Synthesis of Novel Compounds for the Study of Biomolecules using NMR Spectroscopy

A thesis submitted for the degree of Doctor of Philosophy

of

The Australian National University

By

Dharshana Padmakshan

September 2011

THE AUSTRALIAN NATIONAL UNIVERSITY

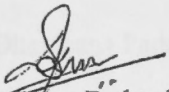
Declaration

This is to declare that the work presented herein represents original work that I carried out during my PhD candidature except for the following.

The cell free synthesis of ubiquitin using (*S*)-γ-fluoroleucine hydrazide was carried out by Dr Madeleine Headlam.

To the best of my knowledge, the work presented in this thesis does not contain material that has been submitted for any other degree or diploma in any university or any other tertiary institution. Established results and methodologies published or written by another person have been acknowledged by citation of the original work throughout the text.

I give consent for a copy of this thesis to be deposited at the University library and be made available for loan and photocopying.



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This thesis is dedicated to my late grandfather

Sri E. N. Vishnu Namboothiri

ABSTRACT

This thesis presents an investigation into the synthesis of compounds *viz.*, bifunctional chelators that could be used as lanthanide shift reagents and fluorinated amino acids that could be used as probes in the study of biomolecules using nuclear magnetic resonance spectroscopy.

Initially, syntheses of acyclic multidentate ligands like DTPA that could function as lanthanide shift reagents were investigated. A DTPA derivative with a cysteine side arm that could bind to biomolecules via a disulfide bond was synthesised. The lanthanum complex of this ligand was synthesised but the NMR spectrum of this coordination compound showed a complex spectrum due to the high conformational flexibility of the compound. Hence the synthesis of other non cyclic ligands with more stereochemically rigid structures, incorporating more stereo centers in the triamine pentaacetic acid backbone was investigated employing dialkylation and reductive amination methods. However, these acyclic ligands could not be synthesised due to steric hindrance in the substrates.

Research was then directed towards the synthesis of another class of bifunctional chelators possessing a polyaza polycarboxylic macrocycle as in TETA. A TETA derivative with a cyclam backbone with three of the four substituents being an acetic

acid moiety and the fourth substituent being a cysteine moiety was synthesised. The ytterbium complex of this ligand however was presumed to exist in a dynamic equilibrium with the uncomplexed ligand. This calls for significant structural modification of the ligand for it to be successfully used as a lanthanide shift reagent.

Synthesis of fluorinated amino acids were investigated that can be incorporated into proteins. A stereoselective synthesis of (*S*)-γ-fluoroleucine was accomplished. An HPLC assay was developed to determine the enantiomeric purity of the amino acid. Gratifyingly, preliminary studies showed that this unnatural amino acid was incorporated significantly in its hydrazide form into the protein *via* cell free protein synthesis. Development of a simple and straightforward general synthetic route for the synthesis of fluorinated amino acids using *N*-phthaloylvaline and *N*-phthaloylleucine and the corresponding hydrazides were investigated. The bromo and chloro substituted analogues were synthesised but the synthesis of the corresponding fluoro substituted analogues failed due to competing intramolecular and elimination side reactions favoured under the reaction conditions.

Abbreviations

Ac	acetyl
ACN	acetonitrile
BINOL	1,1'-Bi-2-naphthol
Boc	*t*-butoxycarbonyl
Bu^t	butyl
BuLi	butyl lithium
Bz	benzoyl
Calcd	calculated
δ	chemical shift
DAST	*N,N'*-diethylaminosulfur trifluoride
DBDMH	1,3-dibromo-5,5-dimethylhydantoin
DCE	dichloroethane
DCM	dichloromethane
DME	dimethyl ether
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid

DOTA	1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid
DTPA	diethylenetriaminepentaacetic acid
EDC	*N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride
EDTA	Ethylenediaminetetraacetic acid
EI	electron impact
ESI	electrospray ionisation
et al.	*et alia*
EtOAc	ethyl acetate
EtOH	ethanol
HCl	hydrochloric acid
His	histidine
HPLC	high performance liquid chromatography
HOBt	hydroxybenzotriazole
HRMS	high resolution mass spectrometry
Hz	hertz
J	coupling constant
lit.	literature
LSR	lanthanide shift reagent

m/z	mass to charge ratio
Me	methyl
MeOH	methanol
mp	melting point
MRI	magnetic resonance imaging
MS	mass spectrometry
MTBE	methyl tertiary butyl ether
NMM	*N*-methyl morpholine
NMR	nuclear magnetic resonance
NPhth	*N*-phthaloyl
Ph	phenyl
Ppm	part per million
t	*tert*
TETA	1,4,8,11-tetraazacyclotetradecane-*N,N',N'',N'''*-tetraacetic acid
TFA	trifluoroacetic acid
TFT	α,α,α-trifluorotoluene
THF	tertahydrofuran
TLC	thin layer chromatography
Tr	triphenyl methyl (trityl)

t_R	retention time (chromatography)
*t*RNA	transfer ribonucleic acid
Ts	*p*-toluenesulfonyl

Contents

Chapter 1. Introduction

Nuclear magnetic resonance (NMR) spectroscopy presents an excellent tool for the structural elucidation of organic compounds. Over the past decades the use of NMR has been effectively extended to the study of complex structures like biomolecules especially with the aid and advance of ^{19}F NMR spectroscopy and NMR shift reagents. The NMR spectrum of a molecule helps to deduce a detailed chemical framework of it from the knowledge of the chemical shifts of the signals and their relative intensities and coupling constants. However measurements can be made with certainty only if the signals are resolved from each other. Proton (^1H) magnetic resonance spectroscopy is most widely used among the NMR techniques for the structural elucidation of organic molecules. Unfortunately, the chemical shifts of hydrogen atoms exhibit an intrinsically low sensitivity to changes in chemical and stereochemical environment. Thus, often in the spectra of complex organic molecules, the signals are bunched together in featureless clusters from which little definitive structural information can be obtained.[1] Among the various recent developments in NMR spectroscopy to obtain better information of molecular structure, like higher frequency spectrometers and 3D-NMR spectroscopy, the methods of using suitable probes to study the complex molecules and the role of NMR shift reagents is significant.

The work presented in this thesis comprises an investigation of the synthesis of compounds that can be used as probes in the study of biomolecules using NMR spectroscopy. The work can be broadly divided into two parts:

1. The study of the synthesis of lanthanide chelates that can be used as lanthanide shift reagents; and

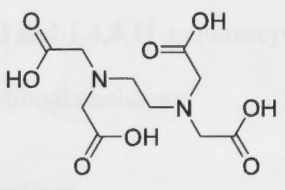
2. The study of syntheses of fluorinated amino acids that be used to probe the structure of biomolecules.

These are detailed in the following sections.

Lanthanide chelating compounds

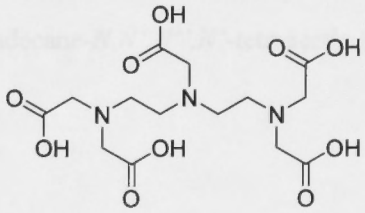
The first part of this thesis describes work on the synthesis of polyaminocarboxylic acids that can be chelated to metals like lanthanides. The syntheses of these compounds were undertaken mainly to employ them as lanthanide shift reagents for biomolecules. As the trivalent ions of lanthanides typically exhibit a coordination number of 8-10, this requirement implies the use of ligands with a denticity between 6-8 in order to obtain complexes with sufficient thermodynamic stability.[2] Polyaminocarboxylic acids present the most studied and widely used chelators of lanthanide ions. Ethylenediaminetetraacetic acid (EDTA) (Figure 1a), diethylenetriaminepentaacetic acid (DTPA) (Figure 1b), 1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid (DOTA) (Figure 1c), and 1,4,8,11-tetraazacyclotetradecane- *N,N',N'',N'''*-tetraacetic acid (TETA) (Figure 1d), and their analogues when complexed with an appropriate

metal, usually a lanthanide, are widely used as contrast reagents in magnetic resonance imaging (MRI),[2] in radiopharmaceuticals for diagnostic[3] and therapeutic[4] purposes, and as X-ray contrast reagents.[5] The first two chapters of this thesis describe the synthetic study of such coordination compounds that can be used as bifunctional chelators. Bifunctional chelators are molecules possessing an array of metal binding groups (chelating function) and an additional moiety through which the chelating function of the molecule may be covalently linked to a biomolecule. The interest was to use these molecules as lanthanide complexes that could be bound to a biomolecule so that they could be used as shift reagents to study proteins using NMR spectroscopy.



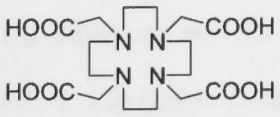
EDTA

(a)



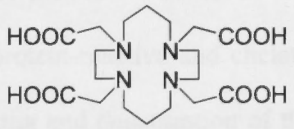
DTPA

(b)



DOTA

(c)



TETA

(d)

Figure 1

In simple words, a lanthanide shift reagent is a lanthanide complex, whose addition to a solution of a compound which possesses an appropriate lone pair of electrons causes the NMR proton resonances to spread out. Lanthanides stand out for their large and varied paramagnetism arising from unpaired electrons in the f orbital of their trivalent ions.[6] The paramagnetism gives rise to pronounced changes in chemical shifts of the nuclear spins located nearby.[7] This effect has been widely exploited in organic chemistry to resolve overlapping NMR signals of compounds with the help of lanthanide-induced shifts. Complexes of lanthanide ions with bulky chelating groups function as the most efficient shift reagents.[1] The exact choice of the chelate is determined primarily by the organic application. Our interest was to use analogues of diethylenetriaminepentaacetic acid (DTPA) and 1,4,8,11-tetraazacyclotetradecane-*N,N',N'',N'*-tetraacetic acid (TETA) as the bifunctional chelators.

DTPA derivatives

Most of the reports of using DTPA as a bifunctional chelator use one of the five carboxyl groups to bind to an antibody via an amide bond while the other four carboxyl groups and the three amines in the backbone are used to chelate to the metal. However as there is no intrinsic difference between the protein-reactive and chelating functions in DTPA such procedures can lead to cross-linking and denaturation of the antibody with concominant degradation of its ability to bind the target antigen.[8] Such cross-linking problems can be avoided using bifunctional chelating agents that incorporate a unique protein-reactive site. One of the earliest syntheses of a DTPA analogue with a unique

protein binding site was reported by Brechbiel and co-workers.[9] Their method involves the synthesis of a DTPA analogue (Figure 2a) that uses a *p*-isothiocyanatobenzyl moiety as the protein linker which is incorporated on a methylene carbon on the polyamine framework. Westerberg and co-workers[8] reported the synthesis of a DTPA analogue (Figure 2b) with the protein linker, a *p*-isothiocyanatobenzyl group on one of the carboxymethyl arms. However it has been reported that the position of this protein linker has significant affects on the conformation and stability of the DTPA derivative.[10,11] The protein linker of the bifunctional chelators described in this thesis is a thiol group of a cysteine residue. This was chosen so that the linkage to the protein could be accomplished via a disulfide bond. This presents a short and efficient linker to the protein that minimises conformational averaging and maximises dipolar couplings.[12] Also, more freedom of lanthanide positioning is achieved by using tags that form a covalent bond with a thiol of the target molecule.[11,13] EDTA analogues that use a cysteine based protein linker have been reported formerly by the groups of Leonov[13] and Ikegami.[14]

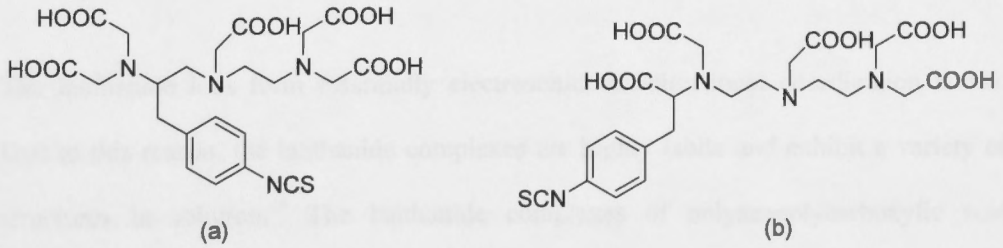


Figure 2

The studies on the synthesis of DTPA derivatives described in this thesis are mainly based on a bis-amination strategy as reported by Williams and Rapoport.[10] The DTPA derivatives prepared via this strategy have the advantage that the protein linker is contained on the central acetic acid moiety of the parent DTPA structure, so that certain symmetry is maintained in the DTPA analogue. Moreover as the central acetic acid moiety is an amino acid derivative, there is accessibility to different functional groups that can be chosen as the protein linker depending on the amino acid chosen. Also starting from an enantiomerically pure amino acid, the synthetic methods can be carefully chosen so that no distortion of stereochemistry occurs resulting in an enantiomerically pure DTPA derivative. Here is described the synthesis of a DTPA analogue with cysteine as the central amino acid. Using cysteine thus provides the thiol group as the covalent linker to the protein via a disulfide bond. The DTPA analogue with a cysteine linker described in Chapter 2 is the first of its kind ever synthesized. The amino group of cysteine is suitably dialkylated to give the desired DTPA analogue.

TETA derivatives

The lanthanide ions form essentially electrostatic, nondirectional coordination bonds. Due to this reason, the lanthanide complexes are highly labile and exhibit a variety of structures in solution.[15] The lanthanide complexes of polyazapolycarboxylic acid macrocycles like 1,4,7,10-tetraazacyclododecane-*N*,*N*',*N*'',*N*'''-tetraacetic acid (DOTA), or 1,4,8,11-tetraazacyclotetradecane- *N*,*N*',*N*'',*N*'''-tetraacetic acid (TETA), are remarkably rigid, highly symmetric and adopt the same geometry in solution and the

solid state. These properties of these macrocyclic complexes make them advantageous over the complexes of similar but noncyclic ligands such as EDTA and DTPA, at least in instances where stability of the lanthanide complexes is important, say, where the lanthanide complexes are used as MRI contrast agents.[16] The DOTA compounds are the most stable lanthanide complexes known to date.[16] The rigidity and conformational properties of complexes of TETA has been extensively studied by Desreux and Loncin.[17] They report that small structural differences between DOTA and TETA bring about drastic changes in the dynamic behavior of the complexes but do not notably alter their rigidity.

The paramagnetic complexes when used *in vivo* must be kinetically inert to cause minimum toxicity to the animal tissue. It has been reported that the Gd-DTPA and ^{90}Y-DTPA complex that are widely used in tumor targeting and radioimmuno-therapy are not totally kinetically inert and that the former complex dissociates measurably leaving deposition of gadolinium in the liver and skeleton.[18] It is generally accepted that the lanthanide complexes of macrocyclic ligands are more kinetically stable *in vivo* than those of DTPA-based ligands and should therefore avert any long-term toxicity problems.[19] Many macrocyclic ligands have hence been studied extensively as bifunctional chelators. For example, a wide variety of analogues of DOTA and DOTA-like systems have been synthesized that can be used as bifunctional chelators.[20] Similarly various analogues of TETA, like 6-[*p*-(bromoacetamido)benzyl]-1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (BAT) (Figure 3a) and 6-[*p*-(isothiocyanato)benzyl]-1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid

(SCN-TETA), (Figure 3b) have been synthesised and studied as bifunctional chelating agents in radiotherapy.[21]

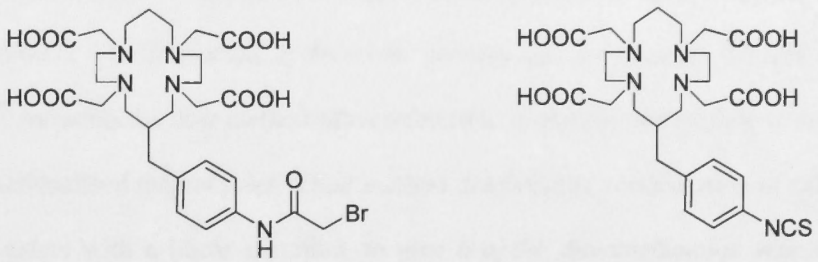


Figure 3

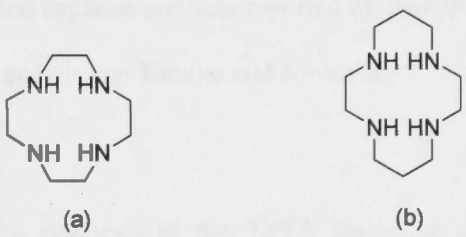


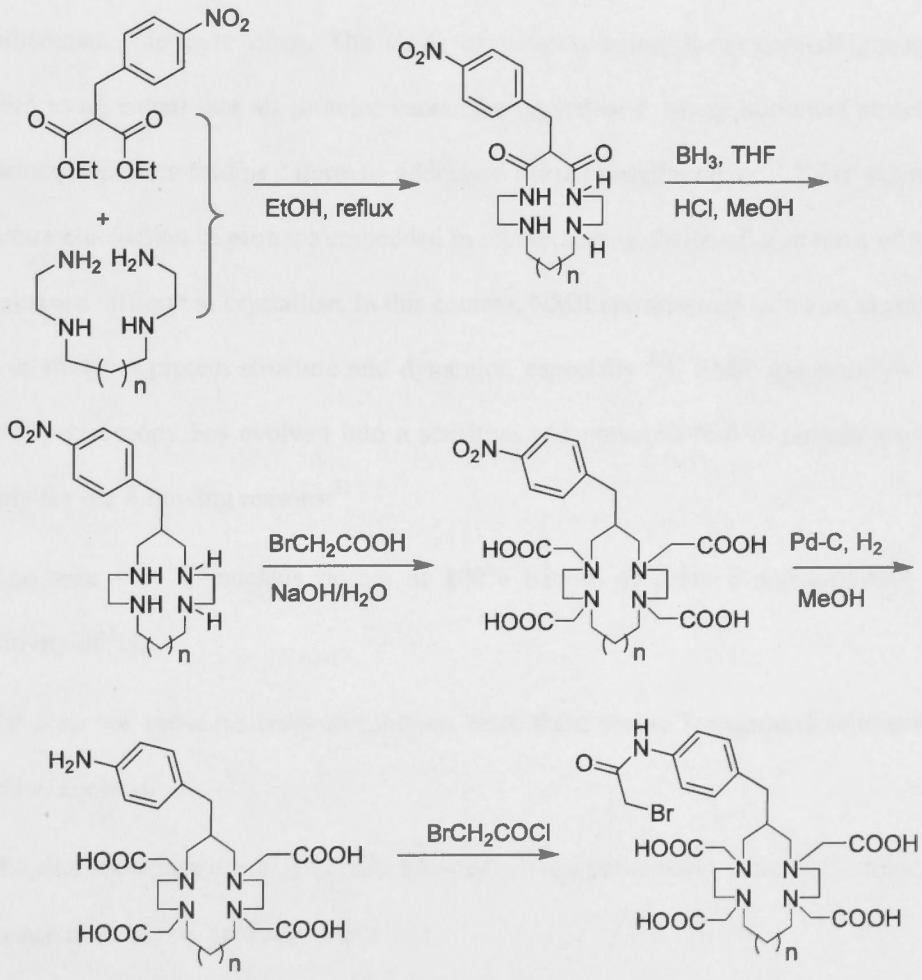
Figure 4

A general method of synthesis of *N*-functionalised tetraaza macrocycles is the reaction of the macrocycle, say a cyclen (Figure 4a) or cyclam (Figure 4b) with excess alkyl bromide in the presence of a base. For example, TETA can be prepared by reaction of cyclam with *tert*-butyl bromoacetate in the presence of a base.[22] The advantage of using *tert*-butyl bromoacetate is that the tetrabutyl ester of cyclam can be easily purified using chromatography. This can be then subjected to acid hydrolysis to give the polydentate

ligand. The introduction of different arms on the secondary amino groups of cyclam however can be done in a few different ways.[22] One method is to protect three nitrogens and then alkylate the remaining one. After alkylation, the protecting groups can be removed. Another method is using a large excess of cyclam to monosubstitute with the alkyl bromide. The unreacted cyclam can be recycled and reused. Meares and co-workers[23] introduced a new method often referred to as the peptide method to synthesise monofunctionalised macrocycles. Their method involves the condensation of substituted malonic esters with a linear tetramine to give a cyclic diaminediamide which is then reduced to the cyclic tetramine (Scheme 1.1). The tetramine is then treated with bromoacetic acid to give the TETA-like structure which has a substituent in one of the propylene bridges derived from the functionalized malonic ester. Other examples of the synthesis of functionalized cyclams are ones reported by Parker and co-workers that are used for radiolabeling[24] and also by Kimura and co-workers.[25]

The method used in the synthesis of the TETA derivative described in this thesis involves the preparation of a monofunctionalised cyclam using an excess of cyclam in the first step. The monofunctionalised cyclam derivative is then used to build up the arms on the other three secondary nitrogens. The monofunctionalised cyclam derivative is prepared via peptide coupling of an amino protected cysteine moiety to the cyclam. The rest of the nitrogens are then alkylated. The resulting compound is deprotected in one step to give the desired TETA derivative. This cyclam derivative has three advantages. Using an amino acid as one of the arms gives access to the introduction of different functional groups depending on the amino acid chosen as discussed earlier. It

possesses a short protein linker of a disulfide bond to the biomolecule via the thiol of cysteine. Using an amino acid as one of the arms instead of an acetic acid moiety increases the denticity of the final ligand as the amine of the amino acid can function as a donor to the metal. The synthesis of the TETA ligand and the investigation of its metal complexation are detailed in Chapter 3.



Scheme 1.1

Fluorinated amino acids

Proteins are biochemical workhorses performing a wide range of tasks that are essential for life.[26] Hence, understanding the structure and dynamics of proteins is very important to help unfold the mysteries of functions of life. There are various methods employed for studying the proteins, mainly X-ray crystallography and NMR spectroscopy, which are complementary to each other. The study of proteins using X-ray crystallography is limited to an extent that all proteins cannot be crystallised. Many important structural questions in protein folding cannot be addressed using crystallography.[27-30] For example, structure elucidation of proteins embedded in membranes is challenging as most of these proteins are difficult to crystallise. In this context, NMR spectroscopy plays an important role in studying protein structure and dynamics, especially ^{19}F NMR spectroscopy. ^{19}F NMR spectroscopy has evolved into a sensitive and powerful tool in protein analysis mainly for the following reasons:[31]

a) The spin 1/2 ^{19}F nucleus occurs at 100% natural abundance and has 83% the sensitivity of ^{1}H;

b) ^{19}F does not occur naturally in proteins, thus, there are no background signals with which to contend;

c) Fluorine incorporation is generally non-perturbing, particularly when substituted for hydrogen in an amino acid side chain;

d) Although the large anisotropy of the chemical shift tensor leads to broader line widths at high field strengths, the ^{19}F chemical shift range is 100-fold larger than that of ^{1}H.

This resolution, coupled with the high detection sensitivity and absence of background signals, generally yields well-resolved ^{19}F resonances in one-dimensional spectra;

e) One-dimensional ^{19}F NMR studies generally require lower protein concentrations and shorter spectral acquisition times than do multidimensional NMR techniques;

f) The ^{19}F chemical shift is controlled primarily by the fluorine lone pair electrons, which provide a large paramagnetic term in the shielding formula. The chemical shift, therefore, is exquisitely sensitive to changes in the local van der Waals environment, as well as to local electrostatic fields; and

g) The exposure of specific fluorine labels to paramagnetic centers, such as a bound or aqueous metal ion, a spin-labeled analogue of a ligand, or a spin-labeled lipid probe, can be easily detected.

The study of protein structure and dynamics using ^{19}F NMR spectroscopy is accomplished via the site specific incorporation of fluorinated amino acids into the biomolecule of interest. Site specific incorporation involves the replacement of all or a fraction of a particular canonical amino acid with a non canonical analog, generally through the use of an auxotrophic bacterial host starved for the natural amino acid and substituted with the analog.[32] Early work of incorporation involved the use of unnatural amino acids with close structural similarity to the natural amino acid, to allow transport into the cell and charging by the appropriate aminoacyl-tRNA synthetase. The position and extent of fluorine substitution within a certain amino acid, peptide or protein affects the properties of these molecules differently. It has been reported that the presence of one fluorinated amino acid in a protein substituted for its hydrocarbon analogue has tremendous impact on protein structure.[32,33] For example, the presence of fluorine in a

protein enhances its structural stability.[30] Introduction of fluorine into a protein does not introduce much steric bulk beyond hydrogen and hence the presence of two or three fluorines in a protein is not expected to significantly perturb its tertiary structure.[30] However, there have been substantial changes in protein structure observed in at least one ^{19}F NMR study, so introduction of fluorine must be carried out judiciously.[34] These fluorinated amino acids are divided into two classes according to the position of fluorine substitution: i) amino acids which contain fluorine in the side chain; and ii) amino acids in which the α-carbon is substituted by a fluoroalkyl group.[35] These two classes of amino acids dictate the properties exerted by the fluorinated building block and also the methods that have to be employed in their synthesis and their incorporation into peptides which will be discussed in detail later.

It is been found that due to the special physico-chemical properties of fluorine its usage has been widely applied in the drug industry. The various drugs that use fluorine and their mode of action are detailed in a review by Purser and co-workers.[36] Fluorine substitution is used in contemporary medicinal chemistry to improve metabolic stability, bioavailability and protein-ligand interactions. Fluoxetine, a popular antidepressant marketed as Prozac has a trifluoromethyl group on one of its aryl rings (Figure 5). Faslodex, an anticancer drug, Flurithromycin, an antibacterial drug, and Efavirenz, an antiviral drug, are others that contain fluorine.

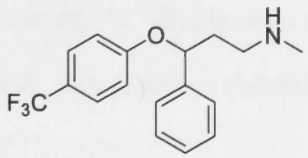


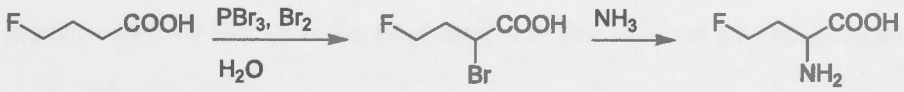
Figure 5

Structure-activity relation studies of Fluoxetine show that the inclusion of the trifluoromethyl group in the phenolic ring increases the potency of the drug 6-fold.[37]

Fluorinated amino acids play an important role in introducing the element fluorine into biomolecules, whether to improve the properties of a peptide-based drug or in the study of the structure of a protein using NMR spectroscopy. Among the fluorinated amino acids, fluorinated α-amino acids have gained extensive attention as synthetic targets due to their importance in biological and peptide chemistry. For example, β-fluoroalanine and *(S)*-α-fluoromethylhistidine are good irreversible inhibitors for the corresponding bacterial alanine racemases and histidine decarboxylase.[38]

Although a number of syntheses of fluorinated amino acids have been reported, the efficient preparation of γ-fluorinated α-amino acids remains a challenge. The earlier reported syntheses of γ-fluoro-α-amino acids followed a limited number of methods. Nucleophilic substitution of halogens with an amino group in fluorinated halocarboxylic acids or nucleophilic substitution of hydroxyl or halogen substituents with fluorine in the corresponding amino acids has mostly been employed. For example, γ-fluoro-α-

aminobutyric acid was synthesised by bromination of γ-fluorobutyric acid and subsequent reaction of the bromide with ammonia (Scheme 1.2).[39]



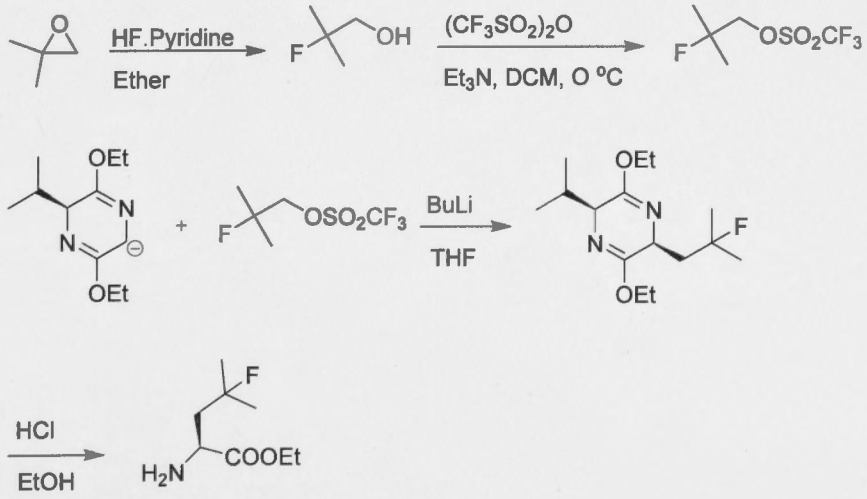
Scheme 1.2

However following a similar procedure for synthesising other amino acid derivatives resulted in low yields or raised the need for multiple steps to achieve the required target.[39] For example, the synthesis of γ-fluoroisoleucine from 4-fluoro-3-methylpentanoic acid resulted in low yields.[40] Employing the direct fluorination of the corresponding amino acid involved hazardous reagents which were difficult to handle. For example, one of the earliest reported syntheses of γ-fluoroglutamic acid involved perchloryl fluoride as the fluorinating agent which had been reported as a very hazardous material.[41] Moreover this and other methods employed for the synthesis of γ-fluoroglutamic acid suffered lack of stereoselectivity.[42] Similarly many syntheses involved other hazardous reagents like HF or HF-derived compounds. Hence a short and mild method for the synthesis of monofluorinated amino acids proves to be relevant.

Chapters 4, 5, 6, 7 and 8 in this thesis deal with studies of the synthesis of fluorinated amino acids and the incorporation of fluorinated amino acids into protein *via* cell-free protein synthesis. Chapter 4 details the synthesis of (*S*)-γ-fluoroleucine. The synthesis is

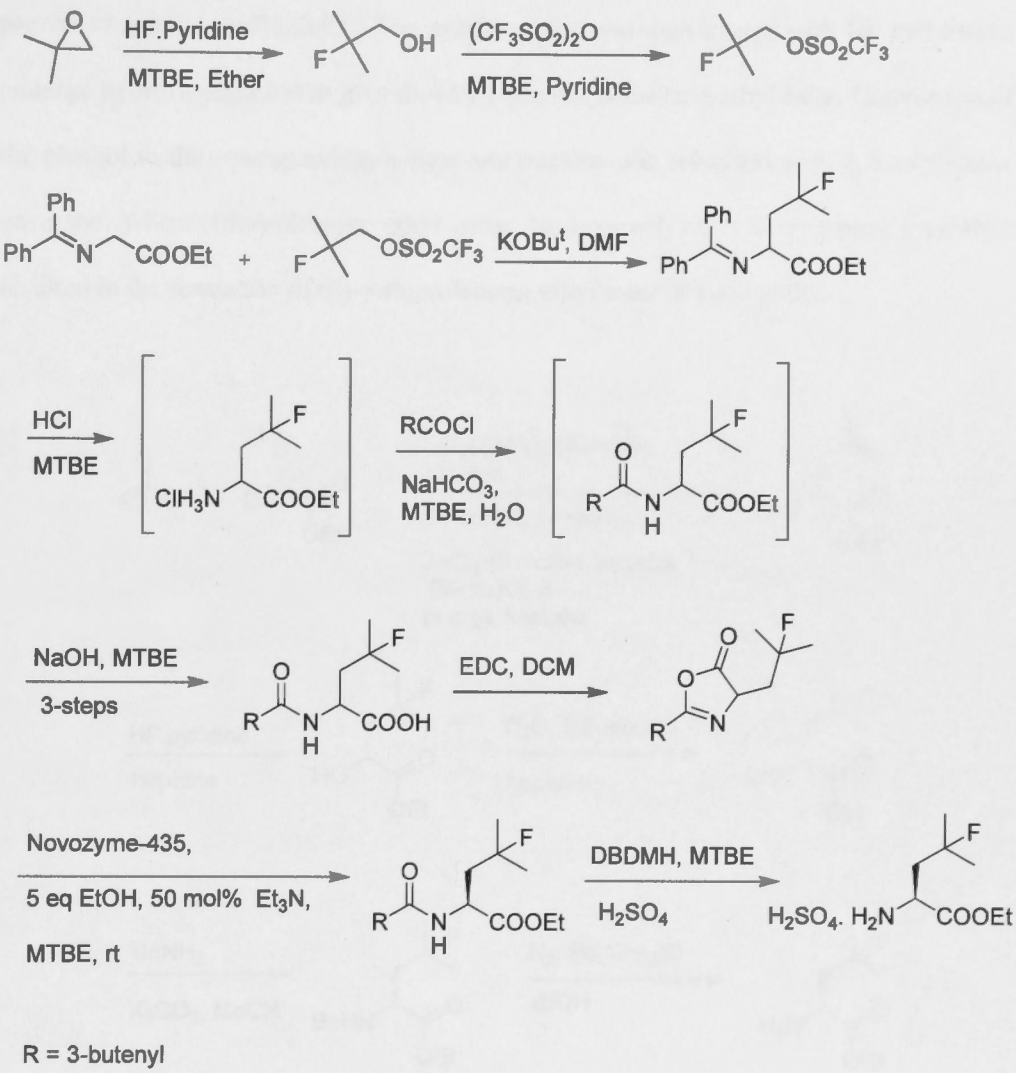
mild, short and stereoselective in that it is based on the direct side chain bromination of a protected leucine which is then substituted with fluorine.

The synthesis of (*S*)-γ-fluoroleucine ethyl ester was first reported by Papageorgiou and Borer in 1994 to investigate the immunosuppressive activity of cyclosporin-A derivatives.[43] The synthesis involved the alkylation of Schollkopf's bis-lactim ether with 1-trifluoromethanesulfonyl-2-fluoro-2-methylpropane (Scheme 1.3). This alkylating agent was synthesised by fluorinating isobutylene oxide with HF and then conversion to the corresponding triflate using triflic oxide. The sulfonate was treated with the bis-lactim ether anion to give the fluorinated derivative of the bis-lactim ether which on acid hydrolysis gave (*S*)-γ-fluoroleucine ethyl ester.



Scheme 1.3

Another synthesis of the ethyl ester was reported in 2005 by Limanto and coworkers,[44] which involved a chemoenzymatic approach (Scheme 1.4). This involved the hydrofluorination of isobutylene oxide with Olah's reagent (Pyridine.HF) to give the fluoro alcohol which was further converted to the corresponding triflate. This fluorine-containing side chain was added to *N*-diphenylmethylene glycine ethyl ester via deprotonation with potassium tertiary butoxide. Subsequent transformation to the amido acid was accomplished in a three-step, one-pot process, involving imine hydrolysis, amide formation under Schotten-Baumman conditions and saponification. Cyclodehydration of the amido acid was then performed using EDC in DCM to give the desired azlactone. This racemic azlactone then was subjected to a lipase-B enzyme catalysed ring opening to the give the (*S*)-isomer of the corresponding *N*-3-butenoyl-γ-fluoroleucine ethyl ester. Oxidative removal of the *N*-3-butenoyl group yielded *(S)*-γ-fluoroleucine ethyl ester in 97% *ee*.

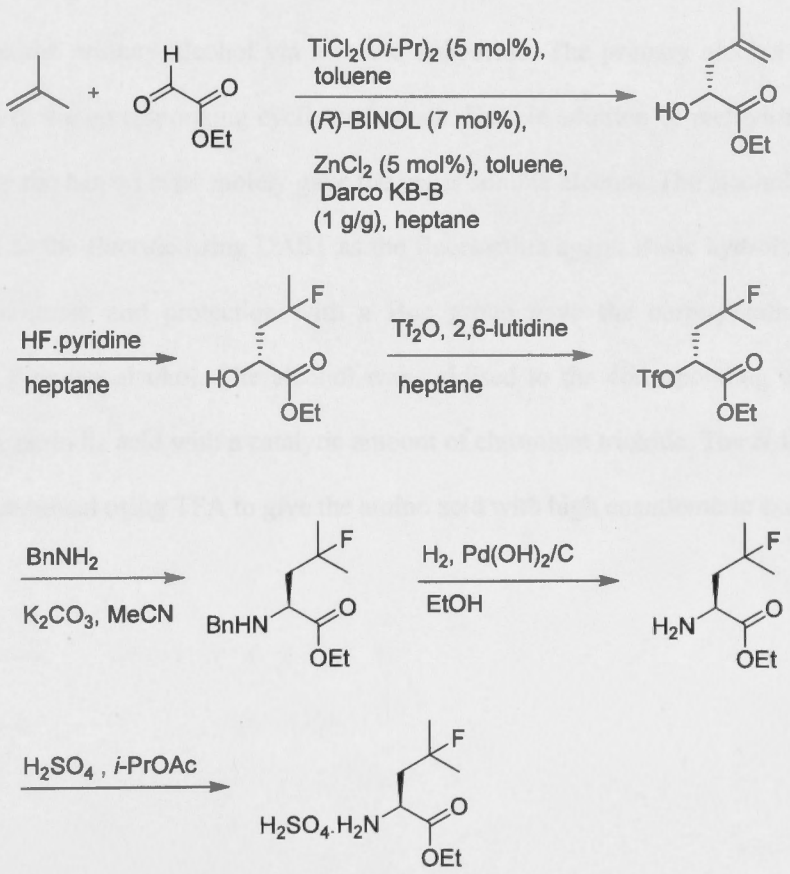


R = 3-butenyl

Scheme 1.4

Yet another synthesis of (*S*)-γ-fluoroleucine ethyl ester was reported in 2006 by Nadeau and co-workers (Scheme 1.5).[45] The synthesis involved the titanium catalysed glyoxylate–ene reaction of ethyl glyoxylate and isobutylene to give the corresponding hydroxy ester. One drawback of this reaction, the repolymerisation of the glyoxylate was

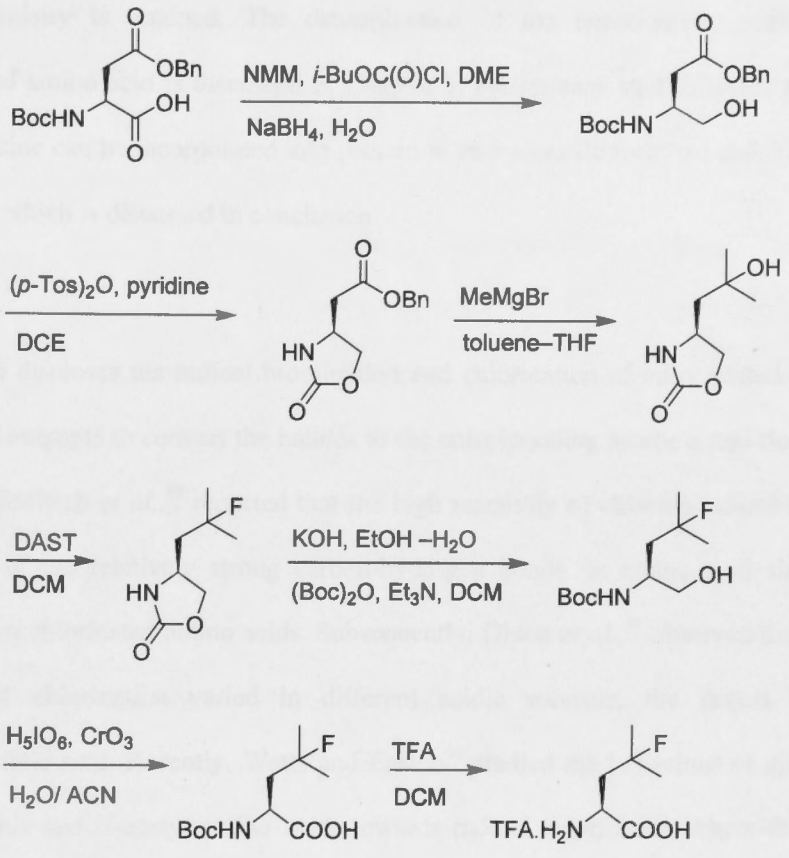
prevented using 5 mol% $ZnCl_2$. The hydroxy ester was then treated with HF.pyridine to undergo hydrofluorination to give the (*R*)-γ-fluoroleucine acid ethyl ester. Conversion of the alcohol to the corresponding triflate and nucleophilic substitution with benzylamine gave the *N*-benzylfluoroleucine ethyl ester. Hydrogenolysis with Pearlman's catalyst resulted in the formation of (*S*)-γ-fluoroleucine ethyl ester in good yield.



Scheme 1.5

All these methods describe the synthesis of (*S*)-γ-fluoroleucine ethyl ester and not the free amino acid itself. Moreover these methods either use corrosive reagents like HF or require an enzymatic resolution for attaining enantiomeric purity.

Recently *(S)*-γ-fluoroleucine was synthesised by Truong and co-workers[46] from *N*-(*tert*-butoxycarbonyl)-(*S*)-aspartic acid 4-benzyl ester (Scheme 1.6). This was selectively reduced to the primary alcohol via a mixed anhydride. The primary alcohol was then converted to the corresponding cyclic carbamate. Double addition of methylmagnesium bromide to the benzyl ester moiety gave the water soluble alcohol. The alcohol was then converted to the fluoride using DAST as the fluorinating agent. Basic hydrolysis of the cyclic carbamate and protection with a Boc group gave the corresponding *N*-Boc protected β-amino alcohol. The alcohol was oxidised to the corresponding carboxylic acid using periodic acid with a catalytic amount of chromium trioxide. The *N*-Boc group was then removed using TFA to give the amino acid with high enantiomeric excess.



