ISOLATION OF APTAMERS FOR GALACTONATE AND SYNTHESIS OF CRYPTOPHANE DERIVATIVE PRECURSORS

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By

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Abstract

Galactonate-binding aptamers were isolated using a technique called Systematic Evolution of Ligands by Exponential Enrichment (SELEX). This iterative process was able to remove unwanted aptamers from a pool of oligonucleotide library. The target molecule (galactonate-Shinkai complex) was eventually reduced to 250 and 10 µM after several rounds of SELEX and observing selectivity. During counter-selection however, it showed that the aptamers were heavily binding to 100 µM Shinkai. Additional SELEX rounds were applied to further enrich the DNA pool. Insertion of the isolated DNAs in *E. coli* did not provide enough bacterial colonies for DNA sequencing.

A 20-step synthetic route was proposed to synthesize two cryptophane molecules. The cryptophane-cage molecule is able to bind with ^{129}Xe which is used in contrast agents to improve signal detection. Precursors for a cryptophane cage molecule were successfully synthesized and characterized. Additional works are needed to complete the proposed steps.

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AND SYNTHESIS OF CRYPTOPHANE DERIVATIVE PRECURSORS

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Introduction

Aptamer Selection for Galactonate

Galactosemia is a genetic disorder wherein the body cannot properly break down galactose sugar into its metabolites.[1-3] Galactose is a sugar molecule that is commonly found in milk and other dairy products. Galactose metabolism is carried out in the Leloir pathway that eventually converts galactose into UDP-glucose (Figure 1).[2-4] The reactions in this pathway are catalyzed by three enzymes: galactokinase (GALK) converts galactose into galactose-1-phosphate (Gal-1-P), galactose-1-phosphate uridyltransferase (GALT) converts Gal-1-P into UDP-galactose, and UDP-galactose 4-epimerase (GALE) converts UDP-galactose into UDP-glucose.[2-4] Molecules produced in this process are utilized in other metabolic pathways such as the conversion of UDP-glucose for energy production. In addition, galactose can undergo a different pathway and convert to form galactitol by aldose reductase enzyme. The oxidation of galactose into galactonate is carried out by the D-galactose-1-dehydrogenase enzyme.

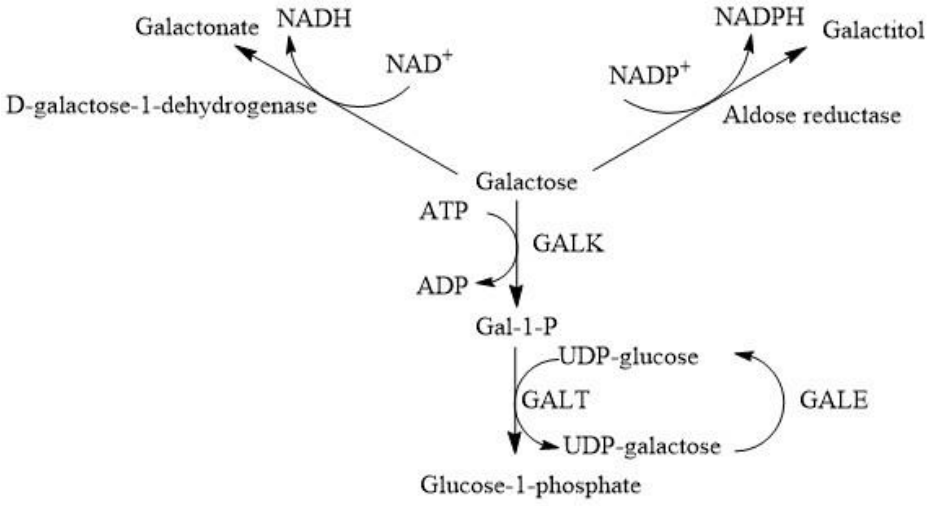


Figure 1. Leloir pathway[2,4] and alternative pathways for galactose metabolism[6].

Patients that have mutations in the enzymes involved in galactose metabolism are diagnosed with galactosemia, where it is classified into three types. Type I/classical involves a mutation in the GALT enzyme, GALK1 in type II and GALE in type III. Deficiencies in these enzymes result in delay in neurological growth, liver diseases, jaundice or cataract.[3] A mutation in the GALT enzyme causes a disruption in the Leloir pathway that leads to an increase in the gal-1-P, galactose, galactitol and galactonate concentrations.[5] Studies have shown a rise in urine galactonate when galactose intake was increased in galactosemic patients which may be used as a marker to monitor galactose metabolism in the body.[6-7] Patients suffering from galactosemia require dietary restrictions to avoid the build-up of galactose and its metabolites in the body and also require them to have routine blood testing.

Developing tools that can detect these markers can be useful for galactosemic patients. This can be used to observe their galactonate level in the body without the need

to go to hospitals. In addition, patients can immediately determine the effect of certain diet restrictions in galactonate concentration in the body. To do this, we aim to isolate an aptamer capable of binding to galactonate (Figure 2). Aptamers are single-stranded DNA molecules capable of binding to a specific target molecules such as ATP, amino acids and other organic molecules with high selectivity and specificity.[8] These oligonucleotides can fold and form complex structures and bind with compatible target molecules through hydrogen bonding, van der Waals, hydrophobic and electrostatic interactions.[8-9] At the same time, the aptamers can bind with certain molecules via these non-covalent forces. The isolated aptamers with high specificity and sensitivity can then be used in the development of a detection kit (Figure 3). The detection kit will be composed of a modified aptamer with a signaling component such as a fluorophore (F) with a quencher (Q) attached to it. The aptamer serves as the targeting moiety that will bind to galactonate (T). This will cause a conformational change in the aptamer that releases the quencher and allow the fluorophore to give an optical signal that can be detected and measured.

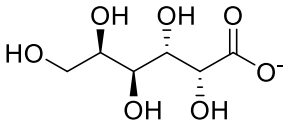


Figure 2. Structure of galactonate molecule.

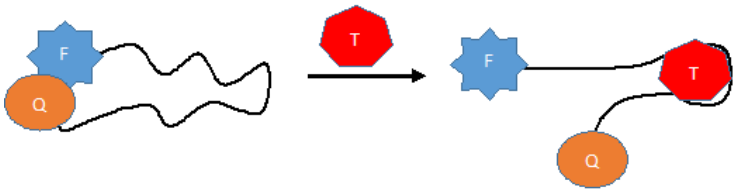


Figure 3. Aptamer-based detection kit concept.

To isolate these oligonucleotides by *in vitro* selection, a technique called Systematic Evolution of Ligands by Exponential Enrichment (SELEX) can be applied. The SELEX mainly revolves around the binding, partition, elution, amplification and regeneration steps (Figure 4).

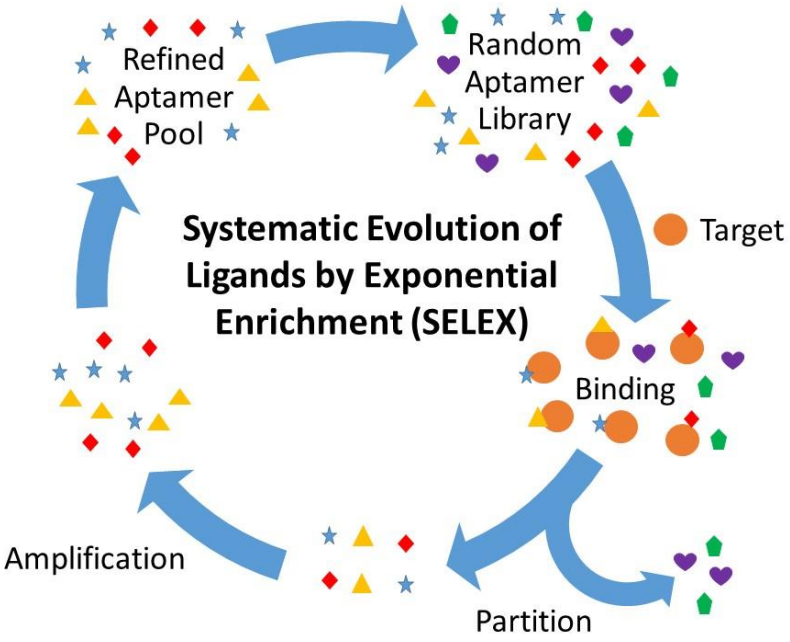


Figure 4. Diagram of SELEX process.

Each round of SELEX undergoes two amplification steps for any of the aptamers collected. A small scale PCR amplification is done to monitor the partitioning of aptamers between those with high binding affinity for the target and unwanted oligonucleotides. The large scale PCR is to ensure that the isolated aptamers are amplified with enough cycles to be used for the next round of SELEX. In this project, the starting point of SELEX is the use of an oligonucleotide library composed of 70-base

long aptamers with 30N random bases (Figure 5). The aptamer is hybridized with a 16-base biotinylated capture sequence (CS) and affixed to a streptavidin column. The bases in the capture sequence are complementary to a section in the aptamer library.

5'-GGA GGC TCT CGG GAC GAC N_{30} GTC GTC CCG ATG CTG CAA TCG T-3' – 70-base long aptamer

5'-GTC CCG AGA GCC TAT A/3BioTEG/-3' – Biotinylated Capture Sequence (CS)

Figure 5. Base composition of oligonucleotide and capture sequence used in SELEX.

The aptamer-CS strands are affixed in a streptavidin agarose gel column for each round of SELEX. The column is washed with a buffer solution to remove any aptamers that did not hybridize well with the CS. The target solution is then introduced into the column and incubated together with the aptamers to allow binding. To improve the interactions between the target and the aptamers, the galactonate is mixed with a Shinkai molecule (Figure 6). Shinkai molecule is a boronic acid that can attach to a target molecule to increase its size and complexity that improves the non-covalent interactions with the aptamers.[10-11]

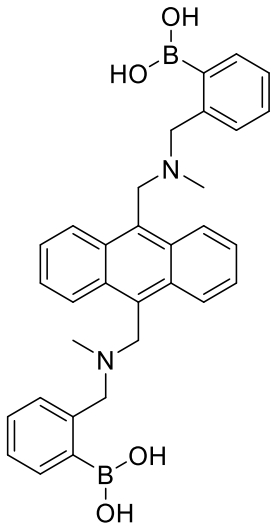


Figure 6. Structure of Shinkai molecule[10].

After the column is washed with the buffer and target solutions, any aptamers that are eluted from the column are collected in separate microcentrifuge tubes. The oligonucleotides from the buffer and target washes are partitioned and subsequently amplified via Polymerase Chain Reaction (PCR). Afterwards, the partitioned aptamers are qualitatively analyzed via gel electrophoresis and ethidium bromide staining. The intensity of the white bands from the buffer and targets washes correlates to the amount of amplifiable aptamers present in those fractions. The aptamers that are washed off from the buffer solution are discarded and only those that bind to the target molecule are used for large scale PCR. Eventually, the oligonucleotide library will be enriched with specifically-binding aptamers after doing several rounds of SELEX. The isolated aptamers from the pool generated from the last round of SELEX can then be cloned for sequencing, binding studies and sensor development.

