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THE DESIGN, SYNTHESIS OF POTENTIAL SIALIDASE INHIBITORS AS ANTI-INFLUENZA DRUGS AND SYNTHESIS OF C-2 SYMMETRIC LIGANDS FOR TRANSITION METAL CATALYZED ASYMMETRIC REDUCTION REACTIONS

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ABSTRACT

THE DESIGN, SYNTHESIS OF POTENTIAL SIALIDASE INHIBITORS AS ANTI-INFLUENZA DRUGS AND SYNTHESIS OF C-2 SYMMETRIC LIGANDS FOR TRANSITION METAL CATALYZED ASYMMETRIC REDUCTION REACTIONS

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The major objective of the work described in this thesis is to develop a strategy for the design and synthesis of new simple nitrogen containing heterocyclic systems for the inhibition of influenza sialidase. Azasugars, a common class of sugar derivatives in which the ring oxygen is replaced by a nitrogen atom, show tremendous potential as therapeutic agents in wide range of diseases, such as HIV, diabetes, and hepatitis because of their effective inhibition towards various glycosidases and glycotransferases. We designed a new azasugar scaffold targeting influenza sialidase which plays an essential role in the virus replication process and explored a short, efficient synthetic route. The ease of this synthetic route may provide access to new commercially available anti-influenza drugs. In the second part of this thesis, the design and synthesis of a new C-2 symmetric ligand is described. C-2 symmetric ligands have shown promising chirality control on transition metal catalyzed reactions. We developed a potentially industrially benign 5-step synthetic route with ubiquitous carbohydrate derivative as starting material. Once the catalyzing capability of this ligand is refined and confirmed, the costs for some industrial process, such as asymmetric ketone reduction, could be much reduced.

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Chapter 1 Background

1.1 Introduction to Influenza Virus and Influenza Neuraminidase

1.1.1 Glycosidase and Neuraminidase

Glycosidase is one kind of enzyme that is responsible for the hydrolysis of poly-and oligosaccharides into monomers or cleavage of bonds between sugars and a non carbohydrate aglycon.[1] This kind of enzyme is involved in several metabolic pathways and plays a key role in various biological processes including remodeling of cell wall, DNA repair etc. The successful inhibition of glycosidase is a way to modulate the cellular interactions and to develop therapeutic agents.

Neuraminidase (NA), also called sialidase, is one type of glycosidase which exists as a mushroom-shape projection on the surface of the influenza virus. It has a head consisting of four co-planar and roughly 4 spherical subunits, and a hydrophobic region that is embedded within the interior of the virus's membrane. It is comprised of a single polypeptide chain that is oriented in the opposite direction to the hemagglutinin antigen. The composition of the polypeptide is a single chain of six conserved polar amino acids, followed by hydrophilic, variable amino acids **(Figure 1.1)**. There are a large number of biological functions ascribed to this enzyme such as cell-cell recognition phenomena and the pathogenicity of some infections by sialidase-bearing microorganisms.[2] Neuraminidase can bind to sialic acid selectively and aid the virus to release from cells efficiently. Neuraminidase cleaves terminal sialic acid residues from carbohydrate moieties on the surfaces of infected cells. This promotes the release of progeny viruses from infected cells.[3, 4]

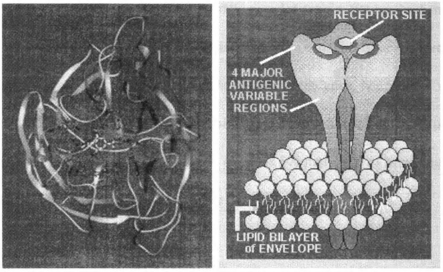


Figure 1.1 Ribbon diagrams of neuraminidase and its active site (Figure adapted from University of Cape Town Medical Virology lecture notes http://web.uct.ac.za/depts/mmi/jmoodie/influen2.html)

1.1.2 Influenza and Influenza Virus

Influenza, also known as the flu, is a contagious disease that is caused by the influenza virus. It attacks the respiratory tract in humans (nose, throat, and lungs) and may cause fever, headache, tiredness (can be extreme), dry cough, sore throat, nasal congestion, body aches. Millions of people in the United States — about 10% to 20% of U.S. residents — will get influenza each year. According to the data from Department of Health and Human Services Centers for Disease Control and Prevention, an average of about 36,000 people per year in the United States die from influenza, and 114,000 per year have to be admitted to the hospital as a result of influenza. Anyone can get the flu (even healthy people), and serious problems from influenza can happen at any age. In the Spanish Flu Pandemic, 50-100 million people were killed within 18 months.

The virion of an influenza virus is generally rounded (about 100 nm in diameter), but may be long and filamentous. The virus is sheathed in a lipid bilayer (derived from the plasma membrane of its host). And on the surface of the virus, two integral membrane proteins stud the lipid bilayer which contains some 3000 molecules of matrix protein and 8 pieces of RNA. The membrane proteins are some 500 molecules of hemagglutinin ("H") and some 100 molecules of neuraminidase ("N") **(Figure 1.2)**.

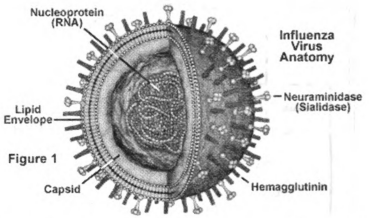


Figure 1.2 Structure of Influenza Virus (Figure adapted from http://micro.magnet.fsu.edu/cells/viruses/influenzavirus.html)

1.1.3 How Influenza Virus Invades Human Cells and the Function of Neuraminidase in This Process

The life cycle of influenza virus can be divided into 4 steps **(Figure 1.3)** in which two proteins on the surface of the influenza virus surface---hemagglutinin (HA) and neuraminidase (NA) play essential role in the invading process of the virus into the cell.[4]

1. Invasion step. The virus infection to the epithelial cells of the upper respiratory tract is initiated by hemagglutinin's binding to the cell surface receptor---sialic acid.
2. Entry to the cell. The attached virus subsequently undergoes endocytosis into the cell.

3. RNA replication step. After the uncoating process, two RNA strands are made in the nucleus. One is exported to the cytoplasm and serves as mRNA, whilst the other (cRNA) is used as a template to synthesize progeny vRNA.
4. Budding step. After virus replication, the progeny virus must be released from the cell to repeat the cell cycle of the infection.

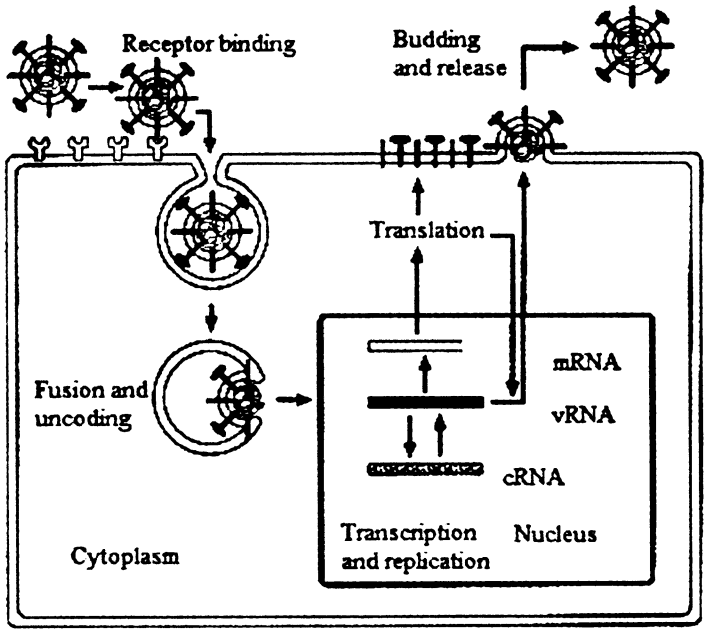


Figure 1.3 The life cycle of influenza virus

Influenza neuraminidase (NA) is involved in the last step of the life cycle of the virus-the budding step in which it mediates the release of the virus from the cell surface. NA cleaves the α-ketosidic linkage between terminal Neu5Ac (sialic acid) and a neighboring saccharide, usually galactose **(Figure 1.4)**. The viral enzyme shows some preference for a 2-3 linkages, but linkage and aglycon specificity is weak.[5, 6]

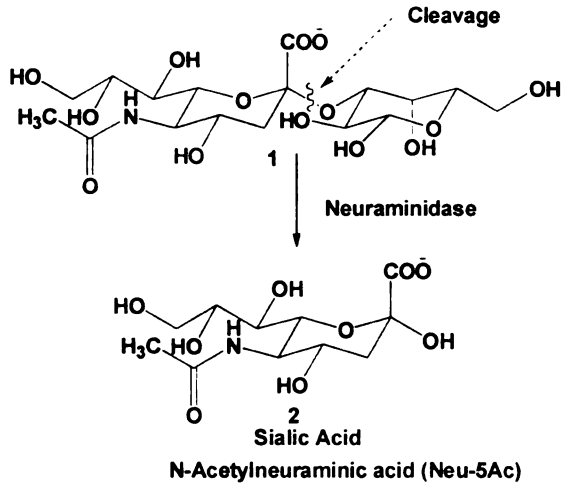


Figure 1.4 Cleavage of sialic acid by neuraminidase

The role of the receptor-destroying enzyme in the viral life cycle is subtle and may not be completely understood. It has been suggested that by cleaving the sialic acid residue, Neuraminidase accomplishes the following functions.[4]

i) Prevents virus aggregation on the cell surface

ii) Releases virus particles from the cells

iii) Destroys cellular receptors recognized by hemagglutinin

iv) Prevents viral inactivation by respiratory mucus

Direct evidence is available to support a role for the enzyme in facilitating release of progeny virions from the surface of infected cells, where they would otherwise aggregate as a result of interactions between the hemagglutinin and sialic acid on the surface of the infected cell and on the progeny virion envelop.[7, 8, 9, 10] NA may also promote viral movement through respiratory tract mucus, thus enhancing viral infectivity.[11, 12]

1.2 Current Solutions to Influenza

1.2.1 Traditional Treatment Methods for Influenza

The main strategy for preventing influenza and its more severe complications is immunoprophylaxis with inactivated (i.e., killed-virus) vaccine. Influenza-specific antiviral drugs for chemoprophylaxis or therapy are an important adjunct to vaccine, but they are not a substitute for influenza vaccine.

Actually, the life cycle of the influenza virus provides several targets for drug development. Those targets include hemagglutinin,[14,15] M2 protein,[16] neuraminidase,[17,18] and endonuclease .[19] In the United States, four antiviral agents are approved for preventing or treating influenza: Amantadine hydrochloride and Rimantadine hydrochloride as well as two recently approved neuraminidase inhibitors, Zanamivir and Oseltamivir.[13]

Amantadine and Rimantadine (Figure 1.5) act by interfering with the M2 protein ion channel function that is found only in influenza A. Besides of the insensitivity of influenza B viruses, clinical use of these agents is also limited because of the rapid emergence of resistance and apparent side effects.

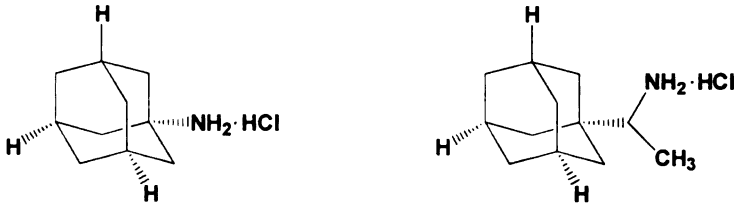


Figure 1.5 The structures of Amantadine and Rimantadine

1.2.2 Neuraminidase Inhibitors as Anti-Influenza Drugs

Neuraminidase offers attractive site for therapeutic intervention in influenza infection. As is said that NA catalyzes removal of terminal sialic acids linked to glycoproteins and glycolipid. It has been postulated that NA activity is necessary in the elution of newly formed viruses from infected cells by digesting sialic acids in the HA receptor.

For influenza A, nine subtypes of neuraminidase have been identified; whereas only one subtype is know for influenza B. Despite this diversity, the catalytic site for all influenza A and B neuraminidases is completely conserved.[18] This gives people a lot of opportunity to develop neuraminidase inhibitors as potent influenza drugs.

1.2.3 Enzyme-Substrate Interaction Study and Mechanism of Neuraminidase

The study of the mechanism of Influenza Neuraminidase received large amount of attention from scientists in the 1990's. Crystallographic studies of the enzyme showed that despite up to 50% sequence variation, the enzymes have similar three-dimensional structures, and the amino acid residues that line the active site are highly conserved in

both influenza A and B virus strains.[20,21,22] Their computational study on the binding between sialic acid and neuraminidase agree with X-ray crystallography very well and showed that the salt bridge between the carboxylate group of 1 and the charged Arg 371 in the active site contributes the greatest to binding. The ring oxygen appears to contribute only marginally to ligand binding through a weak charge-dipole interaction with Arg 292. Each of the hydroxyl groups on C2, C4, C7 and C9 accept protons from bulk solvent and form hydrogen-bond with the active-site. X-ray crystallographic structures of Neu5Ac and its analogues complexed with NAs show that the two terminal hydroxyls of the glycerol side chain form a bidentate interaction with Glu 276.[23, 24] However it is also noted that the C8 of the glycerol chain makes hydrophobic contacts with the hydrocarbon chain of Arg 224.[18] This hydrophobic pocket has been confirmed by scientists by crystallographic study. This hydrophobic pocket and the carboxylic acid-Arg 371 salt bridge are the most important interaction between NA and sialic acid.

The mechanism of the neuraminidase mediated cleavage of the sialic acid attached at the end of glycoprotein has been hypothesized to take place in this shallow pocket model which is shown below **(Figure 1.6)**.[25]

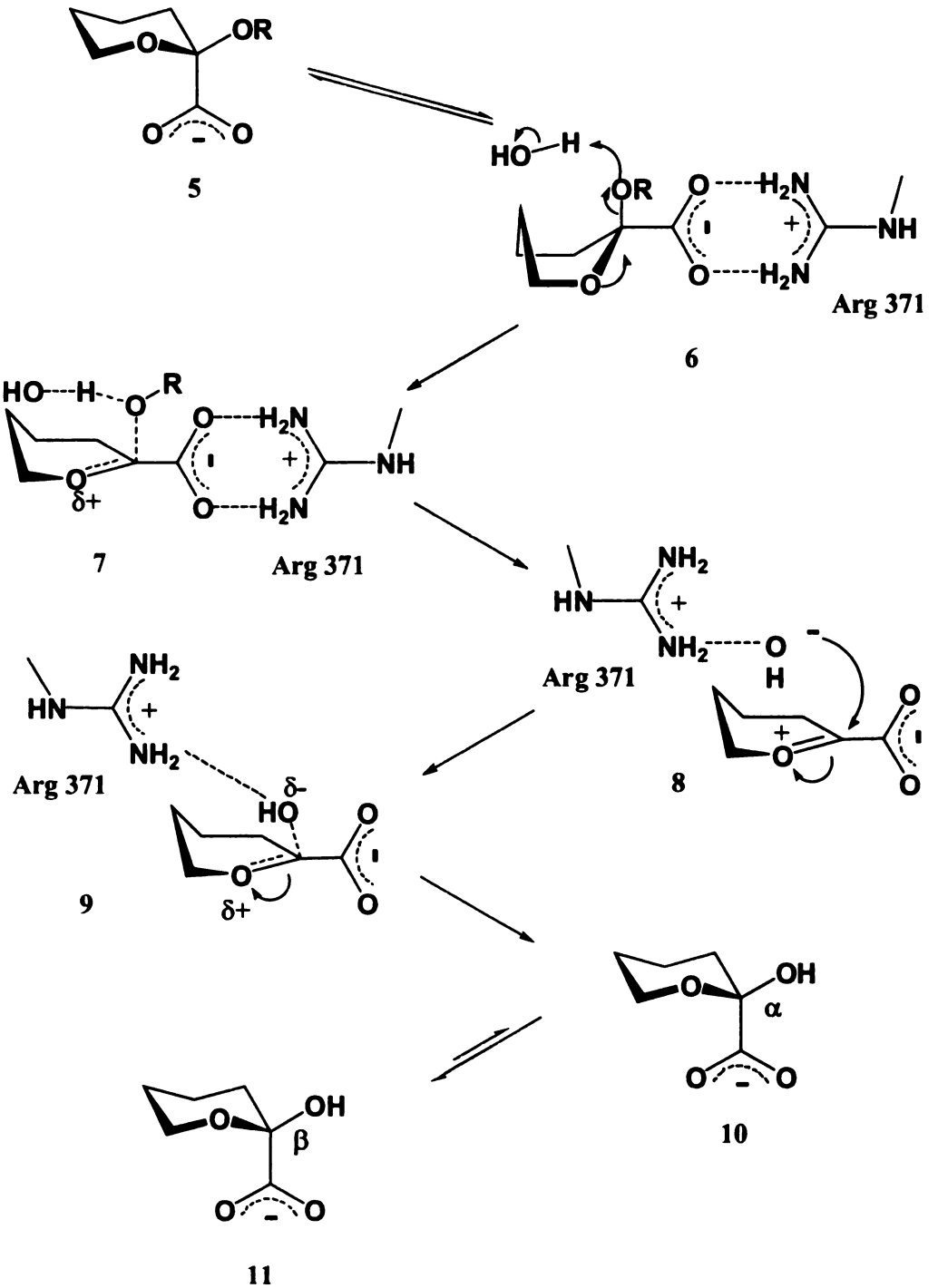


Figure 1.6 Mechanism of neuraminidase catalyzed hydrolysis of sialic acid linked glycoprotein (Figure adapted from von Itzstein, M.; Wu, W. –Y; Kok, G. B. Pegg, M. S.; Dayson, J. C.; Jin, B. *Nature* **1993**, *363*, 418-423)

The catalytic pathway of the sialidase can be regarded as consisting of four major steps.[25]

The first step is the binding event. The binding of sialoside to sialidase involves considerable distortion of the pyranose ring. In solution the Neu5Ac pyranose ring adopts an expected $^2C_5$ chair conformations whereas in the bound state the ring has a pseudo boat conformation. Though this conformation is the result of complex ionic hydrogen bond and steric interactions, it is an unfavorable conformation, in which the C2, C3, and O6 atoms are coplanar. The resulting conformational strain induces the cleavage of the glycosidic bond.[26, 27, 28]

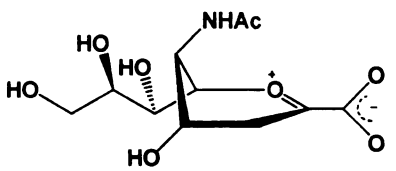
The second step of the catalytic reaction involves proton donation from solvent and formation of the endocyclic sialosyl cation transition-state intermediate. Kinetic isotope studies on influenza virus sialidase with the synthetic substrate 4-(methylumbelliferyl)-Neu5Ac and the corresponding [3, 3-^{2}H]-substituted substrate, an S_N1 type mechanism with proton donation from an activated water molecule and an endocyclic sialosyl cation transition-state intermediate had been postulated.[29] It's almost the same process as the most intensely studied and best known enzyme mechanisms of glycohydrolase, lysozyme.[30] An oxocarbenium ion **8** is believed to form in this step and is stabilized by adjacent Glu 277. This oxocarbenium ion flattens the pyranose ring into a "bed" conformation wherein C2, C3 and O5 are on the same plane.

