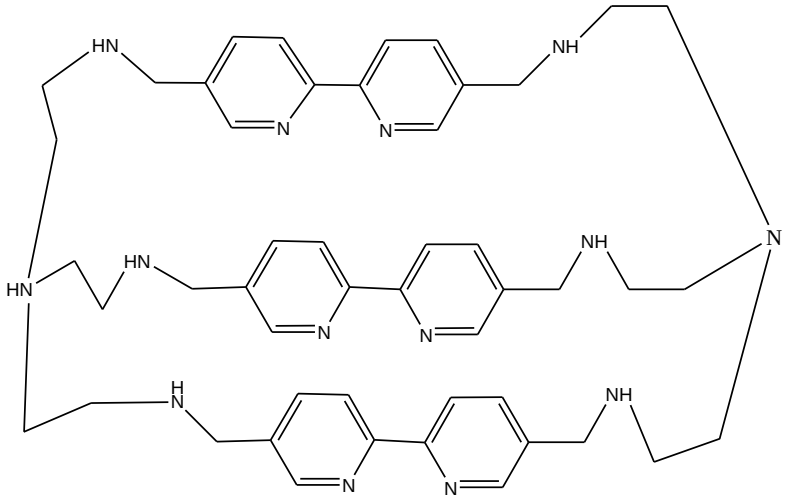

Figure 6: Receptor 2

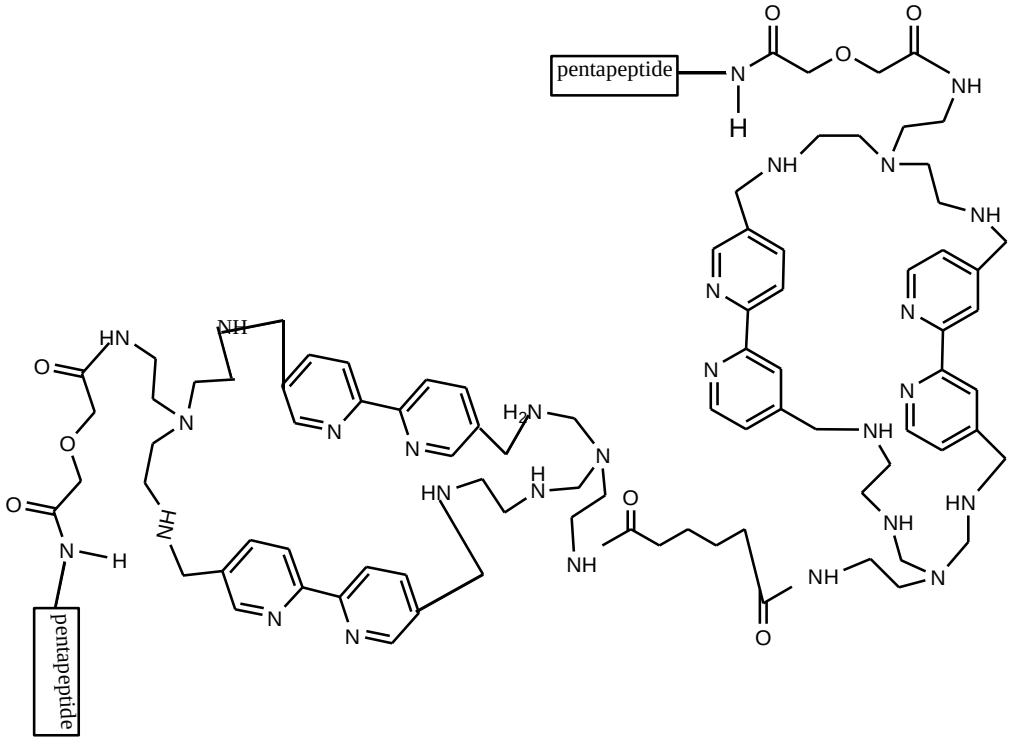

Figure 7: Expected dimerized product of Receptor 1

The macrocyclic bipyridine receptor molecule produced from the dimerization of Receptor 1 (Fig. 3) should be able to recognize and bind to sugars in aqueous media. In the near future, this work is expected to enhance the knowledge into how carbohydrates from cells of body tissues affected by cancer and inflammation can bind to macrocyclic polypyridine molecules which are acting as carbohydrate receptors. These receptors can then be used in other biomedical research works.

The objectives of this project are to:

1. Create a reaction pathway for the synthesis the polypyridine macrocyclic receptor and to use the reaction pathway created to synthesize the macrocyclic receptor.
2. To purify and characterize the receptor using HPLC, NMR and ESI-MS.
3. To study the binding properties of the receptor through spectrophotometric titration of the receptor with sugar substrates.
4. To prepare the divalent version of Receptor 1 using solid phase organic synthesis.

