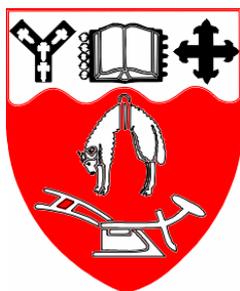


Molecular Switches: The Design, Synthesis and Biological Applications of Photoactive Enzyme Inhibitors

A thesis
submitted in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy in Chemistry
in the
University of Canterbury
By
Nathan A. Alexander



University of Canterbury
2006

Acknowledgements.....	6
Abstract.....	7
Abbreviations.....	9
Introduction.....	12
1.1 Overview.....	12
1.2 Light.....	13
1.3 Photochromism.....	14
1.4 Photoregulation of Enzyme Activity.....	19
1.5 Serine Proteases.....	19
1.6 Designing the inhibitors.....	22
1.7 Fluorine.....	24
1.8 Work described in this thesis.....	28
1.9 References for chapter one.....	28
Synthesis of the Key Trifluoromethyl Carbinol Intermediate.....	31
2.1 Introduction.....	31
2.2 Preparation of fluorinated ketones and related compounds.....	31
2.3 Designing the key trifluoromethyl carbinol intermediate.....	38
2.4 Synthesis of 3-amino-1,1,1-trifluoro-4-phenylbutan-2-ol hydrochloride....	39
2.5 Summary.....	50
2.6 Future work.....	51
2.7 References for chapter two.....	51
Synthesis of Stilbene and Phenanthrene Derivatives.....	55
3.1 Introduction.....	55
3.2 Photochemistry of stilbenes.....	56
3.3 Synthesis of substituted stilbenes.....	58
3.4 Alternate synthetic route for 4-substituted stilbenes.....	63
3.5 Synthesis of phenanthrene based inhibitors.....	65
3.6 Molecular modelling.....	73
3.7 Summary.....	77
3.8 Future work.....	78
3.9 References for chapter three.....	79
Synthesis of <i>E</i> -Azobenzene Derivatives.....	82
4.1 Introduction.....	82

4.2	Synthetic methods for preparing azobenzenes.....	83
4.3	Synthesis of 3- and 4-substituted azobenzenes.....	86
4.4	Synthesis of 4',3- and 4',4-disubstituted azobenzenes.....	93
4.5	Summary and future work.....	104
4.6	References for chapter four.....	104
Isomerisation Studies.....		107
5.1	Introduction.....	107
5.2	<i>E-Z</i> Isomerisation of azobenzenes.....	109
5.3	Experimental setup.....	119
5.4	Isomerisation of the target molecules.....	120
5.5	Summary.....	125
5.6	References for chapter five.....	126
Enzyme Inhibition Studies.....		129
6.1	Introduction.....	129
6.2	Michaelis-Menten kinetics.....	130
6.3	Experimental setup.....	139
6.4	Determination of the Michaelis constant and limiting rate.....	142
6.5	Inhibition studies of trifluoromethyl ketone based inhibitors.....	149
6.6	Summary.....	165
6.7	Future work.....	166
6.8	References for chapter six.....	167
Experimental.....		169
7.1	General Methods and Procedures.....	169
7.2	Experimental work outlined in chapter two.....	180
7.3	Experimental work outlined in chapter three.....	190
7.4	Experimental work outlined in chapter four.....	206
7.5	Experimental work outlined in chapter five.....	265
7.6	References for chapter seven.....	270

Acknowledgements

I would firstly like to thank my supervisor Prof. Andrew Abell for his time and patience during my long stay. Thanks also, for spending some of that Marsden fund on me. Of course a big thanks goes out to my Mum for all the cuppas she has made over the years and for always asking ‘have you finished yet?’

To the rest of my family, thanks for everything. To my uncle Perry, thanks for all the rides. To my sister Jessamae, thanks for the use of the laptop, it was a big help. To Bridget, thanks for Jaymez, he keeps me amused. To Hannah, thanks for the pity money when I was broke. To my Dad, thanks for the drinks, sometimes you need an excuse to get away.

To my friends Reuben and Julian, I thank you for the good times; I don’t know what I would have done without you (Ps, that’s ‘Dr Nathan’ to you now).

To the members of the Abell group past and present, it has been fun. Thanks to the technical staff especially Alistair for all their help over the years, and finally, Kevin (Mr) Tranter, thanks for introducing me to the joys of chemistry, without him, I’d probably be a chef!

Abstract

This thesis examines the design, synthesis and biological applications of a series of inhibitors which incorporate an azobenzene photoswitch, a peptidyl backbone and a trifluoromethyl ketone warhead. The photoswitch can be isomerised by irradiation with UV or visible light and has been employed to modulate the reactivity of the enzyme.

Chapter one gives a brief outline of some of the important areas related to this work. Examples of previously utilised photoswitches as well as some background on serine protease and the uses of fluorine in medicine have been covered.

Chapter two outlines the synthesis of the key trifluoromethyl carbinol **2.6** by two different methods. The condensation of a fluorinated aldehyde with a nitroalkane affords an α -nitro trifluoromethyl carbinol which can be reduced to give the desired amine **2.6**. Treatment of oxazolones with trifluoroacetic anhydride via a modified Dakin-West reaction gives trifluoromethyl ketones which can be reduced to give trifluoromethyl carbinols.

Chapter three investigate the synthesis of substituted stilbenes and phenanthrenes as alternative molecular switches to azobenzenes. Molecular modelling of phenanthrenes suggests they may be suitable mimics of *E*-azobenzenes.

Chapter four outlines the synthesis of a series of mono and disubstituted azobenzenes by direct sulfonation of azobenzene or by condensation of nitroso arenes with aryl amines. The switches incorporate one or two peptidyl residues designed to improve specificity towards the enzyme.

Chapter five examines the photoisomerisation of eight potential inhibitors by irradiating with UV or visible light. Irradiation with UV light enriches the sample to give 78-93 % of the *Z*-isomer. Irradiation with visible light gave photostationary states with 14-21 % *Z*-isomer. Ambient photostationary states are *ca.* 22 % *Z*-isomer.

Chapter six looks at the testing of five trifluoromethyl ketones as potential inhibitors of α -chymotrypsin. The inhibitors vary in substituents, substitution patterns and chain length. The inhibitors were tested at both ambient and *Z*-enriched photostationary states and were found to exhibit slow binding kinetics. In all cases the *Z*-enriched inhibitor solution was at least 3-fold more potent than the ambient solution.

Chapter seven is an experimental chapter and outlines the synthesis of the compounds prepared in this thesis.

Abbreviations

δ	chemical shift
Δ	change or heat (depends on context)
ϵ	molar absorptivity coefficient
Ar	aryl
Az	azobenzene
Boc	<i>tert</i> -butoxycarbonyl
BOP	benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate
br	broad (in NMR)
<i>ca.</i>	circa
Cbz	benzyloxycarbonyl
d	doublet (in NMR)
dd	doublet of doublets (in NMR)
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
DIPEA	<i>N,N</i> -diisopropylethylamine (Hünigs base)
DMA	<i>N,N</i> -dimethylacetamide
DMF	<i>N,N</i> -dimethylformamide
DMSO	dimethylsulphoxide
EDCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EI	electron impact ionisation
eqm	equilibrium
equiv	equivalent(s)
Fmoc	9-fluorenylmethoxycarbonyl
h	hour
HATU	<i>O</i> -(7-Azabenzotriazol-1-yl)- <i>N,N,N',N'</i> -tetramethyluronium hexafluorophosphate
HEH	diethyl 1,4-dihydro-2,6-dimethyl-3,5-pyridine dicarboxylate
HEPES	<i>N</i> -(2-hydroxyethyl)piperazine- <i>N'</i> -2-ethanesulfonic acid
HMPA	hexamethylphosphoric triamide

HOBt	1-hydroxybenotriazole
HRMS	high resolution mass spectrometry
IR	infrared
J	coupling constant (in NMR)
Leu	leucine
LRMS	low resolution mass spectrometry
m	multiplet (in NMR)
min	minute(s)
mp	melting point
NMR	nuclear magnetic resonance
Ph	phenyl
Phe	phenylalanine
Phen	phenanthrene
Phth	phthalimido
ppm	parts per million
PSS	photostationary state
py	pyridine
q	quartet (in NMR)
rt	room temperature
s	second or singlet (in NMR)
t	triplet (in NMR)
TBAF	tetrabutylammonium fluoride
TBDMS	<i>tert</i> -butyldimethylsilyl
TEMPO	2,2,6,6-tetramethylpiperidin-1-oxyl
TFMC	trifluoromethyl carbinol
TFMK	trifluoromethyl ketone
THF	tetrahydrofuran
TRIS	tris(hydroxymethyl)aminoethane
UV	ultraviolet
Val	valine
vis	visible
v/v	volume per volume
w/v	weight per volume

Chapter One

Introduction

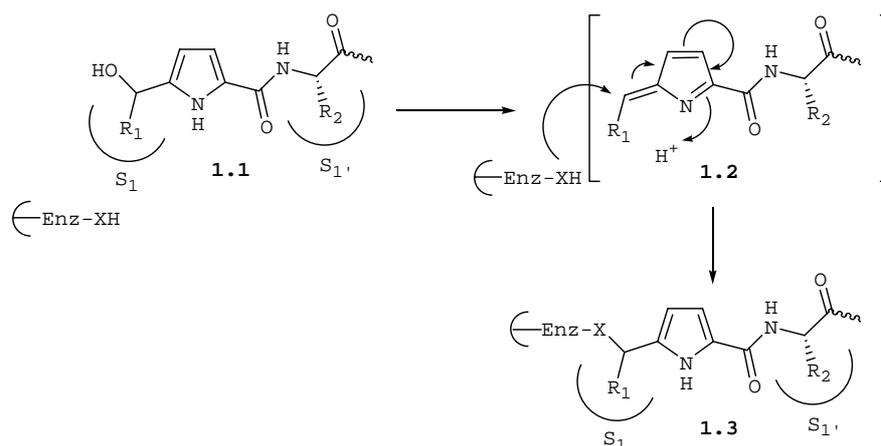
Introduction

1.1 Overview

The focus of this thesis is to prepare a series of inhibitors of α -chymotrypsin which incorporate a molecular switch that can be modulated by external stimuli to alter the reactivity of the target molecule. This modulation can be necessary if the molecule is too reactive, as this can result in undesirable side reactions which lead to depletion of the reactive species. On the other hand, if a molecule is too stable it may not perform its necessary function until some modulation of reactivity has occurred.

An important area in this field is the modulation of enzyme activity by introduction of molecules that can interact with the enzyme. Usually this is in the form of an inhibitor which decelerates or inactivates the function of the enzyme. Inhibitors that are unreactive before reaching the active site of the enzyme but can be activated on demand would be a desirable trait.

Efforts to this effect have been pursued by our group for a number of years to prepare compounds whose latent reactivity has been masked in some way. Under the right conditions the latent reactivity is unleashed within the molecule and the activated species can perform its designated function. One such example is the work on hydroxymethyl pyrole **1.1** which is transformed into the reactive azafulvene **1.2** in the active site of the enzyme. The azafulvene is attacked by a nucleophilic residue on the enzyme and forms a covalent linkage which deactivates the enzyme **1.3** (scheme 1.1).¹



Scheme 1.1. Mechanism based inhibition by hydroxyalkyl pyrrole **1.1**.

Further work by our group has investigated the modification of enzyme reactivity by synthesising inhibitors based around an azobenzene photoswitch.² The conformation of the photoswitch can be altered by irradiation with UV or visible light to give a photostationary state comprised of a mixture of *E*- and *Z*-isomers (fig. 1.1).

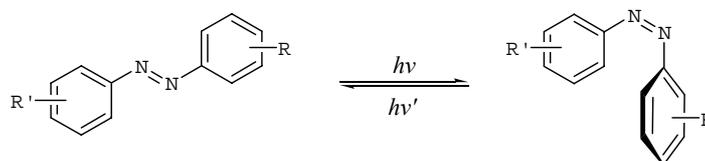


Figure 1.1. *E-Z* isomerisation of azobenzenes.

If one conformation of the azobenzene inhibitor is found to decrease the enzyme activity more than the other we can regulate the enzyme activity by altering the composition of the photostationary state.

1.2 Light

Diverse biological processes are triggered by light signals. Some of the more important processes include photosynthesis, vision, photomorphogenesis and conversion of light energy into chemical energy.³ Bioactive materials have many potential uses such as in optical signal recording, biosensors and biochemical probes and are of particular interest as

target-activated therapeutic substances. An important feature of all these processes is that they all contain a chromophore that upon absorption of light, activates a series of chemical transformations within the chromophore.⁴

As well as biological processes, light plays an important role in organic photochemistry. It is used to effect pericyclic (electrocyclic) reactions⁵ and *cis-trans* isomerisations (fig. 1.2) in compounds that contain C=C, C=N and N=N moieties such as alkenes, imines, oximes and diazo compounds.⁶

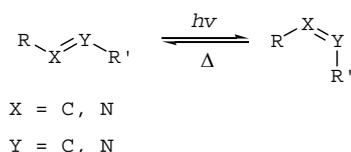


Figure 1.2. *cis-trans* Isomerisation of double bonds.

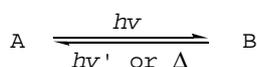
By using these small organic molecules as simple models of more complex biological systems, scientists can examine the interaction of light with synthetic models and the interaction of these synthetic molecules with biomolecules in an attempt to improve our understanding of the truly complex and diverse nature of biological systems.

1.3 Photochromism

1.3.1 Bioswitches

For a molecule to be termed photochromic, certain criteria need to be fulfilled:

- (i) The molecule requires a photoisomerisable chromophore
- (ii) The photoisomerisation must be reversible either by photonic excitation or thermal relaxation



- (iii) The two states must have distinguishably different absorption spectra

The method by which A is transformed into B (*cis-trans* isomerisation, ring opening/closing etc) is unimportant. Provided B reverts exclusively to A the criteria for photochromism is met.

A photochromic molecule can exhibit properties analogous to a switch. Like a switch it has two states, 'on' and 'off'. If this rationale is applied to a photobiological switch that acts on an enzyme the 'on' state allows the enzyme to function as normal, but when the photobiological switch is triggered to the 'off' position the enzyme is deactivated.

Properties that would make the photochromic molecule useful as a biological photoswitch include:

- (i) The dipole moment and molecular structure exhibit significant difference between the 'on' and 'off' states
- (ii) The thermal back reaction is sufficiently slow that the molecule can have a lasting effect on the enzyme
- (iii) The reverse reaction can be induced photochemically
- (iv) The inactive form ('off') contains negligible amounts of the activating ('on') form
- (v) The synthesis of the photochromic molecule is relatively simple and can be obtained in a pure state

1.3.2 Reversible photoisomerisable switches

There are various classes of compounds that undergo reversible photoisomerisation. Many of these fit the criteria for photobiological switches and have been applied to biological systems. A few representative examples have been included below.

Photochemical (4n + 2) π electron ring opening and closing

Diaryl arenes such as stilbene **1.4** undergo reversible *cis-trans* isomerisation about the double bond, however, cyclisation to the dihydrophenanthrene **1.5** can occur. The reverse reaction can occur thermally or photochemically to give a photostationary state comprising *E*- and *Z*- isomers. The major fatigue pathway for this process is the photooxidative reaction which leads to the irreversible formation of phenanthrene **1.6**. The photochemistry of these compounds is discussed further in section 3.2.

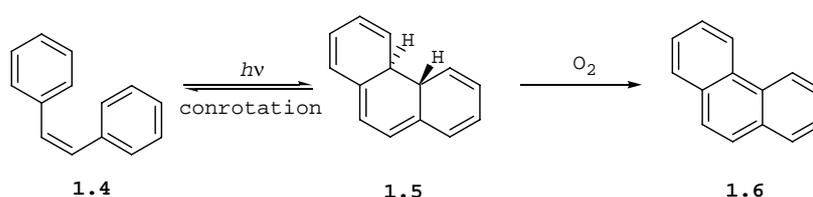
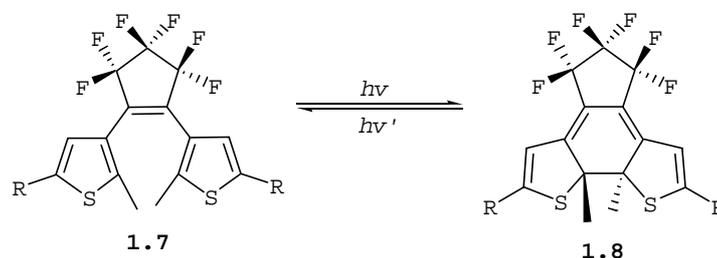


Figure 1.3. Cyclisation of *cis*-stilbene.

Compounds such as **1.7** and **1.9** have been synthesised which undergo light induced ring opening and closing and show greater resistance to photofatigue than stilbenes. The diarylethene **1.7** is a P-type (photoisomerisation only) dye which has a coloured and non-coloured form. The colour develops in the cyclised form due to increased conjugation in the ring. Spirooxazine **1.9** is a T-type (photo or thermal isomerisation) dye which becomes coloured upon ring opening (fig. 1.3).⁷ Compounds based on **1.7** have been used to form molecular ‘tweezers’ and molecular wires.⁸



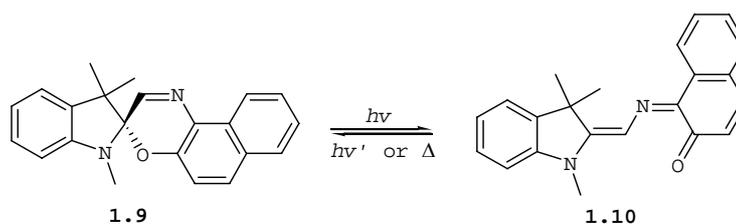


Figure 1.3. Ring opening and closing of 1,2-dithienylperfluorocyclopentene **1.7** or spiropyrans **1.9**.

The thiophenefulgicide **1.11** and nitrospiropyran **1.13** (fig. 1.4) have been bound to lectin concanavalin A to alter the binding properties of two pyranose sugars α -D-mannopyranose and α -D-glucopyranose.

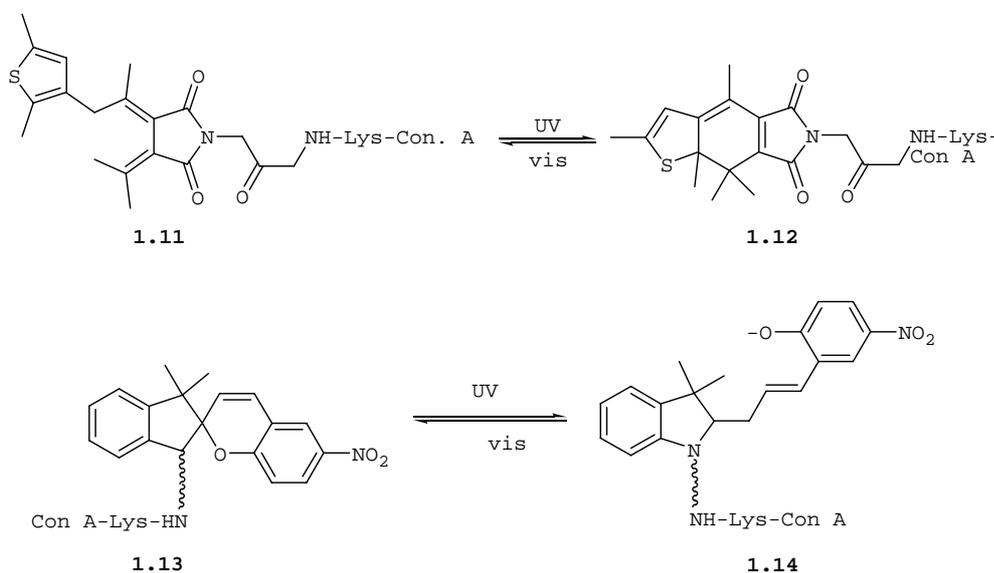


Figure 1.4. Ring opening and closing of thiophenefulgicide **1.11** and nitrospiropyran **1.13**.

Thiophenefulgicide **1.11** electrocycliced after irradiation with UV light where as nitrospiropyran **1.13** ring-opened upon irradiation with UV light. Both processes were reversible upon irradiation with visible light and in both cases the cyclised form exhibited enhanced binding of the monosaccharides.³

Isomerisation about double bonds

Azobenzenes and stilbenes undergo isomerisation about a double bond. The photochemistry is discussed for each in sections 5.2 and 3.2 respectively. Imines **1.15** and oximes **1.16** can undergo *cis-trans* isomerisation across the double bond but neither type of compound would be particularly useful as a biological photoswitch as imines are prone to hydrolysis and oximes can undergo a Beckman-type photorearrangement to give an amide (fig. 1.5).⁶

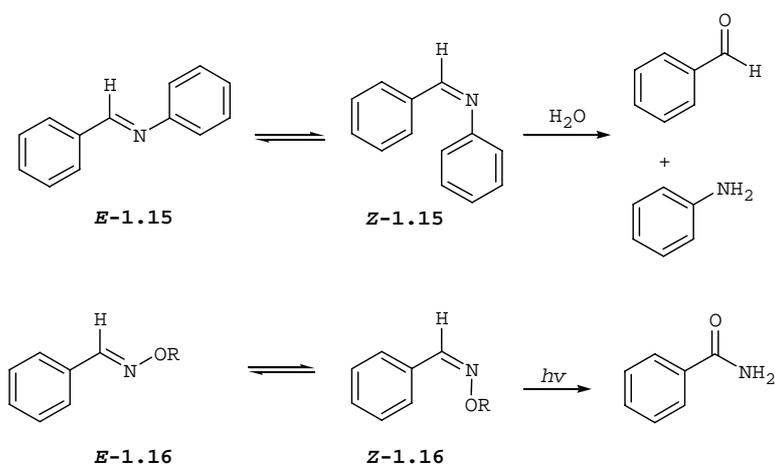


Figure 1.5. Isomerisation and degradation of imines and oximes.

The indigos **1.17** can also isomerise about a carbon-carbon double bond but the overall structural change is minimal and therefore not suitable as a biological photoswitch.

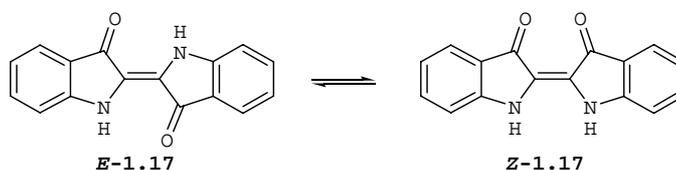


Figure 1.6. Isomerisation of indigo.

1.4 Photoregulation of Enzyme Activity

The aim of this project is to prepare molecules containing a photoswitch that undergoes light induced photoisomerism between different conformations (e.g. *cis* and *trans* isomers) to regulate the binding and release of the photoactive molecule to the target enzyme. The photoactive moiety can be incorporated into the system in several ways:

- (i) Attached to the enzyme through residues in the active site
- (ii) Attached to the protein backbone of the enzyme
- (iii) Covalently linked to the inhibitor that can bind to the enzyme

Methods (i) and (ii) are ways of altering the shape of the enzyme by disrupting folding patterns and changing hydrophobic interactions.

This project looks at option (iii) as it is a way of altering the shape of the inhibitor, hopefully allowing an active and non-active or on/off form of the inhibitor. Examples from the literature are discussed in section 5.2.2. The advantages of this approach are that the native structure of the enzyme is not disturbed. Attaching a photoswitch to the enzyme directly can cause perturbations to the native structure and therefore reduce the native activity of the enzyme irrespective of the conformation of the switch.⁹

1.5 Serine Proteases

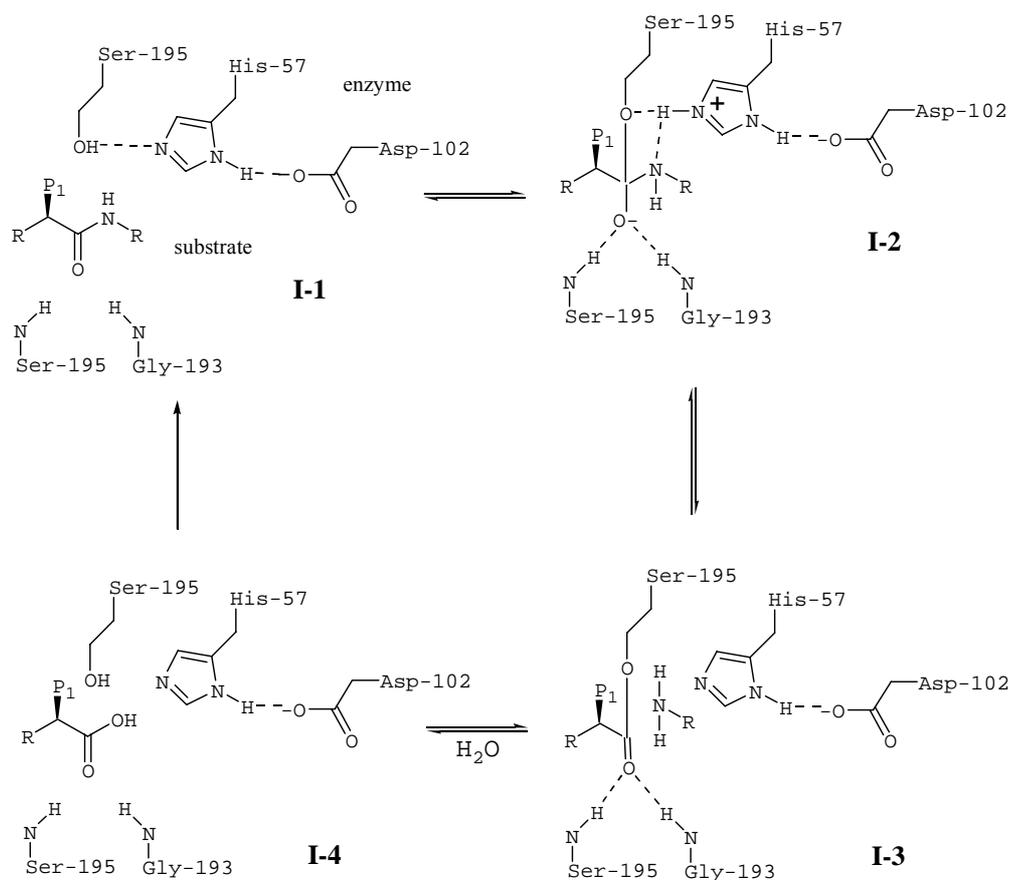
Proteases are a class of enzymes of which serine proteases are the largest and most thoroughly understood. They have important roles in digestion, blood coagulation, complement activation, fibrinolysis, reproduction, development and the release of physiologically active peptides. These processes are initiated by the protease catalysed cleavage of one or more peptide bonds in proteins or peptides and under physiological conditions the cleavage is irreversible. Not only are serine proteases a biological necessity but they are also a potential hazard as uncontrolled proteolysis leads to the destruction of the protein components of cells and tissues. Diseases that arise due to uncontrolled

cleavage include pulmonary emphysema, adult respiratory disease syndrome and pancreatitis. Serine proteases may also be involved in tumour invasion, viral transformations and inflammation.¹⁰

The active site of a serine protease consists of two regions:

- (i) A catalytic site consisting of Ser-195, His-57 and Asp-102 also known as the catalytic triad which is responsible for proton transfer during the hydrolysis of the peptide bond (scheme.1.2)
- (ii) Sub site binding pocket(s) where the amino acid residues of the substrate interact with complementary sites on the enzyme to correctly orientate the substrate for catalysis (fig. 1.7)

The mechanisms for peptide bond cleavage involves nucleophilic attack by the Ser-195 hydroxyl (**I-1**) on the carbonyl of the scissile amide bond of the substrate to give a tetrahedral adduct (**I-2**). The unusual reactivity of the Ser-195 hydroxyl is due to increased nucleophilicity brought about through hydrogen bonding by the imidazole side chain of His-57, which in turn is involved with hydrogen bonding with Asp-102 (**I-1**). An oxyanion hole, formed by the NH groups of Ser-195 and Gly-193, increases the electrophilicity of the carbonyl oxygen before addition of Ser-195 and stabilises the oxyanion of the tetrahedral adduct. The tetrahedral adduct collapses to give an acyl enzyme intermediate and the free amine (**I-3**). Catalytic hydrolysis then affords the free carboxylic acid and regenerates the enzyme to repeat the cycle (**I-4**) (scheme 1.4). The overall sequence results in cleavage of the peptide bond.



Scheme 1.2. Schematic representation of peptide bond hydrolysis by serine proteases.¹¹ Ser-195, His-57, Asp-102 and Gly-193 are all part of the serine protease.

The substrate can increase affinity for the enzyme through binding to amino acid residues extending either side of the scissile bond (fig.1.7). Residues on the C-terminus of the scissile bond are labelled P₁, P₂...P_n and on the N-terminus P₁' , P₂' ...P_n' based on the notation of Schechter and Berger.¹² The corresponding enzyme binding sub sites are designated S₁, S₁' ...S_n'. The binding outside the catalytic site occurs through non-covalent interactions such as hydrogen bonding and hydrophobic forces. The most important being the interaction between the P₁ residue and the S₁ binding site and is the primary determinant of enzyme specificity. The nature of S_{1-n} and S'_{1-n} for a given protease, will determine which P_{1-n} and P'_{1-n} residues will bind and hence this defines the specificity of the different subclasses of serine proteases.

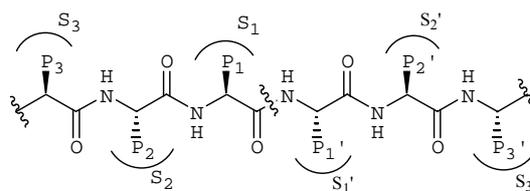


Figure 1.7. Extended binding site of serine proteases.

There are three main types of serine proteases, trypsin-like, chymotrypsin-like and elastase like. The serine protease α -chymotrypsin has been chosen for initial work described in this proposal as its catalytic action is well studied. The S₁ site for chymotrypsin is a narrow crevice that would best accommodate a planar aromatic residue such as phenylalanine, tyrosine or tryptophan. The choice of amino acids in the peptidyl chain is a means to improve the affinity for the S₂ and S₃ sites to increase specificity for the enzyme. Most serine proteases also have extended sub sites on the leaving group (P') side of the scissile bond although these sites have not been studied in as much detail and are deemed less important for enzyme specificity.¹⁰

1.6 Designing the inhibitors

The main aim of this project is to develop a series of inhibitors of α -chymotrypsin that are more active in one conformation than the other and are stable in a physiological environment. The proposed inhibitors consist of three components (fig.1.8):

- (i) A switch that is photoisomerisable about a double bond
- (ii) A peptide chain to increase enzyme specificity (R¹ and R²)
- (iii) A functional group that will act as an inhibitor of the enzyme

There have been extensive studies done on inhibitors of serine proteases employing many types of functional groups¹¹ including heterocycles, electrophilic carbonyl derivatives (diketones and aldehydes, halogenated ketones) and acylating and alkylating agents.

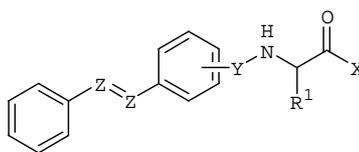


Figure 1.8. General structure of photoswitch based enzyme inhibitors.

Z = C, N; Y = CO, CO-*L*-Leu; SO₂, SO₂-*L*-Leu; X = CF₃, CO₂Me; R = CH₂Ph.

Trifluoromethyl ketones (X = CF₃) have been chosen as our initial target as they are a widely researched species and have been shown to be very potent reversible competitive inhibitors of serine proteases. Attack by a nucleophile forms a very stable tetrahedral adduct which mimics the transition state of enzymatic peptides (scheme 1.2 **I-2**) and therefore, if the nucleophile is the enzymatic Ser-195, trifluoromethyl ketones can act as inhibitors (fig.1.9). The reason for their potency is due to the strong electron withdrawing effects of the three fluorine atoms causing an electron deficiency on the adjacent carbonyl making it very susceptible to nucleophilic attack. The covalent adducts are not only stabilised by fluorine's electronegativity but also by strong hydrogen bonding between fluorine and the acidic enzyme protons.

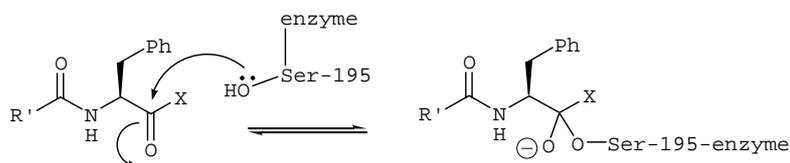


Figure 1.9. Reversible inhibition of α -chymotrypsin.

Another advantage of using fluorinated inhibitors is that ¹⁹F NMR can be used. ¹⁹F NMR can be a powerful tool in synthetic and biological chemistry, with the main applications in this thesis being to use ¹⁹F NMR to determine E/Z ratios, diastereomeric ratios and monitor the progress of trifluoromethyl carbinol oxidations (fig. 1.10). Below is a summary of some of the applications of fluorine and ¹⁹F NMR in medicinal chemistry.



Fig. 1.10. Incomplete oxidation of a trifluoromethyl carbinol. Doublet (TFMC), singlet (ketone), singlet (hydrate).

1.7 Fluorine

1.7.1 Introduction

Fluorine chemistry is a large and very important aspect of modern chemistry. Methods for preparing fluorinated compounds have increased immensely in range and effectiveness. As such it is now possible to prepare fluorinated analogues of most known molecules. The effect of inserting fluorine into a molecule can often have dramatic effects on its function. Fluorine has often been used as a replacement for hydrogen due to its small size. Incorporating fluorine can dramatically alter the properties of the molecule due to its increased electronegativity and additional lone pair electrons. Fluorinated analogues of biologically or agriculturally important molecules often have increased stability towards metabolism and binding to specific receptor sites. Examples of important fluorinated compounds include Diflucan **1.18** for treating thrush, Lefenuron **1.19**, for controlling fleas on household pets and 5-fluorouracil **1.20**, an anti-tumour drug (fig. 1.11).¹³

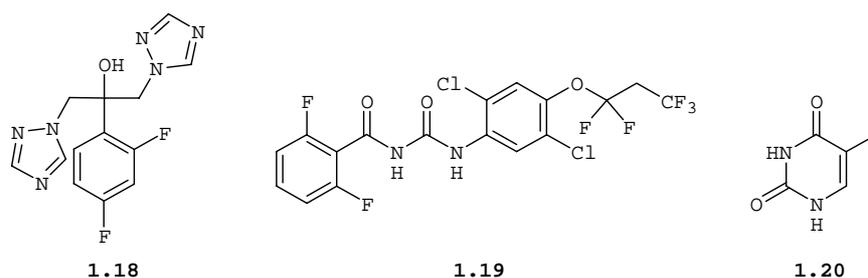


Figure 1.11. Some examples of fluorinated drugs.

1.7.2 ¹⁹F in NMR

¹⁹F is the most stable isotope of fluorine having a natural abundance of 100 %. Like ¹H it has a spin of ½ and is easily detected as its NMR sensitivity is 83 % of ¹H. ¹⁹F NMR is sensitive to changes in Van der Waal interactions, electrostatic fields and long range effects, especially in macromolecules such as proteins. This is illustrated by the broad chemical shift range for organofluorines; $\delta = -267.9$ (CH₃F), 0 (CCl₃F), +147.1 ppm (CF₃OF). Some transition metal fluorides have chemical shifts upwards of + 5000 ppm.¹⁴

1.7.3 ¹⁹F NMR in biomedicine

While ¹⁹F NMR spectroscopy has been used in classical areas of chemistry for some time, it has played a less important role than ¹H and ¹³C NMR as so few naturally occurring fluorine compounds exist. It is however, becoming increasingly more important in biochemistry as unlike hydrogen, which is a major component of the body (mostly as water in tissue), physiological concentrations of fluorine are in the order of less than 10⁻⁶ M and is mainly found immobilised in teeth and bone. This is an advantage as it allows monitoring of administered fluorinated molecules without interference from other fluorine sources. *In vivo* NMR using full body scanners is a non-invasive technique that can be applied to the detection of multiple nuclei. Biological applications which utilise fluorine as an NMR probe have included *in vivo* pharmacokinetics of the anti-tumour drug 5-fluorouracil and its metabolites, *in vivo* studies of perfluorocarbons utilised in blood substitutes by ¹⁹F tomography,¹⁵ monitoring protein structure and function by introduction

of fluorinated amino acids or ligands,¹⁶ fluorinated drug partitioning in lip bilayers¹⁷ and studying the oxygenation of hypoxic tumours by ¹⁹F oximetry.¹⁸ An extensive NMR study on fluorinated carbohydrates has also been reported. A stereospecific synthesis of β^2 -fluorinated amino acids (fig. 1.12) has been reported by our group^ξ for use in the preparation of α -helices.

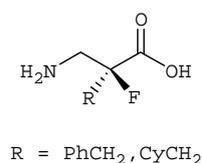


Figure 1.12. An enantiomerically pure β^2 -fluorinated amino acid.

1.7.4 Fluorine in enzyme inhibitors

Due to its strong electron withdrawing ability, fluorine has gained interest from chemists designing peptidyl and non-peptidyl based inhibitors. These inhibitors possess a fluoromethyl ketone warhead comprising one, two or three fluorines covalently bound to the carbon. These fluoromethyl groups increase the electrophilicity of the adjacent carbonyl group thereby increasing the susceptibility of attack from the enzyme. The electrophilicity of the carbonyl group increases with increasing number of adjacent fluorine atoms. When assayed against α -chymotrypsin¹⁹ or acetylcholinesterase²⁰ increased fluorination resulted in a corresponding decrease in K_i (more potent inhibition). Though generally less potent, it can be advantageous to use mono and difluoromethanes to extend the molecule in two dimensions, whereas trifluoromethanes are limited to extension in one direction.^{21,22}

^ξ M. Edmonds; Unpublished work.

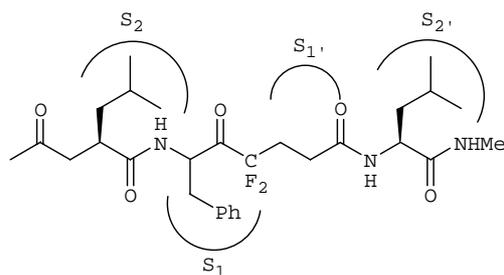


Figure 1.13. Extended binding of a difluoromethyl ketone.

^{19}F NMR has been used to study the degree of hydration mono-, di- and trifluoromethyl ketones undergo in aqueous solution by measuring the integrals of the appropriate spectrum. It was found that di- and trifluoromethyl ketones are essentially completely hydrated while monofluoromethyl ketones are only 50 % hydrated.¹⁹

Studies of hemiketal formation from hydrated ketones^{19,21} have been used in an attempt to ascertain the mode of trifluoromethyl ketones binding with α -chymotrypsin. Addition of methanol to a solution of trifluoroacetone hydrate gave rise to a new peak downfield of the hydrate and upfield of the ketone. Analysis of enzyme solutions containing fluorinated inhibitor by ^{19}F NMR show distinct resonances for the bound and unbound inhibitor, the resonance of the bound inhibitor shifts slightly downfield relative to the hydrate and exhibits typical line broadening associated with high molecular weight complexes. The chemical shift of the bound inhibitor was not inconsistent with the formation of a hemiketal with the serine hydroxyl; however, the chemical shifts of ^{19}F signals are susceptible to solvent polarity and ionic strength and so on there own, are unsuitable for determining the mode of binding.

A new type of phospholipase A_2 (cPLA₂), an enzyme responsible for hydrolysing phospholipids is readily inhibited by arachidonyl trifluoromethyl ketone and has been studied by ^{19}F NMR to determine the stoichiometry of the inhibitor-enzyme complex and determine if its formation was reversible. Measurements with a 1:1 ratio of enzyme to inhibitor showed the disappearance of the free inhibitor with a corresponding increase in the formation of bound inhibitor. Once all free inhibitor had disappeared a second equivalent of inhibitor was added. A new peak appeared which was attributed to the

formation of a non-specific complex with the enzyme. The reversibility of the active inhibitor-enzyme complex was demonstrated by addition of a large excess of a competing difluoro analogue and monitoring by ^{19}F NMR.²³

1.8 Work described in this thesis

The work in this thesis continues with this concept of modulating enzyme activity via photoisomerisation of molecular switches. Work covered in this thesis will look at the design and synthesis of the key components of the enzyme inhibitors, namely the trifluoromethyl carbinol intermediate and the azobenzene switches. The synthesis of stilbene and phenanthrene derivatives as alternative types of molecular switches has been investigated. Incorporating additional functionality within the photoswitch is investigated as a means of improving the photoisomerisation and potency of the trifluoromethyl ketone inhibitors. Isomerisation and enzyme inhibition studies have been performed and certain trends were observed.

1.9 References for chapter one

- (1) Martin, D. C., Thesis; University of Canterbury, 2003.
- (2) Harvey, A. J., Thesis, University of Canterbury, 2000.
- (3) Willner, I.; Rubin, S. *Angewandte Chemie, International Edition in English* **1996**, *35*, 367-385.
- (4) Willner, I. *Accounts of Chemical Research* **1997**, *30*, 347-356.
- (5) Coxon, J. M.; Halton, B. *Organic Photochemistry*; 2nd ed.; Cambridge University Press, 1987.
- (6) Suginome, H. In *CRC Handbook of Organic Photochemistry and Photobiology*; CRC Press, Inc: 1995, p 825-840.
- (7) Evans, R. A.; Such, G. K. *Australian Journal of Chemistry* **2005**, *58*, 825-830.
- (8) Bens, A. T.; Frewert, D.; Kodatis, K.; Kryschi, C.; Martin, H.-D.; Trommsdorff, H. P. *European Journal of Organic Chemistry* **1998**, 2333-2338.

- (9) Amato, C.; Fissi, A.; Vaccari, L.; Balestreri, E.; Pieroni, O.; Felicioli, R. *Journal of Photochemistry and Photobiology, B: Biology* **1995**, *28*, 71-75.
- (10) Harper; Powers *Protease inhibitors*, 1986.
- (11) Edwards, P. D.; Bernstein, P. R. *Medicinal Research Reviews* **1994**, *14*, 127-194.
- (12) Schechter, I.; Berger, A. *Biochemical and Biophysical Research Communications* **1967**, *27*, 157-162.
- (13) Gerig, J. T. Online PDF; 2001.
- (14) Berger, S.; Braun, S.; Kalinowski, H.-O. In *NMR Spectroscopy of the Non-Metallic Elements*; John Wiley and Sons: 1997, p 398-698.
- (15) Bachert, P. *Progress in Nuclear Magnetic Resonance Spectroscopy* **1998**, *33*, 1-56.
- (16) Gakh, Y. G.; Gakh, A. A.; Gronenborn, A. M. *Magnetic Resonance in Chemistry* **2000**, *38*, 551-558.
- (17) Ulrich, A. S. *Progress in Nuclear Magnetic Resonance Spectroscopy* **2005**, *46*, 1-21.
- (18) Robinson, S. P.; Griffiths, J. R. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences* **2004**, *359*, 987-996.
- (19) Imperiali, B.; Abeles, R. H. *Biochemistry* **1986**, *25*, 3760-3767.
- (20) Allen, K. N.; Abeles, R. H. *Biochemistry* **1989**, *28*, 8466-8473.
- (21) Parisi, M. F.; Abeles, R. H. *Biochemistry* **1992**, *31*, 9429-9435.
- (22) Imperiali, B.; Abeles, R. H. *Biochemistry* **1987**, *26*, 4474-4477.
- (23) Trimble, L. A.; Street, I. P.; Perrier, H.; Tremblay, N. M.; Weech, P. K.; Bernstein, M. A. *Biochemistry* **1993**, *32*, 12560-12565.

Chapter Two

Synthesis of the Key Trifluoromethyl Carbinol Intermediate

Synthesis of the Key Trifluoromethyl Carbinol Intermediate

2.1 Introduction

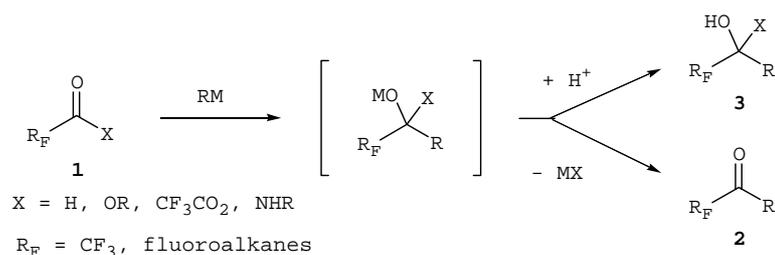
Trifluoromethyl ketones have gained in notoriety over the years due to their growing importance in biological systems and their potential as potent inhibitors of α -chymotrypsin,¹ renin,^{2,3} elastases⁴⁻⁸ and other proteolytic enzymes. A review on the preparation of trifluoromethyl ketones and related fluorinated ketones by Bégué and Bonnet-Delpon⁹ illustrates a wide range of methods by which trifluoromethyl ketones have been synthesised. A brief overview of some of the more pertinent methods has been outlined below.

2.2 Preparation of fluorinated ketones and related compounds

2.2.1 Preparation via organometallics

Early methods of preparing fluoroalkyl ketones were based on two organometallic reactions; (i) reacting a non-fluorinated organometallic reagent with a fluorinated carbonyl compound, (ii) reacting a fluorinated organometallic reagent with a non-fluorinated carbonyl compound.

Reactions between organometallic (RM = Grignard, organolithium, organozinc, organocadmium and organomanganous) reagents and trifluoroacetic acid derivatives **1** (including alkyl trifluoroacetates, amides, trifluoroacetic anhydride and trifluoroacetonitrile) have been widely utilised in the synthesis of trifluoromethyl ketones **2** but suffer from the limitations in the variety of organometallic reagents available and the formation of secondary and tertiary alcohol by-products **3**. It has been shown by Tamborski *et al* that the final product formed is very dependent on the stability of the initial tetrahedral intermediates (scheme 2.1).¹⁰

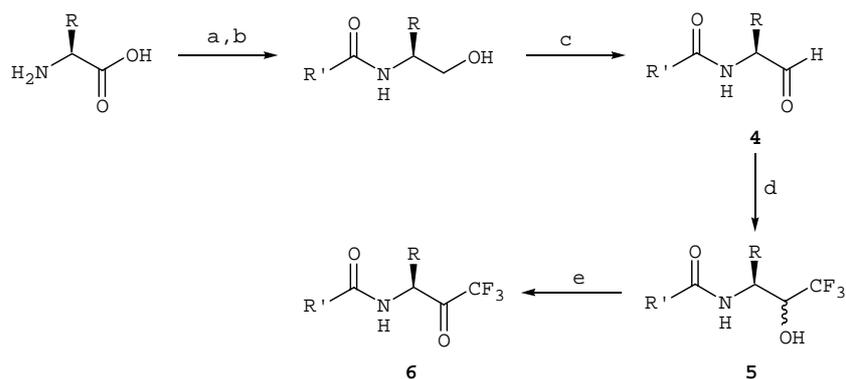


Scheme 2.1. Reaction of organometallics with fluorinated carbonyl compounds.

The second method involves condensations of fluorinated organometallics with aldehydes and ketones to afford secondary and tertiary alcohols respectively. However, the introduction of perfluoroalkyl groups (especially CF_3) to many carbonyl systems is hampered due to decreased nucleophilicity and instability of the fluorinated organometallic reagents used.

Stereospecific synthesis of trifluoromethyl ketones

A stereospecific synthesis of trifluoromethyl ketones employing fluorinated organometallics has been outlined by Edwards. Treatment of peptidyl aldehydes **4** with $\text{CF}_3\text{ZnI}^{11}$ yields secondary trifluoromethyl carbinols **5** which were subsequently oxidised to the corresponding trifluoromethyl ketones **6** (scheme 2.2).^ξ



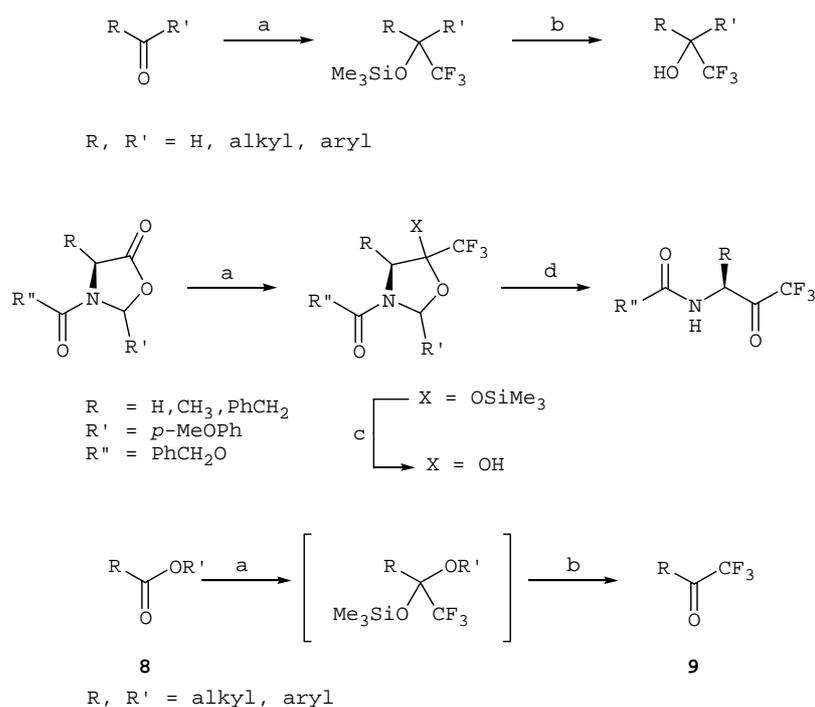
Reagents and conditions: (a) LiAlH_4 ; (b) RCO_2H , $i\text{-BuOCOC}$ Cl , NMM; (c) Swern oxidation; (d) CF_3I , activated Zn, DMF; $-20\text{ }^\circ\text{C}$; (e) Dess-Martin Periodinane.

Scheme 2.2. Zn mediated preparation of trifluoromethyl carbinols.

^ξ This was considered a limitation until the development of successful methods for oxidising secondary trifluoromethyl carbinols.

2.2.2 Silyl reagents

New reagents based on trimethylsilanes, ($\text{CF}_3\text{-TMS}$, Ruppert's reagent) have been reported as efficient fluoroalkylating agents in many systems^{4,12-17} and facilitates the direct conversion of esters **8** into trifluoromethyl ketones **9** (scheme 2.3).^{14,16}



Reagents and conditions: (a) $\text{CF}_3\text{-TMS}$, TBAF or CsF ; (b) 2 M HCl; (c) TBAF; (d) Amberlite[®] IR-120, MeCN.

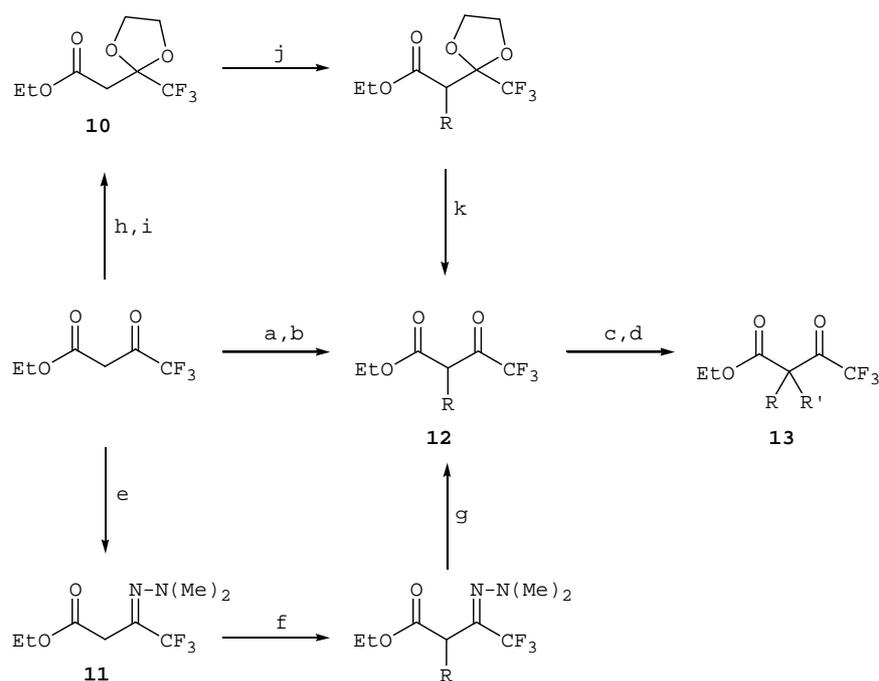
Scheme 2.3. Reactions of carbonyl compounds with $\text{CF}_3\text{-TMS}$.

2.2.3 Ethyl trifluoroacetate

Commercially available ethyl trifluoroacetate has been shown to be a feasible building block for the synthesis of new trifluoromethyl ketones. Mono or dialkylation of the corresponding enol occurs in moderate to good yields provided reactive alkyl halides^ξ are employed.¹⁸

^ξ *O*-alkylation is favoured when non-activated alkyl halides are used.

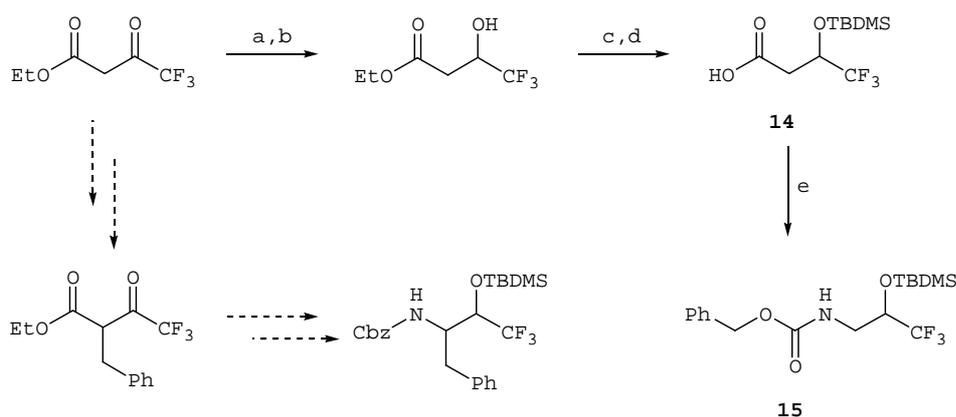
Further work has been undertaken by Bégué *et al* to improve the alkylation of ethyl trifluoroacetate when non-activated halides were used. This involved firstly protecting the trifluoromethyl carbonyl moiety as either the dioxolane **10** or *N,N*-dimethyl hydrazone **11**, thus eliminating the possibility of *O*-alkylation, alkylating the base induced enol and deprotecting the carbonyl to give *C*-alkylated ethyl trifluoroacetate **12**. Treatment of **12** with base and a second equivalent of alkyl halide affords the dialkylated ethyl trifluoroacetate **13** (scheme 2.4).⁹



Reagents and conditions: (a) KH; (b) RX, KI cat., acetone, reflux 24-72 h; (c) KH; (d) R'X, THF-HMPA; (e) $\text{NH}_2\text{N}(\text{Me})_2$; (f) LDA, RX, THF-HMPA; (g) R''X, EtOH, reflux; (h) NaH; (i) $\text{ClCH}_2\text{CH}_2\text{OH}$; (j) LDA, RX, THF-HMPA; (k) BBr_3 , Hexane.

Scheme 2.4. Alkylation of ethyl trifluoroacetate.

Work by Patel *et al* have shown that reduction of ethyl trifluoroacetate with sodium borohydride, followed by treatment with TBDMS-OTf gave the silyl protected trifluoromethyl carbinol **14**. Hydrolysis of the ester allows the molecule to undergo a Curtius rearrangement, in the presence of benzyl alcohol to give the Cbz protected trifluoromethyl carbinol **15** in 75 % overall yield (scheme 2.5).² This work could have been extended to include alkylation of the induced enol with an alkyl halide (benzyl bromide) prior to reduction of the trifluoromethyl ketone.



Reagents and conditions; (a) NaBH_4 ; (b) 1 M NaOH ; (c) Et_3N , TBDMS-OTf; (d) K_2CO_3 ; (e) Et_3N , DPPA, PhCH_2OH , hexane, reflux 3 h.

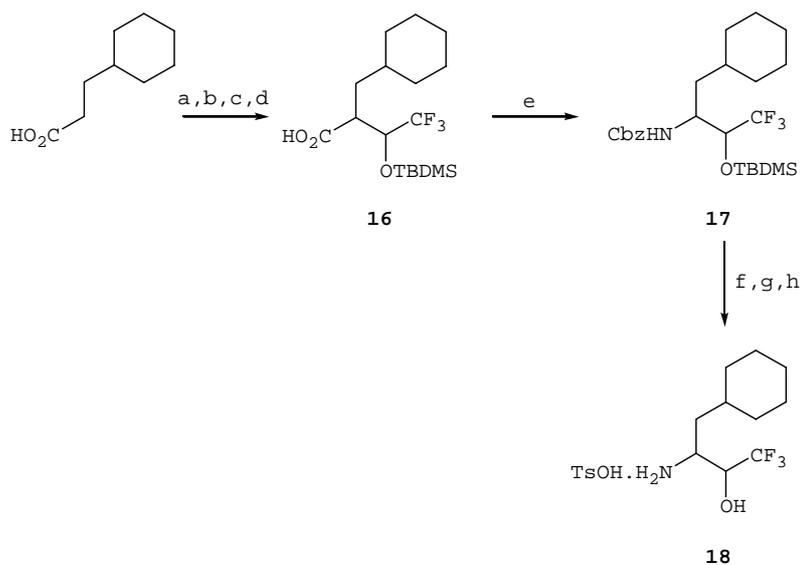
Scheme 2.5. α -Amino trifluoromethyl carbinols via a Curtius rearrangement.

2.2.4 Oxidation of secondary trifluoromethyl carbinols

As previously discussed, trifluoromethyl carbinols can be prepared by treatment of an aldehyde with trifluoromethylating agents. Another approach is to condense stabilised carbanions with fluorinated aldehydes to afford trifluoromethyl carbinols.^{2-4,19} One downside to this method however, is the lack of stereochemical control. Three different groups have reported two important strategies in this area.

Condensation of carbanions with trifluoroacetaldehyde

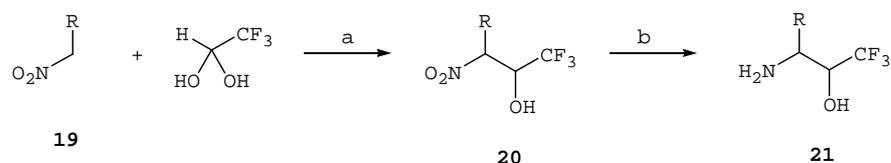
The synthesis of renin inhibitors by Patel *et al* had two key steps. The first step employed the condensation of a carboxylate dianion with trifluoroacetaldehyde followed by treatment with TBDMS-OTf to afford the silyl protected β -hydroxy acid **16**. The second key step involved a Curtius rearrangement of the carboxyl moiety by treatment with diphenylphosphoryl azide (DPPA) and benzyl alcohol affording a Cbz protected α -amino trifluoromethyl carbinol **17**. Double deprotection of the silyl ether (TBAF) and the carbamate (*p*-toluenesulfonic acid/MeOH) afforded the α -amino trifluoromethyl carbinol as the *p*-tosyl salt **18** (scheme 2.6).^{2,3}



Reagents and conditions: (a) LDA, THF, $-20\text{ }^\circ\text{C}$; (b) $[\text{CF}_3\text{CHO}]$, (c) Et_3N , TBDMS-OTf; (d) K_2CO_3 ; MeOH; (e) Et_3N , DPPA, PhCH_2OH , hexane, reflux 14 h.; (f) H_2 , $\text{Pd}(\text{OH})_2/\text{C}$, EtOAc; (g) TBAF; (h) TsOH, MeOH.

Scheme 2.6. Synthesis of an α -amino trifluoromethyl carbinol via a Curtius rearrangement.

The work of Abeles *et al* and Skiles *et al* both utilised the base catalysed condensation of a nitroalkane **19** with trifluoroacetaldehyde (Henry reaction) to give the corresponding α -nitro trifluoromethyl carbinol **20**. Reduction of the nitro group with Raney nickel/ H_2 or LiAlH_4 gave the α -amino trifluoromethyl carbinol **21** (scheme 2.7).^{4,19}



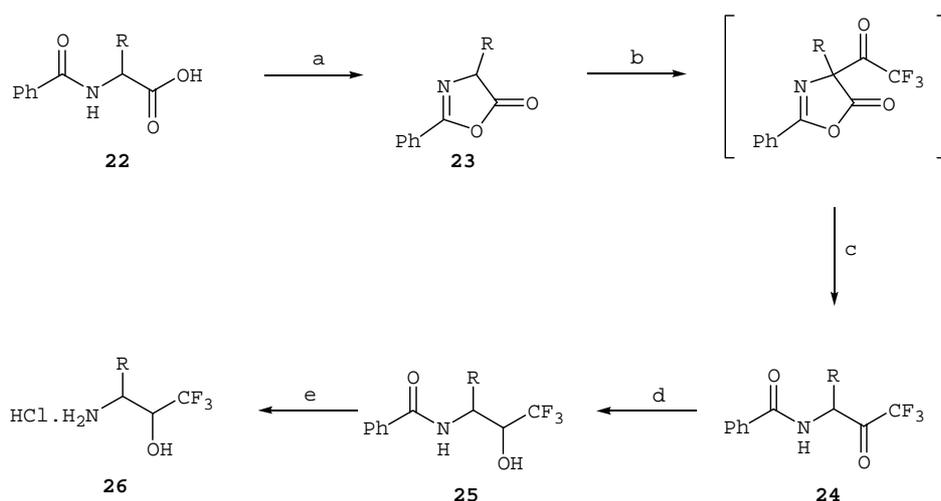
Reagents; (a) K_2CO_3 , $50\text{ }^\circ\text{C}$, 6 h., (b) Raney Ni/ H_2 , EtOH **or** LiAlH_4 , THF.

Scheme 2.7. Synthesis of α -nitro trifluoromethyl carbinols via a Henry reaction.

Trifluoroacetylation of oxazolones

The second strategy is that of Kolb *et al* and involves trifluoroacetylating 5(4*H*)-oxazolones with trifluoroacetic anhydride via a modified Dakin-West reaction. The 5(4*H*)-oxazolones **23** were prepared directly by acetic anhydride induced cyclisation of various

N-benzoyl protected amino acids **22**. The oxazolones were subjected to trifluoroacetylation, decarboxylation and ring opening in a one-pot synthesis that afforded *N*-benzoyl protected trifluoromethyl ketones **24** in moderate to good yields. Reduction of the ketone and deprotection of the amine afforded the trifluoromethyl carbinol **26** (scheme 2.8).^{7,20,21}



Reagents and conditions; (a) Ac₂O; (b) (CF₃CO)₂O; (c) oxalic acid; (d) NaBH₄, EtOH, 0 °C; (e) HCl, EtOH, H₂O, reflux 12 h.

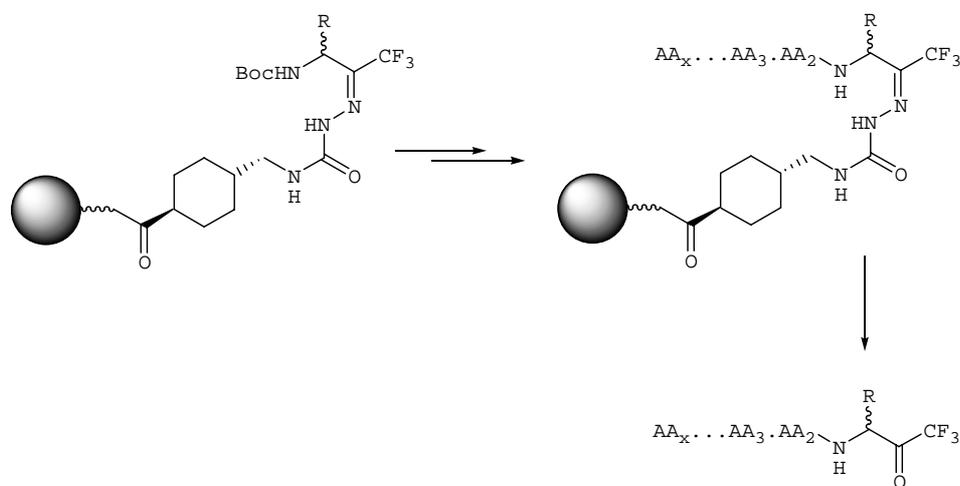
Scheme 2.8. Synthesis of trifluoromethyl carbinols via a modified Dakin-West reaction.

Oxidation of trifluoromethyl carbinols

Once the trifluoromethyl carbinol has been synthesised it can be readily coupled with other compounds to produce extended trifluoromethyl carbinols. Oxidation to the corresponding trifluoromethyl ketone has been achieved by a variety of reagents. These include oxalyl chloride/DMSO/Et₃N (Swern),^{7,17,22} carbodiimide/dichloroacetic acid/DMSO (Moffatt-Pfitzner),^{5,6,11} 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1*H*)-one (Dess-Martin periodinane),^{2-4,11,23} TEMPO/NaOCl/KBr and Ru(II)(biox)₂Cl₂/NaIO₄.²⁴

2.2.5 Solid phase synthesis of polypeptidyl trifluoromethyl ketones

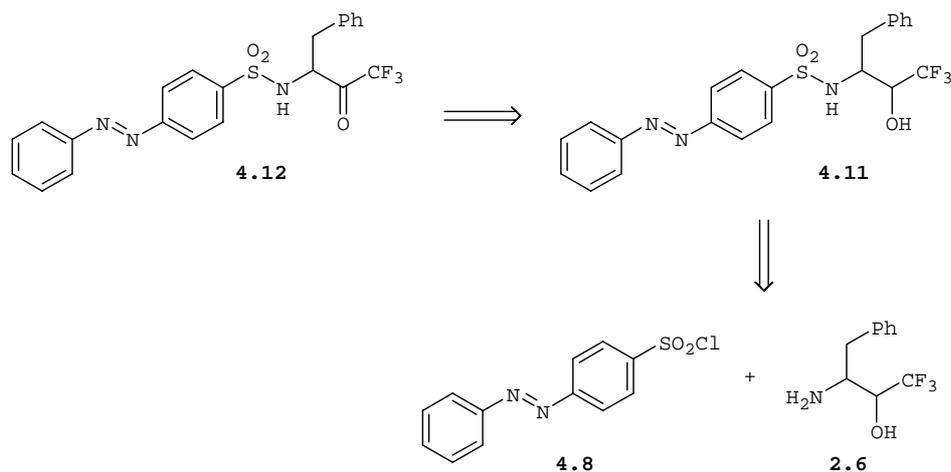
Poupart *et al* have successfully applied this methodology to the synthesis of a series of pentapeptidyl trifluoromethyl ketones using solid phase techniques. The key aspects of their system is the utilisation of a semicarbazone linker bound to a resin which serves as a protecting group for the trifluoromethyl carbonyl moiety and eliminates the need for a final oxidation step at the end of the synthesis (scheme 2.9).²²



Scheme 2.9. Solid phase synthesis of peptidyl trifluoromethyl ketones.

2.3 Designing the key trifluoromethyl carbinol intermediate 2.6

The focus of this thesis is the design and synthesis of potential photoswitchable inhibitors of α -chymotrypsin. After reviewing the literature it was decided that the easiest way to synthesise inhibitors based on trifluoromethyl ketones would be to oxidise the corresponding trifluoromethyl carbinol. Retrosynthetic analysis of attractive target molecules indicated the need to synthesise trifluoromethyl carbinols that mimic amino acids (scheme 2.10), therefore, the method chosen needed to be amenable to the synthesis of α -amino trifluoromethyl carbinols based on *L*-phenylalanine or *L*-tyrosine due to their preference for binding in the S₁ pocket.^{1,25}



Scheme 2.10. Retrosynthetic analysis of a target molecule.

There are a variety of methods outlined for preparing trifluoromethyl carbinols, including the aforementioned reaction of an organometallic reagent with trifluoroacetaldehyde or reaction of an aldehyde with CF_3I /activated zinc,¹¹ or $\text{CF}_3\text{-TMS}$.^{4,17} The method involving CF_3I was discounted immediately due to the difficulties involved with trying to import banned CFC's into the country. The option of using $\text{CF}_3\text{-TMS}$ was not immediately used as the author wished to avoid preparing amino aldehydes.

Modifications to the methods of both Abeles *et al* and Kolb *et al* have been used extensively in the work described in this thesis. This work, and other methods attempted will be discussed in sections 2.4.

2.4 Synthesis of 3-amino-1,1,1-trifluoro-4-phenylbutan-2-ol hydrochloride 2.6

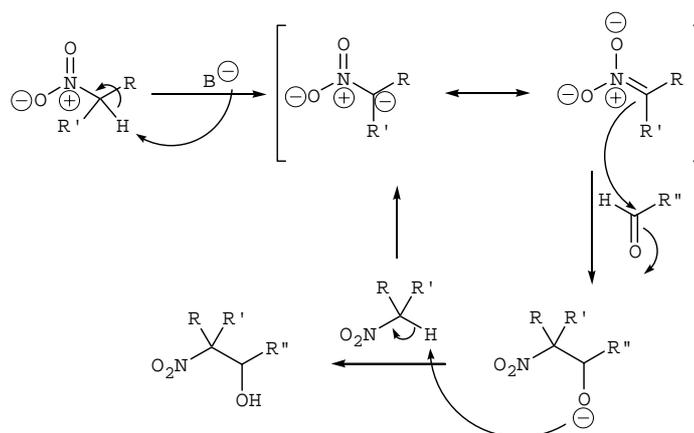
2.4.1 Route 1: Synthesis of 2.6 via trifluoroacetaldehyde

The initial work undertaken in this thesis (scheme 2.12) was based on the synthetic route of Abeles and Imperiali¹⁹ which employs the condensation of floral with a nitroalkane.

The Henry reaction

The Henry reaction, also known as the nitroaldol reaction is an important C-C bond forming reaction utilised in the synthesis of many key targets. The resulting β -nitroalkanol is very versatile as it can undergo many different transformations, including reduction of the nitro group, oxidation of the alcohol and elimination of water to afford nitroalkenes.²⁶ Unfortunately yields are often poor due to the susceptibility for side reactions (Aldol condensation, Cannizzaro reaction) to occur in the presence of base.

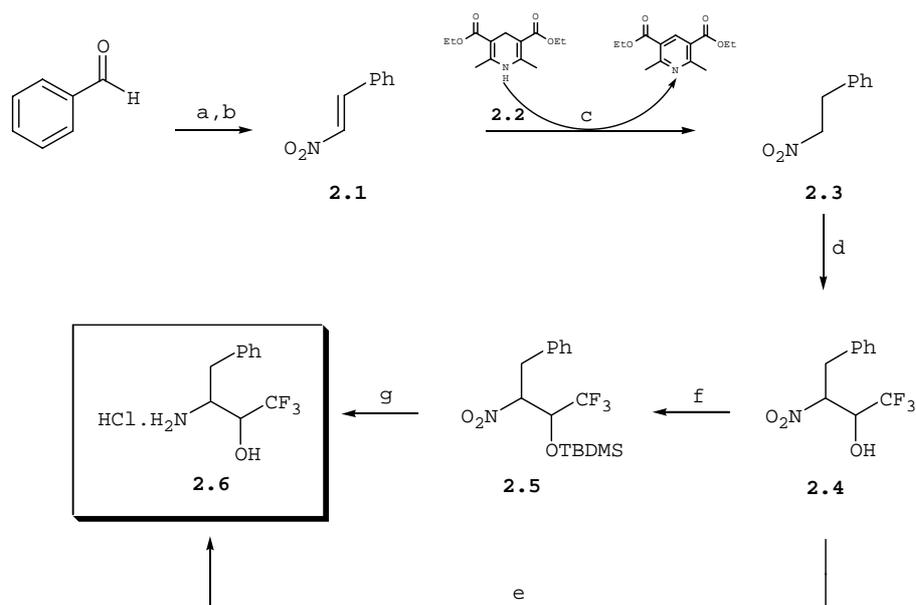
Treatment of nitroalkanes with a catalytic amount of base produces a resonance stabilised anion which then attacks the electrophilic centre of the aldehyde to afford the β -nitroalkoxide. The resulting alkoxide acts as a base, which in turn deprotonates another molecule of nitroalkane to give the desired β -nitroalkanol and another molecule of resonance stabilised anion (scheme 2.11).



Scheme 2.11. Mechanism of the Henry reaction.

Synthetic scheme

In order to prevent complications it was decided that all inhibitors synthesised would be based on the phenylalanine rather than tyrosine core to minimise any potential problems the phenolic hydroxide might cause further down the synthetic pathway.



Reagents and conditions; (a) CH_3NO_2 , NaOH, MeOH, $-10\text{ }^\circ\text{C}$; (b) 4 M HCl; (c) HEH, silica gel, toluene, $65\text{ }^\circ\text{C}$, 50 h; (d) $[\text{CF}_3\text{CHO}]$, K_2CO_3 , $50\text{ }^\circ\text{C}$; (e) Raney Nickel, H_2 , EtOH; (f) TBDMSCl, imidazole, DMF, $35\text{ }^\circ\text{C}$; (g) LiAlH_4 , ether, reflux.

Scheme 2.12. Synthetic scheme for Route 1.

Preparation of nitroalkane **2.3**

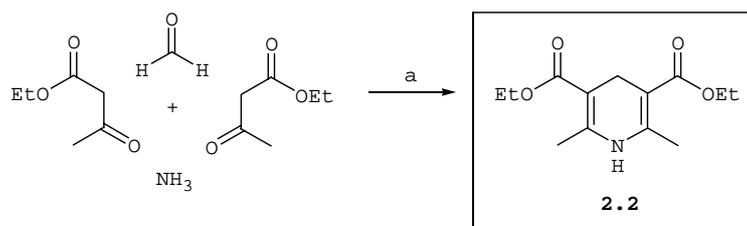
For α -nitro trifluoromethyl carbinols based upon phenylalanine, the required nitroalkane is 1-nitro-2-phenylethane **2.3** which can be prepared by reduction of the corresponding alkene β -nitrostyrene **2.1**. Although **2.1** is commercially available, it is readily prepared in the lab in less than a day from simple starting materials.²⁷ A mixture of benzaldehyde and nitromethane in MeOH was treated with an aqueous solution of sodium hydroxide followed by 4 M aqueous HCl to eliminate water from the molecule and afford crude nitrostyrene **2.1**. Recrystallisation from hot EtOH gave the pure product in 22 % yield.

Reduction of nitrostyrene **2.1**

Nitroalkanes are often obtained by reduction of α,β -unsaturated nitro compounds. Reduction can be achieved by various methods, but these methods are often harsh, result in product dimers and suffer poor chemoselectivity (especially when hydride reagents are employed). An attractive method is the use of NAD(P)H models such as Hantzsch ester

2.2 with an activator to effect reduction under mild conditions with very high chemoselectivity.^{28,29}

Hantzsch ester **2.2** was easily prepared by condensation of ethyl acetoacetate and formaldehyde with ammonia by the method of Singer *et al.*³⁰ Recrystallisation from EtOH gave the pure product in 62 % yield (scheme 2.13).



Reagents and conditions: (a) Diethyl amine, EtOH, 4 °C.

Scheme 2.13. Synthesis of Hantzsch ester **2.2**.

Nitrostyrene **2.1** in toluene was reduced with **2.2** in the presence of silica gel²⁸ over a period of 50 h. in a darkened fumehood. The resulting oil was distilled under reduced pressure and chromatographed to afford the pure nitroalkane **2.3** in 88 % yield (scheme 2.12). It has been shown that reduction also proceeds smoothly when catalytic amounts of acetic acid is used in place of silica gel.²⁹

*α -Nitro trifluoromethyl carbinol **2.4***

The condensation of nitroalkane **2.3** with trifluoroacetaldehyde methyl hemiacetal was catalysed under mild conditions with anhydrous K_2CO_3 in the absence of solvent (scheme 2.12). The outcome of this reaction was often variable and dependant on the amount of base added, temperature of the oil bath and purity of the hemiacetal. Usually a brown oil was obtained which was distilled under reduced pressure to afford **2.4** as a pale yellow oil comprising a variable mixture of diastereomers (1:1-2:1) in *ca.* 40-60% yield (fig. 2.1). In all cases where an excess of one diastereomer existed, the major diastereomer was always same. Freeze drying a sample of the oil resulted in crystallisation of the major diastereomer (fig. 2.2) which has been tentatively assigned as the (2*R*,3*S* + 2*S*,3*R*) isomers based on the coupling constants of the β -H, the stereochemistry of the major isomer of the

nitro reduction and a reported observation that the *anti* isomer crystallised from the oil of a similar compound.⁴ Sometimes the mixture would turn orange and solidify to give an unidentified gum. The problem was rectified when a new sample of hemiacetal was used.

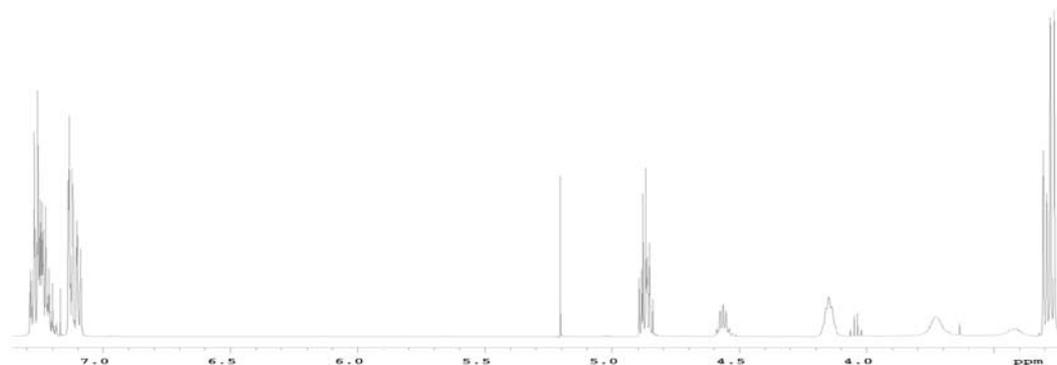


Figure 2.1. ¹H NMR spectrum of the 2:1 diastereomeric mixture of **2.4**. Key chemical shifts; α -H, major + minor, 4.96; β -H, minor, 4.65; β -H, major, 4.24 ppm.

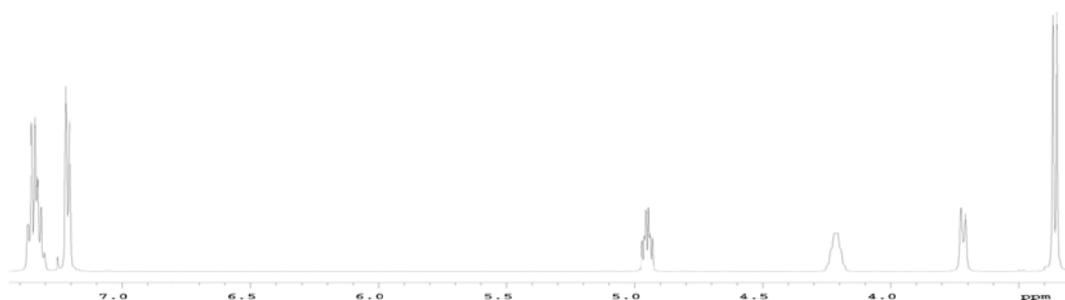


Figure 2.2. ¹H NMR spectrum of (*2R,3S* + *2S,3R*) **2.4**. Key chemical shifts; α -H 4.95, β -H 4.21 ppm.

α -Amino trifluoromethyl carbinol 2.6

The final step in the synthesis of the key trifluoromethyl carbinol was the reduction of the α -nitro trifluoromethyl carbinol to an α -amino trifluoromethyl carbinol. Raney nickel has been successfully utilised to reduce nitrocarbinols to aminocarbinols¹⁹ so Raney nickel W-2 was prepared from Ni-Al alloy according to the method outlined in Vogel's textbook of practical organic chemistry.³¹ A mixture of Raney nickel catalyst and nitrocarbinol **2.4** in EtOH was agitated under an atmosphere of hydrogen in a Parr shaker overnight. The resulting amine was converted to the hydrochloride and recrystallised from hot ethyl

acetate/petroleum ether to afford **2.6** as a single diastereomer in 34 % yield (scheme 2.12). Washing the filtrate with aqueous saturated NaHCO_3 afforded the free amine which consisted of > 90 % of the minor isomer. By comparing the ^1H and ^{19}F NMR of both diastereomers to experimental data obtained by Andrés *et al.*,¹⁷ the major isomer was tentatively assigned as (2*R*,3*S* + 2*S*,3*R*)-3-amino-1,1,1-trifluoro-4-phenylbutan-2-ol (*anti*-**2.6**) and the minor isomer as (2*S*,3*S* + 2*R*,3*R*)-3-amino-1,1,1-trifluoro-4-phenylbutan-2-ol (*syn*-**2.6**) based on the chemical shifts and coupling constants of the $\beta\text{-H}$ (fig. 2.3).