The final two steps of the enzyme mechanism are the formation and release of Neu5Ac. NMR experiments indicate that Neu5Ac is initially released as the α-anomer, which is consistent with the proposed S_N1 mechanism having a high degree of stereofacial

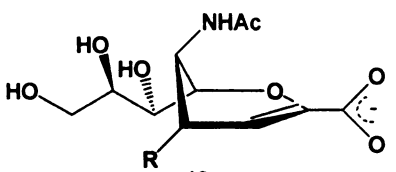
selectivity. It is conceivable that expulsion of product from the active site is favored by the mutarotation of the α-anomer **10** to the thermodynamically more stable β-anomer **11** for Neu5Ac in solution.[31]

1.2.4 Mechanism Based Drug Design

Based on this mechanism, many trials modifying the sialic acid molecule to make analogs as sialidase inhibitors were made both chemically and computationally. In 1991, Itzstein in Monash University, Australia, synthesized the first transition state analogue inhibitor for influenza neuraminidase Neu5Ac2en **(Figure 1.7).**[27]



Transition state of sialic acid residue in neuraminidase catalyzed hydrolysis
12



13

R = a) OH
b) NH_3^+
c) $NHC(NH_2)_2^+$

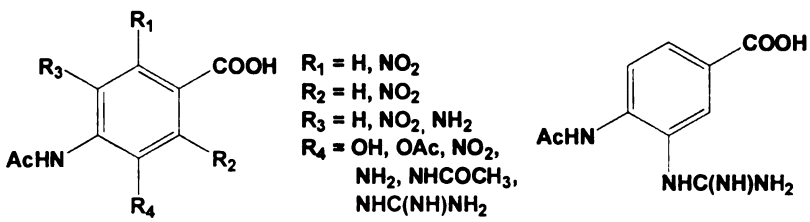
Neu5Ac-2en when R = OH

Inhibitor	K_i (M)
13a	$4X10^{-6}$
13b	$4X10^{-8}$
13c	$4X10^{-9}$

Figure 1.7 von Itszstein's neuraminidase inhibitors, the comparison with sialic acid transition state and their inhibition effect

Comparing these two structures, we can see that Neu5Ac-2en **13a** keep the C-1 carboxylic acid and the use a double bond between C2 and C3. The planarization of the pyranose ring helps mimic the transition state structure. All the other part of the molecule remains the same. The Neu5Ac-2en turned out to be a very good inhibitor of neuraminidase. They also figured out by calculation that when guanidine the group is on C4, a favorable interaction with active site may be obtained. This was confirmed by the best inhibition K_i ($4X10^{-9}$).

After Itszstein, many potential inhibitors towards neuraminidase have been developed.[32] For example, in 1995, Singh in University of Alabama synthesized a series of benzoic acid derivatives as neuraminidase inhibitors.[33] They did the in vitro test of each compound and found inhibitor **15** was the best with an IC_{50} of 0.01mM) **(Figure 1.8)**.



14 15

Figure 1.8 Singh's neuraminidase inhibitors library

On the basis of Singh's work, in 1998, Stevens and his colleagues synthesized a cyclohexane system and tested the effect of different substitute groups on the inhibitor. They focus on the exploration of the alkyl C8-C10 side chain to fit in the hydrophobic pocket of neuraminidase active site. A very efficient inhibitor **17** with IC_{50} as low as

0.5nM was found **(Figure 1.9)**.[34]

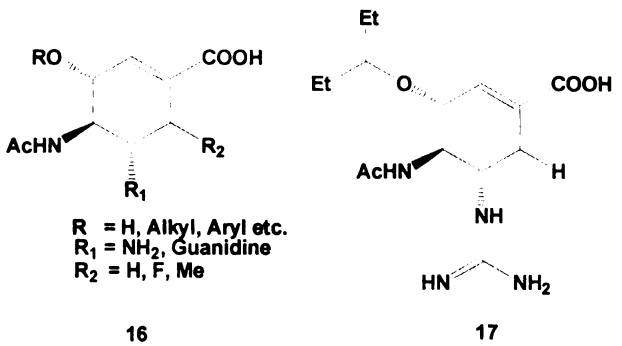


Figure 1.9 Stevens's cyclohexene based neuraminidase inhibitors

So far, scientists have put a great deal of effort in searching for good inhibitors towards neuraminidase. Thousand of compounds have been synthesized and tested.[35] Most of them are transition state analogues. Apart from the scaffold we have discussed, other inhibitors, such as Chand's cyclopentane system **18**, Wang's pyrrolidine systems **19** and Babu's pyridine system **20** have shown good inhibition activity toward neuraminidase **(Figure 1.10)**.[36,37,38]

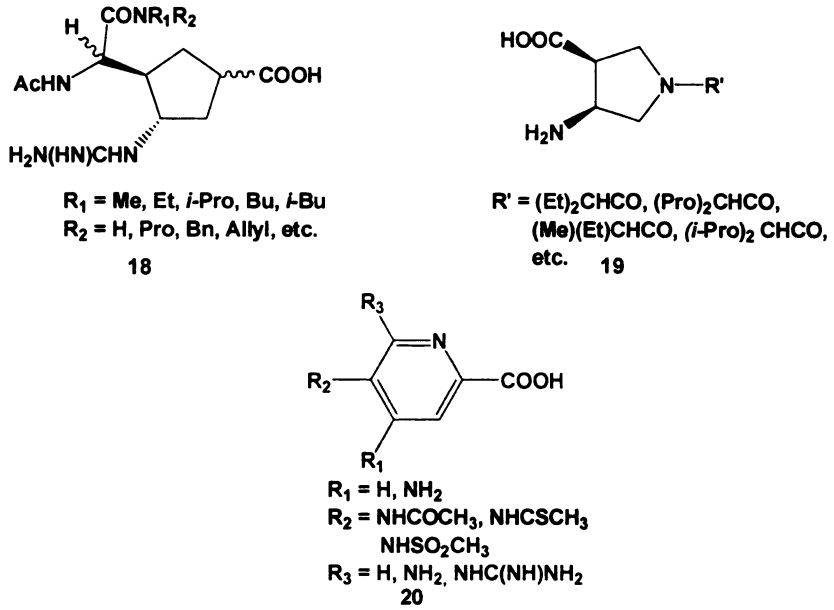
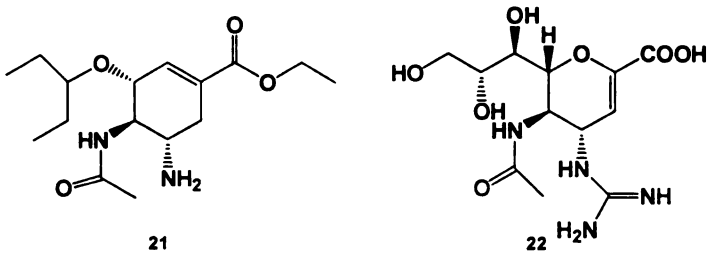


Figure 1.10 Chand's cyclopentane system, Wang's pyrrolidine systems and Babu's pyridine system as neuraminidase inhibitors

Among those compounds, two of the most potent inhibitors, Oseltamivir **21** whose phosphate is referred as Tamiflu and Zanamivir **22** (known as Relenza), are developed to commercially available drugs for influenza **(Figure 1.11).** Zanamivir is approved for treatment of uncomplicated acute illness caused by influenza virus in persons aged greater than or equal to 12 years who have been symptomatic for no more than 2 days. Oseltamivir is approved for treatment of uncomplicated illness caused by influenza infection in adults aged greater than or equal to 18 years who have been symptomatic for no more than 2 days.



21 Oseltamivir

22 Zanamirvir

Figure 1.11 The structures of Zanamirvir and Oseltamivir

Both of those drugs are transition-state analogs of sialic acid. In the Oseltamivir molecule, a carbon atom replaces the in-ring oxygen of the sialic acid and the double bond helps to maintain the coplanar structure mimicking the sialoyl cation intermediate. In the case of Zanamivir, the 2, 3 positions are dehydrogenated to form an olefin; the function of this carbon-carbon double bond is the same as Oseltamivir. Both of those two molecules retain the acetamido group at the C5 position.

Zanamivir can achieve very good binding through appropriate presentation of its four pendent substituents and contains hydrogen bonding glycerol side chain. The guanidino group in Zanamivir is believed to form salt bridges with Glu 119 in the neuraminidase active site and add a strong charge interaction with Glu 227. This interaction anchors Zanamivir into the enzyme active site of neuraminidase. On the other hand, the good inhibition effect of Oseltamivir is believed to be due to that the aliphatic ether branch chain, which on C6, fits into the hydrophobic pocket of the neuraminidase active site.[39]

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Chapter 2 Azasugar as Neuraminidase Inhibitor and Anti-Influenza Drug

2.1 Azasugar as Glycosidase Inhibitor

2.1.1 Azasugar's Potent Inhibition Effect

Since the discovery of nojirimycin, a glycosidase inhibitor, polyhydroxylated piperidines (also called azasugars, the ring O-atom of a carbohydrate is replaced by nitrogen) have attracted considerable attention and have been the targets of numerous synthetic strategies during the last decade. The efficient inhibition capabilities of azasugars toward glycosidases and glycotransferases made them the best candidates for the treatment of a variety of carbohydrate-mediated diseases, such as influenza, diabetes, viral infections including HIV, cancer metastasis, hepatitis, and Gaucher's disease. It is believed that the potent inhibition capability stems from their structural resemblance to the sugar moiety of the natural substrate. Up till now, a large number of azasugars have been developed to defend virus invasion to human body.[1]

In 1966, nojirimycin was discovered as the first alkaloid that mimics a sugar.[2] It is a potent inhibitor of α-and β-glucosidases from a variety of scources. The more stable forms 1-deoxynojirimycin (DNJ) and 1-deoxymannojirimycin (DMJ), were also isolated and showd potent inhibitory activities against glycosidases.[3,4,5,6,7] DNJ is a potent inhibitior of all kinds of glycosidases,[8] but it is more selective to α-glucosidases and its inhibitor effect on mammalian α-glucosidases opened the possiblility of a therapeutic application for DNJ. Apart from DNJ, some natural bicyclic polyhydroxyheterocycles were also discovered and isolated. The inolizidine castanospermine and swainsonine are typical examples.[9,10,11,12] These compounds **(Figure2.1)** have less obvious structure

relationship to monosaccharide but in each case the configuration of hydroxyl groups on the ring can be compared to those of sugars. Castanospermine is an excellent α-glucosidase inhibitor. Swainsonine is believed to be associated with deoxymannojirimycin (DMJ), and showed inhibition against α-mannosidases **(Table 2.1)**.

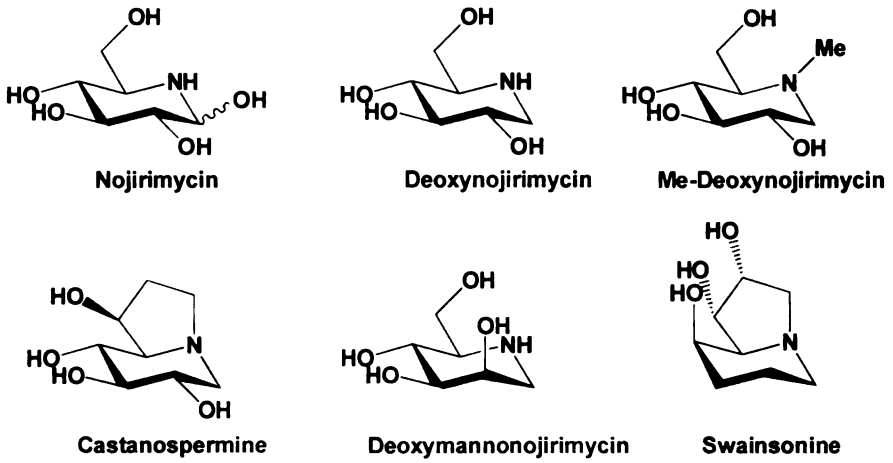


Figure 2.1 Various azasugars which are inhibitors for glycosidases and glycotransferases

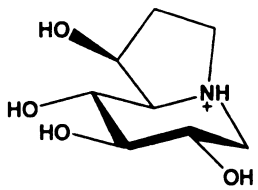
Inhibiton constants Ki(pM) of some natural inhibitors

Enzyme and Source	Nojirimycin	Deoxynojiri -mycin	Castanosper -mine	Swainsoni -ne
a-glucosidases				
Yeast	6.3	12.6	>1500	
Rice	0.01	0.01	0.015	
Sucrase(Rabbit intestine)	0.13	0.032		
b-glucosidases				
Sweet almonds	0.89	47	1.5	
Calf liver(cytosol)	-	210		
Calf spleen(lysosomes)	4.5	180		
a-mannosidases				
Jack beans				0.001

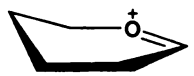
Table 2.1 Inhibition effect of azasugars towards various enzymes

2.1.2 Why Azasugar Has Good Inhibition Capability

Castanospermine and derivatives of deoxynojirimycin are considered selective inhibitors for α-glucosidases because they mimic the positive charge character of the ring oxygen at transition state, which is believed to be an important feature of the α-glucosidase transition state **(Figure 2.2)**.[13,14]



Castanospermine



Oxocarbenium ion transition state in glycosidase active site

Figure 2.2 Castanospermine mimics the positive charge of the oxocarbenium ion

However, it is questionable whether they are real transition state analogues because they do not have the expected half-chair confirmation. Therefore, inhibitors that mimic both the positively charged ring oxygen and the half-chair confirmation have been developed.

In 1994, the five-membered azasugars which is expected to be more flexible than six-membered rings were synthesized by C-H Wong et al.[15] The five-membered ring has more flattened chair confirmation and the positive charge can be mimicked by the positive charged imino group, so compound **1** showed better inhibition against α-glucosidase (K_i = 2.8pM for α-glucosidase) than DNJ and compound **2** showed strong inhibition to α-galactosidase from coffee bean at Ph=5.5 (K_i = 0.05pM). The X-ray

crystal structure of compound **1** indicated an envelop conformation of the five member ring.[16] **Figure 2.3** shows the proposed transition state for α-glucosidase and the positive charged compound **2**. Therefore, fived-membered azasugars are better transition state analogues than six-membered ring azasugars. So, it is also confirmed that for a good inhibitor, both the charge condition and the conformation are required.

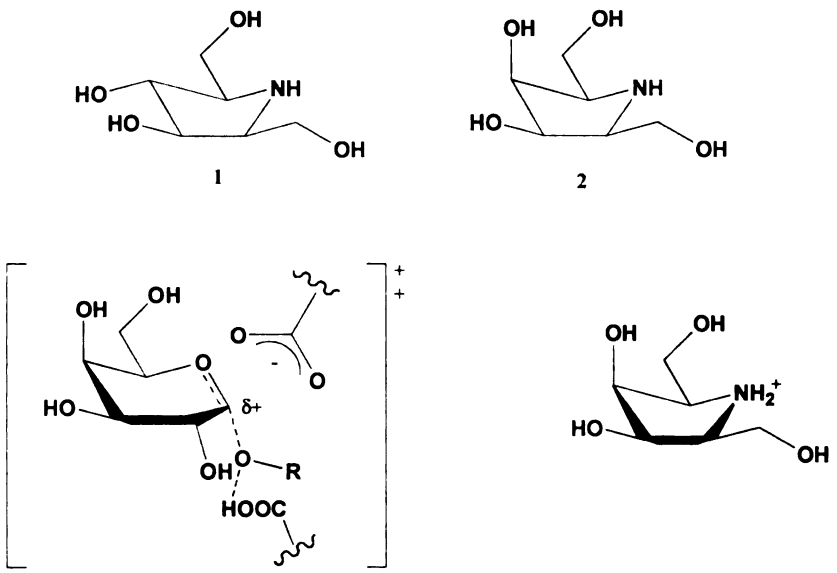


Figure 2.3 5-membered ring azasugar's conformational mimicking to glycosidase transition state (Figure adapted from Wong, Y. F.; Takaoka, Y.; Wong, C. H. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1242-1244)

As we already discussed, Zanamirvir's potent inhibitory activity was due to its 3 most important interactions with the neuraminidase active site. 1. The salt bridge between negative charged C1 carboxylic acid and positive charged Arg 372. 2. The electrostatic interaction between guanidine group on C4 and the carboxylic acids of Asp 152, Glue 229 and Glu 120; 3. C7-C9 polyol fits into the hydrophobic pocket of the active site, as shown by the cartoon below **(Figure 2.4)**.[17] Furthermore, Zanamirvir's coplanar structure

of C1-C2-C3-O5 mimics the NA transition state conformationally.

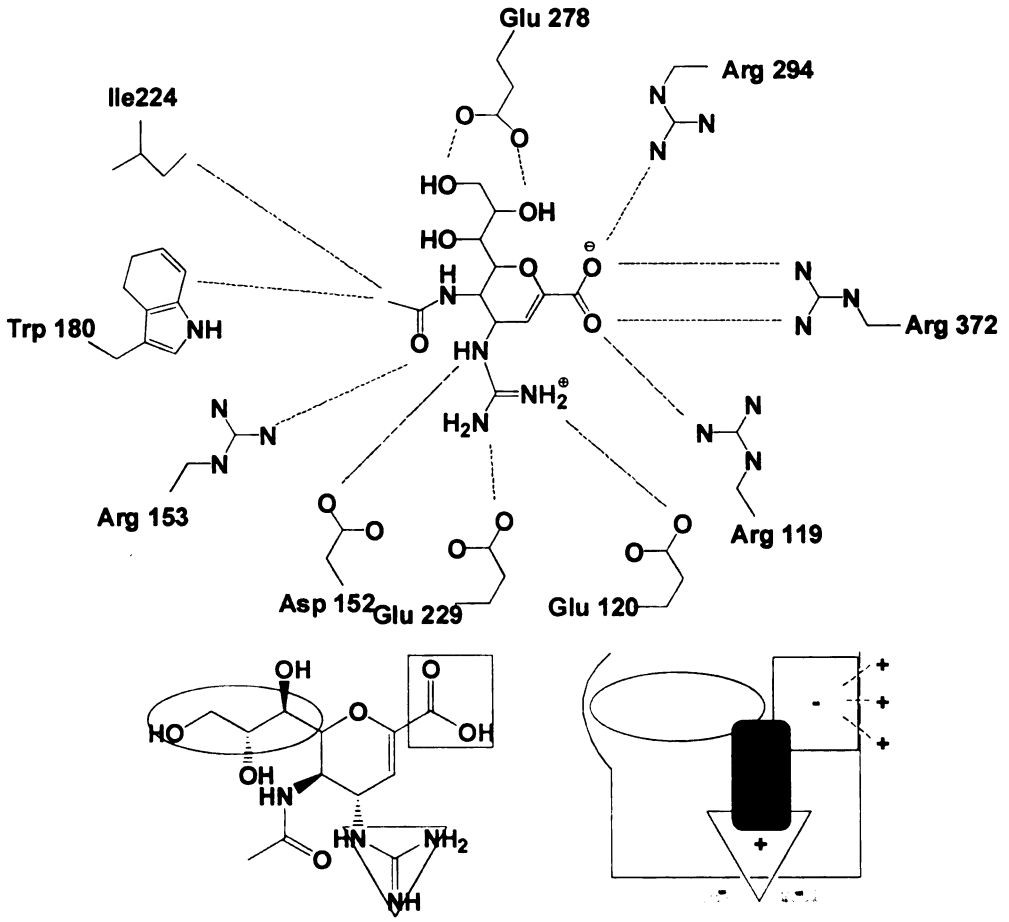


Figure 2.4 3 most important enzyme-substrate interactions (Figure adopted from Kim, C. U.; Lew, W. Williams, M. A. Wu, H. W. *J. Med. Chem.* **1998**, *41*, 2451-2460)

2.2 Rational Design of Azasugar Neuraminidase Inhibitor

Inspired by Zanamirvir and Oseltamivir, in our design of neuraminidase inhibitor for the treatment of Influenza, we try to find molecules that meet both the conformational and the interaction requirements. Obviously, azasugar is a good choice. Compound **3** is our proposed inhibitor scaffold **(Figure 2.5)**.

Three considerations were involved in our design of this piperidine ring as potential

inhibitor: 1. Conformationally, both piperidine and sialic acid intermediate are six-membered ring. A piperidine is appropriate to mimic the conformation of the transition state. 2. The Pk_a of an amine is between 10 to11. It means that under the regular in vitro catalysis condition, almost all the amine is protonated, and the charged nitrogen (compound **5**) will be a good analogue to an oxocarbenium ion (compound **4**) which has a positive charge primarily on the oxygen atom. 3. The third consideration is the bond length. The bond lengths of C-N bond and C-O bond are close. This ensures that our inhibitor does not have a much different special occupation from the sugar transition state. Those favorable interactions with the neuraminidase active site will be much retained. Both proved drugs in the market, Zanamivir and Oseltamivir only mimic the coplanar structure but not the charge. It's definitely possible that the azasugar sialic acid analog could have very good inhibition effect against neuraminidase, probably, even better than Relenza or Tamiflu.

