Synthesis of Novel Fluorescent Heterocyclic-Derived α-Amino Acids and the Total Syntheses of Piperidine Natural Products

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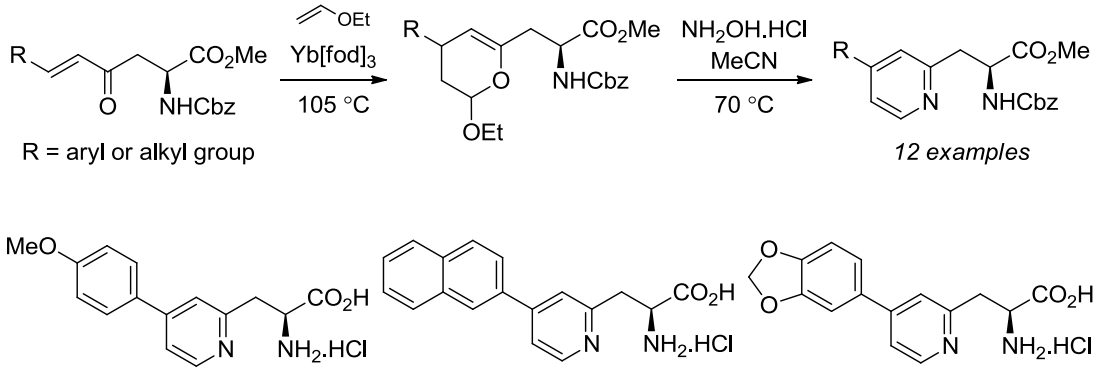
Abstract

During the course of this PhD, methodology for the synthesis of a series of novel, highly fluorescent pyridine-derived α-amino acids was developed. Enone-derived α-amino acids were subjected to an inverse electron demand hetero-Diels-Alder cycloaddition and aromatisation reaction, which led to a twelve-membered library of pyridine analogues. The optical properties of these compounds were analysed, with several exhibiting interesting fluorescent characteristics. One of the analogues was incorporated into a cell penetrating pentapeptide via solid phase peptide synthesis. The resulting hexapeptide was incubated with human fibroblast cells and fluorescence microscopy was used to show accumulation of the peptide in the cells.



The total syntheses of piperidine containing natural products, spruce alkaloid and (+)-241D were also examined during this PhD. A short nine-step linear sequence was developed giving spruce alkaloid and (+)-241D in 21% and 19% overall yield, respectively. A base mediated 6-*endo-trig* cyclisation was employed as the key-step followed by stereoselective ketone reduction to complete the total syntheses. The scope of the cyclisation and reduction was examined with a range of enone side-chains resulting in a small library of novel 4-hydroxy-2,6-disubstituted piperidines.

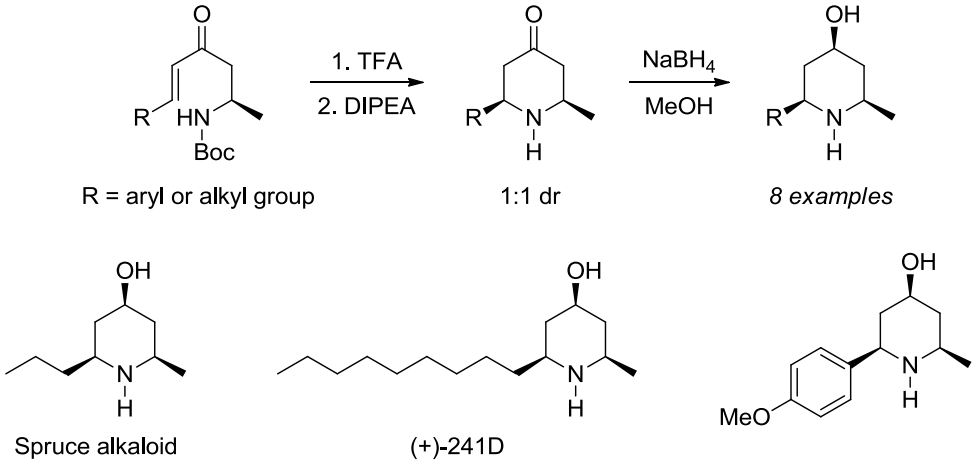


Table of Contents

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Finally, thanks to my lovely girlfriend Kim for putting up with me over the years, coming home late and constantly whining about my research not working. She has always been there for me and has been massively supportive and understanding since I started my PhD, very much appreciated.

"*Boys can do anything, when they put their mind to it.*" - Sally Harkiss

Author's Declaration

I declare that, except where explicit reference is made to the contribution of others, this thesis represents the original work of Alexander H. Harkiss and has not been submitted for any other degree at the University of Glasgow or any other institution. The work upon which is based was carried out at the University of Glasgow in the Loudon laboratory under the supervision of Dr Andrew Sutherland between October 2013 and May 2017. Aspects of the work described herein have previously been published elsewhere as stated below.

L. Gilfillan, R. Artschwager, **A. H. Harkiss**, R. M. L. Liskamp and A. Sutherland, Synthesis of Pyrazole Containing α-Amino Acids via a Highly Regioselective Condensation/Aza-Michael Reaction of β-Aryl α,β-Unsaturated Ketones, *Org. Biomol. Chem.*, 2015, **13**, 4514–4523.

Signature 

Printed name ALEXANDER HUGH HARKISS

Abbreviations

°C	Degrees centigrade
9-BBN	9-Borabicyclo[3.3.1]nonane
Ac	Acetyl
Anap	3-[(6-Acetyl-2-naphthalenyl)amino] alanine
Ar	Aromatic
BADAN	6-Bromoacetyl-2-dimethylaminonaphthalene
BINAP	2,2′-Bis(diphenylphosphino)-1,1′-binaphthalene
Boc	*tert*-Butyloxycarbonyl
BODIPY	Boron-dipyrromethene
br	Broad
c	Concentration
CaM	Calmodulin
Cbz	Carboxybenzyl
CI	Chemical ionisation
COSY	Correlated spectroscopy
CPME	Cyclopentyl methyl ether
CPP	Cell penetrating peptide
d	Doublet
DAPA	Dimethylaminophthalimide
DAST	Diethylaminosulfur trifluoride
DCC	*N*,*N*'-Dicyclohexylcarbodiimide
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DEPT	Distortionless enhancement polarisation transfer
DHFR	Dihydrofolate reductase
DIAD	Diisopropyl azodicarboxylate
DIBAL-H	Diisobutylaluminium hydride
DIC	*N*,*N*′-Diisopropylcarbodiimide
DIPEA	Diisopropylethylamine
DMA	Dimethylacetamide
DMAP	4-Dimethylaminopyridine
DMEM	Dulbecco's Modified Eagle's Medium
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EI	Electron impact

EPL	Expressed protein ligation
ESI	Electrospray ionisation
ESIPT	Excited state intramolecular proton transfer
Et	Ethyl
FAB	Fast atom bombardment
FBS	Fetal bovine serum
Fmoc	Fluorenylmethyloxycarbonyl
FRET	Förster resonance energy transfer
g	Grams
GFP	Green fluorescent protein
GMO	Genetically modified organism
h	Hour
HBTU	(2-(1*H*-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate)
HCTU	*O*-(1*H*-6-Chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HDA	Hetero-Diels-Alder
HDA_{INV}	Inverse electron demand hetero-Diels-Alder
HeLa	Human epithelial cells
HIV	Human immunodeficiency virus
HOMO	Highest occupied molecular orbital
HPLC	High-performance liquid chromatography
HSQC	Heteronuclear single quantum correlation spectroscopy
hTERT	Human telomerase reverse transcriptase
HWE	Horner-Wadsworth-Emmons
Hz	Hertz
IC_{50}	Half maximal inhibitory concentration
IR	Infrared
J	NMR spectra coupling constant
K_d	Dissociation constant
KHMDS	Potassium hexamethyldisilazane
KR	Lysine and arginine rich peptide
LC-MS	Liquid chromatography-mass spectroscopy
LiHMDS	Lithium hexamethyldisilazane
LUMO	Lowest unoccupied molecular orbital
m	Multiplet
M	Molar

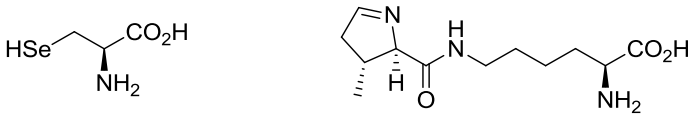
m-	*Meta*-
m/z	Mass to charge
Mcm	7-methoxycoumarinylalanine
Me	Methyl
mg	Milligrams
MHz	Megahertz
mL	Millilitres
mM	Millimolar
mmol	Millimole
mol	Mole
MOM	Methoxymethyl
Ms	Mesyl
MW	Microwave
NBD	4-Fluoro-7-nitrobenzofurazan
NBS	*N*-Bromosuccinimide
NHS	*N*-Hydroxysuccinimide
NIR	Near-infrared
NMM	*N*-Methyl morpholine
NMP	*N*-Methyl-2-pyrrolidone
NOE	Nuclear Overhauser effect
NOESY	Nuclear Overhauser effect spectroscopy
Ns	Nitrobenzenesulfonyl
NVOC	Nitroveratryloxycarbonyl
o-	*Ortho*-
p-	*Para*-
PAF26	Peptide AntiFungal 26
PBS	Phosphate-buffered saline
PC	Phosphatidylcholine
PDC	Pyridinium dichromate
pdCpa	5’-Phospho-2’-deoxyribocytidylriboadenosine
PET	Positron emission tomography
Ph	Phenyl
Pr	Propyl
PTC	Phase transfer catalysis
PTP	Protein tyrosine phosphate
pTyr	Phosphotyrosine

q	Quartet
QY	Quantum yield
RBCs	Red blood cells
rt	Room temperature
s	Singlet
SNAr	Nucleophilic aromatic substitution
SPPS	Solid phase peptide synthesis
t	Triplet
Tat-TAR	Trans-activator of transcription-trans-activating response
TBAF	Tetra-*n*-butylammonium fluoride
TBD	1,5,7-Triazabicyclo[4.4.0]dec-5-ene
TBDMS	*tert*-Butyldimethylsilyl
TBS	Tris-buffered saline
TEA	Triethylamine
Tf	Triflyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TIPS	Triisopropylsilane
TLC	Thin layer chromatography
tRNA	Transfer ribonucleic acid
Ts	Tosyl
TTET	Triplet-triplet energy transfer
UV	Ultraviolet
VMR	Vinylogous Mannich-type reaction
Δ	Reflux
µM	Micromolar

1.0 Introduction

1.1 α-Amino Acids

α-Amino acids are small organic molecules that contain both amino ($–NH_2$) and carboxylic acid ($–CO_2H$) functionality. They exist as zwitterions and possess amphoteric properties, meaning they can act as an acid and a base. They commonly exist in nature as their L-isomeric form, whereby the α-carbon is chiral. These α-amino acids can join to one another to form short peptide chains, longer polypeptides or proteins of varying size. There are 22 genetically encoded proteinogenic amino acids that are incorporated biosynthetically into proteins during translation, 20 in the standard genetic code (Appendix 1) and two of which, selenocysteine and pyrrolysine can be incorporated via special translation mechanisms (Figure 1).[1]



Selenocysteine/Sec/U Pyrrolysine/Pyl/O

Figure 1: Selenocysteine and pyrrolysine.

There are many known non-proteinogenic amino acids and even although they occur in nature they are not used by the translational machinery to assemble proteins. These non-coded amino acids are important, as intermediates of these can be formed during biosynthesis and then the amino acids are synthesised post-translationally within proteins.[1]

Proteins are central to almost every biological process, performing a vast array of functions such as catalysing metabolic reactions, replicating DNA, transporting molecules and responding to stimuli. Unnatural amino acids have been designed in laboratories to provide an important approach to probing the structure and function of proteins and also their medicinal applications.[2]

There are two main methods for unnatural amino acid incorporation to study proteins, residue-specific and site-specific incorporation. The current labelling methods for the latter approach include incorporation of unnatural amino acids with biorthogonal handles that can be modified via chemoselective reactions once incorporated.[3] Another approach includes the incorporation of fluorescent unnatural amino acids and this will be the focus of this chapter, whereby recent syntheses of fluorescent unnatural amino acids will be described in detail and their applications briefly elucidated.[3]

1.2 Fluorescent α-Amino Acids

Fluorescent spectroscopy is a highly sensitive and selective analytical and diagnostic tool, facilitating the development of a number of research fields such as molecular and cellular biology, biophysics, biotechnology, and medicine. It also has a rapid response time and can utilise countless small molecular probes that possess tuneable photophysical properties. Due to the importance of proteins and our understanding of their structure and function, fluorescent spectroscopy can be utilised using a number of strategies.[4]

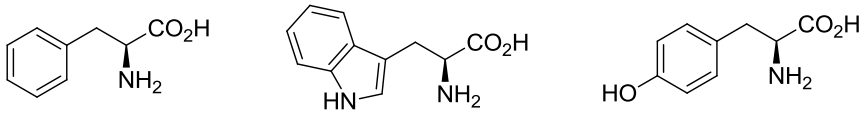
Expression of the protein of interest could be achieved by using a naturally occurring fluorescent protein (GFP)[5] or naturally occurring α-amino acids within the protein itself (tyrosine or tryptophan).[6] These approaches are hampered by limitations. Once a fluorescent protein has been attached to the protein of interest, the presence of this fluorescent protein may alter the form and function of the protein being investigated. In order for the second procedure to work there must be an abundance of the natural α-amino acids in the protein of interest. This might not be possible and combined with the fact that multiple residues may occur in a variety of regions in the protein, the spectroscopy can become complex.[6]

An alternative pathway would be to design and synthesise unnatural fluorescent α-amino acid derivatives and incorporate these into proteins via solid phase peptide synthesis (SPPS), expressed protein ligation (EPL) or unnatural amino acid mutation.[7] There have been several recent reviews that discuss significant developments regarding the design and synthesis of unnatural fluorescent α-amino acids and peptides. Katritzky described the specific structural features required for the use of fluorescent α-amino acids as effective molecular probes.[8] Krueger and Imperiali reported the successful incorporation of unnatural fluorescent α-amino acids into peptides and proteins and their application in chemical biology studies.[7] In this chapter, recent syntheses of novel fluorescent α-amino acids will be described along with their fluorescent properties and applications. Each α-amino acid will also be classified with regard to the type of side-chain fluorophore residue it possesses.

1.3 Natural α-Amino Acids and Fluorescent Analogues

The use of naturally occurring α-amino acids, L-phenylalanine, L-tyrosine and L-tryptophan (Figure 2) for fluorescence imaging reduces the conformational changes in protein structure that may be observed when using unnatural α-amino acids.[9] Regardless of this advantage, the poor optical properties of these natural α-amino acids have limited their use. These residues have distinct absorption and emission wavelengths and differ in quantum yields. The fluorescence quantum yield (QY) value is used to determine the emission efficiency of a given fluorophore. The calculation is

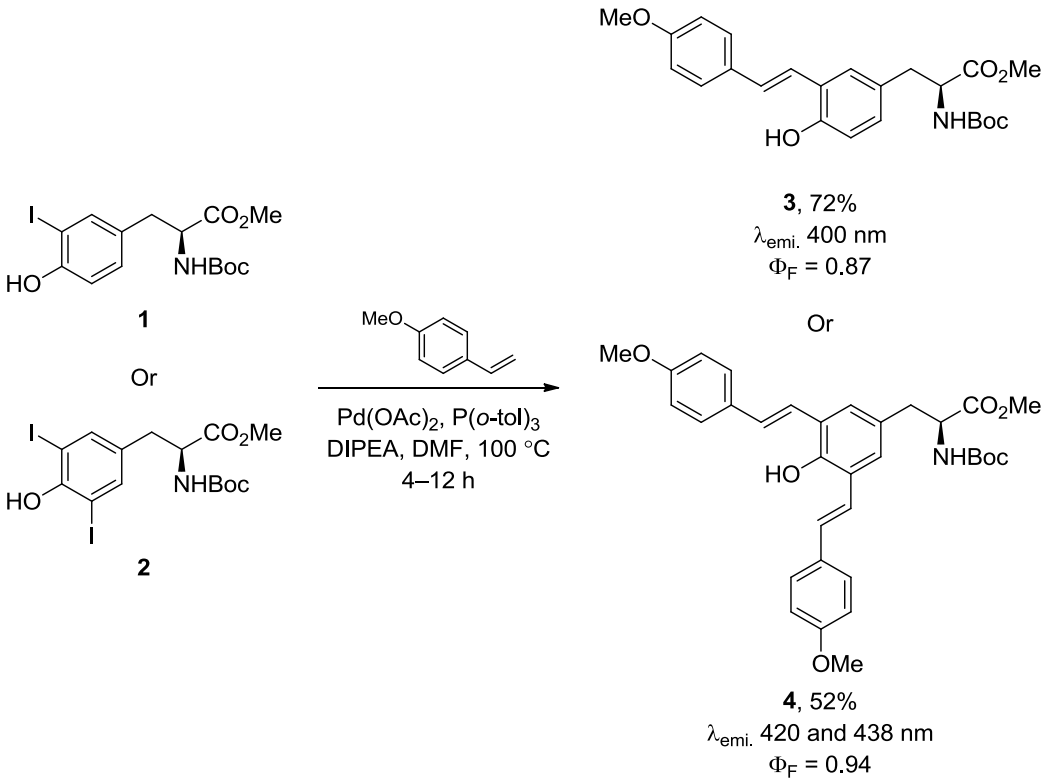
based on the number of photons emitted divided by the number of photons absorbed. Tryptophan is much more fluorescent then either tyrosine or phenylalanine. Due to tryptophan's greater molar extinction coefficient, higher quantum yield and resonance energy transfer, the fluorescence spectrum of a protein containing the three amino acids usually resembles that of tryptophan. This is the main reason for the design and synthesis of novel unnatural fluorescent α-amino acids that have greater emission efficiency and fluoresce in the visible region (380–750 nm) of the electromagnetic spectrum.[10]



Phe	**Trp**	**Tyr**
$\lambda_{abs.}$ = 257 nm	$\lambda_{abs.}$ = 280 nm	$\lambda_{abs.}$ = 274 nm
$\lambda_{emi.}$ = 282 nm	$\lambda_{emi.}$ = 348 nm	$\lambda_{emi.}$ = 303 nm
ε = 200 cm^{-1} M^{-1}	ε = 5,600 cm^{-1} M^{-1}	ε = 1,400 cm^{-1} M^{-1}
Φ_F = 0.04	Φ_F = 0.20	Φ_F = 0.14

Figure 2: Structures and optical properties of L-phenylalanine, L-tryptophan and L-tyrosine.

Derivatives of these naturally occurring amino acids have been investigated thoroughly. Tyrosine derived fluorescent α-amino acids were investigated by Wang and co-workers.[11] Following protection of the amine and carboxylic acid groups, 3-iodo-L-tyrosine **1** and 3,5-diiodo-L-tyrosine **2** were subjected to mono and double Heck couplings between a variety of styrene derivatives to form tyrosine analogues bearing stilbene backbones (Scheme 1). A library of thirteen analogues were synthesised in high yield with varying functionality on the styrene aryl group. The two with the most interesting optical properties were **3** and **4**. The mono-methoxy compound **3** fluoresced at 400 nm and had a high QY of 0.87. The di-methoxy compound **4** had an equally impressive QY of 0.94 and had two emission bands at 420 and 438 nm. This library of tyrosine derived amino acids displayed extended conjugation from the aryl side chain through the vinyl linker to the central phenol ring. The methoxy groups in particular are efficient electron donating groups and this is why **3** and **4** have such desirable optical properties.



Scheme 1: Synthesis of tyrosine stilbene methoxy analogues.

An Fmoc derivative of **4** was synthesised and incorporated into a small cell penetrating pentapeptide (sequence: **4**-VPALK) using SPPS. To assess the cell permeability and fluorescent properties of the hexapeptide **5** (Figure 3), it was incubated with a human epithelial cell line (HeLa) and mouse fibroblast cells (NIH 3T3) for 3 h and visualised using laser scanning confocal microscopy. The images showed that the CPP could accumulate in the cells, suggesting that these types of amino acids could be applied in chemical biology studies as fluorescent probes.

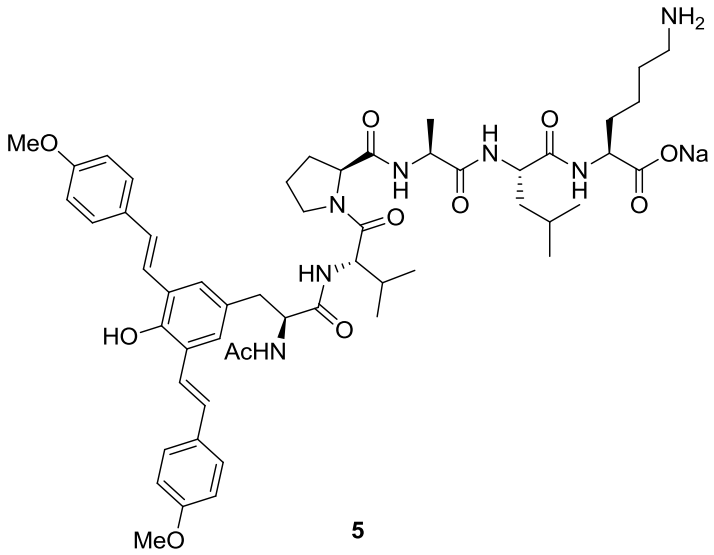
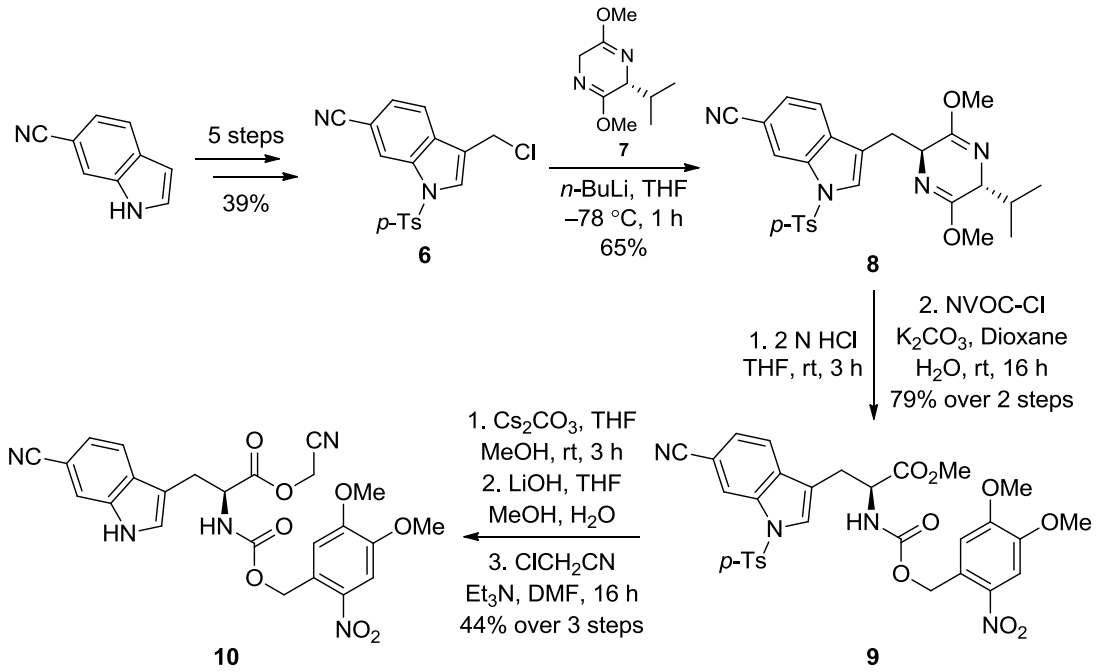


Figure 3: Cell penetrating hexapeptide **5**.

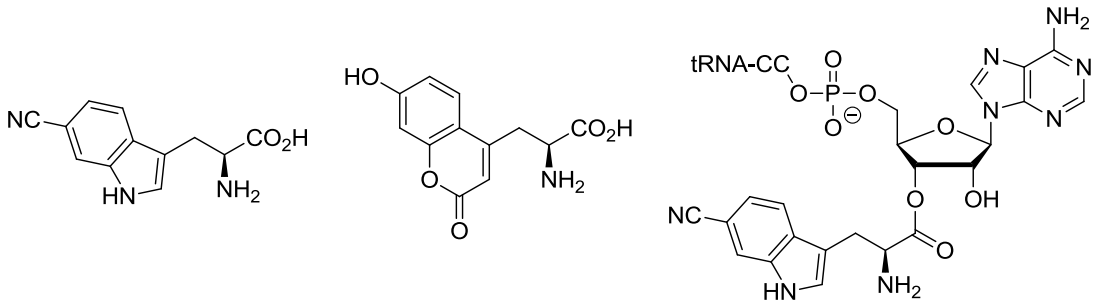
There has also been novel tryptophan derived fluorescent α-amino acids recently synthesised by Hecht and co-workers. His laboratory has reported the synthesis of three novel cyanotryptophans and two quinoline derived tryptophan α-amino acids.[12,13] Both syntheses use similar methodology, taking advantage of the Schöllkopf auxiliary to introduce chirality once the fluorophore portion of the molecule was synthesised (Scheme 2). 6-Cyanoindole was used as the starting material for the synthesis of the fluorophore side-chain and after five transformations produced chloride **6** in 39% overall yield. The lithium enolate of Schöllkopf auxiliary **7** was reacted with **6** to form adduct **8** with high diastereoselectivity. Mild hydrolysis of the auxiliary was followed by protecting the amine as NVOC carbamate **9**. Sequential *N*-detosylation, methyl ester hydrolysis and cyanomethyl ester formation afforded **10** in 44% over three steps. The optical properties of compound **10** were measured in its *N*-acetylated methyl ester form, to mimic the predicted behaviour in a peptide/protein. It displayed an emission maximum value of 370 nm and a QY of 0.53. It should be noted that it possesses an emission maximum wavelength with a substantial red-shift compared with tryptophan. This suggests that the emission signal could be isolated from the natural tryptophan residues found in proteins, making this unnatural α-amino acid a potential imaging agent.



Scheme 2: Schöllkopf bis-lactim amino acid synthesis of **10**.

Hecht and co-workers also investigated the potential use of the cyanotryptophan α-amino acid derivative **11** as a Förster resonance energy transfer (FRET) pair with L-(7-hydroxycoumarin-4-yl)ethylglycine **12** (Figure 4).[12] As discussed, **11** has an emission maximum at 370 nm and **12** has emission maxima values of 345 and 440 nm. Both were incorporated with good efficiency into two different positions (**11** Trp74 and **12** Trp17) of *Escherichia coli* dihydrofolate reductase (*ec*DHFR).

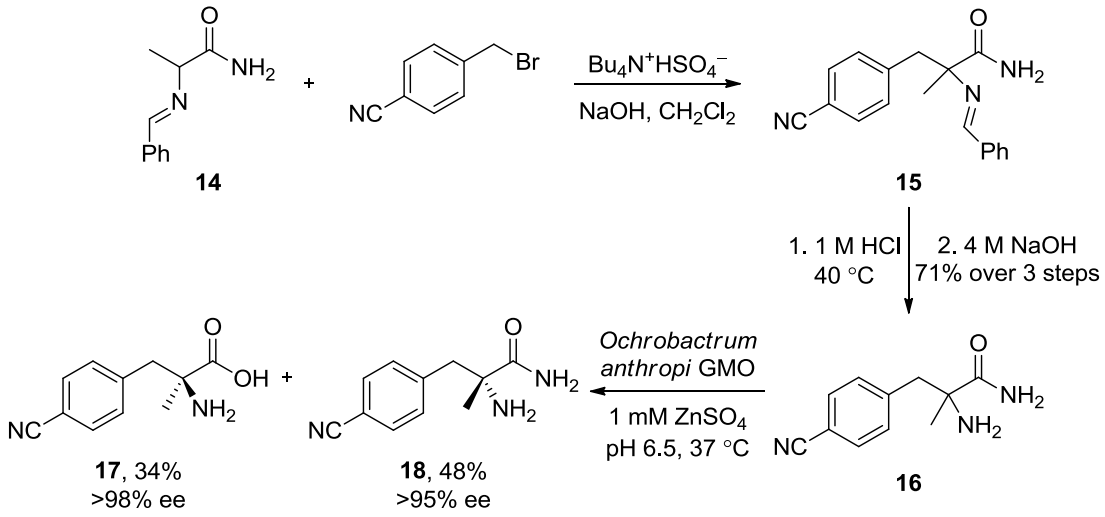
Previous studies suggested the cyanotryptophan derivatives introduction to position 74 were tolerated as well as small fluorescent α-amino acid incorporation into position 17. Calculations determined that the distance between the donor **11** and acceptor **12** pairing was suitable for FRET experiments. Incorporation was done by decoding a four-base codon CGGG with the coumarinyl-$tRNA_{CCCG}$ and the nonsense codon UAG with cyanotryptophanyl-$tRNA_{CUA}$ **13**. The modified DHFR was then excited at 310 nm and an efficient FRET signal was recorded at 460 nm proving that **11** could be used as a FRET partner for studying protein-nucleic acid interactions.



11 **12** **13**

Figure 4: Donor **11** and acceptor **12** FRET pairing, and $tRNA_{CUA}$ with **11** incorporated (**13**).

Phenylalanine derived unnatural α-amino acids have been recently synthesised, particularly by Zotti *et al*.[14] The synthesis starts with the alkylation of N^{α}-benzylidene-DL-Ala-NH_2 **14** with 4-(bromomethyl)benzonitrile using phase transfer catalysis (PTC) to give **15** (Scheme 3). The benzylidene group was hydrolysed to form the racemic amino amide **16**. A genetically modified organism (GMO), *Ochrobactrum anthropi*, was utilised to resolve the racemic mixture, producing the desired phenylalanine derivative **18** and amino amide **17**. Amino acid **18** was incorporated into the decapeptide, trichogin GA IV a membrane active peptaibiotic, to mimic the Aib amino acid commonly found in these types of peptides. The helical conformation was maintained and through exploitation of the *p*-cyanophenyl moiety, the peptide had an emission maximum of 295–305 nm allowing the group to study the interaction of this modified lipopeptaibiotic with model membranes.



Scheme 3: Synthesis of 4-cyanobenzyl chromophores **17** and **18**.

1.4 Coumarin-Derived Fluorescent α-Amino Acids

2*H*-Chromen-2-ones or coumarins are fragrant organic chemical compounds in the benzopyrone chemical class (Figure 5). They have a characteristic ring system that has been used for optical imaging due to the possession of desirable properties such as high quantum yields, an extensive spectroscopic range, photostability and solubility in a variety of solvents.[10,8]

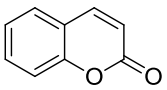
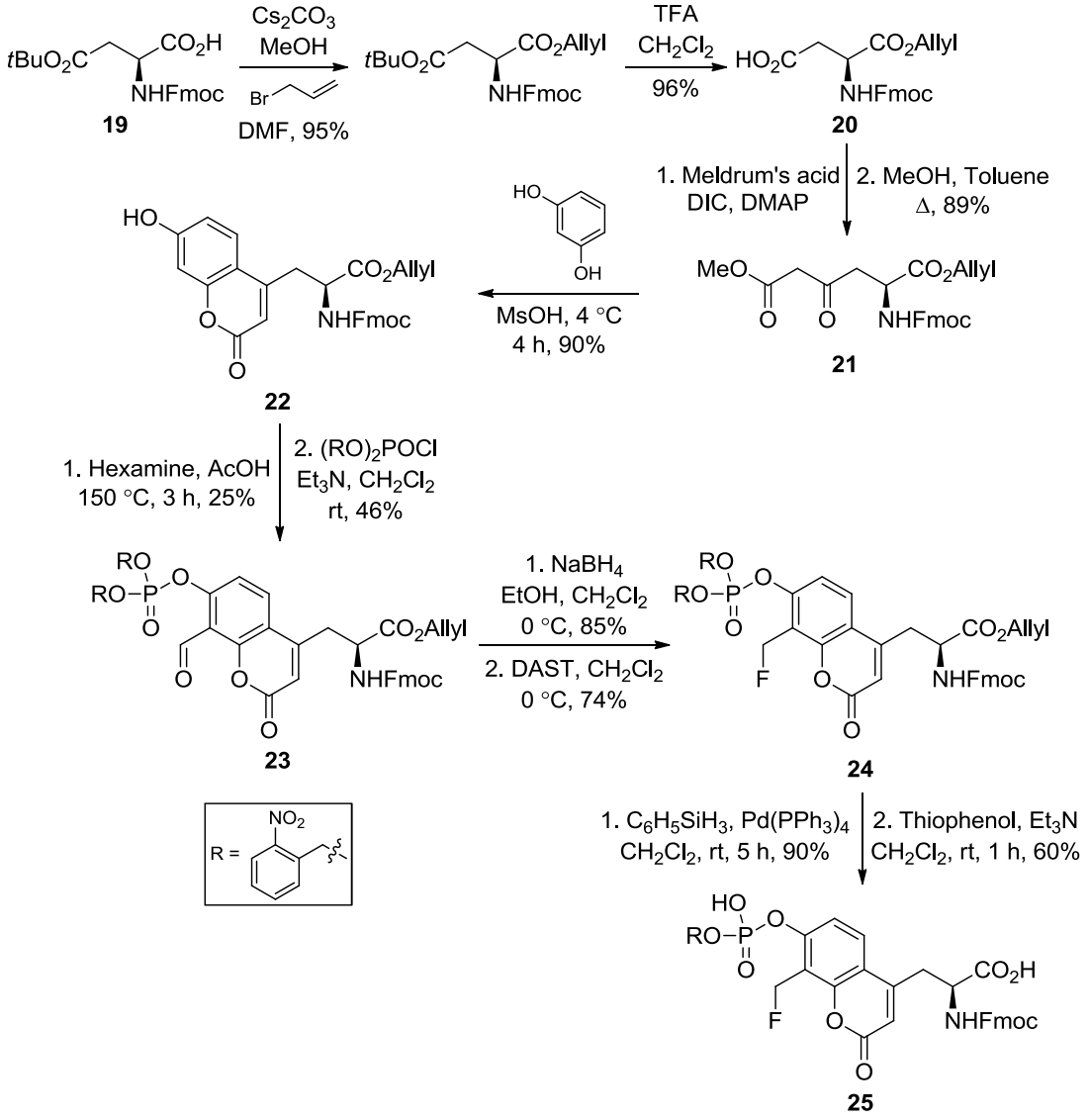


Figure 5: Basic structure of coumarin.

Multiple groups have developed synthetic methodology to access the coumarin ring system and one of the most popular methods to construct coumarin-derived α-amino acids was developed by Garbay and co-workers.[15] They managed to synthesise 7-methoxycoumarin α-amino acid derivatives by applying a Pechmann condensation reaction to construct the ring system. Since then, a number of groups have adopted this Pechmann condensation methodology to prepare coumarin derived α-amino acids.[16] The Yao group synthesised a self-immobilising and fluorogenic phosphotyrosine (pTyr) mimic and used SPPS to incorporate it into peptide-based probes.[17] Fmoc-L-aspartic acid 4-*tert*-butyl ester **19** was used as the starting material for the synthetic route (Scheme 4). Following manipulation of the carboxylic acid protecting groups, compound **20** was treated with Meldrum's acid to form β-ketoester **21**, the substrate for coumarin synthesis. The Pechmann condensation was carried out with resorcinol under acidic conditions to yield 7-hydroxy coumarin α-amino acid derivative **22**. Formylation using the Duff reaction, followed by phosphorylation with bis(*o*-nitrobenzyl) phosphorochloridate produced **23**. A two-step aldehyde reduction and fluorination procedure was used to install the benzylic fluorine **24**. Phenylsilane and

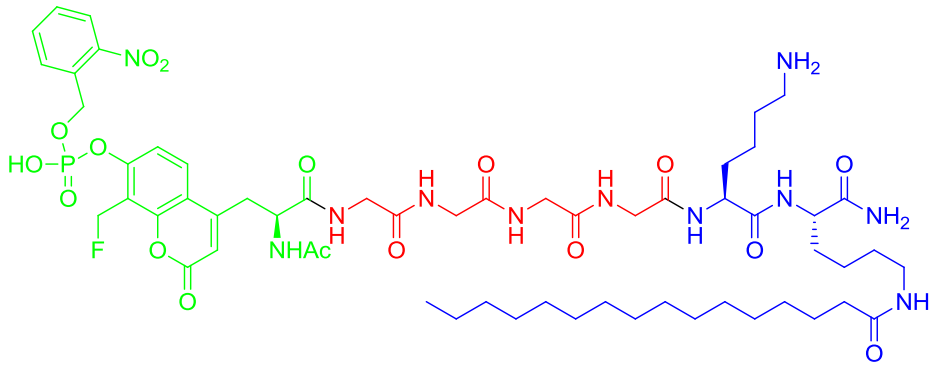
$Pd(PPh_3)_4$ were then used to remove the allyl protecting group before a mixture of TEA/thiophenol was employed to remove one of the 2-nitrobenzyl groups to give **25**. No explanation was provided as to why the selected conditions did not remove both 2-nitrobenzyl groups.



Scheme 4: Synthesis of fluorogenic coumarin-derived α-amino acid **25**.

Yao's group wanted to study the role of protein tyrosine phosphates (PTP), which play an important role in controlling signal transduction processes and regulating the phosphoproteome network.[17] PTP investigations at a molecular level provide unprecedented knowledge of how enzymes work under physiological settings. Once the fluorogenic coumarin derived α-amino acid **25** was synthesised they then incorporated it into three different CPPs via SPPS. Previous work on these localisation peptides have been shown to successfully deliver enzyme inhibitors into subcellular organelles of live cells.[18] A four residue glycine (Gly_4) linker connected the unnatural α-amino acid to the CPP to reduce any interference with the CPP and PTP. CPP **26** was one of the resulting peptide based probes synthesised during this study (Figure 6). It was used to deliver the pTrp mimic to the

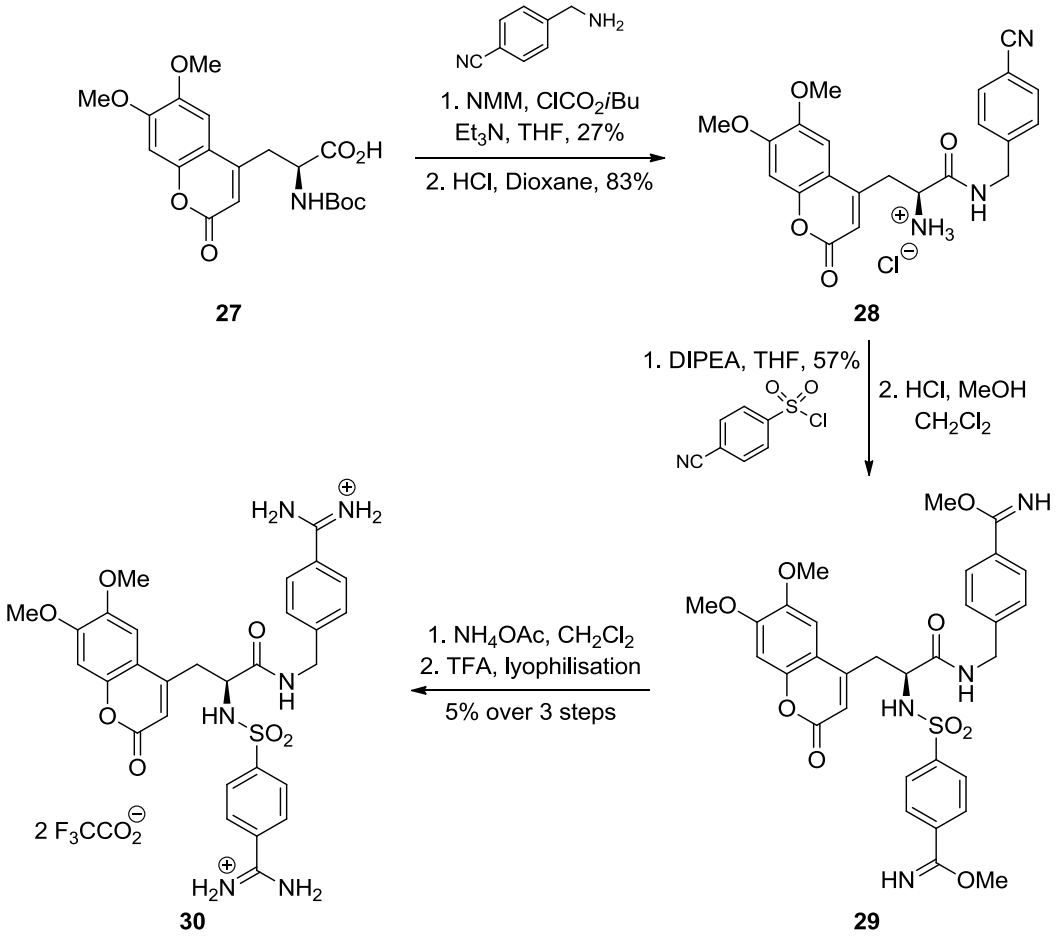
membrane of HeLa cells in order to visualise PTP activities. Successful visualisation was possible via confocal microscopy.



26
pTrp mimic-linker-CPP

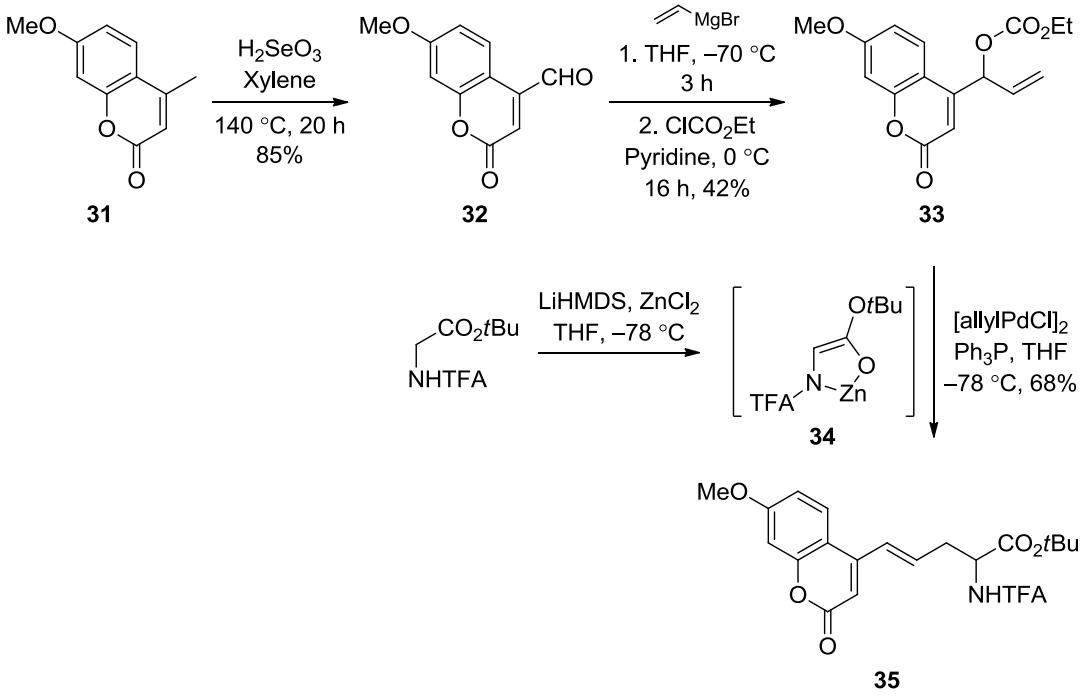
Figure 6: Membrane specific pTrp mimic peptide **26**.

Work carried out by Häußler and Gütschow also focussed on coumarin derived α-amino acids, specifically a 6,7-dimethoxy coumarin analogue.[19] They wanted to synthesise and investigate a route to a coumarin derived α-amino acid with bisbenzamidine functionality in the structure. Due to the benzamidine moiety being capable of binding in the protein active sites via anionic and hydrogen bond interactions it has become of great interest.[20] Bisbenzamidines possess antifungal and antiprotozoal properties because the benzamidine residues can interact with adenine/thymine sites of DNA via insertion into the minor groove.[21,22] These types of interactions could be investigated by using an efficient fluorophore with bisbenzamidine functionality installed and use of fluorescence microscopy for visualisation. Similar conditions as described above were utilised to synthesise **27** using the Pechmann condensation reaction (Scheme 5).[19] The next objective was to install the bisbenzamidine moieties. Carboxylic acid **27** was activated using *N*-methylmorpholine and isobutyl chloroformate followed by subsequent reaction with 4-aminomethylbenzonitrile to form the amino amide. Deprotection of the amine with hydrochloric acid gave HCl salt **28**. The amine salt was treated with DIPEA and 4-cyanobenzenesulfonyl chloride producing the sulfonyl amide and installing the second nitrile group. The Pinner reaction was used to form imidate **29**. Ammonium acetate was used to furnish the desired bisbenzamidine functional groups. Preparative HPLC, treatment with TFA and freeze drying led to the final bisbenzamidine-TFA salt **30**. The excitation maximum for the compound was 350 nm and its emission maximum, 422 nm. Future biological studies are expected to be carried out looking at its interaction with adenine/thymine rich double stranded DNA.



Scheme 5: Synthesis of bisbenzamidine 6,7-dimethoxy coumarin-derived α-amino acid **30**.

An alternative approach to synthesising coumarin derived α-amino acids was recently developed by Kazmaier and co-workers.[23] They synthesised coumarin-derived allylic carbonates and subjected them to a palladium catalysed allylic alkylation with a chelated glycine enolate intermediate (Scheme 6). 4-Methyl derived coumarin **31** was synthesised via Pechmann condensation and *o*-methylation of resorcinol. The methyl group was oxidised using selenious acid, forming 4-formylcoumarin **32**. Reaction with vinylmagnesium bromide led to the allylic alcohol which was directly acylated with ethyl chloroformate to synthesise allylic carbonate **33**. Chelated glycine enolate **34** was generated by deprotonating the protected glycine using lithium hexamethyldisilazide and zinc as the chelating counter ion. Palladium catalysed allylic alkylation completed the synthesis, fusing the coumarin ring with the glycine moiety to give **35**. These coumarin derived allylic carbonates are suitable electrophiles for this final step and when using terminal allylic carbonates, these furnished a single regioisomer and the *E*-alkene exclusively. So far only the racemic approach has been developed but future studies will investigate a stereoselective protocol so that fluorescent labelling of peptides and natural products could be explored.



Scheme 6: Synthesis of coumarin-derived α-amino acids via a Pd-catalysed allylic alkylation.

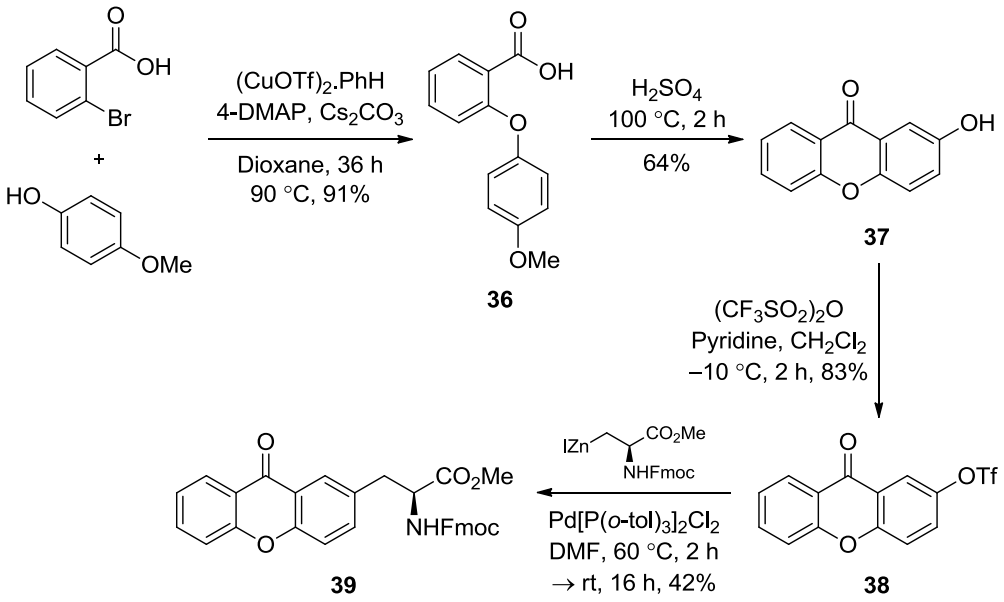
1.5 Xanthone and Acridone-Derived Fluorescent α-Amino Acids

Xanthone derivatives are made up of three fused rings with bridging ketone and ether groups (Figure 7). The structure of acridone is very similar but instead of an oxygen/ether linker, there is an amine bridge. Both have a rich conjugated donor-acceptor relationship within the central B ring. This push-pull motion of electrons allows these fluorophores to exhibit desirable optical properties.

Figure 7: Structures of xanthone and acridone.

There have been recent reports detailing the synthesis of fluorescent α-amino acids with xanthone and acridone frameworks.[24,25] Braun and co-workers designed a synthetic route towards Fmoc and Boc protected α-amino acids with xanthone as the side chain (Scheme 7).[24] Their fluorescent properties were also examined. A xanthone derived α-amino acid was required to investigate the folding mechanisms in polypeptides. A fluorescence triplet-triplet energy transfer (TTET) mechanism takes place between xanthone and naphthylalanine residues in close proximity to one another. In order to study this interaction further an Fmoc or Boc protected xanthone α-amino acid analogue is required, so that direct incorporation can be carried out smoothly, with no post synthesis modifications or protecting groups. To start the synthesis, an Ullmann coupling between 2-bromobenzoic acid and 4-methoxyphenol gave diarylether 2-(4-methoxyphenoxy)benzoic acid

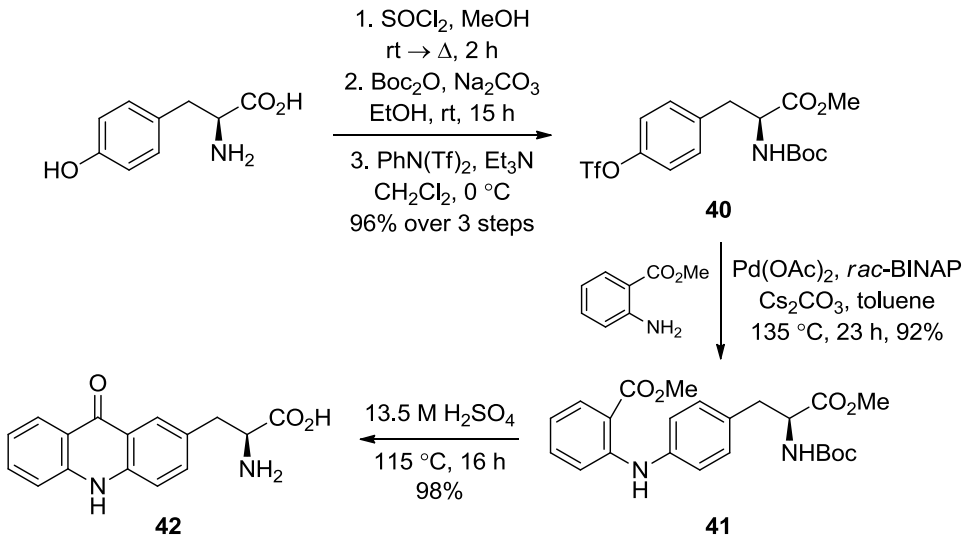
36. Copper triflate benzene complex (2.5 mol%) was the optimal source of copper and 4-dimethylaminopyridine (2.5 mol%) was used as a ligand. The reaction could be scaled up to 40 mmol by switching solvent to toluene, maintaining an excellent 89% yield. Concentrated sulfuric acid was used to form the ring and demethylate the methoxy group to give **37**. Treatment with triflic anhydride led to triflate **38**, necessary for the cross-coupling step. The Negishi coupling proceeded with the organozinc amino acid derivative and $Pd[P(o\text{-tol})_3]_2Cl_2$ (5.4 mol%) producing the xanthone-derived α-amino acid **39**. A Boc-protected organozinc coupling partner was also used and gave the corresponding coupled product in 40% yield. Both Fmoc and Boc protected compounds were hydrolysed using acidic and enzymatic conditions, respectively. The excitation maximum for the Boc protected acid was 340 nm and had a broad emission maximum at 380 nm.



Scheme 7: Synthesis of xanthone-derived α-amino acid **39**.

A five step, high yielding and scaleable route was optimised by Mehl and Petersson's respective groups for the synthesis of an acridone-derived α-amino acid capable of being utilised in ribosomal biosynthesis.[25] This efficient synthetic route started with L-tryptophan, which was fully protected using standard conditions and treated with phenyl triflimide to provide the substrate **40** for the Buchwald-Hartwig coupling (Scheme 8).[26] A solvent, temperature, base and Pd/ligand ratio screen was then carried out to find optimal conditions for the coupling. Palladium acetate, *rac*-BINAP and caesium carbonate, in cyclopentyl methyl ether (CPME) gave the highest yields of the desired product **41.** Toluene was used as the solvent at 135 °C when reactions were carried out on larger scales. A Friedel-Crafts cyclisation was then employed to construct the acridone ring system using concentrated sulfuric acid. This final step also removed the methyl ester and Boc protecting groups to give **42**, with an overall yield of 87%. A route to **42** had already been previously published, however large quantities of the final compound were required for bacterial culture studies, so a

more cost effective route was established.[27,28] The absorption maxima for **42** was 383 and 402 nm, with an emission maxima of 420 and 445 nm and a QY of 0.74 when using methanol as the solvent.



Scheme 8: Synthesis of acridone-derived α-amino acid **42**.

Fluorescent acridone-derived α-amino acid **42** was paired with the common fluorophore 7-methoxycoumarinylalanine (Mcm) for a FRET application.[25] The absorption value for Mcm is around 325 nm and once irradiated it fluoresces at 400 nm. If **42** is in close proximity to Mcm when this occurs it can subsequently be excited and emit at a longer wavelength. The group incorporated both amino acids into calmodulin (CaM) of *Escherichia coli* to study the conformational changes associated with the binding of helical peptides to CaM.

1.6 Flavone-Derived Fluorescent α-Amino Acids

Flavones are a class of flavonoids based on the backbone of 2-phenylchromen-4-one (Figure 8). There are a vast number of natural and synthetic flavones. Several methods exist to synthesise their structure such as the Allan-Robinson reaction,[29] the Auwers synthesis[30] and the Baker-Venkataraman rearrangement.[31] Flavones have antioxidant, anti-proliferative and anti-inflammatory activities and have been an important moiety for the development of new therapeutics.[32]

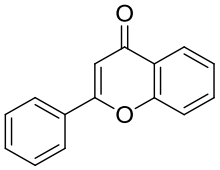
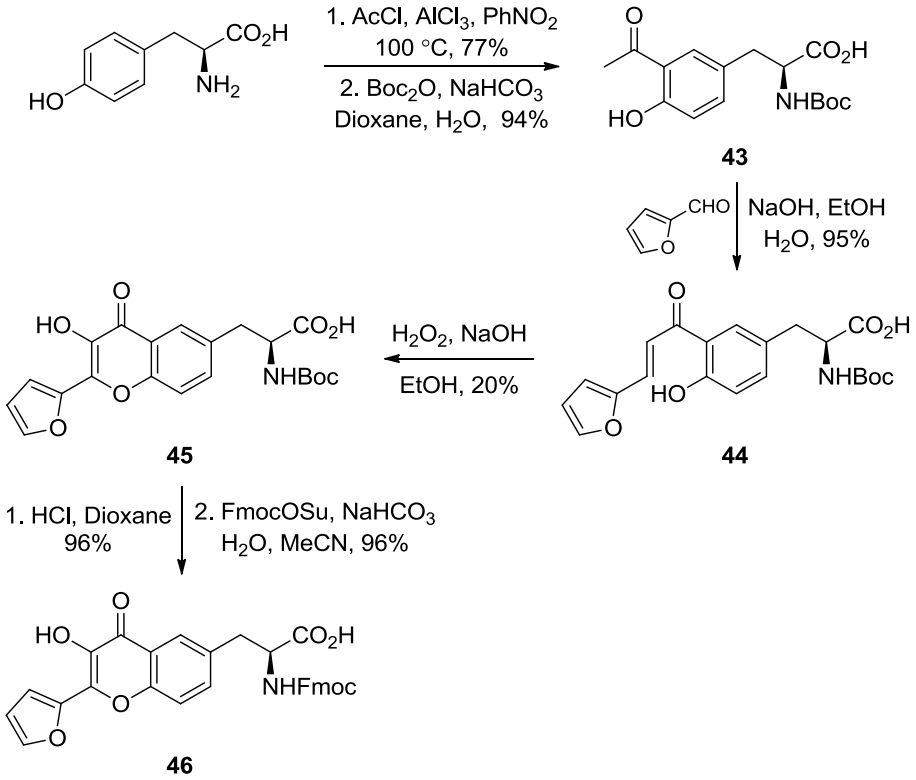


Figure 8: Basic structure of flavone.

Work carried out by the Mély group has focussed on the synthesis of flavone derived α-amino acids and their applications.[33] They described the synthesis of a furyl analogue in the 2-position of the flavone and potential for it to be a tryptophan residue mimic. The six-step synthesis started with L-tyrosine which was treated with acetyl chloride to form the phenolic ester, which then underwent a Fries rearrangement in the presence of $AlCl_3$ (Scheme 9). Subsequent Boc-protection led to the hydroxy acetophenone **43**. This compound was then condensed with furfural to produce chalcone **44** in a 95% yield. The key step in this synthesis was the formation of the flavone backbone where the Algar-Flynn-Oyamada reaction was utilised. Oxidative cyclisation occurred under strong basic and oxidative conditions giving a modest yield of 20% for flavone **45**. The final steps were a deprotection-reprotection procedure to give the final Fmoc protected flavone derived α-amino acid **46**. The optical properties of the Boc-protected flavone were measured showing that it had an absorption maximum of 350 nm and two emission bands at 420 and 525 nm with methanol as the solvent. Using SPPS, **46** was incorporated into the NC(11−55), which is the zinc finger domain of the HIV-1 nucleocapsid protein and displays nucleic acid assembly/disassembly properties.[33] Trp37 and Ala30 were the residues substituted for **46** and although Trp37 plays a key role in the structure and activity of NC(11−55), the fluorescent derivative managed to maintain the folding and chaperone activities of the peptide. Therefore, this flavone-derived α-amino acid has proven to be a tryptophan residue mimic in this particular biological study.



Scheme 9: Synthesis of flavone-derived α-amino acid **46**.

The Mély group went on to make several other analogues using the same synthetic route and apply them to similar biological studies (Figure 9). Compound **47**, a dimethylaminophenyl derivative was incorporated into melitten, a membrane active peptide.[34] The fluorescent analogue replaced Leu9 and Trp19 residues of this peptide. It displayed dual fluorescence bands at 470 and 570 nm via excited state intramolecular proton transfer (ESIPT). Similarly to the furyl analogue, Mély incorporated a methoxyphenyl flavone derivative **48** into a HIV-1 nucleocapsid peptide.[35] A long-lived fluorescence lifetime was observed because of the ESIPT complex and the binding partners of the labelled peptides were visualised through two photon fluorescence lifetime imaging.

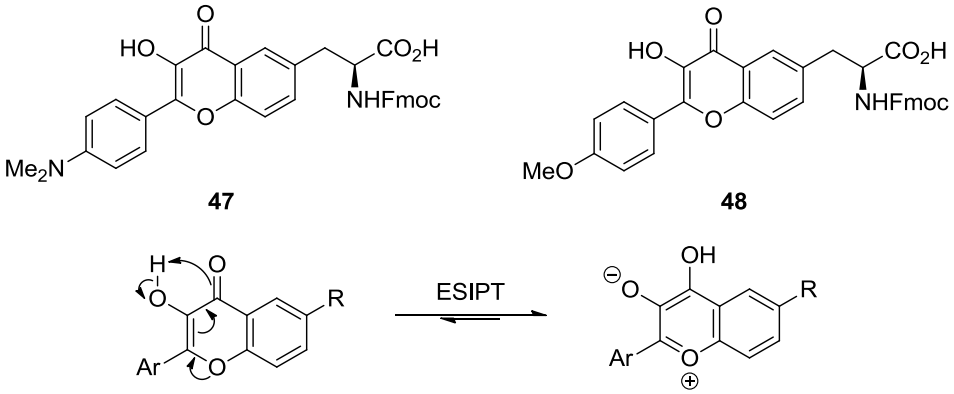


Figure 9: Dimethylaminophenyl **47** and methoxyphenyl **48** flavone-derived α-amino acids and ESIPT reaction in 3-hydroxyflavones.

1.7 BODIPY-Derived Fluorescent α-Amino Acids

Boron-dipyrromethene, commonly known as BODIPY, is dipyrromethene complexed with a disubstituted boron atom, usually a BF_2 unit (Figure 10). Derivatives of BODIPY are known to have small Stokes shifts, high quantum yields, sharp absorption and emission peaks, and high solubility in many organic solvents.[36] This combination of properties makes BODIPY fluorophore an excellent imaging agent. There have been several reviews covering the synthesis and application of BODIPY compounds.[36,37] The general method used to construct BODIPY analogues is treating dipyrromethene with boron trifluoride etherate and a tertiary amine. The dipyrromethene is synthesised from the corresponding pyrroles via the Knorr pyrrole protocol and subsequent oxidation.

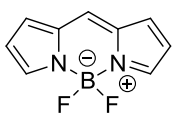
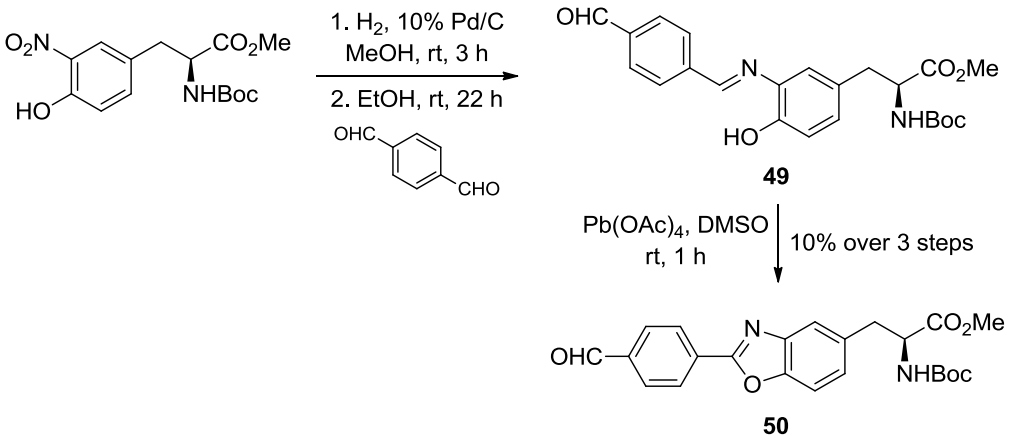


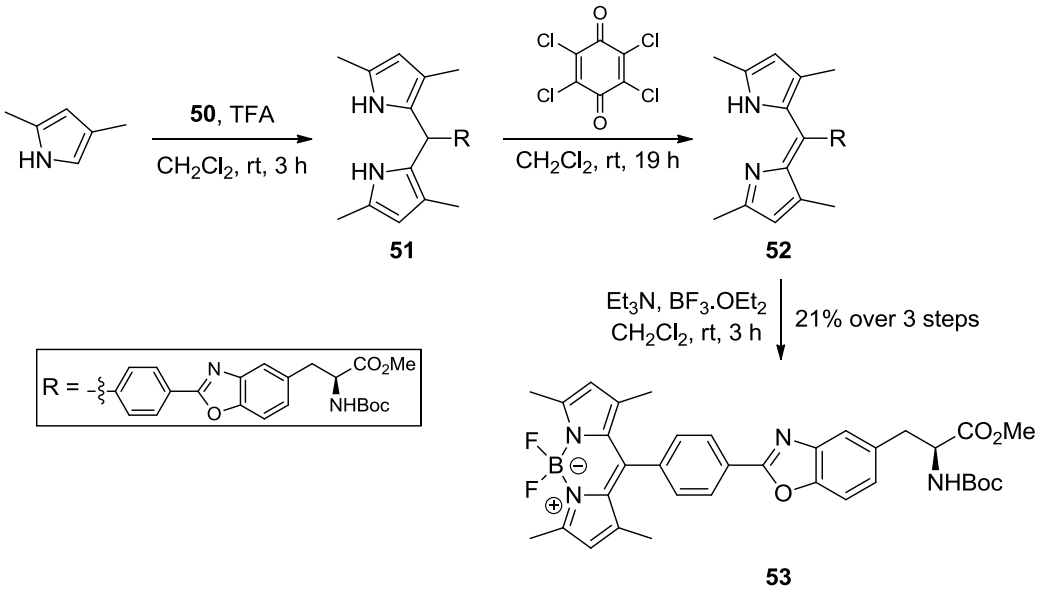
Figure 10: Unsubstituted BODIPY core structure.

Due to the desirable optical properties associated with BODIPY derivatives, several groups have synthesised BODIPY derived α-amino acids and used them in biological studies.[38] Guzow and co-workers reported the synthesis of a BODIPY-derived α-amino acid with a benzoxazole moiety linking the fluorophore with an alanine derivative.[39] Starting with *N*-Boc-3-nitro-L-tyrosine methyl ester, the nitro group was reduced using standard catalytic hydrogenation conditions, followed by treatment with terephthalaldehyde which furnished Schiff base **49** (Scheme 10).[40] Lead(IV) acetate was used as the oxidising agent, inducing cyclisation to benzoxazole **50**.



Scheme 10: Synthesis of benzoxazole-derived α-amino acid **50**.

The next stage in the synthesis was construction of the BODIPY core.[39] Compound **50** was condensed with two equivalents of 2,4-dimethyl-1*H*-pyrrole in the presence of TFA to give dipyrromethane derivative **51** (Scheme 11). *p*-Chloranil, a quinone oxidant was used to generate dipyrromethene **52**. Upon treatment with boron trifluoride etherate and triethylamine, this gave the final BODIPY derivative **53**, in a 21% yield over 3 steps. The spectral and photophysical properties of **53** were described in detail using methanol as the solvent. A strong absorption band was observed at 501 nm with an additional band at 310 nm, resulting from the benzoxazole functionality. When excited at 501 nm it produced an emission band at 516 nm and had a quantum yield of 0.26. The group envisage this BODIPY-derived α-amino acid **53** being incorporated into peptides for biological studies.



Scheme 11: Synthesis of BODIPY-derived α-amino acid **53**.

Another very recent example of a fluorescent BODIPY derived α-amino acid was published by Lavilla and Vendrell's respective groups.[41,42,43] They have developed methodology that allows the efficient arylation of the indole C_2 position of tryptophan through the use of a palladium catalysed C-H activation strategy. In order to utilise this procedure, they synthesised two iodinated BODIPY derivatives, *m*-iodophenyl-BODIPY (41%) **54** and *p*-iodophenyl-BODIPY (29%) **55** (Figure 11).[44] Standard BODIPY synthesis conditions were employed, 3-iodobenzene/4-iodobenzene and dimethylpyrrole condensation, DDQ oxidation and BF_3OEt_2/TEA treatment.

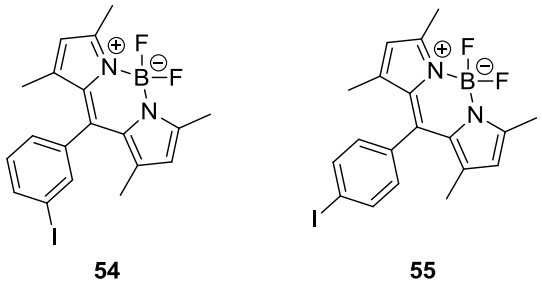
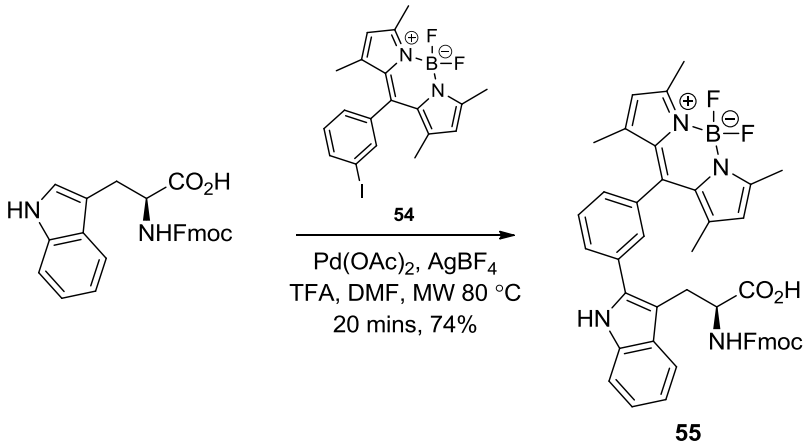


Figure 11: BODIPY iodine substrates for the Pd-catalysed C-H activation reaction.

Fmoc-L-tryptophan was subjected to the C-H activation conditions using **54** as the coupling partner, $Pd(OAc)_2$ as the palladium(II) source, in the presence of $AgBF_4$ and TFA, both were critical for the catalytic cycle (Scheme 12).[44] The reaction was assisted by microwave irradiation, 80 °C for 20 minutes, which made gram-scale synthesis of **55** possible in a high 74% yield. The absorption and emission maximum values for **55** were 503 and 517 nm respectively.



Scheme 12: Synthesis of Trp-BODIPY-derived α-amino acid **55**.

Amino acid **55** was incorporated into Peptide AntiFungal 26 (PAF26).[44] PAF26 is a synthetic antimicrobial hexapeptide with high affinity for fungal cells and selectivity over bacterial and mammalian cells. The *C*-terminal has a hydrophobic domain (Trp-Phe-Trp) and an *N*-terminal cationic domain (Arg-Lys-Lys), both essential for its antifungal properties. As there are two tryptophan residues in this peptide, they decided to use SPPS to synthesise fluorogenic derivatives of PAF26, substituting Trp for Trp-BODIPY **55**. They wanted to assess the impact of **55** in the different positions of the hexapeptide, so four PAF26 analogues were constructed (e.g. Figure 12). The peptides activity towards the fungal pathogen *Aspergillus fumigatus*, various bacterial strains and human red blood cells (RBCs) were determined. Surprisingly, three of the fluorogenic hexapeptides displayed a higher affinity for *A. fumigatus* than the non-labelled PAF26. These results suggest that this novel labelling approach can report the interaction of PAF26 with *A. fumigatus* and does not disrupt the expected molecular interaction between the peptide and fungus.

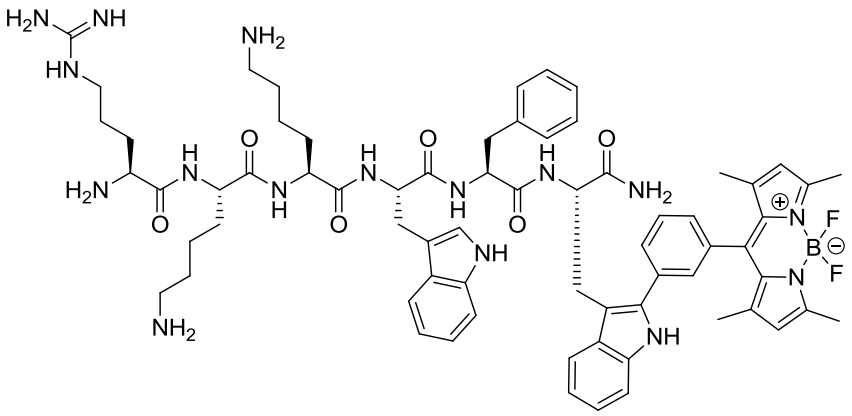


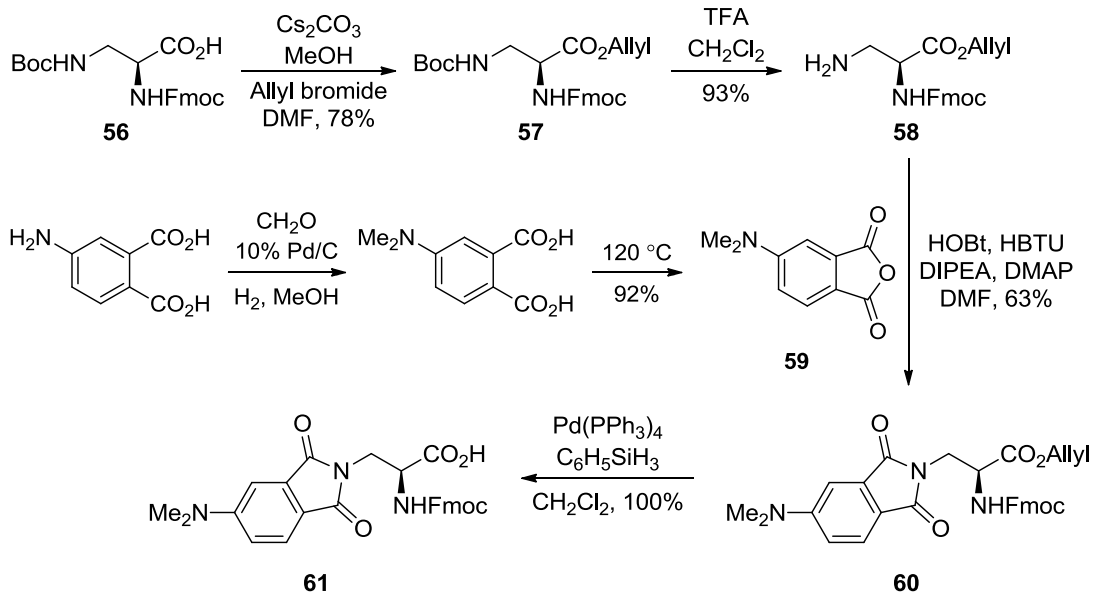
Figure 12: Trp-BODIPY derivative of hexapeptide PAF26.

1.8 Aminophthalimide- and Aminonaphthyl-Derived Fluorescent α-Amino Acids

4-Aminophthalimide is a derivative of phthalic anhydride and 2-naphthylamine is an aromatic amine derived from naphthalene (Figure 13). Careful consideration and design of α-amino acid derivatives of these compounds have led to the synthesis of highly sensitive probes for biological processes. Fluorophores bearing push-pull electronic properties are of great interest and there have been several syntheses and subsequent biological studies carried out on aminophthalimide and aminonaphthyl derived α-amino acids, due to this functionality.

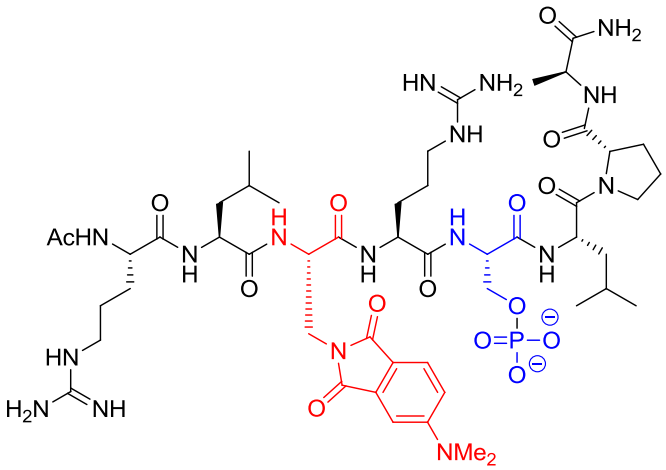
Figure 13: Basic structures of 4-aminophthalimide and 2-naphthylamine.

Imperiali has reported the synthesis of several phthalimide based α-amino acids. 4-Aminophthalimide was of interest because it was recognised as an environmentally sensitive fluorophore that responds to changes in polarity and viscosity.[45] It exhibits solvatochromism and due to its small size, could potentially mimic natural α-amino acids. With that in mind, the synthesis towards a 4-aminophthalimide-derived α-amino acid was developed starting with diaminopropionic acid derivative **56** (Scheme 13).[46] The carboxylic acid group was protected to give allyl ester **57** and following treatment with TFA gave amine **58**. Coupling partner **59** was synthesised from 4-aminophthalic acid via reductive amination and sublimation under reduced pressure. The phthalimide synthesis conditions required activation of the acid intermediate using HBTU and HOBt under basic conditions leading to phthalimide **60**. The allyl ester group was removed quantitatively using $Pd(PPh_3)_4$ and phenylsilane, producing aminophthalimide-derived α-amino acid **61**.



Scheme 13: Synthesis of 4-dimethylaminophthalimide-derived α-amino acid **61**.

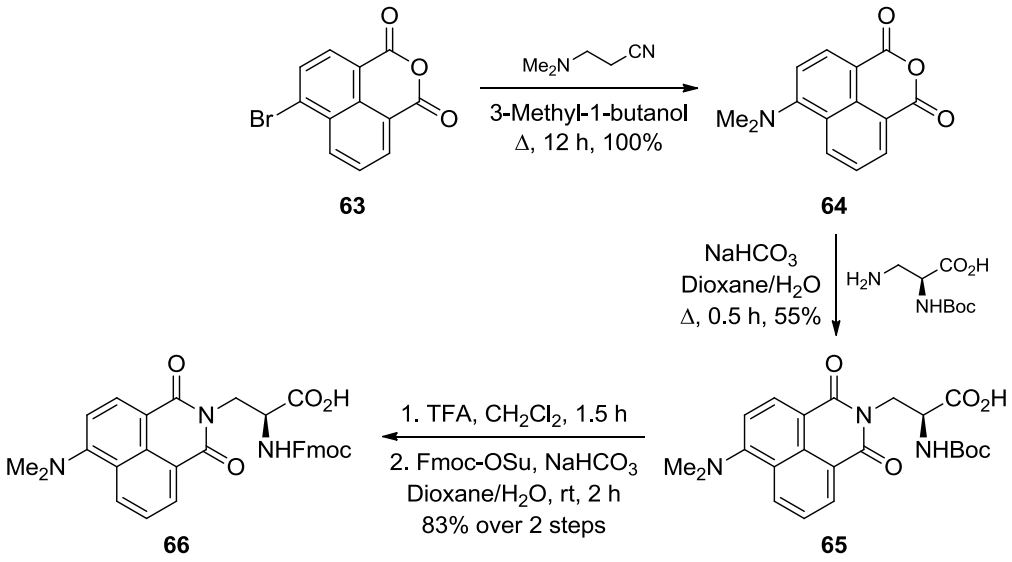
The biological system of interest was the 14-3-3 proteins which are involved in phosphoserine-dependant signalling and essential for cell signal regulation.[46] These proteins are known to bind short phosphopeptides efficiently, therefore compound **61** was incorporated into a short phosphopeptide (14-3-3bp) via standard SPPS protocols (Figure 14). The absorption maximum value for peptide **62** was 395 nm and it had an emission maximum of 510 nm. The fluorescently labelled 14-3-3bp-DAPA peptide was incubated with the 14-3-3 protein. Solutions of the protein-peptide mixture were tested for their fluorescent properties and it was noted that there was a fluorescence intensity increase as well as a blue-shifted emission maximum value from 570 nm (14-3-3bp-DAPA only) to 531 nm upon protein binding. As the peptide binds to the protein, the fluorescent amino acid enters a more hydrophobic environment and is shielded from surrounding water molecules allowing for the observed 6-fold increase in fluorescence intensity. The consequence of the blue-shift suggests that **61** is a highly sensitive fluorescent side-chain that exhibits variable optical properties in different environments.



62

Figure 14: Fluorescently labelled phosphopeptide 14-3-3bp-DAPA **62**.

Imperiali has synthesised several aminophthalimide-derived α-amino acids including a novel 4-*N*,*N*-dimethylamino-1,8-naphthalimide fluorophore.[47] This short synthesis began with anhydride-derived aryl bromide **63** which was transformed to the corresponding dimethylamino compound **64** using 3-dimethylaminopropionitrile in 3-methyl-1-butanol.[48] Anhydride **64** was then condensed with Boc-Dap-OH to form dimethylaminophthalimide **65**. A trivial deprotection/reprotection procedure followed to give Fmoc protected α-amino acid derivative **66**, with an overall yield of 46% over the four steps.



Scheme 14: Synthesis of dimethylaminophthalimide-derived α-amino acid **66**.

Amino acid **66** was incorporated into a lysine and arginine rich peptide, termed KR in order to measure its susceptibility to SPPS, rate of hydrolysis, and optical properties.[47] The results were compared with the other previously synthesised dimethylaminophthalimide α-amino acids (4-DMAP and 6-DMN) prepared by Imperiali as well as other known solvatochromic fluorophores,

BADAN (6-bromoacetyl-2-dimethylaminonaphthalene), dansyl chloride and NBD (4-fluoro-7-nitrobenzofurazan) (Figure 15).[49,50] The previously reported dimethylaminophthalimides were susceptible to hydrolysis when subjected to nucleophilic bases such as the standard base used in SPPS, piperidine. HPLC analysis suggested that there was no reaction for KR_{4DMN} when treated with 20% 4-methylpiperidine/DMF whereas KR_{4DMAP} and KR_{6DMN} had several other peaks, meaning side reactions were taking place. At high pH levels 4-DMAP and 6-DMN have a tendency for the imide ring to hydrolyse over time. A comparison between KR_{4DMAP} and KR_{4DMN} suggested that 4-DMN is able to stay intact whereas 4-DMAP eventually hydrolyses. This was measured via fluorescence spectroscopy as a non-fluorescent by-product is formed from 4-DMAP upon hydrolysis. The solvatochromic properties of 4-DMN were also measured using water and dioxane to investigate the effect of polar and non-polar solvents on the fluorophore.[47] Again the KR peptide motif was utilised to ensure solubility was maintained in both solvent systems. 18-Crown-6 was also present in the dioxane samples to complex with the protonated primary amines on the lysine residues and N-terminus, guaranteeing solubility. The difference in emission maximum values between solvents for all the fluorophores tested was staggering. All dimethylaminophthalimide samples in tris-buffered saline (TBS) displayed no emission maximum bands whereas in dioxane, there were 1200–4500 fold increases in fluorescence intensity. This is attributed to the extremely low fluorescence quantum yields displayed by these dimethylaminophthalimides in polar protic solvents. Overall, the utility of **66** as a fluorescent tag has been demonstrated in this report. It is able to exhibit desirable properties with very few compromises. Excellent attributes include excitation in the visible region, short synthesis of the Fmoc derivative and that 4-DMN is more stable than the previously synthesised dimethylaminophthalimides. However, it is still able to maintain the switch-like fluorescent properties typical for this compound series.

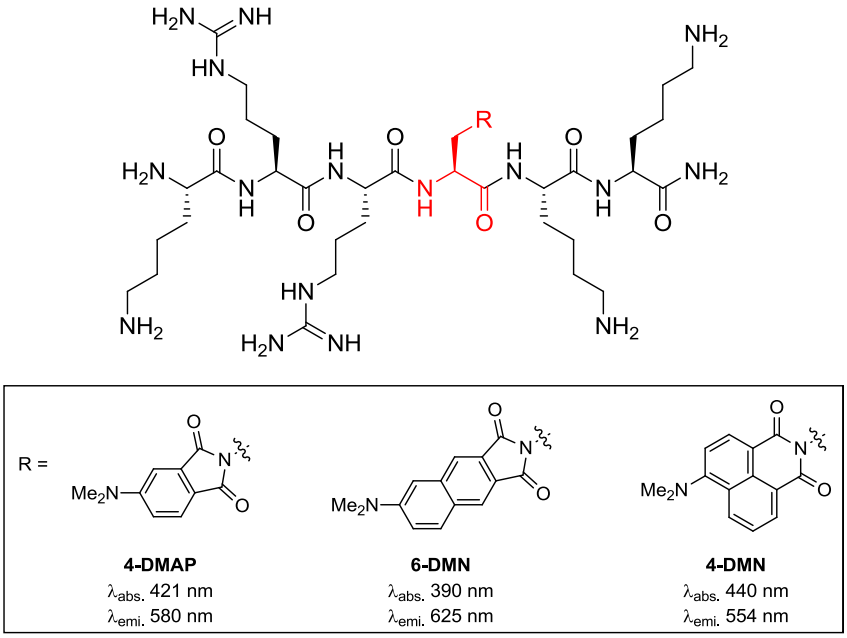
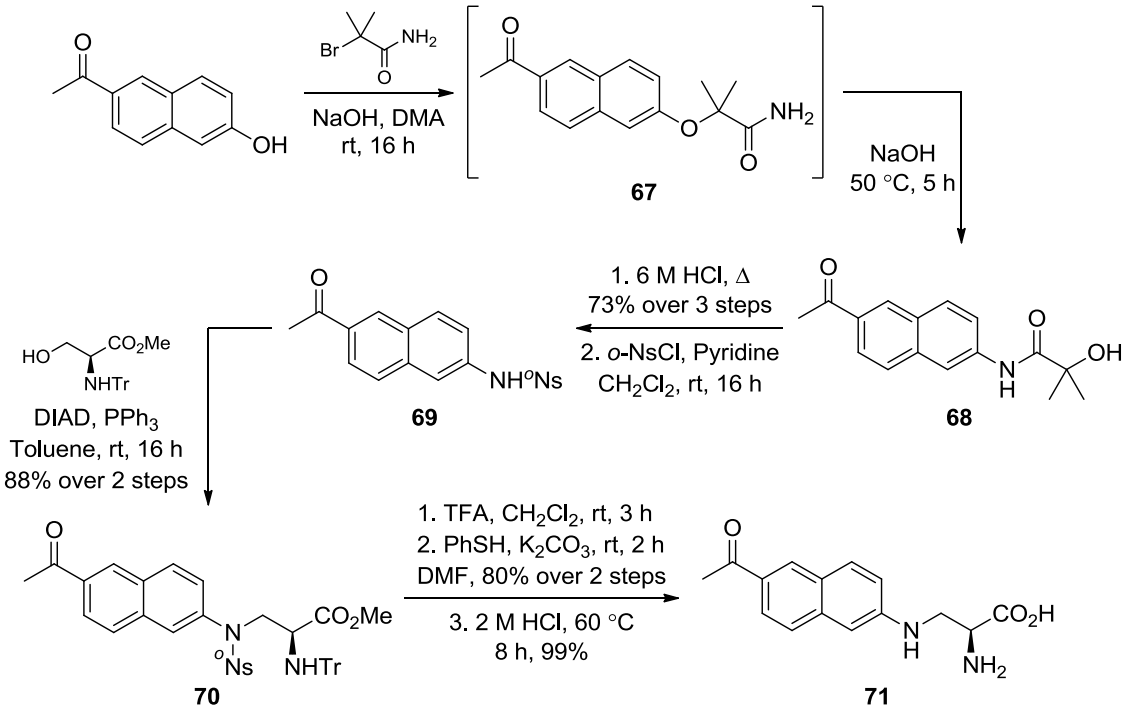


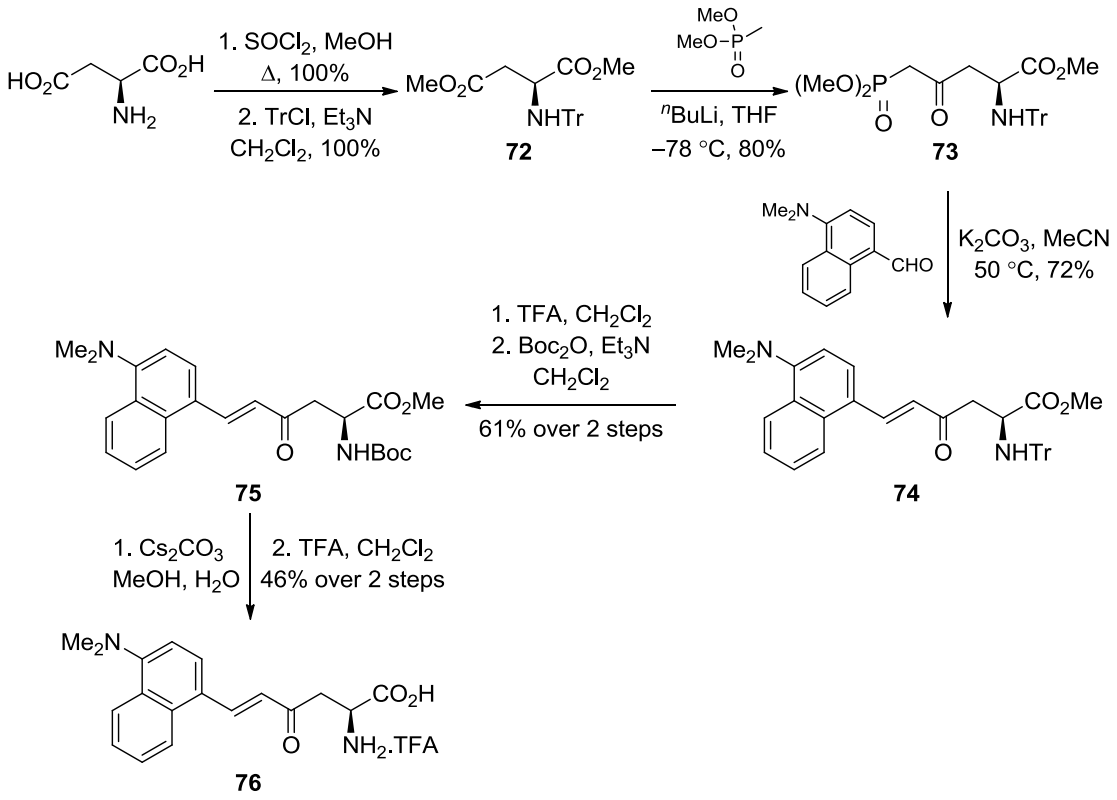
Figure 15: KR peptide sequence and the three dimethylaminophthalimide side-chains.

α-Amino acids with aminonaphthyl side-chains have also been investigated extensively. Anap, a known unnatural α-amino acid, synthesised by Schultz and co-workers, was incorporated into yeast proteins with high efficiency and specificity with response to the amber codon.[51] It was also site-specifically incorporated into *E. coli* glutamine binding proteins and used to probe structural changes caused by ligand binding.[51] However, it was prepared via an inefficient 8-step racemic synthesis with an overall yield of 8.1%. An improved stereospecific synthesis was reported by Wang and co-workers.[52] This began with 6-hydroxy-2-acetylnaphthyl which was subjected to a one-pot alkylation-Smiles rearrangement-hydrolysis sequence, inspired by Mizuno and Yamano's phenol to aniline protocol (Scheme 15).[53] The alkylating agent used was 2-bromo-2-methylpropanamide. After formation of intermediate **67**, the Smiles rearrangement was employed under basic conditions to give amide **68**. Acidic conditions were used to induce hydrolysis and subsequent protection with an *o*-nitrobenzenesulfonyl group produced protected aniline **69**. The key step in this synthesis was the Fukuyama-Mitsunobu reaction which was used to couple the fluorophore and amino acid moieties together to furnish **70**. The final steps in the synthesis were the removal of all protecting groups to provide the parent α-amino acid **71**. This approach is not only higher yielding (overall yield of 51%) but also required fewer steps and allowed the synthesis of the bioactive L-enantiomer. The absorption and emission maximum values for **71** were 350 and 575 nm respectively, with a quantum yield of 0.48 using ethanol as the solvent.