In large scale PCR, the aptamers collected after binding to the target molecule during the SELEX rounds are concentrated and amplified with a certain number of cycles to yield a sufficient amount of DNA that can be used for the next round of SELEX. The amplification products are then separated into aptamers to generate the new library to be used for the next round of SELEX.

Synthesis of Cryptophane Molecule for Xe Contrast Agent

Another project is the synthesis of an organic molecule that can be used for developing MRI contrast agents or biosensors. Magnetic resonance imaging (MRI) is a noninvasive medical imaging technique used for taking pictures of organs and tissues.[13] A major downside of this technique is that it has low sensitivity. To improve this, contrast agents such as hyperpolarized ^{129}Xe were used to increase the signal. Xenon is a non-toxic gas and is easily delivered in biological systems by inhalation.[12-13] This makes it an effective tool in medical diagnosis and sensing.

Xenon has been observed to bind to cryptophane reversibly with high affinity.[14] Cryptophane is an organic cage molecule capable of serving as a host molecule for xenon. The NMR chemical shift in bound and unbound ^{129}Xe in the cryptophane cage is also distinguishable making it an excellent choice for NMR spectroscopy.[17] Previous studies have shown the use of functionalized cryptophane for sensing streptavidin by observing the changes in ^{129}Xe chemical shift or the use of modified cryptophanes to recognize specific receptors.[12-13] This makes Xe-based tool development with the use of a host molecule an interesting potential in molecular imaging and diagnosis.

For this project, we wanted to synthesize two cryptophane derivatives (Figure 7). The cryptophane derivatives **1** and **2** can be functionalized with a linker and a targeting moiety to recognize specific molecules (Figure 8). A Xe-based biosensor with a cryptophane host molecule can be utilized to detect molecules as they undergo reactions inside biological systems that can translate in understanding more about diseases and improve medical diagnosis.

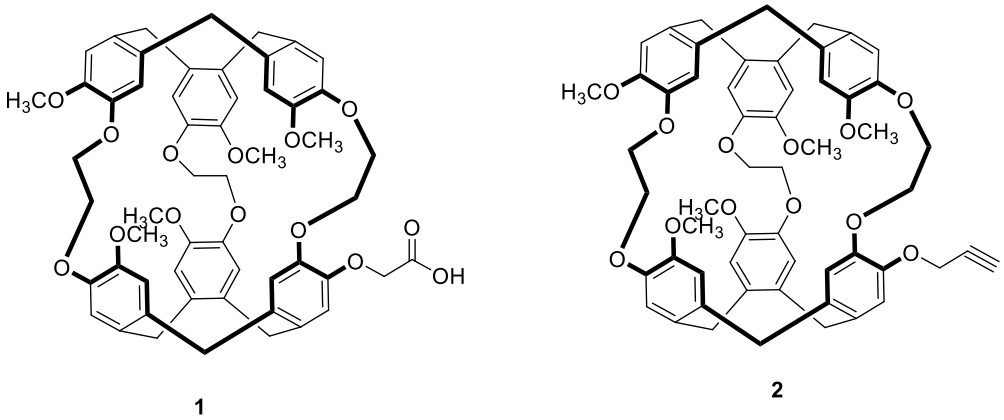


Figure 7. Structure of target cryptophane derivatives.

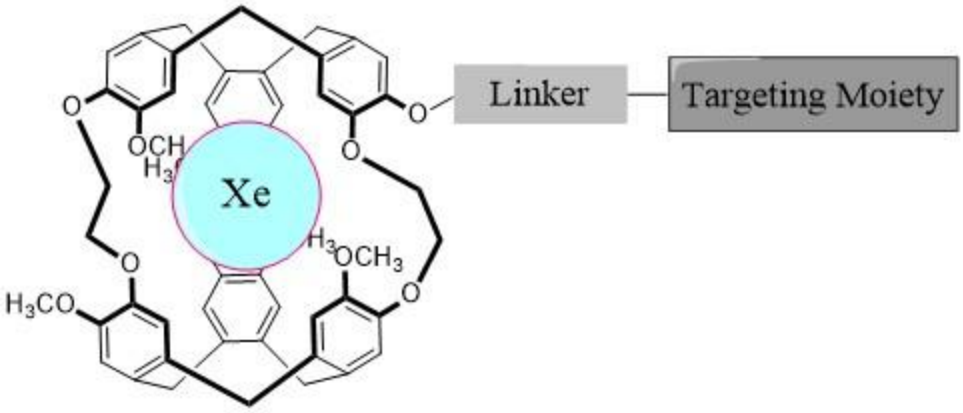
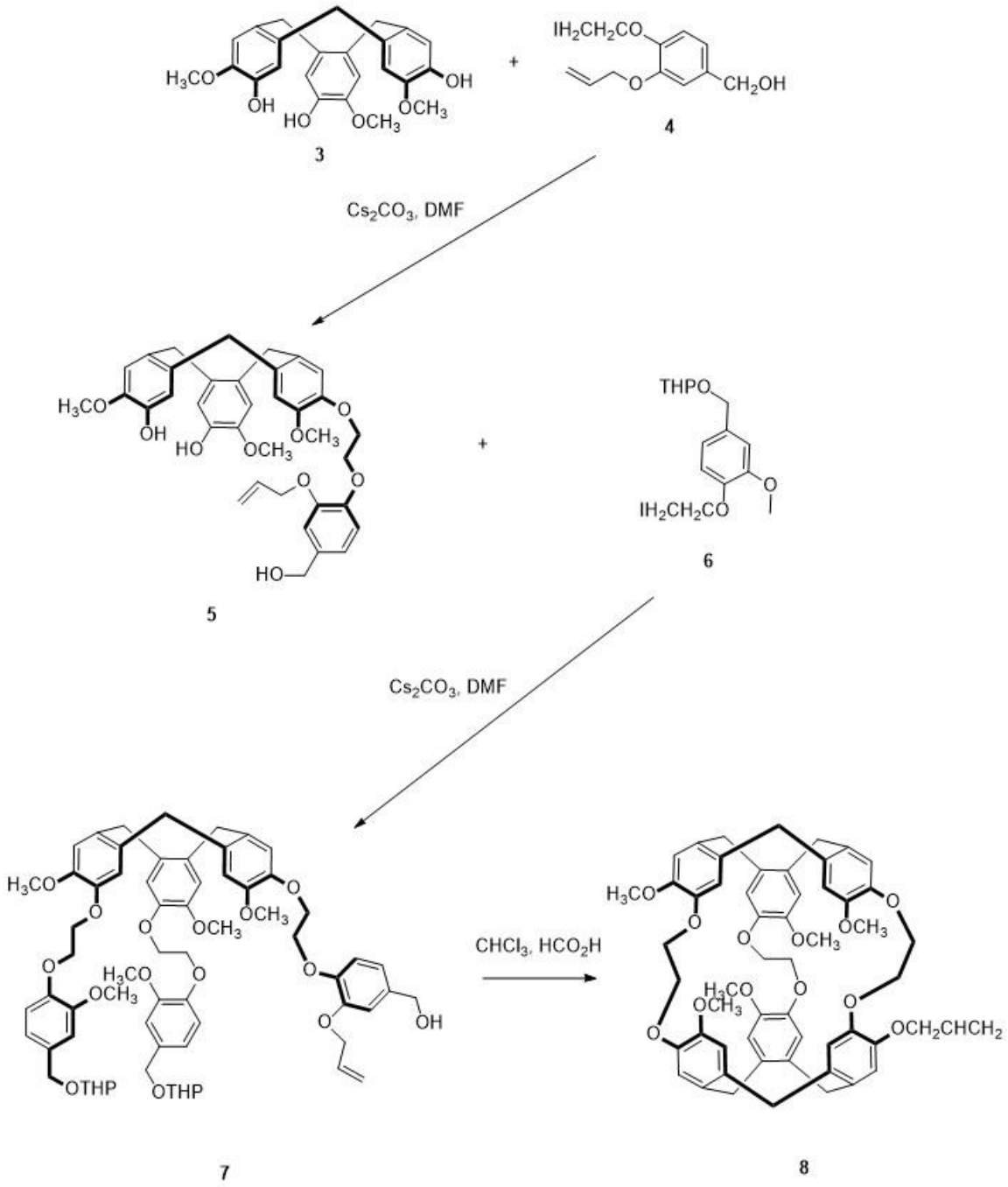


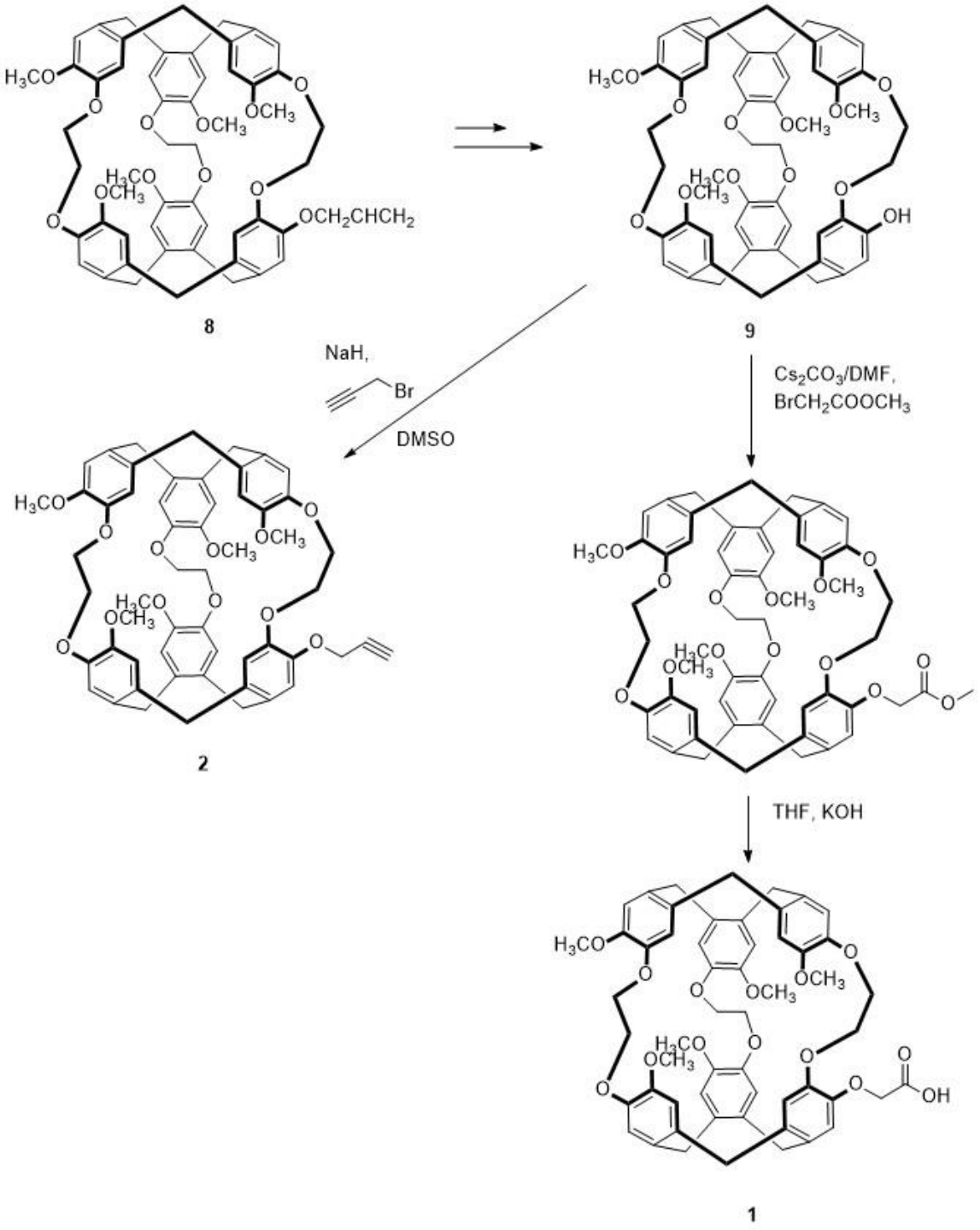
Figure 8. Design concept of a Xe-based biosensor with a functionalized cryptophane cage molecule.