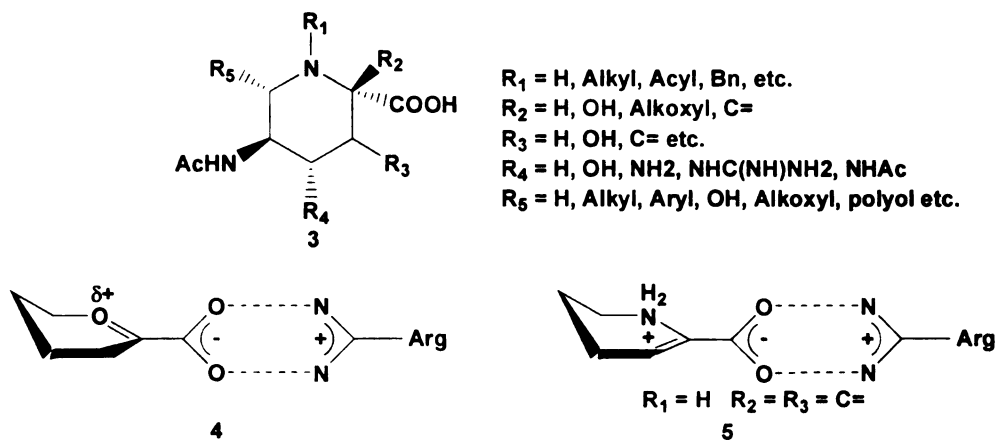


Figure 2.5 Design of neuraminidase inhibitor scaffold and its mimicking to glycoside transition state in the hydrolysis by neuraminidase

2.3 The Synthesis of Azasugars

2.3.1 Background

Though iminoalditols or azasugars, represented by deoxynojirimycin and castanospermine and its derivatives have shown potent glycosidase inhibition capability and have been applied to the medical treatment of numerous diseases,[18, 19] such as antimicrobioals,[20, 21] cancer,[22] and neurological disorders,[23] they have not realized their full clinical potential. This is largely due to the lack of commercially viable syntheses and difficulty in preparing a comprehensive palette of variant structures. So far, many of the possible drug candidates are available only in small exploratory amounts.

Although the only difference between an azasugar and a normal sugar pyranose or furanose is just the replacement of the-ring oxygen by a nitrogen atom, this is a big challenge for organic chemists. A number of research groups are in this field and various chemical and enzymatic syntheses of azasugars have been reported in recent years. These strategies include aminomercuration, double-reductive amination, N-Alkylation, reductive double-alkylation and triple reductive amination, ring closing metathesis, photochemical synthesis and chemo-enzymatic synthesis.[24-39]

Most of the chemo synthesis use readily available and inexpensive chiral-pool starting materials such as carbohydrates, amino acids, and tartaric acids. The obvious similar structural features between azasugars and carbohydrates have made the latter ideal starting materials. Some representative synthetic strategies are described below:

2.3.2 Aminomercuration[40]

Ganem at Cornell University devised an enantioselective synthetic route from readily available chiral monosaccharides. His strategy hinged on breaking open the pyranose (or furanose) ring, and reforming the corresponding piperidine (or pyrrolidine) analogue with retention of the critical stereocenters by the process of intramolecular aminomercuration. The general approach is shown in **Figure 2.6**. A one-pot, reductive ring opening and reductive amination of the pyranose were achieved by heating tri-O-benzyl-6-bromopyranoside with acid-washed zinc dust in propanol-water containing benzylamine and $NaBH_3CN$ to afford amino alkene. When the key intermediate **7** reacted with mercuric trifluoroacetate in anhydrous THF, a 3: 2 mixture of bromomercurials **8** and **9** was isolated. The major cyclization product **9** could be transformed to 1-deoxynojirimycin **10** by reductive oxygenation ($NaBH_4$-DMF-O_2) and hydrogenolytic deprotection. In this synthesis, only one of the bromomercurials **9** can give the desired product, which results in a low yield of deoxynojirimycin **10**. The mercury toxicity is a big problem for this strategy.

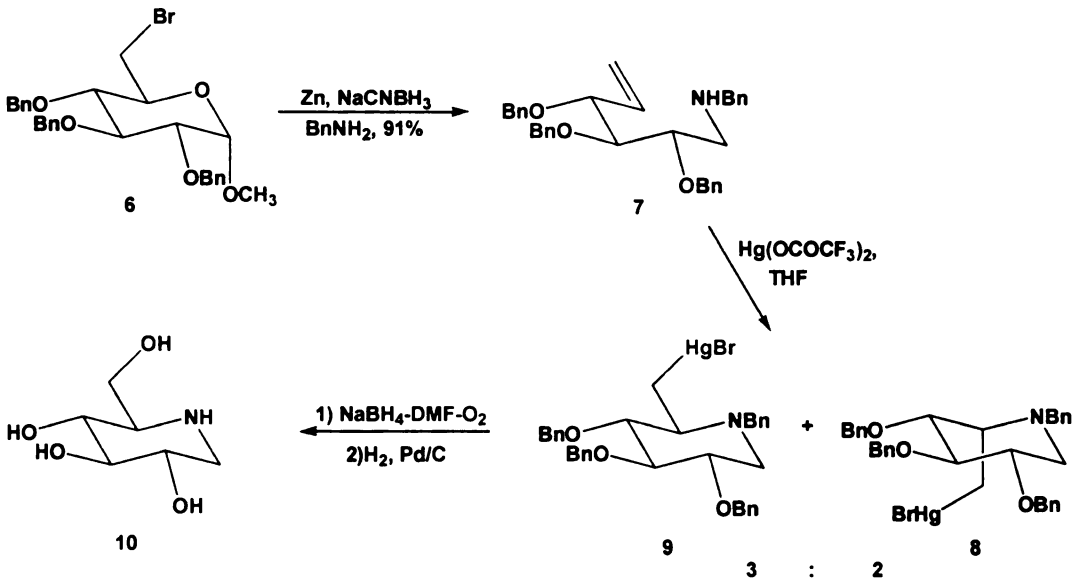


Figure 2.6 Aminomercuration approach for the synthesis of azasugars

2.3.3 Intramolecular N-Alkylation[41]

In this approach, the nitrogen atom is first introduced by the S_N2 reaction of an azide group followed by a reduction reaction to transform it to an amine **(Figure 2.7)**. The amino group, which is a good nucleophile, then kicks out the mesylate leaving group intramolecularly to form a 5 or 6-membered ring. Kibayashi selected diethyl tartrate **11** as starting material and finally synthesized enantiomerically pure nojirimycin. The synthetic route is a little bit lengthy.

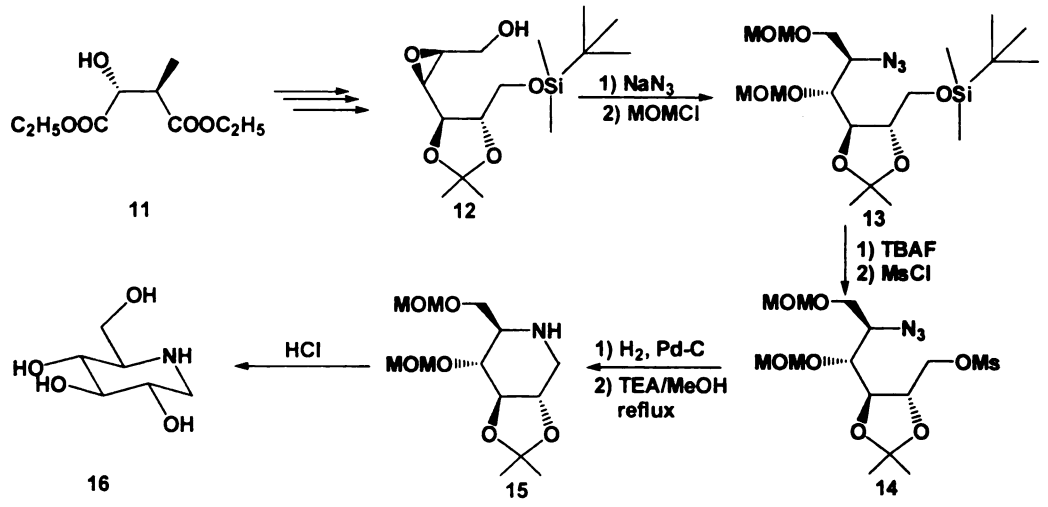


Figure 2.7 Intramolecular N-alkylation approach for the synthesis of azasugars

2.3.4 Reductive Double-Alkylation[42]

This methodology was first applied by Pearson's group for the synthesis of swainsonine in 1996 **(Figure 2.8)**. They started with D-Arabinose **17**, converted it to 2, 3, 4-tri-Obenzyl-D-arabinose **18** followed by Wittig reaction with known phosphonium salt to give the Z-alkene **19**. N_3 was installed by the classical Mitsunobu reaction. An epoxidation reaction provides another electrophilic center apart from the primary chloride. Upon reduction of azide, primary amine was generated, which were cyclized to afford quinolizidines **21** and **22**. The synthesis yields the bicyclic azasugars, however, the diastereoselectivity is not high and it still suffers from the drawback that separation of the diastereoisomers has to be performed.

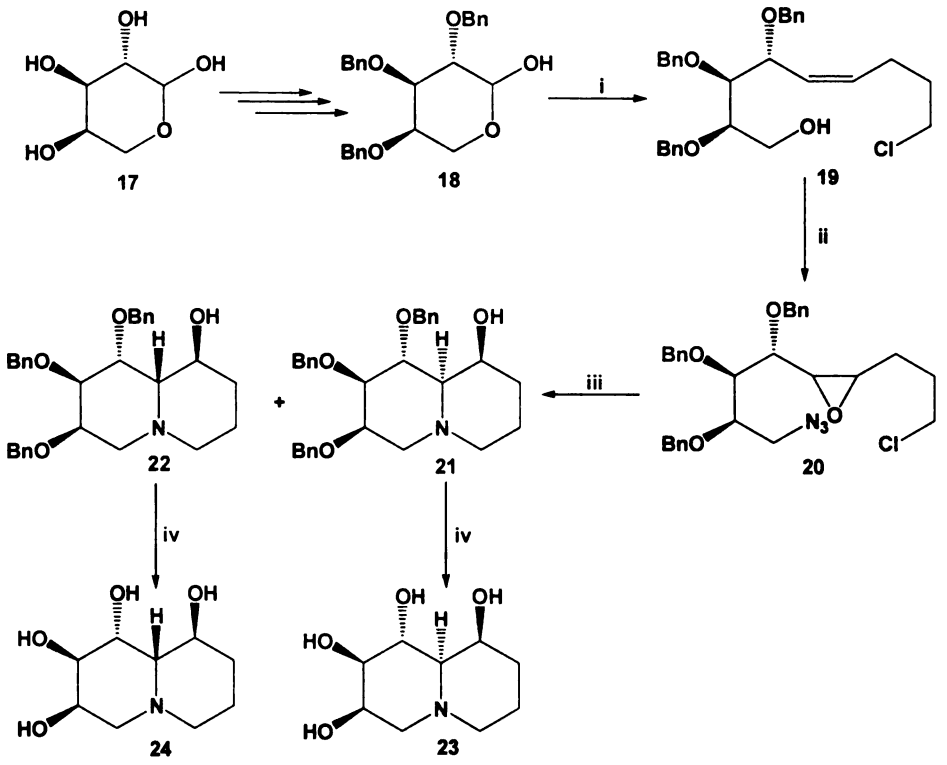


Figure 2.8 Reductive double-alkylation approach for the synthesis of azasugars i. $BrPh_3P(CH_2)_4Cl$, KN $(TMS)_2$, 71%; ii.a) HN_3, PPh_3, DEAD, 84%;b) mCPBA, 88%; iii. a) H_2, Pd/C, EtOH; b) K_2CO_3, EtOH, reflux; c) separate diastereomers; iv. H_2 (45 psl), Pd/C, HCl, MeOH, 99%

2.3.5 Double Reductive Amination[43, 44]

Reductive amination is one of the most popular ways to build carbon-nitrogen bond. By preparing a dicarbonyl compound, nitrogen can easily be introduced into the molecule and at the same time, forming a ring structure. Reitz used a primary amine and sodium cyanoborohydride, successfully synthesized 1-deoxynojirimycin, 1-deoxymannojirimycin and N-alkylated derivatives **(Figure 2.9)**. They tried different amines as nucleophiles and got satisfactory results **(Table 2.2)**. The problem with this methodology is that the stereochemistry of the reduction product is not easily controllable.

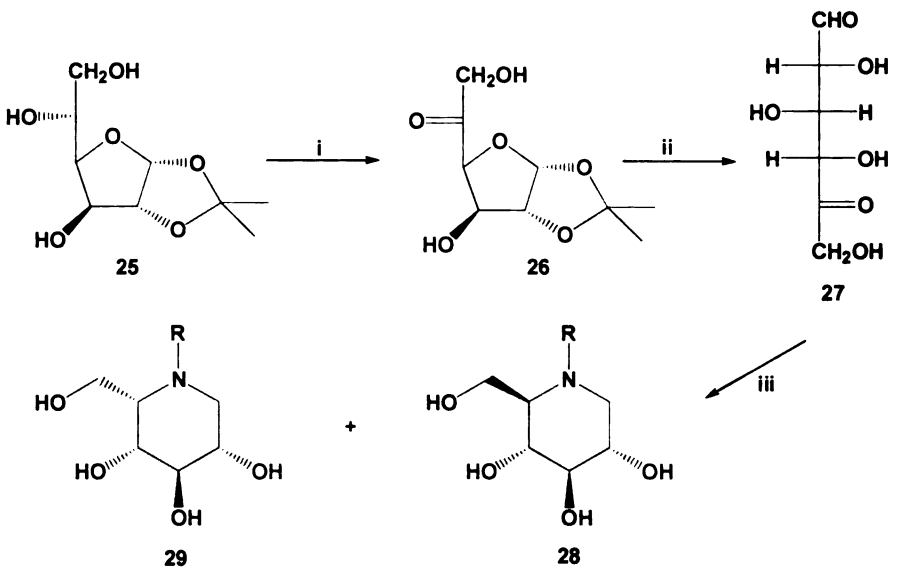


Figure 2.9 Double reductive amination approach for the synthesis of azasugars i. a) Bu_2SnO, MeOH b) Br_2, CH_2Cl_2, 0°C , 48% ii. Dowex-50, H_2O, 70% iii. RNH_2, $NaBH_3CN$, MeOH

RNH_2	Yield	Ratio **29** : **28**
Ph_2CHNH_2	70%	5 : 95
$Ph_2CHCH_2NH_2$	73%	5 : 95
$C_4H_9NH_2$	55%	5 : 95
$C_{12}H_{25}NH_2$	27%	5 : 95

Table 2.2 The overall yields and ratios of the two products formed by the double reductive amination reaction

2.3.6 Triple Reductive Amination[45]

A triple reductive amination reaction involves three carbonyl groups at the same time. It's suitable for the synthesis of bicyclic nitrogen-containing compound such as swainsonine and castanospermine. The allyated monsaccharides are practical precursors for the key tricarbonyl intermediates required for this strategy. A wide range of analogue structures will be possible in view of the number of easily accessible monosaccharide precursors of different configurations and constitution. The triple reductive amination approach is efficient for the synthesis of compounds with bicyclic indolizidine framework (**Figure**

2.10). But, the preparation of the precursor with aldehyde and ketone functionalities is not an easy exercise.

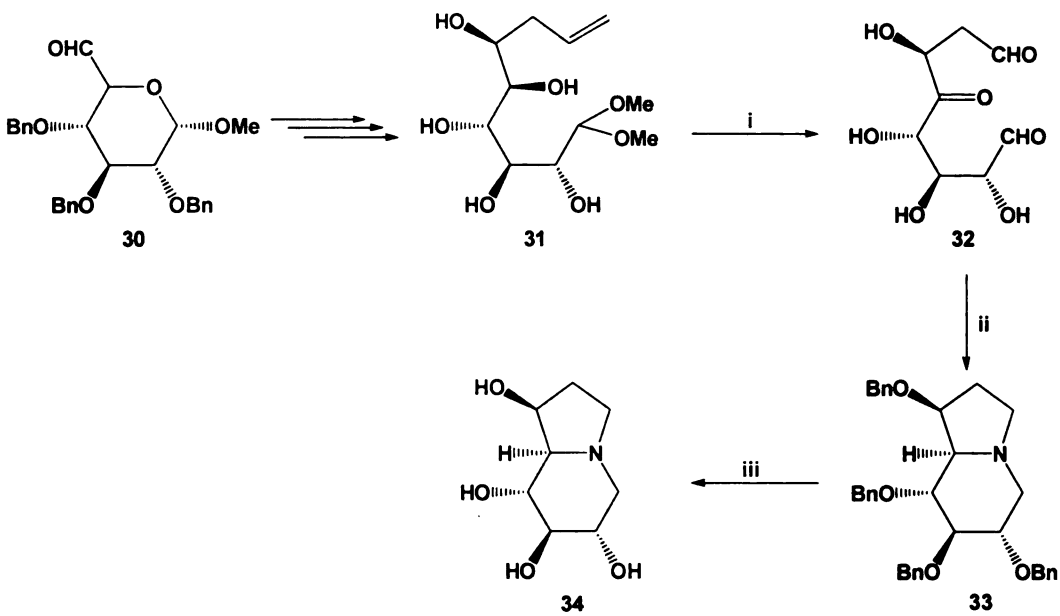


Figure 2.10 Triple reductive amination approach for the synthesis of azasugars i. a) Swern Oxidation; b) O_3, CH_2Cl_2,-78°C then Ph_3P; c) THF-9M HCl, 74% for 3 steps; ii. NH_4HCO_3, $NaCNBH_3$, MeOH, 78%; iii. 10% Pd-C, MeOH-HCOOH, 80%

2.3.7 Ring Closing Metathesis[46, 47]

In the last decade, the ring closing metathesis (RCM) reaction has emerged as an extraordinarily powerful and general method for the construction of nitrogen heterocyclic compounds and has relevant application in the field of alkaloid synthesis. In the field of azasugar synthesis, the double bond formed by RCM reaction is well-suited to install either a cis or trans vicinal diol functionality using a dihydroxylation reaction or an epoxidation reaction followed by subsequent hydrolysis. The synthesis of DGJ and analogues starting from D-Garner's aldehyde **35** (derived from D-serine) with a ring-closing metathesis (RCM) as the key step has been reported by Takahata et al., as shown in the following Scheme **(Figure 2.11)**. The problem with this methodology is that

the catalyst for RCM reaction is too expensive and the cost to maintain the catalysts' activity is too high for industrial production.