METHODS

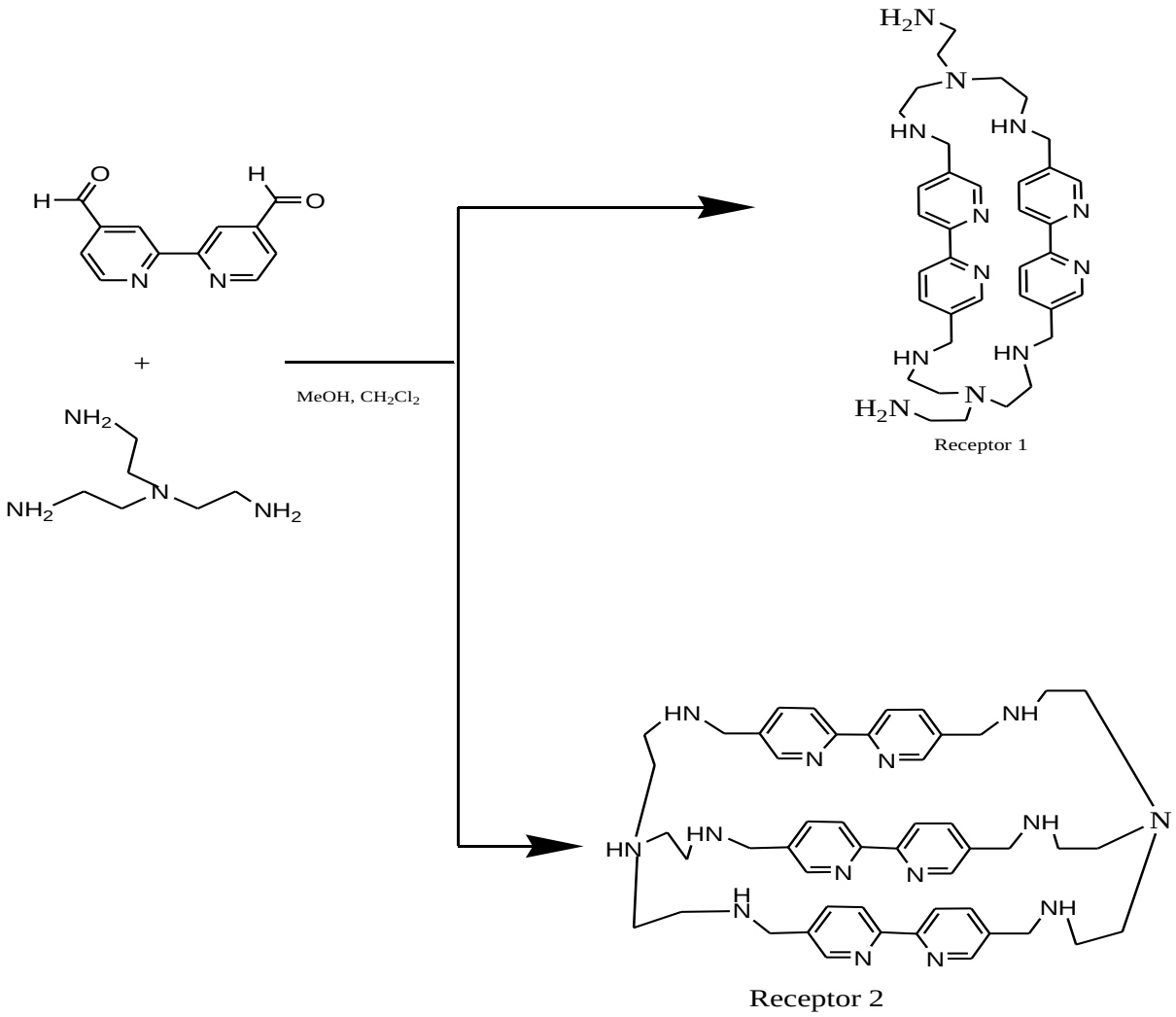

Figure 8: Synthetic scheme for production of Receptor 1 and Receptor 2

3.1.0. Receptor 1

The synthetic process adopted favors the formation of Receptor 1. Receptor 1 with its side amino groups and its symmetrical nature can be used to prepare the multivalent version of

the receptor which is expected to have enhanced binding properties. Receptor 2 on the other hand cannot be used to prepare a multivalent receptor.

3.1.1. Synthesis of Receptor 1

The synthesis of the polypyridine receptors (Receptor 1) was prepared by dropping a solution of Tris(2-aminoethyl)amine in acetonitrile/methanol mixture into a solution of 2,2-bipyridine-4,4-dicarboxyaldehyde in acetonitrile/methanol mixture. To favor the formation of Receptor 1, equal amounts of Tris(2-aminoethyl)amine and the 2,2-bipyridine-4,4-dicarboxyaldehyde were used. The Tris(2-aminoethyl)amine solution was prepared by dissolving a 1.7 mmol of Tris(2-aminoethyl)amine in CH_3CN/CH_3OH (20 mL, 1:1 V/V) mixture. The 2,2-bipyridine-4,4-dicarboxyaldehyde solution was also prepared by dissolving a 1.7 mmol of 2,2-bipyridine-4,4-dicarboxyaldehyde in CH_3CN/CH_3OH (150 mL, 1:1 V/V) mixture. The Tris(2-aminoethyl)amine solution in a dropping funnel was then added drop by drop to the 2,2-bipyridine-4,4-dicarboxyaldehyde solution in a round-bottom flask for a period of 30 minutes, with both solutions at room temperature and under an atmosphere of nitrogen.

The resulting solution was stirred at room temperature for 18 hours using a magnetic stirrer. After that, the solution was evaporated under reduced pressure without heating. The solid formed was washed with diethyl ether. After that, the resulting solution was dried under vacuum to yield a yellow powder. The yellow powder was dissolved in a CH_2Cl_2/CH_3OH (150 mL, 2:1 V/V) mixture and cooled to 0^oC. An 87.0 mg of $NaBH_4$ was added slowly, and the solution was stirred for 4 hours, while maintaining the temperature at 0^oC. The synthetic process was complete after this. The solution was evaporated. The residue left was dissolved in water to

stop the reaction. The solution formed was extracted three times, each with a 50 mL CH_2Cl_2/CH_3OH (9:1 V/V) mixture.

3.1.2. Characterization of the Receptor

Purification was carried out by dissolving the extract in methanol and using HPLC to extract the receptor component. The structure of the receptor formed, the mass, as well as which of the two receptors was formed was determined using HPLC, NMR and ESI-MS on both the organic and aqueous extract of the residue.

3.1.3. Titration of the Receptor with sugars

A 50 mM phosphate buffer with a pH of 6.5 was prepared by weighing 7.05 g of sodium sulfate and dissolving it in distilled water to form 1L of solution. The pH was determined and adjusted to 6.5 by adding drops of HCl or NaOH to the solution. The phosphate buffer was used as a solvent to prepare a solution of the receptor for the titration experiment. 2mL of the receptor solution was used to dissolve a weighed amount of a named sugar to form the sugar solution. This sugar solution was then used for titration.

2 mL of buffer solution was put into the first cuvette to serves as control in the UV-Vis spectrophotometer. 2mL of the receptor solution was then put in the second cuvette. The first reading was taken of the receptor solution. Then aliquots of the sugar solution (in 20µL and 50 µL increments) were added to the receptor solution in cuvette 2. The readings were taken for the solution in cuvette 2 after each aliquot has been added. The readings were recorded, and the data obtained was used in calculating the binding constants. The binding constants (association constants) were calculated using the ReactLab™ equilibria.

The binding constants were used to determine the binding efficiency between the receptor and the sugar. This titration process was repeated for a selected number of sugars. The data produced were compared to find out which sugar binds more effectively to the receptor and those characteristics of the sugar which enable the effective binding was analyzed. The sugars used for the titration were D-fructose, D-Galactose, D-maltose monohydrate, D-glucose-6-phosphate sodium salt, α-D-glucose-1-phosphate disodium salt hydrate and N-acetyl neuraminic acid.

3.2.0. Design of multivalent Receptor

The synthesis of polyvalent or multivalent version of Receptor 1 was made by first synthesizing a polypeptide from individual amino acids using solid phase synthesis. The polypeptide was then linked to Receptor 1 via diglycolic anhydride, also using solid phase synthesis. The resulting substance was then dimerized using adipic acid.

3.2.1. Solid phase synthesis of a pentapeptide on NovaPeg organic resin

The diagram below shows the four main processes involved in the synthesis of the pentapeptide (polypeptide). It involves functionalization of a NovaPeg organic resin by linking the first amino acid to the organic resin. This is then followed by testing for the effectiveness of the coupling reaction. Deprotection of the coupled amino acid follows, and then another amino acid is linked to the first one. The processes of deprotection, coupling and testing the coupling effectiveness are rotated until the five amino acids have been linked together.