Scheme 1.6

The synthesis of (*S*)-γ-fluoroleucine described in this thesis involves a short and concise method based on a direct side chain bromination of *N*-phthaloyl-(*S*)-leucine methyl ester. The phthaloyl and methyl ester groups were used to protect the amino and carboxylic acid functionalities respectively. Previous work revealed that side chain radical bromination using these protecting groups occurred with high regioselectivity.[47] The bromide is then converted to the fluoride using a mild and easy to use reagent, silver fluoride. (*S*)-γ-Fluoroleucine was then obtained after the deprotection of the amino and carboxylic acid groups. The synthesis is accomplished in such a way that

stereochemistry is retained. The determination of the enantiomeric purity of the fluorinated amino acid is discussed in Chapter 5. Preliminary studies reveal that (*S*)-γ-fluoroleucine can be incorporated into protein in its hydrazide form *via* cell-free protein synthesis which is discussed in conclusion.

Chapter 6 discloses the radical bromination and chlorination of other phthaloyl amino acids and attempts to convert the halides to the corresponding alcohols and fluorides. In 1964 Kollonitsch *et al.*,[48] reported that the high reactivity of chlorine radical led to the cleavage of the relatively strong carbon-hydrogen bonds on amino acid side chains resulting in chlorinated amino acids. Subsequently, Dixon *et al.*,[49] observed that the rate of radical chlorination varied in different acidic solvents, the fastest being in trifluoroacetic acid. Recently, Watts and Easton[50] studied the behaviour of different α-amino acids and *N*-acetyl amino acids towards radical chlorination where they report that the backbone and adjacent side chain positions of amino acids and their derivatives are extensively deactivated toward reaction. In Chapter 6 the radical chlorination studies carried out on phthaloyl amino acids are presented for the first time. The further conversion of these chlorides to the corresponding alcohols and fluorides is also investigated. Although there are a few reported methods for the synthesis of hydroxyl-substituted α-amino acids, they are generally either indirect or not enantiospecific or not generally applicable to other amino acids.[51-54] The synthesis of alcohols carried out in the work described in Chapter 6 follows the procedure reported by Easton and co-workers.[55] They report the direct hydroxylation of bromophthaloylamino acids.

Hydroxylation of the phthaloylamino acids presented in this Chapter was studied using both bromo- and chloro-substituted phthaloylamino acids.

Chapter 7 describes the investigation of developing a short and straightforward method for the synthesis of fluorinated amino acid hydrazides. It was reported by Wolman and coworkers[56,57] that *N*-protected-α-amino acid hydrazides or *N*-protected peptide hydrazides are of use for the formation of peptide bonds either *via* the azides or through oxidative coupling. We envisaged that the functionalised amino acid hydrazides could be used for incorporating unnatural amino acids into proteins. The study in this thesis involves the synthesis of *N*-phthaloyl amino acid hydrazides and their conversions to chlorides and bromides using radical halogenation. It was anticipated that these halides could be converted to the corresponding alcohols and fluorides using mild reagents. The fluorides could then be used as is for protein incorporation studies. A detailed study of different functionalisation reactions carried out on *N*-phthaloylvaline and *N*-phthaloylleucine hydrazides is presented in this Chapter.

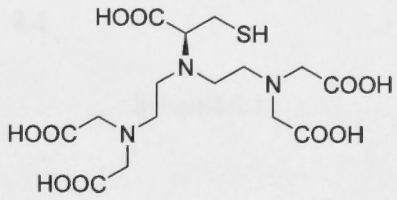
Chapter 2. Synthesis of DTPA analogues as NMR shift reagents

Introduction

This Chapter describes the synthesis of diethylenetriaminepentaacetic acid (DTPA) derivatives and similar compounds that can be used as shift reagents in biomolecular NMR studies when complexed with a lanthanide. Complex formation with a lanthanide results in the introduction of a paramagnetic centre into an otherwise diamagnetic macromolecule. These paramagnetic species can be used as an aid in the study of biomolecules by NMR. Such species provide a rich source of additional structural information, since paramagnetic restraints extend over much larger distances than Nuclear Overhauser Effects.

These paramagnetic entities can be linked to the biomolecules in different ways. For example, incorporation of these molecules into a protein can be achieved using a carboxylic acid group to form an amide linkage or through a thiol group to form a disulfide bond. The first compound **2.1** designed for this study has a DTPA backbone with eight coordination sites provided by the three amines and five carboxylic acid groups. It also contains a free thiol group to enable linkage to a cysteine residue in

protein *via* a disulfide bond. Choi and team reported the synthesis of a cysteine based DTPA derivative that could be used in radioimmunotherapy.[58] More recently, Aime and co-workers reported the synthesis and use of the gadolinium complex of a DTPA derivative with a disulfide side chain derived from cysteine.[59]



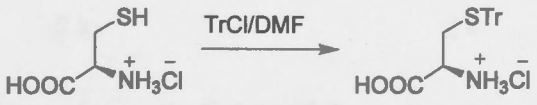
2.1

Synthesis

The synthesis of compound **2.1** was based on the bis-*N*-alkylation strategy reported by Williams and Rapoport[10] using the amino acid cysteine **2.2**. This synthetic strategy required the synthesis of two separate components: a protected amino acid and a protected bis-carboxymethylated amino ethyl bromide.

The carboxylic acid groups and the thiol functionality in cysteine **2.2** were protected so that they did not intervene in any other reactions involved in the synthesis and also so that the crude products could be purified using conventional chromatographic methods prior to the final deprotection to give the ligand **2.1**. Acid labile protecting groups were

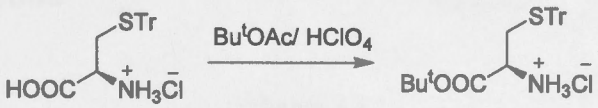
chosen so that basic conditions that might alter the stereochemical integrity of the amino acid **2.2** could be avoided for deprotection.



2.2 2.3

Scheme 2.1

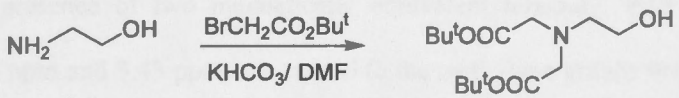
The synthesis commenced with commercially available (*S*)-cysteine **2.2**. Firstly, the thiol group was protected as an *S*-trityl derivative (Scheme 2.1). The thioether was prepared by treating the hydrochloride salt of cysteine with trityl chloride in DMF for 40 hours.[60] Unreacted trityl chloride was then removed and after work up the trityl derivative **2.3** was obtained in 66% yield. The trityl derivative **2.3** was identified using ^{1}H NMR spectroscopy and mass spectrometry. The ESI(+ve) mass spectrum shows peaks at *m/z* 364 and 386 which correspond to the protonated and sodiated ion of a species of molecular weight 363. The increase in mass of 243 units from that of the starting amino acid **2.2** is consistent with the incorporation of a trityl group. The NMR spectrum shows multiplets in the region 7.24-7.38 ppm characteristic of the aromatic hydrogens in the trityl group. All spectroscopic data are in agreement with the literature values for the compound.[60] The melting point of the trityl compound **2.3** also agrees with that reported.[60] This indicates that no racemisation occurred during the reaction. The reaction occurs *via* nucleophilic substitution of the trityl chloride by the thiol. Attack *via* the amine is prevented by maintaining it as the corresponding hydrochloride salt.



2.3 2.4

Scheme 2.2

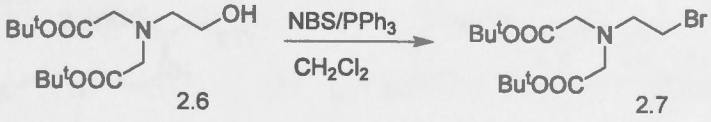
The cysteine derivative **2.3** was then subjected to an acid catalysed esterification with *tert*-butyl acetate to give compound **2.4** as depicted in Scheme 2.2. The ester **2.4** was obtained in 79% yield after chromatographic separation. The compound was identified from the ^{1}H NMR spectrum which showed a singlet resonance at 1.42 ppm integrating to nine hydrogens characteristic of the tertiary butyl ester group.



2.5 2.6

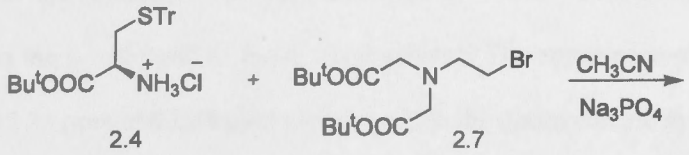
Scheme 2.3

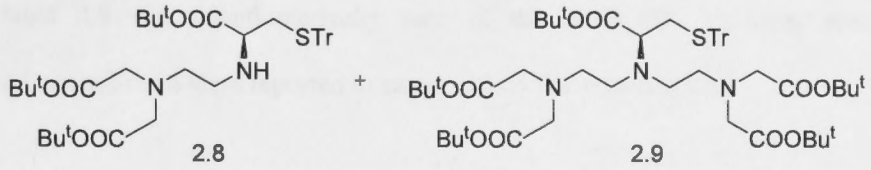
The second component, the bis-carboxymethylated amino ethyl bromide **2.7** was synthesized as follows: firstly the bis-carboxymethylated ethanolamine derivative **2.6** was prepared. Ethanolamine **2.5** was dialkylated using a solution of *tert*-butyl bromoacetate in DMF at 0 °C (Scheme 2.3).



Scheme 2.4

The crude product **2.6** was brominated directly by treating with *N*-bromosuccinimide in the presence of triphenylphosphine[61] to afford the corresponding bromide **2.7** (Scheme 2.4). The bromide **2.7** was obtained in 79% yield after chromatographic separation. It was characterized using mass spectrometry and NMR spectroscopy. The ESI(+ve) mass spectrum shows peaks at *m/z* 352 and 354 of equal intensity. This is a characteristic of a bromide as the two isotopes of bromine, ^{79}Br and ^{81}Br, occur in equal amounts in nature. The peaks correspond to the protonated ion of the bromide **2.7**. The ^{1}H NMR spectrum of the bromide **2.7** displays a singlet at 1.46 ppm integrating to eighteen hydrogens that indicates the presence of two magnetically equivalent *tert*-butyl ester groups. Two triplets at 3.13 ppm and 3.43 ppm correspond to the methylene groups between nitrogen and bromine. The appearance of a singlet at 3.48 ppm that integrates to four hydrogens corresponds to the two methylene groups flanked by the ester groups and the nitrogen. The ^{1}H NMR spectrum is consistent with that reported in the literature.[10]

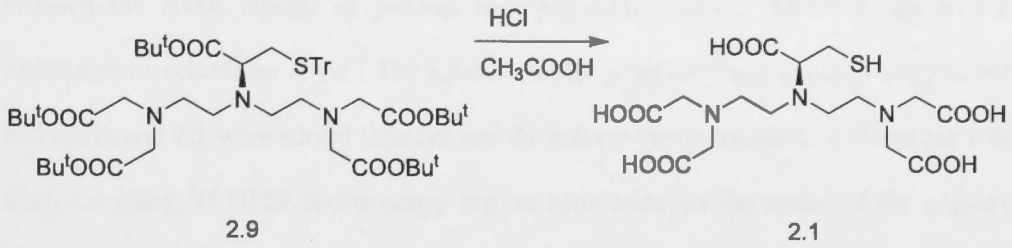




Scheme 2.5

Having prepared the bromide **2.7** and the cysteine derivative **2.4** the bis *N*-alkylation was carried out by treating an excess of the bromide **2.7** with the cysteine derivative **2.4** in phosphate buffer. The reaction furnished the monoalkylated amine **2.8** and the dialkylated amine **2.9** (Scheme 2.5) in 32% and 53% yield respectively, after column chromatography. The unreacted bromide **2.7** was also recovered after reaction. The ESI(+ve) mass spectrum of the monoalkylated amine **2.8** shows a major peak at *m/z* 691 corresponding with the protonated ion. The byproduct, the monoalkylated amine **2.8** was not characterised further. The ESI(+ve) mass spectrum of the dialkylated compound **2.9** shows peaks at *m/z* 962 and 984 that correspond to the protonated and sodiated ion of a species of mass 961 being the calculated mass. The NMR spectrum shows singlet resonances at 1.39 ppm and 1.45 ppm that correspond to the *tert*-butyl groups one on the cysteine residue, and the other four on the side arms of the two terminal nitrogens. The peak integration showed a ratio of 1:4 which indicates the presence of five *tert*-butyl ester groups in the molecule. The appearance of a doublet of doublets at 2.98 ppm corresponds to the α-hydrogen of the cysteine residue. The appearance of two doublets of doublets at 2.35 ppm and 2.48 ppm corresponds to the diastereotopic hydrogens of the SCH_2- group. The appearance of a multiplet between 7.19-7.44 ppm indicates the presence of the aromatic hydrogens of the trityl group. It was assumed that the

dialkylated **2.9** was enantiomerically pure as the dialkylation reaction conditions involved are mild and were reported to cause little or no racemisation.[10]



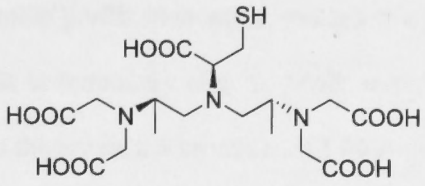
2.9 2.1

Scheme 2.6

The *tert*-butyl ester groups and the trityl group on compound **2.9** were removed through one-step deprotection using HCl (Scheme 2.6). The amine **2.9** was dissolved in minimum amount of acetic acid and treated with 6M hydrochloric acid to yield the diethylenetriaminepentaacetic acid (DTPA) derivative **2.1** as its hydrochloride salt in 67% yield. The DTPA derivative **2.1** was identified using mass spectrometry and ^{1}H NMR spectroscopy. The mass spectrum shows peaks at *m/z* 440 and 462 that correspond to the protonated and sodiated ions. The loss of the trityl group and the *tert*-butyl groups was evident from the reduction of the mass by 522 units. More importantly, the absence of ^{1}H NMR signals corresponding to the *tert*-butyl and trityl groups confirmed the removal of these groups. It was assumed that the ligand **2.1** was enantiomerically pure as in a similar synthesis reported by Williams and Rapoport[10] the acid deprotection caused no racemisation.

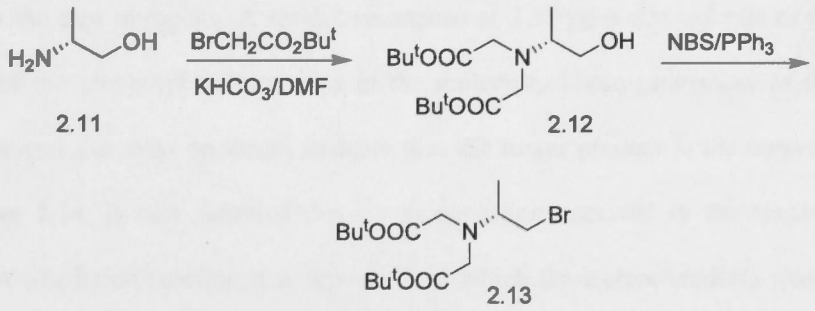
Having obtained the ligand **2.1**, the complexation was carried out with lanthanum. Lanthanum being diamagnetic would allow the observation of the ^{1}H NMR spectrum of the ligand **2.1** in the normal sweep width whereas a paramagnetic lanthanide would broaden the NMR signals of protons near the metal beyond detection due to the paramagnetic relaxation effect.[6] The aqueous solutions of lanthanum sulfate octahydrate and the ligand **2.1** were stirred together and the solvent was evaporated. The residue was analysed using ^{1}H NMR spectroscopy but no conclusion on the nature of the product could be reached as the NMR spectrum was complex. It was presumed that the molecule exhibited several conformations in the NMR time scale. The investigations to deduce the structure of a molecule using a lanthanide shift reagent (LSR) are done with the assumption that the LSR exists in a single conformation.[1] Hence such a conformationally unstable molecule is not suitable as a shift reagent and further investigations on the sample were discontinued.

It was anticipated that introducing alkyl groups on the ethylene bridge between the nitrogens on the DTPA frame would result in steric constraints in the molecule which would in turn lead to more conformational rigidity of the molecule. Thus molecule **2.10** with a similar structure to that of the ligand **2.1** but with alkyl substituents was designed on these grounds.



2.10

The synthesis was based on an *N*-alkylation strategy similar to that used in the synthesis of the ligand **2.1**. (*R*)-2-Amino-1-propanol **2.11** was used as the starting material. Firstly the bis-carboxymethylated derivative **2.12** was prepared. (*R*)-2-Amino-1-propanol **2.11** was dialkylated using a solution of *tert*-butylbromoacetate in DMF (Scheme 2.7). The crude product **2.12** was brominated directly by treating with *N*-bromosuccinimide in the presence of triphenylphosphine[61] to afford the corresponding bromide **2.13**. The mass spectrum show peaks at *m/z* 366 and *m/z* 368 that correspond to the molecular ion of the expected bromide **2.13**.



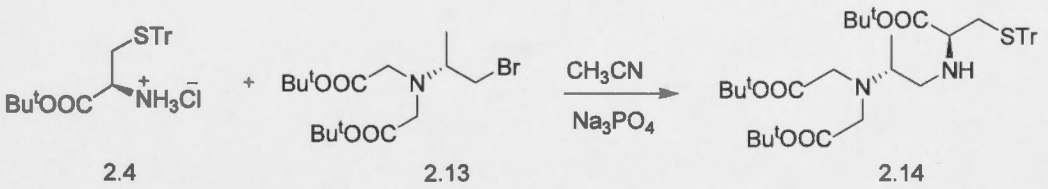
2.11 2.12 2.13

Scheme 2.7

An excess of the bromide **2.13** was treated with the cysteine derivative **2.4** in ACN in the presence of phosphate buffer (Scheme 2.8). After workup, the reaction mixture was

subjected to chromatographic purification which yielded three samples. These samples were analysed using mass spectrometry and ^{1}H NMR spectroscopy. One of the three samples was identified as the unreacted bromide **2.13** by comparing the mass spectrum and ^{1}H NMR spectrum with that of the same compound obtained previously. The ESI(+ve) mass spectrum of the second sample which was a major portion, showed a peak at *m/z* 705 which corresponds to the protonated ion of a species of molecular weight 704. This mass corresponds to the monoalkylated compound **2.14**. The NMR spectrum of the monoalkylated compound **2.14** shows a singlet resonance at 1.45 ppm that corresponds to the *tert*-butyl hydrogens in the molecule. The integration of this peak corresponds to 27 hydrogens which meant that there are only three *tert*-butyl groups in the molecule instead of five that should be present if it was the expected dialkylated compound. The presence of multiplets in the aromatic region between 7.10 ppm and 7.41 ppm shows the presence of a trityl group. A doublet resonance at 0.97 ppm that integrates to three hydrogens corresponds to the methyl group on the ethylene bridge between the two nitrogens. A singlet resonance at 3.37 ppm corresponds to the $-CH_2$ groups of the *tert*-butyl acetate arms in the molecule. These resonances of the NMR spectrum and the mass spectrum indicate that the major product is the monoalkylated derivative **2.14**. It was assumed that no racemisation occurred in the synthesis as a similar *N*-alkylation reaction was reported[10] in which the stereochemistry was retained throughout the process. The mass spectrum of the third sample, which constituted a minute fraction, less than 10% of the product mixture shows a major peak at *m/z* 705 and a minor one at *m/z* 990. The mass of the major peak corresponds to that of the monoalkylated derivative **2.14** as seen in the previous fraction. The minor peak at *m/z*

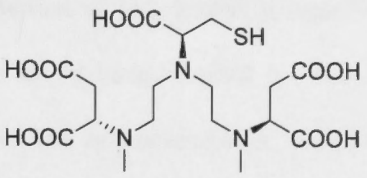
990 corresponds to the expected dialkylated product. However the ^{1}H NMR of this fraction showed similar chemical shifts as that of the monoalkylated derivative **2.14**.



2.4 2.13 2.14

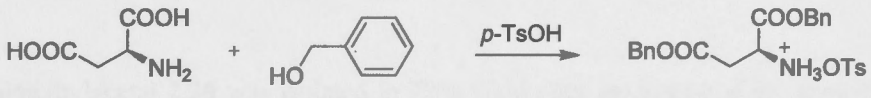
Scheme 2.8

Another attempt to synthesise the dialkylated compound was carried out by treating the monoalkylated compound **2.14** with a large excess of the bromide **2.13**. The mass spectrum of the crude reaction mixture shows a peak at *m/z* 705 that corresponds to the protonated ion of the monoalkylated compound **2.14**. A magnification of the spectrum by 10 times showed a minor peak at *m/z* 990 that corresponds to the protonated ion of the desired dialkylated compound. The reaction was stirred for longer times but did not produce the desired dialkylated compound. Another attempt to improve the reaction was done by heating the mixture to 70 °C in ACN. However this was not fruitful either. The reaction was then repeated using DMF as solvent instead of ACN at room temperature. It was presumed that DMF being a dipolar aprotic solvent would increase the rate of the substitution reaction.[62] However, neither the ^{1}H NMR nor the mass spectrum of the reaction mixture showed any sign of the dialkylated compound. It is presumed that further nucleophilic substitution of the bulky monoalkylated compound **2.14** to give the corresponding dialkylated species does not occur due to steric hindrance.



2.15

Owing to the difficulties faced in the synthesis of the above compound, the ligand with a triamine pentaacetic acid backbone **2.15** was designed. As it was presumed that the bis-alkylation strategy in the above synthesis was restricted due to the bulky nature of the reactants, a different synthetic route was proposed for attaining the ligand **2.15**.

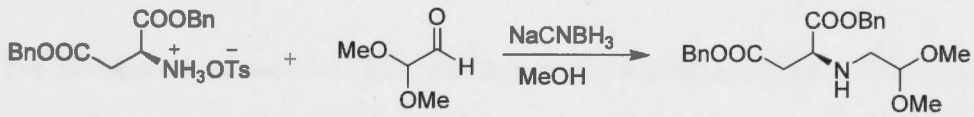


2.16 2.17 2.18

Scheme 2.9

The synthesis was based on a series of reductive aminations of acetals with amino acid derivatives in the presence of the mild reducing agent sodium cyanoborohydride as reported by Watanabe and co-workers.[63] Initially the tosylate salt of (*S*)-Aspartic acid di-benzyl ester **2.18** was synthesised. (*S*)-Aspartic acid was treated with *p*-toluenesulfonic acid in benzyl alcohol at reflux with continuous water separation to afford the di-benzyl ester **2.18** (Scheme 2.9).[64] The di-benzyl ester **2.18** was identified using ESI(+ve) mass spectrometry which shows a peak at *m/z* 314 that corresponds to the protonated

molecular ion. The increase in mass of 180 units from that of the starting amino acid **2.16** is indicative of the addition of two benzyl groups. It is presumed that the high temperature of the reaction might have resulted in some racemisation. However the optical purity of the product was not determined. The di-benzyl ester **2.18** was then subjected to reductive amination by sequentially treating with dimethoxyacetaldehyde **2.19** and sodium cyanoborohydride in methanol as illustrated in Scheme 2.10.

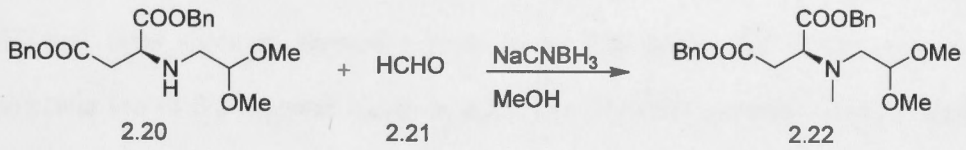


2.18 2.19 2.20

Scheme 2.10

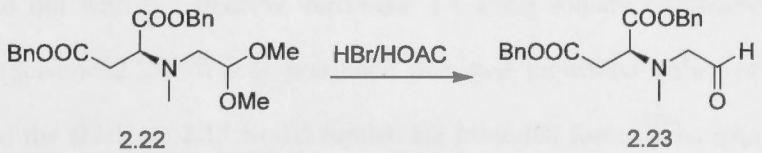
The dimethylacetal **2.20** was isolated in 72% yield after work-up and chromatographic separation and was identified using mass spectrometry and ^{1}H NMR spectroscopy. The ESI(+ve) mass spectrum shows a peak at *m/z* 402 that corresponds to the protonated molecular ion of the expected dimethoxyacetal **2.20**. The NMR spectrum showed a broad resonance at 2.10 ppm that corresponds to the single hydrogen on the nitrogen of the amino acid moiety. The appearance of two triplets at 3.75 ppm and 4.39 ppm corresponds to the α-hydrogen of the di-benzyl aspartate moiety and the hydrogen on the carbon carrying the two methoxy groups. The hydrogens of the methoxy group appear as a singlet resonance at 3.31 ppm. These key features of the NMR spectrum along with the mass spectrum indicate the formation of the expected acetal **2.20**. It is presumed that as the reductive amination was performed under mild conditions, the stereochemistry of the

starting di-benzyl ester **2.18** was retained in the reaction. However the optical purity of the acetal **2.20** was not determined



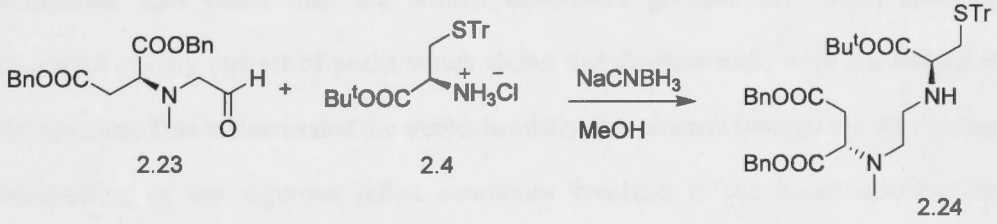
Scheme 2.11

The nitrogen of the aspartate **2.20** was then methylated by reductive amination with formaldehyde **2.21** (Scheme 2.11) to give the *N*-methylated derivative **2.22**. The reaction mixture was subjected to column chromatography and the major fraction was found to be the product **2.22** which was isolated in 85% yield. The ESI(+ve) mass spectrum of the product **2.22** show peaks at *m/z* 416 and 438 which correspond to the protonated and sodiated molecular ions. The appearance of a singlet resonance at 2.40 ppm that integrates to three hydrogens in the ^{1}H NMR spectrum indicates the presence of an *N*-methyl group in the product **2.22**. As in the previous case, it was presumed that the stereochemistry was retained in the reaction due to mild reductive amination conditions. However the optical purity of the product **2.22** was not determined.



Scheme 2.12

The acetal protecting groups of the *N*-methylated species **2.22** were removed by treating with 28% HBr/AcOH[65] to give the corresponding aldehyde **2.23** (Scheme 2.12). After work-up and chromatography the aldehyde **2.23** was isolated in 36% yield. The ESI(+ve) mass spectrum showed a peak at *m/z* 370 correspond to the protonated molecular ion of the expected aldehyde **2.23**. The ^{1}H NMR spectrum shows a singlet resonance at 9.47 ppm which is characteristic of the aldehyde hydrogen. The disappearance of the singlet resonance at 3.31 ppm shows the absence of the methoxy groups. It was believed that the stereochemistry was retained in this reaction as no harsh conditions were employed. The aldehyde **2.23** was found to be unstable at room temperature when kept for a few hours.



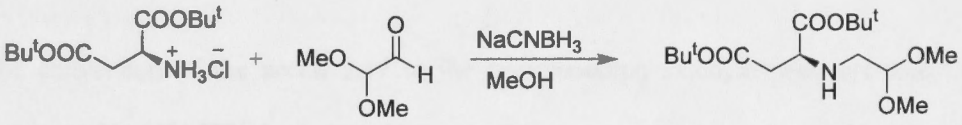
Scheme 2.13

Having obtained the aldehyde **2.23**, the final step in the series of reductive aminations was carried out with the cysteine derivative **2.4** using sodium cyanoborohydride in methanol (Scheme 2.13). It was presumed that two sequential reductive amination reactions of the aldehyde **2.23** would furnish the protected form of the required ligand **2.15**. The reaction product was purified using chromatography and analysed using mass

spectrometry and NMR spectroscopy. The mass spectrum shows two intense peaks at *m/z* 773 and 795 and a minute peak at *m/z* 1126. These molecular masses correspond to that of the protonated and sodiated molecular ion of the monoaminated compound **2.24** and the protonated molecular ion of the corresponding diaminated compound respectively. The ^{1}H NMR spectrum shows a singlet resonance at 1.41 ppm which corresponds to the *tert*-butyl ester group. This shows the presence of the cysteine moiety. The aromatic hydrogens appear as multiplets between 7.18-7.43 ppm that integrate to twenty five hydrogens. This is presumed to comprise the fifteen hydrogens of the trityl group and ten hydrogens of the two benzyl groups in the compound. The singlet resonance at 2.25 ppm that corresponds to the *N*-methyl group integrates to three hydrogens which shows that only one methyl group is present in the molecule. These observations show that the product mixture contains mainly the monoaminated compound **2.24** rather than the desired diaminated product. The NMR spectrum consisted of only one set of peaks which shows that diastereomers were not formed in the reaction. This indicates that the stereochemistry was retained through out the reaction irrespective of the vigorous reflux conditions involved in the initial esterification reaction. The reductive amination reaction was repeated with excess of the aldehyde **2.23** and the monoaminated compound **2.24** as it was presumed that this would improve the yield of the desired dialkylated product. The ESI(+ve) mass spectrum of the crude reaction mixture showed a minute peak at *m/z* 1126 corresponding to the protonated ion of the dialkylated compound, though the major peak was at *m/z* 773 that corresponds to the protonated ion of the monoaminated compound **2.24**. The isolation of the minute amount of dialkylated compound from the monoaminated compound **2.24** was not

successful. It is presumed that the second reductive amination is disfavoured due to the crowded nature of the reactants.

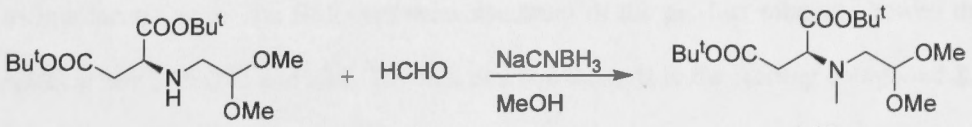
Simultaneously, the reductive amination was also tried using the corresponding *tert*-butyl ester derivative of aspartic acid **2.25** presuming that the removal of the *tert*-butyl ester groups to give the final ligand **2.15** could easily be achieved by simple hydrolysis compared to hydrogenolysis in the case of benzyl esters.



2.25 2.19 2.26

Scheme 2.14

The dimethylacetal **2.19** was reductively aminated with aspartic acid di *tert*-butyl diester **2.25** (Scheme 2.14) which was commercially available. The product **2.26** was isolated in 85% yield after column chromatography and was identified from the ESI(+ve) mass spectrum which showed peaks at *m/z* 334 and 356 that correspond to the protonated and sodiated molecular ions.



2.26 2.21 2.27

Scheme 2.15

The dimethylacetal **2.26** was converted to the corresponding *N*-methylated derivative *via* reductive amination with formaldehyde **2.21** (Scheme 2.15). After work up and chromatography the product **2.27** was collected in 58% yield and identified using mass spectrometry and NMR spectroscopy. The ESI(+ve) mass spectrum of the product **2.27** showed a peak at *m/z* 348 that corresponds to its protonated molecular ion. The ^{1}H NMR spectrum showed a singlet resonance at 2.41 ppm that integrates to three hydrogens corresponding to the *N*-methyl group.

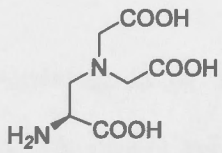
The conversion of the acetal **2.27** to the corresponding aldehyde was attempted by treating with 28% HBr in AcOH. However the mass spectrum of the product mixture was complex. It is presumed that the acidic conditions led to the cleavage of the *tert*-butyl esters and also the decomposition of the molecule.

The conversion of the acetal **2.27** to the corresponding aldehyde was also attempted using trifluoroacetic acid. Initially the reaction was carried out with minimum acid for ten minutes. The ESI(+ve) mass spectrum of the crude product mixture showed no peak that corresponds to the expected product, instead showing a peak at *m/z* 348 that corresponds to the starting material. The reaction was then carried out for 2 hours with trifluoroacetic acid. The ESI(+ve) mass spectrum of the product mixture showed three peaks at *m/z* 348, 292 and 236. The first one corresponds to the starting compound **2.27**. The other two was presumed to correspond to the compounds that resulted from the loss of one and two *tert*-butyl groups respectively from the acetal **2.27**. The ^{1}H NMR

spectrum however showed a peak at 9.79 ppm that corresponds to an aldehydic hydrogen. The integration of the resonance corresponding to the *tert*-butyl groups at 1.45 ppm showed that there were only nine hydrogens which showed that only one ester group is present in the molecule. These observations show that the strong acid conditions lead to the hydrolysis of the *tert*-butyl esters and the dimethoxy acetal simultaneously.

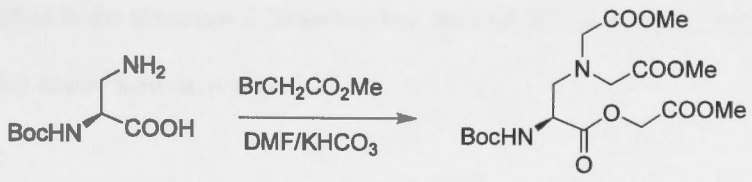
The hydrolysis of the acetal **2.27** was then attempted with lithium tetrafluoroborate in moist acetonitrile.[66] The acetal **2.27** was dissolved in 2% aqueous ACN and lithium tetrafluoroborate was added and the reaction was stirred for an hour at room temperature. The analysis of the crude product mixture using ^{1}H NMR spectroscopy and ESI(+ve) mass spectrometry showed that no reaction occurred and the starting material was recovered. The reaction was then repeated at 80 °C. The mass spectrum of the crude product mixture shows two major peaks at *m/z* 348 and 292. The first one corresponds to the protonated ion of the starting material and the second peak corresponds to the loss of one *tert*-butyl group from the starting compound **2.27** as seen above. A minor peak at *m/z* 334 was assumed to correspond to that of the hemiacetal resulting from partial hydrolysis of the acetal moiety. However the reaction mixture was not characterised any further as no peak corresponding to the desired product was observed in the mass spectrum. It was presumed that the hydrolysis of the ester and the hydrolysis of the acetal take place simultaneously as in the previous instance under the given reaction conditions. Another attempt to improve the reaction was done by heating at 50 °C with excess lithium tetrafluoroborate for 2 days. However no reaction occurred and the starting material was recovered which was evident from the mass spectrum.

As the synthesis of bulkier ligands with a triamine pentaacetic acid backbone was not fruitful as seen in the above instances, the work was redirected towards the synthesis of the simpler molecule **2.28**.



2.28

It was envisaged that this nonnatural amino acid could be incorporated into protein by modifying an amino-acyl-tRNA synthetase that specifically recognizes the amino acid. The main mechanism that keeps nonnatural amino acids from incorporating into proteins is provided by the amino-acyl-tRNA synthetases.[67] If a synthetase's specifity is bypassed then mischarging of an analog onto a tRNA results in the insertion of the analog into a growing polypeptide chain. In this method, a strain auxotrophic for one of the twenty common amino acids found in proteins is used to substitute that amino acid with the nonnatural analog.[68] This results in a new protein that has the canonical amino acid efficiently replaced by its nonnatural analog at all sites. Two amino acids of this type, each that possess the $-N(CH_2\text{-}COOH)_2$ side chain in the protein, would together contribute four carboxylic acid groups that could provide high binding affinity to lanthanides like in EDTA.



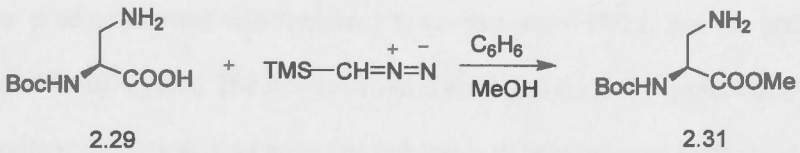
2.29 2.30

Scheme 2.16

The first step of the synthesis involved the amino dialkylation of the amino acid **2.29** that was commercially available. The amino acid **2.29** was treated with methyl bromoacetate in DMF (Scheme 2.16). The product **2.30** was isolated in 70% yield after work-up and chromatography and was characterised using ^{1}H NMR spectroscopy and mass spectrometry. The ESI(+ve) mass spectrum shows a peak at *m/z* 443 that correspond to the sodiated ion of a species of molecular weight 420. The addition of two $-CH_2COOMe$ units to the starting amino acid **2.29** as expected increases the mass by 144 units only. The mass spectrum shows an increase of 216 units which is 72 units greater than that expected. This is presumed to be due to the presence of an extra $-CH_2COOMe$ unit in the molecule. Methyl ester hydrogens appear as a singlet resonance at 3.74 ppm that integrates to six hydrogens in the ^{1}H NMR spectrum. The appearance of another singlet resonance at 3.78 ppm that integrates to three hydrogens shows the presence of another methyl ester in the molecule. The four hydrogens of the two $-CH_2$ groups bridging between the nitrogen and the methyl esters appear as a singlet at 3.64 ppm. The appearance of two singlets, one at 3.78 ppm that integrates to three hydrogens and another at 4.65 ppm that integrates to two hydrogens show the presence of extra methyl ester and $-CH_2$ group in the molecule. These observations led to the conclusion

that the product is the tetraester **2.30** rather than the expected *N,N*-dialkylated derivative of the starting amino acid derivative **2.29**.

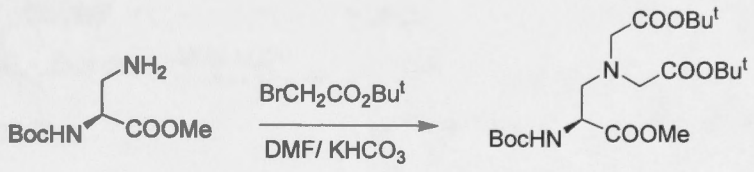
It was decided to protect the acid functionality of the amino acid **2.29** to avoid the alkylation at the oxygen of the carboxylic acid group.



2.29 2.31

Scheme 2.17

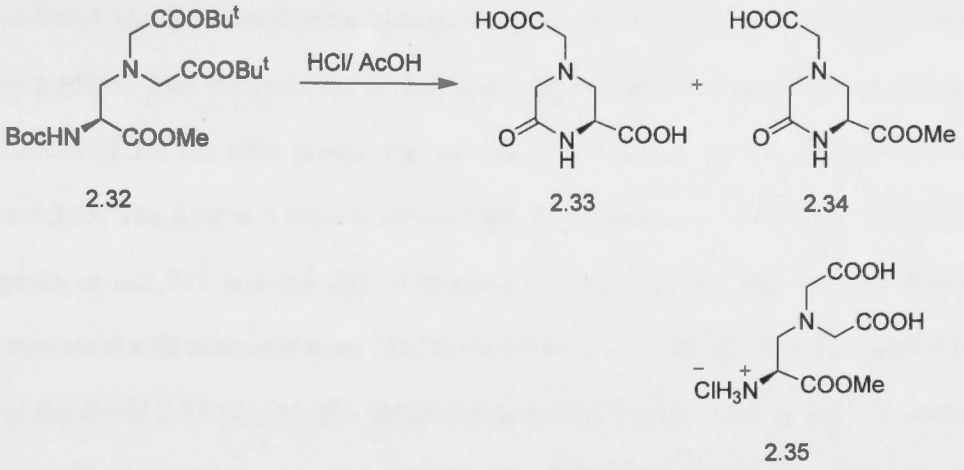
The carboxylic acid group of the amino acid **2.29** was protected as a methyl ester using trimethylsilyldiazomethane[69] in a mixture of methanol and benzene (Scheme 2.17). The residue obtained on evaporation of the solvent was analysed using ^{1}H NMR spectroscopy and mass spectrometry. The mass spectrum shows a peak at *m/z* 219 that corresponds to the protonated ion of a species of molecular weight 218. The increase in 14 mass units above that of the starting compound **2.29** is consistent with methyl ester formation. The NMR spectrum shows a singlet resonance at 3.77 ppm that integrates to three hydrogens which is characteristic of the methyl ester hydrogens. These observations show that the product is the expected methyl ester **2.31**. It was believed that the stereochemistry was retained in the synthesis as it involved mild conditions.



2.31 2.32

Scheme 2.18

The amine **2.31** was then dialkylated using *tert*-butyl bromoacetate in DMF (Scheme 2.18). The product mixture was subjected to reverse phase HPLC and the product **2.32** was isolated in 50% yield. The three *tert*-butyl ester groups in the molecule appear as a broad singlet resonance at 1.44 ppm that integrates to twenty seven hydrogens in the ^{1}H NMR spectrum. The methylene hydrogens that bridge the nitrogen and the ester groups exhibit an AB pattern of doublets at 3.34 and 3.44 ppm that integrate to two hydrogens each. The ESI(+ve) mass spectrum shows two peaks at *m/z* 447 and 469 that correspond to the protonated and sodiated molecular ions of the product **2.32**. Therefore all the spectroscopic data indicates the formation of the expected compound **2.32**. It was assumed that the enantiomeric purity of the starting amino acid **2.29** was retained through out the synthesis as none of the steps involved harsh conditions.



