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The Stereoselective Synthesis of Amino Acids for Biosynthetic Studies

by

Danny Thomas Davis

B.Sc. (Honours), Acadia University, 1991

submitted in partial fulfillment of the requirements for

the degree of Doctor of Philosophy

at

Dalhousie University

Halifax, Nova Scotia

December, 1996

DALHOUSIE UNIVERSITY

FACULTY OF GRADUATE STUDIES

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by Danny Thomas Davis

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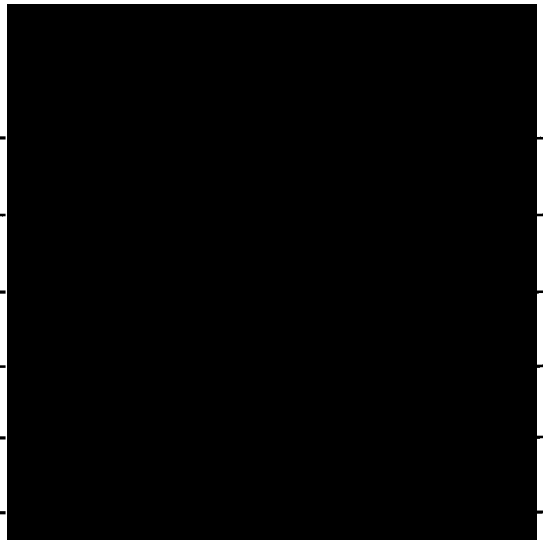

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For

Benjamin, Molly and Sheena

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ABSTRACT

This work provides a new general method for the synthesis of enantiomerically pure (*R*)-amino acids and possibly D-*threo*-β-hydroxy-α-amino acids. (*R*)- and (*S*)-Lysine and D-*threo*-β-(*p*-nitrophenyl)serine are required for future investigations of two metabolic pathways in our laboratory. For example, isotopically labelled (*R*)- and (*S*)-lysine are needed to examine the catabolism of this amino acid in *Fusobacterium* species, anaerobic bacteria associated with the gastrointestinal tract. Also, an investigation of the final steps in the biosynthesis of chloramphenicol, an antibiotic produced by cultures of *Streptomyces venezuelae*, requires the preparation of potential precursors from isotopically labelled D-*threo*-β-(*p*-nitrophenyl)serine.

(*S*)-Amino acids were synthesized by the Belokon method (JCS Perkin I, 1988 and JACS 107, 1985), and the method was extended to include the preparation of (*R*)-amino acids in high yields and enantiomeric purity.

Alkylation of the (*S*)-BPB-complex with a series of cyanoalkylbromides led to a series of cyano (*S*)-amino acids after hydrolysis of the complex. The stereochemistry of the alkylation step was established by x-ray crystallography of an alkylated complex and by optical rotation measurements and hplc analysis of the isolated cyano amino acids. The corresponding series of cyano (*R*)-amino acids was made, demonstrating the first use of the (*R*)-BPB complex for the preparation of enantiomerically pure (*R*)-amino acids. The versatility of the synthetic approach was demonstrated by the preparation of the (*R*) and (*S*) series of acidic and basic amino acids including α-aminoadipic acid and lysine.

Asymmetric routes to other non-protein amino acids, *e.g.*, (2*S*,4*R*)- and (2*S*,4*S*)-dihydroxynorvaline and all the stereoisomers of differentially protected 2,6-diaminopimelic acid are presented. Preliminary experiments suggest that the synthesis of D-*threo*-β-hydroxy-α-aminoacids can be achieved by condensation of the (*R*)-BPB-complex with an aldehyde.

LIST OF ABBREVIATIONS

abs	absolute
AcOH	acetic acid
br	broad
AIDS	acquired immune deficiency syndrome
apci	atmospheric pressure chemical ionization
BPB-complex	Ni(II) complex of glycine Schiff base with 2-[*N*-(*N'*-benzylprolyl)amino]benzophenone
BPB ligand	2-[*N*-(*N'*-benzylprolyl)amino]benzophenone
Cbz	benzyloxycarbonyl
cims	chemical ionization mass spectroscopy
d	doublet
D	sodium D line (578 nm)
DAP	diaminopimelic acid
DAST	diethylaminosulphur trifluoride
DCC	dicyclohexylcarbodiimide
DMAP	4-(*N,N*-dimethylamino)pyridine
DMF	dimethylformamide
d.e.	diastereomeric excess
e.e.	enantiomeric excess
esi	electrospray ionization

EtOH	ethanol
hplc	high performance liquid chromatography
hrms	high resolution mass spectroscopy
Hz	hertz
ir	infra red
J	coupling constant (Hz)
m	multiplet
MeOH	methanol
MeONa	sodium methoxide
mp	melting point
n/a	non-applicable or not available
nmr	nuclear magnetic resonance
opa	*ortho*-phthalaldehyde
ppt	precipitate
psi	pounds per square inch
r.t.	room temperature
s	singlet
t	triplet
TFA	trifluoroacetic acid
THF	tetrahydrafuran
tlc	thin layer chromatography
TMS	tetramethylsilane

tosyl	*p*-toluenesulphonyl
trityl	triphenylmethyl
ms	mass spectrometry
m/z	mass to charge ratio
OAc	acetate
ν_{max}	wavenumbers at an absorption maximum

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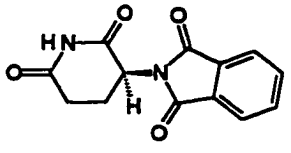
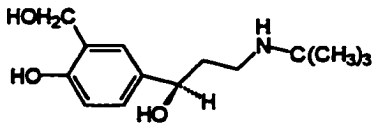
To Sheena I simply say "thanks" because no words can describe the degree of my appreciation of the support, encouragement, patience, and love you have given me over the years. Benjamin Roy, your smiles made it all worthwhile.

INTRODUCTION

The assumption that only one stereoisomer is responsible for the biological activity associated with a compound was poignantly invalidated by the marketing of the morning sickness drug, thalidomide (**1**), as a racemic mixture. After many birth defects it was realized that the (*S*)-(-)-isomer of thalidomide was indeed acting to combat morning sickness in pregnant women while the (*R*)-(+)-isomer was responsible for birth defects in the fetus (1). For another drug, albuterol (**2**), opposing biological effects are produced by each enantiomer; (*R*)-albuterol is an antiasthmatic while (*S*)-albuterol acts to constrict airways (2). As a result of these and other examples of different biological effects exhibited by stereoisomers, pharmaceutical companies have initiated massive efforts to develop single isomer drugs (1-3).



(S)-**1**



(R)-**2**

1.1 Differential Metabolism of Amino Acid Stereoisomers

α-Amino acids, the common building blocks of proteins, are an important group of naturally occurring compounds. More than one stereoisomer exists for each amino acid (except glycine), although (*S*)-amino acids are generally more abundant in nature than the corresponding (*R*)-isomers. Just as drug stereoisomers exhibit different biological effects, amino acid stereoisomers undergo different metabolic reactions. For example the routes employed by bacteria to degrade the stereoisomers of lysine are distinct.

In aerobic bacteria two pathways leading to Δ^1-piperideine-6-carboxylate (**6**) exist for the catabolism of lysine (Figure 1). Δ^1-Piperideine-6-carboxylate formed from lysine *via* saccharopine (**7**) retains the nitrogen in the α-amino group of lysine, whereas the nitrogen of the terminal amino group of lysine is retained when Δ^1-piperideine-6-carboxylate is formed *via* α-oxo-ε-aminocaproic acid (**3**) (4). The pathway involving saccharopine is used primarily for the catabolism of (*S*)-lysine while the pathway involving α-oxo-ε-aminocaproic acid is used for the catabolism of (*R*)-lysine (4).

In anaerobic bacteria lysine is catabolized to acetate (**13**) and butyrate (**14**). Cell resuspension experiments with *Clostridium sticklandii* (strain HF) and with *Clostridium* strain M-E suggest that there are parallel pathways (Figure 2), one for each stereoisomer, operating at different efficiencies (5).

Figure 1 Catabolism of (*R*)- and (*S*)-lysine in aerobic bacteria.

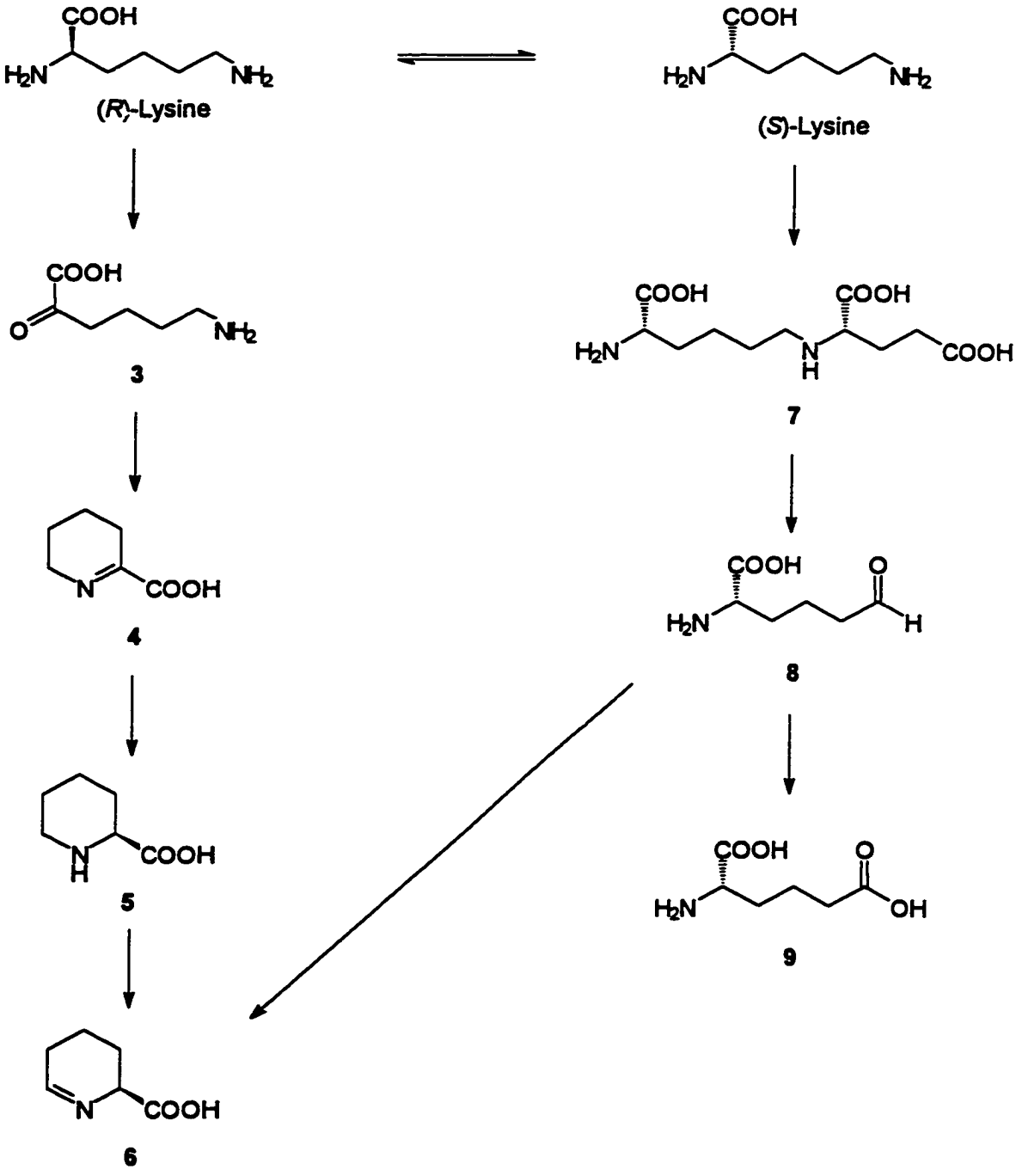
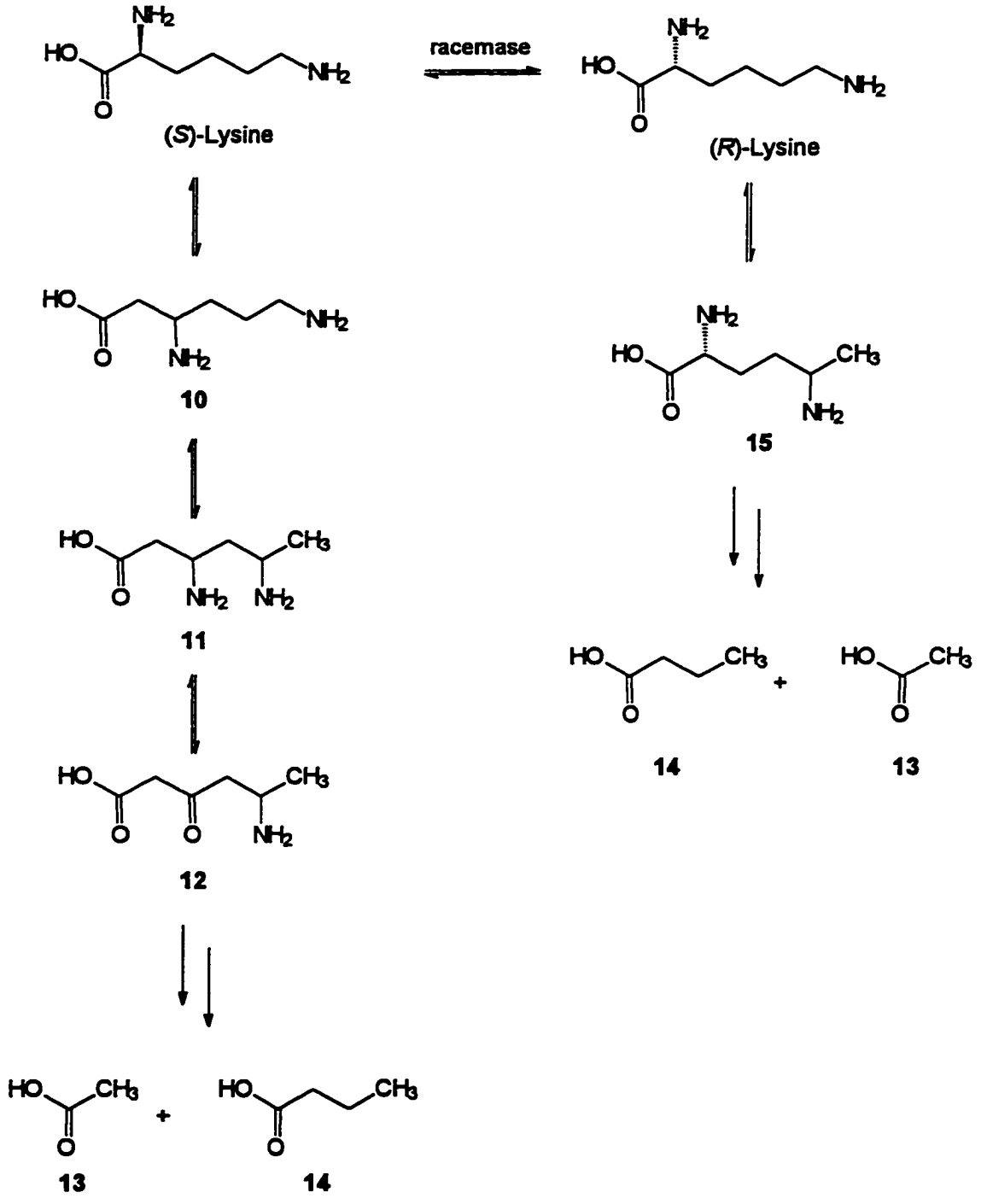


Figure 2 Catabolism of lysine isomers in anaerobic bacteria.



1.2 (*R*)- and (*S*)-Amino Acids as Biosynthetic Precursors

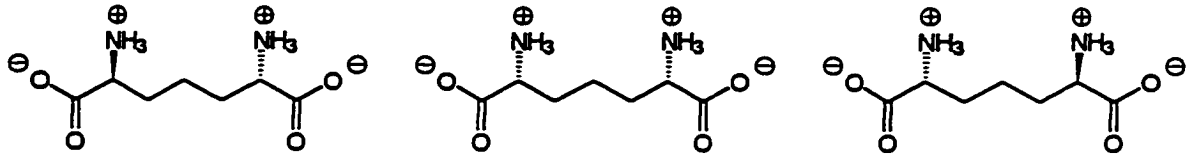
Amino acids, the common building blocks of proteins, are also biosynthetic precursors to many other natural products in plants, animals and microorganisms (6, 7). Often the precursor-product relationship is easily recognized by structural and stereochemical similarities between the amino acid and the biosynthetic product, but extensive modifications of the precursor can also occur, making the recognition of the precursor amino acid (and/or its stereochemistry) from the product's structure less obvious. A number of examples are presented below to illustrate these relationships, particularly the stereochemical aspects.

Proteins are derived from amino acids and the configurations of the (*R*)- and (*S*)-stereocenters along the backbone of the protein correspond directly to the configurations of the precursor amino acids, (*R*)-cysteine and the common (*S*)-amino acids.

Diaminopimelic acid (DAP, **16**), a seven carbon α-amino acid containing two stereocenters (Figure 3), is an essential constituent of the cell walls of most bacteria (8), the only known exceptions being Gram-positive cocci (9). Either the (*R,S*) or the (*S,S*) isomer of DAP is a component of the cell wall (10) and the appropriate precursor is supplied by the reversible epimerization of (*R,S*)-DAP to (*S,S*)-DAP catalyzed by *meso*-DAP epimerase (E.C. 5.1.1.7). In plants and

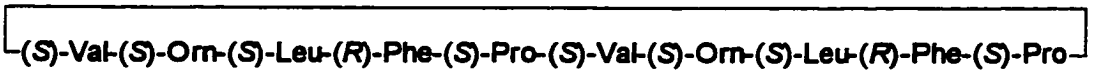
animals, the decarboxylation of (*R,S*)-DAP provides (*S*)-lysine, an essential amino acid in mammals (8, 11).

Figure 3 Stereoisomers of DAP.



(*S,S*)-**16** (*R,S*)-**16** (*R,R*)-**16**

A direct correspondence of precursor-product stereochemistry also holds for most but not all of the amino acid residues in the peptide antibiotic gramicidin S (**17**). In *Bacillus brevis* the gramicidin S synthase 1 (E.C. 5.1.1.11) enzyme complex activates and racemizes (*S*)-phenylalanine for incorporation as (*R*)-phenylalanine into gramicidin S, (**17**), (12).



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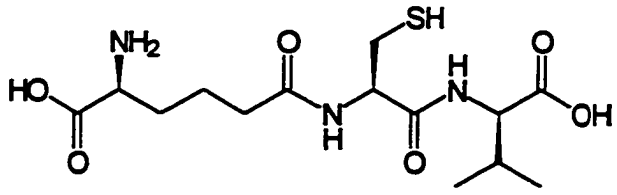
The biosynthesis of penicillin and cephalosporins occurs *via* the tripeptide, δ-((*S*)-α-aminoadipoyl)-(*R*)-cysteinyl-(*R*)-valine (**18**), and the

(*R*)-valine residue is derived from the corresponding (*S*)-amino acid, which is not incorporated *via* α,β-dehydrovaline (13).



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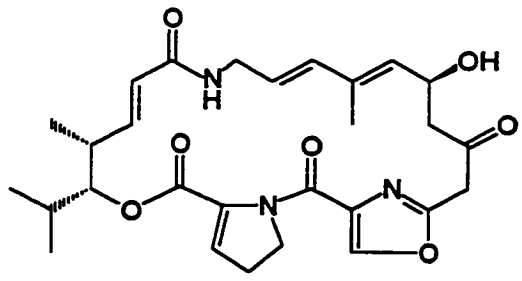
Cultures of *Actinomadura flava* and various *Actinoplanes* produce antibiotic A2315A (**21**), a member of the streptogramin group of antibiotics containing an (*R*)-alanine moiety (14) (Figure 4). Both (*R*)- and (*S*)-alanine are equally efficient precursors to the (*R*)-alanine residue of A2315A, and it has been shown that (*R*)-alanine is not incorporated *via* dehydroalanine (14).

Other members of the streptogramin group of antibiotics, pristinamycin IIA (**19**) and IIB (**20**), are found in cultures of *Streptomyces pristinaespiralis*, and it was suggested that the (*R*)-proline moiety in **20** is derived from the dehydro amino acid. However, the (*R*)-proline residue of pristinamycin IIB is converted into the dehydroproline residue of pristinamycin IIA (15). Both (*R*)- and (*S*)-proline are incorporated to the same degree into the dehydroproline moiety of pristinamycin IIA (15). Pristinamycin IIA is not a precursor to pristinamycin IIB, and a two-enzyme system catalyzing the conversion of the (*R*)-proline residue to the dehydroproline residue has been isolated and purified (16).

Figure 4 Streptogramin antibiotics.



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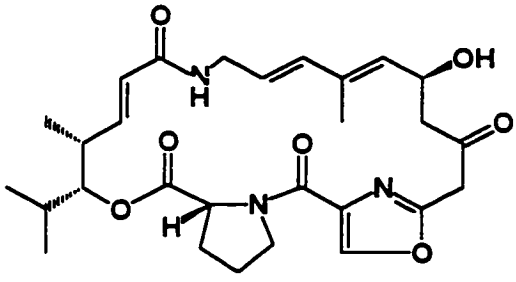

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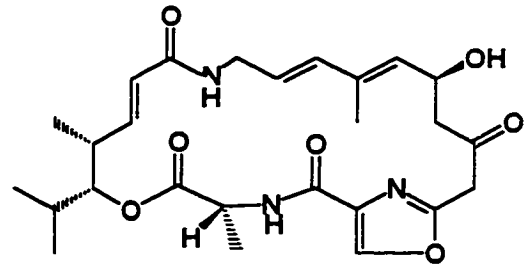

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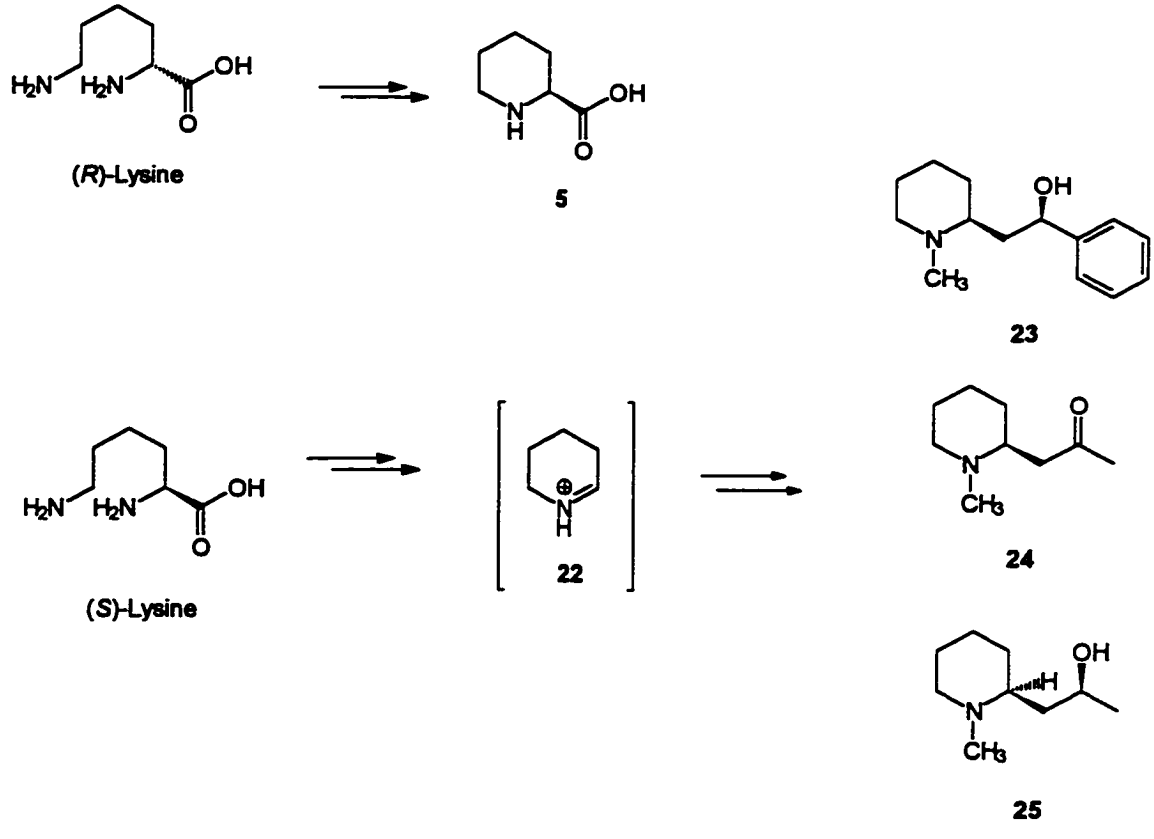
Although there is a strong structural similarity between (*S*)-lysine and (*S*)-pipecolic acid (**5**), (*R*)-lysine is the direct precursor in *Nicotiana glauca R*, *Sedum acre L.* and in *S. sarmentosum* (Figure 5). However, the alkaloids sedamine (**23**), *N*-methylpelletierine (**24**) and *N*-methylallosedridine (**25**) are assembled from (*S*)-lysine *via* Δ^1-piperideine (**22**, Figure 5) in the same plants (17).

Anosmine (**27**), an alkaloid produced by *Dendrobium anosmum* and *Dendrobium parishii*, is biosynthesized from the two stereoisomers of lysine. A current hypothesis (18) (Figure 6) suggests that one of the six membered rings

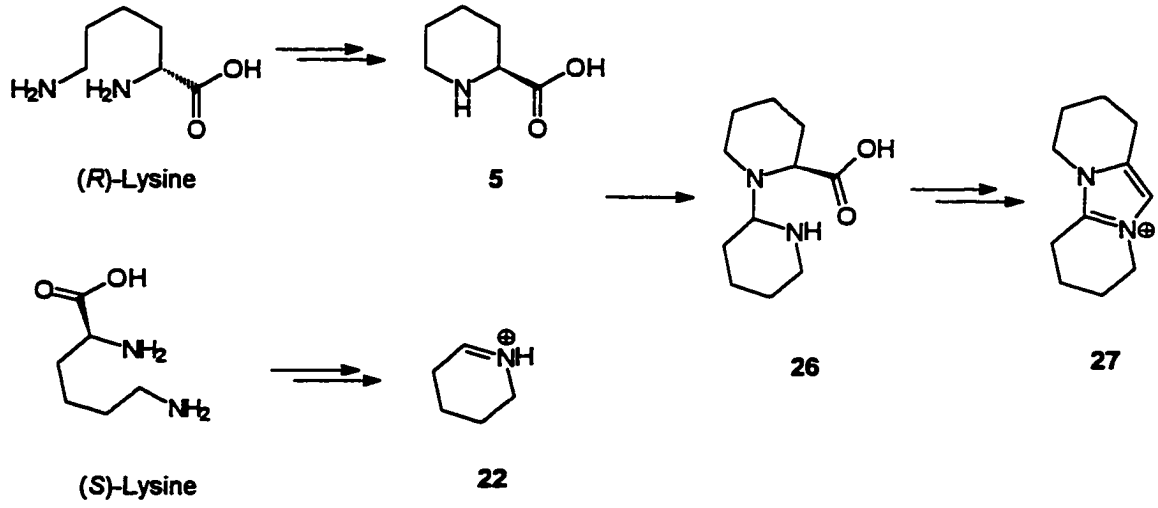
is derived from (*R*)-lysine *via* pipecolic acid (**5**) and the other is derived from (*S*)-lysine *via* Δ^1-piperideine (**22**).

Figure 5 Separate pathways for the utilization of stereoisomers of lysine.

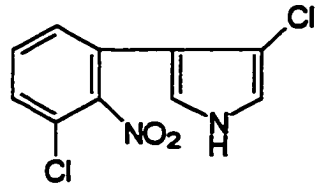


The antibiotic pyrrolnitrin (**28**) and its congeners have been isolated from cultures of *Pseudomonas pyrrocinia* and it is not possible to predict the stereochemistry of the precursor from the structure of the product. (*R*)-Tryptophan is a more efficient precursor than (*S*)-tryptophan (19), and it has

Figure 6 Utilization of both (*R*)- and (*S*)-lysine in the biosynthesis of anosmine.



been conclusively shown that (*S*)-tryptophan is not converted to pyrrolnitrin *via* (*R*)-tryptophan. The involvement of both (*R*)- and (*S*)-tryptophan in the biosynthesis of pyrrolnitrin has not been completely determined.



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The examples presented illustrate that both (*R*)- and (*S*)-amino acids serve as precursors to natural products and that the configuration of a stereocentre in the product is not a reliable indicator of the configuration in the

corresponding precursor amino acid. For example, an (*R*) stereochemistry in an amino acid derived fragment can arise by direct incorporation of an (*R*)-amino acid or by inversion of the configuration of an (*S*)-amino acid, either during or after incorporation. In other cases, a structural comparison to the precursor stereochemistry cannot be made due to the lack of stereocenters in the product. Clearly the stereochemical relationship between precursor and product is not easily deduced from structural comparisons and must be determined by experiments using the individual amino acid stereoisomers.

1.3 Determination of Biosynthetic Precursor-Product Relationship

Structural similarities between a natural product and common, naturally occurring compounds provide the basis for an initial biosynthetic hypothesis, which is tested by administering a possible precursor to an organism and by carrying out an analysis of the end-product of the pathway. The addition of an unlabelled precursor, which is structurally similar to the natural product may lead to an increase in the amount produced, thereby suggesting a precursor-product relationship. However, verification of the incorporation of the precursor into the final product requires additional experiments using substrates labelled with radioactive (*e.g.*, ^{14}C, ^{3}H) or stable (*e.g.*, ^{18}O, ^{15}N, ^{13}C, ^{2}H) isotopes. The detection of a specifically incorporated isotopic label in the isolated and purified product

establishes the labelled substrate as a precursor and provides evidence that can be used to formulate a hypothesis for the biosynthetic pathway.

Methods for the detection of radioactive isotopes have much greater sensitivity than methods commonly employed for stable isotopes (20), but this advantage of increased sensitivity is offset by the requirement for unambiguous chemical degradation of the metabolite to determine the position of the label. The use of radioactive isotopes in feeding studies with plants and microorganisms has declined dramatically due to recent advances in nmr spectroscopy and mass spectrometry that allow stable isotopes to be used routinely (21).

The two stable isotopes ^{13}C and ^{2}H have replaced ^{14}C and ^{3}H, respectively, as the most common isotopes used to test the incorporation of a potential biosynthetic precursor. Stable isotopes are detected primarily by nmr spectroscopy, either directly or indirectly; detection by ms is also employed. If proton and carbon nmr resonances have been assigned during structure elucidation studies of the natural product, the position of an incorporated label is easily determined (22), and avoids the need for chemical degradation of the sample. The latter was necessary when radioactive labels were employed. The use of nmr spectroscopy to determine the position of the label is quicker than chemical degradation and, most importantly, nmr techniques are nondestructive. While ms is a destructive technique it only requires a very small sample.

Several review articles exist which outline the utility of nmr experiments in biosynthetic pathway determinations (21, 23, 24).

Besides ^{13}C and ^{2}H, ^{15}N, a stable isotope of nitrogen, can be detected both directly by ^{15}N nmr spectroscopy and indirectly *via* spin couplings to ^{13}C (25, 26). The greatest utility of the ^{15}N isotope is found when it is bonded to ^{13}C by observing the splitting of the ^{13}C resonance. This method is used to determine whether a ^{13}C-^{15}N bond remains intact (21). The stable isotope of oxygen ^{18}O has no nuclear spin and is therefore not nmr active. However the presence of ^{18}O causes a shift in the resonance of the ^{13}C nucleus to which it is bonded, and hence the presence of ^{18}O can be indirectly determined by nmr spectroscopy (27).

The feeding of a labelled compound that is a true precursor of the metabolite may result in low incorporation if the compound does not readily enter the cell, or if the organism has preferentially converted it to a substance other than the metabolite of interest (28). In biosynthetic studies there is always concern regarding the timing of precursor administration; biosynthesis of the metabolite under investigation should be underway at the time of precursor administration. The many factors affecting a precursor's incorporation exemplify the need to interpret low or no incorporation with discretion. Similarly, wariness should be exercised when the synthetic precursor efficiently labels the metabolite because the synthetic precursor may not be an intermediate of the

pathway. Instead, it may have been converted to a true intermediate by an enzyme-catalyzed step that is not in the main pathway (29).

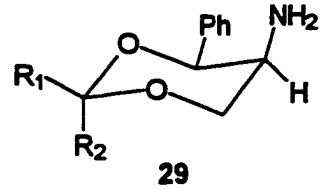
The availability of isotopically labelled substrates is prerequisite to carrying out these biosynthetic experiments, and the determination of the stereochemical relationship between an amino acid precursor and the product requires the isotopically labelled substrate to be stereochemically pure. Both (*R*)- and (*S*)-amino acids can be synthesized according to several methods in the literature.

1.4 Methods for the Stereoselective Synthesis of α-Amino Acids and β-Hydroxy-α-Amino Acids

The asymmetric synthesis of protein amino acids and non-protein amino acids has attracted considerable attention in the literature (30-32), and a complete review is beyond the scope of this thesis. This discussion will focus on examples of the more significant methods for the synthesis of α-amino acids and β-hydroxy-α-amino acids. All approaches other than the homologation of an existing amino acid, require the stereoselective formation of one or more bonds to the α-carbon of the product amino acid.

1.4.1 Strecker

The classical route to racemic amino acids using the Strecker synthesis (aldehyde + NH_3 + CN^-) has been improved to provide enantiomerically pure amino acids. Asymmetric Strecker syntheses usually employ the addition of a cyanide to the Schiff base of a chiral amine (30), and improvements have been made by developing the chiral auxiliary attached to the nitrogen of the Schiff base. One of the earliest asymmetric Strecker syntheses employed α-methylbenzylamine (30) and more recent (33) incarnations have used more elaborate chiral amines such as [4*S*,5*S*]-(+)-5-amino-2,2-alkyl-4-phenyl-1,3-dioxane (**29**).



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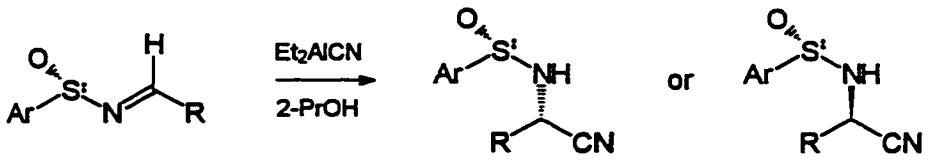
R_1 = Me, H, Ph, or ^tBu R_2 = Me, H, Ph

Recently (34) the chiral amine has been replaced with enantiopure sulfinimines, and cyanide has been delivered *via* diethylaluminum cyanide. The amino acid product is liberated from the newer auxiliaries such as sulfinimines (**31** and **32**) (34) and β-1-amino-tetra-*O*-pivaloyl galactose (35), by acid hydrolysis, instead of the hydrogenation step used to liberate the amino acid in the classical approach.

Figure 7 Asymmetric Strecker synthesis using sulfinimines.



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1.4.2 Gabriel

The Gabriel synthesis (potassium phthalimide + halogenated carboxylic acid), like the Strecker synthesis, is a classical synthetic method for the preparation of amino acids that has seen a resurgence due to recent improvements that allow the stereoselective synthesis of amino acids. The Gabriel method for amino acid synthesis requires as the final step removal of the phthalimide group to provide the free amino group.

