Stony Brook University

Molecular Design and Synthetic Studies of an Amino Acid Receptor

A Thesis Presented

by

JiSun Lee

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Abstract of the Thesis

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Molecular recognition is one of the most important fundamental concepts for much of chemical and biological phenomena. The studies of many synthetic model systems may contribute to the understanding of molecular recognition and its applications. This may offer new perspectives on the development of pharmaceuticals, enantioselective sensors, catalysts and molecular devices.

Synthetic receptors for biologically relevant substrates such as amino acids and peptides are of increasing importance because amino acids and their derivatives are basic building blocks of biological systems. A new amino acid receptor molecule was designed by our research group, using the computer based design program CAVEAT. The general structure containing a guanidine group and a carbonyl group was chosen as an initial target for the receptor design using CAVEAT. Since an amine group and a carboxylate group from an amino acid can bind to an aldehyde group and a guanidinium group of the receptor respectively, the receptor was designed to incorporate each functional group in the proper relative position to form a complex.

We used commercially available 2-bromo benzaldehyde as our starting point to approach our target molecule. The final product could be deprotected, or may be used as an amino acid receptor in the protected form to bind with an amino acid.

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I) Background and Significance

i. Molecular Recognition

Molecular recognition is one of the most important fundamental concepts for much of chemical and biological phenomena.[1] Molecular recognition can be described as the study and development of host-guest systems. Many artificial receptors have been made for recognition of various guests through rational design and synthesis .[2]

Among the first known molecules studied for molecular recognition were the naturally occurring cyclodextrins (CDs), which bind many organic molecules.[2] CDs are cyclic oligosaccaharides consisting of α-1,4-linked D-(+)-glucopyranose units. α-CD, β-CD, and γ-CD are three naturally occurring cyclodextrins consisting of six, seven, and eight glucose units respectively (Figure 1).[2]

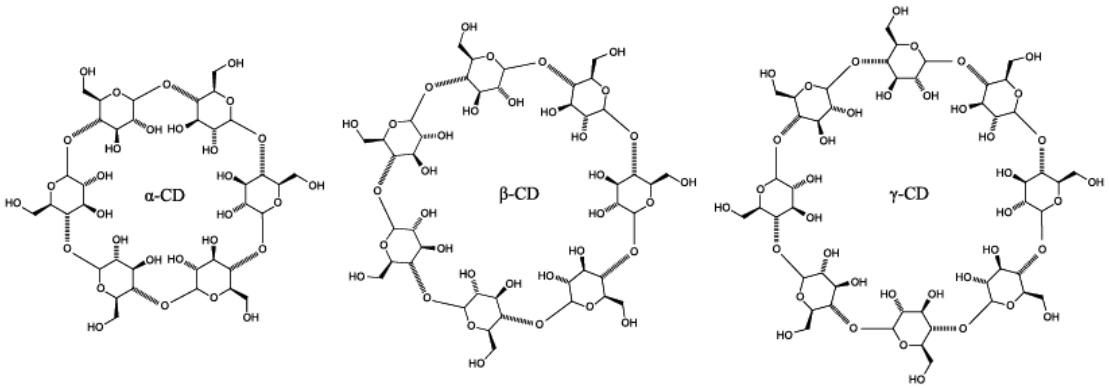


Figure 1. Structures of α-CD, β-CD, and γ-CD[9]

An interesting class of CD receptors with a covalently attached group has been developed. The group that is covalently linked to this type of CD becomes encapsulated by the CD. Then, when the other guests are added to the system, the covalently linked group can be selectively displaced (Figure 2).

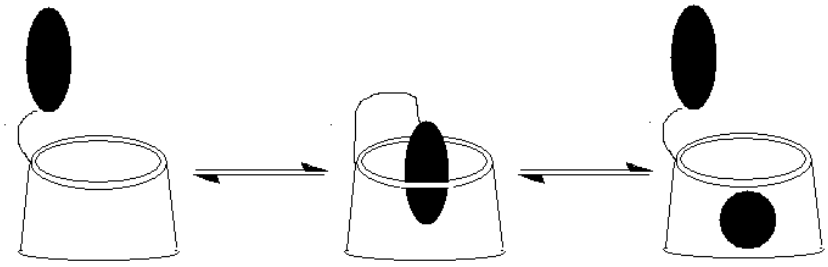
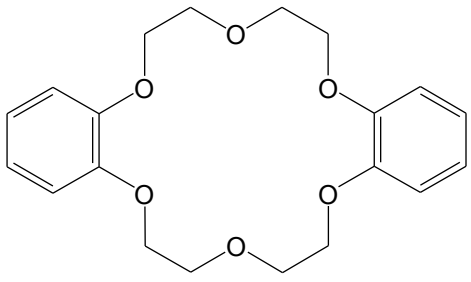


Figure 2. CD Receptor with Covalently Attached Group

Another widely used family of host compounds in supramolecular chemistry are the crown ethers.[2] Figure 3 shows the structure of dibenzo-18-crown-6, which was the first crown ether synthesized by Charles Pedersen, who shared the Nobel Prize in 1987.[3,4] Each adjacent pair of oxygen atoms in a crown ether is linked by a bridge of two or more



1

Figure 3. Dibenzo-18-Crown-6

carbon atoms and arranged in a ring that looks like a crown. Certain metal ions such as sodium or potassium ion bind in the center of the ring by coordination to each of the exposed oxygen atoms, fitting like a key in a lock.[5] The crown ethers have also been extensively studied in binding of biological compounds. For example, Barboiu *et al.* prepared a series of supramolecular complexes of crown ethers with the protonated amine salts of different natural and non-natural amino acids.[2]

The studies of many other synthetic model systems may continue to contribute to the understanding of molecular recognition and its applications. Recent successes in imitating natural phenomena using synthetic artificial receptors have shown that certain biological behavior can be engineered into relatively simple molecules.[6] This may offer new perspectives on the development of pharmaceuticals, enantioselective sensors, catalysts and molecular devices.[1, 27]

ii. **CAVEAT** [7-12]

CAVEAT is a computer program that searches structural databases for molecules that contain bonds satisfying a specified geometric relationship. CAVEAT was developed initially to facilitate the design of constrained peptide analogs as enzyme ligands and has continued to be used as a database search engine for a purely intuitive approach to molecular design.

CAVEAT very rapidly searches its database consisting of a huge number of molecular structures to find connectors that also contain two bond vectors with the same geometrical parameters. The geometrical relationship between the vectors is described with a distance, two angles and dihedral angles, as shown in Figure 4.

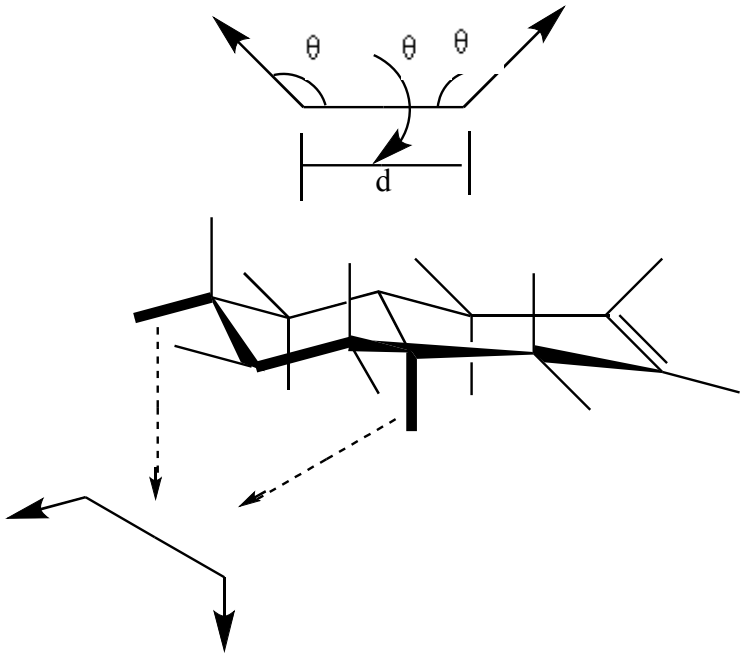
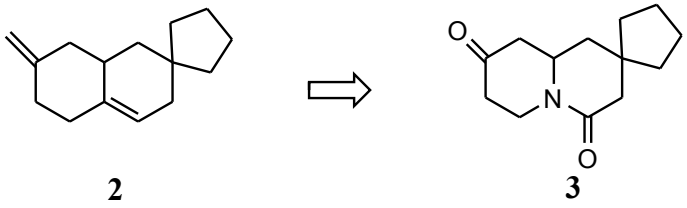