2.32 2.33 2.34 2.35

Scheme 2.19

Having obtained the protected ligand **2.32** its deprotection was attempted using acetic acid and conc. HCl (Scheme 2.19). The reaction mixture was neutralised and evaporated. TLC of the crude product showed a single spot but the ^{1}H NMR spectrum is complex, corresponding to a mixture of products. Passing the mixture through a reverse phase silica column gave two portions. One contained the diacid **2.33** which was identified from mass spectrometry and NMR spectroscopy. The ESI(-ve) spectrum shows an intense peak at *m/z* 201. The decrease in mass by eighteen units relative to that of the expected compound indicates the loss of a water molecule that fits in with the structure of compound **2.33**. The absence of signals in the region 1-2 ppm in the ^{1}H NMR spectrum shows the cleavage of the *tert*-butyl groups. The doublet of doublets at 4.45 ppm corresponds to the α-hydrogen of the amino acid. The HMBC ^{13}C NMR spectrum shows correlation of the α-hydrogen of the amino acid moiety resonating at 4.45 ppm to two carbonyl groups resonating at 170 ppm and at 168 ppm. One of these corresponds to the acid carbonyl group of the amino acid moiety. The other corresponds to the ring

carbonyl. One set of methylene hydrogens of the $-NCH_2$ at 3.95 ppm was also observed to resonate with the carbonyl at 168 ppm. This correlation also indicates the cyclic structure **2.33**. The other portion was presumed to contain a mixture of compounds **2.34** and **2.35**. The ESI(+ve) mass spectrum show three peaks at *m/z* 217, 235 and 239. The peaks at *m/z* 217 and *m/z* 239 correspond to the protonated and sodiated ions of a compound with molecular mass 216. The increase in mass by fifteen units relative to that of the diacid **2.33** suggests the compound to have a similar structure but with a methyl ester. The appearance of a singlet at 3.51 ppm in the ^1H NMR spectrum corresponds to the methyl group of the methyl ester. These data show compound **2.34** to have a cyclic structure. It is presumed that the peak at *m/z* 235 corresponds to compound **2.35**. The calculated mass of the completely deprotected product is 220. The peak at *m/z* 235 is fourteen mass units higher than for the protonated ion of the expected compound. The presence of a signal corresponding to the methyl ester in the NMR spectrum coupled with the mass spectrum is consistent with the structure of the amino acid derivative **2.35**.

The deprotection of compound **2.32** was also investigated using a 1:1 mixture of DCM and TFA. The ^1H NMR and mass spectra of the product were similar to those obtained for the previous reaction (Scheme 2.19). It is presumed that the nucleophilic attack of the primary amine of the amino acid moiety on the carboxylic group attached to the nitrogen at the γ-position, leads to a stable six-membered ring. This intramolecular cyclisation and hydrolysis of the esters are taking place simultaneously under the given reaction conditions.

In summary, synthesis of a DTPA derivative with a thiol as the protein linker *via* a cysteine moiety as the central acid was accomplished. The complexation of this ligand to the metal and its performance as a shift reagent was unsatisfactory due to high conformational flexibility. Syntheses of other similar ligands with more conformational constraints were investigated but could not be achieved. As a result, the synthesis of DTPA derivatives and similar non-cyclic ligands was discontinued and the research was directed towards the synthesis of polyaza polycarboxylic macrocycles, which is described in the following Chapter.

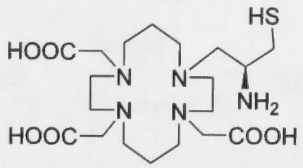
Chapter 3. Synthesis of macrocyclic ligands as NMR shift reagents

Introduction

The lanthanide complexes of polyaza polycarboxylic macrocycles like TETA display a host of unusual properties that are not exhibited by the complexes of similar but non-cyclic ligands like DTPA and EDTA.[16,70] The TETA lanthanide chelates are remarkably rigid, they are highly symmetric and they adopt the same geometry in the solution and the solid state.[17] This Chapter describes the synthesis of the TETA (1,4,8,11-tetraazacyclotetradecane-*N*,*N'*,*N''*,*N'*-tetraacetic acid) derivative **3.1** and the study of its complexation with ytterbium.

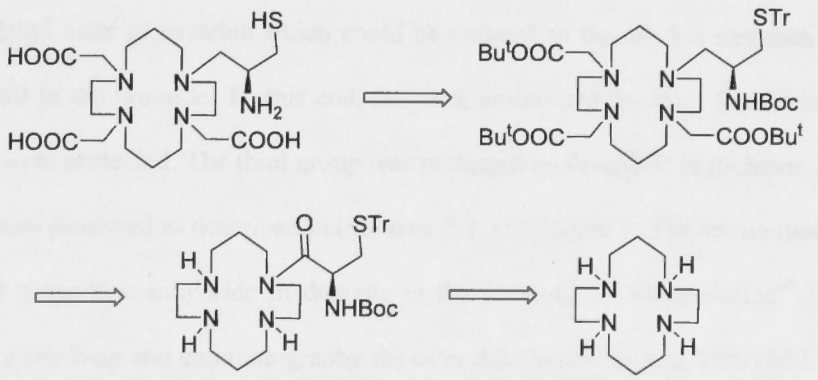
The TETA ligand was designed in such a way that it could be bound to a biomolecule *via* a disulfide bond. To make that possible, the TETA derivative **3.1** was synthesised with a cysteine residue as one of the four arms. Having a cysteine residue in the molecule hence serves two purposes as discussed in the Introduction (page 9). One is that a disulfide linkage can be attained with a biomolecule and the other is that the presence of the cysteine nitrogen on the side arm can be employed to coordinate to the

metal thus preserving the denticity of the ligand to be eight as in the parent molecule TETA.



3.1

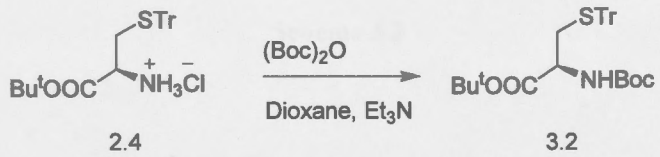
Synthesis of the Ligand



Scheme 3.1

The retrosynthetic plan for the synthesis of compound **3.1** is given above (Scheme 3.1). One of the four nitrogens of the TETA derivative **3.1** contains a structure that is derived from a cysteine moiety. This is accomplished via a peptide coupling of cysteine with cyclam and then the reduction of the amide carbonyl group. The remaining three

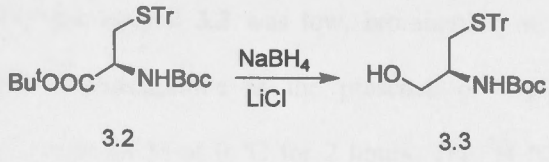
nitrogens are *N*-alkylated using *tert*-butyl bromoacetate to give the protected form of the required ligand, which is then deprotected by acid hydrolysis to give the final ligand **3.1**.



2.4 3.2

Scheme 3.2

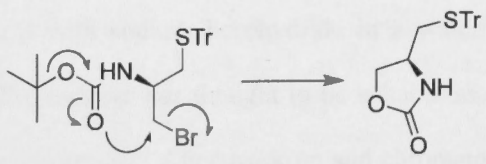
The first step was to obtain (*S*)-cysteine suitably derivatised for reaction with the cyclam. Initially, it was decided to synthesise a bromide of (*S*)-cysteine so that it could be attached to the cyclam employing dehydrohalogenation. The plan was to synthesise the *tert*-butyl ester of cysteine which could be reduced to the alcohol and then convert the alcohol to the bromide. To this end, the acid, amino and the thiol functionalities of cysteine were protected. The thiol group was protected as described in (Scheme 2.1) and the acid was protected as described in (Scheme 2.2) of Chapter 2. The amino moiety was protected using Boc-anhydride in dioxane in the presence of triethylamine[61] (Scheme 3.2). After work-up and chromatography the ester **3.2** was isolated in 95% yield. The ^{1}H NMR spectrum of the product shows a singlet resonance at 1.45 ppm that integrates to 18 hydrogens, corresponding to the two *tert*-butyl groups in the molecule. The ESI(+ve) mass spectrum shows a peak at *m/z* 520 that corresponds to the protonated ion of the expected ester **3.2**.



3.2 3.3

Scheme 3.3

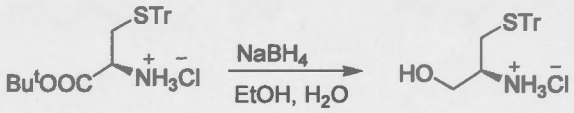
The ester **3.2** was then reduced to the corresponding alcohol **3.3** by treating with sodium borohydride and lithium chloride at ambient temperature in a mixture of ethanol and THF[71] (Scheme 3.3). The product **3.3** was collected in 29% yield after column chromatography. The rest of the product mixture contained unreacted starting compound **3.2**. The ^{1}H NMR spectrum of the alcohol **3.3** shows a multiplet at 3.49 ppm that corresponds to the two hydrogens of the methylene adjacent to the hydroxy group. The singlet resonance at 1.45 ppm that corresponds to the *tert*-butyl hydrogens integrates to nine hydrogens which also show that only one *tert*-butyl group is present in the molecule. The ESI(+ve) mass spectrum shows a peak at *m/z* 472 that corresponds to the sodiated ion of the expected alcohol **3.3**. The mass difference between the starting ester **3.2** and the alcohol **3.3** fits the expected conversion.



3.4 3.5

Scheme 3.4

Although the yield of the alcohol **3.3** was low, bromination of the alcohol **3.3** was attempted using *N*-bromosuccinimide in the presence of triphenylphosphine. The reaction was carried out in DCM at 0 °C for 2 hours. The ^{1}H NMR spectrum of the product mixture was complex. However the absence of signals between 1-2 ppm in the NMR spectrum shows the cleavage of the *tert*-butyl group which was unexpected. The product mixture was not characterised any further as the expected bromide **3.4** was not obtained. It is presumed that the *N*-Boc group participates in the loss of bromide from the initially formed product **3.4** giving the cyclised compound **3.5** as shown in Scheme 3.4.



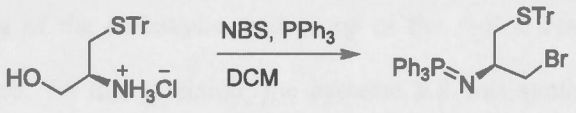
2.4 3.6

Scheme 3.5

To avoid complications associated with the neighbouring group participation of the *N*-Boc-group, the reduction was attempted with the partly protected cysteine **2.4**. The cysteine **2.4** was treated with sodium borohydride in a water/ethanol mixture under reflux (Scheme 3.5). The method was thought to be suitable as no racemisation would occur as claimed in the literature.[72] After work-up and chromatography the product **3.6** was isolated in 26% yield. The absence of signals between 1-2 ppm in the ^{1}H NMR spectrum showed the cleavage of the *tert*-butyl ester. The presence of multiplets in the region 2.85-3.40 ppm that integrate to three protons in the NMR spectrum corresponds

to the methylene hydrogens of the primary alcohol and the α-hydrogen. The ESI(+ve) mass spectrum shows a peak at *m/z* 350 that corresponds to the protonated molecular ion of the alcohol **3.6**.

The reaction was repeated in an attempt to improve the yield of the alcohol **3.6** using an excess of sodium borohydride, but no significant increase in yield was observed. Repeating the reaction at lower temperatures of 40 °C and 60 °C did not improve the yield either.

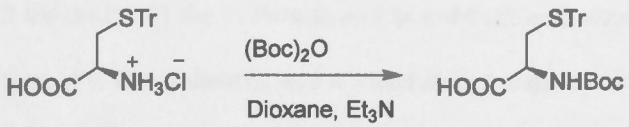


3.6 3.7

Scheme 3.6

Bromination of the alcohol **3.6** was attempted using NBS in the presence of triphenyl phosphine (Scheme 3.6). The ESI(+ve) mass spectrum of the crude product mixture shows two major peaks at m/z 672 and 674. The presence of these peaks of equal intensity in the mass spectrum shows the compound contains a bromine. The mass of the expected bromide is 411 and 413 constituting for the isotopes of bromine. Although the mass spectrum showed the presence of bromine, the observed mass was 262 units higher than the calculated mass of the expected product. The increase in mass of 262 units fits the presence of a triphenylphosphine moiety. The ^{1}H NMR spectrum was complex but the presence of multiplets in the aromatic region around 7-8 ppm integrating to about

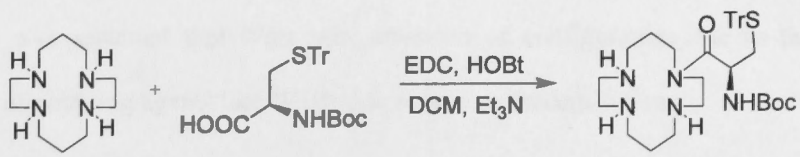
thirty hydrogens is in agreement with the presumed structure of compound **3.7**. An attempt to hydrolyse the bromide **3.7** was not carried out as its yield was very low and this approach was abandoned.



2.3 3.8

Scheme 3.7

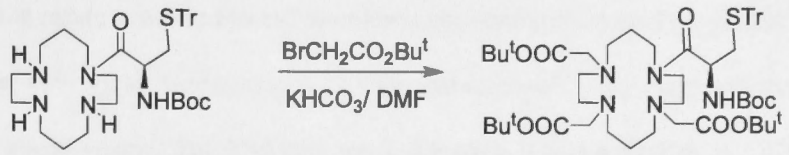
An amide coupling of the carboxylic acid group of the *N*-Boc cysteine **3.8** with the cyclam was planned. To that direction, the cysteine **3.8** was synthesised using Boc-anhydride in dioxane in the presence of triethylamine (Scheme 3.7). The product **3.8** was isolated in 81% yield after column chromatography. The presence of a singlet resonance at 1.45 ppm that integrates to nine hydrogens in the ^{1}H NMR spectrum shows the presence of the required product **3.8**. The ESI(+ve) mass spectrum shows a peak at *m/z* 486 that corresponds to the sodiated ion of the expected *N*-Boc-cysteine **3.8**.



3.9 3.8 3.10

Scheme 3.8

Having obtained the cysteine **3.8**, it was coupled with cyclam **3.9** using *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide (EDC) along with 1-hydroxybenzotriazole hydrate (HOBt).[73] EDC activates the carboxylic acid moiety of the cysteine **3.8** which is then coupled with the amine of the cyclam to produce the desired amide bond. HOBt is used in combination as a rate enhancer and a racemisation suppressant.[74] The reaction was carried out in DCM in the presence of triethylamine at room temperature (Scheme 3.8). The product **3.10** was isolated in 28% yield after column chromatography. The rest of the product mixture was not purified but consisted of unreacted cyclam **3.9** and the corresponding bis-coupled derivative of the cyclam which was evident from the mass spectrum of the mixture. The product **3.10** was characterised using ^{1}H NMR spectroscopy and mass spectrometry. The mass spectrum shows a peak at *m/z* 646 that fits in for the protonated molecular ion of the expected product **3.10**. The NMR spectrum shows a singlet resonance at 1.39 ppm that integrates to nine hydrogens that corresponds to the *tert*-butyl group in the molecule. The appearance of multiplets at 7.28 ppm indicates the presence of the trityl hydrogens. The twenty hydrogens of the ten methylene groups in the cyclam ring appear as multiplet resonances in the region between 2-4 ppm. Analysis of the enantiomeric purity of compound **3.10** was not carried out as it was assumed that there was retention of configuration due to the careful selection of coupling agents like HOBt that suppresses racemisation.

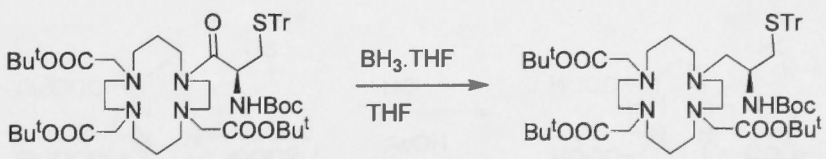


3.10 3.11

Scheme 3.9

The monoalkylated cyclam **3.10** was then *N*-alkylated with *tert*-butyl bromoacetate in DMF (Scheme 3.9). The product **3.11** was isolated in 77% yield after chromatography. The ESI(+ve) mass spectrum shows a peak at *m/z* 988 that corresponds to the protonated ion of the expected tetraalkylated cyclam **3.11**.

It was presumed that the amide carbonyl group on the cysteine moiety would weaken the ability of that nitrogen to take part in the coordination with a metal. Hence it was decided to carry out the reduction of the carbonyl group.

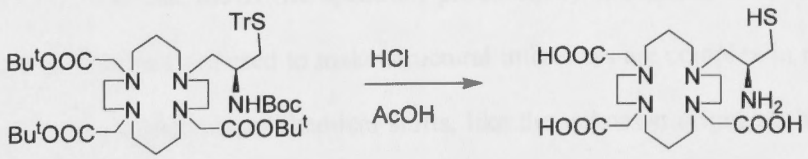


3.11 3.12

Scheme 3.10

The reduction was carried out using BH_3.THF in THF at reflux[75] (Scheme 3.10). The reaction was quenched by refluxing with sodium hydroxide for another thirty minutes.

The product mixture was subjected to column chromatography and the product **3.12** was isolated in 48% yield. Compound **3.12** was characterised using mass spectrometry and ^{1}H NMR spectroscopy. The ESI(+ve) mass spectrum shows a peak at *m/z* 974 which is fourteen mass units less than that of the starting compound **3.11**. This mass difference suggests the reduction of the carbonyl group to a methylene group. The appearance of a multiplet at 2.72 ppm that integrates to 2 hydrogens in the NMR spectrum corresponds to the $-CH_2$ on the cysteine that resulted from the reduction of the carbonyl moiety. These observations show that the compound is the expected cyclam **3.12**. The ESI(+ve) spectrum of the remaining fraction of the product mixture obtained after column chromatography shows peaks at *m/z* 818 and 918. It is presumed that the peak at *m/z* 818 corresponds to a compound with one of the *tert*-butyl esters reduced to the corresponding alcohol and the *N*-Boc group converted to an amine. It is presumed that the peak at *m/z* 918 corresponds to the compound where one of the *tert*-butyl acetate arms is reduced to the corresponding alcohol. However these compounds were not isolated and purified.



3.12 3.1

Scheme 3.11

Compound **3.12** was deprotected using 6N HCl in acetic acid (Scheme 3.11) stirring for thirty hours at room temperature. The ligand **3.1** was obtained in 66% yield as its hydrochloride salt and was analysed using ^{1}H NMR spectroscopy and mass spectrometry. The absence of signals corresponding to the hydrogens of the *tert*-butyl ester groups in the region between 1-1.5 ppm in the NMR spectrum shows the cleavage of these groups from the starting compound **3.12**. The absence of signals in the aromatic region between 7-8 ppm shows the cleavage of the trityl group. The ESI(+ve) mass spectrum shows a peak at *m/z* 464 which fits the protonated molecular ion of the expected ligand **3.1**. It was assumed that no racemisation occurred in the course of synthesis as none of the steps involved harsh basic conditions.

Complexation Studies using Ytterbium

Having obtained the ligand **3.1**, the complexation was investigated with ytterbium. Ytterbium was chosen to acquire wider shifts of resonances in the NMR spectrum.[1] It has been reported that the NMR spectrum produced by lanthanide shift reagents of paramagnetic ions that are used to make structural inferences are complex in nature due to the various contributions to chemical shifts, like the unknown origin of the induced chemical shifts, the low symmetry of the lanthanide complexes and the high lability of these compounds. This can be avoided by using a ytterbium complex, as the shifts induced by ytterbium are dipolar in origin.[17] It is known that the complexes of europium and praseodymium are by far the best shift reagents in general giving shift broadenings of only 0.003 and 0.005 Hz/Hz of shift respectively.[76] However, PrTETA was reported

to show broad and featureless ^{1}H NMR spectra even at 30 °C whereas YbTETA is stereochemically rigid at lower temperatures.[17]

Initially the complexation experiment was carried out using ytterbium chloride hexahydrate. It was treated with the ligand **3.1** in water at basic pH at 80 °C as reported by Desreux.[70] The product mixture was subjected to reverse phase chromatography. Two fractions were obtained. One of the fractions was shown to be the starting ligand **3.1** using ^{1}H NMR spectroscopy. The ^{1}H NMR spectrum of the other fraction showed a singlet resonance at -3 ppm which was presumed to be an indication of the paramagnetic compound, the expected Yb complex. However the NMR spectrum of the fraction contained signals between 1-6 ppm which was presumed to be due to the unbound ligand. The reaction was repeated under nitrogen, stirring for 30 hours. The residue on analysis using ^{1}H NMR spectroscopy shows the signals spread over a large sweep width ranging from 100 to -100 ppm which is characteristic of Yb complexes. However the spectrum also contained signals in the diamagnetic region which is presumed to be due to the presence of the unbound ligand. The ESI(+ve) mass spectrum of the residue shows a peak at *m/z* 464 which corresponds to the protonated ion of the ligand **3.1**. It was presumed that the reaction did not attain complete complexation.

The reaction was then studied using ytterbium triflate at pH 10 and 80 °C. It was presumed that a triflate salt would dissociate easily into the corresponding ions and hence Yb^{3+} ions would be easily available for complexation. The product mixture was filtered to remove unreacted Yb salts. The ^{1}H NMR spectrum of the filtrate shows peaks

ranging from 100 to -100 ppm that shows the presence of the paramagnetic complex in the sample. However the diamagnetic region of 1-6 ppm contained signals as in the previous cases and the ESI(+ve) mass spectrum shows a peak at *m/z* 464 that corresponds to the starting ligand **3.1**. The reaction was repeated with excess ytterbium triflate at neutral pH. The mixture was heated to 70 °C and was stirred for longer. The pH was maintained neutral and the reaction was monitored using ^{1}H NMR. The NMR spectrum showed no significant change over time. After 2 days the pH of the reaction mixture remained stable. The product mixture was purified and was analysed using ^{1}H NMR spectroscopy and mass spectrometry. The NMR spectrum shows signals at a range of -15 to 15 ppm. It also shows signals between 1-10 ppm that were presumed to be due to the starting ligand **3.1**. The ESI(+ve) mass spectrum was complex and did not contain peaks corresponding to the expected complex. The observed NMR and mass spectrum were inconclusive.

It was then decided to study the reaction using ytterbium oxide as per the procedure reported by Parker and co-workers.[19] The ligand **3.1** was treated with ytterbium oxide in deionised water at a slightly acidic pH at reflux for a day. The resulting mixture was filtered and evaporated, and the residue analysed using ^{1}H NMR spectroscopy and mass spectrometry. The ESI(+ve) mass spectrum shows peaks at *m/z* 464, 635 and 657. The peak at *m/z* 464 corresponds to the protonated ion of the ligand **3.1**. The peaks at *m/z* 635 and 657 correspond to the protonated and sodiated ions of a species of molecular mass 634. This increase in mass of 171 units indicates the presence of a ytterbium complex. The ^{1}H NMR spectrum shows signals ranging from 100 to -100 ppm that is

characteristic of the ytterbium complex. However the NMR spectrum also contained signals in the diamagnetic region of 1-10 ppm as seen in previous instances. It was presumed that the unreacted ligand **3.1** was present due to the non-availability of sufficient ytterbium ions to complex with the ligand. As a result the reaction was repeated with excess ytterbium oxide and the reaction mixture was stirred for longer monitoring using mass spectrometry. After 60 hours the mass spectrometry showed significant reduction in the percentage intensity of the peak at *m/z* 464 that corresponds to the starting ligand **3.1**. It also showed peaks at *m/z* 635 and 657 that correspond to the protonated and sodiated ions of the expected ytterbium complex as in the previous instance. The purification of the complex was attempted using reverse-phase chromatography but no separation was achieved which was evident using mass spectrometry. The ^{1}H NMR spectrum was identical to the one observed in previous instances where the diamagnetic region of 1-10 ppm consisted of signals showing the presence of more than 50% of the uncomplexed ligand **3.1**. It is presumed that the complex was unstable on the NMR time scale and that the ligand and the complex exist in equilibrium.

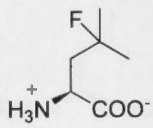
In summary, a macrocyclic ligand with a thiol linker was efficiently synthesised. The complexation of the ligand was studied using different salts of ytterbium at basic and acidic pH. The complex was synthesised, but is believed to exist in equilibrium with the free ligand on the NMR time scale and that it could not be successfully used as a shift reagent. At this juncture, the research into the synthesis of lanthanide shift reagents was discontinued and investigations into the synthesis of nonnatural amino acids that can be

used to probe the protein structure and dynamics were pursued which is detailed in the upcoming Chapters.

Chapter 4. Synthesis of (*S*)-γ-fluoroleucine

Introduction

This Chapter describes a convenient and efficient synthesis of *(S)*-γ-fluoroleucine **4.1** which was developed to be used as an NMR probe for protein structure and dynamic studies. The synthesis was accomplished *via* a direct side-chain bromination of a protected leucine, then halogen exchange with fluorine using silver fluoride, and subsequent deprotection.



4.1

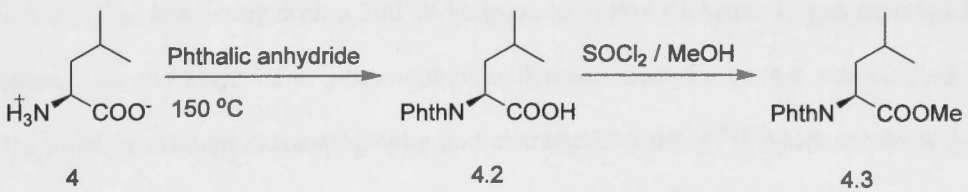
(*S*)-γ-fluoroleucine

(*S*)-γ-Fluoroleucine **4.1** represents a particularly attractive probe because of the simplicity of its spin system and because leucine is an abundant amino acid in proteins, both at positions within the protein structure and on the protein surface. The important role of leucine in hydrophobic and lipophilic interactions of proteins combined with the use of ^{19}F NMR spectroscopy to study protein folding and protein–protein and protein–

drug interactions has resulted in several syntheses of leucine with fluorination in the side chain.[77-79] These syntheses however adopted multistep procedures or had to depend on enzymatic resolutions to attain high enantiomeric purity as described in Chapter 1.

Synthesis of the fluoro amino acid

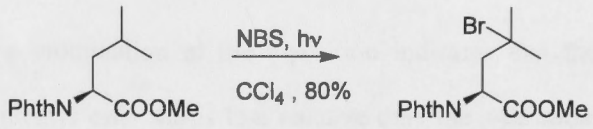
It was envisaged that a direct side-chain substitution of the corresponding amino acid would present a short and stereoselective synthesis of the required fluorinated amino acid. Consequently, the objective was to prepare the bromide **4.4** using literature methods[47] and determine if this could be elaborated to give the fluoroleucine **4.1**.



Scheme 4.1

The first step towards the synthesis was the protection of the amino and carboxylic acid groups of *(S)*-leucine **4**. The amino group was protected as a phthalimide and the acid group as a methyl ester (Scheme 4.1) as described in the literature.[78] *(S)*-Leucine **4** was treated with powdered phthalic anhydride at 150 °C to give *N*-phthaloylleucine **4.2**, which was dissolved in dry methanol and the solution was treated with thionyl chloride

to give the *N*-phthaloylleucine methyl ester **4.3** (Scheme 4.1) in 89% yield. The *N*-phthaloylleucine methyl ester **4.3** was identified from its ^{1}H NMR spectrum. A singlet resonance at 3.72 ppm confirmed the presence of the methyl ester and multiplets at 7.75-7.84 ppm confirmed the presence of the phthaloyl group, both resonance assignments being in agreement with literature values.[79]



4.3 4.4

Scheme 4.2

The *N*-phthaloylleucine methyl ester **4.3** was subjected to radical bromination using NBS in CCl_4, irradiating with a 300 W lamp under reflux (Scheme 4.2) as reported by Easton, Tan and Hay.[47] The γ-bromophthaloylleucine methyl ester **4.4** was isolated in 84% yield by column chromatography and characterised using ^{1}H NMR spectroscopy. The spectrum consists of two singlet resonances at 1.74 ppm and 1.82 ppm corresponding to the two side-chain methyl groups. The β-hydrogens appear as a multiplet at 2.82 ppm and the hydrogens of the methyl ester appear as a singlet resonance at 3.72 ppm. The α-hydrogen appears as a doublet of doublets at 5.22 ppm and the aromatic hydrogens of the phthaloyl group give rise to a multiplet between 7.73 and 7.89 ppm. All these values are in agreement with those reported in the literature.[78]

The radical bromination is presumed to take place *via* hydrogen atom abstraction at the γ-position of the leucine derivative **4.3** by bromine atom to give a tertiary radical and hydrogen bromide.[80] Hydrogen bromide then reacts with NBS to give succinimide and molecular bromine. Reaction of the tertiary radical with bromine gives the γ-bromide **4.4** and bromine atom which propagates the radical chain.

The regioselective bromination at the γ-position indicates that the α-position of *N*-phthaloylleucine methyl ester **4.3** is less reactive than the γ-position. Radical reactions often occur at the α-position of α-amino acid derivatives. This may be attributed to the relative stability and ease of formation of the α-centered radicals.[81] An acylamino acid radical can adopt a planar conformation which is resonance stabilised because there is good overlap of the π-orbitals of the amido substituent with the semi occupied p-orbital of the radical (Figure 6). By comparison, steric interactions distort phthalimido radicals from planarity and limit the extent of the orbital overlap in those cases. In addition, the π-electrons of a phthalimido substituent are less available than those of an amido substituent to stabilize a radical through resonance. The phthaloyl substituent is also more likely to hinder approach of bromine atom to the α-position of the leucine derivative **4.3**. Due to these factors the α-position of the leucine derivative **4.3** is unreactive and the bromination instead occurs at the γ-position, *via* a tertiary radical that is more stable and easily formed than the secondary and primary radicals that would be involved for reaction to occur at the β- and δ-positions, respectively.

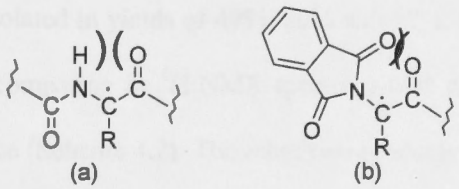
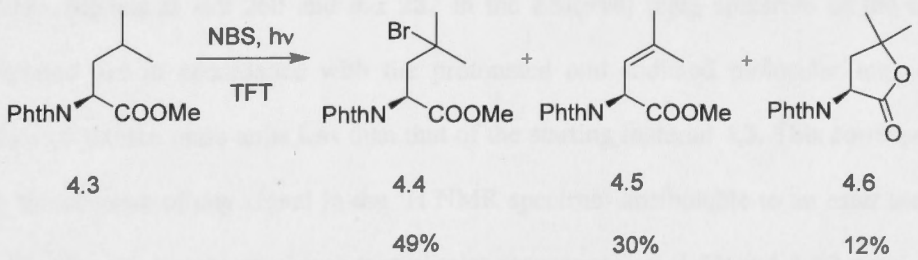


Figure 6

Nonbonding steric interactions associated with planar conformations of acylamino- and phthalimido-substituted radicals.

CCl_4 is an ozone depleting agent and hence its use is highly restricted.[82] Therefore an alternative solvent for the radical bromination of the leucine derivative **4.3** was thought to be desirable. It has been shown that α,α,α-trifluorotoluene (TFT) is suitable for radical halogenations[83-86] because it is unreactive to radical reactions and electrophilic aromatic substitutions. Hence bromination of the leucine derivative **4.3** was also studied in TFT (Scheme 4.3).



Scheme 4.3

The reaction gave a mixture of the γ-bromophthaloylleucine methyl ester **4.4**, the β,γ-dehydrophthaloylleucine methyl ester **4.5** and the lactone **4.6**, which were separated by

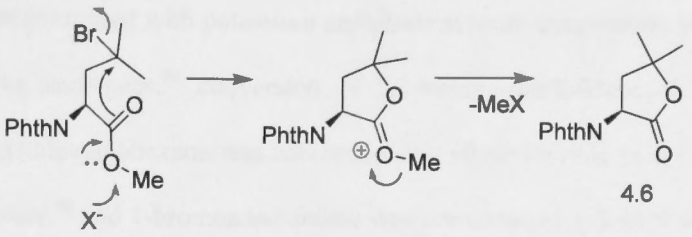
column chromatography and isolated in yields of 49%, 30% and 12%, respectively. The product **4.4** was identified by comparing its ^{1}H NMR spectrum with that of the sample obtained in the previous instance (Scheme 4.2). The other two products **4.5** and **4.6** were identified using NMR spectroscopy and mass spectrometry. The ESI(+ve) mass spectrum of one of the compounds showed signals at *m/z* 274 and *m/z* 296, consistent with the protonated and sodiated molecular ions of a species having a molecular weight of 273, two mass units less than that of the starting material **4.3**. This showed that it was likely to be an alkene. The position of the olefinic double bond was determined from the NMR spectrum. The compound was identified as the β,γ-dehydrophthaloylleucine methyl ester **4.5** because its ^{1}H NMR spectrum consists of two broad singlet resonances at 1.70 and 1.72 ppm corresponding to two olefinic methyl groups. Doublets at 5.64 and 5.82 ppm integrate to one proton each and are attributable to the β- and α-hydrogens respectively. The multiplicity and integrations of these signals show there is only one β-hydrogen and the chemical shift of the signal for this proton is consistent with it being olefinic. Signals at *m/z* 260 and *m/z* 282 in the ESI(+ve) mass spectrum of the other compound are in accordance with the protonated and sodiated molecular ions of a species of sixteen mass units less than that of the starting material **4.3**. This corresponds with the absence of any signal in the ^{1}H NMR spectrum attributable to an ester methyl group. Also the spectrum shows two singlet resonances, at 1.51 and 1.62 ppm each integrating for three hydrogens, corresponding to the methyl groups. The multiplicity and chemical shift of the methyl signals show the absence of a γ-hydrogen and that the γ-carbon is attached to an electronegative atom. These observations combined with the mass spectrum indicate that the other compound is the lactone **4.6**.

When the bromination in TFT was repeated, the yields of the bromide **4.4** and the byproducts **4.5** and **4.6** were found to vary. The bromide **4.4** was found to decompose to the byproducts **4.5** and **4.6** at room temperature on the bench and also on chromatography. The TLC of the crude product mixture before column chromatography showed an intense spot corresponding to the bromide **4.4** whereas after chromatography, the percentage of the isolated bromide **4.4** was less than expected. These observations indicate that the byproducts **4.5** and **4.6** are probably formed from **4.4**, by elimination (Scheme 4.4) and through intramolecular cyclisation (Scheme 4.5), respectively, both during the reaction and on chromatography of the product mixture. In one case the bromide **4.4** was obtained essentially pure and in almost quantitative yield without chromatography. Nevertheless, comparing the reactions in CCl_4 and TFT, those in CCl_4 were more reproducible and gave consistently higher yields of the bromide **4.4**, so this was the solvent of choice.

4.4 4.5

Scheme 4.4

Mechanism of reaction of the bromide 4.4 to give the β,γ-dehydrophthaloylleucine methyl ester 4.5.

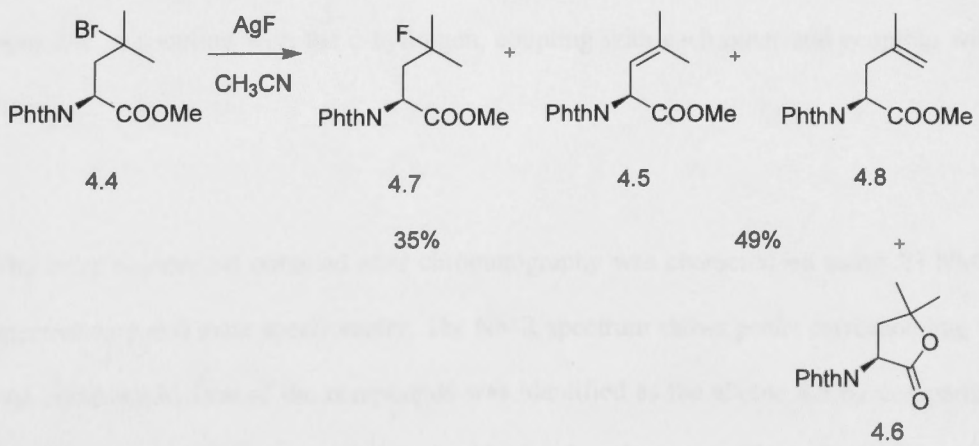


Scheme 4.5

Mechanism of intramolecular cyclisation of the bromide 4.4 to give the lactone 4.6.

Having the bromide **4.4** in hand, the next step was to investigate the possibility of conversion of the bromide **4.4** to the fluoride **4.7** because it was thought that this could then be deprotected to give *(S)*-γ-fluoroleucine **4.1**. The reagent for this conversion was carefully chosen. Most of the reactions in the literature dealing with the making of fluorinated amino acids involve hydrogen fluoride as the fluorinating agent.[87] Taking into consideration the extreme toxicity of the reagent, the difficulty handling it, its property of immediately reacting with the skin before it can be removed or neutralised,[88] the special reaction vessels required to use this reagent and its low boiling point of 19 °C, using this was eliminated. An alternative choice of reagent was silver nitrate,[89-91] to convert the γ-bromophthaloylleucine methyl ester **4.4** to the corresponding alcohol, and then use a deoxofluorinating agent like (diethylamino)sulfur trifluoride (DAST) to make the fluoride **4.7** from the alcohol. This idea of adding another reaction step did not seem worthwhile, however, particularly as each step would involve nucleophilic substitution at a tertiary position, which is normally accompanied by elimination leading to a significant decrease in yield. Instead silver fluoride was selected. It has been used as the fluorinating agent in halogen exchange reactions.[92] For example, silver fluoride in

acetonitrile has been used with potassium carbonate at room temperature in the synthesis of prostacyclin analogues,[90] conversion of 2-bromo-2-methylthiocyclohexane to 2-fluoro-2-methylthiocyclohexane was achieved using silver fluoride in dry acetonitrile at room temperature,[91] and 1-bromoadamantane was converted to 1-fluoroadamatane using silver fluoride in cyclohexane under reflux.[92] Among these reactions, the combination of silver fluoride in dry acetonitrile at room temperature was presumed to be the best one as heating under reflux and using a base were thought likely to lead to elimination accompanying substitution of the γ-bromophthaloylleucine **4.4**. Hence the bromide **4.4** was treated with silver fluoride in dry acetonitrile for 18 hours (Scheme 4.6). The reaction was carried out in the dark as most silver halides are sensitive to light.[93] Silver fluoride when used as a fluorinating agent in halogen exchange reactions forms complex solids with the silver halide formed in the reaction.[88] Hence an excess of this reagent was used to make enough of it available for the fluorination.



Scheme 4.6

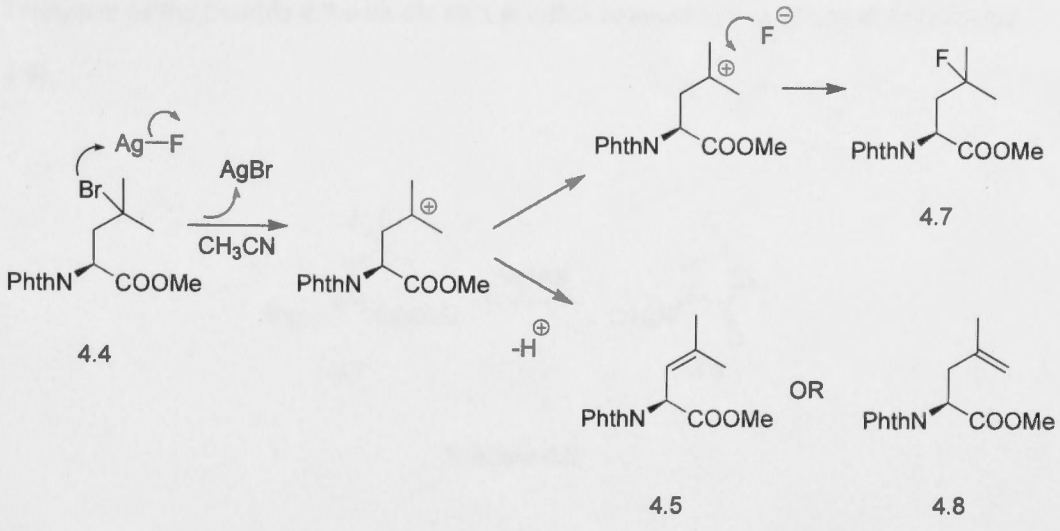
The product mixture was subjected to column chromatography and the fluoride **4.7** was isolated in 35% yield. A 1:2 mixture of the alkenes **4.5** and **4.8** was also obtained in 49% yield after chromatography. The alkenes **4.5** and **4.8** could not be separated from each other as they had the same retention time under the given chromatographic conditions. A small amount of lactone **4.6** was also identified in the crude NMR spectrum of the product mixture, by comparing its values with those obtained in the previous instance.

The fluoride **4.7** was identified using ^{1}H NMR spectroscopy and mass spectrometry. The ESI(+ve) mass spectrum shows peaks at *m/z* 294 and *m/z* 316 which are consistent with the protonated and sodiated molecular ions of a species having a molecular weight of 293. The mass difference from that of the starting bromide **4.4** is consistent with the substitution of fluorine for bromine. The ^{1}H NMR spectrum shows the side chain methyl groups as two doublets at 1.35 and 1.43 ppm with the characteristic large coupling for vicinal hydrogen and fluorine.[88] The β hydrogens appear as multiplets at 2.48 and 2.70 ppm due to coupling with the α-hydrogen, coupling with each other and coupling with fluorine.