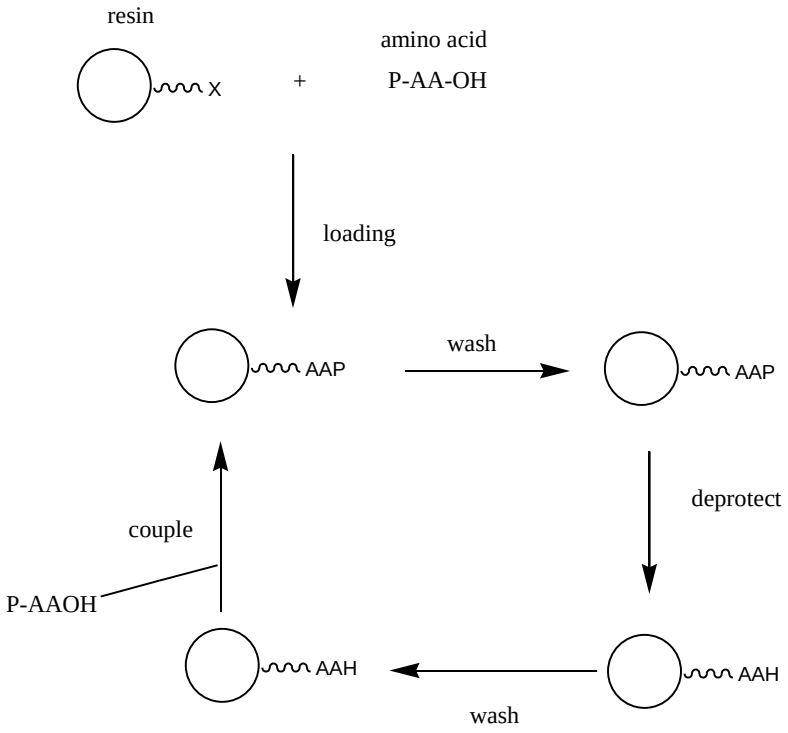

Figure 9: The general scheme for the coupling reaction

3.2.2. Functionalization of the NovaPeg organic resin

The following chemicals were weighed using a balance: 0.3 mmol of NovaPeg organic resin, 1.5 mmol of Fmoc AA_1 (Fmoc-Ala-OH), 1.47 mmol of HBTU (N,N,N',N'-Tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate), 3.0 mmol of DIPEA ((N,N-Diisopropylethylamine).

The weighed NovaPeg™ organic resin was swollen in DMF (N,N-Dimethylformamide) for one hour. The DMF was then drained off. The HBTU and Fmoc AA_1 were dissolved in 6 mL of DMF. The weighed amount of DIPEA was then added to this solution. The resulting solution was added to the swollen NovaPeg™ organic resin.

The resulting mixture was shaken for 3 hours using a Labquake™ shaker. After the coupling reaction, the functionalized resin was washed thoroughly with DMF and then with DCM (Dichloromethane). To remain swollen for subsequent stages of the work, the functionalized resin was stored in 6 mL of DCM.

3.2.3. Testing the effectiveness of the coupling

The effectiveness of the coupling was tested using the UV-VIS spectrophotometer, as well as, performing Kaiser Test on the functionalized resin sample.

First, a piperidine/DMF (2:8 V/V) mixture was prepared and used as the solvent for the testing. 3mL of the solvent was put into the first UV cuvette to serve as control. 1mg of the dry functionalized resin was weighed, and 3 mL of the piperidine/DMF (2:8V/V) mixture was added to the sample for 4 minutes. The solvent was then put in the UV cuvette. The control and the sample were both put into the UV-VIS Spectrophotometer and the absorbance recorded at 301nm. The percentage yield was then calculated using the equation:

$$\textbf{mmol/g} = (Abs_{solution} - Abs_{reference}) / 1.28 \times \text{mg of sample}$$

A yield of more than 90 % was observed.

Second, the Kaiser Test was done on the sample to confirm the results obtained from the UV-VIS Spectrophotometer. The three solutions needed for the Kaiser Test were prepared. The first solution (Kaiser Test Solution A) was prepared by dissolving16.5 mg of potassium cyanide (KCN) in 25 mL of distilled water and 1 mL of this solution was added to 49 mL of pyridine. The final solution was poured into a reagent bottle and labeled as Kaiser Test solution A. The second solution was prepared by dissolving 1.0 mg of ninhydrin in 20 mL of ethanol. The final solution was poured into a reagent bottle and labeled as Kaiser Test solution B. The third solution was

prepared by dissolving 40 g of phenol in 20 mL of ethanol. This solution was poured into a reagent bottle and labeled as Kaiser Test solution C.

The Kaiser Test was then done on some of the functionalized resin using the three Kaiser Test solutions. A few of the functionalized resin beads were placed in an Eppendorf tube. The beads were rinsed five times with ethanol to ensure that no unreacted coupling reagents were present in the sample being tested. Two drops each of the three Kaiser Test solutions were added to the resin and heated at 100^{o}C for 5 minutes. The color of the mixture changed initially from colorless to brown, indicating that the first coupling was complete.

3.2.4. Forming the dipeptide

The stored functionalized resin was taken and the DCM drained off. The functionalized resin first went through deprotection. This was done by treating the functionalized resin with a piperidine/DMF (2:8) mixture for 5 minutes. After that, the solution was drained off and the resin washed two times with DCM. The resin was treated again with the piperidine/DMF mixture for another 5 minutes, and finally, washed four times with DMF, and then, four times again with DCM. A few of the functionalized resin beads was tested by a Kaiser Test. A blue coloration confirmed that the amino (NH_2) group in the alanine was free and available for the next stage of the reaction.

The second Fmoc protected amino acid, Fmoc AA_2 or Fmoc-Lys-D-OH was joined to the first amino acid by a coupling reaction. A 1.5mmol of Fmoc AA_2, 1.47 mmol of HBTU, 1.5 mmol of HCTU (*O*-(6-Chlorobenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate) and 3.0 mmol of DIPEA were weighed. The weighed amounts of Fmoc AA_2, HCTU and HBTU were dissolved in 6mL of DMF. The weighed amount of DIPEA was then added. The final

mixture was added to the swollen functionalized resin. The mixture was shaken for two hours at room temperature, using the Labquake™ shaker. After the coupling reaction, the functionalized resin was washed four times with DMF and then, washed again four times with DCM. The resin was stored in 6 mL of DCM for the subsequent stages of the reaction. A Kaiser test performed on a sample of the resin gave brown coloration showing that the coupling was complete.

3.2.5. Forming the pentapeptide

The three processes: deprotection to remove the Fmoc protection; coupling reaction to link the next amino acid to the previous one; and a Kaiser Test to confirm the effectiveness of the coupling reaction, was repeated three more times in succession to link three more Fmoc protected amino acids (Fmoc –Asp(OALL)-OH, Fmoc-Met-OH and Fmoc-MePhe-OH) to the dipeptide that was formed in 3.2.1.3 to form the pentapeptide chain.

3.2.6. Linking the diglycolic anhydride to the pentapeptide

The functionalized resin, which now consists of the NovaPeg™ organic resin linked to the pentapeptide, was deprotected. A Kaiser test conducted on this polymer to ascertain the availability of the free NH_2 group yielded a blue coloration which showed that a free NH_2 group was available. The following quantities of chemicals were weighed: A 1.5mmol of diglycolic anhydride, 1.47 mmol of HBTU, 1.5 mmol of HCTU, and 3.0 mmol of DIPEA. These substances were dissolved in DMF and added to the functionalized resin. The reaction mixture was shaken for two hours using the Labquake™ shaker. A Kaiser Test on the product showed brown coloration indicating that the coupling was complete.