Figure 4. Vectors in CAVEAT with Distance, Angle, and Dihedral Angle

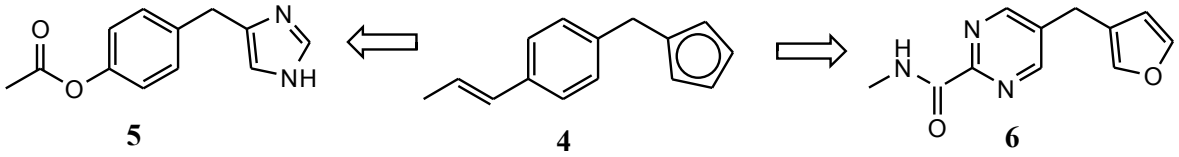
Initially CAVEAT was used to search the Cambridge Structural Database. Later, the databases called TRIAD and ILIAD were developed from the Berkeley group. TRIAD is a collection of 411,000 minimized tricyclic hydrocarbon structures and is comprehensive within the following specifications: tricyclic hydrocarbons containing three- to six-member rings, all possible patterns of unsaturation including one exocyclic methylene group on each ring, and all possible stereoisomers. ILIAD, in turn, is similarly comprehensive as a collection of acyclic molecules built up from combination of five of

the simple building blocks: -CH2-, -CH=CH-, CH2=C<, -C≡C-, -S-, -S-S-, *o-, m-,* and *p*-phenylene, *o*- and *m*-disubstituted cyclopentadienyl, and trimethylenemethane. ILIAD contains vectors for 110,000 linear structure including different functional groups. Structure **2** in Fig. 5 is an example from TRIAD while structure **4** is an example from ILIAD.



2 **3**

Examples for TRIAD



5 **4** **6**

Examples for ILIAD

Figure 5. Some Examples of Structures from TRIAD and ILIAD

The initial core structures may be very challenging for actual synthesis, especially with incorporation of the desired substituents that bind the guest. Therefore, search hits are used to point to frameworks which might be useful as a starting point for designing the target molecule with appropriate heteroatom substituents. For example, **3** is a potentially more synthetically accessible structure with the same geometry as **2**, while **5** and **6** are geometric equivalents of **4**.

In our group, CAVEAT has been extensively incorporated into our research projects. As an example, an arylboronic acid-based glucose sensor was designed with CAVEAT by a previous group member. The molecular design of the structure of the sensor is shown in Figure 6. Initially a conformational search of the bis-*p*-tolyboronate derivative of glucose **7** was performed, followed by a geometry optimization to predict the lowest energy conformation of **7**. Based on this refined structure, a pair of vectors was defined corresponding to the methyl-aryl bonds of **7**. Then, CAVEAT was used to identify polycyclic organic structures that have substituent bonds matching the vector pair from a TRIAD database. After analysis of all possible structures based on several factors, especially ease of synthesis, compound **9** was chosen as the lead structure. Simple replacement of these hydrogen atoms of the C-H bonds matching the defined vectors with phenylboronic acid groups leads to the glucose receptor **10**. The structure was then further modified to improve conformation, stability, ease of synthesis, and aqueous solubility to arrive at the final structure **11**. Compound **11** was synthesized and was shown by NMR, mass spec, and fluorescence studies to bind glucose with high selectivity and affinity to form the complex **12**.

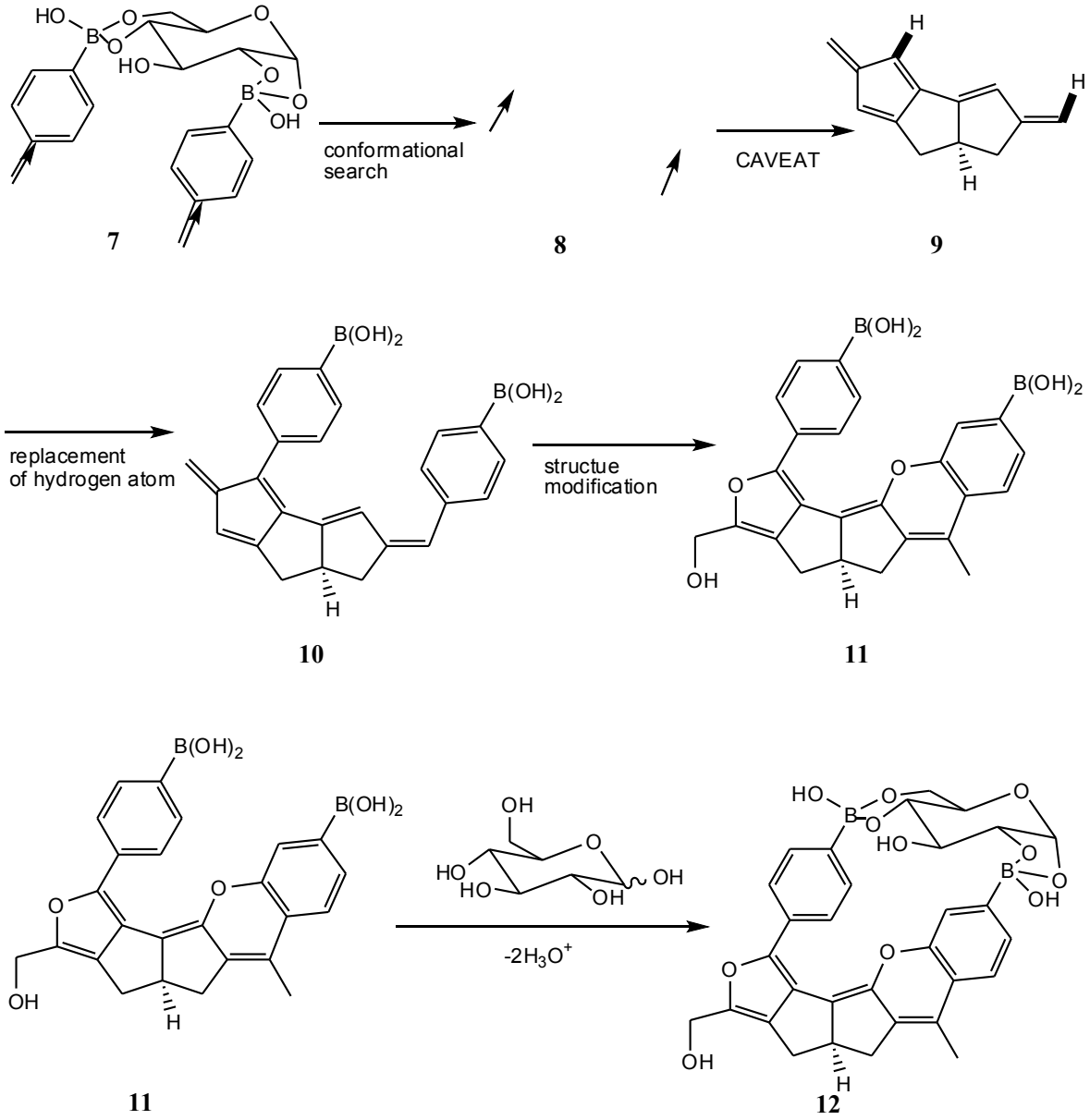
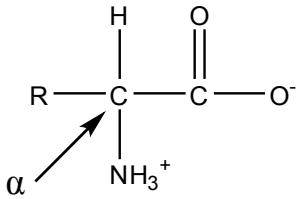


Figure 6. Molecular Design of an Arylboronic Acid-Based Glucose Sensor

iii. Importance of Amino Acids and Amino Acid Receptors

Synthetic receptors for biologically relevant substrates such as amino acids and peptides are of increasing importance because amino acids and their derivatives are basic building blocks of biological systems.[13] Especially, alpha-amino acids (α-amino acids) are the building blocks of proteins which make up the muscles, tendons, organs, glands, nails, and hair. [14] Growth, repair and maintenance of all cells are also dependent upon them.