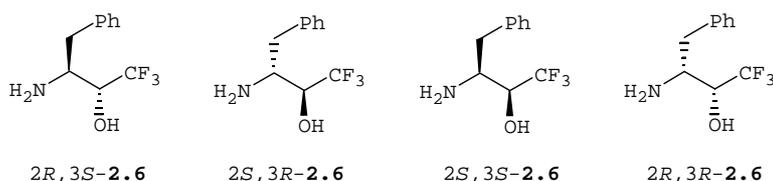


Figure 2.3. The four stereoisomers of **2.6**.

Due to the low yields, fickleness and pyrophoric nature of the Raney nickel reduction, other methods of reduction were investigated to see if the final step could be improved. The most promising of those investigated was reduction via LiAlH_4 . Reports that LiAlH_4 reduction of unprotected α -nitrocarbinols may undergo bond cleavage to yield the starting aldehyde and nitroalkane prompted the protection of the alcohol as a silyl ether before reduction.³²

The α -nitrocarbinol **2.4** was protected as the *tert*-butyldimethylsilyl ether³³ by treating **2.4** with TBDMSCl and imidazole in DMF to afford a 3:2 mixture of **2.5** in 93 % yield. Reduction of **2.5** in a suspension of ethereal LiAlH_4 at reflux, followed by a saturated aqueous Na_2SO_4 work-up gave the free amine. Treatment with HCl and recrystallisation from ethyl acetate/petroleum ether gave (2*R*,3*S* + 2*S*,3*R*) **2.6** in 24 % yield. Evaporation of the filtrate yielded a 1:2 mixture (by ^{19}F NMR) of the (2*R*,3*S* + 2*S*,3*R*) and (2*S*,3*S* + 2*R*,3*R*) isomers in 33 % yield.

This method, while successful, was not ideal as it required an additional synthetic step and purification, made use of the also potentially hazardous LiAlH_4 and the yield of isolated product was not great. This may have been due to difficulties in extracting the product

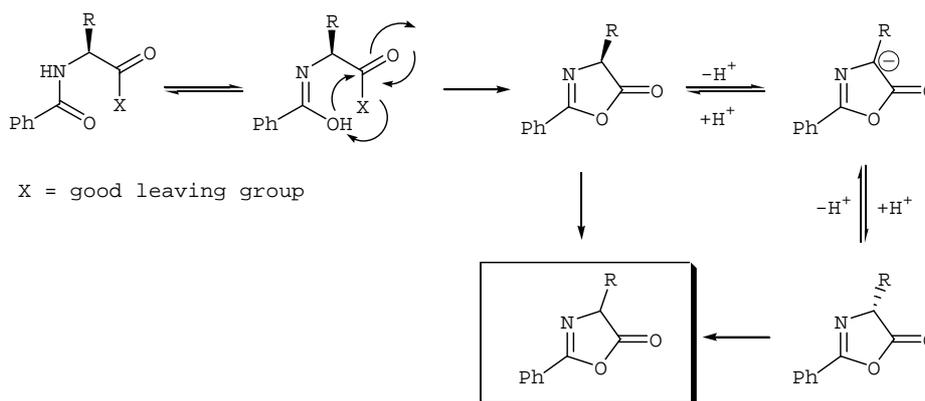
from the resulting white gelatinous mass that formed upon addition of aqueous Na_2SO_4 to the ethereal solution.

Other methods of reduction that were attempted included $\text{NiB}_2/\text{NaBH}_4$ ³⁴, $\text{NiB}_2/\text{hydrazine}$ ³⁵ and $\text{Pd/C}/\text{hydrazine}$ but met with failure and often decomposed the starting material and were therefore discontinued.

2.4.2 Route 2: Synthesis of 2.6 via trifluoroacetylation of oxazolones

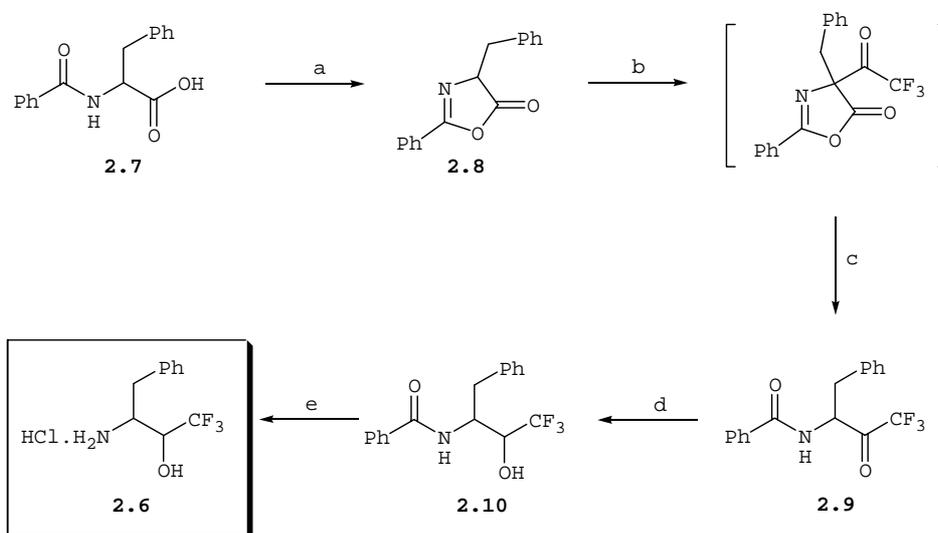
Oxazolone formation

Due to the unreliability of the Abeles method, and the generally mediocre yielding steps, an alternative method was required. The procedure by Kolb⁷ (scheme 2.8) had been previously investigated, but not pursued, as early attempts at preparing oxazolones with acetic anhydride were low yielding and difficult to purify. Further research into oxazolone formation showed that acyl amino acid cyclisation occurs readily during peptide synthesis and is responsible for the racemisation of the chiral centre (scheme 2.14).³⁶ Oxazolone formation is promoted by activation of the carboxylic acid to give a good leaving group, therefore carbodiimide mediated cyclisation was a viable method to try.



Scheme 2.14. Oxazolone formation and racemisation.

The synthesis of trifluoromethyl carbinol **2.6** from benzoyl phenylalanine was undertaken following the method of Kolb *et al* (scheme 2.15)



Reagents and conditions; (a) EDCI, DCM; (b) $(\text{CF}_3\text{CO})_2\text{O}$; (c) oxalic acid; (d) NaBH_4 , EtOH, 0°C ; (e) HCl, EtOH, H_2O , reflux 12 h.

Scheme 2.15. Synthesis of **2.6** via a modified Dakin-West reaction.

Synthesis of oxazolone **2.8**

N-Benzoyl-*L*-phenylalanine **2.7** was prepared by treating *L*-phenylalanine with benzoyl chloride in basic solution followed by acidification to precipitate out the *N*-protected acid **2.7**. The resulting white precipitate was recrystallised from hot water to afford the pure product. Treatment of a suspension of *N*-benzoyl-*L*-phenylalanine in dry dichloromethane with EDCI afforded oxazolone **2.8** in high yields ($> 95\%$) and purity (scheme 2.15).³⁷ Preparation was successful on a 50 mmol scale, but due to the relatively high cost and demand in the lab for EDCI, cheaper methods were investigated. The reaction was repeated using DCC in a THF/dichloromethane solvent system on a 65 mmol scale giving the identical product in 69 % yield. Acetyl chloride (1 equiv) and DMAP in dichloromethane also gave the pure oxazolone **2.8** in 49 % yield.

From these results it can be seen that EDCI gave the highest yields, but is also the most expensive of the reagents used to cyclise *N*-benzoyl-*L*-phenylalanine. Both DCC and

acetyl chloride have shown promise, however the conditions used were not optimised, therefore yields may be improved upon if the conditions were altered somewhat.

Introduction of the trifluoromethyl group

Recrystallised oxazolone **2.8** was stirred for 3 days in trifluoroacetic anhydride. After removal of residual anhydride and trifluoroacetic acid, the trifluoroacetylated oxazolone was treated with anhydrous oxalic acid at 120 °C to promote decarboxylation and subjected to a hydrolytic work-up to afford a 2:3 mixture of trifluoromethyl ketone **2.9** and the corresponding hydrate in 77% yield (scheme 2.15). The ketone and hydrate could be separated by column chromatography but was tedious due to the polar nature of the molecules, especially the hydrate. Chromatography was unnecessary however as the usual yellow impurity was easily removed with an ether wash and both ketone and hydrate were smoothly reduced to the corresponding trifluoromethyl carbinol **2.10**.

Reduction of the trifluoromethyl ketone

Reduction of the ketone/hydrate mixture was achieved using NaBH₄ in EtOH (scheme 2.15). After 12 h the reaction was quenched with 6 M aqueous HCl and worked up. The benzoyl protected trifluoromethyl carbinol **2.10** was isolated in > 95 % yield as a 2:3 mixture of diastereomers (fig. 2.4). No purification was required.

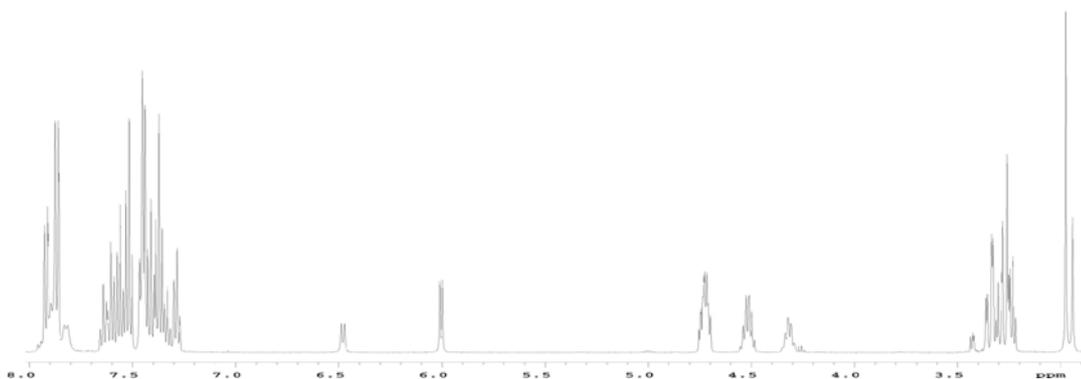


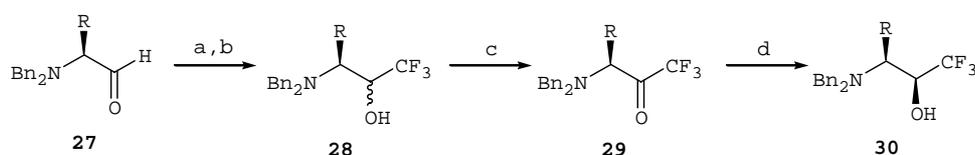
Figure 2.4. ¹H NMR spectrum of the 2:3 diastereomeric mixture of **2.10**. Key chemical shifts; OH, minor, 6.48; OH, major, 6.00; α -H, major + minor, 4.73; β -H, major, 4.52; β -H, minor, 4.32 ppm.

Trifluoromethyl carbinol deprotection

Removal of the benzoyl group was achieved by hydrolysis in concentrated HCl/EtOH/water at reflux (scheme 2.15). Removal of the liberated benzoic acid by extraction, followed by removal of the solvent afforded the crude α -amino trifluoromethyl carbinol as a 3:2 mixture of diastereomers. The crude hydrochloride salts were dissolved in hot ethyl acetate to give a green solution. Addition of cold petroleum ether induced precipitation of a white fluffy solid consisting of mainly the major diastereomer (9:1) in 65 % yield. A second recrystallisation afforded a single diastereomer identical to **2.6**, and therefore assigned as the (2*R*,3*S* + 2*S*,3*R*) isomers.

2.4.3 Attempted synthesis of 2.6 utilising CF₃-TMS

Synthesis of **2.6** via routes 1 and 2 gives rise to mixtures of all 4 possible isomers. Oxidation of the *N*-protected trifluoromethyl carbinol afforded the racemic trifluoromethyl ketone. Synthesis of stereospecific trifluoromethyl ketones would be desirable as that should lead to more potent inhibitors. Several authors^{4,11,17} report methods of synthesising trifluoromethyl ketones stereospecifically by preparing trifluoromethyl carbinols **28** from amino aldehydes **27** with predefined stereochemistry and oxidising the epimeric center to give enantiomerically pure trifluoromethyl ketones **29** (scheme 2.16).



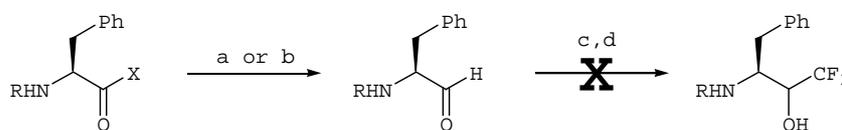
Reagents and conditions: (a) CF₃-TMS, TBAF, THF, 0 °C; (b) aq. NH₄Cl; (c) (COCl)₂, DMSO, Et₃N, DCM, -78 °C; (d) NaBH₄, MeOH, THF, -18 °C.

Scheme 2.16. Diastereoselective synthesis of trifluoromethyl carbinols.

Andés *et al* go even further and have devised a diastereoselective synthesis of α -amino trifluoromethyl carbinols from homochiral α -dibenzyl aldehydes **27** utilising CF₃-TMS. Swern oxidation of the resulting mixtures of *syn* (minor) and *anti* (major) diastereomers **28** afforded enantiomerically pure trifluoromethyl ketones **29**. Reduction of the ketones with

excess NaBH_4 in THF/MeOH afforded the *syn* isomer **30** with high diastereomeric excess (scheme 2.16).

For my work, a series of *N*-protected aminoaldehydes were prepared from *N*-protected *L*-phenylalanine. The aldehydes were synthesised either by LiAlH_4 reduction of Weinreb amides in THF at 0 °C or DIBAL reductions of methyl esters in toluene at -78 °C (scheme 2.17). The reductions usually went smoothly and in high yield, however reductions utilising DIBAL gave products of higher purity. The compounds are summarised in figure 2.5.



Reagents and conditions: (a) X = OMe, DIBAL, toluene, -78 °C; (b) X = N(OCH₃)CH₃, LiAlH_4 , THF, 0 °C; (c) $\text{CF}_3\text{-TMS}$, CsF or TBAF; (d) 4 M aq. HCl.

Scheme 2.17. Attempted trifluoromethylation of aldehydes with $\text{CF}_3\text{-TMS}$.

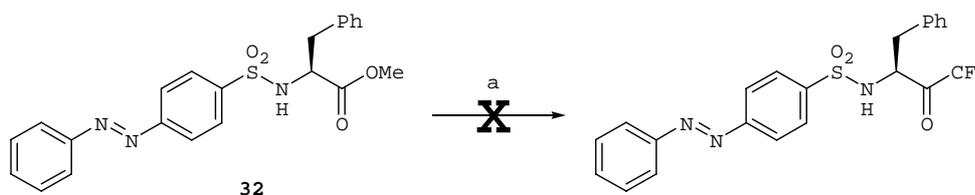
	R	X
31	Cbz	N(OCH ₃)CH ₃
32	Cbz	H
33	4-Az-SO ₂	OMe
34	4-Az-SO ₂	N(OCH ₃)CH ₃
35	4-Az-SO ₂	H

Figure 2.5. Derivatives of *L*-phenylalanine.

Initially, a solution of aldehyde **35** in dimethoxyethane was treated with a catalytic amount of CsF followed by a solution of $\text{CF}_3\text{-TMS}$ in THF (0.5 M, Aldrich[®]) (scheme 2.17).¹⁶ After stirring at rt overnight the solution was analysed by ¹H NMR but only starting materials were detected. Crushed molecular sieves and a second equivalent of $\text{CF}_3\text{-TMS}$ were added to the reaction mixture and stirred for a further 16 h. ¹⁹F NMR of the crude solution showed the presence of a doublet and a multiplet, the doublet being consistent

with fluorine atoms adjacent to a proton. ^1H NMR indicated the loss of the aldehyde proton at 9.55 ppm and the presence of a TMS peak at 0.05 ppm. The solution was filtered to remove the sieves then treated with 4 M aqueous HCl to hydrolyse the silyl ether. ^1H NMR of the hydrolysed product showed a more complex spectrum that did not match the starting aldehyde or expected product. No peaks were observed in the ^{19}F NMR spectrum which indicated no trifluoromethylated product was isolated.

The reaction was repeated in THF with 2 equivalents of $\text{CF}_3\text{-TMS}$ and initiated with TBAF¹² but again no fluorinated product was obtained. Reaction of Cbz-*L*-phenylalanyl (32) with $\text{CF}_3\text{-TMS/TBAF}$ (scheme 2.17) gave similar, disappointing results. Direct conversion of methyl ester 32 to the corresponding trifluoromethyl ketone (scheme 2.18)^{14,16} was unsuccessful and only ester 32 was recovered.



Reagents and conditions: (a) $\text{CF}_3\text{-TMS}$, TBAF, THF.

Scheme 2.18. Attempted direct conversion of an ester into a trifluoromethyl ketone.

It was concluded that the $\text{CF}_3\text{-TMS}$ reagent purchased from Aldrich[®] may have hydrolysed over time to give an inactive mixture of CF_3H and TMS-OH . A fresh supply of neat $\text{CF}_3\text{-TMS}$ was ordered but due to time constraints this area has not been continued.

2.5 Summary

Although two different routes were taken to synthesise the key trifluoromethyl carbinol 2.6, both methods ultimately gave the same product as varying mixtures of (2*R*,3*S*), (2*S*,3*R*), (2*S*,3*S*) and (2*R*,3*R*) isomers. Overall the route based on the oxazolone chemistry was preferred as the starting materials were generally cheaper, the reactions higher yielding, the products easier to purify and consistently more successful. Recrystallisation from ethyl acetate/petroleum ether afforded the major *anti*-diastereomer. This

recrystallisation could be seen as wasteful because ultimately, further down the synthetic route the 2-hydroxyl group was oxidised, removing one of the stereogenic centres and therefore reducing the number of possible isomers. The decision to remove the minor isomers from trifluoromethyl carbinol **2.6** was made to make isolation and purification of compounds discussed in chapters 3 and 4 more straight forward and to simplify the already complex NMR spectra of many azobenzene-trifluoromethyl carbinol based compounds described in this thesis.

2.6 Future work

Continuation in this area should focus on two main aspects. Firstly, optimising the syntheses of the key intermediate **2.6**, in particular, improving the formation of oxazolone **2.8** by cyclisation with cheap activating reagents such as DCC or acetyl chloride should be investigated. Secondly, the preparation of enantiomerically pure trifluoromethyl ketones should be revisited using a fresh supply of Ruppert's reagent.

Both of these areas can be expanded to include the synthesis of trifluoromethyl ketones which can inhibit other classes of enzyme. This could be achieved by altering the nature of the residue at the P₁ position, i.e. change the initial amino acid used from phenylalanine to something more specific to the enzymes' active site.

2.7 References for chapter two

- (1) Imperiali, B.; Abeles, R. H. *Biochemistry* **1986**, *25*, 3760-3767.
- (2) Patel, D. V.; Rielly-Gauvin, K.; Ryono, D. E. *Tetrahedron Letters* **1988**, *29*, 4665-4668.
- (3) Patel, D. V.; Rielly-Gauvin, K.; Ryono, D. E.; Free, C. A.; Smith, S. A.; Petrillo, E. W., Jr. *Journal of Medicinal Chemistry* **1993**, *36*, 2431-2447.

- (4) Skiles, J. W.; Fuchs, V.; Miao, C.; Sorcek, R.; Grozinger, K. G.; Mauldin, S. C.; Vitous, J.; Mui, P. W.; Jacober, S.; et al. *Journal of Medicinal Chemistry* **1992**, *35*, 641-662.
- (5) Warner, P.; Green, R. C.; Gomes, B.; Strimpler, A. M. *Journal of Medicinal Chemistry* **1994**, *37*, 3090-3099.
- (6) Damewood, J. R., Jr.; Edwards, P. D.; Feeney, S.; Gomes, B. C.; Steelman, G. B.; Tuthill, P. A.; Williams, J. C.; Warner, P.; Woolson, S. A.; et al. *Journal of Medicinal Chemistry* **1994**, *37*, 3303-3312.
- (7) Peet, N. P.; Burkhardt, J. P.; Angelastro, M. R.; Giroux, E. L.; Mehdi, S.; Bey, P.; Kolb, M.; Neises, B.; Schirlin, D. *Journal of Medicinal Chemistry* **1990**, *33*, 394-407.
- (8) Veale, C. A.; Bernstein, P. R.; Bohnert, C. M.; Brown, F. J.; Bryant, C.; Damewood, J. R., Jr.; Earley, R.; Feeney, S. W.; Edwards, P. D.; Gomes, B.; Hulsizer, J. M.; Kosmider, B. J.; Krell, R. D.; Moore, G.; Salcedo, T. W.; Shaw, A.; Silberstein, D. S.; Steelman, G. B.; Stein, M.; Strimpler, A.; Thomas, R. M.; Vacek, E. P.; Williams, J. C.; Wolanin, D. J.; Woolson, S. *Journal of Medicinal Chemistry* **1997**, *40*, 3173-3181.
- (9) Bégué, J. P.; Bonnet-Delphon, D. *Tetrahedron* **1991**, *47*, 3207-3258.
- (10) Chen, L. S.; Chen, G. J.; Tamborski, C. *Journal of Fluorine Chemistry* **1981**, *18*, 117-129.
- (11) Edwards, P. D. *Tetrahedron Letters* **1992**, *33*, 4279-4282.
- (12) Prakash, G. K. S.; Krishnamurti, R.; Olah, G. A. *Journal of the American Chemical Society* **1989**, *111*, 393-395.
- (13) Prakash, G. K. S.; Yudin, A. K. *Chemical Reviews* **1997**, *97*, 757-786.
- (14) Weidemann, J.; Heiner, T.; Mloston, G.; Prakash, G. K. S.; Olah, G. A. *Angewandte Chemie International Edition in English* **1998**, *37*, 820-821.
- (15) Walter, M. W.; Adlington, R. M.; Baldwin, J. E.; Schofield, C. J. *Journal of Organic Chemistry* **1998**, *63*, 5179-5192.
- (16) Singh, R. P.; Cao, G.; Kirchmeier, R. L.; Shreeve, J. n. M. *Journal of Organic Chemistry* **1999**, *64*, 2873-2876.
- (17) Andres, J. M.; Pedrosa, R.; Perez-Encabo, A. *European Journal of Organic Chemistry* **2004**, 1558-1566.
- (18) Aubert, C.; Bégué, J.-P.; Charpentier-Morize, M.; Nee, G.; Langlois, B. *Journal of Fluorine Chemistry* **1989**, *44*, 361-376.

- (19) Imperiali, B.; Abeles, R. H. *Tetrahedron Letters* **1986**, 27, 135-138.
- (20) Kolb, M.; Barth, J.; Neises, B. *Tetrahedron Letters* **1986**, 27, 1579-1582.
- (21) Kolb, M.; Neises, B. *Tetrahedron Letters* **1986**, 27, 4437-4440.
- (22) Poupart, M.-A.; Fazal, G.; Goulet, S.; Mar, L. T. *Journal of Organic Chemistry* **1999**, 64, 1356-1361.
- (23) Linderman, R. J.; Graves, D. M. *Tetrahedron Letters* **1987**, 28, 4259-4262.
- (24) Kesavan, V.; Bonnet-Delpon, D.; Begue, J.-P.; Srikanth, A.; Chandrasekaran, S. *Tetrahedron Letters* **2000**, 41, 3327-3330.
- (25) Geiger, R. In *Methods of enzymatic analysis*; Bergmeyer, H. U., Ed. 1984, p 99.
- (26) Ballini, R.; Bosica, G. *Journal of Organic Chemistry* **1997**, 62, 425-427.
- (27) Worrall, D. E. In *Organic Syntheses* 1929; Vol. 9, p 66-68.
- (28) Nakamura, K.; Fujii, M.; Oka, S.; Ohno, A. *Chemistry Letters* **1985**, 523-526.
- (29) Inoue, Y.; Imaizumi, S.; Itoh, H.; Shinya, T. *Bulletin of the Chemical Society of Japan* **1988**, 61, 3020-3022.
- (30) Singer, A.; McElvain, S. M. In *Organic Syntheses* 1934; Vol. XIV, p 30-33.
- (31) In *Vogel's textbook of practical organic chemistry*; 5th ed.; Furniss, B. S., Hannaford, A. J., Smith, S. A., Tatchell, A. R., Eds.; Longman Scientific and Technical: 1989, p 450.
- (32) Colvin, E. W.; Seebach, D. *Journal of the Chemical Society, Chemical Communications* **1978**, 689-691.
- (33) Corey, E. J.; Venkateswarlu, A. *Journal of the American Chemical Society* **1972**, 94, p 6190-6191.
- (34) Osby, J. O.; Ganem, B. *Tetrahedron Letters* **1985**, 26, 6413-6416.
- (35) Lloyd, D. H.; Nichols, D. E. *Journal of Organic Chemistry* **1986**, 51, 4294-4295.
- (36) *The Chemical Synthesis of Peptides*; 3rd ed.; Jones, J., Ed.; Clarendon Press: Oxford, 1994; Vol. 23.
- (37) Chen, F. M. F.; Kuroda, K.; Benoiton, N. L. *Synthetic Communications* **1979**, 230-232.

Chapter Three

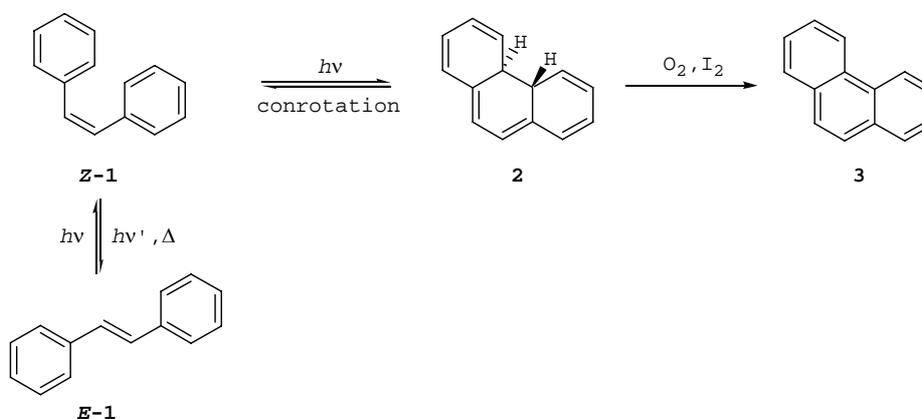
Synthesis of Stilbene and Phenanthrene Derivatives

Synthesis of Stilbene and Phenanthrene Derivatives

3.1 Introduction

Previous work by our group¹ has demonstrated the applicability of incorporating an azobenzene photoswitch into inhibitors of α -chymotrypsin. Introduction of such a photoswitch allows the potential to modify the potency of the inhibitor by inducing conformational change upon irradiation of UV and visible light (section 5.2.2). It was found that azobenzene based inhibitors enriched with the *cis* (*Z*) conformation were 2-3 fold more potent in their inhibition of α -chymotrypsin than the corresponding *trans* (*E*) isomers. Photoisomerisation of azobenzenes in solution are both photochemically and thermally reversible and will revert to a photostationary state comprising a mixture of *cis* and *trans* isomers upon standing, even in the dark.² As a consequence, it can be difficult to study the thermodynamically less stable *cis* isomer in a pure state.

It was envisaged that replacing the azo group with ethylene would afford the corresponding stilbene which are also known to undergo light induced *E-Z* isomerisation. Unlike azobenzenes however, it is possible to ‘trap’ *cis*-stilbenes by oxidative photocoupling to a rigid phenanthrene derivative (scheme 3.1).³ It was hoped that these phenanthrenes could be used as potential *cis* mimics of azobenzenes.



Scheme 3.1. Photoisomerisation, cyclisation and oxidative coupling of stilbene.

3.2 Photochemistry of stilbenes^{3,4}

Photo-induced *cis-trans* isomerisation is a well studied phenomenon that appears to be general for any double bond capable of geometrical isomerisation. Thermal or ground state isomerisation reactions are considered to proceed via a non-planar transition state common to both *cis* and *trans* isomers. The transition state collapses to give the thermodynamically more stable product (usually *trans*).

Photo-induced isomerisations involve population of excited singlet (paired electron spin) states (S_1) and triplet (parallel electron spin) states (T_1) followed by a vibrational cascade (collisional transfer) associated with twisting of the C-C σ -bond. The minimum energy state (*p*-state) occurs when adjacent *p*-orbitals are at 90° to one another. Internal conversion from the S_1 state or intersystem crossing from the T_1 state (fig. 3.1) affords a high energy ground state for the alkene which collapses to give a mixture of *cis* and *trans* isomers which comprise the photostationary state.

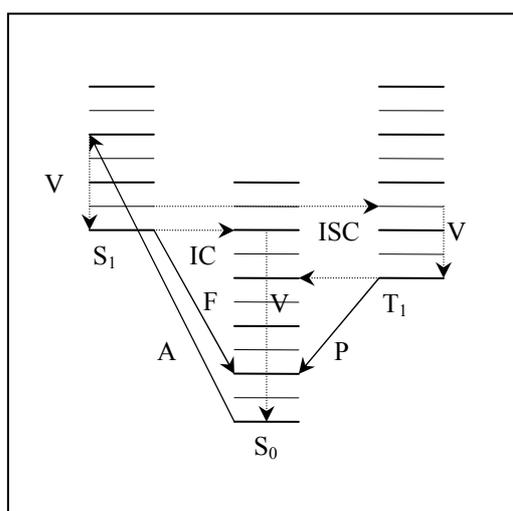


Figure 3.1. Jablonski diagram showing excitation and deactivation routes.

A = absorption; F = fluorescence; P = phosphorescence; V = vibrational cascade; IC = internal conversion; ISC = intersystem crossing

Irradiation of *cis* (**Z-1**) or *trans* (**E-1**) stilbene gives rise to an excited state **Z-1**^{*} or **E-1**^{*} which can undergo radiationless decay by way of a common twisted species **1**^{#*} to give either **E-1** or **Z-1**. Generally mixtures of the two are formed, the exact composition of the

photostationary state being determined by the wavelength of irradiation. For a more detailed discussion of photostationary states see section 5.2.

Excitation of **Z-1** to give the high energy species **Z-1*** can also undergo cyclisation to the excited dihydrophenanthrene **2***. If an oxidant is present **2** can be trapped as the phenanthrene **3**, in the absence of an oxidant **2** thermally or photochemically reverts to **Z-1** (fig. 3.2).⁴

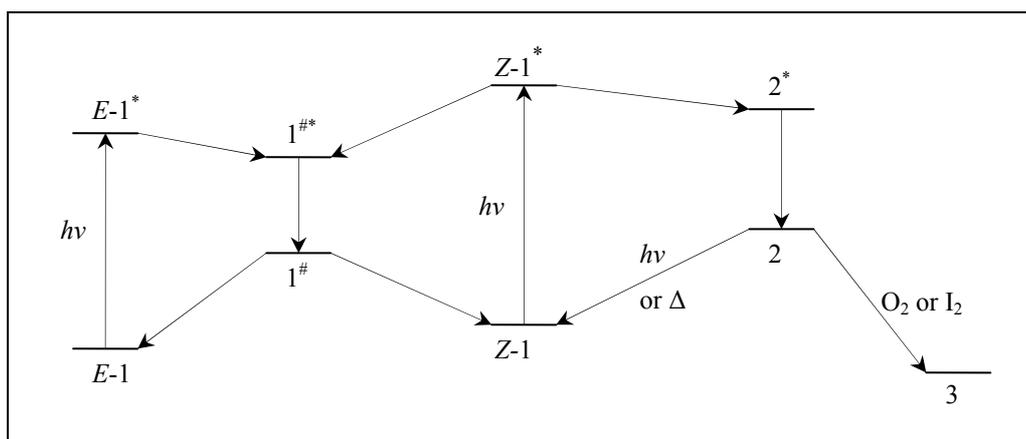


Figure 3.2. Energy level diagram for the photochemical processes of stilbene. Taken from Mallory and Mallory⁴

Absorption characteristics for *cis-trans* isomers are rarely the same; therefore effective filtering of light to give a single wavelength allows preferential absorption by one isomer resulting in complete conversion to the other and is known as optical pumping. Highly conjugated systems, in particular, stabilised alkenes such as stilbene undergo photoisomerisation more readily than non-conjugated alkenes. As a result, diaryl ethenes (stilbenes) have been widely studied. Steric hindrance between the *ortho*-hydrogens of *cis*-stilbene causes one phenyl ring to rotate out of the plane with respect to the rest of the molecule. This results in less effective overlap of the π system which alters the absorption characteristics of the molecule i.e. the *cis* and *trans* stilbenes exhibit different maxima (λ_{\max}) and molar absorptivity coefficients (ϵ) therefore UV/Visible spectroscopy has been widely utilised to monitor the isomerisation of stilbenes.⁵ This was especially important before the widespread use of NMR for interpreting results.

3.3 Synthesis of substituted stilbenes

In order for stilbene to be used as a switch for α -chymotrypsin inhibitors, some sort of linker to allow attachment to the peptidyl portion of the inhibitor needs to be incorporated into the molecule. Amide or sulfonamide linkages have previously been successfully incorporated into azobenzene moieties and allowed coupling of peptides and peptidomimetics in high yield (fig 3.3).

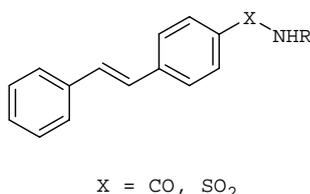
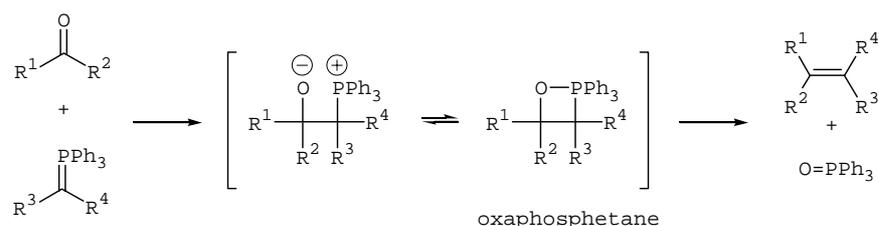


Figure 3.3. Stilbene coupled to an amine by an amide or sulfonamide linker

There are several methods for preparing diarylethenes. Traditional methods include condensation of aryl acetic acids with substituted benzaldehydes⁶ (Perkins reaction), aryl diazonium salts with cinnamic acid derivatives^{6,7} (Meerwein reaction), phosphonium ylides with aldehydes/ketones (Wittig reaction) or phosphonates and aldehydes/ketones (Wittig-Horner).⁸ Recently, a series of *trans*-tetrastilbenemethanes were synthesised via a Wittig-Horner reaction.⁵ More recent methods include use of palladium catalysed C-C coupling of aryl halides and arylenes⁹ (Heck reaction) or arylenyl halides with aryl boric acids¹⁰ (Suzuki reaction).

3.3.1 The Wittig reaction

The Wittig reaction is an important C-C bond forming reaction discovered by Georg Wittig in 1953. It involves replacement of the carbonyl oxygen of aldehydes or ketones with an alkene. The reactive carbonyl is attacked by the nucleophilic phosphorous ylide to give an oxaphosphetane which undergoes rearrangement to displace triphenylphosphine and liberate the alkene (scheme 3.2).⁸



Scheme 3.2. Mechanism of the Wittig reaction.

The driving force behind this reaction is the formation of a phosphorous-oxygen double bond which is stronger than the carbon-oxygen double bond and thus thermodynamically more favourable. The Wittig reaction is generally mild, tolerates a wide range of substituents and leaves no doubt as to the position of the double bond. Non-stabilised ylides ($R^1, R^2 = \text{H, alkyl}$) are more reactive than stabilised ylides ($R^1, R^2 = \text{COR, CN, CO}_2\text{R}$) and are therefore prone to hydrolysis under aqueous conditions. Use of bi-phasic mixtures can be employed to minimise potential loss of product.¹¹

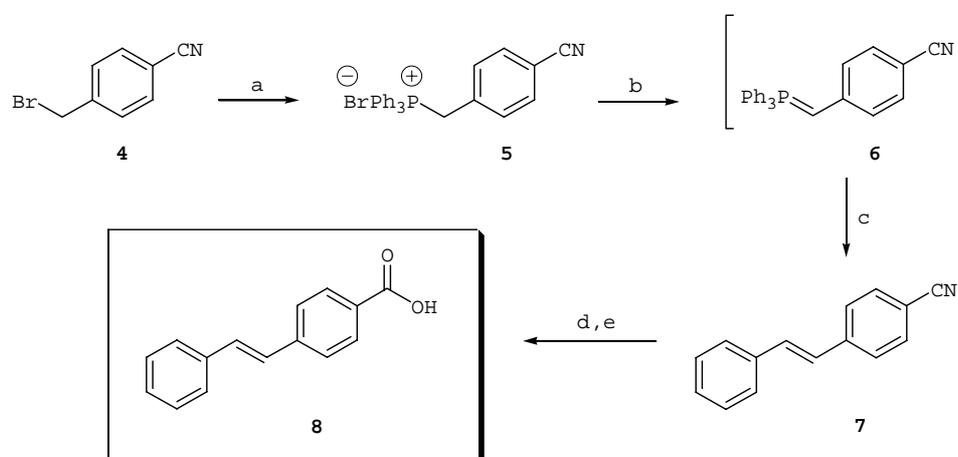
3.3.2 Synthesis of 4-cyanostilbene and 4-stilbenecarboxylic acid

Synthesis of 4-cyanostilbene and 4-stilbenecarboxylic acid (scheme 3.3) was achieved via the Wittig reaction by modification of an old undergraduate lab synthesis of 4-vinylbenzoic acid.¹¹

4-Stilbenecarboxylic acid via 4-cyanostilbene

4-Cyanostilbene **7** was prepared by treating commercially available α -bromo methyl benzonitrile **4** with triphenylphosphine in acetone to give the phosphonium bromide **5** in 79 % yield. A bi-phasic mixture of **5** in water and benzaldehyde in dichloromethane were stirred vigorously and ylide **6** generated *in situ* by slowly treating with concentrated aqueous NaOH. The reaction was stirred for 1 h, the organic layer separated, washed with water then brine, dried over MgSO_4 and concentrated *in vacuo*. The resulting oil was titrated with hot petroleum ether and 4-cyanostilbene **7** filtered off. 4-Cyanostilbene **7** was purified by flash chromatography affording a low melting solid in 24 % yield. Basic

hydrolysis of the nitrile with 10 % KOH in ethylene glycol⁶ followed by acidification gave the carboxylic acid **8** in 15 % yield (scheme 3.3).



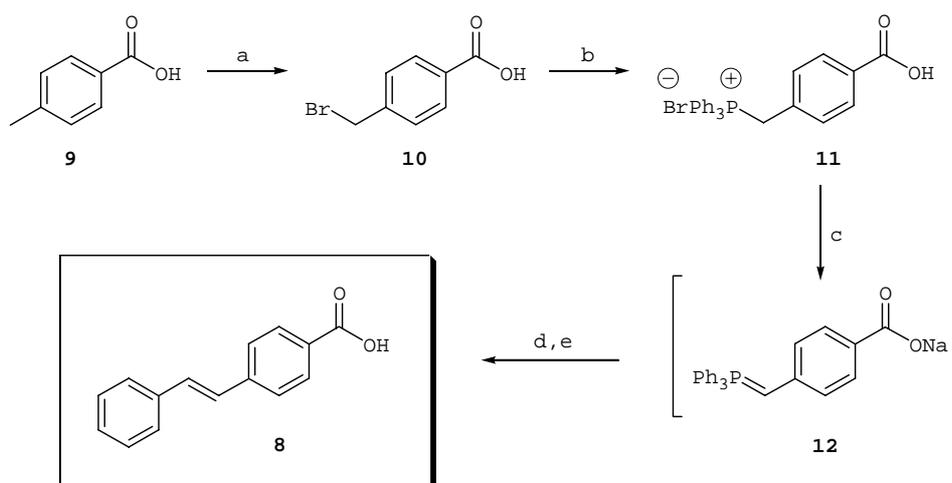
Reagents and conditions: (a) PPh_3 , acetone, reflux; (b) 50 % aqueous NaOH; (c) benzaldehyde, dichloromethane; (d) KOH, ethylene glycol, 165 °C; (e) conc. HCl.

Scheme 3.3. Synthesis of 4-stilbenecarboxylic acid via 4-cyanostilbene.

Direct synthesis of 4-stilbenecarboxylic acid

Due to the low yields of the Wittig reaction and subsequent hydrolysis it was thought that 4-stilbenecarboxylic acid could be made from toluic acid and benzaldehyde. This would reduce the number of synthetic steps by eliminating the hydrolysis of the nitrile and purification could be achieved via an aqueous work-up.

α -Bromo-toluic acid **10** was prepared by radical initiated bromination of toluic acid **9** with NBS in 86 % yield.¹² The phosphonium bromide **11** was prepared by treating **10** with triphenylphosphine in acetone. The phosphonium salt **11** precipitated from solution in 63 % yield. A bi-phasic mixture of benzaldehyde and **11** were treated with concentrated aqueous NaOH to generate ylide **12**. The suspension was filtered through celite, the aqueous layer extracted with dichloromethane to remove residual triphenylphosphine oxide and benzaldehyde then acidified to precipitate the 4-stilbenecarboxylic acid **8** in 31 % yield (scheme 3.4).

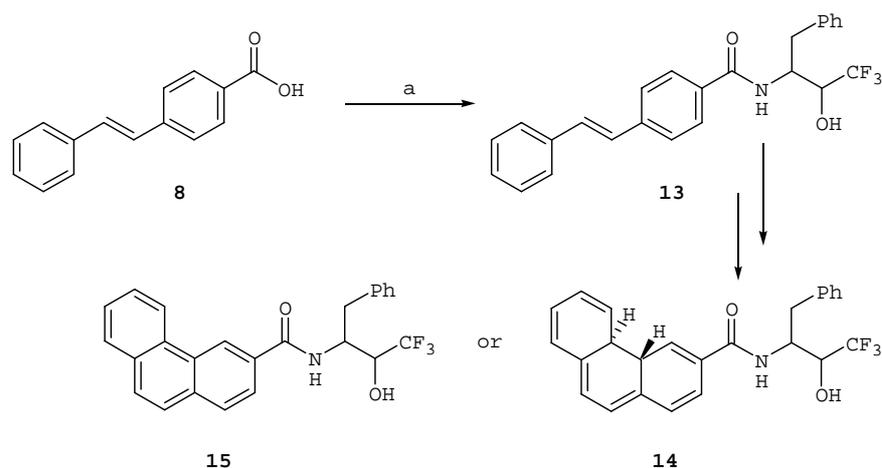


Reagents and conditions: (a) NBS, benzoyl peroxide, chlorobenzene, reflux; (b) PPh_3 , acetone, reflux; (c) 50 % aqueous NaOH; (d) benzaldehyde, dichloromethane; (e) conc. HCl.

Scheme 3.4. Synthesis of 4-stilbenecarboxylic acid via the Wittig reaction.

3.3.3 Attempted synthesis of *E*-[*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)-4-styryl benzamide] 16

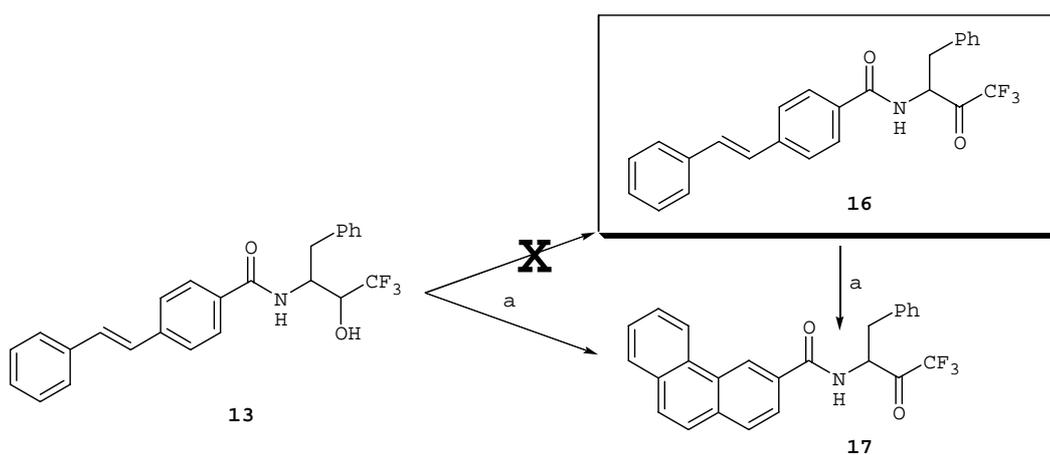
4-Stilbenecarboxylic acid **8** was coupled to trifluoromethyl carbinol **2.6** utilising EDCI methodology to afford the crude stilbene protected trifluoromethyl carbinol **13** (scheme 3.5). Purification by radial chromatography gave the desired product in 50 % yield. A second product was obtained and from the ^1H NMR spectrum it was noted that the olefinic protons at 6.65 ppm normally found in stilbene derivatives had disappeared. A repeat of the reaction utilising the same starting material resulted in *ca.* 1:3 mixture of stilbene **13** and presumably cyclised product (**14** or **15**). This was based on comparing the ratio of integrals of the olefinic protons with the NH, β , α and PhCH_2 proton signals in the ^1H NMR spectrum (1:2:2:2:4).



Reagents and conditions; (a) **2.6**, EDCI, HOBt, DIPEA, DCM, DMF.

Scheme 3.5 EDCI coupling of stilbene **8** with trifluoromethyl carbinol **2.6**.

Oxidation of trifluoromethyl carbinol **13** with Dess-Martin periodinane was unsuccessful, possibly due to poor solubility of the trifluoromethyl carbinol in dichloromethane. TEMPO oxidation in ethyl acetate/dichloromethane afforded a ketone and hydrate (scheme 3.6) which was confirmed by two singlets in the ^{19}F NMR spectrum. However it was also noted that the olefinic protons at 6.65 ppm had completely disappeared indicating that the desired product **16** was not present but instead presumably underwent cyclisation and oxidation to the corresponding phenanthrene **17** (scheme 3.6).



Reagents and conditions: (a) TEMPO, NaOCl, KBr, ethyl acetate/dichloromethane, 0 °C.

Scheme 3.6. TEMPO oxidation of *E*-stilbene **13**.

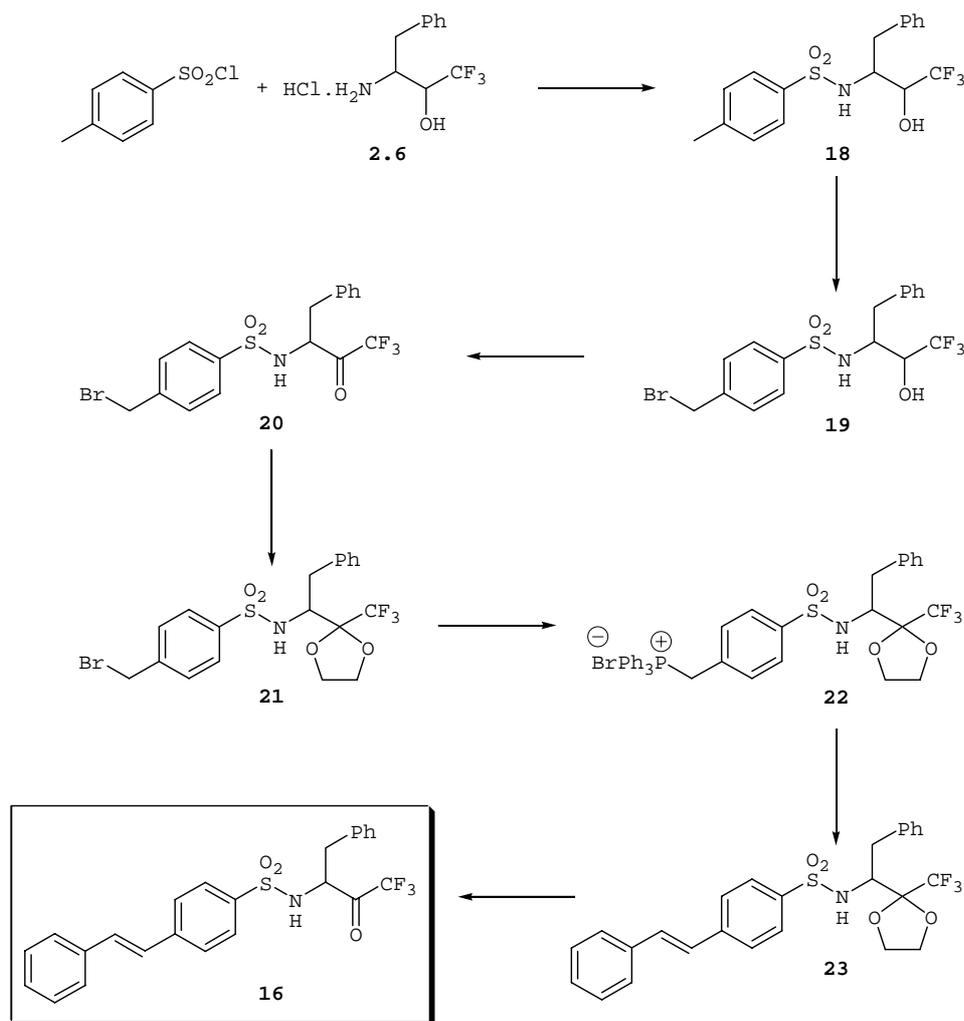
It has been reported that stilbenes incorporating fluoro, chloro, bromo, methoxy, methyl, trifluoromethyl, phenyl and carboxyl substituents undergo cyclisation and oxidation to the phenanthrene in the presence of an oxidant¹³ which gives some credence to the assumption that product **17** is the phenanthrene.

3.4 Alternate synthetic route for 4-substituted stilbenes

To minimise loss of product due to cyclisation, it was decided that a new approach was needed where the double bond is introduced further down the synthetic pathway. The new route was also designed to incorporate a sulfonyl rather than carbonyl linker to increase the similarity between the stilbene and azobenzene inhibitors synthesised in this thesis.^ξ

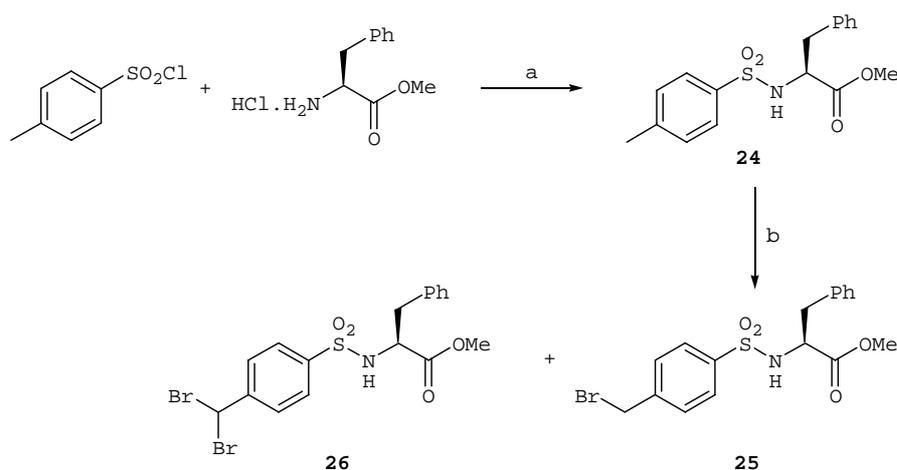
The proposed scheme involved coupling toluenesulfonyl chloride with trifluoromethyl carbinol **2.6** then brominating with NBS. At this point the trifluoromethyl carbinol **19** would be oxidised and the resulting trifluoromethyl ketone protected as the dioxolane **21** or dithiolane due their stability to a wide range of reaction conditions. The protected ketone could then follow a similar procedure already outlined to synthesis stilbene **23**. The final step would involve deprotection of the ketone to afford the desired product **16** (scheme 3.7).

^ξ Attempts at direct sulfonation of stilbene with chlorosulfonic acid had previously failed.

Scheme 3.7. Proposed synthetic route for stilbene **16**.

3.4.1 Bromination of toluenesulfonamides

A model system using *N*-tosyl-*L*-phenylalanine methyl ester was set up to determine whether toluenesulfonamides could undergo successful bromination. Toluenesulfonyl chloride and *L*-PheOMe.HCl were coupled in the presence of Hünigs base to give **24** in 94 % yield. Radical initiated bromination of **24** with NBS afforded a mixture of **24**, mono-brominated **25** and dibrominated **26** products (scheme 3.8). The ratios were difficult to determine from the ^1H NMR spectrum but it appears that **24** was the major component followed by **26** and the desired product **25** being the minor component. Chromatography of the mixture removed some of the unreacted starting material but could not separate the two brominated products, attempts at separation by recrystallisation also failed.



Reagents and conditions: (a) DIPEA, DCM, reflux; (b) NBS, benzoyl peroxide, chlorobenzene, reflux.

Scheme 3.8. Bromination of toluenesulfonamide **24**.

At this point the synthetic route was abandoned in favour of synthesising phenanthrenesulfonyl chlorides directly. This would eliminate the problems of complex mixtures of cyclised and non-cyclised products and allow us to synthesise pure phenanthrene compounds for comparison with analogous *cis*-azobenzenes.

3.5 Synthesis of phenanthrene based inhibitors

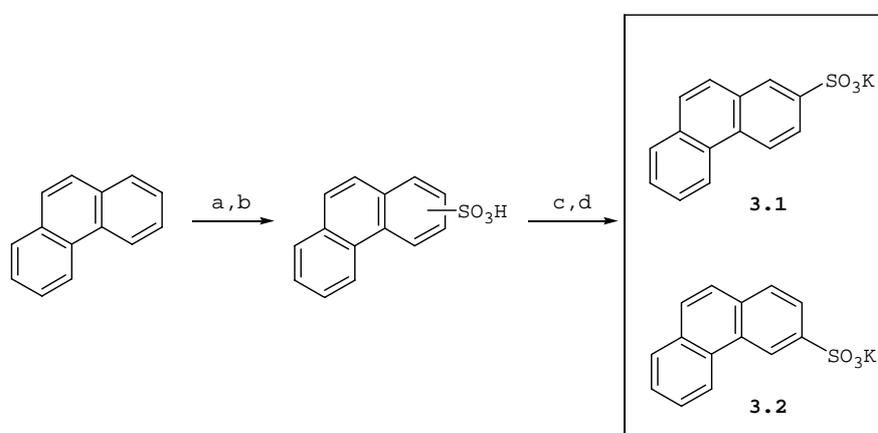
3.5.1 Fuctionalisation of phenanthrene

Due to the failure of preparing substituted stilbenes suitable for photoisomerisation studies it was decided to prepare pure phenanthrene sulfonamides due to their increased stability. The sulfonyl group was chosen as the linker to match the analogous azobenzene derivatives. The sulfonyl chloride is more stable than an acid chloride and does not need activating when reacting with amines. Arylsulfonyl chlorides are readily prepared from aryl sulfonates.¹⁴

2- and 3- Potassium phenanthrene sulfonates

Potassium 2- and 3-phenanthrene sulfonates were synthesised and isolated by the method outlined by Fieser.¹⁵

Recrystallised phenanthrene was heated until molten and treated with concentrated sulfuric acid to afford a mixture of mono and disulfonic acids. Addition of aqueous NaOH precipitated out the 2- and 3-isomers while the highly soluble disulfonates remained in solution. The two isomers were separated by exploiting their differences in solubility (scheme 3.9).

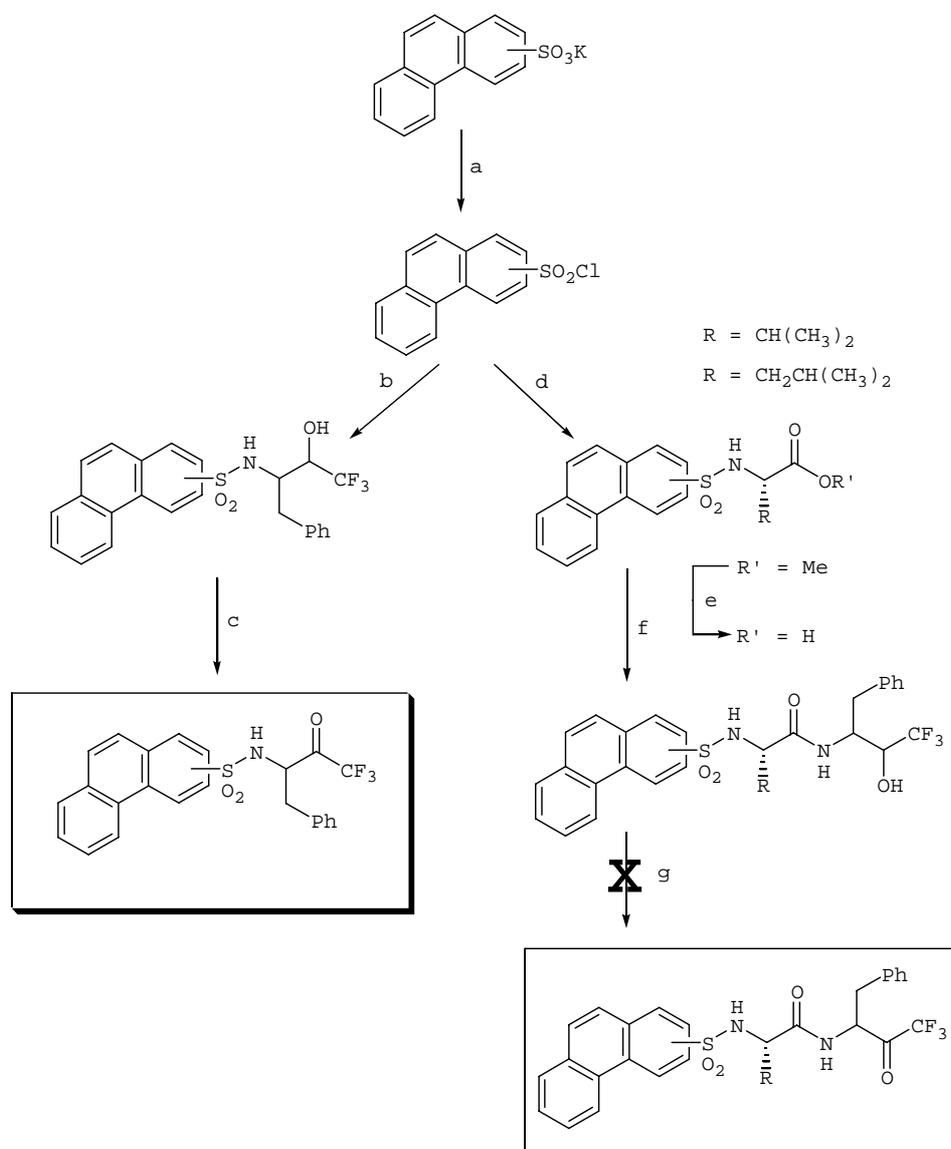


Reagents and conditions: (a) H_2SO_4 ; (b) NaOH; (c) $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$; (d) KOH.

Scheme 3.9. Synthesis of 2- and 3-phenanthrene sulfonates.

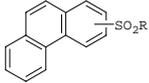
The sodium salts were redissolved in acidified water and concentrated. The less soluble 2-isomer crystallised out upon cooling. The mother liquor containing mostly 3-sulfonate with some 2-sulfonate was retained. The precipitated sodium 2-sulfonate was redissolved in a large volume of boiling water, treated with BaCl_2 and precipitated out as the highly insoluble barium salt. Several digestions of the barium sulfonate mixture followed by filtration removed the remaining traces of the sparingly soluble barium 3-sulfonate which crystallised out of solution upon cooling. The now pure barium 2-sulfonate in boiling water was treated with sulfuric acid and filtered to remove the barium as insoluble BaSO_4 , neutralised with KOH, concentrated and salted out with KCl. The precipitated 2-sulfonate was dissolved in the minimum amount of hot water and allowed to crystallise to give pure potassium 2-phenanthrene sulfonate in 9 % yield. The 3-sulfonate was obtained by

concentrating the original mother liquor and salting out with KCl. The precipitated potassium salts were dissolved in the minimum amount of hot water and cooled to crystallise out pure potassium 3-phenanthrene sulfonate in 10 % yield.



Reagents and conditions; (a) $\text{POCl}_3/\text{sulfolane}$, MeCN, DMA, 70 °C; (b) **2.6**, DIPEA, DCM, reflux; (c) Dess-Martin periodinane, DCM; (d) *L*-ValOMe.HCl or *L*-LeuOMe.HCl, DIPEA, DCM, reflux; (e) K_2CO_3 , MeOH/ H_2O , reflux; (f) EDCI, HOBT, DIPEA, DCM; (g) Dess-Martin periodinane, DCM.

Scheme 3.10. Synthesis of phenanthrenesulfonamides.

	Substitution position	R
3.1	2	OK
3.2	3	OK
3.3	2	Cl
3.4	2	TFMC
3.5	2	TFMK
3.12	3	Cl
3.13	3	TFMC
3.14	3	TFMK

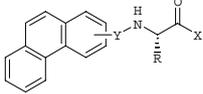
	Y	R	X
3.6	2-SO ₂	CH(CH ₃) ₂	OMe
3.7	2-SO ₂	CH(CH ₃) ₂	OH
3.8	2-SO ₂	CH(CH ₃) ₂	TFMC
3.9	2-SO ₂	CH ₂ CH(CH ₃) ₂	OMe
3.10	2-SO ₂	CH ₂ CH(CH ₃) ₂	OH
3.11	2-SO ₂	CH ₂ CH(CH ₃) ₂	TFMC
3.15	3-SO ₂	CH(CH ₃) ₂	OMe
3.16	3-SO ₂	CH(CH ₃) ₂	OH
3.17	3-SO ₂	CH(CH ₃) ₂	TFMC
3.18	3-SO ₂	CH ₂ CH(CH ₃) ₂	OMe
3.19	3-SO ₂	CH ₂ CH(CH ₃) ₂	OH
3.20	3-SO ₂	CH ₂ CH(CH ₃) ₂	TFMC

Figure 3.5. Compound numbers for 2- and 3-peptidyl phenanthrenesulfonamides from scheme 3.10.

Although the overall yields were low, the chemistry was undertaken on a large scale and resulted in *ca.* 4 g of each phenanthrene sulfonate being obtained. Both were subsequently utilised in the synthesis of a series of phenanthrene based inhibitors (scheme 3.10).

2- and 3-Phenanthrenesulfonyl chloride

The isolated sulfonates were readily converted into the corresponding sulfonyl chlorides by treating a suspension of **3.1** or **3.2** in a mixture of sulfolane and acetonitrile with POCl₃.¹⁴ Addition of a few drops of dimethylacetamide catalysed the reaction and afforded the 2- and 3-sulfonyl chlorides **3.3** and **3.12** in 95 and 98 % yield respectively (scheme 3.10).

NMR characteristics of 2- and 3-phenanthrenes

The phenanthrene isomers were readily identified by ¹H NMR. The ¹H NMR spectra of the 2-isomers have characteristic doublet, doublet, (doublet) singlet patterns (fig. 3.6a) while the ¹H NMR spectra of the 3-isomers shows distinct singlet, doublet (doublet), patterns (fig. 3.6b).

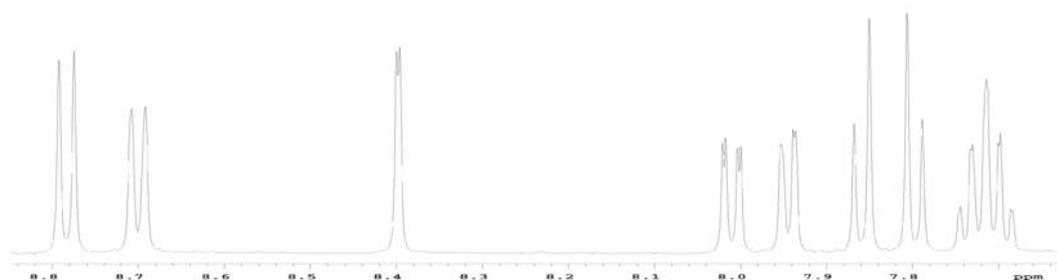


Figure 3.6a. A representative ¹H NMR spectrum for 2-phenanthrene protons. The doublets corresponding to **H9** (7.86 ppm) and **H10** (7.80 ppm) are separated by 31 Hz.

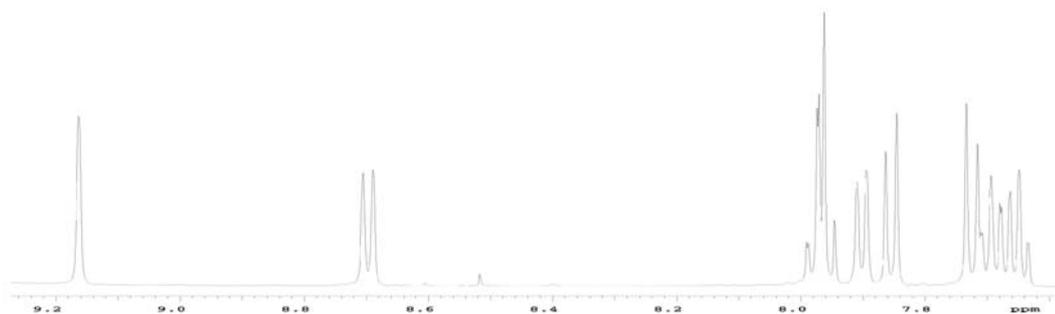


Figure 3.6b. A representative ^1H NMR spectrum for 3-phenanthrene protons. The doublets corresponding to **H9** (7.85 ppm) and **H10** (7.22 ppm) are separated by 64 Hz.

In all cases, the singlet peak of 3-phenanthrenesulfonyl compounds, corresponding to the 4-position of the phenanthrene ring is furthest downfield. In some cases the singlet appears as a minor doublet ($J = 2$ Hz) due to long range coupling with the other proton adjacent to the sulfur, resulting in the appearance of a corresponding doublet of doublets ($J = 2, 9$ Hz). Another common feature is the large separation of the signals corresponding to the doublets associated with **H9** and **H10**. The separation between the two doublets is typically around 30 Hz for the 2-isomer (fig. 3.6a) and 60-80 Hz for the 3-isomer (fig. 3.6b).

3.5.2 Synthesis of 2- and 3-phenanthrene trifluoromethyl carbinols

A series of peptides and peptidyl analogues were smoothly coupled to the 2- and 3-phenanthrenesulfonyl chlorides **3.3** and **3.12** (scheme 3.10) using the Hünigs base method (General method C) in high yields. Initially the standard pyridine coupling method was used (General method A)¹⁶ but this often resulted in oily solids which required further work-up and purification. The ease of coupling, high yield, and crystalline nature of most of the solids demonstrates a great potential as a means of crystallising and identifying unknown amino acids and amines. The chemical shifts of the phenanthrene protons are generally downfield of most normal aromatic protons and when performed in deuterated acetone give well resolved, separated peaks which make coupling constant analysis much more simple.

Oxidation of phenanthrene trifluoromethyl carbinols

Phenanthrenesulfonyl chlorides **3.3** and **3.12** were coupled to trifluoromethyl carbinol **2.6** in quantitative yields. Oxidation of **3.4** and **3.13** to the trifluoromethyl ketones **3.5** and **3.14** was achieved by Dess-Martin periodinane¹⁷ in 56 and 48 % yields respectively (scheme 3.10). The low solubility of **3.4** and **3.13** and periodinane in dichloromethane may have contributed to the moderate yields. A mixture of ketone and hydrate was observed when the NMR was run in CDCl₃; however running the NMR in a hydroscopic solvent such as DMSO or deuterated acetone greatly diminished the amount of ketone **3.5** or **3.14** present. Addition of a drop of D₂O to the sample gave exclusively hydrate, simplifying the spectrum and confirming the rapid formation of the hydrate in aqueous media.

3.5.3 Synthesis of 2- and 3-phenanthrene dipeptidyl analogues

In order to increase the specificity of the inhibitor for α -chymotrypsin it is desirable to increase the number of residues that interact with the binding pockets of the enzyme.

It has been reported that the addition of a single amino acid to the peptidyl core of the inhibitor increased the potency of **27** some 30 fold.¹⁸ For α -chymotrypsin a hydrophobic non-aromatic residue such as *L*-valine or *L*-leucine in the P₂ site is recommended to complement the S₂ subsite (fig. 3.7).¹⁹ Syntheses that incorporate both of these residues were undertaken.

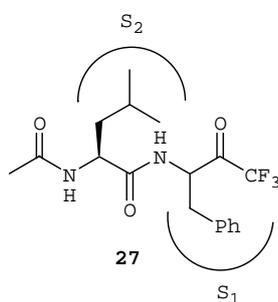


Figure 3.7. A reported inhibitor of α -chymotrypsin.

Ester hydrolysis

Phenanthrenes **3.3** and **3.12** were each coupled to *L*-valine or *L*-leucine methyl ester hydrochloride in the presence of Hünigs base to afford the *N*-protected amino acids **3.6**, **3.9**, **3.15** and **3.18** in excellent yields (scheme 3.10). All four compounds were recrystallised to afford very pure crystalline products. Hydrolysis of the ester was first attempted with 0.25 M LiOH in MeOH/H₂O at rt. Little or no hydrolysis was observed after 18 h. Hydrolysis was eventually achieved with KOH in EtOH/H₂O at reflux but the hydrolysed product contained an unknown yellow impurity that was usually removed with repeated recrystallisation. Repeating the hydrolysis under milder conditions using K₂CO₃ in MeOH/H₂O at reflux afforded the free acids **3.7**, **3.10**, **3.16** and **3.19** in very high yield (scheme 3.10). The free acids contained none of the impurities present using KOH, although longer reaction times were required to effect hydrolysis, especially for valine methyl esters.

Coupling and attempted oxidation of dipeptidyl trifluoromethyl carbinols

Trifluoromethyl carbinol **2.6** was coupled to each of the four free acids **3.7**, **3.10**, **3.16** and **3.19** utilising EDCI methodology to give *ca.* 1:1 mixtures of diastereomers **3.8**, **3.11**, **3.17** and **3.20** in good yields (scheme 3.10). Attempted oxidations of the dipeptidyl trifluoromethyl carbinols **3.8**, **3.11**, **3.17** and **3.20** with Dess-Martin periodinane failed; again the near insolubility of the dipeptides in dichloromethane was probably a contributing factor. It was also suspected that due to the continued failure of the Dess-Martin periodinane to oxidise trifluoromethyl carbinols, even on compounds previously oxidised with Dess-Martin periodinane that this particular batch had deteriorated to give hydrolysed and/or polymeric material. A new batch of Dess-Martin periodinane was synthesised²⁰ but the oxidations were not repeated as it was believed that the poor solubility of the dipeptidyl mimics would limit their effectiveness as inhibitors in aqueous media.

3.6 Molecular modelling

When comparing the structures of *cis*-azobenzene and phenanthrene derivatives drawn in the same orientation, side by side, *cis*-3-azobenzene and 2-phenanthrene derivatives appear to adopt similar conformations as do *cis*-4-azobenzene and 3-phenanthrene derivatives (fig. 3.8).

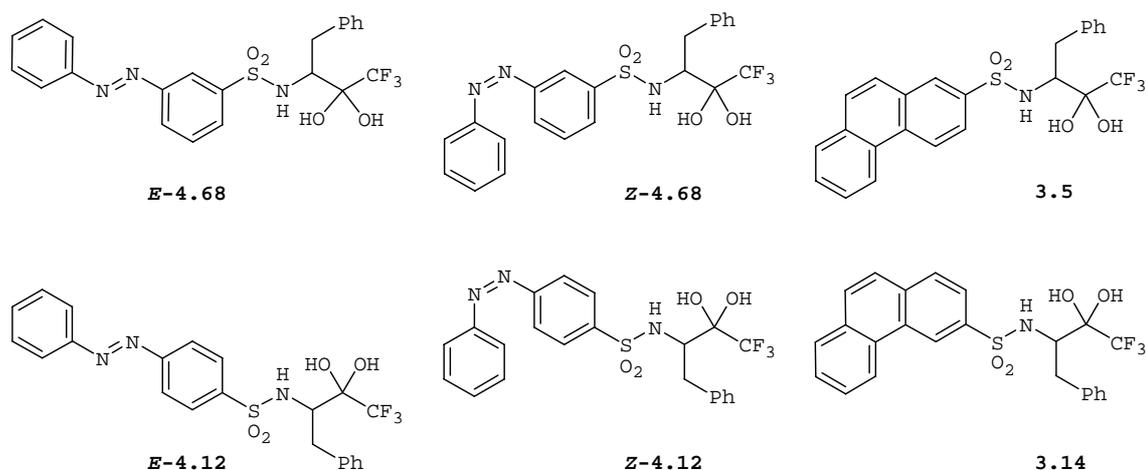


Figure 3.8. Comparisons of phenanthrene and azobenzene conformations.

However, it is known from the crystal structure²¹ that *cis*-azobenzenes adopt a non-planar conformation (fig. 3.9) whereas *trans*-azobenzenes and phenanthrenes are planar and as such will occupy different spatial environments.

Molecular modelling was undertaken by B. Stuart on the hydrate forms of trifluoromethyl ketones *E*-4.68, *Z*-4.68, *E*-4.12, *Z*-4.12, 3.5 and 3.14. The hydrate structure has been modelled as it is the predominant species found in solution.^{18,22} Superimposition of all four models of the azobenzene isomers along the trifluoromethyl ketone backbone of both phenanthrene isomers enabled comparison of the terminal ring systems.

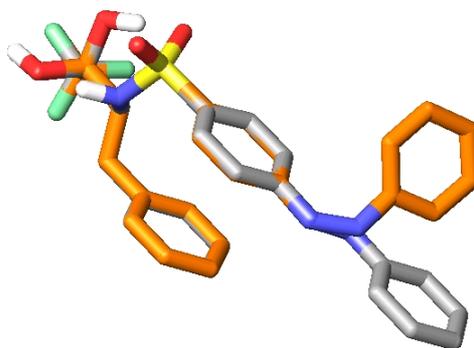


Figure 3.9. Overlapped 3-D models of *E*- and *Z*-4.12

3.6.1 Minimum energy conformation

The six structures were constructed *in silico* using the Maestro build function of the Schrodinger molecular modelling suite. A conformational search of each structure was carried out with MacroModel using the OPLS2001 forcefield with the CHCl₃ solvent model. Constraints were used for *cis*-azobenzenes **Z-4.88** and **Z-4.12** where the C-N-N-C dihedral angle was constrained to 8° with a force constant of 100 kJ/mol.²³ The criteria for convergence of each conformational search was the generation of 3000 starting conformations and a maximum of 5000 iterations in the energy minimisation routine for each conformer, collecting the ensemble of conformers within 12 kJ of the global minima. The lowest energy conformer of each structure was then used for superimposition. Non-polar hydrogens have been removed and the azobenzene carbon skeleton coloured orange for clarity.

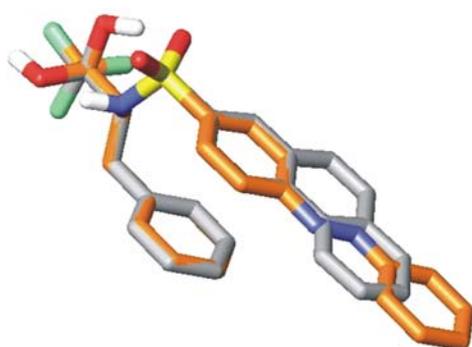
3.6.2 General characteristics of the models

There are several features common to each of the energy minimised structures. The aromatic ring of the phenylalanine mimic is arranged so that complementary π - π stacking, as can be seen in figure 3.9, occurs between the phenyl ring and the first ring of the switch or pseudo switch. The peptidyl backbone has also rearranged to take advantage of intramolecular attractions between the sulfonamide and the diol (2.293 Å), the

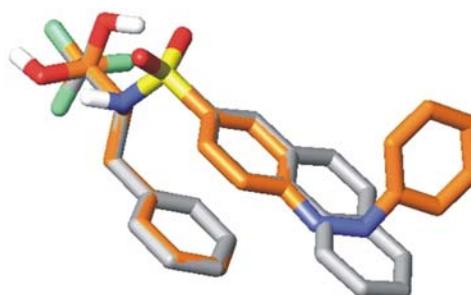
trifluoromethyl moiety and diol (2.375 Å) and a true hydrogen bond between the sulfonyl oxygens and the diol (1.682 Å).

Comparisons of 2-phenanthrene with *E/Z* 3- and 4-azobenzenes

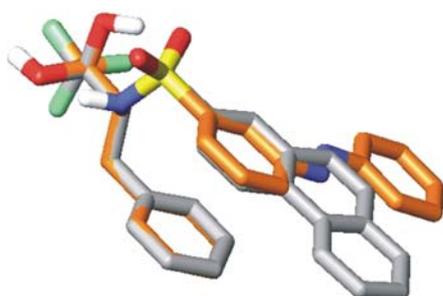
By comparing the overlap of the 2-phenanthrene isomer **3.5** with azobenzenes **4.12** and **4.68** (fig 3.10) it is obvious that *E*-**4.12** is most similar. The phenanthrene ring is somewhat shorter the azobenzene moiety but the overall dimensions and spatial arrangements are comparable. *Z*-**4.12** is comparable in length but the twisting of terminal phenyl ring from planarity significantly alters the spatial arrangement of the azobenzene. In contrast to the structural diagrams, both *E*-**4.68** and *Z*-**4.68** compare poorly as mimics of the 2-phenanthrene derivatives.



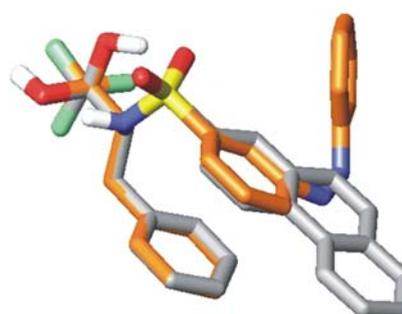
3.5 and *E*-4.12 overlapped.



3.5 and *Z*-4.12 overlapped.



3.5 and *Z*-4.68 overlapped.

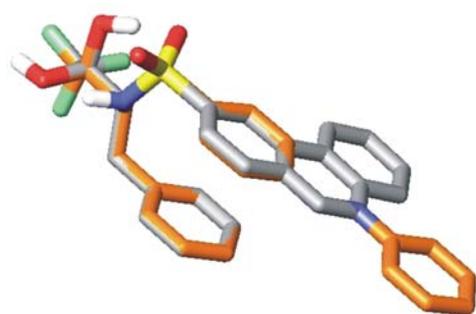


3.5 and *E*-4.68 overlapped.

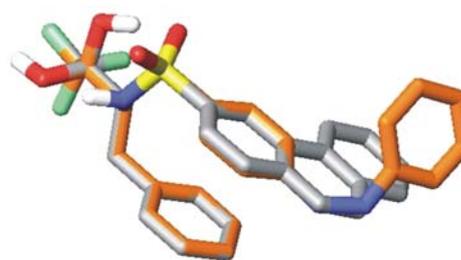
Figure 3.10. Comparison of 3- and 4-azobenzenes with 2-phenanthrenes.

3.6.4 Comparisons of 3-phenanthrene with *E/Z*-3- and 4-azobenzenes

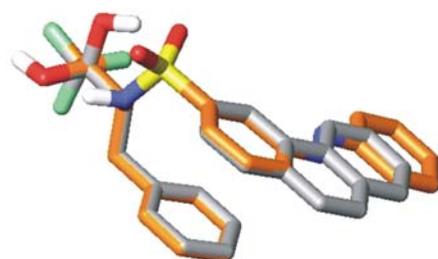
By comparing the overlap of phenanthrene **3.14** with azobenzenes **4.12** and **4.68** (fig. 3.11) showed that *E*-**4.68** occupies a similar spatial environment. Again the phenanthrene moiety is shorter than the azobenzene but the rigid planar structure compliments the *trans* configuration of the azo group nicely. Geometrically, the *Z*-**4.12** compares favourably as the twisted azo group mimics the shape of **3.14** quite well. However, the protrusion of the phenyl ring out of the plane does alter its spatial arrangement. *E*-**4.12** does not fare as well as the planar azobenzene is nearly perpendicular to the phenanthrene ring and *Z*-**3.68** exhibits very poor overlap of the terminal ring systems with the phenyl ring pointing up and away from the phenanthrene ring system.