The other component obtained after chromatography was characterised using ^{1}H NMR spectroscopy and mass spectrometry. The NMR spectrum shows peaks corresponding to two compounds. One of the compounds was identified as the alkene **4.5** by comparing its signals with that of the sample obtained in previous instances. The only characteristic peaks in the mass spectrum, at *m/z* 274 and *m/z* 296, correspond to the alkene **4.5**, so the other compound was assumed to be an isomeric alkene giving similar peaks, consistent

with both compounds having the same chromatographic properties. The position of the double bond of the isomer was determined from the NMR spectrum which showed only one broad singlet resonance at 1.78 ppm integrating to three hydrogens, corresponding to the one methyl group. The chemical shift of this signal and its multiplicity suggests that the methyl group is bonded to a fully substituted olefinic carbon. Two broad singlets at 4.64 and 4.68 ppm integrate to one proton each and are characteristic of the two terminal olefinic hydrogens.

The ratio of the alkenes **4.5** and **4.8** was determined by integration of the NMR spectrum. The fluorination (Scheme 4.6) results in the formation of the two alkenes **4.5** and **4.8** unlike the bromination (Scheme 4.3) where only the alkene **4.5** is formed. Presumably, the bromination gives the more substituted alkene **4.5** which is thermodynamically more stable *via* concerted hydrogen bromide elimination. The fluorination gives a mixture of the alkenes **4.5** and **4.8** presumably because the reaction occurs *via* a different mechanism, through an intermediate carbocation (Scheme 4.7) rather than concerted hydrogen bromide elimination. Statistical formation of the γ,δ-alkene **4.8** is favoured by loss of one of the six methyl hydrogens even though the product is not the most stable.



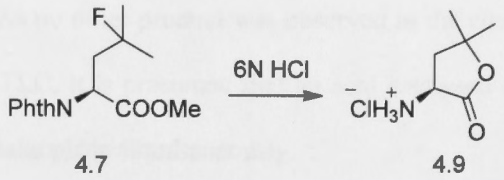
Scheme 4.7

Competition reactions of elimination and substitution

The fluorination reaction was also studied with two equivalents of silver fluoride instead of the ten equivalents used initially, in an attempt to minimise the amount of the reagent used, but this resulted in an incomplete reaction. This showed that a large excess of silver fluoride is necessary. Since the substitution of fluorine in the bromide **4.4** is at a tertiary centre where elimination and substitution invariably compete with each other, it seemed likely that further attempts to improve the yield of the γ-fluoride **4.7** would not be fruitful.

Having synthesised the γ-fluoride **4.7**, what remained was to cleave the protecting phthaloyl and ester groups, to obtain the free amino acid **4.1**. A method that seemed likely to cleave the groups simultaneously was tried initially, with concentrated HCl.[94]

Treatment of the fluoride **4.7** with 6N HCl at reflux resulted in a single product (Scheme 4.8).



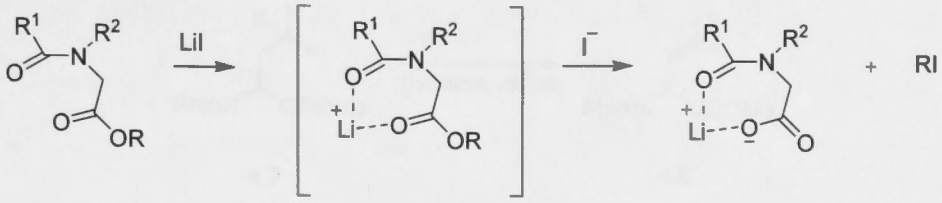
4.7 4.9

Scheme 4.8

The product of the reaction was not the fluoride **4.1**. The lack of a signal at 3.7 ppm corresponding to the methyl ester and at 7-8 ppm corresponding to the phthalimide group in the ^{1}H NMR spectrum of the product showed the absence of these components. However, the side chain methyl groups give rise to singlets at 1.32 and 1.40 ppm without the expected fluorine coupling of around 22 Hz. The appearance of these methyl group signals as singlets shows that the γ-position does not possess a proton or fluorine, due to the absence of the respective coupling that would be seen with these. The chemical shifts of the methyl signals show that the γ-carbon is bound to an electronegative atom. The ESI(+ve) mass spectrum shows a peak at *m/z* 130 instead of *m/z* 150 expected for the free amino acid **4.1**. The difference of 20 mass units corresponds to the loss of hydrogen fluoride from the free amino acid **4.1**. These observations establish that the product is the γ-lactone **4.9**. It seems likely that the fluoride **4.7** undergoes ester hydrolysis then subsequent cyclisation with loss of hydrogen fluoride to give the lactone **4.9**, or a direct cyclisation of the ester. At the same time the hydrolysis of the phthalimide moiety takes place to give the free amine.

The reaction was repeated at room temperature instead with a view of slowing down the reaction. However the ^{1}H NMR spectrum of the product showed the γ-lactone **4.9** as the only species formed. As no other product was observed in the course of reaction, which was monitored using TLC, it is presumed that an acid catalysed ester cleavage and the phthalimide cleavage take place simultaneously.

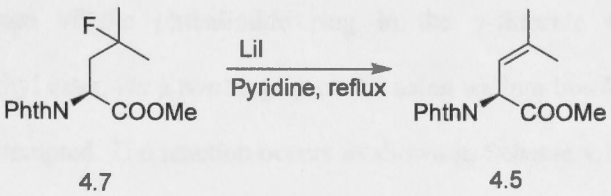
As the attempts to cleave both the phthaloyl and ester groups in the fluoride **4.7** simultaneously did not furnish the required results, it was decided to cleave these protecting groups one by one. A milder method for cleaving methyl esters using lithium iodide in aprotic solvents with dielectric constants less than ten,[95] which is specific for compounds that contain an amide carbonyl at the γ-position to the ester carbonyl (as in *N*-acylamino acid esters and β-lactam derivatives of amino acid esters) was attempted. This arrangement brings in a coordination of the amide carbonyl and the ester carbonyl oxygen with the lithium ion which results in a pulling factor *via* the lithium and a pushing factor *via* the nucleophilic iodide ion, both of which contribute to the ester cleavage as shown in Scheme 4.9.



R = Me, benzyl, *t*-butyl

Scheme 4.9

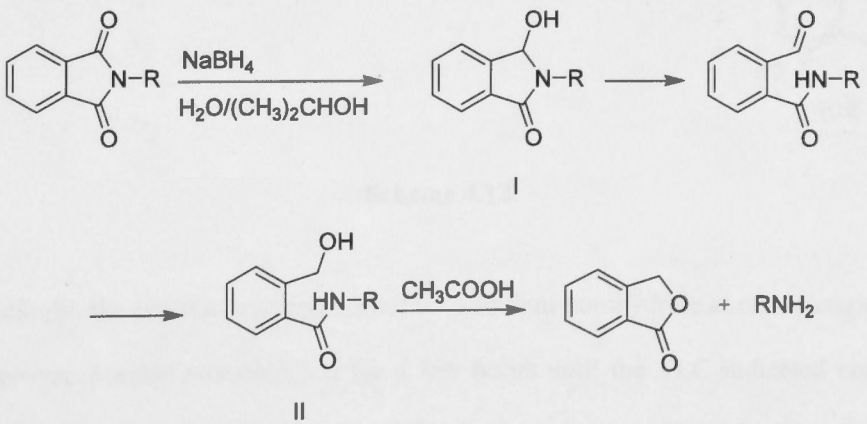
The γ-fluoride **4.7** was stirred with three equivalents of lithium iodide in ethyl acetate at room temperature for a few hours. This reaction gave back mostly the starting material **4.7** which was evident from ^{1}H NMR spectroscopy. The reaction was repeated at reflux for a few hours but led to a complex product mixture which was impractical to purify. Cleavage of the methyl ester was also attempted using excess lithium iodide in refluxing pyridine[96] instead of ethyl acetate as solvent, but this did not furnish the expected product, γ-fluorophthaloylleucine, instead giving traces of β,γ-dehydrophthaloylleucine methyl ester **4.5** as shown in Scheme 4.10 along with a complex mixture that was impractical to separate. The product was identified to be the alkene **4.5** by comparison of its ^{1}H NMR spectrum with that of the samples obtained in previous instances. The presence of a signal in the ^{1}H NMR spectrum corresponding to a methyl ester indicates that the cleavage of the ester group did not happen as expected. It is presumed that the basic conditions led to the formation of the more substituted alkene **4.5** *via* β-elimination.



4.7 4.5

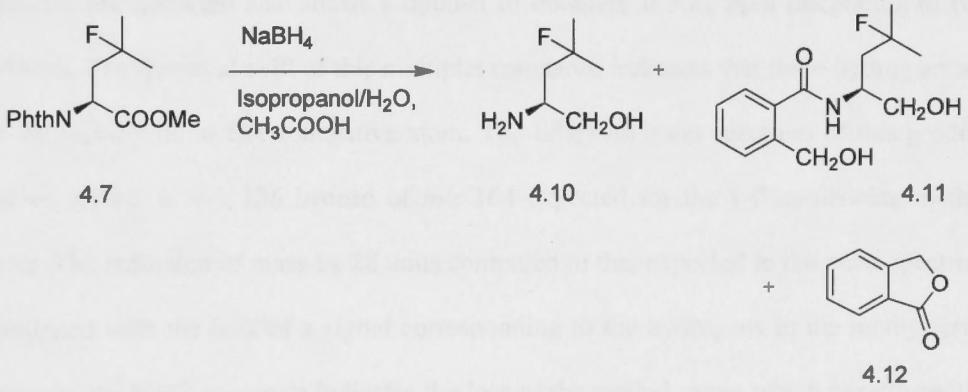
Scheme 4.10

Since basic conditions using pyridine lead to elimination, another attempt of methyl ester cleavage with lithium iodide *via* nucleophilic attack by iodide ion on the methyl group of the ester and the concominant displacement of the carboxylate anion was carried out in the neutral solvent, *N,N*-dimethylformamide (DMF) as suggested by McMurry and Wong.[97] However, heating the fluoride **4.7** with lithium iodide at 75 °C in DMF for 24 hours showed no reaction and the starting material was recovered.



Scheme 4.11

Reductive cleavage of the phthalimide ring in the γ-fluoride **4.7** to yield the fluoroleucine methyl ester, *via* a two stage process, using sodium borohydride and acetic acid[98] was also attempted. The reaction occurs as shown in Scheme 4.11. Treatment of a phthalimide with sodium borohydride in aqueous isopropanol results in the formation of II. It was observed by F. C. Uhle[99] that the use of aqueous isopropanol as solvent was necessary and just isopropanol yielded an isopropyl derivative of the compound I and the reaction did not progress to give the compound II. Compound II then undergoes acid hydrolysis to the free amine and the phthalide.



4.7 4.10 4.11 4.12

Scheme 4.12

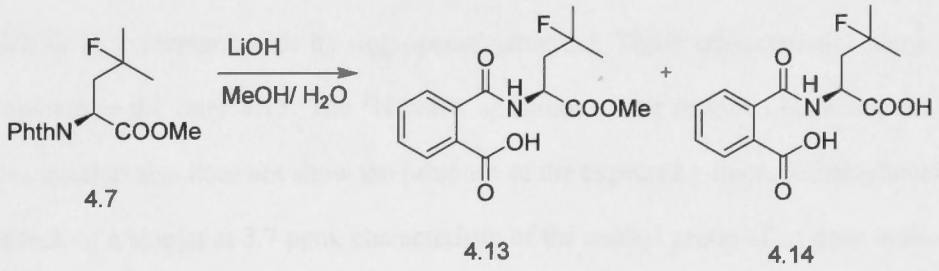
Accordingly, the reaction was carried out with sodium borohydride at room temperature in isopropanol/water mixture (7:3) for a few hours until the TLC indicated complete consumption of the starting material **4.7**. Acetic acid was then added to facilitate hydrolysis and the mixture was stirred at 80 °C for 2 hours (Scheme 4.12). The product mixture was evaporated and the residue was redissolved in ethyl acetate and washed with water. The organic and the aqueous layers were separated and evaporated and the

residues in the two layers were analysed by mass spectrometry and ^{1}H NMR spectroscopy. It was expected that the aqueous layer would contain the γ-fluoroleucine methyl ester as a result of the cleavage of the phthaloyl group in the fluoride **4.7**. The absence of signals between 7-8 ppm shows the cleavage of the phthaloyl group in the product. The lack of signals corresponding to the methyl group of the methyl ester at 3.7 ppm indicated the absence of a methyl ester in the product. The appearance of the δ-methyl groups as two doublets at 1.45 and 1.49 ppm with a coupling of 22 Hz for fluorine and vicinal protons confirms the presence of fluorine in the product. Apart from the β-hydrogens appearing as multiplet resonances at 1.94 ppm integrating to two protons, the spectrum also shows a doublet of doublets at 3.81 ppm integrating to two protons. The chemical shift of this multiplet resonance indicates that these hydrogens are in the vicinity of an electronegative atom. The ESI(+ve) mass spectrum of this product shows a peak at *m/z* 136 instead of *m/z* 164 expected for the γ-fluoroleucine methyl ester. The reduction of mass by 28 units compared to that expected in the mass spectrum combined with the lack of a signal corresponding to the hydrogens in the methyl ester group in the NMR spectrum indicates the loss of the methyl group which constitutes the loss of 15 units. The remaining difference can be attributed to the loss of an oxygen and the addition of three hydrogens indicating the compound to be the primary alcohol **4.10**. The TLC of the organic fraction contained two spots. This showed that it contained a mixture of compounds. The two compounds were expected to be the one corresponding to compound II and the lactone in Scheme 4.8. One of the compounds in the NMR spectrum is identified as the lactone **4.12** by comparing its NMR signals with those reported in the literature.[100] The peak at *m/z* 135 is consistent with the protonated

molecular ion of a species of molecular weight 134 which is in agreement with that of the lactone **4.12**. The other compound shows signals of the side chain methyl groups as two doublet resonances at 1.42 and 1.43 ppm in the NMR spectrum with a coupling of 21.2 Hz which shows the presence of fluorine in the γ-position. The presence of signals in the aromatic region shows that this group has not undergone complete cleavage to the primary amine. The appearance of this group in the NMR spectrum in an unsymmetrical pattern as three multiplets integrating to four protons indicates its ring-opened nature. The singlet at 4.70 ppm is characteristic of the methylene hydrogens in a benzylic alcohol moiety. These NMR features shows that compound **4.11** has a ring opened structure corresponding to compound II in Scheme 4.8. The absence of signals corresponding to a methyl group of a methyl ester shows the cleavage of the methyl ester. The presence of a multiplet at 3.58 ppm integrating to two protons indicates the presence of additional two hydrogens in the product similar to that observed in the case of the alcohol **4.10**. The mass spectrum shows peaks at *m/z* 270 and *m/z* 292, which fits for the molecular ion of a species with a molecular weight of 269 and its sodiated adduct. The mass is 28 units less than the expected product that corresponds to compound II in Scheme 4.8. This mass difference accounts for the loss of a methyl group in the ester as indicated in the NMR spectrum and an oxygen, which indicates the conversion of the ester group to the primary alcohol. Similar to the product **4.10**, this is also presumed to have undergone reduction of the methyl ester to the primary alcohol. These observations indicate the compound to be the alcohol **4.11**. However compounds **4.11** and **4.12** were not purified and characterised further. It was assumed that the use of sodium borohydride would leave esters unaffected[101] but the results show that the methyl

ester in the fluoride **4.7** underwent reduction to the primary alcohol to give compounds **4.10** and **4.11**. Presumably the reaction involves formation of sodium alkoxyborohydride in the presence of an alcohol which is capable of reducing esters.[102]

As the reduction conditions above resulted in the formation of the alcohol **4.10** *via* the reduction of the methyl ester, it was thought that the hydrolysis of the methyl ester in the fluoride **4.7** to the free acid would avoid such a reaction. Then the reaction with sodium borohydride and acetic acid to cleave the phthaloyl group to the free amine would lead to the fluoroleucine **4.1.**



4.7 4.13 4.14

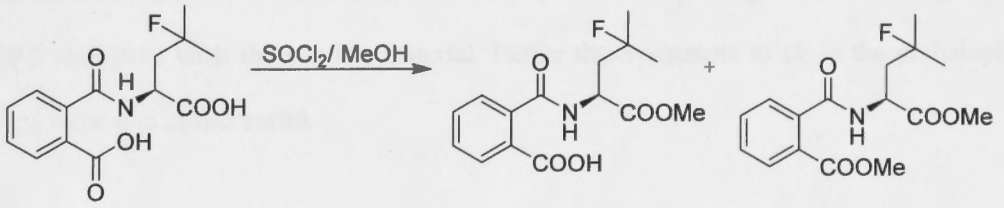
Scheme 4.13

Saponification of the fluoride **4.7** with lithium hydroxide, a mild base (strong basic conditions lead to racemisation, and hence were not tried) was carried out (Scheme 4.13) in a water-methanol mixture (1:2) for a few hours at room temperature.[103] The reaction was monitored by TLC for the disappearance of the starting material. The reaction was expected to produce γ-fluorophthaloylleucine. The product mixture was subjected to reverse phase chromatography and an aqueous and a methanolic fraction were collected

which were characterised by mass spectrometry and ^{1}H NMR spectroscopy. The NMR spectrum of the methanol fraction was complex but certain features of it revealed that it did not contain the expected product. The singlet at 3.74 ppm shows the presence of the methyl ester still in the compound. The signals of the side chain methyl groups at 1.41 ppm and 1.45 ppm with a coupling of 22 Hz indicates the presence of fluorine in the γ-position. The appearances of three multiplets that integrate to four protons altogether in the aromatic region indicate the ring-opened nature of the phthaloyl ring leaving the four protons on the aromatic ring in a non-identical environment. The mass spectrum shows peaks at *m/z* 318 and at *m/z* 334 which fits in for the lithium adduct and sodiated ion of a species with molecular weight 311 which is greater than the fluoride **4.7** by 18 mass units. This accounts for the addition of a water molecule across the phthalimide ring which is in agreement with its ring-opened structure. These characteristics show the compound is the ester **4.13**. The ^{1}H NMR spectrum of the residue obtained from the water fraction also does not show the presence of the expected γ-fluorophthaloylleucine. The lack of a singlet at 3.7 ppm, characteristic of the methyl group of an ester indicates the cleavage of the methyl ester but the aromatic region appears similar to that observed in the ^{1}H NMR spectrum of the compound in the methanol fraction, with three multiplets at 7.40, 7.50 and 7.62 ppm integrating to four protons that indicates the ring-opened nature of the phthaloyl group. The ESI(-ve) mass spectrum shows a peak at *m/z* 296 which is consistent with a species of molecular weight 297. Further, the ESI(+ve) mass spectrum show peaks at *m/z* 298 and 304 which are consistent with the protonated ion and lithium adduct of a compound of molecular weight 297. The increase in mass of eighteen units from the expected γ-fluorophthaloylleucine indicates the addition of a

water molecule across the phthaloyl ring as shown in the NMR spectrum, which suggests the compound to be the diacid **4.14**. The opening of the phthaloyl ring makes the four protons on the benzene ring non-equivalent resulting in different chemical shifts in the ^{1}H NMR spectrum as observed for both compounds **4.13** and **4.14**.

The above reaction (Scheme 4.13) was allowed to stir for a longer time with a view to convert the whole of the fluoride **4.7** to the diacid **4.14** and reclosing the phthaloyl ring and then carrying out the reduction of it to the primary amine using sodium borohydride. Stirring the mixture overnight gave the diacid **4.14** which was isolated after reverse phase chromatography in 57% yield. The product was identified by comparing its ^{1}H NMR spectrum with that of the sample obtained previously.



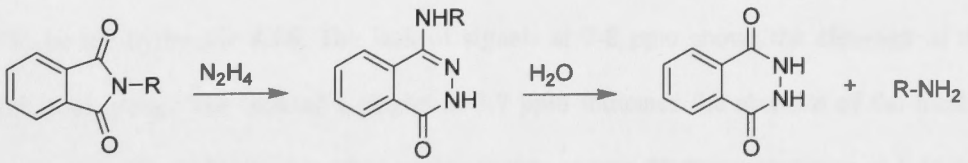
4.14 4.13 4.15

Scheme 4.14

The diacid **4.14** in methanol was treated with thionyl chloride[104] to attempt the ring closure of the phthaloyl moiety. This reaction did not furnish the expected product, instead resulting in a mixture of the mono methyl ester **4.13** and the dimethyl diester **4.15** along with starting material (Scheme 4.14). The mono methyl ester **4.13** was

identified by comparing its ^{1}H NMR signals with those of the sample obtained previously. The dimethyl diester **4.15** was identified by mass spectrometry and ^{1}H NMR spectroscopy of the crude material. The mass spectrum showed a peak at *m/z* 332 which fits the lithium adduct of a species with molecular weight 325. This corresponds to a mass increase of 28 units that indicates the addition of two methyl groups to the starting diacid **4.14**. The appearance of two singlets at 3.77 ppm and 3.83 ppm in the NMR spectrum of the diester **4.15** that integrate to three protons each shows the presence of two ester groups in the molecule. The unsymmetrical pattern of peaks in the aromatic region that integrates to four protons shows the ring-opened structure of the phthaloyl ring as observed in the previous instances.

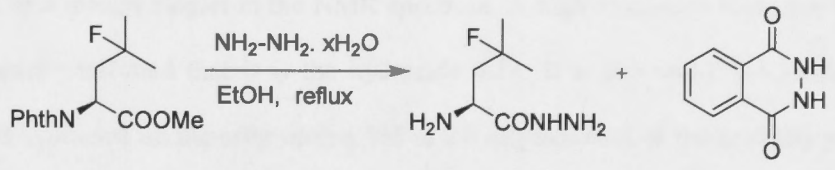
Treating the diacid **4.14** in water with 0.1M HCl for four hours gave back the starting material which was evident from the ^{1}H NMR spectrum. Repeating the reaction with 1M HCl also gave back the starting material. Hence these attempts to close the phthaloyl ring were also unsuccessful.



Scheme 4.15

Another attempt to remove the phthalimide group of the fluoride **4.7** to convert it to the primary amine was carried out using the Ing-Manske[105] procedure. This reaction occurs

via a condensation with hydrazine hydrate and the product can be easily hydrolysed (Scheme 4.15).

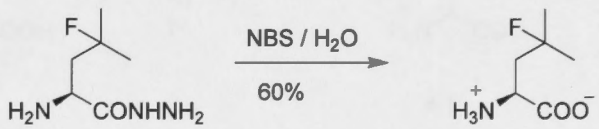


4.7 4.16 4.17

Scheme 4.16

The fluoride **4.7** was treated with hydrazine hydrate in ethanol at reflux (Scheme 4.16). The mixture was monitored using TLC for the disappearance of the starting material, which occurred after about an hour, and a white gelatinous precipitate formed. Acidification of the reaction mixture and repeated centrifugation removed the precipitate.[57,106] The supernatant was evaporated and the residue was subjected to reverse phase HPLC to obtain the pure product. The expected product was the fluoroleucine methyl ester resulting from cleavage of the phthaloyl group. Characterisation of the product using ^{1}H NMR spectrum and mass spectrometry revealed it to be the hydrazide **4.16**. The lack of signals at 7-8 ppm shows the cleavage of the phthaloyl group. The lack of a singlet at 3.7 ppm indicates the absence of the methyl ester group. The side-chain methyl groups appear as two doublet resonances at 1.46 and 1.48 ppm integrating to three protons each and with the vicinal fluorine coupling of 22 Hz. The multiplet at 2.28 ppm integrates to two protons and corresponds to the β-hydrogens coupling with each other being diastereotopic, coupling with fluorine and coupling with the α-hydrogen. The mass spectrum showed peaks at *m/z* 164 and *m/z* 186

under positive ionisation which correspond to the protonated and sodiated ions of a species of molecular weight 163 instead of *m/z* 150 for the free amino acid **4.1**. This matches both the expected ester and the hydrazide **4.16** but the ester is excluded by the absence of a methyl singlet in the NMR spectrum. A high resolution mass spectrum of the product confirmed that it is the hydrazide **4.16**. It is presumed that nucleophilic attack of hydrazine on the ester moiety led to the displacement of the methoxy group to give the corresponding hydrazide. As a result, research was pursued into cleaving the hydrazide to obtain the free amino acid **4.1**.

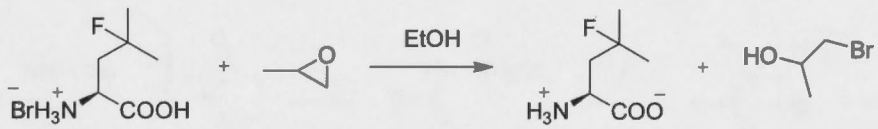


4.16 4.1

Scheme 4.17

Among the few methods available for the cleavage of hydrazides, the method involving thallium(III) nitrate trihydrate[107] was not tried due to the toxicity of thallium salts.[108] The iodine(III) mediated method for cleavage of hydrazides using iodobenzenediacetate was employed.[109] The hydrazide **4.16** was treated with iodobenzenediacetate in a water acetonitrile mixture at room temperature. The solvent was evaporated and the residue was analysed using NMR spectroscopy. The ^{1}H NMR spectrum indicated the presence of the free amino acid **4.1** along with byproducts. Purification of the mixture was not tried as a more straightforward reaction with NBS in water proved to be the mildest for the conversion of the hydrazide **4.16** to *(S)*-γ-fluoroleucine **4.1** (Scheme 4.17). The

reaction was monitored using TLC. Initially the TLC showed two spots, one corresponding to the reactant and the other the product. But as the reaction proceeded, another spot started to appear which corresponds to the lactone. This was confirmed using a sample of the lactone **4.9** obtained previously. HBr, a byproduct in the reaction lead to a highly acidic reaction mixture which on concentration of the solvent resulted in slow decomposition of fluoroleucine **4.1** to the γ-lactone **4.9**, decreasing the overall yield to 60%.



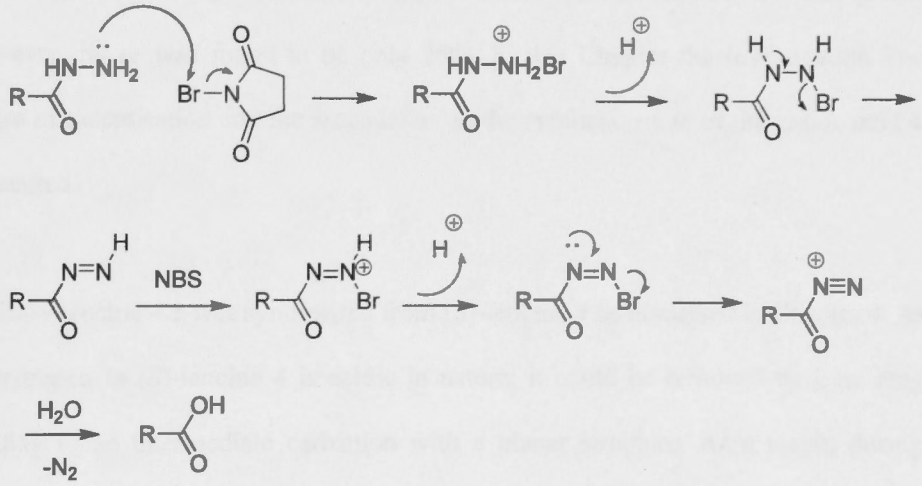
4.1

Scheme 4.18

The pure amino acid was precipitated with propylene oxide in anhydrous ethanol as its zwitterion as shown in Scheme 4.18. The amino acid is present in the reaction mixture as its hydrogen bromide salt. Propylene oxide scavenges HBr from the amino acid that leads to the ring opening of propylene oxide, leaving the zwitterion of the amino acid **4.1**. A reverse phase HPLC method was also developed to purify the amino acid **4.1**. *(S)*-γ-Fluoroleucine **4.1** was identified using ^{1}H NMR spectroscopy and mass spectrometry. The ESI(+ve) mass spectrum of the amino acid **4.1** consist of peaks at *m/z* 150 and *m/z* 172 which fit the protonated ion and sodiated ion of a species with molecular weight 149. The difference of 14 mass units fits the conversion of the hydrazide **4.16** to the amino acid **4.1**. The ^{1}H NMR spectrum shows the side-chain methyl groups as two

doublet resonances at 1.30 and 1.32 ppm integrating to three protons each coupled at 22 Hz characteristic of vicinal fluorine and hydrogens. These characteristic data show the product to be the desired amino acid **4.1**. The enantiomeric excess of the amino acid was determined using a chiral HPLC method. This revealed that the amino acid **4.1** had an *ee* of 76%.

The mechanism of the reaction with NBS is presumed to be as depicted in Scheme 4.19.



Scheme 4.19

In summary, the target of synthesis, γ-fluoroleucine was achieved. However it was found that slight racemisation had occurred in the synthesis. It is presumed that this decrease in the enantiomeric purity must have occurred in one of the synthetic operations. An investigation into how the racemisation occurred and the synthesis of the amino acid **4.1** with higher enantiomeric excess is described in the following Chapter.

Chapter 5. Investigation into the enantiomeric purity of γ-fluoroleucine

Introduction

In the previous Chapter, a concise synthesis of γ-fluoroleucine **4.1** was presented, however, its *ee* was found to be only 76%. In this Chapter the investigation into the cause of racemisation and the amendment in the synthetic route of the amino acid **4.1** is presented.

γ-Fluoroleucine **4.1** was synthesised from (*S*)-leucine **4** as discussed in Chapter 4. As the α-hydrogen in (*S*)-leucine **4** is acidic in nature, it could be removed by base reagents leading to an intermediate carbanion with a planar structure. As a result, during the readdition of the hydrogen to the carbanion, there is an equal probability of both enantiomers being formed. Hence the use of base reagents was avoided in the synthesis. Hence it was expected that the final product γ-fluoroleucine **4.1** would have high enantiomeric purity.

Estimation of enantiomeric purity

The estimation of the enantiomeric purity of the fluoride **4.1** was initially attempted using chiral NMR shift reagents. NMR shift reagents are generally optically active lanthanide chelates. In the presence of optically active chelates, enantiomers generally have non-equivalent NMR spectra. The shift differences are large enough for complete separation of at least one set of enantiotopic signals and enantiomeric compositions can be determined directly from relative peak areas.[110]

An ^1H NMR titration experiment with a europium shift reagent, tris-(3-(trifluoromethylhydroxymethylen)-D-camphorato)europium[110] was attempted. Addition of a dilute, deuterated, methanolic solution of the shift reagent into a solution of the amino acid **4.1** in deuterated water caused precipitation of the shift reagent. Adding acidified fluoroleucine **4.1** to a solution of the shift reagent in deuterated methanol also led to the formation of a turbid solution. Since the amino acid **4.1** and the shift reagent were not miscible with each other, the NMR spectrum was run using deuterated DMSO. The residual solvent peak of DMSO appeared between 3-4 ppm in the ^1H NMR spectrum. As the chemical shift of the α-protons of the two enantiomers of the amino acid also appears in the same region as observed in previous instances resolution of the signals could not be observed. Other resonances also showed little resolution and this reagent proved to be unsuitable.

Other chiral shift reagents of europium like propylenediaminetetra-acetatoeuropium(III)[111] which can be used in aqueous solutions however were not investigated and instead a more precise method using a chiral HPLC assay was developed to measure the enantiomeric purity.

A chiral HPLC column specially designed for enantiomeric separation of aliphatic amino acids[112] manufactured by Daicel Chemical Industries Ltd was used. The column contained a chiral crown ether as the chiral selector. The chiral recognition is achieved when a complex is formed between the crown ether and an ammonium ion derived from the sample. Any compound that has a primary amino group near the chiral centre could be in principle enantiomerically separated using this. According to the manufactures, Daicel Chemical Industries Ltd, the column can even be used to determine the absolute configuration of an amino acid. In this particular column the (*R*)-amino acid always elutes faster than its antipode. An HPLC assay was developed to separate the enantiomers of γ-fluoroleucine **4.1** on the Daicel Crownpak CR(+) chiral column.

To determine the retention time (t_R) of the two enantiomers of γ-fluoroleucine **4.1**, its racemic form was synthesised from racemic leucine **4** using the same procedure as discussed in Chapter 4. The yield of the compounds in each step was similar to that obtained in the synthesis discussed in Chapter 4 and no particular optimising conditions were carried out for the synthesis of the racemic form.

Chiral HPLC was run on racemic γ-fluoroleucine **4.1** obtained and the retention times of (*R*)-γ-fluoroleucine (*R*)-**4.1** and (*S*)-γ-fluoroleucine (*S*)-**4.1** were determined. These were then compared with the HPLC trace of γ-fluoroleucine **4.1** synthesised from (*S*)-leucine **4** described in Chapter 4. The chromatogram showed that the fluoride **4.1** comprised of an enantiomeric mixture of 12% (*R*)-γ-fluoroleucine and 88% (*S*)-γ-fluoroleucine, constituting an enantiomeric excess of only 76%.

The realisation that the fluoride **4.1** had a relatively lower enantiomeric excess than expected led to the investigation of its cause. The starting material in the synthesis, (*S*)-leucine **4** purchased from Sigma Aldrich is claimed to be stereochemically pure from the manufacturers and strong bases that might cause harm to the chirality of the fluoride **4.1** were also avoided in the synthesis. It seemed likely that the high temperature of the phthaloylation reaction to synthesise compound **4.2** caused racemisation. Initially the phthaloylation reaction was carried out by fusing phthalic anhydride and the amino acid together at 150 °C. However no particular care was taken to accurately maintain the temperature at 150 °C. It has been reported that the phthaloylation reaction of amino acids by fusing with phthalic anhydride at 180 °C could result in reacemisation.[113]

To investigate the above, *N*-phthaloylleucine **4.2** was synthesised from racemic leucine **4** fusing with phthalic anhydride. This product was then subjected to chiral HPLC. The enantiomers of *N*-phthaloylleucine (*R*)-**4.2** and (*S*)-**4.2** were well separated at 49 and 52.4 minutes as illustrated in Figure 7.

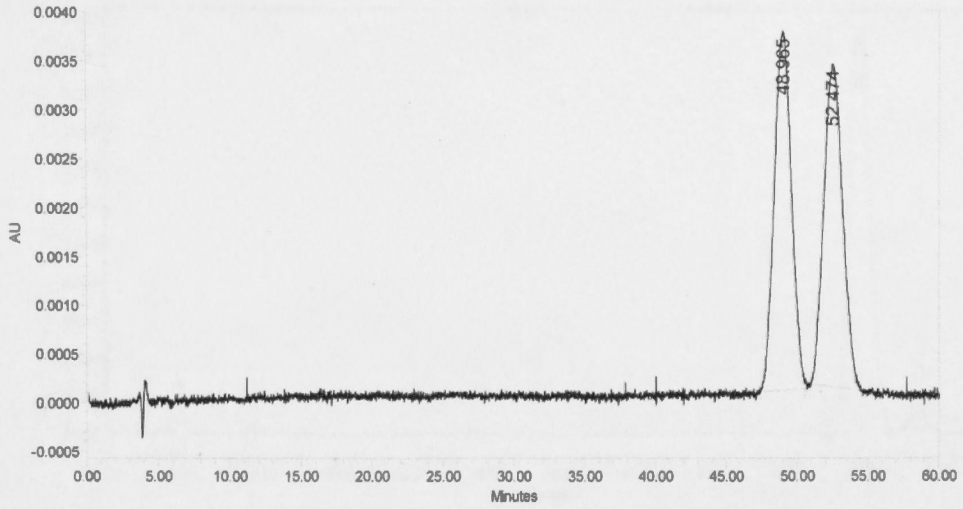
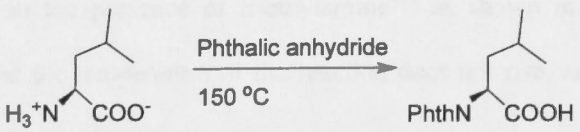


Figure 7

HPLC trace of racemic *N*-phthaloylleucine 4.2 synthesised from racemic leucine 4.

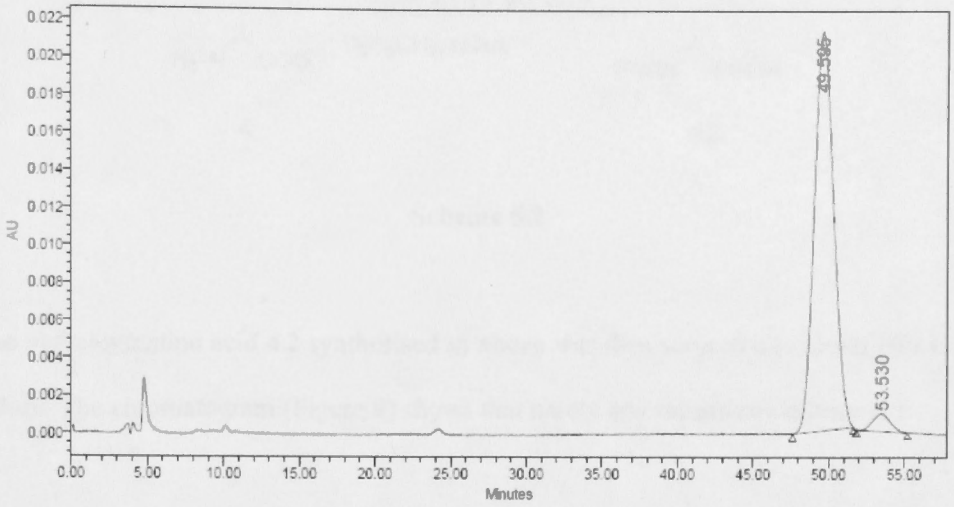
This same HPLC assay was then used to check the enantiomeric purity of *N*-phthaloylleucine **4.2** synthesised by the phthaloylation of (*S*)-leucine **4** (Scheme 5.1).



4 4.2

Scheme 5.1

The chromatogram (Figure 8) reveals that *N*-phthaloylleucine **4.2** synthesised as above undergoes racemisation to a small extent.

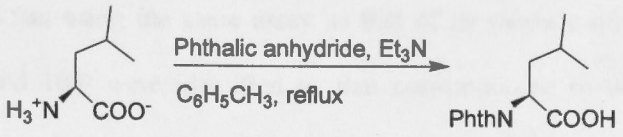


	RT	Area	% Area	Height
1	49.595	1902311	96.21	21319
2	53.530	75006	3.79	937

Figure 8

HPLC trace of *N*-phthaloylleucine 4.2 synthesised using (*S*)-leucine 4

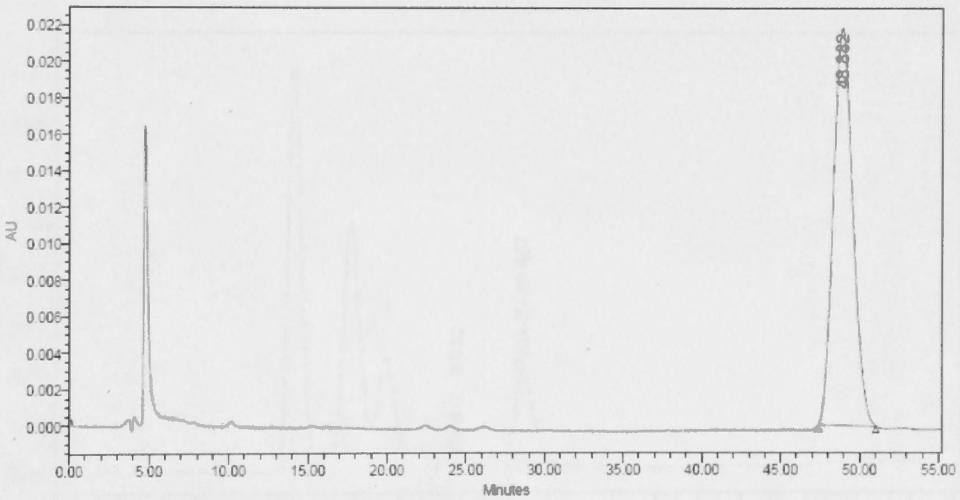
Hence the phthaloylation was repeated using a different procedure, in toluene under reflux conditions, in the presence of triethylamine[114] as shown in Scheme 5.2. This method ensures that the temperature of the reaction does not rise, and is maintained at 110 °C, the boiling point of toluene.



4 4.2

Scheme 5.2

The phthaloylamino acid **4.2** synthesised as above was then subjected to chiral HPLC as before. The chromatogram (Figure 9) shows that hardly any racemisation occurred.