Amino acids exist in neutral aq. solution as zwitterions having a protonated amine group and an ionized carboxyl group. Figure 7 shows the general structure of an α-amino acid. There are 20 α-amino acids commonly found in proteins which can be subdivided into three different groups based on having either a neutral, acidic, or basic side chain.[14, 15]



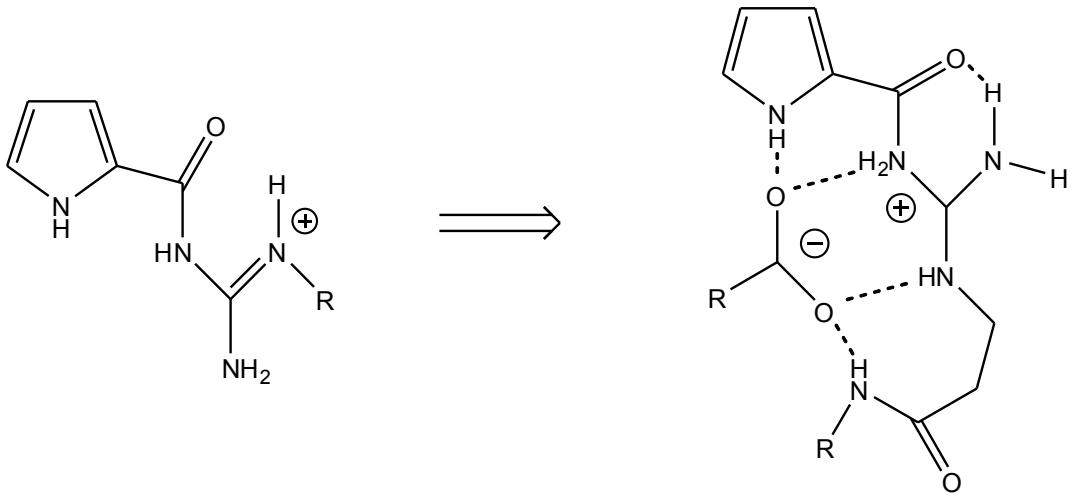
13

Figure 7. The General Structure of α-Amino Acid

Amino acids also can be divided into essential amino acids and nonessential amino acids.[16] Essential amino acids are amino acids that have to be obtained from the diet such as histidine, leucine and methionine. The other amino acids that the body can manufacture from other sources are called nonessential amino acids. Alanine, arginine, and glutamic acid are some examples. These amino acids play very important roles in regulating and maintaining many chemical and biological processes in the human body. For example, leucine works with isoleucine and valine to promote the healing of muscle tissue, skin, and bones. Methionine assists in the breakdown of fats, thus helping to prevent a buildup of fat in the liver and arteries. Alanine plays a major role in the transfer of nitrogen from peripheral tissue to the liver and aids in the metabolism of glucose. [17]

Due to the significance of amino acid in many areas, many researchers have extensively studied and developed artificial receptors for amino acids. Biological recognition of amino acids, amines, and amino alcohols are accomplished by macromolecules and relatively small molecules with binding sites that are complementary to their designated substrates.[18] Complexes of synthetic hosts and amino acid guests can be stabilized by multiple H-bond, particularly in nonpolar solvents.[18] In most of these studies, molecular recognition is based on non-covalent interactions such as hydrogen bonding, metal coordination, hydrophobic hydrogen bonding, and hydrophobic interactions.[13, 20] Individually, such interactions are generally weak, but cumulatively several such interactions can be relatively strong.[18]

Carsten Schmuck and Volker Bickert from the University of Wurzburg in Germany specifically have developed receptors for the carboxylate group, including the carboxylate of amino acids.[20] They designed receptors based on N´-substituted guanidinocarbonyl pyrroles. The carboxylate of the amino acid forms an ion pair with the guandinium moiety with additional H-bonds from the pyrrole NH and the side chain amides to the carboxylate as shown in Figure 8. As a result, they were able to efficiently bind amino acid carboxylates even in water, whereas most other amino acid receptors only form complexes in aprotic organic solvents such as chloroform or acetonitrile.[20, 27] Despite all the progress made in this field and the large number of elegant and sophisticated host systems that have been described over the last two decades, non-covalently controlled phenomena are still not fully understood.[19]



14 **15**

Figure 8. Carboxylic Acid Receptor with N´-Alkylated Guanidiocabonyl Pyrrole

II) Results and Discussion

i. Molecular Design of Target Molecule using CAVEAT

A new amino acid receptor molecule was designed by our research group, using the computer based design program CAVEAT. The general structure **16** containing a guanidine group and a carbonyl group was chosen as an initial target for the receptor design using CAVEAT (Figure 9). The large oval structure in **16** represents an organic framework to be identified using CAVEAT. Since an amine group and a carboxylate group from an amino acid **13** can bind to an aldehyde group and a guanidinium group of the receptor respectively, the receptor was designed to incorporate each functional group in the proper relative position to form a complex represented by structure **17**. Compared to noncovalent interactions, imine bonds are slower to form but have the advantage of being much stronger and structurally well defined. These are features that are particularly desirable for developing amino acid receptors.[21] Computer modeling of the simple complex **18** was used to define the vector pair **19** for the CAVEAT search. A CAVEAT search of the TRIAD and ILIAD databases identified structure **20**, where the C-H bonds in bold match the vector pair **19**. Incorporation of the aldehyde and guanidinium groups into **20** forms the potential amino acid receptor **21**, which is expected to form the amino acid complex **22** (Figure 11). Though **21** has a great deal of conformational flexibility, molecular modeling of **21** and its amino acid complex **22** showed that the lowest energy conformation of **21** matches very well with the amino acid complex **18** and there is almost no change in conformation of **21** upon amino acid binding to form the complex

22. Based on these very positive modeling results, synthetic studies toward **21** were undertaken as described in the next section.