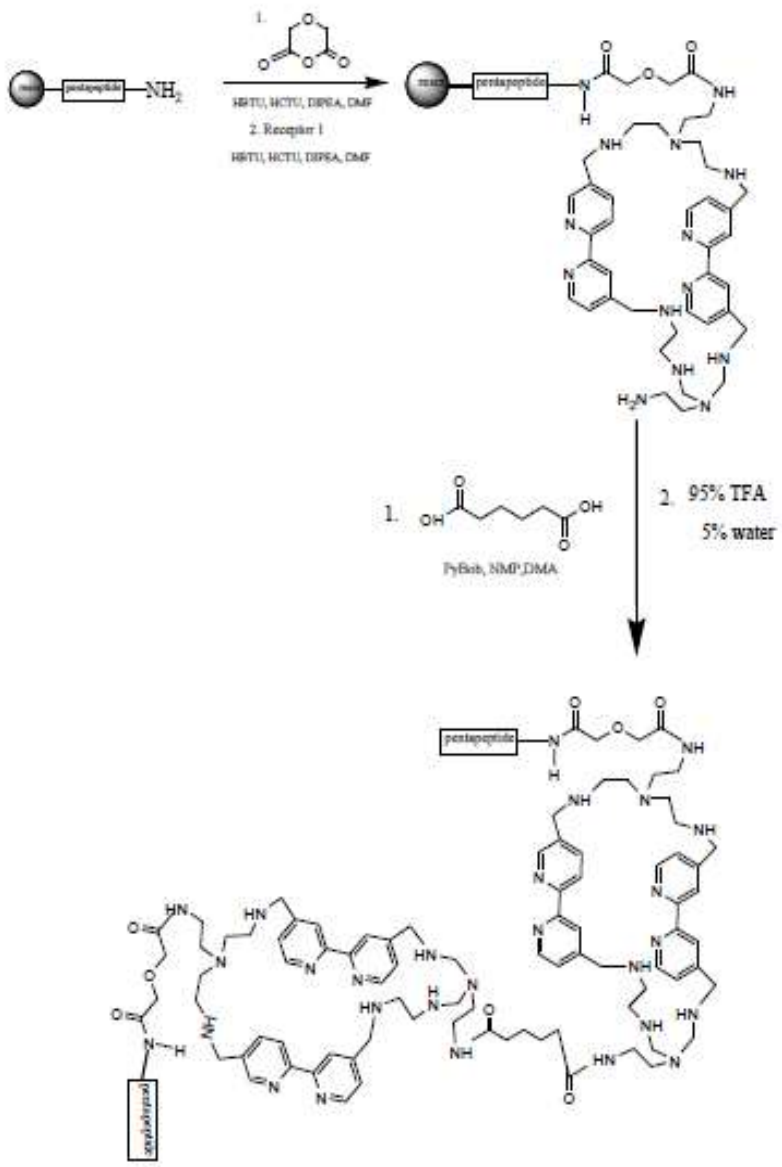

Figure 10: Linking diglycolic anhydride to Receptor 1 and the dimerization of Receptor 1 using adipic acid.

3.2.7. Coupling the pentapeptide to the Receptor 1

Equimolar amounts of Receptor 1 and the polymer produced in 3.2.2.1 were mixed with weighed amounts of HBTU, HCTU and DIPEA. The mixture was dissolved in DMF and taken through a coupling reaction. The product, which was the pentapeptide, the diglycolic anhydride

and Receptor 1 all linked and still linked to the resin, was kept for the next stage of the synthesis.

3.2.8. Dimerization of Receptor 1

Weighed amounts of adipic acid, PyBop, and NMP were dissolved in DMA to form an activated ester solution. The activated ester solution was added to the resin and shaken for four hours. The resin was washed with DMF and then, washed with DCM. Another activated ester solution of adipic acid, PyBop and NMP dissolved in DMA were added to the resin and shaken for 4 hours. The resin was washed with DMF and then with DCM.

3.2.9. Cleavage of the compound from the resin

A TFA/H_2O mixture (95%/5% V:V) was prepared and added to the resin. The mixture was shaken for 2 hours. The solvent was drained into an Erlenmeyer flask. The resin was washed with more of the TFA/H_2O mixture. Cold Et_2O was added to precipitate the product leaving behind the resin.

RESULTS

The main objective in this research was to synthesize Receptor 1, purify and characterize the receptor produced; study the binding properties of the receptor with sugar substrates and to prepare a divalent version of Receptor 1 using solid phase synthesis.

The receptor produced (Receptor 1) was mainly detected in the organic layer during the characterization. Evidence for this was from the analytical results from HPLC, NMR and ESI-MS.

4.1. Result from HPLC

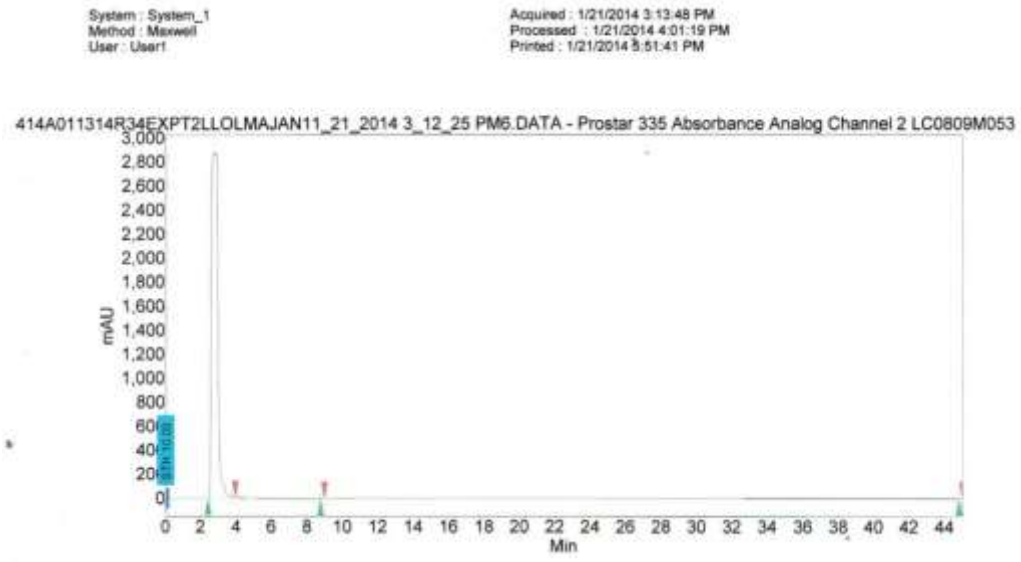

Figure 11: HPLC photograph of Receptor 1

The HPLC showed a single peak after the original crude product from the extraction process had undergone several extraction processes using HPLC. Figure 10 shows a single peak in that HPLC diagram, signifying a pure extract.

4.2. Result from NMR characterization of Receptor

^{1}H NMR (CD_3OD, 400 MHz) δ: 7.79, 7.18 (2-pyridine), 2.75, 3.81 (CH_2 or methylene).