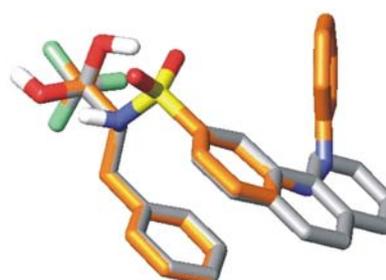
3.14 and *E*-**4.12** overlapped.



3.14 and *Z*-**4.12** overlapped.



3.14 and *E*-**4.68** overlapped.



3.14 and *Z*-**4.68** overlapped.

Figure 3.11. Comparison of 3- and 4-azobenzenes with 3-phenanthrenes

3.7 Summary

Substituted stilbene **13** incorporating the key trifluoromethyl carbinol **2.6** was successfully prepared utilising standard Wittig chemistry to prepare the stilbene **8** followed by routine peptide coupling. Major loss of product was often observed due to probable isomerisation and cyclisation of the stilbene. Oxidation of the trifluoromethyl carbinol to the corresponding trifluoromethyl ketone was achieved with TEMPO/NaOCl but complete oxidative photocoupling of the stilbene occurred as an undesired side reaction. Attempts at introducing the double bond further down the synthetic pathway were abandoned in favour of synthesising phenanthrenesulfonamides.

2- and 3-phenanthrenesulfonyl chloride was readily prepared from the corresponding potassium phenanthrene sulfonates in high yield and purity. Their effectiveness at coupling to primary amines has been demonstrated; although no attempts at regenerating the free amine has been undertaken. The resulting sulfonamides were readily purified by chromatography or recrystallisation. Phenanthrenes incorporating only the trifluoromethyl carbinol **2.6** were successfully oxidised to the trifluoromethyl ketone, but incorporation of another amino acid to increase specificity had a significant impact on their solubilities and thus their ability to be oxidised. Their poor solubility in aqueous media was expected to be detrimental to their use as inhibitors.

Molecular modelling was undertaken to test the hypothesis that phenanthrenes could be used as *cis* mimics of azobenzenes. The models showed that 2-phenanthrenes mimic *E*-4-azobenzenes well, mimic *Z*-4-azobenzenes to a lesser extent and would be poor mimics of 3-azobenzenes, contrary to the two dimensional projections. 3-Phenanthrenes were found to be reasonable mimics of *E*-3-azobenzenes and to a lesser extent, *Z*-4-azobenzenes but poor mimics of *Z*-3 and *E*-4-azobenzenes.

3.8 Future work

From the molecular modelling studies it is apparent that neither 2- or 3-phenanthrene derivatives would be good mimics for *Z*-azobenzenes, they do however show some promise as fused analogues of *E*-3- and 4-substituted azobenzenes. It is unlikely that any planar, fused ring system would make a suitable mimic of *Z*-azobenzenes due to the non-planar nature of the *Z*-azobenzene moiety. However, a better fused mimic of *E*-azobenzenes might be the benzo[*c*]cinnolines (fig. 3.12) which incorporate the azo group fused within the ring. Molecular modelling of the structure should be undertaken to assess the potential of benzo[*c*]cinnoline as mimics of *E*- and *Z*-azobenzene and if the results look promising synthetic methods for the preparation of benzo[*c*]cinnoline based inhibitors should be investigated.

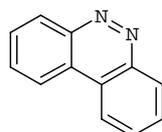


Figure 3.12. Benzo[*c*]cinnoline.

So far several potential inhibitors of α -chymotrypsin incorporating phenanthrene have been limited by their poor solubility in aqueous media. Future work in this field could investigate the incorporation of additional functionality (amines, sulfonic acids, carboxylic acids, glycol units) in the phenanthrene ring system in order to increase water solubility.

Further investigation into the preparation of mono- and disubstituted stilbenes via metal catalysed cross coupling or Wittig-Horner reactions may yield better results and open up new areas of research.

3.9 References for chapter three

- (1) Harvey, A. J., Thesis, University of Canterbury, 2000.
- (2) Zimmerman, G.; Chow, L.-Y.; Paik, U.-J. *Journal of the American Chemical Society* **1958**, *80*, 3528-3531.
- (3) Coxon, J. M.; Halton, B. *Organic Photochemistry*; 2nd ed.; Cambridge University Press, 1987.
- (4) Mallory, F. B.; Mallory, C. W. *Journal of the American Chemical Society* **1972**, *94*, 6041-6048.
- (5) Kim, S.; Seus, P.; Meier, H. *European Journal of Organic Chemistry* **2004**, 1761-1764.
- (6) Bell, F.; Waring, D. H. *Journal of the Chemical Society, Abstracts* **1948**, 1024-1026.
- (7) Bergmann, F.; Schapiro, D. *Journal of Organic Chemistry* **1947**, *12*, 57-66.
- (8) Abell, A. D.; Edmonds, M. K. In *Organophosphorous Reagents*; Murphy, P. J., Ed.; Oxford University Press: 2004, p 99-127.
- (9) Alonso, F.; Beletskaya, I. P.; Yus, M. *Tetrahedron* **2005**, *61*, 11771-11835.
- (10) Berthiol, F.; Doucet, H.; Santelli, M. *European Journal of Organic Chemistry* **2003**, 1091-1096.
- (11) Harwood, L. M.; Moody, C. J. In *Experimental Organic Chemistry; Principles and Practice*; Blackweel Scientific Publications: 1989, p 584-590.
- (12) Harwood, L. M.; Moody, C. J. In *Experimental Organic Chemistry; Principles and Practice*; Blackweel Scientific Publications: 1989, p 481-484.
- (13) Mallory, F. B.; Wood, C. S.; Gordon, J. T. *Journal of the American Chemical Society* **1964**, *86*, 3094-3102.
- (14) Fujita, S. *Synthetic Communications* **1982**, 423-424.
- (15) Fieser, L. F. In *Organic syntheses* 1936; Vol. 16, p 63-67.
- (16) Woolfolk, E. O.; Reynolds, W. E.; Mason, J. L. *Journal of Organic Chemistry* **1959**, *24*, 1445-1450.
- (17) Graves, D. M.; Linderman, R. J. *Journal of Organic Chemistry* **1989**, *54*, 661-668.
- (18) Imperiali, B.; Abeles, R. H. *Biochemistry* **1986**, *25*, 3760-3767.
- (19) Bauer, C. A. *European Journal of Biochemistry* **1980**, *105*, 565-570.
- (20) Ireland, R. E.; Longbin, L. *Journal of Organic Chemistry* **1993**, *58*, 2889.

- (21) Hampson, G. C.; Robertson, J. M. *Journal of the Chemical Society, Abstracts* **1941**, 409-413.
- (22) Allen, K. N.; Abeles, R. H. *Biochemistry* **1989**, 28, 8466-8473.
- (23) Stepanic, V.; Baranovic, G.; Smrecki, V. *Journal of Molecular Structure* **2001**, 569, 89-109.

Chapter Four

Synthesis of Azobenzene

Derivatives

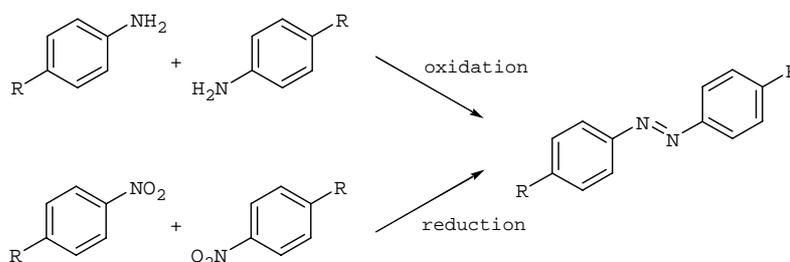
visible light induces a conformational change which enriches the photostationary state with either the *Z*- or *E*-conformation.

This thesis also investigates how increasing the peptide chain by one amino acid, varying the position of substitution of the linking moiety and adding substituents into the terminal ring of the azobenzene switch affects the potency of the enzyme inhibitor and composition of the photostationary state. Described below are the synthetic routes taken to synthesise a range of compounds based on the azobenzene core.

4.2 Synthetic methods for preparing azobenzenes

Symmetrical azobenzenes

Symmetrical azobenzenes are readily prepared by either reducing substituted nitroarenes or oxidising substituted aryl amines (scheme 4.1) and are often produced as unwanted by-products of oxidation or reduction reactions.



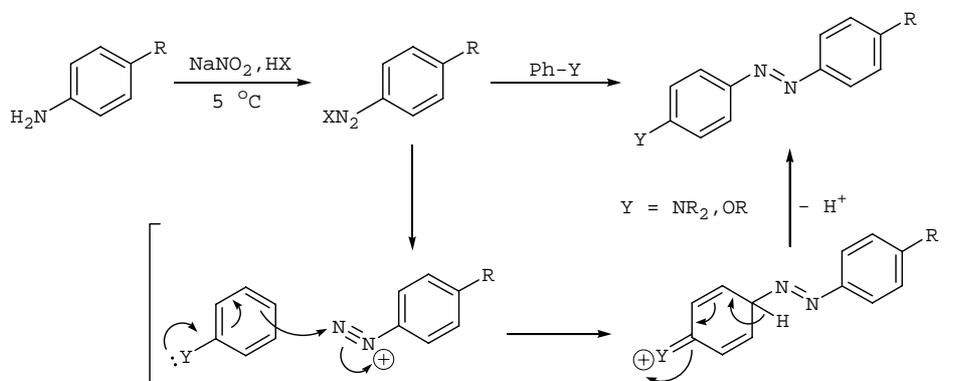
Scheme 4.1. Formation of symmetrical azobenzenes.

Diazonium chemistry

Unsymmetrical polysubstituted azobenzenes can be synthesised via diazonium chemistry. This involves diazotisation of an aryl amine with nitrous acid^v at 0-5 °C to afford a diazonium salt. Diazonium salts undergo typical electrophilic substitution with activated

^v Prepared *in situ* by treating NaNO_2 with mineral acids HCl or H_2SO_4 .

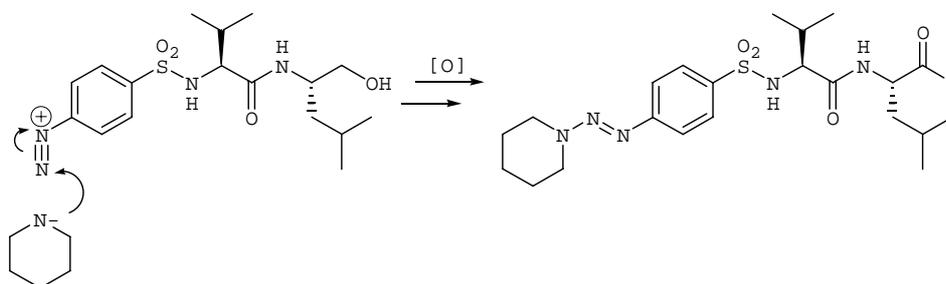
arenes such as aniline or phenol derivatives which results in the rapid formation of aromatic azo compounds. The reaction will generally only proceed when an electron donating group ($Y = \text{NR}_2, \text{OR}$) is present within the aromatic ring (scheme 4.2).



Scheme 4.2. Synthesis of azobenzenes via diazonium chemistry.

The directing effects of $-\text{NR}_2$ and $-\text{OR}$ are activating *ortho-para* directors, *para* substitution invariably occurs unless the position is already blocked, in which case *ortho* substitution occurs. A vast number of azo dyes have been prepared in this manner.³

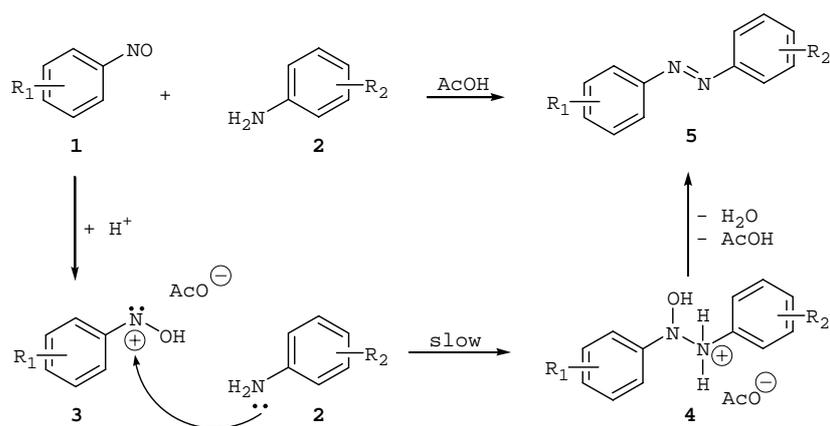
Diazonium chemistry has been utilised to prepare triazenes by reacting sulfonated benzenediazonium salts with cyclic and heterocyclic amines.⁴ Several compounds of this type have been synthesised by our group as potential inhibitors of the cysteine protease calpain (scheme 4.3).⁵



Scheme 4.3. Synthesis of triazene based inhibitors of calpain.

⁵ M. Jones; Unpublished work.

The reaction of nitroso arenes with anilines to form azobenzenes has been known since the late 1800's.⁵ The reaction is acid catalysed and is generally performed in acetic acid or an acetic acid/solvent mixture⁶ as strong inorganic acids lead to the formation of a tarry material.⁷ The postulated mechanism involves protonation of the nitroso arene **1** to give an activated *N*-hydroxylamine **3**. The rate determining step is the attack on the electrophilic nitrogen of **3** by the nucleophilic aryl amine **2** to give an intermediate **4** which undergoes dehydration to give the azobenzene **5** (scheme 4.4).⁷



Scheme 4.4. Postulated mechanism for the condensation of nitroso arenes with aryl amines.

The effect of substituents on the rate of formation of the azobenzene can be summarised as follows: If R_1 is an electron acceptor (e.g. *p*-NO₂) or R_2 is an electron donor (e.g. *p*-methyl) the rate is increased whereas if the substituents are switched around the rate decreases. The kinetic results also indicate that undissociated acetic acid is important in the mechanism (fig. 4.3) which explains the unsatisfactory results obtained with strong acids.

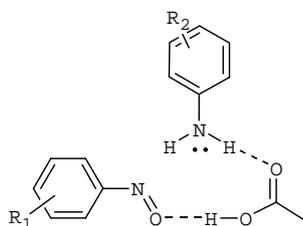


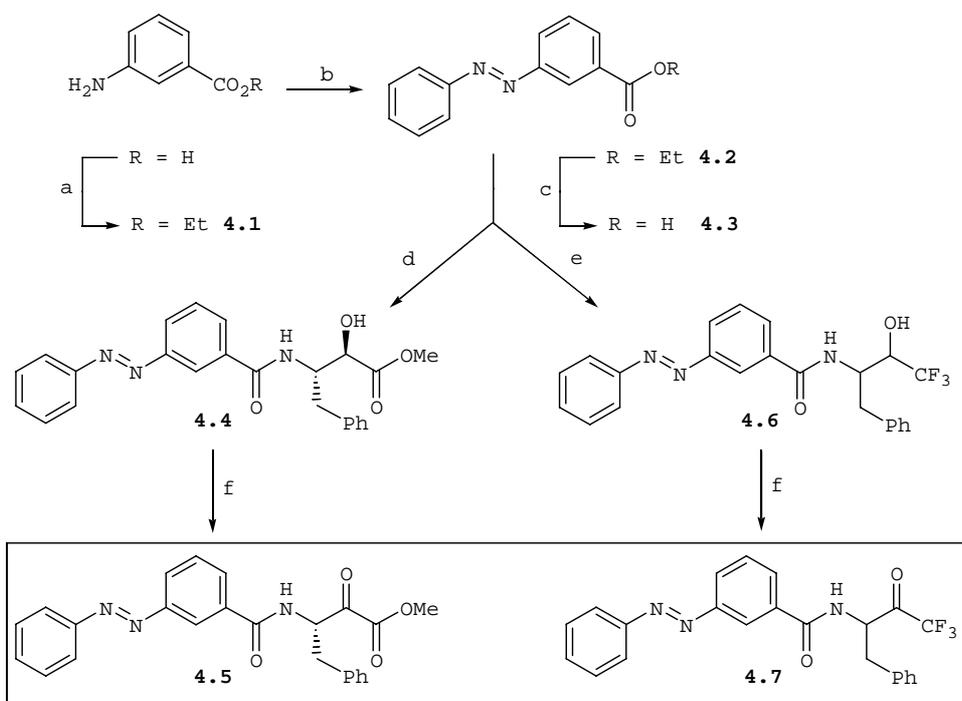
Figure 4.3. Postulated coordination of acetic acid during the reaction.

This is seen as a convenient way of preparing unsymmetrical, unique, unambiguous azobenzenes. The improvements in methods for preparing substituted nitrosobenzenes^{6,8} and aryl amines has opened up a wide range of possibilities for the synthesis of more complex, functionalised azobenzenes. It is this chemistry that has been utilised to prepare a series of 3-azobenzenes and 4',3- and 4',4-disubstituted azobenzenes described in this thesis.

4.3 Synthesis of 3- and 4-substituted azobenzenes

4.3.1 3-Azobenzene amides

Based on the work of Harvey,² 3-phenylazobenzoic acid derivatives incorporating an α -keto ester moiety are potent inhibitors of α -chymotrypsin. Photoswitch **4.3** was synthesised so that compound **4.7**, a trifluoromethyl ketone analogue of Harvey's most potent compound **4.5** could be prepared for comparison (scheme 4.5).



Reagents and conditions; (a) HCl_(g), EtOH, reflux; (b) AcOH, 100 °C, 4 h; (c) KOH, 4:1 EtOH/H₂O, reflux; (d) 3*S*-amino-2*R*-hydroxy-4-phenylbutyric acid methyl ester, BOP, DIPEA, DCM; (e) **2.6**, BOP, DIPEA, DCM; (f) TEMPO, KBr, NaOCl, DCM, 0 °C. Scheme 4.5. Synthesis of 3-azobenzene based inhibitors of α -chymotrypsin.

Synthesis of 3-phenylazobenzoic acid 4.3

Following Harvey's procedure 3-aminobenzoic acid was esterified in acidified EtOH to afford the ester **4.1** in 83 % yield. Condensation of **4.1** with nitrosobenzene in glacial acetic acid at 100 °C gave a black tar. Chromatography on alumina then silica gave the azobenzene **4.2** as an orange red oil in 74 % yield. Hydrolysis of the ester **4.2** with KOH in EtOH afforded 3-phenylazobenzoic acid **4.3** in 83 % yield (scheme 4.5).

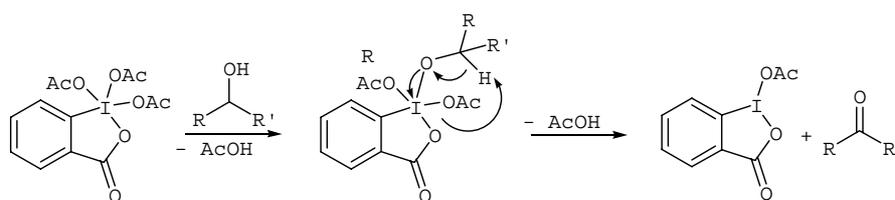
The sequence to prepare **4.3** could possibly be simplified by following an analogous route used to prepare 4-phenylazobenzoic acid by shaking a solution of 4-aminobenzoic acid dissolved in acetic acid with nitrosobenzene for 12 h at room temperature. The resulting product crystallised out of solution eliminating the need for chromatography.⁹

Synthesis of 3-azobenzene amide derivatives

The photoswitch **4.3** was coupled to trifluoromethyl carbinol **2.6** or 3*S*-amino-2*R*-hydroxy-4-phenylbutyric acid methyl ester utilising BOP coupling methodology. The resulting solids were recrystallised from aqueous acetone to afford the amides **4.4** and **4.6** in 66 and 98 % yield respectively (scheme 4.5).

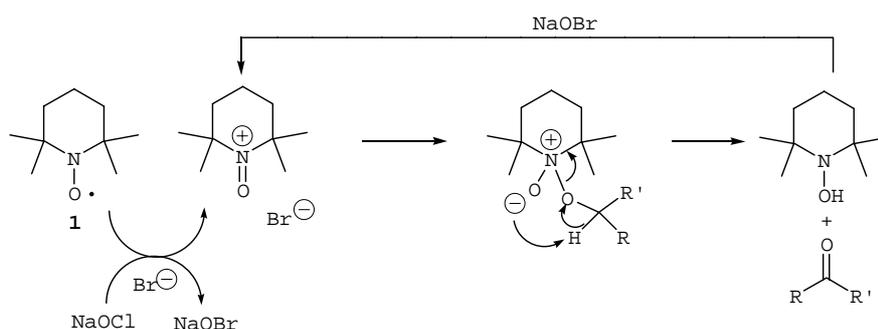
Oxidation of secondary alcohols

Oxidation of secondary alcohols to ketones can be achieved by a variety of reagents¹⁰ (section 2.2.4) with Dess-Martin periodinane being particularly effective on a wide range of substrates. Most alcohols, even sterically hindered secondary alcohols can undergo smooth oxidation in high yield with the periodinane and generally do not suffer from over oxidation, epimerisation or bond isomerisation. Dess-Martin periodinane has shown to be a very effective oxidising agent of electron deficient, weakly nucleophilic alcohols such as α -hydroxy esters, α -hydroxy amides and α -trifluoromethyl alcohols (scheme 4.6).¹¹



Scheme 4.6. Dess-Martine periodinane oxidation of alcohols.

A second reagent that has proved very effective for the oxidation of both α -hydroxy esters and α -trifluoromethyl alcohols is the nitroxide TEMPO **1**. When utilised in a catalytic system with KBr as co-catalyst and buffered NaOCl as a co-oxidant secondary alcohols are smoothly and cleanly oxidised to ketones (scheme 4.7).¹² This method is particularly useful for small scale oxidations.

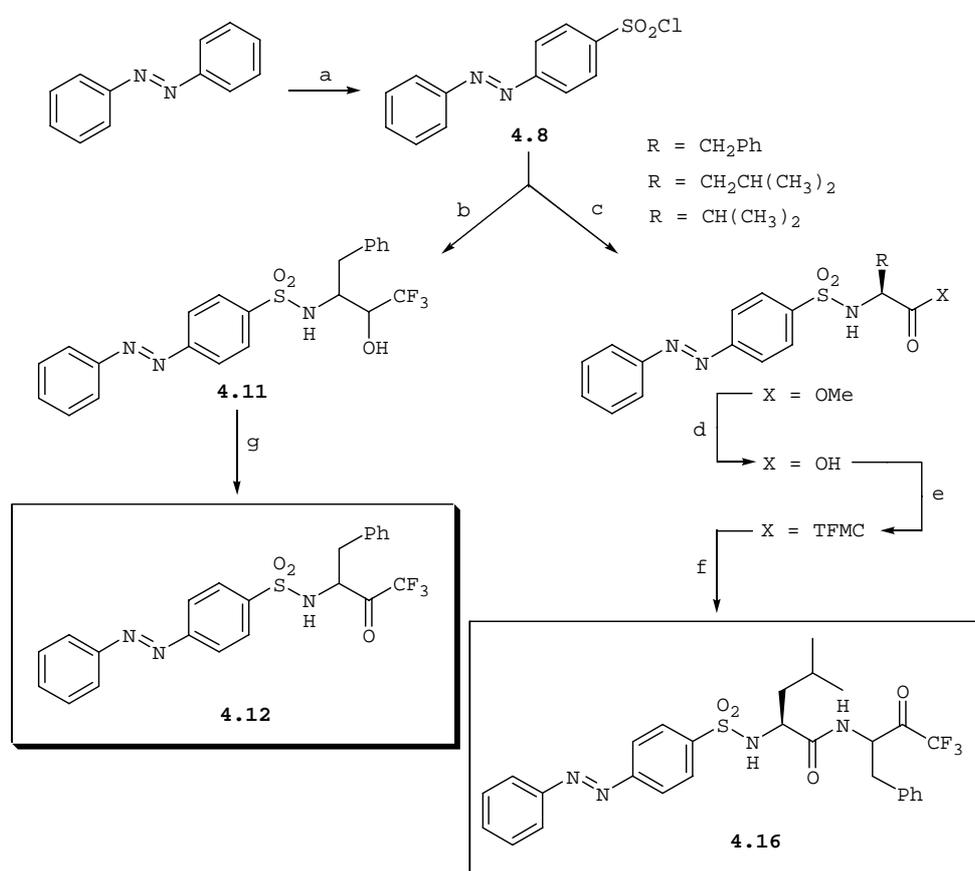


Scheme 4.7. Proposed mechanism for the catalytic TEMPO oxidation of alcohols

α -Hydroxy ester **4.4** and trifluoromethyl carbinol **4.6** were oxidised at 0 °C by TEMPO/KBr/NaOCl to give ketones **4.5** and **4.7** (scheme 4.5). α -Keto ester **4.5** was recrystallised from aqueous acetone to give orange needles as *ca.* 1:1 mixture of ketone and hydrate in 56 % yield. Recrystallisation of trifluoromethyl ketone **4.7** from aqueous MeOH gave an orange solid which was predominantly hydrated in 87 % yield. Similar results were obtained when **4.6** was oxidised with Dess-Martin periodinane.

4.3.2 4-Azobenzenesulfonamides

The sulfonyl linkage was chosen based on its increased stability towards hydrolysis in chemical and biological systems. The photoswitch can be prepared as the sulfonyl chloride which is significantly more stable towards air and moisture compared to the analogous acid chloride.¹³ Another advantage is it does not require coupling reagents to activate the electrophilic centre during amine coupling unlike the corresponding carboxylic acid due to the high reactivity of sulfonyl chlorides with amines. Scheme 4.8 outlines the synthesis of the photoswitch **4.8** and a series of 4-azobenzenesulfonamides (fig. 4.4) prepared by reactions with various amines.



Reagents and conditions; (a) HOSO_2Cl , 100 °C; (b) **2.6**, Py or DIPEA, DCM, reflux; (c) amino acid methyl ester.HCl, Py or DIPEA, DCM; (d) K_2CO_3 , MeOH/ H_2O , reflux; (e) **2.6**, BOP, DIPEA, DCM; (f) Dess-Martin periodinane, DCM; (g) TEMPO, NaOCl, KBr, DCM, 0 °C or Dess-Martin periodinane, DCM.

Scheme 4.8. Synthesis of 4-azobenzenesulfonamides.

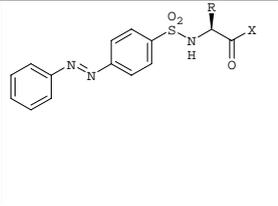
	R	X
4.9	CH ₂ Ph	OMe
4.10	CH ₂ Ph	OH
4.13	CH ₂ CH(CH ₃) ₂	OMe
4.14	CH ₂ CH(CH ₃) ₂	OH
4.15	CH ₂ CH(CH ₃) ₂	TFMC
4.16	CH ₂ CH(CH ₃) ₂	TFMK
4.17	CH(CH ₃) ₂	OMe
4.18	CH(CH ₃) ₂	OH
4.19	CH(CH ₃) ₂	TFMC

Figure 4.4. Amino acid based 4-azobenzenesulfonamides.

Synthesis of 4-azobenzenesulfonyl chloride 4.8

The series of compounds based on the 4-azobenzenesulfonyl core were the easiest to prepare because the switch is readily prepared by direct sulfonation of azobenzene.¹⁴ Recrystallised azobenzene was treated with freshly distilled chlorosulfonic acid to afford the crude product. Repeated extract with hot petroleum ether followed by flash chromatography of the orange product gave the pure 4-azobenzenesulfonyl chloride **4.8** in 41 % yield. The phenylazo group is *ortho-para* directing and as a result only the mono *para* derivative is isolated provided the reaction temperature is kept below 130 °C. At higher temperatures the trisulfonated product is obtained (fig.4.5).¹⁴

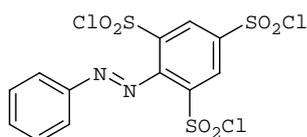


Figure 4.5. Trisulfonated azobenzene.

Sulfonamide coupling

The switch **4.8** has been utilised by Desai¹⁴ for the preparation of sulfanilamide derivatives and Woolfok¹⁵ in the separation, purification and identification of a wide range of amines including primary and secondary aliphatic and aromatic amines, secondary mixed amines and heterocyclic amines.

Sulfonyl chloride **4.8** was readily coupled to *L*-phenylalanine, *L*-leucine and *L*-valine methyl esters and trifluoromethyl carbinol **2.6** affording sulfonamides **4.9**, **4.13**, **4.17** and **4.11** (scheme 4.8) utilising either pyridine (yield > 84 %) or Hünigs base (yield > 97 %). Recrystallisation from aqueous MeOH resulted in crystalline products of high purity.

Hydrolysis of methyl esters

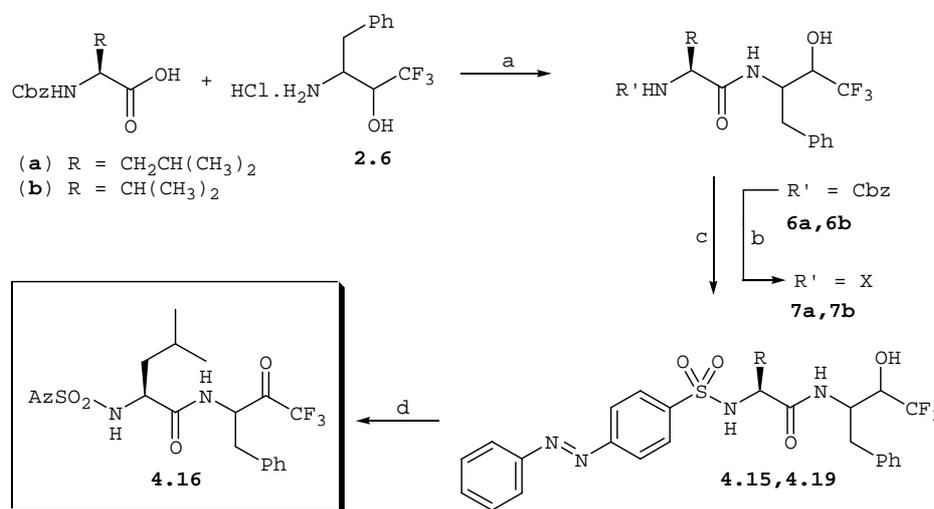
The methyl esters of sulfonamides **4.9**, **4.13**, **4.17** were hydrolysed under mild conditions using K₂CO₃ in aqueous MeOH (scheme 4.8). The reaction proceeded smoothly over a period of 10 h for the phenylalanine derivative but took nearly 24 h for the leucine methyl ester and 48 h for the valine methyl ester. The free acids **4.10**, **4.14** and **4.18** were precipitated from solution by addition of aqueous acid to give orange solids in yields > 97%. Free acids **4.14** and **4.18** were used to prepare dipeptides **4.16** and **4.19** while the phenylalanine derivatives **4.9** and **4.10** were used to prepare amino aldehydes (section 2.4.3).

Synthesis of dipeptidyl sulfonamides

Increases in inhibitor potency have been reported by incorporation of additional amino acids to improve enzyme specificity. Studies have shown that the S₂ binding pocket of α -chymotrypsin favours hydrophobic residues such as *L*-leucine and *L*-valine. Two dipeptides have been synthesised which incorporate one of these residues in addition to the phenylalanine residue required for binding in the S₁ pocket of the enzyme active site. The dipeptides have been introduced in two ways; firstly the peptides were introduced sequentially by using protection-deprotection methodology.

The *O*-protected amino acid *L*-leucine or *L*-valine methyl ester was *N*-protected as the 4-azobenzenesulfonamide **4.13** and **4.17**. The sulfonamide was *O*-deprotected by basic hydrolysis to the *N*-protected free acids **4.14** and **4.18**. Trifluoromethyl carbinol **2.6** was coupled to the free acid via BOP methodology to give the dipeptides **4.15** and **4.19** in 59 and 70 % yield respectively (scheme 4.8). Due to the mixed stereochemistry of trifluoromethyl carbinol **2.6** introduction of another stereogenic centre gives rise to a mixture of diastereomers. The ratio of diastereomers can easily be determined by ^1H and ^{19}F NMR and the agreement between the two methods is generally very good. For **4.15** the ratio of diastereomers was 1:2 while for **4.19** the ratio was 2:3. The diastereomer ratio should be the same for each dipeptide provided the same sample of **2.6** was used in each synthesis. Possible explanations for the difference include racemisation of the α -carbon during chromatography on silica, or more likely, due to the prolonged period of reflux required for the hydrolysis of valine methyl ester **4.17** some base induced racemisation at the α -carbon centre may have occurred.

The second method involved preparing the dipeptides (**6a** and **6b**) separately then coupling the *N*-deprotected dipeptide with 4-azobenzenesulfonyl chloride **4.8** directly (scheme 4.9).



Reagents and conditions; (a) BOP, DIPEA, DCM; (b) Pd/C , H_2 , DCM or 33 % HBr in AcOH; (c) **4.8**, DIPEA, DCM, reflux; (d) Dess Martin periodinane, DCM.

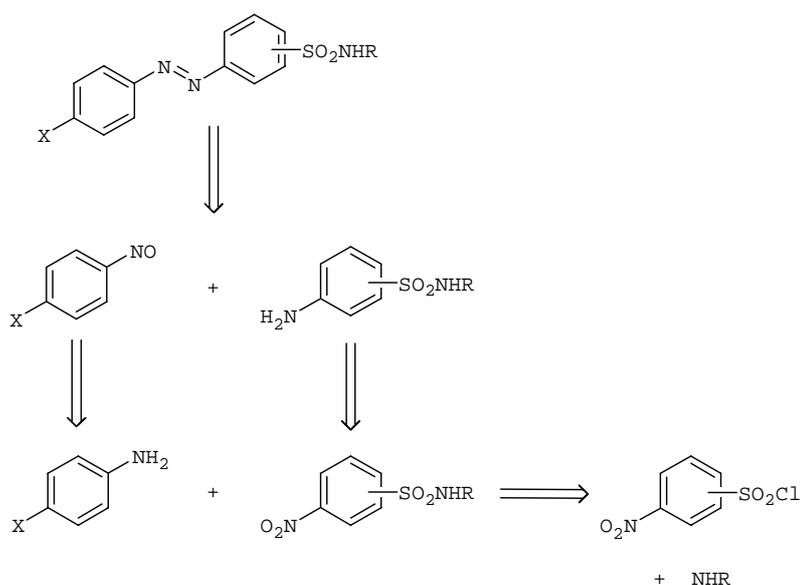
Scheme 4.9. Alternate route for synthesising dipeptidyl 4-azobenzenes.

N-Cbz protected *L*-leucine or *L*-valine was coupled to trifluoromethyl carbinol **2.6** using the BOP coupling methodology to give the *N*-Cbz protected dipeptides **6a** and **6b** in quantitative yields. Deprotection via hydrogenation (> 90 %) or acidolysis (65 %) gave the free amino dipeptides **7a** and **7b**. The dipeptides were coupled to **4.8** in the presence of Hünigs base to give dipeptidyl sulfonamides **4.15** and **4.19** in 61 and 79 % yield respectively.

Deprotection of the Cbz group via hydrogenation over palladium on carbon gave a cleaner product in higher yield than treatment with HBr in acetic acid. This second route would be faster and prevent potential base induced racemisation as the hydrolysis step is no longer required, it does however introduce valuable trifluoromethyl carbinol **2.6** a step earlier in the synthesis so each step would need to be as efficient as possible to minimise potential loss of product.

4.4 Synthesis of 4',3- and 4',4-disubstituted azobenzenes

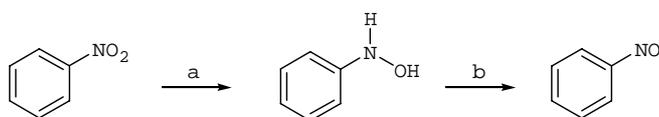
Retrosynthetically, it can be seen that the disubstituted azobenzenes can be synthesised from nitrosobenzenes and metanilamides or sulfanilamides. Nitrosobenzenes can be prepared from the corresponding aryl amine or nitro arene while the metanilamides and sulfanilamides can be prepared from the corresponding 3- or 4-nitrobenzenesulfonyl chloride respectively (scheme 4.10). It was therefore imperative that facile means of synthesising these types of compounds be found.



Scheme 4.10. Retrosynthetic analysis of substituted azobenzenesulfonamides.

4.4.1 Synthesis of 4-substituted nitrosobenzenes

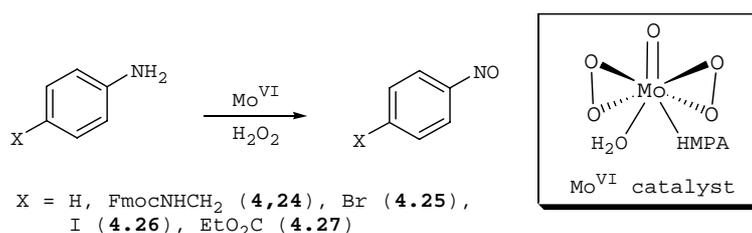
The nitrosobenzene used to synthesise **4.2** was prepared by the reduction of nitrobenzene with Zn dust and NH_4Cl to give the hydroxyl amine followed by oxidation with acidified dichromate to afford the crude nitrosobenzene (scheme 4.11) which required purification by steam distillation.¹⁶



Reagents and conditions; (a) Zn, aq. NH_4Cl , 55 °C; (b) H_2SO_4 , $\text{Na}_2\text{Cr}_2\text{O}_7$, 0 °C.

Scheme 4.11. Synthesis of nitrosobenzene.

This method seemed rather cumbersome so an investigation of the literature revealed that nitrosobenzenes could be obtained by the catalytic oxidation of aryl amines with tungsten or molybdenum species in the presence of hydrogen peroxide (scheme 4.12).^{8,17}



Scheme 4.12. Catalytic oxidation of aryl amines with Mo^{VI} and hydrogen peroxide.

4-Halogenated nitrosobenzenes

4-Bromonitrosobenzene **4.25** and 4-iodonitrosobenzene **4.26** were prepared from commercially available 4-bromoaniline and 4-iodoaniline by oxidation with Mo^{VI}-hydrogen peroxide over 16-18 h in 51 and 19 % yields respectively. The formation of **4.26** was particularly low due to the formation of multiple by-products. At least six different bands were observed on the column. It has been reported that side reactions can be minimised by employing the bi-phasic system developed by Priewisch *et al.*⁶ The advantages of this method are that no catalyst is required, oxidation is achieved with cheap, commercially available Oxone in a mixture of water/dichloromethane, requires much shorter reaction times (3.5 h for the Br derivative) and can be performed on larger scales than previous methods.⁶

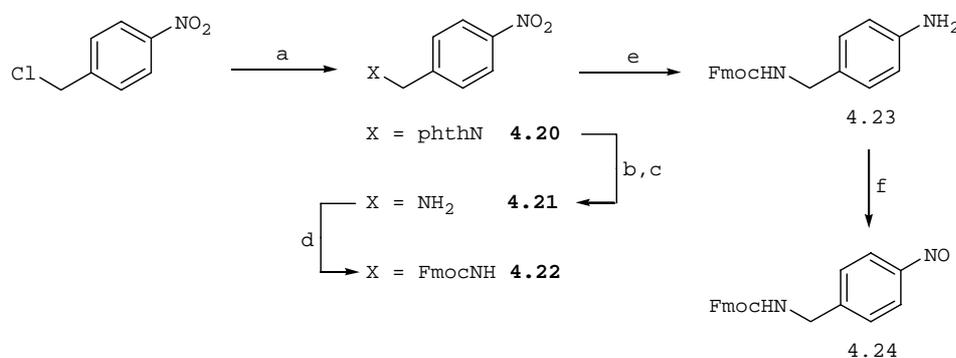
4-Nitrosobenzoic acid ethyl ester **4.27**

4-Nitrosobenzoic acid ethyl ester **4.27** was prepared from 4-aminobenzoic acid which was esterified in acidified EtOH in an analogous manner to **4.1** then oxidised with Mo^{VI}-hydrogen peroxide over *ca.* 50 h to give **4.27** in 64 % yield.

Fmoc protected 4-aminomethylnitrosobenzene **4.24**

The *N*-protected 4-aminomethylaniline **4.23** was prepared from commercially available 4-nitrobenzyl chloride (scheme 4.13). The amino group was introduced by nucleophilic displacement by the phthalimide anion to give phthalimide **4.20** in 89 % yield after recrystallisation. The phthalimido protecting group was removed by treating with

hydrazine hydrate at reflux and acidifying the filtrate to afford the hydrochloride **4.21** in 82 % yield. The amino group was protected with Fmoc to give **4.22** in 71 % yield. The choice to remove the phthalimido group and reprotect as the Fmoc derivative was made because the harsh conditions utilised in removing phthalimides are often incompatible with more sensitive functional groups that may be present within the molecule. Fmoc was chosen due to its stability under acidic and hydrogenation conditions and ease of removal with TBAF or piperidine. The nitrobenzene **4.22** was reduced under a hydrogen atmosphere utilising Adams' catalyst¹⁸ to afford the aniline **4.23** in quantitative yield. The final step in the synthesis was the molybdenum catalysed oxidation over 14 h which was remarkably efficient compared to the other nitroso compounds formed and gave **4.24** in 98 % yield (scheme 4.13).



Reagents and conditions; (a) potassium phthalimide, DMF, 50 °C, (b) hydrazine, EtOH, reflux; (c) AcOH; (d) FmocCl, DIPEA, DCM; (e) PtO₂.H₂O, H₂, EtOAc; (f) Mo^{VI}, H₂O₂, DCM, 14 h.

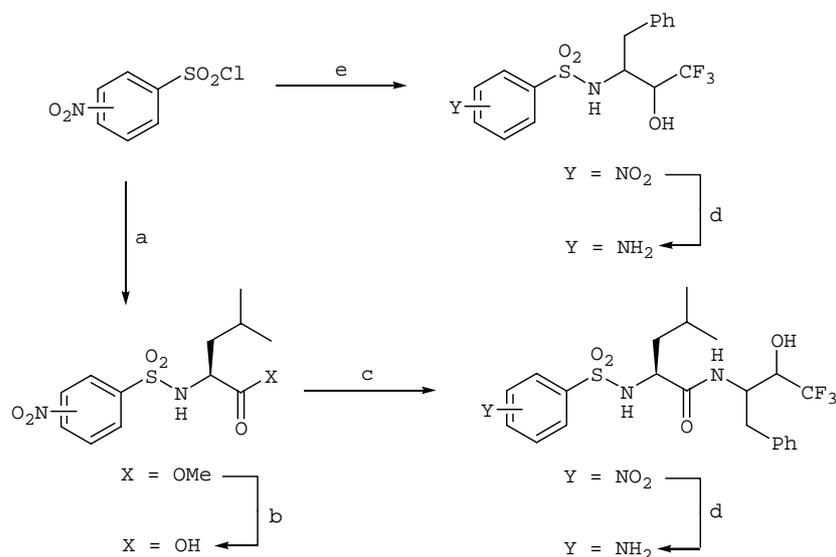
Scheme 4.13. Synthesis of **4.24** from 4-nitrobenzylchloride.

All nitroso compounds synthesised were readily recrystallised from aqueous EtOH. The colour in solution was green but in solid form the colours varied from green to yellow to brown indicating the formation of dimers in solution. The compounds were stored at -18 °C to prevent oxidation and decomposition of the nitrosobenzenes.

4.4.2 Synthesis of metanilamides and sulfanilamides

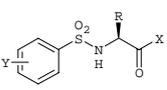
Monopeptidyl metanilamides and sulfanilamides

Amino acids *L*-leucine and *L*-phenylalanine and trifluoromethyl carbinol **2.6** were coupled to 3- and 4-nitrobenzenesulfonyl chloride in the presence of Hünigs base to give sulfonamides **4.28**, **4.30**, **4.32**, **4.35**, **4.37** and **4.39** in excellent yields. The phenylalanine derivatives **4.28**, **4.35** and trifluoromethyl carbinols **4.30**, **4.37** were reduced under a hydrogen atmosphere with Adams' catalyst to afford metanilamides **4.29**, **4.31** and sulfanilamides **4.36**, **4.38** in isolated yields > 95 % (scheme 4.14).



Reagents and conditions; (a) *L*-LeuOMe.HCl, DIPEA, DCM, reflux; (b) K₂CO₃, MeOH/H₂O, reflux; (c) **2.6**, BOP, DIPEA, DCM; (d) PtO₂.H₂O, H₂, EtOAc; (e) **2.6**, DIPEA, DCM, reflux.

Scheme 4.14. Synthesis of metanilamides and sulfanilamides.

	Y	R	X
4.28	3-NO ₂	CH ₂ Ph	OMe
4.29	3-NH ₂	CH ₂ Ph	OMe
4.32	3-NO ₂	CH ₂ CH(CH ₃) ₂	OMe
4.33	3-NO ₂	CH ₂ CH(CH ₃) ₂	OH
4.34	3-NO ₂	CH ₂ CH(CH ₃) ₂	TFMC
4.35	4-NO ₂	CH ₂ Ph	OMe
4.36	4-NH ₂	CH ₂ Ph	OMe
4.39	4-NO ₂	CH ₂ CH(CH ₃) ₂	OMe
4.40	4-NO ₂	CH ₂ CH(CH ₃) ₂	OH
4.41	4-NO ₂	CH ₂ CH(CH ₃) ₂	TFMC
4.42	4-NH ₂	CH ₂ CH(CH ₃) ₂	TFMC

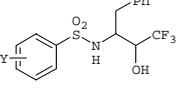
	Y
4.30	3-NO ₂
4.31	3-NH ₂
4.37	4-NO ₂
4.38	4-NH ₂

Figure 4.6. Compounds prepared during the synthesis of metanilamides and sulfanilamides.

Dipeptidyl metanilamides and sulfanilamides

The leucine methyl esters **4.32** and **4.39** were hydrolysed under basic conditions with K₂CO₃ in aqueous MeOH to give the free acids **4.33** and **4.40** in isolated yields of 90 and 72 % respectively (scheme 4.14).

The free acids **4.33** and **4.40** were coupled to trifluoromethyl carbinol **2.6** using the BOP coupling methodology to give the dipeptidyl sulfonamides **4.34** and **4.41** (scheme 4.14) in

isolated yields of 85 and 77 % respectively as 1:1 and 2:3 mixtures of diastereomers. Dipeptide **4.41** was reduced under an atmosphere of hydrogen with Adams' catalyst to give sulfanilamide **4.42** in 83 % yield as a 2:3 mixture of diastereomers. Due to time constraints the metanilamide of **4.34** was not prepared.

The use of hydrogen and Adams' catalyst proved to be a mild and efficient method for the synthesis of aryl amines from nitroarenes and avoided the use of more harsh reductive methods such as metal/acid systems. Little or no by-products were observed, the only major exception was the formation of symmetrical azobenzene **8** (fig. 4.7) when **4.35** was left under hydrogen for 4 days.

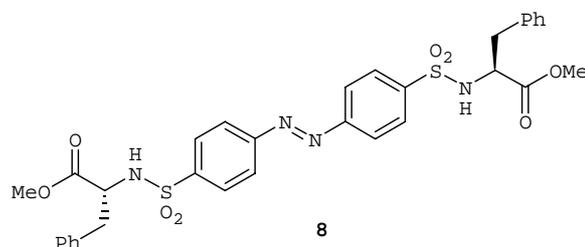


Figure 4.7. Symmetrical azobenzene formed during catalytic hydrogenation of **4.35**.

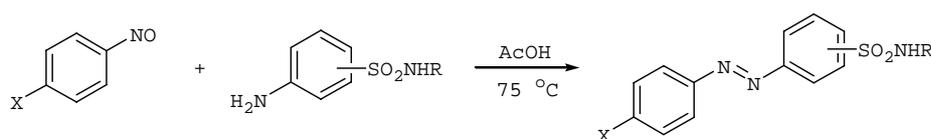
4.4.3 Disubstituted azobenzene formation

4',3-Azobenzenesulfonamides

Metanilamide **4.31** was condensed with nitrosobenzenes **4.25** and **4.26** in acetic acid at 75 °C (scheme 4.15). After chromatography the 4',3-disubstituted azobenzenes **4.43** and **4.45** were isolated in 76 and 77 % yields respectively.

4',4-Azobenzenesulfonamides

Sulfanilamides **4.36**, **4.38** and **4.42** were condensed with various nitrosobenzenes **4.24**, **4.25**, **4.26** and **4.27** in acetic acid at 75 °C (scheme 4.15). After chromatography various 4',4-disubstituted azobenzenes were obtained (fig. 4.8) in yields ranging from 9-69 %.



Scheme 4.15. Synthesis of disubstituted azobenzenes.

Monopeptidyl azobenzenes with halogen or ethyl carboxy groups gave acceptable yields between 64-69 % while the Fmoc protected azobenzene **4.53** was significantly lower at 29 %. The yields of dipeptides **4.60** and **4.61** were especially poor at 9 and 23 % respectively; however significant amounts of starting material were recovered suggesting that the low yields were a result of slower rates of reaction/higher activation energies and not due to decomposition of the starting materials. Longer reaction times and/or higher temperatures may result in improved yields.

	X	Substitution position	R
4.43	Br	3	TFMC
4.45	I	3	TFMC
4.47	Br	4	<i>L</i> -PheOMe
4.48	Br	4	TFMC
4.50	I	4	<i>L</i> -PheOMe
4.51	I	4	TFMC
4.53	FmocNHCH ₂	4	TFMC
4.55	EtO ₂ C	4	TFMC
4.60	Br	4	<i>L</i> -LeuTFMC
4.61	I	4	<i>L</i> -LeuTFMC

Figure 4.8. Synthesised disubstituted azobenzenes by condensation of nitrosobenzenes with aryl amines.

The aryl peaks in the ^1H NMR spectrum for each of the 4'-3-disubstituted azobenzenes were easily assigned based on the integral ratios and multiplicity of the peaks (fig. 4.9).

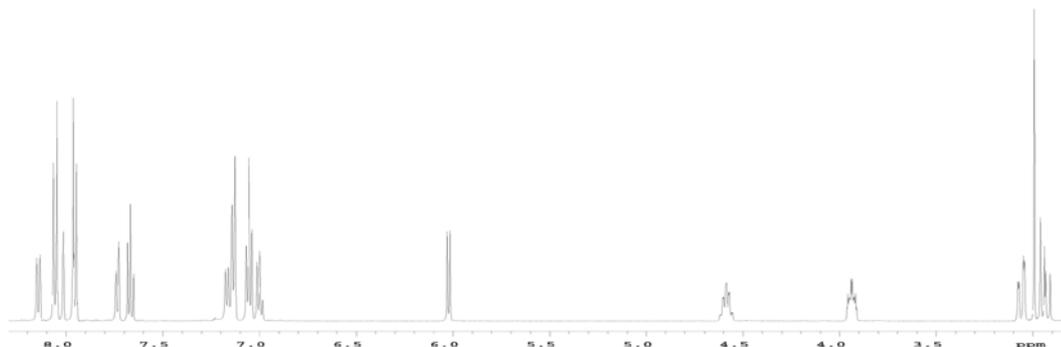


Figure 4.9. ^1H NMR of **4.43**. The key feature is the ability to unequivocally assign the two doublets of the brominated ring. $\mathbf{H2'}$ (8.05 ppm); $\mathbf{H3'}$ (7.95 ppm).

The information gleaned from these spectra simplified the interpretation of the analogous 4',4-disubstituted azobenzenes as the proton chemical shifts of the azobenzene rings had very little variation between derivatives containing the same X substituent (fig. 4.10).

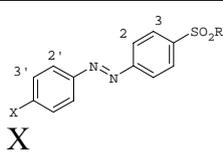
	2	3	2'	3'
Br	7.9	7.7	8.05	7.95
I	7.9	7.7	7.9	8.1
FmocNHCH ₂	7.9	7.7	8.1	7.7
EtO ₂ C	7.95	7.75	8.2	8.4

Figure 4.10. Approximate chemical shifts of the protons of the 4',4-disubstituted azobenzene.

4.4.4 Miscellaneous couplings of functionalised azobenzenes

The potential for extending the azobenzene switch in the terminal direction was investigated. The feasibility of incorporating additional aryl rings by metal catalysed cross couplings were tested by employing the Suzuki coupling of some available boronic acids

with aryl halides. The bromide **4.47** was coupled to 4-methoxyboronic acid and 2-fluoropyridine-5-boronic acid utilising $\text{Pd}^0(\text{PPh}_3)_4$ and KOH under phase transfer conditions. The reactions gave the desired biaryl compounds **4.63** and **4.64** in 23 and 27 % isolated yield. The low yields were due to hydrolysis of the methyl esters resulting in free acids which bound to the silica column. Attempted coupling of bromide **4.48** to the boronic acids under the same conditions failed. The coupling was repeated with 4-methoxyboronic acid via modified procedure¹⁹ which utilised the more reactive aryl iodide **4.51**, a milder base (Na_2CO_3), deoxygenated solvents and bi-phasic conditions. The reaction proceeded smoothly to afford the biaryl **4.65** (fig. 4.11) in 67 % yield.

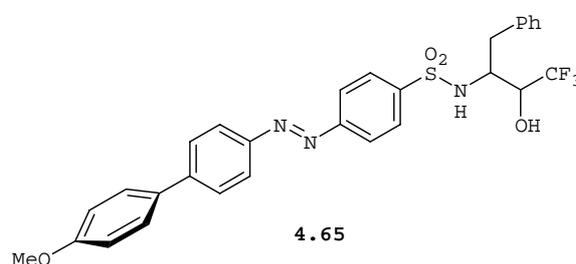


Figure 4.11. Biaryl **4.65** prepared by Suzuki coupling.

Ethyl ester **4.55** was hydrolysed by LiOH in aqueous MeOH to give the water soluble lithium carboxylate. Acidification and recrystallisation afforded the free acid **4.58** in an isolated yield of 98 %. The carboxylic acid moiety can now potentially be used to couple other molecules onto the azobenzene unit. The most obvious choice is to form an amide bond under peptide coupling conditions. To test this out and to make an interesting molecule, half an equivalent of ethylenediamine dihydrochloride was coupled with **4.58** under HATU conditions to give the bridged dimer **4.59** (fig. 4.12) in 96 % yield.

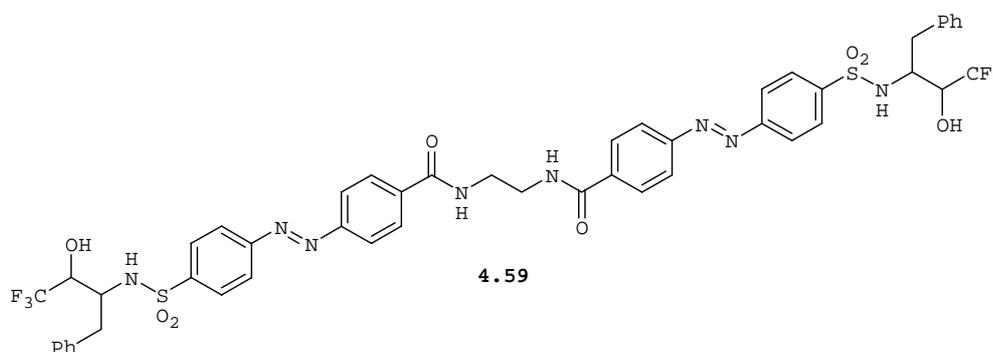


Figure 4.12. Bridged dimer of **4.58**.

The Fmoc protected azobenzene **4.53** was deprotected with TBAF to give the free amine. Due to time restraints no reactions have been performed on the deprotected molecule yet, however similar azo compounds prepared by our group have been successfully coupled to carboxylic acids and amino acids in reasonable yields.^ξ

4.4.5 Trifluoromethyl carbinol oxidation

Oxidation of all 4',3'- and 4',4-disubstituted azobenzene trifluoromethyl carbinols were achieved using Dess-Martin periodinane or TEMPO/NaOCl (fig. 4.13).

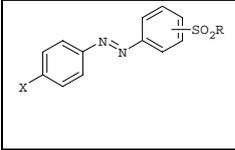
	X	Substitution position	R
4.44	Br	3	TFMK
4.46	I	3	TFMK
4.49	Br	4	TFMK
4.52	I	4	TFMK
4.54	FmocNHCH ₂	4	TFMK
4.57	EtO ₂ C	4	TFMK
4.62	I	4	<i>L</i> -LeuTFMK

Figure 4.13. Synthesised disubstituted azobenzene trifluoromethyl ketones.

Generally, if one method proved unsuccessful, oxidation could be achieved with the other. In cases where solubility was an issue, oxidations were achieved with TEMPO in a 2:1 dichloromethane/ethyl acetate solvent system. The yields were generally 60-85 % with the Fmoc protected **4.53** again proving to be the exception with a yield of 39 %. The trifluoromethyl ketones were found to exist predominantly in a hydrated state (by ¹H and ¹⁹F NMR) and can be completely hydrated upon addition of water to the sample. This is in agreement of studies performed by Abeles *et al* which showed that the ketone/hydrate ratio is 1:500 in aqueous media.²⁰

^ξ D. Pearson. Ph.D student.

4.5 Summary and future work

A series of trifluoromethyl ketones have been synthesised as potential inhibitors of α -chymotrypsin. These inhibitors incorporate a photoswitchable mono or disubstituted azobenzene which has been synthesised by the acid catalysed condensation of nitrosoarene and aryl amine derivatives or by direct coupling to a preformed switch. The position of the sulfonamide has been varied between the 3- and 4- positions of the azobenzene ring while additional substituents (Br, I, EtO₂C, NHRCH₂) have been incorporated into the 4'-position of the terminal ring. These groups were chosen as they have the potential to allow further modification of the azobenzene switch. Some potential modifications which should impart beneficial properties to the inhibitor include; addition of functionalities which improve water solubility (e.g. sugars, polyethylene glycols, *tetraalkylamines*) and increase steric bulk to increase conformational differences between the *E*- and *Z*-isomers and incorporate linkers to allow attachment to solid supports for investigation into photoregulated purification of enzymes. A general method for preparing polysubstituted azobenzenes could be useful for the synthesis of important dye molecules used in digital recording and liquid crystal displays.

4.6 References for chapter four

- (1) Westmark, P. R.; Kelly, J. P.; Smith, B. D. *Journal of the American Chemical Society* **1993**, *115*, 3416-3419.
- (2) Harvey, A. J., Thesis; University of Canterbury, 2000.
- (3) Sawicki, E. *Journal of Organic Chemistry* **1957**, *22*, 1084-1088.
- (4) Kažemekaite, M.; Talaikyte, Z.; Niaura, G.; Butkus, E. *Molecules* **2002**, *7*, 706-711.
- (5) Bamberger, E.; Landsteiner, K. *Berichte der Deutschen Chemischen Gesellschaft* **1893**, *26*, 482-495.
- (6) Priewisch, B.; Rueck-Braun, K. *Journal of Organic Chemistry* **2005**, *70*, 2350-2352.

- (7) Ogata, Y.; Takagi, Y. *Journal of the American Chemical Society* **1958**, *80*, 3591-3595.
- (8) Tollari, S.; Cuscela, M.; Porta, F. *Journal of the Chemical Society, Chemical Communications* **1993**, 1510-1511.
- (9) Anspion, H. D. In *Organic Syntheses; Collective Volume 3*; Horning, E. C., Ed.; John Wiley and Sons, Inc: 1964, p 711-712.
- (10) *Oxidising and Reducing Agents.*; John Wiley and Sons, 1999.
- (11) Linderman, R. J.; Graves, D. M. *Tetrahedron Letters* **1987**, *28*, 4259-4262.
- (12) Anelli, P. L.; Biffi, C.; Montanari, F.; Quici, S. *Journal of Organic Chemistry* **1987**, *52*, 2559-2560.
- (13) Coleman, G. H.; Nichols, G.; McCloskey, C. M.; Anspion, H. D. In *Organic Syntheses: Collective Volume 3*; Horning, E. C., Ed.; John Wiley and Sons, Inc.: 1955, p 712-713.
- (14) Desai, R. D.; Mehta, C. V. *Indian Journal of Pharmacy* **1951**, *13*, 211-213.
- (15) Woolfolk, E. O.; Reynolds, W. E.; Mason, J. L. *Journal of Organic Chemistry* **1959**, *24*, 1445-1450.
- (16) Coleman, G. H.; McCloskey, C. M.; Stuart, F. A. In *Organic Syntheses; Collective Volume 3*; Horning, E. C., Ed.; John Wiley and Sons, Inc.: 1955, p 668-670.
- (17) Bortolini, O.; Bragante, L.; Di Furia, F.; Modena, G. *Can. J. Chem.* **1986**, *64*, 1189-1195.
- (18) In *Vogel's textbook of practical organic chemistry*; Furniss, B. S., Hannaford, A. J., G., S. P. W., Tatchell, A. R., Eds.; Longman Scientific and Technical: 1989, p 897.
- (19) Percec, V.; Bera, T. K.; De, B. B.; Sanai, Y.; Smith, J.; Holerca, M. N.; Barboiu, B.; Grubbs, R. B.; Frechet, J. M. J. *Journal of Organic Chemistry* **2001**, *66*, 2104-2117.
- (20) Brady, K.; Abeles, R. H. *Biochemistry* **1990**, *29*, 7608-1717.

Chapter Five

Isomerisation Studies

Isomerisation Studies

5.1 Introduction

A series of α -chymotrypsin inhibitors have been designed and synthesised which incorporate an azobenzene moiety coupled to a peptidyl core. The azobenzene group is an important feature in the design as it can undergo conformation change between a planar *E*-(*trans*) and a twisted *Z*-(*cis*) conformation of the molecule. The rationale behind the design was to synthesise a series of enzyme inhibitors which can be switched between bioactive and less bioactive forms under the influence of external stimuli. In this case the trigger is irradiation of the azobenzene switch with UV or visible light to induce preferential conversion to one conformation or the other.

5.1.1 Structural properties of azobenzene¹

The azo group is characterised by two nitrogen atoms linked by a double bond with each nitrogen atom having a pair of non-bonding electrons. The photochemical and chemical stability of the azo compound is greatly dependent on the substituents incorporated into the molecule. This stability increases with increasing resonance and inductive effects.² The N=N bond distance varies little between aromatic and aliphatic azo compounds with azobenzene having a bond length of 1.23 Å. The C-N bond length is slightly shorter in *E*-azobenzene than *Z*-azobenzene (1.41 and 1.46 Å respectively) with C-N-N bond angles close to 120° giving rise to geometrical isomers.

E-Azobenzenes adopt a planar conformation through the N=N backbone while one of the phenyl rings of the *Z*-azobenzene is twisted out of the plane of the azo group by *ca.* 56° (section 3.6) due to steric hindrance between the *ortho* protons. This difference in spatial arrangement alters the dipole moments of the two isomers; values of 0 and 3 D have been determined for *E*- and *Z*-azobenzene respectively.

5.1.2 Spectroscopic properties of azobenzenes²

Molecular systems possess the ability to absorb or emit radiation. In this process only discrete quanta ($h\nu$) of characteristic energy are involved. This leads to a change in energy states where the spin state ($S_0 \rightarrow S_1$) must remain the same i.e. any change in multiplicity ($S_0 \rightarrow T_1$) is forbidden (fig. 1.1a). These states can be classed as rotational, vibrational and electronic motion. In solution rotational states are rarely observed and therefore discounted. An interaction between two states leads to the formation of a new high and low energy state (fig. 1.1b). The energy difference (ΔE) depends on the extent of coupling between states.

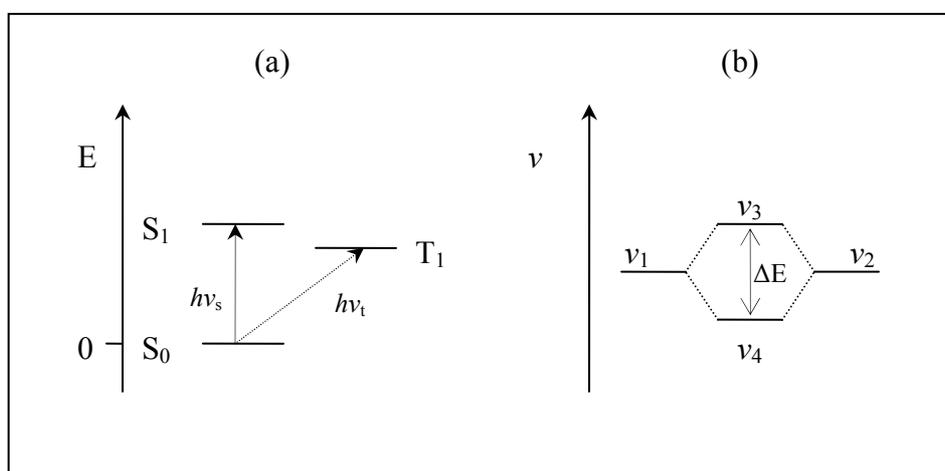


Figure 5.1. (a) Spin allowed and spin forbidden processes; (b) Coupled states.

$(n \rightarrow \pi^*)$ transitions

Azo compounds are characterised spectroscopically by a low energy ${}^1(n, \pi^*)$ state which gives rise to an absorption band at *ca.* 440 nm. This is the result of a weak $(n \rightarrow \pi^*)$ transition. Comparison of a range of aromatic azo compounds indicates that the position of the $(n \rightarrow \pi^*)$ transition is relatively unaffected by substitution of the aryl rings while the intensity of the transition is only slightly affected by the nature of the substituents and unaffected by position within the aromatic ring i.e. $(n \rightarrow \pi^*)$ transitions are determined by local symmetry not molecular geometry. This intensity is greater for *Z*-azobenzenes ($\lambda_{449} = 1250 \text{ M}^{-1} \cdot \text{cm}^{-1}$ for *Z*-azobenzene)³ as the transition to the lowest ${}^1(n \rightarrow \pi^*)$ state is symmetry allowed and approaches oscillator strengths comparable to $(\pi \rightarrow \pi^*)$ transitions.

The same transition for the *E*-isomer is forbidden by selection rules but does occur, albeit at lower intensity, as there is sufficient vibrational coupling with the $^1(\pi \rightarrow \pi^*)$ transition to permit the transition ($\lambda_{449} = 405 \text{ M}^{-1} \cdot \text{cm}^{-1}$ for *E*-azobenzene).³

$(\pi \rightarrow \pi^*)$ transitions

A second characteristic peak in the spectra of azobenzenes is an intense band at *ca.* 340 nm arising from the $(\pi \rightarrow \pi^*)$ transition, which, unlike the $(n \rightarrow \pi^*)$ transition is dependent on the substituents on the phenyl rings of the azo compounds. The highest occupied π orbital is sensitive to substituents, therefore upon substitution the $(\pi \rightarrow \pi^*)$ band is displaced whereas the $(n \rightarrow \pi^*)$ is not. In the case of *E*-azobenzene $(\pi \rightarrow \pi^*)$ vibrational bands are structured and separated at intervals of 220 cm^{-1} and $1300\text{-}1400 \text{ cm}^{-1}$ whereas *Z*-azobenzenes show no structure even at low temperature. A study of solvent effects on the $(\pi \rightarrow \pi^*)$ transition of substituted azobenzenes show that the absorption band red shifts with increasing solvent polarity, the effect being more pronounced with aprotic solvents.⁴

5.2 *E-Z* Isomerisation of azobenzenes

Irradiation of azo compounds with UV or visible light results in the formation of a photoisomeric equilibrium of the *E*- and *Z*-conformations (fig. 5.2).

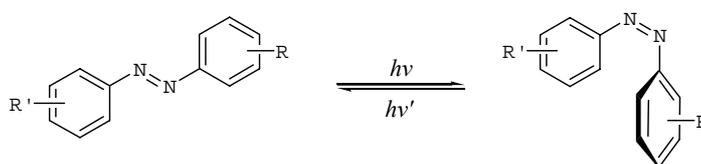


Figure 5.2. *E-Z* isomerisation of aromatic azo compounds.

These photostationary states arise because there are absorbance overlaps for the *E*- and *Z*-isomers at all wavelengths. These conformations exhibit considerable differences in the size, shape and polarity of the two isomers. These differences can be exploited which make azobenzenes ideal candidates for photoregulation. The *E*-isomer is

thermodynamically more stable by 50 kJ.mol^{-1} and while *Z*-azobenzene can thermally revert to the *E*-isomer in the absence of light the activation barrier for this process is 96 kJ.mol^{-1} in solution or 130 kJ.mol^{-1} in the solid state.² The corresponding half life of the *Z*-isomer is in the order of 1 day at room temperature.

The composition of the photostationary is dependant on the ratio of the molar absorptivity coefficients at the wavelength of irradiation. Under these conditions the equilibrium favours the isomer with the lowest ϵ .⁵

Consider the case where a compound can exist as either isomer A or B. Irradiation of the mixture with a specific wavelength of light which favours isomer A will lead to a high energy state A^* which decays to give an excited transition state P^* . Subsequent decay leads to transition state P which then has the choice of relaxing to either ground state A or B by internal conversion followed by loss of excess vibrational energy. If α is the probability of forming A then the probability that B is formed must be $(1-\alpha)$. Due to some overlap of the absorbance of B at the wavelength of irradiation, B is also excited to give B^* which decays to the common transition state P which also relaxes to give either A or B (fig. 5.3).

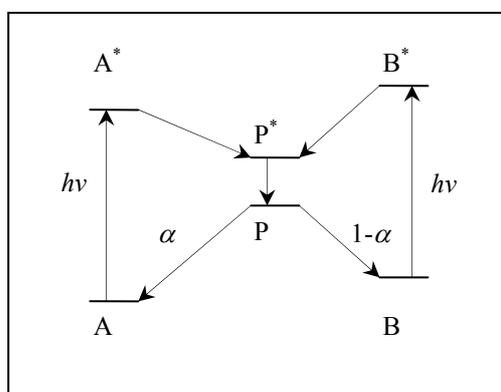


Figure 5.3. Photochemical processes for the photoisomerisation between states A and B.

The photostationary composition of isomers A and B is determined by the steady state equilibrium expressed in equation 5.1.

$$\frac{[A]}{[B]} = \left(\frac{\epsilon_B}{\epsilon_A} \right) \left(\frac{\alpha}{1 - \alpha} \right)$$

Equation 5.1

To put this into practice; if we want to enrich the photostationary state of azobenzene with the *Z*-isomer, the sample is irradiated with UV light ($\lambda < 350$ nm) as ϵ_Z is lower for all wavelengths between 375 and 275 nm. The opposite is true if *E*-enrichment is required, at all wavelengths > 375 nm, ϵ_E is lower so irradiation with visible light ($\lambda > 400$ nm) will afford predominantly *E*-azobenzene (fig. 5.4).

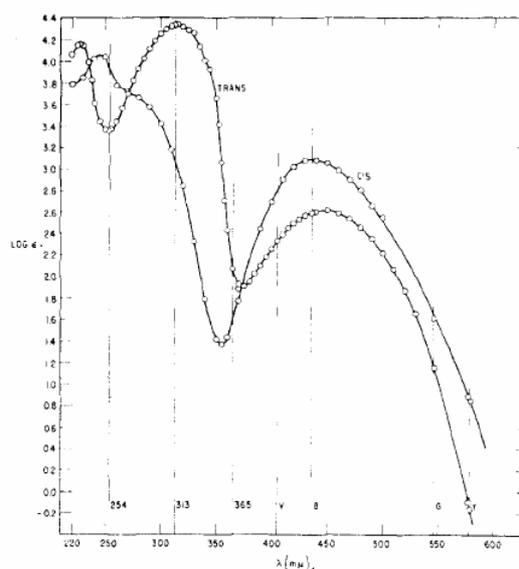


Figure 5.4. A plot of $\log(\epsilon)$ vs. λ for *E*- and *Z*-azobenzene. Taken from Zimmerman *et al* (1957).⁶

If B has a higher ground state energy than A, the activation energy for the thermal back reaction is lower than for the forward reaction and therefore favoured in many cases. Since the thermal $Z \rightarrow E$ isomerisation was first reported in 1937⁷ many studies have been undertaken to determine the isomerisation mechanism and factors which influence the rate. Activation energies for the thermal isomerisation typically range from 88-100 $\text{kJ}\cdot\text{mol}^{-1}$ and are very dependant on substitution, solvent polarity and proton concentration. The thermal isomerisation rate of disubstituted azobenzenes incorporating an electron donating substituent (NR_2 , OR) and an electron withdrawing group (NO_2 , CO_2R) is significantly increased in polar aprotic solvents due to stabilisation of the dipolar transition state.⁴ At low pH the rate of isomerisation is greatly accelerated while at high pH the rate is inhibited.⁸

5.2.1 Mechanism of isomerisation

The proportion of *Z*-isomer formed in solution is dependant on the wavelength of light used and the solvent the photostationary state is obtained in. The final photostationary state is independent of the initial composition, sample concentration and the intensity of light. Only the time taken to reach the photostationary state is dependent on sample concentration and intensity of irradiation which can take from minutes to hours.⁵

The mechanism by which azobenzenes undergo isomerisation has been debated by various groups (Asano,⁹ Bellobono,¹⁰ and Nishimura¹¹). The two favoured mechanisms are inversion of a nitrogen atom which involves the ($n \rightarrow \pi^*$) state and rotation about the N-N σ -bond which involves the ($\pi \rightarrow \pi^*$) state.

The most popular mechanism for isomerisation, 'inversion' proceeds through a transition state where one of the nitrogen atoms has been *sp*-hybridised. Evidence for this was obtained from the facile isomerisation of an azobenzene unit (5.1) incorporated within a ring system (fig. 5.5).

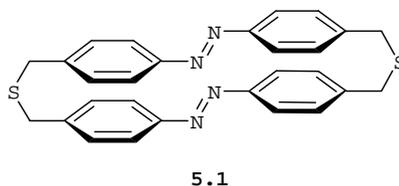


Figure 5.5. *E*-2,19-dithia[3,3](4,4')-diphenyldiazeno-(4)-phane.

Under these circumstances, a rotational mechanism would have been improbable because of the difficulties associated with rotating the phenyl ring around the N-N bond due to steric effects. A study of the mechanism of isomerisation for simple disubstituted azobenzenes lead to some conflicting conclusions. Both Nishimura and Bellobono were in favour of a single path (inversion) mechanism while Asano favoured a mixed inversion-rotation pathway (fig. 5.6).⁹

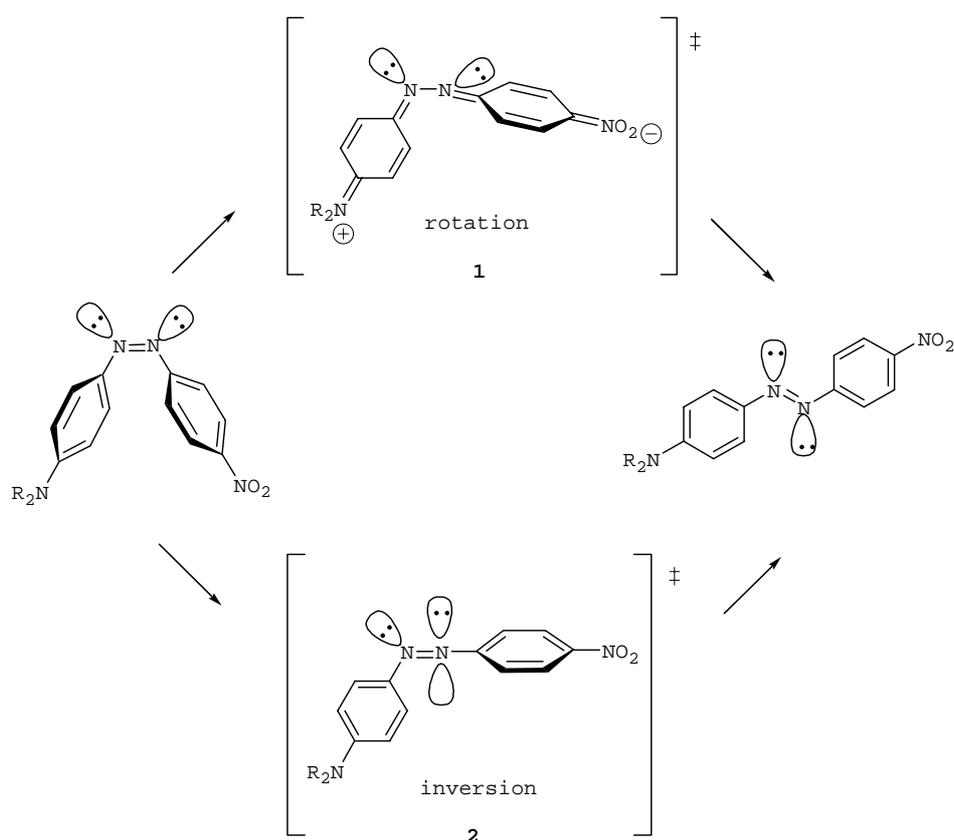


Figure 5.6. Mixed pathway for the isomerisation of 4-(dialkylamino)-4'-nitroazobenzene.

Asano *et al* found that 4-amino-4'-nitroazobenzenes underwent thermal isomerisation by both inversion and rotational routes. This conclusion was based on the effects of solvent, temperature and pressure on the isomerisation kinetics which could not be explained by an inversion only mechanism. It was concluded that the mechanism of isomerisation was dependent on the solvent and nature of the substituents. Rotation around the N-N σ -bond favours polar solvents as the zwitterionic intermediate (1) is stabilised by polar solvents while inversion occurs predominantly in non-polar solvents where the intermediate (2) has no charge and therefore stabilisation of charge is not required.

5.2.2 Practical applications

Azo dyes

Many of the examples of azo compounds in the literature are based on simple mono- and disubstituted azobenzenes which have been synthesised for kinetic and mechanistic studies or as models for more complex dyes. Another niche are the so called azo dyes which range from simple substituted azobenzenes (**5.2**) through to complex macromolecules (**5.4**) which incorporate multiple azo units, aryl rings and substituents and tend to be highly conjugated (fig. 5.7).

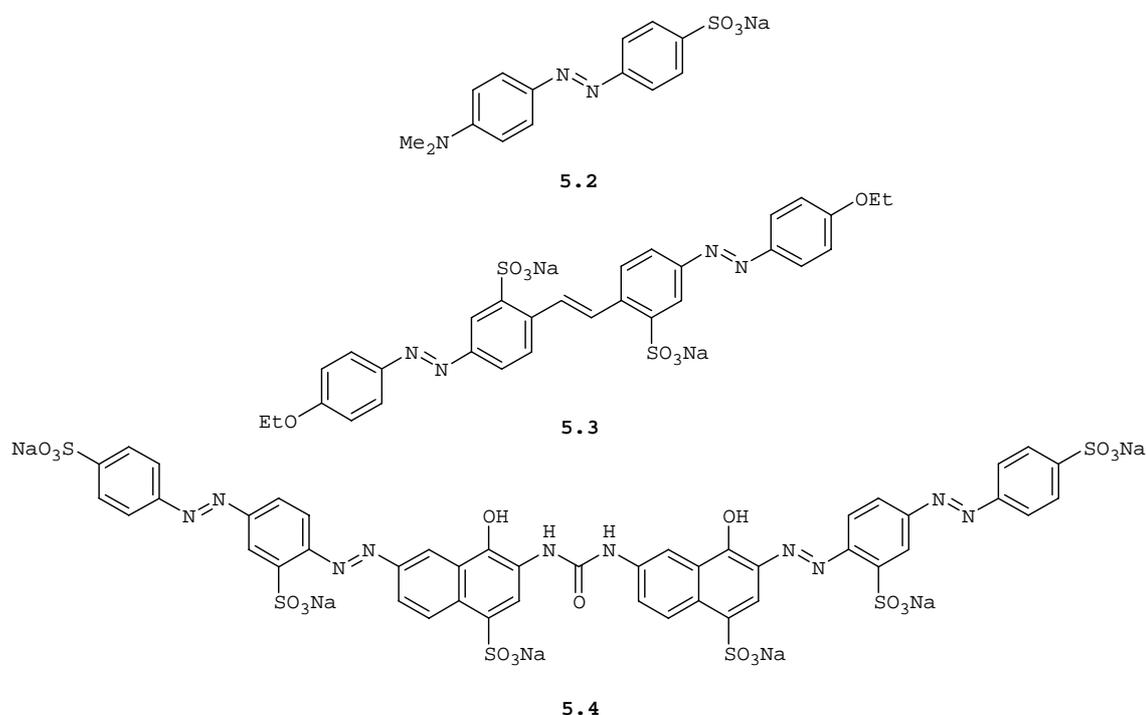


Figure 5.7. Azo dyes Methyl orange **5.2**, Direct yellow 12 **5.3** and Direct red 80 **5.4**.