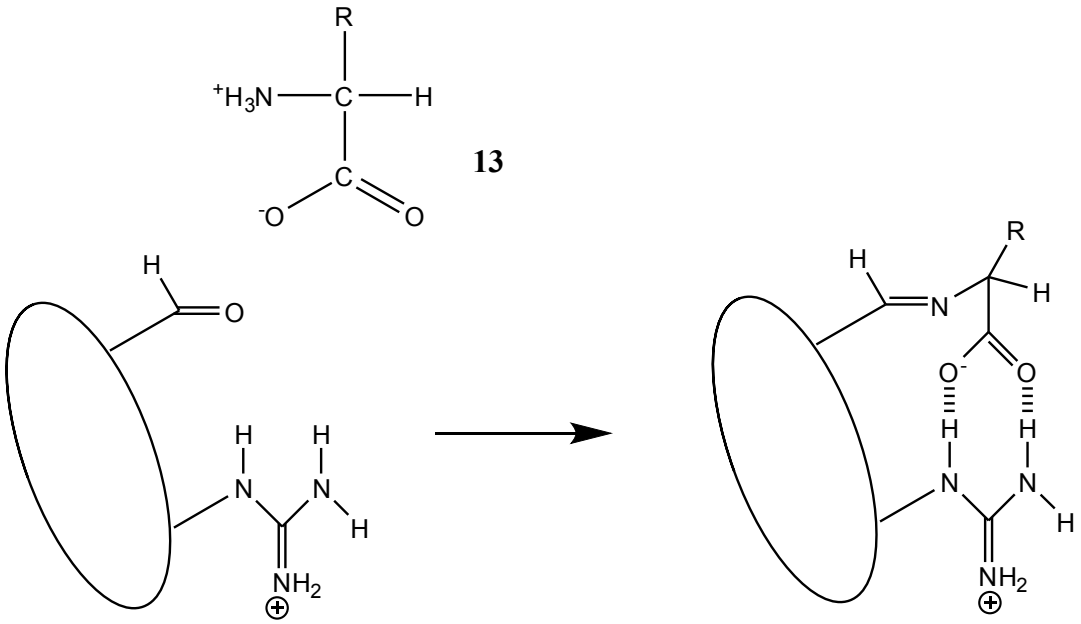


Figure 9. Complex Formation Between an Amino Acid and a Potential Receptor

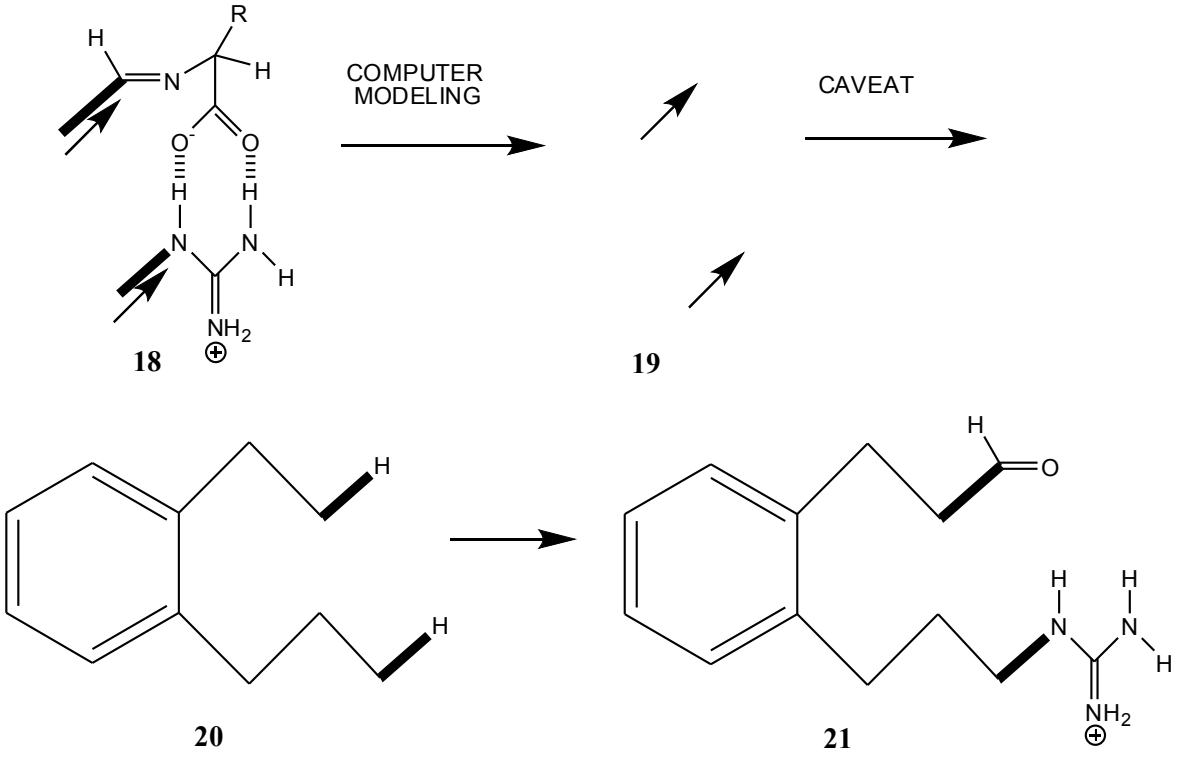


Figure 10. Computer-based Design of an Amino Acid Receptor

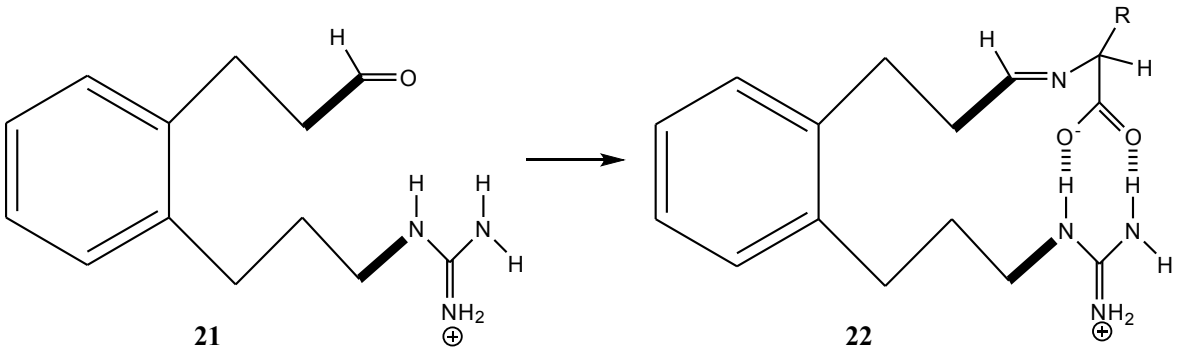


Figure 11. The Designed Amino Acid Receptor **21** and Its Amino Acid Complex **22**

ii. Synthetic Studies of Amino Acid Receptor 33

The synthetic approach toward an amino acid receptor based on structure **21** is shown in Figure 12. The final product **33** could be deprotected to form **21**, or may be used as an amino acid receptor in this protected form. Individual steps of the synthesis are described in the following paragraphs.