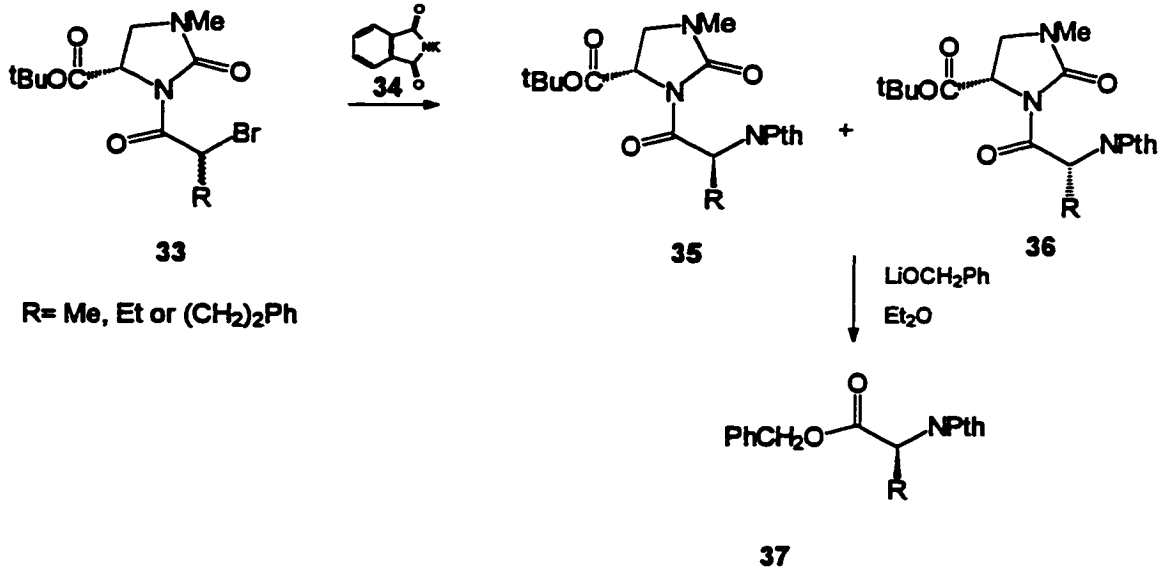
Nunami and coworkers (36) reported the preparation of *N*-phthaloyl-(*S*)-amino acids (Figure 8) and suggested that their method can be used to prepare a range of *(R)*- and (*S*)-amino acids (**36**). The validity of the approach was demonstrated by the preparation of *N*-phthaloyl-(*S*)-alanine (**37**).

1.4.3 Homologation

The elaboration of natural amino acids by homologation at a carbon of the R group is gaining prominence among methods for the asymmetric synthesis of

amino acids (37). Amino acids with R group functionality at the β-carbon have been extensively utilized in this method, *e.g.*, serine, cysteine and aspartic acid.

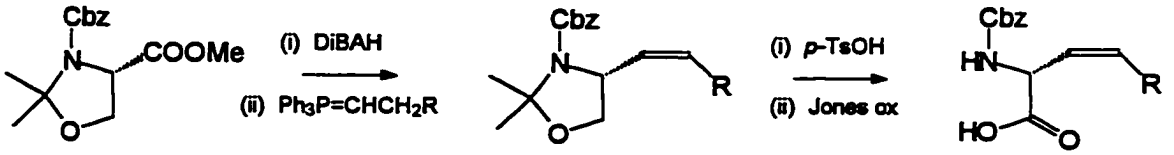
Figure 8 Asymmetric Gabriel synthesis of *N*-phthaloyl-(*S*)-amino acids.



The nucleophilic ring opening of a serine lactone studied by Vederas and coworkers (38) provides product amino acids with minimal loss of enantiomeric purity in most cases. Beaulieu and Schiller (39) converted serine to the cyclic *N,O*-protected methyl ester (**38**) and carried out a Wittig reaction after reduction of the ester functionality to an aldehyde (Figure 9). After reduction of the newly formed double bond the alcohol of serine was oxidized to become the α-carboxylic acid group of the product amino acid. Baldwin and coworkers (40)

employed β-iodoalanine derivatives to prepare highly functional amino acids *via* a free radical reaction with methacryl tin reagents.

Figure 9 Amino acid synthesis from serine.



38 39 40

Homologation of the γ-carbons of methionine, homoserine, aspartic and glutamic acids have also been successfully demonstrated (37). The use of (*S*)-glutamic acid is very attractive since it is currently the cheapest amino acid available. The utility of the glutamic and aspartic acids depend on the selective and differential protection of the carboxylic acid moieties, after which either carboxyl group can be made to undergo selective reactions as desired.

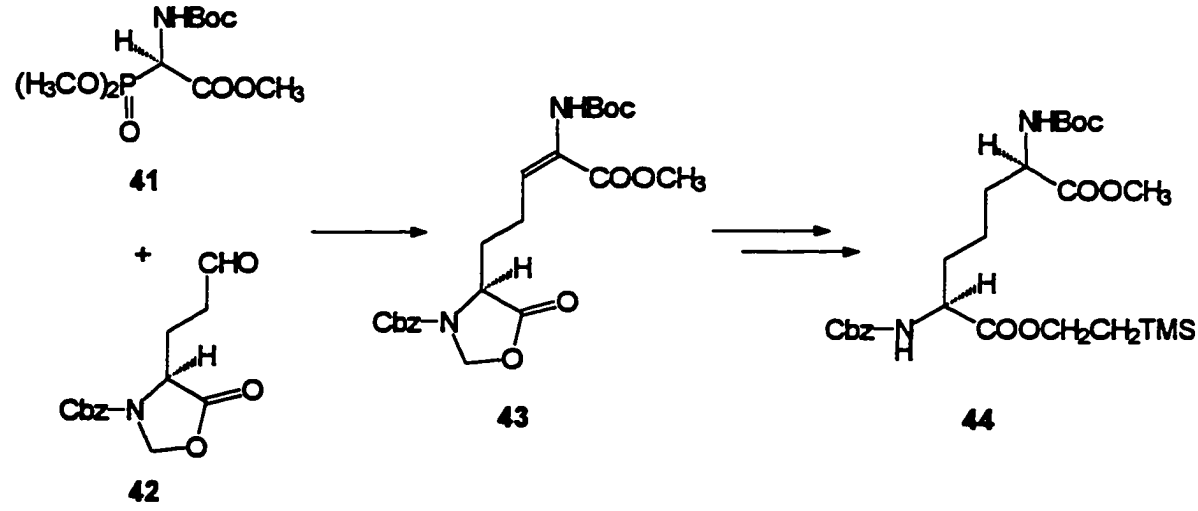
1.4.4 Hydrogenation

Asymmetric hydrogenation methods provide a route to amino acids *via* the corresponding α,β-dehydroamino acids. This approach to amino acid synthesis is usually carried out with a chiral hydrogenation catalyst and an achiral α,β-dehydroamino acid substrate. The complementary approach employing an α,β-

dehydroamino acid attached to a chiral auxiliary is usually carried out with a heterogeneous catalyst.

The asymmetric hydrogenation of dehydro amino acids has been used in the preparation of *meso*-2,4-diaminoglutaric acid (41), and also for the preparation of DAP (42). The preparation of differentially protected DAP reported by Holcomb *et al.* (42) uses glutamic acid as the origin of one stereogenic centre while forming the other by catalytic hydrogenation of the double bond formed by a Wittig reaction (Figure 10).

Figure 10 Key step in the literature synthesis of DAP starting from glutamic acid.



Interest in the development of homogenous chiral catalysts for the asymmetric hydrogenation of dehydroamino acids (and many other types of

unsaturated substrates) has led to the development of many chiral ligands (30). The more successful ligands all contain multiple phenylphosphine moieties that form complexes with the transition metals Pd, Pt, and Rh. The ligands are generally available in both enantiomeric forms and as such provide routes to both stereoisomers of the reduced product. The success of these methods can be measured by the commercial availability of many of these ligands (43).

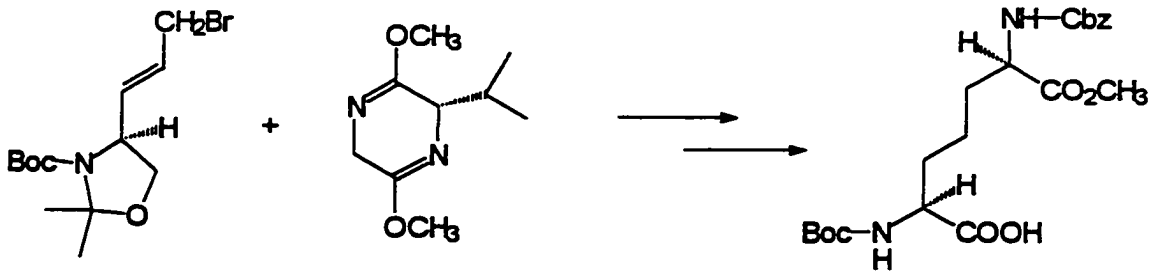
1.4.5 Chiral Glycine Derivatives

Schöllkopf and coworkers (44) introduced the bislactim method for the preparation of (*R*)-α-amino acids. These amino acids are generally obtained in respectable yields (52-89%) and enantiomeric excess (60->95%). The amino acid methyl ester is obtained by mild, but lengthy, acid hydrolysis.

The first stereospecific synthesis of DAP, reported by Jurgens (45) in 1992, employed both the Garner oxazolidine (46) and the Schöllkopf (47) chiral glycine equivalent (Figure 11).

The Williams glycine equivalent (**48**) has seen widespread use for the synthesis of both protein and non-protein amino acids. New methods (48) have been introduced by others for the more efficient preparation of this glycine equivalent. Strong reducing conditions (Li^0/NH_3 or H_2, Pd^0) are required to liberate the amino acid product.

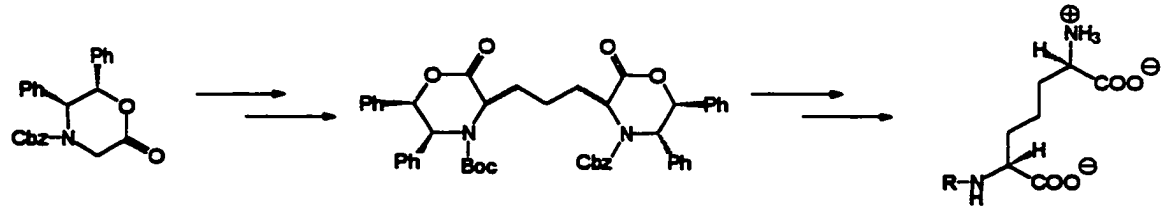
Figure 11 Key step in the literature synthesis of DAP by Jurgens. (45)



45 46 47

The asymmetric synthesis of the three stereoisomers of DAP was reported by Williams and Yuan (49). The (2*S*,6*R*)-isomer was prepared with differentially protected amino groups, but differentially protected carboxylic acid groups cannot be obtained by this approach. This shortcoming could not be avoided since both stereogenic centres of DAP were formed by alkylation of diphenyloxazinones and as a last step the amino acid moieties are generated by catalytic hydrogenation (Figure 12, R=H). Williams and Yuan (50) reported the asymmetric synthesis of a DAP-glutamic acid dipeptide employing the chiral diphenyloxazinones to derive both amino acid centres, (Figure 12, R=Glu).

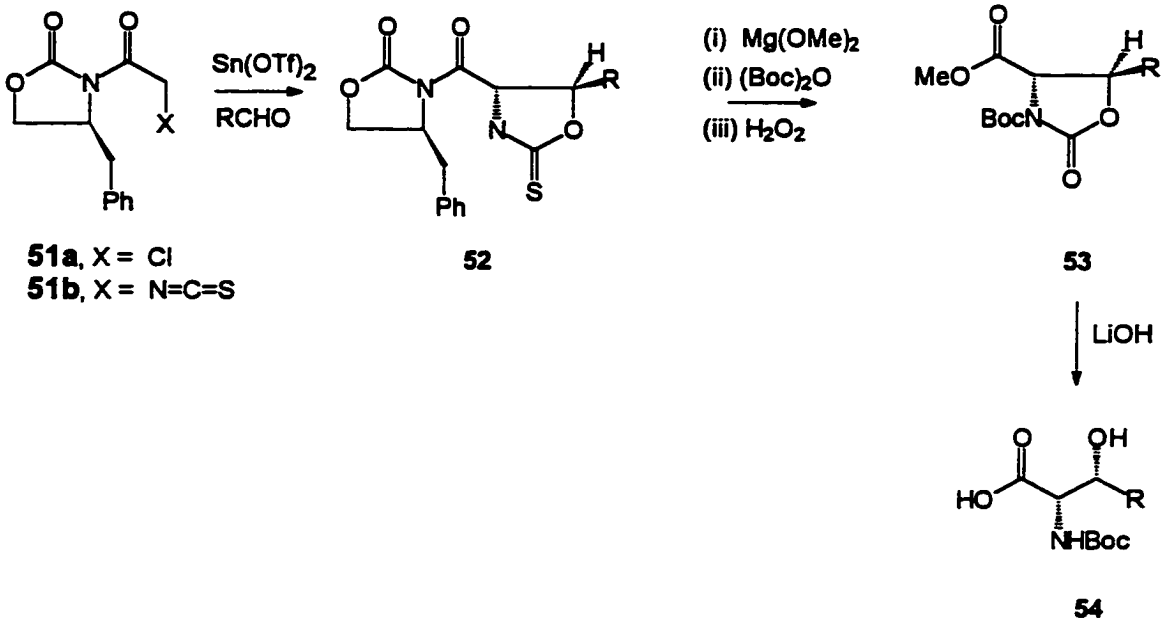
Figure 12 Key step in the synthesis of DAP using diphenyloxazinones.



48 49 50a R = H
50b R = glu

β-Hydroxy-α-amino acids have been synthesized by condensation of aldehydes with the isothiocyanate **51b** in the presence of stannous triflate (51), (Figure 13). This synthetic procedure provides the nitrogen of the β-hydroxy-α-amino acid *via* an azide ion so that the preparation of **51b** from ^{15}N-azide will result in the loss of half of the isotopic label.

Figure 13 Asymmetric synthesis of β-hydroxy-α-amino acids (51).



51a, X = Cl
51b, X = N=C=S

52

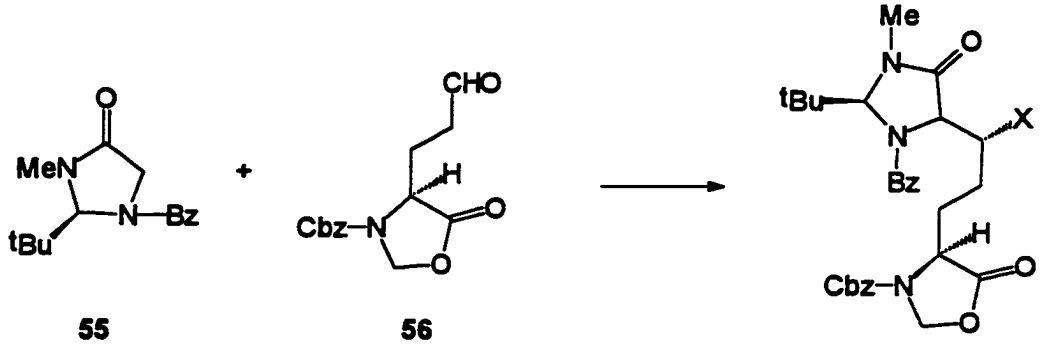
53

54

Seebach and Blaser (52) reported the synthesis of β-hydroxy-α-amino acids *via* the *N*-Boc-oxazolidinone **56**. This method, which has been used to prepare various β-hydroxy-α-amino acids, yields the amino acid by hydrogenation with H_2/Pd-C.

3-Fluorodiaminopimelic acid isomers have been investigated as inhibitors of diaminopimelate epimerase (53). Preparation of these derivatives was achieved by converting β-hydroxy-DAP to the fluoro analogue using DAST. The stereogenic centres of one series of fluoro analogs were derived from an (*S*)-glutamic acid derivative (**56**) and the Seebach (52) imidazolidinone (**55**), (Figure 14), while in the other series the stereocenters were derived from glutamic acid and Schöllkopf's bislactim (47).

Figure 14 Synthesis of fluoro analogs of DAP.



55 **56**

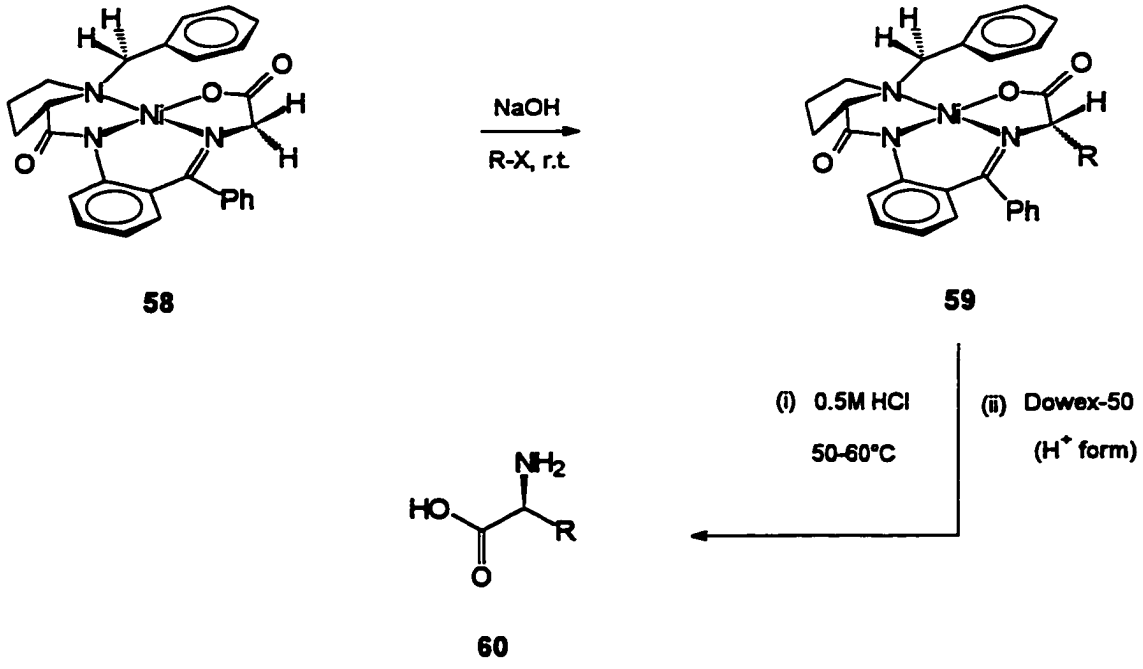
57a X = OH
57b X = F

1.4.6 Use of Glycine Schiff Base Ni(II) Complex for α-Amino Acid and β-Hydroxy-α-amino Acid Syntheses

The chiral glycine equivalent prepared from the nickel (II) complex of the Schiff base of (*S*)-2-[*N*-(*N'*-benzylprolyl)amino]benzophenone and glycine ((*S*)-BPB-complex) has been used for the preparation of (*S*)-amino acids (Figure 15)

(54, 55). The (*S*)-BPB-complex was first used for the synthesis of β-hydroxy-α-amino acids but was later employed with great success in the synthesis of α-amino acids. α-Methyl-α-amino acids were prepared by replacing glycine in the complex with alanine (56). The Michael type addition of nucleophiles to the α,β-dehydroalanine complex (57) allows the use of various nucleophiles to prepare β-substituted alanines. Reaction of the (*S*)-BPB-complex with α,β-dehydro-aminobutanoic acid generated β-substituted (*S*)-2-aminobutanoic acids (58). Since its introduction in 1985 (54) the (*S*)-BPB-complex has been employed by several research groups for the preparation of (*S*)-α-amino acids (59, 60). The

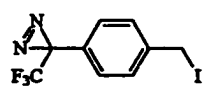
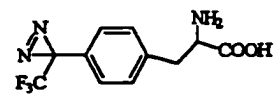
Figure 15 Use of (*S*)-BPB-complex for α-amino acids.



58 59 60

(*S*)-BPB-complex and its precursor (*S*)-BPB-ligand are also available commercially. Table 1 provides a summary of the amino acids prepared using the (*S*)-BPB-complex.

Table 1 Typical literature synthesis of (*S*)-α-amino acids *via* alkylation of (*S*)-BPB-complex.[a]

Alkylating reagent	Product amino acid	Chemical yield (%)	%e.e.[b]	Ref.
MeI	ala	82	70	(56)
$PhCH_2Br$	phe	83	92	(56)
Me_2CHBr	val	81	92	(56)
3,4-dimethoxy-benzylbromide	3,4-dimethoxy-phenylalanine	77	94[c]	(56)
sec-BuBr	ile	n/a	74[d]	(56)
		88	>95[e]	(59)

[a] Reaction in DMF at r.t. [b] %e.e. determined by g.c. analysis. [c] %e.e. determined by polarimetry. [d] *threo/erythro* ratio is 2.5. [e] %d.e. of alkylated complex.

In its only use for the synthesis of isotopically labelled amino acids, Fasth and coworkers (60) used the (*S*)-BPB-complex to prepare ^{11}C labelled amino acids from ^{11}C-labelled alkyl halides, Table 2.

Table 2 Literature synthesis of isotopically labelled (*S*)-α-amino acids using (*S*)-BPB-complex[a] (60).

Alkylating Reagent	Product amino acid	Isotopic yield (%)	%e.e.[b]
$I^{11}CH_3$	[β-^{11}C]-ala	60	80
$I^{11}CH_3$	[β-^{11}C]-ala	40	>99[c]
$I^{11}CH_2Ph$	[β-^{11}C]-phe	30	90
$I^{11}CH_2C_6H_4OMe$	[β-^{11}C]-*O*-methyl tyr	15	90
$I^{11}CH_2C_6H_4OH$	[β-^{11}C]-tyr	12	90

[a] Reaction in acetone at 80°C. [b] %e.e. determined by hplc analysis. [c] After hplc chromatography of diastereomeric complexes.

The anion of the (*S*)-BPB-complex can be added in a Michael type addition to α,β-unsaturated carbonyl compounds. These experiments have been demonstrated by Belokon and coworkers (61) in the synthesis of substituted glutamic acids. This series of reactions also included the Michael type addition reaction of the (*S*)-BPB-complex to acrolein (CH_2=CHCN) to provide (*S*)-2-amino-4-cyanobutyric acid with 92% e.e. and 75% chemical yield. Additional literature reactions demonstrating Michael type additions of the (*S*)-BPB-complex are shown in Table 3.

The (*S*)-BPB complex has been modified to function as an electrophilic glycinate species (62). The conversion of the BPB-complex from a nucleophilic

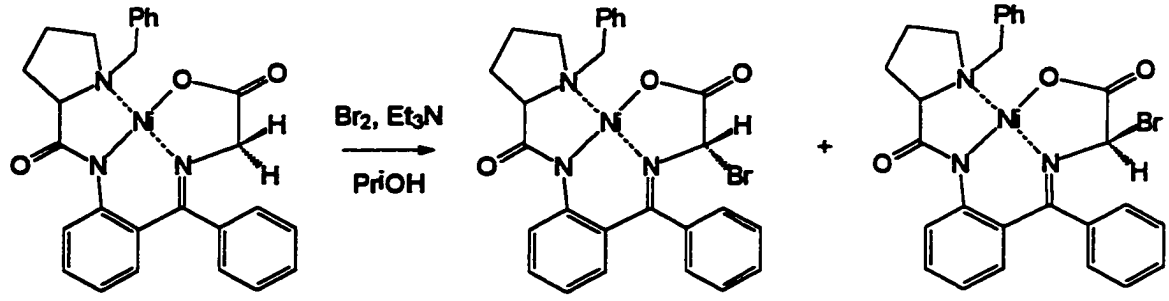
species is done by substitution of one of the α-hydrogens of glycine with bromine (Figure 16).

Table 3 Literature synthesis of (*S*)-α-amino acids employing Michael type additions to (*S*)-BPB-complex[a] (61).

Alkylating reagent	Product amino acid	Chemical yield (%)[b]	%e.e.[c]
CH_2=$CHCO_2Me$	δ-methyl glutamate	57	>95[e]
CH_2=CHCN[d]	2-amino-4-cyanobutanoic acid	62	>95
CH_2=CHCHO	proline[f]	28	>95
CH_2=$C(Me)CO_2Me$	(4*R*)-4-methylglutamate	37	>95
	(4*S*)-4-methylglutamate	17	>95
trans-PhCH=$CHCO_2Me$	(3*R*)-3-phenylglutamate	34	>95
	(3*S*)-3-phenylglutamate	21	>95

[a] Reaction in MeOH/NaOMe at r.t. [b] After SiO chromatography of diastereomeric complexes, yields based on starting (*S*)-BPB-complex. [c] %e.e. based on g.l.c. data. [d] Et_3N used as base. [e] Determined using ^1H nmr spectroscopy and a chiral lanthanide shift reagent. [f] After $NaBH_4$ reduction of the initial product.

Figure 16 Synthesis of nickel Schiff base of α-bromoglycine and 2'-aminobenzophenone.



58 (*S,S*)-61 (*S,R*)-61

Table 4 Literature synthesis of (*S*)-α-amino acids using (*S*)-BPB-complex of α-bromoglycine (62).

Nucleophile	Reaction conditions	Chemical yield (%)[a]	alkylated complex ratio[b]
MeONa	MeOH, 25°C	>90	98:2
PhONa	MeCN, n/a	60	91:9
Me_2NH	MeCN, 25°C	>90	92:8
$CH_2(CO_2Et)_2$	MeCN, ^tBuOH, 25°C	50[c]	90:10
BuZnCl	THF, -70°C	13	80:20

[a] Yield of alkylated complexes. [b] Equilibrium ratio determined by ^1H nmr spectroscopy of mixture. [c] Aspartic acid product (80% e.e.)

Chiral auxiliary transition metal complexes have seen considerable use in the synthesis of β-hydroxy-α-amino acids. Belokon *et al.* (54, 63-65) first

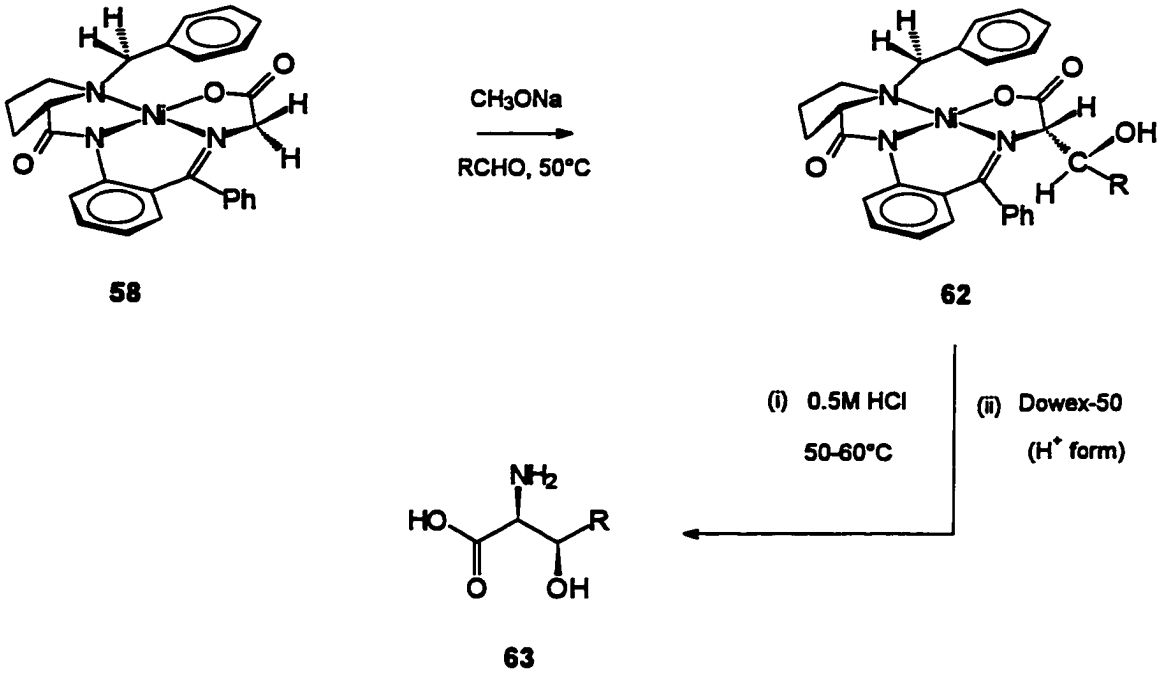
reported the use of the chiral nickel(II) complex of the Schiff base of glycine with (*S*)-2-[*N*-(*N'*-benzylprolyl)amino]benzophenone (**58**) in 1985. Prior to the introduction of **58**, Cu(II) and Ni(II) complexes utilizing ligands incorporating acetophenone and benzaldehyde were investigated. However, the use of these complexes for the preparation of α-amino acids and β-hydroxy-α-amino acids was not as promising as the later developed complex employing Ni(II) and the ligand prepared from 2-aminobenzophenone. The asymmetric condensation of the complex glycyl anion with an aldehyde or ketone provides *threo*-β-hydroxy-α-amino acids with enantiomeric excesses averaging >80% (64). Recently the (*S*)-BPB-complex was used to prepare β-hydroxy-β-trifluoromethyl α-amino acids (66, 67).

1.5 Synthetic Targets

1.5.1 Lysine

The differential metabolism of amino acid stereoisomers was illustrated in Section 1.1 by the separate pathways for the bacterial catabolism of (*R*)- and (*S*)-lysine. The pathway, outlined in Figure 2 was deduced from experiments using *Clostridium* species of anaerobic bacteria (5) and similar routes have been proposed for lysine catabolism in another anaerobe, *Fusobacterium nucleatum* (68).

Figure 17 Asymmetric synthesis of β-hydroxy-α-amino acids using the Ni(II) chiral glycine complex (54).



Amino acids are the primary energy sources for *F. nucleatum*, an opportunistic pathogen implicated in periodontal disease. The catabolism of both (*R*)- and (*S*)-lysine by *F. nucleatum* has been demonstrated (69), but further investigation of lysine catabolism in *Fusobacterium* species requires the synthesis of ^{13}C-labelled lysine in enantiomerically pure forms. Feeding experiments similar to those used to investigate glutamate catabolism (70) will

provide solid evidence to assign the pathways used to degrade (*R*)- and (*S*)-lysine in *F. nucleatum.*

Based on the incorporation of isotopic labels from (*R,S*)-lysine and other possible precursors such as cadaverine and pipecolic acid, it has been suggested (18) that anosmine (**27**) is derived from one molecule of (*R*)-lysine and one of (*S*)-lysine. The incorporation of the lysine stereoisomers has not been verified by separate experiments. Due to the low incorporations observed in feeding experiments with racemic [1,2-$^{13}C_2$]-lysine, stereochemically pure $^{13}C_2$ lysines (*i.e.,* bond labelled) are needed to allow incorporations to be assessed from the ^{13}C satellite signals.

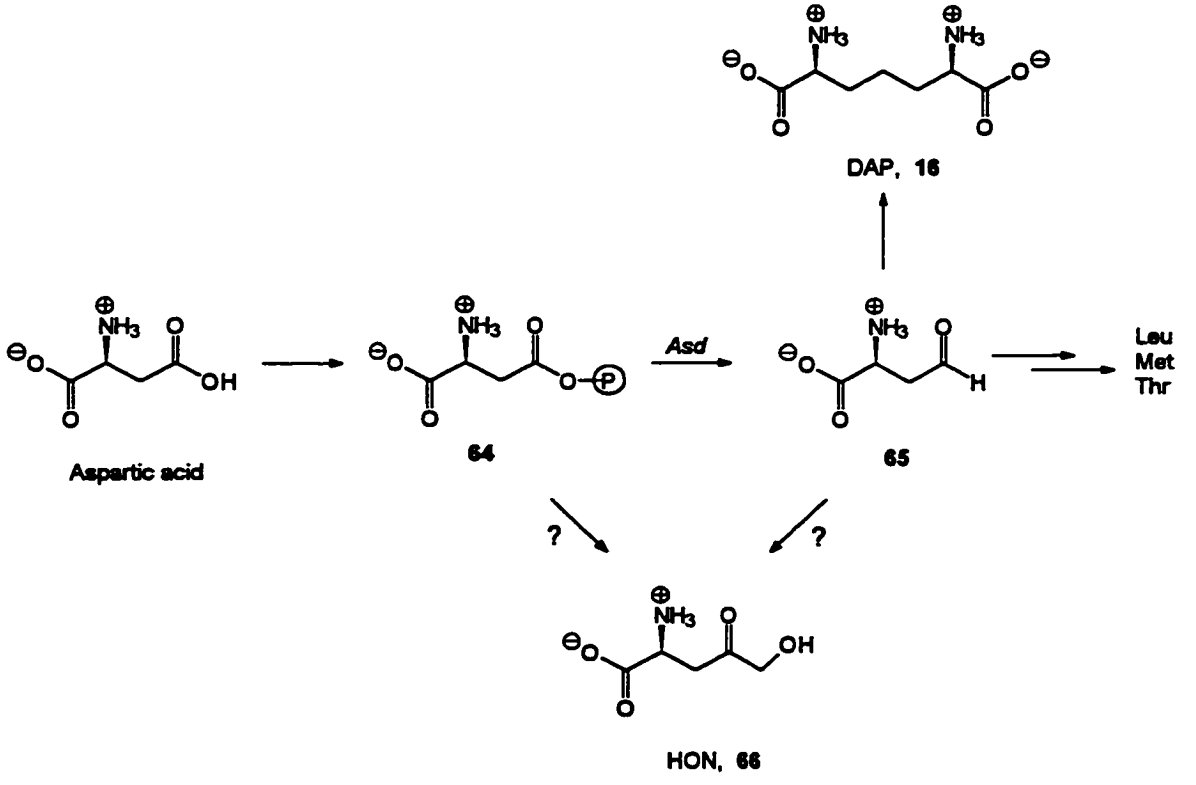
1.5.2 2,6-Diaminopimelic Acid (DAP)

(*R,S*)-DAP and either one or both of the enantiomeric forms (Figure 3) are catabolized by *F. nucleatum* (69). The availability of DAP stereoisomers will permit the stereochemistry of DAP catabolism to be determined and isotopically labelled samples are required to investigate the similarities between the pathways of DAP and lysine catabolism in this anaerobic bacterium.

Aspartic acid has been identified as a precursor to the nonprotein amino acid 5-hydroxy-4-oxonorvaline (**66**, HON), an antibiotic produced by cultures of *Streptomyces akiyoshiensis* (71). To determine whether aspartyl phosphate (**64**) or aspartate semialdehyde (**65**) serves as the branch point of the HON pathway

(Figure 18) in *Streptomyces akiyoshiensis* experiments were undertaken (72) to disrupt the gene coding for the enzyme, aspartate semialdehyde dehydrogenase (Asd, E.C. 1.2.1.11). Mutants deficient in this enzyme would not produce HON if aspartate semialdehyde was a precursor, whereas normal levels of HON production would be observed if aspartyl phosphate was the branch point.

Figure 18 Possible biosynthetic relationship between asp, HON and DAP.



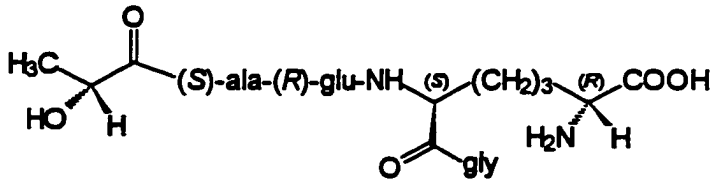
Unfortunately, no viable mutants containing a disrupted Asd gene were obtained (73). Since aspartate semialdehyde is a precursor to DAP, DAP and other amino acids must be made available to cells lacking the ability to make aspartate semialdehyde. Preliminary results suggest that DAP does not enter the cell, and that this is the reason why no viable Asd mutants were isolated. Isotopically labelled samples of DAP are required to test the transport of this amino acid into *S. akiyoshiensis* cells, and the availability of the individual stereoisomers allows the effect of stereochemistry on the transport to be determined.

DAP and its derivatives have also been investigated as antibacterial and antifungal agents (53, 74), particularly derivatives of DAP that inhibit the enzyme diaminopimelate epimerase (E.C.5.1.1.7) (75-77). The recent discovery of the immunostimulating peptide FK-156 (**67**) (78, 79) and its synthetic analog, FK-565 (**68**) (78, 80), containing *meso*-DAP has spawned an increased interest in the chemical synthesis of differentially protected *meso*-DAP (42, 45, 50, 78). These immunostimulating peptides, alone or in combination with other drugs, exhibit the potential for treatment of retroviral infections and therefore are a possible AIDS treatment. It is necessary to prepare DAP differentially protected to allow selective coupling of the required amino acid at the proper end of the DAP molecule.

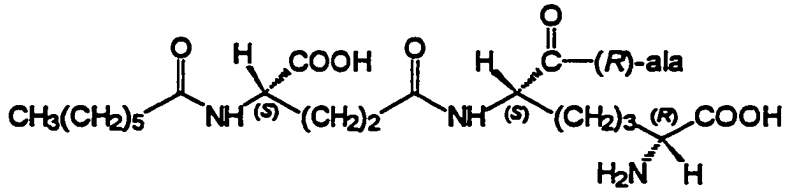
The preparation of a differentially protected DAP would also facilitate the synthesis of dipeptides of *meso*-DAP. The use of peptides to facilitate transport

is shown in the case of the antibiotic alaphosphin (8, 81). Thus di- and tripeptides containing DAP can be used to facilitate the transport of DAP across cell membranes and to provide a means of isolating viable Asd mutants of *S. akiyoshiensis.*

Figure 19 Immunostimulating peptides FK156 and FK565 containing *meso*-DAP (78).



