Solution and Solid Phase Synthesis of Unusual α-Amino Acids From Ortho Ester Protected Synthons

By

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Abstract

Non-proteinacous amino acids are important components of numerous biologically active compounds. As a consequence, there is much interest in the development of straightforward routes for their synthesis. A novel strategy for the synthesis of several classes of non-proteinacous α-amino acids from a variety of α-amino acids is presented in this thesis.

A general strategy of protecting the α-carboxyl group of serine, threonine, aspartic acid and glutamic acid as a cyclic ortho ester (OBO), sufficiently reduces the acidity of the α-proton to allow the use of a variety of basic reagents minimizing racemization at the α-proton.

The sidechain hydroxyl group in Boc/OBO protected serine and threonine is oxidized to the corresponding aldehyde and ketone without enolization. Grignard and Reformatsky additions produced predominantly *threo*-β-hydroxy-α-amino acids in good selectivity with no racemization. Adaptation of the methodology onto the solid phase also gave *threo*-β-hydroxy-α-amino acids albeit with some racemization

The addition of various electrophiles to the enolate of *N*-Cbz-α-OBO-γ-methyl ester glutamic acid gave the 2*S*,4*S* stereoisomers with excellent selectivity and good yield. Alkylation, acylation, Aldol and Claisen reactions and the electrophilic addition of various heteroatoms is described.

Electrophilic additions to the enolate of *N*-Cbz-α-OBO-γ-methyl ester aspartic acid gave the more difficult to synthesize 2*S*,3*S* stereoisomer with varying degrees of stereoselectivity and yields depending on the electrophile.

The stereochemistry of addition for the enolate chemistry is also explored.

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Finally, I cannot begin to express my gratitude to my partner, Jennifer West for her support, encouragement, incredible patience and wit when I needed it. Thank you.

To Julian.

It is not because things are difficult

that we dare not venture. It is because

we dare not venture that they are difficult.

Seneca

Writing a book is an adventure.

To begin with it is a toy and an amusement, and then it becomes a mistress,

and then it becomes a master, and then it becomes a tyrant,

and the last phase is that just as you are about to be reconciled to your servitude

you kill the monster.

Nobel Laureate, Sir Winston Churchill after writing

his monumental six volume history of World War II

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List of Abbreviations

^{1}H-NMR	proton nuclear magnetic resonance
^{13}C-NMR	carbon nuclear magnetic resonance
ABO	2,7,8-trioxabicyclo[3.2.1]octane
Ac	acetyl
ACC	1-Aminocyclopropane-1-carboxylate synthase
AD	asymmetric dihydroxylation
AE	asymmetric epoxidation
Aib	α-aminoisobuytric acid
Ala	alanine
AMPA	2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propanoic acid
Arg	arginine
Asn	asparaginine
Asp	aspartic acid
9-BBN	9-borabicyclo[3.3.1]nonane
BEMP	2-*tert*-butylamino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine
BINAP	binapthol
BMI	*bis*-*N*-methyl imidazolidine
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
BSA	*N*,*O*-bis(trimethysilyl)acetamide
Bu	butyl
CAD	carbamoylphosphate synthetase II, aspartate transcarbamoylase, dihdroorotase complex
Calcd	calculated
Cbz	benzyloxycarbonyl
COD	1,5-cyclooctadiene
CNS	central nervous system
CSA	camphor sulfonic acid

Cys	cysteine
d	doublet
DAST	diethylaminosulfur trifluoride
DBAD	di-*tert*-butyl azodicarboxylate
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	*N,N*-dicyclohexylcarbodiimide
de	diastereomeric excess
DEAD	diethyl azodicarboxylate
dec	decomposed
DIBAL-H	diisobutylaluminium hydride
DIPAMP	((*R,R*)-1,2-ethanediylbis[(o-methoxyphenyl)phenylphosphine])
DIPEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMDO	dimethyldioxirane
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DTBAD	di-*tert*-butylazodicarboxylate
EAA	excitatory amino acid
ee	enantiomeric excess
eq	equivalence
ESI-MS	electrospray ionization mass spectrometry
Et	ethyl
EtOAc	ethyl acetate
EtOH	ethanol
FAB	fast atom bombardment
Fmoc	9-fluorenylmethoxycarbonyl
FT	fourier transform
GC	gas chromatography
GDH	glutamate dehydrogenase
Gla	γ-carboxyglutamic acid
Gln	glutamine

Glu	glutamic acid
Gly	glycine
GOT	glutamic oxalacetic transaminase
h	hour
HMPA	hexamethylphosphoramide
HPLC	high performance/pressure liquid chromatography
HRMS	high resolution mass spectrometry
LDA	lithium diisopropylamide
Leu	leucine
iGlu	ionotropic glutamate receptor
IR	infrared
KA	kainic acid
LiHMDS	lithium hexamethyldisilazane
m	multiplet
M	Molarity
MAS	magic angle spinning
MCPBA	*m*-chloroperoxybenzoic acid
Me	methyl
MeBmt	(*R*)-4[(*E*)-2-butenyl]-4-*N*-dimethyl-L-threonine
MeOH	methanol
Met	methionine
mGlu	metabotrophic glutamate receptor
mhpd ester	2-methyl-2-hydroxymethyl-1,3-propanediol
min	minutes
mp	melting point
n-BuOH	*n*-butanol
NBS	*N*-bromosuccinimide
NFSI	*N*-fluorobenzene sulfonamide
NMDA	*N*-methyl-D-aspartic acid
NMM	*N*-methyl morpholine
NMO	*N*-methylmorpholine-*N*-oxide

NMP	*N*-methyl pyrrolidinone
Nu	nucleophile
OBO ester	2,6,7-trioxabicyclo[2.2.2]octane ortho ester
PEG	polyethyleneglycol
Ph	phenyl
Phe	phenylalanine
PhFl	9-(9-phenylfluorenyl)
Phth	phthaloyl
PLP	pyridoxal phosphate
*p*NB	*p*-nitrobenzoyl
PPTS	pyridinium *p*-toluenesulfonate
Pr	propyl
Pro	proline
PTSA	*p*-toluene sulfonic acid
pyr	pyridine
q	quartet
RBF	round bottom flask
R_f	retention factor
rt	room temperature
SAR	structure activity relationship
Ser	serine
SPOC	solid-phase organic chemistry
t	triplet
TBAF	tetrabutylammonium flouride
TBDMS	*tert*-butyl dimethylsilyl
*t*Bu	*tert*-butyl
Tf	triflate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
THP	tetrahydropyran
Thr	threonine

TMS	trimethylsilyl
TMSE	2-(trimethylsilanyl)-ethyl
TMSI	iodotrimethylsilane
TPAP	tetrapropyl ammonium perruthenate
Tr	trityl
TRAP	(*S*,*S*)-2,2"-bis[(R)-1-(dialkylphosphophino)ethyl]-1,1'-biferrocene
Trp	tryptophan
Ts	*p*-toluenesulfonyl
Tyr	tyrosine
Val	valine
YADH	yeast alcohol dehydrogenase

Chapter One

Introduction: The Synthesis of α-Amino Acids

1.1 General Introduction

α-Amino acids represent a vast array of chiral molecules with considerable structural diversity and importance, both as inherently biologically active compounds and as constituents of larger biomolecules. α-Amino acids are ubiquitous in nature, where 20 common "proteinogenic" amino acids are the standard building blocks for the peptides and proteins that form the structural basis of life. However, over a thousand of the so-called "non-proteinogenic" α-amino acids have been isolated from a variety of natural sources.[1,2] The enormous interest in α-amino acids can be attributed to two factors: their biological activity and their relevance to chemical asymmetric synthesis.

Many non-proteinaceous amino acids (defined as "those amino acids which are not found in protein main chains either for lack of a specific transfer RNA and codon triplet, or because they do not arise from protein amino acids by post-translational modifications")[3] or compounds containing them have been found to possess biological activity. These range from antimicrobial effects to antiepileptic activity to immunosuppressive action. The interest in synthetic routes to these unusual amino acids exists because many are difficult to isolate and/or purify in significant quantities. Consequently, the development of synthetic methods for the practical synthesis of these unusual amino acids allows for the development of structure-activity relationship studies (SAR) in order to identify compounds with optimal therapeutic properties.

Non-proteinogenic amino acids have also been used extensively as probes of biochemical mechanisms. By incorporating amino acids that are conformationally restricted, isotopically labeled or chemically modified into peptides or proteins, insight may be gained into the mechanism of enzymatic action or binding properties of the enzyme. Rationally designed inhibitors may then be developed. Isotopically labeled substrates can also be used to study biosynthetic pathways in order to elucidate the mechanistic details and various steps involved in those pathways. Recent advances in protein engineering use a codon that codes for a transfer RNA containing an unusual amino acid, thus allowing the incorporation of unusual amino acids directly into large proteins.[4]

The other factor governing the increased interest in amino acids is the remarkable growth in asymmetric organic synthesis. Amino acids, as compounds naturally incorporating both chirality and functionality, have been intrinsically involved in the development of asymmetric synthesis as both substrates and targets.[5] The proteinogenic amino acids provide a cheap source of starting materials usually available in both enantiomeric forms, and can be used as chiral auxiliaries,[6] reagents[7] and substrates.[8]

The research presented in this thesis has concentrated on expanding the synthetic use of the 2,6,7-trioxabicyclo[2.2.2]octane protecting group for the α-carboxylate group in amino acids that was pioneered earlier in this laboratory.[9] The use of this group in the synthesis of unusual amino acids will be described.

The volume of literature relevant to amino acid synthesis is immense and a comprehensive review is beyond the scope of this thesis. However, a comparatively brief review of classical routes for the synthesis of both racemic and optically active amino

acids will be presented including a number of selected routes that have proven to be of greatest importance and general utility in leading to enantiomerically pure amino acids. Methods that are specific to classes of amino acids will be discussed in the relevant chapter. For a more detailed review of methods for the stereoselective synthesis of α-amino acids, readers are referred to the excellent reviews by Williams[10] and Duthaler.[11]

1.2 Chemical Synthesis of Racemic α-Amino Acids

Prior to the 1970's, the most widely used procedures for the synthesis of α-amino acids led to racemic compounds. Many of these routes were based on classical syntheses and could be categorized into three groups: addition of the amino or carboxy function to the side chain, alkylation of glycine derivatives and other methods.

1.2.1 Addition of the Amino or Carboxy Function to the Side Chain.

Racemic α-amino acids can be synthesized by the α-amination of a synthon containing a carboxyl group or conversely, by the α-carboxylation of an amine containing synthon. The first reported synthesis of an α-amino acid, in 1850 by the Strecker synthesis is an example of this method (Scheme 1.1).[12]

Scheme 1.1

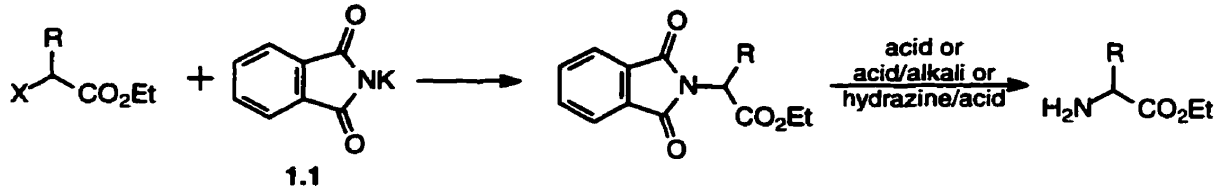


The Strecker synthesis is widely used in industry where the racemic amino acid is *N*-acylated and subsequently enzymatically resolved to give the desired optically pure

product,[13] as in the case of L-α-methyl-dopa.[14] The Bücherer-Bergs synthesis is a modification of the Strecker synthesis proceeding through a hydantoin intermediate.[15]

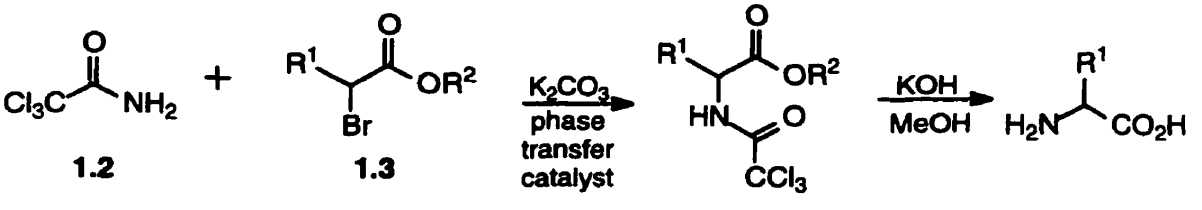
In the Gabriel synthesis, ammonia is replaced with potassium phthalimide **1.1** as the aminating reagent, generally resulting in fewer side reactions (Scheme 1.2).[12]

Scheme 1.2



Racemic syntheses are still commonly used even though the literature on stereospecific syntheses over the past decade has grown immensely. A recent procedure describes the amination of 2-bromocarboxylic esters **1.3** with trichloroacetamide **1.2** (Scheme 1.3) giving the free amino acid in 40-90% yield.[16]

Scheme 1.3



R^1=H, Me, *i*-Pr,*i*-Bu, *n*-C_6H_{13}, *n*-$C_{14}H_{29}$, Ph, Bn, $(CH_3)_2Br$

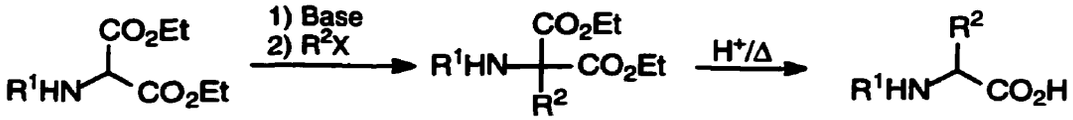
R^2=Me, Et

1.2.2 Alkylation of Glycine Derivatives.

The strategy of adding the side chain of an amino acid to a glycine equivalent was first reported by Sörensen in 1903[12] and since then a number of varied templates have been used. *N*-Acylaminomalonates **1.4** provide a versatile route (Scheme 1.4) where

generation of the enolate and addition of an alkyl halide is followed by decarboxylation to give the racemic amino acid.

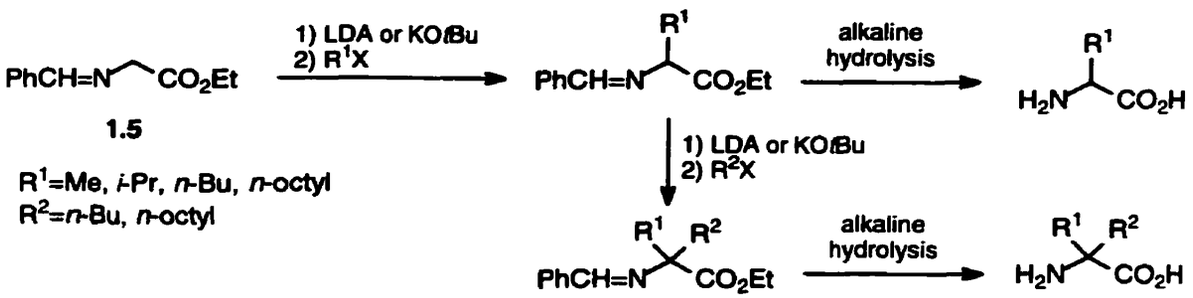
Scheme 1.4



1.4

Similar methodology employs glycine Schiff bases (or α-isocyanoacetates) which are alkylated to a number of mono- and di-alkylated amino acids obtained in 50-90% yield from the benzylidene derivative of glycine ethyl ester **1.5** (Scheme 1.5).[17]

Scheme 1.5



1.5

R^1=Me, *i*-Pr, *n*-Bu, *n*-octyl
R^2=*n*-Bu, *n*-octyl

1.2.3 Other Synthetic Routes to Racemic α-Amino Acids

Numerous specific methods exist for the synthesis of racemic amino acids as a survey of the literature, especially pre-1970's, indicates. Included amongst these is the classic Curtius rearrangement and the many modifications employed to synthesize a large number of both proteinogenic and non-proteinogenic α-amino acids.[12]

1.3 Synthesis of Optically Active α-Amino Acids

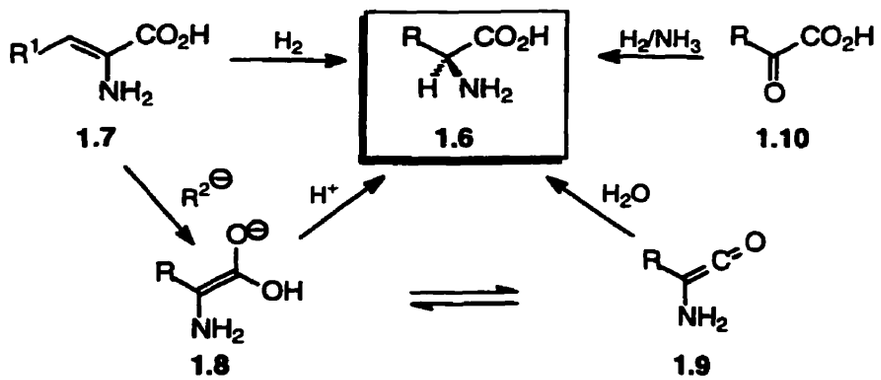
Optically active α-amino acids were initially obtained by the resolution of racemic mixtures, either by crystallization of diastereomeric salts, through derivatization

and separation of diastereomers or through enzymatic methods. Amino acids have also played a pivotal role in the field of stereoselective synthesis, either as targets or reagents (for some reviews of early developments in asymmetric synthesis and the importance of amino acids as targets and reagents see Kagan and Fiaud,[18] Apsimon and Séguin,[19] Halpern,[20] Mosher and Harrison[21] and Bosnich and Fryzuk[22]). A number of elegant approaches for the asymmetric synthesis of various α-amino acids in their optically pure forms have been developed and are summarized below.

1.3.1 Enantioselective Introduction of the α-Hydrogen

The principal transformations leading to α-amino acids **1.6** by the enantioselective introduction of the α-hydrogen are shown in Scheme 1.6.[11] The chiral α-carbon of **1.6** is generated either by hydrogenation of **1.7** or via 1,4-addition of nucleophiles (via **1.8**), protonation of enolates **1.8**, hydration of α-amino-ketenes **1.9** or by reductive amination of α-keto acids **1.10**.

Scheme 1.6



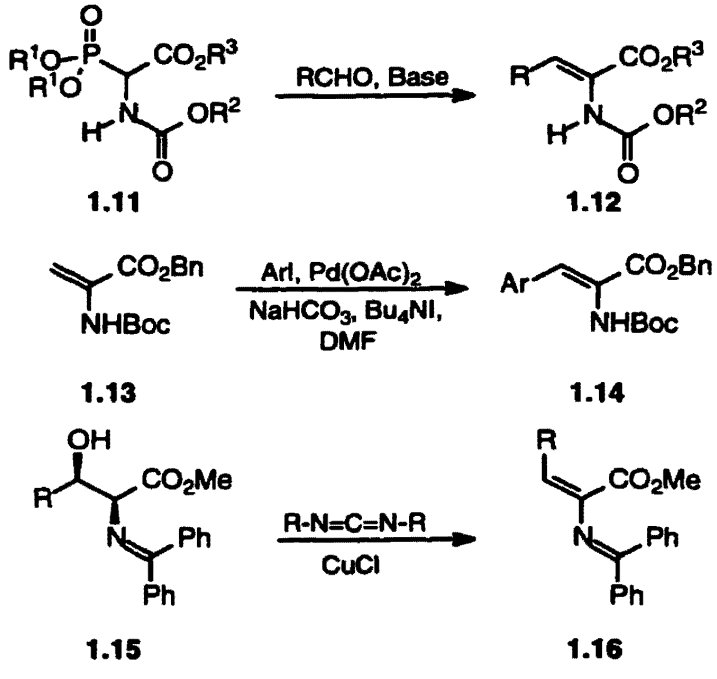
1.3.1.1 Asymmetric Hydrogenation of α,β-Didehydro Amino Acids

The utility of this method in the generation of optically active α-amino acids is twofold; the ease of synthesis of the appropriately functionalized starting material via

classical techniques (Scheme 1.7) and the numerous methods available for the enantioselective hydrogenation of dehydroamino acids.

A number of methods exist for the generation of *E*- and *Z*-olefins, including variations of the Wittig and Horner-Wadsworth-Emmons reactions. Among the most versatile is the reaction of phosphonates **1.11** with various aldehydes to afford Cbz or Boc protected dehydroamino acids **1.12** with a preference for *Z*-isomers (Scheme 1.7).[23] Heck coupling of aryl iodides or bromides to **1.13** gives α-amido acrylates **1.14**, providing an alternative route to various aryl functionalized *Z* isomers. *threo*-β-Hydroxy-α-amino acids **1.15** can be dehydrated into the *E*-isomer **1.16** providing complementarity to the above methods.

Scheme 1.7



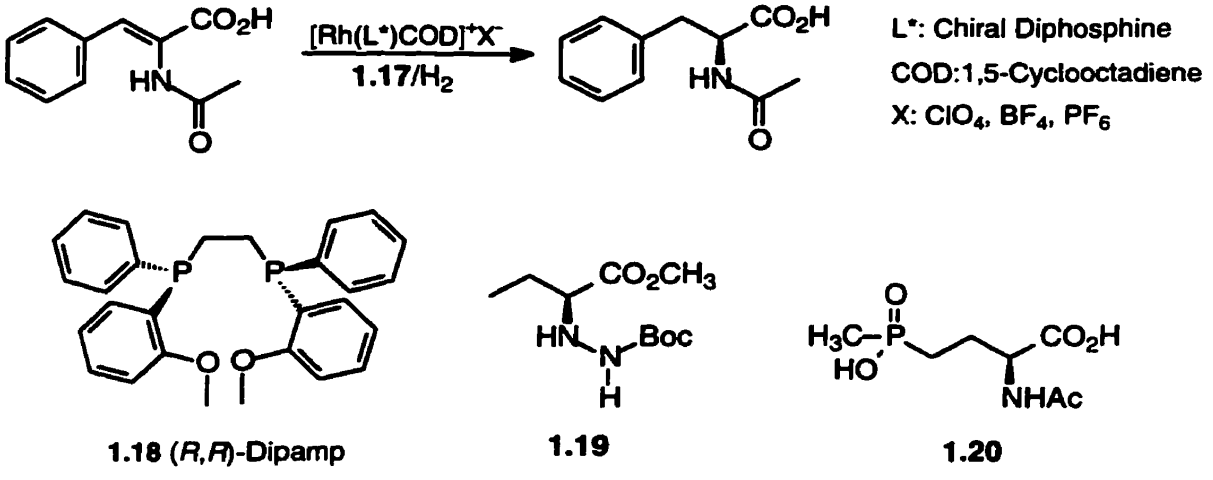
1.11 **1.12** **1.13** **1.14** **1.15** **1.16**

The enantioselective hydrogenation of dehydroamino acids **1.7** is generally catalyzed by a chirally modified Wilkinson catalyst **1.17**, and is such an efficient process for the preparation of chiral amino acids that it is also used industrially (Scheme 1.8).

Higher activity is usually observed for cationic Rh(I) complexes and over 100 chiral *bis*-phosphines have been prepared, the most frequently used being DIPAMP ((*R*,*R*)-1,2-ethanediylbis[(*o*-methoxyphenyl)phenylphosphine]) **1.18**[24] with enantioselectivities typically in the range of 80% to >99%. For example, both **1.19** and **1.20** were generated in 90% ee.[25] β-Hydroxy-α-amino acids have also been synthesized using this methodology.[26] Numerous reviews cover this topic in more detail.[27]

As an alternative to chiral hydrogenation catalysts, reduction of the α,β-unsaturated amino acid can also be controlled by chiral auxiliaries or asymmetric centers in the sidechain. This technique has been used extensively toward the synthesis of substituted glutamic acids and will be covered in more detail in Chapter Four.

Scheme 1.8



1.18 (*R*,*R*)-Dipamp **1.19** **1.20**

1.3.1.2 Reductive Amination of α-Ketoacids

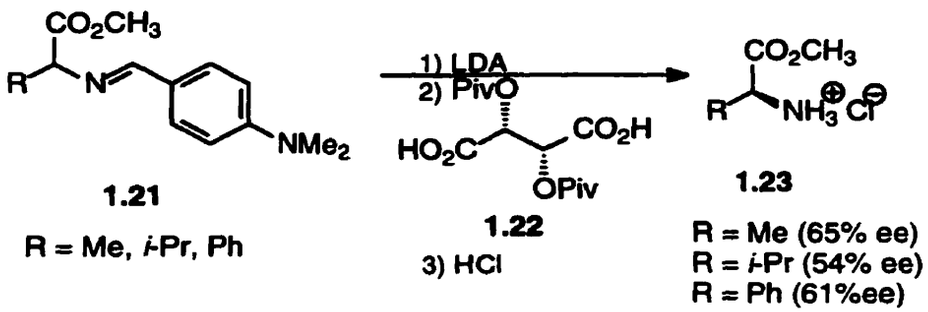
The reductive amination of α-ketoacids is a known biosynthetic step and the corresponding enzymes have been used comprehensively in the synthesis of α-amino acids. Phenylalanine dehydrogenase from *Bacillus sphaericus* has been used to investigate the amination of various aromatic α-ketoacids with varying degrees of

success.[28] The same principle has been used in the synthesis of a number of γ-substituted glutamic acids but will be discussed in further detail in chapter four.

1.3.1.3 Asymmetric Protonation of Enolates

The protonation of enolates generated from Schiff's bases occurs with moderate optical purity and is dependent on the structures of the Schiff base, the lithium base and temperature. For example, the imine **1.21** was deprotonated with lithium diisopropylamide (LDA) and quenched with (*R*,*R*)-dipivaloyl-tartaric acid **1.22** to give predominantly the *L*-configuration of amino acid esters **1.23** (Scheme 1.9)[29]

Scheme 1.9



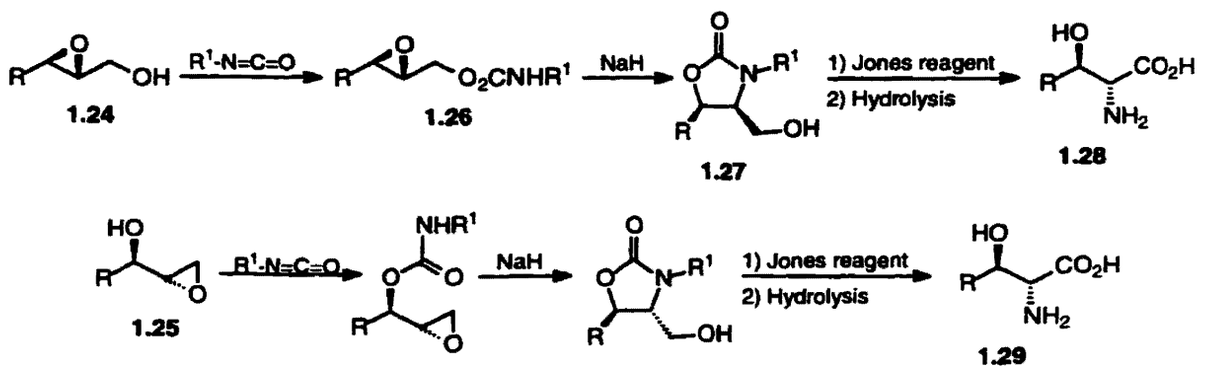
1.3.2 Enantioselective Introduction of the α-Amino Function

Numerous groups have addressed the difficulties of the chiral synthesis of α-amino acids through the concept of enantioselectively introducing the α-amino functionality. Two approaches may be taken; nucleophilic amination based on S_N2 displacement or direct electrophilic amination of enolates both of which rely on previously existing chirality to give the desired selectivity.

1.3.2.1 Nucleophilic Amination

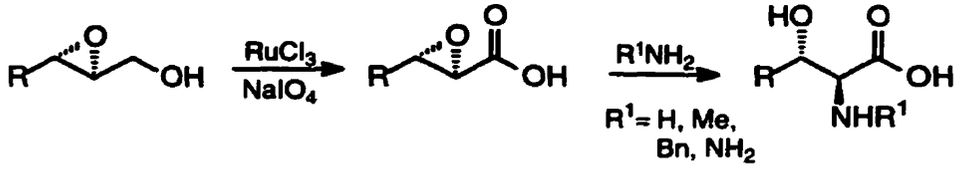
The diastereoselective synthesis of β-hydroxy-α-amino acids from epoxides is particularly amenable to nucleophilic amination (Scheme 1.10). The Sharpless epoxidation provides a convenient route to both stereoisomers of the epoxides **1.24** and **1.25** which are subsequently protected as the carbamate **1.26** and isomerized to the oxazolidinones **1.27** followed by straightforward deprotection to the β-hydroxy-α-amino acids generally in good yields and with high diastereoselectivity.[30] Minor contamination is a result of nonregioselective ring opening of the epoxides **1.26** during oxazolidinones formation.

Scheme 1.10



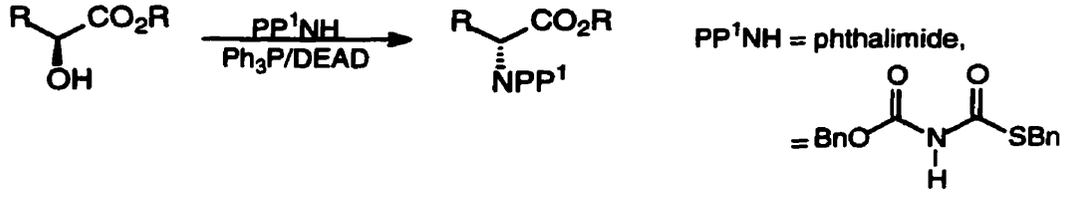
Another route to β-hydroxy-α-amino acids via epoxy allylic alcohols is by oxidation to the corresponding acid followed by epoxide opening by ammonia, primary amines and hydrazines which avoid the regioselectivity problems described in Scheme 1.10 since attack is exclusively at C2 (Scheme 1.11).[31]

Scheme 1.11



α-Hydroxy-carboxylates are also readily available chiral compounds and lend themselves to *N*-nucleophilic substitution (Scheme 1.12). The reactions are generally clean although racemization difficulties have been encountered. Direct substitution by *N*-nucleophiles has been achieved with sodium azide,[32] amines,[32] phthalimide[33] and imides[33] under Mitsunobu conditions. Conversion of the alcohol to the triflate and subsequent direct displacement with di-*N*-Boc-imide has proven to be especially effective[33] and has advantages over the Mitsunobu method, which requires harsh conditions for the unmasking of the nitrogen functionality.

Scheme 1.12

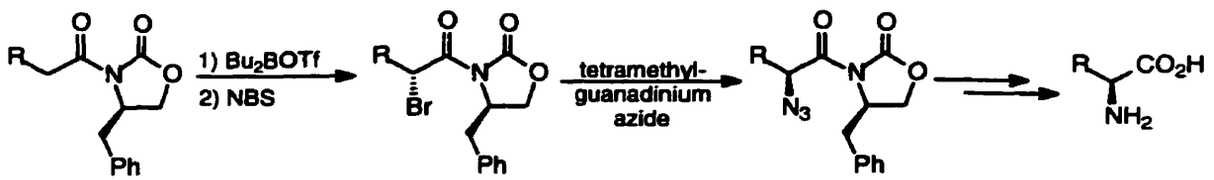


One of the best methods for the generation of α-amino acids by nucleophilic amination has been developed by Evans *et al.*[34] The use of *N*-acyloxazolidinones **1.30** to generate α-bromides followed by S_N2 displacement to the azide gives the desired precursor in high diastereoselectivity (usually >90% de) (Scheme 1.13). Altering the stereochemistry of the N-acyloxazolidinones **1.30** can generate both enantiomers of **1.6**. Furthermore, β-substituted-α-amino acids have been synthesized by first incorporating

the desired β-substituent[35] onto Evans' chiral oxazolidinone **1.30** or by diastereoselective cuprate addition to α,β-unsaturated-*N*-acyloxazolidinones **1.30** followed by α-bromination. The chirality of the β-carbon appears to have little effect on the stereoselectivity of α-bromination.[35]

Several other systems have been adapted to enantioselectively aminate the α-carbon and are based on the use of chiral templates developed for the enantioselective addition to glycine enolates and will be discussed in more detail in Section 1.5.1.1 (*vide infra*).

Scheme 1.13



1.30 **1.6**

1.3.2.2 Electrophilic Amination of Enolates

Perhaps the most direct route to α-amino acids is by amination of enolates with electrophilic reagents. This has only recently been developed due to the scarcity of electrophilic sources of nitrogen that participate in C-N bond forming reactions. One of the first reagents used in this application was di-*t*-butyl azodicarboxylate, which converts Li-enolates to α-hydrazido acids.[36] However, this method suffers from difficulties associated with the harsh conditions required for the cleavage of the N-N bond in the newly formed α-hydrazido acids.

Evans and coworkers have optimized the direct azidation of potassium enolates of *N*-acyloxazolidinones **1.30** with trisyl azide, which occurs in both good yield and high

stereoselectivities.[34b] Numerous examples exist of the synthesis of α-amino acids using this methodology including the recent synthesis of a novel phosphotyrosyl mimetic[37a] and β,γ-dehydrovaline.[37b] Evans' chiral *N*-acyloxazolidinones methodology thus provides access to both *R*- and *S*-α-amino acid enantiomers.

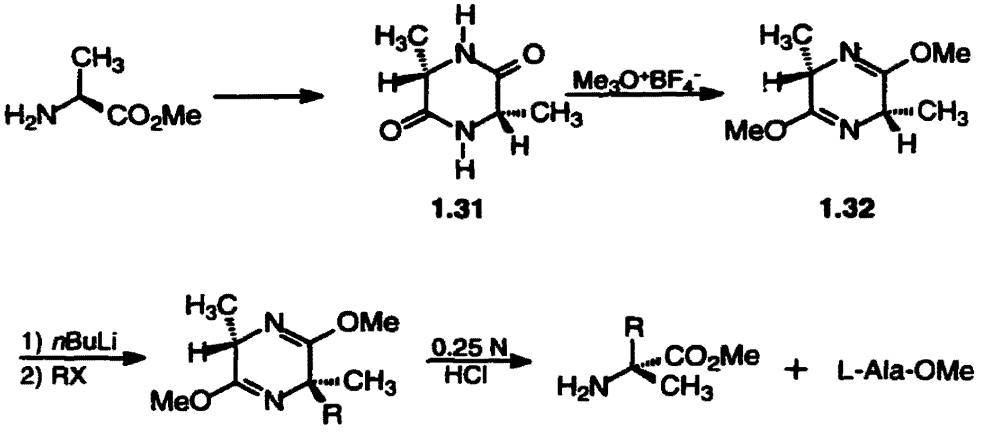
1.3.3 Stereoselective Introduction of the Side Chain

An obvious disconnection in the synthesis of α-amino acids is the stereoselective incorporation of the sidechain to a glycine equivalent. A cyclic chiral template usually induces stereochemical orientation and a review of the literature indicates the majority of methods describing the asymmetric synthesis of α-amino acids employ this approach. Both nucleophilic and electrophilic additions are used in order to introduce a wide range of side chains onto the template. Numerous examples of these chiral synthons exist, however, only the most important and frequently used systems will be discussed.

1.3.3.1 Glycine α-Anion Equivalents

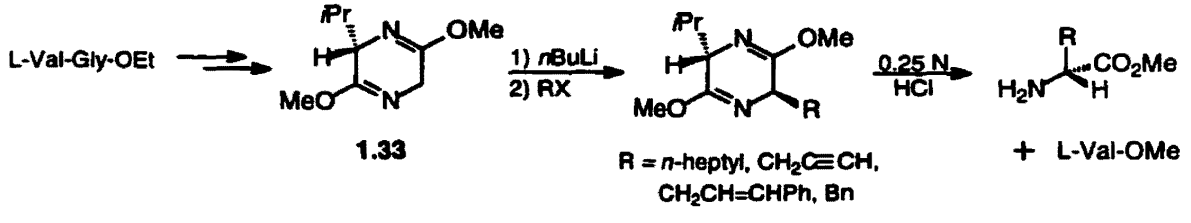
The first cyclic template developed, the most versatile and widely used was the *bis*-lactim ether **1.32** developed by Schöllkopf *et al.* of which several reviews have appeared.[38] The dimethoxy lactim ether **1.32** was formed from the diketopiperazine **1.31** in 70-80% yields and was subsequently used to generate α-methyl amino acids by reaction of its enolate with alkyl halides or carbonyls (Scheme 1.14).

Scheme 1.14



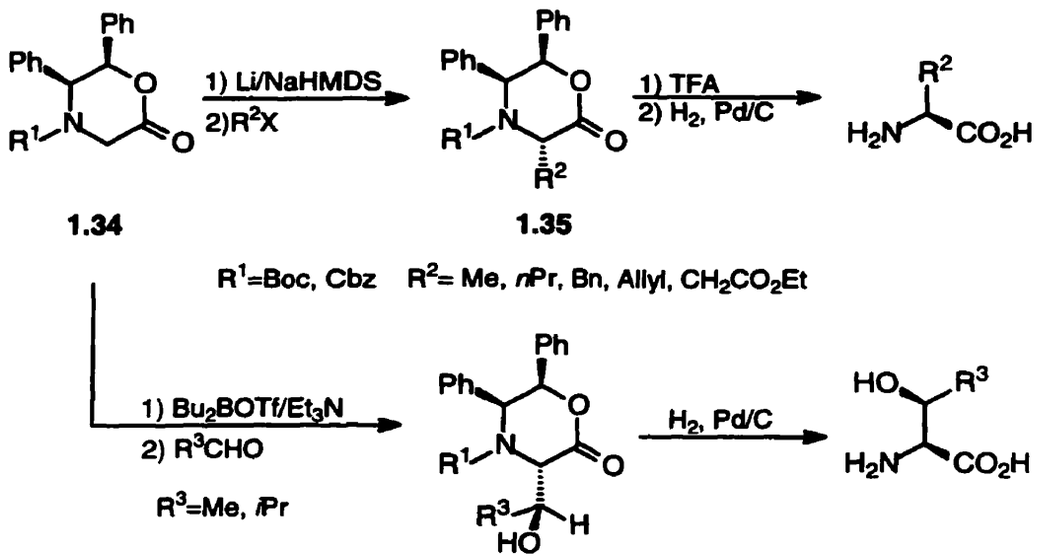
This approach was then applied to the synthesis of α-amino acids by forming a *bis*-lactim ether **1.33** from L-Val-Gly. A number of electrophiles were added, to give after hydrolysis, D-amino acid methyl esters in 75-95% ee, with 62-91% yields for the addition and 52-89% yields for the hydrolysis to the methyl ester (Scheme 1.15).[39] Stereocontrol at the β-carbon is less impressive, with only 22% de when chiral halide electrophiles were used.[40] Furthermore, when the Li-enolate of **1.33** was added to carbonyl electrophiles, only 10-75 % de was achieved, although exchanging the lithium enolate to a titanium derivative increased the diastereoselectivity to >95% de with >95% ee and yields of between 77-93%.[41] A major disadvantage of the method, however, is the necessity to separate the two amino acid methyl esters that are generated as a result of hydrolysis of *bis*-lactim template.

Scheme 1.15



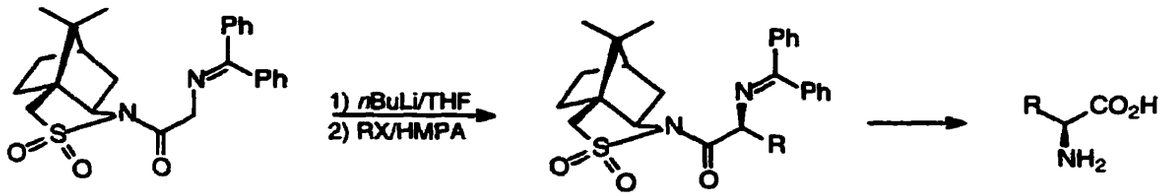
Williams and coworkers have examined electrophilic additions to chiral oxazinones **1.34**. Generation of the enolate and subsequent alkylation gave the *trans* adduct **1.35** in excellent diastereoselectivity (>99%) and reasonable yields (50-90%) (Scheme 1.16 top).[42] Only disilazanes are successful at deprotonating the oxazinone **1.34** and the temperature, mode of addition and many other parameters have to be carefully controlled. Aldol condensations of the boron enolate of **1.34** with aldehydes produce *anti*-β-hydroxy adducts **1.36** in 38-57% yield with diastereoselectivities of approximately 5:1 observed at the carbinol center (Scheme 1.16 bottom).[43]

Scheme 1.16



The camphor sultam **1.37** introduced by Oppolzer has been used to direct the derivatization of glycine enolate equivalents.[44] Deprotonation of the imino sultam synthon **1.37** followed by alkylation in the presence of HMPA gives the amino acid derivatives **1.38** with high diastereoselectivities (90-98% de) and excellent yields (Scheme 1.17). Imine hydrolysis under acidic conditions and removal of the auxiliary with LiOH furnishes α-amino acid **1.6**.

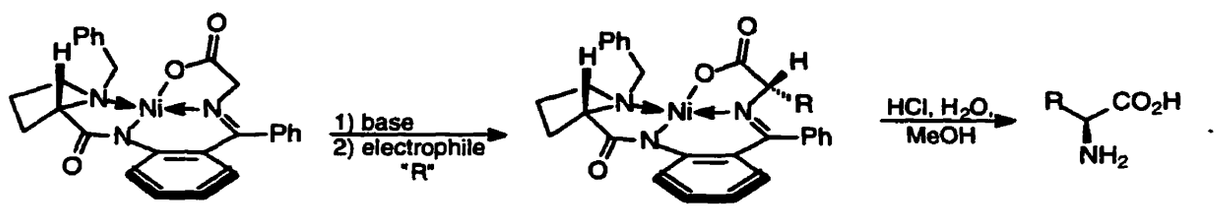
Scheme 1.17



1.37 **1.38** **1.6**

The chiral nickel (II) complex **1.39** reported by Belokon *et al.*,[45] derived from benzyl-proline, *o*-aminoacetophenone and glycine, is one of the best-studied systems for glycine enolate derivatization. Alkyl halides,[46] aldehydes[47] and 1,4-unsaturated carbonyls[48] have all been added to **1.39** and enantioselectivities for addition are typically in the order of >80% ee (Scheme 1.18). β-Hydroxy amino acid derivatives are generated from aldehydes in good yield and with *threo*:*allo* ratios of >20:1 in most cases, however, poor induction of stereochemistry at the β-carbon is achieved with 1,4-unsaturated carbonyl compounds, for example, 2:1 for methyl methacrylate (Section 4.1.4.1).

Scheme 1.18

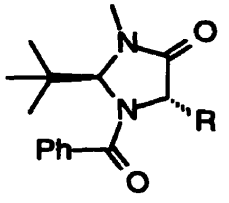


1.39 **1.40** **1.6**

A wide variety of alkylating agents have been reacted with the enolate of **1.39** to give alkylated α-amino acids in yields of 60-90% and generally >98% ee.[46]

Seebach and coworkers have developed the use of imidazolidinones **1.41** prepared from Ala, Met, Val, Phe,[49] Asp and Glu.[50] Deprotonation of the imidazolidinone

template, reaction with alkyl halides and deprotection gave the amino acid in nearly 100% ee at the chiral center.

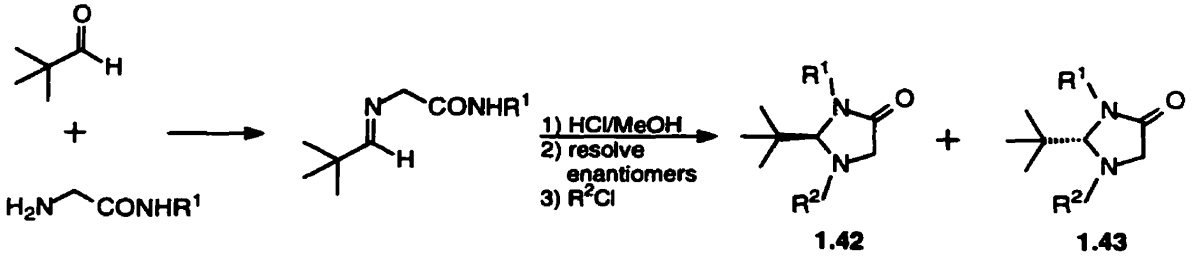


R = Me, *i*Pr, Ph, Bn, $(CH_2)_2SCH_3$, $CH_2CO_2CH_3$, $(CH_2)_2CO_2CH_3$

1.41

The more functional chiral glycine imidazolidinone **1.42** proved to be difficult to synthesize and was initially generated in poor yields (<30%) from the degradation of serine or methionine.[51] Better results were obtained by the recrystallization of the mandelate salt of the racemic imidazolidinone prepared directly from glycine (Scheme 1.19). Both enantiomers can be isolated in >98% ee and the cyclic aminals (abbreviated BMI for the *N*-methyl derivatives) acylated to give Bz, Boc, and Cbz-BMI derivatives **1.42** and **1.43**.[52] The racemic BMI is isolated in <50% yield from glycine methyl amide and upon resolution gives 30% of the *S*-enantiomer and 22% of the *R*-enantiomer.[49a,53]

Scheme 1.19



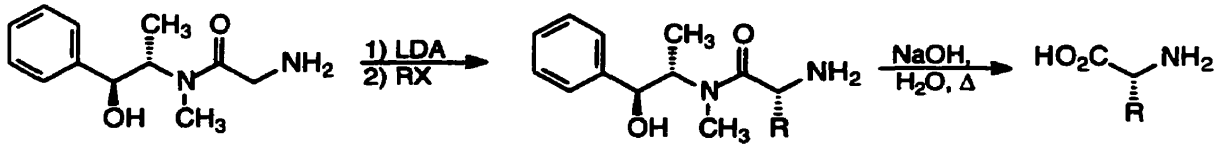
1.42 **1.43**

Deprotonation of **1.42** allows for reaction with various electrophiles including alkyl halides, nitroolefins, carbonyls and epoxides. Products with one new stereocenter (the α-center) gave a single diastereomer whilst secondary halides or nitroolefins give reasonable selectivity at the second center (77–85% ds).[54] Aldol reactions typically occur

in 41-65% yields with 63–96% diastereoselectivity in preference of the *syn* (*threo*) product.[53]

Myers *et al.* have recently reported the use of a pseudoephedrine derivative **1.44** as a chiral auxiliary in the synthesis of various alkylated α-amino acids.[55] The advantages of this template include ease of preparation, high ee and de's and simplicity of hydrolysis off the template. The reported yields ranged between 70-80% for intermediate **1.45** and hydrolysis gives the free α-amino acids in generally >85% yield, however, few reports currently exist utilizing this methodology (Scheme 1.20).

Scheme 1.20



1.44 R=Me, Et, allyl, Bn **1.45**

1.3.3.2 Glycine α-Cation Equivalents

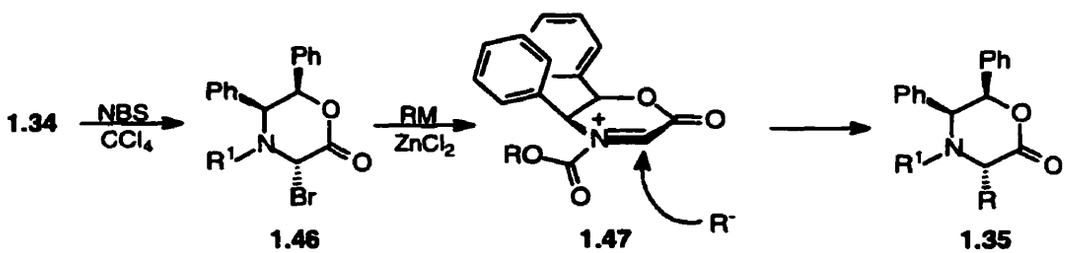
Many of the cyclic chiral templates described in the previous section can be converted to α-cation equivalents simply by the introduction of good leaving groups at the α-position. This approach has been used successfully with Schöllkopf's *bis*-lactim **1.33**, Williams' oxazinone **1.34** and Belokon's Ni-complex **1.39**.

The chlorinated derivative of *bis*-lactim ether **1.33** was converted into aromatic α-amino acids by Friedel-Crafts alkylation with yields in the 60% range and enantioselectivites generally >90% ee and occurs with inversion of stereochemistry at the α-carbon.[56]

The 3-bromo analog of Williams and co-worker's oxazinone **1.34** has been extensively studied and has been substituted efficiently by alkyl thiolates, malonates, alkyl Zn-halides and cuprates in both good yield and high enantioselectivity.[57] Coupling of allylsilanes and silyl enol ethers,[42] Friedel-Crafts alkylations[57a] and trialkylstannylacetylides[58] could also be effected under Lewis-acid catalyzed conditions. The relative stereochemistry of the product remains *anti* and is postulated to proceed through iminium species **1.47** after zinc coordination to the bromide **1.46** (Scheme 1.21). The steric factors controlling addition of the electrophile to **1.34** in Scheme 1.16 exert the same influence in the case of the incoming nucleophile in scheme 1.21, resulting in retention of stereochemistry at the α-carbon.

Belokon *et al.* have investigated the reactivity of the brominated Ni-complex **1.40** which was generated as a 2:1 diastereomeric mixture from **1.39**. However, nucleophilic addition gave the *anti* adducts with excellent stereocontrol (90-98% ee (*S*)) and good yields (60–90%).[59]

Scheme 1.21



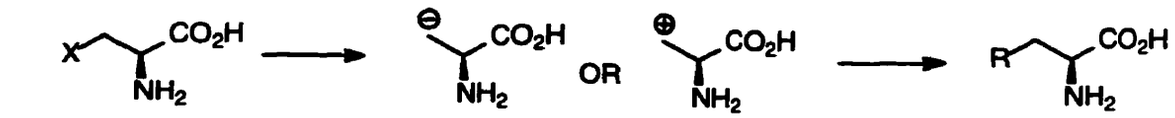
1.46 **1.47** **1.35**

1.3.4 Synthesis from α-Amino Acids

α-Amino acids, with their inherent chirality and functionality, provide an excellent source of starting materials for the synthesis of more complex derivatives.

Countless examples exist of the synthesis of unusual α-amino acids from other amino acid precursors,[10,11,60] but as a whole, general methods for the synthesis of α-amino acids can be separated into three groups: formylglycine equivalents, alanine β-anion equivalents and alanine β-cation equivalents. The basic chiral α-amino acid synthons are the alanine β-anion **1.48** and β-cation **1.49** which are generally derived from serine, cysteine, aspartic acid and to a lesser extent from glutamic acid (Scheme 1.22). Formylglycine, although discussed separately, can be considered an alanine β-cation equivalent.

Scheme 1.22

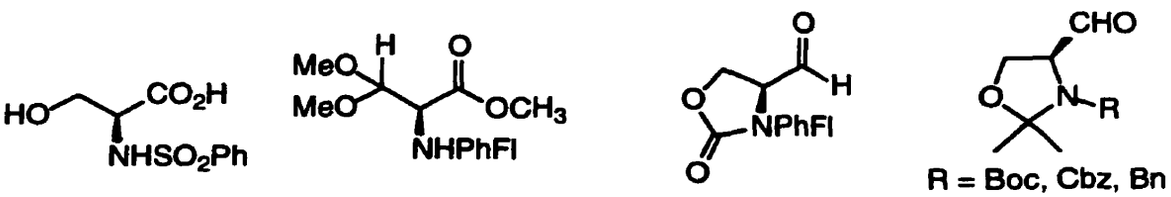


1.3.4.1 Formylglycine Equivalents

The most common formylglycine equivalents are serine aldehydes generally derived directly from serine. As an obvious choice for the derivatization to other α-amino acids, serine suffers from a few notable deficiencies that must necessarily be addressed. Serine is one of the amino acids most prone to racemization and is believed to involve a planar carbanion intermediate produced by the rate-determining removal of the α-proton.[3] This increased rate is due to the hydroxyl group which helps stabilize the intermediate anion. Furthermore, if the side chain is oxidized to a serine aldehyde, enolization results in racemization at the α-center. As such, numerous approaches have been undertaken in order to address this issue. Two routes may be employed in the

formation of serine aldehydes; direct oxidation of the side chain to the aldehyde without altering the oxidation state of the carboxylic acid group, and reduction of the acid to the aldehyde and subsequently oxidizing the side chain to the carboxylic acid in order to regenerate the α-amino acid. These methods include the use of protecting groups that reduce or mask the ability of the α-proton to racemize.

The most important serine-derived aldehydes designed for the synthesis of α-amino acids are illustrated in Figure 1.1.

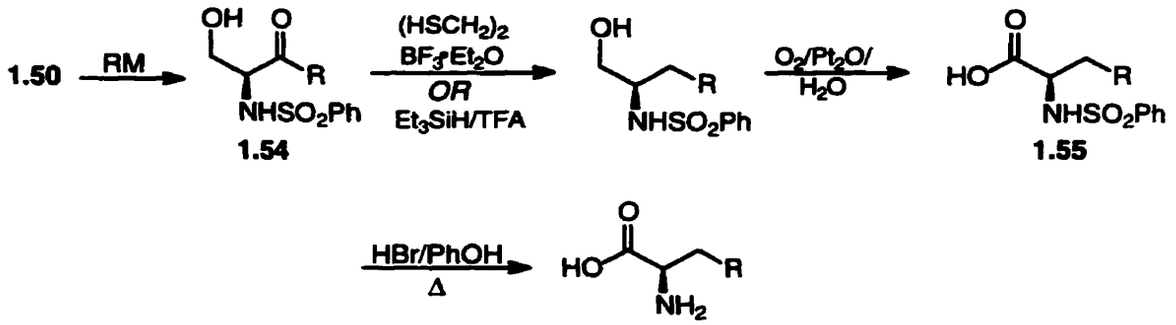


1.50 **1.51** **1.52** **1.53**

Figure 1.1: Important Serine Aldehyde Equivalents.

Rapoport first described the conversion of L-serine to D-amino acids in 1984 using α-sulfonamido acids **1.50** treated with excess organo-Li reagents or Grignard reagents.[61] These could be reduced directly to β-hydroxy-α-amino acids, *erythro* configuration with $NaBH_4$ (7:3 ratio), *threo* with L-selectride (99:1) in good yield or to the alkyl derivative via thioketal desulfurization or triethylsilane reduction. Platinum catalyzed oxidation and subsequent deprotection gave the desired D-amino acids in very good overall yields (Scheme 1.23). However, the conditions used for reduction and oxidation limit the functional groups that can be incorporated. A large scale synthesis has also been developed for the preparation of α-amino ketones **1.54**.[62] This synthon has been applied to the synthesis of various carbacephems[63] and calicogorgin A.[64]

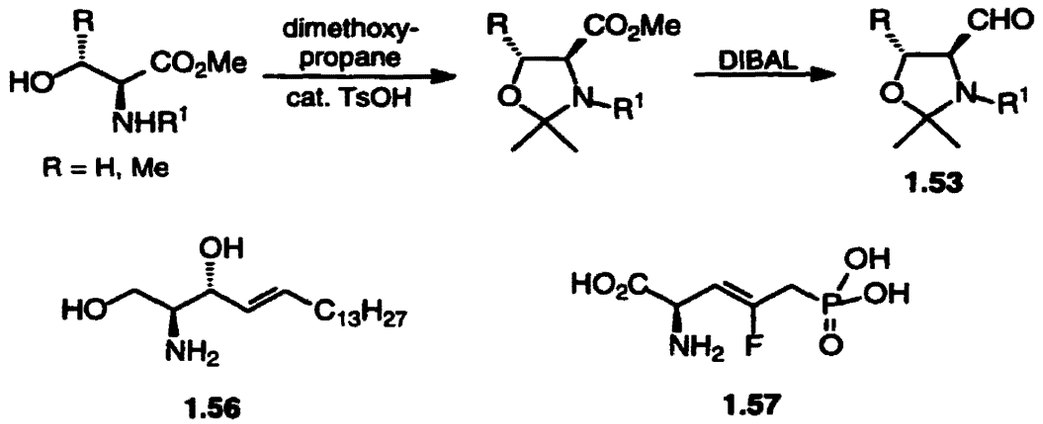
Scheme 1.23



Rapoport has also studied the use of the *N*-(9-phenylfluoren-9-yl) (PhFl) protecting group for preventing racemization in the serine aldehyde equivalents **1.51** and **1.52**.[65] The bulky protecting group is proposed to prevent enolization by obstructing removal of the α-proton.

Probably the most versatile serine aldehyde synthon is Garner's aldehyde **1.53**. First reported in 1984,[66] with an *Organic Syntheses* preparation appearing in 1991,[67] this synthon has been used extensively for the synthesis of various chiral amino alcohols such as sphingosine **1.56** and α-amino acids, particularly β,γ-unsaturated amino acids such as **1.57** (Scheme 1.24).[68] The numerous examples of successful reactions include Wittig olefinations with unstabilized ylides,[69] α-phosphonocarboxylates,[70] Takai olefinations,[71] Reformatsky reactions,[72] cuprate additions[73] and Grignard reactions.[74] Most of the described reactions occur in both good yield and with minimal racemization at the α-carbon even under relatively basic conditions. Stereoselectivity at the β-carbon after various addition reactions to **1.53** varied between 3:1 for vinylmagnesium bromide (*anti*:*syn*) to 98:2 (*syn*:*anti*) for the 1,4-addition of cuprates.[73,74b] Perfect stereocontrol has been obtained with chiral reagents such as allyl-Ti complexes chelated with a diol ligand derived from tartrate.[75]

Scheme 1.24



The main drawback with the Garner aldehyde **1.53** is that an oxidation step is required to generate the α-amino acid after formation of the penultimate β-amino alcohol, limiting the type of side chain functionality that can be present.[76] Racemization has also been observed during the oxidation to α-amino acids.[77]

1.3.4.2 Alanine β-Anion Equivalents

The utilization of the side chain of serine as a nucleophilic entity **1.48** has been accomplished a number of ways with varying degrees of success. Alanine β-anion equivalents are outlined in figure 1.2.

Sasaki *et al.* generated the β-anion of **1.58** derived from serine, and after reaction with various alkyl halides and subsequent desulfurization and reoxidation gave the desired alkyl α-amino acids in good yields with >98% ee.[78]

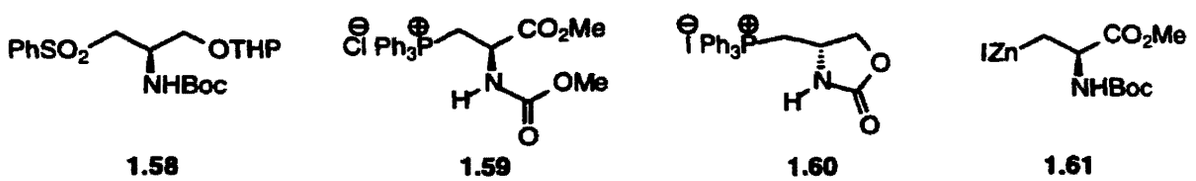


Figure 1.2: Selected Alanine β-Anion Equivalents.

Two similar phosphonium salts have been developed, the serine derived Wittig ylide **1.59** of Itaya *et al.*[79] and the oxazolidinone Wittig ylide **1.60** of Sibi and coworkers.[80] Of the two, **1.60** appears to be the more versatile and has been used in the synthesis of a number of sugar-substituted α-amino acids via a Wittig reaction.[81]

The β-iodozinc alanine derivative **1.61** has been reacted with both acid chlorides and aryl iodides in Pd-catalyzed coupling reactions to give the desired derivatives in moderate yields.[82] Zn/Cu exchange gives the cuprate derivative, which has been reacted with a variety of allyl halides to give a number of unsaturated α-amino acids.[83]

Aspartate, and to a lesser extent glutamate, can be considered as an alanine β-anion equivalent since regioselective β-enolate formation is possible when the α-carbon is either protected with the bulky PhFl group, by deprotonation of the amide or the carboxylate. Following enolate formation, reactions are typically performed with reactive electrophiles and then further derivatized as desired. 1,2-Stereoinduction is generally observed in electrophilic additions to aspartic acids and 1,3-induction in the case of glutamates. Both aspartic acid (chapter five) and glutamic acid (chapter four) will be discussed in more detail in the appropriate section.

1.3.4.3 Alanine β-Cation Equivalents

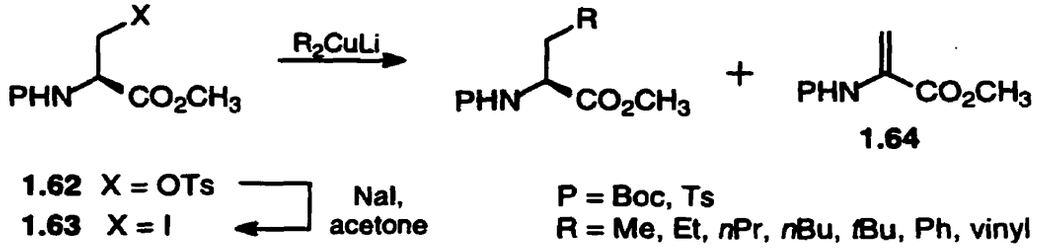
The generation of an alanine β-cation equivalent is realized by the nucleophilic substitution of the serine OH-function after appropriate activation into a good leaving group. However, this immediately raises concerns due to the propensity of serine to undergo elimination as illustrated in the case reported in 1963 by Photaki where displacement of *N*-Cbz-*O*-tosyl-L-serine methyl ester **1.62** (P = Cbz) with the sodium salt

of thiol trityl led to racemic cysteine (Scheme 1.25).[84] On closer examination, the S-trityl anion was shown to cause the elimination of the tosyl group to give dehydroalanine **1.64**, followed by subsequent addition of thiol trityl to the dehydroalanine adduct.

Despite this tendency of activated serine derivatives to eliminate, a number of displacement reactions have been successfully accomplished. For example, both sodium benzyl selenoate[85] and thioacetate[86] displace the tosylate from *O*-tosyl, *N*-Boc or *N*-Cbz serine methyl or benzyl ester to give the enantiomerically pure product.

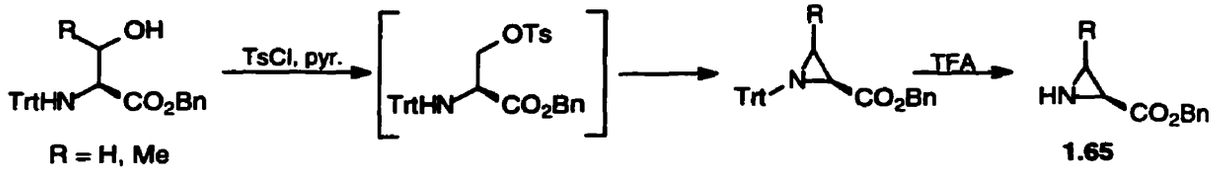
The Cbz-Ser(OTs)-OBn serine derivative can be converted to the β-iodoalanine compound by Finkelstein reaction with NaI. Viallefont and coworkers have successfully used organocuprates for displacement reactions with *O*-tosyl serine **1.62** and iodoalanine **1.63** with 2-70% formation of dehydroalanine **1.64** (Scheme 1.25).[87]

Scheme 1.25



Aziridines are another source of β-cation equivalents described by Okawa and coworkers. Aziridines are generated from serine in much the same manner as used to form **1.62** (P = Cbz) except a *N*-trityl protecting group is used (Scheme 1.26).[88]

Scheme 1.26



Reprotection of the aziridine **1.65** as the Cbz has allowed for various derivatizations. Okawa opened **1.65** (P=Cbz) with alcohols[89] or thiols[90] in the presence of boron trifluoride etherate to give the β-alkoxy or β-thioalkyl-α-amino acids **1.66** in 57-98% yield (Scheme 1.27).

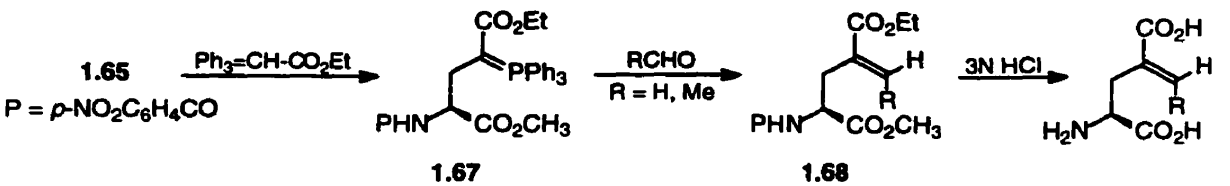
Scheme 1.27



1.65 **1.66**

Baldwin *et al.*[91] have reported opening the *p*-nitrobenzoyl derivative of **1.65** with Wittig ylides to generate a mixture of two new ylides, with the C3 product **1.67** predominating (Scheme 1.28). The ylide was then further reacted with paraformaldehyde or acetaldehyde to give 4-methylene-(2*S*)-glutamate derivatives **1.68**.

Scheme 1.28

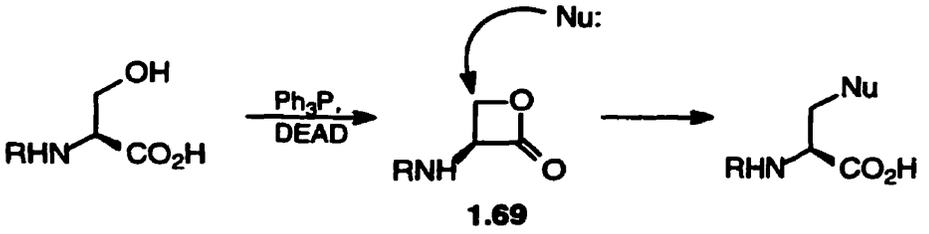


1.67 **1.68**

The most useful method for the synthesis of α-amino acids based on alanine β-cation equivalents is the β-lactone methodology as developed by Vederas and coworkers.[92] Serine is cyclized under modified Mitsunobu conditions without racemization to give *N*-protected α-amino-β-lactones **1.69** in 60-72 % yield (Scheme 1.29). Ring opening with the desired alkyl-oxygen cleavage is achieved with soft

nucleophiles such as acetate and imidazole since hard nucleophiles such as ammonia and methoxide cause unwanted acyl-oxygen cleavage.[93] No racemization is observed.

Scheme 1.29



1.69

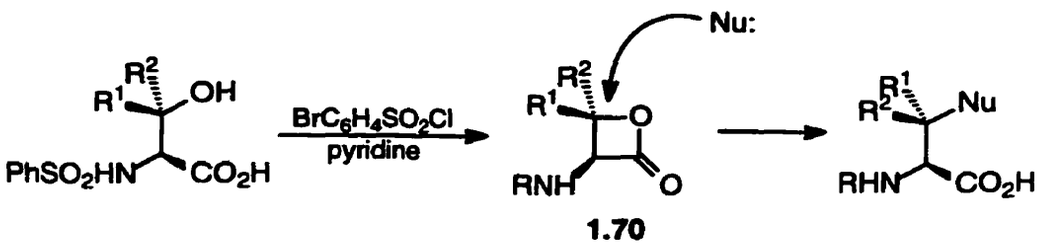
R = Cbz, Boc

Nu = $(CH_3)_3N$, imidazole, NaOAc, $NaSCH_2Ph$, $H_2N\text{-}C(=S)\text{-}NH_2$, $HS(CH_2)_2NH_2$, $MgX_2 Et_2O$ (X = Cl, Br)
= Me, Et, *i*Pr, *n*Bu, *s*Bu, *t*Bu, Ph

Carbon-carbon bonds are formed by reaction of the β-lactone **1.69** with organocuprates since organolithiums and Grignard reagents lead to carbonyl attack. Di-*N*-protected β-lactones were generally found to be better substrates than the corresponding mono-*N*-protected β-lactones with yields in the range 70-80%, however, the deprotected synthons also suffer from increased racemization (10-14% compared with <2% for mono-*N*-protected derivatives).[94]

Threonine and other homologs cannot be transformed into β-lactones **1.70** under Mitsunobu conditions although cyclization is successful using phenylsulfonamide or 2-nitrophenylsulfenyl amides with *p*-bromophenylsulfonyl chloride in pyridine. Unfortunately, the range of nucleophiles that add successfully to **1.70** is limited since organometallic reagents add to the carbonyl. Those that do add, do so with inversion at the β-carbon (Scheme 1.30).[95]

Scheme 1.30



1.70

Nu = Cl, Br, I (MgX_2 Et_2O), thiourea (S attack)

1.4 Description of Thesis

As evident in the methodologies described above, amino acid synthesis is a challenging endeavor due to the chirality and functionality present in this class of molecules. Moreover, a number of criteria should be met by new synthetic routes. The products should be optically active and both enantiomers should be available with other stereocenters in the molecule generated with good stereoselectivity. Several different classes of amino acids should be accessible from a common stable intermediate which is in turn easily obtainable on a large scale from inexpensive starting materials. Finally, it should be possible to generate the deprotected amino acid under conditions mild enough not to cause racemization or decomposition of sensitive functionalities.

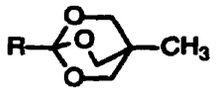
The general methods discussed above each have advantages and shortcomings, addressing some but not all of the optimal yet demanding criteria. Most of the chiral glycine enolate templates require fairly strong hydrolytic conditions to generate free amino acids and suffer from a lack of precise control specifically at the β-carbon since both the α- and β-centers are formed simultaneously. Synthesis via nucleophilic displacement of activated serine derivatives is limited in the type of nucleophiles that

may be employed, while the oxidation step required with the use of serinal equivalents limits the functionalities that may be present.

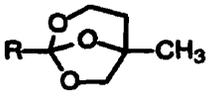
Our approach to the synthesis of non-proteinogenic α-amino acids was based on the elaboration of the current pool of derivatizable α-amino acids using serine, threonine and both aspartic and glutamic acid. Earlier work in our laboratory had shown promise in protecting the carboxylic acid as a base stable cyclic ortho ester which concurrently masked the acidity of the α-proton by preventing enolate formation and thereby anion formation at the α-center. The generation of intermediates particularly susceptible to racemization and the use of strongly basic reaction conditions is possible since the acidity of the α-proton is no longer an issue. We also surmised the steric bulk of the ortho ester would induce stereoselectivity in either nucleophilic or electrophilic additions to the OBO ester protected amino acid.

1.4.1 Ortho Esters

Ortho esters have been used as acid protecting groups for many years and an extensive review of their preparation and use appeared in 1974.[96] Essentially inert under neutral or alkaline conditions, ortho esters are readily hydrolyzed in very mild acidic environments and are one of the few protecting groups that protect the carbonyl against nucleophilic attack by hydroxide or other strong nucleophiles such as Grignard reagents. We selected the 2,6,7-trioxabicyclo[2.2.2]octane ortho ester **1.71** as the carboxyl protecting group since cyclic ortho esters are considerably more stable than the acyclic analogs[96] and a simple route to these ortho esters was available.



1.71

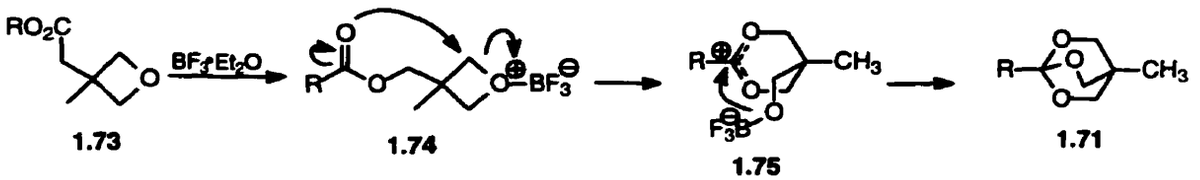


1.72

The 2,6,7-trioxabicyclo[2.2.2]octane protecting group has been used to mask the carboxyl group in several syntheses,[97] most recently in the synthesis of laurencin,[98] a member of the polyether toxins. Wipf *et al.* have recently introduced the 2,7,8-trioxabicyclo[3.2.1]octane (ABO esters) **1.72** as a structurally similar alternative to the OBO esters whose functionality may be incorporated as a desired backbone once deprotection occurs.[99]

General use of 2,6,7-trioxabicyclo[2.2.2]octane began after 1982 when Corey and Raju reported the facile preparation of 4-methyl-2,6,7-trioxabicyclo[2.2.2]octane ortho ester (OBO ester, **1.71**) from the boron trifluoride etherate catalyzed rearrangement of the corresponding 3-methyl-3-hydroxymethyloxetane ester **1.73**.[100] Prior to this, ortho esters had been obtained by the acid catalyzed esterification of the carboxylic acid with 2-substituted-2-hydroxymethyl-1,3-propanediols.[101] Most (acyclic) ortho esters are too thermodynamically unstable relative to their hydrolysis products to allow this method to be used.[96] This increased stability makes the bicyclic ortho esters chromatographically stable compared to their acyclic counterparts.[100] Corey's procedure relies on the Lewis acid catalyzed opening of the oxetane ester by carbonyl participation to form the 6-membered zwitterion **1.75** (Scheme 1.31). Rearrangement generates the ortho ester **1.71**, with the ring angle strain of the oxetane providing the overall thermodynamic driving force.[100]

Scheme 1.31



Several methods for the removal of the ortho ester protecting group have been reported in the literature and may be categorized into two procedures. In the first, acid hydrolysis is employed to ring-open the ortho ester followed by base hydrolysis to generate the acid (H_2SO_4/MeOH then 1N NaOH/MeOH,[97a] DME:H_2O, pH 3 with $NaSO_4$ then LiOH,[97b] catalytic pyridinium PTSA in MeOH/H_2O then LiOH[102]). The other procedure employs transesterification of the ring-opened ortho ester, usually to a methyl ester (with NaOMe/MeOH or K_2CO_3/MeOH) followed by base hydrolysis of the methyl ester to give the deprotected acid.[97]

1.4.2 N-Amine Protection

In developing a strategy for the synthesis of unusual amino acids from amino acid derived OBO ester synthons, we decided to use the benzyloxycarbonyl (carbobenzyloxy or Cbz) and *tert*-butyloxycarbonyl (Boc) protecting groups.

The Cbz group was first introduced into peptide chemistry in 1932[103] and has many advantages over other amino protecting groups. Unlike the fluorenyl-9-ylmethoxycarbonyl (Fmoc) group, the Cbz group is stable to basic reagents, and can be removed by catalytic hydrogenolysis or strong acid hydrolysis. The Cbz group is probably the most popular of the *N*-protecting group and as such, its continued use allows for the comparison of results to previous methods of synthesis.

The Boc group, introduced in 1957 (for a review of Boc and other protecting groups see Carpino),[104] has been used extensively in peptide synthesis for amine protection.[105] The Boc group is not hydrolyzed under basic conditions and is inert to many nucleophilic reagents. Removal is accomplished with trifluoroacetic acid providing a complementary deprotection procedure to that of the ortho ester which is also removed with acid.

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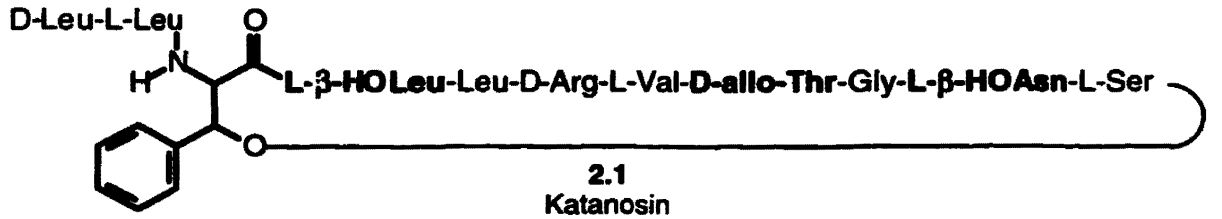
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Chapter Two

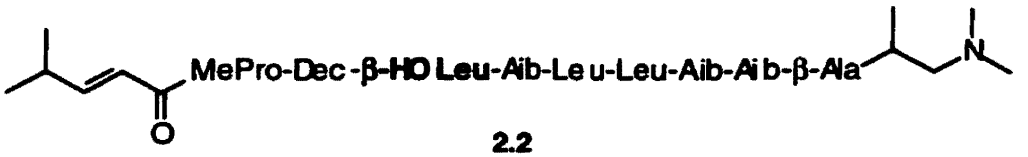
Synthesis of Boc Protected β-Hydroxy-α-Amino Acids

2.1 Introduction

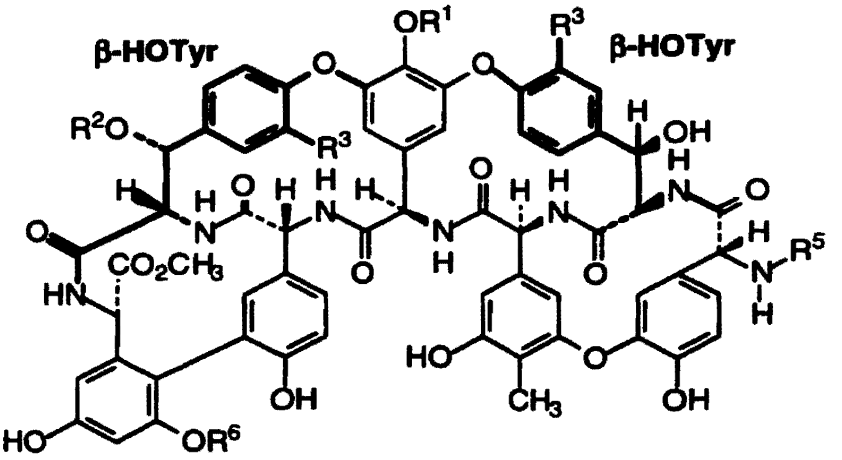
β-Hydroxy-α-amino acids are of immense interest due to either their direct biological action or as structural components of compounds with biological activities (Figure 2.1).[1,2] For example, D-*allo*-threonine is found in antibiotics such as the katanosins **2.1**,[3] while β-hydroxyleucine is found in the leucinostatins **2.2**[4] and the katanosins **2.1**. β-Hydroxytyrosine is present in antibiotics such as vancomycin **2.3**[5] (widely used to treat deep seated Gram negative bacterial infections), the orienticins **2.4**[6] and chloroorienticins **2.5**,[7] risocetins **2.6**[8] and chloramphenicol **2.7**[9] and a number of other cyclic peptides (avoparcin, teicoplanin, actapanin, parvocidin, actinodin, and chloropolysporin).[1] The catecholamine class of neurotransmitters (adrenaline **2.8** and norepinephrine **2.9**) incorporate a β-hydroxytyrosine moiety whereas epinephrine **2.10** contains β-hydroxyphenylalanine. β-Hydroxyproline and β-hydroxyaspartic acid have been isolated from empedopeptin[10] and β-hydroxyaspartic acid is also believed to play a role in hemeostasis proteins.[11] β-Hydroxyglutamine has been identified in antibiotics such as neopeptin.[12] (4*R*)-4-(*E*)-2-Butenyl-4,*N*-dimethyl-L-threonine **2.11**, probably the most well known β-hydroxy-α-amino acid, is a crucial constituent of cyclosporin A, a potent immunosuppressive drug.[13] β-Hydroxy-α-amino acids are also important intermediates in the synthesis of other compounds, for example, many β-lactams (with antimicrobial activity) have been synthesized via β-hydroxy-α-amino acids.[14]



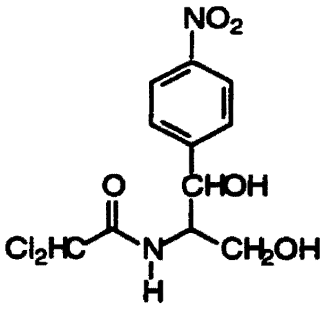
2.1
Katanosin



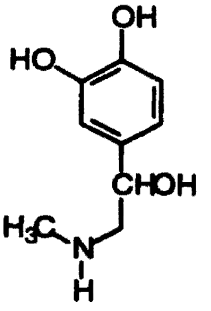
2.2
Leucinostatin A



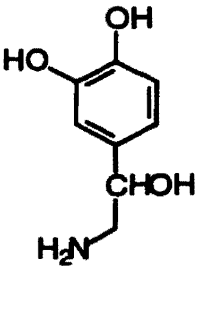
2.3 Vancomycin: R^1=sugar, R^2=R^5=R^6=H, R^3=R^5=Cl
2.4 Orienticins: R^1=R^2=sugar, R^3=Cl, R^4=R^6=H, R^5=H/Me
2.5 Chloroorienticins: R^1=R^2=sugar, R^3=R^4=Cl, R^5=H/Me, R^6=H
2.6 Risocetin: R^1=R^2=R^6=sugar, R^3=R^4=H, R^5=Tyr linkage to Gln



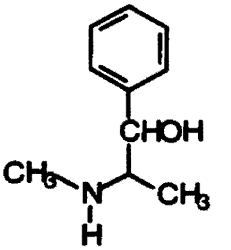
2.7
Chloroamphenicol
2*R*,3*S* active



2.8
Adrenaline
l-form active

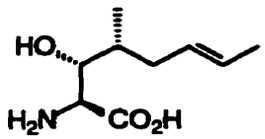


2.9
Norepinephrine
l-form active



2.10
Ephedrine
l-form active

Figure 2.1: β-Hydroxy-α-Amino Acids in Various Biological Compounds.



2.11 MeBmt

2.1.1 Synthesis of β-Hydroxy-α-Amino Acids: General Routes

Many of the general methods described in chapter one are capable of synthesizing β-hydroxy-α-amino acids and other recent methods include Sharpless AE,[1,15] Sharpless AD,[16] electrophilic amination[17] and hydroxylation,[18] stereoselective hydrolysis of aziridine carboxylate esters,[19] aldol condensations[20] from oxazolidinone intermediates[21] and numerous other methods.[22] A brief overview of the synthesis of β-hydroxy-α-amino acids complementing that in chapter one will be described.

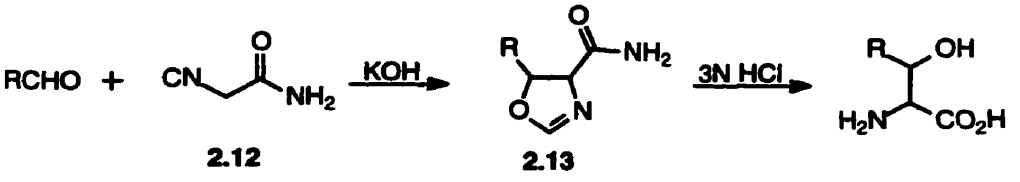
2.1.2 Synthesis of Racemic β-Hydroxy-α-Amino Acids

Racemic β-hydroxy-α-amino acids have traditionally been synthesized by the addition of glycine enolates to aldehydes and ketones. Numerous examples of this methodology exists and are typified by the report of Ozaki *et al.*[23] in which α-isocyanoacetamide **2.12** is reacted with aldehydes in the presence of KOH, forming the *trans*-oxazoline-4-carboxamides **2.13** (Scheme 2.1). Recrystallization followed by acid hydrolysis gave pure *threo*-β-hydroxy amino acids in excellent yields.

Shanzer *et al.* have reported a route to synthesize both *threo* and *erythro* isomers from glycine.[24] Reaction of the *N,N*-bis(trimethylsilyl)glycine trimethylsilyl ester **2.14** with aldehydes or ketones gave pure *erythro* diastereomer, attributable to induction by the bulky disilylated amino group (Scheme 2.2) The *threo* isomers were obtained from

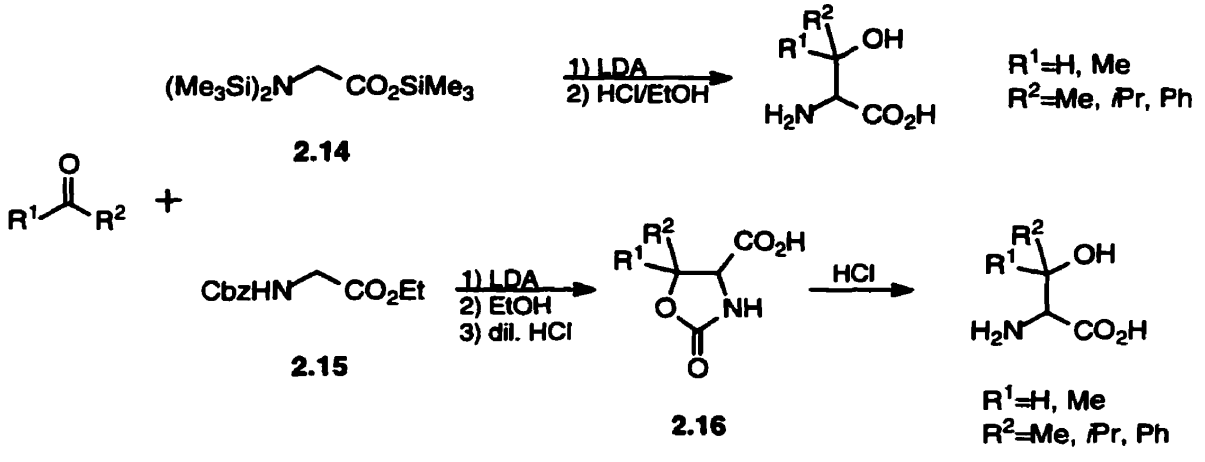
addition of the enolate of Cbz-Gly ethyl ester **2.15** to various carbonyl derivatives and subsequent cyclization to the 2-oxazolidone **2.16** intermediate followed by acid hydrolysis gave the β-alkyl and β-dialkyl-β-hydroxy-*threo*-α-amino acids in excellent yield.

Scheme 2.1



2.12 **2.13**

Scheme 2.2



2.15 **2.16**

2.1.3 Synthesis of Optically Active β-Hydroxy-α-Amino Acids

2.1.3.1 Enzymatic Synthesis of β-Hydroxy-α-Amino Acids

Various groups have tried to use the intrinsic chirospecificity of enzymes to synthesize β-hydroxy-α-amino acids with varying degrees of success. Saeed and Young first reported the use of serine hydroxymethyltransferase (EC 2.1.2.1) in 1992 using the enzyme to couple glycine with various aldehydes.[25] However, the products were

typically generated in both poor yield and *threo:erythro* selectivity and required prolonged reaction times limiting the use of this method.

Wong and coworkers have used L-threonine aldolase (EC 4.1.2.5) to synthesize various substituted β-hydroxy-α-amino acids.[26] Yields and selectivity are dependent on the aldehyde and vary significantly with *threo:erythro* selectivities of <1:99 and 71:29 reported for acetaldehyde and benzaldehyde respectively.

2.1.3.2 Enantioselective Reductions

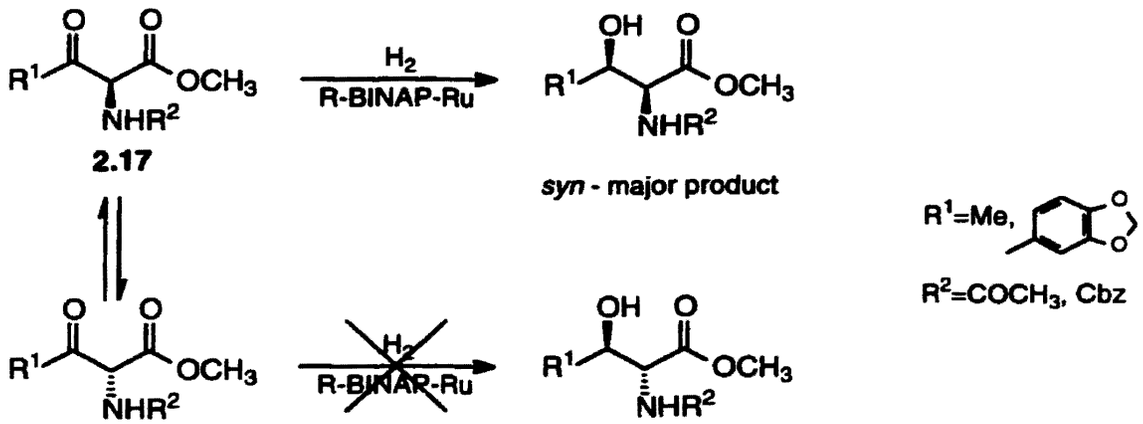
Catalytic asymmetric synthesis of β-hydroxy-α-amino acids has been accomplished by both the dynamic kinetic resolution of reduced carbonyls and the asymmetric hydrogenation of dehydro-α-amino acids.

Noyori and coworkers used a ruthenium-BINAP chiral catalyst in a dynamic kinetic resolution in which racemic keto ester **2.17** was selectively reduced (Scheme 2.3).[27] The stereomutation of the ester and selectivity of the catalyst are both rapid enough that 100% conversion to a single enantiomer occurs, avoiding the 50% loss normally associated with enzymatic resolutions. A number of substrates gave quantative conversion with the product amino acid esters having 93-98% ee and 94:6 to 99:1 *syn* selectivity.

Genêt *et al.* reported a similar methodology using various rhodium and ruthenium catalyzed hydrogenations with a wide variety of ligands.[28] The best results were achieved not only with BINAP-Ru as reported by Noyori but also with CHIRAPHOS®-Ru giving the *syn* product preferentially (97:3) with 85-99% ee. This methodology has been used

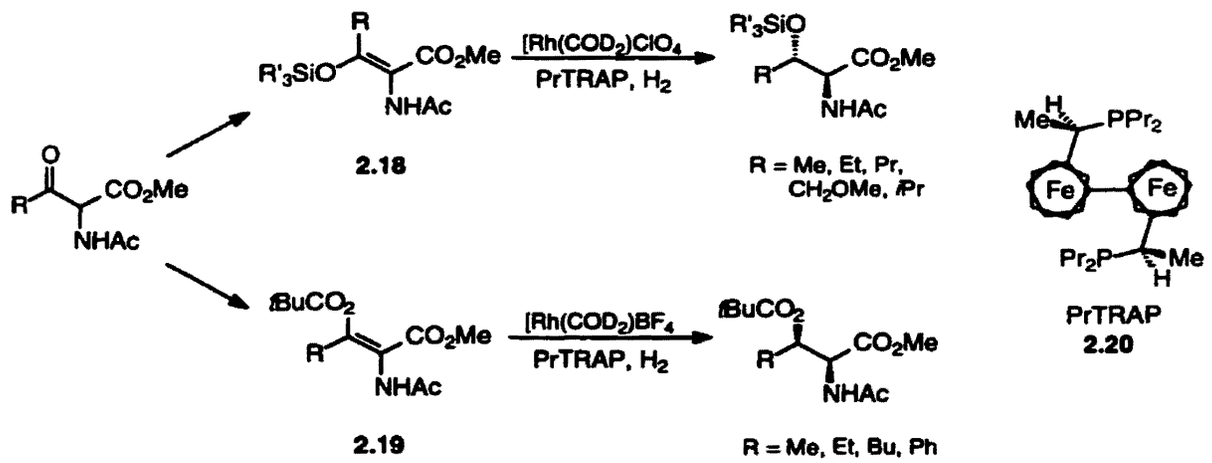
by Genêt *et al.* in the synthesis of *p*-chloro-3-hydroxytyrosine,[29] 3-hydroxylysine[30] and MeBmt **2.11**.[31]

Scheme 2.3



Ito and coworkers have reported the enantioselective synthesis of *erythro*- and *threo*-β-hydroxy-α-amino acids by asymmetric hydrogenation of (*Z*)- and (*E*)-β-oxy-α-acetamidoacrylates **2.18/2.19** with (*S*,*S*)-2,2"-bis[(*R*)-1-(dialkylphosphophino)ethyl]-1,1'-biferrocene (TRAP)-rhodium complexes **2.20** (Scheme 2.4).[32] Yields are generally >90% with 88-97% ee.

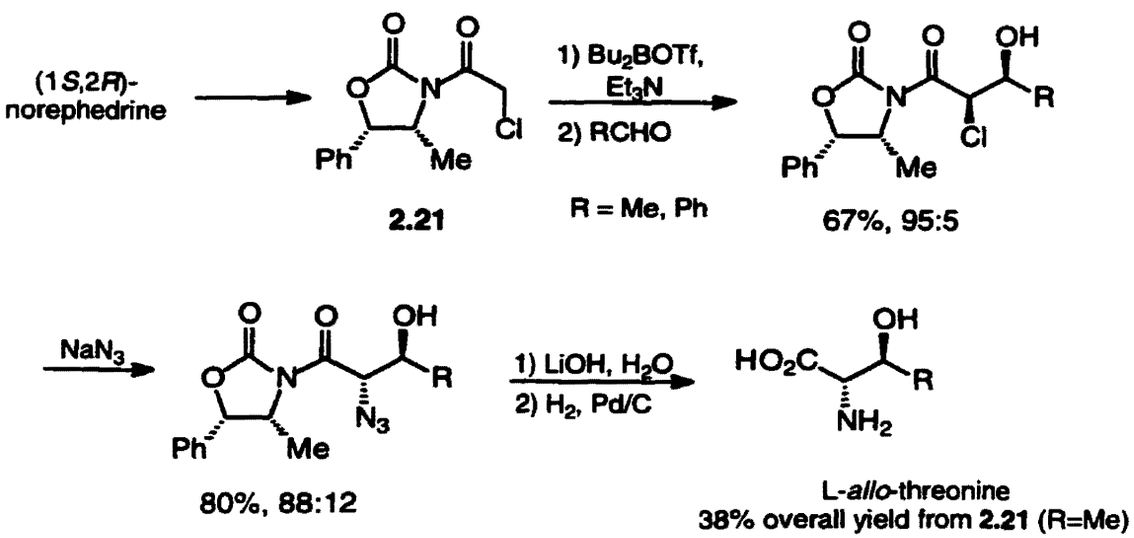
Scheme 2.4



2.1.3.3 Electrophilic/Nucleophilic Amination

Evans' methodology (Scheme 1.13) is capable of producing both 2*S*,3*R*/2*R*,3*S* (*syn* or *threo*) and 2*S*,3*S*/2*R*,3*R* (*anti* or *erythro*) diastereomers. Addition of the enolate of **2.21** to acetaldehyde gives a 95:5 selectivity of the *syn* diastereomer although 10% epimerization occurs at the α-center upon azide displacement (Scheme 2.5).[33] This methodology has been used to synthesize numerous β-hydroxy-α-amino acids including MeBmt **2.11**.[31]

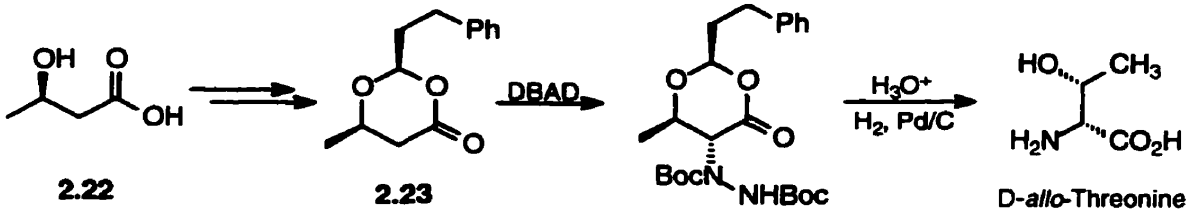
Scheme 2.5



Electrophilic amination has been used to synthesize D-*allo*-threonine from chiral 3-hydroxybutanoate **2.22** protected as a dioxanone **2.23** in 42% yield from the dioxanone and with 99% diastereoselectivity (Scheme 2.6) using di-*tert*-butyl azodicarboxylate (DBAD) as the aminating agent.[34]

Seebach and coworkers have used a similar methodology in the synthesis of trifluorothreonine in both excellent yield and diastereoselectivity using 4,4,4-trifluoro-3-hydroxybutanoate instead of **2.22**.[35]

Scheme 2.6

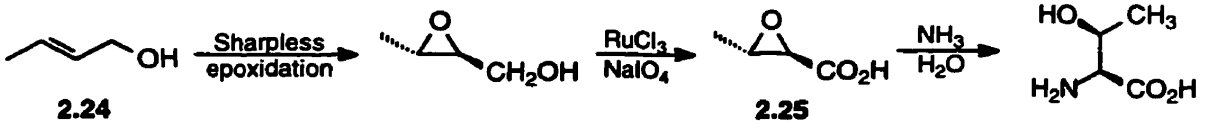


2.22 **2.23** D-*allo*-Threonine

Epoxides are obvious intermediates in the synthesis of β-hydroxy-α-amino acids since nucleophilic opening of the epoxide installs the desired β-hydroxy functionality. Furthermore, Sharpless epoxidation generally provides a convenient route to the desired epoxide chirality. In fact, many of the epoxide-derived methods described in section 1.3.2.1 are capable of the synthesis of β-hydroxy-α-amino acids.

