THE STEREOSELECTIVE SYNTHESIS OF NITRILE AMINO ACIDS USING A CHIRAL SCHIFF BASE COMPLEX

by

Lori C. Connolly

Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
January, 2005

Your file Votre référence
ISBN: 0-494-00835-0
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ISBN: 0-494-00835-0

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Abstract

This thesis focuses on the synthesis of (*S*)- and (*R*)-Schiff base complexes of Ni^{2+}, glycine, and 2-[*N*-(*N'*-benzylprolyl)amino]benzophenone (BPB) and their use in the stereoselective synthesis of a series of nitrile amino acids, a class of non-protein amino acids. Optimized reaction conditions are presented for the alkylation of (*S*)- and (*R*)-BPB complexes with alkylcyano bromides ($Br(CH_2)_nCN$, n = 2-4). Enantiomerically enriched (*S*)- and (*R*)-nitrile amino acids (84-96% e.e.) obtained by acid hydrolysis of the alkylated complexes were recrystallized to yield enantiomerically pure nitrile amino acids (>99% e.e.). The diastereoselectivity of the alkylation reaction and enantiomeric purity of the nitrile amino acids was determined by HPLC analysis of diastereomeric, fluorescent derivatives. Optical rotations were used to assign stereochemical configurations.

The corresponding (*S*)- and (*R*)-diamino acids were produced in a single step by reducing the nitrile group of the nitrile amino acids.

List of Abbreviations and Symbols Used

Ac	acetyl
APCI	atmospheric pressure chemical ionization
app t	apparent triplet
BPB	2-[*N*-(*N'*-benzylprolyl)aminobenzophenone
Cbz	benzyloxycarbonyl
CID	collision induced dissociation
COSY	correlation spectroscopy
d	doublet
dd	doublet of doublets
DCC	dicyclohexylcarbodiimide
DMF	*N,N*-dimethylformamide
d.e.	diastereomeric excess
e.e.	enantiomeric excess
ESI	electrospray ionization
Et	ethyl
EtOH	ethanol
h	hour
HPLC	high performance liquid chromatography
HMBC	heteronuclear multiple bond correlation
HSQC	heteronuclear single quantum correlation
Hz	hertz

^{i}PrOH	isopropanol
J	coupling constant (Hz)
JMOD	*J*-modulated spin echo experiment
m	multiplet
Me	methyl
MeOH	methanol
MeONa	sodium methoxide
min	minute
mp	melting point
MS	mass spectrometry
m/z	mass-to-charge ratio
NMR	nuclear magnetic resonance
NOESY	nuclear overhauser effect spectroscopy
NPS	*ortho*-nitrophenyl sulphenyl
OPA	*ortho*-phthaldehyde
ORD	optical rotary dispersion
s	singlet
t	triplet
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane
Tosyl	*para*-toluenesulphonyl
Trityl	triphenylmethyl

Acknowledgements

First and foremost, I would like to thank my supervisor, Dr. Robert White, for his never-ending guidance and encouragement throughout this work. Thanks are also extended to past and present members of the White lab group for their support and company over the past few years.

In addition, I would like to extend my thanks and appreciation to the staff of the Atlantic Region Magnetic Resonance Center for their helpful suggestions and advice with NMR experiments and also my committee members, Dr. Bearne, Dr. Burnell, and Dr. Thompson for taking time to read my thesis. I would also like to express my gratitude to Xiao Feng and Dr. White for operation of the mass spectrometer as well as Dr. Thompson for allowing me to access the polarimeter through her laboratory.

Many thanks are also extended to Dalhousie University and NSERC for financial support. Finally, I would like to thank my family and friends for their support over these last few years. Most importantly, I would like to thank Jeff and my parents for their patience, never-ending support, and for just understanding.

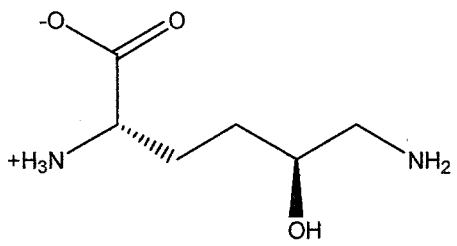
Chapter 1.0 - Introduction

1.1 – Protein/Non-protein Amino Acids

Amino acids are commonly known as the building blocks of proteins. In living organisms, they are found in their free form or bound to one another via amide linkages in peptides and proteins. Currently, there are 20 amino acids encoded by DNA and commonly found in proteins. The structures of protein amino acids were determined after the characterization of glycine and leucine in 1820 by Braconnet. Threonine was the last of these 20 to be isolated and characterized in 1925.[1] Structurally, these amino acids typically consist of a tetrahedral carbon atom which is attached to an amino group, a carboxyl group, a hydrogen atom, and a side chain. The structural diversity of the side chain makes each amino acid unique.

In addition to the 20 common, DNA-encoded protein amino acids, there are others that occur less often in proteins and those that are not found in proteins. For instance, hydroxylysine (**1**) and hydroxyproline (**2**) are mainly found in collagen and gelatin proteins, and aminoadipic acid (**3**) is mainly found in proteins isolated from corn.[2] Naturally occurring amino acids that do not occur in proteins are classified as non-protein amino acids. These amino acids typically form as end products of secondary metabolism, as intermediates in metabolic pathways, or as products from the metabolism or detoxification of foreign compounds. The largest producers of these amino acids are plants and micro-organisms. In higher plants, these non-protein amino acids are present in their free form, or linked as peptides to glutamic acid.

It is interesting that a large number of the non-protein amino acids in plants possess aliphatic chains, usually no longer than 6 carbons, and similar in structure to protein amino acids. The non-protein amino acids from micro-organisms are often more complex in structure, occurring as both D- and L-amino acids, and often linked to other amino acids by peptide bonds. Many of these peptides have antibiotic properties.[3]



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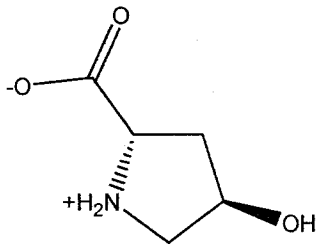

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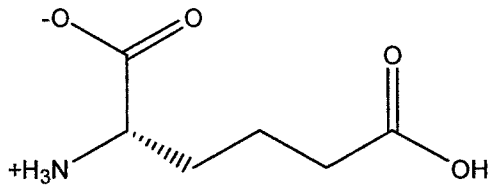

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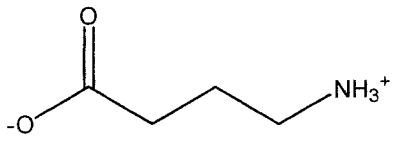
Despite the fact that the non-protein amino acids do not occur in proteins, they may still possess biological importance. For instance, γ-aminobutyric acid (GABA, **4**), the decarboxylation product of glutamic acid, is a potent neurotransmitter, and ornithine (**5**), homocysteine (**6**), and homoserine (**7**) have been recognized as key metabolic intermediates.[2]



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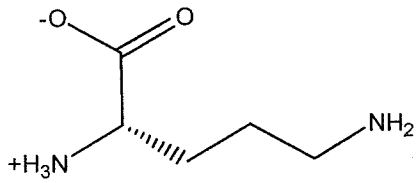

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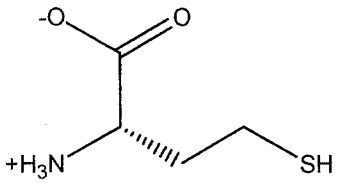

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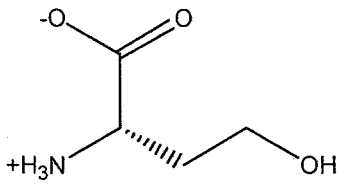

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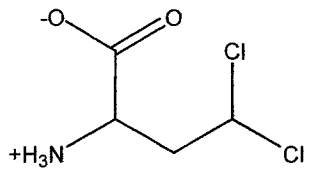
Functional groups not typically associated with biological molecules are frequently found in the side chains of non-protein amino acids. For example, a dichloromethyl group is present in armentomycin (**8**), a low molecular weight amino acid possessing antibiotic and anticancer properties. Armentomycin is produced by *Streptomyces armentosus* var. *armentosus*.[4,5] Phosphinothricin (**9**) is a phosphorus-containing compound that possess herbicidal ctivities. It is a component of bialaphos, a tripeptide metabolite of *Streptomyces hygroscopicus*.[6] A diazo group is found in 6-diazo-5-oxo-L-norleucine (**10**), which is an antitumor antibiotic isolated from *Streptomyces*.[7,8]

This thesis focuses on nitrile amino acids as a specific group of non-protein amino acids. In these amino acids (**11**), the nitrile group is located at the end of an aliphatic side chain of varied lengths. Biological effects of the shorter-chain nitrile amino acids have been documented,[9-12] while fewer investigations have been carried out on the longer-chain amino acids. This thesis presents a

method for the stereoselective synthesis of these non-protein amino acids and demonstrates their utility as intermediates for the synthesis of other amino acids.



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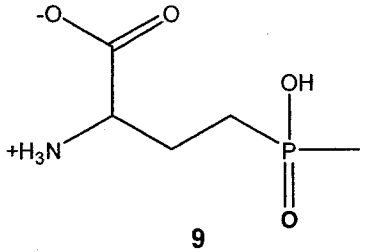

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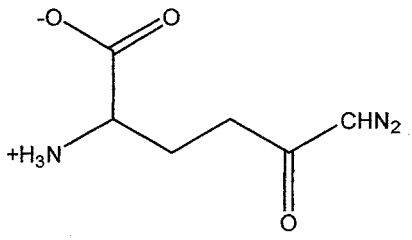

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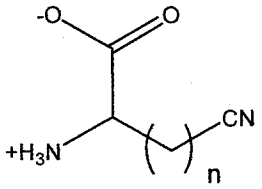

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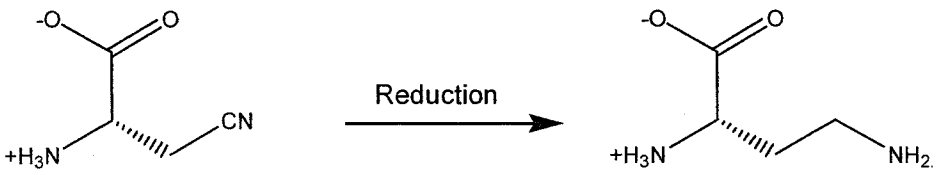
1.2- Nitrile Amino Acids

1.2.1 - Natural occurrence and biosynthesis

3-Cyanoalanine (**12**) was discovered as a naturally occurring amino acid in the early 1960's when it was isolated from a plant species, *Vicia sativa*.[9] It has been found in other common vetch species, such as *Vicia angustifolia,*[9] and also in legumes possessing neurotoxic properties.[13]

The neurotoxic properties of plants led to the search and discovery of several other amino acids structurally related to 3-cyanoalanine. For instance, 2,4-diaminobutyric acid (**13**), a possible reduction product of 3-cyanoalanine (Scheme 1), was found as a neurotoxin in *Lathyrus latifolius*.[9]

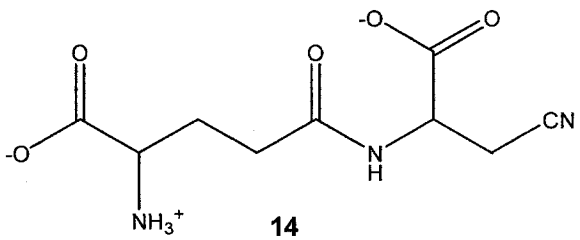


12 **13**

Scheme 1: Possible biosynthesis of (*S*)-2,4-diaminobutyric acid (**13**) by reduction of (*S*)-3-cyanoalanine (**12**).

During feeding experiments to determine the relationship between 3-cyanoalanine and asparagine, a bound form of 3-cyanoalanine was first recognized. This species was later characterized by reduction and hydrolysis experiments[14] as the acidic dipeptide, γ-glutamyl-3-cyanoalanine[15] (**14**), which contains a glutamic acid residue linked through its carboxyl group at C5 to the amino group of 3-cyanoalanine.[14]

In the same vetch and *Lathyrus* species in which 3-cyanoalanine and γ-glutamyl-3-cyanoalanine were discovered, asparagine (**15**) was also found. Because of the structural similarities between 3-cyanoalanine (**12**) and asparagine, it was proposed that 3-cyanoalanine and γ-glutamyl-3-cyanoalanine (**14**) are precursors of asparagine.[16] This was investigated using labeling experiments. [4-^{14}C]-3-Cyanoalanine was fed to seedlings of *Vicia villosa, Lathyrus odoratus*, and *Lathyrus sylvestris W.,* and γ-glutamyl-[4-^{14}C]-3-cyanoalanine was administered to *Lathyrus odoratus*. In all cases, asparagine was formed with the label appearing in the amide carbon. This suggested a direct relationship between the nitrile and the amide carbon of asparagine.[16]



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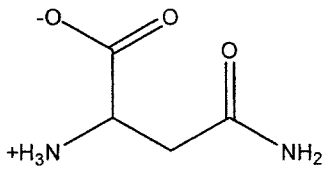

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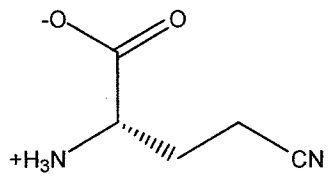
In addition to naturally occurring in plant species, 3-cyanoalanine (**12**) is also produced by micro-organisms. For instance, 3-cyanoalanine forms when cyanide is administered in the presence of serine to *Chromobacterium violaceum* (strain 9).[17] When cyanide is not available as a component, the source of cyanide in this bacterium is believed to be glycine. A labeling experiment conducted with [2-^{14}C]glycine supported this hypothesis as label appeared in the cyano carbon. Label also appeared in the carbon chain of 3-cyanoalanine (in positions 2 & 3) suggesting that glycine provides both the cyano group and a cyanide accepter molecule, such as serine.[17]

In a different strain of *C. violaceum* (D 341), 4-cyano-2-aminobutyric acid (**16**) was formed in addition to 3-cyanoalanine (**12**).[18] The identity of this nitrile amino acid was confirmed by reducing it to ornithine with Na-MeOH-NH_3 and hydrating it to glutamine using HBr / acetic acid. Its formation is explained as the biosynthetic product of cyanide and aspartic acid where cyanide again became the cyano group and aspartic acid supplied C1 to C4. Interestingly, both amino acid nitriles form when this bacterium is incubated with cyanide and either glutamic acid, serine, or threonine. However, when L-asparagine or aspartic acid are administered with cyanide, (*S*)-4-cyano-2-aminobutyric acid (**16**) is the sole product.[18]



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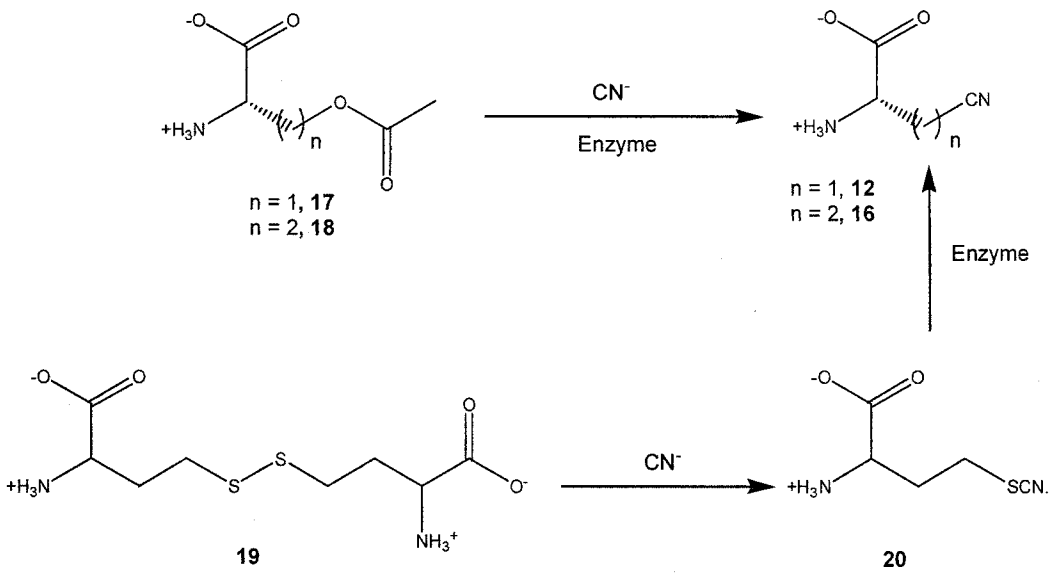
Enzymes catalyzing the formation of 3-cyanoalanine (**12**) and 4-cyano-2-aminobutyric acid (**16**) have been found in *Bacillus stearothermophilus* CN3. 3-Cyano-L-alanine synthase and 4-cyano-2-aminobutyric acid synthase catalyze the synthesis of 3-cyanoalanine and 4-cyano-2-aminobutyric acid from *O*-acetyl-L-serine (**17**) and *O*-acetyl-L-homoserine (**18**), respectively (Scheme 2).[19] Another enzyme (with the same name) is also known to produce 4-cyano-2-aminobutyric acid from homocystine (**19**) and cyanide.[20] The two substances react nonenzymatically to form 4-thiocyano-2-aminobutyric acid (**20**), which reacts with a second cyanide ion in the enzyme-catalyzed reaction to yield 4-cyano-2-L-aminobutyric acid (Scheme 2).

1.2.2 - Biological effects of cyano and diamino acids

3-Cyanonalanine was identified as one of the neurotoxins in lathryus species for studies in rats.[9] Upon administration to rats in the early 1960's, the rats developed several of the characteristics (reflex irritability,

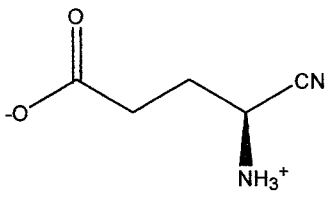


Scheme 2: Two routes for the enzymatic synthesis of 3-cyanoalanine (**12**) and 4-cyano-2-aminobutyric acid (**16**).

weakness, spasticity, rigidity of leg muscles, and in some cases death) observed in humans (often males) after consumption of lathyrus. At three times the dosage level of 3-cyano-L-alanine, 3-cyano-D-alanine also had neurotoxic properties.[9] 2,4-Diaminobutryic acid (**13**) and 4-cyano-2-aminobutyric acid (**16**) were also tested for neurotoxic properties. While L-2,4-diaminobutyric acid is neurotoxic, 4-cyano-2-aminobutyric acid is not.[9]

In addition to its neurotoxic properties, L-2,4-diaminobutyric acid recently has been shown to exhibit antitumor properties.[10] Transport of this amino acid into hepatoma cells leads to hyperosmosis and cell lysis. L-2,4-Diaminobutyric acid was also examined for its ability to inhibit hepatoma cell growth in rats. Experiments showed that the effects of this amino acid were time and dose dependent.

In addition to these properties, 3-cyanoalanine (**12**) and 4-cyano-2-aminobutyric acid (**16**) and their analogues have been investigated as inhibitors of glutamate decarboxylase.[11] This enzyme is present in mammals (primarily in nervous tissue) and modulates nervous tissue by producing γ-aminobutyric acid (the decarboxylation product). While 4-cyano-2-aminobutyric acid and 3-cyanoalanine only slightly inhibited the enzyme, their isomers proved to be significant inhibitors. The most effective nitrile amino acids studied were L-4-cyano-4-aminobutyric acid (**21**) and L-3-cyano-3-alanine (**22**). These results suggested that the α-cyanoamino group was of great importance in inhibiting this enzyme. Furthermore, (*S*)-4-cyano-2-aminobutyric acid is a substructural unit in the cyclic heptadepsipeptide (HUN), which is a potent inhibitor of cell adhesion molecule expression that exhibits anti-inflammatory properties.[12]



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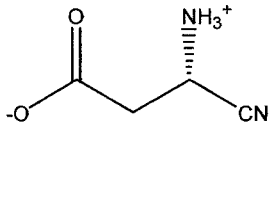

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1.2.3 – IR Probes of Protein Structure

More recently, in another biological application, nitrile amino acids have been recognized as local environment markers to monitor conformation, folding, and binding in proteins.[21] These amino acids were used because of the properties of the CN group. It has a characteristic stretching vibration that is influenced by interactions with other molecules in its environment, making it a

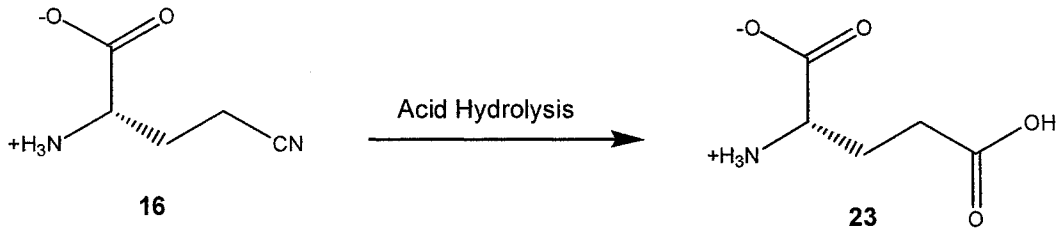
useful IR probe in proteins.[22] In the past, fluorophores (i.e., dyes) were used for similar experiments. However, the dye molecules are large, and they perturbed the native structure of the protein. On the other hand, a nitrile group is a small substituent on an amino acid side chain and will cause little or no perturbation to the protein. In addition, the CN group has an intermediate polarity and should be easily accommodated in the interior of a protein.[21]

Several groups have adopted the CN stretching vibration as a useful tool for studying biological processes. Caughey and coworkers[23] discovered that cyanide could be used as an IR probe in studying ligand binding sites in heme proteins, whereas Andrews and Boxer[24,25] showed that the vibrational stark effects of CN could be used as local probes of electric fields in chemical and biological molecules. Huang and coworkers,[21] as mentioned above, used nitrile amino acids as IR environment probes for studying biological processes such as protein folding and binding.[22] The most common nitrile amino acids employed for this work were 3-cyanoalanine (**12**) and cyanophenylalanine.

1.2.4 – Nitrile amino acids as synthetic intermediates

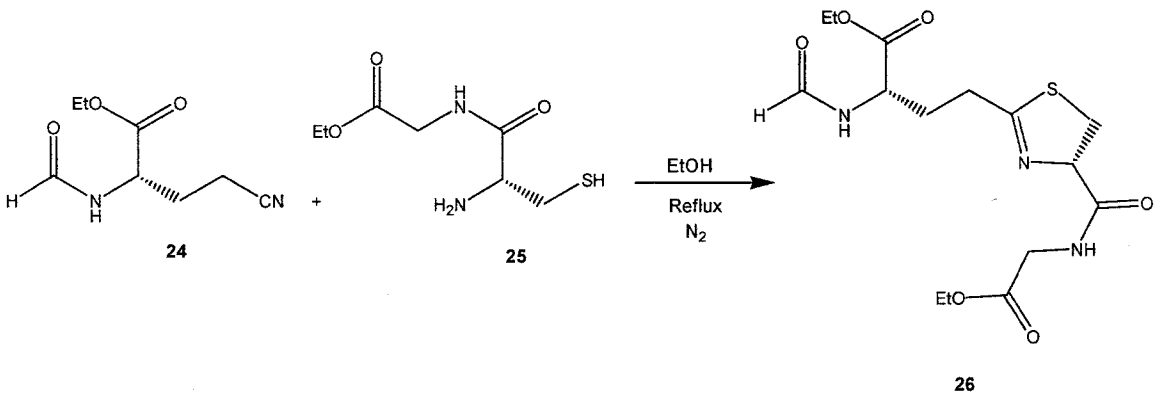
The nitrile functional group is readily converted into many other functional groups by hydrolysis, reduction, or reaction with nucleophiles. Because of this versatility, nitrile amino acids have been valuable intermediates in the syntheses of other amino acids. Examples illustrating the synthetic utility of 4-cyano-2-aminobutyric acid and 5-cyanonorvaline are described below.

Acid hydrolysis of the nitrile group in [5-^{11}C]-(*S*)-4-cyano-2-aminobutyric acid produced in the enzyme-catalyzed reaction (Section 1.1.1) yields [5-^{11}C]-(*S*)-glutamic acid (**23**, Scheme 3).[19] This ^{11}C-labeled product can be used in positron emission tomography (PET), a technique that has received great attention in the field of medical diagnosis. The ^{11}C-labeled compounds have short half lives, and it is important that reactions be done quickly. In this example, the use of an enzyme-catalyzed reaction allows the labeled cyanide to react with an unprotected amino acid, eliminating the need for subsequent deprotection steps.



Scheme 3: Production of [5-^{11}C]-(*S*)-glutamic acid (**23**) by acid hydrolysis of (*S*)-4-cyano-2-aminobutyric acid (**16**).

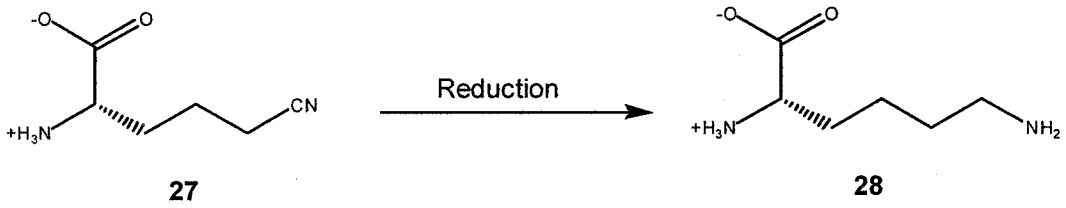
The use of 4-cyano-2-aminobutyric acid in producing thiazolines by condensation with cysteine derivatives (Scheme 4) was illustrated by Ozawa and co-workers[26] in the synthesis of glutathione (GSH), a tripeptide consisting of L-Glu ; L-Cys ; Gly. The direct condensation of *N*-formyl-L-4-cyano-2-aminobutyric acid (**24**) ester with a cysteine-glycine derivative (**25**) yielded the thiazoline compound (**26**) in 62.5% yield.



Scheme 4: Synthesis of a thiazoline by condensation of a cysteine derivative with a 4-cyano-2-aminobutyric acid derivative.

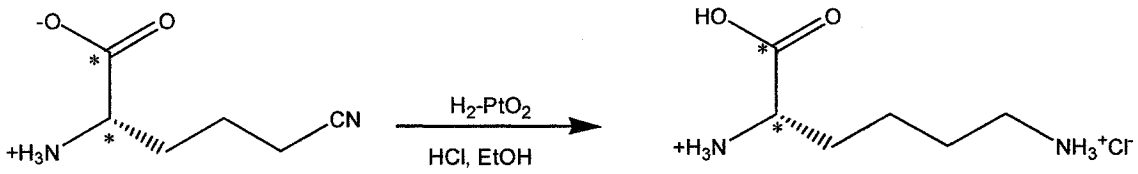
Reduction of the nitrile group to a primary amino group is another important synthetic transformation (Scheme 5). Several groups have used 5-cyanonorvaline (**27**) as an intermediate in syntheses of isotopically labeled lysines (**28**).[27-29]

For instance, Havranek and Mezo[27] produced [6-^{3}H]-(*S*)-lysine monohydrochloride from 5-cyanonorvaline by hydrogenation of the nitrile in acetic acid in the presence of a PtO_2 catalyst. The ^{3}H-label was introduced after the reduction to lysine by catalytic tritiation with carrier-free tritium.

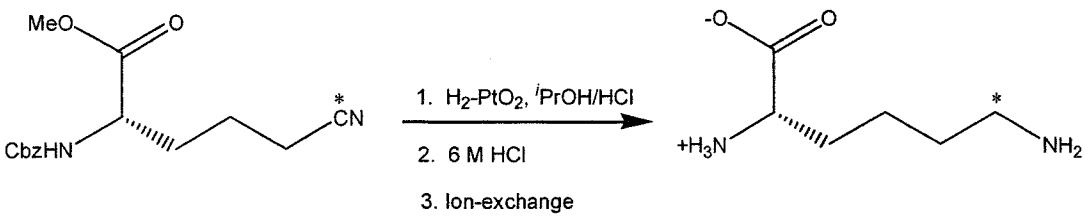


Scheme 5: Production of (*S*)-lysine (**28**) from (*S*)-5-cyanonorvaline (**27**).

Two other groups[28,29] have also used this nitrile amino acid to generate lysine labeled with ^{13}C-nuclei. Yuan and Ajami[28] synthesized [1,2-$^{13}C_2$]-(*S*)-5-cyanonorvaline (see next section) for conversion to doubly labeled (*S*)-lysine (Scheme 6). Here, the free nitrile amino acid was hydrogenated over PtO_2 in a solution of 5 M HCl / EtOH to produce the monohydrochloride salt of [1,2-$^{13}C_2$]-lysine (Scheme 6). On the other hand, Sutherland and Willis[29] used a protected form of the nitrile amino acid. An *N*-benzyloxycarbonyl (Cbz) group was used to protect the amino group of [6-^{13}C]-(*S*)-5-cyanonorvaline methyl ester (see next section for synthesis). Reduction of the nitrile was afforded by catalytic hydrogenation in the presence of a PtO_2 catalyst and hydrolysis of the methyl ester in 6 M HCl yielded the free ^{13}C-labeled amino acid (Scheme 7). By using this nitrile amino acid as an intermediate, label can be incorporated near the end of the synthetic scheme and lysine can be produced in one or two steps from the nitrile amino acid.



Scheme 6: Yuan and Ajami's use of [1,2-$^{13}C_2$]-(*S*)-5-cyanonorvaline for the production of $^{13}C_2$-(*S*)-lysine.



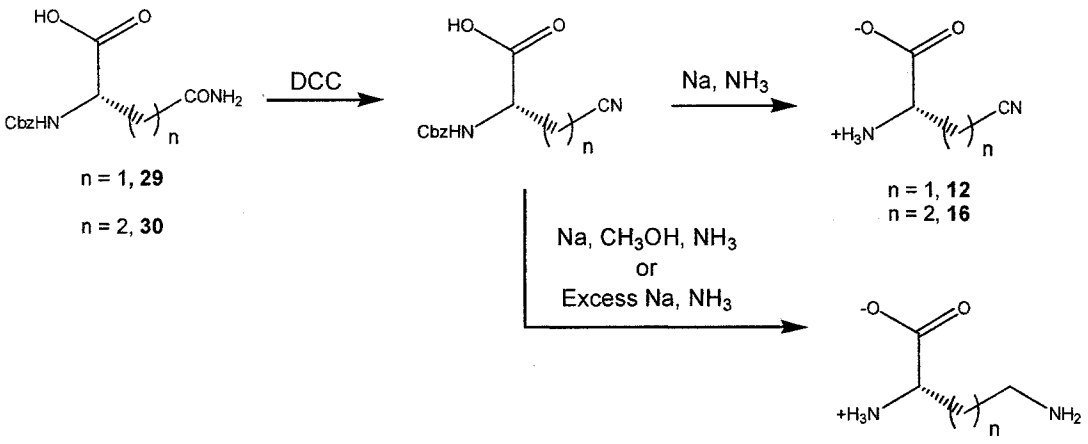
Scheme 7: Sutherland and Willis's use of [6-^{13}C]-(*S*)-5-cyanonorvaline to produce [6-^{13}C]-(*S*)-lysine.