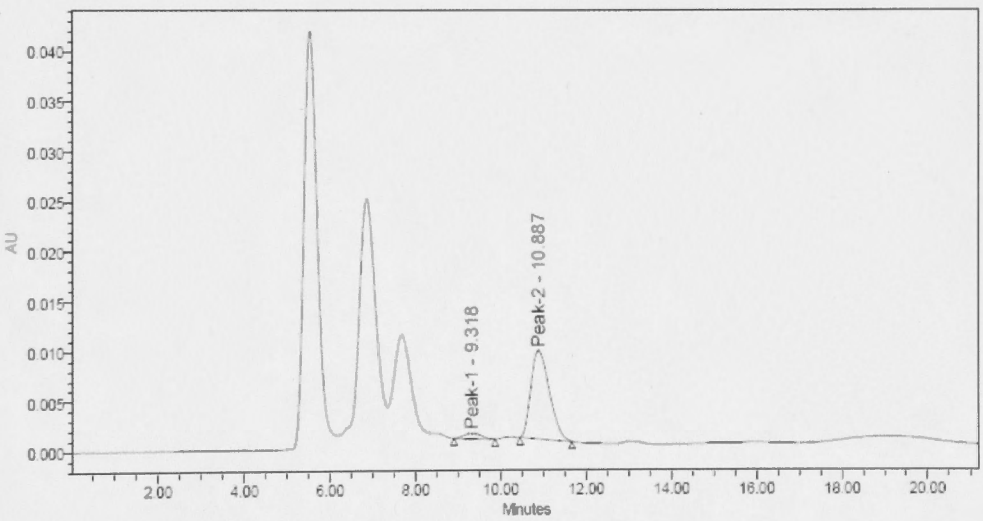
	RT	Area	% Area	Height
1	48.882	1890169	100.00	21869

Figure 9

HPLC trace of *N*-phthaloylleucine 4.2

The synthesis of the fluoride **4.1** was then repeated using *N*-phthaloylleucine **4.2** synthesised at lower temperature as illustrated in Scheme 5.2. A chiral HPLC of this

fluoride **4.1** was run using the same assay as that of its racemic mixture. The peaks eluting at 9.3 and 10.9 were identified as that corresponding to the isomers of γ-fluoroleucine **4.1** using high resolution mass spectrometry. The remaining peaks in the chromatogram were not analysed. The chromatogram below shows that γ-fluoroleucine **4.1** is more than 94% enantiomerically pure. The aim of synthesising the fluoride **4.1** was to use it in incorporation into protein by cell free synthesis. As protein synthesis is enantiospecific, improving the enantiomeric purity further, by examining other steps involved in the synthesis of the fluoride **4.1** as a cause of racemisation was not pursued.



	Peak Name	RT	Area	% Area	Height
1	Peak-1	9.318	15850	5.62	558
2	Peak-2	10.887	266160	94.38	8852

Figure 10

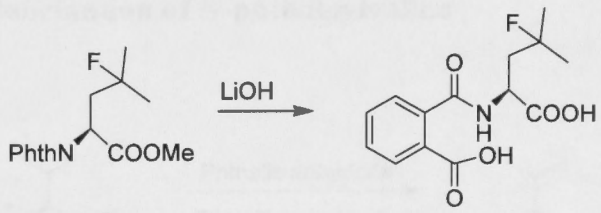
In conclusion, an improved stereospecific synthesis of (*S*)-γ-fluoroleucine **4.1** was achieved. The initial synthetic method of (*S*)-γ-fluoroleucine **4.1** yielded 88% of the (*S*) isomer and 12% of the (*R*) isomer. It was found that about 6% racemisation occurred in the phthaloylation step due the high temperature of the reaction. It was replaced by another method that consisted of using a lower temperature which increased the optical purity of (*S*)-γ-fluoroleucine **4.1** to 94%.

Chapter 6. Studies on the Synthesis of Fluorinated Amino Acids *via* Side Chain Halogenation

Introduction

The development of a short and concise synthetic route to (*S*)-γ-fluoroleucine **4.1** discussed in Chapters 4 and 5 lead to the studies described in this Chapter. This Chapter investigates the possibility of developing a simple synthetic route to fluorinated amino acids from the corresponding bromides and chlorides *via* halogen exchange and the corresponding alcohol of amino acids with the amino moiety in the amino acid protected with a phthaloyl group, and the acid functionality unprotected. Apart from their possible utility as a probe to study biomolecules by NMR spectroscopy as discussed in the Introduction, fluorinated amino acids also form an important moiety in various drugs. It had been found that when the amino acid moieties of certain drugs are fluorinated or even chlorinated, the activity of the drugs were enhanced several fold.[115] Development of a simple method for the side chain fluorination of amino acids hence benefits in many ways.

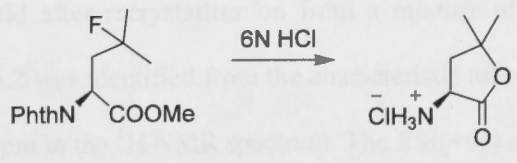
It has been demonstrated that the phthaloyl group is an excellent protecting group for the amino functionality. It is an ideal *N*-protecting group for the side chain functionalisation of an amino acid by a free radical mechanism leaving the α-position unaffected due to the absence of the captodative effect[47] which is discussed in detail in Chapter 4. However a carefully chosen protecting group for the acid is also critical as the deprotection conditions for one group might affect the other adversely. For example, in the case of the γ-fluorophthaloylleucine methyl ester **4.7**, saponification of the methyl ester leads to the opening of the phthaloyl ring as shown in Scheme 6.1 below.



4.7 4.14

Scheme 6.1

An acid catalysed ester hydrolysis of the fluoride **4.7** leads to intramolecular reaction as shown below in Scheme 6.2

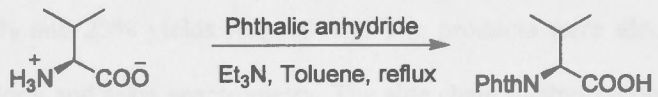


4.7 4.9

Scheme 6.2

In view of such undesired side reactions that may arise during deprotection, it was decided to study the fluorination of *N*-phthaloyl amino acids keeping the acid function free. As the difficulty in the synthesis of the fluoride **4.1** from *N*-phthaloylleucine methyl ester **4.3** was evident from the discussions in Chapter 4, *N*-phthaloylleucine **4.2** was selected for a comparative study. *N*-phthaloylvaline **6.2**, which has structurally similar alkyl group on the α-carbon as in *N*-phthaloylleucine **4.2** was also selected for the study to determine if it reacted in an analogous manner under similar reaction conditions.

Attempts of Fluorination of *N*-phthaloylvaline

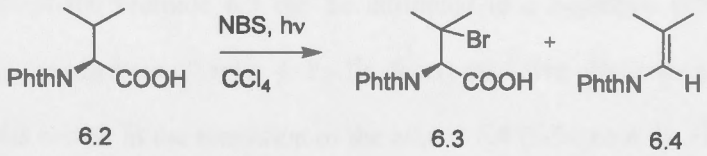


6.1 6.2

Scheme 6.3

N-Phthaloylvaline **6.2** was synthesised from (*S*)-valine **6.1** using phthalic anhydride in the presence of triethylamine in toluene under reflux (Scheme 6.3). The product **6.2** was obtained in 90% yield after recrystallisation from a mixture of dichloromethane and hexane. Compound **6.2** was identified from the characteristic aromatic peaks that appear as multiplets at 7-8 ppm in the ^{1}H NMR spectrum. The ESI(+ve) mass spectrum shows a peak at *m/z* 248 that corresponds to the protonated adduct of a species with a molecular

weight 247. This indicates a mass increase of 130 units from the starting amino acid **6.1** which is consistent with the addition of a phthaloyl group.

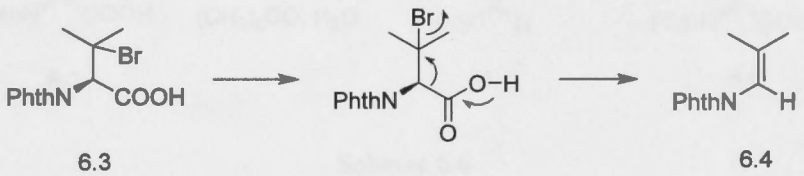


Scheme 6.4

Compound **6.2** was then brominated in CCl_4 using *N*-bromosuccinimide under a 300 W lamp (Scheme 6.4) as reported by Roselt.[116] The product mixture was subjected to column chromatography and the β-bromophthaloylvaline **6.3** and the alkene **6.4** were obtained in 71% and 25% yields respectively. The products were identified using ^1H NMR spectroscopy and mass spectrometry. The side chain methyl groups appear as two singlet resonances at 1.98 and 2.13 ppm in the NMR spectrum of the bromide **6.3** which shows that the adjacent β-carbon does not contain any hydrogen. The hydrogen on the α-carbon appears as a singlet resonance at 5.22 ppm which also shows the absence of any neighbouring protons. The ESI(+ve) mass spectrum show peaks at *m/z* 326 and 328 with equal intensity which is characteristic of compounds containing bromine. The observed increase in mass indicates the substitution of hydrogen for bromine in the starting amino acid **6.2**. The ESI(+ve) mass spectrum of the alkene **6.4**, shows a peak at *m/z* 202 which is in agreement with its structure. The NMR spectrum of the alkene **6.4** shows two doublet resonances corresponding to the methyl groups adjacent to the olefinic bond at 1.64 and 1.91 ppm. The hydrogen on the α-carbon appears as a triplet resonance at 5.88

ppm. All these values are in agreement with those reported in the literature for the alkene **6.4**.[117]

The formation of the bromide **6.3** can be attributed to a hydrogen atom abstraction mechanism as discussed in Chapter 4. Facile decarboxylative elimination of HBr from the bromide **6.3** results in the formation of the alkene **6.4** (Scheme 6.5). This reaction is presumably driven by the release of steric crowding present in the bromide **6.3**.

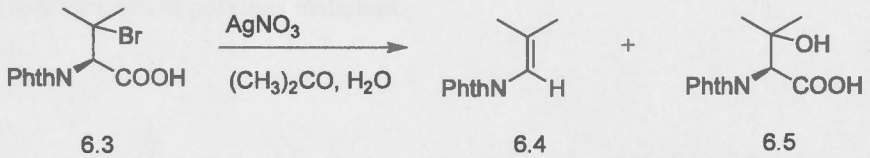


6.3 6.4

Scheme 6.5

Having obtained the bromide **6.3**, further conversion to the fluoride *via* the corresponding alcohol was attempted. Hydroxylation of the bromide **6.3** was carried out using silver nitrate in a 1:1 mixture of acetone and water following the method reported by Easton and coworkers[55] (Scheme 6.6). The product mixture was subjected to reverse phase HPLC which yielded 12% of the β-hydroxyphthaloylvaline **6.5** and the rest contained the alkene **6.4**. The products were identified using ^{1}H NMR spectroscopy and mass spectrometry. The ESI(-ve) mass spectrum of the alcohol **6.5** shows a peak at *m/z* 262 that corresponds to the deprotonated ion of the expected alcohol **6.5**. This was supported by the NMR spectrum where the β-methyl groups appeared as two singlet resonances at 1.32 and 1.58 ppm indicating that the neighbouring position is substituted.

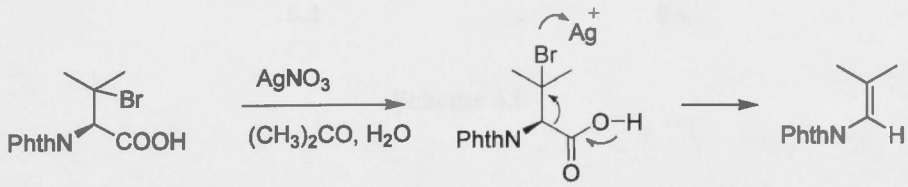
The α-hydrogen appeared as a singlet at 4.94 ppm which also indicates the absence of neighbouring hydrogen. The optical purity of the alcohol **6.5** was not determined, however it is presumed that the reaction did not involve any racemisation as a similar approach for the synthesis of alcohol derivatives of amino acids by Easton and coworkers was reported to be stereospecific.[55] The alkene **6.4** was identified by comparison of its NMR spectrum with that obtained in the previous instance.



6.3 6.4 6.5

Scheme 6.6

Formation of the alkene **6.4** is presumed to be driven by silver ions in this case, it being a good halophile. A proposed mechanism is depicted in Scheme 6.7.



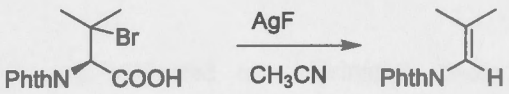
6.3 6.4

Scheme 6.7

Although the conversion of the bromide **6.3** to the alcohol **6.5** could only be achieved in low yield, sufficient quantity of the alcohol **6.5** was generated to attempt further

transformations leading to the corresponding fluoride. Diethylaminosulfur trifluoride (DAST) provides a mild method for the substitution of hydroxy groups with fluorine when compared to other reagents those are generally used for this purpose such as sulfur tetrafluoride, sulfur hexafluoride, pyridine hydrogen fluoride and potassium fluoride.[118] Thus the alcohol **6.5** was treated with DAST at -40 °C for 2 hours in DCM. The reaction resulted in a complex product mixture which contained mostly the alkene **6.4** which was identified by comparing the ^{1}H NMR spectrum of the product mixture with that of the alkene **6.4** obtained in previous instances.

Another attempt to synthesise the corresponding fluoride from the bromide **6.3** employing a halogen exchange reaction with silver fluoride also resulted in the formation of the alkene **6.4** (Scheme 6.8).

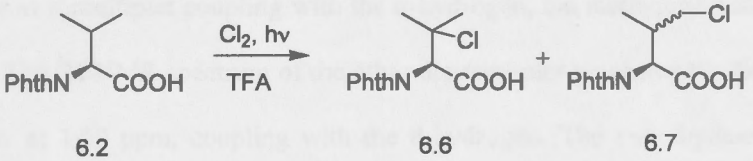


6.3 6.4

Scheme 6.8

It is presumed that the above described decarboxylative elimination to give the alkene **6.4** is favoured in the presence of the halophilic silver (I). Hence fluorination of the bromide **6.3** was attempted using cesium fluoride in *tert*-butanol.[119] It is proposed that the relatively nonpolar protic tertiary alcohol enhances the nucleophilicity of the fluoride ion significantly in the absence of catalysts, increasing the rate of nucleophilic

fluorination and decreasing the formation of side products like alkenes. But this reaction also resulted in a complex product mixture where 80% of the product was shown to be the alkene **6.4** using ^{1}H NMR spectroscopy. As all the substitution reactions using the bromide **6.3** mostly resulted in decarboxylative elimination this approach was discontinued and chlorination of *N*-phthaloylvaline **6.2** was carried out. Since free radical chlorination is less selective than bromination, it was believed that this approach will yield regio-isomeric chlorides thereby providing more opportunities for chlorine displacement reactions.



6.2 6.6 6.7

Scheme 6.9

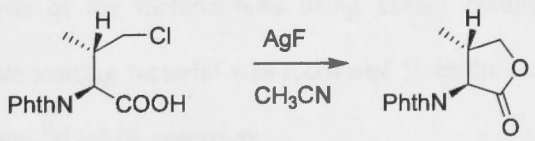
N-Phthaloylvaline **6.2** was subjected to chlorination under a 300 W lamp in trifluoroacetic acid which yielded the β-chlorophthaloylvaline **6.6** and the two diastereomers of γ-chlorophthaloylvaline **6.7** (Scheme 6.9). All the products including the diastereomers were isolated using reverse phase HPLC. The product mixture also consisted of the unreacted starting amino acid **6.2**. The products were analysed using ^{1}H NMR spectroscopy and mass spectrometry. The ESI(+ve) mass spectrum of the β-chlorophthaloylvaline **6.6** shows peaks at *m/z* 304 and 306 in the ratio 3:1 which is characteristic of a compound containing chlorine due to the natural abundance of ^{35}Cl and ^{37}Cl in the ratio 3:1. The ^{1}H NMR spectrum of the chloride **6.6** shows the γ-methyl

groups as two singlets at 1.79 and 1.92 ppm as the neighbouring β-position is substituted. The α-hydrogen appears as a singlet resonance at 5.13 ppm which also shows that the neighbouring position is substituted. The two diastereomers of γ-chlorophthaloylvaline **6.7** were separated from each other and identified using the ^{1}H NMR spectrum. The methyl group at the γ-position of one of the diastereomers appear as a doublet at 1.02 ppm coupling with the β-hydrogen. A doublet of doublets resonance was observed for one γ-methylene hydrogen at 3.71 ppm and another doublet of doublets resonance was observed for the other γ-methylene hydrogen at 3.96 ppm being diastereotopic to each other and coupling with the β-hydrogen. The β-hydrogen appears at 3.04 ppm as a multiplet coupling with the α-hydrogen, the methylene and the methyl hydrogens. The ^{1}H NMR spectrum of the other diastereomer presented the methyl group as a doublet at 1.09 ppm, coupling with the β-hydrogen. The γ-methylene hydrogens appear as two doublets of doublets at 3.37 ppm and 3.70 ppm and the β-hydrogen appears at 3.03 ppm.

The ratio of β-chlorophthaloylvaline **6.6** to γ-chlorophthaloylvaline **6.7** was 35:65 which was determined from the relative peak areas in the ^{1}H NMR spectrum of the crude product mixture. The product distribution ratio is in agreement with that observed by Z. I. Watts[120] in the case of *N*-acylamino acids. According to Watts, the rate of chlorine substitution increases on the carbon further away from the α-carbon centre of the corresponding amino acid. This is due to the fact that polar effects are more important in chlorine substitution than the radical stabilisation effect owing to the electrophilic and reactive nature of the chlorine radical. In the transition state, there is the formation of a

partial positive charge and little radical character on the carbon from where the hydrogen atom is abstracted. As a result of this phenomenon, the degree to which a positive charge can form on the carbon has a greater effect on the reaction rate than the stability of the carbon centered radical. The inductively electron-withdrawing nature of the substituted amino and carboxyl groups result in the carbons situated further away being more able to allow the formation of the partial positive charge. Also, the statistical fact that there are 6 available hydrogens in the γ-position in this case compared to only one in the β-position also accounts for the larger proportion of the γ-substituted product.

A substitution reaction was not carried out with the β-chlorophthaloylvaline **6.6** as it was thought that this would lead to decarboxylative elimination to give the alkene **6.4** as observed in the case of the β-bromophthaloylvaline **6.3**. However substitution reactions were attempted using the diastereomers of the γ-chloride **6.7** to synthesise the corresponding alcohol and the fluoride.



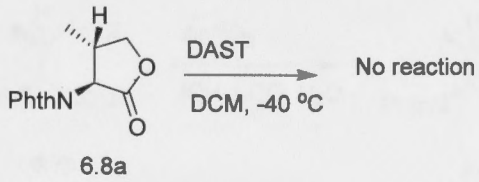
6.7a 6.8a

Scheme 6.10

Fluorination of one of the diastereomers of the γ-chlorophthaloylvaline **6.7a** was attempted by treating with silver fluoride in acetonitrile overnight in the dark (Scheme

6.10). The product mixture was analysed using ^{1}H NMR spectroscopy and mass spectrometry and the product was identified as the γ-lactone **6.8a**. The ESI(+ve) mass spectrum shows a molecular ion at *m/z* 246 consistent with the loss of hydrogen chloride from the starting material **6.7a**. The ^{1}H NMR spectrum shows a doublet at 1.19 ppm integrating to three protons corresponding to the γ-methyl group. The γ-methylene hydrogens appeared as two doublets of doublets at 3.95 and 4.65 ppm integrating to one proton each. The β-hydrogen appears as a multiplet at 3.16 ppm coupling with the neighbouring methyl, methylene and α-hydrogens. The α-hydrogen appears as a doublet at 4.66 ppm coupling with the neighbouring β-hydrogen with a coupling constant J = 11.4 Hz. This rather high value for the coupling constant indicates a *trans* relationship between the α- and β-hydrogens[121] thereby allowing the determination of the relative and absolute stereochemistry of the lactone **6.8a** as well as its precursor **6.7a** as depicted in Scheme 6.10. It also follows from the above discussion that the chloride **6.7b** must be the β-epimer of **6.7a**.

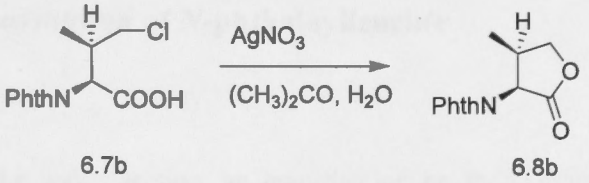
Attempted fluorination of the lactone **6.8a** using DAST resulted in no conversion (Scheme 6.11) and the starting material was recovered from the reaction mixture which was identified using the ^{1}H NMR spectrum.



6.8a

Scheme 6.11

Treatment of the *cis* diastereomer **6.7b** with silver nitrate in an acetone and water mixture in an attempt to synthesise the alcohol led to the formation of the isomeric γ-lactone **6.8b** (Scheme 6.12). The product mixture was characterised using mass spectrometry and ^{1}H NMR spectroscopy. The ESI(+ve) mass spectrum showed two peaks at 246 and 268 that correspond to the protonated and sodiated molecular ions of a species of molecular weight 245. The molecular mass observed accounts for the loss of hydrogen chloride from the chloride **6.7b** as seen in the case of attempted fluorination with the other diastereomer. The γ-methyl hydrogens appeared as doublet at 0.99 ppm integrating to three protons. The γ-methylene hydrogens appeared as two doublets of doublets at 4.23 ppm and 4.60 ppm. The β-hydrogen appears as a multiplet at 2.96 ppm coupling with the neighbouring methyl, methylene and α-hydrogens. The α-hydrogen appears as a doublet at 4.96 ppm coupling with the neighbouring β-hydrogen with a coupling constant of J = 10.5 Hz. This value that is less than that of the *trans* isomer **6.8a** indicates a *cis* relationship between the α- and β-hydrogens thereby allowing the determination of the relative and absolute stereochemistry of the lactone **6.8b** as well as its precursor **6.7b**.



6.7b 6.8b

Scheme 6.12

The basic hydrolysis of the lactone **6.8b** to afford the corresponding alcohol[122] was attempted by refluxing it in 10% sodium hydroxide. The organic portion of the resulting mixture was analysed using ^{1}H NMR spectroscopy. The spectrum presented a complex mixture and there were no peaks corresponding to the phthaloyl group. It is assumed that the lactone **6.8b** decomposed under vigorous conditions.

In summary, *N*-phthaloylvaline **6.2** could be brominated and chlorinated as an initial step for halogen exchange reaction to synthesise the corresponding fluoride. Radical chlorination gave γ-substituted chloro derivatives which were not accessible by bromination and the diastereomers were easily separated using reverse phase HPLC. However further substitution reactions to the corresponding fluoride *via* nucleophilic substitutions proved to be difficult due to decarboxylative eliminations and intramolecular side reactions.

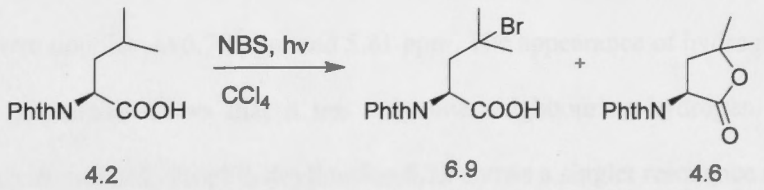
Attempts of fluorination of *N*-phthaloylleucine

As described in the above section, an investigation on the synthesis of halogenated derivatives of *N*-phthaloylvaline **6.2** revealed that although the chloro- and the bromo-derivatives of the former could be accessed, the conversion of these compounds to corresponding fluorides was not trivial. It was surmised that *N*-phthaloylleucine **4.2**, owing to the presence of an extra methylene group, is likely to furnish γ- and δ-halides that may perform better in subsequent fluorination reactions. Therefore a detailed study on the side chain chlorination and bromination reactions of *N*-phthaloylleucine **4.2** was undertaken. The results of the study as well as subsequent attempts to access the corresponding fluorides from the initially generated bromides and chlorides form the subject matter of the discussion in the following pages.

It may be recalled here that a successful synthesis of γ-fluoroleucine **4.1** was achieved in the studies described in Chapter 4. The present investigation focuses on employing *N*-phthaloylleucine **4.2** as a substrate for the side chain halogenation reactions with a view to develop a shorter and efficient protocol to access fluorinated derivatives.

N-Phthaloylleucine **4.2** was prepared from (*S*)-leucine **4** using phthalic anhydride in toluene (Scheme 5.2). The product was recrystallised and was characterised using ^{1}H NMR spectroscopy and mass spectrometry and comparison of the data with that reported in the literature.[79]

N-Phthaloylleucine **4.2** was then subjected to radical bromination in CCl_4 under a 300 W lamp with *N*-bromosuccinimide (Scheme 6.13) as reported by Roselt.[116] The ^1H NMR spectrum of the crude reaction mixture showed that it contained 70% of the bromide **6.9** and 20% of the lactone **4.6**. In the course of purification using normal phase silica chromatography, the bromide **6.9** underwent further conversion to the lactone **4.6** and hence only 50% of analytically pure bromide **6.9** could be isolated. The bromide **6.9** was identified using ^1H NMR spectroscopy and mass spectrometry. The presence of two singlet resonances at 1.74 and 1.83 ppm corresponding to the side chain methyl groups shows that the neighbouring γ-position is substituted. This was supported by ESI(+ve) mass spectrometry which shows molecular ions at *m/z* 362 and 364 in equal intensity which is characteristic of the presence of bromine. The lactone **4.6** was identified by comparing its ^1H NMR spectrum with that of the same compound obtained in previous instances.



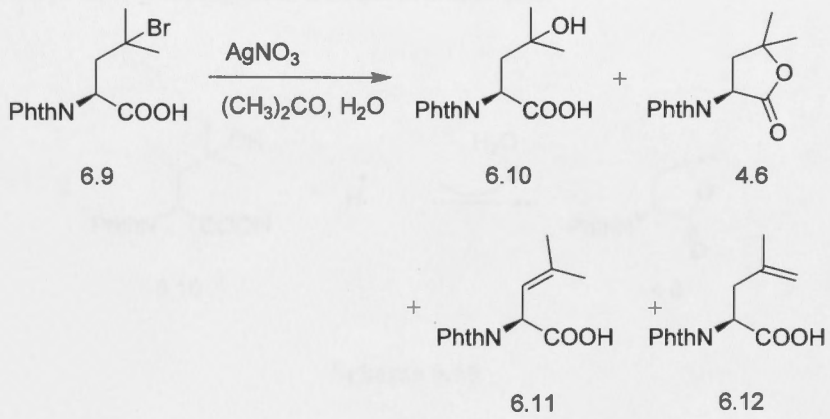
4.2 6.9 4.6

Scheme 6.13

Substitution reactions of the bromide **6.9** to synthesise the corresponding fluoride by halogen exchange and *via* the corresponding alcohol were then attempted. The bromide

6.9 on treatment with silver nitrate in acetone and water gave a mixture of products that consisted of the γ-hydroxyphthaloylleucine **6.10**, the γ-lactone **4.6**, the β,γ-dehydrophthaloylleucine **6.11**, and the γ,δ-dehydrophthaloylleucine **6.12** in the ratio 1:2:2:4 (Scheme 6.14) that were isolated using reverse phase HPLC. The products were characterised using ^{1}H NMR spectroscopy and mass spectrometry. The NMR spectrum of the alcohol **6.10** shows two broad singlet resonances at 1.25 ppm and 1.28 ppm integrating to three protons each corresponding to the two side chain methyl groups. An ABX pattern of doublets of doublets was observed at 2.15, 2.61 and 5.15 ppm consistent with the two diastereotopic β-hydrogens and the α-hydrogen respectively. The ESI(-ve) spectrum shows a peak at *m/z* 276 which is consistent with the deprotonated ion of a species of molecular weight 277. The mass difference is consistent for the substitution of bromine with a hydroxy group. The ^{1}H NMR spectrum and ESI(+ve) mass spectrum of the lactone **4.6** was in agreement with those of the compound obtained in previous instances. The NMR spectrum of the β,γ-dehydrophthaloylleucine **6.11** shows two broad singlet resonances at 1.77 and 1.78 ppm corresponding to the two methyl groups that indicates the lack of neighbouring hydrogens. Hydrogens at the α-position and β-position appear as two doublets at 5.70 ppm and 5.81 ppm. The appearance of hydrogen on the α-carbon as a doublet shows that it has only one neighbouring hydrogen. The NMR spectrum of the γ,δ-dehydrophthaloylleucine **6.12** shows a singlet resonance at 1.74 ppm corresponding to the methyl group. An ABX pattern of doublet of doublets was observed at 2.82 ppm, 3.12 ppm and 5.14 ppm consistent with the two diastereotopic β-hydrogens and the α-hydrogen respectively. The olefinic δ-hydrogens appear as two singlets at 4.65

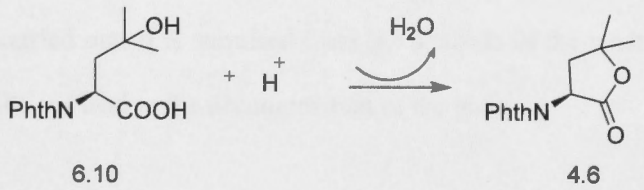
ppm and 4.68 ppm. The ESI(+ve) mass spectrum of these olefins show peaks at *m/z* 260 and 282 which correspond to the protonated and sodiated molecular ions.



Scheme 6.14

The alcohol **6.10** was highly labile and on standing it afforded the lactone **4.6**. The lactonisation was presumably accelerated by the slightly acidic conditions of the HPLC method used in the isolation of the products (Scheme 6.15). It is interesting to note that, in the case of the bromo-phthaloylvaline **6.3** the reaction with silver nitrate leads to the corresponding alcohol **6.5** and the alkene **6.4** whereas in the case of bromo-phthaloylleucine **6.9**, the carboxylic acid is positioned favourably to undergo an intramolecular cyclisation to form the corresponding γ-lactone **4.6**. An intramolecular cyclisation does not occur in the case of the bromide **6.3** as it will lead to a four membered lactone that is energetically not favoured.[123,124] It is not at all surprising that the lactone **4.6** is easily formed in the above reaction as γ-hydroxy acids are known for

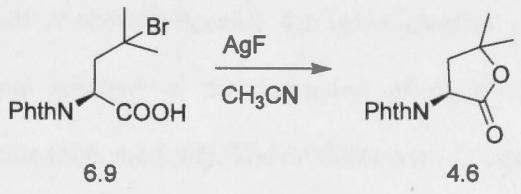
their propensity to afford the corresponding γ-lactone under acidic conditions. Presumably the γ-lactone **4.6** is also formed *via* a silver assisted debromination of the bromide **6.9** to the corresponding tertiary carbocation and its subsequent intramolecular cyclisation with the acid residue. The alkenes **6.11** and **6.12** are formed as a result of silver mediated dehydrohalogenation of the bromide **6.9**.



6.10 4.6

Scheme 6.15

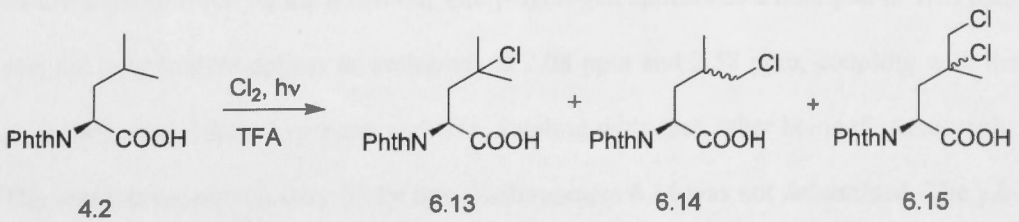
Attempted fluorination of the γ-bromophthaloylleucine **6.9** by treatment with silver fluoride also resulted in the formation of lactone **4.6** as the major product (Scheme 6.16). The silver fluoride reaction is carried out in dry acetonitrile and as the carboxylate anion is not well solvated it is expected to be more reactive and forms the lactone **4.6** whereas the silver nitrate assisted reaction is conducted in acetone-water mixture in which significant solvation of the carboxylic acid/carboxylate moiety reduces its reactivity. As a result the carbocation preferentially undergoes a proton loss to form the isomeric alkenes **6.11** and **6.12**.



Scheme 6.16

An attempt to hydrolyse the lactone **4.6** using 10% sodium hydroxide under reflux conditions was carried out. It is surmised from the analysis of the reaction mixture that the reaction conditions lead to the decomposition of the lactone.

The attempts on synthetic elaboration of the bromide **6.9** proved to be unsuccessful and subsequent investigations were directed towards the preparation of chlorides of *N*-phthaloylleucine **4.2** with a view to access various regio-isomeric chlorides as free radical chlorination takes place randomly compared to analogous bromination. It was envisaged that these regio-isomers could serve as substrates for preparing corresponding fluorides by halogen exchange or *via* the corresponding alcohols.



Scheme 6.17

Radical chlorination of *N*-phthaloylleucine **4.2** using chlorine in trifluoroacetic acid under a 300 W lamp resulted in the formation of regio-isomers of mono and dichlorophthaloylleucine (Scheme 6.17). The products were isolated using reverse phase HPLC. The products were identified using ^{1}H NMR spectroscopy and mass spectrometry. The NMR spectrum of the γ-chlorophthaloylleucine **6.13** shows two singlet resonances at 1.55 ppm and 1.64 ppm corresponding to the side chain methyl groups. The appearance of these methyl group signals as singlets shows that the γ-position is substituted. The relative downfield chemical shifts of the side chain methyl signals show that the γ-carbon is bound to an electronegative atom. The ESI(+ve) mass spectrum shows a peak at *m/z* 318 and 320 in 3:1 ratio which corresponds to the sodiated adducts of a species containing chlorine. The increase in mass from the starting amino acid **4.2** is consistent with the substitution of chlorine for hydrogen. The two diastereomers of δ-chlorophthaloylleucine **6.14** exhibited a similar pattern of ^{1}H NMR spectrum but with slightly different chemical shifts. Certain key features of the NMR spectrum of one of the diastereomers are as follows -The side chain methyl group appears as a doublet at 1.09 ppm coupling to the neighbouring γ-hydrogen. The δ-methylene hydrogens appear as a multiplet at 3.42 ppm. The down field shift shows the chlorine substitution on the δ-carbon. The γ-hydrogen appears as a multiplet at 1.73 ppm and the β-hydrogens appear as multiplets at 2.08 ppm and 2.58 ppm, coupling with the α- hydrogen and the γ-hydrogen and also coupling with each other being diastereotopic. The relative stereochemistry of the two diastereomers **6.14** was not determined. The γ,δ-dichlorophthaloylleucine **6.15** was identified using the ESI(+ve) spectrum, ^{1}H NMR spectra and 2D TOCSY NMR. The ESI(+ve) spectrum show two peaks at *m/z* 352 and

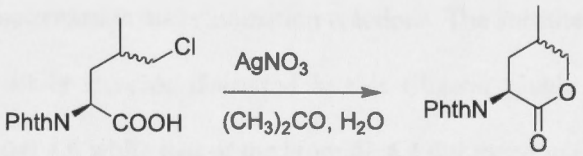
m/z 354 corresponding to the sodium adduct of the isotopic fragments of the dichloride **6.15**. The ^{1}H NMR spectrum shows a singlet resonance at 1.72 ppm for the side chain methyl group which is shifted downfield as the adjacent carbon is substituted with chlorine. The appearance of the β-hydrogens as a multiplet downfield at 2.90 ppm shows the substitution of chlorine on the adjacent γ-carbon compared to δ-chlorophthaloylleucine where the β-hydrogens appear between 2-2.6 ppm. The δ-methylene hydrogens appear as two doublets at 3.61 ppm and 3.67 ppm being diastereotopic. The relative downfield chemical shift of the signals is indicative of a chlorine substitution. The 2D TOCSY NMR shows that the two δ-methylene hydrogens do not have any other cross peaks that show the absence of any neighbouring protons. It is assumed that the diastereomer of the dichloride **6.15** was also formed in the reaction, however it was not separated and characterised.

The product distribution of the γ-isomer **6.13** to the δ-isomer **6.14** to the γ,δ-isomer **6.15** was observed to be 38:30:9 as directly observed from the integration of the peaks in the HPLC corresponding to these isomers. Some unreacted starting amino acid **4.2** was also collected after HPLC. No significant preference for formation of the γ-isomer was observed over the δ-isomer even though the radical stabilization energy of the γ-radical is expected to be high. This is presumed to be due to the polar effect dominating over radical stabilization energies owing to the high electronegativity of chlorine, and the statistical factors (*vide supra*, page 115). The dichloride **6.15** is presumably formed by prolonged chlorination. Inspite of the fact that chlorine substitution retards radical formation on a vicinal carbon due to its negative inductive effect, vicinal chlorination is

favoured due to significant participation of the neighbouring chlorine via a bridged radical transition state for hydrogen abstraction from a tertiary position.[125]

Substitution reactions of the chlorides **6.13** and **6.14** to generate the corresponding alcohol and then to the fluoride were then investigated. Reaction of the γ-chlorophthaloylleucine **6.13** with silver nitrate aimed at the synthesis of the corresponding alcohol was carried out. The resulting mixture was analysed using ESI(+ve) spectrum, which showed a peak at *m/z* 260 that corresponds to the loss of hydrogen chloride from that starting chloride **6.13**. The ^{1}H NMR spectrum was complex consisting of signals corresponding to a mixture of lactone **4.6** and the dehydrohalogenated compounds **6.11** and **6.12** as observed in the previous instance with the bromide **6.9**. However the product mixture was not purified further as it did not contain the expected alcohol.

A few substitution reactions of one of the δ-isomer **6.14** were attempted. Treatment with silver nitrate in acetone and water (Scheme 6.18) to produce the corresponding alcohol was unsuccessful. The ESI(+ve) mass spectrum showed peaks at *m/z* 260 and 282 that correspond to the lactone **6.16** instead of mass 278 expected for the corresponding alcohol. The ^{1}H NMR spectrum of the crude reaction mixture was complex. However some of the peaks of the NMR spectrum *viz*, a doublet at 1.06 ppm, a multiplet at 3.54 ppm and a multiplet at 4.93 ppm were presumed to be that of the lactone **6.16**. Hence it was assumed that the product mixture contained predominantly the δ-lactone **6.16**.



6.14 6.16

Scheme 6.18

Treatment of the γ-chlorophthaloylleucine **6.14** with silver fluoride was carried out which on analysis using mass spectrometry showed peaks at *m/z* 260 and 282. It was presumed that the reaction resulted in lactone formation as observed in the previous case and hence the strategy was not pursued further.

These experiments show that attempts to further substitute the halides of *N*-phthaloylleucine to give the corresponding fluoride leads to intramolecular and elimination side reactions.

In summary, the substitution reactions carried out using the bromides and chlorides of *N*-phthaloylvaline **6.2** and *N*-phthaloylleucine **4.2** to prepare the corresponding fluorides could not be achieved with the carboxylic acid group unprotected. The substitution reactions of β-halide of *N*-phthaloylvaline **6.2** lead to decarboxylative elimination, whereas the corresponding γ-chlorides produce the corresponding lactone **6.8**. An intramolecular cyclisation to give the corresponding lactone does not occur in the case of the β-halides of *N*-phthaloylvaline **6.2** and instead a decarboxylative elimination takes

place. In the case of *N*-phthaloylleucine **4.2**, further substitution reactions are limited due to intramolecular lactonisation and elimination reactions. The substitution reaction of the bromide **6.9** with silver fluoride discussed in this Chapter yields predominantly the corresponding lactone **4.6** while that of the bromide **4.4** discussed in Chapter 4 yields the corresponding fluoride along with the isomeric alkenes **4.5**, **4.8** and the lactone **4.6**. The presence of a free carboxylic acid in the case of **6.9** leads to the intramolecular reaction pathway to dominate over substitution and elimination reactions whereas in the case of the bromide **4.4** (Chapter 4) the carboxylic acid was protected as a methyl ester.

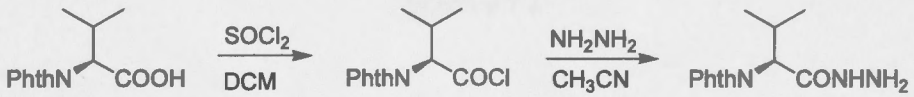
Chapter 7. Investigation into side chain functionalisation of phthaloyl amino acid hydrazides

Introduction

The investigations described in Chapter 6 demonstrated that the elaboration of bromo- and chloro-substituted *N*-phthaloylvaline **6.2** and *N*-phthaloylleucine **4.2** possessing a free carboxylic acid moiety lead to undesired intramolecular cyclisation and elimination reactions. It was surmised that this issue may be addressed by employing a suitable protecting group for the carboxylic acid moiety. Keeping this in mind and inspired by the results described in Chapter 4 on the easy deprotection of (*S*)-γ-fluoroleucine hydrazide **4.16** to (*S*)-γ-fluoroleucine **4.1** we undertook investigations directed at utilizing the hydrazide functionality as an acid masking group. Thus radical substitution reactions of *N*-phthaloylvaline hydrazide **7.1** and *N*-phthaloylleucine hydrazide **7.5** and the further elaboration of these substituted primary products to the corresponding fluorides were explored. The results of these studies form the subject matter of this Chapter.

Functionalisation reactions of *N*-phthaloylvaline hydrazide

N-Phthaloylvaline **6.2** obtained from (*S*)-valine **6.1** (see Scheme 6.3) was converted to the hydrazide **7.1** *via* the acid chloride using excess hydrazine in acetonitrile (Scheme 7.1). The hydrazide **7.1** was purified using reversed phase HPLC and the product was obtained in 70% yield. The compound was identified using ^{1}H NMR spectroscopy and mass spectrometry. The ESI(+ve) mass spectrum showed two peaks at *m/z* 262 and 284 which represents the protonated ion and the sodiated ion of a species of molecular weight 261. The difference of 14 mass units from the starting amino acid derivative **6.2** is consistent with the conversion of the carboxylic acid to the hydrazide.