Genêt and coworkers have used the Sharpless epoxidation of crotyl alcohol **2.24** to establish the epoxide in the desired conformation for the synthesis of L-*allo*-threonine.[36] Oxidation to the acid **2.25** followed by ring opening with ammonia gives L-*allo*-threonine in 29% overall yield (Scheme 2.7). However, the amination takes 10 days limiting the applicability of the method.

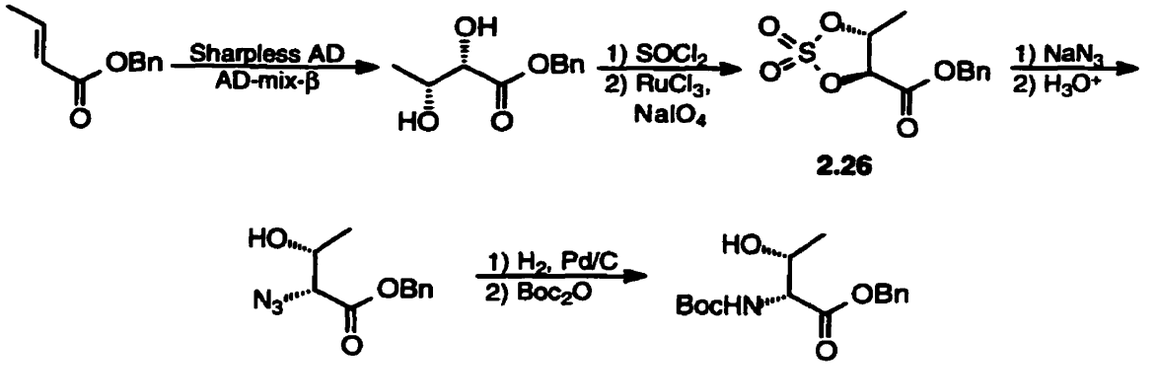
Scheme 2.7



2.24 **2.25**

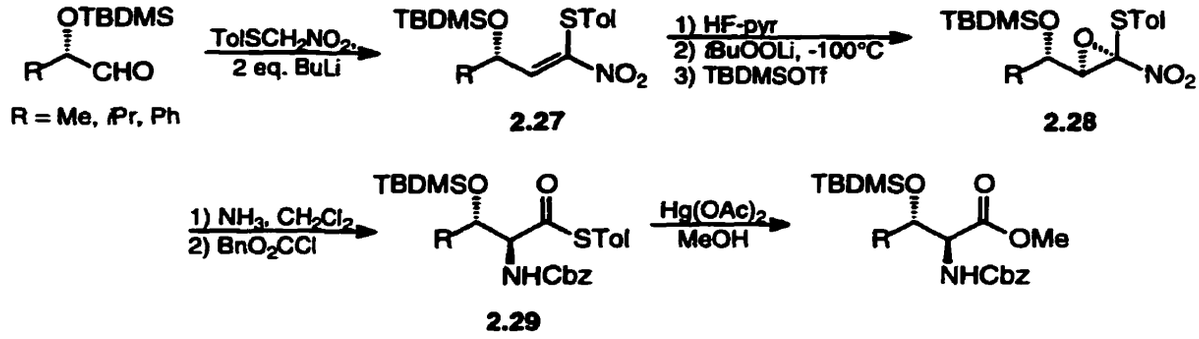
Goodman has used a cyclic sulfate **2.26** (an epoxide equivalent) derived from benzyl crotonate to synthesize *allo*-threonines and β-hydroxyvaline in both good yield and >99% ee (Scheme 2.8).[37]

Scheme 2.8



2-(Arylthio)-2-nitrooxiranes **2.27** have been used as intermediates towards the synthesis of various β-hydroxy-α-amino acids.[38] Either diastereomer can be accessed in the case of β-alkyl-β-hydroxy-α-amino acids while only *anti*-β-aryl-β-hydroxy-α-amino acids may be synthesized (Scheme 2.9). Various aldehydes were condensed with the dianion of (4-methylphenylthio)nitromethane to give the alkenes **2.27** in 52-74% yield (Scheme 2.9). Subsequent deprotection, epoxidation and reprotection gave epoxides **2.28** in 40-55% yield over three steps. *Syn* epoxidation occurs with *t*BuOOLi with 12:1 selectivity whereas *anti* epoxidation with Ph_3OOK gives 15:1 selectivity. Ring opening of the epoxide with aqueous ammonia and trapping with benzyl chloroformate gave the desired protected β-alkyl-β-hydroxy thioester derivatives in 55-68% yield. Treatment with either mercuric acetate or MeOLi in methanol gave the methyl ester protected β-alkyl-β-hydroxy-α-amino acid.

Scheme 2.9



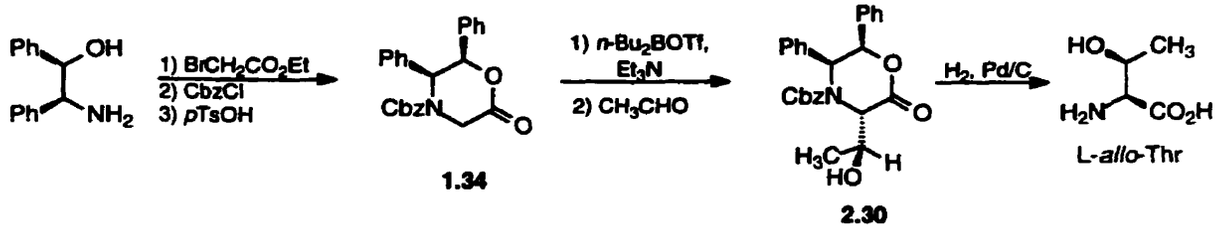
2.1.3.4 Synthesis from Glycine Enolates

Most of the methods of synthesizing α-amino acids via glycine enolates as discussed in chapter one (section 1.3.3) can be used to synthesize β-hydroxy-α-amino acids. The majority of glycine enolate equivalents tend to produce the *syn* or *threo* stereochemistry upon addition to aldehydes with no opportunity of producing the other diastereomer. The stereochemistry at the α-center is usually well defined in these electrophilic additions of aldehydes to glycine anion equivalents, whereas variable selectivity occurs at the β-carbon, in this instance the β-hydroxy functionality.

In general, Schöllkopf's bis-lactim ether **1.33** (Scheme 1.15), the Ni(II) glycine Schiff base **1.39** of Belekon *et al.* (Scheme 1.18) and Seebach's oxazolidinone **1.42** (Scheme 1.19) all add to aldehydes and ketones to give the *threo* diastereomer. Williams' glycine boron enolate equivalent derived from oxazinone **1.34** (Scheme 1.16) gives predominantly the *erythro* isomer.

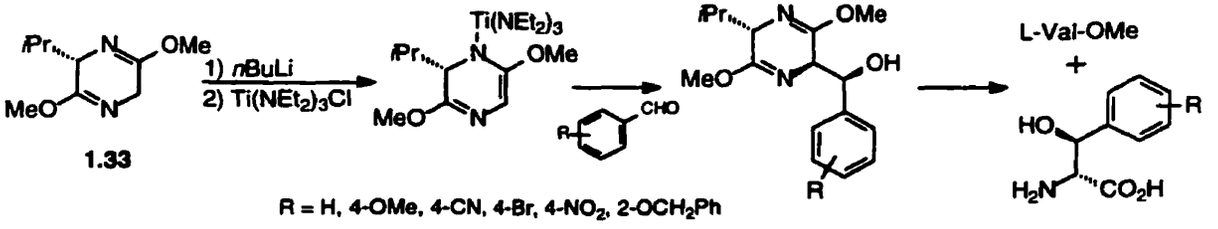
Williams' oxazinone **1.34** has been used as a boron enolate to add to acetaldehyde to give the protected L-*allo*-threonine derivative **2.30** in 85:15 diastereoselectivity and 57% yield (Scheme 2.10).[39]

Scheme 2.10



Schöllkopf *et al.* have synthesized the protected derivatives of D-*threo*-β-phenylserine with 86% ee and 4% de and D-*threo*-β-benzylserine with 89% ee and 66% de from the lithium enolate of the *bis*-lactim ether **1.33**. The titanium enolates generally gave much better diastereoselectivities upon condensation with a number of aromatic aldehydes to give the desired adducts in 65-83% yield (Scheme 2.11).[40] Deprotection by acid hydrolysis gave the methyl esters in 46-66% yields, significantly lower than yields obtained for the aliphatic β-hydroxy-α-amino acids indicating the relative instability of the aromatic compounds. The *bis*-lactim ether **1.33** enolate has also been reacted with acetone to give the β-dimethyl-β-hydroxy adduct in 98% yield and after deprotection obtained in 48% overall yield with >95% ee.[41]

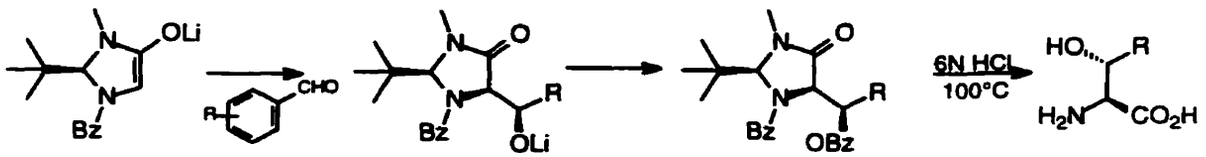
Scheme 2.11



Seebach's oxazolidinone enolate **1.42** has been reacted with aromatic aldehydes to give the *threo* adduct with concomitant transfer of the *N*-benzoyl protecting group to the hydroxyl in 77-85% yield and 88-96% diastereoselectivity (Scheme 2.12).[42] Only

hydrolysis of the L-*threo*-β-phenylserine derivative was reported, giving the free amino acid in 54% yield and 92:8 *threo*:*erythro* ratio with an overall yield of 46% from the imidazolidinone template **1.42**.

Scheme 2.12



1.42 R = H, 4-Me, 3,4(OCH_2O), 3-Ph

Another imidazolidinone-bound enolate **2.31**, described by Craddick *et al.*, has been shown to undergo aldol condensations with aliphatic and aromatic aldehydes in good yield (62-84%) and high stereocontrol (93-95% de) (Scheme 2.13).[43]

Scheme 2.13



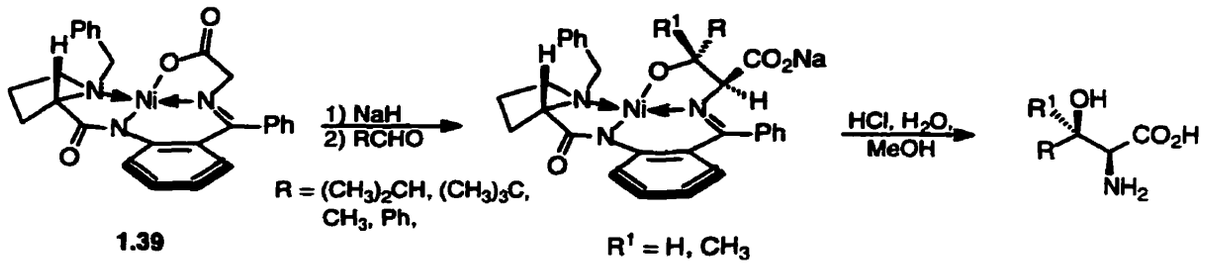
2.31

R = Bu, Ph, 4-MeC_6H_4, 4-FC_6H_4, 4-$NO_2C_6H_4$, 2-furaldehyde

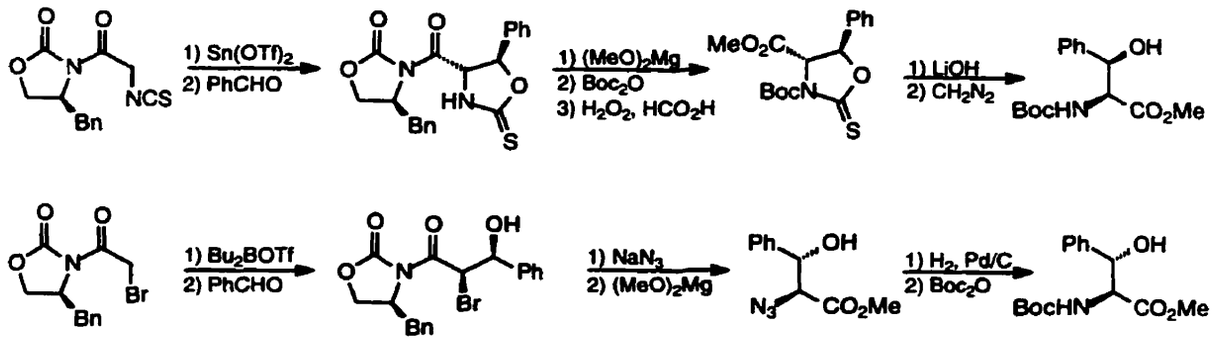
Belokon *et al.* have successfully used the chiral nickel (II) complex **1.39** in the synthesis of *threo*-β-hydroxy-α-amino acids usually with diastereoselectivities >30:1 and yields of 55-87% (Scheme 2.14).[44]

Unlike the glycine enolates described above, Evans' methodology provides access to both *syn* and *anti* diastereomers. β-Phenylserine was synthesized in 50% overall yield with >98% de in the stereocenter forming reactions (Scheme 2.15).[45]

Scheme 2.14



Scheme 2.15

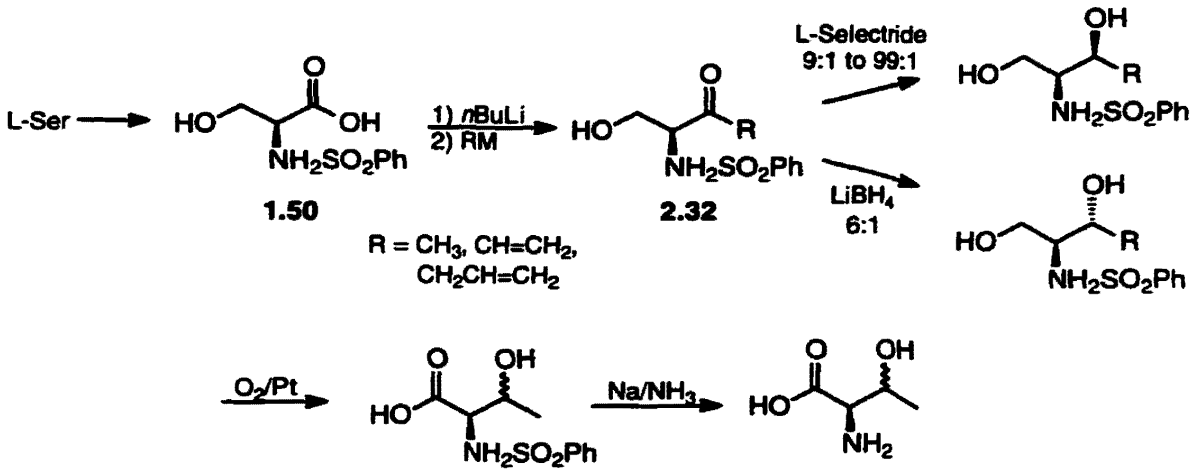


2.1.3.5 Synthesis from α-Amino Acids

Clearly, the most straightforward method for the synthesis of β-hydroxy-α-amino acids is the derivatization of serine and threonine. In particular, the serine aldehyde equivalents **1.50-1.53** described in section 1.3.4.1 are capable of producing 2*S*,3*R*/2*R*,3*S* (*syn* or *threo*) and 2*S*,3*S*/2*R*,3*R* (*anti* or *erythro*) diastereomers.

Rapoport's serinal equivalent **1.50** has been reacted with various organometallic reagents to give ketone **2.32** which is diastereoselectively reduced with L-Selectride to give the *syn* alcohol in ratios of 99:1 to 9:1 depending on the alkyl group or to the *anti* product with $LiBH_4$ in 6:1 selectivities (Scheme 2.16).[46] Oxidation and deprotection to the amino acid was not reported.

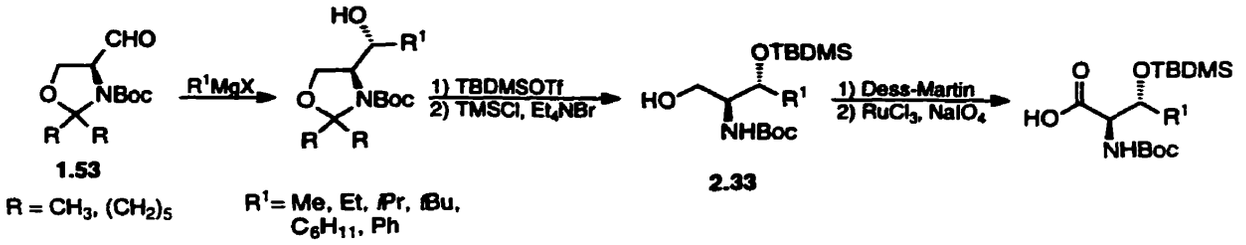
Scheme 2.16



Nucleophilic additions[47] to Garner's aldehyde **1.53** introduces the β-hydroxy motif in the correct position with good selectivity although the method suffers from the limitation that an oxidation step is required to generate the α-carboxylate necessitating a tedious protection/oxidation/deprotection scheme.

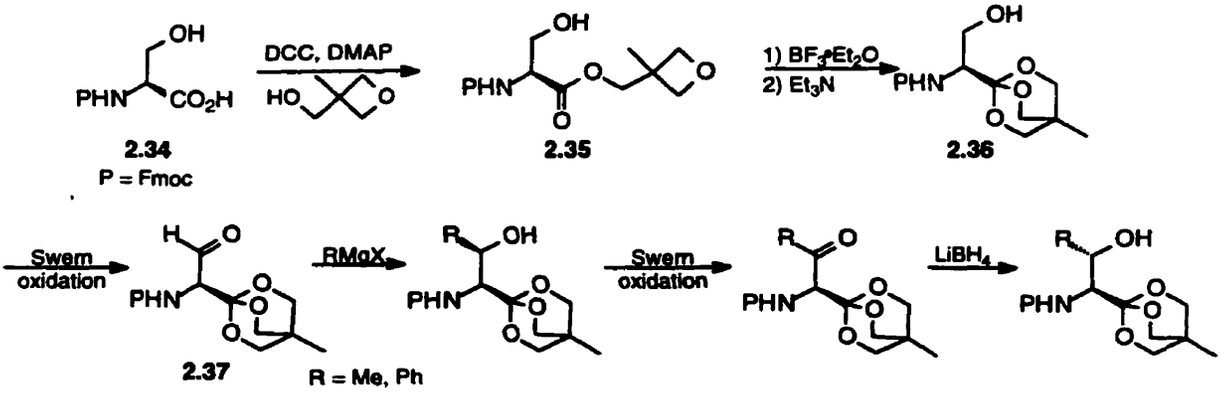
Joullié and coworkers have demonstrated good to high selectivity in the Grignard addition of various nucleophiles to Garner's aldehyde **1.53**. Generally the *erythro* diastereomer predominated (9:1 to 15:1) although in the case of PhMgBr the *threo* diastereomer was generated in excess (8:1) (Scheme 2.17).[48] Deprotection gave the amino alcohol **2.33** in 10-22% yield. The two-step oxidation to the protected β-hydroxy-α-amino acids proved to be difficult, proceeding in 5-22% yield under a number of conditions and demonstrating one of the shortcomings of Garner's aldehyde.

Scheme 2.17



This laboratory previously reported the synthesis of β-hydroxy-α-amino acids using serine protected as an ortho ester.[49] The use of the OBO ester as an α-carboxylate protecting group is an attempt to address the tendency of serinals to racemize for the reasons discussed in Sections 1.3.4.1 and 1.4. Fmoc-L-serine **2.34** was converted to the oxetane ester **2.35** by *N,N*-dicyclohexylcarbodiimide (DCC) and dimethylamino-pyridine (DMAP) mediated esterification with 3-methyl-3-(hydroxymethyl)oxetane (Scheme 2.18). Rearrangement to the OBO ester **2.36** and oxidation to the aldehyde **2.37** followed by Grignard addition gave protected β-hydroxy-α-amino acids in both good yield and excellent diastereoselectivity with a *threo:erythro* ratio of 94:6 (R=Me). The *erythro* diastereomer could then be generated in >98:<2 ratio by an oxidation/reduction sequence.

Scheme 2.18



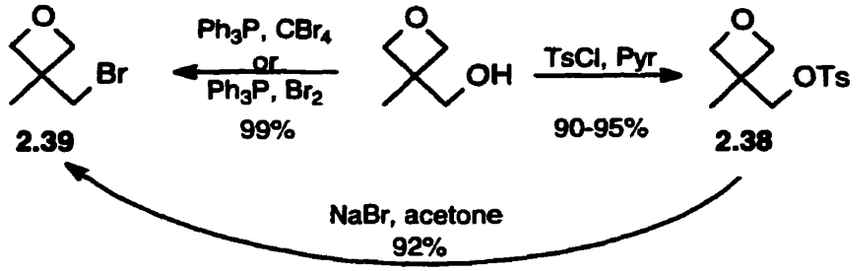
2.2 Results and Discussion

2.2.1 Preparation of Boc-Ser(ald)OBO ester

In an effort to expand the scope of the OBO ester methodology previously reported (Scheme 2.18),[49] we investigated expanding the various nitrogen protecting groups to include the Boc group. We were particularly interested in the synthesis of β-hydroxy-α-amino acids for the eventual incoporation and synthesis of analogs of the aureobasidin class of antifungal agents.[50]

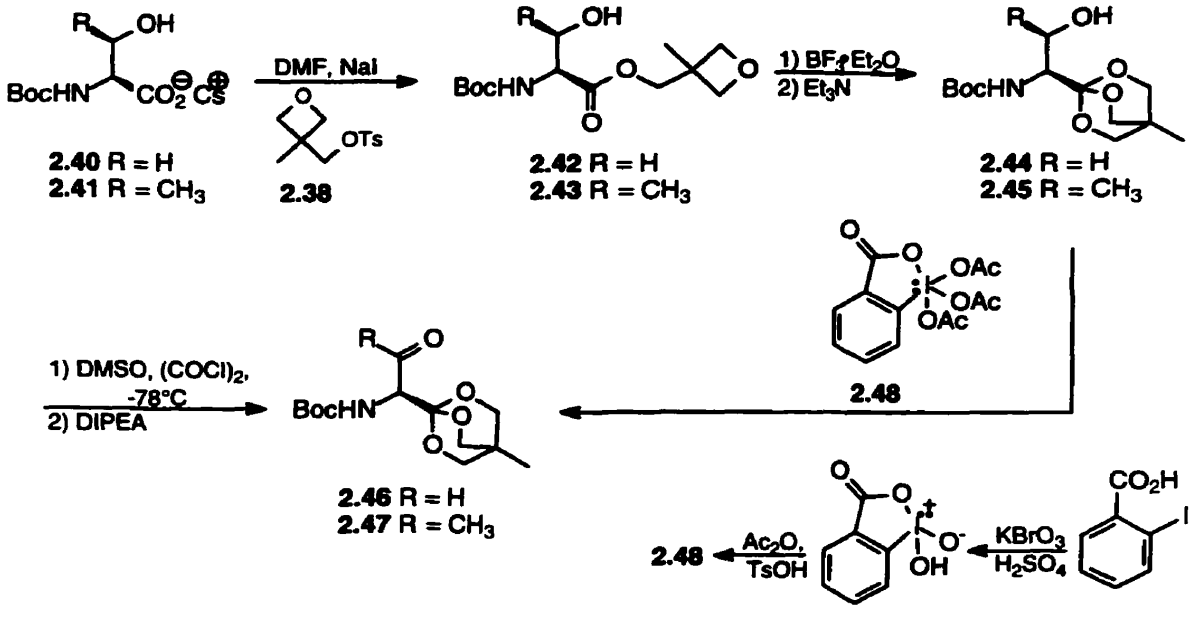
We initially attempted to overcome the difficulties associated with the esterification of oxetane alcohol with DCC and DMAP. Although the yields for the esterification were excellent (80-85%), the reaction required an excess of oxetane alcohol and chromatography on silica to obtain a pure product.[49] Trace amounts of the diimide or urea also affected the yields of the $BF_3{\cdot}Et_2O$ catalyzed rearrangement of the oxetane ester to the OBO ester. We found that the desired oxetane ester is much more conveniently prepared by esterification of the N-protected serine carboxylate by the corresponding oxetane tosylate **2.38** or bromide **2.39**. The oxetane bromide **2.39** can be prepared in a number of ways from the 3-methyl-3-(hydroxymethyl)oxetane with bromine/triphenylphosphine,[51] carbon tetrabromide/tri-phenylphosphine[52] or by displacement of the corresponding tosylate **2.38** with sodium bromide in acetone (Scheme 2.19). The oxetane bromide **2.39** may be distilled from these reactions but slowly decomposes upon standing. The tosylate **2.38** was prepared using standard conditions and then poured into a slurry of ice/cold water at which point the tosylate precipitated out and was isolated as a stable crystalline material in 90-95% yield after filtration and drying under vacuum over P_2O_5.

Scheme 2.19



The best yields for the esterification reaction were obtained using the cesium salt of Boc-L-serine **2.40** or Boc-L-threonine **2.41** with the oxetane bromide **2.39** (85-90%) (Scheme 2.20). However, the preferred method is esterification with the oxetane tosylate **2.38** in DMF in the presence of sodium iodide (10 mol%) as distillation and storage of the sensitive oxetane bromide **2.39** is avoided. In these latter conditions the Boc serine derivative **2.42** was obtained in 66% yield and the Boc threonine derivative **2.43** in 73% yield as oils which crystallized upon standing. The formation of the ortho ester **2.44** and **2.45** from the oxetane esters **2.42** and **2.43** was performed in CH_2Cl_2 with a catalytic amount of $BF_3{\cdot}Et_2O$ (2-5 mol%). The more acid sensitive Boc derivative **2.44**, in comparison to the Fmoc and Cbz derivatives, is obtained in 66% yield after chromatography with similar yields obtained for the threonine derivative **2.45**. Oxidation under Swern conditions gave the aldehyde **2.46** and ketone **2.47** in 90-95% yields. Reduction with $NaBH_4$, deprotection and HPLC analysis of the derivatized amino acid (*vide infra*) indicated no loss of optical activity during the conversion to the aldehyde **2.46** or ketone **2.47**.

Scheme 2.20



The aldehyde was also generated using the Dess-Martin periodinane[53] **2.48**, synthesized by a slight modification of the method described by Ireland.[54] Although Schreiber and coworkers described acceleration of the Dess-Martin oxidation when performed in the presence of water,[55] it was imperative that the reaction be executed under anhydrous conditions due to the sensitivity of the ortho ester to acid hydrolysis. Results initially varied considerably from batch to batch of the periodinane **2.48**, with some batches failing to give the desired aldehyde **2.46** although oxidation of benzyl alcohol proceeded successfully. We determined that residual acetic acid, from the acetylation reaction to give the periodinane **2.48**, was opening the ring of the ortho ester and hence a complex mixture of products. Washing of **2.48** with large volumes of anhydrous diethyl ether under N_2 in a Schlenk tube gave **2.48** with consistent purity for the synthesis of aldehyde **2.46**. An advantage of this method of oxidizing the alcohol is that the reaction is performed at room temperature, although the synthesis of **2.48**

requires careful attention to prevent any contaminating acetic acid which hydrolyzes the OBO ester.

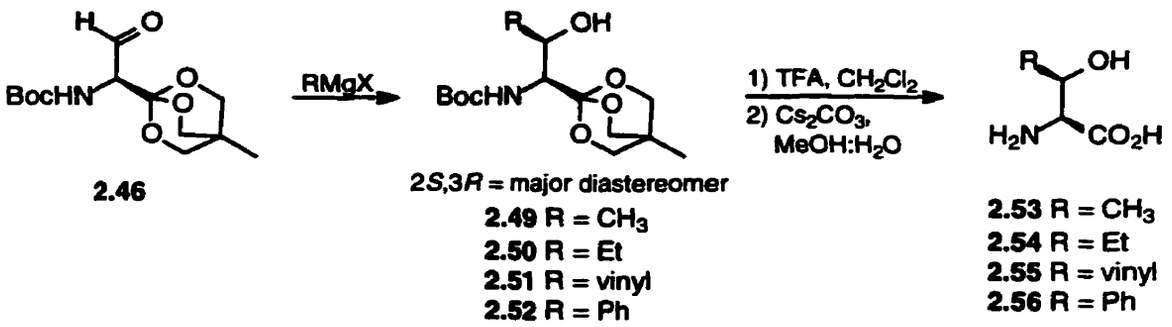
Both Boc-Ser-OBO ester **2.44** and Boc-Thr-OBO ester **2.45** have been stored for over one year at room temperature with the slow loss of optical activity, primarily due to OBO ester ring opening. Aldehyde **2.46** tends to rapidly lose its optical activity due to racemization even when stored at –20°C.

*2.2.2 Addition of Various Grignard Reagents to Boc-Ser(ald)OBO ester **2.46** and Boc-Thr(ket)OBO ester **2.47**.*

In order to investigate the suitability of Boc protected amino acids to the conditions required for the synthesis of OBO ester protected β-hydroxy-α-amino acids, we first examined the Grignard addition of methylmagnesium halides to Boc-Ser(ald)OBO ester **2.46** since the products would correspond to threonine and *allo*-threonine. Standards are available for all four diastereomers (L- and D-threonine and L- and D-*allo*-threonine or 2*S*,3*R*; 2*R*,3*S*; 2*S*,3*S*; 2*R*,3*R* respectively) and therefore the stereoselectivity and extent of racemization could be monitored.

A variety of conditions, based on earlier experience, were examined (Table 2.1).[49] Typically, the Grignard reagent (3 equivalents) was added quickly to a solution of the aldehyde **2.46** at the appropriate temperature (Scheme 2.21). Reaction times generally varied from 0.5-2 hours and the progress of the reaction monitored by TLC. The reaction mixture was quenching by pouring into cold 3% NH_4Cl after a TLC indicated no further progression in the reaction. The yields for all reactions in Table 2.1 are reported for the two step procedure from Boc-Ser-OBO ester **2.44** (Swern oxidation then Grignard reaction) since the crude aldehyde was generally used without further purification.

Scheme 2.21



2.46

2*S*,3*R* = major diastereomer
2.49 R = CH_3
2.50 R = Et
2.51 R = vinyl
2.52 R = Ph

2.53 R = CH_3
2.54 R = Et
2.55 R = vinyl
2.56 R = Ph

As summarized in Table 2.1, the type of Grignard reagent, solvent and temperature were examined in an attempt to optimize the addition. Little difference in yield was obtained with various halide counter ions and thus MeMgBr was used as the reagent of choice due to its commercial availability. Temperature and solvent played a more important role in determining both yield and diastereomeric ratios. Increased temperatures resulted in lower yields due to the formation of complex mixtures of by-products that were not isolated. The moderate increased reactivity of the Grignard reaction in toluene at 0°C (entry 5) is an anomaly, although not without precedent.[56] This enhanced reactivity through the use of a non-polar and noncoordinating solvent has been previously described.[57] However, when the addition was performed in toluene at -78°C, the increased reactivity experienced at 0°C was not observed although the stereoselectivity was increased. The reaction failed to proceed to completion even with extended reaction times (entry 10) for all solvents at -78°C.

The major by-product of the additions was unreacted Boc-Ser(ald)OBO ester **2.46**. One of the most frequent side reactions encountered with Grignard reagents is enolization.[58] Formation of the enolate of the aldehyde **2.46**/ketone **2.47** would compete with the addition reaction and upon work-up produce the aldehyde, albeit racemic. In

order to investigate this as a possible side reaction, once the reaction appeared to have reached completion, it was quenched and the remaining aldehyde reduced with $NaBH_4$ since the aldehyde is known to racemize during exposure to silica gel. The specific rotation of the Boc-Ser-OBO ester **2.44** (-40.2) generated by reduction was found to be close to the specific rotation of pure **2.44** (-41.8) indicating minimal enolization of the unreacted aldehyde occurs during Grignard addition. This consequently eliminated enolization as a possible side reaction preventing reaction of the aldehyde.

Table 2.1: Addition of MeMgX to Boc-Ser(ald)OBO ester 2.46.

Entry	Reagent[a]	Solvent	Temp. (°C)	Yield (%)[b]	diast. ratio[c] *threo*:*erythro*	% ee[c]
1	MeMgCl	THF	0	30	-	-
2	MeMgBr	THF	0	42	70:30[e]	-
3	MeMgI	THF	0	21	-	-
4	MeMgBr	Et_2O[d]	0	39	-	-
5	MeMgBr	toluene	0	55	71:29[e]	-
6	MeMgBr	THF	-78	60 (75)	79:21	96
7	MeMgBr	Et_2O[d]	-78	59 (78)	85:15	96
8	MeMgBr	Et_2O/ CH_2Cl_2	-78	61 (76)	85:15	98
9	MeMgBr	CH_2Cl_2	-78	60 (75)	83:17	96
10	MeMgBr	toluene	-78	51 (85)	82:18	98

[a] 3 Equivalents of Grignard reagent added.
[b] Yield for 2 steps (from **2.44**). Value in parenthesis represents yield based on recovered starting material.
[c] Ratio determined by HPLC analysis unless otherwise stated.
[d] Minimal CH_2Cl_2 was used to dissolve the aldehyde.
[e] Ratio determined by ^{1}H-NMR.

Having optimized the addition of MeMgBr our attention turned to the addition of other Grignard reagents to Boc-Ser(ald)OBO ester **2.46** (Scheme 2.21, Table 2.2). In all cases the addition caused little racemization with the deprotected amino acids all possessing >97% ee. Diastereoselectivity of the crude product ranged from 84:16 to 88:12 *threo*:*erythro* and yields from 40-66%. Purification by flash chromatography and subsequent deprotection and HPLC analysis of the ion-exchange purified amino acid showed a slight increase in diastereoselectivity. In fact, although the R_f for both diastereomers were typically similar, diastereomeric enrichment seemed to be occurring after flash chromatography although the values determined by ^{1}H-NMR integration are subject to experimental error. Steric bulk of the Grignard reagent does not appear to play much of a role in stereoselectivity since similar *threo*:*erythro* ratios are obtained for all reagents.

Table 2.2: Additions of RMgBr to Boc-Ser(ald)OBO ester 2.46.

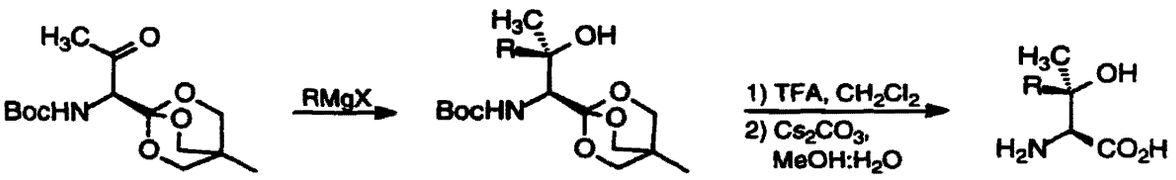
Entry	RMgBr	Product	% yield[a]	protected aa ds ratio (*S*,*R*:*S*,*S*)[b] crude	deprotected aa ds ratio (*S*,*R*:*S*,*S*)[c]	% ee[c]	% overall yield
1	Et	**2.50**	49	84:16	87:13	98	24
2	vinyl	**2.51**	40	87:13	89:11	97	16
3	Ph	**2.52**	66	88:12	90:10	98	40

[a] Yield reported for 2 steps (from **2.44**).
[b] Ratio determined by ^{1}H-NMR
[c] Ratio determined by HPLC analysis

Addition of MeMgBr to Boc-Thr(ket)-OBO ester **2.47** gave protected β-hydroxyvaline **2.57** (Scheme 2.22, Table 2.3). The reaction proceeded in good yield (72%, over two steps) but, as with the additions to the aldehyde **2.46**, TLC analysis indicated remaining ketone **2.47**. The recovered ketone **2.47**, which was stable on silica,

retained its optical activity indicating enolization with the α-hydrogen was not responsible for preventing complete reaction of the ketone, although the enolization of the methyl ketone cannot be disregarded. However, a similar observation of incomplete reaction of Grignard reagent with **2.46** suggests some other phenomena is preventing complete reaction of the ketone.

Scheme 2.22

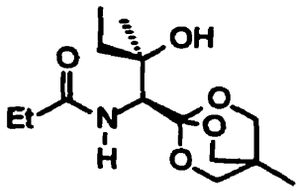


2.47

2*S*,3*R* = major diastereomer
2.57 R = CH_3
2.58 R = Et

2.59 R = CH_3
2.60 R = Et

Excellent stereoselectivity of nucleophilic addition was observed with the bulkier EtMgBr (98:2 *threo*:*erythro*) Grignard reagent when reacted with **2.47**. The stereochemistry of the adduct **2.58** was assigned through spectroscopic comparison to literature values[49] and is consistent with the direction of attack observed with the serine aldehyde **2.46**. This model is discussed in further detail in section 2.2.5. Yields for the addition of both MeMgBr and EtMgBr were generally good; however, the longer reaction times necessary for optimal yields in the case of EtMgBr resulted in the formation of by-product **2.61** in less than 5% yield. This occurs as a result of competitive attack of the Grignard reagent on the carbamate and has been previously reported to take place with bulky Grignard reagents and prolonged reaction times.[59] Although the carbamate most likely exists as the magnesium enolate, slow equilibrium to the carbonyl and subsequent attack of EtMgBr is the probable mechanism of formation.



2.61

Table 2.3: Addition of RMgBr to Boc-Thr(ket)OBO ester 2.47.

Entry	RMgBr	Product	% yield[a]	protected aa ds ratio (*S*,*R*:*S*,*S*)[b] crude	deprotected aa ds ratio (*S*,*R*:*S*,*S*)[c]	% ee[c]	% overall yield
1	Me	**2.57**	72	-	-	99	38
2	Et	**2.58**	65	98:2	99:1	99	37

[a] Yield reported for 2 steps (from **2.45**).
[b] Ratio determined by ^{1}H-NMR
[c] Ratio determined by HPLC analysis

2.2.3 Deprotection of β-Dialkyl- and β-Hydroxy-α-Amino Acids

A necessary part of any synthetic route is the straightforward removal of protecting groups. However, this is the stage at which numerous methods fail for different reasons. Once the various β-hydroxy-α-amino acids had been synthesized, deprotection was required in order to assess both diastereomeric and enantiomeric purity.

2.2.3.1 Boc Removal

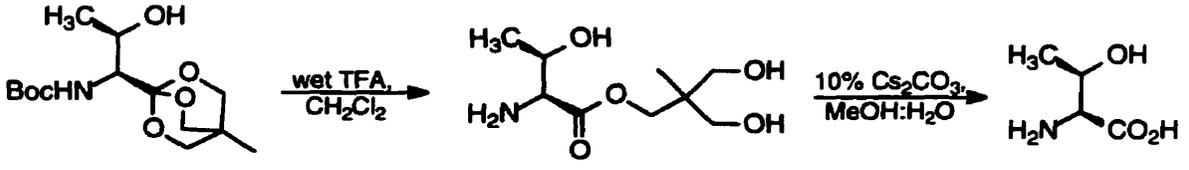
The Boc protecting group was easily removed with wet trifluoroacetic acid (TFA) in CH_2Cl_2 under standard conditions and was typically complete in less than 30 minutes (Scheme 2.23).[60]

2.2.3.2 Ortho Ester Cleavage

Concomitant cleavage of the ortho ester occurred during Boc cleavage with exposure to wet TFA giving the 2-methyl-2-hydroxymethyl-1,3-propanediol ester (mphd) derivative **2.62**.

Several multi-step methods have been reported in the literature for removing bicyclic ortho esters (Section 1.4.1). One employs acid hydrolysis to initially open the ortho ester and base hydrolysis to generate the acid or transesterification of the ring opened ortho ester to a methyl ester followed by base hydrolysis of the methyl ester to give the acid with yields normally <60%.[61] Removal of the mphd ester by the methods described above was found to be unsuccessful.[62] However, alkali carbonates have been shown to be more efficient at ester hydrolysis than bicarbonates[63] and the combination of Cs_2CO_3 in MeOH:H_2O was highly effective in cleaving the ring-opened mhpd ester **2.62** to the acid **2.53**.

Scheme 2.23



2.49 **2.62** **2.53**

The optimized one-pot procedure consists of concurrent cleavage of the Boc protecting group and the OBO ester to the mhpd ester **2.62** with TFA. After evaporation to dryness, the residue was dissolved in methanol to which a solution of 10% Cs_2CO_3 was added in an amount to ensure at least 5 equivalents of base was added.

2.2.3.3 Purification Methods

Once the amino acid was deprotected, ion-exchange chromatography was used to purify the product from the salts of the cleavage reaction. Cation purification was generally used for the Cs_2CO_3 based cleavages and volatile bases (5% Et_3N in H_2O or 0.5 N NH_4OH) employed for elution, allowing for the isolation of the free amino acid. The solution from the Cs_2CO_3 cleavage was acidified before loading onto the resin. Most often, contamination resulted from incomplete hydrolysis of the mhpd ester **2.62**. Anion exchange chromatography effectively removed the mhpd ester during column loading and washing, and elution with either 0.5 N acetic or formic acid allowed the free amino acid to be isolated. Anion exchange chromatography was most often used during purification of the β-dialkyl-β-hydroxy amino acids in order to minimize exposure of the dehydration sensitive β-dialkyl-β-hydroxy moiety to basic conditions. Although strong anion exchange resins incorporate a basic, polymer supported tetra alkylammonium hydroxide, no racemization has been observed in previous reports from this laboratory.[49] Crude yields after ion-exchange chromatography varied from 40-81%, however, generally in the 50% range.

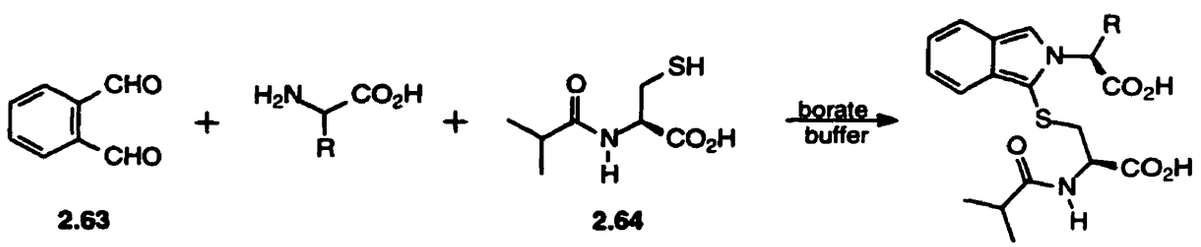
Many of the synthesized amino acids were difficult to recrystallize although spectroscopic techniques indicated high purity after ion-exchange chromatography. Diastereomeric ratios could be determined by ^{1}H-NMR and both diastereomeric and enantiomeric ratios assigned after derivatization and HPLC analysis (Section 2.2.4). Good agreement was found between the two methods.

2.2.4 *Determination of Enantiomeric Purity by HPLC Analysis*

Due to the propensity of serinals to racemize via enolization, evaluation of the enantiomeric purity of the product amino acids was essential in establishing the Boc-Ser(ald)OBO ester **2.46** as a practical alternative to other serinal based synthetic methods. A number of techniques are commonly used to determine enantiomeric purity, all of which rely upon the formation of a diastereomeric complex and subsequent quantitation by spectroscopic or chromatographic methods. However, previous experience within this laboratory has shown the method of Brückner *et al.*[64] to be particularly powerful in resolving α-amino acid diastereomers.[49]

This method relies upon the derivatization of amino acids with *o*-phthalaldehyde **2.63** and *N*-isobutyryl-L-cysteine **2.64** (Scheme 2.24) and their separation on a reverse phase column with detection at 338 nm. The procedure is very rapid (less than 5 minutes) and most importantly is very general.

Scheme 2.24



2.63 **2.64**

The sample is prepared by dissolving crude cleaved product in borate buffer and mixing with *o*-phthalaldehyde **2.63** and *N*-isobutyryl-L-cysteine **2.64**. After 5 minutes, 25μL was injected onto a reverse phase column and eluted under a gradient of 30 mM sodium acetate buffer (pH 6.5) and MeOH. Retention times of the derivatized amino acids **2.53-2.56** and **2.59-2.60** compared favourably to identical previously synthesized

amino acids.[49,65] The extent of racemization caused by oxidation of Boc-Ser-OBO ester **2.44** to the aldehyde **2.46** was determined by comparing deprotected reduced aldehyde to a sample of deprotected Boc-Ser-OBO ester starting material. Enantiomeric purities of >97% ee were routinely assessed for the reduced, deprotected aldehyde.

HPLC analysis conditions were generally established independently for each amino acid. In general, the *threo* and *erythro* diastereomers were well resolved but the L- and D-enantiomers presented more of a challenge. Racemic D,L-*N*-*i*-Bu-cysteine was used to establish conditions for the separation of the amino acid D,L enantiomers.

*2.2.5 Stereoselectivity of Carbonyl Additions to Boc-Ser(ald)OBO ester **2.46** and Boc-Thr(ket)OBO ester **2.47**.*

The nucleophilic carbonyl addition reactions described (*vide supra*) show surprising diastereoselectivity for an acyclic system. Diastereoselectivity ranged from 79:21 to 99:1 (*threo*:*erythro*) for the addition of various Grignard reagents to both Boc-Ser(ald)OBO ester **2.46** and Boc-Thr(ket)OBO ester **2.47** at –78°C. These results compare favourably to those reported by Beaulieu *et al.*[66] in which addition of MeMgBr to Garner's Cbz-protected aldehyde **1.53** gave a 1:1 mixture of diastereomers although better diastereoselectivities have been observed with other nucleophiles (>80% ee).[67]

The stereochemical outcome observed in the carbonyl additions to Boc-Ser(ald)OBO ester **2.46** and Boc-Thr(ket)OBO ester **2.47** is consistent with both a chelation (Figure 2.2A) and non-chelation (Figure 2.2B) controlled Felkin-Anh attack on the aldehyde from the face opposite to the OBO ester group (*re* face attack – see Figure 2.2) (for a review of carbonyl additions see Gawley and Aubé,[68] for Anh's model).[69]

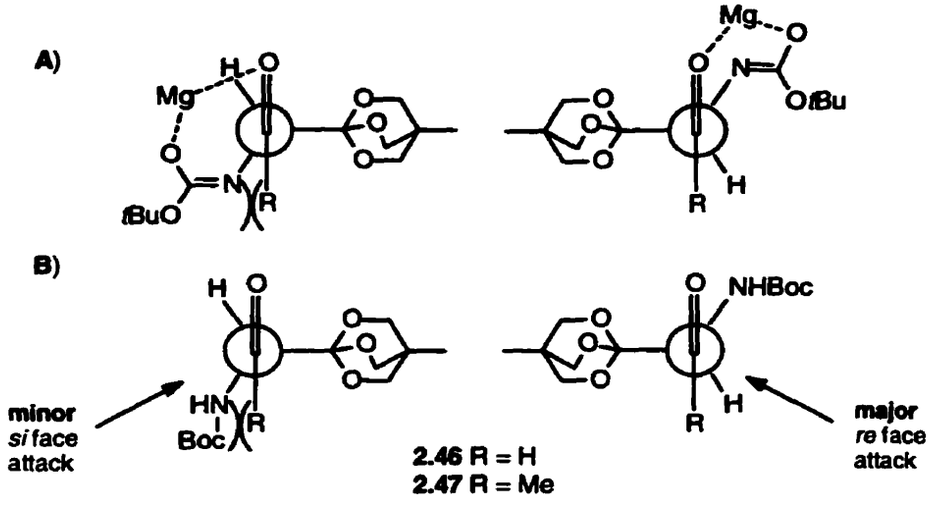


Figure 2.2: Felkin-Anh Model Predicting *re* Face Attack on the Side Chain Carbonyl of 2.46 and 2.47 A) Chelation, B) Non-Chelation models.

The diastereoselectivities observed for the addition of Grignard reagents to Boc protected OBO derivatives are in most cases comparable to those achieved upon nucleophilic addition to Fmoc and Cbz protected OBO derivatives.[49,65] However, the addition of MeMgBr to Boc-Ser(ald)OBO ester **2.46** gave an 85:15 *threo*:*erythro* ratio whereas addition to the Fmoc and Cbz equivalents[65] resulted in 96:4 and 97:3 ratios respectively. This might be attributed to the steric attributes of the Fmoc and Cbz groups compared to Boc, an observation alluded to by the apparently more accessible carbamate in the case of **2.47** due to the absence of formation of side-products **2.61** in the Fmoc and Cbz derivatives.

2.3 Summary

The synthetic strategy described provides a route to various Boc protected *threo*-β-dialkyl- and β-alkyl-β-hydroxy-α-amino acids from the Boc-Ser(ald)-OBO ester **2.46** and Boc-Thr(ket)-OBO ester **2.47** synthons. Overall yields ranged from 16-40% and all products had >96% ee. Diastereomeric purities ranged from 70:30 to 99:1 *threo*:*erythro*; with ratios generally increasing with steric bulk and reduced reaction temperatures.

The overall yields and diastereoselectivity of Grignard additions to Boc-Ser(ald)OBO ester **2.46** and Boc-Thr(ket)OBO ester **2.47** generally correspond to those achieved for the analogous Fmoc and Cbz protected derivatives. In most cases, the diastereoselectivities are comparable to previously published methods (Section 2.1.3) but the method does not require harsh oxidation or deprotection steps. A variety of sidechains have been readily introduced and deprotection to the free amino acid is easily achieved.

2.4 Experimental

2.4.1 General Methods.

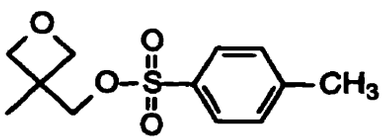
Most reagents were purchased from the Aldrich Chemical Company. Boc-Ser and Boc-Thr were purchased from Advanced ChemTech. CH_2Cl_2 was distilled from CaH_2; toluene, THF and Et_2O from Na/benzophenone. Reactions were carried out under N_2 or Ar in glassware dried overnight at 120°C or flame dried before use.

NMR spectra were recorded in $CDCl_3$ (referenced to TMS at 0.00 ppm for ^1H, to $CDCl_3$ at 77.00 ppm for ^{13}C) or D_2O (referenced to *2,2,3,3-d_4*-3-(trimethylsilyl)propionic acid, sodium salt at 0.00 ppm for both ^1H and ^{13}C) on a Bruker AC-200, AM-250, AM-300, Avance 300 or Avance 500 spectrometer. $CDCl_3$ used for NMR samples containing an ortho ester was prefiltered through basic alumina to remove traces of acid. IR spectra were recorded on a Bomem MB-100 FT-IR spectrophotometer. Optical rotations were measured on a Perkin-Elmer 241 digital polarimeter. Melting points were determined on a Mel-Temp apparatus in an open capillary tube and are uncorrected. Low resolution mass spectral analysis were performed on a VG Quattro II triple quadrupole mass spectrometer (Micromass, UK) equipped with an electrospray source interfaced with an Hewlett-Packard HPLC running 50:50 H_2O/MeCN (0.1 % formic acid) at a flow rate of 30 µl/min at the University of Waterloo. Elemental analyses were performed by MHW Laboratories, Pheonix, Arizona. TLC was carried out on Merck aluminum backed silica gel 60 F_{254}, with visualization by UV, ninhydrin solution (2% in EtOH), or I_2.

HPLC analyses were performed using a Waters 600E System Controller with Waters 600 Multisolvent Delivery System, Model 481 or 486 Variable Wavelength UV/Vis Detector, and Waters 745 Data Module.

2.4.2 2-Methyl-2-(toluenesulfonyloxymethyl)oxetane, Oxetane tosylate, 2.38.

Tosyl chloride (57.20 g, 0.3 mol) was dissolved in dry pyridine (400 mL) under argon. 3-Methyl-3-(hydroxymethyl)oxetane (20.4 g, 0.2 mol) was added slowly and stirred for 1.5 hours. A slurry of cold water (200 mL) and crushed ice (400g) was vigourously stirred and the oxetane tosylate mixture slowly poured in then allowed to stir for an additional 20 minutes. The white precipitate was then collected on Whatman filter paper # 1 and washed with cold H_2O. The product was dried under vacuum over P_2O_5 to obtain the white powder of oxetane tosylate (49.11g, 92%).



mp 49.5-51°C. TLC (3:2, Hex:EtOAc) $R_f = 0.42$; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.81 (d, 2H, J = 8.2Hz, Ar*H*), 7.37 (d, 2H, J = 8.2Hz, Ar*H*), 4.44-4.31 (m, 4H, C*H*$_2$O), 4.11(s, 2H, SO_3CH_2), 2.46 (s, 3H, Ar-C*H*$_3$), 1.31 (s, 3H, CC*H*$_3$); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 145.1 (Ar<u>C</u>SO_3), 132.8 (Ar<u>C</u>CH_3), 129.9, 127.9 (Ar<u>C</u>H), 78.9 (oxetane <u>C</u>H_2O), 74.2 (oxetane SO_3<u>C</u>H_2), 39.3 (oxetane <u>C</u>CH_3), 21.6 (oxetane <u>C</u>CH_3), 20.6 (Ar<u>C</u>H_3). HRMS (FAB) calcd for (M + H^+) $C_{12}H_{16}O_4S$ 256.0769, found 256.0774. Anal. Calcd for $C_{12}H_{16}O_4S$: C, 56.23; H, 6.29. Found: C, 56.33; H, 6.44.

2.4.3 2-Methyl-2-(bromomethyl)oxetane, oxetane bromide, 2.39.

Procedure A : 3-Methyl-3-(hydroxymethyl)oxetane (50.0 mL, 0.50 mol) and carbon tetrabromide (182.9 g, 0.55 mol) were dissolved in CH_2Cl_2 (500 mL) and cooled to 0°C under argon. Triphenylphosphine (157.8 g, 0.60 mol) was added in portions. The reaction was warmed to room temperature and stirred for a further 20 minutes under argon. The CH_2Cl_2 was removed *in vacuo* and Et_2O (500 mL) was added to the mixture.

The crude product was filtered through Celite to remove some of the by-product Ph_3PO. The filtrate was then concentrated to a viscous liquid and hexane (500 mL) was added. The mixture was filtered once more through Celite to remove most of the by-product Ph_3PO. The filtrate was concentrated to obtain a viscous yellow oil. The crude product was distilled under vacuum with a fractionation column. Oxetane bromide **2.39** was collected as a viscous liquid in 95-99% (81.0 g) yield.



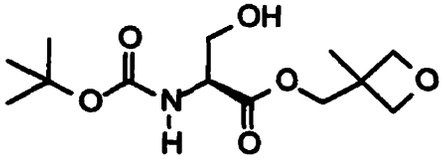
bp 62-64°C/10 mm Hg; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 4.46-4.38 (d+d, 4H, J = 6.3Hz, CH_2O), 3.65 (s, 2H, CH_2Br), 1.44 (s, 3H CCH_3); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 80.5 ($\underline{C}H_2O$), 41.3 ($\underline{C}H_2Br$), 40.5 ($\underline{C}CH_3$), 22.3 ($C\underline{C}H_3$). IR (neat) 2960, 1453, 1233; ESI-MS ($M + H^+$) 157.21, 159.22; Anal. Calcd for C_5H_9BrO : C, 36.39; H, 5.50. Found : C, 36.50; H, 5.70.

Procedure B: To a stirred solution of oxetane alcohol (20.4 g, 0.20 mole), triphenylphosphine (63.0 g, 0.24 mole), and pyridine (39 ml, 0.48 mole) in 200 ml CH_2Cl_2 was slowly added a solution of bromine (10.2 mL, 0.2 mol) in 50 mL of CH_2Cl_2 at 0°C. The mixture was stirred at room temperature for one hour. The solvent was removed under reduced pressure. To the residue was added Et_2O (400 mL) and the mixture then filtered through Celite and the filtrate evaporated *in vacuo*. Hexanes (200 mL) was then used to rinse the remaining solids. The final filtrate was concentrated and distilled to give the product **2.39** (22.7 g, 69%).

Procedure C: 2-Methyl-2-(toluenesulfonyloxymethyl)oxetane **2.38** (25.0 g, 97.53 mmol) and NaBr (50.18 g, 0.49 mol, 5.0 equiv) were suspended in dry acetone (250 mL) under dry nitrogen and refluxed for 30 hours. The solution was first filtered and decolorizing charcoal was added. The solution was filtered and the acetone was evaporated *in vacuo* to give a colorless thick oil (14.8 g, 92%) which was used without further purification.

2.4.4 (2-Methyl-2-oxetanyl)methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-hydroxy-propanoate, Boc-Ser oxetane ester, 2.42.

Boc-L-Ser **2.40** (5.0 g, 0.024 mol) and Cs_2CO_3 (4.69 g, 0.014 mol, 0.6 eq) were combined and dissolved in H_2O (50 mL). The water was then removed *in vacuo* and the resulting oil lyophilized for 12 hours to give a white foam. To this foam was added oxetane tosylate **2.38** (6.46 g, 0.025 mol) and NaI (0.72 g, 4.8 mmol, 0.2 eq) which was then taken up in DMF (250 mL) and allowed to stir under Ar for 48 hours. The DMF was then removed *in vacuo* and the resulting solid dissolved in EtOAc (300 mL) and H_2O (100 mL) and extracted with 10% $NaHCO_3$ (2 × 50 mL), saturated NaCl (1 × 50 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to yield a yellow oil which was purified by flash chromatography (1:1, EtOAc:Hex) to yield the ester **2.42** in 66% yield (4.32 g) as a pale oil.

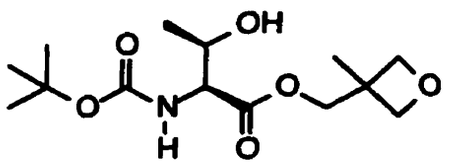


$[\alpha]^{20}_{578}$= -15.4 (c=1.02, EtOAc); TLC (1:1, EtOAc:Hex) R_f = 0.33; ^{1}H NMR ($CDCl_3$,

250 MHz) δ 5.47 (br d, 1H, J = 8.0Hz, N*H*), 4.47 (d, 1H, J = 6.1Hz, β-C*H*H), 4.43 (d, 1H, J = 6.1Hz, β-CH*H*), 4.35-4.27 (m, 4H, 2 oxetane CH_2O), 4.07-3.57 (m, 3H, α-C*H*, CO_2CH_2), 3.01 (br s, 1H, O*H*), 1.36 (s, 9H, $(CH_3)_3C$), 1.24, (s, 3H, oxetane CCH_3); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 171.0 (C=O), 155.6 (CONH), 80.1 ($(CH_3)_3C$) , 79.8 (oxetane CH_2O), 68.8 (CO_2CH_2), 63.1 (β-CH_2), 55.8 (α-CH), 39.3 (oxetane CCH_3), 28.2 ($(CH_3)_3C$), 20.7 (oxetane CCH_3); IR (neat) 3386, 2970, 1746, 1713, 1509, 1367, 1163, 1060; HRMS (FAB) calculated for (M + H^+) $C_{13}H_{24}NO_6$ 290.1604, found 290.1594. Anal. calcd for $C_{13}H_{23}NO_6$: C, 53.78; H, 8.33; N, 4.83. Found: C, 53.97; H, 8.62; N, 5.06.

2.4.5 (2-Methyl-2-oxetanyl)methyl-(2*S*,3*R*)-2-[(*tert*-butoxycarbonyl)amino]-3-hydroxybutanoate, Boc-Thr oxetane ester, 2.43.

Boc-L-Thr **2.41** (5.0 g, 0.023 mol) and Cs_2CO_3 (4.65 g, 0.014 mol, 0.6 eq) were combined and dissolved in H_2O (50 mL). The water was then removed *in vacuo* and the resulting oil was lyophilized for 12 hours to give a white foam. To this foam was added oxetane tosylate **2.38** (6.41 g, 0.024 mol) and NaI (0.71 g, 4.7 mmol, 0.2 eq) which was then taken up in DMF (250 mL) and allowed to stir under Ar for 48 hours. The DMF was then removed *in vacuo* and the resulting solid dissolved in EtOAc (300 mL) and H_2O (100 mL) and extracted with 10% $NaHCO_3$ (2 × 50 mL), saturated NaCl (1 × 50 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to yield a yellow oil which was purified by flash chromatography (1:1, EtOAc:Hex) to yield the ester **2.43** as a white solid in 73% yield (4.32g).

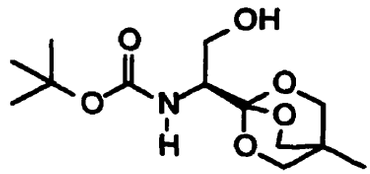


mp 69-71°C; $[\alpha]^{20}_{578}$ = -24.5 (c = 1.04, EtOAc); TLC (1:1, EtOAc:Hex) R_f = 0.21; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 5.40 (br d, 1H, *J* = 9.2Hz, N*H*), 4.54 (d, 2H, *J* = 6.1Hz, oxetane CH_2O), 4.50 (d, 2H, *J* =6.1 Hz, oxetane CH_2O), 4.44 (d, 1H, *J* = 6.1Hz, CO_2CHH), 4.42 (d, 1H, *J* = 6.1Hz, CO_2CHH), 4.40-4.35 (m, 1H, β-C*H*), 4.30 (br d, 1H, *J* = 9.2Hz, α-C*H*), 2.60 (br s, 1H, O*H*), 1.36 (s, 9H, $(CH_3)_3C$), 1.27, (s, 3H, oxetane CCH_3), 1.21 (d, 3H, *J* = 6.3Hz, β-CH_3); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 171.6 (C=O), 155.8 (CONH), 80.1 ($(CH_3)_3C$), 79.5 (oxetane CH_2O), 68.6 (CO_2CH_2), 68.0 (β-CH), 59.0 (α-CH), 39.7 (oxetane CCH_3), 28.3 ($(CH_3)_3C$), 20.8 (oxetane CCH_3), 20.0 (β-CH_3); IR (cast in CH_2Cl_2) 3386, 2975, 1694, 1515, 1367, 1164, 1055; HRMS (FAB) calculated for (M + H^+) $C_{14}H_{26}NO_6$ 304.1761, found 304.1769; Anal. calcd for $C_{14}H_{25}NO_6$: C, 55.43; H, 8.31; N, 4.62. Found: C, 55.74; H, 8.51; N, 4.68.

2.4.6 (1*S*)-1-[1-*N*-(*tert*-Butoxycarbonyl)-(1*S*)-1-amino-2-hydroxyethyl]-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane, Boc-L-Ser-OBO ester, 2.44.

Boc-Ser oxetane ester **2.42** (5.0 g, 0.0183 mol) was dissolved in dry CH_2Cl_2 (250 mL) and cooled to 0°C under Ar. $BF_3 \cdot Et_2O$ (0.11 mL, 0.93 mmol) was diluted in CH_2Cl_2 (5.0 mL) and added to the reaction flask. The reaction was allowed to warm to room temperature and checked by TLC. After 18 hours, Et_3N (0.51 mL, 3.66 mmol) was added and the reaction stirred for an additional 30 minutes before being concentrated to a thick oil. The crude product was redissolved in EtOAc (200 mL) and washed with 3% NH_4Cl (2 × 100 mL), 10% $NaHCO_3$ (100 mL), saturated NaCl (100 mL), dried ($MgSO_4$), and

evaporated to dryness. The residue was purified by flash chromatography (silica gel, 4:1 CH_2Cl_2:EtOAc) to give a light yellow oil (3.35 g) in 67% yield.

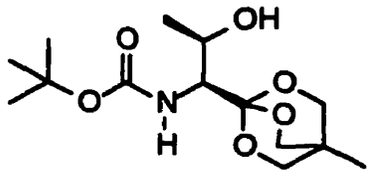


$[\alpha]^{20}_{578}$ = -41.8 (c = 1.00, EtOAc); TLC (4:1 CH_2Cl_2:EtOAc) R_f = 0.47; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 5.05 (d, 1H, *J* = 7.7Hz, N*H*), 3.90 (s, 6H, OBO ester CH_2O), 3.85-3.62 (m, 3H, α-C*H*, β-CH_2), 2.65 (br s, 1H, O*H*), 1.42 (s, 9H, (CH_3)$_3$C), 0.79 (s, 3H, OBO ester CCH_3); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 155.6 (CONH), 108.1 (OBO ester C-O) 79.3 ((CH$_3$)$_3$C), 72.6 (OBO ester CH$_2$O), 67.0 (β-CH$_2$), 62.9 (α-CH), 40.2 (OBO ester CCH$_3$), 27.9 ((CH$_3$)$_3$C), 13.9 (OBO ester CCH$_3$); IR (neat) 3386, 2975, 1694, 1515, 1367, 1164, 1055. Anal. calcd for $C_{13}H_{23}NO_6$: C, 53.78; H, 8.33; N, 4.83. Found: C, 54.02; H, 8.61; N, 5.02.

2.4.7 1-[*N-tert*-Butoxycarbonyl-(1*S*,2*R*)-1-amino-2-hydroxypropyl]-4-methyl-2,6,7-trioxabicyclo [2.2.2]octane, Boc-L-Thr-OBO ester, 2.45.

Boc-L-Thr oxetane ester **2.43** (4.18 g, 13.7 mmol) was dissolved in dry CH_2Cl_2 (250 mL) and cooled to 0 °C under Ar. $BF_3 \cdot Et_2O$ (0.08 mL, 0.69 mmol) was diluted in CH_2Cl_2 (2.0 mL) and added to the reaction flask. The reaction was allowed to warm to room temperature and was checked by TLC. After 24 hours, Et_3N (0.38 mL, 2.76 mmol) was added and the reaction was stirred for an additional 30 minutes before being concentrated to a thick oil. The crude product was redissolved in EtOAc (200 mL) and washed with 3% NH_4Cl (2 × 100 mL), 10% $NaHCO_3$ (100 mL), saturated NaCl (100 mL), dried

($MgSO_4$), and evaporated to dryness. The product was purified by flash chromatography (4:1, CH_2Cl_2:EtOAc) to give a white solid (2.84 g) in 68% yield.

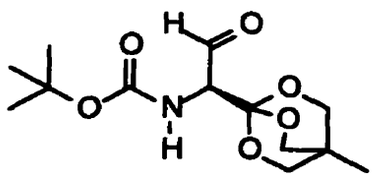


mp 128-129°C; $[\alpha]^{20}_{578}$ = -15.2 (c = 1.04, CH_2Cl_2); TLC (4:1, CH_2Cl_2:EtOAc) R_f = 0.39; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 5.06 (br d, 1H, *J* = 10.4Hz, N*H*), 4.31 (br q, 1H, *J* = 6.4Hz, β-C*H*), 3.89 (s, 6H, OBO ester CH_2O), 3.63 (d, 1H, *J* = 10.4Hz, α-C*H*), 2.88, (br s, 1H, O*H*), 1.42 (s, 9H, (CH_3)$_3$C), 1.06, (d, 3H, *J* = 6.4Hz, β-CH_3), 0.79 (s, 3H, OBO ester CCH_3); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 156.3 (CONH), 108.8 (OBO ester C-O), 79.2 ((CH$_3$)$_3$C), 72.6 (OBO ester CH_2O), 65.3 (β-CH), 57.1 (α-CH), 30.5 (OBO ester CCH$_3$), 28.3 (CH$_3$)$_3$C), 18.9 (β-CH$_3$), 14.3 (OBO ester CCH$_3$); IR (cast from CH_2Cl_2) 3386, 2975, 1715, 1506, 1365, 1169, 1049; HRMS (FAB) calculated for (M + H$^+$) $C_{14}H_{26}NO_6$ 304.1761, found 304.1732; Anal. calcd for $C_{14}H_{25}NO_6$: C, 55.43; H, 8.30; N, 4.61. Found: C, 55.63; H, 8.56; N, 4.92.

2.4.8 1-[*N-tert*-Butoxycarbonyl-(1*S*)-1-amino-2-oxoethyl]-4-methyl-2,6,7-trioxabicyclo [2.2.2]octane, Boc-L-Ser(ald) OBO ester, 2.46.

Boc-Ser OBO ester **2.44** (1.12 g, 3.87 mmol) was dissolved in freshly distilled CH_2Cl_2 (15 mL) under Ar and cooled to -78°C in flask 1. Oxalyl chloride (0.54 mL, 6.19 mmol, 1.60 equiv) was added to CH_2Cl_2 (120 mL) in a separate round bottom flask (flask 2) under Ar, and cooled to -78°C. Dry DMSO (0.91 mL, 12.8 mmol, 3.30 equiv) was added to the oxalyl chloride solution (flask 2) and the mixture was stirred at -78°C for 15

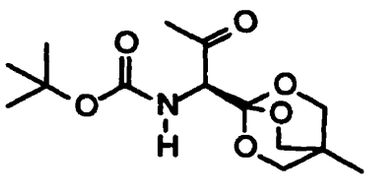
minutes. The alcohol solution was transferred slowly by cannula to flask 2 over a period of 15 minutes and then rinsed with CH_2Cl_2 (5 mL). The resulting cloudy, white mixture was stirred for 1.5 hours at -78°C. DIPEA (3.35 mL, 19.4 mmol, 5.0 equiv) was added and the solution stirred for 30 minutes at -78°C and 10 minutes at 0°C. Ice-cold CH_2Cl_2 (100 mL) was added and the solution was washed with ice-cold 3% NH_4Cl (2 × 80 mL), saturated NaCl (1 × 80 mL), dried ($MgSO_4$), and evaporated to dryness to yield a light coloured foam (1.10g) in 99% yield.



$[\alpha]^{20}_{578}$= -44.8 (c =1.02, CH_2Cl_2); TLC (1:1, EtOAc:Hex) R_f = 0.58; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 9.64 (s, 1H, C*H*O), 5.07 (br d, 1H, *J* = 7.8Hz, N*H*), 4.48 (d, 1H, *J* = 7.8Hz, α-C*H*), 3.91 (s, 6H, OBO ester CH_2O), 1.41 (s, 9H, (CH_3)$_3$C), 0.79 (s, 3H, OBO ester CCH_3); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 195.7 (CHO), 155.6 (CONH), 107.3 (OBO ester C-O), 80.1 ((CH$_3$)$_3$C), 72.8 (OBO ester CH_2O), 63.3 (α-CH), 30.9 (OBO ester CCH$_3$), 28.2 ((CH$_3$)$_3$C), 14.3 (OBO ester CCH$_3$); IR (cast from CH_2Cl_2) 3374, 2973, 1716, 1506, 1166, 1046; HRMS (FAB) calculated for (M + H^+) $C_{13}H_{22}NO_6$ 288.1447, found 288.1425. Anal. calcd for $C_{13}H_{21}NO_6$: C, 53.78; H, 8.33; N, 4.83. Found: C, 53.98; H, 8.56; N, 4.89.

2.4.9 1-[*N-tert*-Butoxycarbonyl-(1*S*)-1-amino-2-oxopropyl]-4-methyl-2,6,7-trioxabicyclo [2.2.2]octane, Boc-L-Thr(ket)OBO ester, 2.47.

Boc-Thr OBO ester **2.45** (0.90 g, 2.97 mmol) was dissolved in freshly distilled CH_2Cl_2 (15 mL) under Ar and cooled to -78°C in flask 1. Oxalyl chloride (0.414 mL, 4.75 mmol, 1.60 equiv) was added to CH_2Cl_2 (20 mL) in a separate round bottom flask (flask 2) under Ar, and cooled to -78°C. Dry DMSO (0.70 mL, 9.8 mmol, 3.30 equiv) was added to the oxalyl chloride solution (flask 2) and the mixture was stirred at -78°C for 15 minutes. The alcohol solution was transferred slowly by cannula to flask 2 over a period of 45 minutes and then rinsed with CH_2Cl_2 (5 mL). The resulting cloudy, white mixture was stirred for 1.5 hours at -78°C. DIPEA (2.57 mL, 14.9 mmol, 5.0 equiv) was added and the solution stirred for 30 minutes at -78°C and 10 minutes at 0°C. Ice-cold CH_2Cl_2 (80 mL) was added and the solution was washed with ice-cold 3% NH_4Cl (2 × 50 mL), saturated NaCl (1 × 50 mL), dried ($MgSO_4$), and evaporated to dryness to yield a light coloured foam (0.885g) in 99% yield.

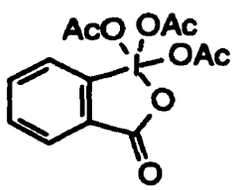


$[\alpha]^{20}_{578}$= -107.1 (c =1.51, EtOAc); TLC (1:1, EtOAc:Hex) R_f = 0.58; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 5.37 (br d, 1H, *J* = 8.3Hz, N*H*), 4.46 (d, 1H, *J* = 8.3Hz, α-C*H*), 3.89 (s, 6H, OBO ester CH_2O), 2.28 (s, 3H, β-CH_3) 1.39 (s, 9H, (CH_3)$_3$C), 0.79 (s, 3H, OBO ester CCH_3); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 201.6 (C=O), 156.6 (CONH), 107.3 (OBO ester C-O) , 80.1 ((CH$_3$)$_3$C), 72.8 (OBO ester CH$_2$O), 63.3 (α-CH), 30.9 (OBO ester CCH$_3$), 29.8 (β-CH$_3$), 28.2 ((CH$_3$)$_3$C), 14.3 (OBO ester CCH$_3$); IR (cast from CH_2Cl_2) 2972, 2883, 1716, 1506, 1362, 1167, 1045; HRMS (FAB) calculated for (M + H$^+$) $C_{14}H_{24}NO_6$

302.1604, found 302.1593; Anal. calcd for $C_{14}H_{23}NO_6$: C, 55.80; H, 7.69; N, 4.64. Found: C, 56.01; H, 7.96; N, 4.86.

2.4.10 1,1,1-Triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1*H*)-one, 2.48.[53]

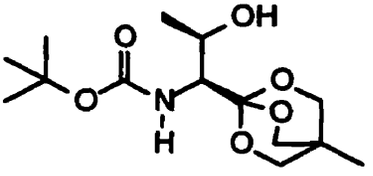
Potassium bromate (19 g, 0.11 mol) was added over a 0.5 hour period to a vigorously stirring mixture of 2-iodobenzoic acid (21.3 g, 0.08 mol) and 200 mL of 0.73 M H_2SO_4. During addition the temperature of the reaction was kept below 55°C but then warmed to 65°C and stirred for an additional 3.5 h. The mixture was then cooled to 0°C and the precipitate filtered off then washed with 700 mL of water and two 30 mL portions of cold ethanol. The precipitate was dried under water aspirator then taken up in Ac_2O (90 mL, 0.85 mol) and AcOH (75 mL, 1.4 mol) under N_2. The vigorously stirring mixture was slowly heated to 85°C over 1 h and the now clear solution stirred for 1.5 h at 85°C then allowed to cool to ambient temperature under N_2. The solution was tightly sealed, wrapped in foil and allowed to slowly crystallized over 2 days. The crystals were transferred under N_2 through a male-male adapter into a Schlenk tube. The white crystals were thoroughly washed with freshly distilled Et_2O (10 × 50 mL) using positive N_2 pressure and vacuum to assist in the filtration. The crystals were dried under N_2 then transferred to dry amber vials and purged with N_2.



mp 125–126.5°C; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.69 (br dd, 1H, Ar*H*), 8.33 (d, 1H, *J* = 8.5Hz, Ar*H*), 8.29 (d, 1H, *J* = 8.5Hz, Ar*H*), 7.90 (br dd, 1H, Ar*H*), 2.27 (s, 3H, C*H*$_3$CO), 2.01 (s, 6H, 2 C*H*$_3$CO).

2.4.11 MeMgBr addition to 2.46: 1-[*N-tert*-Butoxycarbonyl-(1*S*,2*R*)-1-amino-2-hydroxypropyl]-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane, Boc-L-Ser(Me)OBO ester, 2.49.

Crude Boc-L-Ser(ald) OBO ester **2.46** (0.478 g, 1.66 mmol) was dissolved in dry Et_2O (5 mL) and CH_2Cl_2 (5 mL) under N_2. A solution of MeMgBr in Et_2O (1.66 mL, 4.98 mmol) was added quickly by syringe at –78°C and the mixture stirred vigorously. After 2 h the reaction was quenched by pouring into 25 mL of 3% NH_4Cl. CH_2Cl_2 (80mL) was added and the organic layer was separated, washed with 3% NH_4Cl (1 × 50 mL) and brine (1 × 50 mL), dried ($MgSO_4$), and evaporated to dryness. The product was purified by flash chromatography (4:1, CH_2Cl_2:EtOAc) to give 0.307 g of a light oil (61%). A diastereometric ratio of 85:15 *threo*:*erythro* was determined using ^1H NMR integration of the *threo* β-CH_3 at δ 1.12-1.07 ppm and the *erythro* β-CH_3 at δ 1.21-1.14 ppm in the crude product. The two diastereomers were separable by flash chromatography, the *threo* diastereomer with a R_f of 0.39 and *erythro* diastereomer a R_f of 0.34.

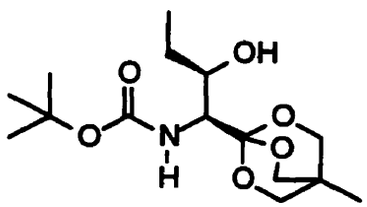


$[\alpha]^{20}_{578}$= -15.4 (c = 1.01, CH_2Cl_2); TLC (4:1, CH_2Cl_2:EtOAc) R_f = 0.39; ^1H NMR ($CDCl_3$, 250 MHz, *threo* isomer) δ 5.06 (br d, 1H, *J* = 10.4Hz, N*H*), 4.31 (br q, 1H, *J* = 6.4Hz, β-C*H*), 3.89 (s, 6H, OBO ester CH_2O), 3.63 (br d, 1H, *J* = 10.4Hz, α-C*H*), 2.88, (br s, 1H, O*H*), 1.42 (s, 9H, (CH_3)$_3$C), 1.06, (d, 3H, *J* = 6.4Hz, β-CH_3), 0.77 (s, 3H, OBO ester CCH_3); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 155.8 (CONH), 108.4 (OBO ester C-O), 79.1 ((CH$_3$)$_3$C), 72.5 (OBO ester CH_2O), 65.6 (β-CH), 59.0 (α-CH), 30.9 (OBO ester

$\underline{C}CH_3$), 28.3 (($\underline{C}H_3$)C), 20.1 (β-$\underline{C}H_3$) 13.9 (OBO ester C$\underline{C}H_3$); IR (cast from CH_2Cl_2) 3386, 2975, 1694, 1515, 1367, 1164, 1055; HRMS (FAB) calculated for (M + H^+) $C_{14}H_{26}NO_6$ 304.1761, found 304.1769; Anal. calcd for $C_{14}H_{25}NO_6$: C, 53.78; H, 8.33; N, 4.83. Found: C, 54.08; H, 8.66; N, 5.16.

2.4.12 EtMgBr addition to 2.46: 1-[N-*tert*-Butoxycarbonyl-(1*S*,2*R*)-1-amino-2-hydroxybutyl]-4-methyl-2,6,7-trioxabicyclo [2.2.2]octane, Boc-L-Ser(Et)OBO ester, 2.50.

Crude Boc-L-Ser(ald) OBO ester **2.46** (0.101 g, 0.35 mmol) was dissolved in dry Et_2O:CH_2Cl_2 (5 mL, 1:1) under N_2. A solution of EtMgBr in Et_2O (0.35 mL, 1.05 mmol) was added quickly by syringe at –78°C and the mixture stirred vigorously. After 2 h the reaction was quenched by pouring into 5 mL of 3% NH_4Cl. CH_2Cl_2 (40 mL) was added and the organic layer separated, washed with 3% NH_4Cl (1 × 10 mL) and brine (1 × 10 mL), dried ($MgSO_4$) then evaporated to dryness. The product was purified by flash chromatography (4:1, CH_2Cl_2:EtOAc) to give 0.072 g of a light oil (65%) with a diastereometric ratio of 84:16 *threo*:*erythro*.

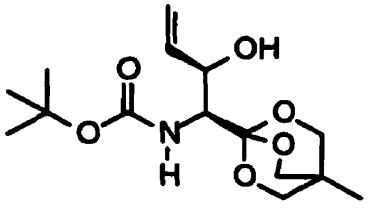


$[\alpha]^{20}_{578}$= -26.3 (c = 0.96, CH_2Cl_2); TLC (4:1, CH_2Cl_2:EtOAc) R_f = 0.45; ^{1}H NMR ($CDCl_3$, 250 MHz) major isomer = *threo* (84%), minor = *erythro* (16%) δ 5.30 (br d, 0.84H, *J* = 10.4Hz, *threo* N*H*), 5.13 (br d, 0.16H, *J* = 10.4Hz, *erythro* N*H*), 4.21 (br t, 1H, *J* = 6.4Hz, β-C*H*), 3.87 (s, 6H, OBO ester CH_2O), 3.69 (br d, 1H, *J* = 10.4Hz, α-C*H*),

2.79, (br s, 1H, O*H*), 1.60-1.34 (m, 2H, CH_3CH_2), 1.42 (s, 9H, $(CH_3)_3C$), 1.01, (t, 3H, J = 7.1Hz, CH_3CH_2), 0.76 (s, 3H, OBO ester CCH_3); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 156.7 (CONH), 108.1 (OBO ester C-O), 79.0 ($(CH_3)_3C$), 72.7 (OBO ester CH_2O), 66.1 (β-CH), 56.8 (α-CH), 46.3 (CH_3CH_2), 30.4 (OBO ester CCH_3), 28.3 ($(CH_3)_3C$), 13.9 (OBO ester CCH_3), 10.1 (CH_3CH_2); IR (cast from CH_2Cl_2) 3386, 2975, 1694, 1515, 1367, 1164, 1055; HRMS (FAB) calculated for (M + H^+) $C_{15}H_{28}NO_6$ 318.3869, found 318.3919; Anal. calcd for $C_{15}H_{27}NO_6$: C, 56.77; H, 8.57; N, 4.41. Found: C, 57.09; H, 8.76; N, 4.49.

2.4.13 VinylMgBr addition to 2.46: 1-[N-*tert*-Butoxycarbonyl-(1*S*,2*R*)-1-amino-2-hydroxybutenyl]-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane, Boc-L-Ser(vinyl)OBO ester, 2.51.

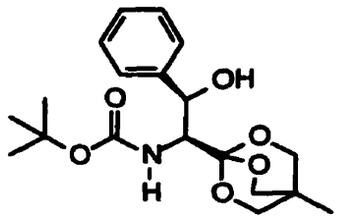
Crude Boc-L-Ser(ald) OBO ester **2.46** (0.097 g, 0.33 mmol) was dissolved in dry Et_2O:CH_2Cl_2 (5 mL, 1:1) under N_2. A solution of VinylMgBr in THF (0.99 mL, 0.99 mmol) was added quickly by syringe at –78°C and the mixture stirred vigorously. After 2 h the reaction was quenched by pouring into 5 mL of 3% NH_4Cl. CH_2Cl_2 (40mL) was added and the organic layer was separated, washed with 3% NH_4Cl (10 mL) and brine (10 mL), dried ($MgSO_4$), and evaporated to dryness. The product was purified by flash chromatography (4:1, CH_2Cl_2:EtOAc) to give 0.041 g of a light oil (40%) with a diastereometric ratio of 87:13 *threo*:*erythro*.



TLC (4:1, CH_2Cl_2:EtOAc) R_f = 0.44; ^1H NMR ($CDCl_3$, 250 MHz) δ 5.79 (ddd, 1H, *J* = 4.9, 11.5, 16.5Hz, CH_2=C*H*), 5.30 (br d, 1H, *J* = 16.5Hz, CH*H*=CH), 5.21 (br d, 1H, *J* = 11.5Hz, C*H*H=CH), 5.33 (br d, 0.87H, *J* = 10.4Hz, *threo* N*H*), 5.10 (br d, 0.13H, *J* = 10.4Hz, *erythro* N*H*), 4.61-4.50 (m, 1H, β-C*H*), 3.88 (s, 6H, OBO ester CH_2O), 3.69 (br d, 1H, *J* = 10.4Hz, α-C*H*), 2.69, (br s, 1H, O*H*), 1.40 (s, 9H, (CH_3)$_3$C), 0.81 (s, 3H, OBO ester CCH_3); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 156.1 (CONH), 135.8 (CH_2=CH), 115.1 (CH_2=CH), 108.5 (OBO ester C-O), 79.1 ((CH_3)$_3$C), 72.6 (OBO ester CH_2O), 67.1 (β-CH), 56.5 (α-CH), 30.4 (OBO ester CCH_3), 28.3 ((CH_3)$_3$C), 13.9 (OBO ester CCH_3); HRMS (FAB) calculated for (M + H^+) $C_{15}H_{26}NO_6$ 316.3709, found 316.3819; Anal. calcd for $C_{15}H_{25}NO_6$: C, 57.13; H, 7.99; N, 4.44. Found: C, 57.34; H, 8.26; N, 4.48.

2.4.14 PhMgBr addition to 2.46: 1-[N-*tert*-Butoxycarbonyl-(1*S*,2*R*)-1-amino-2-hydroxybutenyl]-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane, Boc-L-Ser(Ph)OBO ester, 2.52.

Crude Boc-L-Ser(ald) OBO ester **2.46** (0.122 g, 0.42 mmol) was dissolved in dry Et_2O:CH_2Cl_2 (5 mL, 1:1) under N_2. A solution of PhMgBr in THF (0.42 mL, 1.26 mmol) was added quickly by syringe at –78°C and the mixture stirred vigorously. After 2 h the reaction was quenched by pouring into 5 mL of 3% NH_4Cl. CH_2Cl_2 (50mL) was added and the organic layer was separated, washed with 3% NH_4Cl (10 mL) and brine (10 mL), dried ($MgSO_4$), and evaporated to dryness. The product was purified by flash chromatography (4:1, CH_2Cl_2:EtOAc) to give 0.092 g of a light yellow oil (60%) with a diastereometric ratio of 88:12 *threo*:*erythro*.

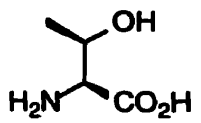


TLC (4:1, CH_2Cl_2:EtOAc) R_f = 0.58; ^1H NMR ($CDCl_3$, 250 MHz) δ 7.51-7.25 (m, 5H, Ar*H*), 5.69 (br d, 0.88H, *J* = 10.3Hz, *threo* N*H*), 5.30 (br d, 0.12H, *J* = 10.3Hz, *erythro* N*H*), 4.93-4.81 (m, 1H, β-C*H*), 3.91 (s, 6H, OBO ester CH_2O), 3.72 (dd, 1H, *J* = 3.4, 10.3Hz, α-C*H*), 1.43 (s, 9H, (CH_3)$_3$C), 0.79 (s, 3H, OBO ester CCH_3); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 156.8 (CONH), 136.7 (Ar=C=), 128.3, 127.7, 127.6 (Ar=CH), 108.1 (OBO ester C-O), 79.3 ((CH$_3$)$_3$C), 72.4 (OBO ester CH$_2$O), 69.1 (β-CH), 57.5 (α-CH), 30.4 (OBO ester CCH$_3$), 28.3 ((CH$_3$)$_3$C), 13.9 (OBO ester CCH$_3$); HRMS (FAB) calculated for (M + H^+) $C_{19}H_{28}NO_6$ 366.4309, found 366.4365; Anal. calcd for $C_{19}H_{27}NO_6$: C, 62.45; H, 7.45; N, 3.83. Found: C, 62.73; H, 7.75; N, 3.91.

2.4.15 Deprotection of Boc-Ser(Me)OBO ester 2.49: (2*S*,3*R*)-2-amino-3-hydroxybutanoic acid, L-Thr, 2.53.

Boc-Ser(Me)OBO ester **2.49** (0.210 g, 0.69 mmol) was dissolved in CH_2Cl_2 (5 mL) to which TFA (1 mL) was added. The mixture was stirred for 30 min and the solvent then removed under reduced pressure. The mixture was rinsed with fresh CH_2Cl_2 then reduced again and repeated once more. The mixture was dissolved in MeOH:H_2O (5 mL, 4:1) then 1 mL of a 10% (wt/vol) Cs_2CO_3 solution was added. The mixture was allowed to stir for 18 h at room temperature before being acidified to pH<3 with 3 N HCl. The solution was then loaded onto a cation exchange resin column (Bio-Rad AG® 50W-X8

100-200 mesh, hydrogen form, 1×10 cm) washed with 0.01 N HCl, H_2O (5 column lengths each) then eluted with 0.5 N NH_4OH. The eluant was reduced in volume then lyophilized to dryness to give 69 mg (81%) of a white, powdery solid. Spectral data identical to authentic Thr/*allo*-Thr.



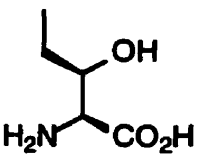
2.4.15a HPLC Analysis of L-Thr 2.53.

A solution of **2.53** (10-50 µL of approximately 1mg/mL) was mixed with borate buffer (0.1 mL of a 0.133 M solution, pH 10.4), *o*-phthalaldehyde **2.63** (40 µL of a 5 mg/mL solution in borate buffer) and *N*-isobutyryl-L-cysteine **2.64** (40 µL of a 20mg/mL solution in borate buffer). After 5 min, 25 µL of the mixture was injected onto a Waters 125-Å 8×100 mm µ-Bondpak C18 Radial-Pak cartridge column (2 mL/min; 100% 30 mM sodium acetate buffer, pH 6.5; linear gradient over 25 min to 60:40 buffer:MeOH; detection at 338 nm). Retention times were identical to those of standards prepared from L-Thr (21.3 min), L-*allo*-Thr (25.5 min), D-Thr (22.2 min), D-*allo*-Thr (26.4 min) showing a *threo*:*erythro* ratio of 85:15 with 98% ee.

2.4.16 Deprotection of Boc-Ser(Et)OBO ester 2.50: (2*S*,3*R*)-2-amino-3-hydroxypentanoic acid, 2.54.

Boc-Ser(Et)OBO ester **2.50** (0.062 g, 0.16 mmol) was deprotected as described in procedure 2.4.15 and lyophilized to give 11 mg (51%) of a white powder. HPLC analysis performed as described in 2.4.15a indicated a *threo*:*erythro* ratio of 87:13 with 97% ee

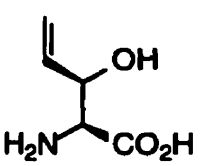
(*threo*-L-**2.54**: 33.0 min, *threo*-D-**2.54**: 34.1 min, *erythro*-L-**2.54**: 37.2 min, *erythro*-D-**2.54**: 38.1 min).



TLC (1:1:1:1, EtOAc:*n*BuOH:MeOH:H_2O) R_f = 0.48; ^{1}H NMR (D_2O, 250 MHz) δ 3.85 (dt, 1H, *J* = 4.9, 8.2Hz, β-C*H*), 3.57 (d, 1H, *J* = 4.4Hz, α-C*H*), 1.57-1.32 (m, 2H, CH_3CH_2), 0.87 (t, 3H, *J* = 7.4Hz, CH_3CH_2); ^{13}C NMR (D_2O, 63 MHz) δ 178.3 ($\underline{C}O_2H$), 75.2 (β-$\underline{C}$H), 62.2 (α-$\underline{C}$H), 30.0 ($CH_3\underline{C}H_2$), 13.1 ($\underline{C}H_3CH_2$); ESI-MS (M + H^+) 134.19.

2.4.17 Deprotection of Boc-Ser(vinyl)OBO ester 2.51: (2*S*,3*R*)-2-amino-3-hydroxy-4-pentenoic acid, 2.55.

Boc-Ser(vinyl)OBO ester **2.51** (0.041 g, 0.13 mmol) was deprotected as described in procedure 2.4.15 and lyophilized to give 8 mg (40%) of a white powder. HPLC analysis performed as described in 2.4.15a indicated a *threo*:*erythro* ratio of 89:11 with 97% ee (*threo*-L-**2.55**: 37.2 min, *threo*-D-**2.55**: 40.1 min, *erythro*-L-**2.55**: 43.6 min, *erythro*-D-**2.55**: 45.2 min).

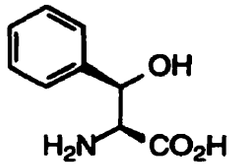


TLC (1:1:1:1, EtOAc:*n*BuOH:MeOH:H_2O) R_f = 0.46; ^{1}H NMR (D_2O, 250 MHz) δ 5.89 (ddd, 1H, *J* = 5.3, 10.9, 16.8Hz, CH_2=C*H*), 5.39 (br d, 1H, *J* = 17.0Hz, C*H*H=CH), 5.22

(br d, 1H, J = 10.6Hz, CH*H*=CH), 4.63-4.52 (m, 1H, β-C*H*), 3.88 (d, 1H, J = 4.0Hz, α-C*H*); ^{13}C NMR (D_2O, 63 MHz) δ 175.3 ($\underline{C}O_2H$), 136.9 (CH_2=$\underline{C}$H), 121.1 ($\underline{C}H_2$=CH), 73.2 (β-$\underline{C}$H), 62.0 (α-$\underline{C}$H); ESI-MS (M + H^+) 131.92.

2.4.18 Deprotection of Boc-Ser(Ph)OBO ester 2.52: (2*S*,3*R*)-2-amino-3-hydroxy-3-phenylpropanoic acid, 2.56.

Boc-Ser(Ph)OBO ester **2.52** (0.073 g, 0.20 mmol) was deprotected as described in procedure 2.4.15 and lyophilized to give 23 mg (66%) of a white powder. HPLC analysis performed as described in 2.4.15a (linear gradient to 50:50 30mM sodium acetate: MeOH over 25 min) indicated a *threo*:*erythro* ratio of 90:10 with 98% ee (*threo*-L-**2.56**: 35.1 min, *threo*-D-**2.56**: 38.1 min, *erythro*-L-**2.56**: 40.6 min, *erythro*-D-**2.56**: 41.3 min).



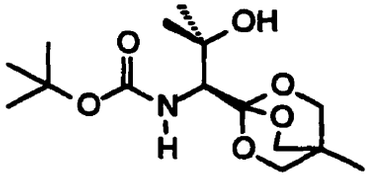
TLC (1:1:1:1, EtOAc:*n*BuOH:MeOH:H_2O) R_f = 0.61; ^{1}H NMR (D_2O, 250 MHz) δ 7.41-7.29 (m, 5H, Ar*H*), 5.31 (d, 0.1H, J = 4.0Hz, *erythro* β-C*H*), 5.25 (d, 0.9H, J = 4.0Hz, *threo* β-C*H*), 4.08 (d, 1H, J = 4.0Hz, α-C*H*); ^{13}C NMR (D_2O, 63 MHz) δ 175.0 ($\underline{C}O_2H$), 140.9 (Ar=$\underline{C}$=), 131.0, 129.9, 128.1 (Ar=$\underline{C}$H), 73.9 (β-$\underline{C}$H), 63.1 (α-$\underline{C}$H); ESI-MS (M + H^+) 182.01.