Scheme 15: Synthesis of aminonaphthyl-derived α-amino acid **71**.

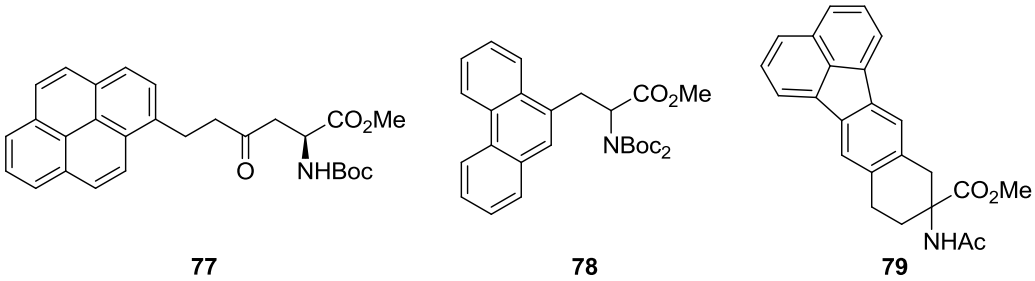
Fowler *et al* have also synthesised a dimethylaminonaphthyl-derived α-amino acid with a conjugated enone group connecting the fluorophore to the α-amino acid.[54] The synthesis began with a chiral pool starting material, L-aspartic acid which was fully protected to give *N*-trityl dimethyl ester **72** (Scheme 16). Dimethyl methylphosphonate was deprotonated using butyl lithium and added to dimethyl ester **72** which transformed into the corresponding β-ketophosphonate ester **73**. The key step in this synthesis was the HWE (Horner-Wadsworth-Emmons) reaction which was used to form a new *E*-alkene between the dimethylaminonaphthyl aldehyde and **73** to furnish enone **74**. To complete the synthesis a deprotection/reprotection strategy was used to isolate the Boc protected amine **75**. Final methyl ester hydrolysis and Boc group removal led to the dimethylaminonaphthyl derived α-amino acid **76**. Compound **76** was tested in a variety of solvents for its optical properties. In water, a polar protic solvent, it exhibited an emission band at 552 nm but in a non-polar solvent like toluene, a hypsochromic shift was observed and emission was recorded at 506 nm. Compound **76** is solvatochromic because it has the ability to change colour due to changes in solvent polarity. It was envisaged that this compound could be used as a fluorescent biomarker, being incorporated into peptides/proteins for biological studies.



Scheme 16: Synthesis of dimethylaminonaphthyl-derived α-amino acid **76**.

1.9 Polyaromatic-Derived Fluorescent α-Amino Acids

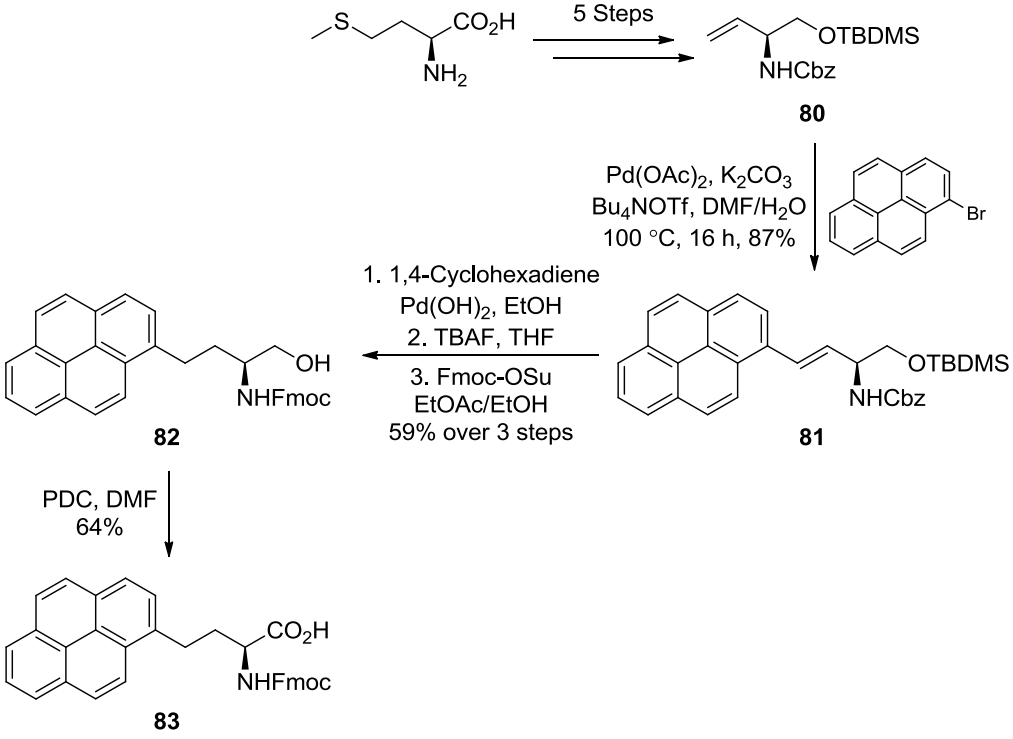
Another class of fluorescent α-amino acids that has been widely investigated has been the use of conjugated polyaromatic groups as the α-amino acid side chain. The optical properties of these polyaromatic side chains may stay consistent when using polar and non-polar solvents, which potentially limits their value as reporting probes. However, their use as standard fluorescent tags is desirable because the fluorescence can be tuned through variation in the functional groups present in the aryl side-chains as well as making the framework of the compound more rigid via the use of fused ring systems. The standard approach involves coupling the fluorescent polyaromatic side chain to an activated amino acid core. Mohite and Bhat developed a Knoevenagel condensation-reductive decarboxylation strategy using aspartic and glutamic acid derivatives to form novel keto α-amino acids with various aromatic side chains such as the pyrene derivative **77** (Figure 16).[55] Ferreira *et al* used a rhodium-catalysed conjugate addition reaction to make β-arylalanine derivatives with fluorescent groups attached such as phenanthrene analogue **78**.[56] An approach towards constrained analogues of phenylalanine was designed by Kotha and Meshram.[57] A Diels-Alder cycloaddition was employed to join the fluoranthene-fused sultine to methyl 2-acetamidoacrylate yielding conformationally constrained fluoranthene α-amino acid derivative **79**.



77 **78** **79**

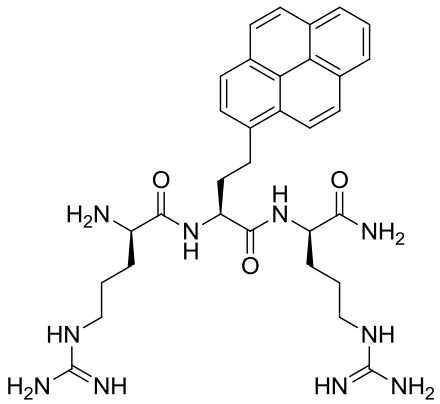
Figure 16: Polyaromatic-derived α-amino acids.

Work carried out by Göbel and his co-workers led to the synthesis of enantiopure α-amino acids with aromatic and heteroaromatic side chains.[58] The synthesis began with L-methionine which was reduced to the alcohol and converted to vinylglycinol **80** via a thermal *syn* elimination reaction previously reported in the literature (Scheme 17).[59] The key step in this synthesis was the Heck coupling which was used to fuse the pyrene framework to the amino acid moiety. The optimised conditions involved quaternary ammonium salts which act as the catalyst promoter. No other ligands were used and high yields were obtained across the substrate scope for this procedure. Furthermore, exclusive formation of *E*-alkene **81** was observed in all cases. Transfer hydrogenation was used to reduce the styrene double bond and remove the Cbz protecting group, without any aromatic side chain reduction. Silyl protecting group removal and subsequent Fmoc amine protection followed to give primary alcohol **82**. The final synthetic step was PDC oxidation to furnish the carboxylic acid **83**.



Scheme 17: Synthesis of pyrene-derived α-amino acid **83**.

The Fmoc protected amino acid was incorporated into a tripeptide **84**, flanked by two arginine residues due to their crucial role in Tat-TAR binding (Figure 17).[58] Previous work in the group has shown that cationic tripeptides can bind to the viral RNA element TAR derived from HIV-1.[60] The complexation of these tripeptides with RNA disrupts the Tat-TAR moiety, which is a molecular switch that regulates the transcriptional efficiency in HIV, effectively halting the spread of HIV in cell culture studies. RNA binding assays were carried out and the IC_{50} and K_d values were obtained for **84** and the other analogues synthesised in this current study. A rhodamine and fluorescein tagged Tat peptide was also used as a binding site competitor as they are able to efficiently quench dyes when RNA is absent. Peptide **84** had an IC_{50} of 0.9 μM which was the lowest value measured and a K_d value of 50 nM. This biological study has proven that tripeptide **84** is one of the most potent small molecule ligands for TAR to date.

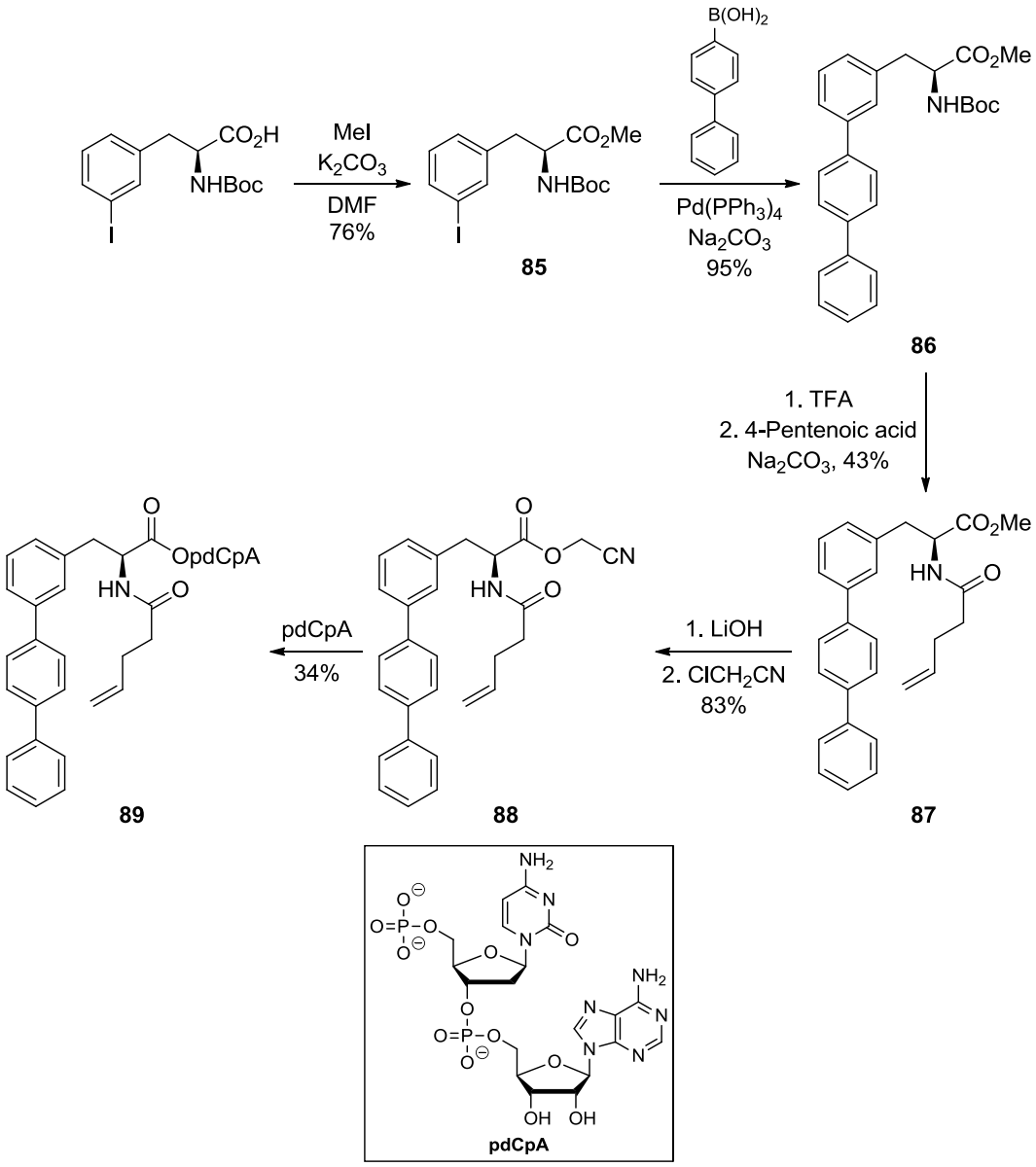


84

Figure 17: Tripeptide **84**.

A popular method for constructing fluorescent polyaromatic α-amino acids is using the Suzuki-Miyaura cross coupling reaction. An example of this is the work carried out by Hecht and co-workers.[61] They describe the synthesis of four biphenyl-phenylalanine derivatives which employ the palladium catalysed coupling reaction as their key synthetic step. The synthesis began with *N*-Boc-3-iodophenylalanine which was esterified with iodomethane under basic conditions to give methyl ester **85** (Scheme 18). Suzuki-Miyaura conditions were then used to couple 4-biphenylboronic acid to aryl iodide **85**. An excellent yield of 95% was obtained, generating terphenylalanine analogue **86**. The Boc group was removed with TFA followed by reprotection to produce pentenoyl protected amine **87**. Methyl ester hydrolysis with lithium hydroxide ensued and the corresponding carboxylic acid was activated as a cyanomethyl ester **88**. Treatment with a solution of tris-(tetrabutylammonium) salt of pdCpA (5'-Phospho-2'-deoxyribocytidylriboadenosine) led to aminoacetyl pdCpA terphenylalanine derivative **89**. The optical properties of this analogue were measured using the *N*-pentenoyl-protected amino acid giving an absorption and emission band at 280 and 342 nm respectively. A high emission efficiency of 0.67 was also calculated. All four of the

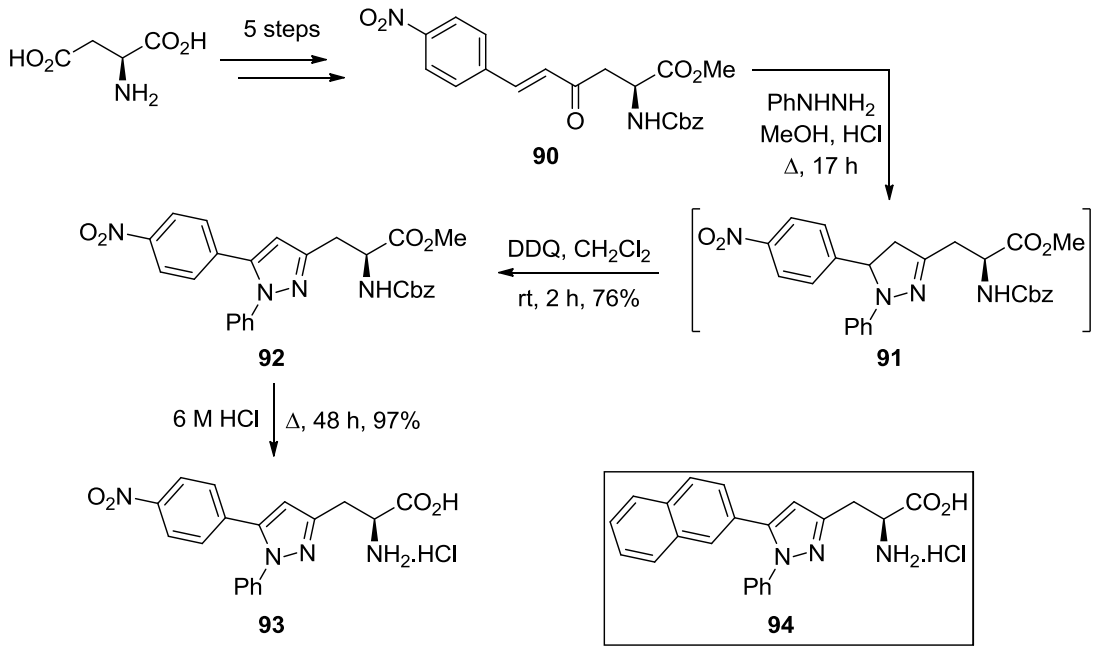
terphenylalanine derivatives were used to activate tRNA transcripts after being incorporated into multiple positions of ecDHFR.[61] Efficient incorporation into position 16 was possible and for all four modified proteins the rate of NADPH consumption doubled when compared with the wild type. The sterically accessible position 49 was also targeted and this led to comparable catalytic function of the four modified proteins with the wild type. A folded region of the protein was of interest to investigate whether the sizeable biphenyl-phenylalanine derivatives could be incorporated with minimal perturbation towards the functionality of DHFR. Two of the four modified proteins were accommodated at the sterically demanding position 115 and **89** was incorporated to one of the proteins that did not perform well in this position. It was hypothesised that aryl groups substituted at the *para* position of the phenylalanine ring introduced the least amount of steric hindrance in proximity to the protein backbone. The group also incorporated these novel fluorescent α-amino acids into ecDHFR along with a 7-hydroxycoumarin α-amino acid. They managed to demonstrate that these compounds can effectively transfer energy to this coumarin via a FRET mechanism.



Scheme 18: Synthesis of biphenyl-phenylalanine-derived α-amino acid **89**.

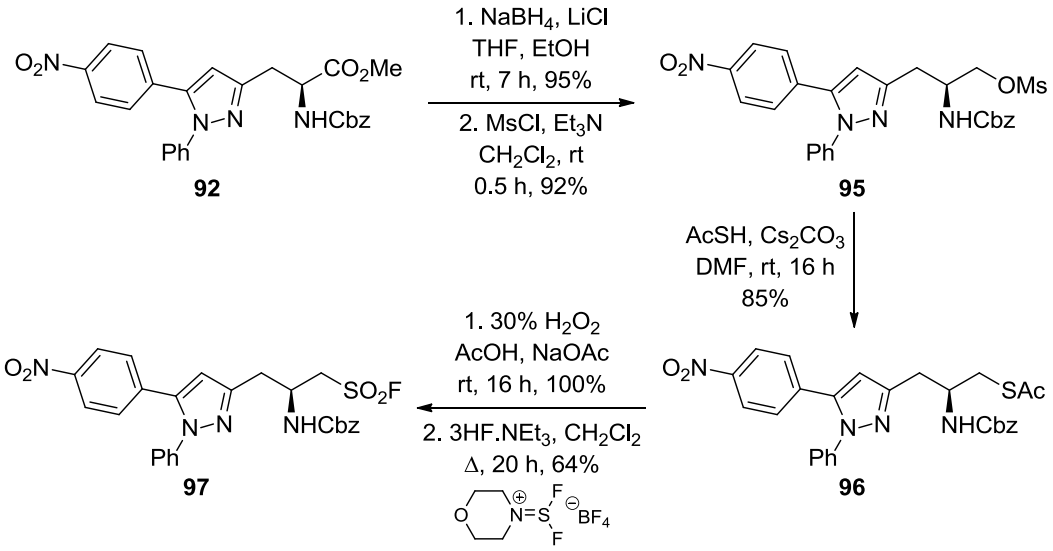
Gilfillan *et al* managed to utilise the α,β-unsaturated ketone functionality on a series of enone derivatives to construct a small five-membered library of pyrazole derived α-amino acids.[62] The synthesis proceeded with L-aspartic acid and a five-step protocol previously developed was employed to access the Cbz protected amine **90** (Scheme 19). Enone **90** was reacted with phenylhydrazine under acidic conditions which efficiently led to the 2-pyrazoline intermediate **91**. Immediate reaction with DDQ oxidised the ring to the fully unsaturated and aromatic pyrazole moiety **92**. Deprotection to the parent α-amino acid was possible using 6 M hydrochloric acid under reflux, producing **93** in an excellent 97% yield. The optical properties for this library of novel α-amino acids were measured. The nitrophenyl derivative **93** had an absorption maximum of 303 nm and displayed an emission band at 415 nm in the visible region of the electromagnetic spectrum. A naphthyl derivative **94** absorbed at 249 nm and emitted strong fluorescence at 356 nm that was blue-shifted in comparison with the nitrophenyl analogue **93**. Naturally occurring tryptophan has

an emission maximum value of 352 nm which is very similar to that of **94**, meaning the potential to use this α-amino acid as a fluorescent probe is limited because it would be difficult to distinguish between the natural and unnatural α-amino acid residues. This is not the case for **93** as it has a red-shifted wavelength in comparison to tryptophan.



Scheme 19: Synthesis of pyrazole-derived α-amino acids.

Due to the promising optical properties displayed by the nitrophenyl derivative **93**, investigation into further functionalisation of this compound was examined. α-Amino acids with sulfonyl fluoride functionality have shown to act as electrophilic traps for the inhibition of protease enzymes.[63] A *N*-Cbz protected tetrapeptide with a sulfonyl fluoride moiety managed to demonstrate antimalarial activity.[64] With this in mind, experiments were carried out to investigate whether **92** could be transformed into a multi-functional compound that could be used as a fluorescent probe as well as a protease enzyme inhibitor. Methyl ester **92** was reduced using sodium borohydride to the primary alcohol and subsequently treated with mesyl chloride to form mesylate **95** (Scheme 20). Cesium thioacetate was generated *in situ* which displaced the mesylate group forming thioacetate **96**. Oxidation with hydrogen peroxide formed the sodium sulfonate salt. The sulfonyl fluoride **97** was generated through the use of triethylamine trihydrofluoride and deoxofluorination agent, XtalFluor-M®. The target compound was converted from **92** in an excellent 48% overall yield. This synthesis has demonstrated the potential for further functionalisation of these pyrazole derived α-amino acids to make duel action fluorescent probes.



Scheme 20: Synthesis of sulfonyl fluoride α-amino acid derivative **97**.

1.10 Summary

The synthesis and biological application of fluorescent unnatural α-amino acids has increased considerably over the last 10–15 years, demonstrating their importance in chemical, biological and medicinal research. This is exemplified by the number of recent reviews on the design of novel fluorescent α-amino acids and their applications.[8,7,65]

Palladium-catalysed coupling reactions have been one of the most popular ways to fuse the conjugated fluorophore to the α-amino acid portion. There have also been examples where construction of the chromophore occurs initially which is followed by late stage asymmetric protocols to introduce the stereocentre, methods such as the Schöllkopf bis-lactim amino acid synthesis. Another favourable method is using readily available chiral pool α-amino acids as starting materials and performing various synthetic transformations to essentially build the novel fluorophores with the α-amino acid unit present throughout the synthesis.

The novel fluorescent α-amino acids described here have exhibited a range of optical properties. Some have blue-shifted emission maximum bands showing up in the UV and purple/blue regions of the electromagnetic spectrum. Many of these fluorophores are easily tuneable through functional groups transformations or extending conjugation through aryl group additions or fusing ring together to increase rigidity. This allows for bathochromic shifts to take place with regards to absorption and fluorescence, allowing emission maximum values to occur in the green and red areas of the visible spectrum, with some even managing to exhibit in the near-infrared (NIR) region (750–2500 nm).

Many of these unnatural α-amino acids have been used as tyrosine and tryptophan mimics allowing a fluorescent tag to be present in peptide and proteins with minimal disruption to structure and conformation. Donor and acceptor FRET pairings allow the investigation into the mechanism of enzymes and a variety of cellular processes.

Optical properties such as sharp absorption and emission peaks in the visible region that are tuneable, with high quantum yield values are the most sought after characteristics of a novel fluorophore. A rapid and high yielding route towards an Fmoc α-amino acid derivative that can be easily incorporated into peptides and proteins is another excellent property. Research is still underway to develop new fluorophores that exhibit all the desirable properties expected of a fluorescent probe to be used for new applications in medical research.[66]

2.0 Synthesis of Novel Fluorescent Heterocyclic-Derived α-Amino Acids

2.1 Previous Work in the Sutherland Group

Previous work carried out by Lindsay Fowler showed that an efficient four-step synthesis towards enone-derived α-amino acids was possible.[54,67] A large substrate scope was carried out to test the robustness of the Horner-Wadsworth-Emmons (HWE) reaction used to form the α,β-unsaturated ketone (Figure 18). Good to excellent yields were achieved using a range of commercially available aryl and alkyl aldehydes. A highly fluorescent dimethylaminonaphthyl analogue **76** was also synthesised during that portion of the research and displayed excellent optical properties. However, the enones were found to be unstable on long-term storage.

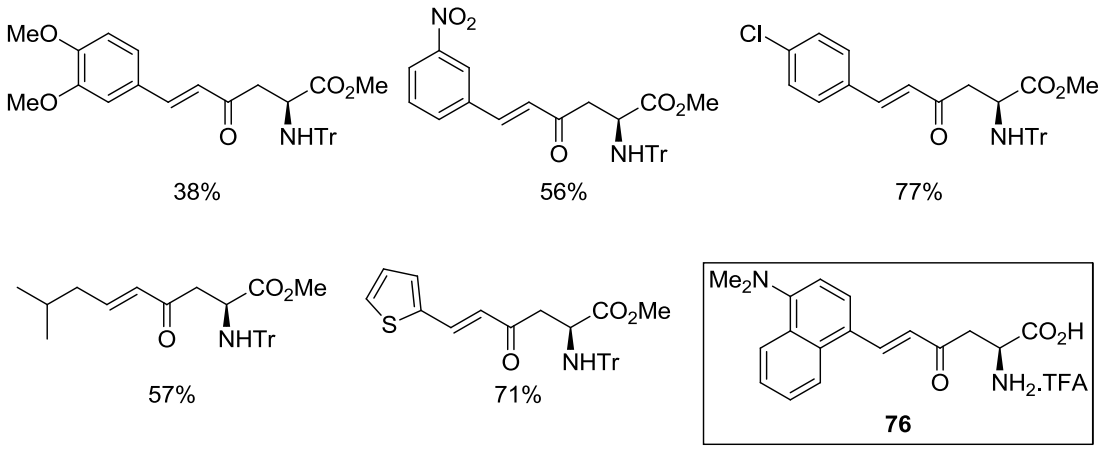


Figure 18: Selection of protected enone-derived α-amino acids and fluorophore **76**.

Lynne Gilfillan was tasked with the challenge of utilising the enone functionality to make a small library of more stable pyrazole-derived α-amino acids (Figure 19).[62,68] Synthesising a more stable heterocycle in place of the reactive enone, as well as extending the conjugation was advantageous. The optical properties of the compounds were tested and results showed that naphthyl **94** and nitrophenyl **93** were the most fluorescent.

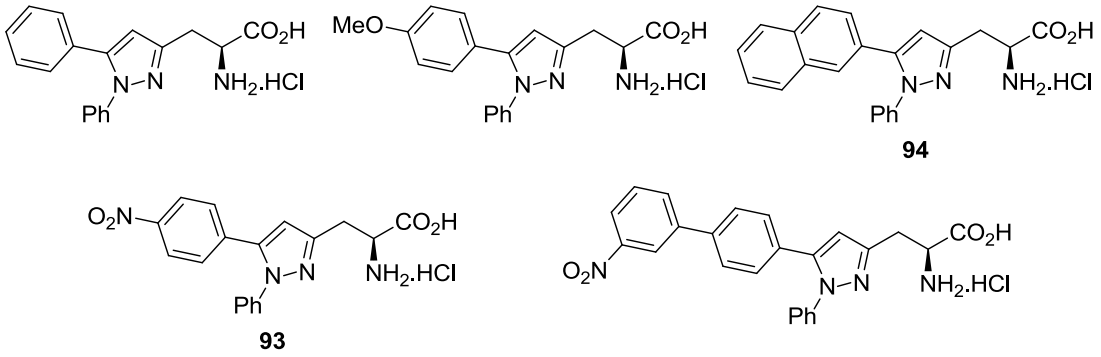
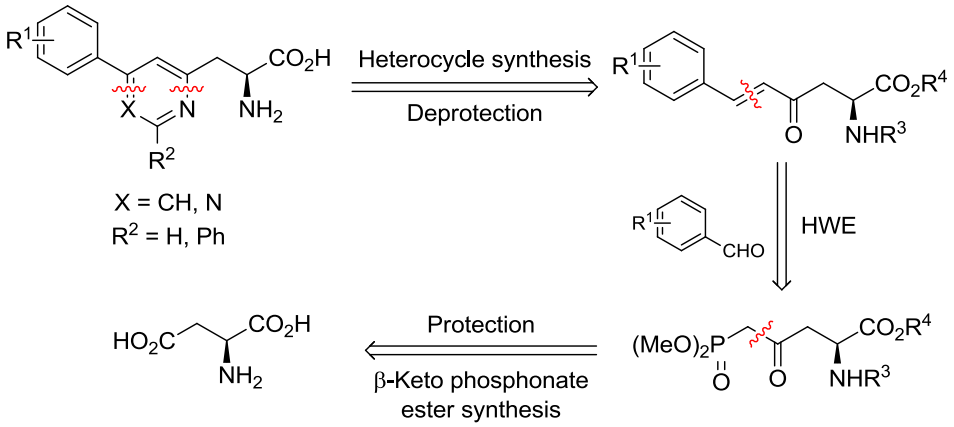


Figure 19: Five-membered library of pyrazole-derived α-amino acids.

2.2 Proposed Research

The aim of this project was to synthesise libraries of heterocyclic α-amino acid derivatives by exploring the reactivity of the corresponding enone functional group (Scheme 21). In order to fully understand the reactivity and scope of these enone derivatives, the idea was to subject them to further heterocycle forming reactions. Heterocycles such as pyridines,[69] functionalised pyridines,[70] pyrimidines,[71] pyridones[72] and functionalised pyrazoles[73] were of interest.



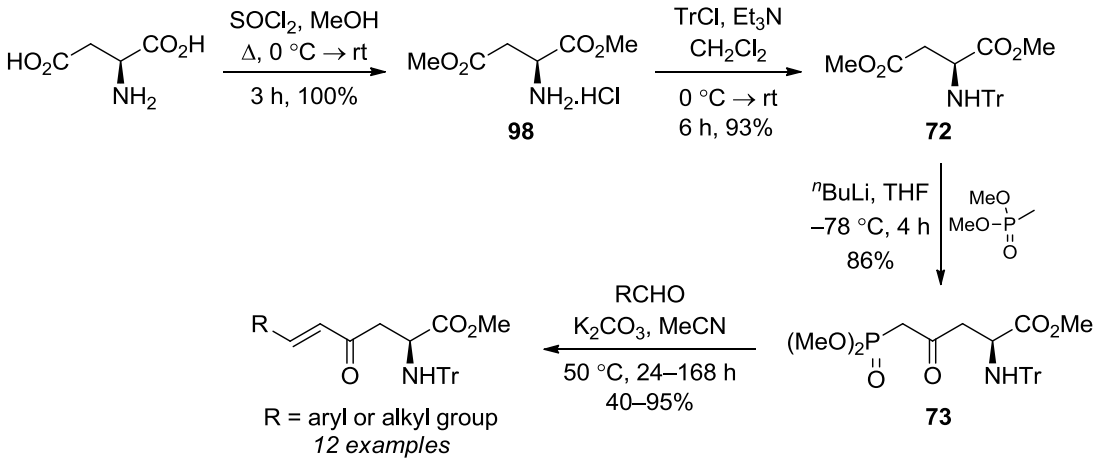
Scheme 21: Retrosynthesis of novel fluorescent α-amino acid derivatives.

Once libraries of these heterocycle-derived α-amino acids were synthesised, they were to be fully analysed with respect to their optical properties. Any that displayed interesting and comparable data to known unnatural α-amino acids that have been exploited as fluorescent probes in biological studies, were to be Fmoc protected. SPPS would be employed to couple the novel fluorescent α-amino acid onto a small CPP. Biological studies using fluorescence microscopy would follow to investigate whether the fluorophore-CPP conjugate could enter and accumulate in cells.

2.3 Synthesis of Pyridine-Derived α-Amino Acids

2.3.1 Synthesis of *N*-Trityl Protected Enone-Derived α-Amino Acids

The synthesis towards the enone derivatives began with the commercially available chiral pool starting material, L-aspartic acid. The carboxylic acid groups were protected using thionyl chloride and methanol to form dimethyl ester hydrochloride salt **98** in quantitative yield (Scheme 22). The primary amine was then protected with a bulky trityl group using standard conditions and excellent yields of 93% were achieved for *N*-trityl dimethyl ester **72**. Addition of the anion of dimethyl methylphosphonate to a solution of **72** in THF at −78 °C led to the regioselective formation of β-keto phosphonate ester **73** in an 86% yield. The reaction gives β-keto phosphonate ester **73** as the sole product due to protection of the α-methyl ester by the steric bulk of the *N*-trityl protecting group. The first key step of this synthesis was the HWE reaction. Previously optimised conditions led to the use of potassium carbonate as the base and acetonitrile as the polar aprotic solvent.[74] The reactions were performed at 50 °C and from one to seven days.



Scheme 22: Synthesis of β-keto phosphonate ester **73** and HWE reaction conditions.

β-Keto phosphonate ester **73** was subjected to the HWE conditions with various aldehydes used as the coupling partner. This led to a twelve-membered library of *N*-trityl enone-derived α-amino acids (Figure 20). The exclusive formation of the *E*-isomer was observed, in good to excellent yield. The identification of the *E*-enone was straightforward due to a vicinal coupling constant of approximately 16.2 Hz in the ^{1}H NMR spectra for these compounds. A range of different aldehydes were submitted to the reaction conditions which included electron-donating/withdrawing aryl groups, a biaryl system, a heterocycle and two alkyl groups. Yields greater than 69% were observed for all analogues apart from **107** which was obtained with a moderate yield of 40%. This was due to the reaction only being attempted once on a small-scale.

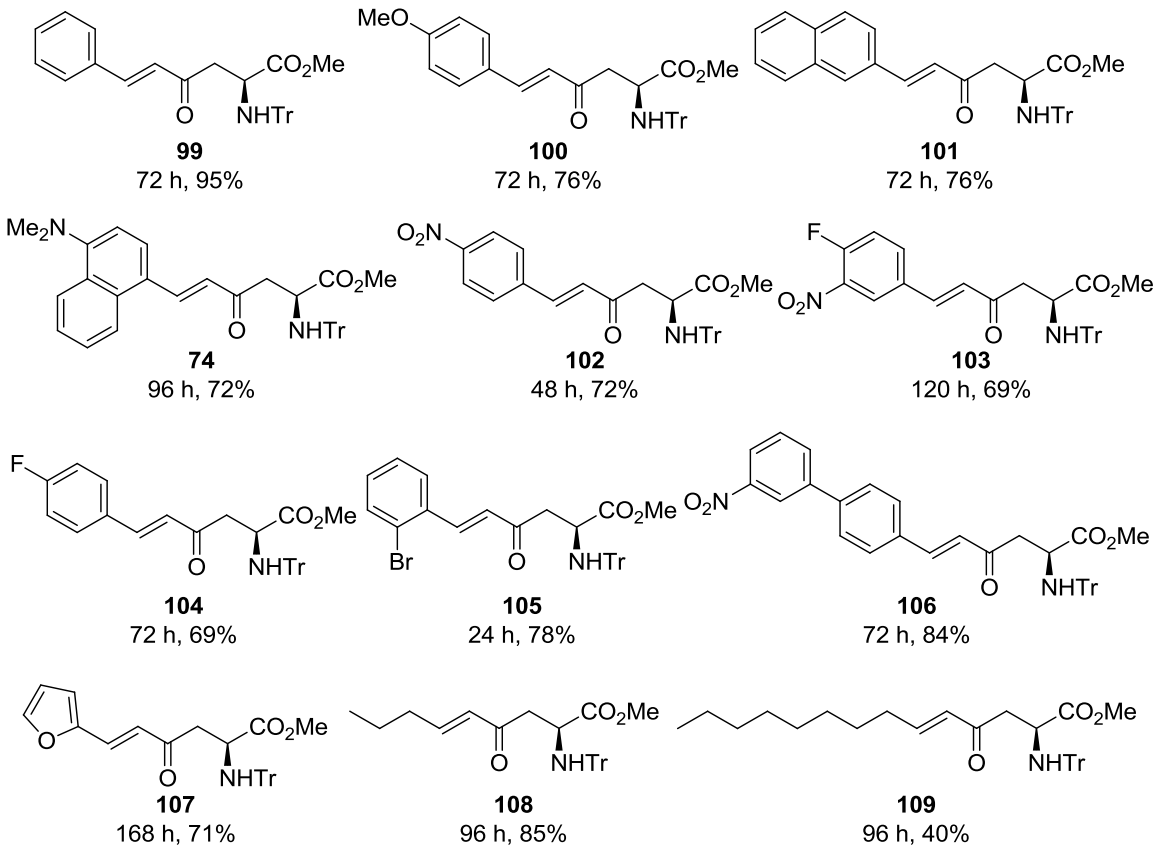
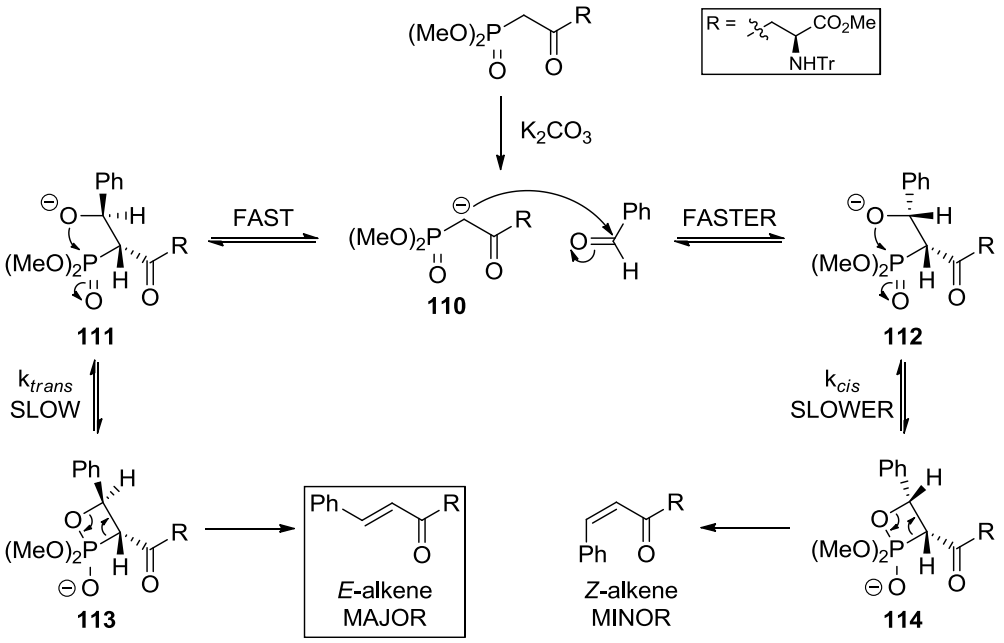


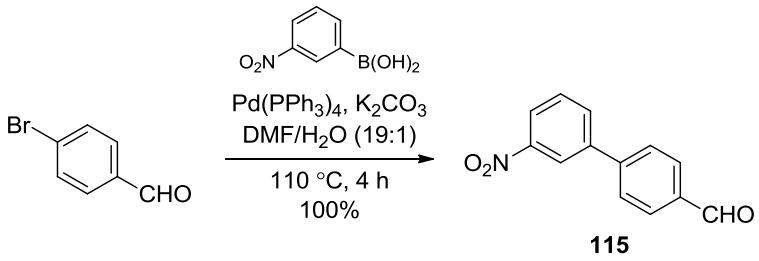
Figure 20: Substrate scope for *N*-trityl protected enone-derived α-amino acids.

The mechanism for the HWE reaction starts with the deprotonation of the most acidic hydrogen atom of the β-keto phosphonate ester (Scheme 23).[75] Reversible, stepwise attack of the phosphoryl stabilised carbanion **110** on benzaldehyde generates interchangeable oxyanions **111** and **112**. The pathway to the *anti*-addition intermediate **112** is faster compared to the *syn*-addition intermediate **111**. Reactive four-membered oxaphosphonates **113** and **114** are then generated, whereby the *trans*-intermediate **113** decomposes leading to the major thermodynamic *E*-alkene product. Both *trans* **113** and *cis* **114** pathways are slow to decompose. Due to the stereoselective nature of this olefination reaction the *cis* pathway must be considerably slower as no *Z*-alkene is observed.



Scheme 23: Mechanism of the HWE olefination.

The majority of the aldehydes used were commercially available, except the aldehyde used to make biaryl analogue **106**. The Suzuki-Miyaura cross-coupling reaction was used to synthesise 4-(3'-nitrophenyl)benzaldehyde **115** in quantitative yield from 4-bromobenzaldehyde and 3-nitrophenylboronic acid (Scheme 24). This protocol had already been reported in the literature.[76]

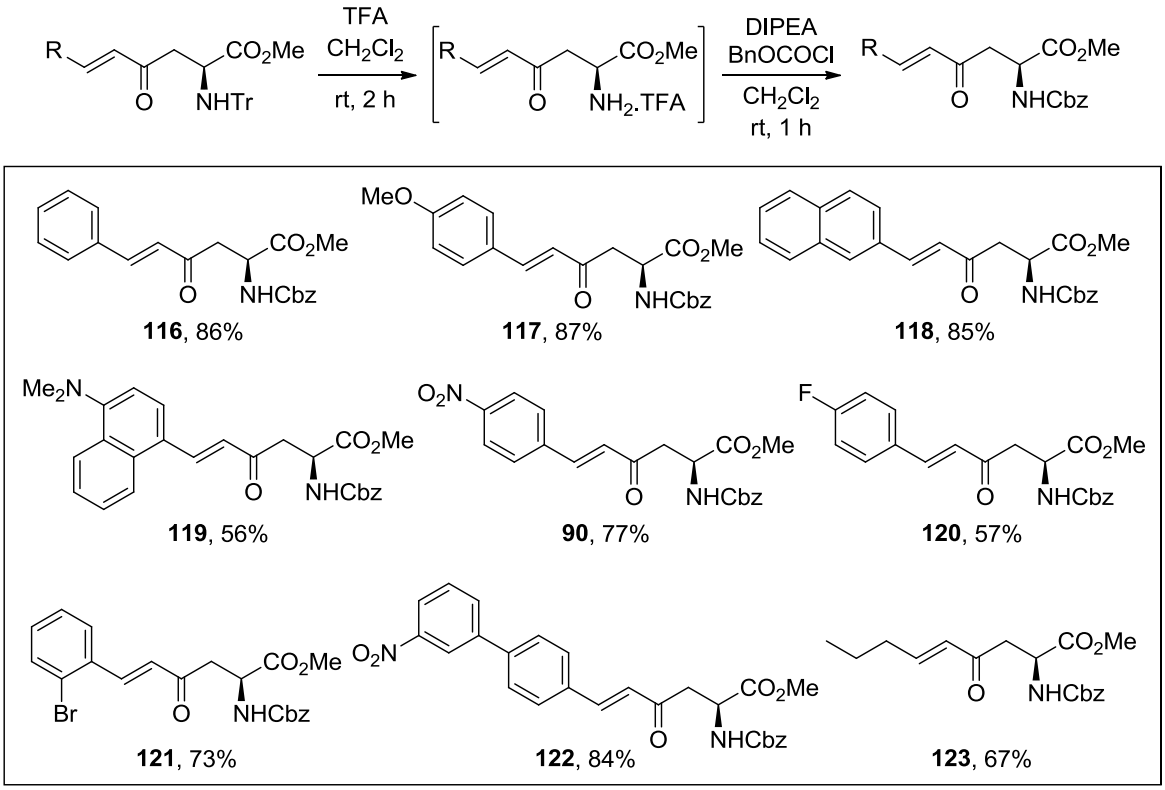


Scheme 24: Synthesis of 4-(3'-nitrophenyl)benzaldehyde **115**.

2.3.2 Synthesis of *N*-Cbz Protected Enone-Derived α-Amino Acids

Now that a library of enone-derived α-amino acids had been synthesised, the next step was to prepare intermediates that could be used in heterocycle forming reactions. Previous work had shown that *N*-trityl derivatives were too sterically hindered to allow reactions at the enone. Therefore, to ensure that heterocycle synthesis was efficient with no side-reaction occurrence, the next step was to remove the acid labile trityl group and reprotect with the Cbz protecting group (Scheme 25). The enone derivatives were treated with trifluoroacetic acid at room temperature, which cleanly removed the trityl group to form the intermediate TFA salt. Hünig's base was then used to neutralise the TFA, followed by benzyl chloroformate addition which led to the *N*-Cbz derivatives. This was a very rapid and clean reaction, allowing good to high yields for the two-step

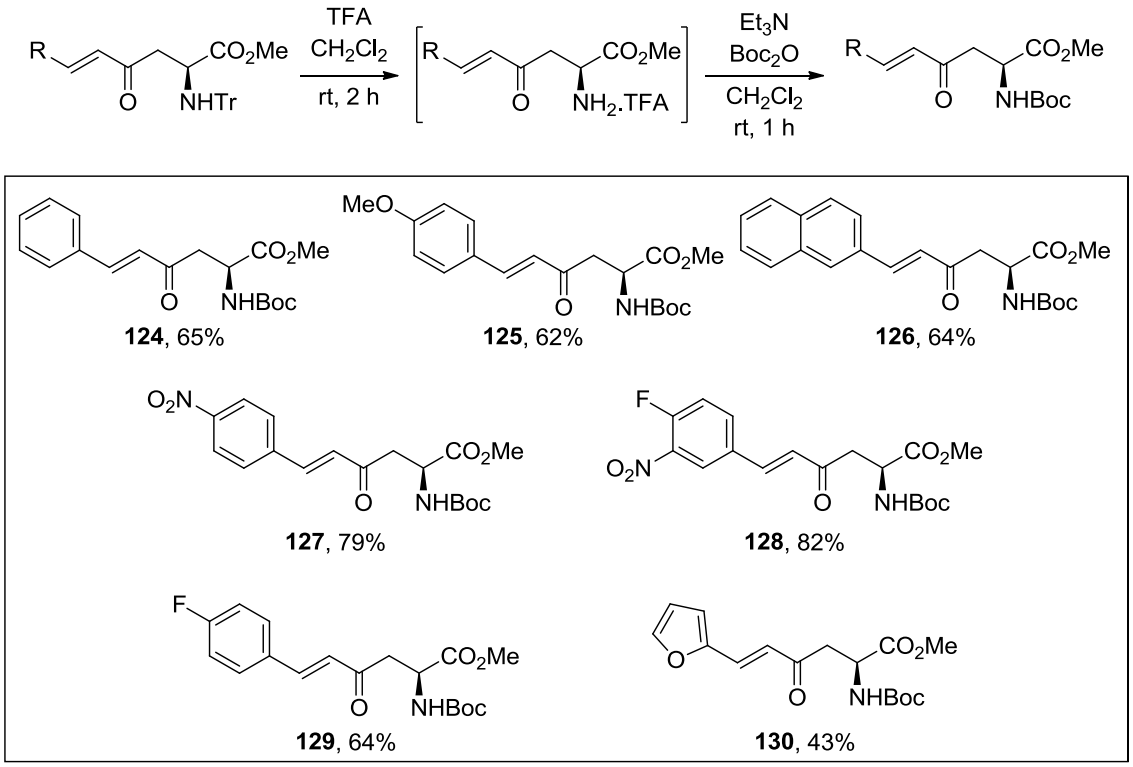
procedure. The substrate scope for this reaction focussed mainly on aryl side-chains. These aryl groups would allow conjugation with the resulting heterocycles giving good potential optical properties. A range of aryl side-chains with electron rich/poor functional groups were investigated. Propyl derivative **123** was also examined to prove that it is possible to subject alkyl side-chain analogues to this protocol.



Scheme 25: Deprotection/reprotection procedure to form *N*-Cbz protected enone-derived α-amino acids.

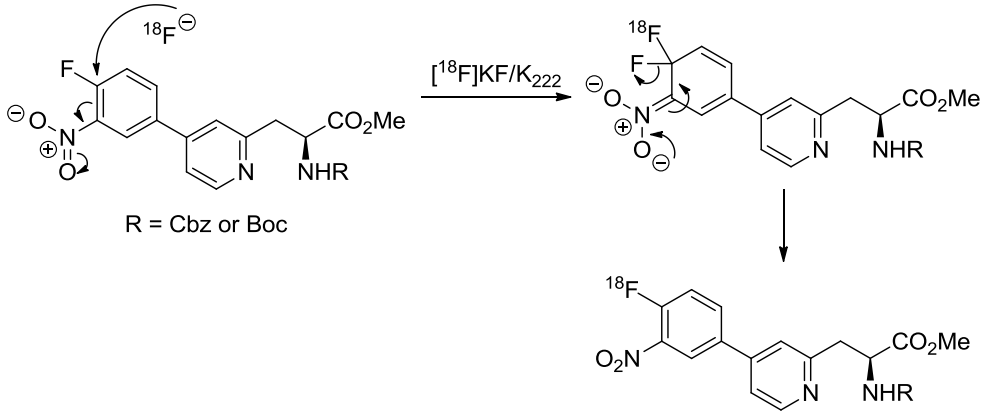
2.3.3 Synthesis of *N*-Boc Protected Enone-Derived α-Amino Acids

It was hypothesised that these *N*-Cbz protected enone derivatives would be stable towards the proposed heterocycle forming conditions. However, as a contingency plan, *N*-Boc protected derivatives were also synthesised. A two-step deprotection/reprotection procedure was employed, similar to that of the *N*-trityl to *N*-Cbz sequence. TFA was used to remove the acid labile trityl group which was followed by *N*-Boc protection using di-*tert*-butyl dicarbonate and TEA (Scheme 26). Aryl group side chains were the main focus of the substrate scope for the *N*-Boc protected series of enones. Yields ranging from 62–82% were achieved across the seven-membered library apart from furan derivative **130** which had only been synthesised once on a small-scale.



Scheme 26: Deprotection/reprotection procedure to form *N*-Boc protected enone-derived α-amino acids.

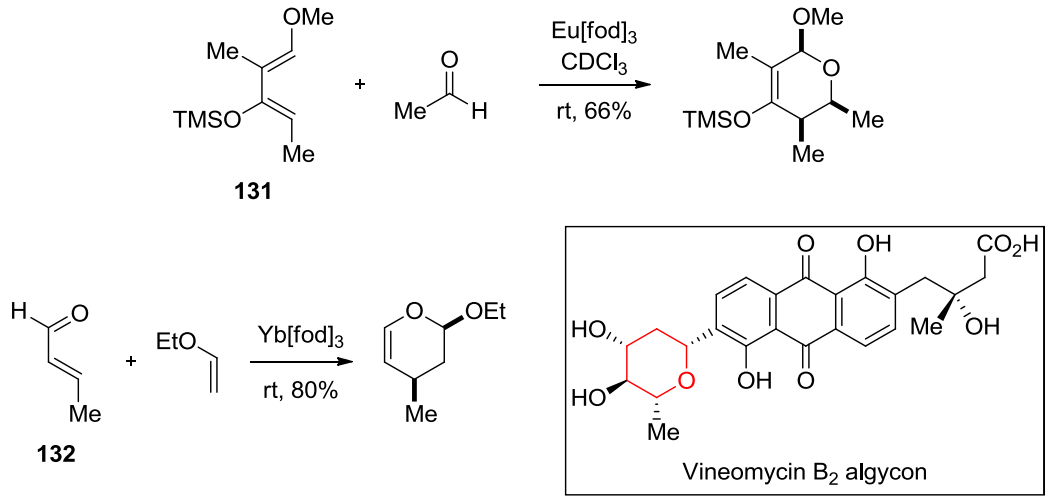
4-Fluoro-3-nitrophenyl derivative **128** was of interest as it could potentially be used in a nucleophilic aromatic substitution (S_NAr) reaction to install fluorine-18 (^{18}F), a radioactive fluorine isotope commonly used in positron emission tomography (PET) imaging.[77] Upon heterocycle synthesis, in this example a functionalised pyridine, the S_NAr reaction and incorporation of the ^{18}F radioisotope could be achieved using ^{18}F potassium fluoride and 2.2.2-cryptand (Scheme 27).[77] The resulting compound could be used as a dual action imaging agent being used in fluorescence or PET imaging.



Scheme 27: Proposed conditions and mechanism for S_NAr ^{18}F-radiolabelling of 4-fluoro-3-nitrophenyl analogue.

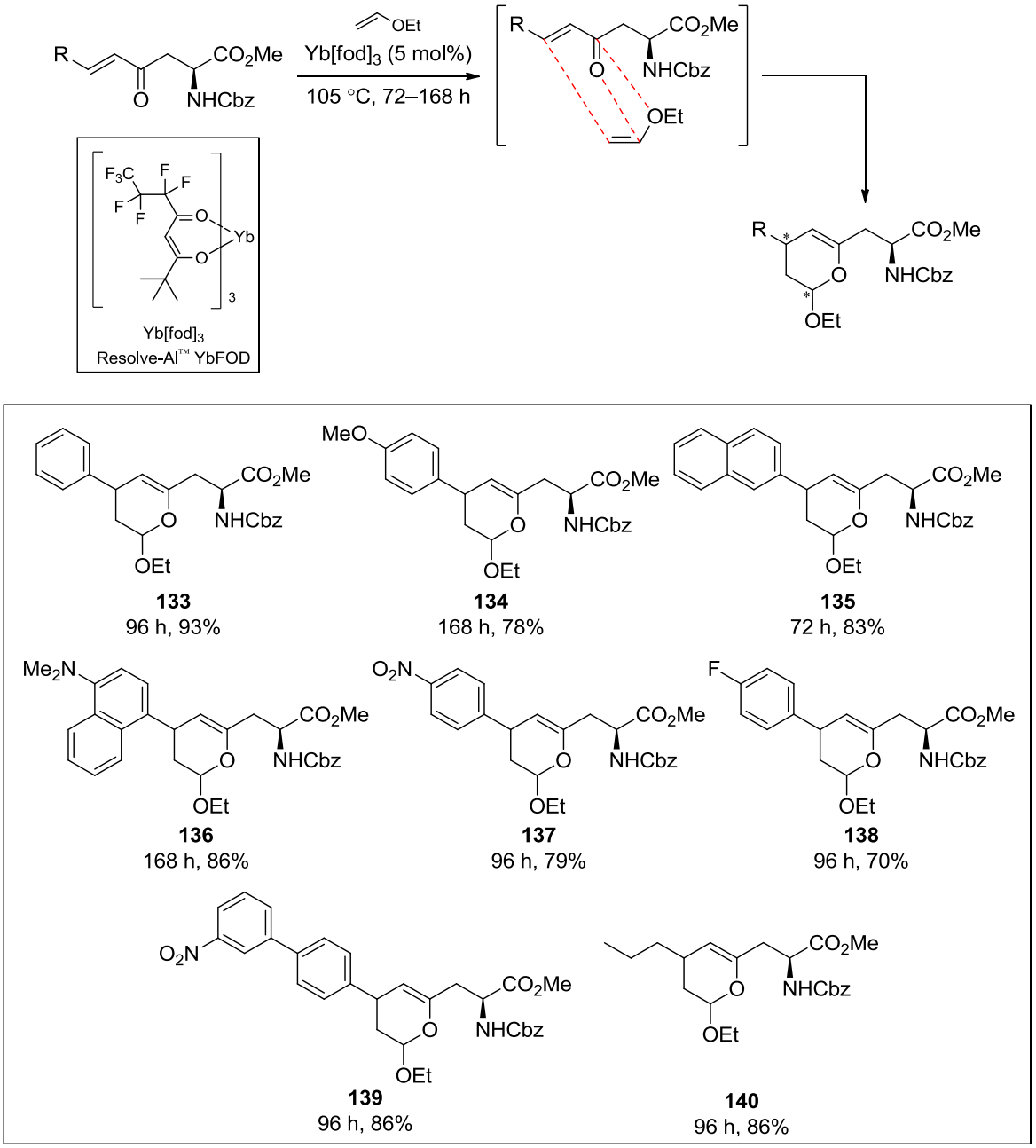
2.3.4 Inverse Electron Demand Hetero-Diels-Alder (HDA_{INV}) Cycloaddition

The second key synthetic step was an inverse electron demand hetero-Diels-Alder (HDA_{INV}) reaction catalysed by a lanthanide Lewis acid catalyst. This work originated from Danishefsky and Bednarski who developed this type of Diels-Alder reaction, employing various lanthanide complexes such as $Eu[fod]_3$ and $Yb[fod]_3$ to catalyse the procedure (Scheme 28). Initial work reported the use of Danishefsky's diene **131** that underwent the HDA reaction with various aldehydes.[78] Focus then shifted to the cycloaddition of α,β-unsaturated aldehydes **132** and vinyl ethers.[79] The protocol was also showcased as a key step in the total synthesis of various natural products such as vineomycin B_2 algycon.[80]



Scheme 28: Initial use of lanthanide catalysts for the HDA reaction.

Throughout all of these literature reports, high *endo* selectivity was observed for this HDA cycloaddition. With that in mind, the next stage in this project was to subject the α,β-unsaturated enones to these conditions. The *N*-Cbz protected enone-derived α-amino acids were chosen as the preferred library for this cycloaddition, due to the ability of the Cbz protecting groups to withstand high temperature and pressure. The enones were heated to 105 °C in neat ethyl vinyl ether with a 5 mol% catalyst loading of $Yb[fod]_3$ (Scheme 29). The enones acted as the electron-withdrawing diene and ethyl vinyl ether was used as the electron-rich dienophile. The reaction provided good to excellent yields of the dihydropyran α-amino acid derivatives. Eight *N*-Cbz protected dihydropyran analogues were synthesised using this procedure with the substrate scope consisting of electron-rich/poor aryl groups, a biaryl system as well as a short alkyl group. Longer reaction times were necessary for the electron rich compounds, methoxyphenyl **134** and dimethylaminonaphthyl **136**.



Scheme 29: General procedure for HDA_{INV} reaction and substrate scope.

This HDA_{INV} reaction furnished two new stereocentres and although the next step in this synthetic sequence results in the loss of both due to aromatisation, it is important to fully understand and predict why the mechanism and resulting regio- and stereoselectivity are observed. The effect of the Lewis acid catalyst was also considered. Standard Diels-Alder reactions involve an electron-rich diene and electron-poor dienophile, whereby two new chemical bonds and a six-membered ring are formed. The roles are reversed for an inverse electron demand Diels-Alder (DA_{INV}) reaction, where the diene is electron-poor and dienophile electron-rich.[81] During a DA_{INV} reaction, three π-bonds are broken, and two σ-bonds and one new π-bond are formed. The regiochemistry can be predicted by considering the best HOMO-LUMO interaction. The LUMO (lowest unoccupied molecular orbital) of the heterodiene reacts with the HOMO (highest occupied molecular orbital)

of the dienophile (Figure 21). The orbitals will align to maximise the bonding interactions and subsequently minimising the anti-bonding interactions. Theoretically, the DA_{INV} obeys a general *endo* selection rule. The DA_{INV} favours an *endo* orientation of the electron-donating substituents on the dienophile as it approaches the diene. This *endo* approach helps maximise secondary orbital overlap.[82] The orbital overlap occurs between the carbonyl carbon of the diene and the ethoxy oxygen of the ethyl vinyl ether dienophile. With the reaction proceeding through the *endo* pathway, the dienophile could approach from the top or bottom face of the diene (Figure 21). The $Yb[fod]_3$ Lewis acid cooridinates to the carbonyl oxygen of the diene, consequently lowering the LUMO energy. A six-membered transition state could also be forming due to the close proximity of the amino group simultaneously coordinating to the Lewis acid. This transition state could be minimising the free rotation around the methylene bridge, locking the conformation in place and blocking the top face of the diene. For all analogues subjected to the HDA_{INV} conditions there was a major product observed, generally with a 9:1 dr. The proposed transition state could explain the observed diastereoselectivity. However, as the next step involved aromatisation, full characterisation was never carried out to determine the major diastereomer.

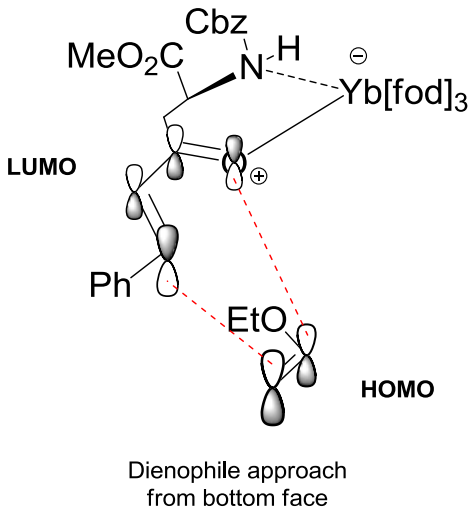


Figure 21: HDA_{INV} potential transition state.

The lanthanide complex used in this reaction was tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)ytterbium ($Yb[fod]_3$) which consists of three bidentate acetylacetonate ligands bound to the metal(III) centre (Figure 22).[83,84] The Lewis acid lowers the LUMO energy, resulting in a smaller energy gap between the HOMO/LUMO and a faster reaction. It also increases the LUMO polarisation allowing for a more regioselective reaction.

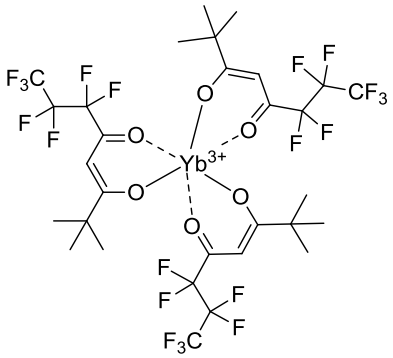
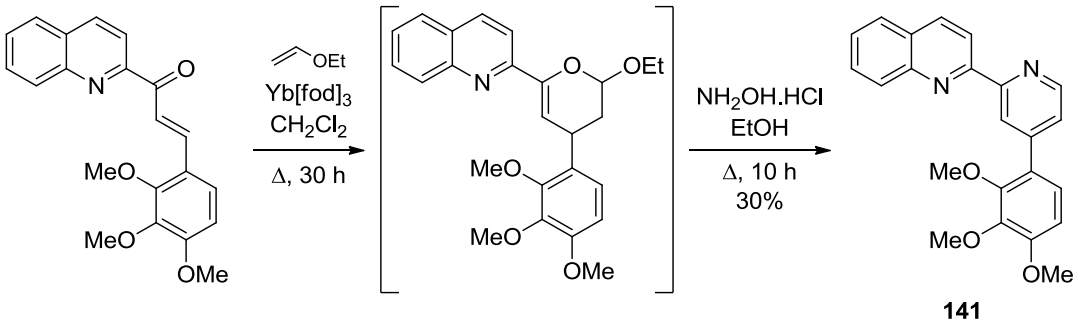


Figure 22: Structure of Yb[fod]$_3$.

2.3.5 Synthesis of *N*-Cbz Protected Pyridine-Derived α-Amino Acids

Following the synthesis of the dihydropyran-derived α-amino acid analogues, the aromatisation step was next investigated. This step combined with the HDA$_{INV}$ reaction was developed by Ciufolini and Byrne, and termed as a modified Knoevenagel-Stobbe condensation reaction. They first reported it for the preparation of 2,4-disubstituted pyridines **141** (Scheme 30).[85]



Scheme 30: Initial use of the two-step HDA$_{INV}$/pyridine synthesis procedure.

Ciufolini described multiple total and formal syntheses of various natural products that contain substituted pyridine rings using this approach (Figure 23). He reported the formal synthesis of lavendamycin methyl ester, a streptonigrin congener.[86] Cystodytin A was also synthesised along with multiple cystodytin natural product analogues.[87,88] This approach was also used to prepare a series of pyridoacridine alkaloids and shermilamine B.[89,90]

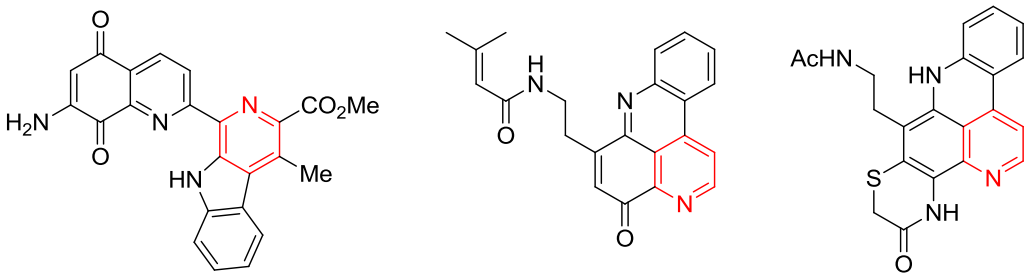
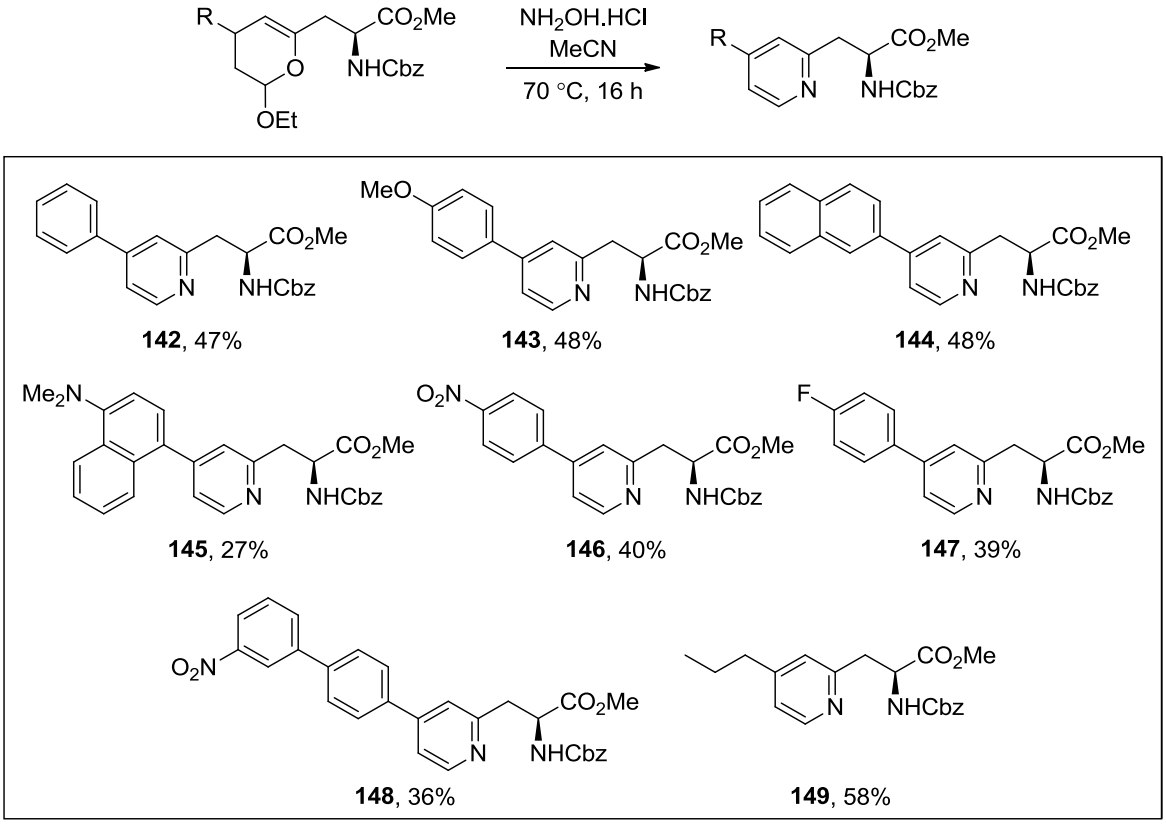


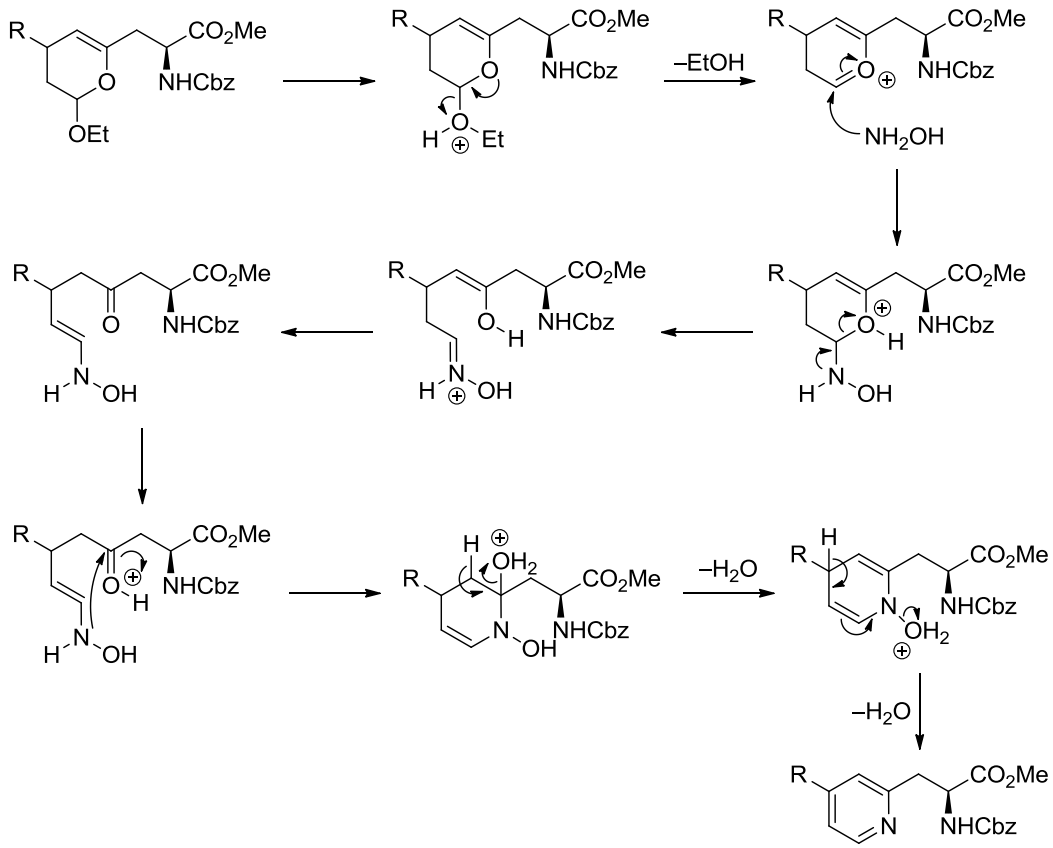
Figure 23: Pyridine-containing natural products synthesised by the Ciufolini group.

Using this approach, each dihydropyran from the eight-membered library was treated with hydroxylamine hydrochloride in acetonitrile and heated to 70 °C overnight to form the desired pyridine heterocycle (Scheme 31). Moderate to good yields were achieved. However, lower yields were observed for the electron-poor nitrophenyl **146**, fluorophenyl **147** and biaryl **148** analogues. Dimethylaminonaphthyl **145** displayed the lowest yield across the series, but its synthesis was only attempted once on a very small-scale. Repeat reactions were carried out on all the other analogues to optimise their yields. Silica chromatography was not the ideal purification method due to retention of these pyridines on the acidic silica. This was also the case when 1% triethylamine was used in the eluting solvents. Neutral alumina was then attempted and proved to be a much more suitable stationary phase for flash column chromatography to purify this series of compounds.



Scheme 31: General conditions for the aromatisation procedure.

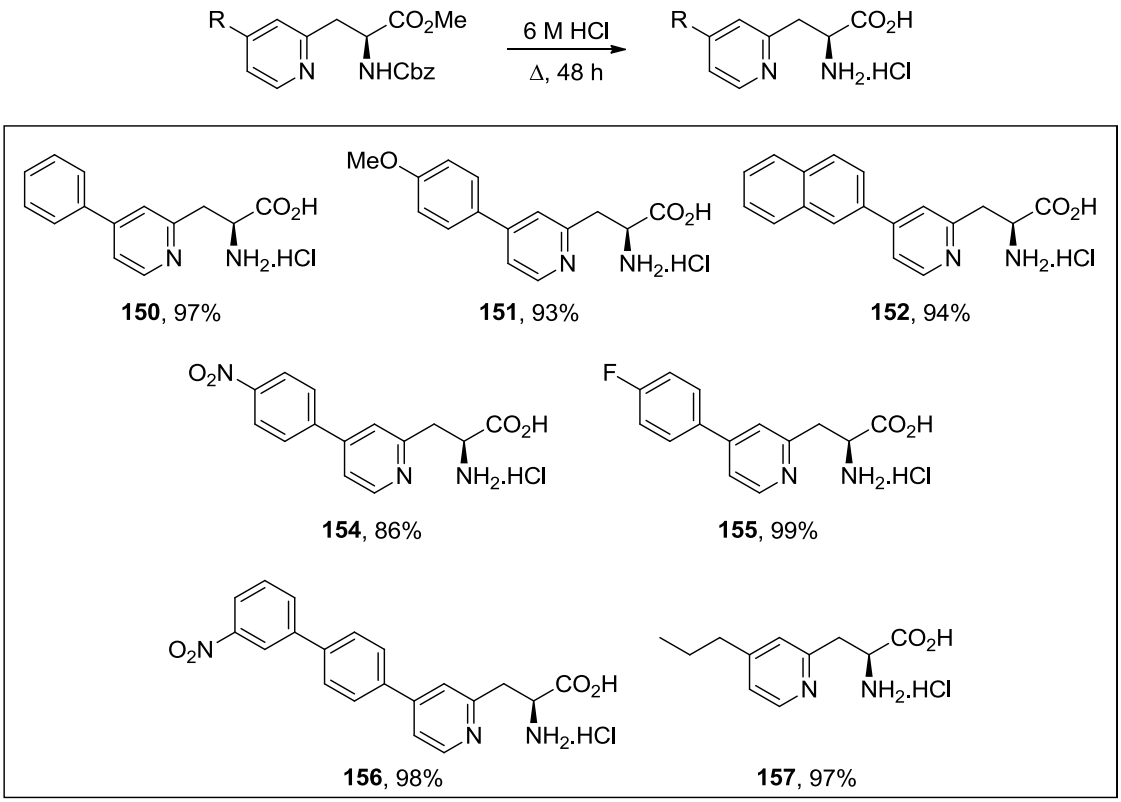
During this transformation, the ethoxy oxygen is protonated and the formation of an oxonium species results in the elimination of ethanol (Scheme 32). Subsequent nuclephilic attack of the hydroxylamine to the electrophilic oxonium ion, proton transfer and ring opening furnishes an oxime/enol intermediate. Tautomerisation of the oxime and enol to their respective enamine and ketone functional groups leads to nucleophilic attack of the enamine nitrogen to the carbonyl group. This is followed by rapid loss of two molecules of water, resulting in the formation of the stable pyridine ring.[91] The driving force of this reaction is the dehydration and ethanol elimination steps, as well as the formation of a stable aromatic ring.



Scheme 32: Possible mechanism for the synthesis of *N*-Cbz protected pyridine-derived α-amino acids.

2.3.6 Deprotection to Form the Parent Pyridine-Derived α-Amino Acids

The final step in this synthetic sequence towards the target molecules was deprotection of the amine and carboxylic acid protecting groups. Acid mediated deprotection using 6 M hydrochloric acid was used to generate the final α-amino acids (Scheme 33). Excellent yields across this series of compounds were obtained.



Scheme 33: Deprotection conditions to form the parent α-amino acids.

2.4 Optical Properties of Pyridine-Derived α-Amino Acids

With the parent α-amino acids available, the next stage of this study was to fully analyse the optical properties of these compounds, including absorption and emission maximum values, molar extinction coefficients and quantum yields. Determination of these properties would allow correlation between structure and fluorescence, generating an understanding of how this information could be used to design further analogues for biological studies.

2.4.1 Absorption and Emission Data for Neutral and Electron-Rich Pyridine Derivatives

The neutral and electron-rich pyridine derivatives displayed the most interesting optical properties. Phenyl analogue **150** had an absorption maximum of 263 nm and an emission maximum of 332 nm (Figure 24). Methyloxyphenyl **151** had an absorption value of 283 nm (Figure 25) and naphthyl **152** a blue-shifted absorption maximum value of 259 nm (Figure 26). When both of these compounds were irradiated at their respective excitation wavelengths, at the standard concentrations, the emission bands were too intense and were off the scale. This suggests that both compounds are highly fluorescent and should be investigated further. Propyl **157** was irradiated at its absorption maximum value of 267 nm and gave a very weak emission maximum of 311 nm (Figure 27). This was predicted due to the limited conjugation of this analogue.

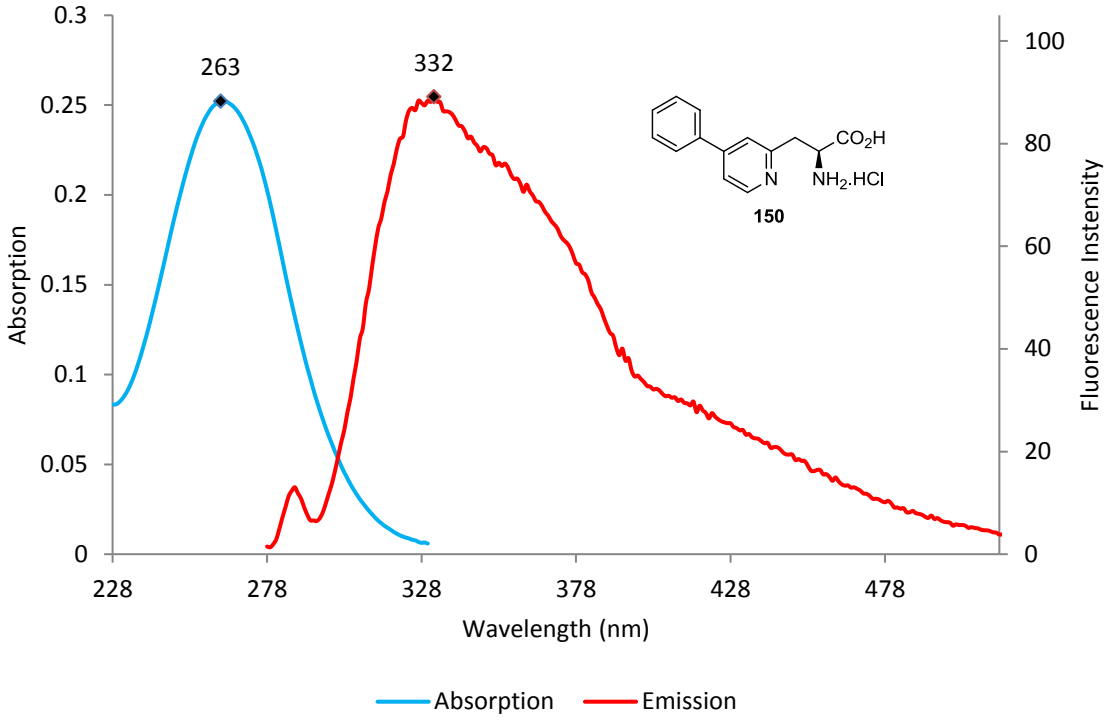


Figure 24: Phenyl **150** absorption and emission data.

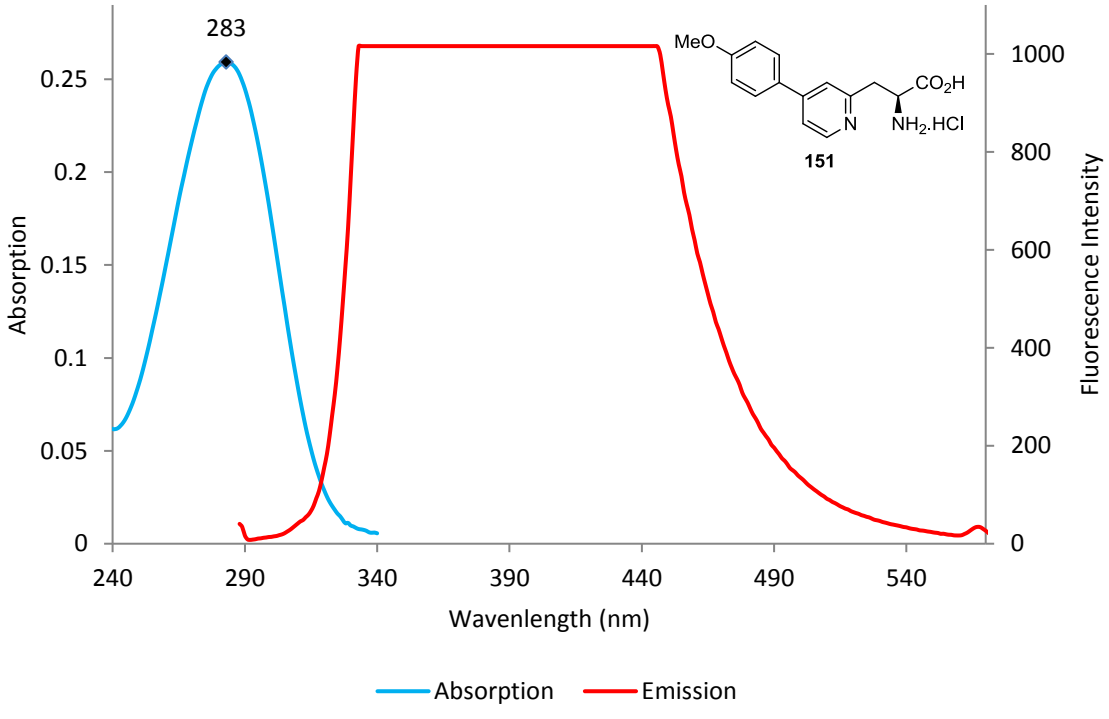


Figure 25: Methoxyphenyl **151** absorption and emission data.

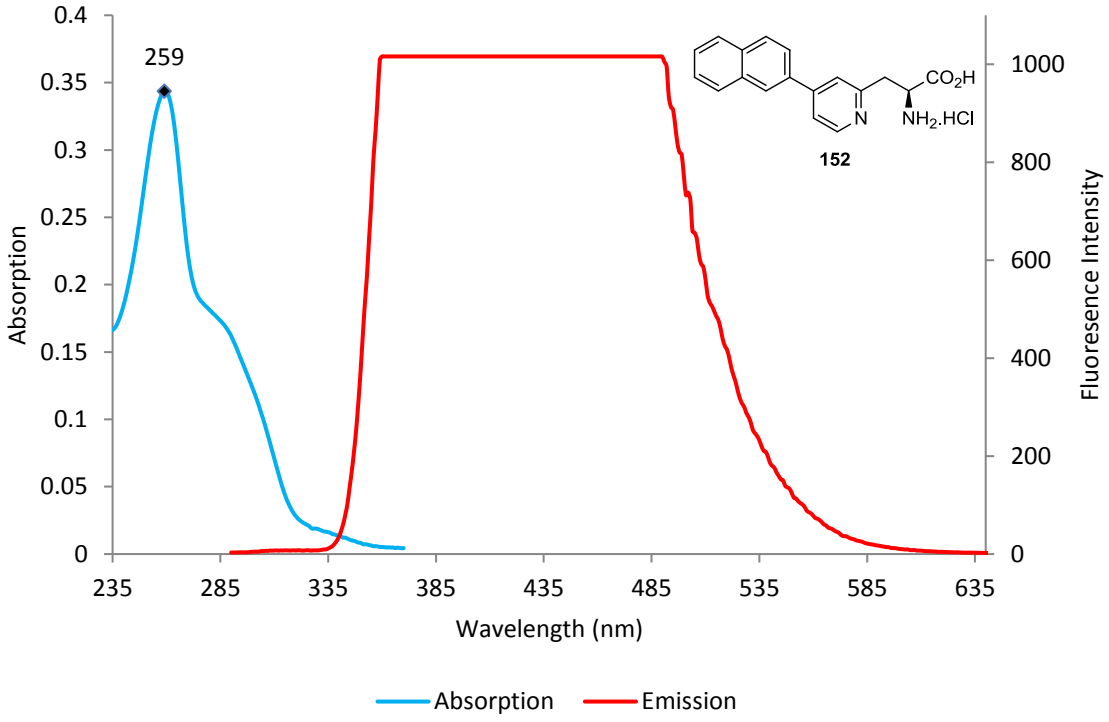


Figure 26: Naphthyl **152** absorption and emission data.

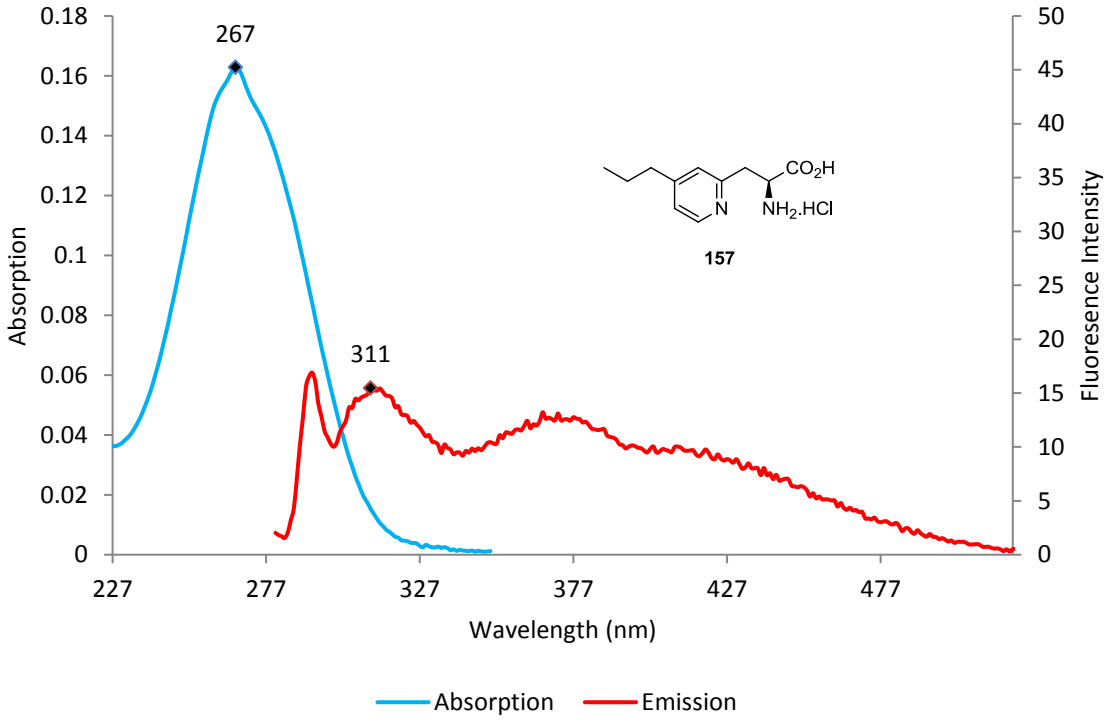


Figure 27: Propyl **157** absorption and emission data.

2.4.2 Absorption and Emission Data for Electron-Poor Pyridine Derivatives

The optical properties for the electron-poor pyridine analogues were not as promising after comparison with the electron-rich series. Nitrophenyl **154** had an absorption value of 277 nm and very weak fluorescence at 381 nm (Figure 28). Fluorophenyl **155** displayed an absorption band at

268 nm and emission at 325 nm (Figure 29). Finally, biaryl **156** had an intense absorption value of 276 nm (Figure 30). Upon irradiation however, a weak emission maximum of 373 nm was observed. The optical properties for this set of compounds was expected due to the electron-deficient properties associated with the aryl side-chains. Generally, good fluorophores exhibit a push-pull electronic relationship but these compounds possess electron-deficient nitro and fluoro functional groups.

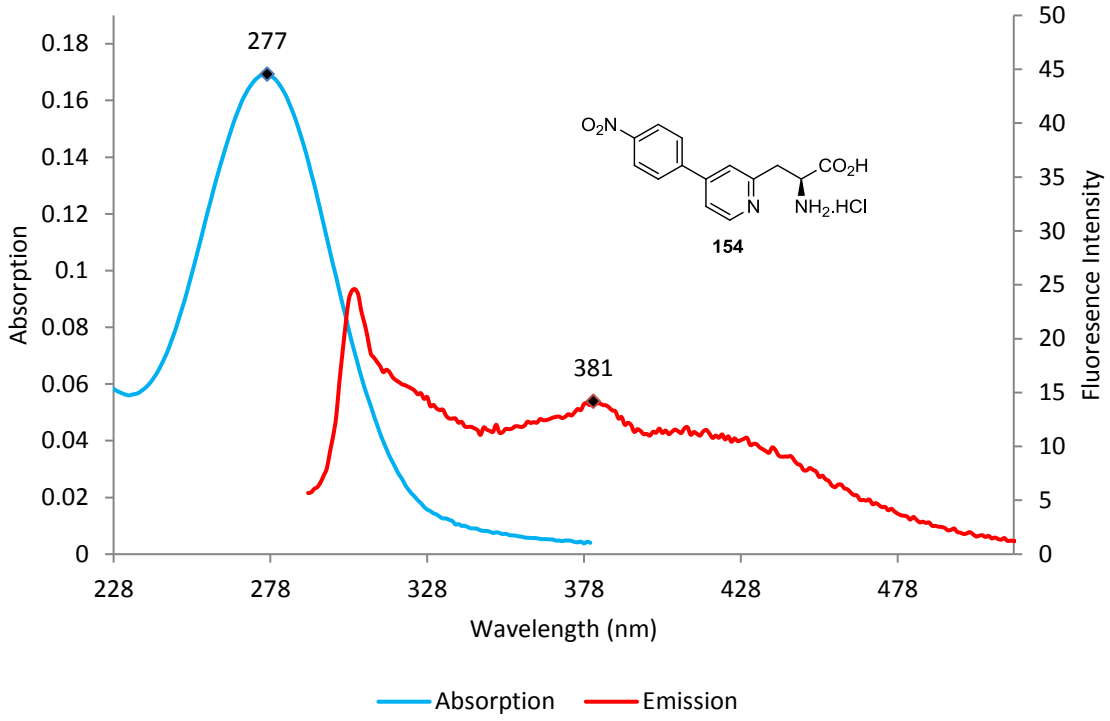


Figure 28: Nitrophenyl **154** absorption and emission data.