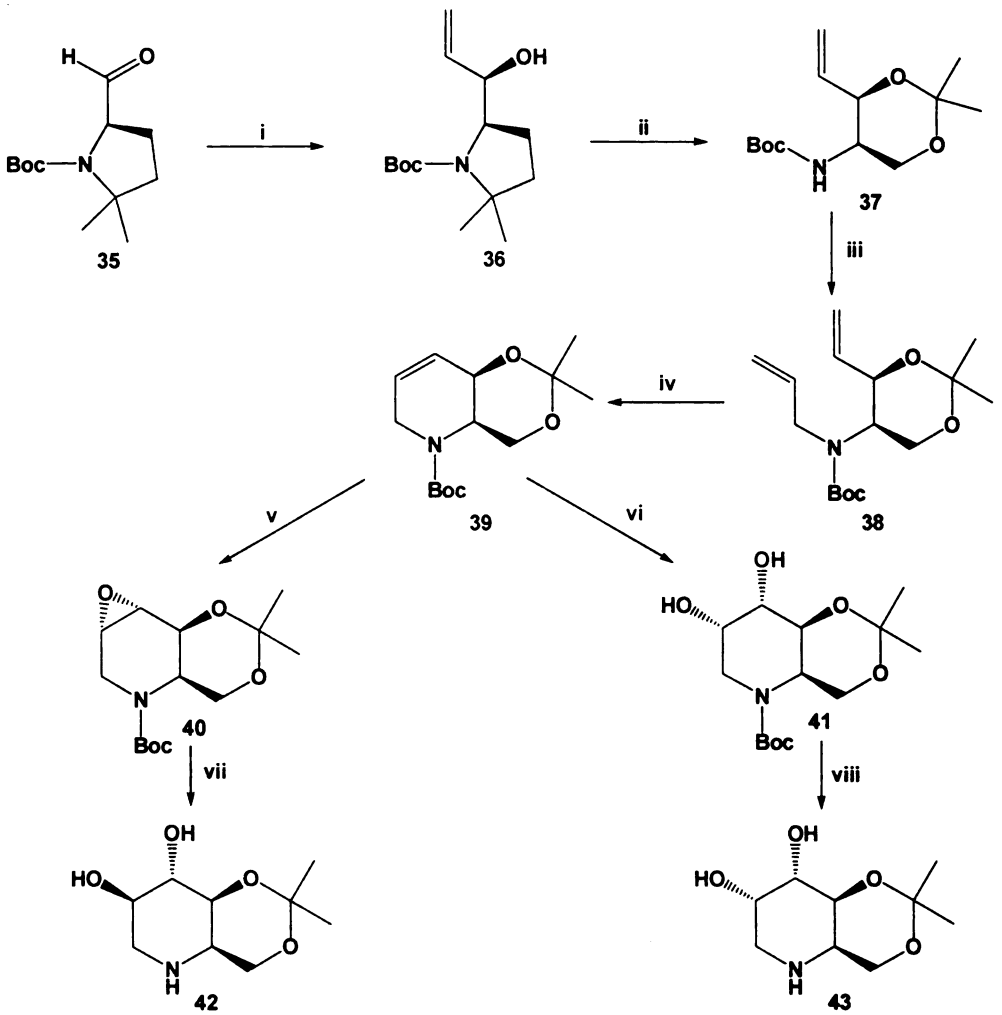


Figure 2.11 Ring closing metathesis approach for the synthesis of azasugars i. VinylZnBr, Et_2O,-78°C to room temp., 2h, chromatography then recystallization form n-hexane/ EtOAc (5: 1), 72%, 92% *de*; ii. HCl gas, $CHCl_3$, room temp., 12h 69%; iii. Allyl iodide, NaH, THF, 0°C, 12h, 76% iv. [Ru], CH_2Cl_2, room temp., 2h, 95%; v. Oxone, CF_3COCH_3, $NaHCO_3$, aqueous Na_2EDTA, CH_3CN, 0°C, 20min 90%; vi. $K_2OsO_4{\cdot}2H_2O$, NMO, acetone, H_2O, 0°C to room temp., 12h, 85%; vii. a) 0.3M KOH, 1,4-dioxane, H_2O, reflux, 26h; b) 6N HCl, MeOH, reflux, 1h, then Amberlite IRA-410(OH-form), 87%(2 steps); viii.6N HCl, MeOH, reflux, 1h, then Dowex 50Wx8(H^+ form), 90%

2.3.8 Photochemical Approach[48]

Photochemical reactions were applied to the synthesis of azasugar too **(Figure 2.12)**. A cyclic amine can be synthesized from D-Tartaric Acid **44**.[49]The piperidine ring was closed by photoinduced electron transfer (PET) reaction. A variety of 1-N-iminosugars are accessible through this approach. But the stereochemistry on the carbon adjacent to the nitrogen atom can not be controlled.

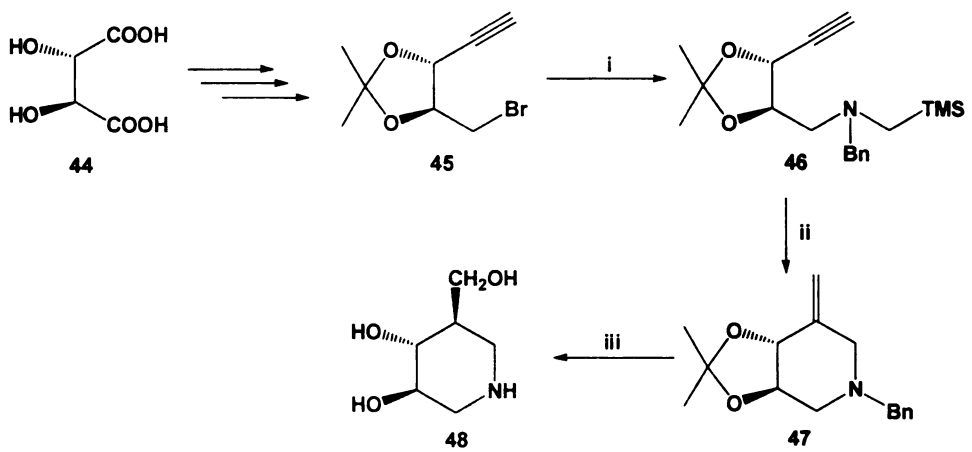


Figure 2.12 Photochemical approach for the synthesis of azasugars i. $PhCH_2NHCH_2TMS$, K_2CO_3, CH_3CN, reflux, 96h, 65%; ii.hv, DCN, 2-PrOH, 90min, 60%; iii. a) 9-BBN, THF, 0°C to room temp., 20h, then NaOH, H_2O_2, 0°C to room temp., 4h, 45%; b) HCl, MeOH, rt, 1h, then NH_4OH, 100%; c) $Pd(OH)_2$ on C, H_2, 75 psi, EtOH, 10h, 95%

2.3.9 Chemo-enzymatic Synthesis[50-53]

This methodology was developed by Wong and his group. It is generally a two step process involving an enzymatic aldol condensation and a catalytic intramolecular reductive amination. The detailed synthetic route is shown below **(Figure 2.13)**. Two azasugars **52** and **53** were obtained as products. However, this method of synthesis is not a general method because the limitation of scale-up of enzymatic reactions.

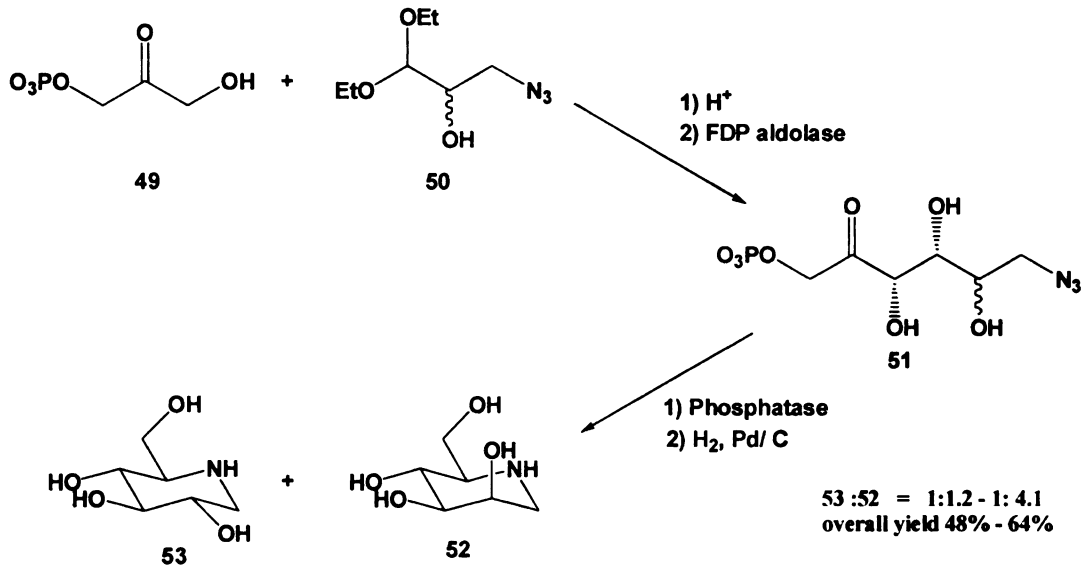


Figure 2.13 Chemo-enzymatic approach for the synthesis of azasugars

2.4 Summary

Although people have developed a lot of synthetic methods towards carbohydrates, the synthesis of azasugar remains troublesome. First, it's really hard to find a route leading to a general precursor for most of the azasugars. Second, as with all carbohydrates, azasugars are multi-hydroxyl compounds. In most of modification processes, protecting these hydroxyl groups is a problem due to limited orthogonal protecting groups. And last, most of the synthetic routes start with a carbohydrate with five to seven carbons. Then, how to make the C-glycoside structure with more than eight carbons is a challenge because of difficulties to form carbon-carbon bond. So, more strategies towards the synthesis of azasugars should be developed in the future.

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Chapter 3 Design and Synthesis of Our Azasugar Target

3.1 Result and Discussion:

3.1.1 Shortcomings of the Currently Available Strategies

Though great effort has been put into the synthesis of azasugar and various approaches have been proposed, they suffer different drawbacks which either prohibit the industrial usage or not be able to give the desired scaffold. The reasons are listed below:

a) The synthesis is too lengthy. A more then 10 steps strategy is very unlikely for large scale production.

b) The synthesis is too costly. For example, the transition metal catalyzed RCM. The expensive catalyst itself significantly reduces the practicality.

c) The reagents are expensive, toxic or corrosive. Organic compounds like HCN etc. should be avoided.

d) The reaction conditions are severe. The desired route should not require extreme conditions such as very high temp, very low temp, or high pressure etc.

e) None of these approaches afford a carboxylic group on the β-position of the piperidine nitrogen.

3.1.2 Our Proposed Synthetic Strategy

We designed a short synthetic pathway toward our target scaffold **6 (Figure 3.1)**. It starts with cheap monosaccharide D-(+)-Mannose **1**. We can protect 2, 3-O and 4, 6-O with two acetal groups leaving anomeric position intact. Then, we plan to apply a Strecker type reaction[1-9] to add cyanide group to anomeric position and open the ring to afford a diol **3**. Because generation of HCN does not obey our industry-friendly principle, we do

not want to do the reaction in acidic condition. In addition, the propylidene protecting groups do not tolerate acid as well. The two hydroxyl group can be converted to good leaving groups by mesyl chloride to form intermediate **4**. An amine (benzylamine for example) is proposed to close the ring to form the piperidine by S_N2 reaction. Then, hydrolysis by acid would transform the cyanide to the carboxylic acid form, and also remove the acetal protecting groups as acetone to afford the proposed scaffold **6**. All the hydroxyl groups are subject to further modification. The Benzyl group can be easily removed by hydrogenation to expose the nitrogen as a secondary amine which can be acylated to become an amide or alkylated to become a tertiary amine. It's a good mother scaffold for an azasugar library.

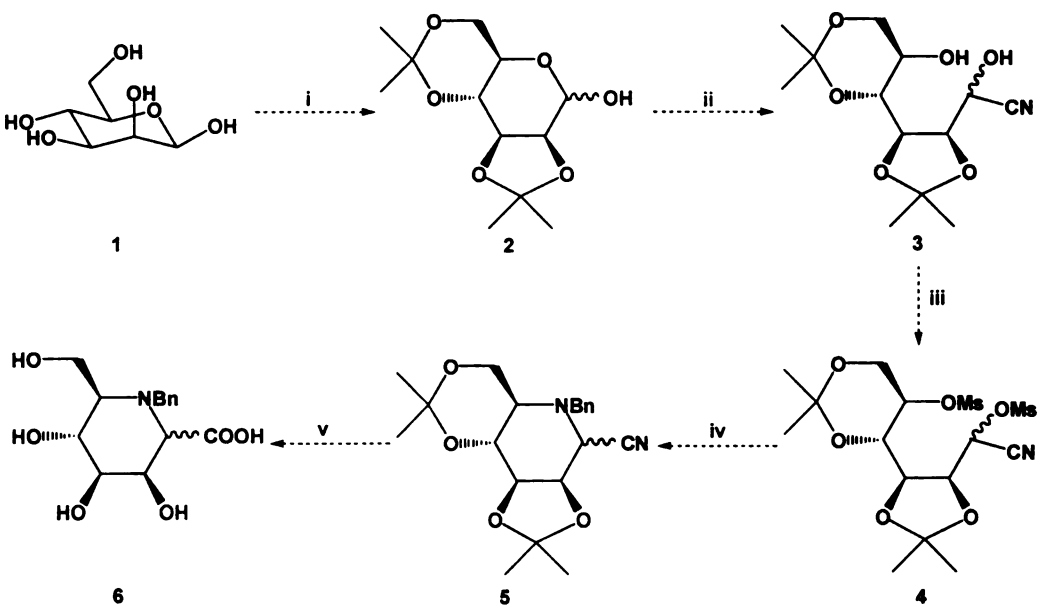
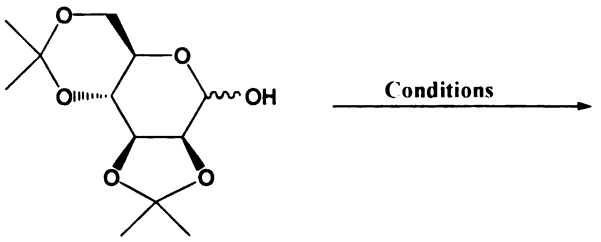


Figure 3.1 Proposed synthetic strategy for our target scaffold i. 2-Methoxypropene, *p*-Toluenesulforic acid, anhydrous DMF; ii. KCN, H_2O, room temp. iii. Mesyl chloride, pyridine, room temp. iv. $BnNH_2$, DMF v. HCl aqueous solution, acetic acid

3.1.3 Progress and Problems with Our Proposed Strategy

In the second step to synthesize compound **3,** by treating the protected mannose with NaCN in neutral condition, we did not obtain the desired product. The results are shown below **(Table 3. 1)**:



2

Conditions	Results
1. NaCN (2 equiv.) , H_2O, room temp, 24 h	No reaction
2. NaCN (2 equiv.), H_2O, 50°C, 24 h	No reaction
3. NaCN (2 equiv.), HCl conc., room temp. 20min	Deprotection product
4. NaCN (2 equiv.), HCl 2%, room temp. 20min	Deprotection product
5. NaCN (2 equiv.), $NaHSO_3$ (2 equiv), room temp. 24h, H_2O	No reaction
6. NaCN (2 equiv.), $NaHCO_3$ (2 equiv), room temp. 24h, H_2O	No reaction

Table 3.1 Trials of different pH conditions and different buffer solutions for the solvent of cyanohydrin formation reaction

Condition 1 and 2 failed to work presumably because: 1. The poor solubility of the 2, 3:4, 6-di-O-isopropylidene-D-mannose **2** in water. 2. Low reactivity of NaCN in neutral or basic conditions. Acidic conditions 3 and 4 remove the isopropylidene quickly as we expected. We also tried to add sodium bisulfite or sodium bicarbonate as buffer reagents to keep the pH value of the solution around 7. Unfortunately, these kinds of buffers did

not help at all.

To solve the problem of solubility, we tried different solvent combinations. The results are shown below **(Table 3. 2):**

Solvent	Result
1. MeOH/ H_2O 1:1	45% starting material reacted after 24h
2. EtOH/ H_2O 1:1	50% starting material reacted after 24h
3. EtOH/ H_2O 1:1 50°C	60% starting material reacted after 24h
4. EtOH/ H_2O 1:1 50 °C, NaCN 3 equiv.	65% starting material reacted after 24h
5. THF/ H_2O 1:1	10% starting material reacted after 24h
6. EtOH/ H_2O 1:2	30% starting material reacted after 24h
7. EtOH/H_2O 2:1	25% starting material reacted after 24h

Table 3.2 Trials of different solvent combinations for the cyanohydrin formation reaction unless noted, all the reactions above were done in room temperature, NaCN (2 equiv.), 5ml solvent for 0.3g 2, 3: 4, 6-di-O-propylidene-D-mannose **2**

Best reaction rate was achieved in 1:1 mixture of ethanol and water. Higher temperature and more NaCN will speed up this reaction, but we did not detect much yield improvement after more than 3 equivalents NaCN is used. Based on these results, we chose condition 4 (EtOH: H_2O 1: 1/ NaCN 2.5-3 equiv. / 50 °C) for all the further research. Though under this condition, the conversion rate for starting protected mannose was only 80%. In the products, we separated compound 7 with 20% yield **(Figure 3.2).** The cyanide was successfully installed and at the same time, and was hydrolyzed to form a carboxylic acid in the solution because of the basic condition (pH ≈10 during the reaction). The other part of the products was complicated. It was an inorganic

salt-organic compound mixture. On the TLC, the spots of organic compounds were covered completely by salt and very hard to observe. We did not use resin to remove the salt because t the acid generated from the resin would destroy acetal protecting groups in the products. Acylation was applied for this mixture in order to reduce the polarity of the organic compounds and make them separable from salts. Compound **8** and **9** were obtained at the end. We assumed that before acylation, they were in their sodium salt forms mixed with inorganic salts. The yields for both of these products were not satisfactory and the e possible mechanisms for the formation of **7** to **9** are discussed afterwards. .

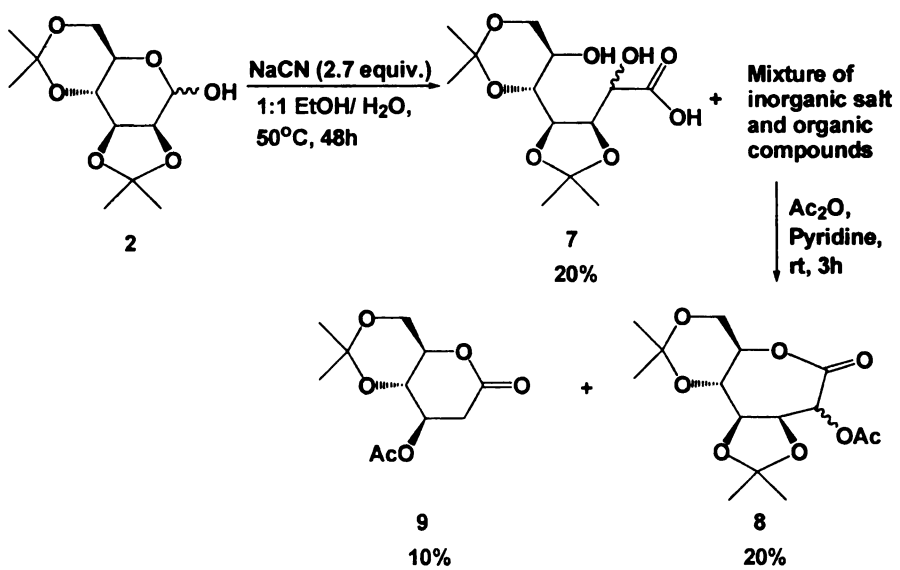


Figure 3.2 Cyanohydrin formation reaction under regular Strecker condition

3.1.4 Possible Mechanism for Product 8

In all the three products obtained, compound **8** is the expected product and the mechanism is the most straightforward one **(Figure 3.3)**. First, cyanide attacks the

electrophilic anomeric carbon to form a cyanohydrin **11**. Under basic condition, the cyanide is hydrolyzed to afford a carboxylic acid salt **12**. This salt is mixed with other inorganic salt and was taken for the acylation reaction. Both the carboxylic group and the α – hydroxyl group are acetylated. Finally, the 5-O attacks carboxylic group, kicks out acetate, and closes the seven member ring to give product **8**.