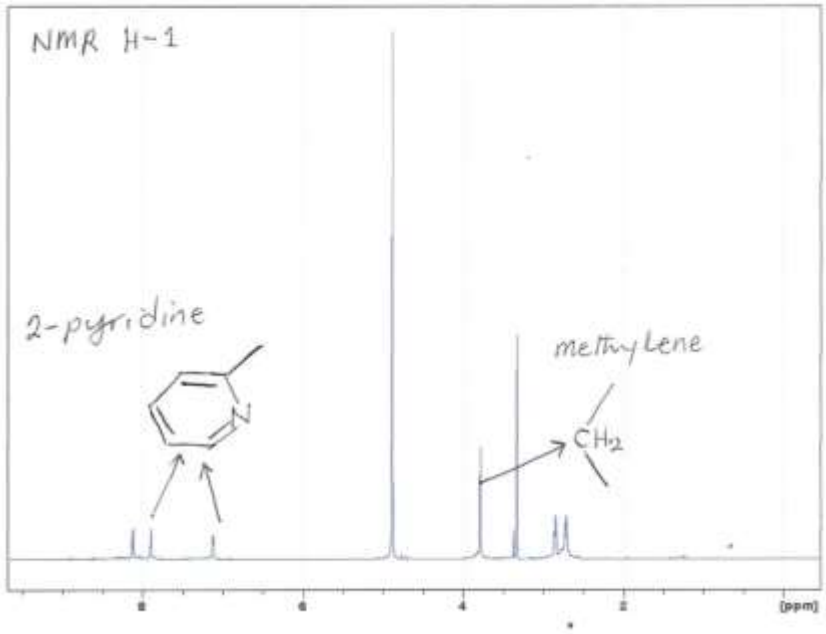

Figure 12: NMR H-1 for Receptor 1 (peaks and structure)

^{13}C NMR (CD_3OD, 400 MHz) 149.8, 123.6 (2-pyridine), 49.1, 51.1 (aliphatic).

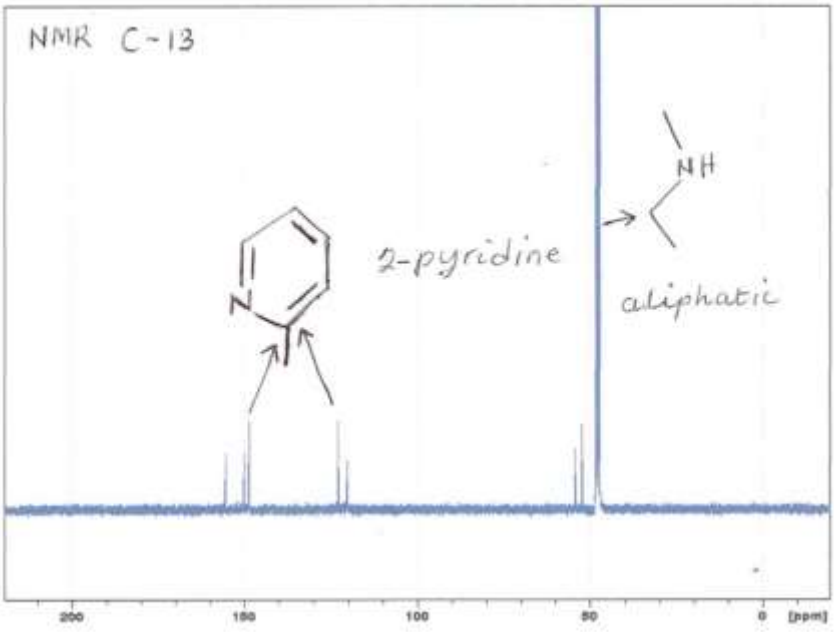

Figure 13: NMR C-13 (Peaks and structures)

There were other peaks in the NMR data. These peaks comes from the solvent (methanol) was used to dissolve the receptor for the analysis.

4.3 Result from Characterization with ESI-MS

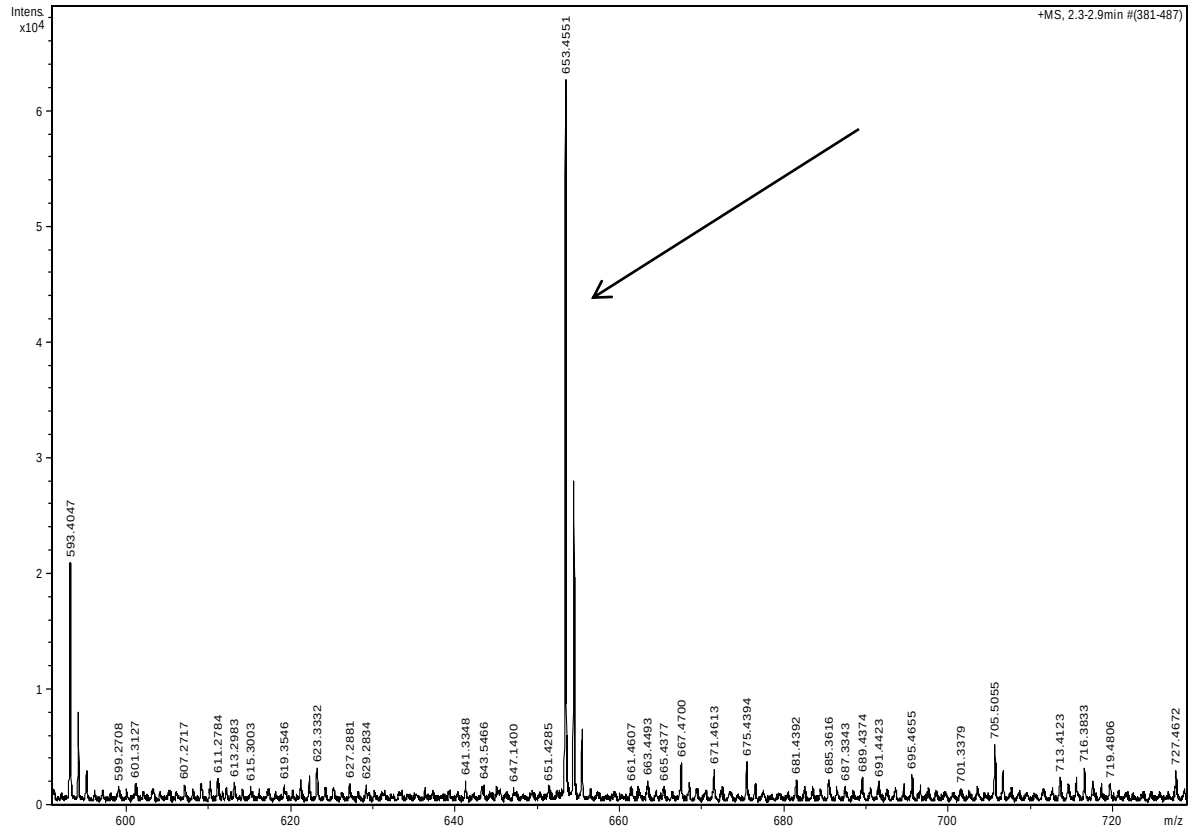

Figure 14: HR-ESI Spectrum of Receptor 1

In Fig. 14, the diagram for the mass spectrum shows a peak at mass 653.4551 [M+1].This mass was in agreement to the calculated mass of Receptor 1 with molecular formula $C_{35}H_{52}N_{12}$.

4.4. Binding Studies

The carbohydrate substrates used for the titration against Receptor 1 were commercially available. The six selected sugars were D-fructose, D-galactose, D-maltose monohydrate, D-

glucose-6-phosphate sodium salt, α-D-glucose-1-phosphate disodium salt hydrate, and N-acetylneuraminic acid. The curve obtained in all cases where the sugar was able to bind to the receptor shows that the absorbance decrease as the volume of the sugar solution added increases (for the raw data). The raw data was further processed to obtain the dissociation constants using the ReactLab™ equilibria.