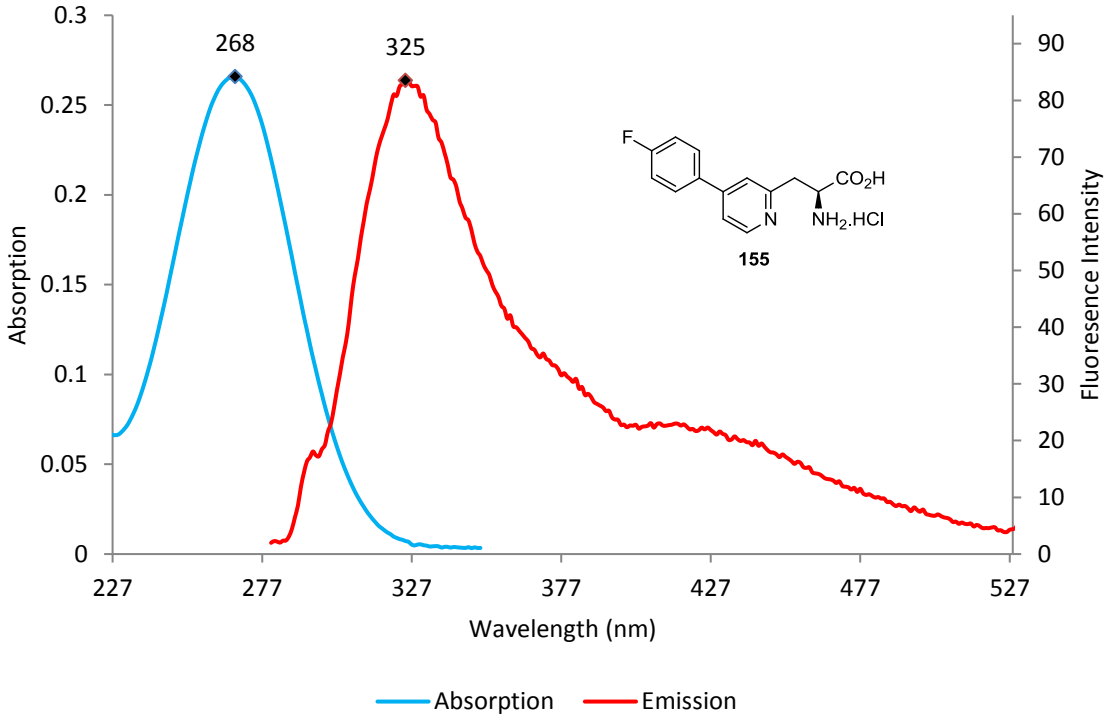


Figure 29: Fluorophenyl **155** absorption and emission data.

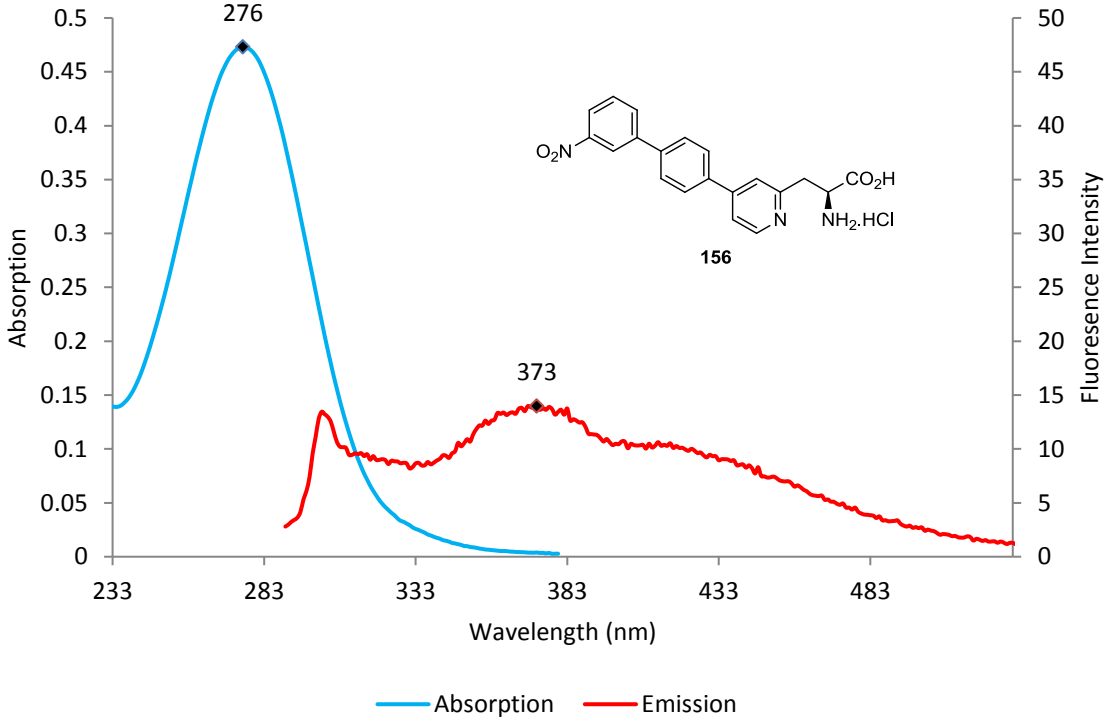


Figure 30: Biaryl **156** absorption and emission data.

2.4.3 Methoxyphenyl 151 and Naphthyl 152 Dilution Study

Due to the intense emission spectra observed for methoxyphenyl **151** and naphthyl **152** when using 10 µM sample concentrations, a dilution study was carried out to investigate the emission maximum values for these analogues. Sample concentrations of 5, 1 and 0.1 µM were analysed

using UV/visible and fluorescence spectroscopy. The absorption maximum value for methoxyphenyl **151** was 283 nm for all three concentrations (Figure 31). Upon irradiation at 283 nm, the 5 µM and 1 µM samples were still too concentrated and the reading was off the scale (Figure 32). The most dilute sample however, displayed an emission maximum value of 366 nm. These results suggest methoxyphenyl **151** exhibits intense emission with a maximum value of 366 nm, which is in the UV-A region of the electromagnetic spectrum.

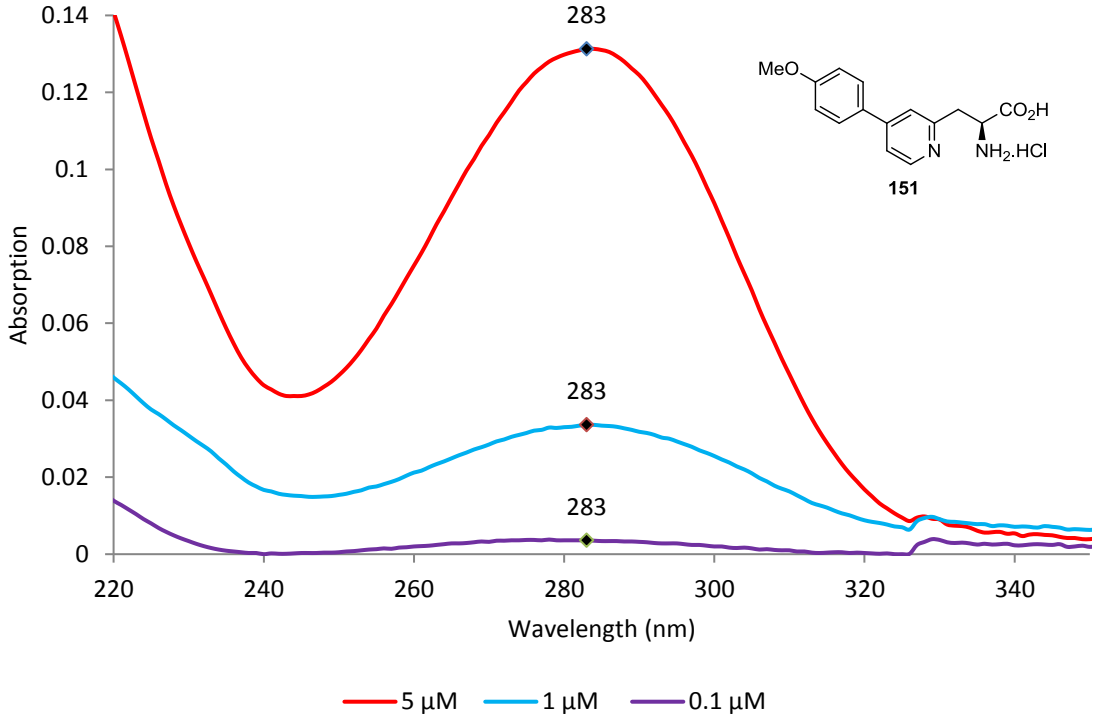


Figure 31: Methoxyphenyl **151** absorption data.

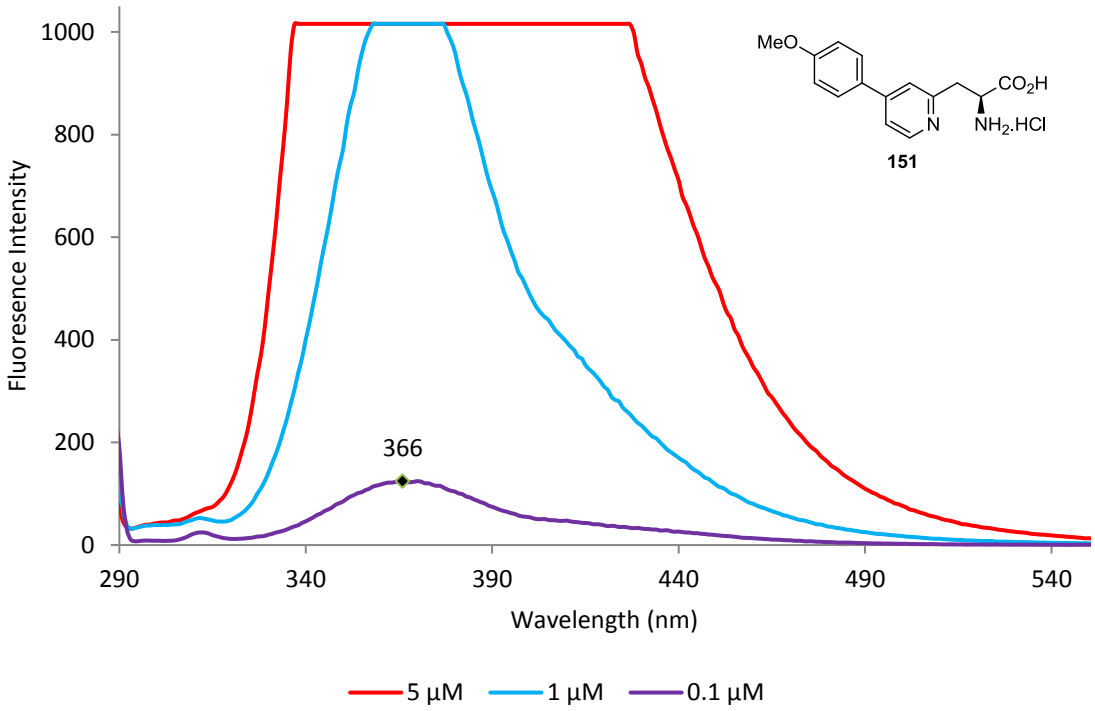


Figure 32: Methoxyphenyl **151** emission data.

The absorption maximum value for naphthyl **152** at the three concentrations was 259 nm (Figure 33). Excitation at this wavelength for each sample followed. The 5 µM sample had two emission maximum bands at 380 and 452 nm (Figure 34). The same result was obtained for the 1 µM sample. However, at 0.1 µM there appeared to be a single emission band at 383 nm. It is unknown why there was a dual band observed for the more concentrated samples. The data obtained from the most dilute sample would be used in future investigations using naphthyl **152** as it was considered the most accurate and reasonable result.

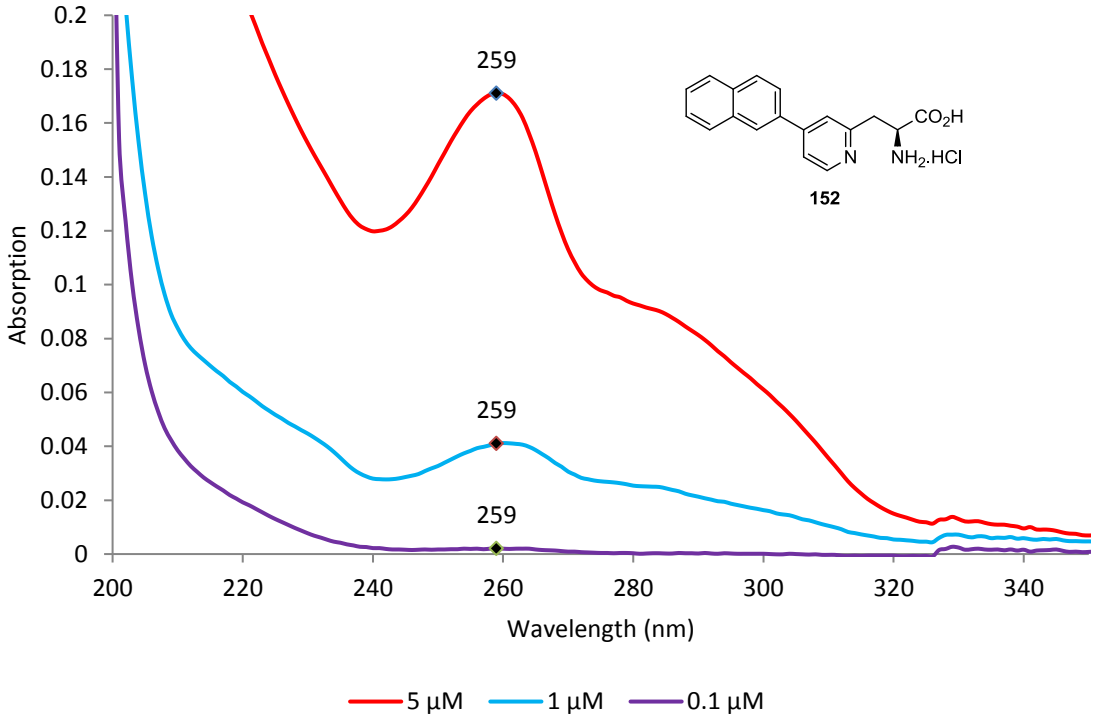


Figure 33: Naphthyl **152** absorption data.

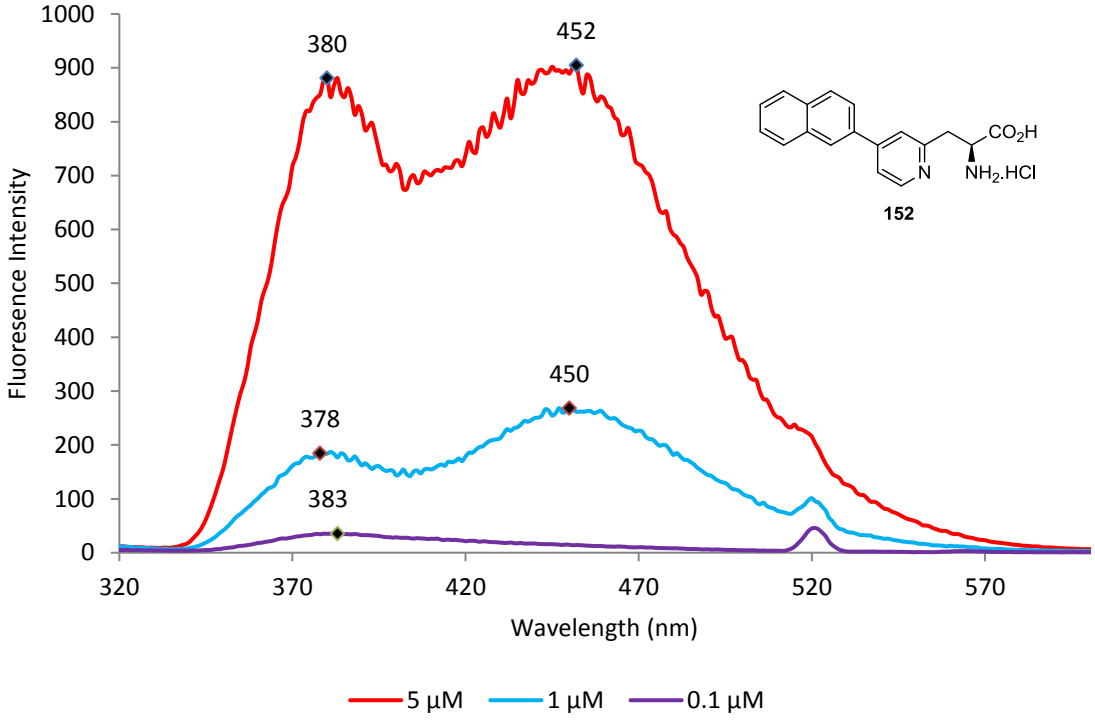


Figure 34: Naphthyl **152** emission data.

2.4.4 Methoxyphenyl 151 pH Study

Due to the intense fluorescence displayed by methoxyphenyl **151**, a pH study was carried out to compare the difference in emission maxima between neutral, acidic and basic solutions. The data recorded for the neutral solution gave an absorption maximum value of 284 nm (Figure 35). The

addition of concentrated hydrochloric acid and sodium hydroxide was used to acidify and basify the stock solutions of methanol to pH 1 and 12 respectively. A red-shifted absorption maximum value of 338 nm was observed for the acidic solution and a minimal blue-shifted absorption maximum of 274 nm for the basic solution.

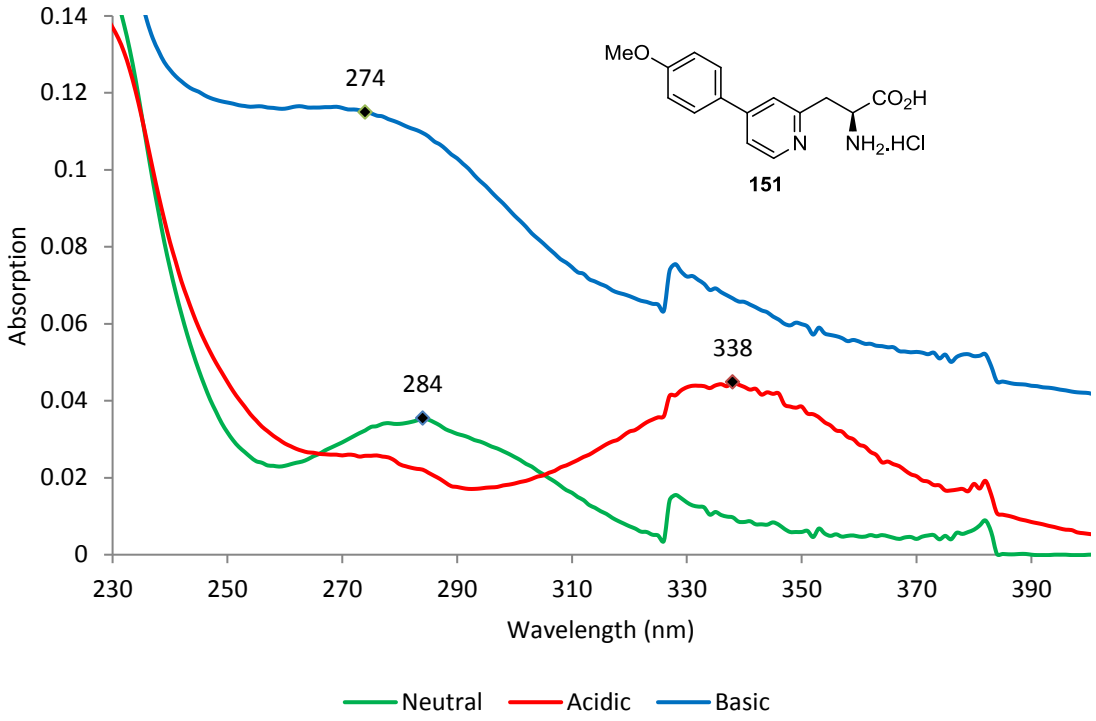


Figure 35: Methoxyphenyl **151** absorption data for pH study.

These samples were then irradiated at their respective absorption maximum values. The neutral sample had the predicted emission value of 368 nm due to previous investigations (Figure 36). Much like the absorption value for the basic solution, a blue-shifted emission maximum reading of 351 nm was obtained, which was considerably less intense than the neutral solution. The acidic solution on the other hand had a predicted emission maximum with a bathochromic shift on comparison with the neutral solution. An emission maximum of 428 nm was observed with about half the fluorescence intensity of the neutral solution. This red-shifted wavelength is located in the purple area of the visible spectrum. The neutral and basic solutions fluoresce in the UV-A region. Upon acidification, the emission band red-shifts to the purple/visible area of the electromagnetic spectrum. These results suggest that methoxyphenyl **151** could potentially be used as a fluorescent pH probe especially when pH levels in the environment switch from neutral/basic to acidic.

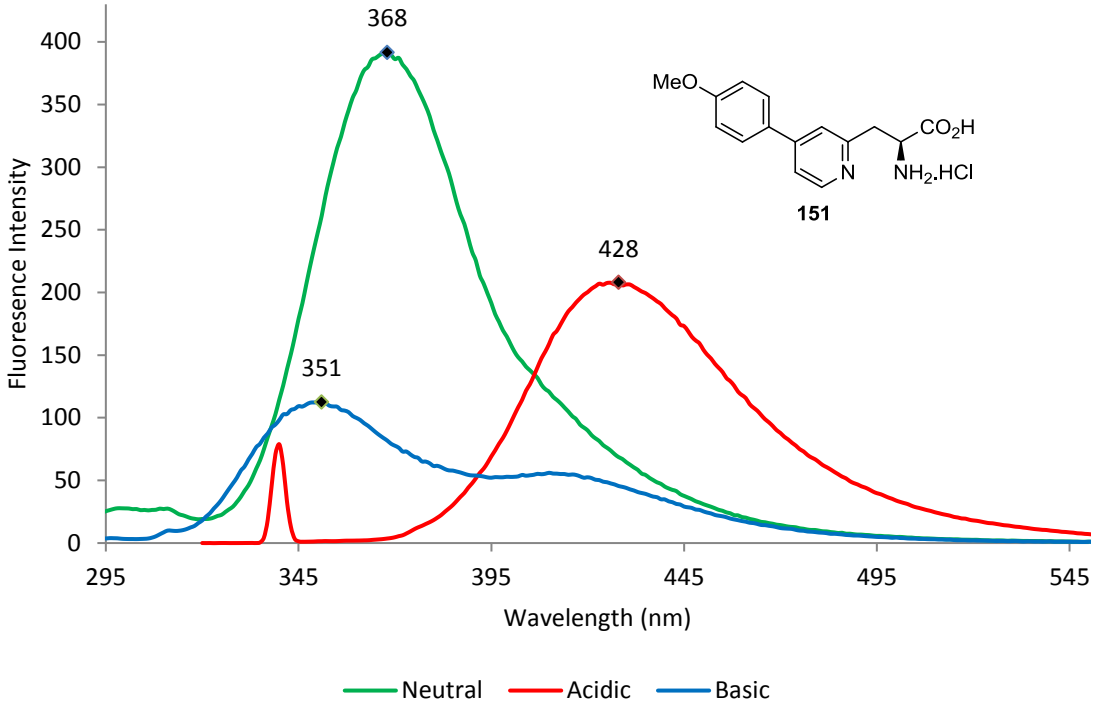


Figure 36: Methoxyphenyl **151** emission data for pH study.

2.4.5 Other Optical Properties

A number of other spectroscopic properties were determined for the library of pyridine-derived α-amino acids using the absorption and emission data. The Stokes shift is the difference in wavelength between the emission and absorption maximum values. It is fundamental to the sensitivity of fluorescence techniques if the Stokes shift is large because it allows emission photons to be detected against a low background, isolated from excited photons.[92] The molar extinction coefficient is the capacity for light absorption at a specific wavelength and is defined by the Beer-Lambert law, A = εcl (A = absorbance, ε = extinction coefficient, c = molar concentration, l = optical pathlength). Fluorescence quantum yield is defined as the number of fluorescence photons emitted per excitation photons absorbed. Experimentally, relative fluorescence quantum yields can be determined by measuring the fluorescence of a fluorophore of known quantum yield with the same experimental parameters as the substance in question. The final property calculated was the brightness or fluorescence output per fluorophore. This is proportional to the product of the extinction coefficient and the fluorescence quantum yield. All of the pyridine derivatives displayed high extinction coefficient values in excess of 16,000 cm^{-1} M^{-1} (Table 1). Biaryl **156** had a molar extinction coefficient of 47,310 cm^{-1} M^{-1}, the highest for this series of compounds. This is due to the conjugation extending over three aryl rings. A good fluorescence quantum yield of 0.18 was observed for naphthyl **152** and an excellent 0.46 was calculated for methoxyphenyl **151**. The results for these two compounds suggest that it is crucial for potential fluorophores to not only have extended conjugation but for electron-donating groups to be present. For propyl **157** and biaryl

156, the fluorescence quantum yields and brightness were not measured due to their extremely low intensity emission graphs.

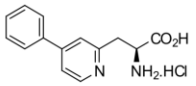
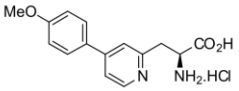
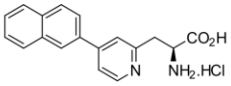
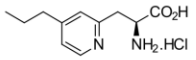
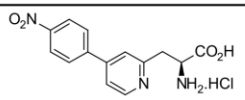
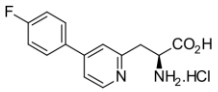
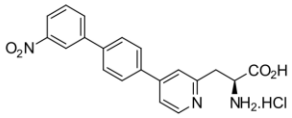
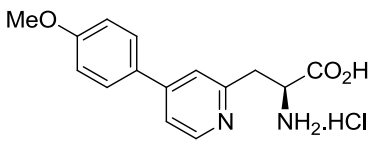
First Generation Pyridine Derivatives	Absorption maximum (nm)	Emission maximum (nm)	Stokes Shift (nm)	Molar extinction coefficient (cm^{-1} M^{-1})	Quantum yield (Φ_F)	Brightness (cm^{-1} M^{-1})
 **150**	263	332	69	25,210	0.005	126
 **151**	283	366	83	25,920	0.46	11,923
 **152**	259	383	124	34,360	0.18	6,184
 **157**	267	311	44	16,280	N/A	N/A
 **154**	277	381	104	16,920	0.0007	11
 **155**	268	325	57	26,580	0.006	159
 **156**	276	373	97	47,310	N/A	N/A

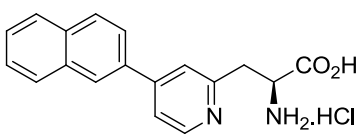
Table 1: Optical properties summary for pyridine-derived α-amino acid library.

2.4.6 Summary

Based on the results from the optical properties investigation, it is clear that the most promising pyridine derivatives that could potentially be used as fluorescent probes are methoxyphenyl **151** and naphthyl **152** (Figure 37). It is apparent that a direct correlation exists between the electron-rich nature of the α-amino acid side-chains and desirable fluorescent properties such as the quantum yield.



151



152

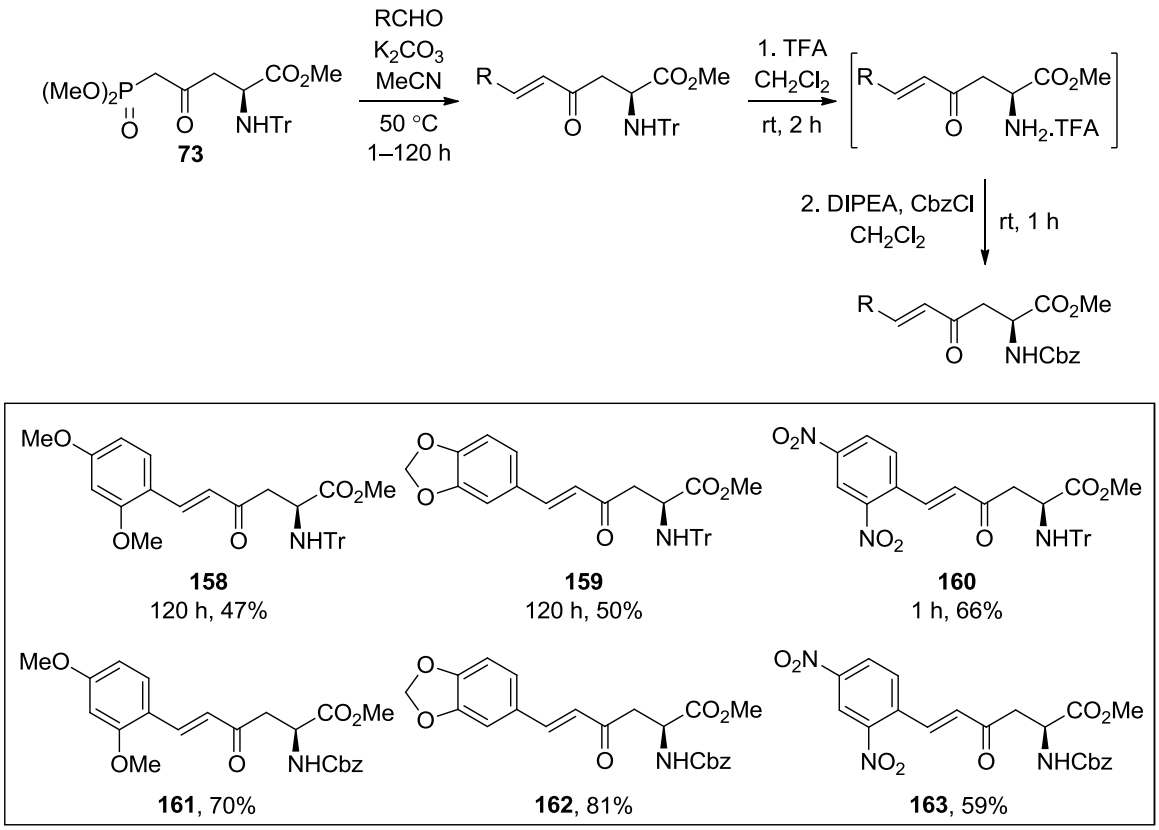
Figure 37: Pyridine-derived fluorophores **151** and **152**.

2.5 Synthesis of Second Generation Pyridine-Derived α-Amino Acids

Having developed an efficient route to pyridine-derived α-amino acids and the synthesis of a seven-membered library of compounds, the next stage of this project was to introduce more electron-donating functionality into the aryl groups. An attempt to improve the optical properties was the main aim. It was hypothesised that a second electron-donating group present on the aryl side-chain might result in a substantial red-shift, with regards to the absorption and emission maximum values, as well as an increase of the fluorescence intensity. Therefore, a library of five compounds was targeted to produce a second generation of pyridine analogues.

2.5.1 Synthesis of *N*-Cbz Protected Enone-Derived α-Amino Acids

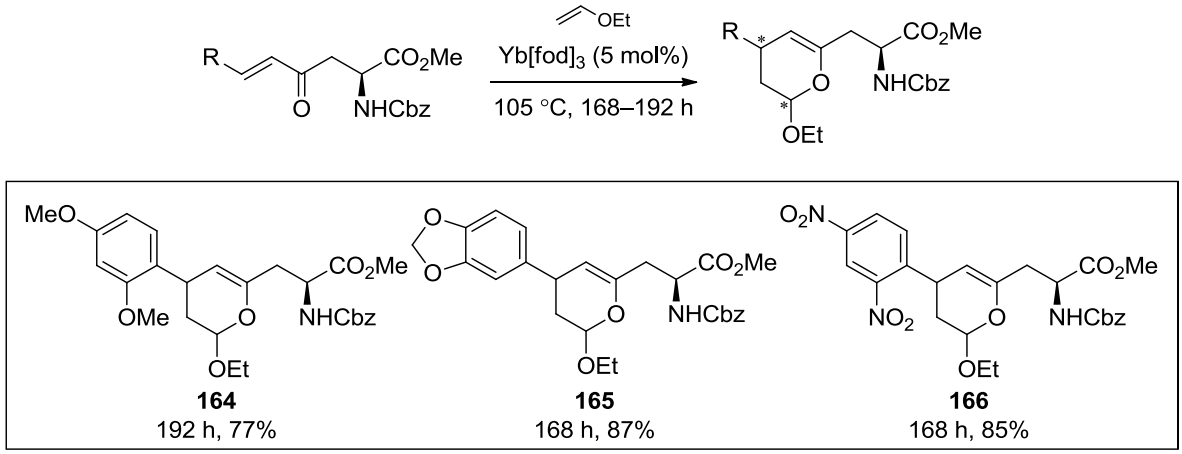
A range of novel enones were prepared using the already developed HWE reaction (Scheme 22). Two electron-donating aldehydes and one electron-withdrawing aldehyde were used to make a small library of enone-derived α-amino acids with disubstituted aryl group functionality (Scheme 34). Long reaction times were required for the HWE reaction with electron rich aldehydes 2,4-dimethoxybenzaldehyde and piperonal, which led to the corresponding enones **158** and **159**. On the contrary, a very short reaction time of 1 h was needed for the 2,4-dinitrobenzaldehyde example, **160**. The standard deprotection/reprotection strategy was used to generate *N*-Cbz protected enones **161**, **162**, and **163**.



Scheme 34: Synthesis of *N*-trityl and *N*-Cbz protected enone-derived α-amino acids.

2.5.2 Inverse Electron Demand Hetero-Diels-Alder (HDA_{INV}) Cycloaddition

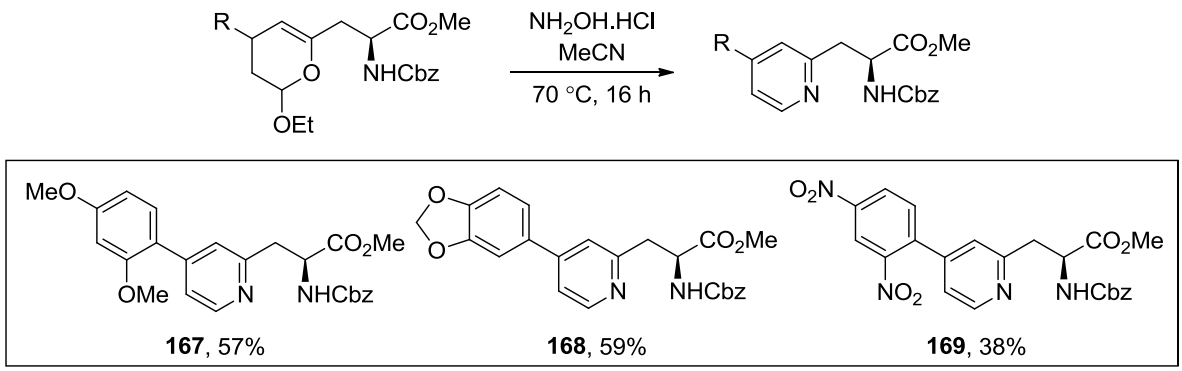
The next step in the synthetic sequence was the HDA_{INV} cycloaddition (Scheme 35). Extended reaction times were imperative for the reaction to reach completion and good yields were achieved for all three analogues.



Scheme 35: HDA_{INV} reaction conditions and analogues synthesised.

2.5.3 Synthesis of *N*-Cbz Protected Pyridine-Derived α-Amino Acids

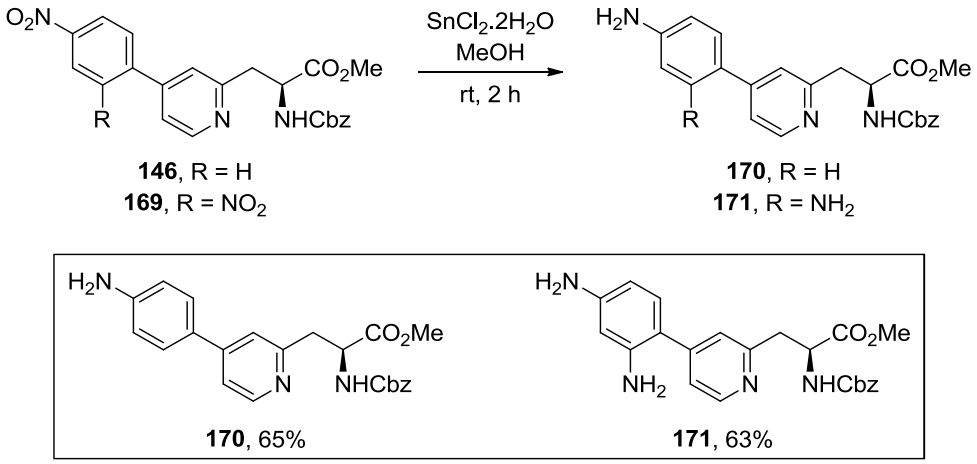
The second part in this two-step procedure was the modified Knoevenagel-Stobbe condensation reaction used to construct the functionalised pyridine ring (Scheme 36). A similar trend was observed regarding the yield obtained for electron-rich and deficient analogues when compared to the first generation pyridine library. Good yields were obtained for the electron donating dimethoxyphenyl **167** and piperonyl **168** whereas a lower 38% yield was acquired for the electron-withdrawing dinitrophenyl derivative **169**. This lower yield could be due to the polar nature of **169** as it was particularly difficult to handle and isolate via column chromatography.



Scheme 36: Pyridine formation conditions and analogues synthesised.

2.5.4 Aryl Nitro Reduction with Tin(II) Chloride Dihydrate

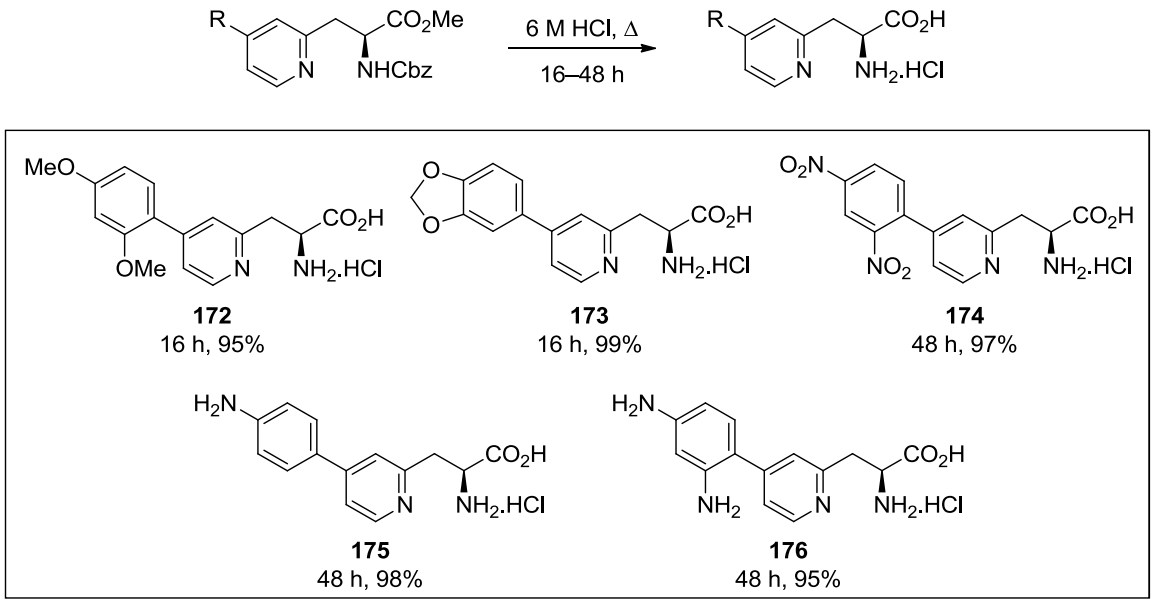
In order to boost the number of pyridine-derived α-amino acids with electron rich aryl side chains, an approach to reducing both nitrophenyl **146** and dinitrophenyl **169** analogues to their corresponding anilines was investigated (Scheme 37). The selective reduction of aromatic nitro groups in the presence of other reducible or acid sensitive groups is possible through the use of tin(II) chloride dihydrate in non-acidic and non-aqueous solvents.[93] Therefore, nitrophenyl **146** was subjected to these mild conditions which resulted in clean conversion to aminophenyl **170** in 65% yield. Dinitrophenyl **169** was also converted to diaminophenyl **171** and a consistent 63% yield was achieved.



Scheme 37: Aryl nitro to aniline reduction conditions.

2.5.5 Deprotection to Form the Parent Pyridine-Derived α-Amino Acids

The final stage in the synthesis of the second generation of pyridine-derived α-amino acids was the deprotection of amine and carboxylic acid protecting groups. The same conditions used for the phase I pyridines was employed, 6 M hydrochloric acid under reflux (Scheme 38). A two day reaction for dimethoxyphenyl **167** and piperonyl **168** resulted in a complex mixture of products, as confirmed by ^{1}H NMR spectroscopy. It is likely that the methoxy and the acetal functional groups were removed along with the Cbz and methyl ester during a long reaction time. Shortening the reaction time to 16 h generated **172** and **173** cleanly.



Scheme 38: Deprotection to form the parent α-amino acid derivatives.

2.6 Optical Properties of Second Generation Pyridine-Derived α-Amino Acids

The optical properties of the second generation of pyridine derivatives were investigated. This was to see if any of the disubstituted analogues had considerably better fluorescent properties than the first generation of pyridines. The analogues with the best combination of emission maximum range and fluorescence intensity would be selected for incorporation into a small CPP and used for subsequent biological investigations.

2.6.1 Absorption and Emission Data for Second Generation Pyridine Derivatives

Dimethoxyphenyl **172** had an absorption maxima at 275 nm and a shoulder peak at 298 nm (Figure 38). Upon irradiation at 275 nm a broad emission band appeared with a maximum value of 384 nm. However, when it was excited at 298 nm, a similar emission spectrum was recorded with exactly the same maximum value of 384 nm but it was considerably more intense than irradiation at 275 nm. This was an unforeseen result as it was predicted that due to the red-shift in absorption maximum, a red-shift in emission maximum was expected. The absorption maximum for piperonyl **173** was 303 nm and it had a red-shifted emission maximum of 460 nm (Figure 39). This was an excellent result as the emission band is located in the blue area of the visible region which expands the potential use of this compound if it was used as a fluorescent probe. Piperonyl **173** does not have comparable fluorescence intensity to methoxyphenyl **151**, naphthyl **152** or dimethoxyphenyl **172**. The only electron-deficient analogue in this library was dinitrophenyl **174**. It had an absorption maximum of 265 nm and weak fluorescence intensity, with an emission maximum of 304 nm (Figure 40). Results for the electron-rich aminophenyl **175** were promising. It displayed an absorption

maximum of 315 nm and a red-shifted emission maximum of 433 nm upon irradiation (Figure 41). This was very interesting as the emission band is located in the purple area of the visible spectrum. Finally, diaminophenyl **176** had an absorption maximum of 328 nm (Figure 42). However, even with an extra amino group donating electrons to the aromatic system it displayed a substantial reduction in fluorescence intensity in comparison with aminophenyl **175**. It was hypothesised that this would have the opposite effect and improve the fluorescent properties. The emission maximum value was 485 nm but due to the poor intensity associated with this analogue it was not further investigated.

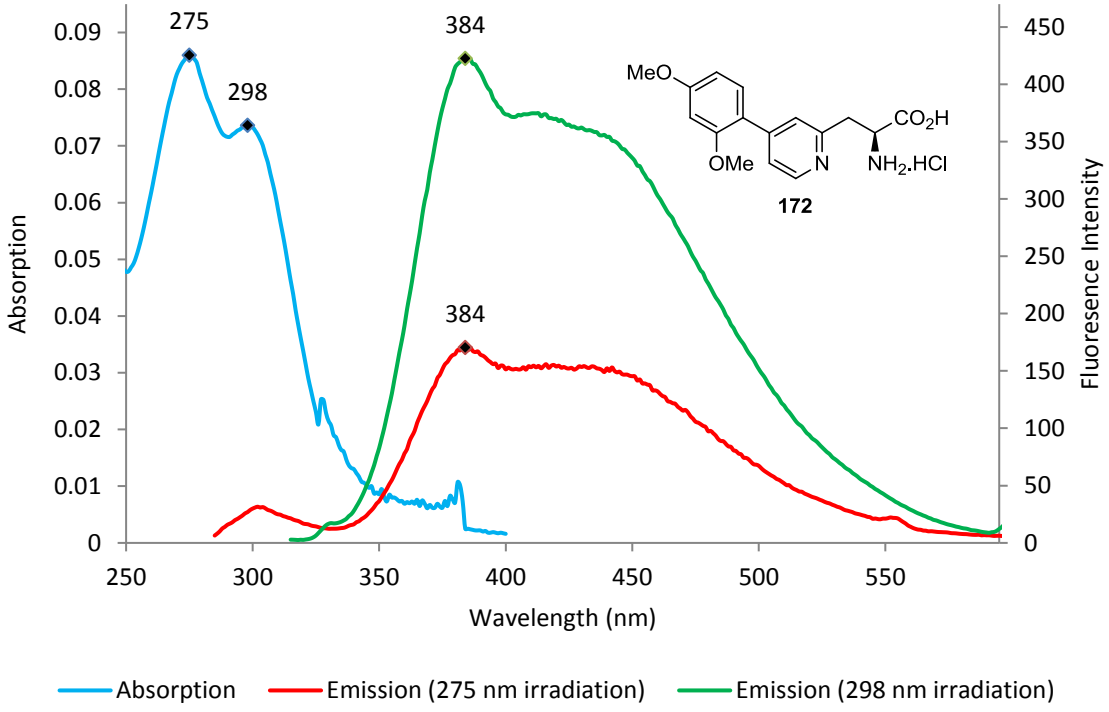


Figure 38: Dimethoxyphenyl **172** absorption and emission data.

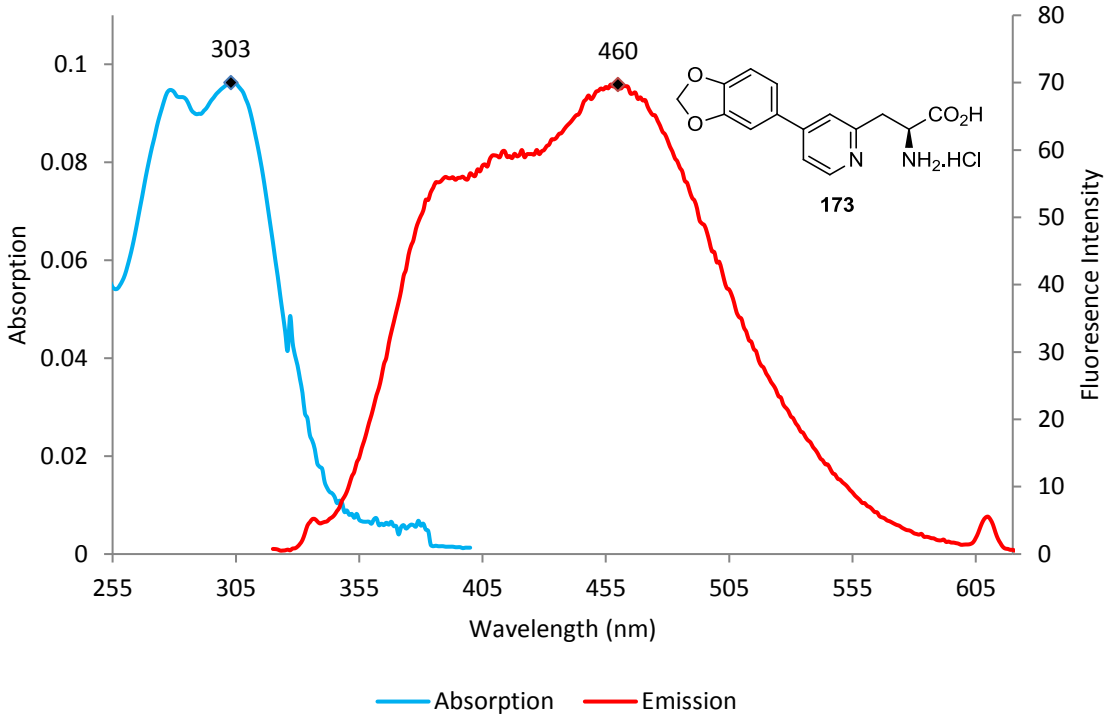


Figure 39: Piperonyl **173** absorption and emission data.

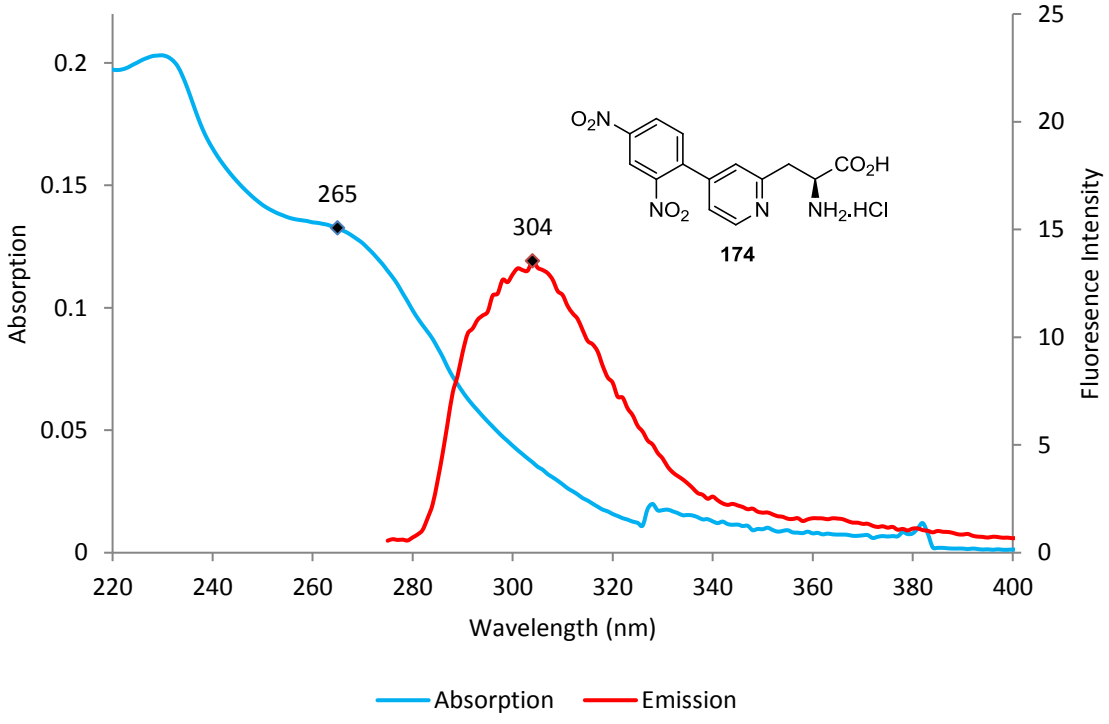


Figure 40: Dinitrophenyl **174** absorption and emission data.

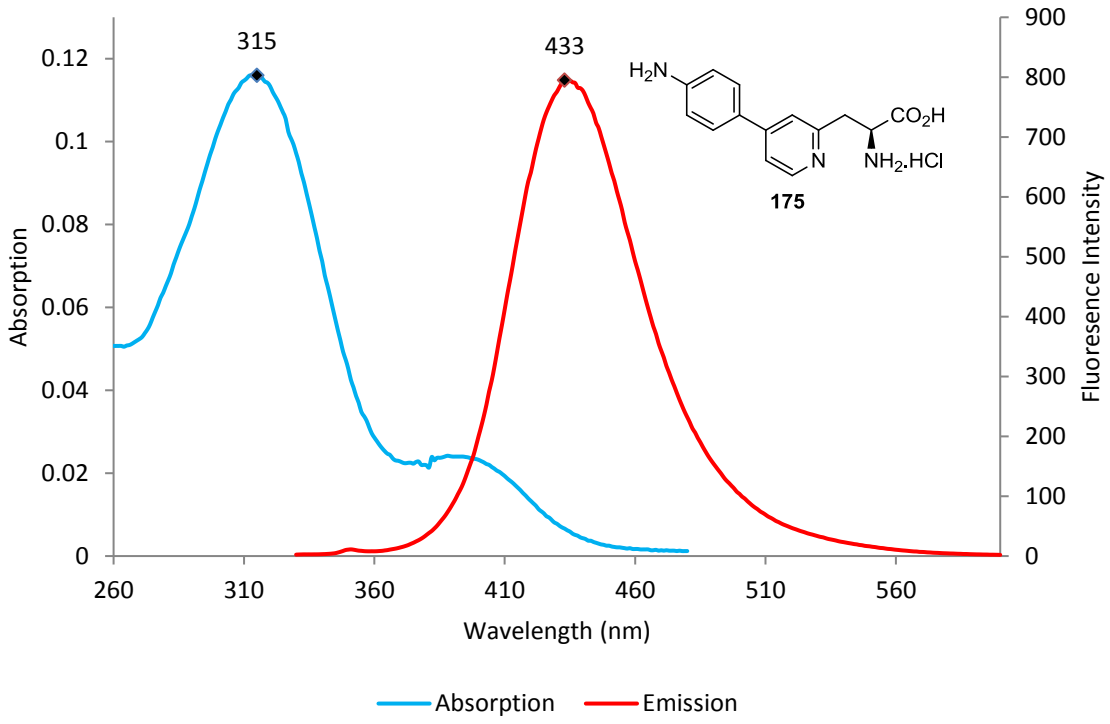


Figure 41: Aminophenyl **175** absorption and emission data.

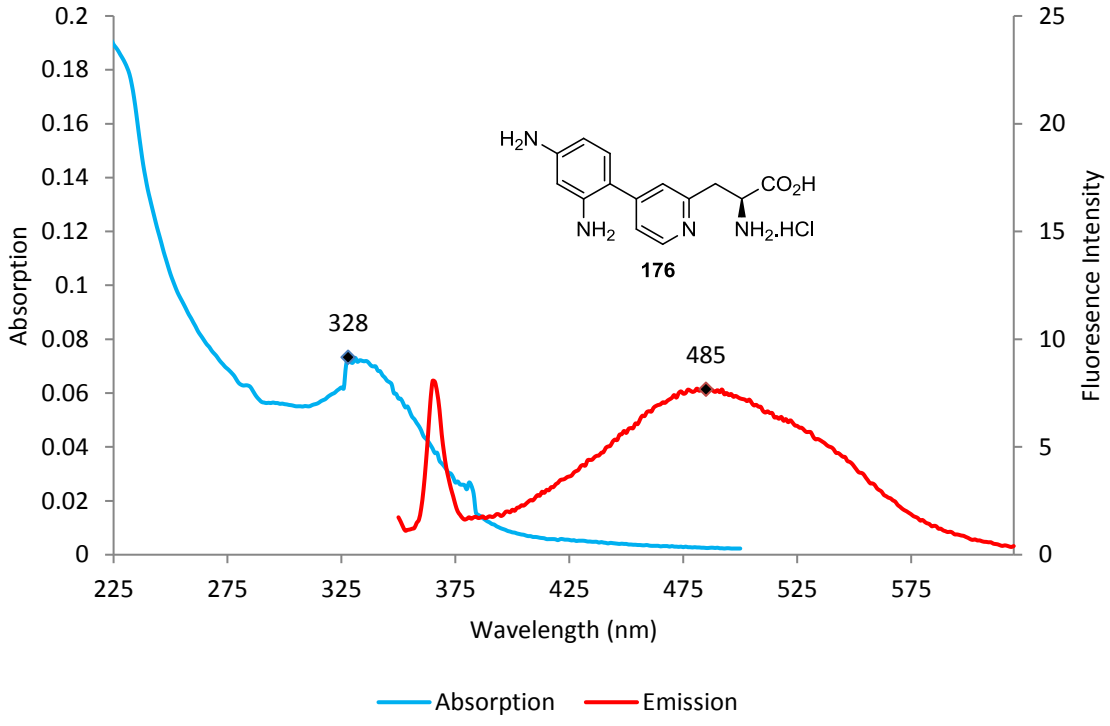


Figure 42: Diaminophenyl **176** absorption and emission data.

2.6.2 Other Optical Properties

Similar to the first library of pyridine derivatives, the next stage was to calculate the Stokes shift, molar extinction coefficient, quantum yield and brightness for each of the second generation pyridine analogues. The most promising derivatives from this library were dimethoxyphenyl **172**

and piperonyl **173**. A good quantum yield of 0.19 was calculated for **172** and it displayed broad and intense fluorescence (Table 2). Even though piperonyl **173** did not exhibit intense fluorescence which was exemplified with a low quantum yield of 0.06, it did however fluoresce in the visible region which would expand the potential for application. The quantum yields and brightness for dinitrophenyl **174** and diaminophenyl **176** were not measured due to the weak emission that each analogue displayed.

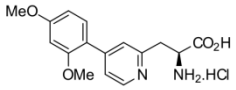
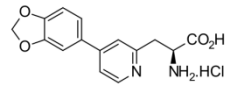
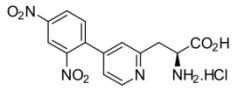
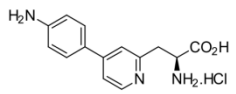
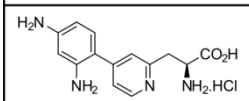
Second Generation Pyridine Derivatives	Absorption maximum (nm)	Emission maximum (nm)	Stokes Shift (nm)	Molar extinction coefficient (cm^{-1} M^{-1})	Quantum yield (ϕ_F)	Brightness (cm^{-1} M^{-1})
 **172**	275 and 298	384 nm × 2	109 and 86	8,600	0.19	1634
 **173**	303	460	157	9,620	0.06	577
 **174**	265	304	39	13,260	N/A	N/A
 **175**	315	433	118	11,600	0.04	464
 **176**	328	485	157	7,330	N/A	N/A

Table 2: Optical properties summary for second generation pyridine-derived α-amino acids.

2.6.3 Summary

From the second generation of pyridine-derived α-amino acids, the most interesting compounds were dimethoxyphenyl **172** and piperonyl **173** (Figure 43). These two compounds along with the first generation methoxyphenyl **151** and naphthyl **152** analogues were the four pyridine derivatives that exhibited the most desirable optical properties. This is likely due to the fact that these four compounds have electron-rich side-chain aryl groups allowing them to possess a push-pull relationship throughout the extended conjugation. They have the most potential to be incorporated into a small CPP and utilised in subsequent cell study investigations.

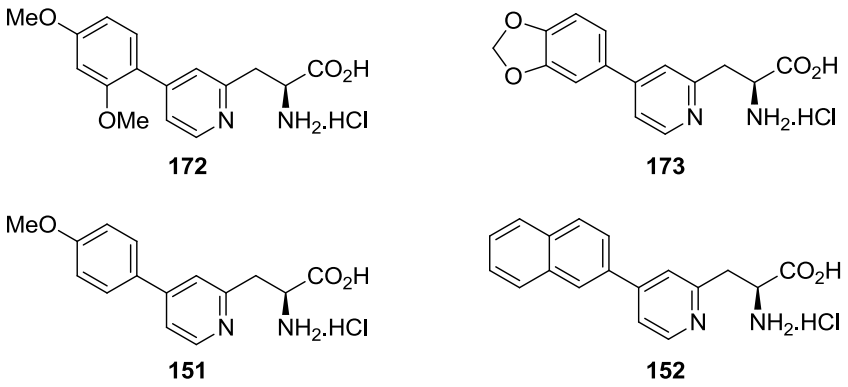


Figure 43: Pyridine-derived fluorophores with interesting optical properties.

2.7 Synthesis of Fmoc Protected Pyridine-Derived α-Amino Acids

2.7.1 Solid Phase Peptide Synthesis

Solid phase peptide synthesis (SPPS) was pioneered by Merrifield in 1963 and it is now the standard method for synthesising peptides and proteins in the laboratory.[94] It allows the synthesis of natural peptides that can be difficult to express in bacteria, the incorporation of unnatural amino acids, peptide/protein backbone modification and the synthesis of proteins that consist of D-amino acids. Porous beads are treated with functional linkers, where peptide chains can be built from. Repeated cycles of deprotection-wash-deprotection-wash take place until the desired peptide has been synthesised. The superiority of this technique is due to the ability to perform wash cycles after each reaction, filtering off excess liquid-phase reagents and by-products while the growing peptide of interest remains covalently attached to the insoluble resin.[95]

2.7.2 SPPS - Boc Protection Method

The original method for the synthesis of peptides and proteins relied upon the Boc group to protect the α-amino group, supressing its nucleophilicity. Acid mediated deprotection with TFA is generally used to remove the N-terminal Boc protecting group, prior to coupling each amino acid to the growing peptide chain. Upon synthesis of the target peptide, the deprotection strategy to remove the side-chain protecting groups, usually benzyl-based groups, requires treatment with hydrogen fluoride. This is extremely hazardous to work with and a harsh final cleavage reagent which can ultimately degrade the peptide. A milder, base labile method for SPPS was developed as an alternative to the Boc protection method.[95]

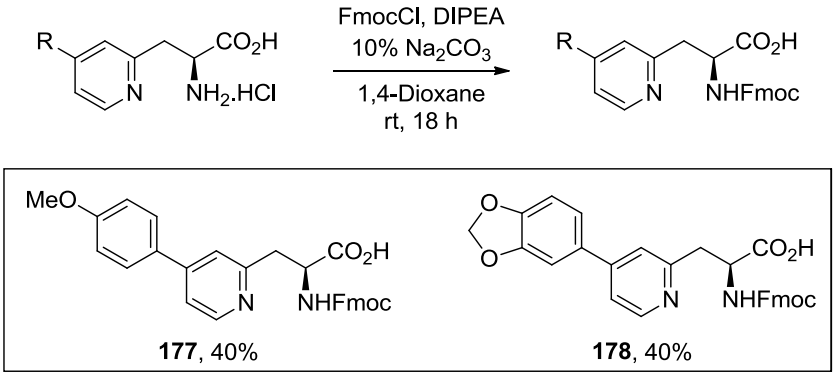
2.7.3 SPPS - Fmoc Protection Method

The Fmoc protection method allows for a milder deprotection strategy using piperidine to remove the Fmoc group, exposing the α-amino group for subsequent coupling. The Fmoc group is stable under acidic conditions which allow the use of mild acid labile protecting groups to protect the side-

chain residues, generally *tert*-butyl based. This orthogonal protecting group strategy is preferred over the Boc method because it is easier to cleave from the resin support. Side-chain protecting group removal and cleavage from the resin is carried out with TFA in the presence of cation scavengers. However, it is less atom economical as the fluorenyl group is much larger than the *tert*-butyl group.[95]

2.7.4 Synthesis of *N*-Fmoc Protected Pyridine-Derived α-Amino Acids

As previously described in the first chapter, a popular application of unnatural fluorescent α-amino acids is incorporation into a peptide or protein to study biological mechanisms via fluorescence microscopy. In order for α-amino acid incorporation to occur via SPPS, the fluorescent analogue must be transformed into a *N*-Fmoc protected derivative. Methoxyphenyl **151** and piperonyl **173** exhibited excellent optical properties and were chosen to be protected as *N*-Fmoc derivatives. Fluorenylmethyloxycarbonyl chloride was used under basic conditions to form methoxyphenyl **177** and piperonyl **178** *N*-Fmoc protected analogues (Scheme 39).[96] Consistent yields of 40% were achieved for both reactions.



Scheme 39: Synthesis of *N*-Fmoc protected pyridine-derived α-amino acids.

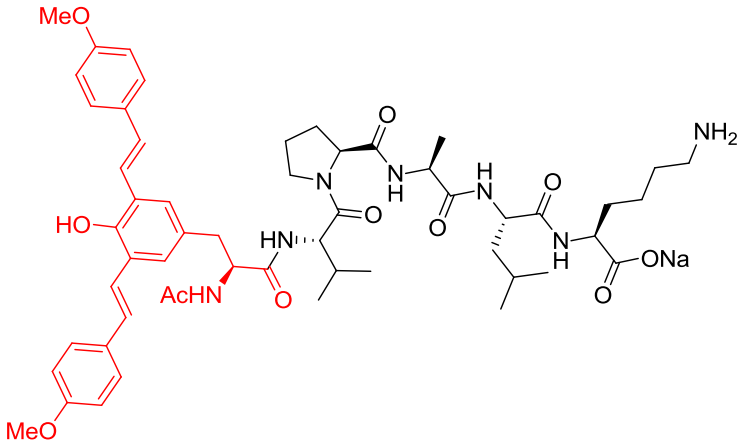
2.8 Synthesis of Novel Fluorescent Cell Penetrating Hexapeptide

As the *N*-Fmoc protected analogues had been successfully synthesised, the next stage of this project was to incorporate them into a known CPP. These fluorescently tagged CPPs would be incubated with cell lines and visualised via fluorescence microscopy to study whether the fluorophore-CPP conjugate could accumulate in the cells.

2.8.1 SPPS of Cell Penetrating Pentapeptide VPTLK

Cheruku *et al* synthesised a series of tyrosine-derived stimuli responsive, fluorescent α-amino acids.[11] During this study they incorporated a novel α-amino acid with the most promising optical properties into a cell penetrating Bax-inhibiting pentapeptide using SPPS.[97] Hexapeptide **5** was successfully synthesised and investigations were carried out to assess the cell permeability and

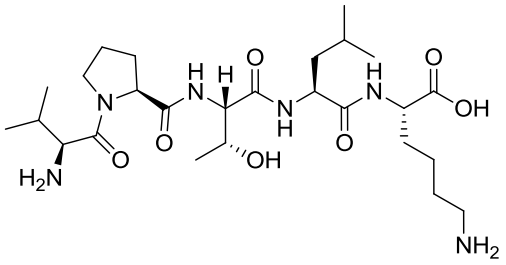
fluorescent properties of this peptide (Figure 44). It was incubated with a human epithelial cell line (HeLa) and mouse fibroblast cells (NIH 3T3) for 3 h. Laser scanning confocal microscopy was used to visualise and confirm that hexapeptide **5** could enter and accumulate in these cell lines. This demonstrates the potential utility of this novel fluorescent α-amino acid proving that it could be used to image cellular processes. Inspired by this work, a known CPP would be selected, synthesised and coupled with *N*-Fmoc protected methyoxyphenyl analogue **177**. This particular pyridine-derived α-amino acid was chosen due to its intense fluorescence as well as its high quantum yield and molar extinction coefficients.



5
Fluorophore-VPALK

Figure 44: Cell penetrating hexapeptide **5**.

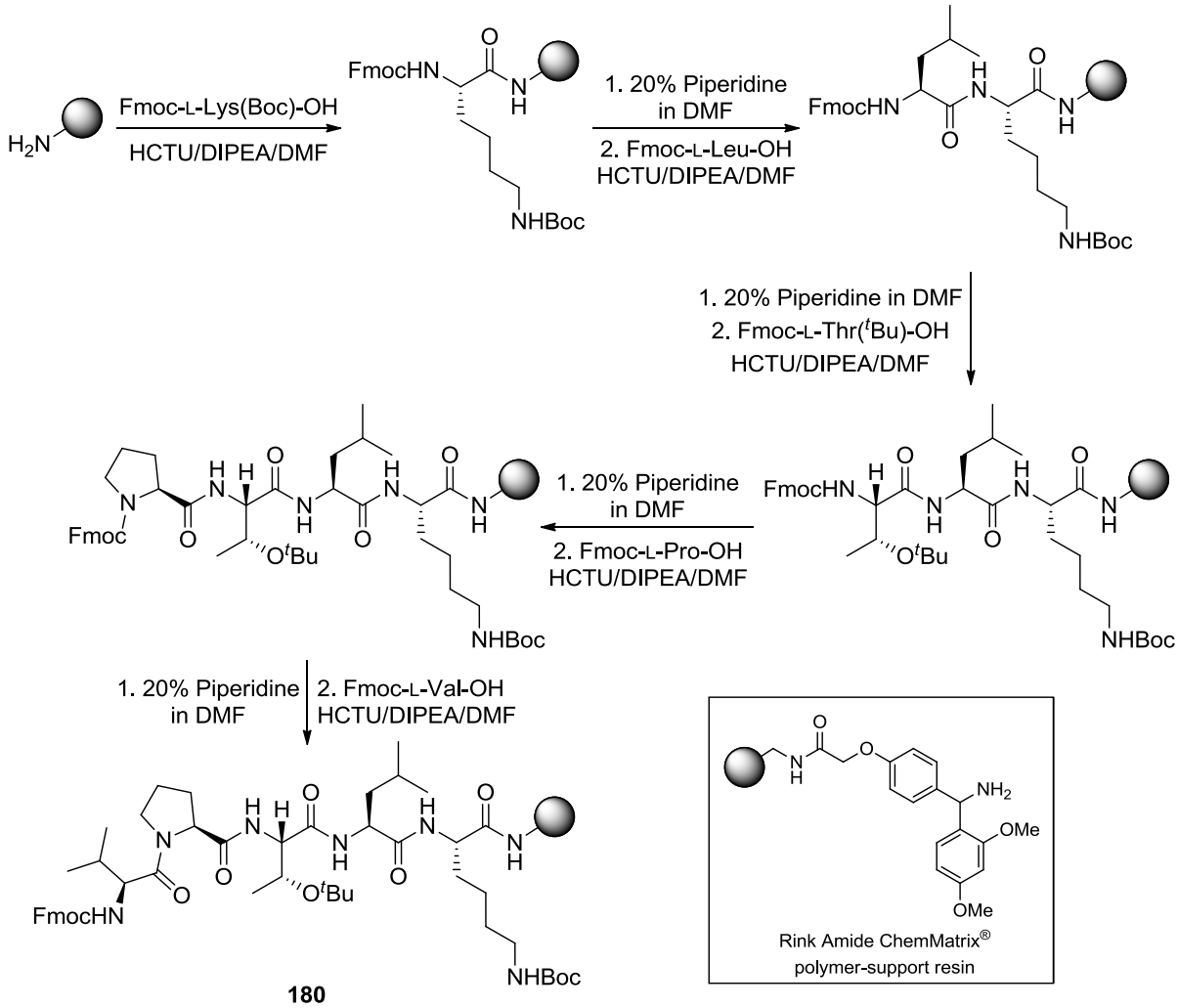
CPPs are a group of peptides that have the ability to penetrate the plasma membrane of living cells.[98–101] Cell penetrating pentapeptides are derived from the protein Ku70 and designed on the Bax-binding domain of Ku70. Ku70 is a multifunctional protein involved in non-homologous end-joining DNA repair and cell-death regulation. Matsuyama and co-workers have developed a series of cell penetrating peptides that were designed based on the Bax-binding domain of Ku70 of various species which include human, rat and mouse.[102] Pentapeptide VPTLK **179** was designed from human Ku70 and displayed excellent cell penetration activity (Figure 45).[97] It was therefore selected as the preferred small CPP that was to be synthesised and coupled with α-amino acid **177**.



179
VPTLK

Figure 45: Structure of cell penetrating pentapeptide VPTLK **179**.

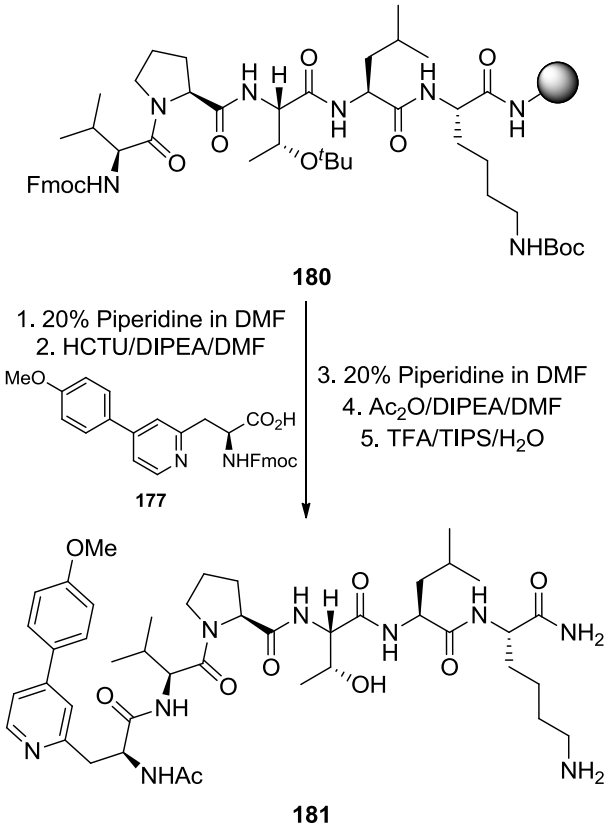
Dr Astrid Knuhtsen and Dr Andrew Jamieson provided invaluable knowledge and expertise when this portion of work was undertaken. SPPS was carried out using Rink Amide ChemMatrix® as the polymer-support. The ChemMatrix® Rink Amide resin does not have any protecting group on the linker therefore the first step was swelling of the resin followed by coupling with Fmoc-L-Lys(Boc)-OH, the C-terminal amino acid (Scheme 40). Following coupling and thorough washing, the resin was subjected to 20% piperidine in DMF to remove the Fmoc-protecting group on the N-terminus. The resin was washed again after which the next amino acid was coupled. These steps were repeated until the synthesis of pentapeptide VPTLK was complete. A test cleavage was carried out on a small amount of resin, in which the resin bound peptide **180** was treated with a TFA/TIPS/H_2O (95:2.5:2.5) cleavage cocktail. This cleaved the peptide from the resin as well as removing the lysine and threonine residue side-chain protecting groups. Triisopropylsilane (TIPS) was used as a cation scavenger, preventing reactive *tert*-butyl cations from generating undesired products. A sample of the resulting Fmoc protected peptide was analysed on the LC-MS to confirm the synthesis of N-terminus Fmoc protected pentapeptide VPTLK. This result suggested that the crude pentapeptide had been successfully synthesised to >95% purity using the peptide synthesiser (Appendix 2).



Scheme 40: SPPS of resin bound pentapeptide **180**.

2.8.2 Synthesis of Hexapeptide 181

In order to couple methoxyphenyl **177** to resin bound pentapeptide **180** the peptide synthesiser was not used. Instead, manual peptide coupling was employed due to the amounts of the novel fluorescent α-amino acid available. Resin bound pentapeptide **180** was treated with 20% piperidine in DMF to remove the N-terminal Fmoc protecting group (Scheme 41). The coupling conditions were the same as above however, only two equivalents of methoxyphenyl **177** were used due to the amount available. Following this manual coupling, a second test cleavage was carried out on a small amount of the resin bound hexapeptide using the same test cleavage conditions as before to ensure the desired fluorophore tagged peptide had formed. Upon LC-MS confirmation that successful coupling had taken place, the Fmoc protected hexapeptide was subjected to 20% piperidine in DMF to form the N-terminus free amine. After which, the N-terminus was acetylated using acetic anhydride/DIPEA/DMF (1:1:8). The final step was cleaveage of the hexameric peptide from the polymer-support and removal of the amino acid side-chain protecting groups, ^{t}Bu and Boc under acidic conditions. Preparative HPLC was used to isolate hexapeptide **181** to >95% purity. Full characterisation was carried out which included analysis by HRMS (Table 4, Figure 53, Figure 54).



Scheme 41: Synthesis of hexapeptide **181**.

2.9 Cell Study with Hexapeptide 181

The final aspect of this project following the successful synthesis of hexapeptide **181** was to carry out a biological investigation, aided by Carol-Anne Smith and Dr Mathis Riehle. Suitable cells would be grown and subsequently incubated with hexapeptide **181** diluted to a known concentration with appropriate growth media. Fluorescence microscopy would then be used to visualise whether **181** manages to enter and accumulate in the cells. Human foreskin fibroblast cells immortalised with hTERT (human telomerase reverse transcriptase) were used for this cell study. The cells were applied and grown on ibidi® µ-Slide VI 0.4 plates for 72 h before visualisation or addition of hexapeptide **181**. Images of control cells with no prior peptide-growth media incubation were taken for comparison (Figure 46). Separate images of each cell were taken with the fluorescence lamp off and again immediately after the fluorescence lamp was turned on (Appendix 3). An overlay of these images is also displayed.

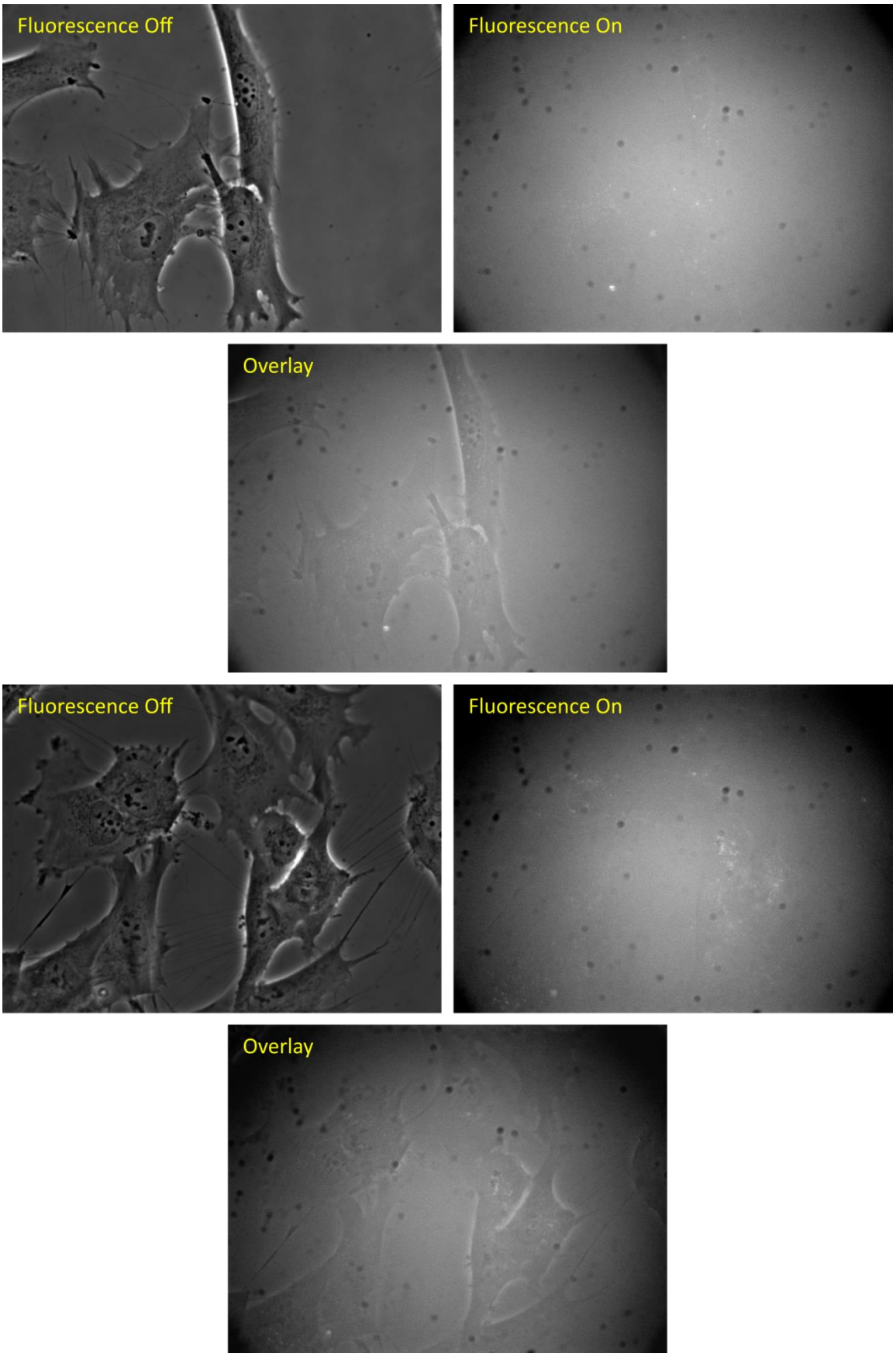


Figure 46: Control cells not incubated with hexapeptide **181**.

Cells were then incubated at room temperature with 120 µL of 100 µM hexapeptide **181** in Dulbecco's Modified Eagle's Medium (DMEM). Like the control cells, two images were taken. One with the fluorescence lamp off and the other with it on, an overlay of these two images was also processed. Pictures were taken from immediate application up to a 70 minute incubation period. The fluorescence on pictures displays a bright, concentrated assembly of hexapeptide **181** (Figure 47, Figure 48, Figure 49 and Figure 50). By superimposing the fluorescence off and on pictures it is clear that hexapeptide **181** has entered the cells. The peptide appears to have entered the cell membrane and accumulated in the cytoplasm, congregating outside the nucleus. By comparing these with the control images there is a considerable difference as there are no bright portions seen in any of the control cells. This excellent result proves that the fluorescently tagged hexapeptide **181** has cell penetrating properties and can be visualised using fluorescence microscopy due to the presence of the novel fluorescent α-amino acid incorporated into the peptide.

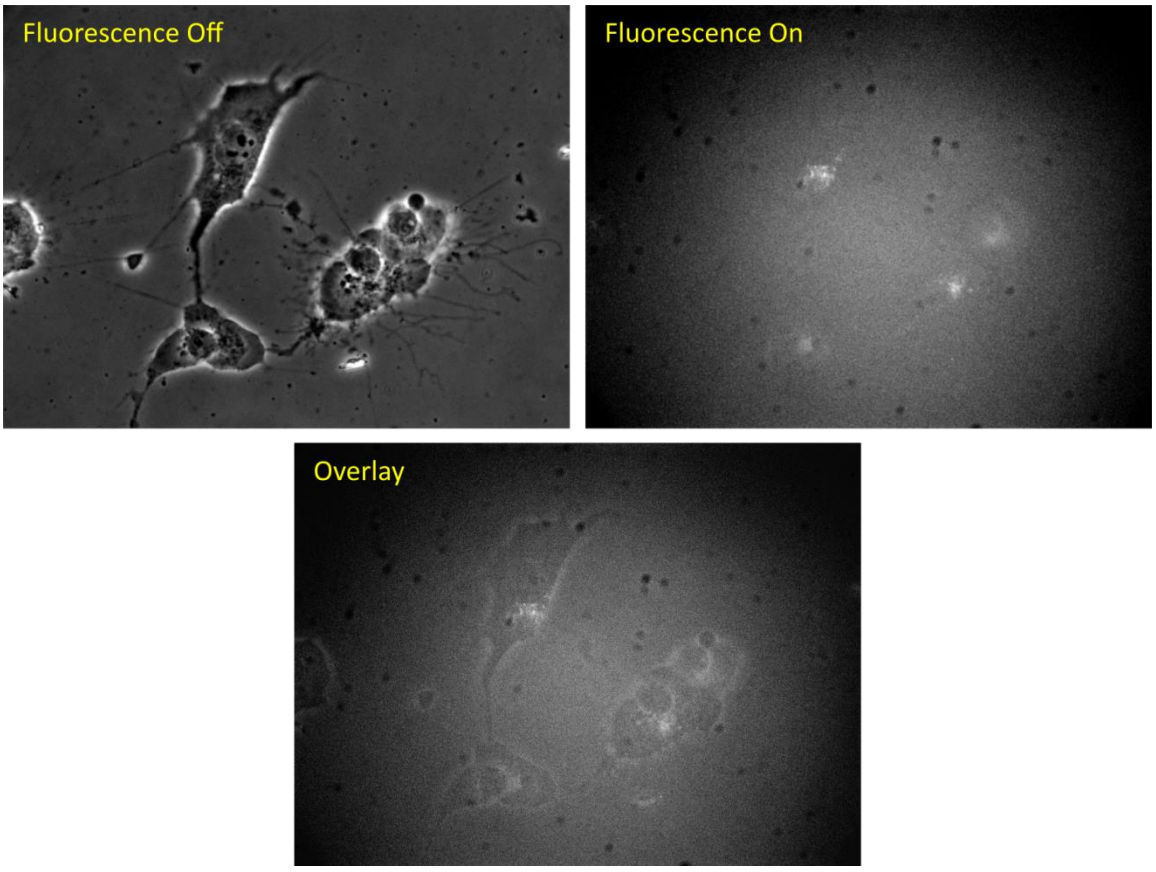


Figure 47: Cells incubated with hexapeptide **181** for 60 minutes.

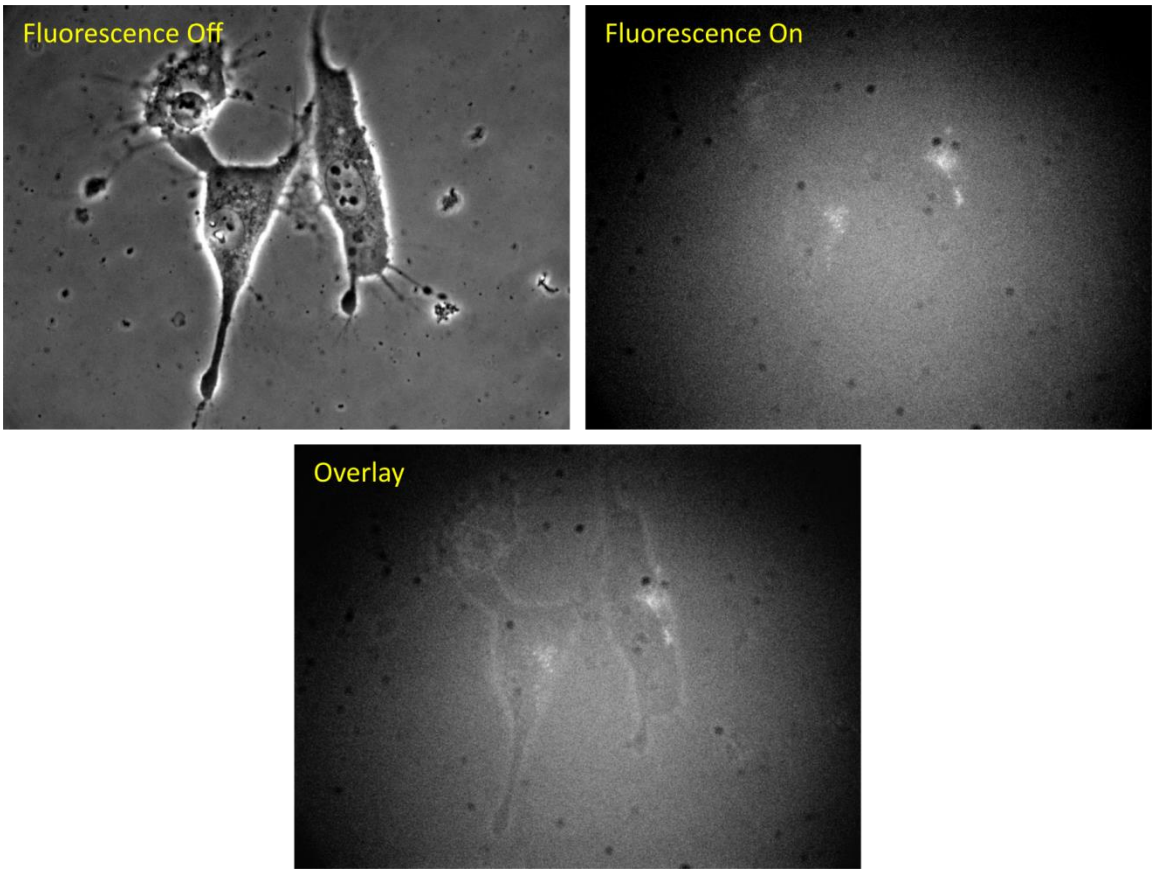


Figure 48: Cells incubated with hexapeptide **181** for 65 minutes.

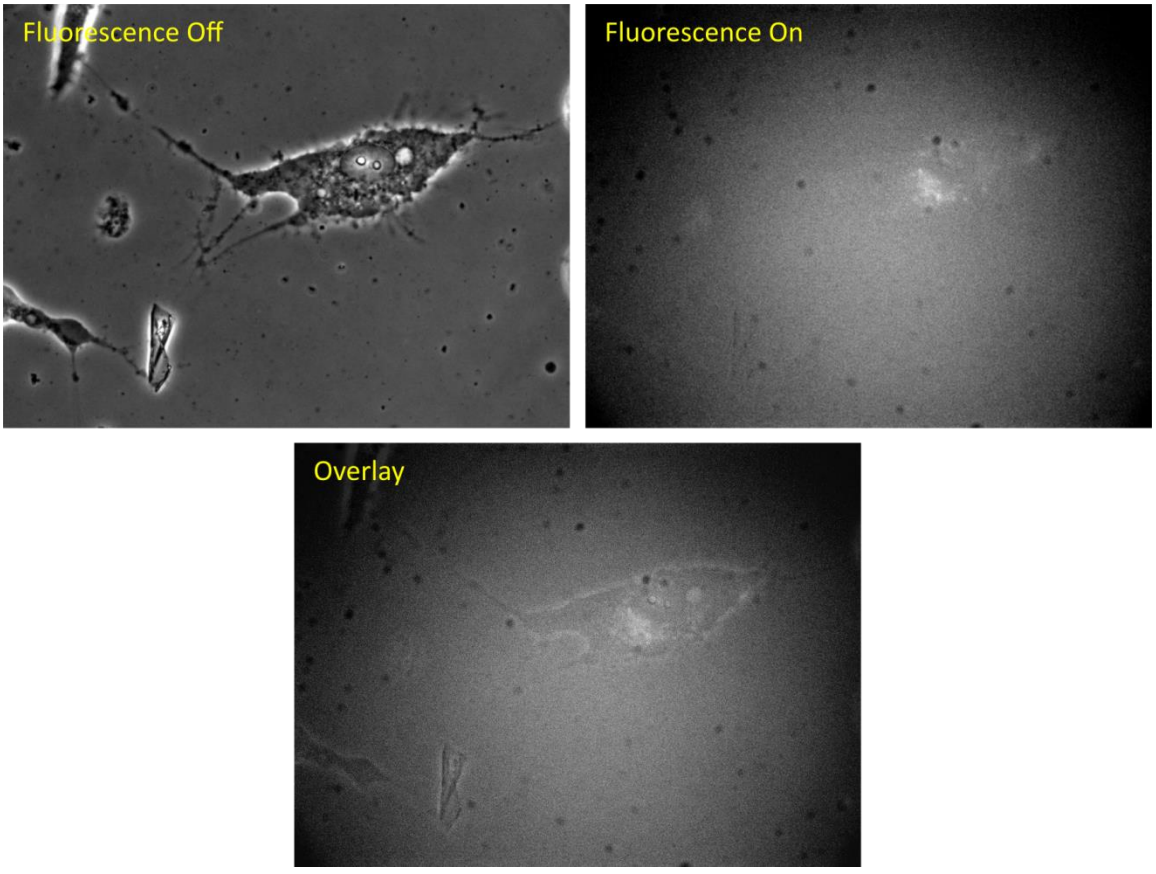


Figure 49: Cells incubated with hexapeptide **181** for 70 minutes.