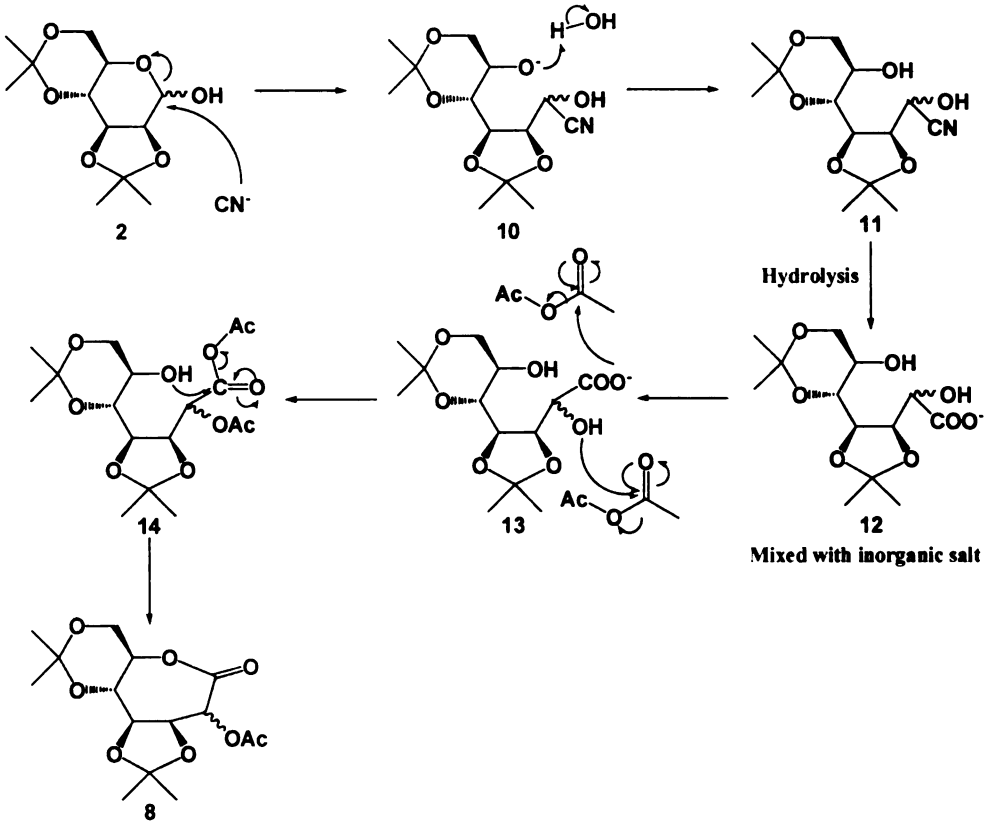


Figure 3.3 Possible mechanism of the formation of compound **8**

3.1.5 Possible Mechanism for Product 7

One of the possible mechanisms for compound **7** is almost the same as compound **8**. After the formation of cyanohydrin **11 (Figure 3.4)**, cyanide group is hydrolyzed to form

a carboxylate **12**. The solution is not basic enough to maintain all the **12** in its ion form. So, after workup, part of the **12** obtains a proton to form acid **7**.

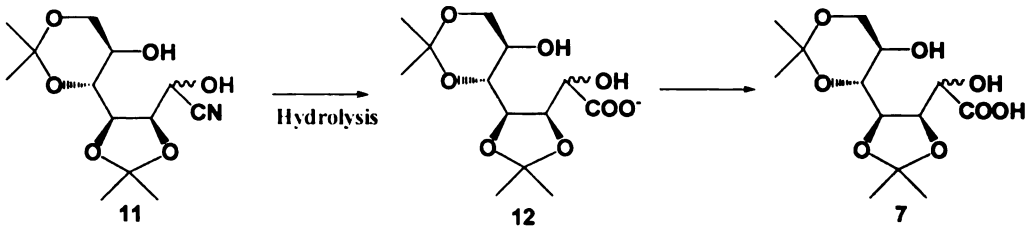


Figure 3.4 Possible mechanism of the formation of compound **7**

3.1.6 Possible Mechanism for Product 9

It's possible that after intermediate **11 (Figure 3.5)**, the C-2 hydrogen leaves as a proton as the strong charge holding capability of cyanide and hydroxyl group are able to stabilize the partial negative charge. This process is similar to the first two steps of the benzoin condensation reaction.[11-14] The lone pair of electron left by the leaving proton pushes the C-3 oxygen away to form enol **17**. Enol is not a stable structure, it automatically tautomerizes to its ketone form **18**. Compound **18** is hydrolyzed and remains in its salt form before the acylation reaction. After the carboxylic group of **18** is acetylated, the C-6 hydroxyl group would easily cyclize the ring to afford lactone **9.**

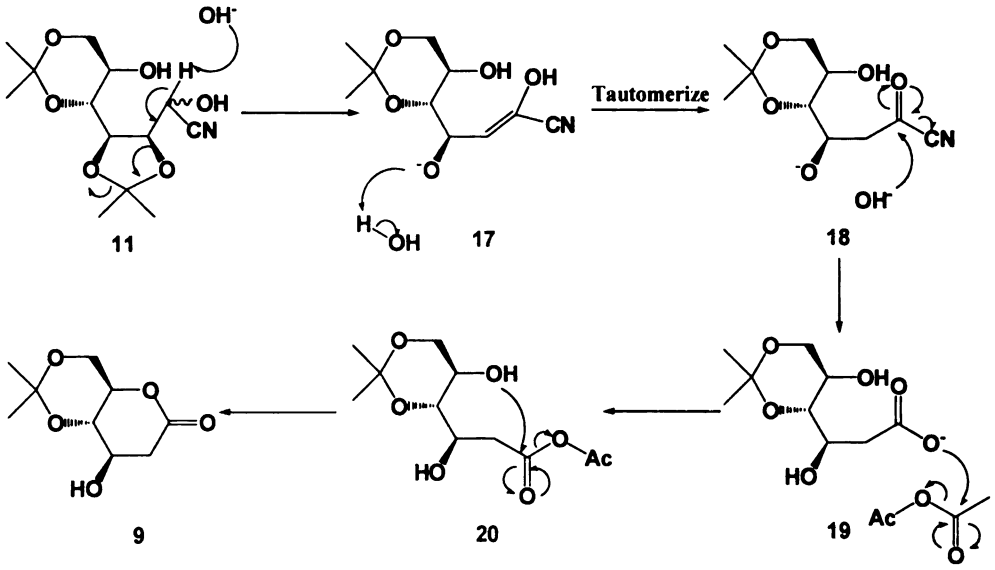


Figure 3.5 Possible mechanism of the formation of compound **9**

3.2 Alternative Cyanohydrin Formation Strategy

After several messy trials of Strecker reaction, we switched to Bucherer-Bergs reaction.[15-26] Bucherer reaction is to treat a ketone with potassium cyanide and sodium carbonate to form a heterocycle **22 (Figure 3.6)**. The mechanism of this reaction is also shown in the following scheme. In the first step, the cyanide attacks the carbonyl group to afford a cyanohydrin **23**. Then the ammonia release by ammonium carbonate in the solution kicks out the hydroxyl group to form structure **24. 24** has an amino group on the α–carbon to a cyanide, which is a carboxylic precursor. This kind of structure is exactly what we need. However, Bucherer reaction does not stop here. The amino group on **24** will attack CO_2 (or carbonate) to form amide **25. 25** experiences a series of transformation to reach the final product lactam **22**.

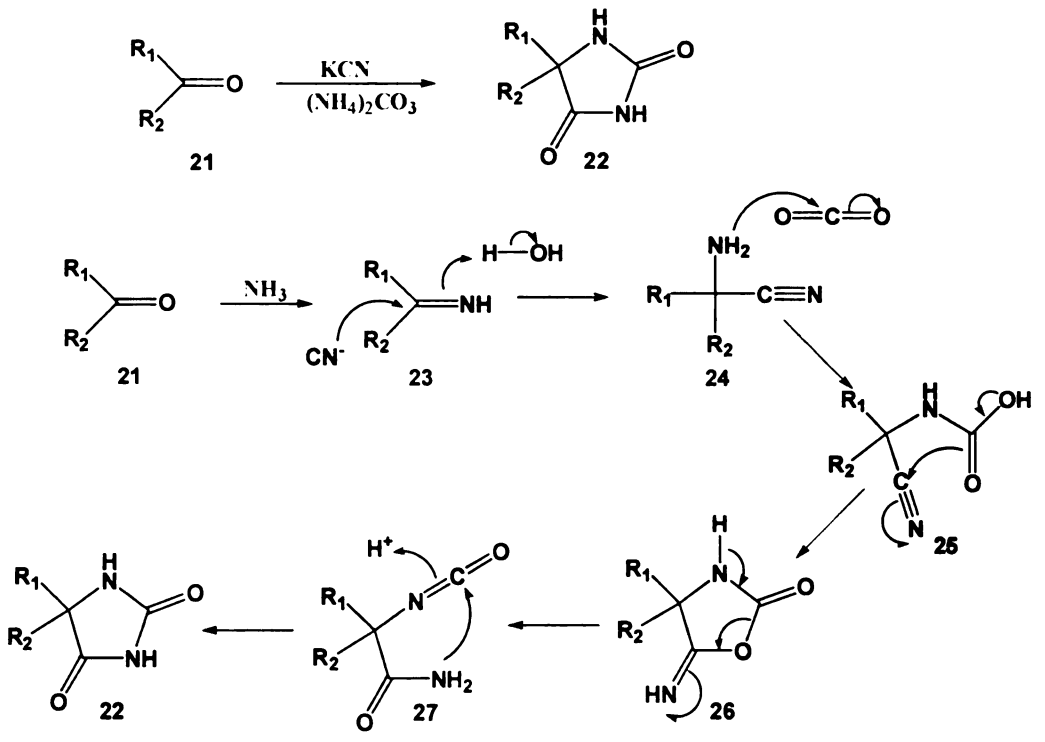


Figure 3.6 Bucherer-Bergs reaction and its mechanism

Inspired by Bucherer reaction, we tried to use ammonium chloride instead of ammonium carbonate, attempting to stop the reaction at compound **24** stage. It was successful **(Figure 3.7)**. We got two series of product: **28, 29** which are cyanoamine and **30, 31** which are cyanohydrin. The ratio of these products depends on the reaction temperature. Higher temperature favors **28**& **29**; Lower temperature favors **30**& **31 (Table 3.3)**. However, in either condition, we can not convert all the starting material after 48h. At 100°C, we got almost pure **28** and **29** in a ratio of 1:2. At room temperature, only **30** and **31** are obtained with a relative ratio of 2:1.

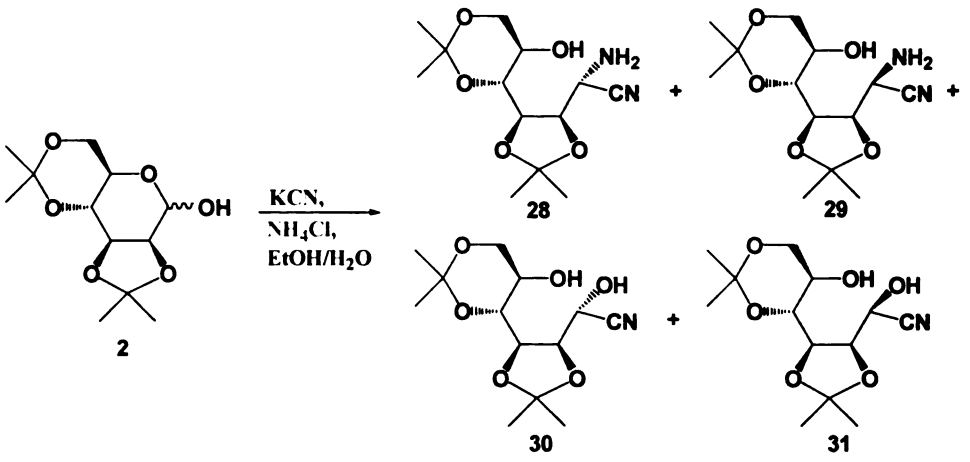


Figure 3.7 Application of Bucherer type reaction to 2, 3: 4, 6-di-O-propylidene-D-mannose

Temperature	**28 + 29 : 30 + 31**	**28 : 29**	**30 : 31**	**Total Yield**
100°C	80 :20	1 : 2	-	75%
55 °C	60: 40	1 : 1	1 : 1	65%
20 °C	90 :10	-	2 : 1	60%

Table 3.3 Products ratio and total yields for Bucherer type reaction of 2, 3: 4, 6-di-O-isopropylidene-D-mannose

3.3 Ring Closure Trials

3.1.1 Intramolecular Mitsunobu Reaction

We took compound **28** and **29** for a classic Mitsunobu reaction in the hope that the C2 amino group would displace the hydroxyl group on C6 to form the piperidine scaffold. But, we got no reaction even after heating **(Figure 3.8)**.

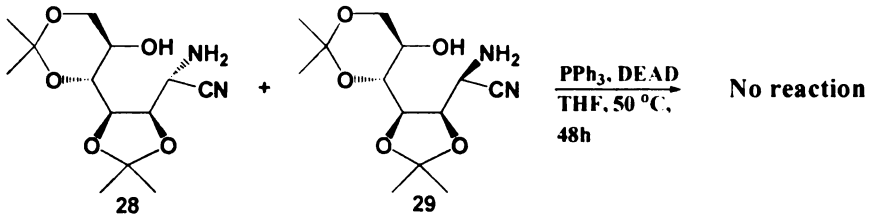


Figure 3.8 Mitsunobu reaction of compound **28** and **29**

3.3.2 Double N-Alkylation Reaction

We took compound **30** and **31 (Figure 3.9)**, converted the C2, C6 hydroxyl groups to mesylates first (compound **32** and **33**). Then we tried to use benzylamine as nucleophile to do a double substitution reaction to built up the 6 member ring. It was really hard to do this alkylation on two secondary carbons. We got only monosubstituted products after bringing the reaction temperature to 150 °C, extending the reaction time to 72 hours.

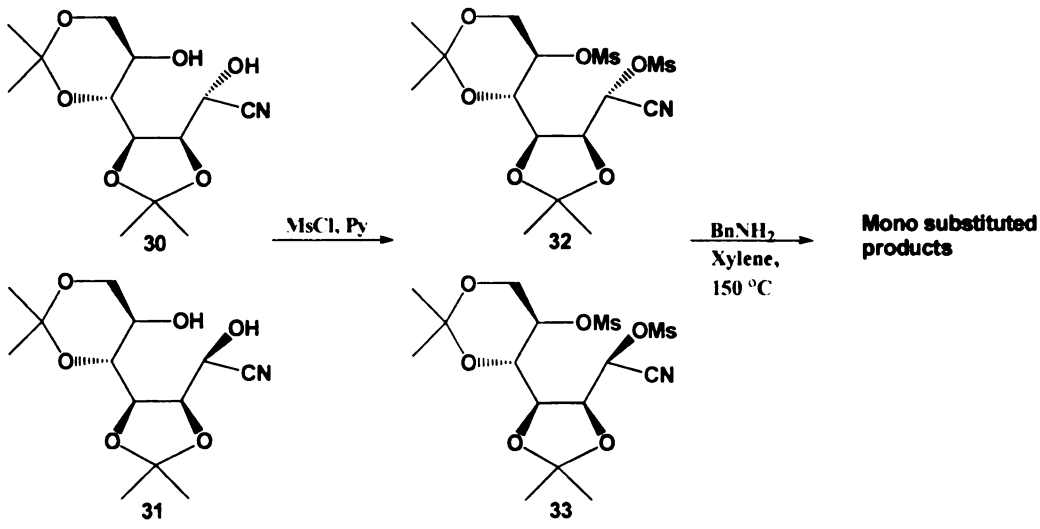


Figure 3.9 Trials on double N-alkylation to cyclize the ring

There are two possible reasons for this unsuccessful S_N2 reaction:

1. Kinetic factors. Generally, S_N2 reaction on a secondary carbon is much more difficult than on a primary carbon. Two S_N2 on two secondary carbons would be even harder. The mesylate on C5 is surrounded by C6 and C4, which are on a

six-membered ring. These groups hinder the incoming benzyl amine to approach C5.

2. Thermodynamic factors. On the C4-C5-C6-isopropylidene ring, both C5 mesylate and the C3 on C4 are on equatorial positions before the substitution reaction. It's a quite stable structure. The amine has to come in from the axial position on C5 to kick the mesylate out. This would result in an axial C4-N bond. One of the two large groups on the ring, C3 or NHR, has to be on axial position even the ring flips. So, this substitution is thermodynamically disfavored.

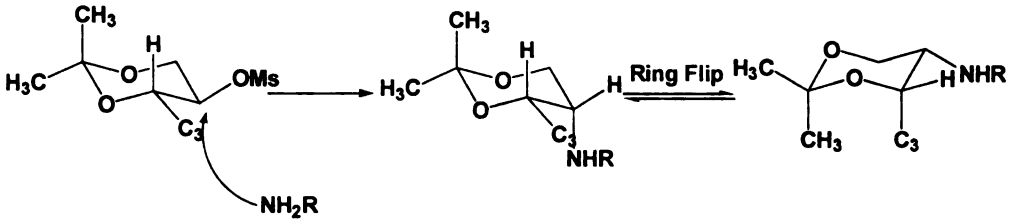


Figure 3.10 Explanation to the unsuccessful ring closing reaction of compound **32**

A possible solution to this substitution reaction is to make a better leaving group than mesylate, for example, triflate.

3.4 Summary

In summary, we have successfully made the cyanohydrins and cyanoamines on the anomeric position of protected mannose **2** by Bucherer reaction. But we failed to close the ring by double S_N2 reaction due to both kinetic and thermodynamic factors. We believe that by switching to a better leaving group, the piperidine ring could be closed and the Influenza inhibitor library could be built.

3.5 Experimental Section

2, 3: 4, 6-di-O-isopropylidene-D-Mannose **2**

1.01g (5.6mmol) D-(+)-Mannose 1 was dissolved in 25ml anhydrous DMF. The solution was cooled in ice bath for 10min, then 0.95g (12.8mmol, 2 equiv.) 2-methoxypropene and 0.05g (3.2mmol) *p*-toluenesulfuric acid two hydrates were added. After 2 hours, the solution was warmed to room temperature, then another portion of 2-methoxypropene (0.95g, 12.8mmol, 2equiv.) was added. The solution was kept in room temperature while stirring overnight before being dumped into a beaker containing 20g ice and 0.1g sodium bicarbonate. The water-DMF solution was extracted with ethyl acetate for three times (30ml *3). The ethyl acetate extracts were combined, dried with sodium sulfate and the solvent was removed. The white solid obtained was recrystallized from ethyl acetate and hexane. The product is the white needle crystal (1.10g, 76%); mp153-154℃, $[a]_D^{20}$-1 (3min)→-16 (5min) →-24° (final, 48h; c 1.2, water);m/e 205 (4.5, M^+-Me), 187 (1.2, 205-H_2O, m^* 170.6), 161(0.7, M^+-Me_2COH), 145 (1.2, 205-AcOH), 131(3.3), 115 (1.5), 103(1.2), 102(1), 101(6.5), 85(2.4), 73(7), 59(30), 58(26) and 43(100)

3, 4: 5, 7-di-O-isopropylidene-D-Manno-heptulopyransonic acid **7**

Isoprpylidene protect Mannose **2** (0.5g, 1.92mmol) was dissolved in 5ml ethanol and 5ml water solution. 231mg NaCN (4.71mmol) was added, and then the solution was kept at 55 degree for 48 hours. The mixture turned reddish. The solvent was removed and column chromatography was applied to separate the products (Eluent: 1:1 Ethyl Acetate: Hexanes). 0.1g starting material 2 was recovered and 0.12g **7** (0.384mmol, 20%) was obtained. Methanol was used to wash the column. After removing the methanol, white solid mixture was obtained. Data for isomer 1: ^{1}H NMR (300MHz, $CDCl_3$) δ 6.86 (1H, s), 6.58 (1H, s), 5.60-5.80 (1H, br.), 4.78 (1H, q, J = 4Hz, 3Hz), 4.59 (1H, d, J = 7Hz), 4.30-4.35 (1H, m), 4.08 (1H, q, J = 5Hz), 3.90-4.00 (2H, m), 1.38 (3H, s), 1.35 (3H,s), 1.27 (3H, s), 1.22 (3H, s); ^{13}C NMR (300MHz, $CDCl_3$) δ 171.0, 113.0, 109.2, 102.3, 86.0, 80.1, 79.6, 72.8, 66.6, 26.8, 25.6, 24.9, 23.9 ppm; Data for Isomer 2: ^{1}H NMR (300MHz, $CDCl_3$) δ 6.71 (1H, s), 6.62 (1H, s), 5.60-5.80 (1H, br.), 4.82 (1H, q, J = 4Hz, 3Hz), 4.71 (1H, d, J = 7Hz), 4.20-4.25 (1H, m), 3.90-4.00 (2H, m), 1.47 (3H, s), 1.33 (3H, s), 1.31 (3H, s), 1.25 (3H, s); ^{13}C NMR (300MHz, $CDCl_3$) δ 172.0, 113.7, 109.2, 99.9, 80.1, 78.8, 77.7, 72.9, 66.8, 26.8, 25.7, 25.0, 24.3 ppm