2.4.19 Reduction of recovered Boc-Ser(ald)-OBO ester 2.46 from section 2.4.11.

Before being purified by flash chromatography, an aliquot (0.093 mg, 0.31 mmol) of the reaction mixture described in section 2.4.11 was dissolved CH_2Cl_2 (20 mL), cooled to -20°C and $NaBH_4$ (0.012 g, 0.31 mmol) then added. The mixture was allowed to warm to room temperature and after 2 h poured into 3% NH_4Cl (10 mL) which was then extracted with CH_2Cl_2 (3 × 10 mL). The organic fractions were pooled, extracted with brine (15 mL) then dried over $MgSO_4$ and evaporated to dryness. After purification by flash chromatography the optical rotation of Boc-Ser-OBO ester **2.44** was determined. $[\alpha]^{20}_{578} = -40.2$; (c = 0.98, EtOAc).

2.4.20 Addition of MeMgBr to Boc-Thr(ket)OBO ester 2.47: 1-[*N-tert*-Butoxycarbonyl-(1*S*)-1-amino-2-butanol]-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane, Boc-L-Thr(Me)-OBO ester, 2.57.

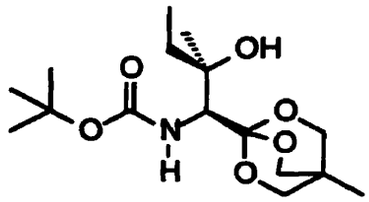
Crude Boc-L-Thr(ket)-OBO ester **2.47** (0.561g, 1.86 mmol) was dissolved in dry Et_2O (15 mL) and CH_2Cl_2 (15 mL) under N_2. A solution of MeMgBr in Et_2O (1.86 mL, 5.58 mmol) was added quickly by syringe at –78°C and the mixture stirred vigorously. After 2 h the reaction was quenched by pouring into 25 mL of 3% NH_4Cl. CH_2Cl_2 (120mL) was added and the organic layer was separated, washed with 3% NH_4Cl (50 mL) and brine (50 mL), dried ($MgSO_4$), and evaporated to dryness. The product was purified by flash chromatography (4:1, CH_2Cl_2:EtOAc) to yield 0.424g (72%) of a white solid.



mp 80-81°C; $[\alpha]^{20}_{578}$= -67.0 (c=1.55, EtOAc); TLC (4:1, CH_2Cl_2:EtOAc) R_f = 0.45; ^1H NMR ($CDCl_3$, 250 MHz) δ 5.02 (br d, 1H, *J* = 10.6Hz, N*H*), 3.89 (s, 6H, OBO ester CH_2O), 3.71 (d, 1H, *J* = 10.6Hz, α-C*H*), 3.38, (br s, 1H, O*H*), 1.41 (s, 9H, (CH_3)$_3$C), 1.28 (s, 3H, β-CH_3), 1.15 (s, 3H, β-CH_3), 0.78 (s, 3H, OBO ester CCH_3); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 156.1 (CONH), 109.3 (OBO ester C-O), 79.1 ((CH$_3$)$_3$C), 72.5 (OBO ester CH_2O), 72.3 (β-C), 59.8 (α-CH), 30.4 (OBO ester CCH_3), 28.3 ((CH_3)$_3$C), 28.1 (β-CH_3), 26.6 (β-CH_3), 14.3 (OBO ester CCH_3); IR (neat) 3386, 2975, 1715, 1506, 1365, 1169, 1049; HRMS (FAB) calcd for (M + H^+) $C_{15}H_{28}NO_6$ 318.1917, found 318.1929. Anal. calcd for $C_{15}H_{27}NO_6$: C, 56.77; H, 8.57; N, 4.41. Found: C, 56.98; H, 8.69; N, 4.73.

2.4.21 Addition of EtMgBr to Boc-Thr(ket)OBO ester 2.47: *tert*-butyl *N*-[(1*S*,2*R*)-2-hydroxy-2-methyl-1-(4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl)butyl]carbamate, Boc-L-Thr(Et)-OBO ester, 2.58.

Crude Boc-L-Thr(ket)-OBO ester **2.47** (0.388g, 1.29 mmol) was dissolved in dry Et_2O (15 mL) and CH_2Cl_2 (15 mL) under N_2. A solution of EtMgBr in Et_2O (1.29 mL, 3.87 mmol) was added quickly by syringe at –78°C and the mixture stirred vigorously. After 2 h the reaction was quenched by pouring into 3% NH_4Cl (25 mL). CH_2Cl_2 (100mL) was added and the organic layer was separated, washed with 3% NH_4Cl (50 mL) and brine (50 mL), dried ($MgSO_4$), and evaporated to dryness. The product was purified by flash chromatography (4:1, CH_2Cl_2:EtOAc) to yield 0.320g (75%) of a white crystalline product.

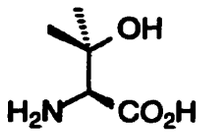


mp 87-88°C; $[\alpha]^{20}_{578}$ = -31.8 (c = 1.06, EtOAc); TLC (4:1, CH_2Cl_2:EtOAc) R_f = 0.55; ^1H NMR ($CDCl_3$, 250 MHz) δ 5.01 (br d, 1H, *J* = 10.3Hz, N*H*), 3.87 (s, 6H, OBO ester CH_2O), 3.75 (d, 1H, *J* = 10.3Hz, α-C*H*), 3.18, (br s, 1H, O*H*), 1.47 (q, 2H, *J* = 7.5Hz, CH_3CH_2), 1.41 (s, 9H, ((CH_3)$_3$C), 1.23 (s, 0.98H, *threo* β-CH_3), 1.19 (s, 0.02H, *erythro* β-CH_3), 0.84 (t, 3H, *J* = 7.5Hz, CH_3CH_2) 0.77 (s, 3H, OBO ester CCH_3); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 156.1 (CONH), 109.6 (OBO ester C-O), 79.1 ((CH_3)$_3$C), 74.7 (β-C), 72.3 (OBO ester CH_2O), 58.3 (α-CH), 31.7 (CH_3CH_2), 30.4 (OBO ester CCH_3), 28.4 ((CH_3)$_3$C), 24.3 (β-CH_3), 14.3 (OBO ester CCH_3), 8.0 (CH_3CH_2); IR (neat) 3380, 2975, 1715, 1505, 1362, 1169, 1051; HRMS (FAB) calculated for (M + H^+) $C_{16}H_{30}NO_6$ 332.2073, found 332.2061; Anal. calcd for $C_{16}H_{29}NO_6$: C, 57.98; H, 8.85; N, 4.22. Found: C, 58.13; H, 9.02; N, 4.38.

2.4.22 Deprotection of Boc-Thr(Me)OBO ester 2.57: (2*S*)-2-amino-3-hydroxy-3-methylbutanoic acid, 2.59.

Boc-Thr(Me)OBO ester **2.57** (0.210 g, 0.66 mmol) was dissolved in CH_2Cl_2 (10 mL) to which TFA (2 mL) was added. The mixture was stirred for 30 min and the solvent then removed under reduced pressure. The mixture was rinsed with fresh CH_2Cl_2 then reduced again and repeated once more. The mixture was dissolved in MeOH:H_2O (5 mL, 4:1) then 1 mL of a 10% (wt/vol) Cs_2CO_3 solution was added. The mixture was allowed to stir for 18 h at room temperature before being directly loaded onto an anion exchange

resin column (Bio-Rad AG® 1-X4 100-200 mesh, chloride form converted to hydroxide form by prewashing with 4N NaOH then rinsed with Millipore Milli-Q H_2O until the rinsings appeared neutral, larger column lengths were required to accommodate the CO_3^{2-} generated). 1N AcOH or 1N formic acid was used to elute the amino acid and the eluant reduced in volume then lyophilized to dryness to give 45 mg (52%) of a white, powdery solid. Recrystallization from H_2O/acetone gave 19 mg (21%) of colourless crystals. HPLC analysis performed as described in 2.4.15a (linear gradient 50:50 30mM sodium acetate: MeOH over 60 min) eluted the diastereomer formed from the L-amino acid at 42.5 min and the D-amino acid at 43.9 min to indicate 99% ee.

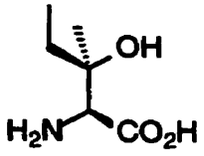


mp 195-198°C (dec); TLC (1:1:1:1, EtOAc:*n*BuOH:MeOH:H_2O) R_f = 0.48; ^{1}H NMR (D_2O, 250 MHz) δ 3.61 (s, 1H, α-C*H*), 1.35 (s, 3H, β-CH_3), 1.11 (s, 3H, β-CH_3); ^{13}C NMR (D_2O, 63 MHz) δ 176.1 (<u>C</u>O_2H), 73.6 (β-<u>C</u>), 63.3 (α-<u>C</u>H), 30.7 (β-<u>C</u>H_3), 26.3 (β-<u>C</u>H_3); ESI-MS (M + H^+) 133.93.

2.4.23 Deprotection of Boc-Thr(Et)OBO ester 2.58: (2*S*,3*R*)-2-amino-3-hydroxy-3-methylpentanoic acid, 2.60.

Boc-Thr(Et)OBO ester **2.58** (0.151 g, 0.46 mmol) was deprotected as described in procedure 2.4.21 and lyophilized to give 37 mg (56%) of a white powder which was recrystallized from H_2O/acetone to give 15 mg (22%) of colourless crystals. HPLC analysis performed as described in 2.4.15a (linear gradient to 80:20 30mM sodium

acetate:MeOH over 5 min then to 60:40 over 100 min) indicated a *threo*:*erythro* ratio of 99:1 with 99% ee (*threo*-L-**2.60**: 73.1 min, *threo*-D-**2.60**: 77.8 min, *erythro*-L-**2.60**: 71.6 min, *erythro*-D-**2.60**: 76.3 min).



mp 220-225°C (dec); TLC (1:1:1:1, EtOAc:*n*BuOH:MeOH:H_2O) R_f = 0.56; ^{1}H NMR (D_2O, 250 MHz) δ 3.51 (s, 1H, α-C*H*), 1.65-1.53 (m, 2H, *J* = 7.7Hz, CH_3CH_2), 1.09 (s, 3H, β-CH_3), 0.79 (t, 3H, *J* = 7.7Hz, CH_3CH_2); ^{13}C NMR (D_2O, 63 MHz) δ 175.1 ($\underline{C}O_2H$), 72.6 (β-$\underline{C}$), 64.5 (α-$\underline{C}H$), 34.7 (β-$CH_3\underline{C}H_2$), 24.1 (β-$\underline{C}H_3$), 10.0 (β-$\underline{C}H_3CH_2$); ESI-MS (M + H^+) 147.93.

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Chapter Three

Synthesis of Vinylglycine

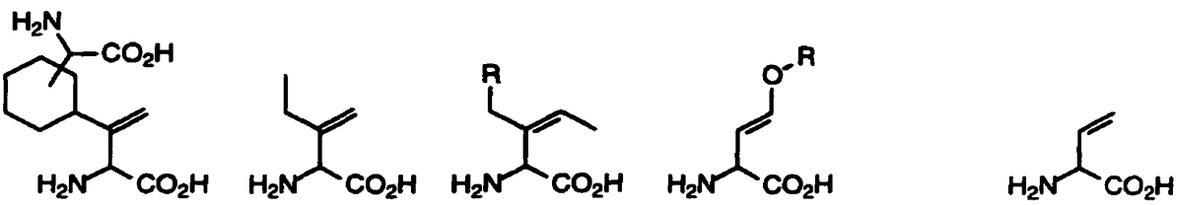
3.1 Introduction

3.1.1 Isolation and Biological Activity

β,γ-Unsaturated amino acids, or vinylglycines, are a class of α-amino acids which have garnered much interest due to both their varied biological activity and as synthetic intermediates.[1] Various substituted vinylglycines have been isolated from natural sources and found to possess antimicrobial activity. The first isolates were of 2-methylene-cyclohept-(4,5 or 6)-ene-1,3-diglycine **3.1** and β-methylene-L-norvaline **3.2** which were found in the mushroom *Lactarius helvus* in 1964[2] and 1967[3,4] respectively. In 1968 two more α,β-unsaturated α-amino acids were isolated from the mushroom *Bankera fuligineoalba*, L-2-amino-3-hydroxymethyl-3-pentenoic acid **3.3** and L-2-amino-3-formyl-3-pentenoic acid **3.4**. Gradually as more and more α,β-unsaturated α-amino acids were isolated their biological activities were determined. For example, L-2-amino-4-methoxy-*trans*-3-butenoic acid **3.5** was extracted from *Pseudomonas aeruginosa*[5] and is active as an antibiotic against both gram positive and negative bacteria by inhibiting methionine biosynthesis.[6] Rhizobitoxine, an antimetabolite isolated from root nodules induced by *Rhizodium japonicum* in the soybean *Glyine max*, was identified as 2-amino-4-(2-amino-3-hydroxypropoxy)-*trans*-but-3-enoic acid **3.6**.[7] Rhizobitoxine's activity occurs through a number of pathways, including the inhibition of the conversion of methionine into ethylene in plants and by irreversibly inactivating β-cystathionase, a

pyridoxal phosphate dependent enzyme catalyzing a step in methionine biosynthesis;[8] it can also catalyze β- and γ-replacement, β- and γ-elimination and α- and β-hydrogen exchange in a number of amino acids.[9] The simplest α,β-unsaturated α-amino acid, 2-amino-3-butenoic acid, also known as vinylglycine **3.7**, was not isolated until 1974 when the D-enantiomer was found in the mushroom *Rhodophyllus nidorosus*.[10]

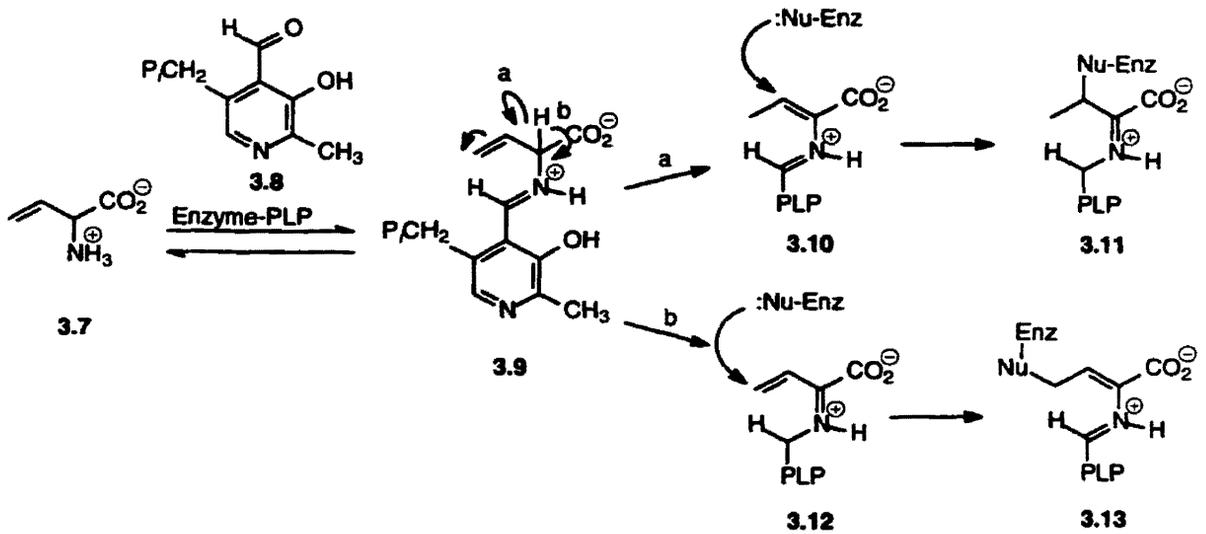
Most of the interest in α,β-unsaturated α-amino acids is due to their ability to act as suicide substrates or mechanistic probes of pyridoxal phosphate (PLP) dependent enzymes. These enzymes are involved in catalyzing chemical changes at the α-, β- or γ-carbons of common amino acids and are a vital link in many biosynthetic pathways. The concept of suicide substrates in general has been extensively reviewed[11] as has the specific use of vinylglycines with respect to PLP dependent enzymes.[12]



Suicide substrates, or enzyme activated inhibitors, are chemically unreactive substances which possess latent reactive groups that are specifically unmasked through the action of the target enzyme. Once generated, the reactive intermediate irreversibly inactivates the enzyme by covalently reacting with an active site residue. Since the suicide substrate must be pre-activated by the enzyme, these substrates can be designed to target specific classes of enzymes. The mechanism for inactivation of PLP enzymes is

generally proposed to proceed as outlined in scheme 3.1[11c,11f] although a more recent article suggests a slightly different version as shown in scheme 3.2.[13]

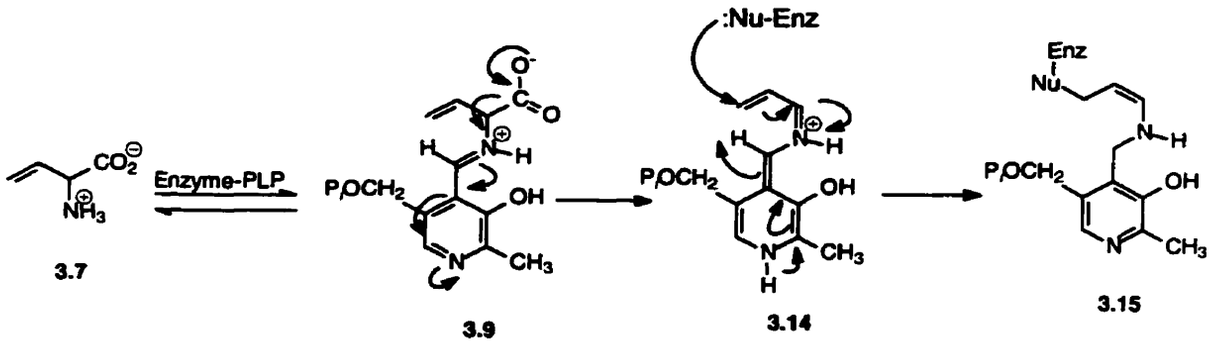
Scheme 3.1



The aldimine **3.9** initially generated is the amino acid-PLP intermediate that the enzyme normally forms with amino acid substrates. However, with suicide substrates, once the reactive 1,4-addition site is unmasked by decarboxylation or proton abstraction, an irreversible addition by an active site nucleophile to give **3.11**, **3.13** or **3.15** occurs. Some of the more recent vinylglycine related inhibitors include α-allenic-α-amino acids (suicide substrates of Vitamin B_6 linked decarboxylase)[14] and a D-vinylglycine containing peptide (a mechanism based inactivator of peptidylglycan α-hydroxylating monooxygenase).[15]

In some pyridoxal phosphate dependent enzymes, vinylglycine analogs are important intermediates in the enzyme mechanism and lead to products instead of inhibition.[11b-11f,12,16] Vinylglycine has been used as a probe of 1-aminocyclopropane-1-carboxylate synthase (ACC)[17] and to determine the mechanism of desulfurization of L-cysteine by the nifS gene product in *Azobacter vinlandii*.[18]

Scheme 3.2



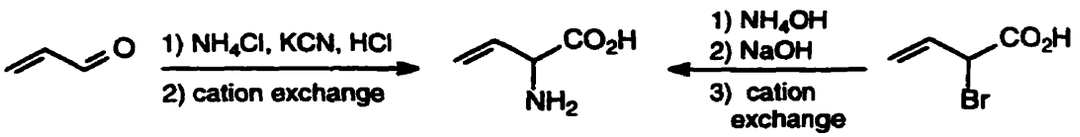
Vinylglycines are also useful as synthons towards more complex molecules. For example, vinylglycine **3.7** itself has been used in the synthesis of antitumor agents (such as acivicin, α-amino-3-bromo-4,5-dihydroisoxazole-5-acetic acid),[19] mitomycin antitumor antibiotics[20], *cis* substituted β-lactams,[21] and a number of other amino acid derivatives.[22] The vinyl amino alcohols that are obtained as intermediates in some synthetic routes to vinylglycines have been used in the synthesis of kainoids,[23] galantinic acid,[24] cyclopropyl amino acids,[25,26] polyhydroxy amino acids[27] and 3-alkylated glutamic acids.[28]

3.1.2 Synthesis of Racemic Vinylglycines

The β,γ-unsaturated-α-amino acids pose a synthetic challenge primarily due to the tendency of these compounds to isomerize to the conjugated α,β-unsaturated derivatives. Racemic vinylglycine was first synthesized in 1974.[29] These authors reported that vinylglycine was unstable to heat, acid and base. Complete decomposition was found after 50 hours at 100°C in 1N HCl or after 22 hours at 100°C in 2N NH_4OH with unidentified decomposition products. When heated in water, vinylglycine was reported to decompose to 2-aminobutyric acid. It was synthesized in 1.1% yield by a Strecker

synthesis (Scheme 3.3, left side) from acrolein **3.16** or in 6.6% yield by bromine displacement from ethyl-2-bromo-3-butenoate **3.17** (Scheme 3.3, right path). D-Vinylglycine was then obtained in approximately 82% ee by resolution with baker's yeast.[29]

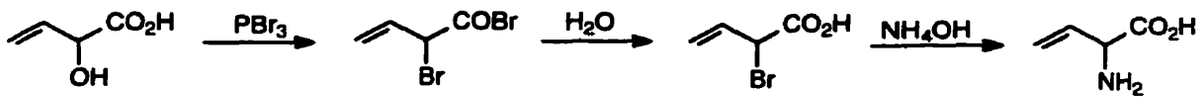
Scheme 3.3



3.16 **3.7** **3.17**

Racemic vinylglycine was also reportedly synthesized in 50% yield from 2-hydroxy-3-butenoic acid,[11c] however, no synthetic details were provided. In 1977, Baldwin *et al.* used the same 2-hydroxy-3-butenoic acid **3.18** to synthesize racemic vinylglycine in 26% overall yield (Scheme 3.4).[30]

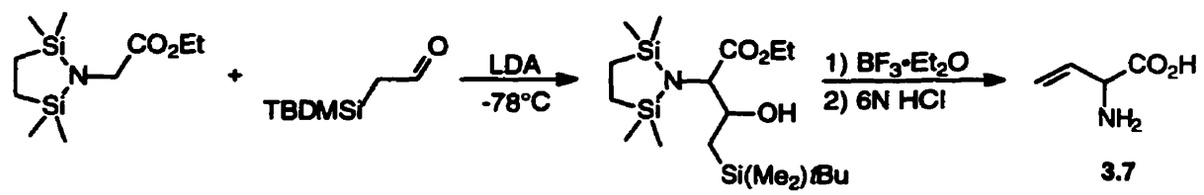
Scheme 3.4



3.18 **3.19** **3.17** **3.7**

A better yield (48% for 3 steps) was achieved in 1981 by β-elimination of a silyl protected glycine enolate adduct **3.20** in a Peterson-type olefination (Scheme 3.5).[31]

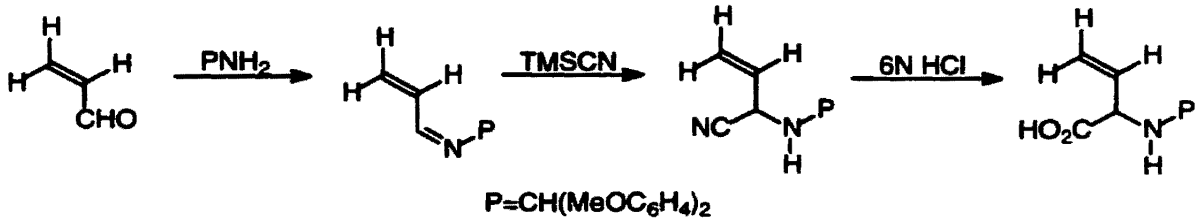
Scheme 3.5



3.20 **3.7**

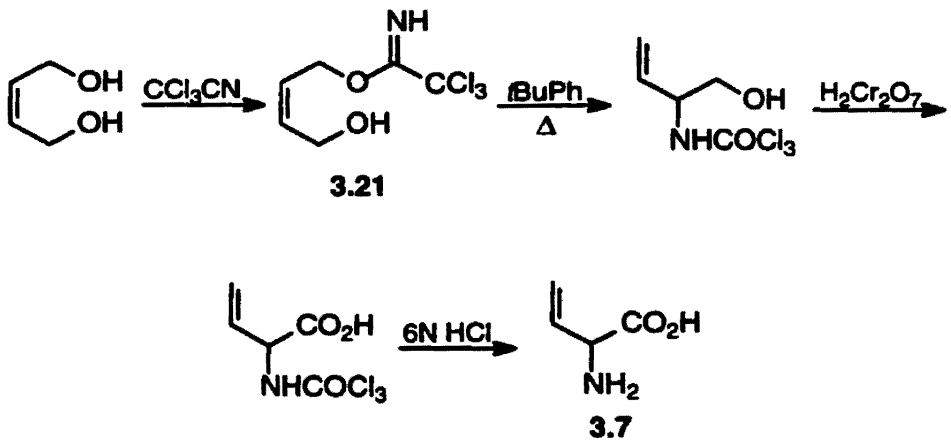
In 1984, a modified Strecker synthesis using TMSCN was used to prepare a variety of racemic β,γ-unsaturated amino acids in 15-68% overall yield although vinylglycine itself was obtained in a low 7% yield (Scheme 3.6). It was noted that cation exchange column elution of vinylglycine with 1M NH_4OH gave a later eluting product identified as 2,4-diaminobutyric acid.[32]

Scheme 3.6



A four step process that makes use of a [3,3] sigmatropic rearrangement of **3.21** gives vinylglycine **3.7** in 26% overall yield (Scheme 3.7) and has potential for industrial application since inexpensive chemicals are used.[33]

Scheme 3.7



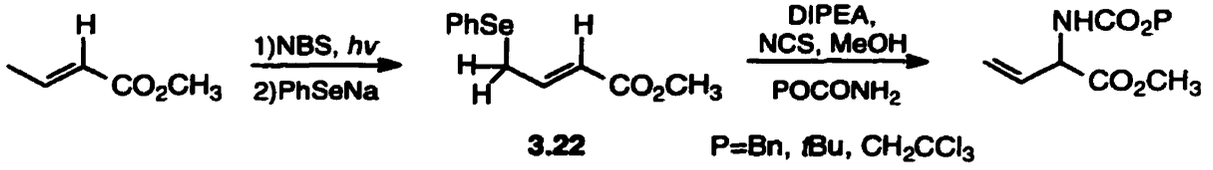
3.21

3.7

A number of racemic β,γ-unsaturated amino acids have been prepared by oxidative rearrangement of allylic selenides **3.22** with yields of 12-87% reported in the

last step (Scheme 3.8). Protected vinylglycine was synthesized in 18% overall yield from methyl crotonate.[34,35]

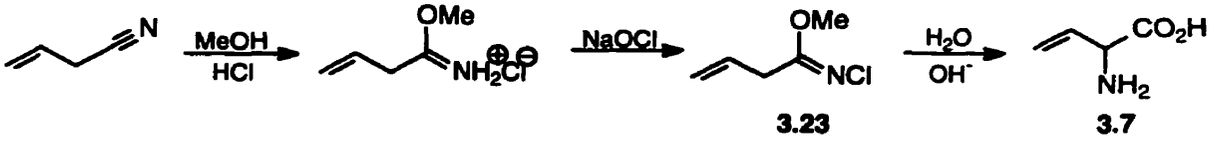
Scheme 3.8



3.22 P=Bn, *t*Bu, CH_2CCl_3

A three step synthesis of vinylglycine via a Neber rearrangement of a *N*-chloroimidate **3.23** intermediate was reported in 50% overall yield.[36] The use of cheap and convenient reagents makes this racemic synthesis of vinylglycine attractive, particularly since an enzymatic resolution method is also described.

Scheme 3.9

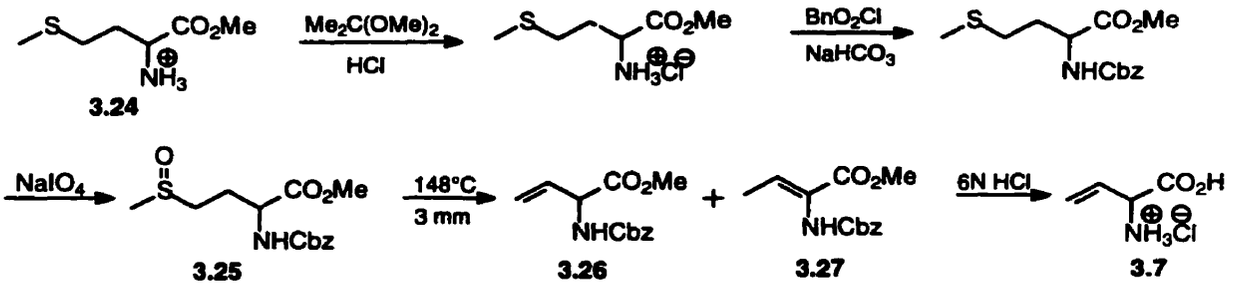


3.23 **3.7**

3.1.3 Synthesis of Optically Active Vinylglycine

Optically active vinylglycine was first synthesized in 1980 in 54% yield[37] via a thermal degradation of L-methionine **3.24** and an improved methodology amenable for large-scale synthesis has since been published (Scheme 3.10).[38]

Scheme 3.10



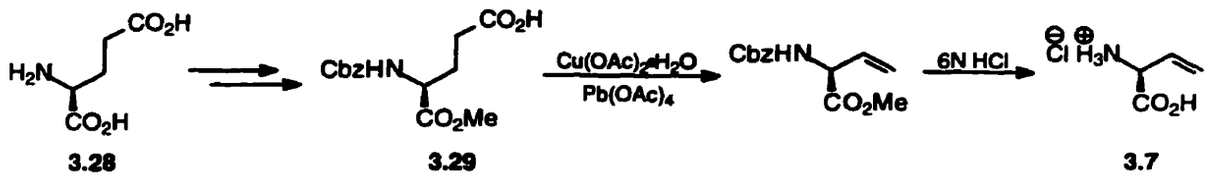
3.24 **3.25** **3.26** **3.27** **3.7**

The main byproduct of elimination was the α,β-unsaturated isomer **3.27**. The protected vinylglcine **3.26** was found to be particularly base sensitive as prolonged stirring with triethylamine or *N*-methylmorpholine in organic solvent or in aqueous 0.5 N LiOH gave quantitative conversion to **3.27**. However, vinylglycine is stable to the acid hydrolysis conditions required for protecting group removal (6N HCl, 1 hour at 100°C).[38]

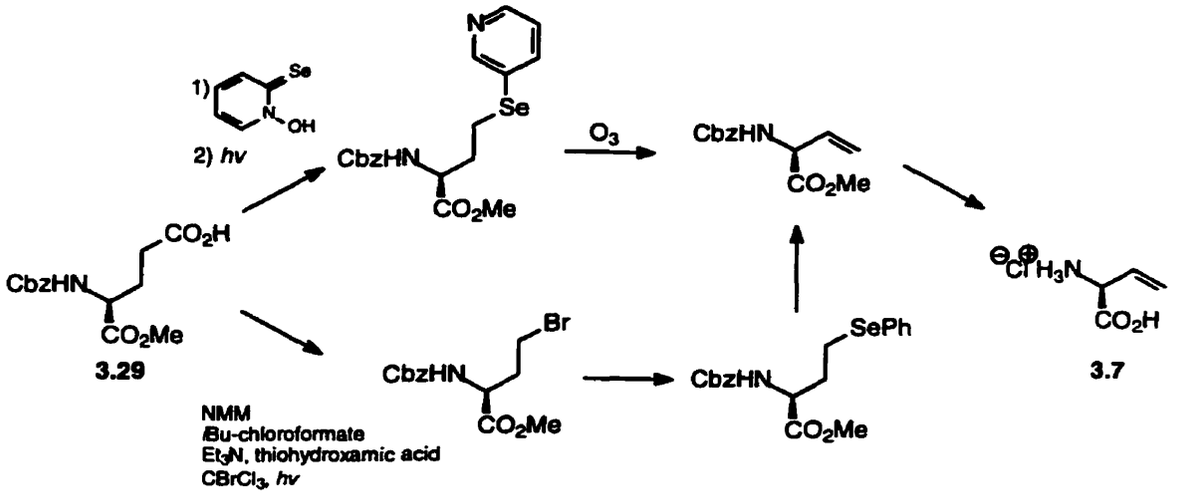
An equally effective method was described in 1984 by Hanessian and Sahoo in which L-vinylglycine was prepared from glutamic acid **3.28** by a decarboxylative elimination of *N*-Cbz-L-glutamic acid monomethyl ester **3.29** (Scheme 3.11).[39] Acid hydrolysis gave L-vinylglycine in 49% yield from the protected glutamic acid **3.29**, which in turn was obtained in 88% yield from Cbz-Glu. An improved version of the Hanessian decarboxylation/elimination procedure was reported in 1991 after the authors found that the synthesis often led to substantial racemization (40-90% ee) when either the *N*-Boc benzhydryl ester, *N*-Cbz benzyl ester or *N*-Cbz methyl ester was prepared.[40] Enantiomerically pure *N*-Cbz benzyl ester could be obtained by crystallization (in 28% yield from *N*-Cbz-L-glutamic acid).

Two other routes to enantiomerically enriched vinylglycine were reported in 1985 (Scheme 3.12), both proceeding through photochemical rearrangements and selenium oxidative eliminations giving comparable overall yields of approximately 45% of vinylglycine **3.7**.

Scheme 3.11



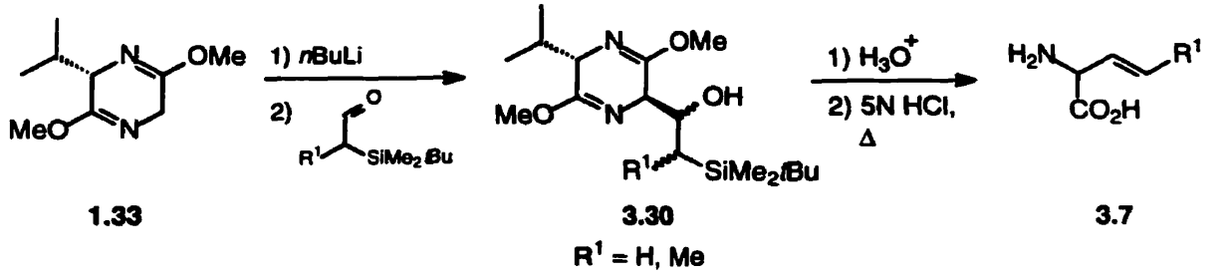
Scheme 3.12



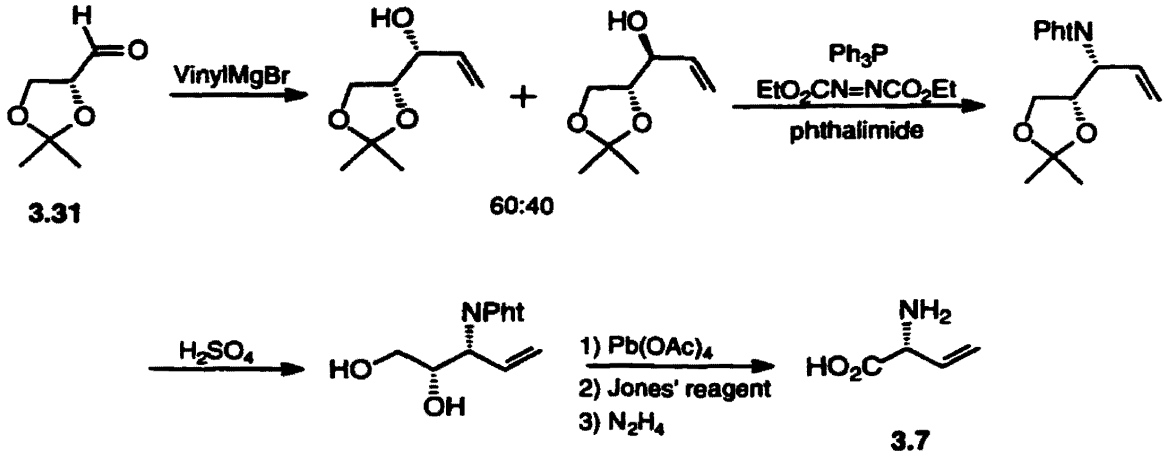
Schöllkopf used a more general approach, reacting the valine derived *bis*-lactam enolate **1.33** (Section 1.3.3.1) with silyl aldehydes. Elimination and hydrolysis of the resulting β-hydroxy adducts **3.30** generated a number of substituted vinylglycines (Scheme 3.13).[41] L-Vinylglycine itself was prepared in 32% overall yield with some racemization (86% ee).

The D-mannitol derived aldehyde **3.31** of Mulzer *et al.*[42] can be elaborated for vinylglycine synthesis. Addition of vinyl Grignard reagent followed by a Mitsunobu reaction with phthalimide on the secondary chiral allylic alcohol and subsequent oxidative cleavage of the protected diol gives D-vinylglycine in approximately 10% yield from the isopropylidieneglyceraldehyde with 97% ee (Scheme 3.14).

Scheme 3.13

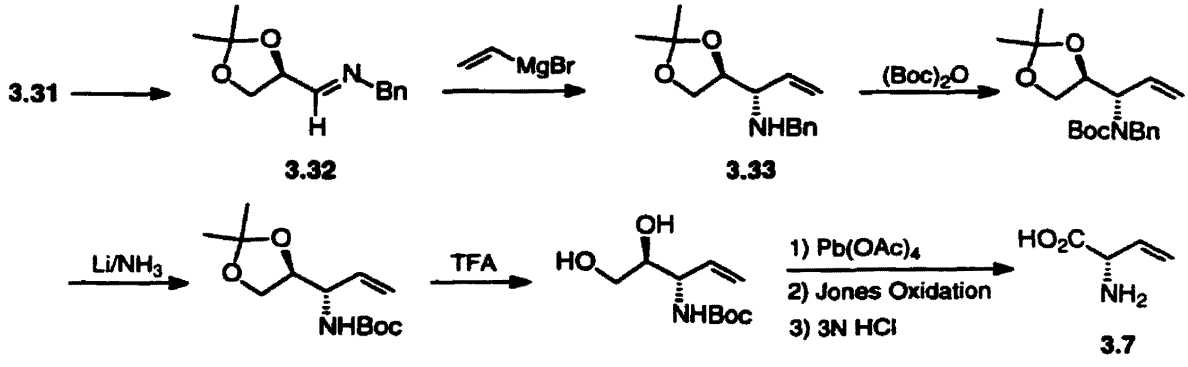


Scheme 3.14



More recently, the same D-mannitol derived aldehyde **3.31** has been used to synthesize L-vinylglycine via imine **3.32** which was reacted with vinylmagnesium bromide to give amine **3.33** as one diastereomer (Scheme 3.25).[43] Subsequent functional group manipulation gave L-vinylglycine in 45% yield over seven steps.

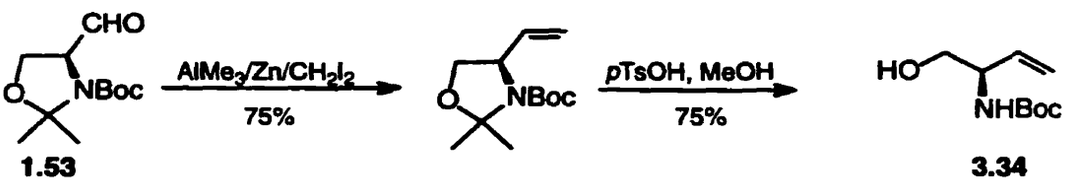
Scheme 3.15



As discussed earlier (Section 1.3.4.2, Scheme 1.23), Garner's aldehyde **1.53** racemizes completely when treated with $Ph_3P^+MeBr^-/KH$.[44] However, the aldehyde may be used to generate β,γ-unsaturated amino acids by Wittig reactions. Garner's aldehyde

1.53 was also successfully methylenylated with $AlMe_3/Zn/CH_2I_2$ in 75% yield with >95% ee (Scheme 3.16).[45] The amino alcohol **3.34** was not oxidized to the amino acid since it was shown to proceed in low yield.

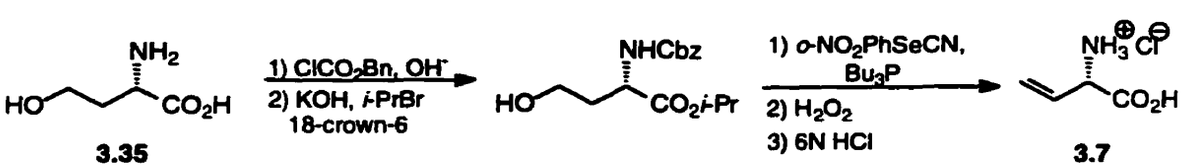
Scheme 3.16



1.53 **3.34**

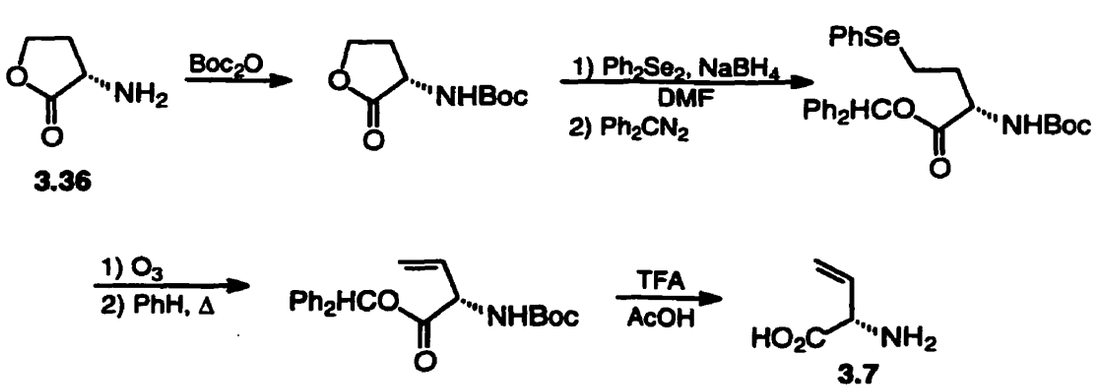
L-vinylglycine has been synthesized from L-homoserine **3.35** in 49% overall yield (Scheme 3.17)[46] and L-homoserine lactone **3.36** (Scheme 3.18)[47] in 72% overall yield with both routes involving dehydroselenoeliminations. L-Homoserine and its lactone are readily available in both enantiomeric forms.

Scheme 3.17



3.35 **3.7**

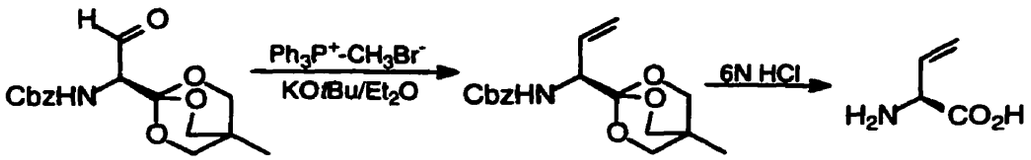
Scheme 3.18



3.36 **3.7**

L-vinylglycine has also been synthesized using methodology previously developed in our laboratory. Cbz-Ser(ald)-OBO ester **3.37** was reacted with methylenetriphenyl-phosphorane to give the vinylglycine intermediate **3.38** which was then deprotected to give vinylglycine in 51% yield over 3 steps albeit in 77% ee.[48]

Scheme 3.19



3.37 **3.38** **3.7**

3.1.4 Synthesis of Labelled β,γ-Unsaturated Amino Acids

A major disadvantage of many of the methods developed for the synthesis of vinylglycines, especially those that rely on degradation of an amino acid, is that isotopic label incorporation is difficult.

Two synthesis leading to racemic products have been described. Chang and Walsh[12f] prepared (*Z*)-[4-^{2}H], (*E*)-[4-^{2}H] and (*E*)-[3,4-$^2H_2$]vinylglycine for mechanistic studies by catalytic hydrogenation of alkyne intermediates. Sawada[49] and co-workers prepared (*E*)-[3,4-$^2H_2$], (*Z*)-[3,4-$^2H_2$], (*E*)-[4-^{2}H] and [3,4,4-$^2H_3$]-vinylglycine *via* a stereoselective reduction of phenyl-2-(TMS)-ethynyl sulfone.

3.2 Results and Discussion: Synthesis of Vinylglycine from Serine

Given that the synthesis of vinylglycine **3.7** via Wittig olefination of Fmoc-Ser(ald)OBO resulted in partial racemization (77% ee) and very poor yields due to Fmoc cleavage,[48] we hypothesized that the basic ylide was responsible for both the racemization

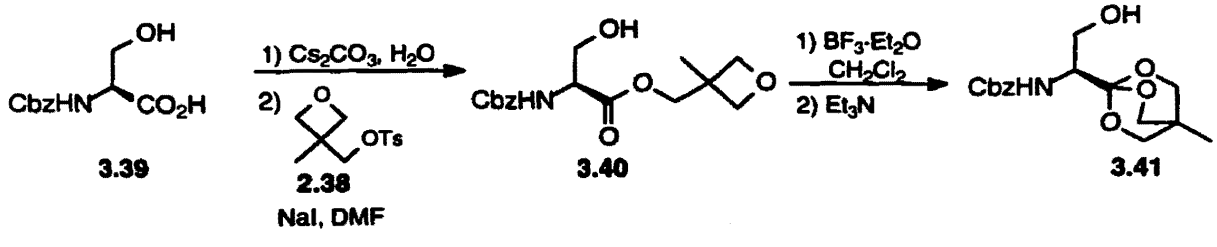
observed and removal of the Fmoc group. Other methylenation methods were therefore investigated using the more robust Cbz-Ser(ald)OBO ester **3.37** in order to demonstrate the utility of the OBO ester protecting group in the synthesis of enantiomerically pure vinylglycine.

3.2.1 Takai Reaction of Cbz-L-Ser(ald)-OBO ester

Nozaki *et al.*[50] in 1978 described a highly electrophilic, mild and non-basic system for methylenation by the *in situ* generation of a metallo-carbon reagent from $AlMe_3/Zn/CH_2I_2$. This reagent was previously used in the synthesis of the β-amino alcohol of vinylglycine **3.34** (Scheme 3.16).

Using the same conditions as described in Section 2.2.1, Cbz-Ser **3.39** was converted to the oxetane ester **3.40** in 78% yield and was recrystallized from EtOAc:Hexanes with relative ease. $BF_3 \cdot Et_2O$ catalyzed rearrangement of the OBO ester proceeded more rapidly than for the Boc-protected serine **2.42** to give Cbz-Ser-OBO ester **3.41** which could be recrystallized from EtOAc:Hexanes in 85% yield.

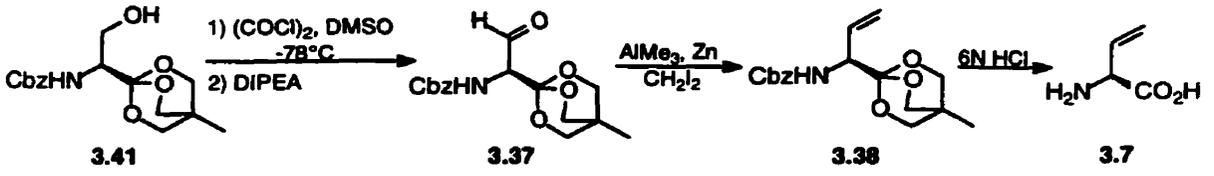
Scheme 3.19



Cbz-Ser-OBO ester **3.41** was oxidized to the aldehyde under Swern conditions as previously described (Section 2.2.1) to give Cbz-Ser(ald)-OBO **3.37** in >98% ee. *In situ* generation of the Takai reagent from $AlMe_3/Zn/CH_2I_2$ followed by addition of Cbz-

Ser(ald)-OBO gave the desired olefin **3.38** in 76% yield over two steps (Swern and olefination). Critical to the success of the reaction was use of freshly purified zinc[51] and rigorous anhydrous conditions to prevent Lewis acid catalyzed opening of the OBO ester. Subsequent acid hydrolysis and ion exchange purification gave L-vinylglycine in 39% yield over five steps and 86% ee determined by derivatization and HPLC analysis as described in section 2.4.15a. The cause of racemization is unclear since the optical purity of the aldehyde **3.37** was known to be >97% ee and vinylglycine has been shown to be stable to the acid hydrolysis conditions required to remove the protecting groups.[37]

Scheme 3.20

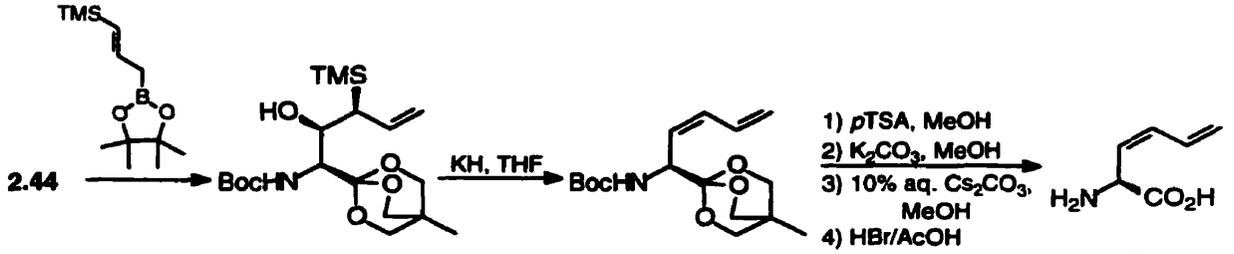


3.41 **3.37** **3.38** **3.7**

3.2.2 Peterson Olefination of Cbz-L-Ser(ald)-OBO ester

The racemization difficulties occurring with the Takai reaction led us to consider other olefination techniques. At that time, a report appeared in the literature detailing the use of the Peterson reaction to synthesize (*S*)-2-amino-(*Z*)-3,5-hexadienoic acid, an isolate from the beetle *Leptinotarsa decemlineata* (Scheme 3.21).[52]

Scheme 3.21

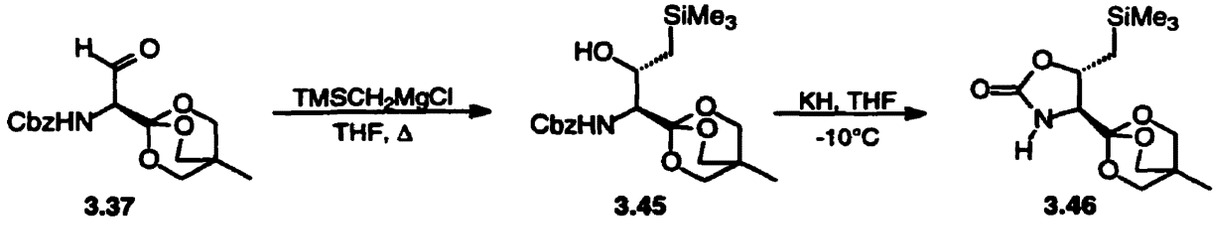


3.42 **3.43** **3.44**

The Peterson olefination has been widely utilized for olefin synthesis as a silicon analog of the Wittig or Horner-Emmons reactions. One of the features of this reaction is that both *E* and *Z* olefins can be obtained stereospecifically from a single diastereomer of β-hydroxyalkylsilane by altering the reaction conditions (acid or base).[53,54]

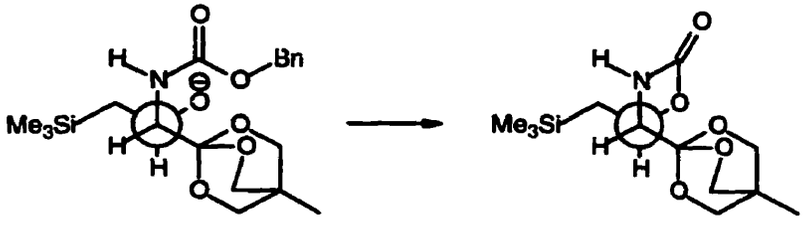
The β-hydroxyalkylsilane **3.45** was generated by reaction of Cbz-Ser(ald)-OBO ester **3.37** with trimethylsilylmethylenemagnesium chloride and proceeded in quantative yield with *threo* stereochemistry as previously observed (Section 2.2.5). In an effort to maintain the integrity of the OBO ester and thereby allow further functionalization, base mediated elimination was attempted. However, those reaction conditions consistently failed to give the desired olefin regardless of base used, temperature or length of reaction, instead forming the oxazolidinone **3.46** as a result of attack of the oxyanion on the carbamate of the Cbz group. Various bases investigated include KH,[52] NaH,[55] KO*t*Bu,[53] LDA, LHMDS, *n*BuLi and KHDMS. Methods pioneered by Chan[56] to convert the alcohol into a better leaving group were also explored including $BF_3 \cdot Et_2O$,[54] MsCl/Et_3N,[54] AcCl,[56] Ac_2O/pyridine, *n*BuLi/MsCl,[57] AcCl/F^-,[58] Ac_2O/cat. TMSCl[59] and Ac_2O/cat. TMSOTf,[60] all of which either resulted in no reaction or total decomposition of the starting material, generally as a result of hydrolysis of the OBO ester.

Scheme 3.22



3.37 **3.45** **3.46**

A *trans* relationship for H2/H3 in the oxazolidinone was assigned on the basis of the ^{1}H NMR coupling constant. The $J_{2,3}$ value of 3.8 Hz was in agreement with those reported for *trans* stereochemistry.[61] In retrospect, formation of the oxazolidinone **3.46** may be explained by the requirement of the β-hydroxyalkylsilane **3.45** to adopt the *syn* conformation which is not easily achieved due to steric considerations. Oxazolidinone formation is a more facile process as seen in the staggered Newman projection of the β-hydroxyalkylsilane **3.45** (Figure 3.1). This is in contrast to the synthesis of (*S*)-2-amino-(*Z*)-3,5-hexadienoic acid **3.43** which does not suffer from this limitation and is driven by the formation of extended conjugation.



3.45 **3.46**

Figure 3.1: Formation of the Oxazolidinone 3.46.

Since base mediated elimination failed to give Cbz-vinylglycine-OBO, our attention turned to the use of acid in order to form vinylglycine **3.7**. This has the advantage that acid hydrolysis in refluxing 6N HCl simultaneously deprotects the ortho ester and Cbz protecting groups as discussed below.

3.2.3 Removal of Protecting Groups

Refuxing 6N HCl has previously been used to deprotect Cbz protected methyl[46] or isopropyl esters[37] of vinylglycine in good yield. When the same conditions were applied to both Cbz-vinylglycine-OBO ester **3.38** and Cbz-Ser(CH_2TMS)-OBO ester **3.45** the free amino acid **3.7** was achieved in 76% and 74% yield respectively.

Since Cbz cleavage is quantitive, purification on an anion exchange column instead of cation exchange column allows for the easy separation of any non-hydrolyzed ester. The anion exchange column also has the advantage that the base-sensitive vinylglycine is only briefly exposed to base during the period the sample is loaded onto the column and is not prone to racemization as previously reported in section 2.2.3.3.

Optical purity was assessed by derivatization of the free amino acid with OPA **2.63** and *N*-*i*-Bu-L-Cys **2.64** as described previously (section 2.2.4) and indicated the vinylglycine synthesize via Takai olefination possessed 86% ee and via Peterson olefination >95% ee (Figure 3.2).

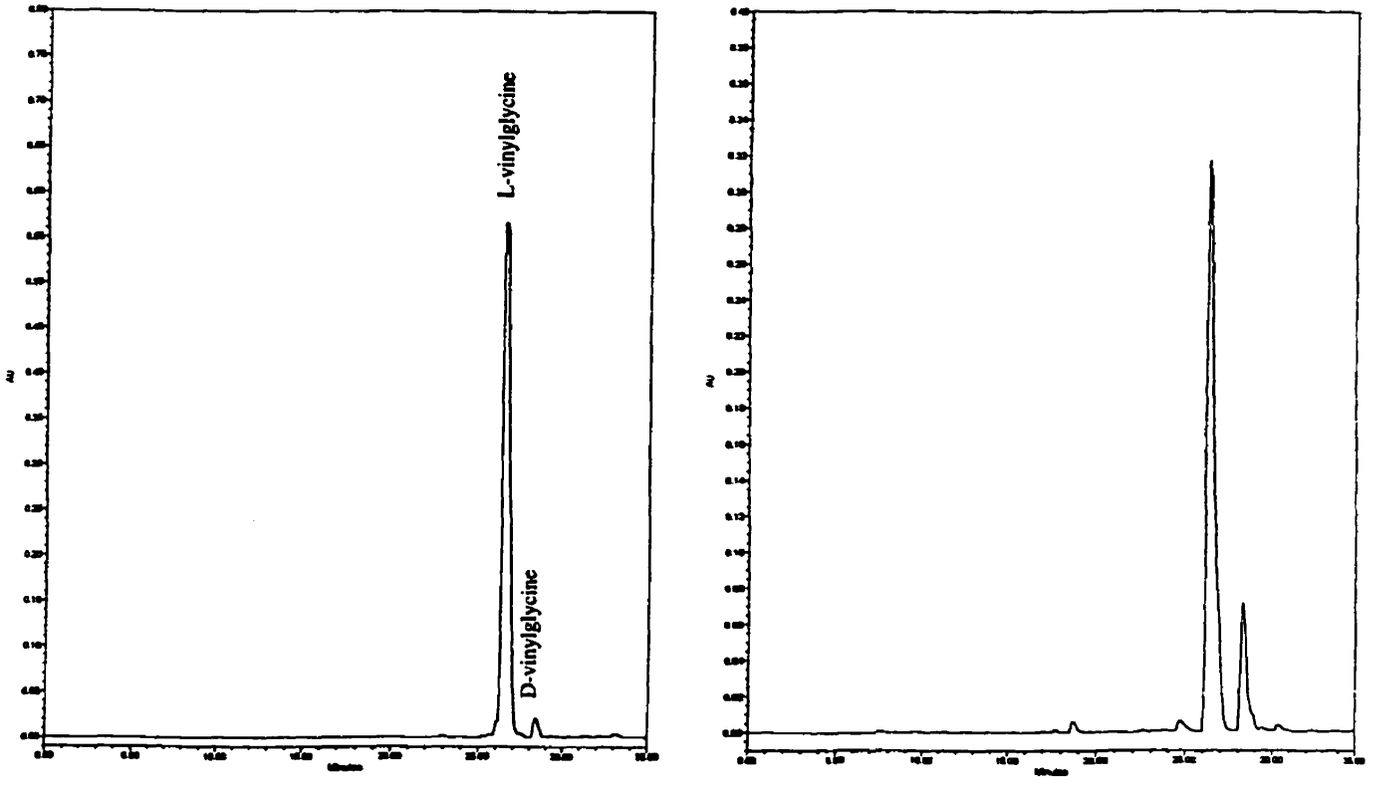


Figure 3.2: Assessment of enantiomeric purity after ion exchange purification of L-vinylglycine obtained after deprotection of Cbz-L-Ser(CH_2TMS)OBO ester 3.45 (left) and Cbz-L-Gly(CH=CH_2)OBO ester 3.38. Amino acid samples prepared as described in Section 2.4.15a.

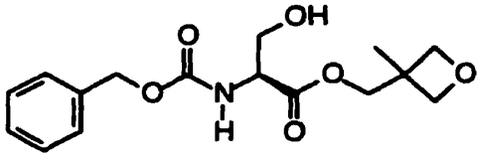
3.3 Summary

The Cbz OBO ester protected serine aldehyde **3.42** can be used to synthesize vinylglycine in high yields (51% over three steps). Unfortunately some racemization occurs in the case of Takai olefination, presumably during the methylenation reaction since the vinylglycine is known to be stable during acid hydrolysis as indicated during the deprotection of the β-hydroxy silane **3.45**. However, the cause of racemization is currently unknown. Simultaneous deprotection of the β-hydroxyalkylsilane **3.45** generates vinylglycine in 53% overall yield and high ee (>95%) and is comparable to other methods used to synthesize vinylglycine.

3.4 Experimental

3.4.1 (1-Methylcyclobutyl)methyl-(2*S*)-2-[(benzyloxy)carbonyl]amino-3-hydroxy-propanoate, Cbz-L-Ser-oxetane ester, 3.40.

Cbz-L-Ser **3.39** (11.36 g, 0.047 mol) and Cs_2CO_3 (9.19 g, 0.028 mol) were combined and dissolved in H_2O (100 mL). The water was then removed *in vacuo* and the resulting oil lyophilized for 12 hours to give a white foam. To this foam was added oxetane tosylate **2.38** (12.65 g, 0.049 mol) and NaI (1.41 g, 9.8 mmol) which was then taken up in DMF (500 mL) and allowed to stir under Ar for 48 hours. The DMF was then removed *in vacuo* and the resulting solid dissolved in EtOAc (600 mL) and H_2O (200 mL) and extracted with 10% $NaHCO_3$ (2 × 100 mL), saturated NaCl (100 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to yield a yellow oil which was recrystallized from ethyl acetate and hexanes to yield colourless rod-like crystals in 78% yield (11.85 g).

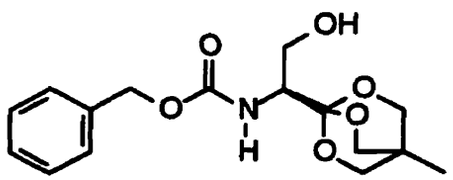


mp 70-70.5 °C; $[\alpha]^{20}_D$ = -8.5 (c = 1.04, EtOAc); TLC (2:1, EtOAc:Hex), R_f = 0.34; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.34-7.28 (br m, 5H, Ar*H*), 5.89 (d, 1H, *J* = 7.9Hz, N*H*), 5.12 (s, 2H, CbzCH_2O), 4.56-4.38 (m, 6H, 3 oxetane ester CH_2O), 4.13-4.04 (br m, 2H, α-C*H*, β-C*H*H), 3.93-3.84 (br m, 1H, β-CH*H*), 3.22 (t, 1H, *J* = 6.0Hz, O*H*), 1.28 (s, 3H, oxetane ester CCH_3); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 170.6 (C=O), 156.2 (CONH), 136.1 (Ar=C=), 128.5, 128.1, 128.0 (Ar=CH), 79.4 (oxetane ester CH_2-O), 68.9 (oxetane ester CO_2CH_2),

67.1 (Ar$\underline{C}H_2O$), 63.2 (β-$\underline{C}H_2$), 56.3 (α-$\underline{C}H$), 39.5 (oxetane ester $\underline{C}CH_3$), 20.7 (oxetane ester C$\underline{C}H_3$); IR (cast from CH_2Cl_2) 3371, 3064, 3034, 2956, 2879, 1723, 1525, 1457, 1339, 1214, 1195, 1061; Anal. calcd. for $C_{16}H_{21}O_6N$: C, 59.43; H, 6.55; N, 4.33. Found: C, 59.44; H, 6.61; N, 4.31.

3.4.2 1-[*N*-(Benzyloxycarbonyl)-(1*S*)-1-amino-2-ethanol]-4-methyl-2,6,7-trioxa-bicyclo[2.2.2]octane, Cbz-L-Ser-OBO ester, 3.41.

Cbz-Ser oxetane ester **3.40** (15.0 g, 46.2 mmol) was dissolved in dry CH_2Cl_2 (450 mL) and cooled to 0°C under Ar. $BF_3 \cdot Et_2O$ (0.11 mL, 0.93 mmol) was diluted in CH_2Cl_2 (5.0 mL) and added to the reaction flask after which the reaction was allowed to warm up to room temperature. After 6 hours, Et_3N (1.29 mL, 9.25 mmol) was added and the reaction stirred an additional 30 minutes before being concentrated to a thick oil. The crude product was redissolved in EtOAc (400 mL) and washed with 3% NH_4Cl (2 × 250 mL), 10% $NaHCO_3$ (100 mL), saturated NaCl (250 mL), dried ($MgSO_4$), and evaporated to dryness. The reaction yielded a colourless thick oil in 95% (14.2 g) yield which was crystallized from EtOAc:Hexanes to give rod-like shiny crystals in 93% (13.6 g) yield.



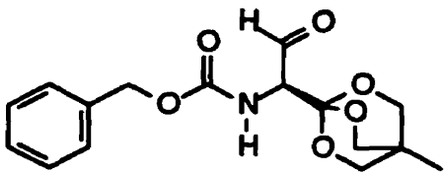
mp 103.5-105.0 °C; $[\alpha]^{20}_D$ = -24.8 (c = 1.00, EtOAc); TLC (3:1 EtOAc:Hex), R_f = 0.37; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.36-7.31 (m, 5H, Ar*H*), 5.34 (br d, 1H, *J* = 8.8Hz, N*H*), 5.17-5.11 (m, 2H, CbzCH_2O), 3.94-3.83 (m, 2H, α-C*H*, β-C*H*H), 3.91 (s, 6H, OBO ester

C*H*$_2$O), 3.71-3.67 (m, 1H, β-CH*H*), 2.60 (br s, 1H, O*H*), 0.81 (s, 3H, OBO ester CC*H*$_3$); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 156.3 (CONH), 136.4 (Ar=C=), 128.4, 128.0, 127.9 (Ar=CH), 108.0 (OBO ester C-O), 72.7 (OBO ester CH_2O), 66.9 (ArCH_2O), 61.9 (β-CH_2), 55.3 (α-CH), 30.5 (OBO ester CCH_3), 14.2 (OBO ester CCH_3); IR (cast from CH_2Cl_2) 3407, 3063, 3033, 2948, 2883, 1716, 1607, 1586, 1527, 1399, 1352, 1237, 1048, 1010 cm^{-1}; HRMS (FAB) calculated for (M + H$^+$) $C_{16}H_{22}NO_6$: 324.1447. Found: 324.1435; Anal. Calcd for $C_{16}H_{21}NO_6$: C, 59.43; H, 6.55; N, 4.33. Found: C, 59.43; H, 6.64; N, 4.34.

3.4.3 1-[*N*-(Benzyloxycarbonyl)-(1*S*)-1-amino-2-oxoethyl]-4-methyl-2,6,7-trioxa-bicyclo[2.2.2]octane, Cbz-L-Ser(ald)-OBO ester, 3.37.

Cbz-Ser OBO ester **3.41** (9.04 g, 27.86 mmol) was dissolved in freshly distilled CH_2Cl_2 (80 mL) under Ar and cooled to -78°C in flask 1. Oxalyl chloride (3.89 mL, 44.58 mmol, 1.60 equiv) was added to CH_2Cl_2 (120 mL) in a separate round bottom flask (flask 2) under Ar, and cooled to -78°C. Dry DMSO (7.03 mL, 91.94 mmol, 3.30 equiv) was added quickly (to prevent freezing in the syringe) to the oxalyl chloride solution (flask 2 – taking care to release the evolved gas) and the mixture was stirred at -78°C for 15 minutes. The alcohol solution was transferred slowly by cannula to flask 2 over a period of 30 minutes and then rinsed with CH_2Cl_2 (50 mL). The resulting cloudy, white mixture was stirred for 1.5 hours at -78°C. DIPEA (24.27 mL, 0.14 mol) was added and the solution stirred for 30 minutes at -78°C and 10 minutes at 0°C. Ice-cold CH_2Cl_2 (250 mL) was added and the solution was washed with ice-cold 3% NH_4Cl (3 × 250 mL), brine (250 mL) and dried over $MgSO_4$ before being evaporated to dryness. The reaction

yielded a slightly yellowish solid in 96% (8.68 g) yield. Recrystallization was possible from EtOAc/hexane but the aldehyde was generally used without further purification.

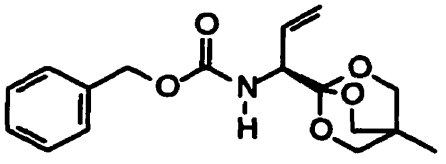


mp 139.5-141.5 °C; $[\alpha]^{20}_{D}$ = -99.3 (c = 1.03, EtOAc); TLC (3:1 EtOAc:Hex), R_f = 0.60; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 9.69 (s, 1H, C*H*O), 7.38-7.30 (m, 5H, Ar*H*), 5.34 (d, 1H, *J* = 9.2Hz, N*H*), 5.15-5.10 (m, 2H, ArCH_2O), 4.61 (d, 1H, *J* = 8.9Hz, α-C*H*), 3.94 (s, 6H, OBO ester CH_2O), 0.83 (s, 3H, OBO ester CCH_3); ^{13}C NMR ($CDCl_3$, 63 MHz); δ 195.6, (CHO) 156.1 (CONH), 136.1 (Ar=C=), 128.4, 128.1, 127.9 (Ar=CH), 107.1 (OBO ester C-O), 72.8 (OBO ester CH_2O), 67.1 (CbzCH_2O), 63.2 (α-CH), 30.8 (OBO ester CCH_3), 14.2 (OBO ester CCH_3); IR (cast from CH_2Cl_2) 3353, 3063, 3033, 2947, 2884, 1723, 1599, 1521, 1355, 1234, 1192, 1071, 1046 cm^{-1}; HRMS (FAB) calculated for (M + H$^+$) $C_{16}H_{21}NO_6$: 322.12906; observed : 322.12854. Anal. Calcd for $C_{16}H_{20}NO_6$: C, 59.81; H, 5.96; N, 4.36. Found: C, 59.72; H, 6.12; N, 4.33.

3.4.4 Takai olefination of Cbz-L-Ser(ald)-OBO ester 3.37: 1-[*N*-(Benzyl-oxycarbonyl)-(1*S*)-1-amino-2-propene]-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane, Cbz-L-Gly(-CH=CH$_2$)-OBO ester, 3.38.

Crude Cbz-Ser(ald)-OBO ester **3.37** (0.50 g, 1.56 mmol assuming 100% yield of the aldehyde from the oxidation) was dissolved in dry THF (10 mL) then added via cannula to a stirring mixture of purified zinc dust[51] (0.918 g, 14.04 mmol), freshly distilled

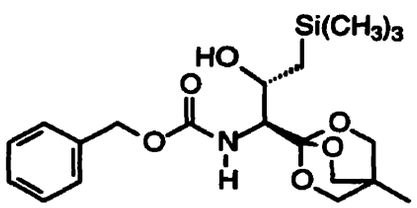
diiodomethane (0.376 g, 4.67 mmol) and trimethylaluminum (0.47 mL, 0.94 mmol, 2.0M in hexanes) in dry THF (10 mL) under Ar. The mixture was stirred at room temperature for 4 h before cold saturated potassium tartrate (10 mL) was added. The resulting aqueous layer was extracted with EtOAc (3 × 40 mL), the organic layers were pooled and extracted with 3% NH_4Cl (2 × 25 mL), 10 % $NaHCO_3$ (25 mL), saturated NaCl (25 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the resulting slightly yellow oil purified by flash chromatography (EtOAc:Hex 1:1) to give a clear oil which was crystallized from Et_2O/hexanes to give 0.35 g of **3.38** (76% yield).



mp 73-74°C; $[\alpha]^{20}_D$ = -66.5 (c = 0.9, EtOAc); TLC (1:1 EtOAc:Hex), R_f = 0.48; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.34-7.25 (m, 5H, Ar*H*), 5.92 (ddd, 1H, *J* = 5.5, 10.5, 17.2Hz, β-C*H*), 5.31-5.06 (m, 3H, N*H*, γ-CH_2), 5.12 (s, 2H, CbzCH_2O), 4.43 (br t, 1H, *J* = 6.9Hz, α-C*H*), 3.89 (s, 6H, OBO ester CH_2O), 0.78 (s, 3H, OBO ester CCH_3); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 156.0 (CONH), 136.5 (Ar=C=), 133.2 (CH=CH_2), 128.4, 128.0, 128.0 (Ar=CH), 116.7 (CH=CH_2), 108.0 (OBO ester C-O), 72.8 (OBO ester CH_2O), 67.8 (CbzCH_2O), 57.1 (α-CH), 30.7 (OBO ester CCH_3), 14.3 (OBO ester CCH_3); IR (cast from CH_2Cl_2) 3371, 3064, 3033, 2944, 2881, 1722, 1646, 1515, 1397, 1336, 1223, 1053 cm^{-1}; ESI-MS (M + H$^+$) 318.91; Anal. Calcd for $C_{17}H_{21}NO_5$: C, 63.94; H, 6.64; N, 4.39. Found: C, 64.09; H, 6.73; N, 4.36.

3.4.5 Grignard addition of trimethylsilylmethylmagnesium chloride to Cbz-L-Ser(ald)-OBO ester 3.37: 1-[*N*-(Benzyloxycarbonyl)-(1*S*)-1-amino-2-hydroxy-3-(1,1,1-trimethylsilyl)-propyl]-4-meth-yl-2,6,7-trioxabicyclo[2.2.2]octane, Cbz-L-Ser-(CH_2TMS)-OBO ester, 3.45.

Crude Cbz-Ser(ald)-OBO ester **3.37** (0.40 g, 1.25 mmol assuming 100% yield of the aldehyde from the oxidation) was dissolved in dry THF (40 mL) to which trimethylsilylmethylmagnesium chloride (5.0 mL, 5.0 mmol, 1.0 M in Et_2O) was added by syringe. The mixture was refluxed under Ar for 6 h before 3% NH_4Cl (20 mL) was added and the mixture then extracted with EtOAc (3 × 50 mL). The organic fractions were pooled and then extracted with with 3% NH_4Cl (2 × 25 mL), 10 % $NaHCO_3$ (25 mL), saturated NaCl (25 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* and the resulting oil purified by flash chromatography to give 0.368 g (72% yield) of a clear oil.

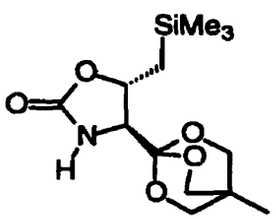


TLC (EtOAC:Hex) R_f = 0.58; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.40-7.26 (m, 5H, Ar*H*), 5.32 (d, 1H, *J* = 10.3Hz, N*H*), 5.12 (s, 2H, CbzCH_2O), 4.31 (dd, 1H, *J* = 4.8, 9.7Hz, β-C*H*), 3.89 (s, 6H, OBO ester CH_2O), 3.68 (d, 1H, *J* = 10.3Hz, α-C*H*), 0.85 (dd, 1H, *J* = 9.6, 14.7Hz, γ-*CH*H), 0.78 (s, 3H, OBO ester CCH_3), 0.60 (dd, 1H, *J* = 4.7, 14.7Hz, γ-CH*H*), 0.02 (s, 9H, Si(CH_3)$_3$); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 157.2 (CONH), 136.7 (Ar=C=), 128.6, 128.5, 128.2 (Ar=CH), 108.9 (OBO ester C-O), 72.8 (OBO ester CH_2O),

67.1 (Cbz$\underline{C}H_2O$), 60.5 (β-$\underline{C}H$), 59.1 (α-$\underline{C}H$), 30.7 (OBO ester $\underline{C}CH_3$), 21.5 (γ-$\underline{C}H_2$), 14.3 (OBO ester C$\underline{C}H_3$), -0.8 (Si($\underline{C}H_3$)$_3$); IR (cast from CH_2Cl_2) 3389, 3064, 3033, 2944, 2881, 1719, 1510, 1387, 1336, 1223, 1053, 995 cm^{-1}; Anal. Calcd for $C_{20}H_{31}NO_6Si$: C, 58.65; H, 7.63; N, 3.42. Found: C, 58.56; H, 7.85; N, 3.21.

3.4.6 Attempted base-mediated Peterson olefination of Cbz-L-Ser(CH_2TMS)-OBO ester, 3.45. (5*S*)-4-(4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl)-5-[(1,1,1-trimethyl-silyl)-methyl]-1,3-oxazolan-2-one, 3.46.

Cbz-L-Ser(CH_2TMS)-OBO ester **3.45** (0.210 g, 0.52 mmol) was dissolved in dry THF (20 mL) and rapidly transferred to a flask containing KH (0.063 g, 0.55 mmol, washed with Et_2O) suspended in THF (10 mL) at –10°C under Ar. One hour later a TLC indicated total conversion to **3.46**. The reaction mixture was poured into Et_2O (40 mL) and extracted with 3% NH_4Cl (10 mL), 10% $NaHCO_3$ (10 mL), brine (10 mL) and dried over $MgSO_4$. The solvent was then removed under reduced pressure and the result slight yellow oil purified by flash chromatography (1:2, EtOAc:Hex) to give 0.139 g (89% yield) of a clear oil.

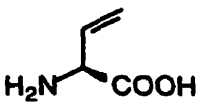


TLC (1:2 EtOAc:Hex), R_f = 0.41; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 5.38 (s, 1H, N*H*), 4.66 (ddd, 1H, *J* = 3.4, 6.8, 7.8Hz, β-C*H*), 3.85 (s, 6H, OBO ester CH_2O), 3.30 (d, 1H, *J* = 3.4Hz, α-C*H*), 1.06 (dd, 1H, *J* = 7.8, 14.5Hz, γ-C*H*H), 0.95 (dd, 1H, *J* = 6.8, 14.5Hz, γ-CH*H*), 0.76 (s, 3H, OBO ester CCH_3), 0.02 (s, 9H, Si(CH_3)$_3$); ^{13}C NMR ($CDCl_3$, 63 MHz)

δ 158.9 (CONH), 107.4 (OBO ester C-O), 76.3 (β-CH), 72.7 (OBO ester CH_2O), 62.8 (CbzCH_2O), 30.8 (OBO ester CCH_3), 24.7 (γ-CH_2), 14.1 (OBO ester CCH_3), -1.1 ($Si(CH_3)_3$); Anal. Calcd for $C_{13}H_{23}NSiO_5$: C, 51.80; H, 7.69; N, 4.65. Found: C, 52.09; H, 7.96; N, 4.34.

3.4.7 Acid Hydrolysis of Cbz-L-Gly(-CH=CH_2)-OBO ester 3.38 with 6N HCl. (2*S*)-2-amino-3-butenoic acid, 3.7.

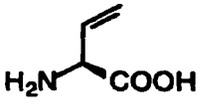
Cbz-L-Gly(-CH=CH_2)-OBO ester **3.38** (0.230 g, 0.720 mmol) was mixed with 6N HCl (2.0 mL) and refluxed for 1 h. The solution was reduced *in vacuo* then cooled, neutralized with a saturated solution of $NaHCO_3$ (approx. 50 mL) and loaded on an anion exchange column (Bio-Rad AG 1-X4 100-200 mesh, chloride form, converted to hydroxide form by prewashing with 4N NaOH). The column was rinsed with H_2O (5 column lengths) and eluted with 1N AcOH, then lyopholized to give 0.0109 g (79%) of a colourless powder. Recrystallization (H_2O/acetone) gave 0.010 g (72%) of a white solid. Derivatisation and analysis by HPLC indicated 86% ee (conditions as described in Section 2.4.15a) (L-vinylglycine 26.5 min; D-vinylglycine 28.4 min).



mp 178-180°C (dec); TLC (1:1:1:1, EtOAc:*n*BuOH:AcOH:H_2O) R_f 0.46; ^{1}H NMR (D_2O, 250 MHz) δ 5.85 (ddd, 1H, *J* = 7.4, 10.1, 17.4Hz, β-C*H*), 5.38 (d, 1H, *J* = 17.2Hz, γ-C*H*H), 5.38 (d, 1H, *J* = 10.4Hz, γ-C*H*H), 4.27 (d, 1H, *J* = 7.3Hz, α-C*H*); ^{13}C NMR (D_2O, 50.3 MHz): δ 174.7 (CO_2H), 132.0 (β-CH), 124.8 (γ-CH_2), 59.1 (α-CH); ESI-MS ($M + H^+$) 102.22.

3.4.8 Acid Hydrolysis of Cbz-L-Ser(CH_2TMS)-OBO Ester 3.45 with 6N HCl. (2*S*)-2-amino-3-butenoic acid, 3.7.

Cbz-L-Ser(CH_2TMS)-OBO ester **3.45** (0.140 g, 0.34 mmol) was mixed with 6N HCl (10.0 mL) and refluxed for 3 h. The solution was cooled extracted twice with ether (2 × 5 mL), neutralized with a saturated solution of $NaHCO_3$ (approx. 50 mL), then loaded on an anion exchange column (Bio-Rad AG 1-X4 100-200 mesh, chloride form, converted to hydroxide form by prewashing with 4N NaOH). The column was rinsed with H_2O (5 cloumn lengths) and eluted with 1N AcOH, then lyopholized to give 0.028 g (81%) of a colourless powder. Recrystallization (H_2O/acetone) gave 0.025 g (74%) of a white solid. Derivatisation with *o*-phthaladehyde and *N*-isobutyryl-L-cysteine, and analysis by HPLC indicated 95% ee (conditions as described in 2.4.15a and retention times as in 3.4.7).



mp 177-180°C (dec); TLC (1:1:1:1, EtOAc:*n*BuOH:AcOH:H_2O) R_f 0.46. ^{1}H NMR (D_2O, 250 MHz) δ 5.85 (ddd, 1H, *J* = 7.4, 10.1, 17.4Hz, β-C*H*), 5.38 (d, 1H, *J* = 17.2Hz, γ-C*H*H), 5.38 (d, 1H, *J* = 10.4Hz, γ-C*H*H), 4.27 (d, 1H, *J* = 7.3Hz, α-C*H*); ^{13}C NMR (D_2O, 50.3 MHz): δ 174.7 ($\underline{C}O_2H$), 132.0 (β-$\underline{C}$H), 124.8 (γ-$\underline{C}H_2$), 59.1 (α-$\underline{C}$H); ESI-MS (M + H^+) 102.29.

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Chapter Four

Stereoselective Synthesis of γ-Substituted Glutamic Acids

4.1 Introduction

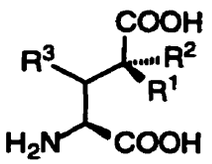
4.1.1 Isolation

γ-Substituted glutamic acids have received significant attention recently due to both their diverse biological activity and synthetic significance. Various glutamic acid derivatives have been isolated from natural sources, beginning with the isolation of γ-methylglutamic acid **4.1** (Scheme 4.1) from the fern *Phyllitis scolopendrium* in 1955 and subsequently from numerous other plants including the mushroom *Mycena pura*.[1] γ-Ethylglutamic acid **4.3** has been found in varying concentrations in *Wagatea spicata* and the seeds of the *Caesalpinia* species.[2]

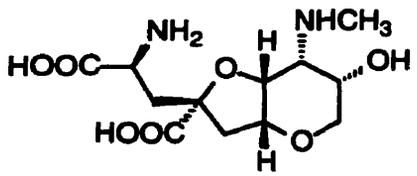
γ-Hydroxyglutamic acid **4.4** was first isolated from the plant *Phlox decussata*[3] and later as a metabolite in the *Hemerocallis* species.[1b] Both (2*S*,4*R*) and (2*S*,4*S*)-γ-hydroxyglutamic acid have been found to be constituents of natural product skeletons, including the potent epileptogenic dysiherbaine **4.5** isolated from the marine sponge *Dysidea herbacea*[4] and the sweetener, monatin **4.6** isolated from *Schlerochiton ilicifolius*.[5] The L-*threo* diastereomer of β-hydroxyglutamic acid **4.7** was first identified in the natural product, antibiotic S-520, obtained from *Streptomyces diastaticos* in 1970.[6] This α-amino acid has been used in the synthesis of other natural products, most noticeably tricolomeric acid **4.8** and its analogs, which have insecticidal activity.[7] β,γ-dihydroxyglutamic acid **4.9** was isolated from the seeds of *Lepidium sativium* and leaves

of *Rheum rhaponticum* over forty years ago[8] and since that time has been shown to be a constituent of a large variety of flowering plants, ferns, mosses and mushrooms.[9]

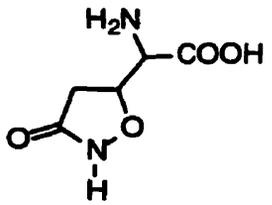
Scheme 4.1



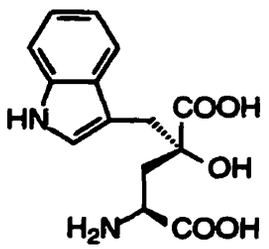
4.1 R^1=Me, R^2=R^3= H
4.2 R^2=Me, R^1=R^3= H
4.3 R^1=Et, R^2=R^3= H
4.4 R^1=OH, R^2=R^3= H
4.7 R^1=R^2=H, R^3=OH
4.9 R^1=R^3= OH, R^2= H
4.10 R^1=CO_2H, R^2=R^3= H
4.11 R^1=NH_2, R^2=R^3= H
4.12 R^1=F, R^2=R^3= H



4.5



4.8



4.6

The best-known γ-substituted-α-amino acid is γ-carboxyglutamic acid **4.10** which is a component of several vitamin K dependent hemostasis proteins, including prothrombin. [10] The γ-dicarboxylate moiety binds Ca^{2+} ions to induce a conformational change in numerous hemostasis proteins providing a biofeedback route for the control of bleeding. First detected in 1974 this calcium binding amino acid has also been discovered in the conantokins, potent *N*-methylaspartate receptor agonist peptides isolated from the venom of the cone snail *Conus geographus*.[11]

γ-Aminoglutamic acid **4.11** and γ-haloglutamic acids **4.12** have not been identified in nature to date but the compounds are of interest therapeutically as will be discussed (*vide infra*).

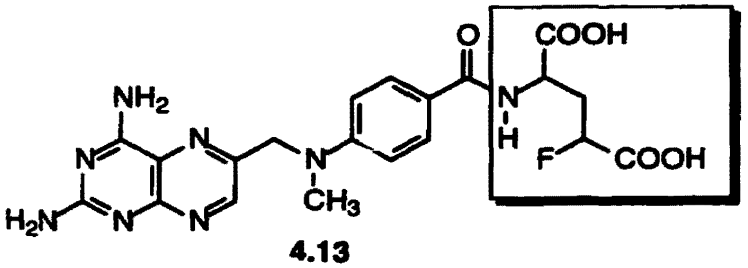
4.1.2 Biological Activity

The main medicinal interest in glutamic acid derivatives is due to the fact that glutamic acid is the major excitatory neurotransmitter in the central nervous system (CNS) and is thought to play a role in a number of pathological processes including epilepsy, pain, neurodegenerative diseases and schizophrenia.[12] Glutamic acid exerts its actions at both the ionotropic and metabotropic excitatory amino acid (EAA) receptors. The metabotropic glutamate receptor (mGlu) and ionotropic glutamate receptor (iGlu) are disparate receptors that are further divided into subclasses of receptors. The mGlu receptors are a family of eight different G-protein coupled receptors: Group I receptors (mGlu 1 and 5) couple to phospholipase C, whereas both group II (mGlu 2 and 3) and group III (mGlu 4,6,7 and 8) are negatively coupled to adenylate cyclase.[13] The iGlu receptors are associated with integral cation-specific channels and include the *N*-methyl-D-aspartate (NMDA), 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propanoic acid (AMPA) and kainic acid (KA) subtypes.[14]

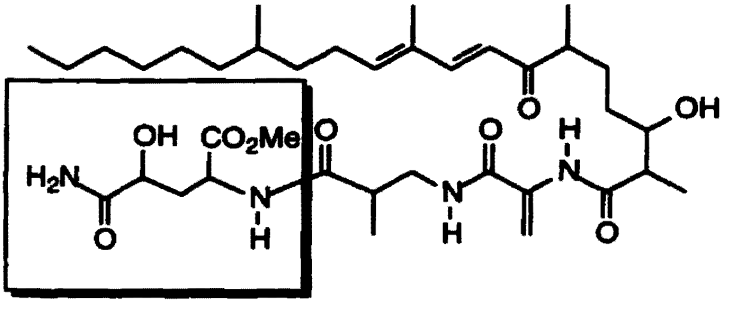
A variety of agonists and antagonists have been described for both iGlu and mGlu receptors, most of which inherently include some type of glutamate motif. For example, (2*S*,4*R*)-methylglutamic acid **4.2** is a potent and selective agonist of the iGlu KA receptor[15] while (2*S*,4*S*)-methylglutamic acid **4.1** is an agonist of the group I mGlu receptors.[16] The medical interest in this class of receptors arose in the 1980's in an attempt to modify various biological processes and/or diseases, and can be associated with the asymmetric methods developed for the synthesis of various glutamate derivatives.

Glutamic acid derivatives are also constituents of numerous natural products and synthetic targets. This includes both discrete incorporations of the glutamic acid motif within the natural product as in the case of dysiherbaine **4.5** or the potent antifolate drug methotrexate **4.13** and the presence of glutamate derivatives within the composition of peptides, as in the lipopeptide antibiotic YM-170320 **4.14** (Scheme 4.2).[17]

Scheme 4.2



4.13



4.14

Derivatives of glutamic acid have been used as substrate analogs in the study of various enzyme mechanisms. In 1984, Weinstein *et al.* used both γ-methyl and γ-fluoroglutamic acid during a study of the stereochemistry of the vitamin K dependent carboxylation of small peptides.[18] γ-Aminoglutamate derivatives have been employed to probe the mechanism of mammalian dihydroorotase, a component of the trifunctional CAD (carbamoylphosphate synthetase II, aspartate transcarbamoylase and dihydroorotase) protein that is involved in the *de novo* pyrimidine nucleotide biosynthesis

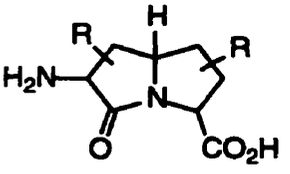
pathway.[19] γ-Difluoroglutamic acid has been designed as an alternative substrate and inhibitor of folate dependent enzymes.[20] γ-Ethylglutamate derivatives have been utilized to probe the mechanism of serine proteases in an attempt to design γ-lactam analogues of potent β-lactam antibiotics.[21]

Glutamic acid has also been used for the preparation of β-turn mimetics in order to introduce restrained backbones and side-chain conformations in peptide-based therapeutics.[22] These conformational restrictions reduced the ability for protease degradation *in vivo*, increase bioavailabilty and generally incorporate atom efficiency. Glutamic acid enjoys a preferred role in the synthesis of these heterocycles because of the existence of an α-amino-δ-carboxy moiety in a five carbon arrangement which is ideally suited for the synthesis of pyrollidines **4.15**, pyyrolizidinones **4.16** and indolizidines **4.17**, all components of β-turn mimetics (Scheme 4.3). However, stereoselective methods for the generation of substituted glutamates for the subsequent incorporation into β-turn mimetics are lacking.

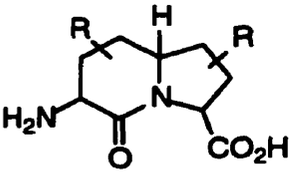
Scheme 4.3



4.15



4.16



4.17

As a result of the varied biological activities discussed above, numerous methodologies have been developed to provide routes to these synthetic targets but none address all the requirements discussed in Section 1.4. In particular, few stereoselective

syntheses exist for the functionalization (Scheme 4.1 where R=alkyl, allyl, acyl, hydroxyl, amino, halo, etc.) of the γ-position of glutamate by a broad range of reagents.

4.1.3 Synthesis of Racemic γ-Substituted Glutamic Acids

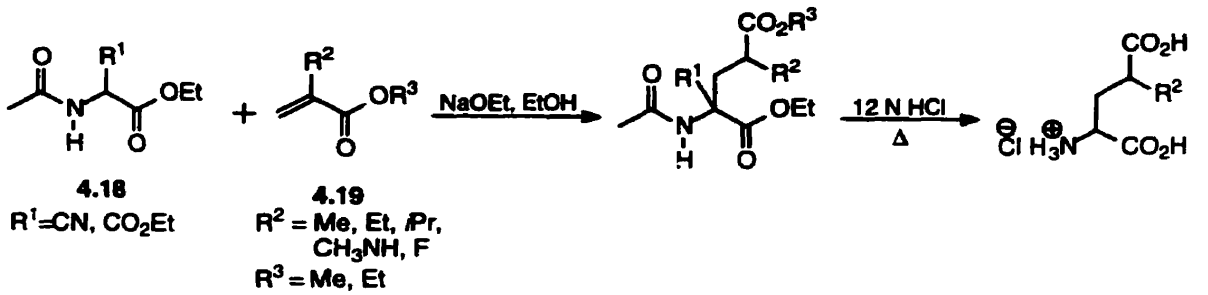
Many of the general synthetic methods described in chapter one can be used to synthesize γ-substituted glutamic acids. The synthetic challenge arises when attempting to induce stereoselectivity in the remote γ-position in glutamic acid and although there are exceptions, these methods generally fail to give high selectivity.

4.1.3.1 Synthesis of γ-Substituted Glutamic Acids: General Racemic Methods

Michael reactions have been used extensively to synthesize racemic γ-substituted glutamates due to the well characterized nature of the reaction and availability of starting materials. The racemic synthesis of γ-methylglutamic was first reported in 1952 by two independent groups. Done and Fowden reacted diethyl acetamidomalonate **4.18** (R^1=CO_2Et) and methyl methacrylate **4.19** (R^2,R^3=Me) in a Michael reaction followed by hydrolysis and decarboxylation to give racemic γ-methylglutamate in 45% overall yield.[23] In a similar reaction, Fillman and Albertson used ethyl acetamidocyanoacetic ester **4.18** (R^1=CN) and methyl methacrylate **4.19** (R^2,R^3=Me) followed by acid hydrolysis and recrystallization to give an unidentified stereoisomer of γ-methylglutamic acid in 17% yield (Scheme 4.4).[24] This procedure was also used to synthesize other racemic γ-alkylsubstituted glutamates.[25]

A similar method was used to synthesize γ-hydroxyglutamic acid **4.4** by reacting ethyl acetamidocyanacetate with ethyl-α-acetoxy-β-chloropropionate. Acid hydrolysis gave γ-hydroxyglutamic acid in 50-60% yield as 1:1 mixture of diastereomers.[26]

Scheme 4.4

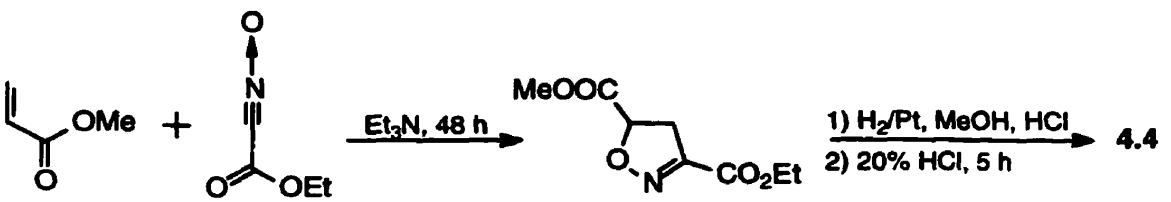


4.18
R^1=CN, CO_2Et

4.19
R^2 = Me, Et, *i*Pr, CH_3NH, F
R^3 = Me, Et

The 1,3-dipolar addition of ethyl cyanoformate *N*-oxide **4.20**, generated from glycine and methyl acrylate gave isoxazoline **4.21** which upon catalytic hydrogenation and acid hydrolysis gave *erythro*-(2*S*,4*R*)-γ-hydroxyglutamate **4.4** in 20% overall yield and the *threo* (2*R*,4*R*) isomer in 40% yield as the lactone (Scheme 4.5).[27]

Scheme 4.5



4.20 **4.21**

γ-Aminoglutamate was synthesized by a Michael reaction between diethyl acetamidomalonate **4.18** (R^1=CO_2Et) and ethyl acetamidoacrylate **4.19** (R^2=CH_3CONH, R^3=Et) then thermally decarboxylated and hydrolyzed to give racemic γ-aminoglutamate **4.12**.[28]

Racemic γ-fluoroglutamate has been synthesized by a variety of methods including the Michael addition between diethyl acetamidoacrylate **4.18** (R^1=CO_2Et) and ethyl-2-fluoroacrylate **4.19** (R^2=F, R^3=Et).[29]

4.1.4 Synthesis of Optically Active γ-Substituted Glutamic Acids

Synthetic methodologies designed specifically for the generation of glutamic acid derivatives center around four main concepts with varying degrees of success: glycine enolate equivalents, enzymatic methods, asymmetric hydrogenation and γ-anion chemistry (including the use of pyroglutamate). Surprisingly few diastereoselective syntheses for γ-substituted glutamic acids exist.