1.2.5 - Literature syntheses of nitrile amino acids

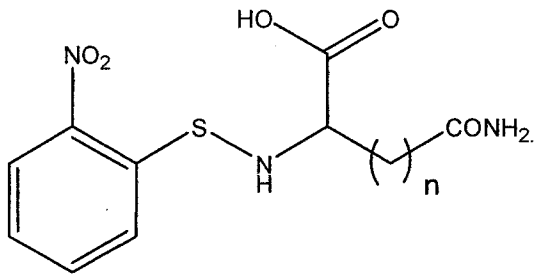
Syntheses of 3-cyanoalanine (**12**)[30] and 4-cyano-2-aminobutyric acid (**16**)[30] dating back as far as the 1950's used carbobenzoxy-L-asparagine (**29**) and carbobenzoxy-L-glutamine (**30**), respectively, as starting materials (Scheme 8). Dehydration of the amide group using *N,N*-dicyclohexylcarbodiimide (DCC) afforded the desired nitrile, and subsequent treatment with sodium in liquid ammonia under anhydrous conditions deprotected the amino acid. If an excess of sodium was employed, or if MeOH was added to the sodium / ammonia mixture, or, if conditions were not perfectly anhydrous, the nitrile was reduced to form the diamino acid, which in some instances could be an inconvenient side product.[30]

The harsh deprotecting conditions required to remove the carbobenzoxy (Cbz) group initially used by Ressler[30] (Na in liquid NH_3) led, a few years later, to the evaluation of other amino protecting groups for this synthetic protocol. The *N*-trityl group used by Liberek, Buczel, and Grzonka[31] gave low yields (< 40%) during the protection process. However, Chimiak and Pastuszak[32] found *o*-nitrophenylsulphenyl (NPS) groups to be advantageous. The NPS-amino acids (**31**) were easily prepared in excellent yields (80-94%) by reaction of the amino

acid with *o*-NPS-chloride in an aqueous alkaline solution. The NPS group was easily removed under mild conditions such as thioacetamide in acetic acid / MeOH.



Scheme 8: Preparation of (*S*)-3-cyanoalanine (**12**) and (*S*)-4-cyano-2-aminobutyric acid (**16**).



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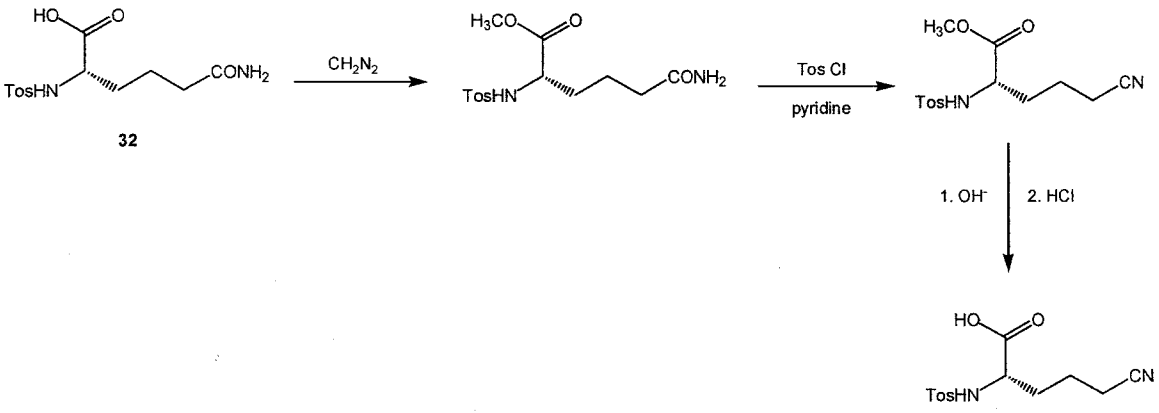
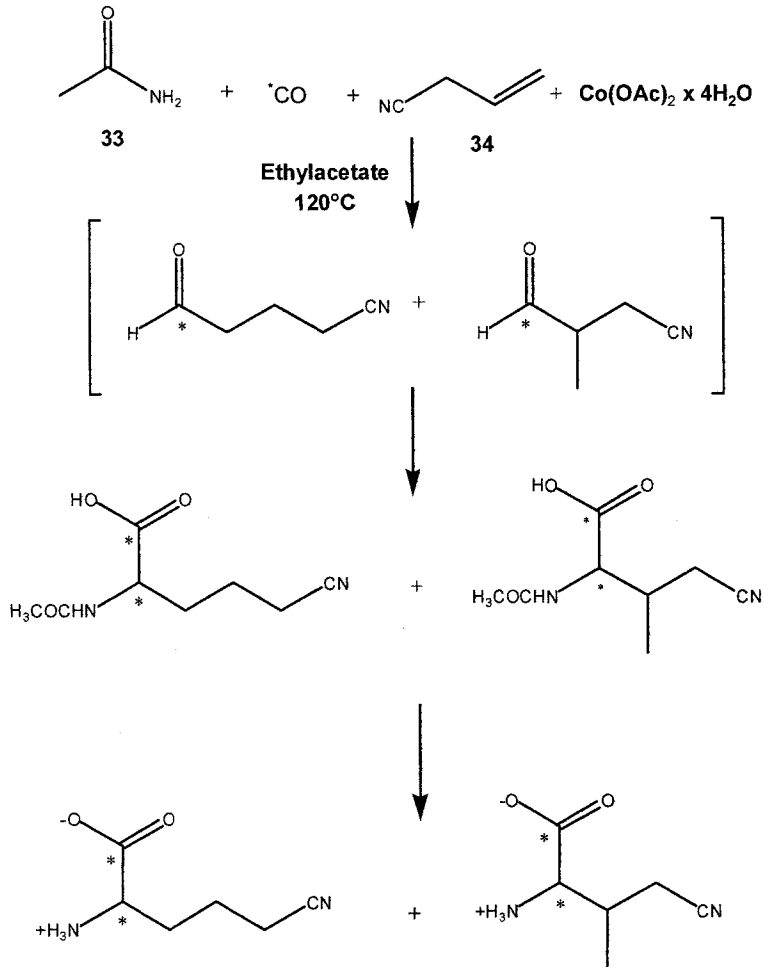
Three years later, Havranek and Mezo[27] also synthesized (*S*)-4-cyano-2-aminobutyric acid (**16**) and (*S*)-5-cyanonorvaline (**27**) by dehydration of an amide. However, they used a tosyl amino protecting group and a different dehydrating reagent. Their synthesis of 5-cyanonorvaline began with tosyl-L-homoglutamine (**32**) (Scheme 9), which was produced from tosyl-L-α-aminoadipic acid. The

amide (**32**) was methylated using diazomethane followed by dehydration to the nitrile in pyridine using tosyl chloride. To obtain the nitrile amino acid, ester hydrolysis was conducted to yield the *N*-tosyl-cyano amino acid. Subsequent cleavage of the tosyl group proved difficult, since methods previously employed (i.e. Na in liquid NH_3) attacked the nitrile group. Therefore, electrolytic reduction was employed, producing free 5-cyanonorvaline in 63% yield.



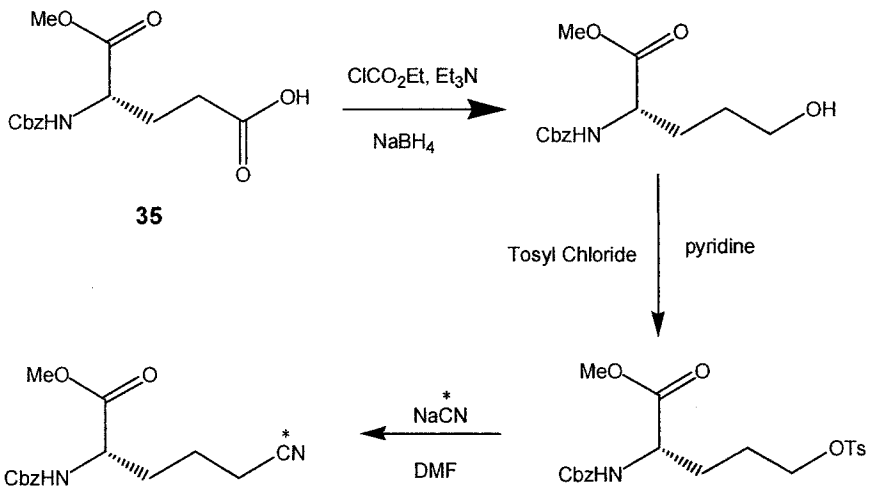
Scheme 9: Production of *N*-tosyl-(*S*)-5-cyanonorvaline.

Several groups[28,29] have also synthesized 5-cyanonorvaline enroute to producing isotopically labeled lysines. Yuan and Ajami[23] synthesized [1,2-$^{13}C_2$]-5-cyanonorvaline (Scheme 10) starting from 3-cyanopropene (**34**). Reaction of **34** with acetamide (**33**) in the presence of ^{13}C-labeled carbon monoxide and cobalt (II) acetate afforded a mixture of the *N*-protected ^{13}C-labeled nitrile amino acid and its isomer (ratio 7:3) in 55% yield. The free nitrile amino acid was obtained by deprotection and recrystallization from H_2O/EtOH. This synthesis incorporates label from carbon monoxide in the initial step and involves several steps in producing the labeled nitrile amino acid.



Scheme 10: Synthesis of [1,2-$^{13}C_2$]-5-cyanonorvaline.

Several years later, Sutherland and Willis[29] also synthesized a protected form of this nitrile amino acid (Scheme 11) for the purpose of producing [6-^{13}C]-lysine. Their synthesis began by reducing the carboxylic acid group in methyl *N*-benzyloxycarbonyl-L-glutamate (**35**) to an alcohol. The alcohol was then activated with *p*-toluenesulfonyl chloride and displaced by ^{13}C-labeled sodium cyanide in DMF at room temperature. By incorporating the label near the end of the synthesis, Sutherland and Willis avoided the loss of label in subsequent reaction steps.

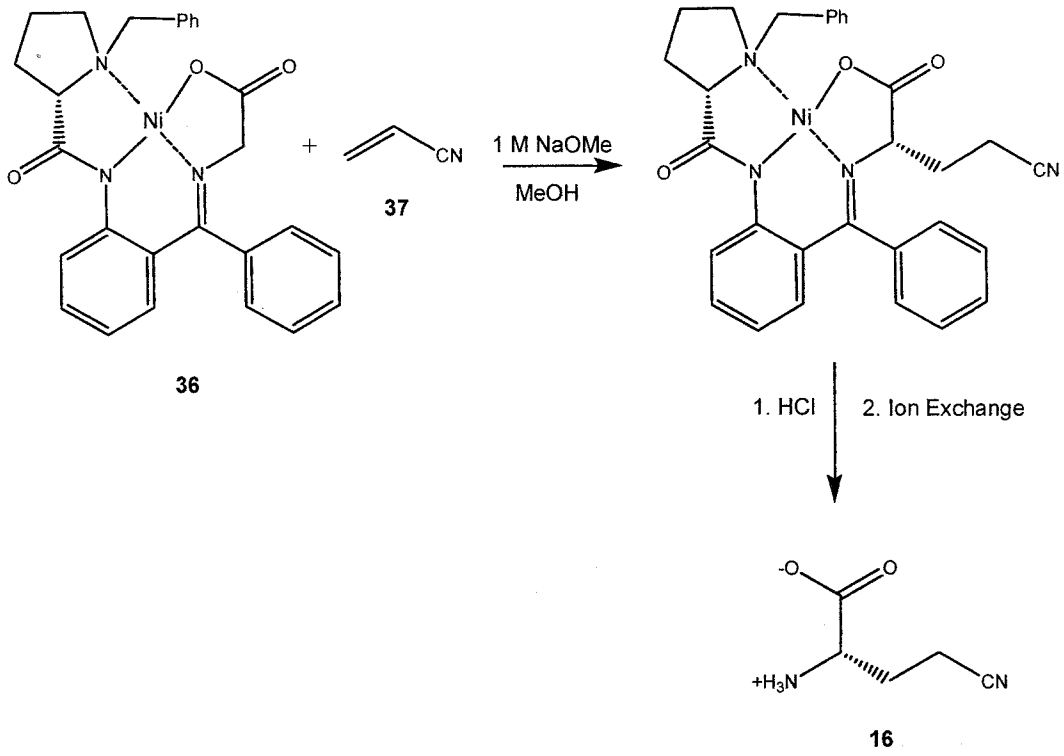


Scheme 11: Preparation of *N*-Cbz-[6-^{13}C]-5-cyanonorvaline.

In the mid 1980's Belokon[33] synthesized (*S*)-4-cyano-2-aminobutyric acid (**16**) using a Ni Schiff base complex (BPB complex, **36**), which acts as a protecting group and chiral auxiliary. In 1 M NaOMe in MeOH the Schiff base complex reacted with acrylonitrile (**37**) (Scheme 12) in a Michael-type addition. Under basic conditions, the conjugate base of the Schiff base complex (**36**) acts as a nucleophile and adds to acrylonitrile, the electrophile. Hydrolysis in mild aqueous acid liberates the free amino acid from the resulting alkylated complex. The free amino acid was isolated by ion-exchange chromatography in 75% yield.

1.2.6 – Disadvantages and advantages of literature syntheses

The preparation of nitrile amino acids by amide dehydration[27,30] involved protecting and deprotecting the amino acid functional groups often using harsh conditions (e.g. Na in liquid NH_3). While the protein amino acids (*S*)-asparagine



Scheme 12: Synthesis of (*S*)-4-cyano-2-aminobutyric acid (**16**) using Belokon's Schiff base complex (**36**).

and (*S*)-glutamine are readily available, other amino acid amides are less available as starting materials. The synthesis of other amino acid amides would add several steps reducing the attractiveness of a dehydration strategy.

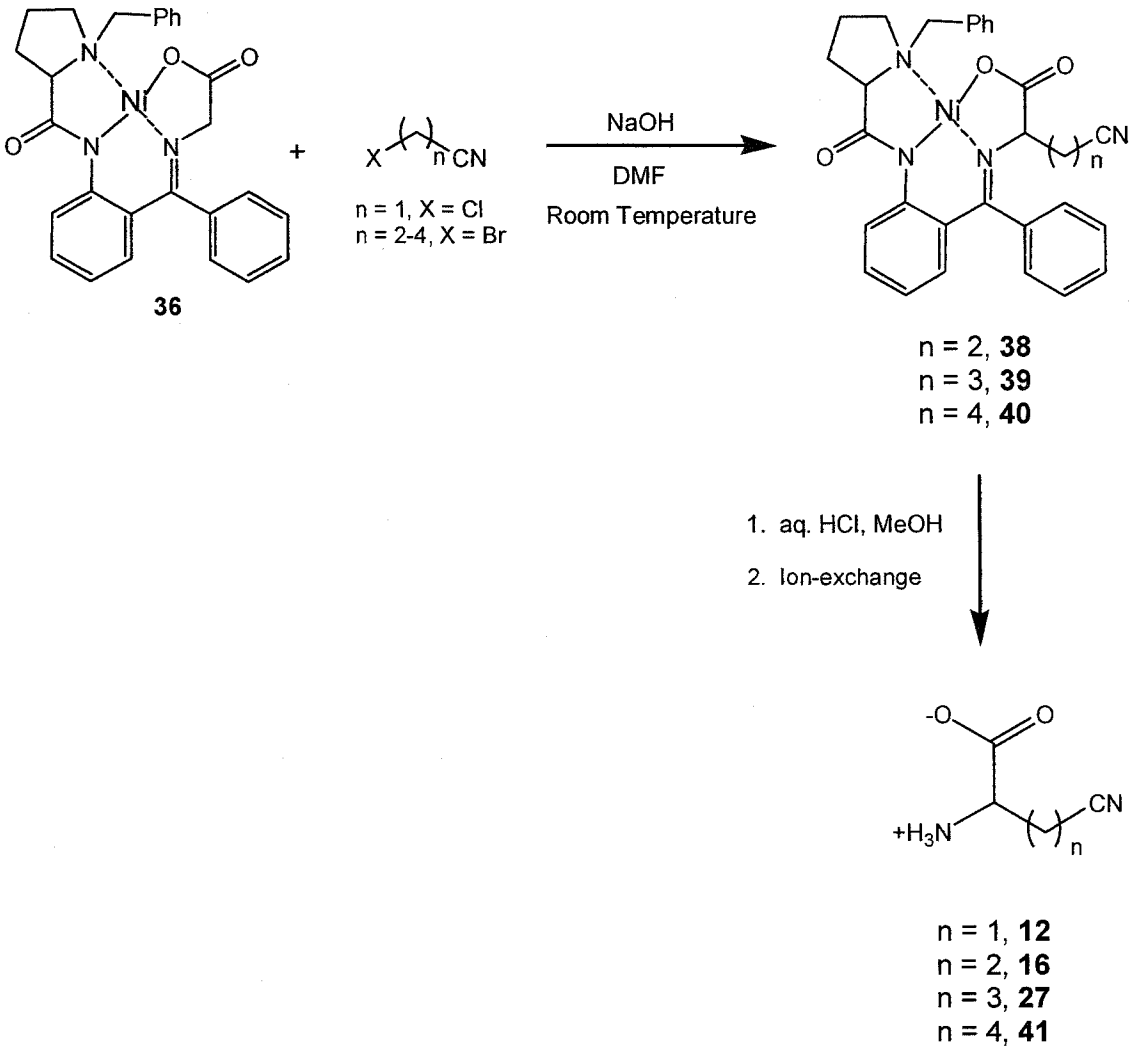
The multi-step sequences used to prepare 5-cyanonorvaline enroute to ^{13}C-labeled lysines[28,29] (Scheme 10 and 11) were designed to yield a specific target. The synthesis of a series of nitrile amino acids of varying chain lengths would require the use of different starting materials and repetition of the entire synthetic sequence. These past procedures are not flexible in allowing different amino acids to be produced by the same method.

Belokon's method for the synthesis of nitrile amino acids, however, is inherently more flexible than other literature routes to nitrile amino acids.[27-30] Belokon's synthesis of (*S*)-4-cyano-2-aminobutyric acid[33] showed that the nitrile functional group was stable under the basic conditions needed to deprotonate the BPB complex. However, his choice of acrylonitrile and a Michael addition limits this approach to the synthesis of amino acids with a single chain length. Alternatively, the choice of an alkylcyano halide, $X(CH_2)_nCN$ and alkylation of the BPB complex (Section 1.2.2) provides a flexible method for the synthesis of the target series of nitrile amino acids, varying in chain length.

In Belokon's approach, the chiral BPB ligand used to make the complex (Section 1.2.1) serves as both a chiral auxiliary and a protecting group for both the amino and carboxyl groups of the glycine starting material. No other amino acid protecting groups are required. After alkylation, the free amino acid is readily obtained by acid hydrolysis. In this thesis, this method will be used for the synthesis of (*S*)- and (*R*)-nitrile amino acids with different side-chain lengths (Scheme 13).

1.3 - Belokon's Complex

Belokon and co-workers[34,35] developed a general method for the production of α-amino acids that relied on the use of readily available chemicals and ambient reaction temperatures. By combining a chiral reagent, 2-[*N*-(*N'*-benzylprolyl)amino]benzophenone (BPB, **42**) with glycine (**43**) and Ni^{2+}, a Schiff



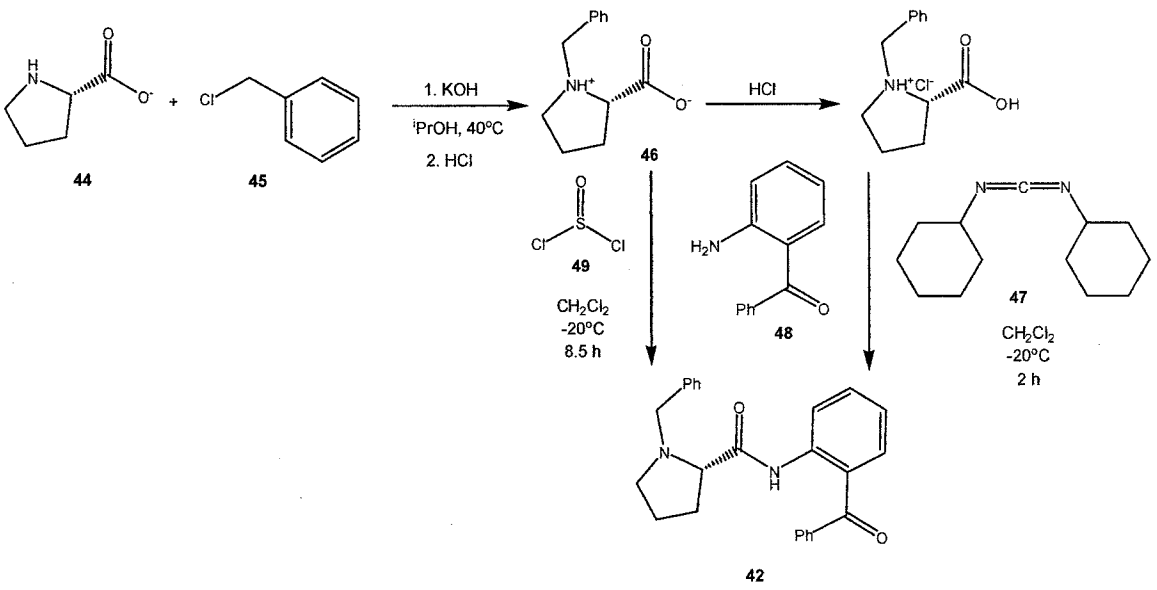
Scheme 13: Alkylation of BPB complex (**36**) with alkylcyano bromides (n = 1-4).

base complex formed (BPB complex). This BPB complex (**36**) has been used in numerous alkylation reactions yielding a variety of amino acids. In addition, the alkylated complex is easily hydrolyzed in aqueous acid to the final free amino acid. The chiral ligand reagent is also regenerated by hydrolysis in good yields (>90%) and can be used several times over without loss of chiral purity.[35]

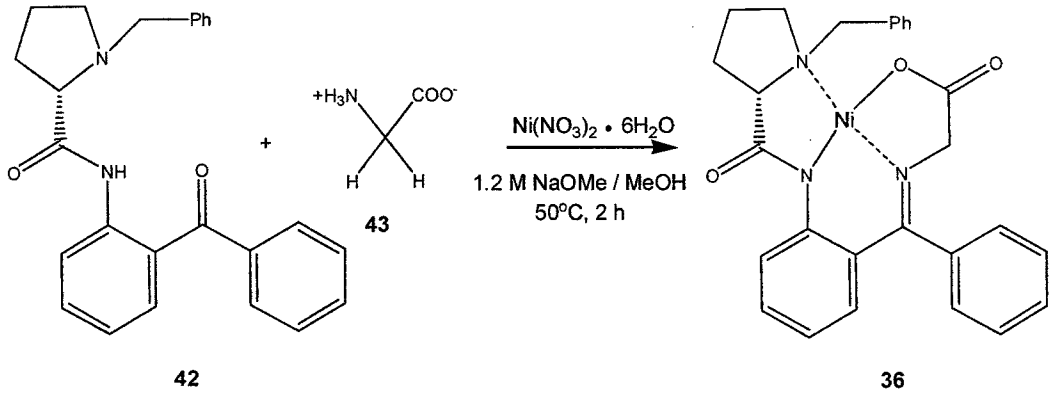
1.3.1 – Synthesis of Belokon's Schiff base complex

Literature syntheses of the chiral ligand auxiliary (BPB) begins with inexpensive L-proline (or (*S*)-proline, **44**) that is coupled to benzyl chloride (**45**) under basic conditions to yield *N*-benzyl-(*S*)-proline (**46**). In Belokon's[36] original synthesis (Scheme 14) of the BPB ligand, *N*-benzylproline was converted to the hydrochloride salt by treatment with HCl, and the carboxyl group of this species was activated using dicyclohexylcarbodiimide (DCC, **47**). The activated species reacted with 2-aminobenzophenone (**48**) to form (*S*)-BPB ligand (**42**). This synthetic protocol was later revised by Belokon[37] since it was not ideal for large scale production of BPB ligand due to formation of a side product, dicyclohexylurea, which was difficult to remove. Therefore, Belokon[37] employed $SOCl_2$ (**49**) to activate the carboxyl group of *N*-benzylproline (**46**) so it could react with 2-aminobenzophenone and form the BPB ligand (Scheme 14). This method is advantageous because there is no unwanted side product to remove, and reactions can be conducted on larger scales (e.g. 1 kg).

The chiral BPB ligand (**42**) is then combined with glycine (**43**) and a source of Ni^{2+} ion (usually $Ni(NO_3)_2 \cdot 6H_2O$) under basic conditions to produce the (*S*)-BPB complex (**36**) (Scheme 15). Originally, Belokon[36] employed a five-fold excess of glycine to BPB ligand and a two-fold excess of $Ni(NO_3)_2 \cdot 6H_2O$ to BPB ligand. This excess was used to drive the equilibrium towards complex formation but also led to excess Ni^{2+} remaining in the aqueous waste after work-up. This posed potential environmental hazards and prompted Nadvornik and Popkov[38] to develop a new, "greener" synthesis of the BPB complex.



Scheme 14: Synthesis of (*S*)-BPB ligand (**42**) using DCC (**47**) and $SOCl_2$ (**49**).



Scheme 15: Synthesis of Belokon's (*S*)-BPB complex (**36**).

The new synthesis[38] used only a two-fold excess of glycine to decrease the amount of Ni-chelating amino acid in the aqueous waste and near stoichiometirc amounts of $Ni(NO_3)_2 \cdot 6H_2O$. Three ratios of $Ni(NO_3)_2 \cdot 6H_2O$ to BPB ligand were studied (1.05, 1.2, and 2) and all gave yields of BPB complex

>70%. The reaction conducted using a 1.2 ratio of $Ni(NO_3)_2 \cdot 6H_2O$ to BPB ligand gave a yield of 88%.

All nickel-BPB complexes are red crystalline solids, which are easily purified by recrystallizaton or silica gel chromatography. The conformation of the complex is square pyramidal with minor distortions, and the charge of the central metal ion is neutralized by the ionized carboxyl group and ionized amide group from glycine (**43**) and the BPB ligand (**42**), respectively. [34,35] One of the most important properties of this complex is that it can be easily hydrolyzed in weak acid to liberate the free amino acid and chiral BPB ligand. [34,35]

The synthesis of Belokon's complex is not only limited to glycine, BPB, and Ni^{2+}. Belokon has also produced complexes using alanine[39] and serine[36] as amino acids and has used 2-[*N*-(*N'*-benzylprolyl)aminoacetophenone as a chiral reagent. Furthermore, Cu^{2+} has also been employed as the central metal but was determined to be inferior to the Ni^{2+} complexes. Complexes made with Ni^{2+} can undergo reactions under conditions in which Cu^{2+} complexes would decompose. Furthermore, Ni^{2+} is diamagnetic, allowing the complexes to be characterized by NMR spectroscopy.[34] For the purpose of this thesis, only the complexes involving glycine, BPB, and Ni^{2+} will be discussed.