FK-156, **67**



FK-565, **68**

1.5.3 4,5-Dihydroxynorvaline

The nonprotein amino acid 4,5-dihydroxynorvaline (**69a**) has been isolated from the seeds of *Lunaria annua L.* as a diastereomeric mixture, and the stereochemistry was not completely assigned (82). The structure of 4,5-

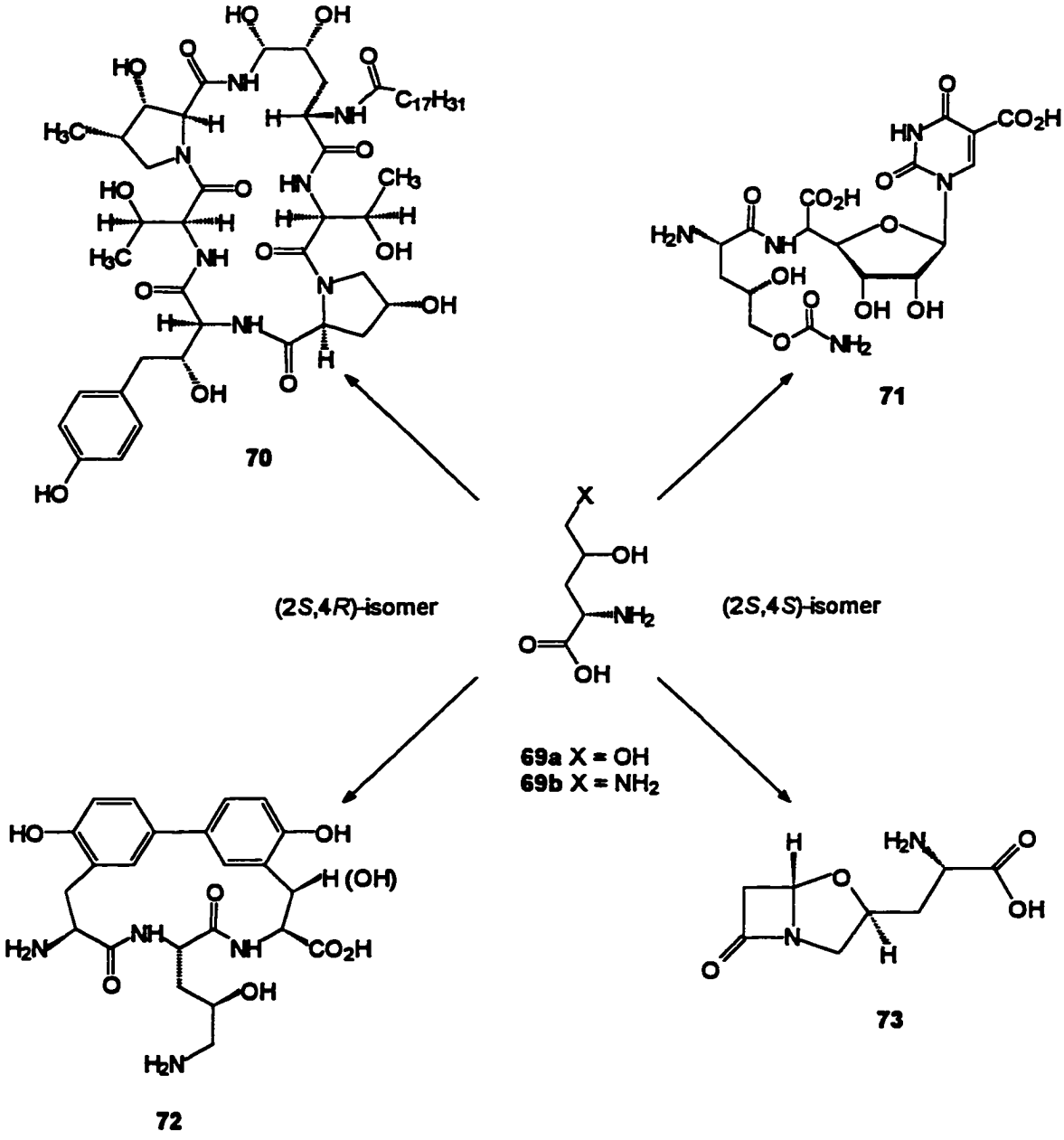
dihydroxynorvaline is closely related to that of HON, (2*S*)-5-hydroxy-4-oxonorvaline, **66**, and it is a potential intermediate in the biosynthesis of HON. The stereoselective synthesis of 4,5-dihydroxynorvaline will facilitate the assignment of the stereochemistry of the natural product and permit the role of 4,5-dihydroxynorvaline in HON biosynthesis to be explored.

Portions of the structures of several antibiotics are analogous to the structure of 4,5-dihydroxynorvaline; (2*S*,4*S*)-4,5-dihydroxynorvaline can be identified in clavalanine (**73**) and polyoxin E (**71**) whereas (2*S*, 4*R*)-dihydroxynorvaline corresponds to the norvaline fragment in biphenomycin (**72**) and echinocandin C (**70**). Recent synthesis of clavalanine and biphenomycin have been carried out from 4,5-dihydroxynorvaline derivatives (83-85), generated by multistep synthetic routes. A simple synthetic route to 4,5-dihydroxynorvaline would facilitate the synthesis of these antibiotics.

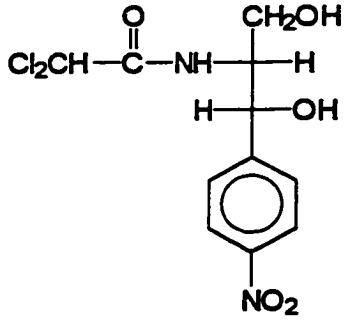
1.5.4 β-(p-Nitrophenyl)serine

β-(*p*-Nitrophenyl)serine is a key precursor for the chemical synthesis of substrates to investigate the final steps of chloramphenicol biosynthesis. Chloramphenicol, D-(-)-*threo*-2,2-dichloroacetamido-1-(*p*-nitrophenyl)-1,3-propanediol (**74**), one of the first broad-spectrum antibiotics discovered, was isolated from the soil bacterium *Streptomyces venezuelae* in the late 1940's by two independent research groups, Ehrlich *et al.* in 1947 (86) and Gottlieb *et al.*

Figure 20 Natural products containing a 4,5-dihydroxynorvaline moiety.



in 1948 (87). The chemical synthesis of chloramphenicol (**74**) has been accomplished on an industrial scale (88), and consequently chloramphenicol became the first antibiotic commercially produced without fermentation (89).



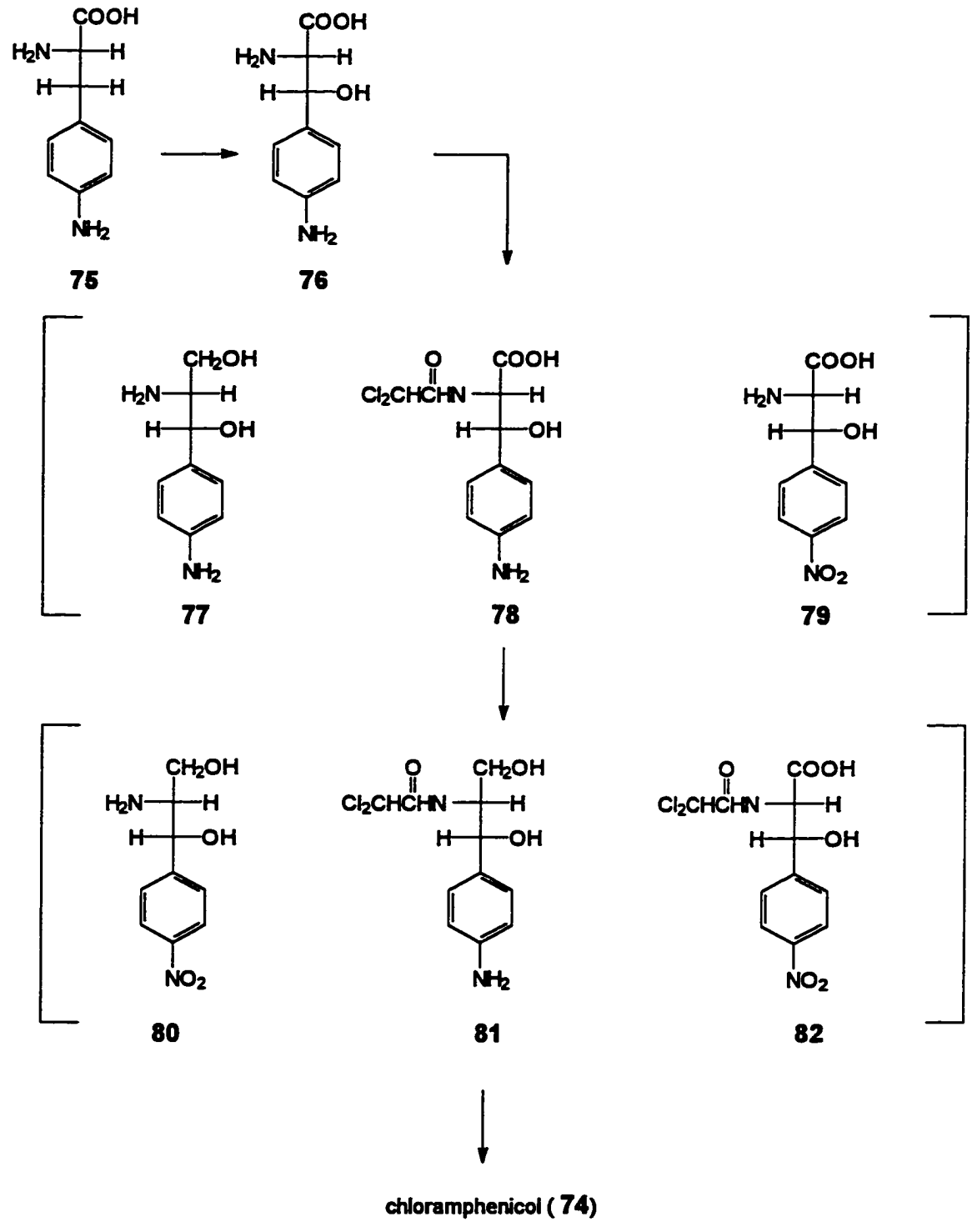
74

The occurrence of aromatic nitro groups and dichloroacetyl groups is quite uncommon in natural products; the presence of both moieties in chloramphenicol has attracted considerable interest in the study of its biosynthesis, but the biosynthetic pathway in *S. venezuelae* has not been completely elucidated.

Confirmation of *p*-aminophenylalanine (**75**) as an intermediate in the biosynthesis of chloramphenicol represented a major step in the elucidation of the biosynthetic pathway in *S. venezuelae* (90). Once **75** was established as a true precursor, the structure of chloramphenicol accommodated the feeding of a defined and limited number of possible intermediates (see Figure 21).

The negligible incorporation into chloramphenicol reported for racemic [2-^{14}C]-*p*-nitrophenylalanine suggest that oxidation of the *p*-amino group in *p*-

Figure 21 Possible biosynthetic pathway to chloramphenicol.



aminophenylalanine is not the next step in the pathway. On the other hand, the efficient incorporation of racemic [1-^{14}C]-*threo*-β-(*p*-aminophenyl)serine (90, 91) strongly supports the hydroxylation at C-3 of D-*threo*-β-(*p*-aminophenyl)alanine as the first step in the conversion of β-(*p*-aminophenyl)alanine to chloramphenicol. Feeding studies of the alternative β-(*p*-aminophenyl)alaninol and *N*-dichloroacetyl-(*p*-aminophenyl)alanine have not been conducted.

Assuming a simple and direct pathway, conversion of **76** to chloramphenicol requires only reduction of the carboxyl group, dichloroacetylation (or acetylation) of the α-amino group, and oxidation of the aromatic amino group. The lack of specific incorporation of both racemic [1-^{14}C]-*threo*-β-(*p*-nitrophenyl)serine (DL-[1-^{14}C]-**79**) (90) and D-[1-^{14}C]-*threo*-β-(*p*-aminophenyl)-serinol (D-[1-^{14}C]-**77**) (91) suggests, by a process of elimination, that acetylation or dichloroacetylation is the next step in the pathway. Since neither D-*threo*-*N*-dichloroacetyl-β-(*p*-aminophenyl)serine (**78**) nor D-*threo*-*N*-acetyl-β-(*p*-aminophenyl)serine has been fed to cultures of *S. venezuelae*, no positive experimental result supports **78** as an intermediate. The inclusion of **78** as an intermediate was based on the aforementioned process of elimination, and is supported in part by the efficient incorporation of L-[1-^{14}C]-*threo*-*N*-dichloroacetyl-β-(*p*-aminophenyl)serinol (**81**) (90) into chloramphenicol. This latter result suggests that oxidation of nitrogen to form the nitro group is the last step in the pathway.

N-Dichloroacetyl-β-(*p*-nitrophenyl)serine (**82**) has been detected in cultures of *S. venezuelae* utilizing proline as its nitrogen source (92), and *N*-acetyl-β-(*p*-aminophenyl)serine and *N*-acetyl-β-(*p*-nitrophenyl)serine were tentatively identified in cultures of *S. venezuelae* using peptone as a nitrogen source (92). The detection of these nitro compounds suggests that a mechanism exists in *S. venezuelae* for oxidation of the aromatic nitro group prior to reduction of the carboxylic acid to the primary alcohol. Feeding studies have not been conducted with *N*-dichloroacetyl-β-(*p*-nitrophenyl)serine (**82**); consequently the order of the last three transformations needed to convert D-*threo*-β-(*p*-aminophenyl)serine (**76**) to chloramphenicol (**74**) cannot be reported with confidence.

The involvement, if any, of compounds **78**, **80**, **81** and **82** in the biosynthesis of chloramphenicol can be determined by feeding appropriately labelled samples and analyzing the product for intact incorporation. Since only the D-*threo* isomer of chloramphenicol is produced, precursors of matching stereochemistry should be employed, and the incorporations of *threo* precursors have been higher than the corresponding *erythro* isomers (90). A stereoselective synthesis of D-*threo*-*p*-nitrophenylserine would provide a common intermediate that could be transformed into several of the untested, potential intermediates of chloramphenicol biosynthesis.

1.6 General Synthetic Approach

To provide a synthetic route to isotopically labelled amino acids, the method of choice must be readily adaptable to allow the incorporation of isotopes. To achieve the highest possible isotopic yield, isotopic labels will be introduced as close as possible to the end of the synthesis, thus reducing isotopic losses to subsequent chemical transformations. These requirements are best met by a convergent synthesis which introduces the isotopic labels near the end of the synthesis. The method of choice should also provide routes to both the targeted α-amino acids and the β-hydroxy-α-amino acid.

Syntheses based on the Strecker method have the disadvantage that the chiral auxiliary is consumed in the reaction. Furthermore, approaches employing alkyl amines require hydrogenation as the final step to provide the free amino group of the amino acid. Hydrogenations have the disadvantage that amino acids with functional groups that are sensitive to these reduction conditions cannot be prepared. Therefore, this method cannot be used to prepare β-(*p*-nitrophenyl)serine.

A synthetic route using a chiral glycine equivalent is the most attractive, since it lends itself to the convergent requirement and is most flexible in terms of providing a route to all of the synthetic targets. The glycine equivalent of Schöllkopf and coworkers (44) has the advantage of having a reusable (*S*)-val-gly-OEt moiety which is used to regenerate the bislactim in two steps. However,

this method and the Williams method do not lend themselves to the preparation of amino acids containing labels in carbons 1 and 2, due to the number of steps required for synthesis of the glycine equivalent. The carbons which ultimately become the "glycine" portion of the molecule are generated early in the synthesis and are thus subjected to the inefficiencies of the subsequent chemical reactions.

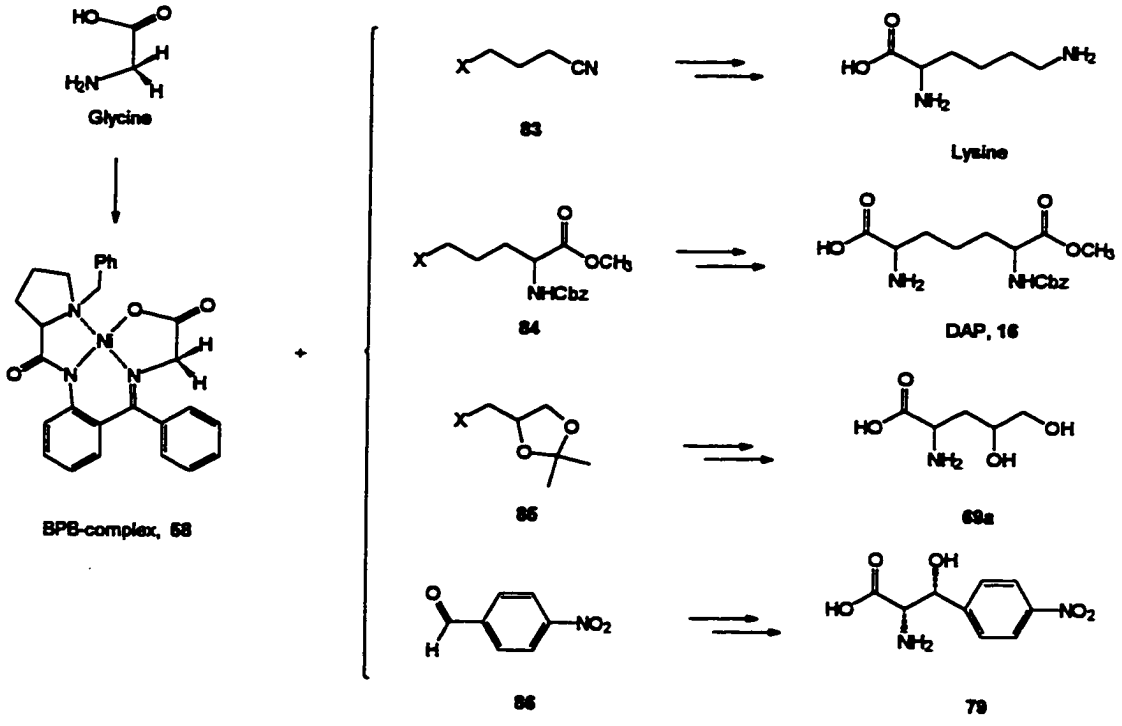
1.6.1 Use of Ni(II) Glycine Schiff Base Complex

A survey of the literature methods for the synthesis of α-amino acids and β-hydroxy-α-amino acids found that the method described by Belokon and coworkers (54, 55) was most applicable to the synthetic targets (Figure 22). This method allows the use of commercially available isotopically labelled glycine, which is utilized in the last bond forming reaction in the preparation of the chiral complex (**58**), and for this reason the isotopic yield is based solely on the final step in the preparation of the complex. This method has the advantage of having a recyclable ligand that can be used to regenerate the complex in one step.

The (*S*)-BPB-complex (**58**) provides (*S*)-amino acids in high yields and stereoselectivity, but the method has not been extended to the synthesis of (*R*)-amino acids. (*R*)-Amino acids have been recovered as minor impurities (usually <10%) during the preparation of (S)-α-amino acids. This work extends the use

of the BPB-complex to include the preparation of (*R*)-amino acids by preparing and using the (*R*)-BPB-complex in alkylation reactions.

Figure 22 Summary of synthetic approaches to synthetic targets.



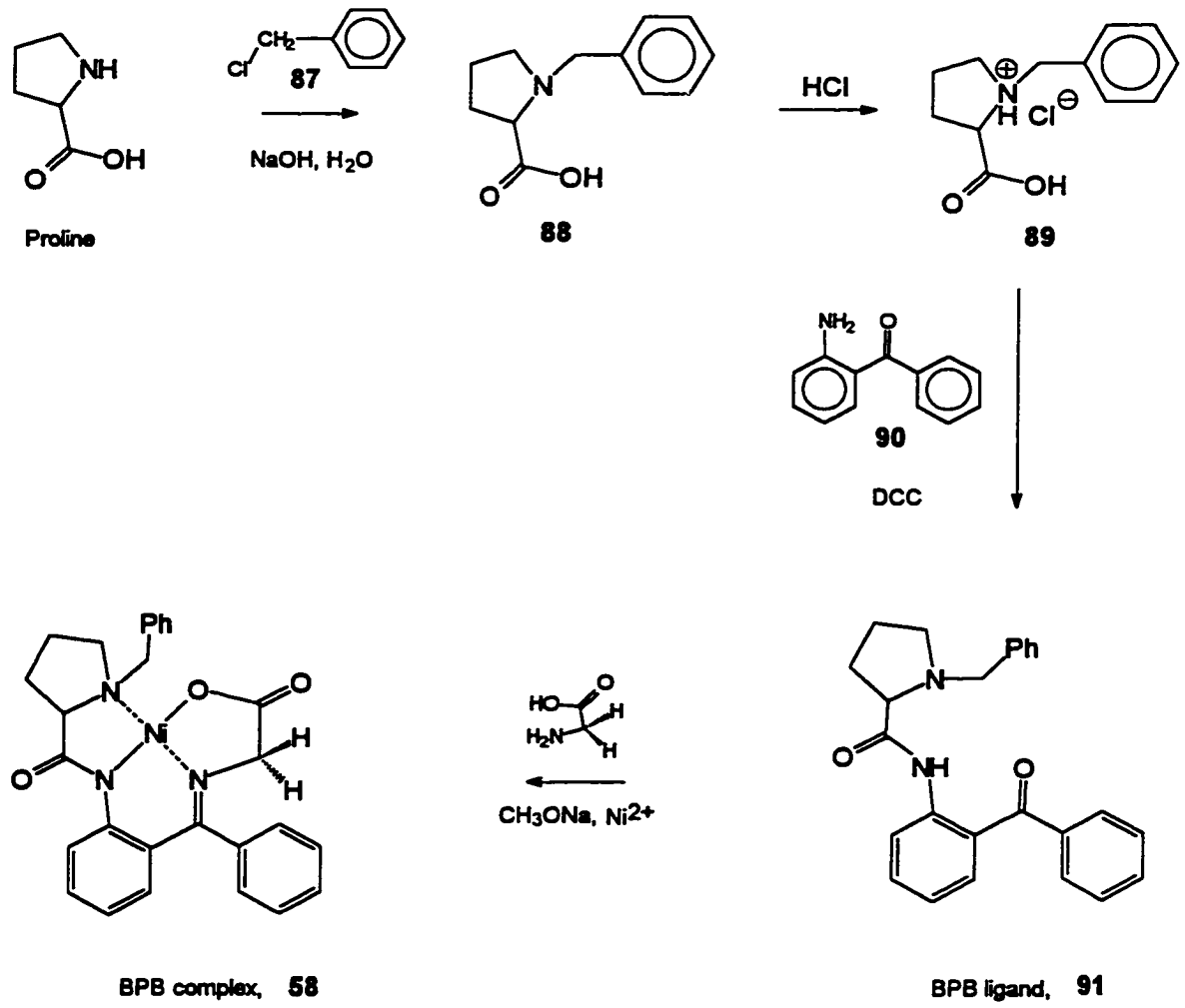
RESULTS AND DISCUSSION

2.1 Synthesis of BPB-Complex

The chiral complexes used for the stereoselective alkylation of glycine were prepared according to literature procedures (54), starting from either (*R*)- or (*S*)-proline (Figure 23). The chiral BPB-ligand (**91**) was assembled by coupling *N*-benzylproline·HCl (**89**) and 2'-aminobenzophenone (**90**), and the required complex (**58**) was formed by mixing the ligand with Ni^{2+} and glycine in the presence of base. The details of the individual steps are described below.

Both stereoisomers of *N*-benzylproline (**88**) and *N*-benzylproline·HCl (**89**) were obtained in yields comparable to those reported in the literature (54, 63) for the (*S*)-isomer. The ^{1}H and ^{13}C nmr spectra of *N*-benzyl-(*S*)-proline (63, 93) and its hydrochloride salt (63) correspond to those reported in the literature. The ^{13}C nmr spectra of the (*R*)-compounds have not been reported, but the spectra are consistent with those for the (*S*) compounds. The major differences between the ^{13}C nmr spectrum of *N*-benzylproline and *N*-benzylproline·HCl is an upfield shift of 2.3 ppm of the carboxyl resonance on forming the hydrochloride. The mass spectra recorded for **88** and **89** are similar, with peaks at *m/z* 205 representing the molecular ion and loss of HCl respectively. The base peak

Figure 23 Preparation of BPB-complex.



(*m/z* 160) in the spectrum of *N*-benzylproline was indicative of α-cleavage at the carbonyl and, not surprisingly, the alternative α-cleavage product representing a loss of 17 mass units (OH) was a very small peak in the mass spectrum. The mass spectra of both compounds had a peak at *m/z* 91 characteristic of benzyl

group fragmentation. The peak at *m/z* 91 represented the base peak in the ms of *N*-benzylproline·HCl.

The specific rotations of (*R*)- and (*S*)-*N*-benzylproline (+27.4° and -28.3°, respectively) compare favorably to the reported value of -29.0° (94). In contrast, the optical rotation determined for *N*-benzyl-(*S*)-proline·HCl (-30.7°) prepared from *N*-benzyl-(*S*)-proline (+32.3°) has the opposite sign to that reported (95) for *N*-benzyl-(*S*)-proline·HCl in the same solvent and concentration. This difference is most easily accounted for by a typographical error in the published article. If this is not the case, then the rotation reported in this thesis is considered correct based on the simplicity of the synthetic method employed and confirmed stereochemistry of the starting materials. The rotation of *N*-benzyl-(*R*)-proline·HCl (+30.3) is of equal magnitude and opposite sign to that measured for *N*-benzyl-(*S*)-proline·HCl.

The ir spectrum (KBr disk) of each product is as expected with the carbonyl stretching frequency shifting from 1644 to 1744 cm^{-1} on going to the hydrochloride salt. A similar shift was observed for the ir spectra of these compounds in $CHCl_3$ (95).

The syntheses of (*R*)- and (*S*)-2-[*N*-(*N'*-benzylprolyl)amino]benzophenone (BPB-ligand, **91**) were accomplished in yields comparable to literature values for the (*S*)-BPB ligand (54). The synthesis of 2-[*N*-(*N'*-benzylprolyl)amino]-benzophenone from *N*-benzylproline·HCl requires the formation of an amide

bond between 2'-aminobenzophenone and *N*-benzylproline·HCl as the last step. The formation of this amide bond proved difficult even when carried out in the presence of DCC and DMAP. Trial reactions showed the best yield and fewest side reactions when the coupling was carried out in the presence of DCC only. The coupling to form the amide bond in 2-[*N*-(*N'*-benzylprolyl)amino]benzophenone is between an aniline and an carboxylic acid; the difficulty of this coupling has been reported (96), and is attributed to the low nucleophilicity of an aniline nitrogen. For the synthesis of 2-[*N*-(*N'*-benzylprolyl)amino]benzophenone the yield obtained is adequate, since the starting materials are relatively inexpensive and no isotopic labels are intended to be used up to this step. The ms recorded for this product is consistent with the proposed structure. The peak at *m/z* 384 represents the molecular ion and the base peak was found at *m/z* 91. The base peak (*m/z* 91) results from characteristic fragmentation of the benzyl group. The ir spectrum shows two carbonyl C=O stretching bonds indicative of the amide bond and the aryl ketone. The C=O stretch of the proline carboxyl group is shifted from 1744 cm^{-1} to 1692 cm^{-1} upon amide formation.

The synthesis of the Ni(II) complex of glycine Schiff base with (*R*)- and (*S*)-2-[*N*-(*N'*-benzylpropyl)amino]benzophenone (BPB-complex, **58**) was achieved in excellent yields (90% and 95% respectively) when excess glycine was present. The spectral and physical data for these products agreed

favorably with those reported in the literature for (*S*)-BPB-complex (54). Electron impact mass spectrometry (eims) was unsuitable for the analysis of the Ni complexes so they were examined by positive ion apci mass spectrometry. The apci mass spectral molecular ion isotope peak ratios of both complexes agreed favorably with the theoretical values, and show intensity ratios indicative of a species containing one Ni atom per molecule (Table 5). The theoretical values were calculated using the Isomass mass spectral data analysis program (97) to determine the contribution from C, H, N, and O; the program was unable to calculate the Ni contribution to the intensities of the isotope peaks so the contributions from C, H, N, and O and the cross products with the Ni isotopes were determined manually.

The literature procedure for the synthesis of **58** requires a 5-fold excess of glycine but this is very inefficient when isotopically labelled glycine is being used in the complex formation. Attempts to prepare **58** using a ligand to glycine ratio of 1:1 resulted in decreased yields (35%) even with extended reaction times. Alternatively, excess glycine could be recovered after isolation of the product. Recovery of unreacted glycine by ion-exchange chromatography was attempted. Assuming a 100% yield of the BPB-complex, isolation of unreacted glycine by cation-exchange chromatography resulted in an 89% recovery of unreacted glycine. This recovery is acceptable and therefore use of excess labelled glycine to prepare the BPB-complex is a feasible approach.

Table 5 Comparison of apci molecular ion isotope peaks with theoretical values for (*R*)- and (*S*)-BPB-complexes.[a]

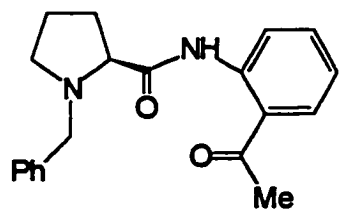
Complex	relative peak intensity (%)[b] [theoretical value (%)]					
	MH+1	MH+2	MH+3	MH+4	MH+5	MH+6
(*S*)-**58**	26.6 [31.6]	45.5 [43.7]	13.4 [14.3]	6.7 [7.9]	2.8 [2.0]	2.2 [1.6]
(*R*)-**58**	30.8 [31.6]	44.1 [43.7]	13.5 [14.3]	7.8 [7.9]	1.9 [2.0]	1.5 [1.6]

[a] molecular formula = $C_{27}H_{25}N_3NiO_3$. [b] intensities measured from one scan and reported relative to the MH^+ peak.

This report represents the first synthesis of the Ni(II) complex of glycine Schiff base with (*R*)-2-[*N*-(*N'*-benzylpropyl)amino]benzophenone ((*R*)-**58**). The early development of the complex (98) saw the examination of copper complexes prepared from ligands resulting from the coupling of (*S*)-proline or a stereoisomer of piperidine-2-carboxylate with *o*-aminobenzophenone ((*R*)-**92** or (*S*)-**93**, Figure 24). The complex prepared with (*R*)-piperidine-2-carboxylate was condensed with acetaldehyde in sodium methoxide to provide a mixture of (*S*)-threonine and (*S*)-allothreonine. When the complex prepared with (*S*)-piperidine-2-carboxylate was used in a similar reaction (*R*)-threonine and (*R*)-allothreonine were isolated. Similar experiments were not conducted with a complex prepared using (*R*)-proline.

The results reported with the complex prepared from (*R*)-piperidine-2-carboxylate suggested that the complex prepared from the (*R*)-proline ligand would yield the enantiomeric amino acid, *i.e.*, (*R*)-α-amino acids instead of (*S*)-α-amino acids, and justify the feasibility of this approach to supply both (*R*) and (*S*) stereoisomers of amino acids.

Figure 24 Ligands used in the early development of Belokon's chiral glycine equivalent (98).



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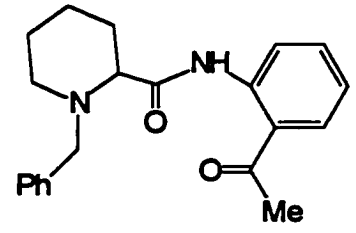

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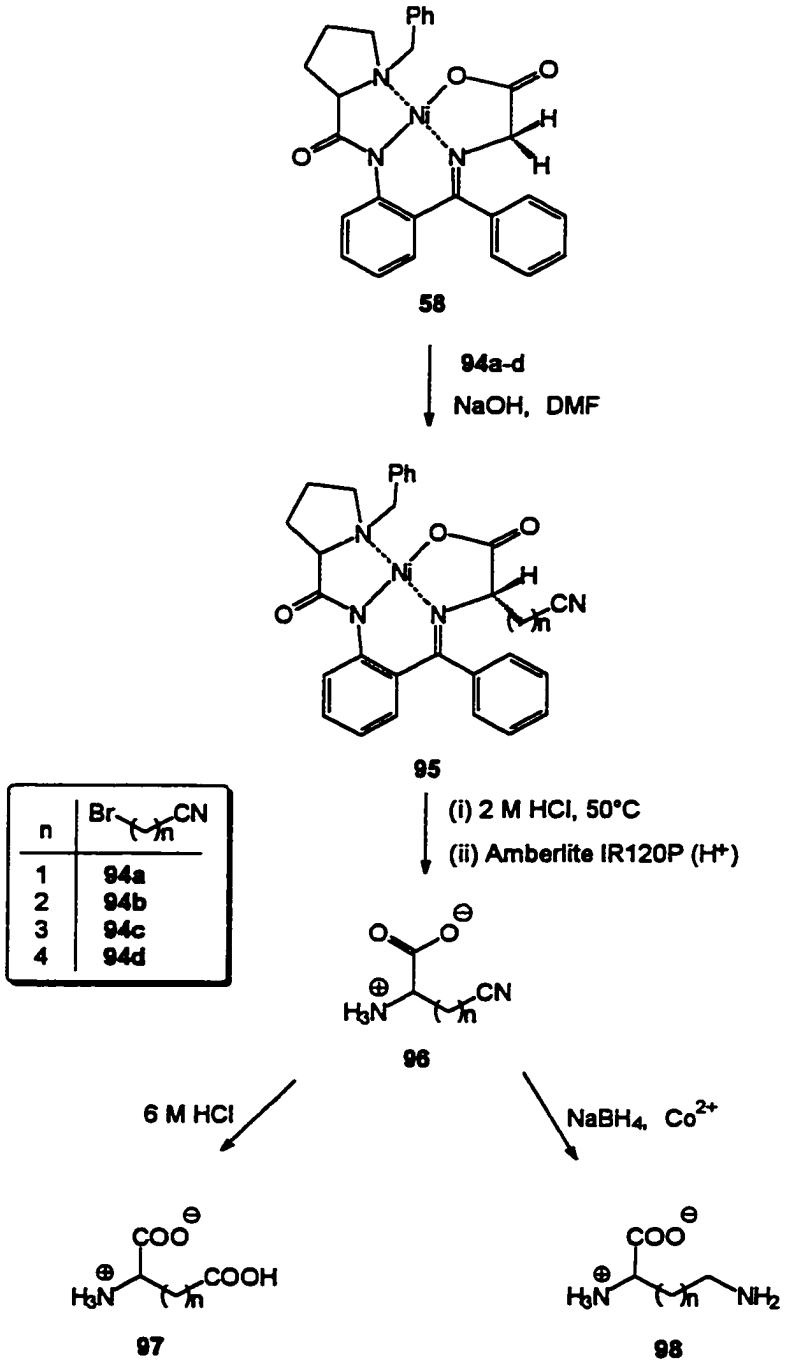
2.2 Synthesis of Lysine and Related Amino Acids

A general synthetic plan was adopted for the synthesis of lysine (Figure 25). Alkylation of the BPB-complexes ((*R*)-**58** and (*S*)-**58**) with halogenated nitriles (**94a-d**) of varying chain lengths generated a series of amino acid nitriles which were subsequently converted to the corresponding acidic (**97**) and basic (**98**) amino acids, *e.g.*, 5-cyanonorvaline can be converted to α-aminoadipic acid and to lysine.

Figure 25 Synthesis of acidic and basic amino acids *via* amino acid nitriles.



The intermediate nitriles are themselves of interest since they exhibit biological activity. The biological occurrence of (*S*)-2-amino-4-cyanobutanoic acid was first discovered (99) in cultures of *Chromobacterium violaceum* (D 341) that were supplemented with cyanide. *C. violaceum* (strain 9) also produced β-cyanoalanine when supplemented with cyanide (99).

β-Cyano-(*S*)-alanine has neurotoxic properties that make investigation of the biological activity of its higher homologs very attractive (100). 5-Cyano-norvaline has attracted considerable attention due to it being a direct synthetic precursor to lysine and 2-aminoadipic acid. This route has been used to prepare labelled lysine by catalytic titration (101) and by $NaBT_4$/Raney Ni reduction (102) of 5-cyanonorvaline.

The literature (103) contains only one account of the preparation of (*S*)-2-amino-6-cyanohexanoic acid, also known as (*S*)-6-cyanonorleucine. The single isomer was prepared by the enzymatic resolution of an acylated racemic mixture by acylase I enzymes isolated from porcine kidney and *Aspergillus* sp (AA) but no physical or spectral data were reported for the resolved product. A literature search found limited and generally unacceptable methods for the enantio-selective preparation of cyanoamino acids.