4.1.4.1 Glycine Enolates

The Ni(II) glycine Schiff base enolate **1.39** of Belekon *et al.*[30] (Scheme 1.3.3.1) was reacted in a Michael addition with methyl methacrylate to give 2:1 mixture of 2*S*:4*R* **4.2** and 2*S*:4*S* methylglutamic acid **4.1** in 75% yield. However, only the methyl adduct was reported.

1,4-Addition of Schöllkopf's *bis*-lactim ether **1.33** (Scheme 1.3.3.1) with methyl methacrylate **4.19** (R^2,R^3=Me) resulted in a 1:1 ratio of 2*R*:4*R* and 2*R*:4*S* methylglutamic acid to the exclusion of any of the 2*S* isomer.[31]

4.1.4.2 Enzymatic Methods

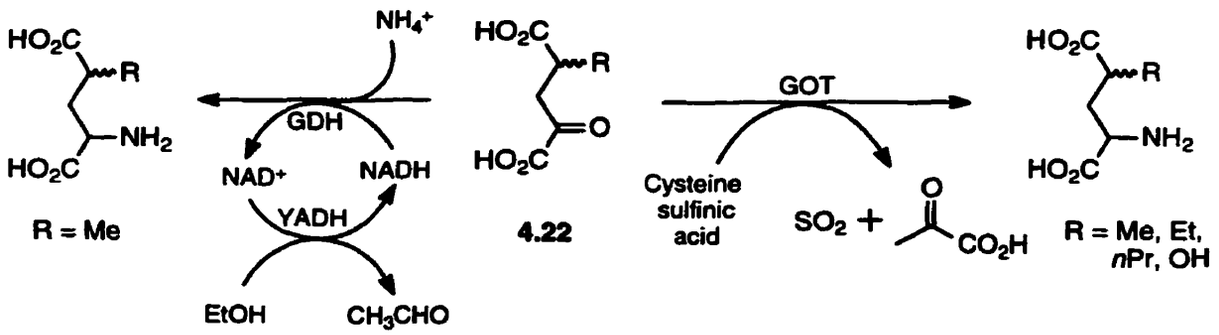
Enzymatic methods have been used to prepare asymmetric molecules due to the inherent nature of enzymes to differentiate between chiral centers. Both of the methods reported to synthesize γ-glutamate derivatives have used appropriately substituted 2-ketoglutaric acids as the substrate. In 1984, Righini-Tapie and Azerad incubated glutamate dehydrogenase (GDH, EC 1.4.1.2) in ammonium phosphate buffer with racemic methyl ketoglutarate **4.22** (R = Me) to give γ-methylglutamate **4.1** in 60 % yield (Scheme 4.6, left-hand side). Surprisingly, no differentiation between the two

enantiomers of methyl ketoglutarate **4.22** (R = Me) was observed since a 1:1 mixture of 2*S*:4*R* and 2*R*:4*S* methylglutamic acid was isolated.[32]

In 1987, Bolte *et al.* reported the large-scale synthesis of γ-hydroxy-L-glutamic acid by the transamination of cysteine sulfinic acid and γ-hydroxy-α-ketoglutarate catalyzed by glutamic oxalacetic transaminase (E.C. 2.6.1.1).[33] In 1993, the same group described the synthesis of methyl and ethyl γ-substituted glutamic acid, and in 1999 the synthesis of *n*-propyl-γ-glutamate (Scheme 4.6, right-hand side). All three alkyl substituents gave 4:1 ratios of 2*S*:4*R* to 2*S*:4*S* γ-alkylglutamates while equal amounts of *threo* and *erythro* γ-hydroxyglutamate were isolated.[34]

Kokuryo *et al.*[35] used a combination of recrystallization and porcine kidney aminoacylase (EC. 3.5.1.14) to prepare enantiomerically pure L-*erythro* and L-*threo*-4-fluoroglutamic acid in poor yield from a racemic mixture of 4-fluoroglutamate **4.12** synthesized via Hudlicky's method.[36]

Scheme 4.6

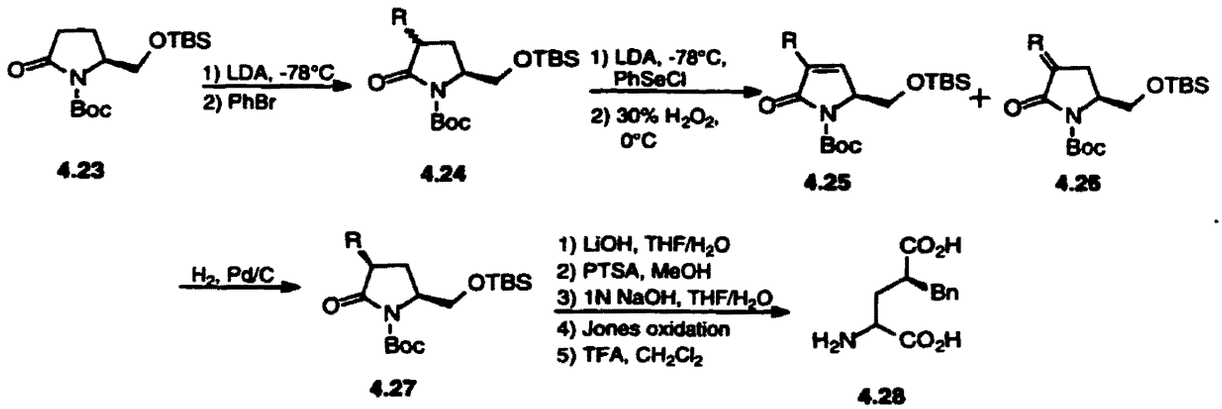


GDH = Glutamate dehydrogenase
YADH = Yeast alcohol dehydrogenase
GOT = Glutamic oxalacetic transaminase

4.1.4.3 Catalytic Hydrogenation

The first use of catalytic hydrogenation to synthesize γ-substituted glutamic acids was reported in 1990 by Hon *et al.*[37] TBDMS protected pyrollidinone **4.23** derived from L-pyroglutamic acid **4.29** was alkylated and then selenenylated/deselenylated to give both endo **4.25** and exo **4.26** α,β-unsaturated lactams. Catalytic hydrogenation gave the (2*S*,4*S*) diastereomer **4.27** as the sole product through steric approach control induced by the TBDMS protecting group. Deprotection eventually gave the desired γ-benzylglutamate **4.28** in 13% overall yield (Scheme 4.7).

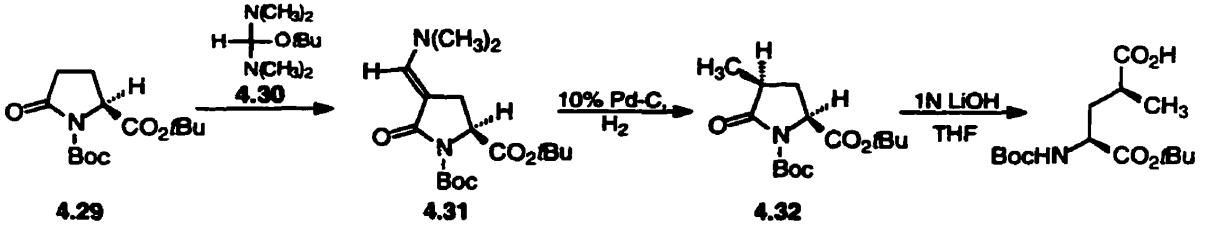
Scheme 4.7



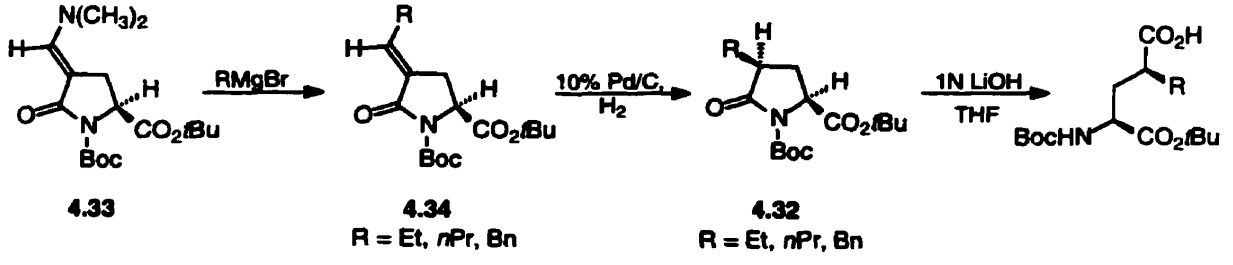
In 1992, Young *et al.*, while preparing isotopically labeled valines, used 2*S*-pyroglutamatic acid as the starting point of their synthesis since it "not only had the desired stereochemistry at C2, but the 1,3 relationship of C2 and C4 in the ring offered the possibility of inducing chirality at C4."[38] The *t*-butyl ester of pyroglutamate **4.29** was converted to the enaminone **4.31** in 91% yield using Bredereck's reagent **4.30**,[39] and the intermediate then reduced by catalytic hydrogenation using 10% Pd-C to give the stereochemically pure *cis*-4-methyl derivative **4.32** (Scheme 4.8).

This methodology was further expanded to include other γ-alkyl groups (R=Et, *n*Pr, Bn)[40] in an elegant 1,4-addition of Grignard reagents to the vinyl amide **4.33** to give the alkylidene derivative **4.34** (Scheme 4.9).[41] Hydrogenation gave one stereoisomer.

Scheme 4.8

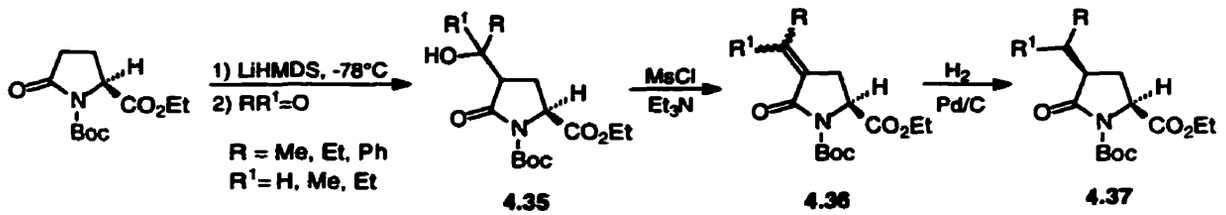


Scheme 4.9



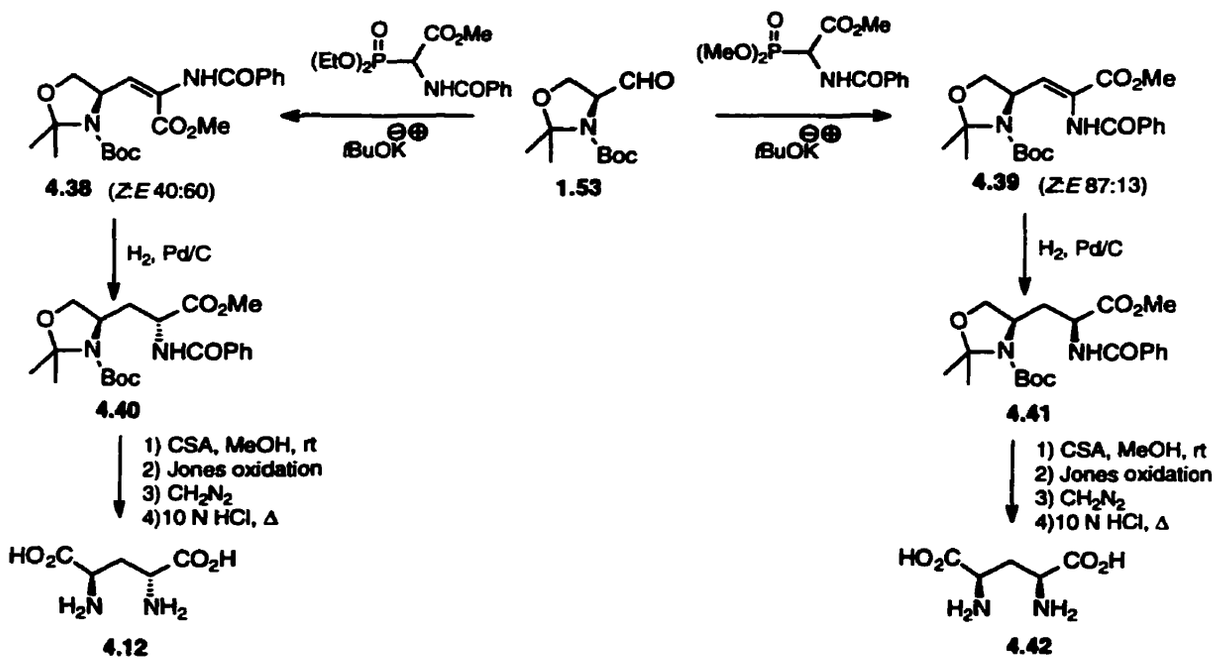
A similar approach has been described to synthesize 4-substituted glutamic acids by using aldol reactions to introduce various substituents.[42] The hydroxylated products **4.35** were dehydrated to the 4-alkylidene pyroglutamates **4.36** and then hydrogenated to give exclusively the 2*S*,4*S* stereoisomer **4.37** (Scheme 4.10). A variety of esters have been used as protecting groups in both this synthesis and by other groups indicating a bulky group is not necessary to achieve stereoselectivity in this step.[43]

Scheme 4.10



Both the *meso* and 2*R*,4*R* forms of γ-aminoglutamic acid have been synthesized using Garner's aldehyde **1.53** (Scheme 1.24) to generate either an *E* **4.38** or *Z* **4.39** olefin which is subsequently hydrogenated to give almost exclusively one stereoisomer.[44] In the case of **4.38**, a ratio of 95:5 for **4.40**:**4.41** (Scheme 4.11, left-hand side) and for **4.39**, a ratio of 94:6 for **4.41**:**4.40** (Scheme 4.11, right-hand side). *meso*-2,4-Diaminoglutamic acid **4.42** was generated in 45% overall yield from the aldehyde **1.53** although no overall yield was given for the 2*R*,4*R* derivative.

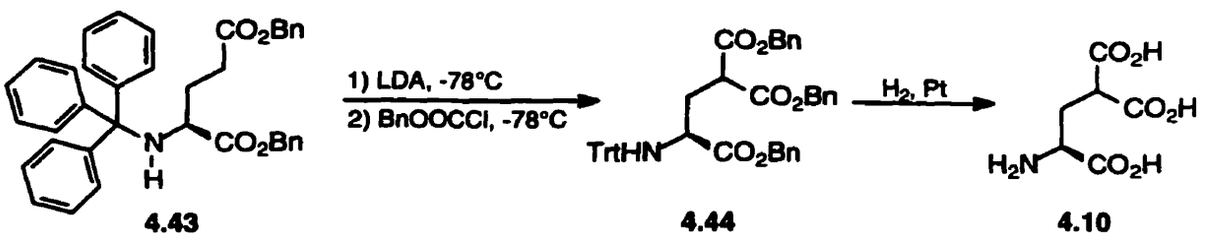
Scheme 4.11



4.1.4.4 γ-Anion Chemistry (Including Pyroglutamic Acid)

Various groups have tried to utilize the chirality of glutamic acid in an attempt to induce stereochemistry in addition reactions. The first report of the use of carbanion chemistry to generate 4-substituted glutamic acids was in 1980 when Robert *et al.* attempted to synthesize γ-carboxyglutamic acid in what they termed an effort to mimic nature.[45] Glutamic acid, protected as the *N*-trityl dibenzyl ester **4.43**, was treated with lithium diisopropylamide (LDA) to generate the enolate. The bulky trityl group was used to decrease the acidity of the α-proton through steric effects and hence reduce racemization. The enolate was treated with benzyl chloroformate, then hydrogenated to give γ-carboxyglutamic acid **4.10** in 50% yield from the fully protected glutamate. The authors reported no detectable racemization occurred at the α-carbon.

Scheme 4.12



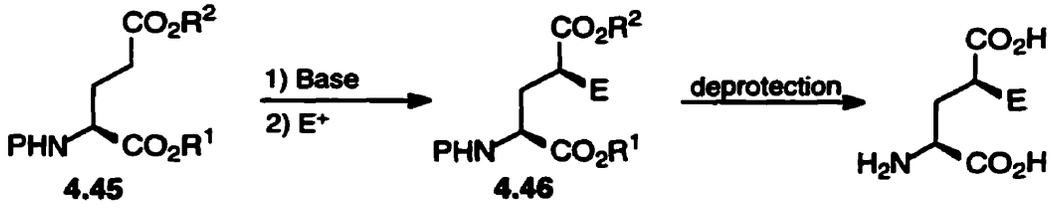
4.43 **4.44** **4.10**

Tanaka *et al.*[46] introduced the use of γ-anion chemistry with pyroglutamate, using the chiral template to synthesize γ-carboxyglutamic acid in a manner identical to that reported by Robert and co-workers six years previously.

Baldwin *et al.*, in the first of a series of papers, described enolate generation and subsequent aldol chemistry on *N*-trityl-glutamic acid-γ-methyl ester α-*t*-butyl ester **4.45** (P=Trt, R^1=*t*Bu, R^2=Me).[47] Although no mention was made concerning any

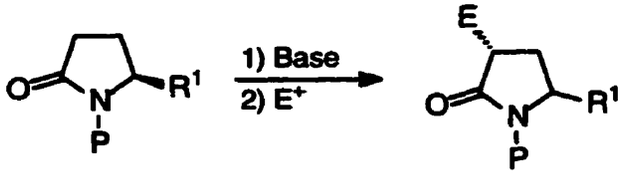
stereochemical induction at the remote γ-position, Baldwin and co-workers noted that the presence of the *N*-trityl group appeared to prevent racemization at the α-carbon.

Scheme 4.13



In rapid succession, articles appeared in which stereoselective γ-anion chemistry was investigated to derivatize both glutamic acid and pyroglutamic acid. In 1989, Ohta *et al.*[48] reported the hydroxylation of *N*-Boc-L-pyroglutamate benzyl ester to give the 2*S*,4*R* derivative of **4.48** (P=Boc, R^1=CO_2Bn, E=OH) with complete stereoselectivity. Baldwin and co-workers experienced similar results when alkylating **4.47** (P=Boc, R^1=CO_2tBu) with benzyl bromide, although both methyl iodide and bromopropane failed to alkylate.[49] Ezquerra *et al.* observed the same selectivity for larger aromatic substitutes but poor selectivities for smaller groups such as allyl and cyanomethyl.[50] This methodology has been used to synthesize 4-allyl and 4-cinnamyl derivatives for structure activity relationship (SAR) studies on mGlu 5 kinate receptor agonists.[51]

Scheme 4.14



4.47 **4.48**

Rapoport *et al.*, in the first reported stereoselective synthesis of 4-substituted glutamic acids used the 9-(9-phenylfluorenyl)(PhFl) group for nitrogen protection **4.45** (P=PhFl, R^1=*t*Bu, R^2=Me), which also prevents epimerization of the α-carbon due to the size of PhFl.[52] Minimal selectivity, in the order of 2:1 (2*S*,4*S*:2*S*,4*R*) for MeI were achieved and 3:1 with propyl triflate, **4.46** (P=PhFl, R^1=*t*Bu, R^2=Me, E = Me, *n*Pr).

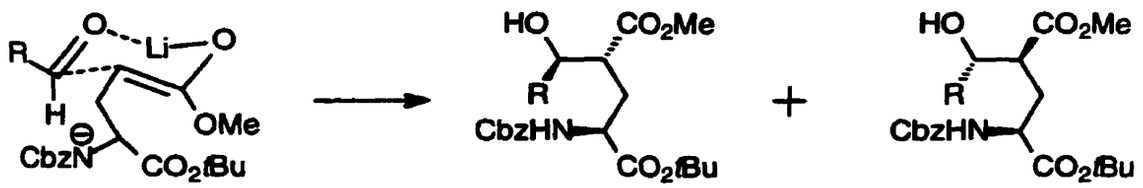
In the same paper in which they describe the synthesis of 2*S*,4*S* γ-substituted glutamic acid derivatives via catalytic hydrogenation (Section 4.1.4.3), Hon *et al.* describe the alkylation of pyroglutamate **4.47** (P=Boc, R^1=CH_2OTBDMS) with benzyl bromide to give the 2*S*,4*R*-pyroglutamate with 15:1 selectivity.[37] Pyroglutamate **4.47** (P=TBDMS, R^1=CO_2Bn) was also alkylated with ethyl iodide to give the 2*S*,4*R*:2*S*:4*S* product in a 10:1 ratio.[53]

In 1993, Hanessian and Vanasse reported the 9:1 (2*S*,4*S*;2*S*,4*R*) hydroxylation of Cbz-glutamate dimethyl ester **4.45** (P = Cbz, R^1=R^2=Me) with Davis' oxaziridine to give the γ-hydroxy derivative in 70% yield.[54]

The 2,4-diamino derivative of glutamic acid was synthesized via azide displacement of a protected glutamate (**4.46**, P=PhFl, R^1=*t*Bu, R^2=Me, E=OH) in a 3:1 ratio (2*S*,4*S*:2*S*,4*R*) and 54% yield followed by reduction for subsequent incorporation into a benzodiazepine side-chain of a tripeptide.[55]

Johnstone *et al.* reported the successful γ-methylation of **4.45** (P=Cbz, R^1=*t*Bu, R^2=Me) in 1994 in a ratio of 2:1 (2*S*,4*S*:2*S*,4*R*) and 1:1 for allylation, however aldol reactions with benzaldehyde proceeded with high selectivities at the γ-carbon since only two of four possible stereoisomers were formed in a 1:1 ratio.[56] The lack of stereoselectivity during alkylation was attributed to the fact that the dianion of the

protected glutamate did not adopt a chelated, cyclic structure since both faces of the enolate were obviously accessible whereas the benzaldehyde adduct formed a six-member, chelated, cyclic transition state **4.49** where all substituents adopt equatorial positions (Figure 4.1). The authors commented "the chiral center at the α-carbon appears to have little effect on the induced stereochemistry at the chiral centers formed during the reaction."



4.49

Figure 4.1: Six-member chelated cyclic transition state.[56a]

Shortly thereafter, Gu and co-workers published the stereoselective synthesis of *p*-nitrobenzoyl *N*-protected 2*S*,4*S*-methylglutamate **4.46** (P=*p*NB, R^1=R^2=E=Me) obtained by dianion chemistry and isolated as a single isomer.[15,57] However, in contrast to those observations reported by Johnstone and co-workers,[56] Gu *et al.* explained their dramatic results by way of a chelated dianion similar to those reported for the alkylation of succinimides.[58] The use of potassium as a counter-ion reportedly negated any stereoselectivity, strengthening the argument of chelation controlled alkylation. Gu *et al.* also reported the synthesis of 2*S*,4*R*-methylglutamate by the alkylation of protected pyroglutamate **4.47** (P=Boc, R^1=CH_2OTBDPS) with methyl iodide to give the 2*S*,4*R*;2*S*,4*S* derivative in a 6:1 ratio.[59] Hanessian and Margarita reported the allylation of similarly protected glutamic acid **4.45** (P=Cbz, R^1=R^2=Me) in remarkable

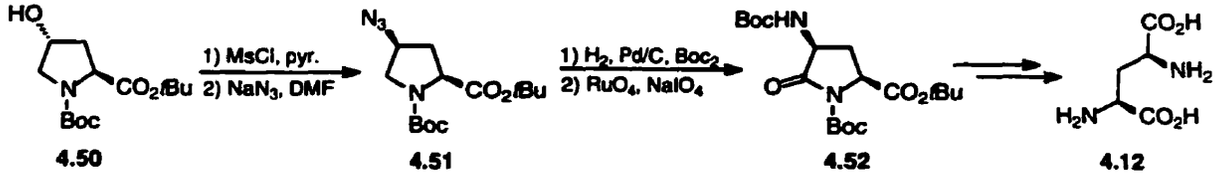
stereoselectivities of >99:1 for 2*S*,4*S*:2*S*,4*R*.[60] However, in our hands, methylation and allylation consistently gave selectivities of 6:1 as described later.

4.1.4.5 Other Methods for the Synthesis of γ-Substituted Glutamic Acids

Numerous methods exist for the synthesis of γ-substituted glutamic acids that do not fit into the categories described above. These are methods usually designed to synthesize a specific amino acid.

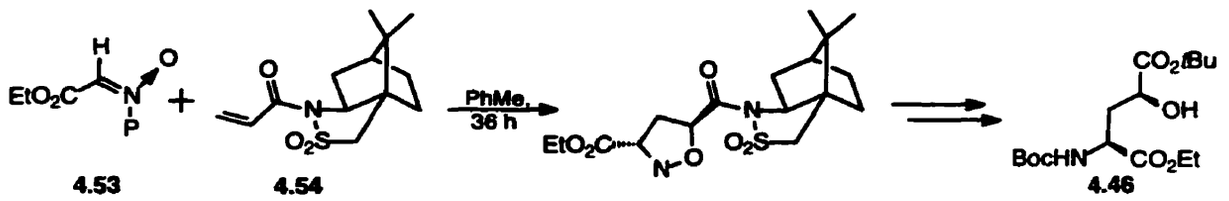
Protected 4-hydroxy-L-proline **4.50** has been used in a synthesis of 2*S*,4*S*-diaminoglutamate **4.11**.[61] Conversion of the alcohol to the mesylate was followed by S_N2 displacement with azide to give **4.51** which was then reduced and trapped as the Boc protected amine **4.52** (Scheme 4.15). Hydrolytic ring-opening and deprotection yielded 2*S*,4*S*-diaminoglutamate **4.11** as one diastereomer in 20% overall yield. 4-Fluroglutamate **4.12** has also been synthesized using similar methods with DAST as the fluorinating agent.[36]

Scheme 4.15



Oppolzer's chiral sultam (Section 1.3.3.1) has been employed in the 1,3-dipolar cycloaddition of nitrone **4.53** with acrylamide **4.54**. After removal of the chiral synthon and reprotection, protected (2*S*,4*S*)-hydroxyglutamate **4.46** (P=Cbz, R^1=Et, R^2=*t*Bu, E=OH) was isolated in 52% overall yield with a diastereomeric ratio of 20:1 (Scheme 4.16).[62]

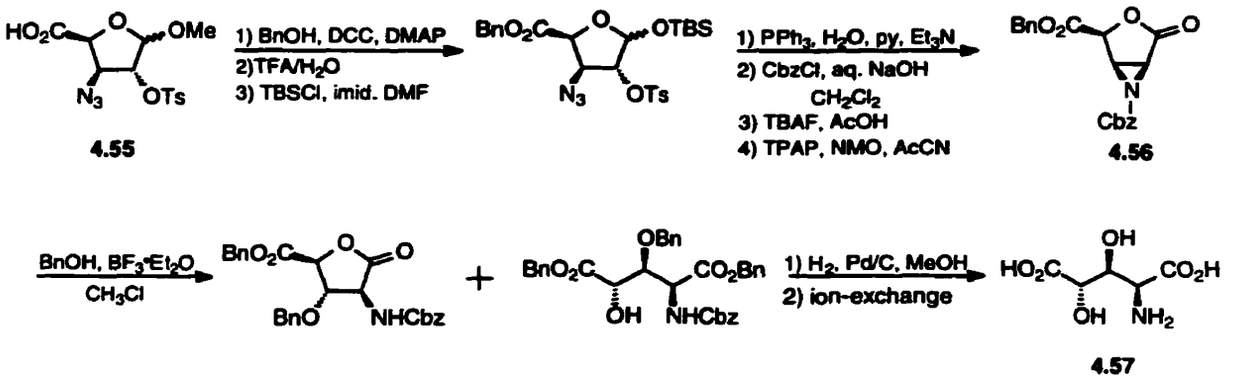
Scheme 4.16



4.53 4.54 4.46

The first synthesis of β,γ-dihydroxyglutamic acid **4.9** has only recently been reported.[63] 2*S*,3*S*,4*S*-Dihydroxyglutamic acid **4.57** was synthesized from a D-ribose derivative **4.55** via an aziridino-γ-lactone **4.56** in a lengthy procedure and 3% overall yield (15 steps) (Scheme 4.17). Pharmacological studies have shown **4.57** to be an agonist against mGlu 1 and weak agonist of mGlu 4.[64]

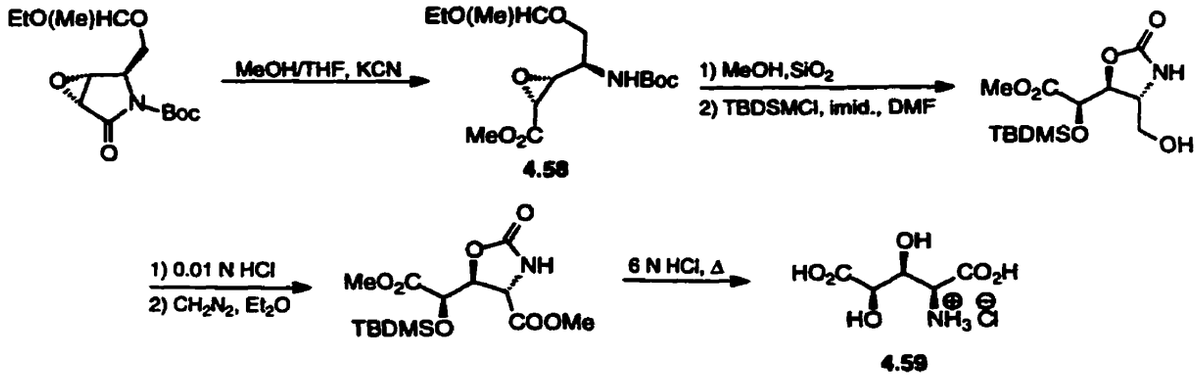
Scheme 4.17



4.55 4.56 4.57

The synthesis of (2*S*,3*S*,4*R*)-dihydroxyglutamic acid **4.59** followed shortly thereafter using a strategy essentially opposite that used for the synthesis of the 4*S* stereoisomer.[65] An epoxy-pyrrolidinone template **4.58** derived from S-pyroglutaminol (**4.48**, E=OH, R^1=CO_2H) gave the desired product **4.59** in 50% overall yield over 7 steps.

Scheme 4.18



4.2 Results and Discussion: Synthesis of γ-Substituted Glutamates

4.2.1 Methylation of Cbz-Glu(OMe)OBO 4.69: Optimization and Determination of Diastereoselectivity.

Since the OBO ester protecting group had shown its usefulness in both maintaining the chiral integrity of the α-carbon and acting as a directing group through steric constraints in various nucleophilic additions (Chapter 2), we decided to investigate its utility with electrophilic reactions in the context of amino acid chemistry. Aspartic and glutamic acid provided the ideal opportunity to explore this area since both are inexpensive, readily available and amenable to electrophilic chemistry. Furthermore, the additional methylene unit in the sidechain of glutamate provides an opportunity to explore not only 1,2-induction as with aspartate but also 1,3-induction of stereochemistry (aspartic acid is described in chapter five). We were also eager to develop a broadly applicable route with both high yields and diastereoselectivities towards the synthesis of γ-substituted glutamates since all the reports in the literature describe methods that provide access to only a few of the potential γ-substituted glutamates. We decided to protect the glutamic acid sidechain as the methyl ester in order to ensure any

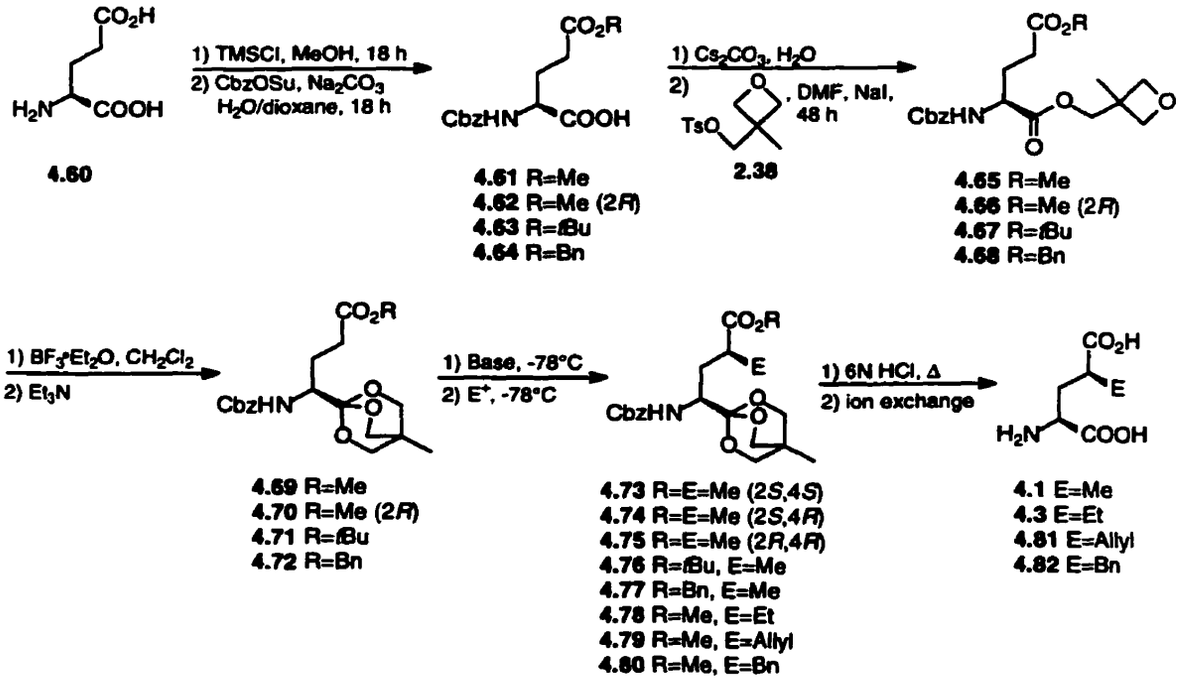
stereoselectivity during electrophilic additions would be due to 1,3-asymmetric induction of the chiral center and not a result of a bulky γ-carboxy protecting group. The α-amino group was protected as the Cbz group since it is typically more robust that than the Boc group and the use of strong base to generate the enolate prohibited the use of the Fmoc group.

Regioselective esterification of glutamate **4.60** was achieved using a similar method to that reported earlier by our group.[66] Chlorotrimethylsilane (TMSCl) was added in two separate aliquots to glutamic acid **4.60** stirring in dry methanol (Scheme 4.19). After the removal of any residual solvent, CbzOSu was used to protect the amine as the Cbz carbamate to give **4.61**. Purification at this point was laborious, therefore **4.61** was esterified using oxetane tosylate **2.38** to give the fully protected glutamate **4.65** in 68% and **4.66** in 62% yield over three steps. The *t*-butyl ester **4.67** was synthesized in 78% yield from commercially available **4.63** and the benzyl ester **4.68** in 75% yield from **4.64**. Boron trifluoride etherate mediated rearrangement of **4.65-4.68** to the OBO esters **4.69-4.72** gave crystalline products in 72%, 69%, 70% and 70% yield respectively after flash chromatography. Larger amounts (15-20 mol%) of boron trifluoride etherate could be used to rapidly promote the rearrangement of the oxetane ester **4.65**, conditions which caused an increase in decomposition of the OBO ester in the serine derivative **3.41** previously reported.[67] This is presumably due to the lack of interaction of a hydroxyl sidechain with the Lewis acid.

Lithium hexamethyldisilazane (LiHMDS) was chosen as the base to generate the dianion since other groups have reported inconsistent results and decomposition of the enolate with LDA.[49,52] However, careful control of the reaction conditions is necessary.

Inverse addition was used to generate the dianion (based on results described in chapter 5) and addition of electrophile were both carried out at –78°C to prevent decomposition of the enolate and erosion of diastereoselectivity which occurred upon warming to –30°C. Methyl iodide was chosen as the alkylating agent since an easily identifiable doublet would be formed which could be integrated by NMR methods to determine diastereoselectivity.

Scheme 4.19



At first, after generation of the enolate and addition of methyl iodide, the γ-methylglutamate **4.73** derivative was seemingly generated in approximately 10:1 ratio based upon ^{1}H-NMR integration of the new methyl group at δ1.15 ppm (Figure 4.2) although no other signals corresponding to the other diastereomer were observed. High field NMR analysis (≥ 300 MHz) was essential since examination of the methyl doublets on lower field instruments failed to distinguish between diastereomers (*vide infra*). In

fact, when we repeated the synthesis of 2*S*,4*S*-Cbz-Glu(γ-Me)(OMe)OMe **4.46** (P=Cbz, R^1=R^2=E=Me) reported by Hanessian and Margarita,[60] we observed (500 MHz ^{1}H-NMR) diastereomeric contamination of the crude product as a 6:1 mixture of the 2*S*,4*S*:2*S*,4*R* diastereomers. However, the 2*S*,4*R* isomer was not observed when using conditions identical to those by Hanessian and Margarita to analyze their product (200 MHz ^{1}H-NMR).

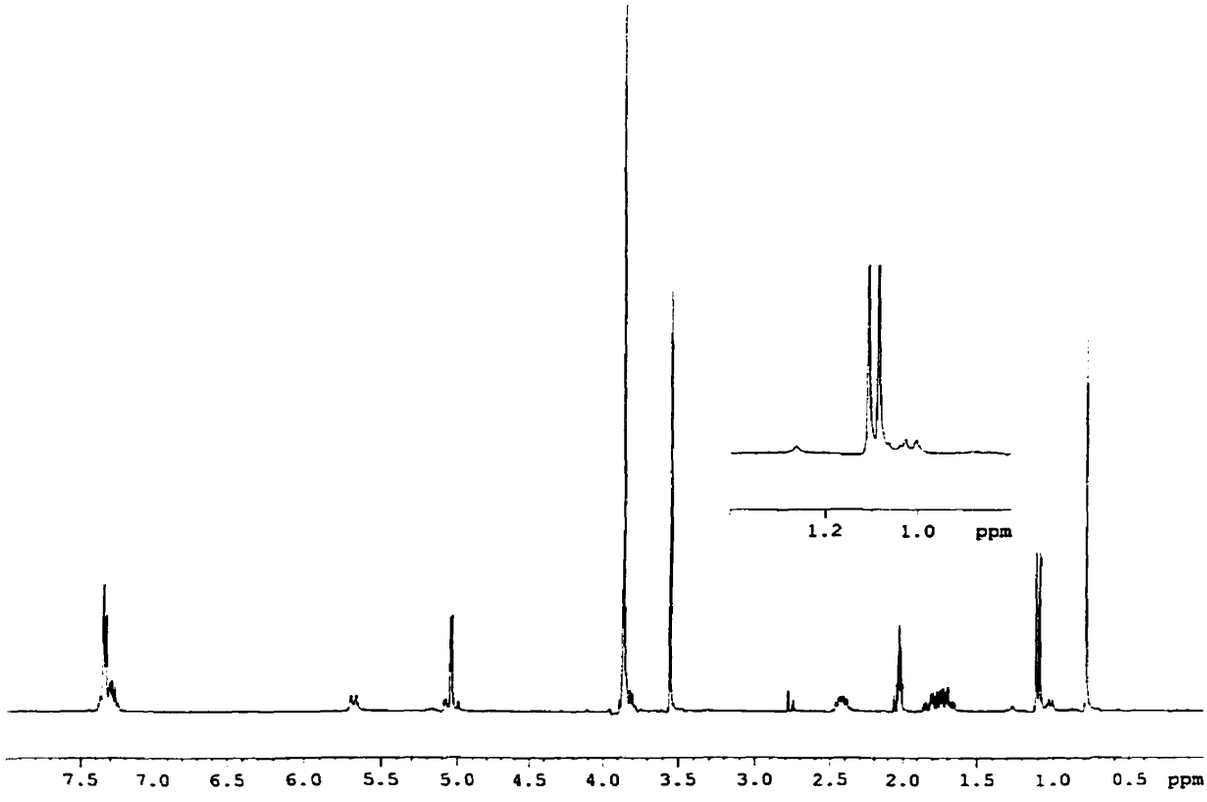


Figure 4.2: 300 MHz ^{1}H-NMR of Cbz-L-Glu(γ-Me)(OMe)OBO ester 4.73.

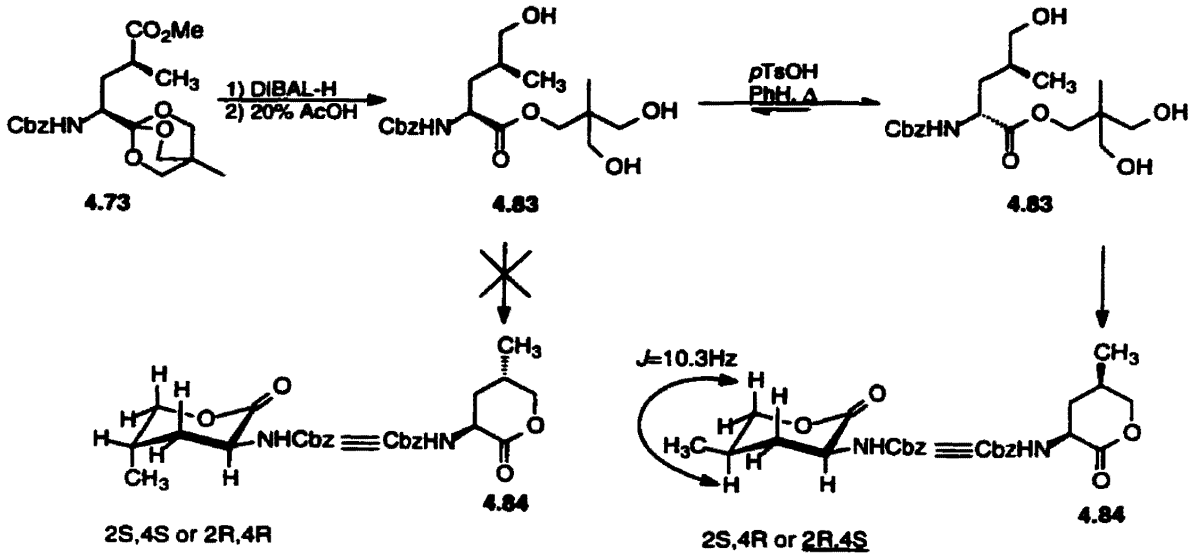
Numerous attempts at optimization failed to alter the 10:1 ratio, suggesting the existence of some other phenomena. ^{1}H-NMR studies in $CDCl_3$, CD_2Cl_2, acetone-d_6, methanol-d_4, THF-d_8, DMF-d_7 and DMSO-d_6 all showed the same ratio of methyl

doublets. Variable temperature ^{1}H-NMR studies in DMF-d_7 failed to disprove the notion that the minor doublet was due to the presence of rotamers.

In an effort to rationalize the modest selectivity we were encountering, we attempted to identify the stereochemistry of methylation. Lactones have been used extensively[68] to determine relative stereochemistry and so **4.73** was reduced with DIBAL-H and the OBO ester ring-opened with 20% acetic acid to the mhpd ester **4.83**, then lactonized under acidic conditions (*p*-TsOH in refluxing benzene) to give lactone **4.84** (Scheme 4.20). The relative stereochemistry was initially assigned as 2*S*,4*R* after decoupling ^{1}H-NMR experiments showed the proton at C4 to be axial with a coupling constant of 10.3Hz ($J_{4ax,5ax}$). Typical values for the ABX axial, axial proton coupling in lactones are 5-10Hz.[68] However, the assignment of this stereochemistry was determined to be incorrect since a crystal structure of **4.73** was eventually obtained (Figure 4.3) and X-ray analysis indicated 2*S*,4*S* stereochemistry (Appendix A). Presumably, under the thermodynamic conditions used to lactonize **4.83**, epimerization occurs at the α-carbon of 2*S*,4*S*-**4.83** to convert the dihydroxy ester to 2*R*,4*S*-**4.83** which then lactonizes to 2*R*,4*S*-**4.84**. An alternative mechanism involves the lactone 2*S*,4*S*-**4.84** racemizing to the more stable 2*R*,4*S*-lactone **4.84** in which both CH_3 and CbzHN are equatorial.[68c]

In an effort to irrefutably determine the diastereomeric ratio of methylation, we unsuccessfully attempted to epimerize the γ-carbon of γ-methylglutamate **4.73** with excess LiHMDS; however, excess LDA at –78°C followed by aqueous work-up successfully epimerized the γ-carbon to give a 1:1 mixture of the 2*S*,4*S*:2*S*,4*R* γ-methylglutamate derivative. Although the γ-methyl protons had essentially identical chemical shifts for both 2*S*,4*S*-**4.73** and 2*S*,4*R*-**4.74** diastereomers, the β- (1.65-1.85 ppm

Scheme 4.20



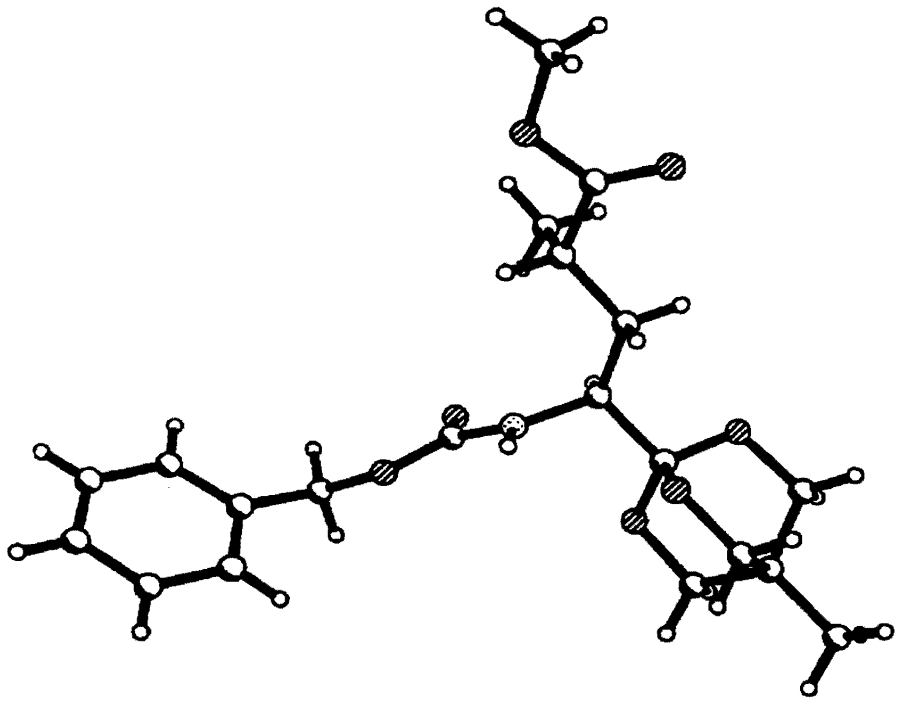


Figure 4.3: X-Ray Crystal structure of Cbz-L-Glu(γ-Me)(OMe)OBO ester 4.73.

vs. 1.40-1.55 ppm and 2.00-2.10 ppm) and γ-protons (2.36-2.50 ppm vs. 2.50-2.61 ppm) were clearly distinguishable allowing for the assignment of diastereoselectivity in the alkylation (Figure 4.4). It is interesting to note that 2*S*,4*R*-Cbz-Glu(γ-Me)(OMe)OBO ester **4.74** does not have the corresponding minor γ-methyl doublet. GC-MS studies also clearly indicated that methylation occurred stereospecifically (Figure 4.5). The two diastereoisomers were also separable by TLC, although 2*S*,4*R*-Cbz-Glu(γ-Me)(OMe)OBO ester **4.74** had an identical R_f to that of the Cbz-L-Glu(OMe)OBO ester **4.69** synthon.

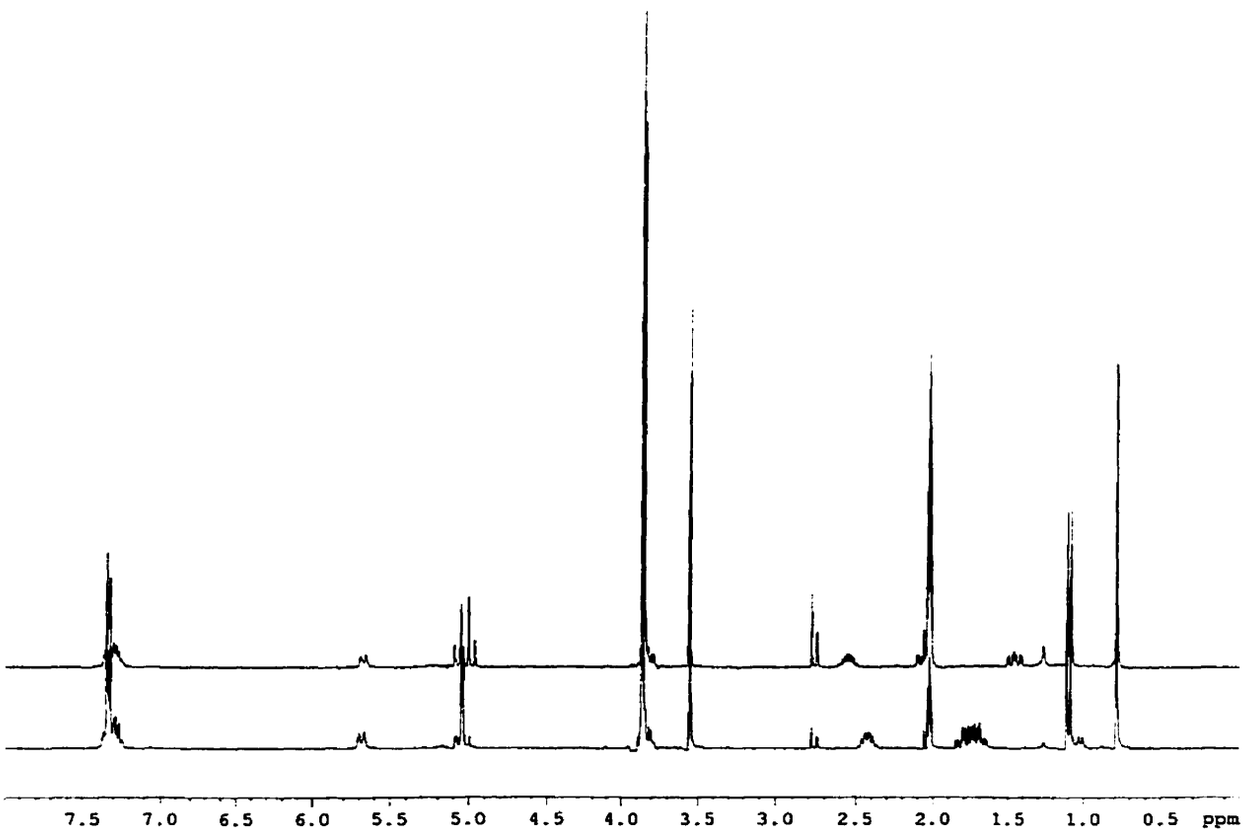


Figure 4.4: 300 MHz ^{1}H-NMR of 2*S*,4*R* 4.74 (top) and 2*S*,4*S* 4.73 (bottom) Cbz-L-Glu(γ-Me)(OMe)-OBO ester.

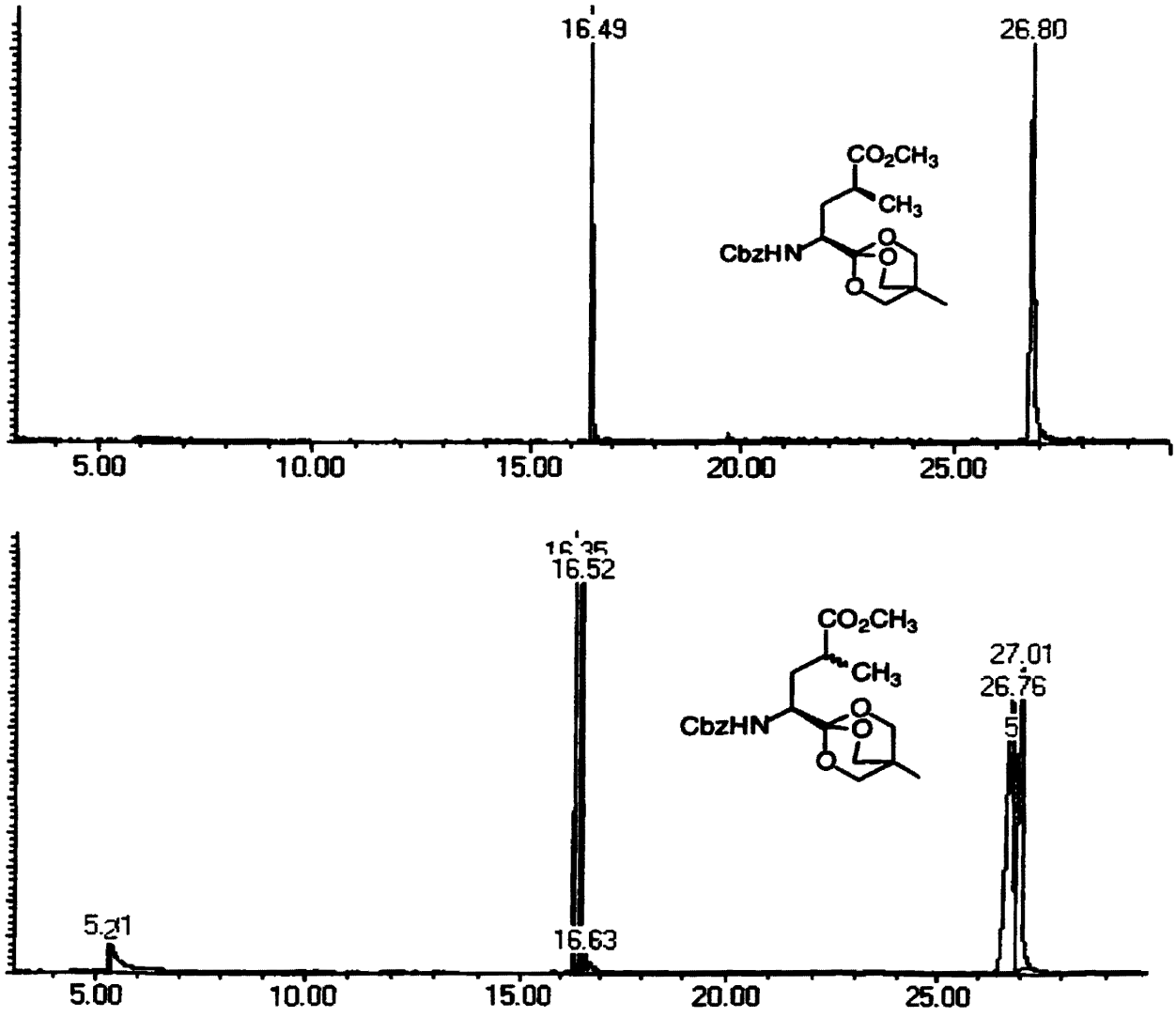


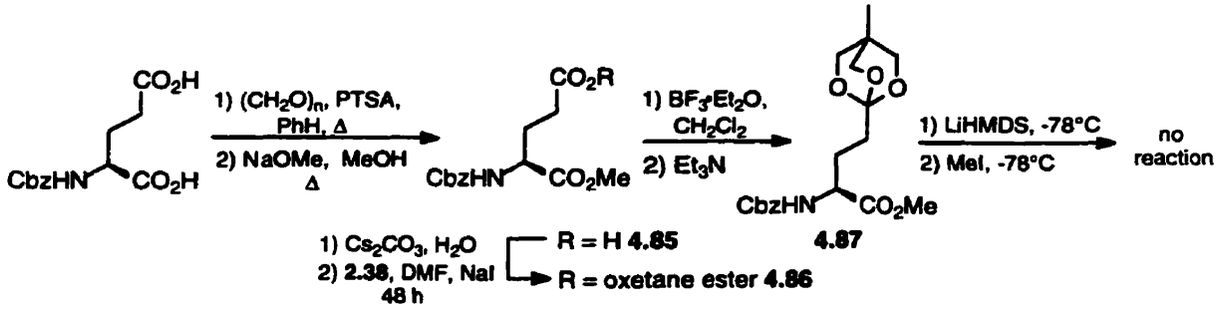
Figure 4.5: GCMS of 2*S*,4*S*-Cbz-L-Glu(γ-CH_3)(OMe)OBO ester 4.73 (top) and epimerized 2*S*,4*RS*-Cbz-L-Glu(γ-CH_3)(OMe)OBO ester 4.73/4.74 (bottom). The isocyanate (rt 16.5 min) is a result of pyrolysis of the carbamate on the column.

Extensive attempts to purify the 2*S*,4*S*-Cbz-Glu(γ-Me)(OMe)OBO ester **4.73** by both flash chromatography and HPLC failed to remove the unknown doublet at δ1.05 ppm. In order to dismiss the possibility that racemization had occurred at the α-carbon, Cbz-L-Glu(OMe)OBO ester **4.69** was treated with excess LiHMDS for 6 hours then

quenched by aqueous work-up. After acid hydrolysis in 6N HCl and ion exchange purification, the rotation of the resulting glutamic acid **4.60** was comparable to that of the starting material, indicating no racemization had occurred during esterification, amine protection, rearrangement to give Cbz-L-Glu(OMe)OBO ester **4.69** or after prolonged exposure to base. Cbz-D-Glu(OMe)OBO **4.70** was also methylated to give 2*R*,4*R*-Cbz-Glu(γ-Me)(OMe)OBO ester **4.75** which had an identical ^{1}H-NMR spectra to the 2*S*,4*S* product **4.73** as expected, including the minor doublet at 1.05 ppm.

To eliminate the possibility of the methyl ester regioisomer **4.85** being formed in the initial esterification and being carried through, Cbz-L-Glu(OBO)-OMe was synthesized via the oxazolidinone[69] and subsequently γ-esterified to the oxetane ester **4.86** followed by rearrangement to the OBO ester **4.87** (Scheme 4.21). Exposure of **4.87** to conditions identical to those used for the methylation of **4.69** failed to produce any product after 6 hours as expected.

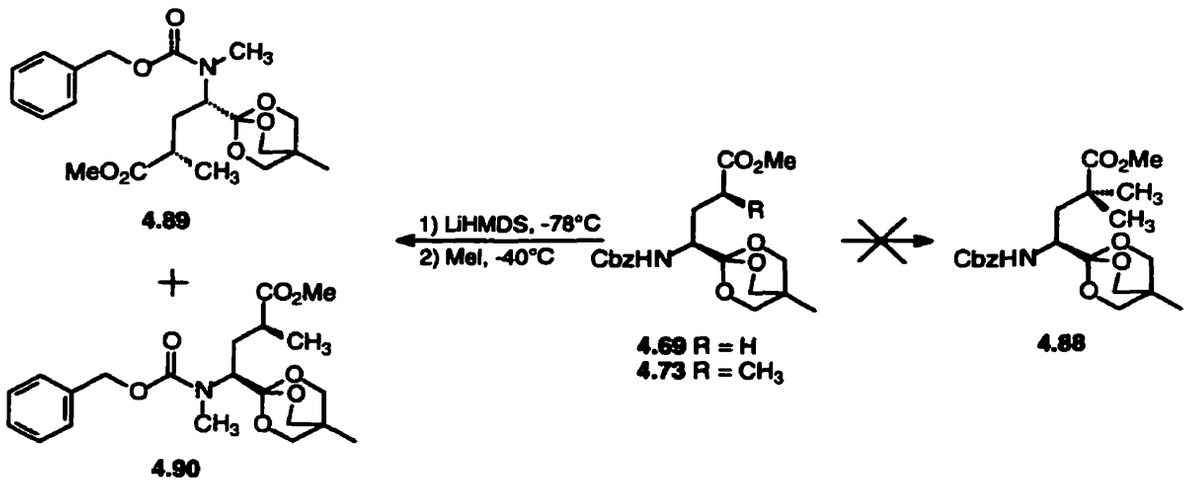
Scheme 4.21



Kawahata *et al.* were able to dimethylate PhFl-Asp(OMe)O*t*Bu **5.11** by sequential treatment with KHMDS and MeI.[70] In order to dismiss γ-dimethylglutamate as a possible contaminant, (whose two methyl singlets might be mistaken as the minor doublet) we tried to γ-dimethylate Cbz-L-Glu(OMe)-OBO **4.69** and γ-monomethylate 2*S*,4*S*-Cbz-

Glu(γ-Me)(OMe)OBO ester **4.73**. However, both routes failed to give the dimethylated product **4.88** instead giving *cis* **4.89** and *trans* **4.90** 2*S*,4*S*-Cbz-*N*-Me-Glu(γ-Me)(OMe)OBO (Scheme 4.22) in 36% yield with remaining starting material regardless of base used. This is presumably due to the sterically hindered nature of the γ-carbon once monomethylated, leading to *N*-methylation at the elevated temperatures (-40°C to 0°C) required to drive the reaction.

Scheme 4.22



Serendipitously, while exploring the methylation of **4.69** in non-polar solvents, the γ-methyl derivative was found to be quite soluble in toluene. The use of a non-polar solvent with a broad temperature range was ideal for variable temperature NMR studies. Experiments over the range of 233 K (–40°C) to 363 K (90°C) in toluene-d_8 showed coalescence of the minor methyl doublet at 330K (57°C) indicating the existence of rotamers in Cbz-L-Glu(γ-Me)(OMe)OBO ester **4.73** (Figure 4.6). The free energy difference (ΔG°) was calculated to be 1.31 kcal· mol^{-1} and the free-energy of activation

($\Delta G_c^{\ddagger}$) 17.3 kcal· mol^{-1}, calculated from the coalescence temperature using the graphical method of Shanan-Atidi and Bar-Eli (Appendix B).[71]

Conformational isomers have been reported for amides[72] and carbamates[73] as the C-N bond adopts a less stable *syn* conformation (Figure 4.7a) and have been observed in aspartic acid derivatives.[74] Typical values for rotation about the C-N bond are 22 kcal· mol^{-1} in the case of amides (*N*,*N*-dimethylformamide)[75] and 16 kcal· mol^{-1} for carbamates (*N*,*N*-dimethylbenzylcarbamate).

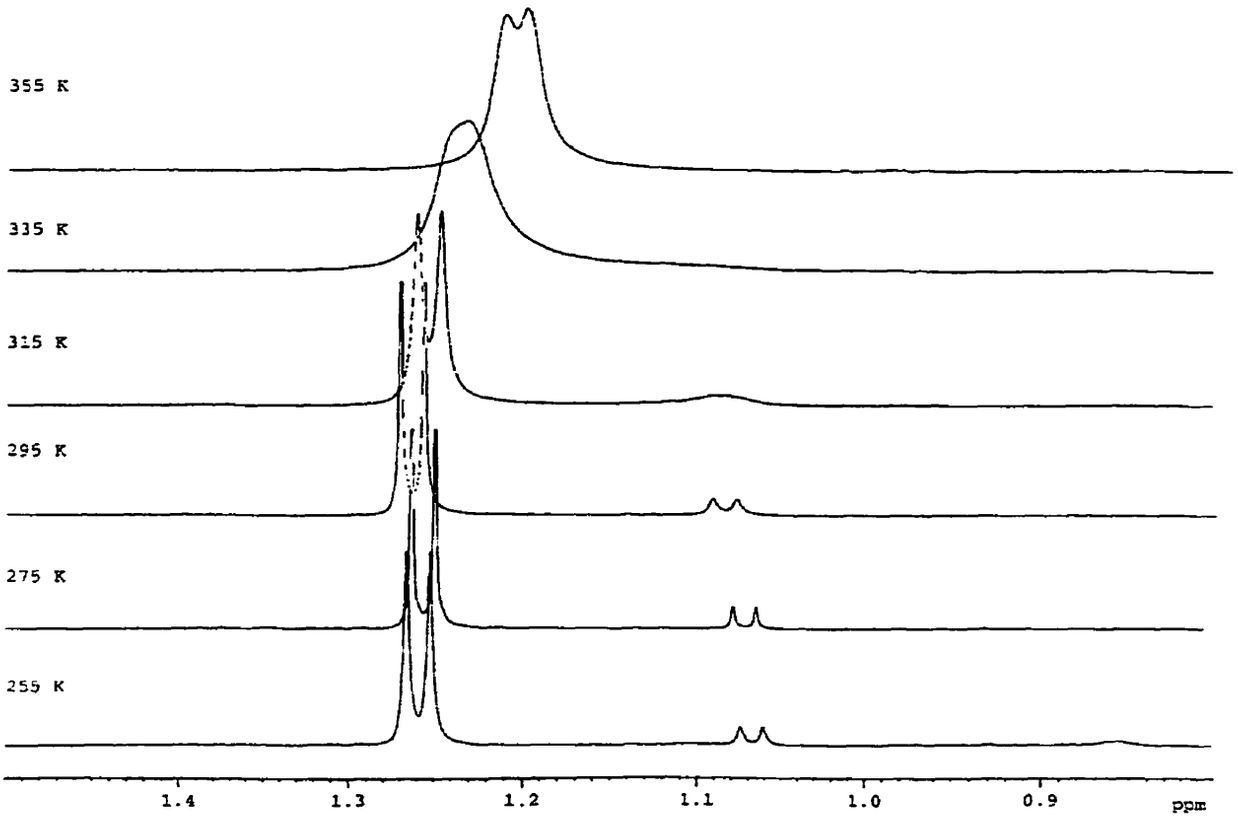


Figure 4.6: Variable-Temperature 500 MHz ^{1}H-NMR studies in toulene-d_8 of Cbz-L-Glu(γ-Me)(OMe)OBO ester 4.73.

This conformational isomerism is observed in the methyl ester **4.73**, the *t*-butyl ester **4.76** and benzyl ester **4.77** derivatives and also in 2*S*,4*S*-Boc-Glu(γ-Me)(OMe)-

OBO ester. Moreover, the stereochemistry at the γ-carbon appears to induce this effect since the minor rotamer seems to be absent in the 2*S*,4*R*-Cbz-Glu(γ-Me)(OMe)OBO ester **4.74** isomer (Figure 4.4 bottom) regardless of solvent. Since *anti* carbamate rotamers are more stable than *syn* rotamers, presumably the *anti* rotamer corresponds to the major doublet at δ 1.26 ppm and the *syn* rotamer the minor doublet at δ 1.08 ppm. Some intrinsic property present in 2*S*,4*S*-Cbz-Glu(γ-Me)(OMe)OBO ester **4.73** is therefore responsible for the observed rotamer. Intramolecular hydrogen bonding may stabilize the conformation depicted in Figure 4.7b in which both the OBO and methyl groups are equatorial in the *syn*-2*S*,4*S* conformer whereas the *syn*-2*S*,4*R* conformer has the methyl group axial, perhaps sufficiently disfavoured to explain the absence of the *syn*-2*S*,4*R* isomer in the 2*S*,4*R*-Cbz-Glu(γ-Me)(OMe)OBO ester **4.74** ^{1}H-NMR spectrum. Similar seven-membered hydrogen bond interactions have been shown to be responsible for the configurational stability in sugar thioureas,[76] *N*-(α-hydroperoxyalkyl)amides,[77] and macrocyclic polypeptides.[78] Corey and Lee also recently discussed the importance of hydrogen-bonding as an organizing element in the restriction of rotation leading to enantioselectivity in many reactions.[79] Polar protic solvents should disrupt the propensity of the 2*S*,4*S*-methyl derivative to intramolecular hydrogen bond which would be detected in the ^{1}H-NMR spectrum, however, the rotamer doublet is observed with solvents such as methanol-d_4. If this phenomenon is indeed due to intramolecular hydrogen bonding, the seven-member hydrogen bond must be sufficiently strong to overcome the disruptive nature of methanol-d_4.

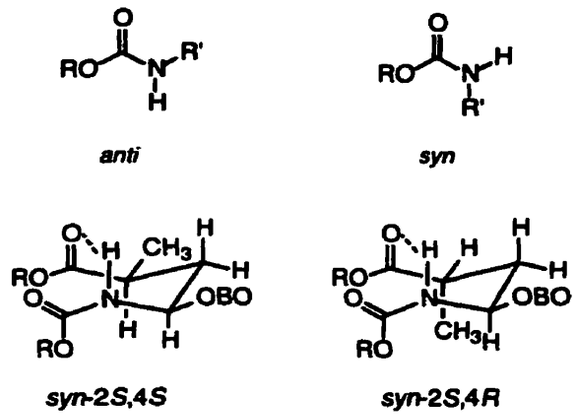


Figure 4.7. (a) *Anti* versus *Syn* carbamate isomers (b) Hydrogen bonding in the *Syn* conformational isomer of 2*S*,4*S* and 2*S*,4*R* Cbz-L-Glu(γ-Me)(OMe)OBO ester 4.73 and 4.74.

Once the high diastereoselectivity of addition had been established, our attention turned to optimizing alkylation (Table 4.1). Other counterions were investigated for the generation of the enolate. NaHMDS gave the same results as LiHMDS, however, the potassium enolate resulted in a 1:1 mixture of 4-methylated product. This is in agreement with results reported by Gu and Hesson[57] (Scheme 4.13, **4.46** P=*p*NB, R^1=R^2= E=Me) where a chelated dianion mechanism was proposed and subsequently described by Hanessian and Schuam (Section 4.2.10). An excess of base was also required since 2 equivalents of LiHMDS, titrated according to the method of Love and Jones,[80] gave a lower yield of product (entry 8). Inverse addition (i.e. addition of the substrate dropwise to a mixture of base) was also necessary, implying that the reaction is under kinetic control.[81] In order to identify whether an *E* or *Z* enolate was formed we attempted to trap the enolate with TMSCl as described by Humphrey *et al.*[82] and compare the chemical shift of the silyl ketene acetal to literature values described by Ireland and Daub.[83] However, all trapping experiments failed and the addition of HMPA had no effect on either yield or diastereoselectivity.

Table 4.1: Optimization of Methylation of Cbz-L-Glu(OR)OBO ester.

Entry	γ-CO_2R R	Base[a]	Temp[b] (°C)	Reaction Time	Yield[c] (%)	Ratio[d] 2*S*,4*S*:2*S*,4*R*
1	Me	LiHMDS	-78°C	4 h	84(93)	>95:5
2	Me	LiHMDS/HMPA	-78°C	4 h	78(92)	>95:5
3	Me	KHMDS	-78°C	8 h	85	1:1
4	Me	LiHMDS	-100°C	8 h	48(94)	>95:5
5	Me	LiHMDS	-50°C	8 h	71	>95:5
6	Me	LiHMDS	-30°C	4 h	59	>95:5
7	Me	LiHMDS	-78°C	24 h	88	>95:5
8	Me	LiHMDS[e]	-78°C	4 h	55	>95:5
9	Me	NaHMDS	-78°C	8 h	84	>95:5
10	Me	LiHMDS/LiCl	-78°C	15 h	66(94)	>95:5
11	*t*Bu	LiHMDS	-78°C	4 h	80(89)	>95:5
12	Bn	LiHMDS	-78°C	4 h	82(89)	>95:5
13	Me	LiHMDS[f]	-78°C	8 h	62	>95:5
14	Me	LiHMDS[g]	-78°C	8 h	75	>95:5

[a] Reaction performed in THF unless otherwise noted, with 3 equivalents of base and inverse addition
[b] Temperature during addition of alkylating agent
[c] Value in parenthesis denotes yield base on recovered starting material
[d] Determined by ^{1}H-NMR and/or HPLC analysis
[e] Two equivalents of base
[f] Reaction performed in toluene
[g] Reaction performed in diethyl ether

4.2.2 Addition of Other Alkylating Agents to Cbz-Glu(OMe)OBO 4.69.

Confident that we had assigned both the stereochemistry and diastereoselectivity of methylation, we then investigated other alkylations. Ethyl iodide, allyl bromide and benzyl bromide were all used as alkylating agents following optimized conditions developed with LiHMDS and MeI. In all cases, alkylation occurred in both good yield

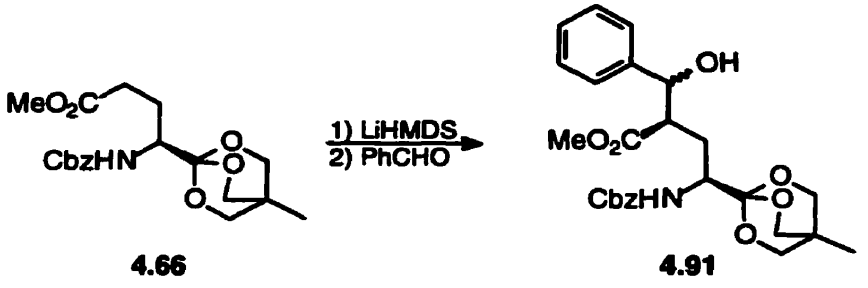
and excellent diastereoselectivity with no observable racemization. Only one stereoisomer was visible within the detection limits of TLC, ^{1}H-NMR and ^{13}C-NMR and was tentatively assigned as the 2*S*,4*S* isomer due to the observed results for the γ-methyl derivative. This was confirmed upon deprotection, derivatization and HPLC analysis (*vide infra*) and comparison to literature results. The major by-product of the additions was unreacted Cbz-Glu(OMe)OBO ester **4.69**. Care was taken to ensure diastereomeric enrichment did not occur after purification.

Ethyl iodide gave the γ-ethyl derivative **4.78** in 75% yield, allyl bromide the γ-allyl derivative **4.79** in 83% yield and benzyl bromide the γ-benzyl derivative **4.80** in 76% yield. The reactivity, leaving group and bulk of the electrophile had little effect on the yield and selectivity.

4.2.3 Aldol Reaction of Cbz-Glu(OMe)OBO 4.69.

The aldol reaction of benzaldehyde with the enolate of **4.69** gave only two of the four possible stereoisomers of **4.91** in 71% yield and ratio of 3:1 (determined by ^{1}H-NMR and ^{13}C-NMR) compared to the aldol reaction of **4.45** (P=Cbz, R^{1}=*t*Bu, R^{2}=Me) in which a 1:1 ratio of diastereomers was reported (Scheme 4.23).[56] The ratio was determined by the integration of well resolved peaks in the ^{1}H-NMR spectrum. The stereochemistry of the major diastereomer was not identified although, based on previous results, presumably the 2*S*,4*S* stereoisomer predominates.

Scheme 4.22

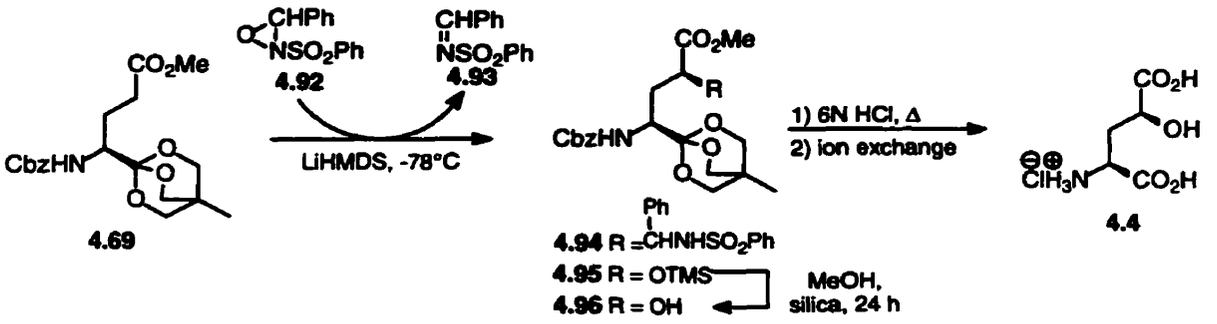


4.66 **4.91**

4.2.4 γ-Hydroxylation of Cbz-Glu(OMe)OBO 4.69.

The 2-sulfonyloxaziridine **4.92** of Davis and coworkers[84] has been used extensively to α-hydroxylate ketones and enolates due to its convenient preparation. However, only one account of its use to synthesize γ-hydroxyglutamic acids has been reported giving 2*S*,4*S*-Cbz-Glu(γ-OH)(OMe)OMe **4.46** (P=Cbz, R^1=R^2=Me, E=OH) in 70% yield as a 9:1 mixture of inseparable diastereomers.[54] Using identical conditions as those optimized for the alkylation of Cbz-Glu(OMe)OBO **4.69**, Davis' oxaziridine **4.92** was added to the lithium enolate resulting in a complex mixture of products that were not isolated. However, when 3 equivalents of LiHMDS was added to a stirring mixture of the oxaziridine **4.92** and Cbz-L-Glu(OMe)OBO ester **4.69** at -78°C, three distinct products were isolated. The sulfonamide **4.94**, an adduct of the sulfonimine **4.93** and enolate of **4.69**, the silyl ether **4.95**, presumably generated through trimethylsilylation of the oxyanion by hexamethyldisilazane, and the desired product **4.96** (Scheme 4.24).

Scheme 4.24



Davis *et al.* have previously observed the formation of the sulfonamide **4.94**, albeit in low yield.[84a] Its formation was reportedly not significant until the reaction was performed at room temperature and both the use of KHMDS and maintaining the reaction at –78°C alleviated this side reaction. A variety of conditions were investigated to optimize γ-hydroxylation, (Table 4.2) including those reported by Davis' group,[84a] *in situ* reduction of the sulfonamide **4.93** with $NaBH_4$ (entries 5 and 6),[85] and the use of LDA with varying amounts of oxaziridine **4.92** to avoid both sulfonamide **4.94** and TMS ether **4.95** formation (entries 7 and 8).

Silylation of alcohols with hexamethyldisilazane has been previously described[86] and the secondary nature of the silyl ether probably explains its stability during aqueous work-up. The TMS ether **4.95** could be quantitively converted to the desired α-hydroxyester **4.96** derivative with stirring in methanol over silica for 24 hours, conditions attempted due to the observation of decomposition of **4.95** to **4.96** on TLC.

Table 4.2: Optimization of Hydroxylation of Cbz-L-Glu(OMe)OBO ester 4.69.

Entry	Conditions[a]	Yield (%)		
		4.94	**4.95**	**4.96**
1	LiHMDS(3 eq.), **4.92** (1.5 eq.)	28	46	20
2	LiHMDS(3 eq.), **4.92** (5 eq.)	5	64	23
3	KHMDS (3 eq.), **4.92** (1.25 eq.)	30	34	22
4	NaHMDS (3 eq.), **4.92** (1.25 eq.)	19	39	45
5	LiHMDS(3 eq.), **4.92** (1.5 eq.), $NaBH_4$ (20 eq.)	5	69	10
6	LiHMDS(3 eq.), **4.92** (1.5 eq.), $NaBH_4$ (20 eq.)[b]	6	58	9
7	LDA (3 eq.), **4.92** (1.1 eq.)	35	0	55
8	LDA (3 eq.), **4.92** (5 eq.)	12	0	79

[a] Reaction performed in THF at –78°C unless otherwise noted.
[b] Performed at –40°C

Only one stereoisomer of Cbz-Glu(γ-OH)(OMe)OBO ester **4.96** could be detected by TLC, ^{1}H-NMR and ^{13}C-NMR and subsequent HPLC analysis. An X-ray structure of **4.96** was obtained confirming the same stereochemical outcome (2*S*,4*S*) as observed with alkylation (Figure 4.8) and showing a strong hydrogen-bond between the hydrogen of the γ-hydroxyl group and the double bonded oxygen in the carbamate (Appendix C). The excellent diastereoselectivity observed in the electrophilic γ-hydroxylation of Cbz-Glu(OMe)OBO ester **4.69** is in contrast to that reported by Hanessain and Vanasse in which a 9:1 (2*S*,4*S*:2*S*,4*R*) stereoselectivity was observed.[54] This is presumably due to the higher steric demand of the OBO ester protecting group over that of the methyl ester in the chelated transition state model described in Figure 4.9 (Section 4.2.10).

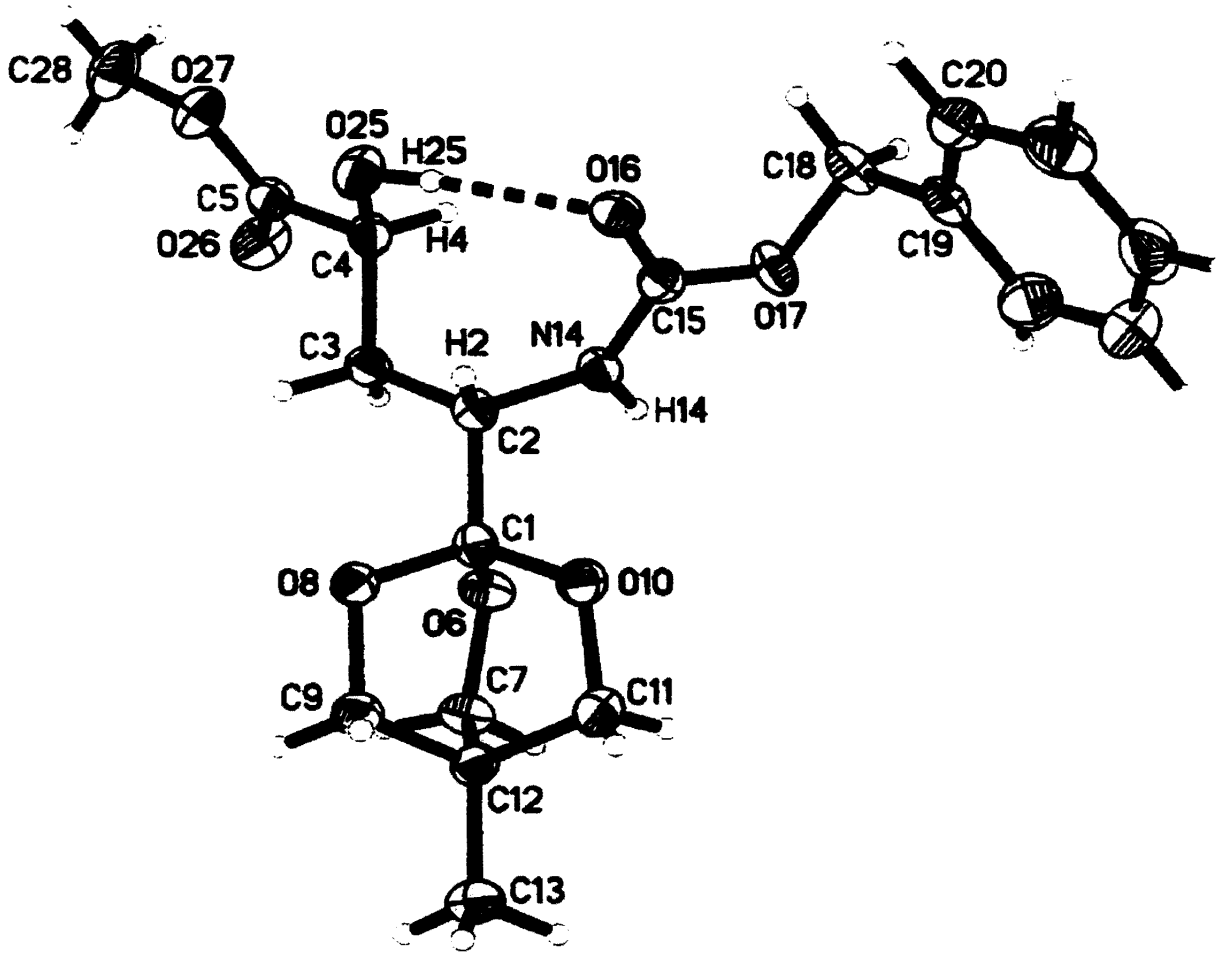
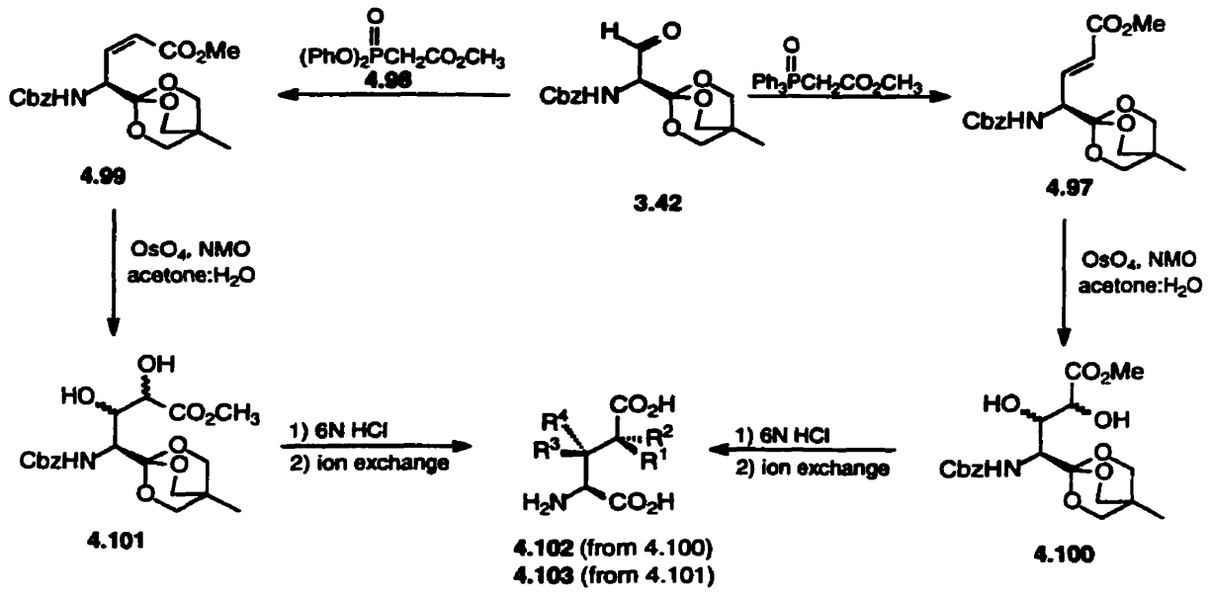


Figure 4.8: X-Ray Crystal structure of Cbz-L-Glu(γ-OH)(OMe)OBO ester 4.96.

Dihydroxylation to give Cbz-Glu(β,γ-dihydroxy)(OMe)OBO esters was accomplished by the osmylation of both the *E* **4.97** and *Z* **4.99** isomers of Cbz-Glu(β,γ-dehydro)(OMe)OBO ester. *E*-Cbz-Glu(β,γ-dehydro)(OMe)OBO ester **4.97** was synthesized from Cbz-Ser(ald)OBO ester **3.42** and methyl(triphenylphosphoranylidene) in good yield (78%) and with 98:2 *E*:*Z* ratio (Scheme 4.25). Cbz-Ser(ald)OBO ester **3.42** was reacted with Ando's reagent[87] **4.98** to give *Z*-Cbz-Glu(β,γ-dehydro)(OMe)OBO ester **4.99** as a 10:1 *Z*:*E* ratio. The *E*-isomer could be separated by flash chromatography to give **4.99** in 61% yield.

Scheme 4.25

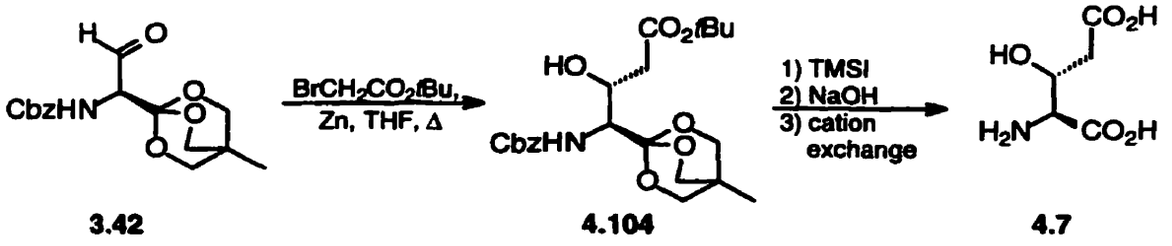


Initially, dihydroxylation of **4.97** and **4.99** with stoichiometric amounts of OsO_4 in pyridine and reduction with refluxing methanolic sodium sulfite or sodium bisulfite gave a complex mixture of decomposed materials that were not isolated.[88] However, *N*-methylmorpholine *N*-oxide catalyzed osmylation[89] gave the *cis*-diols **4.100** and **4.101** in 54% and 48% yield respectively. Determining the stereochemistry of dihydroxylation proved to be problematic since attempts at crystallizing **4.102**, its *N*-trityl derivative and *bis*-3,5-dinitrobenzoyl ester derivative failed to give crystals capable of X-ray analysis. With optical rotations reported for only two of the eight possible isomers of dihydroxyglutamic acid,[63,65] deprotection of **4.102** and **4.103** and comparison of optical rotations held little promise for identifying the stereochemistry of addition, especially due to the small amount of material available. Efforts are continuing in an attempt to grow crystals for identification of stereochemistry by X-ray analysis. Deprotection of the Cbz

and subsequent formation of the δ-lactam is a well known method which would provide the relative stereochemistry of the dihydroxy derivatives **4.100** and **4.101**.

The β-hydroxyglutamic acid derivative **4.104** was synthesized by Reformatsky reaction of **3.42** with the organozinc derivative of *t*-butyl bromoacetate to give a 93:7 ratio of *threo* (2*S*,3*R*):*erythro* (2*S*,3*S*) β-hydroxyglutamic acid derivative **4.104** in 75% yield over two steps (Scheme 4.26).[90] The addition of lithium *t*-butyl acetate[91] failed to furnish the desired product, instead giving a complex mixture of products that were not isolated. All three groups were simultaneously deprotected upon treatment with TMSI to give 2*S*,3*R*-β-hydroxyglutamic acid **4.7** in 82% yield and >98% ee.

Scheme 4.26



3.42 **4.104** **4.7**

Epoxidation of the α,β-unsaturated ester **4.97** was attempted as an alternative means of fuctionalizing the protected glutamate but was unsuccessful. Both nucleophilic and non-nucleophilic conditions were explored including 10% $NaHCO_3$ and H_2O_2, K_2CO_3 and *t*BuOOH, dimethyldioxirane (DMDO) and *m*-chloroperoxybenzoic acid (MCPBA) and $NaHCO_3$. Previous attempts from our group to epoxidize Fmoc-Glu(β,γ-dehydro)(OMe)OBO ester also reportedly failed.[92] Presumably the unreactivity of **4.97** to epoxidation is due to a combination of the electron deficiency of the α,β-unsaturated ester **4.97** and steric factors.

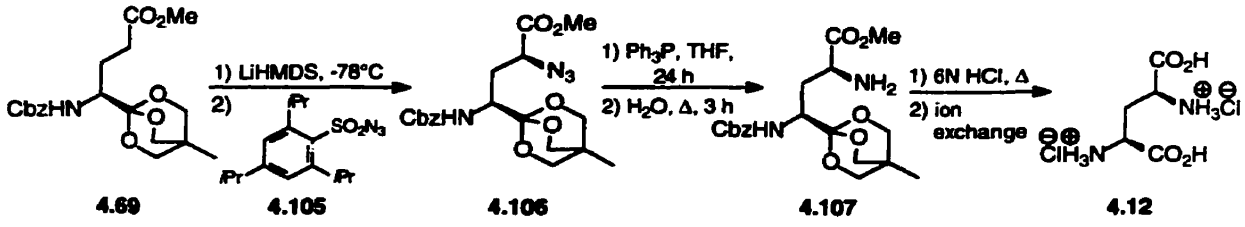
4.2.5 γ-Amination of Cbz-Glu(OMe)OBO 4.69.

The direct electrophilic azidization of enolates has previously been described, primarily in a landmark study by Evans *et al.* showing that treatment of the potassium enolate of chiral oxazolidinones with trisyl azide produced the corresponding 2-azido derivatives in good yield and excellent selectivity (Section 1.3.2.2).[93] Hanessian *et al.* have investigated the azidation of 3-substituted-butyrolactones although no diastereoslectivities were reported.[94] Encouraged by the excellent selectivity we encountered in the γ-hydroxylation of Cbz-Glu(OMe)OBO ester **4.69**, we attempted the azidation of Cbz-Glu(OMe)OBO ester **4.69** under conditions previously reported by Evans *et al.*[93]

Initially trisyl azide[95] **4.105** was added to the potassium enolate of Cbz-Glu(OMe)OBO ester **4.69** as described by Evans *et al.*[93] with little success. However, reaction of the lithium dianion gave the desired azide **4.106** although prolonged reaction times resulted in a decrease of product as shown in Figure 4.9. This was monitored by both quenching the reaction and isolating the product and by following the progress of the reaction by ESI-MS. A variety of conditions were investigated including counter-ion, temperature and length of reaction, however, after optimization 2*S*,4*S*-Cbz-Glu(γ-N_3)(OMe)OBO ester **4.106** was synthesized in 54% yield with 32% recovery of starting material (Scheme 4.27). Only one stereoisomer was detected by TLC, ^{1}H-NMR and ^{13}C-NMR, presumably the 2*S*,4*S* isomer based on earlier results. The reasons for apparent decomposition of γ-azide **4.106** during prolonged reaction times are unclear. Evans *et al.*[93] have reported the use of acetic acid to quench the reaction and decompose the diazonium salt formed, however, the sensitivity of the OBO ester to acidic conditions

precludes the use of any acid although the addition of potassium acetate may have the desired effect if **4.106** is formed according to the mechanism described by Evans *et al.* The yield is however consistent with previous attempts by other groups at generating γ-azidoglutamates[55] and pyroglutamates.[94] The azide **4.106** was then reduced to the amine **4.107** in 78% yield under Staudinger type conditions.[96]

Scheme 4.27



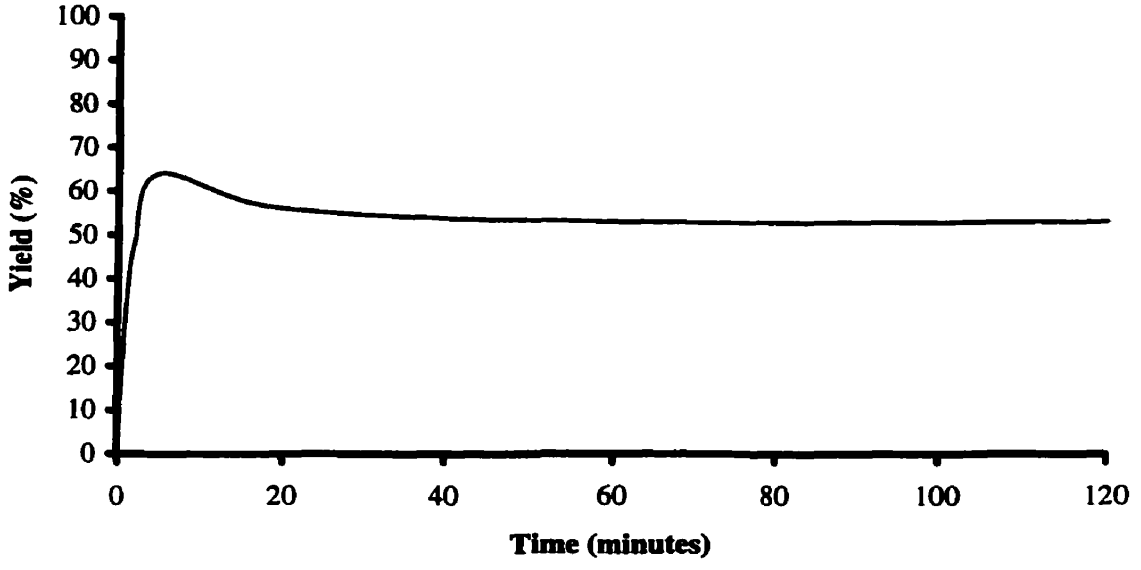
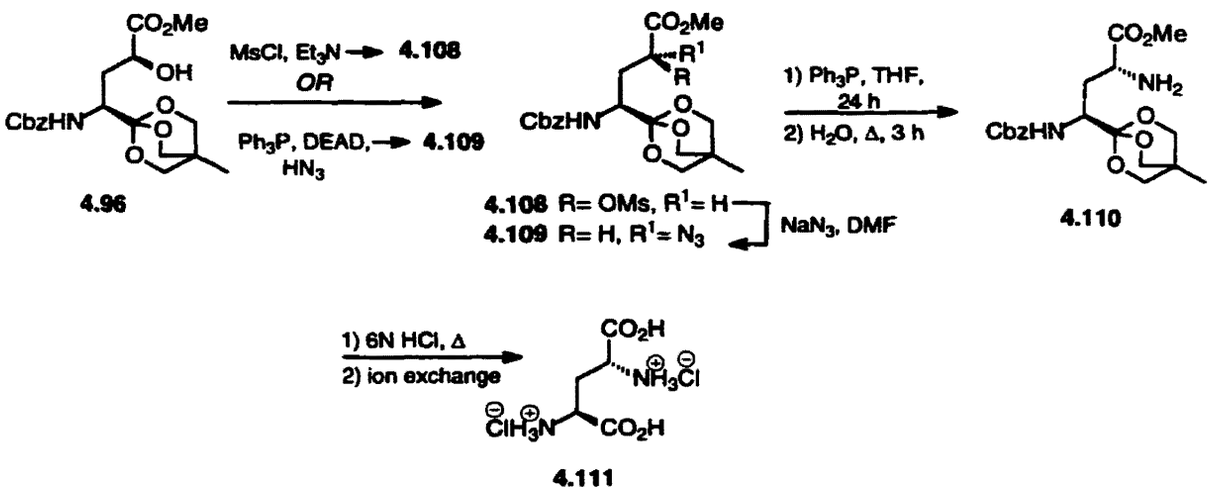


Figure 4.9: Plot of Yield (%) versus Time (minutes) after quenching of electrophilic azidation of Cbz-Glu(OMe)OBO ester 4.69 with Trisyl Azide 4.105.