1.3.2 – Alkylation reactions using Belokon's BPB-complex

The acidity (pKa) of the glycine protons of the BPB-complex (**36**) was measured to be ~18 in DMSO which is a value comparable to mono-nitrocompounds.[40] This high acidity allows common bases (strong or weak) such

as NaOH, KOH, NaOMe, and Et_3N to be used in alkylation reactions with the BPB complex. Deprotonation of the complex under basic conditions creates a carbanion complex (nucleophile), which can react with a wide variety of electrophiles such as alkyl halides, aldehydes, ketones, and activated olefins (Figure 1).[33-47] An equilibrium is established during the alkylation reaction creating diastereomers differing in configuration at the asymmetric glycine center.[35] In many cases, one diastereomer is preferred over the other (usually the (*S,S)* diastereomer when (*S*)-proline is used) and the stereochemical outcome is thermodynamically controlled. In general, only monoalkylation occurs because the other glycine proton is protected by the phenyl substituent at the C=N position of the BPB ligand. Dialkylation would place a great deal of steric stress on the system.[34]

1.3.3 – Alkylation of BPB-complex with alkyl halides

One of the most studied reactions with the BPB-complex (**36**) is its alkylation with alkyl halides.[35,40] These reactions are straightforward and are driven by base (usually in solid form of NaOH or KOH) in a solution of DMF (or MeCN) at room temperature. Monoalkylation occurs (no dialkylation is observed)

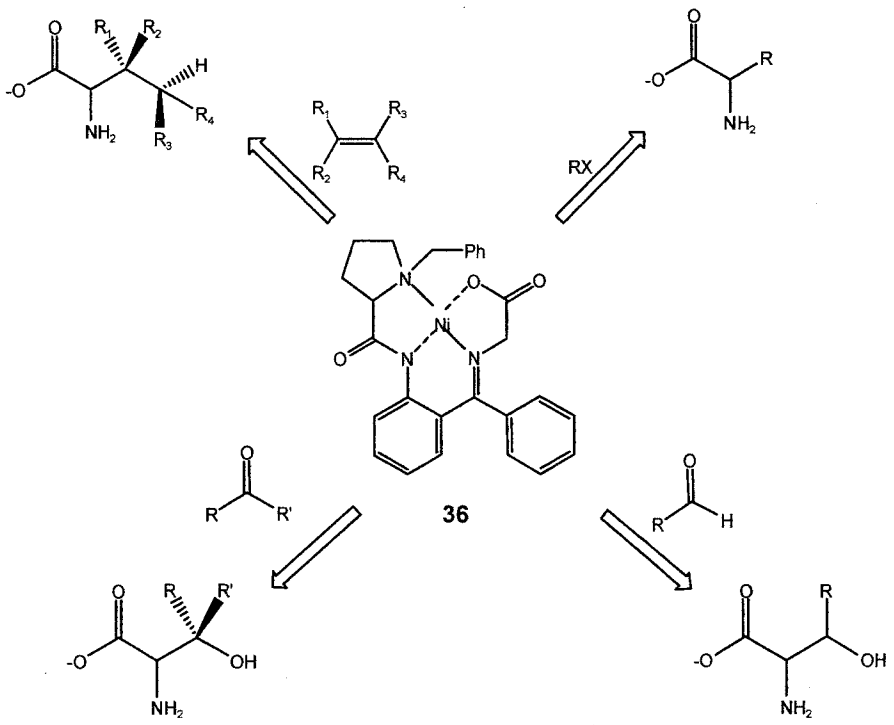


Figure 1: Reactions with Ni-Gly-BPB complex (**36**).

in good yields (70-90%) with an excess of the thermodynamically favored (*S,S*)-diastereomer usually in short reaction times.[40] A wide range of alkylating agents (CH_3I, 2-naphthyl-CH_2Br, $PhCH_2Br$)[40] can be applied to this reaction without limitations of the structure of the alkyl halide. However, if the alkyl halide is base-sensitive, the base can be replaced by sodium or potassium carbonates.[35]

α-Amino-ω-phosphonocarboxylic acids are an example of a unique class of amino acids that have been produced by this type of alkylation.[41,42] Phosphinothricin (**9**) and its analogues have been produced by Belokon and co-workers[41] by alkylating the BPB-complex with ω-haloalkylphosphonates. These

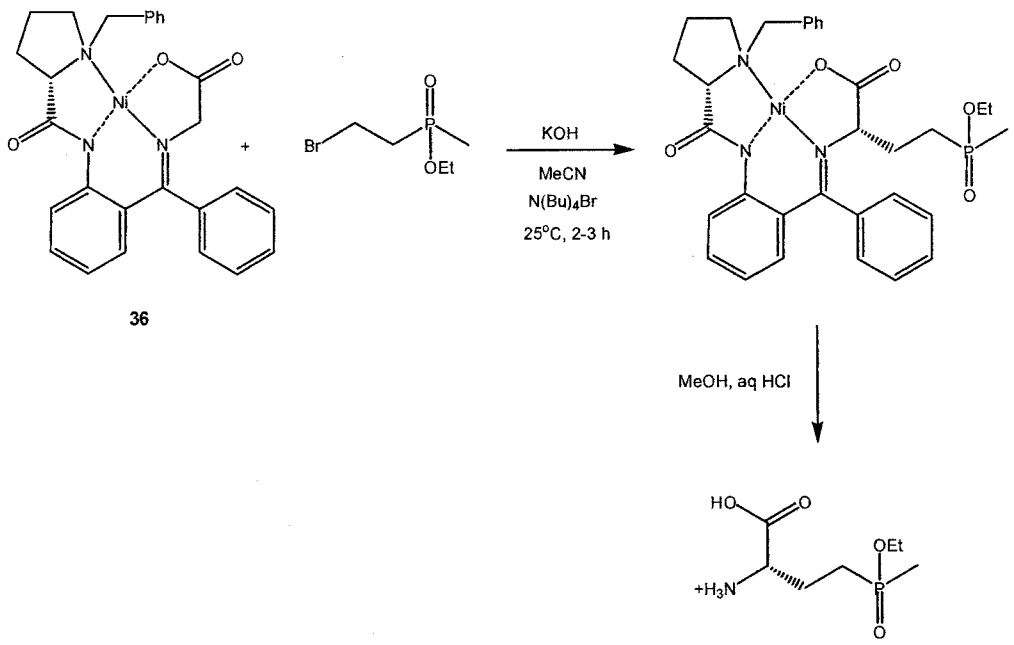
compounds have been studied due to their biological activity, potentially acting as false substrates and interfering with biological mechanisms.[41,42]

Scheme 16 shows an example of an alkylation with a haloalkylphosphonate,[41] which is added to a solution of the (*S*)-BPB-complex (**36**) and KOH in acetonitrile under argon. Tetrabutylammonium bromide acts as a phase transfer catalyst to promote product formation, and the reaction medium is stirred at room temperature until the initial BPB complex is consumed. This particular reaction proceeded in 73.5% yield and produced 95% of the thermodynamically favored (*S,S*) diastereomer.[41] The free amino acid was obtained by hydrolysis in aqueous acid in MeOH (83.5%).

A series of haloalkylphosphonates was studied[42,43] and in every case the thermodynamically favored (*S,S*)-diastereomer was formed in excess. At a minimum, the alkylation reactions proceeded with a diastereomer excess (d.e.) of 86%. This is an indication that despite the functional groups present in the side chain of the alkylating agent, the alkylation reaction proceeds in good yields and with high stereochemical purity.

1.3.4 – Alkylation of Belokon's BPB-complex with olefins

Belokon's BPB complex (**36**) can also participate in Michael addition reactions with activated olefins.[34,35,40] These reactions are also straightforward and have been extensively studied. Belokon's synthesis[33]of (*S*)-4-cyano-2-aminobutyric acid (**16**) in Section 1.1.5 is an example of a Michael-type addition. Similar to alkylation with alkyl halides, a carbanion complex forms under basic



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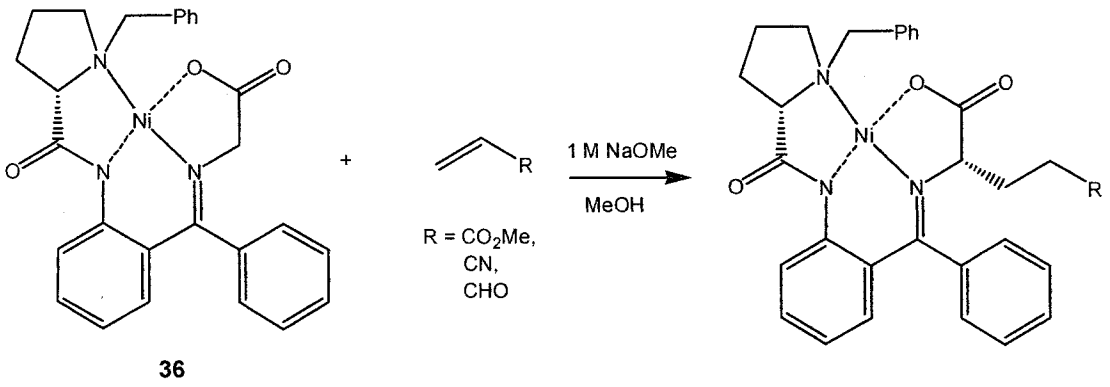
Scheme 16: Alkylation of (*S*)-BPB complex (**36**) with haloalkylphosphonates.

conditions and attacks the double bond of the olefin to form the product (Scheme 17). Belokon[34,35,40] studied many olefins as electrophiles and found that, in general, the best solvent and base combination for this sort of alkylation was NaOMe and MeOH.[40] The alkylation reactions in Scheme 17 proceeded to produce the thermodynamically favored (*S,S*)-diastereomers with diastereoselectivities greater than 70%.

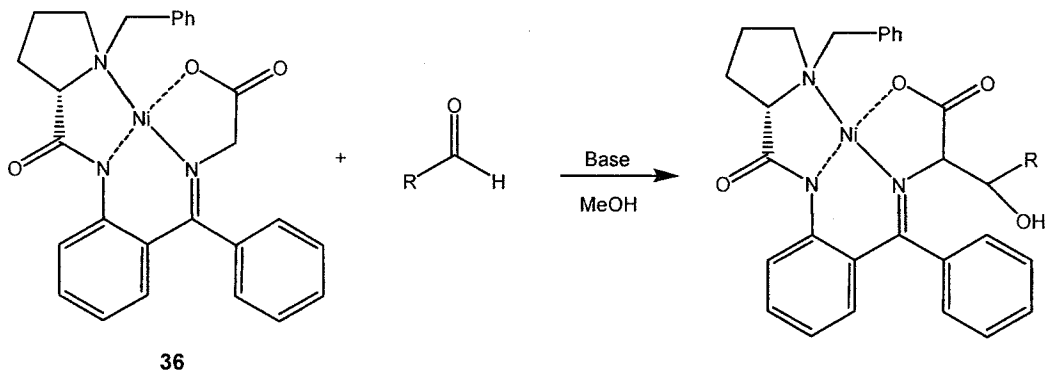
1.3.5 – Aldol condensations with Belokon's BPB-complex

Aldol condensations with aldehydes and ketones were also studied[34,541,43-46] using Belokon's BPB-complex (**36**). These reactions were found to be much more complicated than the two alkylations discussed above. In



Scheme 17: Olefin addition to (*S*)-BPB complex (**36**).

particular, the stereochemcial outcome of these alkylations did not follow the same general pattern as above; the diastereomer with the (*S*)-configuration at the proline and glycine α-carbon was not always the thermodynamically favored product. It was found that the stereochemical outcome highly depended on the basicity of the reaction mixture.[43,44] Reactions with aliphatic aldehydes[43,44] (e.g. formaldehyde and acetaldehyde, Scheme 18) reacted reversibly with the BPB complex to yield an excess of the diastereomeric complex having the (S)-configuration at the α-carbons of the amino acids when Et_3N was used as base in MeOH. For reactions under these conditions, an excess of the aldehyde was needed to drive the reaction towards completion. The formation of the (*S*)-configuration at the α-carbon of the serine moiety was explained as being the more thermodynamically favored product.



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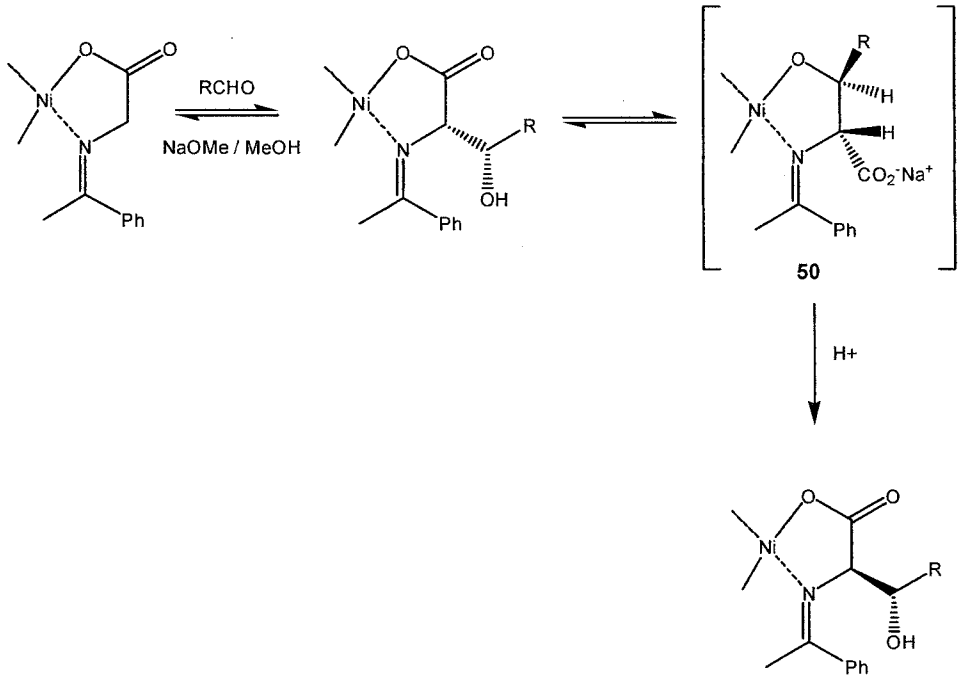
Scheme 18: Aldol condensations with (*S*)-BPB complex (**36**).

On the other hand, when the condensations[44] with these aldehydes were conducted using NaOMe as the base in MeOH, the rate of the reaction and thermodynamic equilibration of the products was greatly increased. The stereochemical outcome in these cases was opposite. An overwhelming excess of the (*R*)-configuration occurred at the glycine α-carbon. The reason for this was attributed to the stronger base present in the reaction mixture. Under highly basic conditions, the hydroxyl group (formed upon condensation of the BPB complex with an aldehyde) is ionized. This ionized species is now more basic than the carboxylate group and thus has a higher affinity for nickel. Therefore, a rearrangement takes place to produce the more thermodynamically stable species (**50**, Scheme 19). Under these conditions the carboxylate group adopts a pseudoaxial orientation (the (*R*)-configuration), which is energetically more favorable than the pseudoequatorial orientation (the (*S*)-configuration), due to a repulsive interaction of the carboxylate with the phenyl group at the C=N position. Neutralization of the reaction mixture would cause protonation of the hydroxyl ion, which in turn gives the carboxylate group the higher affinity for the



Scheme 19: Rearrangement of the BPB-complex under highly basic reaction conditions in aldol condensations with aldehydes.

nickel ion, promoting rearrangement of the complex back into the usual form (now with the α-(*R*)-configuration).[43,44]

To investigate the highly basic reaction further, Soloshonok and co-workers[43] examined the stereochemical composition of the reaction with acetaldehyde over time. It was found that after 1 h the (*S*)-configuration at the α-carbon was dominant (90% d.e. of (*S*)-threonine), but over time the diastereomer with the (*R*)-configuration at the glycine α-carbon increased to 68% d.e. after 2 h and to 90% d.e. after 24 h. A study[43] of the steric bulk of the aldehyde side chain was also carried out, but the same general trend was observed. One exception, however, was 2,2-dimethyl-1-propanal which, after 24 h, still showed the (*S*)-amino acid as the dominant product. After 20 days, there was a slight excess of the (*R*)-amino acid (57.5% (*R*) to 42.5% (*S*). These results suggest that the

stereochemical outcome of the aldol condensation with aldehydes depends on reaction time. In addition, the steric bulk of the aldehyde side chain can affect the equilibrium ratio of the diastereomeric complexes.

If the (*R*)-α-amino-β-hydroxyamino acid were desired, this method would be very useful. However, if the (*S*)-amino acid were desired under reproducible, thermodynamically controlled conditions the enantiomeric (*R*)-BPB chiral auxiliary would be required. As mentioned above, the (*S*)-amino acid formed first in excess, but the reaction is difficult to stop at that stage since within minutes the kinetic product (the (*S*)-product) is transformed into the thermodynamically favored (*R*)-product.[46]

As mentioned earlier, by conducting the alkylation reaction using a weaker base, the (*S*)-BPB complex produces (*S*)-amino acids.[43,44] Belokon and co-workers[47] developed another method for achieving (*S*)-amino acids by changing the solvent used in the reaction. They determined that by using an aprotic solvent such as THF, the (*S,S*)-complex was produced in excess.

Numerous aldol reactions[43-45, 47] were also conducted to examine the effect of fluorosubstituents on the aldehydes and ketones. This new class of amino acids has potential biological activity making them interesting to study. In general, the addition of fluorosubstituents influences the stereochemical outcome of the reaction. Depending on the reaction conditions, the stereochemical outcome can be opposite to their aliphatic analogues.[44] For instance, reaction of (*S*)-BPB complex with trifluoroacetaldehyde yielded the complex with (*S*)-configuration at both amino acid α-carbons in 92% d.e under high pH conditions

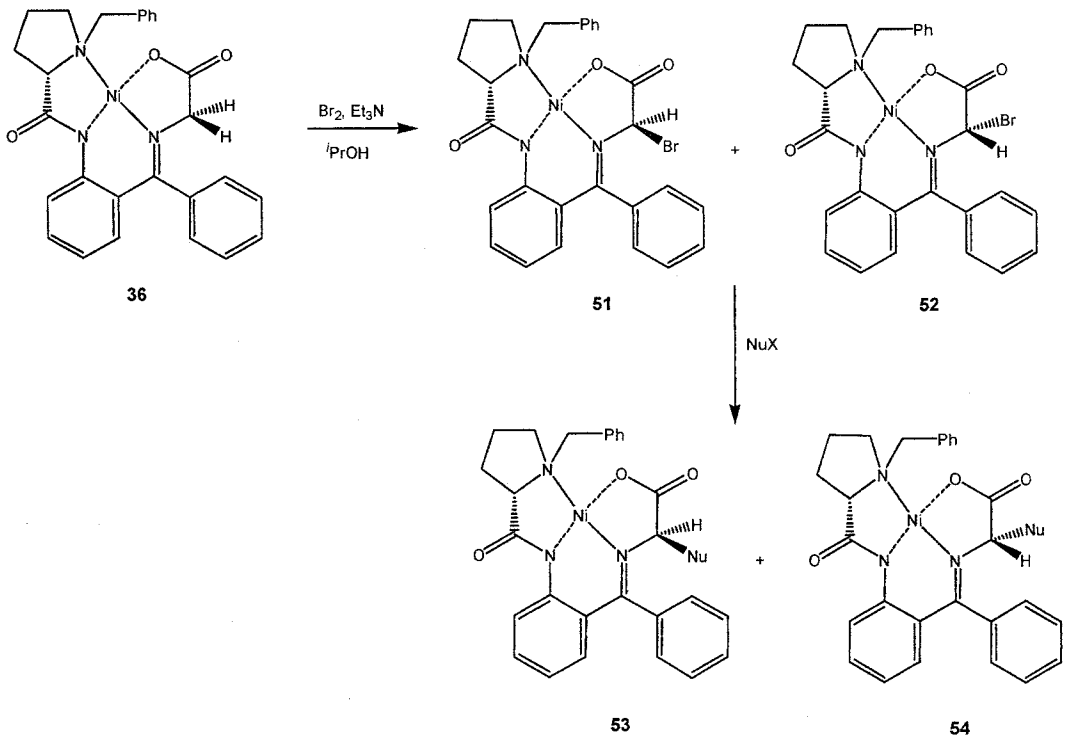
(NaOMe / MeOH) whereas reaction with acetaldehyde yielded the (*S,R*)-complex.[43] This effect on stereochemical outcome was attributed to the strong electron withdrawing effects of the fluorine atoms.

Aldol reactions have also been conducted with acetone,[44] but they have been less widely studied. Reaction with acetone is quite slow and is not as stereoselective as the reactions involving aldehydes. In contrast, trifluoroacetone reacts very quickly with the (*S*)-BPB-complex (**36**) to yield the diastereomer with the (*S*)-configuration at the amino acid α-carbons. However, due to the basic reaction conditions trifluoroacetone decomposes, and a ten-fold excess is needed for a complete conversion of the BPB-complex to product.

1.3.6 – BPB-complex as an electrophile

In the above reactions, the BPB-complex (**36**) acts as a nucleophile. It is also possible for the BPB-complex to be converted to an electrophile. This can be achieved by converting the BPB-complex into a bromoglycine complex(**51** and **52**) using base-promoted bromination (Scheme 20).[48] This complex can then react with numerous nucleophiles (**53** and **54**) such as phenolates, methoxide anions, and malonic esters. The reaction conditions employed for the alkylations vary depending on the nucleophile used. In general, the thermodynamically more stable (*S,S*)- complex is obtained in good yield (60-90%).

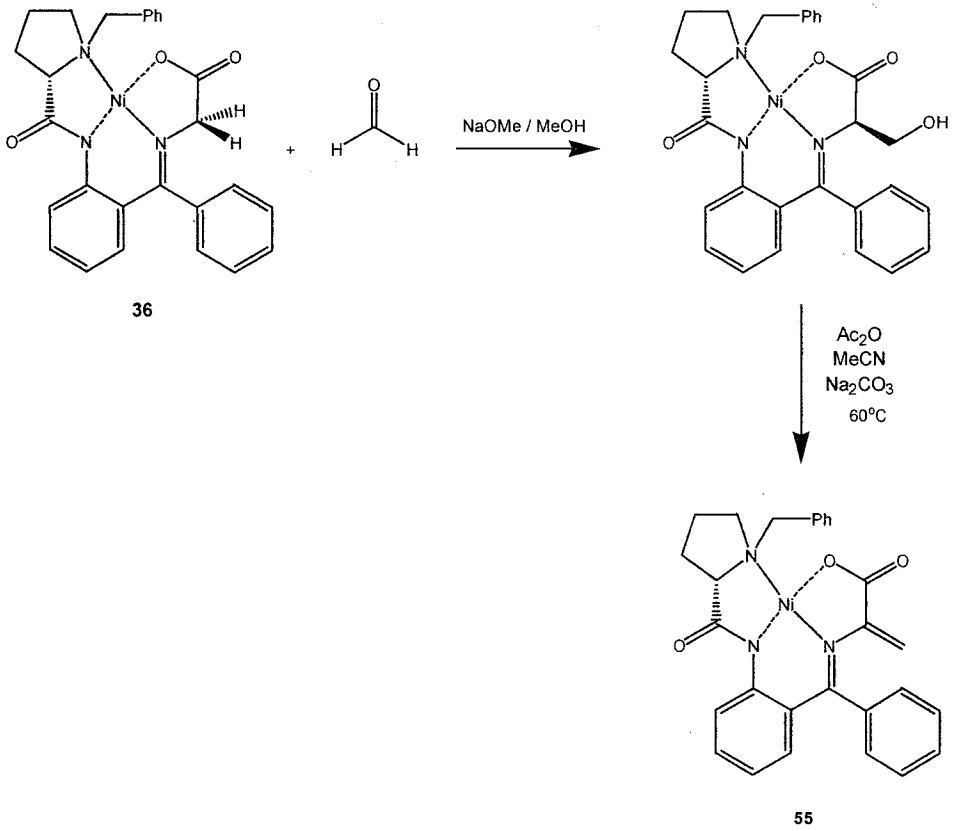
The BPB-complex can also be converted to a dehydroalanine complex[40,49,50] (**55**, Scheme 21) and act as an electrophile in this manner. This



Scheme 20: Bromination of Belokon's (*S*)-BPB complex (**36**).

complex can be achieved via an aldol condensation with formaldehyde (under highly basic conditions) to yield the corresponding (*R*)-serine complex. This is then dehydrated using acetic anhydride in acetonitrile. Na_2CO_3 is used as the base in this reaction to yield the dehydroalanine complex (Scheme 21). Like the bromoglycine complex, this complex can also react with a variety of nucleophiles, such as amines, alcohols, and thiols via a typical Michael-type addition reaction.[40]

Belokon's BPB-complex (**36**) offers several advantages for producing α-amino acids such as simplicity of operation, ambient reaction temperatures, the use of common bases, high rates of alkylations, and facile recovery of the free

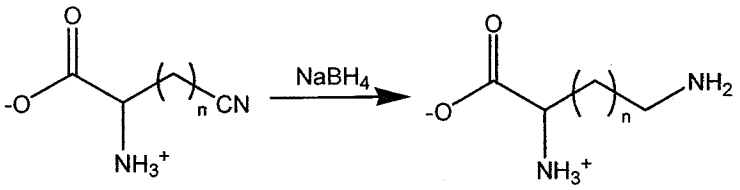


Scheme 21: Formation of a dehydroalanine complex (**55**).

amino acid and the chiral auxiliary.[34,40] The properties of the BPB complex allow it to enter into numerous reactions such as alkylations with alkyl halides,[35,40-42] Michael additions with activated olefins,[34,40] and condensations with aldehydes and ketones.[37,43-47] In addition, the BPB-complex can also be converted to an electrophile[40,48-50] and react with various nucleophiles. The stereochemical outcome of the reactions is thermodynamically controlled and can be affected by the reaction conditions and the addition of electron withdrawing groups (such as fluoro groups) within the reactants. Utilization of the BPB complex provides a convenient way for producing enantiomerically pure free α-amino acids without the use of other protecting groups.

1.4 - Thesis Goals

The work outlined in this thesis focuses on the stereoselective synthesis of (*S*) and (*R*)-nitrile amino acids (n = 2-4) and their use as intermediates for producing diamino acids. The synthesis will be accomplished by alkylating Belokon's BPB-complex with alkylcyano bromides (n = 2-4) in DMF using NaOH as base (Scheme 13). Belokon reported high stereoselectivity[40] of the alkylation reactions, and stereochemical purity will be evaluated throughout the synthetic protocol. To obtain the free nitrile amino acids, the alkylated BPB-complexes will be hydrolyzed in aqueous acid,[40] and the amino acids will be isolated by ion-exchange chromatography. Once pure nitrile amino acids are obtained, reduction[51] to their basic analogues will be conducted (Scheme 22).



n = 1, **12**
n = 2, **16**
n = 3, **27**
n = 4, **41**

n = 1, **13**
n = 2, **5**
n = 3, **28**
n = 4, **56**

Scheme 22: Reduction of nitrile amino acids.

Chapter 2.0 – Experimental – General Procedures

All chemicals from commercial sources were used as supplied unless otherwise stated. High boiling petroleum ether refers to the fraction that boils at 60-80°C. All H_2O used was purified by a Barnstead Nanopure Ultrapure Water system; MeOH was dried by refluxing in the presence of magnesium metal and iodine and distilled;[52] DMF was dried over CaO and distilled under vacuum,[53] and CH_2Cl_2 was dried by distillation from P_2O_5.[53]

Melting points were measured in open capillary tubes using a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were recorded on a Digipod 781 Automatic Polarimeter. NMR spectra were acquired on either the Bruker Spectrospin AC250 or the Bruker AV500. All ^{13}C NMR spectra were recorded using the AV500 spectrometer. Chemical shifts of samples run in $CDCl_3$ and D_2O are relative to TMS (0 ppm) and HOD (4.80 ppm), respectively. Fractions collected from ion-exchange columns were tested for the presence of amino acid by subjecting them to a ninhydrin spot test.[54] A portion of the fraction was spotted onto filter paper and heated (~100°C, 5 min) to remove any ammonia solution present. Ninhydrin solution (0.25% w/v in acetone) was applied to the paper, and the paper heated (~100°C, 5 min). Positive tests were indicated by a purple spot.

2.1 – HPLC determination of amino acids

HPLC determination of amino acids was carried out using two Beckman Model 110B solvent delivery modules and a Beckman 157 fluorescence detector. Samples were applied to the column via a 20 µL injection loop. A Beckman Ultrasphere ODS (4.6 x 45 mm) column was employed for achiral amino acid derivatives, and a Phenomenex nucleosil 5 C_{18} (250 x 4.6 mm) column for separation of diastereomeric amino acid derivatives.

Fluorescent derivatives of amino acids were prepared by mixing a portion of the sample solution (40 µL) with an equal volume of commercial *o*-phthaldehyde (OPA) reagent (Fluoraldehyde, Pierce Chemical Co.). After 1 min at ambient temperature, 120 µL of HPLC solvent was added, and a portion (20 µL) was injected onto a reversed-phase column. Gradients as described in Tables 10 and 11 were used to elute amino acid derivatives from the columns.

Table 1: HPLC gradient for achiral OPA HPLC analysis of cyanoamino acids.[a]

Time (min)	Solvent A[b] (%)	MeOH (%)
0.0	100	0
0.5	90	10
1.5	90	10
2.0	55	45
3.0	55	45
3.5	0	100
4.0	0	100
4.5	100	0
5.0	100	0

[a] Total flow rate, 2.5 mL/min, [b]NaOAc (0.1 M), filtered and degassed, pH 6.2 with 6 M HCl, 10% THF/MeOH (5 mL/95 mL).

Table 2: HPLC gradient for chiral OPA HPLC analysis of cyano amino acids.[a]

Time (min)	Solvent A[b] (%)	MeCN (%)
0.0	0	100
3.0	8	92
10.0	8	92
11.0	12	88
20.0	12	88
22.0	0	100

[a] Total flow rate, 2.00 mL/min, [b]CuOAc (2.5 mM), (*S*)-proline (5 mM), NH_4OAc (2 g), filtered and degassed, pH 7.0 with 5 M NaOH, 5% MeCN.

2.2 - Preparation of *N*-Benzylproline

A mixture of (*S*) or (*R*)-proline (7.5 g, 65 mmol) and KOH (11.0 g, 196 mmol) in isopropanol (45 mL) was stirred at 40°C. Once dissolved, freshly distilled benzylchloride (9.0 g, 71 mmol) was added dropwise, and stirring was continued at 40°C for 6 h. The reaction mixture was adjusted to pH 7-8 using concentrated HCl. $CHCl_3$ (18 mL) was added to the neutralized mixture, which was then stirred overnight at room temperature.

The reaction mixture was filtered by gravity to remove a white precipitate. The filtrate was concentrated *in vacuo* to yield an oil, which solidified upon addition of acetone. The white solid was crystallized from $CHCl_3$/ether.

***N*-Benzyl-(*S*)-proline** (6.81 g, 51%); mp 165-166°C (dec) lit.[55] 164°C; $[\alpha]_D^{21}$ -26.5°(EtOH, c = 1.0), lit.[55] $[\alpha]_D^{20}$ -28.4°(EtOH, c = 0.01); ^1H NMR (500 MHz, D_2O) δ: 7.53 (s, ArH, 5H), 4.41 (AB quartet J = 4, 13 Hz), $BnCH_2$, 2H), 4.02 (dd, J = 7, 9.5 Hz, α-ProCH, 1H), 3.68-3.64 (m, $ProCH_2$, 1H), 3.37-3.29 (m, $ProCH_2$, 1H), 2.54-2.49 (m, $ProCH_2$, 1H), 2.18-2.14 (m, $ProCH_2$, 1H), 2.11-2.05 (m, $ProCH_2$, 1H), 2.04-1.97 (m, $ProCH_2$, 1H); ^{13}C NMR (125.8 MHz, D_2O) δ: 173.8 (C=O),

130.7, 130.2 (ArCH), 130.1 (ArC), 129.3 (ArCH), 68.3 (α-CH), 58.4 ($BnCH_2$), 54.8, 28.9, 22.9 ($proCH_2$).

***N*-Benzyl-(*R*)-proline** (4.27 g, 32%); mp 160-165°C (dec); $[\alpha]_D^{22}$ 27.2° (EtOH, c = 1.0); ^1H NMR (500 MHz, D_2O) δ: 7.53 (s, ArH, 5H), 4.46-4.40 (AB quartet *J* = 4 13 Hz, $BnCH_2$ 2H), 4.02 (dd, *J* = 7, 9.5 Hz, α-ProCH, 1H), 3.70-3.65 (m, $ProCH_2$, 1H), 3.36-3.30 (m, $ProCH_2$, 1H), 2.56-2.49 (m, $ProCH_2$, 1H), 2.21-2.15 (m, $ProCH_2$, 1H), 2.11-2.06 (m, $ProCH_2$, 1H), 2.06-1.97 (m, $ProCH_2$, 1H); ^{13}C NMR (125.8 MHz, D_2O) δ: 173.8 (C=O), 130.7 (ArCH), 130.2 (ArC), 130.1, 129.3 (ArCH), 68.3 (α-CH), 58.5 ($BnCH_2$), 54.8, 28.9, 22.9 ($ProCH_2$).