(*S*)-2-Aminoadipic acid is found at the carboxy terminus of the ACV tripeptide (**18**) used in the biosynthesis of penicillins and cephalosporins, and it is also the end-product of (*S*)-lysine catabolism in aerobic bacteria (**9**, Figure 1).

(*R*)-2-Aminoadipic acid is not available commercially whereas neither (*R*)- nor (*S*)-2-aminopimelic acid is available; both compounds are available as racemic mixtures. Therefore, a convenient route to enantiomerically pure samples of these amino acids is necessary to facilitate further investigation of their biological function.

The basic amino acids are of importance since they are found in biologically active peptides (104) and are used for preparing conformationally restricted cyclic peptides (105). There is interest in the literature towards the preparation of basic amino acids having (*R*) stereochemistry; these amino acids have been introduced into cyclic peptides (39) and the resulting conformational changes studied.

The BPB-complex (**58**) was alkylated with the bromonitriles (**94b-d**) in good yields of 75-94% based on the starting complex (Figure 25). These yields are in the range for similar alkylations of (*S*)-BPB-complex reported in the literature. The yield for the alkylation of the (*R*)-BPB-complex ((*R*)-**58**) with 5-bromovaleronitrile (**94d**) was 94% which was considerably higher than the yield of the other members of the series. This high yield when viewed in conjunction with the relatively low specific rotation suggest that the product may have been of a lower purity than others of the series. The specific rotation of the alkylated (*R*)-complex was always negative while that of the corresponding alkylated (*S*)-complex was always positive. The specific rotation of the alkylated complexes

can only be used as measures of the relative optical purity of each enantiomeric pair of alkylated complexes (**95b-d**).

The diastereomeric purity of the alkylated complexes **94b-d** were assessed by hydrolysis of a sample of the crude reaction mixture and determination of the enantiomeric purity of the cyanoamino acids by chiral opa hplc analysis (Table 6). The diastereoselectivity of the alkylation was also assessed by mass analysis (numbers in parentheses, Table 6) of those products that were purified by flash chromatography, and the one product that crystallized

Table 6 Diastereoselectivity of BPB-complex alkylation.

Cyano amino acid	Enantiomeric purity[a]			
	from (*R*)-**58**[b]		from (*S*)-**58**[b]	
	(*R*)	(*S*)	(*R*)	(*S*)
96b[c]	97 (95)	3 (5)	6 (11)	94 (89)
96c[c]	84	16	7 (5)	93 (95)
96d[d]	(94)	(6)	–	–

[a] Determined by chiral opa hplc, (determined by mass after silica gel chromatography). [b] Starting BPB-complex. [c] Obtained by hydrolysis of alkylated complexes prior to chromatographic purification. [d] major diastereomer crystallized during workup.

during the workup thereby removing the need for the chromatographic step (**95d**, Table 6). This is consistent with similar analyses of the diastereoselectivity of the alkylation reported in the literature (54, 55, 60).

The alkylation of (*R*)-BPB-complex with bromoacetonitrile (**94a**) resulted in a mulitcomponent mixture by tlc analysis. Analysis by ^{13}C nmr of the mixture showed no peaks consistent with that expected for the desired product. Silica gel flash chromatography of the product mixture seemed unlikely to produce clean fractions so the mixture was hydrolyzed to produce whatever amino acids present that could then be analyzed by the better resolution of hplc. One difficulty was that no hplc standards were available to allow chromatographic identification of any β-cyanoalanine produced. To circumvent this problem the aqueous layer from the mild acid hydrolysis (1.5 M HCl, 50°C, 15 min) of the alkylated BPB-complex mixture was subjected to strong acid hydrolysis (6 M HCl, 100°C, 90 min) such that any nitriles in the mixture would be converted to carboxylic acids; this would result in the formation of aspartic acid from any β-cyanoalanine which could then be identified by opa hplc analysis. Achiral hplc analysis of the acid hydrolysate gave several peaks, none of which corresponded to the aspartic acid standard. The major peak in the chromatogram corresponded to that of glycine. When the same reaction was carried out using the (*S*)-BPB-complex ((*S*)-**58**) and **94a** the strong acid hydrosylate contained a peak attributed to aspartic acid. This result suggests

that while the current alkylating conditions are unsuitable for the alkylation of (*S*)-**58** with **94a** the potential does exist for optimum conditions to be found. This result is consistent with the very recently reported (67) failure of benzyltrifluoromethyl ketone to react with (*S*)-BPB-complex which was attributed to the highly enolizable nature of the ketone.

The ^{13}C nmr data for the complexes formed from the other bromonitriles (**94b-d**) were as expected; the resonance due to CH_2 of glycine in the starting BPB-complex typically moved approximately 9 ppm downfield upon becoming the α-carbon of the amino acid moiety in the alkylated complex. The new resonance (118-119 ppm) due to the carbon of the nitrile had the expected chemical shift. The ^1H nmr spectra of the alkylated complexes had many overlapping complex multiplets but the resonance due to the AB system of the benzyl CH_2 group remained distinct although its chemical shift was shifted slightly from that in the starting BPB-complex. The resonance due to the α-CH of the newly formed cyano amino acid was distinct and typically appeared in a region (~3.8 ppm) that was free of other peaks.

The most diagnostic vibration in the ir spectra of the alkylated complexes is the vibration at 2224 cm^{-1} due to the CN nitrile stretch; this vibration was absent in the ir spectrum of the starting BPB-complex (**58**). The alkylated complexes were analyzed by positive ion apci and the intensity of the molecular ion isotope cluster had intensities consistent with the assigned structures (Table

7). The theoretical intensities were calculated as described for the BPB-complexes in Section 2.1 (Page 48). The favorable correlations between the actual intensities and the theoretical intensities are consistent with one Ni atom

Table 7 Comparison of apci molecular ion isotope peaks with theoretical values for alkylated complexes in the cyanoamino acid series..

Compound, Formula	relative peak intensity[a] (%) [theoretical value (%)]					
	MH+1	MH+2	MH+3	MH+4	MH+5	MH+6
(*S*)-**95b** $C_{30}H_{29}N_4NiO_3$	34.2 [35.4]	43.9 [44.9]	15.5 [16.0]	8.2 [8.5]	2.4 [2.3]	1.8 [1.7]
(*R*)-**95b** $C_{30}H_{29}N_4NiO_3$	34.2 [35.4]	42.1 [44.9]	15.2 [16.0]	8.8 [8.5]	2.0 [2.3]	1.8 [1.7]
(*S*)-**95c** $C_{31}H_{31}N_4NiO_3$	38.6 [36.5]	43.7 [45.3]	16.4 [16.5]	8.5 [8.6]	1.5 [2.4]	1.1 [1.7]
(*R*)-**95c** $C_{31}H_{31}N_4NiO_3$	34.2 [36.5]	42.1 [45.3]	15.2 [16.5]	8.8 [8.6]	2.0 [2.4]	1.8 [1.7]
(*S*)-**95d** $C_{32}H_{33}N_4NiO_3$	33.9 [37.7]	44.2 [45.7]	16.3 [17.0]	9.8 [8.8]	3.9 [2.5]	1.3 [1.8]
(*R*)-**95d** $C_{32}H_{33}N_4NiO_3$	36.8 [37.7]	49.2 [45.7]	16.6 [17.0]	7.8 [8.8]	3.1 [2.5]	1.3 [1.8]

[a] Based on one scan and reported relative to the MH^+ peak.

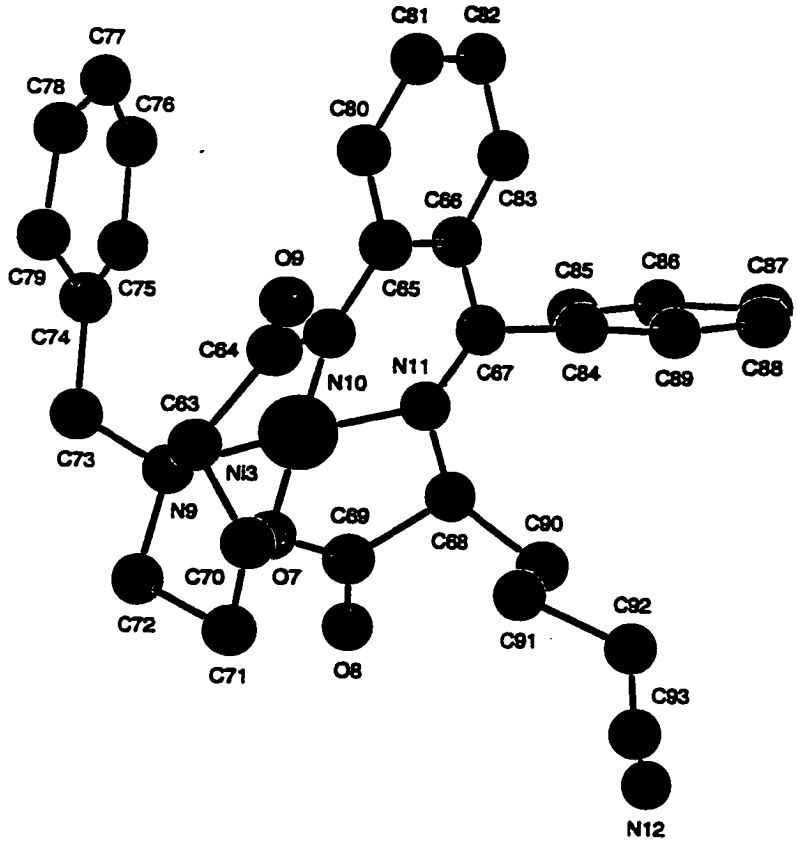
per molecule. The alkylated complexes showed very little fragmentation under the conditions employed for the apci analysis. Occasionally a small peak at m/z 385 was observed that can be attributed to protonated BPB-ligand.

Alkylations of the (*S*)-BPB-complex with simple alkyl halides have generally resulted in producing (*S*)-amino acids. There are only a few examples of alkylation of the (*S*)-BPB-complex with electrophiles containing modest functionality (Table 1). The stereochemical outcome of the condensation of (*S*)-BPB-complex with aldehydes and ketones depends on the base used in the reaction (54, 55) and the structure of the electrophile (66, 67). X-ray crystal structure analysis of the condensation product of trifluoromethyl ketones with (*S*)-BPB-complex in the presence of NaOMe shows a product having the (*S*,2*S*,3*S*) configuration, these 3-trifluoromethyl-3-substituted ketones have the highest diastereomeric selectivity (90-98%) reported to date (67). The X-ray structures reported for the alkylation of (*S*)-BPB-complex with aliphatic aldehydes indicated that the alkylated complexes had the (*S*,2*R*,3*S*) configuration. This influence of electrophiles prompted a crystal structure determination of (*S*)-**95c** to provide a definitive answer to the stereochemistry of the alkylated complexes. It is assumed that the other members of the series will form with the same stereochemistry.

The x-ray crystal structure determined for the (*S*)-BPB-complex alkylated with 4-bromobutylnitrile confirms the stereochemistry of the proline α-carbon

centre (C63, Figure 26) as (*S*) while showing the newly formed stereocentre (C68, Figure 26) to also have a (*S*) configuration. This is consistent with the stereochemistry predicted based on similar literature alkylations and shows that hydrolysis of the complex should provide (*S*)-cyanoamino acids. This result confirms the stereochemistry of the new stereocentre in the alkylated product. Thus (*S*)-cyano amino acids are formed from the complex prepared from (*S*) ligand.

Figure 26 X-Ray crystal structure of (*S*)-BPB-complex alkylated with 4-bromobutylnitrile.



2.2.1 Cyano α-Amino Acids

The amino acid product (**96**) was released from the alkylated BPB-complex (**95**) by mild acid hydrolysis which makes the preparation of amino acids with sensitive functionality possible. This is of particular importance in the preparation of cyanoamino acids where the cyano function is sensitive to reducing reagents and to hydrolysis by strongly acidic conditions. The mild hydrolytic conditions allow the BPB-ligand to be recycled in high yields with no loss of enantiomeric purity and recycling of the BPB-ligand makes the synthesis very economical. The BPB-ligand was typically recovered in good yields of 79-89%. No additional purification of the BPB-ligand was necessary, and the recovered BPB-ligand was converted to the BPB-complex in one step.

The purity of the cyano amino acids (**96**) was determined by chiral hplc and by comparison to literature optical rotations where applicable. The enantiomeric excess was determined to be >99% for (*S*)-2-amino-4-cyanobutanoic acid when determined by either chiral hplc (Figure 27c) or comparison of the measured optical rotation to the literature value. Chiral hplc analysis of (*R*)-2-amino-4-cyanobutanoic acid support a >99% e.e. (*e.g.* Figure 27a) but the measured optical rotation (-33.9°) was larger than the literature value (+26.5°) for its enantiomer. The enantiomeric purities of (*R*)- and (*S*)-5-cyanonorvaline ((*R*)- and (*S*)-**96c**) were 92.8 and 96.4% e.e., respectively, by hplc analysis, but comparisons of the measured optical rotations (-10.1° and

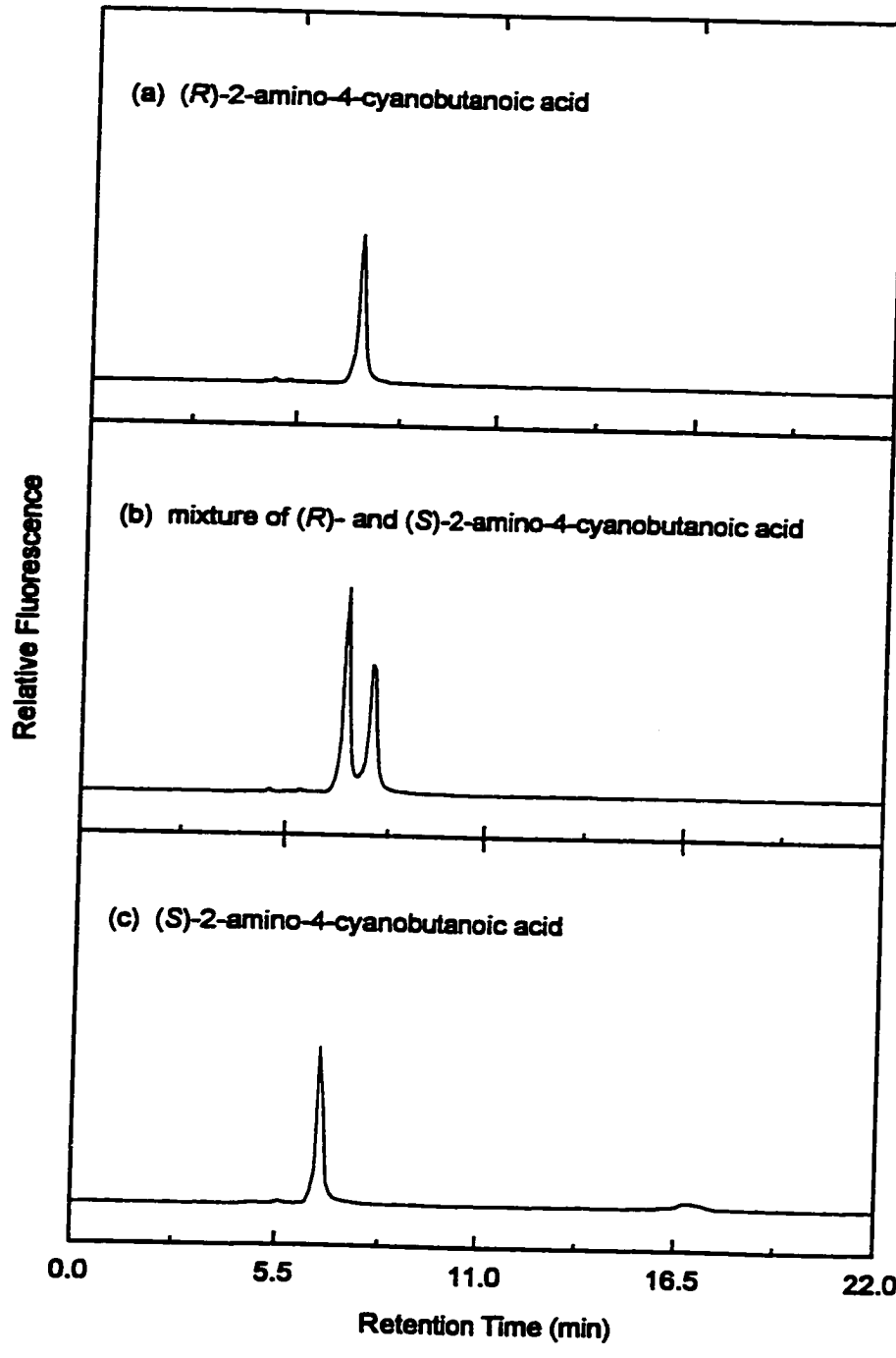
+7.7°, respectively) to the literature value (+10°) for (*S*)-5-cyanonorvaline suggest optical purity of 100% and 77%, respectively. The optical rotation measurements recorded for (*S*)-5-cyanonorvaline suggest that the compound could have an impurity that is not hplc active, *i.e.,* not a primary amine and does not contain any hydrogens since the ^{1}H nmr spectrum of the compound does not show any resonances not attributed to the product. Another candidate for this type of impurity is an inorganic salt but the procedure used to purify and recrystallize (*S*)-5-cyanonorvaline was identical to that employed for the purification and recrystallization of the other cyano amino acids in the series. Thus the most likely candidate responsible for the low optical rotation is water due to insufficient drying of the (*S*)-5-cyanonorvaline sample before the optical rotation measurements were carried out. The ^{1}H nmr spectrum of this compound was acquired in D_2O so the presence of residual water in the sample would not be observed due to masking by the residual water in D_2O.

Table 8 Enantiomeric purity of cyano, acidic and basic amino acid products.

	Cyanoamino Acid[a]		Acidic Amino Acid[a]		Basic Amino Acid[b]	
	(*R*)-**96**	(*S*)-**96**	(*R*)-**97**	(*S*)-**97**	(*R*)-**98**	(*S*)-**98**
b	>99	>99	>99	>99	–	–
c	93	96	95	>99	90.1	95.5
d	>99	>99	>99	>99	–	–

[a] Determined by chiral opa hplc. [b] Calculated from optical rotations.

Figure 27 Chiral opa-hplc analysis of hydrolysis products from alkylation of BPB-complex with 3-bromopropionitrile.



The specific rotation of (*R*)-2-amino-6-cyanohexanoic acid ((*R*)-**96d**) was approximately half of that recorded for (*S*)-2-amino-6-cyanohexanoic ((*S*)-**96d**). The literature value for the rotations of these compounds have not been reported so no comparisons can be made. The isomers of 2-amino-6-cyanohexanoic acid were enantiomerically pure (>99% e.e.) by chiral hplc analysis. The inherent error in the small actual rotation measured may be the main reason for the inconsistency in the specific rotations. There is only one account in the literature of the preparation of (*S*)-2-amino-6-cyanohexanoic acid, also known as (*S*)-6-cyanonorleucine (103). The single isomer was prepared by the enzymatic resolution of an acylated racemic mixture by acylase I enzymes isolated from porcine kidney and *Aspergillus* sp (AA) but no physical or spectral data were reported for the resolved product (103).

The ir of the cyano amino acids all had the expected CN stretch vibration at 2250 cm^{-1}. The ^{1}H and ^{13}C nmr spectra were as expected with a characteristic resonance at 123-124 ppm due to the carbon of the cyano group.

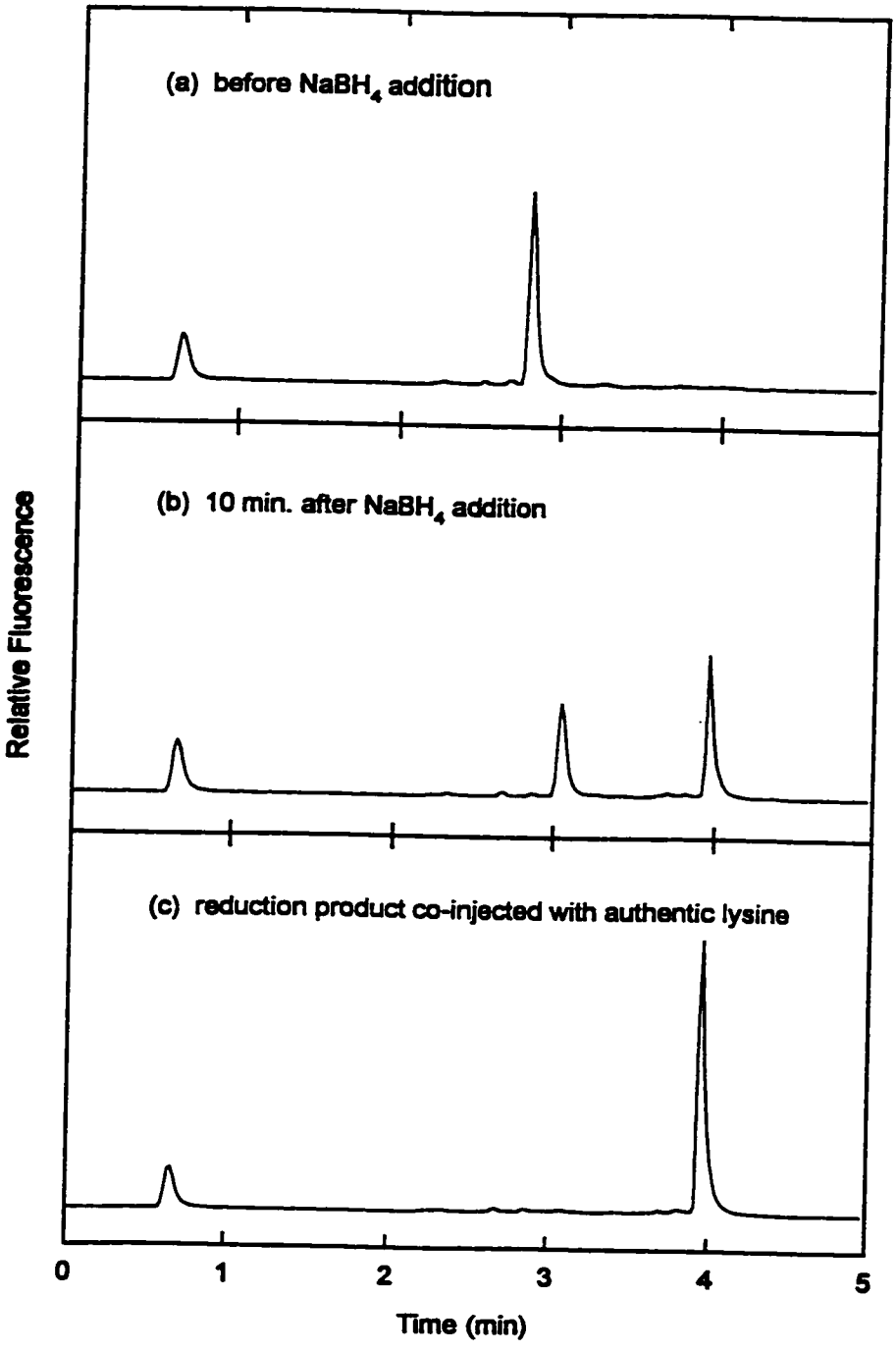
2.2.2 Basic α-Amino Acids

Catalytic hydrogenation is usually employed for the reduction of a cyano group to a primary amino function and has been demonstrated for the preparation of lysine from (*S*)-5-cyanonorvaline (101, 102). Labelled lysine was prepared by catalytic tritiation (101) and by $NaBT_4$/Raney Ni reduction (102) of

5-cyanonorvaline. In this work the nitriles (**96**) were reduced using $NaBH_4$ in the presence of Co^{2+} in water according to a modified literature procedure (106). It was found that fewer side reactions occurred, as determined by hplc, when water rather than methanol was used as the solvent. The reduction was carried out on the recrystallized cyanoamino acids but trial experiments demonstrated that the reduction could have just as easily been carried out on the neutralized aqueous layer obtained by hydrolysis of the alkylated complex. The aqueous layer contained Ni^{2+} ions which proved sufficient to effect the desired reduction. In practice the reduction was not carried out in this fashion since the purified cyano amino acids were used for spectral and physical characterization. The reduction was followed by achiral opa-hplc, and the starting material was converted to a single product. For example, the reduction of 5-cyanonorvaline produces a single product that co-elutes with authentic *(RS)*-lysine (Figure 28).

The basic amino acids were prepared in yields of 70-80% from their respective alkylated complex, but difficulties were encountered in the crystallization of these amino acids. (*R*)- and (*S*)-Lysine monohydrochloride were obtained as white crystals after 2-3 weeks at 4°C but the other basic amino acids were not recovered as recrystallized solids. Only small amounts of impurities were detected in the crude amino acids by ^1H nmr spectral and hplc analysis, and the stereochemical purity of the basic amino acids could not be determined by the current chiral opa method.

Figure 28 Opa-hplc analysis of the reduction of 5-cyanonorvaline with $NaBH_4$ and Co(II).



The mono hydrochloride salts of (*R*)- and (*S*)-lysine had optical rotations indicative of 90.1% e.e. and 95.5% e.e., respectively. These rotations suggest that no racemization occurred during the reduction reaction. The mp and the ^{1}H and ^{13}C nmr data were consistent with those reported in the literature for these compounds.

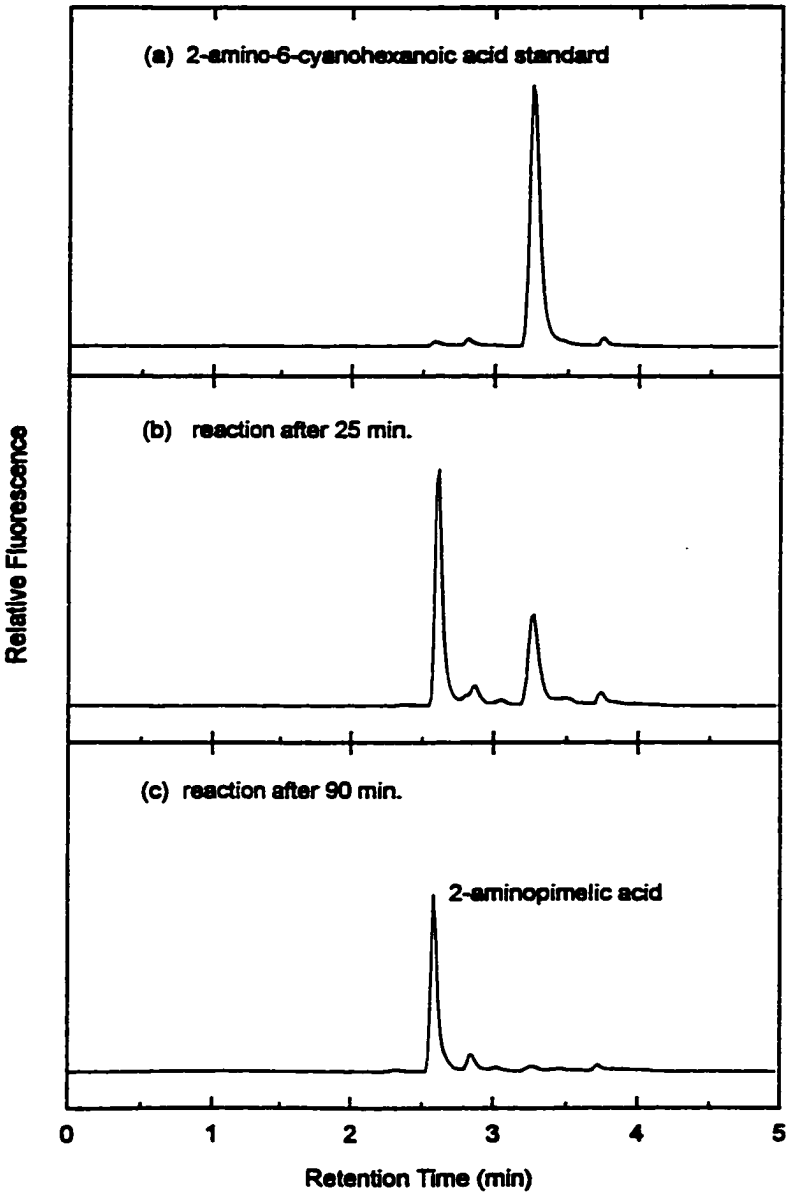
2.2.3 Acidic α-Amino Acids

Acid hydrolysis of the cyanoamino acids (**96**) provided a convenient route to the corresponding acidic amino acids (**97**). The hydrolysis was followed by achiral opa hplc (*e.g.* Figure 29); the chromatograms showed the disappearance of the starting cyanoamino acid peak along with the appearance of a single product peak. The hydrolysis of the cyanoamino acids proceeded with high isolated yields (50-80%) of the acidic amino acid, but lower recoveries were obtained after recrystallization.

The ^{1}H and ^{13}C nmr spectra of the acidic amino acids were as expected for these compounds and consistent with those reported in the literature (107). On going from the cyano amino acid to the acidic amino acid the disappearance of the CN resonance and the appearance of an additional resonance due to the carbon of the newly formed COOH is most noticeable. Likewise, a comparison of the ir spectra of the cyano amino acids *vs* the acidic amino acids shows an

absence of the CN stretching vibration at 2250 cm^{-1} and the appearance of an additional C=O carbonyl stretching vibration in the latter.

Figure 29 Opa-hplc analysis of the acid hydrolysis reaction of 2-amino-6-cyanohexanoic acid.

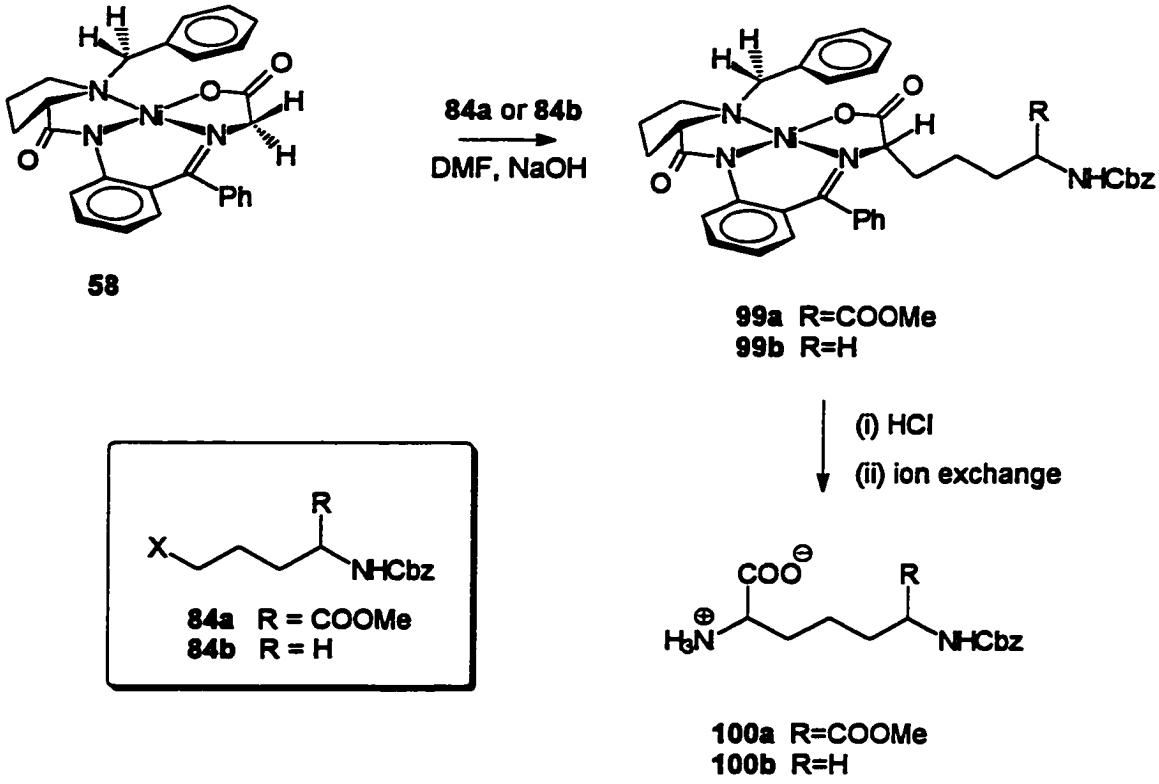


2.3 Attempted Synthesis of Diaminopimelic Acid (DAP)

The stereoselective synthesis of the acidic, basic and cyano amino acids (Section 2.2) demonstrated that the alkylation of the BPB-complex proceeded with high stereoselectivity and that both *(R)*- and (S)-α-amino acids can be formed. Given the appropriate alkyl halide, the reaction could be employed to synthesize many different amino acids. Indeed Belokon and coworkers and other groups have prepared various amino acids (Tables 1 and 2) with alkyl or aromatic side chains, but the usefulness of the alkylation reaction has not been demonstrated for more complicated alkyl halides, *i.e.*, those containing other functional groups in a protected form. The stereoselective alkylation of the BPB-complex can be used as the key step in a synthesis of DAP (Figure 30), if a protected form of 5-halonorvaline (*e.g.*, **84a**) is available.

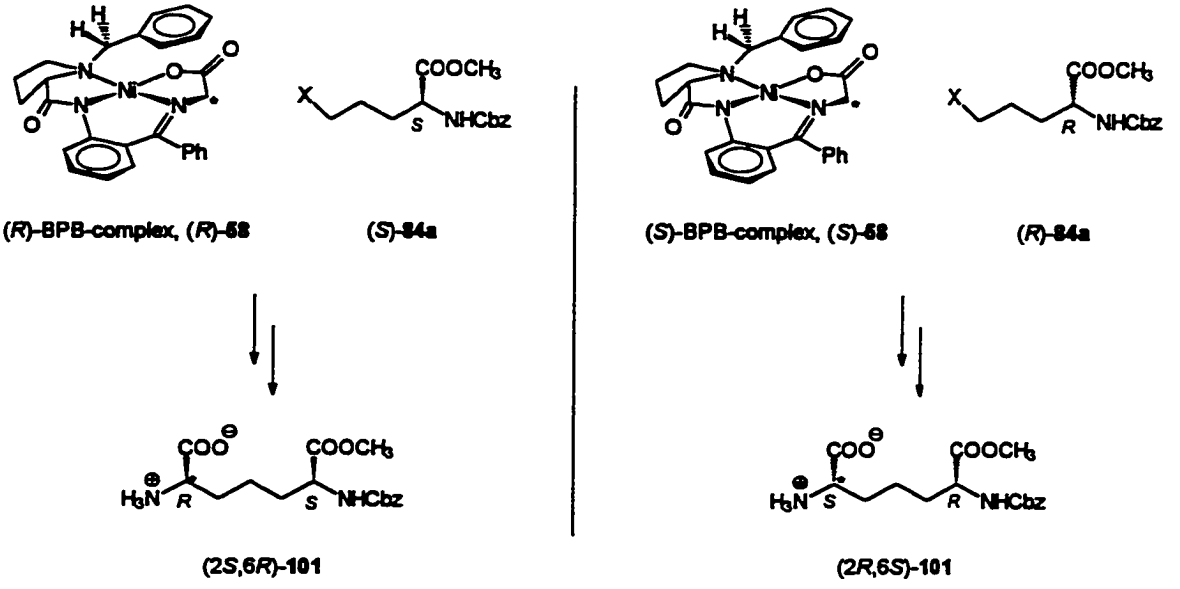
The three stereoisomers of DAP (**16**, Figure 3) can be prepared *via* the proposed route (Figure 30) by choosing the stereochemistry of the starting materials. (*R,R*)-DAP would be obtained from the (*R*)-complex with the (*R*)-norvaline derivative, whereas reaction of the (*S*)-BPB-complex with the (*S*)-norvaline derivative would yield (*S,S*)-DAP. On the other hand, reaction of the (*R*) and (*S*) forms of the BPB-complex and the appropriate norvaline derivative would yield stereospecifically labelled or differentially protected forms of (*R,S*)-DAP (Figure 31). These forms of (*R,S*)-DAP are needed for the incorporation of (*R,S*)-DAP into peptides or to use isotopic labeling to study its metabolism.

Figure 30 Synthetic plan for the synthesis of DAP.



A key factor with the current experimental approach to the preparation of DAP (Figure 30, **100a**) is the stability of protecting groups to the alkylation and mild acid conditions used for the hydrolysis of the complex. A model study was undertaken to examine the stability of the Cbz protecting group, and three routes were examined for the preparation of the key intermediate, *N*-Cbz-5-halonorvaline-1-methyl ester, from glutamic acid.