The other diastereomer, 2*S*,4*R*-Cbz-Glu(γ-N_3)(OMe)OBO ester **4.109** was synthesized by conversion of the alcohol of 2*S*,4*S*-Cbz-Glu(γ-OH)(OMe)OBO ester **4.96**

to the mesylate **4.108** in 80% yield and subsequent S_N2 displacement with NaN_3 to give the desired product **4.109** in 56% yield (Scheme 4.28). The only other product was unreacted starting material which was recovered. The azide **4.109** was then reduced under Staudinger conditions as described for **4.107**. Alternatively, Mitsunobu reaction of **4.96** with HN_3 also gave the desired azide **4.109**. TLC analysis indicated only one product that was contaminated with diethylamidodicarboxylate. However, after reduction under Staudinger conditions, the γ-amino derivative **4.110** was isolated in 76% yield for the mesylate method and 54% yield (for two steps) for the Mitsunobu method in >95:5 (2*S*,4*R*:2*S*,4*S*) diastereoselectivity. With the 2*S*,4*R*-Cbz-Glu(γ-N_3)(OMe)OBO ester **4.110** in hand, confirmation of the diastereoselectivity of the direct electrophilc azidation to give **4.107** by ^1H and ^{13}C-NMR was possible.

Scheme 4.28

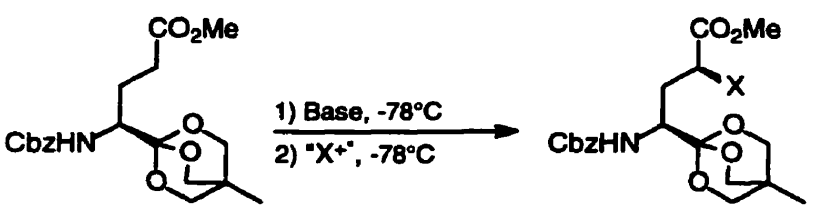


4.2.6 γ-Halogenation of Cbz-Glu(OMe)OBO 4.69.

Electrophilic bromination was performed in order to introduce a good leaving group in the γ-position of the Cbz-Glu(OMe)OBO ester **4.69**. The lithium enolate of

Cbz-Glu(OMe)OBO ester **4.69** was added to *N*-bromosuccinimide (NBS) under conditions similar to that reported by Evans *et al.*[93] After generation of a deep purple colour, the reaction was quenched to give the protected γ-bromoglutamate **4.112** (X=Br) in 64% yield (Scheme 4.29). A number of side-reactions occurred since only 8% of Cbz-Glu(OMe)OBO ester **4.66** was recovered, although none of these contaminants were isolated. ^{1}H-NMR integration gave a 90:10 ratio of diastereomers, the major product presumably the 2*S*,4*S* stereoisomer based on previous electrophilic additions. The reduced diastereoselectivity is perhaps due to displacement at the α-bromoester with a bromine anion to give the 2*S*,4*R* stereoisomer by an S_N2 reaction. Higher diastereoselectivities (95:5) were achieved after shorter reaction times although yields were drastically lower (<20%), quenching the reaction after six hours was a compromise between diastereoselectivity and yield.

Scheme 4.29



4.69 X⁺=NBS, DAST, NFSI, Selectfluor **4.112** X=Br

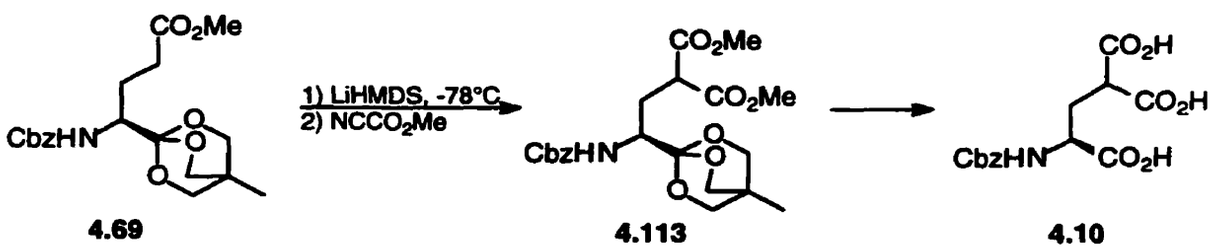
All attempts at synthesizing the γ-fluoro derivative **4.112** (X=F) failed although lithium, sodium and potassium enolates were reacted with a number of fluorinating reagents including diethylaminosulfur trifluoride (DAST), Selectfluor™ and *N*-fluorobenzene sulfonamide (NFSI) using methods previously reported for the successful electrophilic fluorination of enolates.[97] Complex mixtures of products generally resulted

which were not isolated. ESI-MS and ^{19}F-NMR analysis of the crude reaction mixture indicated Cbz-Glu(OMe)OBO ester **4.69** remained although no mono- or di-fluoronated glutamic acid derivatives were identified.

4.2.7 γ-Carboxylation of Cbz-Glu(OMe)OBO 4.69.

In order to synthesize the important glutamic acid derivative, γ-carboxyglutamic acid **4.10** via electrophilic addition it was necessary to select conditions to prevent the competing side-reaction of O-acylation, known to occur with acyl halides or anhydrides.[98] Methyl cyanoformate, also known as Mander's Reagent,[99] was used to trap the lithium enolate of Cbz-Glu(OMe)OBO ester **4.69** to give the malonate derivative **4.113** in 68% yield (Scheme 4.30). Initial attempts to deprotect **4.113** to γ-carboxyglutamic acid **4.10** by first opening the OBO ester to the mhpd ester followed by alkaline hydrolysis of the esters with Cs_2CO_3 then hydrogenation of the Cbz group failed to give any **4.10**, but rather a complex mixture of products.

Scheme 4.30



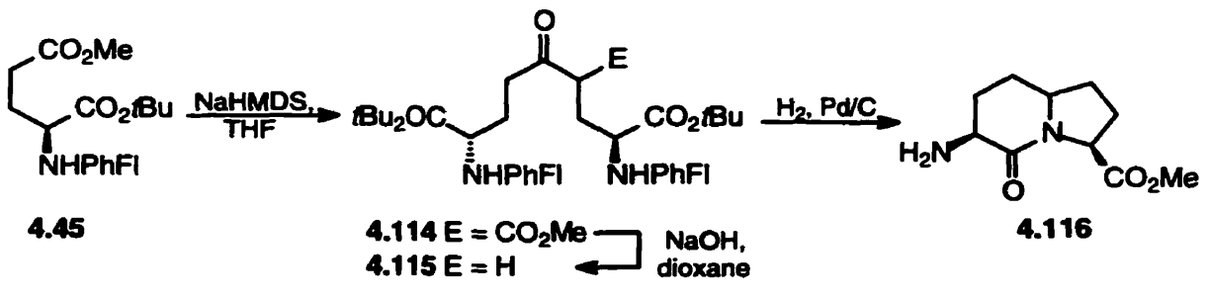
4.69 **4.113** **4.10**

4.2.8 Claisen Condensation of Cbz-Glu(OMe)OBO 4.69.

Another classical reaction of enolates is the Claisen condensation. In particular, in the case of glutamates, the condensation product provides access to various substituted

β-turn mimetics such as the pyrollidines **4.15**, pyyrolizidinones **4.16** and indolizidines **4.17** (Scheme 4.3). Lubell and coworkers have reported the Claisen condensation of PhFl glutamates **4.45** (P=PhFl, R_1=*t*Bu, R_2=Me) giving the adduct **4.114** which was subsequently decarboxylated **4.115** and cyclized to give indolizidinone **4.116** in good overall yield (Scheme 4.31).[100] However, the stereoselective introduction of various substituents proved to be problematic, with diastereoselectivities ranging from 1:1 to 15:1 depending on substrate, base and electrophile.[101]

Scheme 4.31



A variety of conditions were examined for the Claisen condensation of Cbz-L-Glu(OMe)OBO ester **4.69** including various addition conditions, concentrations, counter-ion and temperature (Table 4.3). NaHMDS, the preferred base reported by Lubell and Lombart,[100a] failed to give any of the desired product **4.117**, instead giving a complex mixture of products including **4.69** (Scheme 4.32). Cbz-L-Glu(OBn)OBO ester **4.72** is presumably formed by transesterification with benzyl alcohol liberated from the Cbz of **4.69** by adventitious hydrolysis (entry 2 and 3). LiHMDS (entry 5) gave the desired Claisen condensation product **4.117** in 48% yield. Decarboxylation of the β-keto ester **4.117** to the ketone **4.118** failed under a number of thermodynamic conditions including NaOH in dioxane,[100] NaCl in DMSO/H_2O,[102] and Cs_2CO_3 in MeOH/H_2O.[103] The highly

concentrated reaction mixture of **4.114** (1.0 M) reported by Lubell and Lombart was not necessary for the formation of **4.117**.

Scheme 4.32

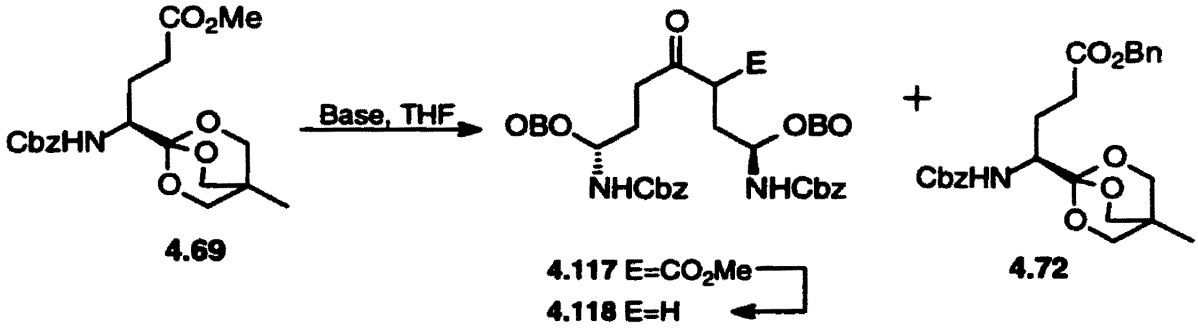


Table 4.3: Claisen Condensation of 4.69

Entry	Base	Temp.(°C)	Time (h)	[4.69](M)	Yield 4.69	Yield 4.117	Yield 4.72
1	NaHMDS (1.5 eq)	-78°C	2	0.5	95	-	-
2	NaHMDS (1.5 eq)	-30°C	2	0.5	81	-	12
3	NaHMDS (3 eq)	-30°C→ rt	18	1.0	48	-	32
4	LiHMDS (1.5 eq)	-78°C	18	0.5	18	33	-
5	LiHMDS (1.5 eq)	-30°C	12	0.15	-	48	-
6	LiHMDS (1.5 eq)	0°C	2	0.1	25	31	-

4.2.9 Deprotection of γ-Substituted Glutamic Acids

Acid hydrolysis has been used extensively to deprotect γ-substituted glutamic acid derivatives.[30,32a,105] In our case refluxing 6N HCl gave γ-substituted glutamates in good yield. Reflux periods of between 2-4 hours were required to fully hydrolyze the

dihydroxyester **2.62**, generated after ring opening of the OBO ester, although both the benzyl carbamate and methyl ester were rapidly cleaved.[104] However, this method has been reported to cause up to 3% racemization during 6 hours in refluxing 6 N HCl.[30,32a,105] Purification by cation exchange (gradient elution with 0.1N to 1N NH_4OH) or anion exchange (elution with 0.1N to 1N formic acid or 0.1N to 0.5 HCl) typically gave the desired γ-substituted glutamates in 50-80% yield. Formation of the γ-lactam of 4-substituted glutamates during prolonged hydrolysis has been observed,[106] especially in the case of diaminoglutamic acids (17%) **4.12** and **4.111**.[44a] γ-Lactonization has also been observed with 2*S*,4*S*-γ-hydroxyglutamic **4.4** after prolonged bubbling of HCl gas through an aqueous solution (18 hours).[26,33] No cyclized product was isolated in our case, although ESI-MS analysis of **4.11** indicated formation of a polymeric substance although in insufficient yield for full characterization. Only the β-hydroxyglutamic acid derivative **4.104** was cleaved by TMSI, based on a previously reported synthesis from our laboratory.[67a]

Crystallization of the γ-substituted glutamates proved difficult and was generally unsuccessful with the exception of the hydroxyglutamic acids **4.4** and **4.7**. However, diastereomeric and enantiomeric purities, established by derivatization and HPLC analysis of the lyophilized powders after ion exchange, were excellent. In most cases, the values reported for the diastereoselectivity of addition of various electrophiles to the enolate of Cbz-Glu(OMe)OBO ester **4.69** are based upon HPLC analysis of the derivatives after deprotection. Since a minimal amount of racemization occurs (<3%) during hydrolysis,[30,32c,105] these values are reported as >95:5 since one cannot distinguish between racemization occurring during addition of the electrophile in basic conditions

and during acid hydrolysis. HPLC analysis conditions for all the γ-alkyl glutamates were identical to those described previously (section 2.4.15a) and gave excellent separation. HPLC analysis of the diaminoglutamic acids was unsuccessful due to the partial reaction of both amino groups with the derivatizing agent to give two peaks for *meso*-diaminoglutamic acid **4.111** and three for 2*S*,4*S*-diaminoglutamic **4.11** acid.

It was also difficult to obtain samples that were analytically pure by elemental analysis although spectroscopic techniques all indicated high purity. This might be caused by small amounts of residual salts and the fact that recrystallizations were generally unsuccessful. However, optical activity for all the isolated γ-substituted glutamic acids agreed with literature results.

4.2.10 Discussion of the Stereoselectivity of Electrophilic addition to the Cbz-Glu(OMe)OBO ester 4.69 Enolate

The model depicted in figure 4.10 has been previously proposed to explain the 1,3-induction occurring in the addition of various electrophiles to the enolates of protected glutamates.[107] Gu *et al.* have also explained their high diastereoselectivities by way of a chelated dianion, similar to those reported for the alkylation of succinimides.[58] Indeed, our results further support these models since the increased steric bulk of the OBO ester correlates well with the increase in diastereoselectivity observed as predicted by the model (Figure 4.10). For example, the hydroxylation of protected glutamic acid **4.45** (P=Cbz, R^1=R^2=Me), where a methyl ester replaces the OBO ester proceeds with 9:1 2*S*,4*S*;2*S*,4*R* selectivity[54] compared to >95:5 in the case of Cbz-Glu(OMe)OBO ester **4.69** to give **4.96**.

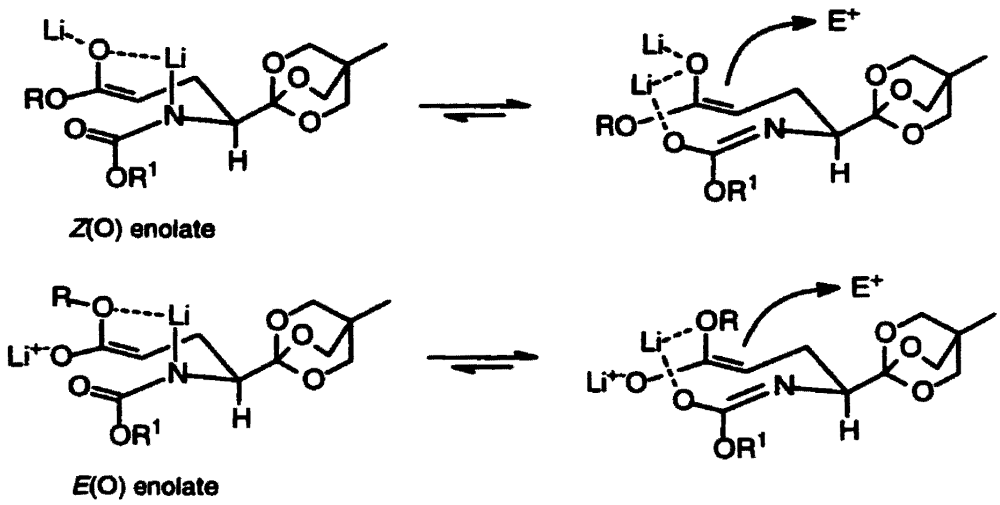


Figure 4.10: Possible transition states of the lithium enolate of Cbz-Glu(OMe)OBO ester 4.66.[107]

The 1:1 selectivity observed in the electrophilic addition of methyl iodide to the potassium enolate of **4.69** (Table 4.1, entry 3) also supports the chelation transition state depicted in figure 4.10 since larger counter-ions (i.e. $K^+ > Na^+ > Li^+$) are known to possess a reduced ability to chelate.[108] Furthermore, the ability of the Li-enolate of **4.69** to chelate and form a seven-membered transition state may be implied from the *syn* rotamer of **4.73** depicted in figure 4.7. Presumably, if hydrogen bonding can induce the formation of a stable seven-membered ring, then the stronger ionic interaction between the lithium cation and the oxygen anion may also do so.

4.3 Summary

A general method for the synthesis of a wide variety of 2*S*,4*S* γ-substituted glutamates has been developed with diastereoselectivities generally >95:5 (2*S*,4*S*;2*S*,4*R*). This methodology represents a significant step in the synthesis of γ-substituted glutamic acids due to its high selectivity and flexibility since a wide variety of substituents may be incorporated. Overall yield of the free γ-substituted glutamic acids from glutamic acid **4.60** range from 15-33% over six steps. The fully protected glutamate **4.69** is conveniently crystalline as are a number of the γ-substituted derivatives.

The 4-alkyl substitutents are easily generated in high yield (75-84% yield) and excellent diastereoselectivity (>95:5) with the only contaminant starting material, which may be recovered and recycled.

γ-Hydroxyglutamic acid **4.4** is synthesized in good overall yield (25%) and excellent diastereomeric purity (>95:5) and is a noteworthy improvement over current methods used to synthesize this amino acid. The synthesis of the two unidentified diastereomers of dihydroxyglutamic acid (**4.102/4.103**) represents a significant improvement of current methods in both length and overall yield. β-Hydroxyglutamic acid **4.7** was synthesized in both good yield and high diastereoselectivity, comparable to other reported methods. Furthermore, crystalline 2*S*,4*S*-Cbz-Glu(γ-OH)(OMe)OBO ester **4.96** provides access to a number of other potential derivatives via functionalization of the hydroxyl group as illustrated in the synthesis of the 2*S*,4*R* isomer of diaminoglutamic acid.

Protected 2*S*,4*S*-azidoglutamic acid **4.106** was obtained in moderate yield (54%) but excellent diastereomeric purity (>95:5). Reduction and deprotection gave 2*S*,4*S*-

diaminoglutamic acid **4.11** in eight steps and 12% overall yield from glutamic acid. 2*S*,4*R*-Cbz-Glu(γ-N_3)(OMe)OBO ester **4.109** was synthesized via S_N2 displacement with azide from the corresponding 2*S*,4*S*-Cbz-Glu(γ-OH)(OMe)OBO ester **4.96**. Reduction and deprotection gave 2*S*,4*R*-diaminoglutamic **4.110** in 10% (nine steps) and 12% (eight steps) overall yield depending on the method of azide displacement. Excellent diastereoselectivity in the electrophilic azidation (>95:5) was also achieved and could be unambiguously assigned since both diastereomers were in hand.

Both 2*S*,4*S*-Cbz-Glu(γ-Br)(OMe)OBO ester **4.112** and 2*S*-Cbz-Glu(γ-CO_2Me)(OMe)-OBO ester **4.113** were synthesized in good overall yield (33 and 32% respectively) and good selectivity, 90:10 for **4.112**.

The Claisen condensation product, although by no means an optimized synthesis, has promise in providing a synthetic route towards indolizidines with the possible incorporation of γ-substituted glutamic acid derivatives.

The overall yields and diastereoselectivities of the products synthesized by our route are comparable, if not significantly better to most reported synthesis for γ-substituted glutamates.

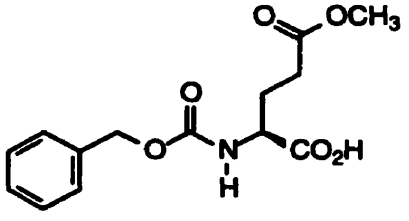
4.4 Experimental Results

General Procedure for Removal of Protecting Groups. Cbz-L-Glu(γ-Me)(OMe)-OBO ester **4.73** (72 mg, 0.18 mmol) was combined with doubly distilled 6N HCl (2 ml) and refluxed for 4 hours. The solvent was then removed *in vacuo*, rinsed with distilled water, reduced again and then lyophilized to a white powder. The white powder was dissolved in a minimum of distilled water and placed on Dowex 50X8-100 ion-exchange resin. The column was then rinsed with 5 column lengths of distilled water then 5 column lengths of 0.5 N NH_4OH that were collected. The solvent was removed under reduced pressure and lyophilized to give a white powder which was then crystallized from acetone:water or ethanol:water.

4.4.1 (2*S*)-2-[(Benzyloxy)carbonyl]amino-5-methoxy-5-oxopentanoic acid, Cbz-L-Glu-(OMe)-OH, 4.61.

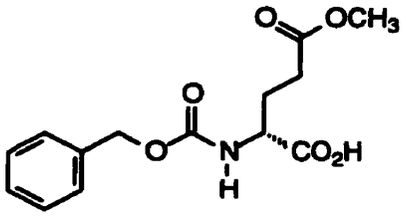
Glutamic acid **4.60** (20.0 g, 0.136 mol) was suspended in freshly distilled MeOH (400 mL) and cooled to 0°C whilst stirring under Ar. Chlorotrimethylsilane (15 mL, 0.119 mol) was slowly added by dropping funnel and after 30 min allowed to warm to ambient temperature. After 2 hours the mixture was cooled to 0°C and a second aliquot of chlorotrimethylsilane (15 mL, 0.119 mol) slowly added. After 24 hours the solvent was removed *in vacuo*, revealing an oil which was placed under high vacuum. De-ionized water (200 mL) was added to the oil followed by slow addition of Na_2CO_3 (20.23 g, 0.163 mol) to prevent excessive frothing. Once the oil was completely in solution it was cooled to 0°C and *N*-(benzyoxycarbonyl)-succinimide (33.89 g, 0.136 mol), pre-

dissolved in 1,4-dioxane (200 mL), slowly added to the stirring mixture which was then allowed to warm to room temperature. After 24 hours, the volume was reduced to approximately 200 mL under vacuum, then de-ionized water (100 mL) added and the final pH of the mixture adjusted to 3 with 1M HCl. This was then extracted with CH_2Cl_2 (3 × 150 mL), the organic extracts were then pooled and extracted with saturated $NaHCO_3$ (3 × 100 mL). The aqueous extracts were pooled, chilled to 0°C, acidified to pH 3 with 1M HCl then extracted with CH_2Cl_2 (4 × 100 mL). The organic extracts were pooled, extracted with brine (50 mL) then dried over $MgSO_4$ and the solvent removed under reduced pressure to yield a colourless oil which solidified upon standing and was used without further purification.



TLC (1:1, $CHCl_3$:EtOAc, 1% AcOH) R_f = 0.18; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.72 (br s, 1H, CO_2H), 7.31-7.26 (m, 5H, Ar*H*), 5.61 (d, 1H, *J* = 7.5Hz, N*H*), 5.08 (s, 2H, CbzCH_2O), 4.60-4.42 (m, 1H, α-C*H*), 3.63 (s, 3H, CO_2CH_3), 2.50-2.33 (m, 2H, γ-CH_2), 2.30-2.16 (m, 1H, β-C*H*H), 2.09-1.91 (m, 1H, β-CH*H*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 176.1 (C=O), 173.6 (C=O), 156.3 (CONH), 136.1 (Cbz=C=), 128.6, 128.3, 128.2 (Cbz=CH=), 67.3 (CbzCH$_2$O), 53.3 (α-CH), 52.0 (CO_2CH_3), 30.1 (γ-CH_2), 27.3 (β-CH_2); Anal. calcd for $C_{14}H_{17}NO_6$: C, 56.95; H, 5.80; N, 4.74. Found: C, 57.19; H, 5.61; N, 4.81.

4.4.2 (2*R*)-2-[(Benzyloxy)carbonyl]amino-5-methoxy-5-oxopentanoic acid, Cbz-D-Glu-(OMe)-OH, 4.62.

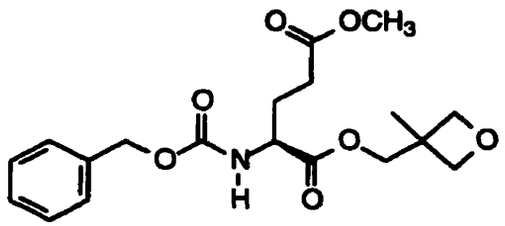


Same procedure as in 4.4.1. TLC (1:1, $CHCl_3$:EtOAc, 1% AcOH) $R_f = 0.18$; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.74 (br s, 1H, CO_2H), 7.36-7.28 (m, 5H, Ar*H*), 5.52 (d, 1H, J = 7.8Hz, N*H*), 5.08 (s, 2H, CbzCH_2O), 4.46-4.36 (m, 1H, α-C*H*), 3.63 (s, 3H, CO_2CH_3), 2.48-2.34 (m, 2H, γ-CH_2), 2.30-2.16 (m, 1H, β-C*H*H), 2.08-1.94 (m, 1H, β-CH*H*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 176.1 (C=O), 173.6 (C=O), 156.3 (CONH), 136.1 (Cbz=C=), 128.6, 128.3, 128.2 (Cbz=CH=), 67.3 (CbzCH_2O), 53.3 (α-CH), 52.0 (CO_2CH_3), 30.1 (γ-CH_2), 27.3 (β-CH_2); Anal. calcd for $C_{14}H_{17}NO_6$: C, 56.95; H, 5.80; N, 4.74. Found: C, 57.19; H, 5.61; N, 4.81.

4.4.3 5-Methyl-1-[(3-methyl-3-oxetanyl)methyl]-(2*S*)-2-(benzyloxy)carbonyl]-aminopentanedioate, Cbz-L-Glu(OMe) oxetane ester, 4.65.

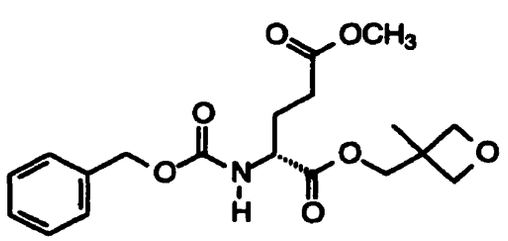
Cbz-L-Glu(OMe)-OH **4.61** (6.14 g, 0.0208 mol) was combined with Cs_2CO_3 (4.06 g, 0.0125 mol) then dissolved in de-ionized water (100 mL) and lyophilized overnight. To the resulting solid was added oxetane tosylate **2.38** (5.59 g, 0.0218 mol) and NaI (0.62 g, 4.16 mmol) and then taken up in DMF (300 mL). The mixture was allowed to stir for 48 hours before the DMF is then removed *in vacuo* (0.5 mm Hg, bath temperature 50°C) and the resulting solid dissolved in EtOAc (300 mL) and H_2O (100 mL) and extracted with 10% $NaHCO_3$ (2 × 50 mL), saturated NaCl (50 mL) and dried over $MgSO_4$. The solvent

was removed under reduced pressure and the resulting oil purified by flash chromatography (1:1 EtOAc:Hex)to give a clear oil in 78% yield (6.23 g).



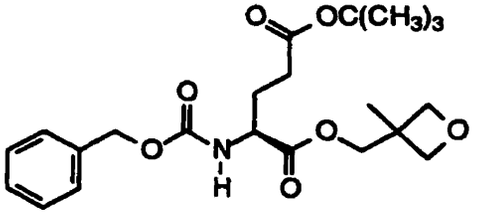
$[\alpha]^{20}_D$ = +1.4 (c = 1.0, CH_2Cl_2); TLC (1:1, EtOAc:Hex), R_f = 0.31; ^{1}H NMR ($CDCl_3$, 300MHz) δ 7.35-7.28 (m, 5H, Ar*H*), 5.41 (d, 1H, *J* = 7.9Hz, N*H*), 5.07 (s, 2H, CbzCH_2O), 4.50-4.34 (m, 5H, 2 oxetane ester CH_2O, α-C*H*), 4.21 (br s, 2H, CO_2CH_2), 3.62 (s, 3H, CO_2CH_3), 2.46-2.32 (m, 2H, γ-CH_2), 2.27-2.13 (m, 1H, β-C*H*H), 2.03-1.92 (m, 1H, β-CH*H*), 1.29 (s, 3H, oxetane ester CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.1 (C=O), 172.1 (C=O), 156.1 (CONH), 136.2 (Cbz=C=), 128.3, 128.2, 128.1 (Cbz=CH), 79.5 (oxetane ester CH_2O), 69.8 (CO_2CH_2), 67.1 (CbzCH_2O), 53.5 (α-CH), 51.9(CO_2CH_3), 39.1 (oxetane ester CCH_3), 30.0 (γ-CH_2), 27.5 (β-CH_2), 21.1 (oxetane ester CCH_3); IR (cast from $CHCl_3$) 3342, 2956, 2875, 1736, 1526, 1263, 1213, 1053, 982; Anal. calcd for $C_{19}H_{25}NO_7$: C, 60.15; H, 6.64; N, 3.69. Found: C, 59.88; H, 6.48; N, 3.71.

4.4.4 5-Methyl-1-[(3-methyl-3-oxetanyl)methyl]-(2*R*)-2-[(benzyloxy)carbonyl]-aminopentanedioate, Cbz-D-Glu(OMe) oxetane ester, 4.66.



Same procedure as in 4.4.3. $[\alpha]^{20}_D$ = -1.3 (c = 1.0, CH_2Cl_2); TLC (1:1, EtOAc:Hex), R_f = 0.37; ^{1}H NMR ($CDCl_3$, 300MHz) δ 7.35-7.28 (m, 5H, Ar*H*), 5.41 (d, 1H, *J* = 7.9Hz, N*H*), 5.07 (s, 2H, CbzCH_2O), 4.50-4.34 (m, 5H, 2 oxetane ester CH_2O, α-C*H*), 4.21 (br s, 2H, CO_2CH_2), 3.62 (s, 3H, CO_2CH_3), 2.49-2.32 (m, 2H, γ-CH_2), 2.28-2.16 (m, 1H, β-C*H*H), 2.03-1.92 (m, 1H, β-CH*H*), 1.31 (s, 3H, oxetane ester CCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.1 (C=O), 172.1 (C=O), 156.1 (CONH), 136.2 (Cbz=C=), 128.6, 128.3, 128.2 (Cbz=CH), 79.4 (oxetane ester CH_2O), 69.7 (CO_2CH_2), 67.1 (CbzCH_2O), 53.5 (α-CH), 51.9(CO_2CH_3), 39.1 (oxetane ester CCH_3), 30.0 (γ-CH_2), 27.5 (β-CH_2), 21.1 (oxetane ester CCH_3); IR (cast from $CHCl_3$) 3342, 2956, 2875, 1736, 1526, 1263, 1213, 1053, 982; Anal. calcd for $C_{19}H_{25}NO_7$: C, 60.15; H, 6.64; N, 3.69. Found: C, 60.32; H, 6.78; N, 3.74.

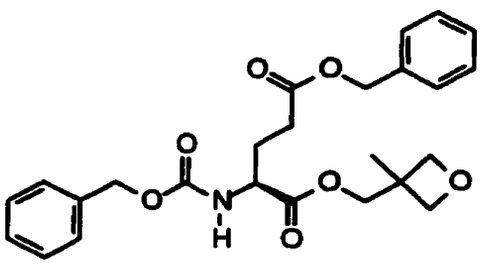
4.4.5 5-(*tert*-Butyl)-1-[(3-methyl-3-oxetanyl)methyl]-(2*S*)-2-[(benzyloxy)carbonyl]-aminopentanedioate, Cbz-L-Glu(OtBu)-oxetane ester, 4.67.



Same procedure as in 4.4.3. $[\alpha]^{20}_D$ = -11.5 (c = 1.45, EtOAc); TLC (1:1, EtOAc:Hex), R_f = 0.53; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.34-7.26 (m, 5H, Ar*H*), 5.52 (d, 1H, *J* = 7.9Hz, N*H*), 5.06 (s, 2H, CbzCH_2O), 4.49-4.42 (m, 2H, oxetane ester CH_2O), 4.39-4.30 (m, 3H, oxetane CH_2O, α-C*H*), 4.20 (s, 2H, CO_2CH_2), 2.34-2.24 (m, 2H, γ-CH_2), 2.18-2.08 (m, 1H, β-C*H*H), 2.00-1.86 (m, 1H, β-CH*H*), 1.39 (s, 9H, C(CH_3)$_3$), 1.28 (s, 3H, oxetane

ester CCH_3); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 172.2 (C=O), 172.0 (C=O), 156.1 (CONH), 136.2 (Cbz=C=), 128.6, 128.3, 128.2 (Cbz=CH=), 81.0 ($C(CH_3)_3$), 79.4 (oxetane ester CH_2O), 69.7 (CO_2CH_2), 67.1 ($CbzCH_2O$), 53.7 (α-CH), 39.1 (oxetane ester CCH_3), 31.5 (γ-CH_2), 28.1 ($C(CH_3)_3$), 27.4 (β-CH_2), 21.1 (oxetane ester CCH_3); IR (cast from CH_2Cl_2) 3336, 2969, 2875, 1728, 1539, 1456, 1258, 1153, 1053, 982; Anal. calcd for $C_{22}H_{31}NO_7$:C, 62.69; H, 7.41; N, 3.32. Found: C, 62.85; H, 7.61; N, 3.39.

4.4.6 5-Benzyl-1-[(3-methyl-3-oxetanyl)methyl]-(2*S*)-2-(benzyloxy)carbonyl]amino-pentanedioate, Cbz-L-Glu(OBn)-oxetane ester, 4.68.

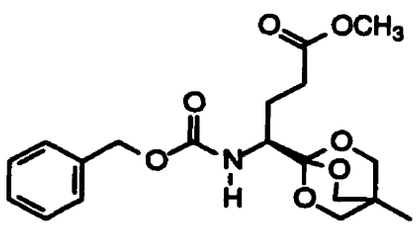


Same procedure as in 4.4.3. $[\alpha]^{20}_D$ = -12.5 (c = 1.05, EtOAc); TLC (1:1, EtOAc:Hex), R_f = 0.48; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.36-7.27 (m, 10H, Ar*H*), 5.44 (d, 1H, *J* = 7.8Hz, N*H*), 5.10-5.06 (m, 4H, CbzCH_2O, PhCH_2O), 4.47-4.32 (m, 5H, 2 oxetane ester CH_2O, α-C*H*), 4.20 (s, 2H, CO_2CH_2), 2.55-2.36 (m, 2H, γ-CH_2), 2.30-2.17 (m, 1H, β-C*H*H), 2.07-1.92 (m, 1H, β-CH*H*), 1.28 (s, 3H, oxetane ester CCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.5 (C=O), 172.0 (C=O), 156.0 (CONH), 136.2 (Cbz=C=), 135.7 (Ph=C=), 128.7, 128.6, 128.4, 128.4, 128.3, 128.2 (Cbz=CH=, Ph=CH=), 79.4 (oxetane ester CH_2O), 69.8 (CO_2CH_2C), 67.2 ($CbzCH_2O$), 66.7 (CO_2CH_2Ph), 53.5 (α-CH), 39.1 (oxetane ester CCH_3), 30.3 (γ-CH_2), 27.6 (β-CH_2), 21.1 (oxetane ester CCH_3); IR (cast

from CH_2Cl_2) 3338, 2962, 2875, 1732, 1522, 1455, 1262, 1214, 1176, 1053, 981; Anal. calcd for $C_{25}H_{29}NO_7$: C, 65.92; H, 6.42; N, 3.07. Found: C, 65.65; H, 6.27; N, 3.06.

4.4.7 5-Methyl-(2*S*)-2-[(benzyloxy)carbonyl]amino-1-(4-methyl-2,6,7-trioxabicyclo-[2.2.2]oct-1-yl)pentanoate, Cbz-L-Glu(OMe)-OBO ester, 4.69.

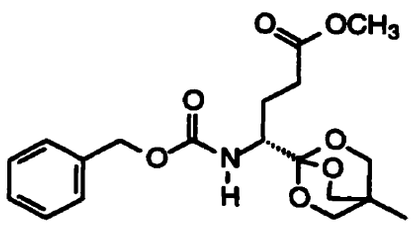
Cbz-L-Glu(OMe)-oxetane ester **4.65** (5.40 g, 14.2 mmol) was dissolved in dry CH_2Cl_2 (225 mL) while stirring under Ar. The mixture was cooled to 0°C then $BF_3 \cdot Et_2O$ (90μL, 0.64 mmol) was added by syringe. The mixture was allowed to warm to room temperature and stir for 6 hours after which a TLC indicated the reaction was complete. Et_3N (0.39 mL, 2.58 mmol) was then added and the mixture stirred an additional 30 min before the solvent was removed *in vacuo*. The resulting oil was dissolved in EtOAc (150 mL) and extracted with 3% NH_4Cl (2 × 25 mL), saturated $NaHCO_3$ (25 mL), brine (25 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to reveal a light coloured oil which was purified by flash chromatography (1:1 EtOAc:Hex) to give a clear oil in 91% yield (4.45 g) which crystallized upon standing. The product can be recrystallized from a mixture of diethyl ether:petroleum ethers (30-60):hexanes to give 3.54 g (65.5% yield) of white, needles.



m.p. 50.5-51.5 °C, $[\alpha]^{20}_D$ = -33.0 (c = 1.0, EtOAc); TLC (1:1, EtOAc:Hex), R_f = 0.56; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.38-7.26 (m, 5H, Ar*H*), 5.74 (d, 1H, *J* =10.3Hz, N*H*), 5.10 (d, 1H, *J* = 12.7Hz, CbzC*H*HO), 5.03 (d, 1H, *J* = 12.7Hz, CbzCH*H*O), 3.89 (s, 6H,

OBO ester C*H*$_2$O), 3.79 (dt, 1H, *J* = 3.9, 10.3Hz, α-C*H*), 3.59 (s, 3H, CO_2CH_3), 2.38-2.28 (m, 2H, γ-C*H*$_2$), 2.10-1.98 (m, 1H, β-C*H*H), 1.74-1.60(m, 1H, β-CH*H*), 0.81 (s, 3H, OBO ester CC*H*$_3$); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 173.1 (C=O), 156.4 (CONH), 137.6 (Cbz=C=), 128.3, 127.7, 127.6 (Cbz=CH=), 108.2 (OBO ester C-O), 72.3 (OBO ester OCH$_2$), 65.7 (CbzCH$_2$O), 54.5 (α-CH), 50.7 (CO_2CH_3), 30.3 (OBO ester CCH$_3$), 30.1 (γ-CH$_2$), 25.2 (β-CH$_2$), 13.3 (OBO ester CCH$_3$); IR (cast from $CHCl_3$) 3354, 2953, 2881, 1727, 1521, 1452, 1233, 1049, 1011; HRMS (FAB) calcd for (M + H$^+$) $C_{19}H_{26}NO_7$ 380.17093, found 380.17034; Anal. calcd for $C_{19}H_{25}NO_7$: C, 60.15; H, 6.64; N, 3.69. Found: C, 60.36; H, 6.62; N, 3.71.

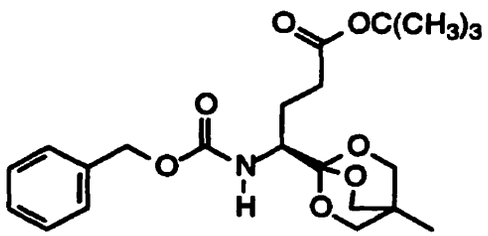
4.4.8 5-Methyl-(2*R*)-2-[(benzyloxy)carbonyl]amino-1-(4-methyl-2,6,7-trioxabicyclo-[2.2.2]oct-1-yl)pentanoate, Cbz-D-Glu(OMe)-OBO ester, 4.70.



Same procedure as in 4.4.7. $[\alpha]^{20}_D$ = +30.1 (c = 0.98, EtOAc); TLC (1:1, EtOAc:Hex), R_f = 0.47; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.38-7.26 (m, 5H, Ar*H*), 5.74 (d, 1H, *J* =10.3Hz, N*H*), 5.07 (d, 1H, *J* = 12.7Hz, CbzC*H*HO), 5.00 (d, 1H, *J* = 12.7Hz, CbzCH*H*O), 3.87 (s, 6H, OBO ester C*H*$_2$O), 3.79 (dt, 1H, *J* = 3.9, 10.3Hz, α-C*H*), 3.56 (s, 3H, CO_2CH_3), 2.35-2.27 (m, 2H, γ-C*H*$_2$), 2.10-1.98 (m, 1H, β-C*H*H), 1.72-1.58 (m, 1H, β-CH*H*), 0.79 (s, 3H, OBO ester CC*H*$_3$); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 173.1 (C=O), 156.4 (CONH), 137.6 (Cbz=C=), 128.3, 127.7, 127.6 (Cbz=CH=), 108.2 (OBO

ester C-O), 72.4 (OBO ester $\underline{C}H_2O$), 65.7 (Cbz$\underline{C}H_2O$), 54.5 (α-$\underline{C}H$), 50.7 ($CO_2\underline{C}H_3$), 30.4 (OBO ester $\underline{C}CH_3$), 30.1 (γ-$\underline{C}H_2$), 25.2 (β-$\underline{C}H_2$), 13.2 (OBO ester C$\underline{C}H_3$); IR (cast from $CHCl_3$) 3354, 2953, 2881, 1727, 1521, 1452, 1233, 1049, 1011; HRMS (FAB) calcd for (M + H^+) $C_{19}H_{26}NO_7$ 380.17093, found 380.17038; Anal. calcd for $C_{19}H_{25}NO_7$: C, 60.15; H, 6.64; N, 3.69. Found: C, 60.28; H, 6.76; N, 3.72.

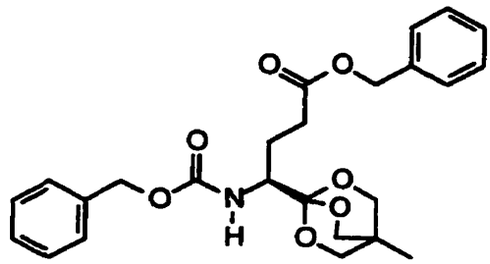
4.4.9 5-*tert*-Butyl-(2*S*)-2-[(benzyloxy)carbonyl]amino-1-(4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl)pentanoate, Cbz-L-Glu(O*t*Bu)-OBO ester, 4.71.



Same procedure as in 4.4.7. m.p. 86-87°C $[\alpha]^{20}_D$ = -29.0 (c = 1.00, EtOAc); TLC (1:1, EtOAc:Hex), R_f = 0.59; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.38-7.23 (m, 5H, Ar*H*), 5.72 (d, 1H, *J* = 9.8Hz, N*H*), 5.08 (d, 1H, *J* = 12.7Hz, CbzC*H*HO), 5.03 (d, 1H, *J* = 12.7Hz, CbzCH*H*O), 3.86 (s, 6H, OBO ester CH_2O), 3.77 (dt, 1H, *J* = 3.4, 10.8Hz, α-C*H*), 2.26-2.16 (m, 2H, γ-CH_2), 2.04-1.92 (m, 1H, β-C*H*H), 1.64-1.54 (m 1H, β-CH*H*), 1.39 (s, 9H, C(CH_3)$_3$), 0.78 (s, 3H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 172.1 ($\underline{C}$=O), 156.3 ($\underline{C}$=O), 137.6 (Cbz=$\underline{C}$=), 128.4, 127.7, 127.6 (Cbz=$\underline{C}$=), 108.3 (OBO ester $\underline{C}$-O), 79.3 ($CO_2\underline{C}(CH_3)_3$), 72.3 (OBO ester $\underline{C}H_2O$), 65.7 (Cbz$\underline{C}H_2O$), 54.5 (α-$\underline{C}H$), 31.5 (γ-$\underline{C}H_2$), 30.3 (OBO ester $\underline{C}CH_3$), 27.5 (C($\underline{C}H_3$)$_3$), 25.2 (β-$\underline{C}H_2$), 13.4 (OBO ester C$\underline{C}H_3$); IR (cast from $CHCl_3$) 3361, 2974, 2934, 2880, 1727, 1520, 1456, 1367, 1153, 1047,

1013; HRMS (FAB) calcd for (M + H^+) $C_{22}H_{32}NO_7$ 422.21786, found 422.21631. Anal. calcd for $C_{22}H_{31}NO_7$: C, 62.69; H, 7.41; N, 3.32. Found: C, 62.50; H, 7.28; N, 3.23.

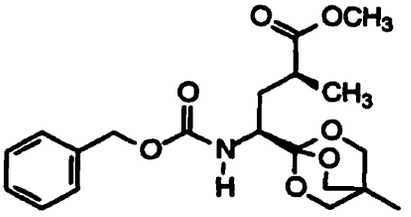
4.4.10 5-Benzyl-(2*S*)-2-[(benzyloxy)carbonyl]amino-1-(4-methyl-2,6,7-trioxabicyclo-[2.2.2]oct-1-yl)pentanoate, Cbz-L-Glu(OBn)-OBO ester, 4.72.



Same procedure as in 4.4.7. $[\alpha]^{20}_D$ = -27.2 (c = 1.6, EtOAc); TLC (1:1, EtOAc:Hex), R_f = 0.55; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.39-7.24 (m, 10H, Ar*H*), 5.73 (d, 1H, *J* = 10.1Hz, N*H*), 5.10-4.97 (m, 4H, CbzCH_2O, PhCH_2O), 3.87 (s, 6H, OBO ester CH_2O), 3.80 (dt, 1H, *J* = 3.9, 10.1Hz, α-C*H*), 2.42-2.34 (m, 2H, γ-CH_2), 2.11-2.00 (m, 1H, β-C*H*H), 1.74-1.62 (m, 1H, β-CH*H*), 0.78 (s, 3H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 172.6 (C=O), 156.4 (CONH), 137.6 (Cbz=C=), 136.8 (Ph=C=), 128.4, 128.3, 128.2, 128.1, 127.9, 127.7 (Cbz=CH=, Ph=CH=), 108.2 (OBO ester C-O), 72.3 (OBO ester CH_2O), 65.7 (CbzCH_2O), 65.5 (CO_2CH_2Ph), 54.5 (α-CH), 30.3 (γ-CH_2), 30.1 (OBO ester CCH_3), 25.2 (β-CH_2), 13.4 (OBO ester CCH_3); IR (cast from $CHCl_3$) 3359, 2982, 2930, 2879, 1723, 1514, 1455, 1367, 1143, 1045, 1010; HRMS (FAB) calcd for (M + H^+) $C_{25}H_{30}NO_7$ 456.20224, found 456.20102. Anal. calcd for $C_{25}H_{29}NO_7$: C, 65.92; H, 6.42; N, 3.07. Found: C, 66.12; H, 6.54; N, 3.10.

4.4.11 5-Methyl-(2*S*,4*S*)-2-[(benzyloxy)carbonyl]amino-4-methyl-1-(4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl)pentanoate, Cbz-L-Glu(γ-Me)(OMe)-OBO ester, 4.73.

Cbz-L-Glu(OMe)-OBO ester **4.69** (0.440 g, 1.12 mmol) was dissolved in dry THF (40 mL) then cooled to –78°C whilst stirring under Ar. In a second flask, LiHMDS (3.48 mL, 3.48 mmol, 1.0M in THF) was added to dry THF (10 mL) then cooled to –78°C whilst stirring under Ar. The Cbz-L-Glu(OMe)-OBO ester **4.69** was then transferred dropwise to the second flask via cannula. The mixture was allowed to stir at –78°C for 1 hour before methyl iodide (0.28 mL, 5.60 mmol) was added by syringe. The mixture was allowed to stir for 4 hours at –78°C before being poured into 3% NH_4Cl (20 mL) and extracted with Et_2O (100 mL). The organic layer was then extracted with 3% NH_4Cl (20 mL), saturated $NaHCO_3$ (20 mL), brine (20 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* to reveal a yellow oil which was further purified by flash chromatography (1:1 EtOAc:Hex, 0.5% Et_3N) to give a clear oil in 82% yield (0.358 g). The product was crystallized from EtOAc:Hexanes to give 0.316 g of white needles (72% yield after crystallization).

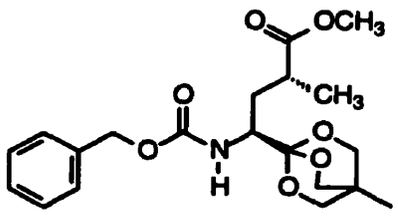


mp 75-76°C, $[\alpha]^{20}_D$ = -25.6 (c = 1.02, CH_2Cl_2); TLC (1:1, EtOAc:Hex), R_f = 0.60; ^1H NMR (Acetone-d_6, 300 MHz) δ 7.43-7.26 (m, 5H, Ar*H*), 5.71 (d, 1H, *J* = 10.2Hz, N*H*), 5.15-5.05 (m, 2H, CbzCH_2O), 3.91-3.85 (s + m, 7H, OBO ester CH_2O, α-C*H*), 3.58 (s, 3H, CO_2CH_3), 2.50-2.38 (m, 1H, γ-C*H*), 1.84 (ddd, 1H, *J* = 5.1, 11.3, 14.0Hz, β-C*H*H),

1.73 (ddd, 1H, J = 3.6, 9.3, 14.0Hz, β-CH*H*), 1.16 (d, 3H, J = 6.7Hz, γ-CH_3), 0.82 (s, 3H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 176.5 (C=O), 156.4 (CONH), 137.6 (Cbz=C=), 128.3, 127.7, 127.7 (Cbz=CH=), 108.3 (OBO ester C-O), 72.4 (OBO ester CH_2O), 65.8 (CbzCH_2O), 53.0 (α-CH), 50.9 (CO_2CH_3), 35.8 (γ-CH), 33.3 (OBO ester CCH_3), 30.3 (β-CH_2), 15.9 (γ-CH_3), 13.4 (OBO ester CCH_3); IR (cast from $CHCl_3$) 3330, 2958, 2877, 1714, 1531, 1214, 1061, 978, 752; HRMS (FAB) calcd for (M + H^+) $C_{20}H_{28}NO_7$ 394.18658, found 394.18465. Anal. calcd for $C_{20}H_{27}NO_7$: C, 61.06; H, 6.84; N, 3.56. Found: C, 60.87; H, 6.84; N, 3.43.

4.4.12 5-Methyl-(2*S*,4*R*)-2-[(benzyloxy)carbonyl]amino-4-methyl-1-(4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl)pentanoate, Cbz-L-Glu(γ-Me)(OMe)-OBO ester, 4.74

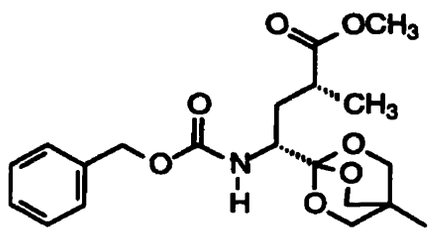
Cbz-L-Glu(γ-Me)(OMe)OBO ester **4.73** (0.177 g, 0.45 mmol) was dissolved in dry THF (25 mL) and cooled to –78°C under Ar. LDA (9 mL, 9.00 mmol) was added by syringe and the mixture stirred for 1 h at –78°C before being poured into Et_2O (100 mL) and extracted with 3% NH_4Cl (25 mL), 10%$NaHCO_3$ (25 mL), brine (25 mL) and the dried over $MgSO_4$. The solvent was removed under reduced pressure to give a light yellow oil which was purified by flash chromatography (1:1 EtOAC:hexanes) to give 79 mg of **4.73** (44% yield) and 85 mg of **4.74** (46% yield).



TLC (1:1, EtOAc:Hex), R_f = 0.55; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.38-7.22 (m, 5H, Ar-*H*), 5.71 (d, 1H, J = 10.3Hz, N*H*), 5.07 (d, 1H, J = 12.7Hz, CbzC*H*HO), 4.98 (d, 1H, J

= 12.7Hz, CbzC*H*HO), 3.87-3.78 (s + m, 7H, OBO ester CH_2O, α-C*H*), 3.55 (s, 3H, CO_2CH_3), 2.60-2.48 (m, 1H, γ-C*H*), 2.10-2.00 (m, 1H, β-C*H*H), 1.45 (ddd, 1H, *J* = 3.9, 10.7, 14.5Hz, β-CH*H*), 1.16 (d, 3H, *J* = 7.3Hz, γ-CH_3), 0.78 (s, 3H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 176.4 (C=O), 156.3 (CONH), 135.1 (Cbz=C=), 128.3, 127.6, 127.6 (Cbz=CH=), 108.8 (OBO ester C-O), 72.3 (OBO ester CH_2O), 65.6 (CbzCH_2O), 53.3 (α-CH), 50.8 (CO_2CH_3), 35.7 (γ-CH), 33.5 (OBO ester CCH_3), 30.3 (β-CH_2), 17.6 (γ-CH_3), 13.4 (OBO ester CCH_3); Anal. calcd for $C_{20}H_{27}NO_7$: C, 61.06; H, 6.84; N, 3.56. Found: C, 61.29; H, 7.01; N, 3.66.

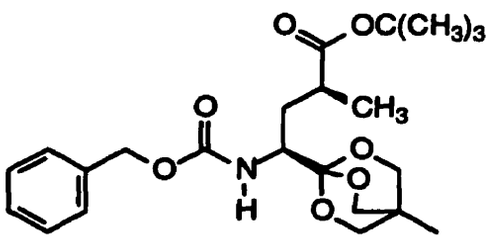
4.4.13 5-Methyl-(2*R*,4*R*)-2-[(benzyloxy)carbonyl]amino-4-methyl-1-(4-methyl-2,6,7-trioxa-bicyclo[2.2.2]oct-1-yl)pentanoate, Cbz-D-Glu(γ-Me)(OMe)-OBO ester, 4.75



Same procedure as in 4.4.11. $[\alpha]^{20}_D$ = +24.3 (c = 1.1, CH_2Cl_2); TLC (1:1, EtOAc:Hex), R_f = 0.59; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.38-7.26 (m, 5H, Ar*H*), 5.71 (d, 1H, *J* = 9.8Hz, N*H*), 5.10-5.00 (m, 2H, CbzCH_2O), 3.89 (s, 6H, OBO ester CH_2O), 3.90-3.82 (m, 1H, α-C*H*), 3.55 (s, 3H, CO_2CH_3), 2.49-2.28 (m, 1H, γ-C*H*), 1.88-1.64 (m, 2H, β-CH_2), 1.10 (d, 3H, *J* = 6.7Hz, γ-CH_3), 0.78 (s, 3H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 176.5 (C=O), 156.4 (CONH), 137.6 (Cbz=C=), 128.3, 127.7, 127.7 (Cbz=CH=), 108.3 (OBO ester C-O), 72.4 (OBO ester CH_2O), 65.8 (CbzCH_2O), 53.0 (α-CH), 50.9 (CO_2CH_3), 35.9 (γ-CH), 33.3 (OBO ester CCH_3), 30.3 (β-CH_2), 15.8 (γ-CH_3),

13.5 (OBO ester C<u>C</u>H$_3$);IR (cast from CHCl$_3$) 3330, 2958, 2877, 1714, 1531, 1214, 1061, 978, 752; HRMS (FAB) calcd for (M + H$^+$) $C_{20}H_{28}NO_7$ 394.18658, found 394.18563. Anal. calcd for $C_{20}H_{27}NO_7$: C, 61.06; H, 6.84; N, 3.56. Found: C, 61.17; H, 6.98; N, 3.61.

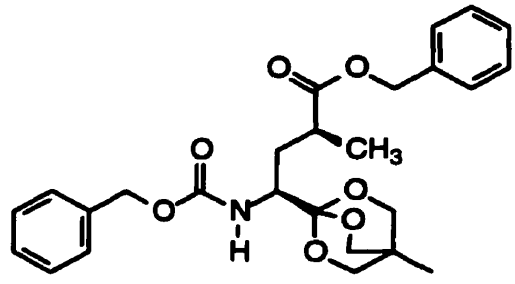
4.4.14 5-*tert*-Butyl-(2*S*,4*S*)-2-[(benzyloxy)carbonyl]amino-4-methyl-1-(4-methyl-2,6,7-trioxa-bicyclo[2.2.2]oct-1-yl)pentanoate, Cbz-L-Glu(γ-Me)(O*t*Bu)-OBO ester, 4.76.



Procedure same as in 4.4.11. $[\alpha]^{20}_D$ = -21.2 (c = 0.94, EtOAc); TLC (1:1, EtOAc:Hex), R_f = 0.56; ^{1}H NMR (Acetone-d$_6$, 300 MHz) δ 7.37-7.25 (m, 5H, Ar*H*), 5.67 (d, 1H, *J* = 10.8Hz, N*H*), 5.08-5.00 (m, 2H, CbzC*H*$_2$O), 3.87 (s, 6H, OBO ester C*H*$_2$O), 3.79 (ddd, 1H, *J* = 3.9, 10.8, 21.0Hz, α-C*H*), 2.36-2.26 (m, 1H, γ-C*H*), 2.23-2.16 (m, 1H, β-C*H*H), 1.80-1.60 (m, 1H, β-CH*H*), 1.38 (s, 9H, C(C*H*$_3$)$_3$), 1.07 (d, 3H, *J* = 7.3 Hz, γ-C*H*$_3$), 0.78 (s, 3H, OBO ester CC*H*$_3$); ^{13}C NMR (Acetone-d$_6$, 75 MHz) δ 172.0 (<u>C</u>=O), 156.3 (<u>C</u>ONH), 137.6 (Cbz=<u>C</u>=), 128.4, 127.7, 127.6 (Cbz=<u>C</u>H=), 108.3 (OBO ester <u>C</u>-O), 78.3 (CO$_2$<u>C</u>(CH$_3$)$_3$), 72.3 (OBO ester <u>C</u>H$_2$O), 65.7 (Cbz<u>C</u>H$_2$O), 54.5 (α-<u>C</u>H), 31.5 (γ-<u>C</u>H), 30.3 (OBO ester <u>C</u>CH$_3$), 27.5 (C(<u>C</u>H$_3$)$_3$), 25.2 (β-<u>C</u>H$_2$), 16.0 (γ-<u>C</u>H$_3$), 13.4 (OBO ester C<u>C</u>H$_3$); IR (cast from CHCl$_3$) 3354, 2952, 2880, 1731, 1520, 1456, 1244, 1045,

1016; HRMS (FAB) calcd for (M + H^+) $C_{23}H_{34}NO_7$ 436.23352, found 436.23647. Anal. calcd for $C_{23}H_{33}NO_7$: C, 63.43; H, 7.64; N, 3.22. Found: C, 63.58; H, 7.87; N, 3.29.

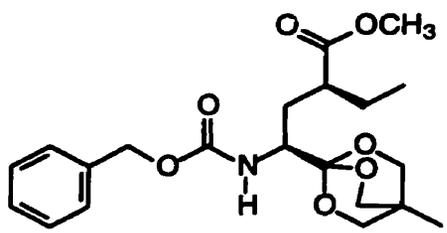
4.4.15 5-Benzyl-(2*S*,4*S*)-2-[(benzyloxy)carbonyl]amino-4-methyl-1-(4-methyl-2,6,7-trioxa-bicyclo[2.2.2]oct-1-yl)pentanoate, Cbz-L-Glu(γ-Me)(OBn)-OBO ester, 4.77.



Procedure same as in 4.4.11. $[\alpha]^{20}_D$ = -22.5 (c = 1.08, EtOAc); TLC (1:1, EtOAc:Hex), R_f = 0.62; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.37-7.23 (m, 10H, Ar*H*), 5.73 (d, 1H, *J* = 10.3Hz, N*H*), 5.00-5.11 (m, 4H, CbzCH_2O, PhCH_2O), 3.86-3.82 (s + m, 7H, OBO ester CH_2O, α-C*H*), 2.56-2.42 (m, 1H, γ-C*H*), 1.85 (ddd, 1H, *J* = 4.9, 11.2, 14.2Hz, β-C*H*H) 1.72 (ddd, 1H, *J* = 3.9, 13.7, 14.2Hz, β-CH*H*), 1.13 (d, 3H, *J* = 6.8Hz, γ-CH_3), 0.78 (s, 3H, OBO ester CCH_3); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 175.8 (C=O), 156.4 (CONH), 137.6, 136.8 (Cbz=C=, Ph=C=), 128.5, 128.3, 128.0, 127.9, 127.7, 127.6 (Cbz=CH=, Ph=CH=), 108.3 (OBO ester C-O), 72.4 (OBO ester CH_2O), 65.7, 65.6 (CbzCH_2O, PhCH_2O), 52.9 (α-CH), 35.9 (γ-CH), 33.2 (OBO ester CCH_3), 30.3 (β-CH_2), 15.9 (γ-CH_3), 13.3 (OBO ester CCH_3); IR (cast from $CHCl_3$) 3350, 2952, 2880, 1732, 1519, 1451, 1239, 1040, 1016; HRMS (FAB) calcd for (M + H^+) $C_{26}H_{32}NO_7$ 470.21786, found 470.21705. Anal. calcd for $C_{26}H_{31}NO_7$: C, 66.51; H, 6.65; N, 2.98. Found: C, 66.70; H, 6.42; N, 3.01.

4.4.16 5-Methyl-(2*S*,4*S*)-2-[(benzyloxy)carbonyl]amino-4-ethyl-1-(4-methyl-2,6,7-tri-oxabicyclo[2.2.2]oct-1-yl)pentanoate, Cbz-L-Glu(γ-Et)(OMe)-OBO ester, 4.78.

Cbz-L-Glu(OMe)-OBO ester **4.69** (0.325 g, 0.85 mmol) was dissolved in dry THF (30 mL) then cooled to –78°C whilst stirring under Ar. In a second flask, LiHMDS (2.57 mL, 2.57 mmol, 1.0M in THF) was added to dry THF (5 mL) then cooled to –78°C whilst stirring under Ar. The Cbz-L-Glu(OMe)-OBO ester **4.69** was then transferred dropwise to the second flask via cannula. The mixture was allowed to stir at –78°C for 1 hour before ethyl iodide (0.34 mL, 4.25 mmol) was added by syringe. The mixture was allowed to stir for 6 hours at –78°C before being poured into 3% NH_4Cl (20 mL) and extracted with Et_2O (100 mL). The organic layer was then extracted with 3% NH_4Cl (20 mL), saturated $NaHCO_3$ (20 mL), brine (20 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* to reveal a yellow oil which was further purified by flash chromatography (1:1 EtOAc:Hex, 0.5% Et_3N) to give a clear oil in 68% yield (0.234 g).

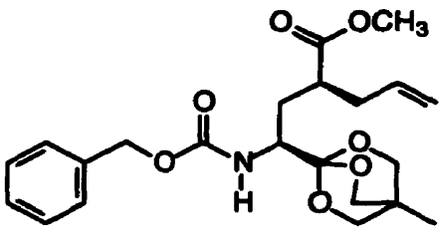


$[\alpha]^{20}_D$ = -25.6 (c = 1.24, EtOAc); TLC (1:1, EtOAc:Hex), R_f = 0.61; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.42-7.24 (m, 5H, Ar*H*), 5.63 (d, 1H, *J* = 10.3Hz, N*H*), 5.10-4.98 (m, 2H, CbzCH_2O), 3.86 (s, 6H, OBO ester CH_2O), 3.88-3.78 (m, 1H, α-C*H*), 3.53 (s, 3H, CO_2CH_3), 2.36-2.26 (m, 1H, γ-C*H*), 1.82 (ddd, 1H, *J* = 3.6, 8.0, 14.1Hz, β-C*H*H), 1.71 (ddd, 1H, *J* = 6.4, 11.3, 14.1Hz, β-CH*H*), 1.64-1.44 (m, 2H, CH_2CH_3), 0.79 (t, 3H, *J* =

7.3Hz, CH_2CH_3), 0.78 (s, 3H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 175.9 (C=O), 156.3 (CONH), 137.6 (Cbz=C=), 128.3, 127.7, 127.7 (Cbz=CH=), 108.3 (OBO ester C-O), 72.4 (OBO ester CH_2O), 65.7 (CbzCH_2O), 53.3 (α-CH), 50.7 (CO_2CH_3), 43.2 (γ-CH), 31.6 (β-CH_2), 30.3 (CCH_3), 24.3 (CH_2CH_3), 13.4 (OBO ester CCH_3), 10.8 (CH_2CH_3); IR (cast from $CHCl_3$) 3359, 2962, 2879, 1730, 1517, 1457, 1230, 1047, 1009; HRMS (FAB) calcd for (M + H^+) $C_{21}H_{30}NO_7$ 408.20224, found 408.20133. Anal. calcd for $C_{21}H_{29}NO_7$: C, 61.90; H, 7.17; N, 3.44. Found: C, 61.70; H, 7.16; N, 3.45.

4.4.17 5-Methyl-(2*S*,4*S*)-2-[(benzyloxy)carbonyl]amino-4-[1-propene]-(4-methyl-2,6,7-trioxa-bicyclo[2.2.2]oct-1-yl)ethyl]-pentanoate, Cbz-L-Glu(γ-Allyl)(OMe)-OBO ester, 4.79.

Cbz-L-Glu(OMe)-OBO ester **4.69** (0.232 g, 0.61 mmol) was dissolved in dry THF (30 mL) then cooled to –78°C whilst stirring under Ar. In a second flask, LiHMDS (1.83 mL, 1.83 mmol, 1.0M in THF) was added to dry THF (5 mL) then cooled to –78°C whilst stirring under Ar. The Cbz-L-Glu(OMe)-OBO ester **4.69** was then transferred dropwise to the second flask via cannula. The mixture was allowed to stir at –78°C for 1 hour before allyl bromide (0.25 mL, 3.05 mmol) was added by syringe. The mixture was allowed to stir for 6 hours at –78°C before being poured into 3% NH_4Cl (20 mL) and extracted with Et_2O (100 mL). The organic layer was then extracted with 3% NH_4Cl (20 mL), saturated $NaHCO_3$ (20 mL), brine (20 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* to reveal a yellow oil which was further purified by flash chromatography (1:1 EtOAc:Hex, 0.5% Et_3N) to give a clear oil in 78% yield (0.199 g).

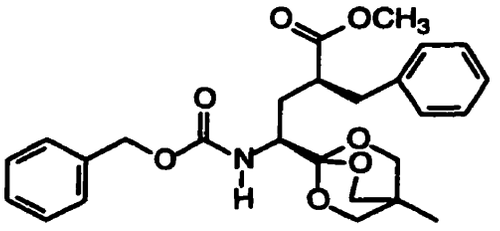


$[\alpha]^{20}_D$ = -20.0 (c = 1.1, EtOAc); TLC (1:1, EtOAc:Hex), R_f = 0.56; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.41-7.26 (m, 5H, Ar*H*), 5.78-5.60 (m, 1H, C*H*=CH_2), 5.15-4.95 (m, 4H, CbzCH_2O, CH=CH_2), 4.73 (d, 1H, *J* = 10.5Hz, N*H*) 3.95-3.85 (m, 1H, α-C*H*), 3.85 (s, 6H, OBO ester CH_2O), 3.50 (s, 3H, CO_2CH_3), 2.55-2.45 (m, 1H, γ-C*H*), 2.36-2.25 (m, 2H, CH_2CH=CH_2), 1.95-1.73 (m, 2H, β-CH_2), 0.76 (s, 3H, OBO ester CCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 176.3 (C=O), 155.4 (CONH), 136.5 (Cbz=C=), 134.9 (CH=CH_2), 128.4, 128.1, 128.0 (Cbz=CH=), 117.1 (CH=CH_2), 108.2 (OBO ester C-O), 72.7 (OBO ester CH_2O), 66.7 (CbzCH_2O), 53.7 (α-CH), 51.4 (CO_2CH_3), 42.1 (γ-CH), 36.2 (CH_2CH=CH_2), 31.2(β-CH_2), 30.5 (OBO ester CCH_3), 14.3 (OBO ester CCH_3); IR (cast from $CHCl_3$) 3355, 2952, 2881, 1732, 1519, 1448, 1340, 1227, 1047, 1013; Anal. calcd for $C_{22}H_{29}NO_7$: C, 62.99; H, 6.97; N, 3.34. Found: C, 62.78; H, 7.15; N, 3.33.

4.4.18 5-Methyl-(2*S*,4*S*)-4-benzyl-2-[(benzyloxy)carbonyl]amino-1-(4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl)pentanoate, Cbz-L-Glu(γ-Bn)(OMe)-OBO ester, 4.80.

Cbz-L-Glu(OMe)-OBO ester **4.69** (0.135 g, 0.36 mmol) was dissolved in dry THF (10 mL) then cooled to –78°C whilst stirring under Ar. In a second flask, LiHMDS (1.07 mL, 1.07 mmol, 1.0M in THF) was added to dry THF (5 mL) then cooled to –78°C whilst stirring under Ar.. The Cbz-L-Glu(OMe)-OBO ester **4.69** was then transferred dropwise to the second flask via cannula. The mixture was allowed to stir at –78°C for 1

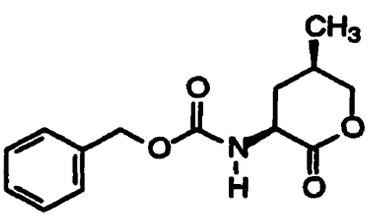
hour before benzyl bromide (0.21 mL, 1.80 mmol) was added by syringe. The mixture was allowed to stir for 6 hours at –78°C before being poured into 3% NH_4Cl (20 mL) and extracted with Et_2O (100 mL). The organic layer was then extracted with 3% NH_4Cl (20 mL), saturated $NaHCO_3$ (20 mL), brine (20 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* to reveal a yellow oil which was further purified by flash chromatography (1:1 EtOAc:Hex, 0.5% Et_3N) to give a clear oil in 75% yield (0.199 g).



$[\alpha]^{20}_D$ = -51.3 (c = 1.00, EtOAc); TLC (1:1, EtOAc:Hex), R_f = 0.60; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.40-7.02 (m, 10H, Ar*H*), 5.75 (d, 1H, *J* = 10.3Hz, N*H*), 5.13 (d, 1H, *J* = 12.7Hz, CbzC*H*HO), 5.00 (d, 1H, *J* = 12.7Hz, CbzCH*H*O), 3.97 (dt, 1H, *J* = 3.4, 10.3Hz, α-C*H*), 3.87 (s, 6H, OBO ester CH_2O), 3.41 (s, 3H, CO_2CH_3), 2.97-2.89 (m, 1H, γ-C*H*), 2.78-2.60 (m, 2H, PhCH_2), 1.93-1.85 (m, 1H, β-C*H*H), 1.80-1.69 (m, 1H, β-CH*H*), 0.76 (s, 3H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 175.3 (C=O), 156.5 (CONH), 139.5, 139.5 (Cbz=C=, Ph=C=), 129.0, 128.4, 128.2, 127.8, 127.8, 126.2 (Cbz=CH=, Ph=CH=), 108.3 (OBO ester C-O), 72.4 (OBO ester CH_2O), 65.9 (Cbz=CH_2O), 53.2 (α-CH), 50.6 (CO_2CH_3), 44.6 (γ-CH), 37.1 (CH_2Ph), 32.3 (OBO ester CCH_3), 30.3 (β-CH_2), 13.3 (OBO ester CCH_3); IR (cast from $CHCl_3$) 2949, 2880, 1732, 1516, 1455, 1220, 1048, 1012; HRMS (FAB) calcd for (M + H^+) $C_{26}H_{32}NO_7$ 470.21786,

found 470.21822. Anal. calcd for $C_{26}H_{31}NO_7$: C, 66.51; H, 6.65; N, 2.98. Found: C, 66.60; H, 6.62; N, 3.04.

4.4.19 Benzyl-*N*-[(3*R*,5*S*)-5-methyl-2-oxotetrahydro-2*H*-3-pyranyl]carbamate, 4.84. Cbz-L-Glu(OMe)-OBO ester **4.69** (0.077 g, 0.20 mmol) was dissolved in THF (10 mL) then cooled to –30°C whilst stirring under Ar. DIBAL-H (0.60 mL, 0.60 mmol) was then added and the mixture allowed to warm to room temperature. After 6 h a TLC indicated the reaction complete and so 20% AcOH (4 mL) was added and the mixture stirred for 30 min before saturated potassium sodium tartrate (50 mL) was added and the mixture stirred for 1 h. The mixture was extracted with EtOAc (3 × 100 mL), the organic fractions pooled then extracted with brine (50 mL) and dried over $MgSO_4$. The solvent was then removed under reduced pressure and the resulting oil dried under high vacuum. The light yellow oil was taken-up in benzene to which *p*-TsOH (10 mg) was added and the mixture stirred overnight at 60°C. The solvent was removed *in vacuo* and the resulting oil taken up in EtOAC:Hexanes (1:1) then passed through a silica plug to remove any polar by-products to give 21 mg of crude **4.84** (40% yield).

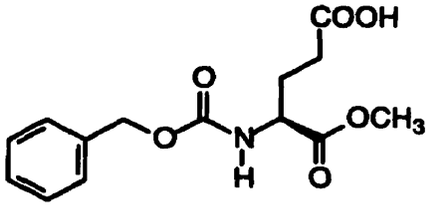


^{1}H NMR ($CDCl_3$, 300MHz) δ 7.39-7.25 (m, 5H, Ar*H*), 5.56 (br s, 1H, N*H*), 5.10 (s, 2H, CbzCH_2O), 4.56-4.43 (m, 1H, α-C*H*), 4.23 (dd, 1H, *J* = 5.3, 11.3Hz, CO_2CHH), 3.97 (dd,

1H, J = 10.3Hz (determined by decoupling experiments), 11.3Hz, CO_2CHH,) 2.38-2.12 (m, 2H, $CHCH_3$, β-CHH), 1.89-1.71 (m, 1H, β-CHH), 1.04 (d, 3H, J = 6.4Hz, $CHCH_3$).

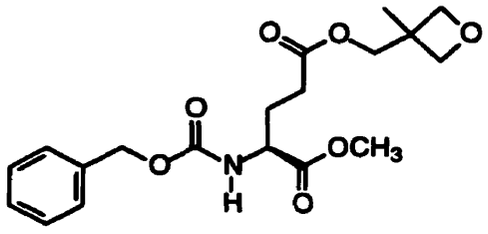
4.4.20 (*S*)-2-[(Benzyloxy)carbonyl]amino-1-methoxy-1-oxopentanoic acid, Cbz-L-Glu-(OH)OMe, 4.85.

Cbz–L-Glu(OH)OH (5.00 g, 17.7 mmol) and *p*-toluenesulfonic acid (0.16 g, 0.81 mmol) and paraformaldehyde (0.66 g) were refluxed in benzene (100 mL) in a Dean-Stark apparatus. After 6 h., the solvent was removed under reduced pressure and the residue suspended in EtOAc (150 mL) and extracted with H_2O (2 × 50 mL). The organic layer was then washed twice with 10% $NaHCO_3$ (2 × 50 mL) and the aqueous layers pooled and acidified to pH 3 with 1 N HCl. These were then extracted with EtOAc (3 × 50 mL) and the organic layers pooled, extracted with brine (50 mL) then dried over $MgSO_4$. The solvent was then removed under reduced pressure. The residue was dried under high vacuum then dissolved in MeOH (50 mL) and added to methanol (100 mL) to which sodium metal (0.81 g, 35.4 mmol) had been added. The mixture was then refluxed for 6 h, cooled and the solvent removed *in vacuo*. The residue was taken-up in chloroform (100 mL) and extracted with 0.1 N HCl (2 × 20 mL), H_2O (20 mL), brine (20 mL) then dried over $MgSO_4$. The solvent was removed under reduced pressure to give 4.18 g (81% yield) of **4.85** as an oil that was used without further purification.



TLC (1:1, $CHCl_3$:EtOAc, 1% AcOH) R_f = 0.14; NMR ($CDCl_3$, 300MHz) δ 10.4 (br s, 1H, COO*H*), 7.32 (br s, 5H, Ar*H*), 6.25 (br s, 1H, N*H*), 5.10 (s, 2H, CbzCH_2O), 4.41 (m, 1H, α-C*H*), 3.70 (s, 3H, CO_2CH_3), 2.50-2.31 (m, 2H, γ-CH_2), 2.27-2.10 (m, 1H, β-C*H*H), 2.04-1.84 (m, 1H, β-CH*H*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 178.1 (C=O), 172.8 (C=O), 156.1 (CONH), 136.1 (Cbz=C=), 128.6, 128.3, 128.2 (Cbz=CH), 67.3 (CbzCH$_2$O), 53.2 (α-CH), 52.7 (CO_2CH_3), 29.9 (γ-CH$_2$), 27.5 (β-CH$_2$); MS (ESI) (M + H$^+$) 296.12.

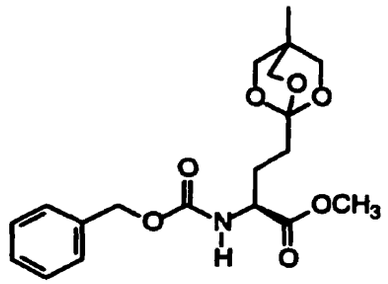
4.4.21 1-Methyl-5-[(3-methyl-3-oxetanyl)methyl]-(2*S*)-2-[(benzyloxy)carbonyl]-aminopentanedioate, Cbz-L-Glu(oxetane ester)OMe, 4.86.



Same as described in 4.4.3. TLC (1:1, EtOAc:Hex), R_f = 0.35; ^{1}H NMR ($CDCl_3$, 300MHz) δ 7.37-7.28 (m, 5H, Ar*H*), 5.41 (d, 1H, *J* = 8.3Hz, N*H*), 5.08 (s, 2H, CbzCH_2O), 4.47 (d, 2H, *J* = 5.9Hz, oxetane ester C*H*HO), 4.44-4.38 (m, 1H, α-C*H*), 4.35 (d, 2H, *J* = 5.9Hz, oxetane CH*H*O), 4.14 (s, 2H, CO_2CH_2), 3.73 (s, 3H, CO_2CH_3), 2.52-2.38 (m, 2H, γ-CH_2), 2.28-2.13 (m, 1H, β-C*H*H), 2.04-1.90 (m, 1H, β-CH*H*), 1.29 (s, 3H, oxetane ester CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.8 (C=O), 172.3 (C=O), 156.0 (CONH), 136.2 (Cbz=C=), 128.6, 128.2, 128.1 (Cbz=CH), 79.6 (oxetane ester CH$_2$O), 69.0 (CO_2CH_2), 67.2 (CbzCH$_2$O), 53.3 (α-CH), 52.7 (CO_2CH_3), 39.0 (oxetane ester CCH$_3$), 30.0 (γ-CH$_2$), 27.7 (β-CH$_2$), 21.2 (oxetane ester CCH$_3$); ESI-MS (M + H$^+$)

378.22; Anal. calcd for $C_{19}H_{25}NO_7$: C, 60.15; H, 6.64; N, 3.69. Found: C, 60.31; H, 6.78; N, 3.71.

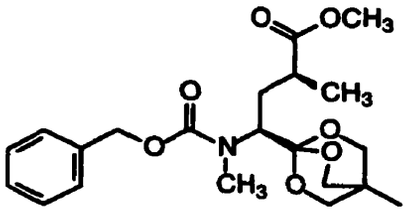
4.4.22 Methyl-(2*S*)-2-[(benzyloxy)carbonyl]amino-4-(4-methyl-2,6,7-trioxabicyclo-[2.2.2]oct-1-yl)butanoate, Cbz-L-Glu(OBO)OMe, 4.87.



Same as described in 4.4.7. Recrystallized from diethyl ether:hexanes:petroleum ethers 30-60. m.p. 75-76°C, $[\alpha]^{20}_D$ = +0.7 (c = 0.97, CH_2Cl_2); TLC (1:1, EtOAc:Hex), R_f = 0.41; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.36-7.24 (m, 5H, Ar*H*), 6.62 (d, 1H, *J* = 8.3Hz, N*H*), 5.04 (d, 1H, *J* = 12.7Hz, CbzC*H*HO), 4.20 (dt, 1H, *J* = 4.9, 8.3 Hz, α-C*H*), 3.82 (s, 6H, OBO ester OCH_2), 3.65 (s, 3H, CO_2CH_3), 2.00-1.86 (m, 2H, γ-CH_2), 1.84-1.64 (m, 2H, β-CH_2), 0.76 (s, 3H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 174.5 (C=O), 158.0 (CONH), 139.1 (Cbz=C=), 130.2, 129.6, 129.5 (Cbz=CH=), 110.2 (OBO ester C-O), 73.9 (OBO ester CH_2O), 67.7 (CbzCH_2O), 55.7 (α-CH), 53.2 (CO_2CH_3), 34.8 (OBO ester CCH_3), 31.8 (γ-CH_2), 27.6 (β-CH_2), 15.3 (OBO ester CCH_3); IR (cast from $CHCl_3$) 3343, 2954, 2879, 1723, 1529, 1454, 1214, 1057, 993; Anal. calcd for $C_{19}H_{25}NO_7$: C, 60.15; H, 6.64; N, 3.69. Found: C, 60.11; H, 6.60; N, 3.73.

4.4.23 Methyl-(2*S*)-2-[[(benzyloxy)carbonyl](methyl)amino]-1-(4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl)pentanoate, Cbz-*N*-Me-L-Glu(γ-Me)(OMe)OBO ester 4.89/4.90.

Cbz-L-Glu(γ-Me)(OMe)OBO ester **4.73** (61 mg, 0.15 mmol) was dissolved in dry THF (5 mL) and then transferred via cannula to a second flask containing LDA (0.45 mL, 0.45 mmol) in dry THF (3 mL) at –40°C. After one hour, MeI (38 μL, 0.75 mmol) was added and the mixture allowed to warm to room temperature. After 4 hours, the mixture was poured into ether (25 mL) and extracted with 3% NH_4Cl (10 mL), 10% $NaHCO_3$ (10 mL), brine (10 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to give a light yellow oil which was purified by flash chromatography (1:1, EtOAc:Hex) to give a clear oil as a mixture of *cis* **4.89** and *trans* **4.90** isomers in 54% yield (32 mg).

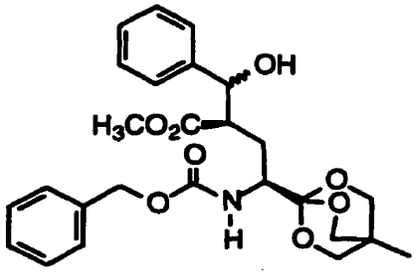


TLC (1:1, EtOAc:Hex), R_f = 0.58; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.42-7.26 (m, 5H, Ar*H*), 5.09 (s, 2H, CbzCH_2O), 4.44 (dd, 0.53H, *J* = 3.4, 12.2Hz, α-C*H* cis/trans), 4.28 (dd, 0.47H, *J* = 3.4, 12.2Hz, α-C*H* cis/trans), 3.86 (s, 6H, OBO ester CH_2O), 3.55 (s, 1.41H, CO_2CH_3 cis/trans), 3.53 (s, 1.59H, CO_2CH_3 cis/trans), 2.72 (s, 1.5H, *N*-CH_3), 2.69 (s, 1.5H, *N*-CH_3), 2.30-2.20 (m, 1H, γ-C*H*), 2.11-1.98 (m, 1H, β-C*H*H), 1.64-1.52 (m, 1H, β-CH*H*), 1.08 (d, 1.59H, *J* = 6.8Hz, γ-CH_3 cis/trans), 1.02 (d, 1.41H, *J* = 6.8Hz, γ-CH_3 cis/trans), 0.76 (s, 3H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 175.9

(C=O), 164.0 (CONH), 143.3 (Cbz=C=), 128.3, 127.7, 127.6 (Cbz=CH=), 107.7 (OBO ester C-O), 72.2 (OBO ester CH_2O), 65.8 (CbzCH_2O), 53.0 (α-CH), 50.9 (CO_2CH_3), 32.8 (γ-CH), 33.1 (OBO ester CCH_3), 29.3 (β-CH_2), 17.9, 16.3 (γ-CH_3), 16.2 (OBO ester CCH_3).

4.4.24 Methyl-(4*S*)-4-[(benzyloxy)carbonyl]amino-2-[hydroxy(phenyl)methyl]-4-(4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl)butanoate, Cbz-L-Glu(γ-OHPh)(OMe)-OBO ester, 4.91.

Cbz-L-Glu(OMe)-OBO ester **4.69** (0.107 g, 0.28 mmol) was dissolved in dry THF (10 mL) then cooled to –78°C whilst stirring under Ar. In a second flask, LiHMDS (0.87 mL, 0.87 mmol, 1.0M in THF) was added to dry THF (5 mL) then cooled to –78°C whilst stirring under Ar. The Cbz-L-Glu(OMe)-OBO ester **4.69** was then transferred dropwise to the second flask via cannula. The mixture was allowed to stir at –78°C for 1 hour before benzaldehyde (0.14 mL, 1.40 mmol) was added by syringe. The mixture was allowed to stir for 6 hours at –78°C before being poured into 3% NH_4Cl (10 mL) and extracted with Et_2O (50 mL). The organic layer was then extracted with 3% NH_4Cl (15 mL), saturated $NaHCO_3$ (15 mL), brine (15 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* to reveal a yellow oil which was further purified by flash chromatography (1:1 EtOAc:Hex, 0.5% Et_3N) to give a clear oil in 71% yield (0.096 g).

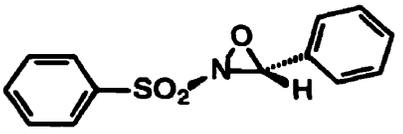


TLC (1:1, EtOAc:Hex), R_f = 0.38; ^{1}H NMR (Acetone-d_6, 300 MHz) mixture of 3:1 stereoisomers δ 8.06-8.00 (m, 2H, Ar*H*), 7.65-7.57 (m, 1H, Ar*H*), 7.51-7.44 (m, 2H, Ar*H*), 7.40-7.22 (m, 5H, ArH), 5.61 (d, 0.25H, *J* = 10.3Hz, N*H*), 5.51 (d, 0.75H, *J* = 10.3Hz, N*H*), 5.10-4.98 (m, 2H, CbzCH_2O), 4.77 (d, 075H, *J* = 6.8Hz, δ-C*H*OH), 4.71 (d, 0.25H, *J* = 8.3Hz, δ-C*H*OH), 3.79 (s, 4.5H, OBO ester CH_2O), 3.77 (s, 1.5H, OBO ester CH_2O), 3.84-3.72 (m, 1H, α-C*H*), 3.55 (s, 0.75H, CO_2CH_3), 3.44 (s, 2.25H, CO_2CH_3), 2.96 (ddd, 0.25H, *J* = 3.4, 8.3, 11.7Hz, γ-C*H*), 2.81 (ddd, 0.75H, *J* = 5.3, 6.8, 8.3Hz, γ-C*H*), 1.96-1.84 (m, 1H, β-C*H*H), 1.78-1.64 (m, 1H, β-CH*H*), 0.75 (s, 2.25H, OBO ester CCH_3), 0.73 (s, 0.75H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 174.7, 174.3 (C=O), 156.2 (CONH), 143.4, 143.0 (Cbz=C=), 137.7, 137.4 (Ar=C=), 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8, 127.6, 127.6, 127.5, 127.3, 127.3, 127.0, 126.6 (Cbz=CH=), 108.2, 108.1 (OBO ester C-O), 74.8, 74.2 (δ-CHOH), 72.2, 72.2 (OBO ester OCH_2), 65.8, 65.5 (CbzCH_2O), 53.8, 53.7 (α-CH), 50.6, 50.4 (CO_2CH_3), 50.0, 49.6 (γ-CH), 30.2 (OBO ester CCH_3), 29.2, 29.1 (β-CH_2), 13.3, 13.3 (OBO ester CCH_3); ESI-MS (M + H^+) 486.31; Anal. calcd for $C_{26}H_{31}NO_8$: C, 64.32; H, 6.44; N, 2.88. Found: C, 64.79; H, 6.70; N, 2.97.

4.4.25 *trans*-2-(Phenylsulfonyl)-3-phenyloxaziridine, 4.92.

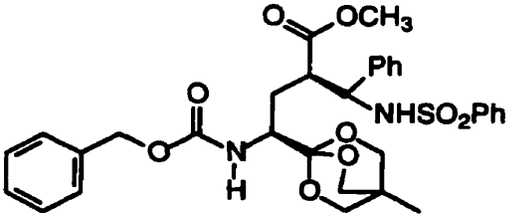
N-Benzylidenebenzenesulfonamide (5.00 g, 2.2 mmol), saturated $NaHCO_3$ (20 mL) and chloroform (30 mL) were placed in a flask and cooled to 0°C. MCPBA (4.9 g, 22 mmol) in chloroform (30 mL) was then slowly added by dropping funnel and the mixture stirred for 1 hour. The organic layer was washed with water (25 mL), 10% sodium sulfite (25 mL), water (25 mL), brine (25 mL) then dried over anhydrous potassium carbonate for 30

min. The solvent was removed under reduced pressure, ensuring the bath temperature remained below 40°C, to give 0.55 g of the desired product as a white solid in quantative yield. The white solid was washed with pentane and either used without further purification or recrystallized from EtOAc:pentane.



^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.05 (br d, 2H, J = 7.1Hz, Ar*H*), 7.82-7.61 (m, 3H, Ar*H*), 7.55-7.35 (m, 5H, J = 10.3Hz, Ar*H*), 5.50 (s, 1H, C*H*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 135.2 (Ar=C̲H=), 134.7 (Ar=C̲=), 131.6 (Ar=C̲H=), 130.5 (Ar=C̲=), 129.5, 129.5, 128.9, 128.4 (Ar=C̲H=), 75.5 (PhC̲H).

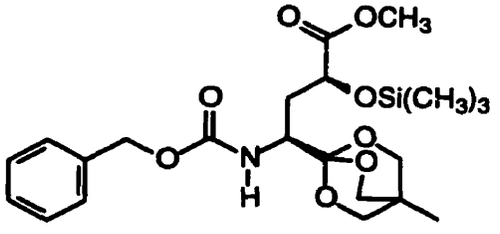
4.4.26 Methyl-(2*S*,4*R*)-2-[(benzyloxy)carbonyl]amino-1-(4-methyl-2,6,7-trioxabicyclo-[2.2.2]oct-1-yl)-4-(*R*)-1-phenyl-1-[(phenylsulfonyl)amino]methyl-butanoate, 4.94.



Isolated from 4.4.28. TLC (1:1, EtOAc:Hex), R_f = 0.37; ^{1}H NMR (Acetone-d_6, 300 MHz) 7.92-7.02 (m, 15H, Ar*H*), 5.60 (d, 1H, J = 10.3Hz, glu-N*H*), 5.09 (d, 1H, J = 12.3Hz, CbzC*H*HO), 5.03 (d, 1H, J = 12.3Hz, CbzCH*H*O), 4.73 (d, 1H, J = 6.8Hz, SO_2N*H*), 4.16-

4.10 (m, 1H, sulfonamide C*H*), 3.91-3.81 (m, 1H, α-C*H*), 3.79 (s, 6H, OBO ester OCH_2), 3.38 (s, 3H, CO_2CH_3), 2.85-2.77 (m, 1H, γ-C*H*), 1.94-1.80 (m, 1H, β-C*H*H), 1.78-1.64 (m, 1H, β-CH*H*), 0.75 (s, 3H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 174.2 (C=O), 156.3 (CONH), 141.8 (Ar=C=), 139.6 (Ar=C=), 137.4 (Cbz=C=), 131.9, 131.8, 128.9, 128.5, 128.4, 128.1, 126.9, 126.8, 126.0 (Cbz=CH=, Ar=CH=) 108.1 (OBO ester C-O), 72.3 (OBO ester CH_2O), 65.9 (CbzCH_2O), 58.6 (ArCHNH), 51.9 (α-CH), 51.1 (CO_2CH_3), 49.3 (γ-CH), 35.0 (OBO ester CCH_3), 30.2 (β-CH_2), 13.3 (OBO ester CCH_3)

4.4.27 Methyl-(2*S*,4*S*)-2-[(benzyloxy)carbonyl]amino-1-(4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl)-4-[(1,1,1-trimethylsilyl)oxy]pentanoate, Cbz-L-Glu(γ-OTMS)-(OMe)-OBO ester, 4.95.

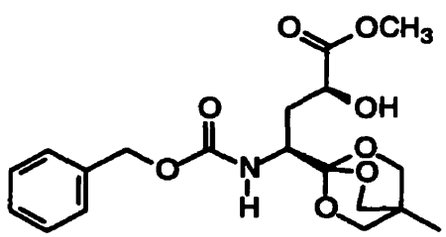


Isolated from 4.4.28. TLC (1:1, EtOAc:Hex), R_f = 0.76; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.38-7.24 (m, 5H, Ar*H*), 5.85 (d, 1H, *J* = 10.3Hz, N*H*), 5.09 (d, 1H, *J* = 12.7Hz, CbzC*H*HO), 5.01 (d, 1H, *J* = 12.7Hz, CbzCH*H*O), 4.20 (dd, 1H, *J* = 1.9, 11.4Hz, γ-C*H*), 4.10-4.00 (m, 1H, α-C*H*), 3.85 (s, 6H, OBO ester CH_2O), 3.64 (s, 3H, CO_2CH_3), 1.97 (ddd, 1H, *J* = 1.9, 10.7, 14.1Hz, β-C*H*H), 1.74 (ddd, 1H, *J* = 2.4, 11.4, 14.1Hz, β-CH*H*), 0.76 (s, 3H, OBO ester CCH_3), 0.06 (s, 9H, $(CH_3)_3$Si); ^{13}C NMR (Acetone-d_6, 75 MHz) δ

173.8 (C=O), 156.3 (CONH), 137.6 (Cbz=C=), 128.4, 127.7, 127.7 (Cbz=CH=), 108.5 (OBO ester C-O), 72.4 (OBO ester CH_2O), 68.8 (CbzCH_2O), 65.7 (γ-CH), 51.6 (α-CH), 51.2 (CO_2CH_3), 35.2 (OBO ester CCH_3), 30.3 (β-CH_2), 13.5 (OBO ester CCH_3), -0.7 ($Si(CH_3)_3$); ESI-MS (M + H^+) 468.32. Anal. calcd for $C_{22}H_{38}NO_7Si$: C, 56.51; H, 7.11; N, 3.00. Found : C, 56.71; H, 7.29; N, 3.04.