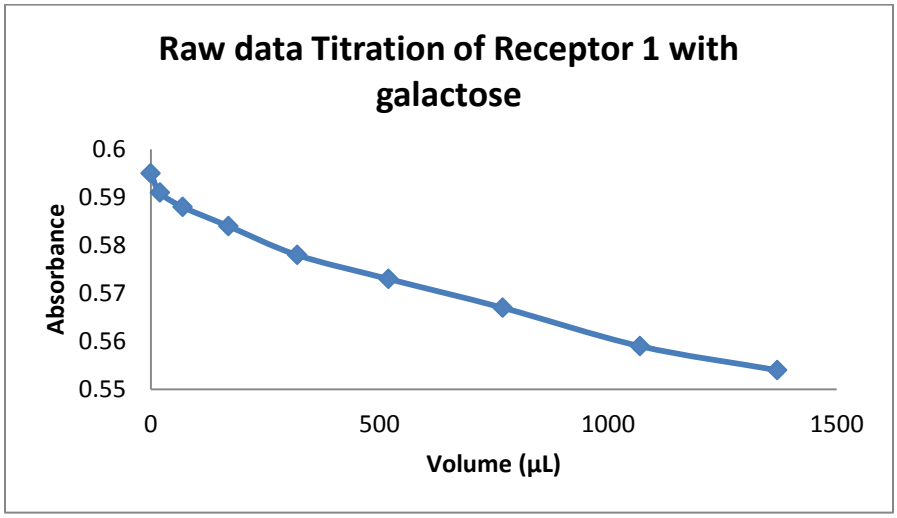

Figure 15: A plot of absorption against volume of sugar solution for the titration of Receptor 1 using D-Galactose (0.0278 molar) @ 284.5 nm.

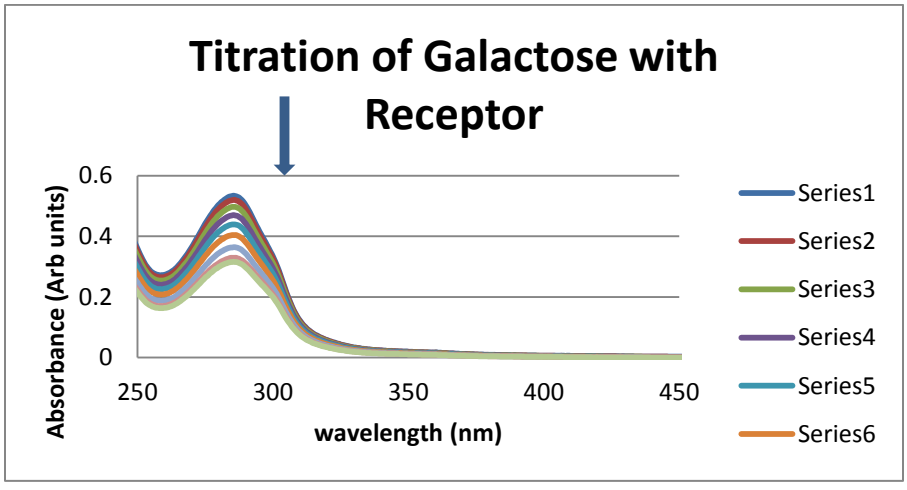

Figure 16: A plot of absorption against wavelength for the titration of Receptor 1 using D-Galactose (0.0278 molar)

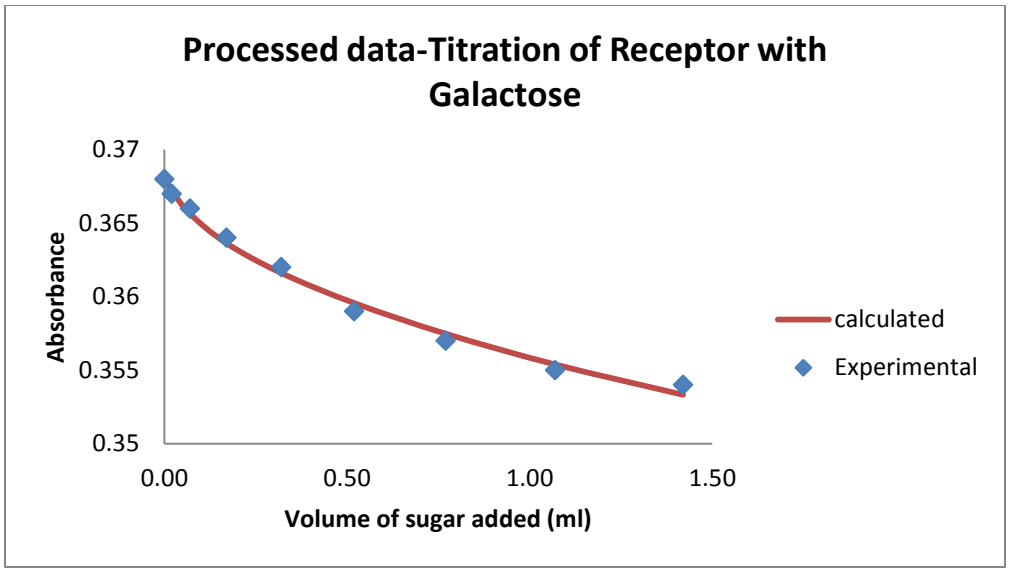

Figure 17: A plot of absorption against volume for the titration of Receptor 1 using D-Galactose (0.0278 M) @ 284.5 nm.

Table 1: Binding (dissociation) constants for the titration between receptor 1 and the six selected sugars

Sugars	Average K_d /M
D-fructose	1.5488×10^{-4}
D-galactose	3.6539×10^{-4}
D-maltose monohydrate	ND
D-glucose-6-phosphate sodium salt	ND
α-D-glucose-1-phosphate disodium salt hydrate	ND
N-acetylneuraminic acid	2.6903×10^{-4}

Table 1 shows that three out of the six sugars titrated against the receptor were able to bind to the receptor. The three sugars that were able to bind to Receptor 1 are D-Fructose, D-Galactose and N-acetylneuraminic acid (also known as sialic acid).

DISCUSSION

5.1. Synthesis of Receptor

The main reactants for the synthesis of the receptor are Tris(2-aminoethyl)amine and 2,2-bipyridine-4,4-dicarboxyaldehyde. The reaction follows the general nucleophilic addition reaction between an aldehyde and a primary amine followed by elimination of water. The Tris(2-aminoethyl)amine is behaving as a typical primary amine whiles the 2,2-bipyridine-4,4-dicarboxyaldehyde is behaving as an aldehyde.

$$RCOH + RH^{I} + R^{II}NH_2 \longrightarrow RCH{=}NR^{II} + H_2O$$

The reaction lead to the formation of an imine in the yellow powder obtained. The imine formed was then reduced when the reducing agent sodium borohydride was added to form another amine, this time, an amine with the aromatic groups in the 2,2-bipyridine-4,4-dicarboxyaldehyde in it to give us the desired receptor.

$$RCH{=}NR^{II} \xrightarrow{\text{Sodium borohydride}} RCHNHR^{II}$$

The synthetic process was not without challenges. There is the possibility of forming Receptor 1 or 2 depending on the mole ratio. Since Receptor I was preferred because of it amino group side chains, its flexibility in conformation as well as it symmetrical nature, the reaction scheme had to be manipulated to a 1:1 ratio. Nevertheless, some amount of Receptor 2 was formed

and the mixture had to be purified. HPLC was used to purify and this involved several runs to obtain enough sample for characterization and titration.