A series of steps based from previous literature was proposed in synthesizing the target molecules (Schemes 1-2).[12-16]

Scheme 1. Schematic diagram for the synthesis of cryptophane molecule.



Scheme 2. Schematic diagram for the synthesis of final cryptophane molecules **1** and **2**.



Chapter Two

Results and Discussion

Isolation of Aptamers for Galactonate via SELEX

To increase the chance of binding of the aptamers to the target molecule, we started with a target concentration that is higher than the desired final concentration. After the library is enriched with aptamers with a high-binding affinity to the target, indicated by more intense bands of the target washes relative to the buffer washes, the concentration of the target solution was reduced. A 10 mM galactonate solution was used in the early rounds of SELEX. This was reduced to 1 mM galactonate mixed with 100 µM Shinkai. Selectivity was observed at each experimental concentrations after doing several rounds of SELEX (Figure 9).

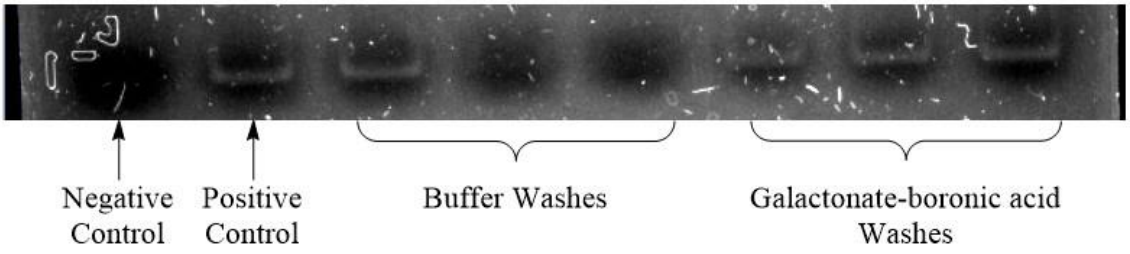


Figure 9. Gel image from a SELEX round. White bands indicated the presence of DNA in the partitioned eluents, with the intensity correlated to amount of DNA.

The negative control that had no band on the gel image above indicated that the results obtained during the specific round had no contamination or external DNA source that can interfere with the imaging process. The positive control that had an intense band

showed that DNAs were present in the library used in the SELEX round. The more intense bands of the target washes relative to the buffer washes showed that aptamers with high binding affinity to the target were collected. This indicated that aptamers were eluted from the column after binding to the galactonate-boronic acid complex compared to washing the column with a buffer solution. The aptamers in the target washes were collected, concentrated and amplified via PCR (Figure 10).

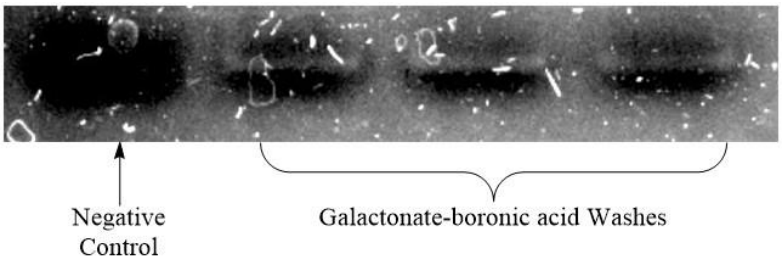


Figure 10. Gel image of the aptamers collected from the target washes. Intense bands indicated sufficient number of amplification cycles were applied during PCR.

After doing several rounds of SELEX and collecting aptamers with high binding affinity to the target at the given concentration, the galactonate-Shinkai solutions were gradually reduced to a final concentration of 250 and 10 µM. Counter-selection against 100 µM Shinkai was performed. The more intense bands of the boronic acid washes relative to the target washes showed that the counter-selection target was also binding to the isolated aptamers (Figure 11). Several rounds of SELEX were applied to wash away the oligonucleotides that were binding to the Shinkai molecule.

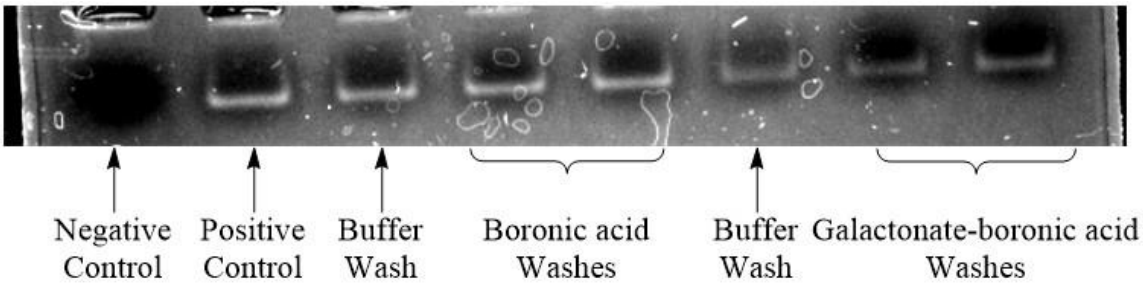


Figure 11. Counter-selection of galactonate-Shinkai against 100 µM Shinkai.

After removing any aptamers that were binding to the boronic acid by doing more counter-SELEX rounds, the enriched oligonucleotide pool was tested for its binding affinity with galactonate of increasing concentrations (50, 100, 250, 500 µM and 1 mM) (Figure 12). It showed that the oligonucleotides were also binding to the galactonate.

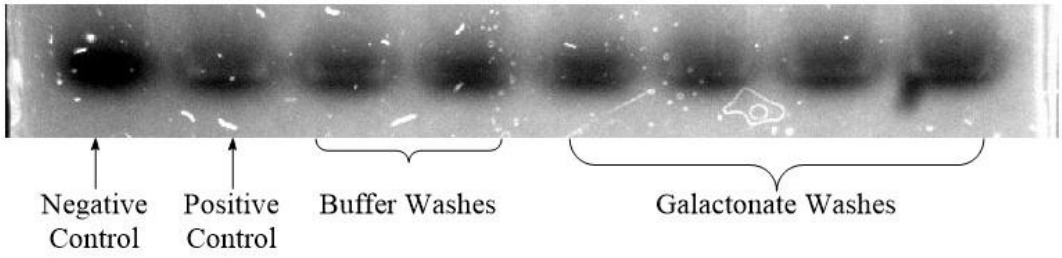


Figure 12. Gel image from small scale SELEX with galactonate target and buffer washes.

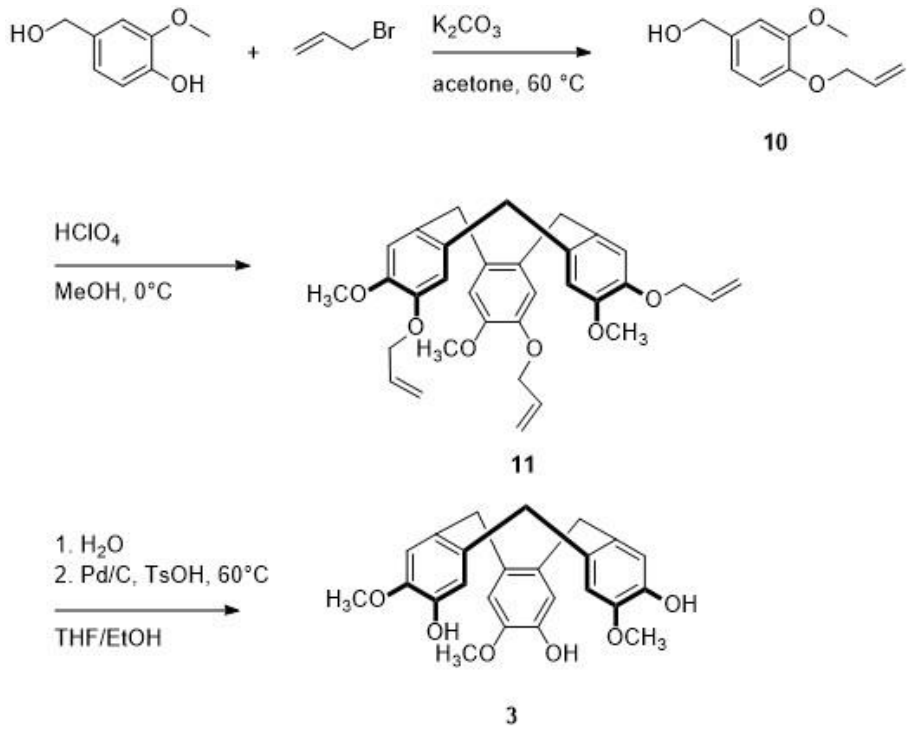
After enriching the aptamer pool with galactonate-binding aptamers by doing several SELEX rounds, the oligonucleotides were collected, concentrated and amplified for DNA cloning in *E. coli*. The pool was cloned twice following manufacturer's protocol. However, the bacteria was not able to yield sufficient number of bacterial colonies that can be used for DNA sequencing.

Synthesis of Precursors for Cryptophane

To synthesize **1** and **2**, sections of the cryptophane cage molecule were initially synthesized. The reaction work-ups were monitored by TLC analysis and the products were characterized using ^{1}H NMR.

We were able to synthesize **3** following a previous procedure (Scheme 3).[13] The hydroxyl group in the vanillyl alcohol was replaced with an allyl group from allyl bromide and eventually coupled together to produce a polyaromatic ring structure **3** (4 g, 53% yield).