2.3 - Preparation of 2-[*N*-(*N'*-benzylprolyl)amino]benzophenone (BPB)

Thionyl chloride (1.46 g, 12.3 mmol) was added to a stirred solution of *N*-benzylproline (2.46 g, 12.0 mmol) in CH_2Cl_2 (12.5 mL) at approximately -20°C. Stirring was continued until the reaction mixture became transparent at which point a solution of 2-aminobenzophenone (1.86 g, 9.41 mmol) in CH_2Cl_2 (6 mL) was added. Stirring was continued at -20°C for approximately 8.5 h.

After stirring, the mixture was allowed to warm to room temperature and neutralized with aqueous sodium carbonate. The aqueous layer was extracted with CH_2Cl_2 (2 x 2.5 mL). The organic layer was dried over $MgSO_4$, and evaporated *in vacuo* to yield a yellow oil, which crystallized on addition of isopropanol. The resulting yellow solid was recrystallized from high boiling petroleum ether.

***(S)*-BPB** (1.76 g, 57%): mp 98.5-100.5°C lit.[36] 101-102°C; $[\alpha]_D^{21}$ -132° (MeOH, c = 1.0) lit.[36] $[\alpha]_D^{20}$ -134.5° (MeOH, c = 0.5); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.51 (s, NH, 1H), 8.57-7.08 (m, ArH, 14H), 3.92 (d, *J* = 12.5 Hz, BnCH, 1H), 3.59 (d, *J* = 13 Hz, BnCH, 1H), 3.31 (dd, *J* = 5, 10.5 Hz, α-CH, 1H), 3.23-3.20 (m, $ProCH_2$, 1H), 2.43-2.38 (m, 1H, $ProCH_2$), 2.29-2.21 (m, $ProCH_2$, 1H), 1.99-1.93 (m, $ProCH_2$, 1H), 1.94-1.74 (m, $ProCH_2$, 2H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ: 198.2 (C=O), 174.9 (C=O), 138.4, 138.8, 138.4 (ArC), 133.6, 132.8, 132.7, 130.3, 129.3, 128.5, 128.4, 127.3 (ArCH), 125.6 (ArC), 122.4, 121.7 (ArCH), 68.5 (α-CH), 60.1 ($BnCH_2$), 54.1 ($ProCH_2$), 31.2 ($ProCH_2$), 24.4 ($ProCH_2$).

***(R)*-BPB** (1.72 g, 51%): mp 98.5-100.5°C; $[\alpha]_D^{21}$ 135.8° (MeOH, c = 1.0); ^{1}H NMR (250 MHz, $CDCl_3$) δ: 11.53 (s, NH, 1H), 8.59-7.05 (m, ArH, 14H), 3.92 (d, *J* = 15, BnCH, 1H), 3.59 (d, *J* = 12.5, BnCH, 1H), 3.32 (dd, *J* = 2.5, 7.5 Hz, α-CH, 1H), 3.25-3.18 (m, $ProCH_2$, 1H), 2.46-2.34 (m, $ProCH_2$, 1H), 2.30-2.18 (m, $ProCH_2$, 1H), 2.00-1.71 (m, $ProCH_2$, 3H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ: 198.3 (C=O), 174.9 (C=O), 139.4, 138.8, 138.3 (ArC), 133.6, 132.8, 132.7, 130.3, 129.3, 128.5, 128.4, 127.3 (ArCH), 125.6 (ArC), 122.4, 121.8 (ArCH), 68.5 (α-CH), 60.1 ($BnCH_2$), 54.1 ($ProCH_2$), 31.2 ($ProCH_2$), 24.4 ($ProCH_2$).

2.4 - Preparation of BPB-Ni-Gly Complex

NaOMe (2.5 M, 24 mL) was added to a mixture of BPB (1.5 g, 3.9 mmol), $Ni(NO_3)_2 \cdot 6H_2O$ (1.36 g, 4.68 mmol), and glycine (0.59 g, 7.9 mmol) in dry MeOH (12 mL), under N_2 gas at 50°C. The mixture immediately became red and the volume was increased to 45 mL with dry MeOH. The reaction mixture was stirred for 0.5 h and determined complete by TLC, then cooled and neutralized by adding chilled glacial acetic acid dropwise. MeOH was removed from the mixture *in vacuo,* and the resulting solid was partitioned between $CHCl_3$ (55 mL) and water (25 mL). The $CHCl_3$ solution was retained and dried over $MgSO_4$. The solvent was removed *in vacuo* to yield a red foam, which was crystallized from acetone.

***(S)*-BPB Complex** (1.53 g, 78%); mp 213-216°C (dec) lit.[36] 208-212°C (dec); $[\alpha]_D^{23}$ 2167° (c = 0.022, $CHCl_3$) lit.[37] $[\alpha]_D$ 2006° (c = 0.1, MeOH); ^1H NMR (500 MHz, $CDCl_3$) δ: 8.31-6.69 (m, ArH, 14H), 4.49 (d, *J* = 12.5 Hz, $BnCH_2$, 1H), 3.67 (d, *J* = 12.5 Hz, $BnCH_2$, 1H), 3.80-3.70 (m, $ProCH_2$, 1H and $GlyCH_2$, 2H), 3.47 (dd, *J* = 5, 10.5 Hz, α-ProCH, 1H), 3.39-3.30 (m, $ProCH_2$, 1H), 2.59-2.56 (m, $ProCH_2$, 1H, 2.46-2.38 (m, $ProCH_2$, 1H), 2.18-2.13 (m, $ProCH_2$, 1H), 2.10-2.06 (m, $ProCH_2$, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ: 181.6 (C=O), 177.4 (C=O), 171.8 (C=N), 142.8, 134.8, 133.5 (ArC), 133.4, 132.4, 131.9 (2C), 129.9, 129.7, 129.5, 129.3, 129.1 (2C), 126.4, 125.9 (ArCH), 125.4 (ArC), 124.4, 121.0 (ArCH), 70.1 (α-ProCH), 63.3 (BnCH2), 61.5 ($GlyCH_2$), 57.7 ($ProCH_2$), 30.9 ($ProCH_2$), 23.9 ($ProCH_2$).

***(R)*-BPB Complex** (4.03 g, 80%); mp 214-215 °C (dec); $[\alpha]_D^{22}$ -2167° (c = 0.022, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ: 8.31-6.70 (m, ArH, 14H), 4.49 (1/2 ab *J* = 10 Hz, $BnCH_2$, 1H), 3.80-3.70 (m, 1 $ProCH_2$, 2 $GlyCH_2$, 3H), 3.67 (1/2 ab *J* = 10 Hz, $BnCH_2$, 1H), 3.47 (dd *J* = 5.5, 11 Hz, α-ProCH, 1H), 3.38-3.31 (m, $ProCH_2$, 1H), 2.59-2.55 (m, $ProCH_2$, 1H), 2.46-2.40 (m, $ProCH_2$, 1H), 2.18-2.13 (m, $ProCH_2$, 1H), 2.10-2.05 (m, $ProCH_2$, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ: 181.6 (C=O), 177.5 (C=O), 171.9 (C=N), 142,8, 134.9, 133.5 (ArC), 133.4, 132.4, 132.0 (2C), 129.8, 129.6, 129.3, 129.1 (2C), 126.5, 125.9 (ArCH), 125.4 (ArC), 124.5, 121.1 (ArCH), 70.1 (α-ProCH), 63.3 ($BnCH_2$,), 61.5 ($GlyCH_2$), 57.7, 30.9, 23.9 ($ProCH_2$).

2.5 - Alkylation of BPB Complex with Alkylcyano bromides

2.5.1 – HPLC analysis of alkylation reaction mixtures

Samples of the reaction mixture (20 µL), potentially containing a mixture of alkylated and unalkylated complex were mixed with 3 M HCl (50 µL) and heated at 53°C until the red color had disappeared. The acidic reaction mixture was extracted with $CHCl_3$ (1 mL) to remove BPB ligand formed by hydrolysis. The aqueous portion was neutralized using concentrated NH_3 solution and diluted to (~1 mL).

2.5.2 – Alkylation reaction conditions

Alkylcyano bromide (0.27 g, 1.7 mmol) and powdered NaOH (0.15 g, 3.8 mmol) were added to a stirred solution of BPB-complex (0.75 g, 1.5 mmol) in DMF (4 mL). The mixture was placed under nitrogen and stirred at room temperature until all of the complex had reacted as determined by OPA HPLC (~ 25 min).

After 40 min, the reaction mixture was neutralized by pouring it into cooled acetic acid (0.5 M, 20 mL). The product was extracted into $CHCl_3$ (4 x 4 mL). The combined organic layers were washed with water (8 mL), dried over $MgSO_4$, and evaporated to dryness to yield an oily liquid (DMF remained). The complexes were purified either by dry flash chromatography or crystallization from acetone.

BPB Complex alkylated with 3-propionitrile:

***(S,S)*-Complex** (0.40 g, 48%); mp 209-210°C (dec) lit.[33] mp 220-224°C (dec); $[\alpha]_D^{22}$ 2608° (c = 0.0249, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ: 8.15 (d, *J* = 8.5 Hz, 1H, ArH), 8.06 (d, *J* = 7 Hz, 2H ArH), 7.57-7.52 (m, 3H, ArH), 7.35 (app t, *J* = 7.5, 8 Hz, 2H, ArH), 7.29-7.27 (m, 1H, ArH), 6.96 (d, *J* = 7.5 Hz, 1H, ArH), 6.68-6.53 (m, 2H, ArH), 4.41 (d, *J* = 13 Hz, 1H, $BnCH_2$), 3.86 (dd, *J* = 3.5, 10.5 Hz, 1H, α-CH), 3.57 (d, *J* = 12.5 Hz, 1H, $BnCH_2$), 3.53-3.47 (m, 3H, $ProCH_2$), 2.77-2.70 (m, 2H), 2.57-2.50 (m, 2H), 2.38-2.31 (m, 1H), 2.24-2.22 (m, 1H), 2.18-2.06 (m, 1H), 1.94-1.91 (m, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ: 180.6 (C=O), 178.1 (C=O), 172.0 (C=N), 142.7, 133.4, 133.4 (ArC), 133.6, 132.9, 131.7, 130.5,

129.6, 129.2, 129.2, 129.4, 127.2 (ArCH), 126.2 (ArC), 124.1, 121.1 (ArCH), 118.4 (CN), 70.4 (α-ProCH), 68.5 (α-CH), 63.5 ($BnCH_2$), 57.5 ($ProCH_2$), 31.5, 30.9, 24.4 (CH_2), 14.0 (CH_2CN).

***(R,R)*-Complex** (0.30 g, 36%); mp 208-210°C (dec); $[\alpha]_D^{18}$ -2995° (c = 0.0216, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ: 8.14 (d, *J* = 8.5 Hz, 1H, ArH), 9.07 (d, *J* = 7.5 Hz, 2H, ArH), 7.57-7.50 (m, 3H, ArH), 7.36 (t, *J* = 7.5 Hz, 2H, ArH), 7.29-7.27 (m, 1H, ArH), 7.19 (t, *J* = 7.5 Hz, 1H, ArH), 7.17-7.13 (m, 1H, ArH), 6.96 (d, *J* = 7 Hz, 1H, ArH), 6.68-6.63 (m, 2H, ArH), 4.39 (d, *J* = 12.5 Hz, 1H, $BnCH_2$), 3.86 (dd, *J* = 3.5, 10.5 Hz, 1H, α-CH), 3.55 (d, *J* = 13 Hz, 1H, BnCH2), 3.53-3.47 (m, 3H, proCH2), 2.76-2.70 (m, 2H), 2.58-2.47 (m, 2H), 2.38-2.32 (m, 1H), 2.25-2.20 (m, 1H), 2.18-2.06 (m, 1H), 1.95-1.89 (m, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ: 180.5 (C=O), 177.9 (C=O), 171.8 (C=N), 142.6, 133.4, 133.3 (ArC), 133.5, 131.5, 130.3, 129.4, 129.1, 129.0, 127.8, 127.2 (ArCH), 126.1 (ArC), 123.9, 120.9 (ArCH), 118.2 (CN), 70.3 (α-ProCH), 68.3 (α-CH), 63.3 ($BnCH_2$), 54.0, 31.5, 30.8, 24.2 (CH_2), 13.9 (CH_2CN).

BPB Complex alkylated with 4-bromobutyronitrile:

(*S,S*)-Complex (0.44 g, 73%); mp 195-197°C (dec); $[\alpha]_D^{22}$ 2474° (c = 0.023, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ: 8.15 (d, *J* = 8.5 Hz, 1H, ArH7), 8.06 (d, *J* = 10 Hz, 2H, ArH 23/27), 7.55-7.50 (m, 3H, ArH 15, 16, 17), 7.36 (t, *J* = 8, 7.5 Hz, 2H, ArH 24/26), 7.27-7.25 (m, 1H, ArH 14), 7.20 (t, *J* = 7.5 Hz, 1H, ArH 25), 7.17-7.13 (m, 1H, ArH 8), 7.02 (dd, *J* = 2, 5.5 Hz, 1H, ArH 18), 6.69-6.64 (m, 2H, ArH

9, 10), 4.42 (d J = 12.5 Hz, 1H, $BnCH_2$), 3.57 (d J = 12.5 Hz, 1H, $BnCH_2$), 3.52-3.47 (m, 3H, H1, H2, H4), 2.77-2.74 (m, 1H, H3), 2.56-2.51 (m, 1H, H3), 2.36-2.27 (m, 2H, H28, H30), 2.26-2.18 (m, 2H, H2, H29), 2.10-2.04 (m, 2H, H1, H30), 1.79-1.68 (m, 2H, H28, H29); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ: 180.6 (C5), 178,9 (C20), 171.2 (C12), 142.6 (C6), 133.7 (C13), 133.5 (C10), 133.4 (C22), 132.6 (C8) , 131.7 (C23 & 27), 130.1 (C16), 129.6 (C17), 129.2 (C25), 129.2 (C15), 129.1 (C24 & 26), 127.7 (C14), 127.2 (C18), 126.3 (C11), 124.0 (C7), 121.0 (C9), 119.1 (C31), 70.4 (C4), 69.3 (C19), 63.4 (C21), 57.4 (C1), 34.8 (C28), 30.9 (C3), 24.2 (C2), 21.8 (C29), 16.8 (C30).

***(R,R)*-Complex** (0.55 g, 63%); mp 195-197°C (dec); $[\alpha]_D^{22}$ (c = 0.023, $CHCl_3$) -2605°; ^1H NMR (500 MHz, $CDCl_3$) δ: 8.15 (d, J = 8.5 Hz, 1H, ArH), 8.05 (d, J = 7 Hz, 2H, ArH), 7.55-7.52 (m, 3H, ArH), 7.36 (t, J = 7.5, 8 Hz, 2H, ArH), 7.27-7.25 (m, 1H, ArH), 7.21 (t, J = 7.5 Hz, 1H, ArH), 7.17-7.14 (m, 1H, ArH), 7.00 (dd, J = 1.5, 5 Hz, 1H, ArH), 6.68-6.64 (m, 2H, ArH), 4.43 (d, J = 13 Hz, 1H, $BnCH_2$), 3.82 (dd, J = 3.5, 9.5 Hz, 1H α-CH), 3.60 (d, J = 10.5 Hz, 1H, $BnCH_2$), 3.57-3.47 (m, 3H, $ProCH_2$), 2.76-2.74 (m, 1H, $ProCH_2$), 2.56-2.54 (m, 1H, $ProCH_2$), 2.36-2.26 (m, 2H, CH_2), 2.21-2.17 (m, 2H, CH_2), 2.09-2.04 (m, 2H, CH_2), 1.77-1.61 (m, 2H, CH_2), ^{13}C NMR (125.8 MHz, $CDCl_3$) 180.7 (C=O), 179.0 (C=O), 171.2 (C=N), 142.6, 133.7 (ArC), 133.5 (ArCH), 133.4 (ArC), 132.7, 131.8, 130.2, 129.7, 129.2, 129,2, 129.2, 127.7, 127.3 (ArCH), 126.4 (ArC), 124.0, 121.1 (ArCH), 119.1 (CN), 70.4 (α-ProCH), 69.3 (α-CH), 63.4 ($BnCH_2$), 57.4 (α-CH), 34.8 (CH_2), 31.0 ($ProCH_2$), 24.2 ($proCH_2$), 21.9 (CH_2), 16.0 (CH_2CN); ^{13}C NMR (125.8 MHz,

$CDCl_3$) δ: 190.7 (C=O), 179.0 (C=O), 171.2 (C=N), 142.6, 133.7, 133.4, 126.4 (ArC), 133.5, 132.7, 131.8, 130.2, 129.7, 129.2, 129.2, 129.2, 127.7, 127.3, 124.0, 121.1 (ArCH), 119.1 (CN), 70.4 (α-pro CH), 69.3 (α-CH), 63.4 ($BnCH_2$), 57.4, 34.8, 31.0, 24.2, 21.9 (CH_2), 16.9 (CH_2CN).

BPB Complex alkylated with 5-bromovaleronitrile:

***(S,S)*-Complex** (0.55 g, 63%); mp 182-185°C; $[\alpha]_D^{22}$ (c = 0.0288, $CHCl_3$) 2446°; ^1H NMR (500 MHz, $CDCl_3$) δ: 8.13 (d, *J* = 8 Hz, 1H, ArH), 8.05 (d, *J* = 7 Hz, 2H, ArH), 7.55-7.49 (m, 3H, ArH), 7.35 (t, *J* = 7.5, 8 Hz, 2H, ArH), 7.29-7.26 (m, 1H, ArH), 7.20 (t, *J* = 7.5 Hz, 1H, ArH), 7.16-7.13 (m, 1H, ArH), 6.94 (d, *J* = 7.5 Hz, 1H, ArH), 6.67-6.62 (m, 2H, ArH), 4.44 (d, *J* = 13 Hz, 1H, $BnCH_2$), 3.87 (dd, J = 3.5, 9.5 Hz, 1H,), 3.58 (d, *J* = 12.5 Hz, 1H, $BnCH_2$), 3.54-3.46 (m, 3H, $ProCH_2$), 2.76-2.74 (m, 1H, $ProCH_2$), 2.56-2.53 (m, 1H, $ProCH_2$), 2.23-2.15 (m, 4H, CH_2), 2.11-2.05 (m, 1H, CH_2), 2.00-1.95 (m, 1H, CH_2), 1.66-1.59 (m, 2H, CH_2), (1.51-1.43 (m, 1H, CH_2), (1.40-1.34 (m, 1H, CH_2); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ: 180.6 (C=O), 179.2 (C=O), 170.8 (C=N), 142.6, 133.8, 133.2 (ArC), 133.2, 132.3, 131.6, 130.0, 129.1, 129.1, 129.0, 129.0, 127.5, 127.3 (ArCH), 126.4 (ArC), 123.8, 120.8 (ArCH), 119.2 (CN), 70.4 (α-ProCH), 69.7 (α-CH), 63.3 ($BnCH_2$), 57.3 ($ProCH_2$), 35.1, 31.0, 24.9, 24.8, 24.2, (CH_2), 17.1 (CH_2CN).

***(R,R)*-Complex** (0.72 g, 82%); mp 181-183°C; $[\alpha]_D^{21}$ (c = 0.0279, $CHCl_3$) -2529°; ^1H NMR (500 MHz, $CDCl_3$) δ: 8.13 (d, *J* = 10 Hz, 1H, ArH), 8.06 (d, *J* = 10 Hz, 2H, ArH), 7.56-7.47 (m, 3H, ArH), 7.35 (app t, splittings of 7.5, 8 Hz, 2H, ArH), 7.28-7.27 (m, 1H, ArH), 7.20 (t, J = 7.5 Hz, 1H, ArH), 7.16-7.13 (m, 1H, ArH), 6.94 (d, *J* = 7.5 Hz, 1H, ArH), 6.68-6.62 (m, 2H, ArH), 4.43 (d *J* = 12.5 Hz, 1H, $BnCH_2$), 3.87 (dd *J* = 3, 9 Hz, 1H, α-CH), 3.57 (d *J* = 13 Hz, 1H, $BnCH_2$), 3.52-3.47 (m, 3H, $ProCH_2$), 2.78-2.73 (m, 1H, $ProCH_2$), 2.26-2.15 (m, 4H, CH_2), 2.11-2.05 (m, 1H, CH_2), 2.03-1.95 (m, 1H, CH_2), 1.68-1.59 (m, 2H, CH_2), 1.52-1.44 (m, 1H, CH_2), 1.41-1.32 (m, 1H, CH_2); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ: 180.6 (C=O), 179.1 (C=O), 170.7 (C=N), 142.5, 133.9, 133.4 (ArC), 133.4, 132.4, 131.7, 130.1, 129.2, 129.2, 129.1, 129.1, 127.6, 127.4 (ArCH), 126.5 (ArC), 123.9, 120.9 (ArCH), 119.4 (CN), 70.4 (α-ProCH), 69.6 (α-CH), 63.3 ($BnCH_2$), 57.3 ($ProCH_2$), 35.0 (alkyl CH_2), 30.9 ($ProCH_2$), 24.9 (alkyl CH_2), 24.7 (alkyl CH_2), 24.1 ($proCH_2$), 17.0 (CH_2CN).

2.6 – Preparation of Cyanoamino Acids

Alkylated complex (0.62 mmol) was dissolved in MeOH (5.5 mL). H_2O (2.5 mL) and 3 M HCl (2.5 mL) were added, and the red solution was heated at 53°C until the red color disappeared and a green color remained. The aqueous mixture was extracted with $CHCl_3$ (3 x 4 mL) to remove BPB ligand, and adjusted to a neutral pH using concentrated NH_3 solution. The resulting aqueous layer was applied to an Amberlite IR 120 ion-exchange column (H^+ form). Regenerated ion-exchange resin was washed with H_2O until the effluent was

neutral before loading the sample. A H_2O wash followed and the amino acid was eluted with 0.5 M NH_3. Product was isolated as an off-white residue by evaporation of the basic fractions containing amino acid (as determined by ninhydrin stain). The cyanoamino acid was further purified by crystallization from H_2O/EtOH.

(*S*)-2-Amino-4-cyanobutyric Acid (58.8 mg, 91%); mp 214-216°C (dec) lit.[27] 230-235°C (dec); $[\alpha]_D^{19}$ 28.9° (c = 0.075, H_2O) lit.[27] $[\alpha]_D^{25}$ 27.4° (c = 0.5, H_2O); >99% ee by HPLC; ^{1}H NMR (500 MHz, H_2O) δ: 3.83 (t, *J* = 6 Hz, 1H, α-CH), 2.74-2.72 (m, 2H, CH_2), 2.29-2.24 (m, 2H, CH_2CN); ^{13}C NMR (125.8 MHz, H_2O) δ: 173.5 (C=O), 120.3 (CN), 53.6 (α-CH), 26.6 (CH_2), 13.7 (CH_2CN); ESI$^+$ MS (MeOH/H_2O, 20 µL/min) *m/z* 129 $[M+H]^+$: CID of *m/z* 129, *m/z* 83 (100%).

(*R*)-2-Amino-4-cyanobutyric Acid (0.031 g, 85%); mp 213-216°C (dec); $[\alpha]_D^{20}$ -26.3° (c = 0.035, H_2O); >99% ee by HPLC; ^{1}H NMR (500 MHz, D_2O) δ: 3.83 (t, *J* = 6 Hz, 1H, α-CH), 2.74 (m, 2H, CH_2CN), 2.30-2.24 (m, 2H, CH_2); ^{13}C NMR (125.8 MHz, D_2O) δ: 173.6 (C=O), 120.3 (CN), 52.8 (α-CH), 26.6 (CH_2), 13.7 (CH_2CN); ESI$^+$ MS (MeOH/H_2O, 20 µL/min) *m/z* 129 $[M+H]^+$: CID of *m/z* 129, *m/z* 83 (100%).

(*S*)-5-Cyanonorvaline (51.0 mg, 75%); mp 223-227°C (dec) lit.[27] mp 230-237°C (dec); $[\alpha]_D^{20}$ 10.1° (c = 0.11, H_2O) lit.[27] $[\alpha]_D$ 10° (c = 0.5, H_2O); >99% ee by HPLC; ^{1}H NMR (500 MHz, D_2O) δ: 3.81 (app t, splittings of 6, 6.5 Hz, 1H, α-CH), 2.61

(t, *J* = 7 Hz, 2H, CH_2CN), 2.06-2.00 (m, 2H, CH_2), 1.87-1.76 (m, 2H, CH_2); ^{13}C NMR (125.8 MHz, D_2O) δ: 174.4 (C=O), 121.3 (CN), 54.3 (α-CH), 29.7 (CH_2), 20.8 (CH_2), 16.4 (CH_2CN); ESI^+ MS (MeOH/H_2O, 20 μL/min) *m/z* 143 $[M+H]^+$: CID of *m/z* 143, *m/z* 97 (100%).

(*R*)-5-Cyanonorvaline (62.8 mg, 72%); mp 227-231°C (dec); $[\alpha]_D^{20}$ -13.0°; >99% ee by HPLC; ^1H NMR (500 MHz, D_2O) δ: 3.80 (t, J = 6 Hz, 1H, α-CH), 2.61 (app t, splittings of 7 Hz, 2H, CH_2CN), 2.04-2.01 (m, 2H, CH_2), 1.87-1.78 (m, 2H, CH_2); ^{13}C NMR (500 MHz, D_2O) δ: 174.4 (C=O), 121.3 (CN), 54.3 (α-CH), 29.7 (CH_2), 20.8 (CH_2), 16.4 (CH_2CN); ESI^+ MS (MeOH/H_2O, 20 μL/min) *m/z* 143 $[M+H]^+$: CID of *m/z* 143, *m/z* 97 (100%).

(*S*)-2-Amino-6-cyanohexanoic Acid (85.2 mg, 63%); mp 226-227°C (dec); $[\alpha]_D^{21}$ 6.7° (c = 0.115, H_2O); >99% ee by HPLC; ^1H NMR (500 MHz, D_2O) δ: 3.78 (t, J = 6 Hz, 1H, α-CH); 2.56 (app t, splittings of 7.5, 7 Hz, 2H, CH_2CN), 1.97-1.90 (m, 2H, CH_2), 1.78-1.74 (m, 2H, CH_2), 1.60-1.55 (m, 2H, CH_2); ^{13}C NMR (125.8 MHz, D_2O) δ: 174.8 (C=O), 121.9 (CN), 54.7 (α-CH), 29.9 (CH_2), 24.3 (CH_2), 23.8 (CH_2), 16.3 (CH_2CN); ESI^+ MS (MeOH/H_2O, 20 μL/min) *m/z* 157 $[M+H]^+$: CID of *m/z* 157, *m/z* 111 (100%).

(*R*)-2-Amino-6-cyanohexanoic Acid (93.7 mg, 65%); mp 228-230°C (dec); $[\alpha]_D^{21}$ -5.3° (c = 0.175, H_2O); >99% ee by HPLC; ^1H NMR (500 MHz, D_2O) δ: 3.78 (t, *J* = 8 Hz, 1H, α-CH), 2.56 (app t, splittings of 7, 7.5 Hz, 2H, -CH_2CN),

1.94-1.92 (m, 2H, CH_2), 1.79-1.73 (m, 2H, CH_2), 1.61-1.53 (m, 2H, CH_2); ^{13}C NMR (125.8 MHz, D_2O) δ: 174.9 (C=O), 121.9 (CN), 54.7 (α-CH), 29.9 (CH_2), 24.3 (CH_2), 23.8 (CH_2), 16.3 (CH_2CN); ESI^+ MS (MeOH/H_2O, 20 μL/min) *m/z* 157 $[M+H]^+$: CID of *m/z* 157, *m/z* 111 (100%).

2.7 - Preparation of Basic Amino Acids:

$CoCl_2 \cdot 6H_2O$ (0.124 g, 0.52 mmol) and $NaBH_4$ (0.10 g, 2.6 mmol) were added to a solution of cyanoamino acid (0.26 mmol) in H_2O (1 mL). A black precipitate formed upon addition of $NaBH_4$ and the resulting mixture was stirred at room temperature until the reaction was complete (~20 min), as determined by OPA HPLC. The reaction mixture was quenched with 1 M HCl (1 mL) and acetone (1 mL), and filtered. The resulting filtrate was applied to an Amberlite IR 120 ion-exchange column. The column was washed with water (~200 mL) and eluted with 0.5 M aqueous NH_3. Product was isolated as an off white residue after concentration *in vacuo.* The dihydrochloride salts of the basic amino acids were made by the addition of 1 M HCl (2.2 equivalents). Concentration of this solution yielded an oil which was crystallized from MeOH/ether.

(*S*)-Ornithine Dihydrochloride (0.015 g, 72%); ^1H NMR (500 MHz, D_2O) δ: 3.93 (t, *J* = 6 Hz, 1H, α-CH), 3.10 (t, *J* = 7.5 Hz, 2H, CH_2NH_2), 2.04-1.98 (m, 2H, CH_2), 1.92-1.86 (m, 1H, CH_2), 1.83-1.78 (m, 1H, CH_2); ^{13}C NMR (125.8 MHz, D_2O) δ: 173.4 (C=O), 53.7 (α-CH), 39.0 (CH_2NH_2), 27.4, 22.9 (CH_2); ESI^+ MS

(MeOH/H_2O, 20 µL/min) *m/z* 133 $[M+H]^+$: CID of *m/z* 133, *m/z* 116 (32%), 115 (100%).

(*S*)-Lysine Dihydrochloride (0.072 g, 94%); mp 193-195°C (dec) lit.[56] 193°C (dec); $[\alpha]_D^{19}$ 18.3° (c = 0.035, 5 M HCl); lit.[57] $[\alpha]_D^{25}$ 15.3° (c = 0.5, H_2O); ^1H NMR (500 MHz, D_2O) δ: 4.06 (app t, splittings of 6, 6.5 Hz, 1H, α-CH), 3.07 (app t, splittings of 7.5, 8 Hz, 2H, CH_2NH_2), 2.05-1.98 (m, 2H, CH_2), 1.79-1.74 (m, 2H, CH_2), 1.60-1.52 (m, 2H, CH_2); ^{13}C NMR (125.8 MHz, D_2O) δ: 172.7 (C=O), 53.2 (α-CH), 39.2 (CH_2NH_2), 29.5, 26.5, 21.6 (CH_2); ESI^+ MS (MeOH/H_2O, 20 µL/min) *m/z* 147 $[M+H]^+$: CID of *m/z* 147, *m/z* 130 (100%), 129 (15%).