The coupling process in the production of the pentapeptide was difficult because amino acids require multiple coupling before they could link to the resin or the functionalized resin. This could have been eliminated by acquiring effective coupling apparatus which were expensive and unavailable to me at the time of this research.

The sugars selected based on certain reasons. The six sugars were available and economical to obtain. Also, these sugars have already been tested on the 1,8-Naphthyridine receptors 3 and 4 with good results. So, their selectivity was to be confirmed with Receptor 1 and more importantly, their binding efficiency was to be studied with Receptor 1.

5.2. Selection of Sugar substrates

The dissociation constants (K_d) shows that Receptor 1 binds effectively to neutral monosaccharides such as galactose and fructose as well as sialic acid which is negatively charged. Affinities towards other sugar substrates tested, which include maltose monohydrate (neutral), Glucose-6-phosphate sodium salt and glucose-1-phosphate disodium salt hydrate (negatively charged) were non-determinable.

5.3. Binding

The binding constants of Receptor 1 and the selected sugars were compared with those of two naphthyridine receptors. These two receptors have been named as Receptor 3 and Receptor 4.

Table 2: Comparison of the dissociation constants for 2,2-bipyridine and 1,8-naphthyridine receptors. (NT means “not tested” and ND means “tested but not determined”).

Sugar substrate	K_d (M) value with Receptor 1 (a bipyridine receptor)	K_d (M) value with Receptor 3 (a 1,8-naphthyridine Receptor	K_d (M) value with Receptor 4 (a 1,8-naphthyridine Receptor
D-galactose	3.7×10^{-4}	1.6×10^{-3} (± 0.1)	$\geq 1.0 \times 10^{-2}$
D-glucose	NT	8.9×10^{-3} (± 0.9)	$\geq 1.0 \times 10^{-2}$
1,5-anhydroglucitol	NT	$\geq 1.0 \times 10^{-2}$	$\geq 1.0 \times 10^{-2}$
2-deoxy-D-glucose	NT	$\geq 1.0 \times 10^{-2}$	$\geq 1.0 \times 10^{-2}$
3-deoxy-D-glucose	NT	$\geq 1.0 \times 10^{-2}$	$\geq 1.0 \times 10^{-2}$
D-fructose	1.6×10^{-4}	6.2×10^{-3} (± 0.5)	$\geq 1.0 \times 10^{-2}$
D-xylose	NT	$\geq 1.0 \times 10^{-2}$	$\geq 1.0 \times 10^{-2}$
D-ribose	NT	$\geq 1.0 \times 10^{-2}$	$\geq 1.0 \times 10^{-2}$
D-glucose-1-phosphate	ND	5.6×10^{-3} (± 0.1)	4.3×10^{-3} (± 0.1)
D-glucose-6-phosphate	ND	1.9×10^{-3} (± 0.1)	1.4×10^{-3} (± 0.05)
sialic acid	2.7×10^{-4}	0.3×10^{-3} (± 0.01)	5.6×10^{-3} (± 0.3)
muramic acid	NT	$\geq 1.0 \times 10^{-2}$	2.5×10^{-3} (± 0.3)
D-trehalose	NT	3.2×10^{-3} (± 0.1)	$\geq 1.0 \times 10^{-2}$
D-gentiobiose	NT	6.3×10^{-3} (± 0.6)	$\geq 1.0 \times 10^{-2}$
D-maltose monohydrate	ND	NT	NT

Multipyridine macroreceptors are known to bind to sugars, especially monosaccharides and oligosaccharides in aqueous solution (Mazik et al., 2000). These receptors behave like natural lectins. They mimic lectins by binding to these sugars through charge-charge

interactions, hydrogen bonding and hydrophobic interactions. The hydrogen bonding interactions occurs between the hydroxyl groups in the monosaccharides and the amino groups in the Receptor. The charge-charge interaction occurs between protonated amino groups and lone pairs of electrons on the hydroxyl groups in sugars. In sialic acids, there are negative charges due to the carboxylic functional group. The hydrophobic interaction occurs from the pi-pi stacking interactions created by the aromatic rings with the CH of the sugars (Mazik et al., 2000, Mensah & Cudic, 2011).

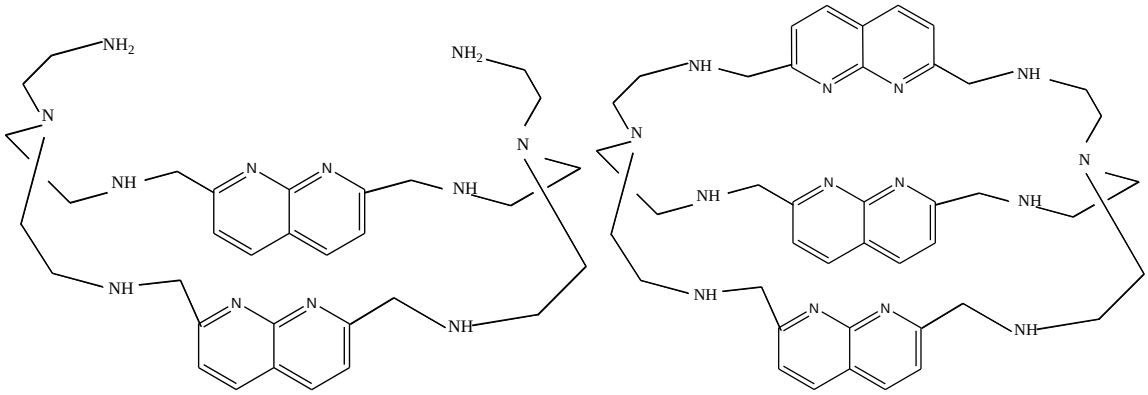

Figure 18: **Receptor 3 (**1,8-Naphthyridine dimer) and **Receptor 4** (1,8-Naphthyridine trimer). These are two other pyridine-based carbohydrate receptors from a different research. They were found to bind to similar sugars that also bind to receptor 1 (Mensah & Curic, 2011).

Natural lectins are carbohydrate-binding structures. Lectins are highly selective in their carbohydrate substrates (Berg et al., 2002; Sharon & Lis, 1998). Receptor 1, serving as a lectin mimic, was able to bind to three out of the six monosaccharides. This shows that Receptor 1 exhibits selectivity. However, it also shows that Receptor 1 exhibits low selectivity because it was able to bind to three different monosaccharides instead of one. Binding to only one monosaccharide could have would have made Receptor 1 highly selective.

Previous work on the binding effects of 1,8-naphthyridine receptors on similarly selected monosaccharides showed that the conformational flexibility of the receptor is of importance in discussing the degree of selectively of the receptor in question. The more flexible the receptor in its conformational structure, the less selective the receptor is expected to be towards monosaccharide substrates (Mensah & Cudic, 2011). For example, Figs. 16 and17 show two different receptors made from 1,8 naphthyridine. Receptor 3 is more flexible than Receptor 4 in terms of conformational structure. Table 2 shows that out of 14 monosaccharides titrated against the two receptors, Receptor 3 was able to bind to 8, compared to receptor 4 which was able to bind to 4 (Mensah & Cudic, 2011). Receptor 3, being more flexible in terms of conformational structure, was able to bind to more sugars than Receptor 4.