Scheme 3.



The ^{1}H NMR for **11** showed that the correct product was obtained (Figure 13). All of **11** was used in synthesizing **3** which was also successfully acquired, based on the ^{1}H NMR, although the product still had some solvent residue (Figure 14).

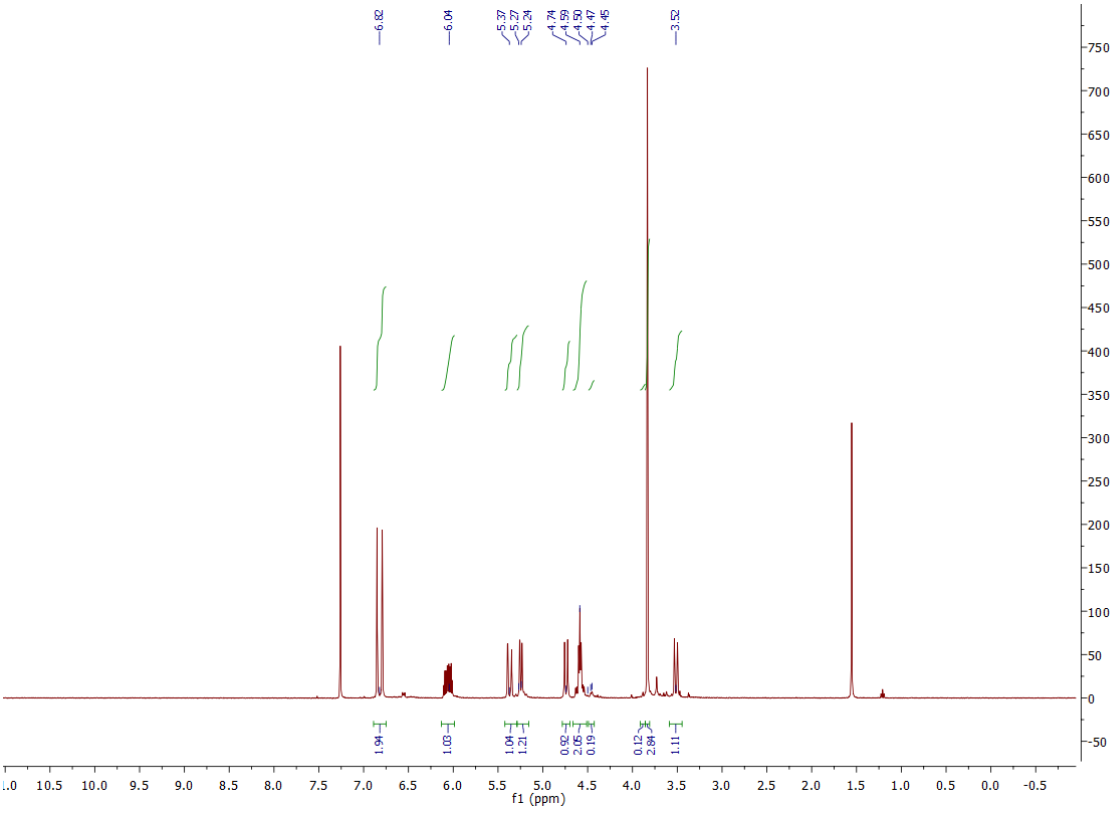


Figure 13. ^{1}H NMR of **11**.

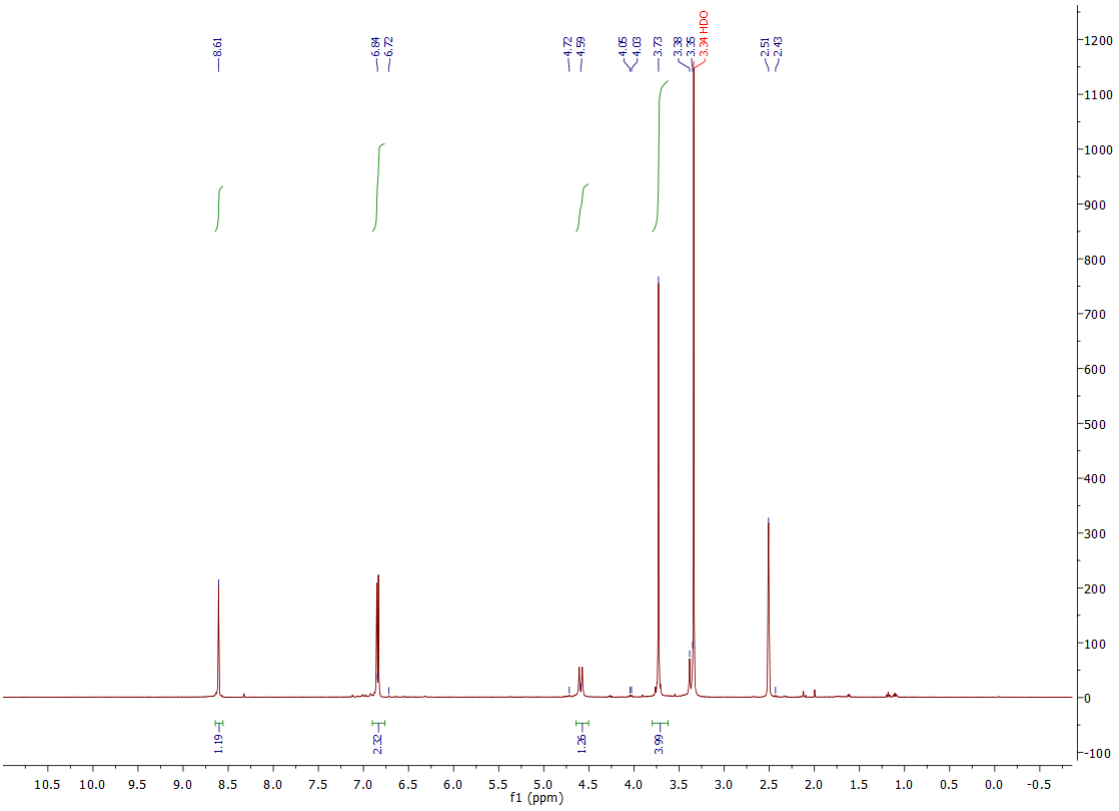
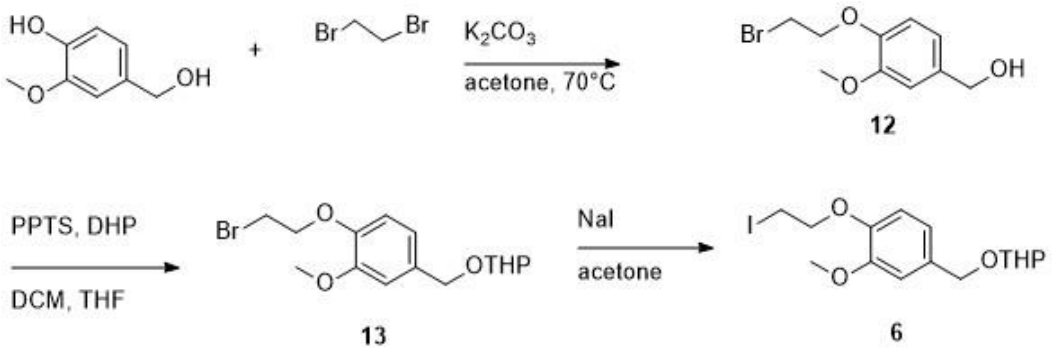


Figure 14. ^{1}H NMR of **3**.

Another precursor for the cryptophane molecule was synthesized following literature procedure (Scheme 4).[17]

Scheme 4.



Molecule **12** was synthesized and characterized using ^{1}H NMR and comparing it with literature values (Figure 15). Unreacted starting material was removed from the reaction mixture by column chromatography.

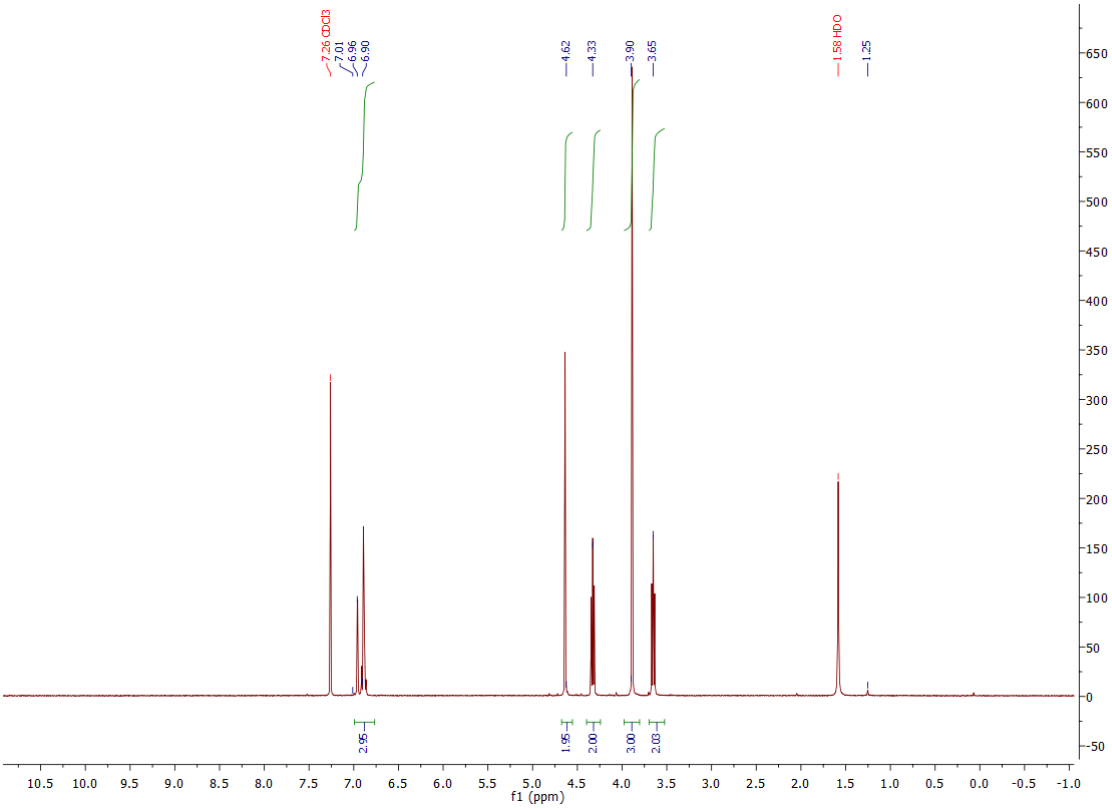


Figure 15. ^{1}H NMR of **12**.

After **12** was obtained, the hydroxyl group was replaced with tetrahydropyranyl group (THP) that served as a protecting group during the cyclization in the proposed synthesis route. ^{1}H NMR on the product showed that **13** was synthesized (Figure 16).