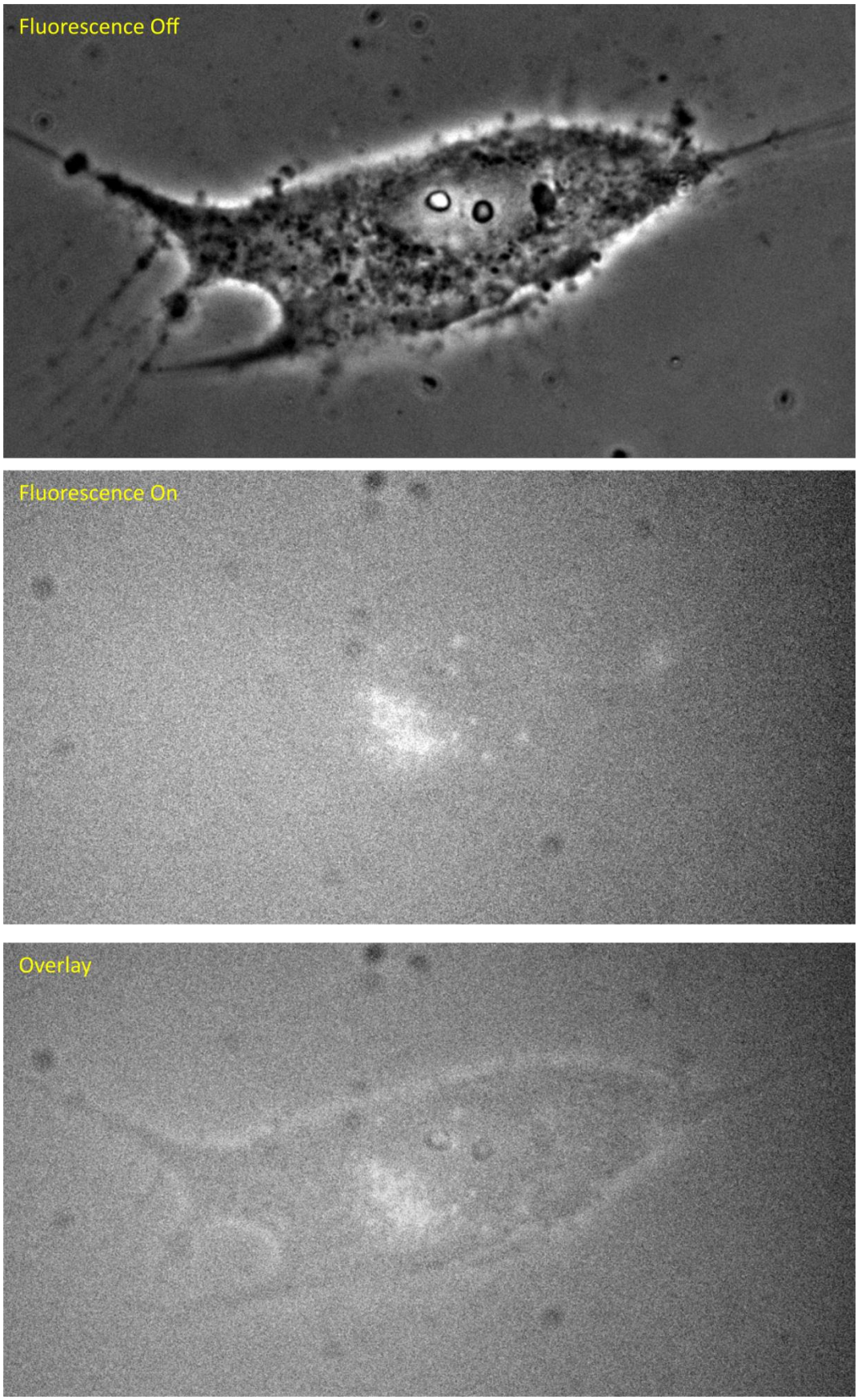


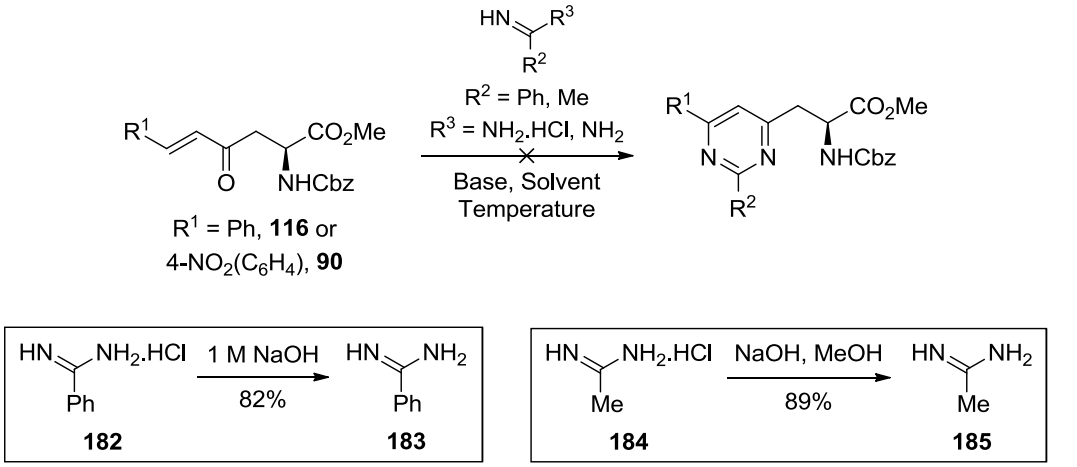
Figure 50: Cells incubated with hexapeptide **181** for 10 minutes (enlarged and cropped).

2.10 Synthesis of Pyrimidine-Derived α-Amino Acids

2.10.1 Initial Synthesis of Pyrimidine-Derived α-Amino Acids

In order to fully understand the reactivity of the enone-derived α-amino acids, a variety of heterocycle forming reactions were considered and an investigation into the synthesis of pyrimidine-derivatives was undertaken. Treating enones with various amidines under acidic[103] or basic[104] conditions can lead to substituted pyrimidine rings. However, there have been no previous syntheses of pyrimidine-derived α-amino acids using an enone substrate.

Phenyl enone **116** and nitrophenyl enone **90** were used as the model substrates and subjected to a number of various reaction conditions in an attempt to synthesise the desired pyrimidine ring (Scheme 42 and Table 3). For entries 1 and 2, sodium carbonate was used as the base which was required to neutralise the HCl salt of benzamidine hydrochloride. Acetonitrile and methanol were used as the respective solvents under reflux. Unfortunately, a complex mixture of products formed for both reactions. Benzamidine hydrochloride **182** was treated with 1 M NaOH to form benzamidine **183** as the corresponding free amine. This was used as the amidine reagent for entry 3 with methanol as the solvent and no base. Again decomposition took place. It was hypothesised that pyrimidine ring formation was not taking place due to the electron-rich nature of benzamidine and that switching to a more electron-deficient amidine coupling partner would help the reaction proceed. Acetamidine hydrochloride **184** was used for the next attempt along with sodium carbonate and methanol but no reaction took place (entry 4). The formation of acetamidine **185** as the free amine was carried out and used for the next reaction (entry 5). All the starting material was consumed but there was no formation of any major products. For the next attempt, potassium carbonate and benzamidine hydrochloride **182** was used in toluene under reflux (entry 6). All the reactions used polar protic or aprotic solvents so an attempt with non-polar toluene was investigated but decomposition ensued (entry 6). Lewis acids have been employed in heterocycle forming reactions such as a ytterbium triflate catalysed method for the preparation of substituted imidazoles.[105] Ytterbium triflate was used catalytically along with potassium carbonate, benzamidine hydrochloride **182** in THF under reflux (entry 7). Nitrophenyl enone **90** was also used as the substrate for this reaction (entry 7) as no phenyl enone **116** remained due to the number of previous test reactions. A major product had formed however, the methyl ester group had also hydrolysed. Therefore, the crude reaction mixture was treated with thionyl chloride and methanol and, the desired product **186** was isolated in 44% yield.

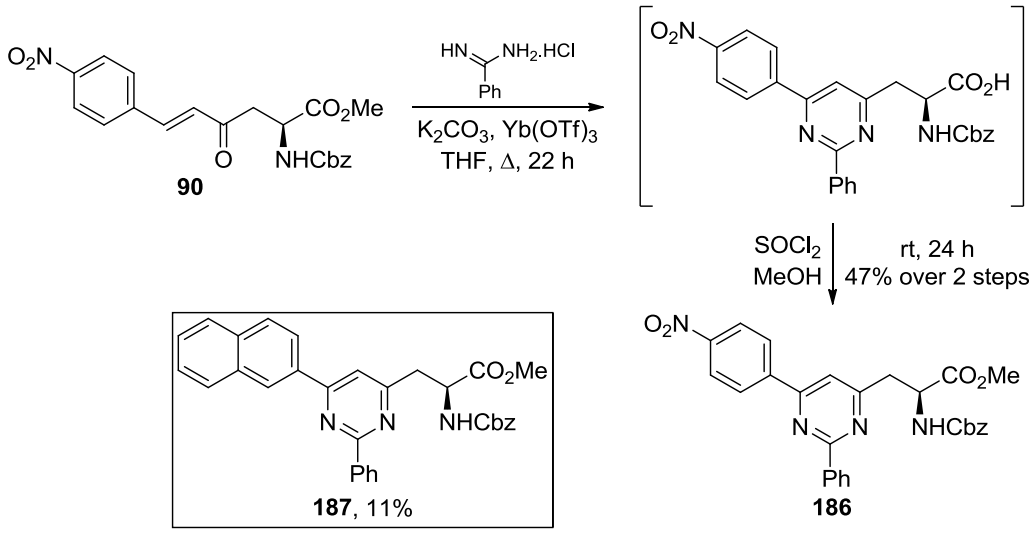


Scheme 42: General conditions for pyrimidine ring synthesis.

Entry	Base	Solvent	Temperature	Amidine	Catalyst	Outcome
1[a]	Na_2CO_3	MeCN	Δ/82 °C	**182**	/	Decomposition
2[a]	Na_2CO_3	MeOH	Δ/65 °C	**182**	/	Decomposition
3[a]	/	MeOH	Δ/65 °C	**183**	/	Decomposition
4[a]	Na_2CO_3	MeOH	Δ/65 °C	**184**	/	Decomposition
5[a]	/	MeOH	Δ/65 °C	**185**	/	Decomposition
6[a]	K_2CO_3	Toluene	Δ/110 °C	**182**	/	Decomposition
7[b]	K_2CO_3	THF	Δ/66 °C	**182**	$Yb(OTf)_3$	44%

Table 3: General conditions for pyrimidine ring synthesis. [a] Phenyl **116** as starting material enone [b] Nitrophenyl **90** as starting material enone.

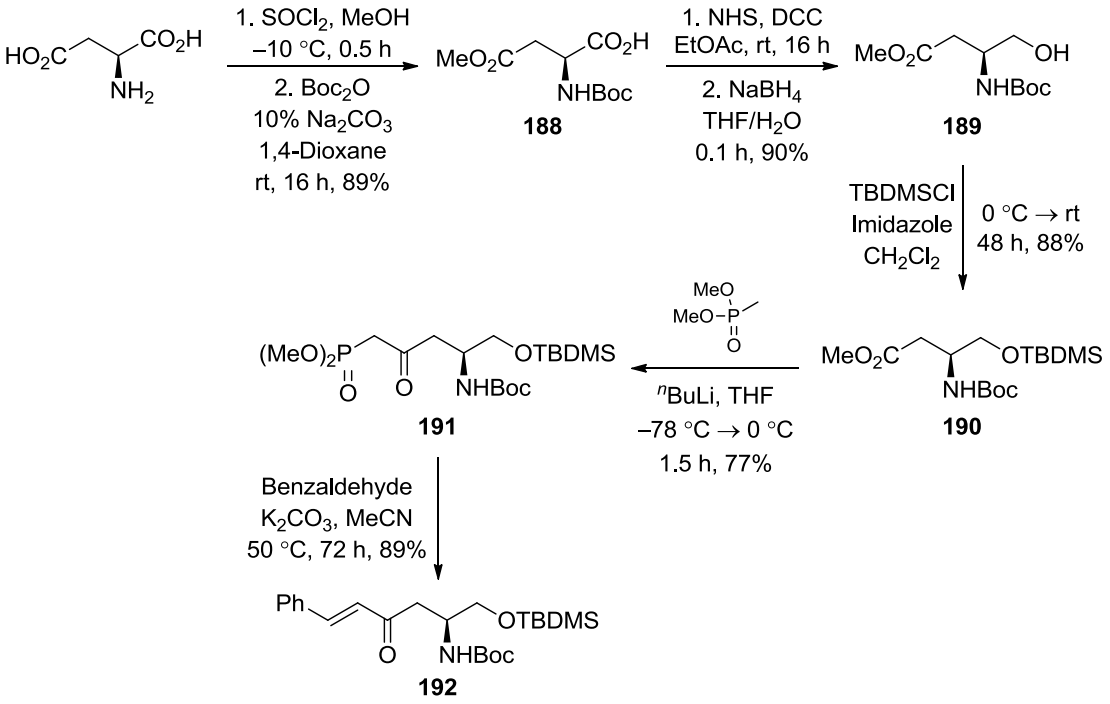
The reaction conditions were repeated in an attempt to improve the yield of this two-step procedure (Scheme 43). The synthesis of nitrophenyl pyrimidine **186** proceeded with a 47% yield. A second analogue was synthesised, naphthyl pyrimidine **187** from the corresponding enone. Unfortunately a low 11% yield was observed for this compound and after several attempts, no improvement to the yield was achieved. A potential reason for the observed low yields for this reaction is due to the amidine reagent hydrolysing the methyl ester protecting group. With the free carboxylic acid moiety unprotected, side-reactions could be taking place leading to a complex mixture of intermediates and eventual decomposition. It was observed by Bartlett and co-workers that a guanidine/ethanol mixture was able to hydrolyse the methyl ester present in their substrate.[106] Due to this finding and the results so far, investigations towards the synthesis of pyrimidine-derived α-amino acids using this particular synthetic route was halted.



Scheme 43: Synthesis of pyrimidine-derived α-amino acids **186** and **187**.

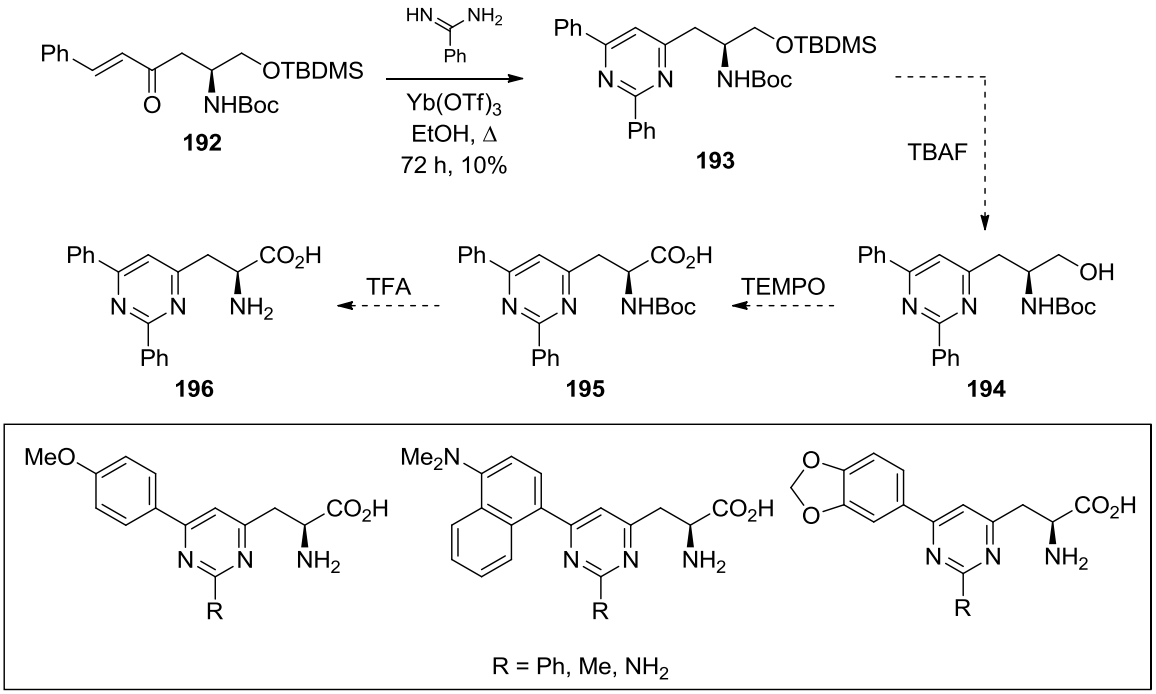
2.10.2 Alternative Synthesis Towards Pyrimidine-Derived α-Amino Acids

Due to the inefficient synthesis of the naphthyl **187** derivative using the conditions described, an alternative approach was investigated. An alternative functional group and protection strategy was investigated due to the earlier problems with hydrolysis of the methyl ester by the amidine reagents. L-Aspartic acid was used as the starting material for the new synthetic route (Scheme 44). The β-acid was protected as a methyl ester and subsequent Boc-protection led to carboxylic acid **188**.[107,108] The α-acid was activated using *N*-hydroxysuccinimide and *N*,*N*'-dicyclohexylcarbodiimide followed by reduction with sodium borohydride to furnish primary alcohol **189**.[109] It was then protected to form *tert*-butyldimethylsilyl ether **190**.[110] Now that the methyl ester had been replaced with a silyl ether moiety, the next step was to synthesise the corresponding β-keto phosphonate ester **191** using the standard conditions previously described in this chapter.[111,112] Benzaldehyde was used in the HWE reaction along with **191** to synthesise *E*-alkene **192** in an excellent 89% yield.



Scheme 44: Synthesis of enone derivative **192**.

With phenyl enone **192** available, the next step was to subject it to pyrimidine synthesis conditions. Unfortunately no reaction was observed when using potassium carbonate, THF and ytterbium triflate. In an attempt to force the reaction, the free base of benzamidine was formed and treated with the enone in ethanol under reflux (Scheme 45). The reaction yielded the desired pyrimidine **193** in 10%, but due to the scale of the reaction only characterisation via ^{1}H NMR spectroscopy and mass spectrometry was possible. Therefore this only proves the concept of this proposed synthesis. Upon reaction optimisation, **193** could be treated with TBAF to remove the silyl protecting group, forming primary alcohol **194**.[113] Oxidation with TEMPO and sodium hypochlorite could provide carboxylic acid **195** and trivial Boc-removal with TFA could furnish the parent α-amino acid **196**. A number of analogues could be synthesised by differentiating the side-chain aryl groups as well as varying the amidine coupling partner used. There are a range of commercially available amidines such as benzamidine, acetamidine and guanidine. If successful, there would be three aryl groups in conjugation and by strategically placing electron-donating functional groups in optimal positions these compounds could have the potential to display interesting optical properties.

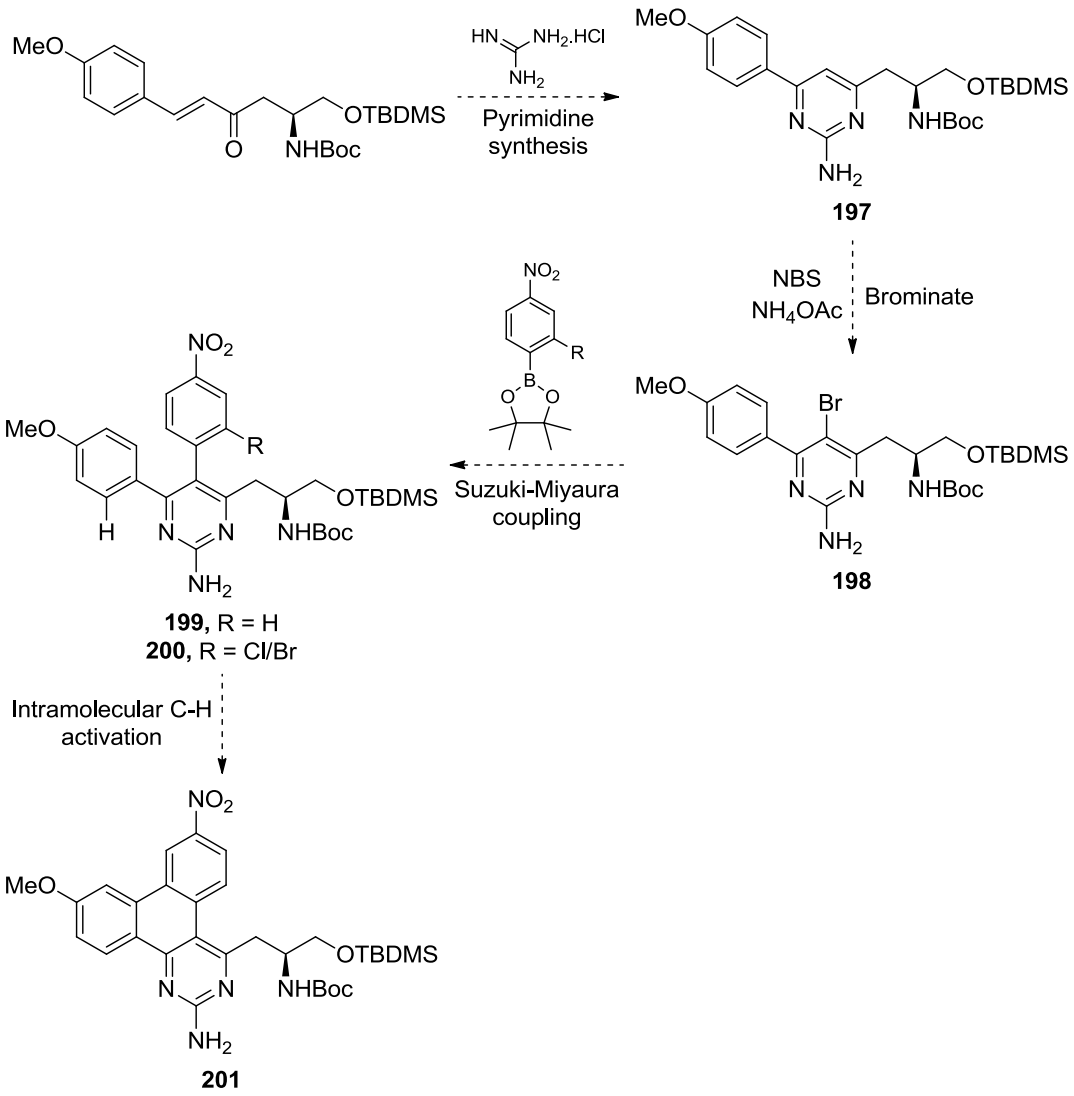


Scheme 45: Pyrimidine ring formation conditions, proposed synthesis to parent α-amino acid **196** and potential pyrimidine-derived α-amino acid analogues.

2.11 Future Work

2.11.1 Proposed Synthesis of Pyrimidine-Derived α-Amino Acids

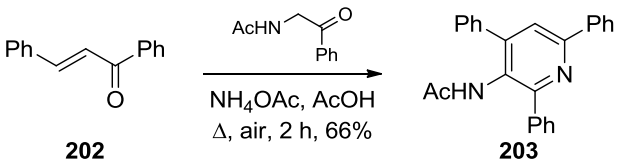
The potential synthesis of pyrimidine derived α-amino acids was discussed earlier in this chapter. If the ring forming reaction was optimised and a guanidine derivative **197** was synthesised, there could be potential to brominate *para* to the amino group using NBS, creating a brominated derivative **198**.[114] A commercially available or synthetically modified boronic acid could then be cross coupled with **198** to form arylated **199** or **200**.[115] If unsubstituted **199** was formed then the standard deprotection procedure could be used to form the parent α-amino acid. However, if the chlorinated or brominated derivative **200** was synthesised then an intramolecular C-H activation reaction could be investigated to fuse both aryl groups together to form tetracycle **201**.[116,117] An appropriate catalyst such as palladium acetate could potentially undergo oxidative addition with the aryl halide moiety and after approach of the other aryl side-chain a concerted metalation-deprotonation transition state could form. Subsequent reductive elimination would then take place forming a new carbon-carbon bond. This would rigidify the overall structure and in this example there are two electron-donating groups (methoxy and amino) and one electron-withdrawing nitro group. Effective fluorophores display this push-pull electronic relationship. Investigation into this proposed route could lead to a library of highly fluorescent derivatives that could exhibit emission bands further into the visible region of the electromagnetic spectrum. This increases their potential to be used as fluorescent probes and a variety of biological mechanisms could be explored.



Scheme 46: Proposed halogenation-Suzuki coupling-intramolecular C-H activation sequence on pyrimidine α-amino acid analogues.

2.11.2 Proposed Synthesis of β-Carboline-Derived α-Amino Acids

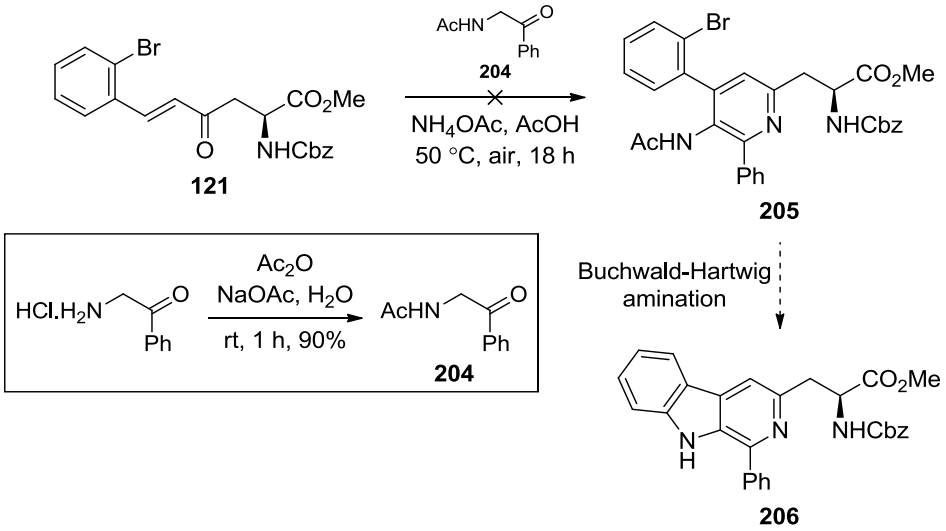
Synthesis towards β-carboline-derived α-amino acids was proposed and an initial proof of concept investigation was carried out. Robinson and co-workers synthesised 2,3,4,6-tetrasubstituted pyridine **203** by condensing enone **202** with acetophenone derivatives (Scheme 47).[118]



Scheme 47: Synthesis of 2,3,4,6-tetrasubstituted pyridine **203**.

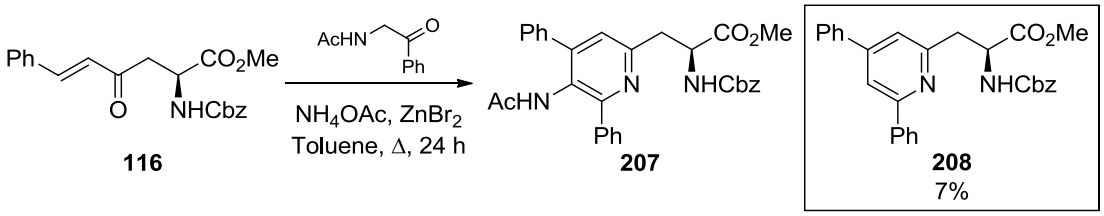
Using this literature precedent as inspiration, 2-bromophenyl enone **121** was subjected to the reported conditions. 2-Aminoacetophenone hydrochloride was acetylated prior, using acetic

anhydride under basic conditions to form **204**.[119] Unfortunately there was no observed product after 18 h at 50 °C and decomposition started to take place when the temperature was increased. If the reaction had been successful then **205** could be subjected to an intramolecular Buchwald-Hartwig coupling to fuse the pyridine and aryl group together to form the desired β-carboline **206**.[120]



Scheme 48: Proposed synthesis of β-carboline-derived α-amino acid **206**.

A second attempt was carried out with phenyl enone **116** using a catalytic amount of zinc bromide and toluene under reflux (Scheme 49). The desired 2,3,4,6-tetrasubstituted pyridine **207** was not observed however, 2,4,6-trisubstituted pyridine **208** was isolated albeit with a low 7% yield after a single small-scale attempt. An efficient synthesis of 2,3,4,6-tetrasubstituted pyridines could be possible following optimisation of the Lewis acid and solvent. Buchwald-Hartwig coupling conditions could then be used to access the β-carboline framework. A series of analogues would then be synthesised with particular focus on electron-donating groups.

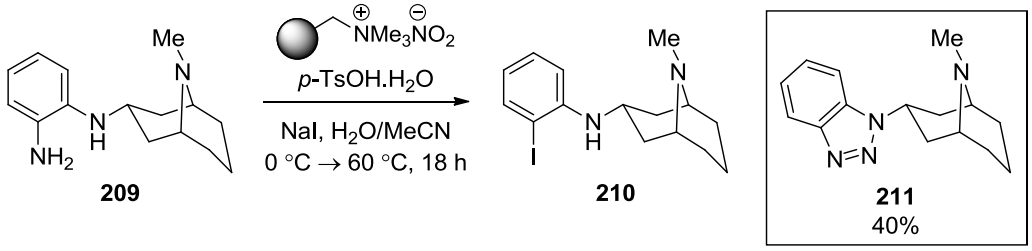


Scheme 49: Attempted synthesis of 2,3,4,6-tetrasubstituted pyridine **207**.

2.11.3 Proposed Synthesis of Benzotriazole-Derived α-Amino Acids

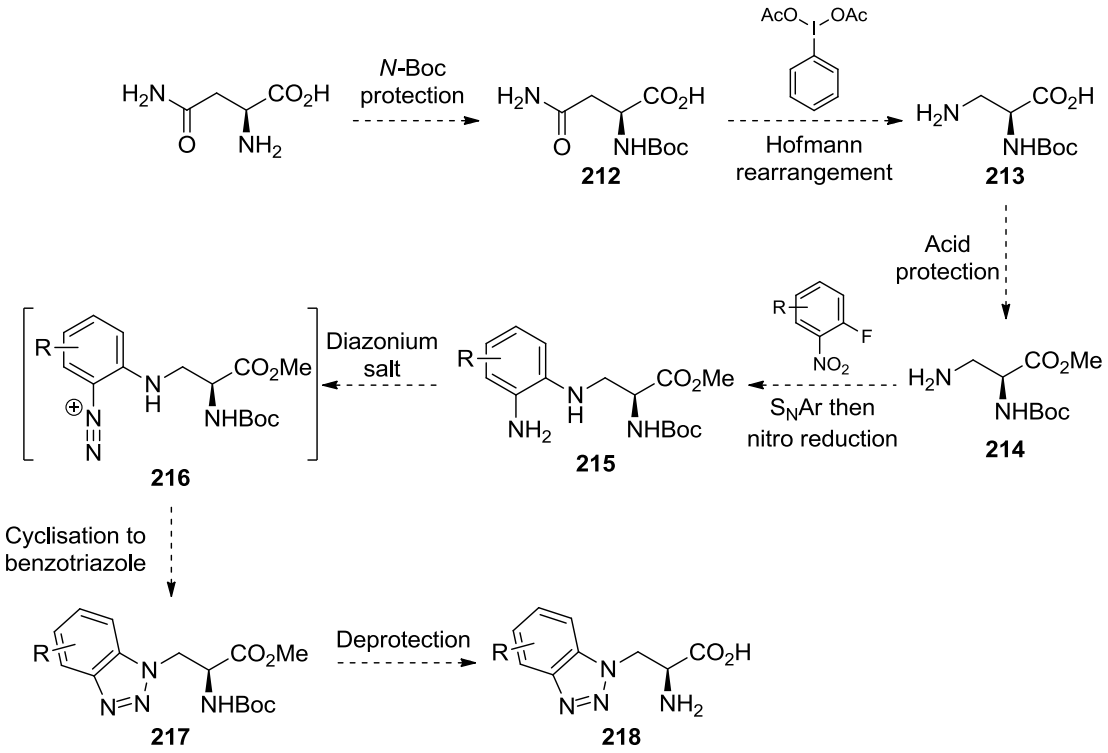
In unrelated research to this fluorescent α-amino acid project, Nikki Sloan from the group developed a one-pot iodination of aryl amines via stable diazonium salts.[121] The standard method for the synthesis of aryl iodides is through diazonium salt formation of aryl amines by using

Sandmeyer-type reaction conditions.[122] Harsh acidic conditions are often required for the preparation of diazonium salts and unwanted side-reactions can occur due to this. Therefore a new general method for the iodination of aryl compounds that could use a wide array of commercially available starting materials as well as mild reaction conditions was developed. However, during the project an unusual result was observed. Aniline **209** was treated with *p*-toluenesulfonic acid in the presence of polymer-supported nitrite reagent (Scheme 50). Sodium iodine was used as the iodinating reagent which was supposed to displace the diazonium salt and furnish aryl iodine **210**. However, a competing intramolecular reaction took place whereby the secondary amine *ortho* to the aryl amine attacked the diazonium salt species and gave benzotriazole **211** in 40% yield.



Scheme 50: Unexpected benzotriazole cyclisation.

A proposed route towards the synthesis of benzotriazole-derived α-amino acids was devised that would utilise this unexpected cyclisation. L-Asparagine would be used as the chiral pool starting material which would be Boc-protected initially to give carboxamide **212** (Scheme 51).[123] A Hofmann rearrangement could then be employed, using a reagent such as (diacetoxyiodo)benzene to form amine **213**.[124] Treatment with $TMSCHN_2$/MeOH would produce methyl ester **214**.[125] Various 1-fluoro-2-nitrobenzene derivatives could then be used in nucleophilic aromatic substitution reactions with amine **214** to construct a library of aryl nitro derivatives.[126] Aryl nitro reduction using Pd/C or tin(II) chloride dihydrate would furnish aniline **215**, the substrate for the cyclisation reaction. Preparation of the corresponding diazonium salt intermediate **216** would follow, whereby the intramolecular cyclisation would take place to form benzotriazole **217**. Deprotection to parent α-amino acid **218** would conclude the synthesis. This seven-step proposed synthesis could access novel benzotriazole-derived α-amino acids that could potentially have interesting optical properties. Numerous S_NAr coupling partners are commercially available which could lead to late-stage synthesis of a large library of analogues.



Scheme 51: Proposed synthesis of benzotriazole-derived α-amino acids.

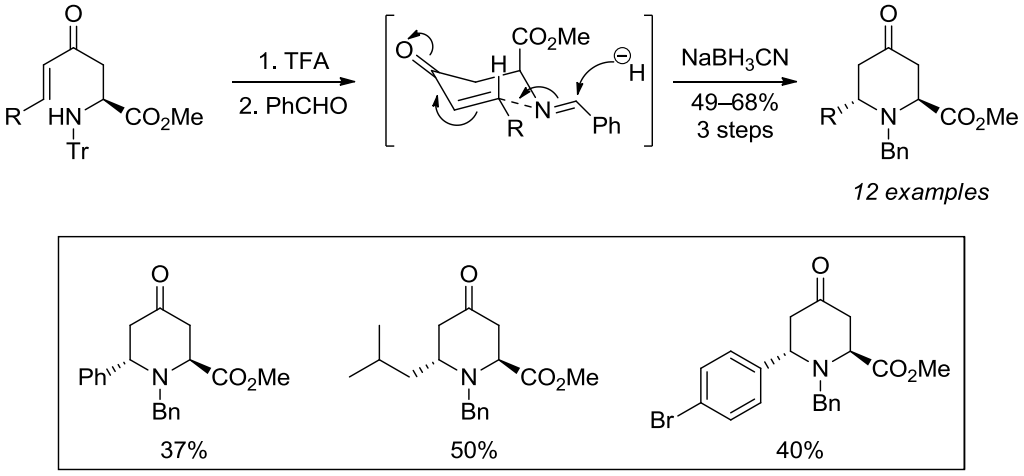
2.13 Conclusions

A synthetic route was developed and optimised for the synthesis of pyridine-derived α-amino acids. An initial seven-membered library was constructed with a varied substrate scope. An in-depth analysis of their optical properties was investigated, which resulted in two analogues being considered for further analysis, methoxyphenyl **151** and naphthyl **152**. A second generation of analogues was also synthesised, focussing mainly on electron-rich targets. This was based on the correlation observed between good fluorescent properties and electron-donating side-chain aryl groups. The optical properties were measured for these compounds and interesting fluorescent characteristics was exhibited by dimethoxyphenyl **172** and piperonyl **173**. An *N*-Fmoc derivative of methoxyphenyl **151** was synthesised and SPPS was carried out to construct a cell penetrating hexapeptide tagged with the fluorescent α-amino acid. Human fibroblast cells were incubated with this fluorophore tagged peptide and visualisation of the peptide in the cells was possible using fluorescence microscopy. This project shows that by carefully constructing α-amino acid side-chains with conjugated aryl groups and strategically placing electron-rich functional groups in positions that boost the charge transfer of the compound, it is possible to incorporate them into CPPs and confirm cell penetration via fluorescence microscopy. Potential future work has also been proposed that takes advantage of the enone functionality. This could lead to more α-amino acid derivatives that possess highly fluorescent side-chains and display red-shifted absorption and emission maxima as well as excellent quantum yield values.

3.0 Total Syntheses of 4-Hydroxy-2,6-Disubstituted Piperidine Natural Products

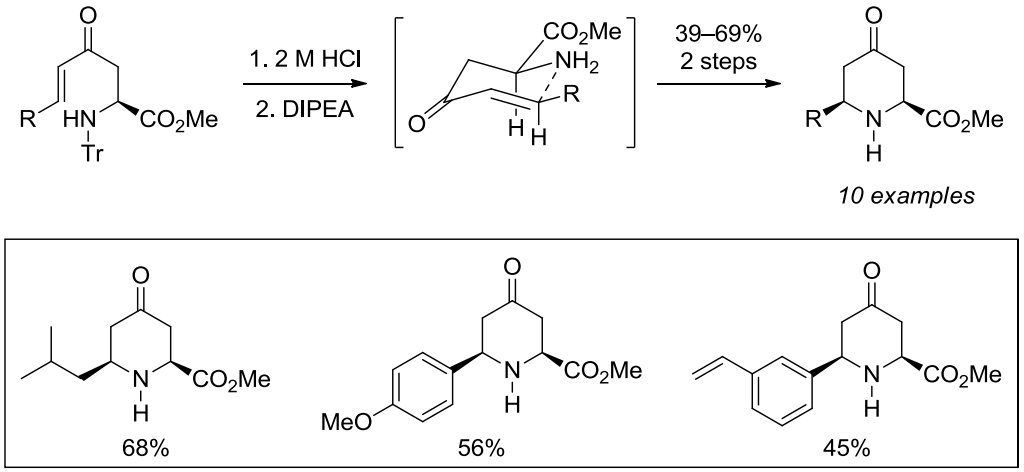
3.1 Previous Work in the Sutherland Group

Previous work carried out by Lindsay Fowler showed the first stereoselective synthesis of 2,6-*trans*-6-substituted-4-oxo-L-pipecolic acids using a tandem reductive amination/6-*endo-trig* cyclisation (Scheme 52).[127,67] The sequential reduction and cyclisation mediated by sodium cyanoborohydride allowed the preparation of a series of highly functionalised 6-alkyl and 6-aryl analogues. During the 6-*endo-trig* cyclisation, it was proposed that the enones adopted a Zimmerman-Traxler chair-like transition state with both the R and *N*-benzyl groups in equatorial positions.[128]



Scheme 52: Reductive amination/6-*endo-trig* cyclisation.

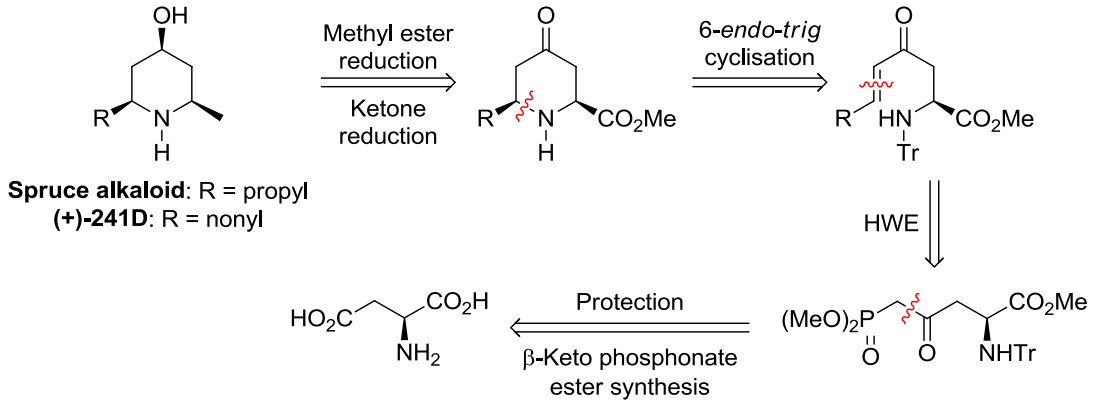
Following on from this portion of work, Mark Daly managed to develop conditions that could switch the stereochemical outcome of the 6-*endo-trig* cyclisation to provide an efficient synthesis of 2,6-*cis*-6-substituted-4-oxo-L-pipecolic acids (Scheme 53).[129,130] A more direct intramolecular conjugate addition reaction took place that proceeded under basic conditions. Using a free amine, it was believed an alternative Zimmerman-Traxler chair-like conformer was generated with the R and methyl ester groups occupying pseudoequatorial positions, which essentially controlled the cyclisation. Molecular modelling of this cyclisation allowed some insight as to how these compounds were formed.



Scheme 53: Base mediated 6-*endo-trig* cyclisation.

3.2 Proposed Research

The aim of this research was to showcase the previously developed base-mediated 6-*endo-trig* cyclisation as a key-step in the synthesis of 4-hydroxy-2,6-disubstituted piperidines natural products (Scheme 54).[129] Spruce alkaloid and (+)-241D were the targeted natural products, as both contain a 2,4,6-*cis* substitution pattern which could be easily accessed using the 6-*endo-trig* cyclisation followed by subsequent functional group manipulations.

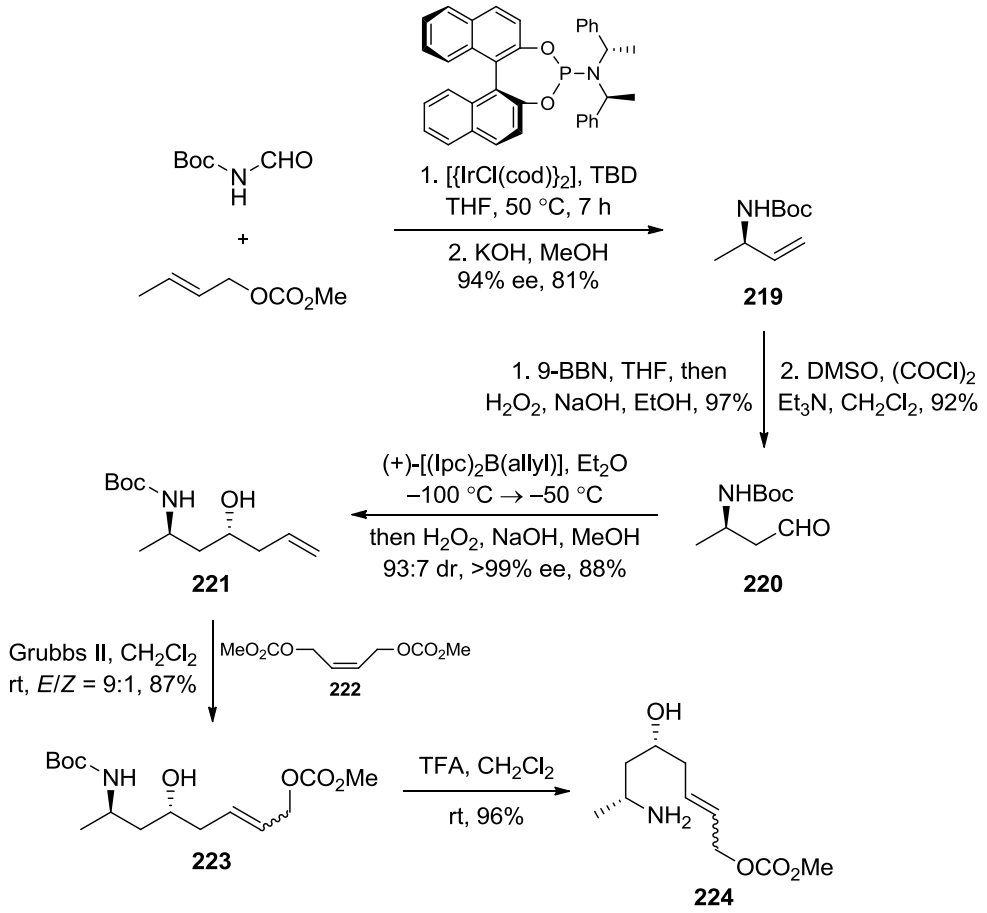


Scheme 54: Retrosynthesis of natural products spruce alkaloid and (+)-241D.

On optimisation of cyclisation conditions, the next challenge would be the reduction of the methyl ester to the methyl group with as few synthetic steps as possible. This was to ensure that a rapid and efficient synthetic route was possible to these natural products with a low step count and high overall yield. The scope of this cyclisation would be explored further to discover if more complex frameworks and natural products intermediates could be accessed.

3.3 Previous Synthesis of Spruce Alkaloid

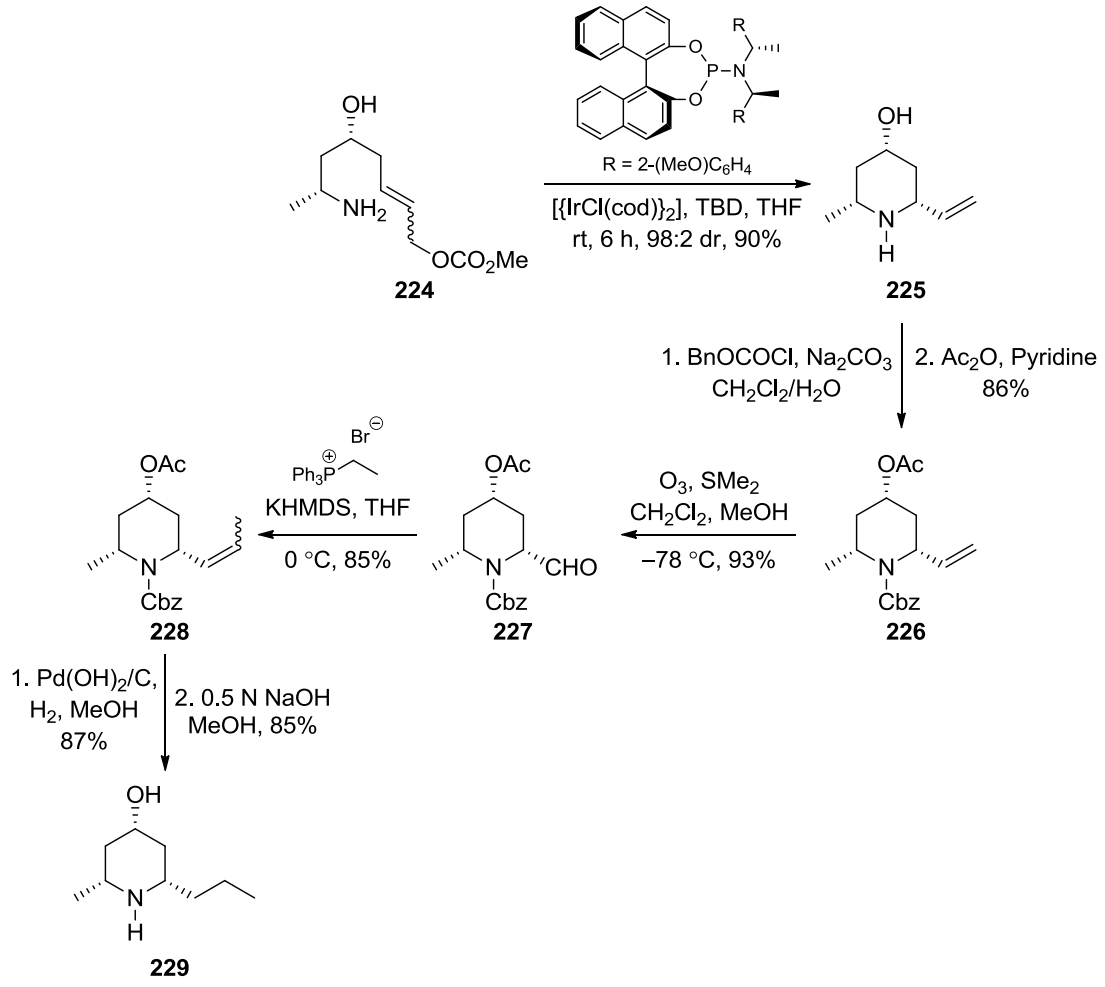
Spruce alkaloid was identified as a trace alkaloid in extracts from the Colorado blue spruce, *Picea pungens* by Stermitz and co-workers.[131] The structure and relative configuration was determined via NOESY and by comparing NMR spectra with other known alkaloids with similar structures. The only previous synthesis of spruce alkaloid was carried out by Gnamm *et al*.[132,133] The total synthesis started with the known iridium-catalysed amination of *trans*-crotyl methyl carbonate with *N*-Boc formamide (Scheme 55).[134] Treatment with potassium hydroxide removed the formyl group to furnish Boc-protected amine **219**. Hydroboration/oxidation and subsequent Swern oxidation produced aldehyde **220**. (+)-*B*-Allyldiisopinocampheylborane was employed at low temperatures to give a 93:7 mixture of homoallylic alcohols *anti*-**221** and *syn*. The desired alcohol *anti*-**221** was then subjected to a cross-metathesis reaction with biscarbonate **222** to yield a 9:1 *E*/*Z* mixture of allylic carbonate **223**. Cleavage of the Boc protecting group gave cyclisation precursor **224**.



Scheme 55: Synthesis of cyclisation precursor **224**.

The next stage of the synthesis was initiated with another iridium-catalysed cyclisation of amine **224** which proceeded smoothly to form compound **225** in 90% yield and dr of 98:2 (Scheme 56). Acetyl and Cbz protecting groups were employed to protect the secondary alcohol and amine respectively to give carbamate **226**. Ozonolysis was used to cleave the alkene to form aldehyde **227**.

A Wittig olefination was employed to introduce the two extra carbon atoms needed for the propyl chain found in the natural product. Alkene **228** was subjected to hydrogenation conditions which removed the Cbz protecting group and reduced the alkene. Finally, basic conditions were used to remove the acetyl protecting group to yield the natural product, spruce alkaloid **229**. The group also managed to synthesise (+)-241D from compound **226**. Cross metathesis with 1-nonene, hydrogenation and treatment with sodium hydroxide produced (+)-241D. This fourteen-step synthesis of spruce alkaloid was possible in a 24% overall yield. Excellent selectivity was observed for the iridium-catalysis steps and homoallylic alcohol formation steps. However, the reagents needed to perform these asymmetric induction reactions are very expensive and difficult to handle, particularly the air sensitive iridium catalyst. There were no examples of selective substrate controlled reactions and there were a number of protecting group removal and reprotection steps which was detrimental to the step count and overall yield.

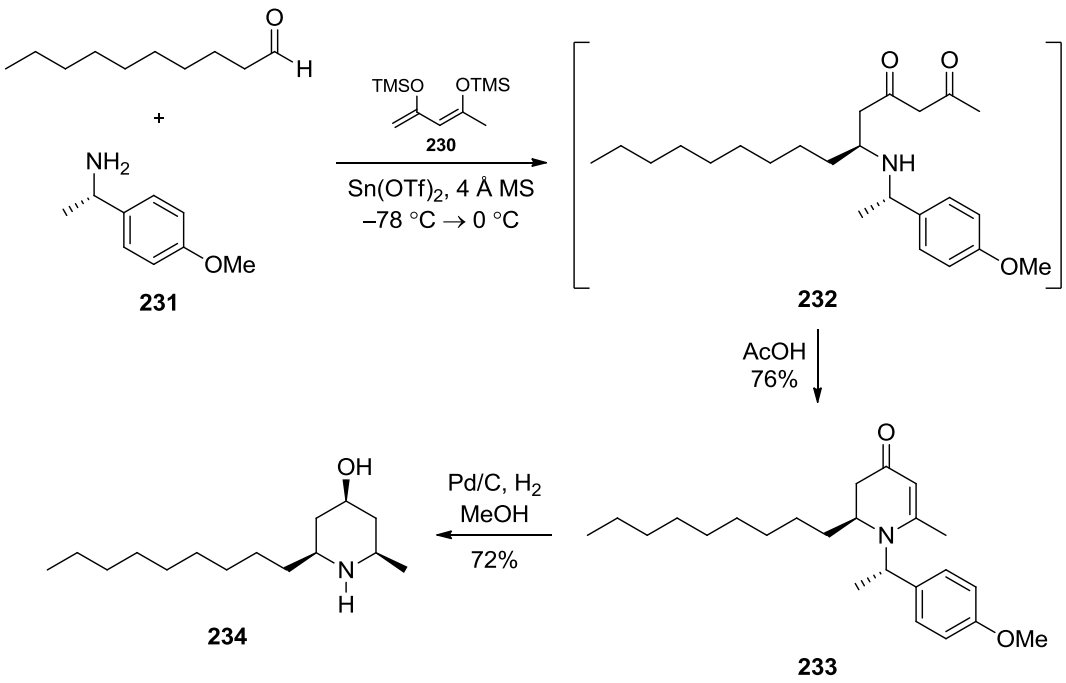


Scheme 56: Synthesis of spruce alkaloid **229**.

3.4 Previous Syntheses of (+)-241D

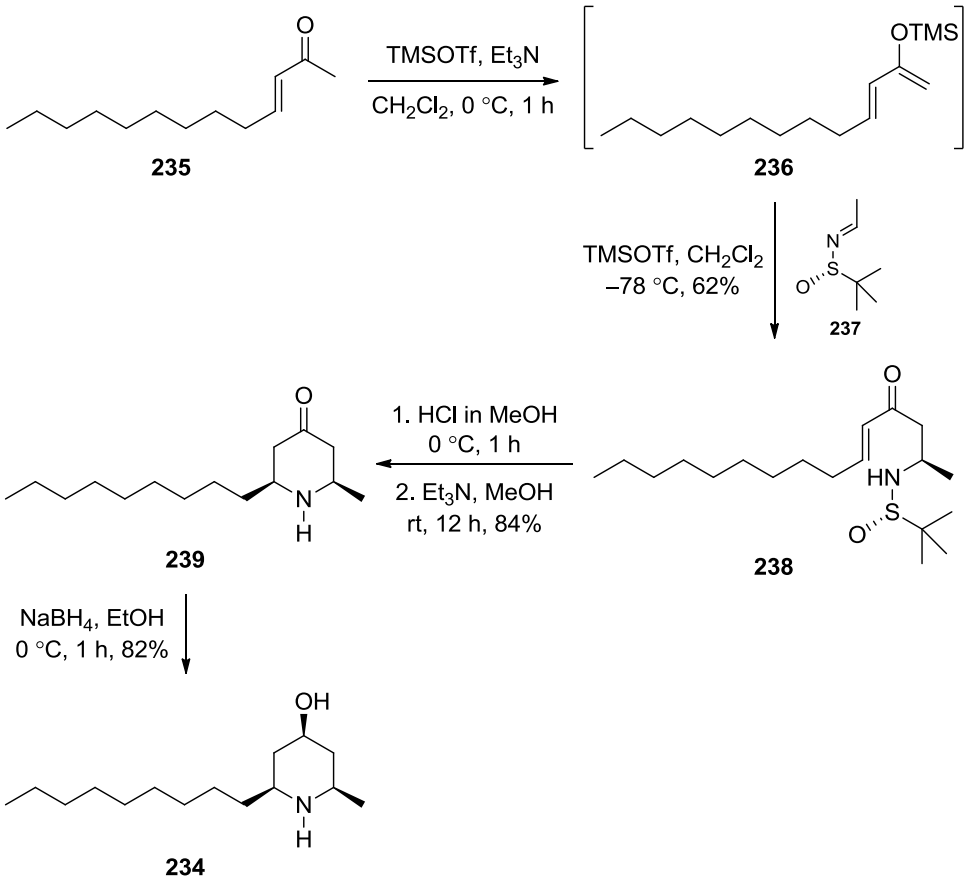
Alkaloid (+)-241D was isolated by Daly and co-workers, from skin extracts of *Dendrobates speciosus*, a rare poison frog located in the highlands of western Panama.[135,136] A racemic synthesis was carried out by Daly and his group a few years later and the compound was found to be a potent inhibitor of the binding of [^{3}H]perhydrohistrionicotoxin to nicotinic acetylcholine receptor channels.[137] Since then (+)-241D has become a very popular natural product, with numerous groups reporting their total synthesis of this small 4-hydroxy-2,6-disubstituted piperidine alkaloid.[138–144] Troin and co-workers reported the first enantioselective synthesis of (+)-241D and the C-4 epimer.[145] He reported another total synthesis of this natural product years later to showcase the scope and limitations of the diastereoselective preparation of 2,6-*cis* and 2,6-*trans* disubstituted piperidines through an intramolecular reaction of chiral β'-carbamate-α,β-unsaturated ketones.[112]

A recent synthesis of (+)-241D was reported by Yang.[146] A general method to assemble multi-substituted chiral piperidines was developed which was inspired by the biosynthesis of piperidine natural products. A stereoselective three-component vinylogous Mannich-type reaction (VMR) of 1,3-bis-trimethylsilyl enol ether **230** was investigated (Scheme 57). To ensure stereoselective control in the VMR, the commercially available chiral α-methyl benzylamine **231** was used to form chiral aldimines *in situ*. The three-component VMR between benzylamine **231**, bis-trimethylsilyl enol ether **230** and decanal proceeded smoothly to form acyclic intermediate **232**, which upon treatment with a catalytic amount of acetic acid cyclised to produce the desired chiral adduct **233** in 76% yield. Palladium catalysed hydrogenation was used to remove the chiral auxiliary and reduce the enone to the corresponding saturated alcohol, completing the total synthesis of (+)-241D **234**. This is a very concise two-stage synthesis of (+)-241D, with an overall yield of 55%. A number of other alkaloid natural products were generated using this protocol such as (−)-241D, isosolenopsin A and (−)-epimyrtine.



Scheme 57: Yang's synthesis of alkaloid (+)-241D **234**.

The most recent synthesis of (+)-241D was carried out by Prasad and co-workers.[147] They reported the synthesis of β-amino-substituted enones with excellent selectivity, via the addition of silyl enol ethers to chiral sulfinimines. Further synthetic manipulations led to the total synthesis of several natural products. The synthesis of (+)-241D began with enone **235** which was transformed into the corresponding silyl enol ether **236** and immediately treated with sulfinimine **237** to form β-sulfinamido ketone **238** in 62% yield (Scheme 58). Acidic conditions were required to remove the sulfinyl group. Upon cleavage, TEA was used to induce the cyclisation to form piperidinone **239** in 82% yield as a single diastereomer. Standard conditions to reduce the ketone were employed to furnish (+)-241D **234**. An overall yield of 43% was achieved for this three-step total synthesis of (+)-241D. The synthesis of (+)-lasubine II and (−)-lasubine I were also described. A surprising aspect surrounding this report was the result obtained for the cyclisation reaction. Prasad's group only observe a single diastereomer **239** and with a high isolated yield of 82%. The only functional group providing any facial selectivity is the methyl group. It is highly unlikely that the small group would induce diastereoselectivity of this magnitude. Inspired by this finding, an attempt to recreate this highly diastereoselective cyclisation, particularly the base-mediated step, in our laboratory was unsuccessful. Only a 1:1 dr was achieved after numerous attempts. These findings will be described in detail later in this chapter.

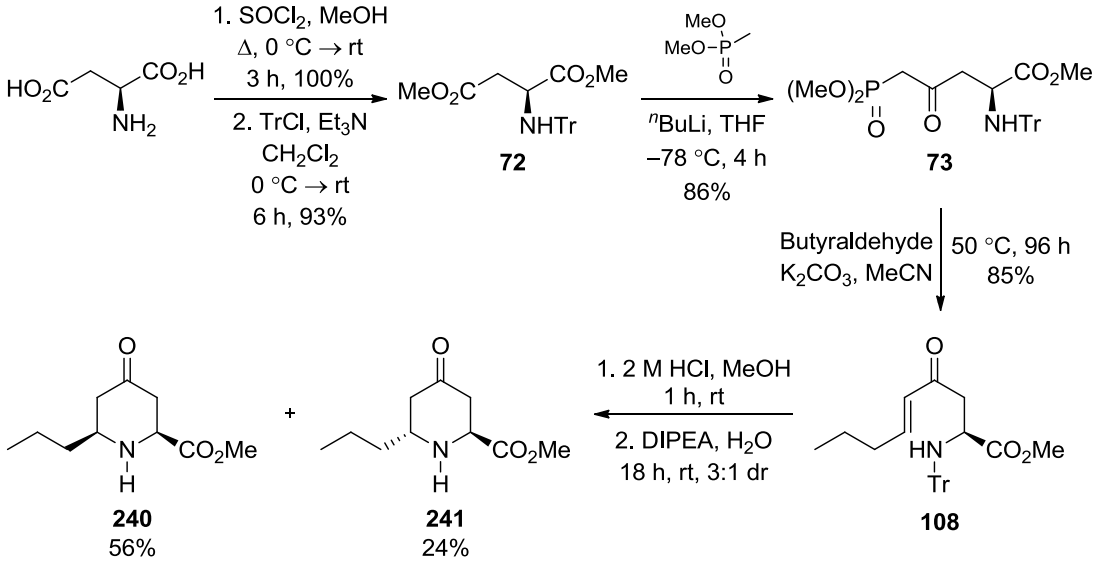


Scheme 58: The Prasad group synthesis of alkaloid (+)-241D **234**.

3.5 Towards the Total Synthesis of Spruce Alkaloid

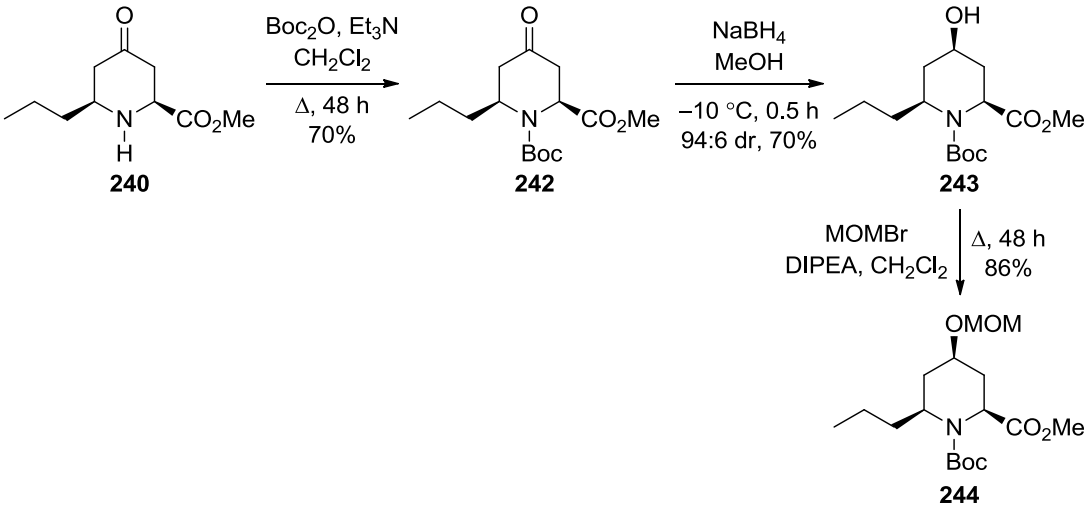
3.5.1 First Approach Towards the Total Synthesis of Spruce Alkaloid

The first stage towards the total synthesis of spruce alkaloid was to use the previously developed β-keto phosphonate ester synthesis followed by the HWE reaction to install the appropriate alkyl side-chain. The synthesis began with L-aspartic acid which was protected to form dimethyl ester hydrochloride salt (Scheme 59). Amine protection followed to give *N*-trityl **72**. β-Keto phosphonate ester **73** was then synthesised and used as a substrate for the HWE reaction with butyraldehyde, producing *E*-alkene **108** in an excellent 85% yield. This compound was then treated with 2 M hydrochloric acid to remove the acid labile trityl group, forming the HCl salt.[129] The reaction mixture was basified (pH 8/9) by addition of Hünig's base. This induced the 6-*endo*-*trig* cyclisation to produce *cis*-piperidinone **240** and *trans*-piperidinone **241** as a 3:1 mixture of diastereomers. The major product of this stereoselective 6-*endo*-*trig* cyclisation, the desired *cis* diastereomer **240** was isolated in 56% yield. The synthesis of this compound had previously been developed by Mark Daly.[129,130] Compounds **240** and **241** ^{1}H and ^{13}C NMR spectra were an exact match to the data already published in the Sutherland group.



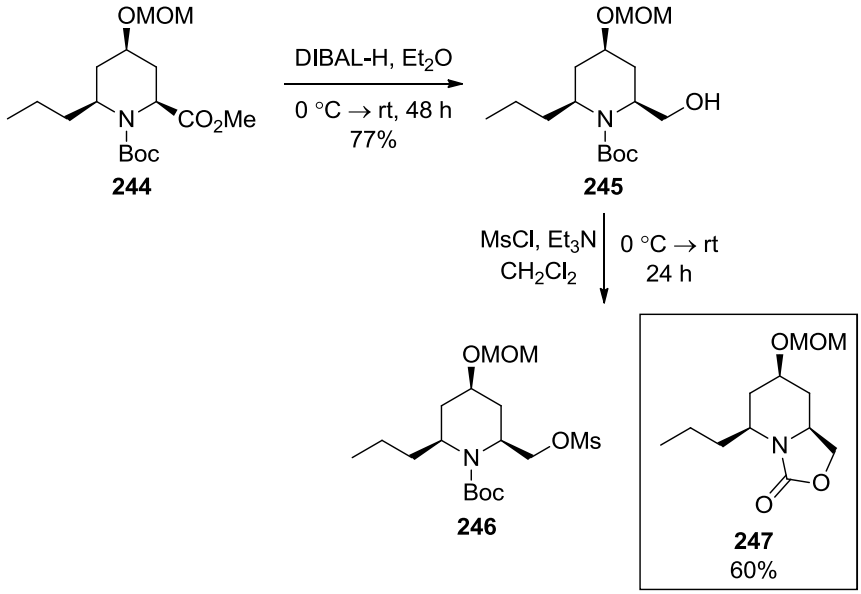
Scheme 59: Synthesis of cyclised piperidinone derivatives **240** and **241**.

With the desired *cis*-piperidinone **240** available, the next stage was to reduce the ketone to the alcohol. Secondary amine **240** was Boc-protected, furnishing *N*-Boc piperidinone **242** in 70% yield (Scheme 60). The following step involved stereoselective reduction of the ketone to the corresponding alcohol. Substrate control and class of reducing agent was used to dictate the facial selectivity of the reductant. In this case sodium borohydride was chosen and the reaction was carried out at −10 °C in methanol to produce 4-hydroxy-2,6-disubstituted piperidine **243** with high diastereoselectivity (94:6 dr). Although no selective NOE experiments were used to confirm the relative stereochemistry of **243**, work reported by Renault shows that a *tert*-butyl ester analogue of **243** was reduced using the same conditions to form the corresponding 4-hydroxy-2,6-disubstituted piperidine with a 9:1 dr and in 84% yield.[148] Due to the similar result obtained in our laboratory, we were satisfied that the relative stereochemistry was correct. The resulting secondary alcohol **243** was protected using bromomethyl methyl ether in the presence of DIPEA to yield *O*-MOM protected piperidinol **244**.



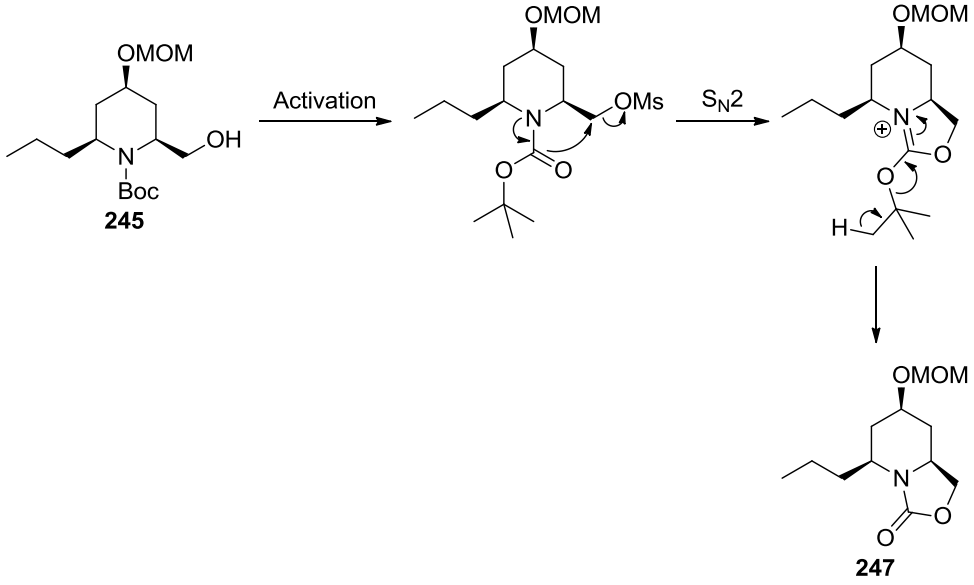
Scheme 60: Synthesis of protected 4-hydroxy-2,6-disubstituted piperidine **244**.

As all the stereocentres were set in the correct configuration for the natural product, the next stage was reduction of the methyl ester to the methyl group before removal of the protecting groups to complete the total synthesis of spruce alkaloid. Attempts to directly reduce the methyl ester to methyl group were unsuccessful. For example, attempted reduction of **244** with $B(C_6F_5)_3$ and triethylsilane showed no reaction. Reduction with $B(C_6F_5)_3$ of aldehydes, acyl chlorides, esters and carboxylic functional groups with hydrosilanes was reported by Gevorgyan and Yamamoto.[149,150] These reports only show examples of this methodology working with simple substrates, not complex natural product intermediates such as **244**. Instead, methyl ester **244** was treated with two equivalents of diisobutylaluminium hydride (DIBAL-H) which effectively reduced the ester to the corresponding primary alcohol **245** in 77% yield (Scheme 61). Attempts to tosylate the resulting alcohol only returned starting material. A possible reason is due to steric hinderance. The chair-like conformation of the 6-membered ring may bring both bulky protecting groups (Boc and MOM) in close proximity to the primary alcohol, restricting access to it. It was decided instead to use the smaller mesyl chloride reagent. Alcohol **245** was treated with mesyl chloride and TEA for 24 h. Analysis of the reaction by NMR spectroscopy revealed that none of the desired mesylate **246** was present however, there was a major product, which was isolated. The ^{1}H NMR spectrum of this product showed that there was no singlet *tert*-butyl peak from the Boc group present. This unknown compound was fully characterised and analysis showed that a cyclisation had taken place to form carbamate **247** in 60% yield.



Scheme 61: Attempted mesylation which led to cyclised carbamate **247**.

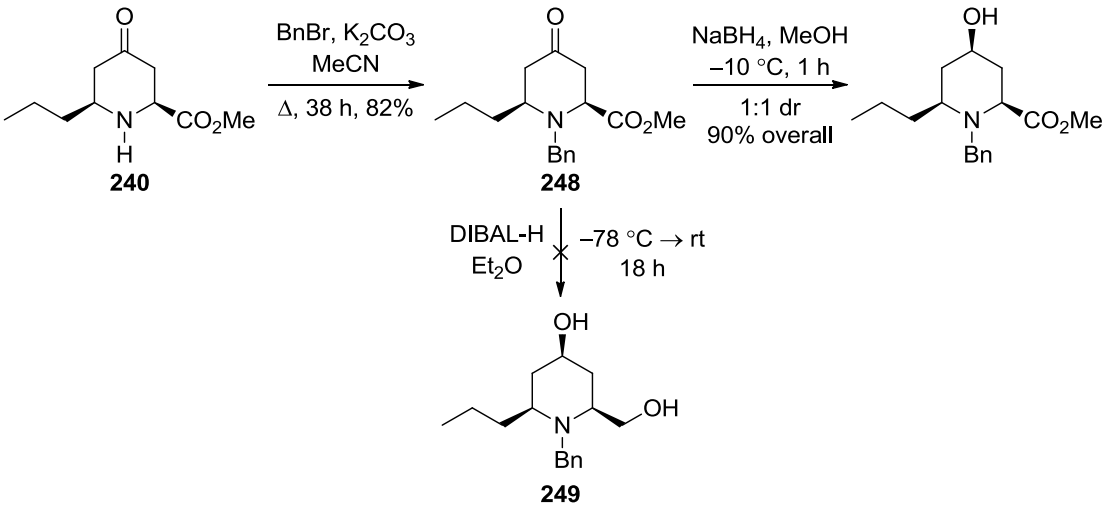
A plausible mechanism for this unexpected cyclisation was proposed involving a S_N2 intramolecular displacement (Scheme 62). Benedetti and co-workers have shown that when the hydoxy group of chiral *N*-Boc-β-amino alcohols is converted into a suitable leaving group, cyclisation to oxazolidinones can take place with inversion of configuration.[151] Gao and Renslo applied this reaction for the practical synthesis of differentially protected 2-(hydroxymethyl)piperazines.[152] Primary alcohol **245** was activated and intramolecular S_N2 displacement by the carbonyl oxygen of the neighbouring carbamate took place to form oxazolidinone **247**, after concomitant loss of isobutene.



Scheme 62: Proposed intramolecular cyclisation mechanism.

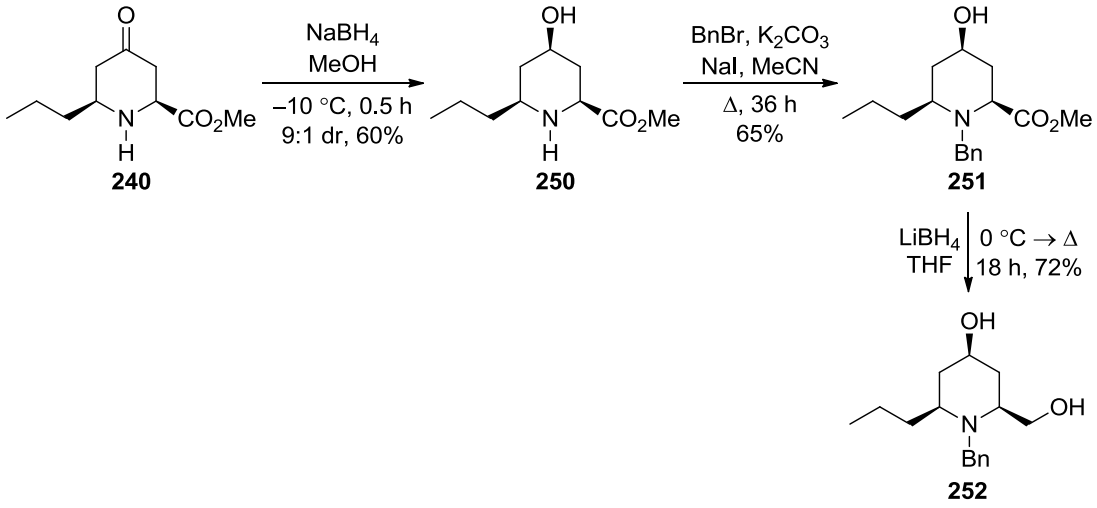
3.5.2 Revised Approach Towards the Total Synthesis of Spruce Alkaloid

Due to this outcome, an alternative approach was developed. A major change in this route was proposed, which involved switching the secondary amine protecting group from a carbamate to a benzyl group. This was in order to avoid any unwanted side-reactions. The 2,6-*cis* piperidinone **240** was protected with the benzyl group on treatment with benzyl bromide under basic conditions (Scheme 63). This gave **248** in 82% yield. An attempt to reduce both the ketone and methyl ester groups to diol **249** was unsuccessful after reaction with DIBAL-H. A complex mixture of products was observed after serval attempted reactions. Sodium borohydride in methanol was then employed to reduce the ketone however, poor diastereoselectivity was observed and an inseparable mixture of diastereomers was produced.



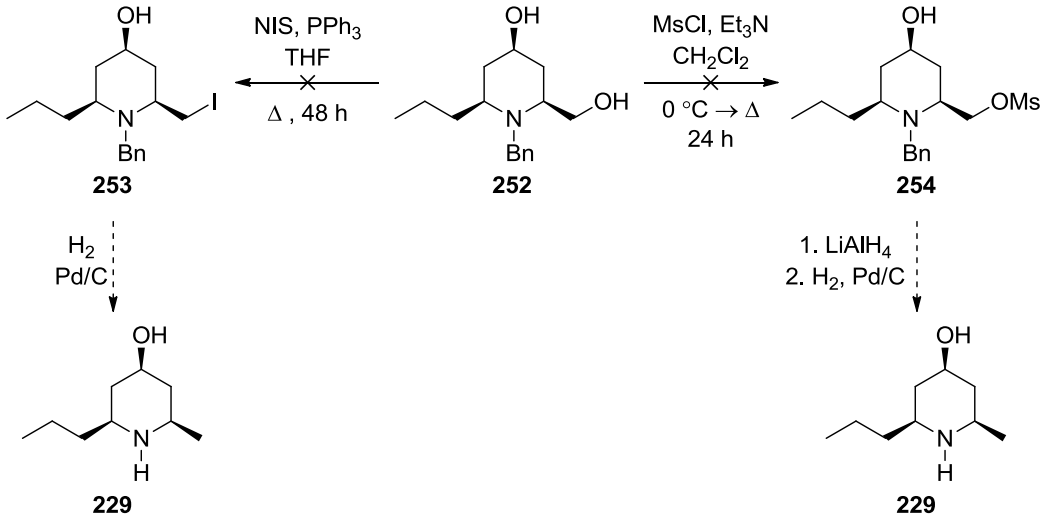
Scheme 63: *N*-Benzyl protection and unsuccessful ketone/ester reductions.

Due to the lack of selectivity of ketone reduction using the *N*-benzyl protecting group, a change in the reaction sequence was investigated. Directly reducing ketone **240** led to secondary alcohol **250** in 60% yield with a 9:1 dr (Scheme 64). Although no selective NOE experiments were used to confirm the relative stereochemistry of **250**, work previously developed in the Sutherland group reported the stereoselective reduction of an isopropyl analogue of **250** using sodium tris(acetoxy)borohydride to produce the corresponding 4-hydroxy-2,6-disubstituted piperidine with similar results.[129,130] Conditions to install the benzyl group were employed, leading to *N*-benzyl **251**. Lithium borohydride was then used to reduce methyl ester **251** to the corresponding primary alcohol **252**, in 72% yield.



Scheme 64: Synthesis of diol **252**.

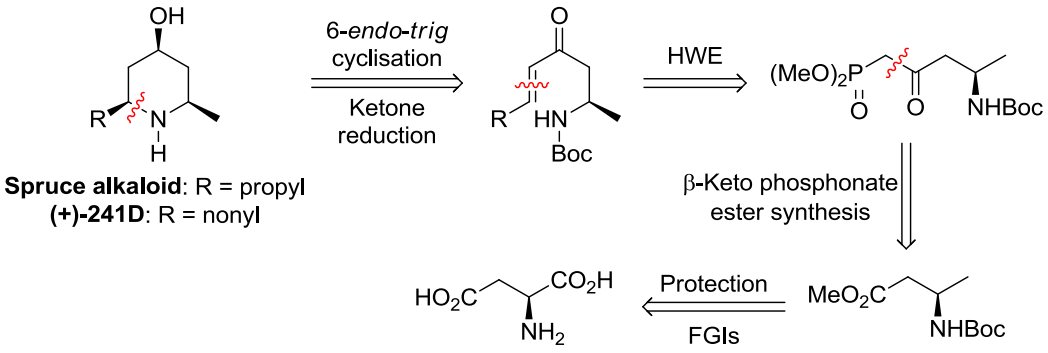
With a route to diol **252** now established, the next step was to selectively transform the primary alcohol into a leaving group in the presence of the secondary alcohol.[153] The first proposed approach was the conversion of primary alcohol **252** to iodide **253** using the Appel reaction. The newly iodinated compound would then be subjected to catalytic hydrogenation conditions to generate the methyl group as well as remove the benzyl protecting group.[154,155] This would complete the total synthesis of spruce alkaloid **229**. Unfortunately the iodination was unsuccessful and no reaction took place (Scheme 65). Switching from THF to the higher boiling point solvent, 1,4-dioxane and heating the reaction to 80 °C was not enough to force the reaction. Due to the large size of the resulting phosphonium iodide salt formed as well as the benzyl protecting group being in close proximity to the primary alcohol, steric clashes between the substrate and reagent may have hindered any reaction taking place. With this in mind, a final attempt to transform the primary alcohol into a leaving group was carried out. Standard conditions to mesylate alcohol **252** were attempted but no reaction took place to form mesylate **254**. If successful, lithium aluminium hydride reduction followed by hydrogenation could have completed the total synthesis.[156] Due to the failed attempts to reduce the alcohol to the methyl group, a completely revised synthetic route was devised to work around the impeding reduction issue.



Scheme 65: Unsuccessful attempts to iodinate and mesylate diol **252**.

3.6 Total Synthesis of Spruce Alkaloid and (+)-241D

The next stage of this total synthesis project was to design a new route that would successfully access the desired natural products, spruce alkaloid and (+)-241D. Instead of utilising the 6-*endo*-*trig* methodology early in the synthesis and attempting to perform late stage methyl ester to methyl group reduction, the plan was to form the methyl group during the early stages of the synthesis and then cyclise towards the end of the route (Scheme 66).

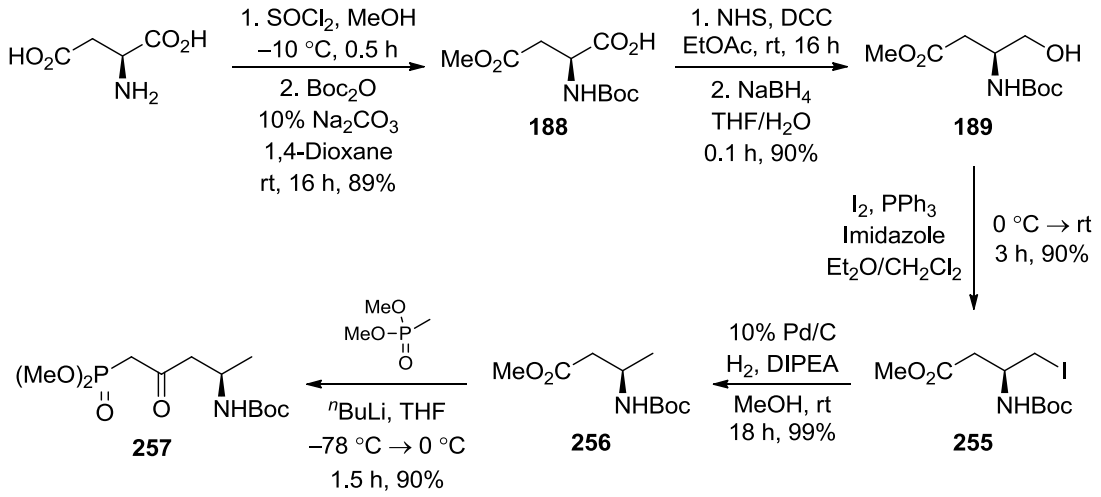


Scheme 66: Retrosynthesis of natural products spruce alkaloid and (+)-241D.

3.6.1 Synthesis of Enones

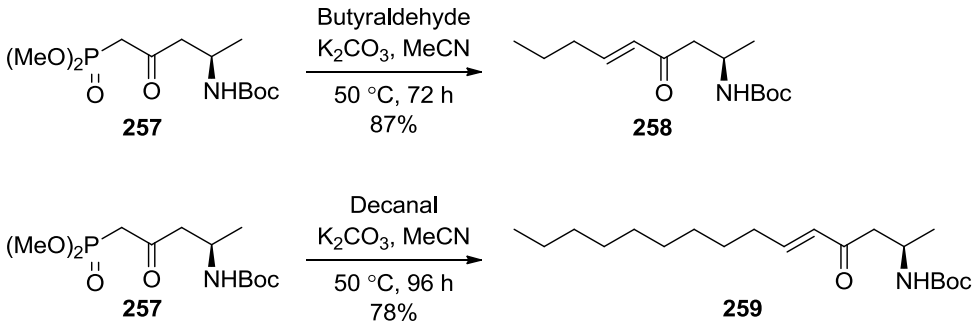
The revised route started with L-aspartic acid which was treated with thionyl chloride and methanol at −10 °C to selectively protect the more accessible β-carboxylic acid (Scheme 67).[107] Subsequent *N*-Boc protection led to 89% yield of **188**.[108] Compound **188** was reacted with *N*-hydroxysuccinimide and *N*,*N*'-dicyclohexylcarbodiimide to form the activated ester, which was rapidly reduced upon treatment with sodium borohydride to yield primary alcohol **189**.[109] Mild Appel reaction conditions were employed to convert alcohol **189** to iodide **255** in 90% yield.[157] An attempt to use the crude material from the iodination reaction for direct hydrogenation was

unsuccessful. It was necessary for iodide **255** to be isolated prior to hydrogenation. Standard catalytic hydrogenation conditions under neutral conditions did not furnish the desired methyl derivative **256**. Hydrogenation under pressure was also unsuccessful. Reports in the literature state that in order to cleave an alkyl C-I bond, the presence of base is pivotal for the reaction to proceed.[154,155] It was proposed that the formation of HI poisons the palladium catalyst rendering it inactive. Under basic conditions, the HI is neutralised allowing the reaction to proceed. Therefore, Hünig's base was added to the reaction mixture, which allowed clean formation of methyl derivative **256** in an excellent 99% yield. Formation of the β-keto phosphonate ester **257** was carried out using a previously developed protocol.[111,112] An efficient 6-step synthesis of the β-keto phosphonate ester **257** was optimised with an overall yield of 64%.



Scheme 67: Synthesis of β-keto phosphonate ester **257**.

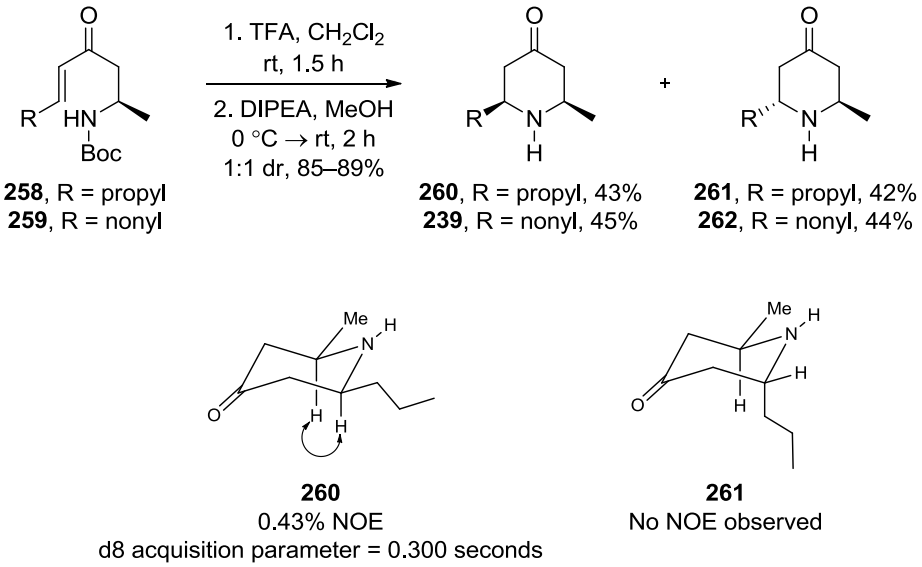
With the β-keto phosphonate ester **257** available, the next phase of this project was to synthesise *E*-enones that have the required alkyl side-chains that correspond to the targeted natural products. Therefore, butyraldehyde was used in the first HWE reaction and an 87% yield of enone **258** was achieved (Scheme 68). Enone **259** was synthesised in 78% yield from the HWE reaction between β-keto phosphonate ester **257** and decanal.



Scheme 68: HWE reaction conditions and synthesis of enone derivatives **258** and **259**.

3.6.2 6-*Endo*-*Trig* Cyclisation of Enones 258 and 259

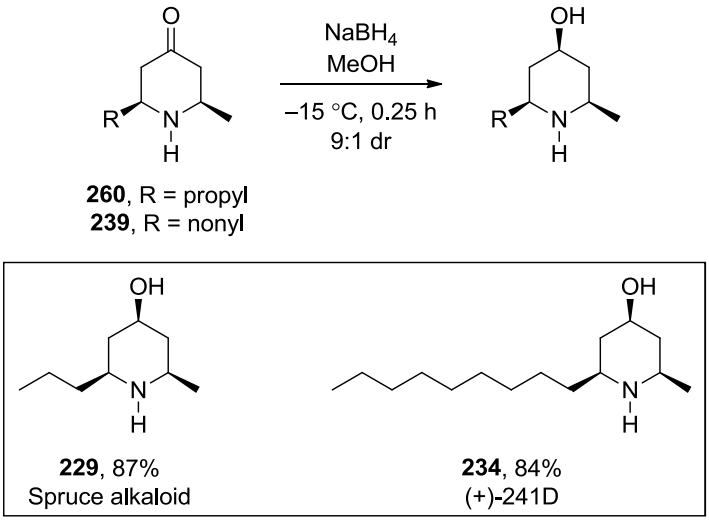
The problematic step in the earlier attempts to synthesise the natural products was the formation of the methyl group at a late stage in the synthesis. One of the main objectives of the revised route was to efficiently access methyl-derived enones **258** and **259** as early as possible. The final key-steps in the total synthesis would be the 6-*endo*-*trig* cyclisation followed by ketone reduction to produce the natural products. With enones **258** and **259** available, the next step was to apply the 6-*endo*-*trig* cyclisation that had previously been developed in the Sutherland group.[129] Enones **258** and **259** were treated with TFA, allowing clean Boc protecting group removal and formation of the TFA salt intermediates (Scheme 69). The crude material was then dissolved in methanol and DIPEA was added to induce the 6-*endo*-*trig* cyclisation. Propyl derivatives **260** and **261** were synthesised with good isolated yields of 43% for the *cis* and 42% for the *trans* diastereomer. There have been no reports in the literature regarding the synthesis of these two compounds, therefore selective NOE experiments were carried out to confirm the relative configuration of each diastereomer. The selective NOE spectrum confirmed that compound **260** was in fact the *cis* diastereomer and no NOE enhancement was observed for **261** which was predicted due to the hydrogen atoms not being in close proximity to one another. Similar yields were obtained for the nonyl derivatives. *Cis*-**239** was isolated in 45% yield and *trans*-**262** in 44% yield. Compound **239** has been previously synthesised by Hurvois and co-workers.[144] The ^{1}H and ^{13}C NMR spectra recorded in our laboratory matched the literature data exactly. Unfortunately there was no substrate controlled stereoselectivity observed for both compounds. The previously described method (Scheme 59) had a larger methyl ester substituent that induced the lower energy Zimmerman-Traxler transition state, which led to the *cis* diastereomer predominating. With a smaller methyl group there is no stereoselectivity as a 1:1 dr is observed. However, high isolated yields for both diastereomers were achieved upon optimisation. Although *cis* isomers **260** and **239** would be used to complete the total syntheses, the *trans* compounds **261** and **262** could also be used to access the natural product epimers.