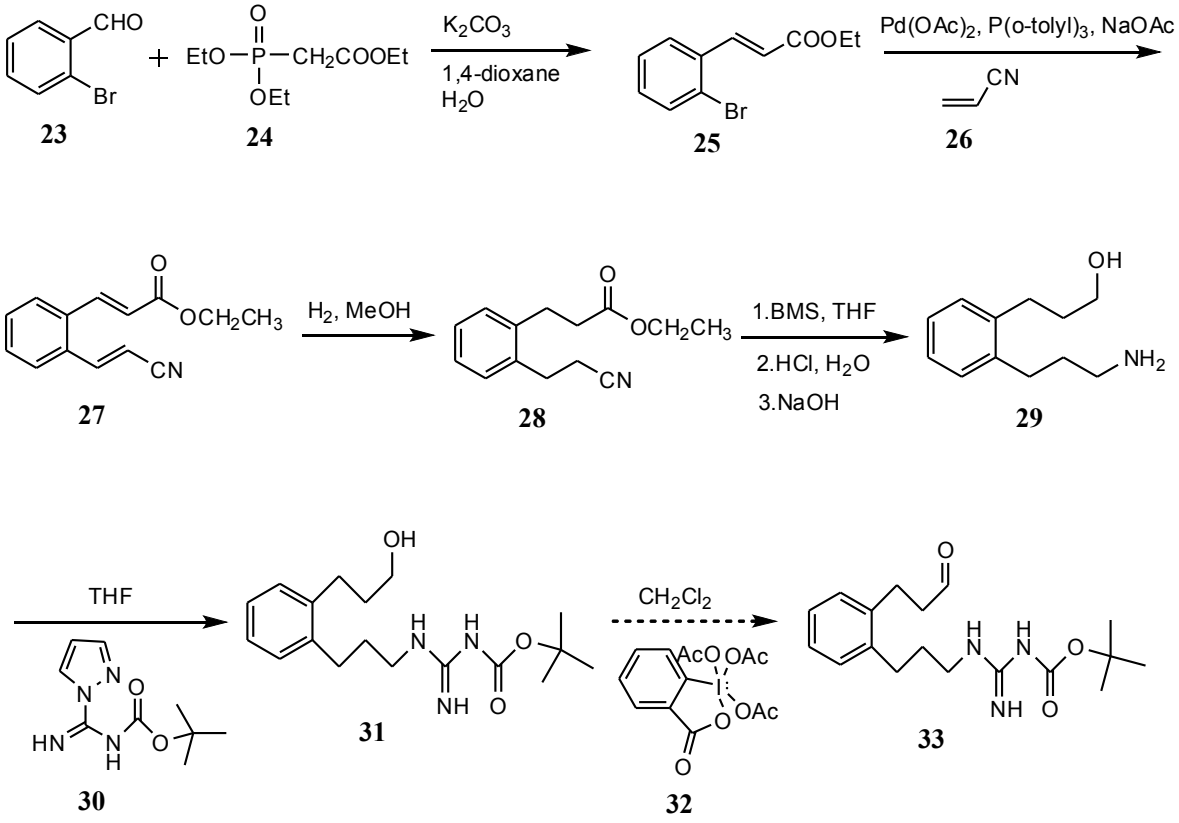
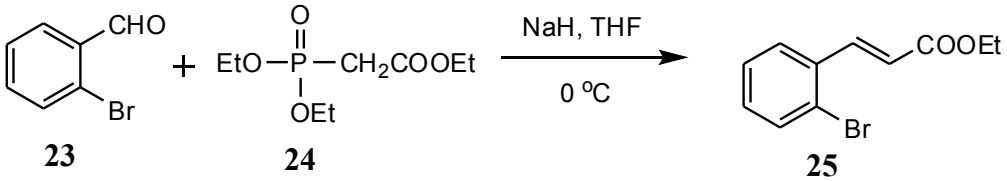


Figure 12. Overall Synthetic Route Taken to Target Molecule **33**

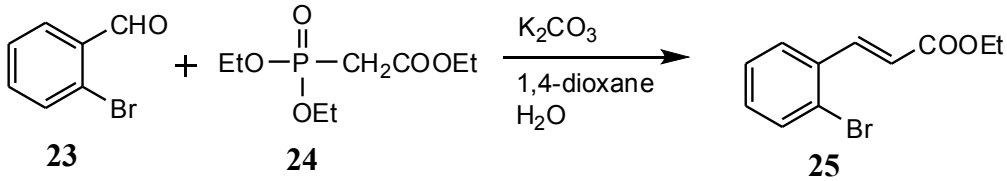
The synthesis of compound **25** was achieved by the Horner-Emmons reaction of commercially available 2-bromo benzaldehyde **23** with triethyl phosphonoacetate, **24** (Figure 13). The Wittig reaction and the Horner-Emmons reaction are used to convert aldehydes and ketones to carbon-carbon double bonds. However, in contrast to phosphonium ylides used in the Wittig reaction, phosphonate-stabilized carbanions are more nucleophilic and more basic.[22] When we synthesized compound **25** for the first time we followed method A, using NaH in THF as the base and solvent.[25] However, reaction with method A had to be done with several temperature changes. Also, sodium hydride is a highly reactive reagent that requires extra care and caution. Since method A was a relatively intricate procedure to follow, we decided to attempt the method B procedure. Method B did not require any temperature change during the reaction other than refluxing of the mixture at 80^{o} C for 18 hours. After reaction was done, the 1,4-dioxane solvent was evaporated prior to workup by extraction. Since triethyl phosphonoacetate was not visible under UV light, it was stained with 10% phosphomolybdic acid to do TLC. TLC showed that excess triethy phosphonoacetate remained in the reaction. After the subsequent workup, the crude product was purified by column chromatography using 8:1 and then 6: 1 ethyl acetate to hexanes. ^{1}H-NMR and ^{13}C-NMR data obtained after the column showed that the unsaturated ester product was a mixture of E & Z isomers. However, the E form was the major product in this experiment as expected. Since the double bond is to be reduced in a later step, the isomeric mixture at this point was not a problem.

Method A



23 **24** **25**

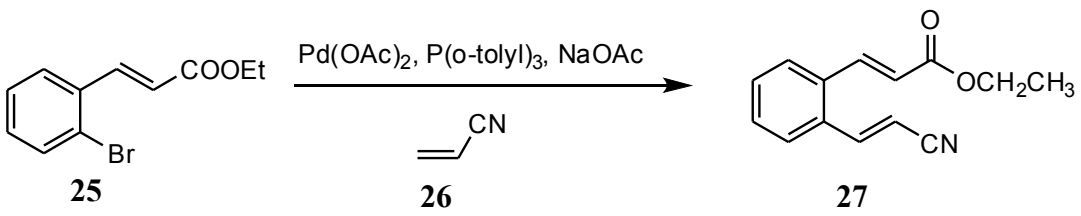
Method B



23 **24** **25**

Figure 13. Horner-Emmons Reaction

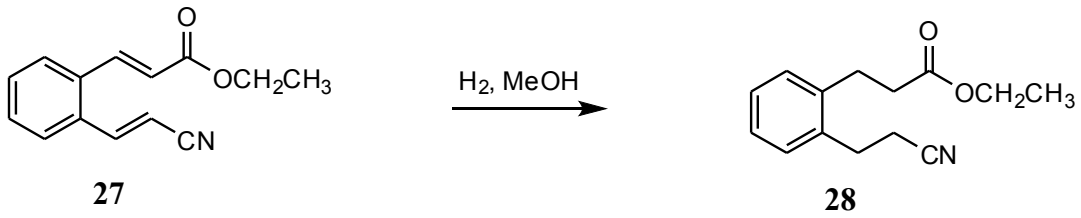
Compound **27** was then synthesized by a Heck reaction with a palladium catalyst and sodium acetate as base to form the α,β-unsaturated nitrile (Figure 14). The reaction was carried out under nitrogen to exclude any moisture from the reaction. Before the reaction was started, the solvent, dry DMF, was tested for the presence of water or other impurity by ^{1}H-NMR. Initially, the yield of the reaction was really low and TLC still showed a spot for the starting material **25,** even after many hours of the reaction. After increasing the amount of catalyst used, the yield was increased with no trace of the starting material shown in TLC. The Heck reaction has a tendency to form a mixture of E and Z isomers as product.[25] When palladium (II) acetate and tri-o-tolyphosphine were used as catalysts, the major product was the E isomer **27**.[25] ^{1}H-NMR and ^{13}C-NMR both confirmed that the product was a mixture of E and Z isomers.