2-O-acetyl-3, 4: 5, 7-di-O-isopropylidene-D-Manno-1, 6-heptanolactone **8**

The white solid mixture from the preparation of **7** was mixed with 10 ml anhydrous pyridine to form syrup. The syrup was cooled to 0 degree, and then excessive amount of acetic anhydride was dropped in. The solution was kept stirring and warmed up to room temperature in 3 hours. The syrup after removal of the solvent was distributed into water and ethyl acetate. The ethyl acetate extract was dried with sodium sulfate. Column chromatography (Eluent: 1:2 ethyl acetate to 1:1 ethyl acetate) was used to separate product **8** (0.127g 20%). ^{1}H NMR (300MHz, $CDCl_3$) δ 5.48 (1H, d, J = 5Hz), 4.81 (2H, dd, J = 8Hz), 4.41 (1H,m J = 3Hz), 4.13 (3H, m, J=4Hz),2.28 (3H, s), 2.18 (1H, d, J = 3Hz), 1.49 (3H, s), 1.46 (3H, s), 1.41 (3H, s), 1.39 (3H, s); ^{13}C NMR (300MHz, $CDCl_3$)

δ 170.2, 165.1, 112.3, 110.0, 73.5, 72.8, 72.4, 69.5, 66.5, 27.0, 25.8, 25, 24.4, 20.5 ppm

3-O-acetyl-4, 6-O-isopropylidene-2-deoxy-D-gluconic-1, 5-lactone **9**

The procedure is the same as the preparation of compound **8.** Lactone **9** was obtained in yellow oil (94 mg, 20%). ^{1}H NMR (300MHz, $CDCl_3$) δ 5.52 (1H, td, J = 9Hz), 4.38 (2H, m, J= 6Hz), 4.10 (1H, t, J = 6Hz), 4.00 (1H, q, J = 4Hz), 2.87 (1H, q, J = 4Hz), 2.58 (1H, q, J = 2Hz), 2.07 (3H, s), 1.37 (3H, s), 1.30 (3H, s); ^{13}C NMR(300MHz, $CDCl_3$) δ 173.5, 169.4, 119.4, 82.0, 71.9, 69.3, 67.3, 36.5, 26.8, 25.2, 20.0 ppm

1-amino-1-cyano-1-deoxy-2, 3: 4, 6-di-O-isopropylidene-D-Mannitol **28&29**

To a solution of protected mannose **2** (1.0 g, 3.85mmol) in 60ml 1:1 Ethanol and water, KCN (0.75g 11.5mmol) and NH_4Cl (1.235g, 23.1mmol) were added. The solution was stirred at 100 °C for 24h. The solvent is removed and the solid is dissolved in 20 ml water. Ether was used to extract the water solution (20ml *3). All the ether layers were combined and dried. Column chromatography (1:2 Ethyl acetate to hexanes 1:2 ethyl acetate to hexanes) was used to separate the starting material and compound **28** and **29** (0.83g, 75% overall) were obtained as a 2:1 mixture. Data for **28:** ^{1}H NMR (300MHz, $CDCl_3$) δ 4.56 (1H, d, J = 7Hz), 4.26 (1H, t, J = 8Hz), 4.10 (m, 1H), 3.82-3.91 (3H, m), 3.60-3.70 (1H, m), 1.90-2.20 (2H, br.), 1.52 (3H, s), 1.48 (3H, s), 1.36 (3H, s), 1.38 (3H, s); ^{13}C NMR(300MHz, $CDCl_3$) δ 119.8, 109.7, 99.0, 78.8, 74.6, 72.0, 64.5, 63.0, 44.0, 28.8, 26.7, 25.7, 19.0 ppm; Data for **29:** ^{1}H NMR (300MHz, $CDCl_3$) δ 4.47 (1H, q, J = 6Hz, 2Hz), 4.19 (1H, t, J = 8Hz), 3.95-4.10 (2H, m), 3.80-3.90 (2H, m), 3.60-3.70 (1H, m), 1.90-2.20 (2H, br.), 1.53 (3H, s), 1.51 (3H, s), 1.48 (3H, s), 1.46 (3H, s); ^{13}C NMR(300MHz, $CDCl_3$) δ 121.9, 109.8, 98.9, 77.2, 75.0, 71.3, 64.7, 63.6, 44.9, 28.2, 26.8, 25.4, 19.5 ppm

1-cyano-2, 3: 4, 6-di-O-isopropylidene-D-Mannitol **30&31**

To a solution of protected mannose **2** (1.0 g 3.85mmol) in 60ml 1:1 Ethanol and water, KCN (0.75g, 11.5mmol) and NH_4Cl (1.235g, 23.1 mmol) were added. The solution was stirred in room temperature for 24h. The solvent is removed and the solid is dissolved in 20 ml water. Ether was used to extract the water solution (20ml *3). All the ether layers were combined and dried. Column chromatography (1:2 Ethyl acetate to hexanes 1:2 ethyl acetate to hexanes) was used to separate the starting material and compound **30** and **31** (0.66g, 60% overall) were obtained as a 1:2 mixture. Data for **30**: ^{1}H NMR (300MHz, $CDCl_3$) δ 4.55-4.60 (2H, m), 4.24 (1H, t, J = 6Hz), 4.02-4.08 (1H, m), 3.83-3.90 (2H, m), 3.64 (1H, t, J = 5Hz), 1.99 (1H, s), 1.53 (3H, s), 1.46 (3H, s), 1.39 (3H, s), 1.32 (3H, s); ^{13}C NMR(300MHz, $CDCl_3$) δ 118.7, 109.4, 99.8, 76.9, 74.2, 69.8, 64.3, 62.7, 61.8, 28.3, 26.3, 25.2, 19.0 ppm; Data for **31**: ^{1}H NMR (300MHz, $CDCl_3$) δ 4.55-4.60 (2H, m), 4.29 (1H, t, J = 6Hz), 4.02-4.08 (1H, m), 3.83-3.90 (2H, m), 3.64 (1H, t, J = 5Hz), 1.99 (1H, s), 1.50 (3H, s), 1.36 (3H, s), 1.34 (3H, s), 1.32 (3H, s); ^{13}C NMR (300MHz, $CDCl_3$) δ 118.0, 109.8. 99.4, 77.4, 74.0, 71.3, 64.5, 62.8, 61.4, 28.0, 26.2, 25.3, 19.1 ppm

1-cyano-2, 3: 4, 6-di-O-isopropylidene-1, 5-di-O-mesyl-D-Mannitol **32& 33**

Cyanohydrin mixture of **30& 31** (100mg, 0.35 mmol) was dissolved in 2ml anhydrous

pyridine, cooled in ice bath while stirring. 0.06ml (0.77mmol) mesyl chloride was dropped into the solution in 5 min. The solution was kept stirring while it warmed up to room temperature in 3 hours. The reaction was quenched by 5ml anhydrous ether and 100 mg sodium bicarbonate. Another 2 portions (10ml) of ether was used to extract the water solution. All the ether extracts were combined, dried with sodium sulfate. After the ether was removed, mixture of **32** and **33** as a white solid was obtained (134mg, 90%). Data for isomer 1: ^{1}H NMR (300MHz, $CDCl_3$) δ 5.21 (1H, d, 2Hz), 4.70 (1H, q, 3Hz), 4.42-4.50 (2H, m), 4.0 (2H, m), 3.78 (1H, m), 3.12 (3H, s), 2.96 (3H, s), 1.49 (3H, s), 1.40 (3H, s), 1.31 (3H, s), 1.25 (3H, s); ^{13}C NMR(300MHz, $CDCl_3$) δ 114.4, 110.5, 100.1, 75.6, 73.5, 71.3, 67.8, 67.2, 61.9, 39.2, 37.5, 26.6, 25.9, 24.7, 19.8 ppm; Data for isomer 2: ^{1}H NMR (300MHz, $CDCl_3$) δ 5.48 (1H, d, J = 7Hz), 4.70 (1H, q, 3Hz), 4.42-4.50 (2H, m), 4.0 (2H, m), 3.78 (1H, m), 3.05 (3H, s), 2.98 (3H,s), 1.44 (3H, s), 1.39 (3H, s), 1.29 (3H, s), 1.24 (3H, s); ^{13}C NMR (300MHz, $CDCl_3$) δ 114.2, 110.5, 99.6, 75.0, 73.1, 70.2, 68.6, 65.6, 61.7, 38.7, 37.6, 27.4, 26.2, 25.0, 18.9 ppm

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Chapter 4 Design and Synthesis of C-2 Symmetric Ligands for Asymmetric Ketone Reduction Reactions

4.1 Introduction to C-2 Symmetric Ligands

More than ever, synthetic chemists are faced with the requirement of preparing materials in enantioselective fashion. While there are several options for entering this optically active world, the one with the greatest benefit is the use of asymmetric catalysts. The key feature responsible for the success of these catalysts is the chiral ligands that surround the catalytic core. They create a chiral environment in which the reaction proceeds. The enantioselectivity of these catalysts ultimately can be traced back to the ligands, especially C-2 symmetric ligands.

C-2 symmetric ligands are chiral and exhibit an axis of symmetry which make them bidentate ligands in many the cases. Some of the most common C-2 symmetric ligands are shown below **(Figure 4.1)**.

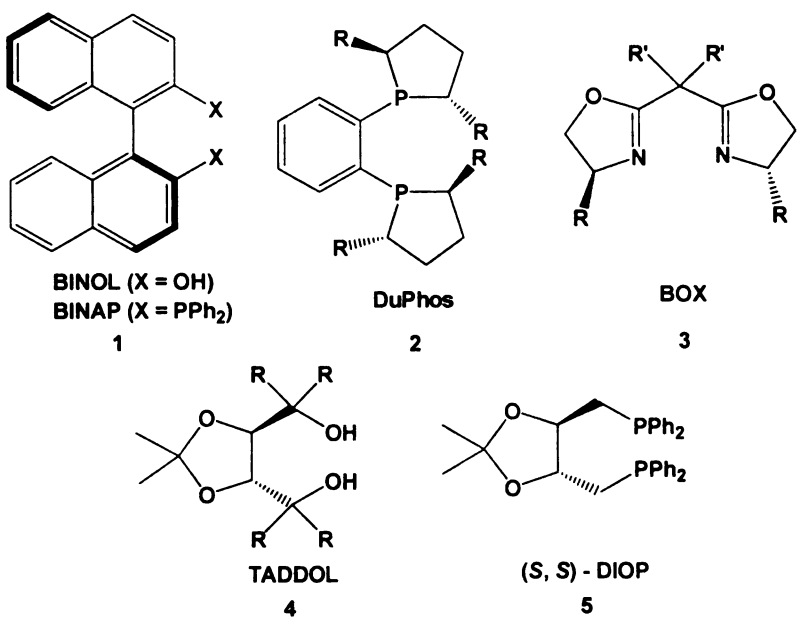

Figure 4.1 Most common C-2 symmetrical ligands

Because C-2 symmetric ligands allow high levels of enantiocontrol in many different metal-catalyzed reactions, they stand out from thousands of chiral ligands prepared so far and were called "privileged ligands".

4.2 Typical C-2 Symmetrical Ligands and Their Developments

4.2.1 (4*R*, 5*R*)-trans-4, 5-bis [(diphenylphosphino) methyl]-2, 2-dimethyl-1, 3-dioxolane (DIOP) Family

The C-2 symmetric ligand DIOP was first introduced by Dang and Kagan in 1971. They found its good catalytic activity in the asymmetric hydrogenation in the synthesis of hydratropic acid and amino-acids **(Figure 4.2)**.[1, 2]

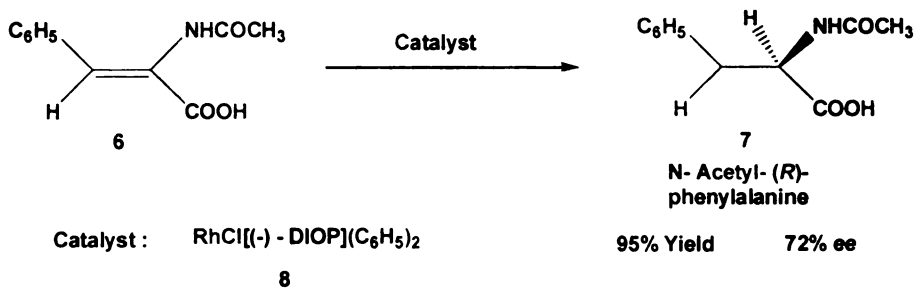

Figure 4.2 The asymmetric reduction of amino acid by Rhodium catalyst with C-2 symmetric DIOP as ligand

The reason for choosing a C-2 symmetric ligand with two equivalent phosphine atoms was to reduce the number of possible isomeric metal complexes, as well as the number of different substrate-catalyst arrangements and reaction pathways, when compared with a nonsymmetrical ligand. This consequence of C-2 symmetry can have a beneficial effect on enantioselectivity because the competing less-selective pathways are possibly

eliminated. Because fewer reaction intermediates to take into account, C-2 symmetry is of particular advantage in mechanistic studies because it facilitates analysis of the ligand-substrate interactions that may be responsible for enantioselection.

Based on Kagan's work, scientists tried to make modifications on DIOP to discover more efficient C-2 symmetric ligands. Zhang[3] and Rajanbabu[4, 5] have independently reported that the (*S*, *R*, *R*, *S*)-DIOP which has two alkyl substituents at α positions of the diphenylphosphine groups on the DIOP, provides excellent enantioselectivity in Rh-catalyzed hydrogenation for arylenamides **(Figure 4.3)**.

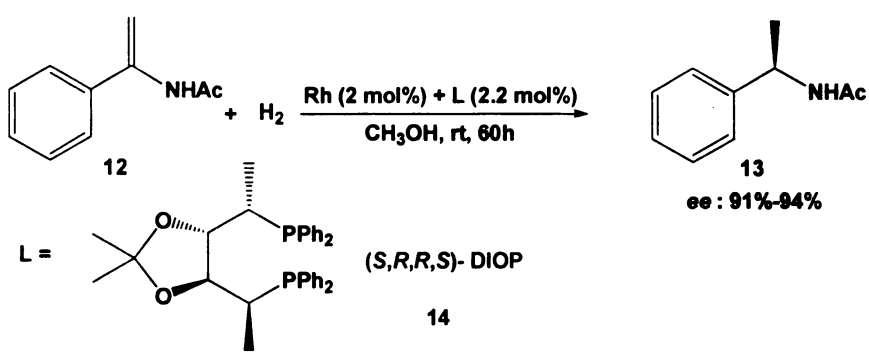

Figure 4.3 (*S*, *R*, *R*, *S*)-DIOP coordinated Rhodium catalyzed asymmetric hydrogenation of alkene

The capability of enantiocontrol offered by (*S*, *R*, *R*, *S*)-DIOP is much better than the (*S*, *S*)-DIOP prepared by Kagan. It is believed that the two methyl groups of (*S*, *R*, *R*, *S*)-DIOP are oriented at pseudoequatorial postions in the "effective" conformer of the DIOP metal complex, therefore stabilizing the "effective" conformer to promote high enantioselectivity.

Apart from those two DIOP derivatives, other DIOP analog have been developed. They showed satisfactory catalytic activities. For example, (2*R*, 2'*R*)-bis(diphenylphosphino)-(1*R*, 1'*R*)-dicyclopentane (BICP) has been found efficient for hydrogenation of α-dehydroamino acids, β-dehydroamino acids, arylenamides, and MOM-protected β-hydroxy enamides.[6-9] Also, Lee's 1, 4 diphosphane ligands (4*S*, 5*S*)-4,5-bis(dipenylphosphinomethyl)imidazolidin-2-one (BDPMI) which has a imidazolidin-2-one backbone have been successfully applied in Rh-catalyzed hydrogenation of arylenamides with up to 99% *ee*'s .[10-12]

4.2.2 2, 2'-bis (diphenylphosphino)-1, 1'-binaphthyl (BINAP) Family

Another important family of C-2 symmetrical ligands is the BINAP family. BINAP was first synthesized by Noyori in 5 steps from BINOL. Noyori brought BINAP into the spotlight by applying it to his asymmetric ketone reduction. BINAP become one of the most popular ligands for transition metals including Ru (II), Rh (I), Pd and Ir. Many of these species have been found to promote asymmetric transformations including hydrogenation, isomerisation, hydrosilylation, hydroboration, allylic alkylation and the Heck reaction **(Figure 4.4)**.[13] Now, BINAP is being assessed for several industrial and pharmaceutical applications.

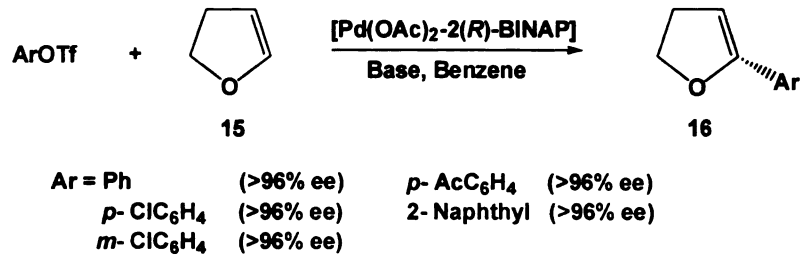

Figure 4.4 BINAP-Pd complex catalyzed Heck reaction

After screening lots of different ligands, Tanaka and Takeaki reported in 2005 that BINAP is the only ligand to enable Rh to efficiently catalyze the following propargylic alcohols to α, β-enal transformation **(Figure 4.5)**.[14]

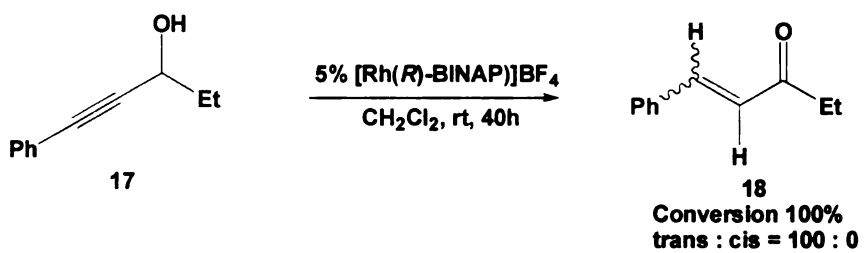

Figure 4.5 BINAP-Rhodium complex catalyzed propargylic alcohol reaction

1, 1'-bi-2-naphthol (BINOL), another C-2 symmetric ligand in BINAP family, also showed very promising catalytic activities. It has been used for asymmetric expoxidation,[15] asymmetric Diel-Alder reaction,[16] asymmetric Michael addition,[17, 18] and 1, 3-dipolar cycloaddition reactions.[19, 20]

4.3 Summary of C-2 symmetric Ligands

In summary, C-2 symmetric ligands have been widely used in transition metal catalyzed asymmetric reactions. Their highly ordered structures create steric hindrance so that the

substrate can only approach the core metal from a specific direction with a specific conformation. We believe that as more C-2 symmetric ligands are developed, people will have more power to control the chirality in a compound.

4.4 Asymmetric Ketone Reduction Reaction

4.4.1 Introduction

The reduction of ketones to enantiomerically enriched alcohols is a pivotal transformation in synthetic organic chemistry.[21-23] In recent years, the demand for optically active secondary alcohols in the area of pharmaceuticals and advanced materials increased dramatically.[24] In response to that, a series of asymmetric reductions of prochiral ketones has been developed.[25] Two major methodologies have been developed so far. One is introduced by E. J. Corey; he used asymmetric boranes to do the reduction; another is transition metal catalyzed reduction which has also been studied by Noyori intensively after 1995.