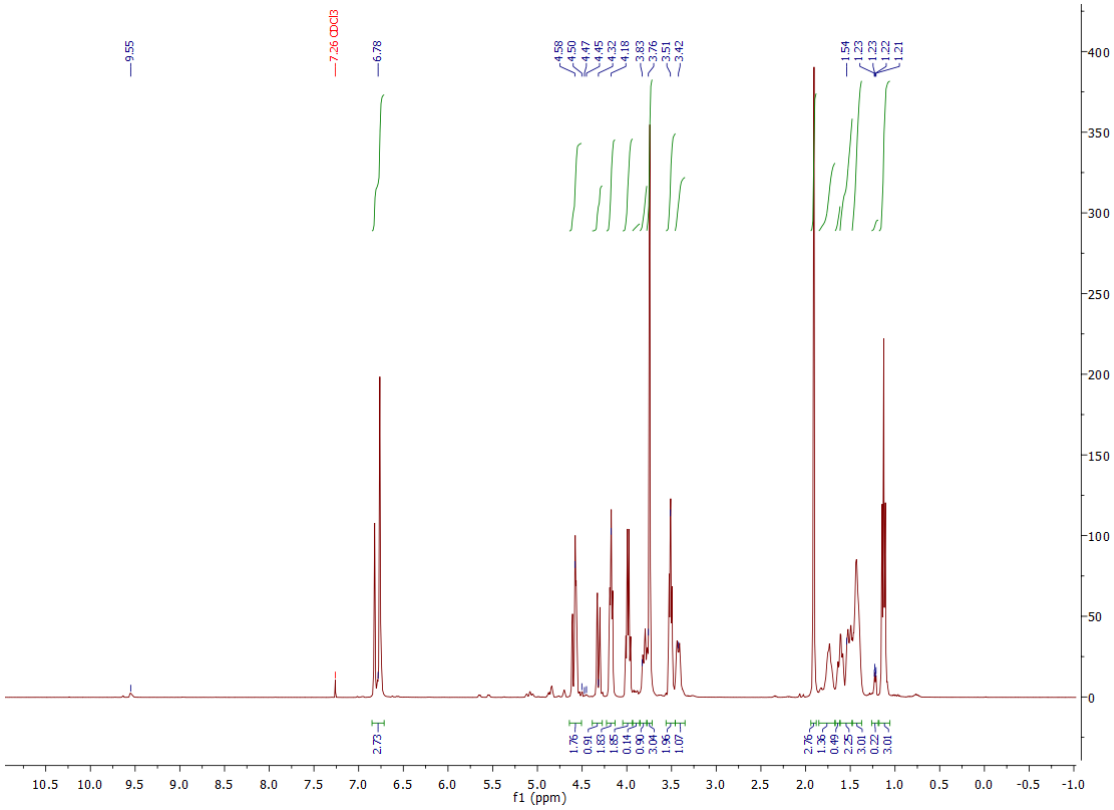


Figure 16. ^{1}H NMR of **13**.

The last step in scheme 4 was a substitution reaction, replacing the bromine with iodine. The reaction mixture was monitored by TLC analysis. ^{1}H NMR between the product **6** and the starting material **13** was very similar with small changes in their chemical shifts. This is most likely due to the small difference in electronegativity values of bromine and iodine (Figure 17).

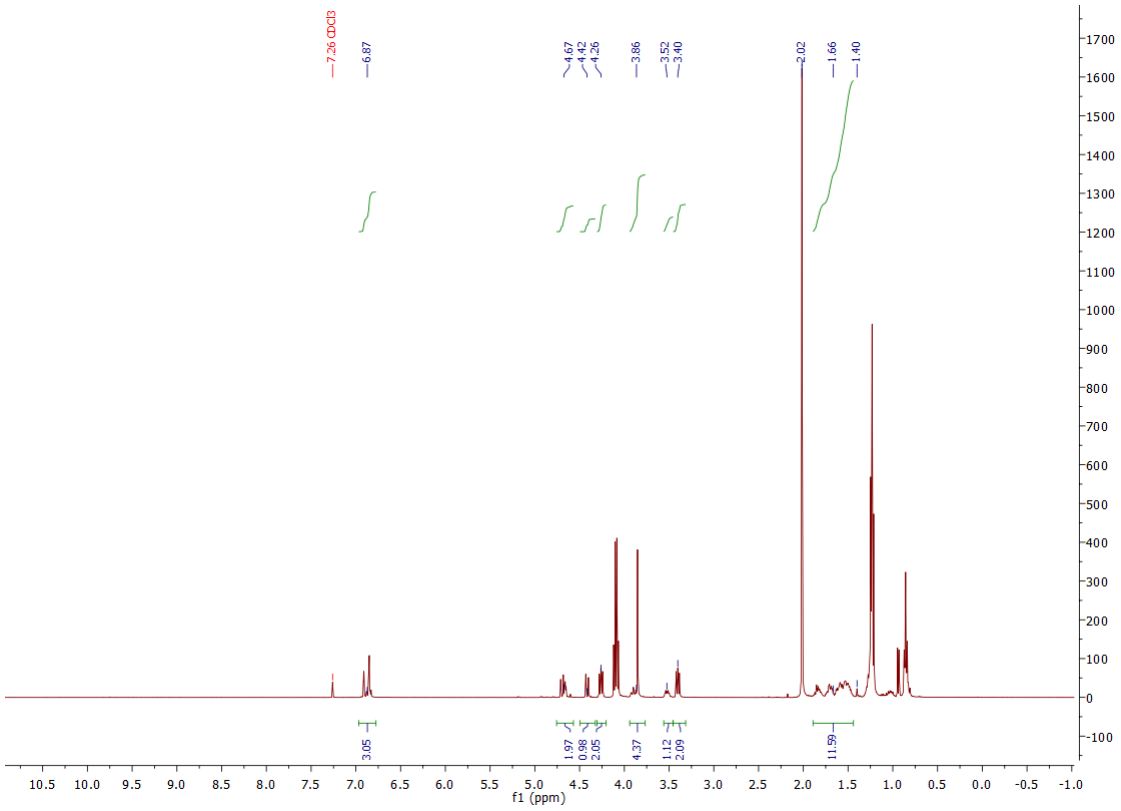
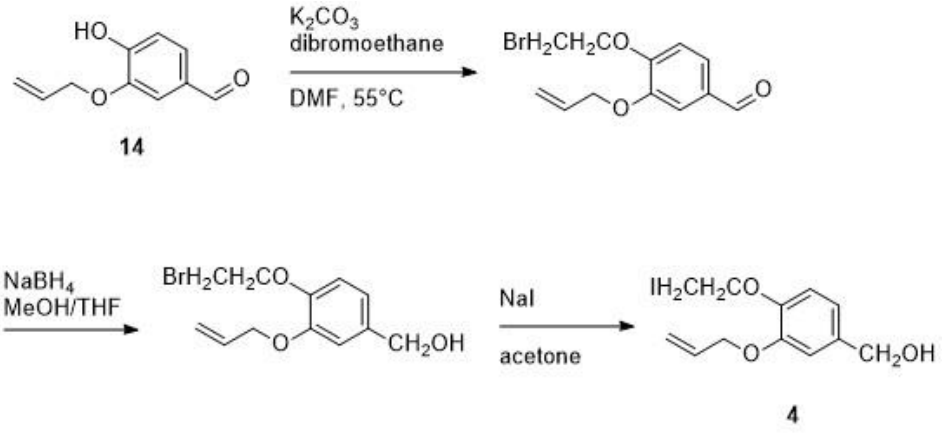


Figure 17. ^{1}H NMR of **6**.

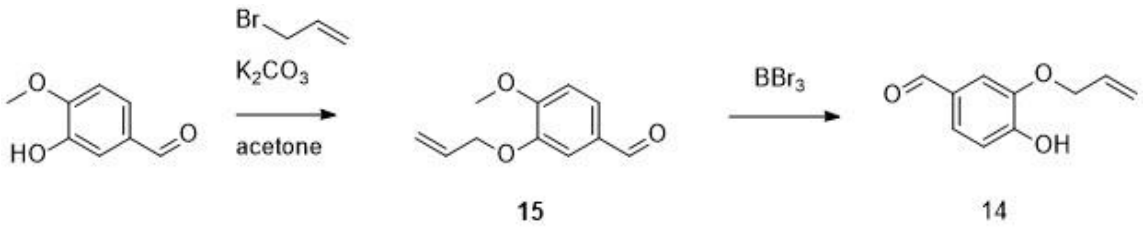
To synthesize **4**, we proposed a synthetic route based on previous literature (Scheme 5). [14]

Scheme 5.

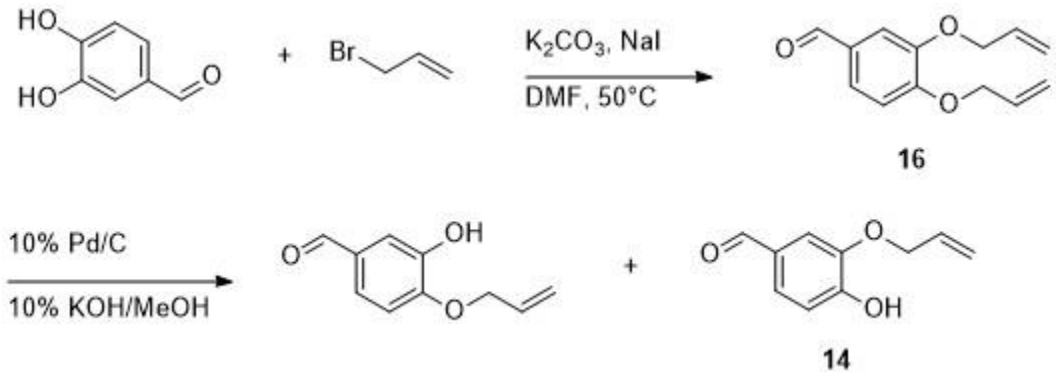


Two routes were proposed for the synthesis of **14**, which is a precursor to compound **4** (Scheme 6-7).

Scheme 6.



Scheme 7.



Following scheme 6, **15** was successfully synthesized following literature procedure.[13] However, the following reaction to produce **14** from **15** was not successful. The reaction mixture of **15** with BBr_3 yielded a dark green solution which turned into a dark purple solution after the addition of water and $NaHCO_3$. The organic layer was extracted and the crude product was characterized by ^1H NMR that showed that both the allyl and methyl groups were cleaved (Figure 18).