25 **26** **27**

Figure 14. Heck Reaction

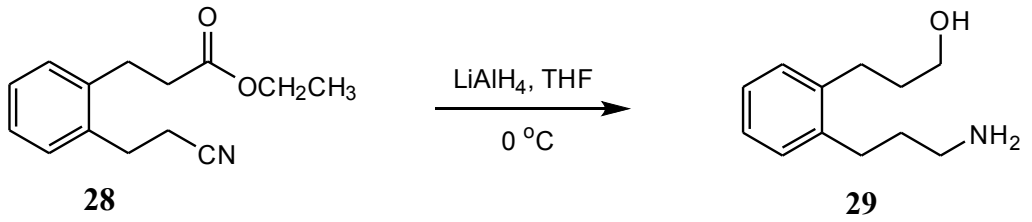
After the successful synthesis of compound **27,** the reduction of the two double bonds to form compound **28** was done by hydrogenation. In hydrogenation, alkenes react quantitatively with molecular hydrogen, H_2 in the presence of a transition metal catalyst to give alkanes.[25] Although the addition of hydrogen to an alkene is exothermic, reduction is immeasurably slow in the absence of a catalyst. In our experiment, 10% palladium on carbon powder was used as a catalyst to enhance the reaction. We used a three neck round bottom flask to make a connection to each of hydrogen gas, nitrogen gas and the vacuum pump. Since palladium on carbon is a pyrophoric substance, it may ignite when it makes contact with water or humid air. Therefore, the addition of catalyst was done under nitrogen with extra caution. Also during the reaction, all the connecting parts were carefully sealed with parafilm to prevent any hydrogen gas leaking during the reaction. The reaction was monitored by TLC and was complete after 7 hours, in about 75 % yield. The crude material was purified by column chromatography with an ethyl acetate and hexane mixture to give pure compound **28**.



27 **28**

Figure 15. Reduction of Two Double bonds

For the next step of synthesis, the ester and nitrile groups of **28** were reduced as shown in Figure 16. In this reaction, the ester group is reduced to the alcohol with two equivalents of hydride ion from lithium aluminum hydride. Also, the cyano group is reduced by lithium aluminum hydride to a primary amino group. Because both ester and nitrile groups are present in compound **28**, we had to use a large excess of lithium aluminum hydride to ensure enough hydride ion to reduce both groups. However, the reaction gave only low and variable yield even when though the reaction was done carefully under nitrogen condition. The dry tetrahydrofuran solvent was tested for any moisture that might have interfered with the reagent. Also, the reactivity of lithium aluminum hydride was tested with water to assure that the reagent was not oxidized. The difficulty in monitoring the reaction by TLC was also problematic. Another possibility is that the nitrile group may be first reduced and then react with the ester group to form a lactam instead of our desired product **29**.



28 **29**

Figure 16. Reduction of Ester and Nitrile Groups with $LiAlH_4$

In more recent work, borane-dimethylsulfide complex has been explored as the reducing agent for this reaction as shown in Figure 17. Since the literature only provided us the procedures for the reducing each ester and nitrile groups separately, we had to try few different work up steps to find one would work with both functional groups. First we did extraction with NaOH solution and ether followed by addition of HCl in 1,4-dioxane solution to the initial reaction. However, ^{1}H-NMR showed large impurity peaks with relatively small product peaks. When we did not employ any extraction step to bypass use of any water, ^{1}H-NMR showed cleaner data but very broad peaks that made it hard to interpret integrations. Finally, we decided to do a two step extraction procedure to isolate any neutral impurity before we actually extract our desired product. After adding HCl and heating the reaction mixture, we extracted the mixture with ether to remove neutral byproducts. Then, we added NaOH to neutralize the desired amine and extracted with ether to isolate the product. ^{1}H-NMR clearly showed that ester group from compound **28** had disappeared to form the alcohol and the new N-H peak and the peaks for the methylene group attached to the amine indicated that the nitrile group had been converted to the amine group as desired.

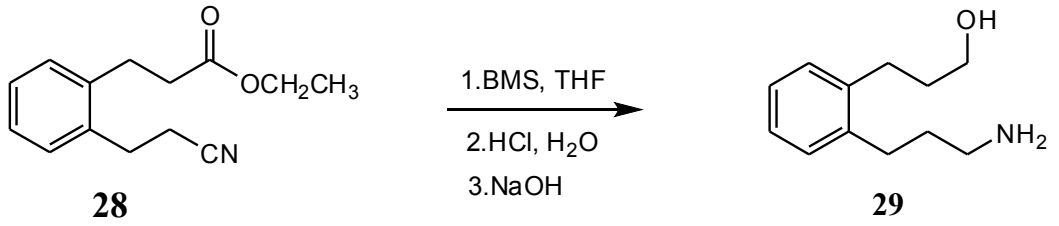


Figure 17. Reduction of Ester and Nitrile Groups with Borane-dimethylsulfide Complex

The commercially available reagent *N*-Boc-1*H*-Pyrazole-1-carboxamidine **30** was chosen for incorporation of the Boc-protected guanidine group into compound **29**. The Boc group should facilitate the reaction and product isolation, and while not present in the initially designed receptor **21**, its removal for amino acid binding may not actually be necessary. Before attempting the reaction with **29**, it was decided to first test the reaction with a simpler readily available amine. Thus benzylamine **34** was chosen and reacted with **30** in THF at room temperature, to form the product **35** (Figure 18). The ^{1}H-NMR spectrum of the crude product confirmed that the Boc-protected guanidine group was indeed successfully attached to the amine group of benzylamine.

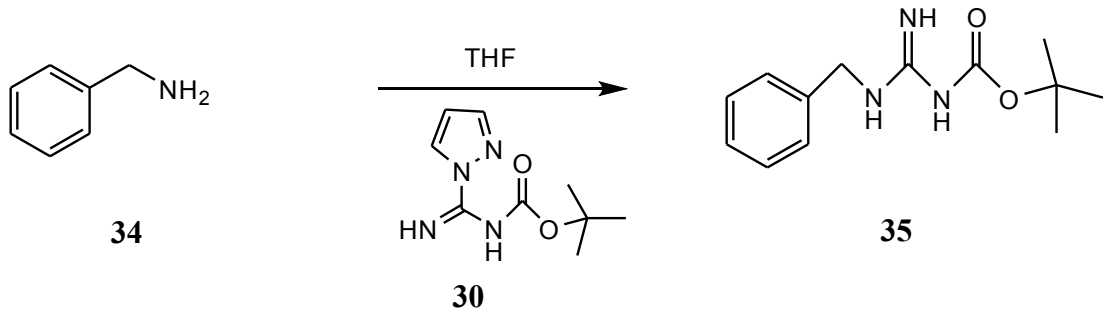


Figure 18. Test Reaction with Monoprotected Boc **30**

Based upon this successful test reaction, the same reaction conditions were applied to the reaction of the reagent **30** with our amine compound **29** (Figure 19). The reaction was done at room temperature and monitored by TLC, and appeared to proceed. However, integration of the ^{1}H-NMR signals did not agree with the expected numbers. Especially, the tert-butyl group integrated to only 5 hydrogen atoms instead of 9 relative to other assignable peaks in the spectrum. We thought that these observations may be due to the possible presence of three tautomers of the protected guanidine group of compound **31**, that interconvert slowly enough to be detected individually in the ^{1}H-NMR spectrum. However, the ^{13}C-NMR spectrum does not support this explanation. It may be necessary to obtain larger quantities of the product for further separation and characterization.