(*R*)-Lysine Dihydrochloride (0.013 g, 45%); mp 193-196°C (dec); $[\alpha]_D^{19}$ -15.4° (c = 0.065, 5 M HCl); ^1H NMR (500 MHz, D_2O) δ: 4.02 (app t, splittings of 6, 6.5 Hz, 1H, α-CH), 3.06 (app t, splittings of 7.5, 8 Hz, 2H, CH_2NH_2), 2.04-1.97 (m, 2H, CH_2), 1.79-1.74 (m, 2H, CH_2), 1.59-1.52 (m, 2H, CH_2); ^{13}C NMR (500 MHz, D_2O) δ: 173.0 (C=O), 53.4 (α-CH), 39.2 (CH_2NH_2), 29.5, 26.5, 21.6 (CH_2); ESI^+ MS (MeOH/H_2O, 20 µL/min) *m/z* 147 $[M+H]^+$: CID of *m/z* 147, *m/z* 130 (100%), 129 (15%).

(*S*)-2,7-Diaminoheptanoic Acid Dihydrochloride (0.036 g, 94%); mp 198-201°C (dec); lit.[58] mp 239-242°C; $[\alpha]_D^{22}$ 16.9° (c = 0.5, 5 M HCl); lit.[58] $[\alpha]_D^{23}$ 14.4° (c = 0.5, 1 M HCl); ^1H NMR (500 MHz, D_2O) δ: 3.95 (app t, splittings of 6.5, 6 Hz, 1H, α-CH), 3.04 (app t, splittings of 7, 7.5 Hz, 2H, CH_2NH_2), 1.07-1.96

(m, 2H, CH_2), 1.74-1.71 (m, 2H, CH_2). 1.49-1.47 (m, 4H, CH_2); ^{13}C NMR (125.8 MHz, D2O) δ: 173.7 (C=O), 53.9 (α-CH), 39.4 (CH_2NH_2), 30.4, 26.5, 25.3, 23.8 (CH_2); ESI^+ MS (MeOH/H_2O, 20 μL/min) *m/z* 161 $[M+H]^+$: CID of *m/z* 161, *m/z* 144 (100%), 143 (13%), 115 (16%), 98 (20%).

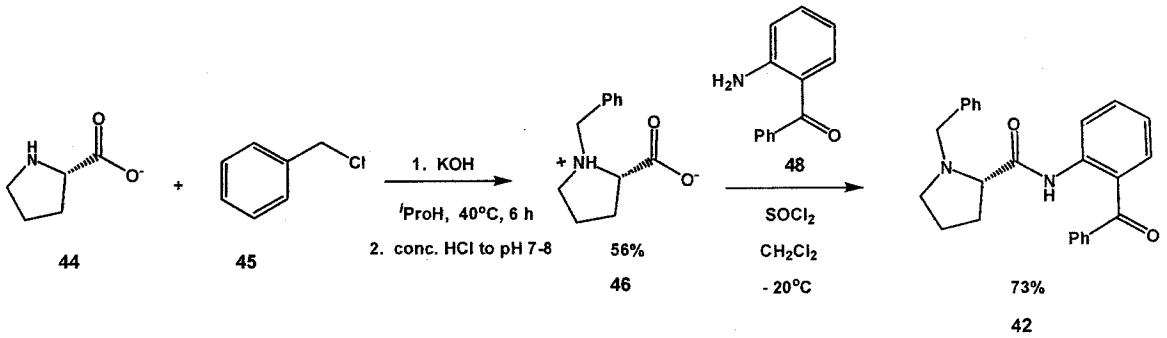
(*R*)-2,7-Diaminoheptanoic Acid Dihydrochloride (0.037 g, 90%); mp 195-200°C (dec); $[\alpha]_D^{22}$ -15.6° (c = 0.17, 5 M HCl); ^1H NMR (500 MHz, D_2O) δ: 3.94 (app t, splittings of 6 Hz, 1H, α-CH), 3.04 (app t, splittings of 7, 8 Hz, 2H, CH_2NH_2), 1.99-1.95 (m, 2H, CH_2), 1.74-1.72 (m, 2H, CH_2), 1.53-1.48 (m, 4H, CH_2); ^{13}C NMR (125.8 MHz, D_2O) δ: 173.7 (C=O), 54.0 (α-CH), 39.4 (CH_2NH_2), 29.9, 26.5, 25.3, 23.8 (CH_2); ESI^+ MS (MeOH/H_2O, 20 μL/min) *m/z* 161 $[M+H]^+$: CID of *m/z* 161, *m/z* 144 (100%), 143 (15%), 115 (15%), 98 (23%).

Chapter 3.0 - Results and Discussion

3.1 - Synthesis of 2-[*N*-(*N'*-Benzylprolyl)amino]benzophenone (BPB)

The synthesis of (*S*)-BPB ligand (**42**) and the use of the BPB complex (**36**) to synthesize L-amino acids from glycine (**43**) was introduced by Belokon[36] in the mid 1980's. With widespread interest in the synthesis of L-amino acids, the (*S*)-forms of the BPB ligand (**42**) and BPB complex have become commercially available. For the synthesis of D-amino acids, the corresponding (*R*)-isomers of BPB ligand and BPB complex are required.[59-61] The syntheses of (*R*)-BPB ligand and (*R*)-BPB complex were achieved in this thesis from D-proline after reaction conditions were established for the corresponding (*S*)-series, starting from the less expensive L-proline.

The synthesis of the chiral BPB ligand (**42**) auxiliary (Scheme 23) was based on Belokon's most recent published procedure.[37] *N*-Benzylproline (**46**) was produced and coupled to 2-aminobenzophenone (**48**) to form the chiral ligand auxiliary, which was subsequentually used in the synthesis of the BPB complex (**36**).



Scheme 23: (*S*)-BPB ligand (**42**) synthesis.

In the first step, proline (**44**) was alkylated under basic conditions using a slight excess of freshly distilled benzyl chloride (**45**). After neutralizing the reaction mixture using concentrated HCl, $CHCl_3$ was added and the heterogeneous mixture was filtered. Evaporation of the filtrate yielded a yellow oil, which crystallized upon the addition of acetone at room temperature. The yield at this stage (56%) was lower than that reported (89%),[37] and the formation of unwanted side products was observed. For instance, *N,N*-dibenzylproline was detected in the reaction mixture using TLC (by comparison to a standard), and formation of benzyl alcohol was also possible. While benzyl alcohol would be removed by the acetone treatment, crystallization was used to remove *N,N*-dibenzylproline and purify *N*-benzylproline (**46**) for the next reaction step. It is interesting to note that Belokon's product prepared by this method[37] was used in the next step without purification.

During initial attempts to couple *N*-benzylproline (**46**) with aminobenzophenone (**48**) (see later), a more significant problem with the purity of *N*-benzylproline was discovered. Low yields (~10%) of BPB ligand (**42**) correlated with the detection of *N*-benzylproline hydrochloride in three lots of *N*-benzyl-(*S*)-proline and one sample of *N*-benzyl-(*R*)-proline. The hydrochloride salt was detected by dissolving a portion of the *N*-benzylproline sample in H_2O and adding a solution of $AgNO_3$ in H_2O to precipitate Cl^- ion as AgCl.

The Cl^- ion was removed by ion-exchange on Amberlite IR 120. Lyophilization of the aqueous column effluent yielded *N*-benzylproline (**46**) as an off-white solid, which produced very little or no precipitate in the $AgNO_3$ test.

These samples were recrystallized from $CHCl_3$/ether and gave significantly increased yields (~50%) of BPB ligand under non-optimized conditions for the coupling reaction.

Detection of the hydrochloride salt in *N*-benzylproline (**46**) by other means is not obvious. ^1H NMR analysis was carried out in D_2O, conditions under which *N*-benzylproline and its HCl salt are in equilibrium. Also, *N*-benzylproline and its hydrochloride salt have similar melting points (164°C[55] vs 170-170.5°C[62]) and specific rotations (-28.4[55] vs -30.4[63]). The *N*-benzyl-(*S*)-proline samples contaminated with HCl produced here resulted in melting points between 168°C and 173°C while the rotations obtained ranged from –25° to –30°. Belokon's sample of *N*-benzylproline prepared by this method[37] melted at 174-175°C, a value close to the melting point of the hydrochloride salt.

N-Benzylproline HCl formed when HCl was added to the reaction mixture. In the published procedure,[37] HCl was added to a pH of 5-6. To avoid producing the HCl salt in subsequent reactions, less HCl was added, by stopping at a higher pH range, 7-8. This proved to be beneficial as subsequent *N*-benzylproline samples did not test positive in the $AgNO_3$ test, and these samples were successfully coupled to 2-aminobenzophenone in the next step.

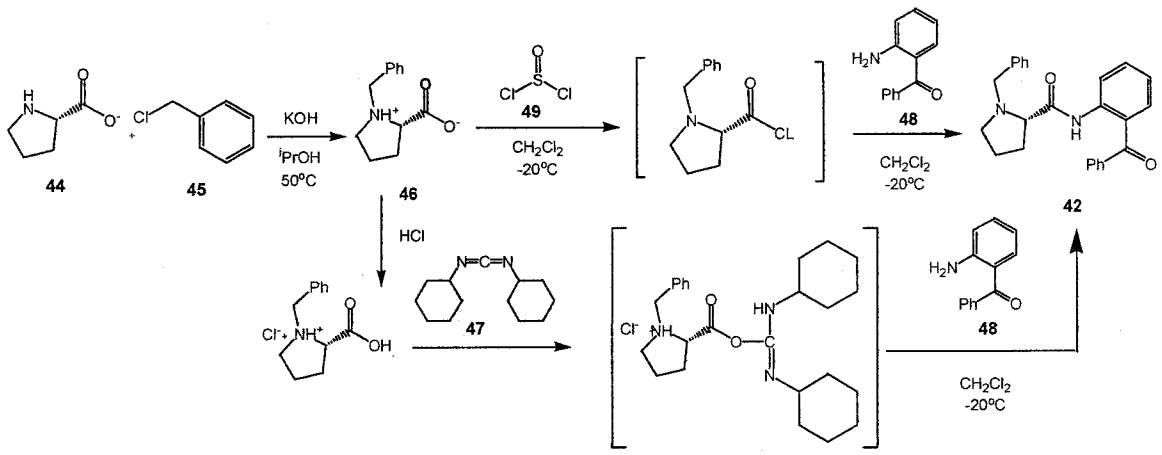
N-Benzyl-(*S*)-proline was produced four times while *N*-benzyl-(*R*)-proline was isolated once using the optimized reaction conditions. Each recrystallized product was characterized by melting point, NMR spectroscopy, and specific rotation. Melting point (165-166°C), ^1H, and ^{13}C NMR spectra were consistent with published data.[36,55] Data obtained for the (*R*)-isomer agreed with those

obtained for the (*S*)-isomer. Enantiomeric purity was assessed by specific rotation. Rotations obtained for the *(S)*-isomer ranged between –24.5° to –26.4° giving an average rotation of -25.5°. The negative rotation value verified the configuration of the *(S)*-isomer. Two rotation values were obtained for the *(R)*-isomer: +27.2° and +29.4° giving an average of +28.3°. The opposite, positive rotation values confirm the configuration of the *(R)*-isomer. Overall, the rotation values matched well with the literature rotation of -28.4°,[55] indicating 90% or better e.e.

The synthesis of *(S)*- and *(R)*-2-[*N*-(*N'*-benzylprolyl)amino]benzophenone (**42**, BPB) requires coupling of *N*-benzylproline (**46**) and aminobenzophenone (**48**) (Scheme 24). Initially, Belokon[36] used DCC (dicyclohexylcarbodiimide, **47**) to activate the carboxyl group in *N*-benzylproline for the amide bond formation step with 2-aminobenzophenone. This protocol was attempted, but proved to be unsatisfactory because formation of a side product, dicyclohexylurea, made product isolation difficult.

The purpose of DCC is to convert the oxygen in the carboxyl group of *N*-benzylproline into a good leaving group, allowing the activated carbonyl to readily form an amide with the aniline nitrogen of 2-aminobenzophenone. In Belokon's more recent protocol,[37] thionyl chloride (**49**) was used in place of DCC. The new conditions were advantageous, enabling the reaction to be conducted on a large scale without worrying about the removal of dicyclohexylurea, the unwanted side product from DCC coupling.

In this coupling procedure (Scheme 24), thionyl chloride was added to a cooled suspension of *N*-benzylproline in CH_2Cl_2. After a short period, the amino acid dissolved, indicating conversion to an activated, carboxylic acid derivative. 2-Aminobenzophenone (**48**) was added at this stage, and the reaction mixture was kept cold for several hours. BPB ligand (**42**) was isolated by extraction after the reaction mixture was neutralized with Na_2CO_3.



Scheme 24: Alternative routes for (*S*)-BPB ligand synthesis.

The molar equivalents of thionyl chloride (**49**) used in the published procedure[37] were unclear: Belokon reports that 0.5 mol (35.5 g) of thionyl chloride, 0.4 mol (78 g) *N*-benzylproline (**46**) and 0.25 mol (50 g) of aminobenzophenone (**48**) were employed. However, 0.5 mol of thionyl chloride is not equal to 35.5 g; it is 59.5 g. On the other hand, 35.5 g is 0.3 mol, a substoichiometric amount relative to *N*-benzylproline, but greater than the number of moles of the limiting reactant, 2-aminobenzophenone.

Initially, the BPB reaction was attempted using a 25% excess of thionyl chloride to *N*-benzylproline (the ratio of *N*-benzylproline to aminobenzophenone

was unchanged). Within 2-4 h a precipitate formed in the reaction mixture, and the product was isolated in low yields, 20-40%. The samples of *N*-benzylproline in these reactions were subsequently tested using $AgNO_3$ and found to be HCl-free. Other reactions were also conducted using the same ratio of thionyl chloride, but the *N*-benzylproline samples were not assessed by $AgNO_3$. Low yields, 10-20% were obtained, and a precipitate formed during the early stages, (0.5-1 h) of these reactions. In reactions later conducted using samples now known to contain *N*-benzylproline HCl, a precipitate formed in the reaction mixture after the *N*-benzylproline had dissolved, but before 2-aminobenzophenone had been added. If 2-aminobenzophenone was added and the procedure was carried through, the yields of the BPB ligand (**42**) were negligible (<10%).

The correlation of the low yields with the timing of precipitate formation and the variable HCl content of the *N*-benzylproline (4**6**) samples suggested that *N*-benzylproline precipitated from the reaction mixture as its HCl salt, and therefore was unavailable to couple with 2-aminobenzophenone (**48**). Furthermore, the use of thionyl chloride in excess could result in the formation of large amounts of HCl which could lead to *N*-benzylproline precipitation.

To reduce the amount of HCl in the reaction mixture, thionyl chloride (**49**) was added in a 1:1 molar ratio to *N*-benzylproline, and HCl- free samples of *N*-benzylproline were used. Under these conditions, no precipitate formed during the course of the reaction, and yields of BPB ligand improved to ~70%, or ~ 50% after recrystallization from high boiling petroleum ether.

While Belokon[37] used ethanol to isolate the chiral BPB ligand, ethanol was found to dissolve BPB ligand as well as the aminobenzophenone remaining in the mixture. Isopropanol was substituted for the ethanol in the literature treatment. BPB ligand was found to be less soluble in isopropanol and, importantly, aminobenzophenone (**48**) remained very soluble. This treatment proved to be more successful at separating the BPB ligand (**42**) from 2-aminobenzophenone and in turn promoted more ligand to crystallize.

With the above changes, the reaction yields were as good as those reported by Belokon[37] for six repetitions for the reactions forming *(S)*-BPB ligand and three for *(R)*-BPB ligand. The yields of isolated BPB ligand ranged from 60-88%, and the average yield of isolated *(S)*-BPB ligand (73%) was comparable to Belokon's yield of 81%.[37] Furthermore, the yield of *(S)*-BPB ligand after recrystallization (57%) was slightly higher than the 50% reported by Belokon for the DCC method.[36] This yield is also higher than the 44% obtained by a previous student in this laboratory[63] using the DCC method.

Recrystallization from high boiling petroleum ether gave a crystalline solid. Melting points were in the range of 98-100°C, which is very close to the literature[36] value of 101-102°C. Signals in the ^{1}H and ^{13}C NMR spectra closely matched with those reported in the literature.[36] The enantiomeric purity of the BPB ligand was determined by measuring its specific rotation. Rotations obtained for the *(S)*-BPB ligand ranged between -129.5° and -139° to yield an average rotation of -133.9°. This value agrees well with the literature value for the (*S*)-isomer (-134.5°).[36] The (*R*)-BPB ligand, synthesized three times, gave

rotation values ranging from +133° to +141°. The average rotation obtained for this isomer was +136.6° which is very close to that reported[37] for the (*S*)-BPB ligand.

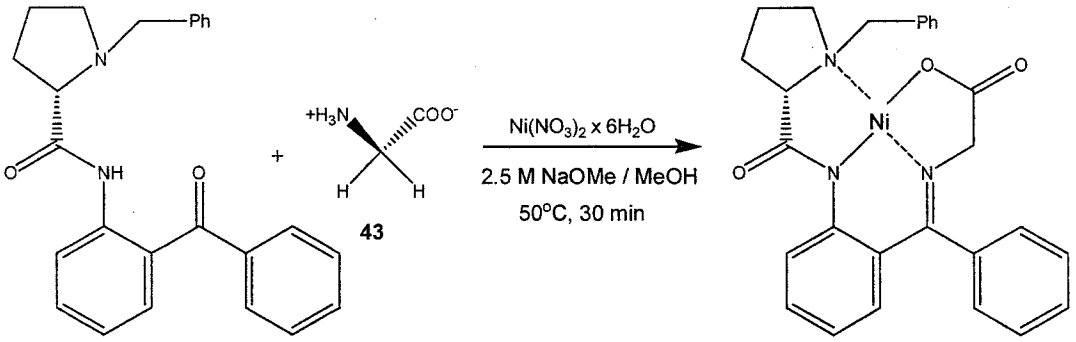
3.2 - Synthesis of BPB-Complex

In Belokon's original synthesis of the BPB complex (**36**),[36] a 5:1 ratio of glycine (**43**) to BPB ligand (**42**) and a 2:1 ratio of Ni ion to BPB ligand was used. This approach is inefficient, and leaves excess Ni^{2+} ions and glycine as waste in the aqueous wash of the work up. In Nadvornik and Popkov's[38] modified method, almost equal equivalents of Ni ion to BPB ligand (1.2:1) and only a 2:1 excess of glycine to BPB ligand were employed to conserve reagents and to create a "greener" method.

The reduction in the amount of glycine required for BPB complex formation is also particularly advantageous for syntheses incorporating isotopically labeled glycines. For this reason, Nadvornik and Popkov's recent protocol[38] was adopted as a starting point in the synthesis of BPB complex (Scheme 25). Upon the addition of NaOMe to a solution of BPB ligand, glycine, and $Ni(NO_3)_2 \cdot 6H_2O$, the mixture was transformed into a deep red color, which is characteristic of Ni (II) complexes. In this case, the reaction was complete in approximately 30 min, whereas in Belokon's synthesis,[36] the reaction was complete only after 2 h. This is possibly due to the difference in concentration of the solutions used. Belokon's original protocol[36] employed 1.2 M NaOMe,

whereas Nadvornik and Popkov[38] used 2.5 M NaOMe. Therefore, by increasing the concentration of base, the reaction time is decreased significantly.

Similar to the above reactions, (*S*)-BPB complex was synthesized and characterized seven times before moving on to producing the (*R*)-BPB complex. Yields following recrystallization from acetone ranged from 63-79% for the (*S*)-complex and 80% for the (*R*)-BPB complex. These yields are comparable to the literature yield of 88%,[38] but were slightly lower than yields obtained when a five-fold excess of glycine was employed by Belokon[36] (93%). Nevertheless, this method is a "greener" and more efficient way to produce the BPB complex (**36**) because it conserves reagents and reduces the amount of nickel in the water waste.



Scheme 25: Synthesis of (*S*)-BPB complex.

Melting points obtained for each isomer were in the range of 208-212°C which agrees with the literature melting point of 208-212°C.[36] The ^{1}H NMR spectra obtained for both isomers of the BPB complexes integrated for 11 aliphatic and 14 aromatic protons while the carbon spectrum showed a total of 25

signals (5 CH_2, 13 CH, 7C). The spectrum obtained here are consistent with the data obtained by Nadvornik et al.[64,65] who characterized the (*S*)-BPB complex by 2-D NMR spectroscopy.

Enantiomeric purity of the *(S)*- and *(R)* -BPB complex (**36**) was assessed by measuring the specific rotation in $CHCl_3$, a solvent used for specific rotation measurements by a previous student.[63] Rotations obtained for the (*S*)-BPB complex ranged from +1982° to +2168° to give an average rotation of +2121°. The rotation obtained for the (*R*)-BPB complex (-2167°) was consistent with the values obtained for its enantiomer but opposite in sign. These values are consistent with the literature value reported by Belokon of +2006° for *(S)*-BPB complex in MeOH.[37]

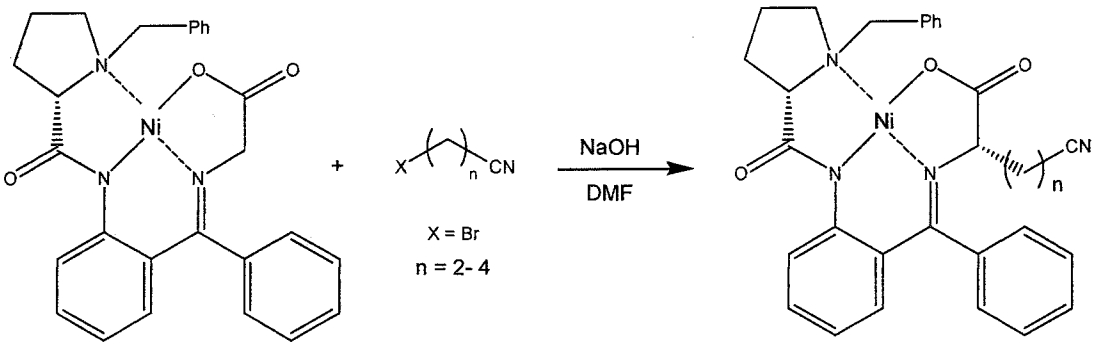
To ensure that the rotations were comparable in the two different solvents, rotations were measured for several samples of (*S*)-BPB complex in MeOH. The values obtained ranged from +2053° to +2495°, averaging to +2205°, indicating that similar values are obtained in MeOH and $CHCl_3$. Furthermore, Belokon's literature rotation[37] was conducted at a concentration of c = 1.0 whereas the present rotations were obtained at a much lower concentration, c = 0.022. The lower concentration was required for use with the polarimeter employed in the present study.

3.3 - Alkylation of BPB complex

Alkylation of the BPB complex (**36**) using powdered NaOH and a series of bromonitriles was conducted in DMF under nitrogen (Scheme 26). The alkylated

complex was purified by using either recrystallization or chromatography on silica gel. Under the optimized conditions, the yields of alkylated BPB complexes (**38-40**) were 77-93% before purification. The alkylated complexes readily hydrolyzed in mild acid liberating the nitrile amino acids, which were isolated in yields of 60-90% after ion-exchange chromatography.

The initial alkylation reaction conditions were modeled on procedures used by previous students in this laboratory.[63,66] While these students successfully carried out the alkylation reaction without incorporating the precautions of cooling and the use of an inert gas given in the published literature procedure,[40] closer inspection of their results revealed significant variations in their yields and varying amounts of a side-product accompanying the alkylated BPB complex. A series of experiments in the present study demonstrated that the amount of base, the order of addition, and the use of nitrogen were key for the alkylation reaction to proceed efficiently.



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n = 2, **38**
n = 3, **39**
n = 4, **40**

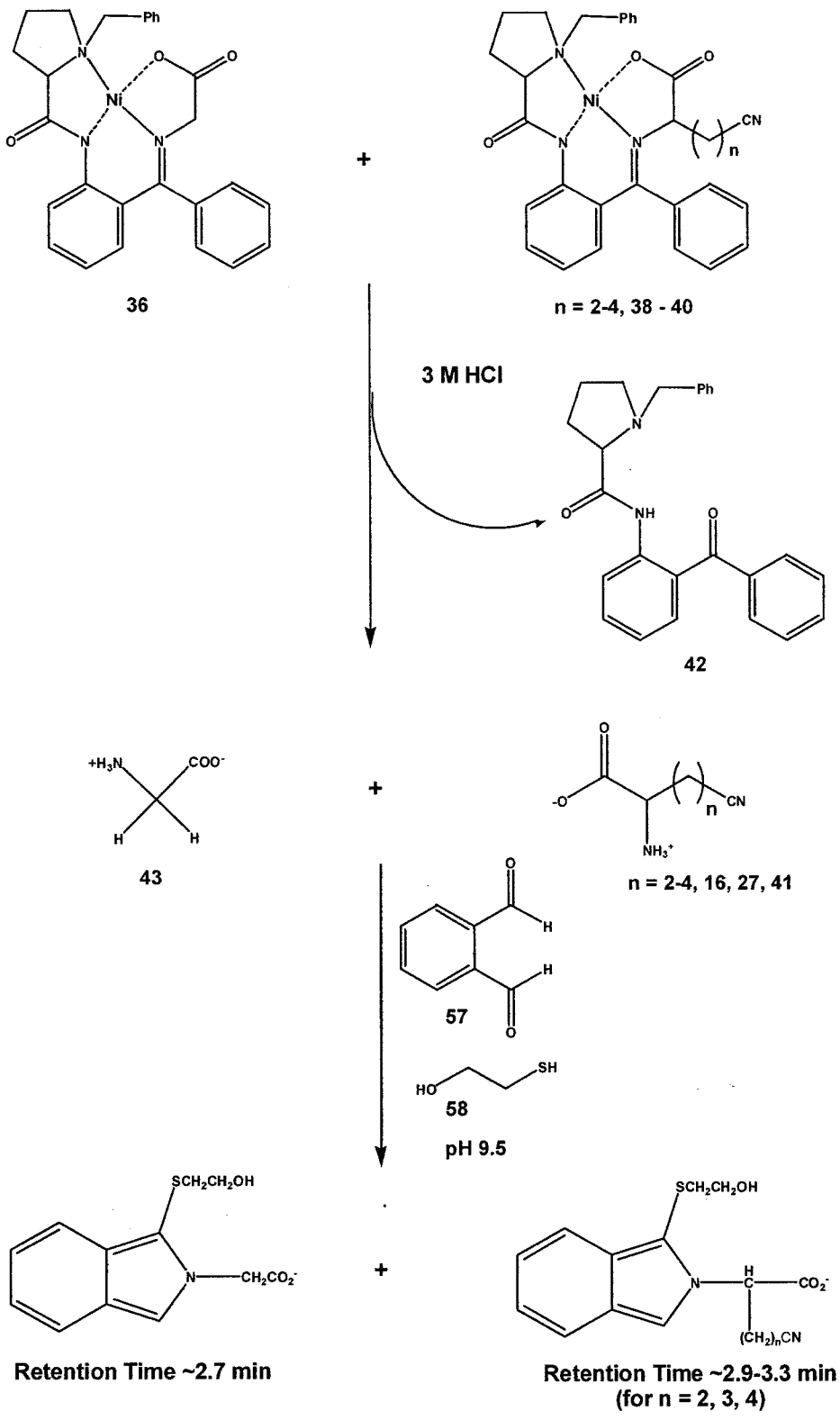
Scheme 26: Alkylation of the (*S*)-BPB complex by bromonitriles (n = 2, 3, 4).

3.3.1 –Monitoring reaction progress by HPLC

Small portions of the alkylation reaction mixture were removed at various times during the course of the reaction and mixed with 3 M HCl to hydrolyze alkylated and unalkylated BPB complex to the nitrile amino acid and glycine (**43**), respectively (Scheme 27). Liberated BPB ligand (**42**) was extracted into chloroform, and the amino acids present in the aqueous layer were reacted with *o*-phthaldehyde (**57**) and mercaptoethanol (**58**). In this precolumn derivatization reaction, the amino acids were converted to fluorescent isoindole derivatives for detection and separation using reversed-phase HPLC.[67] This analytical method is also selective because only primary amines react to form isoindole derivatives. The isoindole derivatives of the nitrile amino acids were retained on the reversed-phase column to a greater extent than the glycine derivative (Figure 2). The presence of glycine represents un-reacted BPB complex (**36**) in the reaction mixture, and the reaction was judged complete when the OPA-derivatized glycine peak was indistinguishable from the baseline (Figure 3).

3.3.2 – Alkylation of BPB complex: Initial attempts

The importance of monitoring the alkylation reaction was demonstrated in the first alkylation reaction conducted. Conditions for the alkylation were reproduced from a previous student's protocol[63] in which 5-bromovaleronitrile (n = 4) and 2.5 equivalents of NaOH were added to 225 mg of *(S)*-BPB complex (**36**) in DMF. The reaction was allowed to proceed at room temperature for 45 min before work-up. The work-up consisted of neutralization with acetic



Retention Time ~2.7 min

Retention Time ~2.9-3.3 min (for n = 2, 3, 4)

Scheme 27: Hydrolysis of alkylated BPB complex and conversion of amino acids to fluorescent isoindole derivatives.

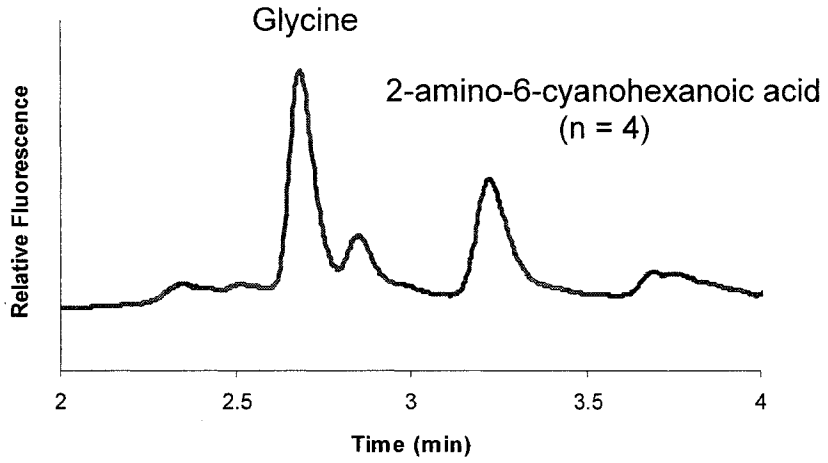


Figure 2: Characterization of isoindole derivatives formed from an alkylation reaction mixture at ~ 1 min, showing incomplete reaction.