Figure 31 Proposed synthesis of differentially protected or stereospecifically labelled *meso*-DAP (* indicates a ^{13}C label).

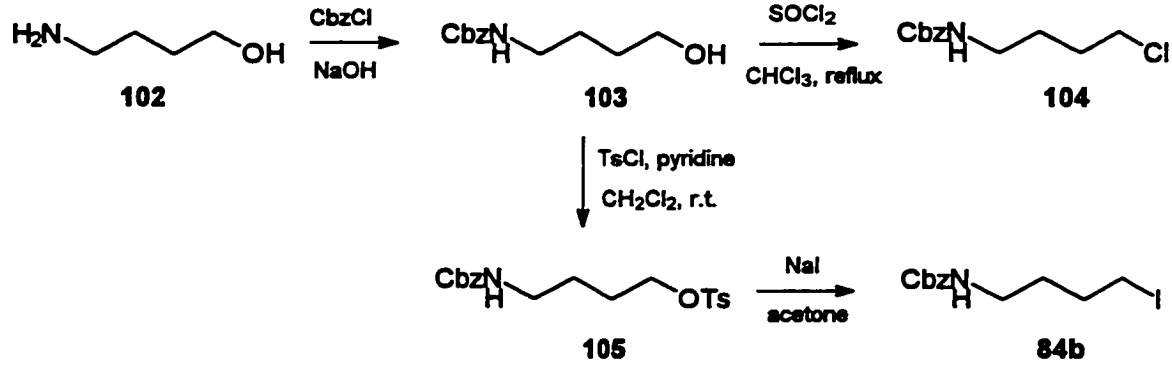


2.3.1 Synthesis of N^6-Cbz-Lysine (Model Study for the Synthesis of DAP)

To investigate the feasibility of the proposed alkylation and halogenation steps the synthesis of N^6-Cbz-lysine was chosen as a model system for DAP synthesis. The synthesis of N^6-Cbz-lysine (Figure 30, **100b**) differs from the proposed synthesis of differentially protected DAP (Figure 30, **100a**) only by the presence of the methyl ester, and thus it allows the investigation of the stability of the Cbz protecting group to alkylation and to the acid hydrolysis conditions of the alkylated complex.

The synthetic route to the required alkylating agent (**84b**) from *N*-Cbz-4-aminobutanol (**102**) is shown in Figure 32. The preparation of the alkylating agent starts with commercially available 4-aminobutanol (**102**), and the amino group was protected with benzylchloroformate using the method described by Keirs and coworkers (108). The yields and melting point range reported by Keirs for alcohol **103** (64% and 58-61°C, respectively) were both considerably lower than those obtained in this study (84% and 78.5-80.0°C, respectively). The melting point of 78.5-80.0°C corresponds favorably to other reports for alcohol **103** in the literature (109-111).

Figure 32 Synthesis of *N*-Cbz-4-aminobutyl iodide (**84b**).



The alcohol was subsequently converted to the chloride with $SOCl_2$ (Figure 32) but attempted alkylations of (*S*)-BPB-complex ((*S*)-**58**) with *N*-Cbz-4-aminobutyl chloride (**104**) at room temperature in DMF (55) or at 80°C in acetone (60) did not yield an alkylated complex. The lack of examples in the

literature (55) of alkylation of the (*S*)-BPB-complex with alkylchlorides suggest that the use of alkylbromides or iodides is imperative for successful alkylation. Attempts to prepare the corresponding bromide using commonly employed methods, *e.g.*, PPh_3/CBr_4, met with limited success.

N-Cbz-4-Aminobutyl iodide (**84b**) was successfully prepared from the alcohol *via* the tosylate **105** in a moderate yield of 56%. Only one report of the synthesis of *N*-Cbz-4-aminobutyl iodide is reported in the literature (109), and the preparation of *N*-Cbz-4-aminobutyl tosylate has not been reported. Roberts and Elmore (109) prepared the iodide *via* *N*-Cbz-aminobutyl naphthalene-2'-sulphonate. Only a melting point range and elemental analysis were used to characterize the iodide; this thesis represents the first complete characterization of *N*-Cbz-4-aminobutyl iodide.

(*S*)-BPB-Complex was successfully alkylated with *N*-Cbz-4-aminobutyl iodide in the presence of NaOH. Hydrolysis of the alkylated complex provided N^6-Cbz-(*S*)-lysine (92% e.e. by hplc, 53% e.e. by optical rotation) in 56% chemical yield. The measured optical rotation was determined at 1% in 2 M HCl while the literature concentration was 1.5% in 2 M HCl. It is unlikely that this small change in concentration is responsible for the large discrepancy observed. The literature value is supported by independent measurements. While this poor correlation remains unexplained the salient result is the successful preparation of N^6-Cbz-(*S*)-lysine showed that the approach to DAP was a

feasible one, and that the *N*-Cbz group is stable to the alkylation reaction conditions. The experiment also demonstrated that chloride was not a good enough leaving group to facilitate alkylation of the BPB-complex in DMF.

2.3.2 Synthesis of a Halogenated Norvaline Derivative

The synthesis of the required halogenated norvaline derivative **84a** can most easily be envisioned as proceeding from a suitably protected 5-hydroxy-norvaline derivative. Of the amino acids in the chiral pool, glutamic acid most closely resembles **84a**. The intact carbon skeleton of glutamic acid directly fits that of **84a**, all that is required is the protection of the α-amino and carboxyl groups along with reduction of the terminal carboxylic acid.

Similarly derivatized 5-hydroxynorvalines have received considerable attention for their use in the synthesis of chlamydocin (112), clavulanic acid (113), and foroxymithine (114), and the intact 5-hydroxynorvaline structure is found in humilixanthin (115) and polyoxins (116, 117). A recent report described the synthesis and biological activity of a cyclosporin where the second amino acid residue, 2-aminobutanoic acid, was replaced with 5-hydroxynorvaline (118). To prepare the 5-hydroxynorvaline moiety of the cyclosporin the linear peptide containing glutamic acid was reduced using ethylchloroformate and $NaBH_4$.

Literature (112, 114, 116, 119) synthetic approaches to 5-hydroxynorvaline derivatives have been plagued with difficulties which we hoped

to avoid in our approach to DAP. Baldwin and coworkers (119) indicated the preparation of the fully protected 5-iodonorvaline, but only a description of the preparation of 5-chloronorvaline was reported in a subsequent publication (112).

Miller and Kolasa (116) prepared the *N*-Cbz-1-methyl ester of 5-hydroxynorvaline by reduction of the methyl ester of *N*-Cbz-pyroglutamate. The required (*S*)-*N*-Cbz-pyroglutamate methyl ester is commercially available, but the (*R*)-isomer is also necessary for our work. Preparation of the required (*R*)-*N*-Cbz-pyroglutamate was not an attractive option, since it is well documented that formation of the glutamate anhydride required to prepare the pyroglutamate resulted in substantial racemization at the alpha carbon of the amino acid which necessitated successive recrystallizations to obtain an enantiomerically pure product (116).

Sutherland and Willis (120) synthesized 5-hydroxynorvaline from another commercially available product that is only available as the (*S*)-isomer. This approach provided the 5-hydroxynorvaline derivative from reduction of *N*-Cbz-1-methyl glutamate in only modest yields with *N*-Cbz-proline methyl ester as a significant by-product. The reduction of a *N*-protected 1-alkyl ester of glutamic acid *via* the mixed anhydride method has also been employed by Miller and Kolasa (116) to prepare 5-hydroxynorvaline derivatives. The problem with this approach lies in preparation of the *N*-protected-1-alkylglutamic acid ester.

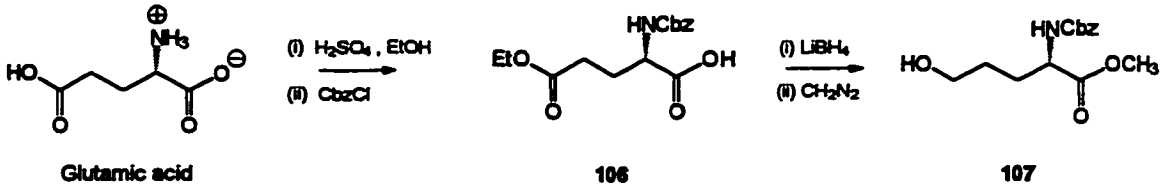
Barlos *et al.* (113) reported the synthesis of *N*-trityl-5-hydroxynorvaline from $LiAlH_4$ reduction of the 5-methyl monoester of *N*-tritylglutamate. Even though $LiAlH_4$ is capable of reducing carboxylic acid groups it was postulated that in this case the α-carboxylic acid is not reduced due to the steric influence of the bulky *N*-trityl group. The preparation of *N*-trityl-5-hydroxynorvaline by the method reported by Barlos (113) was attempted but the second step of the sequence, preparation of *N*-trityl-5-ethylglutamate from 5-ethylglutamate, resulted in quantitative recovery of the starting 5-ethylglutamate ester. Preparation of the ammonium salt of (*S*)-5-hydroxynorvaline was recently reported (121) starting from 2-amino-2-deoxy-D-gluconic acid.

Route 1

The first approach taken towards the synthesis of 5-iodonorvaline is shown in Figure 33. (*S*)-*N*-Cbz-5-Ethylglutamate (**106**) was prepared from (*S*)-glutamic acid and ethanol with H_2SO_4 as catalyst in acceptable yields (122). The selective reduction of the ester moiety of *N*-Cbz-glutamate-5-ethyl ester with $LiBH_4$ resulted in very low yields (< 1%) of the desired alcohol (**107**) accompanied by loss of the Cbz protecting group. The reduction product could not be recovered as the free acid but treatment of the reduction product mixture with CH_2N_2 allowed recovery of ester **107** after silica gel and reverse phase (C-18) chromatography. The other components of the reaction mixture were not

characterized since they were very likely to be proline derivatives; more importantly a sample of *N*-Cbz-5-hydroxynorvaline methyl ester was required as an analytical standard necessary for impending optimization reactions. Note that these experiments were carried out prior to the publication of any spectral or physical data on *N*-Cbz-5-hydroxynorvaline methyl ester.

Figure 33 Synthesis of protected 5-halonorvaline derivative employing Cbz protection.



Glutamic acid 106 107

Reports exist in the literature showing both the feasibility (123, 124) and unreliability (125) of hydride reduction in the presence of urethane protecting groups. It was evident that in our case the urethane could not survive the reduction conditions. Modifications to the reduction procedure including variations in reaction duration and temperature all failed to provide reasonable yields of the required 5-hydroxynorvaline derivative. Trial reactions were carried out on a small scale and evaluated using uv achiral hplc analysis. The experiments clearly showed that when the reaction was carried out in THF the temperature must be at least 60°C before any substrate is converted to product.

Higher reaction temperatures were investigated using mixed solvent systems containing 25% (v/v) toluene in THF. Under these reaction conditions, temperatures above 80°C for more than 15 min resulted in a decrease in the relative area of the product peak and in some runs the relative areas of other non-assigned peaks in the chromatogram increased. This can be interpreted as loss of the *N*-Cbz group or further reaction of the product. The effect of equivalents of $LiBH_4$ was also investigated; these experiments were conducted in parallel to the experiments probing the effect of duration and temperature. When reactions conducted at temperatures below 60°C were supplemented with additional $LiBH_4$ the substrate was consumed to produce an increase in the number and area of the impurity peaks but not the product peak. $LiBH_4$ was always added at a rate that kept gas evolution at a manageable rate. Reactions that were allowed to react overnight, whether at reflux or at a temperature less than 60°C all resulted in an increase in impurity peaks without a significant increase in the peak due to the desired product. In summary, short reaction times and/or low reaction temperatures usually resulted in quantitative recovery of the starting material whereas increasing the temperature and/or reaction duration usually resulted in reduction of both the ester and the carboxyl group, and loss of the Cbz protecting group. A compromise of duration and temperature could not be achieved. This approach was therefore abandoned. The reduction of *N*-urethane protected glutamic acid derivatives to provide 5-

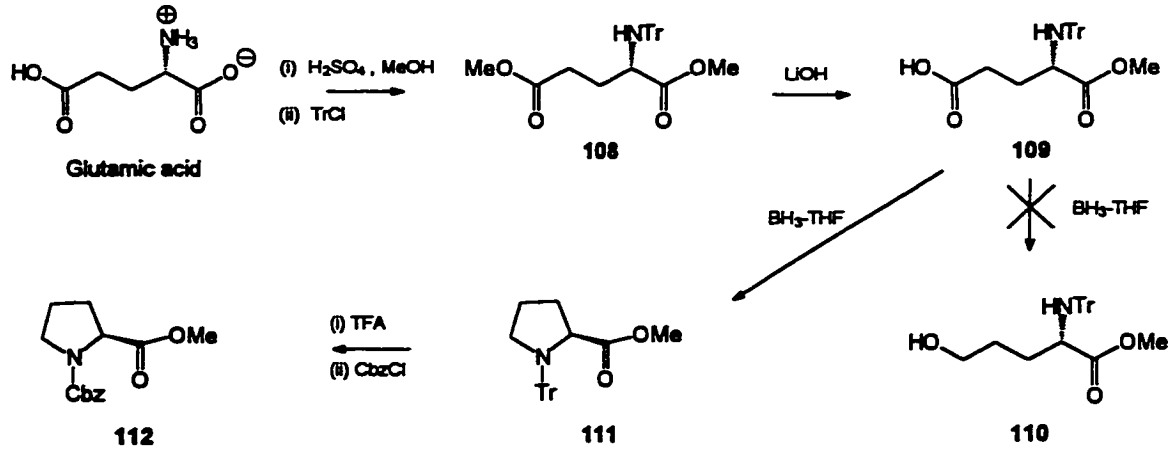
hydroxynorvaline derivatives is difficult as documented by many literature attempts (113, 120, 125, 126).

Route 2

An alternative route to 5-hydroxynorvaline was designed that would avoid the above complication arising from hydride reduction in the presence of the Cbz protecting group (Figure 34). It seemed likely that other urethane type protecting groups would come to the same fate as observed for the *N*-Cbz-protecting group. *N*-Trityl protection of the amino group should allow the reduction to be completed but the trityl group would be readily cleaved during the mild acid hydrolysis of the alkylated BPB-complex. Switching the trityl group after the reduction with the *N*-Cbz group would provide a route to the desired methyl ester of *N*-Cbz-5-hydroxynorvaline (**107**).

The 1,5-dimethylglutamate was prepared from glutamic acid in methanol in the presence of a mineral acid catalyst. The diester was used immediately to prepare *N*-trityl-1,5-dimethylglutamate. The resonance of the protons of the methyl group of the α-carboxylate of *N*-trityl-1,5-dimethyl-(*S*)-glutamate ester shifted downfield to 3.66 ppm from 3.15 ppm in the unprotected diester. This shift is consistent with that reported in the literature (127, 128). The isolated product of *N*-trityl-1,5-dimethyl glutamate contained trityl alcohol as a minor impurity that could be removed by silica gel

Figure 34 Synthesis of protected 5-halonorvaline employing trityl protection.



chromatography. In practice the crude product was carried on to the next step in the reaction sequence (base hydrolysis) where separation of the trityl alcohol from the monoester **109** is easily accomplished with an aqueous $NaHCO_3$ extraction. Differentiation of the two carboxyl functionalities was accomplished by base hydrolysis of the 5-methyl ester of **108**. The trityl protecting group exhibits sufficient steric hindrance that selective hydrolysis of the 5-methyl ester was easily accomplished using LiOH in MeOH (113, 129).

There exists in the literature only one synthesis of ester **109** (113) but no spectral or physical data were reported. *N*-Trityl-1-ethyl-glutamate was reported by Amiard *et al.* (130) but a crystalline product could not be obtained so the product was isolated as the corresponding D-*threo*-1-(*p*-nitrophenyl)-2-amino-1,3-propanediol salt for which a melting point and optical rotation were reported.

The closely related *N*-trityl-1-^{t}butyl glutamate was reported (129) but a comparison of these two compounds is of no utility.

The step that follows preparation of the trityl monoester **109** is reduction to yield *N*-trityl-1-methyl-5-hydroxynorvaline (**110**). Again no reports exist in the literature of the spectral or physical properties of this compound but Barlos *et al.* (113) reported the synthesis and melting point for the diethylammonium (DEA) salt of *N*-trityl-5-hydroxynorvaline. However, no spectral data were provided.

The mono ester (**109**) was reduced with BH_3-THF to provide a white solid which after recrystallization from hot methanol had a melting point range of 115-117°C. No physical or spectral data have been published for the desired *N*-trityl-5-hydroxynorvaline methyl ester product. A multiplicity analysis (DEPT) experiment aided the assignment of the ^{13}C signals at 62.73 (d, α-CH), 51.57 (q, OCH_3) and 49.91 (t, CH_2N) ppm; the chemical shift of the CH_2 at 49.91 ppm is more consistent with CH_2N than CH_2O. The ms of the product showed a weak (2%) molecular ion at m/z 371 due to the facile loss of the trityl moiety represented by the base peak at m/z 243. These results strongly suggest that the reduction product was *N*-trityl-proline methyl ester. The literature (128) melting point of *N*-trityl-proline methyl ester (118-120°C) corresponds favorably to the mp of the isolated reduction product (115-117°C).

The *N*-trityl-proline methyl ester (**111**) was quantitatively converted to its trifluoroacetic acid salt by treatment with TFA at 0°C. The ^{13}C nmr spectrum of

the product fits that expected for (*S*)-proline methyl ester and not the methyl ester of (*S*)-5-hydroxynorvaline. It thus appears that cyclization occurs at the BH_3-THF reduction step of the sequence to provide *N*-trityl-proline methyl ester (**111**). It follows that the actual synthetic sequence is as shown in Figure 34.

A mechanism describing the formation of methyl proline can be envisaged as occurring *via* nucleophilic attack by the nitrogen onto C-5 which is activated as the boronate ester. Alternatively, the cyclization can occur if the ester group was first reduced to the aldehyde followed by attack of the nitrogen on the aldehyde carbonyl carbon (126). The intermediate Schiff base is then reduced to yield the protected proline.

Treatment of the TFA salt of the methyl ester of proline with benzylchloroformate under alkaline conditions gave the methyl ester of *N*-Cbz-proline (**112**) as a colourless oil. The nmr showed a species which appeared as a 1:1 mixture of rotamers. The presence of rotamers in the nmr spectrum is consistent with the reported ^1H nmr spectra (120) and urethane protected prolines in general (131, 132).

The literature (120) chemical ionization mass spectrum of *N*-Cbz-(*S*)-proline methyl ester (**112**) correlates favorably with our eims spectrum. Sutherland and Willis (120) reported physical and spectral data for alcohol **107** but the reported characteristics do not match those of our isolated product. Alcohol **107** does not exist as distinguishable rotamers on the nmr time scale at

r.t. as does its cyclic counterpart **112** (120). It can thus be concluded that the isolated compound after benzylchloroformate treatment was *N*-Cbz-proline methyl ester (**112**) and not the desired *N*-Cbz-5-hydroxynorvaline methyl ester (**107**).

Route 3

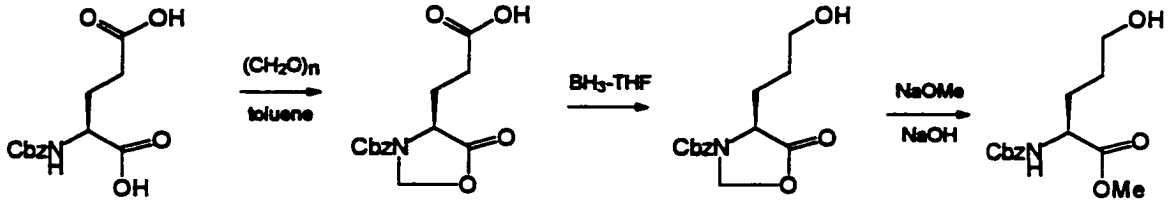
Protection of the α-amino and carboxyl groups of glutamic acid as part of an oxazolidinone ring provides several advantages over the methods tried in Routes 1 and 2. Formation of the oxazolidinone **114** provides one step protection for both the amino and carboxyl groups of glutamic acid (Figure 35).

The approach to DAP employing the oxazolidinone is quite similar to that used by Vederas and co-workers (53) to prepare 3-fluorodiaminopimelic acid. The aldehyde oxazolidinone (**56**) was used for condensation with the Seebach imidazolidinone (**55**) (Figure 14, Page 23), whereas we are using the 5-iodonorvaline and the Belokon chiral glycine equivalent (55).

The prospect of cyclization during the reduction (as in Route 2) of the 5-carboxyl group is greatly reduced since cyclization would result in an unfavorable fused ring product. There is literature precedence (133) for the reduction of acid **114** to alcohol **115**. Noteworthy is the fact that facile cyclization (this work and (126)) did not occur to form a proline derivative confirming the ability of the oxazolidinone to protect the amino group. Several

literature (126, 134) synthetic schemes employing **115** as an intermediate failed because **115** readily cyclizes to give the 6 membered lactone. Attempted synthesis of the *N*-tosylate analogue of oxazolidinone **115** *via* reduction of the corresponding acid chloride with $NaBH_4$ also resulted in formation of the 6-membered lactone (134).

Figure 35 Synthesis of *N*-Cbz-5-hydroxynorvaline *via* oxazolidinone protection.



113 **114** **115** **116**

The oxazolidinone is not stable to the strong basic conditions (135) necessary for alkylation of the BPB-complex (**58**) so the protection of the carboxyl group has to be switched to the methyl ester before alkylation. While several methods exist for removal of the oxazolidinone (136-139), opening of the oxazolidinone with methanolic NaOMe was very attractive since it simultaneously forms the required methyl ester at C-1. This approach also opens any lactones to provide the same product. This method (1.05 M NaOMe in refluxing benzene) while providing the α-methyl ester product in high yields

(95%) has also been touted as a way to prepare racemic *N*-Cbz-1-methyl-glutamate from (*S*)-5-oxo-3-*N*-Cbz-4-oxazolidinepropanoic acid (**114**) (140). Milder methods (0.1 M NaOMe in MeOH, 0°C, 1-2 h) exist (138) for which racemization does not seem to be a problem. The lithium alkoxide of trimethylsilyl ethanol has also been used (0°C) to open the diastereomeric mixture of the oxazolidine protected intermediate (**42**, Figure 10) used in the preparation of DAP (42). The differentially protected DAP product (**43**) was reported as a mixture of diastereomers that was separated by flash chromatography but no mention was made concerning the assessment of the enantiomeric purity of the diastereomers.

An azeotropic reflux/distillation of commercially available *N*-Cbz-glutamic acid (**113**) with paraformaldehyde in toluene provided oxazolidinone **114** as a light yellow oil in 91% yield. The ^{1}H and ^{13}C nmr spectra correlate favorably with published spectra (141). Compound **114** is reported (141) as a solid (mp 68-69°C) but as in other reports of this compound (139) only an oil was obtained in this work.

Acid **114** was reduced with BH_3-THF at -20°C using the method described by Aebi *et al.* (133) to give **115** as an oil. The ^{1}H nmr spectrum of the crude product compares favorably to the literature (133). A new resonance at 3.64 ppm is consistent with the newly formed CH_2OH moiety. The crude material

was used without further purification to check the suitability of NaOMe to effect the desired oxazolidinone ring opening reaction.

The opening of the oxazolidinone of **115** with NaOMe was carried out in MeOH at 0°C to yield a two component mixture containing the desired *N*-Cbz-5-hydroxynorvaline methyl ester (**107**) as the minor component as determined by ^{13}C nmr. The ^{13}C nmr spectrum of the product mixture has a resonance at 49.62 ppm which is reminiscent of C-5 of a proline type structure, however the product was not the previously isolated *N*-Cbz-proline methyl ester (**112**). The ^{13}C nmr of the product mixture also had peaks attributable to the second species in the mixture still containing the *N*-Cbz protecting group, but the characteristic doubling of signals for *N*-urethane protected prolines (120, 131, 132) was not observed. Further investigation of this result is necessary.

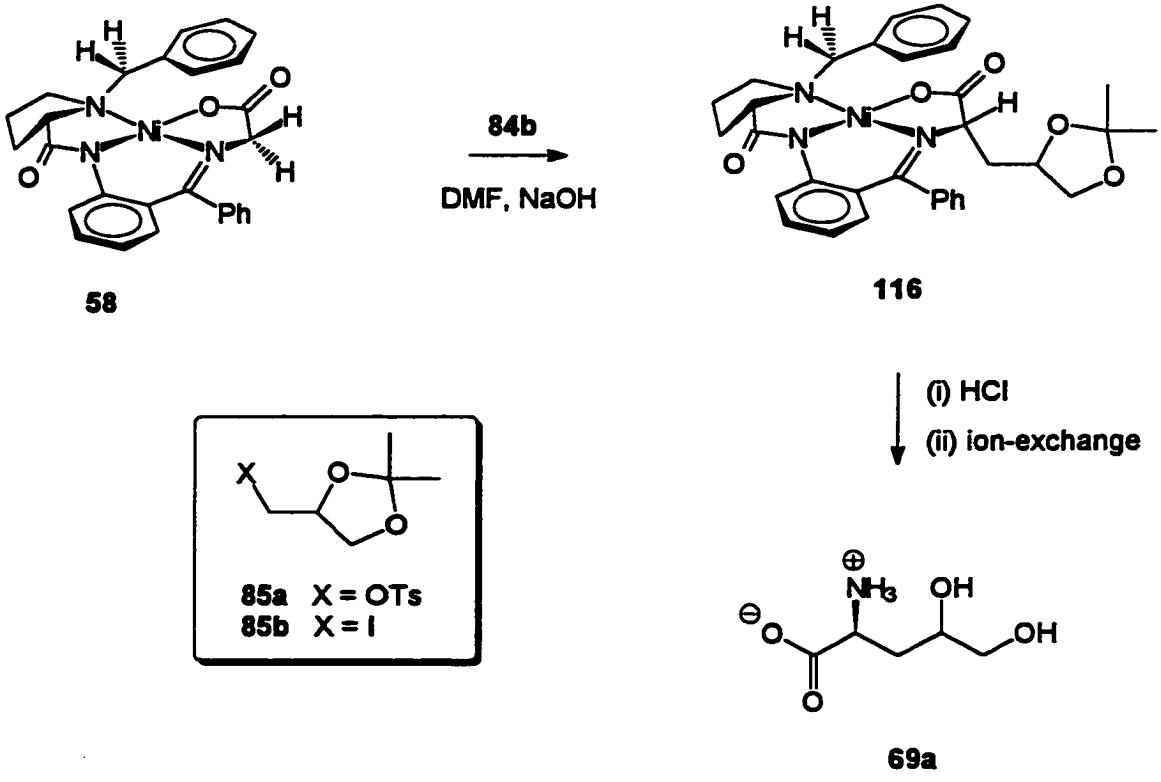
2.4 Synthesis of (2*S*, 4*R*)- and (2*S*, 4*S*)-4,5-Dihydroxynorvaline

The literature syntheses of both isomers of (2*S*,4*R*)- and (2*S*,4*S*)-4,5-dihydroxynorvaline ((2*S*,4*R*)- and (2*S*,4*S*)-**69a**) have been multistep syntheses some even requiring separation of diastereomers. Schmidt (85) employed 11 steps to prepare the required precursor in his synthesis of biphenomycin. The lactone of 4,5-dihydroxynorvaline was prepared in 4 steps from γ,δ-dehydronorvaline and used in the synthesis of echinocandin. Ariza and coworkers (83) prepared a diastereotopic mixture of (2*S*,4*R*) and (2*S*,4*S*)-4,5-

dihydroxynorvaline in 7 steps from D-ribonolactone. (2*S*)-5-Hydroxy-4-oxonorvaline (HON, **66**) isolated from cultures of *Streptomyces akiyoshiensis* was reduced by $NaBH_4$ to provide a diastereomeric mixture of (2*S*,4*R*)- and (2*S*,4*S*)-4,5-dihydroxynorvaline (142). Assignment of the stereochemistry at C-4 was attempted by separation of the diastereomeric mixture and lactonization of each isomer but nuclear Overhauser effect nmr experiments on the lactones were inconclusive (142).

Alkylation of BPB-complex (**58**) with 2,2-dimethyl-1,3-dioxolane-4-methyliodide (**85b**) to prepare (2*S*,4*R*)- and (2*S*,4*S*)-4,5-dihydroxynorvaline ((2*S*,4*R*)- and (2*S*,4*S*)-**69a**) was similar to that of the other alkylations reported in this thesis (Figure 36). Conversion of the commercially available tosylates ((4*R*)- and (4*S*)-**85a**) to the corresponding iodides (4*R*)- and (4*S*)-**85b** was readily accomplished with NaI in refluxing acetone. (*R*)- and (*S*)-2,2-Dimethyl-1,3-dioxolane-4-methyliodide (**85b**) were isolated as colourless oils in yields (68-71%) comparable to literature (143) values (60%). The ^1H and ^{13}C nmr spectra were in agreement with those reported in the literature (143). The mass spectra of both (*R*)- and (*S*)-2,2-dimethyl-1,3-dioxolane-4-methyliodide (**85b**) did not show a molecular ion peak but instead the peak at highest mass (m/z 227) was due to loss of one methyl group. The base peak (m/z 42) is attributed to $(C_3H_6)^+$ the acetal moiety.

Figure 36 Synthesis of (2*S*,4*R*)- and (2*S*,4*S*)-4,5-dihydroxynorvaline.



Chromatographic purification of the alkylated complexes was done using alumina and not silica gel chromatography as with the other complexes and as reported in the literature (55). Since the dihydroxy moiety was protected as the acetal, chromatography was carried out on alumina to prevent hydrolysis of the acetal protecting group.

An analysis of the apci mass spectral molecular ion isotope intensities confirmed the presence of one Ni atom per complex molecule (Table 9), and

confirmed the retention of the acetal group through the alkylation step. The theoretical intensities were calculated as described in Section 2.1.0, Page 48.

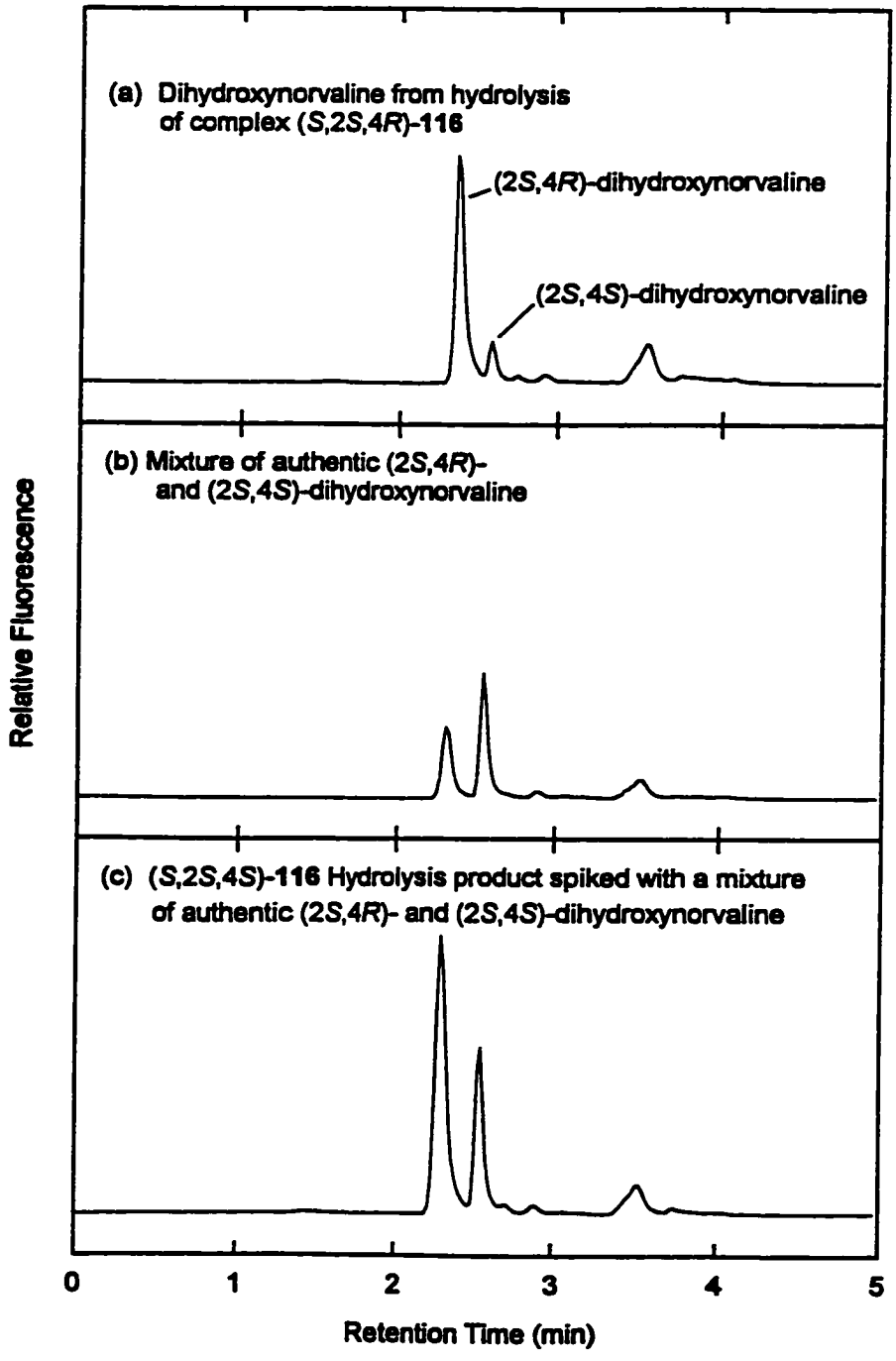
Table 9 Comparison of apci molecular ion isotope peaks with theoretical values for alkylated (*S*)-BPB-complexes in the 4,5-dihydroxynorvaline series.[a]

Complex	relative peak intensity[b] (%) [theoretical value (%)]					
	MH+1	MH+2	MH+3	MH+4	MH+5	MH+6
(4*R*)-**116**	34.0 [38.5]	41.4 [46.5]	16.9 [17.5]	9.0 [9.2]	2.0 [2.6]	1.9 [1.8]
(4*S*)-**116**	37.8 [38.5]	42.9 [46.5]	15.6 [17.5]	5.8 [9.2]	2.5 [2.6]	1.2 [1.8]

[a] molecular formula = $C_{33}H_{35}N_3NiO_5$. [b] intensities based on one scan and reported relative to MH^+ peak

For the alkylation of (*S*)-BPB-complex with (*R*)-2,2-dimethyl-1,3-dioxolane-4-methyliodide (**85b**) the crude alkylation mixture was hydrolyzed and the resulting diastereomeric amino acid product analyzed by achiral hplc (Figure 37). This analysis showed the amino acid product mixture contained 88% of the (2*S*,4*R*) isomer and 12% of the (2*R*,4*R*) isomer of 4,5-dihydroxynorvaline (67). The chromatograms show no other peaks confirming that only a minimal amount of amino acid impurities were generated during the alkylation step. The small

Figure 37 Diastereomeric excess determination of 4,5-dihydroxynorvaline by achiral opa hplc analysis.



peak at 2.74 min represents 1% glycine, this glycine represents unreacted BPB-complex.

The ^{13}C nmr spectrum of the alkylated complex matches that expected for the compound, and the resonances at 109.0 ($\underline{C}(CH_3)_2$), 26.85 (CH_3) and 24.31 (CH_3) ppm provide direct evidence that the acetal protecting group survived the alkylation step. Hydrolysis of alkylated complex was not completed.

2.5 Synthesis of β-(*p*-Nitrophenyl)serine

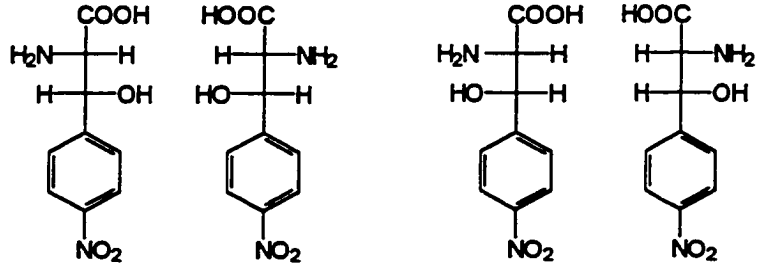
Compound **79**, required for the proposed feeding studies in the investigation of the biosynthesis of chloramphenicol, has two stereogenic centres, and thus is one of four possible stereoisomers (Figure 38). There are a very limited number of accounts in the literature for the synthesis of D-*threo*-β-(*p*-nitrophenyl)serine. D-*threo*-β-(*p*-Nitrophenyl)serine has been prepared by enzymatic hydrolysis of *N*-dichloroacetyl-DL-*threo*-β-(*p*-nitrophenyl)serine. Only two attempts of the asymmetric chemical synthesis of D-*threo*-**79** have been reported (52, 144). Blaser and Seebach (52) reported the first attempt of an asymmetric synthesis of β-(*p*-nitrophenyl)serine in 1991 using a *N*-Boc-oxazolidinone (see Figure 14 for similar oxazolidinone). Difficulties were encountered in the preparation of the free amino acid so the *N*-dichloroacetyl methyl ester of β-(*p*-nitrophenyl)serine was prepared instead. This method involves a chromatographic resolution and thus is unsuitable for the preparation

of the required isotopically labelled sample of D-*threo*-**79**; moreover this method provides the undesired L-*threo*-**79** isomer.