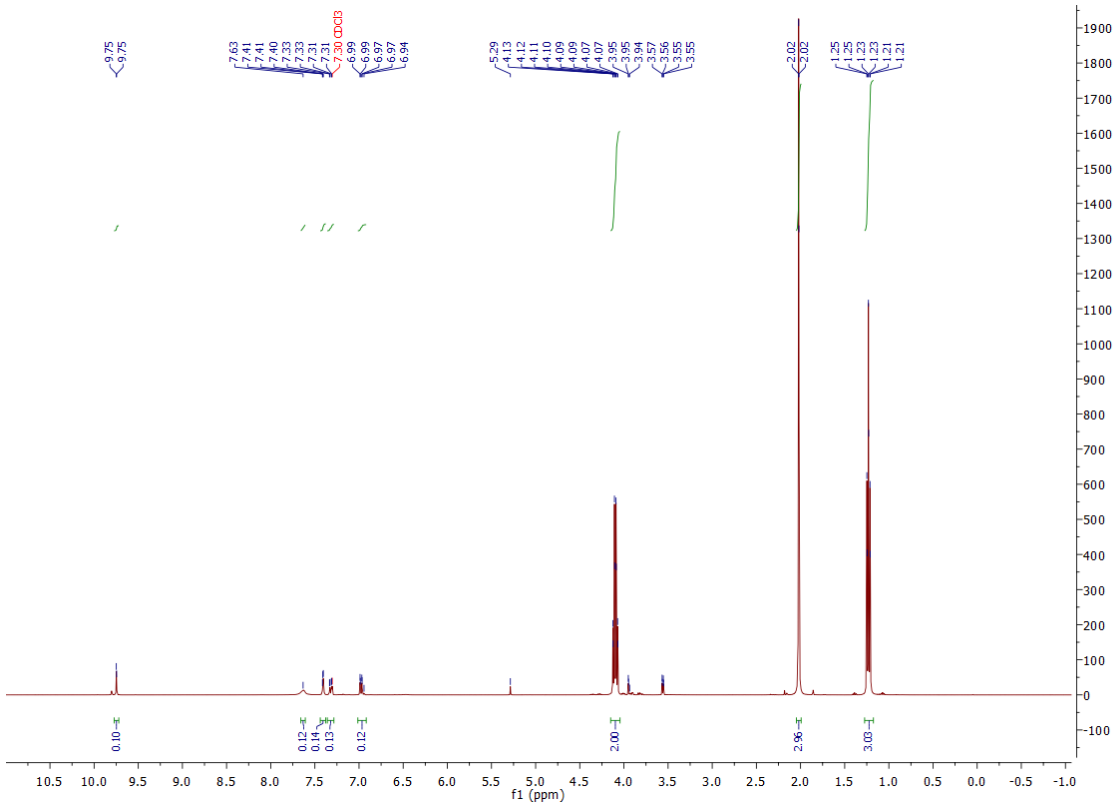


Figure 18. ^{1}H NMR of crude product obtained from the reaction of **15** with BBr_3.

Scheme 7 was followed instead to produce **14**, which will be eventually used to make **4**. In this reaction, the diallylcatechol **16** was successfully prepared using literature procedures.[18] The product obtained after the work-up was characterized by ^{1}H NMR to confirm its identity (Figure 19).

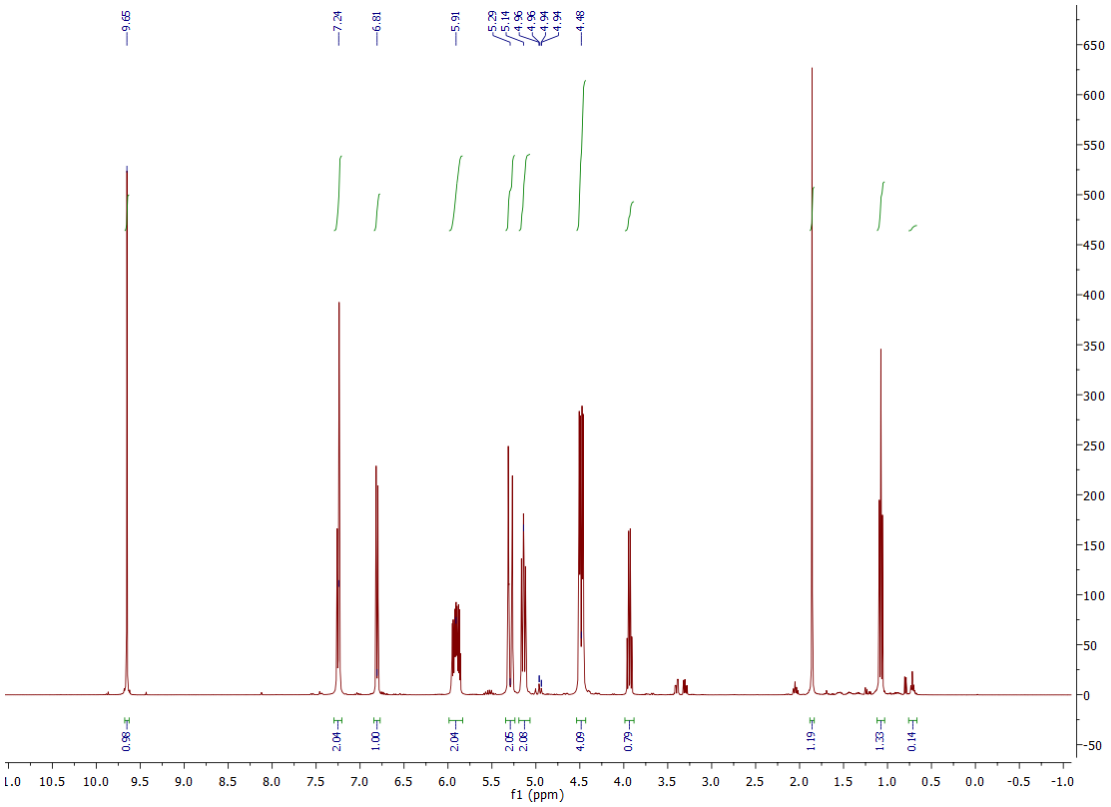


Figure 19. ^{1}H NMR of **16**.

The deallylation step on **16** to convert it into **14** using 10% KOH-MeOH catalyzed by 10% Pd/C was unsuccessful. Initial TLC analysis between the starting material and the reaction mixture showed no change. The reaction was performed again and was left to react for a longer period of time to ideally obtain the product. The reaction was performed a few more times but the product was still not obtained, even on a small-scale set-up. TLC analysis on the reaction mixture and comparing it with the starting material **16** showed spots that had similar R_f values. ^{1}H NMR on the product obtained after running the experiment for 2 days confirmed that the starting material was still present in the reaction mixture and was not converted into the target product (Figure 20).

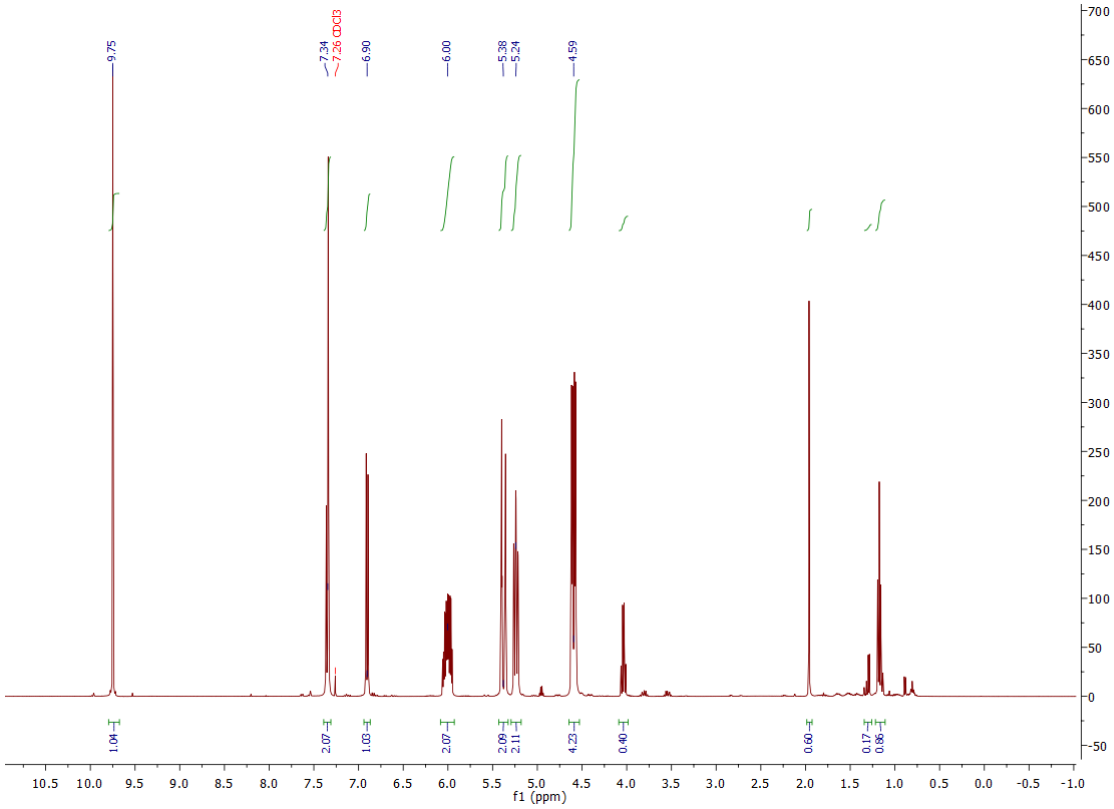


Figure 20. ^{1}H NMR of product obtained from the reaction of **16** with 10% KOH-MeOH.

Chapter Three

Conclusions

After doing several rounds of SELEX, the aptamer pool was enriched with oligonucleotides binding to 250 µM galactonate with 10 µM Shinkai. During counter-selection with 100 µM Shinkai, the aptamers were binding to the counter-selection target. Additional rounds of SELEX were applied to remove unwanted aptamers. The aptamers in the enriched pool was also binding to galactonate solutions (50, 100, 250, 500 µM and 1 mM). At the last round of SELEX, the aptamer pool was cloned in *E. coli*. However, there were not enough bacterial colonies produced for DNA sequencing and binding studies.

A 20-step synthesis route was proposed to synthesize two cryptophane molecules. Currently, some of the precursors for the cryptophane were successfully synthesized and characterized with ^{1}H NMR. The removal of one of the allyl group in one of the products with 10% KOH-MeOH was not successful even after prolonged reaction.

Chapter Four

Future Directions

An aptamer capable of binding to galactonate can be isolated using a new pool of random aptamers. Once the aptamer pool has been reduced after doing several rounds of SELEX and that selectivity to galactonate is observed, counter-selection against other target molecules such as Shinkai molecule, galactitol or galactose-1-phosphate (Gal-1-P) will be tested to ensure specificity of the aptamer to galactonate. If the counter-selection targets exhibit binding to the isolated aptamers, specificity may still be achieved by applying more rounds of SELEX to wash off those that are not target-specific. This hopefully would then further reduce the aptamer pool that will only contain the preferred strands. At the last round of SELEX, the aptamer pool will be cloned in *E. coli* to provide bacterial colonies to be used for DNA sequencing. From the DNA sequences identified, binding studies can then be tested. Afterwards, a fluorophore and quencher can be attached. When a target molecule binds to the aptamer, this causes a change in the structure and the quencher is released. The fluorophore gives a fluorescence signal that can be detected. The intensity of the signal can then be correlated with the target concentration.

