New methods for the stereoselective synthesis of fluorescent amino acids, natural products and biomolecules

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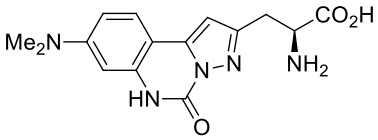
Abstract

The stereoselective synthesis of fluorescent amino acids, biomolecules and natural products was achieved using a chiral pool strategy with α-amino acids. The use of L-aspartic acid gave access to a small library of enone derived amino acids via a Horner-Wadsworth-Emmons (HWE) reaction. Transformation of the enones via a number of steps gave a series of pyrazoloquinazoline derived amino acids. Due to the rigid structure of these amino acids, high quantum yields were observed. The lead compound from this series, a dimethylamino substituted pyrazoloquinazoline also displayed high excitation and emission wavelengths. It was found that this compound could be excited either by a one or a two-photon process. This amino acid was also incorporated into a short cell-penetrating pentapeptide in high yield, with no loss of fluorescence.

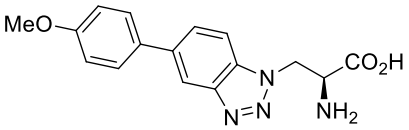
A second series of fluorescent amino acids bearing benzotriazole side-chains was synthesised from L-asparagine in five steps. The conjugation of these was extended using a Suzuki-Miyaura reaction that led to a highly fluorescent 4-methoxyphenyl substituted analogue. This new chromophore was also rigidified using a copper-catalysed C-H insertion reaction, which yielded a new carbazole derived amino acid.

The HWE reaction used in the first project was investigated as a new approach for bioconjugation. The aspartic acid derived phosphonate ester was coupled with alanine, and this model dipeptide was subjected to successful HWE reactions with various aldehydes, showing this as a possible new method for incorporating chromophores within peptides and proteins.

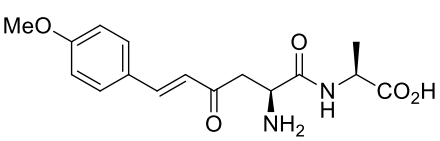
Finally, L-aspartic acid was also used as a starting material to synthesis a small library of enones, using a HWE reaction. These were found to be excellent substrates for an acid-mediated 6-*endo*-*trig* cyclisation for the stereoselective synthesis of a series of 2,6-dialkyl-4-oxopiperidines. This transformation was then used as the key step for the synthesis of the alkaloids, (+)-myrtine and (−)-solenopsin A.



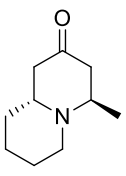
Pyrazoloquinazoline derived amino acid



Benzotriazole derived amino acid



Horner-Wadsworth-Emmons bioconjugation adduct



(+)-Myrtine

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Thanks to E-Number 512 for helping me complete this PhD and BBC radio 4 for teaching me the importance of hesitation, repetition and deviation.

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Author's Declaration

I declare that, except where explicit reference is made to the contribution of others, this thesis represents the original work of Jonathan D. Bell and has not been submitted for any other degree at the University of Glasgow or any other institution. The research was carried out at the University of Glasgow in the Loudon Laboratory under the supervision of Dr Andrew Sutherland between October 2015 to September 2019. Aspects of the work described herein have been published elsewhere as listed below.

J. D. Bell, T. E. F. Morgan, N. Buijs, A. H. Harkiss, C. R. Wellaway and A. Sutherland, Synthesis and Photophysical properties of Benzotriazole-Derived Unnatural α-Amino Acids, *J. Org. Chem.*, 2019, **84**, 10436–10448.

A. H. Harkiss, J. D. Bell, A. Knuhtsen, A. G. Jamieson and A. Sutherland, Synthesis and Fluorescent Properties of β-Pyridyl α-Amino Acids, *J. Org. Chem.*, 2019, **84**, 2879–2890.

J. D. Bell, A. H. Harkiss, C. R. Wellaway and A. Sutherland, Stereoselective Synthesis of 2,6-*trans*-4-Oxopiperidines using an Acid-Mediated 6-*endo*-*trig* Cyclisation, *Org. Biomol. Chem.*, 2018, **16**, 6410–6422.