4.4.28 Methyl-(2*S*,4*S*)-2-[(benzyloxy)carbonyl]amino-4-hydroxy-1-(4-methyl-2,6,7-trioxa-bicyclo[2.2.2]oct-1-yl)pentanoate, Cbz-L-Glu(γ-OH)(OMe)-OBO, 4.96.

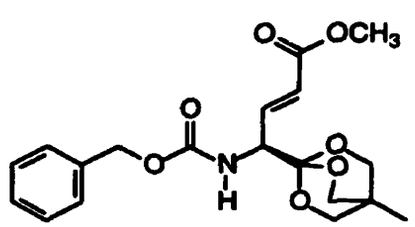
n-Butyllithium (0.72 mL, 1.08 mmol) was slowly added to diisopropylamine (0.165 mL, 1.18 mmol) in dry THF (5 mL) at 0°C under Ar. After 30 minutes the mixture was transferred via cannula to a second flask containing Cbz-L-Glu(OMe)OBO **4.69** (0.135 g, 0.36 mmol) and (±)-*trans*-2-(phenylsulfonyl)-3-phenyloxaziridine **4.92** (0.47 g, 1.80 mmol) in dry THF (10 mL) at –78°C under Ar. After 3 h the mixture was poured into ether (50 mL) and extracted with 3% NH_4Cl (2 × 10 mL), 10% $NaHCO_3$ (10 mL), brine (10 mL) and dried over $MgSO_4$. The resulting light yellow oil was purified by flash chromatography (1:1 EtOAc:Hex) to yield 0.11g (79% yield) of a clear oil which could be crystallized from EtOAc:hexanes to give 95 mg (68 % yield) of clear needles.



m.p. 103-104°C; $[\alpha]^{20}_D$ = -29.3 (c = 1.01, CH_2Cl_2); TLC (1:1, EtOAc:Hex), R_f = 0.17; ^1H NMR (Acetone-d_6, 300 MHz) δ 7.36-7.25 (m, 5H, Ar*H*), 5.89 (d, 1H, *J* = 10.0Hz, N*H*),

5.10-5.00 (m, 2H, CbzCH_2O), 4.24-4.04 (m, 3H, γ-C*H*, α-C*H*, O*H*) 3.86 (s, 6H, OBO ester CH_2O), 3.64 (s, 3H, CO_2CH_3), 1.92 (ddd, 1H, J = 2.9, 11.3, 14.2 Hz, β-C*H*H), 1.75 (ddd, 1H, J = 2.9, 11.7, 14.2Hz, β-CH*H*), 0.79 (s, 3H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 174.6 (C=O), 156.6 (CONH), 137.6 (Cbz=C=), 128.3, 127.7, 127.7 (Cbz=CH=), 108.4 (OBO ester C-O), 72.4 (OBO ester CH_2O), 67.4 (γ-CH), 65.8 (CbzCH_2O), 51.9 (α-CH), 51.2 (CO_2CH_3), 35.1 (OBO ester CCH_3), 30.3 (β-CH_2), 13.4 (OBO ester CCH_3); IR (cast from $CHCl_3$) 3370, 2953, 2881, 1730, 1523, 1234, 1049, 1012; MS (ESI) (M + H^+) 396.22. Anal. calcd for $C_{19}H_{25}NO_8$: C, 57.72; H, 6.37; N, 3.54. Found: C, 57.90; H, 6.35; N, 3.65.

4.4.29 Methyl-(*E*,2*S*)-2-[(benzyloxy)carbonyl]amino-1-(4-methyl-2,6,7-trioxabicyclo[2.2.2]-oct-1-yl)-3-butenoate, Cbz-L-Glu(*E*-β,γ-dehydro)(OMe)OBO ester 4.97. Cbz-Ser(ald)OBO ester **3.42** (0.517 g, 1.61 mmol) and methyl(triphenylphosphoranylidene)acetate (0.646 g, 1.93 mmol) were combined then dissolved in dry CH_2Cl_2 (25 mL) and stirred under Ar. After 1.5 h, the solvent was removed under reduced pressure and then purified by flash chromatography (1:1, EtOAc:Hexanes) to give the desired product in 86% yield (1.62 g) which was recrystallized from diethyl ether:hexanes:petroleum ethers 30-60 to give 1.46g (74% yield)of white needles.

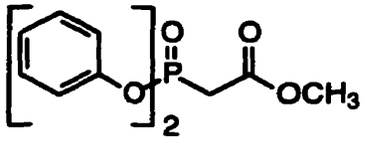


mp. 116-116.5°C; $[\alpha]^{20}_D$ = -21.0 (c = 0.95, CH_2Cl_2); TLC (1:1, EtOAc:Hex), R_f = 0.51; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.38-7.24 (m, 5H, Ar*H*), 6.94 (dd, 1H, *J* = 4.9, 15.7Hz, C*H*=CHCO$_2$CH$_3$), 6.30 (d, 1H, *J* = 9.3Hz, N*H*), 5.96 (dd, 1H, *J* = 1.5, 15.7Hz, CH=C*H*CO$_2$CH$_3$), 5.07 (s, 2H, CbzC*H*$_2$O), 4.49 (ddd, 1H, *J* = 1.5, 4.9, 9.3Hz, α-C*H*), 3.89 (s, 6H, OBO ester C*H*$_2$O), 3.66 (s, 3H, CO$_2$C*H*$_3$), 0.80 (s, 3H, OBO ester CC*H*$_3$); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 165.4 (C=O), 155.3 (CONH), 142.5 (CH=CHCO$_2$CH$_3$), 137.4 (Cbz=C=), 128.4, 127.9, 127.9 (Cbz=CH=), 108.1 (OBO ester C-O), 72.5 (OBO ester CH$_2$O), 65.9 (CbzCH$_2$O), 51.8 (α-CH), 50.7 (CO$_2$CH$_3$), 30.5 (OBO ester CCH$_3$), 13.5 (OBO ester CCH$_3$); HRMS (FAB) calcd for (M + H$^+$) $C_{21}H_{24}NO_7$ 378.14746, found 378.13431. Anal. calcd for $C_{19}H_{23}N_2O_7$: C, 60.47; H, 6.14; N, 3.71. Found: C, 60.24; H, 6.16; N, 3.71.

4.4.30 Methyl Diphenylphosphonoacetate, 4.98.[87]

Triphenyl phosphite (4.65 g, 15.0 mmol) and methyl iodide (0.76 mL, 15.0 mmol) were combined and refluxed for 24 h. After cooling, ethanol (20 mL) was added to the thick oil and stirred to homogeneity then distilled to remove ethanol and ethyl iodide. The temperature was then raised until phenol stopped crystallizing in the distillation apparatus. The dark oil was then cooled to room temperature and diluted with Et_2O (80 mL) and extracted with 1N NaOH (2 × 20 mL) then dried over $MgSO_4$. The solvent was removed under reduced pressure to give a brown coloured oil. The oil was then dissolved in dry THF (20 mL) and combined with methyl chloroformate (1.16 mL, 15.0 mmol) and cooled to –78°C then transferred to a flask containing LiHMDS (34.5 mL, 34.5 mmol) at –78°C. After stirring at –78°C for 1 hour the mixture was quenched with 3% NH_4Cl and

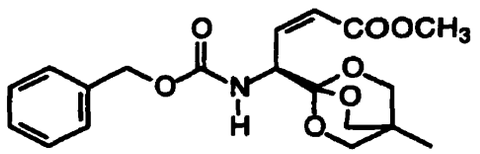
extracted with EtOAc (2 × 20 mL). The organic extracts were combined and washed with water (2 × 20 mL), brine (20 mL) the dried over $MgSO_4$ and then concentrated to a pale yellow residue which was purified by flash chromatography (5:1 to 3:1 hexanes:EtOAc) to give **4.98** (3.22 g, 67% yield) as a clear oil.



^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.37-7.31 (m, 4H, Ar*H*), 7.25-7.17 (m, 6H, Ar*H*), 3.77 (s, 3H, CO_2CH_3), 3.28 (d, 2H, J = 22Hz, CH_2CO_2).

4.4.31 Methyl-(*Z*,2*S*)-2-[(benzyloxy)carbonyl]amino-1-(4-methyl-2,6,7-trioxabicyclo[2.2.2]-oct-1-yl)-3-butenoate, Cbz-L-Glu(Z-β,γ-dehydro)(OMe)OBO ester 4.99. Sodium hydride (0.220 g, 4.56 mmol) was suspended in dry THF (20 mL) to which methyl diphenylphosphonoacetate **4.98** (1.32 g, 4.32 mmol) pre-dissolved in THF (25 mL) was added. After 30 min. the mixture was cooled to –78°C and Cbz-Ser(ald)OBO ester **3.42** (1.55 g, 4.8 mmol) dissolved in dry THF (15 mL) added by syringe. After 2 h the mixture was quenched with 3% NH_4Cl and extracted with EtOAc (2 × 50 mL). $NaBH_4$ (0.5g) was then taken-up in H_2O (30 mL) then added to the vigourously stirring EtOAc extracts to reduce any remaining **3.42**. After 10 min. the organic layer was separated and washed with 10% $NaHCO_3$ (2 × 15 mL), brine (15 mL) then dried over $MgSO_4$ and concentrated to a white residue which was purified by flash chromatography

(3:2 EtOAc:hexanes) to give **4.99** (1.10 g, 61% yield) as an oil which could be recrystallized from EtOAc:hexanes to give white, dense crystals.

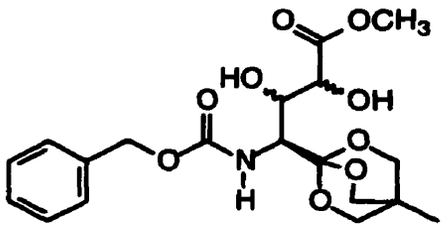


mp. 128-130°C; $[\alpha]^{20}_{D}$ = -48.8 (c = 1.03, EtOAc); TLC (1:1, EtOAc:Hex), R_f = 0.51; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.38-7.20 (m, 5H, Ar*H*), 6.04 (dd, 1H, *J* = 9.8, 11.4Hz, C*H*=CHCO$_2$CH$_3$), 5.86 (d, 1H, *J* = 11.4Hz, CH=C*H*CO$_2$CH$_3$), 5.74 (br dd, 1H, *J* = 9.4, 9.8Hz, N*H*), 5.00 (s, 2H, CbzC*H*$_2$O), 3.86 (s, 6H, OBO ester C*H*$_2$O), 3.66 (s + m, 4H, α-C*H*, CO$_2$C*H*$_3$), 0.79 (s, 3H, OBO ester CC*H*$_3$); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 165.4 (C=O), 156.3 (CONH), 142.5 (CH=CHCO$_2$CH$_3$), 137.5 (Cbz=C=), 128.4, 127.9, 127.9 (Cbz=CH=), 108.1 (OBO ester C-O), 72.5 (OBO ester CH$_2$O), 65.9 (CbzCH$_2$O), 51.8 (α-CH), 50.7 (CO$_2$CH$_3$), 30.5 (OBO ester CCH$_3$), 13.5 (OBO ester CCH$_3$).

4.4.32 Methyl-(2*S*)-2-[(benzyloxy)carbonyl]amino-3,4-dihydroxy-1-(4-methyl-2,6,7-trioxa-bicyclo[2.2.2]oct-1-yl)pentanoate, Cbz-L-Glu(β,γ-dihydroxy)(OMe)OBO, 4.100.

Cbz-L-Glu(*E*-β,γ-dehydro)(OMe)OBO **4.97** (0.426, 1.13 mmol) and *N*-methylmorpholine *N*-oxide (0.139 g, 1.19 mmol) were combined and dissolved in acetone:water (4:1, 15 mL) to which OsO_4 (15 mg, 0.05 mmol) was added. After the mixture had stirred for 48 hours at room temperature the solvent was reduced *in vacuo*, dissolved in methanol (20 mL), reduced again then dissolved in a minimum of methanol to which an equal amount of EtOAc:Hex (2:1) was added and then flash chromatographed to give 0.264 g

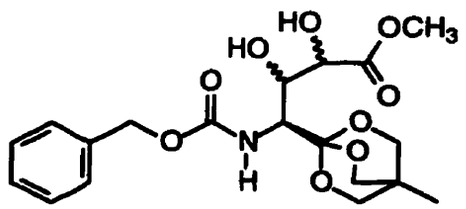
(57% yield) of the desired product which was recrystallized from EtOAc:hexanes to give 0.20 g (44% yield) of small dense white crystals.



mp. 121-122.5°C; $[\alpha]^{20}_{D}$ = -38.3 (c = 1.07, CH_2Cl_2); TLC (2:1, EtOAc:Hex), R_f = 0.21; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.40-7.26 (m, 5H, Ar*H*), 6.09 (d, 1H, *J* = 9.6Hz, N*H*), 5.06 (s, 2H, CbzCH_2O), 4.47 (m, 1H, γ-C*H*), 4.20 (m, 1H, β-C*H*), 4.12 (dd, 1H, *J* = 7.3, 10.3Hz, α-C*H*) 3.97-3.93 (s + m, 7H, OBO ester CH_2O, β-O*H*), 3.86 (d, 1H, J = 7.9Hz, γ-O*H*), 3.70 (s, 3H, CO_2CH_3), 0.84 (s, 3H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 173.3 (C=O), 156.3 (CONH), 137.5 (Cbz=C=), 128.3, 127.7, 127.7 (Cbz=CH=), 108.3 (OBO ester C-O), 72.3 (OBO ester CH_2O), 71.7 (γ-CH), 70.0 (β-CH), 65.8 (CbzCH_2O), 56.8 (α-CH), 51.4 (CO_2CH_3), 30.4 (OBO ester CCH_3), 13.3 (OBO ester CCH_3); IR (cast from CH_2Cl_2) 3429, 2955, 2883, 1731, 1520, 1231, 1046, 1013; ESI-MS (M + H^+) 412.15; Anal. calcd for $C_{19}H_{25}NO_9$: C, 55.47; H, 6.12; N, 3.04. Found: C, 55.64; H, 6.26; N, 3.09.

4.4.33 Methyl-(2*S*)-2-[(benzyloxy)carbonyl]amino-3,4-dihydroxy-1-(4-methyl-2,6,7-trioxa-bicyclo[2.2.2]oct-1-yl)pentanoate, Cbz-L-Glu(β,γ-dihydroxy)(OMe)OBO, 4.101.

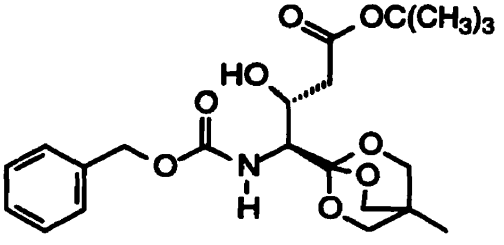
Cbz-L-Glu(Z-β,γ-dehydro)(OMe)OBO **4.99** (0.150 g, 0.40 mmol) and *N*-methylmorpholine (0.049 g, 0.42 mmol) were combined and dissolved in acetone:water (4:1, 5 mL) to which OsO_4 (1 grain) was added. After the mixture had stirred for 48 hours at room temperature the solvent was reduced *in vacuo*, dissolved in methanol (20 mL), reduced again then dissolved in a minimum of methanol to which an equal amount of EtOAc:Hex (2:1) was added and then flash chromatographed to give 0.074 g (45% yield) of **4.101** as a light coloured oil.



$[\alpha]^{20}_D$ = +4.4 (c = 0.9, CH_2Cl_2); TLC (2:1, EtOAc:Hex), R_f = 0.21; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.40-7.26 (m, 5H, Ar*H*), 6.09 (d, 1H, *J* = 10.3Hz, N*H*), 5.07 (s, 2H, CbzCH_2O), 4.66 (br d, 1H, *J* = 7.3Hz, γ-C*H*), 4.16 (dd, 1H, J = 9.8, 10.3Hz, α-C*H*), 3.91 (s, 6H, OBO ester CH_2O), 3.83 (dd, 1H, J = 7.3, 9.8Hz, β-C*H*) 3.66 (s, 3H, CO_2CH_3), 0.80 (s, 3H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 172.8 (C=O), 156.7 (CONH), 137.4 (Cbz=C=), 128.4, 127.8, 127.8 (Cbz=CH=), 108.6 (OBO ester C-O), 72.4 (OBO ester CH_2O), 71.5 (β-CH), 70.8 (γ-CH), 66.1 (CbzCH_2O), 53.8 (α-CH), 50.9 (CO_2CH_3), 30.4 (OBO ester CCH_3), 13.2 (OBO ester CCH_3); IR (cast from CH_2Cl_2) 3447, 2953, 2883, 1734, 1717, 1521, 1228, 1048; ESI-MS (M + H^+) 412.15; Anal. calcd for $C_{19}H_{25}NO_9$: C, 55.47; H, 6.12; N, 3.04. Found: C, 55.69; H, 6.24; N, 3.06.

4.4.34 Reformatsky addition to Cbz-L-Ser(ald)-OBO ester 3.42: *tert*-butyl(1S,2*R*)-1-[(benzyloxy)carbonyl]amino-2-hydroxy-1-(4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl)butanoate, Cbz-L-Glu(β-OH)(O*t*Bu)OBO ester, 4.104.

Purified zinc powder (0.188g, 2.88 mmol) and iodine (1 small crystal) were dissolved in dry THF (10 mL) then refluxed for 20 min before a solution of Cbz-Ser(ald)OBO ester **3.42** (0.153g, 0.48 mmol) and *t*-butylbromoacetate (0.35 mL, 2.40 mmol) in THF (5 mL) was quickly added to the refluxing solution. After 30 min the zinc was filtered off and the filtrate poured into cold 3% NH_4Cl (50 mL) then extracted with Et_2O (3 × 50 mL). The organic layers were combined and extracted with 10% $NaHCO_3$ (15 mL), brine (15 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* and the resulting oil purified by flash chromatography to give 0.157 g of **4.104** (75% yield over 2 steps) as a pale oil.

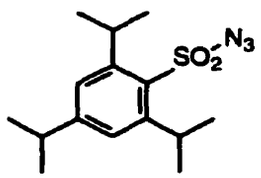


$[\alpha]^{20}_D$ = -7.3 (c = 0.9, CH_2Cl_2); TLC (1:1, EtOAc:Hex), R_f = 0.32; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.40-7.24 (m, 5H, Ar*H*), 5.78 (d, 1H, *J* = 10.3Hz, N*H*), 5.10 (d, 1H, *J* = 12.7Hz, CbzC*H*HO), 5.03 (d, 1H, *J* = 12.7Hz, CbzCH*H*O), 4.51-4.44 (m, 1H, β-C*H*), 3.86 (s, 6H, OBO ester CH_2O), 3.79 (dd, 1H, *J* = 2.0, 10.3Hz, α-C*H*), 2.31-2.24 (m, 2H, γ-CH_2), 1.39 (s, 9H, (CH_3)$_3$C), 0.80 (s, 3H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 170.1 (C=O), 156.4 (CONH), 137.5 (Cbz=C=), 128.4, 127.7, 127.6 (Cbz=CH=), 108.4 (OBO ester C-O), 79.5 ((CH$_3$)$_3$C), 72.3 (OBO ester OCH$_2$), 66.3 (β-CH), 66.0

(Cbz$\underline{C}H_2O$), 57.4 (α-$\underline{C}H$), 39.8 (γ-$\underline{C}H_2$), 30.4 (OBO ester $\underline{C}CH_3$), 28.5 (($\underline{C}H_3)_3C$) 13.7 (OBO ester C$\underline{C}H_3$); IR (cast from CH_2Cl_2) 3515, 3452, 2973, 2934, 2881, 1726, 1513, 1366, 1279, 1225, 1153, 1047; ESI-MS (M + H^+) 438.21; Anal. calcd for $C_{22}H_{31}NO_8$: C, 60.40; H, 7.14; N, 3.20. Found: C, 60.63; H, 7.34; N, 3.26.

4.4.35 1-[(2,4,6-Triisopropylphenyl)sulfonyl]-1,2-triazadien-2-ium, Trisyl azide, 4.105.

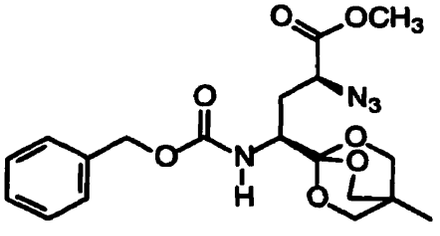
2,4,6-Triisopropylbenzenesulfonyl chloride (3.94 g, 13.0 mmol) was added to a stirring mixture of NaN_3 (2.53 g, 39.0 mmol) in H_2O (20 mL). Acetone (40 mL) was immediately added and the solution stirred for 5 hours. The mixture was then extracted with Et_2O (3 × 50 mL), the organic layers pooled and then extracted with H_2O (30 mL), brine (30 mL) and then dried over Na_2SO_4. The solvent was removed *in vacuo* to reveal a cream coloured oil which crystallized on standing to give 3.40 g (85% yield) of **4.105** which was used without further purification.



^{1}H NMR (Acetone-d_6, 300 MHz) 7.43 (s, 2H, Ar*H*), 4.06 (septet, 2H, *J* = 6.8Hz, $(CH_3)_2C\underline{H}$), 3.03 (septet, 1H, *J* = 7.1Hz, $(CH_3)_2C\underline{H}$), 1.29 (d, 12H, *J* = 6.8Hz, $(C\underline{H}_3)_2CH$), 1.26 (d, 6H, J = 7.1Hz, $(C\underline{H}_3)_2CH$); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 155.7 (Ar=$\underline{C}$=) 151.2 (Ar=$\underline{C}$=), 132.3 (Ar=C=), 124.8 (Ar=$\underline{C}$H=), 34.5 (($CH_3)_2\underline{C}H$), 30.2 (($CH_3)_2\underline{C}H$), 24.4 (($\underline{C}H_3)_2CH$), 23.2 (($\underline{C}H_3)_2CH$).

4.4.36 1-[(2*S*,4*S*)-3-[(Benzyloxy)carbonyl]amino-1-(methoxycarbonyl)-3-(4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl)propyl]-1,2-triazadien-2-ium, Cbz-L-Glu(γ-N_3)-(OMe)-OBO ester, 4.106.

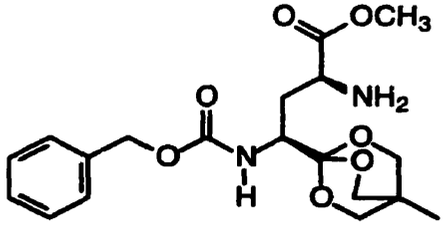
Cbz-L-Glu(OMe)-OBO ester **4.69** (0.600 g, 1.58 mmol) was dissolved in dry THF (5 mL) then cooled to –78°C whilst stirring under Ar. In a second flask, LiHMDS (4.74 mL, 4.74 mmol, 1.0M in THF) was added to dry THF (15 mL) then cooled to –78°C whilst stirring under Ar. The Cbz-L-Glu(OMe)-OBO ester **4.69** was then transferred dropwise to the second flask via cannula. The mixture was allowed to stir at –78°C for 1 hour before trisyl azide **4.105** (0.825 g, 3.16 mmol) was added, pre-dissolved in dry THF (10 mL), by syringe. The mixture was allowed to stir for 6 hours at –78°C before being poured into 3% NH_4Cl (20 mL) and extracted with Et_2O (100 mL). The organic layer was then extracted with 3% NH_4Cl (20 mL), saturated $NaHCO_3$ (20 mL), brine (20 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* to reveal a yellow oil which was further purified by flash chromatography (1:1 EtOAc:Hex, 0.5% Et_3N) to give a clear oil in 54% yield (0.081 g).



TLC (1:1, EtOAc:Hex), R_f = 0.40; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.38-7.26 (m, 5H, Ar*H*), 5.12-5.02 (m, 2H, CbzCH_2O), 4.92 (d, 1H, *J* = 10.1Hz, N*H*), 4.08 (dt, 1H, *J* = 2.9, 10.1 Hz, α-C*H*), 3.96 (dd, 1H, *J* = 3.7, 10.7 Hz, γ-C*H*), 3.85 (s, 6H, OBO ester CH_2O), 3.71 (s, 3H, CO_2CH_3), 2.08 (ddd, 1H, *J* = 2.9, 10.9, 14.5Hz, β-C*H*H), 1.85 (ddd, 1H, *J* =

3.7, 10.7, 14.5Hz, β-CH*H*), 0.76 (s, 3H, OBO ester CC*H*$_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.1 (C=O), 156.4 (CONH), 136.3 (Cbz=C=), 128.5, 128.2, 128.1 (Cbz=CH=), 108.0 (OBO ester C-O), 72.7 (OBO ester O*C*H$_2$), 67.0 (CbzCH$_2$O), 59.3 (γ-CH), 52.6 (α-CH), 52.1 (CO_2CH_3), 32.2 (OBO ester CCH_3), 30.5 (β-CH_2), 14.2 (OBO ester CCH_3); IR (cast from $CHCl_3$) 3432, 2954, 2883, 2121, 1727, 1515, 1227, 1048, 1016, 909; ESI-MS (M + H^+) 421.03. Anal. calcd for $C_{19}H_{25}N_4O_7$: C, 54.28; H, 5.75; N, 13.33. Found: C, 54.48; H, 5.98; N, 13.36.

4.4.37 Methyl-(2*S*,4*S*)-4-amino-2-[(benzyloxy)carbonyl]amino-1-(4-methyl-2,6,7-trioxabicyclo-[2.2.2]oct-1-yl)butanoate, Cbz-L-Glu(γ-NH$_2$)(OMe)-OBO ester, 4.107. Cbz-L-Glu(γ-N_3)(OMe)-OBO ester **4.106** (0.032 g, 0.07 mmol) was combined with PPh_3 (24 mg, 0.09 mmol) and dissolved in dry THF (50 mL) then stirred at ambient temperature for 24 hours. Distilled water (15 μl) was then added and the mixture refluxed for 3 hours. The solvent was then removed under reduced pressure to give a white solid which was purified by flash chromatography to give the desired product in 76% yield (21 mg).

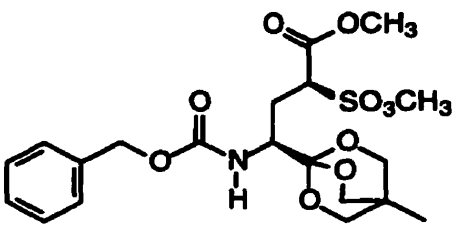


TLC (9:1, EtOAc:MeOH:0.5 % NH_4OH). R_f = 0.60; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.36-7.21 (m, 5H, Ar*H*), 5.78 (d, 1H, *J* = 10.4Hz, N*H*), 5.06 (d, 1H, *J* = 12.7Hz, CbzC*H*HO), 4.96 (d, 1H, *J* = 12.7Hz, CbzCH*H*O), 4.11 (br dd, 1H, *J* = 3.4, 10.7Hz, γ-

C*H*), 3.85 (s, 6H, OBO ester CH_2O), 3.68 (ddd, 1H, J = 2.9, 10.4, 12.2Hz, α-C*H*), 3.57 (s, 3H, CO_2CH_3), 2.86 (br s, 2H, γ-NH_2), 2.24 (ddd, 1H, J = 2.4, 10.7, 13.7Hz, β-C*H*H), 1.88 (ddd, 1H, J = 3.4, 12.2, 13.7Hz, β-CH*H*), 0.76 (s, 3H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 172.0 (C=O), 156.1 (CONH), 137.7 (Cbz=C=), 128.4, 127.7, 127.7 (Cbz=CH=), 108.5 (OBO ester C-O), 72.3 (OBO ester CH_2O), 65.6 (CbzCH_2O), 59.5 (γ-CH), 51.8 (α-CH), 51.1 (CO_2CH_3), 33.0 (OBO ester CCH_3), 30.3 (β-CH_2), 13.4 (OBO ester CCH_3); IR (cast from $CHCl_3$) 3374, 2949, 2880, 1730, 1516, 1224, 1046, 1011; ESI-MS (M + H^+) 394.90. Anal. calcd for $C_{19}H_{26}N_2O_7$: C, 57.86; H, 6.64; N, 7.10. Found: C, 58.08; H, 6.75; N, 7.12.

4.4.38 Methyl-(2*S*,4*S*)-2-[(benzyloxy)carbonyl]amino-4-[(methylsulfonyl)oxy]-1-(4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl)pentanoate, Cbz-L-Glu(γ-OMs)(OMe)OBO ester, 4.108.

Cbz-L-Glu(γ-OH)(OMe)OBO ester **4.96** was dissolved in dry CH_2Cl_2 (5 mL) then cooled to 0°C. Et_3N (57 μL, 0.42 mmol) was then added followed by mesyl chloride (16 μL, 0.21 mmol). The mixture was allow to stir for 12 h before the solvent was removed *in vacuo* after which the desired product could be purified by flash chromatography to give **4.108** in 80% yield (76 mg). However, the mesylate was generally displaced by azide without isolation.

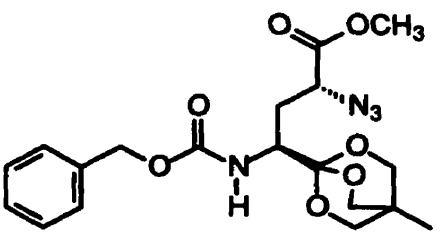


TLC (1:1, EtOAc:Hex), $R_f = 0.35$; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.38-7.24 (m, 5H, Ar*H*), 6.22 (d, 1H, *J* = 10.3Hz, N*H*), 5.10 (d, 1H, *J* = 12.7Hz, CbzC*H*HO), 5.03 (d, 1H, *J* = 12.7Hz, CbzCH*H*O), 4.83 (dd, 1H, *J* = 2.9, 11.7Hz, γ-C*H*), 3.96 (ddd, 1H, *J* = 2.4, 10.3, 11.7Hz, α-C*H*), 3.88 (s, 6H, OBO ester OCH_2), 3.71 (s, 3H, CO_2CH_3), 2.19 (ddd, 1H, *J* = 2.9, 11.7, 15.1Hz, β-C*H*H), 1.89 (ddd, 1H, *J* = 2.4, 11.7, 15.1Hz, β-CH*H*), 0.76 (s, 3H, OBO ester CCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.0 (C=O), 157.1 (CONH), 137.7 (Cbz=C=), 128.5, 128.1, 128.1 (Cbz=CH=), 108.3 (OBO ester C-O), 75.4 (γ-CH) 72.7 (OBO ester CH_2O) 66.4 ($CbzCH_2O$), 52.4 (α-CH), 51.8 (CO_2CH_3), 38.4 (SO_3CH_3), 33.1 (β-CH_2), 30.7 (OBO ester CCH_3), 14.0 (OBO ester CCH_3); ESI-MS (M + H^+) 474.21.

4.4.39 1-[(1*R*,3*S*)-3-[(benzyloxy)carbonyl]amino-1-(methoxycarbonyl)-3-(4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl)propyl]-1,2-triazadien-2-ium, Cbz-L-Glu(γ-N_3)-(OMe)-OBO ester, 4.109.

By displacement of the mesylate 4.108. Cbz-L-Glu(γ-OMs)(OMe)OBO ester **4.108** (0.076 g, 0.16 mmol) was dissolved in dry DMF (5 mL) to which NaN_3 (21 mg, 0.32 mmol) was added and the mixture stirred for 48 h. The solvent was reduced in vacuo and the purified by flash chromatography to give 37 mg (56% yield) of **4.109**.

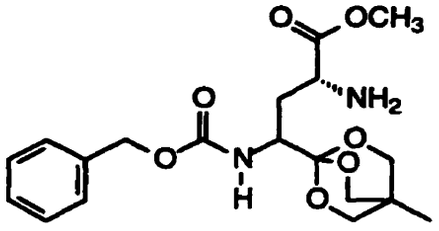
By Mitsunobu reaction. Cbz-L-Glu(γ-OH)(OMe)OBO ester **4.96** (0.100 g, 0.25 mmol) and Ph_3P (0.098 g, 0.38 mmol) were combined and dissolved in dry THF (5 mL) and stirred at 0°C under Ar. DEAD (60 μL, 0.38 mmol) was added dropwise followed by HN_3 (0.97 mL, 0.75 mmol). The reaction was allowed to warm to room temperature and stir for 18 h before a TLC indicated the reaction complete. The solvent was removed under reduced pressure with NaOH trapping the residual HN_3. The oily residue could be purified by flash chromatography (1:1 EtOAc:hexanes) to give pure **4.109** (26 mg, 26% yield) with the remainder contaminated with diethyl amidodicarboxylate.



TLC (1:1, EtOAc:Hex), $R_f = 0.52$; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.38-7.23 (m, 5H, Ar*H*), 5.88 (d, 1H, *J* = 9.7Hz, N*H*), 5.08 (d, 1H, CbzC*H*HO), 5.00 (d, 1H, CbzCH*H*O), 4.18 (br dd, 1H, *J* = 6.3, 6.9Hz, N*H*), 3.97 (ddd, 1H, *J* = 4.4, 9.3, 9.7Hz, α-C*H*), 3.87 (s, 6H, OBO ester CH_2O), 3.69 (s, 3H, CO_2CH_3), 2.08 (ddd, 1H, *J* = 4.4, 6.9, 14.1Hz, β-C*H*H), 1.80 (ddd, 1H, *J* = 6.3, 9.3, 14.1Hz, β-CH*H*), 0.76 (s, 3H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 170.8 (C=O), 156.3 (CONH), 137.8 (Cbz=C=), 128.6, 128.0, 127.9 (Cbz=CH=), 108.5 (OBO ester C-O), 72.7 (OBO ester OCH_2), 66.1 (CbzCH_2O), 59.9 (γ-CH), 52.5 (α-CH), 52.3 (CO_2CH_3), 32.2 (β-CH_2), 30.7 (OBO ester CCH_3), 13.6 (OBO ester CCH_3); IR (cast from $CHCl_3$) 3432, 2954, 2883, 2121, 1727,

1515, 1227, 1048, 1016, 909; ESI-MS (M + H^+) 421.12. Anal. calcd for $C_{19}H_{25}N_4O_7$: C, 54.28; H, 5.75; N, 13.33. Found: C, 54.41; H, 5.93; N, 13.39.

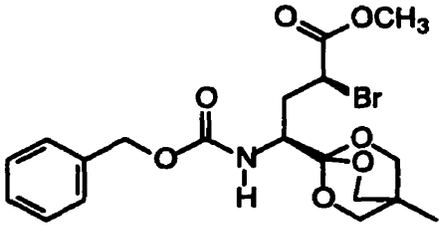
4.4.40 Methyl-(2*S*,4*R*)-4-amino-2-[(benzyloxy)carbonyl]amino-1-(4-methyl-2,6,7-trioxabicyclo-[2.2.2]oct-1-yl)pentanoate, Cbz-L-Glu(γ-NH_2)(OMe)-OBO ester, 4.110.



As described in 4.4.36. TLC (9:1, EtOAc:MeOH:0.5 % NH_4OH), R_f = 0.57; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.39-7.28 (m, 5H, Ar*H*), 6.85 (d, 1H, *J* = 8.9Hz, N*H*), 5.07 (s, 2H, CbzCH_2O), 4.22 (m, 1H, γ-C*H*), 3.86 (s, 6H, OBO ester CH_2O), 3.68-3.55 (m + s, 4H, α-C*H*, CO_2CH_3), 2.82 (br s, 2H, γ-NH_2), 2.54-2.46 (m, 1H, β-CH*H*), 2.30-2.22 (m, 1H, β-C*H*H), 0.79 (s, 3H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 171.8 (C=O), 156.0 (CONH), 137.5 (Cbz=C=), 128.4, 127.7, 127.7 (Cbz=CH=), 108.4 (OBO ester C-O), 72.2 (OBO ester OCH_2), 65.3 (CbzCH_2O), 59.9 (γ-CH), 51.7 (α-CH), 51.1 (CO_2CH_3), 33.0 (OBO ester CCH_3), 29.9 (β-CH_2), 13.4 (OBO ester CCH_3); ESI-MS (M + H^+) 394.89. Anal. calcd for $C_{19}H_{26}N_2O_7$: C, 57.86; H, 6.64; N, 7.10. Found : C, 58.16; H, 6.81; N, 7.21.

4.4.41 Methyl-(2*S*,4*S*)-2-[(benzyloxy)carbonyl]amino-4-bromo-1-(4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl)pentanoate, Cbz-L-Glu(γ-Br)(OMe)-OBO ester, 4.112.

Cbz-L-Glu(OMe)-OBO ester **4.69** (0.179 g, 0.47 mmol) was dissolved in dry THF (10 mL) then cooled to –78°C whilst stirring under Ar. In a second flask, LiHMDS (1.42 mL, 1.42 mmol, 1.0M in THF) was added to dry THF (15 mL) then cooled to –78°C whilst stirring under Ar. The Cbz-L-Glu(OMe)-OBO ester **4.69** was then transferred dropwise to the second flask via cannula. The mixture was allowed to stir at –78°C for 1 hour. *N*-bromosuccinimide (0.092 g, 0.52 mmol) was suspended in THF (10 mL) in a third flask and cooled to –78°C. The enolate was then added quickly by cannula to give a deep purple coloured mixture which was then stirred for approximately 6 hours at –78°C before being poured into Et_2O (70 mL) and extracted with 3% NH_4Cl (20 mL), 10% sodium sulfite (20 mL), saturated $NaHCO_3$ (20 mL), brine (20 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* to reveal a light yellow oil which was further purified by flash chromatography (1:1 EtOAc:Hex) to give a clear oil in 64% yield (0.137 g).

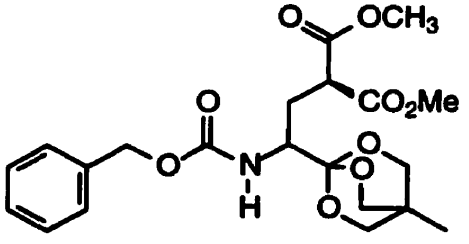


$[\alpha]^{20}_D$ = +21.2 (c = 3.00, CH_2Cl_2); TLC (1:1, EtOAc:Hex), R_f = 0.50; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.34-7.28 (m, 5H, Ar*H*), 5.09-5.04 (m, 2H, CbzCH_2O), 4.79 (d, 1H, *J* = 10.3Hz, N*H*), 4.29 (dd, 1H, *J* = 6.1, 8.5Hz, γ-C*H*), 4.10 (dt, 1H, *J* = 3.5, 10.3Hz, α-C*H*), 3.85 (s, 6H, OBO ester CH_2O), 3.66 (s, 3H, CO_2CH_3), 2.41 (ddd, 1H, *J* = 3.6, 8.6, 15.0Hz, β-C*H*H), 2.18 (ddd, 1H, *J* = 3.6, 6.1, 15.0Hz, β-CH*H*), 0.77 (s, 3H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 170.8 (C=O), 156.4 (CONH), 136.3

(Cbz=C=), 128.5, 128.2, 128.1 (Cbz=CH=), 107.9 (OBO ester C-O), 72.7 (OBO ester CH_2O), 67.0 (CbzCH_2O), 53.4 (α-CH), 52.8 (CO_2CH_3), 42.3 (γ-CH), 35.6 (OBO ester CCH_3), 30.6 (β-CH_2), 14.2 (OBO ester CCH_3); ESI-MS (M + H^+) 457.89, 459.90. Anal. calcd for $C_{19}H_{24}BrNO_7$: C, 49.79; H, 5.28; N, 3.06. Found: C, 50.12; H, 5.48; N, 3.10.

4.4.42 Dimethyl-2-[(2*S*)-2-[(benzyloxy)carbonyl]amino-2-(4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl)ethyl]malonate, Cbz-L-Glu(γ-COOMe)(OMe)-OBO, 4.113.

Cbz-L-Glu(OMe)-OBO ester **4.69** (0.140 g, 0.37 mmol) was dissolved in a flask in dry THF (5 mL) and then transferred via cannula to a second flask containing LiHMDS (1.11 mL, 1.11 mmol) in THF (5 mL) at –78°C under Ar. After 1 hour, methyl cyanoformate (35 μL, 0.44 mmol) was added by syringe. After 2 hours the reaction mixture was poured into Et_2O (80 mL) and extracted with 3% NH_4Cl (20 mL), saturated $NaHCO_3$ (20 mL), brine (20 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* to reveal a light oil which was further purified by flash chromatography (1:1 EtOAc:Hex, 0.5% Et_3N) to give a clear oil in 68% yield (0.110 g).

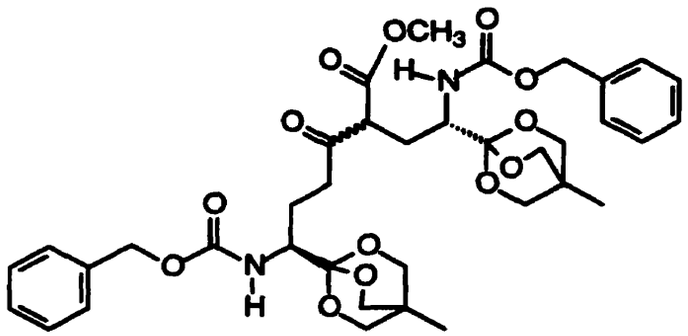


$[\alpha]^{20}_D$ = -24.4 (c = 1.2, CH_2Cl_2); TLC (1:1, EtOAc:Hex), R_f = 0.46; ^1H NMR (Acetone-d_6, 300 MHz) δ 7.40-7.27 (m, 5H, Ar*H*), 5.78 (d, 1H, *J* = 10.2Hz, N*H*), 5.10 (d, 1H, *J* = 12.6Hz, CbzC*H*HO), 5.00 (d, 1H, *J* = 12.6Hz, CbzCH*H*O), 3.92-3.86 (s + m, 7H, OBO ester CH_2O, α-C*H*), 3.65 (s, 3H, CO_2CH_3), 3.63 (s, 3H, CO_2CH_3), 3.50 (dd, 1 H, *J* = 5.6,

9.6Hz, γ-C*H*) 2.36 (ddd, 1H, *J* = 3.8, 9.6, 14.3Hz, β-C*H*H), 1.94 (ddd, 1H, *J* = 5.6, 11.3, 14.3Hz, β-CH*H*), 0.81 (s, 3H, OBO ester C*CH*$_3$); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 169.8 (C=O), 169.1 (C=O), 156.3 (CONH), 137.5 (Cbz=C=), 128.3, 127.7, 127.7 (Cbz=CH=), 108.1 (OBO ester C-O), 72.4 (OBO ester CH_2O), 65.8 (CbzCH_2O) 53.2 (α-CH), 51.9 (CO_2CH_3), 51.8 (CO_2CH_3), 48.2 (γ-CH), 30.3 (OBO ester CCH_3), 28.3 (β-CH_2), 13.3 (OBO ester CCH_3); IR (cast from $CHCl_3$) 2954, 2881, 1734, 1522, 1438, 1241, 1047, 1014; HRMS (FAB) calcd for (M + H^+) $C_{21}H_{28}NO_9$ 438.17639, found 438.17564. Anal. calcd for $C_{21}H_{27}NO_9$: C, 57.66; H, 6.22; N, 3.37. Found : C, 57.83; H, 6.29; N, 3.37.

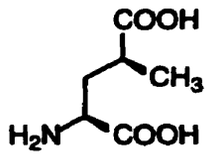
4.4.43 Methyl-(2*S*)-2-[(benzyloxy)carbonyl]amino-6-[(2*S*)-2-[(benzyloxy)carbonyl]-amino-2-(4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl)ethyl]-1-(4-methyl-2,6,7-trioxa-bicyclo[2.2.2]oct-1-yl)-5-oxoseptanoate, Cbz-L-Glu(Cbz-L-Glu(keto)OBO ester)-(OMe)-OBO ester 4.117.

Cbz-L-Glu(OMe)OBO ester **4.69** (0.110 g, 0.29 mmol) was dissolved in dry THF (2 mL) and cooled to –30°C under Ar. LiHMDS (0.43 mL, 0.43 mmol) was then added by syringe. After 12 h at –30°C the mixture was poured into Et_2O (25 mL) and extracted with 3% NH_4Cl (5 mL), 10% $NaHCO_3$ (5 mL), brine (5 mL) then dried over $MgSO_4$. The solvent was removed under reduced pressure to reveal a light yellow oil which was purified by flash chromatography to give **4.117** in 48% yield (51 mg) as a mixture of approximately 1:1 6*R*,*S*-diastereomers.



TLC (1:1, EtOAc:Hex), $R_f = 0.17$; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.43-7.25 (m, 10H, Ar*H*), 5.75-5.68 (br m, 2H, 2 N*H*), 5.08-5.02 (m, 4H, 2 CbzCH_2O), 3.94-3.86 (s + m, 13H, OBO ester CH_2O, COC*H*CO_2CH_3), 3.78-3.64 (m, 2H, 2 α-C*H*), 3.62 (s, 1.65H, CO_2CH_3), 3.60 (s, 1.35H, CO_2CH_3), 2.67-2.60 (m, 2H, CH_2CO), 2.43-2.36 (m, 1H, β-C*H*HCH), 2.34-2.26 (m, 1H, β-CH*H*CH), 1.92-1.78 (m, 1H, β-C*H*HCH_2), 1.92-1.78 (m, 1H, β-CH*H*CH_2), 0.80 (s, 6H, 2 OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 125 MHz) δ 204.7, 204.2 (C=O), 170.2, 169.8 (C=O), 156.8, 156.8, 156.6, 156.6 (CONH), 137.9, 137.9, 137.8, 137.7 (Cbz=C=), 128.7, 128.7, 128.2, 128.1 128.0, 128.0, 127.9 (Cbz=CH=), 108.5, 108.5, 108.4 (OBO ester C-O), 72.6, 72.6 (OBO ester OCH_2), 66.3, 66.2, 66.1, 66.0 (CbzCH_2O), 55.4, 54.7, (α-CH), 54.7, 53.6 (COCHCO_2CH_3), 52.0, 51.9 (CO_2CH_3), 39.4, 38.5 (CH_2CO), 30.6, 30.6 (OBO ester CCH_3), 28.7, 28.5 (β-CH_2CH), 24.2, 24.1 (β-CH_2CH_2), 13.7, 13.6, 13.6 (OBO ester CCH_3); ESI-MS (M + H^+) 727.27; Anal. calcd for $C_{37}H_{46}N_2O_{13}$: C, 61.15; H, 6.38; N, 3.85. Found : C, 61.42; H, 6.72; N, 3.99.

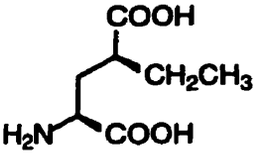
4.4.44 (2*S*,4*S*)-2-Amino-4-methylpentanedioic acid, (2S,4S)-Methylglutamic acid, 4.1.



See General Procedure for Removal of Protecting Groups.

$[\alpha]^{20}_D$ = +34.0 (c = 0.71, 6 N HCl), (lit.[57] $[\alpha]^{20}_D$ = +34.7 (c = 0.79, 6 N HCl)); ^{1}H NMR (D_2O, 300 MHz) δ 3.84-3.76 (m, 1H, α-C*H*), 2.70-2.61 (m, 1H, γ-C*H*), 2.12-2.01 (m, 1H, β-C*H*H), 1.93-1.84 (m, 1H, β-CH*H*), 1.12 (t, 3H, *J* = 6.8 Hz, β-CH_3); ^{13}C NMR (D_2O, 75 MHz) δ 183.5 (C=O), 175.0 (C=O), 55.0 (α-CH), 39.1 (β-CH_2), 36.6 (γ-CH), 19.6 (γ-CH_3); ESI-MS (M + H^+) 161.91. Anal. calcd for $C_6H_{12}NO_4Cl$: C, 36.47; H, 6.12; N, 7.09. Found: C, 36.72; H, 6.32; N, 7.19.

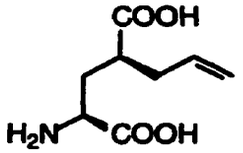
4.4.45 (2*S*,4*S*)-2-Amino-4-ethylpentanedioic acid, (2S,4S)-Ethylglutamic acid, 4.3.



See General Procedure for Removal of Protecting Groups.

$[\alpha]^{20}_D$ = +23.0 (c = 0.78, 6N HCl) (lit.[57] $[\alpha]^{20}_D$ = +28.4 (c = 1.0, 6N HCl)); TLC (1:1:1:1, EtOAc:H_2O:nBuOH:AcOH), R_f = 0.75; ^{1}H NMR (D_2O, 300 MHz) δ 4.08-3.84 (m, 1H, α-C*H*), 2.62-2.44 (m, 1H, γ-C*H*), 2.12-2.00 (m, 1H, β-C*H*H), 1.98-1.85 (m, 1H, β-CH*H*) 1.58-1.45 (m, 2H, CH_2CH$_3$), 0.77 (t, 3H, *J* = 7.3Hz, CH_2CH_3); ^{13}C NMR (D_2O, 75 MHz) δ 183.8 (C=O), 175.1 (C=O), 54.0 (α-CH), 47.5 (γ-CH), 33.7 (β-CH_2), 25.4 (CH_2CH_3), 10.9 (CH_2CH_3); ESI-MS (M + H^+) 176.30. Anal. calcd for $C_7H_{14}NO_4Cl$: C, 39.73; H, 6.67; N, 6.62. Found: C, 40.11; H, 6.99; N, 6.75.

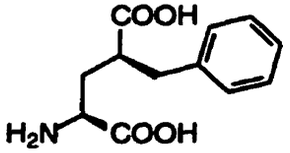
4.4.46 (2*S*,4*S*)-2-Allyl-4-aminopentanedioic acid, (2S,4S)-Allylglutamic acid, 4.81.



See General Procedure for Removal of Protecting Groups using 1N HCl.

$[\alpha]^{20}_D$ = +36.7 (c = 0.65, 6N HCl); ^{1}H NMR (D_2O, 300 MHz) δ 5.72-5.56 (m, 1H, C*H*=CH_2), 5.05-4.96 (m, 2H, CH=C*H*$_2$), 4.07-3.95 (m, 1H, α-C*H*), 2.74-2.63 (m, 2H, β-C*H*H, γ-C*H*) 2.55-2.44 (m, 1H, β-CH*H*), 2.14-1.88 (m, 2H, C*H*$_2$CH=CH_2); ^{13}C NMR (D_2O, 75 MHz) δ 182.8 (C=O), 173.5 (C=O), 133.2 (CH=CH_2), 119.1 (CH=CH_2), 54.1 (α-CH), 41.0 (γ-CH), 35.4 (β-CH_2), 31.0 (CH_2CH=CH_2); ESI-MS (M + H^+) 187.85. Anal. calcd for $C_8H_{14}NO_4Cl$: C, 42.96; H, 6.31; N, 6.26. Found: C, 43.29; H, 6.64; N, 6.35.

4.4.47 (2*S*,4*S*)-2-Amino-4-benzylpentanedioic acid, (2S,4S)-Benzylglutamic acid, 4.82.

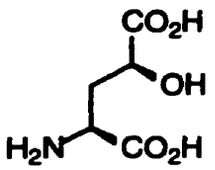


See General Procedure for Removal of Protecting Groups.

$[\alpha]^{20}_D$ = +24.4 (c = 0.55, 6N HCl); ^{1}H NMR (D_2O, 300 MHz) δ 7.28-7.10 (m, 5H, Ar*H*), 3.85 (dd, 1H, *J* = 5.2, 9.0 Hz, α-C*H*), 2.98-2.80 (m, 3H, γ-C*H*, ArC*H*$_2$), 2.05 (ddd, 1H, *J* = 5.2, 9.8, 14.2 Hz, β-C*H*H) 1.93 (ddd, 1H, *J* = 4.0, 9.0, 14.2 Hz, β-CH*H*); ^{13}C NMR (D_2O, 75 MHz) δ 178.1 (C=O), 171.7 (C=O), 137.9 (Ar=C=), 129.1, 128.8, 126.8 (Ar=CH=), 51.4 (α-CH), 43.5 (γ-CH), 38.1 (ArCH_2), 31.5 (β-CH_2); ESI-MS (M + H^+)

238.3; Anal. calcd for $C_{12}H_{16}NO_4Cl$: C, 52.66; H, 5.89; N, 5.12. Found: C, 52.90; H, 6.02; N, 5.14.

4.4.48 (2*S*,4*S*)-2-Amino-4-hydroxypentanedioic acid, (2S,4S)-Hydroxyglutamic acid, 4.4.



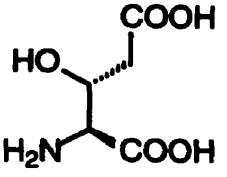
See General Procedure for Removal of Protecting Groups.

$[\alpha]^{20}_D$ = +60.3 (c = 0.9, 6N HCl) (lit.[26] $[\alpha]^{20}_D$ = +61 (c = 1, 5N HCl); ^{1}H NMR (D_2O, 300 MHz) δ 4.03 (dd, 1H, *J* = 4.4, 7.8 Hz, γ-C*H*), 3.73 (dd, 1H, *J* = 3.4, 7.9 Hz, α-C*H*), 2.14-2.00 (m, 2H, β-CH_2); ^{13}C NMR (D_2O, 75 MHz) δ 182.6 (C=O), 178.7 (C=O), 69.9 (γ-CH), 51.8 (α-CH), 39.5 (β-CH_2); ESI-MS (M + H^+) 163.87. Anal. calcd for $C_5H_{10}NO_5Cl$: C, 30.09; H, 5.05; N, 7.02. Found: C, 30.41; H, 5.35; N, 7.24.

4.4.49 (2*S*,3*R*)-2-amino-3-hydroxypentanedioic acid, 4.7.

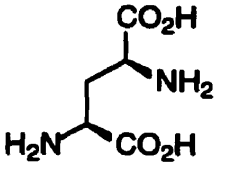
Cbz-L-Glu(β-OH)(O*t*Bu)OBO ester **4.104** (0.098 g, 0.22 mmol) was stirred in fresh TMSI (500 μL, 3.5 mmol) at 75°C for 24 h. After cooling, Et_2O (3 mL) was slowly added followed by the dropwise addition of 0.5N NaOH (5 mL). The organic layer was carefully removed by pipette and washed with 0.5N NaOH (2 × 3 mL). The aqueous fractions were combined, washed with Et_2O (2 × 5 mL) then acidified to pH<3 with 2N HCl. The sample was purified by cation exchange as described in section 2.4.15 to give 0.029 g (82%) of the monoammonium salt which was recrystallized from EtOH/H_2O to

give 0.020 g (54%) of the monoammonium salt. Derivatization and HPLC analysis as described in section 2.4.15a showed a 93:7 ratio of *threo*:*erythro* L-β-hydroxyglutamic acid with 99% ee (retention times: *threo*-L-**4.7** (22.1 min), *threo*-D-**4.7** (22.9 min), *erythro*-L-**4.7** (26.9 min), *erythro*-D-**4.7** (33.1 min).



mp 202-205°C (dec); (lit.[67a] mp 205-206°C (dec)), (lit.[6] mp 193°C (dec)); ^{1}H NMR (300 MHz, D_2O) δ 4.20-4.42 (m, 1H, β-C*H*), 3.91 (d, 0.07H, *J* = 3.4Hz, *erythro* α-C*H*), 3.73 (d, 0.93H, *J* = 3.4Hz, *threo* α-C*H*), 2.62 (dd, 1H, *J* = 4.7, 15.4Hz, γ-C*H*H), 2.47 (dd, 1H, *J* = 8.7, 15.4Hz, γ-CH*H*), ESI-MS ($M + H^+$) 164.22.

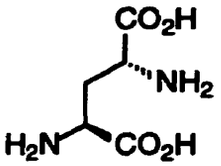
4.4.50 (2*S*,4*S*)-2,4-diaminopentanedioic acid, (2S,4S)-Aminoglutamic acid, 4.11.



See General Procedure for Removal of Protecting Groups.

$[\alpha]^{20}_D$ = +36.3 (c = 0.4, 6N HCl); (lit.[61] $[\alpha]^{20}_D$ = +45.4 (c = 1, 1N HCl); ^{1}H NMR (D_2O, 300 MHz) δ 3.90 (t, 2H, *J* = 6.7 Hz, β-CH_2), 2.26 (t, 2H, *J* = 6.7 Hz, α-C*H*, γ-C*H*); ^{13}C NMR (D_2O, 75 MHz) δ 176.0 (C=O), 55.9 (α-CH, γ-CH), 34.3 (β-CH_2); ESI-MS 163.21. Anal. calcd for $C_5H_{12}N_2O_4Cl_2$: C, 25.55; H, 5.15; N, 11.92. Found : C, 25.83; H, 5.42; N, 12.15.

4.4.51 (2*R*,4*S*)-2,4-diaminopentanedioic acid, (2S,4*R*)-Aminoglutamic acid, 4.111



See General Procedure for Removal of Protecting Groups.

$[\alpha]^{20}_D = 0$ (c = 0.55, 6N HCl); (lit.[44] $[\alpha]^{20}_D = 0$ (c = 5.83, H_2O); ^1H NMR (D_2O, 300 MHz) δ 3.99 (t, 2H, *J* = 6.9 Hz, α-C*H*, γ-C*H*), 2.46-2.31 (m, 1H, β-C*H*H), 2.12-1.99 (m, 1H, β-CH*H*); ^{13}C NMR (D_2O, 75 MHz) δ 172.0 (C=O) , 53.9, 51.1 (α-CH, γ-CH), 30.3 β-CH_2) ; ESI-MS (M + H^+) 163.27. Anal. calcd for $C_5H_{12}N_2O_4Cl_2$: C, 25.55; H, 5.15; N, 11.92. Found: C, 25.83; H, 5.42; N, 12.15.

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Chapter Five

Stereoselective Synthesis of β-Substituted Aspartic Acids

5.1 Introduction

5.1.1 Isolation

The biological activity and synthetic importance of β-substituted aspartic acids has led to considerable interest in their properties and synthesis. These aspartic acid derivatives have been isolated from a wide variety of sources including plants, bacteria and humans. β-Methylaspartic acid **5.1** has been isolated from *Clostridium tetanomorphum* and as a by-product of glutamic acid degradation in humans.[1] β-Methyl aspartic acid has also been identified as a constituent of numerous biological molecules including aspartocin,[2] amphomycin,[3] glumamycin[4] and the cyclic peptides nodularin,[5] microcystin-LR,[6] cyanogenosin –LA and –RR[7] and motuporin.[8] Various β-alkylaspartates have been used in the synthesis of numerous biologically active molecules including derivatives of the dipeptide antibiotic alahopcin **5.2**,[9] the β-lactam antibiotic thienamycin **5.3**[10] and the anti-glaucoma agent pilocarpin **5.4**[11] to name a few (Scheme 5.1).

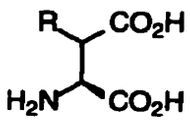
β-Hydroxyaspartic acid **5.5** was first reported as a natural product in the extracellular material of *Azobacter* cultures[12] and has been isolated from numerous plant seeds and extracts,[1] *Streptomyces* cultures,[13] rat liver extracts[14] and human urine.[15] The diastereomers of β-hydroxyaspartic acid are also key intermediates in the Krebs cycle in some microorganisms.[16] This amino acid has been found as a constituent of several blood

clotting cascade proteins[17] and as a constituent of the peptide antibiotic lysobactin.[18] L-*erythro*-β-Hydroxyaspartic acid has been used in the synthesis of β-lactam antibiotics.[19]

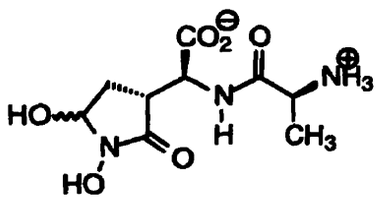
β-Amino aspartic acid **5.6** is less common and has been isolated from *Streptomyces rimosus* cultures. It has been used to construct the core constituent of lactam **5.7** of the streptothricin class of antibiotics[20] and structurally similar biotin.[21] The well-known metal-complexing properties[22] of diaminoacids have resulted in the investigation of various diamino derivatives in the anti-tumor drug *cis*-platin.[23]

Although not naturally occurring, 5-fluoroaspartic acid has been used as an intermediate in the synthesis of 5-fluorouracil.[24]

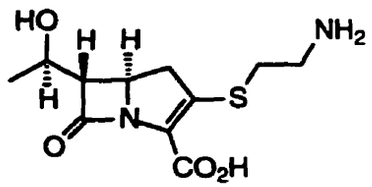
Scheme 5.1



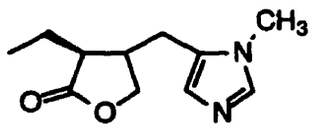
5.1 R=CH_3
5.5 R=OH
5.6 R=NH_2



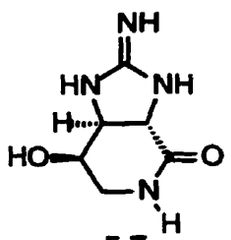
5.2



5.3



5.4



5.7

5.1.2 Biological Activity

Unlike glutamate (Chapter 4), aspartate has no direct role in humans other than a constituent of factor X in humans.[17a] However, derivatized aspartic acids have been used as probes into the mechanistic action of various enzymes and cofactors. The stereochemistry of coenzyme B_{12} isomerization has been investigated in the conversion

of β-methylaspartate **5.1** to glutamate.[25] Molecular biology techniques have been used to replaced aspartic acid with *threo*- or *erythro*-β-methylaspartic acid in the active site of the enzymes dihydrofolate reductase,[26] asparagine synthetase B[27] and HIV-1 protease[28] in order to elucidate the mechanism of action of these enzymes.

Erythro-β-hydroxyaspartate **5.5** has been used as an inhibitor and co-crystallized with aspartate aminotransferase.[29]

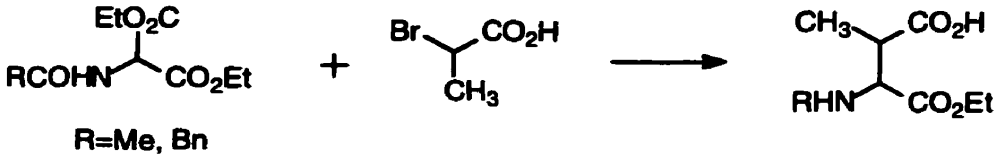
5.1.3 Synthesis of β-Substituted Aspartic Acids

Most of the general synthetic methods described in chapter one can be used to synthesize β-substituted aspartic acids. These include Schöllkopf's bis-lactim ether **1.33**,[30] Williams' oxazinones **1.34**[31] and various expoxide based methods (*vide infra*). However, as described previously, the synthetic challenge arises whilst attempting to stereospecifically introduce β-substituents by 1,2-induction. Although there are exceptions most of the general methods for the synthesis of α-amino acids fail in this regard and therefore specific methodologies have been developed for β-substituted aspartic acids.

5.1.3.1 Synthesis of β-Substituted Aspartic Acids: General Racemic Methods

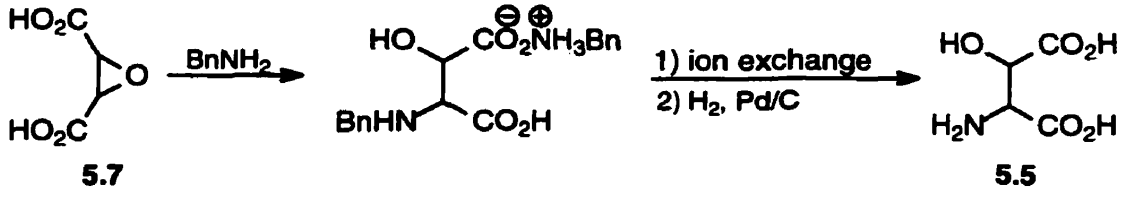
β-Methylaspartic acid **5.1** was first prepared in 1941 by Dakin who condensed ethyl α-bromopropionate with diethyl benzamidomalonate (Scheme 5.2).[32] Subsequent racemic syntheses have also been described using diethyl acetamidomalonate[33] and ethyl acetamidocyanoacetate.[34] Fractional crystallization and ion-exchange chromatography have been used to resolve *threo*- and *erythro*-DL-β-methylaspartate.

Scheme 5.2



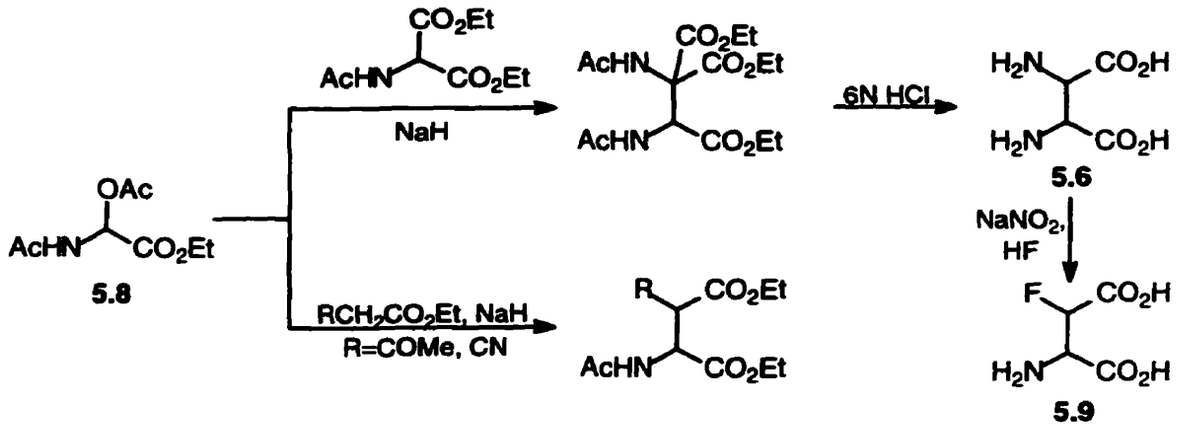
Threo- and *erythro*-β-hydroxy aspartic acid **5.5** were prepared by Kornguth and Sallach in 1960 by the condensation of glyoxylic acid with copper glycinate; the two isomers were then separated by ion-exchange chromatography.[14] Liwschitz *et al.* reported the synthesis of *threo-* and *erythro*-DL-β-hydroxyaspartic acid by the benzylamination and subsequent hydrogenolysis of *cis-* and *trans*-2,3-epoxysuccinic acid **5.7** (Scheme 5.3).[35] A variation of this methodology was later applied to the chiral synthesis of *erythro*-L-β-hydroxyaspartic acid using chiral (-)-*trans*-epoxysuccinic acid **5.7**.[36] *erythro*-DL-β-Hydroxyaspartic acid has also been synthesized from fumaric acid via the ammonolysis of chloromalic acid intermediate.[37]

Scheme 5.3



Racemic β-aminoaspartate **5.6** and various other β-substituted aspartic acids have been synthesized using ethyl 2-acetoxyglycinate **5.8** as a cationic synthon (Scheme 5.4).[38] The same group later synthesized racemic β-fluoroaspartic acid **5.9** after diazotization of **5.6** in the presence of neat HF.[39]

Scheme 5.4



5.1.4 Synthesis of Optically Active β-Substituted Aspartic Acids

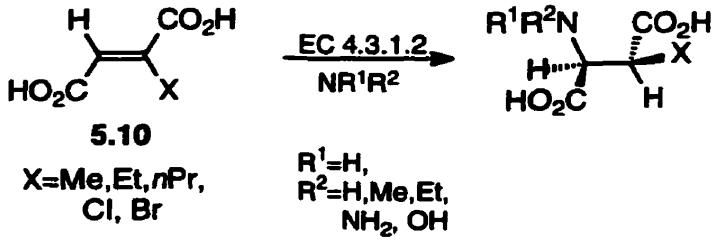
A number of routes exist for the stereoselective synthesis of β-substituted aspartic acids. These can be divided into two main groups: enzymatic methods and β-anion (including the use of β-lactams) methods which will be discussed in detail. Numerous other specific syntheses of desired β-aspartic acid derivatives exist and will be explored briefly.

5.1.4.1 Enzymatic Methods

β-Methylaspartic acid has been isolated from extracts of *Clostridium tetanomorphum* produced by the enzyme 3-methylaspartate ammonia lyase (EC 4.3.1.2) which catalyzes the α,β-elimination of ammonia from 2*S*,3*S*-3-methylaspartic acid to give mesaconic acid. First isolated in 1960,[40] the enzyme has been used in a retro-physiological reaction with various substituted fumaric acid derivatives **5.10** and ammonia to give β-substituted aspartic acids (Scheme 5.5).[41] Yields were generally in the 50-60% range although reactions times were described as fast to very slow. The same

group has also added various substituted amines to **5.10** although yields were typically less than 50%.[42] Monne *et al.* have performed similar experiments with β-methylaspartase in expanding the functionalities that may be incorporated.[43]

Scheme 5.5



5.10

X=Me,Et,*n*Pr, Cl, Br

R^1=H, R^2=H,Me,Et, NH_2, OH

Aspartate aminotransferase (EC 2.6.1.1) has also been used for the synthesis of both *threo*- and *erythro*-L-β-hydroxyaspartic acid from dihydroxyfumarate and cysteine sulfinate.[44] It was necessary to separate the diastereomers by ion exchange chromatography giving 72% of the *erythro* derivative and 26% of *threo*-L-β-hydroxyaspartic acid.

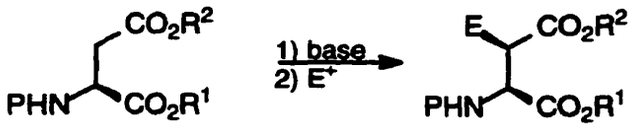
5.1.4.2 β-Anion Chemistry (including β-Lactams)

The obvious, and indeed most popular, method for the stereoselective synthesis of β-substituted aspartic acids is to utilize the inherent chirality of aspartic acid for 1,2-aymmetric induction reactions. This is most easily accomplished through the β-anion of aspartic acid.

Seebach *et al.* first reported the stereoselective alkylation of aspartic acid derivatives in 1981.[45] Di-*t*-butyl-*N*-formyl-aspartate **5.11** (P=CHO, R^1=R^2=*t*Bu) was doubly deprotonated with lithium diethylamide then alkylated with a variety of electrophiles to give a mixture of α- and β-substituted aspartic acid derivatives in a ratio

of 2:7 respectively, both of which were diastereomerically and enantiomerically pure (Scheme 5.6). The predominant β-methyl product was identified as the *anti* or 2*S*,3*R*-3-methylaspartic acid **5.12** (E=Me).

Scheme 5.6



5.11 **5.12**

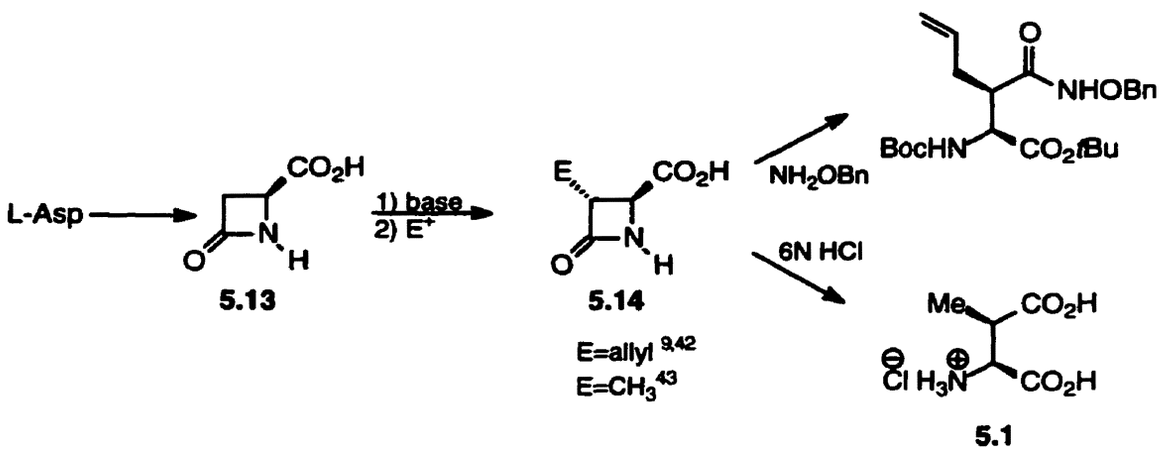
Baldwin, in an attempt to overcome the α-deprotonation experienced by Seebach replaced the *N*-formyl protecting group of aspartic acid with Cbz.[46] However, although no α-addition products were detected, diastereoselectivities of 3:1 to 5:1 were obtained in the reaction of **5.11** (P=Cbz, R^1=*t*Bu, R^2=Me) with benzyl and allylbromide. When methyl iodide was used, a mixture of *N*-methyl and β-methyl products was isolated. The stereochemistry of the addition product was not reported.

Rapoport and coworkers successfully addressed the problems associated with *N*- and α-alkylation by protecting the amine in **5.11** as the PhFl (P=PhFl, R^1=*t*Bu,Me, R^2=Me).[47] As discussed in chapter four, this protecting group insulates the α-center and prevents removal of the α-proton. However, generation of the β-enolate and subsequent addition of various electrophiles resulted in poor diastereoselectivities, ranging from 3:2 (*syn*:*anti*) for the β-methyl derivative to 5:1 (*syn*:*anti*) for the β-isopropyl derivative. In the case of more bulky substitutents, alkyl triflates were used as electrophiles. Careful control of the addition conditions, including type and amount of base and alkylation reagent resulted in an increase in selectivity to 18:1 (*syn*:*anti*) for the addition of ethyl triflate.[48] Rapoport was able to further augment the diastereoselectivity to 30:1 by

increasing the steric bulk on the amine, protecting it as the PhFl/Bn derivative **5.11** (P=PhFl, Bn, R^1=*t*Bu, R^2=Me).[49]

Baldwin and coworkers approached the moderate stereoselectivity previously reported by his group through the use of azetidinone **5.13** (Scheme 5.7).[9,50] Stereospecific "*anti*" allylation was achieved in >95% in 95% yield. The substituted azetidinone **5.14** was opened with *O*-benzylhydroxylamine to give the 2*S*,3*R* derivative *en route* to the synthesis of alahopcin **5.2**.[9] Hanessian *et al.* used the same methodology to synthesize β-methylaspartate **5.1** by hydrolyzing **5.14** (E=Me) with 6N HCl.[51]

Scheme 5.7



The 3-bromo analog of Williams and co-worker's oxazinone **1.34** was condensed with a dibenzyl malonate derivative towards the synthesis of β-carboxyaspartate (Section 1.3.3.2).[52] Hydrogenolysis gave β-carboxyaspartic acid in 30% overall yield with >98% ee.

The first report of the general addition of heteroatoms at the β-position of aspartate was by Sardina *et al.* in 1992.[53] Using Rapoport's PhFl protected aspartic acid derivative **5.11** (P=PhFl, R^1=*t*Bu,Me, R^2=Me), the enolate was quenched with solid MoOPH to give the β-hydroxyaspartate derivative **5.12** (P=PhFl, R^1=*t*Bu,Me, R^2=Me,

E=OH). Changing the reaction conditions and base altered the stereoselectivity from 1:11 *anti*:*syn* (2*S*,3*R*:2*S*,3*S*) to 20:1 *anti*:*syn* (2*S*,3*R*:2*S*,3*S*). The same conditions were used to synthesize the β-azide **5.11** (P=PhFl, R^1=*t*Bu,Me, R^2=Me) using trisyl azide **4.105** and di-*t*-butylazodicarboxylate (DTBAD). Trisyl azide consistently gave a 1:1 mixture of β-epimers whereas the use of DTBAD to trap the enolate generated by LiHMDS in HMPA gave a 30:1 ratio of *anti*:*syn* (2*S*,3*R*:2*S*,3*S*).[54]

Hanessian's group used similar methodology in reacting **5.11** (P=Ts, R^1=R^2=*t*Bu) with Davis' oxaziridine **4.92** to give the β-hydroxyaspartate derivative **5.12** (P=Ts, R^1=R^2=*t*Bu, E=OH) in 45:1 *anti*:*syn* (2*S*,3*R*:2*S*,3*S*), although this methodology suffers from the relatively harsh conditions required for removal of the sulfonamide *N*-protecting group.[55] Hanessian *et al.* also reported the allylation of various protected derivatives of **5.11** (P=Cbz, R^1=Me, TMSE, R^2=Me, TMSE) in generally greater than 95:5 (2*S*,3*R*:2*S*,3S) diastereoselectivity.[56]

Chamberlin and coworkers used the synthon **5.11** (P=PhFl, Bn, R^1=*t*Bu, R^2=Me) to generate both β-alkyl diastereomers as a function of enolate geometry.[57] The potassium enolate gave selectivities of 1:10 *syn*:*anti* (2*R*,3*S*:2*R*,3*R*) whereas lithium enolates gave ratios of 23:1 *syn*:*anti* (2*R*,3*S*:2*R*,3*R*) in the addition of methyl iodide. Chelation and non-chelation controlled addition models were used to describe the observed results.

Parr *et al.* reported in 1999 the first concise study of the numerous factors influencing 1,2-asymmetric induction in dianionic functionalization of L-aspartic acid diesters.[58] This study was based upon earlier findings in which the enolate of **5.11** (P=Cbz, R^1=*t*Bu, R^2=Me) was methylated in poor diastereoselectivity while allylation

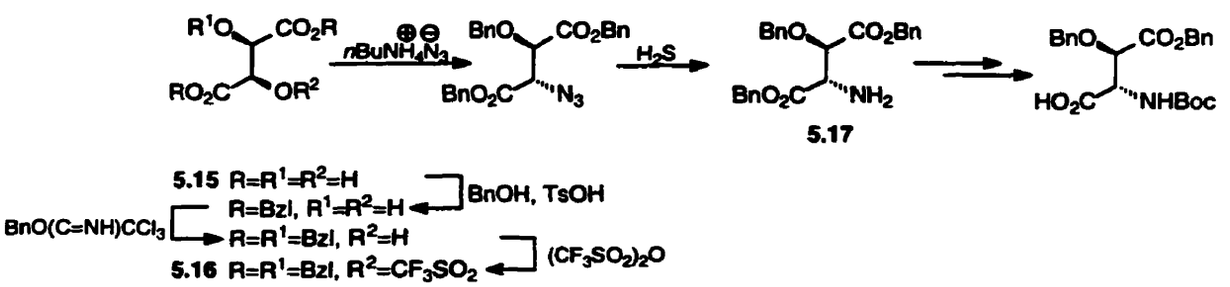
occurred in high diastereoselectivity.[59] The various factors influencing alkylation of **5.11** (P=Cbz, R^1=*t*Bu, R^2=Me) that were studied included temperature, solvent, various additives, concentration, ester protecting group, base and electrophile. The observed preference for *anti* alkylation in most of the previously described syntheses by other groups was rationalized by chelation of the lithium counter-ion in a (*Z*)-lithium ester enolate (discussed in more detail in section 5.2.5).

5.1.4.3 Other Methods for the Synthesis of β-Substituted Aspartic Acids

Many methods exist for the synthesis of specific β-substituted aspartic acids that do not fit into the categories described above.

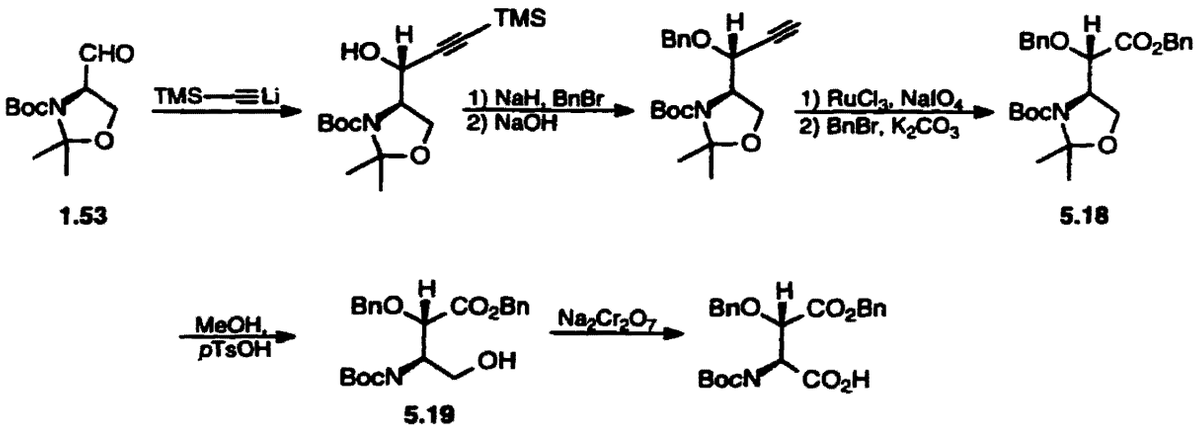
(*R*,*R*)-(+)-Tartrate **5.15** was used to synthesize L-*erythro*-β-hydroxyaspartate (2*S*,3*R*) by selective protection and formation of triflate **5.16** which was then displaced with azide and reduced with H_2S to give amine **5.17**.[60] Subsequent deprotection/reprotection gave the desired product in 15% overall yield (Scheme 5.8). In 1990, Wagner *et al.* reported a modification of the tartrate method described above, which although gave lower yields the authors reported was more amenable to scale-up.[61] The key step was a copper-mediated regioselective saponification of **5.17** and subsequent protection of the amine.

Scheme 5.8



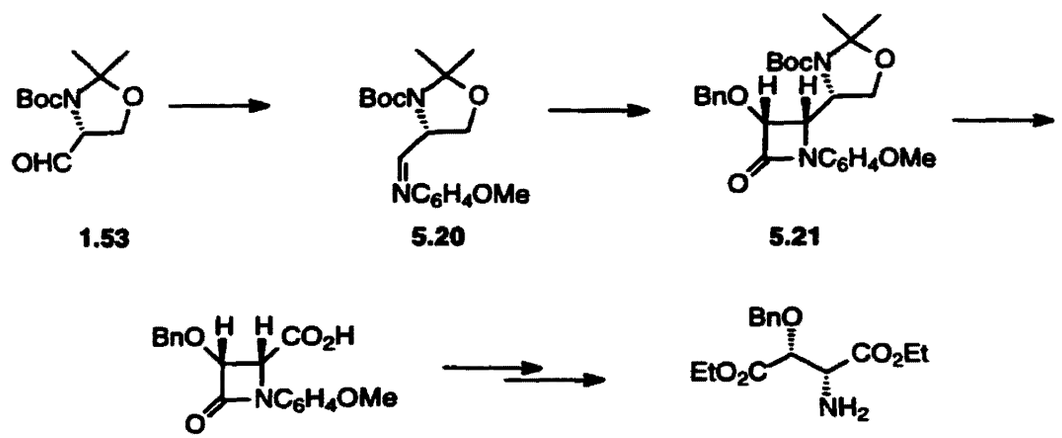
Garner's aldehyde **1.53** was also used to synthesize L-*threo*-β-hydroxyaspartate (2*S*,3*S*) via an acetylenic intermediate which was oxidized to benzyl ester **5.18**.[62] The acetonide was then hydrolyzed to alcohol **5.19** that was oxidized to give the desired product in 12% overall yield (Scheme 5.9).

Scheme 5.9

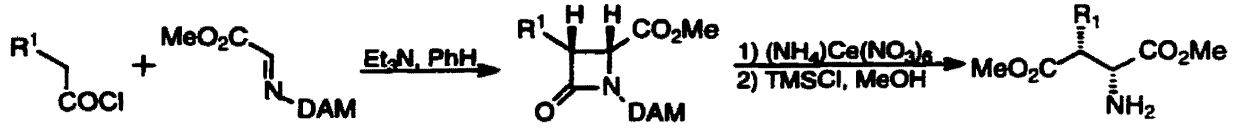


Garner's aldehyde **1.53** has also been used in an asymmetric [2+2] cycloaddition in a five step synthesis of protected 2*R*,3*R*-β-hydroxyaspartate (Scheme 5.10).[63] The same group also reported a short synthesis of a variety of (2*R*,3*R*)-β-alkylated aspartates via β-lactam **5.22** in high diastereoselectivity and good yield (Scheme 5.11).[64]

Scheme 5.10



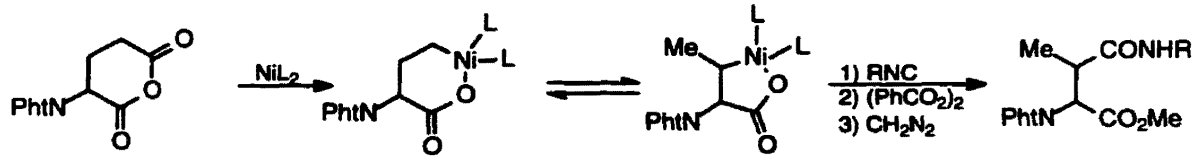
Scheme 5.11



R^1=Me, Et, *i*Pr, Ph, Bn DAM = di-*p*-anisylmethylamine

β-Methylaspartate has been synthesized from *N*-phthaloylglutamic anhydride **5.22** via a six-member nickelacycle **5.23** to give aspartate amides in a 3:1 mixture (2*S*,3*S*:2*S*,3*R*) in 55% overall yield (Scheme 5.12).[65]

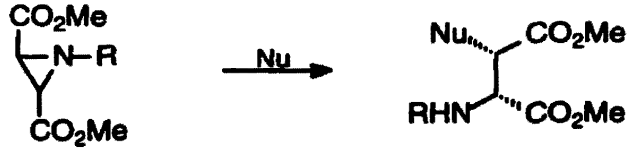
Scheme 5.12



5.22 **5.23**

A variety of β-heteroatom aspartate derivatives were synthesized from the dicarboxylate aziridine **5.24** to give 2*R*,3*S*-β-substituted aspartates in both excellent diastereoselectivity and enantioselectivity and generally high yields (Scheme 5.13).[66]

Scheme 5.13



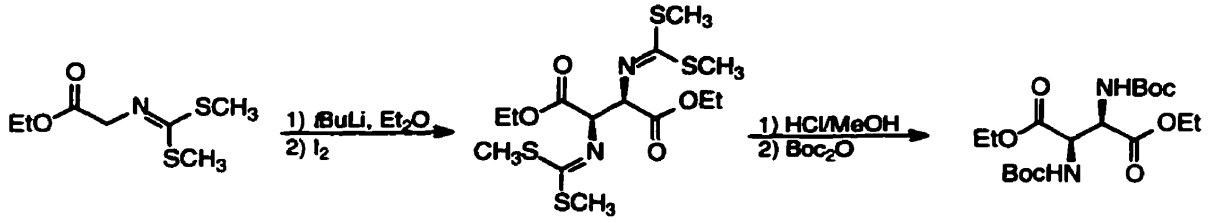
5.24

R=H, CH_3CO, CO_2CH_3, SO_2CH_3

Nu = Cl, I, N_3, BnS, CF_3COO, CH_3O, CH_3COO

Protected β-aminoaspartate **5.6** was synthesized by the oxidative dimerization of glycinate **5.25** in the presence of iodine to give *threo* derivative **5.26**.[67] This was then deprotected and reprotected to give protected β-aminoaspartate in excellent diastereoselectivity, enantioselectivity and yield (Scheme 5.14).

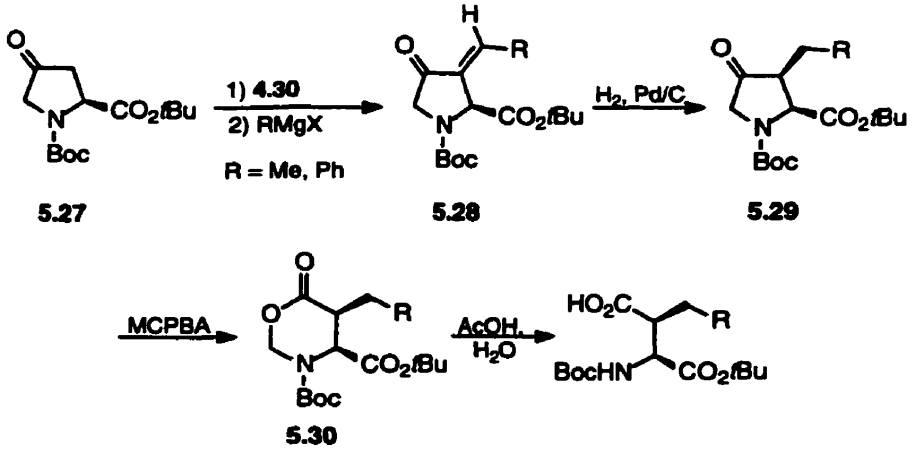
Scheme 5.14



5.25 **5.26**

A variety of β-alkylated aspartate derivatives were synthesized from protected 4-ketoproline **5.27**.[68] Bredereck's reagent **4.30** gave an enaminone which when reacted with a Grignard reagent gave enone **5.28** that was catalytically hydrogenated to give only the *cis*-isomer **5.29** (Scheme 5.15). Baeyer-Villiger oxidation gave **5.30** and after hydrolysis protected β-alkylaspartic acid derivatives were isolated in good yield as single diastereomers. Direct alkylations on the enolate of **5.30** were also attempted, however, diastereoselectivities ranged from 1:1 to 4:1 of the *trans*:*cis* isomers.

Scheme 5.15



5.27 **5.28** **5.29**

5.30

5.2 Results and Discussion

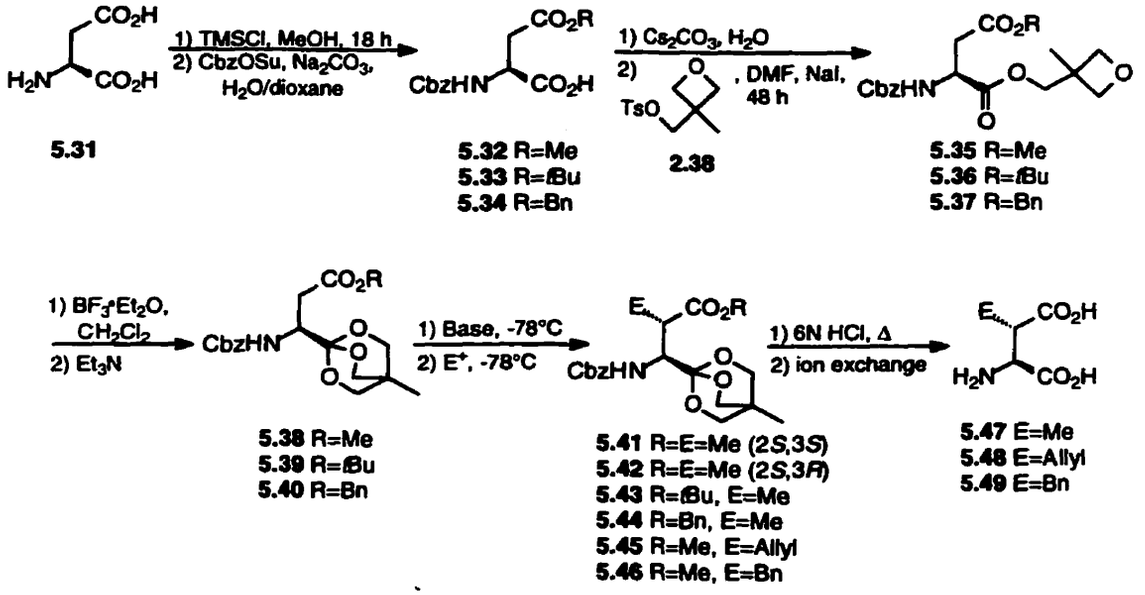
5.2.1 Methylation of Cbz-Asp(OMe)OBO ester 5.38: Optimization and Determination of Diastereoselectivity

While examining the 1,3-asymmetric induction of the addition of various electrophiles to glutamic acid (chapter four) we concurrently investigated the 1,2-induction in aspartic acid **5.31**. Regioselective esterification of **5.31** was achieved under similar conditions to those described in chapter four.[69] TMSCl was added in two separate aliquots to aspartic acid **5.31** stirring in freshly distilled methanol and after 18 hours the solvent was evaporated to give a white solid of the monomethyl ester (Scheme 5.16). N-Protection of the crude product as the Cbz derivative was accomplished with CbzOSu to give **5.32**. Purification was difficult and so the α-carboxylate was esterified with oxetane tosylate **2.38** to give the fully protected aspartic acid derivative **5.35** in 66% overall yield for three steps. Commercially available γ-*t*-butyl ester **5.33** and γ-benzyl ester **5.34** were esterified under identical conditions to give **5.36** and **5.37** in 99% and 97% yields respectively, after column chromatography. All three oxetane esters **5.35-5.37** could be stored for prolonged periods at room temperature without loss of optical activity. Boron trifluoride etherate mediated rearrangement of the oxetane esters **5.35-5.37** gave the ortho esters **5.38-5.40** which were recrystallized from EtOAC:hexanes in 91%, 68% and 78% yields respectively.

At first, both LiHMDS and LDA were used as bases in the generation of enolates, although the use of LDA was later abandoned due to inconsistent results. Baldwin *et al.*[46a] and Seebach *et al.*[45] also observed increased substrate decomposition and racemization with LDA. Three equivalents of base were used, titrated according to the method of Love and Jones,[70] since the use of two equivalents resulted in a significant

amount of unreacted starting material. Methyl iodide was chosen as the initial alkylating agent since the easily identifiable doublet could be integrated by NMR methods to determine diastereoselectivity.

Scheme 5.16



Addition of LiHMDS to **5.38** at –30°C and quenching with methyl iodide disappointingly gave a 1:1 ratio of β-methylaspartate **5.41** diastereomers, based upon ^{1}H NMR integration of the new methyl doublet at δ 1.12 ppm (major) and 1.08 ppm (minor) and the α- and β-protons. All attempts to increase the diastereoselectivity failed until the order of addition was altered. Inverse addition of **5.38** to base at –30°C followed by the addition of methyl iodide gave a 3:1 ratio of diastereomers (Table 5.1, entries 1-3). Cooling to –78°C resulted in an increase of diastereoselectivity to 10:1 in 81% yield after 8 hours (entry 5). However, although a number of other conditions were investigated including the use of other counterions, additives (HMPA and LiCl) and alternative β-ester protecting groups (*t*Bu **5.43**, Bn **5.44**), no further increase in diastereoselectivity was

achieved. The major contaminant was remaining starting material (resulting in a reaction yield of 92% based on starting material) and the reaction failed to go to completion even with prolonged reaction times. Raising the temperature drove the reaction to completion at the expense of diastereoselectivity.

Table 5.1: Optimization of Methylation of Cbz-Asp(OR)OBO ester 5.38-5.40.

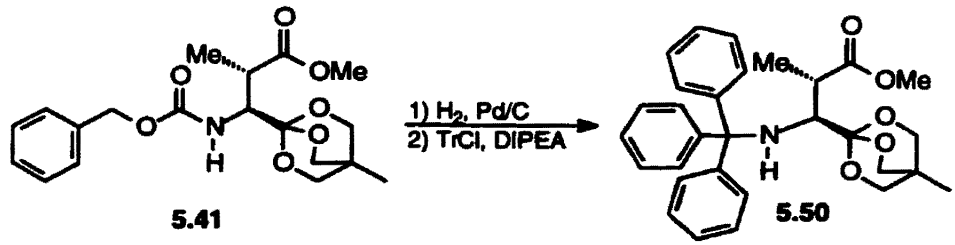
Entry	β-CO_2R R	Base[a]	Temp (°C)[b]	Yield[c] (%)	Ratio[d] 2*S*,3*S*:2*S*,3*R*
1	Me	LDA	-30°C	45	2.5:1
2	Me	LiHMDS	-30°C	63	3:1
3	Me	NaHMDS	-30°C	55	2.7:1
4	Me	KHMDS	-30°C	52	2.5:1
5	Me	LiHMDS	-78°C	81 (92)	10:1
6	Me	LiHMDS[e]	-78°C	55 (94)	10:1
7	Me	LiHMDS[f]	-78°C	49 (85)	9:1
8	Me	NaHMDS	-78°C	61 (86)	9:1
9	Me	KHMDS	-78°C	59 (88)	8:1
10	Me	LiHMDS/ HMPA	-78°C	76	9:1
11	Me	LiHMDS/ LiCl	-78°C	66	8:1
12	*t*Bu	LiHMDS	-78°C	74	10:1
13	Bn	LiHMDS	-78°C	71	10:1

[a] Reaction performed in THF (unless otherwise noted) with 3 eq. of base and inverse addition.
[b] Temperature during addition of alkylating agent.
[c] Value in parenthesis denotes yield based on recovered starting material.
[d] Determined by ^{1}H-NMR.
[e] Two eq. of base
[f] THF/Hexanes (1:1)

In an attempt to rationalize the modest diastereoselectivities we were encountering, we tried to identify the stereochemistry of methylation. Although attempts to crystallize **5.41** failed, conversion to the *N*-trityl derivative **5.50** (Scheme 5.17)

provided crystals of sufficient quality for X-ray analysis which assigned the stereochemistry of addition as 2*S*,3*S* (Figure 5.1). Crystallization also removed the minor diastereomer to give optically pure 2*S*,3*S*-**5.50** in 73% yield. Subsequent deprotection of the purified 2*S*,3*S*-β-methylaspartate derivative **5.41** with refluxing 6N HCl (*vide infra*) gave β-methylaspartate **5.47** in 68% yield after purification by cation exchange, confirming the stereochemical assignment by comparison of optical rotation to literature values.