Azo dyes generally exist in a full *E*-configuration but can undergo *E-Z* isomerisation which often results in colour change or changes in colour intensity. Potentially, due to multiple double bonds within the structure, the photochemistry can be quite complex and result in mixtures of *E*- and *Z*-substituted double bonds within the molecule. An interesting observation noted that fibre affinity can be affected by isomerisation. Applications of Direct yellow 12 include dyeing of cellulose fibres. Upon irradiation of an aqueous solution

of the dye the hue visibly intensified and the affinity for the cellulose decreased. This was presumably because the non-linear geometry of **Z-5.3** no longer complimented the rigid cellulose structure.¹²

Host-Guest chemistry

Azobenzene units have been utilised to enforce reversible changes in larger molecules such as crown ethers, cryptands, cyclodextrins and polymers and have proved useful as agents for reversibly binding host ions and molecules during extraction.¹³ For example, cryptand **E-5.4** was found to bind heavy metal ions such as Cu^{2+} , Ni^{2+} , Co^{2+} and Hg^{2+} from aqueous media and extract them into organic solutions. Conversely, **Z-5.5** was unable to bind the metal ions due to expansion of the cavity. Photoswitching of the cryptand allowed the metals to be released from the complex upon isomerisation to the Z-isomer (fig. 5.8).

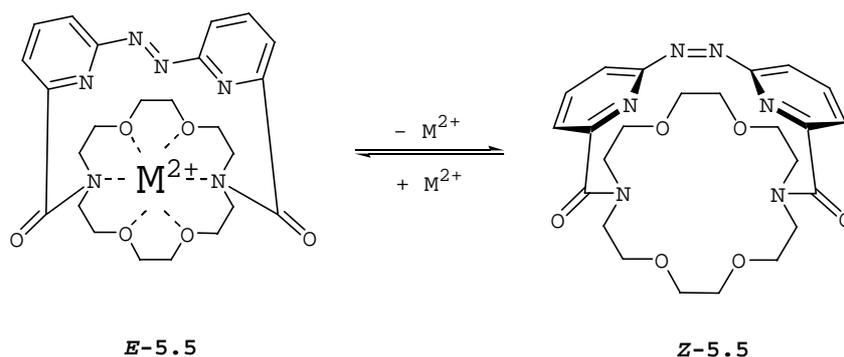


Figure 5.8. Specific binding of heavy metals by an azo bridged cryptand.

Photoregulation of enzyme activity

The work in this field has focused on two main areas; modification of the enzyme or enzyme analogue by covalently linking the photoactive molecule through a side chain residue¹⁴ or polymer matrix,¹⁵ or by synthesis of specific molecules incorporating the photoactive moiety which can interact with the enzyme or macromolecule.¹⁶⁻²²

Photoregulation of enzyme inhibition with small molecules is the basis for this thesis. A series of inhibitors have been designed which incorporate three key features; molecular

switching, enzyme specificity and an inhibitory warhead. The design of these inhibitors has been discussed in greater detail in section 4.1.

Previous work in this area began in the late sixties when Kaufman *et al*²³ and later Bieth *et al*¹⁶ modified known acetylcholinesterase inhibitors by incorporating azobenzene into the structure. In this way the activity of *N*-diphenylcarbamyl halides **5.6** were compared with the activity of *N*-4-phenylazophenyl-*N*-phenylcarbamyl halides **5.7**.

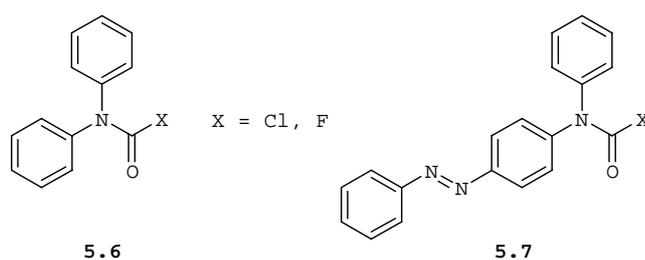


Figure 5.9. Acetylcholinesterase inhibitors.

Compounds **5.7** exhibited significant photoswitching and upon irradiation with UV light gave enriched mixtures consisting of 85-90 % *Z*-isomer. Kinetic studies showed the *Z*-isomers to be 3-6 fold more active than the corresponding *E*-isomers and in all cases the introduction of an azobenzene moiety improved the potency of the inhibitor compared with the parent carbamate.

Westmark *et al* designed transition state analogue inhibitors of cysteine and serine proteases which incorporated an azobenzene photoswitch and either an aldehyde **5.8** or boronic acid **5.9** warhead.¹⁷ Boronic acid warheads have also been incorporated into some inhibitors synthesised in our laboratories.

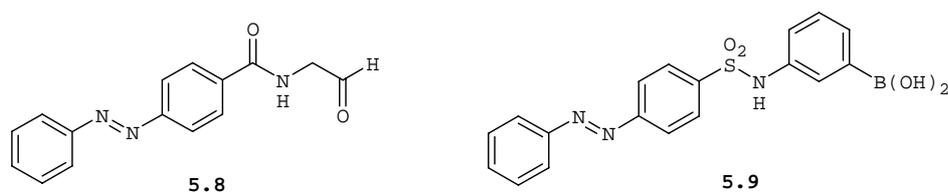


Figure 5.10. Cysteine **5.8** and serine **5.9** protease inhibitors.

Irradiation of **5.8** and **5.9** with UV light ($330 < \lambda < 370$ nm) gave 83 and 80 % *Z*-enrichment respectively while irradiation of **5.8** with visible light ($\lambda > 400$ nm) gave complete restoration of the *E*-isomer. Unlike the acetylcholinesterase inhibitors it was found that both *E*-isomers were more active against their respective enzymes than the *Z*-isomers (fig. 5.11).

compound	K_i (μM)	
	<i>E</i> -enriched	<i>Z</i> -enriched
5.8	2.1	8.8
5.9	11	41

Figure 5.11. Apparent K_i for the *E*- and *Z*-enriched conformations of **5.8** and **5.9**.

Work by Amato *et al* showed that alfalfa leaf protease was inhibited by interlinked positively charged amino groups. By separating the charges with an azobenzene linker (fig. 5.12) they demonstrated biological activity of the enzyme depended on the conformation of the azobenzene.

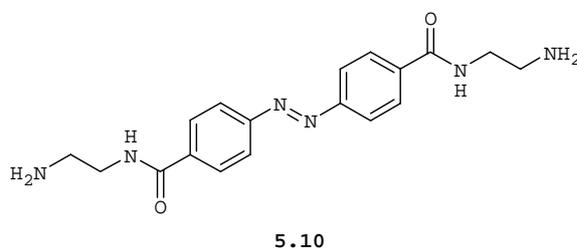


Figure 5.12. An alfalfa leaf protease inhibitor.

The symmetrical disubstituted azobenzene **5.10** was synthesised and when irradiated at 340 nm gave 60 % *Z*-isomer while irradiation at 417 nm reduced the proportion of *Z*-isomer to 5 %. It was found that *E*-**5.10** was a competitive inhibitor of the enzyme ($K_i = 5.7 \times 10^{-4}$ M) while *Z*-**5.10** was completely inactive under the experimental conditions. The difference in biological activity was attributed to the change in distance between the two amino groups as a result of the different conformations.¹⁸

Tyrosinase is an enzyme with a binuclear copper active site responsible for converting *L*-tyrosine into melanin and may also be useful for detoxifying phenolic compounds. It is inhibited by benzoic acid derivatives and as such deemed a potential target for photoregulation. Komori *et al* prepared 4-azobenzene carboxylic acid **5.11** and 4,4'-azobenzene dicarboxylic acid **5.12** as photoswitchable analogues of benzoic acid.²²

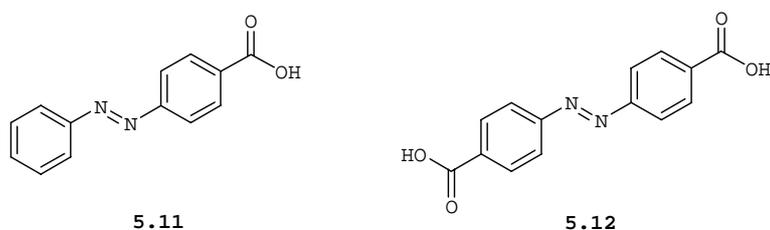
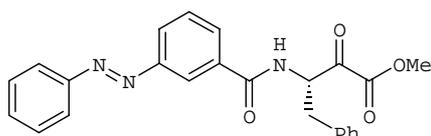


Figure 5.13 Tyrosinase inhibitors.

Irradiation of **5.11** and **5.12** with UV light ($\lambda < 370$ nm) gave 65 and 68 % *Z*-isomer respectively. Irradiation of *Z*-**5.11** with visible light ($\lambda > 400$ nm) gave 67 % *E*-**5.11**. Interestingly, *Z*-**5.11** was more potent than the *E*-isomer while *Z*-**5.12** was less inhibitory than the *E*-isomer. Under their experimental conditions the changes in enzyme activity were 2-fold or less. The changes in the active conformation were attributed to different electron densities of the carboxylic acid groups which coordinate to the copper in the active site and differences in steric hindrance; **5.12** was expected to be more sterically hindered due to the extra carboxylic acid.

The work of Harvey examined the effects of substitution patterns of the azobenzene moiety as well as connectivity order within the molecule for a series of α -keto esters based on *L*-phenylalanine. It was found that the most active order of connectivity is based on switch-peptide- α -keto ester and that for substitution the order of potency was *m*- > *p*- > *o*-substituted azobenzenes.²⁰ The most active compound synthesised was the 3-substituted azobenzene **5.13** (fig. 14).



5.13

Figure 5.14. An azobenzene based α -keto ester.

The ambient photostationary state for **5.13** was 28 % *Z*-isomer while irradiation with UV light ($330 > \lambda > 370$ nm) increased the proportion to 54 %. Assays against α -chymotrypsin showed that **Z-5.13** ($K_i = 40$ nM) was 2-fold more active than **E-5.13** ($K_i = 80$ nM) which was consistent with the 2-fold increase in the proportion of *Z*-isomer upon irradiation with UV light.²⁰ It was trying to improve this work which much of my thesis was based on.

From work described in the literature it is clear that biological activity can be influenced by the conformation of the photoactive inhibitor and as such photoregulation is possible. The active conformation varies, for some the *Z*-isomers are more bioactive while for others it is the *E*-isomer. Obviously the active conformation of the inhibitor must be dependent on interactions and steric requirements within the enzymes active site. Currently the differences in activity are of the order of 2-6 fold but as mechanistic understanding improves these differences may be increased.

5.3 Experimental setup

Eight potential inhibitors were chosen for the isomerisation studies. The samples were prepared as *ca.* 10 mM solutions in deuterated acetonitrile or DMSO and analysed by ^1H NMR in a 5 mm quartz NMR tube. Samples were irradiated with a 500 W high pressure mercury arc lamp filtered to allow either UV or visible light transmission. A Schott UG11 filter ($230 < \lambda < 410$ nm) or a Corning 7-37 filter ($310 < \lambda < 390$ nm) afforded *Z*-isomer enrichment while a Corning 0-51 filter ($\lambda > 380$ nm) afforded enrichment of the *E*-isomer. The filter was mounted at the end of a plastic tube 30 cm in length which contained a 17 cm water cell with quartz glass windows to allow passage of UV and visible light but

reduce sample heating from infrared radiation. Samples were placed an additional 5 cm from the filter.

5.4 Isomerisation of the target molecules

A typical experiment involved recording an NMR spectrum of the freshly prepared sample to ensure 100 % *E*-enrichment. If significant amounts of ketone were present, 10 μL of H_2O was added to give hydrate exclusively. The use of H_2O rather than D_2O was important to prevent deuterium exchange of the NH and OH peaks which were necessary for integration. The sample was then irradiated with filtered UV light for 75-90 min in a darkened fume hood to enrich the sample with the *Z*-isomer. The sample was encased with foil to protect from ambient light and analysed by ^1H NMR immediately after irradiation. *E/Z* ratios were determined by measurement of the average *E/Z* integrals of appropriate non-overlapping proton resonances, typically the hydrate OH, α -H and NH signals were used depending on the degree of overlap. The sample was then irradiated with visible light for 75-90 min and analysed in an analogous manner. The photostationary state was determined by leaving the sample sitting on a window sill for one or two days then measuring the *E/Z* ratio by ^1H NMR. All experiments were repeated on the same samples and agree within $\pm 2\%$. The average value has been reported, rounded to the nearest 1 %.

5.4.1 Schott UG11 UV filter

Initially, the UG11 filter was used for the UV irradiations, however it was found the *Z*-enrichment reached a maximum of *ca.* $50 \pm 5\%$ for the mono substituted azobenzenes and the alkyl substituted azobenzene **4.54**, for the halogenated derivatives *Z*-enrichment was very poor at only *ca.* 30 % (fig. 5.15).

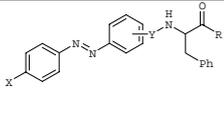
	X	Y	R	PSS ambient (% Z)	PSS uv (% Z)
4.4	H	3-CO	CO ₂ Me	26	56
4.7	H	3-CO	CF ₃	21	47
4.12	H	4-SO ₂	CF ₃	21	50
4.49	Br	4-SO ₂	CF ₃	17	32
4.52	I	4-SO ₂	CF ₃	17	31
4.54	FmocNHCH ₂	4-SO ₂	CF ₃	15	49

Figure 5.15. *Z*-Enrichment of substituted azobenzenes with a UG filter.

Comparison of other *E/Z* photostationary states after *Z*-enrichment with the reported literature (section 5.2.2) suggested these values were lower than should be expected. Analysis of the UV spectrum of the UG11 filter showed a broad, intense UV profile between 230 and 410 nm. This band was found to overlap with the band of *Z*-azobenzenes corresponding to the ($n \rightarrow \pi^*$) transition and resulted in conversion of *Z* \rightarrow *E* giving lower than expected *Z*-enrichment. A new Corning 7-37 filter was purchased and was found to have a narrower, less intense UV profile with minimal overlap (fig. 5.16).

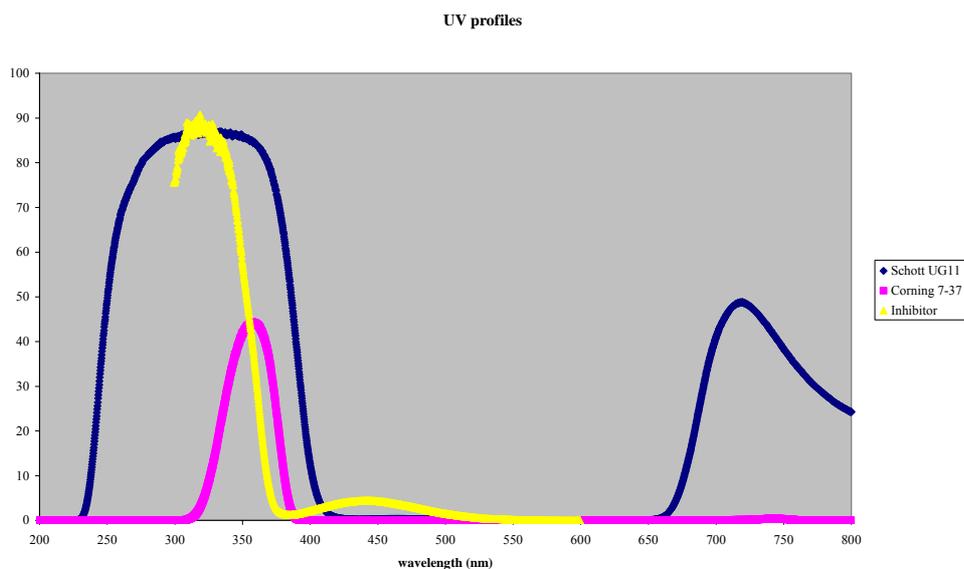


Figure 5.16. UV profiles for the Schott and Corning filters overlaid against the UV profile of an inhibitor at an ambient photostationary state. The inhibitor profile is not to scale.

5.4.2 Corning 7-37 UV filter

The isomerisation experiments were repeated using the Corning 7-37 filter on eight samples deemed most suitable as inhibitors of α -chymotrypsin. Z-Enrichment was improved to the point where most samples exhibited 80-90 % enrichment. The biggest improvements were for the halogenated azobenzenes **4.49** and **4.52**, increasing from *ca.* 30 to *ca.* 90 %. The results were not so impressive for **4.7**; Z-enrichment only went from 47 to 66 % (fig. 5.17). A representative ^1H NMR spectrum of **4.52** after irradiation for 90 min with UV light is shown in figure 5.18.

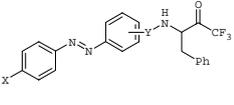
	X	Y	PSS ambient (% Z)	PSS uv (% Z)	PSS vis (% Z)
4.12	H	4-SO ₂	22	78	18
4.12 [#]	H	4-SO ₂	23	80	19
4.16	H	4-SO ₂ -Leu	23	66	20
4.44	Br	3-SO ₂	23	91	18
4.46	I	3-SO ₂	22	92	18
4.49	Br	4-SO ₂	19	87	17
4.52	I	4-SO ₂	17	87	14
4.62	I	4-SO ₂ -Leu	16	83	14
4.7	H	3-CO	27	66	21

Figure 5.17. *E-Z* Isomerisations of substituted azobenzenes with Corning filters. # Sample dissolved in DMSO-*d*₆

5.4.3 Corning 0-51 visible filter

Irradiations of a sample with the Corning 0-51 filter typically gave a photostationary state within 5 % of the ambient photostationary state (fig. 5.17). As a result it was decided that the ambient photostationary state could be used as a good approximation for the visible photostationary state during the enzyme assays. A representative ^1H NMR spectrum of **4.52** after irradiation for 90 min with visible light is shown in figure 5.19.

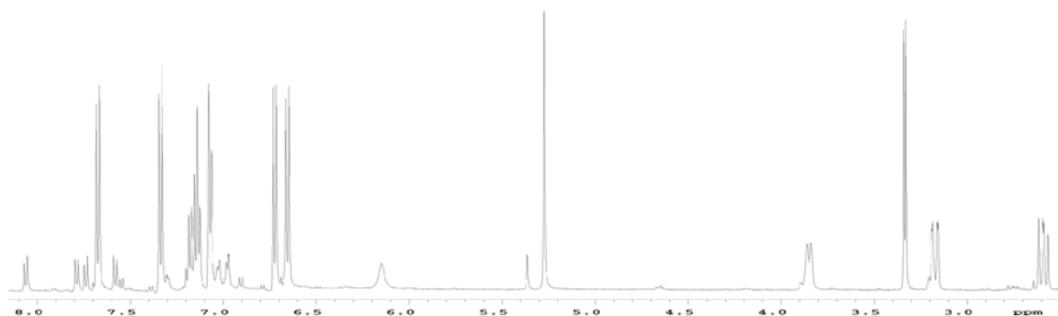


Figure 5.18. ^1H NMR spectrum of **4.52** after irradiation for 90 min with UV light. The composition was determined by measuring the integrals of the OH protons at 5.37 (*E*) and 5.27 (*Z*) ppm.

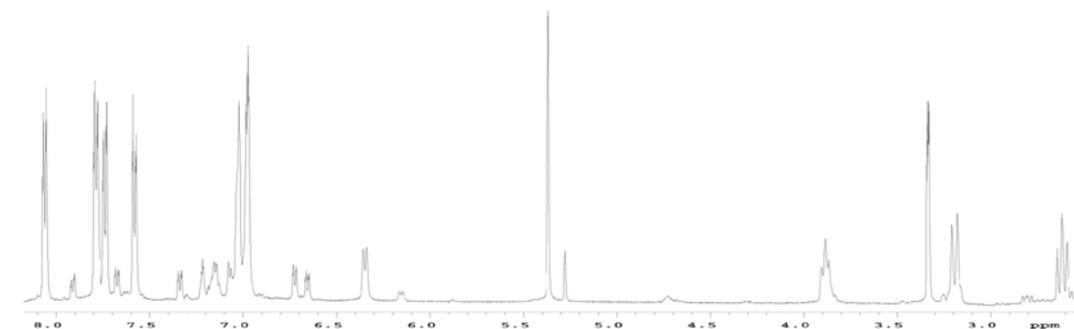


Figure 5.18. ^1H NMR spectrum of **4.52** after irradiation for 90 min with visible light. The composition was determined by measuring the integrals of the OH protons at 5.37 (*E*) and 5.27 (*Z*) ppm.

A second experiment was performed to illustrate the dependence of wavelength and independence of composition on the degree of isomerisation. A solution of **4.12** in deuterated acetonitrile was allowed to reach its ambient photostationary state comprising 23 % *Z*-isomer. The sample was first irradiated with visible light for 90 min to reduce the *Z*-isomer to 18 % then with UV light for 90 min to enrich the *Z*-isomer to 78 %. Irradiation with visible light once again gave 18 % *Z*-isomer and after 1 day sitting under fluorescent lights the photostationary state reverted to 22 % *Z*-isomer. From these results it can be concluded the *E*- and *Z*-isomer compositions for the three photostationary states are fixed for specific wavelengths and are independent of the initial composition of *E*- and *Z*-isomers.

5.4.4 Isomerisation in deuterated DMSO

Originally, the inhibitors used in the enzyme assays were dissolved in acetonitrile and added to the assay media such that the final concentration of acetonitrile in the assay was 5 % v/v. Due to solubility issues of the inhibitors in the aqueous environment of the assay medium; it became necessary to change the organic solvent from acetonitrile to DMSO in an attempt to enhance solubility. As most of the isomerisation studies had already been performed in deuterated acetonitrile, it became prudent to investigate whether a model inhibitor underwent the same degree of isomerisation in DMSO as for acetonitrile.

It has been reported that the photostationary state of a sample is dependent on the polarity of the solvent. Polar solvents stabilise the *Z*-isomer and therefore it is expected that the percentage of *Z*-isomer will increase with increasing polarity.²⁴ The dielectric constant²⁵ and dipole moment²⁵ for acetonitrile is 37.5 and 3.92 respectively and a relative polarity²⁶ of 0.460 compared to water. For DMSO the values are 47, 3.96 and 0.444 respectively. These values compare favourably and as such it was expected that the results obtained in either solvent would be similar.

A sample of **4.12** was dissolved in deuterated DMSO from a freshly opened ampoule in an attempt to minimise the amount of water in the solvent. The ¹H NMR spectrum showed resonances for the NH and OH peaks had shifted significantly downfield from 6.35 and 5.40 ppm to 8.10 and 7.20 ppm respectively. This was seen as a problem due to the potential for overlap with the aromatic protons. Fortunately, at least for **4.12**, the *E*- and *Z*-NH resonances were situated between aromatic peaks and as such could be integrated with only minor overlap. The hydrate OH peaks could not be integrated due to significant overlap of the aromatic protons. The α -H was free from overlap from other protons however minor overlap occurred between the resonances for the *E*- and *Z*-isomers. As such, the estimates for the ratios of *E*/*Z* incur an error of ± 5 %. UV irradiation with the Corning 7-37 filter gave *Z*-enrichment of 80 % (78).[§] The enriched sample was encased in foil and placed in a dark cupboard over the weekend. After two days in the dark the sample had only reverted to 39 % *Z*. Irradiation with UV light gave 81 % *Z*. Subsequent irradiation

[§] Percentage of *Z*-isomer in CD₃CN is included in brackets for comparison.

with visible light gave 19 % (18) *Z* and after exposure to sunlight for a day reached a photostationary state comprising 23 % (22) *Z*-isomer.

From these model results the assumption that the photostationary state of the inhibitors in DMSO should approximate those seen in acetonitrile is a fair one. The results also show that even in the absence of light, *Z* → *E* isomerisation occurs. From the literature it has been shown that isomerisation in the absence of light occurs via a thermal route (section 5.2).

Selected ¹H NMR data for the isomerisation studies can be found in section 7.5.3.

5.5 Summary

All the inhibitors synthesised exhibit excellent photoswitching which can be cycled by irradiation with UV or visible light. The ambient photostationary state for each inhibitor was found to be within 5 % of the visible photostationary state and as such made a good approximation. Compound **4.12** exhibited very similar photochemistry in both DMSO and acetonitrile, and again the approximation that the isomerisation in either solvent is the same appears valid. Incorporation of a halogen into the azobenzene ring system increased the percentage of *Z*-isomer after UV irradiation for **4.49** and **4.52** by 9 % compared with the analogous non-halogenated azobenzene **4.12**. In the case of the dipeptide **4.62** the increase was 17 % compared to **4.16**. Interestingly, the percentage of *Z*-isomer for the ambient and visible photostationary states decreased compared with the non-halogenated molecule. The higher percentages of halogenated *Z*-isomers could be rationalised as being stabilised to a greater degree by the polar solvent due to the increased dipole moments caused by the electronegative effects of the halogens.

5.6 References for chapter five

- (1) Hampson, G. C.; Robertson, J. M. *Journal of the Chemical Society, Abstracts* **1941**, 409-413.
- (2) Rau, H. *Angewandte Chemie International Edition* **1973**, *12*, 224-235.
- (3) Rau, H.; Dürr, H., Bouas-Laurent, H., Eds.; Elsevier: Amsterdam: **1990**, p 165-192.
- (4) Schanze, K. S.; Mattox, T. F.; Whitten, D. G. *Journal of Organic Chemistry* **1983**, *48*, 2808-2813.
- (5) Fischer, E.; Frankel, M.; Wolovsky, R. *Journal of Chemical Physics* **1955**, *23*, 1367.
- (6) Zimmerman, G.; Chow, L.-Y.; Paik, U.-J. *Journal of the American Chemical Society* **1958**, *80*, 3528-3531.
- (7) Hartley, G. S. *Nature (London, United Kingdom)* **1937**, *140*, 281.
- (8) Sanchez, A. M.; Rossi, R. H. *Journal of Organic Chemistry* **1995**, *60*, 2974-2976.
- (9) Asano, T.; Okada, T. *Journal of Organic Chemistry* **1986**, *51*, 4454-4458.
- (10) Marcandalli, B.; Pellicciari-Di Liddo, L.; Di Fede, C.; Bellobono, I. R. *Journal of the Chemical Society, Perkin Transactions 2: Physical Organic Chemistry (1972-1999)* **1984**, 589-593.
- (11) Nishimura, N.; Tanaka, T.; Asano, M.; Sueishi, Y. *Journal of the Chemical Society, Perkin Transactions 2: Physical Organic Chemistry (1972-1999)* **1986**, 1839-1845.
- (12) Saunders, K. H.; Allen, R. L. M. In *Aromatic Diazo Compounds*; 3rd ed.; Edward Arnold: **1985**, p 256-367.
- (13) Suginome, H. In *CRC Handbook of Organic Photochemistry and Photobiology*; CRC Press, Inc: **1995**, p 825-840.
- (14) Willner, I.; Rubin, S. *Angewandte Chemie, International Edition in English* **1996**, *35*, 367-385.
- (15) Willner, I.; Rubin, S.; Zor, T. *Journal of the American Chemical Society* **1991**, *113*, 4013-4014.
- (16) Bieth, J.; Vratsanos, S. M.; Wassermann, N. H.; Cooper, A. G.; Erlanger, B. F. *Biochemistry* **1973**, *12*, 3023-3027.
- (17) Westmark, P. R.; Kelly, J. P.; Smith, B. D. *Journal of the American Chemical Society* **1993**, *115*, 3416-3419.

-
- (18) Amato, C.; Fissi, A.; Vaccari, L.; Balestreri, E.; Pieroni, O.; Felicioli, R. *Journal of Photochemistry and Photobiology, B: Biology* **1995**, *28*, 71-75.
- (19) Harvey, A. J., Thesis; University of Canterbury, **2000**.
- (20) Harvey, A. J.; Abell, A. D. *Tetrahedron* **2000**, *56*, 9763-9771.
- (21) Harvey, A. J.; Abell, A. D. *Bioorganic and Medicinal Chemistry Letters* **2001**, *11*, 2441-2444.
- (22) Komori, K.; Yatagai, K.; Tatsuma, T. *Journal of Biotechnology* **2004**, *108*, 11-16.
- (23) Kaufman, H.; Vratsanos, S. M.; Erlanger, B. F. *Science* **1968**, *162*, 1487-1489.
- (24) Zacharias, P. S.; Ameerunisha, S.; Korupoju, S. *Journal of the Chemical Society, Perkin Transactions 2* **1998**, 2055-2059.
- (25) *CRC Handbook of Chemistry and Physics*; 73rd ed.; CRC Press, **1992-1993**.
- (26) Reichardt, C. *Solvents and Solvent Effects in Organic Chemistry*; 2nd ed.; VCH Publishers, **1988**.

Chapter Six

Enzyme Inhibition Studies

Enzyme Inhibition Studies

6.1 Introduction

A selection of trifluoromethyl ketones were assayed against α -chymotrypsin in order to ascertain their potential as protease inhibitors. Each inhibitor was assayed at its ambient and UV photostationary state and the level of inhibition determined for each photostationary state. The aim of this study was to confirm the mode of inhibition and observe how K_i is affected by *E/Z*-conformations, substitution patterns and peptide chain length.

Enzyme catalysed reactions are very important in nature due to their remarkable specificity towards a substrate, exceptional levels of catalytic turnover and efficiency. In order to study enzyme kinetics methods need to be devised in which either the disappearance of substrate or formation of product is monitored. Importantly, the use of UV/Vis spectroscopy allows one to continuously measure the change in absorbance of the analyte of interest. A common method for studying enzyme kinetics involves hydrolysis of a peptidyl substrate which incorporates a chromophore that upon cleavage from the substrate liberates a compound with a high molar absorptivity coefficient ($\epsilon > 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$) at a wavelength not strongly absorbed by the enzyme or substrate. Some chromophore examples include 4-nitroaniline, 7-amino-4-methylcoumarin, 2-naphthylamine and substituted phenols (fig. 6.1).³

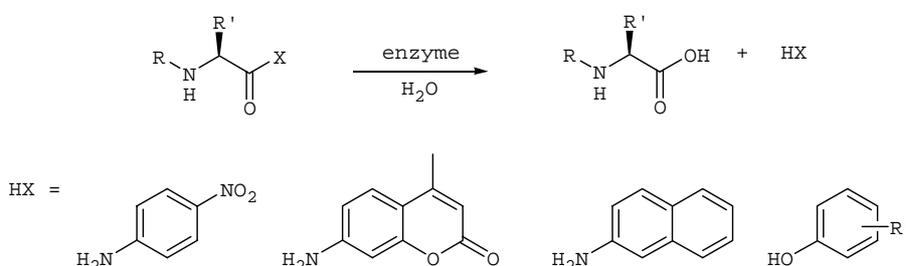
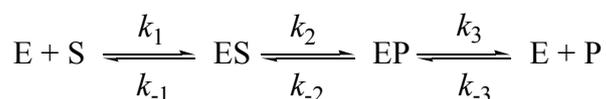


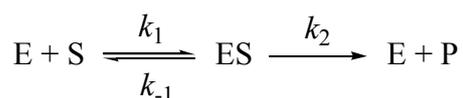
Figure 6.1. Enzymatic hydrolysis of peptidyl substrates.

6.2 Michaelis-Menten kinetics^{1,2}

If we look at a simple model where the enzyme acts on a single substrate to give a single product we can write an expression as follows:



However, this equation can be simplified if we only consider the enzyme kinetics at the onset of the reaction i.e. measure initial velocities (V_0) when $[S] \approx 100\%$ and $[P] \approx 0\%$. While $[P]$ remains low the back reaction is negligible and the above expression may be simplified to:



In this model, substrate (S) binds reversibly with the enzyme (E) to form an enzyme-substrate complex (ES) which undergoes conversion to give product (P) and free enzyme. From this expression two equations can be derived depending on the assumption made. If one assumes that the equilibrium of $E + S \rightleftharpoons ES$ is only slightly perturbed by the conversion of $ES \rightarrow P$ ($k_{-1} \gg k_2$) then equation 6.1 takes the form:

$$V_0 = \frac{V_{\max} [S]}{K + [S]} \quad \text{where } K = \frac{k_{-1}}{k_1} \quad \text{and } V_{\max} = [E]_T k_2 \quad \text{Equation 6.1}$$

The value V_{\max} refers to the maximal velocity or limiting rate and K a true dissociation constant. This dissociation constant is a true measure of binding affinity, the smaller the value of K, the stronger the binding of substrate to enzyme.

Unfortunately, the assumption that enzymes achieve rapid equilibrium is invalid in most cases so a second model involving a steady state assumption for the ES complex was postulated by Briggs and Haldane.

In this model [S] and [P] are changing but [ES] remains relatively static after an initial burst phase (pre-steady state) provided $[S] \gg [E]$ i.e. $\frac{d[ES]}{dt} \approx 0$

By a similar derivation equation 6.2 is obtained.

$$V_o = \frac{V_{\max} [S]}{\left(\frac{k_{-1} + k_2}{k_1}\right) + [S]} \quad \text{Equation 6.2}$$

This is more often expressed in the form:

$$V_o = \frac{V_{\max} [S]}{K_m + [S]} \quad \text{where } K_m = \left(\frac{k_{-1} + k_2}{k_1}\right) \quad \text{and } V_{\max} = [E]_T k_2 \quad \text{Equation 6.3}$$

This equation is commonly referred to as the Michaelis-Menten equation even though they actually developed equation 6.1. However, irrespective of whichever assumption is made, the fundamental equation is the same. The rapid equilibrium assumption can be seen as a limiting case of the steady state assumption when $k_{-1} \gg k_2$. The Michaelis constant K_m is not an equilibrium constant as it is obtained under steady-state rather than equilibrium conditions but is often mistakenly used to describe enzyme affinity. $K_m = K$ is only true when k_2 is very small relative to k_{-1} .

6.2.1 Linearisation of the Michaelis-Menten equation

If a graph of V_o is plotted against [S] a non-linear curve is obtained (fig. 6.2). At high [S] saturation of the enzyme occurs (i.e. all the active sites of the enzyme are bound to substrate) and V_o approaches V_{\max} . A graph of this type is not useful as V_{\max} can never be achieved in practice; [S] would theoretically need to reach infinity before V_{\max} is reached. As a consequence, K_m , which is defined as the substrate concentration at half the maximal velocity, can not be measured accurately from the graph. To overcome these difficulties, equation 6.3 can be reformulated in several ways to give a linear fit of the Michaelis-Menten equation. The four most popular forms are described below:

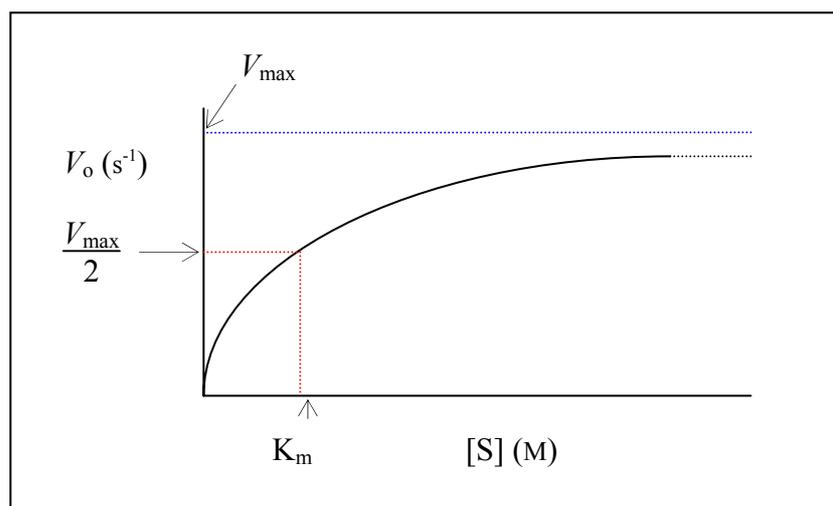


Figure 6.2. Dependence of initial rates on increasing substrate concentration.

Double reciprocal plot

Taking the reciprocal of both sides of equation 6.3 gives:

$$\frac{1}{V_o} = \frac{K_m}{V_{\max}} \frac{1}{[S]} + \frac{1}{V_{\max}} \quad \text{Equation 6.4}$$

A plot of $\frac{1}{V_o}$ vs. $\frac{1}{[S]}$ gives a straight line with a slope of $\frac{K_m}{V_{\max}}$ and intercepts on the x and y -axes of $-\frac{1}{K_m}$ and $\frac{1}{V_{\max}}$ respectively (fig. 6.3). This double reciprocal plot is referred to as the Lineweaver-Burk plot and is often preferred due to its ease of interpretation. Experimentally however it is flawed as the data tends to get more crowded as $[S]$ increases while at low $[S]$ and V_o values errors are often exaggerated. Of the three methods for obtaining a straight line the double reciprocal plot is the least accurate for obtaining kinetic parameters.

Modified double reciprocal plot

The Eadie-Hofstee equation is obtained if both sides of equation 6.4 are multiplied by $V_o \cdot V_{\max}$ then rearranged in terms of $\frac{V_o}{[S]}$ and V_o .

$$\frac{V_o}{[S]} = \frac{V_{\max}}{K_m} - \frac{1}{K_m} V_o \quad \text{Equation 6.5}$$

A plot of $\frac{V_o}{[S]}$ vs. V_o gives a straight line with a slope of $-\frac{1}{K_m}$ and an x -axis intercept of V_{\max} (fig. 6.3). An advantage of this graph is the ability to detect deviations from normal Michaelis-Menten behaviour. The errors associated with the Lineweaver-Burk plot are lessened by eliminating one reciprocal variable but now any errors in V_o are expressed along both axes.

The Hanes-Woolf plot

Multiplying both sides of the double reciprocal equation 6.4 by $[S]$ gives:

$$\frac{[S]}{V_o} = \frac{1}{V_{\max}} [S] + \frac{K_m}{V_{\max}} \quad \text{Equation 6.6}$$

A plot of $\frac{[S]}{V_o}$ vs. $[S]$ affords a straight line with a slope of $\frac{1}{V_{\max}}$ and an x -intercept of $-K_m$ (fig. 6.3). This is the preferred of the three linear plots with errors being more evenly distributed across a range of substrate concentrations.

Modified direct linear plots

The Hanes-Woolf equation 6.6 can be rearranged to show the dependence of $\frac{1}{V_{\max}}$ on $\frac{K_m}{V_{\max}}$.

$$\frac{1}{V_{\max}} = \frac{1}{V_o} - \frac{1}{[S]} \frac{K_m}{V_{\max}} \quad \text{Equation 6.7}$$

$\frac{1}{V_{\max}}$ and $\frac{K_m}{V_{\max}}$ are treated as variables while $\frac{1}{V_o}$ and $\frac{[S]}{V_o}$ are treated as constants. For any set of $\frac{1}{V_o}$ and $\frac{[S]}{V_o}$ there are an infinite set of values for $\frac{1}{V_{\max}}$ and $\frac{K_m}{V_{\max}}$. If a second line is drawn with a different set of $\frac{1}{V_o}$ and $\frac{[S]}{V_o}$ values the point at which the two lines intersect must be a unique value of $\frac{1}{V_{\max}}$ and $\frac{K_m}{V_{\max}}$ which satisfies both sets of data.

A plot of $\frac{1}{V_{\max}}$ vs. $\frac{K_m}{V_{\max}}$ for n values of $\frac{1}{V_o}$ and $\frac{[S]}{V_o}$ (fig. 6.3) will afford $\frac{n(n-1)}{2}$ estimates for $\frac{1}{V_{\max}}$ and $\frac{K_m}{V_{\max}}$. It is common practice that the median value is taken as it has the statistical advantage of being unaffected by outlying values.

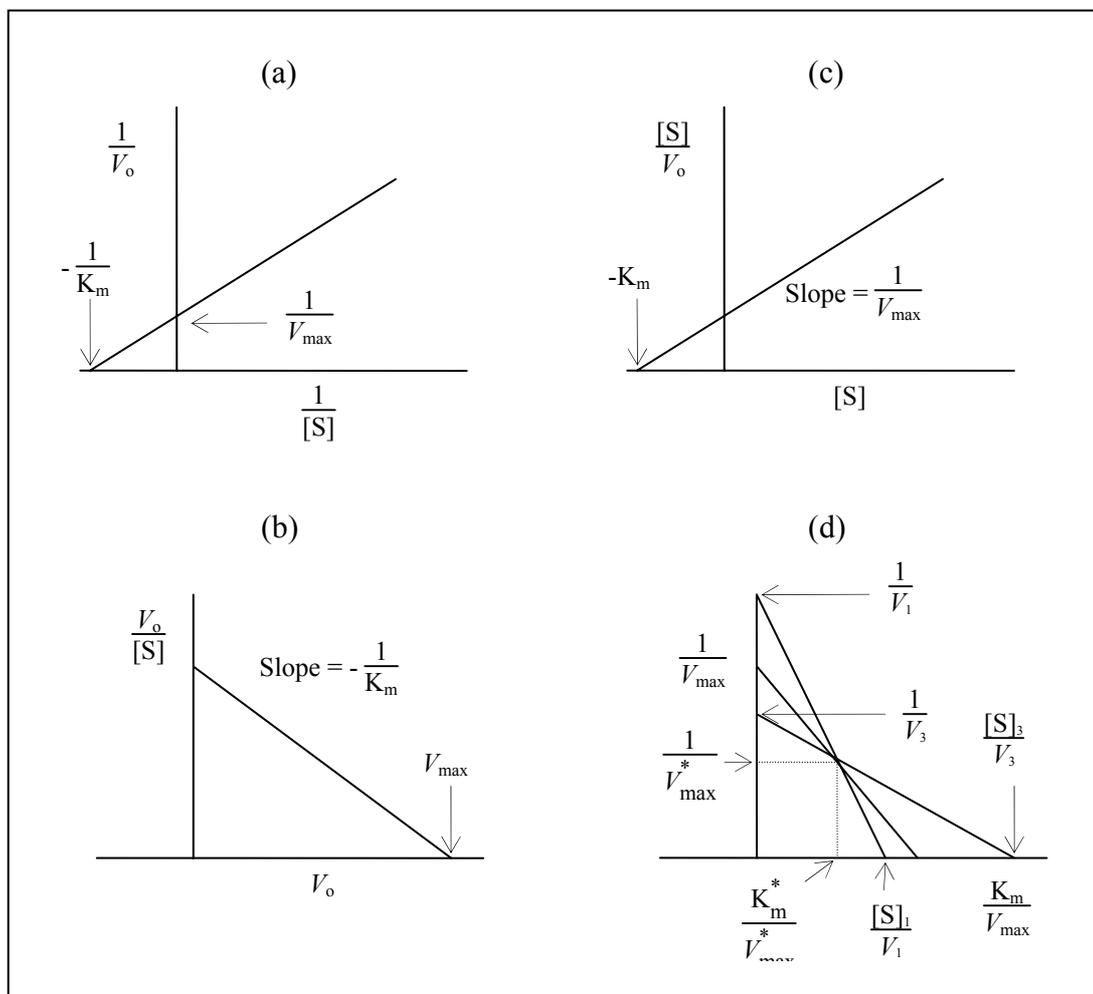


Figure 6.3. Graphical representations of the Michaelis-Menten equation; (a) Lineweaver-Burk; (b) Eadie-Hofstee; (c) Hanes-Woolf; (d) Modified direct linear plot.

Non-Linear regression

While graphical representations of data fitted to the Michaelis-Menten equation are useful, especially in the laboratory they have been superseded by computational methods which utilise non-linear regression. Using computer software such as Enzfitter the data can be fitted to the Michaelis-Menten equation 6.3 directly affording the desired kinetic parameters.

6.2.2 Types of inhibition

In the presence of a reversible inhibitor the observed effects on the initial rate will fall within one of four categories; competitive, mixed, non-competitive (a special case of mixed inhibition) and uncompetitive inhibition. The type of inhibition can be categorised by the effect the inhibitor has on the apparent V_{\max} and $\frac{V_{\max}}{K_m}$ (V_{\max}^* and $\frac{V_{\max}^*}{K_m^*}$ respectively) but does not necessarily imply a particular mechanism (fig. 6.4).

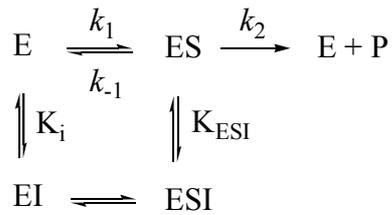
Inhibition type	V_{\max}^*	$\frac{V_{\max}^*}{K_m^*}$
Competitive	unaffected	decreased
Mixed	decreased	decreased
Non-competitive	decreased	decreased
Uncompetitive	decreased	unaffected

Figure 6.4. Effects of inhibition type on V_{\max}^* and $\frac{V_{\max}^*}{K_m^*}$

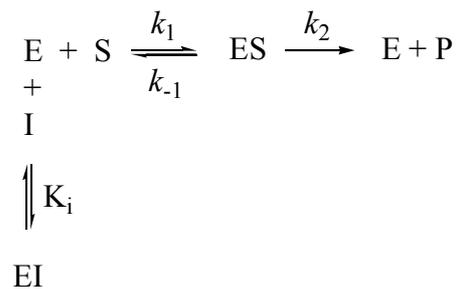
Simple mechanisms have been postulated for each type of inhibition which is *consistent* with the observed kinetics but only the mechanism and corresponding plots for reversible competitive inhibitors is outlined as previous studies have determined that trifluoromethyl ketones are reversible competitive inhibitors of α -chymotrypsin.⁴

Reversible competitive inhibition

A simple model for an enzyme catalysed reaction in the presence of an inhibitor can be expressed as follows:



For a competitive inhibitor we can assume $\text{K}_{\text{ESI}} = \infty$ (therefore ES complex cannot combine with I nor EI with S) which simplifies to:



A rate equation can be written in the form of the Michaelis-Menten equation:

$$V_o = \frac{V_{\text{max}} [\text{S}]}{\left(1 + \frac{[\text{I}]}{\text{K}_i}\right) \text{K}_m + [\text{S}]} \qquad \text{Equation 6.8}$$

This is more commonly expressed as:

$$V_o = \frac{V_{\text{max}} [\text{S}]}{\alpha \text{K}_m + [\text{S}]} \quad \text{where } \alpha = \left(1 + \frac{[\text{I}]}{\text{K}_i}\right) \qquad \text{Equation 6.9}$$

The characteristic feature of competitive inhibition is an apparent increase in K_m but no apparent change in V_{max} with increasing $[\text{I}]$, however based on the recommendations of the International Union of Biochemistry and Molecular Biology⁵ the inhibition type should be classified by the effects of the inhibitor on $\frac{V_{\text{max}}}{\text{K}_m}$ and V_{max} . A competitive inhibitor decreases the apparent affinity of the substrate for the enzyme thus increasing K_m , but does not alter the reactivity of the enzyme hence V_{max} remains unchanged. If V_{max} is unchanged and K_m increases by a factor of $\left(1 + \frac{[\text{I}]}{\text{K}_i}\right)$ then $\frac{V_{\text{max}}}{\text{K}_m}$ should decrease with increasing $[\text{I}]$.

$$V_{\max}^* = V_{\max} \quad \text{and} \quad K_m^* = \left(1 + \frac{[I]}{K_i}\right) K_m \quad \text{Equation 6.10}$$

One mechanism devised to explain these observations is that the inhibitor competes with the substrate to bind to the active site of the enzyme. This process is reversible as increasing [S] or decreasing [I] (dilution or dialysis) acts to ‘wash out’ the inhibitor from the active site.

The kinetic parameters K_m , V_{\max} and K_i can be determined graphically by applying the same methodologies previously outlined for the Michaelis-Menten equation 6.3. Taking the inverse of both sides of equation 6.8 gives:

$$\frac{1}{V_o} = \left(1 + \frac{[I]}{K_i}\right) \frac{K_m}{V_{\max}} \frac{1}{[S]} + \frac{1}{V_{\max}} \quad \text{Equation 6.11}$$

A plot of $\frac{1}{V_o}$ vs. $\frac{1}{[S]}$ for n different [I] gives a series of n straight lines with a slope of $\frac{\alpha K_m}{V_{\max}}$ and intercepts on the x and y -axes of $-\frac{1}{\alpha K_m}$ and $\frac{1}{V_{\max}}$ respectively (fig. 6.5). A replot of the slope $\left(\frac{\alpha K_m}{V_{\max}}\right)$ vs. [I] should afford a straight line with the x and y -intercepts giving the values of $-K_i$ and $\frac{K_m}{V_{\max}}$ respectively (fig. 6.6). It should be noted that the same inaccuracies outlined for the double reciprocal plots hold for kinetic parameters derived from this type of analysis.

The same modification used in equation 6.6 can be employed here to reduce inaccuracy. Multiplying the double reciprocal equation by [S] gives:

$$\frac{[S]}{V_o} = \frac{1}{V_{\max}} [S] + \left(1 + \frac{[I]}{K_i}\right) \frac{K_m}{V_{\max}} \quad \text{Equation 6.12}$$

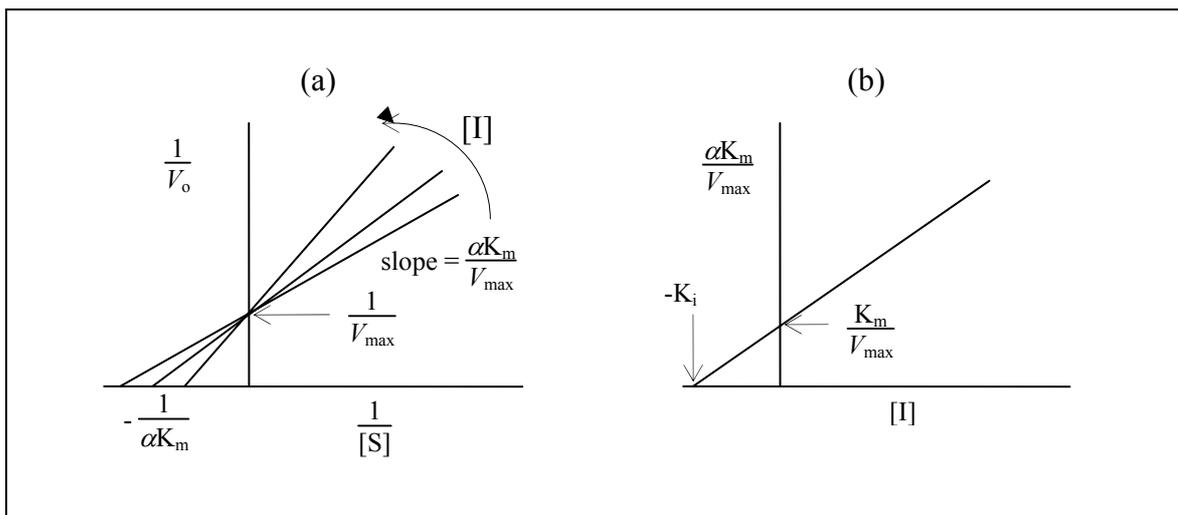


Figure 6.5. (a) A double reciprocal plot of a competitive inhibitor; (b) A replot of $\frac{\alpha K_m}{V_{max}}$ vs. $[I]$ to determine the kinetic parameters K_i and $\frac{K_m}{V_{max}}$.

A plot of $\frac{1}{V_0}$ vs. $[I]$ (Dixon Plot) or $\frac{[S]}{V_0}$ vs. $[I]$ (Modified Dixon plot) avoids some of the inaccuracy incurred by the double reciprocal plot and affords the kinetic parameters and inhibition type from the same graph. Lines from a Dixon plot intersect in the second quadrant giving an estimate for $-K_i$ and $\frac{1}{V_{max}}$ on the x and y -axes respectively while the Modified Dixon plot afford a series of parallel lines for competitive inhibition (fig.6.6).

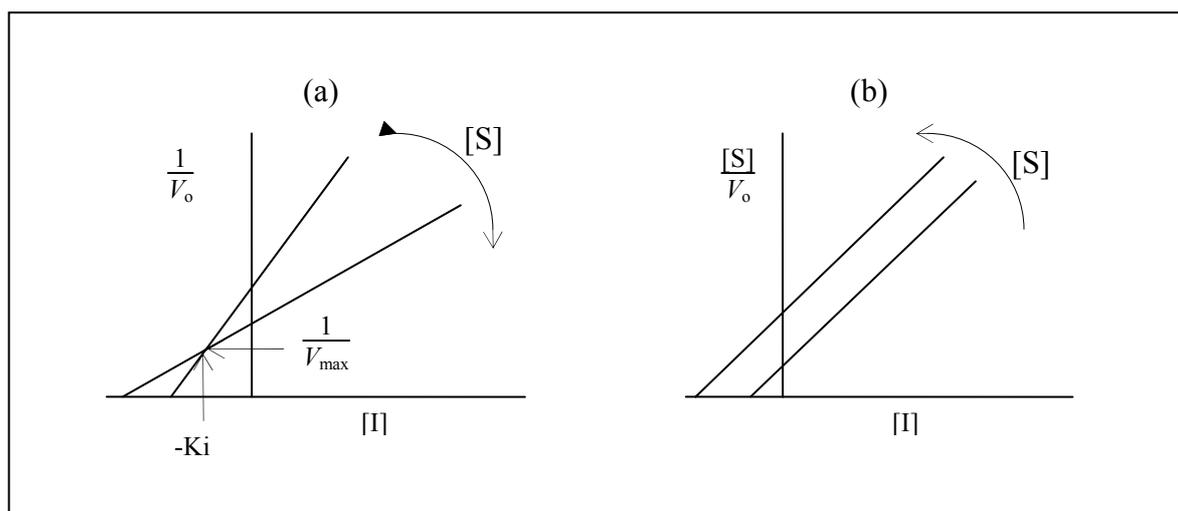
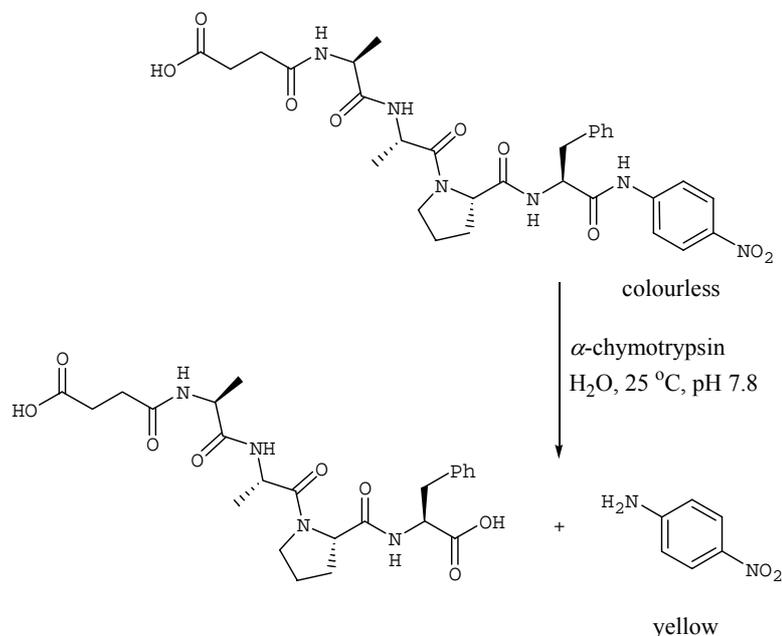


Figure 6.6. (a) Dixon plot of a competitive inhibitor; (b) Modified Dixon plot of a competitive inhibitor.

6.3 Experimental setup

6.3.1 Designing the assay



Scheme 6.1. Cleavage of *N*-Suc-Ala-Ala-Pro-Phe-4-NA by α -chymotrypsin.

The activity of α -chymotrypsin was measured by monitoring the formation of 4-nitroaniline (4-NA) by the enzymatic hydrolysis of *N*-Suc-Ala-Ala-Pro-Phe-4-NA at 25 °C and pH 7.8. The absorbance of 4-nitroaniline was measured at 405 nm as a function of time for a period of 5 or 10 min. 4-Nitroaniline was chosen as the chromophore as it is well studied, strongly coloured and the inhibitors ($\lambda_{\text{max}} \approx 330, 445 \text{ nm}$, fig. 5.16) do not absorb strongly at 405 nm. Assays were initiated by addition of enzyme rather than by addition of substrate in order to get a true measure of the competition between the binding of substrate and inhibitor to the active site of the enzyme.

Rates of reaction (V or ν) were obtained from the linear portions of the absorbance vs. time profiles and converted to the appropriate units. Results were analysed by a combination of graphical methods and non-linear regression to obtain the kinetic parameters K_m , V_{max} and K_i . Errors from the graphs were determined at the 95 % confidence level (2 standard deviations from the mean) using the Microsoft Excel LINEST function.

6.3.2 Equipment used in the enzyme assays

Absorbance measurements were made on a GBC 918 UV/Vis spectrophotometer running the default GBC Scientific v2.01 software package. Plastic cuvettes (1.8 mL) were held in a water block regulated at 25 ± 0.2 °C by circulating water. The buffer and substrate solutions were stored in a water bath heated to 25 ± 0.2 °C with a Tempette TE-8A water bath thermostat. Small volume transfers were made with Eppendorf micropipettes (100, 200, 1000 μ L). Substrate and inhibitor solutions were made up in acetonitrile or DMSO (analytical grade or better).

6.3.3 Buffer solutions

Phosphate buffer; Phosphate, 0.078 M; K^+ , 0.18 M; Triton X-100, 0.05 %; pH 7.8.

K_2HPO_4 (13.6 g), KCl (2.2 g) and Triton X-100 (0.5 g) were dissolved in double distilled water (750 mL), acidified with aqueous HCl (1 M) until the pH reached 7.8 and made up to 1 L. The solution was stored at 4 °C when not in use.

TRIS buffer; TRIS (0.1 M); Ca^{2+} , 0.02 M; Triton X-100, 0.05 %; pH 7.8.

TRIS.HCl (12.2 g), $CaCl_2$ (2.2 g) and Triton X-100 (0.5 g) were dissolved in Milli Q water (800 mL). The solution was adjusted to pH 7.8 by addition of aqueous HCl (0.1 M) then made up to 1 L with Milli Q water. The buffer was stored at 4 °C when not in use.

6.3.4 Substrate solutions

Substrate solution 1; N-Suc-Ala-Ala-Pro-Phe-4-NA (ca. 3.4 mM); TRIS, 100 mM; pH 7.0.

A neutral TRIS buffer was prepared by dissolving a commercially available sachet in Milli Q water (1 L)

N-Suc-Ala-Ala-Pro-Phe-4-NA (Sigma[®], 21 mg, 34 μmol) was suspended in TRIS buffer (10 mL) and sonicated at *ca.* 5 °C for 10 min to complete dissolution. The solution was stored below 0 °C for up to a week. The concentration of the substrate was determined each day by measuring the absorbance at 315 nm of a 1 % substrate solution in buffer ($\epsilon_{315} = 14000 \text{ M}^{-1}.\text{cm}^{-1}$).

Substrate solution 2; N-Suc-Ala-Ala-Pro-Phe-4-NA (*ca.* 20 mM).

N-Suc-Ala-Ala-Pro-Phe-4-NA (25 mg, 40 μmol) was dissolved in DMSO (2 mL) and stored below 0 °C when not in use. The concentration of the substrate solution was determined by diluting a 25 μL aliquot of substrate with DMSO (25 μL) and phosphate buffer (950 μL). The solution was mixed by inversion and a 50 μL aliquot of the solution further diluted with phosphate buffer (950 μL). The absorbance was measured at 315 nm and the concentration determined ($\epsilon_{315} = 14000 \text{ M}^{-1}.\text{cm}^{-1}$).

Substrate solution 3; N-Suc-Phe-4-NA (40 mM).

N-Suc-Phe-4-NA (Sigma[®], 7.7 mg, 20 μmol) was dissolved in DMSO (500 μL). The solution was prepared fresh each day.

6.3.5 Enzyme solutions

Enzyme solution 1; α -Chymotrypsin, 6.8 $\mu\text{g}.\text{mL}^{-1}$ (272 nM); HCl, 2.5 mM; Triton X-100, 0.05 % (w/v).

A stock enzyme solution was prepared by dissolving α -chymotrypsin (3.4 mg, 136 nmol, Sigma[®], Type II: from bovine pancreas 3 x crystallised from 4 x crystallised chymotrypsinogen) in Milli Q water (1 mL). The stock solution (100 μL , 13.6 nmol) was further diluted in a 50 mL volumetric flask with Milli Q water (30 mL) to which was added HCl (analytical reagent grade, 10 μL , 1.2 mM) and Triton X-100 (25 mg, 0.05 % w/v). The contents were thoroughly mixed by inversion then made up to 50 mL with Milli Q water. The enzyme solution was made fresh each day.

Enzyme solution 2; α -Chymotrypsin, $4.0 \text{ mg}\cdot\text{mL}^{-1}$, ($160 \text{ }\mu\text{M}$); HCl, 2.5 mM , Triton X-100, 0.05 \% (w/v) .

A stock acid solution was prepared by diluting concentrated HCl ($216 \text{ }\mu\text{L}$, 2.5 mmol) to 750 mL with double distilled water, adding Triton X-100 (0.5 g , 0.05 \% w/v) and mixing thoroughly by inversion. The solution was made up to 1 L and stored at $4 \text{ }^\circ\text{C}$ when not in use.

A stock enzyme solution was prepared by dissolving α -chymotrypsin (3.0 mg , 120 nmol , Sigma[®], Type II: from bovine pancreas $3 \times$ crystallised from $4 \times$ crystallised chymotrypsinogen) in the above acid solution ($750 \text{ }\mu\text{L}$). The enzyme solution was prepared fresh each day.

6.4 Determination of the Michaelis constant (K_m) and limiting rate (V_{\max})

The assay procedure was adapted from the methods outlined by Geiger³ and Harvey.⁶ TRIS buffer was used in accordance with Geiger as HEPES (Harvey) was found to hydrolyse the substrate before addition of the enzyme.

To a plastic cuvette (1.8 mL) was added TRIS buffer ($(1020 - x - y) \text{ }\mu\text{L}$), *substrate solution 1* ($x \text{ }\mu\text{L}$, $x = 30\text{-}160 \text{ }\mu\text{L}$) and acetonitrile ($y \text{ }\mu\text{L}$, $y = 0, 50 \text{ }\mu\text{L}$). The assay solution was mixed by inversion and incubated at $25 \text{ }^\circ\text{C}$ for 5 min . The assay was initiated by the addition of the enzyme ($30 \text{ }\mu\text{L}$) and the progress monitored by measuring the absorbance of 4-nitroaniline at 405 nm over a period of 5 min ($\epsilon_{405} = 10200 \text{ M}^{-1}\cdot\text{cm}^{-1}$). A plot of absorbance vs. time gave a linear slope over the entire course of the reaction in most cases, the exception being at low substrate concentrations, for which the initial rate was determined from the linear portion of the graph. Each measurement was performed in duplicate and the raw data for the measurements can be found in figure 6.7.

0 % MeCN		4.76 % MeCN
----------	--	-------------

[S] (μM)	V_o ($\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{mg}^{-1}$)		[S] (μM)	V_o ($\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{mg}^{-1}$)	
87	0.765	0.822	84	0.893	0.913
173	0.905	0.907	168	1.066	1.047
260	0.945	0.970	252	1.167	1.195
346	1.003	1.052	336	1.135	1.192
433	0.996	1.039	420	1.185	1.217

Figure 6.7. Kinetic data for K_m and V_{\max} determination utilising *N*-Suc-Ala-Ala-Pro-Phe-4-NA as the substrate.

The initial rate V_o was determined from the slope of the absorbance vs. time profiles at five different substrate concentrations by using equation 6.13.

$$V_o = \left(\frac{V_T}{m_{\text{enz}} \epsilon_{405} l} \right) \text{slope} \quad \text{Equation 6.13}$$

V_o = initial rate ($\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{mg}^{-1}$)

slope is the linear portion of the profile (s^{-1})

V_T = total volume of solution in the assay run (μL)

m_{enz} = mass of enzyme present in assay run (mg)

ϵ_{405} = Molar absorptivity coefficient of 4-nitroaniline at 405 nm ($\text{M}^{-1}\cdot\text{cm}^{-1}$)

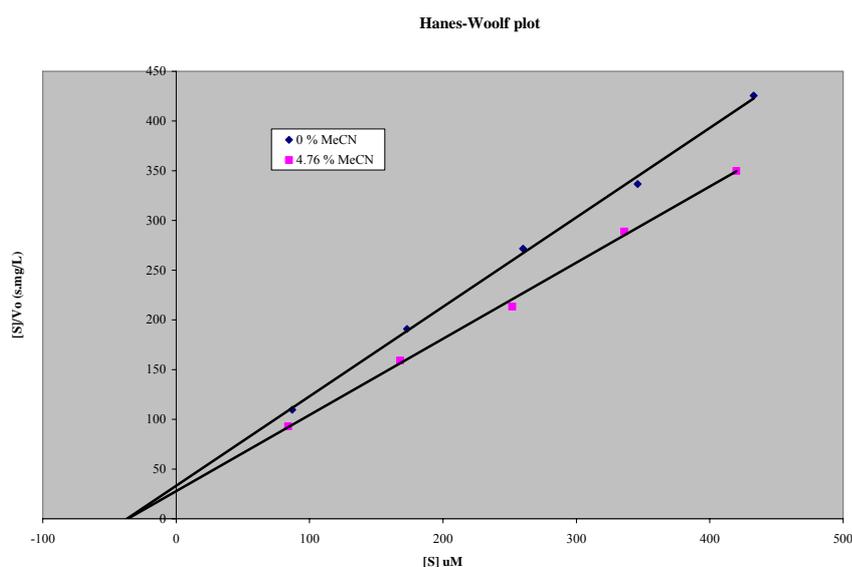
l = path length of the cell (cm)

The initial rates were processed by four graphical methods to give the kinetic parameters K_m and V_{\max} (fig. 6.8).

		K_m (μM)		V_{max} ($\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{mg}^{-1}$)	
		Run 1	Run 2	Run 1	Run 2
Lineweaver-Burk	0 % MeCN	35.2	31.4	1.079	1.107
	4.76 % MeCN	38.0	39.8	1.300	1.337
Hanes-Woolf	0 % MeCN	34.1	36.1	1.074	1.118
	4.76 % MeCN	35.9	39.8	1.290	1.338
Eadie-Hofstee	0 % MeCN	34.9	32.0	1.078	1.111
	4.76 % MeCN	37.7	39.9	1.300	1.338
Modified direct linear	0 % MeCN	34.6	44.0	1.078	1.019
	4.76 % MeCN	40.8	45.7	1.318	1.430

Figure 6.8. Kinetic parameters determined by four graphical methods.

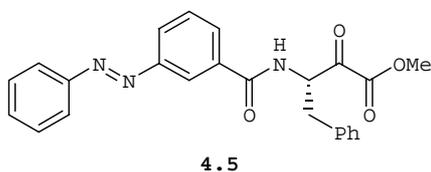
By averaging the results the kinetic parameters for α -chymotrypsin were estimated in the presence and absence of acetonitrile. When a solution of buffer only was used, K_m and V_{max} were determined to be approximately $35 \pm 8 \mu\text{M}$ and $1080 \pm 60 \text{ nmol}\cdot\text{s}^{-1}\cdot\text{mg}^{-1}$ respectively. When acetonitrile ($50 \mu\text{L}$) was added to the assay media, the values of K_m and V_{max} increased to approximately $39 \pm 6 \mu\text{M}$ and $1330 \pm 90 \text{ nmol}\cdot\text{s}^{-1}\cdot\text{mg}^{-1}$ respectively. A Hanes-Woolf plot of the experimental data gives a common intersection on the x -axis but different y -intercepts (fig. 6.9).

Figure 6.9. Modified double reciprocal plots of *N*-Suc-Ala-Ala-Pro-Phe-4-NA hydrolysis in the presence and absence of acetonitrile.

A common x -intercept implies that K_m is unchanged by the addition of acetonitrile while the differing slopes imply the rate is affected by acetonitrile. Within experimental error the value of K_m is unaffected acetonitrile ($K_m \approx 37 \pm 8 \mu\text{M}$) whereas V_{max} and $\frac{V_{\text{max}}^*}{K_m^*}$ increase in the presence of acetonitrile. Comparing these observations to those in figure 6.4 indicates the opposite effect of uncompetitive inhibition i.e. acetonitrile activates α -chymotrypsin rather than inhibiting the enzyme. The results with the addition of acetonitrile offer a better estimation of the kinetic parameters as the conditions more accurately mimic the conditions used when an inhibitor is added to the assay.

6.4.1 Comparison with a reported inhibitor

The assays testing enzyme inhibition were first run using a reported inhibitor **4.5** at an ambient photostationary state following the procedure of Harvey, with the exceptions that TRIS rather than HEPES buffer was used and the concentration of acetonitrile was kept constant.



A stock solution of inhibitor (*ca.* $0.1 \text{ mg}\cdot\text{mL}^{-1}$) was prepared by dissolving **4.5** (5.0 mg, $12.0 \mu\text{mol}$) in acetonitrile (50 mL). A solution suitable for assay was prepared by diluting stock solution (1 mL) to 10 mL with acetonitrile to give a final concentration of $10.0 \mu\text{g}\cdot\text{mL}^{-1}$ ($24.0 \mu\text{M}$).

Initially, 0, 10, 20, 30, 40 and 50 μL aliquots of the diluted solution were added to the assay media and the acetonitrile concentration maintained at 4.76 % by making the total volume of acetonitrile 50 μL . The results obtained for these assays were variable and it was believed the problem was due to low accuracy of the micropipettes when dispensing volumes less than 20 μL . To overcome these problems five inhibitor solutions of different concentrations ($1.5\text{-}18 \mu\text{M}$) were prepared by further dilution of the stock inhibitor solution with acetonitrile.

The assay was run at five different substrate concentrations and six different inhibitor concentrations to give a large data set suitable for analysis. Into a plastic cuvette was added TRIS buffer ((970- x) μL), *substrate solution 1* (x μL , $x = 40\text{-}140$ μL) and inhibitor solution (50 μL). The assay solution was mixed by inversion and incubated at 25 °C. After 5 min the reaction was initiated by addition of *enzyme solution 1* (30 μL) and absorbance of 4-nitroaniline measured over 5 min. The slopes of the graphs were used to determine the initial rates of reaction and the averaged raw data is given in figure 6.10.

	4.5 [I] (μM)						$V_{\text{initial}} (\mu\text{mol/L}\cdot\text{s})$
[S] (μM)	0	0.072	0.143	0.287	0.573	0.860	
113.9	1.143	0.840	0.745	0.552	0.320	0.218	
185.1	1.269	0.966	0.922	0.726	0.458	0.324	
256.3	1.320	1.181	1.042	0.846	0.563	0.431	
327.5	1.370	1.238	1.130	0.947	0.663	0.513	
398.7	1.408	1.314	1.181	1.048	0.739	0.609	

Figure 6.10. Inhibition of α -chymotrypsin by the ambient photostationary state of 4.5.

The kinetic parameters K_m^* and V_{max}^* were determined by double reciprocal (fig. 6.11) and modified double reciprocal plots (fig. 6.12).

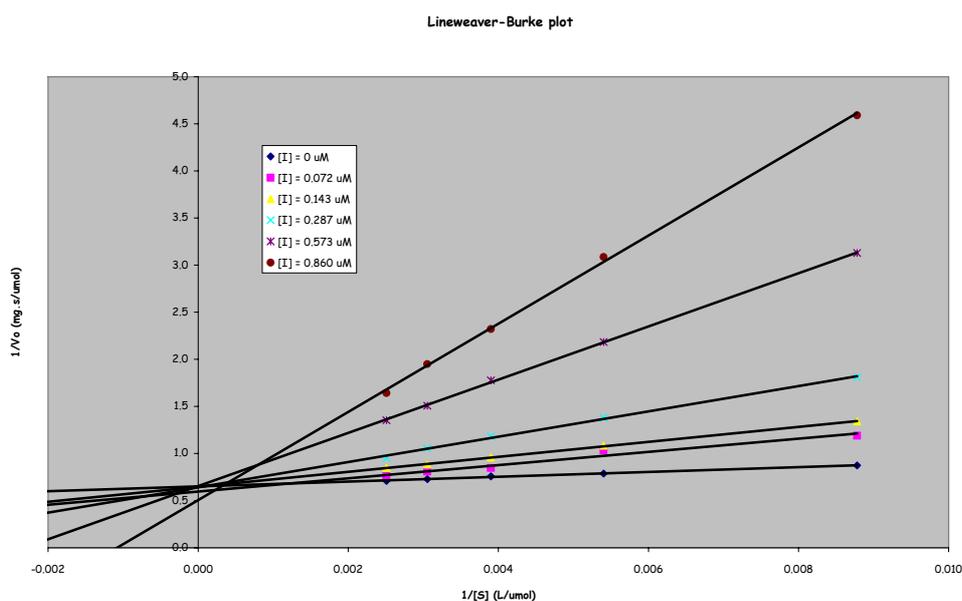


Figure 6.11 Double reciprocal plots for the ambient photostationary state of 4.5.

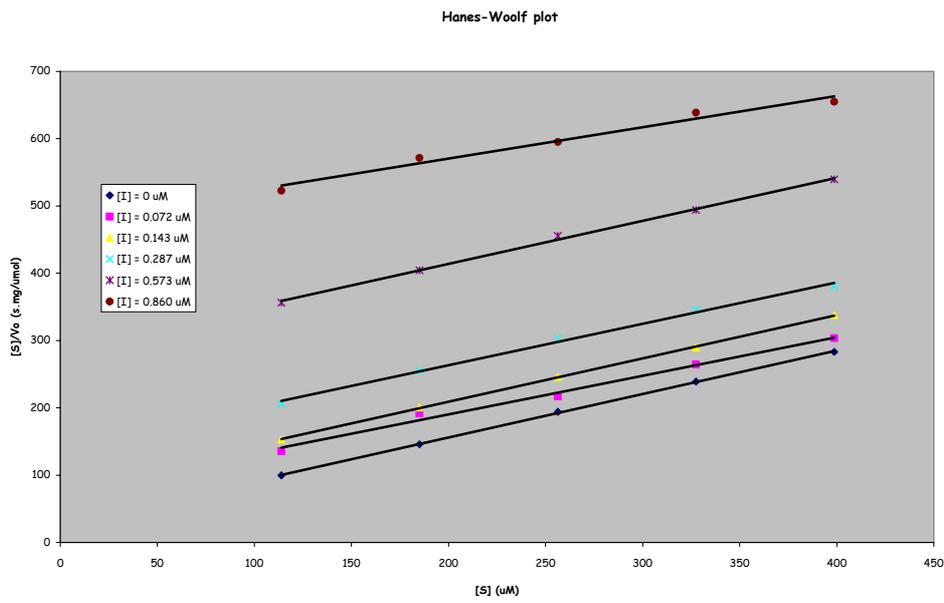


Figure 6.12. Modified double reciprocal plot for the ambient photostationary state of **4.5**.

Replotting the slopes of the double reciprocal graphs (fig 6.11) against $[I]$ gave an estimated value for K_i (fig. 6.13).

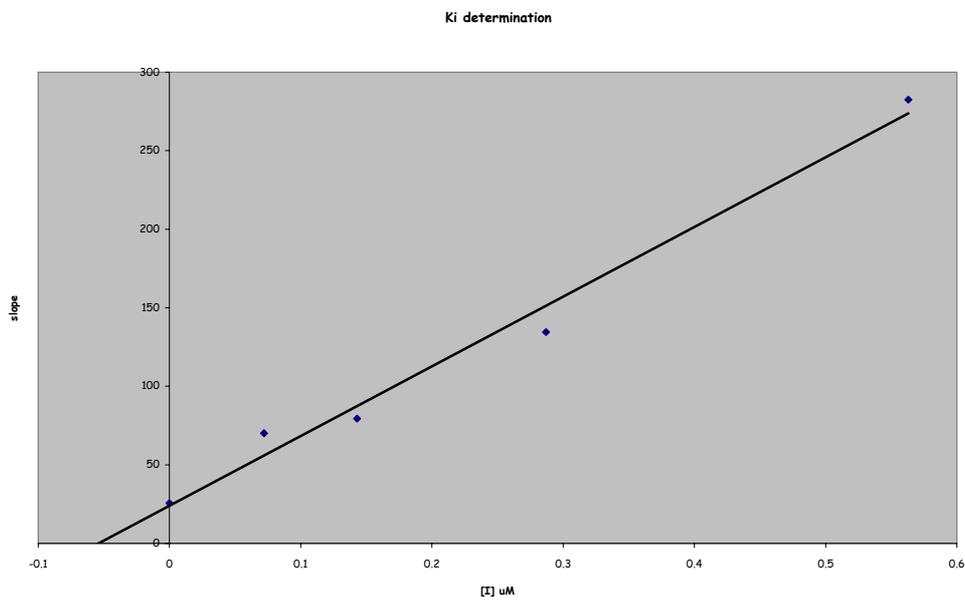


Figure 6.13. Estimation of K_i for the ambient photostationary state of **4.5**.

Analysis of the results clearly indicates that **4.5** acts as a competitive inhibitor of α -chymotrypsin because K_m^* increases with increasing $[I]$, $\frac{V_{max}^*}{K_m}$ decreases with increasing $[I]$ while V_{max}^* remains constant (fig. 6.14).

Harvey					Alexander				
[I] (μM)	K_m^* (μM)	V_{max}^*	$\frac{V_{max}^*}{K_m^*}$	K_i (μM)	[I] (μM)	K_m^* (μM)	V_{max}^*	$\frac{V_{max}^*}{K_m^*}$	K_i (μM)
0.11	130	1.10	8.46	0.063	0.072	118	1.68	14.24	0.041
0.22	220	1.10	5.00	0.069	0.143	123	1.54	12.52	0.067
0.34	250	1.10	4.40	0.089	0.287	210	1.56	7.43	0.066
0.44	410	1.30	3.17	0.089	0.573	431	1.53	3.55	0.056
0.55	390	1.10	2.82	0.096	0.860	926	1.98	2.14	0.049

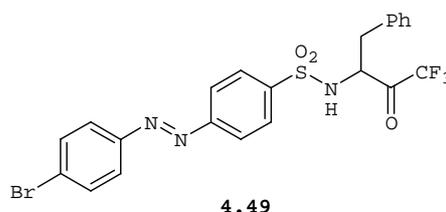
Figure 6.14. Comparison of kinetic data for the ambient photostationary state for **4.5**.

Units for V_{max}^* ($\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{mg}^{-1}$), units for $\frac{V_{max}^*}{K_m}$ ($\text{mL}\cdot\text{s}^{-1}\cdot\text{mg}^{-1}$).

The estimated value for K_i (*ca.* 0.05 μM) is comparable to that obtained by Harvey (0.08 μM).⁶ The activation of α -chymotrypsin observed by Harvey when acetonitrile was used in conjunction with the enzyme assays was confirmed and accounted for by using a constant concentration of the organic solvent.

6.5 Inhibition studies of trifluoromethyl ketone based inhibitors

Inhibitor **4.49** was initially chosen as a test compound due to its high purity (> 99 %) and availability.



6.5.1 Assays in TRIS buffer

A solution of **4.49** in acetonitrile (0.12 mg.mL^{-1} , 0.22 mM) was prepared in an analogous manner to that of **4.49** and allowed to reach an ambient photostationary state. No inhibition of the enzyme was observed at $[I] = 10.5 \text{ }\mu\text{M}$ so a new, more concentrated solution was prepared. **4.49** (4.2 mg , 7.8 mmol) was dissolved in acetonitrile (2 mL) to give a stock inhibitor solution (2.1 mg.mL^{-1} , 3.9 mM) and allowed to equilibrate for 24 h. Diluted solutions were prepared in the range $263\text{-}2100 \text{ }\mu\text{M}$ to give final assay concentrations of $12.5\text{-}100 \text{ }\mu\text{M}$. At $[I] < 50 \text{ }\mu\text{M}$, precipitation occurred resulting in lower than expected concentrations of inhibitor in solution and generally poor absorbance measurements obtained due to the turbidity of the solution. As a consequence only solutions where $[I] \leq 50 \text{ }\mu\text{M}$ were used.

The assays were performed as per normal; however, a plot of absorbance vs. time for assays containing inhibitor did not give the expected linear graphs at any substrate concentration. The assays were repeated several times but the results were not particularly reproducible. Under these conditions the results were unsatisfactory; precipitation, poor reproducibility and non-linearity of the curves prompted the investigation of a new assay system.

6.5.2 Assays in Phosphate buffer

A new assay protocol was set up which incorporated aspects of the procedure outlined by Abeles *et al* for the assay of trifluoromethyl ketones and the procedure of Harvey.

The buffer was changed in accordance with reported studies of trifluoromethyl ketones which use phosphate buffer predominantly. The substrate was kept the same due to its high specificity for α -chymotrypsin but the organic solution was changed to DMSO in an attempt to increase solubility of the inhibitors in aqueous media. As per the previous assays the concentration of organic solvent was kept constant.

Aliquots of *substrate solution 2* were diluted with DMSO to give substrate solutions ranging from *ca.* 2-16 mM. The inhibitor **4.49** (4.4 mg, 8.1 mmol) was dissolved in DMSO (2 mL) to give a solution of 4.1 mM which was allowed to reach an ambient photostationary state. Aliquots of stock inhibitor solution were diluted to give 1, 2 and 3 mM solutions.