Scheme 69: Base mediated 6-*endo*-*trig* cyclisation of **258** and **259** and selective NOE data.

3.6.3 Stereoselective Reduction

As an efficient cyclisation procedure had been optimised to access the 2,6-*cis* piperidinone derivatives, the final step to complete the total syntheses was the stereoselective reduction of the ketone to the corresponding secondary alcohol. Ketones **260** and **239** were treated with sodium borohydride in methanol at −15 °C (Scheme 70). A substrate and reagent controlled stereoselective reduction took place and a diastereomeric ratio of 9:1 was observed for both reactions. High isolated yields of the natural products were achieved, 87% for spruce alkaloid **229** and 84% for (+)-241D **234**. The ^{1}H and ^{13}C spectra for these compounds matched the literature data for spruce alkaloid[132,133] and (+)-241D,[144] confirming the synthesis of the correct structures and relative stereochemistry.



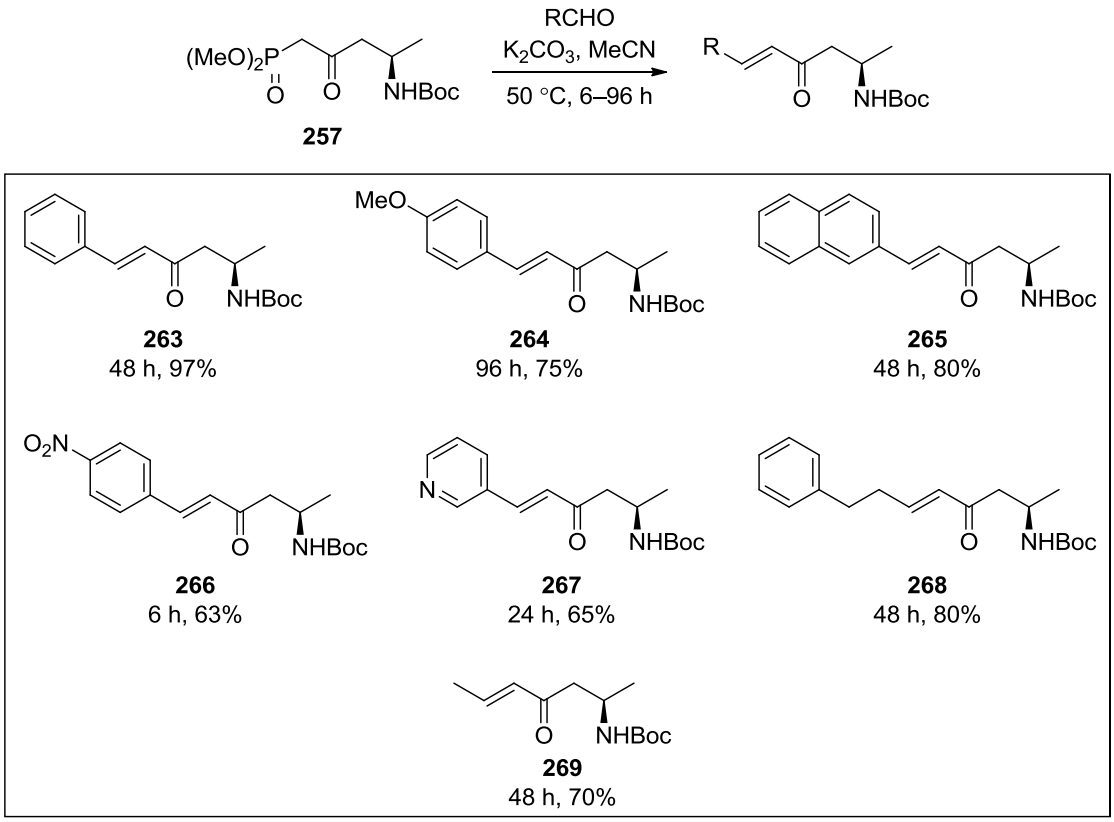
Scheme 70: Stereoselective reduction to spruce alkaloid **229** and (+)-241D **234**.

3.7 Expanding the Scope of 4-Hydroxy-2,6-Disubstituted Piperidine Synthesis

As both natural products had been synthesised successfully, focus turned to the preparation of a small library of cyclised 4-hydroxy-2,6-disubstituted piperidine derivatives. The sole aim of this was to expand the substrate scope of the cyclisation and reduction steps to examine whether various side-chain aryl groups could be tolerated.

3.7.1 Synthesis of Enones

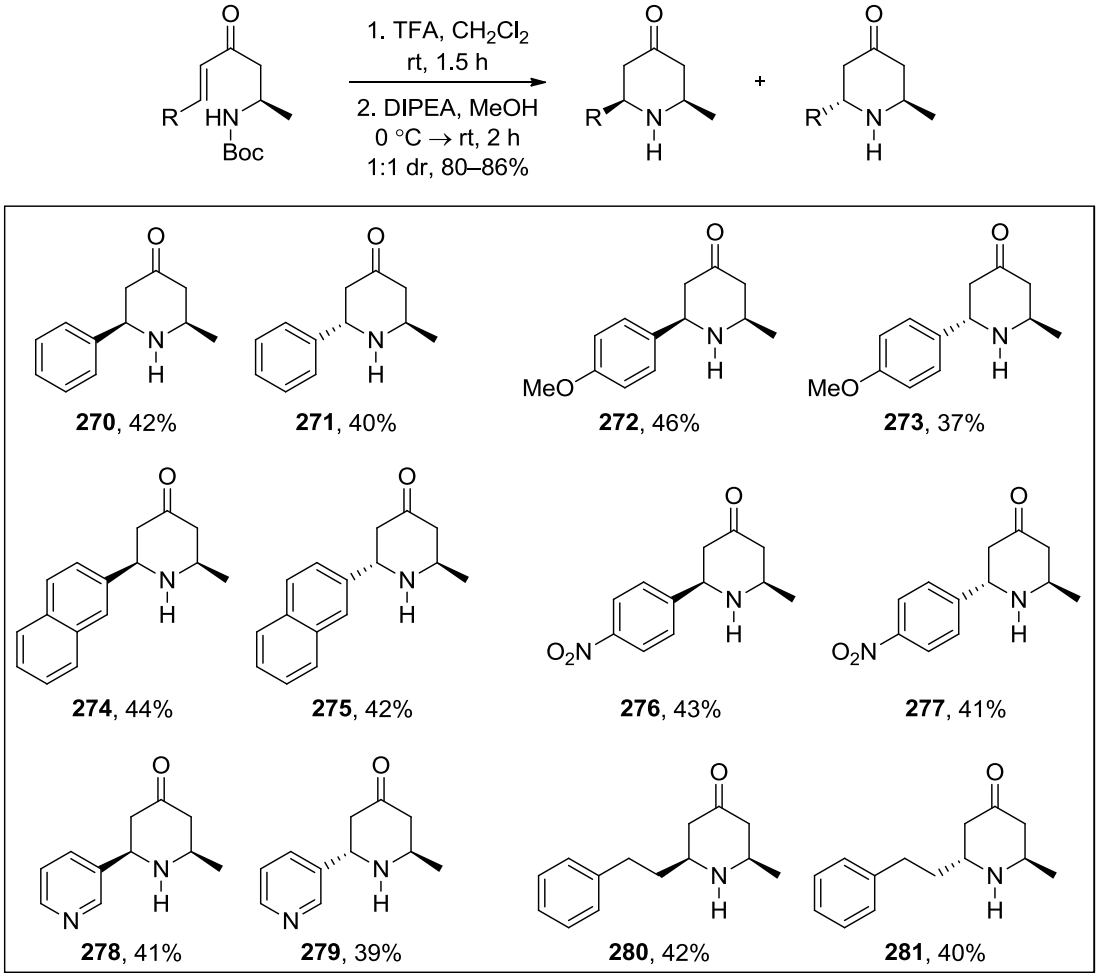
β-Keto phosphonate ester **257** was reacted with a number of aldehydes under the previously optimised HWE reaction conditions (Scheme 71). Various electron-rich and electron-poor analogues were synthesised as well as phenethyl **268** and methyl **269** derivatives. This gave good to high yields for all of the examples and displayed a clear trend between reaction time and electronic nature of the aldehyde used. Electron-poor nitrophenyl **266** and pyridine **267** took 6 h and 24 h respectively. Significantly longer reaction times were required for the more electron-rich phenyl **263**, methoxyphenyl **264** and naphthyl **265** derivatives.



Scheme 71: HWE conditions and seven-membered library of enones.

3.7.2 Scope of 6-*Endo*-*Trig* Cyclisation

With access to a small library of *E*-enones, the next stage was to submit these compounds to the 6-*endo*-*trig* cyclisation conditions (Scheme 72). A 1:1 dr for each example was observed, comparable to the propyl and nonyl side-chain analogues that were used in the natural product syntheses project. Aryl groups were well tolerated and very consistent overall yields of 80–86% were achieved for each example. The isolation of each diastereomer was difficult but after extensive TLC solvent elution investigations, clean separation was possible using column chromatography. Methyl-derived enone **269** was cyclised under the standard conditions giving the two products in a 1:1 dr however, an attempt to separate the diastereomers was unsuccessful.



Scheme 72: Cyclisation conditions and twelve-membered library of 2,6-substituted 4-piperidinone derivatives.

3.7.3 Selective NOE Assignments

Selective NOE experiments were carried out to confirm the relative stereochemistry of each diastereomer. Compound **270** has been previously synthesised by Davis and co-workers.[139] The ^{1}H and ^{13}C NMR spectra recorded in our laboratory matched the literature data exactly. The other 2,6-piperidinones were examined to confirm that the correct diastereomers had been identified (Figure

51). All of the 2,6-*cis* compounds displayed NOE enhancements when each hydrogen atom was individually irradiated confirming their close proximity to one another. No NOE enhancements were observed between the two hydrogen atoms in question for the 2,6-*trans* compounds when each was irradiated respectively. This data provides further confirmation that each diastereomer had been correctly assigned.

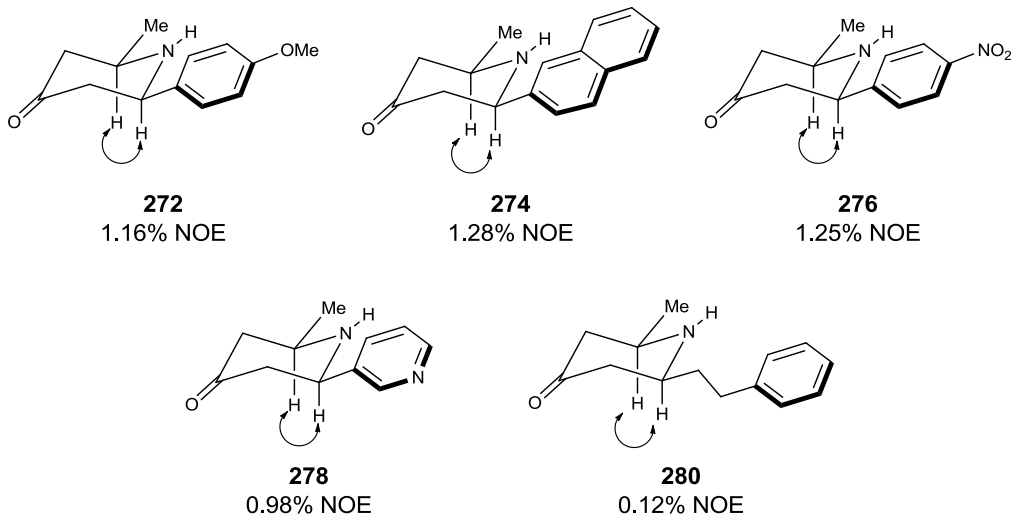
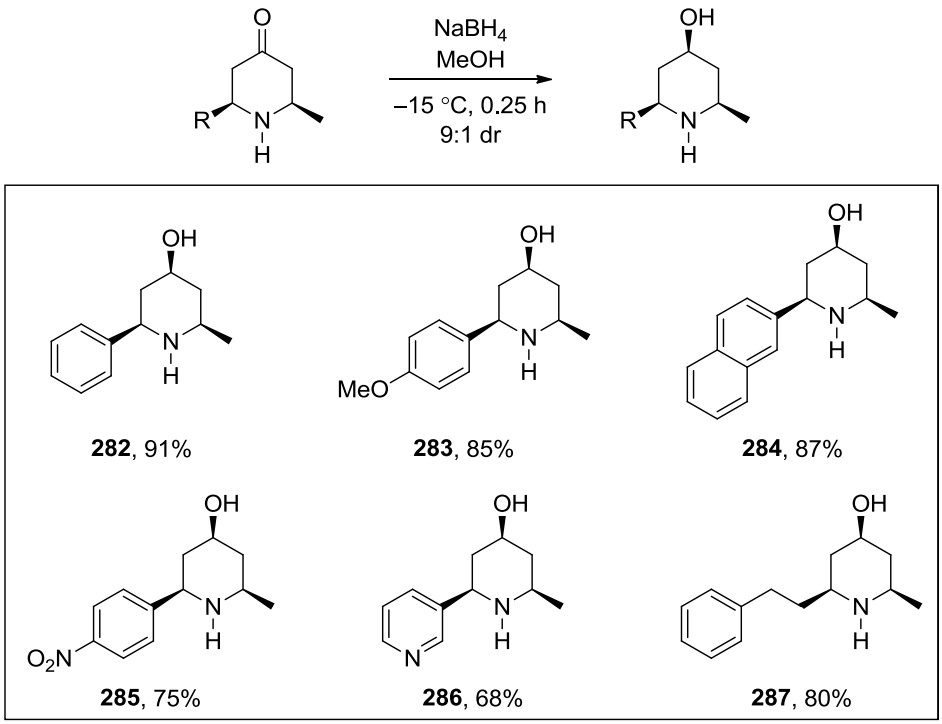


Figure 51: Selective NOE data for the 2,6-*cis* piperidinone library (d8 acquisition parameter for all compounds was 0.300 seconds).

3.7.4 Stereoselective Reduction

The final stage of this investigation was the stereoselective reduction of the 4-piperidinones using the previously developed conditions (Scheme 73). The six-membered library of 2,6-*cis* piperidinones were subjected to these conditions which led to the synthesis of the corresponding 4-hydroxy-2,6-disubstituted piperidine analogues. A very good 9:1 diastereoselectivity was achieved along with isolated yields of 68–91% for the major diastereomers.



Scheme 73: Reduction conditions and six-membered library of 4-hydroxy-2,6-disubstituted piperidine derivatives.

3.7.5 Selective NOE Assignments

Selective NOE experiments were carried out to confirm the relative stereochemistry of each compound. Piperidinol **282** has been previously synthesised by the Ma and Davis groups.[138,158] Compound **287** has also been reported in the literature by Yang.[146] The ^{1}H and ^{13}C NMR spectra recorded in our laboratory matched both sets of literature data. The other 4-hydroxy-2,6-disubstituted piperidines were examined to confirm that the correct diastereomers had been identified (Figure 52). Compounds **283** and **286** displayed distinctive signals in the ^{1}H NMR spectrum for the C-2, C-4 and C-6 hydrogen atoms. Upon irradiation at each hydrogen signal, there were NOE enhancements for the other two hydrogen atoms in close proximity. For compounds **284** and **285**, the hydrogen signals at the 4 and 6 positions overlapped therefore selective NOE was only possible for the C-2 hydrogen atom and the C-4/6 overlap signal. This data establishes further confirmation that each diastereomer had been correctly assigned regarding the relative stereochemistry.

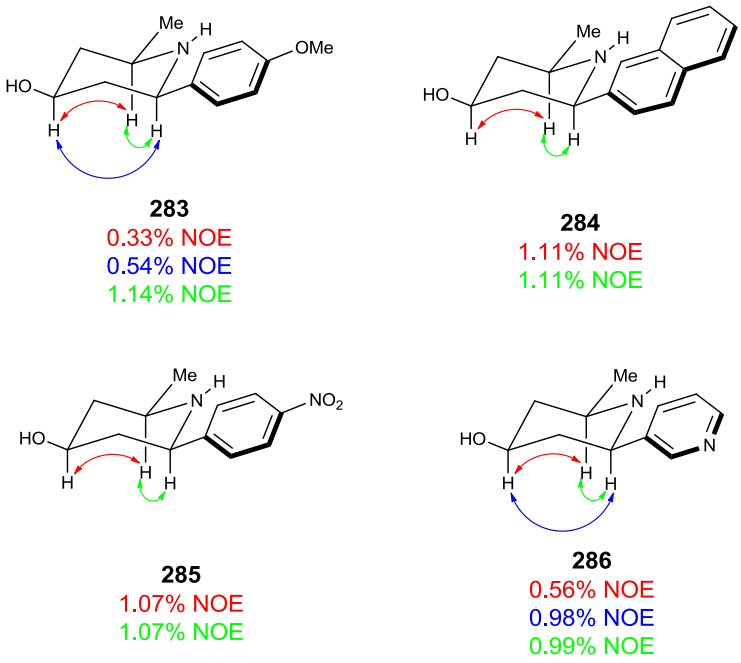
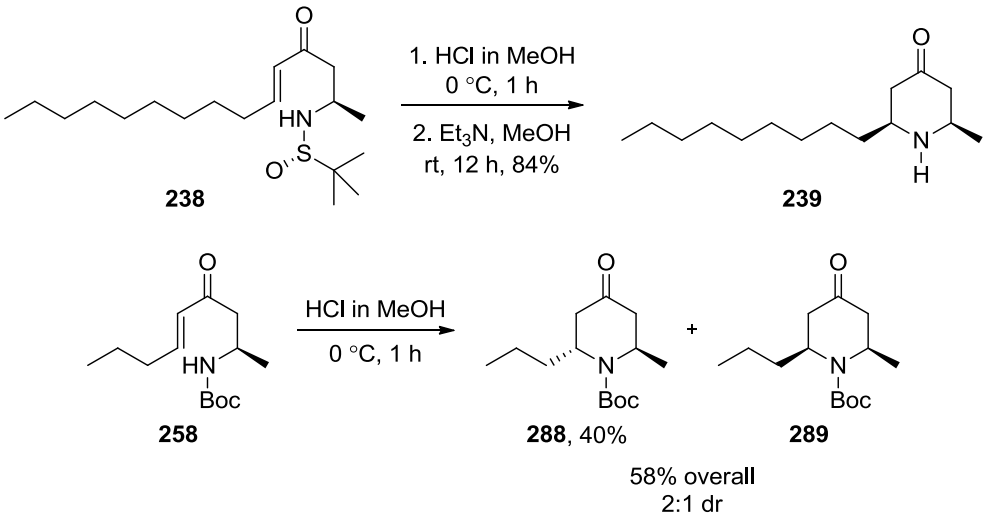


Figure 52: Selective NOE data for the 4-hydroxy-2,6-disubstituted piperidine library (d8 acquisition parameter for all compounds was 0.300 seconds).

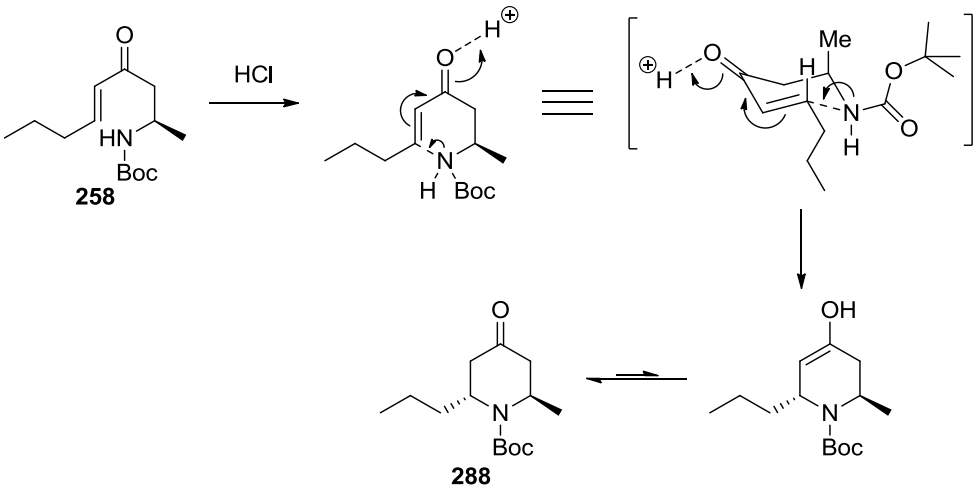
3.8 Stereoselective Acid Mediated 6-*Endo*-*Trig* Cyclisation

While attempting to synthesise natural products spruce alkaloid and (+)-241D, a recent paper by Reddy *et al* reported a highly selective 6-*endo*-*trig* cyclisation that was used in their total synthesis of (+)-241D.[147] β-Sulfinamido ketone **238** was treated with hydrochloric acid in methanol to remove the sulfinyl group (Scheme 74). Neutralisation with TEA induced the 6-*endo*-*trig* cyclisation to produce *cis*-piperidinone **239** as a single diastereomer in 84% yield. In an attempt to replicate this excellent result in our laboratory, enone **258** was subjected to the same reaction conditions as described above, to form the expected *cis*-piperidinone **260**. However, when following the first stage of this reaction via TLC, two new spots started to appear above the starting material. The expected baseline spot indicating that the Boc group had been cleaved and that the HCl salt had formed was not present. ^{1}H NMR spectroscopy clearly showed that the *tert*-butyl singlet peak was still present and that cyclisation had taken place. Silica chromatography was used to isolate both spots together in order to get an accurate diastereomeric ratio and an overall yield. A 6-*endo*-*trig* cyclisation had taken place to form *trans*-piperidinone **288** and *cis*-piperidinone **289** with a 2:1 dr and 58% overall yield. The structure of *cis*-piperidinone **289** was confirmed by comparison with the literature NMR data.[159] Therefore, an acid-mediated 6-*endo*-*trig* cyclisation had converted enone **258** into piperidinone diastereomers but selectively for *trans*-*N*-Boc protected piperidinone **288**. Major compound **288** was then isolated in 40% yield and fully characterised.



Scheme 74: Acid mediated 6-*endo*-*trig* cyclisation.

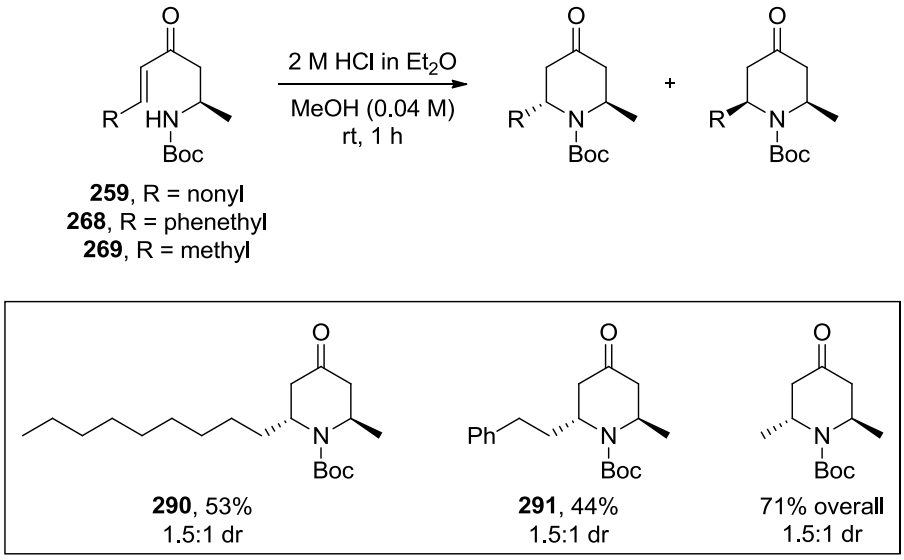
A proposed mechanism for this cyclisation is shown in Scheme 75. A stoichiometric amount of acid was added and likely acted as a Brønsted acid. The proton could have coordinated to the enone oxygen, making the alkene more electrophilic and susceptible to conjugate addition. The 6-*endo*-*trig* cyclisation then took place and a similar Zimmerman-Traxler transition state that was previously described (Scheme 52) induced the diastereoselective nature of this cyclisation. Both the propyl and *N*-Boc groups would prefer equatorial positions, the *N*-Boc group particularly due to its size. The resulting enol would then rapidly tautomerise to its keto form **288**.



Scheme 75: Proposed mechanism for 6-*endo*-*trig* cyclisation.

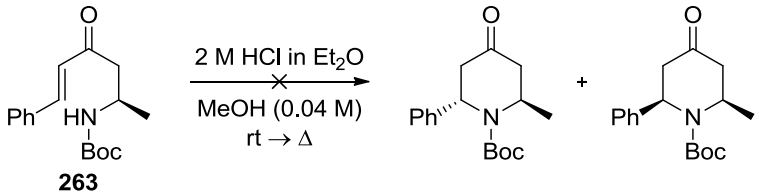
In order to ensure one equivalent of hydrochloric acid was being added, commercially available 2 M ethereal hydrochloric acid was used in subsequent reactions. Several other alkyl side-chain analogues were subjected to these conditions (Scheme 76). Nonyl-derived enone **259** was converted to the corresponding cyclised products with a 1.5:1 dr in favour of *trans*-piperidinone **290** which was isolated in 53% yield. By comparing the ^{1}H NMR spectrum of the crude reaction

mixture with the *cis*-piperidinone that has previously been published, it was clear that the major product was 2,6-*trans* piperidinone **290**.[142] A 1.5:1 dr was observed after phenethyl-derived enone **268** was transformed into the piperidinone analogues. A 44% isolated yield was achieved for the major product, *trans*-piperidinone **291**. A selective NOE experiment was carried out to prove that the isolated compound was *trans*-piperidinone **291**. Upon irradiation of the C-2 and C-6 hydrogen atoms, there were no corresponding NOE enhancements, suggesting they are not in close proximity to one another. This confirms that the relative stereochemistry is correct. Methyl-derived enone **269** was also subjected to the acid mediated cyclisation and a 1.5:1 dr was observed however, attempts to separate the cyclised products via column chromatography were unsuccessful.



Scheme 76: Cyclisation conditions and *trans*-piperidinone major compounds.

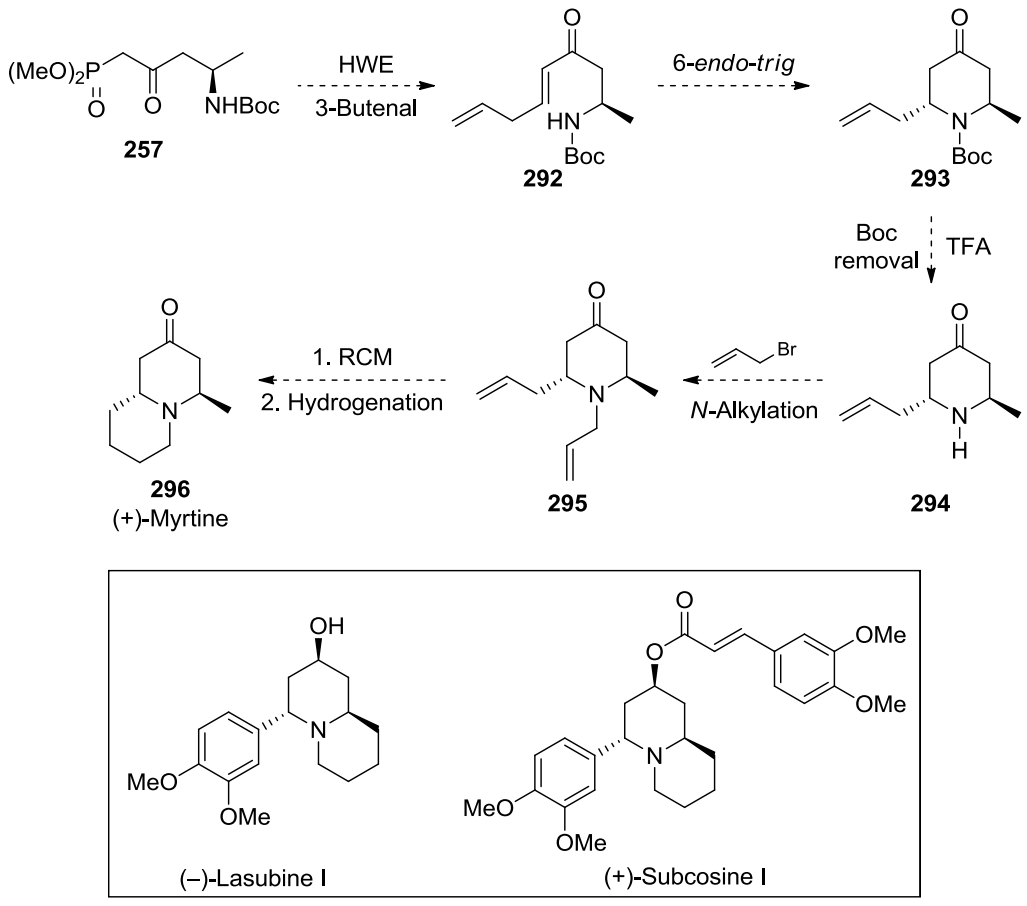
Phenyl-derived enone **263** was also subjected to these conditions in an attempt to investigate whether enones with aryl side-chains would also cyclise (Scheme 77). Unfortunately no reaction took place and when the reaction was heated to try and force the cyclisation to work, Boc protecting group removal ensued. A plausible reason for no reaction occurring for the aryl side-chain analogues is due to more stable, less reactive, highly conjugated aryl enone. Although the sp^2 secondary amino group is nucleophilic enough to undergo conjugate addition with the activated alkyl-derived enones, it appears to not possess the nucleophilicity to disrupt the conjugated aryl-enone, resulting in no reaction taking place.



Scheme 77: Attempt to cyclise aryl-derived enone derivative **263**.

3.9 Future Work

Having successfully utilised the base mediated 6-*endo*-*trig* cyclisation as a key-step in the total synthesis of spruce alkaloid and (+)-241D, the future work in this project will involve the optimisation and application of the acid mediated cyclisation. The substrate scope for this procedure will be thoroughly investigated and natural products with *trans*-piperidinone functionality will be targeted. Natural products such as (+)-myrtine **296** could be accessed using the acid mediated 6-*endo*-*trig* cyclisation as a key-step.[160,161] β-Keto phosphonate ester **257** could be converted to enone **292** using the HWE reaction (Scheme 78). Acid mediated cyclisation would then be used and *trans*-piperidinone **293** isolated. The Boc group would be removed to give secondary amine **294** which would then be alkylated with allyl bromide to form tertiary amine **295**. Ring closing metathesis and hydrogenation would be employed to complete the total synthesis of (+)-myrtine **296**. (−)-Lasubine I[162,163] and (+)-subcosine I[164,165] are also natural products that contain a 2,6-*trans* piperidinone ring system and could also be targeted for total synthesis.



Scheme 78: Proposed synthesis of (+)-myrtine **295** and other potential targets.

3.10 Conclusions

Spruce alkaloid **229** and (+)-241D **234** were synthesised from cheap commercially available starting materials. There are three stereocentres present in these compounds, two of which were constructed from substrate controlled reactions and the third was already present in the chiral pool starting material, L-aspartic acid. After the initial unsuccessful attempts to synthesise spruce alkaloid, a new approach was taken which included formation of the methyl group early on in the synthesis and late stage base mediated 6-*endo*-*trig* cyclisation to construct the 2,6-*cis* piperidinone framework. Throughout the syntheses there was no need for expensive reagents such as catalyst/ligand combinations to generate the stereocentres, or the use of over-elaborate protocols. The syntheses began with L-aspartic acid and a total of nine synthetic steps were required to reach spruce alkaloid **229** and (+)-241D **234** with overall yields of 21% and 19%, respectively. The key-step in the syntheses was the 6-*endo*-*trig* cyclisation. Although no stereoselectivity was observed in favour of the desired *cis* diastereomer during the cyclisation, the high isolated yields for both *cis* and *trans* compounds would allow access to the natural product epimers upon reduction of the *trans* isomer. A small substrate scope was also carried out to display that a variety of substrates could tolerate the cyclisation conditions. A serendipitous result also allowed access to 2,6-*trans* piperidinones diastereoselectively using acidic conditions. Once this procedure has been optimised, a substrate scope will be investigated and the reaction will be used as a key-step in the total synthesis of multiple natural products.

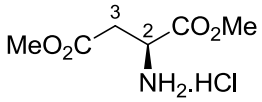
4.0 Experimental

4.1 General Experimental

All reagents and starting materials were obtained from commercial sources and used as received. Dry solvents were purified using a PureSolv 500 MD solvent purification system. All reactions were performed under an atmosphere of argon unless otherwise mentioned. Brine refers to a saturated solution of sodium chloride. Flash column chromatography was carried out using Fisher matrix silica 60. Macherey-Nagel aluminium-backed plates pre-coated with silica gel 60 (UV_{254}) were used for thin layer chromatography and visualised by staining with $KMnO_4$, vanillin or ninhydrin. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX 400 or 500 spectrometer with chemical shift values in ppm relative to TMS (δ_H 0.00 and δ_C 0.0), or residual chloroform (δ_H 7.26 and δ_C 77.2), dimethylsulfoxide (δ_H 2.50 and δ_C 39.52) or methanol (δ_H 3.31 and δ_C 49.00) as standard. Proton and carbon assignments are based on two-dimensional COSY and DEPT experiments, respectively. Mass spectra were obtained using a JEOL JMS-700 spectrometer for EI and CI or Bruker Microtof-q for ESI. Infrared spectra were obtained neat using a Shimadzu IR Prestige-21 spectrometer. Melting points were determined on a Reichert platform melting point apparatus. Optical rotations were determined as solutions irradiating with the sodium D line (λ= 589 nm) using an Autopol V polarimeter. $[\alpha]_D$ values are given in units 10^{-1} deg cm^2 g^{-1}. UV-Vis spectra were recorded on a Pekin Elmer Lamda 25 instrument. Fluoresence spectra were recorded on a Shimadzu RF-5301PC spectrofluorophotometre. Emission data were measured using an excitation slit width of 3 nm and emission slit width of 5 nm. Quantum yield data were measured using anthracene and L-tryptophan as standard references. Emission data were measured using an excitation slit width of 3 nm and emission slit width of 3 nm. Fluoresence microscopy was carried out using a Zeiss Axiovert 200 M fluorescence microscope.

4.2 Experimental Procedures

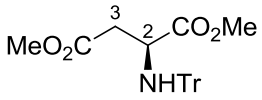
Dimethyl (2*S*)-2-aminobutandioate hydrochloride (98)[166]



To a suspension of L-aspartic acid (5.00 g, 37.6 mmol) in dry methanol (100 mL) at 0 °C under argon was added thionyl chloride (3.80 mL, 52.6 mmol). The mixture was allowed to warm to room temperature and stirred under reflux for 3 h. The solution was allowed to cool to room temperature and concentrated *in vacuo*, azeotroping with toluene-dichloromethane to produce dimethyl (2*S*)-

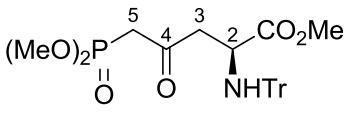
2-aminobutandioate hydrochloride (**98**) (7.41 g, 100%) as a white solid. Mp 115–116 °C (lit.,[166] Mp 114–115 °C); $[\alpha]_D^{24}$ +22.0 (*c* 1.0, MeOH); δ_H (400 MHz, DMSO-d_6) 2.99 (1H, dd, *J* 18.0, 5.5 Hz, 3-*H*H), 3.05 (1H, dd, *J* 18.0, 5.5 Hz, 3-H*H*), 3.66 (3H, s, OCH_3), 3.74 (3H, s, OCH_3), 4.35 (1H, t, *J* 5.5 Hz, 2-H), 8.72 (3H, s, CHNH_3^+); δ_C (100 MHz, DMSO-d_6) 34.0 (CH_2), 48.4 (CH), 52.2 (CH_3), 53.0 (CH_3), 168.7 (C), 169.6 (C); *m/z* (CI) 162 (MH^+, 100%), 148 (5), 102 (20).

Dimethyl (2*S*)-2-(tritylamino)butandioate (72)[167]



To a suspension of dimethyl (2*S*)-2-aminobutandioate hydrochloride (**98**) (7.41 g, 37.5 mmol) in dichloromethane (300 mL), at 0 °C under argon was added triethylamine (11.0 mL, 78.8 mmol) dropwise, followed by triphenylmethyl chloride (12.6 g, 45.0 mmol). The mixture was allowed to warm to room temperature and stirred for 6 h. The mixture was diluted with dichloromethane (50 mL), washed with 2 M citric acid (300 mL), water (150 mL), brine (150 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel, eluting with 30% diethyl ether in petroleum ether (40–60) to produce dimethyl (2*S*)-2-(tritylamino)butandioate (**72**) (14.1 g, 93%) as a white solid. Mp 71–72 °C (lit.,[167] Mp 70–71 °C); R_f 0.15 (30% diethyl ether in petroleum ether); $[\alpha]_D^{24}$ +36.6 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.51 (1H, dd, *J* 14.7, 7.0 Hz, 3-*H*H), 2.66 (1H, dd, *J* 14.7, 5.4 Hz, 3-H*H*), 2.93 (1H, d, *J* 10.1 Hz, NH), 3.25 (3H, s, OCH_3), 3.67 (3H, s, OCH_3), 3.68–3.73 (1H, m, 2-H), 7.15–7.20 (3H, m, 3 × ArH), 7.23–7.28 (6H, m, 6 × ArH), 7.46–7.51 (6H, m, 6 × ArH); δ_C (100 MHz, $CDCl_3$) 39.0 (CH_2), 50.5 (CH), 50.7 (CH_3), 52.4 (CH_3), 69.9 (C), 125.2 (3 × CH), 126.6 (6 × CH), 127.5 (6 × CH), 144.4 (3 × C), 169.7 (C), 172.6 (C); *m/z* (EI) 403 (M^+, 1%), 326 (35), 243 (100), 165 (30), 83 (70).

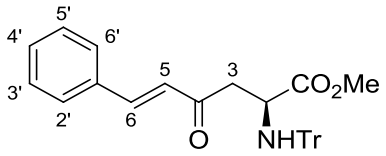
Methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (73)[167]



To a solution of dimethyl methylphosphonate (9.44 mL, 87.5 mmol) in dry tetrahydrofuran (70 mL) at −78 °C under argon was added *n*-butyllithium (34.3 mL, 85.8 mmol, 2.5 M in hexanes) dropwise. After stirring at −78 °C for 1 h, the solution was added via cannula to a stirring solution of dimethyl (2*S*)-2-(tritylamino)butandioate (**72**) (14.1 g, 35.0 mmol) in dry tetrahydrofuran (100 mL) at −78 °C. After stirring at −78 °C under argon for 3 h, the reaction was quenched by the addition of a saturated

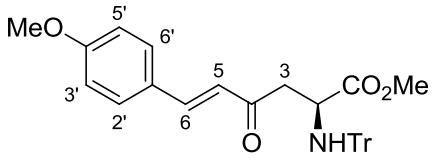
solution of ammonium chloride (80 mL). The solution was then concentrated *in vacuo*, redissolved in ethyl acetate (200 mL), washed with water (100 mL), brine (100 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel, eluting with 90% ethyl acetate in petroleum ether (40–60) to produce methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**73**) (14.9 g, 86%) as a white solid. Mp 117–118 °C (lit.,[167] Mp 117–118 °C); R_f 0.20 (90% ethyl acetate in petroleum ether); $[\alpha]_D^{24}$ +31.1 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.78 (1H, dd, *J* 16.7, 6.9 Hz, 3-*H*H), 2.85–2.95 (2H, m, 3-H*H* and NH), 3.06 (2H, d, $J_{H–C–P}$ 22.7 Hz, 5-H_2), 3.29 (3H, s, OCH_3), 3.65–3.73 (1H, m, 2-H), 3.76 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 7.15–7.21 (3H, m, 3 × ArH), 7.24–7.28 (6H, m, 6 × ArH), 7.45–7.49 (6H, m, 6 × ArH); δ_C (100 MHz, $CDCl_3$) 41.8 (d, $J_{C–P}$ 128 Hz, CH_2), 48.7 (CH_2), 52.0 (CH_3), 52.9 (CH_3), 53.0 (CH_3), 53.1 (CH), 71.2 (C), 126.5 (3 × CH), 127.9 (6 × CH), 128.7 (6 × CH), 145.6 (3 × C), 174.0 (C), 199.2 (C); *m/z* (CI) 496 (MH^+, 1%), 301 (5), 254 (90), 243 (100), 237 (55), 167 (45).

Methyl (2*S*,5*E*)-4-oxo-6-phenyl-2-(tritylamino)hex-5-enoate (99)



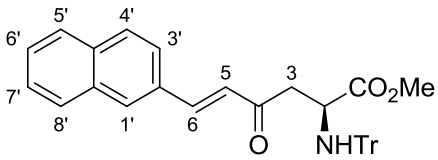
Methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**73**) (0.598 g, 1.21 mmol) was dissolved in anhydrous acetonitrile (12 mL) and potassium carbonate (0.200 g, 1.45 mmol) was added. The mixture was stirred at room temperature for 0.5 h followed by addition of benzaldehyde (0.246 mL, 2.42 mmol). The temperature was increased to 50 °C and the mixture was stirred for 72 h. Once the reaction was complete, the solution was concentrated *in vacuo*, redissolved in ethyl acetate (20 mL) and washed with water (2 × 15 mL) and brine (15 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel, eluting with 30% diethyl ether in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-4-oxo-6-phenyl-2-(tritylamino)hex-5-enoate (**99**) (0.546 g, 95%) as a yellow oil. R_f 0.17 (30% diethyl ether in petroleum ether); ν_{max}/cm^{-1} (NaCl) 3023 (NH), 2950 (CH), 1737 (C=O), 1657 (C=C), 1608, 1205; $[\alpha]_D^{25}$ +111.0 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.80 (1H, dd, *J* 15.2, 7.0 Hz, 3-*H*H), 2.88–2.97 (2H, m, 3-H*H* and NH), 3.28 (3H, s, OCH_3), 3.79–3.89 (1H, m, 2-H), 6.69 (1H, d, *J* 16.2 Hz, 5-H), 7.14–7.29 (10H, m, 6-H and 9 × ArH), 7.37–7.41 (3H, m, 3 × ArH), 7.44–7.53 (8H, m, 8 × ArH); δ_C (100 MHz, $CDCl_3$) 43.6 (CH_2), 50.0 (CH_3), 51.7 (CH), 69.2 (C), 124.5 (3 × CH), 125.7 (6 × CH), 126.1 (CH), 126.3 (2 × CH), 127.4 (6 × CH), 127.8 (2 × CH), 128.6 (CH), 133.0 (C), 141.3 (3 × C), 143.7 (CH), 172.4 (C), 195.5 (C); *m/z* (FAB) 476.2231 (MH^+. $C_{32}H_{30}NO_3$ requires 476.2226), 398 (15%), 259 (6), 243 (100), 232 (25), 166 (23), 132 (24).

Methyl (2*S*,5*E*)-6-(4'-methoxyphenyl)-4-oxo-2-(tritylamino)hex-5-enoate (100)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-4-oxo-6-phenyl-2-(tritylamino)hex-5-enoate (**99**) using methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**73**) (0.495 g, 1.00 mmol) and 4-methoxybenzaldehyde (0.25 mL, 2.00 mmol) for 72 h. The crude product was purified via flash column chromatography on silica gel, eluting with 30% ethyl acetate in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-6-(4'-methoxyphenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**100**) (0.384 g, 76%) as a colourless oil. R_f 0.20 (30% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 3320 (NH), 2951 (CH), 1736 (C=O), 1595 (C=C), 1510, 1447, 1252, 1171, 1028; $[\alpha]_D^{23}$ +54.1 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.78 (1H, dd, *J* 15.0, 7.0 Hz, 3-*H*H), 2.84–2.99 (2H, m, 3-H*H* and NH), 3.27 (3H, s, OCH_3), 3.71–3.93 (4H, m, 2-H and OCH_3), 6.59 (1H, d, *J* 16.1 Hz, 5-H), 6.92 (2H, d, *J* 8.7 Hz, 2 × ArH), 7.11–7.35 (9H, m, 9 × ArH), 7.39–7.59 (9H, m, 6-H and 8 × ArH); δ_C (100 MHz, $CDCl_3$) 45.7 (CH_2), 52.0 (CH_3), 54.0 (CH), 55.4 (CH_3), 71.3 (C), 114.5 (2 × CH), 124.3 (CH), 126.6 (3 × CH), 127.1 (C), 128.0 (6 × CH), 128.9 (6 × CH), 130.2 (2 × CH), 143.2 (CH), 145.9 (3 × C), 161.8 (C), 174.6 (C), 197.5 (C); m/z (FAB) 506.2329 (MH^+. $C_{33}H_{32}NO_4$ requires 506.2331), 428 (5%), 262 (11), 243 (100), 162 (18), 86 (5).

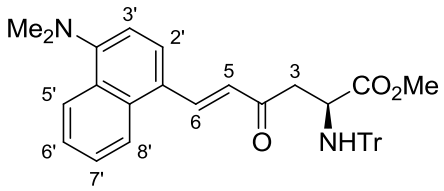
Methyl (2*S*,5*E*)-6-(naphthalen-2'-yl)-4-oxo-2-(tritylamino)hex-5-enoate (101)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-4-oxo-6-phenyl-2-(tritylamino)hex-5-enoate (**99**) using methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**73**) (1.09 g, 2.20 mmol) and 2-naphthaldehyde (0.687 g, 4.40 mmol) for 72 h. The crude product was purified via flash column chromatography on silica gel, eluting with 30% diethyl ether in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-6-(naphthalen-2'-yl)-4-oxo-2-(tritylamino)hex-5-enoate (**101**) (1.03 g, 89%) as a yellow solid. Mp 62–63 °C; R_f 0.15 (30% diethyl ether in petroleum ether); ν_{max}/cm^{-1} (NaCl) 3055 (NH), 2982 (CH), 1734 (C=O), 1655 (C=C), 1604, 1593, 1489, 1172; $[\alpha]_D^{24}$ +64.1 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.84 (1H, dd, *J* 15.1, 7.0 Hz,

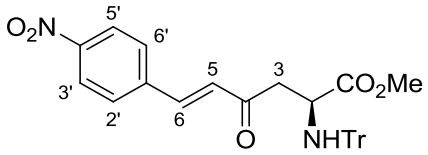
3-*H*H), 2.92–3.00 (2H, m, 3-H*H* and NH), 3.29 (3H, s, OCH_3), 3.79–3.86 (1H, m, 2-H), 6.80 (1H, d, *J* 16.2 Hz, 5-H), 7.15–7.30 (9H, m, 9 × ArH), 7.50–7.55 (8H, m, 6'-H, 7'-H and 6 × ArH), 7.62–7.69 (2H, m, 6-H and 3'-H), 7.84–7.89 (3H, m, 4'-H, 5'-H and 6'-H), 7.94 (1H, br s, 1'-H); δ_C (100 MHz, $CDCl_3$) 45.9 (CH_2), 52.1 (CH), 54.0 (CH_3), 71.4 (C), 123.6 (CH), 126.6 (CH), 126.6 (3 × CH), 126.9 (CH), 127.6 (CH), 128.0 (CH), 128.0 (6 × CH), 128.2 (CH), 128.7 (CH), 128.9 (6 × CH), 130.7 (CH), 132.0 (C), 133.4 (C), 134.5 (C), 143.5 (CH), 145.9 (3 × C), 174.6 (C), 197.6 (C); *m/z* (FAB) 526.2388 (MH^+. $C_{36}H_{32}NO_3$ requires 526.2382), 448 (7%), 273 (8), 243 (100), 181 (19), 165 (24).

Methyl (2*S*,5*E*)-6-[4'-(dimethylamino)naphthalen-1'-yl]-4-oxo-2-(tritylamino)hex-5-enoate (74)



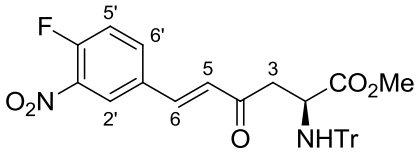
The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-4-oxo-6-phenyl-2-(tritylamino)hex-5-enoate (**99**) using methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**73**) (0.451 g, 0.911 mmol) and 4-dimethylamino-1-naphthaldehyde (0.363 g, 1.82 mmol) for 96 h. The crude product was purified via flash column chromatography on silica gel, eluting with 40% diethyl ether in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-6-[4'-(dimethylamino)naphthalen-1'-yl]-4-oxo-2-(tritylamino)hex-5-enoate (**74**) (0.372 g, 72%) as an orange oil. R_f 0.18 (40% diethyl ether in petroleum ether); ν_{max}/cm^{-1} (NaCl) 3448 (NH), 3019, 2947 (CH), 1737 (C=O), 1651 (C=C), 1569, 1449, 1390; $[\alpha]_D^{18}$ +73.7 (*c* 0.4, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.83 (1H, dd, *J* 14.8, 6.8 Hz, 3-*H*H), 2.88–3.04 (8H, m, 3-H*H*, NH and NMe_2), 3.30 (3H, s, OCH_3), 3.78–3.88 (1H, m, 2-H), 6.71 (1H, d, *J* 15.7 Hz, 5-H), 7.04 (1H, d, *J* 8.0 Hz, 3'-H), 7.14–7.29 (9H, m, 9 × ArH), 7.49–7.60 (8H, m, 8 × ArH), 7.74 (1H, d, *J* 8.0 Hz, 2'-H), 8.13–8.26 (2H, m, 2 × ArH), 8.30 (1H, d, *J* 15.7 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 44.9 (2 × CH_3), 45.8 (CH_2), 52.0 (CH_3), 54.0 (CH), 71.3 (C), 113.4 (CH), 123.6 (CH), 125.2 (CH), 125.3 (CH), 125.4 (C), 125.8 (CH), 126.5 (3 × CH), 126.6 (CH), 126.9 (CH), 127.9 (6 × CH), 128.4 (C), 128.9 (6 × CH), 133.1 (C), 140.2 (CH), 145.9 (3 × C), 153.8 (C), 174.7 (C), 197.4 (C); *m/z* (FAB) 569.2799 (MH^+. $C_{38}H_{37}N_2O_3$ requires 569.2804), 491 (3%), 460 (4), 325 (52), 243 (100), 224 (19), 85 (56).

Methyl (2*S*,5*E*)-6-(4'-nitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (102)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-4-oxo-6-phenyl-2-(tritylamino)hex-5-enoate (**99**) using methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**73**) (2.17 g, 4.38 mmol) and 4-nitrobenzaldehyde (1.32 g, 8.76 mmol) for 48 h. The crude product was purified via flash column chromatography on silica gel, eluting with 30% ethyl acetate in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-6-(4'-nitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**102**) (1.64 g, 72%) as an off-white solid. Mp 139–141 °C; R_f 0.33 (30% ethyl acetate in petroleum ether); ν_{max} (neat)/cm^{-1} 2951 (CH), 1742 (C=O), 1712 (C=O), 1490, 1509, 1341; $[\alpha]_D^{25}$ +43.3 (*c* 0.2, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.80 (1H, dd, *J* 15.5, 6.9 Hz, 3-*H*H), 2.91 (1H, dd, J 15.5, 5.1 Hz, 3-H*H*), 2.95 (1H, br s, NH), 3.31 (3H, s, OCH_3), 3.81 (1H, m, 2-H), 6.77 (1H, d, *J* 16.2 Hz, 5-H), 7.17–7.51 (16H, m, 6-H and 3 × Ph), 7.66 (2H, d, *J* 8.8 Hz, 2'-H and 6'-H), 8.26 (2H, d, *J* 8.8 Hz, 3'-H and 5'-H); δ_C (101 MHz, $CDCl_3$) 46.2 (CH_2), 52.1 (CH_3), 53.7 (CH), 71.3 (C), 124.3 (CH), 126.6 (3 × CH), 128.0 (6 × CH), 128.8 (6 × CH), 128.9 (2 × CH), 129.6 (2 × CH), 139.9 (CH), 140.6 (C), 145.7 (3 × C), 148.6 (C), 174.3 (C), 197.0 (C); *m/z* (FAB + NaI) 543.1903 (MNa^+. $C_{32}H_{28}NO_5Na$ requires 543.1896), 443 (9%), 413 (9), 351 (19), 243 (100), 176 (78).

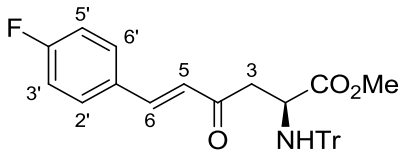
Methyl (2*S*,5*E*)-6-(4'-fluoro-3'-nitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (103)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-4-oxo-6-phenyl-2-(tritylamino)hex-5-enoate (**99**) using methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**73**) (0.466 g, 0.941 mmol) and 4-fluoro-3-nitrobenzaldehyde (0.318 g, 1.882 mmol) for 120 h. The crude product was purified via flash column chromatography on silica gel, eluting with 50% diethyl ether in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-6-(4'-fluoro-3'-nitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**103**) (0.391 g, 69%) as a pale yellow solid. Mp 54–57 °C; R_f 0.13 (50% diethyl ether in petroleum ether); ν_{max}/cm^{-1} (neat) 3545 (NH), 2985 (CH), 1612 (C=O), 1537 (C=C), 1350, 1084, 707; $[\alpha]_D^{26}$ +9.3 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 2.77 (1H, dd, *J* 15.4, 7.0 Hz, 3-*H*H), 2.87–2.98 (2H, m, 3-H*H* and NH), 3.32 (3H, s, OCH_3), 3.80 (1H, dt, *J*

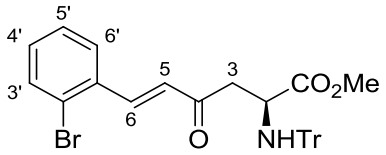
9.3, 7.0 Hz, 2-H), 6.68 (1H, d, *J* 16.2 Hz, 5-H), 7.17–7.21 (3H, m, 3 × ArH), 7.24–7.28 (7H, m, 7 × ArH), 7.34 (1H, dd, *J* 10.2, 8.7 Hz, 5'-H), 7.41 (1H, d, *J* 16.2 Hz, 6-H), 7.47–7.52 (5H, m, 5 × ArH), 7.76 (1H, ddd, *J* 8.7, 4.1, 2.3 Hz, 6'-H), 8.20 (1H, dd, *J* 7.0, 2.3 Hz, 2'-H); δ_C (126 MHz, $CDCl_3$) 46.4 (CH_2), 52.2 (CH_3), 53.8 (CH), 71.5 (C), 119.4 (d, $J_{C\text{-}C\text{-}F}$ 21.4 Hz, CH), 125.5 (d, $J_{C\text{-}C\text{-}C\text{-}F}$ 2.3 Hz, CH), 126.8 (3 × CH), 128.1 (6 × CH), 128.5 (d, $J_{C\text{-}C\text{-}C\text{-}C\text{-}F}$ 2.1 Hz, C), 128.9 (CH), 128.9 (6 × CH), 131.9 (d, $J_{C\text{-}C\text{-}F}$ 4.4 Hz, C), 134.8 (d, $J_{C\text{-}C\text{-}C\text{-}F}$ 8.8 Hz, CH), 138.9 (CH), 145.8 (3 × C), 156.3 (d, $J_{C\text{-}F}$ 269.7 Hz, C), 174.4 (C), 196.8 (C); *m/z* (ESI) 561.1773 (MNa^+. $C_{32}H_{27}FN_2NaO_5$ requires 561.1796).

Methyl (2*S*,5*E*)-6-(4-fluorophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (104)



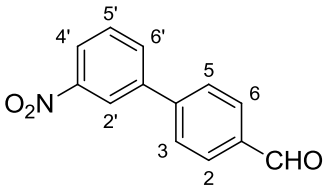
The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-4-oxo-6-phenyl-2-(tritylamino)hex-5-enoate (**99**) using methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**73**) (0.571 g, 1.15 mmol) and 4-fluorobenzaldehyde (0.247 mL, 2.30 mmol) for 72 h. The crude product was purified via flash column chromatography on silica gel, eluting with 40% diethyl ether in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-6-(4-fluorophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**104**) (0.391 g, 69%) as a yellow oil. R_f 0.25 (40% diethyl ether in petroleum ether); ν_{max}/cm^{-1} (NaCl) 3057 (NH), 2950 (CH), 1738 (C=O), 1612, 1598, 1233; $[\alpha]_D^{25}$ +128.8 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.78 (1H, dd, *J* 15.2, 7.0 Hz, 3-*H*H), 2.87–2.95 (2H, m, 3-H*H* and NH), 3.28 (3H, s, OCH_3), 3.78–3.85 (1H, m, 2-H), 6.61 (1H, d, *J* 16.1 Hz, 5-H), 7.05–7.11 (2H, m, 3'-H and 5'-H), 7.15–7.19 (3H, m, 3 × ArH), 7.21–7.29 (7H, m, 7 × ArH), 7.44 (1H, d, *J* 16.1 Hz, 6-H), 7.47–7.53 (7H, m, 7 × ArH); δ_C (100 MHz, $CDCl_3$) 44.9 (CH_2), 51.1 (CH_3), 52.9 (CH), 70.4 (C), 115.3 (d, $J_{C\text{-}C\text{-}F}$ 22.1 Hz, 2 × CH), 125.1 (CH), 127.0 (3 × CH), 127.2 (6 × CH), 128.2 (CH), 129.4 (6 × CH), 129.7 (2 × CH), 141.1 (C), 144.8 (3 × C), 163.2 (d, $J_{C\text{-}F}$ 251.5 Hz, C), 173.6 (C), 196.4 (C); *m/z* (FAB) 494.2128 (MH^+. $C_{32}H_{29}FNO_3$ requires 494.2131), 416 (31%), 258 (6), 243 (100), 166 (34), 150 (35).

Methyl (2*S*,5*E*)-6-(2'-bromophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (105)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-4-oxo-6-phenyl-2-(tritylamino)hex-5-enoate (**99**) using methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**73**) (1.11 g, 2.25 mmol) and 2-bromobenzaldehyde (0.525 mL, 4.50 mmol) for 24 h. The crude product was purified via flash column chromatography on silica gel, eluting with 30% diethyl ether in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-6-(2'-bromophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**105**) (0.973 g, 78%) as a colourless oil. R_f 0.17 (30% diethyl ether in petroleum ether); ν_{max}/cm^{-1} (NaCl) 3021 (NH), 2950 (CH), 1737 (C=O), 1437, 1204, 1027; $[\alpha]_D^{25}$ +48.6 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.82 (1H, dd, *J* 15.4, 6.8 Hz, 3-*H*H), 2.88–2.97 (2H, m, 3-H*H* and NH), 3.30 (3H, s, OCH_3), 3.78–3.83 (1H, m, 2-H), 6.60 (1H, d, *J* 16.2 Hz, 5-H), 7.13–7.33 (11H, m, 4'-H, 5'-H and 9 × ArH), 7.48–7.53 (6H, m, 6 × ArH), 7.59 (2H, td, *J* 8.0, 1.1 Hz, 3'-H and 6'-H), 7.85 (1H, d, *J* 16.2 Hz, 6-H); δ_C (100 MHz, $CDCl_3$) 45.3 (CH_2), 52.1 (CH), 53.8 (CH_3), 71.3 (C), 125.9 (C), 126.6 (3 × CH), 127.8 (CH), 127.9 (CH), 128.0 (6 × CH), 128.9 (6 × CH), 129.1 (CH), 131.6 (CH), 133.6 (CH), 134.4 (C), 141.7 (CH), 145.8 (3 × C), 174.4 (C), 197.5 (C); *m/z* (FAB) 554.1322 (MH^+. $C_{32}H_{29}{}^{79}BrNO_3$ requires 554.1331), 478 (7%), 312 (13), 243 (100), 209 (7), 165 (19).

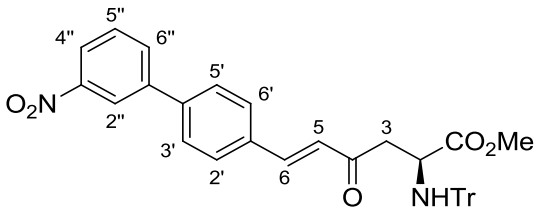
4-(3'-Nitrophenyl)benzaldehyde (115)[76]



To a solution of 4-bromobenzaldehyde (0.111 g, 0.599 mmol) in *N,N*-dimethylformamide and water (3 mL, 19:1) was added 3-nitrophenylboronic acid (0.148 g, 0.887 mmol), potassium carbonate (0.204 g, 1.476 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.033 g, 0.029 mmol). The reaction mixture was heated to 110 °C and stirred for 4 h. After cooling to room temperature, the solution was concentrated *in vacuo*, redissolved in chloroform (10 mL), filtered through Celite® and concentrated *in vacuo*. The resulting solid was dissolved in diethyl ether (20 mL), washed with water (3 × 10 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel, eluting with 20% ethyl acetate in petroleum ether (40–60) to

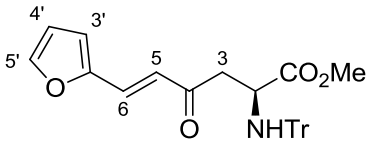
give 4-(3'-nitrophenyl)benzaldehyde (**115**) as an off-white solid (0.139 g, 100%). Mp 113−114 °C (lit.,[76] 114−116 °C); R_f 0.27 (20% ethyl acetate in petroleum ether); δ_H (400 MHz, $CDCl_3$) 7.68 (1H, t, *J* 8.0 Hz, 5'-H), 7.81 (2H, d, *J* 8.3 Hz, 3-H and 5-H), 7.95−8.00 (1H, m, 6'-H), 8.03 (2H, d, *J* 8.3 Hz, 2-H and 6-H), 8.26−8.31 (1H, m, 4'-H), 8.51 (1H, t, *J* 2.0 Hz, 2'-H), 10.10 (1H, s, CHO); δ_C (101 MHz, $CDCl_3$) 122.4 (CH), 123.3 (CH), 128.0 (2 × CH), 130.2 (CH), 130.7 (2 × CH), 133.4 (CH), 136.2 (C), 141.6 (C), 144.5 (C), 149.0 (C), 191.8 (CH); *m/z* (CI) 228 (MH^+. 100%), 198 (10).

Methyl (2*S*,5*E*)-6-(3''-nitrobiphen-4'-yl)-4-oxo-2-(tritylamino)hex-5-enoate (106)



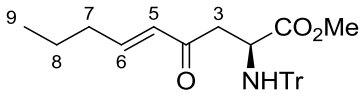
The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-4-oxo-6-phenyl-2-(tritylamino)hex-5-enoate (**99**) using methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**73**) (0.550 g, 1.11 mmol) and 4-(3'-nitrophenyl)benzaldehyde (**115**) (0.355 g, 1.56 mmol) for 72 h. The crude product was purified via flash column chromatography on silica gel, eluting with 20% ethyl acetate in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-6-(3''-nitrobiphen-4'-yl)-4-oxo-2-(tritylamino)hex-5-enoate (**106**) (0.556 g, 84%) as a yellow foam. R_f 0.12 (20% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 3030 (NH), 1736 (C=O), 1657 (C=C), 1603, 1530, 1514, 1348; $[\alpha]_D^{23}$ +61.7 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.82 (1H, dd, *J* 15.2, 6.9 Hz, 3-*H*H), 2.90–3.02 (2H, m, 3-H*H* and NH), 3.30 (3H, s, OCH_3), 3.77–3.88 (1H, m, 2-H), 6.75 (1H, d, *J* 16.2 Hz, 5-H), 7.12–7.32 (9H, m, 9 × ArH), 7.45–7.73 (12H, m, 6-H and 11 × ArH), 7.93 (1H, d, *J* 7.9 Hz, 6''-H), 8.23 (1H, d, *J* 7.9 Hz, 4''-H), 8.48 (1H, br s, 2''-H); δ_C (100 MHz, $CDCl_3$)45.9 (CH_2), 52.1 (CH_3), 53.8 (CH), 71.3 (C), 121.9 (CH), 122.7 (CH), 126.6 (3 × CH), 127.0 (CH), 127.7 (2 × CH), 128.0 (6 × CH), 128.9 (6 × CH), 129.2 (2 × CH), 130.0 (CH), 132.9 (CH), 134.7 (C), 140.6 (C), 141.7 (C), 142.2 (CH), 145.8 (3 × C), 148.8 (C), 174.5 (C), 197.4 (C); *m/z* (FAB) 597.2384 (MH^+. $C_{38}H_{33}N_2O_5$ requires 597.2389), 519 (23%), 419 (5), 353 (32), 243 (100), 194 (9), 166 (54).

Methyl (2*S*,5*E*)-6-(furan-2-yl)-4-oxo-2-(tritylamino)hex-5-enoate (107)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-4-oxo-6-phenyl-2-(tritylamino)hex-5-enoate (**99**) using methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**73**) (0.308 g, 0.622 mmol) and 2-furaldehyde (0.103 mL, 1.24 mmol) for 96 h. The crude product was purified via flash column chromatography on silica gel, eluting with 40% diethyl ether in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-6-(furan-2-yl)-4-oxo-2-(tritylamino)hex-5-enoate (**107**) (0.234 g, 81%) as a light brown solid. Mp 97–98 °C; R_f 0.20 (40% diethyl ether in petroleum ether); ν_{max}/cm^{-1} (NaCl) 3058 (NH), 2951 (CH), 1737 (C=O), 1607; $[\alpha]_D^{23}$ +42.9 (*c* 0.3, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.72 (1H, dd, *J* 15.1, 7.0 Hz, 3-*H*H), 2.82–2.94 (2H, m, 3-H*H* and NH), 3.27 (3H, s, OCH_3), 3.74–3.81 (1H, m, 2-H), 6.47–6.50 (1H, m, 4'-H), 6.59 (1H, d, *J* 15.8 Hz, 5-H), 6.66 (1H, d, *J* 3.3 Hz, 3'-H), 7.14–7.30 (11H, m, 6-H, 5'-H and 9 × ArH), 7.46–7.52 (6H, m, 6 × ArH); δ_C (101 MHz, $CDCl_3$) 46.2 (CH_2), 51.9 (CH_3), 53.8 (CH), 71.3 (C), 112.6 (CH), 116.0 (CH), 123.6 (CH), 126.5 (3 × CH), 128.2 (6 × CH), 128.6 (6 × CH), 129.2 (CH), 145.1 (CH), 145.8 (3 × C), 151.0 (C), 174.4 (C), 197.0 (C); *m/z* (FAB) 488.1833 (MNa^+. $C_{30}H_{27}NO_4Na$ requires 488.1838), 388 (7%), 352 (3), 243 (100), 166 (18).

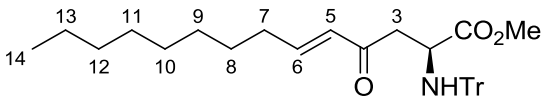
Methyl (2*S*,5*E*)-2-(tritylamino)-4-oxonon-5-enoate (108)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-4-oxo-6-phenyl-2-(tritylamino)hex-5-enoate (**99**) using methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**73**) (1.01 g, 2.04 mmol) and butyraldehyde (0.367 mL, 4.08 mmol) for 96 h. The crude product was purified via a plug of silica gel, eluting with 20% ethyl acetate in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-2-(tritylamino)-4-oxonon-5-enoate (**108**) (0.765 g, 85%) as a yellow oil. R_f 0.18 (20% ethyl acetate in petroleum ether); ν_{max} (neat)/cm^{-1} 3316 (NH), 2955 (CH), 1736 (C=O), 1667, 1443, 1204, 1173; $[\alpha]_D^{27}$ +28.6 (*c* 0.5, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.93 (3H, t, *J* 7.3 Hz, 9-H_3), 1.44–1.53 (2H, m, 8-H_2), 2.18 (2H, dtd, *J* 7.4, 7.0, 1.5 Hz, 7-H_2), 2.65 (1H, dd, *J* 15.3, 7.1 Hz, 3-*H*H), 2.79 (1H, dd, *J* 15.3, 5.2 Hz, 3-H*H*), 2.85 (1H, d, *J* 9.8 Hz, NH), 3.27 (3H, s, OCH_3), 3.67–3.74 (1H, m, 2-H), 6.04 (1H, dt, *J* 16.0, 1.5 Hz, 5-H), 6.74 (1H, dt, *J* 16.0, 7.0 Hz, 6-H),

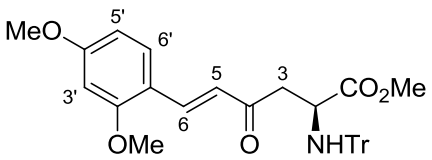
7.08–7.19 (9H, m, 9 × ArH), 7.39–7.41 (6H, m, 6 × ArH); δ_C (101 MHz, $CDCl_3$) 13.7 (CH_3), 21.3 (CH_2), 34.5 (CH_2), 44.9 (CH_2), 51.9 (CH), 53.6 (CH_3), 71.2 (C), 126.5 (3 × CH), 127.9 (6 × CH), 129.1 (6 × CH), 130.7 (CH), 145.8 (3 × C), 148.3 (CH), 174.6 (C), 198.0 (C); *m/z* (FAB) 442.2378 (MH^+. $C_{29}H_{32}NO_3$ requires 442.2382), 364 (60%), 243 (100), 198 (64), 165 (43).

Methyl (2*S*,5*E*)-2-(tritylamino)-4-oxotetradec-5-enoate (109)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-4-oxo-6-phenyl-2-(tritylamino)hex-5-enoate (**99**) using methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**73**) (0.243 g, 0.574 mmol) and nonanal (0.188 mL, 1.09 mmol) for 96 h. The crude product was purified via flash column chromatography on silica gel, eluting with 20% diethyl ether in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-2-(tritylamino)-4-oxotetradec-5-enoate (**109**) (0.117 g, 40%) as a colourless oil. R_f 0.19 (20% diethyl ether in petroleum ether); ν_{max}/cm^{-1} (neat) 3316 (NH), 2955 (CH), 1736 (C=O), 1667, 1443, 1204, 1173; $[\alpha]_D^{26}$ +9.8 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.88 (3H, t, *J* 6.9 Hz, 14-H_3), 1.21–1.34 (10H, m, 9-H_2, 10-H_2, 11-H_2, 12-H_2 and 13-H_2), 1.39–1.49 (2H, m, 8-H_2), 2.15–2.23 (2H, m, 7-H_2), 2.65 (1H, dd, *J* 15.3, 7.0 Hz, 3-*H*H), 2.76–2.92 (2H, m, 3-H*H* and NH), 3.26 (3H, s, OCH_3), 3.68–3.75 (1H, m, 2-H), 6.03 (1H, dt, *J* 15.9, 1.4 Hz, 5-H), 6.74 (1H, dt, *J* 15.9, 6.9 Hz, 6-H), 7.14–7.20 (3H, m, 3 × ArH), 7.21–7.29 (6H, m, 6 × ArH), 7.45–7.52 (6H, m, 6 × ArH); δ_C (101 MHz, $CDCl_3$) 14.1 (CH_3), 22.7 (CH_2), 28.1 (CH_2), 29.2 (CH_2), 29.2 (CH_2), 29.4 (CH_2), 31.8 (CH_2), 32.5 (CH_2), 44.9 (CH_2), 51.9 (CH_3), 53.7 (CH), 71.2 (C), 126.5 (3 × CH), 127.9 (6 × CH), 128.8 (6 × CH), 130.6 (CH), 145.8 (3 × C), 148.6 (CH), 174.6 (C), 197.7 (C); *m/z* (ESI) 534.2955 (MNa^+. $C_{34}H_{41}NNaO_3$ requires 534.2979).

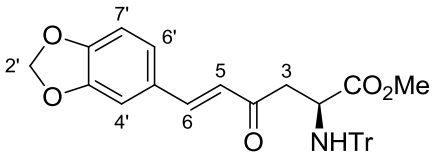
Methyl (2*S*,5*E*)-6-(2',4'-dimethoxyphenyl)-4-oxo-2-(tritylamino)hex-5-enoate (158)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-4-oxo-6-phenyl-2-(tritylamino)hex-5-enoate (**99**) using methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**73**) (1.11 g, 2.24 mmol) and 2,4-dimethoxybenzaldehyde (0.743 g, 4.48 mmol) for 120 h. The crude product was purified via flash column chromatography on silica

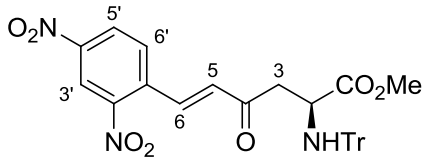
gel, eluting with 30% ethyl acetate in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-6-(2',4'-dimethoxyphenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**158**) (0.564 g, 47%) as a yellow foam. Mp 66–70 °C; R_f 0.15 (30% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 2948 (CH), 1736 (C=O), 1599 (C=C), 1266, 1210, 1160, 1028, 734, 699; $[\alpha]_D^{26}$ +52.3 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 2.83 (1H, dd, *J* 14.9, 6.8 Hz, 3-*H*H), 2.87–2.95 (2H, m, 3-H*H* and NH), 3.25 (3H, s, OCH_3), 3.75–3.86 (7H, m, 2-H and 2 × OCH_3), 6.45 (1H, d, *J* 2.3 Hz, 3'-H), 6.51 (1H, dd, *J* 8.6, 2.3 Hz, 5'-H), 6.69 (1H, d, *J* 16.3 Hz, 5-H), 7.12–7.19 (3H, m, 3 × ArH), 7.20–7.27 (6H, m, 6 × ArH), 7.44–7.54 (7H, m, 6'-H and 6 × ArH), 7.82 (1H, d, *J* 16.3 Hz, 6-H); δ_C (126 MHz, $CDCl_3$) 45.3 (CH_2), 51.8 (CH_3), 53.9 (CH), 55.5 (CH_3), 55.5 (CH_3), 71.3 (C), 98.4 (CH), 105.5 (CH), 116.5 (C), 124.8 (CH), 126.4 (3 × CH), 127.8 (6 × CH), 128.9 (6 × CH), 130.2 (CH), 138.8 (CH), 145.9 (3 × C), 160.1 (C), 163.2 (C), 174.6 (C), 198.0 (C); *m/z* (ESI) 558.2227 (MNa^+. $C_{34}H_{33}NNaO_5$ requires 558.2251).

Methyl (2*S*,5*E*)-6-(2*H*-1',3'-benzodioxol-5'-yl)-4-oxo-2-(tritylamino)hex-5-enoate (159)



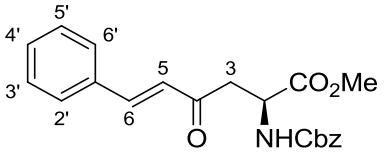
The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-4-oxo-6-phenyl-2-(tritylamino)hex-5-enoate (**99**) using methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**73**) (0.215 g, 0.434 mmol) and piperonal (0.130 g, 0.868 mmol) for 120 h. The crude product was purified via flash column chromatography on silica gel, eluting with 20% ethyl acetate in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-6-(2*H*-1',3'-benzodioxol-5'-yl)-4-oxo-2-(tritylamino)hex-5-enoate (**159**) (0.113 g, 50%) as a yellow oil. R_f 0.12 (20% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 3055 (NH), 2898 (CH), 1734 (C=O), 1594 (C=C), 1489, 1446, 1253, 1034, 699; $[\alpha]_D^{26}$ +49.2 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.75 (1H, dd, *J* 15.1, 6.8 Hz, 3-*H*H), 2.84–2.95 (2H, m, 3-H*H* and NH), 3.27 (3H, s, OCH_3), 3.78 (1H, dt, *J* 9.3, 6.8 Hz, 2-H), 5.98 (2H, s, 2'-H_2), 6.52 (1H, d, *J* 16.1 Hz, 5-H), 6.81 (1H, d, *J* 7.9 Hz, 7'-H), 6.97–7.04 (2H, m, 4'-H and 6'-H), 7.13–7.19 (3H, m, 3 × ArH), 7.20–7.29 (6H, m, 6 × ArH), 7.39 (1H, d, *J* 16.1 Hz, 6-H), 7.47–7.53 (6H, m, 6 × ArH); δ_C (101 MHz, $CDCl_3$) 45.8 (CH_2), 51.9 (CH_3), 53.8 (CH), 71.3 (C), 101.6 (CH_2), 106.6 (CH), 108.6 (CH), 124.5 (CH), 125.0 (CH), 126.5 (3 × CH), 127.9 (6 × CH), 128.1 (C), 128.8 (6 × CH), 143.1 (CH), 145.8 (3 × C), 148.5 (C), 150.0 (C), 174.5 (C), 197.2 (C); *m/z* (ESI) 542.1915 (MNa^+. $C_{33}H_{29}NNaO_5$ requires 542.1938).

Methyl (2*S*,5*E*)-6-(2',4'-dinitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (160)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-4-oxo-6-phenyl-2-(tritylamino)hex-5-enoate (**99**) using methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**73**) (1.06 g, 2.15 mmol) and 2,4-dinitrobenzaldehyde (0.842 g, 4.29 mmol) for 1 h. The crude product was purified via flash column chromatography on silica gel, eluting with 60% diethyl ether in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-6-(2',4'-dinitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**160**) (0.801 g, 66%) as an orange solid. Mp 58–61 °C; R_f 0.25 (60% diethyl ether in petroleum ether); ν_{max}/cm^{-1} (neat) 3057 (NH), 2952 (CH), 1734 (C=O), 1530 (C=C), 1447, 1266, 834, 705; $[\alpha]_D^{26}$ +37.7 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 2.79 (1H, dd, *J* 15.8, 6.8 Hz, 3-*H*H), 2.85–3.05 (2H, m, 3-H*H* and NH), 3.35 (3H, s, OCH_3), 3.78–3.84 (1H, m, 2-H), 6.60 (1H, d, *J* 16.1 Hz, 5-H), 7.15–7.28 (9H, m, 9 × ArH), 7.47–7.52 (6H, m, 6 × ArH), 7.78 (1H, d, *J* 8.6 Hz, 6'-H), 7.86 (1H, d, *J* 16.1 Hz, 6-H), 8.46 (1H, dd, *J* 8.6, 2.3 Hz, 5'-H), 8.90 (1H, d, *J* 2.3 Hz, 3'-H); δ_C (126 MHz, $CDCl_3$) 45.5 (CH_2), 52.2 (CH_3), 53.5 (CH), 71.3 (C), 120.7 (CH), 126.6 (3 × CH), 127.7 (CH), 128.0 (6 × CH), 128.8 (6 × CH), 130.6 (CH), 133.4 (CH), 136.0 (CH), 136.6 (C), 145.6 (3 × C), 148.1 (C), 148.2 (C), 174.1 (C), 196.5 (C); *m/z* (ESI) 588.1714 (MNa^+. $C_{32}H_{27}N_3NaO_7$ requires 588.1741).

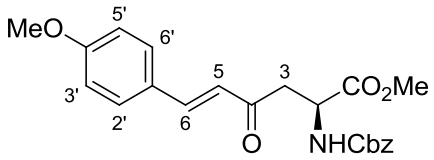
Methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxo-6-phenylhex-5-enoate (116)



To a solution of methyl (2*S*,5*E*)-4-oxo-6-phenyl-2-(tritylamino)hex-5-enoate (**99**) (0.347 g, 0.730 mmol) in dichloromethane (7 mL) was added trifluoroacetic acid (0.112 mL, 1.46 mmol). The reaction mixture was stirred at room temperature for 2 h before concentrating *in vacuo*. The resulting residue was dissolved in chloroform (2 mL) and petroleum ether (40–60) was added until an orange oil formed, at which point the solvent was decanted off. The resulting oil was dissolved in dichloromethane (7 mL) and *N*,*N*'-diisopropylethylamine (0.320 mL, 1.83 mmol) was added, followed by benzyl chloroformate (0.158 mL, 1.10 mmol). The reaction mixture was stirred at room temperature for 1 h, before diluting with water (20 mL). The mixture was then extracted with

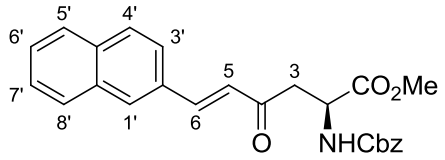
dichloromethane (3 × 10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel, eluting with 30% ethyl acetate in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxo-6-phenylhex-5-enoate (**116**) (0.230 g, 86%) as a white solid. Mp 77−78 °C; R_f 0.17 (30% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 3333 (NH), 3059, 2951 (CH), 1734 (C=O), 1688 (C=O), 1533 (C=C), 1435, 1343, 1254, 1090, 980, 748; $[\alpha]_D^{26}$ +26.5 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.26 (1H, dd, *J* 17.9, 4.2 Hz, 3-*H*H), 3.48 (1H, dd, *J* 17.9, 4.2 Hz, 3-H*H*), 3.75 (3H, s, OCH_3), 4.68 (1H, dt, *J* 8.4, 4.2 Hz, 2-H), 5.12 (2H, s, OCH_2Ph), 5.85 (1H, d, *J* 8.4 Hz, NH), 6.69 (1H, d, *J* 16.2 Hz, 5-H), 7.26−7.43 (8H, m, 8 × ArH), 7.51−7.59 (3H, m, 6-H and 2 × ArH); δ_C (101 MHz, $CDCl_3$) 42.4 (CH_2), 50.2 (CH), 52.9 (CH_3) 67.2 (CH_2), 125.6 (CH), 128.2 (2 × CH), 128.3 (CH), 128.6 (4 × CH), 129.2 (2 × CH), 131.0 (CH), 134.2 (C), 136.4 (C), 144.2 (CH), 156.2 (C), 171.7 (C), 197.6 (C); *m/z* (CI) 368.1506 (MH^+. $C_{21}H_{22}NO_5$ requires 368.1498), 326 (8%), 260 (23), 234 (21), 219 (18), 181 (6), 147 (17), 107 (16), 85 (100).

Methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(4'-methoxyphenyl)-4-oxohex-5-enoate (117)



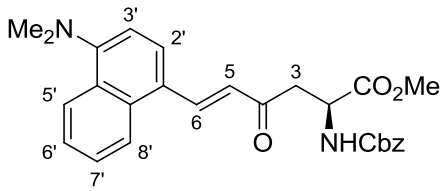
The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxo-6-phenylhex-5-enoate (**116**) using methyl (2*S*,5*E*)-6-(4'-methoxyphenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**100**) (0.932 g, 1.84 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 40% ethyl acetate in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(4'-methoxyphenyl)-4-oxohex-5-enoate (**117**) as a colourless oil (0.636 g, 87%). R_f 0.21 (40% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 3347 (NH), 2953 (CH), 1717 (C=O), 1655 (C=O), 1597 (C=C), 1510 (C=C), 1248, 1208, 1169, 1026, 816; $[\alpha]_D^{29}$ +30.3 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.23 (1H, dd, *J* 17.9, 4.2 Hz, 3-*H*H), 3.47 (1H, dd, *J* 17.9, 4.2 Hz, 3-H*H*), 3.75 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 4.67 (1H, dt, *J* 8.5, 4.2 Hz, 2-H), 5.12 (2H, s, OCH_2Ph), 5.88 (1H, d, *J* 8.5 Hz, NH), 6.58 (1H, d, *J* 16.2 Hz, 5-H), 6.92 (2H, d, *J* 8.8 Hz, 3'-H and 5'-H), 7.27−7.38 (5H, m, Ph), 7.49 (2H, d, *J* 8.8 Hz, 2'-H and 6'-H), 7.52 (1H, d, *J* 16.2 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 42.3 (CH_2), 50.3 (CH), 52.8 (CH_3), 55.6 (CH_3), 67.1 (CH_2), 114.6 (2 × CH), 123.4 (CH), 126.9 (C), 128.1 (2 × CH), 128.2 (CH), 128.6 (2 × CH), 130.4 (2 × CH), 136.4 (C), 144.0 (CH), 156.2 (C), 162.1 (C), 171.8 (C), 197.4 (C); *m/z* (EI) 397.1526 (M^+. $C_{22}H_{23}NO_6$ requires 397.1525), 336 (10%), 289 (19), 262 (19), 243 (45), 182 (34), 161 (100), 91 (32).

Methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(naphthalen-2'-yl)-4-oxohex-5-enoate (118)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxo-6-phenylhex-5-enoate (**116**) using methyl (2*S*,5*E*)-6-(naphthalen-2'-yl)-4-oxo-2-(tritylamino)hex-5-enoate (**101**) (0.736 g, 1.40 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 40% ethyl acetate in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(naphthalen-2'-yl)-4-oxohex-5-enoate (**118**) (0.497 g, 85%) as a colourless oil. R_f 0.26 (40% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 3339 (NH), 2951 (CH), 1717 (C=O), 1659 (C=O), 1505 (C=C), 1207, 1057, 976, 812; $[\alpha]_D^{28}$ +27.5 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.31 (1H, dd, *J* 18.0, 4.2 Hz, 3-*H*H), 3.54 (1H, dd, *J* 18.0, 4.2 Hz, 3-H*H*), 3.77 (3H, s, OCH_3), 4.71 (1H, dt, *J* 8.6, 4.2 Hz, 2-H), 5.13 (2H, s, O*CH*$_2$Ph), 5.89 (1H, d, *J* 8.6 Hz, NH), 6.82 (1H, d, *J* 16.2 Hz, 5-H), 7.28−7.39 (5H, m, Ph), 7.51−7.58 (2H, m, 6'-H and 7'-H), 7.67 (1H, dd, *J* 8.6, 1.5 Hz, 3'-H), 7.73 (1H, d, *J* 16.2 Hz, 6-H), 7.82−7.90 (3H, m, 4'-H, 5'-H and 8'-H), 7.96 (1H, br s, 1'-H); δ_C (101 MHz, $CDCl_3$) 42.5 (CH_2), 50.2 (CH), 52.9 (CH_3), 67.2 (CH_2), 123.6 (CH), 125.7 (CH), 127.0 (CH), 127.7 (CH), 127.9 (CH), 128.1 (2 × CH), 128.3 (CH), 128.6 (2 × CH), 128.8 (CH), 129.0 (CH), 130.9 (CH), 131.7 (C), 133.4 (C), 134.6 (C), 136.4 (C), 144.3 (CH), 156.2 (C), 171.7 (C), 197.5 (C); *m/z* (CI) 418.1653 (MH^+. $C_{25}H_{24}NO_5$ requires 418.1654), 383 (32%), 310 (48), 275 (20), 147 (30), 107 (29).

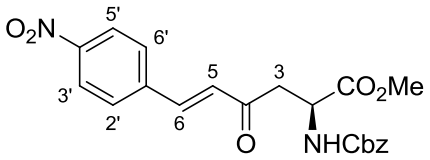
Methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-[4'-(dimethylamino)naphthalen-1'-yl]-4-oxohex-5-enoate (119)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxo-6-phenylhex-5-enoate (**116**) using methyl (2*S*,5*E*)-6-[4'-(dimethylamino)naphthalen-1'-yl]-4-oxo-2-(tritylamino)hex-5-enoate (**74**) (0.188 g, 0.331 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 30% ethyl acetate in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-

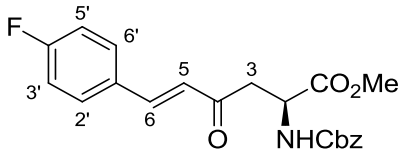
6-[4'-(dimethylamino)naphthalen-1'-yl]-4-oxohex-5-enoate (**119**) (0.086 g, 56%) as a yellow oil. R_f 0.12 (30% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 3423 (NH), 2953 (CH), 1720 (C=O), 1558 (C=C), 1209, 1045, 732; $[\alpha]_D^{24}$ +18.7 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.94 (6H, s, NMe_2), 3.31 (1H, dd, *J* 17.8, 4.2 Hz, 3-*H*H), 3.53 (1H, dd, *J* 17.8, 4.2 Hz, 3-H*H*), 3.76 (3H, s, OCH_3), 4.72 (1H, dt, *J* 8.6, 4.2 Hz, 2-H), 5.13 (2H, s, OC*H*$_2$Ph), 5.94 (1H, d, *J* 8.6 Hz, NH), 6.73 (1H, d, *J* 15.8 Hz, 5-H), 7.01 (1H, d, *J* 8.0 Hz, 3'-H), 7.25–7.37 (5H, m, Ph), 7.48–7.59 (2H, m, 6'-H and 7'-H), 7.74 (1H, d, *J* 8.0 Hz, 2'-H, 8.16 (1H, d, *J* 8.0 Hz, ArH), 8.20–8.26 (1H, m, ArH), 8.39 (1H, d, *J* 15.8 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 42.5 (CH_2), 44.8 (2 × CH_3), 50.2 (CH), 52.7 (CH_3), 67.0 (CH_2), 113.3 (CH), 123.4 (CH), 124.9 (C), 125.3 (2 × CH), 125.3 (CH), 126.0 (CH), 126.9 (CH), 128.0 (2 × CH), 128.1 (CH), 128.3 (C), 128.5 (2 × CH), 133.1 (C), 136.3 (C), 140.9 (CH), 154.0 (C), 156.1 (C), 171.8 (C), 197.2 (C); *m/z* (EI) 460.2003 (M^+. $C_{27}H_{28}N_2O_5$ requires 460.1998).

Methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(4'-nitrophenyl)-4-oxohex-5-enoate (90)



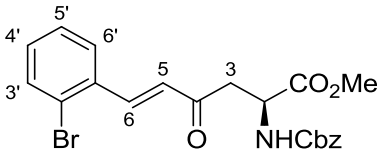
The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxo-6-phenylhex-5-enoate (**116**) using methyl (2*S*,5*E*)-6-(4'-nitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**102**) (1.55 g, 2.97 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 40% ethyl acetate in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(4'-nitrophenyl)-4-oxohex-5-enoate (**90**) (0.943 g, 77%) as an yellow solid. Mp 73−74 °C; R_f 0.41 (40% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 3331 (NH), 2953 (CH), 1730 (C=O), 1686 (C=O), 1512 (C=C), 1343, 1202, 1059, 978, 860; $[\alpha]_D^{28}$ +20.6 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.30 (1H, dd, *J* 18.1, 4.2 Hz, 3-*H*H), 3.48 (1H, dd, *J* 18.1, 4.2 Hz, 3-H*H*), 3.76 (3H, s, OCH_3), 4.70 (1H, dt, *J* 8.4, 4.2 Hz, 2-H), 5.12 (2H, s, OC*H*$_2$Ph), 5.82 (1H, d, *J* 8.4 Hz, NH), 6.80 (1H, d, *J* 16.2 Hz, 5-H), 7.29−7.37 (5H, m, Ph), 7.58 (1H, d, *J* 16.2 Hz, 6-H), 7.69 (2H, d, *J* 8.8 Hz, 2'-H and 6'-H), 8.26 (2H, d, *J* 8.8 Hz, 3'-H and 5'-H); δ_C (101 MHz, $CDCl_3$) 43.0 (CH_2), 50.2 (CH), 53.0 (CH_3), 67.3 (CH_2), 124.4 (2 × CH), 128.2 (2 × CH), 128.4 (CH), 128.7 (2 × CH), 128.9 (CH), 129.1 (2 × CH), 136.3 (C), 140.4 (C), 140.9 (CH), 149.0 (C), 156.2 (C), 171.5 (C), 197.0 (C); *m/z* (CI) 413.1352 (MH^+. $C_{21}H_{21}N_2O_7$ requires 413.1349), 383 (42%), 348 (38), 305 (30), 275 (30), 257 (23), 137 (68), 91 (68), 69 (100).

Methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(4'-fluorophenyl)-4-oxohex-5-enoate (120)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxo-6-phenylhex-5-enoate (**116**) using methyl (2*S*,5*E*)-6-(4-fluorophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**104**) (0.367 g, 0.744 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 30% ethyl acetate in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(4'-fluorophenyl)-4-oxohex-5-enoate (**120**) (0.174 g, 57%) as a white solid. Mp 66–69 °C; R_f 0.20 (30% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 3333 (NH), 2951 (CH), 1685 (C=O), 1508 (C=C), 1236, 1161, 981, 829, 698; $[\alpha]_D^{27}$ +26.5 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.24 (1H, dd, *J* 17.9, 4.2 Hz, 3-*H*H), 3.46 (1H, dd, *J* 17.9, 4.2 Hz, 3-H*H*), 3.75 (3H, s, OCH_3), 4.68 (1H, dt, *J* 8.5, 4.2 Hz, 2-H), 5.12 (2H, s, O*C*H_2Ph), 5.84 (1H, d, *J* 8.5 Hz, NH), 6.62 (1H, d, *J* 16.2 Hz, 5-H), 7.06–7.13 (2H, m, 3'-H and 5'-H), 7.28–7.39 (5H, m, Ph), 7.49–7.58 (3H, m, 6-H, 2'-H and 6'-H); δ_C (101 MHz, $CDCl_3$) 42.5 (CH_2), 50.2 (CH), 52.9 (CH_3), 67.2 (CH_2), 116.4 (d, $J_{C\text{-}C\text{-}F}$ 22.0 Hz, 2 × CH), 125.3 (CH), 128.2 (2 × CH), 128.3 (CH), 128.7 (2 × CH), 130.5 (C), 130.5 (d, $J_{C\text{-}C\text{-}C\text{-}F}$ 8.5 Hz, 2 × CH), 136.4 (C), 142.9 (CH), 156.2 (C), 164.4 (d, $J_{C\text{-}F}$ 252.6 Hz, C), 171.7 (C), 197.4 (C); *m/z* (ESI) 408.1201 (MNa^+. $C_{21}H_{20}FNNaO_5$ requires 408.1218).

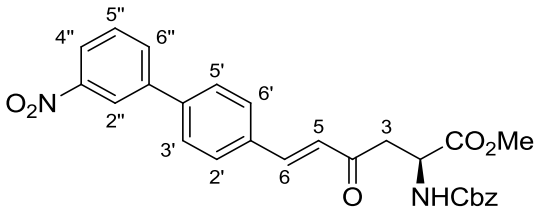
Methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(2'-bromophenyl)-4-oxohex-5-enoate (121)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxo-6-phenylhex-5-enoate (**116**) using methyl (2*S*,5*E*)-6-(2'-bromophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**105**) (0.223 g, 0.402 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 30% ethyl acetate in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(2'-bromophenyl)-4-oxohex-5-enoate (**121**) (0.131 g, 73%) as a yellow oil. R_f 0.18 (30% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 3338 (NH), 2960 (CH), 1687 (C=O), 1533 (C=C), 1286, 1087, 979, 746; $[\alpha]_D^{27}$ +22.1 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 3.30 (1H, dd, *J* 18.0, 4.1 Hz, 3-*H*H), 3.52 (1H, dd,

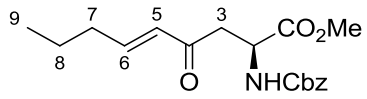
J 18.0, 4.1 Hz, 3-H*H*), 3.76 (3H, s, OCH_3), 4.71 (1H, dt, *J* 8.5, 4.1 Hz, 2-H), 5.12 (2H, s, OCH_2Ph), 5.85 (1H, d, *J* 8.5 Hz, NH), 6.62 (1H, d, *J* 16.3 Hz, 5-H), 7.23–7.38 (7H, m, 4'-H, 5'-H and Ph), 7.62 (2H, td, *J* 8.1, 0.9 Hz, 3'-H and 6'-H), 7.94 (1H, d, *J* 16.3 Hz, 6-H); δ_C (126 MHz, $CDCl_3$) 42.4 (CH_2), 50.2 (CH), 52.9 (CH_3), 67.2 (CH_2), 126.0 (C), 127.9 (CH), 128.0 (CH), 128.2 (3 × CH), 128.3 (CH), 128.7 (2 × CH), 131.9 (CH), 133.7 (CH), 134.2 (C), 136.4 (C), 142.6 (CH), 156.2 (C), 171.7 (C), 197.5 (C); *m/z* (ESI) 468.0402 (MNa^+. $C_{21}H_{20}{}^{79}BrNNaO_5$ requires 468.0417).

Methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(3''-nitrobiphen-4'-yl)-4-oxohex-5-enoate (122)



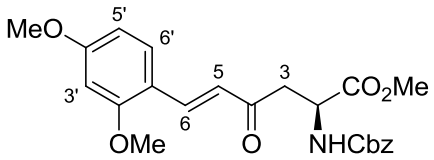
The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxo-6-phenylhex-5-enoate (**116**) using methyl (2*S*,5*E*)-6-(3''-nitrobiphen-4'-yl)-4-oxo-2-(tritylamino)hex-5-enoate (**106**) (0.324 g, 0.543 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 50% ethyl acetate in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(3''-nitrobiphen-4'-yl)-4-oxohex-5-enoate (**122**) (0.221 g, 84%) as a pale yellow solid. Mp 102−104 °C; R_f 0.50 (50% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 3325 (NH), 2953 (CH), 1742 (C=O), 1683 (C=O), 1664 (C=O), 1529 (C=C), 1342, 1179, 1057, 970, 802; $[\alpha]_D{}^{27}$ +17.2 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.29 (1H, dd, *J* 18.0, 4.2 Hz, 3-*H*H), 3.51 (1H, dd, *J* 18.0, 4.2 Hz, 3-H*H*), 3.77 (3H, s, OCH_3), 4.71 (1H, dt, *J* 8.5, 4.2 Hz, 2-H), 5.13 (2H, s, OCH_2Ph), 5.87 (1H, d, *J* 8.5 Hz, NH), 6.77 (1H, d, *J* 16.2 Hz, 5-H), 7.28−7.39 (5H, m, Ph), 7.61 (1H, d, *J* 16.2 Hz, 6-H), 7.64−7.70 (5H, m, 2'-H, 3'-H, 5'-H, 6'-H and 5"-H), 7.94 (1H, ddd, *J* 7.8, 2.0, 1.0 Hz, 6"-H), 8.24 (1H, ddd, *J* 8.2, 2.0, 1.0 Hz, 4"-H), 8.48 (1H, t, *J* 2.0 Hz, 2"-H); δ_C (101 MHz, $CDCl_3$) 42.6 (CH_2), 50.2 (CH), 52.9 (CH_3), 67.2 (CH_2), 122.0 (CH), 122.8 (CH), 126.2 (CH), 127.9 (2 × CH), 128.2 (2 × CH), 128.3 (CH), 128.7 (2 × CH), 129.4 (2 × CH), 130.1 (CH), 133.0 (CH), 134.5 (C), 136.4 (C), 141.0 (C), 141.8 (C), 143.1 (CH), 149.0 (C), 156.2 (C), 171.7 (C), 197.4 (C); *m/z* (CI) 489.1664 (MH^+. $C_{27}H_{25}N_2O_7$ requires 489.1662), 459 (8%), 418 (10), 381 (42), 351 (23), 338 (32), 310 (19), 275 (10), 181 (15), 147 (26), 91 (100).

Methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxonon-5-enoate (123)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxo-6-phenylhex-5-enoate (**116**) using methyl (2*S*,5*E*)-2-(tritylamino)-4-oxonon-5-enoate (**108**) (0.398 g, 0.902 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 30% ethyl acetate in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxonon-5-enoate (**123**) (0.201 g, 67%) as a white solid. Mp 40–42 °C; R_f 0.29 (30% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 3352 (NH), 2958 (CH), 1723 (C=O), 1502 (C=C), 1209, 1060, 978, 736, 698; $[\alpha]_D^{27}$ +23.7 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.94 (3H, t, *J* 7.4 Hz, 9-H_3), 1.44–1.55 (2H, m, 8-H_2), 2.20 (2H, qd, *J* 7.2, 1.4 Hz, 7-H_2), 3.11 (1H, dd, *J* 18.0, 4.2 Hz, 3-*H*H), 3.34 (1H, dd, *J* 18.0, 4.2 Hz, 3-H*H*), 3.73 (3H, s, OCH_3), 4.62 (1H, dt, *J* 8.5, 4.2 Hz, 2-H), 5.11 (2H, s, O*C*H_2Ph), 5.79 (1H, d, *J* 8.5 Hz, NH), 6.06 (1H, dt, *J* 15.8, 1.4 Hz, 5-H), 6.86 (1H, dt, *J* 15.8, 7.2 Hz, 6-H), 7.28–7.39 (5H, m, Ph); δ_C (101 MHz, $CDCl_3$) 13.8 (CH_3), 21.4 (CH_2), 34.7 (CH_2), 41.7 (CH_2), 50.1 (CH), 52.8 (CH_3), 67.2 (CH_2), 128.2 (2 × CH), 128.3 (CH), 128.7 (2 × CH), 130.1 (CH), 136.4 (C), 149.4 (CH), 156.2 (C), 171.8 (C), 197.8 (C); *m/z* (ESI) 356.1455 (MNa^+. $C_{18}H_{23}NNaO_5$ requires 356.1468).

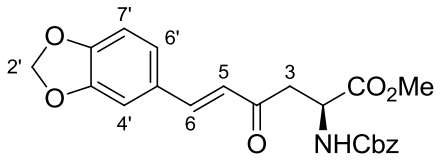
Methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(2',4'-dimethoxyphenyl)-4-oxohex-5-enoate (161)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxo-6-phenylhex-5-enoate (**116**) using methyl (2*S*,5*E*)-6-(2',4'-dimethoxyphenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**158**) (0.550 g, 1.03 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 40% ethyl acetate in petroleum ether (40–60) to give methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(2',4'-dimethoxyphenyl)-4-oxohex-5-enoate (**161**) (0.168 g, 70%) as a yellow oil. R_f 0.16 (40% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 3362 (NH), 2949 (CH), 1722 (C=O), 1600 (C=C), 1504, 1210, 1028, 698; $[\alpha]_D^{26}$ +19.6 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.23 (1H, dd, *J* 17.9, 4.2 Hz, 3-*H*H), 3.47 (1H, dd, *J* 17.9, 4.2 Hz, 3-H*H*), 3.73 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 4.67

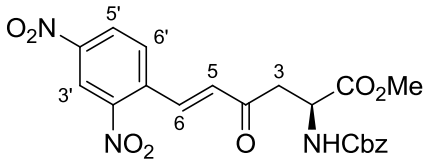
(1H, dt, *J* 8.7, 4.2 Hz, 2-H), 5.11 (2H, s, OC*H*$_2$Ph), 5.91 (1H, d, *J* 8.7 Hz, NH), 6.44 (1H, d, *J* 2.3 Hz, 3'-H), 6.50 (1H, dd, *J* 8.6, 2.3 Hz, 5'-H), 6.66 (1H, d, *J* 16.4 Hz, 5-H), 7.27–7.39 (5H, m, Ph), 7.45 (1H, d, *J* 8.6 Hz, 6'-H), 7.84 (1H, d, *J* 16.4 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 41.8 (CH_2), 50.2 (CH), 52.6 (CH_3), 55.5 (CH_3), 55.5 (CH_3), 66.9 (CH_2), 98.4 (CH), 105.6 (CH), 116.1 (C), 123.6 (CH), 128.0 (2 × CH), 128.1 (CH), 128.5 (2 × CH), 130.3 (CH), 136.3 (C), 139.4 (CH), 156.1 (C), 160.1 (C), 163.4 (C), 171.8 (C), 197.8 (C); *m/z* (ESI) 450.1503 (MNa^+. $C_{23}H_{25}NNaO_7$ requires 450.1523).

Methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(2*H*-1',3'-benzodioxol-5'-yl)-4-oxohex-5-enoate (162)



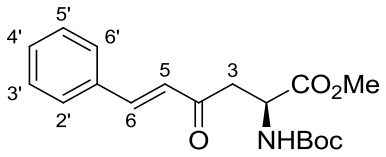
The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxo-6-phenylhex-5-enoate (**116**) using methyl (2*S*,5*E*)-6-(2*H*-1',3'-benzodioxol-5'-yl)-4-oxo-2-(tritylamino)hex-5-enoate (**159**) (1.51 g, 2.91 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 40% ethyl acetate in petroleum ether (40–60) to give methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(2*H*-1',3'-benzodioxol-5'-yl)-4-oxohex-5-enoate (**162**) (0.972 g, 81%) as a yellow oil. R_f 0.20 (40% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 3368 (NH), 2953 (CH), 1720 (C=O), 1502 (C=C), 1252, 980, 928, 736, 698; $[\alpha]_D^{26}$ +28.6 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.21 (1H, dd, *J* 17.9, 4.2 Hz, 3-*H*H), 3.43 (1H, dd, *J* 17.9, 4.2 Hz, 3-H*H*), 3.74 (3H, s, OCH_3), 4.67 (1H, dt, *J* 8.6, 4.2 Hz, 2-H), 5.11 (2H, s, OC*H*$_2$Ph), 5.88 (1H, d, *J* 8.6 Hz, NH), 6.01 (2H, s, 2'-H_2), 6.52 (1H, d, *J* 16.1 Hz, 5-H), 6.81 (1H, d, *J* 8.1 Hz, 7'-H), 6.99–7.05 (2H, m, 4'-H and 6'-H), 7.28–7.38 (5H, m, Ph), 7.46 (1H, d, *J* 16.1 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 42.3 (CH_2), 50.1 (CH), 52.7 (CH_3), 67.0 (CH_2), 101.7 (CH_2), 106.6 (CH), 108.7 (CH), 123.5 (CH), 125.3 (CH), 128.0 (2 × CH), 128.1 (CH), 128.5 (2 × CH), 128.5 (C), 136.3 (C), 143.9 (CH), 148.5 (C), 150.2 (C), 156.1 (C), 171.7 (C), 197.2 (C); *m/z* (ESI) 434.1192 (MNa^+. $C_{22}H_{21}NNaO_7$ requires 434.1210).

Methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(2',4'-dinitrophenyl)-4-oxohex-5-enoate (163)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxo-6-phenylhex-5-enoate (**116**) using methyl (2*S*,5*E*)-6-(2',4'-dinitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**160**) (0.620 g, 1.10 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 40% ethyl acetate in petroleum ether (40–60) to give methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(2',4'-dinitrophenyl)-4-oxohex-5-enoate (**163**) (0.293 g, 59%) as a brown solid. Mp 88–91 °C; R_f 0.15 (40% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 3382 (NH), 2955 (CH), 1719 (C=O), 1527 (C=C), 1343, 1211, 835, 735; $[\alpha]_D^{27}$ +19.7 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 3.34 (1H, dd, *J* 18.1, 4.3 Hz, 3-*H*H), 3.49 (1H, dd, *J* 18.1, 4.3 Hz, 3-H*H*), 3.76 (3H, s, OCH_3), 4.74 (1H, dt, *J* 8.5, 4.3 Hz, 2-H), 5.10 (2H, s, OCH_2Ph), 5.91 (1H, d, *J* 8.5 Hz, NH), 6.70 (1H, d, *J* 16.1 Hz, 5-H), 7.26–7.36 (5H, m, Ph), 7.85 (1H, d, *J* 8.6 Hz, 6'-H), 8.01 (1H, d, *J* 16.1 Hz, 6-H), 8.49 (1H, dd, *J* 8.6, 2.3 Hz, 5'-H), 8.89 (1H, d, *J* 2.3 Hz, 3'-H); δ_C (126 MHz, $CDCl_3$) 42.7 (CH_2), 49.9 (CH), 52.9 (CH_3), 67.0 (CH_2), 120.7 (CH), 127.8 (CH), 128.0 (2 × CH), 128.2 (CH), 128.5 (2 × CH), 130.7 (CH), 132.4 (CH), 136.1 (C), 136.4 (C), 136.9 (CH), 148.1 (C), 148.2 (C), 156.0 (C), 171.3 (C), 196.5 (C); *m/z* (ESI) 480.0997 (MNa^+. $C_{21}H_{19}N_3NaO_9$ requires 480.1014).

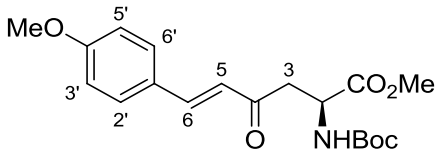
Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxo-6-phenylhex-5-enoate (124)



To a solution of methyl (2*S*,5*E*)-4-oxo-6-phenyl-2-(tritylamino)hex-5-enoate (**99**) (0.214 g, 0.449 mmol) in dichloromethane (5 mL) was added trifluoroacetic acid (0.344 mL, 4.490 mmol). The reaction mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated *in vacuo*, and then the residue was dissolved in water (10 mL) and washed with diethyl ether (2 × 10 mL). The aqueous layer was concentrated *in vacuo*, azeotroping with ethyl acetate-chloroform to give the TFA salt. The resulting TFA salt was dissolved in dichloromethane (5 mL) and triethylamine (0.124 mL, 0.898 mmol) was added followed by di-*tert*-butyl dicarbonate (0.196 g, 0.898 mmol). The reaction mixture was stirred at room temperature for 3 h before diluting with

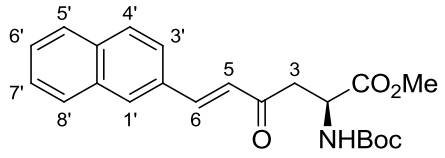
water (10 mL). The mixture was then extracted with dichloromethane (3 × 10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel, eluting with 40% diethyl ether in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxo-6-phenylhex-5-enoate (**124**) (0.097 g, 65%) as a colourless oil. R_f 0.22 (40% diethyl ether in petroleum ether); ν_{max}/cm^{-1} (NaCl) 3368 (NH), 2979 (CH), 1747 (C=O), 1713 (C=O), 1663 (C=C), 1496, 1367, 1168; $[\alpha]_D^{17}$ +56.9 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.44 (9H, s, 3 × CH_3), 3.33 (1H, dd, *J* 17.8, 4.3 Hz, 3-*H*H), 3.44 (1H, dd, *J* 17.8, 4.1 Hz, 3-H*H*), 3.75 (3H, s, OCH_3), 4.62 (1H, ddd, *J* 8.5, 4.3, 4.1 Hz, 2-H), 5.60 (1H, d, *J* 8.5 Hz, NH), 6.71 (1H, d, *J* 16.1 Hz, 5-H), 7.38–7.42 (3H, m, 3 × ArH), 7.52–7.55 (2H, m, 2 × ArH), 7.57 (1H, d, *J* 16.1 Hz, 6-H); δ_C (100 MHz, $CDCl_3$) 28.3 (3 × CH_3), 42.4 (CH_2), 49.6 (CH), 52.6 (CH_3), 79.9 (C), 125.6 (CH), 128.4 (2 × CH), 129.2 (2 × CH), 130.9 (CH), 134.1 (C), 143.9 (CH), 155.6 (C), 172.0 (C), 197.6 (C); *m/z* (CI) 334.1653 (MH^+. $C_{18}H_{24}NO_5$ requires 334.1654), 320 (4%), 278 (100), 234 (13).

Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(4'-methoxyphenyl)-4-oxohex-5-enoate (125)



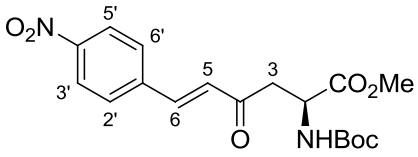
The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxo-6-phenylhex-5-enoate (**124**) using methyl (2*S*,5*E*)-6-(4'-methoxyphenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**100**) (0.132 g, 0.261 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 40% diethyl ether in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(4'-methoxyphenyl)-4-oxohex-5-enoate (**125**) (0.058 g, 62%) as a colourless oil. R_f 0.11 (40% diethyl ether in petroleum ether); ν_{max}/cm^{-1} (neat) 3367 (NH), 2970 (CH), 1708 (C=O), 1597 (C=C), 1512, 1249, 1112, 1026, 734; $[\alpha]_D^{26}$ +42.4 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.44 (9H, s, 3 × CH_3), 3.19 (1H, dd, *J* 17.7, 4.2 Hz, 3-*H*H), 3.41 (1H, dd, *J* 17.7, 4.2 Hz, 3-H*H*), 3.74 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 4.60 (1H, dt, *J* 8.5, 4.2 Hz, 2-H), 5.59 (1H, d, *J* 8.5 Hz, NH), 6.59 (1H, d, *J* 16.2 Hz, 5-H), 6.89–6.94 (2H, m, 3'-H and 5'-H), 7.46–7.56 (3H, m, 6-H, 2'-H and 6'-H); δ_C (101 MHz, $CDCl_3$) 28.5 (3 × CH_3), 42.4 (CH_2), 49.9 (CH), 52.7 (CH_3), 55.6 (CH_3), 80.1 (C), 114.7 (2 × CH), 123.5 (CH), 127.0 (C), 130.4 (2 × CH), 143.9 (CH), 155.7 (C), 162.1 (C), 172.2 (C), 197.7 (C); *m/z* (ESI) 386.1557 (MNa^+. $C_{19}H_{25}NNaO_6$ requires 386.1574).

Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(naphthalen-2'-yl)-4-oxohex-5-enoate (126)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxo-6-phenylhex-5-enoate (**124**) using methyl (2*S*,5*E*)-6-(naphthalen-2'-yl)-4-oxo-2-(tritylamino)hex-5-enoate (**101**) (0.191 g, 0.363 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 40% diethyl ether in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(naphthalen-2'-yl)-4-oxohex-5-enoate (**126**) (0.088 g, 64%) as a white solid. Mp 73–75 °C; R_f 0.14 (40% diethyl ether in petroleum ether); ν_{max}/cm^{-1} (neat) 3475 (NH), 2951 (CH), 1724 (C=O), 1481, 1367, 1155, 1091, 817, 750; $[\alpha]_D^{26}$ +40.8 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.45 (9H, s, 3 × CH_3), 3.27 (1H, dd, *J* 17.7, 4.1 Hz, 3-*H*H), 3.49 (1H, dd, *J* 17.7, 4.1 Hz, 3-H*H*), 3.76 (3H, s, OCH_3), 4.61–4.67 (1H, m, 2-H), 5.61 (1H, d, *J* 8.5 Hz, NH), 6.82 (1H, d, *J* 16.2 Hz, 5-H), 7.50–7.57 (2H, m, 6'-H and 7'-H), 7.67 (1H, dd, *J* 8.6, 1.5 Hz, 3'-H), 7.73 (1H, d, *J* 16.2 Hz, 6-H), 7.82–7.90 (3H, m, 4'-H, 5'-H and 8'-H), 7.96 (1H, br s, 1'-H); δ_C (126 MHz, $CDCl_3$) 28.5 (3 × CH_3), 42.7 (CH_2), 49.9 (CH), 52.8 (CH_3), 80.2 (C), 123.6 (CH), 125.9 (CH), 127.0 (CH), 127.7 (CH), 128.0 (CH), 128.8 (CH), 129.0 (CH), 130.9 (CH), 131.8 (C), 133.5 (C), 134.7 (C), 144.2 (CH), 155.8 (C), 172.2 (C), 197.7 (C); *m/z* (ESI) 406.1609 (MNa^+. $C_{22}H_{25}NNaO_5$ requires 406.1625).

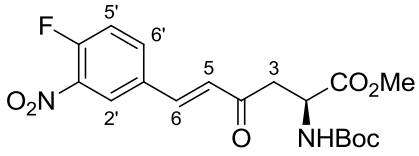
Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(4'-nitrophenyl)-4-oxohex-5-enoate (127)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxo-6-phenylhex-5-enoate (**124**) using methyl (2*S*,5*E*)-6-(4'-nitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**102**) (0.152 g, 0.292 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 60% diethyl ether in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(4'-nitrophenyl)-4-oxohex-5-enoate (**127**) (0.087 g, 79%) as an pale yellow oil. R_f 0.23 (60% diethyl ether in petroleum ether); ν_{max}/cm^{-1} (neat) 3365 (NH), 2953 (CH), 1705 (C=O), 1518 (C=C), 1342, 1161, 862, 744; $[\alpha]_D^{26}$ +36.7 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.44 (9H, s, 3 × CH_3), 3.27 (1H, dd, *J*

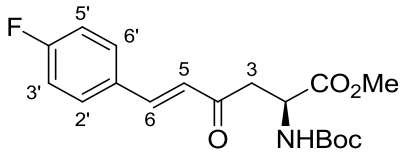
17.9, 4.2 Hz, 3-*H*H), 3.44 (1H, dd, *J* 17.9, 4.2 Hz, 3-H*H*), 3.75 (3H, s, OCH_3), 4.63 (1H, dt, *J* 8.3, 4.2 Hz, 2-H), 5.55 (1H, d, *J* 8.3 Hz, NH), 6.81 (1H, d, *J* 16.3 Hz, 5-H), 7.58 (1H, d, *J* 16.3 Hz, 6-H), 7.69 (2H, d, *J* 8.8 Hz, 2'-H and 6'-H), 8.26 (2H, d, *J* 8.8 Hz, 3'-H and 5'-H); δ_C (126 MHz, $CDCl_3$) 28.3 (3 × CH_3), 43.1 (CH_2), 49.6 (CH), 52.7 (CH_3), 80.2 (C), 124.3 (2 × CH), 128.9 (CH), 129.0 (2 × CH), 140.3 (C), 140.6 (CH), 148.8 (C), 155.5 (C), 171.8 (C), 197.1 (C); *m/z* (ESI) 401.1305 (MNa^+. $C_{18}H_{22}N_2NaO_7$ requires 401.1319).

Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(4'-fluoro-3'-nitrophenyl)-4-oxohex-5-enoate (128)



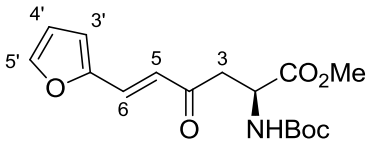
The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxo-6-phenylhex-5-enoate (**124**) using methyl (2*S*,5*E*)-6-(4'-fluoro-3'-nitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**103**) (0.272 g, 0.505 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 60% diethyl ether in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(4'-fluoro-3'-nitrophenyl)-4-oxohex-5-enoate (**128**) (0.165 g, 82%) as a yellow solid. Mp 90–93 °C; R_f 0.18 (60% diethyl ether in petroleum ether); ν_{max}/cm^{-1} (neat) 3367 (NH), 2989 (CH), 1739 (C=O), 1533 (C=C), 1153, 989, 825; $[\alpha]_D^{26}$ +37.5 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.44 (9H, s, 3 × CH_3), 3.24 (1H, dd, *J* 17.9, 4.2 Hz, 3-*H*H), 3.42 (1H, dd, *J* 17.9, 4.2 Hz, 3-H*H*), 3.75 (3H, s, OCH_3), 4.62 (1H, dt, *J* 8.3, 4.2 Hz, 2-H), 5.54 (1H, d, *J* 8.3 Hz, NH), 6.73 (1H, d, *J* 16.2 Hz, 5-H), 7.35 (1H, dd, *J* 10.1, 8.7 Hz, 5'-H), 7.52 (1H, d, *J* 16.2 Hz, 6-H), 7.79 (1H, ddd, *J* 8.7, 4.0, 2.2 Hz, 6'-H), 8.24 (1H, dd, *J* 7.0, 2.2 Hz, 2'-H); δ_C (126 MHz, $CDCl_3$) 28.5 (3 × CH_3), 43.3 (CH_2), 49.8 (CH), 52.9 (CH_3), 80.3 (C), 119.5 (d, $J_{C\text{-}C\text{-}F}$ 21.5 Hz, CH), 125.7 (d, $J_{C\text{-}C\text{-}C\text{-}F}$ 2.1 Hz, CH), 127.9 (CH), 131.6 (d, $J_{C\text{-}C\text{-}F}$ 4.4 Hz, C), 134.8 (d, $J_{C\text{-}C\text{-}C\text{-}F}$ 8.9 Hz, CH), 138.0 (C), 139.6 (CH), 155.7 (C), 156.5 (d, $J_{C\text{-}F}$ 270.0 Hz, C), 171.9 (C), 197.0 (C); *m/z* (CI) 397.1412 (MH^+. $C_{18}H_{22}FN_2O_7$ requires 397.1411), 367 (4%), 340 (100), 311 (13), 296 (14), 267 (5), 157 (3), 71 (13).

Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(4'-fluorophenyl)-4-oxohex-5-enoate (129)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxo-6-phenylhex-5-enoate (**124**) using methyl (2*S*,5*E*)-6-(4-fluorophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**104**) (0.107 g, 0.217 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 40% diethyl ether in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(4'-fluorophenyl)-4-oxohex-5-enoate (**129**) (0.047 g, 64%) as a pale yellow oil. R_f 0.15 (40% diethyl ether in petroleum ether); ν_{max}/cm^{-1} (neat) 3362 (NH), 2970 (CH), 1708 (C=O), 1508 (C=C), 1365, 1230, 1159, 817, 736; $[\alpha]_D^{26}$ +41.7 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.44 (9H, s, 3 × CH_3), 3.22 (1H, dd, *J* 17.8, 4.1 Hz, 3-*H*H), 3.42 (1H, dd, *J* 17.8, 4.1 Hz, 3-H*H*), 3.74 (3H, s, OCH_3), 4.56–4.66 (1H, m, 2-H), 5.57 (1H, d, *J* 8.5 Hz, NH), 6.63 (1H, d, *J* 16.3 Hz, 5-H), 7.05–7.15 (2H, m, 3'-H and 5'-H), 7.48–7.61 (3H, m, 6-H, 2'-H and 6'-H); δ_C (126 MHz, $CDCl_3$) 28.3 (3 × CH_3), 42.5 (CH_2), 49.7 (CH), 52.6 (CH_3), 80.0 (C), 116.2 (d, $J_{C\text{-}C\text{-}F}$ 22.0 Hz, 2 × CH), 125.3 (CH), 130.4 (d, $J_{C\text{-}C\text{-}C\text{-}F}$ 8.5 Hz, 2 × CH), 137.8 (C), 142.6 (CH), 155.6 (C), 164.2 (d, $J_{C\text{-}F}$ 252.5 Hz, C), 171.9 (C), 197.4 (C); *m/z* (ESI) 374.1361 (MNa^+. $C_{18}H_{22}FNNaO_5$ requires 374.1374).

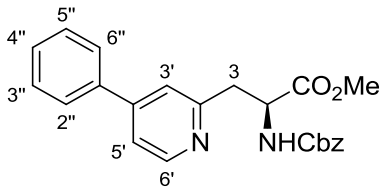
Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(furan-2-yl)-4-oxohex-5-enoate (130)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxo-6-phenylhex-5-enoate (**124**) using methyl (2*S,*5*E*)-6-(furan-2-yl)-4-oxo-2-(tritylamino)hex-5-enoate (**107**) (0.136 g, 0.292 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 50% diethyl ether in petroleum ether (40–60) to produce methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(furan-2-yl)-4-oxohex-5-enoate (**130**) (0.040 g, 43%) as a yellow oil. R_f 0.23 (50% diethyl ether in petroleum ether); ν_{max}/cm^{-1} (NaCl) 3362 (NH), 2978 (CH), 1747 (C=O), 1709 (C=O), 1609 (C=O), 1495 (C=C); $[\alpha]_D^{24}$ +48.6 (c 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.44 (9H, s, 3 × CH_3), 3.16 (1H, dd, *J* 18.0, 4.4 Hz, 3-*H*H), 3.37 (1H, dd, *J* 18.0, 4.4 Hz, 3-H*H*), 3.74 (3H, s, OCH_3), 4.59 (1H, m, 2-H), 5.58 (1H, d, *J* 8.8 Hz, NH), 6.50 (1H, dd, *J* 3.6, 1.6 Hz,

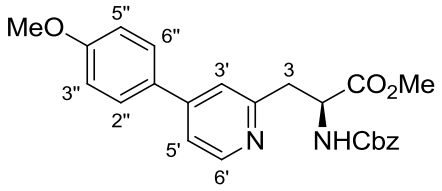
4'-H), 6.60 (1H, d, *J* 16.0 Hz, 5-H, 6.69 (1H, d, *J* 3.6 Hz, 3'-H), 7.32 (1H, d, *J* 16.0 Hz, 6-H), 7.51 (1H, d, *J* 1.6 Hz, 5'-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 42.8 (CH_2), 49.6 (CH), 52.6 (CH_3), 78.0 (C), 112.7 (CH), 116.5 (CH), 122.7 (CH), 126.7 (CH), 145.4 (CH), 150.7 (C), 155.6 (C), 172.0 (C), 197.2 (C); *m/z* (ESI) 323.1349 (MNa^+. $C_{16}H_{21}NNaO_6$ requires 323.1363).

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-phenylpyridin-2'-yl)propanoate (142)



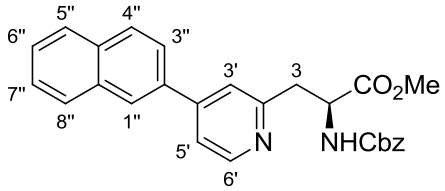
To a solution of methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxo-6-phenylhex-5-enoate (**116**) (0.186 g, 0.506 mmol) in ethyl vinyl ether (5 mL) was added tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)ytterbium (0.0269 g, 0.0253 mmol) and the sealed tube purged with argon. The reaction mixture was stirred at 110 °C for 96 h. The mixture was then allowed to cool to room temperature and concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel, eluting with 20% ethyl acetate in petroleum ether (40–60) to produce dihydropyran intermediate **133** (0.208 g, 93%). Intermediate **133** (0.208 g, 0.476 mmol) was then treated with hydroxylamine hydrochloride (0.166 g, 2.38 mmol) in acetonitrile (5 mL) at 70 °C for 16 h. The crude product was purified via flash column chromatography on neutral alumina (Brockmann V grade), eluting with 1% methanol in dichloromethane to produce methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-phenylpyridin-2'-yl)propanoate (**142**) (0.132 g, 71%) as a yellow oil. R_f 0.79 (1% methanol in dichloromethane on alumina); ν_{max}/cm^{-1} (neat) 3345 (NH), 2951 (CH), 1718 (C=O), 1506 (C=C), 1212, 1060, 763, 697; $[\alpha]_D^{22}$ +27.6 (*c* 1.1, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 3.36 (1H, dd, *J* 14.9, 5.2 Hz, 3-*H*H), 3.44 (1H, dd, *J* 14.9, 5.2 Hz, 3-H*H*), 3.70 (3H, s, OCH_3), 4.81 (1H, dt, *J* 8.3, 5.2 Hz, 2-H), 5.10 (1H, d, *J* 12.4 Hz, OC*H*HPh), 5.13 (1H, d, *J* 12.4 Hz, OCH*H*Ph), 6.35 (1H, d, *J* 8.3 Hz, NH), 7.29–7.37 (7H, m, 3'-H, 5'-H and Ph), 7.42–7.50 (3H, m, 3 × ArH), 7.56–7.63 (2H, m, 2 × ArH), 8.51 (1H, d, *J* 5.1 Hz, 6'-H); δ_C (126 MHz, $CDCl_3$) 39.2 (CH_2), 52.5 (CH_3), 53.6 (CH), 67.0 (CH_2), 120.1 (CH), 121.8 (CH), 127.1 (2 × CH), 128.2 (3 × CH), 128.6 (2 × CH), 129.2 (2 × CH), 129.3 (CH), 136.6 (C), 138.1 (C), 149.3 (C), 149.7 (CH), 156.2 (C), 157.6 (C), 172.2 (C); *m/z* (ESI) 413.1461 (MNa^+. $C_{23}H_{22}N_2NaO_4$ requires 413.1472).

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-methoxyphenyl)pyridin-2'-yl]propanoate (143)



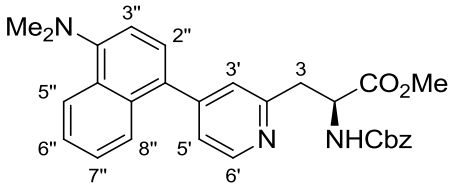
The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-phenylpyridin-2'-yl)propanoate (**142**) using methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(4'-methoxyphenyl)-4-oxohex-5-enoate (**117**) (0.602 g, 1.515 mmol) for 168 h. The crude product was purified via flash column chromatography on silica gel, eluting with 30% ethyl acetate in petroleum ether (40–60) to produce dihydropyran intermediate **134** (0.590 g, 83%). Intermediate **134** (0.590 g, 1.26 mmol) was then treated with hydroxylamine hydrochloride (0.438 g, 6.30 mmol) in acetonitrile (12 mL) at 70 °C for 16 h. The crude product was purified via flash column chromatography on neutral alumina (Brockmann V grade), eluting with 1% methanol in dichloromethane to produce methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-methoxyphenyl)pyridin-2'-yl]propanoate (**143**) (0.254 g, 48%) as a yellow oil. R_f 0.81 (1% methanol in dichloromethane on alumina); ν_{max}/cm^{-1} (neat) 3340 (NH), 2953 (CH), 1718 (C=O), 1602, 1516 (C=C), 1250, 1179, 1026, 825, 698; $[\alpha]_D^{25}$ +23.0 (*c* 0.8, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 3.33 (1H, dd, *J* 14.9, 5.2 Hz, 3-*H*H), 3.42 (1H, dd, *J* 14.9, 5.2 Hz, 3-H*H*), 3.70 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 4.80 (1H, dt, *J* 8.2, 5.2 Hz, 2-H), 5.09 (1H, d, *J* 12.3 Hz, OC*H*HPh), 5.13 (1H, d, *J* 12.3 Hz, OCH*H*Ph), 6.36 (1H, d, *J* 8.2 Hz, NH), 6.97–7.01 (2H, m, 3''-H and 5''-H), 7.29–7.35 (7H, m, 3'-H, 5'-H and Ph), 7.55 (2H, d, *J* 8.7 Hz, 2''-H and 6''-H), 8.47 (1H, d, *J* 5.2 Hz, 6'-H); δ_C (126 MHz, $CDCl_3$) 39.2 (CH_2), 52.5 (CH_3), 53.6 (CH), 55.6 (CH_3), 67.0 (CH_2), 114.7 (2 × CH_2), 119.6 (CH), 121.2 (CH), 128.2 (3 × CH), 128.3 (2 × CH), 128.6 (2 × CH), 130.4 (C), 136.6 (C), 148.8 (C), 149.6 (CH), 156.3 (C), 157.5 (C), 160.8 (C), 172.3 (C); *m/z* (ESI) 443.1556 (MNa^+. $C_{24}H_{24}N_2NaO_5$ requires 443.1577).

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(naphthalen-2''-yl)pyridin-2'-yl]propanoate (144)



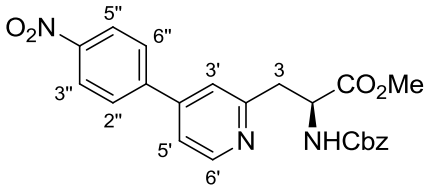
The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-phenylpyridin-2'-yl)propanoate (**142**) using methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(naphthalen-2'-yl)-4-oxohex-5-enoate (**118**) (0.110 g, 0.264 mmol) for 72 h. The crude product was purified via flash column chromatography on silica gel, eluting with 40% ethyl acetate in petroleum ether (40–60) to produce dihydropyran intermediate **135** (0.129 g, 83%). Intermediate **135** (0.129 g, 0.264 mmol) was then treated with hydroxylamine hydrochloride (0.0917 g, 1.32 mmol) in acetonitrile (3 mL) at 70 °C for 16 h. The crude product was purified via flash column chromatography on neutral alumina (Brockmann V grade), eluting with 1% methanol in dichloromethane to produce methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(naphthalen-2''-yl)pyridin-2'-yl]propanoate (**144**) (0.056 g, 48%) as a yellow solid. Mp 56–60 °C; R_f 0.84 (1% methanol in dichloromethane on alumina); ν_{max}/cm^{-1} (neat) 3345 (NH), 3054 (CH), 2951 (CH), 1716 (C=O), 1597 (C=C), 1503, 1437, 1210, 1056, 1028; $[\alpha]_D^{25}$ +26.1 (*c* 0.9, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 3.39 (1H, dd, *J* 15.0, 5.2 Hz, 3-*H*H), 3.48 (1H, dd, *J* 15.0, 5.2 Hz, 3-H*H*), 3.71 (3H, s, OCH_3), 4.84 (1H, dt, *J* 8.4, 5.2 Hz, 2-H), 5.10 (1H, d, *J* 12.4 Hz, OC*H*HPh), 5.13 (1H, d, *J* 12.4 Hz, OCH*H*Ph), 6.37 (1H, d, *J* 8.4 Hz, NH), 7.27–7.37 (5H, m, Ph), 7.45–7.49 (2H, m, 3'-H and 5'-H), 7.52–7.56 (2H, m, 2 × ArH), 7.70 (1H, dd, *J* 8.6, 1.5 Hz, 3''-H), 7.86–7.92 (2H, m, 2 × ArH), 7.94 (1H, d, *J* 8.6 Hz, 4''-H), 8.07 (1H, br s, 1''-H), 8.55 (1H, d, *J* 5.1 Hz, 6'-H); δ_C (126 MHz, $CDCl_3$) 39.3 (CH_2), 52.6 (CH_3), 53.6 (CH), 67.1 (CH_2), 120.3 (CH), 121.9 (CH), 124.7 (CH), 126.6 (CH), 126.9 (CH), 127.0 (CH), 127.9 (CH), 128.2 (3 × CH), 128.6 (CH), 128.6 (2 × CH), 129.1 (CH), 133.6 (C), 133.6 (C), 135.4 (C), 136.5 (C), 149.2 (C), 149.8 (CH), 156.3 (C), 157.7 (C), 172.3 (C); *m/z* (EI) 440.1738 (M^+. $C_{27}H_{24}N_2O_4$ requires 440.1736), 381 (17%), 332 (45), 305 (43), 273 (76), 245 (96), 219 (100).

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-{4'-[4''-(dimethylamino)naphthalen-1''-yl]pyridin-2'-yl}propanoate (145)



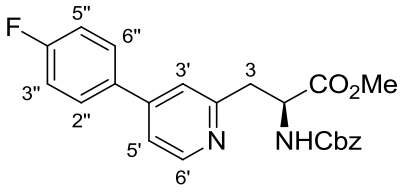
The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-phenylpyridin-2'-yl)propanoate (**142**) using methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-[4'-(dimethylamino)naphthalen-1'-yl]-4-oxohex-5-enoate (**119**) (0.0419 g, 0.0910 mmol) for 168 h. The crude product was purified via flash column chromatography on silica gel, eluting with 30% ethyl acetate in petroleum ether (40–60) to produce dihydropyran intermediate **136** (0.048 g, 89%). Intermediate **136** (0.0480 g, 0.0901 mmol) was then treated with hydroxylamine hydrochloride (0.0313 g, 0.451 mmol) in acetonitrile (2 mL) at 70 °C for 16 h. The crude product was purified via flash column chromatography on neutral alumina (Brockmann V grade), eluting with 1% methanol in dichloromethane to produce methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-{4'-[4''-(dimethylamino)naphthalen-1''-yl]pyridin-2'-yl}propanoate (**145**) (0.0118 g, 27%) as a yellow oil. R_f 0.82 (1% methanol in dichloromethane on alumina); ν_{max}/cm^{-1} (neat) 3349 (NH), 2947 (CH), 1722 (C=O), 1580 (C=C), 1208, 1048, 771; $[\alpha]_D^{24}$ +10.5 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.94 (6H, s, NMe_3), 3.35 (1H, dd, *J* 14.9, 5.1 Hz, 3-*H*H), 3.44 (1H, dd, *J* 14.9, 5.1 Hz, 3-H*H*), 3.72 (3H, s, OCH_3), 4.82 (1H, dt, *J* 8.4, 5.1 Hz, 2-H), 5.10 (1H, d, *J* 12.6 Hz, OC*H*HPh), 5.14 (1H, d, *J* 12.6 Hz, OCH*H*Ph), 6.40 (1H, d, *J* 8.4 Hz, NH), 7.10 (1H, d, *J* 7.7 Hz, 2''-H), 7.23–7.38 (8H, m, 3'-H, 5'-H, 3''-H and Ph), 7.40–7.46 (1H, m, ArH), 7.51 (1H, ddd, *J* 8.4, 6.8, 0.9 Hz, ArH), 7.79 (1H, d, *J* 8.4 Hz, ArH), 8.31 (1H, dd, *J* 8.4, 0.9 Hz, ArH), 8.56 (1H, d, *J* 5.0 Hz, 6'-H); δ_C (101 MHz, $CDCl_3$) 39.0 (CH_2), 45.1 (2 × CH_3), 52.4 (CH_3), 53.5 (CH), 66.9 (CH_2), 113.4 (CH), 123.5 (CH), 124.8 (CH), 125.1 (CH), 125.4 (CH), 125.6 (CH), 126.5 (CH), 127.0 (CH), 128.1 (CH), 128.1 (2 × CH), 128.5 (2 × CH), 128.8 (C), 131.7 (C), 132.0 (C), 136.4 (C), 149.1 (CH), 149.8 (C), 151.7 (C), 156.1 (C), 156.9 (C), 172.2 (C); *m/z* (ESI) 484.2211 (MH^+. $C_{29}H_{30}N_3O_4$ requires 484.2231).

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-nitrophenyl)pyridin-2'-yl]propanoate (146)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-phenylpyridin-2'-yl)propanoate (**142**) using methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(4'-nitrophenyl)-4-oxohex-5-enoate (**90**) (0.104 g, 0.252 mmol) for 96 h. The crude product was purified via flash column chromatography on silica gel, eluting with 30% ethyl acetate in petroleum ether (40–60) to produce dihydropyran intermediate **137** (0.097 g, 79%). Intermediate **137** (0.0968 g, 0.199 mmol) was then treated with hydroxylamine hydrochloride (0.0691 g, 0.995 mmol) in acetonitrile (2 mL) at 70 °C for 16 h. The crude product was purified via flash column chromatography on neutral alumina (Brockmann V grade), eluting with 1% methanol in dichloromethane to produce methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-nitrophenyl)pyridin-2'-yl]propanoate (**146**) (0.035 g, 40%) as a yellow solid. Mp 79–82 °C; R_f 0.79 (1% methanol in dichloromethane on neutral alumina); ν_{max}/cm^{-1} (neat) 3349 (NH), 2952 (CH), 1718 (C=O), 1595 (C=C), 1346 (CH), 1265, 1211, 1175, 1108, 1051; $[\alpha]_D^{25}$ +25.7 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 3.40 (1H, dd, *J* 15.1, 5.1 Hz, 3-*H*H), 3.48 (1H, dd, *J* 15.1, 5.1 Hz, 3-H*H*), 3.72 (3H, s, OCH_3), 4.84 (1H, dt, *J* 8.4, 5.1 Hz, 2-H), 5.09 (1H, d, *J* 12.3 Hz, OC*H*HPh), 5.13 (1H, d, *J* 12.3 Hz, OCH*H*Ph), 6.21 (1H, d, *J* 8.4 Hz, NH), 7.28–7.39 (7H, m, 3'-H, 5'-H and Ph), 7.74 (2H, d, *J* 8.7 Hz, 2''-H and 6''-H), 8.33 (2H, d, *J* 8.7 Hz, 3''-H and 5''-H), 8.59 (1H, d, *J* 5.1 Hz, 6'-H); δ_C (126 MHz, $CDCl_3$) 39.2 (CH_2), 52.5 (CH_3), 53.3 (CH), 67.0 (CH_2), 120.0 (CH), 121.8 (CH), 124.4 (2 × CH), 128.1 (2 × CH), 128.1 (2 × CH), 128.2 (CH), 128.5 (2 × CH), 136.3 (C), 144.4 (C), 146.7 (C), 148.3 (C), 150.0 (CH), 156.0 (C), 158.2 (C), 172.0 (C); *m/z* (ESI) 434.1347 ($[M-H]^-$. $C_{23}H_{20}N_3O_6$ requires 434.1358).

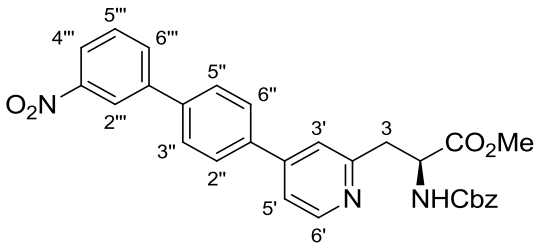
Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-fluorophenyl)pyridin-2'-yl]propanoate (147)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-phenylpyridin-2'-yl)propanoate (**142**) using methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxo-6-phenylhex-5-enoate (**120**) (0.126 g, 0.310 mmol) for 96 h. The

crude product was purified via flash column chromatography on silica gel, eluting with 30% ethyl acetate in petroleum ether (40–60) to produce dihydropyran intermediate **138** (0.098 g, 70%). Intermediate **138** (0.0872 g, 0.191 mmol) was then treated with hydroxylamine hydrochloride (0.0616 g, 0.955 mmol) in acetonitrile (2 mL) at 70 °C for 16 h. The crude product was purified via flash column chromatography on neutral alumina (Brockmann V grade), eluting with 1% methanol in dichloromethane to produce methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-fluorophenyl)pyridin-2'-yl]propanoate (**147**) (0.047 g, 60%) as a white solid. Mp 48–52 °C; R_f 0.79 (1% methanol in dichloromethane on alumina); ν_{max}/cm^{-1} (neat) 3345 (NH), 2952 (CH), 1718 (C=O), 1605 (C=C), 1513, 1222, 1058, 827, 698; $[\alpha]_D^{22}$ +25.5 (*c* 0.6, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 3.35 (1H, dd, *J* 15.0, 5.2 Hz, 3-*H*H), 3.44 (1H, dd, *J* 15.0, 5.2 Hz, 3-H*H*), 3.70 (3H, s, OCH_3), 4.81 (1H, dt, *J* 8.3, 5.2 Hz, 2-H), 5.09 (1H, d, *J* 12.3 Hz, OC*H*HPh), 5.13 (1H, d, *J* 12.3 Hz, OCH*H*Ph), 6.31 (1H, d, *J* 8.3 Hz, NH), 7.13–7.19 (2H, m 3''-H and 5''-H), 7.28–7.36 (7H, m, 3'-H, 5'-H and Ph), 7.53–7.60 (2H, m, 2''-H and 6''-H), 8.51 (1H, d, *J* 5.1 Hz, 6'-H); δ_C (126 MHz, $CDCl_3$) 39.2 (CH_2), 52.5 (CH_3), 53.5 (CH), 67.1 (CH_2), 116.3 (d, $J_{C\text{-}C\text{-}F}$ 21.8 Hz, 2 × CH), 120.0 (CH), 121.6 (CH), 128.2 (3 × CH), 128.6 (2 × CH), 128.9 (d, $J_{C\text{-}C\text{-}C\text{-}F}$ 8.3 Hz, 2 × CH), 134.3 (d, $J_{C\text{-}C\text{-}C\text{-}C\text{-}F}$ 3.2 Hz, C), 136.5 (C), 148.2 (C), 149.8 (CH), 156.2 (C), 157.8 (C), 163.62 (d, $J_{C\text{-}F}$ 249.4 Hz, C), 172.2 (C); *m/z* (ESI) 431.1364 (MNa^+. $C_{23}H_{21}FN_2NaO_4$ requires 431.1378).

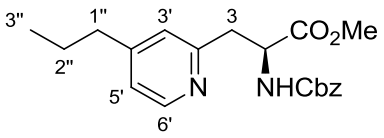
Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-{4'-[4''-(3'''-nitrophenyl)phenyl]pyridin-2'-yl}propanoate (148)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-phenylpyridin-2'-yl)propanoate (**142**) using methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(3''-nitrobiphen-4'-yl)-4-oxohex-5-enoate (**122**) (0.103 g, 0.211 mmol) for 96 h. The crude product was purified via flash column chromatography on silica gel, eluting with 30% ethyl acetate in petroleum ether (40–60) to produce dihydropyran intermediate **139** (0.095 g, 86%). Intermediate **139** (0.100 g, 0.191 mmol) was then treated with hydroxylamine hydrochloride (0.0664 g, 0.955 mmol) in acetonitrile (2 mL) at 70 °C for 16 h. The crude product was purified via flash column chromatography on neutral alumina (Brockmann V grade), eluting with 1% methanol in dichloromethane to produce methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-{4'-[4''-(3'''-nitrophenyl)phenyl]pyridin-2'-yl}propanoate (**148**) (0.035 g, 36%) as an off-white solid. Mp 48–

50 °C; R_f 0.70 (1% methanol in dichloromethane on alumina); ν_{max}/cm^{-1} (neat) 3339 (NH), 2951 (CH), 1718 (C=O), 1601 (C=C), 1518, 1349, 1212, 1061, 730; $[\alpha]_D^{24}$ +22.1 (*c* 0.6, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 3.38 (1H, dd, *J* 14.9, 5.2 Hz, 3-*H*H), 3.47 (1H, dd, *J* 14.9, 5.2 Hz, 3-H*H*), 3.72 (3H, s, OCH_3), 4.83 (1H, dt, *J* 8.3, 5.2 Hz, 2-H), 5.10 (1H, d, *J* 12.3 Hz, OC*H*HPh), 5.13 (1H, d, *J* 12.3 Hz, OCH*H*Ph), 6.34 (1H, d, *J* 8.3 Hz, NH), 7.27–7.36 (5H, m, Ph), 7.37–7.43 (2H, m, 3'-H and 5'-H), 7.65 (1H, t, *J* 8.0 Hz, 5'''-H), 7.71–7.78 (4H, m, 2''-H, 3''-H, 5''-H and 6''-H), 7.96 (1H, ddd, *J* 8.0, 1.9, 1.0 Hz, 6'''-H), 8.24 (1H, ddd, *J* 8.0, 1.9, 1.0 Hz, 4'''-H), 8.49–8.51 (1H, m, 2'''-H), 8.55 (1H, d, *J* 5.1 Hz, 6'-H); δ_C (126 MHz, $CDCl_3$) 39.2 (CH_2), 52.6 (CH_3), 53.5 (CH), 67.0 (CH_2), 120.0 (CH), 121.6 (CH), 122.0 (CH), 122.6 (CH), 127.9 (2 × CH), 128.0 (2 × CH), 128.2 (2 × CH), 128.2 (2 × CH), 128.6 (CH), 130.0 (CH), 133.0 (CH), 136.5 (C), 138.2 (C), 139.5 (C), 142.0 (C), 148.2 (C), 149.0 (CH), 149.9 (C), 156.2 (C), 157.8 (C), 172.2 (C); *m/z* (EI) 511.1717 (M^+. $C_{29}H_{25}N_3O_6$ requires 511.1743), 452 (18%), 376 (25), 344 (45), 279 (100), 108 (71), 79 (62).

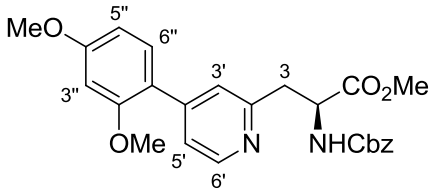
Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-propylpyridin-2'-yl]propanoate (149)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-phenylpyridin-2'-yl)propanoate (**142**) using methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxonon-5-enoate (**123**) (0.103 g, 0.309 mmol) for 96 h. The crude product was purified via flash column chromatography on silica gel, eluting with 20% ethyl acetate in petroleum ether (40–60) to produce dihydropyran intermediate **140** (0.108 g, 86%). Intermediate **140** (0.107 g, 0.264 mmol) was then treated with hydroxylamine hydrochloride (0.0917 g, 1.32 mmol) in acetonitrile (3 mL) at 70 °C for 16 h. The crude product was purified via flash column chromatography on neutral alumina (Brockmann V grade), eluting with 1% methanol in dichloromethane to produce methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-propylpyridin-2'-yl]propanoate (**149**) (0.054 g, 58%) as a pale yellow oil. R_f 0.85 (1% methanol in dichloromethane on alumina); ν_{max}/cm^{-1} (neat) 3343 (NH), 2958 (CH), 1720 (C=O), 1605 (C=C), 1506, 1209, 1055, 737, 697; $[\alpha]_D^{22}$ +18.0 (*c* 0.7, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 0.92 (3H, t, *J* 7.3 Hz, 3''-H), 1.57–1.67 (2H, m, 2''-H), 2.49–2.56 (2H, m, 1''-H), 3.23 (1H, dd, *J* 14.8, 5.2 Hz, 3-*H*H), 3.32 (1H, dd, *J* 14.8, 5.2 Hz, 3-H*H*), 3.67 (3H, s, OCH_3), 4.74 (1H, dt, *J* 8.2, 5.2 Hz, 2-H), 5.08 (1H, d, *J* 12.4 Hz, OC*H*HPh), 5.12 (1H, d, *J* 12.4 Hz, OCH*H*Ph), 6.38 (1H, d, *J* 8.2 Hz, NH), 6.91–6.97 (2H, m, 3'-H and 5'-H), 7.28–7.37 (5H, m, Ph), 8.33 (1H, d, *J* 5.0 Hz, 6'-H); δ_C (126 MHz, $CDCl_3$) 13.8 (CH_3), 23.5 (CH_2), 37.3 (CH_2), 38.9 (CH_2), 52.4 (CH_3), 53.6 (CH), 66.9 (CH_2), 122.3 (CH), 124.0 (CH), 128.2 (2 × CH), 128.2 (CH), 128.6 (2 × CH),

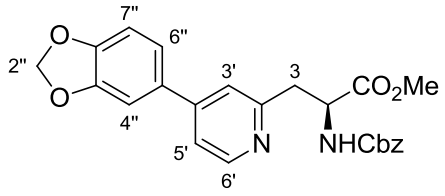
136.6 (C), 149.1 (CH), 152.3 (C), 156.2 (C), 156.9 (C), 172.2 (C); *m/z* (EI) 356.1729 (M^+. $C_{20}H_{24}N_2O_4$ requires 356.1736), 297 (36%), 207 (21), 189 (48), 135 (95), 91 (100).

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(2'',4''-dimethoxyphenyl)pyridin-2'-yl]propanoate (167)



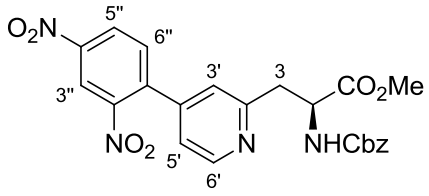
The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-phenylpyridin-2'-yl)propanoate (**142**) using methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(2',4'-dimethoxyphenyl)-4-oxohex-5-enoate (**161**) (0.140 g, 0.327 mmol) for 192 h. The crude product was purified via flash column chromatography on silica gel, eluting with 30% ethyl acetate in petroleum ether (40–60) to produce dihydropyran intermediate **164** (0.135 g, 83%). Intermediate **164** (0.135 g, 0.270 mmol) was then treated with hydroxylamine hydrochloride (0.0938 g, 1.35 mmol) in acetonitrile (5 mL) at 70 °C for 16 h. The crude product was purified via flash column chromatography on neutral alumina (Brockmann V grade), eluting with 1% methanol in dichloromethane to produce methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(2'',4''-dimethoxyphenyl)pyridin-2'-yl]propanoate (**167**) (0.069 g, 57%) as a pale yellow oil. R_f 0.78 (1% methanol in dichloromethane on alumina); ν_{max}/cm^{-1} (neat) 3342 (NH), 2951 (CH), 1718 (C=O), 1508 (C=C), 1207, 1028, 736, 698; $[\alpha]_D^{27}$ +22.9 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 3.30 (1H, dd, *J* 14.8, 5.2 Hz, 3-*H*H), 3.40 (1H, dd, *J* 14.8, 5.2 Hz, 3-H*H*), 3.70 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 4.77 (1H, dt, *J* 8.2, 5.2 Hz, 2-H), 5.09 (1H, d, *J* 12.6 Hz, OC*H*HPh), 5.12 (1H, d, *J* 12.6 Hz, OCH*H*Ph), 6.39 (1H, d, *J* 8.2 Hz, NH), 6.52–6.60 (2H, m, 3''-H and 5''-H), 7.22–7.38 (8H, m, 3'-H, 5'-H, 6''-H and Ph), 8.44 (1H, d, *J* 5.2 Hz, 6'-H); δ_C (126 MHz, $CDCl_3$) 39.0 (CH_2), 52.3 (CH_3), 53.5 (CH), 55.5 (CH_3), 55.5 (CH_3), 66.9 (CH_2), 99.1 (CH), 105.0 (CH), 120.3 (C), 122.4 (CH), 124.0 (CH), 128.0 (CH), 128.1 (2 × CH), 128.5 (2 × CH), 131.1 (CH), 136.4 (C), 146.8 (C), 148.8 (CH), 156.1 (C), 156.4 (C), 157.7 (C), 161.5 (C), 172.2 (C); *m/z* (ESI) 451.1846 (MH^+. $C_{25}H_{27}N_2O_6$ requires 451.1840).

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(2*H*-1'',3''-benzodioxol-5''-yl)pyridin-2'-yl]propanoate (168)



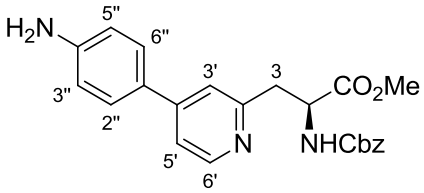
The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-phenylpyridin-2'-yl)propanoate (**142**) using methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(2*H*-1',3'-benzodioxol-5'-yl)-4-oxohex-5-enoate (**162**) (0.972 g, 2.36 mmol) for 168 h. The crude product was purified via flash column chromatography on silica gel, eluting with 30% ethyl acetate in petroleum ether (40–60) to produce dihydropyran intermediate **165** (0.990 g, 87%). Intermediate **165** (0.990 g, 2.05 mmol) was then treated with hydroxylamine hydrochloride (0.711 g, 10.2 mmol) in acetonitrile (20 mL) at 70 °C for 16 h. The crude product was purified via flash column chromatography on neutral alumina (Brockmann V grade), eluting with 1% methanol in dichloromethane to produce methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(2*H*-1'',3''-benzodioxol-5''-yl)pyridin-2'-yl]propanoate (**168**) (0.530 g, 59%) as an orange oil. R_f 0.80 (1% methanol in dichloromethane on alumina); ν_{max}/cm^{-1} (neat) 3358 (NH), 2953 (CH), 1720 (C=O), 1506 (C=C), 1475, 1236, 1039, 808; $[\alpha]_D^{27}$ +28.0 (*c* 1.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.35 (1H, dd, *J* 15.0, 5.1 Hz, 3-*H*H), 3.44 (1H, dd, *J* 15.0, 5.1 Hz, 3-H*H*), 3.72 (3H, s, OCH_3), 4.82 (1H, dt, *J* 8.2, 5.1 Hz, 2-H), 5.11 (1H, d, *J* 12.5 Hz, OC*H*HPh), 5.15 (1H, d, *J* 12.5 Hz, OCH*H*Ph), 6.05 (2H, s, 2''-H_2), 6.35 (1H, d, *J* 8.2 Hz, NH), 6.92 (1H, d, *J* 8.0 Hz, 7''-H), 7.07–7.15 (2H, m, 4''-H and 6''-H), 7.26–7.40 (7H, m, 3'-H, 5'-H and Ph), 8.49 (1H, d, *J* 5.2 Hz, 6'-H); δ_C (101 MHz, $CDCl_3$) 39.1 (CH_2), 52.4 (CH_3), 53.4 (CH), 66.9 (CH_2), 101.5 (CH_2), 107.2 (CH), 108.9 (CH), 119.6 (CH), 121.0 (CH), 121.2 (CH), 128.1 (3 × CH), 128.5 (2 × CH), 132.1 (C), 136.4 (C), 148.5 (C), 148.6 (C), 148.7 (C), 149.5 (CH), 156.1 (C), 157.4 (C), 172.1 (C); *m/z* (ESI) 457.1355 (MNa^+. $C_{24}H_{22}N_2NaO_6$ requires 457.1370).

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(2'',4''-dinitrophenyl)pyridin-2'-yl]propanoate (169)



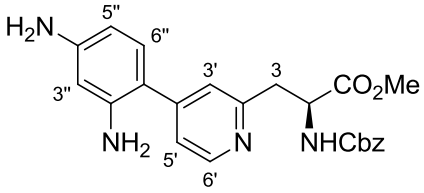
The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-phenylpyridin-2'-yl)propanoate (**142**) using methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(2',4'-dinitrophenyl)-4-oxohex-5-enoate (**163**) (0.198 g, 0.432 mmol) for 168 h. The crude product was purified via flash column chromatography on silica gel, eluting with 30% ethyl acetate in petroleum ether (40–60) to produce dihydropyran intermediate **166** (0.193 g, 84%). Intermediate **166** (0.193 g, 0.365 mmol) was then treated with hydroxylamine hydrochloride (0.127 g, 1.83 mmol) in acetonitrile (5 mL) at 70 °C for 16 h. The crude product was purified via flash column chromatography on neutral alumina (Brockmann V grade), eluting with 1% methanol in dichloromethane to produce methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(2'',4''-dinitrophenyl)pyridin-2'-yl]propanoate (**169**) (0.068 g, 38%) as an orange oil. R_f 0.87 (1% methanol in dichloromethane on alumina); ν_{max}/cm^{-1} (neat) 3363 (NH), 2953 (CH), 1720 (C=O), 1531 (C=C), 1346, 1211, 1055, 833, 740; $[\alpha]_D^{27}$ +19.5 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 3.38 (1H, dd, *J* 15.1, 5.2 Hz, 3-*H*H), 3.45 (1H, dd, *J* 15.1, 5.2 Hz, 3-H*H*), 3.72 (3H, s, OCH_3), 4.79–4.86 (1H, m, 2-H), 5.10 (1H, d, *J* 12.3 Hz, OC*H*HPh), 5.13 (1H, d, *J* 12.3 Hz, OCH*H*Ph), 6.16 (1H, d, *J* 8.3 Hz, NH), 7.07–7.11 (2H, m, 3'-H and 5'-H), 7.27–7.39 (5H, m, Ph), 7.59 (1H, d, *J* 8.4 Hz, 6''-H), 8.50 (1H, dd, *J* 8.4, 2.1 Hz, 5''-H), 8.60 (1H, d, *J* 5.7 Hz, 6'-H), 8.83 (1H, d, *J* 2.1 Hz, 3''-H); δ_C (126 MHz, $CDCl_3$) 39.2 (CH_2), 52.6 (CH_3), 53.1 (CH), 67.0 (CH_2), 120.2 (CH), 120.4 (CH), 122.2 (CH), 127.1 (CH), 128.2 (2 × CH), 128.2 (CH), 128.5 (2 × CH), 132.9 (CH), 136.3 (C), 139.6 (C), 144.2 (C), 147.8 (C), 148.5 (C), 149.7 (CH), 156.0 (C), 158.2 (C), 171.8 (C); *m/z* (ESI) 503.1154 (MNa^+. $C_{23}H_{20}N_4NaO_8$ requires 503.1173).

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-aminophenyl)pyridin-2'-yl]propanoate (170)



Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-nitrophenyl)pyridin-2'-yl]propanoate (**146**) (0.0206 g, 0.0473 mmol) was dissolved in anhydrous methanol (2 mL) and tin(II) chloride dihydrate (0.0530 g, 0.235 mmol) was added. The reaction was stirred at room temperature for 2 h. Once the reaction was complete, the solution was diluted with ethyl acetate (15 mL) and washed with saturated potassium fluoride solution (3 × 10 mL) and brine (10 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel, eluting with 40% ethyl acetate/1% triethylamine in dichloromethane to produce methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-aminophenyl)pyridin-2'-yl]propanoate (**170**) (0.0127 g, 65%) as a yellow oil. R_f 0.24 (40% ethyl acetate/1% triethylamine in dichloromethane); ν_{max}/cm^{-1} (neat) 3363 (NH), 2947 (CH), 1720 (C=O), 1597 (C=C), 1519, 1211, 1056, 825; $[\alpha]_D^{26}$ +25.6 (*c* 0.5, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 3.31 (1H, dd, *J* 14.8, 5.2 Hz, 3-*H*H), 3.41 (1H, dd, *J* 14.8, 5.2 Hz, 3H*H*), 3.69 (3H, s, OCH_3), 3.89 (2H, br s, NH_2), 4.78 (1H, dt, *J* 8.3, 5.2 Hz, 2-H), 5.09 (1H, d, *J* 12.4 Hz, OC*H*HPh), 5.12 (1H, d, *J* 12.4 Hz, OCH*H*Ph), 6.40 (1H, d, *J* 8.3 Hz, NH), 6.74 (2H, d, *J* 8.6 Hz, 3''-H and 5''-H), 7.27–7.37 (7H, m, 3'-H, 5'-H and Ph), 7.44 (2H, d, *J* 8.6 Hz, 2''-H and 6''-H), 8.42 (1H, d, *J* 5.2 Hz, 6'-H); δ_C (126 MHz, $CDCl_3$) 38.7 (CH_2), 52.4 (CH_3), 53.5 (CH), 66.9 (CH_2), 115.3 (2 × CH), 119.0 (CH), 120.6 (CH), 127.2 (C), 128.1 (2 × CH), 128.1 (CH), 128.1 (2 × CH), 128.5 (2 × CH), 136.4 (C), 147.9 (C), 148.7 (CH), 149.4 (C), 156.2 (C), 156.8 (C), 172.1 (C); *m/z* (ESI) 406.1745 (MH^+. $C_{23}H_{24}N_3O_4$ requires 406.1761).

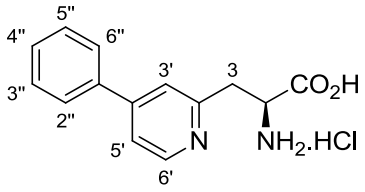
Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(2'',4''-diaminophenyl)pyridin-2'-yl]propanoate (171)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-aminophenyl)pyridin-2'-yl]propanoate (**170**) using methyl

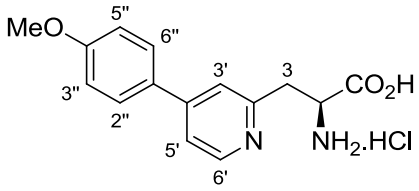
(2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(2'',4''-dinitrophenyl)pyridin-2'-yl]propanoate (**169**) (0.359 g, 0.0747 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 50% ethyl acetate/1% triethylamine in dichloromethane to produce methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(2'',4''-diaminophenyl)pyridin-2'-yl]propanoate (**171**) (0.0198 g, 63%) as a yellow oil. R_f 0.13 (50% ethyl acetate/1% triethylamine in dichloromethane); ν_{max}/cm^{-1} (neat) 3364 (NH), 2952 (CH), 1712 (C=O), 1599 (C=C), 1515, 1266, 1213, 1055, 698; $[\alpha]_D^{26}$ +15.8 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 3.30 (1H, dd, *J* 14.7, 5.3 Hz, 3-*H*H), 3.35 (1H, dd, *J* 14.7, 5.3 Hz, 3-H*H*), 3.63–3.78 (7H, m, OCH_3 and 2 × NH_2), 4.78 (1H, dt, *J* 8.3, 5.3 Hz, 2-H), 5.07 (1H, d, *J* 12.3 Hz, OC*H*HPh), 5.11 (1H, d, *J* 12.3 Hz, OCH*H*Ph), 6.06 (1H, d, *J* 2.2 Hz, 3''-H), 6.18 (1H, dd, *J* 8.2, 2.2 Hz, 5''-H), 6.30 (1H, d, *J* 8.3 Hz, NH), 6.91 (1H, d, *J* 8.2 Hz, 6''-H), 7.20–7.24 (2H, m, 3'-H and 5'-H), 7.28–7.36 (5H, m, Ph), 8.45 (1H, d, *J* 5.1 Hz, 6'-H); δ_C (126 MHz, $CDCl_3$) 39.1 (CH_2), 52.4 (CH_3), 53.5 (CH), 66.9 (CH_2), 101.8 (CH), 106.6 (CH), 115.2 (C), 122.0 (CH), 123.6 (CH), 128.1 (2 × CH), 128.1 (CH), 128.5 (2 × CH), 131.3 (CH), 136.4 (C), 144.7 (C), 148.2 (C), 148.6 (C), 149.5 (CH), 156.1 (C), 157.1 (C), 172.2 (C); *m/z* (ESI) 421.1853 (MH^+. $C_{23}H_{25}N_4O_4$ requires 421.1870).

(2*S*)-2-Amino-3-(4'-phenylpyridin-2'-yl)propanoic acid hydrochloride (150)



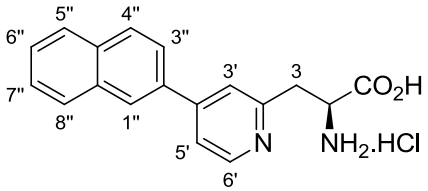
Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-phenylpyridin-2'-yl)propanoate (**142**) (0.0637 g, 0.163 mmol) was suspended in 6 M hydrochloric acid (5 mL) and heated under reflux for 48 h. The mixture was cooled to room temperature and concentrated *in vacuo*. The crude material was purified by trituration with diethyl ether (5 mL) to produce (2*S*)-2-amino-3-(4'-phenylpyridin-2'-yl)propanoic acid hydrochloride (**150**) (0.044 g, 97%) as a brown solid. Mp 110–113 °C; ν_{max}/cm^{-1} (neat) 3375 (NH), 2931 (CH), 1633 (C=O), 1479, 1392, 1024, 844, 765; $[\alpha]_D^{22}$ +28.6 (*c* 1.1, MeOH); δ_H (500 MHz, CD_3OD) 3.73–3.84 (2H, m, 3-H_2), 4.76 (1H, dd, *J* 8.4, 6.5 Hz, 2-H), 7.62–7.67 (3H, m, 3 × ArH), 8.00–8.07 (2H, m, 2 × ArH), 8.32 (1H, dd, *J* 6.3, 1.7 Hz, 5'-H), 8.50 (1H, d, *J* 1.7 Hz, 3'-H), 8.82 (1H, d, *J* 6.3 Hz, 6'-H); δ_C (126 MHz, CD_3OD) 35.0 (CH_2), 52.9 (CH), 124.3 (CH), 126.6 (CH), 129.3 (2 × CH), 130.9 (2 × CH), 133.3 (CH), 135.8 (C), 143.1 (CH), 152.3 (C), 159.4 (C), 169.8 (C); *m/z* (ESI) 277.0744 ($[M-H]^-$. $C_{14}H_{14}{}^{35}ClN_2O_2$ requires 277.0749).

(2*S*)-2-Amino-3-[4'-(4''-methoxyphenyl)pyridin-2'-yl]propanoic acid hydrochloride (151)



The reaction was carried out according to the above procedure for the synthesis of (2*S*)-2-amino-3-(4'-phenylpyridin-2'-yl)propanoic acid hydrochloride (**150**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-methoxyphenyl)pyridin-2'-yl]propanoate (**143**) (0.0610 g, 0.145 mmol) for 48 h. The crude material was purified by trituration with diethyl ether (5 mL) to produce (2*S*)-2-amino-3-[4'-(4''-methoxyphenyl)pyridin-2'-yl]propanoic acid hydrochloride (**151**) (0.043 g, 93%) as a brown solid. Mp 165–167 °C; ν_{max}/cm^{-1} (neat) 3340 (NH), 2926 (CH), 1737 (C=O), 1369, 1224, 823; $[\alpha]_D^{21}$ +34.0 (*c* 0.8, MeOH); δ_H (500 MHz, CD_3OD) 3.67–3.77 (2H, m, 3-H_2), 3.91 (3H, s, OCH_3), 4.71 (1H, t, *J* 7.3 Hz, 2-H), 7.17 (2H, d, *J* 8.9 Hz, 3''-H and 5''-H), 8.04 (2H, d, *J* 8.9 Hz, 2''-H and 6''-H), 8.22 (1H, dd, *J* 6.3, 1.3 Hz, 5'-H), 8.39 (1H, d, *J* 1.3 Hz, 3'-H), 8.70 (1H, d, *J* 6.3 Hz, 6'-H); δ_C (126 MHz, CD_3OD) 35.1 (CH_2), 52.9 (CH), 56.2 (CH_3), 116.4 (2 × CH), 122.8 (CH), 125.0 (CH), 127.6 (C), 131.1 (2 × CH), 143.1 (CH), 152.1 (C), 158.3 (C), 164.8 (C), 170.0 (C); *m/z* (ESI) 307.0847 ($[M-H]^-$. $C_{15}H_{16}{}^{35}ClN_2O_3$ requires 307.0855).

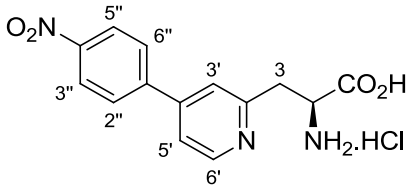
(2*S*)-2-Amino-3-[4'-(naphthalen-2''-yl)pyridin-2'-yl]propanoic acid hydrochloride (152)



The reaction was carried out according to the above procedure for the synthesis of (2*S*)-2-amino-3-(4'-phenylpyridin-2'-yl)propanoic acid hydrochloride (**150**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(naphthalen-2''-yl)pyridin-2'-yl]propanoate (**144**) (0.0260 g, 0.0590 mmol) for 48 h. The crude material was purified by trituration with diethyl ether (5 mL) to produce (2*S*)-2-amino-3-[4'-(naphthalen-2''-yl)pyridin-2'-yl]propanoic acid hydrochloride (**152**) (0.019 g, 94%) as a brown solid. Mp 120–124 °C; ν_{max}/cm^{-1} (neat) 2924 (CH), 1739 (C=O), 1622 (C=C), 1456, 1365, 1228, 817, 754; $[\alpha]_D^{21}$ +35.9 (*c* 0.9, MeOH); δ_H (500 MHz, CD_3OD) 3.72–3.82 (2H, m, 3-H_2), 4.72–4.80 (1H, m, 2-H), 7.59–7.68 (2H, m, 6''-H and 7''-H), 7.97 (1H, d, *J* 7.8 Hz, ArH), 8.03–8.13 (3H, m, 3''-H, 4''-H and ArH), 8.38 (1H, br d, *J* 4.8 Hz, 5'-H), 8.56 (1H, br s, 3'-H), 8.61 (1H, br s, 1''-H), 8.81 (1H, d, *J* 4.8 Hz, 6'-H); δ_C (126 MHz, CD_3OD) 35.3 (CH_2), 53.0 (CH), 124.1 (CH), 125.1 (CH),

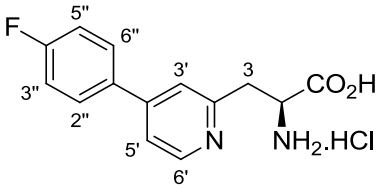
126.3 (CH), 128.4 (CH), 128.9 (CH), 129.6 (CH), 130.2 (CH), 130.3 (CH), 130.8 (CH), 133.1 (C), 134.8 (C), 136.2 (C), 143.9 (CH), 152.8 (C), 158.4 (C), 170.0 (C); *m/z* (ESI) 327.0898 ([M−H]$^-$. $C_{18}H_{16}{}^{35}ClN_2O_2$ requires 327.0906).

(2*S*)-2-Amino-3-[4'-(4''-nitrophenyl)pyridin-2'-yl]propanoic acid hydrochloride (154)



The reaction was carried out according to the above procedure for the synthesis of (2*S*)-2-amino-3-(4'-phenylpyridin-2'-yl)propanoic acid hydrochloride (**150**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-nitrophenyl)pyridin-2'-yl]propanoate (**146**) (0.0263 g, 0.0604 mmol) for 48 h. The crude material was purified by trituration with diethyl ether (5 mL) to produce (2*S*)-2-amino-3-[4'-(4''-nitrophenyl)pyridin-2'-yl]propanoic acid hydrochloride (**154**) (0.017 g, 86%) as a brown solid. Mp 140–143 °C; ν_{max}/cm^{-1} (neat) 3221 (NH), 2853 (CH), 1718 (C=O), 1635 (C=C), 1529, 1479, 1349, 1263, 1246; $[\alpha]_D{}^{25}$ +31.9 (*c* 0.9, MeOH); δ_H (500 MHz, CD_3OD) 3.78 (2H, d, *J* 6.5 Hz, 3-H_2), 4.75 (1H, t, *J* 6.5 Hz, 2-H), 8.22 (2H, d, *J* 8.5 Hz, 2''-H and 6''-H), 8.30 (1H, d, *J* 5.7 Hz, 5'-H), 8.46 (2H, d, *J* 8.5 Hz, 3''-H and 5''-H), 8.48 (1H, br s, 3'-H), 8.89 (1H, d, *J* 5.7 Hz, 6'-H); δ_C (126 MHz, CD_3OD) 35.4 (CH_2), 52.9 (CH), 124.7 (CH), 125.6 (2 × CH), 126.9 (CH), 130.6 (2 × CH), 142.4 (C), 144.8 (CH), 151.0 (C), 153.9 (C), 155.7 (C), 170.0 (C); *m/z* (ESI) 322.0593 ([M−H]$^-$. $C_{14}H_{13}{}^{35}ClN_3O_4$ requires 322.0600).

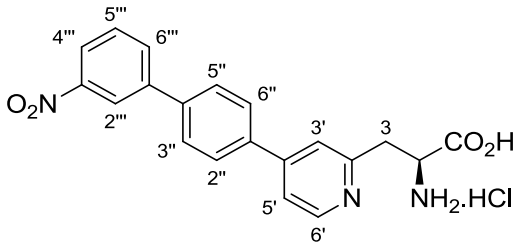
(2*S*)-2-Amino-3-[4'-(4''-fluorophenyl)pyridin-2'-yl]propanoic acid hydrochloride (155)



The reaction was carried out according to the above procedure for the synthesis of (2*S*)-2-amino-3-(4'-phenylpyridin-2'-yl)propanoic acid hydrochloride (**150**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-fluorophenyl)pyridin-2'-yl]propanoate (**147**) (0.0139 g, 0.0341 mmol) for 48 h. The crude material was purified by trituration with diethyl ether (5 mL) to produce (2*S*)-2-amino-3-[4'-(4''-fluorophenyl)pyridin-2'-yl]propanoic acid hydrochloride (**155**) (0.010 g, 99%) as a yellow solid. Mp 80–84 °C; ν_{max}/cm^{-1} (neat) 3360 (NH), 2920 (CH), 1602 (C=O),

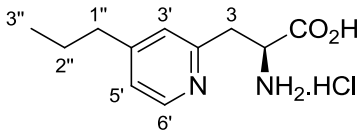
1516 (C=C), 1236, 1163, 827; $[\alpha]_D^{25}$ +30.8 (*c* 1.0, MeOH); δ_H (500 MHz, CD_3OD) 3.69–3.79 (2H, m, 3-H_2), 4.72 (1H, t, *J* 7.2 Hz, 2-H), 7.38 (2H, t, *J* 8.8 Hz, 3''-H and 5''-H), 8.07 (2H, dd, *J* 8.8, 5.1 Hz, 2''-H and 6''-H), 8.22 (1H, dd, *J* 6.1, 1.1 Hz, 5'-H), 8.38 (1H, br s, 3'-H), 8.78 (1H, d, *J* 6.1 Hz, 6'-H); δ_C (126 MHz, CD_3OD) 35.3 (CH_2), 52.9 (CH), 117.8 (d, $J_{C\text{-}C\text{-}F}$ 22.3 Hz, 2 × CH), 123.8 (CH), 125.9 (CH), 131.7 (d, $J_{C\text{-}C\text{-}C\text{-}F}$ 9.1 Hz, 2 × CH), 132.5 (d, $J_{C\text{-}C\text{-}C\text{-}C\text{-}F}$ 3.0 Hz, C), 144.1 (CH), 153.1 (C), 157.2 (C), 166.5 (d, $J_{C\text{-}F}$ 252.3 Hz, C), 170.0 (C); *m/z* (ESI) 283.0843 (MNa^+. $C_{14}H_{13}FN_2NaO_2$ requires 283.0853).

(2*S*)-2-Amino-3-{4'-[4''(3'''-nitrophenyl)phenyl]pyridin-2'-yl]}propanoic acid hydrochloride (156)



The reaction was carried out according to the above procedure for the synthesis of (2*S*)-2-amino-3-(4'-phenylpyridin-2'-yl)propanoic acid hydrochloride (**150**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-{4'-[4''-(3'''-nitrophenyl)phenyl]pyridin-2'-yl)propanoate (**148**) (0.0240 g, 0.0469 mmol) for 48 h. The crude material was purified by trituration with diethyl ether (5 mL) to produce (2*S*)-2-amino-3-{4'-[4''(3'''-nitrophenyl)phenyl]pyridin-2'-yl]}propanoic acid hydrochloride (**156**) (0.018 g, 98%) as a brown solid. Mp 140–142 °C; ν_{max}/cm^{-1} (neat) 3414 (NH), 2922 (CH), 1633 (C=O), 1521 (C=C), 1348, 806, 748; $[\alpha]_D^{27}$ +14.1 (*c* 0.1, MeOH); δ_H (400 MHz, CD_3OD) 3.72–3.83 (2H, m, 3-H_2), 4.72–4.80 (1H, m, 2-H), 7.77 (1H, t, *J* 7.8 Hz, ArH), 7.87–8.04 (2H, m, 3'-H and ArH), 8.13–8.23 (3H, m, 3 × ArH), 8.24–8.38 (2H, m, 5'-H and ArH), 8.47–8.60 (2H, m, 2 × ArH), 8.83 (1H, s, 6'-H); δ_C (126 MHz, CD_3OD) 35.3 (CH_2), 53.0 (CH), 122.8 (CH), 124.0 (CH), 124.1 (CH), 126.3 (CH), 129.6 (2 × CH), 130.2 (2 × CH), 131.6 (CH), 134.4 (CH), 136.0 (C), 142.5 (C), 143.4 (C), 144.0 (CH), 150.4 (C), 153.0 (C), 157.9 (C), 170.0 (C); *m/z* (ESI) 398.0899 ($[M-H]^-$. $C_{20}H_{17}{}^{35}ClN_3O_4$ requires 398.0913).

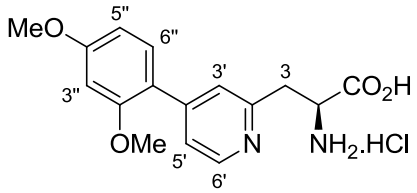
(2*S*)-2-Amino-3-(4'-propylpyridin-2'-yl]propanoic acid hydrochloride (157)



The reaction was carried out according to the above procedure for the synthesis of (2*S*)-2-amino-3-(4'-phenylpyridin-2'-yl)propanoic acid hydrochloride (**150**) using methyl (2*S*)-2-

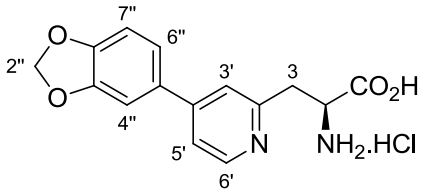
[(benzyloxycarbonyl)amino]-3-(4’-propylpyridin-2’-yl]propanoate (**149**) (0.0230 g, 0.0645 mmol) for 48 h. The crude material was purified by trituration with diethyl ether (5 mL) to produce (2*S*)-2-amino-3-(4’-propylpyridin-2’-yl]propanoic acid hydrochloride (**157**) (0.015 g, 97%) as a brown solid. Mp 112–116 °C; ν_{max}/cm^{-1} (neat) 3360 (NH), 2924 (CH), 1635 (C=O), 1506 (C=C), 1226, 1062, 819; $[\alpha]_D^{22}$ +27.5 (*c* 0.9, MeOH); δ_H (500 MHz, CD_3OD) 1.03 (3H, t, *J* 7.3 Hz, 3’’-H_3), 1.77–1.86 (2H, m, 2’’-H_2), 2.91–2.97 (2H, m, 1’’-H_2), 3.66 (1H, dd, *J* 14.8, 8.9 Hz, 3-*H*H), 3.72 (1H, dd, *J* 14.8, 6.2 Hz, 3-H*H*), 4.65 (1H, dd, *J* 8.9, 6.2 Hz, 2-H), 7.89 (1H, d, *J* 5.9 Hz, 5’-H), 8.05 (1H, s, 3’-H), 8.69 (1H, d, *J* 5.9 Hz, 6’-H); δ_C (126 MHz, CD_3OD) 13.9 (CH_3), 24.1 (CH_2), 34.7 (CH_2), 38.9 (CH_2), 52.8 (CH), 127.3 (CH), 129.5 (CH), 142.1 (CH), 151.4 (C), 166.6 (C), 169.7 (C); *m/z* (ESI) 243.0902 ([M−H]$^-$. $C_{11}H_{16}{}^{35}ClN_2O_2$ requires 243.0906).