For the cryptophane synthesis project, it is possible to re-do some of the reactions that failed in the earlier synthesis steps. The conversion of **15** with BBr_3 to produce **14** may be performed again as the reaction may still work. Other synthetic routes may be present in literature and could be tested to get the target compound.

Chapter Five

Experimental

Materials and Methods. Solvents and reagents were purchased from Fisher Scientific and Sigma-Aldrich. ^{1}H NMR spectra were obtained at 400 MHz on a *Bruker 400 FT-NMR* instrument. DMSO-d_6 or $CDCl_3$ were used as solvent and residual solvent peaks in ^{1}H NMR were used for reference during data interpretation. Taq Polymerase enzyme was purchased from Promega. DNA cloning kit was purchased from ThermoFischer Scientific. All chemicals and reagents were obtained from Sigma-Aldrich, ThermoFischer Scientific, Acros or JT Baker unless specified.

Buffer Preparation. The 1x SELEX buffer was prepared by the following: 20 mM HEPES, 140 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$ with pH adjusted to 7.2-7.4 with NaOH or HCl. The 2x SELEX buffer was made in a similar manner with the 1x SELEX buffer with twice the concentration. The strand separation buffer was prepared using 20 mM HEPES, 140 mM NaCl, pH 7.2-7.4. All buffers were made using nuclease-free water (HyClone HyPure™ Molecular Biology Grade Water).

Systematic Evolution of Ligands by Exponential Enrichment (SELEX).

Small Scale and Counter SELEX. The column was prepared by adding streptavidin agarose gel (Pierce, ThermoScientific, 200 µL) in a filtered gravity column and conditioned by running a series of 1x SELEX buffer washes. First round of SELEX started with 0.3 nmol oligonucleotide library and 1.5 nmol biotinylated capture sequence (CS). The aptamer-biotinylated capture sequence (CS) were mixed and heated to 95 °C and cooled to rt using a thermocycler. Subsequent SELEX rounds used aptamer

concentrations ranging from 0.18 – 0.36 nmol and mixed with the capture sequence in a 1:5 mole ratio. The hybridized aptamer-biotinylated CS mix was then diluted to a final volume of 700 µL with 1x SELEX buffer. Three portions of 200 µL aptamer library were passed into the column, with each portion incubated for about 2 min. Afterwards, the column was washed with 1x SELEX buffer (8-10 x 200 µL) and the target solution (3 x 200 µL). All eluents were collected in separate microcentrifuge tubes. A 30 mM galactonate solution was prepared by dissolving D-galactonic acid (Sigma-Aldrich, 50 mg) in 1x SELEX buffer (3.87 mL). A 10 mM galactonate solution, prepared by diluting the 30 mM galactonate stock solution with 1x SELEX buffer, was initially used in the early rounds of SELEX. After selectivity was observed and the aptamer pool was enriched with oligonucleotides at the given concentration, the target concentration was reduced to 1 mM mixed with 100 µM Shinkai molecule (made from a 2.5 mM stock solution). The 2.5 mM Shinkai stock solution was prepared by dissolving Shinkai molecule (5 mg) in methanol (MeOH, 3.87 mL). Eventually, the target concentration was decreased to 250 µM galactonate with 10 µM Shinkai. Any aptamers present collected in the eluent in the buffer and target washes were used for polymerase chain reaction (PCR).

After several rounds of SELEX and observing selectivity, counter-selection against other molecules were performed. The counter-selection target was passed through the column (3 x 200 µL) in between the aptamer-cs and target washes.

PCR Protocol and Analysis. Any DNA collected in the microcentrifuge tubes were amplified via Polymerase Chain Reaction (PCR). A PCR elution profile was prepared by mixing 5x Taq enzyme buffer (50 µL), 10 mM dNTP (5 µL), 100 µM

forward and biotinylated end primers (IDT, 2.5 µL), 5 u/µL Taq Polymerase enzyme (2 µL) and diluting it with DNA-free water to obtain a final volume of 250 µL. The primers used in the amplification process had the following sequences: forward primer (fp) – 5'GGA GGC TCT CGG GAC GAC -3', biotin end primer (BiotEP) – 5'/5Biosg/ACG ATT GCA GCA TCG GGA CGA C-3'. A 1 mL total volume of the PCR profile was used for the large scale PCR portion of SELEX using the same ratios. The PCR mixture was equally divided among 10 PCR wells. First PCR well had 3 µL nuclease-free water that served as the negative control, 2nd well is the positive control that had 3 µL aptamer-CS fraction. The other wells contained eluents from the buffer, target or counter-selection washes. Amplification settings were 95 °C for 2 min, variable number of cycles of 94 °C for 15 s, 55 °C for 15 s and 72 °C for 30 s, held constant at 72 °C for 3 min after the last cycle and the temperature is then lowered to 4 °C. The number of cycles can vary from 10-32.

To analyze the PCR products, the samples were loaded with a bromophenol blue loading dye (2X) in 3% agarose gel which is submerged in 0.5x TBE buffer. The agarose gels were prepared by dissolving agarose powder (1.5 g) in 0.5x TBE buffer (50 mL). The samples are ran in the gel and stained with ethidium bromide for imaging. The gel was imaged using a Trans-UV illuminator and Image Lab software (BioRad). The presence of DNA in the eluents were analyzed by qualitatively comparing the band intensities between the target and buffer washes.

Large Scale PCR. The eluents collected after passing the target solution in the column were filtered using an EMD Millipore centrifugal 10 kDa filter to concentrate any

aptamers. The DNA concentrate was added in the PCR large scale profile template. The aptamers were amplified via PCR until a significant amount of oligonucleotides had been obtained.

Strand Separation of PCR Products. The PCR product from the large scale SELEX was then filtered using an EMD Millipore Centrifugal 30 kDa filter to obtain about 30 µL and diluted to a final volume of 200 µL with DNA-free water. During strand separation of the DNA product, a streptavidin agarose gel column was prepared in a similar manner as described earlier but conditioned with the strand separation buffer (5 x 200 µL). The PCR product was passed through the conditioned column at least 3x. Two portions of 0.5 M NaOH (150 µL) were passed through the column with 1 min incubation and collected in a microcentrifuge tube. The pH was adjusted using 1 M HCl to obtain a final pH of 7.2-7.4 and diluted with 2x SELEX buffer to achieve a final volume of 700 µL. The aptamer concentration was obtained by getting the absorbance at 260 nm using NanoDrop UV-Vis spectrophotometer.

DNA Cloning. After reducing the aptamer library, the DNA samples were cloned using TOPO *E. coli* bacteria using manufacturer's protocols.

3-methoxy-4-(2-propenyloxy) benzenemethanol (10). In a 500 mL round bottom flask, vanillyl alcohol (25 g, 162 mmol) was mixed with allyl bromide (16 mL, 185 mmol) in acetone (110 mL). Afterwards, K_2CO_3 (22.5 g, 163 mmol) was added into the reaction mixture and temperature was increased and stirred overnight under reflux. Solvent was removed under vacuum leaving a yellowish-white residue. The residue was dissolved in dichloromethane (DCM, 200 mL) and H_2O (200 mL). The organic layer was

collected and washed with 1M NaOH (3 x 50 mL) and brine. The organic layer was again collected and dried with $MgSO_4$. The solution was filtered and solvent was removed under vacuum. The remaining residue was re-dissolved in ethyl acetate (EtOAc, 250 mL) and solvent was removed under vacuum to remove any excess allyl bromide to obtain an off white product (28 g, yield: 89%).

2,7-12-trimethoxy-3,8,12-tris(2-propenyloxy)-10,15-dihydro-5H-tribenzo[a,d,g] cyclonone (11). Compound **10** (28 g, 141 mmol) was dissolved in MeOH (220 mL) in a flask that was cooled to 0 °C while stirring. Afterwards, perchloric acid ($HClO_4$, 90 mL) was added dropwise. Reaction flask was left to stir overnight to produce a pinkish solution. The solution was neutralized with 5 M NaOH (200 mL) which was added over a period of time followed by saturated $NaHCO_3$ (200 mL). This converted the reaction mixture to a yellow-colored solution. Sample was then filtered off and solvent was removed from the filtrate under vacuum to obtain a reddish-pink residue. The residue was mixed with diethyl ether (70 mL) and sonicated for 1 hr. while replacing any evaporated ether to obtain an oatmeal-like solution that was stirred overnight. Solution was filtered to obtain the product (7 g, yield: 36%).^{1}H NMR ($CDCl_3$) δ 6.82 (d, 10H), 6.06 (m, 6H), 5.37 (dq, 6H), 5.24 (dq, 7H), 4.74 (d, 5H), 4.66-4.51 (m, 11H), 4.46 (d, 1H).

2,7,12-Trihydroxy-3,8,13-trimethoxy-10,15-5H-tribenzo[a,d,g] cyclononene (3). In a 500 mL round bottom flask, **11** (14 g, 26 mmol) was mixed in tetrahydrofuran (THF, 250 mL) and ethanol (EtOH, 270 ml). While stirring, 10% Pd/C (2.4 g) and p-Toluenesulfonic acid (p-TsOH, 0.5 g) were added and the temperature was increased to

60 °C. The resulting solution was filtered with Celite and washed with THF. The solvent was removed under vacuum to afford a brownish residue. The residue was washed and extracted with THF (200 mL), EtOAc (200 mL) and finally with brine. The organic layer was collected and dried with $MgSO_4$. The solution was filtered and solvent was removed under pressure. The residue was digested in chloroform (160 mL), and stirred for 1 hr, and subsequently filtered to afford a yellowish powder (4 g, yield: 53%). ^{1}H NMR (DMSO-d_6) δ 8.60 (s, 1H), 6.83 (d, 2H), 4.58 (d, 1H), 3.72 (s, 3H), 3.36 (d, 5H).

(4-(2-bromoethoxy)-3-methoxyphenyl)methanol (12). Vanillyl alcohol (6 g, 38.9 mmol), K_2CO_3 (10.4 g, 75.2 mmol), dibromoethane (12 mL, 139 mmol) were mixed in acetone (100 mL) in a round bottom flask. The reaction mixture was stirred under reflux overnight. The solvent was removed under vacuum and residue was dissolved in EtOAc and washed with water then brine. The organic layer was collected and dried under vacuum. The residue was purified by silica gel column chromatography (20:80 EtOAc:DCM) to give **12** (4 g, yield: 39%) as white solid. ^{1}H NMR ($CDCl_3$) δ 6.96 (d, 1H), 6.94-6.84 (m, 2H), 4.64 (s, 2H), 4.33 (t, 2H), 3.89 (s, 3H), 3.65 (t, 2H).