4.4.2 Asymmetric Ketones Reduction by Boranes

The introduction of aluminum and boron hydrides for the reduction of carbonyl groups half a century ago had an enormous impact on the field of synthetic chemistry, and helped to usher in a golden age for the rationally planned multistep construction of complex organic molecules.[26-28] In 1981, Itsuno and his collaborators achieved promising result with mixtures of chiral 1, 2-amino alcohols and borane. Itsuno reported that by mixing (*S*)-valinol and BH_3 • THF in a ratio of 1:2 in THF at 20°C, the enantioselective

reduction of a number of achiral ketones to chiral secondary alcohols could achieve nearly 100% yield with enantiomeric excesses in the range of 10-73% *ee* **(Figure 4.6)**. This discovery led to the world of borane-mediated enantioselective reduction of a wide variety of prochiral ketones (CBS reduction).[29-32] On screening numerous amino alcohols, it was discovered that the tertiary amino alcohol **21** derived from (*S*)-Valine, in most of the cases was the best ligand for BH_3 • THF at-78 °C to 0 °C.

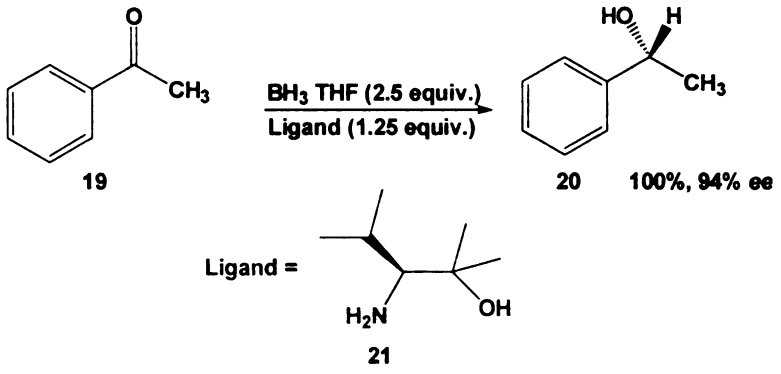

Figure 4.6 Itsono's approach of asymmetric reduction of ketones

Based on Itsuno's work, E. J. Corey[33-36] and his collaboraters did a more detailed study on this asymmetric reduction reaction. In 1986-1987, Bakshi and Shibata from Corey's group discovered that the effective reagent for this reaction was the oxazoborolidine which is created when amino alcohol meets two equivalents of BH_3 in THF. This was confirmed by further IR and NMR studies **(Figure 4.7)**.

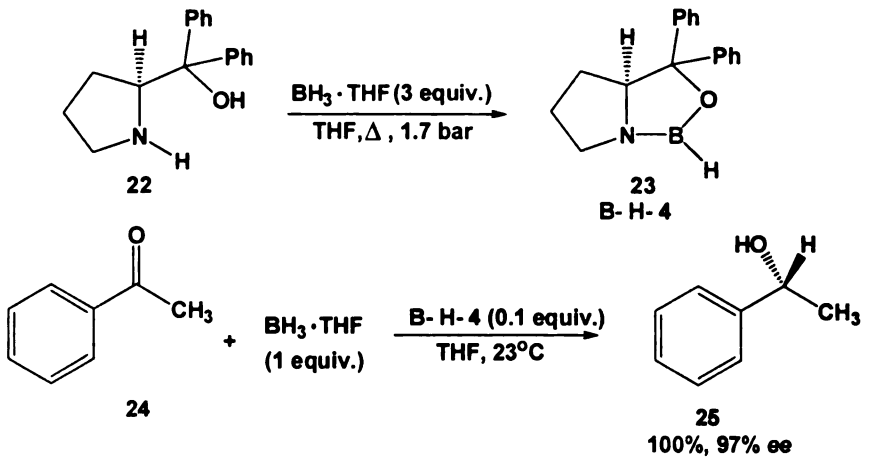

Figure 4.7 Corey-Bakshi-Shibata reduction (CBS reduction)

Though Corey-Bakshi-Shibata reduction (CBS reduction) achieved some success on specific types of asymmetric ketone reduction, this methodology has some drawbacks that prevent it from being used in industrial production. Most of the CBS reduction reagents are difficult to handle and require a tedious workup, involving hydrolysis and separations, plus the disposal of the large amounts of inorganic hydroxides produced. Now, this methodology is losing popularity to transition metal catalyzed asymmetric ketone reductions.

4.4.3 Transition Metal Catalyzed Asymmetric Ketone Reduction Reactions

The transition metal catalyzed asymmetric reactions have been used in organic synthesis for a long time. Chemists also tried to explore the possibility of reducing prochiral ketones to secondary alcohol by applying transition metal catalysts. Different metal complexes have been used, such as Pd, Ru, and some other Lanthanide metals. Among them, Ru has the best catalytic activity.[37-40] Based on the mechanisms, this kind of

reduction can be divided into two categories: 1. transfer hydrogenation reduction; 2. catalytic hydrogenation.

4.4.3.1 Transition Metal Catalyzed Transfer Hydrogenation Reduction

In the 1990s, two new Ruthenium systems based on transfer hydrogenation for asymmetric reduction of prochiral ketones were developed by professors Noyori and Ikariya. [41, 42] The ketone was reduced to alcohol while the isopropanol, working as solvent, was oxidized to actone. These systems enable catalytic asymmetric reduction to provide a route for the generation of enantiomerically pure secondary alcohols in a highly efficient, simple and economic way.[43] However, Noyori found that the direct hydrogenation reduction would be much more efficient and faster.

4.4.3.2 Ruthenium Catalyzed Asymmetric Ketone Hydrogenation

In 1995, Noyori and his co-workers[44] expanded the scope of Phosphine-Ru(II) catalyst. They found that by adding 1 equivalent of ethylenediamine and a >2.8M solution of KOH in isopropanol to the already known $RuCl_2$ [P $(C_6H_5)_3]_3$ system, the turnover frequency (TOF, defined as moles of the product per mole of the catalyst per hour) of the reduction was increased to 6700 from less than 5. Noyori examined the catalytic activities of different phosphine ligands and found BINAP **26** and diamine **27** to be good catalytic couples **(Figure 4.8)**. This methodology has a great industrial potential because it does not involve a great deal of workup. In addition, excess H_2 can be used in the reaction. At the end, both H_2 and the catalyst are recycled. It is much superior to CBS

reduction because of total atom economy, low cost, and effortless separation on degassing. Also, it beats transfer hydrogenation by its fast reaction rate and high yield. Noyori was awarded the Nobel Prize in 2001 due to this work.

A variety of aromatic ketones can be hydrogenated enantioselectively by the BINAP-Ru (II)-diamine-inorganic base catalyst system, where the C-2 symmetric BINAP and C-2 or pseudo C-2 symmetric diamine **28-30** act as the most effective chiral controllers **(Table 4.1)**.

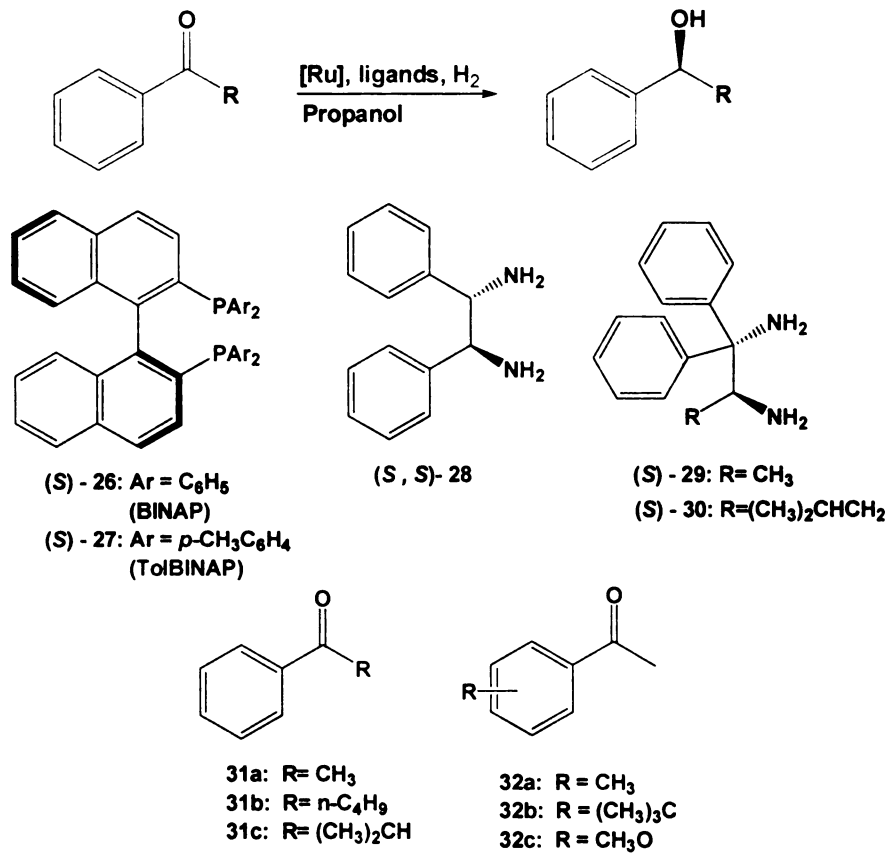

Figure 4.8 Noyori's asymmetric ketone reduction and his screening of ligands for Ruthenium catalysts

Ketones	Catalyst combination		Conditions		Alcohol product		
	Phosphine	Diamine	H_2, atm	Time, h	Yield %	*ee* %	Config
31a	(*S*)-**26**	(*S*)-**30**	4	3	>99	87	*R*
31b	(*S*)-**26**	(*S*)-**29**	4	3	>99	90	*R*
o-**32a**	(*S*)-**27**	(*S,S*)-**28**	4	5	>99	94	*R*
p-**32b**	(*S*)-**27**	(*S,S*)-**28**	4	1.5	>99	96	*R*

Table 4.1 Experimental result of Noyori's screening of ligands

We can see from the result **(Table 4.1)** that the combination of **27** and **28**, both of them are C-2 symmetric ligands, showed the best catalytic activity. It opens a door to the research on the development of new, more C-2 symmetric ligands for the Ru catalyzed ketone reduction reaction.

4.5 Why Do We Need to Develop More C-2 Symmetric Ligands?

Though C-2 symmetric ligands have been widely applied to organic synthesis, they still suffer from the following drawbacks:

1. C-2 symmetric ligands are quite expensive. Asymmetric synthesis depends on high purity ligands. However, the synthesis of C-2 symmetric ligands itself is asymmetric synthesis involving lengthy and costly chirality control, separation and purification steps. The high price is a big barrier for its industrial application.
2. No general synthetic strategies for C-2 symmetric ligands are available. In most of the cases, each synthetic route is applicable to only one type of ligands.
3. C-2 symmetric ligands are still limited. There are thousands of possible C-2

symmetric structures in nature of which only a small number have been synthesized and tested. By exploring more compounds, it's very likely to find more efficient ligands for asymmetric catalysis.

4. Most of the C-2 symmetric ligands are bidentate. In fact, by introducing another binding atom, we can create a tridentate C-2 symmetric ligand. It is believed that tridentate ligands have tighter interactions with the boundmetal, thus be able to exert stronger enantiocontrol during reactions.

4.6 Design and Synthesis of C-2 Symmetric Ligand

4.6.1 Design of C-2 Symmetric Ligand

To solve the problems above, we designed a C-2 symmetric furan ligand scaffold which is very easy to synthesize and modify.

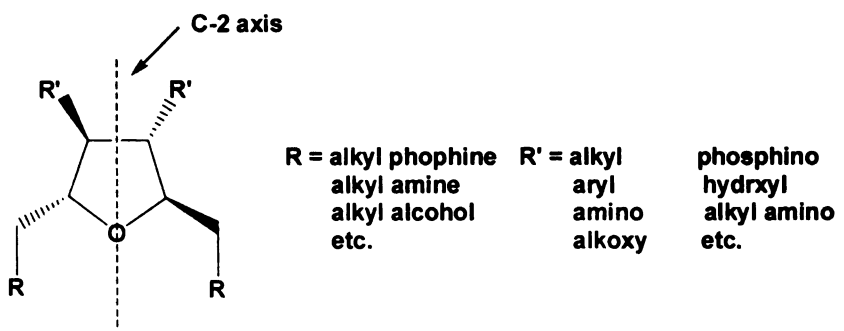

Figure 4.9 Furan based C-2 symmetric ligand scaffold

The advantages of this scaffold are:

1. It's a perfect C-2 symmetric system with a furan ring; the oxygen on the furan may provide another coordination center.

2. There are two possible sets of coordination sites---1, 6 or 3, 4 positions. We can develop at least two types of ligands based on that.

3. The flexibility of the furan ring is adjustable. When R's on C3 and C4 positions are big, the bulky repulsion between them will twist the furan more and lock the C-2 symmetry. On the other hand, if they are smaller, the furan ring would gain more flexibility

4. By using different substituents on the furan, we can vary the polarity of the ligands. For example, when R's are alkyl groups, the ligand tends to be hydrophobic; if R's are carboxylic acids, the ligand may be soluble in aqueous solution. By adding hydroxyl groups on the side chain, we can manipulate the solubility of the ligand elaborately and even obtain phase transfer characteristics of the ligands.

5. As shown later, we use the ubiquitous cheap carbohydrate as starting material to synthesize this molecule. The synthetic route is less than 6 steps and does not involve extreme high temp, low temp, high pressure, toxic or environment-hostile reagents. All those offer this kind of ligands big advantages on their industrial scale application.

4.6.2 Synthesis of C-2 Symmetric Ligands

Carbohydrate is cheap and pure natural product that offers multiple chiralities. We decided to take advantage of it **(Figure 4.10)**.

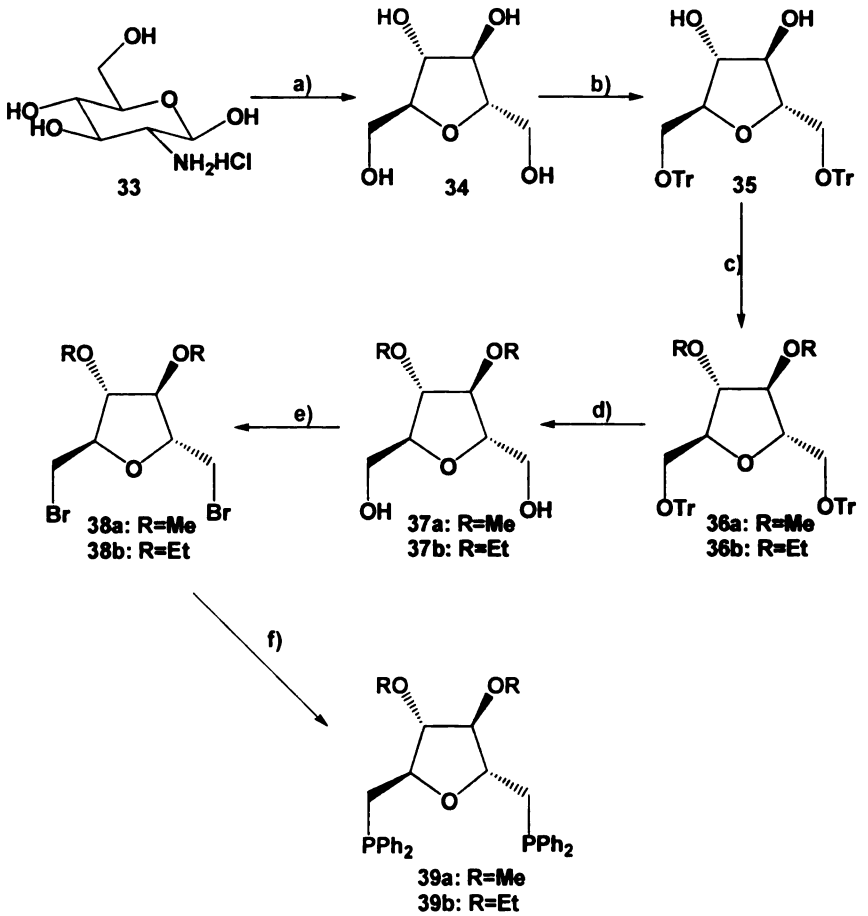

Figure 4.10 The synthetic route towards our C-2 symmetric ligands a) i. Acetic acid, Na NO_2; ii. $NaBH_4$; b) TrCl, pyridine, room temperature; c) NaH, RI, THF; d) Pd/C, H_2, CH_2Cl_2; e) CBr_4, Ph_3P, pyridine; f) Ph_2PK, THF

We started from 2-glucosamine hydrochloric acid **33**, transform it to C-2 symmetric tetraol **34** within one step. The detailed mechanism is shown below **(Figure 4.11)**. The nitrous acid oxidizes the C2 amino group to form N_2, leaving C2 an electrophilic site. The O5 then attacks C2 to close the furan ring and form an intermediate aldehyde **40**. Sodium borohydride reduces the carbonyl group to afford **34**.

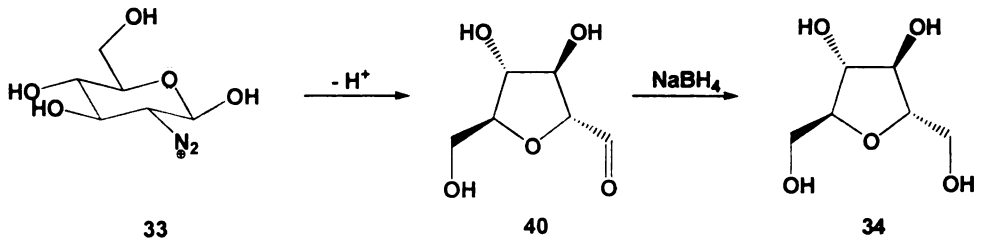

Figure 4.11 Mechanism of the one step reaction from 2-glucosamine salt to tetraol

Next, we modified the substituents on the hydroxyl groups. The bulky triphenyl methyl protecting group was used to selectively protect two primary alcohols. Two secondary hydroxyls of C3 and C4 were alkylated by methyl or ethyl groups. The trityl groups were removed by hydrogen gas/Pd. Then, dibromide is prepared through a classic Mitsunobu reaction. The last step is the replacement of bromides with diphenyl phosphine to afford the diphosphine ligand **39a** or **39b**.

4.6.3 Result and Discussion

The whole synthesis route has six steps. It turned out that the third step, which is protection of C3 and C4 hydroxyl groups with alkyls is the yield limiting step **(Figure 4.12)**.

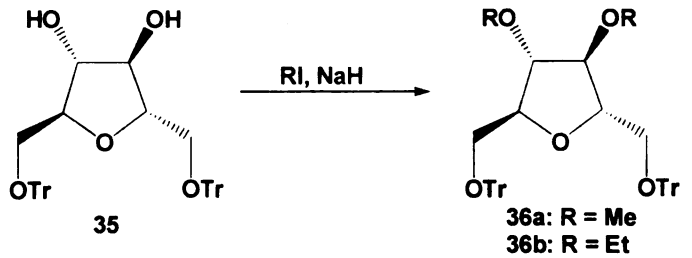

Figure 4.12 C3, C4 hydroxyl group protection of compound **35**

Reagents	Conditions	Yields of **36**
MeI	NaH (4 equiv.), room temp., 24h, DMF	87%
EtI	NaH (4 equiv.), 50 °C., 24h, DMF	45%
n-BuI	NaH (4 equiv.), 75 °C, 24h, DMSO	Trace amount
n-HexI	NaH (4 equiv.), 85 °C, 48h, DMSO	No reaction

Table 4.2 Reaction conditions and yields of the alkylation of C3, C4 postions of our furan scaffold

When R is methyl group, the yield was pretty good (85%). However, if the size of the alkyl groups goes bigger, the yields drop dramatically. We got no hexyl substituted product even when the reaction temperature was increased to above 85°C and the reaction time was extended to 2 days. We think the reason is the bulkiness of the trityl groups on C1 and C6. They stretch into the space and prevent oxygen anion from attacking the alkyl iodides. It's a dilemma situation because if we used smaller protecting group than trityl to protect the C1 and C6 hydroxyl groups, the selectivity for primary hydroxyl groups would decrease.

Another handicap is the deprotection of trityl groups. After we got fully protected tetraol **36a/ 36b (Figure 4.12)**, the two most common ways to deprotect the trityl groups were tried: 1. Acidic deprotection. Compound **36a** was thrown into a 1:1 ether and formic acid mixture and stirred in room temperature for 10 minutes. We found that the acid not only removes the trityl group, but opens the furan ring as well. The yield of the desired product **37a** remained below 30% even after we shortened the reaction time. So, we switched to solution 2: Catalytic Hydrogenation. We used 5% Pd/ C and 2 atm of H_2 gas, stirred in room temperature. The catalyst lost its activity after 3 hour. To get 1 gram of

36a converted, a huge amount of catalyst (0.3 g) was consumed. After further trials, we found that a couple of drops of acetic acid would keep the catalyst active. After mixing 2 drops of acetic acid in 20ml solution, we obtained 93% yield on the conversion from **36a** to **37a**. Also, the reaction time was significantly decreased from 2 days to 8 hours.