To a plastic cuvette was added phosphate buffer (925 μ L), substrate (25 μ L) and inhibitor or DMSO (25 μ L). The total volume of DMSO in the aqueous media was 50 μ L (5 % v/v). After incubating 5 min at 25 °C the hydrolysis was initiated by addition of *enzyme solution 1* (25 μ L) and the progress monitored over 5 min at 405 nm. The lines were non-linear for runs containing inhibitor at all substrate concentrations assayed against. All curves were characteristic in that there was little change in slope for the first minute followed by deviations from linearity (fig. 6.15).

Kinetic parameters were determined by using the slope of the linear portion of the last 100 seconds of each run and using that data as an estimation of V_o . The assays were run in duplicate and the raw data is presented in figure 6.16.

The stock inhibitor solution (4 mM, 1 mL) was irradiated in a quartz cell with UV light for 1 h then aliquots were diluted to give 1 and 2 mM solutions which were protected from light as much as possible. The assays were run as for the ambient inhibitor photostationary state, in duplicate and the raw data presented in figure 6.16.

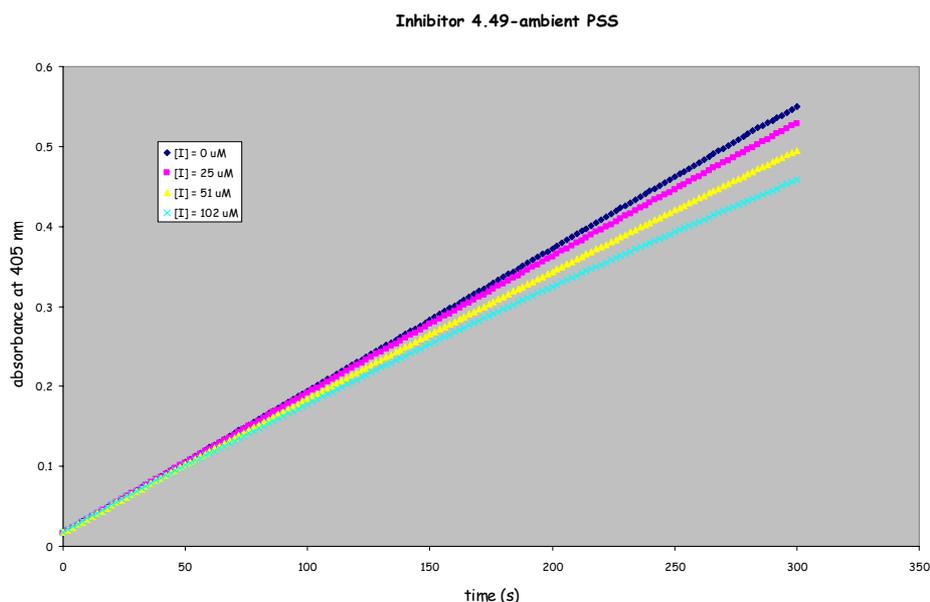


Figure 6.15. Absorbance vs. time plots for the ambient photostationary state of **4.49**. $[S] = 475 \mu\text{M}$.

		4.49 [I] (μM)						V_i ($\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{count}$)
[S]	(μM)	0	25.4	50.9	101.8			
		-	eqm	Z-enriched	eqm	Z-enriched	eqm	
95		0.95	0.77	-	0.62	-	0.41	-
190		1.20	1.06	0.91	0.94	0.70	0.76	0.40
285		1.26	-	1.06	1.06	0.87	0.89	0.62
380		1.34	1.25	1.13	1.15	0.97	1.01	0.73
475		1.39	1.30	1.18	1.19	1.04	1.06	0.81

Figure 6.16. Inhibition of α -chymotrypsin by **4.49**.

The data was analysed by graphical methods (Lineweaver-Burke and Hanes-Woolf plots) and non-linear regression (Enzfitter) to determine the kinetic parameters K_m^* , V_{\max}^* and K_i for the ambient and Z-enriched photostationary states of **4.49** (fig. 6.17).

	PSS _{ambient}			PSS _{UV}		
	K _m (μ M)	V _{max} [*]	K _i (μ M)	K _m (μ M)	V _{max} [*]	K _i (μ M)
LW-B	61 \pm 18	1.55 \pm 0.4	52 \pm 14	53 \pm 23	1.52 \pm 0.7	15 \pm 4
H-W	62 \pm 8	1.53 \pm 0.2	50 \pm 6	62 \pm 38	1.51 \pm 0.9	16 \pm 6
Enzfitter	61 \pm 12	1.56 \pm 0.1	40 \pm 9	52 \pm 24	1.54 \pm 0.1	15 \pm 6

Figure 6.17. Kinetic parameters for **4.49**.

The kinetic parameters determined graphically are comparable to those determined by the enzfitter program which should be considered the more accurate method as it does not propagate errors associated with reformulating the Michaelis-Menten equation to produce linear graphs (section 6.2.1).

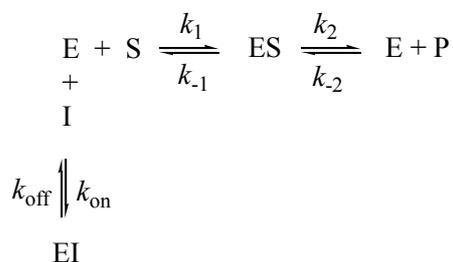
Further research into kinetic studies of trifluoromethyl ketones indicated that mono-peptidyl trifluoromethyl ketones often adhered to Michaelis-Menten type kinetics whereas di-peptidyl and higher trifluoromethyl ketones exhibit slow binding kinetics which are characterised by an initial uninhibited rate of hydrolysis followed by a gradual decrease in the rate of reaction. This strongly resembled the curves obtained for the assays of ambient and Z-enriched **4.49**. Kinetics of this type are routinely analysed by the method of Cha⁷ and relates both the initial (v_0) and steady state (v_s) velocities as a function of time (t).

6.5.3 Slow binding kinetics

In cases where association and/or dissociation of the enzyme-inhibitor complex are slow in comparison to ordinary enzyme-substrate processes, it becomes obvious that standard steady-state rate equations (i.e. the Michaelis-Menten equation) are inadequate for determining kinetic parameters.

A model describing the kinetics of an enzyme and substrate in the presence of an inhibitor can be constructed in an analogous manner to that of the Michaelis-Menten model; however, for slow binding inhibitors the association and dissociation rate constants k_{on} and

k_{off} (using the notation of Abeles and Imperiali)⁴ are significantly slower than k_1 and k_2 so a near steady-state is achieved for E and ES much faster than E and EI.⁷



Slow binding inhibitors exhibit the defining characteristics that upon initiation of the reaction by addition of enzyme the rate proceeds at an initial uninhibited rate followed by a gradual decrease in reaction rate until a steady-state velocity is reached (i.e. equilibrium occurs slowly). This can be expressed by the equation:

$$v = v_s + (v_o - v_s)e^{-k't} \quad \text{Equation 6.14}$$

Integration of equation 6.14 gives equation 6.15 in terms of absorbance and time:

$$A = v_s t - \frac{(v_s - v_o)[1 - e^{-k't}]}{k'} + A_o \quad \text{Equation 6.15}$$

From the standard UV profile of A vs. t , v_o and v_s can be determined from the initial and final slopes of the curve respectively (fig. 6.18).

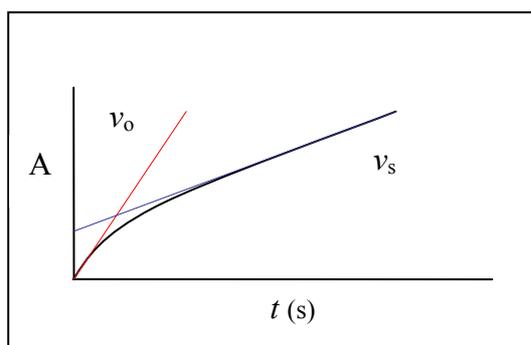


Figure 6.18. Plot of absorbance vs. time

The values of absorbance and time can be fitted to equation 6.15 using non-linear regression to obtain estimates of k' at different $[I]$. A plot of k' vs. $[I]$ should afford a straight line where k_{on} and k_{off} can be evaluated from the slope and y -intercept respectively (equation 6.16).

$$k' = k_{\text{off}} + \frac{k_{\text{on}} [I]}{\left(1 + \frac{[S]}{K_m}\right)} \quad \text{Equation 6.16}$$

The inhibition constant K_i for a competitive inhibitor can be determined from single values using equation 6.17

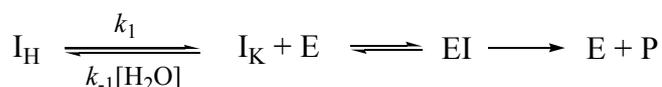
$$K_i = \frac{v_s [I]}{(v_o - v_s) \left(1 + \frac{[S]}{K_m}\right)} \quad \text{Equation 6.17}$$

However to get a better estimation of K_i , equation 6.17 can be rearranged in terms of known variables. A plot of $\frac{(v_o - v_s)}{v_s}$ vs. $[I]$ should afford a straight line with the slope being inversely proportional to K_i .

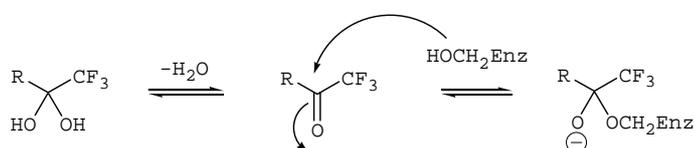
$$\frac{(v_o - v_s)}{v_s} = \frac{[I]}{K_i \left(1 + \frac{[S]}{K_m}\right)} \quad \text{Equation 6.18}$$

6.5.4 Hydration of trifluoromethyl ketones

It is well established that trifluoromethyl ketones are equilibrium with their hydrates in aqueous media. The formation of hydrate is fast ($k_{-1} = 0.25 \text{ s}^{-1}$) and the equilibrium heavily favours the hydrate (500:1). The dissociation of water from the hydrate is several orders of magnitude slower ($k_1 = 5 \times 10^{-4} \text{ s}^{-1}$) resulting in $K_{\text{hyd}} \left(= \frac{k_{-1}}{k_1 [\text{H}_2\text{O}]}\right) = 9 \text{ M}^{-1}$.⁸



The predominance of hydrate in aqueous acetone ($> 95\%$ by ^1H and ^{19}F NMR) was observed in all cases for trifluoromethyl ketones synthesised in this thesis. Trifluoromethyl ketones that possess additional electron withdrawing groups such as amides and sulfonamides are shown to decrease the amount of ketone present by about a factor of nine, therefore the ratio of hydrate to ketone is potentially 4500:1. Studies have shown that the ketone, not hydrate is the reactive species that undergoes nucleophilic attack by the Ser-195 hydroxyl group to give an ionised hemiketal (scheme 6.2); therefore dehydration of the inhibitor must occur before formation of the ES complex is permitted. At high $[\text{E}]$, ketone may be consumed faster than it is formed from the hydrate, thus the dehydration of the hydrate (k_1) can be rate limiting.



Scheme 6.2. Inhibition of α -chymotrypsin by trifluoromethyl ketones.

In some cases authors have applied correction factors to their values of K_i and k_{on} to account for the true concentration of reactive species in solution giving K_i values in the nanomolar rather than micromolar range.

6.5.5 Assay of the target compounds

Representative targets (fig. 6.19) were chosen based on the degree of E/Z isomerisation, purity and structural type. Initial work was performed on the simplest target **4.12** which exhibited a high degree of Z -enrichment (78%). Other inhibitors were tested to examine the effects of increasing peptide chain length, increased substitution of the azobenzene switch and different substitution patterns and were compared to **4.12**.

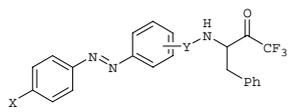
	X	Y
4.12	H	4-SO ₂
4.16	H	4-SO ₂ -L-Leu
4.46	I	3-SO ₂
4.52	I	4-SO ₂
4.62	I	4-SO ₂ -L-Leu

Figure 6.19. Inhibitors chosen for enzyme inhibition studies.

Initially *substrate solution 2* was to be used as it is very a sensitive substrate for α -chymotrypsin; however it was found that its purity was questionable. The solid *N*-Suc-Ala-Ala-Pro-Phe-4-NA was distinctly yellow (it should be white) and when dissolved in aqueous media or DMSO gave a deep yellow solution. Determining the concentration of the solution by UV/Vis spectroscopy gave confusing results as the values obtained changed non-systematically. A new supply of substrate was ordered from the more reputable Sigma-Aldrich chemical company but due to time constraints could not wait for it to arrive. As a result, the less sensitive *N*-Suc-Phe-4-NA was used instead. A typical experiment is outlined below.

The kinetic parameters K_m and V_{max} were determined for the *N*-Suc-Phe-4-NA/ α -chymotrypsin system in the presence of 5 % DMSO in a manner analogous to determining kinetic parameters for *N*-Suc-Ala-Ala-Pro-Phe-4-NA (section 6.4). The DMSO concentration was held constant to account for any activation/deactivation effects. The concentration of substrate was varied from 0.5 to 5 mM while $[E]$ was held constant at 4 μ M. Analysis of the raw data by a Hanes-Woolf plot gave $K_m = 1.65 \pm 0.20$ mM and $V_{max} = (7.5 \pm 0.1) \times 10^{-4}$ nmol.s⁻¹.mg⁻¹. From these results it can be seen that the specificity and catalytic activity ($k_{cat} = V_{max}[E]_T$) is greatly decreased compared to the *N*-Suc-Ala-Ala-Pro-Phe-4-NA/ α -chymotrypsin system (fig. 6.20) and as such required a greater concentration of enzyme to affect hydrolysis.

	K_m (μM)	V_{max} ($\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{mg}^{-1}$)	k_{cat} (s^{-1})
<i>N</i> -Suc-Ala-Ala-Pro-Phe-4-NA	35	1.08	27
<i>N</i> -Suc-Phe-4-NA	1650	7.46×10^{-4}	0.0187

Figure 6.20. Comparison of kinetic data for two different substrates.

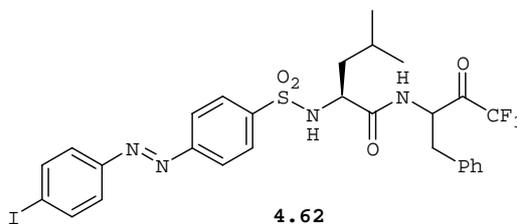
To a plastic cuvette was added *substrate solution 3* (25 μL), phosphate buffer (925 μL) and inhibitor solution or DMSO (25 μL). The contents were mixed by inversion and incubated at 25 $^{\circ}\text{C}$ for 5 min. The reaction was initiated by addition of *enzyme solution 2* (25 μL) and the progress of the reaction monitored at 405 nm for 10 min. Each measurement was performed in duplicate.

Stock inhibitor solutions were irradiated with UV radiation for 1 h. Aliquots were diluted with DMSO to give concentrations of 0.4 - 2 mM and the solutions protected from light as much as possible.

Initial velocities (v_o) were determined from 15-20 data points at the beginning of the reaction curve while steady state velocities (v_s) were determined from the linear portion of the curve between 400-600 s. The pseudo first-order rate constant k' was determined by non-linear regression for five different inhibitor concentrations and a plot of k' vs. $[\text{I}]$ was obtained (equation 6.16). In most cases the graph was scattered but with linear trends which could be used to give an estimation of k_{on} and k_{off} . Unfortunately due to a miscalculation during the assay design, $[\text{E}]_{\text{T}}$ is at least 10-fold more concentrated than desired and as a consequence $[\text{E}] \approx [\text{I}]$ at low $[\text{I}]$ rather than $[\text{E}] \leq 10 [\text{I}]$ as is required for pseudo first order conditions. The inhibition constant K_i was determined by plotting $\frac{(v_o - v_s)}{v_s}$ vs. $[\text{I}]$ (equation 6.18).

6.5.6 A representative data set: Full kinetic data for 4.62

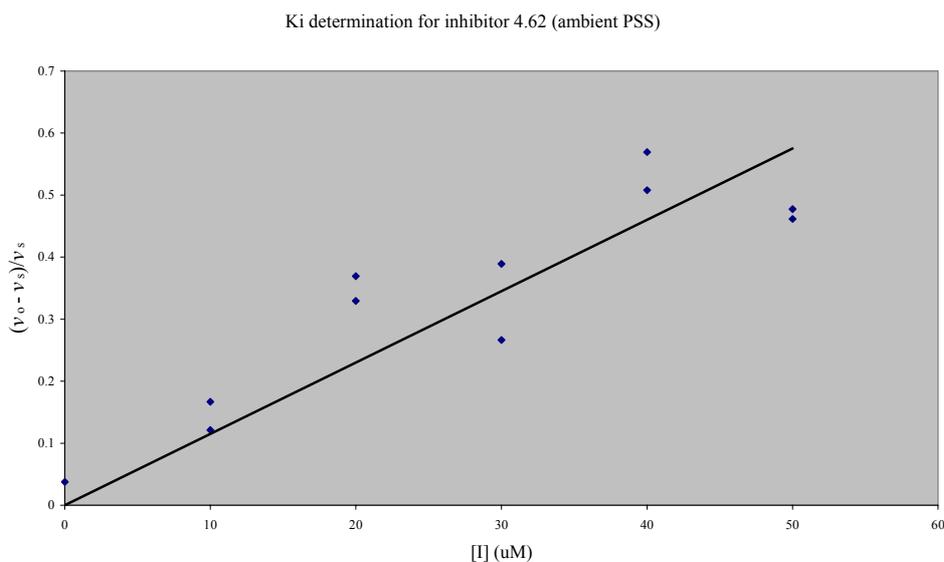
Outlined below is the full kinetic data for the dipeptide **4.62**.



The raw data for v_s and v_o and k' is tabulated in figure 6.21.

A plot of $\frac{(v_o - v_s)}{v_s}$ vs. $[I]$ for the ambient (fig. 6.22) and Z-enriched (fig. 6.23) photostationary states afforded straight lines from which K_i could be determined. Estimates for the association and dissociation rate constants for the ambient (fig. 6.24) and Z-enriched (fig. 6.25) photostationary state of **4.62** were achieved by plotting k' vs. $[I]$. The second order rate constant k_{on} was obtained from the slope while k_{off} was determined from the y-intercept.

PSS _{ambient}				PSS _{Z-Enriched}			
[I] (μM)	v_s ($\times 10^{-5} \text{ s}^{-1}$)	v_o ($\times 10^{-5} \text{ s}^{-1}$)	k' ($\times 10^{-3} \text{ M}^{-1} \cdot \text{s}^{-1}$)	[I] (μM)	v_s ($\times 10^{-5} \text{ s}^{-1}$)	v_o ($\times 10^{-5} \text{ s}^{-1}$)	k' ($\times 10^{-3} \text{ M}^{-1} \cdot \text{s}^{-1}$)
0	26.2	25.4	-	0	27.7	28.0	-
	27.5	28.6			27.7	28.0	
10	25.0	28.0	2.86	5	24.2	31.8	3.70
	22.6	26.3			18.3	26.7	4.18
20	22.8	31.3	3.97	10	16.4	26.7	3.01
	18.4	24.4			15.0	26.4	3.43
30	18.7	25.9	4.93	15	13.9	28.1	5.13
	20.4	25.9			12.1	24.3	4.25
40	18.2	27.4	4.97	20	11.1	26.4	4.23
	18.7	29.4			9.1	24.6	3.90
50	18.5	27.4	7.77	25	9.0	24.1	4.53
	20.1	29.4			8.4	20.9	4.35

Figure 6.21. Raw data for v_s , v_o and k' for **4.62**.Figure 6.22. Determination of K_i for the ambient photostationary state of **4.62**. $K_i = 50 \pm 12 \mu\text{M}$.

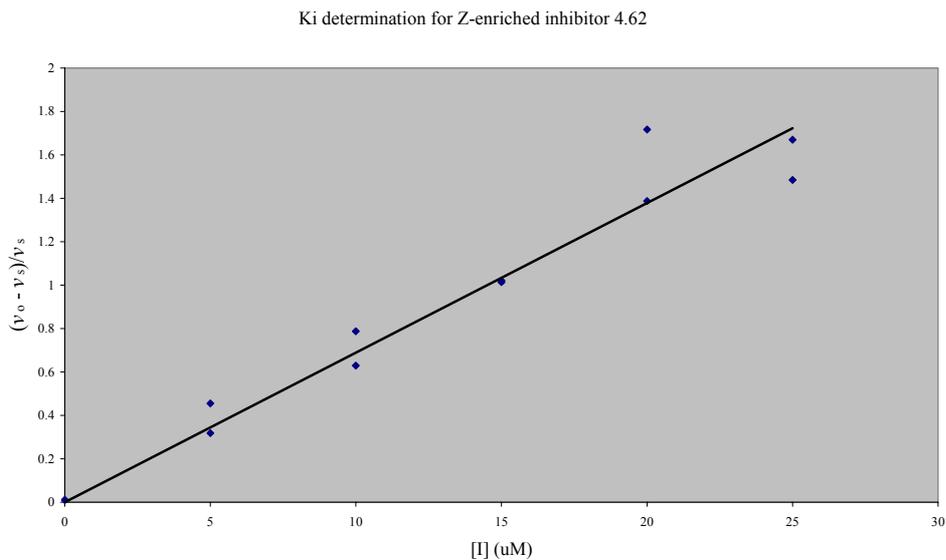


Figure 6.23. Determination of K_i for Z-enriched **4.62**. $K_i = 9 \pm 1 \mu\text{M}$.

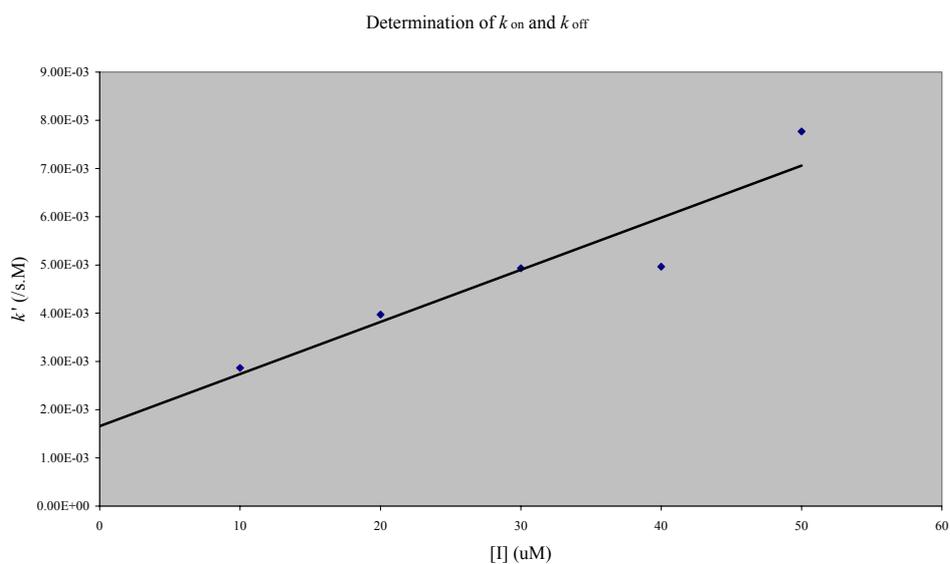


Figure 6.24. Determination of k_{on} and k_{off} for ambient **4.62**. $k_{\text{on}} = 170 \pm 70 \text{ M}^{-1} \cdot \text{s}^{-1}$; $k_{\text{off}} = (1.7 \pm 1.7) \times 10^{-3} \text{ s}^{-1}$.

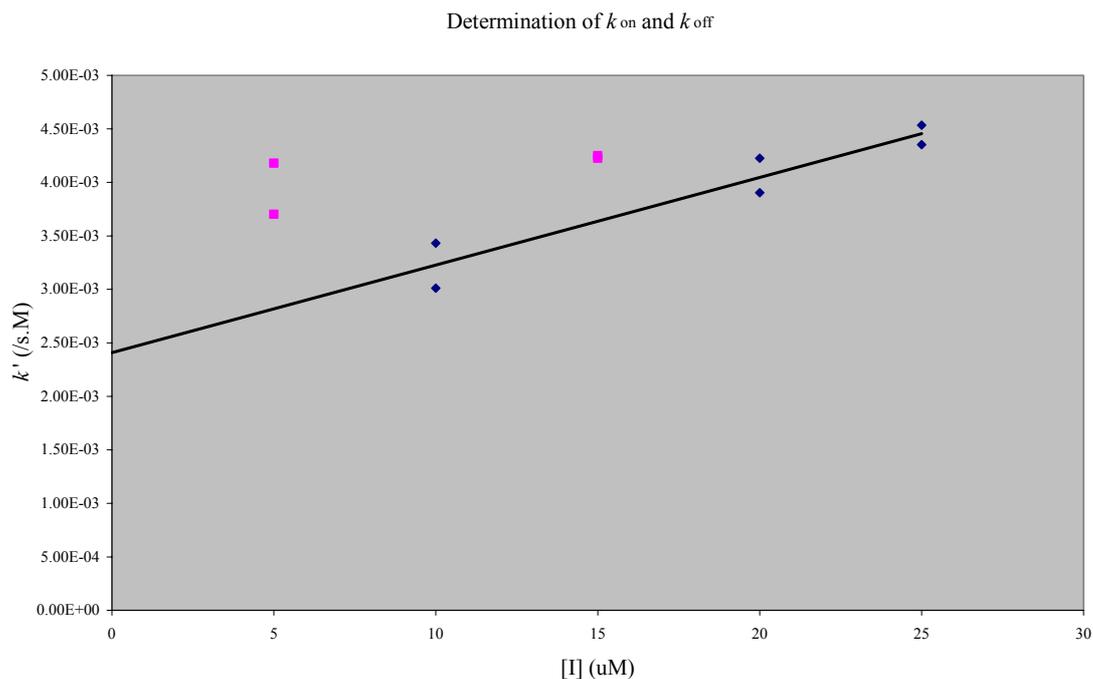


Figure 6.25. Determination of k_{on} and k_{off} for Z-enriched **4.62**. $k_{\text{on}} = 130 \pm 40 \text{ M}^{-1} \cdot \text{s}^{-1}$; $k_{\text{off}} = (2.4 \pm 0.5) \times 10^{-3} \text{ s}^{-1}$.

By analysing the data obtained from figures 6.19-6.23 three important parameters could be obtained for each of the photostationary states. The inhibition constant K_i was determined to be $50 \pm 12 \text{ } \mu\text{M}$ for the ambient photostationary state and $9 \pm 1 \text{ } \mu\text{M}$ for the UV photostationary state. From this it is apparent that the Z-isomer is the more active form of the inhibitor and represents a 5-fold increase in potency. The dissociation constant k_{off} was determined from the y-intercept to be $(1.7 \pm 1.7) \times 10^{-3} \text{ s}^{-1}$ for the ambient photostationary state and $(2.4 \pm 0.5) \times 10^{-3} \text{ s}^{-1}$ for the UV photostationary state, therefore, within experimental error, the value of k_{off} is constant. This implies that the rate of dissociation is independent of conformation. The association rate constant k_{on} was determined from the slope to be $170 \pm 70 \text{ M}^{-1} \cdot \text{s}^{-1}$ for the ambient photostationary state and $130 \pm 40 \text{ M}^{-1} \cdot \text{s}^{-1}$ for the UV photostationary state. Though the errors overlap and one could state that k_{on} is independent of conformation, comparison with the other four inhibitors indicates that k_{on} is larger for the UV photostationary state. This would not be unexpected as the Z-isomer has shown to be the more potent inhibitor therefore it seems plausible that it might bind faster than the less potent conformation.

Based on the obtained data it can be concluded that the dipeptidyl inhibitor **4.62** is a competitive reversible slow binding inhibitor of α -chymotrypsin. The kinetic data for the other inhibitors has been summarised in figure 6.26.

6.5.7 Problems with the assays

It has been pointed out by Cha that the significance of k' (equation 6.16) only holds true when depletion of the free inhibitor by binding (i.e. formation of EI) is negligible. For this to be valid the total enzyme concentration must be at least an order of magnitude less than the total inhibitor concentration ($[E]_T \leq 10 [I]_T$) and the inhibitor concentration must also be kept sufficiently low compared to K_i to ensure the enzyme is not completely inhibited.⁷

In such cases better estimates for the association and dissociation can be determined by pre-incubation of enzyme with inhibitor^{4,9} and assaying for remaining activity (k_{on}) and by completely binding the enzyme with excess inhibitor, separating the EI complex from residual inhibitor by gel filtration and measuring the recovery of enzyme activity (k_{off}).^{10,11}

At high $[E]$ there is significant depletion of the ketone species which results in decreased k_{on} . Studies performed on Ac-Phe-CF₃ show that as $[E]$ increases, k_{on} decreases.¹⁰ Equations which take in to account the depletion of inhibitor and slow rates of association have been derived by Cha which resemble equation 6.15 but include extra terms comprising K_i , $[E]_T$ and $[I]_T$ (equation 6.19).¹²

$$P = v_{st} + \frac{(1 - \gamma)(v_o - v_s)}{k\gamma} \ln\left(\frac{1 - \gamma e^{-kt}}{1 - \gamma}\right) \quad \text{where } k = \frac{k_{on}\{(K_i + [E]_T + [I]_T)^2 - 4[E]_T[I]_T\}^{1/2}}{\left(1 + \frac{[S]}{K_m}\right)} \quad \text{and}$$

$$\gamma = \frac{K_i + [E]_T + [I]_T - \{(K_i + [E]_T + [I]_T)^2 - 4[E]_T[I]_T\}^{1/2}}{K_i + [E]_T + [I]_T + \{(K_i + [E]_T + [I]_T)^2 - 4[E]_T[I]_T\}^{1/2}} \quad \text{Equation 6.19}$$

Analyses of this type would be very complex and thus deemed beyond the scope of this thesis.

Assay results

The constants K_i , k_{on} and k_{off} for the five inhibitors have been summarised in figure 6.26.

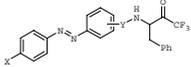
	X	Y	PSS _{ambient}			PSS _{UV}		
			K_i (μM)	k_{on} ($\text{M}^{-1} \cdot \text{s}^{-1}$)	k_{off} $\times 10^{-3}$	K_i (μM)	k_{on} ($\text{M}^{-1} \cdot \text{s}^{-1}$)	k_{off} $\times 10^{-3}$
4.12	H	4-SO ₂	11 ± 2	190 ± 60	2.7 ± 1.2	2* ± 0.4	740 ± 180	3.3 ± 1.4
4.46	I	3-SO ₂	19 ± 3	100 ± 50	3.1 ± 0.7	1* ± 0.3	290 ± 60	3.0 ± 0.7
4.52	I	4-SO ₂	47 ± 11	#	#	6* ± 1	270 ± 200	4.2 ± 2.2
4.16	H	4-SO ₂	18 ± 2	60 ± 40	3.5 ± 0.6	6 ± 1	170 ± 50	3.2 ± 0.6
4.62	I	4-SO ₂	50 ± 12	170 ± 70	1.7 ± 1.5	9 ± 1	130 ± 40	2.4 ± 0.5

Figure 6.26. Kinetic parameters K_i , k_{on} and k_{off} estimated for the ambient and Z-enriched photostationary state of α -chymotrypsin inhibitors. * K_i has been estimated from the slope of a best fit line through a curve. # Data was too scattered to obtain a straight line.

Plots of $\frac{(v_o - v_s)}{v_s}$ vs. $[I]$ (equation 6.18) were linear for all inhibitors measured at an ambient photostationary state and for Z-enriched dipeptides **4.16** and **4.62**. The plots for Z-enriched monopeptides **4.12**, **4.46** and **4.52** were distinctly parabolic and a replot of $\frac{(v_o - v_s)}{v_s}$ vs. $[I]^2$ gave a linear slope. Potentially this could occur because a ternary complex is formed (i.e. two molecules of inhibitor bind to the enzyme giving mixed inhibition), more work would be needed to determine this, or because of experimental error due to the high $[E]$ used in conjunction with inhibitors possessing a relatively low K_i .

Kinetic results obtained for slow binding inhibitors in this thesis should be viewed with some caution. Data was obtained under less the optimal conditions and the resulting analyses are comparable to each other but not necessarily comparable to other published results in this field.^{4,8-11}

While the validity of some of the results maybe questionable, certain trends between the different inhibitors have been noted. All inhibitors (mono and dipeptidyl) at both ambient and *Z*-enriched photostationary state exhibited slow-binding kinetics; this is in contrast to the results of Abeles *et al* who report that Ac-Phe-CF₃ shows fast reversible competitive inhibition whereas Ac-Leu-Phe-CF₃ exhibit slow-binding kinetics. This phenomenon had been attributed to slow but catalytically relevant van der Waals interactions between His-57 and Isl-99 of the enzyme and the leucine residue of the inhibitor. A study of various dipeptide ester and amide substrates showed that increasing the bulk of the substituents at the P₂ subsite resulted in tighter binding and slower association rates.^{9,10} Because of the slow-binding kinetics observed for all azobenzene based mono- and dipeptidyl inhibitors it was postulated that the azobenzene moiety arranges itself to take advantage of complementary columbic and electrostatic interactions within the enzyme.

For the five inhibitors tested it was found that the *Z*-enriched inhibitors had lower values of K_i and were therefore more potent than the corresponding inhibitor at an ambient photostationary state. This follows the same trend observed by Harvey when investigating α -keto ester based inhibitors of α -chymotrypsin.¹³ The increases in potency were 3-6 fold which is consistent with the degree of isomerisation i.e. a 3-fold increase in ratio of the percentage of *Z*-isomer resulted in *ca.* 3-fold decrease in the ratio of K_i values (fig. 6.27).

compound	$\frac{K_i \text{ (Z-enriched)}}{K_i \text{ (ambient)}}$	$\frac{\% Z \text{ (Z-enriched)}}{\% Z \text{ (ambient)}}$
4.16	3.0 ± 0.8	2.9
4.12	5.5 ± 2.1	3.5
4.46	19.0 ± 8	4.2
4.52	7.8 ± 3.1	5.1
4.62	5.6 ± 2.0	5.2

Figure 6.27. Comparison of K_i and % *Z* ratios for the ambient and *Z*-enriched photostationary state.

The result for **4.46** appears to be an anomaly and is due to the difficulty in determining the K_i from poor data. It is probable that the true ratio of K_i values is closer to 4-5 than 19-fold difference in potency.

The association rates (k_{on}) for the ambient photostationary state of the inhibitors was found to be lower than that of the corresponding *Z*-enriched inhibitors and both dipeptidyl inhibitors appear to have lower association rates than the three mono-peptidyl inhibitors. It is presumed that like Ac-Leu-Phe-CF₃, dipeptidyl inhibitors **4.16** and **4.62** require a delay period to setup secondary interactions of the leucine with the enzyme in addition to any interactions which may occur between the switch and the enzyme. The dissociation rate (k_{off}) remained relatively constant ($3 \times 10^{-3} \text{ s}^{-1}$) for all inhibitors at both photostationary states.

The effects of incorporating an iodine atom into the azobenzene switch are somewhat variable. Comparison of K_i values for **4.16** (18 and 6 μM) and **4.62** (50 and 9 μM) indicated that while the K_i for the *Z*-enriched inhibitors are similar, there were noticeable differences between the K_i of the ambient photostationary states which can be attributed to the differences in the degree of photoisomerisation (fig. 6.27). The effect is less pronounced when comparing **4.12** and **4.52** although similar trends are observed. Due to the anomalous result obtained for **4.46** it is difficult to draw any sound conclusions other than it a potent inhibitor. This was not unexpected as Harvey found that 3-substituted azobenzene peptidyl α -keto esters were more potent than the equivalent 4-substituted azobenzene inhibitors.⁶ Based on the observation that bulkier substituents at the P₂ subsite decreases k_{on} it was not surprising that k_{on} was lower for disubstituted azobenzenes **Z-4.16** ($170 \pm 50 \text{ M}^{-1} \cdot \text{s}^{-1}$) and **Z-4.62** ($130 \pm 40 \text{ M}^{-1} \cdot \text{s}^{-1}$) compared to **4.12** ($740 \pm 180 \text{ M}^{-1} \cdot \text{s}^{-1}$), especially for the *Z*-enriched inhibitors as the twisted conformation of the iodine substituted phenyl ring may be in closer proximity to the P₂ subsite than the corresponding planar *E*-conformation.

6.6 Summary

Five inhibitors were assayed against α -chymotrypsin at both ambient and *Z*-enriched photostationary states. In all cases slow binding kinetics were observed and the *Z*-enriched inhibitors were at least 3-fold more potent than the same inhibitor at an ambient photostationary state. These increases compared well to the degree of *Z*-enrichment exhibited by each inhibitor. Increasing the peptide chain length to improve specificity and

thus improve binding did not lower the K_i as expected, however incorporation of leucine into the peptide chain did decrease the association rate and increase the potency of the *Z*-enriched inhibitors **4.16** and **4.62** 3- and 5-fold respectively. Incorporation of iodine into the molecule had little effect on the value of K_i but did increase the ratio of K_i for ambient and *Z*-enriched inhibitors **4.46**, **4.52** and **4.62** and decreased the association rate. The effect of altering the position of substitution from 4- to 3- is inconclusive but general observations suggest that 3-substituted azobenzenes would be as good, if not better inhibitors of α -chymotrypsin.

6.7 Future work

Azobenzene based peptidyl trifluoromethyl ketones have shown great promise as potent inhibitors of α -chymotrypsin. Effective switching between active and less active forms of the inhibitors has been achieved. Addition of bulky substituents to the unsubstituted phenyl ring stabilises the *Z*-conformation of the azobenzene switch and increases the proportion of *Z*-isomer present after irradiation. The assays performed in this thesis need to be repeated with a better substrate than *N*-Suc-Phe-4-NA in order to reduce the concentration of enzyme used and get more definitive values for K_i , k_{on} and k_{off} .

Testing of some of the other inhibitors which incorporate different moieties such as bromine, carboxyl, amino and additional carbocycles needs to be done. Potentially some of the inhibitors already synthesised could be modified to incorporate functional groups designed to improve the solubility of azobenzene based inhibitors in aqueous media. Currently we are limited to an upper limit of *ca.* 50-60 μ M therefore improved solubility would be advantageous and allow comprehensive testing over a wider range of inhibitor concentrations.

Comparison of a wider selection of mono- and disubstituted azobenzenes with varying substitution patterns would better illustrate various trends and perhaps help elucidate potential mechanisms of inhibition. Testing of the phenanthrene derivatives could also be of use to see how well they compare to similar azobenzene derivatives and whether they could potentially mimic either *E*- or *Z*-azobenzenes.

Further investigation needs to be undertaken to confirm whether the mechanism is purely competitive or whether other mechanisms such as mixed inhibition is occurring in some instances.

6.8 References for chapter six

- (1) Price, N. C.; Stevens, L. In *Fundamentals of Enzymology*; Oxford University Press: New York, 1999, p 118-153.
- (2) Cornish-Bowden, A. *Fundamentals of Enzyme Kinetics*; Revised ed.; Portland Press: London, 1995.
- (3) Geiger, R. In *Methods of enzymatic analysis*; Bergmeyer, H. U., Ed. 1984, p 99.
- (4) Imperiali, B.; Abeles, R. H. *Biochemistry* 1986, 25, 3760-3767.
- (5) *European Journal of Biochemistry* 1982, 128, 281-291.
- (6) Harvey, A. J., Thesis; University of Canterbury, 2000.
- (7) Cha, S. *Biochemical Pharmacology* 1975, 24, 2177-2185.
- (8) Allen, K. N.; Abeles, R. H. *Biochemistry* 1989, 28, 8466-8473.
- (9) Brady, K.; Liang, T. C.; Abeles, R. H. *Biochemistry* 1989, 28, 9066-9070.
- (10) Brady, K.; Abeles, R. H. *Biochemistry* 1990, 29, 7608-7617.
- (11) Stein, R. L.; Strimpler, A. M.; Edwards, P. D.; Lewis, J. J.; Mauger, R. C.; Schwartz, J. A.; Stein, M. M.; Trainor, D. A.; Wildonger, R. A.; Zottola, M. A. *Biochemistry* 1987, 26, 2682-2689.
- (12) Williams, J. W.; Morrison, J. F. *Methods in enzymology* 1979, 63, 437-467.
- (13) Harvey, A. J.; Abell, A. D. *Bioorganic and Medicinal Chemical Letters* 2001, 11, 2441-2444.

Chapter Seven

Experimental

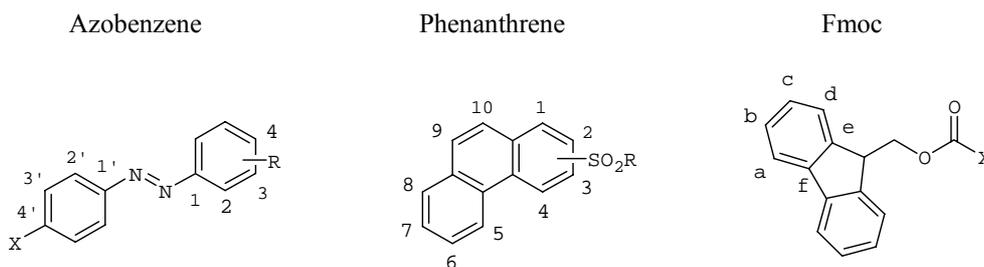
Experimental

7.1 General Methods and Procedures

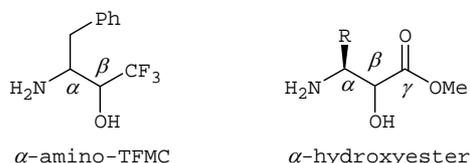
7.1.1 General practice

Compound numbering

Compounds described in this thesis were numbered for NMR characterisation in the following manner and do not necessarily follow IUPAC standards.



When reporting NMR data α refers to the carbon (or hydrogen) adjacent to the nitrogen functionality, β refers to the second carbon (or hydrogen) from the nitrogen and so on. This is irrespective of priority rules and applies to NMR assignments only.



NMR reporting

Invariably, synthesis of azobenzene compounds result in minor amounts of the *Z*-isomer being present. Recrystallisation of the purified material usually gave the *E*-isomer

exclusively. Unless otherwise stated, all reported data is for the *E*-isomer only. A solution of an azobenzene derivative reached an ambient photo-stationary state (a mixture of *E*- and *Z*-isomers) after exposure to light for *ca.* one day.

For compounds containing two diastereomers, NMR data associated with the minor isomer in the mixture has been labelled with an asterisk (*). For spectra that contain both ketone and hydrate peaks, the hydrate data has been labelled with an asterisk (*).

Concentrating *in vacuo*

Concentrating *in vacuo* refers to the bulk removal of solvent and volatiles under reduced pressure using a Büchii rotary evaporator (low vacuum pump). More rigorous drying was achieved under high vacuum (oil pump) for at least 30 min.

Elemental Analysis

Elemental analyses were performed for carbon, hydrogen, nitrogen and occasionally sulphur at the University of Otago Microanalytical laboratory and agree within ± 0.4 % of the calculated value.

Flash Chromatography

Flash chromatography (positive pressure) and column chromatography (atmospheric pressure) were performed on 230-400 mesh Merck silica 60 based on the guidelines of Still *et al.*¹ The eluting solvents, petroleum ether 50/70,[§] dichloromethane, and ethyl acetate were distilled in bulk from calcium hydride chips and stored in brown bottles away from light.

[§] A mixture of hexanes with a boiling point range between 50-70 °C.

Melting points

All melting points were obtained on an Electrothermal apparatus and are uncorrected. Melting points are not reported for diastereomeric or ketone/hydrate mixtures.

Mass Spectrometry

Electron impact ionisation (EI) mass spectroscopy was performed on a Kratos MS80 Mass Spectrometer operating at 4 kV (accelerating potential) and 70 eV (ionising potential). Electrospray ionisation (EI) mass spectroscopy was performed on a Micromass LCT TOF Mass Spectrometer operating with a probe voltage of 3.2 kV at 150 °C and a source temperature of 80 °C.

Nuclear Magnetic Resonance

^1H NMR spectra were obtained on either a Varian Unity 300 or Inova 500 spectrometer operating at 300 and 500 MHz respectively. ^{19}F NMR were obtained on the Varian Unity 300 spectrometer operating at 282 MHz. ^{13}C NMR were obtained on the Varian Unity 300 spectrometer operating at 75 MHz. All spectra were obtained at 23 °C with a delay (D_1) of at least 1 s. ^1H and ^{19}F NMR experiments had an acquisition time (A_t) of 2 s, ^{13}C NMR experiments had an acquisition time of 1-2 s. All two-dimensional NMR experiments, which include; COSY, HSQC and CIGAR were run on the Inova 500 spectrometer with a delay of 1 s.

Chemical shifts are reported in parts per million (ppm) on the δ scale and were referenced to the appropriate residual solvent peaks: CDCl_3 referenced to $(\text{CH}_3)_4\text{Si}$ at δ_{H} 0.00 ppm (^1H) and (CDCl_3) at δ_{C} 77.01 ppm (^{13}C); D_2O referenced to 1,4-dioxane as an internal standard at δ_{H} 3.70 ppm (^1H) and δ_{C} 67.4 ppm (^{13}C); methanol- d_4 referenced to CHD_2OD at δ_{H} 3.30 ppm (^1H) and CD_3OD at δ_{C} 49.3 ppm (^{13}C); acetone- d_6 referenced to $(\text{CHD}_2)(\text{CD}_3)\text{CO}$ at δ_{H} 2.17 ppm (^1H) and $(\text{CD}_3)_2\text{CO}$ at δ_{C} 29.2 ppm (^{13}C); acetonitrile- d_3 referenced to CHD_2CN at δ_{H} 2.00 ppm (^1H) and CD_3CN at δ_{C} 1.30 ppm; DMSO- d_6

referenced to $(\text{CHD}_2)(\text{CD}_3)\text{SO}$ at δ_{H} 2.60 ppm (^1H) and $(\text{CD}_3)_2\text{SO}$ at δ_{C} 39.6 ppm (^{13}C). All ^{19}F NMR spectra were referenced against an internal standard (fluorobenzene $\text{C}_6\text{H}_5\text{F}$) at δ_{F} -113.15 ppm (^{19}F).

Optical Rotary Dispersion

Optical rotation measurements were performed on a Perkin Elmer polarimeter Model 341 with a 1.00 dm pathlength. Measurements were taken at 20 °C in HPLC grade MeOH ($\lambda = 589$ nm). $[\alpha]_{\text{D}}$ values are given in $^{\circ}\cdot\text{mL}\cdot\text{g}^{-1}\cdot\text{dm}^{-1}$ and the sample concentration given in units of 10^1 $\text{mg}\cdot\text{mL}^{-1}$.

Ultraviolet Spectroscopy

UV/Visible spectroscopy was performed on a GBC UV/Vis 918 spectrometer running the default GBC Scientific v 2.01 software package.

Thin Layer Chromatography

Analytical TLC was performed on plastic-backed Merck-Kieselgel KG60F₂₅₄ or Polygram Sil G/UV₂₅₄ plates. Traces were visualised with UV light and a suitable dip, typically, basic potassium permanganate (general use), ninhydrin (amines), Mary's Dip^ξ (oxazolones, free acids) and ammonium molybdate-ceric^{IV} sulphate dip (azobenzenes).

Reagents and solvents

Reagents purchased from Aldrich or Sigma were used as supplied, with the exceptions of 3- and 4-nitrobenzene sulfonyl chloride, which required further purification.^ψ All other reagents and solvents were purified according to well established procedures.²

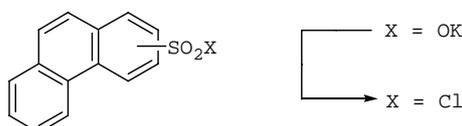
^ξ 4, 4'-Bis(dimethylamino)benzhydrol in acetone.

^ψ Recrystallisation from petroleum ether or petroleum ether/dichloromethane.

Triethylamine, dichloromethane, pyridine, benzene and toluene were distilled from calcium hydride powder immediately prior to use. Diethyl ether and THF were distilled from sodium benzophenone ketyl immediately prior to use. Super dry ethanol and methanol were distilled from magnesium ethoxide or magnesium methoxide respectively and stored under argon over 4 Å molecular sieves.

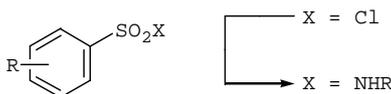
7.1.2 General methods

General Method A: Phenanthrenesulfonyl chlorides from phenanthrene sulfonates ³



The phenanthrene sulfonate (1.0 equiv) was suspended in a mixture of acetonitrile and sulfolane (2:1, 1.5 mL/mmol). Freshly distilled POCl_3 (0.38 mL/mmol) and dimethyl acetamide (1-3 drops) were added and the reaction mixture stirred for 75-90 min at 70 °C. Cooling of the reaction mixture to *ca.* 5 °C, followed by addition of cold water induced precipitation of the sulfonyl chloride. The precipitate was filtered, washed with distilled water and dried under vacuum. The crude sulfonyl chloride was purified by flash chromatography where required.

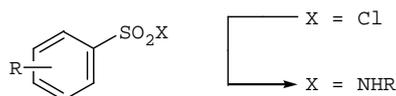
General Method B: Sulfonamide coupling (pyridine) ⁴



The sulfonyl chloride (1.0 equiv) and the amine hydrochloride (1.1 equiv) were dissolved in dry pyridine (11 mL/mmol) and heated at reflux for 2-4 h under an inert atmosphere. After cooling, the mixture was poured into a cold, saturated solution of aqueous NaHCO_3

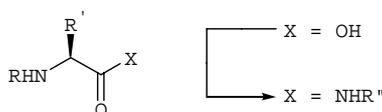
and the resulting precipitate collected by vacuum filtration, washed twice with 10 % aqueous HCl and distilled water then air-dried. Further purification of the crude sulfonamide was achieved by flash chromatography or recrystallisation.

General Method C: Sulfonamide coupling (Hünigs base)



To a stirred suspension of sulfonyl chloride (1.0 equiv) and the amine hydrochloride (1.1 equiv) in dry dichloromethane (5-10 mL/mmol) was added Hünigs base (2.2 equiv) to complete dissolution. The resulting solution was heated at reflux under an inert atmosphere for 2-4 h, cooled, and the solvent removed *in vacuo*. The residue was redissolved in ethyl acetate, successively extracted with 10 % aqueous HCl, saturated aqueous NaHCO₃ and brine, dried over MgSO₄, filtered and concentrated *in vacuo*. Further purification of the crude sulfonamide was achieved by recrystallisation or flash chromatography where required.

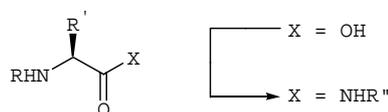
General Method D: EDCI coupling of free amines and *N*-protected amino acids^{5,6}



A suspension of the *N*-protected amino acid (1.0 equiv), amine hydrochloride (1.1 equiv), EDCI (1.3 equiv), and HOBT (1.5 equiv) in dry dichloromethane (25 mL/mmol) was stirred at rt under an inert atmosphere. After 5 min Hünigs base (2.2 equiv) was added dropwise to complete dissolution. The resulting solution was stirred for a further 16 h then diluted with a 4 x volume of ethyl acetate. The organics were successively extracted with 10 % aqueous HCl, saturated aqueous NaHCO₃ and brine, dried over MgSO₄, filtered and concentrated *in vacuo*. Further purification of the crude product was achieved by

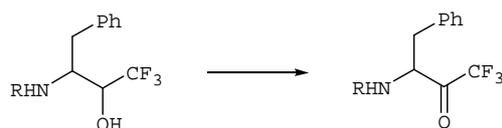
recrystallisation or flash chromatography.

General Method E: BOP coupling of free amines and *N*-protected amino acids⁵

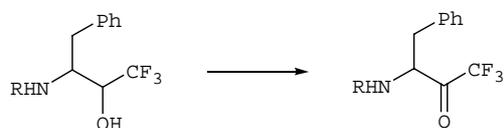


A suspension of the *N*-protected amino acid (1.0 equiv), amine hydrochloride (1.1 equiv), and BOP (1.1 equiv) in dry dichloromethane (5-20 mL/mmol) was stirred at rt under an inert atmosphere. After 5 min Hünigs base (2.2 equiv) was added dropwise to complete dissolution. The resulting solution was stirred for a further 16 h then diluted with a 4 x volume of ethyl acetate. The organics were successively extracted with 10 % aqueous HCl, saturated aqueous NaHCO₃ and brine, dried over MgSO₄, filtered and concentrated *in vacuo*. Further purification of the crude product was achieved by recrystallisation or flash chromatography.

General Method F: Trifluoromethyl carbinol oxidation via Dess-Martin Periodinane⁷



Trifluoromethyl carbinol (1.0 equiv) and Dess-Martin periodinane (3.7 equiv) were suspended in dry dichloromethane (0.5-1 mL/ μmol) and stirred at rt under an inert atmosphere for 3-16 h. The reaction mixture was diluted with 3-5 x volume of diethyl ether or ethyl acetate followed by addition of an equal volume of a solution containing 10 % w/v Na₂S₂O₃ in saturated aqueous NaHCO₃. The bi-phasic mixture was stirred for a further 20 min then separated. The aqueous layer was back extracted with dichloromethane and the combined organics washed with brine, dried over MgSO₄, filtered and concentrated *in vacuo*. Further purification of the crude product was achieved by recrystallisation or column chromatography where necessary.

General Method G: Trifluoromethyl carbinol oxidation via TEMPO- NaOCl ⁸

A buffered solution of sodium hypochlorite (5.25 %, 9.7 mL commercial bleach, 15.3 mL distilled water) was prepared by addition of solid NaHCO_3 (*ca.* 300 mg) until the pH lay within the range 8.6-9.5.^ψ

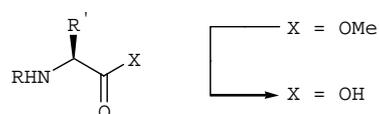
A solution of trifluoromethyl carbinol (1.0 equiv), KBr (0.2 equiv) and TEMPO (0.1 equiv) in dichloromethane (35 mL/mmol) was cooled to 0 °C.^ξ Buffered NaOCl (0.5 mL/mL dichloromethane) was added drop-wise and the reaction mixture stirred rapidly for 30-120 min. The bi-phasic mixture was diluted with ethyl acetate, separated, washed with brine, dried over MgSO_4 , filtered and concentrated *in vacuo*. Further purification of the crude product was achieved by recrystallisation or column chromatography.

Modified General Method G

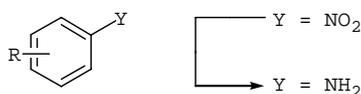
To a cooled solution of trifluoromethyl carbinol (1.0 equiv) in dichloromethane (12.5 mL/mmol) was added 1.25 equiv of buffered aqueous NaOCl solution (2.6 %, 0.35 M, pH 8.6), 0.1 equiv of aqueous KBr solution (50 mM) and 0.01 equiv of a solution of TEMPO dissolved in dichloromethane (400 μM). The bi-phasic mixture was rapid stirred at *ca.* 0 °C for 2 h, diluted with a 4 x volume ethyl acetate, washed with water then brine, dried over MgSO_4 , filtered and concentrated *in vacuo*. Further purification of the crude product was achieved by recrystallisation or column chromatography.

^ψ The required pH range for HOCl distribution between both phases.⁸

^ξThe temperature of the solution must be maintained at around 0 °C to minimise decomposition of the TEMPO catalyst.⁸

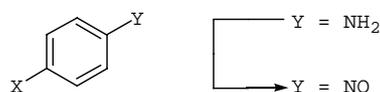
General Method H: K₂CO₃ mediated hydrolysis of methyl esters

A suspension of the methyl ester (1.0 equiv) and K₂CO₃ (1.5 equiv) in a mixture of methanol/water (9:1, 10 mL/mmol) was heated at reflux until hydrolysis complete by TLC (9-72 h). The reaction mixture was cooled, acidified with 10 % aqueous HCl and the resulting precipitate taken up in ethyl acetate. The separated aqueous layer was back extracted with dichloromethane and the combined organics washed with brine, dried over MgSO₄, filtered and concentrated *in vacuo*. Further purification of the free acid was achieved by recrystallisation when necessary.

General Method I: Reduction of substituted nitrobenzenes⁹

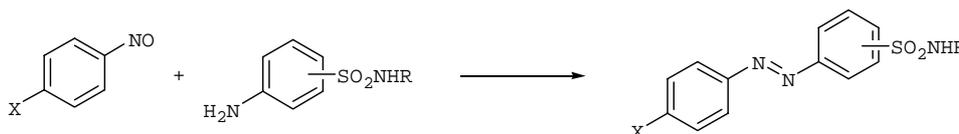
In a two necked flask fitted with a hydrogen balloon was placed the substituted nitrobenzene. Ethyl acetate (half volume of flask) was added followed by Adams' catalyst (PtO₂.H₂O, catalytic amount). The suspension was rapidly stirred under vacuum for 3-5 min then stirred under a hydrogen atmosphere for a further 16 h. The suspension was filtered through a mixture of celite and MgSO₄ and concentrated *in vacuo*. Further purification of the crude product was achieved by flash chromatography where necessary.

General Method J: Molybdenum catalysed oxidation of substituted anilines to nitrosobenzenes¹⁰



To a solution (or suspension) of 4-substituted aniline (1.0 equiv) in dichloromethane (5 mL/mmol) was added 30 % aqueous H_2O_2 (5.0 equiv) followed by the catalyst ($[\text{Mo}^{\text{VI}}(\text{O})\text{O}_2(\text{H}_2\text{O})(\text{HMPA})]$,¹¹ 0.1 equiv in 1 mL dichloromethane). After a few minutes the solution became tinged with green indicating the formation of the desired nitroso compound. The reaction mixture was stirred under an inert atmosphere for 12-48 h until no nitrobenzene remained by TLC.[§] The aqueous solvent was removed by addition of solid Na_2SO_4 , filtered, and concentrated *in vacuo*. The crude nitrosobenzene was purified by flash chromatography.

General Method K: Synthesis of (4-substituted phenylazo)benzenesulfonamidyl derivatives¹²

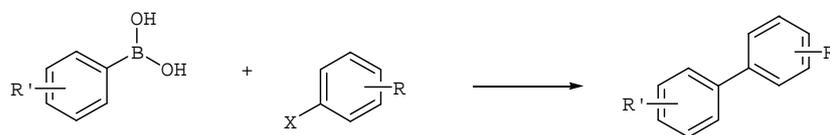


A suspension of 4-substituted nitrosobenzene (1.0 equiv) and sulfanilamide **OR** metanilamide (1.0 equiv) in glacial acetic acid (4 mL/mmol) was stirred at 70-100 °C for 4 h. The reaction mixture was cooled to rt then diluted with water/dichloromethane (1:1, 50 mL). Solid Na_2CO_3 was added cautiously until effervescence ceased and the aqueous layer basic to litmus. The aqueous layer was back extracted with dichloromethane and the combined organics washed successively with 10 % aqueous HCl, saturated aqueous NaHCO_3 and brine, dried over MgSO_4 , filtered and concentrated *in vacuo*. Further

[§] The reaction was very time dependent. Prolonged stirring lead to the formation of undesirable azobenzenes.

purification of the crude azobenzene was achieved by column chromatography.

General Method L: Suzuki coupling of aryl boronic acids ¹³



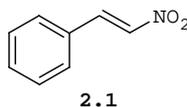
A suspension of aryl boronic acid (1.0 equiv), aryl bromide (1.2 equiv), *tetrakis*(triphenylphosphine)palladium(0) (0.05 equiv), tetrabutylammonium bromide (1.0 equiv) and KOH (3.0 equiv) in dry THF (30 mL/mmol) were heated at reflux under an inert atmosphere for 16 h. The cooled reaction mixture was diluted with ethyl acetate, washed with distilled water and brine, dried over MgSO₄, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography.

7.2 Experimental work outlined in chapter two

Synthesis of the key intermediate ($2R^*$, $3S^*$)-3-amino-1,1,1-trifluoro-4-phenylbutan-2-ol hydrochloride

7.2.1 Route 1: Condensation of 1-nitro-2-phenylethane with trifluoroacetaldehyde¹⁴

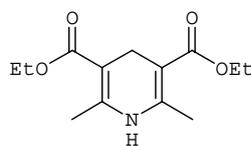
β -trans-Nitrostyrene (**2.1**)^{15,16}



β -Nitrostyrene was prepared by the condensation of nitromethane (30.5 g, 0.5 mol) and freshly distilled benzaldehyde (53.6 g, 0.5 mol) according to literature methods.^{15,16} Recrystallisation of the crude product from hot EtOH afforded pale yellow needles (16.3 g, 22 %). **Severe irritant!**

¹H NMR (CDCl₃, 500 MHz); δ 7.98 (d, 1H, J = 13.7 Hz, PhCH), 7.58 (d, 1H, J = 13.7 Hz, CHNO₂), 7.53 (d, 2H, J = 7.3 Hz, *o*-Ph), 7.48 (d, 1H, J = 7.1 Hz, *p*-Ph), 7.44 (t, 2H, J = 7.4 Hz, *m*-Ph).

¹³C NMR (CDCl₃, 75 MHz); δ 139.0, 137.1, 132.1, 130.0, 129.4, 129.1.

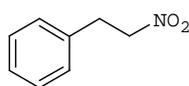
Diethyl 1,4-dihydro-2,6-dimethyl-3,5-pyridine dicarboxylate (2.2, Hantzsch ester)^{17,18}**2.2**

Hantzsch ester was prepared by condensation of freshly distilled ethyl acetoacetate (75.0 g, 0.58 mol) and 37 % aqueous formaldehyde (24.3 g, 0.30 mol), and subsequent condensation with gaseous ammonia following literature methods.^{17,18} Recrystallisation of the crude product from the minimum amount of hot EtOH afforded **2.2** as fluffy yellow crystals (45.0 g, 62 %).

mp 181-185 °C, (Lit. 181-183 °C)

¹H NMR (DMSO-*d*₆, 300 MHz); δ 8.38 (s, 1H, **NH**), 4.15 (q, 4H, J = 7.2 Hz, CH₃CH₂), 3.20 (s, 2H, CH₂), 2.20 (s, 6H, CH₃), 1.28 (t, 6H, J = 7.1 Hz, CH₃CH₂).

¹³C NMR (DMSO-*d*₆, 75 MHz); δ 167.2 (CO), 146.6 (C2), 97.2 (C3), 59.1 (CH₃CH₂), 24.9 (C4), 18.1 (CH₃), 14.6 (CH₃CH₂).

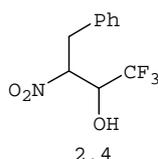
1-Nitro-2-phenylethane (2.3)¹⁹**2.3**

Nitrostyrene **2.1** (14.9 g, 100 mmol), Hantzsch ester **2.2** (28.0 g, 110 mmol) and silica (16.0 g) were suspended in dry toluene (300 mL). The reaction mixture was stirred under an inert atmosphere at 65 °C in a darkened fumehood. The progress of the reaction was monitored by TLC and after 50 h the reaction mixture was cooled to rt, filtered and concentrated *in vacuo* to ca. 150 mL. The organics were continually extracted with 40 % aqueous HCl until the aqueous layer no longer appeared green. The organics were washed with saturated aqueous NaHCO₃ and brine, dried over MgSO₄ and concentrated *in vacuo* to give a yellowish oil. The residue was rigorously dried on the high vacuum to remove toluene then distilled under reduced pressure to give **2.3** as a pale green oil (13.3 g, 88 %).

^1H NMR (CDCl_3 , 500 MHz); δ 7.33-7.18 (m, 5H, *o/m/p*-Ph), 4.58 (t, 2H, $J = 7.3$ Hz, CH_2NO_2), 3.29 (t, 2H, $J = 7.3$ Hz, PhCH_2).

^{13}C NMR (CDCl_3 , 75 MHz); δ 135.6 (*i*-Ph), 128.7 (*m*-Ph), 128.4 (*o*-Ph), 127.2 (*p*-Ph), 76.0 (CH_2NO_2), 33.1 (PhCH_2).

($2R^*$, $3S^*$)-1,1,1-Trifluoro-3-nitro-4-phenylbutan-2-ol (2.4**)^{14,20}**



2-Nitro-1-phenylethane **2.3** (9.63 g, 63.7 mmol), trifluoroacetaldehyde methyl hemiacetal[‡] (9.60 g, 70.1 mmol) and K_2CO_3 (0.35 g, 2.5 mmol) were stirred under an inert atmosphere for 12 h at 50 °C and 8 h at rt. The resulting brown oil was diluted with ethyl acetate (50 mL) and washed with 10 % aqueous HCl (3 x 50 mL) and brine (50 mL), dried over MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified by distillation under reduced pressure, followed by flash chromatography (dichloromethane) to give **2.4** as a pale yellow oil (9.50 g, 60 %) as a mixture of diastereomers (2:1 by ^1H and ^{19}F NMR). Freeze drying of a small amount of the oil under vacuum initiated crystallisation of one diastereomer as fine white crystals. It was tentatively assigned the stereochemistry ($2R^*$, $3S^*$) based on comparisons of coupling constants, the resulting stereochemistry of the reduction product and the crystallisation from oil reported on a similar compound.²⁰ Recrystallisation from hot petroleum ether removed traces of the minor diastereomer.

mp 59-60 °C

^1H NMR (CDCl_3 , 500 MHz); Mixture (2:1) δ 7.38-7.29 (m, 6H, *m/p*-Ph, major + minor), 7.22 (d, 2H, $J = 6.5$ Hz, *o*-Ph), 7.19* (d, 2H, $J = 6.8$ Hz, *o*-Ph), 4.96 (m, 2H, α -H, major + minor), 4.65* (m, 1H, β -H), 4.24 (m, 1H, β -H), 3.82 (br s, 1H, OH), 3.51* (br s, 1H, OH), 3.39* (d, 2H, $J = 7.3$ Hz, PhCH_2), 3.36 (d, 2H, $J = 7.8$ Hz, PhCH_2).

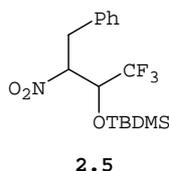
[‡] Lancaster, 80% technical grade, distilled through a 15-cm vigereux column prior to use.

^1H NMR (CDCl_3 , 500 MHz); Major (isolated) δ 7.37-7.30 (m, 3H, *m/p*-Ph), 7.21 (d, 2H, $J = 7.8$ Hz, *o*-Ph), 4.95 (m, 1H, α -H), 4.21 (m, 1H, β -H), 3.72 (d, 1H, $J = 8.8$ Hz, OH), 3.36 (d, 2H, $J = 7.8$ Hz, PhCH_2).

^{13}C NMR (CDCl_3 , 75 MHz); Major (isolated) δ 133.2 (*i*-Ph), 129.2, 128.9, 128.2 (*p*-Ph), 123.3 (q, $J = 283.2$ Hz, CF_3), 86.6 (α -C), 70.1 (q, $J = 30.5$ Hz, β -C), 36.5 (PhCH_2); Minor (from mixture) δ 134.4 (*i*-Ph), 129.0, 128.1, 127.7 (*p*-Ph), 87.6 (α -C), 70.6 (q, $J = 28.0$ Hz, β -C), 34.4 (PhCH_2). The CF_3 quartet could not be detected over the baseline noise.

^{19}F NMR (CDCl_3 , 282 MHz); Mixture (2:1) δ -76.4 (d, $J = 6.6$ Hz), -76.9* (d, $J = 6.4$ Hz).

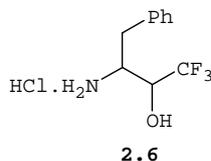
tert-Butyldimethyl-(2-nitro-3-phenyl-1-trifluoromethylpropoxy)silane (**2.5**)²¹



The nitrocarbinol **2.4** (5.80 g, 23.3 mmol, 3:2 mixture of diastereomers), TBDMSCl (4.39 g, 96 %, 28.0 mmol) and imidazole (3.97 g, 58.3 mmol) were dissolved in dry DMF (10 mL) and stirred at 35 °C under an inert atmosphere for 18 h. The reaction mixture was diluted with ethyl acetate (50 mL) and successively washed with water (5 x 30 mL), saturated aqueous NaHCO_3 (2 x 30 mL) and brine (30 mL), dried over MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified by flash chromatography (dichloromethane) to give **2.5** as a bright yellow oil (7.87 g, 93 %) comprising a mixture of diastereomers (3:2 by ^1H NMR).

^1H NMR (CDCl_3 , 300 MHz); Mixture (3:2) δ 7.46-7.24 (m, 10H, *o/m/p*-Ph, major + minor), 5.07-5.00 (m, 2H, α -H, major + minor), 4.82* (m, 1H, β -H), 4.63 (m, 1H, β -H), 3.55-3.32 (m, 4H, PhCH_2 , major + minor), 1.08* (s, 9H, $\text{C}(\text{CH}_3)_3$), 0.99 (s, 9H, $\text{C}(\text{CH}_3)_3$), 0.29* (s, 6H, CH_3), 0.27 (s, 6H, CH_3).

^{13}C NMR (CDCl_3 , 75 MHz); Mixture δ 135.4 (*i*-Ph), 134.2 (*i*-Ph), 129.0, 128.8, 128.7, 127.8 (*p*-Ph, major), 127.5* (*p*-Ph), 123.5 (q, $J = 283.8$ Hz, CF_3), 123.5* (q, $J = 284.1$ Hz, CF_3), 90.7, 88.8, 72.7* (q, $J = 32.1$ Hz, β -C), 72.5 (q, $J = 31.1$ Hz, β -C), 35.4, 33.3, 25.5* ($\text{C}(\text{CH}_3)_3$), 25.4 ($\text{C}(\text{CH}_3)_3$), 18.1 ($\text{C}(\text{CH}_3)_3$), 18.0 ($\text{C}(\text{CH}_3)_3$), Silyl CH_3 peaks were outside spectral window.

(2*R*^{*},3*S*^{*})-3-Amino-1,1,1-trifluoro-4-phenylbutan-2-ol hydrochloride (2.6)*Method 1: Reduction via Raney nickel*¹⁴

The nitrocarbinol **2.3** (2.51 g, 10.1 mmol, 2:1 mixture of diastereomers) was dissolved in EtOH.[§] Raney nickel²² was added, the reaction vessel flushed with hydrogen, pressurised to 50 psi and then shaken in a Parr reactor for 12 h. The suspension was filtered through celite, washed with distilled EtOH and concentrated *in vacuo*. The residue was redissolved in dichloromethane, extracted with 10 % aqueous HCl (5 x 25 mL) and the combined aqueous extracts concentrated *in vacuo* to give an off-white solid. Recrystallisation from hot ethyl acetate/petroleum ether gave the hydrochloride salt **2.6** as very fine, fluffy white needles (0.88 g, 34 %, single diastereomer).

mp 232-235 °C

¹H NMR (D₂O, 500 MHz); δ 7.37-7.27 (m, 5H, *o/m/p*-Ph), 4.44 (m, 1H, **β-H**), 3.90 (m, 1H, **α-H**). 3.19 (dd, 1H, J = 3.7, 14.9 Hz, Ph**CH_a**), 2.90 (dd, 1H, J = 10.7, 14.6 Hz, Ph**CH_b**).

¹³C NMR (D₂O, 75 MHz); δ 135.4 (*i*-Ph), 130.2, 130.1, 128.7 (*p*-Ph), 124.9 (q, J = 282.4 Hz, CF₃), 69.1 (q, J = 31.3 Hz, **β-C**), 53.6 (**α-C**), 33.4 (PhCH₂).

¹⁹F NMR (D₂O, 282 MHz); δ -74.3 (d, J = 7.4 Hz).

HRMS (EI) 220.0957 (M-HCl)⁺; C₁₀H₁₃F₃NO requires 220.0949.

*Method 2: Reduction via lithium aluminium hydride*²³

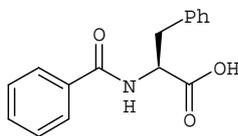
A suspension of LiAlH₄ (0.70 g, 18.4 mmol) in dry ether (25 mL) was heated to reflux for 30 min under an inert atmosphere. A solution of the silyl protected nitrocarbinol **2.5** (2.99 g, 8.23 mmol, 3:2 mixture of diastereomers) in dry ether (25 mL) was added dropwise to the hydride suspension and the reaction mixture continued to reflux for 30 min. After

[§] EtOH was distilled from Raney nickel to remove catalyst poisons prior to use.

stirring at rt for a further 16 h, the LiAlH_4 was cautiously decomposed with a solution of saturated aqueous Na_2SO_4 (10 mL). The resulting white precipitate was filtered off, washed with ether and the washings combined with the filtrate. The aqueous layer was separated and back extracted with ether (25 mL). The combined ether layers were washed with brine then stirred with 10 % aqueous HCl for 10 min. The separated ether layer was further extracted with 10 % aqueous HCl (3 x 20 mL) and the combined aqueous acid extracts concentrated *in vacuo* to give a white solid. Recrystallisation from hot ethyl acetate/petroleum ether gave a single diastereomer identical to **2.6** isolated from the Raney nickel reduction (0.51 g, 24 %). The filtrate was concentrated *in vacuo* to afford a brown oil that solidified under high vacuum to give an off white solid (0.69 g, 33 %) as a mixture of diastereomers (1:2 by ^{19}F NMR).

7.2.2 Route 2: Trifluoromethyl carbinol synthesis via a modified Dakin-West reaction²⁴

***N*-Benzoyl-*L*-phenylalanine (2.7)**



2.7

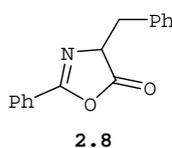
L-Phenylalanine (17.4 g, 105 mmol) was dissolved in a stirred solution of NaOH (8.4 g, 210 mmol) in water (250 mL) at 0 °C. Distilled benzoyl chloride (11.6 mL, 100 mmol) was added dropwise to the solution. The reaction mixture was stirred for a further 2 h then acidified with 6 M aqueous HCl. The resulting precipitate was filtered off, washed with 10 % aqueous HCl, distilled water and air-dried to give a white solid that retained significant amounts of water. The sample was recrystallised from hot water in batches to give **2.7** as fluffy white needles (24.5 g, 91 %).

mp 141-142 °C

^1H NMR (acetone- d_6 , 500 MHz); δ 7.95 (d, 3H, $J = 7.3$ Hz, *o*-Bz, **NH**), 7.62 (t, 1H, $J = 7.4$ Hz, *p*-Bz), 7.54 (m, 2H, $J = 7.5$ Hz, *m*-Bz), 7.47 (d, 2H, $J = 7.4$ Hz, *o*-Ph), 7.39 (m, 2H, $J = 7.5$ Hz, *m*-Ph), 7.31 (t, 1H, $J = 7.3$ Hz, *p*-Ph), 5.05 (m, 1H, α -**H**), 3.46 (dd, 1H, $J = 4.8, 13.9$ Hz, **PhCH_a**), 3.29 (dd, 1H, $J = 9.3, 13.9$ Hz, **PhCH_b**).

4-Benzyl-2-phenyl-4H-oxazol-5-one (2.8)

*Method 1: Cyclisation via EDCI*²⁵



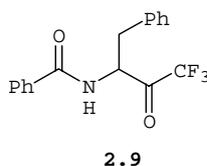
N-Benzoyl-*L*-phenylalanine **2.7** (13.5 g, 50.0 mmol) and EDCI (9.8 g, 50.0 mmol) were dissolved in dry dichloromethane (30 mL) and stirred at 0 °C under an inert atmosphere for 3 h. The reaction mixture was successively washed with ice water, saturated aqueous NaHCO_3 and brine, dried over MgSO_4 , filtered and concentrated *in vacuo* to give the oxazolone as a colourless oil. Rigorous drying on a high vacuum pump yielded a white solid (12.3 g, 98 %). Purified by flash chromatography (1:3 ethyl acetate/petroleum ether) where required. Recrystallisation from hot petroleum ether afforded **2.8** as fine white needles.

^1H NMR (acetone- d_6 , 500 MHz); δ 8.03 (d, 2H, $J = 7.3$ Hz, *o*-Ar), 7.74 (t, 1H, $J = 7.5$ Hz, *p*-Ar), 7.65 (t, 2H, $J = 7.7$ Hz, *m*-Ar), 7.42 (d, 2H, $J = 7.2$ Hz, *o*-Ph), 7.37 (t, 2H, $J = 7.4$ Hz, *m*-Ph), 7.31 (t, 1H, $J = 7.2$ Hz, *p*-Ph), 5.01 (dd, 1H, $J = 5.0, 6.9$ Hz, α -**H**), 3.48 (dd, 1H, $J = 5.0, 14.0$ Hz, **PhCH_a**), 3.30 (dd, 1H, $J = 6.9, 14.1$ Hz, **PhCH_b**).

^{13}C NMR (acetone- d_6 , 75 MHz); δ 177.6, (**CO**), 161.2 (**N=CO**), 136.4, 132.8, 129.8, 129.1, 128.3, 127.7, 127.1, 126.5, 66.4 (α -**C**), 37.0 (**PhCH₂**).

Method 2: Cyclisation via DCC

To a cooled (0 °C), stirred solution of *N*-benzoyl-*L*-phenylalanine **2.7** (17.5 g, 65.0 mmol) in dry THF (30 mL) under an inert atmosphere was added dropwise, a solution of dicyclohexylcarbodiimide (13.4 g, 65.0 mmol) in dry dichloromethane (100 mL). The reaction mixture was stirred a further 30 min then filtered to remove the precipitated dicyclohexylurea. The solution was concentrated *in vacuo*, redissolved in ethyl acetate and filtered to remove residual traces of the urea by-product. The ethyl acetate solution was successively washed with saturated aqueous NaHCO₃, water and brine, dried over MgSO₄, filtered and concentrated *in vacuo* to give the oxazolone as an oil which solidified upon cooling (11.2 g, 44.6 mmol, 69 %). Recrystallisation from hot petroleum ether afforded **2.8** as fine white needles identical to the above product.

***N*-(1*S**-Benzyl-3,3,3-trifluoro-2-oxopropyl)benzamide (2.9)**

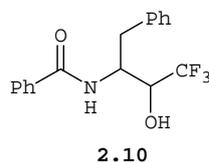
To a stirred solution of trifluoroacetic anhydride (8.0 mL, 57.0 mmol) was added, in small portions, recrystallised oxazolone **2.8** (11.0 g, 43.8 mmol). The thick slurry was heated at 40 °C for 3 days to give a yellow solution. Trifluoroacetic acid and excess trifluoroacetic anhydride was removed *in vacuo* at 80 °C before increasing the temperature to 120 °C. The resulting oil was treated, portionwise, with recrystallised anhydrous oxalic acid (5.9 g, 65.8 mmol). Once the slurry had become too thick to stir, the mixture was diluted with ethyl acetate (50 mL) and the remaining oxalic acid added. The solution was heated until effervescence ceased, cooled to rt and further diluted with ethyl acetate (250 mL). The organics were washed with water (3 x 100 mL) and brine, dried over MgSO₄ and concentrated *in vacuo* to give a pale yellow solid. Washing the solid with dry ether (3 x 50 mL) afforded racemic **2.9** as a fluffy white solid (10.8 g, 77 %) comprising a mixture of ketone and hydrate (2:3 by ¹H NMR). An analytical sample was obtained by recrystallisation from aqueous acetone to afford the hydrate as a white crystalline solid (>95 % by ¹⁹F NMR).

^1H NMR (acetone- d_6 , 500 MHz); From mixture δ 8.56 (br s, 1H, **NH**), 8.20* (d, 1H, $J = 6.9$ Hz, **NH**), 7.94 (d, 2H, $J = 7.5$ Hz, ***o*-Bz**), 7.83* (d, 2H, $J = 7.5$ Hz, ***o*-Bz**), 7.69-7.28 (m, 16H, ***m/p*-Bz**, ***o/m/p*-Ph**, ketone + hydrate), 5.30 (m, 1H, **α -H**), 4.43* (m, 1H, **α -H**), 3.50-3.32 (m, 4H, **PhCH₂**, ketone + hydrate).

^{13}C NMR (acetone- d_6 , 75 MHz); Hydrate (isolated) δ 170.2 (PhCO), 139.0 (***i*-Ph**), 134.0 (***i*-Bz**), 132.0 (***p*-Bz**), 129.5, 128.5 (x2), 127.6, 126.6 (***p*-Ph**), 124.3 (q, $J = 289.2$ Hz, **CF₃**), 94.8 (q, $J = 29.9$ Hz, **β -C**), 58.4 (**α -C**), 32.9 (PhCH₂).

^{19}F NMR (acetone- d_6); From Mixture (5:95) δ -74.9 (s, 3F), -80.7* (s, 3F).

***N*-(1-Benzyl-3,3,3-trifluoro-2-hydroxypropyl)benzamide (2.10)**



To a cooled (0 °C), stirred suspension of the ketone/hydrate mixture **2.9** (4.45 g, 13.4 mmol) in EtOH (100 mL) was added NaBH₄ (0.52 g, 13.7 mmol). The reaction mixture was stirred under an inert atmosphere for 12 h then quenched by the addition of 6 M aqueous HCl (30 mL). The EtOH was removed *in vacuo* and the residue taken up in ethyl acetate (50 mL). The ethyl acetate was successively washed with 10 % aqueous HCl, saturated aqueous NaHCO₃ and brine, dried over MgSO₄ and concentrated *in vacuo* to give **2.10** as a white solid (4.24 g, mmol, 98 %) comprising a mixture of diastereomers (2:3 by ^1H NMR).