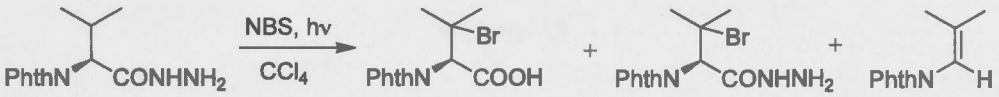


6.2 7.1

Scheme 7.1

The hydrazide **7.1** was then subjected to radical bromination with *N*-bromosuccinimide in carbon tetrachloride using a 300 W lamp in a nitrogen atmosphere. The reaction mixture was filtered and purified using reverse phase HPLC. The analysis of product collected after HPLC showed that there was 10% of the β-bromophthaloylvaline hydrazide **7.2**, 30% of the β-bromophthaloylvaline **6.3** and 60% of the alkene **6.4** formed (Scheme 7.2). The products were identified using ^{1}H NMR spectroscopy. The side chain methyl groups of β-bromophthaloylvaline hydrazide **7.2** appear as singlet resonances at

1.99 ppm and 2.14 ppm. The singlet resonances and relative downfield shift of the signals compared to the starting material indicates the presence of bromine in the β-position. The α-hydrogen appears as a singlet at 5.22 ppm which implies that its neighbouring position is substituted. The ^{1}H NMR spectra of the β-bromophthaloylvaline **6.3** and the alkene **6.4** were in agreement with those of these compounds obtained in previous instances. The bromo-hydrazide **7.2** was highly unstable and decomposed on standing.

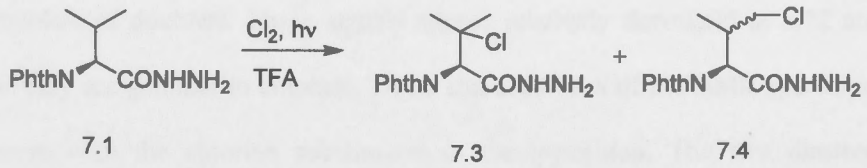


7.1 6.3 7.2 6.4

Scheme 7.2

It is reasonable to assume that the α-substituents of the hydrazide **7.1** present a considerable steric barrier for the abstraction of a hydrogen atom at the β-position resulting in a low yield of the desired bromo-hydrazide **7.2**. The fact that bromination of *N*-phthaloylvaline methyl ester attempted under similar reaction conditions also lead to the formation of the alkene **6.4** as a major product as reported by Roselt also support the above.[116] The sluggish nature of β-hydrogen abstraction lead to side reactions such as an oxidative hydrolysis of the hydrazide **7.1** to the acid **6.2**.[57] NBS mediated β-bromination of the latter to afford the bromide **6.3** is sterically less demanding. The water for the hydrolysis of the hydrazide **7.1** is assumed to be available from the inadvertent moisture present in NBS.

Functionalisation reactions could not be studied using the bromide **7.2** due to its highly unstable nature. Alternatively, the hydrazide **7.1** was subjected to radical chlorination expecting that the γ-substituted isomer if formed could be used for further reactions.



7.1 7.3 7.4

Scheme 7.3

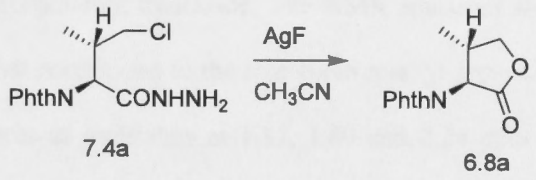
Chlorination of *N*-phthaloylvaline hydrazide **7.1** was carried out using chlorine in trifluoroacetic acid[49] under a 300 W lamp (Scheme 7.3). The products were isolated using reverse phase HPLC. The ratio of the β-isomer **7.3** to the γ-isomer **7.4** was 1:2 as determined from the area of the respective peaks in the HPLC. The product distribution ratio is in agreement with that observed by Z. I. Watts[120] in the case of chlorination of *N*-acyl amino acids. The dominance of polar effects over radical stabilization and statistical factors account for the preferential formation of the γ-isomer **7.4** as described in the case of *N*-phthaloylvaline **6.2** (*vide supra*, page 118). The products were identified by ^{1}H NMR analysis as β-chlorophthaloylvaline hydrazide **7.3** and the diastereomers of γ-chlorophthaloylvaline hydrazide **7.4**. The side chain methyl groups appear as two singlet resonances at 1.80 and 1.94 ppm in the ^{1}H NMR spectrum of the β-chloro derivative **7.3.** The relatively downfield chemical shift and their appearance as singlet resonances are consistent with chlorine substitution in the β-position. The appearance of the α-hydrogen

as a singlet at 5.13 ppm also shows that the neighbouring carbon does not contain any hydrogen. The diastereomers of the γ-chloride were isolated. The NMR spectrum of one of the diastereomeric γ-chlorides **7.4** shows a doublet at 1.03 ppm corresponding to the methyl group, coupling to the β-hydrogen. The α-hydrogen appears as a doublet at 5.08 ppm coupling with the neighbouring β-hydrogen. The γ-methylene hydrogens appear as two doublets of doublets. These signals appear relatively downfield at 3.72 and 3.97 ppm as they are geminal to chlorine. These characteristics of the NMR spectrum are in agreement with the chlorine substitution in the γ-position. The two diastereomers exhibited similar patterns of ^{1}H NMR spectra but showed considerable difference in the chemical shifts and the data is presented in the experimental section.

The β-chloride **7.3** showed a propensity for decomposition on standing and hence further substitution reactions were not carried out with it. The conversion of the γ-chloride **7.4** to the corresponding fluoride was investigated by means of halogen exchange reactions and also through the corresponding alcohol.

Treatment of one of the diastereomer of the γ-chlorophthaloylvaline hydrazide **7.4** with silver fluoride in dry acetonitrile resulted in the formation of the corresponding γ-lactone **6.8a** (Scheme 7.4). The product was characterised using ^{1}H NMR spectrum and the data is in agreement with that of the γ-lactone **6.8a**. The observation that the lactone is a *trans* isomer as discussed in Chapter 6, allows the determination of the relative and absolute stereochemistry of its precursor, the chloride **7.4a** to be the *trans* isomer. It also follows from the above discussion that the other isomer is the β-epimer of **7.4a**. The reaction is

presumed to proceed *via* the acid formed by the oxidative hydrolysis of the hydrazide by silver oxide[57] which may have formed in the course of the reaction.



7.4a 6.8a

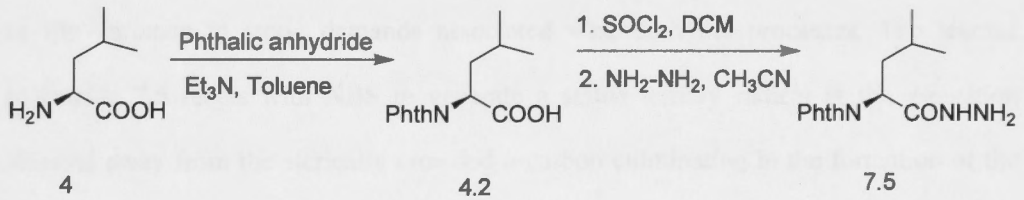
Scheme 7.4

The reaction of γ-chlorophthaloylvaline hydrazide **7.4** with silver nitrate to synthesise the corresponding hydroxy derivative was not carried out as the reaction involved a mixture of water and acetone as solvent. It is apparent from the above described reactions that treatment of the chloride **7.4** with silver (I) under aqueous conditions is likely to lead to an oxidative hydrolysis lactonisation sequence.

Functionalisation reactions of *N*-phthaloylleucine hydrazide

A comparative study of radical substitution and elaboration of the radically substituted product was performed using *N*-phthaloylleucine hydrazide **7.5**. *N*-Phthaloylleucine **4.2** was treated with a large excess of hydrazine in THF in acetonitrile to give the corresponding hydrazide **7.5** (Scheme 7.5). *N*-Phthaloylleucine hydrazide **7.5** was obtained in 60% yield after purification using reversed phase HPLC. The product was

identified using mass spectrometry and ^{1}H NMR spectroscopy. The ESI(+ve) mass spectrum shows a peak at *m/z* 276 which corresponds to the molecular ion of a species of mass 275. The difference of 14 mass units from the starting compound **4.2** indicates the formation of the corresponding hydrazide. The NMR spectrum shows two doublets at 0.86 and 0.88 ppm that correspond to the side chain methyl groups. The γ-hydrogen and the β-hydrogens appear as multiplets at 1.35, 1.80 and 2.24 ppm respectively. The α-hydrogen appears as a doublet of doublets at 5.04 ppm. The multiplets between 7.72-7.84 ppm represents the phthaloyl hydrogens. These values are in agreement with the structure of the expected hydrazide **7.5**.

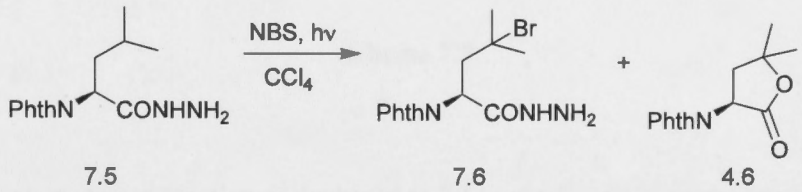


4 4.2 7.5

Scheme 7.5

Radical bromination of *N*-phthaloylleucine hydrazide **7.5** using *N*-bromosuccinimide in CCl_4 under a 300 W lamp generated 80% of the corresponding γ-bromo derivative **7.6** (Scheme 7.6) inferred from the ^{1}H NMR analysis of the crude reaction mixture. The attempted purification on the HPLC however led to the conversion of the bromo derivative **7.6** to the corresponding γ-lactone **4.6** decreasing the isolated yield to 60%. The ^{1}H NMR spectrum of the bromide **7.6** shows two singlet resonances for the side

chain methyl groups at 1.74 ppm and 1.83 ppm instead of two doublet resonances in the starting material which indicates the bromine substitution at the γ-position.

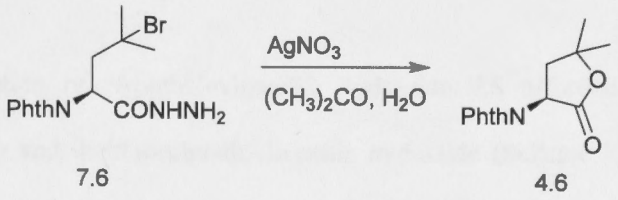


7.5 7.6 4.6

Scheme 7.6

The rather facile formation of the bromide **7.6** is in stark contrast to the reaction involving *N*-phthaloylvaline hydrazide **7.1**. This difference in reactivity can be attributed to the variance in steric demands associated with both the processes. The leucine hydrazide **7.5** reacts with NBS to generate a stable tertiary radical at the γ-position situated away from the sterically crowded α-carbon culminating in the formation of the γ-bromide **7.6**. The formation of a tertiary carbon centered radical in the case of the valine hydrazide **7.1** on the other hand, involves steric repulsion and thus does not proceed efficiently.

Treatment of the bromide **7.6** with silver nitrate in acetone water (1:1) mixture resulted in the formation of the γ-lactone **4.6** instead of the corresponding alcohol (Scheme 7.7). The reaction resulted in the complete conversion of the bromide **7.6** to the γ-lactone **4.6** which was evident from the ^{1}H NMR spectrum of the reaction mixture.

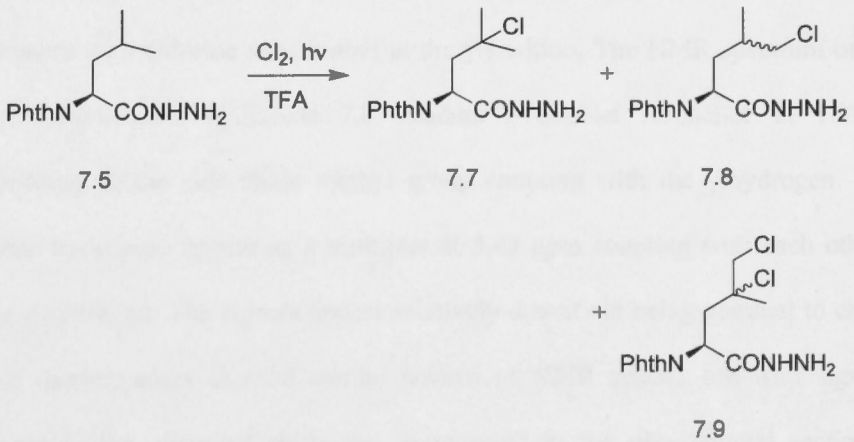


7.6 4.6

Scheme 7.7

The halogen exchange reaction of the bromide **7.6** to the fluoride was not attempted as it was presumed that this would also lead to lactonisation.

As the elaboration of the bromide **7.6** to the corresponding fluoride proved to be difficult, this attempt was discontinued. The radical chlorination of the hydrazide **7.5** was investigated so that different regio isomers of chlorides accessed could serve as substrates to attain the fluorides.



7.5 7.7 7.8

7.9

Scheme 7.8

Radical chlorination of *N*-phthaloylleucine hydrazide **7.5** afforded different regio-isomers of mono and dichlorophthaloylleucine hydrazide (Scheme 7.8). The yield of each isomer as determined from the area of the corresponding peaks in HPLC is 40:36:16 for the γ-chloride **7.7**, the δ-chloride **7.8**, and the dichloride **7.9** respectively. The distribution of the regio-isomers, where there is almost equal amount of the γ-isomer and the δ-isomer can be explained as that in the case of chloro-phthaloylvaline derivatives as explained by Watts[120] (*vide supra*, page 118). The products were isolated using reverse phase HPLC and characterised using ^{1}H NMR spectroscopy. In the course of purification, γ-chlorophthaloylleucine hydrazide **7.7** underwent decomposition to the corresponding lactone to some extent which was evident from the NMR spectrum. The ^{1}H NMR spectrum of γ-chlorophthaloylleucine hydrazide **7.7** shows two singlet resonances at 1.56 and 1.65 ppm corresponding to the side chain methyl groups. This indicates that the neighbouring γ-carbon does not contain any hydrogen. The relative downfield shift of the methyl groups compared to that of the starting material **7.5** is also in agreement with chlorine substitution at the γ-position. The NMR spectrum of the δ-chlorophthaloylleucine hydrazide **7.8** exhibits a doublet resonance at 1.09 ppm corresponding to the side chain methyl group coupling with the γ-hydrogen. The δ-methylene hydrogens appear as a multiplet at 3.43 ppm coupling with each other and also the γ-hydrogen. The signals appear relatively downfield being geminal to chlorine. The two diastereomers showed similar pattern of NMR spectra but with significant differences in the chemical shifts that is reported in the experimental section. The relative and absolute stereochemistries of these diastereomers were not determined. The γ,δ-dichlorophthaloylleucine hydrazide **7.9** shows a singlet resonance at downfield of

1.72 ppm corresponding to the side chain methyl group which ensures that the γ-position is substituted with an electronegative chlorine. The δ-methylene hydrogens appear as two doublets at 3.62 ppm and 3.68 ppm. The relative downfield chemical shift of the signals is due to chlorine substitution. It is likely that a diastereomer of the dichloride **7.9** was formed in the reaction; however it was not isolated and characterised. Interestingly the chemical shifts of the chlorides derived from the hydrazide **7.5** and that derived from *N*-phthaloylleucine **4.2** exhibited almost identical chemical shifts in the ^{1}H NMR spectrum. However these compounds had different retention times on HPLC under identical conditions which helped to distinguish the compounds from each other.

Further substitution of the chloride **7.8** with silver nitrate resulted the lactone **6.16** which was evident from the ^{1}H NMR spectrum of the same compound obtained in the previous instance.

It was hence presumed that the chlorides of *N*-phthaloylleucine hydrazide **7.5** are very likely to suffer similar problems of hydrolysis and subsequent lactonisation/ elimination as observed in the case of *N*-phthaloylvaline hydrazide **7.1**.

In summary, it has been shown that *N*-Phthaloylvaline hydrazide **7.1** and *N*-Phthaloylleucine hydrazide **7.5** can undergo radical substitution reactions leading to the formation of the corresponding halide derivatives. Subsequent functionalisation reactions of these halides to the corresponding fluorides however turned out to be

problematic. Major undesired side reactions encountered under the various conditions employed included oxidative hydrolysis of the hydrazides to the corresponding acid, lactonisation and decarboxylative elimination.

Chapter 8. Conclusion

The investigations described in this thesis are primarily directed at developing methods to access chemical entities that would be potentially useful in the study and manipulation of biomolecules such as proteins. Synthesis and subsequent screening of novel DTPA and TETA analogues for use as NMR shift reagents for the analysis of biomolecules was the focus of a detailed study. Development of convenient synthetic protocols for the stereo- and regioselective introduction of fluorine into amino acids (*S*)-leucine **4** and (*S*)-valine **6.1** was the goal of another set of investigations. The fluorinated amino acids, once synthesised, were to be employed in cell free protein synthesis to access proteins with fluorine incorporated at well-defined points. It was presumed that these shift reagents and the fluorinated amino acids would serve as tools for carrying out detailed structural investigations of proteins. Relevant observations made during these investigations are presented in the following passages along with some proposals for addressing the issues in future studies.

Various acyclic bifunctional chelators possessing a triamine pentaacetic acid backbone were selected as targets for accessing potential NMR shift reagents. A simple and straightforward synthesis of a DTPA derivative **2.1** with a thiol functional group was developed and the results are described in Chapter 2. The lanthanum complex of this compound was prepared and its utility as a shift reagent was investigated using NMR

spectroscopy studies. These investigations revealed that compound **2.1** possesses a high degree of conformational flexibility and thus it is not suitable to be used as a shift reagent. In order to address this issue other stereochemically rigid compounds with a similar backbone were designed and their syntheses were investigated. However a viable synthetic route to these compounds could not be developed, presumably due to the high degree of steric hindrance present in the substrates.

The search for NMR shift reagents with improved properties was then extended to a macrocyclic bifunctional chelator, *viz.*, TETA derivative **3.1**. The ligand **3.1** was believed to be capable of binding the lanthanides more strongly than the DTPA derivatives due to its macrocyclic structure and could possibly be used as an NMR shift reagent. The optimum condition for the preparation of the ytterbium complex of this compound was developed during the investigations detailed in Chapter 3. This complex however was observed to exist in dynamic equilibrium with the free ligand in the NMR time scale thus rendering it unsuitable for use as a shift reagent.

The substitution of a hydrogen with fluorine in amino acids brings about changes in certain physical and chemical aspects of proteins formed from such amino acids and these changes can be monitored rather easily by virtue of the presence of the fluorine atom using NMR spectroscopy. Thus development of synthetic methods for the introduction of fluorine into amino acids assume great significance and efforts aimed at this goal form the subject matter of chapters 4-7. In Chapter 4, a method for the stereoselective synthesis of (*S*)-γ-fluoroleucine **4.1** is presented. This was accomplished

by the side chain radical bromination of *N*-phthaloylleucine methyl ester **4.3** followed by fluorination of the resulting bromide **4.4** under mild conditions. Once minor issues in the deprotection of the *N*-phthaloyl and the carboxylic ester units are addressed, the radical bromination-fluorination sequence may find application in the synthesis of other fluorinated amino acids. It may also be beneficial to investigate the utility of other carboxylic acid-protecting groups in this sequence.

The observation that (*S*)-γ-fluoroleucine **4.1** accessed by the above-mentioned method had an *ee* of only 76% necessitated further investigations into the cause of racemisation. It was found that stereochemical integrity was partially lost during *N*-phthaloylation carried out at a high temperature. A milder set of conditions for *N*-phthaloylation resulted in the formation of a sample of **4.1** having a better optical purity

Cell free protein synthesis

A preliminary study aimed at the incorporation of fluorinated amino acid **4.1** into a protein *via* cell-free synthesis was carried out by Dr Madeleine Headlam. The details of this study and an interesting serendipitous observation made during it are presented below

Cell free protein synthesis is the synthesis of a protein *in-vitro* in the laboratory using the nucleotides, amino acids and enzymes required for the synthesis of a protein. The basic requirements for cell free protein synthesis are first a DNA or RNA and second, a source

of enzymes and cofactors, usually from a crude bacterial extract capable of catalysing the synthesis of the protein.[126]

The incorporation of different labelled amino acids into proteins *via* cell free protein synthesis has several advantages. Cell-free protein synthesis uses the amino acid of interest much more economically than *in vivo* expression systems.[127] In addition, since the target protein is the only protein synthesized during the reaction, the NMR experiments allow selective observation of the target protein directly in the reaction mixture without prior purification of the protein by chromatography or concentration of the sample.[128,129] Cell-free protein synthesis coupled with selective labelling and NMR analysis thus presents a powerful combination by which proteins can be synthesised using DNA and structurally characterized within 24 hours.[130]

The basic method for a cell free protein synthesis involves the preparation of a bacterial extract, in this case, an S30 cell extract derived from *Escherichia coli*, and preparation of stock solutions of amino acids and nutrients. Usually, the unnatural amino acid is added later to the final reaction mixture from a stock of concentration adjusted for the economical usage of the unnatural amino acid and then the cell free reaction is carried out.[127,131]

Inadvertent incorporation of fluoroleucine hydrazide

The S30 extract was prepared from the *E.* coli strain A19.[132] The extract was dialysed against an amino acid mixture that contained all the amino acids except (*S*)-leucine **4**. At this stage, however, (*S*)-γ-fluoroleucine hydrazide **4.16** was inadvertently employed instead of (*S*)-γ-fluoroleucine **4.1**. This anomaly occurred because the deprotection of the *N*-phthaloyl group in the fluoride **4.7** by treatment with hydrazine hydrate provided the hydrazide **4.16** as the product. As the stability of the fluoride possessing a free amino group was a major concern, the purported acid was employed immediately in cell free protein synthesis. Careful characterisation of **4.16** revealed its true identity, however, only after the experiments of cell-free protein synthesis was completed. Interestingly, this experiment led to an intriguing serendipitous observation which is described in the following passages.

The cell free reaction was carried out using the plasmid DNA for 6His-Ubiquitin. Ubiquitin was used as a model protein, because it is readily expressed using *in-vitro* protein synthesis (for the *E. coli* system used) from plasmid, it has many leucine residues for monitoring incorporation of leucine analogs and it is small and soluble and amenable to preparation for mass spectrometry analysis with the instrumentation that was available. Ubiquitin contains nine leucines as shown in Figure 11

Met[1]-Gln[2]-Ile[3]-Phe[4]-Val[5]-Lys[6]-Thr[7]-Leu[8]-Thr[9]-Gly[10]-Lys[11]-Thr[12]-Ile[13]-Thr[14]-Leu[15]-Glu[16]-Val[17]-Glu[18]-Pro[19]-Ser[20]-Asp[21]-Thr[22]-Ile[23]-Glu[24]-Asn[25]-Val[26]-Lys[27]-Ala[28]-Lys[29]-Ile[30]-Gln[31]-Asp[32]-Lys[33]-Glu[34]-Gly[35]-Ile[36]-Pro[37]-Pro[38]-Asp[39]-Gln[40]-Gln[41]-Arg[42]-Leu[43]-Ile[44]-Phe[45]-Ala[46]-Gly[47]-Lys[48]-Gln[49]-Leu[50]-Glu[51]-Asp[52]-Gly[53]-Arg[54]-Thr[55]-Leu[56]-Ser[57]-Asp[58]-Tyr[59]-Asn[60]-Ile[61]-Gln[62]-Lys[63]-Glu[64]-Ser[65]-Thr[66]-Leu[67]-His[68]-Leu[69]-Val[70]-Leu[71]-Arg[72]-Leu[73]-Arg[74]-Gly[75]-Gly[76]

Primary structure of Ubiquitin

Figure 11

The cell free reaction was performed overnight at 37 °C. The protein was extensively dialysed in 0.1% (v/v) formic acid prior to mass spectrometric analysis. The protein purity was assessed from SDS/PAGE gels that were stained with Coomassie blue. The protein concentrations were determined by the method of Bradford[133] using bovine serum albumin as standard.

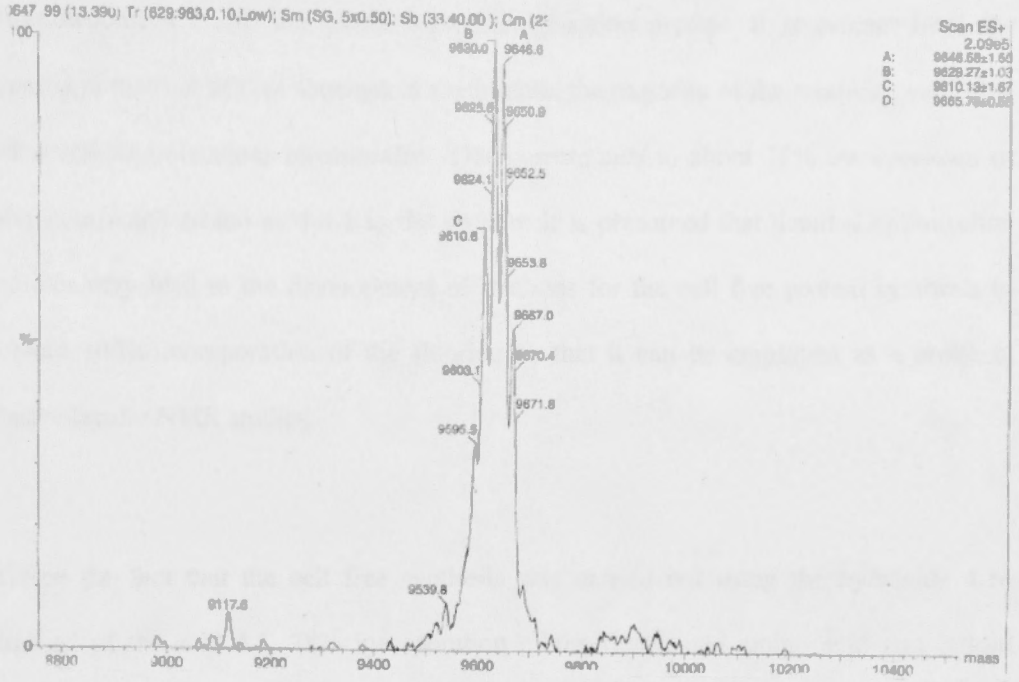


Figure 12

The molecular masses of purified protein were confirmed by ESI mass spectrometry (Figure 12). The peaks corresponding to the protein synthesised are A,B,C,D listed on the RHS of the spectrum and those average masses are 9646.58, 9629.27, 9610.13 and 9665.78 respectively. The observed spectrum can be explained as follows. The theoretical mass for formyl-Met-6His-Ubiqutin without fluorine incorporation is 9502. The observed masses correspond to the theoretical masses for formyl-Met-6His-Ubiquitin with 8, 7, 6 and 9 equivalents of (*S*)-γ-fluoroleucine **4.1** respectively, incorporated in place of leucine within the mass accuracy shown for A-D. Given the mass accuracy of the protein spectrum, as listed for the components A-D, the data are consistent with having a majority distribution of 6-9 leucine moieties replaced by (*S*)-γ-

fluoroleucine **4.1** into the formyl-Met-6His-Ubiquitin protein. It is evident from the spectrum that out of nine leucines in the protein, the majority of the mixture constituted of seven fluoroleucines incorporated. This corresponds to about 78% incorporation of the fluorinated amino acid **4.1** in the protein. It is presumed that detailed optimisation studies may lead to the development of methods for the cell free protein synthesis to obtain 100% incorporation of the fluoride so that it can be employed as a probe in biomolecular NMR studies.

Given the fact that the cell free synthesis was carried out using the hydrazide **4.16** instead of the acid **4.1,** 78% incorporation of the fluorinated amino acid was indeed surprising. This result points to the possibility that hydrazides may be employed as surrogates of amino acids in cell-free protein synthesis. This may be especially useful in cases of unnatural amino acids where stability of the free amino acid is an issue.

Although the exact order of events through which the hydrazide participated in the protein synthesis is not known at present, two scenarios may be considered tentatively. One involves the hydrolysis of the hydrazide **4.16** under the conditions of cell-free protein synthesis to generate the amino acid **4.1** and subsequent intake of the latter. A more interesting possibility is that the enzymes at work may not distinguish between the hydrazide **4.16** and the acid **4.1** and the former is consumed directly in the protein synthesis as an amino acid surrogate. In the case of the hydrazide, the peptide bond formation will be completed with the ejection of a molecule of hydrazine whereas a molecule of water is eliminated in the case of the acid. It is important to note that a

recognition system that may rely on hydrogen bond formations may not be able to distinguish effectively between the hydrazide and the corresponding acid. Additionally, it may be noted that as the same enzymes are incapable of distinguishing between the fluorinated amino acids from the natural one. If the second scenario is indeed operative, it may have implications in drug delivery. For example, hydrazide based ligands possessing improved properties may be designed to target biological receptors for amino acids and peptides. Therefore, future studies directed at understanding the mechanism of hydrazide participation in cell free protein synthesis are likely to be rewarding.

In Chapter 6 the attempts to develop a simple synthetic route to the synthesis of fluorinated amino acids using *N*-phthaloyl amino acids were described. Bromides of *N*-phthaloylvaline **6.2** and *N*-phthaloylleucine **4.2** were synthesized and further conversions were attempted. The corresponding hydroxides were also synthesised but attempts to convert them to the fluorides were not successful. The radical chlorination of the *N*-phthaloyl amino acids was carried out and the different regio-isomers were isolated. The hydroxylation and fluorination of the chloro-derivatives of these amino acids however were not fruitful. This revealed that these *N*-phthaloyl amino acids can undergo radical substitutions as reported by Roselt[116] but attempted subsequent nucleophilic substitutions result in intramolecular cyclisations. Hence it is important that the acid group is suitably protected.

The various studies attempted for the synthesis of alcohol and fluoride derivatives of *N*-phthaloylvaline hydrazide **7.1** and *N*-phthaloylleucine hydrazide **7.5** are presented in

Chapter 7. The conversion of the bromo-substituted hydrazides to the hydroxy and fluoro analogs using silver salts however failed due to the oxidative hydrolysis of the hydrazide under the reaction conditions. The chloro-substituted derivatives of these amino acid hydrazides were synthesised and the different regio-isomers were isolated. These reactions revealed that *N*-phthaloyl amino acid hydrazides undergo radical substitutions but attempted nucleophilic substitutions of these derivatives invariably lead to undesirable side chain reactions.

In summary this thesis presents studies aimed at the synthesis of novel compounds that can be used as probes to study biomolecules using NMR spectroscopy. The emphasis is on the development of a simple synthetic route to two classes of compounds: bifunctional chelators that can be used as NMR shift reagents and fluorinated amino acids that can be used as probes to study biomolecules by incorporating them into proteins. The studies carried out on the synthesis of bifunctional chelators show that the ligands must be conformationally rigid, should possess a symmetrical structure, and bind the lanthanides efficiently so that these compounds could be used as NMR shift reagents. The studies carried out on the synthesis of fluorinated amino acids demonstrated that side chain fluorination of *N*-phthaloyl amino acids can be achieved by the nucleophilic substitution of the corresponding bromides if the carboxylic acid moiety is appropriately protected. Fluorination by the nucleophilic substitution of the halide derivatives of *N*-phthaloyl amino acids using hydrazide as a masking group for the carboxylic acid or leaving the carboxylic group unprotected leads to undesired side reactions.

Chapter 9. Experimental

Melting points were determined using a Stanford Research Systems MPA 100 Optimelt Automated Melting Point apparatus.

^{1}H and ^{13}C NMR spectra were recorded on either a Varian Mercury 300 spectrometer operating at 300 MHz for proton and 75 MHz for carbon; or a Varian Inova 500 spectrometer operating at 500 MHz for proton or 125 MHz for carbon. Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (J) are reported in Hertz. The multiplicities of NMR signals are reported as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet and br, broad. Deuterated solvents were purchased from Cambridge Isotope Laboratories. Chloroform-*d* ($CDCl_3$) was referenced to 7.26 ppm for proton and 77.35 ppm for carbon spectra, methanol-d_4 (MeOD) was referenced to 3.35 ppm for proton and 49.0 ppm for carbon spectra, deuterated water (D_2O) was referenced externally with a coaxial insert containing 3-(trimethylsilyl)-3,3,2,2-tetradeuteropropionic acid sodium salt (d_4-TSPA) to 0.00 ppm for both proton and carbon spectra.

Electrospray ionisation mass spectrometry (ESI) was performed using a Micromass VG Quattro II mass spectrometer and a Waters LCT premier XE spectrometer. Mass spectral data are reported as mass-to-charge ratios (*m/z*) with their percentage abundance.

Elemental analyses were carried out by the Australian National University Microanalytical Service and were performed using a Carlo Erba 1106 Autoanalyser.

Analytical high performance liquid chromatography (HPLC) was carried out on a Waters Alliance 2695 Separations Module in conjunction with a Waters 2996 photodiode array detector. An Alltech Alltima HP C18 5 μM (4.6 x 250 mm) column, was used for analysis in general. The chiral separation of amino acids was carried out using a Crownpak CR(+) (4.0 x 150 mm) column and that of *N*-phthaloyl amino acid derivatives was carried out using a Daicel chiralpak-OD-RH (4.6 x 150 mm). For semi-preparative high performance liquid chromatography, a Waters 600 Controller, Waters 717plus autosampler and a Waters Alliance 2996 photodiode array detector were used. An Alltech Alltima C18 5 μM (250 x 22 mm) column was used for semi-preparative separations. The HPLC data were collected and processed using the Empower pro-Empower-2 software.

Thin layer chromatography (TLC) was performed using Merck Kieselgel 60 F_{254} aluminium-backed silica TLC plates. TLC plates were visualised under a UV lamp (254

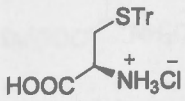
nm) or developed with potassium permanganate or ninhydrin stains. Flash column chromatography was performed with Scharlau silica gel 60 (230-400 mesh ASTM).

Ultraviolet-visible spectra were recorded using a Shimadzu UV-2450 UV-Visible spectrophotometer.

All chemicals were purchased from Sigma-Aldrich. HPLC solvents were purchased from Merck and purified water was obtained using an Elga Purelab Classic UV water purification system.

Experimental for Chapter 2

S-Trityl-*(S)*-cysteine 2.3



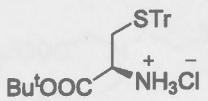
2.3

To HCl (2 mL, 1 M) was added (*S*)-cysteine (3.00 g, 19.05 mmol) and the mixture was stirred for one minute. The solution was concentrated *in vacuo* to yield the corresponding hydrochloride salt. To a solution of this salt in DMF (11 mL) was added trityl chloride (7.14 g, 25.6 mmol). The mixture was stirred for forty hours at room temperature. A solution of sodium acetate (10.66 g, 13.00 mmol) in water (130 mL) was

then added to form a white precipitate which was separated by filtration and washed with distilled water. The residue was then suspended in acetone and stirred for one hour at 80 °C. The resulting suspension was cooled and the precipitate was separated by filtration. The crude precipitate was washed with acetone and diethyl ether to furnish the title compound **2.3** (5.0 g, 66%) as a white powder.

Mp. 193 °C (decomp) (lit.,[60] 195 °C decomp). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 2.40 (dd, J = 9.0, 12.4 Hz, 1H), 2.56 (dd, J = 4.3, 12.4 Hz, 1H), 2.95 (dd, J = 4.3, 9.0 Hz, 1H), 7.24-7.38 (m, 15H). **MS (ESI)**(+ve): *m/z* 364 ($[M+H]^+$, 60%), *m/z* 386 ($[M+Na]^+$, 100%). **HRMS (ESI)** calcd. for $C_{22}H_{21}NO_2S$ $[M+Na]^+$ *m/z* 386.1207, found 386.1191. The acquired ^{1}H NMR data are consistent with the literature values.[60]

***tert*-Butyl-*S*-trityl-(*S*)-cysteinate hydrochloride 2.4**



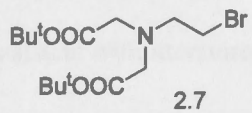
2.4

To a vigorously stirred suspension of compound **2.3** (3.00 g, 7.51 mmol) in *tert*-butyl acetate (44.20 mL, 329 mmol), at 0 °C was added $HClO_4$ (70%, 2.0 mL) dropwise. The mixture was stirred at room temperature for one hour and then ethyl acetate (20 mL) was added. The pH of the mixture was adjusted to 8 by the dropwise addition of saturated $NaHCO_3$ solution. The resulting precipitate was separated from the solution and discarded. The filtrate was washed with HCl (0.5 M, 2 x 2 mL) and then brine solution

(2 x 2 mL) and the organic fraction was dried over sodium sulfate and concentrated under reduced pressure. The residue was subjected to flash column chromatography eluting with diethyl ether/hexanes (1:1) to give the title compound **2.4** (2.46 g, 79%) as a colourless oil.

^{1}H NMR (300 MHz, CDCl3): δ 1.42 (s, 9H) 2.48 (m, 2H), 3.15 (dd, J = 4.3, 12.0 Hz, 1H), 7.24-7.38 (m, 15H). **^{13}C NMR** (75 MHz, CDCl3): δ 28.1, 36.9, 54.4, 66.9, 81.8, 126.9, 128.1, 129.7, 144.7, 169.5. **MS (ESI)** (+ve): *m/z* 420 ($[M+H]^+$, 100%). **HRMS (ESI)** (+ve) calcd. for $C_{26}H_{29}NO_2S$ $[M+H]^+$ *m/z* 420.1997, found 420.1995.

***N,N*-Bis(*tert*-butoxycarbonylmethyl)-2-bromoethylamine 2.7**



2.7

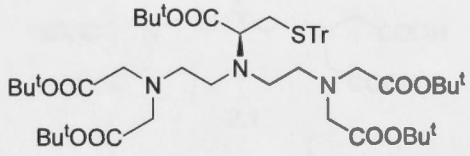
To a solution of *tert*-butyl bromoacetate (2.75 mL, 18.6 mmol) in DMF (14 mL) was added $KHCO_3$ (2.10 g, 21 mmol). The suspension was cooled to 0 °C and ethanolamine (5.00 mL, 82.8 mmol) was added over a 20 minute period. The mixture was stirred at 0 °C for 30 minutes and then at room temperature for 22 hours. The mixture was diluted with diethyl ether and washed successively with saturated $NaHCO_3$ solution (2 x 3 mL)

and brine solution (2 x 3 mL). The organic layer was dried over sodium sulfate and concentrated *in vacuo* to give the dialkylated ethanolamine **2.6** as a colourless oil.

The crude dialkylated ethanolamine **2.6** was dissolved in DCM (26 mL) and triphenyl phosphine (2.39 g, 9.11 mmol) was added. The solution was cooled to 0 °C and NBS (1.62 g, 9.11 mmol) was added in portions over 15 minutes. The solution was stirred at 0 °C for 2.5 hours and then concentrated under reduced pressure to produce a crude amorphous mass. This was triturated with diethyl ether and the residue was subjected to flash column chromatography eluting with n-hexane/ethyl acetate (3:1) to furnish the desired bromide **2.7** (2.31 g, 79%) as a pale yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (s, 18H), 3.13 (t, J = 7.7 Hz, 2H), 3.43 (t, J = 7.7 Hz, 2H), 3.48 (s, 4H). **^{13}C NMR** (75 MHz, $CDCl_3$): δ 28.3, 30.4, 56.6, 56.7, 81.4, 170.6. **MS (ESI)** (+ve): *m/z* 352 ($[M+H]^+$, ^{79}Br, 48%), *m/z* 354 ($[M+H]^+$, ^{81}Br, 50%). The acquired ^{1}H NMR signals are consistent with literature values.[10]

***N,N*-Bis[2-[-bis(tert-butoxycarbonylmethyl)]amino]ethyl-*S*-trityl-(*S*)-cysteine-*tert*-butyl ester 2.9**

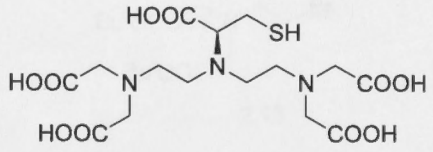


2.9

To the bromide **2.7** (1.60 g, 4.54 mmol) dissolved in CH_3CN (4.6 mL) was added phosphate buffer (10 mL, 2 M, pH 8) and the cysteine derivative **2.4** (1.05 g, 2.3 mmol). The mixture was stirred for 26 hours at room temperature. The acetonitrile layer was filtered and diluted with ethyl acetate and then washed successively with phosphate buffer (2 x 3 mL, pH 8) and brine (2 x 3 mL). The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The residue was subjected to flash column chromatography with hexanes/ethyl acetate/acetic acid (6:3:1) which furnished the title compound **2.9** (1.01 g, 42%) as a colourless oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39 (s, 9H), 1.45 (s, 36H), 2.35 (dd, J = 8.7, 12.6 Hz, 1H) 2.48 (dd, J = 6.6, 12.6 Hz, 1H), 2.59-2.61 (m, 4H), 2.66-2.68 (m, 4H), 2.98 (dd, J = 6.6, 8.7 Hz, 1H), 3.42 (s, 8H), 7.19-7.44 (m, 15H). **^{13}C NMR** (75 MHz, $CDCl_3$): δ 28.3, 32.1, 50.7, 54.3, 56.2, 66.6, 66.7, 80.9, 81.2, 126.6, 128.0, 129.8, 144.0, 170.8, 171.9. **MS (ESI)** (+ve): *m/z* 962 ($[M+H]^+$, 100%), 984 ($[M+Na]^+$, 20%). **HRMS (ESI)** calcd. for $C_{54}H_{80}N_3O_{10}S$ $[M+H]^+$ *m/z* 962.5564, found 962.5569.

***N,N*-Bis[2-[bis(carboxymethylamino)]ethyl]-(*S*)-cysteine 2.1**

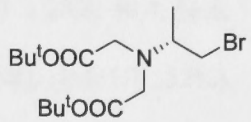


2.1

To the solution of the compound **2.9** (0.23 g, 0.24 mmol) in acetic acid (2 mL) was added hydrochloric acid (14 mL, 6 M). The mixture was stirred for 2 days at room temperature. The solvent was then removed under reduced pressure and the residue was triturated with ethyl acetate (3 x 1 mL). The residue was passed through a reverse phase silica column washing with water to furnish the desired compound **2.1** (0.06 g, 67%) as a white solid.