4.6.4 Why Our Strategy is Superior to Others'

Compared to other synthetic approaches towards C-2 symmetric ligands, our approach shows obvious advantages on cost and potential for industrial production:

1. Our approach is short. In 6 steps, we converted a carbohydrate to a C2 symmetric diphosphine ligand.
2. Cost advantage. None of the reagents used in our synthesis are very expensive. Especially, the starting material D-glucosamine hydrochloride is very cheap.
3. Ease of operation. In the 6 step synthesis, column chromatography is not required until the fifth step. Furthermore, in some steps, even quantitative control of reagents is not indispensable. In step from **34** to **35** and from **35a/35b** to **36/36b**, excessive amount of triphenyl methyl chloride and methyl iodide/ ethyl iodide were used respectively. The unreacted trityl chloride was recovered by recrystallation and the unreacted methyl iodide/ ethyl iodide was removed by rotavapor. All those operations can be done in large scale.
4. General applicability. As we have already discussed, apart from the 1, 6 position, we can also develop 3, 4 position to be the coordination sites. In addition, by varying substitution groups, we can synthesize a library of ligands of different

polarities.

4.7 Future Directions

1. Find out the solution for the low yield in the protection step of O4 and O5 in compound **34**. To solve this problem, we should avoid bulky protecting groups on O1 and O6 when we alkylate O3 and O4 **(Figure 4.13)**. Two primary hydroxyl groups in **34** may be converted to bromide first to form **41**. Alkylation of **41** could produce intramolecular substitution side product **43** and intermolecular substitution product **42**. Due to the high strach of the four-membered ring and the reaction rate superiority of alkyl iodides over bromides, we believe that these two side products will be minor. By applying this approach, we are even able to make our synthetic pathway one step shorter.

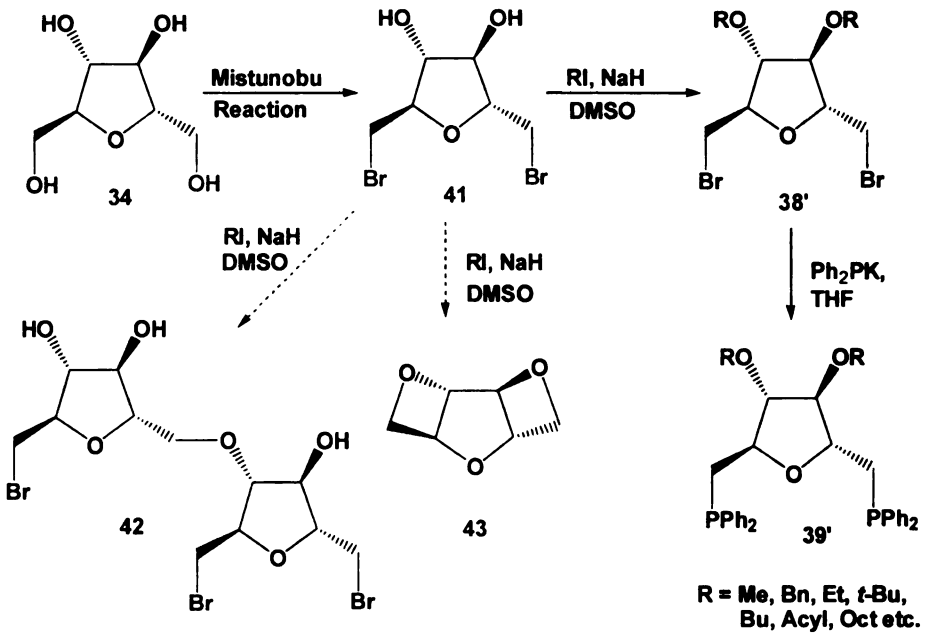

Figure 4.13 Alternative synthetic route to avoid O3, O4 alkylation obstacle in compound **34**

2. By using different nucleophiles at the last step of the synthetic pathway, we can obtain not only diphosphine ligands, but also diamine ligands like scaffold **44** **(Figure 4.14)**. Carbon nucleophiles may extend the "arm" of the ligand to make the ligand looser and more flexible. For example, we can treat the dibromide **38** with nitromethane and sodium methoxide to extend one carbon on C1, C6 positions. After a reduction reaction, a diamine ligand **47** could be obtained **(Figure 4.15)**.

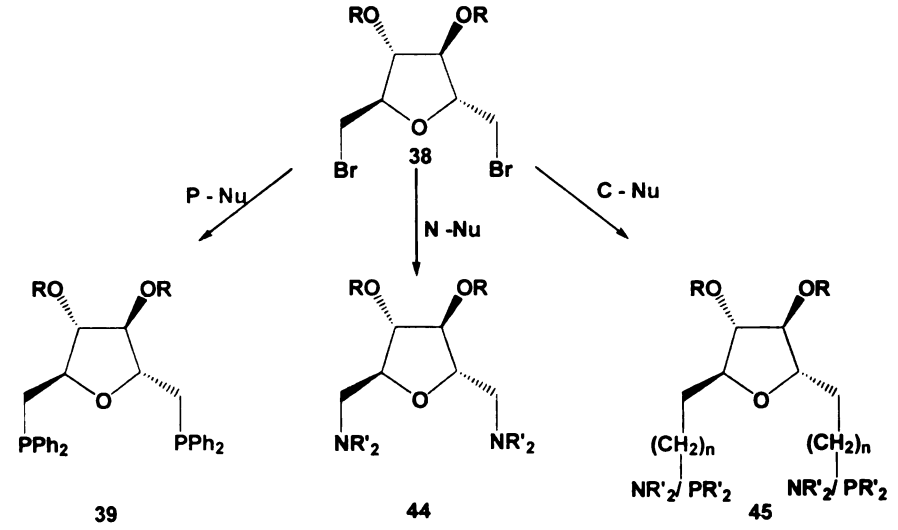

Figure 4.14 Synthetic proposal for different kinds of ligands based on intermediate **38**

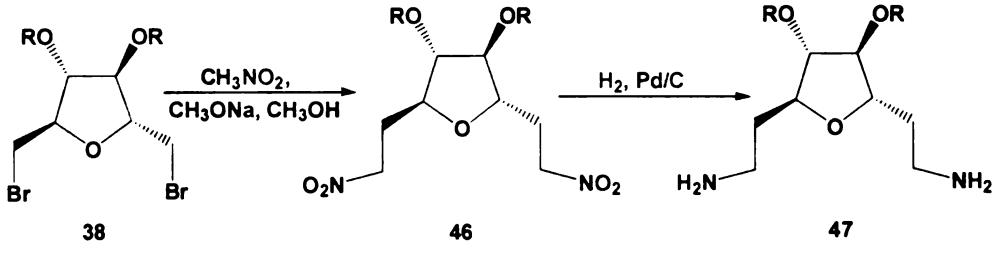

Figure 4.15 Proposed synthesis of diamine ligand **47**

3. A ligand is made to catalyze. So, we want to apply the ligand to reactions and examine the catalytic activity **(Figure 4.16)**:

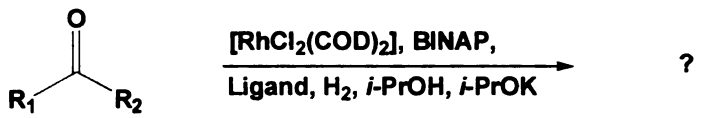

Figure 4.16 The reaction system to test the activities of our ligands

The catalyst can be prepared by simply mixing 0.5mol% Ru (II) complex, 1.5 mol% of BINAP and 3 mol% of the ligands we prepared in isopropanol. A little bit of base (5 mol %) and dihydrogen are also needed.[45] This system is suitable for testing our ligands's activity in asymmetric ketone reductions.

The application of our C-2 symmetric ligand library is not limited to ketone reduction reaction. We can try our ligands on other transition metal catalyzed asymmetric reactions, such as asymmetric Michael addition reaction, Asymmetric Heck reaction etc.

4.8 Experimental Section

2, 5-anhydro-D-mannitol **34**

A solution of D-glucosamine hydrochloride (10g, 46.4 mmol) in 150ml water was cooled to 0 °C in ice bath. After 30 minutes, sodium nitrate (13.6g, 197.1 mmol) was added to the solution and 8.3 ml acetic acid was slowly added dropwise within 3 hours. The mixture was warmed up to room temperature while stirring, and then air was bubbled into the solution to get rid of the yellow color. Cationic Resin DOW-50 was used to remove the inorganic salt. The solution was concentrated to 30ml, cooled in ice bath again; sodium borohydride (3g, 81mmol) was added slowly. After 4 hours, 2N hydrochloric acid was used to remove the unreacted sodium borohydride. The salt was removed by the DOW-50 again. A yellowish sticky liquid (6.13g, 82%) was obtained after the solvent was rotavapored. $[\alpha]_D$ = +50 (c=1.0); H_2O); ^1H NMR (D_2O) δ 3.70 (2H, dd, $J_{1b,2} = J_{5,6b}$= 5.6 Hz, H-1b and H-6b), 3.79 (2H, dd, $J_{1a,2} = J_{5,6a}$= 3.1 Hz, $J_{1a,1b} = J_{6a,6b}$ = 12.4 Hz, H-1a and H-6a), 3.90 (m, 2H, H-2 and H-5), 4.07 (2H, m, $J_{2,3}$ = 7.3 Hz, H-3 and H-4). ^{13}C NMR (D_2O) δ 61.5 (2C, C-1 and C-6), 76.6 (2C, C-3 and C-4), 82.6 (2C, C-2 and C-5); MS (IS, MeOH +5-10% H_2O) m/z =165 $[M+H]^+$, 187.0 $[M+ Na]^+$; calcd. 187.0582; found 187.0576 (HR-ESI-TOF-MS)

2, 5-anhydro-1, 6-di-O-trityl-D-mannitol **35**

To a solution of 2, 5-anhydro-D-mannitol **34** (0.207g, 1.26mmol) in 2ml anhydrous pyridine, triphenyl methyl chloride (0.773g, 2.77mmol) was added. The mixture was stirred in room temperature for overnight. Another portion of trityl chloride was added (0.387g, 1.38mmol). After 5 hours, pyridine solvent was removed and excessive trityl chloride was recrystallized from the solution (solvent: Ethyl acetate and Hexanes). A white solid of **35** (0.69g, 85%) was obtained after the solvent is removed. mp= 92-93 °C; $[\alpha]^{25}_D$ 8.75° ($CHCl_3$, c 0.93); ^{1}H NMR ($CDCl_3$ 300MHz) δ 3.23 (s, 2H), 3.40 (2H, dd, J = 3.8, 9.8Hz), 3.48 (2H, dd, J = 5.4, 9.8Hz), 4.25 (2H, d, J = 2.8Hz), 4.41 (2H, m), 7.0 (30H, m); ^{13}C NMR ($CDCl_3$) δ 62.8, 78.6, 78.8, 87.2, 127.0-143.2 ppm; Anal. Calcd for $C_{44}H_{40}O_5$: C, 81.46; H, 6.21. Found: C, 81.19, H, 6.19.

2, 5-anhydro-3, 4-di-O-methyl-1, 6-di-O-trityl-D-mannitol **36a**

To a solution of 2, 5-anhydro-1, 6-di-O-trityl-D-mannitol **35** (0.3g, 0.46mmol) in 5ml anhydrous THF, sodium hydride (0.028g, 1.16mmol) was added under 0°C. The solution was stirred in ice bath with drying tube contain calcium chloride on the flask. Then, methyl iodide was dropped into the mixture (0.210g, 1.38mmol). After 3 hours, all the solvent was removed. 10 ml water was used to dissolve the remaining sodium hydride. The water layer was extracted by ether (3* 20ml). The product was got as a reddish solid (0.264g, 87%) after all the ether is evaporated. mp 163-164 °C, $[\alpha]_D$ +4.3° (c 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$ 300MHz) δ 3.15-3.40 (10H, m, 2* OMe, H-1 1, 1', 6, 6'), 3.80 (2H, m, H-3 and H-4), 4.13 (2H, m, H-2 and H-5), 7.15-7.60 (30H, m, Ar); ^{13}C NMR ($CDCl_3$) δ 143.9, 128.6, 127.3, 126.8, 86.9, 81.4, 64.0, 57.3 ppm; m/z 676 (0.5, M^+), 599 (3, M-Ph), 433 (5, M-Tr), 403 (11, M-CH_2OTr), 243 (100, Tr^+), 165 (38, Ph_2C^+); Anal. Calc. for $C_{46}H_{44}O_5$: C, 81.60; H, 6.51; Found C, 81.60; H, 6.49

2, 5-anhydro-3, 4-di-O-ethyl-1, 6-di-O-trityl-D-mannitol **36b**

To a solution of 2, 5-anhydro-1, 6-di-O-trityl-D-mannitol **35** (0.3g, 0.46mmol) in 5ml anhydrous THF, sodium hydride (0.028g, 1.16mmol) was added under 0 °C. The solution was stirred in ice bath with drying tube contain calcium chloride on the flask. Then, ethyl iodide was dropped into the mixture (0.229g, 1.38mmol). After 3 hours, all the solvent was removed. 10 ml water was used to dissolve the remaining sodium hydride. The water layer was extracted by ether (3* 20ml). The product was got as a reddish solid (0.051g, 45%) after all the ether is evaporated. ^{1}H NMR ($CDCl_3$ 300MHz) δ 7.3-7.7 (30H, m, Ar), 4.32 (2H, d, J = 4Hz), 4.08 (2H,d, J = 3Hz), 3.60 (4H, m), 3.44 (4H, d, J = 7Hz), 1.22 (6H, t, 5Hz); ^{13}C NMR ($CDCl_3$) δ 143.9, 128.6, 127.5, 126.7, 86.5, 85.4, 81.9, 65.0, 64.1, 29.7 ppm

2, 5-anhydro-3, 4-di-O-methyl-D-mannitol **37a**

2, 5-anhydro-3, 4-di-O-methyl-1, 6-di-O-trityl-D-mannitol **36a** (1g, 1.55mmol) was dissolved in 5 ml dichloromethane. 5% Pd/ C catalyst (0.05g 5%) and 3 drops of acetic acid were added. Hydrogen gas was applied while stirring in room temperature overnight. The Pd /C catalyst was filtered out. The filtrate was rotavapored and the solid left was

run through a flash column (Eluent 5:1 hexanes: ethyl acetate). The product is a white solid (0.277g, 93%). ^{1}H NMR ($CDCl_3$ 300MHz) δ 4.15-4.01 (2H, m), 3.80-3.63 (6H, m), 3.40 (6H,-OCH_3, s), 2.95-2.73 (2H, br, s,-OH); ^{13}C NMR ($CDCl_3$) δ 85.80 (CH), 83.21(CH), 62.70 (-CH_2OH), 57.59 (-OCH_3) ppm

2, 5-anhydro-3, 4-di-O-ethyl-D-mannitol **37b**

2, 5-anhydro-3, 4-di-O-ethyl-1, 6-di-O-trityl-D-mannitol **36b** (1g, 1.42mmol) was dissolved in 5 ml dichloromethane. 5% Pd/ C catalyst (0.05g, 5%) and 3 drops of acetic acid were added. Hydrogen gas was applied while stirring in room temperature overnight. The Pd /C catalyst was filtered out. The filtrate was rotavapored and the solid left was run through a flash column (Eluent 5:1 hexanes: ethyl acetate). The product is a white solid (0.280g, 90%). ^{1}H NMR ($CDCl_3$ 300MHz) 4.30 (2H, d, J = 4Hz), 4.08 (2H, d, J = 3Hz), 3.60-3.62 (4H, m), 3.41 (4H, d, J = 7Hz), 1.22 (6H, t, 5Hz); ^{13}C NMR ($CDCl_3$) δ 85.5, 82.9, 64.2, 60.8, 29.6 ppm

2, 5-anhydro-1, 6-dibromo-1, 6-dideoxy-3, 4-di-O-methyl-D-mannitol **38a**

The solution of 2, 5-anhydro-3, 4-di-O-methyl-D-mannitol **37a** (600mg, 3.13mmol) in 10ml anhydrous pyridine was cooled to 0 °C in ice bath. Triphenyl phosphine (2.46g, 9.38 mmol) and carbon tetrabromide (1.55g, 4.70mmol) was put into the solution. After kept in ice bath for 30 min, the reaction flask was warmed up to 75 °C and stired for another 2 hours. The reaction was cooled down and column chromatography (Eluent 4:1 hexanes: ethyl acetate) was applied to separate the product out (0.90g, 90%). ^{1}H NMR ($CDCl_3$ 300MHz) δ 4.24 (2H, t, J = 4Hz), 3.82 (2H, d, 2Hz), 3.37-3.39 (2H, m), 3.36 (6H, s); ^{13}C NMR ($CDCl_3$) δ 87.5, 83.0, 57.3, 32.2 ppm

2, 5-anhydro-1, 6-dibromo-1, 6-dideoxy-3, 4-di-O-ethyl-D-mannitol **38b**

The solution of 2, 5-anhydro-3, 4-di-O-ethyl-D-mannitol **37a** (689mg, 3.13mmol) in 10ml anhydrous pyridine was cooled to 0 °C in ice bath. Triphenyl phosphine (2.46g, 9.38 mmol) and carbon tetrabromide (1.55g, 4.70mmol) was put into the solution. After kept in ice bath for 30 min, the reaction flask was warmed up to 75 °C and stired for another 2 hours. The reaction was cooled down and column chromatography (Eluent 4:1 hexanes: ethyl acetate) was applied to separate the product out (0.98g, 90%). ^{1}H NMR ($CDCl_3$ 300MHz) δ 4.31 (2H, t, J = 4Hz), 4.0 (2H, s), 3.42-3.66 (8H, m), 1.24 (6H, J = 10 Hz); ^{13}C NMR ($CDCl_3$) δ 84.5, 83.8, 65.0, 22.1, 15.4 ppm

2, 5-anhydro-1, 6-di (diphenyl phosphenyl)-1, 6-dideoxy-3, 4-di-O-methyl-D-mannitol **39a**

Dibromide **38a** (410mg, 1.29mmol) was put into 3ml 0.5 M diphenyl phosphomium patasium THF solution. The mixture was stirred in room temperature overnight. The solvent is removed and the product (0.50g, 73%) was separated out by column chromatography (Eluent 10:1 hexanes: ethyl acetate). ^{1}H NMR ($CDCl_3$ 300MHz) δ 7.3-7.5 (20H, m, Ar), 4.34 (2H, q, J = 4Hz), 3.71(2H, d, J = 2Hz), 3.28 (6H, s), 2.35-2.55 (4H, m); ^{13}C NMR ($CDCl_3$) δ 138.2 (q), 132.8 (q), 128.6, 128.4 (d), 89.6 (d), 80.5 (d), 57.2, 33.8(d) ppm

2, 5-anhydro-1, 6-di (diphenyl phosphenyl)-1, 6-dideoxy-3, 4-di-O-ethyl-D-mannitol **39b** Dibromide **38b** (449mg, 1.29mmol) was put into 3ml 0.5 M diphenyl phosphomium patasium THF solution. The mixture was stirred in room temperature overnight. The solvent is removed and the product (0.230g, 32%) was separated out by column chromatography (Eluent 10:1 hexanes: ethyl acetate). ^{1}H NMR ($CDCl_3$ 300MHz) δ 7.3-7.5 (20H, m, Ar), 4.10 (2H, q, J = 4Hz), 3.43 (4H, m), 2.42 (4H, m), 1.18(6H, t, J = 5Hz); ^{13}C NMR ($CDCl_3$) δ 138.2 (q), 132.8 (q), 128.6 (d), 128.3 (d), 88.2 (d), 80.8 (d), 67.1, 32.9 (d), 15.3 ppm

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