2-((4-(2-bromoethoxy)-3-methoxybenzyl)oxy)tetrahydro-2H-pyran (13). Compound **12** (4 g, 16.5 mmol) was mixed with dihydropyran (DHP, 2.4 mL, 26 mmol) and THF (40 mL) and pyridinium p-toluenesulfonate (PPTS, 0.44 g, 1.75 mmol). The reaction mixture was stirred overnight at room temperature. Afterwards, the solvent was removed under vacuum and the residue was dissolved in ether (3 x 50 mL) and washed with water and brine. The organic layer was then collected and dried with $MgSO_4$. The solution was filtered and solvent removed under vacuum to obtain an oil-like product.

The residue was purified by silica gel column chromatography (30:70 EtOAc:hexane) to obtain **13** (3 g, yield: 46%). ^{1}H NMR ($CDCl_3$) δ 6.79 (dt, 6H), 4.64-4.51 (m, 4H), 4.32 (dt, 2H), 4.18 (td, 4H), 3.99 (qd, 4H), 3.85-3.78 (m, 2H), 3.78-3.72 (m, 6H), 3.51 (td, 4H), 3.46-3.35 (m, 2H), 1.91 (d, 6H), 1.85-1.67 (m, 3H), 1.67-1.61 (m, 1H), 1.61-1.48 (m, 5H), 1.48-1.37 (m, 6H), 1.13 (m, 6H).

2-((4-(2-iodoethoxy)-3-methoxybenzyl)oxy)tetrahydro-2H-pyran (6). In a 250 mL round bottom flask, **7** (6 g, 17.4 mmol) was dissolved with NaI (26 g, 173 mmol) in acetone (55 mL). The reaction mixture was stirred under reflux overnight. Solvent was then removed under vacuum and the product was purified by silica gel column chromatography (30:70 EtOAc:hexane). The product was concentrated under vacuum to obtain **6** (5.6 g, yield: 82%) as a light yellow residue. ^{1}H NMR ($CDCl_3$) δ 6.96-6.80 (m, 3H), 4.74-4.63 (m, 2H), 4.42 (d, 1H), 4.30-4.22 (m, 2H), 3.90 (ddd, 1H), 3.85 (s, 3H), 3.58-3.47 (m, 1H), 3.45-3.36 (m, 2H), 1.90-1.78 (m, 2H), 1.78-1.64 (m, 3H), 1.64-1.44 (m, 7H), 1.33-1.19 (m, 21H), 1.07-0.97 (m, 1H).

3-(allyloxy)-4-methoxybenzaldehyde (15). Using a 500 mL round bottom flask, isovanillin (12 g, 78.9 mmol), K_2CO_3 (13.6 g, 98.4 mmol), allyl bromide (8.5 mL, 98.4 mmol) were dissolved in acetone (100 mL). The reaction was heated to 70 °C and stirred for 3.5 hr. The reaction mixture was filtered and the filtrate was concentrated under vacuum. The residue was dissolved in EtOAc (3 x 40 mL) and washed with H_2O (40 mL) and brine. The organic layer was dried, filtered and solvent was removed under vacuum to afford 10.9 (72%) **15**. ^{1}H NMR ($CDCl_3$) δ 9.73 (d, 2H), 7.40-7.28 (m, 4H), 6.89 (d, 2H), 6.07-5.92 (m, 2H), 5.35 (dq, 2H), 5.22 (dt, 2H), 4.56 (dd, 4H), 3.85 (d, 6H).

3,4-bis(allyloxy)benzaldehyde (16). In a 250 mL round bottom flask, 3,4-dihydroxybenzaldehyde (6 g, 43.4 mmol) was mixed with dimethylformamide (DMF, 30 mL), allyl bromide (9.4 mL, 109 mmol), K_2CO_3 (18.0 g, 130 mmol) and NaI (0.65 g, 4.3 mmol). The solution was stirred for 1.5 hr at room temperature. It was filtered and the filtrate was diluted with H_2O (40 mL) and extracted with ether (3 x 40 mL). The organic layer was washed with water and brine, dried with $MgSO_4$. Solvent was removed under vacuum and purified by silica gel column chromatography (30:70 EtOAc:hexane). The product was concentrated under vacuum to obtain **16** (2.04 g, yield: 22%). ^{1}H NMR ($CDCl_3$) δ 9.65 (s, 1H), 7.30-7.21 (m, 2H), 6.81 (d, 1H), 5.91 (m, 2H), 5.29 (dq, 2H), 5.14 (m, 2H), 4.48 (m, 4H).

3-(allyloxy)-4-hydroxybenzaldehyde (14). All of **15** previously prepared was dissolved in DCM in an ice bath. Slowly, BBr_3 (13 mL, 137 mmol) was added into the reaction mixture. The reaction was allowed to stir for 3.5 hr and left to warm to room temperature. Afterwards, 60 mL H_2O and 100 mL $NaHCO_3$ were added into the reaction flask. The organic layer was extracted with EtOAc (3 x 50mL), dried with $MgSO_4$ and filtered. The solvent was removed under vacuum to obtain a dark crude product. ^{1}H NMR ($CDCl_3$) δ 4.05 (qd, 2H), 1.98 (d, 3H), 1.19 (td, 3H).

In a different method, **16** (0.1 g, 0.458 mmol) was dissolved in 10 mL 10% (w/v) KOH-MeOH in a round-bottom flask. After which, 10% Pd/C (0.02g) was carefully added in the reaction flask and stirred for 48 hr under room temperature. The solution was filtered under Celite and solvent was removed under vacuum. The concentrate was washed with 1 M HCl (10 mL) and the organic layer was extracted with EtOAc (3 x 10

mL). The organic layer was washed with water and brine, dried with $MgSO_4$ and filtered. The solvent was removed under vacuum to afford a red-orange residue. ^1H NMR ($CDCl_3$) δ 9.75 (s, 1H), 7.34 (s, 2H), 6.90 (s, 1H), 6.00 (m, 2H), 5.38 (dq, 2H), 5.24 (m, 2H), 4.59 (m, 4H).

References

1. Berry, G. T. Classic Galactosemia and Clinical Variant Galactosemia. 2000 Feb 4 [Updated 2014 Apr 3]. In: Pagon RA, Adam MP Ardinger HH, et al., editors. GeneReviews ® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2017. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK1518.

2. Timson, D. J. (2015). The Molecular Basis of Galactosemia – Past, Present and Future. *Gene*. Retrieved from http://dx.doi.org/10.1016/j.gene.2015.06.077.

3. Holden, H. M.; Rayment, I.; Thoden, J. B. Structure and Function of Enzymes of the Leloir Pathway for Galactose Metabolism. *The Journal of Biological Chem.* **2003**, *45*, 43885-43888.

4. Ficicioglu, C.; Hussa, C.; Gallagher, P. R.; Thomas, N.; Yager, C. Monitoring of Biochemical Status in Children with Duarte Galactosemia: Utility of Galactose, Galactitol, Galactonate, and Galactose 1-Phosphate. *Clinical Chem*. **2010**. *56*. 1177-1182.

5. Daenzer, J. M. I.; Jumbo-Lucioni, P. P.; Hopson, M. L.; Garza, K. R.; Ryan, E. L.; Fridovich-Keil, J. L. Acute and Long-term Outcomes in a *Drosophilia melanogaster* Model of Classic Galactosemia Occur Independently of Galactose-1-phosphate Accumulation. *Disease Models & Mechanisms*. **2016**. *9(11)*. 1375-1382.

6. Cuatrecasas, P.; Segal, S. Galactose Conversion to D-Xylulose: An Alternate Route of Galactose Metabolism. *Science*. **1966**. *153*. 549-551.

7. Wehrli, S. L.; Berry, G. T.; Palmieri, M.; Mazur, A.; Elsas III, L.; Segal, S. Urinary Galactonate in Patients with Galactosemia: Quantitation by Nuclear Magnetic Resonance Spectroscopy. *Pediatric Research*. **1997**. *42*. 855-861.

8. Stoltenburg, R.; Reinemann, C.; Strehlitz, B. SELEX – A (r)evolutionary Method to Generate High-Affinity Nucleic Acid Ligands. *Biomolecular Engineering*. **2007**, *24*, 381-403.

9. Hermann, T.; Patel, D. J. Adaptive Recognition by Nucleic Acid Aptamers. *Science*. **2000**. *287*. 820-825.

10. James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. A Glucose-Selective Molecular Fluorescence Sensor. *Angew. Chem. Int. Ed. Engl.* **1994**. *33*. 2207-2209.

11. Yang, K.; Barbu, M.; Halim, M.; Pallavi, P.; Kim, B.; Kolpashchikov, D. M.; Pecic, S.; Taylor, S.; Worgall, T. S.; Stojanovic, M. N. Recognition and Sensing of Low-epitope Targets via Ternary Complexes with Oligonucleotides and Synthetic Receptors. *Nature*. **2014**. *6*. 1003-1008.

12. Khan, N. S.; Riggle, B. A.; Seward, G. K.; Bai, Y.; Dmochowski, I. J. A Cryptophane-Folate Biosensor for ^{129}Xe NMR. *Bioconjugate Chem*. **2014**. *26*. 101-109.

13. Wei, Q.; Seward, G.K.; Hill, P. A.; Patton, B.; Dimitrov, I. E.; Kuzma, N. N.; Dmochowski, I. J.; Designing ^{129}Xe NMR Biosensors for Matrix Metalloproteinase Detection. *J. Am. Chem. Soc.* **2006**. *128*. 13274-13283.

14. Brotin, T.; Cavagnat, D.; Jeanneau, E.; Buffeteau, T. Synthesis of Highly Substituted Cryptophane Derivatives. *J. Org. Chem*. **2013**. *78*. 6143-6153.

15. Cavagnat, D.; Buffeteau, T.; Brotin, T. Synthesis and Chiropractical Properties of Cryptophanes Having C_1-Symmetry. *J. Org. Chem*. **2008**. *73*. 66-75.

16. Darzac, M.; Brotin, T.; Bouchu, D.; Dutasta, J.P. Cryptophanols, New Versatile Compounds for the Synthesis of Functionalized Cryptophanes and Polycryptophanes. *Chem. Commun*. **2002**. 48-49.

17. Brotin, T.; Devic, T.; Lesage, A.; Emsley, L.; Collet, A. Synthesis of Deuterium-Labeled Cryptophane-A and Investigation of Xe@Cryptophane Complexation Dynamics by 1D-EXSY NMR Experiments. *Chem. Eur. J*. **2001**. *7*. 1561-1573.

18. Hayashida, M.; Ishizaki, M.; Hara, H. Investigation of Selective Mono-deallylation of O,O'-Diallycatechols and 3-Methylene-1,5-benzodioxepanes. *Chem. Pharm. Bull*. **2006**. *54.* 1299-1303.