The second attempt to **79** is that reported by Belokon *et al.*, (144) for the synthesis of L-*threo*-**79**. (*S*)-BPB-Complex ((S)-**58**) was condensed with *p*-nitrobenzaldehyde but hydrolysis of the complex was not carried out to liberate the free amino acid. The fact that the complex was not hydrolyzed may be due to difficulties similar to those reported by Blaser and Seebach (52).

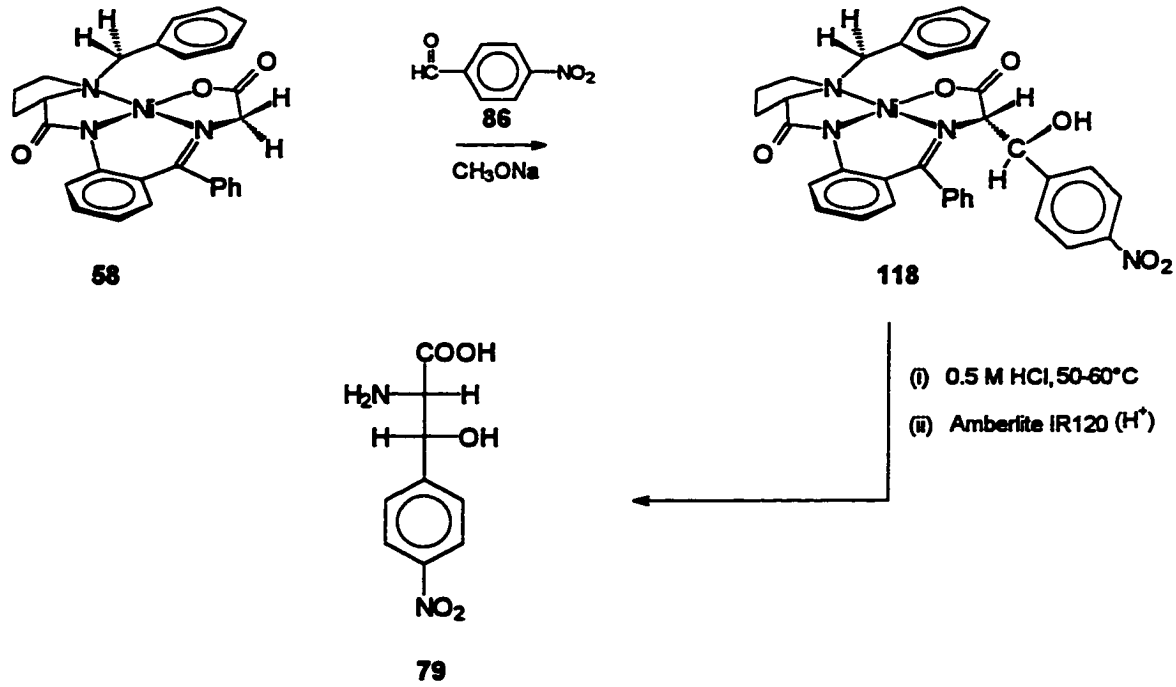
Condensation of (*S*)-BPB-complex with aldehydes as reported in the literature (54) produced β-hydroxy-α-amino acids having a L-*threo* configuration. To continue the extension of the use of (*R*)-BPB-complex to prepare (*R*)-amino acids it was predicted that the condensation of (*R*)-BPB-complex with β-(*p*-nitrophenyl)serine would produce β-(*p*-nitrophenyl)serine of the desired D-*threo* configuration (Figure 39).

Figure 38 Stereoisomers of β-(*p*-nitrophenyl)serine.



(2*S*, 3*R*)-**79** (D-*threo*) (2*R*, 3*S*)-**79** (L-*threo*) (2*S*, 3*S*)-**79** (L-*erythro*) (2*R*, 3*R*)-**79** (D-*erythro*)

Figure 39 Preparation of D-*threo*-β-(*p*-nitrophenyl)serine.



The condensation of *p*-nitrobenzaldehyde (**86**) and (*R*)-BPB-complex (**58**) was carried out in methanolic NaOMe according to the general procedure outlined in the literature (144) for the condensation of aldehydes with (*S*)-BPB-complex (Figure 39).

Positive ion electrospray ionization (esi) mass spectral analysis of the crude crystals showed the molecular ion and isotopic cluster at m/z 649 which is consistent with the proposed structure. The agreement between the theoretical molecular ion isotope cluster intensities (Table 10) and the measured intensities do not correlate as well as those intensities calculated for apci spectra above. The theoretical values were calculated as in Section 2.1, Page 48. It was

necessary to add formic acid (trace amount) to the esi ms samples in order to obtain a spectrum. There was a peak at m/z 498 that had an intensity 37% of the molecular ion but this was not attributed to a species containing a Ni atom because its isotope peaks did not resemble that of a Ni containing species.

Table 10 Comparison of esi molecular ion isotope peaks with theoretical values for (*R*)-BPB-complex condensation product with *p*-nitrobenzaldehyde.[a]

Complex	relative peak intensity[b] (%) [theoretical value (%)]					
	MH+1	MH+2	MH+3	MH+4	MH+5	MH+6
(4*R*)-**118**	33.0 [40.0]	37.2 [46.5]	13.8 [17.5]	5.9 [9.5]	0.0 [2.8]	0.0 [1.9]

[a] molecular formula = $C_{34}H_{31}N_4NiO_6$. [b] intensities based on one scan and reported relative to MH^+ peak

The yield after chromatography was 77%. The melting point range was 157-160°C which differs substantially from the literature value of 122-128°C for the complex (**118**) formed from condensation of *p*-nitrobenzaldehyde and (*S*)-BPB-complex.

The ^1H nmr spectra (in $CDCl_3$) of the reported complex exactly matched that of the crude complex from this work also recorded in $CDCl_3$. Since the

literature product is L-*threo*, this correlation is also consistent with the predicted D-*threo* configuration of the *p*-nitrophenylserine moiety in this work. The stereochemistry (*S*,2*R*,3*S*) of the literature condensation product supports the enantiomeric configuration (*R*,2*S*,3*R*) for the condensation product reported here otherwise the relationship would be diastereomeric and the nmr spectra would not match. Surprisingly the recrystallized condensation complex product was not soluble in $CDCl_3$ so the ^1H and ^{13}C nmr spectra were recorded in DMSO-d^6; this change of solvent caused a sufficient shift in the spectra to invalidate any comparison to the published ^1H data. The ^{13}C nmr showed a set of peaks due to a minor component; this component is not starting (*R*)-**58** as determined by an absence of a molecular ion cluster at m/z 489 in the esi mass spectrum. The minor component could be a diastereomer of **118**; interestingly the ^{13}C nmr spectrum of the crude material in $CDCl_3$ did not show any impurities. The change in solubility behavior and the appearance of the minor component suggest that some degradation of **118** occurred during the reaction workup, probably during the silica gel flash chromatography. The condensation product has not been hydrolyzed pending its further characterization.

These apparent discrepancies between this work and the literature should be weighed cautiously since the condensation product of this work is only predicted to be an enantiomer of the literature condensation product. The

product reported here could easily be a diastereomer of the literature product in which instance no correlation of physical and spectral data should be expected.

2.6 Summary and Conclusions

The synthesis of both isomers of BPB-complex was accomplished in very good yields and high optical purity. The preparation of (*R*)-BPB-complex demonstrates a new method for the preparation of (*R*)-amino acids and possibility for (2*S*,3*R*)-β-hydroxy-α-aminoacids. The high yield of the last step in the preparation of the BPB-complex lends itself well to the proposed preparation of the BPB-complex with isotopically labelled glycine. The isotopic yield as demonstrated by this work could be as high as 90%. One limiting factor in the preparation of the (*R*)-BPB-complex is the cost associated with (*R*)-proline.

A method has been demonstrated for the synthesis of cyano, basic and acidic amino acids that is readily adaptable to the preparation of isotopically labelled enantiomerically pure cyano basic and acidic amino acids in high chemical yields and high isotopic yields.

The proposed synthesis of DAP was shown to be feasible by the model study synthesis of (*S*)-N^6-Cbz-lysine. Attempts to prepare the necessary norvaline derivative for DAP synthesis did not yield significant quantities of product. However, one of the routes could be used to prepare both (*R*)- and (*S*)-proline with any combination of nitrogen and carboxyl protection in high yields.

The cost of (*R*)-proline may be restrictive in the preparation of (*R*)-BPB-complex as noted above but the simple preparation of proline demonstrated in this work from the very inexpensive glutamic acid makes the use of the chemistry developed with the (*R*)-BPB-complex very attractive.

The alkylation of (*S*)-BPB-complex with both (*R*)- and (*S*)-2,2-dimethyl-1,3-dioxolane-4-methyliodide demonstrates the preparation of (2*S*, 4*R*)- and (2*S*, 4*S*)-4,5-dihydroxynorvaline as feasible. The successful alkylation of the (*S*)-BPB-complex with the acetal protected electrophile demonstrates the synthesis of another highly functionalized amino acid using the BPB-complex.

FUTURE WORK

While the successful recovery of excess glycine from the preparation of BPB-complex has been demonstrated, it is necessary to reinvestigate the preparation of the BPB-complex using smaller amounts of glycine. The recovery step becomes significantly undesirable if the glycine contains radioactive labels. Higher yields of BPB-complex using close to one equivalent of glycine might be achieved by extending the reaction time and/or by increasing the temperature in conjunction with decreasing the solvent volume thereby increasing the concentration of the reactants.

The problems encountered in the attempted preparation of β-cyanoalanine *via* the alkylation the BPB-complex with bromoacetonitrile could be reinvestigated using a different base or by using a less reactive alkyl halide like chloroacetonitrile. The effect of solvent on the reaction could also be investigated.

Yield optimization studies should be conducted for the preparation of the bond labelled lysine samples. The ultimate goal of the synthesis is the preparation of bond labelled lysine to investigate amino acid catabolism in the anaerobic bacterium *Fusobacterium nucleatum* as noted in the introduction. Feeding studies with these substrates should allow irrefutable determination of the degradation pathway of (*R*)- and (*S*)-lysine in *F. nucleatum*. The expected

degradation products, butyrate and acetate, will be isolated from the cultures as their *p*-bromophenacyl derivatives (145). Mass spectral and nmr analysis of these derivatives will allow the degree to which each pathway is operating to be determined.

The methods developed for the synthesis of labelled amino acids in this thesis place labels from glycine at C-1 and/or C-2 of the amino acid. To study lysine catabolism labels must be present at other positions, thereby requiring labels in 4-bromobutylnitrile. Since these species are not commercially available their synthesis has to be completed from a commercially available, precursor before preparation of the required labelled lysine samples can be undertaken. This work has shown the task of preparing these labelled electrophiles is warranted and that their use in the alkylation of (*R*)- and (*S*)-BPB-complex to prepare (*R*)- and (*S*)-lysine is possible.

The feasibility of the present synthetic approach for the synthesis of differentially protected DAP was demonstrated. The successful preparation of DAP hinges upon the successful preparation of the key protected 5-hydroxynorvaline derivative. The easiest route to this intermediate may be the exploitation of one of the low yielding literature routes.

Attempted (134) synthesis of 5-oxo-3-*N*-Cbz-4-oxazolidinepropanol *via* reduction of 5-oxo-3-*N*-Cbz-4-oxazolidinepropanoic acid chloride with $NaBH_4$ resulted in reduction and formation of the 6-membered lactone. This was

problematic for Lee and Miller but for our work opening this lactone with sodium methoxide should give the required protected norvaline derivative.

The further investigation of the biosynthesis of chloramphenicol in *Streptomyces venezuelae* requires the preparation of labelled intermediates for use in feeding studies. The key intermediate in the synthesis of these intermediates is the β-(*p*-nitrophenyl)serine with the correct stereochemistry, *i.e.*, D-*threo*. This work has demonstrated for the alkylation reaction that the stereochemistry of the product amino acid changes from (*R*) to (*S*) when (*R*)-BPB-complex is used instead of the (*S*)-BPB-complex. An extrapolation of this idea to the synthesis of β-hydroxy-α-amino acids suggests that the desired absolute configuration at C-2 will be obtained by using the (*R*)-BPB-complex together with the *threo* relative stereochemistry.

The immediate task is to determine the stereochemistry of the crystals isolated from the condensation of (*R*)-BPB-complex and *p*-nitrobenzaldehyde by x-ray crystallography. Once the stereochemistry of the product is established, the synthesis of labelled substrates *e.g.*, [α-^{15}N]-β-(*p*-nitrophenyl)serine from *p*-nitrobenzaldehyde and (*R*)-[$^{15}N_{gly}$]-BPB-complex can be undertaken. Synthesis of D-*threo*-[4-^{13}C, α-^{15}N]-*N*-dichloroacetyl-β-(*p*-aminophenyl)serine by [1-^{13}C]-dichloroacetylation and nitro group reduction will generate the key substrate for feeding studies in the investigation of chloramphenicol biosynthesis.

EXPERIMENTAL

4.1 General Procedures

All chemicals were used as supplied unless stated otherwise. Hexanes (68-70°C, Anachemia) contained 92.8% n-hexane. High boiling petroleum ether refers to the fraction boiling at 60-80°C. Pyridine was washed with KOH then distilled from CaO (146); CH_2Cl_2 and $CHCl_3$ were dried by distillation from P_2O_5 (147). THF was dried by distillation from $LiAlH_4$. All other solvents were purified and dried by published procedures (147). All solids were dried *in vacuo* over P_2O_5 and organic extracts were dried with anhydrous salts ($MgSO_4$ or $CaCl_2$) as noted.

Melting points (uncorrected) were determined in open tubes using a Gallenkamp melting point apparatus (MF-370). Optical rotations were recorded using a Perkin-Elmer 141 polarimeter. Electron impact mass spectral data were recorded using a CEC Model 21-110 mass spectrometer at 70 eV. Positive ion atmospheric pressure chemical ionization (apci) and positive ion electrospray (esi) mass spectra were acquired on a VG Quattro II triple quadrupole mass spectrometer. Samples (20-40 µL, 0.5 mg/mL) were dissolved in CH_3CN and injected at a flow rate of 0.2 mL/min. Nitrogen was used as the bath, nebulizer and sheath gas. Probe temperature and cone voltage were 200°C and 20 V, respectively.

Nmr spectra were acquired on Bruker Spectrospin AC250F (^{1}H at 250 MHz and ^{13}C at 62.9 MHz) and a Bruker AMX400 (^{1}H at 400 MHz and ^{13}C at 100 MHz) spectrometers; J values are given in Hz. ^{1}H and ^{13}C chemical shifts of samples run in $CDCl_3$ and D_2O are relative to TMS (0 ppm) and HOD (4.80 ppm), respectively. Assignments are based on 2D homonuclear (COSY) and heteronuclear (HECTOR) correlation spectra and multiplicity analysis (DEPT) spectra. All nmr spectra were acquired using standard Bruker programs. Infrared spectra were recorded on a Nicolet 510P Fourier transform infrared spectrometer.

Thin layer chromatography was carried out on silica gel (Whatman PE SILG/UV, 250 μm). The following solvent systems were used for elutions: system A: 100% CH_2Cl_2; system B: CH_2Cl_2:EtOAc, 5:1 (v/v); system C: CH_2Cl_2:EtOAc, 1:1 (v/v); system D: $CHCl_3$:acetone, 5:1 (v/v); system E: hexanes:EtOAc, 3:1 (v/v). All developed tlc's were visualized by I_2 chamber, uv light (254 nm) and/or ninhydrin (0.25% w/v in acetone). Fractions collected from ion-exchange columns were checked for the presence of amino acids by spotting (10 μL) on filter paper, heating (100°C, 5 min), and spraying with ninhydrin (0.25% w/v in acetone). Positive tests were indicated by purple spots after heating (100°C, 5 min).

4.1.1 Amino Acid Analysis by HPLC

Synthetic samples were analyzed using two Beckman Model 110B solvent delivery modules and either uv (Hewlett Packard 1050 Diode Array UV detector) or fluorescence detection (Beckmann 157 fluorescence detector). Samples were applied *via* a 20-µL injection loop and an Upchurch Scientific Model C-130B guard column (2 x 20 mm). Usage of chromatographic columns is described in Table 11. Data acquisition and pump flow rates were controlled as described in the literature (148). Peak areas were obtained by electronic integration using in-house software (see Appendix 1). The binary solvent system gradients are described in Tables 12, 13 and 14.

Table 11 Summary of hplc chromatographic columns.

Method	Column Type	Size (mm)	Gradient
Achiral OPA	Beckman Ultrasphere ODS	45 x 4.6	Ref (148)
Achiral uv	Phenomenex Spherisorb 5ODS	150 x 4.6	see Table 12
Chiral OPA	Phenomenex Nucleosil 5	250 x 4.6	see Tables 13 and 14

Table 12 Hplc gradient for achiral uv analysis.

Time (min)	Flow Rate (mL/min)	Composition	
		Solvent A[a](%)	Solvent B[b] (%)
0.0	1.25	95.0	5.0
2.0	1.25	95.0	5.0
9.0	1.25	--	--
9.5	1.50	--	--
10.0	1.50	100.0	0.0
13.0	1.50	100.0	0.0
14.0	1.50	--	--
14.5	1.25	--	--
16.0	1.25	95.0	5.0

[a] NaOAc (0.1 M), pH 4.0, degassed and filtered (0.45 μm); [b] MeOH (100%), glass distilled.

Table 13 Hplc gradient for chiral opa analysis of cyanoamino acids

Time	Composition	
(min)	Solvent A[a] (%)	Solvent B[b] (%)
0.0	0.0	100.0
3.0	8.0	92.0
10.0	8.0	92.0
11.0	12.0	88.0
20.0	12.0	88.0
22.0	0.0	100.0

[a] $Cu(OAc)_2$ (2.5 mM), (*S*)-Proline (5.0 mM), NH_4OAc (38.5 M), pH 7.0, degassed and filtered (0.45 µm), 5% (v/v) MeCN; [b] MeCN (100%), glass distilled; [c] constant flow rate of 2.00 mL/min.

Table 14 Hplc gradient for chiral opa analysis of acidic amino acids

Time	Composition	
(min)	Solvent A[a] (%)	Solvent B[b] (%)
0.0	0.0	100.0
3.0	6.0	84.0
10.0	6.0	84.0
11.0	9.0	81.0
15.0	9.0	81.0
16.0	0.0	100.0

[a] $Cu(OAc)_2$ (2.5 mM), (*S*)-Proline (5.0 mM), degassed and filtered (0.45 µm), pH adjusted to 6.0 with NH_4OAc, MeCN (2%, v/v); [b] MeCN (100%), glass distilled; [c] constant flow rate of 2.00 mL/min.

4.2 Synthesis of Ni(II) Complex of Glycine Schiff base with 2-[*N*-(*N'*-Benzylprolyl)amino]benzophenone (BPB-complex)

4.2.1 N-Benzylproline (**88**) (94)

Aqueous NaOH (80 mL, 4 M) was added to a stirred solution of proline (12.6 g, 109 mmol) and freshly distilled benzyl chloride (15.0 mL, 183 mmol) in EtOH (120 mL, 95%, v/v). The solution was refluxed for 2.5 h. Benzyl alcohol was removed by steam distillation, which was carried out until 750 mL of distillate had been collected. The cooled reaction mixture was adjusted to pH 5.9 with glacial AcOH and concentrated to dryness *in vacuo* to yield a white solid. The solid was extracted with hot $CHCl_3$ (200 mL), and the extract was evaporated to dryness *in vacuo*. The resulting solid was recrystallized from $CHCl_3$/ether.

(*R*)-*N*-Benzylproline (5.31 g, 59%): mp 165-166°C (dec); $[\alpha]_D^{23}$ +26.70° (c=1.0, EtOH); ν_{max} (KBr): 3435, 1640 cm^{-1}; ^1H nmr (250 MHz, D_2O) δ: 7.30 (s, 5H, ArH), 4.17 (s, 2H, $BzCH_2$), 3.78 (ABX, 1H, ProCH), 3.47-3.39 (m, 1H, $ProCH_2$), 3.13-3.02 (m, 1H, $ProCH_2$), 2.38-2.20 (m, 1H, ProCH), 1.99-1.70 (m, 3H, $ProCH_2$); ^{13}C nmr (62.9 MHz, D_2O) δ: 176.13 (C=O), 133.02 (2 x ArCH), 132.53, 132.42 (ArC), 131.69 (ArCH), 70.63 (α-CH_2), 60.74 ($BzCH_2$), 57.07 (5-CH_2), 31.28 (3-CH_2), 25.24 (4-CH_2); m/z: 205 (M^+, 5%), 160 (100), 91 (72).

(*S*)-*N*-Benzylproline (13.55 g, 60%): mp 160-163°C (dec) lit. (63) 164-165°C; $[\alpha]_D^{21}$ -28.3° (c=1.0, EtOH) lit (63, 94) -29.07° and -28.4° (c=0.01, EtOH) respectively; ν_{max} (KBr): 3435, 1644 cm^{-1}; ^1H nmr (250 MHz, D_2O) δ: 7.42 (s, 5H, ArH), 4.08 (s, 2H, $BzCH_2$), 3.90 (ABX, 1H, ProCH), 3.56-3.47 (m, 1H, $ProCH_2$), 3.22-3.11 (m, 1H, $ProCH_2$), 2.49-2.37 (m, 1H, $ProCH_2$), 2.09-1.80 (m, 3H, $ProCH_2$); ^{13}C nmr (250 MHz, D_2O) δ: 176.13 (C=O), 133.27, 131.98 (ArCH), 132.76 (ArC), 132.68 (ArCH), 70.88 (α-CH), 60.88 ($BzCH_2$), 57.23 (5-CH_2), 31.49 (3-CH_2), 25.48 (4-CH_2); m/z: 205 ($M^{+\cdot}$, 2.5%), 160 (100), 91 (99).

4.2.2 N-Benzylproline·HCl (**89**) (54)

N-Benzylproline (13.40 g, 55.5 mmol) was stirred with aqueous HCl (60 mL, 1 M) for 10 min and concentrated to dryness under reduced pressure to yield a light pink solid which was dried over P_2O_5.

(*R*)-*N*-Benzylproline·HCl (5.70 g, 93%): $[\alpha]_D^{21}$ +30.3° (c=2.0, MeOH); ^1H nmr (250 MHz, D_2O) δ: 7.30 (s, 5H, ArH), 4.27 (one half AB, J_{AB}=12.97, 1H, $BzCH_2$), 4.21 (one half AB, J_{AB}=12.97, 1H, $BzCH_2$), 4.13 (ABX, 1H, α-CH), 3.49-3.40 (m, 1H, 5-CH_2), 3.21-3.10 (m, 1H, 5-CH_2), 2.43-2.31 (m, 1H, 3-CH_2), 2.05-1.87 (m, 2H, 4-CH_2), 1.84-1.76 (m, 1H, 3-CH_2); ^{13}C nmr (62.8 MHz, D_2O) δ: 173.97 (C=O), 133.47, 132.96 (ArCH), 132.33 (ArC), 132.05 (ArCH), 69.00 (α-CH), 61.36 ($BzCH_2$), 57.66, 30.98, 25.04 ($ProCH_2$); m/z: 205 (M-36, 4%), 160 (97), 91 (100).

(*S*)-*N*-Benzylproline·HCl (13.24 g, 99%): mp 184-186°C lit. (54) 179-180°C; $[\alpha]_D^{21}$ -30.38° (c=2.0, MeOH) lit (95) +32.3° (c=0.02, MeOH); ν_{max} (KBr): 3430, 1744 cm^{-1}; ^1H nmr (250 MHz, D_2O) δ: 7.26 (s, 5H, ArH), 4.24 (one half AB, J_{AB}=12.51, 1H, $BzCH_2$), 4.17 (one half AB, J_{AB}=12.51, 1H, $BzCH_2$), 4.10 (ABX, 1H, α-CH), 3.45-3.56 (m, 1H, 5-CH_2), 3.17-3.06 (m, 1H, 5-CH_2), 2.43-2.28 (m, 1H, 3-CH_2), 2.03-1.87 (m, 2H, 4-CH_2), 1.85-1.73 (m, 1H, 3-CH_2); ^{13}C nmr (62.8 MHz, D_2O) δ: 173.84 (C=O), 133.44, 132.93, 132.28, 132.02 (ArC), 68.88 (α-CH) 61.31 ($BzCH_2$), 57.64 (5-CH_2), 30.94 (3-CH_2), 25.01 (4-CH_2); m/z: 205 (M-36, 3%), 160 (95), 91 (100).

4.2.3 2-[N-(N'-Benzylprolyl)amino]benzophenone (**91**) (54)

N-Benzylproline·HCl (10.04 g, 41.4 mmol) and 2'-aminobenzophenone (8.17 g, 41.4 mmol) were dissolved in dry CH_2Cl_2 (80 mL) and cooled to -10°C. DCC was added in three equal portions (3 x 4.3 g, 62.5 mmol total) over a period of 1 h. The reaction was stirred at -20°C for 2.5 h and allowed to warm to room temperature overnight. Water (150 mL) and CH_2Cl_2 (100 mL) were added and the pH was adjusted to 10 with solid Na_2CO_3. Both layers were filtered to remove precipitated dicyclohexylurea, and the aqueous layer was extracted with CH_2Cl_2 (2 x 50 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated to dryness. The resulting oil was washed with high boiling (60-

80°C) pet. ether (200 mL) at r.t.; the oil solidified and was recrystallized from hot high boiling (60-80°C) pet. ether.

(*R*)-2-[*N*-(*N'*-Benzylprolyl)amino]benzophenone (3.49 g, 42%): mp 98-99°C; $[\alpha]_D^{21}$ +129.87° (c=0.5, MeOH); ν_{max} (KBr): 1690, 1644 cm^{-1}; ^1H nmr (250 MHz, $CDCl_3$) δ: 11.54 (s, 1H, NH), 8.59-7.05 (m, 14H, ArH), 3.92 (one half AB, J_{AB}=12.81, 1H, $BzCH_2$), 3.58 (one half AB, J_{AB}=12.82, 1H, $BzCH_2$), 3.31 (ABX, 1H, α-CH), 3.25-3.18 (m, 1H, $ProCH_2$), 2.45-2.16 (m, 2H, $ProCH_2$), 2.01-1.73 (m, 3H, $ProCH_2$); ^{13}C nmr (62.8 MHz, $CDCl_3$) δ: 198.07 (C=O), 174.71 (C(O)N), 139.20, 138.57, 138.16 (ArC), 133.42, 132.60, 132.55 (ArCH), 130.16 (2 x ArCH), 129.15, 128.35, 128.18 (2 x ArCH), 127.09 (ArCH), 125.32 (ArC), 122.23, 121.53 (ArCH), 68.27 (α-CH), 59.87 ($BzCH_2$), 53.90 (5-CH_2), 31.04 (3-CH_2), 24.20, (4-CH_2); m/z: 384 (M^+, 6%), 160 (98), 91 (100).

(*S*)-2-[*N*-(*N'*-Benzylprolyl)amino]benzophenone (6.92 g, 44%): mp 97.0-98.5°C lit. (54) 101-102°C; $[\alpha]_D^{21}$ -132.66° (c=0.5, MeOH) lit (54) $[\alpha]_D^{21}$ -134.5° (c=0.5, MeOH); ν_{max} (KBr) 1692, 1647 cm^{-1}; ^1H nmr (250 MHz, $CDCl_3$) δ: 11.55 (s, NH, 1H), 8.6-7.0 (m, 14H, ArH), 3.91 (one half AB, J_{AB}=12.82, 1H, $BzCH_2$), 3.58 (one half AB, J_{AB}=12.82, 1H, $BzCH_2$), 3.31 (ABX, 1H, α-CH), 3.25-3.18 (m, 1H, $ProCH_2$), 2.44-1.76 (m, 4H, CH_2); ^{13}C nmr (62.8 MHz, $CDCl_3$) δ: 198.04 (C=O), 174.66 (NC=O), 139.25, 138.57, 138.17, 125.32 (ArC), 133.43, 132.65, 132.55, 130.17, 129.18, 128.38, 128.22, 127.13, 122.26, 121.53,

(ArCH), 68.31 (α-CH), 59.90 ($BzCH_2$), 53.93 (5-CH_2), 31.06 (3-CH_2), 24.23 (4-CH_2); m/z: 384 ($M^{\cdot+}$, 49%), 160 (97), 91(100).

4.2.4 Ni(II) Complex of Glycine Schiff Base with 2-[N-(N'-Benzylprolyl)amino]benzophenone (BPB-complex) (**58**) (54, 58)

2-[*N*-(*N'*-Benzylprolyl)amino]benzophenone (2.65 g, 6.89 mmol), $Ni(NO_3)_2 \cdot 6H_2O$ (3.70 g, 12.7 mmol), and a solution of glycine (2.45 g, 34.5 mmol) in methanolic NaOMe (48 mL, 1 M) were added to MeOH (freshly glass distilled). The reaction mixture was heated to 60°C for 4 h. The cooled reaction mixture was adjusted to pH 6.8 with HCl (1 M) and concentrated *in vacuo* to remove MeOH. The resulting suspension was partitioned between H_2O (50 mL) and $CHCl_3$ (100 mL). The organic layer was further washed with H_2O (2 x 50 mL), dried ($MgSO_4$), and concentrated to dryness *in vacuo.* Recrystallization from acetone provided the product as bright red crystals.

(*R*)-BPB-Complex (3.68 g, 90%): mp 216-221°C (dec); $[\alpha]_D^{21}$ -2155° (c=0.024, $CHCl_3$); ν_{max} (KBr): 3430, 1674, 1642 cm^{-1}; ^1H nmr (250 MHz, $CDCl_3$) δ: 8.30-6.66 (m, 15H, ArH), 4.47 (one half AB, J_{AB}=12.51, 1H, $BzCH_2$), 3.67 (one half AB, J_{AB}=12.51, 1H, $BzCH_2$), 3.50-3.26 (m, 2H, $ProCH_2$), 3.83-3.63 (m, 4H, $ProCH_2$), 3.46 (dd, J=10.68 and 5.50, 1H, $ProCH_2$), 3.37-3.26 (m, 2H, $GlyCH_2$), 2.59-2.33 (m, 2H, 3-CH_2), 2.20-2.05 (m, 2H, $ProCH_2$); ^{13}C nmr (62.8 MHz, $CDCl_3$) δ: 181.39 (C=O), 177.36 (C=O), 171.64 (C=N), 142.52, 134.62, 133.34 (ArC),

133.21, 132.24 (ArCH), 131.75 (2x ArCH), 129.77, 129.63, 129.37, 129.15 (ArCH), 128.96 (2x ArCH), 126.28, 125.68 (ArCH), 124.20 (ArC), 124.28, 120.88 (ArCH), 69.89 (Pro α-CH), 63.14 ($BzCH_2$), 61.30 (Gly α-CH_2), 57.51 (5-CH_2), 30.74 (3-CH_2), 23.73 (4-CH_2).

(*S*)-BPB-Complex (6.18 g, 95%): mp 216-220°C (dec) lit (54) 208-212°C (dec); $[\alpha]_D^{21}$ +2164° (c=0.022, $CHCl_3$); ν_{max} (KBr): 3251, 1692, 1647 cm^{-1}; ^1H nmr (250 MHz, $CDCl_3$) δ: 8.4-6.6 (m, 15H, ArH), 4.49 (one half AB, J_{AB}=12.52, 1H, $BzCH_2$), 3.67 (one half AB, J_{AB}=12.67, 1H, $BzCH_2$), 3.50-3.26 (m, 2H, ProCH_2), 3.9-3.6 (m, 4H, ProCH_2), 3.46 (dd, J=10.68 and 5.50, 1H, ProCH_2), 3.37-3.26 (m, 2H, GlyCH_2), 2.59-2.33 (m, 2H, 3-CH_2), 2.20-2.05 (m, 2H, ProCH_2); ^{13}C nmr (62.8 MHz, $CDCl_3$) δ: 181.38 (C=O), 177.29 (C=O), 171.57 (C=N), 142.51, 134.58, 133.44 (ArC), 133.16, 132.16 (ArCH), 131.72 (2x ArCH), 129.74, 129.60, 129.35, 129.10 (ArCH), 128.92 (2x ArCH) 126.26, 125.66 (ArCH), 125.16 (ArC) 124.25, 120.83 (ArCH), 69.88 (Pro α-CH), 63.14 ($BzCH_2$), 61.26 (Gly α-CH_2), 57.56 (5-CH_2), 30.72 (3-CH_2), 23.71 (4-CH_2).

4.2.5 Recovery of Excess Glycine

The aqueous layer from the synthesis of (*S*)-BPB-complex (4.63 mmol) was adjusted to pH 2.0 with HCl (6 M) and applied to an Amberlite IR120P (H^+) column (2.5 x 26 cm). The column was washed with H_2O until the effluent was neutral, and the amino acid was eluted with aqueous NH_3 (5%, w/v). Fractions

containing glycine (hplc analysis) were combined, concentrated to dryness *in vacuo*, and recrystallized from H_2O/EtOH to provide glycine as off-white crystals. (1.13 g, 89% recovery): decomposition started at 230°C, lit. (149) decomposition started at 233°C; ^1H nmr (250 MHz, D_2O) δ: 3.33 (s, CH_2); ^{13}C nmr (62.8 MHz, D_2O) δ: 175.17 (C=O), 44.04 (CH_2).

4.3 Synthesis of Lysine and Related Amino Acids

4.3.1 General Procedure for Alkylation of BPB-Complex with Alkylcyanobromides

The alkylcyanobromide (0.78 mmol) and finely ground NaOH (1.94 mmol) were added to a suspension of BPB-complex (403 mg, 0.78 mmol) in DMF (2.0 mL). After stirring for 0.5 h, the reaction mixture was neutralized by pouring it onto AcOH (10 mL, 0.5 M); the product was extracted with $CHCl_3$ (2 x 2 mL). The combined organic layers were washed with H_2O (1 x 4 mL), dried ($MgSO_4$), and evaporated to dryness. The residue was purified by dry flash chromatography (150), and fractions containing the desired product were pooled and concentrated to yield a red oil which was crystallized from acetone. The precipitate formed when larger scale reactions (>3 mmol) were neutralized by pouring into AcOH was collected and recrystallized from acetone. Additional product was recovered by extracting the aqueous filtrate with $CHCl_3$.

Complex Alkylated with 3-Bromopropionitrile (**95b**)

(*R,R*)-Complex (1200 mg, 75%): mp 218-220°C; $[\alpha]_D^{19}$ -2754° (c=0.023, $CHCl_3$); λ_{max} (MeOH): 414, 332, 264, 230 nm; ν_{max} (KBr): 2969, 2246, 1675, 1637cm^{-1}; ^{1}H nmr (400 MHz, $CDCl_3$) δ: 8.14-8.06 (m, 3H, ArCH), 7.59-7.49 (m, 4H, ArCH), 7.36 (t, 2H, J=7.83, ArCH), 7.21-7.13 (m, 2H, ArCH), 6.96 (d, J=6.84, 1H, ArCH), 6.69-6.63 (m, 2H, ArCH), 4.40 (one half AB, J_{AB}=12.72, 1H, $BzCH_2$), 3.86 (ABX, 1H, α-CH), 3.56 (one half AB, 1H, J_{AB}=12.72, $BzCH_2$), 3.52-3.47 (m, 3H, $ProCH_2$), 2.77-2.69 (m, 2H, CH_2), 2.59-2.47 (m, 2H, CH_2), 2.39-2.31 (m, 1H, CH_2), 2.26-2.19 (m, 1H, $ProCH_2$), 2.12-2.05 (m, 1H, CH_2), 1.96-1.88 (m, 1H, CH_2); ^{13}C nmr (100 MHz, $CHCl_3$) δ: 180.44 (C=O), 177.85 (C=O), 171.78 (C=N), 142.56, 133.41, 133.28, 132.63, 131.73, 131.49, 131.15, 130.23, 129.33, 129.01, 128.95, 127.16, 127.00, 126.03, 123.84, 120.86 (ArC), 118.16 (CN), 70.28 (CH), 68.28 (CH), 63.29 ($BzCH_2$), 57.33 ($ProCH_2$), 31.39 (CH_2), 30.72 ($ProCH_2$), 24.13 ($ProCH_2$), 13.80 ($\underline{C}H_2CN$).