(2*S*)-2-Amino-3-[4’-(2’’,4’’-dimethoxyphenyl)pyridin-2’-yl]propanoic acid hydrochloride (172)



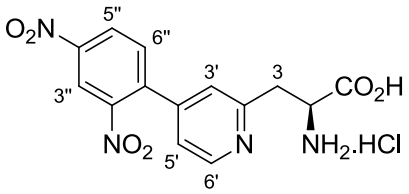
The reaction was carried out according to the above procedure for the synthesis of (2*S*)-2-amino-3-(4’-phenylpyridin-2’-yl)propanoic acid hydrochloride (**150**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4’-(2’’,4’’-dimethoxyphenyl)pyridin-2’-yl]propanoate (**167**) (0.0470 g, 0.108 mmol) for 16 h. The crude material was purified by trituration with diethyl ether (5 mL) to produce (2*S*)-2-amino-3-[4’-(2’’,4’’-dimethoxyphenyl)pyridin-2’-yl]propanoic acid hydrochloride (**172**) (0.308 g, 95%) as a dark brown solid. Mp 180–183 °C; ν_{max}/cm^{-1} (neat) 3410 (NH), 2931 (CH), 1743 (C=O), 1597 (C=C), 1211, 1018, 833; $[\alpha]_D^{27}$ +10.1 (*c* 0.1, MeOH); δ_H (400 MHz, CD_3OD) 3.67–3.81 (2H, m, 3-H_2), 3.91 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 4.63–4.74 (1H, m, 2-H), 6.71–6.82 (2H, m, 3’’-H and 5’’-H), 7.71 (1H, d, *J* 8.3 Hz, 6’’-H), 8.26 (1H, d, *J* 6.1 Hz, 5’-H), 8.36 (1H, br s, 3’-H), 8.67 (1H, d, *J* 6.1 Hz, 6’-H); δ_C (101 MHz, CD_3OD) 34.9 (CH_2), 53.1 (CH), 56.4 (CH_3), 56.6 (CH_3), 100.0 (CH), 108.0 (CH), 117.4 (C), 126.1 (CH), 128.0 (CH), 133.8 (CH), 141.7 (CH), 150.7 (C), 158.1 (C), 160.9 (C), 166.1 (C), 169.9 (C); *m/z* (ESI) 303.1338 (MH^+. $C_{16}H_{19}N_2O_4$ requires 303.1339).

(2*S*)-2-Amino-3-[4'-(2*H*-1'',3''-benzodioxol-5''-yl)pyridin-2'-yl]propanoic acid hydrochloride (173)



The reaction was carried out according to the above procedure for the synthesis of (2*S*)-2-amino-3-(4'-phenylpyridin-2'-yl)propanoic acid hydrochloride (**150**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(2*H*-1'',3''-benzodioxol-5''-yl)pyridin-2'-yl]propanoate (**168**) (0.244 g, 0.562 mmol) for 16 h. The crude material was purified by trituration with diethyl ether (5 mL) to produce (2*S*)-2-amino-3-[4'-(2*H*-1'',3''-benzodioxol-5''-yl)pyridin-2'-yl]propanoic acid hydrochloride (**173**) (0.179 g, 99%) as a dark brown solid. Mp 197–200 °C; ν_{max}/cm^{-1} (neat) 3410 (NH), 2808 (CH), 1735 (C=O), 1612 (C=C), 1111, 1033, 810; $[\alpha]_D^{27}$ +38.5 (*c* 0.1, MeOH); δ_H (400 MHz, CD_3OD) 3.69 (1H, dd, *J* 12.8, 5.7 Hz, 3-*H*H), 3.74 (1H, dd, *J* 12.8, 5.7 Hz, 3-H*H*), 4.71 (1H, dd, *J* 8.0, 5.7 Hz, 2-H), 6.12 (2H, s, 2''-H_2), 7.07 (1H, d, *J* 8.2 Hz, 7''-H), 7.55 (1H, d, *J* 1.9 Hz, 4''-H), 7.63 (1H, dd, *J* 8.2, 1.9 Hz, 6''-H), 8.20 (1H, dd, *J* 6.4, 1.9 Hz, 5'-H), 8.35 (1H, d, *J* 1.9 Hz, 3'-H), 8.71 (1H, d, *J* 6.4 Hz, 6'-H); δ_C (101 MHz, CD_3OD) 35.1 (CH_2), 52.9 (CH), 103.9 (CH_2), 108.8 (CH), 110.4 (CH), 123.4 (CH), 125.0 (CH), 125.5 (CH), 129.5 (C), 142.9 (CH), 150.9 (C), 152.0 (C), 153.1 (C), 158.7 (C), 169.9 (C); *m/z* (ESI) 287.1029 (MH^+. $C_{15}H_{15}N_2O_4$ requires 287.1026).

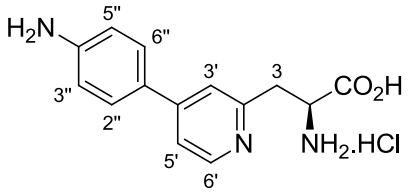
(2*S*)-2-Amino-3-[4'-(2'',4''-dinitrophenyl)pyridin-2'-yl]propanoic acid hydrochloride (174)



The reaction was carried out according to the above procedure for the synthesis of (2*S*)-2-amino-3-(4'-phenylpyridin-2'-yl)propanoic acid hydrochloride (**150**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(2'',4''-dinitrophenyl)pyridin-2'-yl]propanoate (**169**) (0.0237 g, 0.0493 mmol) for 48 h. The crude material was purified by trituration with diethyl ether (5 mL) to produce (2*S*)-2-amino-3-[4'-(2'',4''-dinitrophenyl)pyridin-2'-yl]propanoic acid hydrochloride (**174**) (0.175 g, 97%) as a yellow solid. Mp 130–134 °C; ν_{max}/cm^{-1} (neat) 3402 (NH), 3039 (CH), 1743 (C=O), 1527 (C=C), 1342, 1080, 833, 740; $[\alpha]_D^{26}$ +9.3 (*c* 1.0, MeOH); δ_H (500 MHz, CD_3OD) 3.55 (1H, dd, *J* 16.3, 7.1 Hz, 3-*H*H), 3.62 (1H, dd, *J* 16.3, 5.4 Hz, 3-H*H*), 4.57 (1H, dd, *J* 7.1, 5.4 Hz, 2-H), 7.53 (1H, dd, *J* 5.3, 1.6 Hz, 5'-H), 7.63 (1H, br s, 3'-H), 7.84 (1H, d, *J* 8.4 Hz, 6''-H), 8.64 (1H, dd, *J* 8.4, 2.3 Hz, 5''-

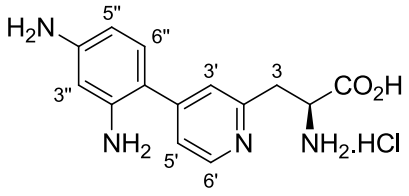
H), 8.71 (1H, d, *J* 5.3 Hz, 6'-H), 8.95 (1H, d, *J* 2.3 Hz, 3''-H); δ_C (126 MHz, CD_3OD) 36.5 (CH_2), 52.9 (CH), 121.4 (CH), 123.6 (CH), 124.9 (CH), 128.7 (CH), 128.7 (CH), 134.5 (C), 139.9 (CH), 149.1 (C), 149.7 (C), 149.8 (C), 155.0 (C), 170.9 (C); *m/z* (ESI) 333.0822 (MH^+. $C_{14}H_{13}N_4O_6$ requires 333.0830).

(2*S*)-2-Amino-3-[4'-(4''-aminophenyl)pyridin-2'-yl]propanoic acid hydrochloride (175)



The reaction was carried out according to the above procedure for the synthesis of (2*S*)-2-amino-3-(4'-phenylpyridin-2'-yl)propanoic acid hydrochloride (**150**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-aminophenyl)pyridin-2'-yl]propanoate (**170**) (0.0136 g, 0.0335 mmol) for 48 h. The crude material was purified by trituration with diethyl ether (5 mL) to produce (2*S*)-2-amino-3-[4'-(4''-aminophenyl)pyridin-2'-yl]propanoic acid hydrochloride (**175**) (0.00970 g, 98%) as a dark orange solid. Mp 151–154 °C; ν_{max}/cm^{-1} (neat) 3379 (NH), 2831 (CH), 1743 (C=O), 1589 (C=C), 1473, 1311, 817; $[\alpha]_D^{27}$ +34.9 (*c* 0.1, MeOH); δ_H (400 MHz, CD_3OD) 3.70 (2H, d, *J* 7.3 Hz, 3-H_2), 4.69 (1H, t, *J* 7.3 Hz, 2-H), 7.30 (2H, d, *J* 8.7 Hz, 3''-H and 5''-H), 8.04 (2H, d, *J* 8.7 Hz, 2''-H and 6''-H), 8.15 (1H, dd, *J* 6.3, 1.7 Hz, 5'-H), 8.33 (1H, d, *J* 1.7 Hz, 3'-H), 8.68 (1H, d, *J* 6.3 Hz, 6'-H); δ_C (101 MHz, CD_3OD) 35.3 (CH_2), 53.0 (CH), 121.5 (2 × CH), 122.6 (CH), 124.7 (CH), 131.1 (C), 131.1 (2 × CH and C), 143.8 (CH), 152.6 (C), 157.1 (C), 170.1 (C); *m/z* (ESI) 258.1236 (MH^+. $C_{14}H_{16}N_3O_2$ requires 258.1237).

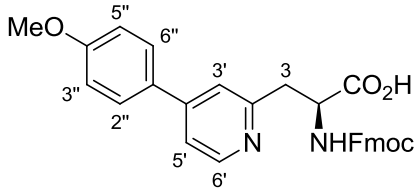
(2*S*)-2-Amino-3-[4'-(2'',4''-diaminophenyl)pyridin-2'-yl]propanoic acid hydrochloride (176)



The reaction was carried out according to the above procedure for the synthesis of (2*S*)-2-amino-3-(4'-phenylpyridin-2'-yl)propanoic acid hydrochloride (**150**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(2'',4''-diaminophenyl)pyridin-2'-yl]propanoate (**171**) (0.0163 g, 0.0388 mmol) for 48 h. The crude material was purified by trituration with diethyl ether (5 mL) to produce (2*S*)-2-amino-3-[4'-(2'',4''-diaminophenyl)pyridin-2'-yl]propanoic acid hydrochloride (**176**) (0.0113 g, 95%) as a dark brown solid. Mp 159–162 °C; ν_{max}/cm^{-1} (neat) 3332 (NH), 2846 (CH), 1743

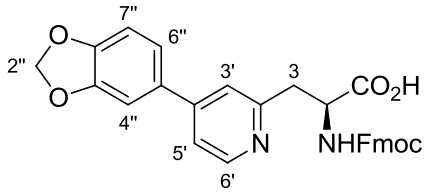
(C=O), 1620 (C=C), 1473, 1234, 1072, 840; $[\alpha]_D^{26}$ +15.7 (*c* 1.0, MeOH); δ_H (400 MHz, CD_3OD) 3.70–3.84 (2H, m, 3-H_2), 4.72 (1H, t, *J* 7.3 Hz, 2-H), 6.94 (1H, dd, *J* 8.3, 1.9 Hz, 5''-H), 7.04 (1H, d, *J* 1.9 Hz, 3''-H), 7.51 (1H, d, *J* 8.3 Hz, 6''-H), 8.16 (1H, br d, *J* 6.0 Hz, 5'-H), 8.35 (1H, br s, 3'-H), 8.82 (1H, d, *J* 6.0 Hz, 6'-H); δ_C (126 MHz, CD_3OD) 35.0 (CH_2), 52.9 (CH), 112.2 (CH), 114.0 (CH), 122.6 (C), 127.0 (CH), 129.2 (CH), 133.8 (CH), 137.1 (C), 143.2 (CH), 145.9 (C), 152.2 (C), 158.6 (C), 169.8 (C); *m/z* (ESI) 273.1346 (MH^+. $C_{14}H_{17}N_4O_2$ requires 273.1346).

(2*S*)-2-[(9*H*-Fluoren-9-ylmethoxycarbonyl)amino]-3-[4'-(4''-methoxyphenyl)pyridin-2'-yl]propanoic acid (177)



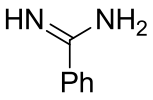
(2*S*)-2-Amino-3-[4'-(4''-methoxyphenyl)pyridin-2'-yl]propanoic acid hydrochloride (**151**) (0.264 g, 0.0855 mmol) was dissolved in 1,4-dioxane (1.5 mL) followed by the addition of 10% sodium carbonate solution (1.5 mL), *N*,*N*-diisopropylethylamine (0.0164 mL, 0.0941 mmol) and 9-fluorenylmethoxycarbonyl chloride (0.0243 g, 0.0941 mmol). The reaction was stirred at room temperature for 18 h. Once the reaction was complete, the solution was concentrated *in vacuo*, redissolved in water (5 mL) and acidified to pH 1 with hydrochloric acid (2 M). The aqueous solution was washed with dichloromethane (3 × 10 mL) and the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel, eluting with 3% methanol/1% acetic acid in dichloromethane to produce (2*S*)-2-[(9*H*-fluoren-9-ylmethoxycarbonyl)amino]-3-[4'-(4''-methoxyphenyl)pyridin-2'-yl]propanoic acid (**177**) (0.0171 g, 40%) as a brown solid. Mp 90–93 °C; R_f 0.09 (3% methanol/1% acetic acid in dichloromethane); ν_{max}/cm^{-1} (neat) 3326 (NH), 2935 (CH), 1716 (C=O), 1602 (C=C), 1518, 1251, 1182, 1047, 826, 739; $[\alpha]_D^{21}$ +60.1 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.37–3.56 (2H, m, 3-H_2), 3.87 (3H, s, OCH_3), 4.24 (1H, t, *J* 7.3 Hz, CO_2CH_2CH), 4.39 (2H, d, *J* 7.3 Hz, CO_2CH_2CH), 4.47–4.57 (1H, m, 2-H), 6.31 (1H, d, *J* 3.4 Hz, NH), 7.00 (2H, d, *J* 8.6 Hz, 3''-H and 5''-H), 7.32 (2H, t, *J* 7.4 Hz, 2 × ArH), 7.41 (2H, t, *J* 7.4 Hz, 2 × ArH), 7.55 (1H, d, *J* 5.6 Hz, 5'-H), 7.58–7.67 (5H, m, 3'-H, 2''-H, 6''-H and 2 × ArH), 7.77 (2H, d, *J* 7.4 Hz, 2 × ArH), 8.49 (1H, d, *J* 5.6 Hz, 6'-H); δ_C (101 MHz, $CDCl_3$) 38.4 (CH_2), 47.2 (CH), 53.2 (CH), 55.5 (CH_3), 67.2 (CH_2), 114.8 (2 × CH), 120.0 (2 × CH), 120.3 (CH), 122.5 (CH), 125.2 (2 × CH), 127.1 (2 × CH), 127.7 (2 × CH), 128.6 (2 × CH), 141.3 (2 × C), 143.8 (2 × C), 143.9 (C), 145.6 (CH), 152.0 (C), 155.7 (C), 156.1 (C), 161.5 (C), 172.8 (C); *m/z* (ESI) 495.1901 (MH^+. $C_{30}H_{27}N_2O_5$ requires 495.1914).

(2*S*)-2-[(9*H*-Fluoren-9-ylmethoxycarbonyl)amino]-3-[4'-(2*H*-1'',3''-benzodioxol-5''-yl)pyridin-2'-yl]propanoic acid (178)



The reaction was carried out according to the above procedure for the synthesis of (2*S*)-2-[(9*H*-fluoren-9-ylmethoxycarbonyl)amino]-3-[4'-(4''-methoxyphenyl)pyridin-2'-yl]propanoic acid (**177**) using (2*S*)-2-amino-3-[4'-(2*H*-1'',3''-benzodioxol-5''-yl)pyridin-2'-yl]propanoic acid hydrochloride (**173**) (0.349 g, 1.13 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 5% methanol/1% acetic acid in dichloromethane to produce (2*S*)-2-[(9*H*-fluoren-9-ylmethoxycarbonyl)amino]-3-[4'-(2*H*-1'',3''-benzodioxol-5''-yl)pyridin-2'-yl]propanoic acid (**178**) (0.304 g, 40%) as a yellow solid. Mp 96–100 °C; R_f 0.24 (5% methanol/1% acetic acid in dichloromethane); ν_{max}/cm^{-1} (neat) 2970 (CH), 1716 (C=O), 1604 (C=C), 1473, 1234, 1033, 738; $[\alpha]_D^{26}$ +25.7 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.38–3.57 (2H, m, 3-H_2), 4.22 (1H, t, *J* 7.2 Hz, CO_2CH_2CH), 4.33–4.46 (2H, m, CO_2CH_2CH), 4.54 (1H, s, 2-H), 6.03 (2H, br d, *J* 5.1 Hz, 2''-H_2), 6.26 (1H, d, *J* 4.4 Hz, NH), 6.88 (1H, d, *J* 8.1 Hz, 7''-H), 7.06–7.19 (2H, m, 4''-H and 6''-H), 7.27–7.45 (4H, m, 4 × ArH), 7.46–7.66 (4H, m, 3'-H, 5'-H and 2 × ArH), 7.77 (2H, d, *J* 7.4 Hz, 2 × ArH), 8.51 (1H, d, *J* 5.5 Hz, 6'-H); δ_C (101 MHz, $CDCl_3$) 38.5 (CH_2), 47.2 (CH), 53.0 (CH), 67.2 (CH_2), 101.8 (CH_2), 107.3 (CH), 109.1 (CH), 120.0 (2 × CH), 120.5 (CH), 121.7 (CH), 122.7 (CH), 125.2 (2 × CH), 127.1 (2 × CH), 127.7 (2 × CH), 130.6 (C), 141.3 (2 × C), 143.8 (2 × C), 145.8 (CH), 148.9 (C), 149.6 (C), 151.9 (C), 155.7 (C), 156.3 (C), 172.6 (C); *m/z* (ESI) 531.1517 (MNa^+. $C_{30}H_{24}N_2NaO_6$ requires 531.1527).

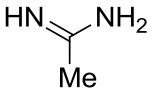
Benzamidine (183)[168,169]



Benzamidine hydrochloride (**182**) (1.09 g, 6.99 mmol) was dissolved in 1 M sodium hydroxide solution (10 mL) and left to stir at room temperature for 2 h. The solution was then diluted with dichloromethane (15 mL), extracted with dichloromethane (3 × 10 mL), dried over $MgSO_4$ and concentrated *in vacuo* to produce benzamidine (**183**) (0.689 g, 82%) as a white solid. Spectroscopic data was consistent with the literature.[168,169] Mp 79–82 °C (lit.,[170] Mp 77–79 °C); δ_H (400 MHz, $CDCl_3$)

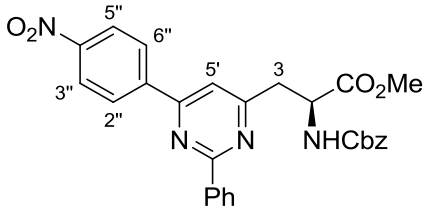
5.28 (3H, s, NH and NH_2), 7.42–7.62 (5H, m, Ph); δ_C (101 MHz, $CDCl_3$) 125.9 (2 × ArH), 128.5 (2 × ArH), 130.2 (ArH), 136.7 (C), 166.3 (C); *m/z* (ESI) 143 (MNa^+, 100%).

Acetamidine (185)[171]



Acetamidine hydrochloride (**184**) (1.07 g, 11.35 mmol) was dissolved in methanol (28 mL, 0.4 M) followed by addition of sodium hydroxide (0.45 g, 11.35 mmol) and was left to stir a room temperature for 2 h. The solution was then filtered through a pad of Celite® and concentrated *in vacuo*. The crude solid was then triturated with 15% isopropyl alcohol in dichloromethane (10 mL), filtered and concentrated *in vacuo* to give acetamidine (**185**) (0.59 g, 89%) as a colourless solid. Spectroscopic data was consistent with the literature.[171] Mp 64–66 °C (lit.,[171] Mp 66–67 °C); δ_H (500 MHz, $CDCl_3$) 1.99 (3H, s, CH_3), 4.88 (1H, s, NH), 7.26 (2H, s, 2 × NH), deuterium exchange with hydrogen, the sum of integrals δ 4.88 and δ 7.26 equal to that of δ 1.99; δ_C (126 MHz, $CDCl_3$) 23.2 (CH_3), 165.4 (C); *m/z* (ESI) 81 (MNa^+, 100%).

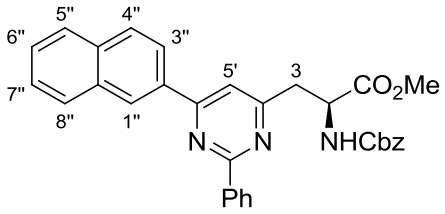
Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[6'-(4''-nitrophenyl)-2'-phenylpyrimidin-4'-yl]propanoate (186)



Methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(4'-nitrophenyl)-4-oxohex-5-enoate (**90**) (0.0456 g, 0.111 mmol) was dissolved in tetrahydrofuran (1.5 mL) followed by sequential addition of benzamidine hydrochloride **182** (0.0262 g, 0.167 mmol), potassium carbonate (0.0184 g, 0.133 mmol) and ytterbium triflate (0.00680 g, 0.0111 mmol). The mixture was heated under reflux for 22 h. The mixture was concentrated *in vacuo* and redissolved in methanol (5 mL) followed by addition of thionyl chloride (0.0107 mL, 0.147 mmol). The mixture was stirred at room temperature for 24 h. Upon completion, the mixture was concentrated *in vauco*, redissolved in dichloromethane (10 mL), washed with a saturated solution of sodium hydrogen carbonate (5 mL), brine (5 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel, eluting with 30% ethyl acetate in petroleum ether (40–60) to produce

methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[6'-(4''-nitrophenyl)-2'-phenylpyrimidin-4'-yl]propanoate (**186**) (0.0267 g, 47%) as an orange solid. Mp 66–70 °C; R_f 0.17 (30% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 3338 (NH), 2953 (CH), 1720 (C=O), 1541 (C=C), 1348, 1211, 1058, 748, 694; $[\alpha]_D^{24}$ +44.8 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.50 (1H, dd, *J* 16.2, 4.7 Hz, 3-*H*H), 3.63 (1H, dd, *J* 16.2, 4.7 Hz, 3-H*H*), 3.77 (3H, s, OCH_3), 4.90–4.97 (1H, m, 2-H), 5.09 (1H, d, *J* 12.5 Hz, OC*H*HPh), 5.13 (1H, d, *J* 12.5 Hz, OCH*H*Ph), 6.05 (1H, d, *J* 8.2 Hz, NH), 7.26–7.37 (5H, m, Ph), 7.48–7.55 (4H, m, 5'-H, 2''-H, 6''-H, ArH), 8.31–8.39 (4H, m, 4 × ArH), 8.48–8.55 (2H, m, 3''-H and 5''-H); δ_C (101 MHz, $CDCl_3$) 39.0 (CH_2), 52.0 (CH), 52.7 (CH_3), 67.1 (CH_2), 115.0 (CH), 124.1 (2 × CH), 128.1 (2 × CH), 128.2 (3 × CH), 128.4 (2 × CH), 128.5 (2 × CH), 128.7 (2 × CH), 131.3 (CH), 136.1 (C), 137.1 (C), 142.8 (C), 149.3 (C), 156.0 (C), 161.8 (C), 164.5 (C), 167.2 (C), 171.8 (C); *m/z* (EI) 535.1563 (MNa^+. $C_{28}H_{24}N_4NaO_6$ requires 535.1588).

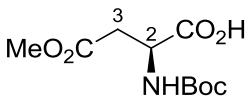
Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[6'-(naphthalen-2''-yl)-2-phenylpyrimidin-4'-yl]propanoate (187)



The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[6'-(4''-nitrophenyl)-2'-phenylpyrimidin-4'-yl]propanoate (**186**) using methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(naphthalen-2'-yl)-4-oxohex-5-enoate (**118**) (0.0822 g, 0.197 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 30% ethyl acetate in petroleum ether (40–60) to produce methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[6'-(naphthalen-2''-yl)-2-phenylpyrimidin-4'-yl]propanoate (**187**) (0.0112 g, 11%) as a colourless oil. R_f 0.20 (30% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 3345 (NH), 3054 (CH), 2951 (CH), 1716 (C=O), 1597 (C=C), 1503, 1437, 1210, 1056, 1028; $[\alpha]_D^{24}$ +22.1 (*c* 0.6, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 3.48 (1H, dd, *J* 16.0, 4.7 Hz, 3-*H*H), 3.64 (1H, dd, *J* 16.0, 4.7 Hz, 3-H*H*), 3.76 (3H, s, OCH_3), 4.90–4.96 (1H, m, 2-H), 5.12 (2H, s, OC*H*$_2$Ph), 6.19 (1H, d, *J* 8.3 Hz, NH), 7.23–7.37 (5H, m, Ph), 7.49–7.62 (6H, m, 5'-H, 6''-H, 7''-H and 3 × ArH), 7.88–7.94 (1H, m, ArH), 7.97–8.04 (2H, m, 4''-H and ArH), 8.30 (1H, dd, *J* 8.6, 1.5 Hz, 3''-H), 8.55–8.60 (2H, m, 5''-H and 8''-H), 8.69 (1H, br s, 1''-H); δ_C (126 MHz, $CDCl_3$) 38.8 (CH_2), 52.1 (CH), 52.7 (CH_3), 67.0 (CH_2), 114.5 (CH), 124.1 (CH), 126.6 (CH), 127.5 (CH), 127.6 (CH), 127.8 (CH), 128.0 (2 × CH), 128.1 (CH), 128.4 (2 × CH), 128.5 (2 × CH), 128.6 (2 × CH), 128.7 (CH), 129.1 (CH), 130.9 (CH), 133.3 (C), 134.2 (C), 134.7

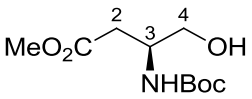
(C), 136.2 (C), 137.7 (C), 156.1 (C), 164.2 (C), 166.2 (C), 172.0 (C), 194.4 (C); *m/z* (EI) 540.1885 (MNa^+. $C_{32}H_{27}N_3NaO_4$ requires 540.1894).

_N_-Boc-L-Aspartic acid 4-methyl ester (188)[172]



L-Aspartic acid (5.00 g, 37.6 mmol) was dissolved in methanol (250 mL) and cooled to −10 °C. Thionyl chloride (4.77 mL, 41.4mmol) was added dropwise and the resultant reaction mixture was stirred for 0.5 h. The solution was concentrated *in vacuo* and triturated with diethyl ether to give L-aspartic acid 4-methyl ester as a white solid (8.27 g, 89%). This was then dissolved in 1,4-dioxane (100 mL) and cooled to 0 °C followed by the addition of di-*tert*-butyl dicarbonate (9.03 g, 41.4 mmol) and a 10% aqueous solution of sodium carbonate (100 mL). The resulting reaction mixture was allowed to warm to room temperature and stirred for 16 h. Once the reaction had reached completion, the mixture was concentrated *in vacuo* and the residue was then dissolved in saturated aqueous ammonium chloride (100 mL), washed with dichloromethane (3 × 50 mL) and acidified to pH 1 with 1 M aqueous hydrochloric acid. This was extracted with dichloromethane (3 × 50 mL), the organic fractions were combined, washed with brine (100 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give *N*-Boc-L-aspartic acid 4-methyl ester (**188**) as a white solid (8.27 g, 89%). Mp 54–56 °C (lit.,[172] 56 °C); δ_H (500 MHz, $CDCl_3$) 1.45 (9H, s, 3 × CH_3), 2.85 (1H, dd, *J* 17.2, 3.8 Hz, 3-*H*H), 3.04 (1H, dd, *J* 17.2, 3.8 Hz, 3-H*H*), 3.72 (3H, s, OCH_3), 4.55–4.67 (1H, m, 2-H), 5.55 (1H, d, *J* 8.0 Hz, NH); δ_C (126 MHz, $CDCl_3$) 28.4 (3 × CH_3), 36.4 (CH_2), 49.9 (CH), 52.3 (CH_3), 80.8 (C), 155.8 (C), 171.8 (C), 175.1 (C); *m/z* (ESI) 270 (MNa^+, 100%).

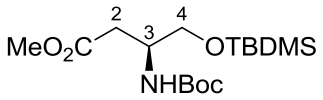
Methyl (3*S*)-3-(*tert*-butoxycarbonylamino)-4-hydroxybutanoate (189)[173]



To a solution of *N*-Boc-L-aspartic acid 4-methyl ester (**188**) (8.27 g, 33.5 mmol) in ethyl acetate (200 mL) at 0 °C was added *N*-hydroxysuccinimide (4.24 g, 36.9 mmol) and then *N*,*N*'-dicyclohexylcarbodiimide (7.05 g, 34.2 mmol) in ethyl acetate (20 mL) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 16 h. Once the reaction was complete, the reaction mixture was filtered through Celite®. The filtrate was washed with saturated sodium carbonate solution (100 mL), brine (100 mL), dried ($MgSO_4$) and concentrated *in*

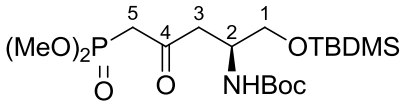
vacuo. The resulting residue was then dissolved in tetrahydrofuran (20 mL) and added dropwise to a solution of sodium borohydride (2.03 g, 53.6 mmol) in a mixture of tetrahydrofuran and water (7.5:1, 85 mL). The reaction mixture was stirred for 0.1 h before quenching with saturated aqueous ammonium chloride (5 mL). The reaction mixture was extracted with dichloromethane (3 × 50 mL). The organic fractions were combined and washed with brine (100 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel, eluting with 30% ethyl acetate in dichloromethane to produce methyl (3*S*)-3-(*tert*-butoxycarbonylamino)-4-hydroxybutanoate (**189**) as a clear oil (7.03 g, 90%). R_f 0.22 (40% ethyl acetate in dichloromethane); $[\alpha]_D^{26}$ +5.6 (*c* 1.0, $CHCl_3$), lit.,[173] $[\alpha]_D^{23}$ +6.3 (*c* 0.5, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.42 (9H, s, 3 × CH_3), 2.62 (2H, d, *J* 6.1 Hz, 2-H_2), 2.82 (1H, br s, OH), 3.65–3.73 (5H, m, OCH_3 and 4-H_2), 3.92–4.04 (1H, m, 3-H), 5.25 (1H, br s, NH); δ_C (126 MHz, $CDCl_3$) 28.4 (3 × CH_3), 35.9 (CH_2), 49.4 (CH), 52.0 (CH_3), 64.4 (CH_2), 79.9 (C), 155.9 (C), 172.4 (C); *m/z* (ESI) 256 (MNa^+, 100%).

Methyl (3*S*)-3-(*tert*-butoxycarbonylamino)-4-(*tert*-butyldimethylsilyloxy)butanoate (190)[174]



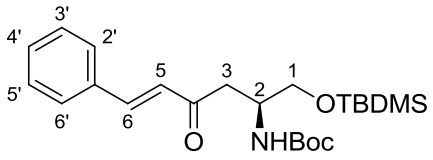
Methyl (3*S*)-3-(*tert*-butoxycarbonylamino)-4-hydroxybutanoate (**189**) (0.0508 g, 0.218 mmol) was dissolved in anhydrous dichloromethane (5 mL) and cooled to 0 °C followed by addition of *tert*-butyldimethylsilyl chloride (0.0454 mL, 0.262 mmol) and imidazole (0.0223 g, 0.327 mmol). The reaction was warmed to room temperature and stirred for 48 h. Once the reaction was complete, the solution was diluted with dichloromethane (5 mL) and washed with brine (10 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel eluting with 10% ethyl acetate in petroleum ether (40–60) to produce methyl (3*S*)-3-(*tert*-butoxycarbonylamino)-4-(*tert*-butyldimethylsilyloxy)butanoate (**190**) (0.0662 g, 88%) as a colourless oil. R_f 0.18 (10% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 3363 (NH), 2955 (CH), 1716 (C=O), 1365, 1251, 1168, 835, 777; $[\alpha]_D^{25}$ +13.1 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.02 (6H, s, $Si(CH_3)_2$), 0.87 (9H, s, 3 × CH_3), 1.42 (9H, s, 3 × CH_3), 2.49–2.64 (2H, m, 2-H_2), 3.56–3.71 (5H, m, OCH_3 and 4-H_2), 4.00 (1H, s, 3-H), 5.07 (1H, d, *J* 7.8 Hz, NH); δ_C (101 MHz, $CDCl_3$) −5.6 (CH_3), −5.5 (CH_3), 18.3 (C), 25.8 (3 × CH_3), 28.4 (3 × CH_3), 35.6 (CH_2), 48.9 (CH), 51.6 (CH_3), 64.1 (CH_2), 79.4 (C), 155.2 (C), 172.1 (C); *m/z* (ESI) 370.2008 (MNa^+. $C_{16}H_{33}NNaO_5Si$ requires 370.2020).

(2*S*)-1-(*tert*-Butyldimethylsilyloxy)-2-(*tert*-butoxycarbonylamino)-5-(dimethyloxyphosphoryl)pentan-2-one (191)



Dimethyl methylphosphonate (0.270 mL, 2.49 mmol) was dissolved in tetrahydrofuran (5 mL) and cooled to −78 °C under an argon atmosphere. *n*-Butyl lithium (2.5 M in hexane, 0.997 mL, 2.493 mmol) was added dropwise and the mixture was stirred for 0.3 h. A solution of methyl (3*S*)-3-(*tert*-butoxycarbonylamino)-4-(*tert*-butyldimethylsilyloxy)butanoate (**190**) (0.346 g, 0.997 mmol) in tetrahydrofuran (5 mL) was added dropwise. The resulting reaction mixture was then stirred at −78 °C for 0.5 h and allowed to warm to 0 °C over a period of 1 h. The reaction was quenched with a saturated aqueous solution of ammonium chloride (2 mL). Extraction was performed with ethyl acetate (2 × 10 mL). The combined organic layers were then combined, washed with brine (10 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude product was purified via a plug of silica gel eluting with 100% ethyl acetate to produce (2*S*)-1-(*tert*-butyldimethylsilyloxy)-2-(*tert*-butoxycarbonylamino)-5-(dimethyloxyphosphoryl)pentan-2-one (**191**) (0.338 g, 77%) as a colourless oil. R_f 0.38 (100% ethyl acetate); ν_{max}/cm^{-1} (neat) 3315 (NH), 2955 (CH), 1712 (C=O), 1251, 1170, 1112, 835, 777; $[\alpha]_D^{26}$ +3.6 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.04 (6H, s, $Si(CH_3)_2$), 0.88 (9H, s, 3 × CH_3), 1.42 (9H, s, 3 × CH_3), 2.85 (2H, d, *J* 6.2 Hz, 3-H_2), 3.09 (2H, dd, *J* 22.1, 13.1 Hz, 5-*H*H), 3.18 (1H, dd, *J* 21.4, 13.1 Hz, 5-H*H*), 3.58–3.69 (2H, m, 1-H_2), 3.76 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 4.04 (1H, br s, 2-H), 5.01 (1H, br d, *J* 6.7 Hz, NH); δ_C (101 MHz, $CDCl_3$) -5.5 (CH_3), -5.5 (CH_3), 18.3 (C), 25.9 (3 × CH_3), 28.4 (3 × CH_3), 41.8 (d, $J_{C\text{-}P}$ 127.9 Hz, CH_2), 45.1 (CH_2), 48.6 (CH), 53.0 (d, $J_{C\text{-}O\text{-}P}$ 6.5 Hz, CH_3), 53.1 (d, $J_{C\text{-}O\text{-}P}$ 6.5 Hz, CH_3), 64.2 (CH_2), 79.4 (C), 155.3 (C), 200.6 (d, $J_{C\text{-}C\text{-}P}$ 6.4 Hz, C); *m/z* (ESI) 462.2029 (MNa^+. $C_{18}H_{38}NNaO_7PSi$ requires 462.2047).

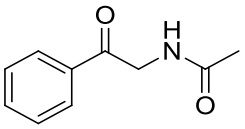
(2*S*,5*E*)-6-Phenyl-1(*tert*-butyldimethylsilyloxy)-2-(*tert*-butoxycarbonylamino)-4-oxo-hex-5-ene (192)



(2*S*)-1-(*tert*-butyldimethylsilyloxy)-2-(*tert*-butoxycarbonylamino)-5-(dimethyloxyphosphoryl)pentan-2-one (**191**) (0.324 g, 0.737 mmol) was dissolved in anhydrous acetonitrile (8 mL) and potassium carbonate (0.122 g, 0.884 mmol) was added. The mixture was

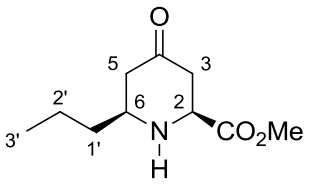
stirred at room temperature for 0.5 h followed by addition of benzaldehyde (0.149 mL, 1.474 mmol). The temperature was increased to 50 °C and stirred for 72 h. Once the reaction was complete, the solution was concentrated *in vacuo*, redissolved in ethyl acetate (10 mL) and washed with water (2 × 10 mL) and brine (10 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel eluting with 20% diethyl ether in petroleum ether (40–60) to produce (2*S*,5*E*)-6-phenyl-1(*tert*-butyldimethylsilyloxy)-2-(*tert*-butoxycarbonylamino)-4-oxo-hex-5-ene (**192**) (0.277 g, 89%) as a white solid. Mp 50–53 °C; R_f 0.16 (20% diethyl ether in petroleum ether); ν_{max}/cm^{-1} (neat) 3448 (NH), 2929 (CH), 1710 (C=O), 1494 (C=C), 1251, 1168, 835, 748; $[\alpha]_D^{26}$ +30.6 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.04 (6H, s, $Si(CH_3)_2$), 0.88 (9H, s, 3 × CH_3), 1.43 (9H, s, 3 × CH_3), 2.90 (1H, dd, *J* 16.1, 6.8 Hz, 3-*H*H), 3.00 (1H, dd, *J* 16.1, 3.9 Hz, 3-H*H*), 3.65 (1H, dd, *J* 10.0, 5.5 Hz, 1-*H*H), 3.72 (1H, dd, *J* 10.0, 3.9 Hz, 1-H*H*), 4.05–4.16 (1H, m, 2-H), 5.16 (1H, br d, *J* 8.0 Hz, NH), 6.73 (1H, d, *J* 16.2 Hz, 5-H), 7.37–7.42 (3H, m, 3 × ArH), 7.52–7.61 (3H, m, 2 × ArH and 6-H); δ_C (101 MHz, $CDCl_3$) -5.5 (CH_3), -5.4 (CH_3), 18.3 (C), 25.9 (3 × CH_3), 28.4 (3 × CH_3), 41.2 (CH_2), 49.1 (CH), 63.9 (CH_2), 79.3 (C), 126.4 (CH), 128.4 (2 × CH), 129.0 (2 × CH), 130.6 (CH), 134.4 (C), 143.1 (CH), 155.3 (C), 199.0 (C); *m/z* (ESI) 442.2367 (MNa^+. $C_{23}H_{37}NNaO_4Si$ requires 442.2384).

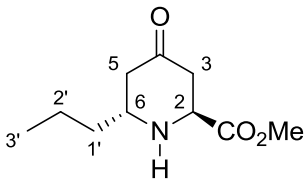
2-Amidoacetophenone (204)[119]



2-Aminoacetophenone hydrochloride (0.0510 g, 0.297 mmol) was suspended in a solution of acetic anhydride (0.199 mL, 1.49 M). Sodium acetate (0.0463 g, 0.564 mmol) was added to the mixture and the solution was stirred at room temperature for 0.5 h. Water (0.401 mL, 0.74 M) was then added dropwise to the solution, which was left to stir for 1 h. The mixture was then diluted with ethyl acetate (10 mL), washed with saturated sodium hydrogen carbonate (2 × 5 mL), brine (5 mL), dried over $MgSO_4$ and concentrated *in vacuo* to produce 2-amidoacetophenone (**204**) (0.047 g, 90%) as a white solid. Spectroscopic data was consistent with the literature.[119] Mp 84–85 °C (lit., [119] Mp 86–87 °C); δ_H (400 MHz, $CDCl_3$) 2.09 (3H, s, H_3), 4.75 (2H, d, *J* 4.3 Hz, H_2), 6.66 (1H, br s, NH), 7.43–7.52 (2H, m, 2 × ArH), 7.57–7.65 (1H, m, 1 × ArH), 7.91–8.01 (2H, m, 2 × ArH); δ_c (101 MHz, $CDCl_3$) 23.1 (CH_3), 46.5 (CH_2), 127.9 (2 × CH), 128.9 (2 × CH), 134.2 (CH), 134.3 (C), 170.2 (C), 194.2 (C); *m/z* (ESI) 200 (MNa^+, 100%).

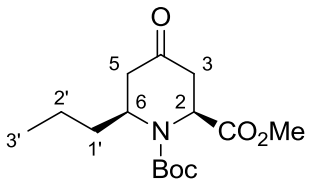
Methyl (2*S*,6*S*)-4-oxo-6-propylpiperidine-2-carboxylate (240)[129] and methyl (2*S*,6*R*)-4-oxo-6-propylpiperidine-2-carboxylate (241)[130]





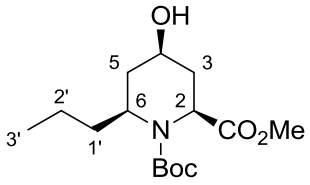
To a solution of methyl (2*S*,5*E*)-2-(tritylamino)-4-oxonon-5-enoate (**108**) (0.103 g, 0.23 mmol) in methanol (10 mL) was added 2 M hydrochloric acid solution (2.5 mL). The mixture was stirred for 1 h, then diluted with water (10 mL) and basified to pH 8 with *NN*-diisopropylethylamine (1.5 mL). The mixture was stirred for 18 h then partitioned between ethyl acetate (20 mL) and brine (20 mL). The aqueous layer was separated and re-extracted with ethyl acetate (20 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel, eluting with 40% ethyl acetate/1% triethylamine in petroleum ether (40–60) to produce methyl (2*S*,6*S*)-4-oxo-6-propylpiperidine-2-carboxylate (**240**) (0.026 g, 56%) as a colourless oil. Further elution yielded methyl (2*S*,6*R*)-4-oxo-6-propylpiperidine-2-carboxylate (**241**) (0.011 g, 24%) as a colourless oil. Data for methyl (2*S*,6*S*)-4-oxo-6-propylpiperidine-2-carboxylate (**240**): R_f 0.22 (40% ethyl acetate/1% triethylamine in petroleum ether); ν_{max} (neat)/cm^{-1} 3330 (NH), 2959, (CH), 2359, 1740 (C=O), 1715 (C=O), 1437, 1217, 1009; $[\alpha]_D^{25}$ −20.9 (*c* 0.5, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.94 (3H, t, *J* 7.0 Hz, 3'-H_3), 1.36–1.63 (4H, m, 1'-H_2 and 2'-H_2), 2.09 (2H, dd, *J* 14.1, 11.7 Hz, 5-H_2), 2.39–2.46 (2H, m, 3-*H*H and NH), 2.69 (1H, dddd, *J* 14.4, 3.4, 2.1, 0.6 Hz, 3-H*H*), 2.83–2.90 (1H, m, 6-H), 3.64 (1H, dd, *J* 12.2, 3.4 Hz, 2-H), 3.78 (3H, s, OCH_3); δ_C (101 MHz, $CDCl_3$) 14.0 (CH_3), 18.8 (CH_2), 38.9 (CH_2), 44.5 (CH_2), 48.4 (CH_2), 52.5 (CH_3), 55.6 (CH), 57.9 (CH), 171.9 (C), 207.3 (C); *m/z* (EI) 199.1212 (M^+. $C_{10}H_{17}NO_3$ requires 199.1208), 156 (95%), 140 (97), 114 (70), 98 (96). Data for methyl (2*S*,6*R*)-4-oxo-6-propylpiperidine-2-carboxylate (**241**): R_f 0.11 (40% ethyl acetate/1% triethylamine in petroleum ether); ν_{max}/cm^{-1} (neat) 3325 (NH), 2957 (CH), 1732 (CO), 1717 (CO), 1435, 1120, 1163; $[\alpha]_D^{24}$ −7.5 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.94 (3H, t, *J* 7.3 Hz, 3'-H_3), 1.22–1.51 (4H, m, 1'-H_2 and 2'-H_2), 2.16 (1H, ddd, *J* 14.5, 9.9, 0.9 Hz, 5-*H*H), 2.43 (1H, ddd, *J* 14.5, 3.7, 1.7 Hz, 5-H*H*), 2.64 (1H, ddd, *J* 15.0, 6.6, 0.9 Hz, 3-*H*H), 2.72 (1H, ddd, *J* 15.0, 3.9, 1.7 Hz, 3-H*H*), 3.06–3.10 (1H, m, 6-H), 3.74 (3H, s, OCH_3), 4.04 (1H, dd, *J* 6.6, 3.9 Hz, 2-H); δ_C (101 MHz, $CDCl_3$) 13.9 (CH_3), 18.7 (CH_2), 38.2 (CH_2), 42.1 (CH_2), 47.7 (CH_2), 52.3 (CH), 52.4 (CH_3), 55.8 (CH), 173.1 (C), 207.1 (C); *m/z* (CI) 200.1292 (MH^+. $C_{10}H_{18}NO_3$ requires 200.1287), 181 (9%), 164 (7), 156 (4), 140 (5).

Methyl (2*S*,6*S*)-1-*tert*-butoxycarbonyl-4-oxo-6-propylpiperidine-2-carboxylate (242)



Methyl (2*S*,6*S*)-4-oxo-6-propylpiperidine-2-carboxylate (**240**) (0.134 g, 0.673 mmol) was dissolved in dichloromethane (7 mL) and cooled to 0 °C. Triethylamine (0.469 mL, 3.37 mmol) was added followed by di-*tert*-butyl dicarbonate (0.294 g, 1.34 mmol). The solution was heated under reflux and stirred for 48 h. Upon completion, the mixture was quenched with an aqueous saturated ammonium chloride solution (5 mL), diluted with dichloromethane (5 mL), washed with water (10 mL), brine (10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel, eluting with 30% ethyl acetate in petroleum ether (40–60) to produce methyl (2*S*,6*S*)-1-*tert*-butoxycarbonyl-4-oxo-6-propylpiperidine-2-carboxylate (**242**) (0.140 g, 70%) as a yellow oil. R_f 0.28 (30% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 2961 (CH), 1690 (C=O), 1395, 1364, 1167, 773; $[\alpha]_D^{27}$ −90.5 (*c* 1.1, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 0.91 (3H, t, *J* 7.2 Hz, 3'-H_3), 1.33–1.50 (12H, m, 3 × CH_3, 2'-H_2 and 1'-*H*H), 1.64–1.73 (1H, m, 1'-H*H*), 2.36–2.48 (1H, m, 5-*H*H), 2.57–2.73 (2H, m, 3-*H*H and 5-H*H*), 2.79–2.93 (1H, m, 3-H*H*), 3.74 (3H, s, OCH_3), 4.32–4.64 (1H, m, 6-H), 4.71–5.24 (1H, m, 2-H); δ_C (126 MHz, $CDCl_3$) 14.0 (CH_3), 19.8 (CH_2), 28.4 (3 × CH_3), 38.1 (CH_2), 39.8 (CH_2), 44.0 (CH_2), 51.8 (CH), 52.6 (CH_3), 53.6 (CH), 81.3 (C), 154.9 (C), 172.5 (C), 205.9 (C); *m/z* (ESI) 322.1624 (MNa^+. $C_{15}H_{25}NNaO_5$ requires 322.1625).

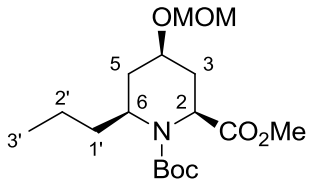
Methyl (2*S*,4*R*,6*S*)-1-*tert*-butyloxycarbonyl-4-hydroxy-6-propylpiperidine-2-carboxylate (243)



Methyl (2*S*,6*S*)-1-*tert*-butoxycarbonyl-4-oxo-6-propylpiperidine-2-carboxylate (**242**) (0.140 g, 0.468 mmol) was dissolved in methanol (5 mL) and cooled to −10 °C. Sodium borohydride (0.0195 g, 0.515 mmol) was then added. The mixture was stirred for 0.5 h and quenched with water (3 mL). The solution was diluted with ethyl acetate (10 mL) and washed with water (5 mL), brine (5 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel, eluting with 30% ethyl acetate in dichloromethane to produce methyl (2*S*,4*R*,6*S*)-1-*tert*-butyloxycarbonyl-4-hydroxy-6-propylpiperidine-2-carboxylate (**243**) (0.098 g,

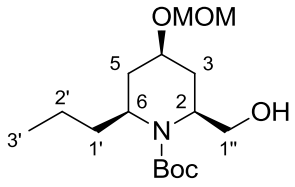
70%) as a colourless oil. R_f 0.38 (30% ethyl acetate in dichloromethane); ν_{max}/cm^{-1} (neat) 3502 (OH), 2958 (CH), 1670 (C=O), 1365, 1170, 1074, 734; $[\alpha]_D^{27}$ −56.4 (*c* 0.9, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 0.90 (3H, t, *J* 7.4 Hz, 3'-H_3), 1.29–1.39 (2H, m, 2'-H), 1.46 (9H, s, 3 × CH_3), 1.55–1.65 (1H, m, 1'-*H*H), 1.71–1.82 (3H, m, 1'-H*H* and 5-H_2), 1.94 (1H, ddd, *J* 14.3, 7.2, 3.5 Hz, 3-*H*H), 2.08 (1H, d, *J* 2.3 Hz, OH), 2.40 (1H, dt, *J* 14.3, 3.5 Hz, 3-H*H*), 3.71 (3H, s, OCH_3), 4.08–4.17 (2H, m, 4-H and 6-H), 4.87 (1H, d, *J* 3.5 Hz, 2-H); δ_C (126 MHz, $CDCl_3$) 14.2 (CH_3), 20.5 (CH_2), 28.5 (3 × CH_3), 32.4 (CH_2), 33.6 (CH_2), 38.0 (CH_2), 49.9 (CH), 50.0 (CH), 52.3 (CH_3), 64.1 (CH), 80.3 (C), 155.6 (C), 174.7 (C); *m/z* (ESI) 324.1792 (MNa^+. $C_{17}H_{26}NNaO_5$ requires 324.1781).

Methyl (2*S*,4*R*,6*S*)-1-*tert*-butyloxycarbonyl-4-(methoxymethoxy)-6-propylpiperidine-2-carboxylate (244)



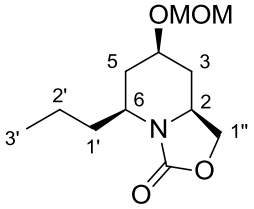
Methyl (2*S*,4*R*,6*S*)-1-*tert*-butyloxycarbonyl-4-hydroxy-6-propylpiperidine-2-carboxylate (**243**) (0.172 g, 0.572 mmol) was dissolved in dichloromethane (10 mL) at room temperature and bromomethyl methyl ether (0.0700 mL, 0.858 mmol) was added, followed by *N*,*N*-diisopropylethylamine (0.149 mL, 0.858 mmol). The mixture was allowed to stir at reflux for 48 h. Upon completion, the mixture was diluted with dichloromethane (10 mL), washed with water (2 × 10 mL), brine (10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel, eluting with 20% ethyl acetate in petroleum ether (40–60) to produce methyl (2*S*,4*R*,6*S*)-1-*tert*-butyloxycarbonyl-4-(methoxymethoxy)-6-propylpiperidine-2-carboxylate (**244**) (0.169 g, 86%) as a colourless oil. R_f 0.30 (20% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 2954 (CH0, 1685 (C=O), 1365, 1174, 1098, 1035, 917, 734; $[\alpha]_D^{24}$ +13.0 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 0.92 (3H, t, *J* 7.4 Hz, 3'-H_3), 1.30–1.39 (2H, m, *J* 14.8, 7.4 Hz, 2'-H_2), 1.45 (9H, s, 3 × CH_3), 1.56–1.67 (1H, m, 1'-*H*H), 1.72–1.88 (3H, m, 1'-H*H* and 5-H_2), 1.93 (1H, ddd, *J* 14.0, 7.5, 3.3 Hz, 3-*H*H), 2.40 (1H, dt, *J* 10.2, 4.6 Hz, 3-H*H*), 3.33 (3H, s, OCH_2OCH_3), 3.69 (3H, s, OCH_3), 3.91 (1H, ddd, *J* 9.2, 5.3, 4.1 Hz, 4-H), 4.10 (1H, td, *J* 8.6, 2.5 Hz, 6-H), 4.52 (1H, d, *J* 6.8 Hz, OC*H*HOCH_3), 4.61 (1H, d, *J* 6.8 Hz, OCH*H*OCH_3), 4.70–4.77 (1H, m, 2-H); δ_C (126 MHz, $CDCl_3$) 14.3 (CH_3), 20.4 (CH_2), 28.5 (3 × CH_3), 29.8 (CH_2), 31.7 (CH_2), 38.3 (CH_2), 50.0 (CH), 51.0 (CH), 51.9 (CH_3), 55.4 (CH_3), 67.8 (CH), 80.20 (C), 94.1 (CH_2), 155.6 (C), 173.3 (C); *m/z* (ESI) 368.2027 (MNa^+. $C_{17}H_{31}NNaO_6$ requires 368.2044).

(2*S*,4*R*,6*S*)-1-*tert*-Butyloxycarbonyl-4-(methoxymethoxy)-6-propylpiperidine-2-hydroxymethyl (245)



Methyl (2*S*,4*R*,6*S*)-1-*tert*-butyloxycarbonyl-4-(methoxymethoxy)-6-propylpiperidine-2-carboxylate (**244**) (0.078 g, 0.226 mmol) was dissolved in diethyl ether (10 mL) and cooled to 0 °C. Diisobutylaluminium hydride solution (0.497 mL, 0.497 mmol, 1.0 M in THF) was added and the mixture was allowed to stir for 48 h while warming to room temperature. Upon completion the reaction was quenched with saturated potassium sodium tartrate solution (10 mL) and allowed to stir vigorously for 1 h. The mixture was diluted with diethyl ether (10 mL), washed with water (5 mL), brine (5 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel, eluting with 40% ethyl acetate in petroleum ether (40–60) to produce (2*S*,4*R*,6*S*)-1-*tert*-butyloxycarbonyl-4-(methoxymethoxy)-6-propylpiperidine-2-hydroxymethyl (**245**) (0.055 g, 77%) as a colourless oil. R_f 0.21 (40% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 3454 (OH), 2957 (CH), 2930 (CH), 1685 (C=O), 1663, 1466, 1366, 1039; $[\alpha]_D^{23}$ −24.6 (*c* 1.2, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.90 (3H, t, *J* 7.3 Hz, 3'-H_3), 1.23–1.40 (2H, m, 2'-H_2), 1.45 (9H, s, 3 × CH_3), 1.59–1.81 (4H, m, 1'-H_2, 3-*H*H and 5-*H*H), 1.87–1.98 (2H, m, 3-H*H* and 5-H*H*), 2.79 (1H, s, OH), 3.35 (3H, s, OCH_3), 3.69 (1H, dd, *J* 10.8, 6.2 Hz, 1''-*H*H), 3.76 (1H, dd, *J* 10.8, 8.3 Hz, 1''-H*H*) 3.84 (1H, tt, *J* 6.2, 4.1 Hz, 4-H), 4.12 (1H, ddd, *J* 15.1, 7.9, 3.5 Hz, 6-H), 4.35 (1H, tdd, *J* 8.0, 6.3, 4.4 Hz, 2-H), 4.63 (2H, s, O*CH*$_2$OCH_3); δ_C (101 MHz, $CDCl_3$) 14.2 (CH_3), 20.2 (CH_2), 28.6 (3 × CH_3), 30.1 (CH_2), 32.1 (CH_2), 40.1 (CH_2), 49.8 (CH), 51.8 (CH), 55.5 (CH), 67.5 (CH_2), 69.8 (CH), 80.3 (C), 95.1 (CH_2), 157.0 (C); *m/z* (ESI) 340.2085 (MNa^+. $C_{16}H_{31}NNaO_5$ requires 340.2094).

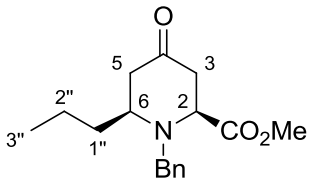
(5*S*,7*R*,8a*S*)-7-(Methoxymethoxy)-5-propyl-hexahydro-1*H*-[1,3]oxazolo[3,4-a]pyridin-3-one (247)



(2*S*,4*R*,6*S*)-1-*tert*-Butyloxycarbonyl-4-(methoxymethoxy)-6-propylpiperidine-2-hydroxymethyl (**245**) (0.0276 g, 0.0870 mmol) was dissolved in dichloromethane (5 mL) and cooled to 0 °C. 4-

(Dimethylamino)pyridine (0.00160 g, 0.0131 mmol), triethylamine (0.0279 mL, 0.200 mmol) and methanesulfonyl chloride (0.0108 mL, 0.139 mmol) was added sequentially. The mixture was allowed to stir at room temperature for 24 h. Upon completion the mixture was diluted with dichloromethane (10 mL), washed with water (5 mL), brine (5 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel, eluting with 40% ethyl acetate/1% triethylamine in petroleum ether (40–60) to produce (5*S*,7*R*,8a*S*)-7-(methoxymethoxy)-5-propyl-hexahydro-1*H*-[1,3]oxazolo[3,4-a]pyridin-3-one (**247**) (0.012 g, 60%) as a colourless oil. R_f 0.13 (40% ethyl acetate/1% triethylamine in petroleum ether); ν_{max}/cm^{-1} (neat) 2958 (CH), 1743 (C=O), 1365, 1224, 1033, 734; $[\alpha]_D^{23}$ −30.7 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.95 (3H, t, *J* 7.4 Hz, 3'-H_3), 1.33–1.49 (4H, m, 2'-H_2, 3-*H*H and 5-*H*H), 1.74 (1H, ddt, *J* 13.8, 8.9, 6.6 Hz, 1'-*H*H), 2.01–2.07 (1H, m, 5-H*H*), 2.07–2.15 (1H, m, 3-H*H*), 2.41 (1H, ddt, *J* 12.9, 9.7, 6.6 Hz, 1'-H*H*), 3.05–3.14 (1H, m, 6-H), 3.34–3.40 (3H, m, OCH_3), 3.53–3.64 (1H, m, 2-H), 3.68 (1H, tt, *J* 11.2, 4.3 Hz, 4-H), 3.87 (1H, dd, *J* 8.4, 4.8 Hz, 1''-*H*H), 4.30 (1H, dd, *J* 8.4, 7.5 Hz, 1''-H*H*), 4.67 (1H, d, *J* 7.0 Hz, OC*H*HCH_3), 4.70 (1H, d, *J* 7.0 Hz, OCH*H*CH_3); δ_C (101 MHz, $CDCl_3$) 14.1 (CH_3), 19.8 (CH_2), 33.4 (CH_2), 36.7 (CH_2), 37.9 (CH_2), 55.0 (CH), 55.6 (CH_3), 56.1 (CH), 66.7 (CH_2), 73.2 (CH), 94.9 (CH_2), 156.1 (C); *m/z* (ESI) 266.1356 (MNa^+. $C_{12}H_{21}NNaO_4$ requires 266.1363).

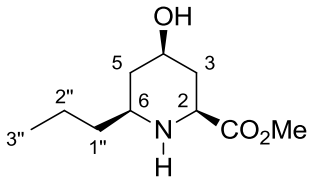
Methyl (2*S*,6*S*)-1-benzyl-4-oxo-6-propylpiperidine-2-carboxylate (248)



Methyl (2*S*,6*S*)-4-oxo-6-propylpiperidine-2-carboxylate (**240**) (0.0414 g, 0.208 mmol) was dissolved in acetonitrile (3 mL) followed by addition of potassium carbonate (0.0431 g, 0.312 mmol) and benzyl bromide (0.0296 mL, 0.249 mmol). The solution was heated under reflux and stirred for 38 h. Upon completion, the mixture was diluted with ethyl acetate (5 mL), washed with water (10 mL), brine (10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel, eluting with 30% ethyl acetate in petroleum ether (40–60) to produce methyl (2*S*,6*S*)-1-benzyl-4-oxo-6-propylpiperidine-2-carboxylate (**248**) (0.049 g, 82%) as a yellow oil. R_f 0.29 (30% ethyl acetate in petroleum ether); $[\alpha]_D^{23}$ −28.7 (*c* 1.2, $CHCl_3$); ν_{max}/cm^{-1} (neat) 2956 (CH), 1732 (C=O), 1454, 1197, 1166, 1028, 734, 698; δ_H (400 MHz, $CDCl_3$) 0.83 (3H, t, *J* 7.1 Hz, 3''-H_3), 1.15–1.40 (3H, m, 2''-H_2 and 1''-*H*H), 1.54–1.67 (1H, m, 1''-H*H*), 2.38 (1H, ddd, *J* 15.4, 7.4, 0.9 Hz, 5-*H*H), 2.48–2.58 (2H, m, 3-*H*H and 5-H*H*), 2.78 (1H, ddd, *J* 15.4, 6.1, 0.9 Hz, 3-H*H*), 3.12 (1H, tt, *J* 7.4, 4.8 Hz, 6-H), 3.57 (3H, s, OCH_3), 3.79 (1H, t, *J* 6.1 Hz, 2-H), 3.92 (1H, d, *J* 14.6 Hz, C*H*HPh), 4.00 (1H, d, *J* 14.6 Hz, CH*H*Ph), 7.22–7.43 (5H, m, Ph); δ_C (101 MHz, $CDCl_3$) 14.0

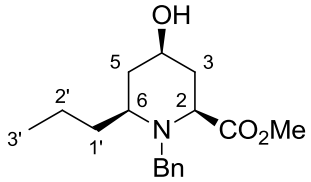
(CH_3), 19.5 (CH_2), 35.5 (CH_2), 40.2 (CH_2), 43.6 (CH_2), 52.0 (CH_3), 55.5 (CH_2), 59.6 (CH), 60.8 (CH), 127.2 (CH), 128.3 (2 × CH), 128.6 (2 × CH), 139.0 (C), 173.1 (C), 207.8 (C); *m/z* (ESI) 312.1558 (MNa^+. $C_{17}H_{23}NNaO_3$ requires 312.1570).

Methyl (2*S*,4*R*,6*S*)-4-hydroxy-6-propylpiperidine-2-carboxylate (250)



Methyl (2*S*,6*S*)-4-oxo-6-propylpiperidine-2-carboxylate (**240**) (0.0728 g, 0.366 mmol) was dissolved in methanol (4 mL) and cooled to −10 °C. Sodium borohydride (0.0145 g, 0.384 mmol) was then added. The mixture was stirred for 0.5 h and quenched with water (3 mL). The solution was diluted with ethyl acetate (10 mL) and washed with water (5 mL), brine (5 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel, eluting with 5% methanol/1% triethylamine in dichloromethane to produce methyl (2*S*,4*R*,6*S*)-4-hydroxy-6-propylpiperidine-2-carboxylate (**250**) (0.044 g, 60%) as a yellow oil. R_f 0.26 (5% methanol/1% triethylamine in dichloromethane); $[\alpha]_D^{23}$ −15.5 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} (neat) 3336 (NH and OH), 2955 (CH), 1737 (C=O), 1435, 1205, 1159, 1047, 754; δ_H (500 MHz, $CDCl_3$) 0.92 (3H, t, *J* 7.1 Hz, 3''-H_3), 1.01 (1H, q, *J* 11.9 Hz, 5-*H*H), 1.28–1.54 (5H, m, 2''-H_2, 1''-H_2 and 3-*H*H), 1.99 (1H, ddt, *J* 11.9, 4.6, 2.5 Hz, 5-H*H*), 2.11 (2H, br s, NH and OH), 2.31 (1H, ddt, *J* 11.9, 4.6, 2.5 Hz, 3-H*H*), 2.57 (1H, dtd, *J* 8.8, 6.2, 2.5 Hz, 6-H), 3.38 (1H, dd, *J* 11.9, 2.5 Hz, 2-H), 3.66–3.76 (4H, m, 4-H and OCH_3); δ_C (126 MHz, $CDCl_3$) 14.1 (CH_3), 19.1 (CH_2), 38.4 (CH_2), 38.7 (CH_2), 41.5 (CH_2), 52.2 (CH_3), 54.0 (CH), 57.3 (CH), 68.9 (CH), 173.0 (C); *m/z* (ESI) 224.1254 (MNa^+. $C_{10}H_{19}NNaO_3$ requires 224.1257).

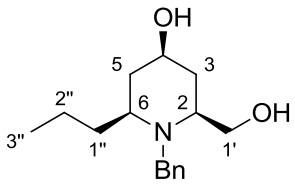
Methyl (2*S*,4*R*,6*S*)-1-benzyl-4-hydroxy-6-propylpiperidine-2-carboxylate (251)



Methyl (2*S*,4*R*,6*S*)-4-hydroxy-6-propylpiperidine-2-carboxylate (**250**) (0.103 g, 0.512 mmol) was dissolved in acetonitrile (5 mL) followed by addition of potassium carbonate (0.283 g, 2.05 mmol), sodium iodide (0.575 g, 3.84 mmol) and benzyl bromide (0.609 mL, 5.12 mmol). The solution was heated under reflux and stirred for 36 h. Upon completion, the mixture was diluted with ethyl

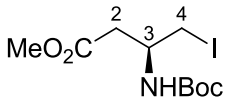
acetate (10 mL), washed with water (10 mL), brine (10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel, eluting with 5% methanol in dichloromethane to produce methyl (2*S*,4*R*,6*S*)-1-benzyl-4-hydroxy-6-propylpiperidine-2-carboxylate (**251**) (0.123 g, 63%) as a yellow oil. R_f 0.28 (5% methanol in dichloromethane); $[\alpha]_D^{21}$ +2.6 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} (neat) 3385 (NH), 2955 (CH), 1735 (C=O), 1452, 1281, 1164, 1027, 732, 699; δ_H (400 MHz, $CDCl_3$) 0.78 (3H, t, *J* 7.3 Hz, 3'-H), 1.13–1.28 (1H, m, 2'-*H*H), 1.29–1.44 (3H, m, 2'-H*H*, 1'-H_2 and 5-*H*H), 1.60–1.79 (2H, m, 1'-H_2 and 3-*H*H), 1.92–2.00 (1H, m, 5-H*H*), 2.00–2.11 (2H, m, 3-H*H* and OH), 2.45 (1H, ddt, *J* 11.1, 8.1, 3.1 Hz, 6-H), 3.30 (1H, dd, *J* 11.7, 2.9 Hz, 2-H), 3.48 (3H, s, OCH_3), 3.56–3.80 (3H, m, CH_2Ph and 4-H), 7.17–7.23 (1H, m, ArH), 7.27–7.35 (4H, m, ArH); δ_C (101 MHz, $CDCl_3$) 14.3 (CH_3), 19.0 (CH_2), 36.3 (CH_2), 36.9 (CH_2), 38.7 (CH_2), 51.9 (CH_3), 54.3 (CH_2), 59.8 (CH), 64.8 (CH), 68.0 (CH), 126.8 (CH), 128.0 (2 × CH_2), 128.8 (2 × CH_2), 139.0 (C), 173.5 (C); *m/z* (ESI) 314.1718 (MNa^+. $C_{17}H_{25}NNaO_3$ requires 314.1727).

(2*S*,4*R*,6*S*)-1-Benzyl-2-(hydroxymethyl)-6-propylpiperidine-4-ol (252)



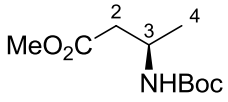
Methyl (2*S*,4*R*,6*S*)-1-benzyl-4-hydroxy-6-propylpiperidine-2-carboxylate (**251**) (0.0522 g, 0.179 mmol) was dissolved in acetonitrile (2 mL) and cooled to 0 °C. Lithium borohydride (0.00897 g, 0.412 mmol) was added and the solution was heated under reflux for 18 h. Upon completion, the mixture was diluted with ethyl acetate (10 mL), washed with water (10 mL), brine (10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel, eluting with 10% methanol in dichloromethane to produce (2*S*,4*R*,6*S*)-1-benzyl-2-(hydroxymethyl)-6-propylpiperidine-4-ol (**252**) (0.034 g, 72%) as an off-white solid. Mp 87–90 °C; R_f 0.26 (10% methanol in dichloromethane); $[\alpha]_D^{23}$ +17.2 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} (neat) 3190 (OH), 2932 (CH), 1454, 1043, 732, 697; δ_H (400 MHz, $CDCl_3$) 0.87 (3H, t, *J* 7.2 Hz, 3''-H_3), 1.20–1.49 (4H, m, 2''-H_2 and 1''-H_2), 1.55 (1H, q, *J* 11.7 Hz, 3-*H*H), 1.61–1.80 (2H, m, 5-*H*H and 3-H*H*), 1.91–2.28 (3H, m, 5-H*H*, NH and OH), 2.62 (1H, ddd, *J* 11.7, 7.1, 3.5 Hz, 6-H), 2.73–2.83 (1H, m, 2-H), 3.35 (1H, dd, *J* 11.7, 5.7 Hz, 1'-*H*H), 3.50 (1H, dd, *J* 11.7, 4.1 Hz, 1'-H*H*), 3.61–3.80 (3H, m, 4-H and CH_2Ph), 7.21–7.26 (1H, m, ArH), 7.30–7.42 (4H, m, 4 × ArH); δ_C (101 MHz, $CDCl_3$) 14.3 (CH_3), 19.6 (CH_2), 34.5 (CH_2), 37.0 (CH_2), 37.9 (CH_2), 50.5 (CH_2), 60.9 (CH), 63.0 (CH), 63.7 (CH_2), 68.7 (CH), 126.9 (CH), 127.5 (2 × CH), 128.7 (2 × CH), 141.4 (C); *m/z* (ESI) 286.1768 (MNa^+. $C_{16}H_{25}NNaO_2$ requires 286.1778).

Methyl (3*S*)-3-(*tert*-butoxycarbonylamino)-4-iodobutanoate (255)[109]



To a suspension of imidazole (4.11 g, 60.4 mmol) and triphenylphosphine (11.9 g, 45.3 mmol) in a mixture of diethyl ether and dichloromethane (2:1, 100 mL) at 0 °C was added iodine (11.5 g, 45.3 mmol) in three portions over 0.5 h. After stirring for a further 0.2 h, a solution of methyl (3*S*)-3-(*tert*-butoxycarbonylamino)-4-hydroxybutanoate (**189**) (7.03 g, 30.2 mmol) in a mixture of diethyl ether and dichloromethane (2:1, 50 mL) was added and the resulting reaction mixture was stirred for 3 h at room temperature. The reaction mixture was filtered through Celite® and the filtrate was concentrated *in vacuo.* The crude product was purified via flash column chromatography on silica gel, eluting with 30% diethyl ether in petroleum ether (40–60) to produce methyl (3*S*)-3-(*tert*-butoxycarbonylamino)-4-iodobutanoate (**255**) (9.33 g, 90%) as a clear oil. Spectroscopic data were consistent with the literature.[109] R_f 0.27 (20% ethyl acetate in petroleum ether); $[\alpha]_D^{33}$ +7.3 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.40 (9H, s, 3 × CH_3), 2.60 (1H, dd, *J* 16.4, 6.1 Hz, 2-*H*H), 2.70 (1H, dd, *J* 16.4, 5.6 Hz, 2-H*H*), 3.28–3.45 (2H, m, 4-H_2), 3.66 (3H, s, OCH_3), 3.81–3.95 (1H, m, 3-H), 5.11 (1H, d, *J* 7.2 Hz, NH); δ_C (101 MHz, $CDCl_3$) 11.1 (CH_2), 28.4 (3 × CH_3), 38.6 (CH_2), 47.7 (CH), 52.0 (CH_3), 80.0 (C) 154.7 (C), 171.2 (C); *m/z* (ESI) 366 (MNa^+, 100%).

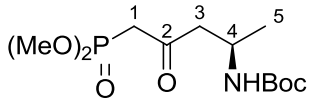
Methyl (3*R*)-3-(*tert*-butoxycarbonylamino)butanoate (256)[175]



A solution of methyl (3*S*)-3-(*tert*-butoxycarbonylamino)-4-iodobutanoate (**255**) (9.33 g, 27.2 mmol), *N*,*N*-diisopropylethylamine (7.11 mL, 40.8 mmol), 10% Pd/C (2.89 g, 2.72 mmol) in methanol (50 mL) was purged with hydrogen for 0.5 h. The reaction mixture was stirred under an atmosphere of hydrogen for 18 h at room temperature. It was then filtered through Celite® and the filtrate was concentrated *in vacuo*. The resulting residue was dissolved in dichloromethane (100 ml) and washed with a saturated solution of sodium hydrogen carbonate (50 mL), 1 M hydrochloric acid (50 ml), brine (50 mL), dried over $MgSO_4$, filtered and the dichloromethane was removed *in vacuo* to give methyl (3*R*)-3-(*tert*-butoxycarbonylamino)butanoate (**256**) as a clear oil (5.87 g, 99%). Spectroscopic data were consistent with the literature.[175] R_f 0.17 (20% ethyl acetate in petroleum ether); $[\alpha]_D^{26}$ +21.5 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.20 (3H, d, *J* 6.8 Hz, 4-H_3), 1.43 (9H, s, 3 × CH_3), 2.47 (1H, dd, *J* 15.5, 6.0 Hz, 2-*H*H), 2.52 (1H, dd, *J* 15.5, 5.4 Hz, 2-H*H*), 3.68 (3H, s, OCH_3), 4.03

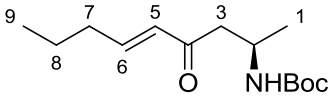
(1H, br s, 3-H), 4.91 (1H, br s, NH); δ_C (126 MHz, $CDCl_3$) 20.5 (CH_3), 28.4 (3 × CH_3), 40.6 (CH_2), 43.4 (CH), 51.6 (CH_3), 79.3 (C), 155.0 (C), 172.0 (C); *m/z* (ESI) 240 (MNa^+, 100%).

(4*R*)-4-(*tert*-Butoxycarbonylamino)-1-(dimethyloxyphosphoryl)pentan-2-one (257)



Dimethyl methylphosphonate (3.74 mL, 34.5 mmol) was dissolved in tetrahydrofuran (100 mL) and cooled to −78 °C under an argon atmosphere. *n*-Butyl lithium (2.5 M, in hexane, 13.8 mL, 34.5 mmol) was added dropwise and the mixture was stirred for 0.3 h. A solution of methyl (3*R*)-3-(*tert*-butoxycarbonylamino)butanoate (**256**) (3.00 g, 13.8 mmol) in tetrahydrofuran (20 mL) was added dropwise. The resulting reaction mixture was then stirred at −78 °C for 0.5 h and allowed to warm to 0 °C over a period of 1 h. The reaction was quenched with a saturated aqueous solution of ammonium chloride (4 mL). Extraction was performed with ethyl acetate (2 × 50 mL). The combined organic layers were then combined, washed with brine (100 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel, eluting with 40% ethyl acetate in dichloromethane to produce (4*R*)-4-(*tert*-butoxycarbonylamino)-1-(dimethyloxyphosphoryl)pentan-2-one (**257**) (2.99 g, 70%) as a clear oil. R_f 0.19 (100% ethyl acetate); ν_{max}/cm^{-1} (neat) 3316 (NH), 2976 (CH), 1704 (C=O), 1700 (C=O), 1248, 1167, 1023; $[\alpha]_D^{31}$ +38.3 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.17 (3H, d, *J* 6.7 Hz, 5-H_3), 1.39 (9H, s, 3 × CH_3), 2.71 (1H, dd, *J* 16.9, 5.9 Hz, 3-*H*H), 2.80 (1H, dd, *J* 16.9, 5.9 Hz, 3-H*H*), 3.03 (1H, dd, *J* 22.6, 13.6 Hz, 1-*H*H), 3.12 (1H, dd, *J* 22.6, 13.6 Hz, 1-H*H*), 3.74 (3H, d, *J* 0.8 Hz, OCH_3), 3.77 (3H, d, *J* 0.8 Hz, OCH_3), 3.93–4.09 (1H, m, 4-H), 4.92 (1H, br s, NH); δ_C (101 MHz, $CDCl_3$) 20.6 (CH_3), 28.3 (3 × CH_3), 41.8 (d, $J_{C\text{-}P}$ 127.5 Hz, CH_2), 43.3 (CH), 50.2 (CH_2), 53.1 (d, $J_{C\text{-}O\text{-}P}$ 6.5 Hz, CH_3), 53.2 (d, $J_{C\text{-}O\text{-}P}$ 6.5 Hz, CH_3), 79.3 (C), 155.2 (C), 200.6 (C); *m/z* (ESI) 332.1225 (MNa^+. $C_{12}H_{24}NNaO_6P$ requires 332.1233).

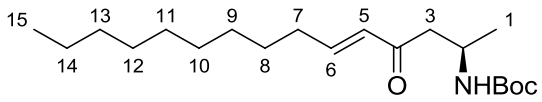
(2*R*,5*E*)-2-(*tert*-Butoxycarbonylamino)-4-oxonona-5-ene (258)



(4*R*)-4-(*tert*-Butoxycarbonylamino)-1-(dimethyloxyphosphoryl)pentan-2-one (**257**) (0.421 g, 1.36 mmol) was dissolved in anhydrous acetonitrile (14 mL) and potassium carbonate (0.225 g, 1.63 mmol) was added. The mixture was stirred at room temperature for 0.5 h followed by addition of butyraldehyde (0.250 mL, 2.72 mmol). The temperature was increased to 50 °C and the mixture

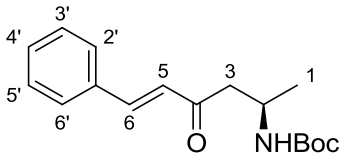
was stirred for 72 h. Once the reaction was complete, the solution was concentrated *in vacuo*, redissolved in ethyl acetate (20 mL) and washed with water (2 × 15 mL) and brine (15 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified via a plug of silica gel, eluting with 20% ethyl acetate in petroleum ether (40–60) to produce (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxonona-5-ene (**258**) as a clear colourless oil (0.301 g, 87%). R_f 0.24 (20% ethyl acetate/petroleum ether); ν_{max}/cm^{-1} (neat) 3350 (NH), 2968 (CH), 1689 (C=O), 1516, 1365, 1166, 1053, 977; $[\alpha]_D^{26}$ +9.3 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 0.94 (3H, t, *J* 7.3 Hz, 9-H_3), 1.20 (3H, d, *J* 6.9 Hz, 1-H_3), 1.43 (9H, s, 3 × CH_3), 1.46–1.55 (2H, m, 8-H_2), 2.20 (qd, *J* 6.9, 1.5 Hz, 7-H_2), 2.64 (1H, dd, *J* 15.9, 6.5 Hz, 3-*H*H), 2.86 (1H, dd, *J* 15.9, 4.5 Hz, 3-H*H*), 3.98–4.09 (1H, m, 2-H), 5.02 (1H, br s, NH), 6.09 (1H, dt, *J* 15.9, 1.5 Hz, 5-H), 6.86 (1H, dt, *J* 15.9, 6.9 Hz, 6-H); δ_C (126 MHz, $CDCl_3$) 13.7 (CH_3), 20.5 (CH_3), 21.4 (CH_2), 28.4 (3 × CH_3), 34.5 (CH_2), 43.7 (CH), 45.7 (CH_2), 79.1 (C), 130.8 (CH), 148.2 (CH), 155.2 (C), 199.2 (C); *m/z* (ESI) 278.1725 (MNa^+. $C_{14}H_{25}NNaO_3$ requires 278.1727).

(2*R*,5*E*)-2-(*tert*-Butoxycarbonylamino)-4-oxopentadec-5-ene (259)



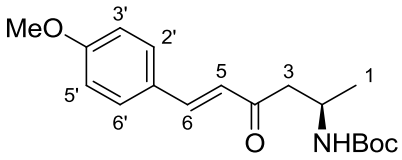
The reaction was carried out according to the above procedure for the synthesis of (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxonona-5-ene (**258**) using (4*R*)-4-(*tert*-butoxycarbonylamino)-1-(dimethyloxyphosphoryl)pentan-2-one (**257**) (0.405 g, 1.31 mmol) and decanal (0.5 mL, 2.618 mmol) for 96 h. The crude product was purified via flash column chromatography on silica gel, eluting with 30% diethyl ether in petroleum ether (40–60) to produce (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxopentadec-5-ene (**259**) (0.345 g, 78%) as a colourless oil. R_f 0.25 (30% diethyl ether in petroleum ether); ν_{max}/cm^{-1} (neat) 3327 (NH), 2958 (CH), 1693 (C=O), 1365, 1170, 1053; $[\alpha]_D^{25}$ +4.2 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.88 (3H, t, *J* 6.3 Hz, 15-H_3), 1.20 (3H, d, *J* 6.7 Hz, 1-H_3), 1.23–1.34 (12H, m, 9-H_2, 10-H_2, 11-H_2, 12-H_2, 13-H_2 and 14-H_2), 1.39–1.50 (11H, m, 8-H_2 and 3 × CH_3), 2.20 (2H, q, *J* 7.0 Hz, 7-H_2), 2.63 (1H, dd, *J* 15.7, 6.6 Hz, 3-*H*H), 2.85 (1H, dd, *J* 15.7, 4.5 Hz, 3-H*H*), 3.96–4.08 (1H, m, 2-H), 4.95 (1H, br s, NH), 6.08 (1H, d, *J* 15.6 Hz, 5-H), 6.85 (1H, dt, *J* 15.6, 7.0 Hz, 6-H); δ_C (126 MHz, $CDCl_3$) 14.1 (CH_3), 20.5 (CH_3), 22.7 (CH_2), 28.1 (CH_2), 28.4 (3 × CH_3), 29.2 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 31.9 (CH_2), 32.5 (CH_2), 43.7 (CH), 45.7 (CH_2), 79.2 (C), 130.6 (CH), 148.5 (CH), 155.2 (C), 199.2 (C); *m/z* (ESI) 362.2649 (MNa^+. $C_{20}H_{37}NNaO_3$ requires 362.2666).

(2*R*,5*E*)-6-Phenyl-2-(*tert*-butoxycarbonylamino)-4-oxohex-5-ene (263)



The reaction was carried out according to the above procedure for the synthesis of (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxonona-5-ene (**258**) using (4*R*)-4-(*tert*-butoxycarbonylamino)-1-(dimethyloxyphosphoryl)pentan-2-one (**257**) (0.251 g, 0.810 mmol) and benzaldehyde (0.160 mL, 1.62 mmol) for 48 h. The crude product was purified via flash column chromatography on silica gel, eluting with 20% ethyl acetate in petroleum ether (40–60) to produce (2*R*,5*E*)-6-phenyl-2-(*tert*-butoxycarbonylamino)-4-oxohex-5-ene (**263**) (0.227 g, 97%) as a white solid. Mp 59–62 °C; R_f 0.18 (20% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 3345 (NH), 2976 (CH), 1687 (C=O), 1655 (C=O), 1608 (C=C), 1495, 1365, 1247, 1166; $[\alpha]_D^{23}$ +10.0 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.25 (3H, d, *J* 6.8 Hz, 1-H_3), 1.43 (9H, s, 3 × CH_3), 2.77 (1H, dd, *J* 15.8, 6.8 Hz, 3-*H*H), 3.00 (1H, dd, *J* 15.8, 4.6 Hz, 3-H*H*), 4.05–4.18 (1H, m, 2-H), 5.04 (1H, br s, NH), 6.73 (1H, d, *J* 16.2 Hz, 5-H), 7.36–7.41 (3H, m, 3 × ArH), 7.52–7.54 (2H, m, 2 × ArH), 7.56 (1H, d, *J* 16.2 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 20.6 (CH_3), 28.4 (3 × CH_3), 43.8 (CH), 46.6 (CH_2), 79.2 (C), 126.4 (CH), 128.4 (2 × CH), 129.0 (2 × CH), 130.6 (CH), 134.4 (C), 143.2 (CH), 155.2 (C), 198.9 (C); *m/z* (ESI) 312.1558 (MNa^+. $C_{17}H_{23}NNaO_3$ requires 312.1570).

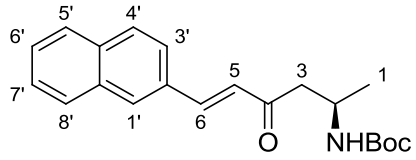
(2*R*,5*E*)-6-(4'-Methoxyphenyl)-2-(*tert*-butoxycarbonylamino)-4-oxohex-5-ene (264)



The reaction was carried out according to the above procedure for the synthesis of (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxonona-5-ene (**258**) using (4*R*)-4-(*tert*-butoxycarbonylamino)-1-(dimethyloxyphosphoryl)pentan-2-one (**257**) (0.241 g, 0.781 mmol) and anisaldehyde (0.190 mL, 1.56 mmol) for 96 h. The crude product was purified via flash column chromatography on silica gel, eluting with 20% ethyl acetate in petroleum ether (40–60) to produce (2*R*,5*E*)-6-(4'-methoxyphenyl)-2-(*tert*-butoxycarbonylamino)-4-oxohex-5-ene (**264**) (0.186 g, 75%) as a white solid. Mp 102–105 °C; R_f 0.10 (20% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 3375 (NH), 2980 (CH), 1682 (C=O), 1600 (C=C), 1511 (C=C), 1324, 1248, 1163, 1030; $[\alpha]_D^{23}$ +50.1 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.24 (3H, d, *J* 6.8 Hz, 1-H_3), 1.43 (9H, s, 3 × CH_3), 2.74 (1H, dd, *J* 15.7, 6.8 Hz, 3-

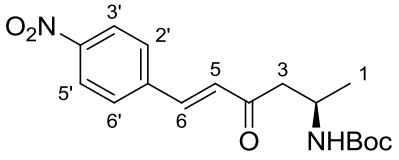
*H*H), 2.97 (1H, dd, *J* 15.7, 4.5 Hz, 3-H*H*), 3.84 (3H, s, OCH_3), 4.04–4.16 (1H, m, 2-H), 5.03 (1H, br s, NH), 6.62 (1H, d, *J* 16.1 Hz, 5-H), 6.91 (2H, d, *J* 8.7 Hz, 3'-H and 5'-H), 7.50 (2H, d, *J* 8.7 Hz, 2'-H and 6'-H), 7.54 (1H, d, *J* 16.1 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 20.6 (CH_3), 28.4 (3 × CH_3), 43.9 (CH), 46.4 (CH_2), 55.4 (CH_3), 79.2 (C), 114.4 (2 × CH), 124.2 (CH), 127.0 (C), 130.1 (2 × CH), 143.1 (CH), 155.2 (C), 161.7 (C), 198.8 (C); *m/z* (ESI) 342.1661 (MNa^+. $C_{18}H_{25}NNaO_4$ requires 342.1676).

(2*R*,5*E*)-6-(Naphthalen-2'-yl)-2-(*tert*-butoxycarbonylamino)-4-oxohex-5-ene (265)



The reaction was carried out according to the above procedure for the synthesis of (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxonona-5-ene (**258**) using (4*R*)-4-(*tert*-butoxycarbonylamino)-1-(dimethyloxyphosphoryl)pentan-2-one (**257**) (0.262 g, 0.849 mmol) and 2-naphthaldehyde (0.265 g, 1.70 mmol) for 48 h. The crude product was purified via flash column chromatography on silica gel, eluting with 30% ethyl acetate in petroleum ether (40–60) to produce (2*R*,5*E*)-6-(naphthalen-2'-yl)-2-(*tert*-butoxycarbonylamino)-4-oxohex-5-ene (**265**) (0.231 g, 80%) as a white solid. Mp 103–106 °C; R_f 0.35 (30% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 3358 (NH), 2972 (CH), 1683 (C=O), 1518 (C=C), 1364, 1247, 1168, 1050; $[\alpha]_D^{23}$ +30.4 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.27 (3H, d, *J* 6.7 Hz, 1-H_3), 1.44 (9H, s, 3 × CH_3), 2.81 (1H, dd, *J* 15.9, 6.7 Hz, 3-*H*H), 3.04 (1H, dd, *J* 15.9, 4.4 Hz, 3-H*H*), 4.07–4.21 (1H, m, 2-H), 5.00 (1H, br s, NH), 6.85 (1H, d, *J* 16.1 Hz, 5-H), 7.48–7.56 (2H, m, 6'-H and 7'-H), 7.68 (1H, dd, *J* 8.6, 1.2 Hz, 3'-H), 7.75 (1H, d, *J* 16.1 Hz, 6-H), 7.81–7.90 (3H, m, 4'-H, 5'-H and 8'-H), 7.97 (1H, br s, 1'-H); δ_C (101 MHz, $CDCl_3$) 20.6 (CH_3), 28.4 (3 × CH_3), 43.9 (CH), 46.7 (CH_2), 79.2 (C), 123.5 (CH), 126.4 (CH), 126.8 (CH), 127.4 (CH), 127.8 (CH), 128.6 (CH), 128.7 (CH), 130.5 (CH), 131.9 (C), 133.3 (C), 134.4 (C), 143.2 (CH), 155.2 (C), 198.8 (C); *m/z* (ESI) 362.1710 (MNa^+. $C_{21}H_{25}NNaO_3$ requires 362.1727).