The flexibility in the structure of the 1,2-bipyridine, due to the allowed rotation in it, makes Receptor 1 even more flexible than either of the two 1,8-Naphthyridine receptors 3 and 4. This implies that Receptor 1 was expected to be less selective based on this phenomenon alone. However, Table 2 shows that certain sugars were able to bind to Receptor 3 but were not able to bind to Receptor 1 despite the fact that structural conformation makes Receptor 1 more flexible, and hence, less selective than Receptor 3. D-glucose-6-phosphate sodium salt and α-D-glucose-1-phosphate disodium salt hydrate were two monosaccharides that were able to bind to Receptor 3 but were not able to bind to Receptor 1. This shows that apart from structural conformation, other factors play a role in the Receptor's (Receptor 1) selectivity.

Previous research has shown that the three sugars that were able to bind with Receptor 1, also binds to selected natural lectins which have N-terminal amino acid. Galactose binds to a lectin isolated from the venom of *Bothrops jararaca*, a venomous snake. This lectin has an N-

terminal amino acid and is a C-type lectin (Ozeki et al., 1994). Fructose also binds to a lectin isolated from *Musa acuminate* (Del Monte banana) and this lectin also has an N-terminal amino acid. (Cheung et al., 2009). N-acetyl neuraminic acid (Sialic acid) in nature binds to selectins, which also have an N-terminal amino acid (Varki et al., 2009). Other research works also shows that Galactose and Sialic acid binds to C-type lectins which include selectins (Varki et al., 2009).

Receptor 1 was able to bind to fructose and galactose but was not able to bind to glucose. This phenomenon may be explained by the fact that, in aqueous solutions, both galactose and fructose have a significant percentage of the furanose form (64% for galactose and 22% for fructose, as compared to 0.3% for glucose) in equilibrium with the pyranose form (Neuman, 2013). It is likely that Receptor 1 forms a stable structure with the furanose form as compared with the pyranose form.

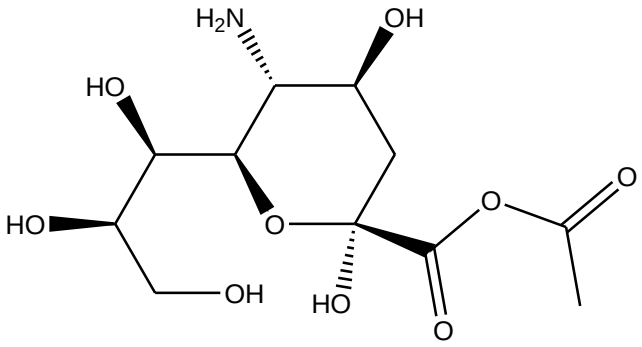

Figure 19: structure of acetyl neuraminic acid

Acetyl-neuraminic acid was able to bind to Receptor 1. This may be due to the fact that there was an extensive hydrogen bonding between Acetyl-neuraminic acid and the receptor because of the NH, NH_2 and OH groups. The structure of acetyl-neuraminic acid (Fig. 17) shows OH groups and one NH_2 group. The carbonyl groups in the structure of acetyl-neuraminic acid

can possess charges due to resonance. Receptor 1 also shows NH and NH_2 groups in its structure. So, there can be charge-charge interaction between the negative charges generated by the carbonyl groups in Acetyl-neuraminic acid and Receptor 1. Also, hydrophobic interactions could have resulted from the aromatic groups in the receptor and the CH structures in Acetyl-neuraminic acid. It is reasonable that Acetyl-neuraminic acid was able to bind to receptor 1 with all these three forces, and especially, the intensive hydrogen bonding in play.

The three sugars that were able to bind to Receptor 1 are also known to play vital roles in the studying of certain ailments in humans. Fructose, as a carbohydrate, is known to fuel cancer growth by providing an alternative carbon source for glycolysis (Ava et al., 2012). Fructose is, therefore, known to induce pancreatic cancer proliferation (Liu et al., 2010). There seems to be no evidence that fructose is a common cell-surface carbohydrate in cancer tissues. Fructose just provides energy for cancer cell growth. The main idea in this research was to design and synthesize a carbohydrate receptor that can bind to cell surface carbohydrates. This phenomenon can then be used to study early detection of cancer growth in human. Receptor 1, although it binds to fructose, cannot be used predict cancer growth based on its ability to bind to fructose. However, galactose, one of the sugars that was able to bind to Receptor 1, is known to play a role in cancer of the liver. Tumor cells in the liver express high galactose concentration on their surface. The galactose expression is known to be directly proportional to the liver metastasis (Yeatman et al., 1989). This means that Receptor 1 can be developed and used as a target marker to study the occurrence and growth of cancer in the liver. N-acetylneuraminic acid (or sialic acid), was another sugar that was able to bind to Receptor 1. It is known to occur

in high concentrations in malignant intracranial tumors and oral cancer (Xing et al., 1994). Receptor 1 can be developed to be used in early diagnosis and to study the development of these two types of cancer.

CONCLUSION

The experimental results from the HPLC, NMR, and ESI-MS show that Receptor 1, a macrocyclic 1,2 – bipyridine macromolecule, was synthesized from its reactants and under specific conditions already stated above. The experimental results also show that Receptor 1 was able to bind to three sugars – fructose, galactose and sialic acid. Receptor 1, therefore, can selectively recognize and bind to monosaccharides in aqueous solutions through intermolecular forces and other noncovalent interactions. The dissociation constants of receptor-sugar complexes ranged from 0.16 mM to 0.37 mM. Receptor 1 displayed selectivity which may be based on factors like flexibility of the receptor (Mensah & Cudic, 2011). Other factors like Receptor 1 having amine groups at both ends and the structure of the sugar in aqueous solution (pyranose or furanose) might have also played a role in the selectivity. The dissociation constant values show strong binding affinities between Receptor 1 and the three sugars. The strongest binding was between Receptor 1 and fructose. The K_d values were also consistent with binding constants between those sugars and some naturally occurring lectins. A galactose binding lectin from *Ricinus communis* beans has a K_d value of 1.2 mM when it binds to galactose (Podder et al., 1974). Del Monte™ Banana lectin, which is known to bind to fructose, does so at a K_d value of 3.12 mM (Cheung et al., 2009). Hemolymph lectin from the cricket *Teleogryllus commodus*, has a K_d value of 1×10^{-2} M when it binds to free sialic acid (Urich, 1990).

Based on the findings in this research, I conclude that Receptor 1 shows a new carbohydrate receptor which can have promising applications in bio-chemical and bio-medicinal fields in the future. Similar research on the design and synthesis of macrocyclic molecules are going on in

different parts of the world with an aim to find an alternative to the detection and treatment of cancer apart from the current chemotherapy.

Part of this research was to synthesize dimers of Receptor 1 via solid phase organic synthesis (Fig. 7). Part of this was done. Time did not allow for the dimer produced to be characterized and tested on its binding capabilities with sugars. In the future, a follow up to this research will be to re-synthesize the dimer (Fig. 7) and test its binding capability with sugars.

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