(*S,S*)-Complex (677 mg, 63%): mp 219-220°C; $[\alpha]_D^{19}$ -2989° (c=0.019, $CHCl_3$); λ_{max} (MeOH): 416, 332, 264, 230 nm; ν_{max} (KBr): 2968, 2246, 1675, 1637 cm^{-1}; ^{1}H nmr (400 MHz, $CDCl_3$) δ: 8.14-8.05 (m, 3H, ArCH), 7.56-7.49 (m, 4H, ArCH), 7.36 (t, 2H, J=7.83, ArCH), 7.21-7.13 (m, 2H, ArCH), 6.96 (d, 1H, J=6.84, ArCH), 6.69-6.63 (m, 2H, ArCH), 4.40 (one half AB, J_{AB}=12.72, 1H, $BzCH_2$), 3.86 (ABX, 1H, α-CH), 3.56 (one half AB, 1H, J_{AB}=12.72, $BzCH_2$), 3.53-3.46 (m, 3H,

$ProCH_2$), 2.77-2.69 (m, 2H, CH_2), 2.59-2.47 (m, 2H, CH_2), 2.39-2.31 (m, 1H, CH_2), 2.26-2.19 (m, 1H, $ProCH_2$), 2.12-2.05 (m, 1H, $ProCH_2$), 1.96-1.87 (m, 1H, CH_2); ^{13}C nmr (100 MHz, $CDCl_3$) δ: 180.44 (C=O), 177.88 (C=O), 171.77 (C=N), 142.54, 133.40, 133.27, 132.63, 131.49, 130.23, 129.34, 128.96, 127.14, 127.00, 126.03, 123.84, 120.88 (ArC), 118.17 (CN), 70.23 (CH), 68.26 (CH), 63.26 ($BzCH_2$), 57.31 ($ProCH_2$), 31.41 (CH_2), 30.71 ($ProCH_2$), 24.15 ($ProCH_2$), 13.8 ($\underline{C}H_2CN$).

Complex Alkylated with 4-Bromobutylnitrile (**95c**)

(*R,R*)-Complex (432 mg, 76%): mp 180-183°C; $[\alpha]_D^{21}$ -2937° (c=0.020, $CHCl_3$); λ_{max} (MeOH): 412, 332, 262, 234 nm; ν_{max} (KBr): 3445, 2243, 1678, 1637 cm^{-1}; ^1H nmr ($CHCl_3$) δ: 8.17-8.04 (m, 3H, ArCH), 7.56-6.98 (m, 9H, ArCH), 6.71-6.63 (m, 2H, ArCH), 4.43 (one half AB, J_{AB}=12.67, 1H, $BzCH_2$), 3.82 (ABX, 1H, CH), 3.58 (AB, J_{AB}=12.67, 1H, $BzCH_2$), 3.60-3.45 (m, 3H, CH and $ProCH_2$), 2.75-1.66 (m, 10H, CH_2); ^{13}C nmr (62.8 MHz, $CHCl_3$) δ: 180.54 (C=O), 178.93 (C=O), 171.00 (C=N), 142.27, 133.45, 133.32, 132.43, 131.54, 129.98, 129.47, 128.96, 127.50, 127.06, 126.19, 123.84, 120.92 (ArC), 119.04 (CN), 70.22 (CH), 69.13 (CH), 63.20 ($BzCH_2$), 57.25 ($ProCH_2$), 34.59 (CH_2), 30.75 (CH_2), 24.04 (CH_2), 21.63 (CH_2), 16.67 ($\underline{C}H_2CN$).

(*S,S*)-Complex (340 mg, 75%): mp 198-200°C (dec); $[\alpha]_D^{21}$ +2943°(c=0.023, MeOH); λ_{max} (MeOH): 420, 334, 266, 244 nm; ν_{max} (KBr): 2961, 2248, 1676, 1636 cm^{-1}; ^1H nmr (250 MHz, $CDCl_3$) δ: 8.15-8.05 (m, 3H, ArCH), 7.54-6.99 (m, 9H, ArCH), 6.71-6.65 (m, 2H, ArCH), 4.43 (one half AB, J_{AB}=12.81, 1H, $BzCH_2$), 3.82 (ABX, 1H, CH), 3.57 (AB, J_{AB}=12.81, 1H, $BzCH_2$), 3.60-3.46 (m, 3H, CH and $ProCH_2$), 2.75-1.71 (m, 10H, CH_2); ^{13}C nmr (62.8 MHz, $CDCl_3$) δ: 180.47 (C=O), 178.81 (C=O), 170.97 (C=N), 142.34, 133.48, 133.33, 133.23, 132.45, 131.56, 129.97, 129.48, 129.02, 128.96, 127.51, 127.06, 126.18, 123.84, 120.89 (ArC), 118.96 (CN), 70.19 (CH), 69.10 (CH), 63.15 ($BzCH_2$), 57.18 ($ProCH_2$), 34.62 (CH_2), 30.76 (CH_2), 24.04 (CH_2), 21.64 (CH_2), 16.66 ($\underline{C}H_2CN$).

Complex Alkylated with 5-Bromovaleronitrile (**95d**)

(*R,R*)-Complex (1.63 g, 94%): mp 181-183°C; $[\alpha]_D^{21}$ -2328° (c=0.020, $CHCl_3$); λ_{max} (MeOH): 412, 332, 264, 210 nm; ν_{max} (KBr): 2295, 2243, 1667, 1639 cm^{-1}; ^1H nmr (250 MHz, $CHCl_3$) δ: 8.13-8.05 (m, 3H, ArH), 7.56-7.46 (m, 3H, ArH), 7.38-7.11 (m, 6H, ArH), 6.96-6.92 (m, 1H, ArH), 6.70-6.61 (m, 2H, ArH), 4.43 (one half AB, 2H, J_{AB}=12.66, $BzCH_2$), 3.87 (ABX, 1H, CH), 3.57 (one half AB, J_{AB}=12.66, 2H, $BzCH_2$), 3.67-3.45 (m, 3H, CH and $ProCH_2$), 2.80-2.69 (m, 1H, CH_2), 2.62-2.45 (m, 1H, CH_2), 2.24-1.92 (m, 6H, CH_2), 1.72-1.30 (m, 4H, CH_2); ^{13}C nmr (62.8 MHz, $CHCl_3$) δ: 180.43 (C=O),179.04 (C=O), 170.54 (C=N), 142.27, 133.66 (ArC), 133.24, 132.28, 131.56, 129.96, 129.07, 128.93, 127.46, 127.27 (ArCH),

126.35 (ArC), 123.79, 120.82 (ArCH), 119.31 (CN), 70.20 (CH), 69.43 (CH), 63.14 (CH_2), 57.18 (CH_2), 34.84 (CH_2), 30.76 (CH_2), 24.67 (CH_2), 24.53 (CH_2), 24.00 (CH_2), 16.88 ($\underline{C}H_2CN$).

(*S,S*)-Complex (869 mg, 75%): mp 191.0-192.5°C; $[\alpha]_D^{21}$ +2447° (c=0.034, $CHCl_3$); ν_{max} (KBr): 2957, 2248, 1671, 1642 cm^{-1}; λ_{max} (MeOH): 412, 330, 264, 236, 210 nm; ^1H nmr (250 MHz, $CHCl_3$) δ: 8.13-8.05 (m, 3H, ArH), 7.58-7.45 (m, 3H, ArH), 7.38-7.11 (m, 6H, ArH), 6.96-6.93 (m, 1H, ArH), 6.70-6.60 (m, 2H, ArH), 4.44 (one half AB, 2H, J_{AB}=12.67, $BzCH_2$), 3.57 (one half AB, 2H, J_{AB}=12.67, $BzCH_2$), 3.87 (AB$\underline{X}$, 1H, CH), 3.66-3.45 (m, 3H, CH and $ProCH_2$), 2.80-2.69 (m, 1H, CH_2), 2.58-2.49 (m, 1H, CH_2), 2.24-1.92 (m, 6H, CH_2), 1.69-1.32 (m, 4H, CH_2); ^{13}C nmr (62.8 MHz, $CHCl_3$) δ:180.43 (C=O), 179.03 (C=O), 170.53 (C=N), 142.28, 133.68 (CH), 133.24, 132.30, 131.57, 129.97, 129.07, 128.93, 127.47, 127.29 (ArCH), 126.36 (ArC), 123.80, 120.82 (ArCH), 119.32 (CN), 70.17 (CH), 69.43 (CH), 63.12 (CH_2), 57.15 (CH_2), 34.86 (CH_2), 30.77 (CH_2), 24.68 (CH_2), 24.54 (CH_2), 24.01 (CH_2), 16.90 ($\underline{C}H_2CN$).

4.3.2 Preparation of Cyanoamino Acids

Water (2.2 mL) and aqueous HCl (2.2 mL, 3 M) was added to a solution of the alkylated complex (302 mg, 0.52 mmol) in MeOH (4.8 mL) and the resulting solution was heated in a sealed tube at 53°C for 20 min until the red colour of

the complex completely disappeared (60). Alkylated complexes from larger (> 2 mmol) runs were heated in flasks equipped with a reflux condenser. The resulting solution was extracted with $CHCl_3$ (2 x 5.5 mL) to recover BPB-ligand and the combined organic layers were washed with H_2O (2 x 5.5 mL) (55, 60). The aqueous layer was acidified to pH 2 with HCl (3 M), and applied to an Amberlite IR120P (H^+) column. The column was washed with water until the effluent was neutral, and the amino acid product was eluted with aqueous NH_3. Fractions containing the amino acid product were combined and concentrated *in vacuo*. The resulting white solid was recrystallized from H_2O/EtOH.

(*R*)-2-Amino-4-Cyanobutanoic acid ((*R*)-**96b**) (118 mg, 46%): mp 212-213°C; >99% e.e. by hplc; $[\alpha]_D^{21}$ -33.9° (c=0.46, H_2O); ν_{max} (KBr): 3462, 2255, 1612 cm^{-1}; ^1H nmr (400 MHz, D_2O) δ: 3.80 (apparent t, splitting of 6.36 and 6.85, 1H, α-CH), 2.78-2.64 (m, 2H, CH_2), 2.31-2.17 (m, 2H, CH_2); ^{13}C nmr (100 MHz, D_2O) δ: 175.63 (COOH), 122.74 (CN), 55.96 (α-CH), 28.91 (CH_2), 16.13 (CH_2).

(*S*)-2-Amino-4-Cyanobutanoic acid ((*S*)-**96b**) (97.3 mg, 68%): mp 212-214°C (dec) lit. (151) 220-235°C (dec); >99% e.e. by hplc; $[\alpha]_D^{19}$ +26.5° (c=0.5, H_2O) lit (151) $[\alpha]_D^{21}$ +26.5° (c=0.5, H_2O); ν_{max} (KBr): 3464, 2255, 1613 cm^{-1}; ^1H nmr (400 MHz, D_2O) δ: 3.79 (apparent t, splitting of 6.36 and 6.85, 1H, α-CH), 2.76-2.63

(m, 2H, CH_2), 2.30-2.15 (m, 2H, CH_2); ^{13}C nmr (100 MHz, D_2O) δ: 175.62 (COOH), 122.76 (CN), 55.96 (α-CH), 28.90 (CH_2), 16.14 (CH_2).

(*R*)-5-Cyanonorvaline ((*R*)-**96c**) (356 mg, 62%): mp 226-228°C (dec); 92.8% e.e. by hplc; $[\alpha]_D^{21}$ -10.1° (c=0.50, H_2O); ν_{max} (KBr): 3430, 2946, 2248, 1591 cm^{-1}; ^1H nmr (250 MHz, D_2O) δ: 3.73 (apparent t, 1H, CH), 2.53 (t, J=6.9, 2H, 5-CH_2), 2.00-1.81 (m, 2H, 3-CH_2), 1.78-1.63 (m, 2H, 4-CH_2); ^{13}C nmr (62.8 MHz, D_2O) δ: 176.87 (C=O), 123.79 (CN), 56.82 (CH), 32.22 (CH<u>C</u>H_2), 23.38 (<u>C</u>H_2CH_2CN), 18.96 (<u>C</u>H_2CN).

(*S*)-5-Cyanonorvaline ((*S*)-**96c**) (265 mg, 76%): mp 225-227°C lit (101) 230-237°C (dec); 96.4% e.e. by hplc; $[\alpha]_D^{21}$ +7.7° (c=0.52, H_2O) lit (101) $[\alpha]_D^{21}$ +10° (c=0.50, H_2O); ν_{max} (KBr): 3430, 2946, 2248, 1586 cm^{-1}; ^1H nmr (250 MHz, D_2O) δ: 3.73 (apparent t, J=5.80 and 6.10, 1H, α-CH), 2.53 (t, J=6.9, 2H, 5-CH_2), 2.01-1.91 (m, 2H, 3-CH_2), 1.84-1.65 (m, 2H, 4-CH_2); ^{13}C nmr (62.8 MHz, D_2O) δ: 176.89 (COOH), 123.80 (CN), 56.85 (CH), 32.24 (CH_2), 23.41 (CH_2), 18.98 (<u>C</u>H_2CN).

(*R*)-2-Amino-6-Cyanohexanoic acid ((*R*)-**96d**) (268 mg, 77%): mp 232-234°C; >99% e.e. by hplc; $[\alpha]_D^{21}$ -4.5° (c=0.5, H_2O); ν_{max} (KBr): 3426, 2248, 1584 cm^{-1}; ^1H nmr (D_2O, 400 MHz) δ: 3.77 (apparent t, 1H, α-CH), 2.54 (t, J=7.1, 2H, 6-CH_2),

1.99-1.84 (m, 2H, 3-CH_2), 1.78-1.71 (m, 2H, 5-CH_2), 1.63-1.45 (m, 2H, 4-CH_2); ^{13}C nmr (D_2O, 100 MHz) δ: 177.12 (C=O), 124.36 (CN), 57.13 (α-CH), 32.24 (3-CH_2), 26.74 (5-CH_2), 26.21 (4-CH_2), 18.68 (6-CH_2).

(*S*)-2-Amino-6-Cyanohexanoic acid ((*S*)-**96d**) (54 mg, 68%): mp 228-230°C; >99% e.e. by hplc; $[\alpha]_D^{21}$ +2.5° (c=0.52, H_2O); ν_{max} (KBr): 3424, 2248, 1584 cm^{-1}; ^1H nmr (400 MHz, D_2O) δ: 3.75 (apparent t, 1H, α-CH), 2.53 (t, J=7.1, 2H, 6-CH_2), 1.97-1.83 (m, 2H, 3-CH_2), 1.76-1.69 (m, 2H, 5-CH_2), 1.62-1.44 (m, 2H, 4-CH_2); ^{13}C nmr (100 MHz, D_2O) δ: 177.21 (C=O), 124.45 (CN), 57.20 (α-CH), 32.31 (3-CH_2), 26.78 (5-CH_2), 26.26 (4-CH_2), 18.71 (6-CH_2).

4.3.3 Preparation of Basic Amino Acids

The cyanoamino acid (0.704 mmol) and $CoCl_2.6H_2O$ (335 mg, 1.41 mmol) were dissolved in H_2O (3 mL), and $NaBH_4$ (267 mg, 7.04 mmol) was added in two portions over 20 min. The nitrile was completely reduced within 30 min at r.t. as determined by opa-hplc.

Alternatively, the cyanoamino acid was reduced directly as the aqueous layer from hydrolysis of the cyanoamino acid complex. The aqueous layer was first neutralized with $NaHCO_3$ before addition of $NaBH_4$.

The reduction mixture was quenched with aqueous HCl (1 M) and acetone (3 mL) and applied to an Amberlite IR120P (H^+) column (1.5 x 29 cm).

The column was washed with H_2O until the effluent was neutral; elution with aqueous ammonia (0.3 M) and concentration *in vacuo* provided the basic amino acid as a oily residue. The residue was redissolved in H_2O and the pH adjusted to 3-4 with HCl (0.5 M), concentration *in vacuo* provided the amino acid monohydrochloride as off-white solid (lysine) or tan coloured oil.

(*R*)-Ornithine ((*R*)-**98b**) (17.9 mg, 69%): ^{1}H nmr (250 MHz, D_2O) δ: 3.96 (apparent t, splittings of 5.80 and 6.41 Hz, 1H, α-CH), 2.93 (t, J=7.29, 2H, CH_2N), 1.98-1.67 (m, 4H, $C\underline{H}_2C\underline{H}_2CH_2N$); ^{13}C nmr (62.8 MHz, D_2O) δ: 174.59 (COOH), 55.32 (α-CH), 41.53 (CH_2N), 29.67 (CH_2), 25.48 (CH_2).

(*S*)-Ornithine ((*S*)-**98b**) (11.8 mg, 75%): ^{1}H nmr (250 MHz, D_2O) δ: 3.96 (apparent t, splittings of 5.79 and 6.41 Hz, 1H, α-CH), 2.93 (t, J=7.33, 2H, CH_2N), 1.97-1.62 (m, 4H, $C\underline{H}_2C\underline{H}_2CH_2N$); ^{13}C nmr (62.8 MHz, D_2O) δ: 174.63 (COOH), 55.32 (α-CH), 41.56 (CH_2N), 29.67 (CH_2), 25.50 (CH_2).

(*R*)-Lysine ((*R*)-**98c**) (72 mg, 75%): mp 257-260°C; $[\alpha]_D^{21}$ -13.1° (c=1.9, 0.6 M HCl); ^{1}H nmr (400 MHz, D_2O) δ: 3.62 (apparent t, 1H, α-CH), 2.86 (t, J=7.33, 2H, 6-CH_2), 1.80-1.70 (m, 2H, CH_2), 1.60-1.52 (m, 2H, CH_2), 1.40-1.22 (m, 2H, CH_2); ^{13}C nmr (100 MHz, D_2O) δ: 176.98 (C=O), 57.04 (CH), 41.72 (CH_2), 32.44 (CH_2), 28.97 (CH_2), 24.06 (CH_2).

(*S*)-Lysine ((*S*)-**98c**) (47.4 mg, 77%): mp 256-258°C (dec) lit (149) 263-264°C; $[\alpha]_D^{21}$ +13.9° (c=2, 0.6 M HCl) lit (149) $[\alpha]_D^{21}$ +14.6 (c=2, 0.6 M HCl); ^{1}H nmr (250 MHz, D_2O) δ: 3.54 (apparent t, splitting of 6.10 and 6.11 Hz, 1H, α-CH), 2.81 (t, J=7.48, 2H, 6-CH_2), 1.74-1.65 (m, 2H, CH_2), 1.58-1.46 (m, 2H, CH_2), 1.33-1.22 (m, 2H, CH_2); ^{13}C nmr (62.8 MHz, D_2O) δ: 177.38 (C=O), 57.22 (CH), 41.82 (CH_2), 32.63 (CH_2), 29.14 (CH_2), 24.17 (CH_2).

(*R*)-2,7-Diaminoheptanoic acid ((*R*)-**98d**) (35.5 mg, 81%): ^{1}H nmr (250 MHz, D_2O) δ: 3.80 (apparent t, splitting of 5.80 and 6.10 Hz, 1H, α-CH), 2.86 (t, J=7.63, 2H, 7-CH_2), 1.91-1.65 and 1.61-1.49 (m, 8H, 4 x CH_2); ^{13}C nmr (62.8 MHz, D_2O) δ: 176.26 (COOH), 56.57 (α-CH), 41.62 (7-CH_2), 29.94 (CH_2), 29.12 (CH_2), 26.50 (CH_2), 25.53 (CH_2).

4.3.4 Preparation of Acidic Amino Acids

The cyanoamino acid (0.704 mmol) was dissolved in aqueous HCl (6 M, 10 mL) and refluxed until all of the starting cyanoamino acid was consumed as determined by achiral hplc analysis (~90 min). The reaction mixture was cooled, diluted to 500 mL with H_2O, and adjusted to pH 2.0 with aqueous NH_3. The solution was applied to an Amberlite IR120P (H^+) column. The column was washed with H_2O until the effluent was neutral and eluted with aqueous NH_3 (0.5

M). Fractions containing the amino acid were combined and concentrated *in vacuo* to yield a white solid which was recrystallized from H_2O/EtOH.

(*R*)-Glutamic acid ((*R*)-**97b**) (23 mg, 50%): mp 185-187°C; >99% e.e. by hplc $[\alpha]_D^{21}$ -13° (c=0.5, H_2O); ν_{max} (KBr): 3434, 3059, 1644, 1514, 1513 cm^{-1}; ^1H nmr (250 MHz, D_2O) δ: 3.68 (apparent t, 1H, 2-CH), 2.45-2.39 (m, 2H, CH_2), 2.06-1.96 (m, 2H, CH_2); ^{13}C nmr (62.8 MHz, D_2O) δ: 179.88 (C=O), 176.60 (C=O), 56.66 (CH), 32.85 (CH_2), 28.32 (CH_2).

(*S*)-Glutamic acid ((*S*)-**97b**) (29 mg, 63%): mp 185-187°C; >99% e.e. by hplc; $[\alpha]_D^{21}$ +8.5° (c=0.5, H_2O) (9.6° for a standard sample, lit (152) $[\alpha]_D^{21}$ +17.7 (H_2O)); ν_{max} (KBr): 3435, 3065, 1642 cm^{-1}; ^1H nmr (250 MHz, D_2O) δ: 3.68 (apparent t, 1H, 2-CH), 2.45-2.39 (m, 2H, CH_2), 2.06-1.96 (m, 2H, CH_2); ^{13}C nmr (62.8 MHz, D_2O) δ: 179.91 (C=O), 176.62 (C=O), 56.67 (CH), 32.88 (CH_2), 28.34 (CH_2).

(*R*)-2-Aminoadipic acid ((*R*)-**97c**) (91 mg, 80%): mp 203-205°C lit. (107) 205-207°C; 94.8% e.e. by hplc; $[\alpha]_D^{21}$ -20.8° (c=1.9, H_2O) lit (153) $[\alpha]_D^{25}$ -25.0° (c=2, H_2O); ν_{max} (KBr): 3455, 1674, 1652 cm^{-1}; ^1H nmr (250 MHz, D_2O/NaOD) δ: 3.48 (ABX, 1H, CH), 2.19 (t, J=7.01, 2H, 5-CH_2), 1.79-1.51 (m, 4H, 3-$C\underline{H}_2$ and 4-$C\underline{H}_2$); ^{13}C nmr (62.8 MHz, D_2O/NaOD) δ: 185.83 (COO^-), 181.52 (COO^-), 57.88 (CH), 39.93 (CH_2), 35.00 (CH_2), 24.61 (CH_2).

(*S*)-2-Aminoadipic acid ((*S*)-**97c**) (23 mg, 52%): mp 194-195°C; >99% e.e. by hplc; $[\alpha]_D^{21}$ +21.2°(c=0.9, 5 M HCl) lit (153) $[\alpha]_D^{25}$ +24.6° (c=2, 5 M HCl); ν_{max} (KBr): 3452, 1673, 1649 cm^{-1}; ^1H nmr (250 MHz, D_2O) δ: 3.59 (ABX, 1H, CH), 2.10 (t, J=7.32, 2H, 5-CH_2), 1.82-1.41 (m, 4H, 3-CH_2 and 4-CH_2); ^{13}C nmr (62.8 MHz, D_2O) δ: 185.45 (COOH), 177.62 (COOH), 57.41 (CH), 39.70 (CH_2), 33.09 (CH_2), 24.32 (CH_2).

(*R*)-2-Aminopimelic acid ((*R*)-**97d**) (28 mg, 53%): mp 225-227°C lit (107) 205-207°C; >99% e.e. by hplc; $[\alpha]_D^{21}$ -24.2° (c=1.0, 5 M HCl) lit (154) $[\alpha]_D^{21}$ -21.0° (c=1 5 M HCl); ν_{max} (KBr): 3440, 2931, 1669, 1642 cm^{-1}; ^1H nmr (D_2O/NaOD, 400 MHz) δ: 3.03 (apparent t, 1H, α-CH), 1.99 (t, J=7.53, 2H, 6-CH_2), 1.45-1.32 (m, 4H, 3-CH_2 and 5-CH_2), 1.15-1.08 (m, 2H, 4-CH_2); ^{13}C nmr (D_2O/NaOD, 100 MHz) δ: 186.54 (C=O), 186.41 (C=O), 58.62 (α-CH), 40.15 (6-CH_2), 37.25 (3-CH_2), 28.53 (5-CH_2), 27.75 (4-CH_2).

(*S*)-2-Aminopimelic acid ((*S*)-**97d**) (18 mg, 70%): mp 219-201°C; >99% e.e. by hplc; $[\alpha]_D^{21}$ 1.0° (c=1, H_2O) lit (154) $[\alpha]_D^{21}$ +21.6° (c=1, 5 M HCl); ^1H nmr (250 MHz, D_2O) δ: 3.62 (apparent t, 1H, α-CH), 2.27 (t, J=7.32, 2H, 6-CH_2), 1.80-1.70 (m, 2H, CH_2), 1.58-1.46 (m, 2H, CH_2), 1.38-1.21(m, 2H, CH_2); ^{13}C nmr (62.8 MHz, D_2O) δ: 181.86 (C=O), 177.45 (C=O), 57.38 (α-CH), 36.43 (6-CH_2), 32.85 (3-CH_2), 26.76 and 26.63 (4-CH_2 and 5-CH_2).

4.4 Synthesis of N^{ε}-Cbz-Lysine (Model Study for the Synthesis of DAP)

4.4.1 N-Cbz-4-Aminobutanol (103)

CbzCl (7.0 mL, 49 mmol) was added to a cooled (0°C) solution of aqueous NaOH (6.5 mL, 5 M) and 4-amino-1-butanol (3.00 mL, 32 mmol). A flocculent white precipitate formed almost immediately. MeOH (12 mL) and H_2O (6.5 mL) were added, and after stirring for 50 min, the reaction mixture was warmed to r.t. and acidified (HCl, 3 M). The precipitate was collected by filtration, and the mother liquors were concentrated and extracted with EtOAc (1 x 10 mL). The extract was dried ($MgSO_4$), concentrated to dryness *in vacuo,* and combined with the initial precipitate. The combined solids were recrystallized from $CHCl_3$:ether (1:1, v/v) to yield a white solid. (6.07 g, 84%): mp 78.5-80.0°C lit (109-111) 80-81°C and 78-79°C; ν_{max} (KBr): 3333, 2956, 1693 cm^{-1}; ^1H nmr (250 MHz, $CDCl_3$) δ: 7.34 (s, 5H, ArCH), 5.09 (s, 2H, $BzCH_2$), 4.98 (br, 1H, NH), 3.64 (d, J=3.05, 2H, $C\underline{H}_2OH$), 3.22 (d, J=5.80, 2H, CH_2N) 1.93 (br, 1H, OH), 1.58 (s, 4H, $C\underline{H}_2C\underline{H}_2CH_2OH$); ^{13}C nmr (62.8 MHz, $CDCl_3$) δ: 156.58 (C=O), 136.58 (ArC), 128.52 (3 x ArCH), 128.11 (2 x ArCH), 66.64 ($BzCH_2$), 62.32 (CH_2OH), 40.78 (CH_2NH), 29.61 and 26.50 ($\underline{C}H_2\underline{C}H_2CH_2OH$); m/z: 223 (11%, $M^{\cdot+}$), 108 (44), 91 (100); hrms calcd. for $C_{12}H_{17}NO_3$: 223.1202; found: 223.1208.

4.4.2 N-Cbz-4-Amino-1-butyl chloride (**104**)

A solution of *N*-Cbz-4-aminobutanol (0.82 g, 3.7 mmol), $SOCl_2$ (1.6 mL, 21.9 mmol) in $CHCl_3$ (16.0 mL) was refluxed for 9 h. The reaction mixture was cooled to r.t. and washed successively with H_2O (3 x 10 mL), $NaHCO_3$ (1 x 10 mL, 5%, w/v), and H_2O (2 x 10 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to yield a colourless oil. (0.62 g, 71%;). ν_{max} (KBr): 3338, 2976, 2875, 1703 cm^{-1}; ^1H nmr (250 MHz, $CDCl_3$) δ: 7.37 (s, 5H, ArCH), 5.13 (s, 2H, $BzCH_2$), 3.64 (m, 4H, $C\underline{H}_2Cl$ and $C\underline{H}_2NH$), 1.58 (m, 4H, $C\underline{H}_2C\underline{H}_2CH_2Cl$); ^{13}C nmr (62.8 MHz, $CDCl_3$) δ: 154.95 (C=O), 137.16 (ArC), 128.44 (2 x ArCH), 128.11 (ArCH), 127.84 (2 x ArCH), 66.58 ($BzCH_2$), 46.26 and 45.82 ($\underline{C}H_2Cl$ and $\underline{C}H_2NH$), 25.76, 24.97 ($\underline{C}H_2\underline{C}H_2CH_2Cl$); hrms calcd. for $C_{12}H_{16}ClNO_2$: 241.0866; found: 241.0869.

4.4.3 N-Cbz-4-Amino-1-butyl tosylate (**105**) (155)

Tosyl chloride (3.28 g, 17.2 mmol) was added to a solution of *N*-Cbz-4-aminobutanol (3.50 g, 15.7 mmol) in dry CH_2Cl_2 (18.6 mL) and dry pyridine (2.48 mL); the solution was stirred at r.t. for 17 h. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed successively with aqueous HCl (2 x 25 mL, 1 M), and H_2O (2 x 25 mL). The organic layer was dried ($CaCl_2$) and concentrated *in vacuo* to yield a colourless oil which crystallized on standing in the cold (-20°C). The solid was recrystallized from EtOAc:hexanes (1.5:1, v/v) 3.80 g,

64%. The mother liquors provided a 2nd crop as white crystals (0.41 g) for an overall yield of 71%. Mp 41-42°C; ν_{max} (KBr): 3333, 3032, 1688, 1540, 1347 cm^{-1}; ^1H nmr (250 MHz, $CDCl_3$) δ: 7.77 (d, J=8.24, 2H, ArCH (Ts)), 7.33 (s, 5H, ArCH (Cbz)), 7.32 (d, J=7.94, 2H, ArCH (Ts)), 5.06 (s, 2H, $BzCH_2$), 4.89 (br, 1H, NH), 4.01 (t, J=6.10, 2H, $C\underline{H}_2OTs$), 3.14 (m, 2H, $C\underline{H}_2NH$), 2.43 (s, 3H, CH_3), 1.71-1.61 (m, 2H, $C\underline{H}_2CH_2OTs$), 1.57-1.46 (m, 2H, $C\underline{H}_2CH_2NH$); ^{13}C nmr (62.8 MHz, $CDCl_3$) δ: 156.46 (C=O), 144.88, 136.54, 132.93 (ArC), 129.93, 128.55, 128.14, 128.09, 127.88 (ArCH), 70.11 ($\underline{C}H_2OTs$), 66.64 ($BzCH_2$), 40.26 ($\underline{C}H_2NH$), 26.02 and 25.99 ($C\underline{H}_2C\underline{H}_2CH_2OTs$), 21.67 ($Ts\underline{C}H_3$); m/z 377 ($M^{\cdot+}$, 7%), 205 (54), 172 (14), 172 (18), 160 (17), 91 (100); hrms calcd. for $C_{19}H_{23}NO_5S$: 377.1296; found: 377.1297.

4.4.4 N-Cbz-4-Amino-1-butyl iodide (**84b**)

N-Cbz-4-Amino-1-butyl tosylate (1.00 g, 2.6 mmol) and NaI (0.98 g, 6.5 mmol) were stirred in refluxing dry acetone (10 mL) for 0.5 h. The reaction mixture was concentrated *in vacuo*. The resulting solid was suspended in CH_2Cl_2 (20 mL) and filtered. The filtrate was washed with aqueous Na_2SO_3 (2 x 20 mL, 5%, w/v), H_2O (2 x 20 mL), dried ($MgSO_4$) and concentrated *in vacuo* to yield a colourless liquid that crystallized in the cold (-20°C). The solid was recrystallized from EtOAc:hexanes to provide white crystals (0.58 g, 65%). The mother liquors provided a 2nd crop as white crystals (0.14 g) for an overall yield

of 82%. Mp 33.0-34.5°C lit (109) 35-37°C; ν_{max} (KBr): 3282, 1172 cm^{-1}; ^1H nmr (250 MHz, $CDCl_3$) δ: 7.35 (s, 5H, ArCH), 5.09 (s, 2H, $BzCH_2$), 4.86 (br, 1H, NH), 3.25-3.16 (m, 4H, ($\underline{CH_2}NH$ and $C\underline{H_2}I$), 1.84 (quint, J=7.32 and 6.71, 2H, $C\underline{H_2}CH_2I$), 1.60 (quint, J=7.32 and 6.71, 2H, $C\underline{H_2}CH_2N$); ^{13}C nmr (62.8 MHz, $CDCl_3$) δ: 156.47 (C=O), 136.57 (ArC), 128.57 (ArCH), 128.16 (ArCH), 66.70 ($BzCH_2$), 39.97 (CH_2NH), 30.95 and 30.56 ($\underline{C}H_2C\underline{H_2}CH_2I$), 6.23 ($CH_2I$); m/z 333 ($M^{\cdot+}$, 8%), 206 (5), 205 (9), 108 (21), 91 (100), 65 (5); hrms calcd. for $C_{12}H_{16}INO_2$: 333.0212; found: 333.0225;

4.4.5 N^6-Cbz-Lysine

N-Cbz-4-Amino-1-butyliodide (1.12 g, 4.01mmol) and finely ground NaOH (0.40 g, 10.0 mmol) were added to a suspension of *(S)*-BPB complex (2.0 g, 4.01 mmol) in DMF (4.0 mL). After stirring for 20 min the reaction was neutralized with AcOH (40 mL, 0.5 M) and extracted with $CHCl_3$ (2 x 25 mL). The combined organic layers were washed with H_2O (5 x 50 mL), dried ($MgSO_4$), and concentrated to yield a red liquid still containing DMF.

The alkylated complex was dissolved in MeOH (22 mL) and HCl (20 mL, 2 M) and refluxed for 10 min. The hydrolysis solution was concentrated to approx. 10 mL *in vacuo* and the precipitate of (*S*)-BPB ligand·HCl was collected and washed with cold H_2O. The filtrate was adjusted to pH 8.0 with aqueous NH_3 (2 M) and washed with $CHCl_3$ (2 x 15 mL). The aqueous layer was acidified with

HCl (3 M) and applied to an Amberlite IR120P (H^+) column. The column was washed with H_2O until the effluent was neutral, and the amino acid was eluted with aqueous NH_3 (0.3 M). 625 mg, 56%; mp 233-235°C; $[\alpha]_D^{21}$ +7.7° (c=1, 2 M HCl) lit (156) $[\alpha]_D$ +14.4° (c=1.6, 2 M HCl); ν_{max} (KBr): 3343, 1693, 1586 cm^{-1}; ^1H nmr (250 MHz, D_2O/DCl) δ: 7.40 (s, 5H, ArCH), 5.07 (s, 2H, $BzCH_2$), 4.08 (apparent t, 1H, α-CH), 3.10 (t, J=6.41, 2H, 6-CH_2), 1.99-1.88 (m, 2H, CH_2), 1.54-1.34 (m, 4H, CH_2CH_2); ^{13}C nmr (62.8 MHz, D_2O/DCl) δ: 173.46 (COOH), 160.26 (N-C=O), 138.22 (ArC), 130.60 (2 x ArCH), 130.16 (ArCH), 129.43 (2 x ArCH), 68.78 ($BzCH_2$), 54.62 (α-CH), 41.99 (6-CH_2), 31.09 (5-CH_2), 30.14 (3-CH_2), 23.30 (4-CH_2).

4.5 Synthesis of *N*-Cbz-5-Hydroxynorvaline Methyl Ester (Route 1)

4.5.1 5-Ethylglutamate

Glutamic acid (15.00 g, 100 mmol) was added to a solution of conc. H_2SO_4 (6.6 mL) in EtOH (148 mL), and the resulting colourless solution was heated to 45°C with stirring for 24 h. Diethylamine (25 mL) dissolved in EtOH (70 mL) was added, and the product was allowed to crystallize at 4°C overnight.