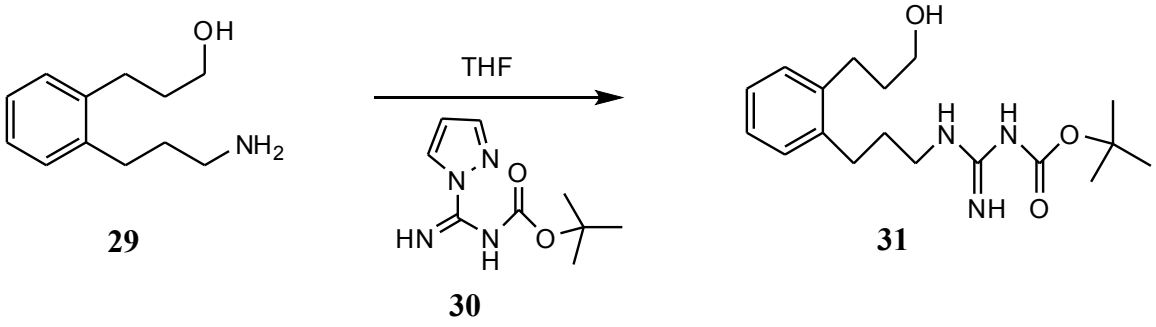
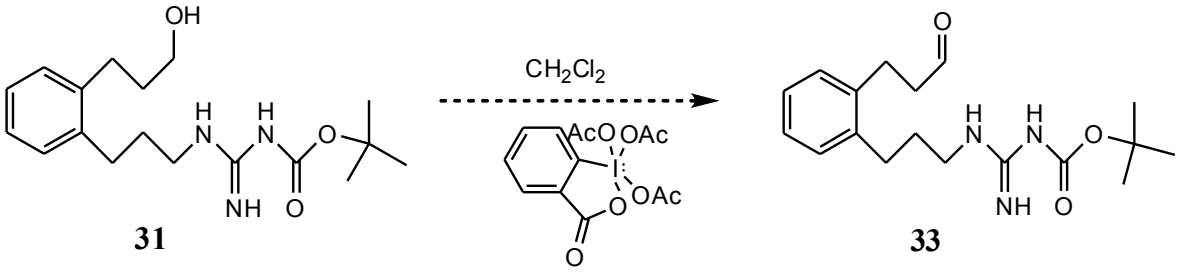


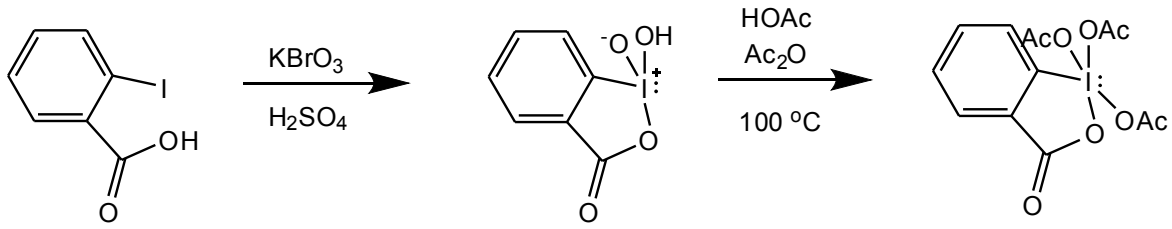
Figure 19. Synthesis of the Boc-protected guanidine **31**

For the last step to make our target molecule **33**, we will be oxidizing the alcohol group of compound **31** to an aldehyde group using oxidizing reagent **32** (Figure 20). We first prepared the reagent **32** from 2-iodobenzoic acid **36** in two steps as shown in Figure 21. Compound **37** was reported to be explosive by the Meyer group, therefore extra caution was required.[26] To prevent any bromine or impurities in compound **37** that might induce explosion, we washed the product with both water and ethanol. The melting point of compound **37** agreed with the literature value of 233° C.



31 **32** **33**

Figure 20. Oxidation of Alcohol Group of Compound **31**



36 **37** **32**

Figure 21. Synthesis of Oxidizing Agent **32**

Then, intermediate compound **37** was reacted with acetic acid and acetic anhydride at 100^{o} C to form our oxidizing reagent **32**. After the reaction was completed, ^{1}H-NMR confirmed the tris-acetylated product. IR data also confirmed all functional groups in compound **32**.

iii. Future Studies

The initial goal of future work will be to complete the synthesis of the designed amino acid receptor **33**. After **33** is successfully synthesized, the binding of compound **33** to alanine or other amino acid will be studied by ^{1}H-NMR analysis. It may also be desirable to remove the Boc protecting group from **33** to study amino acid binding to the deprotected compound. Depending on solubility of **33** and the amino acid, possible solvents for ^{1}H-NMR are D_2O, CD_3OD, and DMSO-d_6 .

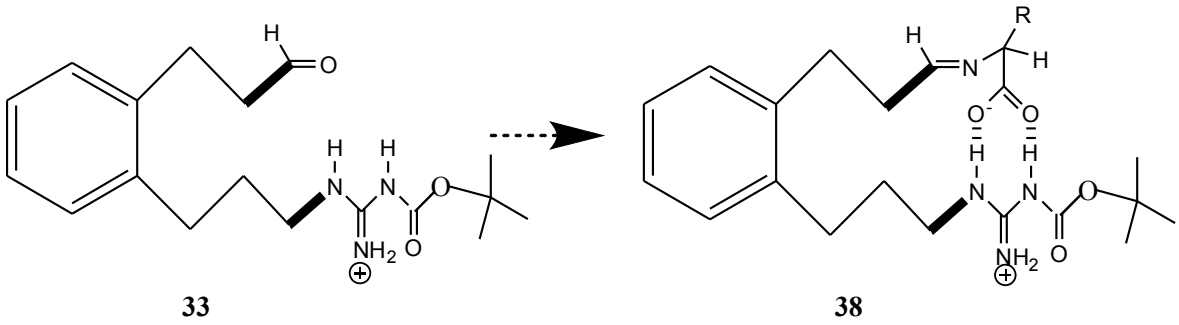


Figure 22. Binding Study of Target Molecule **33** and an Amino Acid

When the guanidinium group of the compound **33** binds to carboxylate through a combination of ion pairing and hydrogen bonding, ^{1}H-NMR in an aprotic solvents such as DMSO should show large downfield shifts of the guanidinium NH protons. Upon imine formation between the amine group of the amino acid and the aldehyde group of **33**, ^{1}H-NMR will no longer show the aldehyde peak at near $\delta = 9.7$ but instead should show the corresponding imine peak in the range of 6-7 ppm. From the ratio of bound and unbound amino acid and/or receptor at known concentrations of each, the equilibrium

constant for complex formation will be determined. If complex formation is sufficiently slow, it may also be possible to determine the rate constant for formation of the complex using appropriately designed NMR experiments.

A more distant objective may be to use **33** or the deprotected form as the starting point for a receptor for a specific amino acid. This might be achieved by introducing additional functionality to interact with a complementary functional group of the targeted amino acid. Also of potential future interest could be the incorporation of an environmentally sensitive fluorophore to convert the amino acid receptor into a fluorescence-based amino acid sensor.

iv. Conclusion

A new amino acid receptor has been designed for recognition of an amino acid by using computer based design program CAVEAT. We used commercially available 2-bromo benzaldehyde as our starting point to approach our target molecule. Each aldehyde and bromine group was converted to ester and acrylonitrile group with Horner-Emmons reaction and Heck reaction respectively. Then, ester group was reduced into alcohol group while nitile group was reduced into amine group. The monoprotected Boc group was then attached to the amine group to facilitate the reaction and product isolation. The last step of synthesizing our target molecule, oxidation of alcohol to aldehyde, will be done with the oxidizing reagent we made previously. The final product could be deprotected, or may be used as an amino acid receptor in the protected form to bind with an amino acid.