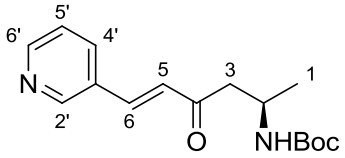
(2*R*,5*E*)-6-(4'-Nitrophenyl)-2-(*tert*-butoxycarbonylamino)-4-oxohex-5-ene (266)



The reaction was carried out according to the above procedure for the synthesis of (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxonona-5-ene (**258**) using (4*R*)-4-(*tert*-butoxycarbonylamino)-1-

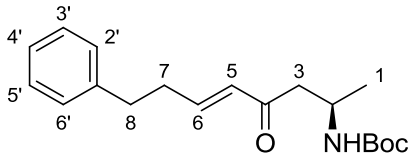
(dimethyloxyphosphoryl)pentan-2-one (**257**) (0.209 g, 0.676 mmol) and 4-nitrobenzaldehyde (0.204 g, 1.35 mmol) for 6 h. The crude product was purified via flash column chromatography on silica gel, eluting with 30% ethyl acetate in petroleum ether (40–60) to produce (2*R*,5*E*)-6-(4'-nitrophenyl)-2-(*tert*-butoxycarbonylamino)-4-oxohex-5-ene (**266**) (0.143 g, 63%) as a pale yellow solid. Mp 122–126 °C; R_f 0.19 (30% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 3365 (NH), 2980 (CH), 1683 (C=O), 1612 (C=O), 1514, 1348, 1168, 1047; $[\alpha]_D^{26}$ +14.7 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.27 (3H, d, *J* 6.7 Hz, 1-H_3), 1.43 (9H, s, 3 × CH_3), 2.80 (1H, dd, *J* 15.8, 6.7 Hz, 3-*H*H), 3.06 (1H, dd, *J* 15.8, 4.2 Hz, 3-H*H*), 4.07–4.20 (1H, m, 2-H), 4.94 (1H, br s, NH), 6.86 (1H, d, *J* 16.2 Hz, 5-H), 7.63 (1H, d, *J* 16.2 Hz, 6-H), 7.72 (2H, d, *J* 8.8 Hz, 2'-H and 6'-H), 8.26 (2H, d, *J* 8.8 Hz, 3'-H and 5'-H); δ_C (101 MHz, $CDCl_3$) 20.6 (CH_3), 28.4 (3 × CH_3), 43.8 (CH), 47.4 (CH_2), 79.4 (C), 124.2 (2 × CH), 128.9 (2 × CH), 129.6 (CH), 140.0 (CH), 140.7 (C), 148.6 (C), 155.2 (C), 198.2 (C); *m/z* (ESI) 357.1405 (MNa^+. $C_{17}H_{22}N_2NaO_5$ requires 357.1421).

(2*R*,5*E*)-6-(Pyridin-3'-yl)-2-(*tert*-butoxycarbonylamino)-4-oxohex-5-ene (267)



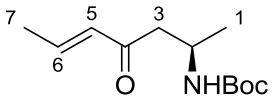
The reaction was carried out according to the above procedure for the synthesis of (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxonona-5-ene (**258**) using (4*R*)-4-(*tert*-butoxycarbonylamino)-1-(dimethyloxyphosphoryl)pentan-2-one (**257**) (0.212 g, 0.687 mmol) and 3-pyridinecarboxaldehyde (0.130 mL, 1.37 mmol) for 24 h. The crude product was purified via flash column chromatography on silica gel, eluting with 40% ethyl acetate in dichloromethane to produce (2*R*,5*E*)-6-(pyridin-3'-yl)-2-(*tert*-butoxycarbonylamino)-4-oxohex-5-ene (**267**) (0.130 g, 65%) as an off-white solid. Mp 93–96 °C; R_f 0.1 (40% ethyl acetate in dichloromethane); ν_{max}/cm^{-1} (neat) 3362 (NH), 2970 (CH), 1678 (C=O), 1519 (C=C), 1365, 1251, 1165, 1057, 977; $[\alpha]_D^{26}$ +6.0 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.26 (3H, d, *J* 6.8 Hz, 1-H_3), 1.43 (9H, s, 3 × CH_3), 2.78 (1H, dd, *J* 15.8, 6.8 Hz, 3-*H*H), 3.03 (1H, dd, *J* 15.8, 4.6 Hz, 3-H*H*), 4.06–4.19 (1H, m, 2-H), 5.03 (1H, br s, NH), 6.81 (1H, d, *J* 16.3 Hz, 5-H), 7.35 (1H, dd, *J* 7.9, 4.1 Hz, 5'-H), 7.59 (1H, d, *J* 16.3 Hz, 6-H), 7.85–7.91 (1H, m, 4'-H), 8.62 (1H, d, *J* 4.1 Hz, 6'-H), 8.77 (1H, br s, 2'-H); δ_C (101 MHz, $CDCl_3$) 20.6 (CH_3), 28.4 (3 × CH_3), 43.8 (CH), 47.1 (CH_2), 79.3 (C), 123.8 (CH), 128.0 (CH), 130.3 (C), 134.4 (CH), 139.3 (CH), 150.1 (CH), 151.2 (CH), 155.2 (C), 198.3 (C); *m/z* (ESI) 313.1513 (MNa^+. $C_{16}H_{22}N_2NaO_3$ requires 313.1523).

(2*R*,5*E*)-8-Phenyl-2-(*tert*-butoxycarbonylamino)-4-oxooct-5-ene (268)



The reaction was carried out according to the above procedure for the synthesis of (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxonona-5-ene (**258**) using (4*R*)-4-(*tert*-butoxycarbonylamino)-1-(dimethyloxyphosphoryl)pentan-2-one (**257**) (0.349 g, 1.13 mmol) and hydrocinnamaldehyde (0.300 mL, 2.26 mmol) for 48 h. The crude product was purified via flash column chromatography on silica gel, eluting with 20% ethyl acetate in petroleum ether (40–60) to produce (2*R*,5*E*)-8-phenyl-2-(*tert*-butoxycarbonylamino)-4-oxooct-5-ene (**268**) (0.288 g, 80%) as a pale yellow oil. R_f 0.19 (20% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 3353 (NH), 2976 (CH), 1692 (C=O), 1496 (C=C), 1247, 1221, 1054; $[\alpha]_D^{23}$ +4.1 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.17 (3H, d, *J* 6.7 Hz, 1-H_3), 1.43 (9H, s, 3 × CH_3), 2.53 (2H, q, *J* 6.8 Hz, 7-H_2), 2.60 (1H, dd, *J* 15.7, 7.8 Hz, 3-*H*H), 2.77 (2H, t, *J* 6.8 Hz, 8-H_2), 2.84 (1H, dd, *J* 15.7, 4.5 Hz, 3-H*H*), 3.95–4.08 (1H, m, 2-H), 4.99 (1H, br s, NH), 6.09 (1H, d, *J* 15.9 Hz, 5-H), 6.87 (1H, dt, *J* 15.9, 6.8 Hz, 6-H), 7.14–7.23 (3H, m, 3 × ArH), 7.25–7.32 (2H, m, 2 × ArH); δ_C (101 MHz, $CDCl_3$) 20.5 (CH_3), 28.4 (3 × CH_3), 34.2 (CH_2), 34.3 (CH_2), 43.7 (CH), 45.8 (CH_2), 79.1 (C), 126.2 (CH), 128.3 (2 × CH), 128.5 (2 × CH), 131.0 (CH), 140.6 (C), 146.9 (CH), 155.1 (C), 198.9 (C); *m/z* (ESI) 340.1868 (MNa^+. $C_{19}H_{27}NNaO_3$ requires 340.1883).

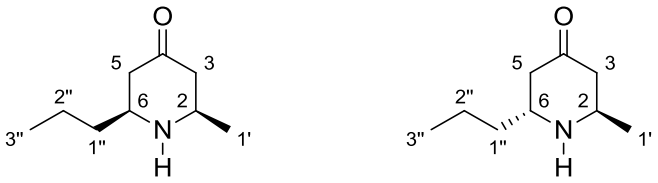
(2*R*,5*E*)-2-(*tert*-Butoxycarbonylamino)-4-oxohepta-5-ene (269)



The reaction was carried out according to the above procedure for the synthesis of (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxonona-5-ene (**258**) using (4*R*)-4-(*tert*-butoxycarbonylamino)-1-(dimethyloxyphosphoryl)pentan-2-one (**257**) (0.386 g, 1.25 mmol) and acetaldehyde (0.140 mL, 2.49 mmol) for 48 h. The crude product was purified via a plug of silica gel, eluting with 30% ethyl acetate in petroleum ether (40–60) producing (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxohepta-5-ene (**269**) (0.197 g, 70%) as a pale yellow oil. R_f 0.27 (30% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 3359 (NH), 2975 (CH), 1690 (C=O), 1517, 1365, 1247, 1166, 1052, 970; $[\alpha]_D^{23}$ +2.6 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.20 (3H, d, *J* 6.7 Hz, 1-H_3), 1.43 (9H, s, 3 × CH_3), 1.91 (3H, d, *J* 6.8 Hz, 7-H_3), 2.62 (1H, dd, *J* 15.7, 6.7 Hz, 3-*H*H), 2.86 (1H, dd, *J* 15.7, 4.6 Hz, 3-H*H*), 3.96–4.09 (1H, m, 2-H), 5.02 (1H, br s, NH), 6.12 (1H, d, *J* 15.8 Hz, 5-H), 6.88 (1H, dq, *J* 15.8, 6.8 Hz, 6-H); δ_C (101 MHz,

$CDCl_3$) 18.3 (CH_3), 20.5 (CH_3), 28.4 (3 × CH_3), 43.8 (CH), 45.7 (CH_2), 79.1 (C), 132.3 (CH), 143.5 (CH), 155.2 (C), 198.9 (C); *m/z* (ESI) 250.1405 (MNa^+. $C_{12}H_{21}NNaO_3$ requires 250.1414).

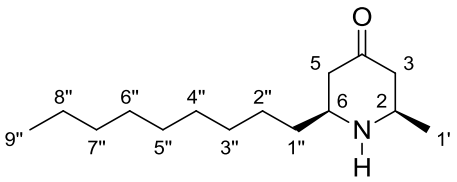
(2*R*,6*S*)-2-Methyl-6-propylpiperidin-4-one (260) and (2*R*,6*R*)-2-methyl-6-propylpiperidin-4-one (261)

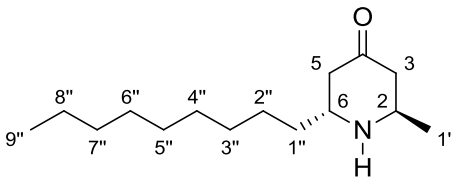


(2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxonona-5-ene (**258**) (0.298 g, 1.17 mmol) was dissolved in dichloromethane (12 mL) and trifluoroacetic acid (0.890 mL, 11.7 mmol) was added dropwise. The mixture was stirred for 1.5 h at room temperature. Once the reaction was complete, the mixture was concentrated *in vacuo* and the crude residue was redissolved in methanol (12 mL) and cooled to 0 °C. *N*,*N*-Diisopropylethylamine (0.300 mL, 1.75 mmol) was added dropwise and the mixture was allowed to warm to room temperature and left to stir for 2 h. Upon completion, the mixture was diluted with ethyl acetate (15 mL) and washed with a saturated aqueous solution of sodium hydrogen carbonate (10 mL) and brine (10 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel (soaked with 1% triethylamine/dichloromethane), with a gradient elution from 40% ethyl acetate/10% dichloromethane/1% triethylamine in petroleum ether (40–60) to 40% ethyl acetate/30% dichloromethane/1% triethylamine in petroleum ether (40–60) to produce (2*R*,6*S*)-2-methyl-6-propylpiperidin-4-one (**260**) (0.0780 g, 43%) as a dark orange oil. Further elution yielded (2*R*,6*R*)-2-methyl-6-propylpiperidin-4-one (**261**) (0.0760 g, 42%) as a dark orange oil. Data for (2*R*,6*S*)-2-methyl-6-propylpiperidin-4-one (**260**): R_f 0.47 (40% ethyl acetate/30% dichloromethane/1% triethylamine in petroleum ether); ν_{max}/cm^{-1} (neat) 3302 (NH), 2962 (CH), 1658 (C=O), 1527, 1458, 1257; $[\alpha]_D^{26}$ +9.8 (*c* 0.2, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.93 (3H, t, *J* 7.2 Hz, 3''-H_3), 1.21 (3H, d, *J* 6.2 Hz, 1'-H_3), 1.29–1.68 (5H, m, 1''-H_2, 2''-H_2 and NH), 1.99–2.12 (2H, m, 3-*H*H and 5-*H*H), 2.30–2.39 (2H, m, 3-H*H* and 5-H*H*), 2.85 (1H, tdd, *J* 9.1, 6.2, 2.9 Hz, 6-H), 2.96 (1H, dqd, *J* 12.4, 6.2, 2.9 Hz, 2-H); δ_C (101 MHz, $CDCl_3$) 14.1 (CH_3), 18.9 (CH_2), 22.7 (CH_3), 39.2 (CH_2), 48.2 (CH_2), 50.2 (CH_2), 52.1 (CH), 56.3 (CH), 209.6 (C); *m/z* (ESI) 156.1377 (MH^+. $C_9H_{18}NO$ requires 156.1383). Data for (2*R*,6*R*)-2-methyl-6-propylpiperidin-4-one (**261**): R_f 0.23 (40% ethyl acetate/30% dichloromethane/1% triethylamine in petroleum ether); ν_{max}/cm^{-1} (neat) 3271 (NH), 2954 (CH), 1720 (C=O), 1535, 1458, 964; $[\alpha]_D^{26}$ −7.9 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.92 (3H, t, *J* 7.1 Hz, 3''-H_3), 1.16 (3H, d, *J* 6.5 Hz, 1'-H_3), 1.23–1.52 (4H, m, 1''-H_2 and 2''-H_2), 1.61 (1H, br s, NH), 2.14 (2H, dddd, *J* 13.6, 11.6, 6.0, 1.6 Hz, 3-*H*H and 5-*H*H), 2.47 (2H, dddd, *J* 15.6, 14.0, 5.2, 1.6 Hz, 3-H*H* and

5-H*H*),3.27–3.36 (1H, m, 6-H), 3.39–3.49 (1H, m, 2-H); δ_C (101 MHz, $CDCl_3$) 13.9 (CH_3), 19.3 (CH_2), 21.6 (CH_3), 36.9 (CH_2), 47.8 (CH_2 and CH), 49.6 (CH_2), 52.3 (CH), 210.0 (C); *m/z* (ESI) 156.1378 (MH^+. $C_9H_{18}NO$ requires 156.1383).

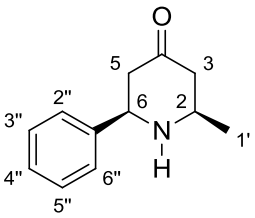
(2*R*,6*S*)-2-Methyl-6-nonylpiperidin-4-one (239)[144] and (2*R*,6*R*)-2-methyl-6-nonylpiperidin-4-one (262)

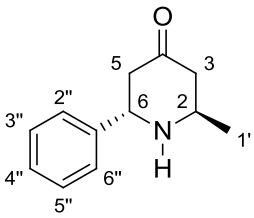




The reaction was carried out according to the above procedure for the synthesis of (2*R*,6*S*)-2-methyl-6-propylpiperidin-4-one (**260**) and (2*R*,6*R*)-2-methyl-6-propylpiperidin-4-one (**261**) using (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxopentadec-5-ene (**259**) (0.302 g, 0.889 mmol). The crude product was purified via flash column chromatography on silica gel, (soaked with 1% triethylamine/petroleum ether) eluting with 20% ethyl acetate/1% triethylamine in petroleum ether (40–60) to produce (2*R*,6*S*)-2-methyl-6-nonylpiperidin-4-one (**239**) (0.0950 g, 45%) as an orange oil. Further elution yielded (2*R*,6*R*)-2-methyl-6-nonylpiperidin-4-one (**262**) (0.0930 g, 44%) as an orange oil. Data for (2*R*,6*S*)-2-methyl-6-nonylpiperidin-4-one (**239**): R_f 0.22 (20% ethyl acetate/1% triethylamine in petroleum ether); $[\alpha]_D^{26}$ −2.4 (*c* 1.0, $CHCl_3$), lit.,[144] $[\alpha]_D^{22}$ −1.5 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.88 (3H, t, *J* 6.2 Hz, 9''-H_3), 1.21 (3H, d, *J* 5.8 Hz, 1'-H_3), 1.23–1.61 (17H, m, 1''-H_2, 2''-H_2, 3''-H_2, 4''-H_2, 5''-H_2, 6''-H_2, 7''-H_2, 8''-H_2 and NH), 1.99–2.12 (2H, m, 3-*H*H and 5-*H*H), 2.24–2.45 (2H, m, 3-H*H* and 5-H*H*), 2.77–2.89 (1H, m, 6-H), 2.90–3.04 (1H, m, 2-H); δ_C (101 MHz, $CDCl_3$) 14.1 (CH_3), 22.7 (CH_3 and CH_2), 25.7 (CH_2), 29.3 (CH_2), 29.5 (2 × CH_2), 29.6 (CH_2), 31.9 (CH_2), 37.1 (CH_2), 48.2 (CH_2), 50.2 (CH_2), 52.1 (CH), 56.6 (CH), 209.6 (C); *m/z* (ESI) 240 (MH^+, 100%). Data for (2*R*,6*R*)-2-methyl-6-nonylpiperidin-4-one (**262**): R_f 0.07 (20% ethyl acetate/1% triethylamine in petroleum ether); ν_{max}/cm^{-1} (neat) 3300 (NH), 2958 (CH), 1710 (C=O), 1458, 1377, 1327; $[\alpha]_D^{26}$ −3.5 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.88 (3H, t, *J* 6.3 Hz, 9''-H_3), 1.16 (3H, d, *J* 6.4 Hz, 1'-H_3), 1.20–1.54 (17H, m, 1''-H_2, 2''-H_2, 3''-H_2, 4''-H_2, 5''-H_2, 6''-H_2, 7''-H_2, 8''-H_2 and NH), 2.09–2.20 (2H, m, 3-*H*H and 5-*H*H), 2.42–2.54 (2H, m, 3-H*H* and 5-H*H*), 3.24–3.34 (1H, m, 6-H), 3.39–3.49 (1H, m, 2-H); δ_C (101 MHz, $CDCl_3$) 14.1 (CH_3), 21.7 (CH_3), 22.7 (CH_2), 26.1 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 31.9 (CH_2), 34.8 (CH_2), 47.8 (CH), 47.8 (CH_2), 49.6 (CH_2), 52.7 (CH), 210.0 (C); *m/z* (ESI) 240.2316 (MH^+. $C_{15}H_{30}NO$ requires 240.2322).

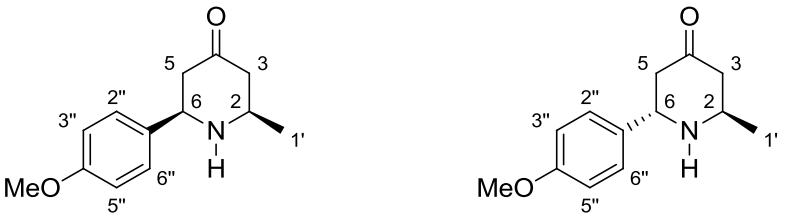
(2*R*,6*R*)-2-Methyl-6-phenylpiperidin-4-one (270)[139] and (2*R*,6*S*)-2-methyl-6-phenylpiperidin-4-one (271)





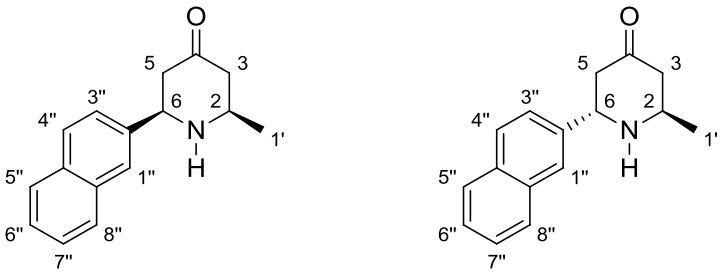
The reaction was carried out according to the above procedure for the synthesis of (2*R*,6*S*)-2-methyl-6-propylpiperidin-4-one (**260**) and (2*R*,6*R*)-2-methyl-6-propylpiperidin-4-one (**261**) using (2*R*,5*E*)-6-phenyl-2-(*tert*-butoxycarbonylamino)-4-oxohex-5-ene (**263**) (0.105 g, 0.365 mmol), dichloromethane (4 mL) and trifluoroacetic acid (0.279 mL, 3.65 mmol). The crude product was purified via flash column chromatography on silica gel (soaked with 1% triethylamine/petroleum ether), with a gradient elution from 30% ethyl acetate/1% triethylamine in petroleum ether (40–60) to 60% ethyl acetate/1% triethylamine in petroleum ether (40–60) to produce (2*R*,6*R*)-2-methyl-6-phenylpiperidin-4-one (**270**) (0.0290 g, 42%) as an orange solid. Further elution yielded (2*R*,6*S*)-2-methyl-6-phenylpiperidin-4-one (**271**) (0.0280 g, 40%) as an orange solid. Data for (2*R*,6*R*)-2-methyl-6-phenylpiperidin-4-one (**270**): Mp 58–61 °C; R_f 0.30 (30% ethyl acetate/1% triethylamine in petroleum ether); $[\alpha]_D^{26}$ +63.4 (*c* 0.9, $CHCl_3$), lit.,[139] $[\alpha]_D^{20}$ +72.2 (*c* 0.9, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.26 (3H, d, *J* 6.1 Hz, 1'-H_3), 1.80 (1H, br s, NH), 2.23 (1H, dd, *J* 14.0, 11.9 Hz, 3-*H*H), 2.41 (1H, dd, *J* 14.0, 2.7 Hz, 3-H*H*), 2.47–2.52 (2H, m, 5-H_2), 3.12 (1H, dqd, *J* 11.9, 6.1, 2.7 Hz, 2-H), 3.96 (1H, dt, *J* 11.9, 8.0 Hz, 6-H), 7.27–7.43 (5H, m, Ph); δ_C (101 MHz, $CDCl_3$) 22.7 (CH_3), 49.8 (CH_2), 50.0 (CH_2), 52.4 (CH), 61.1 (CH), 126.5 (2 × CH), 127.9 (CH), 128.8 (2 × CH), 142.7 (C), 208.9 (C); *m/z* (ESI) 190 (MH^+, 100%). Data for (2*R*,6*S*)-2-methyl-6-phenylpiperidin-4-one (**271**): Mp 65–69 °C; R_f 0.06 (30% ethyl acetate/1% triethylamine in petroleum ether); ν_{max}/cm^{-1} (neat) 3309 (NH), 2962 (CH), 1712 (C=O), 1450 (C=C), 1242, 756, 702; $[\alpha]_D^{26}$ +8.2 (*c* 0.2, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.19 (3H, d, *J* 6.4 Hz, 1'-H_3), 1.82 (1H, br s, NH), 2.23 (1H, ddd, *J* 14.2, 6.4, 1.1 Hz, 3-*H*H), 2.59–2.73 (3H, m, 3-H*H* and 5-H_2), 3.41–3.51 (1H, m, 2-H), 4.48 (1H, t, *J* 8.0 Hz, 6-H), 7.25–7.41 (5H, m, Ph) ; δ_C (101 MHz, $CDCl_3$) 21.2 (CH_3), 47.8 (CH), 47.8 (CH_2), 48.9 (CH_2), 55.6 (CH), 126.9 (2 × CH), 127.6 (CH), 128.7 (2 × CH), 142.7 (C), 209.5 (C); *m/z* (ESI) 190.1226 (MH^+. $C_{12}H_{16}NO$ requires 190.1226).

(2*R*,6*R*)-2-Methyl-6-(4''-methoxyphenyl)piperidin-4-one (272) and (2*R*,6*S*)-2-methyl-6-(4''-methoxyphenyl)piperidin-4-one (273)



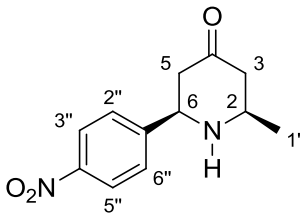
The reaction was carried out according to the above procedure for the synthesis of (2*R*,6*S*)-2-methyl-6-propylpiperidin-4-one (**260**) and (2*R*,6*R*)-2-methyl-6-propylpiperidin-4-one (**261**) using (2*R*,5*E*)-6-(4'-methoxyphenyl)-2-(*tert*-butoxycarbonylamino)-4-oxohex-5-ene (**264**) (0.178 g, 0.558 mmol). The crude product was purified via flash column chromatography on silica gel (soaked with 1% triethylamine/petroleum ether), with a gradient elution from 40% ethyl acetate/1% triethylamine in petroleum ether (40–60) to 80% ethyl acetate/1% triethylamine in petroleum ether (40–60) to produce (2*R*,6*R*)-2-methyl-6-(4''-methoxyphenyl)piperidin-4-one (**272**) (0.0560 g, 46%) as an orange solid. Further elution yielded (2*R*,6*S*)-2-methyl-6-(4''-methoxyphenyl)piperidin-4-one (**273**) (0.0450 g, 37%) as a red solid. Data for (2*R*,6*R*)-2-methyl-6-(4''-methoxyphenyl)piperidin-4-one (**272**): Mp 83–85 °C; R_f 0.22 (40% ethyl acetate/1% triethylamine in petroleum ether); ν_{max}/cm^{-1} (neat) 3300 (NH), 2966 (CH), 1708 (C=O), 1510 (C=C), 1301, 1242, 1028, 823, 788; $[\alpha]_D^{26}$ +65.3 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.25 (3H, d, *J* 6.2 Hz, 1'-H_3), 1.79 (1H, br s, NH), 2.21 (1H, dd, *J* 14.0, 11.9 Hz, 3-*H*H), 2.39 (1H, dd, *J* 14.0, 2.6 Hz, 3-H*H*), 2.44–2.51 (2H, m, 5-H_2), 3.10 (1H, dqd, *J* 11.9, 6.2, 2.6 Hz, 2-H), 3.80 (3H, s, OCH_3), 3.90 (1H, dd, *J* 8.6, 6.2 Hz, 6-H), 6.87–6.91 (2H, m, 3''-H and 5''-H), 7.30–7.34 (2H, m, 2''-H and 6''-H); δ_C (126 MHz, $CDCl_3$) 22.6 (CH_3), 49.8 (CH_2), 50.1 (CH_2), 52.3 (CH), 55.3 (CH_3), 60.5 (CH), 114.1 (2 × CH), 127.7 (2 × CH), 134.9 (C), 159.2 (C), 209.0 (C); *m/z* (ESI) 220.1327 (MH^+. $C_{13}H_{18}NO_2$ requires 220.1332). Data for (2*R*,6*S*)-2-methyl-6-(4''-methoxyphenyl)piperidin-4-one (**273**): Mp 78–80 °C; R_f 0.070 (40% ethyl acetate/1% triethylamine in petroleum ether); ν_{max}/cm^{-1} (neat) 3300 (NH), 2823 (CH), 1705 (C=O), 1512 (C=C), 1141, 1026, 825, 786; $[\alpha]_D^{26}$ +34.7 (*c* 0.2, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.18 (3H, d, *J* 6.5 Hz, 1'-H_3), 1.76 (1H, br s, NH), 2.22 (1H, ddd, *J* 14.2, 6.5, 1.1 Hz, 3-*H*H), 2.58–2.70 (3H, m, 3-H*H* and 5-H_2), 3.39–3.48 (1H, m, 2-H), 3.80 (3H, s, OCH_3), 4.44 (1H, t, *J* 6.5 Hz, 6-H), 6.85–6.90 (2H, m, 3''-H and 5''-H), 7.23–7.29 (2H, m, 2''-H and 6''-H); δ_C (101 MHz, $CDCl_3$) 21.2 (CH_3), 47.7 (CH), 48.0 (CH_2), 48.9 (CH_2), 55.1 (CH_3), 55.3 (CH), 114.0 (2 × CH), 128.0 (2 × CH), 134.8 (C), 158.9 (C), 209.7 (C); *m/z* (ESI) 220.1331 (MH^+. $C_{13}H_{18}NO_2$ requires 220.1332).

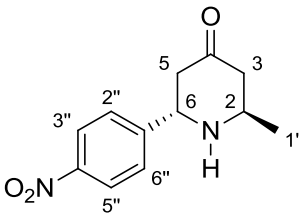
(2*R*,6*R*)-2-Methyl-6-(naphthalen-2''-yl)piperidin-4-one (274) and (2*R*,6*S*)-2-methyl-6-(naphthalen-2''-yl)piperidin-4-one (275)



The reaction was carried out according to the above procedure for the synthesis of (2*R*,6*S*)-2-methyl-6-propylpiperidin-4-one (**260**) and (2*R*,6*R*)-2-methyl-6-propylpiperidin-4-one (**261**) using (2*R*,5*E*)-6-(naphthalen-2'-yl)-2-(*tert*-butoxycarbonylamino)-4-oxohex-5-ene (**265**) (0.132 g, 0.390 mmol). The crude product was purified via flash column chromatography on silica gel (soaked with 1% triethylamine/petroleum ether), with a gradient elution from 40% ethyl acetate/1% triethylamine in petroleum ether (40–60) to 1% triethylamine in ethyl acetate to produce (2*R*,6*R*)-2-methyl-6-(naphthalen-2''-yl)piperidin-4-one (**274**) (0.0410 g, 44%) as an orange oil. Further elution yielded (2*R*,6*S*)-2-methyl-6-(naphthalen-2''-yl)piperidin-4-one (**275**) (0.0390 g, 42%) as an orange oil. Data for (2*R*,6*R*)-2-methyl-6-(naphthalen-2''-yl)piperidin-4-one (**274**): R_f 0.39 (40% ethyl acetate/1% triethylamine in petroleum ether); ν_{max}/cm^{-1} (neat) 3311 (NH), 2970 (CH), 1714 (C=O), 1373, 1303, 1232, 860; $[\alpha]_D^{26}$ +65.9 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.29 (3H, d, *J* 6.2 Hz, 1'-H_3), 1.91 (1H, br s, NH), 2.27 (1H, dd, *J* 14.0, 11.9 Hz, 3-*H*H), 2.44 (1H, dd, *J* 14.0, 2.7 Hz, 3-H*H*), 2.57 (2H, d, *J* 8.0 Hz, 5-H_2), 3.17 (1H, dqd, *J* 11.9, 6.2, 2.7 Hz, 2-H), 4.08–4.15 (1H, m, 6-H), 7.45–7.51 (2H, m, 6''-H and 7''-H), 7.52 (1H, dd, *J* 8.6, 1.5 Hz, 3''-H), 7.80–7.86 (4H, m, 1''-H, 4''-H, 5''-H and 8''-H); δ_C (126 MHz, $CDCl_3$) 22.7 (CH_3), 49.8 (CH_2), 49.9 (CH_2), 52.4 (CH), 61.1 (CH), 124.7 (CH), 125.1 (CH), 126.0 (CH), 126.3 (CH), 127.7 (CH), 127.9 (CH), 128.5 (CH), 133.1 (C), 133.4 (C), 140.0 (C), 208.7 (C); *m/z* (ESI) 240.1379 (MH^+. $C_{16}H_{18}NO$ requires 240.1383). Data for (2*R*,6*S*)-2-methyl-6-(naphthalen-2''-yl)piperidin-4-one (**275**): R_f 0.10 (40% ethyl acetate/1% triethylamine in petroleum ether); ν_{max}/cm^{-1} (neat) 3315 (NH), 2964 (CH), 1708 (C=O), 1506 (C=C), 1305, 1236, 858, 619; $[\alpha]_D^{26}$ −58.7 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.20 (3H, d, *J* 6.6 Hz, 1'-H_3), 1.86 (1H, br s, NH), 2.26 (1H, ddd, *J* 14.2, 6.6, 1.4 Hz, 3-*H*H), 2.65 (1H, ddd, *J* 14.2, 4.9, 1.4 Hz, 3-H*H*), 2.74 (1H, ddd, *J* 14.4, 5.3, 1.4 Hz, 5-*H*H), 2.81 (1H, ddd, *J* 14.4, 6.9, 1.4 Hz, 5-H*H*), 3.41–3.49 (1H, m, 2-H), 4.65 (1H, dd, *J* 6.9, 5.3 Hz, 6-H), 7.45–7.51 (3H, m, 3''-H, 6''-H and 7''-H), 7.75 (1H, br s, 1''-H), 7.79–7.86 (3H, m, 4''-H, 5''-H and 8''-H); δ_C (101 MHz, $CDCl_3$) 21.1 (CH_3), 47.4 (CH_2), 47.8 (CH), 48.8 (CH_2), 55.7 (CH), 125.1 (CH), 125.6 (CH), 126.1 (CH), 126.4 (CH), 127.6 (CH), 128.0 (CH), 128.6 (CH), 132.8 (C), 133.2 (C), 139.5 (C), 208.6 (C); *m/z* (ESI) 240.1385 (MH^+. $C_{16}H_{18}NO$ requires 240.1383).

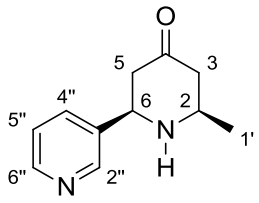
(2*R*,6*R*)-2-Methyl-6-(4''-nitrophenyl)piperidin-4-one (276) and (2*R*,6*S*)-2-methyl-6-(4''-nitrophenyl)piperidin-4-one (277)

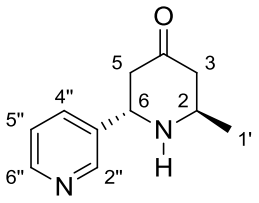




The reaction was carried out according to the above procedure for the synthesis of (2*R*,6*S*)-2-methyl-6-propylpiperidin-4-one (**260**) and (2*R*,6*R*)-2-methyl-6-propylpiperidin-4-one (**261**) using (2*R*,5*E*)-6-(4'-nitrophenyl)-2-(*tert*-butoxycarbonylamino)-4-oxohex-5-ene (**266**) (0.103 g, 0.309 mmol). The crude product was purified via flash column chromatography on silica gel (soaked with 1% triethylamine/petroleum ether), eluting with 40% ethyl acetate/1% triethylamine in petroleum ether (40–60) to produce (2*R*,6*R*)-2-methyl-6-(4''-nitrophenyl)piperidin-4-one (**276**) (0.0310 g, 43%) as a red solid. Further elution yielded (2*R*,6*S*)-2-methyl-6-(4''-nitrophenyl)piperidin-4-one (**277**) (0.0300 g, 41%) as a brown solid. Data for (2*R*,6*R*)-2-methyl-6-(4''-nitrophenyl)piperidin-4-one (**276**): Mp 117–120 °C; R_f 0.21 (40% ethyl acetate/1% triethylamine in petroleum ether); ν_{max}/cm^{-1} (neat) 3321 (NH), 2968 (CH), 1705 (C=O), 1510, 1346, 1305, 1292, 858; $[\alpha]_D^{26}$ +62.4 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.29 (3H, d, *J* 6.1 Hz, 1'-H_3), 1.86 (1H, br s, NH), 2.25 (1H, ddd, *J* 14.0, 12.0, 0.8 Hz, 3-*H*H), 2.37–2.54 (3H, m, 3-H*H* and 5-H_2), 3.15 (1H, dqd, *J* 12.0, 6.1, 2.9 Hz, 2-H), 4.09 (1H, dd, *J* 11.8, 3.2 Hz, 6-H), 7.58–7.64 (2H, m, 2''-H and 6''-H), 8.19–8.26 (2H, m, 3''-H and 5''-H); δ_C (126 MHz, $CDCl_3$) 22.6 (CH_3), 49.6 (CH_2), 49.7 (CH_2), 52.2 (CH), 60.3 (CH), 124.1 (2 × CH), 127.4 (2 × CH), 147.5 (C), 149.9 (C), 207.4 (C); *m/z* (ESI) 235.1073 (MH^+. $C_{12}H_{15}N_2O_3$ requires 235.1077). Data for (2*R*,6*S*)-2-methyl-6-(4''-nitrophenyl)piperidin-4-one (**277**): Mp 88–91 °C; R_f 0.07 (40% ethyl acetate/1% triethylamine in petroleum ether); ν_{max}/cm^{-1} (neat) 3300 (NH), 2962 (CH), 1707 (C=O), 1508, 1344, 1236, 1170, 854, 700; $[\alpha]_D^{26}$ +11.1 (*c* 0.2, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.22 (3H, d, *J* 6.6 Hz, 1'-H_3), 1.82 (1H, br s, NH), 2.26 (1H, ddd, *J* 14.0, 6.1, 1.0 Hz, 3-*H*H), 2.61–2.72 (3H, m, 3-H*H* and 5-H_2), 3.42–3.50 (1H, m, 2-H), 4.59 (1H, t, *J* 5.0 Hz, 6-H), 7.55–7.60 (2H, m, 2''-H and 6''-H), 8.18–8.23 (2H, m, 3''-H and 5''-H); δ_C (126 MHz, $CDCl_3$) 21.1 (CH_3), 47.7 (CH_2), 48.2 (CH), 48.9 (CH_2), 55.2 (CH), 123.9 (2 × CH), 127.8 (2 × CH), 147.3 (C), 150.0 (C), 208.3 (C); *m/z* (ESI) 235.1075 (MH^+. $C_{12}H_{15}N_2O_3$ requires 235.1077).

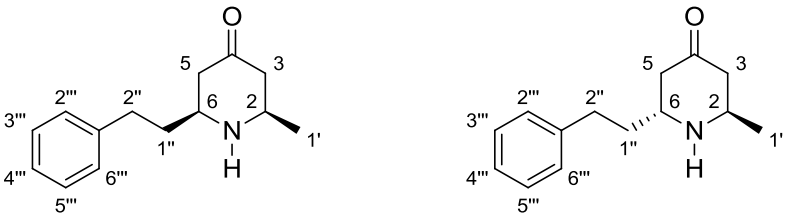
(2*R*,6*R*)-2-Methyl-6-(pyridin-3''-yl)piperidin-4-one (278) and (2*R*,6*S*)-2-methyl-6-(pyridin-3''-yl)piperidin-4-one (279)





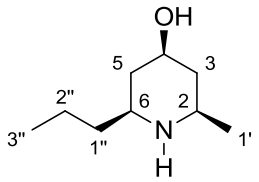
The reaction was carried out according to the above procedure for the synthesis of (2*R*,6*S*)-2-methyl-6-propylpiperidin-4-one (**260**) and (2*R*,6*R*)-2-methyl-6-propylpiperidin-4-one (**261**) using (2*R*,5*E*)-6-(pyridin-3'-yl)-2-(*tert*-butoxycarbonylamino)-4-oxohex-5-ene (**267**) (0.0961 g, 0.331 mmol). The crude product was purified via flash column chromatography on silica gel (soaked with 1% triethylamine/dichloromethane), eluting with 2% methanol/50% dichloromethane/1% triethylamine in petroleum ether (40–60) to produce (2*R*,6*R*)-2-methyl-6-(pyridin-3''-yl)piperidin-4-one (**278**) (0.0260 g, 41%) as an orange oil. Further elution yielded (2*R*,6*S*)-2-methyl-6-(pyridin-3''-yl)piperidin-4-one (**279**) (0.0250 g, 39%) as an orange oil. Data for (2*R*,6*R*)-2-methyl-6-(pyridin-3''-yl)piperidin-4-one (**278**): R_f 0.22 (2% methanol/50% dichloromethane/1% triethylamine in petroleum ether); ν_{max}/cm^{-1} (neat) 3285 (NH), 2965 (CH), 1709 (C=O), 1426, 1304, 1026, 713; $[\alpha]_D^{26}$ +70.5 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.27 (3H, d, *J* 6.1 Hz, 1'-H_3), 1.85 (1H, br s, NH), 2.23 (1H, dd, *J* 14.0, 10.6 Hz, 3-*H*H), 2.40–2.54 (3H, m, 3-H*H* and 5-H_2), 3.14 (1H, dqd, *J* 12.2, 6.1, 2.9 Hz, 2-H), 4.01 (1H, dd, *J* 10.6, 4.4 Hz, 6-H), 7.31 (1H, dd, *J* 7.9, 4.8 Hz, 5''-H), 7.77 (1H, dt, *J* 7.9, 1.9 Hz, 4''-H), 8.56 (1H, dd, *J* 4.8, 1.9 Hz, 6''-H), 8.64 (1H, br d, *J* 1.9 Hz, 2''-H); δ_C (126 MHz, $CDCl_3$) 22.6 (CH_3), 49.6 (CH_2), 49.6 (CH_2), 52.4 (CH), 58.6 (CH), 123.7 (CH), 134.2 (CH), 138.0 (C), 148.5 (CH), 149.5 (CH), 207.9 (C); *m/z* (ESI) 191.1181 (MH^+. $C_{11}H_{15}N_2O$ requires 191.1179). Data for (2*R*,6*S*)-2-methyl-6-(pyridin-3''-yl)piperidin-4-one (**279**): R_f 0.11 (2% methanol/50% dichloromethane/1% triethylamine in petroleum ether); ν_{max}/cm^{-1} (neat) 3263 (NH), 2962 (CH), 1705 (C=O), 1419, 1026, 802, 709; $[\alpha]_D^{26}$ +38.9 (*c* 0.2, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.22 (3H, d, *J* 6.4 Hz, 1'-H_3), 1.69 (1H, br s, NH), 2.26 (1H, dd, *J* 14.1, 6.4 Hz, 3-*H*H), 2.62–2.70 (3H, m, 3-H*H* and 5-H_2), 3.42–3.52 (1H, m, 2-H), 4.54 (1H, t, *J* 6.2 Hz, 6-H), 7.28 (1H, dd, *J* 7.9, 4.8 Hz, 5''-H), 7.70 (1H, dt, *J* 7.9, 1.8 Hz, 4''-H), 8.54 (1H, dd, *J* 4.8, 1.8 Hz, 6''-H), 8.64 (1H, d, *J* 1.8 Hz, 2''-H); δ_C (101 MHz, $CDCl_3$) 21.0 (CH_3), 47.5 (CH_2), 48.1 (CH), 48.8 (CH_2), 53.6 (CH), 123.5 (CH), 134.4 (CH), 137.8 (C), 148.9 (CH), 149.1 (CH), 208.4 (C); *m/z* (ESI) 191.1178 (MH^+. $C_{11}H_{15}N_2O$ requires 191.1179).

(2*R*,6*S*)-2-Methyl-6-(2''-phenylethyl)piperidin-4-one (280) and (2*R*,6*R*)-2-methyl-6-(2''-phenylethyl)piperidin-4-one (281)



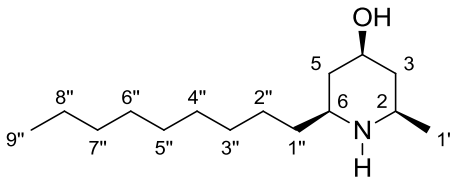
The reaction was carried out according to the above procedure for the synthesis of (2*R*,6*S*)-2-methyl-6-propylpiperidin-4-one (**260**) and (2*R*,6*R*)-2-methyl-6-propylpiperidin-4-one (**261**) using (2*R*,5*E*)-8-phenyl-2-(*tert*-butoxycarbonylamino)-4-oxooct-5-ene (**268**) (0.133 g, 0.420 mmol). The crude product was purified via flash column chromatography on silica gel (soaked with 1% triethylamine/petroleum ether), with a gradient elution from 60% ethyl acetate/1% triethylamine in petroleum ether (40–60) to 90% ethyl acetate/1% triethylamine in petroleum ether (40–60) to produce (2*R*,6*S*)-2-methyl-6-(2''-phenylethyl)piperidin-4-one (**280**) (0.0380 g, 42%) as a brown oil. Further elution yielded (2*R*,6*R*)-2-methyl-6-(2''-phenylethyl)piperidin-4-one (**281**) (0.0360 g, 40%) as a brown oil. Data for (2*R*,6*S*)-2-methyl-6-(2''-phenylethyl)piperidin-4-one (**280**): R_f 0.32 (60% ethyl acetate/1% triethylamine in petroleum ether); ν_{max}/cm^{-1} (neat) 3300 (NH), 2960 (CH), 1714 (C=O), 1454, 1365, 1217, 750; $[\alpha]_D^{26}$ +2.2 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.20 (3H, d, *J* 6.2 Hz, 1'-H_3), 1.67 (1H, br s, NH), 1.75–1.91 (2H, m, 1''-H_2), 2.04–2.14 (2H, m, 3-*H*H and 5-*H*H), 2.34 (1H, dt, *J* 15.0, 5.0 Hz, 3-H*H*), 2.41 (1H, dt, *J* 15.0, 5.0 Hz, 5-H*H*), 2.70 (2H, t, *J* 8.0 Hz, 2''-H_2), 2.84–2.98 (2H, m, 2-H and 6-H), 7.15–7.32 (5H, m, Ph); δ_C (126 MHz, $CDCl_3$) 22.6 (CH_3), 32.2 (CH_2), 38.6 (CH_2), 48.1 (CH_2), 50.2 (CH_2), 52.0 (CH), 56.1 (CH), 126.1 (CH), 128.3 (2 × CH), 128.5 (2 × CH), 141.4 (C), 209.2 (C); *m/z* (ESI) 218.1540 (MH^+. $C_{14}H_{20}NO$ requires 218.1539). Data for (2*R*,6*R*)-2-methyl-6-(2''-phenylethyl)piperidin-4-one (**281**): R_f 0.11 (60% ethyl acetate/1% triethylamine in petroleum ether); ν_{max}/cm^{-1} (neat) 3300 (NH), 2926 (CH), 1708 (C=O), 1454, 1379, 1232, 748; $[\alpha]_D^{26}$ +6.0 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.14 (3H, d, *J* 6.5 Hz, 1'-H_3), 1.68–1.86 (3H, m, 1''-H_2 and NH), 2.13 (1H, ddd, *J* 13.8, 7.3, 1.4 Hz, 3-*H*H), 2.21 (1H, ddd, *J* 13.8, 6.0, 1.4 Hz, 5-*H*H), 2.47 (1H, ddd, *J* 13.8, 4.6, 1.4 Hz, 3-H*H*), 2.52 (1H, ddd, *J* 13.8, 5.1, 1.4 Hz, 5-H*H*), 2.61–2.73 (2H, m, 2''-H_2), 3.31–3.38 (1H, m, 6-H), 3.40–3.48 (1H, m, 2-H), 7.15–7.31 (5H, m, Ph); δ_C (126 MHz, $CDCl_3$) 21.6 (CH_3), 32.5 (CH_2), 36.2 (CH_2), 47.8 (CH_2), 47.8 (CH), 49.7 (CH_2), 52.3 (CH), 126.0 (CH), 128.3 (2 × CH), 128.5 (2 × CH), 141.4 (C), 209.6 (C); *m/z* (ESI) 218.1537 (MH^+. $C_{14}H_{20}NO$ requires 218.1539).

(2*R*,4*S*,6*S*)-2-Methyl-6-propylpiperidin-4-ol (229)[132]



(2*R*,6*S*)-2-Methyl-6-propylpiperidin-4-one (**260**) (0.094 g, 0.061 mmol) was dissolved in anhydrous methanol (2 mL) and cooled to −15 °C. Sodium borohydride (0.0046 g, 0.12 mmol) was added and the solution was stirred rapidly for 0.25 h. Once the reaction was complete, brine (1 mL) was added to quench the reaction and the mixture was diluted with ethyl acetate (10 mL). The organic layer was washed with brine (5 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel, eluting with 30% methanol/1% triethylamine in ethyl acetate to produce (2*R*,4*S*,6*S*)-2-methyl-6-propylpiperidin-4-ol (**229**) (0.080 g, 87%) as an off-white solid. Spectroscopic data was coJnsistent with the literature.[132] Mp 74–76 °C; R_f 0.25 (30% methanol/1% triethylamine in ethyl acetate); $[\alpha]_D^{26}$ +9.0 (*c* 0.7, MeOH), lit.,[132] $[\alpha]_D^{20}$ +8.8 (*c* 0.4, MeOH); δ_H (400 MHz, $CDCl_3$) 0.91 (3H, t, *J* 7.1 Hz, 3''-H_3), 0.98 (1H, q, *J* 11.8 Hz, 5-*H*H), 1.03 (1H, q, *J* 11.8 Hz, 3-*H*H), 1.12 (3H, d, *J* 6.3 Hz, 1'-H_3), 1.29–1.46 (4H, m, 1''-H_2 and 2''-H_2), 1.86 (2H, br s, NH and OH), 1.91–2.01 (2H, m, 3-H*H* and 5-H*H*), 2.52–2.61 (1H, m, 6-H), 2.69 (1H, dqd, *J* 12.6, 6.3, 2.4 Hz, 2-H), 3.65 (1H, tt, *J* 11.0, 4.5 Hz, 4-H); δ_C (101 MHz, $CDCl_3$) 14.2 (CH_3), 19.2 (CH_2), 22.4 (CH_3), 39.0 (CH_2), 41.6 (CH_2), 43.9 (CH_2), 50.2 (CH), 54.6 (CH), 69.3 (CH); *m/z* (ESI) 158 (MH^+, 100%).

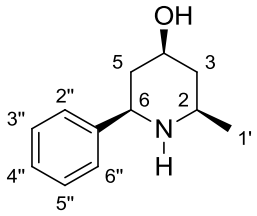
(2*R*,4*S*,6*S*)-2-Methyl-6-nonylpiperidin-4-ol (234)[142]



The reaction was carried out according to the above procedure for the synthesis of (2*R*,4*S*,6*S*)-2-methyl-6-propylpiperidin-4-ol (**229**) using (2*R*,6*S*)-2-methyl-6-nonylpiperidin-4-one (**239**) (0.028 g, 0.12 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 10% methanol/1% triethylamine in ethyl acetate to produce (2*R*,4*S*,6*S*)-2-methyl-6-nonylpiperidin-4-ol (**234**) (0.023 g, 84%) as an off-white solid. Spectroscopic data was consistent with the literature.[142] Mp 86–88 °C; R_f 0.16 (10% methanol/1% triethylamine in ethyl acetate); $[\alpha]_D^{26}$ +7.9 (*c* 1.0, MeOH), lit.,[142] $[\alpha]_D^{25}$ +7.0 (*c* 1.0, MeOH); δ_H (500 MHz, $CDCl_3$) 0.88 (3H, t, *J* 7.0 Hz, 9''-

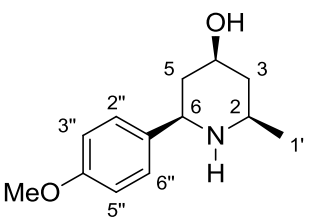
H_3), 0.93–1.06 (2H, m, 3-*H*H and 5-*H*H), 1.12 (3H, d, *J* 6.3 Hz, 1'-H_3), 1.20–1.48 (16H, m, 1''-H_2, 2''-H_2, 3''-H_2, 4''-H_2, 5''-H_2, 6''-H_2, 7''-H_2 and 8''-H_2), 1.63 (2H, br s, NH and OH), 1.97 (1H, q, *J* 11.8 Hz, 3-H*H*), 2.02 (1H, q, *J* 11.8 Hz, 5-H*H*), 2.50–2.58 (1H, m, 6-H), 2.68 (1H, dqd, *J* 11.8, 6.3, 2.4 Hz, 2-H), 3.65 (1H, tt, *J* 11.0, 4.6 Hz, 4-H); δ_C (126 MHz, $CDCl_3$) 14.1 (CH_3), 22.5 (CH_3), 22.7 (CH_2), 26.1 (CH_2), 29.3 (CH_2), 29.6 (CH_2), 29.6 (CH_2), 29.8 (CH_2), 31.9 (CH_2), 36.8 (CH_2), 41.8 (CH_2), 43.9 (CH_2), 50.2 (CH), 54.9 (CH), 69.4 (CH); *m/z* (ESI) 242 (MH^+, 100%).

(2*R*,4*S*,6*R*)-2-Methyl-6-phenylpiperidin-4-ol (282)[158]



The reaction was carried out according to the above procedure for the synthesis of (2*R*,4*S*,6*S*)-2-methyl-6-propylpiperidin-4-ol (**229**) using (2*R*,6*R*)-2-methyl-6-phenylpiperidin-4-one (**270**) (0.0146 g, 0.0770 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 1% methanol/1% triethylamine in dichloromethane to produce (2*R*,4*S*,6*R*)-2-methyl-6-phenylpiperidin-4-ol (**282**) (0.013 g, 91%) as an off white solid. Spectroscopic data was consistent with the literature.[158] Mp 92–94 °C; R_f 0.19 (1% methanol/1% triethylamine in dichloromethane); $[\alpha]_D^{26}$ +27.6 (*c* 1.0, $CHCl_3$), lit.,[158] $[\alpha]_D^{20}$ +29.0 (*c* 0.5, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.10–1.21 (4H, m, 1'-H_3 and 3-*H*H), 1.45 (1H, q, *J* 11.8 Hz, 5-*H*H), 1.58 (2H, br s, NH and OH), 2.00 (1H, ddt, *J* 11.8, 4.6, 2.4 Hz, 3-H*H*), 2.10 (1H, ddt, *J* 11.8, 4.6, 2.4 Hz, 5-H*H*), 2.86 (1H, dqd, *J* 11.8, 6.3, 2.4 Hz, 2-H), 3.69 (1H, dd, *J* 11.8, 2.4 Hz, 6-H), 3.81 (1H, tt, *J* 11.8, 4.6 Hz, 4-H), 7.23–7.41 (5H, m, Ph); δ_C (101 MHz, $CDCl_3$) 22.5 (CH_3), 43.4 (CH_2), 43.4 (CH_2), 50.7 (CH), 59.8 (CH), 69.8 (CH), 126.8 (2 × CH), 127.3 (CH), 128.5 (2 × CH), 144.0 (C); *m/z* (ESI) 192.1385 (MH^+. $C_{12}H_{18}NO$ requires 192.1383).

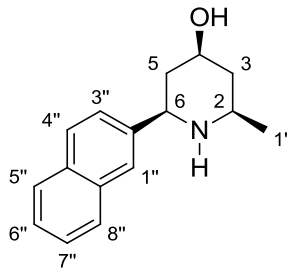
(2*R*,4*S*,6*R*)-2-Methyl-6-(4''-methoxyphenyl)piperidin-4-ol (283)



The reaction was carried out according to the above procedure for the synthesis of (2*R*,4*S*,6*S*)-2-methyl-6-propylpiperidin-4-ol (**229**) using (2*R*,6*R*)-2-methyl-6-(4''-methoxyphenyl)piperidin-4-one

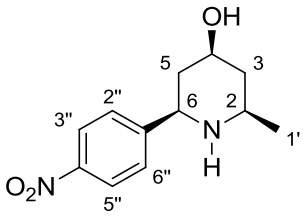
(**272**) (0.0170 g, 0.0780 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 70% ethyl acetate/1% triethylamine in dichloromethane to produce (2*R*,4*S*,6*R*)-2-methyl-6-(4''-methoxyphenyl)piperidin-4-ol (**283**) (0.015 g, 85%) as a white solid. Mp 85–90 °C; R_f 0.11 (70% ethyl acetate/1% triethylamine in dichloromethane); ν_{max}/cm^{-1} (neat) 3340 (NH and OH), 2933 (CH), 1514 (C=C), 1303, 1244, 1178, 1033, 827, 734; $[\alpha]_D^{26}$ +21.3 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.09–1.19 (4H, m, 1'-H_3 and 3-*H*H), 1.43 (1H, q, *J* 12.0 Hz, 5-*H*H), 1.61 (2H, br s, NH and OH), 1.99 (1H, ddt, *J* 12.0, 4.4, 2.3 Hz, 3-H*H*), 2.07 (1H, ddt, *J* 12.0, 4.4, 2.3 Hz, 5-H*H*), 2.84 (1H, dqd, *J* 12.0, 6.2, 2.3 Hz, 2-H), 3.64 (1H, dd, *J* 12.0, 2.3 Hz, 6-H), 3.75–3.83 (4H, m, OCH_3 and 4-H), 6.83–6.89 (2H, m, 3''-H and 5''-H), 7.27–7.32 (2H, m, 2''-H and 6''-H); δ_C (126 MHz, $CDCl_3$) 22.5 (CH_3), 43.4 (2 × CH_2), 50.7 (CH), 55.3 (CH_3), 59.1 (CH), 69.8 (CH), 113.8 (2 × CH), 127.8 (2 × CH), 136.3 (C), 158.8 (C); *m/z* (ESI) 222.1487 (MH^+. $C_{13}H_{20}NO_2$ requires 222.1489).

(2*R*,4*S*,6*R*)-2-Methyl-6-(naphthalen-2''-yl)piperidin-4-ol (284)



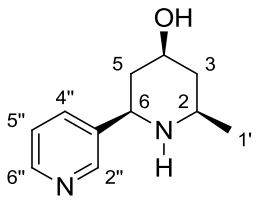
The reaction was carried out according to the above procedure for the synthesis of (2*R*,4*S*,6*S*)-2-methyl-6-propylpiperidin-4-ol (**229**) using (2*R*,6*R*)-2-methyl-6-(naphthalen-2''-yl)piperidin-4-one (**274**) (0.0363 g, 0.152 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 80% ethyl acetate/1% triethylamine in dichloromethane to produce (2*R*,4*S*,6*R*)-2-methyl-6-(naphthalen-2''-yl)piperidin-4-ol (**284**) (0.032 g, 87%) as a white solid. Mp 157–160 °C; R_f 0.21 (80% ethyl acetate/1% triethylamine in dichloromethane); ν_{max}/cm^{-1} (neat) 3284 (NH and OH), 2926 (CH), 1599 (C=C), 1371, 1303, 1087, 1035, 817, 746; $[\alpha]_D^{26}$ +6.9 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.12–1.26 (4H, m, 1'-H_3 and 3-*H*H), 1.53 (1H, q, *J* 12.2 Hz, 5-*H*H), 1.68 (2H, br s, NH and OH), 2.03 (1H, ddt, *J* 12.2, 4.5, 2.3 Hz, 3-H*H*), 2.18 (1H, ddt, *J* 12.2, 4.5, 2.3 Hz, 5-H*H*), 2.90 (1H, dqd, *J* 12.2, 6.3, 2.3 Hz, 2-H), 3.80–3.90 (2H, m, 4-H and 6-H), 7.41–7.53 (3H, m, 3''-H, 6''-H and 7''-H), 7.77–7.86 (4H, m, 1''-H, 4''-H, 5''-H and 8''-H); δ_C (101 MHz, $CDCl_3$) 22.5 (CH_3), 43.5 (CH_2), 43.5 (CH_2), 50.8 (CH), 59.8 (CH), 69.8 (CH), 125.0 (CH), 125.3 (CH), 125.6 (CH), 126.0 (CH), 127.6 (CH), 127.9 (CH), 128.1 (CH), 132.9 (C), 133.5 (C), 141.5 (C); *m/z* (ESI) 242.1538 (MH^+. $C_{16}H_{20}NO$ requires 242.1539).

(2*R*,4*S*,6*R*)-2-Methyl-6-(4''-nitrophenyl)piperidin-4-ol (285)



The reaction was carried out according to the above procedure for the synthesis of (2*R*,4*S*,6*S*)-2-methyl-6-propylpiperidin-4-ol (**229**) using (2*R*,6*R*)-2-methyl-6-(4''-nitrophenyl)piperidin-4-one (**276**) (0.0192 g, 0.0820 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 70% ethyl acetate/1% triethylamine in petroleum ether (40–60) to produce (2*R*,4*S*,6*R*)-2-methyl-6-(4''-nitrophenyl)piperidin-4-ol (**285**) (0.015 g, 75%) as a yellow oil. R_f 0.12 (70% ethyl acetate/1% triethylamine in petroleum ether); ν_{max}/cm^{-1} (neat) 3298 (NH and OH), 2935 (CH), 1516, 1344, 1087, 1033, 854, 698; $[\alpha]_D^{26}$ +20.0 (*c* 0.9, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.12–1.22 (4H, m, 1'-H_3 and 3-*H*H), 1.37 (1H, q, *J* 12.3 Hz, 5-*H*H), 1.58 (2H, br s, NH and OH), 2.03 (1H, ddt, *J* 12.3, 4.5, 2.4 Hz, 3-H*H*), 2.10 (1H, ddt, *J* 12.3, 4.5, 2.4 Hz, 5-H*H*), 2.89 (1H, dqd, *J* 12.3, 6.2, 2.4 Hz, 2-H), 3.79–3.88 (2H, m, 4-H and 6-H), 7.54–7.58 (2H, m, 2''-H and 6''-H), 8.16–8.21 (2H, m, 3''-H and 5''-H); δ_C (126 MHz, $CDCl_3$) 22.5 (CH_3), 43.1 (CH_2), 43.5 (CH_2), 50.5 (CH), 59.2 (CH), 69.4 (CH), 123.8 (2 × CH), 127.6 (2 × CH), 147.2 (C), 151.6 (C); *m/z* (ESI) 237.1239 (MH^+. $C_{12}H_{17}N_2O_3$ requires 237.1234).

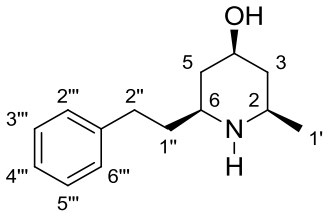
(2*R*,4*S*,6*R*)-2-Methyl-6-(pyridin-3''-yl)piperidin-4-ol (286)



The reaction was carried out according to the above procedure for the synthesis of (2*R*,4*S*,6*S*)-2-methyl-6-propylpiperidin-4-ol (**229**) using (2*R*,6*R*)-2-methyl-6-(pyridin-3''-yl)piperidin-4-one (**278**) (0.0176 g, 0.0920 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 5% methanol/1% triethylamine in dichloromethane to produce (2*R*,4*S*,6*R*)-2-methyl-6-(pyridin-3''-yl)piperidin-4-ol (**286**) (0.012 g, 68%) as a pale yellow oil. R_f 0.23 (5% methanol/1% triethylamine in dichloromethane); ν_{max}/cm^{-1} (neat) 3254 (NH and OH), 2933 (CH), 1425, 1305, 1089, 1028, 804, 711; $[\alpha]_D^{26}$ +17.7 (*c* 0.8, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.10–1.23 (4H, m, 1'-H_3 and 3-*H*H), 1.44 (1H, q, *J* 12.2 Hz, 5-*H*H), 1.87 (2H, br s, NH and OH), 2.02 (1H, ddt, *J* 12.2, 4.5,

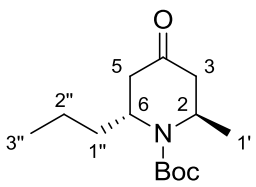
2.3 Hz, 5-H*H*), 2.09 (1H, ddt, *J* 12.2, 4.5, 2.3 Hz, 3-H*H*), 2.88 (1H, dqd, *J* 12.2, 6.2, 2.3 Hz, 2-H), 3.74 (1H, dd, *J* 12.2, 2.3 Hz, 6-H), 3.82 (1H, tt, *J* 12.2, 4.5 Hz, 4-H), 7.26 (1H, dd, *J* 7.9, 4.8 Hz, 5''-H), 7.74 (1H, dt, *J* 7.9, 1.8 Hz, 4''-H), 8.50 (1H, dd, *J* 4.8, 1.8 Hz, 6''-H), 8.58 (1H, d, *J* 1.8 Hz, 2''-H); δ_C (101 MHz, $CDCl_3$) 22.5 (CH_3), 43.2 (2 × CH_2), 50.7 (CH), 57.3 (CH), 69.4 (CH), 123.6 (CH), 134.4 (CH), 139.4 (C), 148.7 (CH), 148.8 (CH); *m/z* (ESI) 193.1339 (MH^+. $C_{11}H_{17}N_2O$ requires 193.1335).

(2*R*,4*S*,6*S*)-2-Methyl-6-(2''-phenylethyl)piperidin-4-ol (287)[146]



The reaction was carried out according to the above procedure for the synthesis of (2*R*,4*S*,6*S*)-2-methyl-6-propylpiperidin-4-ol (**229**) using (2*R*,6*S*)-2-methyl-6-(2''-phenylethyl)piperidin-4-one (**280**) (0.0136 g, 0.0630 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 1% methanol/1% triethylamine in dichloromethane to produce (2*R*,4*S*,6*S*)-2-methyl-6-(2''-phenylethyl)piperidin-4-ol (**287**) (0.011 g, 80%) as an off-white solid. Spectroscopic data was consistent with the literature.[146] Mp 98–102 °C; R_f 0.19 (1% methanol/1% triethylamine in dichloromethane); $[\alpha]_D^{26}$ +7.0 (*c* 0.6, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.02 (1H, q, *J* 11.7 Hz, 5-*H*H), 1.04 (1H, q, *J* 11.7 Hz, 3-*H*H), 1.12 (3H, d, *J* 6.3 Hz, 1'-H_3), 1.47 (2H, br s, NH and OH), 1.67–1.83 (2H, m, 1''-H_2), 1.95 (1H, ddt, *J* 11.7, 4.5, 2.2 Hz, 5-H*H*), 2.03 (1H, ddt, *J* 11.7, 4.5, 2.2 Hz, 3-H*H*), 2.56–2.72 (4H, m, 2-H, 6-H and 2''-H_2), 3.66 (1H, tt, *J* 11.7, 4.5 Hz, 4-H), 7.16–7.21 (3H, m, 3 × ArH), 7.25–7.31 (2H, m, 2 × ArH); δ_C (101 MHz, $CDCl_3$) 22.6 (CH_3), 32.6 (CH_2), 38.6 (CH_2), 41.8 (CH_2), 44.1 (CH_2), 50.3 (CH), 54.5 (CH), 69.5 (CH), 126.0 (CH), 128.5 (2 × CH), 128.6 (2 × CH), 142.1 (C); *m/z* (ESI) 220 (MH^+, 100%).

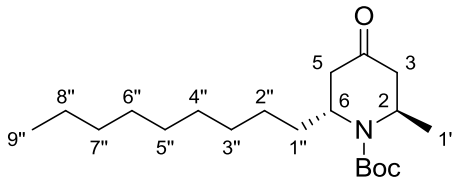
***tert*-Butyl (2*R*,6*R*)-2-methyl-4-oxo-6-propylpiperidine-1-carboxylate (288)**



(2*R*,5*E*)-2-(*tert*-Butoxycarbonylamino)-4-oxonona-5-ene (**258**) (0.101 g, 0.396 mmol) was dissolved in methanol (9.9 mL, 0.04 M). Addition of 2 M hydrochloric acid in diethyl ether (0.198 mL, 0.396

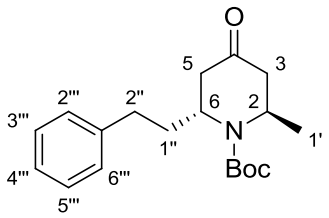
mmol) followed and the solution was allowed to stir at room temperature for 1 h. Upon completion, the mixture was concentrated *in vacuo*. The crude product was purified via flash column chromatography on silica gel, eluting with 30% diethyl ether in petroleum ether (40–60) to produce *tert*-butyl (2*R*,6*R*)-2-methyl-4-oxo-6-propylpiperidine-1-carboxylate (**288**) (0.0404 g, 40%) as a colourless oil. R_f 0.21 (30% diethyl ether in petroleum ether); ν_{max}/cm^{-1} (neat) 2962 (CH), 1658 (C=O), 1527, 1458, 1257; $[\alpha]_D^{24}$ +30.1 (*c* 0.9, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.91 (3H, t, *J* 7.2 Hz, 3''-H_3), 1.23–1.39 (6H, m, 1'-H_3, 1''-*H*H and 2''-H_2), 1.50 (9H, s, 3 × CH_3), 1.66–1.79 (1H, m, 1''-H*H*), 2.36 (1H, dd, *J* 17.7, 2.0 Hz, 3-*H*H), 2.53 (1H, dd, *J* 17.9, 2.0 Hz, 5-*H*H), 2.72 (1H, ddd, *J* 17.9, 6.3, 1.4 Hz, 5-H*H*), 2.82 (1H, dd, *J* 17.7, 6.3 Hz, 3-H*H*), 4.11–4.23 (1H, m, 6-H), 4.30–4.41 (1H, m, 2-H); δ_C (101 MHz, $CDCl_3$) 13.8 (CH_3), 19.9 (CH_2), 22.7 (CH_3), 28.5 (3 × CH_3), 39.3 (CH_2), 41.3 (CH_2), 44.6 (CH_2), 46.5 (CH), 50.9 (CH), 79.9 (C), 154.6 (C), 208.1 (C); *m/z* (ESI) 278.1730 (MNa^+. $C_{14}H_{25}NNaO_3$ requires 278.1727).

tert-Butyl (2*R*,6*R*)-2-methyl-4-oxo-6-nonylpiperidine-1-carboxylate (290)



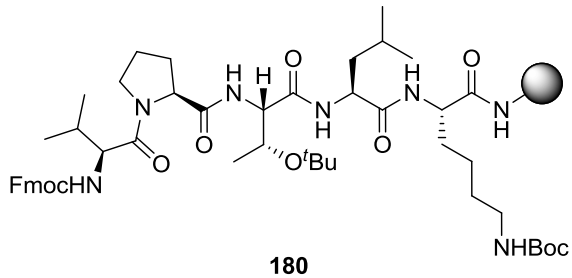
The reaction was carried out according to the above procedure for the synthesis of *tert*-butyl (2*R*,6*R*)-2-methyl-4-oxo-6-propylpiperidine-1-carboxylate (**288**) using (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxopentadec-5-ene (**259**) (0.0504 g, 0.148 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 20% diethyl ether in petroleum ether (40–60) to produce *tert*-butyl (2*R*,6*R*)-2-methyl-4-oxo-6-nonylpiperidine-1-carboxylate (**290**) (0.0269 g, 53%) as a colourless oil. R_f 0.14 (20% diethyl ether in petroleum ether); ν_{max}/cm^{-1} (neat) 2958 (CH), 1710 (C=O), 1458, 1377, 1327; $[\alpha]_D^{24}$ +19.6 (*c* 1.5, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.88 (3H, t, *J* 6.8 Hz, 9''-H_3), 1.17–1.36 (18H, m, 1'-H_3, 1''-*H*H, 2''-H_2, 3''-H_2, 4''-H_2, 5''-H_2, 6''-H_2, 7''-H_2 and 8''-H_2), 1.50 (9H, s, 3 × CH_3), 1.69–1.81 (1H, m, 1''-H*H*), 2.36 (1H, dd, *J* 17.7, 1.8 Hz, 3-*H*H), 2.53 (1H, dd, *J* 18.0, 1.8 Hz, 5-*H*H), 2.72 (1H, dd, *J* 18.0, 5.5 Hz, 5-H*H*), 2.81 (1H, dd, *J* 17.7, 6.4 Hz, 3-H*H*), 4.09–4.20 (1H, m, 6-H), 4.30–4.42 (1H, m, 2-H); δ_C (101 MHz, $CDCl_3$) 14.1 (CH_3), 22.7 (CH_2), 22.7 (CH_3), 26.8 (CH_2), 28.5 (3 × CH_3), 29.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 31.9 (CH_2), 37.2 (CH_2), 41.3 (CH_2), 44.6 (CH_2), 46.5 (CH), 51.2 (CH), 79.9 (C), 154.6 (C), 208.1 (C); *m/z* (ESI) 362.2662 (MNa^+. $C_{20}H_{37}NNaO_3$ requires 362.2666).

tert-Butyl (2_R_,6_R_)-2-methyl-4-oxo-6-(2''-phenylethyl)piperidine-1-carboxylate (291)



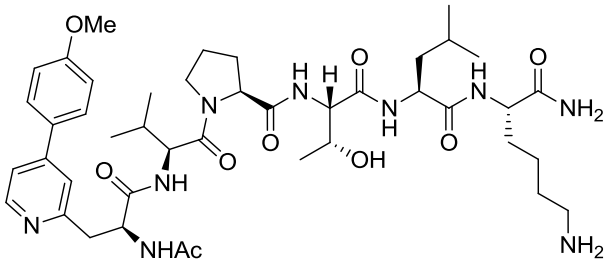
The reaction was carried out according to the above procedure for the synthesis of *tert*-butyl (2*R*,6*R*)-2-methyl-4-oxo-6-propylpiperidine-1-carboxylate (**288**) using (2*R*,5*E*)-8-phenyl-2-(*tert*-butoxycarbonylamino)-4-oxooct-5-ene (**268**) (0.0354 g, 0.111 mmol). The crude product was purified via flash column chromatography on silica gel, eluting with 20% ethyl acetate in petroleum ether (40–60) to produce *tert*-butyl (2*R*,6*R*)-2-methyl-4-oxo-6-(2''-phenylethyl)piperidine-1-carboxylate (**291**) (0.0156 g, 44%) as a white solid. Mp 88–90 °C; R_f 0.15 (20% ethyl acetate in petroleum ether); ν_{max}/cm^{-1} (neat) 2958 (CH), 1710 (C=O), 1458, 1377, 1327; $[\alpha]_D^{24}$ +19.6 (*c* 1.5, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.27 (3H, d, *J* 6.7 Hz, 1'-H_3), 1.49 (9H, s, 3 × CH_3), 1.64 (1H, dtd, *J* 13.4, 9.9, 6.1 Hz, 1''-*H*H), 2.04–2.16 (1H, m, 1''-H*H*), 2.38 (1H, dd, *J* 17.8, 2.0 Hz, 3-*H*H), 2.54–2.69 (3H, m, 5-*H*H), 2.72–2.86 (2H, m, 3-H*H* and 5-H*H*), 4.20–4.30 (1H, m, 6-H), 4.31–4.42 (1H, m, 2-H), 7.12–7.32 (5H, m, Ph); δ_C (101 MHz, $CDCl_3$) 22.7 (CH_3), 28.5 (3 × CH_3), 33.2 (CH_2), 39.0 (CH_2), 41.4 (CH_2), 44.6 (CH_2), 46.6 (CH), 50.9 (CH), 80.1 (C), 126.1 (CH), 128.3 (2 × CH), 128.5 (2 × CH), 141.0 (C), 154.5 (C), 207.7 (C); *m/z* (ESI) 340.1876 (MNa^+. $C_{19}H_{27}NNaO_3$ requires 340.1883).

4.3 Experimental for the Synthesis of Hexapeptide 181



180

Pentapeptide **180** was synthesised on a Biotage Initiator+ Alstra peptide synthesiser using an Fmoc/^{t}Bu protecting group strategy on a 0.1 mmol synthetic scale using Rink Amide ChemMatrix® resin. Resin bound peptide **180** was synthesised by first loading Fmoc-Lys(Boc)-OH to the resin and by introducing the amino acids (4 equivalents) successively with a combination of 0.5 M HCTU in DMF (4 equivalents) and 2 M DIPEA in NMP (8 equivalents). Fmoc groups were removed using 20% piperidine in DMF.



181

Resin bound pentapeptide **180** (0.0889 g, 0.0300 mmol) was treated with 20% piperidine in DMF (2 mL) and shaken for 0.25 h. The solution was decanted and the resin bound peptide was washed with dichloromethane (3 × 2 mL), isopropanol (3 × 2 mL) and dimethylformamide (3 × 2 mL). The resin bound peptide was suspended in dimethylformamide (2 mL) followed by addition of (2*S*)-2-[(9*H*-fluoren-9-ylmethoxycarbonyl)amino]-3-[4'-(4''-methoxyphenyl)pyridin-2'-yl]propanoic acid (**177**) (0.0297 g, 0.0600 mmol), diisoproptlethylamine (0.0209 mL, 0.120 mmol), 0.5 M 2-(6-chloro-1-*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate in dimethylformamide (0.120 mL, 0.0600 mmol). The mixture was shaken for 4 h. The solution was decanted and the resin bound peptide was washed with dichloromethane (3 × 2 mL), isopropanol (3 × 2 mL) and dimethylformamide (3 × 2 mL). The resin bound peptide was treated with 20% piperidine in DMF (2 mL) and shaken for 0.25 h. The solution was decanted and the resin bound peptide was washed with dichloromethane (3 × 2 mL), isopropanol (3 × 2 mL) and dimethylformamide (3 × 2 mL). The resin bound peptide was suspended in dimethylformamide (1.50 mL) followed by sequential addition of acetic anhydride (0.190 mL) and diisopropylethylamine (0.190 mL). The mixture was

shaken for 1 h. The solution was decanted and the resin bound peptide was washed with dichloromethane (3 × 2 mL), isopropanol (3 × 2 mL) and dimethylformamide (3 × 2 mL). The resin bound peptide was treated with trifluoroacetic acid/water/triisopropylsilane (2 mL, 95:2.5:2.5) and shaken for 2 h. The cleavage cocktail was evaporated using a stream of nitrogen and peptide **181** was precipitated from a solution of ice cold diethyl ether (2 mL), centrifuged at 3700 rpm for 5 minutes and the precipitate was washed with ice cold diethyl ether (3 × 2 mL). Peptide **181** was purified on a reverse-phase Dionex HPLC system equipped with Dionex P680 pumps and a Dionex UVD170U UV-Vis detector (monitoring at 214 nm and 280 nm), using a Phenomenex, Gemini, C18, 5 um, 250 × 21.2 mm column. Gradients were run using a solvent system consisting of A (H_2O + 0.1% TFA) and B (MeCN + 0.1% TFA), and collected fractions were lyophilised on a Christ Alpha 2-4 LO plus freeze dryer. Pure peptide **181** was analysed on a Shimadzu reverse-phase HPLC (RP-HPLC) system equipped with Shimadzu LC-20AT pumps, a Shimadzu SIL-20A autosampler and a Shimadzu SPD-20A UV-Vis detector (monitoring at 214 nm and 280 nm) using a Phenomenex, Aeris, 5 µm, peptide XB-C18, 150 × 4.6 mm column at a flow rate of 1 mL/minute. RP-HPLC gradients were run using a solvent system consisting of solution A (5% MeCN in H_2O + 0.1% TFA) and B (5% H_2O in MeCN + 0.1% TFA). Two gradients were used to characterise peptide **181**; a gradient from 0–100% solution B over 20 minutes (Figure 53) and a gradient from 0–100% solution B over 50 minutes (Figure 54). Analytical RP-HPLC data is reported as column retention time (t_R) in minutes (Table 4). High resolution mass spectrometry (HRMS) was performed on a Bruker microTOF-Q II (ESI^+). HRMS data are reported as mass to charge ratio (*m/z*) = observed / MW.

20 Minute Gradient		**50 Minute Gradient**		**Calculated MW**	**Observed MW**
T_R (min)	Purity (%)	T_R (min)	Purity (%)	852.4969 $[M+H]^+$	852.4978 $[M+H]^+$
11.86	98	20.95	98		

Table 4: RP-HPLC gradients and HRMS data.

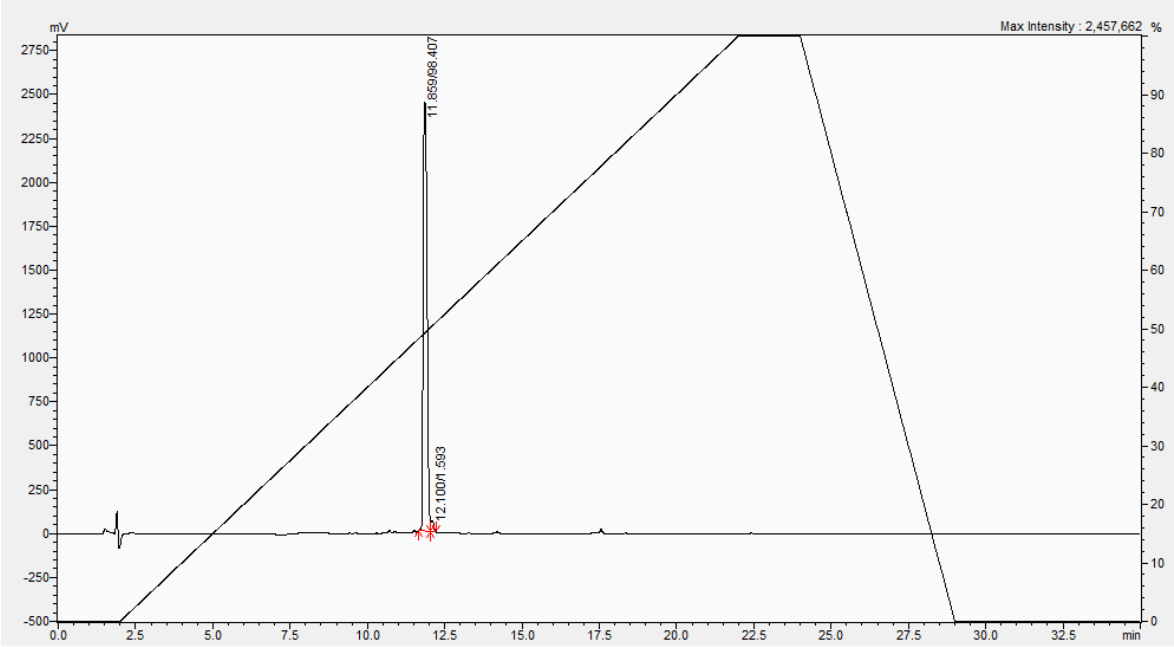


Figure 53: Analytical HPLC 20 minute gradient.

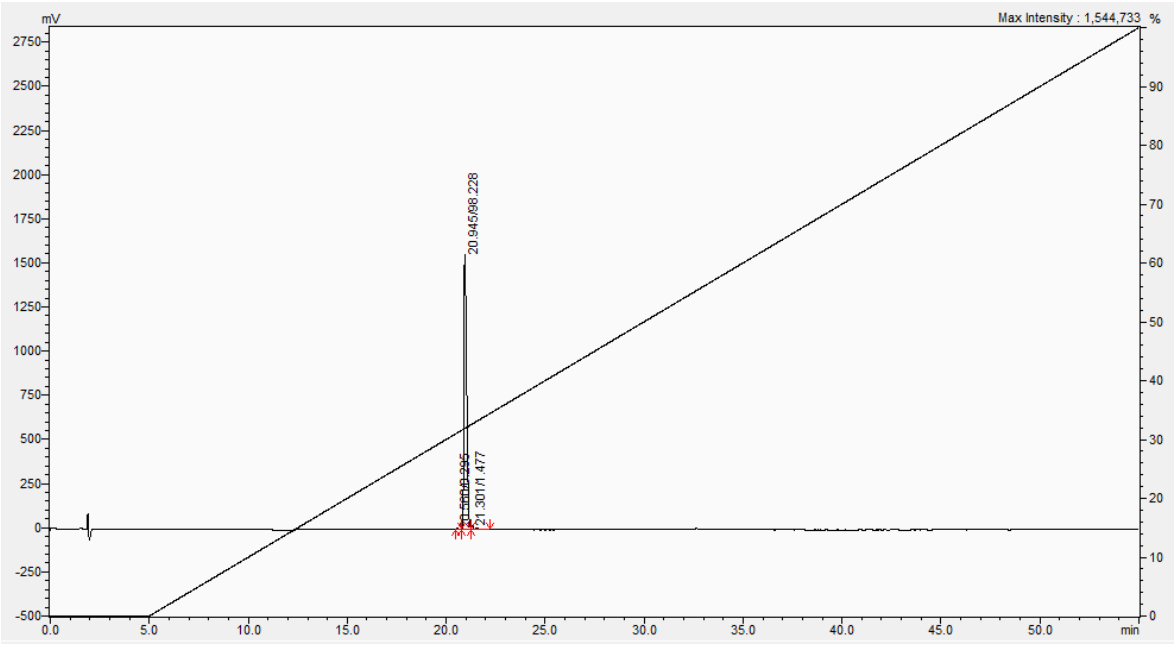


Figure 54: Analytical HPLC 50 minute gradient.

4.4 Cell-Hexapeptide 181 Study

15 × 10^4 human foreskin fibroblast cells immortalised with hTERT (human telomerase reverse transcriptase) were plated onto ibidi® µ-Slide VI 0.4 six-chamber plates and grown for 72 h in Dulbecco's Modified Eagle's Medium (DMEM) complemented with medium 199 (18%), fetal bovine serum (FBS) (9%), penicillin/streptomycin (2%) and sodium pyruvate (1%) in a 5% CO2 atmosphere at 37 °C. Fresh growth media was applied every 24 h. Cells were treated with 120 µL of 100 µM hexapeptide **181** and were visualised immediately using a fluorescence microsope.

5.0 References

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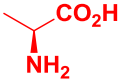
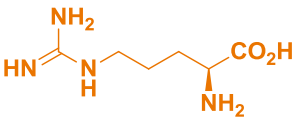
6.0 Appendices

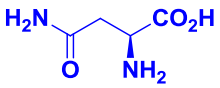
Appendix 1 - Genetically Encoded Proteinogenic α-Amino Acids



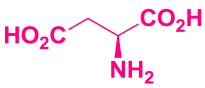
Alanine/Ala/A



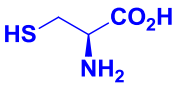
Arginine/Arg/R



Asparagine/Asn/N

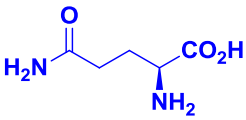


Aspartic Acid/Asp/D

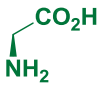


Cysteine/Cys/C

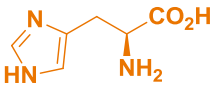
Glutamic Acid/Glu/E



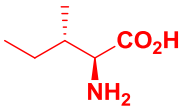
Glutamine/Gln/Q



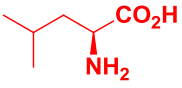
Glycine/Gly/G



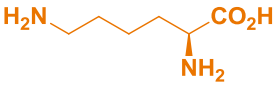
Histidine/His/H



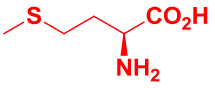
Isoleucine/Ile/I



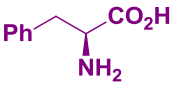
Leucine/Leu/L



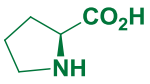
Lysine/Lys/K



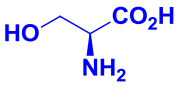
Methionine/Met/M



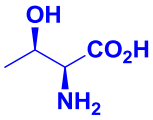
Phenylalanine/Phe/F



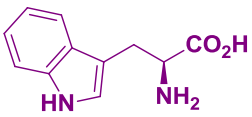
Proline/Pro/P



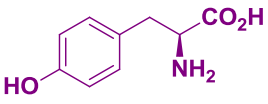
Serine/Ser/S



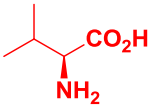
Threonine/Thr/T



Tryptophan/Trp/W



Tyrosine/Tyr/Y

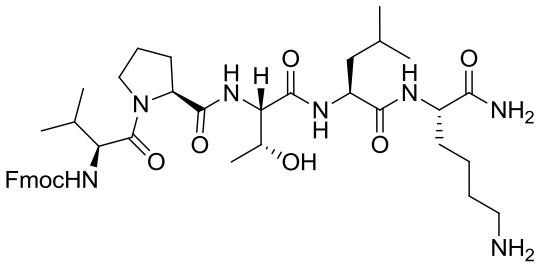


Valine/Val/V

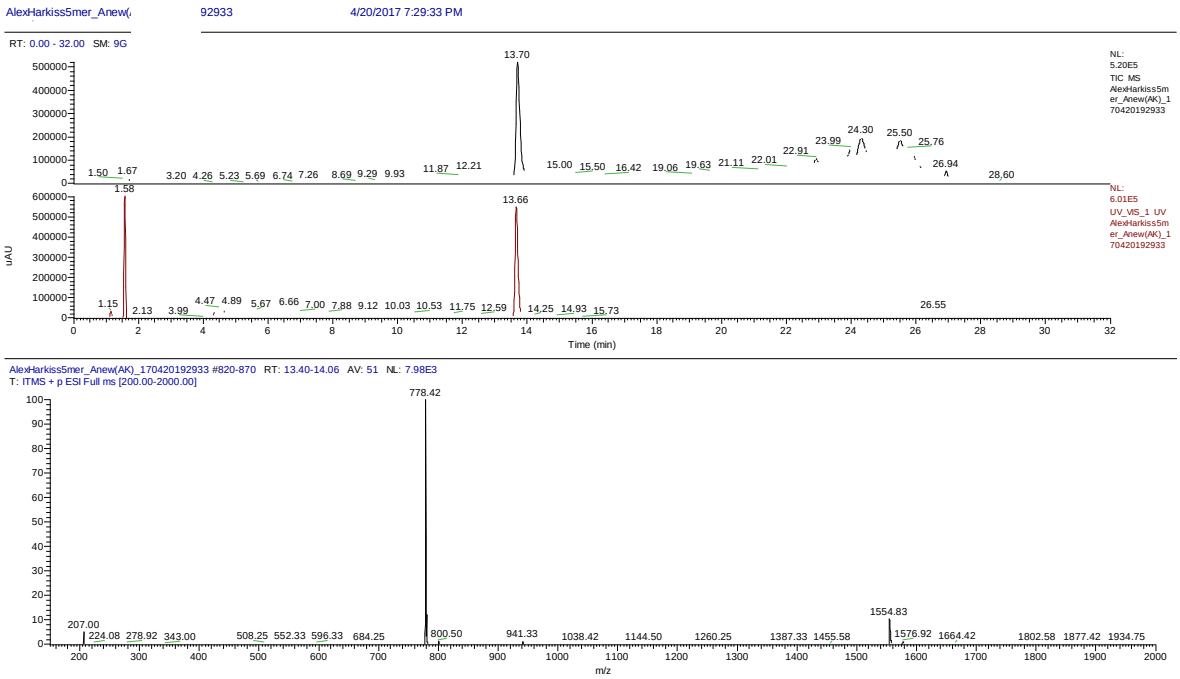
Hydrophobic - Aliphatic
Hydrophobic - Aromatic
Polar Neutral
Electrically charged - Acidic
Electrically charged - Basic
Unique

Appendix 1

Appendix 2 - Pentapeptide Test Cleavage Data

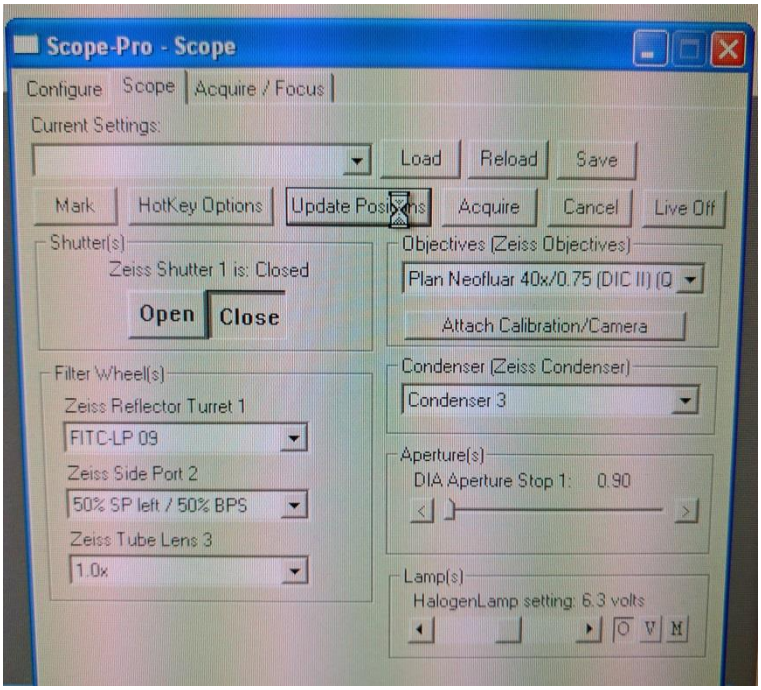


Exact Mass: 777.4425



Appendix 2

Appendix 3 - Fluorescence Lamp Settings



Appendix 3