Mp. 176-178 ° C (decomp). **^{1}H NMR** (300 MHz, D_2O) δ 2.60 (dd, J = 6.6, 14.1 Hz, 1H), 2.79 (dd, J = 7.2, 14.1 Hz, 1H), 2.97 (t, J = 5.7 Hz, 4H), 3.37 (t, J = 5.7 Hz, 4H), 3.47 (dd, J = 6.6, 7.2 Hz, 1H), 4.08 (br s, 8H). **^{13}C NMR** (75 MHz, $CDCl_3$): δ 21.5, 38.4, 45.3, 47.7, 56.9, 160.8, 165.7. **MS (ESI)** (+ve): *m/z* 440 ($[M+H]^+$, 100%), *m/z* 462 ($[M+Na]^+$, 25%). **HRMS (ESI)** calcd. for $C_{15}H_{25}N_3O_{10}S$ $[M+H]^+$ *m/z* 440.1339, found 440.1343.

***N,N*-Bis(*tert*-butoxycarbonyl)methyl-1-methyl-2-bromoethylamine 2.13**



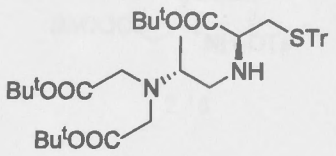
2.13

To a solution of *tert*-butyl bromoacetate (1.50 mL, 10.2 mmol) in DMF (6.5 mL) was added $KHCO_3$ (0.40 g, 4.00 mmol). The suspension was cooled to 0 °C and (*R*)-2-amino-1-propanol **2.11** (0.29 g, 3.87 mmol) was added over a 5 minute period. The reaction mixture was stirred at 0 °C for a further 5 minute period and then at room temperature for 22 hours. The mixture was then diluted with ether and washed successively with saturated $NaHCO_3$ solution (2 x 2 mL) and brine (2 x 2 mL). The organic layer was then dried and concentrated.

The crude dialkylated 1-methylethanolamine **2.12** was dissolved in DCM (12.66 mL) and triphenylphosphine (1.11 g, 4.23 mmol) was added. The solution was cooled to 0 °C and NBS (0.76 g, 4.23 mmol) was added in portions over 5 minutes. The solution was stirred at 0 °C for 2 hours and then concentrated *in vacuo* to produce a crude amorphous mass. The residue was triturated with diethyl ether (2 x 3 mL) and then subjected to flash column chromatography eluting with hexanes/ethyl acetate (6:1) to furnish the desired bromide **2.13** (0.99 g, 70%) as a pale yellow oil

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.42 (s, 18H), 1.66 (d, J = 6.6 Hz, 3H), 2.85 (dd, J = 7.8, 14.1 Hz, 1H), 3.13 (dd, J = 6.3, 14.1 Hz, 1H) 3.42 (m, 4H), 4.10 (dd, J = 6.3, 7.8 Hz, 1H). **^{13}C NMR** (75 MHz, $CDCl_3$): δ 28.3, 30.4, 56.6, 56.7, 81.4, 170.6. **MS (ESI)** (+ve): *m/z* 366.0 ($[M+H]^+$, 48%), *m/z* 368.0 ($[M+H]^+$, 52%).

***N*-[2-[*N'*,*N'*-bis(tert-butoxycarbonyl)methyl]amino]-2-methyl-ethyl-*S*-trityl-(*S*)-cysteine-*tert*-butyl ester 2.14**

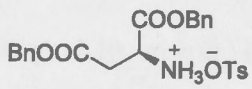


2.14

To a solution of the bromide **2.13** (0.60 g, 1.65 mmol) in ACN (1.7 mL) was added phosphate buffer (4 mL, 2 M, pH 8) and the cysteine derivative **2.4** (0.50 g, 1.10 mmol). The reaction mixture was stirred for 24 hours at room temperature. The mixture was filtered and the filtrate was collected. This was then diluted with ethyl acetate and washed successively with phosphate buffer (2 x 10 mL pH 8) and brine (2 x 10 mL). The organic layer was dried over sodium sulfate and concentrated under reduced pressure to give a solid which was purified using flash column chromatography eluting with hexanes/ethyl acetate (3:1) to yield the compound **2.14** (0.93 g, 80%) as a colourless oil.

^{1}H NMR (300 MHz, CDCl3): δ 0.97 (d, J = 6.3 Hz, 3H), 1.45 (s, 27H), 2.11 (m, 2H) 2.43 (dd, J = 8.1, 12.9 Hz, 1H), 2.63 (dd, J = 6.3, 12.9 Hz, 1H,), 2.71 (m. 1H), 2.96 (dd, J = 6.6, 8.1 Hz, 1H), 3.37 (s, 8H), 7.10-7.41 (m, 15H). **^{13}C NMR** (75 MHz, $CDCl_3$): δ 14.0, 28.1, 31.9, 50.5, 54.1, 56.0, 64.4, 66.5, 80.6, 81.0, 126.4, 127.8, 129.6, 144.9, 170.6, 170.9. **MS (ESI)** (+ve): *m/z* 705 ($[M+H]^+$, 80%). **HRMS (ESI)** calcd. for $C_{41}H_{57}N_2O_6S$ $[M+H]^+$ *m/z* 705.3937, found 705.3937

(*S*)-Aspartic acid di-benzyl ester-*p*-toluenesulfonate 2.18



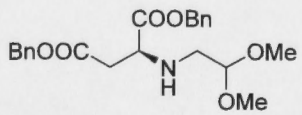
2.18

To a solution of *p*-toluenesulfonic acid (7.86 g, 41.3 mmol) in benzyl alcohol **2.17** (38.95 mL, 375.6 mmol) and benzene (75 mL) was suspended (*S*)-aspartic acid **2.16** (5.00 g, 37.6 mmol). The resulting mixture was refluxed for 20 hours by continuous water extraction. The reaction mixture was filtered and the filtrate discarded. The residue was washed with diethyl ether (3 x 50 mL) and was dried *in vacuo* to obtain the title compound **2.18** (18 g, 98.7%).as a white solid.

Mp. 158-160 °C.(lit.,[64] 156-158 °C). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 3.05 (dd, J = 4.2, 12.0 Hz, 1H) 3.17 (dd, J = 6, 12.6 Hz, 1H), 4.45 (dd, J = 4.2, 6 Hz, 1H), 4.90 (d, J =

11.2 Hz, 2H), 5.01 (d, J = 15.1 Hz, 2H), 7.02 (d, J = 8.1 Hz, 2H) 7.27 (m, 6H), 7.74-7.81 (d, J = 8.4 Hz, 2H). **MS (ESI)** (+ve): *m/z* 314 ($[M+H]^+$, 40%).

(*S*)-Di-benzyl-2-(2,2-dimethoxyethylamino)succinate 2.20



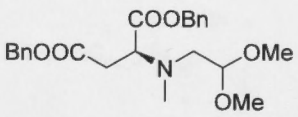
2.20

To a solution of **2.18** (1.50 g, 3.10 mmol) in MeOH (3 mL) was added dimethoxyacetaldehyde **2.19** (60% solution in water, 0.59 mL, 3.41 mmol) and the mixture was stirred for one hour. To this mixture, $NaBH_3CN$ (0.19 g, 3.10 mmol) was added and stirred for another 2.5 hours. The resulting mixture was partitioned between DCM (20 mL) and brine (20 mL). The organic layer was washed with water (2 x 20 mL) and dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified using flash column chromatography eluting with 2% methanol in chloroform to give the title compound **2.20** (0.90 g, 72%) as a colourless oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ 2.10 (br s, 1H), 2.60-2.71 (m, 2H) 2.75-2.85 (m, 2H), 3.31 (s, 6H), 3.75 (dd, J = 4.8, 6.3 Hz, 1H), 4.39 (t, J = 4.8 Hz, 1H), 5.09 (s, 2H), 5.13 (s, 2H), 7.33 (s, 10H). **^{13}C NMR** (75 MHz, $CDCl_3$): δ 38.1, 49.1, 53.2, 53.8, 57.7, 66.5,

66.9, 103.6, 128.6, 135.6, 170.6, 173.2. **MS (ESI)** (+ve): *m/z* 402 ($[M+H]^+$, 80%). **HRMS (ESI)** calcd. for $C_{22}H_{27}NO_6$ $[M+Na]^+$ *m/z* 424.1736, found 424.1734.

(*S*)-Di-benzyl-2-(*N*-(2,2-dimethoxyethyl)-*N*-methylamino)succinate 2.22



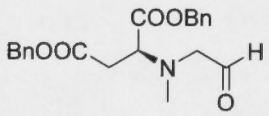
2.22

To a solution of **2.20** (0.87 g, 2.17 mmol) in MeOH (2 mL) was added formaldehyde **2.21** (37% in acetic acid, 0.88 mL, 10.9 mmol). The resulting solution was cooled to 0 °C under ice and $NaBH_3CN$ (0.20 g, 3.25 mmol) was added. The solution was gradually warmed to room temperature and was stirred for 2 hours. The resulting mixture was extracted with DCM (2 x 10 mL) and washed with brine (2 x 10 mL). The organic layer was dried over sodium sulfate and filtered and was concentrated under reduced pressure. The residue was purified using flash column chromatography in 2% MeOH in $CHCl_3$ to give the title compound **2.22** (0.77 g, 85%) as a colourless oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ 2.40 (s, 3H), 2.64-2.72 (m, 2H), 2.88-2.97 (m, 2H), 3.30 (s, 3H), 3.95 (dd, *J* = 5.1, 5.8 Hz, 1H), 4.36 (t, *J* = 6 Hz, 1H), 5.11 (d, *J* = 11.1 Hz, 2H), 5.13 (d, *J* = 15.6 Hz, 2H) 7.29-7.36 (m, 10H). **MS (ESI)** (+ve): *m/z* 416.3 ($[M+H]^+$,

40%), *m/z* 438.3 ($[M+Na]^+$, 50%). **^{13}C NMR** (75 MHz, $CDCl_3$): δ 35.4, 39.9, 53.7, 54.0, 56.5, 63.5, 66.6, 103.7, 127.1, 128.6, 135.8, 171.2, 171.3.

(*S*)-Di-benzyl-2-[*N*-(formylmethyl)-*N*-methylamino]succinate 2.23



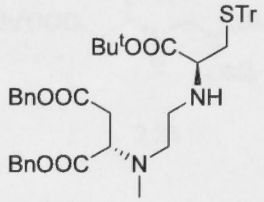
2.23

To the compound **2.22** (0.62 g, 1.49 mmol) was added HBr-HOAc (45% in HOAc, 2.69 mL, 14.9 mmol) dropwise at 0 °C. The mixture was slowly warmed to room temperature and stirred for one hour. The resulting mixture was extracted with ethyl acetate (2 x 5 mL) and was washed successively with saturated $NaHCO_3$ solution (2 x 5 mL), water (2 x 5 mL) and brine (2 x 5 mL). The solution was dried over sodium sulfate and filtered and was concentrated under reduced pressure. The residue was purified using flash column chromatography eluting with hexanes/ethyl acetate (1:1) to give the title compound **2.23** (0.20 g, 36%) as a colourless oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ 2.35 (s, 3H), 2.73 (dd, J = 8.4, 11.2 Hz, 1H), 2.91 (dd, J = 6.6, 11.2 Hz, 1H), 3.33 (s, 2H), 3.91 (dd, J = 6.6, 8.4 Hz, 1H), 5.11 (d, J = 12.3 Hz, 2H), 5.13 (d, J = 13.8 Hz, 2H) 7.34-7.38 (m, 10H), 9.47 (t, J = 1.8 Hz, 1H). **^{13}C NMR**

(75 MHz, $CDCl_3$): δ 35.6, 39.7, 63.4, 64.5, 66.9, 128.6, 135.6, 135.8, 170.7, 170.8, 202.1. **MS (ESI)** (+ve): *m/z* 370.4 ($[M+H]^+$, 60%). **HRMS (ESI)** calcd. for $C_{21}H_{23}NO_5$ $[M+H]^+$ *m/z* 370.1654, found 370.1658.

(*S*)-Di-benzyl-2-(*N*-((*S*)-tert-butyl-2-amino-3-(tritylthio)propanoic)-*N*-methylamino)succinate 2.24

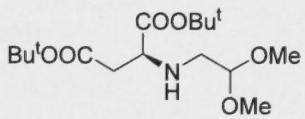


2.24

To a solution of **2.23** (0.20g, 0.54 mmol) in MeOH (2 mL) was added the cysteine derivative **2.4** (0.09 g, 0.20 mmol). The resulting solution was cooled to 0 °C and $NaBH_3CN$ (0.02 g, 0.32 mmol) was added. The resulting solution was warmed to room temperature and stirred for a day. The mixture was partitioned between DCM and water. The DCM layer was collected and dried over sodium sulfate and concentrated under reduced pressure. The residue was subjected to flash column chromatography in 5% AcOH in DCM to give the title compound **2.24** as a colourless oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41 (s, 9H), 2.25 (s, 3H), 2.31-2.36 (m, 2H), 2.39-2.43 (m, 2H), 2.60-2.70 (m, 2H), 2.88-2.98 (m, 2H), 3.68-3.75 (m, 1H), 3.85-3.92 (m, 1H), 5.08 (d, J = 13.5 Hz, 2H), 5.15 (d, J = 12.3 Hz, 2H) 7.18-7.43 (m, 25H). **MS (ESI)** (+ve): *m/z* 773.6 ($[M+H]^+$, 100%).

(*S*)-Di-*tert*-butyl-2-(2,2-dimethoxyethylamino)succinate 2.26

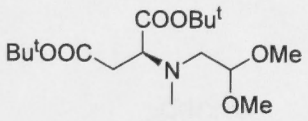


2.26

Compound **2.26** was synthesised using (*S*)-aspartic acid di-*tert*-butyl ester **2.25** (1.5 g, 5.3 mmol) as per the procedure given for the compound **2.20**. The product mixture was subjected to flash column chromatography in ethylacetate/hexane (1:1) to give the title compound **2.26** (1.5 g, 85 % yield).

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.42 (s, 18H), 2.5-2.54 (m, 2H), 2.65 (dd, J = 4.8, 11.1 Hz, 1H), 2.82 (dd, J = 6.3, 11.1 Hz, 1H), 3.34 (s, 6H), 3.47 (dd, J = 4.8, 6.3 Hz, 1H), 4.44 (t, J = 6.1 Hz, 1H). **MS (ESI)** (+ve): *m/z* 334.4 ($[M+H]^+$, 50%), *m/z* 356.2 ($[M+Na]^+$, 40%).

(*S*)-Di-*tert*-butyl-2-(*N*-(2,2-dimethoxyethyl)-*N*-methylamino)succinate 2.27

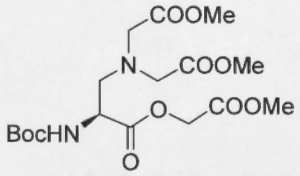


2.27

Compound **2.27** was synthesised using **2.26** (1.00 g, 3.00 mmol) as per the procedure given for the synthesis of compound **2.22**. The product mixture was subjected to flash column chromatography eluting with 1% MeOH in chloroform to give the title compound **2.27** (0.6 g, 58% yield)

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (s, 18H), 2.41 (s, br, 3H), 2.62-2.78 (m, 4H), 3.35 (s, 6H), 3.64-3.74 (m, 1H), 4.34-4.48 (m, 1H). **^{13}C NMR** (75 MHz, $CDCl_3$): δ 28.3, 36.8, 40, 53.7, 54.1, 64.5, 80.7, 104, 170.8, 173.0. **MS (ESI)** (+ve): *m/z* 348.3 ($[M+H]^+$, 70%).

***tert*-Butyl-(*S*)-1-[((methoxycarbonyl)methoxy)carbonyl)-2-(bis((methoxycarbonyl)methyl)amino)]ethylcarbamate 2.30**

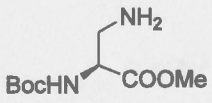


2.30

To a solution of methyl bromoacetate (0.07 mL, 0.29 mmol) in DMF (0.24 mL) was added $KHCO_3$ (0.04 g, 0.37 mmol). The suspension was cooled to 0 °C and was added Boc-Dap-OH **2.29** (0.03 g, 0.15 mmol) in tiny amounts. The mixture was stirred at 0 °C for 30 minutes and was slowly warmed to room temperature and was stirred over night. The mixture was extracted with ethyl acetate (5 mL) and washed with brine (2 x 5 mL) and concentrated under reduced pressure. The residue was subjected to flash chromatography in EtOAc/hexane (1:1) to give the title compound **2.30** (0.43 g, 70% yield).

^{1}H NMR (300 MHz, CD_3OD): δ 1.45 (s, 9H), 3.15 (dd, J = 7.5, 4.1 Hz, 1H), 3.26 (dd, J = 5.2, 4.1 Hz, 1H), 3.64 (s, 4H), 3.74 (s, 6H), 3.78 (s, 3H), 4.32 (dd, J = 5.2, 7.5 Hz, 1H) 4.65 (s, 2H). **MS (ESI)** (+ve): *m/z* 443.1 ($[M+Na]^+$, 100%).

***tert*-Butyl-(*S*)-1-(methoxycarbonyl)-2-aminoethylcarbamate 2.31**

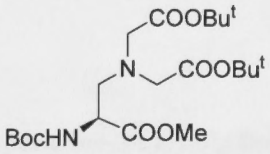


2.31

To a stirred solution of Boc-Dap-OH **2.29** (0.10 g, 0.49 mmol) in a mixture of benzene (3.5 mL) and MeOH (1 mL) was added trimethyl silane (0.10 mL, 0.64 mmol) dropwise. The mixture was stirred at room temperature for 15 hours. The disappearance of the yellow colour of the reaction mixture shows the complete consumption of trimethyl silane. The reaction mixture was concentrated under reduced pressure and dried *in vacuo* to obtain the title compound **2.31** as a colourless oil (0.10 g, 93% yield).

^{1}H NMR (300 MHz, CD_3OD): δ 1.48 (s, 9H), 2.91 (dd, J = 6.3, 13.2 Hz, 1H), 3.03 (dd, J =, 4.8, 13.2 Hz 1H), 3.77 (s, 3H), 4.22 (dd, J = 4.8, 6.3 Hz, 1H). **MS (ESI)** (+ve): *m/z* 219.3 ($[M+H]^+$, 20%).

***tert*-Butyl-(*S*)-1-(methoxycarbonyl)-2-(bis((*tert*-butoxycarbonyl)methyl)amino)ethylcarbamate 2.32**



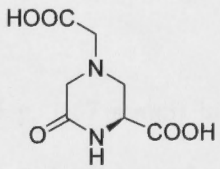
2.32

To a suspension of *tert*-butylbromo acetate (0.15 mL, 1.03 mmol) in DMF (0.75 mL) was added $KHCO_3$ (0.115 g, 1.15 mmol). The suspension was cooled to 0 °C and **2.31** was added dropwise. The mixture was stirred at 0 °C for 30 minutes and slowly warmed to room temperature and stirred for 18 hours. The mixture was extracted with ethyl acetate (5 mL) and washed with brine (2 x 5 mL). The combined organics were concentrated under reduced pressure. The residue was subjected to preparative high performance chromatography to give the title compound **2.32** (0.10 g, 50%) as a colourless oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.44 (s, br, 27 H), 3.08- 3.15 (m, 2H) 3.34 (d, J = 18 Hz, 2H), 3.44 (d, J = 18 Hz, 2H), 3.71 (s, 3H), 4.20 (dd, J = 5.7, 6.3 Hz, 1H), 6.20 (s, br, 1H). **^{13}C NMR** (75 MHz, CD_3OD): δ 28.4, 28.7, 52.8, 54.5, 56.2, 57.3, 80.7, 82.6, 157.9, 172.4, 173.9. **MS (ESI)** (+ve): *m/z* 447.2 ($[M+H]^+$, 100%). **HRMS (ESI)** calcd. for $C_{21}H_{38}N_2O_8$ $[M+H]^+$ *m/z* 447.2706, found 447.2721

HPLC: t_R 16.3 (column: Alltech Alltima C18 5u, 250 x 10 mm; (20:80) water 0.1% TFA: acetonitrile; flow rate: 11.5 mL min^{-1})

(*S*)-4-(Carboxymethyl)-6-oxopiperazine-2-carboxylicacid 2.33



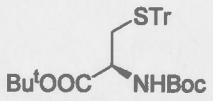
2.33

To a solution of **2.32** (0.04 g, 0.09 mmol) in acetic acid (1 mL) was added HCl (4 mL, 6M). The resulting solution was stirred for 2 hours at room temperature until the TLC showed a ninhydrin charring spot. The solution was evaporated under reduced pressure and the residue was triturated with ethyl acetate (2 x 1 mL) to remove the organic impurities. The residue was then subjected to reverse phase silica column chromatography eluting with water to obtain the title compound.

^{1}H NMR (500 MHz, D_2O): δ 3.65 (dd, J = 7, 13 Hz, 1H), 3.73 (dd, J = 8, 13 Hz, 1H), 3.95 (s, 2H), 4.03 (s, 2H), 4.45 (dd, J = 7, 8 Hz, 1H). **^{13}C NMR** (75 MHz, D_2O): δ 49.4, 50.9, 52.3, 56.6, 164.9, 168.2, 170. **MS (ESI)** (-ve): *m/z* 201.1 ($[M-H]^-$, 100%).

Experimental for chapter 3

tert-Butyl-(*S*)-1-(*tert*-butoxycarbonyl)-2-(tritylthio)ethylcarbamate 3.2

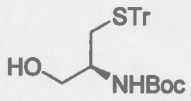


3.2

To a stirred solution of **2.4** (0.85 g, 1.87 mmol) in dioxane (30 mL) was added boc anhydride (0.631 g, 2.89 mmol) and triethylamine (0.80 mL, 5.78 mmol) and the reaction mixture was stirred over night at room temperature. The resulting mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was extracted with ethyl acetate (10 mL) and washed with HCl (2 x 2 mL, 0.5 M) and then with brine (2 x 3 mL). The organic layer was concentrated under reduced pressure and the residue was purified using flash column chromatography eluting with ethyl acetate/hexanes (10% in hexanes) to give the title compound **3.2** (0.92 g, 95%) as a yellowish oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (s, 18 H), 2.43-2.54 (m, 2H) 4.20 (dd, J = 5.1, 7.8 Hz, 1H), 5.1 (d, br, 1H), 7.25-7.42 (m, 15H). **MS (ESI)** (+ve): *m/z* 520.2 ($[M+H]^+$, 30%).

***tert*-Butyl (*S*)-1-hydroxy-3-(tritylthio)propan-2-ylcarbamate 3.3**

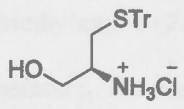


3.3

To a solution of **3.2** (0.27 g, 0.52 mmol) in THF (1 mL) was added LiCl (0.04 g, 1.04 mmol) and $NaBH_4$ (0.04 g, 1.04 mmol) and then ethanol (2 mL). The reaction mixture was stirred over night at room temperature. Water was added to the resulting mixture to quench the excess $NaBH_4$ and then extracted with ethyl acetate. The ethyl acetate layer was flushed through on a flash chromatographic column to eliminate the polar impurities in the reaction mixture. The eluent was collected and concentrated under reduced pressure and the crude residue was purified using flash column chromatography eluting with ethyl acetate/hexanes (1:5) to give the title compound **3.3** (0.07 g, 29%) as an oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (s, 9 H), 2.36-2.45 (m, 2H) 3.45-3.52 (m, 2H), 4.70 (s, br, 1H), 7.20-7.45 (m, 15H). **^{13}C NMR** (75 MHz, $CDCl_3$): δ 28.6, 33.4, 52.0, 64.8, 67.2, 80.1, 127.1, 128.3, 129.8, 144.8, 156.1. **MS (ESI)** (+ve): *m/z* 472.2 ($[M+H]^+$, 20%).

(*S*)-2-Amino-3-(tritylthio)propan-1-ol 3.6



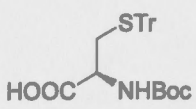
3.6

To a suspension of **2.4** (0.20 g, 0.44 mmol) in water (1.5 mL) and ethanol (2.25 mL) was added $NaBH_4$ (0.17 g, 4.49 mmol) and the mixture was stirred overnight at reflux. The resulting mixture was evaporated under reduced pressure. The residue was extracted with ethylacetate (2 x 10 mL) and washed with water (2 x 10 mL). The combined organics were collected and was concentrated under reduced pressure and the residue was purified using flash column chromatography eluting with methanol/DCM (10% in DCM) to give the title compound **3.6** (0.04 g, 26%) as an oil.

^{1}H NMR (300 MHz, $CDCl_3$): 2.19-2.30 (m, 2H) 2.85-3.40 (m, 3H), 7.27-7.43 (m, 15H).

MS (ESI) (+ve): *m/z* 350.2 ($[M+H]^+$, 10%).

(*S*)-2-*tert*-Butyloxycarbonylamino-3-(tritylthio)propanoic acid 3.8

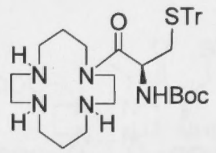


3.8

To a stirred solution of **2.3** (2.75 g, 6.88 mmol) in dioxane (20 mL) was added boc anhydride (2.33 g, 10.7 mmol) and triethylamine (2.96 mL, 21.3 mmol) and the mixture was stirred over night at room temperature. The resulting mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was extracted with ethyl acetate (20 mL) and washed with HCl (2 x 10 mL, 0.5 M) and then with brine (2 x 10 mL). The organic layer was concentrated under reduced pressure and the residue was subjected to flash column chromatography eluting with ethyl acetate/hexanes (3:1) to give the title compound **3.8** (2.60 g, 81%) as a yellowish oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (s, 9 H), 2.52- 2.70 (m, 2H) 4.29 (dd, J = 5.1, 7.8 Hz, 1H), 5.07 (d, J = 7.8 Hz, 1H), 7.28-7.44 (m, 15H), 11.70 (s, br, 1H). **^{13}C NMR** (75 MHz, $CDCl_3$): δ 28.5, 34.0, 52.6, 67.2, 80.6, 127.1, 128.3, 129.7, 144.5, 155.5, 175.6. **MS (ESI)** (+ve): *m/z* 486.1 ($[M+H]^+$, 50%). **HRMS** (ESI) calcd. for $C_{27}H_{29}NO_4S$ $[M+H]^+$ *m/z* 486.1715, found 486.1719.

1-(*tert*-Butyl-(*S*)-2-carbamato-3-oxo-1-(tritylthio)-propanyl)-1,4,8,11-tetraazacycloteradecane 3.10

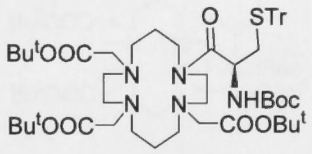


3.10

To a stirred solution of **3.8** (0.77 g, 1.66 mmol) in DCM (6 mL) was added EDC (0.49 g, 1.66 mmol) and HOBt (0.225 g, 1.66 mmol) and the mixture was stirred for 10 minutes at room temperature. Cyclam **3.9** (0.50 g, 2.50 mmol) and triethylamine (0.02 mL, 1.7 mmol) was then added and the mixture was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure and the residue was extracted with ethyl acetate (20 mL) and washed with saturated $NaHCO_3$ solution (2 x 20 mL), water (2 x 20 mL) and then with brine (2 x 20 mL). The organics were combined and concentrated under reduced pressure and the residue was subjected to flash column chromatography eluting with methanol/chloroform/acetic acid (3:6.5:0.5 → 1:2) to give the title compound **3.10** (0.30 g, 28%) as a light yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39 (s, 9 H), 1.6-1.8 (m, 2H), 2.01-2.2 (m, 2H), 2.2- 2.8 (m. 9H), 3.18-3.60 (m, 6H), 4.4- 4.5 (m, 8H), 7.22-7.36 (m, 15H). **^{13}C NMR** (75 MHz, $CDCl_3$): δ 28.5, 35.2, 45.4, 46.9, 47.7, 48.0, 48.7, 49.8, 52.6, 67.0, 79.9, 126.9, 128.1, 129.7, 144.6, 155.2, 171.0. **MS (ESI)** (+ve): *m/z* 646.5 ($[M+H]^+$, 100%). **HRMS (ESI)** calcd. for $C_{37}H_{51}N_5O_3S$ $[M+H]^+$ *m/z* 646.3791, found 646.3790.

1-(*tert*-Butyl-(*S*)-2-carbamato-3-oxo-1-(tritylthio)-propanyl)-1,4,8,11-tetraazacycloteradecane-4,8,11-triacetic acid, Tris-(1,1-dimethylethyl)ester 3.11

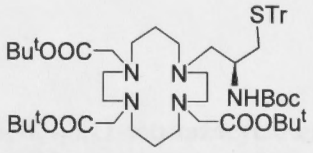


3.11

To a solution of **3.10** (2.20 g, 3.4 mmol) in DMF (50 mL) was added *tert*-butylbromo acetate (3.02 g, 20.5 mmol) and K_2CO_3 (1.88 g, 13.6 mmol) and the mixture was stirred for 5 hours at room temperature. The resulting mixture was extracted with ethylacetate (50 mL) and washed with water (2 x 20 mL). The organics were combined and concentrated under reduced pressure and the residue was subjected to flash column chromatography eluting with 2% MeOH in chloroform to give the title compound **3.11** (2.60 g, 77%) as a yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.38 (s, 36 H), 1.50-1.60 (m, 2H), 2.35-2.88 (m, 14H), 3.10-3.55 (m, 12H), 4.51 (t, J = 6 Hz, 1H), 5.13 (s, br, 1H) 7.24-7.39 (m, 15H). **^{13}C NMR** (75 MHz, $CDCl_3$): δ 25.4, 25.8, 28.2, 28.3, 49.2, 50.5, 52.1, 55.0, 57.0, 66.7, 79.6, 80.8, 126.7, 127.9, 129.6, 144.5, 162.6, 170.9. **MS (ESI)** (+ve): *m/z* 988.8 ($[M+H]^+$, 100%). **HRMS (ESI)** calcd. for $C_{55}H_{81}N_5O_9S$ $[M+H]^+$ *m/z* 988.5833, found 988.5831.

1-(*tert*-Butyl-(*S*)-2-carbamato-1-(tritylthio)-propanyl)-1,4,8,11-tetraazacycloteradecane-4,8,11-triacetic acid, Tris-(1,1-dimethylethyl)ester - 3.12



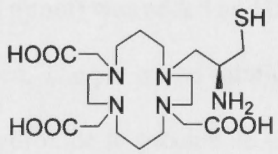
3.12

To a stirred solution of borane-dimethylsulfide complex (2.0 M in dimethylsulfide, 3.29 mL, 6.59 mmol) at 0 °C for 10 minutes was added a solution of **3.11** (1.3 g, 1.3 mmol) in THF (3.88 mL) dropwise. The mixture was refluxed for one hour under a nitrogen atmosphere and treated with sodium hydroxide (1.1 mL, 2 M) and refluxed for another 30 minutes. The resulting mixture was poured into water (4 mL) and extracted with ethyl acetate (3 x 15 mL). The organic extracts were dried on sodium sulfate, filtered and concentrated under reduced pressure. The residue was subjected to flash column chromatography eluting with 5% MeOH in $CHCl_3$ to give the title compound **3.12** (0.62 g, 48%) as a colourless oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.44 (s, 36 H), 1.63 (m, 2H), 1.82 (m, 2H), 2.32-2.38 (m, 8H), 2.54-2.58 (m, 8H), 2.62-2.80 (m, 2H) 3.20-3.70 (m, 10H) 7.18-7.48(m, 15H). **^{13}C NMR** (75 MHz, $CDCl_3$): δ 22.5, 24.2, 28.1, 28.3, 34.2, 49.8, 50.6, 51.0, 51.4, 52.8, 53.0, 55.1, 55.2, 67.5, 79.7, 81.2, 81.4, 126.7, 127.9, 129.6, 144.6, 155.6, 169.8, 170.2,

176.0. **MS (ESI)** (+ve): *m/z* 974.5 ($[M+H]^+$, 100%). **HRMS (ESI)** calcd. for $C_{55}H_{83}N_5O_9S$ $[M+H]^+$ *m/z* 974.6041, found 974.6094.

1-((*S*)-2-Amino-1-thio-propyl)-1,4,8,11-tetraazacycloteradecane-4,8,11-triacetic acid, 3.1



3.1

To a stirred solution of **3.12** (0.39 g, 0.40 mmol) in acetic acid (2 mL) was added hydrochloric acid (12 mL, 6 M) and the mixture was stirred for 30 hours at room temperature. The mixture was concentrated under reduced pressure and the residue was triturated with ethyl acetate (1 mL) several times to remove the organic impurities. The residue was subjected to reverse phase column chromatography eluting with water/ACN (1:1) to give the title compound **3.1** (0.17 g, 66%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.87-1.92 (m, 4 H), 2.54-2.78 (m, 8H), 2.94-2.98 (m, 3H), 3.25-3.42 (m, 10H) 3.65 (s, 2H) 3.90-3.92 (m, 4H). **^{13}C NMR** (75 MHz, D_2O): δ 20.4, 21.4, 24.7, 47.3, 48.4, 50.2, 50.6, 50.8, 51.3, 52.5, 55.2, 55.9, 169.2. **MS (ESI)**

(+ve): *m/z* 464.4 ([M+H]$^+$, 100%). **HRMS (ESI)** calcd. for $C_{19}H_{37}N_5O_9S$ [M+H]$^+$ *m/z* 464.2543, found 464.2543.

Complexation experiment with $YbCl_3.6H_2O$

The ligand **3.1** (0.03 g, 0.046 mmol) was dissolved in deionised water (1 mL). The pH of the solution was adjusted to 8 by the dropwise addition of sodium borohydride and then ytterbium chloride (0.04 g, 0.10 mmol) was added and the reaction mixture was stirred at 80 °C for 30 hours under nitrogen. The pH of the solution was then increased to 9 by the dropwise addition of sodium hydroxide to precipitate out the unreacted ytterbium as its hydroxide. The resulting mixture was filtered and evaporated. The residue was passed through a reverse phase column and the eluent was collected and evaporated.

Complexation experiment with $Yb(OTF)_3$

The ligand **3.1** (0.06 g, 0.09 mmol) was dissolved in deionised water (2 mL). The pH of the solution was adjusted to 7 by the dropwise addition of sodium hydroxide (0.1 M) and then ytterbium triflate (0.12 g, 0.18 mmol) was added and the reaction mixture was stirred at 70 °C for 2 days under nitrogen. The pH of the solution was then increased to 9 by the dropwise addition of sodium hydroxide to precipitate out the unreacted ytterbium as its hydroxide. The resulting mixture was filtered through celite and the filtrate was collected and evaporated.

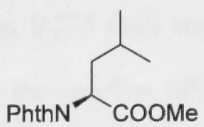
Complexation experiment with Yb_2O_3

The ligand **3.1** (0.05 g, 0.08 mmol) was dissolved in deionised water (3 mL) and was added to ytterbium oxide (0.016 g, 0.040 mmol). The pH of the solution was adjusted to 5 by the dropwise addition of sodium hydroxide and the reaction mixture was stirred for 18 hours at reflux. Then the pH was increased to 6 using sodium hydroxide and the mixture was refluxed for another hour. The resulting mixture was filtered and the filtrate was evaporated under reduced pressure.

MS (ESI) (+ve): *m/z* 634.6 ($[M+H]^+$, 50%), *m/z* 656.4 ($[M+Na]^+$, 100%).

Experimental for chapter 4

(*S*)-*N*-Phthaloylleucine methyl ester 4.3

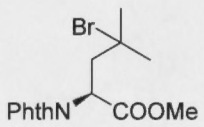


4.3

To a solution of (*S*)-*N*-phthaloylleucine **4.2** (11.5 g, 44.1 mmol) in dry methanol (38 mL) thionylchloride (13.52 mL, 132.2 mmol) was added drop wise at 0 °C. The reaction was allowed to stir at room temperature for 22 hours. The resulting mixture was concentrated under reduced pressure. The residue was subjected to flash column chromatography in hexane/ether (3:7) to give the title compound **4.3** (10.77 g, 89%) as a viscous oil.

^{1}H NMR (300 MHz, $CDCl_3$): 0.91, (d, J = 6.6 Hz, 3H), 0.94 (d, J = 6.6 Hz, 3H), 1.40-1.50 (m, 1H), 1.90-1.97 (m, 1H), 2.31-2.40 (m, 1H) 3.72 (s, 1H), 4.94 (dd, J = 4.5, 11.7 Hz, 1H) 7.75-7.84 (m, 4H). **^{13}C NMR** (75 MHz, $CDCl_3$): 21.0, 23.2, 25.1, 37.3, 50.6, 52.8, 123.6, 131.9, 134.3, 167.8, 170.3. **MS (ESI)** (+ve): *m/z* 276.35 ($[M+H]^+$, 100%). All the ^{1}H NMR signals are consistent with those reported in the literature.[79]

***N*-Phthaloyl-γ-bromoleucine methyl ester 4.4**



4.4

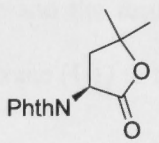
To a solution of *N*-phthaloylleucine methyl ester **4.3** (25.0 g, 91.0 mmol) in CCl_4 was added *N*-bromosuccinimide (48.6 g, 0.273 mol) and the solution was stirred at reflux under nitrogen for 4 hours, with the reaction initiated by irradiation with a 300W ultraviolet lamp. The reaction mixture was cooled and the filtered washing with diethyl ether, and the filtrate was concentrated under reduced pressure and then flushed through a silica gel plug eluting with diethyl ether/n-hexane (1:1) to give the bromide **4.4** (27 g, 84 %).

^{1}H NMR (300 MHz, $CDCl_3$) 1.74 (s, 3H) 1.82 (s, 3H), 2.82-2.85 (m, 2H) 3.72 (s, 3H) 5.22 (dd, J = 4.2, 8.4 Hz, 1H) 7.73-7.89 (m, 4H). **^{13}C NMR** (75 MHz, $CDCl_3$): 34.0

34.9, 44.4, 50.2, 53.3, 63.8, 123.8, 132.0 134.4, 167.8, 169.7. **MS (ESI)** (+ve): *m/z* 354.3 ($[M+H]^+$, 55%). and 356.2 ($[M+H]^+$, 45%). **HRMS (ESI)** calcd. for $C_{15}H_{16}BrNO_4$ $[M+H]^+$ *m/z* 354.0340, found 354.0336. All the NMR data are consistent with those previously reported.[78]

The reaction was also performed in α,α,α-trifluorotoluene at reflux, instead of CCl_4 which resulted in the alkene **4.5** and the lactone **4.6** as byproducts.

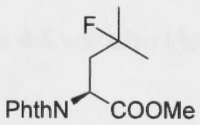
2-((*S*)-Tetrahydro-5,5-dimethyl-2-oxofuran-3-yl)isoindoline-1,3-dione



4.6

^{1}H NMR (300 MHz, $CDCl_3$) 1.51 (s, 3H), 1.62 (s, 3H), 2.43 (dd, J = 11.7, 12.3 Hz, 1H), 2.58 (dd, J = 9.9, 12.3 Hz, 1H), 4.22 (dd, J = 9.9, 11.7 Hz, 1H), 7.73-7.87 (m, 4H). **MS (ESI)** (+ve): *m/z* 260.1 ($[M+H]^+$, 100%). and 282.1 ($[M+H]^+$, 60%).

***N*-Phthaloyl-γ-fluoroleucine methyl ester 4.7**

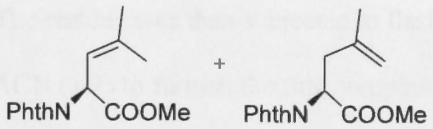


4.7

To a solution of *N*-phthaloyl-γ-bromoleucine methyl ester **4.4** (15.0 g, 42.4 mmol) in dry acetonitrile (278 mL) was added silver fluoride (53.75 g, 423.7 mmol). The mixture was stirred at room temperature in the dark for 18 hours. The insoluble silver salts were filtered off on a celite plug using ethyl acetate. The filtrate was collected and concentrated under reduced pressure and the resulting residue was subjected to flash chromatography in diethyl ether/*n*-hexane (1:1) to furnish the desired compound **4.7** (4.3 g, 35%) as a colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$): 1.35 (d, J = 21.6 Hz, 3H), 1.43 (d, J = 21.3 Hz, 3H), 2.39-2.57 (m, 1H), 2.66-2.74 (m, 1H), 3.73, (s, 3H) 5.15 (dd, J = 2.7, 11.1 Hz, 1H) 7.72-7.79 (m, 4H). **^{13}C NMR** (75 MHz, $CDCl_3$): 25.5 (d, J = 24.6), 28.0 (d, J = 92.4), 38.6 (d, J = 21.2), 48.3 (d, 1.74 Hz), 53.1, 94.6 (d, J = 161.7), 123.6, 131.9, 134.2, 167.6, 169.8. **MS (ESI)** (+ve): *m/z* 294.0 ($[M+H]^+$, 100%). **HRMS (ESI)** calcd. for $C_{15}H_{16}FNO_4$ $[M+H]^+$ *m/z* 294.1142, found 294.1144.