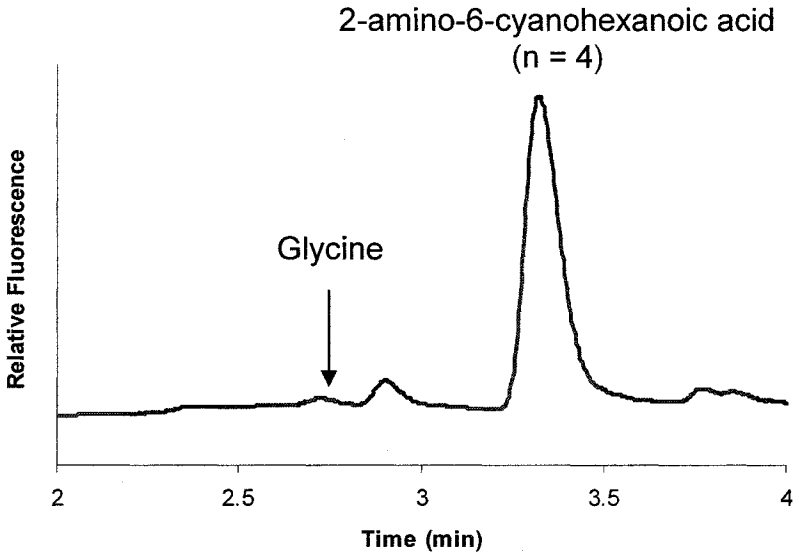


Figure 3: Characterization of isoindole derivatives formed from an alkylation reaction mixture at ~ 25 min, showing almost complete utilization of glycine.

acid and extraction of the alkylated BPB complex into $CHCl_3$. A portion of the $CHCl_3$ extract was removed for OPA HPLC analysis. The chromatogram showed two peaks of approximately equal intensity. One corresponded to the OPA-derivatized nitrile amino acid and the other to OPA-derivatized glycine (derived from unalkylated complex). This result indicated that the reaction was incomplete, and that it was important to monitor the reaction to determine when it was complete since reaction times obviously vary. To increase the rate of the reaction, the amount of NaOH was increased to a three-fold excess.

Therefore, in subsequent alkylation reactions, three equivalents of powdered NaOH and one equivalent of *(S)*-BPB complex (conducted on scales ranging from 225 mg to 500 mg) were employed. Also, the amount of electrophile in the reaction was increased to 1.1 equivalents to ensure that the reaction would proceed to completion. Each time, the reaction was monitored at 25 min by HPLC (Figure 4) and a large peak at retention time 3.2 min was observed and attributed to the OPA-derivatized nitrile amino acid. No peak at 2.7 min corresponding to OPA-derivatized glycine was present indicating a complete reaction. Therefore, the reaction was worked-up, and the alkylated complex was hydrolyzed to liberate the nitrile amino acid. Amino acid product was isolated as a white powder after freeze drying the fractions collected from the ion-exchange column. Yields (~30%), however, were less than half of the 60-85% reported for this procedure by a previous student.[63] The isolated product was confirmed as 2-amino-6-cyanohexanoic acid (**41**, n = 4) by comparison of its retention time with a standard sample through OPA HPLC analysis.

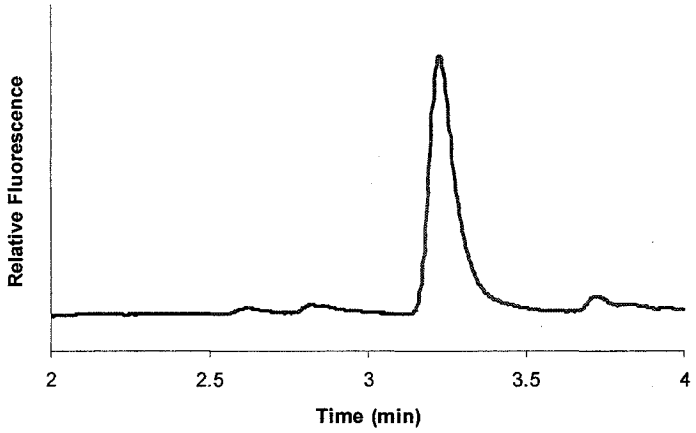
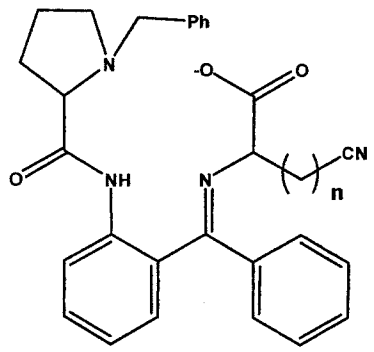


Figure 4: HPLC analysis of the hydrolyzed reaction mixture at 25 min from the alkylation of BPB complex with 5-bromovaleronitrile (n = 4).

3.3.3 – Development of reaction conditions: Discovery of a side product

To determine why the yield of nitrile amino acid isolated from the alkylation reaction was so low, several possibilities were examined. First, the water wash and sample effluent from the ion-exchange column were examined to determine whether any amino acid did not bind to the ion-exchange resin. Ninhydrin stain indicated the presence of amino acid in these aqueous fractions, but evaporation of the water recovered only a small amount (~5%) of material. The organic layer from the hydrolysis reaction was evaporated and the amount of BPB ligand (**42**) recovered (0.15 g) was less than expected (0.38 g). From this discovery, it was thought that BPB ligand and amino acid were still bound (**59**) and present in the aqueous layer. If this were true, then this species would bind tightly to the ion-exchange resin due to the phenyl rings of the BPB ligand and not elute from the column under the conditions employed. This would account for low yields of both BPB ligand and amino acid.



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To test this hypothesis, another alkylation reaction was conducted under identical conditions as above. After hydrolysis of the alkylated complex, the aqueous layer was analyzed by OPA HPLC. By comparing the integrated peak area obtained for the product to that of a known amount of a standard amino acid, a yield of 10% was calculated. Base (NH_3) was added to the aqueous layer to neutralize it and to free the amino acid and BPB ligand. As base was added, BPB ligand (**42**) was recovered and extracted into $CHCl_3$. Once the pH of the aqueous layer was neutral, HPLC was conducted again but results showed no increase in the amount of amino acid. This experiment showed that BPB ligand was present in the aqueous layer (most likely in the hydrochloride salt form) but was not bound to the amino acid and most importantly, not contributing to the low yields.

Attention was then shifted to the possibility that the solubility properties of the current amino acid under study was the reason for low yields. In particular, the amino acid being liberated contains four methylene groups, which perhaps contributes to lower solubility in H_2O than other amino acids and therefore forces it to stay in the organic layer. To test this, the next series of alkylations were conducted using 4-bromobutyronitrile (n = 3). The amino acid from these

reactions, 5-cyanonorvaline (**27**), is known to be quite soluble in H_2O. Therefore, if yields improved, the low recoveries obtained thus far could be attributed to the solubility properties of the amino acid.

An alkylation reaction mixture containing *(S)*-BPB complex (500 mg), 4-bromobutyronitrile and NaOH (3 equivalents) was monitored by OPA HPLC at 25 min. The chromatogram (Figure 5) showed the reaction to be complete as almost no glycine (**43**) was present after 25 min. The worked-up reaction mixture (oily residue, 86%) was immediately hydrolyzed to the amino acid. In the hydrolysis work-up, concentrated NH_3 was added to the aqueous portion, and 86% of the BPB ligand (**42**) was recovered. However, the yield of amino acid in the aqueous layer calculated from HPLC data was only 35%. Therefore, similar yields were obtained for two different amino acids, and the solubility of an individual amino acid is not the main cause of the low yield.

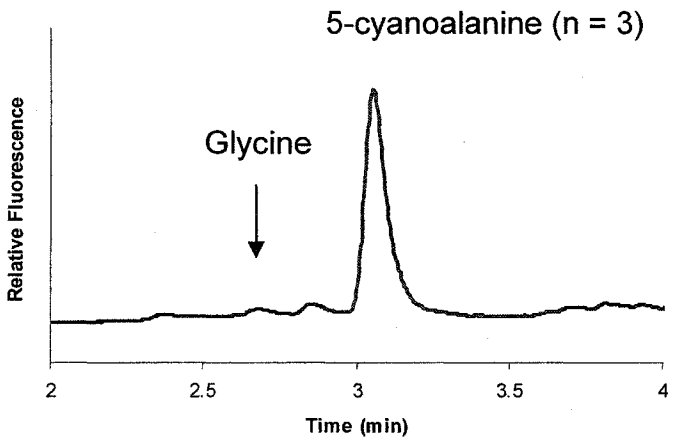


Figure 5: HPLC analysis of the hydrolyzed, 25 min reaction mixture from the alkylation of BPB complex with 4-bromobutyronitrile.

To examine the alkylation reaction in more detail, another alkylation was conducted under identical conditions, and judged to be complete after 25 min using OPA HPLC. The alkylated complex was isolated as an oily residue (DMF still present), and applied to a reversed-phase silica column. The isolated product (DMF free) was obtained in low yield (30%). Its ^{1}H NMR spectrum integrated for 14 aromatic protons and 16 aliphatic protons, while the carbon spectrum showed 9 aliphatic resonances and 16 aromatic resonances. These data were consistent with the product being the alkylated complex. For further details on this characterization and 2-D NMR studies, see Section 2.3.8.

The purified alkylated complex was hydrolyzed in aqueous acid, and the nitrile amino acid was isolated in 80% yield, suggesting that low yields were not associated with the hydrolysis step. The ^{1}H NMR spectrum of the product was consistent with 5-cyanonorvaline (**27**).

When the above alkylation reaction was repeated, the worked-up reaction mixture was applied to a reversed-phase silica column, and the alkylated complex was obtained in 29% yield. BPB ligand, **42** (0.174 g), was also eluted from this column. Together, the isolated alkylated complex and recovered BPB ligand accounted for ~74% of the initial BPB complex (**36**). If hydrolysis of BPB complex or alkylated BPB complex occurred during the reaction or work-up, glycine and nitrile amino acid would be produced. HPLC analysis of the aqueous layer from the alkylation reaction, however, showed no amino acid peaks. Therefore, it is very unlikely that simple hydrolysis of the BPB complex or alkylated BPB complex was occurring.

The question was then posed that if neither the unalkylated or alkylated complex was hydrolyzing, what was producing the ligand? The 0.5 M acetic acid used for neutralization in the work-up was not a strong enough acid to hydrolyze the complex so it was hypothesized that the excess base in the reaction mixture could breakdown the BPB complex and release BPB ligand (**42**). To test this hypothesis, a reaction was conducted (250 mg of BPB complex) with 5-bromovaleronitrile (n = 4), and the reaction pot was cooled during the addition of base (in the hope of preventing a hydrolysis from taking place) and then allowed to warm to room temperature after the addition was complete. The reaction was monitored using OPA HPLC at 55 min and worked-up in a similar manner. Before applying the reaction mixture to a reversed-phase column, a TLC was conducted on a reversed-phase silica plate (solvent: MeOH/H_2O, 80/20), which did not show any spot corresponding to alkylated BPB complex. Instead, a spot at a very low Rf value was present in addition to a BPB ligand spot.

Despite not forming the desired product, the product obtained was chromatographed on a reversed-phase silica column, and 0.110 g of a reddish/brown solid was obtained. This color indicated that a Ni^{2+} complex was still being formed. The fact that no desired product formed was surprising since the chromatogram showed the presence of the corresponding OPA-derivatized nitrile amino acid. Therefore, a side product must have formed after the formation of the alkylated BPB complex. The ^{13}C NMR spectrum obtained, did not contain a nitrile resonance, nor was there a signal corresponding to $-CH_2CN$, which has a distinct chemical shift in the aliphatic region of the spectrum.

Comparison of the aliphatic region with a ^{13}C NMR spectrum obtained by a previous student[63] showed differences in chemical shifts of the carbons in the aliphatic region, but the same number of signals. This suggested that the electrophile had added, but the complex had changed somehow after the production of the alkylated BPB complex.

A sample of this unknown product was submitted for analysis using APCI mass spectrometry. By comparison of the mass spectra obtained by previous students in this laboratory[63,66] it was clear that this product had formed before. Previous mass spectra contained similar peaks (*m/z* 366.1 and 440.1) in varying amounts in several of the alkylated complexes produced. This could account for the varying yields obtained by previous students.[63,66] The structure of this unknown compound was not determined, as it was felt that it was more important to concentrate on working out the optimal conditions for the alkylation reaction.

3.3.4 – Development of reaction conditions: Effect of base

The possibility that excess base in the reaction mixture led to breakdown of BPB complex (**36**) and release of BPB ligand (**42**) was investigated by conducting three alkylation reactions (on 100 mg scales of BPB-complex) using 5-bromovaleronitrile (n = 4) and different amounts of NaOH (3, 2.5, and 2 equivalents). In each experiment, the reaction progress was monitored by sampling the mixture 10 times over the course of 60 min, and subjecting these samples to HPLC analysis.

In the reaction mixture containing three equivalents of NaOH to BPB complex (**36**), the chromatograms (Figure 6) showed that no glycine (**43**) was present after ~ 10 min (chromatogram B). After 15 min, the prominent OPA-derivatized nitrile amino acid peak began to decrease, and by 40 min (chromatogram C, Figure 6) the area of the OPA-derivatized nitrile amino acid peak was only ~30% of the corresponding peak in chromatogram B. While the product amino acid peak decreased significantly, no new peak appeared in the chromatogram.

When 2.5 equivalents of NaOH were employed (Figure 7), the reaction was judged complete by 10 min (similar to when 3 equivalents of NaOH was used) and, at ~25 min, the OPA-derivatized nitrile amino acid peak had only decreased to 80% of the peak area measured at 10 min. After 25 min, there was no further decrease in nitrile amino acid as can be seen in chromatogram C (intensity is approximately equal to chromatogram B). When 2 equivalents of NaOH were used, the OPA-derivatized glycine peak did not decrease to baseline levels until ~25 min (the OPA-derivatized nitrile amino acid peak grew in over this time) indicating that the alkylation reaction was not complete until that time.

These experiments indicated that excess base promotes the conversion of alkylated complex to a side product that does not liberate a primary amino acid upon acid hydrolysis. To minimize this side reaction, BPB complex (**36**) was alkylated with 2.5 equivalents of NaOH. After 10-15 min the reaction was worked-up, and only a 30% yield of alkylated BPB complex was obtained. HPLC analysis showed 10% glycine present in the product. It was concluded that the

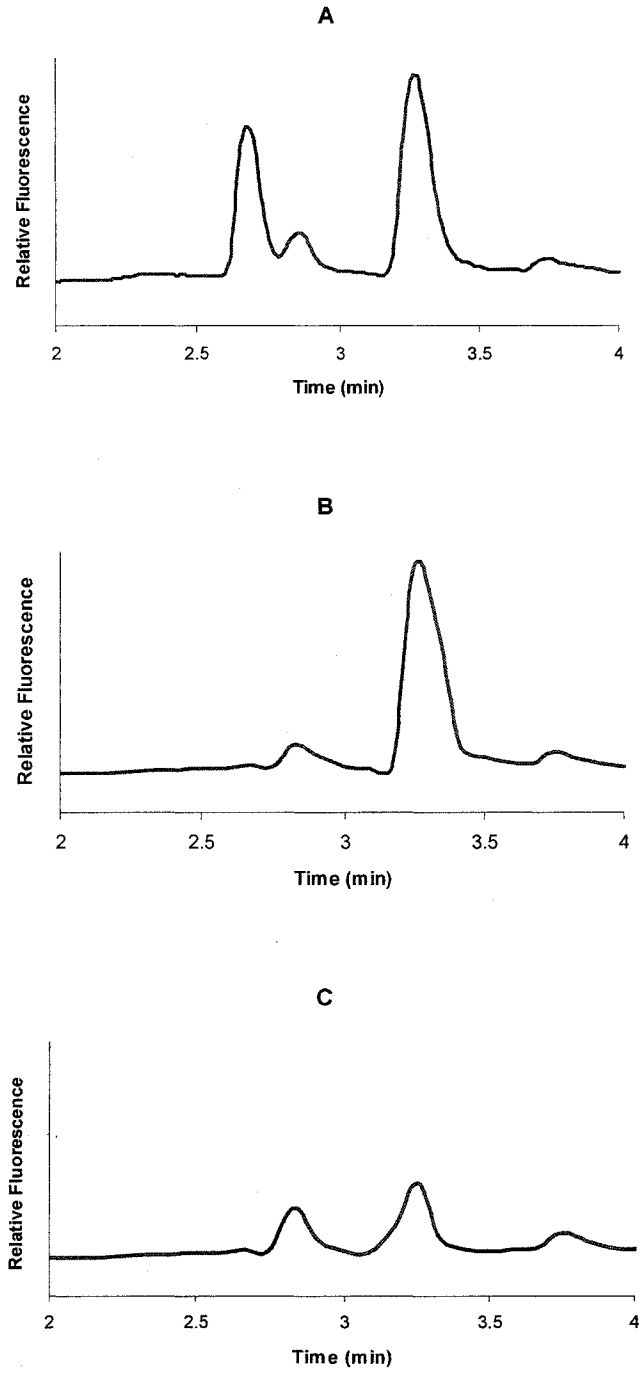


Figure 6: HPLC analysis of the alkylation reaction mixture containing three equivalents of NaOH. A: Reaction at 1 min, B: Reaction at 10 min, C: Reaction at 40 min.

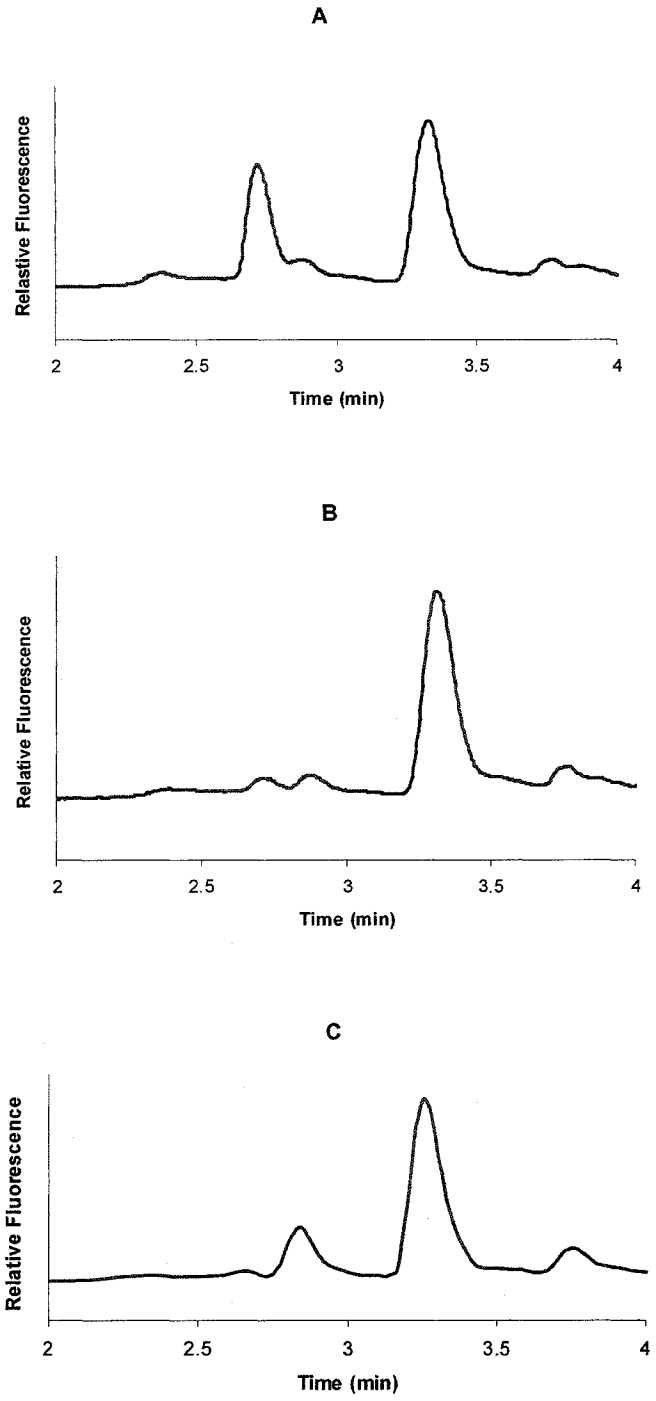


Figure 7: HPLC analysis of the alkylation reaction mixture containing 2.5 equivalents of NaOH. A: Reaction at 1 min, B: Reaction at 10 min, C: Reaction at 40 min.

peak corresponding to the OPA-derivatized nitrile amino acid decreased to a greater extent over time when the reaction was conducted with three equivalents of NaOH. This decrease in the OPA-derivatized nitrile amino acid peak suggests that the side reaction is taking place after the alkylated complex forms. However, it is also possible that the side reaction could be taking place on unalkylated complex, as well.

3.3.5 – Development of reaction conditions: Effect of oxygen

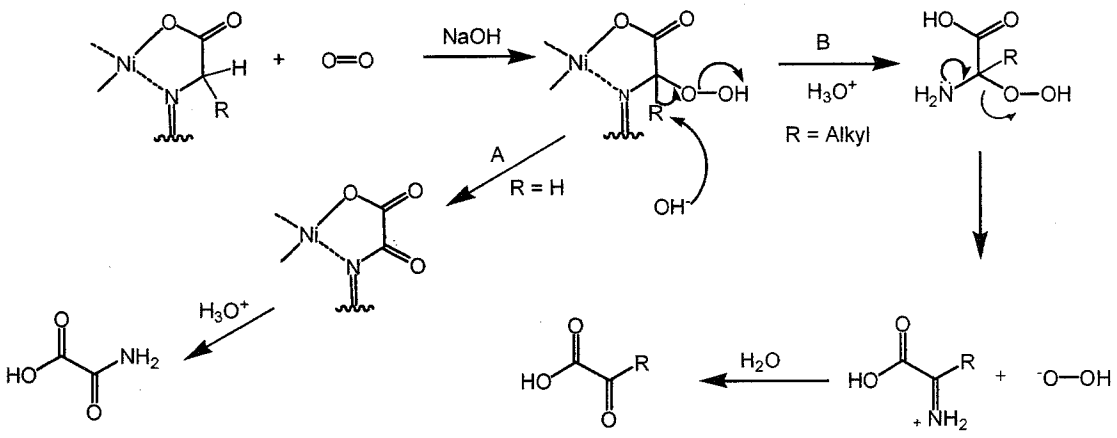
Since the previous reaction did not go to completion, the next few reactions contained the NaOH (3 and 2.5 equivalents) dissolved in the BPB complex (**36**) / DMF solution. The difference with these reactions was that NaOH was stirred in the BPB complex / DMF solution for approximately 30 min before electrophile was added. This was done to dissolve NaOH completely and get all reactants into solution to help the reaction proceed more efficiently and eliminate possible variations in reaction times. In each instance, no alkylated complex formed, but the side product formed as determined by reversed-phase TLC. A final reaction conducted under these same conditions was monitored as above by OPA HPLC (10 times over 60 min), but the chromatograms showed no peaks corresponding to OPA-derivatized glycine (**43**) or nitrile amino acid. This suggested that the BPB complex was reacting with something to change it before any other reaction could occur as it was unlikely that the side product was formed completely within 1 min. This suggests that the side product formation can

occur from BPB complex as well. The obvious explanation for this was the prolonged exposure to NaOH.

To determine if NaOH was reacting with the BPB complex (**36**), a small amount (50 mg) of the BPB complex was dissolved in DMF and 10 mg (0.25 mmol) of NaOH was added. The reaction was immediately sampled for HPLC analysis and also after NaOH had dissolved (~25 min). It was observed that glycine (**43**) was present initially; however after 25 min the peak corresponding to OPA-derivatized glycine was no longer present. This indicated that prolonged exposure to NaOH was changing the complex in such a way that a primary amine was no longer present after acid hydrolysis.

Belokon's literature procedure[40] was then examined and I noticed that his reaction was conducted under argon. This led to the idea that oxygen was reacting with the carbanion complex as suggested in Scheme 28. Scheme 28 shows a possible mechanism for what could be happening to the complex under basic conditions. This possible mechanism is consistent with not seeing any peaks in the HPLC chromatograms as no primary amine is present in the product. Note that only route B is available for the alkylated complex.

To test this hypothesis, the reaction was conducted under nitrogen. (*S*)-BPB complex (**36**, 50 mg) was dissolved in DMF and NaOH (10 mg) was added (already dissolved in DMF). This solution was placed under nitrogen and sampled twice over 30 min. Glycine (**43**) did not decrease over that time frame. However, the amount of glycine present seemed less than expected for the unalkylated complex (by comparison to hydrolyzed unalkylated BPB complex).



Scheme 28: Possible reaction of oxygen and BPB complex under basic conditions.

This indicated that the reaction in Scheme 28 may be happening to a small extent. Therefore, to eliminate the possibility of this side reaction, the order in which reactants were added was changed.

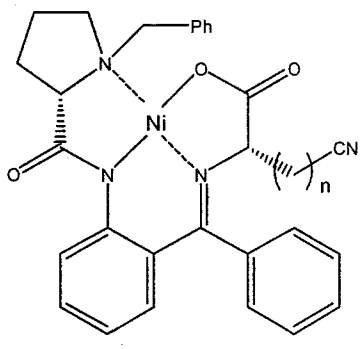
3.3.6 – Development of reaction conditions: Order of addition

The addition of NaOH as the last reagent to the reaction mixture (not dissolving it completely) was explored since NaOH would form the carbanion complex and be forced react with electrophile (already in solution) to form alkylated BPB complex. To test these conditions, 100 mg of BPB complex (**36**) was reacted, and the reaction was sampled 10 times over 60 min. No significant decrease in nitrile amino acid was observed. After the reaction was complete and worked-up, it was applied to a reversed-phase column for purification. A yield of 60% was recovered from this reaction. It should be noted that 200 μL (1/3 of the reaction mixture) was removed to monitor the reaction. The yield

taking into consideration the removed portion would be ~90%. The reaction was repeated (this time using 250 mg of BPB complex) and an 80% yield was obtained. This time, the reaction was not monitored 10 times but just once, at 25 min. This suggested that the alkylation reaction conditions were optimized. Alkylations were then conducted on 750 mg-scales using either the (*S*)- or the (*R*)-BPB complex.

3.3.7 – Alkylation with cyanoalkyl bromides (n = 2-4)

The optimized reaction conditions discussed in the previous sections were used to alkylate the (*S*)- and (*R*)-BPB complexes with alkylcyano bromides (n = 2-4) on 1.5 mmol-scales (i.e., 750 mg of BPB complex, **36**). In one case, 1.0 mmol (500 mg) of (*S*)-BPB complex was alkylated with 4-bromobutyronitrile (n = 3). In each case, the reaction was monitored at 25 min by OPA HPLC. The chromatograms (Figure 8) showed one major peak (retention time 2.9-3.3 min) corresponding to the isoindole derivatives of the product nitrile amino acids (**38**, **39**, **40**) and little or no response at the retention time (2.7 min) expected for the glycine derivative. These results indicated complete reaction of the BPB



n = 2, **38**
n = 3, **39**
n = 4, **40**

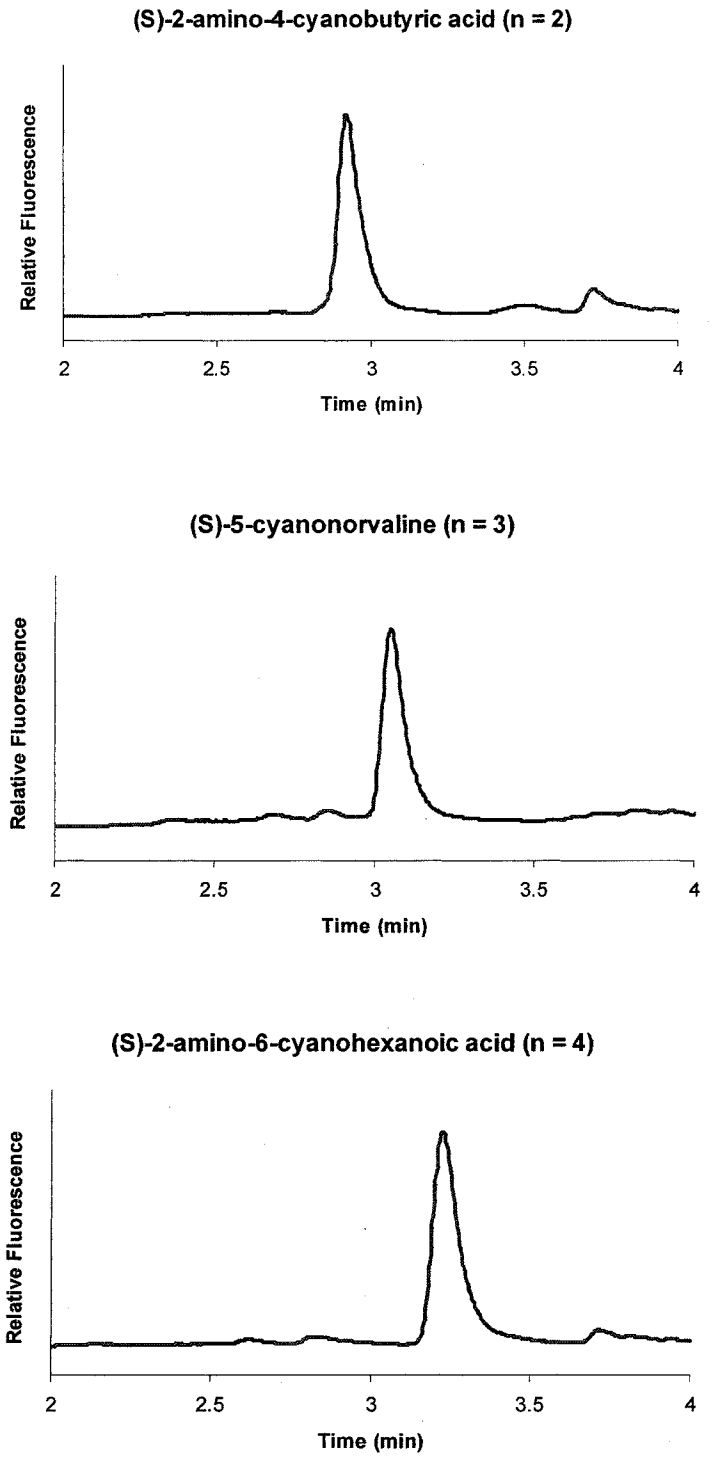


Figure 8: Isoindole derivatives of the nitrile amino acids (**16**, **27**, **41,** n = 2-4) obtained from alkylation reaction mixtures at 25 min showing completed reactions.

complex, and the resulting reaction mixtures were worked up. The complexes alkylated with 3-propionitrile (n = 2, **38**) and 4-bromobutyronitrile (n = 3, **39**) were first purified using dry flash chromatography on reversed-phase silica (eluted with 60/40 MeOH/H_2O). This gave a separation of the diastereomers; however, it was found that by using silica (normal-phase) and eluting with 14:1 $CHCl_3$: acetone, a much better separation was achieved. The complex **39** also was crystallized from acetone, while the shortest complex (**38**) did not crystallize from acetone. The complexes alkylated with 5-bromovaleronitrile (n = 4, **40**) were separated from their diastereomers by crystallization from acetone.