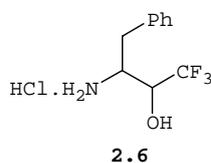
$[\alpha]_{\text{D}} = -0.40$ ($c = 1.0$, MeOH)

^1H NMR (acetone- d_6 , 500 MHz); Mixture (2:3) δ 7.92* (d, 2H, $J = 7.3$ Hz, ***o*-Bz**), 7.87 (d, 3H, $J = 7.5$ Hz, ***o*-Bz**, minor, **NH**, major), 7.80* (d, 1H, $J = 7.9$ Hz, **NH**), 7.66-7.27 (m, 16H, ***m/p*-Bz**, ***o/m/p*-Ph**, major + minor), 6.48* (d, 1H, $J = 7.7$ Hz, **OH**), 6.00 (d, 1H, $J = 6.7$ Hz, **OH**), 4.73 (m, 2H, **α -H**, major + minor), 4.52 (m, 1H, **β -H**), 4.32* (m, 1H, **β -H**), 3.36-3.21 (m, 4H, **PhCH₂**, major + minor).

^{13}C NMR (acetone- d_6 , 75 MHz); Mixture δ 167.5* (CO), 167.2 (CO), 138.7, 138.3*, 134.8, 134.8*, 131.6*, 131.4, 129.5, 129.5*, 128.6, 128.5, 128.4 (x2), 127.4, 127.4*, 126.7*, 126.5, 125.8 (q, J = 283.3 Hz, CF_3), 125.5* (q, J = 282.2 Hz, CF_3), 71.4 (q, J = 28.6 Hz, $\beta\text{-C}$), 69.9* (q, J = 29.5 Hz, $\beta\text{-C}$), 52.0 ($\alpha\text{-C}$), 51.6* ($\alpha\text{-C}$), 37.1* (PhCH_2), 34.9 (PhCH_2).

^{19}F NMR (acetone- d_6 , 282 MHz) Mixture (57:43) δ -74.2 (d, J = 7.6 Hz), -75.6* (d, J = 7.5 Hz).

(2*R,3*S**)-3-Amino-1,1,1-trifluoro-4-phenylbutan-2-ol hydrochloride (2.6)**

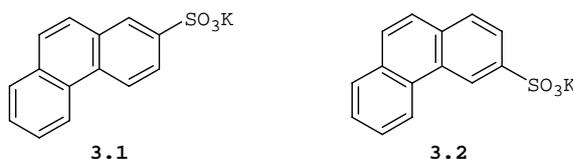


Trifluoromethyl carbinol **2.10** (2.50 g, 7.73 mmol, 2:3 mixture of diastereomers) was suspended in a mixture of HCl/EtOH/H₂O (2:1:1, 350 mL) and heated at reflux for 16 h. The solvents were removed *in vacuo* and the residue taken up in water (100 mL). Liberated benzoic acid was removed by extraction with dichloromethane (3 x 50 mL) and the aqueous layer concentrated *in vacuo* to give an off-white solid. Recrystallisation from hot ethyl acetate/petroleum ether afforded the hydrochloride salt as fine fluffy white needles (1.38 g, 70 %, 9:1 mixture of diastereomers). Further recrystallisation from ethyl acetate/petroleum ether afforded the major *anti* diastereomer which is identical to **2.6** by NMR.

7.3 Experimental work outlined in chapter three

Synthesis of phenanthrenesulfonyl derivatives

Potassium 2- and 3-phenanthrene sulfonates²⁶



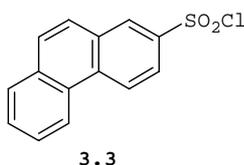
The sulfonates were prepared by direct sulfonation of recrystallised phenanthrene (25.0 g, 140 mmol) with concentrated sulfuric acid (17 mL) following the procedure outlined by Fieser²⁶ and isolated as the potassium salts. Recrystallisation of the crude sulfonates from hot water gave potassium phenanthrene-2-sulfonate (3.6 g, 9 %) as a very pale tan flakes and potassium phenanthrene-3-sulfonate (4.1 g, 10 %) as yellow flakes.

2-Phen-SO₃K; HRMS (ES) 257.0274 (M-K)⁻; C₁₄H₉O₃S requires 257.0272.

3-Phen-SO₃K; HRMS (ES) 257.0269 (M-K)⁻; C₁₄H₉O₃S requires 257.0272.

7.3.1 Phenanthrene-2-sulfonyl derivatives

Phenanthrene-2-sulfonyl chloride (3.3)



Potassium sulfonate **3.1** (2.96 g, 10.0 mmol) was treated with POCl₃ (3.80 mL, 41.0 mmol) following General Method A to give the desired product as a dull yellow solid. The crude sulfonyl chloride was subjected to flash chromatography (dichloromethane) to afford **3.3**

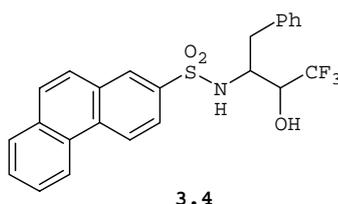
as a bright yellow powder (2.63 g, 95 %). An analytical sample was obtained by recrystallisation from aqueous acetone.

^1H NMR (CDCl_3 , 300 MHz); δ 8.83 (d, 1H, $J = 8.9$ Hz, ArH4), 8.68 (d, 1H, $J = 9.5$ Hz, ArH5), 8.57 (d, 1H, $J = 2.1$ Hz, ArH1), 8.17 (dd, 1H, $J = 2.1, 8.9$ Hz, ArH3), 7.95 (d, 1H, $J = 9.3$ Hz, ArH8), 7.90 (d, 1H, $J = 8.9$ Hz, ArH9), 7.81 (d, 1H, $J = 8.9$ Hz, ArH10), 7.75 (m, 2H, ArH6, ArH7).

^{13}C NMR (CDCl_3 , 75 MHz); δ 141.5, 134.4, 133.3, 131.2, 129.7, 129.1, 129.0, 128.9, 128.3, 127.7, 126.5, 124.6, 123.5, 122.7.

HRMS (EI) 276.0016 (M^+); $\text{C}_{14}\text{H}_9\text{ClO}_2\text{S}$ requires 276.0012.

Phenanthrene-2-sulfonic acid ($1S^*, 2R^*$)-(1-benzyl-3,3,3-trifluoro-2-hydroxypropyl) amide (3.4)



Sulfonyl chloride **3.3** (100 mg, 0.36 mmol) and **2.6** (98 mg, 0.38 mmol) were coupled together in the presence of Hünigs base (0.15 mL, 0.86 mmol) following General Method C to give the desired product **3.4** as a pale yellow solid (165 mg, quantitative). Recrystallisation of the crude trifluoromethyl carbinol from aqueous EtOH gave a very pale yellow crystalline product as a single diastereomer.

mp 176-177 °C.

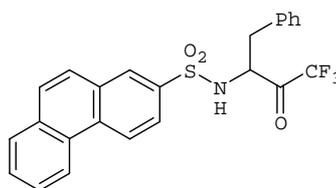
^1H NMR (acetone- d_6 , 500 MHz); δ 8.96 (d, 1H, $J = 8.0$ Hz, ArH5), 8.80 (d, 1H, $J = 8.8$ Hz, ArH4), 8.22 (s, 1H, ArH1), 8.18 (d, 1H, $J = 7.6$ Hz, ArH8), 8.07 (d, 1H, $J = 8.9$ Hz, ArH9), 7.95 (d, 1H, $J = 8.9$ Hz, ArH10), 7.89 (m, 2H, ArH6, ArH7), 7.74 (d, 1H, $J = 8.8$ Hz, ArH3), 7.06 (m, 3H, NH, *o*-Ph), 6.84 (t, 2H, $J = 7.5$ Hz, *m*-Ph), 6.76 (t, 1H, $J = 7.3$ Hz, *p*-Ph), 5.99 (d, 1H, $J = 6.9$ Hz, OH), 4.60 (m, 1H, β -H), 3.96 (m, 1H, α -H), 3.04 (dd, 1H, $J = 2.7, 14.4$ Hz, PhCH_a), 2.93 (dd, 1H, $J = 10.7, 14.4$ Hz, PhCH_b).

^{13}C NMR (acetone- d_6 , 75 MHz); δ 138.6, 137.4, 133.2, 132.5, 131.5, 129.8, 129.2, 129.0, 128.4, 128.1 (x 2), 127.5 (x 2), 127.2, 126.1, 125.4 (q, $J = 283.0$ Hz, CF₃), 124.2, 123.6, 123.5, 73.0 (q, $J = 28.6$ Hz, β -C), 56.0 (α -C), 34.2 (PhCH₂).

^{19}F NMR (acetone- d_6 , 282 MHz); δ -74.2 (d, 3F, $J = 8.0$ Hz).

HRMS (EI) 459.1128 (M^+); $\text{C}_{24}\text{H}_{20}\text{F}_3\text{NO}_3\text{S}$ requires 459.1116.

Phenanthrene-2-sulfonic acid ($1S^*$ -benzyl-3,3,3-trifluoro-2-oxopropyl)amide (3.5)



3.5

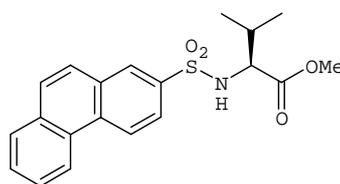
Trifluoromethyl carbinol **3.4** (23 mg, 50 μmol) was oxidised with Dess-Martin periodinane (78 mg, 190 μmol) over 3 h following General Method F. The resulting brown oil was titrated with aqueous MeOH to give **3.5** as a white solid (13 mg, 56 %) comprising a racemic mixture of hydrate and ketone. One drop of D_2O was added to the NMR sample to give the hydrate exclusively.

^1H NMR (acetone- d_6 , 500 MHz); Hydrate δ 8.93 (d, 1H, $J = 8.1$ Hz, Ar**H5**), 8.74 (d, 1H, $J = 8.8$ Hz, Ar**H4**), 8.16 (d, 1H, $J = 7.5$ Hz, Ar**H8**), 8.11 (s, 1H, Ar**H1**), 8.05 (d, 1H, $J = 8.8$ Hz, Ar**H9**), 7.91-7.83 (m, 3H, Ar**H6**, Ar**H7**, Ar**H10**), 7.74 (dd, 1H, $J = 2.0, 8.8$ Hz, Ar**H3**), 7.01 (d, 2H, $J = 7.3$ Hz, *o*-Ph), 6.65 (t, 2H, $J = 7.6$ Hz, *m*-Ph), 6.45 (t, 1H, $J = 7.4$ Hz, *p*-Ph), 4.05 (dd, 1H, $J = 2.3, 10.8$ Hz, α -H), 3.27 (dd, 1H, $J = 2.5, 14.3$ Hz, Ph**CH_a**), 2.75 (dd, 1H, $J = 11.0, 14.3$ Hz, Ph**CH_b**).

^{13}C NMR (acetone- d_6 , 75 MHz); Hydrate δ 138.9, 137.6, 132.9, 132.3, 131.3, 129.5, 129.0, 128.8, 128.1, 128.0, 127.7, 127.4, 127.1 (x 2), 125.6, 123.9 (q, $J = 289.2$ Hz, CF_3), 123.9, 123.4, 123.3, 93.5 (q, $J = 30.0$ Hz, β -C), 60.8 (α -C), 35.4 (Ph**CH₂**).

^{19}F NMR (acetone- d_6 , 282 MHz); Hydrate δ -79.0 (s, 3F).

HRMS (ES) 458.1044 (MH^+); $\text{C}_{24}\text{H}_{19}\text{F}_3\text{NO}_3\text{S}$ requires 458.1038.

3-Methyl-2S-(phenanthrene-2-sulfonylamino)butyric acid methyl ester (3.6)

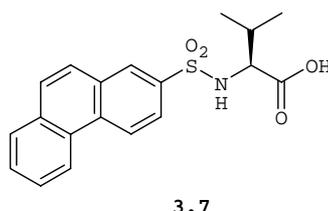
Sulfonyl chloride **3.3** (150 mg, 0.54 mmol) and *L*-valine methyl ester hydrochloride (100 mg, 0.60 mmol) were coupled together utilising Hünigs base (0.21 mL, 1.21 mmol) following General Method C to give the desired ester **3.6** as a yellow powder (201 mg, quantitative). An analytical sample of the ester was obtained by recrystallisation from aqueous MeOH resulting in pale yellow needles.

mp 206-207 °C.

¹H NMR (CDCl₃, 500 MHz); δ 8.79 (d, 1H, J = 8.8 Hz, ArH4), 8.71 (d, 1H, J = 7.8 Hz, ArH5), 8.40 (s, 1H, ArH1), 8.01 (dd, 1H, J = 2.0, 8.8 Hz, ArH3), 7.95 (d, 1H, J = 8.8 Hz, ArH8), 7.86 (d, 1H, J = 8.9 Hz, ArH9), 7.80 (d, 1H, J = 8.9 Hz, ArH10), 7.72 (m, 2H, ArH6, ArH7), 5.20 (d, 1H, J = 10.3 Hz, NH), 3.85 (m, 1H, α-H), 3.23 (s, 3H, OCH₃), 2.04 (m, 1H, ValCH), 0.93 (d, 3H, J = 6.8, Hz, ValCH₃), 0.89 (d, 3H, J = 6.8 Hz, ValCH₃).

¹³C NMR (CDCl₃, 75 MHz); δ 171.7 (CO), 137.0 (ArC2), 133.0, 132.9, 131.3, 129.4, 128.8 (x 2), 128.4, 128.1, 127.3, 126.7, 123.9, 123.7, 123.3, 61.2 (α-C), 52.1 (OCH₃), 31.7 (ValCH), 19.0 (ValCH₃), 17.5 (ValCH₃).

HRMS (EI) 371.1206 (M⁺); C₂₀H₂₁NO₄S requires 371.1191.

3-Methyl-2S-(phenanthrene-2-sulfonylamino)butyric acid (3.7)

Methyl ester **3.6** (118 mg, 0.32 mmol) was treated with K₂CO₃ (69 mg, 0.50 mmol) in MeOH/H₂O for 3 days following General Method H to give the free acid **3.7** as a white

solid. Recrystallisation of **3.7** from aqueous MeOH afforded a white powder (101 mg, 89 %).^ξ

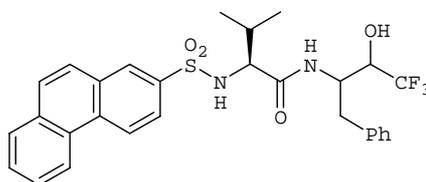
mp 195-196 °C

¹H NMR (CDCl₃, 500 MHz); δ 8.72 (d, 1H, J = 8.8 Hz, ArH4), 8.63 (d, 1H, J = 7.9 Hz, ArH5), 8.36 (d, 1H, J = 1.9 Hz, ArH1), 8.01 (dd, 1H, J = 1.9, 8.8 Hz, ArH3), 7.88 (d, 1H, J = 8.7 Hz, ArH8), 7.79 (d, 1H, J = 8.9 Hz, ArH9), 7.76 (d, 1H, J = 8.9 Hz, ArH10), 7.69-7.60 (m, 2H, ArH6, ArH7), 5.57 (d, 1H, J = 9.7 Hz, NH), 3.75 (m, 1H, α-H), 2.05 (m, 1H, ValCH), 0.96 (d, 3H, J = 6.7 Hz, ValCH₃), 0.82 (d, 3H, J = 6.7 Hz, ValCH₃).

¹³C NMR (acetone-*d*₆, 75 MHz); δ 171.8 (CO), 139.2 (ArC2), 133.2, 131.6, 129.9, 129.7, 129.0, 128.7, 128.2, 128.1, 127.6, 127.1, 124.3, 124.1, 123.6, 61.4 (α-C), 31.3 (ValCH), 18.9 (ValCH₃), 17.3 (ValCH₃).

HRMS (ES) 358.1119 (MH⁺); C₁₉H₂₀NO₄S requires 358.1113.

(1*S,2*R**)-N-(1-Benzyl-3,3,3-trifluoro-2-hydroxypropyl)-3-methyl-2*S*-(phenanthrene-2-sulfonylamino)butyramide (3.8)**



3.8

Phenanthrene **3.7** (100 mg, 0.28 mmol), **2.6** (75 mg, 0.29 mmol), EDCI (70 mg, 0.36 mmol), HOBt (57 mg, 0.42 mmol) and Hünigs base (0.11 mL, 0.63 mmol) were reacted together following General Method D to give the dipeptide as a yellowish solid. Flash chromatography (1:9 ethyl acetate/dichloromethane) of the crude trifluoromethyl carbinol, followed by recrystallisation from aqueous MeOH afforded **3.8** as a white powder (112 mg, 70 %) comprising a mixture of diastereomers (44:56 by ¹H NMR).

[α]_D = +8.4 (c = 0.25, MeOH)

¹H NMR (acetone-*d*₆); Mixture (44:56) δ 9.09 (d, 1H, J = 8.8 Hz, ArH5), 9.04* (d, 1H, J = 8.8 Hz, ArH5), 8.99 (d, 2H, J = 9.0 Hz, ArH4, major + minor), 8.58* (s, 1H, ArH1), 8.54

^ξ Contains traces of a plasticiser impurity from the ethyl acetate used during extraction.

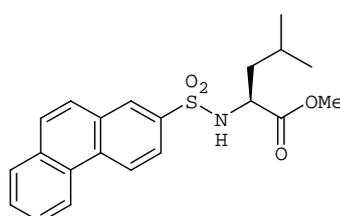
(s, 1H, Ar**HI**), 8.17-8.05 (m, 8H, Ar**H3**, Ar**H8**, Ar**H9**, Ar**HI0**, major + minor), 7.87 (m, 4H, Ar**H6**, Ar**H7**, major + minor), 7.54 (m, 2H, TFMC **NH**, major + minor), 7.34-7.04 (m, 10H, *o/m/p*-Ph, major + minor), 6.72* (d, 1H, J = 8.4 Hz, Val**NH**), 6.52 (d, 1H, J = 9.1 Hz, Val**NH**), 5.83* (br s, 1H, **OH**), 5.51 (br s, 1H, **OH**), 4.42 (m, 1H, TFMC α -**H**), 4.32* (m, 1H, TFMC α -**H**), 4.01* (m, 1H, TFMC β -**H**), 3.88 (m, 2H, Val α -**H**, major + minor), 3.75 (m, 1H, TFMC β -**H**), 3.07 (m, 2H, Ph**CH_a**, major + minor), 2.86 (m, 2H, Ph**CH_b**, major + minor), 2.05* (m, 1H, Val**CH**), 1.84 (m, 1H, Val**CH**), 0.90* (d, 3H, J = 6.8 Hz, Val**CH₃**), 0.81 (m, 6H, Val**CH₃**, major + minor), 0.52 (d, 3H, J = 6.8 Hz, Val**CH₃**).

¹³C NMR (acetone-*d*₆, 75 MHz); Mixture δ 170.7* (**CO**), 170.2 (**CO**), 138.1, 137.5*, 133.2, 133.2*, 132.8*, 132.7, 131.5, 129.7, 129.4, 129.3*, 129.0, 129.0*, 128.8*, 128.8, 128.4, 128.3, 128.2 (x 2), 128.0, 127.6*, 127.5, 127.0*, 126.9, 126.5, 126.4*, 124.2, 124.1, 123.7*, 123.7, 61.9*, 61.7, 51.1*, 50.6, 34.6*, 34.3, 31.7, 31.5*, 19.1, 19.0*, 16.4*, 16.2. The quartets for the β -C and CF₃ carbons could not be accurately measured over the baseline noise.

¹⁹F NMR (acetone-*d*₆, 282 MHz); Mixture (35:65) δ -74.1* (d, 3F, J = 7.6 Hz), -74.6 (d, 3F, J = 7.8 Hz).

HRMS (EI) 558.1787 (M⁺); C₂₉H₂₉F₃N₂O₄S requires 558.1800.

4-Methyl-2S-(phenanthrene-2-sulfonylamino)pentanoic acid methyl ester (**3.9**)



Sulfonyl chloride **3.3** (159 mg, 0.58 mmol) and *L*-leucine methyl ester hydrochloride (115 mg, 0.63 mmol) were coupled together utilising Hünigs base (0.22 mL, 1.26 mmol) following General Method C to give the ester **3.9** as a fluffy yellow solid (222 mg, quantitative). An analytical sample was obtained by recrystallisation from aqueous MeOH affording pale yellow fluffy needles.

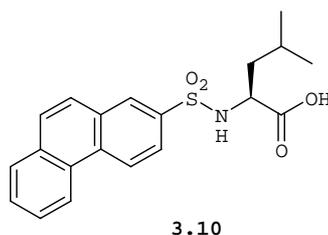
mp 187-189 °C.

^1H NMR (CDCl_3 , 500 MHz); δ 8.79 (d, 1H, $J = 8.8$ Hz, ArH4), 8.71 (d, 1H, $J = 8.1$ Hz, ArH5), 8.41 (d, 1H, $J = 1.9$ Hz, ArH1), 8.02 (dd, 1H, $J = 1.9, 8.8$ Hz, ArH3), 7.95 (d, 1H, $J = 7.4$ Hz, ArH8), 7.87 (d, 1H, $J = 8.9$ Hz, ArH9), 7.81 (d, 1H, $J = 8.9$ Hz, ArH10), 7.72 (m, 2H, ArH6, ArH7), 5.13 (d, 1H, $J = 10.1$ Hz, NH), 4.05 (m, 1H, $\alpha\text{-H}$), 3.21 (s, 3H, OCH₃), 1.81 (m, 1H, LeuCH), 1.50 (m, 2H, LeuCH₂), 0.91 (d, 3H, $J = 7.0$ Hz, LeuCH₃), 0.89 (d, 3H, $J = 6.8$ Hz, LeuCH₃).

^{13}C NMR (CDCl_3 , 75 MHz); δ 172.6 (CO), 137.0 (ArC2), 133.0, 132.9, 131.3, 129.4, 128.9 (x 2), 128.4, 128.1, 127.4, 126.7, 123.9, 123.7, 123.3, 54.5 ($\alpha\text{-C}$), 52.2 (OCH₃), 42.4 (LeuCH), 24.3 (LeuCH₂), 22.7 (LeuCH₃), 21.4 (LeuCH₃).

HRMS (EI) 385.1358 (M^+); $\text{C}_{21}\text{H}_{23}\text{NO}_4\text{S}$ requires 385.1348.

4-Methyl-2S-(phenanthrene-2-sulfonylamino)pentanoic acid (3.10)



Methyl ester **3.9** (136 mg, 0.35 mmol) was treated with K_2CO_3 (74 mg, 0.54 mmol) in MeOH/ H_2O for 3 days following General Method H to give the free acid **3.10** as a white solid. Recrystallisation of **3.10** from aqueous MeOH gave a white crystalline solid (117 mg, 93 %).^ξ

mp 157-162 °C.

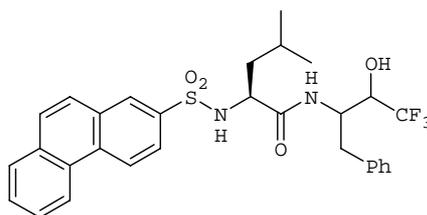
^1H NMR (CDCl_3 , 500 MHz); δ 8.73 (d, 1H, $J = 8.8$ Hz, ArH4), 8.68 (d, 1H, $J = 7.9$ Hz, ArH5), 8.37 (s, 1H, ArH1), 7.95 (t, 2H, $J = 8.3$ Hz, ArH3, ArH8), 7.85 (d, 1H, $J = 8.9$ Hz, ArH9), 7.77 (d, 1H, $J = 8.8$ Hz, ArH10), 7.72 (m, 2H, ArH6, ArH7), 4.93 (d, 1H, $J = 9.9$ Hz, NH), 3.94 (m, 1H, $\alpha\text{-H}$), 1.73 (m, 1H, LeuCH), 1.31 (m, 2H, LeuCH₂), 0.85 (d, 3H, $J = 6.6$, LeuCH₃), 0.79 (d, 3H, $J = 6.5$, LeuCH₃).

^ξ Contains traces of a plasticiser impurity from the ethyl acetate used during extraction.

^{13}C NMR (acetone- d_6 , 75 MHz); δ 172.8 (CO), 139.3 (ArC2), 133.2, 132.7, 131.6, 129.7, 129.0, 128.7, 128.2, 128.0, 127.6, 127.2, 124.3, 124.2, 123.7, 54.6 (α -C), 42.0 (LeuCH), 24.4 (LeuCH₂), 22.4 (LeuCH₃), 20.9 (LeuCH₃).

HRMS (ES) 372.1268 (MH⁺); C₂₀H₂₂NO₄S requires 372.1270.

4-Methyl-2S-(phenanthrene-2-sulfonylamino)pentanoic acid (1S*,2R*)-(1-benzyl-3,3,3-trifluoro-2-hydroxypropyl)amide (3.11)



3.11

Phenanthrene **3.10** (100 mg, 0.27 mmol), **2.6** (72 mg, 0.28 mmol), EDCI (67 mg, 0.35 mmol), HOBt (55 mg, 0.41 mmol) and Hünigs base (0.10 mL, 0.57 mmol) were reacted together following General Method D to give the dipeptide as a yellowish solid. Flash chromatography (1:9 ethyl acetate/dichloromethane) of the crude trifluoromethyl carbinol, followed by recrystallisation from aqueous MeOH afforded **3.11** as a white powder (127 mg, 80 %) comprising a mixture of diastereomers (45:55 by ^1H NMR).

$[\alpha]_{\text{D}} = -29.8$ ($c = 0.51$, MeOH)

^1H NMR (acetone- d_6); Mixture (45:55) δ 9.10 (d, 1H, $J = 8.8$ Hz, ArH5), 9.07* (d, 1H, $J = 8.8$ Hz, ArH5), 8.99 (d, 2H, $J = 8.0$ Hz, ArH5, major + minor), 8.59* (s, 1H, ArH1), 8.56 (s, 1H, ArH1), 8.16-8.07 (m, 8H, ArH3, ArH8, ArH9, ArH10, major + minor), 7.91-7.84 (m, 4H, ArH6, ArH7, major + minor), 7.61 (m, 2H, TFMC NH, major + minor), 7.37-7.16 (m, 10H, *o/m/p*-Ph, major + minor), 6.95* (d, 1H, $J = 8.0$ Hz, LeuNH), 6.81 (d, 1H, $J = 8.7$ Hz, LeuNH), 5.86* (br s, 1H, OH), 5.65 (br s, 1H, OH), 4.40 (m, 1H, TFMC α -H), 4.30* (m, 1H, TFMC α -H), 4.04* (m, 1H, TFMC β -H), 3.98 (m, 2H, Leu α -H, major + minor), 3.90 (m, 1H, TFMC β -H), 3.10-2.90 (m, 4H, PhCH_a + PhCH_b, major + minor), 1.68* (m, 1H, LeuCH), 1.52 (m, 1H, LeuCH), 1.39* (m, 2H, LeuCH₂), 1.17 (m, 2H, LeuCH₂), 0.83 (d, 3H, $J = 6.7$ Hz, LeuCH₃), 0.78 (d, 3H, $J = 6.7$ Hz, LeuCH₃), 0.69 (d, 3H, $J = 6.5$ Hz, LeuCH₃), 0.67 (d, 3H, $J = 6.5$ Hz, LeuCH₃).

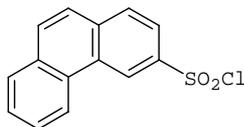
^{13}C NMR (acetone- d_6 , 75 MHz); Mixture (45:55) δ 171.9* (CO), 171.4 (CO), 139.3, 138.8*, 138.1, 137.8*, 133.2, 133.2*, 132.8*, 132.7, 131.5, 131.5*, 129.7, 129.6 (x 2), 129.5, 129.0, 129.0*, 128.9*, 128.8, 128.4, 128.4*, 128.2 (x 3), 128.0, 127.6*, 127.5, 127.0*, 126.9, 126.5, 126.5*, 124.3, 124.1 (x 3), 123.7*, 123.6, 71.5 (q, $J = 29.0$ Hz, $\beta\text{-C}$), 70.9* (q, $J = 28.9$ Hz, $\beta\text{-C}$), 55.6*, 55.4, 51.2*, 50.5, 42.2, 42.2*, 34.6*, 34.3, 24.1, 24.1*, 22.6, 22.6*, 20.9, 20.8*. The two CF_3 quartets could not be accurately determined as the signals were buried amongst the aromatic carbon peaks.

^{19}F NMR (acetone- d_6 , 282 MHz); Mixture (46:54) δ -74.2* (d, 3F, $J = 7.4$ Hz), -74.5 (d, 3F, $J = 7.7$ Hz).

HRMS (EI) 572.1957 (M^+); $\text{C}_{30}\text{H}_{31}\text{F}_3\text{N}_2\text{O}_4\text{S}$ requires 572.1967.

7.3.2 Phenanthrene-3-sulphonyl derivatives

Phenanthrene-3-sulfonyl chloride (3.12)



Potassium sulfonate **3.2** (2.96 g, 10.0 mmol) was treated with POCl_3 (3.80 mL, 41.0 mmol) following General Method A to give the desired product as a dull yellow solid. The crude sulfonyl chloride was subjected to flash chromatography (1:4 ethyl acetate/ petroleum ether) to afford **3.12** as a pale yellow powder (2.71 g, 98 %). An analytical sample was obtained by recrystallisation from aqueous acetone.

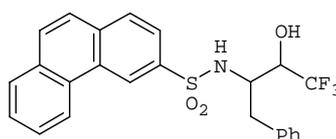
mp 110-111 °C

^1H NMR (CDCl_3 , 500 MHz); δ 9.27 (d, 1H, $J = 1.4$ Hz, ArH4), 8.64 (d, 1H, $J = 8.1$ Hz, ArH5), 8.11 (dd, 1H, $J = 1.8, 8.5$ Hz, ArH2), 8.02 (d, 1H, $J = 8.5$ Hz, ArH3), 7.92 (d, 1H, $J = 8.6$ Hz, ArH8), 7.95 (d, 1H, $J = 8.8$ Hz, ArH9), 7.77 (d, 1H, $J = 8.8$ Hz, ArH10), 7.70 (m, 2H, ArH6, ArH7).

^{13}C NMR (CDCl_3 , 75 MHz); δ 141.4, 135.6, 132.2, 131.7, 130.2, 129.8, 129.7, 128.9, 128.1, 128.0, 125.6, 122.9, 122.7 (x 2).

HRMS (EI) 276.0008 (M^+); $C_{14}H_9ClO_2S$ requires 276.0012.

Phenanthrene-3-sulfonic acid ($1S^*,2R^*$)-(1-benzyl-3,3,3-trifluoro-2-hydroxypropyl) amide (3.13**)**



3.13

Sulfonyl chloride **3.12** (101 mg, 0.37 mmol) and **2.6** (98 mg, 0.38 mmol) were coupled together utilising Hünigs base (0.15 mL, 0.86 mmol) following General Method C to give the desired product as a pale yellow solid. Recrystallisation of the crude trifluoromethyl carbinol from aqueous EtOH gave **3.13** as a very pale yellow powder (165 mg, quantitative, single diastereomer).

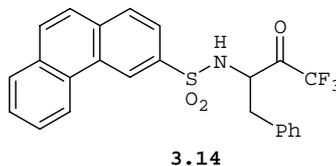
mp 167-169 °C.

1H NMR (acetone- d_6 , 500 MHz); δ 9.01 (s, 1H, Ar**H4**), 8.80 (d, 1H, $J = 8.2$ Hz, Ar**H5**), 8.18 (d, 1H, $J = 7.8$ Hz, Ar**H8**), 8.13 (d, 1H, $J = 8.8$ Hz, Ar**H9**), 8.00 (d, 2H, $J = 8.8$ Hz, Ar**H1**, Ar**H10**), 7.89 (m, 2H, Ar**H6**, Ar**H7**), 7.74 (dd, 1H, $J = 1.2, 8.3$ Hz, Ar**H2**), 7.06 (d, 1H, $J = 8.0$ Hz, **NH**), 7.02 (d, 2H, $J = 7.5$ Hz, *o*-Ph), 6.73 (t, 3H, $J = 7.6$ Hz, *m*-Ph), 6.59 (t, 1H, $J = 7.4$ Hz, *p*-Ph), 5.99 (br s, 1H, **OH**), 4.57 (m, 1H, β -**H**), 3.99 (m, 1H, α -**H**), 3.02 (m, 1H, Ph**CH_a**), 2.90 (m, 1H, Ph**CH_b**).

^{13}C NMR (acetone- d_6 , 75 MHz); δ 138.7, 137.3, 134.2, 132.6, 130.4, 129.9, 129.7 (x 2), 129.0, 128.0, 127.7, 127.6 (x 2), 126.4, 126.0, 125.4 (q, $J = 282.8$ Hz, CF_3), 123.6, 123.1, 122.0, 72.9 (q, $J = 28.7$ Hz, β -**C**), 56.0 (α -**C**), 34.3 (Ph**CH₂**).

^{19}F NMR (acetone- d_6 , 282 MHz); δ -74.2 (d, 3F, $J = 7.8$ Hz).

HRMS (EI) 459.1113 (M^+); $C_{24}H_{20}F_3NO_3S$ requires 459.1116.

Phenanthrene-3-sulfonic acid (1*S-benzyl-3,3,3-trifluoro-2-oxopropyl)amide (3.14)**

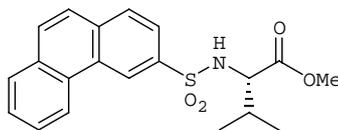
Phenanthrene **3.13** (23 mg, 50 μmol) was oxidised with Dess-Martin periodinane (78 mg, 190 μmol) following General Method F. The crude ketone was recrystallised from aqueous MeOH to give **3.14** as pale brown needles (11 mg, 48 %) comprising a racemic mixture of hydrate and ketone (9:1 by ^1H NMR). One drop of D_2O was added to the NMR sample to give the hydrate exclusively.

^1H NMR (acetone- d_6 , 500 MHz); Hydrate δ 8.87 (s, 1H, Ar**H4**), 8.75 (d, 1H, $J = 8.2$ Hz, Ar**H5**), 8.14 (d, 1H, $J = 7.8$ Hz, Ar**H8**), 8.09 (d, $J = 8.8$ Hz, Ar**H9**), 7.95 (m, 2H, Ar**H1**, Ar**H10**), 7.85 (m, 2H, Ar**H6**, Ar**H7**), 7.75 (dd, 1H, $J = 1.7, 8.3$ Hz, Ar**H2**), 6.97 (d, 2H, $J = 7.3$ Hz, *o*-Ph), 6.51 (t, 2H, $J = 7.7$ Hz, *m*-Ph), 6.26 (t, 1H, $J = 7.4$ Hz, *p*-Ph), 4.10 (dd, 1H, $J = 2.3, 10.8$ Hz, α -H), 3.26 (dd, 1H, $J = 2.6, 14.4$ Hz, Ph**CH_a**), 2.72 (dd, 1H, $J = 10.9, 14.4$ Hz, Ph**CH_b**).

^{13}C NMR (acetone- d_6 , 75 MHz); Hydrate δ 138.9, 137.6, 134.2, 132.5, 130.4, 129.9 (x 2), 129.7, 129.5, 128.9, 127.7, 127.6, 127.5, 126.4, 125.6, 124.1 (q, $J = 289.2$ Hz, CF_3), 123.6, 123.2, 121.9, 61.1 (α -C), 35.6 (Ph**CH₂**). The quartet due to the β -C could not be measured over the baseline noise.

^{19}F NMR (acetone- d_6 , 282 MHz); Hydrate δ -78.8 (s, 3F).

HRMS (ES) 458.1031 (MH^+); $\text{C}_{24}\text{H}_{19}\text{F}_3\text{NO}_3\text{S}$ requires 458.1038.

3-Methyl-2S-(phenanthrene-3-sulfonylamino)butyric acid methyl ester (3.15)**3.15**

Sulfonyl chloride **3.12** (150 mg, 0.54 mmol) and *L*-valine methyl ester hydrochloride (107 mg, 0.64 mmol) were coupled together utilising Hünigs base (0.22 mL, 1.26 mmol) following General Method C to give the desired ester **3.15** as a white solid. Recrystallisation of the ester from aqueous MeOH afforded a white crystalline solid (175 mg, 88 %).

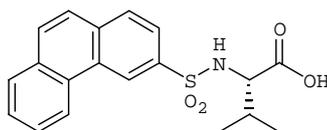
$[\alpha]_D = -50.7$ ($c = 0.68$, MeOH)

mp 131-132 °C.

^1H NMR (CDCl_3 , 500 MHz); δ 9.16 (s, 1H, Ar**H4**), 8.74 (d, 1H, $J = 8.3$ Hz, Ar**H5**), 7.98 (m, 2H, Ar**H1**, Ar**H2**), 7.94 (d, 1H, $J = 7.8$ Hz, Ar**H8**), 7.86 (d, 1H, $J = 8.8$ Hz, Ar**H9**), 7.73 (d, 1H, $J = 8.8$ Hz, Ar**H10**), 7.72 (m, 2H, Ar**H6**, Ar**H7**), 5.26 (d, 1H, $J = 10.2$ Hz, **NH**), 3.87 (m, 1H, α -**H**), 3.18 (s, 3H, **OCH**₃), 2.04 (m, 1H, Val**CH**), 0.97 (d, 3H, $J = 6.8$ Hz, Val**CH**₃), 0.90 (d, 3H, $J = 6.8$ Hz, Val**CH**₃).

^{13}C NMR (CDCl_3 , 75 MHz); δ 171.7 (**CO**), 137.0 (Ar**C3**), 134.2, 132.1, 130.1, 129.9, 129.7, 129.5, 128.7, 127.6, 127.5, 125.9, 123.7, 122.8 (x 2), 61.2 (α -**C**), 52.0 (**OCH**₃), 31.6 (Val**CH**), 18.9 (Val**CH**₃), 17.5 (Val**CH**₃).

HRMS (EI) 371.1200 (M^+); $\text{C}_{20}\text{H}_{21}\text{NO}_4\text{S}$ requires 371.1191.

3-Methyl-2S-(phenanthrene-3-sulfonylamino)butyric acid (3.16)**3.16**

The methyl ester **3.15** (171 mg, 0.46 mmol) was treated with K_2CO_3 (115 mg, 0.83 mmol) in MeOH/ H_2O for 2 days following General Method H to give free acid **3.16** as a pale

yellow solid. Recrystallisation of **3.16** from aqueous EtOH gave a white powder (160 mg, 97 %).

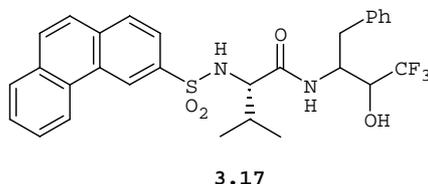
mp 91-92 °C.

^1H NMR (CDCl_3 , 500 MHz); δ 9.08 (s, 1H, Ar**H4**), 8.65 (d, 1H, $J = 8.2$ Hz, Ar**H5**), 7.92-7.86 (m, 2H, Ar**H1**, Ar**H2**), 7.82 (d, 1H, $J = 7.9$ Hz, Ar**H8**), 7.77 (d, 1H, $J = 8.8$ Hz, Ar**H9**), 7.64 (m, 2H, Ar**H10**, Ar**H6**), 7.56 (t, 1H, Ar**H7**), 5.95 (d, 1H, $J = 9.6$ Hz, **NH**), 3.69 (m, 1H, α -**H**), 2.00 (m, 1H, Val**CH**), 0.90 (d, 3H, $J = 6.7$ Hz, Val**CH**₃), 0.78 (d, 3H, $J = 6.7$ Hz, Val**CH**₃).

^{13}C NMR (CDCl_3 , 75 MHz); δ 172.6 (**CO**), 137.5, 133.8, 131.8, 129.9, 129.5, 129.4, 129.1, 128.4, 127.3, 127.3, 125.8, 123.9, 122.8, 122.4, 60.8 (α -**C**), 31.0 (Val**CH**), 18.9 (Val**CH**₃), 17.0 (Val**CH**₃).

HRMS (ES) 358.1109 (MH^+); $\text{C}_{19}\text{H}_{20}\text{NO}_4\text{S}$ requires 358.1113.

(1*S,2*R**)-N-(1-Benzyl-3,3,3-trifluoro-2-hydroxypropyl)-3-methyl-2*S*-(phenanthrene-3-sulfonylamino)butyramide (3.17)**



Phenanthrene **3.16** (100 mg, 0.28 mmol), **2.6** (75 mg, 0.29 mmol), EDCI (70 mg, 0.36 mmol), HOBt (57 mg, 0.42 mmol) and Hünig's base (0.11 mL, 0.63 mmol) were reacted together following General Method D to give the dipeptide as a yellowish solid. Flash chromatography (1:9 ethyl acetate/dichloromethane) of the crude trifluoromethyl carbinol, followed by recrystallisation from aqueous MeOH afforded **3.17** as a white clumping solid (113 mg, 70 %) comprising a mixture of diastereomers (1:1 by ^1H NMR).

$[\alpha]_{\text{D}} = +73.3$ ($c = 0.54$, MeOH)

^1H NMR (acetone- d_6 , 500 MHz); Mixture (1:1) δ 9.39 (s, 1H, Ar**H4**), 9.37 (s, 1H, Ar**H4**), 8.99 (d, 1H, $J = 9.2$ Hz, Ar**H5**), 8.97 (d, 1H, $J = 9.3$ Hz, Ar**H5**), 8.25-7.83 (m, 14H, 2 x (Ar**H1**, Ar**H2**, Ar**H6**, Ar**H7**, Ar**H8**, Ar**H9**, Ar**H10**)), 7.52 (m, 2H, 2 x TFMC **NH**), 7.34-7.26 (m, 5H, Ph), 7.07 (m, 3H, Ph), 6.95 (d, 2H, $J = 7.8$ Hz, Ph), 6.82 (d, 1H, $J = 8.5$ Hz, Val**NH**), 6.66 (d, 1H, $J = 9.1$ Hz, Val**NH**), 5.82 (br s, 1H, **OH**), 5.41 (br s, 1H, **OH**), 4.42

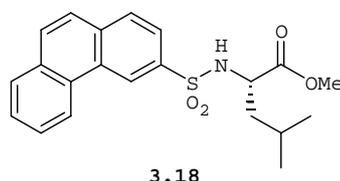
(m, 1H, TFMC α -H), 4.34 (m, 1H, TFMC α -H), 4.00 (m, 1H, TFMC β -H), 3.93 (dd, 1H, J = 4.6, 8.5 Hz, Val α -H), 3.88 (dd, 1H, J = 4.6, 9.1 Hz, Val α -H), 3.69 (m, 1H, TFMC β -H), 3.07 (br m, 6H, 2 x PhCH_a, 2 x H₂O), 2.80 (m, 2H, 2 x PhCH_b), 2.06 (m, 1H, ValCH), 1.84 (m, 1H, ValCH), 0.93 (d, 3H, J = 6.8 Hz, ValCH₃), 0.85 (d, 3H, J = 6.8 Hz, ValCH₃), 0.82 (d, 3H, J = 6.8 Hz, ValCH₃), 0.54 (d, 3H, J = 6.8 Hz, ValCH₃).

¹³C NMR (acetone-*d*₆, 75 MHz); δ 170.8, 170.3, 139.2, 139.0, 138.1, 137.3, 134.4, 134.3, 132.7, 132.6, 130.4, 130.3, 130.0, 129.9, 129.8 (x 2), 129.7, 129.5, 129.4, 129.3, 129.1 (x 2), 128.4, 128.2, 127.9, 127.8, 127.7 (x 2), 126.5, 126.4 (x 2), 126.3, 124.2 (x 2), 123.2 (x 2), 122.6 (x 2), 71.6 (q, J = 29.1 Hz, β -C), 70.5 (q, J = 28.9 Hz, β -C), 61.8, 61.7, 50.9, 50.5, 34.5, 34.2, 31.7, 31.6, 19.1 (x 2), 16.4, 16.2. The peaks for the two CF₃ quartets could not be identified as they were obscured by other aromatic carbon signals.

¹⁹F NMR (acetone-*d*₆, 282 MHz); Mixture (47:53) δ -74.1 (d, 3F, J = 7.5 Hz), -74.7 (d, 3F, J = 7.7 Hz).

HRMS (EI) 558.1781 (M⁺); C₂₉H₂₉F₃N₂O₄S requires 558.1800.

4-Methyl-2S-(phenanthrene-3-sulfonylamino)pentanoic acid methyl ester (**3.18**)



Sulfonyl chloride **3.12** (153 mg, 0.55 mmol) and *L*-leucine methyl ester hydrochloride (115 mg, 0.63 mmol) were coupled together utilising Hünigs base (0.22 mL, 1.26 mmol) following General Method C to give the desired product as a white solid. Recrystallisation of the ester from aqueous MeOH afforded **3.18** as a fluffy white solid (212 mg, quantitative).

$[\alpha]_D = -66.8$ (c = 0.85, MeOH)

mp 91-92 °C.

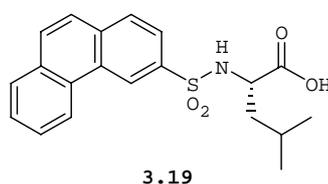
¹H NMR (CDCl₃, 500 MHz); δ 9.17 (s, 1H, ArH₄), 8.74 (d, 1H, J = 8.3 Hz, ArH₅), 7.98 (m, 2H, ArH₁, ArH₂), 7.94 (d, 1H, J = 7.8 Hz, ArH₈), 7.90 (d, 1H, J = 8.8 Hz, ArH₉), 7.77 (d, 1H, J = 8.8 Hz, ArH₁₀), 7.75 (m, 1H, ArH₆), 7.69 (t, 1H, J = 7.4 Hz, ArH₇),

5.18 (d, 1H, $J = 9.8$ Hz, **NH**), 4.08 (m, 1H, α -**H**), 3.16 (s, 3H, **OCH₃**), 1.82 (m, 1H, **LeuCH**), 1.54 (m, 2H, **LeuCH₂**), 0.90 (t, 6H, $J = 6.3$ Hz, **LeuCH₃**).

¹³C NMR (CDCl₃, 75 MHz); δ 172.6 (**CO**), 137.0, 134.3, 132.2, 130.1, 130.0, 129.7, 129.6, 128.8, 127.6, 127.5, 125.9, 123.7, 122.9, 122.8, 54.5 (α -**C**), 52.1 (**OCH₃**), 42.3 (**LeuCH**), 24.3 (**LeuCH₂**), 22.7 (**LeuCH₃**), 21.4 (**LeuCH₃**).

HRMS (EI) 385.1349 (M^+); C₂₁H₂₃NO₄S requires 385.1348.

4-Methyl-2S-(phenanthrene-3-sulfonylamino)pentanoic acid (**3.19**)



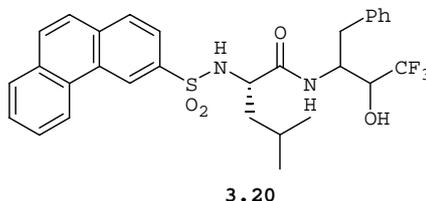
The methyl ester **3.18** (207 mg, 0.54 mmol) was treated with K₂CO₃ (153 mg, 1.11 mmol) in MeOH/H₂O for 24 h following General Method H to afford a white solid containing minor traces of the ester. Recrystallisation of the free acid from aqueous MeOH gave pure acid **3.19** as a white crystalline solid (179 mg, 90 %).

¹H NMR (CDCl₃, 500 MHz); δ 9.13 (s, 1H, **ArH₄**), 8.69 (d, 1H $J = 7.8$ Hz, **ArH₅**), 7.98-7.90 (m, 2H, **ArH₂**, **ArH₈**), 7.85 (d, 1H, $J = 7.9$ Hz, **ArH₁₁**), 7.80 (d, 1H, $J = 8.9$ Hz, **ArH₉**), 7.69 (d, 1H, $J = 8.9$ Hz, **ArH₁₀**), 7.67 (m, 1H, **ArH₆**), 7.60 (m, 1H, **ArH₇**), 5.90 (d, 1H, $J = 9.9$ Hz, **NH**), 3.93 (m, 1H, α -**H**), 1.81 (m, 1H, **LeuCH**), 1.48 (m, 2H, **LeuCH₂**), 0.83 (dd, 6H, $J = 6.6, 10.4$ Hz, 2 x **LeuCH₃**).

¹³C NMR (CDCl₃, 75 MHz); δ 173.9 (**CO**), 137.5, 134.0, 131.9, 130.0, 129.6, 129.5, 129.3, 128.5, 127.4, 127.4, 125.9, 123.9, 122.9, 122.5, 54.3 (α -**C**), 42.1 (**LeuCH**), 24.1 (**LeuCH₂**), 22.7 (**LeuCH₃**), 21.2 (**LeuCH₃**).

HRMS (ES) 372.1271 (MH^+); C₂₀H₂₂NO₄S requires 372.1270.

4-Methyl-2S-(phenanthrene-3-sulfonylamino)pentanoic acid (1S*,2R*)-(1-benzyl-3,3,3-trifluoro-2-hydroxypropyl)amide (3.20)



Phenanthrene **3.19** (60 mg, 0.16 mmol), **2.6** (43 mg, 0.17 mmol), EDCI (40 mg, 0.21 mmol), HOBt (33 mg, 0.24 mmol) and Hünigs base (60 μ L, 0.34 mmol) were reacted together following General Method D to give the dipeptide as a yellowish solid. Flash chromatography (1:9 ethyl acetate/dichloromethane) of the crude trifluoromethyl carbinol, followed by recrystallisation from aqueous EtOH afforded **3.20** as fine white needles (74 mg, 78 %) comprising a mixture of diastereomers (1:1 by ^1H NMR).

$[\alpha]_{\text{D}} = +33.2$ ($c = 0.43$, MeOH)

^1H NMR (acetone- d_6 , 500 MHz); Mixture (1:1) δ 9.40 (s, 1H, Ar**H4**), 9.39 (s, 1H, Ar**H4**), 8.98 (d, 1H, $J = 8.1$ Hz, Ar**H5**), 8.97 (d, 1H, $J = 8.1$ Hz, Ar**H5**), 8.27-8.02 (m, 10H, 2 x (Ar**H1**, Ar**H2**, Ar**H8**, Ar**H9**, Ar**H10**)), 7.89 (m, 4H, 2 x (Ar**H6**, Ar**H7**)), 7.62 (d, 1H, $J = 8.4$ Hz, TFMC **NH**), 7.58 (d, 1H, $J = 8.8$ Hz, TFMC **NH**), 7.31 (m, 5H, Ph), 7.21 (m, 3H, Ph), 7.11 (d, 2H, $J = 7.5$ Hz, Ph), 7.01 (d, 1H, $J = 8.1$ Hz, Leu**NH**), 6.89 (d, 1H, $J = 8.7$ Hz, Leu**NH**), 4.40 (m, 1H, TFMC α -**H**), 4.30 (m, 1H, TFMC α -**H**), 4.00 (m, 3H, 2 x Leu α -**H**, TFMC β -**H**), 3.84 (m, 1H, TFMC β -**H**), 3.07 (dd, 2H, $J = 3.5, 14.3$ Hz, 2 x Ph**CH_a**), 2.90 (m, 4H, 2 x Ph**CH_b**, **H₂O**), 1.71 (m, 1H, Leu**CH**), 1.52 (m, 1H, Leu**CH**), 1.41 (m, 2H, Leu**CH₂**), 1.17 (m, 2H, Leu**CH₂**), 0.83 (d, 3H, $J = 6.7$ Hz, Leu**CH₃**), 0.77 (d, 3H, $J = 6.6$ Hz, Leu**CH₃**), 0.68 (dd, 6H, $J = 3.7, 6.5$ Hz, Leu**CH₃**).

^{13}C NMR (acetone- d_6 , 75 MHz); Mixture (1:1) δ 171.9 (**CO**), 171.5 (**CO**), 139.4, 139.0, 138.2, 137.7, 134.4, 134.3, 132.7 (x 2), 130.3 (x 2), 130.0, 129.9, 129.8 (x 2), 129.6 (x 2), 129.5, 129.1 (x 2), 128.4, 128.3, 127.9 (x 2), 127.8 (x 2), 126.5, 126 (x 2), 126.3, 124.2 (x 2), 123.2, 123.1, 122.6, 71.5 (q, $J = 29.2$ Hz, β -**C**), 70.8 (q, $J = 29.0$ Hz, β -**C**), 55.6, 55.4, 51.1, 50.5, 42.3 (x 2), 34.5, 34.2, 24.1 (x 2), 22.6 (x 2), 21.0, 20.8.

^{19}F NMR (acetone- d_6 , 282 MHz); Mixture (1:1) δ -74.2 (d, 3F, $J = 7.5$ Hz), -74.6 (d, 3F, $J = 7.8$ Hz).

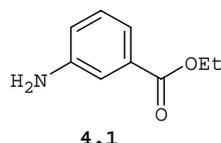
HRMS (EI) 572.1963 (M^+); $\text{C}_{30}\text{H}_{31}\text{F}_3\text{N}_2\text{O}_4\text{S}$ requires 572.1957.

7.4 Experimental work outlined in chapter four

Synthesis of *E*-azobenzene derivatives

7.4.1 *E*-Azobenzene-3-carbonyl derivatives⁶

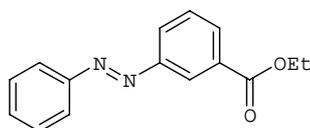
Ethyl-3-aminobenzoate (4.1)



3-Aminobenzoic acid (10.3 g, 75.0 mmol) was added to a stirred solution of dry EtOH saturated with gaseous HCl (100 mL) and heated at reflux for 36 h. Water (100 mL) was added to the cooled solution and treated with solid K₂CO₃ until basic to litmus. The reaction mixture was further cooled by the addition of ice, which induced the ester to separate out as an oil. The oil was taken up in dichloromethane and the aqueous layer extracted with dichloromethane (4 x 25 mL). The combined organic extracts were washed with brine, dried over MgSO₄ and concentrated *in vacuo* to give **4.1** as a brown oil (10.3 g, 83 %).

¹H NMR (CDCl₃, 500 MHz); δ 7.42 (d, 1H, J = 7.8 Hz, Ar**H4**), 7.35 (s, 1H, Ar**H2**), 7.19 (t, 1H, J = 7.8 Hz, Ar**H5**), 6.83 (d, 1H, J = 7.8 Hz, Ar**H6**), 4.34 (q, 2H, J = 7.1 Hz, CH₃CH₂), 3.84 (b, 2H, NH₂), 1.36 (t, 3H, J = 7.2 Hz, CH₃CH₂).

¹³C NMR (CDCl₃, 75 MHz); 166.7 (CO), 146.5 (Ar**C1**), 131.2 (Ar**C3**), 129.0 (Ar**C5**), 119.4, 119.1, 115.5 (Ar**C2**), 60.7 (CH₂), 14.2 (CH₃).

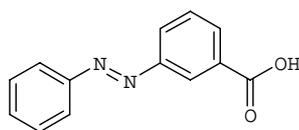
***E*-3-Phenylazobenzoic acid ethyl ester (4.2)**

4.2

Nitrosobenzene (5.00 g, 46.7 mmol) and ethyl-3-aminobenzoate **4.1** (7.71 g, 46.7 mmol) in glacial acetic acid (187 mL) were condensed following General Method K to give an almost black oil. Flash chromatography of the crude azobenzene on alumina (1:1 dichloromethane/petroleum ether) removed most of the black impurities affording an orange oil. Further purification by flash chromatography on silica (1:9 ethyl acetate/petroleum ether) gave **4.2** as an orange/red oil (8.80 g, 74 %).

^1H NMR (CDCl_3 , 500 MHz); δ 8.57 (s, 1H), 8.15 (d, 1H, $J = 7.7$ Hz), 8.08 (d, 1H, $J = 7.9$ Hz), 7.94 (d, 2H, $J = 7.1$ Hz), 7.57 (t, 1H, $J = 7.8$ Hz), 7.51 (m, 3H), 4.42 (q, 2H, $J = 7.1$ Hz, CH_3CH_2), 1.42 (t, 3H, $J = 7.1$ Hz, CH_3CH_2).

^{13}C NMR (CDCl_3 , 75 MHz); δ 165.9 (CO_2Et), 152.5, 152.4, 131.6 ($\text{ArC4}'$), 131.3 (ArC4), 129.1 (x 2), 126.5, 124.2, 123.0, 61.2 (CH_3CH_2), 14.3 (CH_3CH_2).

***E*-3-Phenylazobenzoic acid (4.3)**

4.3

The ester **4.2** (7.63 g, 30.0 mmol) and KOH (3.37 g, 60.0 mmol) were suspended in a solution of EtOH/ H_2O (4:1, 300mL) and heated at reflux for 12 h. The reaction mixture was cooled to rt and the solvent removed *in vacuo*. The orange residue was suspended in water and acidified with 10 % aqueous H_2SO_4 . The precipitated free acid was taken up in ethyl acetate, washed with distilled water and brine, dried over MgSO_4 , filtered and concentrated *in vacuo*. The resulting solid was resuspended in water, vigorously stirred for 1 h, filtered, washed with water, and air-dried to give **4.3** as a fluffy orange solid (5.60 g,

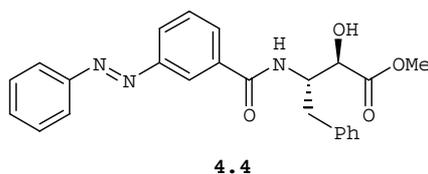
83 %). An analytical sample was obtained by recrystallisation from aqueous EtOH to give fine orange needles.

mp 169-170 °C

^1H NMR (acetone- d_6 , 500 MHz); δ 8.67 (s, 1H, ArH2), 8.32 (dd, 2H, $J = 7.6, 13.9$ Hz, ArH4, ArH6), 8.11 (dd, 2H, $J = 1.5, 8.1$ Hz, ArH2'), 7.87 (t, 1H, $J = 7.8$ Hz, ArH5), 7.76-7.70 (m, 3H, ArH3', ArH4').

^{13}C NMR (acetone- d_6 , 75 MHz); δ 166.4, 152.7, 152.6, 141.4, 132.1, 131.9, 129.9, 129.5, 127.2, 123.6, 123.1.

***E*-[2*R*-Hydroxy-4-phenyl-3*S*-(3-phenylazobenzoylamino)butyric acid methyl ester]**
(4.4)



3-Phenylazobenzoic acid **4.3** (77 mg, 0.34 mmol) and 3*S*-amino-2*R*-hydroxy-4-phenylbutyric acid methyl ester[§] (102 mg, 0.35 mmol) were coupled together utilising BOP (155 mg, 0.35 mmol) and Hünigs base (122 μL , 0.70 mmol) in dry dichloromethane (5 mL) following General Method E. The crude α -hydroxy ester was subjected to flash chromatography (1:4 ethyl acetate/dichloromethane) followed by recrystallisation from aqueous acetone to afford **4.4** as fine orange needles (93 mg, 66 %).

$[\alpha]_{\text{D}} = -113.0$ ($c = 0.39$, MeOH)

mp 141-142 °C

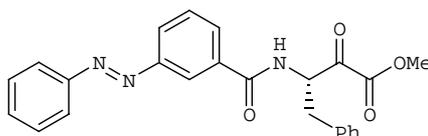
^1H NMR (CDCl_3 , 500 MHz); δ 8.18 (s, 1H, ArH2), 8.03 (d, 1H, $J = 7.8$ Hz, ArH6), 7.94 (d, 2H, $J = 6.8$ Hz, ArH2'), 7.82 (d, 1H, $J = 7.8$ Hz, ArH4), 7.58-7.50 (m, 4H, ArH3', ArH4', ArH5), 7.37-7.32 (m, 4H, *o/m*-Ph), 7.25 (t, 1H, $J = 6.9$ Hz, *p*-Ph), 6.58 (d, 1H, $J = 9.2$ Hz, NH), 4.84 (m, 1H, α -H), 4.23 (m, 1H, β -H), 3.76 (s, 3H, OCH₃), 3.44 (d, 1H, $J = 2.4$ Hz, OH), 3.12 (dd, 1H, $J = 6.7, 13.5$ Hz, PhCH_a), 3.05 (dd, 1H, $J = 9.3, 13.5$ Hz, PhCH_b).

[§] Kindly gifted by A Harvey.

^{13}C NMR (CDCl_3 , 75 MHz); δ 174.1 (CO_2Me), 166.7 (CONH), 152.2 ($\text{ArC1}'$), 137.2 (*i*-Ph), 135.2 (ArC3), 131.3 ($\text{ArC4}'$), 129.3, 129.3, 129.0, 128.7, 128.6, 126.7, 125.3, 122.9 ($\text{ArC2}'$), 121.4 (ArC2), 70.2 ($\beta\text{-C}$), 53.5 ($\alpha\text{-C}$), 52.9 (OCH_3), 37.7 (PhCH_2).

HRMS (ES) 418.1755 (MH^+); $\text{C}_{24}\text{H}_{24}\text{N}_3\text{O}_4$ requires 418.1767.

***E*-[2-Oxo-4-phenyl-3*S*-(3-phenylazobenzoylamino)butyric acid methyl ester] (4.5)**



4.5

α -Hydroxy ester **4.4** (65 mg, 156 μmol) was oxidised with TEMPO/ NaOCl over 2.5 h following General Method G. Recrystallisation of the α -keto ester from aqueous acetone gave **4.5** as fluffy orange needles (36 mg, 56 %) comprising a mixture of ketone and hydrate (45:55 by ^1H NMR). After 1 day in a solution of CDCl_3 all hydrate had reverted to the ketone form.

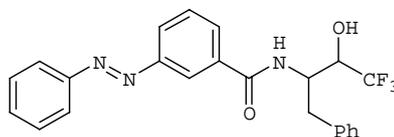
$[\alpha]_{\text{D}} = -106.8$ ($c = 0.22$, MeOH), Lit. (-53 , $c = 0.67$, acetonitrile)⁶

^1H NMR (CDCl_3 , 500 MHz); From mixture δ 8.21 (s, 1H), 8.07 (d, 1H, $J = 8.0$ Hz) 8.03* (d, 2H, $J = 6.7$ Hz), 7.94 (m, 4H), 7.84 (d, 1H, $J = 7.6$ Hz), 7.70 (d, 1H, $J = 7.7$ Hz), 7.59 (t, 1H, $J = 7.8$ Hz), 7.56-7.51 (m, 6H), 7.34-7.22 (m, 9H), 7.18 (d, 2H, $J = 7.1$ Hz), 6.72 (d, 1H, $J = 6.9$ Hz, **NH**), 6.48* (d, 1H, $J = 8.2$ Hz, **NH**), 5.66 (m, 1H, $\alpha\text{-H}$), 5.49* (s, 1H, **OH**), 4.84* (m, 1H, $\alpha\text{-H}$), 4.79* (s, 1H, **OH**), 3.91 (s, 3H, OCH_3), 3.83* (s, 3H, OCH_3), 3.41 (dd, 1H, $J = 6.2, 14.1$ Hz, PhCH_a), 3.26 (dd, 1H, $J = 6.7, 14.1$ Hz, PhCH_b), 3.14* (dd, 1H, $J = 4.4, 14.3$ Hz, PhCH_a), 2.92* (dd, 1H, $J = 10.3, 14.3$ Hz, PhCH_b).

^{13}C NMR (CDCl_3 , 75 MHz); Ketone (eqm) δ 191.5 (COCO_2Me), 166.4 (CONH), 160.6 (CO_2Me), 152.5 (ArC1), 152.4 ($\text{ArC1}'$), 134.9 (*i*-Ph), 134.3 (ArC3), 131.5 ($\text{ArC4}'$), 129.6 (ArC4), 129.4 ($\text{ArC3}'$, ArC5), 129.2 (*m*-Ph), 128.9 (*o*-Ph), 127.5 (ArC6), 126.4 (*p*-Ph), 123.0 ($\text{ArC2}'$), 121.0 (ArC2), 57.0 ($\alpha\text{-C}$), 53.4 (OCH_3), 36.9 (PhCH_2).

HRMS (ES) 416.1608 (MH^+); $\text{C}_{24}\text{H}_{22}\text{N}_3\text{O}_4$ requires 416.1610.

***E*-(1*S*^{*},2*R*^{*})-[*N*-(1-Benzyl-3,3,3-trifluoro-2-hydroxypropyl)-3-phenylazobenzamide] (4.6)**



4.6

3-Phenylazobenzoic acid **4.3** (88 mg, 0.39 mmol) and **2.6** (102 mg, 0.40 mmol) were coupled together utilising BOP (176 mg, 0.40 mmol) and Hünigs base (139 μ L, 0.80 mmol) in dry dichloromethane (5 mL) following General Method E. Recrystallisation of the crude trifluoromethyl carbinol from aqueous acetone gave **4.6** as an orange powder (162 mg, 98 %, single diastereomer).

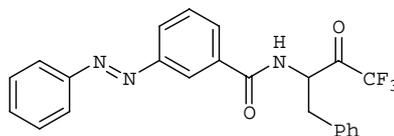
mp 187-188 °C

¹H NMR (acetone-*d*₆, 500 MHz); δ 8.40 (t, 1H, *J* = 1.6 Hz, Ar**H**₂), 8.15 (dd, 2H, *J* = 1.7, 7.8 Hz, Ar**H**₆, NH), 8.07 (dd, 2H, *J* = 1.2, 8.0 Hz, Ar**H**₂'), 8.04 (d, 1H, *J* = 7.8 Hz, Ar**H**₄), 7.76-7.69 (m, 4H, Ar**H**₃', Ar**H**₄', Ar**H**₅), 7.48 (d, 2H, *J* = 7.4 Hz, *o*-Ph), 7.39 (t, 2H, *J* = 7.6 Hz, *m*-Ph), 7.29 (t, 1H, *J* = 7.4 Hz, *p*-Ph), 4.79 (m, 1H, α -H), 4.56 (m, 1H, β -H), 3.38 (dd, 1H, *J* = 3.6, 14.2 Hz, Ph**CH**_a), 3.28 (dd, 1H, *J* = 10.8, 14.2 Hz, Ph**CH**_b).

¹³C NMR (acetone-*d*₆); δ 166.4 (CO), 152.6 (Ar**C**₁, Ar**C**₁'), 138.6 (*i*-Ph), 136.3 (Ar**C**₃), 131.9, 130.0, 129.6, 128.5 (*o*-Ph), 126.5 (Ar**C**₆), 125.8 (q, *J* = 283.2 Hz, CF₃), 125.3 (*p*-Ph), 123.0 (Ar**C**₂'), 121.8 (Ar**C**₂), 71.3 (q, *J* = 28.6 Hz, β -C), 52.3 (α -C), 35.0 (PhCH₂).

¹⁹F NMR (CDCl₃, 282 MHz); δ -74.4 (d, 3F, *J* = 7.4 Hz).

HRMS (ES) 428.1595 (MH⁺); C₂₃H₂₁F₃N₃O₂ requires 428.1586.

***E*-[*N*-(1*S**-Benzyl-3,3,3-trifluoro-2-oxopropyl)-3-phenylazobenzamide] (**4.7**)****4.7**

Trifluoromethyl carbinol **4.6** (43 mg, 0.10 mmol) was oxidised with TEMPO/NaOCl following General Method G. The crude product was purified by column chromatography (1:9 ethyl acetate/dichloromethane) followed by recrystallisation from aqueous MeOH to afford **4.7** as an orange solid (37 mg, 87 %). One drop of D₂O was added to the NMR sample to give exclusively hydrate in solution.

mp 154-156 °C

¹H NMR (acetone-*d*₆, 500 MHz); Hydrate δ 8.30 (s, 1H, Ar**H2**), 8.11 (d, 1H, J = 7.9 Hz, Ar**H6**), 8.02 (dd, 2H, J = 1.5, 8.2 Hz, Ar**H2'**), 7.95 (d, 1H, J = 7.7 Hz, Ar**H4**), 7.76-7.65 (m, 4H, Ar**H3'**, Ar**H4'**, Ar**H5**), 7.44 (d, 2H, J = 7.2 Hz, *o*-Ph), 7.32 (t, 2H, J = 7.5 Hz, *m*-Ph), 7.23 (t, 1H, J = 7.4 Hz, *p*-Ph), 6.91 (d, J = 8.5 Hz, OH), 4.73 (dd, 1H, J = 2.6, 11.8 Hz, α-**H**), 3.47 (dd, J = 3.0, 14.1 Hz, Ph**CH_a**), 3.19 (dd, J = 12.3, 13.7 Hz, Ph**CH_b**).

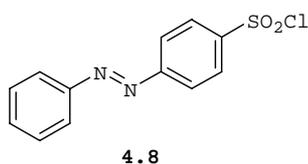
¹³C NMR (acetone-*d*₆, 75 MHz); Hydrate δ 167.9 (CONH), 152.4 (Ar**C1**, Ar**C1'**), 138.7 (*i*-Ph), 135.6 (Ar**C3**), 131.8 (Ar**C4'**), 130.0 (Ar**C4**), 129.6 (Ar**C5**), 129.5 (Ar**C3'**, Ph), 128.3 (Ph), 126.3 (Ar**C6**), 125.4 (*p*-Ph), 124.2 (q, J = 289.7 Hz, CF₃), 122.9 (Ar**C2'**), 121.7 (Ar**C2**), 94.1 (q, J = 29.9 Hz, β-**C**), 56.2 (α-**C**), 33.4 (PhCH₂).

¹⁹F NMR (acetone-*d*₆, 282 MHz); Hydrate δ -80.5 (s, 3F).

HRMS (ES) 444.1528 (MH⁺); C₂₃H₂₁F₃N₃O₃ requires 444.1535.

7.4.2 *E*-4-Azobenzenesulfonamide derivatives

E-[4-Phenylazobenzenesulfonyl chloride] (**4.8**)²⁷



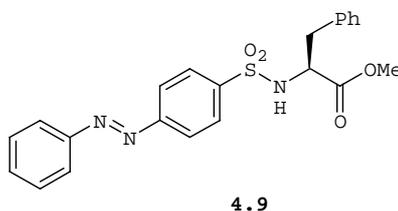
Finely ground recrystallised azobenzene (13.7 g, 75.0 mmol) was added slowly in small portions to a stirred solution of freshly distilled chlorosulfonic acid (45 mL) at rt then heated to 100 °C until evolution of HCl had ceased (*ca.* 4 h). The mixture was cooled to rt, then poured into a 1L beaker half filled with water and ice. The resulting brown precipitate was filtered, washed with copious amounts of water and air-dried. The soil-like solid was ground up and repeatedly extracted with hot petroleum ether to isolate the sulfonyl chloride. The solvent was removed *in vacuo*, and the crude product purified through a silica plug (dichloromethane) to give pure azobenzenesulfonyl chloride **4.8** as an orange solid (8.7 g, 41 %). An analytical sample was obtained by recrystallisation from petroleum ether to afford orange/red flakes.

mp 120-121 °C

¹H NMR (CDCl₃, 500 MHz) δ 8.15 (d, 2H, J = 8.8 Hz, ArH₃), 8.03 (d, 2H, J = 8.8 Hz, ArH₂), 7.95 (m, 2H, ArH₂'), 7.54 (m, 3H, ArH₃', ArH₄').

¹³C NMR (CDCl₃, 75 MHz); δ 155.9 (ArC₁), 152.3 (ArC₂), 145.0 (ArC₄), 132.6 (ArC₄'), 129.3 (ArC₃'), 128.2 (ArC₂'), 123.7 (ArC₂), 123.5 (ArC₂').

Microanalysis; C, 51.49; H, 2.98; N, 10.01; Cl, 12.80; S, 11.54; C₁₂H₉ClN₂O₂S requires C, 51.34; H, 3.23; N, 9.98; Cl 12.63; S, 11.42.

***E*-[3-Phenyl-2*S*-(4-phenylazobenzenesulfonylamino)propionic acid methyl ester] (**4.9**)***Method 1: Pyridine*

A solution of **4.8** (1.26 g, 4.49 mmol) and *L*-phenylalanine methyl ester hydrochloride (1.00 g, 4.63 mmol) in dry pyridine (10 mL) were heated at reflux following General Method B to give the ester **4.9** as an orange solid (1.79 g, 94 %). An analytical sample was obtained by recrystallisation from aqueous MeOH affording fluffy orange needles.

$[\alpha]_D = +20.7$ ($c = 0.51$, MeOH)

mp 140-142 °C

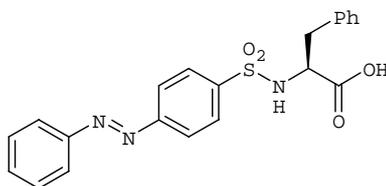
^1H NMR (acetone- d_6 , 500 MHz); δ 8.10 (m, 4H, Ar**H2'**, Ar**H**), 8.00 (d, 2H, $J = 8.5$ Hz, Ar**H**), 7.75 (m, 3H, Ar**H3'**, Ar**H4'**), 7.32 (m, 6H, *o/m/p*-Ph, NH), 4.36 (m, 1H, α -**H**), 3.58 (s, 3H, OCH₃), 3.19 (dd, 1H, $J = 6.1, 13.7$ Hz, PhCH_a), 3.07 (dd, 1H, $J = 8.4, 13.7$ Hz, PhCH_b), 2.96 (s, 4H, 2 x H₂O).

^{13}C NMR (acetone- d_6 , 75 MHz); δ 171.3 (CO), 154.4, 152.6, 143.1 (Ar**C4**), 136.4 (*i*-Ph), 132.3 (Ar**C4'**), 129.6, 129.5, 128.5, 128.3, 127.0 (*p*-Ph), 123.2, 123.1, 57.9 (α -**C**), 51.7 (OCH₃), 38.7 (PhCH₂).

HRMS (EI) 423.1265 (M^+); C₂₂H₂₁N₃O₄S requires 423.1253.

Method 2: Hünigs base

A solution of **4.8** (1.00 g, 3.56 mmol), *L*-phenylalanine methyl ester hydrochloride (845 mg, 3.92 mmol) and Hünigs base (1.40 mL, 8.03 mmol) in dry dichloromethane (50 mL) were heated at reflux following General Method C to give the ester **4.9** as an orange solid (1.47 g, 97 %). The product and NMR data were identical to the above sample.

***E*-[3-Phenyl-2*S*-(4-phenylazobenzenesulfonylamino)propionic acid] (4.10)****4.10**

Hydrolysis of methyl ester **4.9** (1.47 g, 3.46 mmol) with K_2CO_3 (725 mg, 5.25 mmol) in MeOH/H₂O over a period of 9 h following General Method H gave the free acid as an orange solid. Recrystallisation of the free acid from aqueous acetone afforded **4.10** as fine orange needles (1.37 g, 97 %).

$[\alpha]_D = +10.8$ (c = 0.23, MeOH)

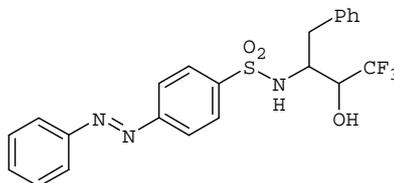
mp 169-170 °C

¹H NMR (acetone-*d*₆, 500 MHz); δ 8.11 (dd, 2H, J = 1.8, 7.7 Hz, ArH_{2'}), 8.06 (d, 2H, J = 8.5 Hz, ArH), 8.00 (d, 2H, J = 8.5 Hz, ArH), 7.75 (m, 3H, ArH_{3'}, ArH_{4'}), 7.31 (m, 5H, *o/m/p*-Ph), 7.16 (d, 1H, J = 8.7 Hz, NH), 4.36 (m, 1H, α-H), 3.26 (dd, 1H, J = 5.4, 13.8 Hz, PhCH_a), 3.08 (dd, 1H, J = 8.4, 13.8 Hz, PhCH_b).

¹³C NMR (acetone-*d*₆, 75 MHz); δ 171.8 (CO), 154.3, 152.5, 143.1 (ArC₄), 136.6 (*i*-Ph), 132.3 (ArC_{4'}), 129.6 (x 2), 128.4, 128.2, 126.9 (*p*-Ph), 123.2, 123.1, 57.6 (α-C), 38.7 (PhCH₂).

HRMS (EI) 409.1091 (M⁺); C₂₁H₁₉N₃O₄S requires 409.1096.

***E*-(1*S*^{*},2*R*^{*})-[*N*-(1-Benzyl-3,3,3-trifluoro-2-hydroxypropyl)-4-phenylazobenzene sulfonamide] (4.11)**



Method 1: Pyridine

A solution of **4.8** (203 mg, 0.72 mmol) and **2.6** (204 mg, 0.80 mmol) in dry pyridine (8 mL) were heated at reflux as described in General Method B to give the desired product **4.11** as an oily orange solid (280 mg, 84 %). An analytical sample was obtained by recrystallisation from aqueous MeOH to afford fluffy orange needles (single diastereomer).

mp 143-144 °C

¹H NMR (CDCl₃, 500 MHz); δ 8.12 (dd, 2H, J = 1.7, 7.9 Hz, ArH₂'), 7.90 (d, 2H, J = 8.5 Hz, ArH), 7.74 (m, 5H, ArH, ArH₃', ArH₄'), 7.18 (m, 3H, *m*-Ph, NH), 7.14 (m, 3H, *o/p*-Ph), 6.02 (d, 1H, J = 6.9 Hz, OH), 4.57 (m, 1H, β-H), 3.93 (m, 1H, α-H), 3.08 (dd, 1H, J = 3.1, 14.4 Hz, PhCH_a), 2.96 (dd, 1H, J = 10.8, 14.4 Hz, PhCH_b).

¹³C NMR (acetone-*d*₆, 75 MHz); δ 154.1, 152.7, 142.7 (ArC_I), 137.5 (ArC_I'), 132.3 (ArC₄'), 129.6, 129.4, 128.4, 127.7, 126.5 (*p*-Ph), 125.4 (q, J = 283.5 Hz, CF₃), 123.2, 123.1, 73.0 (q, J = 28.8 Hz, β-C), 56.1 (α-C), 34.2 (PhCH₂).

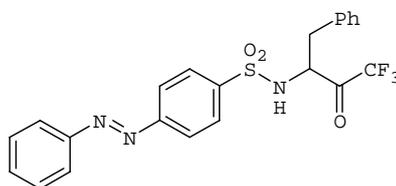
¹⁹F NMR (acetone-*d*₆, 282 MHz); δ -74.2 (d, 3F, J = 8.0 Hz).

HRMS (EI) 463.1191 (M⁺); C₂₂H₂₀F₃N₃O₃S requires 463.1178.

Method 2: Hünigs base

A solution of **4.8** (285 mg, 1.02 mmol), **2.6** (288 mg, 1.13 mmol) and Hünigs base (0.39 mL, 2.24 mmol) in dry dichloromethane (10 mL) were heated at reflux following General Method C to give the desired product **4.11** as an orange solid (473 mg, quantitative, single diastereomer). The product and NMR data were identical to the above sample.

***E*-[*N*-(1*S**-Benzyl-3,3,3-trifluoro-2-oxopropyl)-4-phenylazobenzenesulfonamide]**
(4.12)



4.12

Method 1: TEMPO oxidation

A solution of **4.11** (50 mg, 0.11 mmol) in dichloromethane (4 mL) was oxidised with TEMPO/NaOCl in 2 h following General Method G. Flash chromatography (3:7 ethyl acetate/petroleum ether) of the crude product afforded an oily solid. Recrystallisation of the racemic ketone/hydrate mixture from aqueous MeOH gave **4.12** as fluffy orange needles (40 mg, 80 %).

¹H NMR (acetone-*d*₆, 500 MHz); Hydrate δ 8.13 (dd, 2H, *J* = 1.5, 7.7 Hz, Ar**H2'**), 7.85 (d, 2H, *J* = 8.5 Hz, Ar**H2**), 7.77 (m, 3H, Ar**H3'**, Ar**H4'**), 7.71 (d, 2H, *J* = 8.4 Hz, Ar**H3**), 7.35 (d, 1H, *J* = 9.0 Hz, **NH**), 7.18 (m, 2H, *m*-Ph), 7.06 (m, 3H, *o/p*-Ph), 6.40 (s, 1H, **OH**), 6.35 (s, 1H, **OH**), 4.05 (m, 1H, *α*-**H**), 3.36 (dd, 1H, *J* = 2.3, 14.3 Hz, Ph**CH_a**), 2.86 (dd, 1H, *J* = 11.3, 14.3 Hz, Ph**CH_b**).

¹³C NMR (acetone-*d*₆, 75 MHz); Hydrate δ 154.0 (Ar**C1**), 152.8 (Ar**C1'**), 143.1 (Ar**C4**), 137.9 (*i*-Ph), 132.2 (Ar**C4'**), 129.7, 129.4, 128.4, 127.7, 126.4 (*p*-Ph), 124.1 (q, *J* = 289.2 Hz, **CF₃**), 123.2, 123.0, 93.6 (q, *J* = 29.4 Hz, *β*-**C**), 61.1 (*α*-**C**), 35.6 (Ph**CH₂**).