III) Experiment

Ethyl 3-(2-bromophenyl)acrylate (25)

Method A: To a stirred suspension of NaH (6.5mmol, 0.156g) in 7ml THF, triethylphosphonoacetate (6mmol, 1.34g) was added at 0^{o} C to give a white foam. The mixture was allowed to warm to room temperature and after 30 minutes was re-cooled in an ice bath and 2-bromo benzaldehyde (5mmol, 0.925g) was added as a solution in 7ml THF. After 20 minutes the reaction mixture was allowed to warm to room temperature and stirred for overnight. Saturated aqueous NH_4Cl was then added to the mixture. Diethyl ether was added and the organic phases were combined and washed with water, dried with magnesium sulfate and the solvent removed by evaporation to give light yellow oil. (Yield: 2.75mmol, 0.698g, 55%)

Method B: To a mixture of 2-bromo benzaldehyde (0.087mol, 16.43g), triethyl phosphonoacetate (0.108mol, 24.38g) and potassium carbonate (0.126mol, 17.44g), 1,4-dioxane (65ml) was added . Then, 2ml water was added to the reaction mixture. Then, the mixture was stirred under reflux for overnight at 80^{o} C. The resulting solution was concentrated by evaporation. Then, extracted with ethyl acetate and washed with water and brine. The combined organic layer was dried over magnesium sulfate and concentrated by evaporation. Further purification was carried out by preparative column chromatography with ethyl acetate and hexane on silica to give light yellow oil. (Yield: 3.35mmol, 0.85g, 67%) ^{1}H-NMR ($CDCl_3$, 300MHz): 1.32 (t, 3H), 4.27(q, 2H), 6.14 (d, 1H), 7.19 (t, 1H), 7.29(t, 1H), 7.57 (d, 2H), 8.04 (d, 1H); ^{13}C-NMR ($CDCl_3$, 300MHz): 14.2, 60.5, 76.9, 120.9, 125.1, 127.4, 130.9, 133.2, 142.6, 166.1

Ethyl 3-(2-(2-cyanovinyl)phenyl)acrylate (27): To dry DMF (70ml) in a round bottom flask, compound **25** (20mmol, 4.502g), acrylonitrile (34mmol, 1.805g), tri-o-tolyphosphine (1.8mmol, 0.56g), palladium(II) acetate (1.4mmol, 0.314g), and sodium acetate (60mmol, 4.921g) were added. The mixture was stirred under reflux for 48 hours at 130^{o} C. After adding 20ml of water to the resulting solution and extracted with diethyl ether (80ml). Then, it was washed with water and brine. Further purification was carried out by preparative column chromatography with 6:1 ethyl acetate and hexane (6:1) on silica to give compound **27**. (Yield: 13mmol, 2.95g, 65%) ^{1}H-NMR ($CDCl_3$, 300MHz): 1.36 (t, 3H), 4.28 (q, 2H), 5.80 and 5.64 (d, 1H), 6.30 (d, 1H), 7.40-7.92 (m, 6H); ^{13}C-NMR ($CDCl_3$, 300MHz): 14.2, 60.7, 70.8, 99.2, 116.3, 117.5, 122.1, 122.7, 126.6, 127.7, 129.3, 130.8, 132.8, 133.9, 140.1, 140.5, 146.8, 147.3, 165.8, 166.0

Ethyl 3-(2-(2-cyanoethyl)phenyl)propanoate (28): To a solution of compound **27** (2.11mmol, .48g) in methanol (40ml), 10% pd/c was added under nitrogen. The solution was stirred for overnight under hydrogen. The resulting solution was filtered then concentrated by evaporation. Further purification was carried out by preparative column chromatography with 8:1 ethyl acetate and hexane on silica to give compound **28**. (Yield: 1.47mmol, 0.341g, 70%) ^{1}H-NMR ($CDCl_3$, 300MHz): 1.23 (t, 3H), 2.60 (t, 4H), 3.01 (m, 4H), 4.11 (q, 2H), 7.19 (s, 4H); ^{13}C-NMR ($CDCl_3$, 300MHz): 14.1, 18.5, 27.2, 28.1, 35.1, 60.4, 76.6, 118.8, 126.7, 128.9, 135.6, 138.0, 172.2

3-(2-(3-aminopropyl)phenyl)propan-1-ol (29): Under nitrogen, $LiAlH_4$ (8.72mmol, 0.331g) were transferred to a round bottom flask containing 10ml of THF. Then compound **28** (1.09mmol, 0.25g) in dry THF (10ml) were added slowly at 0^o C, and the temperature was increased to room temperature. After the mixture was stirred for 5 hours, it was quenched at 0^o C by slowly adding water (0.45ml), 15% NaOH (0.45ml), and water (1.35ml) in order then was filtered. The filtered solution was extracted with ether (2 x 50ml). The organic phase was separated, dried, and concentrated in vacuo to yield compound **29**. (Yield: 0.218mmol, 0.042g, 20%)

3-(2-(3-aminopropyl)phenyl)propan-1-ol (29): To a solution of 1ml 2 N BMS in THF, compound **28** (1mmol, 0.231g) was added to be reduced. The reaction mixture was heated to reflux and the dimethyl sulfide collected as it was distilled. After 0.5 hours the reaction was cooled to room temperature and 1ml 6 N HCl was added dropwise. The reaction mixture was then heated under reflux for 0.5 hours. After it was cooled to room temperature, the mixture was extracted with ether (2 x 10ml). Combined aqueous layer was then extracted with 2ml 4 N NaOH and ether (3 x 10ml). The organic layer was dried over magnesium sulfate and concentrated by evaporation. (Yield: 0.91mmol, 0.175g, 91%) ^{1}H-NMR ($CDCl_3$, 300MHz): 1.31 (t, 1H), 1.94 (m, 2H), 2.58 (s, 1H), 2.84 (m, 6H), 3.79 (m, 2H), 7.25 (m, 4H)

Compound 35: To a solution of benzylamine (1mmol, 0.107g) in 1ml THF, N-Boc-1H-pyrazole-1-carboxamidine (1.2mmol, 0.252g) was added and stirred at room temperature for overnight. The resulting mixture was extracted with ethyl acetate (3 x 10ml). It was dried over magnesium sulfate and concentrated in vacuo.

Compound 31: To a solution of compound **29** (1mmol, 0.193g) in 1ml THF, N-Boc-1H-pyrazole-1-carboxamidine (1.5mmol, 0.315g) was added and stirred at room temperature for overnight. The resulting mixture was extracted with ethyl acetate (3 x 10ml). It was dried over magnesium sulfate and concentrated in vacuo. It was purified by column chromatography 3:1 (hexane: ethyl acetate) and the result was a white powder. (Yield: 0.52mmol, 0.174g, 52%)

1-Hydroxy-1,2-benziodoxol-3(1*H*)-one 1-Oxide (37): Potassium bromate (4.5mmol, 0.75g) was added slowly to a vigorously stirred mixture of 2-iodobenzoic acid (0.34mmol, 1g) and 7.3ml of 0.73 M H2SO4 in a 55^{o} C bath. The mixture was stirred for 4 hours at 68^{o} C and then cooled with an ice bath. Filtration and washing of the solid with 10ml of water and 5ml ethanol gave compound **37**. (Yield: 0.29mmol, 0.081g, 85%) mp 232-233^{o} C

1,1,1-Triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1H)-one (32): A slurry of small crystals of **37** (0.0189mol, 5.28g) in a mixture of acetic anhydride (0.189mol, 19.33g) and acetic acid (0.315mol, 18.95g) was stirred for 15 hours at room temperature and the solvent was removed under vacuum until a thick slurry remained. Filtering the slurry in an inert atmosphere and washing with 30ml of anhydrous ether gave a white solid. (Yield: 0.0176mol, 7.45g, 93%) ^{1}H-NMR ($CDCl_3$, 300MHz): 2.00 (s, 3H), 2.09 (s, 3H), 2.32 (s, 3H), 7.90-8.32 (m, 6H) mp 134^{o} C

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