The yields of the purified alkylated BPB complexes ranged from 36-82%. The yields of the alkylated BPB complexes obtained by alkylating with 3-propionitrile (**38**) were low because chromatography on several dry flash chromatography columns were needed to completely free the complex of its diastereomer.

Each purified, alkylated complex was characterized by melting point, specific rotation (Table 3), and NMR spectroscopy. ^{1}H NMR spectroscopy was used to assess the diastereomeric purity of complexes **38** and **39** after purification on silica gel. Doublets for H7 and H23/27 of the (*S,S*) and (*S,R*) alkylated BPB complexes appeared at ~8.5 and 8.7 ppm as distinct signals from other aromatic resonances. The assignment of these resonances for the (*S,S*)-**39** is described in Section 2.3.8.

Table 3: Characterization data for alkylated BPB complexes (n = 2-4).

BPB complex	(*S,S*)			(*R,R*)		
	Yield (%)	mp (°C)	$[\alpha]_D$ (°)	Yield (%)	mp (°C)	$[\alpha]_D$ (°)
38	48	209-210	+2608	36	208-210	-2995
39	73	195-197	+2474	63	195-197	-2605
40	63	182-185	+2449	82	181-183	-2529

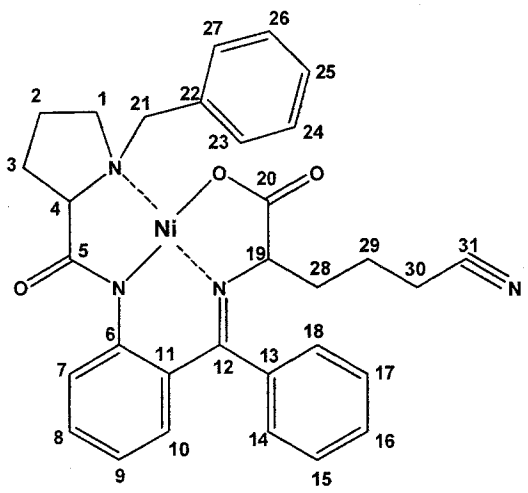
A literature melting point for the (*S,S*)-**38** was available.[33] In the mid-1980's Belokon[33] produced this complex using a different electrophile, acrylonitrile (**37**). The values reported by Belokon[33] (220-224°C) and a previous Student[63] (219-220°C) are ~10°C higher than those obtained for the (*S,S*) and (*R,R*)-**38** in this study. Melting points obtained for **39** and **40** agreed well with values obtained by a previous student.[63]

Similar to the BPB complex (**36**), the alkylated complexes gave very large specific rotations. Consequently, dilute solutions (~0.2 mg / mL, prepared by weighing small amounts (<1 mg) of the purified solids) were required for polarimetry. The reason for the difference in rotation values for the n = 2 (*S,S*) and (*R,R*) complexes (Table 3) may reflect weighing errors in the preparation of the rotation samples. The values obtained in all cases are comparable to those obtained by a previous student.[63]

The (*S,S*)-**39** complex was characterized by two-dimensional NMR (COSY, HSQC, HMBC, NOESY). This complex was chosen for more detailed analysis because of the availability of other complementary characterization data.[63] The one dimensional NMR data collected for the (*R,R*)-**39** complex agrees with that

obtained for the (*S,S*)-complex. NMR data for the other two complexes (**38** and **40**) are consistent to those obtained for (*S,S*)-**39**.

3.3.8 - NMR structural assignment of (S)-complex alkylated with 4-bromobutyronitrile:



The aliphatic regions of the 1-D ^{1}H and ^{13}C NMR spectra of the *(S)*-complex alkylated with 4-bromobutyronitrile showed nine carbon resonances (2 CH and 7 CH_2 as determined by 1-D JMOD) and ^{1}H resonances integrating to 16 protons. The proton resonances originate from two seven-spin systems, each composed of CH-CH_2-CH_2-CH_2, and one benzylic CH_2. The 1-D NMR data correlations observed in 2-D NMR spectra are listed in Table 4.

Correlation between an aliphatic carbon (δ 63.4) and aromatic protons (δ 8.06, 2H, d) in the HMBC spectrum assigns the resonance at δ 63.4 to C21, the only aliphatic carbon bonded directly to an aromatic ring. The protons directly attached to C21 (H21a, H21b) show long range correlations to an aromatic CH at δ 131.7 and an aliphatic CH at δ 70.4 (C4) and a CH_2 at δ 57.4 (C1). The HSQC

correlations at these aliphatic carbons identify the ^{1}H NMR resonances of the terminal protons in the seven-spin proline system. The protons H2 and H3 were assigned by their COSY correlations with H1 and H4. HSQC correlations were used to assign the carbon chemical shifts of C2 and C3.

As a result of shielding by the nitrile groups, C30 was assigned to the lowest frequency at δ16.9. The protons directly attached to C30 (δ 2.12-2.03 (2H, m) and 2.38-2.26 (2H, m) were assigned through HSQC correlations. In the HMBC spectrum, these protons correlated with a quaternary carbon at δ 119.1, assigned to the nitrile carbon. COSY correlations with H30 were used to assign protons attached to C28 and C29. To further support these assignments, correlations with the α-CH of the nitrile amino acid portion of the complex were used. Of the two aliphatic CH groups in the molecule, one at δ 70.4 has been assigned to C4, and the other at δ 68.3 to C19. By HSQC correlations, H19 was found to resonate at δ 3.82 of the ^{1}H NMR spectrum. In the COSY spectrum, this proton correlates to resonances at δ 1.79-1.68 and 2.38-2.26 H28a and H28b, leaving δ 1.79-1.68 and δ 2.24-2.14 for H29, and completing the aliphatic spin system.

The aromatic region of the ^{13}C NMR spectrum contains 16 resonances to assign to three ring systems (one disubstituted ring, and two monosubstituted rings). The benzylic C21 (δ 63.4) shows HMBC correlations to aromatic protons (δ 8.06, 2H) assigned to H23/27. COSY correlations between H23/H27, H24/H26, and H25, and HSQC correlations indicated only 3 different proton resonances and 4 carbon resonances. This is consistent with a monosubstituted

ring with free rotation about the C21-C22 bond. The quaternary carbon, C22, was identified at δ 133.4 through its HMBC correlation with H21 and H23/27.

The two other aromatic rings were well separated from the aliphatic spin systems. No HMBC correlation between C5 and H7 was observed: however, a long-range correlation between H19 assigned C12 to the resonance at δ 171.2. A second correlation between C12 and an aromatic proton identified the proton resonance as either H10 or H14, but it could not distinguish between the ring systems. On the other hand, a long range NOSEY correlation between H28 and H29 and an aromatic proton at δ 7.01-6.99 was observed. This aromatic proton was assigned to the monosubstituted ring. Previous NMR studies on the BPB complex[64,65] have indicated that this ring is not free to rotate. For this reason the proton resonance was assigned to position 18. COSY correlations were used to assign the remaining protons on this ring and HSQC correlations assigned the respective carbons. H15, H16, and H17 were not resolved. Hence it was very difficult to assign the appropriate carbons by HSQC correlations. Also, the H14 and H18 showed NOESY correlations with H24, H25, H26 in the benzylic aromatic ring, indicating that the benzylic aromatic ring is positioned over the molecule, directing the substituent at C19 to the opposite face. A NOESY correlation between H14 and δ 6.69-6.64 (m, 2H), assigned these resonances to H9/H10. COSY and HSQC correlations were used to complete the remaining ^{1}H and carbon assignments

Unfortunately, there were no NOESY correlation between H19 and H4 as indicated by the literature report for the BPB complex.[64] This would have

confirmed the stereochemistry of the center of chirality created in the alkylation reaction. However, NOESY correlations between H4 and H19 and H23/H27 and H25, respectively, in the benzylic aromatic ring indicate that H4 and H19 are on the same side of the molecule, giving the two centers the same (*S*)-configuration.

Table 4: NMR characterization data for (S,S)-**39**.

Position	Group[a]	δ_C	δ_H	COSY	HMBC	NOESY
1	CH_2	57.4	2.12-2.03 (2H, m)	H1b, H2a, H2b	H2b, H21a, H21b	H21a, H21b, H1b/2b/4
			3.51-3.47 (2H, m)	H1a, H2b		H21a, H23/27
2	CH_2	24.2	2.24-2.14 (2H, m)	H1a, H2b, H3a, H3b H1a,	H1a, H4	H3a, H21b, H11b/2b/4
			3.65-3.56 (2H, m)	H1b, H2a, H3a, H3b		H23/27
3	CH_2	30.9	2.56-2.51 (1H, m)	H2a, H2b, H3b, H4	H2b, H4	H2a, H3b, H1b/2b/4
			2.77-2.74 (1H, m)	H2b, H3a, H4		H3a, H21b
4	CH	70.4	3.51-3.47 (2H, m)	H3a, H3b	H1a, H1b, H21a, H21b	H23/27

[a]Multiplicity determined by *J*-MOD

Table 4: Continued.

Position	Group[a]	δC	δH	COSY	HMBC	NOESY
5	C=O	180.6	--	--	--	--
6	C	142.6	--	--	H8, H10	--
7	CH	124.0	8.15 (1H, d, *J* = 8.5 Hz)	H8	H9	H8
8	CH	132.6	7.17-7.13 (1H, m)	H7, H9	H7, H9	H7, H9/10, H14
9	CH	121.0	6.69-6.64 (2H, m)	H8, H10	H7	H8, H14, H15/16/17, H24/26, H25
10	CH	133.5	6.69-6.64 (2H, m)	H9	H9	H8, H14, H15/16/17, H24/26, H25
11	C	126.3	--	--	H7, H10	--
12	C=N	171.2	--	--	H10, H19	--
13	C	133.7	--	--	H14, H15,H17	--

[a]Multiplicity determined by *J*-MOD

Table 4: Continued.

Position	Group[a]	δC	δH	COSY	HMBC	NOESY
14	CH	127.7	7.02 (1H, dd *J* = 2, 5.5 Hz)	H15	H18	H8, H9/10, H15/16/17
15	CH	129.2	7.55-7.50 (3H, m)	H14, H16	H14	H9/10, 14, 18, 24/26, 23/27, 25
16	CH	130.1	7.55-7.50 (3H, m)	H15, H17	H14, H18	H9/10, 14, 18, 24/26, 23/27, 25
17	CH	129.6	7.55-7.50 (3H, m)	H16	H18	H9/10, 14, 18, 24/26, 23/27, 25
18	CH	127.2	7.27-7.25 (1H, m)	H17	H14, H16, H17	H9/10, 14, 15/16/17 24/26, 25
19	CH	69.3	3.82 (1H, dd, *J* = 3.5, 9.5 Hz)	H28a, 28b	H28b	H25, H28a, H28b
20	C=O	178.9	--	--	H19	--

[a]Multiplicity determined by *J*-MOD

Table 4: Continued.

Position	Group[a]	δC	δH	COSY	HMBC	NOESY
21	CH_2	63.4	3.57 (1H, d, *J* = 12.5 Hz)	H21b	H1a, H1b, H4, H23/H27	H1a, H23, H27
			4.42 (1H, d, *J* = 12.5 Hz)	H21a		H1a, H2a, H23, H27
22	C	133.4	--	--	H21a, H21b, H23, H27	--
23	CH	131.7	8.06 (2H, d, *J* = 10 Hz)	H24	H21a, H21b, H24, H26	H1a, 1b/2b/4, H7, H15/16/17, H24/26
24	CH	129.1	7.36 (2H, app t, splitting of 8, 7.5 Hz)	H23, H25	H23, H27, H25	H14, H25, H23/27
25	CH	129.2	7.20 (1H, t, *J* = 7.5 Hz)	H24, H26	H23, H24, H26, H27	H24/26, 23/27
26	CH	129.1	7.36 (2H, app t, splittings of 8, 7.5 Hz)	H25, H27	H23, H25, H27	H14, H25, H23/27

[a]Multiplicity determined by *J*-MOD

Table 4: Continued.

Position	Group[a]	δC	δH	COSY	HMBC	NOESY
27	CH	131.7	8.06 (2H, d, *J* = 10 Hz)	H26	H21a, H21b, H24, H26	H1a, 1b/2b/4, H7, H15/16/17, H24/26
28	CH_2	34.8	1.79-1.68 (2H, m)	H19, H28b, H29a, H29b	H19, H30a, H30b	H18, H19, H29b
			2.38-2.26 (2H, m)	H19, H28a, H29a, H29b		H28a/29b, H30a/29, H30b
29	CH_2	21.8	1.79-1.68 (2H, m)	H28a, 28b, H29b, 30a, H30b	H19, H28b, H30a, H30b	H18
			2.24-2.14 (2H, m)	H28a, 28b, H29a, H30a, H30b		H28/29a, H28b/30b
30	CH_2	16.8	2.12-2.03 (2H, m)	H29a, 29b, H30b	H29	H28/29a, H28/30b
			2.38-2.26 (2H, m)	H29a, 29b, H30a		H28/29a, H30a, H29b, H28b
31	CN	119.1	--	--	H30a, H30b	--

[a]Multiplicity determined by *J*-MOD

3.3.9 - Alkylation with chloroacetonitrile (n = 1)

Similar to the above alkylation reactions, akylations using chloroacetonitrile (n = 1) were conducted in DMF using (*S*)-BPB complex on scales ranging from 50 to 100 mg. In each case, the reaction was done in the presence of 3 equivalents of NaOH and a 10% excess of electrophile (base was added to a homogeneous solution of the BPB complex (**36**) and electrophile in DMF). Furthermore, each experiment was done at room temperature and open to the atmosphere.

Alkylation reactions were monitored by OPA HPLC. The observed disappearance of glycine (**43**) was consistent with alkylation of the BPB complex, but no peak corresponding to the desired nitrile amino acid (**12**) arose (expected retention time ~ 2 min). A peak, at ~2.6 min, however, did grow over time. Work-up of this experiment to isolate alkylated complex or amino acid was not carried out.

The next alkylation experiment was conducted under identical reaction conditions, and the progress of the reaction at 25 and 50 min was monitored using HPLC. These chromatograms showed the same results as above: no peak corresponding to the desire nitrile amino acid (**12**) was apparent. The alkylated complex from this reaction was isolated as a red oil (0.073 g), and hydrolyzed in 3 M HCl solution to liberate amino acid products. The aqueous layer from hydrolysis, which yielded a positive response in the ninhydrin test, was applied to an ion-exchange column. A small amount of product (~5%) eluted from the column, and it was analyzed by ^{1}H NMR spectroscopy. The spectrum

showed signals of a mixture of compounds, believed to be glycine (**43**) and serine (**60**). A singlet at 3.53 ppm was attributed to the methylene protons in glycine. A triplet at 4.05 ppm and a singlet at ~4.4 ppm were also present in the spectrum. These signals possibly correspond to serine.

As a next step, the effect of base on reaction progress was investigated. This was done because it was believed that the product did not accumulate when excess base was present. In particular, one equivalent of base was added in increments every 30 min to a total of 3 equivalents, instead of adding all of the NaOH at the beginning. HPLC analysis was conducted at 30 min intervals. The resulting chromatograms revealed no reaction products before 2 equivalents were added (just glycine present). Once two equivalents of NaOH had been added, the OPA-derivatized glycine peak decreased over time and a peak at ~ 2.6 min increased (Figure 9). The peak at 2.6 min was confirmed as serine (**60**) by injecting a standard and comparing retention times. Unfortunately, no peak grew at 2 min. HPLC did not provide evidence for the formation of the desired product, and no attempt was made to isolate the alkylated complex.

This result led to the question as to why serine was being produced. As a shorter chain analogue of electrophiles used in previously described successful alkylation reactions, alkylation of the BPB complex (**36**) was reasonable to account for the disappearance of BPB complex (i.e., glycine) from the reaction mixture. However, since this shorter chain length of the electrophile places only one methylene group between the second acidic proton on the α-carbon of the

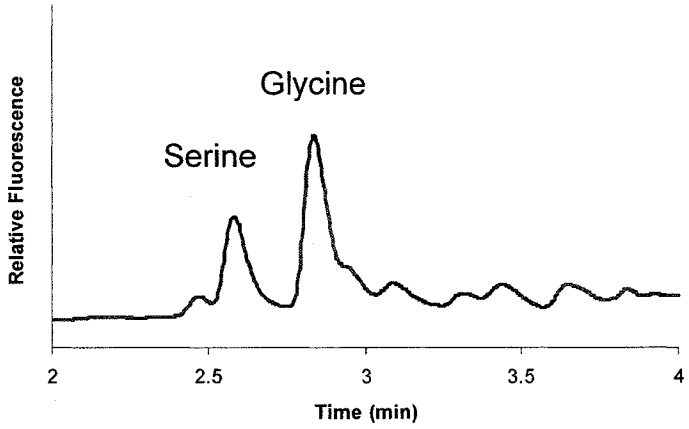
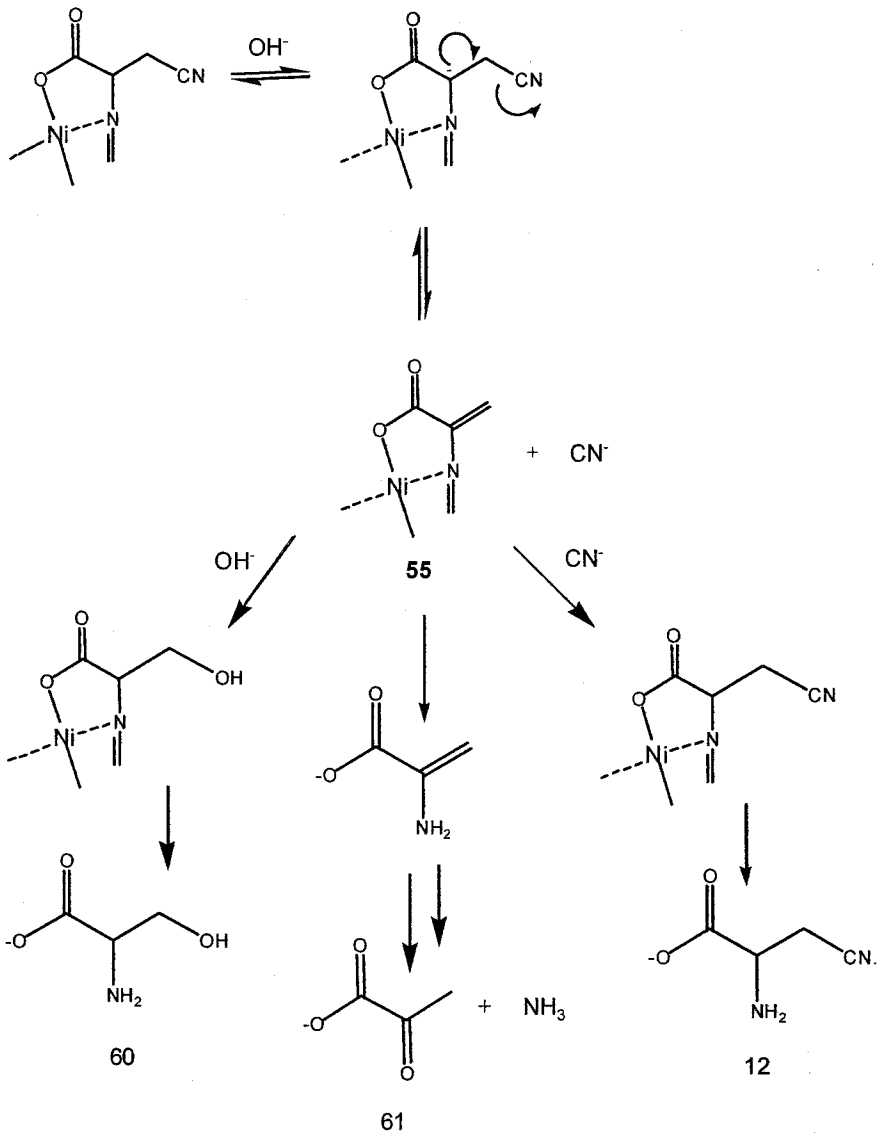


Figure 9: Alkylation with chloroacetonitrile: Formation of serine at 2.6 min.

glycine portion and the nitrile, the nitrile can act as a potential leaving group. It is possible that excess base deprotonates the alkylated complex, and leads to the formation of a dehydroalanine complex (**55**) by releasing CN^- ion (Scheme 29). In the presence of excess base, hydroxide ion would be able to react as a nucleophile with the dehydroalanine complex to form a complex identical with one synthesized from serine. Furthermore, if the dehydroalanine complex itself hydrolyzed, it would yield pyruvate (**61**) which is not detectable by OPA HPLC (no primary amine, Scheme 29).

Previously, dehydroalanine complexes have been produced by Belokon[40,49,50] and reacted with various nucleophiles in Michael addition-type reactions (Section 1.2.6). These complexes were produced by an aldol condensation with formaldehyde or acetaldehyde[49,50] to form the corresponding serine or threonine complexes, respectively. Acetylation of the hydroxyl groups



Scheme 29: Formation and reaction of a dehydroalanine complex.

followed by elimination of acetic acid under basic conditions yielded the dehydroalanine and methyl substituted dehydroalanine complexes. If the dehydroalanine complex could be isolated from the above reaction mixture, this would be a new, one-step method for producing this complex.

To test the intermediacy of the dehydroalanine complex further, several trapping agents were added to separate portions of the reaction mixture containing complex, electrophile, and three equivalents of NaOH. In each instance, 4-8 mg of $NaBH_4$, KCN, potassium phthalimide, and cysteine were added to 100-150 μL of the reaction mixture.

A reaction mixture supplemented with $NaBH_4$ (which would yield alanine if successful) was stirred for 2 h at room temperature, but $NaBH_4$ did not dissolve under this condition. Similarly, solubility was a problem when potassium phthalimide and cysteine were added to stirred reaction mixtures. To determine if any of these trapping agents reacted, OPA HPLC analysis was conducted on the hydrolyzed samples. No additional peaks were observed in chromatograms of these reaction mixtures, suggesting that the low solubility prevented these nucleophiles from trapping the dehydroalanine complex.

The other nucleophile used in this study, KCN, was also not very soluble. However, an effort was made to overcome this technical problem since reaction of cyanide with the dehydroalanine complex would yield the desired nitrile amino acid. To aid in dissolving KCN, a crown ether was added to the solution to complex the K^+ and allow CN^- to dissolve. This was not successful, so several drops of MeOH were added. When this mixture was monitored by HPLC, a small peak was observed at 2.1 min, the expected retention time of the desired OPA-derivatized nitrile amino acid (Figure 10). The formation of this nitrile amino acid in this mixture is consistent with the addition of cyanide to the intermediate dehydroalanine complex (Scheme 29).

The amino acids produced in this study are consistent with the formation of a dehydroalanine complex when chloroacetonitrile (n = 1) is used as the electrophile in the alkylation reaction. Elimination of HCN from the alkylated complex creates another electrophile, available to react in Michael addition-type reactions. Although the stereochemistries of the amino acid products were not determined, it is likely that the complex acts as a chiral auxiliary resulting in a stereoselective Michael addition. This approach could be developed as a method for the stereoselective synthesis of amino acids containing a nucleophilic group at C3.

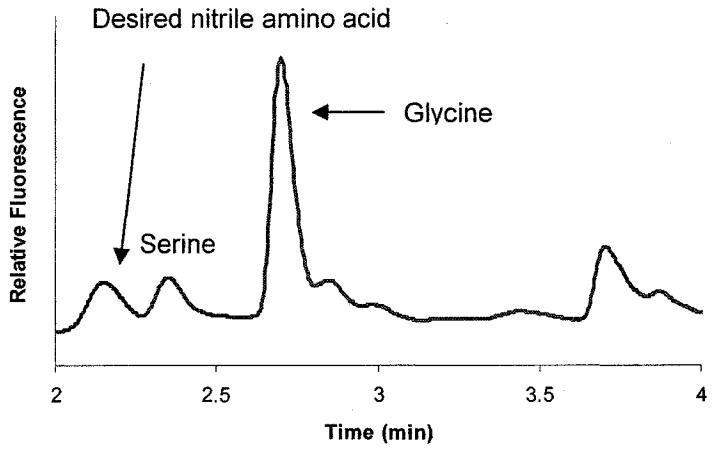
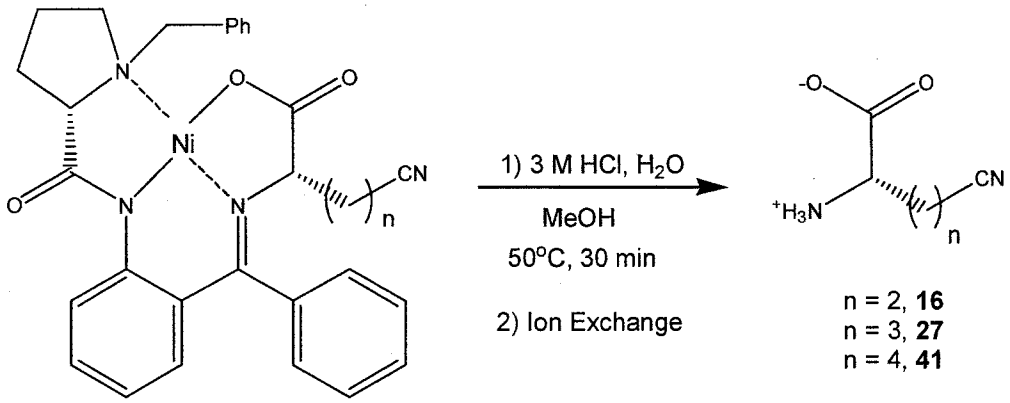


Figure 10: Appearance of a chromatographic peak at 2.1 min upon addition of KCN to the reaction mixture of BPB complex and chloroacetonitrile.

3.4 – Cyanoamino Acids

The target nitrile amino acids were obtained by heating the alkylated complexes (**38-40**) in aqueous acid until the red color of the alkylated complex disappeared and a green color remained (Scheme 30). BPB ligand (**42**) was extracted from the hydrolysate in good yields (~80%), similar to literature

reports.[36] The liberated nitrile amino acids (**16**, **27**, **41**) were isolated from the hydrolysate using ion-exchange chromatography in yields ranging from 63-93%. One portion of the amino acid was crystallized for characterization purposes, and the second portion was reduced to produce the diamino acid (see next section).



Scheme 30: Hydrolysis of (*S,S*)-alkylated complex to (*S*)-nitrile amino acids.

Upon completion of the reaction (as determined by the disappearance of the red color), it was originally worked-up according to a previous student's protocol,[63] which was simply the extraction of the BPB ligand (**42**) into $CHCl_3$ and application of the aqueous portion to an ion-exchange column. In order to improve the recovery of BPB ligand, the aqueous layer was neutralized with NH_3.[40] The NH_3 solution was added carefully to avoid the addition of excess base. During the addition of NH_3, BPB ligand was removed as it came out of the aqueous portion (determined by the appearance of a yellow color) because the alkylated complex reformed in the presence of BPB ligand, Ni^{2+}, and amino acid

under basic conditions. If the alkylated complex reforms, it must be extracted into $CHCl_3$, concentrated, and rehydrolyzed.

After removing BPB ligand (**42**), the neutralized aqueous portion was applied to an Amberlite IR 120 ion-exchange column (H^+ form). The column was washed with H_2O, and the free amino acid was eluted using 0.5 M aqueous NH_3. The amino acid in the fractions was detected by ninhydrin. In general, the fraction before, and a few after the first basic one, contained the bulk of the product. In all cases, the combined amino acid fractions were concentrated to a small volume (~20 mL), then split into two portions of equal volume and concentrated to dryness. One portion of each compound (**16**, **27**, **41**) was crystallized from H_2O/EtOH for characterization purposes while the other was reduced (see next section). The crystallized nitrile amino acids were characterized by melting point, specific rotation (Table 5), NMR spectroscopy, and mass spectrometry.

Table 5: Characterization data for cyanoamino acids.

Cyanoamino Acid	(*S*)			(*R*)		
	Yield (%)	**mp (°C)**	**$[\alpha]_D$ (°)**	**Yield (%)**	**mp (°C)**	**$[\alpha]_D$ (°)**
16	91	214-216	+28.9	85	213-216	-26.3
27	75	223-227	+10.1	72	227-231	-13.0
41	63	226-227	+6.7	65	228-230	-5.3

In all cases the melting points and rotations for the amino acid enantiomers agreed with one another. The melting points for (*S*)- and (*R*)-4-cyano-2-aminobutyric acid (**16**) were close to the values on the lower end of the range 217-235°C, which agreed with several literature values.[7,18,27] In the literature,[7,18,27] there are reports that after one crystallization, the melting points are in the range of 212-215°C (values very close to those reported in Table 5). After several recrystallizations, the melting points increased. Furthermore, the values in Table 5 agree with those of a previous student.[63]

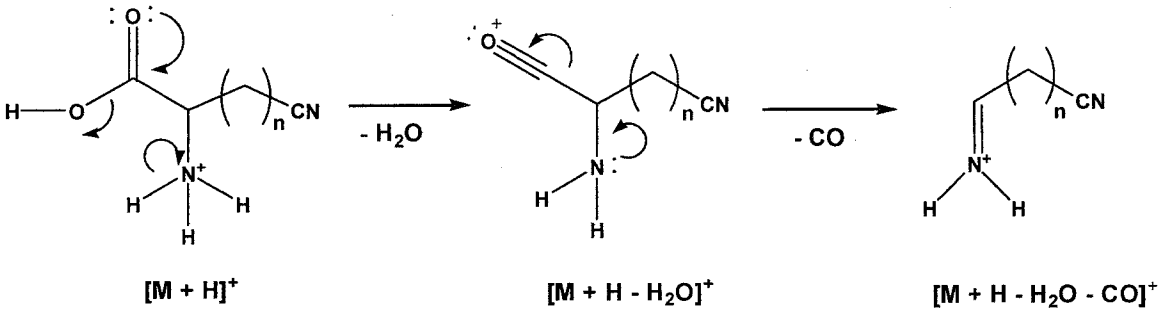
The rotation value obtained for (*S*)-**16** falls within the range of literature values[18,27] (+27.4 - +32.2°) and the (*R*)-enantiomer of **16** produced a value similar in magnitude but opposite in sign. ^{1}H and ^{13}C NMR spectra obtained for these amino acids agree with data reported.[33]

(*S*)- and (*R*)-5-cyanonorvaline (**27**) had melting points slightly lower than the literature[27] melting point for the (*S*)-enantiomer (230-237°C) while the rotation values are similar in magnitude (+10.0°).[27] ^{1}H and ^{13}C NMR spectra for the enantiomers agree well with those obtained by a previous student.[63]

Data collected for the (*S*)- and (*R*)-**41** were compared to those obtained by a previous student[63] as no literature data were available for comparison purposes. Melting points and NMR spectra agreed well with those of a previous student,[57] however, the specific rotations are larger.