The reaction also resulted in isomeric alkenes, the β,γ- dehydroleucine derivative **4.5** and γ,δ- dehydroleucine derivative **4.8** which were isolated as an inseparable mixture in 49% yield. A small amount of the lactone **4.6** was also identified in the NMR spectrum.

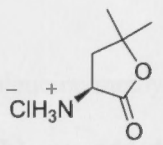


4.5 4.8

β,γ-dehydroleucine derivative **4.5**: **^{1}H NMR** (300 MHz, $CDCl_3$) 1.70 (s, br, 3H), 1.72 (s, br, 3H), 3.76 (s, 3H) 5.64 (d, J = 9.0 Hz, 1H) 5.82 (d, br, J = 9.2 Hz, 1H), 7.72-7.79 (m, 4H).

γ,δ-dehydroleucine derivative **4.8**: **^{1}H NMR** (300 MHz, $CDCl_3$) 1.78 (s, br, 3H), 2.84 (dd, br, J = 4.2, 14.1 Hz, 1H), 3.09 (dd, br, J = 12.1, 14.1 Hz, 1H), 3.76 (s, 3H) 4.64 (s, br, 1H) 4.68 (s, br, 1H) 5.08 (dd, J = 4.2, 12.1 Hz, 1H) 7.72-7.79 (m, 4H). **MS (ESI)** (+ve): *m/z* 274.0 ($[M+H]^+$, 100%).

(*S*)-3-Amino-dihydro-5,5-dimethylfuran-2(3H)-one 4.9

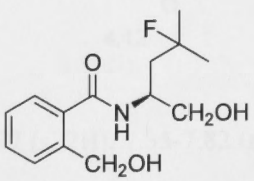


4.9

To a solution of *N*-phthaloyl-γ-fluoroleucine methyl ester **4.7** (0.06 g, 0.20 mmol) in acetic acid (0.75 mL) was added HCl (5 mL, 6 N) dropwise. The resulting mixture was stirred at room temperature for 2 hours. The pH of the resulting solution was adjusted to 7 by the dropwise addition of sodium hydroxide (1 M) and the solution was evaporated under reduced pressure. The residue was then subjected to flash column chromatography eluting with *n*-propanol/ACN (1:2) to furnish the title compound **4.9** (0.019 g, 60%) as a white solid.

^{1}H NMR (300 MHz, D_2O) 1.32 (s, 3H), 1.40 (s, 3H), 2.09 (dd, br J = 8.7, 12.3 Hz, 1H), 2.60 (dd, br, J = 9.0, 12.3 Hz, 1H), 4.49 (dd, J = 8.7, 9.0 Hz, 1H). **MS (ESI)** (+ve): *m/z* 130.0 ($[M+H]^+$, 100%).

_N_-((_S_)-4-Fluoro-1-hydroxy-4-methylpentan-2-yl)-2-(hydroxymethyl)benzamide 4.11



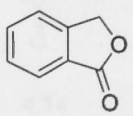
4.11

To a solution of *N*-phthaloyl-γ-fluoroleucine methyl ester **4.7** (0.10 g, 0.34 mmol) in iso-propanol:water mixture (7:3, 8.5 mL) was added sodium borohydride (0.065 g, 1.71 mmol) portion wise and the reaction stirred under nitrogen maintaining the temperature at 20 °C until the TLC showed complete consumption of the starting material. Acetic

acid (0.85 mL) was then added to the reaction mixture and was stirred at 80 °C for 2 hours. The resulting solution was evaporated under reduced pressure and the residue was extracted with ethyl acetate and washed with dilute HCl (2 x 2 mL) and then with brine (2 x 2 mL). The ethyl acetate layer was collected and was dried over sodium sulfate and evaporated under reduced pressure to give the compounds **4.11** and **4.12** as a mixture. Evaporation of the aqueous phase gave the compound **4.10**.

^{1}H NMR (300 MHz, $CDCl_3$) 1.42 (d, J = 21.3 Hz, 3H), 1.43 (d, J = 21.0 Hz, 3H), 1.93 (m, 2H), 3.58 (m, 2H) 4.35 (m, 1H), 4.70 (s, 2H), 7.35-7.82 (m, 4H). **MS (ESI)** (+ve): *m/z* 270.1 ($[M+H]^+$, 100%).

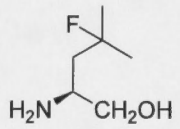
Isobenzofuran-1(3H)-one 4.12



4.12

^{1}H NMR (300 MHz, $CDCl_3$): 5.37 (s, 2H), 7.35-7.82 (m, 4H). **MS (ESI)** (+ve): *m/z* 135 ($[M+H]^+$, 100%). The ^{1}H NMR data is consistent with that reported in literature.[100]

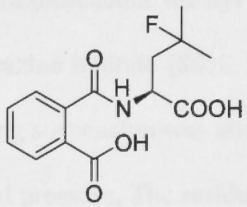
(*S*)-2-Amino-4-fluoro-4-methylpentan-1-ol 4.10.



4.10

^{1}H NMR (300 MHz, CD_3OD) 1.45 (d, J = 21.6 Hz, 3H), 1.49 (d, J = 21.3 Hz, 3H), 1.94 (m, 2H), 3.81 (dd, br, J = 2.7, 11.1 Hz, 2H). **MS (ESI)** (+ve): *m/z* 136.1 ($[M+H]^+$, 60%), 158.2 ($[M+Na]^+$, 80%).

2-((*S*)-1-Carboxy-3-fluoro-3-methylbutylcarbamoyl)benzoic acid 4.14

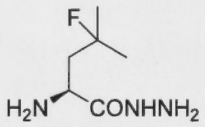


4.14

To a solution of *N*-phthaloyl-γ-fluoroleucine methyl ester **4.7** (0.10 g, 0.34 mmol) in a mixture of dissolved in methanol: water (2:1, 0.4 mL) was added lithium hydroxide (0.016 g, 0.38 mmol) and the resulting mixture was stirred at room temperature overnight. The reaction mixture was neutralized with HCl (1M) and the resulting solution was evaporated under reduced pressure. The residue was then subjected to flash chromatography in *n*-propanol/water (3:7) to give the title compound **4.14** (60 mg, 57%).

^{1}H NMR (300 MHz, CD_3OD) 1.45 (d, J = 21.6 Hz, 3H), 1.46 (d, J = 21.3 Hz, 3H), 2.22 (m, 2H), 4.61 (dd, J = 3.3, 9.6 Hz, 1H) 7.38-7.65 (m, 4H). **MS (ESI)** (+ve): *m/z* 298.0 ($[M+H]^+$, 70%), *m/z* 304.0 ($[M+Li]^+$, 30%).

(*S*)-γ-Fluoroleucine hydrazide 4.16



4.16

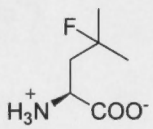
To a solution of *N*-phthaloyl-γ-fluoroleucine methyl ester **4.7** (2.0 g, 6.8 mmol) in ethanol (25 mL) was added hydrazine hydrate (80%, 2.94 g, 91.8mmol) dropwise and refluxed for one hour. The resulting suspension was allowed to cool to room temperature and was evaporated under reduced pressure. The residue was dissolved in dry ethanol (2 mL) and centrifuged. The supernatant was collected and evaporated. The residue was redissolved in ethanol (2 mL) and the processes of centrifugation, evaporation and redissolving was repeated at least 5 times to remove the insoluble phthalimide. The supernatant was then evaporated under reduced pressure and the residue was subjected to reverse phase HPLC to give the title compound **4.16** (0.9 g, 81%) as a white solid.

Mp.78-80 °C. **^{1}H NMR** (300 MHz, D_2O): 1.46 (d, J = 23.1, 3H), 1.48 (d, J = 22.2, 3H), 2.24-2.34 (m, 2H), 4.40 (dd, J = 7.2, 13.2 Hz, 1H). **^{13}C NMR** (75 MHz, D_2O): 27.3 (d, J

= 23.48 Hz), 29.5 (d, J = 23.48 Hz), 43.6 (d, J = 15.86 Hz), 51.7, 99.2 (d, J = 162.1 Hz) 171.02. **MS (ESI)** (+ve): *m/z* 164.1 ($[M+H]^+$, 100%). **HRMS (ESI)** calcd. for $C_6H_{14}FN_3O$ $[M+H]^+$ *m/z* 164.1201, found 164.1190.

HPLC: t_R 13.6 min (column: Alltech Alltima C18 5u, 250 x 22 mm; (gradient conditions: 100:0 to 0:100 over 25 minutes, (water 0.1% TFA: acetonitrile) flow rate:12.5 mL min^{-1}).

(*S*)-γ-Fluoroleucine 4.1



4.1

To a vigorously stirring solution of *N*-bromosuccinimide (1.94 g, 10.9 mmol) in water (2 mL), was added a concentrated solution of the hydrazide **4.16** (0.89 g, 5.5 mmol) in water (3 mL), dropwise and stirred at room temperature for 30 minutes. The resulting solution was concentrated under reduced pressure and dried under vacuum to give a quantitative yield of the amino acid **4.1** as its hydrogen bromide salt. The residue was then dissolved in dry ethanol (14 mL) and propylene oxide (1.8 mL) was added and refrigerated for 24 hours. The resultant precipitate was filtered to give the title compound **4.1** (0.3 g, 45 %) as colorless granules.

Mp. 200-202°C; **^{1}H NMR** (300 MHz, D_2O) 1.30 (d, J = 22.8 Hz, 3H), 1.32 (d, J = 22.5 Hz, 3H), 2.02-2.16 (m, 2H), 3.81 (dd, J = 3, 9.3 Hz, 1H). **^{13}C NMR** (75 MHz, D_2O): 27.0 (d, J = 24.08 Hz), 30.2 (d, J = 23.48 Hz), 43.7 (d, J = 20.61 Hz), 54.6, 100.4 (d, J = 160.44 Hz), 177.3. **MS (ESI)** (+ve): *m/z* 150.0 ($[M+H]^+$, 100%). **HRMS (ESI)** calcd. for $C_6H_{12}FNO_2$ $[M+H]^+$ *m/z* 150.0930, found 150.0932. $[\alpha]D$ = -19.2° (c = 0.0399 in MeOH)

An HPLC method was also developed to purify the amino acid instead of precipitating as the zwitterion.

HPLC: t_R 6.6 min (column: Alltech Alltima C18 5u, 250 x 4.6 mm; 95:5, (water 0.1% TFA: acetonitrile) flow rate: 1 mL min^{-1}).

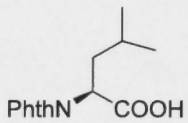
Experimental for chapter 5

The chiral HPLC method to separate the enantiomers of fluoroleucine **4.1** was done using a Daicel crownpak CR(+) chiral column (150 x 4.0 mm). The assay consisted of a flow of 0.2 mL min^{-1} of the mobile phase, pH = 2 perchloric acid solution. The detection was done at 200 nm using a photo diode array detector. (*R*)-γ-fluoroleucine (*R*)-**4.1** eleuted at t_R = 9.17 minutes and (*S*)-γ-fluoroleucine (*S*)-**4.1** eleuted at t_R = 10.89 minutes.

The chiral HPLC method to separate the enantiomers of racemic *N*-phthaloylleucine involved a Daicel chiralpak OD-RH column (150 x 4.6 mm). The mobile phase consisted of acetonitrile and 0.1% TFA in water at a ratio of 75:25 at a flow rate of 0.5

mL min^{-1}. The compound had a λ_{max} of 254 nm and the detection was done using a photo diode array detector. The *R* and *S* configurations of *N*-phthaloylleucine **4.2** was separated at 48.97 and 52.5 minutes respectively.

N-Phthaloylleucine 4.2



4.2

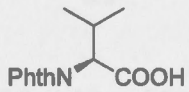
To a solution of (*S*)-leucine **4** (9.00 g, 68.6 mmol) in toluene (527 mL) was added phthalic anhydride (11.2g, 75.5 mmol) and then triethylamine (9.56 mL, 68.6 mmol) drop wise at room temperature. The resulting mixture was stirred at 112 °C for 4 hours in a round bottomed flask fitted with a Dean stark apparatus. The resulting solution was concentrated under reduced pressure. The resulting residue was dissolved in DCM. This was then washed with dil. HCl (4 x 2 mL) and then with water (4 x 2 mL) and the organic portion was filtered and dried over sodium sulfate. This was then filtered and concentrated under reduced pressure. The residue was subjected to flash column chromatography eluting with hexanes/diethyl ether (3:7) to give the title compound **4.2** (17 g, 95%) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$): 0.91, (d, *J* = 6.3 Hz, 3H), 0.93 (d, *J* = 6.6 Hz, 3H), 1.42-1.56 (m, 1H), 1.86-1.98 (m, 1H), 2.30-2.42 (m, 1H) 5.0 (dd, *J* = 4.3, 11.1 Hz, 1H), 7.70-

7.85 (m, 4H), 11.4 (s, br, 1H). **^{13}C NMR** (75 MHz, $CDCl_3$): 21.1, 23.2, 25.2, 37.1, 50.5, 122.9, 131.8, 134.4, 167.8, 176.1. **MS (ESI)** (+ve): *m/z* 262.2 ($[M+H]^+$, 60%). The NMR data is consistent with that reported in literature.[79]

Experimental for chapter 6

N-Phthaloylvaline 6.2



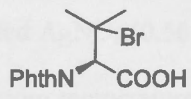
6.2

To a solution of (*S*)-valine **6.1** (5.00 g, 42.7 mmol) in toluene (328 mL) was added triethylamine (0.003 g, 3.31 mmol) and phthalic anhydride (6.96 g, 47.0 mmol) and the resulting solution was stirred under reflux using a Dean Stark apparatus for 4 hours. The solution was concentrated under reduced pressure and the resulting residue was redissolved in DCM (12 mL), washed with dilute HCl (2 x 4 mL) and brine (2 x 3 mL) and was concentrated to dryness *in vacuo* to give the title compound **6.2** (9.5 g, 90%). It was then recrystallised from a mixture of DCM and hexane.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.91 (d, J = 6.6, 3H), 1.16 (d, J = 6.6, 3H), 2.71-2.79 (m, 1H), 4.62 (d, J = 6.8 Hz, 1H) 7.73-7.88 (m, 4H). **^{13}C NMR** ($CDCl_3$, 75 MHz): δ 19.6,

21.1, 28.5, 57.6, 123.8, 131.7, 134.4, 167.9, 174.9. **HRMS**: $[M+H]^+$ (FTICR) calcd for $C_{13}H_{13}NO_4$, *m/z* 248.0917, found, 248.0941. All the ^1H NMR signals are consistent with those reported previously.[116]

N-Phthaloyl-β-bromovaline 6.3

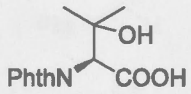


6.3

To a solution of *N*-phthaloylvaline **6.2** (1.00 g, 4.05 mmol) in CCl_4 (81 mL), was added NBS (2.16 g, 12.2 mmol) and the resulting solution was stirred under reflux for 4 hours while photo irradiated with a 300W mercury lamp. The resulting mixture was then cooled and filtered and the filtrate was collected and evaporated under reduced pressure. The residue was then purified by flash column chromatography eluting with DCM/ 2% acetic acid in MeOH to give the title compound **6.3** (0.94 g, 71%) as a yellow oil.

^1H NMR ($CDCl_3$, 300 MHz): δ 1.98 (s, 3H), 2.13 (s, 3H), 5.22 (s, 1H), 7.76-7.92 (m, 4H). **^{13}C NMR** ($CDCl_3$, 75 MHz): δ 28.5, 32.0, 32.9, 60.4, 61.0, 63.9, 124.1, 134.7, 131.6, 167.4, 170.2. **MS (ESI)** (+ve): *m/z* 326.2 ($[M+H]^+$, 52%), **MS (ESI)** (+ve): *m/z* 328.2 ($[M+H]^+$, 48%). The NMR data obtained is consistent with that reported in literature.[116]

***N*-Phthaloyl-β-hydroxyvaline 6.5**



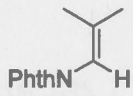
6.5

To a solution of *N*-phthaloyl-γ-bromovaline **6.3** (0.60 g, 1.8 mmol) in a 1:1 mixture of acetone and water (26 mL), was added $AgNO_3$ (0.56 g, 3.31 mmol) and the solution was stirred in the dark for 24 hours at room temperature. The reaction mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was then purified using HPLC to give the title compound **6.5** (0.06 g, 12%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.32 (s, 3H), 1.58 (s, 3H), 4.94 (s, 1H), 7.74-7.91 (m, 4H). **^{13}C NMR** ($CDCl_3$, 75 MHz): δ 27.9, 28.1, 29.8, 54.7, 123.8, 132.7, 134.5, 168.4, 170.0. **HRMS**: $[M]^-$ (FTICR) calcd for $C_{13}H_{13}NO_5$, *m/z* 262.0715, found, 262.0698.

HPLC: t_R 12.7 min (column: Alltech Alltima C18 5u, 250 x 22 mm; (gradient conditions: 90:10 to 0:100 over 20 minutes, (water 0.1% TFA: acetonitrile) flow rate: 15 mL min^{-1}).

The reaction also yielded ***N*-phthalimido methyl propene 6.4.**

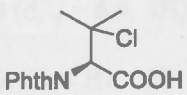


6.4

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.64 (d, J = 1.2, 3H), 1.91 (d, J = 1.5, 3H), 5.88 (t, J = 1.5, 1H), 7.74-7.91 (m, 4H). **^{13}C NMR** ($CDCl_3$, 75 MHz): δ 19.0, 22.9, 112.2, 123.5, 134.2, 139.2, 167.5. The ^{1}H NMR data is consistent with that reported in literature.[117]

HPLC: t_R 18.3 min (column: Alltech Alltima C18 5u, 250 x 22 mm; (gradient conditions: 90:10 to 0:100 over 20 minutes, (water 0.1% TFA: acetonitrile) flow rate: 15 mL min^{-1}).

***N*-Phthaloyl-β-chlorovaline 6.6**



6.6

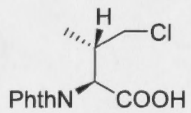
To a solution of *N*-phthaloylvaline **6.2** (0.50 g, 2.02 mmol) in TFA (10 mL), chlorine gas was bubbled under moderate pressure for 3 min while irradiated with a 300W mercury lamp. The solution was then bubbled with nitrogen gas to remove the excess chlorine gas and evaporated to dryness under reduced pressure. The residue was then subjected to

HPLC to obtain the isomeric chlorides **6.6** (0.096 g, 17%), **6.7a** (0.112 g, 20%), and **6.7b** (0.108 g, 19%)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.79 (s, 3H), 1.92 (s, 3H), 5.13 (s, 1H), 7.76-7.91 (m, 4H). **^{13}C NMR** ($CDCl_3$, 75 MHz): δ 30.1, 31.4, 59.8, 69.6, 124.1, 131.5, 134.7, 167.6, 170.6. **MS (ESI)** (+ve): *m/z* 304.0 ($[M+H]^+$, 60%).

HPLC: t_R 27.3 min (column: Alltech Alltima C18 5u, 250 x 22 mm; 60:40, (0.1% TFA: acetonitrile) flow rate 12 mL min^{-1}).

The reaction also yielded the diastereomers ***N*-phthaloyl-*γ*-chlorovaline 6.7a**

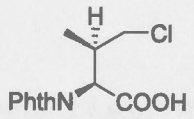


6.7a

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.09 (d, J = 6.9, 3H), 3.03 (m, J = 3.9, 1H), 3.37 (dd, J = 6.6, 11.1 Hz, 1H), 3.70 (dd, J = 3.9, 11.4 Hz, 1H), 4.93 (d, J = 7.8, 1H) 7.76-7.91 (m, 4H). **^{13}C NMR** ($CDCl_3$, 75 MHz): δ 16.2, 35.7, 48.0, 54.4, 123.9, 131.6, 134.6, 167.8, 173.5.

HPLC: t_R 24.1 min (column: Alltech Alltima C18 5u, 250 x 22 mm; 60:40, (0.1% TFA: acetonitrile) flow rate: 12 mL min^{-1}).

***N*-Phthaloyl-γ-chlorovaline 6.7b**

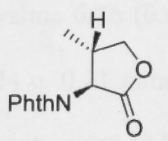


6.7b

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.02 (d, J = 6.9, 3H), 3.04 (m, J = 3.3, 1H), 3.71 (dd, J = 3.9, 11.1 Hz, 1H), 3.96 (dd, J = 4.8, 11.6 Hz, 1H), 5.07 (d, J = 8.0, 1H) 7.76-7.91 (m, 4H). **^{13}C NMR** ($CDCl_3$, 75 MHz): δ 15.3, 35.3, 48.8, 52.4, 123.9, 131.6, 134.6, 167.7, 173.8.

HPLC: t_R 25.3 min (column: Alltech Alltima C18 5u, 250 x 22 mm; 60:40, (0.1% TFA: acetonitrile) flow rate: 12 mL min^{-1}).

2-((3*S*,4*S*)-tetrahydro-4-methyl-2-oxofuran-3-yl)isoindoline-1,3-dione 6.8a



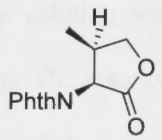
6.8a

To solution of *N*-phthaloyl-γ-chlorovaline **6.7a** (0.02 g, 0.07 mmol) in dry acetonitrile (1 mL) was added AgF (0.09 g, 0.71 mmol) and the reaction was stirred in the dark at room temperature for 18 hours. The resulting reaction mixture was filtered to get rid of the

insoluble silver salts and the filtrate was evaporated under reduced pressure to give the lactone **6.8a** (0.015 g, 85%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.19 (d, J = 6.6, 3H), 3.16 (m, J = 3.3, 1H), 3.95 (t, J = 9.9, 1H), 4.65 (t, J = 8.7, 1H), 4.66 (d, J = 11.4, 1H) 7.76-7.91 (m, 4H). **^{13}C NMR** ($CDCl_3$, 75 MHz): δ 14.9, 34.8, 54.6, 123.9, 131.7, 134.6, 167.1, 171.9. **HRMS**: $[M+H]^+$ (FTICR) calculated for $C_{13}H_{11}NO_4$, *m/z* 246.0766, found, 246.0767.

(3*S*,4*R*)-dihydro-3-(isoindolin-2-yl)-4-methylfuran-2(3H)-one 6.8b

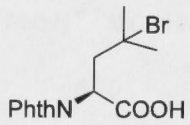


6.8b

To solution of *N*-phthaloyl-γ-chlorovaline **6.7b** (0.04 g, 0.14 mmol) in acetone (2 mL) was added a solution of $AgNO_3$ (0.04 g, 0.21 mmol) in water (2 mL) and the reaction was stirred in the dark at room temperature for 48 hours. The resulting reaction mixture was filtered to get rid of the insoluble silver salts and the filtrate was evaporated under reduced pressure to give the lactone **6.8b** (0.03 g, 87%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.99 (d, J = 7.2 Hz, 3H), 2.96 (m, J = 3.3 Hz, 1H), 4.23 (t, J = 7.8 Hz, 1H), 4.60 (t, J = 8.1 Hz, 1H), 4.96 (d, J = 10.5 Hz, 1H) 7.75-7.90 (m, 4H). **MS (ESI)** (+ve): *m/z* 246, ($[M+H]^+$, 100%).

_N_-Phthaloyl-γ-bromoleucine 6.9



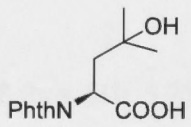
6.9

To a solution of *N*-phthaloylleucine **4.2** (1.5 g, 5.8 mmol) in CCl_4 (115 mL), NBS was added (3.06 g, 17.2 mmol) and the solution was refluxed for 4 hours while photo irradiated with a 300W mercury lamp. The reaction mixture was then cooled under ice, filtered and the filtrate was concentrated under reduced pressure. The residue was subjected to flash column chromatography in DCM/2% acetic acid in ACN to give the title compound **6.9** (1.36 g, 70%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.74 (s, 3H), δ 1.83 (s, 3H), 2.75-2.89 (m, 2H), 5.29 (dd, J = 9.3, 12.3 Hz, 1H), 7.73-7.89 (m, 4H). **^{13}C NMR** ($CDCl_3$, 75 MHz): δ 33.8, 34.7, 44.2, 50.0, 63.7, 123.7, 131.7, 134.4, 167.7, 173.7. **MS (ESI)** (+ve): *m/z* 361.9, 363.9 ($[M+Na]^+$, 60%). **HRMS**: $[M+H]^+$ (FTICR) calcd for $C_{14}H_{15}NO_4{}^{79}Br$ *m/z* 340.0184,

found, 340.0179, calcd for $C_{14}H_{15}NO_4{}^{81}Br$ *m/z* 342.0164, found 342.0159. The NMR data obtained is consistent with that in literature.[116]

_N_-Phthaloyl-γ-hydroxyleucine 6.10



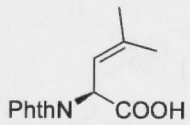
6.10

To a solution of *N*-phthaloyl-γ-bromoleucine **6.9** (0.30 g, 0.88 mmol) in acetone (6 mL), a solution of $AgNO_3$ (0.30 g, 1.76 mmol) in water (6 mL) was added and the solution was stirred in the dark for 24 hours at room temperature. The reaction mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was then purified by HPLC to give the title compound **6.10** (0.72 g, 30%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.25 (br s, 3H), 1.28 (br s, 3H), 2.08-2.18 (m, 1H), 2.54-2.63 (m, 1H), 5.02-5.2 (m, 1H), 7.68-7.83 (m, 4H). **MS (ESI)** (-ve): *m/z* 276.0, ($[M-H]^-$, 60%). **HRMS**: $[M-H]^-$ (FTICR) calcd for $C_{14}H_{14}NO_5$ *m/z* 276.0872, found, 276.0872.

HPLC: t_R 25.3 min (column: Alltech Alltima C18 5u, 250 x 22 mm; 60:40, (0.1% TFA: acetonitrile) flow rate: 15 mL min^{-1}).

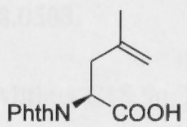
The reaction also yielded ***N*-phthaloyl-β,γ-dehydroleucine 6.11.**



6.11

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.78 (br s, 6H), 5.70 (d, J = 9.3 Hz, 1H), 5.81 (d, J = 9.0 Hz,1H), 7.71-7.87 (m, 4H). **MS (ESI)** (+ve): *m/z* 260.0, ($[M+H]^+$, 60%), *m/z* 282.0, ($[M+Na]^+$, 30%).

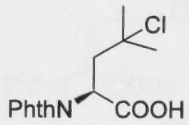
The reaction also yielded ***N*-phthaloyl-γ,δ-dehydroleucine 6.12.**



6.12

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.74 (br s, 3H), 2.82 (dd, J = 4.2, 14.1 Hz, 1H), 3.12 (dd, J = 2.1, 14.1 Hz, 1H), 4.65 (s, 1H), 4.68(s, 1H), 5.14 (dd, J = 2.1, 4.2 Hz, 1H), 7.70-7.85 (m, 4H). **^{13}C NMR** ($CDCl_3$, 75 MHz): δ 29.8, 36.8, 50.2, 114.7, 123.7, 131.8, 134.3, 140.6, 167.6, 175.0.

***N*-Phthaloyl-γ-chloroleucine 6.13**



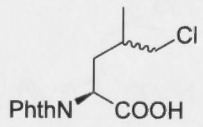
6.13

Procedure is same as given in the synthesis of the chloride **6.6**. The product mixture was purified using HPLC to give the chlorides **6.13** (0.22 g, 38%), **6.14** (0.17 g, 30%), the dichloride **6.15** (0.06 g, 9%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.55 (s, 3H), 1.64 (s, 3H), 2.65-2.85 (m, 2H), 5.27 (dd, *J* = 2.4, 9.3 Hz, 1H), 7.71-7.87 (m, 4H). **^{13}C NMR** ($CDCl_3$, 75 MHz): δ 32.2, 33.4, 43.0, 49.4, 68.4, 124.0, 132.0, 134.6, 167.9, 175.2. **HRMS**: $[M+Na]^+$ (FTICR) calcd for $C_{14}H_{14}NO_4Cl$ *m/z* 318.0509, found, 318.0503.

HPLC: t_R 28.5 min (column: Alltech Alltima C18 5u, 250 x 22 mm; 60:40, (0.1% TFA: acetonitrile) flow rate: 15 mL min^{-1}).

The above reaction also yielded the diastereomers of ***N*-phthaloyl-δ-chloroleucine 6.14**



6.14

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.09 (d, J = 6.3 Hz, 3H), 1.69-1.76 (m, 1H), 2.01-2.11 (m, 1H), 2.53-2.63 (m, 1H), 3.42 (m, 2H), 5.00 (dd, J = 4.2, 11.7 Hz, 1H), 7.78-7.95 (m, 4H). **^{13}C NMR** ($CDCl_3$, 75 MHz): δ 21.1, 23.3, 25.2, 32.8, 50.9, 123.8, 131.7, 134.5, 167.8, 174.3.

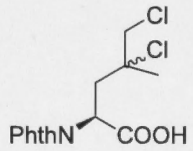
HPLC: t_R 30.5 min (column: Alltech Alltima C18 5u, 250 x 22 mm; 60:40, (0.1% TFA: acetonitrile) flow rate: 15 mL min^{-1}).

The other diastereomer of ***N*-phthaloyl-δ-chloroleucine 6.14**

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.05 (d, J = 6.6 Hz, 3H,), 1.45-1.51 (m, 1H), 1.88-1.98 (m, 1H), 2.30-2.41 (m, 1H), 3.48-3.61 (m, 2H), 4.92-5.00 (m, 1H), 7.73-7.87 (m, 4H).

HPLC: t_R 32.5 min (column: Alltech Alltima C18 5u, 250 x 22 mm; 60:40, (0.1% TFA: acetonitrile) flow rate: 15 mL min^{-1}).

The above reaction also yielded ***N*-phthaloyl-γ,δ-dichloroleucine 6.15**



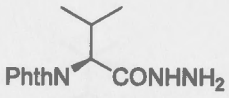
6.15

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.72 (s, 3H), 2.90 (m, 2H), 3.61 (d, J = 11.4 Hz, 1H), 3.67 (d, J = 11.4 Hz, 1H), 5.22 (t, J = 6.3 Hz, 1H), 7.73-7.88 (m, 4H). **^{13}C NMR** ($CDCl_3$, 75 MHz): δ 28.4, 38.5, 48.7, 52.4, 69.6, 124.1, 132.0, 134.7, 167.9, 174.5. **MS (ESI)** (+ve): *m/z* 352.0, 354.0 ($[M+Na]^+$, 40%). **HRMS**: $[M-H]^-$ (FTICR) calcd for $C_{14}H_{12}NO_4{}^{35}Cl_2$ *m/z* 328.0143, found, 328.0143. Calcd for $C_{14}H_{12}NO_4{}^{35}Cl{}^{37}Cl$ *m/z* 330.0114 found 330.0124

HPLC: t_R 38.0 min (column: Alltech Alltima C18 5u, 250 x 22 mm; 60:40, (0.1% TFA: acetonitrile) flow rate: 15 mL min^{-1}).

Experimental for chapter 7

N-Phthaloylvaline hydrazide 7.1



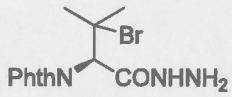
7.1

To a solution of *N*-phthaloylvaline **6.2** (0.6 g, 2.4 mmol) in DCM (20 mL) thionyl chloride (6.00 mL, 0.08 mmol) was added slowly and the mixture was stirred for 5 hours at room temperature. The reaction mixture was evaporated under reduced pressure, and dried *in vacuo*. The residue was diluted with dry acetonitrile (300 mL) and the resulting solution was slowly added through a dropping funnel over a few hours to a solution of 1 M hydrazine in THF (24.3 mL, 24.29 mmol) in dry acetonitrile (20 mL). The reaction mixture was then purified by reverse phase HPLC to give the title compound **7.1** (0.44 g, 70%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.82 (d, J = 6.6 Hz, 3H), δ 1.03 (d, J = 7.2 Hz, 3H) 2.80 (m, J = 4.5 Hz, 1H), 4.51 (d, J = 10.5 Hz, 1H), 7.80 (m, 4H). **^{13}C NMR** ($CDCl_3$, 75 MHz): δ 19.1, 19.7, 27.3, 58.8, 123.9, 131.1, 134.7, 168.3, 170.5. **MS (ESI)** (+ve): *m/z* 262.2, ($[M+H]^+$, 60%), *m/z* 284.2, ($[M+Na]^+$, 30%). **HRMS**: $[M+H]^+$ (FTICR) calcd for $C_{13}H_{15}N_3O_3$, *m/z* 262.1192, found, 262.1185.

HPLC: t_R 6 min (column: Alltech Alltima C18 5u, 250 x 22 mm; 60:40, (0.1% TFA: acetonitrile) flow rate: 15 mL min^{-1}).

***N*-Phthaloyl-β-bromovaline hydrazide 7.2**



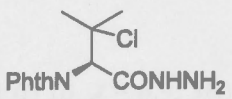
7.2

Procedure is as given in the synthesis of compound **6.3**. The reaction resulted in 10% yield of the title compound **7.2**.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.99 (s, 3H), 2.14 (s, 3H) 5.22 (s, 1H), 7.73-7.89 (m, 4H).

HPLC: t_R 28 min (column: Alltech Alltima C18 5u, 250 x 22 mm; 60:40, (0.1% TFA: acetonitrile) flow rate: 15 mL min^{-1}).

***N*-Phthaloyl-β-chlorovaline hydrazide 7.3**



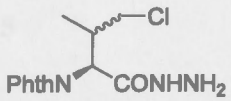
7.3

To a solution of *N*-phthaloylvaline hydrazide **7.1** (0.15 g, 0.57 mmol) in TFA (4 mL), chlorine gas was bubbled under moderate pressure for 4 minutes while irradiated with a 300W mercury lamp. The solution was then passed with nitrogen gas to remove the excess of chlorine gas and then evaporated to dryness under reduced pressure. The residue was then purified by reverse phase HPLC to obtain the title compound **7.3** (0.03 g, 18%) and the two diastereomers of the corresponding γ derivative **7.4** (0.07 g, 42%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.80 (s, 3H), 1.94 (s, 3H), 5.13 (s, 1H), 7.76-7.91 (m, 4H). **^{13}C NMR** ($CDCl_3$, 75.45 MHz): δ 30.1, 31.5, 59.9, 69.6, 124.1, 131.6, 134.7, 167.5, 170.3.

HPLC: t_R 31.2 min (column: Alltech Alltima C18 5u, 250 x 22 mm; 60:40, (0.1% TFA: acetonitrile) flow rate: 15 mL min^{-1}).

***N*-Phthaloyl-γ-chlorovaline hydrazide 7.4**



7.4

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.03 (d, J = 6.9, 3H), 3.01-3.09 (m, 1H), 3.72 (dd, J = 3.9, 11.1 Hz, 1H), 3.97 (dd, J = 4.8, 11.1 Hz, 1H), 5.08 (d, J = 8.4, 1H) 7.75-7.91 (m,

4H). **^{13}C NMR** ($CDCl_3$, 75 MHz): δ 15.3, 35.4, 48.8, 52.4, 124.0, 131.6, 134.6, 167.7, 173.8.

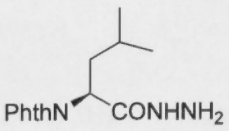
HPLC: t_R 27.6 min (column: Alltech Alltima C18 5u, 250 x 22 mm; 60:40, (0.1% TFA: acetonitrile) flow rate: 15 mL min^{-1}).

The other diastereomer

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.30 (d, J = 6.9, 3H), 3.00-3.08 (m, 1H), 3.38 (dd, J = 6.3, 11.4 Hz, 1H), 3.71 (dd, J = 3.9, 11.1 Hz, 1H), 4.94 (d, J = 7.8 Hz, 1H) 7.75-7.91 (m, 4H). **^{13}C NMR** ($CDCl_3$, 75 MHz): δ 16.3, 35.7, 48.0, 54.4, 124.0, 131.7, 134.6, 167.8, 173.4.

HPLC: t_R 29.0 min (column: Alltech Alltima C18 5u, 250 x 22 mm; 60:40, (0.1% TFA: acetonitrile) flow rate: 15 mL min^{-1}).

***N*-Phthaloylleucine hydrazide 7.5**



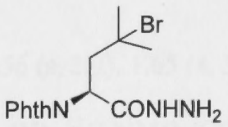
7.5

Procedure is as given for **7.1**. The product mixture was purified using HPLC to give the title compound in 60% yield.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.86 (d, J = 6 Hz, 3H), δ 0.88 (d, J = 6 Hz, 3H), 1.31-1.39 (m, 1H), 1.76-1.83 (m, 1H), 2.20-2.28 (m, 1H) 5.04 (dd, J = 3.9, 11.1 Hz, 1H), 7.72-7.82 (m, 4H). **^{13}C NMR** ($CDCl_3$, 75 MHz): δ 21.0, 23.2, 25.0, 29.8, 36.8, 123.9, 131.6, 134.6, 168.3, 170.4. **MS (ESI)** (+ve): *m/z* 276.1 ($[M+H]^+$, 100%). **HRMS**: $[M+H]^+$ (FTICR) calcd for $C_{14}H_{18}N_3O_3$ *m/z* 276.1348, found, 276.1348.

HPLC: t_R 6.8 min (column: Alltech Alltima C18 5u, 250 x 22 mm; 60:40, (0.1% TFA: acetonitrile) flow rate: 17 mL min^{-1}).

***N*-Phthaloyl-γ-bromoleucine hydrazide 7.6**



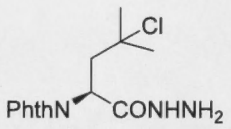
7.6

Procedure is as given in the synthesis of compound **7.2**. HPLC purification resulted in 80% yield of the bromide **7.6**.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.74 (s, 3H), δ 1.83 (s, 3H), 2.75-2.85 (m, 2H), 5.25 (dd, J = 3.9, 8.4 Hz, 1H) 7.68-7.78 (m, 4H). **^{13}C NMR** ($CDCl_3$, 75 MHz): δ 34.0, 34.9, 44.4, 50.1, 64.0, 123.8, 131.9, 135.4, 168.0, 178.3.

HPLC: t_R 36.4 min (column: Alltech Alltima C18 5u, 250 x 22 mm; 60:40, (0.1% TFA: acetonitrile) flow rate: 17 mL min^{-1})

***N*-Phthaloyl-γ-chloroleucine hydrazide 7.7**



7.7

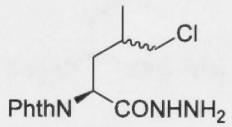
Procedure is as given in the synthesis of compound **7.3**. The yield calculated from the relative area of the HPLC chromatogram for **7.7** is 40% and that of **7.8** is 36% and **7.9** is 16%.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.56 (s, 3H), 1.65 (s, 3H), 2.68-2.90 (m, 2H), 5.24 (dd, *J* = 1.8, 9.6 Hz, 1H), 7.73-7.87 (m, 4H). **^{13}C NMR** ($CDCl_3$, 75 MHz): δ 32.3, 33.5, 38.8, 48.9, 68.5, 124.1, 132.1, 134.8, 167.9, 171.9.

HPLC: t_R 107.9 min (column: Alltech Alltima C18 5u, 250 x 22 mm; 70:30, (0.1% TFA: acetonitrile) flow rate: 15 mL min^{-1}).

The above reaction yielded the two diastereomers of the chloride **7.8** and the dichloride **7.9**

***N*-Phthaloyl-δ-chloroleucine hydrazide 7.8**



7.8

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.09 (d, J = 6.6 Hz, 3H), 1.70-1.77 (m, 1H), 2.02-2.13 (m, 1H), 2.54-2.64 (m, 1H), 3.43 (m, 2H), 5.00 (dd, J = 4.2, 12.0 Hz, 1H), 7.73-7.88 (m, 4H).

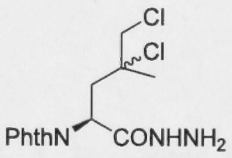
HPLC: t_R 115.0 min (column: Alltech Alltima C18 5u, 250 x 22 mm; 70:30, (0.1% TFA: acetonitrile) flow rate: 15 mL min^{-1}).

The other diastereomer of ***N*-Phthaloyl-δ-chloroleucine hydrazide 7.8**

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.05 (d, J = 6.6 Hz, 3H), 1.84-1.96 (m, 1H), 2.18-2.31 (m, 1H), 2.36-2.47 (m, 1H), 3.58 (m, 2H), 4.95 (dd, J = 4.8, 10.5 Hz, 1H), 7.73-7.87 (m, 4H).**^{13}C NMR** ($CDCl_3$, 75 MHz): δ 21.3, 23.4, 25.4, 32.3, 49.6, 124.1, 131.9, 134.8, 167.9, 174.5.

HPLC: t_R 128.8 min (column: Alltech Alltima C18 5u, 250 x 22 mm; 70:30, (0.1% TFA: acetonitrile) flow rate: 15 mL min^{-1}).

***N*-Phthaloyl-γ,δ-dichloroleucine hydrazide 7.9**



7.9

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.72 (s, 3H), 2.91 (m, J = 6.9 Hz, 2H), 3.62 (d, J = 11.7 Hz, 1H), 3.68 (d, J = 11.7 Hz, 1H), 5.23 (dd, J = 4.2, 6.9 Hz, 1H), 7.73-7.89 (m, 4H).

HPLC: t_R 158.7 min (column: Alltech Alltima C18 5u, 250 x 22 mm; 70:30, (0.1% TFA: acetonitrile) flow rate: 15 mL min^{-1}).

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