5-Ethyl-(*R*)-glutamate was prepared as above except the product was allowed to crystallize at 4°C for 2 days before collection by filtration and recrystallization from H_2O/EtOH (2.53 g, 71%). mp 181-182°C; $[\alpha]_D^{19}$ -13.8° (c=1.0, H_2O); ^1H nmr (250 MHz, $CDCl_3$) δ: 4.01 (q, J=7.15, 2H, $OC\underline{H}_2CH_3$), 3.61

(apparent t, 1H, α-CH), 2.43-2.36 (m, 2H, 4-CH_2), 2.04-1.94 (m, 2H, 3-CH_2), 1.09 (t, J=7.15, 3H, $OCH_2C\underline{H}_3$); ^{13}C nmr (62.8 MHz, $CDCl_3$) δ: 177.67(C=O), 176.68 (C=O), 64.73 ($O\underline{C}H_2CH_3$), 56.74 (CH), 32.75 (4-CH_2), 28.25 (3-CH_2), 16.06 ($OCH_2\underline{C}H_3$).

(*S*)-5-Ethylglutamate: The crude product was collected by centrifugation (14 749*g*, 20 min) and recrystallized from EtOH (25 mL, 95%, v/v):H_2O (25 mL) to provide a white solid (10.0 g, 58%). mp 183-184°C lit. (157) 194°C; $[\alpha]_D^{19}$ +12.6° (c=1.0, H_2O) lit (157) $[\alpha]_D$ +14° (c=1.0, H_2O); ^1H nmr (250 MHz, $CDCl_3$) δ: 4.00 (q, J=7.17, 2H, $OC\underline{H}_2CH_3$), 3.61 (apparent t, 1H, α-CH), 2.43-2.36 (m, 2H, 4-CH_2), 2.04-1.94 (m, 2H, 3-CH_2), 1.09 (t, J=7.17, 3H, $OCH_2C\underline{H}_3$); ^{13}C nmr (62.8 MHz, $CDCl_3$) δ: 177.65 (C=O), 176.66 (C=O), 64.71 ($O\underline{C}H_2CH_3$), 56.72 ($\underline{C}H$), 32.73 (4-CH_2), 28.23 (3-CH_2), 16.05 ($OCH_2\underline{C}H_3$).

4.5.2 (S)-N-Cbz-5-Ethylglutamate (106)

5-Ethyl-*(S)*-glutamate (9.53 g, 54.4 mmol) was added to a suspension of $NaHCO_3$ (11.4 g, 136 mmol) in H_2O (66 mL). Benzylchloroformate (12.0 mL, 84 mmol) was added slowly over 30 min. The reaction mixture was stirred at r.t. for an additional 90 min and extracted with ether (50 mL). The aqueous layer was adjusted to pH 3.0 with HCl (45 mL, 3 M) and extracted with ether (3 x 100 mL). The ethereal extracts were combined, dried ($MgSO_4$), and concentrated to dryness *in vacuo*. The resulting white solid was recrystallized from CCl_4 (150

mL) to give the product as white crystals (8.56 g, 59%): mp 79-80°C lit. (158) 87°C; ^{1}H nmr (250 MHz, $CDCl_3$) δ: 10.05 (s, 1H, COOH), 7.32 (s, 5H, ArH), 5.71 (d, J=7.93, 1H, NH), 5.10 (s, 2H, $BzCH_2$), 4.41 (ABX, 1H, α-CH), 4.10 (q, J=7.14, 2H, OCH_2CH_3), 2.50-2.39 (m, 2H, 4-CH_2), 2.30-1.94 (m, 2H, 3-CH_2), 1.22 (t, J=7.14, 3H, OCH_2CH_3); ^{13}C nmr (62.8 MHz, $CDCl_3$) δ: 175.92 (COOH), 173.21 (COOEt), 156.29 (C(O)N), 136.04 (ArC), 128.57, 128.27, 128.15 (ArCH), 67.25 ($BzCH_2$), 60.94 (OCH_2CH_3), 53.27 (α-CH), 30.35 (4-CH_2), 27.27 (3-CH_2), 14.14 (OCH_2CH_3); m/z: 309 (M^+, 9%), 220 (12), 130 (13), 108 (29), 92 (14), 91 (100).

4.5.3 Synthesis of (S)-N-Cbz-5-hydroxynorvaline Methyl Ester (**107**)

(S)-N-Cbz-5-Ethylglutamate (5.03 g, 16.2 mmol) was dissolved in dry THF (80 mL) and toluene (25 mL). A slurry of $LiBH_4$ (1.00 g, 45.9 mmol) in THF (50 mL) was added slowly to the reaction mixture. The reaction was heated and THF (50 mL) distilled. The reaction was quenched with the addition of HCl (1 M, 100 mL), and the resulting biphasic solution was separated. The aqueous layer was extracted with EtOAc (2 x 50 mL). The combined organic layers were washed with $NaHCO_3$ (2 x 75 mL, 5%, w/v), dried ($MgSO_4$), and concentrated *in vacuo* to yield a colourless oil (3.96 g) consisting of a complex mixture of products as determined by nmr, hplc and tlc.

A portion (2.15 g) of the oily mixture was dissolved in EtOAc (25 mL) and MeOH (1 mL) and treated with an ethereal solution of CH_2N_2. Ethereal CH_2N_2

was freshly prepared from Diazald using standard procedures (159). After standing overnight at r.t., the reaction mixture was quenched with AcOH (1 M) and concentrated *in vacuo* to yield an oily residue. The residue was dissolved in a minimum volume of CH_2Cl_2 and applied to a silica gel (Merck 70-230 mesh) column (2.5 x 23 cm) and eluted with CH_2Cl_2:EtOAc (5:2). Fractions containing the *N*-Cbz-5-hydroxynorvaline methyl ester also contained a second component as determined by hplc analysis. The two component mixture (100 mg) was applied to a C-18 PrepSep™ (Fisher Scientific) column and eluted with a H_2O/MeOH gradient. Fractions containing *N*-Cbz-5-hydroxynorvaline methyl ester were combined and evaporated *in vacuo* to yield a colourless oil (11.9 mg, <1% from starting ester): ^{1}H nmr (250 MHz, $CDCl_3$) δ: 7.35 (s, 5H, ArCH), 5.50 (d, J=7.94, 1H, NH), 5.11 (s, 2H, $BzCH_2$), 4.42 (AB$\underline{X}$, 1H, α-CH), 3.74 (s, 3H, OCH_3), 3.66 (t, J=6.10, 2H, $C\underline{H}_2OH$), 2.02-1.59 (m, 5H, $C\underline{H}_2C\underline{H}_2CH_2O\underline{H}$); ^{13}C nmr (62.8 MHz, $CDCl_3$) δ: 172.90 ($\underline{C}OOCH_3$), 156.02 (N-C=O), 136.18 (ArC), 128.54, 128.22, 128.12, (ArCH), 67.05 ($BzCH_2$), 62.05 ($\underline{C}H_2OH$), 53.54 (α-CH), 52.45 (OCH_3), 29.36 (CH_2), 28.11 (CH_2); m/z: 281 (M^+, 5%), 222 (11), 178 (9), 108 (16), 91 (100).

4.6 Attempted Synthesis of *N*-Cbz-5-Hydroxynorvaline methyl ester (Route 2)

4.6.1 1,5-Dimethylglutamate

Conc. H_2SO_4 (2.6 mL) was added to a solution of glutamic acid (6.00 g, 40.8 mmol) in dry MeOH (60 mL) and the solution was stirred at reflux overnight. The reaction mixture was concentrated *in vacuo* to a colourless oil, which was dissolved in $CHCl_3$ (50 mL) and extracted with aqueous $NaHCO_3$. Solid $NaHCO_3$ was added to the aqueous layer to maintain an alkaline pH, and it was extracted with $CHCl_3$ (1 x 50 mL). The combined organic layers were washed with H_2O (50 mL), dried ($MgSO_4$), and concentrated *in vacuo* to yield a colourless oil (4.06 g, 57%): ^{1}H nmr (250 MHz, $CDCl_3$) δ: 3.73 (s, 3H, α-OC$\underline{H}_3$), 3.68 (s, 3H, γ-OC$\underline{H}_3$), 3.49 (AB$\underline{X}$, 1H, α-CH), 2.48 (t, J=7.48, 2H, C$\underline{H}_2$), 2.15-2.02 and 1.91-1.77 (m, 1H, CH_2), 1.52 (s, 2H, NH_2); ^{13}C nmr (62.8 MHz, $CDCl_3$) δ: 176.1 (C=O), 173.6 (C=O), 53.7 (α-$\underline{C}$H), 52.1 (α-OC$\underline{H}_3$), 51.7 (γ-OC$\underline{H}_3$), 30.3 (4-CH_2) 29.7 (3-CH_2); m/z: 175 (M^+, 0.8%), 144 (18), 143 (10), 116 (100), 88 (11), 84 (62).

4.6.2 N-Trityl-1,5-Dimethylglutamate (**108**)

Tritylchloride (9.80 g, 35 mmol) was added to a solution of dimethyl-(*S*)-glutamate (4.06 g, 23.2 mmol) in $CHCl_3$ (100 mL) and Et_3N (4.6 mL). The reaction mixture was stirred at r.t. overnight and washed with H_2O (2 x 50 mL), aqueous citric acid (2 x 50 mL, 5%, w/v), aqueous $NaHCO_3$ (2 x 50 mL, 5%, w/v),

and H_2O (2 x 50 mL). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo* to yield a mixture of a brown solid and oil. Tritylalcohol was removed by repeated precipitation with dry MeOH. The methanolic mother liquors were pooled, concentrated to dryness and recrystallized from MeOH to provide the product as a white solid (4.35 g, 45%). mp 68-73°C lit. (128) 94-96°C; ν_{max} (KBr): 3445, 3037, 2951, 1734 cm^{-1}; ^1H nmr (250 MHz, $CDCl_3$) δ: 7.50-7.13 (m, ArC<u>H</u>, 15H), 3.67 (s, 3H,OCH_3), 3.41 (AB<u>X</u>, 1H, α-C<u>H</u>), 3.14 (s, 3H,OCH_3), 2.68 (d, J=10.68, 1H, NH), 2.56-2.30 (m, 2H, 3-CH_2), 2.13-2.04 (m, 2H, 4-CH_2); ^{13}C nmr (62.8 MHz, $CDCl_3$) δ: 174.83 (C=O), 173.48 (C=O), 145.77 (Ar<u>C</u>), 128.76 (2 x Ar<u>C</u>H), 127.81 (2 x Ar<u>C</u>H), 126.43 (Ar<u>C</u>H), 71.11 (Ph_3<u>C</u>), 55.19 (α-<u>C</u>H), 51.69 (OCH_3), 51.58 (OCH_3), 30.46 (CH_2), 29.89 (CH_2); hrms calcd. for $C_{26}C_{27}NO_4$: 417.1940; found: 417.1939; m/z: 417 ($M^{+\cdot}$, 3%), 358 (8), 340 (50), 260 (23), 243 (100), 183 (38), 165 (37) The product still contained tritylalcohol as a minor impurity.

4.6.3 N-Trityl-1-Methylglutamate (109) (129)

A saturated solution of LiOH in MeOH:H_2O (9:1, 42.0 mL) was added to *N*-trityl-dimethyl-*(S)*-glutamate (1.77 g, 4.24 mmol) in MeOH (32 mL), and the resulting solution was stirred for 6.5 h at r.t. The reaction was concentrated *in vacuo* and the residue was partitioned between H_2O (75 mL) and EtOAc (75 mL). The aqueous layer was acidified to pH 6.0 with aqueous citric acid (5%, w/v) and

extracted with EtOAc (2 x 50 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to yield a yellow oil which crystallized on standing at r.t. (2.18 g, quant.). mp 123-125°C lit. (160) 140-141°C; ν_{max} (KBr): 3022, 2948, 1740, 1705 cm^{-1}; ^1H nmr (250 MHz, $CDCl_3$) δ: 7.50-7.13 (m, 15H, ArC<u>H</u>), 3.44 (t, J=6.25, 1H, α-C<u>H</u>), 3.16 (s, 3H,OCH_3), 2.60-2.35 (m, 2H, 3-CH_2), 2.12-2.03 (m, 2H, 4-CH_2); ^{13}C nmr (62.8 MHz, $CDCl_3$) δ: 179.25 (C=O), 174.80 (C=O), 145.64 (Ar<u>C</u>), 128.74 (2 x Ar<u>C</u>H), 127.84 (2 x Ar<u>C</u>H), 126.48 (Ar<u>C</u>H), 71.21 ($Ph_3\underline{C}$), 55.14 (α-CH), 51.68 (OCH_3), 30.05 (CH_2), 29.95 (CH_2); m/z: 403 ($M^{\cdot+}$, 2.6%), 326 (25), 260 (12), 244 (17), 243 (47) 241 (6), 197 (14), 183 (10), 165 (20), 162 (24), 154 (8), 144 (11), 143 (23), 105 (20), 84 (100).

4.6.4 N-Trityl Proline Methyl Ester

BH_3 in THF (Aldrich, 2.49 mL,1 M, 2.49 mmol) was added to a flask containing *N*-trityl-1-methyl-*(S)*-glutamate (1.00 g, 2.49 mmol) at 0°C *via* a rubber septum. An additional portions of BH_3/THF (1 mL, 1 M) were added after stirring for 0.5 h and 3 h. After stirring for a total of 6 h at 0°C, $NaHCO_3$ (10 mL, 5%, w/v) was added and the product was extracted with EtOAc (2 x 15 mL). The combined EtOAc layers were washed with H_2O (2 x 10 mL), dried ($MgSO_4$), and concentrated *in vacuo* to yield *N*-trityl-proline methyl ester as a white solid. The product was recrystallized from MeOH (0.63 g, 65%). mp 115-117°C lit. (128) 118-120°C; $[\alpha]_D^{22}$ -23.7° (c=1.4, $CHCl_3$); ν_{max} (KBr): 1736 cm^{-1}; ^1H nmr (250 MHz,

$CDCl_3$) δ: 7.57 (d, J=7.32, 6H, ArCH), 7.27-7.12 (m, 9H, ArCH), 3.90 (ABX, 1H, α-CH), 3.68 (s, 3H, OCH_3), 3.44-3.37 (m, 1H, 5-CH_2), 2.89-2.79 (m, 1H, 5-CH_2), 1.63-1.45 (m, 2H, 4-CH_2), 1.09-0.86 (m, 2H, 3-CH_2); ^{13}C nmr (62.8 MHz, $CDCl_3$) δ: 177.21 (C=O), 144.69 (ArC), 129.23, 127.64, 126.15 (ArCH), 77.39 (Ph_3C), 62.73 (α-CH), 51.57 (OCH_3), 49.91 (5-CH_2), 31.22 (3-CH_2), 24.26 (4-CH_2); m/z: 371 ($M^{\cdot+}$, 2%), 294 (5), 244 (11), 243 (100), 165 (11).

4.6.5 Trifluoroacetate Salt of Proline Methyl Ester

TFA (0.115 mL, 1.5 mmol) was added to a cooled solution (0°C) of *N*-tritylproline methyl ester (55.7 mg, 0.143 mmol) in $CHCl_3$:MeOH (0.20 mL, 1:1, v/v) and the resulting solution was stirred for 2 h. The reaction was concentrated and residual TFA was removed by repeated evaporation *in vacuo* of portions of ether. The residue was dissolved in ether (0.5 mL), and extracted into H_2O (3 x 0.5 mL). The combined aqueous layers were concentrated to yield a colourless oil (38.7 mg, 99%). ^1H nmr (250 MHz, $CDCl_3$) δ: 4.47 (ABX, 1H, α-CH), 3.83 (s, 3H, OCH_3), 3.50-3.33 (m, 2H, CH_2N), 2.50-2.36 (m, 1H, 3-CH), 2.24-1.99 (m, 3H, 3-CH, 4-CH_2); ^{13}C nmr (62.8 MHz, $CDCl_3$) δ: 173.16 (Pro C=O), 165.32 (TFA C=O), 116.7 (F_3C), 62.20 (α-CH), 56.45 (CH_2N), 48.96 (OCH_3), 30.78 and 25.97 (3-CH_2, 4-CH_2). This product was immediately taken to the next step.

4.6.6 N-Cbz-Proline Methyl Ester

A cooled (0°C) solution of the TFA salt of proline methyl ester (38.7 mg, 0.142 mmol) in EtOAc:H_2O (2 mL, 2:5, v/v) was made alkaline (pH paper) by adding solid $NaHCO_3$. Benzylchloroformate (0.020 mL, 0.142 mmol) was added, and the solution was stirred for 1.5 h at 0°C. The aqueous layer was separated and washed with EtOAc (1 x 1 mL). The combined organic layers were washed with H_2O (1 x 1 mL), dried ($MgSO_4$) and concentrated *in vacuo* to yield a colourless oil. Column chromatography (silica gel, system B) provided the product as a colourless oil (33.1 mg, 82%). ^1H nmr (all peaks, except OCH_3 singlets, appear as complex multiplets due to the presence of rotamers, 250 MHz, $CDCl_3$) δ: 7.37-7.28 (m, 5H, ArCH), 5.21-5.02 (m, 2H, $BzCH_2$), 4.42-4.32 (m, 1H, α-CH), 3.74 and 3.58 (s, 3H total, OCH_3), 3.68-3.43 (m, 2H, CH_2), 2.28-2.16 (m, 1H, CH_2), 2.02-1.85 (m, 3H, CH_2); ^{13}C nmr (62.9 MHz, $CDCl_3$) δ: 173.26 (C=O), 154.89 (C(O)N), 136.74, 128.40, 127.90, 127.79 (ArC), 67.02 ($BzCH_2$), 59.20 and 58.86 (rotamers, α-CH), 52.22 and 52.04 (rotamers, OCH_3), 46.93 and 46.43 (rotamers, 5-CH_2), 30.93 and 29.92 (rotamers, 3-CH_2), 24.34 and 23.55, (rotamers, 4-CH_2); m/z: 263 ($M^{\cdot+}$, 18%), 204 (20), 160 (44), 92 (9), 91 (100).

4.7 Attempted Synthesis of N-Cbz-5-Hydroxynorvaline methyl ester (Route 3)

4.7.1 N-Cbz-5-oxo-4-Oxazolidine Propionic Acid (**114**)

A mixture of commercially available *N*-Cbz-glutamic acid (1.70 g, 6.0 mmol), paraformaldehyde (12.3 mg, 0.41 mmol), *p*-toluenesulfonic acid (0.078 g, 0.45 mmol) and toluene (42 mL) was refluxed (7.5 h) using a Dean-Stark apparatus for the azeotropic removal of water. The product was extracted into $NaHCO_3$ (2 x 50 mL, 5%, w/v). The combined aqueous layers were acidified to pH 2.2 with aqueous HCl (3 M), extracted with EtOAc (3 x 20 mL), dried ($MgSO_4$) and concentrated *in vacuo* to yield a light yellow oil (91%). ^{1}H nmr (250 MHz, $CDCl_3$) δ: 7.36 (s, 5H, ArH), 5.53 (br, 1H, N-CH_2O), 5.22 (d, J=4.57, 1H, N-CH_2O), 5.18 (s, 2H, BzCH_2), 4.40 (apparent t, splitting of 6.11 and 5.79 Hz, 1H, α-CH), 2.57-2.42 (m, 2H, CH_2), 2.38-2.10 (m, 2H, CH_2); ^{13}C nmr (62.8 MHz, $CDCl_3$) δ: 177.39 (COOH), 171.77 (α-C=O), 153.15 (N-C=O), 135.20 (ArC), 128.73, 128.69, 128.38 (ArCH), 77.92 (N-CH_2O), 68.23 (BzCH_2), 53.94 (α-CH), 29.09 (CH_2), 25.74 (CH_2).

4.7.2 N-Cbz-5-oxo-4-Oxazolidine Propanol (**115**)

BH_3 in THF (Aldrich, 9 mL, 1 M, 9 mmol) was added to a flask containing *N*-Cbz-5-oxo-4-oxazolidine propionic acid (0.89 g, 3.0 mmol) at -30°C *via* a rubber septum. After stirring for 1 h at -30°C, the reaction was warmed to 0°C

and stirred for an additional 4.5 h. The excess BH_3 was quenched at -30°C by the addition of methanolic acetic acid (10%, 3 mL). The reaction mixture was concentrated *in vacuo*. The residue was dissolved in EtOAc (6 mL) and washed with HCl (1 x 3 mL, 1 M), H_2O (2 x 4 mL), $NaHCO_3$ (1 x 4 mL, 5%, w/v), H_2O (1 x 4 mL) dried ($MgSO_4$), and concentrated *in vacuo* to yield the crude product as a light yellow oil (0.91 g, 105%). ν_{max} (neat) 3330, 2959, 1800, 1714 cm^{-1}; ^1H nmr (250 MHz, $CDCl_3$) δ: 7.36 (s, 5H. ArH), 5.52 (br, 1H, one half of N-CH_2-O), 5.22 (d, 1H, one half of N-CH_2-O), 5.18 (s, 2H, BzCH_2), 4.40 (apparent t splittings of 5.80 and 5.79 Hz, 1H, 4-CH), 3.64 (t, 2H, CH_2), 2.48-2.11 (m, 4H, C$\underline{H_2}$C$\underline{H_2}$OH); ^{13}C nmr (62.8 MHz, $CDCl_3$) δ: 171.56 (C=O), 155.22 (C=O), 72.46 (CH_2), 71.57 (CH_2), 69.45 (CH_2), 56.24 (4-CH), 25.52 (CH_2), 22.52 (CH_2).

4.7.3 N-Cbz-5-Hydroxynorvaline Methyl Ester

A solution of *N*-Cbz-5-oxo-4-oxazolidine propanol (0.85 g) in methanolic NaOMe (60 mL, 0.2 M) was stirred at 0°C for 2.5h. The reaction was quenched with the addition of HCl (7 mL, 1.5 M) and concentrated to dryness *in vacuo*. The residue was partitioned between $CHCl_3$ (10 mL) and H_2O (5 mL), The resulting biphasic solution was separated and the organic layer washed with H_2O (1 x 5 mL), dried ($MgSO_4$) and concentrated *in vacuo* to yield a colourless oil (0.19 g, 23%). ^1H nmr (250 MHz, $CDCl_3$) δ: unresolved undistinguishable multiplets; ^{13}C nmr (62.8 MHz, $CDCl_3$) δ: 173.14 (C=O), 156.02 (N-C=O), 136.20 (ArC), 128.58, 128.26,

128.12 (ArCH), 67.06 ($BzCH_2$), 61.79 (CH_2OH), 53.54 (α-CH), 52.49 (OCH_3), 29.07 (CH_2), 28.08 (CH_2); Unidentified major component(s): 174.83, 172.48, 156.44, 156.26, 136.08, 128.55, 128.19, 67.64, 67.15, 53.67, 49.62, 28.89, 27.83, 25.45, 21.05.

4.8 Synthesis of 4,5-Dihydroxynorvaline

4.8.1 2,2-Dimethyl-1,3-dioxolan-4-ylmethyliodide (143)

NaI (524 mg, 3.5 mmol) was added to a solution of 2,2-dimethyl-1,3-dioxolan-4-ylmethyl *p*-toluenesulfonate (250 mg 0.87 mmol) in acetone (2.4 mL) and the resulting solution was refluxed overnight. The reaction mixture was diluted with CH_2Cl_2 (4 mL) and washed with aqueous $NaSO_3$ (1 x 4 mL, 5%, w/v), and H_2O (2 x 4 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to yield a colourless oil.

(R)-2,2-Dimethyl-1,3-dioxolane-4-methyliodide ((*R*)-**85b**) (152 mg, 68%). ^1H nmr (400 MHz, $CDCl_3$) δ: 4.30-4.24 (m, 1H, CH), 4.16-4.12 (m, 1H, CH_2O), 3.80-3.76 (m, 1H, CH_2I), 3.27-3.24 (m, 1H, CH_2I), 3.16-3.12 (m, 1H, CH_2O); ^{13}C nmr (100 MHz, $CDCl_3$) δ: 110.29 (C), 75.50 (CH), 69.46 (CH_2), 27.02 and 25.49 (2 x CH_3), 6.62 (CH_2I); m/z: 227 ($M^{\cdot+}$-15, 46%), 213 (5), 171 (5), 56 (14), 42 (100).

(S)-2,2-Dimethyl-1,3-dioxolane-4-methyliodide ((*S*)-**85b**) (161 mg, 71%). ^{1}H nmr (400 MHz, $CDCl_3$) δ: 4.30-4.24 (m, 1H, CH), 4.16-4.12 (m, 1H, CH_2O), 3.80-3.76 (m, 1H, CH_2I), 3.27-3.23 (m, 1H, CH_2I), 3.16-3.11 (m, 1H, CH_2O); ^{13}C nmr (100 MHz, $CDCl_3$) δ: 110.29 (C), 75.50 (CH), 69.46 (CH_2), 27.02 and 25.49 (2 x CH_3), 6.62 (CH_2I); m/z: 227 (M^+-15, 60%), 127 (6), 42 (100).

4.8.2 4,5-Dihydroxynorvaline

2,2-Dimethyl-1,3-dioxolane-4-methyliodide (138 mg, 0.54 mmol) in a solution of DMF (1.0 mL) was added to a suspension of finely ground NaOH (53 mg, 1.34 mmol) and (*S*)-BPB-complex (261 mg, 0.54 mmol) in DMF (1.0 mL). After stirring for 0.5 h at r.t., the reaction was neutralized with AcOH (0.5 M) and extracted with $CHCl_3$ (2 x 2.0 mL). The combined organic layers were washed with H_2O (5 x 3.0 mL), dried ($MgSO_4$) and concentrated to dryness *in vacuo.* The residue was purified by column chromatography on alumina. The column was washed with CH_2Cl_2 and the desired product eluted $CHCl_3$. Fractions containing the desired product were pooled and concentrated *in vacuo* to yield a red oil.

Complex Alkylated with 2,2-Dimethyl-1,3-dioxolane-4-methyliodide

(*S*,2*S*,4*R*)-Complex ((*S*,2*S*,4*R*)-**116**) (117 mg, 43%): ^{13}C nmr (62.8 MHz, $CDCl_3$) δ: 180.42, 178.72, 170.54, 142.36, 133.60, 133.23, 132.29, 131.58, 129.99,

129.12, 129.05, 128.92, 127.73, 127.21, 126.29, 123.82, 120.78, 109.04, 72.28, 70.16, 68.55, 67.93, 63.12, 57.20, 39.44, 30.74, 26.93, 25.75, 23.99.

(*S*, 2*S*, 4*S*)-Complex ((*S*,2*S*,4*S*)-**116**): (153 mg, 45%): ^{13}C nmr (100 MHz, $CDCl_3$) δ: 180.34, 178.70, 170.73, 133.59, 133.32, 133.27, 132.24, 132.15, 131.54, 129.79, 129.26, 129.07, 129.03, 128.87, 128.13, 127.61, 127.37, 123.66, 120.71, 71.96, 70.30, 69.30, 67.96, 63.14, 57.21, 40.81, 30.74, 26.85, 25.33, 24.02.

4.9 Synthesis of *threo*-β-(*p*-Nitrophenyl)serine

*4.9.1 (R, 2S, 3R)-Complex (R, 2S, 3R-**118**)* (144)

p-Nitrobenzaldehyde (0.52 g, 3.5 mmol) was added to a stirred solution of (*R*)-BPB-complex (0.56 g, 1.1 mmol) in methanolic MeONa (3 mL, 1 M). The reaction mixture was stirred at ambient temperature for 45 min and quenched by slow addition into cold aqueous acetic acid (15 mL, 0.5 M). The precipitate was collected and purified by flash silica gel chromatography (150). A portion of the product was recrystallization from acetone to provide red crystals (0.66 mg, 90%): mp 157-160°C lit (144) 122-128°C; ^{13}C nmr (62.8 MHz, DMSO-d^6) δ: 180.27 (C=O), 173.27 (C=O), 169.91 (C=N), 149.28, 146.24, 142.91, 134.72 (ArCH), 134.11 (ArC), 132.63, 131.57, 131.36, 129.98, 129.51, 129.37, 128.43,

128.28, 126.52 (ArCH), 126.11 (ArC), 123.42, 123.27, 122.62, 120.03 (ArCH), 75.72, 73.64, 69.72 (CH), 62.49, 57.56, 30.72, 22.73 (CH_2).

4.9.2 L-threo-β-(p-Nitrophenyl)serine (L-*threo*-**79**) (54)

To a stirred solution of (*S*)-BPB-complex (0.47 g, 0.92 mmol) in methanoic NaOMe (1 M, 9 mL) was added *p*-nitrobenzaldehyde (0.44 g, 2.9 mmol) followed by an additional portion of methanolic NaOMe (1 M, 9.8 mL), and the resulting solution was allowed to stir for 30 min at 22°C. The reaction was quenched by the addition of hot (50°C) HCl (5.5 M, 50 mL), and stirred until the red colour had disappeared. The solution was concentrated to ~10 mL *in vacuo*, adjusted to pH 9.0 with aqueous NH_3 (5%, w/v), and extracted with $CHCl_3$ (4 x 50 mL). The aqueous layer was applied to an Amberlite IR120 (H^+) column (2.5 x 50 cm), and the amino acids were eluted with aqueous NH_3 (5%, w/v) after the column was washed with H_2O, (1 L). Fractions containing product were combined and lyophilized. The resulting solid was redissolved in H_2O (1.5 mL), applied to an Amberlite XAD-2 column (1.5 x 27 cm), and eluted with water. Fractions containing product were lyophilized to yield an off white powder (14.3 mg, 7%). $[\alpha]_D^{21}$ +13.12° (c=0.64, 0.5 M HCl); ^{1}H nmr (250 MHz, D_2O) δ: 8.30 (d, J=7.32, 2H, ArCH), 7.69 (d, J=7.94, 2H, ArCH), 5.41 (d, J=3.97, 1H, β-CH), 3.97 (d, J= 4.72, 1H, α-CH); ^{13}C nmr (62.8 MHz, D_2O) δ: 171.00 (COOH),147.12 (ArC), 146.50 (ArC), 126.64 (ArCH), 123.56 (ArCH), 70.22 (β-CH), 59.97 (α-CH).

APPENDIX 1

Modification of Computer Program for Integration of Chromatograms

The original computer program used for the integration of hplc chromatographic data was rewritten to include convenience features that would reduce the time spent manipulating chromatographic data. The major changes resulting from the most recent modifications are detailed below. The program was renamed Display2 to reflect the changes.

Original code by Brian Millier (1988)

- Compiled using Borland Turbo Pascal version 3.0

Modified by Kevin C. Smith (August 1990)

- Contains HP III printer driver
- Quick integration
- Automatic file call up

Rewritten by Danny T. Davis (January 1993)

- Automatic monitor detection:

Automatic monitor detection allows program to automatically detect the type of monitor in use and supports all of the popular video formats including SVGA. The original version of the program was

only capable of operating with Hercules and Hercules compatible video adapter cards.

- Full colour display

The new Display2 is now capable of full colour display but it also works with monochrome monitors.

- Fully mouse driven

All functions of the program are available by using a mouse. All functions can still be accessed by using the function keys thereby providing full downward compatibility with the original version. The original program was not able to utilize a mouse.

- Undo feature

Pressing the right mouse button cancels the drawing of integration lines and the drawing of zoom boxes.

- Smart integration file creation

Integration files are not created until the first peak is integrated thus avoiding the creation of empty files when simply viewing chromatograms.

- Integration file appending

It is now possible to append current integration data to an existing integration file.

- Integration file viewing and renaming

Existing integration files can now be viewed from within the program to allow the user to decide whether to rename, overwrite or append the existing integration file.

- ASCII file export feature

The ASCII export feature allows the user to create an ASCII text file consisting of two columns containing the time and signal data thus allowing the user to output publication quality chromatograms from a suitable graphics program.

- Multiple page input capability

The modification made by Kevin Smith (1990) allowed the user to input a series of chromatograms differing only by the last character of their filename. The program is now capable of accepting multiple series of chromatograms for batchwise integrations, printing or exporting.

- Vertical line draw feature

The user is now able to 'drop' a line to the baseline to allow more accurate integration of peaks not fully resolved. The feature was not available in the original program.

- File name displayed on-screen

The name of the file being processed is displayed onscreen along with the detector sensitivity during the acquisition.

- User friendly code

All constants are contained in a separate file so that changing general aspects of the program, *e.g.*, screen colours or mouse sensitivity, are readily available to a novice Pascal programmer.

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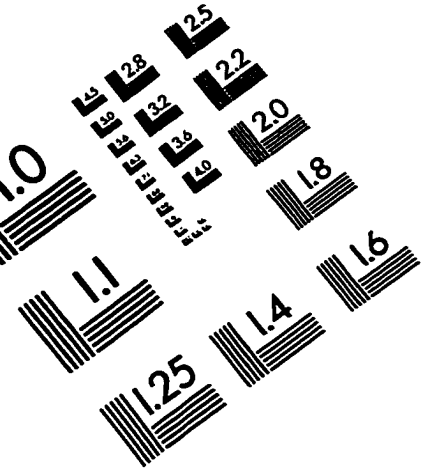
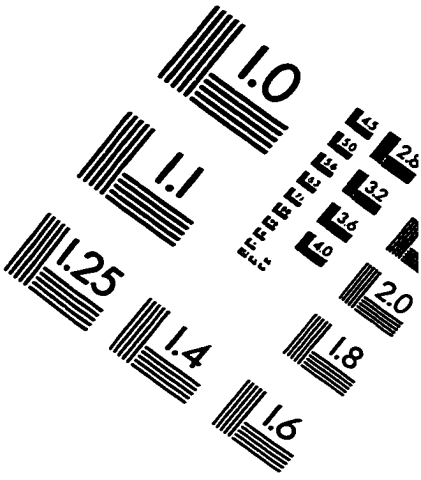
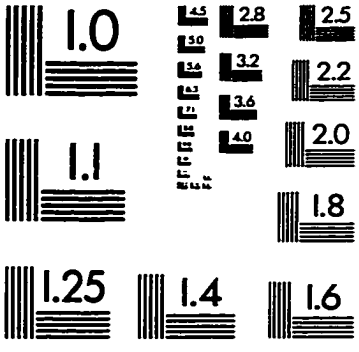
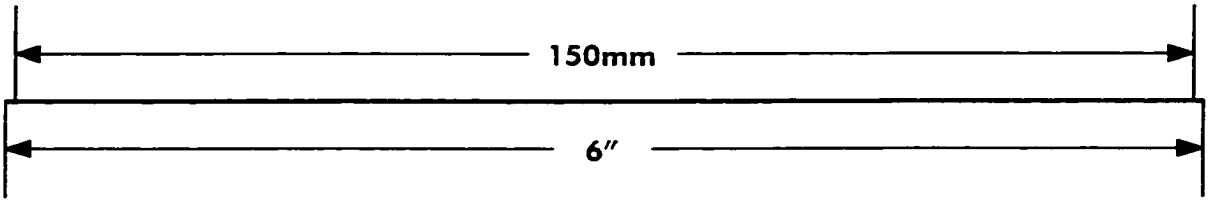
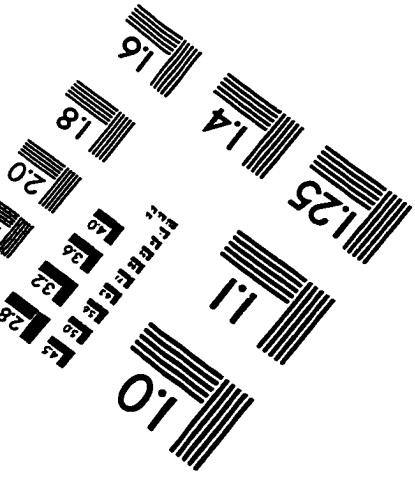
IMAGE EVALUATION TEST TARGET (QA-3)

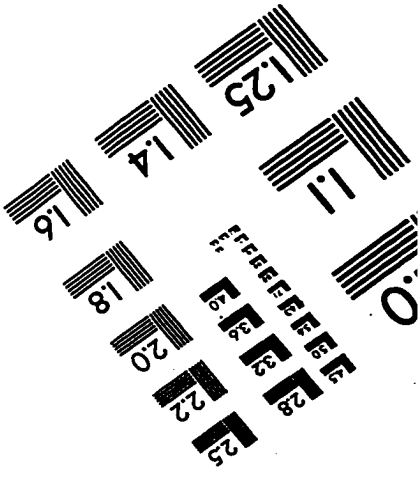












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