Scheme 5.17



5.41 **5.50**

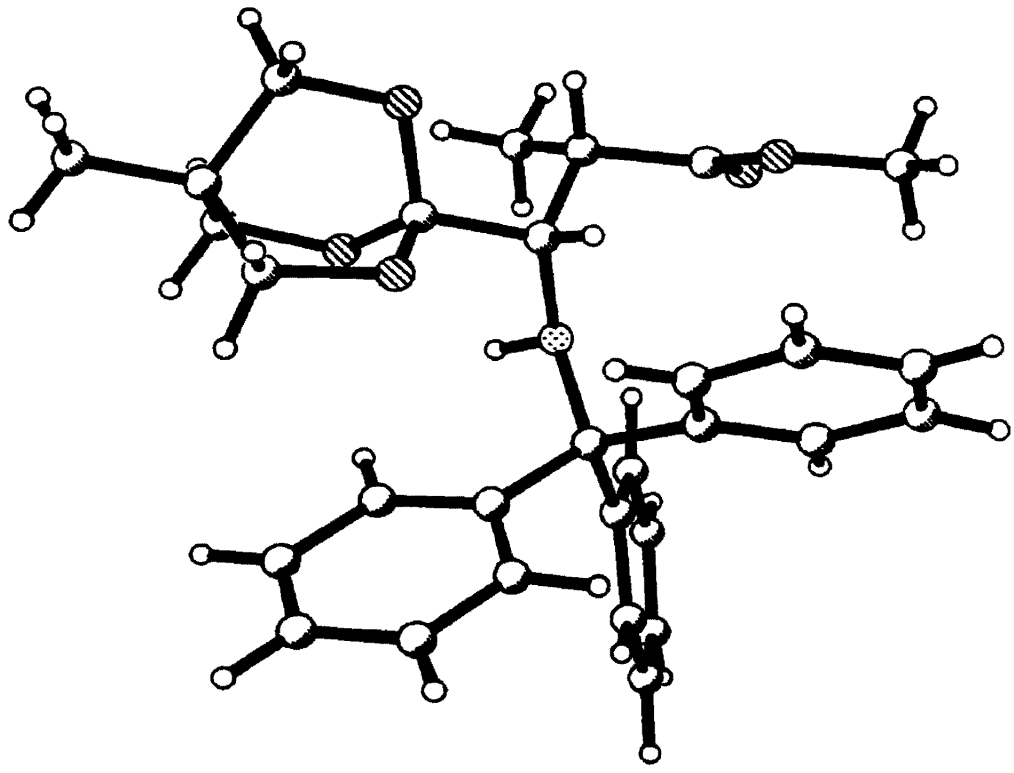


Figure 5.1: X-Ray Crystal structure of Tr-L-Asp(β-Me)(OMe)OBO ester 5.50.

In order to confirm the ^{1}H-NMR assignments, the β-carbon of **5.41** was epimerized with excess LDA to give a 1:1 mixture of the 2*S*,3*S*:2*S*,3*R* β-methyl derivatives **5.41** and **5.42** respectively (Figure 5.2) that were separable after labourious flash chromatography. The 2*S*,3*S* diastereomer had a lower R_f than 2*S*,3*R*-**5.41**. Although the α- and β-protons of **5.41** and **5.42** were distinguishable at 300 MHz, higher fields (500 MHz) were necessary to clearly differentiate between the 3*S* and 3*R* β-methyl protons in order to assign diastereoselectivities for addition. ^{1}H-NMR in solvents other than acetone-d_6 did not particularly influence the chemical shifts of the resolved peaks except that a rotamer became apparent in toluene-d_8.

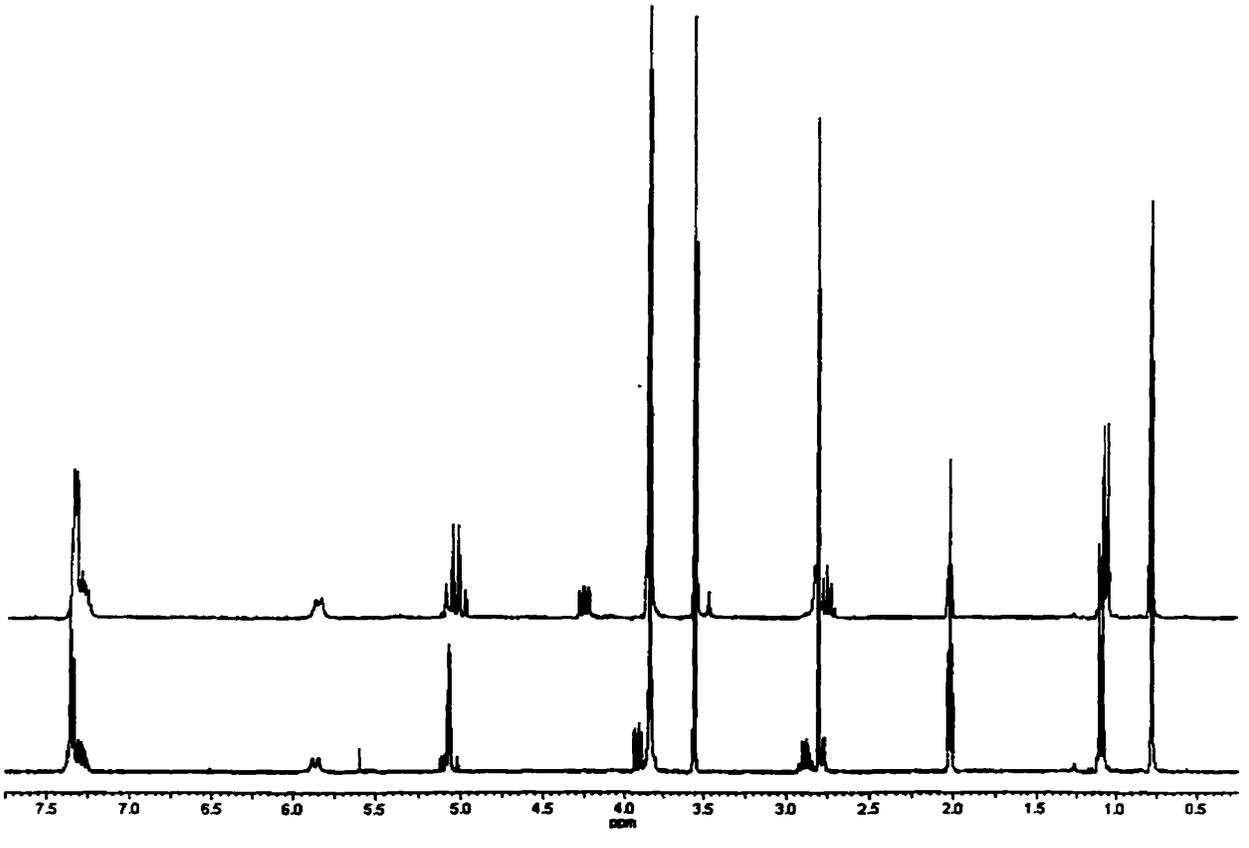


Figure 5.2: 300 MHz ^{1}H-NMR of 2*S*,3*S* 5.41 (top) and 2*S*,3*R* 5.42 (bottom) Cbz-L-Asp(β-Me)(OMe)OBO ester.

5.2.2 Addition of Other Alkylation Agents to Cbz-Asp(OMe)OBO 5.38.

Having optimized the methylation of **5.41**, our attention turned to investigating the addition of other alkylating reagents. Allyl bromide was added to the enolate of **5.38** under the optimized conditions determined for **5.41**. Allylation to give **5.45** occurred with a 4:1 diastereoselectivity, determined by ^{1}H-NMR comparison of the β-protons, and in a yield of 68% after 12 hours (Scheme 5.16). The diastereomers were readily separated by flash chromatography and the starting material could be recovered. The main diastereomer had a lower R_f than the minor isomer, which corresponds with the observation for **5.41**. As with **5.41** the reaction failed to go to completion even after prolonged reaction times and the addition of HMPA. The yield based on recovered starting material was 88%. Deprotection of the separated diastereomers in refluxing 6N HCl gave both 2*S*-diastereomers of **5.48** in approximately 35% yield after purification by cation exchange.

Benzyl bromide was used to benzylate **5.38** to give **5.46** in 3:1 diastereoselectivity and 61% yield. Diastereomeric ratios were determined by ^{1}H-NMR integration of the β-protons. The main contaminant was starting material although the reaction was maintained at –78°C for 16 hours. The yield, based on recovered starting material, was 88% and the reaction could be driven to completion with warming although diastereoselectivity decreased to 1.5:1. Both diastereomers were easily separated by flash chromatography and subsequently deprotected in refluxing 6N HCl to give both 2*S*-diastereomers of **5.49** in approximately 55% yield.

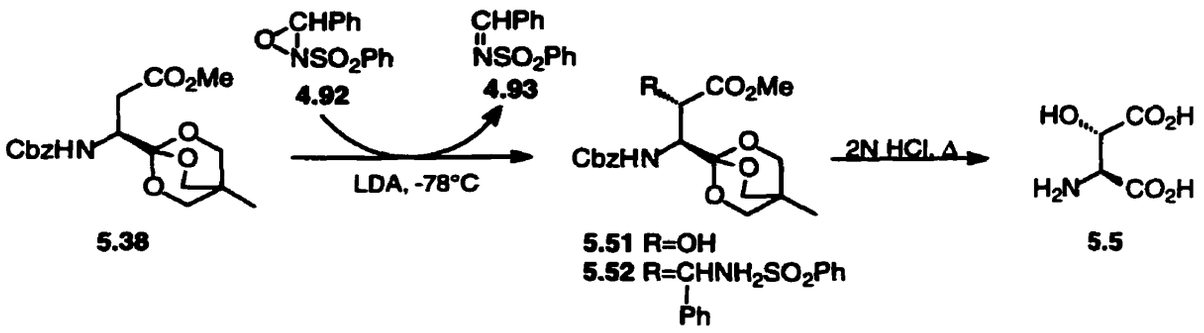
Presumably, addition of allyl bromide and benzyl bromide to **5.38** occurs with the same stereochemistry as **5.41**, the stereochemistry of the addition products thereby tentatively assigned as 2*S*,3*S*.

5.2.3 Addition of Heteroatoms to Cbz-Asp(OMe)OBO 5.38.

In an effort to expand the methodology to incorporate a variety of heteroatoms in the β-position of **5.38,** the enolate of **5.38** was reacted with various heteroatomic electrophilic reagents.

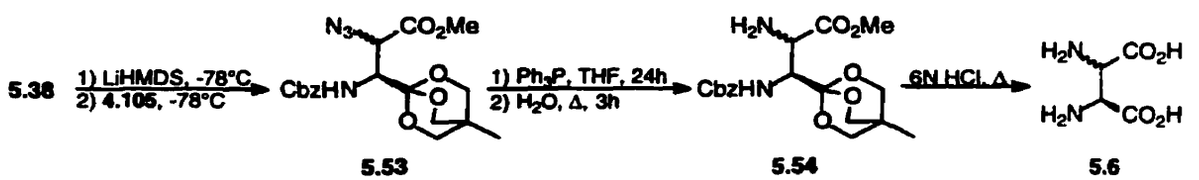
Davis' 2-sulfonyloxaziridine **4.92** has been used previously to hydroxylate aspartic acid derivatives giving β-hydroxyaspartate derivative **5.12** (P=Ts, R^1=R^2=*t*Bu, E=OH) in 45:1 *anti*:*syn* (2*S*,3*R*:2*S*,3*S*). Using conditions previously optimized for the hydroxylation of Cbz-Glu(OMe)OBO ester **4.69** (Section 4.2.4), LDA (3 eq.) was added to a mixture of Cbz-Asp(OMe)OBO ester **5.38** and Davis' oxaziridine **4.92** (5 eq.) in THF at –78°C to give Cbz-Asp(β-OH)(OMe)OBO ester **5.51** in 51% yield and 3:1 ratio of inseparable diastereomers after 18 h at –78°C (Scheme 5.18). The predominant stereoisomer was tentatively assigned as 2*S*,3*S*-Cbz-Asp(β-OH)(OMe)OBO **5.51** based on the stereochemical assignment of **5.41**. Contaminants in the optimized synthesis of **5.51** included remaining starting material **5.38** (15%) and the sulfonamide **5.52** (26%), probably in significant quantities due to the prolonged reaction time as compared to **4.96** (Section 4.2.4). Deprotection of **5.51** with refluxing 2N HCl gave β-hydroxyaspartate **5.5** in 62% yield after purification by cation exchange as a mixture of diastereomers.

Scheme 5.18



We also investigated azidation of the enolate of **5.38**. The addition of trisyl azide **4.105** to the enolate of **5.38** consistently gave the β-azide **5.53** with poor diastereoselectivity since a mixture of approximately 1:1 inseparable epimers was obtained under all the conditions examined (LiHMDS with or without HMPA, LiHMDS/*n*BuLi, NaHMDS and KHMDS) (Scheme 5.19). The azide **5.53** was also generated in poor yield (15-28%) even after warming to room temperature after 16 hours at –78°C and 12 hours at –40°C. Previous reports for the synthesis of **5.12** (P=PhFl, R^1=*t*Bu,Me, R^2=Me, E=N_3) stated the importance of short reaction times (<5 min.). However, the formation of **5.53** was not detected by ESI-MS after 5 and 10 minute periods. The diastereomers of **5.53** were reduced to the amine **5.54** in 67% yield, in an unsuccessful attempt to facilitate isolation of both diastereomers. Hydrolysis of **5.54** gave the β-aminoaspartate **5.6** as a mixture of diastereomers in 32% yield.

Scheme 5.19



5.2.4 Deprotection and HPLC Analysis of β-substituted Aspartic Acids

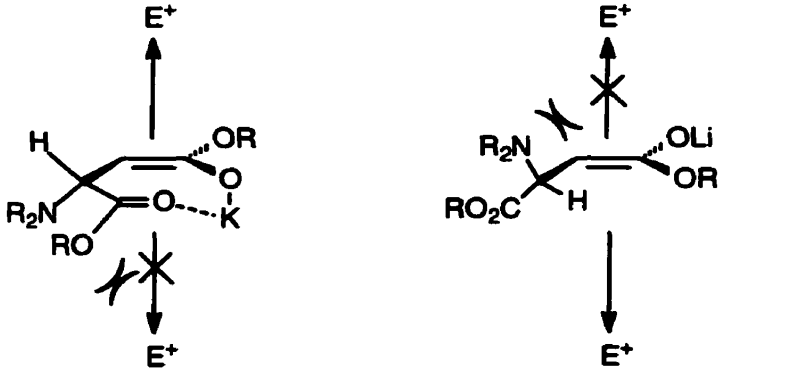
Acid hydrolysis has been used extensively to deprotect β-substituted aspartic acid derivatives.[17b,49,51,67] The diastereomers of β-alkylaspartic acids **5.41**, **5.45** and **5.46** and the diastereomeric mixtures of **5.51** and **5.54** were refluxed in 6N HCl for a period of 2-4 hours. The prolonged hydrolysis conditions were necessary to fully hydrolyze the dihydroxyester **2.62**, generated after ring opening of the OBO ester. The β-substituted aspartic acids were purified by cation exchange (gradient elution with 0.1 N to 1.0 N NH_4OH) in yields of 32-68%.

Crystallization of the β-substituted aspartic acids was unsuccessful with the exception of both 2*S*,3*S*- and 2*S*,3*R*-methyl aspartate **5.1**. However, diastereomeric purities established after lyophilization of the purified β-substituted aspartates and subsequent derivatization followed by HPLC analysis agreed well with ratios determined by ^{1}H-NMR. In general, HPLC analysis of the derivatized β-alkyl aspartic acids occurred with good separation. The diastereomers of β-hydroxyaspartic acid **5.5** proved to be difficult to separate with significant overlap of the 2*S*,3*S* and 2*S*,3*R* derivatives by HPLC, regardless of conditions. Separation of the diaminoaspartic acid diastereomers **5.6** by HPLC was unsuccessful, presumably due to the formation of multiple derivatives of the *o*-phthalaldehyde **2.64**/N-*i*-Bu-L-Cys **2.65** product.

The optical rotation of 2*S*,3*S*-β-methylaspartate was comparable to literature values. Optical rotations for 2*S*,3*S*-β-allylaspartate **5.48** and 2*S*,3*S*-β-benzyl-aspartate **5.49** have not been reported in the literature. The optical rotations for *threo*- and *erythro*-L-β-hydroxaspartic acid **5.5** are very small and cannot be used reliably to ascertain optical purity.[44,71] All the β-substituted aspartic acids were easily identifiable by ESI-MS.

5.2.5 Discussion of the Stereochemistry of Addition to Cbz-Asp(OMe)OBO 5.38.

The majority of stereoselective electrophilic additions to aspartic acid derivatives **5.11** occur with predominantly 2*S*,3*R* stereochemistry. Numerous models have been proposed and in general employ a lithium chelation based model to explain the observed stereochemistry.[57,58] However, the factors responsible for this selectivity, or lack thereof, are complex and include numerous variables such as type of ester, electrophile reactivity, base used, enolate geometry etc.[57,58] In fact, opposite stereoselectivity of addition has been observed for similar aspartate systems (i.e. P=Cbz[56] vs. P=PhFl[48] in **5.12** gives opposite stereochemistry) to those as described above. The only reports in which 2*S*,3*S* stereochemistry was observed (Rapoport,[48] Chamberlin[57a]) both used KHMDS in formation of the enolate. Furthermore, Chamberlin and co-workers were able to invert stereochemistry to 2*S*,3*R* by use of a Li enolate, and afterward identified the (*E*)-potassium enolate and (*Z*)-lithium enolate by trapping studies and subsequently proposed the model depicted in figure 5.3 to explain their observations.



E-potassium enolate to give 2*S*,3*S* stereochemistry

Z-lithium enolate to give 2*S*,3*R* stereochemistry

Figure 5.3: Chamberlin's proposed enolate models.[57a]

In our system, the diastereoselective alkylations of the Li-enolate of **5.38** gave predominantly 2*S*,3*S* stereochemistry. Ireland *et al.* have reported that LiHMDS/THF systems produce (*E*)-lithium ester enolates.[72] Trapping experiments with the enolate of Cbz-Asp(OMe)OBO **5.38** failed to provide any insight to the enolate being formed, and therefore the lithium enolate cannot be assumed to be the *E*-enolate, especially with the observation that the addition of HMPA has no effect on stereoselectivity or yield and Chamberlin's observations.

Adapting previously proposed models for chelation in which the (*E*)-lithium enolate of **5.38** chelates to the Cbz protected nitrogen, the predicted stereochemistry is 2*S*,3*R* which is inconsistent with the observed results. However, if the (*E*)-lithium enolate chelates in a 7-member ring with an oxygen in the OBO ester, the system is locked in a conformation in which alkylation of the enolate gives 2*S*,3*S* stereochemistry in the product (Figure 5.4a). Additionally, the Li bonded to nitrogen cannot chelate to the ester enolate since this would also give the incorrect stereochemistry. However, the N-Li bond may also chelate to an oxygen in the OBO ester to give a 5-membered ring (Figure 5.4a) This model is an adaptation of that proposed by Seebach and Wasmuth.[45] Fredriksen and Dale[73] have shown that polyether ligands complex with Li^+ and Na^+, in some cases in slightly more stable complexes with Li^+ which may explain the small increase in selectivity when LiHMDS is used over NaHMDS and KHMDS.

Conversely, in a non-chelation controlled model, if the (*Z*)-lithium enolate is considered it becomes apparent that the OM^+ group cannot form a cyclic chelate because of its (*Z*)-geometry and as such adopts a hydrogen-in-plane conformation (Figure 4b) that is attacked opposite the OBO group which is bulkier than the Cbz group (i.e. from the *re*

face) to give rise to the 2*S*,3*S* stereoisomer. The model in figure 5.4b is similar to that proposed by Chamberlin and co-workers who used bulkier N-protection (**5.12** P=PhFl,Bn, R^1=R^2=Me) which may explain the increased stereoselectivity observed in their system.[57a]

The size of the β-ester protecting group has no effect on stereoselectivity, and as both models (Figure 5.4) indicate, have little effect on the direction of attack on the enolate. However, the size of the incoming electrophile alters the stereoselectivty in addition from 10:1 (2*S*,3*S*:2*S*,3*R*) in the case of methyl iodide to 1:1 with trisyl azide. The reason for this are currently unclear although two possible explanations exist. If the enolate is particularly sterically constrained, the enolate intermediate may have to adopt some other higher energy transition state in order to react with the electrophile,[58] hence the increased reaction times. Alternatively, the longer reaction times may result in epimerization of the β-carbon by the excess base present, although entries 5 and 6 in Table 5.1 suggest otherwise.

Seebach has described the complexity of enolate structures and the dramatic effect these have on organic reactions.[74] The observation that the addition of base to substrate at –78°C and subsequent trapping with an electrophile results in 1:1 selectivity in the case of **5.38** versus inverse addition which gives 10:1 selectivity with methyl iodide may be a result of various aggregates in solution.

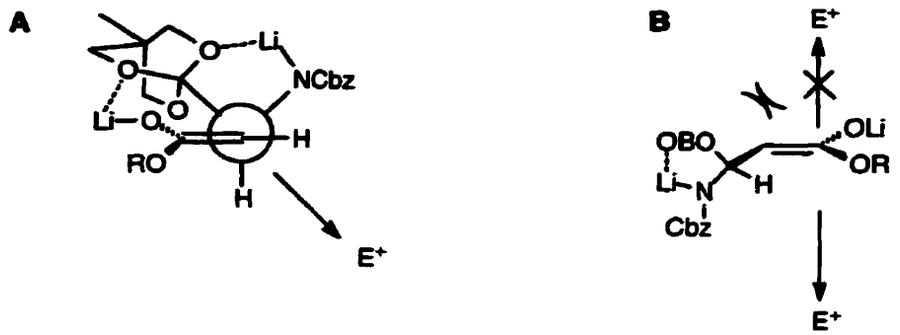


Figure 5.4: A) Chelation controlled model of (*E*)-lithium enolate of 5.38. B) Non-chelation model with (*Z*)-lithium enolate of 5.38.

Unexpectedly, the 1,3-addition to glutamates described in chapter four occurs in higher selectivity than in the case of 1,2-addition to aspartate derivatives. Of significance, the extra methylene unit in glutamate **4.69** allows rotation of the (*Z*)-enolate described in figure 4.10 into a position in which it may chelate with the N-Li bond. As such, both the (*E*)- and (*Z*)-enolate may adopt the same transition state to give high selectivity in which the geometry of the enolate is irrelevant.

5.3 Summary

The synthesis of *threo*-L-β-substituted aspartic acids has been described, with selectivities ranging from as high as 10:1 to 1:1 and in overall yields of 5-28% in six steps.

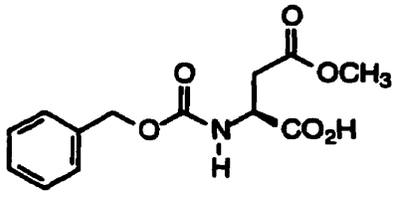
The aspartate **5.38** was synthesized in good overall yield and its crystalline nature made its use as a synthon particularly convenient. The stereoselectivity of methylation of Cbz-Asp(OMe)OBO **5.38** occurred in a 10:1 ratio and is comparable to the best reported procedure[57a] for the synthesis of 2*S*,3*S*-methylaspartate, but is preferable in terms of atom efficiency. Inverse addition of the substrate to base at –78°C is necessary for high selectivity. Disappointingly a rapid decrease in stereoselectivity occurs with bulkier reagents, from 4:1 with allyl bromide to 1:1 selectivity with trisyl azide, the reasons for which are still unclear.

Deprotection in refluxing 6N HCl and cation exchange gave the β-substituted aspartic acids in 32-68% yield.

5.4 Experimental

5.4.1 (2*S*)-2-[(Benzyloxy)carbonyl]amino-4-methoxy-4-oxobutanoic acid, Cbz-L-Asp-(OMe)-OH, 5.32.

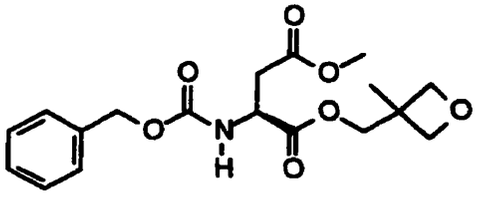
Aspartic acid **5.31** (10.0 g, 0.075 mol) was suspended in freshly distilled MeOH (250 mL) and cooled to 0°C whilst stirring under Ar. Chlorotrimethylsilane (11.8 mL, 0.094 mol) was slowly added by dropping funnel and after 30 min allowed to warm to ambient temperature. After 2 hours the mixture was cooled to 0°C and a second aliquot of chlorotrimethylsilane (11.8 mL, 0.094 mol) slowly added. After 24 hours the solvent was removed *in vacuo*, revealing an oil which was placed under high vacuum. De-ionized water (150 mL) was added to the oil followed by slow addition of Na_2CO_3 (10.23 g, 0.083 mol) to prevent excessive frothing. Once the oil was completely in solution it was cooled to 0°C and *N*-(benzyoxycarbonyl)-succinimide (18.7 g, 0.075 mol), pre-dissolved in 1,4-dioxane (150 mL), slowly added to the stirring mixture which was then allowed to warm to room temperature. After 24 hours, the volume was reduced to approximately 200 mL under vacuum, then de-ionized water (100 mL) added and the final pH of the mixture adjusted to 3 with 1M HCl. This was then extracted with CH_2Cl_2 (3 ×150 mL), the organic extracts were then pooled and extracted with saturated $NaHCO_3$ (3 × 100 mL). The aqueous extracts were pooled, chilled to 0°C, acidified to pH 3 with 1M HCl then extracted with CH_2Cl_2 (4 × 100 mL). The organic extracts were pooled, extracted with brine (50 mL) then dried over $MgSO_4$ and the solvent removed under reduced pressure to yield a colourless oil which was used without further purification.



TLC (1:1, $CHCl_3$:EtOAc, 1% AcOH) R_f = 0.18; ^1H NMR ($CDCl_3$, 250 MHz) δ 10.07 (br s, 1H, CO_2H), 7.38-7.26 (m, 5H, ArH), 5.86 (d, 1H, J = 8.5Hz, NH), 5.12 (s, 2H, CbzCH_2O), 4.69-4.61 (m, 1H, α-CH), 3.69 (s, 3H, CO_2CH_3), 3.05 (dd, 1H, J = 4.4, 17.4Hz, β-CHH), 2.87 (dd, 1H, J = 4.6, 17.4Hz, β-CHH); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 175.9 (C=O), 173.7 (C=O), 156.0 (CONH), 136.3 (Cbz=C=), 128.5, 128.3, 128.2 (Cbz=CH=), 67.2 (CbzCH_2O), 54.1 (α-CH), 52.1 (CO_2CH_3), 31.3 (β-CH_2); ESI-MS (M + H^+) 281.9.

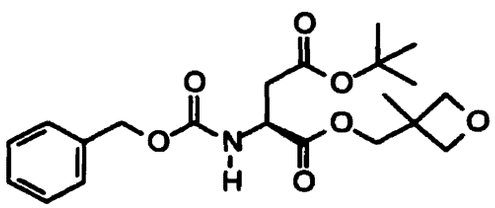
5.4.2 4-Methyl 1-[(3-methyl-3-oxetanyl)methyl] (2*S*)-2-[(benzyloxy)carbonyl]-aminobutanedioate, Cbz-L-Asp(OMe) oxetane ester, 5.35.

Cbz-L-Asp(OMe)-OH **5.32** (5.63 g, 0.022 mol) was combined with Cs_2CO_3 (4.30 g, 0.013 mol) then dissolved in de-ionized water (100 mL) and lyophilized overnight. To the resulting solid was added oxetane tosylate **2.38** (5.92 g, 0.023 mol) and NaI (0.66 g, 4.40 mmol) and then taken up in DMF (300 mL). The mixture was allowed to stir for 48 hours before the DMF is then removed *in vacuo* (0.5 mm Hg, bath temperature 50°C) and the resulting solid dissolved in EtOAc (300 mL) and H_2O (100 mL) and extracted with 10% $NaHCO_3$ (2 × 50 mL), saturated NaCl (50 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the resulting oil purified by flash chromatography (1:1 EtOAc:Hex) to give a clear oil in 66% yield from **5.31** (5.34 g).



TLC (1:1, EtOAc:Hex), R_f = 0.39; ^{1}H NMR ($CDCl_3$, 250MHz) δ 7.48-7.25 (m, 5H, Ar*H*), 5.82 (d, 1H, *J* = 8.2Hz, N*H*), 5.12 (s, 2H, CbzCH_2O), 4.74-4.60 (m, 1H, α-CH), 4.53-4.12 (m, 6H, 2 oxetane ester CH_2O, CO_2CH_2), 3.67 (s, 3H, CO_2CH_3), 3.05 (dd, 1H, *J* = 4.3, 17.2Hz, β-CH*H*), 2.86 (dd, 1H, *J* = 4.5, 17.2Hz, β-C*H*H), 1.29 (s, 3H, oxetane ester CH_3); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 170.5 (C=O), 170.3 (C=O), 155.3 (CONH), 135.9 (Cbz=C=), 129.3, 127.6, 127.3 (Cbz=CH), 77.9 (oxetane ester CH_2O), 73.6 (CO_2CH_2), 66.1 (CbzCH_2O), 51.8 (α-CH), 51.1 (CO_2CH_3), 38.4 (oxetane ester CCH_3), 35.4 (β-CH_2), 20.7 (oxetane ester CCH_3); FT-IR (cast from $CDCl_3$) 3341, 2958, 2876, 1730, 1521, 1439, 1372, 1338, 1215, 1048; ESI-MS (M + H^+) 365.95; Anal. calcd for $C_{18}H_{23}NO_7$: C, 59.17; H, 6.34; N, 3.83. Found: C, 59.38; H, 6.58; N, 3.81.

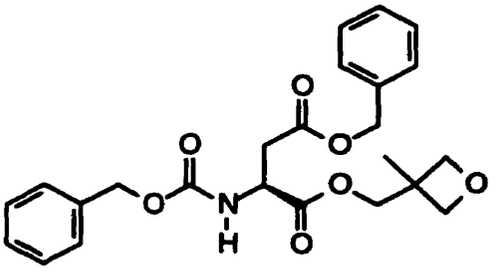
5.4.3 4-(*tert*-Butyl)-1-[(3-methyl-3-oxetanyl)methyl]-(2*S*)-2-[(benzyloxy)carbonyl]-aminobutanedioate, Cbz-L-Asp(O*t*Bu) oxetane ester, 5.36.



Same procedure as in section 5.4.2 using Cbz-L-Asp(O*t*Bu)OH · H_2O **5.33** to give 11.81 g (98.9% yield) of **5.36**. TLC (1:1, EtOAc:Hex), R_f = 0.51; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.38-7.26 (m, 5H, Ar*H*), 5.78 (d, 1H, *J* = 8.7Hz, N*H*), 5.09 (s, 2H, CbzCH_2O), 4.62-4.55 (m, 1H, α-C*H*), 4.50-4.16 (m, 6H, 2 oxetane ester CH_2O, CO_2CH_2), 2.93 (dd, 1H, *J* = 4.6, 17.1Hz, β-C*H*H), 2.74 (dd, 1H, , *J* = 4.4, 17.1Hz, β-CH*H*), 1.39 (s, 9H, C(CH_3)$_3$), 1.28 (s, 3H, oxetane ester CCH_3); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 170.9 (C=O), 169.9 (C=O),

156.0 (CONH), 136.2 (Cbz=C=), 128.5, 128.1, 128.0 (Cbz=CH=), 81.9 ($C(CH_3)_3$), 79.3 (oxetane ester CH_2O), 69.7 (CO_2CH_2), 67.0 ($CbzCH_2O$), 50.6 (α-CH), 39.1 (oxetane ester CCH_3), 37.6 (β-CH_2), 28.0 ($C(CH_3)_3$), 20.9 (oxetane ester CCH_3); Anal. calcd for $C_{21}H_{29}NO_7$: C, 61.90; H, 7.17; N, 3.44. Found: C, 62.19; H, 7.31; N, 3.49.

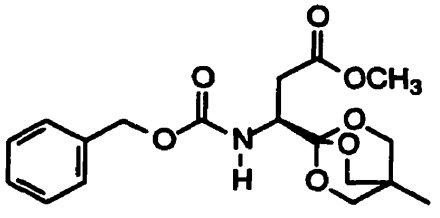
5.4.4 4-Benzyl-1-[(3-methyl-3-oxetanyl)methyl]-(2*S*)-2-[(benzyloxy)carbonyl]-aminobutanedioate, Cbz-L-Asp(OBn) oxetane ester, 5.37.



Same procedure as in section 5.4.2 to give 5.98 g (96.8%) of **5.37**. TLC (1:1, EtOAc:Hex), $R_f = 0.47$; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.44-7.20 (m, 10H, Ar*H*), 5.87 (d, 1H, *J* = 8.8Hz, N*H*), 5.12-5.07 (m, 4H, $CbzCH_2O$, $PhCH_2O$), 4.74-4.62 (m, 1H, α-C*H*), 4.45-4.06 (m, 6H, 2 oxetane ester CH_2O, CO_2CH_2), 3.09 (dd, 1H, *J* = 4.5 , 17.1Hz, β-C*H*H), 2.92 (dd, 1H, *J* = 4.6 , 17.1Hz, β-CH*H*), 1.28 (s, 3H, oxetane ester CH_3); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 170.5 (C=O), 170.4 (C=O), 155.8 (CONH), 136.0 (Cbz=C=), 135.2 (Ph=C=), 129.7, 128.4, 128.4, 128.3, 128.4, 128.0 (Cbz=CH=, Ph=CH=), 79.1 (oxetane ester CH_2O), 69.6 (CO_2CH_2C), 67.0 ($CbzCH_2O$), 66.7 (CO_2CH_2Ph), 50.4 (α-CH), 38.9 (oxetane ester CCH_3), 36.5 (β-CH_2), 20.8 (oxetane ester CCH_3); Anal. calcd for $C_{24}H_{27}NO_7$: C, 65.30; H, 6.16; N, 3.17. Found: C, 65.65; H, 6.32; N, 3.24.

5.4.5 4-Methyl-(2*S*)-2-[(benzyloxy)carbonyl]amino-1-(4-methyl-2,6,7-trioxabicyclo-[2.2.2]oct-1-yl)propanoate, Cbz-L-Asp(OMe)OBO ester, 5.38.

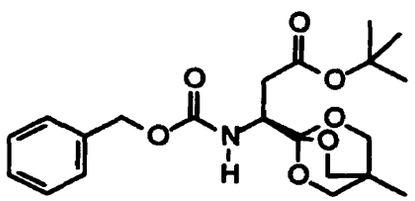
Cbz-L-Asp(OMe)-oxetane ester **5.35** (10.97 g, 30.0 mmol) was dissolved in dry CH_2Cl_2 (400 mL) while stirring under Ar. The mixture was cooled to 0°C then $BF_3 \cdot Et_2O$ (0.38 mL, 3.0 mmol) was added by syringe. The mixture was allowed to warm to room temperature and stir for 4 hours after which a TLC indicated the reaction was complete. Et_3N (0.84 mL, 6.0 mmol) was then added and the mixture stirred an additional 30 min before the solvent was removed *in vacuo*. The resulting oil was dissolved in EtOAc (600 mL) and extracted with 3% NH_4Cl (2 × 100 mL), saturated $NaHCO_3$ (100 mL), brine (100 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to reveal a light coloured oil which was purified by flash chromatography (1:1 EtOAc:Hex) to give a clear oil which recrystallized from EtOAc:Hexanes to give 7.45 g (68%) of long white crystals.



m.p. 79-80°C, $[\alpha]^{20}_D$ = -37.4 (c = 1.15, CH_2Cl_2); TLC (1:1, EtOAc:Hex), R_f = 0.44; ^1H NMR ($CDCl_3$, 250 MHz) δ 7.39-7.26 (m, 5H, Ar*H*), 5.21-5.02 (m, 3H, N*H*, CbzCH_2), 4.33 (ddd, 1H, *J* = 5.3, 7.9, 13.0Hz, α-C*H*), 3.85 (s, 6H, OBO ester CH_2O), 3.61 (s, 3H, CO_2CH_3), 2.68 (dd, 1H, *J* = 5.3, 15.1Hz, β-C*H*H), 2.42 (dd, 1H, *J* = 7.8, 15.1Hz, β-CH*H*) 0.77 (s, 3H, OBO ester CCH_3); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 171.4 (C=O), 155.9 (CONH), 136.6 (Cbz=C=), 128.3, 128.0, 127.9 (Cbz=CH=), 107.9 (OBO ester C-O), 72.2 (OBO ester CH_2O), 66.7 (CbzCH_2O), 52.2 (α-CH), 51.6 (CO_2CH_3), 35.6 (OBO

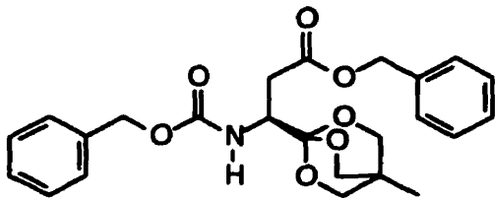
ester $\underline{C}CH_3$), 30.6 (β-$\underline{C}H_2$), 14.1 (OBO ester C$\underline{C}H_3$); IR (cast from $CHCl_3$) 3362, 2953, 2882, 1734, 1520, 1456, 1399, 1352, 1293, 2133, 1052; HRMS (FAB) calcd for ($M + H^+$) $C_{18}H_{24}NO_7$ 366.15527, found 366.15538; Anal. calcd for $C_{18}H_{23}NO_7$: C, 59.17; H, 6.34; N, 3.83. Found: C, 59.33; H, 6.58; N, 3.87.

5.4.6 4-*tert*-Butyl-(2*S*)-2-[(benzyloxy)carbonyl]amino-1-(4-methyl-2,6,7-trioxa-bicyclo[2.2.2]oct-1-yl)butanoate, Cbz-L-Asp(O*t*Bu)OBO ester, 5.39.



Same procedure as in section 5.4.5 to give 7.35 g (68.1%) of **5.39**. m.p. 77-78°C; $[\alpha]^{20}_D$ = -4.8 (c = 1.36, CH_2Cl_2); TLC (1:1, EtOAc:Hex), R_f = 0.45; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.32-7.22 (m, 5H, Ar*H*), 5.97 (d, 1H, *J* = 8.8Hz, N*H*), 5.06 (s, 2H, CbzCH_2), 4.59-4.51 (dt, 1H, *J* = 4.8, 8.8Hz, α-C*H*), 3.81 (s, 6H, OBO ester CH_2O), 2.87 (dd, 1H, *J* = 4.8, 17.1Hz, β-C*H*H), 2.72 (dd, 1H, *J* = 4.8, 17.1Hz, β-CH*H*), 1.36 (s, 9H, (CH_3)$_3$C), 0.77 (s, 3H, OBO ester CCH_3); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 170.1 ($\underline{C}$=O), 156.1 ($\underline{C}$ONH), 135.9 (Cbz=$\underline{C}$=), 128.3, 128.0, 127.9 (Cbz=$\underline{C}$H=), 107.9 (OBO ester $\underline{C}$-O), 81.9 ((CH_3)$_3\underline{C}$), 72.4 (OBO ester $\underline{C}H_2$O), 66.9 (Cbz$\underline{C}H_2$O), 50.5 (α-$\underline{C}$H), 40.5 (OBO ester $\underline{C}CH_3$), 37.4 (β-$\underline{C}H_2$), 16.5 (OBO ester C$\underline{C}H_3$); Anal. calcd for $C_{21}H_{29}NO_7$: C, 61.90; H, 7.17; N, 3.44. Found: C, 62.23; H, 7.39; N, 3.96.

5.4.7 Benzyl-(2*S*)-2-[(benzyloxy)carbonyl]amino-1-(4-methyl-2,6,7-trioxabicyclo-[2.2.2]oct-1-yl)butanoate, Cbz-L-Asp(OBn)OBO ester, 5.40.

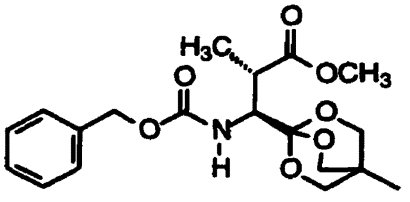


Same procedure as in section 5.4.5 to give 7.35 g (68.1%) of **5.40**. $[\alpha]^{20}_{D}$ = -16.0 (c = 1.00, CH_2Cl_2); TLC (1:1, EtOAc:Hex), R_f = 0.49; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.42-7.24 (m, 10H, Ar*H*), 5.18 (d, 1H, *J* = 10.5Hz, N*H*), 5.15-5.04 (m, 4H, CbzCH_2O, PhCH_2O), 4.36 (ddd, 1H, *J* = 5.4, 7.4, 13.2Hz, α-C*H*), 3.81 (s, 6H, OBO ester OCH_2), 2.71 (dd, 1H, *J* = 5.4, 15.0Hz, β-C*H*H), 2.48 (dd, 1H, *J* = 7.4, 15.0Hz, β-CH*H*), 0.74 (s, 3H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 171.1 (C=O), 156.0 (CONH), 136.6, 136.0 (Cbz=C=, Ph=C=), 128.7, 128.7, 128.5, 128.4, 128.3, 128.1 (Cbz=CH=, Ph=CH=), 108.0 (OBO ester C-O), 72.8 (OBO ester OCH_2), 66.9, 66.4 (CbzCH_2O, CO_2CH_2Ph), 52.3 (α-CH), 36.1 (OBO ester CCH_3), 30.7 (β-CH_2), 14.3 (OBO ester CCH_3); HRMS (FAB) calcd for (M + H^+) $C_{24}H_{28}NO_7$ 442.18658, found 442.18221. Anal. calcd for $C_{24}H_{27}NO_7$: C, 65.30; H, 6.16; N, 3.17. Found: C, 66.57; H, 6.43; N, 3.23.

5.4.8 Methyl-(2*S*,3*S*)-2-[(benzyloxy)carbonyl]amino-3-methyl-2-(4-methyl-2,6,7-tri-oxabicyclo[2.2.2]oct-1-yl)propanoate, Cbz-L-Asp(β-Me)(OMe)OBO ester, 5.41.

Cbz-Asp(OMe)OBO ester **5.38** (0.240 g, 0.66 mmol) was dissolved in dry THF (5 mL) then added via cannula to a flask containing LiHMDS (1.98 mL, 1.98 mmol) in THF (5 mL) at –78°C under Ar. After 1 h, methyl iodide (0.168 mL, 3.30 mmol) was added and the mixture stirred for 8 h at –78°C. The mixture was poured into 3% NH_4Cl (5 mL) and

extracted with Et_2O (3 × 25 mL), the organic fractions pooled and extracted with 10% $NaHCO_3$ (10 mL), brine (10 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure to reveal a light yellow oil which was purified by flash chromatography to give 0.202 g (81% yield) of a clear oil.

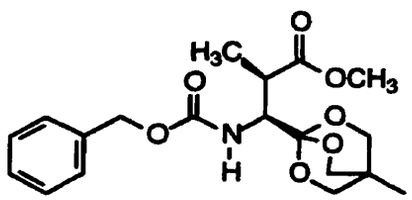


$[\alpha]^{20}_D$ = -37.1 (c = 1.52, CH_2Cl_2); TLC (1:1, EtOAc:Hex), R_f = 0.48; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.38-7.24 (m, 5H, Ar*H*), 5.84 (d, 1H, *J* = 10.1Hz, N*H*), 5.10 (d, 1H, *J* = 12.6Hz, CbzC*H*HO), 5.03 (d, 1H, *J* = 12.6Hz, CbzC*H*HO), 4.29 (dd, 1H, *J* = 7.3, 10.1Hz, α-C*H*), 3.87 (s, 6H, OBO ester CH_2O), 3.57 (s, 3H, CO_2CH_3), 2.78 (dt, 1H, *J* = 7.3, 7.4Hz, β-C*H*), 1.08 (d, 3H, *J* = 7.3Hz, β-CH_3), 0.81 (s, 3H, OBO ester CCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.9 (C=O), 156.7 (CONH), 136.6, (Cbz=C=), 128.3, 127.9, 127.7 (Cbz=CH=) 108.1 (OBO ester C-O), 72.6 (OBO ester CH_2O), 66.8 (CbzCH_2O), 56.1 (α-C*H*), 51.6 (CO_2CH_3), 40.0 (β-CH), 30.5 (OBO ester CCH_3), 14.1 (OBO ester CCH_3), 12.7 (β-CH_3); FT-IR (cast from $CDCl_3$) 2994, 2879, 1731, 1515, 1225, 1049; ESI-MS (M + H^+) 379.95; Anal. calcd for $C_{19}H_{25}NO_7$: C, 60.15; H, 6.64; N, 3.69. Found: C, 60.33; H, 6.89; N, 3.75.

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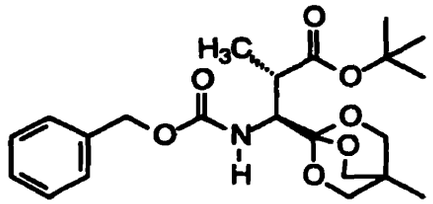
5.4.9 Methyl-(2*S*,3*R*)-2-[(benzyloxy)carbonyl]amino-3-methyl-2-(4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl)propanoate, Cbz-L-Asp(β-Me)(OMe)OBO ester, 5.42.

Cbz-Asp(β-Me)(OMe)OBO ester **5.41** (0.200 g, 0.53 mmol) was dissolved in THF (10 mL) and cooled to –78°C while stirring under Ar. LDA (7.0 mL, 10.5 mmol) was added and the mixture stirred for 1 h before being poured into Et_2O (50 mL) and extracted with 3% NH_4Cl (10 mL), 10% $NaHCO_3$ (10 mL), brine (10 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* and the resulting oil purified by flash chromatography to give 0.052 g of **5.41** (26% yield) and 0.046 g of **5.42** (23% yield).



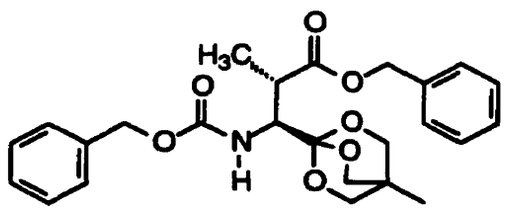
TLC (1:1, EtOAc:Hex), $R_f = 0.51$; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.40-7.28 (m, 5H, Ar*H*), 5.88 (d, 1H, *J* = 10.4Hz, N*H*), 5.11 (d, 1H, *J* = 12.6Hz, CbzC*H*HO), 5.07 (d, 1H, *J* = 12.6Hz, CbzCH*H*O), 3.94 (dd, 1H, *J* = 3.8, 10.4Hz, α-C*H*), 3.86 (s, 6H, OBO ester CH_2O), 3.58 (s, 3H, CO_2CH_3), 2.91 (ddd, 1H, *J* = 3.6, 6.9, 10.7Hz, β-C*H*), 1.12 (d, 3H, *J* = 7.3Hz, β-CH_3), 0.80 (s, 3H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 174.6 (C=O), 156.4 (CONH), 136.6, (Cbz=C=), 128.3, 127.7, 127.7 (Cbz=CH=) 108.0 (OBO ester C-O), 72.3 (OBO ester CH_2O), 66.8 (CbzCH_2O), 57.2 (α-CH), 50.8 (CO_2CH_3), 38.2 (β-CH), 30.5 (OBO ester CCH_3), 14.5 (OBO ester CCH_3), 13.3 (β-CH_3).

5.4.10 *tert*-Butyl-(2*S*,3*S*)-2-[(benzyloxy)carbonyl]amino-3-methyl-2-(4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl)propanoate, Cbz-L-Asp(β-Me)(*t*Bu)OBO ester, 5.43.
Same procedure as in section 5.4.8 to give 0.184 g (74%) of **5.43**.



TLC (1:1, EtOAc:Hex), R_f = 0.48; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.39-7.24 (m, 5H, Ar*H*), 5.71 (d, 1H, *J* = 10.2Hz, N*H*), 5.10-5.03 (m, 2H, CbzCH_2O), 4.19 (m, 1H, α-C*H*), 3.83 (s, 6H, OBO ester CH_2O), 2.78 (m, 1H, β-C*H*), 1.38 (s, 9H, (CH_3)$_3$C), 1.07 (d, 3H, *J* = 7.1Hz, β-CH_3), 0.79 (s, 3H, OBO ester CCH_3); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 175.5 (C=O), 156.6 (CONH), 136.3, (Cbz=C=), 128.3, 127.9, 127.7 (Cbz=CH=) 108.3 (OBO ester C-O), 81.8 ((CH$_3$)$_3$C), 72.4 (OBO ester CH$_2$O), 66.7 (CbzCH$_2$O), 55.6 (α-CH), 39.8 (β-CH), 30.5 (OBO ester CCH$_3$), 14.3 (OBO ester CCH$_3$), 12.9 (β-CH$_3$); ESI-MS (M + H$^+$) 421.98; Anal. calcd for $C_{22}H_{31}NO_7$: C, 62.69; H, 7.31; N, 3.32. Found: C, 62.99; H, 7.56; N, 3.41.

5.4.11 Benzyl-(2*S*,3*S*)-2-[(benzyloxy)carbonyl]amino-3-methyl-2-(4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl)propanoate, Cbz-L-Asp(β-Me)(OMe)OBO ester, 5.44. Same procedure as in section 5.4.8 to give 0.127 g (71%) of **5.44**.

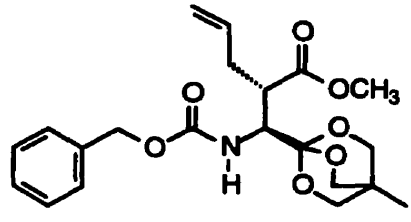


TLC (1:1, EtOAc:Hex), R_f = 0.54; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.45-7.23 (m, 10H, Ar*H*), 5.94 (d, 1H, *J* = 10.2Hz, N*H*), 5.22-5.03 (m, 4H, CbzCH_2O, PhCH_2O), 4.36 (dd,

1H, J = 7.3, 10.1Hz, α-C*H*), 3.86 (s, 6H, OBO ester CH_2O), 2.87-2.73 (m, 1H, β-C*H*), 1.15 (d, 3H, J = 7.3Hz, β-CH_3), 0.80 (s, 3H, OBO ester CCH_3); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 175.3 (C=O), 156.4 (CONH), 136.3, 136.1 (Cbz=C=, Ph=C=), 128.7, 128.7, 128.5, 128.4, 127.9, 127.7 (Cbz=CH=, Ar=CH=), 108.1 (OBO ester C-O), 72.3 (OBO ester CH_2O), 66.7, 66.3 (CbzCH_2O, ArCH_2O), 52.4 (α-CH), 39.0 (β-CH), 31.1 (OBO ester CCH_3), 14.1 (OBO ester CCH_3), 12.8 (β-CH_3); ESI-MS (M + H^+) 456.30; Anal. calcd for $C_{25}H_{29}NO_7$: C, 65.92; H, 6.42; N, 3.07. Found: C, 66.24; H, 6.64; N, 3.14.

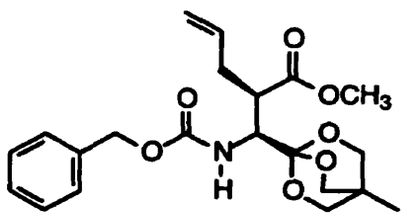
5.4.12 Methyl-(3*S*)-2-[(*S*)-1-[(benzyloxy)carbonyl]amino-1-(4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl)methyl]-3-pentenoate, Cbz-L-Asp(β-Allyl)(OMe)OBO ester 5.45.

Same procedure as in section 5.4.8 using allyl bromide to give 0.112 g (68%) of **5.45** as a 4:1 mixture of diastereomers that were separated by flash chromatography (1:1, EtOAc:Hex).



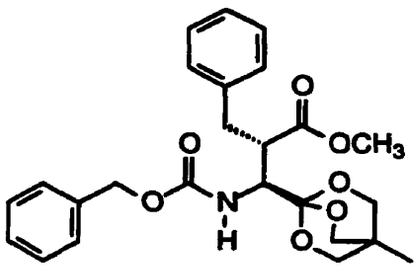
2*S*,3*S*-**5.45**. TLC (1:1, EtOAc:Hex), R_f = 0.52; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.41-7.27 (m, 5H, Ar*H*), 5.92 (d, 1H, J = 10.3Hz, N*H*), 5.78-5.64 (m, 1H, CH_2=C*H*), 5.11 (s, 2H, CbzCH_2O), 5.05-4.97 (m, 2H, CH_2=CH), 4.24 (dd, 1H, J = 8.8, 10.4Hz, α-C*H*), 3.86 (s, 6H, OBO ester CH_2O), 3.58 (s, 3H, CO_2CH_3), 2.74 (ddd, 1H, J = 4.0, 8.7, 11.3Hz, β-C*H*), 2.31-2.21 (m, 2H, CH_2CH=CH_2), 0.80 (s, 3H, OBO ester CCH_3); ^{13}C NMR ($CDCl_3$,

63 MHz) δ 175.9 (C=O), 156.0 (CONH), 136.3 (Cbz=C=), 134.1 (CH_2=CH), 128.4, 127.9, 127.7 (Cbz=CH=), 117.1 (CH_2=CH), 108.2 (OBO ester C-O), 72.2 (OBO ester CH_2O), 66.7 (CbzCH_2O), 52.8 (α-CH), 51.3 (CO_2CH_3), 41.5 (β-CH), 36.0 (CH_2CH=CH_2), 30.8 (OBO ester CCH_3), 14.0 (OBO ester CCH_3); ESI-MS (M + H^+) 405.96; Anal. calcd for $C_{21}H_{27}NO_7$: C, 62.21; H, 6.71; N, 3.45. Found: C, 62.44; H, 7.01; N, 3.53.

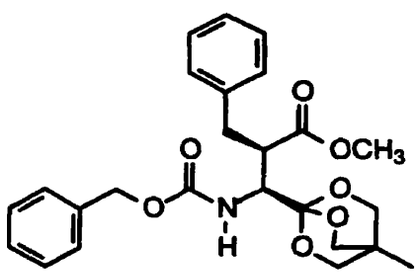


2*S*,3*R*-**5.45**. TLC (1:1, EtOAc:Hex), R_f = 0.56; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.41-7.27 (m, 5H, Ar*H*), 5.87 (d, 1H, *J* = 9.8Hz, N*H*), 5.85-5.74 (m, 1H, CH_2=C*H*), 5.09 (s, 2H, CbzCH_2O), 5.00-4.88 (m, 2H, CH_2=CH), 4.00 (dd, 1H, *J* = 3.2, 10.4Hz, α-C*H*), 3.86 (s, 6H, OBO ester CH_2O), 3.56 (s, 3H, CO_2CH_3), 2.91 (ddd, 1H, J = 3.2, 6.8, 8.3Hz, β-C*H*), 2.46-2.39 (m, 2H, CH_2CH=CH_2), 0.80 (s, 3H, OBO ester CCH_3).

5.4.13 Methyl-(2*S*,3*S*)-3-benzyl-2-[(benzyloxy)carbonyl]amino-2-(4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl)propanoate, Cbz-L-Asp(β-Bn)(OMe)OBO ester, 5.46. Same procedure as in section 5.4.8 using benzyl bromide to give 0.102 g (61%) of **5.46** as a 3:1 mixture of diastereomers that were separated by flash chromatography (1:1, EtOAc:Hex).



2*S*,3*S*-**5.46**. TLC (1:1, EtOAc:Hex), R_f = 0.54; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.41-7.21 (m, 10H, Ar*H*), 5.79 (d, 1H, *J* = 10.1Hz, N*H*), 5.11 (s, 2H, CbzCH_2O), 4.24-4.11 (m, 1H, α-C*H*), 3.84 (s, 6H, OBO ester CH_2O), 3.59 (s, 3H, CO_2CH_3), 2.76-2.49 (m, 3H, β-C*H*, PhCH_2O), 0.79 (s, 3H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 175.3 (C=O), 155.6 (CONH), 136.1 (Cbz=C=), 128.3, 127.9, 127.7 (Cbz=CH=), 108.1 (OBO ester C-O), 72.3 (OBO ester CH_2O), 66.7 (CbzCH_2O), 53.1 (α-CH), 51.0 (CO_2CH_3), 43.5 (β-CH), 36.9 (PhCH_2), 31.8 (OBO ester CCH_3), 13.8 (OBO ester CCH_3); ESI-MS (M + H^+) 456.27; Anal. calcd for $C_{25}H_{29}NO_7$: C, 65.92; H, 6.42; N, 3.07. Found: C, 66.27; H, 6.77; N, 3.13.

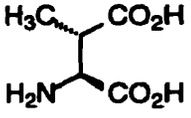


2*S*,3*R*-**5.46**. TLC (1:1, EtOAc:Hex), R_f = 0.58; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.41-7.26 (m, 10H, Ar*H*), 5.61 (d, 1H, *J* = 10.1Hz, N*H*), 5.11-5.02 (m, 2H, CbzCH_2O), 4.20-4.07 (m, 1H, α-C*H*), 3.86 (s, 6H, OBO ester CH_2O), 3.56 (s, 3H, CO_2CH_3), 2.67-2.41 (m, 3H, β-C*H*, PhCH_2O), 0.79 (s, 3H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ

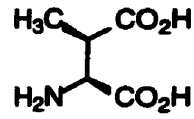
175.1 (C=O), 155.8 (CONH), 136.1 (Cbz=C=), 128.1, 127.8, 127.7 (Cbz=CH=), 108.2 (OBO ester C-O), 72.3 (OBO ester CH_2O), 66.6 (CbzCH_2O), 53.7 (α-CH), 51.9 (CO_2CH_3), 43.3 (β-CH), 36.1 (PhCH_2), 31.0 (OBO ester CCH_3), 13.3 (OBO ester CCH_3); ESI-MS (M + H^+) 456.22; Anal. calcd for $C_{25}H_{29}NO_7$: C, 65.92; H, 6.42; N, 3.07. Found: C, 66.27; H, 6.77; N, 3.13.

5.4.14 (2*S*,3*S*)-2-Amino-3-methylbutanedioic acid, Asp(β-Me)OH, 5.47.

Cbz-Asp(β-Me)(OMe)OBO ester **5.41** (0.189 g, 0.49 mmol) was taken-up in doubly distilled 6N HCl (5 mL) and refluxed for 4 hours. The solvent was removed under reduced pressure, rinsed with distilled water, reduced again and lyophilized to give 0.048 g of a white powder (68% yield). The white powder was dissolved in a minimum of water and placed on Dowex 50X8-100 ion-exchange resin. The column was rinsed with 5 column lengths of water then eluted with 0.5 N NH_4OH. The fractions were collected and combined then reduced *in vacuo*, lyophilized to give a white powder which was then crystallized from acetone:water to give 0.021 g of clear crystals.



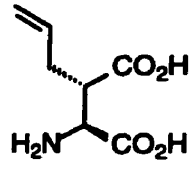
2*S*,3*S*-**5.47**: mp 270-274 (dec.); $[\alpha]^{20}_D$ = +12.8 (c = 0.5, 1N HCl), (lit.[59] $[\alpha]^{20}_D$ = +13.6 (c = 0.46, 1N HCl); ^{1}H NMR (D_2O, 300 MHz) δ 3.97 (d, 1H, *J* = 3.6Hz, α-C*H*), 3.15 (dq, 1H, *J* = 3.6, 7.4Hz, β-C*H*), 1.18 (d, 3H, *J* = 7.5Hz, β-CH_3); ^{13}C NMR (D_2O, 75 MHz) δ 177.2 (C=O), 172.9 (C=O), 55.5 (α-CH), 39.1 (β-CH), 11.6 (β-CH_3); ESI-MS 147.89.



2*S*,3*R*-**5.47**: mp 220-232 (dec.); $[\alpha]^{20}_D$ = -3.2 (c = 2.4, 1M HCl), (lit.[59] $[\alpha]^{20}_D$ = +5.6 (c = 3.1, H_2O); ^{1}H NMR (D_2O, 300 MHz) δ 4.00 (d, 1H, *J* = 4.3Hz, α-C*H*), 3.20 (dq, 1H, *J* = 4.3, 7.4Hz, β-C*H*), 1.29 (d, 3H, *J* = 7.5Hz, β-CH_3); ^{13}C NMR (D_2O, 75 MHz) δ 176.6 (C=O), 171.2 (C=O), 56.5 (α-CH), 39.6 (β-CH), 12.5 (β-CH_3); ESI-MS 147.91.

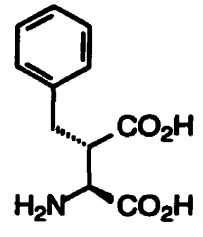
5.4.15 (2*S*,3*S*)-3-Allyl-2-aminobutanedioic acid, Asp(β-allyl)OH, 5.48.

Same procedure as in section 5.4.14 to give 0.021 g (34%) of **5.48**.



^{1}H-NMR (D_2O, 300 MHz) δ 5.93-5.81 (m, 1H, CH_2=C*H*), 5.32-5.15 (m, 2H, CH_2=CH), 4.21-4.01 (m, 1H, α-C*H*), 3.11-2.68 (m, 3H, β-C*H*, CH_2CH=CH_2); ^{13}C NMR (D_2O, 75 MHz) δ 176.7 (C=O), 173.3 (C=O), 132.1 (CH_2=CH), 117.0 (CH_2=CH), 54.5 (α-CH), 39.9 (β-CH), 29.9 (CH_2CH=CH_2); ESI-MS 173.87.

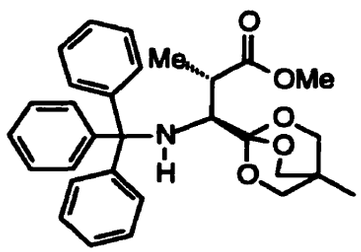
5.4.16 (2*S*,3*S*)-2-amino-3-benzylbutanedioic acid, Asp(β-Bn)OH, 5.49.



^{1}H-NMR (D_2O, 300 MHz) δ 7.26-7.03 (m, 5H, Ar*H*), 4.19-4.07 (m, 1H, α-C*H*), 3.11-2.99 (m, 1H, β-C*H*), 2.78-2.56 (m, 2H, Ph<u>C</u>H_2); ^{13}C NMR (D_2O, 75 MHz) δ 176.3 (<u>C</u>=O), 174.7 (<u>C</u>=O), 139.7 (Ar=<u>C</u>=), 131.0, 129.9, 128.9 (Ar=<u>C</u>H), 56.5 (α-<u>C</u>H), 41.8 (β-<u>C</u>H), 34.2 (Ph<u>C</u>H_2); ESI-MS (M + H^+) 223.94.

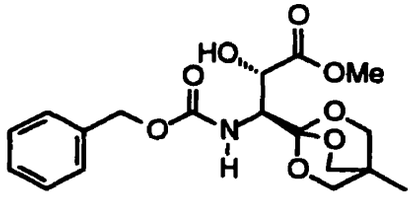
5.4.17 Methyl-(2*S*,3*S*)-3-methyl-2-(4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl)-2-(tritylamino)propanoate, Tr-L-Asp(β-Me)(OMe)OBO ester, 5.50.

Cbz-Asp(β-Me)(OMe)OBO ester **5.41** (0.360 g, 0.95 mmol) was dissolved in EtOAc:EtOH (1:1, 40 mL) to which Pd/C (0.5 g, Degussa type E101 NE/W) was added. The mixture was then evacuated and purged with H_2 which was repeated twice. The mixture was stirred for 12 h before being filtered through celite and the filtrate reduced in vacuo to reveal a yellow oil. Dry CH_2Cl_2 (10 mL) and DIPEA (0.26 mL, 1.88 mmol) was added followed by trityl chloride (0.262 g, 0.95 mmol). The mixture was allowed to stir for 6 h before being poured into Et_2O (100 mL) and extracted with 3% NH_4Cl (3 × 20 mL), 10 % $NaHCO_3$ (20 mL), brine (20 mL) then dried over $MgSO_4$. The solvent was removed under reduced pressure to reveal a white foam which was recrystallized from Et_2O:Hex to give 0.332 g (73%) of small cube shaped crystals.



^{1}H-NMR ($CDCl_3$, 250 MHz) δ 7.53-7.18 (m, 15H, Ar*H*), 3.82-3.61 (m, 6H, OBO ester CH_2O), 3.55 (dd, 1H, J = 3.5, 10.5Hz, α-C*H*), 3.25 (s, 3H, CO_2CH_3), 2.94 (d, 1H, J = 10.5Hz, N*H*), 2.69 (dq, 1H, J = 3.5, 7.1Hz, β-C*H*), 1.24 (d, 3H, J = 7.1Hz, β-CH_3), 0.77 (s, 3H, OBO ester CCH_3); ESI-MS 487.97.

5.4.18 Methyl-(2*S*)-2-[(benzyloxy)carbonyl]amino-3-hydroxy-2-(4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl)propanoate, Cbz-L-Asp(β-OH)(OMe)OBO ester, 5.51. *n*-Butyllithium (0.78 mL, 1.08 mmol) was slowly added to diisopropylamine (0.163 mL, 1.17 mmol) in dry THF (5 mL) at 0°C under Ar. After 30 min the mixture was transferred via cannula to a second flask containing Cbz-Asp(OMe)OBO ester **5.38** (0.130 g, 0.36 mmol) and Davis' oxaziridine **4.92** (0.470 g, 1.80 mmol) in dry THF (5 mL) at –78°C under Ar. The mixture was stirred for 18 h at –78°C then poured into 3% NH_4Cl (5 mL) and extracted with Et_2O (3 × 25 mL), the organic fractions pooled and extracted with 10% $NaHCO_3$ (10 mL), brine (10 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure to reveal a light yellow oil which was purified by flash chromatography to give 0.069 g (51%) of **5.51** as a 3:1 mixture of inseparable diastereomers.

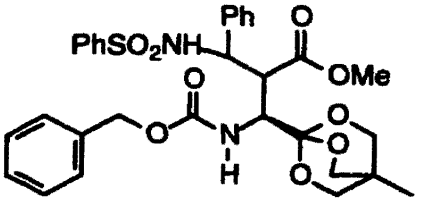


$[\alpha]^{20}_D$ = -18.1 (c = 0.95, CH_2Cl_2); TLC (1:1, EtOAc:Hex), R_f = 0.23; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.38-7.22 (m, 5H, Ar*H*), 5.88 (d, 1H, J = 10.1Hz, N*H*), 5.11 (d, 1H, J =

12.7Hz, CbzC*H*HO), 5.02 (d, 1H, *J* = 12.7Hz, CbzCH*H*O), 4.29-4.08 (m, 2H, α-C*H*, β-C*H*), 3.83 (s, 6H, OBO ester CH_2O), 3.59 (s, 3H, CO_2CH_3), 0.80 (s, 3H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 174.1 (C=O), 156.4 (CONH), 136.2, (Cbz=C=), 128.0, 127.7, 127.7 (Cbz=CH=), 108.1 (OBO ester C-O), 72.3 (OBO ester CH_2O), 66.1 (CbzCH_2O), 58.9, 58.5 (α-CH, β-CH), 51.1 (CO_2CH_3), 30.7 (OBO ester CCH_3), 13.9 (OBO ester CCH_3); ESI-MS (M + H^+) 381.97; Anal. calcd for $C_{18}H_{23}NO_8$: C, 56.69; H, 6.08; N, 3.67. Found: C, 56.99; H, 6.37; N, 3.75.

5.4.19 Methyl-(2*S*)-2-[(benzyloxy)carbonyl]amino-2-(4-methyl-2,6,7-trioxabicyclo-[2.2.2]oct-1-yl)-3-phenyl[(phenylsulfonyl)amino]methylpropanoate, 5.52.

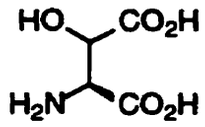
Isolated from 5.4.18.



TLC (1:1, EtOAc:Hex), R_f = 0.73; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.87-7.11 (m, 15H, Ar*H*), 5.74 (d, 1H, *J* = 10.2Hz, glu-N*H*), 5.11 (s, 2H, CbzCH_2O), 4.61 (d, 1H, *J* = 7.2Hz, SO_2NH), 4.20-4.01 (m, 2H, α-C*H*, sulfonamide-C*H*), 3.81 (s, 6H, OBO ester CH_2O), 3.42 (s, 3H, CO_2CH_3), 2.90-2.79 (m, 1H, β-C*H*), 0.84 (s, 3H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 174.5 (C=O), 156.0 (CONH), 140.9 (Ar=C=), 139.6 (Ar=C=), 136.9, (Cbz=C=), 132.0, 131.9, 128.9, 128.9, 128.5, 128.3, 127.9, 127.7, 126.9 (Cbz=CH=, Ar=CH=), 108.1 (OBO ester C-O), 72.6 (OBO ester CH_2O), 65.8 (CbzCH_2O), 58.9 (ArCHNH-), 52.1 (CO_2CH_3), 51.5 (α-CH), 49.0 (β-CH), 30.5 (OBO ester CCH_3), 13.7 (OBO ester CCH_3); ESI-MS (M + H^+) 611.40.

5.4.20 (2*S*)-2-Amino-3-hydroxybutanedioic acid, Asp(β-OH)OH, 5.5.

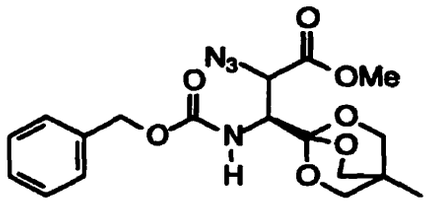
Same procedure as in section 5.4.14 to give 0.029 g (62%) of **5.5**.



2*S*,3*S*-**5.5**: $[\alpha]^{20}_{D}$ = +0.4 (c = 1.5, 1N HCl), (lit.[71] $[\alpha]^{27}_{D}$ = +1.3 (c = 3.12, 1N HCl); ^{1}H NMR (D_2O, 300 MHz) δ 4.89 (d, 1H, *J* = 3.6Hz, β-C*H*) 3.82 (d, 1H, *J* = 3.6Hz, α-C*H*); ^{13}C NMR (D_2O, 75 MHz) δ 178.2 (C=O), 173.7 (C=O), 69.3 (β-CH), 61.5 (α-CH); ESI-MS 149.91.

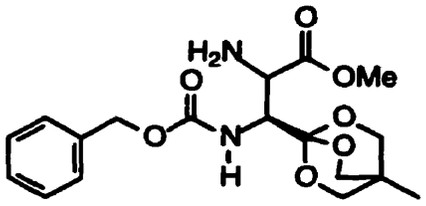
5.4.21 1-[(2*S*)-2-[(Benzyloxy)carbonyl]amino-1-(methoxycarbonyl)-2-(4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl)ethyl]-1,2-triazadien-2-ium, Cbz-L-Asp(β-N_3)(OMe)OBO ester, 5.53.

Cbz-Asp(OMe)OBO ester **5.38** (0.370 g, 1.01 mmol) was dissolved in dry THF (3 mL) then added by cannula to a flask containing LiHMDS (3.03 mL, 3.03 mmol) in dry THF (5 mL) at –78°C under Ar. After 1 h, trisyl azide **4.105** (0.624 g, 2.02 mmol) dissolved in dry THF (3 mL) was slowly added by syringe. After 18 h at –78°C, the mixture was allowed to warm to room temperature then poured into Et_2O (50 mL) and extracted with 3% NH_4Cl (2 × 20 mL), 10% $NaHCO_3$ (20 mL), brine (20 mL) and dried over $MgSO_4$ and the solvent removed under reduced pressure to give a bright yellow oil which was purified by flash chromatography to give **5.53** in 28% yield (0.114 g).



TLC (1:1, EtOAc:Hex), R_f = 0.40; ^{1}H NMR (Acetone-d_6, 300 MHz) δ 7.37-7.22 (m, 5H, Ar*H*), 5.44 (d, 1H, *J* = 10.2Hz, N*H*), 5.07 (m, 2H, CbzCH_2O), 4.19 (dd, 1H, *J* = 3.7, 10.2Hz, β-C*H*), 3.95-3.79 (s + m, 7H, OBO ester CH_2O, α-CH), 3.67 (s, 3H, CO_2CH_3), 0.80 (s, 3H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 172.3 (C=O), 156.6 (CONH), 136.4, (Cbz=C=), 128.2, 127.9, 127.6 (Cbz=CH=), 108.1 (OBO ester C-O), 72.3 (OBO ester CH_2O), 66.8 (CbzCH_2O), 59.9 (β-CH), 52.1 (CO_2CH_3), 52.2 (α-CH), 30.4 (OBO ester CCH_3), 14.1 (OBO ester CCH_3); FT-IR (cast from CH_2Cl_2) 3432, 2962, 2884, 2122, 1729, 1515, 1048; ESI-MS (M + H^+) 406.91; Anal. calcd for $C_{18}H_{22}N_4O_7$: C, 53.20; H, 5.46; N, 13.79. Found: C, 53.54; H, 5.85; N, 13.85.

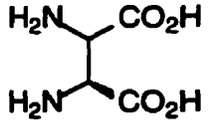
5.4.22 Methyl-(2*S*)-3-amino-2-[(benzyloxy)carbonyl]amino-2-(4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl)propanoate, Cbz-L-Asp(β-NH_2)(OMe)OBO ester, 5.54.
Cbz-L-Asp(β-N_3)(OMe)OBO ester **5.53** (0.114 g, 0.28 mmol) was combined with Ph_3P (0.088 g, 0.33 mmol) and dissolved in dry THF (5 mL) then stirred at room temperature for 24 hours. Distilled water (20 μL) was then added and the mixture refluxed for 3 hours. The solvent was removed under reduced pressure to give a white solid that was purified by flash chromatography (9:1, EtOAc:MeOH 0.5% NH_4OH) to give **5.54** in 67% yield (0.071 g).



TLC (9:1, EtOAc:MeOH 0.5% NH_4OH); ^1H NMR (Acetone-d_6, 300 MHz) δ 7.37-7.24 (m, 5H, Ar*H*), 5.64 (d, 1H, *J* = 10.1Hz, N*H*), 5.11 (m, 2H, CbzCH_2O), 4.21-3.75 (m + s, 8H, α-C*H*, β-C*H*, OBO ester CH_2O), 3.69 (s, 3H, CO_2CH_3), 0.83 (s, 3H, OBO ester CCH_3); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 172.3 (C=O), 156.1 (CONH), 137.4, (Cbz=C=), 128.3, 127.9, 127.7 (Cbz=CH=), 108.4 (OBO ester C-O), 72.3 (OBO ester CH_2O), 65.8 (CbzCH_2O), 59.8 (β-CH), 52.3 (α-CH), 51.2 (CO_2CH_3), 32.4 (OBO ester CCH_3), 13.6 (OBO ester CCH_3); ESI-MS (M + H^+) 380.94; Anal. calcd for $C_{18}H_{24}N_2O_7$: C, 56.84; H, 6.36; N, 7.36. Found: C, 57.13; H, 6.85; N, 7.42.

5.4.23 (2*S*)-2,3-diaminobutanedioic acid, Asp(β-NH_2)OH, 5.6.

Same procedure as in section 5.4.14 to give 0.029 g (62%) of **5.5**.



^1H NMR (D_2O, 300 MHz) δ 4.09, 4.00, 3.75, 3.69 (d, 1H, *J* = 3.4Hz, β-C*H*, α-C*H*); ^{13}C NMR (D_2O, 75 MHz) δ 174.0 (C=O), 173.8 (C=O), 62.0, 61.5 (α-CH, β-CH); ESI-MS 148.87.

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Chapter Six

Solid Phase Synthesis of β-Hydroxy-β-Substituted-α-Amino Acids

6.1 Introduction

Traditionally, the goal of medicinal chemists has been to produce single compounds in as pure a form as possible for submission to biological testing and evaluation as drug candidates. This methodical approach to the development of new drug entities is tedious, expensive and unavoidably time-consuming.

A combination of necessity as the mother of invention and economic factors thereby directed the development of a new method for the rapid synthesis and screening of compounds, evolving into the field of combinatorial chemistry. In essence, combinatorial chemistry involves both the synthesis and screening of large groups of compounds known as libraries. These techniques have revolutionized the pharmaceutical industry and are gradually expanding to other fields of the physical sciences. In fact, this method has been used to produce or optimize lead compounds in many different research areas and has progressed from its early strategy in generating random libraries into a powerful design approach for developing and optimizing potential candidates.[1]

The primary goal of combinatorial chemistry is the generation of compounds with a broad structural diversity, the synthesis of which should occur in a precise manner in order to facilitate screening. Solid-phase organic synthesis, which has many similarities to solid-phase peptide techniques, therefore uses well-defined organic reactions to minimize side-reactions on the solid phase. As such, the purity of the final product after cleavage off the resin is of paramount importance.

One of the challenges of solid-phase organic chemistry (SPOC) is the monitoring of reactions on the solid phase. Classical methods, such as TLC, are obviously incompatible with resin-bound synthons, and as such a number of specialized techniques have been developed.[2] These include single-bead FT-IR and magic angle spinning NMR (MAS-NMR) as well as a number of pulse programs that take advantage of the difference in relaxation rates between the resin and the "epitope" of interest. However, rather than provide a detailed review of the field of combinatorial chemistry and all of its peculiarities, a few key points of particular relevance to the solid phase synthesis of unusual α-amino acids will be discussed. Readers are directed to the following excellent references by Wilson and Czarnik,[3] Dolle[4] and Baldino[5] for further reading.

In order to exploit the benefits of solid-phase organic chemistry (SPOC), solution phase organic reactions should be shown to possess the same characteristics on the solid-phase. Having described the synthesis of various β-hydroxy-α-amino acids in some detail,[6] we attempted to transfer the synthetic methodology onto the solid-phase as described in this chapter.

6.1.1 Solid-Phase Synthesis of α-Amino Acids

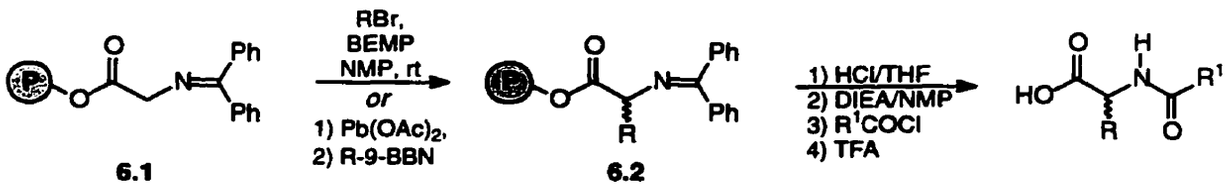
Considering that solid-phase organic chemistry was born from the synthesis of peptides on the solid phase first reported by Merrifield,[7] remarkably few syntheses of unusual α-amino acids on the solid phase have been reported, especially since the therapeutic value of amino acid based drugs is well documented.[8] α-Amino acids are also useful intermediate chiral building blocks and have been used toward the synthesis of peptides,[9] small molecule combinatorial libraries[10] and as components in condensation

reactions such as the Ugi condensation.[11] Furthermore, the inherent functionality of α-amino acids provides a convenient handle for additional derivatization.[12]

O'Donnell *et al.*[13] first reported the synthesis of unnatural α-amino acids on the solid phase with the goal of synthesizing unnatural peptides and a number of other groups have expanded on O'Donnell's chemistry. A resin-bound Schiff base **6.1**, generated with the non-ionic base 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphos-phorine (BEMP), was alkylated with either alkyl halides[13,14,15] or organoboranes[16] to give the resin-bound products **6.2** in 51-99% yield and high purity (>75%) (Scheme 6.1). Enantiomeric excesses range from 64-99% ee depending on the alkyl group but generally increasing with size. The peptide could then be extended through peptide coupling. The same authors extended the Schiff base method towards the synthesis of α,α-disubstituted-amino acids by starting with various amino acids and alkylating with benzyl, allyl or 2-naphthylmethyl bromide.[17]

Miyabe and co-workers took a similar approach to unnatural α-amino acids, using alkyl radicals to alkylate polymer-supported glyoxylic oxime ethers.[18]

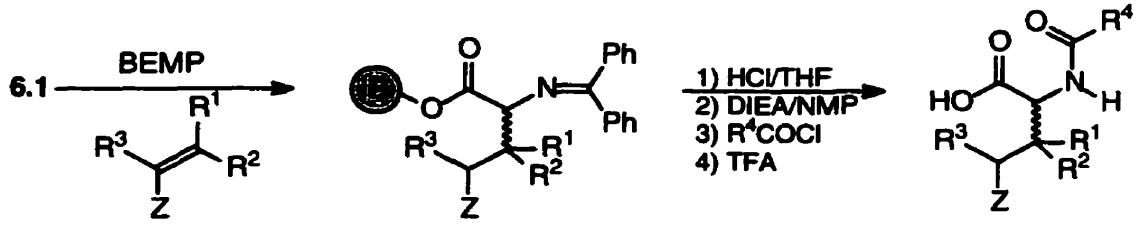
Scheme 6.1



O'Donnell has also used the same Schiff base methodology in the synthesis of racemic glutamic acid derivatives via Michael reactions. The Schiff base of **6.1** was condensed with the Michael acceptor to give the resin-bound glutamic acid derivatives

6.3 in 61-88% yield and purities of 70-95% (Scheme 6.2).[19] This methodology was later expanded by using chiral phase transfer catalysts to synthesize simple glutamic acid analogs in 34-82% ee.[20]

Scheme 6.2

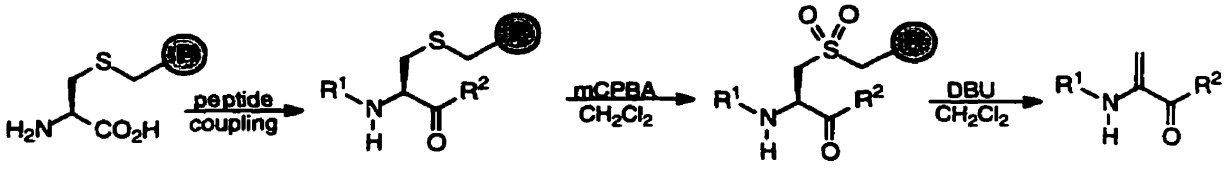


6.3

R^1,R^2,R^3=CO_2Me, Me, Ph
Z=CO_2Me, CN, SO_2Ph

The recognized therapeutic properties of dehydroamino acids **6.6**[21] and their incorporation in peptide libraries have been addressed by the DBU catalyzed elimination of the sulfone **6.5** generated by oxidation of the derivatized cysteine **6.4** attached to the resin via the sidechain (Scheme 6.3). The dehydro-peptides **6.6** were generated in 31-86% yield over 4 steps and generally >90% purity. Cycloadditions have also been performed on the solid-phase on Fmoc-dehydroalanine generated through a similar oxidation/elimination strategy in which cysteine is attached to the resin via the α-carboxylate.[22]

Scheme 6.3



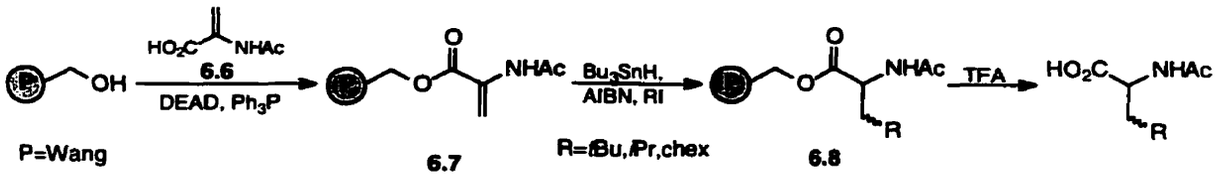
6.4 **6.5** **6.6**

P=Merrifield

R^1=Boc, Cbz, Cbz-Phe, 1-Nap
R^2=OMe, OBn, NHBn, PheOEt

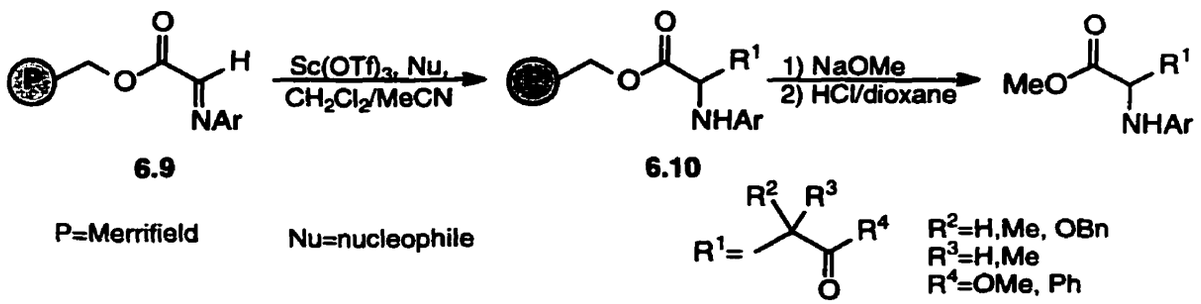
Dehydroamino acids have also been used as intermediates towards the solid-phase synthesis of α-amino acids.[23] Radical additions to resin-bound dehydroamino acids **6.7** provided various racemic α-amino acids **6.8** in both high yield and purity (Scheme 6.4).

Scheme 6.4



Polymer supported α-imino acetates **6.9** have been used to synthesize α-amino libraries via a Mannich-type reaction with various silyl nucleophiles to afford resin-bound γ-oxo-α-amino acids **6.10** (Scheme 6.5).[24] Cleavage off the resin gave the desired products in 69-94% yield and high purity although no comments were made regarding enantioselectivity in the Mannich-type reaction.

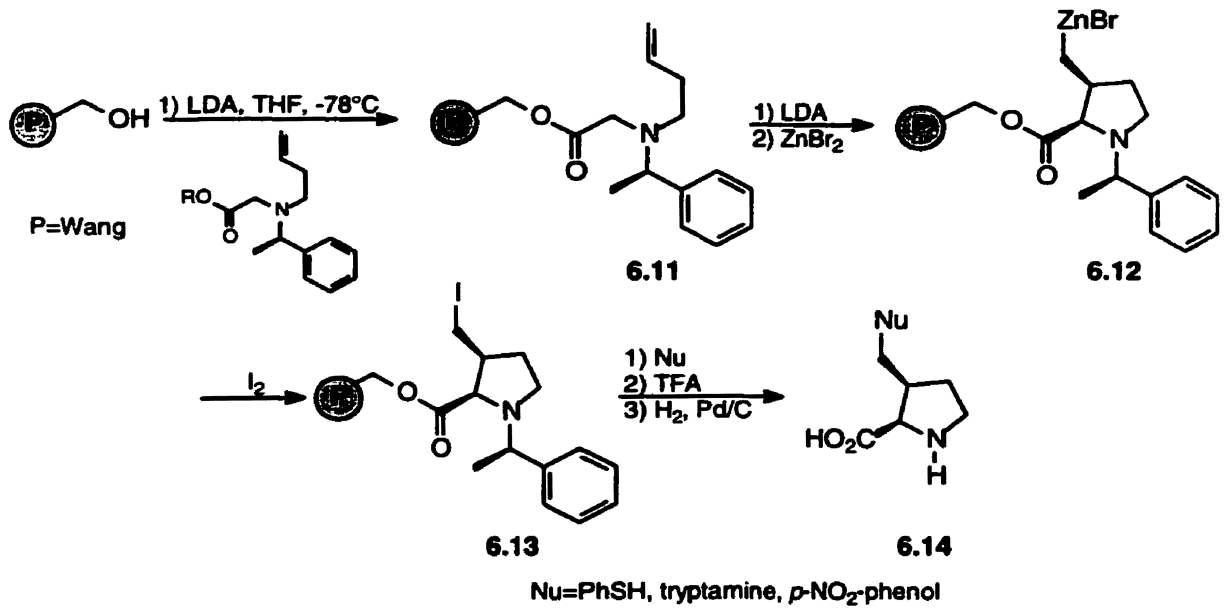
Scheme 6.5



3-Substituted prolines **6.14** have been synthesized diastereoselectively by amino-zinc-enolate cyclization chemistry (Scheme 6.6).[25] Esterification with the oxy anion gave **6.11** which was cyclized to give the cyclic organozinc derivative **6.12**. Iodonolyzation gave **6.13** which could be further derivatized via nucleophilic substitution then cleaved

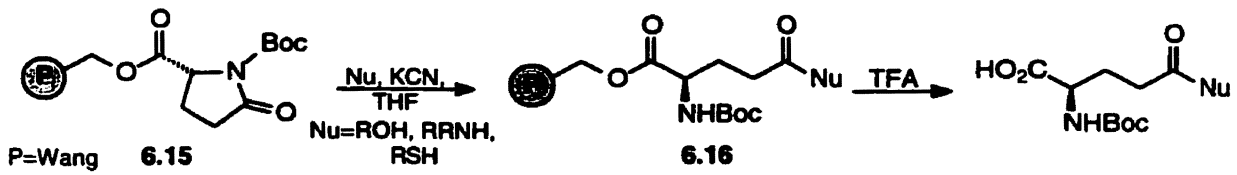
off the resin under classical conditions and N-debenzylated to give the 3-substituted prolines **6.14**. However, no comments were made regarding the final yield or purity of **6.14** after cleavage off the resin.

Scheme 6.6



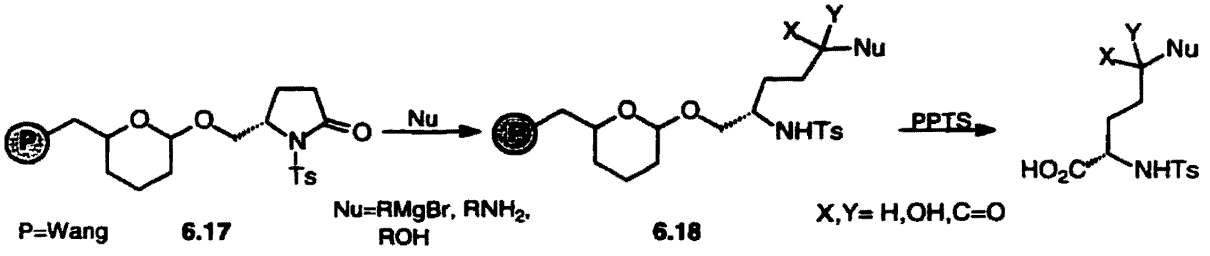
Various nucleophiles have been added to pyroglutamate **6.15** attached to Wang resin to give glutamate derivatives **6.16** which were cleaved off the resin with TFA (Scheme 6.7).[26] The use of a catalyst (KCN) was required with most of the reactions occurring in good yield and high purity.

Scheme 6.7



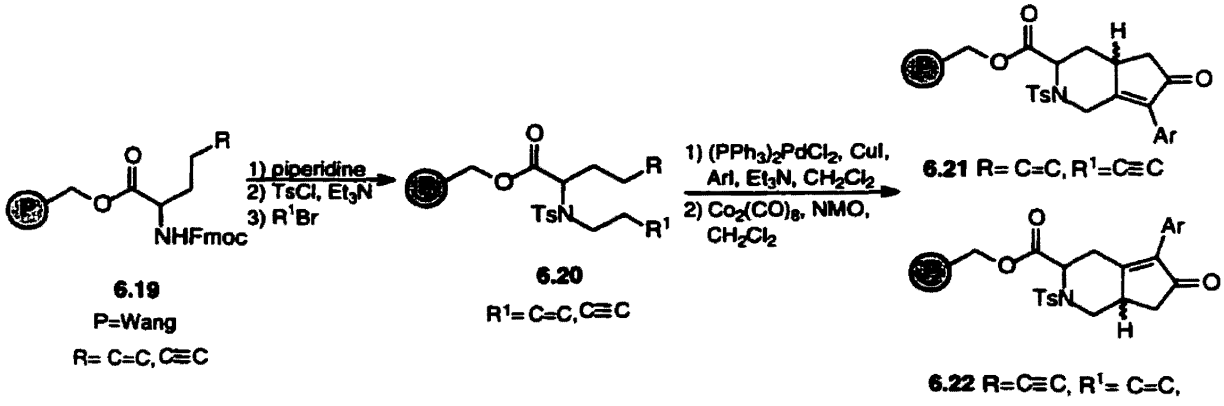
Pyroglutamate was reduced into the pyrollidinone alcohol that was then attached to Ellman's resin-bound tetrahydropyran (THP) to give the N-tosyl lactam **6.17**.[27] Treatment with various nucleophiles opened the lactam to give resin-bound derivatives **6.18** which after cleavage off the resin gave N-tosyl amines in both high yield and purity.

Scheme 6.8



Functionalized fused bicyclic amino acids **6.21/6.22** have been synthesized by an intramolecular Pauson-Khand cyclization (Scheme 6.9).[28] The position of various functional groups within the hexahydro-1H-[2]pyrindinone construct is determined by the order of addition of various alkenes and alkynes in the intermediate **6.20**. Good overall yields and purities were achieved as well as excellent diastereoselectivities during the Pauson-Khand cyclization.

Scheme 6.9



The distinct lack of solid-phase methods for the synthesis of unusual α-amino acids prompted us to investigate our previously described serine aldehyde methodology for incorporation onto the solid-phase. We herein describe the stereoselective synthesis of β-hydroxy- and β-disubstituted-β-hydroxy-α-amino acids on solid supports.

6.2 Results and Discussion.

The solution-phase synthesis of β-hydroxy- and β-disubstituted-β-hydroxy-α-amino acids via a serine aldehyde equivalent has been described in detail in chapter two. Incorporating the β-hydroxy functionality on α-amino acids on the solid phase provides an opportunity to further functionalize the amino acid and extend peptide chains off the sidechain of the newly synthesized solid-supported β-hydroxy-α-amino acid (Figure 6.1). Consequently, a number of both cyclic and linear peptides incorporating a modified β-hydroxy moiety may be envisioned. The well characterized nature of the reaction, having been performed with a variety of solvents, protecting groups, temperatures and nucleophiles prompted us to transfer the serine and threonine methodology onto the solid-phase.