ESI^{+}-MS/MS was also used to characterize the nitrile amino acids. In all cases the $[M+H]^{+}$ ion fragmented to yield an ion of 46 mass units less. This is consistent with successive neutral losses of H_2O and CO from the protonated

species (Scheme 31).[68] Initial loss of H_2O from an unstable acylium ion ($[M+H-H_2O]^+$) was not observed in the mass spectrum, possibly due to it quickly fragmenting to form an iminium ion by loss of CO ($[M+H-H_2O-CO]^+$). No further fragmentation for these amino acids was observed.

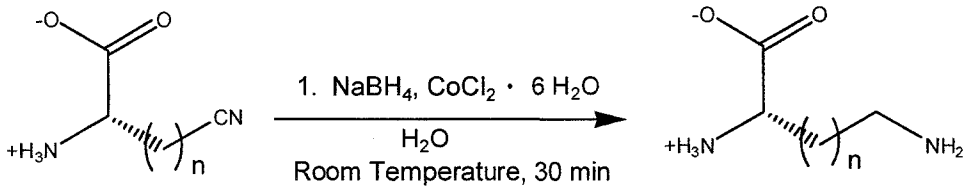


Scheme 31: Neutral losses of H_2O and CO for the nitrile amino acids.

3.5 – Basic Amino Acids

The basic amino acids (or diamino acids) were produced from the second portion of the aqueous solution of nitrile amino acid obtained after ion-exchange chromatography. The nitrile group was reduced[51] by adding a ten-fold excess of $NaBH_4$ to an aqueous solution of $CoCl_2$ and the nitrile amino acid (Scheme 30). Upon addition of $NaBH_4$, a black precipitate formed in the reaction mixture. This was attributed to the formation of Co_2B,[69] which assists $NaBH_4$ in the reduction of the nitrile. The presence of $CoCl_2$ was therefore needed since $NaBH_4$ cannot reduce the nitrile group on its own.[51] To determine when the reaction was complete (25-30 min), OPA HPLC was done on a 50 µL portion of the reaction mixture. The mixture was quenched with 1 M HCl and acetone and filtered to

remove the black precipitate. The basic amino acids were isolated using ion-exchange chromatography. After applying the sample, the ion-exchange column was washed with water, and the basic amino acids were eluted using 0.5 M NH_3. The fractions containing the amino acid were concentrated to yield an off-white solid in yields ranging from 45-94%.



n = 2, **16**
n = 3, **27**
n = 4, **41**

n = 2, **5**
n = 3, **28**
n = 4, **62**

Scheme 32: $NaBH_4$ reduction of (*S*)-nitrile amino acids to produce (*S*)-diamino acids.

In order to produce samples that could be more easily crystallized than the free base, the basic amino acids were converted to their dihydrochloride salts by treatment with 2.2 equivalents of 1 M HCl.[70] Crystallization of these samples were attempted using MeOH / ether. **28** and **62** produced a small amount of an off-white solid, which was characterized by melting point, specific rotation (Table 6), NMR spectroscopy, and mass spectrometry.

The ornithine samples (**5**), however, did not produce workable samples. Therefore, the dihydrochloride salts (in MeOH) were treated with 1 equivalent of pyridine in an attempt to produce the monohydrochloride salt.[71] The monohydrochloride salt, in theory, would precipitate out of the MeOH solution leaving pyridine hydrochloride in solution. This attempt did not yield enough

sample to work with. Note that ^{1}H and ^{13}C NMR spectra were obtained for (*S*)-ornithine dihydrochloride salt before its conversion to the monohydrochloride.

Table 6: Characterization data for the basic amino acids.

Basic Acid	(*S*)			(*R*)		
	Yield (%)	mp (°C)	$[\alpha]_D$ (°)	Yield (%)	mp (°C)	$[\alpha]_D$ (°)
5	72	-	-	98	-	-
28	94	193-196	+18.3	45	193-196	-15.4
62	94	187-190	+6.3	90	195-200	-5.3

The yields in Table 6 represent the yield of the amino acid in its free base form (after ion-exchange). The melting points and specific rotations reported were measured on the dihydrochloride salts. The melting points for (*S*) and (*R*)-lysine dihydrochloride agree with the literature value of 193°C.[56] The specific rotation of (*S*)-lysine also is similar to that reported for the (*S*)-dihydrochloride salt (15.3°).[57] The ^{1}H and ^{13}C NMR spectra were consistent with signals expected for lysine.

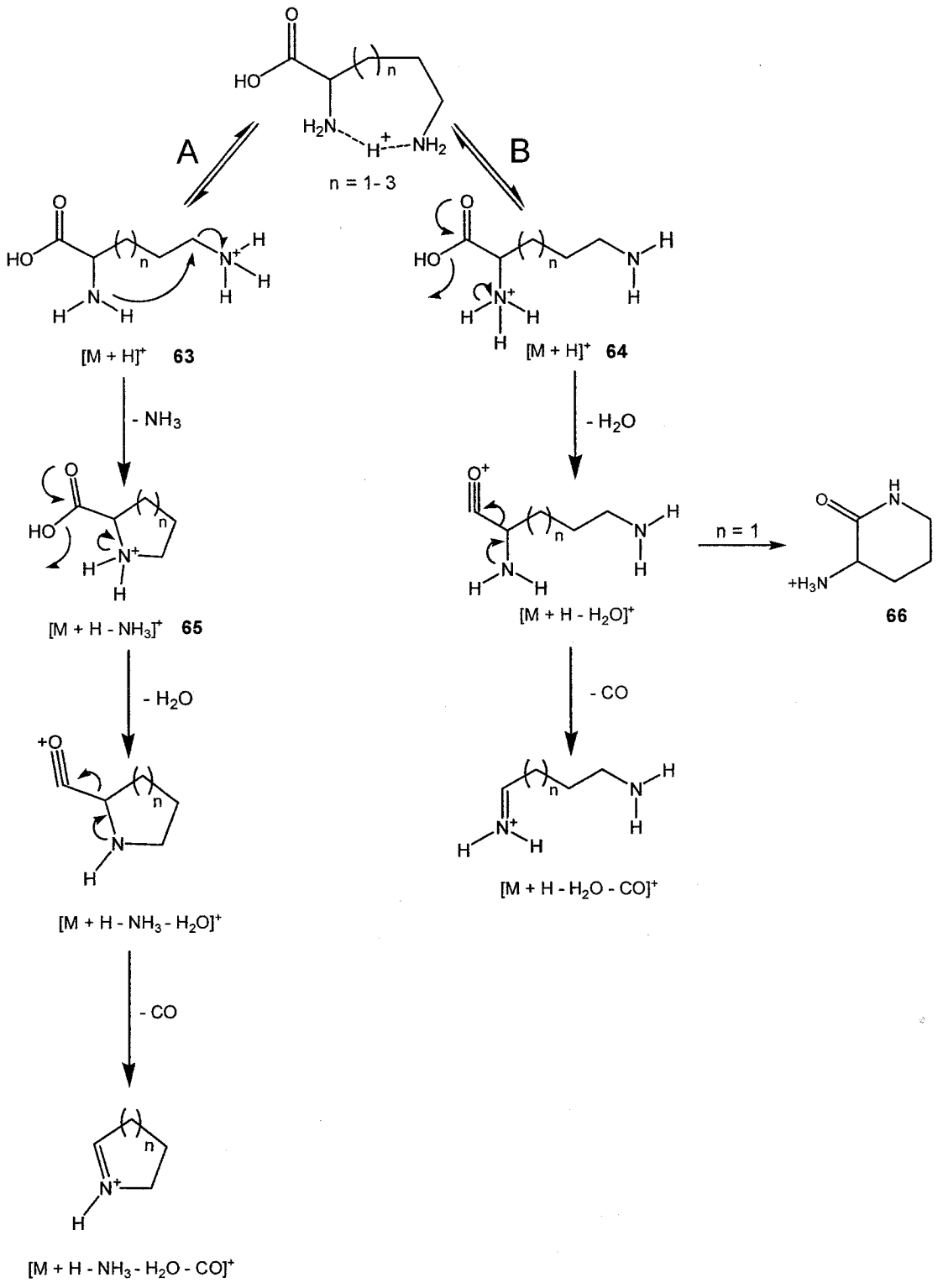
The melting points obtained for (*S*) and (*R*)-**62** were consistent with one another but lower than the literature value of 239-242°C.[58] This was possibly due to a mixture of mono- and dihydrochloride salts or impurities in the sample. The specific rotations, however, are consistent with the value reported for (*S*)-2,7-diaminoheptanoic acid (+14.4°).[58]

The ESI^{+}-MS spectra of the diamino acids **5**, **28**, **62**) show a dominant peak corresponding to the singly protonated ion $[M+H]^{+}$. The formation of only

the monoprotonated species is consistent with the formation of intramolecular hydrogen bonding in $[M+H]^+$. The high basicity of gas phase, aliphatic diamines has been attributed to similar interactions.[72]

In the ESI^+ MS/MS spectra obtained for the basic amino acids, two fragmentation pathways were observed (Scheme 33). Pathway A involves the loss of the amino group in the side chain, while pathway B involves the loss of water and carbon monoxide to form an iminium ion (similar to the nitrile amino acids). The two pathways can be viewed as originating from two separate $[M+H]^+$ species; one having the side chain amino group protonated (**63**) and the other having the α-amino group protonated (**64**). The two $[M+H]^+$ species (**63** and **64**) would interconvert via the hydrogen bonded species shown in Scheme 33, but different ratios would be present depending on the amino acid.

For two of the three amino acids studied, lysine dihydrochloride and 2,7-diaminoheptanoic acid dihydrochloride, pathway A was the major fragmentation pathway. When the more basic side chain amino group is protonated, it is lost as ammonia in an intramolecular nucleophilic substitution reaction when it is displaced by the α-amino group.[68,73] Further fragmentation of this species (**65**) for (*S*) and (*R*)-lysine dihydrochloride by loss of H_2O and CO occurred to a very small extent. A peak (~2% intensity) present at 46 mass units less than $[M+H-NH_3]^+$ was attributed to the subsequent loss of H_2O and CO ($[M+H-NH_3-H_2O-CO]^+$). This suggested that fragmentation, for the most part, stopped after formation of the cyclic species (**65**). Loss of ammonia from the side chain, is consistent with retention of ^{15}N label in [α-^{15}N]-lysine.[68]



Scheme 33: MS/MS fragmentation pathways proposed for the basic amino acids.

Similar fragmentation patterns were observed for $[M+H]^+$ from (*S*)- and (*R*)-2,7-diaminoheptanoic acid dihydrochloride. However, in this case, a peak with greater intensity (~20%) was observed for the loss of H_2O and CO from the cyclic species (**65**). A MS/MS experiment was conducted on the $[M+H-NH_3]^+$ fragment, and only a peak with mass equal to $[M+H-H_2O-CO]^+$ was observed. This observation further supports the initial loss of ammonia from the side chain. Loss of H_2O and CO from the $[M+H-NH_3]^+$ species, according to the mechanism in Scheme 33, would require retention of the α-nitrogen.

The minor pathway (pathway B) for these amino acids involved the initial loss of H_2O and CO from $[M+H]^+$ protonated on the α-amino group (**64**). In this pathway, loss of H_2O results in the formation of an acylium ion ($[M+H-H_2O]^+$), which rapidly fragments to an iminium ion ($[M+H-H_2O-CO]^+$) by loss of CO. The pKa values for the two amino groups suggest that fewer molecules are protonated on the α-amino group, and that the relative importance of the two pathways is consistent with the relative amounts of the two possible $[M+H]^+$ ions (**63** and **64**).

Pathways A and B were also observed for (*S*)-ornithine hydrochloride, but with reversed relative importance; pathway B was the major fragmentation pathway. While H_2O was lost readily, further fragmentation of this species (i.e., loss of CO) was a minor process (<1%). Further fragmentation after the loss of H_2O may indicate cyclization of the acylium ion with the nucleophilic side chain amino group to form a stable ion (e.g. **66**). In the minor pathway (pathway A), loss of NH_3 would lead to the formation of a five-.membered ring instead of the

six- and seven-membered rings formed from lysine and 2,7-diaminoheptanoic acid, respectively. A slower cyclization to a five-membered ring would allow the facile loss of H_2O to become the dominant fragmentation pathway. Fragmentation of this cyclic species was also not detected to any significant extent.

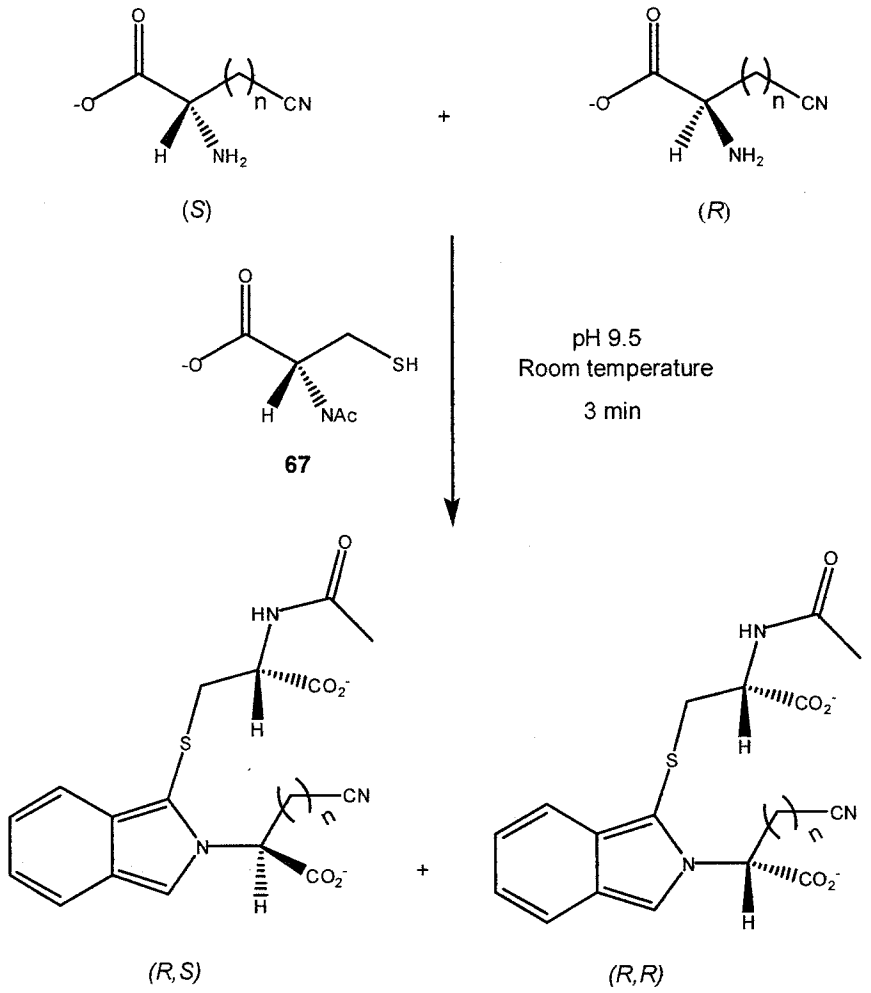
3.6 – Assessment of Stereochemistry

3.6.1 – Analysis of nitrile amino acids by chiral OPA HPLC

Similar to the method described in Section 2.3.1, a precolumn derivatization reaction was employed to convert the nitrile amino acids into fluorescent isoindole derivatives. By substituting *N*-acetyl-L-cysteine (**67**) for mercaptoethanol (**58**) in the derivatization reagent, enantiomeric amino acids are transformed into diastereomeric isoindole derivatives for separation on a reversed-phase HPLC column (Scheme 34, Figure 11).[74] The retention of the isoindole derivatives on the reversed-phase column correlated with chain length of the amino acid; the longest amino acid (n = 4) was retained to the greatest extent. The enantiomeric composition was calculated from the areas of the peaks assigned to the amino acid enantiomers.

3.6.2 – Assignment of Configuration in the Alkylated BPB complex

In Belokon's method,[34,35,40] the stereochemistry of the chiral auxiliary synthesized from proline (**44**) controls the configuration of the new center of



Scheme 34: Conversion of nitrile amino acids to fluorescent, diastereomeric isoindole derivatives.

chirality formed in the alkylation reaction. The two series of alkylated BPB complexes formed from (*S*)- and (*R*)-BPB complexes show complementary specific rotations (Table 7), indicating that the enantiomeric BPB complexes react with bromonitriles to yield enantiomeric alkylated complexes of similar stereochemical purity.

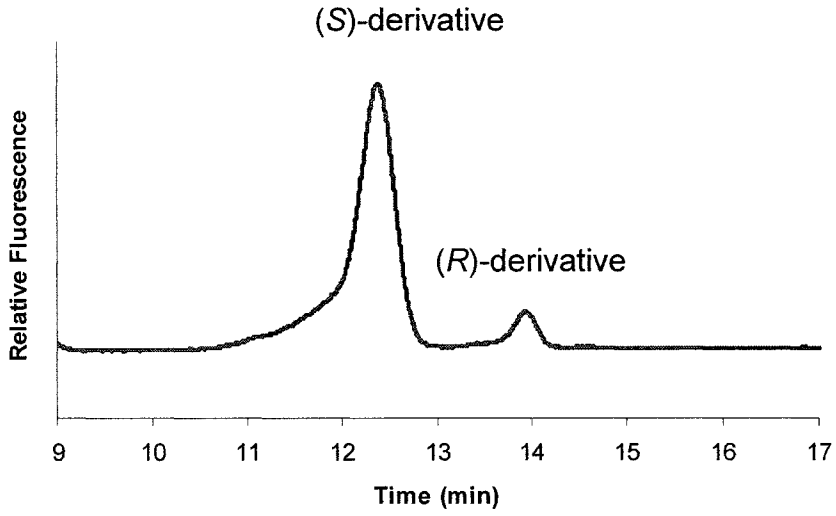


Figure 11: Isoindole derivatives of a 95/5 mixture of (*S*)-2-amino-6-cyanohexanoic acid and (*R*)-2-amino-6-cyanohexanoic acid.

Of the six alkylated complexes prepared in this thesis, only one ((*S*)-**38** complex) is reported in the literature.[33] The stereochemistry of this complex was assigned as (*S,S*) by Belokon using ORD spectra.

At 589 nm, the mole rotation read from Belokon's published ORD spectrum[33] is ~1.68 x 10^4 M corresponding to a specific rotation of 3049° at an unspecified concentration. The sign of this specific rotation agrees with the present value (Table 7) and the magnitude is similar, indicating that alkylation of the (*S*)-BPB complex with 3-bromopropionitrile (**38**) in these studies leads to the same (*S,S*)-complex. An X-ray structure obtained for the (*S*)-complex alkylated with 4-bromobutyronitrile (**39**) also supports this conclusion.[63]

Table 7: Specific rotations of BPB complex alkylated with alkylcyano bromides.

Alkylated complex	$[\alpha]_D$ (°)	
	(*S*)-configuration	(*R*)-configuration
38	2608	-2995
39	2474	-2605
40	2446	-2529

An NOE interaction between the proline α-proton and the α-proton of the bromoglycine fragment in a complex formed with bromoglycine was used to establish the stereochemistry of a bromoglycine BPB complex.[48] The NOESY NMR experiment done on the complex **39** (Section 2.3.9), however, did not show a direct correlation between the methines of the two amino acid subunits that would help assign the relative configurations of the α-protons in the amino acid subunits. While these protons are nominally on the same side of the molecule, they are pointed away from each other in the X-ray structure.[63]

The center of chirality formed in the alkylation reaction becomes the center of chirality in the nitrile amino acid released upon hydrolysis of the alkylated BPB complex (**36**). The nitrile amino acids and hydrochloride salts of the corresponding diamino acids synthesized from the (*S*)-BPB complex have positive specific rotations, and the corresponding series formed by alkylation of the (*R*)-BPB complex have negative specific rotations (Table 8). The complementary relationship of these specific rotations confirm that the configuration of the chiral auxiliary determines the chirality of the amino acid product.

Table 8: Characterization of the stereochemistry of nitrile amino acids.

	Product obtained by alkylating:			
	(*S*)-BPB complex		(*R*)-BPB complex	
Nitrile Amino Acid	**$[\alpha]_D$ (°)**	**Retention time[a] (min)**	**$[\alpha]_D$ (°)**	**Retention time[a] (min)**
16	+28.9	7.0	-26.3	7.7
27	+10.1	8.4	-13.0	9.5
41	+6.7	13.2	-5.3	14.5

[a]chiral HPLC

The (*S*)-enantiomer of 2-amino-4-cyanobutyric acid (**16**) and (*S*)-5-cyanonorvaline (**27**) have been synthesized by dehydrating the corresponding (*S*)-amino acid amides.[27] Their published rotations are positive, indicating that the nitrile amino acids (**16** and **27**, Table 8) prepared by alkylating the (*S*)-BPB complex have the (*S*)-configuration. No literature rotations are available in the literature for 2-amino-6-cyanohexanoic acid (**41**, n = 4).

Reduction of the nitrile amino acids gave diamino acids,[51] two of which, ornithine and lysine, are very well known and readily available. The positive rotation measured for the lysine sample agrees with the literature value,[57] again supporting the formation of (*S*)-amino acid nitriles by alkylation of (*S*)-BPB complex.

Under chiral HPLC conditions the nitrile amino acids obtained by hydrolysis of the alkylated (*S*)-BPB complexes elute before their isomers prepared using the (*R*)-BPB complex (Table 8). In this technique, (*S*)-amino acids typically elute before their (*R*)-enantiomers,[75] again indicating that (*S*)-nitrile amino acids are formed by alkylating the (*S*)-BPB complex.

The stereochemical evidence collected for the alkylated complexes, as well as the nitrile amino and diamino acids, supports the conclusion that (*S*)-amino acids are formed by alkylating the (*S*)-BPB complex and (*R*)-amino acids by alkylating the enantiomeric (*R*)-BPB complex.

3.6.3 – Stereoselectivity of the alkylation reaction

The stereoselectivity of the alkylation reactions was investigated by removing a small portion of the alkylated complex (before separation of diastereomers). After hydrolysis, the nitrile amino acid in the aqueous portion was subjected to chiral OPA HPLC. This analysis provides the stereochemical purity of the nitrile amino acid formed by alkylating the BPB complex. The enantiomeric purity calculated from the resulting chromatograms relates to the diastereomeric purity of the alkylated complexes.

The chromatographic results (Table 9) showed that in three of the six reactions 92% of the major diastereomer and 8% of the minor diastereomer was produced. In the other reactions, the ratios were greater, and the ratios of the major diastereomer to minor diastereomer ranged between 95/5 to 98/2 (or a range of 90% to 96% diastereomeric excess). These results are consistent with Belokon's report of diastereomeric ratios ranging from 92:8 up to 98:2.[34,35,40]

An assumption in relating the above analysis to the diastereoselectivity of the reaction is the enantiomeric purity of the BPB complex (**36**) used in the alkylation reaction. The synthesis started from commercial samples of (*R*)- and (*S*)-proline (**44**), and the purity of the BPB complex and the intermediates, *N*-

Table 9: Enantiomeric purity of nitrile amino acids from crude alkylated complex.

Cyanoamino Acid	From *(R)* Complex		From *(S)* Complex	
	% *(R)*	% *(S)*	% *(R)*	% *(S)*
16	96	4	8	92
27	92	8	8	92
41	98	2	5	95

benzylproline (**46**) and BPB ligand (**42**), were assessed by measuring specific rotations. The magnitudes of the specific rotations for the (*S*)- and (*R*)-BPB complexes (2167° and -2167°, respectively) are greater than the one literature value[37] reported for the (*S*)-BPB complex. Also, the magnitude of the specific rotations of the (*S*)- and (*R*)-BPB ligands (-132° and 135.8°, respectively) are very close to the literature[36] value of -134.5° for the (*S*)-BPB ligand. These comparisons indicate a 99% or greater e.e. for the (*S*) and (*R*)-BPB complexes used in the alkylation reactions. This is reasonable because recrystallization was carried out at each step of the synthesis to remove small amounts of enantiomeric impurities.

3.6.4 – Enantiomeric purity of nitrile amino acids

The mixture of diastereomeric complexes isolated from the alkylation reaction mixture were purified by chromatography (for **38** and **39**) or recrystallization (**40**) to improve the stereochemical purity of the nitrile amino acid products. In all cases, the enantiomeric purities of the amino acids after purification were improved, as expected (Table 10). In the chromatograms of (*R*)-**16** and **41** no (*S*)-amino acid enantiomer was detected, even on enlarged

chromatograms. In the other chromatograms, a small peak corresponding to the minor enantiomer was detected; in all but one instance the minor enantiomer was <1%. Given measurements on the purified nitrile amino acids (Table 11), the limit of detection was set at 0.2%.

Table 10: Enantiomeric purity of nitrile amino acids from purified alkylated complex.

Cyanoamino Acid	From *(R)* Complex		From *(S)* Complex	
	% *(R)*	% *(S)*	% *(R)*	% *(S)*
16	>99.8	<1	2.2	97.8
27	99.7	0.3	0.6	99.4
41	>99.8	<1	0.8	99.2

The data given in Table 9 and 10 demonstrate the effectiveness of the purification. This improvement in purity of the alkylated complex, however, comes at the price of lower yields, because only a partial separation of the diastereomers was achieved on silica gel and even less of a separation was achieved on reversed-phase silica gel. The chromatographic step decreased yields of the complex from 77% - 93% to 36 - 82%.

The enantiomeric purity of the recrystallized nitrile amino acids was determined also by chiral OPA HPLC. Five of the six amino acids had no detectable minor enantiomer, and therefore, are indicated as >99.8% in Table 11. The (*S*)-**16** amino acid did have a detectable peak on the baseline at the expected retention time of the (*R*)-enantiomer on the enlarged chromatogram, but this was only 0.2% of the peak area of the (*S*)-amino acid. The high enantiomeric purity of the nitrile amino acids shows that enantiomeric purity is not

lost through hydrolysis of the alkylated complex and that recrystallization further improves the stereochemical purity of the nitrile amino acids.

Table 11: Enantiomeric purity of purified nitrile amino acids.

Cyanoamino Acid	From *(R)* Complex		From *(S)* Complex	
	% *(R)*	% *(S)*	% *(R)*	% *(S)*
16	>99.8	<1	0.2	99.8
27	>99.8	<1	<1	>99.8
41	>99.8	<1	<1	>99.8

Chapter 4.0 – Conclusions and Future Work

The syntheses of (*S*) and (*R*)-BPB complex (**36**) were accomplished in good yields starting from (*S*) and (*R*)-proline (**44**), respectively. A more recent procedure[38] for the synthesis of BPB complex was employed which used almost stoichiometric amounts of Ni^{2+} to BPB ligand (**42**) and only a two-fold excess of glycine (**43**) to BPB ligand. When using isotopically labeled glycine, this method offers a clear advantage over the previous method,[37] which employed a five-fold excess of glycine to BPB ligand. The latter conditions would require the recovery of the unused labeled glycine after isolation of the BPB complex.

Alkylation of the (*S*) and (*R*)-BPB complexes with alkylcyano bromides (n = 2-4) were accomplished after optimization of the reaction conditions. It was determined that the amount of base, the use of nitrogen, and the order of addition of reagents was important to avoid the production of a side product and for the reaction to proceed successfully. Stereochemically pure alkylated complexes were obtained after separation of the diastereomers (**38**, **39**) on silica gel or by recrystallization (**40**). Purification on silica gel was successful but came at the expense of lower yields. The pure alkylated complexes were used to produce their respective nitrile amino acids in good yields. The nitrile groups of these amino acids were reduced using $NaBH_4$ in the presence of Co (II)[52] to produce the basic amino acids. Future work in this area should involve the production of (*R*)-$^{13}C_2$-lysine to investigate lysine catabolism by the anaerobe, *Fusobacterium nucleatum*. To evaluate the degradation pathway, (*R*)-[2,3-$^{13}C_2$]-lysine and (*R*)-[4,5-$^{13}C_2$]-lysine are required. The synthesis of (*R*)-[2,3-$^{13}C_2$]-

lysine requires a ^{13}C label to be incorporated into the BPB complex through the C_2 of glycine and a label through the electrophile while (*R*)-[4,5-$^{13}C_2$]-lysine, requires two labels from the electrophile. Since ^{13}C labeled 4-bromobutyronitrile (n = 3) is not commercially available, it must be synthesized.

Once labeled alkylated complex is produced, its hydrolysis to ^{13}C labeled 5-cyanonorvaline can be accomplished in one step and its further reduction to lysine can also be done in a single step. This procedure offers efficient use of the ^{13}C label as only two synthetic steps are done to produce the final product after label is incorporated.

The alkylation with chloroacetonitrile (n = 1) also requires further investigation. Further experiments to determine if cyanide ion is released need to be done. Also, if the dehydroalanine complex (**55**) could be isolated from the reaction mixture, a Michael addition reaction with cyanide may yield the desired alkylated complex. Furthermore, by altering the reaction conditions (by using a weaker base such as Na_2CO_3 or Et_3N or a different solvent) the alkylation might proceed successfully.

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