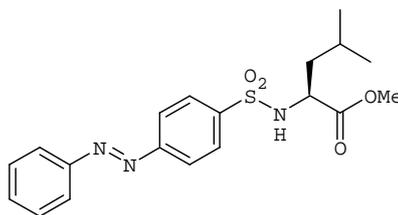
¹⁹F NMR (acetone-*d*₆, 282 MHz); Hydrate δ -78.9 (s, 3F).

HRMS (EI) 461.1031 (*M*⁺, ketone); C₂₂H₁₈F₃N₃O₃S requires 461.1021.

Method 2: Dess-Martin oxidation

A solution of **4.11** (20 mg, 43 μmol) in dichloromethane (10 mL) was oxidised with Dess-Martin periodinane (69 mg, 162 μmol) following General Method F. The crude product was purified down a silica plug (1:9 ethyl acetate). Recrystallisation of the racemic ketone/hydrate mixture from aqueous MeOH gave **4.12** as fluffy orange needles (15 mg, 74 %). The product and NMR data were identical to the above sample.

***E*-[4-Methyl-2*S*-(4-phenylazobenzenesulfonylamino)pentanoic acid methyl ester]**
(4.13)



4.13

Method 1: Pyridine

A solution of **4.8** (702 mg, 2.50 mmol) and *L*-leucine methyl ester hydrochloride (556 mg, 3.06 mmol) in dry pyridine (15 mL) were heated at reflux following General Method B to give ester **4.13** as an orange solid (820 mg, 84 %). An analytical sample of the ester was obtained by recrystallisation from aqueous MeOH (fluffy orange needles).

$[\alpha]_D = +23.8$ ($c = 0.99$, MeOH)

mp 135-136 °C

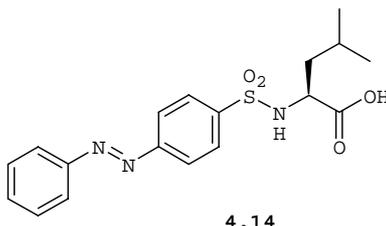
^1H NMR (CDCl_3 , 500 MHz); δ 8.00 (m, 4H, Ar**H2**, Ar**H3**), 7.96 (dd, 2H, $J = 2.1, 7.5$ Hz, Ar**H2'**), 7.55 (m, 3H, Ar**H3'**, Ar**H4'**), 5.12 (d, 1H, $J = 10.1$ Hz, **NH**), 4.01 (m, 1H, α -**H**), 3.45 (s, 3H, **OCH**₃), 1.80 (m, 1H, Leu**CH**), 1.52 (t, 2H, $J = 7.1$ Hz, Leu**CH**₂), 0.92 (t, 6H, $J = 6.0$ Hz, Leu**CH**₃).

^{13}C NMR (CDCl_3 , 75 MHz); δ 172.6, 154.7, 152.3, 141.0, 132.1, 129.2, 128.4, 123.3, 123.2, 54.5(α -**C**), 52.4 (**OCH**₃), 42.3 (Leu**CH**), 24.3 (Leu**CH**₂), 22.7 (Leu**CH**₃), 21.4 (Leu**CH**₃).

HRMS (EI) 389.1408 (M^+); $\text{C}_{19}\text{H}_{23}\text{N}_3\text{O}_4\text{S}$ requires 389.1409.

Method 2: Hünigs base

A solution of **4.8** (152 mg, 0.54 mmol), *L*-leucine methyl ester hydrochloride (107 mg, 0.59 mmol) and Hünigs base (0.20 mL, 1.15 mmol) in dry dichloromethane (10 mL) were heated at reflux following General Method C to give ester **4.13** as an orange solid (207 mg, 98 %). The product and NMR data were identical to the above sample.

***E*-[4-Methyl-2*S*-(4-phenylazobenzenesulfonylamino)pentanoic acid] (**4.14**)**

Hydrolysis of methyl ester **4.13** (812 mg, 2.08 mmol) with K_2CO_3 (450 mg, 3.26 mmol) in MeOH/H₂O over a period of 24 h following General Method H gave the free acid **4.14** as an orange solid (759 mg, 97 %).

$[\alpha]_D = +35.6$ ($c = 0.30$, MeOH)

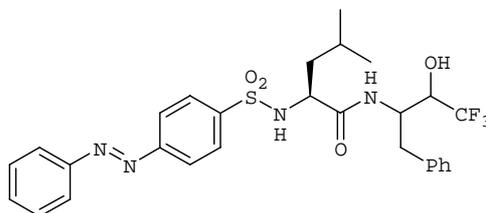
mp 149 °C

¹H NMR (acetone-*d*₆, 500 MHz); δ 8.18 (m, 4H, Ar**H**₂, Ar**H**₃), 8.11 (dd, 2H, $J = 1.9, 7.7$ Hz, Ar**H**_{2'}), 7.75 (m, 3H, Ar**H**_{3'}, Ar**H**_{4'}), 7.14 (d, 1H, $J = 8.2$ Hz, **NH**), 4.12 (m, 1H, α -**H**), 1.94 (m, 1H, Leu**CH**), 1.70 (m, 2H, Leu**CH**₂), 1.03 (d, 1H, $J = 6.6$ Hz, Leu**CH**₃), 0.99 (d, 1H, $J = 6.6$ Hz, Leu**CH**₃).

¹³C NMR (acetone-*d*₆, 75 MHz); δ 172.7 (**CO**), 154.5, 152.6, 143.4 (Ar**C**₄), 132.4 (Ar**C**_{4'}), 129.7, 128.5, 123.2, 123.2, 54.5 (α -**C**), 41.9 (Leu**CH**), 24.4 (Leu**CH**₂), 22.5 (Leu**CH**₃), 20.9 (Leu**CH**₃).

HRMS (EI) 375.1261 (M^+); C₁₈H₂₁N₃O₄S requires 375.1253.

***E*-[4-Methyl-2*S*-(4-phenylazobenzenesulfonylamino)pentanoic acid (1*S**,2*R**)-(1-benzyl-3,3,3-trifluoro-2-hydroxypropyl)amide] (4.15)**



4.15

Method 1:

The free acid **4.14** (76 mg, 0.20 mmol), **2.6** (57 mg, 0.22 mmol), BOP (100 mg, 0.22 mmol) and Hünigs base (80 μ L, 0.46 mmol) in dry dichloromethane (8 mL) were reacted together following General Method E to give the dipeptide as an orange solid. Column chromatography (1:9 ethyl acetate/dichloromethane) of the crude trifluoromethyl carbinol gave **4.15** as an orange solid (69 mg, 59 %) comprising a mixture of diastereomers (1:2 by ^1H and ^{19}F NMR).

$[\alpha]_{\text{D}} = -19.7$ ($c = 0.97$, MeOH)

^1H NMR (acetone- d_6 , 500 MHz); Mixture (1:2) δ 8.17-8.08 (m, 12H, ArH2, ArH2', ArH3, major + minor), 7.75 (m, 6H, ArH3', ArH4', major + minor), 7.38-7.29 (m, 10H, *o/m/p*-Ph, major + minor), 7.66* (d, 1H, $J = 8.4$ Hz, TFMC NH), 7.62 (d, 1H, $J = 8.8$ Hz, TFMC NH), 6.96* (br s, 1H, LeuNH), 6.81 (br s, 1H, LeuNH), 5.89* (d, 1H, $J = 6.5$ Hz, OH), 5.73 (d, 1H, $J = 6.4$ Hz, OH), 4.44 (m, 1H, TFMC α -H), 4.35* (m, 1H, TFMC α -H), 4.13* (m, 1H, TFMC β -H), 3.96 (m, 3H, Leu α -H, major + minor, TFMC β -H, major), 3.08 (m, 2H, PhCH_a, major + minor), 2.95 (m, 2H, PhCH_b, major + minor), 1.73* (m, 1H, LeuCH), 1.57 (m, 1H, LeuCH), 1.41* (m, 2H, LeuCH₂), 1.14 (m, 2H, LeuCH₂), 0.90* (d, 3H, $J = 6.7$ Hz, LeuCH₃), 0.84 (d, 3H, $J = 6.6$ Hz, LeuCH₃), 0.80 (t, 6H, $J = 6.2$ Hz, LeuCH₃, major + minor).

^{13}C NMR (acetone- d_6 , 75 MHz); Mixture δ 171.7* (CO), 171.2 (CO), 154.6, 154.5*, 152.6, 152.6*, 143.3 (ArC4), 143.0* (ArC4), 138.2 (*i*-Ph), 138.0* (*i*-Ph), 132.3 (ArC4'), 132.3* (ArC4'), 129.6 (m), 128.5 (m), 128.4, 126.6* (*p*-Ph), 126.5 (*p*-Ph), 125.5* (q, $J = 283.4$ Hz, CF₃), 125.3 (q, $J = 284.4$ Hz, CF₃), 123.2 (m), 123.1, 71.6 (q, $J = 29.0$ Hz, β -C), 71.0* (q, J

= 28.8 Hz, β -C), 55.6*, 55.4, 51.3*, 50.4, 42.3, 42.3*, 34.6*, 34.2, 24.2*, 24.1, 22.6, 22.6*, 20.9, 20.8*.

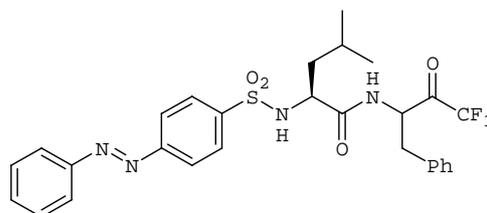
^{19}F NMR (acetone- d_6 , 282 MHz); Mixture (1:2) δ -74.2* (d, 3F, J = 7.7 Hz), -74.4 (d, 3F, J = 7.9 Hz).

HRMS (EI) 576.2025 (M^+); $\text{C}_{28}\text{H}_{31}\text{F}_3\text{N}_4\text{O}_4\text{S}$ requires 576.2018.

Method 2:

A solution of **4.8** (150 mg, 0.53 mmol), *L*-Leu-TFMC.HCl (230 mg, 0.62 mmol) and Hünigs base (0.24 mL, 1.38 mmol) in dry dichloromethane (15 mL) were heated to reflux for 16 h following General Method C. Column chromatography (1:9 ethyl acetate/dichloromethane) of the crude trifluoromethyl carbinol gave **4.15** as an orange solid (179 mg, 61 %) as a mixture of diastereomers. The product and NMR data was identical to the above compound.

E-[4-Methyl-2*S*-(4-phenylazobenzenesulfonylamino)pentanoic acid (1*S**-benzyl-3,3,3-trifluoro-2-oxopropyl)amide] (**4.16**)



4.16

A solution of **4.15** (29 mg, 50 μmol) in dichloromethane (10 mL) was oxidised with Dess-Martin periodinane (79 mg, 186 μmol) following General Method F. The crude product was purified down a silica plug (1:4 ethyl acetate/dichloromethane). Recrystallisation of the ketone/hydrate mixture from aqueous MeOH gave **4.16** as an orange solid (20 mg, 70 %) comprising a mixture of diastereomers (65:35 by ^1H NMR).

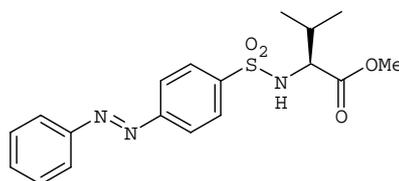
^1H NMR (acetone- d_6 , 500 MHz); Hydrate, mixture of diastereomers (65:35) δ 8.14-8.08 (m, 8H, ArH₂, ArH₃, major + minor), 7.84 (d, 2H, J = 8.7 Hz, TFMK NH, major + minor), 7.74 (m, 6H, ArH₃', ArH₄', major + minor), 7.38 (m, 8H, *o/m*-Ph, major + minor), 7.30 (m, 2H, *p*-Ph, major + minor), 7.13* (d, 1H, J = 7.1 Hz, LeuNH), 7.07 (d, 1H,

$J = 8.5$ Hz, LeuNH), 6.72* (s, 1H, OH), 6.59 (s, 2H, OH, major + minor), 6.54 (s, 1H, OH), 4.43 (m, 2H, TFMK α -H, major + minor), 4.03 (m, 1H, Leu α -H), 3.95* (m, 1H, Leu α -H), 3.39 (d, 2H, $J = 14.1$ Hz, PhCH₂, major + minor), 2.96 (m, 2H, PhCH₂, major + minor), 1.62* (m, 1H, LeuCH), 1.55 (m, 1H, LeuCH), 1.40* (m, 1H, LeuCH_a), 1.31* (m, 1H, LeuCH_b), 1.22 (m, 1H, LeuCH_a), 1.12 (m, 1H, LeuCH_b), 0.84* (d, 3H, $J = 6.7$ Hz, LeuCH_{3a}), 0.81 (d, 3H, $J = 6.6$ Hz, LeuCH_{3a}), 0.75 (d, 3H, $J = 6.5$ Hz, LeuCH_{3b}), 0.70* (d, 3H, $J = 6.5$ Hz, LeuCH_{3b}).

¹³C NMR (acetone-*d*₆, 75 MHz); δ 173.8, 173.5*, 154.5*, 154.5, 152.6*, 152.6, 143.3, 142.7*, 138.6*, 138.5, 132.3*, 132.3, 129.6*, 129.6, 129.5, 129.5*, 128.5, 128.4 (x2), 128.3, 126.5, 126.4*, 124.1* (q, $J = 289.0$ Hz, CF₃), 123.9 (q, $J = 289.1$ Hz, CF₃), 123.3, 123.2*, 123.2*, 123.2, 94.4* (q, $J = 30.3$ Hz, β -C), 94.2 (q, $J = 30.0$ Hz, β -C), 56.4, 56.0*, 55.7*, 55.2, 42.2, 42.0*, 34.3, 34.1*, 24.1*, 24.0, 22.6, 22.5*, 20.8*, 20.7.

LRMS (ES) (MH⁺) 575.2; C₂₈H₃₀F₃N₄O₄S requires 575.2.

***E*-[3-Methyl-2*S*-(4-phenylazobenzenesulfonylamino)butyric acid methyl ester] (4.17)**



4.17

Method 1: Pyridine

A solution of **4.8** (563 mg, 2.00 mmol) and *L*-valine methyl ester hydrochloride (369 mg, 2.20 mmol) in dry pyridine (15 mL) were heated at reflux following General Method B to give ester **4.17** as an orange solid (752 mg, quantitative). An analytical sample of the ester was obtained by recrystallisation from aqueous MeOH to afford fine orange crystals.

$[\alpha]_D = +28.2$ ($c = 1.0$, MeOH)

mp 145-146 °C

¹H NMR (CDCl₃, 500 MHz); δ 8.00 (s, 4H, ArH₂, ArH₃), 7.95 (dd, 2H, $J = 1.9, 7.2$ Hz, ArH_{2'}), 7.54 (m, 3H, ArH_{3'}, ArH_{4'}), 5.39 (d, 1H, $J = 10.1$ Hz, NH), 3.83 (dd, 1H, $J = 5.1,$

10.1 Hz, α -**H**), 3.46 (s, 3H, OCH₃), 2.07 (m, 1H, Val**CH**), 0.98 (d, 3H, J = 6.8 Hz, Val**CH**₃), 0.90 (d, 3H, J = 6.8 Hz, Val**CH**₃).

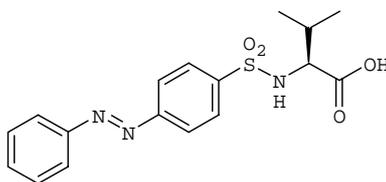
¹³C NMR (CDCl₃, 75 MHz); δ 171.6 (CO), 154.6, 152.3, 141.0 (Ar**C4**), 132.0 (Ar**C4'**), 129.2, 128.3, 123.2, 123.1, 61.1 (α -**C**), 52.3 (OCH₃), 31.6 (Val**CH**), 18.9 (Val**CH**₃), 17.4 (Val**CH**₃).

HRMS (EI) 375.1266 (M⁺); C₁₈H₂₁N₃O₄S requires 375.1253.

Method 2: Hünigs base

A solution of **4.8** (152 mg, 0.54 mmol), *L*-valine methyl ester hydrochloride (117 mg, 0.79 mmol) and Hünigs base (0.20 mL, 1.15 mmol) in dry dichloromethane (10 mL) were heated at reflux following General Method C to give ester **4.17** as an orange solid (203 mg, quantitative). The product and NMR data were identical to the above sample.

E-[3-Methyl-2*S*-(4-phenylazobenzenesulfonylamino)butyric acid] (**4.18**)



4.18

Hydrolysis of the methyl ester **4.17** (206 mg, 0.55 mmol) with K₂CO₃ (114 mg, 0.83 mmol) in MeOH/H₂O over a period of 48 h following General Method H gave free acid **4.18** as an orange solid (196 mg, 99 %).

$[\alpha]_D = +23.6$ (c = 0.22, MeOH)

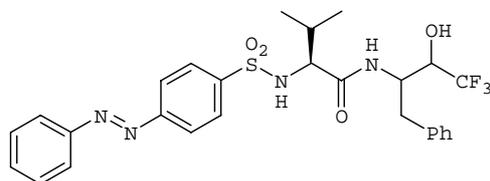
mp 230 °C

¹H NMR (acetone-*d*₆, 500 MHz); δ 8.17 (m, 4H, Ar**H2**, Ar**H3**), 8.10 (d, 2H, J = 6.0 Hz, Ar**H2'**), 7.74 (m, 3H, Ar**H3'**, Ar**H4'**), 6.96 (d, 1H, J = 9.4 Hz, **NH**), 3.97 (m, 1H, α -**H**), 2.25 (m, 1H, Val**CH**), 1.11 (d, 3H, J = 6.6 Hz, Val**CH**₃), 1.04 (d, 3H, J = 6.7 Hz, Val**CH**₃).

¹³C NMR (acetone-*d*₆, 75 MHz); δ 171.7 (CO), 154.5, 152.7, 143.3 (Ar**C4**), 132.4 (Ar**C4'**), 129.7, 128.6, 123.2, 123.1, 61.5 (α -**C**), 31.3 (Val**CH**), 18.9 (Val**CH**₃), 17.3 (Val**CH**₃).

HRMS (EI) 361.1113 (M⁺); C₁₇H₁₉N₃O₄S requires 361.1096.

***E*-[(1*S*^{*},2*R*^{*})-*N*-(1-Benzyl-3,3,3-trifluoro-2-hydroxypropyl)-3-methyl-2*S*-(4-phenyl azobenzenesulfonylamino)butyramide] (4.19)**



4.19

Method 1:

The free acid **4.18** (73 mg, 0.20 mmol), **2.6** (57 mg, 0.22 mmol), BOP (100 mg, 0.22 mmol) and Hünigs base (80 μ L, 0.46 mmol) in dry dichloromethane (8 mL) were reacted together following General Method E to give the dipeptide as an oily orange solid. Column chromatography (1:9 ethyl acetate/dichloromethane) of the crude trifluoromethyl carbinol, followed by recrystallisation from aqueous MeOH gave **4.19** as an orange powder (79 mg, 70 %) comprising a mixture of diastereomers (2:3 by ^1H and ^{19}F NMR).

$[\alpha]_{\text{D}} = +4.9$ ($c = 0.97$, MeOH)

^1H NMR (acetone- d_6 , 500 MHz); Mixture (2:3) δ 8.15-8.07 (m, 12H, ArH2, ArH2', ArH3, major + minor), 7.75 (m, 6H, ArH3', ArH4', major + minor), 7.62* (d, 1H, $J = 8.3$ Hz, TFMC NH), 7.57 (d, 1H, $J = 8.9$ Hz, TFMC NH), 7.37-7.24 (m, 10H, *o*/*m*/*p*-Ph, major + minor), 6.75* (br s, 1H, ValNH), 6.54 (d, 1H, $J = 8.6$ Hz, ValNH), 5.87* (d, 1H, $J = 6.5$ Hz, OH), 5.66 (d, 1H, $J = 6.4$ Hz, OH), 4.48 (m, 1H, TFMC α -H), 4.39* (m, 1H, TFMC α -H), 4.12* (m, 1H, TFMC β -H), 3.89* (m, 1H, Val α -H), 3.85 (m, 2H, TFMC β -H, major, Val α -H, major), 3.18-2.87 (m, 4H, PhCH_a, PhCH_b, major + minor), 2.08* (m, 1H, ValCH), 1.83 (m, 1H, ValCH), 0.94* (d, 3H, $J = 6.8$ Hz, ValCH₃), 0.84 (dd, 6H, $J = 6.9$, 8.7 Hz, ValCH₃, major + minor), 0.53 (d, 3H, $J = 6.8$ Hz, ValCH₃).

^{13}C NMR (acetone- d_6 , 75 MHz); Mixture δ 170.6 (CO), 170.0 (CO), 154.6*, 154.5, 152.7, 152.7*, 143.2 (ArC4), 143.0* (ArC4), 138.2 (*i*-Ph), 137.9* (*i*-Ph), 132.3 (ArC4'), 132.3* (ArC4'), 129.6, 129.6*, 129.5*, 129.4, 128.6 (x 2), 128.5 (x 2), 126.6* (*p*-Ph), 126.5 (*p*-Ph), 125.5* (q, $J = 283.5$ Hz, CF₃), 125.2 (q, $J = 284.0$ Hz, CF₃), 123.2 (x 3), 123.0, 71.8 (q, $J = 29.1$ Hz, β -C), 71.3* (q, $J = 29.0$ Hz, β -C), 61.9*, 61.7, 51.3*, 50.5, 34.5*, 34.2, 31.7, 31.6*, 19.1, 19.1*, 16.5*, 16.2.

^{19}F NMR (acetone- d_6 , 282 MHz) Mixture (2:3) δ -74.2* (d, 3F, J = 7.6 Hz), -74.5 (d, 3F, J = 7.9 Hz).

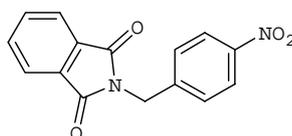
HRMS (EI) 562.1875 (M^+); $\text{C}_{28}\text{H}_{31}\text{F}_3\text{N}_4\text{O}_4\text{S}$ requires 562.1862.

Method 2:

A solution of **4.8** (38 mg, 0.14 mmol), *L*-Val-TFMC (46 mg, 0.15 mmol) and Hünigs base (30 μL , 0.17 mmol) in dry dichloromethane (10 mL) were heated at reflux for 16 h following General Method C. Column chromatography (1:9 ethyl acetate/dichloromethane) of the crude trifluoromethyl carbinol gave **4.19** as an orange solid (60 mg, 79 %) comprising a mixture of diastereomers. The product and NMR data was identical to the above compound.

7.4.3 Synthesis of 4-substituted nitrosobenzenes

4-Nitrobenzylphthalimide (**4.20**)²⁸

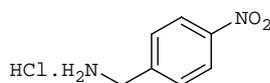


4.20

A suspension of recrystallised 4-nitrobenzyl chloride (17.1 g, 99.7 mmol) and potassium phthalimide (18.5 g, 99.9 mmol) in DMF (300 mL) was stirred at 50 °C for 2 h. The resulting purple mixture was poured into cold water (1 L) to precipitate out a pale yellow solid. The crude product was recrystallised from EtOH to afford **4.20** as fine pale yellow needles (25.1 g, 89 %).

^1H NMR (CDCl_3 , 500 MHz); δ 8.18 (d, 2H, J = 8.7 Hz), 7.88 (dd, 2H, J = 3.0, 5.4 Hz), 7.75 (dd, 2H, J = 3.0, 5.4 Hz), 7.59 (d, 2H, J = 8.7 Hz), 4.94 (s, 2H, CH_2).

^{13}C NMR (CDCl_3 , 75 MHz); δ 167.7, 147.5, 143.3, 134.3, 131.8, 129.3, 123.9, 123.6, 40.8.

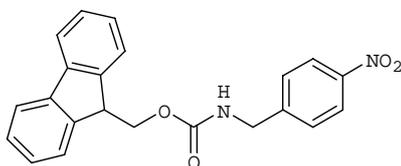
4-Nitrobenzylamine hydrochloride (4.21)²⁸

4.21

4-Nitrobenzylphthalimide **4.20** (5.64 g, 20.0 mmol) and hydrazine hydrate (4.40 mL, 91.0 mmol) in EtOH (350 mL) were heated at reflux for 16 h.^ξ The suspension was cooled to rt and most of the solvent removed *in vacuo* before dissolving the precipitate in water (600 mL) and acidifying with acetic acid. The resulting precipitate of phthalazine-1,4-dione was removed by filtration through a celite plug. The filtrate was concentrated *in vacuo* and crystallisation induced by scratching. Filtering off the solid proved difficult so the solid was redissolved in distilled water, basified with NaOH, extracted with dichloromethane (5 x 50 mL), dried over MgSO₄ and filtered. The volume of solution was reduced to *ca.* 100 mL and cooled in an ice bath. Dry, gaseous HCl was bubbled into the solution for 30 min. The resulting precipitate was filtered, washed with dry ether and petroleum ether and air dried to afford **4.21** as a pale brown solid (3.11 g, 82 %).

¹H NMR (D₂O, 300 MHz); δ 8.24 (d, 2H, J = 8.6 Hz, ArH₂), 7.64 (d, 2H, J = 8.6 Hz, ArH₃), 4.30 (s, 2H, CH₂).

¹³C NMR (D₂O, 75 MHz); δ 148.7, 140.7, 130.7, 125.1, 43.1.

(4-Nitrobenzyl)carbamic acid 9H-fluoren-9-ylmethyl ester (4.22)⁶

4.22

A suspension of hydrochloride **4.21** (2.03 g, 11.0 mmol) and FmocCl (2.66 g, 10.0 mmol) in dry dichloromethane (15 mL) was cooled in an ice bath. Hünigs base (3.80 mL, 22.0

^ξ After *ca.* 1 h a thick yellow precipitate began to form.

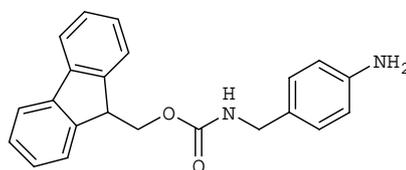
mmol) was added dropwise to complete dissolution and the resulting solution stirred for 5 h under an inert atmosphere at rt.^ξ The solvent was removed *in vacuo*, redissolved in ethyl acetate (300 mL) and successively extracted with 10 % aqueous HCl, saturated aqueous NaHCO₃ and brine, dried over MgSO₄, filtered and concentrated *in vacuo* to afford **4.22** as a pale yellow solid (2.66 g, 71 %). An analytical sample was recrystallised from aqueous EtOH.

mp 155-156 °C (lit. 155-156 °C)⁶

¹H NMR (acetone-*d*₆, 500, MHz); δ 8.32 (d, 2H, J = 8.6 Hz, Ar**H2**), 7.99 (d, 2H, J = 7.6 Hz, Fmoc**H_a**), 7.82 (d, 2H, J = 7.5 Hz, Fmoc**H_d**), 7.68 (d, 2H, J = 8.5 Hz, Ar**H3**), 7.54 (t, 2H, J = 7.5 Hz, Fmoc**H_b**), 7.45 (t, 2H, J = 7.4 Hz, Fmoc**H_c**), 7.29 (br m, 1H, CO₂**NH**), 4.61 (d, 2H, J = 6.3 Hz, NH**CH₂**), 4.56 (d, 2H, J = 6.8 Hz, Fmoc**CH₂**), 4.37 (t, 1H, J = 6.7 Hz, Fmoc**CH**).

¹³C NMR (acetone-*d*₆, 75 MHz); δ 156.8 (CO₂**NH**), 148.0, 144.4, 141.5 (x 2), 128.3, 127.9, 127.3, 125.4, 123.7, 120.2, 66.2, 47.5, 44.0.

(4-Aminobenzyl)carbamic acid 9H-fluoren-9-ylmethyl ester (**4.23**)⁶



4.23

A suspension of **4.22** (2.35 g, 6.28 mmol) in ethyl acetate was reduced under an atmosphere of hydrogen for 36 h following General Method I to afford **4.23** as a white solid containing a minor impurity (2.16 g, quantitative). Recrystallisation from EtOH yielded the pure product as fine white needles.

mp 152-153 °C (lit. 147-148 °C)⁶

¹H NMR (acetone-*d*₆, 500 MHz); δ 7.97 (d, 2H, J = 7.6 Hz, Fmoc**H_a**), 7.82 (d, 2H, J = 7.4 Hz, Fmoc**H_d**), 7.52 (t, 2H, J = 7.5 Hz, Fmoc**H_b**), 7.43 (t, 2H, J = 7.2 Hz, Fmoc**H_c**), 7.13 (d, 2H, J = 8.1 Hz, Ar**H3**), 6.86 (br m, 1H, CO₂**NH**), 6.73 (d, 2H, J = 8.3 Hz, Ar**H2**), 4.66 (br

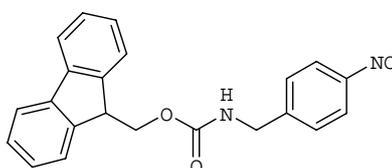
^ξ During the course of the reaction a precipitate of diisopropylethylamine hydrochloride formed.

s, 2H, **NH**₂), 4.46 (d, 2H, J = 7.2 Hz, Fmoc**CH**₂), 4.34 (t, 1H, J = 7.1 Hz, Fmoc**CH**), 4.30 (d, 2H, J = 6.1 Hz, NH**CH**₂).

¹³C NMR (acetone-*d*₆, 75 MHz); δ 156.6 (CO₂NH), 147.7 (Ar**CI**), 144.5, 141.4, 128.7 (Ar**C3**), 127.9 (Ar**C4**), 127.8, 127.2, 125.5, 120.1, 114.4 (Ar**C2**), 66.2 (Fmoc**CH**₂), 47.5 (NH**CH**₂), 44.4 (Fmoc**CH**).

HRMS (EI) 344.1527 (M⁺); C₂₂H₂₀N₂O₂ requires 344.1525.

(4-Nitrosobenzyl)carbamic acid 9H-fluoren-9-ylmethyl ester (**4.24**)



4.24

A suspension of **4.23** (1.03 g, 3.00 mmol) in dichloromethane (15 mL) was oxidised with Mo^{VI}/hydrogen peroxide over 14 h following General Method J. The crude nitrosobenzene was subjected to flash chromatography (1:9 ethyl acetate/dichloromethane) to afford **4.24** as a green solid (1.06 g, 98 %). An analytical sample was obtained by recrystallisation from aqueous EtOH resulting in bright green crystals.

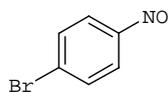
The ¹H and ¹³C NMR's had extra aromatic peaks that correspond to the nitro derivative **4.22**, presumably due to over-oxidation of the aniline or atmospheric oxidation of the nitrosobenzene. These peaks have been omitted from the data below.

mp 134 °C (dec)

¹H NMR (CDCl₃, 500 MHz); δ 7.86 (d, 2H, J = 7.9 Hz, Ar**H2**), 7.77 (d, 2H, J = 7.5 Hz, Fmoc**H**_a), 7.60 (d, 1H, J = 7.2 Hz, Fmoc**H**_a), 7.42 (m, 4H, Ar**H3**, Fmoc**H**_b), 7.32 (t, 2H, J = 7.3 Hz, Fmoc**H**_c), 5.19 (br s, 1H, **NH**), 4.53 (d, 2H, J = 6.4 Hz, Fmoc**CH**₂), 4.44 (d, 2H, J = 5.9 Hz, NH**CH**₂), 4.22 (m, 1H, Fmoc**CH**).

¹³C NMR (CDCl₃, 75 MHz); δ 165.4 (Ar**CI**), 156.5 (CO₂NH), 146.6, 143.7, 141.3, 127.7, 127.0, 124.9, 123.8, 121.4, 120.0, 66.6, 47.3, 44.5.

HRMS (ES) 359.1401 (MH⁺); C₂₂H₁₉N₂O₃ requires 359.1396.

1-Bromo-4-nitrosobenzene (4.25)**4.25**

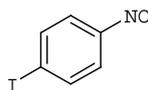
A solution of 4-bromoaniline (4.30 g, 25.0 mmol) in dichloromethane (125 mL) was oxidised with Mo^{VI}/hydrogen peroxide over 18 h following General Method J. The crude nitrosobenzene was subjected to flash chromatography (dichloromethane). Only the green band of interest was collected. A second column (1:3 ethyl acetate/petroleum ether) afforded **4.25** as a yellowish solid (2.36 g, 51 %). An analytical sample was obtained by recrystallisation from aqueous EtOH to give pale tan crystals.

mp 93-94 °C

¹H NMR (CDCl₃, 500 MHz); δ 7.78 (br s, 4H, Ar**H2**, Ar**H3**).

¹³C NMR (CDCl₃, 75 MHz); δ 163.8 (C**NO**), 132.7 (Ar**C2**), 131.7 (Ar**C3**), 122.1 (Ar**C1**).

HRMS (EI) 184.9480 (M⁺); C₆H₄BrNO requires 184.9476.

1-Iodo-4-nitrosobenzene (4.26)**4.26**

A solution of 4-iodoaniline (4.99 g, 22.8 mmol) in dichloromethane (120 mL) was oxidised with Mo^{VI}/hydrogen peroxide over 16 h according to General Method J. The crude nitrosobenzene was subjected to flash chromatography, eluting with progressively more polar solvent systems (petroleum ether to 1:1 dichloromethane/petroleum ether), to remove multiple by-products. Only the green band of interest was collected. A second column (1:1 dichloromethane/petroleum ether) afforded **4.26** as a green solid (1.00 g, 19 %). An analytical sample was obtained by recrystallisation from aqueous EtOH resulting in green needles.

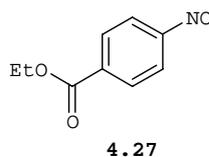
mp 101-102 °C

^1H NMR (CDCl_3 , 500 MHz); δ 8.03 (d, 2H, $J = 8.5$ Hz, ArH2), 7.60 (d, 2H, $J = 8.5$ Hz, ArH3).

^{13}C NMR (CDCl_3 , 75 MHz); δ 164.2 (CNO), 138.7 (ArC2), 121.8 (ArC3), 105.5 (ArC1).

HRMS (EI) 232.9346 (M^+); $\text{C}_6\text{H}_4\text{INO}$ requires 232.9338.

4-Nitrosobenzoic acid ethyl ester (4.27)



A suspension of 4-aminobenzoic acid ethyl ester[§] (825 mg, 5.00 mmol) in dichloromethane (25 mL) was oxidised with Mo^{VI} /hydrogen peroxide following General Method J. The crude nitrosobenzene was subjected to flash chromatography (1:9 ethyl acetate/dichloromethane) to afford **4.27** as a yellow/green solid (569 mg, 64 %). An analytical sample was recrystallised from aqueous EtOH affording a yellow solid.

mp 79-80 °C

^1H NMR (CDCl_3 , 500 MHz); δ 8.30 (d, 2H, $J = 8.0$ Hz, ArH3), 7.93 (d, 2H, $J = 8.0$ Hz, ArH2), 4.44 (dd, 2H, $J = 6.7, 13.7$ Hz, CH₂), 1.44 (t, 3H, $J = 6.8$ Hz, CH₃).

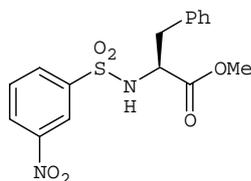
^{13}C NMR (CDCl_3 , 75 MHz); δ 165.2, 164.2, 130.9 (ArC3), 120.3 (ArC2), 61.8 (CH₂), 14.2 (CH₃).

HRMS (EI) 179.0590 (M^+); $\text{C}_9\text{H}_9\text{NO}_3$ requires 179.0582.

[§] Kindly gifted by T. Cain.

7.4.4 Synthesis of Metanilamides

2*S*-(3-Nitrobenzenesulfonylamino)-3-phenylpropionic acid methyl ester (**4.28**)



3-Nitrobenzenesulfonyl chloride (1.08 g, 4.87 mmol), *L*-phenylalanine methyl ester hydrochloride (1.21 g, 5.61 mmol) and Hünigs base (1.96 mL, 11.2 mmol) in dry dichloromethane (20 mL) were heated at reflux following General Method C to give the desired product as a pale yellow oil. Flash chromatography (dichloromethane) of the crude ester afforded **4.28** as a pale yellow solid (1.71 g, 96 %).

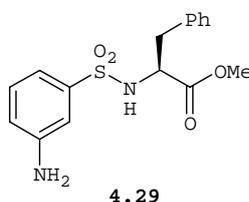
$[\alpha]_D = -28.5$ ($c = 0.78$, MeOH)

mp 91-92 °C

^1H NMR (CDCl_3 , 500 MHz); δ 8.46 (s, 1H, Ar**H2**) 8.33 (dd, 1H, $J = 2.3, 8.3$ Hz, Ar**H6**) 8.00 (d, 1H, $J = 7.8$ Hz, Ar**H4**), 7.60 (t, 1H, $J = 8.0$ Hz, Ar**H5**), 7.16 (m, 3H, *o/p*-Ph), 7.04 (dd, 1H, $J = 1.8, 7.4$ Hz, *m*-Ph), 5.63 (d, 1H, $J = 9.4$ Hz, NH), 4.27 (m, 1H, α -H), 3.63 (s, 3H, OCH₃), 3.11 (dd, 1H, $J = 5.2, 13.9$ Hz, PhCH_a), 2.95 (dd, 1H, $J = 7.8, 13.8$ Hz, PhCH_b).

^{13}C NMR (CDCl_3 , 75 MHz); δ 171.2 (CO₂Me), 148.0 (Ar**C3**), 141.9 (Ar**C1**), 134.8 (*i*-Ph), 132.4 (Ar**C6**) 130.2 (Ar**C5**), 129.2 (*m*-Ph), 128.6 (*o*-Ph), 127.2, 126.9, 122.1 (Ar**C2**), 57.2 (α -C), 52.7 (OCH₃), 39.0 (PhCH₂).

HRMS (ES); 365.0820 (MH⁺); C₁₆H₁₇N₂O₆S requires 365.0807.

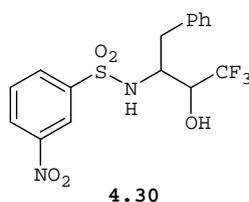
2*S*-(3-Aminobenzenesulfonylamino)-3-phenylpropionic acid methyl ester (4.29)

Ester **4.28** (1.46 g, 4.00 mmol) was reduced under an atmosphere of hydrogen as described in General Method I to give metanilamide **4.29** as a glassy off-white solid (1.28g, 96 %).

^1H NMR (acetone- d_6 , 500 MHz); δ 7.38-7.27 (m, 6H, *o/m/p*-Ph, **NH**), 7.16 (t, 1H, $J = 1.9$ Hz, **ArH2**), 7.05 (d, 1H, $J = 7.7$ Hz, **ArH6**), 6.97 (dd, 1H, $J = 2.1, 8.0$ Hz, **ArH5**), 6.85 (d, 1H, $J = 9.3$ Hz, **ArH4**), 5.20 (br s, 2H, **ArNH2**), 4.23 (m, 1H, α -**H**), 3.56 (s, 3H, **OCH3**), 3.11 (dd, 1H, $J = 6.9, 13.6$ Hz, **PhCH_a**) 3.03 (dd, 1H, $J = 7.4, 13.6$ Hz, **PhCH_b**).

^{13}C NMR (acetone- d_6 , 75 MHz); δ 171.4 (**CO₂Me**), 149.3 (**ArC3**), 141.7 (**ArC1**), 136.5 (*i*-Ph), 129.7 (**ArC5**), 129.5 (*m*-Ph), 128.5 (*o*-Ph), 127.0 (*p*-Ph), 118.0 (**ArC4**), 114.7 (**ArC6**), 112.1 (**ArC2**), 57.8 (α -**C**), 51.5 (**OCH₃**), 38.9 (**PhCH₂**).

HRMS (ES); 335.1060 (MH^+); $\text{C}_{16}\text{H}_{19}\text{N}_2\text{O}_4\text{S}$ requires 335.1066.

(1*S,2*R**)-N-(1-Benzyl-3,3,3-trifluoro-2-hydroxypropyl)-3-nitrobenzenesulfonamide (4.30)**

3-Nitrobenzenesulfonyl chloride (222 mg, 1.00 mmol), **2.6** (269 mg, 1.05 mmol) and Hünigs base (0.37 mL, 2.12 mmol) in dry dichloromethane (10 mL) were heated at reflux following General Method C to give the desired product as a pale brown oil. Flash chromatography (dichloromethane) of the crude trifluoromethyl carbinol gave **4.30** as a pale yellow solid (400 mg, 99 %, single diastereomer).

mp 116-118 °C

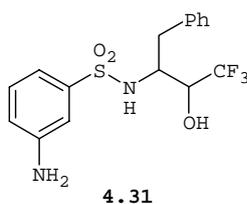
^1H NMR (CDCl_3 , 500 MHz); δ 8.26 (d, 1H, $J = 8.2$ Hz, ArH6), 8.12 (s, 1H, ArH2), 7.78 (d, 1H, $J = 7.9$ Hz, ArH4), 7.48 (t, 1H, $J = 8.0$ Hz, ArH5), 6.97-6.94 (m, 3H, *o/p*-Ph), 6.85 (dd, 2H, $J = 2.8, 6.3$ Hz, *m*-Ph), 5.44 (d, 1H, $J = 8.1$ Hz, NH), 4.55 (m, 1H, β -H), 3.71 (m, 1H, α -H), 3.70 (br s, OH), 2.97 (dd, 1H, $J = 3.3, 14.4$ Hz, PhCH_a), 2.68 (dd, 1H, $J = 11.6, 14.4$ Hz, PhCH_b).

^{13}C NMR (CDCl_3 , 75 MHz); δ 148.2 (ArC3), 140.6 (ArC1), 136.0 (*i*-Ph), 132.1 (ArC6), 130.0 (ArC5), 128.8 (Ph), 128.6 (Ph), 127.0, 126.7, 124.1 (q, $J = 283.3$ Hz, CF₃), 122.0 (ArC2), 72.7 (q, $J = 30.1$ Hz, β -C), 56.3 (α -C), 34.2 (PhCH₂).

^{19}F NMR (CDCl_3 , 282 MHz); δ -74.7 (d, 3F, $J = 7.4$ Hz).

Could not obtain LRMS or HRMS using ES.

3-Amino-(1*S,2*R**)-N-(1-Benzyl-3,3,3-trifluoro-2-hydroxypropyl)benzenesulfonamide (4.31)**



A solution of **4.30** (302 mg, 0.75 mmol) was reduced under an atmosphere of hydrogen as described in General Method I to give the metanilamide **4.31** as a foamy off-white solid (267 mg, 95 %, single diastereomer). Recrystallisation of the crude metanilamide from ethyl acetate gave pale yellow shingles.

mp 131-134 °C

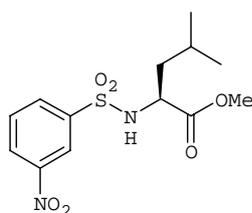
^1H NMR (CDCl_3 , 500 MHz); δ 7.02 (m, 3H, *m/p*-Ph), 6.92 (t, 1H, $J = 7.9$ Hz, ArH5), 6.88 (dd, 2H, $J = 2.5, 6.0$ Hz, *o*-Ph), 6.70 (d, 1H, $J = 7.7$ Hz, ArH6), 6.61 (d, 1H, $J = 8.0$ Hz, ArH4), 6.57 (s, 1H, ArH2), 5.92 (d, 1H, $J = 8.0$ Hz, NH), 5.84 (br s, 1H, OH), 4.22 (m, 1H, β -H), 3.94 (b, 2H, ArNH₂), 3.58 (m, 1H, α -H), 2.81 (dd, 1H, $J = 3.6, 14.4$ Hz, PhCH_a), 2.64 (dd, 1H, $J = 10.7, 14.4$ Hz, PhCH_b).

^{13}C NMR (CDCl_3 , 75 MHz); δ 147.1 (ArC3), 139.9 (ArC1), 136.7 (*i*-Ph), 129.3 (ArC5), 128.9 (Ph), 128.0 (Ph), 126.2 (*p*-Ph), 124.5 (q, $J = 283.6$ Hz, CF₃), 118.4 (ArC4), 115.7 (ArC6), 112.1 (ArC2), 71.7 (q, $J = 29.2$ Hz, β -C), 55.6 (α -C), 33.9 (PhCH₂).

^{19}F NMR (CDCl_3 , 282 MHz); δ -74.1 (d, 3F, $J = 8.0$ Hz).

HRMS (ES); 375.0991 (MH^+); $\text{C}_{16}\text{H}_{18}\text{F}_3\text{N}_2\text{O}_3\text{S}$ requires 375.0990.

4-(Methyl-2*S*-(3-nitrobenzenesulfonylamino)pentanoic acid methyl ester (4.32)

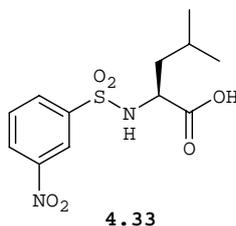


3-Nitrobenzenesulfonyl chloride (2.22 g, 10.0 mmol), *L*-leucine methyl ester hydrochloride (2.04 g, 11.0 mmol) and Hünigs base (3.83 mL, 22.0 mmol) in dry dichloromethane (50 mL) were heated at reflux following General Method C to give the desired product as a thick yellow oil. Flash chromatography (2:3 ethyl acetate/petroleum ether) of the crude ester gave **4.32** as a very pale yellow solid (3.10 g, 94 %, single enantiomer).

$[\alpha]_{\text{D}} = +2.8$ ($c = 1.01$, MeOH)

^1H NMR (CDCl_3 , 500 MHz); δ 8.67 (s, 1H, Ar**H2**), 8.42 (d, 1H, $J = 8.1$ Hz, Ar**H6**), 8.18 (d, 1H, $J = 7.9$ Hz, Ar**H4**), 7.73 (t, 1H, $J = 8.0$ Hz, Ar**H5**), 5.49 (d, 1H, $J = 9.9$ Hz, **NH**), 4.04 (dd, 1H, $J = 7.3, 17.2$ Hz, α -**H**), 3.48 (s, 3H, **OCH₃**), 1.76 (m, 1H, Leu**CH**), 1.53 (t, 2H, $J = 7.2$ Hz, Leu**CH₂**), 0.89 (dd, 6H, $J = 6.6, 9.5$ Hz, Leu**CH₃**).

^{13}C NMR (CDCl_3 , 75 MHz); δ 172.3 (**CO₂Me**), 148.0 (Ar**C3**), 142.1 (Ar**C1**), 132.7 (Ar**C6**), 130.4 (Ar**C5**), 127.1 (Ar**C4**), 122.3 (Ar**C2**), 54.5 (α -**C**), 52.4 (**OCH₃**), 41.9 (Leu**CH₂**), 24.2 (Leu**CH**), 22.6 (Leu**CH₃**), 21.2 (Leu**CH₃**).

4-Methyl-2S-(3-nitrobenzenesulfonylamino)pentanoic acid (4.33)

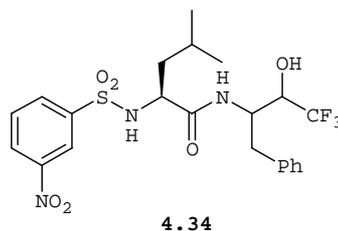
Methyl ester **4.32** (2.31 g, 6.99 mmol) was hydrolysed with K_2CO_3 (1.45 g, 10.5 mmol) in MeOH/ H_2O as described in General Method H affording the free acid **4.33** as a white solid (2.00 g, 90 %).

$[\alpha]_D = +12.4$ ($c = 1.01$, MeOH)

mp 144-146 °C

1H NMR (acetone- d_6 , 500 MHz); δ 8.76 (s, 1H, Ar**H2**), 8.60 (d, 1H, $J = 8.3$ Hz, Ar**H6**), 8.39 (d, 1H, $J = 7.8$ Hz, Ar**H4**), 8.02 (t, 1H, $J = 8.0$ Hz, Ar**H5**), 7.40 (d, 1H, $J = 9.5$ Hz, **NH**), 4.16 (m, 1H, α -**H**), 1.92 (m, 1H, Leu**CH**), 1.71 (m, 2H, Leu**CH**₂), 1.02 (dd, 6H, $J = 6.6, 16.5$ Hz, Leu**CH**₃).

^{13}C NMR (acetone- d_6 , 75 MHz); 172.4 (**CO**₂**H**), 148.4 (Ar**C3**), 143.4 (Ar**C1**), 133.0 (Ar**C6**), 131.0 (Ar**C5**), 127.0 (Ar**C4**), 122.1 (Ar**C2**), 54.6 (α -**C**), 41.6 (Leu**CH**₂), 24.4 (Leu**CH**), 22.4 (Leu**CH**₃), 20.8 (Leu**CH**₃).

4-Methyl-2S-(3-nitrobenzenesulfonylamino)pentanoic acid (1S*-benzyl-3,3,3-trifluoro-2-hydroxypropyl)amide (4.34)

The free acid **4.33** (1.58 g, 5.00 mmol) and **2.6** (1.40 g, 5.48 mmol) were coupled together utilising BOP (2.21 g, 5.00 mmol) and Hünigs base (1.92 mL, 11.0 mmol) in dichloromethane (20 mL) following General Method E to give the desired product as a

yellow solid. The crude trifluoromethyl carbinol was purified by flash chromatography (2:3 ethyl acetate/petroleum ether) to afford **4.34** as an off-white solid (2.21 g, 85 %) comprising a mixture of diastereomers (49:51 by ^1H NMR).

$[\alpha]_{\text{D}} = +4.9$ ($c = 1.01$, MeOH)

mp 162-163 °C

^1H NMR (acetone- d_6 , 500 MHz); Mixture (49:51) δ 8.73 (t, 1H, $J = 1.9$ Hz, Ar**H2**), 8.71* (t, 1H, $J = 1.9$ Hz, Ar**H2**), 8.57 (m, 2H, Ar**H4**, major + minor), 8.33* (d, 1H, $J = 7.8$ Hz, Ar**H6**), 8.23 (d, 1H, $J = 7.8$ Hz, Ar**H6**), 7.99* (t, 1H, $J = 8.0$ Hz, Ar**H5**), 7.89 (t, 1H, $J = 8.0$ Hz, Ar**H5**), 7.66 (d, 1H, $J = 8.2$ Hz, TFMC **NH**), 7.59* (d, 1H, $J = 8.7$ Hz, TFMC **NH**), 7.40-7.29 (m, 10H, *o/m/p*-Ph, major + minor), 7.21 (d, 1H, $J = 8.5$ Hz, Leu**NH**), 7.12* (d, 1H, $J = 9.2$ Hz, Leu**NH**), 5.85 (d, 1H, $J = 6.6$ Hz, **OH**), 5.69* (d, 1H, $J = 6.7$ Hz, **OH**), 4.42* (m, 1H, TFMC α -**H**), 4.31 (m, 1H, TFMC α -**H**), 4.14 (m, 1H, TFMC β -**H**), 4.01 (m, 2H, Leu α -**H**, major + minor), 3.89* (m, 1H, β -**H**), 3.16-2.88 (m, 4H, Ph**CH**₂, major + minor), 1.73 (m, 1H, Leu**CH**), 1.56* (m, 1H, Leu**CH**), 1.44 (m, 2H, Leu**CH**₂), 1.12* (m, 1H, Leu**CH**₂), 0.90 (d, 3H, $J = 6.7$ Hz, Leu**CH**₃), 0.84* (d, 3H, $J = 6.6$ Hz, Leu**CH**₃), 0.81 (d, 3H, $J = 6.5$ Hz, Leu**CH**₃), 0.80* (d, 3H, $J = 6.6$ Hz, Leu**CH**₃).

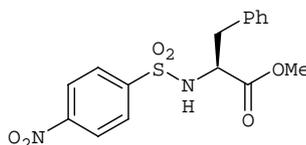
^{13}C NMR (acetone- d_6 , 75 MHz); Mixture δ 171.4 (**CONH**), 171.0* (**CONH**), 148.3 (Ar**C3**) 148.3* (Ar**C3**), 143.5* (Ar**C1**), 143.3 (Ar**C1**), 138.1* (*i*-Ph), 138.0 (*i*-Ph), 132.9 (Ar**C6**), 132.9* (Ar**C6**), 131.0 (Ar**C5**), 130.9* (Ar**C5**), 129.6, 129.6*, 128.5, 128.5*, 127.0, 127.0*, 126.6, 126.6*, 125.5 (q, $J = 283.4$ Hz, **CF**₃), 125.2* (q, $J = 283.6$ Hz, **CF**₃), 122.1 (Ar**C2**), 122.1* (Ar**C2**), 71.7* (q, $J = 29.1$ Hz, β -**C**), 70.9 (q, $J = 28.9$ Hz, β -**C**), 55.6, 55.3*, 51.3, 50.6*, 42.3* (Leu**CH**₂), 42.2 (Leu**CH**₂), 34.5 (Ph**CH**₂), 34.5* (Ph**CH**₂), 24.2, 24.1*, 22.6 (Leu**CH**₃), 22.6* (Leu**CH**₃), 20.8* (Leu**CH**₃), 20.7 (Leu**CH**₃).

^{19}F NMR (acetone- d_6 , 282 MHz); Mixture (48:52) δ -74.3* (d, 3F, $J = 7.1$ Hz), -74.5 (d, 3F, $J = 7.3$ Hz).

HRMS (ES) 518.1569 (MH^+); $\text{C}_{22}\text{H}_{27}\text{F}_3\text{N}_3\text{O}_6\text{S}$ requires 518.1573.

7.4.5 Synthesis of Sulfanilamides

2*S*-(4-Nitrobenzenesulfonylamino)-3-phenylpropionic acid methyl ester (**4.35**)



4-Nitrobenzenesulfonyl chloride (886 mg, 4.00 mmol), *L*-phenylalanine (950 mg, 4.40 mmol) and Hünigs base (1.53 mL, 8.80 mmol) in dry dichloromethane (25 mL) were heated at reflux following General Method C. Flash chromatography (dichloromethane) of the crude ester, followed by recrystallisation from aqueous MeOH gave **4.35** as a pale yellow solid (1.18 g, 81 %).

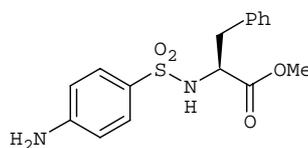
$[\alpha]_D = -17.5$ ($c = 0.50$, MeOH)

mp 151-152 °C

^1H NMR (acetone- d_6 , 500 MHz); δ 8.39 (d, 2H, $J = 8.7$ Hz, ArH₃), 8.00 (d, 2H, $J = 8.7$ Hz, ArH₂), 7.59 (d, 1H, $J = 9.3$ Hz, NH), 7.29 (m, 5H, *o/m/p*-Ph), 4.38 (m, 1H, α -H), 3.65 (s, 3H, OCH₃), 3.21 (dd, 1H, $J = 5.4, 13.8$ Hz, PhCH_a), 3.02 (dd, 1H, $J = 9.1, 13.8$ Hz, PhCH_b).

^{13}C NMR (acetone- d_6 , 75 MHz); δ 171.3 (CO₂Me), 150.0 (ArC₄), 146.9 (ArC₁), 136.5 (*i*-Ph), 129.5, 128.5, 128.2, 127.0 (*p*-Ph), 124.3 (ArC₃), 58.0 (α -C), 51.8 (OCH₃), 38.5 (PhCH₂).

HRMS (ES); 365.0812 (MH⁺); C₁₆H₁₇N₂O₆S requires 365.0807.

2S-(4-Aminobenzenesulfonylamino)-3-phenylpropionic acid methyl ester (4.36)

4.36

Ester **4.35** (729 mg, 2.00 mmol) was reduced in an atmosphere of hydrogen following General Method I to give sulfanilamide **4.36** as a pale yellow solid. Flash chromatography (1:9 ethyl acetate/dichloromethane gave a white solid (655 mg, 98 %).

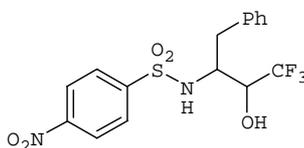
$[\alpha]_D = +8.8$ (c = 1.0, MeOH)

mp 111-112 °C

^1H NMR (acetone- d_6 , 500 MHz); δ 7.54 (d, 2H, J = 8.7 Hz, ArH₂), 7.34 (m, 2H, *m/p*-Ph), 7.27 (d, 2H, J = 6.9 Hz, *o*-Ph), 6.79 (d, 2H, J = 8.7 Hz, ArH₃), 6.59 (d, 1H, J = 9.3 Hz, NH), 5.58 (s, 2H, ArNH₂), 4.16 (m, 1H, α -H), 3.54 (s, 3H, OCH₃), 3.08 (dd, 1H, J = 6.9, 13.6 Hz, PhCH_a), 3.04 (m, 1H, PhCH_b).

^{13}C NMR (acetone- d_6 , 75 MHz); δ 171.6 (CO₂Me), 152.7 (ArC₄), 136.6 (*i*-Ph), 129.5, 129.2, 128.5, 127.1 (ArC₁), 126.9 (*p*-Ph), 113.2 (ArC₃), 57.6 (α -C), 51.5 (OCH₃), 38.9 (PhCH₂).

HRMS (ES); 335.1067 (MH⁺); C₁₆H₁₉N₂O₄S requires 335.1066.

(1S*,2R*)-N-(1-Benzyl-3,3,3-trifluoro-2-hydroxypropyl)-4-nitrobenzenesulfonamide (4.37)

4.37

4-Nitrobenzenesulfonyl chloride (228 mg, 1.00 mmol), **2.6** (281 mg, 1.10 mmol) and Hünigs base (0.38 mL, 2.18 mmol) in dry dichloromethane (10 mL) were heated at reflux following General Method C. Flash chromatography (dichloromethane) of the crude

trifluoromethyl carbinol gave **4.37** as a pale yellow solid (403 mg, quantitative, single diastereomer).

mp 129-131 °C

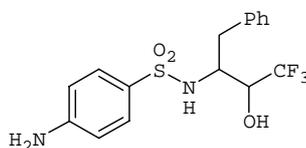
^1H NMR (acetone- d_6 , 500 MHz); δ 8.17 (d, 2H, $J = 8.9$ Hz, ArC3), 7.73 (d, 2H, $J = 8.9$ Hz, ArH2), 7.40 (d, 1H, $J = 8.3$ Hz, NH), 7.13-7.07 (m, 5H, *o/m/p*-Ph), 6.05 (d, 1H, $J = 6.9$ Hz, OH), 4.56 (m, 1H, β -H), 3.92 (m, 1H, α -H), 3.06 (dd, 1H, $J = 2.9, 14.4$ Hz, PhCH_a), 2.90 (dd, 1H, $J = 11.2, 14.4$ Hz, PhCH_b).

^{13}C NMR (acetone- d_6 , 75 MHz); δ 149.6 (ArC4), 146.4 (ArC1), 137.4 (*i*-Ph), 129.4, 128.4, 127.7, 126.3 (*p*-Ph), 125.2 (q, $J = 283.5$, CF₃), 124.2 (ArC3), 73.3 (q, $J = 29.0$ Hz, β -C), 56.5 (α -C), 34.1 (PhCH₂).

^{19}F NMR (acetone- d_6 , 282 MHz); δ -74.2 (d, 3F, $J = 8.0$ Hz).

Could not obtain LRMS or HRMS using ES.

4-Amino-(1*S**,2*R**)-*N*-(1-benzyl-3,3,3-trifluoro-2-hydroxypropyl)benzenesulfonamide (4.38)



A solution of **4.37** (1.32 g, 3.62 mmol) was reduced in an atmosphere of hydrogen following General Method I to give the sulfanilamide **4.38** as a pale yellow solid (1.20 g, 99 %, single diastereomer).

mp 175-180 °C

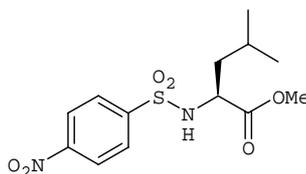
^1H NMR (acetone- d_6 , 500 MHz); δ 7.29-7.24 (m, 5H, ArH2, *o/p*-Ph), 7.17 (dd, 2H, $J = 3.0, 6.5$ Hz, *m*-Ph), 6.63 (d, 2H, $J = 8.7$ Hz, ArH3), 6.32 (d, 1H, $J = 7.8$ Hz, NH), 5.85 (d, 1H, $J = 6.9$ Hz, OH), 5.50 (s, 2H, ArNH₂), 4.52 (m, 1H, β -H), 3.77 (m, 1H, α -H), 3.04 (dd, 1H, $J = 3.5, 14.5$ Hz, PhCH_a), 2.92 (dd, 1H, $J = 10.2, 14.5$ Hz, PhCH_b).

^{13}C NMR (acetone- d_6 , 75 MHz); δ 152.5 (ArC4), 137.6 (*i*-Ph), 129.4 (ArC2), 128.8, 128.4, 126.6 (ArC1), 126.4 (*p*-Ph), 125.5 (q, $J = 283.3$ Hz, CF₃), 113.3 (ArC3), 71.1 (q, $J = 28.8$ Hz, β -C), 55.2 (α -C), 34.3 (PhCH₂).

^{19}F NMR (acetone- d_6 , 282 MHz); δ -74.1 (d, 3F, $J = 8.1$ Hz).

HRMS (EI) 374.0900 (M^+); $C_{16}H_{17}F_3N_2O_3S$ requires 374.0912.

4-(Methyl-2*S*-(4-nitrobenzenesulfonylamino)pentanoic acid methyl ester (4.39)



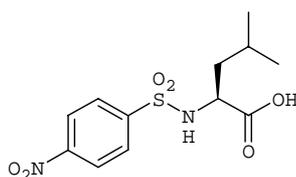
4-Nitrobenzenesulfonyl chloride (2.22 g, 10.0 mmol), *L*-leucine methyl ester hydrochloride (2.04 g, 11.0 mmol) and Hünigs base (3.83 mL, 22.0 mmol) in dry dichloromethane (50 mL) were heated at reflux following General Method C to give the desired product as a pale brown solid. Flash chromatography (2:3 ethyl acetate/petroleum ether) of the crude ester gave **4.39** as a pale yellow solid (3.24 g, 98 %).

$[\alpha]_D = +5.8$ ($c = 1.02$, MeOH)

mp 91-92 °C

^1H NMR (CDCl_3 , 500 MHz); 8.34 (d, 2H, $J = 8.9$ Hz, Ar**H3**), 8.03 (d, 2H, $J = 8.9$ Hz, Ar**H2**), 5.32 (d, 1H, $J = 9.9$ Hz, NH), 4.02 (m, 1H, α -**H**), 3.49 (s, 3H, O**CH**₃), 1.76 (m, 1H, Leu**CH**) 1.53 (m, 2H, Leu**CH**₂), 0.90 (dd, 6H, $J = 6.7, 8.0$ Hz, Leu**CH**₃).

^{13}C NMR (CDCl_3 , 75 MHz); δ 171.9 (**CO**₂Me), 150.3 (Ar**C4**), 147.1 (Ar**C1**), 128.6 (Ar**C2**), 124.4 (Ar**C3**), 54.8 (α -**C**), 51.7 (O**CH**₃), 41.5 (Leu**CH**₂), 24.4 (Leu**CH**), 22.3 (Leu**CH**₃), 20.8 (Leu**CH**₃).

4-(Methyl-2*S*-(4-nitrobenzenesulfonylamino)pentanoic acid (4.40)

4.40

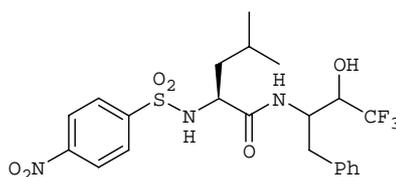
Methyl ester **4.39** (2.31 g, 6.99 mmol) was hydrolysed with K_2CO_3 (1.45 g, 10.5 mmol) in MeOH/H₂O following General Method H to afford the free acid **4.40** as a white solid (1.57 g, 72 %).[§]

$[\alpha]_D = +16.7$ ($c = 1.01$, MeOH)

mp 160-162 °C

¹H NMR (acetone-*d*₆, 500 MHz); δ 8.53 (d, 2H, $J = 8.9$ Hz, Ar**H3**), 8.25 (d, 2H, $J = 8.9$ Hz, Ar**H2**), 7.37 (d, 1H, $J = 9.6$ Hz, NH), 4.16 (m, 1H, α -**H**), 1.92 (m, 1H, Leu**CH**), 1.70 (m, 1H, Leu**CH**₂), 1.03 (d, 3H, $J = 6.6$ Hz, Leu**CH**₃), 1.00 (d, 3H, $J = 6.6$ Hz, Leu**CH**₃).

¹³C NMR (acetone-*d*₆, 75 MHz); δ 172.4 (CO₂H), 150.2 (Ar**C4**), 147.1 (Ar**C1**), 128.7 (Ar**C2**), 124.3 (Ar**C3**), 54.6 (α -**C**), 41.6 (Leu**CH**₂), 24.4 (Leu**CH**), 22.4 (Leu**CH**₃), 20.7 (Leu**CH**₃).

4-Methyl-2*S*-(4-nitrobenzenesulfonylamino)pentanoic acid (1*S*^{*},2*R*^{*})-(1-benzyl-3,3,3-trifluoro-2-hydroxypropyl)amide (4.41)

4.41

The free acid **4.40** (1.00 g, 3.16 mmol) and **2.6** (0.89 g, 3.48 mmol) were coupled together utilising BOP (1.40 g, 3.17 mmol) and Hünigs base (1.21 mL, 6.32 mmol) in dichloromethane (20 mL) following General Method E to give the desired product as a

[§] Lower than expected yield due to spillage of reaction mixture!

pale orange solid. The crude trifluoromethyl carbinol was purified twice by flash chromatography (1:9 ethyl acetate/dichloromethane) to afford **4.41** as an off-white solid (1.26 g, 77 %) comprising a mixture of diastereomers (2:3 by ^1H NMR).

$[\alpha]_{\text{D}} = +3.5$ ($c = 1.0$, MeOH)

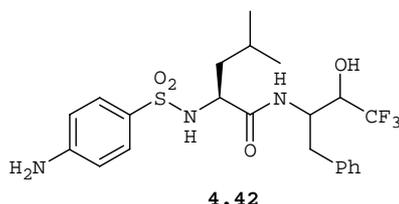
^1H NMR (acetone- d_6 , 500 MHz); Mixture (2:3) δ 8.50 (d, 2H, $J = 8.8$ Hz, Ar**H3**), 8.38* (d, 2H, $J = 8.8$ Hz, Ar**H3**), 8.18 (d, 2H, $J = 8.8$ Hz, Ar**H2**), 8.09* (d, 2H, $J = 8.8$ Hz, Ar**H2**), 7.69* (d, 1H, $J = 8.0$ Hz, TFMC **NH**), 7.62 (d, 1H, $J = 8.7$ Hz, TFMC **NH**), 7.42-7.28 (m, 10H, *o/m/p*-Ph, major + minor), 7.19* (d, 1H, $J = 9.3$ Hz, Leu**NH**), 7.10 (d, 1H, $J = 9.2$ Hz, Leu**NH**), 5.88* (d, 1H, $J = 6.4$ Hz, **OH**), 5.77 (d, 1H, $J = 6.5$ Hz, **OH**), 4.44 (m, 1H, TFMC α -**H**), 4.29* (m, 1H, TFMC α -**H**), 4.15* (m, 1H, TFMC β -**H**), 4.02 (m, 2H, Leu α -**H**, major + minor), 3.91 (m, 1H, TFMC β -**H**), 3.12 (m, 1H, Ph**CH_a**), 3.02* (m, 1H, Ph**CH₂**), 2.92 (dd, 1H, $J = 11.7, 13.9$ Hz, Ph**CH_b**), 1.76* (m, 1H, Leu**CH**), 1.56 (m, 1H, Leu**CH**), 1.47* (m, 1H, Leu**CH_a**), 1.41* (m, 1H, Leu**CH_b**), 1.19 (m, 1H, Leu**CH_a**), 1.06 (m, 1H, Leu**CH_b**), 0.92* (d, 3H, $J = 6.7$ Hz, Leu**CH₃**), 0.83 (m, 9H, Leu**CH₃**, major + minor).

^{13}C NMR (acetone- d_6 , 75 MHz); Mixture δ 171.4* (**CONH**), 171.0 (**CONH**), 150.2 (Ar**C4**), 150.1* (Ar**C4**), 147.3 (Ar**CI**), 147.1* (Ar**CI**), 138.2* (*i*-Ph), 138.1 (*i*-Ph), 129.6 (x2), 128.6 (x2), 128.5 (x2), 126.6* (*p*-Ph), 126.6 (*p*-Ph), 125.5* (q, $J = 285.3$ Hz, **CF₃**), 125.3 (q, $J = 283.5$ Hz, **CF₃**), 124.3* (Ar**C3**), 124.3 (Ar**C3**), 71.7 (q, $J = 29.1$ Hz, β -**C**), 71.0* (q, $J = 28.8$ Hz, β -**C**), 55.6*, 55.4, 51.5*, 50.6, 42.3 (Leu**CH₂**), 42.3* (Leu**CH₂**), 34.5* (Ph**CH₂**), 34.4 (Ph**CH₂**), 24.2* (Leu**CH**), 24.1 (Leu**CH**), 22.6 (Leu**CH₃**), 22.6* (Leu**CH₃**), 20.8 (Leu**CH₃**), 20.7* (Leu**CH₃**).

^{19}F NMR (acetone- d_6 , 282 MHz); Mixture (2:3) δ -74.3 (d, 3F, $J = 7.6$ Hz), -74.5 (d, 3F, $J = 7.8$ Hz).

HRMS (ES) 518.1576 (MH^+); $\text{C}_{22}\text{H}_{27}\text{F}_3\text{N}_3\text{O}_6\text{S}$ requires 518.1573.

4-Methyl-2*S*-(4-aminobenzenesulfonylamino)pentanoic acid (1*S*^{*},2*R*^{*})-(1-benzyl-3,3,3-trifluoro-2-hydroxypropyl)amide (4.42)



A solution of **4.41** (1.05 g, 2.03 mmol,) was reduced under an atmosphere of hydrogen following General Method I. Flash chromatography (1:4 ethyl acetate/dichloromethane) of the crude sulfanilamide afforded **4.42** as a white solid (858 mg, 87 %) comprising a mixture of diastereomers (2:3 by ¹H NMR).

$[\alpha]_D = -26.2$ ($c = 1.0$, MeOH)

¹H NMR (acetone-*d*₆, 500 MHz); Mixture (2:3) δ 7.61 (dd, 4H, $J = 3.3, 8.7$ Hz, ArH₂, major + minor), 7.51 (m, 2H, TFMC NH, major + minor), 7.40-7.30 (m, 10H, *o/m/p*-Ph, major + minor), 6.84 (t, 4H, $J = 8.3$ Hz, ArH₃, major + minor), 6.36* (d, 1H, $J = 7.0$ Hz, LeuNH), 6.14 (d, 1H, $J = 8.5$ Hz, LeuNH), 5.86* (d, 1H, $J = 6.5$ Hz, OH), 5.65 (d, 1H, $J = 6.3$ Hz, OH), 5.60* (s, 2H, ArNH₂), 5.57 (s, 2H, ArNH₂), 4.46 (m, 2H, TFMC α -H, major + minor), 4.19* (m, 1H, TFMC β -H), 4.07 (m, 1H, TFMC β -H), 3.73 (m, 1H, Leu α -H), 3.68* (m, 1H, Leu α -H), 3.09 (m, 2H, PhCH_a, major + minor), 2.98 (m, 2H, PhCH_a, major + minor), 1.61* (m, 1H, LeuCH), 1.53 (m, 1H, LeuCH), 1.31* (m, 2H, LeuCH₂), 1.15 (m, 2H, LeuCH₂), 0.83* (d, 3H, $J = 6.7$ Hz, LeuCH₃), 0.81 (d, 3H, $J = 6.6$ Hz, LeuCH₃), 0.74 (d, 3H, $J = 6.5$ Hz, LeuCH₃), 0.69* (d, 3H, $J = 6.5$ Hz, LeuCH₃).

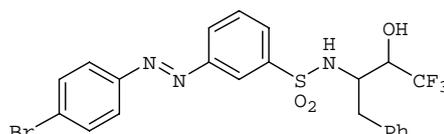
¹³C NMR (acetone-*d*₆, 75 MHz); Mixture δ 172.3* (CONH), 172.0 (CONH), 152.9* (ArC₄), 152.7 (ArC₄), 138.2 (*i*-Ph), 138.0* (*i*-Ph), 129.6* (ArC₂), 129.6 (ArC₂), 129.4, 129.4*, 128.4, 128.4*, 127.2 (ArC₁), 127.2* (ArC₁), 126.5 (*p*-Ph) 126.5* (*p*-Ph), 125.5* (q, $J = 283.2$ Hz, CF₃), 125.4 (q, $J = 283.4$ Hz, CF₃), 113.2 (ArC₃), 113.2* (ArC₃), 71.5 (q, $J = 29.1$ Hz, β -C), 71.0* (q, $J = 28.9$ Hz, β -C), 55.5*, 55.1, 50.9*, 50.5, 42.3 (LeuCH₂), 42.1* (LeuCH₂), 34.5* (PhCH₂), 34.2 (PhCH₂), 24.1* (LeuCH), 24.0 (LeuCH), 22.6 (LeuCH₃), 22.5* (LeuCH₃), 21.0 (LeuCH₃), 20.9* (LeuCH₃).

¹⁹F NMR (acetone-*d*₆, 282 MHz); Mixture (43:57) δ -74.2* (d, 3F, $J = 7.3$ Hz), -74.4 (d, 3F, $J = 7.8$ Hz).

HRMS (EI) 487.1757 (M^+); $C_{22}H_{28}F_3N_3O_4S$ requires 487.1753.

7.4.6 Synthesis of 4',3-disubstituted azobenzenes

E-[(1*S**,2*R**)-*N*-(1-Benzyl-3,3,3-trifluoro-2-hydroxypropyl)-3-(4-bromophenylazo)benzenesulfonamide] (**4.43**)



4.43

Metanilamide **4.31** (103 mg, 0.30 mmol) and 4-bromonitrosobenzene **4.25** (56 mg, 0.30 mmol) in glacial acetic acid (1.2 mL) were condensed following General Method K. Column chromatography of the crude azobenzene (1:9 ethyl acetate/dichloromethane) gave **4.43** as an orange solid (123 mg, 76 %, single diastereomer).

mp 140-141 °C

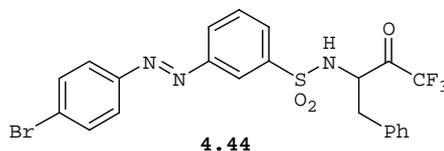
1H NMR (acetone- d_6 , 500 MHz); δ 8.14 (d, 1H, $J = 7.9$ Hz, ArH4), 8.05 (d, 2H, $J = 8.7$ Hz, ArH2'), 8.01 (s, 1H, ArH2), 7.95 (d, 2H, $J = 8.7$ Hz, ArH3'), 7.73 (d, 1H, $J = 7.9$ Hz, ArH6), 7.67 (t, 1H, $J = 6.1$ Hz, ArH5), 7.17 (d, 1H, $J = 8.2$ Hz, NH), 7.13 (d, 2H, $J = 7.2$ Hz, *o*-Ph), 7.05 (t, 2H, $J = 7.3$ Hz, *m*-Ph), 7.00 (m, 1H, $J = 7.3$ Hz, *p*-Ph), 6.04 (d, 1H, $J = 6.9$ Hz, OH), 4.58 (m, 1H, β -H), 3.94 (m, 1H, α -H), 3.06 (dd, 1H, $J = 3.0, 14.4$ Hz, PhCH_a) 2.94 (dd, 1H, $J = 10.9, 14.4$ Hz, PhCH_b).

^{13}C NMR (acetone- d_6 , 75 MHz); δ 152.3, 151.5, 142.4 (ArC3), 137.4 (*i*-Ph), 132.9 (ArC3'), 130.3, 129.3 (Ph), 129.1, 128.3 (Ph), 127.1, 126.7 (*p*-Ph), 125.9 (ArC4'), 125.4 (q, $J = 283.5$ Hz, CF₃), 124.9 (ArC2'), 120.0 (ArC2), 73.0 (q, $J = 28.8$ Hz, β -C), 56.2 (α -C), 34.2 (PhCH₂).

^{19}F NMR (acetone- d_6 , 282 MHz); δ -74.2 (d, 3F, $J = 8.0$ Hz).

HRMS (EI) 541.0278 (M^+); $C_{22}H_{19}BrF_3N_3O_3S$ requires 541.0283.

***E*-[*N*-(1*S**-Benzyl-3,3,3-trifluoro-2-oxopropyl)-3-(4-bromophenylazo)benzene sulfonamide] (**4.44**)**



A solution of **4.43** (22 mg, 40 μ mol) in dichloromethane (0.5 mL) was oxidised with TEMPO/NaOCl in 2 h following Modified General Method G. Flash chromatography (1:9 ethyl acetate/dichloromethane) of the crude product, followed by recrystallisation from aqueous MeOH gave the hydrate of **4.44** as an orange solid (18 mg, 83 %, racemic).

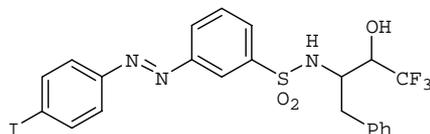
mp 86-88 °C

^1H NMR (acetone- d_6 , 500 MHz); Hydrate δ 8.06 (d, 1H, $J = 7.9$ Hz, Ar**H4**), 8.01 (d, 2H, $J = 8.7$ Hz, Ar**H2'**), 7.91 (d, 3H, $J = 8.8$ Hz, Ar**H3'**, Ar**H2**), 7.74 (d, 1H, $J = 7.9$ Hz, Ar**H6**), 7.62 (t, 1H, $J = 7.9$ Hz, Ar**H5**), 7.10 (d, 2H, $J = 7.2$ Hz, *o*-Ph), 6.95 (t, 2H, $J = 7.4$ Hz, *m*-Ph), 6.89 (t, 1H, $J = 7.3$ Hz, *p*-Ph), 4.08 (dd, 1H, $J = 2.5, 11.0$ Hz, α -**H**), 3.31 (dd, 1H, $J = 2.6, 14.3$ Hz, Ph**CH_a**), 2.76 (dd, 1H, $J = 11.1, 14.3$ Hz, Ph**CH_b**).

^{13}C NMR (acetone- d_6 , 75 MHz); Hydrate δ 151.8, 151.2, 143.2 (Ar**C3**), 137.8 (*i*-Ph), 132.7 (Ar**C3'**), 130.0, 129.2 (Ph), 128.9, 128.1 (Ph), 126.8, 126.4, 125.7 (Ar**C4'**), 124.7 (Ar**C2'**), 119.6 (Ar**C2**), 93.5 (q, $J = 29.7$ Hz, β -**C**), 60.8 (α -**C**), 35.4 (Ph**CH₂**). Couldn't detect CF_3 quartet over baseline noise.

^{19}F NMR (acetone- d_6 , 282 MHz); Hydrate δ -78.9 (s, 3F).

***E*-[(1*S*^{*},2*R*^{*})-*N*-(1-Benzyl-3,3,3-trifluoro-2-hydroxypropyl)-3-(4-iodophenylazo)benzenesulfonamide] (4.45)**



4.45

Metanilamide **4.31** (103 mg, 0.30 mmol) and 4-iodonitrosobenzene **4.26** (70 mg, 0.30 mmol) in glacial acetic acid (1.2 mL) were condensed following General Method K. Column chromatography of the crude azobenzene (1:9 ethyl acetate/dichloromethane) gave the **4.45** as an orange solid (137 mg, 77 %, single diastereomer).

mp 134-137 °C

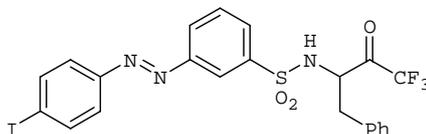
¹H NMR (acetone-*d*₆, 500 MHz); δ 8.16 (d, 2H, *J* = 8.7 Hz, ArH3'), 8.14 (d, 1H, *J* = 7.9 Hz, ArH4), 8.01 (s, 1H, ArH2), 7.89 (d, 2H, *J* = 8.6 Hz, ArH2'), 7.73 (d, 1H, *J* = 7.8 Hz, ArH6), 7.66 (t, 1H, *J* = 7.8 Hz, ArH5), 7.17 (d, 1H, *J* = 8.2 Hz, NH), 7.13 (d, 2H, *J* = 7.1 Hz, *o*-Ph), 7.05 (t, 2H, *J* = 7.3 Hz, *m*-Ph), 6.99 (t, 1H, *J* = 7.2 Hz, *p*-Ph), 6.02 (d, 1H, *J* = 6.9 Hz, OH), 4.59 (m, 1H, β-H), 3.94 (m, 1H, α-H), 3.06 (dd, 1H, *J* = 2.9, 14.4 Hz, PhCH_a), 2.94 (dd, 1H, *J* = 10.9, 14.4 Hz, PhCH_b).