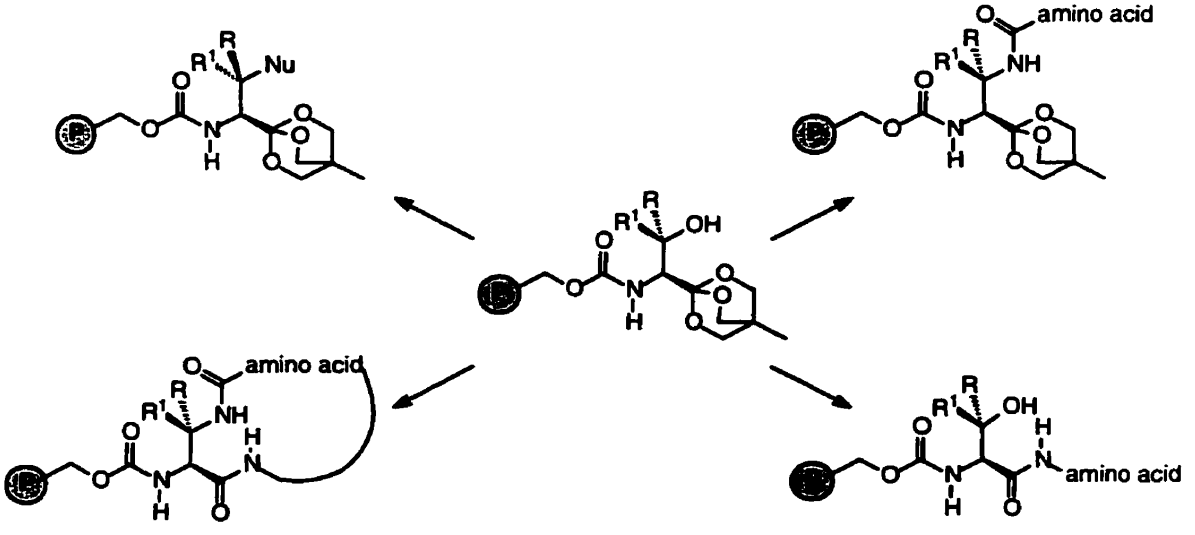


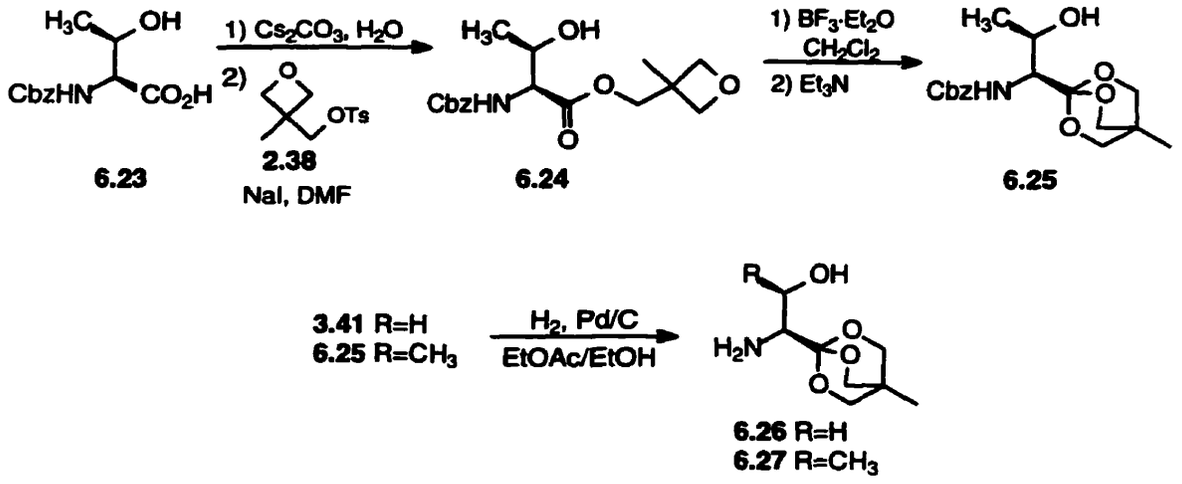
Figure 6.1: Possible Functionalization of Solid-Supported β-Hydroxy-α-Amino Acids.

6.2.1 Synthesis of Resin-Bound β-Hydroxy-β-Substituted-α-Amino Acids

We studied two common polymers, Wang resin and TentaGel PHB® resin, on which to perform the sequence of reactions described in detail in chapter two. Wang resin is generally the resin of choice for SPOC due to its excellent swelling characteristics, straightforward cleavage of substrates off the resin and low price. TentaGel PHB® resin incorporates a polyethylene glycol (PEG) spacer, and as such possess swelling characteristics and reaction kinetics more typical of solution-phase chemistry.[29] We choose the Cbz-equivalent linker introduced by Hauske and Dorff[30] to attach the α-amino group since manipulation of the α-carboxylate into the OBO ester was necessary to prevent racemization during Grignard reaction on the serine aldehyde.

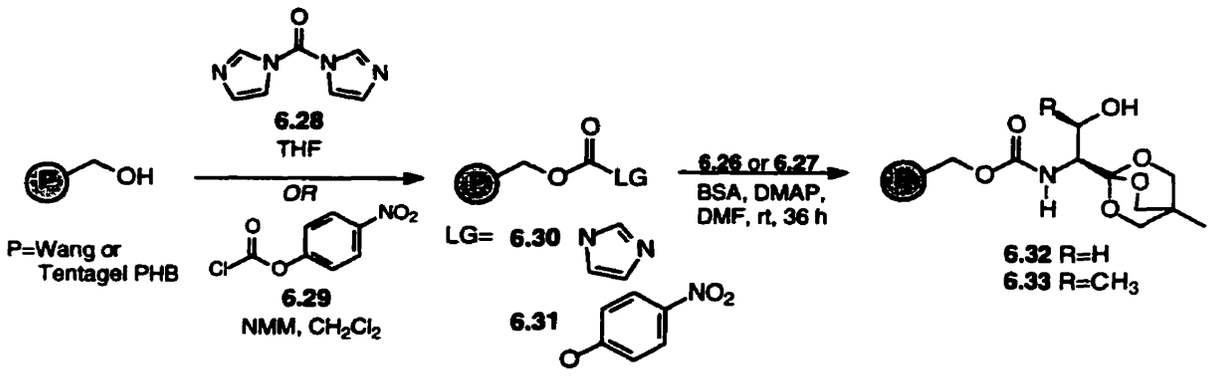
The serinal equivalent was incorporated onto the resin as the OBO ester since preliminary studies indicated $BF_3 \cdot Et_2O$ mediated rearrangement on the resin to be problematic. Cbz-Thr-OBO ester **6.25** was synthesized in 92% yield over two steps, in an identical manner to that described for Cbz-Ser-OBO ester **3.41** (Scheme 6.10). The Cbz protecting group was easily removed from both **6.25** and **3.41** by hydrogenolysis to give the free amino OBO esters **6.26** and **6.27** in quantitative yield. Of note is the fact that both **6.26** and **6.27** were stored at room temperature for over three years without lost of optical purity, presumably due to the acid sequestering nature of the free amine.

Scheme 6.10



In order to attach the amino acids **6.26** and **6.27** onto the resin via their N-termini, conversion of the methylene hydroxyl moiety in both Wang (0.74 mmol/g loading) and TentaGel PHB® (0.30 mmol/g loading) resins into a carbamate linker was necessary. The resins were activated as either the imidazolide carbamate **6.30** using *N,N'*-carbonyldiimidazole **6.28** in THF or the *p*-nitrophenol carbonate **6.31** using *p*-nitrophenolchloroformate **6.29** and *N*-methyl morpholine (NMM) in CH_2Cl_2 (Scheme 6.11). Loading was monitored by elemental analysis for nitrogen and was generally quantitative for both carbonyldiimidazole **6.28** and *p*-nitrophenolchloroformate **6.29** (see Appendix E for calculation) on both Wang and TentaGel PHB® resin. The imidazolide and carbonate resins were both stable for six months without loss of activity.

Scheme 6.11



The amino acid derivatives **6.26** and **6.27** were coupled to the resin in the presence of *N,O*-bis(trimethysilyl)acetamide (BSA) and DMAP to give the resin-bound amino acid OBO esters **6.32** and **6.33**. After 36 h, the remaining reactants were thoroughly washed off the resin with DMF and CH_2Cl_2 till the rinsing was clear. Loading was determined by either elemental analysis or in the case of **6.31**, the rinsings combined, reduced in volume then assayed using spectrophotometric techniques for *p*-nitrophenol content. Substitution generally occurred in >95% yield but varied from batch to batch. The resin was then capped by rinsing with copious amounts of methanol, rinsed with Et_2O then dried under vacuum.

The progress of reactions could be monitored by a number of techniques specially developed for SPOC. FT-IR is particularly useful due to changes in functional groups. For example, **6.31** to **6.32** is characterized by a 1761 cm^{-1} to 1720 cm^{-1} shift. MAS-NMR was used when pronounced changes occurred in the ^1H NMR, such as the appearance of the OBO ester CH_2-O in **6.32** using a 2D TOCSY,[31] spin-echo[32] or presaturation pulse experiment (Figure 6.2).[33]

Oxidation to the aldehyde **6.34** and ketone **6.35** was more difficult than in solution phase for a number of reasons. Swern oxidation required transfer of the alcohol into a –78°C mixture of the generated chlorosulfonium salt which proved to be difficult since the alcohol was bound to the resin. TentaGel PHB® resin was particularly troublesome due to its "sticky" nature (a consequence of the PEG spacer) in comparison to Wang resin. However, after numerous different experimental setups, the arrangements depicted in figure 6.3 were found to be most convenient: (a) for additions of resin <500 mg and (b) for >500 mg.

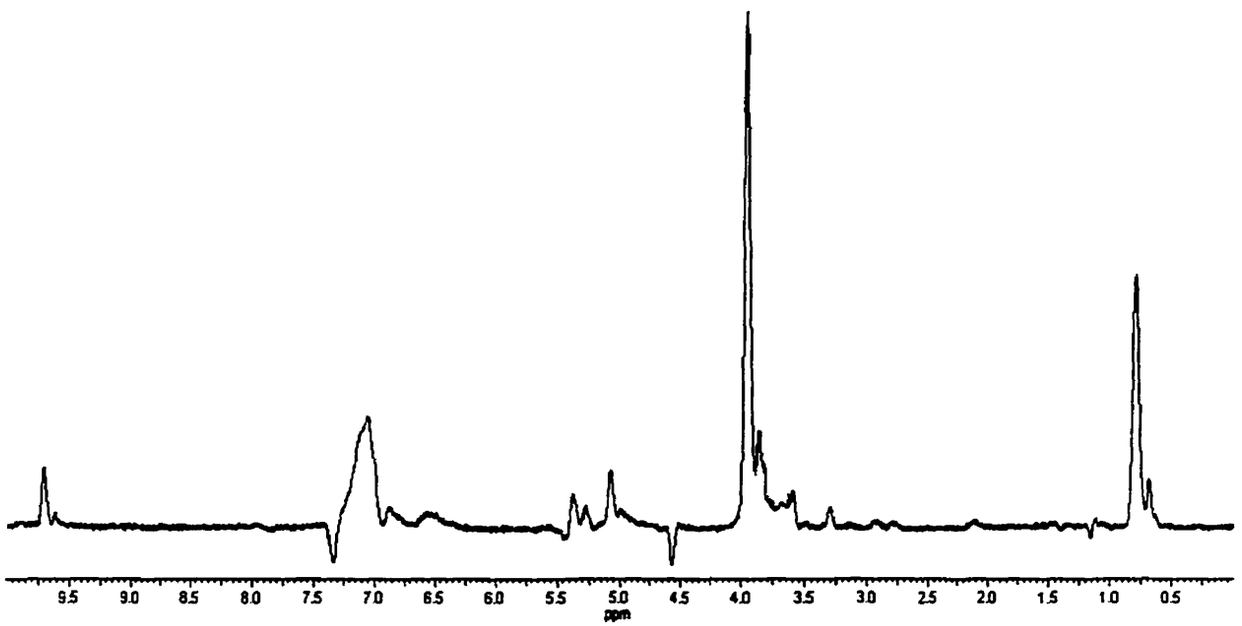


Figure 6.2: MAS ^{1}H-NMR (500 MHz) with spin-echo pulse program of 6.34 (P=Wang).

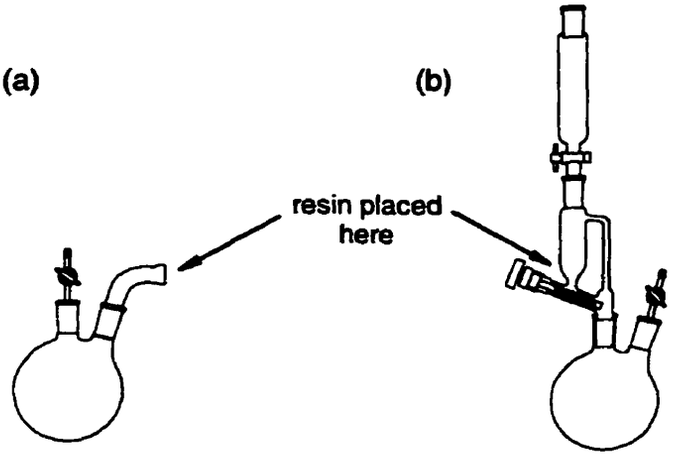
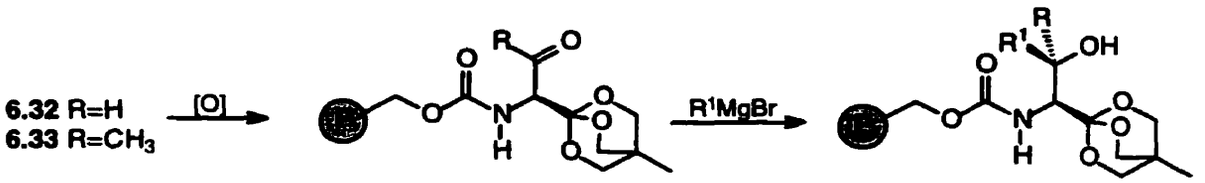


Figure 6.3: Experimental setup for Swern Oxidation. (a) Requires tilting of the RBF to transfer the resin into the mixture (b) resin is added to the mixture and any remaining beads washed into solution.

The optimized conditions for oxidation were determined by observing the appearance of the aldehyde peak for **6.34** or the methyl ketone for **6.35** in the MAS ^{1}H NMR (Figure 6.2). The requirement of high purity in the final product necessitated quantitative conversion to the aldehyde **6.34**/ketone **6.35** to prevent contamination with remaining serine or threonine. Large excesses of reagents for the Swern oxidation were required, (16-50 eq.) since performing the reaction on a scale comparable to that in solution phase (1.6-5 eq.) failed to give any oxidation product. Eight hours at –78°C was required for complete conversion to the aldehyde **6.34**/ketone **6.35** (Scheme 6.12). Other methods of oxidation were also investigated including the Doering oxidation (pyr·SO_3/DMSO),[34] Dess-Martin oxidation with **2.48**,[35] and TPAP (tetrapropyl ammonium perruthenate)/NMO[36] with little success.

Scheme 6.12



6.34 R=H
6.35 R=CH_3

6.36 R=H, R^1=Me
6.37 R=H, R^1=$^{13}CH_3$
6.38 R=H, R^1=Et
6.39 R=H, R^1=Ph
6.40 R=H, R^1=Bn
6.41 R=H, R^1=vinyl
6.42 R=R^1=Me
6.43 R=Me, R^1=$^{13}CH_3$
6.44 R=Me, R^1=Et
6.45 R=Me, R^1=Ph

Grignard addition to aldehyde **6.34** and ketone **6.35** was performed using essentially identical conditions as those reported in chapter two. Prolonged reaction times and a twenty-fold excess of Grignard reagent was required. The appearance of the desired resin-bound β-hydroxy-α-amino acids **6.36-6.44** was monitored using MAS ^{1}H NMR to follow the disappearance of the aldehyde peak **6.34** at δ 9.67 ppm and the methyl ketone **6.35** at δ 2.30 ppm (Scheme 6.12). MAS ^{13}C NMR was used to follow **6.37** and **6.43**, labeled by $^{13}CH_3MgI$ (Figure 6.4). The use of Et_2O in the reaction mixture was also minimized since it caused significant shrinkage of the resin, particularly TentaGel PHB®. After approximately 6 h, acetone was added and the solvents were removed under anhydrous conditions. The resin was then washed with dry CH_2Cl_2 and Et_2O.

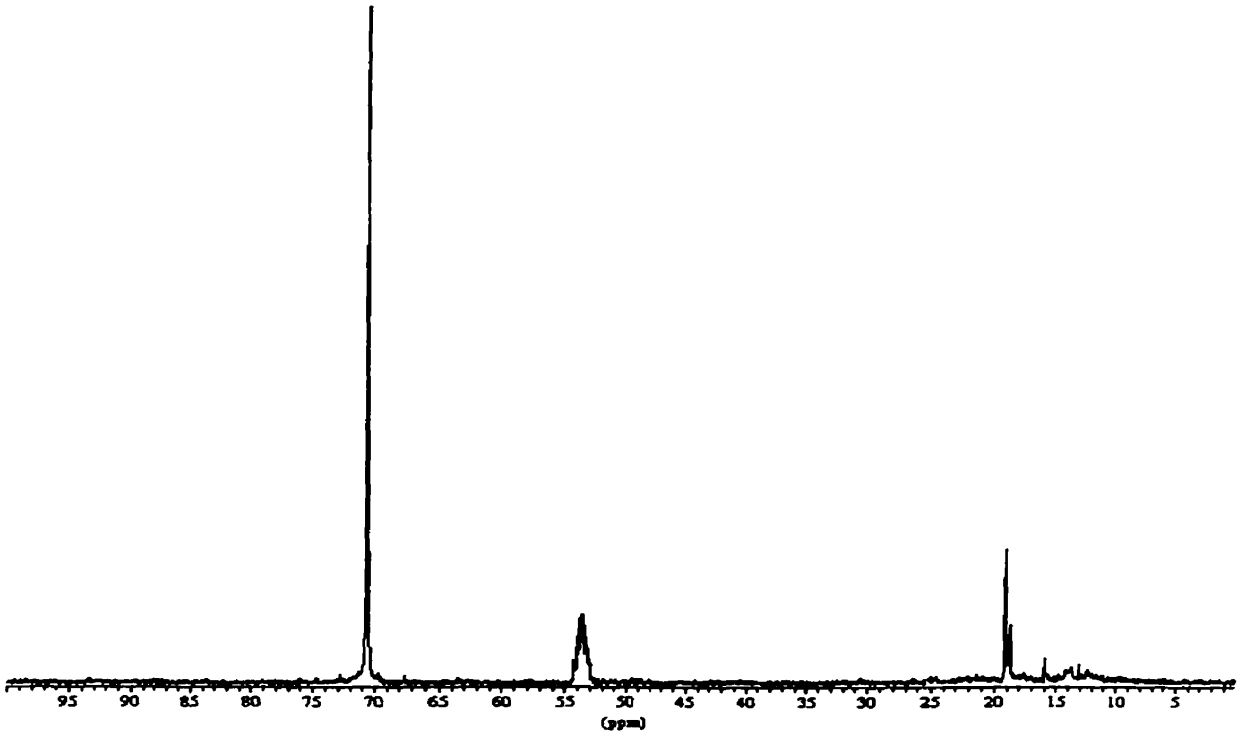
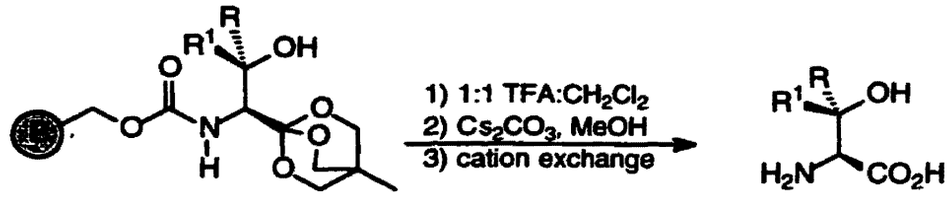


Figure 6.4: MAS ^{13}C-NMR (500 MHz) using spin-echo program of 6.37 (P=TentaGel® PHB resin).

Cleavage of **6.36-6.45** off the resin was accomplished with TFA and subsequent hydrolysis of the mhpd ester **2.62** with Cs_2CO_3 gave the free β-hydroxy-α-amino acids **6.46-6.55** (Scheme 6.13). The free amino acids cleaved off Wang resin generally showed purities of >90% as indicated by ^{1}H NMR and HPLC analysis and were observable by ESI-MS with the exception of **6.55** which was not isolated. Unfortunately, the amino acids cleavage off TentaGel PHB® resin were contaminated with up to 30% PEG from the spacer on the resin. Lower concentrations of TFA decreased the amount of PEG contamination but prolonged reaction times were required for complete cleavage off the resin. Cation exchange chromatography was necessary for the removal of contaminating PEG and cleavage salts and is describe in section 2.4.15. No significant difference in yield or purity exists between Wang and TentaGel PHB® resin as indicated in table 6.1.

Scheme 6.13



6.36-6.45

6.46 R=H, R^1=Me
6.47 R=H, R^1=$^{13}CH_3$
6.48 R=H, R^1=Et
6.49 R=H, R^1=Ph
6.50 R=H, R^1=Bn
6.51 R=H, R^1=vinyl
6.52 R=R^1=Me
6.53 R=Me, R^1=$^{13}CH_3$
6.54 R=Me, R^1=Et
6.55 R=Me, R^1=Ph

Overall yields of the β-hydroxy-α-amino acids after purification ranged from 19-41% on Wang or TentaGel PHB® resin. Derivatization and HPLC analysis (as described in section 2.4.15a) indicated a slight reduction in the diastereoselectivity of addition when compared to Grignard addition in the solution phase to Boc, Cbz and Fmoc protected Ser(ald)-OBO ester **2.46**, **3.42** and **2.37** respectively (Table 6.1).[6] The optimized solvent conditions for the addition of Grignard reagents described in chapter two is a 1:1 mixture of Et_2O:CH_2Cl_2. However, as previously described, the swelling problems associated with the use of Et_2O meant it was only added to the reaction mixture during addition of the Grignard reagent. Of note, is the observation in chapter two that the use CH_2Cl_2 as the reaction solvent results in an erosion of diastereoselectivity.

HPLC analysis also indicated a significant amount of racemization (15-18%) was occurring. Two likely sources are during Swern oxidation or after addition of the Grignard reagent. Racemization has been shown to occur during the Swern oxidation,[37]

Table 6.1: Addition of R^1MgBr to 6.34 and 6.35.

Entry	Resin	R	R^1	Product	Yield (%)[a]	ds[b] *threo:erythro*	Racemization (%)[b]
1	Wang	H	Me	**6.46**	30	80:20	18
2	Wang	H	$^{13}CH_3$	**6.47**	32	80:20	17
3	Wang	H	Et	**6.48**	29	83:17	17
4	Wang	H	Ph	**6.49**	25	87:13	16
5	Wang	H	Bn	**6.50**	24	64:36	18
6	Wang	H	vinyl	**6.51**	25	81:19	18
7	Wang	CH_3	Me	**6.52**	27	-	15
8	Wang	CH_3	$^{13}CH_3$	**6.53**	26	-	15
9	Wang	CH_3	Et	**6.54**	29	85:15	15
10	Wang	CH_3	Ph	**6.55**	ni[c]	-	-
11	TentaGel	H	Me	**6.46**	41	81:19	18
12	TentaGel	H	Et	**6.48**	40	82:18	16
13	TentaGel	H	Ph	**6.49**	22	85:15	16
14	TentaGel	H	Bn	**6.50**	29	69:31	15
15	TentaGel	H	vinyl	**6.51**	19	81:19	17
16	TentaGel	CH_3	Me	**6.52**	38	-	18
17	TentaGel	CH_3	Et	**6.54**	32	83:17	13
18	TentaGel	CH_3	Ph	**6.55**	ni[c]	-	-

[a] After purification by cation exchange chromatography.
[b] Determined by derivatization and HPLC analysis.
[c] Not isolated.

but is typically dependent on individual systems and cannot be predicted. The prolonged exposure to base under conditions required to drive the oxidation to completion might be responsible for causing racemization. On the other hand, enolization is frequently encountered during Grignard reactions[38] and would cause racemization if occurring with **6.34** and **6.35**. Although the Grignard reaction time on the solid phase is longer than in

solution, enolization was previously dismissed as a source of racemization for Boc and Fmoc protected Ser(ald)-OBO esters **2.46** and **2.47** as described in chapter two. Reducing the reaction time during Swern oxidation resulted in significant deterioration of purity, a result of incomplete oxidation since HPLC analysis indicated serine or threonine contamination after cleavage off the resin.

The stereochemistry of addition is identical to that observed in solution phase chemistry, confirmed by the comparison of HPLC products derived after cleavage off the resin to those reported previously[6] and in chapter two. HPLC analysis generally showed all four diastereomers although the very minor *erythro*-D-isomer was usually difficult to identify. MAS ^{13}C NMR of **6.37** also clearly showed two stereoisomers (Figure 6.2).

β-Dialkyl-β-hydroxy-α-amino acid **6.55** was not isolated and is consistent with the reported instability of this class of compounds.[39]

6.3 Summary

Attachment of Ser-OBO ester and Thr-OBO ester onto the solid phase via a carbamate linker allowed for the oxidation and subsequent Grignard addition of various nucleophiles towards the synthesis of β-substituted-β-hydroxy-α-amino acids. Moderate diastereoselectivity for Grignard addition was observed to give predominantly the *threo* isomer. Good overall yields (19-41%) were achieved over six steps after purification by cation exchange

The synthesis of β-hydroxy-α-amino acids on the solid phase has been shown to possess similar characteristics as solution phase chemistry, albeit with an increased occurrence of racemization.

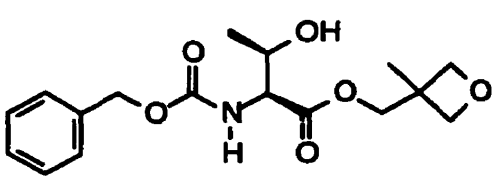
To our knowledge, this represents the first report of β-substituted-β-hydroxy-α-amino acids on the solid phase.

6.4 Experimental

Wang (1.04 mmol/g to 0.74 mmol/g) and TentaGel PHB® (0.33 mmol/g to 0.29 mmol/g) resin were purchased from Advanced ChemTech or Novabiochem. All NMR spectra were acquired using a Bruker AMX-500 NMR. Approximately 1-2 mg of resin was placed in a 4 mm MAS zirconia rotor with Kel-F cap and a spherical insert (diameter 2.6 mm). Samples were spun at the magic angle at a rate of 4 kHz and were run in CD_2Cl_2 and referenced internally to residual CH_2Cl_2.

6.4.1 (3-Methyl-3-oxetanyl)methyl (2*S*,3*R*)-2-[(benzyloxy)carbonyl]amino-3-hydroxybutanoate, Cbz-Thr oxetane ester, 6.24.

Cbz-L-Thr **6.23** (5.06 g, 0.02 mol) and Cs_2CO_3 (3.91 g, 0.012 mol) were combined and dissolved in H_2O (100 mL). The water was then removed *in vacuo* and the resulting oil lyophilized for 12 hours to give a white foam. To this foam was added oxetane tosylate **2.38** (5.38 g, 0.021 mol) and NaI (0.6 g, 4.0 mmol) which was then taken up in DMF (200 mL) and allowed to stir under Ar for 48 hours. The DMF was then removed *in vacuo* and the resulting solid dissolved in EtOAc (300 mL) and H_2O (100 mL) and extracted with 10% $NaHCO_3$ (2 × 50 mL), saturated NaCl (50 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to yield a yellow oil which was recrystallized from ethyl acetate to give long, clear crystals in 96.8% yield (6.80 g).

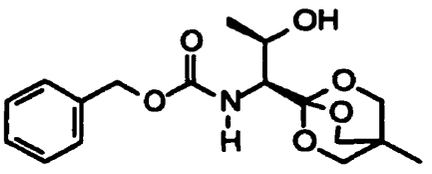


mp 49-51°C; $[\alpha]^{20}_D$ = -18.2 (c = 1.12, EtOAc); TLC (3:1 EtOAc:hexane), R_f = 0.46; ^1H NMR ($CDCl_3$, 250 MHz) δ 7.32-7.36 (m, 5H, Ar*H*), 5.66 (d, 1H, *J* = 8.9 Hz, N*H*), 5.14

(s, 2H, CbzCH_2O), 4.57-4.40 (m, 7H, 3 oxetane ester CH_2O, β-C*H*), 4.13 (d, 1H, *J* = 11.2Hz, α-C*H*), 2.82 (br d, 1H, *J* = 4.9Hz, O*H*), 1.28 (s, 3H, oxetane ester CCH_3), 1.26 (d, 3H, *J* = 8.0Hz, β-CH_3); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 171.2 (C=O), 156.7 (C=O), 136.0 (Cbz=C=), 128.5, 128.2, 128.0 (Cbz=CH), 79.4 (oxetane ester CH_2O), 68.8 (β-CH), 67.9 (CO_2CH_2), 67.1 (CbzCH_2O), 59.5 (α-CH), 39.6 (oxetane ester CCH_3), 20.7 (oxetane ester CCH_3), 19.9 (β-CH_3); Anal. calcd for $C_{17}H_{23}NO_6$: C, 60.52; H, 6.87; N, 4.15. Found : C, 60.42; H, 7.04; N, 4.18.

6.4.2 Benzyl-*N*-[(2*S*,3*R*)-3-hydroxy-1-(4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl)-propyl]carbamate, Cbz-L-Thr-OBO ester, 6.25.

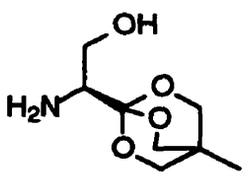
Cbz-Thr oxetane ester **6.24** (15.0 g, 46.2 mmol) was dissolved in dry CH_2Cl_2 (450 mL) and cooled to 0°C under Ar. $BF_3 \cdot Et_2O$ (0.29 mL, 2.31 mmol) was diluted in CH_2Cl_2 (5.0 mL) and added to the reaction flask. The reaction was allowed to warm up to room temperature and was checked by TLC. After 6 hours, Et_3N (1.29 mL, 9.25 mmol) was added and the reaction was stirred for an additional 30 minutes before being concentrated to a thick oil. The crude product was redissolved in EtOAc (400 mL) and washed with 3% NH_4Cl (2 × 250 mL), saturated NaCl (250 mL), dried ($MgSO_4$), and evaporated to dryness. The reaction yielded a colourless thick oil in 95% (14.2 g) yield. The clear colourless oil was crystallized from EtOAc to give rod-like shiny crystals in 93% (13.6 g) yield.



mp 117.0-118.0°C; $[\alpha]^{20}_D$ = -12.1 (c = 1.06, EtOAc); TLC (3:1 EtOAc:hexane), R_f = 0.40; ^{1}H NMR ($CDCl_3$) δ 7.27-7.38 (m, 5H, Ar*H*), 5.33 (d, 1H, *J* = 10.3Hz, N*H*), 5.07-5.20 (m, 2H, CbzCH_2O), 4.36 (m, 1H, β-C*H*), 3.93 (s, 6H, OBO ester CH_2O), 3.75 (m, 1H, α-C*H*), 2.88 (br s, 1H, O*H*), 1.12 (d, 3H, *J* = 6.4Hz, β-CH_3), 0.82 (s, 3H, OBO ester CCH_3); ^{13}C NMR ($CDCl_3$) δ 156.9 (C=O), 136.5 (Cbz=C=), 128.4, 127.9, 127.9 (Cbz=CH), 108.7 (OBO ester C-O), 72.7 (OBO ester CH_2O), 66.8 (CbzCH_2O), 65.1 (β-CH), 57.9 (α-CH), 30.6 (OBO ester CCH_3), 18.9 (β-CH_3), 14.3 (OBO ester CCH_3); Anal. calcd for $C_{17}H_{23}NO_6$: C, 60.52; H, 6.87; N, 4.15. Found: C, 60.29; H, 6.82; N, 3.94.

6.4.3 (2*S*)-2-Amino-1-(2,6,7-trioxabicyclo[2.2.2]oct-1-yl)ethan-3-ol, Ser-OBO ester, 6.26.

Cbz-Ser-OBO **3.41** (5.0 g, 15.3 mmol) was dissolved in EtOAc:EtOH (1:1, 100 mL) to which Pd/C (2 g) was added. The mixture was evacuated, purged with H_2, evacuated once more then purged with H_2 and allowed to stir overnight. The solution was filtered through Celite and the filtrate reduced *in vacuo* to reveal 2.87 g (99% yield) of a slight yellow solid.

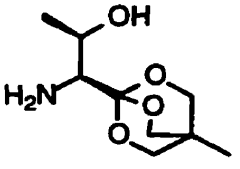


mp 98-99°C; $[\alpha]^{20}_D$ = -20.8 (c = 1.02, EtOH); TLC (9:1 EtOAc:MeOH) R_f = 0.29; ^{1}H NMR (DMSO-d_6, 250 MHz) δ 3.80 (s, 6H, OBO ester CH_2O), 3.51 (dd, 1H, *J* = 3.4, 10.7Hz, β-CH*H*), 3.12 (dd, 1H, *J* = 8.7, 10.5Hz, β-C*H*H), 2.66 (dd, 1H, J = 3.4, 8.6Hz, α-C*H*), 0.72 (s, 3H, OBO ester CCH_3); ^{13}C NMR (DMSO-d_6, 63 MHz) δ 108.2 (OBO ester

C-O), 71.9 (OBO ester $\underline{C}H_2O$), 61.7 (β-$\underline{C}H$), 57.9 (α-$\underline{C}H$), 29.9 (OBO ester $\underline{C}CH_3$), 13.9 (OBO ester $C\underline{C}H_3$); ESI-MS (M + H^+) 189.93; Anal. calcd for $C_8H_{15}NO_4$: C, 50.78; H, 7.99; N, 7.40. Found: C, 50.99; H, 8.23; N, 7.47.

6.4.4 (2*S*,3*R*)-2-Amino-1-(2,6,7-trioxabicyclo[2.2.2]oct-1-yl)propan-3-ol, Thr-OBO ester, 6.27.

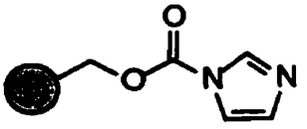
Cbz-Thr-OBO ester **6.25** (6.11 g, 18.11 mmol) was dissolved in EtOAc:EtOH (1:1, 100 mL) to which Pd/C (2 g) was added. The mixture was evacuated, purged with H_2, evacuated once more then purged with H_2 and allowed to stir overnight. The solution was filtered through Celite and the filtrate reduced *in vacuo* to reveal 3.63 g (99% yield) of a yellow oil that solidified upon standing.



$[\alpha]^{20}_D$ = -5.8 (c = 1.09, EtOH); TLC (9:1 EtOAc:MeOH) R_f = 0.34; ^{1}H NMR (DMSO-d_6, 250 MHz) δ 4.12 (m, 1H, β-C*H*), 3.81 (s, 6H, OBO ester CH_2O), 2.59 (d, 1H, *J* = 8.4Hz, α-C*H*), 1.22 (d, 3H, *J* = 6.7Hz, β-CH_3), 0.79 (s, 3H, OBO ester CCH_3); ^{13}C NMR (DMSO-d_6, 63 MHz) δ 108.1 (OBO ester $\underline{C}$-O), 71.9 (OBO ester $\underline{C}H_2O$), 64.7 (β-$\underline{C}H$), 58.9 (α-$\underline{C}H$), 29.9 (OBO ester $\underline{C}CH_3$), 18.3 (β-$\underline{C}H_3$), 13.9 (OBO ester $C\underline{C}H_3$); ESI-MS (M + H^+) 203.84; Anal. calcd for $C_9H_{17}NO_4$: C, 53.19; H, 8.43; N, 6.89. Found: C, 53.43; H, 8.67; N, 6.96.

6.4.5 Wang/TentaGel PHB® resin imidazolide, 6.30.

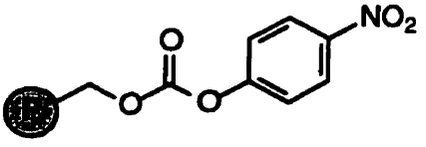
***N,N'*-Carbonyldiimidazole 6.28 (0.60 g, 3.7 mmol) was combined with Wang resin (1.0 g, 0.74 mmol, 0.74 mmol/g) then taken up in dry THF (10 mL). After 4 h, the mixture was poured into a sintered glass funnel and the solvent removed under vacuum. The resin was washed alternatively with THF (5 × 25 mL) and CH_2Cl_2 (5 × 25 mL) then with Et_2O (2 × 25 mL) and dried under high vacuum.**



IR (KBr) 1755 cm^{-1}. Anal. calcd for N, 0.94% corresponds to 98% substitution.

6.4.6 Wang/TentaGel PHB® resin *p*-nitrophenol carbonate, 6.31.

p-Nitrophenolchloroformate **6.29** (1.31 g, 6.5 mmol) was added to a stirring mixture of Wang resin (1.75 g, 1.3 mmol, 0.74 mmol/g) in *N*-methyl morpholine (1.43 mL, 13.0 mmol) and dry CH_2Cl_2 (30 mL) whilst under Ar at 0°C. The mixture was allowed to reach ambient temperature and after 12 h the resin was filtered off and washed with CH_2Cl_2 (5 × 50 mL), Et_2O (2 × 50 mL) and dried under high vacuum.

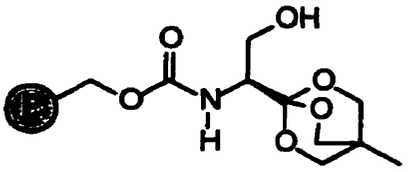


IR (KBr) 1761 cm^{-1}. Anal. calcd for N, 0.92% corresponds to 99% substitution.

6.4.6 Wang/TentaGel PHB® resin Ser-OBO carbamate, 6.32.

Ser-OBO **6.26** (0.600 g, 3.17 mmol) and DMAP (0.15 g, 1.26 mmol) were dissolved in *N*-methyl pyrrolidinone (5 mL) and *N,O*-bis(trimethylsilyl)acetamide (0.94 mL, 3.8 mmol) to which Wang *p*-nitrophenol carbonate resin **6.31** (1.00 g, 0.63 mmol/g) was added. The mixture was stirred for 24 h before the resin was filtered off and the resin

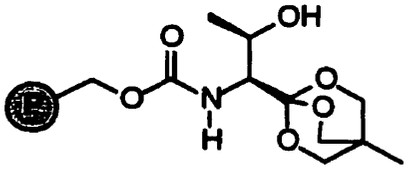
rinsed, alternating with dry DMF (5 × 20 mL) and dry CH_2Cl_2 (5 × 20 mL) before being rinsed with Et_2O (2 × 20 mL) and dried under high vacuum.



IR (KBr) 1742 cm^{-1}; MAS ^1H NMR (Spin-Echo-60 ms): δ 3.91 (OBO ester C*H*$_2$O), 0.71 (OBO ester C*H*$_3$).

6.4.7 Wang/TentaGel PHB® resin Thr-OBO carbamate, 6.33.

Thr-OBO **6.27** (0.304 g, 1.50 mmol) and DMAP (0.074 g, 0.6 mmol) were dissolved in *N*-methyl pyrrolidinone (5 mL) and *N,O*-bis(trimethylsilyl)acetamide (0.47 mL, 1.9 mmol) to which Wang *p*-nitrophenol carbonate resin **6.31** (0.478 g, 0.63 mmol/g) was added. The mixture was stirred for 24 h before the resin was filtered off and the resin rinsed, alternating with dry DMF (5 × 20 mL) and dry CH_2Cl_2 (5 × 20 mL) before being rinsed with Et_2O (2 × 20 mL) and dried under high vacuum.

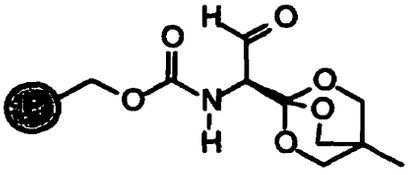


IR (KBr) 1742 cm^{-1}; MAS ^1H NMR (Spin-Echo-60 ms): δ 3.88 (OBO ester C*H*$_2$O), 0.70 (OBO ester C*H*$_3$).

6.4.8 Wang/TentaGel PHB® resin Ser(ald)-OBO carbamate, 6.34.

Oxalyl chloride (0.233 mL, 2.72 mmol) was added to dry CH_2Cl_2 (20 mL) in a 50 mL 2-neck RBF and stirred under Ar at –78°C. DMSO (0.385 mL, 5.44 mmol) was quickly

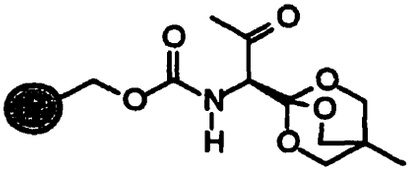
added and the mixture stirred an additional 15 min. The 2-neck 50 mL RBF was tilted (see figure 6.1a) to add the Wang Ser-OBO carbamate **6.33** (0.200 g, 0.14 mmol, 0.68 mmol/g) to the stirring mixture at –78°C. After 8 h at –78°C, DIPEA (1.41 mL, 8.2 mmol) was added and the mixture stirred an additional 2 h while warming to room temperature. The solvent was removed under anhydrous conditions and copiously rinsed with dry CH_2Cl_2, Et_2O (2 × 25 mL) then dried under high vacuum.



MAS ^{1}H NMR (Spin-Echo-60 ms): δ 9.71 (*CH*O), 3.91 (OBO ester C*H*$_2$O), 0.80 (OBO ester C*H*$_3$).

6.4.9 Wang/TentaGel PHB® resin Thr(ket)-OBO carbamate, 6.35.

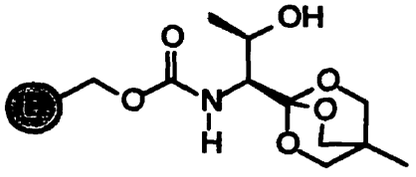
Oxalyl chloride (0.293 mL, 3.36 mmol) was added to dry CH_2Cl_2 (20 mL) in a 50 mL 2-neck RBF and stirred under Ar at –78°C. DMSO (0.491 mL, 6.93 mmol) was quickly added and the mixture stirred an additional 15 min. The 2-neck 50 mL RBF was tilted (see figure 6.1a) to add the Wang Thr-OBO carbamate **6.34** (0.320 g, 0.21 mmol, 0.64 mmol/g) to the stirring mixture at –78°C. After 8 h at –78°C, DIPEA (1.82 mL, 10.5 mmol) was added and the mixture stirred an additional 2 h while warming to room temperature. The solvent was removed under anhydrous conditions and copiously rinsed with dry CH_2Cl_2, Et_2O (2 × 25 mL) then dried under high vacuum.



MAS ^{1}H NMR (Spin-Echo-60 ms): δ 3.87 (OBO ester C*H*$_2$O), 2.34 (C*H*$_3$CO), 0.73 (OBO ester C*H*$_3$).

6.4.10 General method for the addition of Grignard reagents to Wang/TentaGel PHB® resin 6.34 or 6.35.

Wang Ser(ald)-OBO ester resin **6.34** (0.18 g, 0.12 mmol, 0.62 mmol/g) was suspended in CH_2Cl_2 (5 mL) and cooled to –78°C. Methylmagnesium bromide (0.3 mL, 0.9 mmol) was then added by syringe and the mixture stirred for 6 h. Acetone (1 mL) was then added and the mixture stirred for 30 min before the resin was filtered off and rinsed with CH_2Cl_2 (5 × 25 mL), DMF (5 × 25 mL), CH_2Cl_2 (5 × 25 mL), Et_2O (2 × 25 mL) then dried under high vacuum.



MAS ^{1}H NMR (Spin-Echo-60 ms): δ 3.82 (OBO ester C*H*$_2$O), 0.73 (OBO ester C*H*$_3$).
MAS ^{13}C NMR (Spin-Echo-60 ms) (^{13}C labeled **6.37**) 19.1 (β-$\underline{C}H_3$).

6.4.11 General method for the cleavage of 6.36 off Wang resin, (2*S*,3*R*)-2-amino-3-hydroxybutanoic acid, 6.46.

Wang Thr-OBO ester **6.36** (0.15 g, 0.1 mmol, 0.62 mmol/g) was stirred in TFA:CH_2Cl_2 (1:1, 3 mL) for 30 min before the resin was filtered off and the filtrate reduced under a gentle stream of N_2. The light brown residue was dissolved in CH_2Cl_2 (2 mL) and reduced again before Cs_2CO_3:MeOH (10% wt/vol, 2 mL) was added and the mixture stirred for 18 hours. The mixture was acidified with 2N HCl and purified as described in

section 2.4.15 then lyophilized to give 4 mg (30% overall yield) of **6.45** as a white powder. Spectral data identical to authentic Thr. HPLC conditions as in section 2.4.15a with retention times for L-Thr **6.45** (21.4 min), L-*allo*-Thr (25.5 min), D-Thr (22.5 min).

6.4.12 (2*S*,3*R*)-2-amino-3-hydroxypentanoic acid, 6.48.

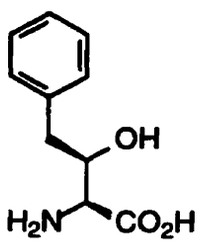
Same procedure as in 6.4.11. Spectral data identical to that in 2.4.16.

6.4.13 (2*S*,3*R*)-2-amino-3-hydroxy-3-phenylpropanoic acid, 6.49.

Same procedure as in 6.4.11. Spectral data identical to that in 2.4.18.

6.4.14 (2*S*,3*R*)-2-amino-3-hydroxy-4-phenylbutanoic acid, 6.50.

Same procedure as in 6.4.11.



TLC (1:1:1:1, EtOAc:*n*BuOH:MeOH:H_2O) R_f = 0.73; ^{1}H NMR (D_2O, 250 MHz) major *threo* isomer δ 7.35-7.19 (m, 5H, Ar*H*), 4.25-4.08 (m. 1H, β-C*H*), 3.64 (d, 1H, *J* = 4.8Hz, α-C*H*), 3.00-2.71 (m, 2H, BnCH_2); ^{13}C NMR (D_2O, 63 MHz) δ 174.9 ($\underline{C}O_2H$), 140.2 (Ar=$\underline{C}$=), 131.0, 129.5, 128.0 (Ar=$\underline{C}$H), 73.1 (β-$\underline{C}$H), 62.7 (α-$\underline{C}$H), 41.0 (Bn$\underline{C}H_2$); ESI-MS (M + H^+) 195.98. HPLC (*threo*-L-**6.50**: 66.1 min, *threo*-D-**6.50**: 67.3 min, *erythro*-L-**6.50**: 71.0 min, *erythro*-D-**6.50**: 75.3 min)

6.4.15 (2*S*,3*R*)-2-amino-3-hydroxy-4-pentenoic acid, 6.51.

Same procedure as in 6.4.11. Spectral data identical to that in 2.4.17.

6.4.16 (2*S*)-2-amino-3-hydroxy-3-methylbutanoic acid, 6.52.

Same procedure as in 6.4.11. Spectral data identical to that in 2.4.22.

6.4.17 (2*S*,3*R*)-2-amino-3-hydroxy-3-methylpentanoic acid, 6.54.

Same procedure as in 6.4.11. Spectral data identical to that in 2.4.23.

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Chapter Seven

Conclusions and Suggestions for Future Research

7.1 Conclusions

Synthesis of the Boc serinal OBO ester synthon occurs in moderate yield and is isolated as an oil. The Grignard addition to the Boc serinal OBO ester derivative, in the synthesis of β-hydroxy-α-amino acids, occurred in both high yield and good stereoselectivity. Although the selectivity of addition was not as high as the Fmoc and Cbz derivatives, the Boc protected β-hydroxy-α-amino acids have the advantage of a more facile deprotection with TFA to the mhpd ester before hydrolysis to the free β-hydroxy-α-amino acids.

The synthesis of vinylglycine in excellent enantiomeric excess was accomplished by the acid hydrolysis of the β-silanol Peterson olefination intermediate. The fully protected vinylglycine derivative was synthesized in 86% ee via Takai olefination, compared to 81% ee via the Wittig reaction previously described by this group.[1]

A variety of γ-substituted glutamic acid derivatives were synthesized in both excellent stereoselectivity (2*S*,4*S*) and overall yield from a Cbz glutamate OBO ester synthon. The enolate of the glutamate synthon is amenable to alkylation, acylation, Aldol and Claisen reactions and the electrophilic addition of various heteroatoms. The protected γ-hydroxyglutamate derivative was activated as the triphenylphosphine derivative and could be displaced by the addition of a nucleophile in good yield and stereoselectivity. This methodology represents a significant contribution to the synthesis

of these important derivatives due to the excellent diastereoselectivity, good yields, variety of electrophiles that may be incorporated and ease of synthesis of the synthon.

β-Substituted aspartic acid derivatives were synthesized in varying yields and diastereoselectivities, depending on the nature of the electrophile. The stereoselectivity of the addition was the more unusual 2S,3S-stereoisomer and the diastereoselectivity in the synthesis of β-methylaspartate is comparable to the best current procedure. However, the methodology described here is preferable in terms of atom efficiency. Disappointingly, the addition of various other electrophiles occurred with lower diastereoselectivty than other reported methods. Various models have been described in an attempt to explain the previously unreported stereoselectivity observed with the lithium enolate of the Cbz/OBO aspartate derivative.

To our knowledge, the synthesis of β-hydroxy-α-amino acids on the solid phase described herein is the first report of the synthesis of this class of α-amino acids on the solid phase. Although not enantiomerically pure, the functionality contained within the β-hydroxy-α-amino acids makes them a particularly attractive target for synthesis on the solid phase due to the potential for derivitization.

7.2 Suggestions for Future Research

Current syntheses of the vinylglycine derivatives described in this thesis result in some racemization at the α-carbon. Eliminating this source of racemization is necessary in order to use the OBO vinylglycine derivatives as intermediates in various syntheses. However, the protected vinylglycine derivative should be investigated in various [2+2] cycloaddition reactions towards various constrained α-amino acids.

The success in the optimized synthesis of alkyl and hydroxy glutamic acid derivatives provides the opportunity to expand the methodology into other aspects of glutamic acid chemistry. However, the synthesis of the fully protected glutamate derivative **4.69**, although currently optimized, would be advantageous if column purification could be avoided. The crystalline derivative is stable making it particularly amenable for use as a general synthon.

Confirmation of the model predicting the stereochemistry of addition of various electrophiles with glutamic acid could be achieved by di-N-protection. The N-Li bond, thus removed, could no longer participate in chelation, which is critical in the proposed model in section 4.2.10. Replacing the OBO ester protecting group with the ABO ester protecting group as described by Wipf *et al.*[2] and thus increasing the size of the directing group may have a beneficial effect on stereoselectivity.

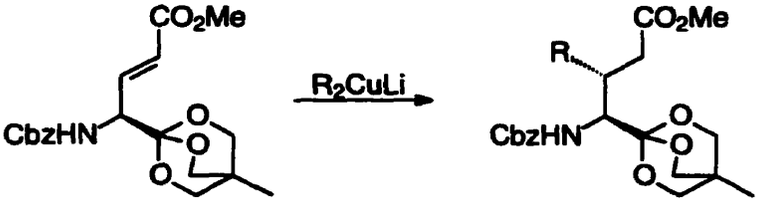
The successful synthesis of 2*S*,4*R*-aminoglutamic acid via the nucleophilic addition of azide illustrates the synthetic utility of the activated hydroxyl derivative of glutamic acid. Numerous other nucleophilic additions may be envisioned, providing access to 2*S*,4*R* derivatives of glutamic acid and hence complementarity to the synthesis of 2*S*,4*S* substituted glutamates described in this thesis. The γ-bromoglutamate derivative should also be investigated as a γ-cationic glutamate derivative.

Preliminary results indicate the Claisen condensation described in section 4.2.8 to be particularly promising. Current methods for the synthesis of substituted indolizidines in a stereoselective manner are severely lacking. Decarboxylation of the glutamate dimer **4.117** to the ketone **4.118** would provide the opportunity for 1,3-stereochemical induction in **4.118**. This may allow for the stereoselective introduction of various substituents into

the glutamate dimer. It may also be possible to introduce γ-substituted glutamate derivatives (such as **4.73**) into pre-generated mixtures of the enolate of **4.69**, ensuring the high stereochemistry is pre-installed.

1,4-Additions to *E* and *Z* dehydroglutamic acids would provide access to β-substituted glutamic acids, of immense interest in the synthesis of kainic acid analogues which possess potent neuroexcitatory activity.[3] Current synthetic methods used in the synthesis of these derivatives suffer from a lack of generality in the incorporation of various substituents and/or poor stereoselectivity. 1,2-Stereoinduction has been used with great success in a number of Michael additions and the proximity of the OBO ester may ensure high stereoselectivity in these reactions (Scheme 7.1).

Scheme 7.1



Epoxidation of the dehydroglutamic acids, although unsuccessful under conditions reported in this thesis, warrants further investigation due to its synthetic utility. Nucleophilic ring-opening reactions to give β-substituted-γ-hydroxy-glutamic acids are of interest not only as potential iGlu and mGlu agonists/antagonists but also as intermediates in natural product syntheses due to their highly functionalized nature.

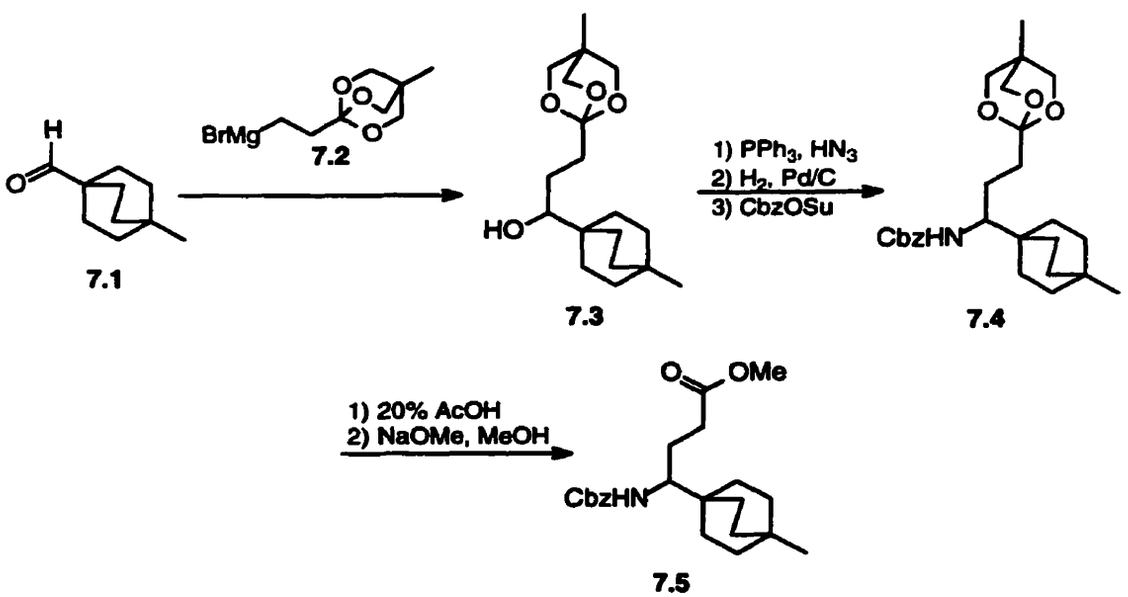
As a natural extension of the high 1,3-induction of addition to protected glutamic acid derivatives, investigation of the potential 1,4-induction of L-α-aminoadipic acid should be explored. Various analogs of L-α-aminoadipic acid have been incorporated in

the synthesis of penicillin analogs,[4] iGlu and mGlu agonists/antagonists[5] and metalloprotease inhibitors.[6]

The synthesis of 2*S*,3*S*-aspartic acid derivatives and the role of the numerous factors involved in the stereochemical outcome of addition of electrophiles requires additional investigation. The use of more reactive electrophiles has been shown to increase diastereoselectivity[7] and needs to be examined. Furthermore, the effect of larger N-protecting groups on stereoselectivity, and the corresponding effect of the observations on the proposed models should be investigated.

The OBO ester has been implicated in both directing nucleophilic attack and chelation. Investigating the role of the oxygens of the OBO ester in chelation could be accomplished by using a bicyclo[2.2.2]octane derivative. The derivative could be synthesized as outlined in Scheme 7.2 although the separation of the enantiomers might prove challenging. Synthesis of **7.1**[8] and **7.2**[9] have been previously reported as has the transesterification to **7.5**.[10]

Scheme 7.2



7.3 References

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Appendices

Appendix A

X-Ray Crystallographic Data for Compound 4.73

Table 1. Crystal data and structure refinement for gl907.

Identification code	gl907
Empirical formula	$C_{20}H_{27}NO_7$
Formula weight	393.43
Temperature	180(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a = 5.4681(6) Å alpha = 90° b = 18.8646(15) Å beta = 90° c = 19.4478(16) Å gamma = 90°
Volume, Z	2006.1(3) $Å^3$, 4
Density (calculated)	1.303 Mg/m^3
Absorption coefficient	0.098 mm^{-1}
F(000)	840
Crystal size	0.20 x 0.20 x 0.14 mm
θ range for data collection	2.09 to 30.00°
Limiting indices	$0 \le h \le 7$, $0 \le k \le 26$, $0 \le l \le 27$
Reflections collected	3361
Independent reflections	3361
Completeness to θ = 30.00°	100.0 %
Absorption correction	Integration
Max. and min. transmission	0.9794 and 0.9717
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3361 / 0 / 269
Goodness-of-fit on F^2	1.616
Final R indices [$I>2\sigma(I)$]	R1 = 0.0390, wR2 = 0.0609
R indices (all data)	R1 = 0.0511, wR2 = 0.0616
Extinction coefficient	0.0195(4)
Largest diff. peak and hole	0.212 and -0.183 $eÅ^{-3}$

Table 2. Atomic coordinates [x 10^4] and equivalent isotropic displacement parameters [$Å^2$ x 10^3] for gl907. U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U(eq)
C(1)	2557(3)	1955(1)	1254(1)	24(1)
C(2)	1754(4)	2512(1)	1776(1)	26(1)
C(3)	2862(4)	3232(1)	1607(1)	29(1)
C(4)	2180(4)	3812(1)	2117(1)	29(1)
C(5)	3417(4)	4500(1)	1926(1)	32(1)
O(6)	1781(3)	2178(1)	600(1)	31(1)
C(7)	2440(4)	1669(1)	75(1)	32(1)
O(8)	1446(2)	1308(1)	1423(1)	31(1)
C(9)	2171(4)	755(1)	956(1)	32(1)
O(10)	5098(2)	1895(1)	1276(1)	32(1)
C(11)	5984(4)	1390(1)	779(1)	37(1)
C(12)	3829(4)	1064(1)	408(1)	29(1)
C(13)	4615(4)	504(1)	-112(1)	41(1)
N(14)	2419(3)	2284(1)	2466(1)	30(1)
C(15)	709(4)	2071(1)	2917(1)	33(1)
O(16)	-1478(3)	2103(1)	2844(1)	41(1)
O(17)	1830(3)	1825(1)	3493(1)	42(1)
C(18)	204(5)	1522(1)	4008(1)	53(1)
C(19)	1579(4)	1484(1)	4674(1)	36(1)
C(20)	662(5)	1811(1)	5250(1)	47(1)
C(21)	1839(6)	1758(2)	5868(1)	65(1)
C(22)	3979(6)	1388(2)	5918(1)	69(1)
C(23)	4924(5)	1056(1)	5347(1)	57(1)
C(24)	3713(5)	1106(1)	4727(1)	45(1)
C(25)	-592(4)	3945(1)	2156(1)	42(1)
O(26)	4120(3)	4658(1)	1362(1)	48(1)
O(27)	3630(3)	4926(1)	2467(1)	53(1)
C(28)	4712(7)	5617(1)	2334(1)	68(1)

Table 3. Bond lengths [Å] and angles [°] for g1907.

C(1)-O(10)	1.395(2)	C(1)-O(8)	1.403(2)
C(1)-O(6)	1.405(2)	C(1)-C(2)	1.526(3)
C(2)-N(14)	1.456(2)	C(2)-C(3)	1.522(2)
C(3)-C(4)	1.523(2)	C(4)-C(5)	1.510(3)
C(4)-C(25)	1.538(3)	C(5)-O(26)	1.201(2)
C(5)-O(27)	1.328(2)	O(6)-C(7)	1.447(2)
C(7)-C(12)	1.515(2)	O(8)-C(9)	1.439(2)
C(9)-C(12)	1.515(3)	O(10)-C(11)	1.442(2)
C(11)-C(12)	1.512(3)	C(12)-C(13)	1.525(2)
N(14)-C(15)	1.343(3)	C(15)-O(16)	1.206(3)
C(15)-O(17)	1.358(2)	O(17)-C(18)	1.456(2)
C(18)-C(19)	1.500(3)	C(19)-C(24)	1.371(3)
C(19)-C(20)	1.374(3)	C(20)-C(21)	1.367(3)
C(21)-C(22)	1.366(4)	C(22)-C(23)	1.376(4)
C(23)-C(24)	1.379(3)	O(27)-C(28)	1.455(3)

O(10)-C(1)-O(8)	110.74(15)	O(10)-C(1)-O(6)	110.72(16)
O(8)-C(1)-O(6)	110.03(14)	O(10)-C(1)-C(2)	108.71(16)
O(8)-C(1)-C(2)	108.59(14)	O(6)-C(1)-C(2)	107.98(14)
N(14)-C(2)-C(3)	111.25(15)	N(14)-C(2)-C(1)	109.77(15)
C(3)-C(2)-C(1)	110.94(15)	C(2)-C(3)-C(4)	113.80(15)
C(5)-C(4)-C(3)	110.34(16)	C(5)-C(4)-C(25)	108.28(17)
C(3)-C(4)-C(25)	112.93(18)	O(26)-C(5)-O(27)	123.02(17)
O(26)-C(5)-C(4)	125.61(16)	O(27)-C(5)-C(4)	111.37(16)
C(1)-O(6)-C(7)	111.35(13)	O(6)-C(7)-C(12)	108.83(13)
C(1)-O(8)-C(9)	111.24(13)	O(8)-C(9)-C(12)	109.29(14)
C(1)-O(10)-C(11)	111.49(15)	O(10)-C(11)-C(12)	109.07(15)
C(11)-C(12)-C(7)	106.84(15)	C(11)-C(12)-C(9)	106.68(15)
C(7)-C(12)-C(9)	106.85(16)	C(11)-C(12)-C(13)	112.21(17)
C(7)-C(12)-C(13)	112.29(15)	C(9)-C(12)-C(13)	111.60(15)
C(15)-N(14)-C(2)	121.11(18)	O(16)-C(15)-N(14)	126.7(2)
O(16)-C(15)-O(17)	124.2(2)	N(14)-C(15)-O(17)	109.04(19)
C(15)-O(17)-C(18)	115.17(17)	O(17)-C(18)-C(19)	107.86(18)
C(24)-C(19)-C(20)	118.8(2)	C(24)-C(19)-C(18)	121.1(2)
C(20)-C(19)-C(18)	120.0(2)	C(21)-C(20)-C(19)	120.9(2)
C(22)-C(21)-C(20)	120.2(2)	C(21)-C(22)-C(23)	119.7(2)
C(22)-C(23)-C(24)	119.7(2)	C(19)-C(24)-C(23)	120.6(2)
C(5)-O(27)-C(28)	115.89(15)		

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters [$Å^2$ x 10^3] for gl907.
The anisotropic displacement factor exponent takes the form:
$-2\pi^2$ [$(ha^*)^2 U_{11}$ + ... + $2hka^*b^*U_{12}$]

	U11	U22	U33	U23	U13	U12
C(1)	25(1)	24(1)	25(1)	2(1)	0(1)	-1(1)
C(2)	29(1)	25(1)	23(1)	2(1)	0(1)	0(1)
C(3)	36(1)	25(1)	26(1)	-1(1)	3(1)	-2(1)
C(4)	38(1)	25(1)	25(1)	-1(1)	-2(1)	0(1)
C(5)	38(1)	24(1)	34(1)	-2(1)	-7(1)	2(1)
O(6)	42(1)	27(1)	23(1)	-1(1)	-3(1)	8(1)
C(7)	38(1)	31(1)	27(1)	-4(1)	2(1)	-1(1)
O(8)	40(1)	21(1)	31(1)	-2(1)	6(1)	-6(1)
C(9)	34(1)	24(1)	38(1)	-3(1)	4(1)	-1(1)
O(10)	25(1)	33(1)	39(1)	-9(1)	-2(1)	0(1)
C(11)	27(1)	36(1)	46(1)	-8(1)	3(1)	2(1)
C(12)	27(1)	26(1)	34(1)	-4(1)	4(1)	-1(1)
C(13)	44(1)	34(1)	46(1)	-10(1)	11(1)	1(1)
N(14)	32(1)	31(1)	25(1)	4(1)	-2(1)	-2(1)
C(15)	47(1)	26(1)	25(1)	-3(1)	1(1)	-4(1)
O(16)	38(1)	52(1)	34(1)	3(1)	3(1)	-8(1)
O(17)	49(1)	51(1)	27(1)	14(1)	0(1)	-7(1)
C(18)	58(2)	67(2)	34(1)	18(1)	-2(1)	-22(1)
C(19)	44(1)	33(1)	30(1)	7(1)	2(1)	-11(1)
C(20)	51(2)	43(1)	48(1)	-1(1)	5(1)	-3(1)
C(21)	76(2)	83(2)	35(1)	-16(1)	6(2)	-15(2)
C(22)	71(2)	98(2)	38(1)	14(1)	-17(2)	-23(2)
C(23)	53(2)	50(1)	70(2)	18(1)	-7(2)	-2(1)
C(24)	52(2)	41(1)	42(1)	-1(1)	10(1)	-9(1)
C(25)	45(1)	38(1)	42(1)	-4(1)	5(1)	2(1)
O(26)	72(1)	35(1)	36(1)	1(1)	2(1)	-17(1)
O(27)	97(1)	27(1)	35(1)	-5(1)	-11(1)	-10(1)
C(28)	119(3)	25(1)	61(2)	-3(1)	-31(2)	-15(2)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$ x 10^3) for gl907.

	x	y	z	U(eq)
H(3X)	2326	3374	1152	35
H(3Y)	4628	3187	1596	35
H(7X)	3452	1894	-271	38
H(7Y)	978	1489	-147	38
H(9X)	736	548	743	39
H(9Y)	3027	385	1205	39
H(11X)	6916	1022	1009	44
H(11Y)	7049	1624	452	44
H(13X)	5928	686	-388	62
H(13Y)	3256	386	-402	62
H(13Z)	5157	87	127	62
H(18X)	-313	1052	3868	64
H(18Y)	-1238	1816	4060	64
H(20)	-778	2071	5219	57
H(21)	1181	1976	6255	78
H(22)	4795	1361	6337	83
H(23)	6372	800	5379	69
H(24)	4349	880	4341	54
H(25X)	-1198	4062	1707	63
H(25Y)	-912	4330	2465	63
H(25Z)	-1396	3525	2320	63
H(28X)	6417	5561	2230	102
H(28Y)	4533	5912	2733	102
H(28Z)	3899	5835	1950	102
H(2)	-50(30)	2511(8)	1752(8)	15(4)
H(4)	2750(30)	3685(8)	2565(8)	23(5)
H(14)	3940(40)	2214(9)	2549(9)	20(5)

Appendix B

Calculation of Free-Energy Difference and Free- Energy of Activation for Compound 4.73

Calculation of Free Energy Difference (ΔG)

$$\Delta G = RT \ln\left(\frac{1+\Delta P}{1-\Delta P}\right)$$

$$\Delta G = (1.987)(295)\ln\left(\frac{1+0.808}{1-0.808}\right)$$

$$\Delta G = 1.314 \text{ kcal mol}^{-1}$$

$$\Delta P = P_A - P_B$$

where P_A = Population of A
P_B = Population of B
$R = 1.987$ cal K^{-1} mol^{-1}
$T = 295$ K

$$\Delta P = \left(\frac{3.0660}{3.3844}\right) - \left(\frac{0.3244}{3.3844}\right)$$

$$\Delta P = 0.808$$

Calculation of Free Energy of Activation ($\Delta G^{\ddagger}_C$)

$$\Delta G^{\ddagger}_A = 4.57\ T_c \left[10.62 + \log\frac{X}{2\pi(1-\Delta P)} + \log\frac{T_c}{\delta\nu}\right]$$

$$\Delta G^{\ddagger}_A = 4.57(330)\left[10.62 + \log\frac{2.6389}{2\pi(1-0.808)} + \log\frac{330}{89}\right]$$

$$\Delta G^{\ddagger}_A = 17.38 \text{ kcal mol}^{-1}$$

where:
T_c = temperature of coalescence
$\delta\nu$ = shift between the lines at very slow exchange
$X = 2\pi\delta\nu\tau$
τ = correlation time - extrapolated from a plot of $\delta\nu\tau$ vs. ΔP (Figure 1)

$$\Delta G^{\ddagger}_B = 4.57\ T_c \left[10.62 + \log\frac{X}{2\pi(1+\Delta P)} + \log\frac{T_c}{\delta\nu}\right]$$

$$\Delta G^{\ddagger}_B = 4.57(330)\left[10.62 + \log\frac{2.6389}{2\pi(1+1.808)} + \log\frac{330}{89}\right]$$

$$\Delta G^{\ddagger}_B = 17.22 \text{ kcal mol}^{-1}$$

Appendix C

X-Ray Crystallographic Data for Compound 4.96

Table 1. Crystal data and structure refinement for gl955xm.

Identification code	gl955xm
Empirical formula	$C_{19}H_{25}NO_8$
Formula weight	395.40
Temperature	150(1) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a = 6.0244(3) Å alpha = 90° b = 13.7314(8) Å beta = 90° c = 23.1172(13) Å gamma = 90°
Volume, Z	1912.33(18) $Å^3$, 4
Density (calculated)	1.373 Mg/m^3
Absorption coefficient	0.107 mm^{-1}
F(000)	840
Crystal size	0.48 x 0.25 x 0.18 mm
θ range for data collection	1.72 to 35.01°
Limiting indices	$-9 \le h \le 9$, $-20 \le k \le 21$, $-37 \le l \le 36$
Reflections collected	31312
Independent reflections	8213 (R_{int} = 0.0461)
Completeness to θ = 35.01°	98.8 %
Absorption correction	Empirical
Max. and min. transmission	0.983 and 0.961
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	8213 / 0 / 354
Goodness-of-fit on F^2	1.293
Final R indices [I>2σ(I)]	R1 = 0.0513, wR2 = 0.0830
R indices (all data)	R1 = 0.0596, wR2 = 0.0847
Extinction coefficient	0.0014(5)
Largest diff. peak and hole	0.325 and -0.296 $eÅ^{-3}$

Table 2. Atomic coordinates [x 10^4] and equivalent isotropic displacement parameters [$Å^2$ x 10^3] for gl955xm. U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U(eq)
C(1)	4450(2)	3536(1)	961(1)	21(1)
C(2)	5780(2)	4468(1)	887(1)	21(1)
C(3)	6983(2)	4773(1)	1441(1)	24(1)
C(4)	8295(2)	5710(1)	1356(1)	24(1)
C(5)	9466(2)	5988(1)	1913(1)	28(1)
O(6)	2897(1)	3649(1)	1410(1)	26(1)
C(7)	1866(2)	2721(1)	1547(1)	28(1)
O(8)	5951(1)	2782(1)	1095(1)	28(1)
C(9)	4852(2)	1844(1)	1065(1)	29(1)
O(10)	3364(2)	3339(1)	439(1)	26(1)
C(11)	1831(2)	2539(1)	504(1)	28(1)
C(12)	2359(2)	2007(1)	1063(1)	23(1)
C(13)	1063(2)	1064(1)	1120(1)	31(1)
N(14)	4361(2)	5235(1)	662(1)	23(1)
C(15)	4970(2)	5766(1)	206(1)	23(1)
O(16)	6811(1)	5754(1)	-21(1)	27(1)
O(17)	3310(2)	6353(1)	32(1)	31(1)
C(18)	3812(3)	7008(1)	-445(1)	31(1)
C(19)	3215(2)	6571(1)	-1020(1)	27(1)
C(20)	4747(3)	6599(1)	-1464(1)	32(1)
C(21)	4197(3)	6258(1)	-2010(1)	42(1)
C(22)	2126(3)	5893(1)	-2117(1)	45(1)
C(23)	592(3)	5842(1)	-1675(1)	48(1)
C(24)	1129(2)	6180(1)	-1126(1)	40(1)
O(25)	9831(2)	5647(1)	897(1)	32(1)
O(26)	8489(2)	6039(1)	2368(1)	40(1)
O(27)	11610(2)	6186(1)	1845(1)	33(1)
C(28)	12745(3)	6538(1)	2356(1)	42(1)

Table 3. Bond lengths [Å] and angles [°] for gl955xm.

C(1)-O(10)	1.3990(13)	C(1)-O(6)	1.4059(13)
C(1)-O(8)	1.4087(14)	C(1)-C(2)	1.5197(16)
C(2)-N(14)	1.4528(15)	C(2)-C(3)	1.5306(16)
C(3)-C(4)	1.5240(17)	C(4)-O(25)	1.4099(14)
C(4)-C(5)	1.5185(17)	C(5)-O(26)	1.2064(15)
C(5)-O(27)	1.3293(15)	O(6)-C(7)	1.4522(15)
C(7)-C(12)	1.5179(17)	O(8)-C(9)	1.4490(15)
C(9)-C(12)	1.5185(18)	O(10)-C(11)	1.4428(15)
C(11)-C(12)	1.5174(17)	C(12)-C(13)	1.5185(18)
N(14)-C(15)	1.3332(15)	C(15)-O(16)	1.2275(14)
C(15)-O(17)	1.3455(14)	O(17)-C(18)	1.4552(15)
C(18)-C(19)	1.5023(18)	C(19)-C(20)	1.3809(18)
C(19)-C(24)	1.388(2)	C(20)-C(21)	1.386(2)
C(21)-C(22)	1.367(2)	C(22)-C(23)	1.379(2)
C(23)-C(24)	1.390(2)	O(27)-C(28)	1.4477(17)

O(10)-C(1)-O(6)	110.27(9)	O(10)-C(1)-O(8)	110.37(9)
O(6)-C(1)-O(8)	110.22(9)	O(10)-C(1)-C(2)	108.16(9)
O(6)-C(1)-C(2)	109.97(9)	O(8)-C(1)-C(2)	107.79(9)
N(14)-C(2)-C(1)	109.90(9)	N(14)-C(2)-C(3)	112.36(9)
C(1)-C(2)-C(3)	112.64(9)	C(4)-C(3)-C(2)	111.56(9)
O(25)-C(4)-C(5)	110.45(10)	O(25)-C(4)-C(3)	112.72(10)
C(5)-C(4)-C(3)	110.02(9)	O(26)-C(5)-O(27)	124.43(12)
O(26)-C(5)-C(4)	121.85(11)	O(27)-C(5)-C(4)	113.70(11)
C(1)-O(6)-C(7)	110.43(8)	O(6)-C(7)-C(12)	108.76(9)
C(1)-O(8)-C(9)	110.44(9)	O(8)-C(9)-C(12)	108.73(10)
C(1)-O(10)-C(11)	110.93(9)	O(10)-C(11)-C(12)	108.69(10)
C(11)-C(12)-C(7)	106.05(10)	C(11)-C(12)-C(9)	106.32(11)
C(7)-C(12)-C(9)	106.63(11)	C(11)-C(12)-C(13)	112.18(10)
C(7)-C(12)-C(13)	112.68(10)	C(9)-C(12)-C(13)	112.49(10)
C(15)-N(14)-C(2)	121.18(10)	O(16)-C(15)-N(14)	125.37(11)
O(16)-C(15)-O(17)	123.50(10)	N(14)-C(15)-O(17)	111.10(10)
C(15)-O(17)-C(18)	116.28(10)	O(17)-C(18)-C(19)	111.93(11)
C(20)-C(19)-C(24)	119.00(12)	C(20)-C(19)-C(18)	119.08(12)
C(24)-C(19)-C(18)	121.85(13)	C(19)-C(20)-C(21)	120.48(14)
C(22)-C(21)-C(20)	120.48(15)	C(21)-C(22)-C(23)	119.71(14)
C(22)-C(23)-C(24)	120.24(15)	C(19)-C(24)-C(23)	120.06(15)
C(5)-O(27)-C(28)	115.49(12)		

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters [$Å^2$ x 10^3] for gl955xm.
The anisotropic displacement factor exponent takes the form:
$-2\pi^2$ [$(ha^*)^2U_{11}$ + ... + $2hka^*b^*U_{12}$]

	U11	U22	U33	U23	U13	U12
C(1)	22(1)	23(1)	19(1)	1(1)	0(1)	3(1)
C(2)	22(1)	22(1)	20(1)	1(1)	1(1)	3(1)
C(3)	28(1)	23(1)	22(1)	3(1)	-1(1)	-2(1)
C(4)	22(1)	25(1)	24(1)	2(1)	2(1)	1(1)
C(5)	28(1)	24(1)	31(1)	-1(1)	-4(1)	1(1)
O(6)	32(1)	23(1)	23(1)	-2(1)	8(1)	-1(1)
C(7)	34(1)	23(1)	26(1)	1(1)	7(1)	-3(1)
O(8)	23(1)	21(1)	42(1)	1(1)	-5(1)	1(1)
C(9)	26(1)	21(1)	41(1)	-1(1)	-4(1)	1(1)
O(10)	30(1)	30(1)	19(1)	0(1)	-3(1)	-5(1)
C(11)	25(1)	34(1)	26(1)	0(1)	-3(1)	-5(1)
C(12)	22(1)	23(1)	24(1)	-1(1)	-1(1)	-1(1)
C(13)	30(1)	27(1)	36(1)	-2(1)	0(1)	-4(1)
N(14)	21(1)	25(1)	23(1)	4(1)	3(1)	4(1)
C(15)	27(1)	21(1)	20(1)	-2(1)	-1(1)	-1(1)
O(16)	27(1)	30(1)	24(1)	3(1)	5(1)	-1(1)
O(17)	33(1)	36(1)	25(1)	11(1)	5(1)	10(1)
C(18)	46(1)	24(1)	24(1)	6(1)	1(1)	5(1)
C(19)	33(1)	22(1)	26(1)	7(1)	-2(1)	5(1)
C(20)	36(1)	32(1)	29(1)	3(1)	2(1)	-1(1)
C(21)	57(1)	40(1)	29(1)	1(1)	6(1)	-4(1)
C(22)	67(1)	37(1)	31(1)	4(1)	-14(1)	-6(1)
C(23)	42(1)	49(1)	53(1)	4(1)	-14(1)	-9(1)
C(24)	32(1)	47(1)	41(1)	6(1)	3(1)	-1(1)
O(25)	22(1)	49(1)	24(1)	3(1)	2(1)	-2(1)
O(26)	38(1)	55(1)	28(1)	-9(1)	2(1)	-7(1)
O(27)	25(1)	39(1)	36(1)	-6(1)	-5(1)	-1(1)
C(28)	34(1)	46(1)	47(1)	-14(1)	-13(1)	0(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$ x 10^3) for gl955xm.

	x	y	z	U(eq)
H(2)	6870(20)	4346(10)	577(5)	20(3)
H(3X)	7970(20)	4266(11)	1570(6)	35(4)
H(3Y)	5960(30)	4899(11)	1757(6)	38(4)
H(4)	7260(20)	6267(10)	1288(6)	28(4)
H(7X)	260(30)	2831(10)	1593(6)	30(4)
H(7Y)	2480(20)	2490(11)	1915(6)	31(4)
H(9X)	5360(20)	1503(11)	720(6)	36(4)
H(9Y)	5250(20)	1488(10)	1397(6)	32(4)
H(11X)	2040(20)	2118(9)	175(5)	19(3)
H(11Y)	480(30)	2762(12)	516(6)	40(4)
H(13X)	1560(30)	567(12)	834(6)	39(4)
H(13Y)	1290(20)	764(11)	1497(7)	39(4)
H(13Z)	-520(30)	1168(12)	1049(7)	48(4)
H(14)	3080(30)	5282(11)	781(6)	30(4)
H(18X)	2940(20)	7583(11)	-365(6)	30(4)
H(18Y)	5460(20)	7198(10)	-435(6)	29(4)
H(20)	6280(30)	6853(11)	-1383(6)	38(4)
H(21)	5200(30)	6262(13)	-2299(7)	55(5)
H(22)	1760(30)	5676(13)	-2505(7)	54(5)
H(23)	-810(30)	5599(13)	-1745(7)	55(5)
H(24)	210(30)	6201(13)	-827(7)	58(5)
H(25)	9120(30)	5627(13)	596(7)	50(5)
H(28X)	14130(30)	6610(13)	2273(8)	60(6)
H(28Y)	12300(30)	7187(15)	2439(8)	64(6)
H(28Z)	12450(30)	6084(14)	2697(8)	70(6)

Appendix D

X-Ray Crystallographic Data for Compound 5.50

Table 1. Crystal data and structure refinement for gl902.

Identification code	gl902
Empirical formula	$C_{30}H_{33}NO_5$
Formula weight	487.57
Color; Habit	Colorless prism
Temperature	180(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a = 11.6843(7) Å alpha = 90° b = 14.5133(9) Å beta = 90° c = 14.9638(11) Å gamma = 90°
Volume, Z	2537.5(3) $Å^3$, 4
Density (calculated)	1.276 Mg/m^3
Absorption coefficient	0.086 mm^{-1}
F(000)	1040
Crystal size	0.14 x 0.20 x 0.20 mm
θ range for data collection	2.21 to 29.00°
Limiting indices	$0 \le h \le 15$, $0 \le k \le 19$, $0 \le l \le 20$
Reflections collected	3773
Independent reflections	3773 (R_{int} = 0.0000)
Completeness to θ = 29.00°	100.0 %
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3773 / 0 / 458
Goodness-of-fit on F^2	1.164
Final R indices [$I>2\sigma(I)$]	R1 = 0.0350, wR2 = 0.0487
R indices (all data)	R1 = 0.0500, wR2 = 0.0501
Extinction coefficient	0.0055(2)
Largest diff. peak and hole	0.163 and -0.152 $eÅ^{-3}$

Table 2. Atomic coordinates [x 10^4] and equivalent isotropic displacement parameters [$Å^2$ x 10^3] for gl902. U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U(eq)
C(1)	-466(2)	630(1)	1340(1)	26(1)
C(2)	111(2)	240(1)	509(1)	24(1)
C(3)	-383(2)	-721(1)	273(1)	31(1)
C(4)	55(2)	-1017(1)	-630(1)	33(1)
O(5)	-350(1)	1593(1)	1341(1)	29(1)
C(6)	-726(2)	1987(1)	2182(1)	35(1)
O(7)	55(1)	247(1)	2103(1)	30(1)
C(8)	-538(2)	498(2)	2914(1)	36(1)
O(9)	-1641(1)	401(1)	1328(1)	33(1)
C(10)	-2248(2)	849(2)	2048(1)	36(1)
C(11)	-1387(2)	1255(1)	2698(1)	31(1)
C(12)	-1967(2)	1624(2)	3533(2)	40(1)
N(13)	1357(1)	170(1)	580(1)	24(1)
C(14)	2182(2)	890(1)	309(1)	23(1)
C(15)	1853(2)	1212(1)	-636(1)	22(1)
C(16)	2372(2)	861(1)	-1396(1)	30(1)
C(17)	1996(2)	1123(1)	-2243(1)	36(1)
C(18)	1095(2)	1727(2)	-2335(1)	36(1)
C(19)	578(2)	2090(1)	-1587(1)	32(1)
C(20)	962(2)	1841(1)	-746(1)	27(1)
C(21)	3375(2)	413(1)	304(1)	25(1)
C(22)	4369(2)	938(2)	271(1)	35(1)
C(23)	5442(2)	546(2)	314(2)	42(1)
C(24)	5549(2)	-399(2)	390(1)	41(1)
C(25)	4585(2)	-934(2)	388(1)	39(1)
C(26)	3498(2)	-540(1)	343(1)	31(1)
C(27)	2287(2)	1717(1)	967(1)	25(1)
C(28)	2252(2)	1558(1)	1883(1)	31(1)
C(29)	2417(2)	2265(2)	2493(2)	41(1)
C(30)	2627(2)	3148(2)	2198(2)	46(1)
C(31)	2685(2)	3322(1)	1293(2)	42(1)
C(32)	2534(2)	2609(1)	681(1)	32(1)
C(33)	-151(2)	-1465(2)	966(2)	46(1)
O(34)	-239(1)	-415(1)	-1269(1)	34(1)
O(35)	568(2)	-1712(1)	-788(1)	57(1)
C(36)	121(2)	-644(2)	-2168(2)	41(1)

Table 3. Bond lengths [Å] and angles [°] for gl902.

C(1)-O(5)	1.405(2)	C(1)-O(7)	1.407(2)
C(1)-O(9)	1.413(2)	C(1)-C(2)	1.523(2)
C(2)-N(13)	1.464(2)	C(2)-C(3)	1.551(3)
C(3)-C(4)	1.508(3)	C(3)-C(33)	1.521(3)
C(4)-O(35)	1.197(2)	C(4)-O(34)	1.340(2)
O(5)-C(6)	1.449(2)	C(6)-C(11)	1.524(3)
O(7)-C(8)	1.445(2)	C(8)-C(11)	1.516(3)
O(9)-C(10)	1.443(2)	C(10)-C(11)	1.518(3)
C(11)-C(12)	1.519(3)	N(13)-C(14)	1.479(2)
C(14)-C(15)	1.537(2)	C(14)-C(21)	1.556(2)
C(14)-C(27)	1.557(2)	C(15)-C(16)	1.386(2)
C(15)-C(20)	1.394(2)	C(16)-C(17)	1.394(3)
C(17)-C(18)	1.378(3)	C(18)-C(19)	1.376(3)
C(19)-C(20)	1.385(3)	C(21)-C(22)	1.390(3)
C(21)-C(26)	1.391(3)	C(22)-C(23)	1.379(3)
C(23)-C(24)	1.381(3)	C(24)-C(25)	1.369(3)
C(25)-C(26)	1.395(3)	C(27)-C(28)	1.391(3)
C(27)-C(32)	1.395(3)	C(28)-C(29)	1.387(3)
C(29)-C(30)	1.377(3)	C(30)-C(31)	1.380(3)
C(31)-C(32)	1.392(3)	O(34)-C(36)	1.449(2)

O(5)-C(1)-O(7)	110.46(14)	O(5)-C(1)-O(9)	109.08(14)
O(7)-C(1)-O(9)	109.77(14)	O(5)-C(1)-C(2)	109.11(15)
O(7)-C(1)-C(2)	108.93(13)	O(9)-C(1)-C(2)	109.47(14)
N(13)-C(2)-C(1)	114.04(15)	N(13)-C(2)-C(3)	108.88(15)
C(1)-C(2)-C(3)	110.78(15)	C(4)-C(3)-C(33)	110.42(18)
C(4)-C(3)-C(2)	109.50(16)	C(33)-C(3)-C(2)	114.64(17)
O(35)-C(4)-O(34)	122.47(18)	O(35)-C(4)-C(3)	126.0(2)
O(34)-C(4)-C(3)	111.53(16)	C(1)-O(5)-C(6)	111.37(14)
O(5)-C(6)-C(11)	108.56(15)	C(1)-O(7)-C(8)	112.01(13)
O(7)-C(8)-C(11)	108.47(16)	C(1)-O(9)-C(10)	111.30(14)
O(9)-C(10)-C(11)	109.06(15)	C(8)-C(11)-C(10)	106.85(17)
C(8)-C(11)-C(12)	111.81(17)	C(10)-C(11)-C(12)	111.62(17)
C(8)-C(11)-C(6)	106.34(16)	C(10)-C(11)-C(6)	106.39(16)
C(12)-C(11)-C(6)	113.41(17)	C(2)-N(13)-C(14)	125.39(14)
N(13)-C(14)-C(15)	107.68(14)	N(13)-C(14)-C(21)	105.67(13)
C(15)-C(14)-C(21)	110.79(14)	N(13)-C(14)-C(27)	115.00(14)
C(15)-C(14)-C(27)	111.53(14)	C(21)-C(14)-C(27)	106.02(14)
C(16)-C(15)-C(20)	118.02(17)	C(16)-C(15)-C(14)	122.24(16)
C(20)-C(15)-C(14)	119.64(16)	C(15)-C(16)-C(17)	120.59(18)
C(18)-C(17)-C(16)	120.31(19)	C(19)-C(18)-C(17)	119.87(19)
C(18)-C(19)-C(20)	119.81(19)	C(19)-C(20)-C(15)	121.37(19)
C(22)-C(21)-C(26)	117.36(18)	C(22)-C(21)-C(14)	120.28(16)
C(26)-C(21)-C(14)	122.36(17)	C(23)-C(22)-C(21)	122.14(19)
C(22)-C(23)-C(24)	119.8(2)	C(25)-C(24)-C(23)	119.2(2)
C(24)-C(25)-C(26)	121.09(19)	C(21)-C(26)-C(25)	120.3(2)
C(28)-C(27)-C(32)	117.54(17)	C(28)-C(27)-C(14)	119.58(16)
C(32)-C(27)-C(14)	122.54(16)	C(29)-C(28)-C(27)	121.5(2)
C(30)-C(29)-C(28)	120.2(2)	C(29)-C(30)-C(31)	119.6(2)
C(30)-C(31)-C(32)	120.2(2)	C(31)-C(32)-C(27)	120.99(19)
C(4)-O(34)-C(36)	116.02(16)		

Table 4. Anisotropic displacement parameters [$Å^2$ x 10^3] for gl902.
The anisotropic displacement factor exponent takes the form:
$-2\pi^2 [(ha^*)^2 U_{11} + ... + 2hka^* b^* U_{12}]$

	U11	U22	U33	U23	U13	U12
C(1)	24(1)	28(1)	27(1)	2(1)	0(1)	-1(1)
C(2)	24(1)	23(1)	25(1)	2(1)	0(1)	0(1)
C(3)	32(1)	28(1)	32(1)	-3(1)	4(1)	-5(1)
C(4)	32(1)	25(1)	41(1)	-5(1)	3(1)	-6(1)
O(5)	36(1)	24(1)	28(1)	0(1)	6(1)	3(1)
C(6)	38(1)	35(1)	33(1)	-6(1)	10(1)	4(1)
O(7)	31(1)	35(1)	24(1)	4(1)	4(1)	7(1)
C(8)	35(1)	46(1)	27(1)	2(1)	5(1)	6(1)
O(9)	23(1)	43(1)	34(1)	-6(1)	6(1)	-2(1)
C(10)	27(1)	46(1)	34(1)	-2(1)	7(1)	2(1)
C(11)	28(1)	38(1)	27(1)	-1(1)	6(1)	6(1)
C(12)	37(1)	53(1)	30(1)	-2(1)	8(1)	7(1)
N(13)	25(1)	24(1)	24(1)	7(1)	0(1)	3(1)
C(14)	23(1)	21(1)	24(1)	-1(1)	2(1)	2(1)
C(15)	24(1)	19(1)	23(1)	0(1)	1(1)	-5(1)
C(16)	34(1)	26(1)	29(1)	-1(1)	1(1)	-1(1)
C(17)	47(1)	36(1)	26(1)	-5(1)	3(1)	-3(1)
C(18)	49(1)	36(1)	23(1)	7(1)	-8(1)	-8(1)
C(19)	34(1)	27(1)	36(1)	9(1)	-6(1)	-4(1)
C(20)	28(1)	24(1)	27(1)	1(1)	2(1)	0(1)
C(21)	26(1)	27(1)	21(1)	-1(1)	0(1)	3(1)
C(22)	32(1)	34(1)	41(1)	-9(1)	0(1)	1(1)
C(23)	28(1)	52(1)	45(1)	-16(1)	0(1)	2(1)
C(24)	31(1)	62(2)	30(1)	-5(1)	2(1)	18(1)
C(25)	50(1)	35(1)	33(1)	4(1)	11(1)	18(1)
C(26)	34(1)	28(1)	30(1)	1(1)	8(1)	5(1)
C(27)	22(1)	25(1)	28(1)	-5(1)	0(1)	6(1)
C(28)	24(1)	36(1)	33(1)	-5(1)	-3(1)	4(1)
C(29)	31(1)	58(2)	33(1)	-15(1)	-5(1)	8(1)
C(30)	41(1)	41(1)	57(2)	-29(1)	-11(1)	10(1)
C(31)	43(1)	26(1)	57(2)	-11(1)	-11(1)	6(1)
C(32)	32(1)	28(1)	35(1)	-4(1)	-6(1)	3(1)
C(33)	60(2)	27(1)	51(1)	5(1)	8(1)	-10(1)
O(34)	37(1)	36(1)	30(1)	-8(1)	1(1)	-2(1)
O(35)	76(1)	35(1)	59(1)	-3(1)	17(1)	13(1)
C(36)	38(1)	50(1)	37(1)	-15(1)	5(1)	-7(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$ x 10^3) for gl902.

	x	y	z	U(eq)
H(2)	-73(12)	660(10)	28(10)	8(4)
H(3)	-1216(15)	-642(12)	211(11)	26(5)
H(6X)	-1224(16)	2518(14)	2019(13)	44(6)
H(6Y)	-10(20)	2192(12)	2524(14)	46(6)
H(8X)	61(17)	707(13)	3366(14)	54(6)
H(8Y)	-925(16)	-66(13)	3169(12)	42(6)
H(10X)	-2725(16)	1358(13)	1769(12)	41(6)
H(10Y)	-2723(18)	363(13)	2320(13)	47(6)
H(12X)	-2582(18)	2114(15)	3396(14)	58(7)
H(12Y)	-1441(18)	1839(14)	3953(14)	50(7)
H(12Z)	-2420(20)	1100(16)	3870(16)	75(8)
H(13)	1557(14)	-30(11)	1132(11)	26(5)
H(16)	2967(14)	442(11)	-1324(11)	24(5)
H(17)	2404(16)	874(13)	-2799(13)	40(6)
H(18)	844(15)	1883(12)	-2906(12)	31(5)
H(19)	-70(16)	2531(13)	-1651(12)	40(6)
H(20)	634(15)	2091(11)	-258(12)	24(5)
H(22)	4292(17)	1609(14)	211(13)	44(6)
H(23)	6146(19)	917(15)	331(14)	57(7)
H(24)	6380(19)	-645(15)	443(14)	58(7)
H(25)	4609(18)	-1570(14)	431(13)	47(6)
H(26)	2817(16)	-930(13)	308(12)	31(5)
H(28)	2137(15)	945(12)	2108(11)	23(5)
H(29)	2380(17)	2127(14)	3141(14)	49(7)
H(30)	2738(17)	3644(13)	2639(13)	42(6)
H(31)	2810(16)	3942(13)	1060(12)	36(5)
H(32)	2631(15)	2728(11)	32(12)	25(5)
H(33X)	680(19)	-1536(14)	1048(14)	53(7)
H(33Y)	-497(19)	-2034(15)	777(14)	57(7)
H(33Z)	-542(18)	-1281(14)	1572(15)	59(7)
H(36X)	-269(19)	-1226(15)	-2328(14)	55(7)
H(36Y)	922(16)	-752(13)	-2151(13)	36(6)
H(36Z)	-154(18)	-106(14)	-2554(13)	45(6)

Appendix E

Calculation to Determine Resin Loading

Theoretical Substitution ($S_{(th)}$):

$$\frac{S_{(s)}}{1+\frac{(S_{(s)} \times Wt_{(add)})}{1000}} = S_{(th)}$$

$$\frac{0.8}{1+\left(\frac{0.8+(188.08-138.06)}{1000}\right)} = 0.769 mmol/g$$

for substitution of Wang p-nitrophenol resin **6.31** with Ser-OBO **6.26**.

$Wt_{(g)}$ = weight gained by resin in g
$Wt_{(add)}$ = g/mol added to resin
$Wt_{(t)}$ = total new weight of resin in g
$S_{(s)}$ = starting substitution mmol/g