¹³C NMR (acetone-*d*₆, 75 MHz); δ 152.2, 151.9, 142.4 (ArC3), 139.0 (ArC3'), 137.4 (*i*-Ph), 130.2, 129.2 (Ph), 129.1, 128.3 (Ph), 127.1, 126.6, 125.4 (q, *J* = 283.4 Hz, CF₃), 124.8 (ArC2'), 120.0 (ArC2), 98.5 (ArC4'), 73.0 (q, *J* = 28.7 Hz, β-C), 56.2 (α-C), 34.1 (PhCH₂).

¹⁹F NMR (acetone-*d*₆, 282 MHz); δ -74.2 (d, 3F, *J* = 8.0 Hz).

HRMS (EI) 589.0146 (M⁺); C₂₂H₁₉F₃IN₃O₃S requires 589.0144.

***E*-[*N*-(1*S**-Benzyl-3,3,3-trifluoro-2-oxopropyl)-3-(4-iodophenylazo)benzene sulfonamide] (4.46)**



4.46

A solution of **4.45** (24 mg, 40 μmol) in dichloromethane (0.5 mL) was oxidised with TEMPO/NaOCl in 2 h following Modified General Method G. Flash chromatography (1:9 ethyl acetate/dichloromethane) of the crude product, followed by recrystallisation from aqueous MeOH gave the ketone/hydrate mixture **4.46** as an orange solid (19 mg, 81 %, racemic). One drop of D_2O was added to the NMR mixture to give the hydrate exclusively. mp 109-112 $^\circ\text{C}$

^1H NMR (acetone- d_6 , 500 MHz); Hydrate δ 8.14 (d, 2H, $J = 8.5$ Hz, Ar**H3'**), 8.07 (d, 1H, $J = 7.8$ Hz, Ar**H4**), 7.92 (s, 1H, Ar**H2**), 7.87 (d, 2H, $J = 8.5$ Hz, Ar**H2'**), 7.73 (d, 1H, $J = 7.9$ Hz, Ar**H6**), 7.63 (t, 1H, $J = 7.8$ Hz, Ar**H5**), 7.10 (d, 2H, $J = 7.4$ Hz, *o*-Ph), 6.96 (t, 2H, $J = 7.5$ Hz, *m*-Ph), 6.89 (t, 1H, $J = 7.4$ Hz, *p*-Ph), 4.08 (dd, 1H, $J = 2.2, 11.0$ Hz, α -H), 3.30 (dd, 1H, $J = 2.4, 14.2$ Hz, Ph**CH_a**), 2.76 (dd, 1H, $J = 11.3, 14.1$ Hz, Ph**CH_b**).

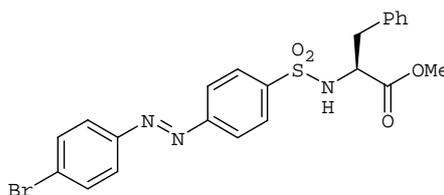
^{13}C NMR (acetone- d_6 , 75 MHz); Hydrate δ 152.0, 151.9, 143.0 (Ar**C3**), 138.9 (Ar**C3'**), 137.8 (*i*-Ph), 130.1, 129.3 (Ph), 129.0, 128.2 (Ph), 127.0, 126.6, 124.7 (Ar**C2'**), 124.0 (q, $J = 289.2$ Hz, CF_3), 119.8 (Ar**C2**), 98.4 (Ar**C4'**), 93.8 (q, $J = 29.6$ Hz, β -C) 61.0 (α -C), 35.5 (Ph**CH₂**).

^{19}F NMR (acetone- d_6 , 282 MHz); Hydrate δ -78.9 (s, 3F).

HRMS (ES) 606.0165 (MH^+); $\text{C}_{22}\text{H}_{20}\text{F}_3\text{IN}_3\text{O}_4\text{S}$ requires 606.0171.

7.4.7 Synthesis of 4',4-disubstituted azobenzenes

E-{2*S*-[4-(4-Bromophenylazo)benzenesulfonylamino]-3-phenylpropionic acid methyl ester} (**4.47**)



Sulfanilamide **4.36** (251 mg, 0.75 mmol) and 4-bromonitrosobenzene **4.25** (140 mg, 0.75 mmol) in glacial acetic acid (3.0 mL) were condensed following General Method K. Column chromatography of the crude azobenzene (1:9 ethyl acetate/dichloromethane), followed by recrystallisation from aqueous MeOH gave **4.47** as an orange crystalline solid (261 mg, 69 %).

$[\alpha]_D = +20.0$, ($c = 0.20$, MeOH)

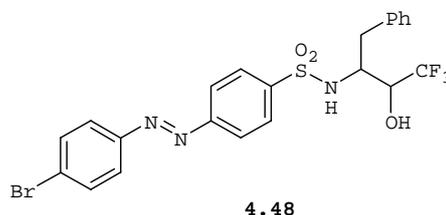
mp 163-165 °C

$^1\text{H NMR}$ (acetone- d_6 , 500 MHz); 8.10 (d, 2H, $J = 8.5$ Hz, ArH₂), 8.05 (d, 2H, $J = 8.6$ Hz, ArH₂'), 8.00 (d, 2H, $J = 8.5$ Hz, ArH₃), 7.95 (d, 2H, $J = 8.6$ Hz, ArH₃'), 7.32 (m, 6H, *o/m/p*-Ph, NH), 4.36 (m, 1H, α -H), 3.58 (s, 3H, OCH₃), 3.19 (dd, 1H, $J = 6.2, 13.6$ Hz, PhCH_a), 3.06 (dd, 1H, $J = 8.4, 13.7$ Hz, PhCH_b).

$^{13}\text{C NMR}$ (acetone- d_6 , 75 MHz); δ 171.3 (CO₂Me), 154.3, 151.5, 143.4 (ArC₄), 136.5 (*i*-Ph), 132.9 (ArC₃'), 129.5, 128.6, 128.3, 127.0 (*p*-Ph), 126.2 (ArC₄'), 125.0 (ArC₂'), 123.3 (ArC₂), 57.9 (α -C), 51.7 (OCH₃), 38.7 (PhCH₂).

HRMS (ES) 502.0447 (MH⁺); C₂₂H₂₁BrN₃O₄S requires 502.0436.

***E*-[(1*S*^{*},2*R*^{*})-*N*-(1-Benzyl-3,3,3-trifluoro-2-hydroxypropyl)-4-(4-bromophenylazo)benzenesulfonamide] (4.48)**



Sulfanilamide **4.38** (206 mg, 0.60 mmol, single diastereomer) and 4-bromonitrosobenzene **4.25** (113 mg, 0.60 mmol) in glacial acetic acid (2.4 mL) were condensed following General Method K. Column chromatography of the crude azobenzene (1:9 ethyl acetate/dichloromethane) gave **4.48** as an orange solid (208 mg, 64 %, single diastereomer). An analytical sample was recrystallised from aqueous MeOH to afford an orange crystalline solid.

mp 135-138 °C

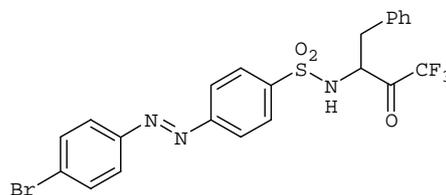
¹H NMR (acetone-*d*₆, 500 MHz); δ 8.06 (d, 2H, *J* = 8.6 Hz, ArH2'), 7.95 (d, 2H, *J* = 8.6 Hz, ArH3'), 7.90 (d, 2H, *J* = 8.5 Hz, ArH2), 7.72 (d, 2H, *J* = 8.5 Hz, ArH3), 7.16 (m, 3H, *m*-Ph, NH), 7.12 (m, 3H, *o/p*-Ph), 6.03 (d, 1H, *J* = 6.9 Hz, OH), 4.56 (m, 1H, β-H), 3.93 (m, 1H, α-H), 3.07 (dd, 1H, *J* = 3.0, 14.4 Hz, PhCH_a), 3.01 (s, 2H, H₂O), 2.95 (dd, 1H, *J* = 10.9, 14.6 Hz, PhCH_b).

¹³C NMR (acetone-*d*₆, 75 MHz); δ 154.0, 151.6, 143.1 (ArC4), 137.5 (*i*-Ph), 132.9 (ArC3'), 129.4, 128.5, 127.8, 126.5 (*p*-Ph), 126.2 (ArC4'), 125.4 (q, *J* = 283.5 Hz, CF₃), 125.0 (ArC2'), 123.3 (ArC2), 73.0 (q, *J* = 28.9 Hz, β-H), 56.1 (α-C), 34.2 (PhCH₂).

¹⁹F NMR (acetone-*d*₆, 282 MHz); δ -74.2 (d, 3F, *J* = 8.0 Hz).

HRMS (EI) 541.0280 (M⁺); C₂₂H₁₉BrF₃N₃O₃S requires 541.0283.

***E*-[*N*-(1*S**-Benzyl-3,3,3-trifluoro-2-oxopropyl)-4-(4-bromophenylazo)benzene sulfonamide] (4.49)**



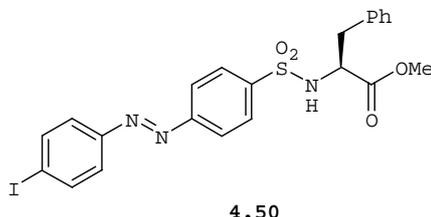
Trifluoromethyl carbinol **4.48** (81 mg, 0.15 mmol, single diastereomer) was oxidised with TEMPO/NaOCl over 3 h in a mixture of ethyl acetate/dichloromethane (2:1, 15 mL) following General Method G. The crude product was recrystallised from aqueous MeOH to afford the hydrate of **4.49** as an orange solid (57 mg, 70 %, racemic).

^1H NMR (acetone- d_6 , 500 MHz); Hydrate δ 8.08 (d, 2H, $J = 8.7$ Hz, Ar**H2'**), 7.96 (d, 2H, $J = 8.7$ Hz, Ar**H3'**), 7.86 (d, 2H, $J = 8.6$ Hz, Ar**H2**), 7.71 (d, 2H, $J = 8.6$ Hz, Ar**H3**), 7.35 (br s, 1H, **NH**), 7.17 (dd, 2H, $J = 1.9, 7.1$ Hz, *m*-Ph), 7.05 (m, 3H, *o/p*-Ph), 6.37 (br s, 1H, **OH**), 4.05 (d, 1H, $J = 9.9$ Hz, α -**H**), 3.37 (dd, 1H, $J = 2.4, 14.3$ Hz, Ph**CH_a**), 2.85 (dd, 1H, $J = 11.3, 14.3$ Hz, Ph**CH_b**).

^{13}C NMR (acetone- d_6 , 75 MHz); Hydrate δ 153.5, 151.4, 143.7 (Ar**C4**), 137.9 (*i*-Ph), 132.8, 129.4, 128.2, 127.5, 126.2 (*p*-Ph), 125.9 (Ar**C4'**), 124.8, 123.9 (q, $J = 289.1$ Hz, **CF₃**), 123.0, 93.5 (q, $J = 29.9$ Hz, β -**C**), 60.8 (α -**C**), 35.4 (Ph**CH₂**).

^{19}F NMR (acetone- d_6 , 282 MHz); Hydrate δ -79.0 (s, 3F).

HRMS (ES) 558.0311 (MH^+); $\text{C}_{22}\text{H}_{20}\text{BrF}_3\text{N}_3\text{O}_4\text{S}$ requires 558.0310.

***E*-{2*S*-[4-(4-Iodophenylazo)benzenesulfonylamino]-3-phenylpropionic acid methyl ester} (4.50)**

Sulfanilamide **4.36** (100 mg, 0.30 mmol) and 4-iodonitrosobenzene **4.26** (70 mg, 0.30 mmol) in glacial acetic acid (1.2 mL) were condensed following General Method K. Column chromatography of the crude azobenzene (1:9 ethyl acetate/dichloromethane), followed by recrystallisation from aqueous MeOH gave **4.50** as an orange crystalline solid (105 mg, 64 %).

$[\alpha]_{\text{D}} = +16.3$, ($c = 0.22$, MeOH)

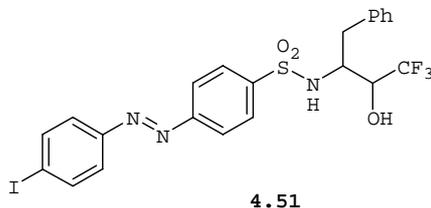
mp 177-179 °C

^1H NMR (acetone- d_6 , 500 MHz): δ 8.16 (d, 2H, $J = 8.5$ Hz, ArH3'), 8.09 (d, 2H, $J = 8.4$ Hz, ArH2), 8.00 (d, 2H, $J = 8.4$ Hz, ArH3), 7.89 (d, 2H, $J = 8.5$ Hz, ArH2'), 7.31 (m, 6H, *o/m/p*-Ph, NH), 4.35 (m, 1H, α -H), 3.58 (s, 3H, OCH₃), 3.19 (dd, 1H, $J = 6.2, 13.6$ Hz, PhCH_a), 3.06 (dd, 1H, $J = 8.4, 13.6$ Hz, PhCH_b).

^{13}C NMR (acetone- d_6 , 75 MHz): δ 171.3 (CO₂Me), 154.3, 152.0, 143.4 (ArC4), 139.0 (ArC3'), 136.5 (*i*-Ph), 129.5, 128.6, 128.3, 127.0 (*p*-Ph), 124.9 (ArC2'), 123.3 (ArC2), 98.9 (ArC4'), 57.9 (α -C), 51.7 (OCH₃), 38.7 (PhCH₂).

HRMS (EI) 550.0313 (MH⁺); C₂₂H₂₁IN₃O₄S requires 550.0297.

***E*-[(1*S*^{*},2*R*^{*})-*N*-(1-Benzyl-3,3,3-trifluoro-2-hydroxypropyl)-4-(4-iodophenylazo)benzenesulfonamide] (4.51)**



Sulfanilamide **4.38** (127 mg, 0.339 mmol, single diastereomer) and 4-iodonitrosobenzene **4.26** (79 mg, 0.34 mmol) in glacial acetic acid (1.5 mL) were condensed following General Method K. Column chromatography of the crude azobenzene (1:9 ethyl acetate/dichloromethane) gave **4.51** as an orange solid (134 mg, 67 %, single diastereomer).

mp 149-150 °C

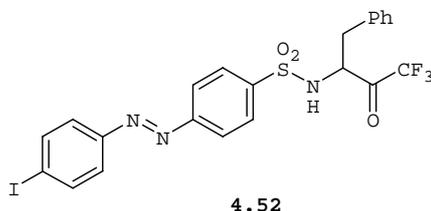
¹H NMR (acetone-*d*₆, 500 MHz); δ 8.17 (d, 2H, *J* = 8.5 Hz, ArH3'), 7.91 (d, 2H, *J* = 8.5 Hz, ArH2'), 7.90 (d, 2H, *J* = 8.4 Hz, ArH2), 7.72 (d, 2H, *J* = 8.4 Hz, ArH3), 7.16 (m, 3H, *m*-Ph, NH), 7.12 (m, 3H, *o/p*-Ph), 4.56 (m, 1H, β-H), 3.92 (m, 1H, α-H), 3.07 (dd, 1H, *J* = 2.9, 14.4 Hz, PhCH_a), 2.95 (dd, 1H, *J* = 10.9, 14.4 Hz, PhCH_b).

¹³C NMR (acetone-*d*₆, 75 MHz); δ 153.9, 152.0, 143.1 (ArC4), 139.0 (ArC3'), 137.5 (*i*-Ph), 129.4, 128.4, 127.7, 126.5 (*p*-Ph), 125.4 (q, *J* = 283.4 Hz, CF₃), 124.9 (ArC2'), 123.2 (ArC2), 98.7 (ArC4'), 73.0 (q, *J* = 28.7 Hz, β-C), 56.1 (α-C), 34.2 (PhCH₂).

¹⁹F NMR (acetone-*d*₆, 282 MHz); -74.2 (d, 3F, *J* = 7.9 Hz).

HRMS (EI) 589.0155 (M⁺); C₂₂H₁₉F₃IN₃O₃S requires 589.0144.

E*-[*N*-(1*S**-Benzyl-3,3,3-trifluoro-2-oxopropyl)-4-(4-iodophenylazo)benzene sulfonamide] **4.52*



Trifluoromethyl carbinol **4.51** (88 mg, 0.15 mmol, single diastereomer) was oxidised with TEMPO/NaOCl over 3 h in a mixture of ethyl acetate/dichloromethane (2:1, 15 mL) following General Method G. The crude product was recrystallised from aqueous MeOH to afford the hydrate of **4.52** as an orange solid (53 mg, 60 %, racemic).

mp 154-156 °C

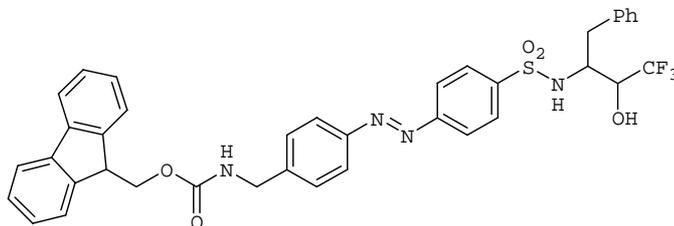
¹H NMR (acetone-*d*₆, 500 MHz); Hydrate δ 8.17 (d, 2H, *J* = 8.5 Hz, Ar*H*3'), 7.91 (d, 2H, *J* = 8.5 Hz, Ar*H*2'), 7.85 (d, 2H, *J* = 8.6 Hz, Ar*H*2), 7.70 (d, 2H, *J* = 8.6 Hz, Ar*H*3), 7.34 (br s, 1H, NH), 7.17 (dd, 2H, *J* = 1.8, 7.0 Hz, *m*-Ph), 7.05 (m, 3H, *o/p*-Ph), 6.38 (br s, 1H, OH), 4.05 (d, 1H, *J* = 10.5 Hz, α-*H*), 3.36 (dd, 1H, *J* = 2.2, 14.3 Hz, PhCH_a), 2.85 (dd, 1H, *J* = 11.3, 14.3 Hz, PhCH_b).

¹³C NMR (acetone-*d*₆, 75 MHz); Hydrate δ 153.4, 151.8, 143.7 (ArC4), 138.8 (ArC3'), 137.8 (*i*-Ph), 129.3, 128.2, 127.4, 126.1 (*p*-Ph), 124.6 (ArC2'), 124.8 (q, *J* = 289.2, CF₃), 122.9 (ArC2), 98.6 (ArC4'), 93.4 (q, *J* = 29.8 Hz, β-C), 60.6 (α-C), 35.3 (PhCH₂).

¹⁹F NMR (acetone-*d*₆, 282 MHz); Hydrate δ -79.0 (s, 3F).

HRMS (EI) 586.9992 (M⁺); C₂₂H₁₇F₃IN₃O₃S requires 586.9987.

***E*-{4-[4-(1*S*^{*},2*R*^{*})-(1-Benzyl-3,3,3-trifluoro-2-hydroxypropyl)sulfamoyl]phenylazo} benzyl] carbamic acid 9*H*-fluoren-9-yl methyl ester} (4.53)**



Sulfanilamide **4.38** (205 mg, 0.60 mmol, single diastereomer) and nitrosobenzene **4.24** (215 mg, 0.60 mmol) in glacial acetic acid (2.4 mL) were condensed following General Method K. Column chromatography of the crude azobenzene (1:4 ethyl acetate/dichloromethane) gave **4.53** as an orange solid (126 mg, 29 %, single diastereomer).

mp 110-112 °C

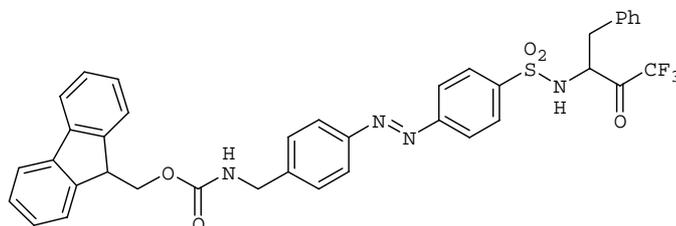
¹H NMR (acetone-*d*₆, 500 MHz); δ 8.08 (d, 2H, *J* = 8.1 Hz, Ar*H*2'), 7.99 (d, 2H, *J* = 7.4 Hz, Fmoc*H*_a), 7.89 (d, 2H, *J* = 8.4 Hz, Ar*H*2), 7.84 (d, 2H, *J* = 7.4 Hz, Fmoc*H*_a), 7.72 (d, 2H, *J* = 8.4 Hz, Ar*H*3), 7.68 (d, 2H, *J* = 8.1 Hz, Ar*H*3'), 7.54 (t, 2H, *J* = 7.4 Hz, Fmoc*H*_b), 7.45 (t, 2H, *J* = 7.4 Hz, Fmoc*H*_c), 7.26 (t, 1H, *J* = 5.6 Hz, FmocNH), 7.17 (m, 3H, *m*-Ph, NH), 7.13 (m, 3H, *o/p*-Ph), 6.03 (d, 1H, *J* = 6.8 Hz, OH), 4.60 (d, 2H, *J* = 6.2 Hz, NHCH₂), 4.56 (d, 3H, *J* = 6.9 Hz, β-*H*, FmocCH₂), 4.39 (t, 1H, *J* = 6.9 Hz, FmocCH), 3.93 (m, 1H, α-*H*), 3.08 (dd, 1H, *J* = 2.7, 14.4 Hz, PhCH_a), 2.95 (t, 1H, *J* = 7.2 Hz, PhCH_b).

¹³C NMR (acetone-*d*₆, 75 MHz); δ 154.2, 151.8, 144.7, 144.5, 142.7, 141.5, 137.5, 129.4, 128.5, 128.4, 127.9, 127.8, 127.3, 126.5, 125.4, 125.4 (q, *J* = 282.6 Hz, CF₃), 123.4, 123.1, 120.2, 73.0 (q, *J* = 28.8 Hz, β-*C*), 66.3 (FmocCH₂), 56.1 (α-*C*), 47.5 (NHCH₂), 44.3 (FmocCH), 34.2 (PhCH₂). Could not detect the carbamate peak over the baseline noise.

¹⁹F NMR (acetone-*d*₆, 282 MHz); δ -74.2 (d, 3F, *J* = 8.0 Hz).

LRMS (ES) 715.2 (MH⁺); C₃₈H₃₄F₃N₄O₅S requires 715.2.

E*-{4-[4-(1*S**-Benzyl-3,3,3-trifluoro-2-oxopropylsulfamoyl)phenylazo)benzyl] carbamic acid 9*H*-fluoren-9-ylmethyl ester} **4.54*



4.54

Trifluoromethyl carbinol **4.53** (72 mg, 100 μ mol, single diastereomer) was oxidised with TEMPO/NaOCl over 3 h in a mixture of ethyl acetate/dichloromethane (2:1, 15 mL) following General Method G. The crude product was recrystallised from aqueous MeOH to afford the hydrate of **4.54** as an orange solid (28 mg, 39 %, racemic).

mp 147-148 °C

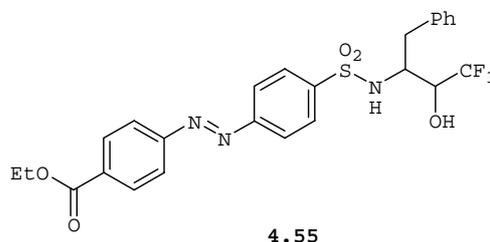
^1H NMR (acetone- d_6 , 500 MHz); Hydrate δ 8.09 (d, 2H, $J = 7.8$ Hz, Ar**H2'**), 7.99 (d, 2H, $J = 7.4$ Hz, Fmoc**H_a**), 7.84 (d, 4H, $J = 7.2$ Hz, Ar**H2**, Fmoc**H_d**), 7.69 (m, 4H, Ar**H3**, Ar**H3'**), 7.55 (t, 2H, $J = 7.3$ Hz, Fmoc**H_b**), 7.46 (t, 2H, $J = 7.2$ Hz, Fmoc**H_c**), 7.34 (d, 1H, $J = 8.9$ Hz, **NH**), 7.26 (br m, 1H, Fmoc**NH**), 7.17 (d, 2H, $J = 4.4$ Hz, Ph), 7.05 (s, 3H, Ph), 6.38 (d, 1H, $J = 18.5$ Hz, 2 x **OH**), 4.60 (s, 2H, **NHCH₂**), 4.56 (d, 2H, $J = 6.8$ Hz, Fmoc**CH₂**), 4.39 (t, 1H, $J = 6.6$ Hz, Fmoc**CH**), 4.05 (t, 1H, $J = 9.2$ Hz, α -H), 3.37 (d, 1H, $J = 14.2$ Hz, Ph**CH_a**), 2.86 (dd, 1H, $J = 12.4, 13.1$ Hz, Ph**CH_b**).

^{13}C NMR (acetone- d_6 , 75 MHz); Hydrate δ 156.9 (OCONH), 154.0 (Ar**CI**), 151.8 (Ar**CI'**), 144.6, 144.4, 142.9, 141.4, 137.8, 129.4, 128.4 (x 2), 127.8, 127.7, 127.2, 126.4, 125.4, 124.1 (q, $J = 289.2$ Hz, **CF₃**), 123.3, 123.0, 120.1, 93.6 (q, $J = 29.7$ Hz, β -**C**), 66.3 (Fmoc**CH₂**), 61.1 (α -**C**), 47.4 (**NHCH₂**), 44.1 (Fmoc**CH**), 35.6 (Ph**CH₂**).

^{19}F NMR (acetone- d_6 , 282 MHz); Hydrate δ -79.0 (s, 3F).

LRMS (ES) 731.3 (MH^+); $\text{C}_{38}\text{H}_{34}\text{F}_3\text{N}_4\text{O}_6\text{S}$ requires 731.2.

***E*-{4-[4-(1*S**,2*R**)-(1-Benzyl-3,3,3-trifluoro-2-hydroxypropyl)sulfamoyl]phenylazo] benzoic acid ethyl ester} (4.55)**



Sulfanilamide **4.38** (264 mg, 0.70 mmol, single diastereomer) and 4-nitrosobenzoic acid ethyl ester **4.27** (126 mg, 0.70 mmol) in glacial acetic acid (2.8 mL) were condensed following General Method K for 5.5 h. Column chromatography of the crude azobenzene (3:7 ethyl acetate/petroleum ether) gave **4.55** as an orange solid (247 mg, 66 %, single diastereomer). An analytical sample was recrystallised from aqueous MeOH to give orange needles.

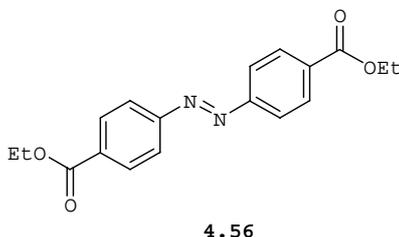
mp 187-188 °C

¹H NMR (acetone-*d*₆, 500 MHz); δ 8.38 (d, 2H, *J* = 8.3 Hz, ArH3'), 8.21 (d, 2H, *J* = 8.3 Hz, (ArH2')), 7.94 (d, 2H, *J* = 8.3 Hz, ArH2), 7.74 (d, 2H, *J* = 8.3 Hz, ArH3), 7.20 (br s, 1H, NH), 7.17 (m, 2H, *m*-Ph), 7.13 (m, 3H, *o/p*-Ph), 6.05 (d, 1H, *J* = 6.1 Hz, OH), 4.54 (m, 3H, β-H, CH₃CH₂), 3.93 (m, 1H, α-H), 3.07 (dd, 1H, *J* = 2.8, 14.4 Hz, PhCH_a), 2.95 (dd, 1H, *J* = 10.8, 14.3 Hz, PhCH_b), 1.53 (t, 1H, *J* = 7.1 Hz, CH₃CH₂).

¹³C NMR (acetone-*d*₆, 75 MHz); δ 165.4 (CO₂Et), 155.1, 154.0, 143.4 (ArC4), 137.5 (*i*-Ph), 133.3 (ArC4'), 130.8, 129.4, 128.5, 127.8, 126.6 (*p*-Ph), 125.4 (q, *J* = 283.8 Hz, CF₃), 123.5, 123.2, 73.1 (q, *J* = 29.1 Hz, β-H), 61.3 (CH₃CH₂O), 56.2 (α-C), 34.2 (PhCH₂), 13.9 (CH₃CH₂O).

¹⁹F NMR (acetone-*d*₆, 282 MHz); δ -74.2 (d, 3F, *J* = 8.0 Hz).

HRMS (EI) 535.1380 (M⁺); C₂₅H₂₅F₃N₃O₅S requires 535.1389.

***E*-[Azo-4,4'-bis(benzoic acid ethyl ester)] (4.56)**

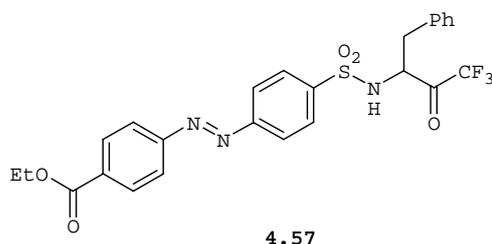
A second orange product was isolated from the reaction mixture. From the ^1H and ^{13}C NMR spectra, the structure has been proposed to be the symmetrical azobenzene formed by self-condensation of **4.27**. Recrystallisation from aqueous MeOH afforded **4.56** as fine orange needles (76 mg, 33 %)

mp 137-138 °C

^1H NMR (acetone- d_6 , 500 MHz); δ 8.36 (d, 4H, $J = 8.4$ Hz, Ar**H3**), 8.19 (d, 4H, $J = 8.4$ Hz, Ar**H2**), 4.53 (q, 4H, $J = 7.1$ Hz, **CH**₂), 1.53 (t, 6H, $J = 7.1$ Hz, **CH**₃).

^{13}C NMR (acetone- d_6 , 75 MHz); δ 165.3 (**CO**₂Et), 155.0 (Ar**C1**), 133.2 (Ar**C4**), 130.7 (Ar**C3**), 123.1 (Ar**C2**), 61.2 (**CH**₃**CH**₂), 13.9 (**CH**₃**CH**₂).

HRMS (ES) 327.1356 (MH^+); $\text{C}_{18}\text{H}_{19}\text{N}_2\text{O}_4$ requires 327.1345.

***E*-{4-[4-(1*S**-Benzyl-3,3,3-trifluoro-2-oxopropylsulfamoyl)phenylazo]benzoic acid ethyl ester} (4.57)**

A solution of trifluoromethyl carbinol **4.56** (30 mg, 56 μmol) in dichloromethane (10 mL) was oxidised with Dess-Martin periodinane (*ca.* 60 %, 147 mg, 207 μmol) following General Method F. The crude product was purified down a silica plug (1:4 ethyl acetate/dichloromethane) and concentrated *in vacuo* to give **4.57** as an orange solid (25 mg, 84 %, racemic).

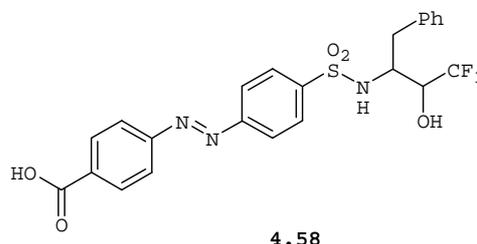
^1H NMR (acetone- d_6 , 500 MHz); Hydrate δ 8.38 (d, 2H, $J = 8.5$ Hz, ArH3'), 8.21 (d, 2H, $J = 8.5$ Hz, ArH2'), 7.90 (d, 2H, $J = 8.5$ Hz, ArH2), 7.73 (d, 2H, $J = 8.5$ Hz, ArH3), 7.36 (d, 1H, $J = 8.9$ Hz, NH), 7.18 (m, 2H, *m*-Ph), 7.05 (m, 3H, *o/p*-Ph), 6.40 (s, 1H, OH), 6.36 (s, 1H, OH), 4.54 (q, 2H, $J = 7.1$ Hz, CH₃CH₂), 4.06 (t, 1H, $J = 8.7$ Hz, α -H), 3.37 (dd, 1H, $J = 2.2, 14.4$ Hz, PhCH_a), 2.86 (dd, 1H, $J = 11.3, 14.2$ Hz, PhCH_b), 1.53 (t, 3H, $J = 7.1$ Hz, CH₃CH₂).

^{13}C NMR (acetone- d_6 , 75 MHz); Hydrate δ 165.6 (CO₂Et), 154.9, 153.5, 144.0 (ArC4), 137.8 (*i*-Ph), 132.9 (ArC4'), 130.6 (ArC3'), 129.3, 128.2, 127.5, 126.2 (*p*-Ph), 123.9 (q, $J = 289.8$ Hz, CF₃), 123.2, 122.9, 93.5 (q, $J = 29.8$ Hz, β -C), 61.4 (CH₃CH₂O), 60.8 (α -C), 35.4 (PhCH₂), 13.8 (CH₃CH₂O).

^{19}F NMR (acetone- d_6 , 282 MHz); Hydrate δ -79.1 (s, 3F).

HRMS (ES) 552.1395 (MH⁺); C₂₅H₂₅F₃N₃O₆S requires 552.1416.

***E*-{4-[4-(1*S**,2*R**)-(1-Benzyl-3,3,3-trifluoro-2-hydroxypropyl)sulfamoyl]phenylazo]benzoic acid} (4.58)**



A solution of ester **4.55** (31 mg, 58 μmol , single diastereomer) in MeOH (2.5 mL) was hydrolysed with 0.25 M LiOH in MeOH/water (2:1, 350 μL , 87.5 μmol) at 70 °C until hydrolysis was complete by TLC. The solvent was removed *in vacuo* and the residue redissolved in water. The solution was acidified with 6 M aqueous HCl and the resulting precipitate taken up in ethyl acetate. The organic layer was washed with brine, dried over MgSO₄ and concentrated *in vacuo* to afford an orange solid. Recrystallisation from aqueous acetone gave **4.58** as orange needles containing one water of crystallisation (30 mg, 98 %, single diastereomer).

mp 201-211 °C

^1H NMR (acetone- d_6 , 500 MHz); δ 8.40 (d, 2H, $J = 8.5$ Hz, ArH3'), 8.21 (d, 2H, $J = 8.5$ Hz, ArH2'), 7.94 (d, 2H, $J = 8.5$ Hz, ArH2), 7.74 (d, 2H, $J = 8.5$ Hz, ArH3), 7.18 (m, 3H,

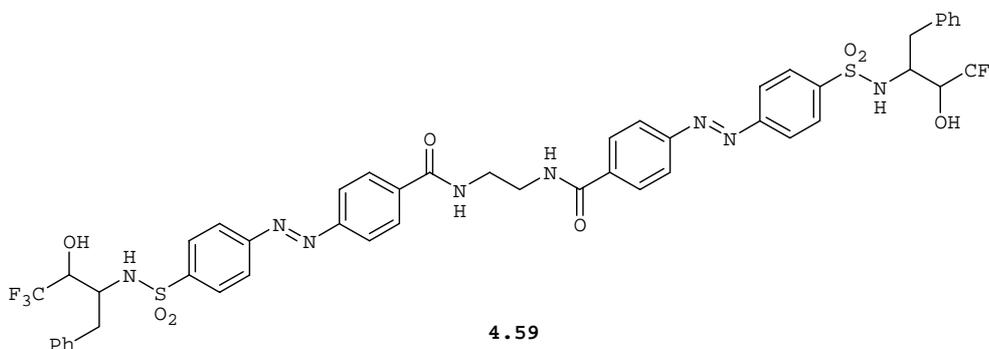
o-Ph, **NH**), 7.13 (m, 3H, *m/p*-Ph), 6.03 (br s, 1H), 4.57 (m, 1H, β -H), 3.94 (m, 1H, α -H), 3.08 (dd, 1H, $J = 2.9, 14.4$ Hz, PhCH_a), 2.95 (dd, 1H, $J = 10.8, 14.4$ Hz, PhCH_b).

¹³C NMR (acetone-*d*₆, 75 MHz); δ 166.2 (COOH), 155.2 (ArC1'), 154.0 (ArC1), 143.4 (ArC4), 137.5 (*i*-Ph), 133.4 (ArC4'), 131.1 (ArC3'), 129.4, 128.5, 127.8, 126.6 (*p*-Ph), 125.4 (q, $J = 288.5$ Hz, CF₃), 123.5, 123.1, 73.1 (q, $J = 28.9$ Hz, β -C), 56.2 (α -C), 34.2 (PhCH₂).

¹⁹F NMR (acetone-*d*₆, 282 MHz); δ -74.2 (d, 3F, $J = 8.0$ Hz).

HRMS (ES) 508.1150 (MH⁺); C₂₃H₂₁F₃N₃O₅S requires 508.1154.

Ethylenediamine bridged dimer of **4.48** (**4.59**)



To a stirred solution of **4.48** (10.5 mg, 20 μ mol) and ethylenediamine dihydrochloride (1.3 mg, 10 μ mol) in DMF (2 mL) was added HATU (8.4 mg, 22 μ mol) and Hünigs base (7.6 μ L, 44 μ mol). The solution was stirred overnight at rt under an inert atmosphere. The solution was diluted with ethyl acetate (20 mL) and washed with water, 10 % aqueous HCl, saturated aqueous NaHCO₃ and brine, dried over MgSO₄ and concentrated *in vacuo* to afford an orange solid (10 mg, 96 %). An analytical sample was recrystallised from MeOH.

mp > 250 °C

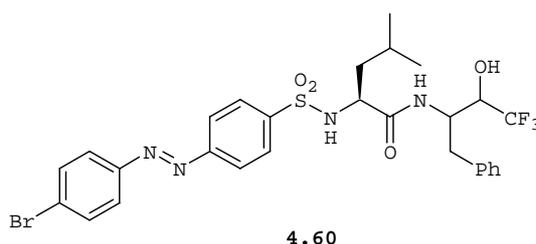
¹H NMR (acetone-*d*₆, 500 MHz); 8.48 (s, 2H, **NHCO**), 8.29 (d, 4H, $J = 8.5$ Hz, Ar**H3'**), 8.18 (d, 4H, $J = 8.6$ Hz, Ar**H2'**), 7.92 (d, 4H, $J = 8.6$ Hz, Ar**H2**), 7.81 (d, 2H, $J = 7.96$, **NH**), 7.73 (d, 4H, $J = 8.6$ Hz, Ar**H3**), 7.18 (m, 4H, *m*-Ph), 7.13 (m, 6H, *o/p*-Ph), 6.52 (d, 2H, $J = 6.80$ Hz, **OH**), 4.56 (m, 2H, β -H), 3.93 (m, 2H, α -H), 3.85 (m, 4H, NHCH₂), 3.07 (dd, 2H, $J = 2.9, 14.5$ Hz, PhCH_a), 2.95 (dd, 2H, $J = 10.9, 14.6$ Hz, PhCH_b).

^{13}C NMR (acetone- d_6 , 75 MHz); δ 166.4 (CONH), 154.0, 153.8, 143.4 (ArC4), 137.8 (*i*-Ph), 137.8 (ArC4'), 129.4, 128.8, 128.3, 127.7, 126.3 (*p*-Ph), 125.4 (q, $J = 283.9$ Hz, CF₃), 123.2, 123.0, 73.0 (q, $J = 28.5$ Hz, β -C), 56.2 (α -C), 40.12 (NHCH₂), 34.1 (PhCH₂).

^{19}F NMR (acetone- d_6 , 282 MHz); δ -74.2 (d, 6F, $J = 7.9$ Hz).

LRMS (ES) 1039.4 (MH⁺); C₄₈H₄₅F₆N₈O₈S₂ requires 1039.3.

***E*-{2*S*-[4-(4-Bromophenylazo)benzenesulfonylamino]-4-methylpentanoic acid (1*S*^{*},2*R*^{*})-(1-benzyl-3,3,3-trifluoro-2-hydroxypropyl)amide} (4.60)**



Sulfanilamide **4.42** (244 mg, 0.50 mmol, 2:3 mixture of diastereomers) and 4-bromonitrosobenzene **4.25** (93 mg, 0.50 mmol) in glacial acetic acid (2.0 mL) were condensed following General Method K. Column chromatography of the crude azobenzene (1:9 ethyl acetate/dichloromethane) gave **4.60** as an orange solid (29 mg, 9 %) comprising a mixture of diastereomers (2:3 by ^1H NMR).

^1H NMR (acetone- d_6 , 500 MHz); Mixture (2:3) δ 8.17-8.10 (m, 8H, ArH2, ArH3, major + minor), 8.04 (dd, 4H, $J = 5.4, 8.3$ Hz, ArH2', major + minor), 7.95 (d, 4H, $J = 8.5$ Hz, ArH3', major + minor), 7.65* (d, 1H, $J = 8.1$ Hz, TFMC NH), 7.60 (d, 1H, $J = 8.8$ Hz, TFMC NH), 7.38-7.28 (m, 10H, *o/m/p*-Ph, major + minor), 6.96* (d, 1H, $J = 6.9$ Hz, LeuNH), 6.82 (d, 1H, $J = 8.7$ Hz, LeuNH), 5.86* (d, 1H, $J = 6.4$ Hz, OH), 5.70 (d, 1H, $J = 6.4$ Hz, OH), 4.44 (m, 1H, TFMC α -H), 4.34* (m, 1H, TFMC α -H), 4.12* (m, 1H, TFMC β -H), 3.97 (m, 2H, Leu α -H, major + minor), 3.93 (m, 1H, TFMC β -H), 3.13-2.91 (m, 4H, PhCH₂, major + minor), 1.74* (m, 1H, LeuCH), 1.57 (m, 1H, LeuCH), 1.43* (m, 1H, LeuCH₂), 1.14 (m, 1H, LeuCH₂), 0.90* (d, 2H, $J = 6.7$ Hz, LeuCH₃), 0.84 (d, 2H, $J = 6.7$ Hz, LeuCH₃), 0.80 (dd, 4H, $J = 4.1, 6.3$ Hz, LeuCH₃, major + minor).

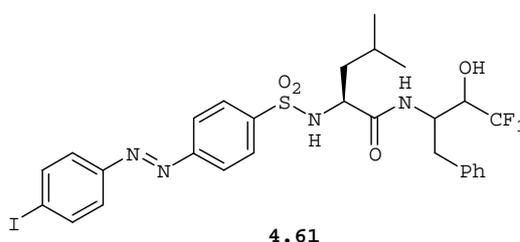
^{13}C NMR (acetone- d_6 , 75 MHz); Mixture δ 171.6* (CONH), 171.1 (CONH), 154.4, 154.3*, 151.5, 151.5*, 143.7, 143.7*, 138.2, 138.0*, 132.9, 132.9*, 129.5, 129.5*, 128.5 (x 3), 128.4, 126.6*, 126.5, 126.2 (ArC4'), 126.2* (ArC4'), 124.9, 124.9*, 123.3*, 123.2, 71.7 (q,

$J = 29.3$ Hz, β -C), 71.0^* (q, $J = 29.3$ Hz, β -C), 55.5^* , 55.2 , 51.3^* , 50.4 , 42.3 , 42.3^* , 34.6^* , 34.2 , 24.2^* , 24.1 , 22.6 , 22.6^* , 20.9 , 20.8^* . The quartets due to the CF_3 could not be detected over the baseline noise.

^{19}F NMR (acetone- d_6 , 282 MHz); Mixture (2:3) δ -74.2^* (d, 3F, $J = 7.5$ Hz), -74.4 (d, 3F, $J = 8.0$ Hz).

HRMS (ES) 655.1207 (MH^+); $\text{C}_{28}\text{H}_{31}\text{BrF}_3\text{N}_4\text{O}_4\text{S}$ requires 655.1201 .

***E*-{2*S*-[4-(4-Iodophenylazo)benzenesulfonylamino]-4-methylpentanoic acid (1*S*^{*},2*R*^{*})-(1-benzyl-3,3,3-trifluoro-2-hydroxypropyl)amide} (4.61)**



Sulfanilamide **4.42** (122 mg, 0.25 mmol, 2:3 mixture of diastereomers) and 4-iodo-nitrosobenzene **4.26** (58 mg, 0.25 mmol) in glacial acetic acid (1.0 mL) were condensed following General Method K. Column chromatography of the crude azobenzene (1:9 ethyl acetate/dichloromethane) gave **4.61** as an orange solid (40 mg, 23 %) comprising a mixture of diastereomers (2:3 by ^1H NMR).

$[\alpha]_{\text{D}} = -10.7$, ($c = 0.28$, MeOH)

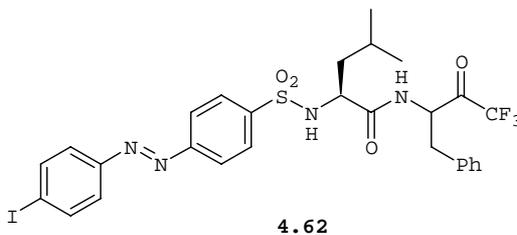
^1H NMR (acetone- d_6 , 500 MHz); δ 8.16-8.10 (m, 12H, ArH2, ArH3 ArH3', major + minor), 7.88 (dd, 4H, $J = 5.4, 8.4$ Hz, ArH2', major + minor), 7.65^* (d, 1H, $J = 8.3$ Hz, TFMC NH), 7.60 (d, 1H, $J = 8.9$ Hz, TFMC NH), 7.38-7.28 (m, 10H, *o/m/p*-Ph, major + minor), 6.96^* (d, 1H, $J = 6.8$ Hz, LeuNH), 6.82 (d, 1H, $J = 8.8$ Hz, LeuNH), 5.86^* (d, 1H, $J = 6.5$ Hz, OH), 5.70 (d, 1H, $J = 6.4$ Hz, OH), 4.44 (m, 1H, TFMC α -H), 4.34^* (m, 1H, TFMC α -H), 4.13^* (m, 1H, TFMC β -H), 3.97 (m, 2H, Leu α -H, major + minor), 3.93 (m, 1H, TFMC β -H), 3.13-2.92 (m, 4H, PhCH₂, major + minor), 1.74^* (m, 1H, LeuCH), 1.57 (m, 1H, LeuCH), 1.43^* (m, 1H, LeuCH₂), 1.14 (m, 1H, LeuCH₂), 0.90^* (d, 2H, $J = 6.7$ Hz, LeuCH₃), 0.83 (d, 2H, $J = 6.7$ Hz, LeuCH₃), 0.80 (dd, 4H, $J = 4.1, 6.5$ Hz, LeuCH₃, major + minor).

^{13}C NMR (acetone- d_6 , 75 MHz); Mixture δ 171.7* (CONH), 171.1 (CONH), 154.4, 154.4*, 152.0, 152.0*, 143.7 (ArC4), 143.3* (ArC4), 139.0 (ArC3'), 139.0* (ArC3'), 138.2 (*i*-Ph), 138.1* (*i*-Ph), 129.6, 126.9*, 128.6 (x 3), 128.5, 126.6* (*p*-Ph), 126.5 (*p*-Ph), 125.5* (q, J = 283.3 Hz, CF₃), 125.3 (q, J = 283.6 Hz, CF₃), 124.9, 124.9*, 123.4* (ArC2), 123.2 (ArC2), 98.9* (ArC4'), 98.8 (ArC4'), 71.7 (q, J = 29.1 Hz, β -C), 71.0* (q, J = 29.0 Hz, β -C), 55.6*, 55.4, 51.4*, 50.4, 42.3, 42.3*, 34.6*, 34.2, 24.2*, 24.1, 22.6, 22.6*, 20.9, 20.8*.

^{19}F NMR (acetone- d_6 , 282 MHz); Mixture (2:3) δ -74.1* (d, 3F, J = 7.7 Hz), -74.4 (d, 3F, J = 7.7 Hz).

HRMS (ES) 703.1076 (MH⁺); C₂₈H₃₁F₃IN₄O₄S requires 703.1063.

***E*-{2*S*-[4-(4-Iodophenylazo)benzenesulfonylamino]-4-methylpentanoic acid (1*S**-benzyl-3,3,3-trifluoro-2-oxopropyl)amide} (4.62)**



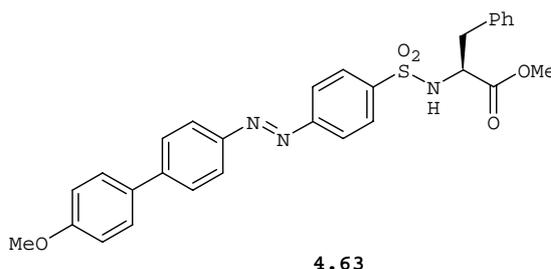
A solution of trifluoromethyl carbinol **4.61** (12 mg, 17 μmol) in dichloromethane (10 mL) was oxidised with Dess-Martin periodinane (*ca.* 60 %, 46 mg, 65 μmol) following General Method F. The crude product was purified down a silica plug (1:3 ethyl acetate/dichloromethane) and concentrated *in vacuo* to give **4.62** as an orange solid (9 mg, 76 %) comprising a mixture of diastereomers (1:1 by ^1H NMR). One drop of D₂O was added to the NMR sample to give exclusively hydrate.

^1H NMR (acetone- d_6 , 500 MHz); Hydrate, mixture of diastereomers (2:3) δ 8.12 (br s, 4H, 2 x ArH2), 8.07 (br s, 4H, 2 x ArH3), 8.01 (d, 4H, J = 8.7 Hz, 2 x ArH3'), 7.91 (dd, 4H, J = 1.2, 8.8, Hz, 2 x ArH2'), 7.35-7.33 (m, 8H, 2 x *o/m*-Ph), 7.29-7.22 (m, 2H, 2 x *p*-Ph), 4.43 (m, 2H, 2 x TFMC α -H), 3.92 (m, 2H, 2 x Leu α -H), 3.34 (m, 2H, 2 x PhCH_a), 2.85 (m, 2H, 2 x PhCH_b), 1.58-0.85 (m, 6H, 2 x LeuCH, 2 x LeuCH₂), 0.80 (d, 3H, J = 6.7 Hz, LeuCH₃), 0.77 (d, J = 6.7 Hz, LeuCH₃), 0.70 (d, J = 6.5 Hz, LeuCH₃), 0.67 (d, J = 6.5 Hz, LeuCH₃).

^{13}C NMR (acetone- d_6 , 75 MHz); Hydrate, mixture of diastereomers (2:3) δ 173.8 (CONH), 173.6* (CONH), 154.9 (ArC1), 154.9* (ArC1), 152.6 (ArC1'), 152.6* (ArC1'), 144.1 (ArC4), 143.7* (ArC4), 139.6 (ArC3'), 139.6* (ArC3'), 139.1 (*i*-Ph), 138.9* (*i*-Ph), 130.2, 130.2*, 129.1, 129.0, 129.0, 129.0, 127.1 (*p*-Ph), 127.0* (*p*-Ph), 125.5* (ArC2'), 125.5 (ArC2'), 124.1 (ArC2), 124.0* (ArC2), 99.5* (ArC4'), 99.4 (ArC4'), 94.6* (q, $J = 29.0$ Hz, β -C), 94.5 (q, $J = 30.1$ Hz, β -C), 56.2, 56.2*, 56.0*, 55.8, 42.7 (LeuCH₂), 42.5* (LeuCH₂), 35.0 (PhCH₂), 35.0* (PhCH₂), 24.7* (LeuCH), 24.6 (LeuCH), 23.3 (LeuCH₃), 23.1* (LeuCH₃), 21.4 (LeuCH₃), 21.4* (LeuCH₃).

^{19}F NMR (acetone- d_6 , 282 MHz); Hydrate, mixture (53:47) δ -80.0 (s, 3F), -80.2* (s, 3F).

***E*-{2*S*-[4-(4'-Methoxybiphenyl-4-ylazo)benzenesulfonylamino]-3-phenylpropionic acid methyl ester} (4.63)**



4-Methoxybenzene boronic acid (15 mg, 0.10 mmol), aryl bromide **4.47** (60 mg, 0.12 mmol), *tetrakis*(triphenylphosphine)palladium⁰ (2.2 mg, 5.0 μmol), tetrabutyl ammonium bromide (33 mg, 0.10 mmol) and KOH (17 mg, 0.30 mmol) in dry THF (3 mL) were reacted together following General Method L to give the desired product as an orange brown solid. Flash chromatography of the crude product (1:9 ethyl acetate/dichloromethane) followed by recrystallisation from aqueous MeOH afforded **4.63** as an orange solid (12 mg, 23 %).

$[\alpha]_{\text{D}} = +34.5$, ($c = 0.067$, MeOH)

mp 192-194 °C

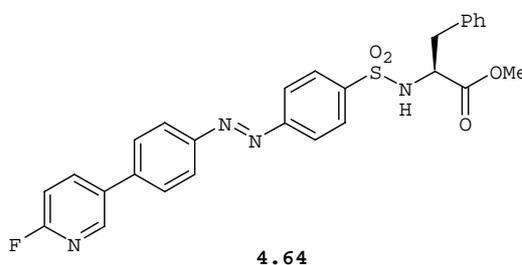
^1H NMR (CDCl₃, 500 MHz); δ 8.01 (d, 2H, $J = 8.5$ Hz), 7.95 (d, 2H, $J = 8.6$ Hz,), 7.88 (d, 2H, $J = 8.6$ Hz) 7.73 (d, 2H, $J = 8.5$ Hz), 7.63 (d, 2H, $J = 8.7$ Hz, MeOArH2), 7.25 (m, 3H, *o/p*-Ph), 7.08 (dd, 2H, $J = 1.7, 7.2$ Hz, *m*-Ph), 7.02 (d, 2H, $J = 8.7$ Hz, MeOArH3), 5.16 (d,

^1H , $J = 9.2$ Hz, **NH**), 4.28 (m, 1H, $\alpha\text{-H}$), 3.88 (s, 3H, ArOCH_3), 3.52 (s, 3H, CO_2CH_3), 3.07 (dq, 2H, $J = 6.0, 13.8$ Hz, PhCH_2).

^{13}C NMR (CDCl_3 , 75 MHz); δ 171.1 (CO_2Me), 159.9 (*i*-MeOAr), 154.8, 151.1, 144.5, 140.7, 134.8, 132.5, 132.3, 129.4, 128.7, 128.3, 128.2, 127.3, 123.9, 123.2, 114.4 (*o*-MeOAr), 56.7 (CH_3OAr), 55.4 ($\alpha\text{-C}$), 52.5 (CO_2CH_3), 39.4 (PhCH_2).

HRMS (ES) 530.1739 (MH^+); $\text{C}_{29}\text{H}_{27}\text{N}_3\text{O}_5\text{S}$ requires 530.1749.

***E*-{2*S*-[4-[4-(Fluoropyridin-3-yl)phenylazo]benzenesulfonylamino]-3-phenyl propionic acid methyl ester} (4.64)**



2-Fluoropyridine-5-boronic acid[§] (14 mg, 100 μmol), aryl bromide **4.47** (60 mg, 120 μmol), *tetrakis*(triphenylphosphine)palladium⁰ (2.2 mg, 5.0 μmol), tetrabutylammonium bromide (33 mg, 100 μmol) and KOH (17 mg, 300 μmol) in dry THF (3 mL) were reacted together following General Method L to give the desired product as an orange brown solid. Flash chromatography of the crude product (1:9 ethyl acetate/dichloromethane) followed by recrystallisation from aqueous MeOH afforded **4.64** as an orange solid (14 mg, 27 %).

mp 177-179 °C

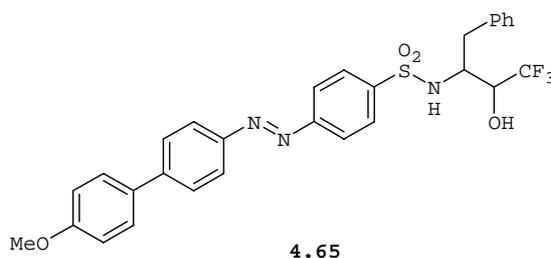
^1H NMR (CDCl_3 , 500 MHz); δ 8.52 (s, 1H, **PyH2**), 8.07 (m, 3H, **PyH4**, **ArH2'**), 7.96 (d, 2H, $J = 8.2$ Hz, **ArH2**), 7.90 (d, 2H, $J = 8.2$ Hz, **ArH3'**), 7.73 (d, 2H, $J = 8.1$ Hz, **ArH3''**), 7.25 (m, 3H, *m/p*-Ph), 7.07 (m, 3H, **PyH5**, *o*-Ph), 5.18 (d, 1H, $J = 9.1$ Hz, **NH**), 4.29 (m, 1H, $\alpha\text{-H}$), 3.53 (s, 3H, OCH_3), 3.07 (m, 2H, PhCH_2).

^{13}C NMR (CDCl_3 , 75 MHz); δ 171.1 (CO_2Me), 165.3 (d, $J = 240.6$ Hz, **PyH6**), 154.6, 151.9, 146.0 (d, $J = 15.1$ Hz, **PyC2**), 141.2 (**ArC4**), 140.1 (*i*-Ph), 139.8 (d, $J = 7.9$ Hz,

[§] Kindly gifted by A. McCarthy.

PyC4), 134.8 (ArC4'), 133.7 (d, J = 4.9 Hz, PyC3), 129.4, 128.7, 128.2, 127.9, 127.4 (*p*-Ph), 124.1, 123.3, 109.8 (d, J = 37.5 Hz, PyC5), 56.7 (α -C), 52.5 (OCH₃), 39.4 (PhCH₂). HRMS (ES) 519.1483 (MH⁺); C₂₇H₂₄FN₄O₄S requires 519.1502.

***E*-{*N*-((1*S*^{*},2*R*^{*})-1-Benzyl-3,3,3-trifluoro-2-hydroxypropyl)-4-(4'-methoxybiphenyl-4-ylazo)benzenesulfonamide} (4.65)**



To a deoxygenated solution of aryl iodide **4.51** (51 mg, 87 μ mol, single diastereomer) and *tetrakis*(triphenylphosphine)palladium⁰ (9 mg, 8 μ mol) in toluene under nitrogen was added deoxygenated aqueous Na₂CO₃ (2 M, 300 μ L) and 4-methoxybenzene boronic acid (12 mg, 79 μ mol) dissolved in deoxygenated EtOH.²⁹ The solution was heated at reflux for 16 h, cooled to rt and poured into a 1:1 mixture of ether/water. The aqueous layer was separated, back extracted with dichloromethane and the combined organics washed with water and brine, dried over MgSO₄ and concentrated *in vacuo*. Column chromatography (1:9 ethyl acetate/dichloromethane) of the crude product gave **4.65** as an orange solid (30 mg, 67 %, single diastereomer).

mp 190-195 °C

¹H NMR (acetone-*d*₆, 500 MHz); δ 8.19 (d, 2H, J = 8.6 Hz, ArH2'), 8.01 (d, 1H, J = 8.6 Hz, ArH3'), 7.91 (d, 2H, J = 8.6 Hz, ArH3), 7.87 (d, 2H, J = 8.8 Hz, MeOArH2), 7.73 (d, 2H, J = 8.6 Hz, ArH2), 7.21 (d, 2H, J = 8.8 Hz, MeOArH3), 7.20-7.16 (m, 3H, NH, *m*-Ph), 7.16-7.13 (m, 3H, *o/p*-Ph), 6.02 (d, 1H, J = 6.5 Hz, OH), 4.57 (m, 1H, β -H), 4.00 (s, 3H, ArOCH₃), 3.94 (d, 1H, J = 10.6 Hz, α -H), 3.08 (dd, 1H, J = 3.0, 14.3 Hz, PhCH_a), 2.96 (dd, 1H, J = 10.6, 14.3 Hz, PhCH_b).

¹³C NMR (acetone-*d*₆, 75 MHz); δ 160.5 (MeOArC4), 154.3, 151.4, 144.5, 142.6, 137.5, 132.1, 129.4, 128.5, 127.8, 127.4, 126.6 (*p*-Ph), 125.9 (q, CF₃), 124.0, 123.1, 114.7 (MeOArC3), 73.0 (q, J = 28.7, Hz β -C), 56.1, 55.1, 34.2 (PhCH₂).

^{19}F NMR (acetone- d_6 , 282 MHz); δ -74.2 (d, 3F, $J = 7.9$ Hz).

HRMS (ES) 570.1678 (MH^+); $\text{C}_{29}\text{H}_{27}\text{F}_3\text{N}_3\text{O}_4\text{S}$ requires 570.1674.

7.5 Experimental work outlined in chapter five

Isomerisation studies of the target compounds.

7.5.1 Experimental setup

Eight potential inhibitors were chosen for the isomerisation studies. The samples were prepared as *ca.* 10 mM solutions in deuterated acetonitrile or DMSO and analysed by ^1H NMR in a 5 mm quartz NMR tube. Samples were irradiated with a 500 W high pressure mercury arc lamp filtered to allow either UV or visible light transmission. A Schott UG11 filter ($230 < \lambda < 410$ nm) or a Corning 7-37 filter ($310 < \lambda < 390$ nm) afforded *Z*-isomer enrichment while a Corning 0-51 filter ($\lambda > 380$ nm) afforded enrichment of the *E*-isomer. The filter was mounted at the end of a plastic tube 30 cm in length which contained a 17 cm water cell with quartz glass windows to allow passage of UV and visible light but reduce sample heating from infrared radiation. Samples were placed an additional 5 cm from the filter.

7.5.2 Isomerisation of the target molecule

A typical experiment involved recording an NMR spectrum of the freshly prepared sample to ensure 100 % *E*-enrichment. If significant amounts of ketone were present, 10 μL of H_2O was added to give hydrate exclusively. The use of H_2O rather than D_2O was important to prevent deuterium exchange of the NH and OH peaks which were necessary for integration. The sample was then irradiated with filtered UV light for 75-90 min in a darkened fumehood to enrich the sample with the *Z*-isomer. The sample was encased with foil to protect from ambient light and analysed by ^1H NMR immediately after irradiation.

E/Z ratios were determined by measurement of the average *E/Z* integrals of appropriate non-overlapping proton resonances. Typically, the hydrate OH, α -H and NH signals were used depending on the degree of overlap. The sample was then irradiated with visible light for 75-90 min and analysed in an analogous manner. The photostationary state was determined by leaving the sample sitting on a window sill for one or two days then measuring the *E/Z* ratio by ^1H NMR. All experiments were repeated on the same samples and agree within $\pm 2\%$. The average value has been reported, rounded to the nearest 1 %.

7.5.3 Selected data from ^1H NMR studies

Selected NMR data for the UV irradiated samples dissolved in deuterated acetonitrile have been listed below. Data for the initial *ca.* 100 % *E*-hydrate samples have also been included for comparison. Only the NH, OH and α -H protons has been assigned as they are the peaks of interest. Resonances for the *Z*-isomer are found upfield of the corresponding *E*-isomer peak.

N-(1*S**-Benzyl-3,3,3-trifluoro-2-oxopropyl)-4-phenylazobenzenesulfonamide (**4.12**)

^1H NMR (CD_3CN , 500 MHz); **E-4.12** δ 8.04 (dd, 2H, $J = 1.7, 7.7$ Hz), 7.74 (d, 2H, $J = 8.5$ Hz), 7.68 (m, 3H), 7.59 (d, 2H, $J = 8.5$ Hz), 7.03 (m, 2H), 6.99 (m, 3H), 6.36 (d, 1H, $J = 9.1$ Hz, **NH**), 5.40 (d, 2H, $J = 4.2$ Hz, **OH**), 3.89 (m, 1H, α -**H**), 3.20 (dd, 1H, $J = 2.4, 14.4$ Hz), 2.62 (dd, 1H, $J = 11.5, 14.3$ Hz).

^1H NMR (CD_3CN , 500 MHz); **Z-4.12** from mixture δ 7.32 (m), 7.14 (m), 7.06 (d, $J = 7.5$ Hz), 6.86 (d, $J = 7.8$ Hz), 6.71 (d, $J = 8.3$ Hz), 6.17 (d, $J = 9.2$ Hz, **NH**), 5.32 (d, $J = 3.1$ Hz, **OH**), 3.84 (m, α -**H**), 3.18 (m), 2.58 (dd, $J = 11.3, 14.1$ Hz).

^1H NMR ($\text{DMSO-}d_6$, 500 MHz); **E-4.12** δ 8.10 (d, 1H, $J = 9.2$ Hz, **NH**), 8.05 (dd, 2H, $J = 1.4, 7.1$ Hz), 7.81 (d, 2H, $J = 8.3$ Hz), 7.74 (m, 3H), 7.63 (d, 2H, $J = 8.4$ Hz), 7.21 (s, 1H, **OH**), 7.12 (s, 1H, **OH**), 7.09 (d, 2H, $J = 7.2$ Hz), 7.02 (m, 3H), 3.97 (m, 1H, α -**H**), 3.20 (d, 1H, $J = 12.5$ Hz), 2.63 (d, 1H, $J = 10.8$ Hz).

^1H NMR (DMSO- d_6 , 500 MHz); **Z-4.12** from mixture δ 7.87 (d, $J = 9.4$ Hz, **NH**), 7.37 (m), 7.14 (m), 7.09 (d, $J = 7.8$ Hz), 6.93 (d, $J = 7.9$ Hz), 6.82 (d, $J = 8.4$ Hz), 3.90 (m, α -**H**), 3.17 (m), 2.53 (dd, $J = 10.3, 14.1$ Hz).

4-Methyl-2S-(4-phenylazobenzenesulfonylamino)pentanoic acid (1S*-benzyl-3,3,3-trifluoro-2-oxopropyl)amide (4.16)

^1H NMR (CD $_3$ CN, 500 MHz); **E-4.16** (2:3 mixture of diastereomers) δ 8.01 (m), 7.66 (dd, $J = 3.1, 6.9$ Hz), 7.29 (m), 6.19* (d, $J = 6.6$ Hz, **NH**), 6.13 (d, $J = 8.3$ Hz, **NH**), 5.64* (s, **OH**), 5.57* (s, **OH**), 5.50 (s, **OH**), 5.45 (s, **OH**), 4.24 (m), 3.71 (m), 3.63* (m), 3.23 (m), 2.87* (m), 2.77 (m).

^1H NMR (CD $_3$ CN, 500 MHz); **Z-4.16** (2:3 mixture of diastereomers) δ 7.74 (d, $J = 8.4$ Hz), 7.00 (d, $J = 8.4$ Hz), 6.92 (m), 6.11* (d, $J = 6.6$ Hz, **NH**), 6.03 (d, $J = 8.1$ Hz, **NH**), 3.59 (m), 3.50* (m).

N-(1S*-Benzyl-3,3,3-trifluoro-2-oxopropyl)-3-(4-bromophenylazo)benzene sulfonamide (4.44)

^1H NMR (CD $_3$ CN, 500 MHz); **E-4.44** δ 8.01 (d, 1H, $J = 7.9$ Hz), 7.94 (d, 2H, $J = 8.7$ Hz), 7.84 (m, 3H), 7.63 (d, 1H, $J = 7.8$ Hz), 7.53 (t, 1H, $J = 7.9$ Hz), 7.00 (d, 2H, $J = 7.4$ Hz), 6.92 (t, 2H, $J = 7.3$ Hz), 6.87 (t, 1H, $J = 7.2$ Hz), 6.31 (d, 1H, $J = 9.0$ Hz, **NH**), 5.33 (d, 2H, $J = 7.8$ Hz, **OH**), 3.91 (t, 1H, $J = 9.8$ Hz, α -**H**), 3.18 (d, 1H, $J = 14.1$ Hz), 2.60 (dd, 1H, $J = 11.9, 13.9$ Hz).

^1H NMR (CD $_3$ CN, 500 MHz); **Z-4.44** from mixture δ 7.50 (d, $J = 8.4$ Hz), 7.19 (m), 7.11 (m), 7.04 (d, $J = 8.8$ Hz), 6.89 (dd, $J = 1.5, 7.0$ Hz), 6.79 (d, $J = 8.3$ Hz), 6.13 (d, $J = 9.2$ Hz, **NH**), 5.26 (s, **OH**), 5.22 (s, **OH**), 3.82 (t, $J = 8.8$ Hz, α -**H**), 3.16 (dd, $J = 2.5, 14.2$ Hz), 2.58 (dd, $J = 11.2, 14.2$ Hz).

***N*-(1*S*^{*}-Benzyl-3,3,3-trifluoro-2-oxopropyl)-3-(4-iodophenylazo)benzenesulfonamide (4.46)**

¹H NMR (CD₃CN, 500 MHz); ***E*-4.46** δ 8.06 (d, 2H, J = 7.5 Hz), 8.01 (d, 1H, J = 7.9 Hz), 7.82 (s, 1H), 7.78 (d, 2H, J = 8.4 Hz), 7.63 (d, 1H, J = 7.0 Hz), 7.53 (t, 1H, J = 7.9 Hz), 7.00 (d, 2H, J = 7.6 Hz), 6.92 (t, 2H, J = 7.4 Hz), 6.87 (t, 1H, J = 7.3 Hz), 6.34 (d, 1H, J = 8.9 Hz, ***NH***), 5.37 (d, 2H, J = 4.7 Hz, ***OH***), 3.91 (t, 1H, J = 9.7 Hz, ***α-H***), 3.18 (dd, 1H, J = 1.4, 14.6 Hz), 2.60 (dd, 1H, J = 11.7, 14.0 Hz).

¹H NMR (CD₃CN, 500 MHz); ***Z*-4.46** from mixture δ 7.70 (d, J = 8.5 Hz), 7.18 (d, J = 8.2 Hz), 7.11 (d, J = 6.7 Hz), 7.03 (d, J = 7.2 Hz), 6.87 (dd, J = 1.5, 7.1 Hz), 6.65 (d, J = 8.6 Hz), 6.15 (d, J = 9.2 Hz, ***NH***), 5.30 (s, ***OH***), 5.27 (s, ***OH***), 3.82 (t, J = 8.9 Hz, ***α-H***), 3.16 (dd, J = 2.6, 14.2 Hz), 2.58 (dd, J = 11.1, 14.3 Hz).

***N*-(1*S*^{*}-Benzyl-3,3,3-trifluoro-2-oxopropyl)-4-(4-bromophenylazo)benzenesulfonamide (4.49)**

¹H NMR (CD₃CN, 500 MHz); ***E*-4.49** δ 7.94 (d, 2H, J = 8.1 Hz), 7.85 (d, 2H, J = 8.2 Hz), 7.74 (d, 2H, J = 8.1 Hz), 7.58 (d, 2H, J = 8.2 Hz), 7.03 (d, 2H, J = 4.9 Hz), 6.99 (m, 3H), 6.33 (d, 1H, J = 9.0 Hz, ***NH***), 5.35 (d, 2H, J = 2.74 Hz, ***OH***), 3.89 (t, 1H, J = 9.3 Hz, ***α-H***), 3.19 (d, 1H, J = 14.4 Hz), 2.62 (dd, 1H, J = 11.9, 13.8 Hz).

¹H NMR (CD₃CN, 500 MHz); ***Z*-4.49** from mixture δ 7.47 (d, J = 7.8 Hz), 7.34 (d, J = 7.7 Hz), 7.15 (m), 7.07 (d, J = 7.3 Hz), 6.79 (d, J = 7.7 Hz), 6.72 (d, J = 7.8 Hz), 6.29 (d, J = 9.2 Hz, ***NH***), 5.57 (d, J = 7.3 Hz, ***OH***), 3.86 (t, J = 9.4 Hz, ***α-H***), 3.17 (d, J = 13.8 Hz), 2.58 (dd, J = 11.7, 13.5 Hz).

***N*-(1*S*^{*}-Benzyl-3,3,3-trifluoro-2-oxopropyl)-4-(4-iodophenylazo)benzenesulfonamide (4.52)**

¹H NMR (CD₃CN, 500 MHz); ***E*-4.52** δ 8.06 (d, 2H, J = 8.4 Hz), 7.79 (d, 2H, J = 8.4 Hz), 7.74 (d, 2H, J = 8.4 Hz), 7.58 (d, 2H, J = 8.4 Hz), 7.02 (d, 2H, J = 5.3 Hz), 6.97 (m, 3H),

6.35 (br s, 1H, **NH**), 5.37 (s, 2H, **OH**), 3.88 (d, 1H, $J = 11.0$ Hz, α -**H**), 3.19 (d, 1H, $J = 14.4$ Hz), 2.62 (dd, 1H, $J = 11.8, 13.9$ Hz).

^1H NMR (CD_3CN , 500 MHz); **Z-4.52** from mixture δ 7.67 (d, $J = 8.6$ Hz), 7.34 (d, $J = 8.6$ Hz), 7.16 (m), 6.72 (d, $J = 8.5$ Hz), 6.66 (d, $J = 8.6$ Hz), 6.15 (br s, **NH**), 5.27 (s, **OH**), 3.85 (d, $J = 10.6$ Hz, α -**H**), 3.17 (dd, $J = 2.7, 14.3$ Hz), 2.59 (dd, $J = 11.1, 14.1$ Hz).

2S-[4-(4-Iodophenylazo)benzenesulfonylamino]-4-methylpentanoic acid (1S*-benzyl-3,3,3-trifluoro-2-oxopropyl)amide (4.62)

^1H NMR (CD_3CN , 500 MHz); **E-4.62** (2:3 mixture of diastereomers) δ 8.06-7.96 (m), 7.93 (d, $J = 7.5$ Hz), 7.84 (d, $J = 7.7$ Hz), 7.32 (m), 7.26 (m, $J = 8.1$ Hz), 7.09* (d, 1H, $J = 8.5$ Hz, **NH**), 6.95 (d, 1H, $J = 8.3$ Hz, **NH**), 6.15* (br s, **NH**), 6.08 (br s, **NH**), 5.60* (br s, **OH**), 5.52* (br s, **OH**), 5.46 (br s, **OH**), 5.41 (br s, **OH**), 4.22 (m), 3.70 (d, $J = 7.8$ Hz), 3.63* (dd, $J = 4.2, 9.5$ Hz), 3.23 (d, $J = 14.2$ Hz), 2.86* (m), 2.76 (t, $J = 13.1$ Hz).

^1H NMR (CD_3CN , 500 MHz); **Z-4.62** (2:3 mixture of diastereomers) δ 7.76 (d, $J = 8.4$ Hz), 7.69 (d, $J = 8.4$ Hz), 7.51 (d, $J = 8.4$ Hz), 7.34-7.21 (m), 7.01 (d, $J = 8.4$ Hz), 6.96 (d, $J = 8.3$ Hz), 6.84 (t, $J = 8.0$ Hz), 6.28* (d, $J = 6.5$ Hz, **NH**), 6.19 (d, $J = 8.2$ Hz, **NH**), 5.94* (s, **OH**), 5.89 (s, **OH**), 5.85* (s, **OH**), 5.78 (s, **OH**), 4.26 (m), 3.59 (m), 3.51* (m), 3.26 (m), 3.23 (m), 2.84* (m), 2.76 (m).

N-(1S*-Benzyl-3,3,3-trifluoro-2-oxopropyl)-3-phenylazobenzamide (4.7)

^1H NMR (CD_3CN , 500 MHz); **E-4.7** δ 8.10 (t, 2H), 8.00 (dd, 2H, $J = 1.4, J = 8.0$ Hz), 7.79 (d, 1H, $J = 7.9$ Hz), 7.69-7.63 (m, 4H), 7.45 (d, 1H, $J = 8.2$ Hz, **NH**), 7.34 (m, 4H), 7.26 (t, 1H, $J = 6.7$ Hz), 6.30 (s, **OH**), 5.85 (s, **OH**), 4.39 (dd, 1H, $J = 7.1, 10.2$ Hz, α -**H**), 3.35 (m, 1H), 3.18 (m, 1H).

^1H NMR (CD_3CN , 500 MHz); **Z-4.7** from mixture δ 7.40 (d, $J = 7.8$ Hz), 7.16 (d, $J = 8.1$ Hz), 7.11 (s), 6.93 (d, $J = 7.7$ Hz), 6.86 (d, $J = 8.2$ Hz), 6.16 (s, **OH**), 5.75 (s, **OH**), 4.32 (t, $J = 9.9$ Hz, α -**H**), 3.32 (m), 3.08 (t, 1H, $J = 13.0$ Hz).

7.6 References for chapter seven

- (1) Still, W. C.; Kahn, M.; Mitra, A. *Journal of Organic Chemistry* **1978**, *43*, 2923-2925.
- (2) Armarego, W. L. F.; Chai, C. L. L. *Purification of laboratory chemicals.*; 5th ed.; Butterworth-Heinemann, 2003.
- (3) Fujita, S. *Synthetic Communications* **1982**, 423-424.
- (4) Woolfolk, E. O.; Reynolds, W. E.; Mason, J. L. *Journal of Organic Chemistry* **1959**, *24*, 1445-1450.
- (5) *The Chemical Synthesis of Peptides*; 3rd ed.; Jones, J., Ed.; Clarendon Press: Oxford, 1994; Vol. 23.
- (6) Harvey, A. J., Thesis, University of Canterbury, 2000.
- (7) Graves, D. M.; Linderman, R. J. *Journal of Organic Chemistry* **1989**, *54*, 661-668.
- (8) Anelli, P. L.; Biffi, C.; Montanari, F.; Quici, S. *Journal of Organic Chemistry* **1987**, *52*, 2559-2560.
- (9) In *Vogel's textbook of practical organic chemistry*; Furniss, B. S., Hannaford, A. J., G., S. P. W., Tatchell, A. R., Eds.; Longman Scientific and Technical: 1989, p 897.
- (10) Tollari, S.; Cuscela, M.; Porta, F. *Journal of the Chemical Society, Chemical Communications* **1993**, 1510-1511.
- (11) Bortolini, O.; Bragante, L.; Di Furia, F.; Modena, G. *Canadian Journal of Chemistry* **1986**, *64*, 1189-1195.
- (12) Anspion, H. D. In *Organic Syntheses; Collective Volume 3*; Horning, E. C., Ed.; John Wiley and Sons, Inc: 1964, p 711-712.
- (13) Hartmann, R. W.; Reichert, M. *Archiv der Pharmazie (Weinheim, Germany)* **2000**, *333*, 145-153.
- (14) Imperiali, B.; Abeles, R. H. *Tetrahedron Letters* **1986**, *27*, 135-138.
- (15) Worrall, D. E. In *Organic Syntheses* 1929; Vol. 9, p 66-68.
- (16) In *Vogel's textbook of practical organic chemistry*; Furniss, B. S., Hannaford, A. J., Smith, S. A., Tatchell, A. R., Eds.; Longman Scientific and Technical: 1989, p 1035.
- (17) Singer, A.; McElvain, S. M. In *Organic Syntheses* 1934; Vol. XIV, p 30-33.

- (18) In *Vogel's textbook of practical organic chemistry*; Furniss, B. S., Hannaford, A. J., G., S. P. W., Tatchell, A. R., Eds.; Longman Scientific and Technical: 1989, p 1168.
- (19) Nakamura, K.; Fujii, M.; Oka, S.; Ohno, A. *Chemistry Letters* **1985**, 523-526.
- (20) Skiles, J. W.; Fuchs, V.; Miao, C.; Sorcek, R.; Grozinger, K. G.; Mauldin, S. C.; Vitous, J.; Mui, P. W.; Jacober, S.; et al. *Journal of Medicinal Chemistry* **1992**, 35, 641-662.
- (21) Corey, E. J.; Venkateswarlu, A. *Journal of the American Chemical Society* **1972**, 94, 6190-6191.
- (22) In *Vogel's textbook of practical organic chemistry*.; 5th ed.; Furniss, B. S., Hannaford, A. J., Smith, S. A., Tatchell, A. R., Eds.; Longman Scientific and Technical: 1989, p 450.
- (23) Colvin, E. W.; Seebach, D. *Journal of the Chemical Society, Chemical Communications* **1978**, 689-691.
- (24) Peet, N. P.; Burkhart, J. P.; Angelastro, M. R.; Giroux, E. L.; Mehdi, S.; Bey, P.; Kolb, M.; Neises, B.; Schirlin, D. *Journal of Medicinal Chemistry* **1990**, 33, 394-407.
- (25) Chen, F. M. F.; Kuroda, K.; Benoiton, N. L. *Synthetic Communications* **1979**, 230-232.
- (26) Fieser, L. F. In *Organic syntheses* 1936; Vol. 16, p 63-67.
- (27) Desai, R. D.; Mehta, C. V. *Indian Journal of Pharmacy* **1951**, 13, 211-213.
- (28) Andrews, A. F.; Smith, D. M.; Hodson, H. F.; Thorogood, P. B. *Journal of the Chemical Society, Perkin Transactions 1: Organic and Bio-Organic Chemistry (1972-1999)* **1982**, 2995-3006.
- (29) Percec, V.; Bera, T. K.; De, B. B.; Sanai, Y.; Smith, J.; Holerca, M. N.; Barboiu, B.; Grubbs, R. B.; Frechet, J. M. J. *Journal of Organic Chemistry* **2001**, 66, 2104-2117.