Abbreviations

°C	Degrees centigrade
Ac	Acetyl
Ar	Aromatic
Boc	*tert*-Butyloxycarbonyl
BINAP	2,2'-Bis(diphenylphosphino)-1,1'-binaphthyl
Br	Broad
C	Concentration
Cbz	Carboxybenzyl
CI	Chemical ionisation
COSY	Correlated spectroscopy
D	Doublet
DCC	*N*,*N*'-Dicyclohexylcarbodiimide
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DEPT	Distortionless enhancement polarisation transfer
DIAD	Diisopropyl azodicarboxylate
DIPEA	Diisopropylethylamine
DMA	Dimethylacetamide
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
EI	Electron impact
ESI	Electrospray ionisation
ESIPT	Excited state intramolecular proton transfer
Fmoc	Fluorenylmethyloxycarbonyl
FWHM	Full width at half maximum
g	Grams
GFP	Green fluorescent protein
GMO	Genetically modified organism
h	Hour
HATU	1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium-3-oxide hexafluorophosphate
HBTU	3-[Bis(dimethylamino)methyliumyl]-3*H*-benzotriazol-1-oxide hexafluorophosphate
HCTU	*O*-(1*H*-6-Chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

HeLa	Human epithelial cells
HIV	Human immunodeficiency virus
HOMO	Highest occupied molecular orbital
HPLC	High-performance liquid chromatography
HSQC	Heteronuclear single quantum correlation spectroscopy
HWE	Horner-Wadsworth-Emmons
Hz	Hertz
ICT	Intramolecular charge transfer
IR	Infrared
J	NMR spectra coupling constant
Kd	Dissociation constant
KHMDS	Potassium 1,1,1-trimethyl-*N*-(trimethylsilyl)silanaminide
LC-MS	Liquid chromatography-mass spectroscopy
LE	Locally excited
LUMO	Lowest unoccupied molecular orbital
m	Multiplet
M	Molar
m-	*Meta*-
m/z	Mass to charge
mg	Milligrams
MHz	Megahertz
mL	Millilitres
mM	Millimolar
mmol	Millimole
mol	Mole
NBS	*N*-Bromosuccinimide
NHS	*N*-Hydroxysuccinimide
NMR	Nuclear Magnetic Resonance
NIR	Near-infrared
Ns	Nitrobenzenesulfonyl
o-	*Ortho*-
p-	*Para*-
PBS	Phosphate-buffered saline
PET	Positron emission tomography
PIDA	(Diacetoxyiodo)benzene
PIFA	Bis-[(trifluoroacetoxy)iodo)benzene

PPA	Polyphosphoric acid
PTC	Phase transfer catalysis
PYBOP®	Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
q	Quartet
ϕ	Quantum yield
rt	Room temperature
s	Singlet
SNAr	Nucleophilic aromatic substitution
SPECT	Single photon emission computed tomography
SPPS	Solid-phase peptide synthesis
t	Triplet
TBAF	Tetra-*n*-butylammonium fluoride
TBDMS	*tert*-Butyldimethylsilyl
Tf	Trifluoromethanesulfonyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TIPS	Triisopropylsilane
TLC	Thin layer chromatography
Tr	Triphenylmethyl
Ts	4-Methylbenzenesulfonyl
TsCl	4-Methylbenzenesulfonyl chloride
UV	Ultraviolet
Vis	Visible
Δ	Reflux
µM	Micromolar

1.1 Introduction

1.1.1 Fluorescence imaging

Fluorescence imaging is a non-invasive technique that can offer much insight into biological processes.[1] The success of fluorescence imaging is due in part to the range of different fluorescent probes that are available, such as small organic molecules, quantum dots and proteins. The use of two-photon excitation techniques have allowed living cells to be imaged with minimal morphological change.[2] Fluorescence imaging has inherent advantages over other imaging techniques such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT) that relies upon radioactive nuclei.[3] No special handling and disposal procedures are required and fluorescent probes are radiologically stable. Carbon-11 that is used for PET imaging has a half-life of 20 minutes. Therefore, the shelf life of PET probes based upon ^{11}C is severely limited.[4] Recently, fluorescent probes have also recently found use in surgery.[5] It has been found that the use of fluorescence-guided surgery can significantly increase the survival rate of patients with malignant glioma.

1.1.2 Introduction to photoluminescence

Electrons within the ground state of a compound can be excited to a higher energy level. As the electron returns back to the ground state, it can emit a photon as luminescence. There are many methods in which an electron can be excited for luminescence to occur, including chemiluminescence, bioluminescence, electroluminescence, radioluminescence, sonoluminescence, thermoluminescence and photoluminescence.[6,7] Photoluminescence is the absorption of a photon of light, leading to excitation and then luminescence as the electron relaxes back to the ground state.[8] This is summarised with a Perrin-Jablonski diagram (**Figure 1**).[7] Luminescence occurs either through fluorescence or phosphorescence. Fluorescence is the electronic transition from an excited singlet state (S_1) back to the ground singlet state (S_0). As this is an allowed transition, it has a short excited-state lifetime with retention of spin multiplicity.[9] Phosphorescence occurs with intersystem crossing to an excited triplet state (T_1) then, transition back to the ground singlet state (S_0) and release of a photon of light. As this is a forbidden transition, this results in a long excited-state lifetime and involves a change of spin multiplicity. With photoluminescence, the photon emitted is at a longer wavelength than the photon that was absorbed. This is due to energy losses within the compound after excitation resulting in the light emitted being lower in energy. This difference between excitation and emission wavelengths is known as the Stokes shift.

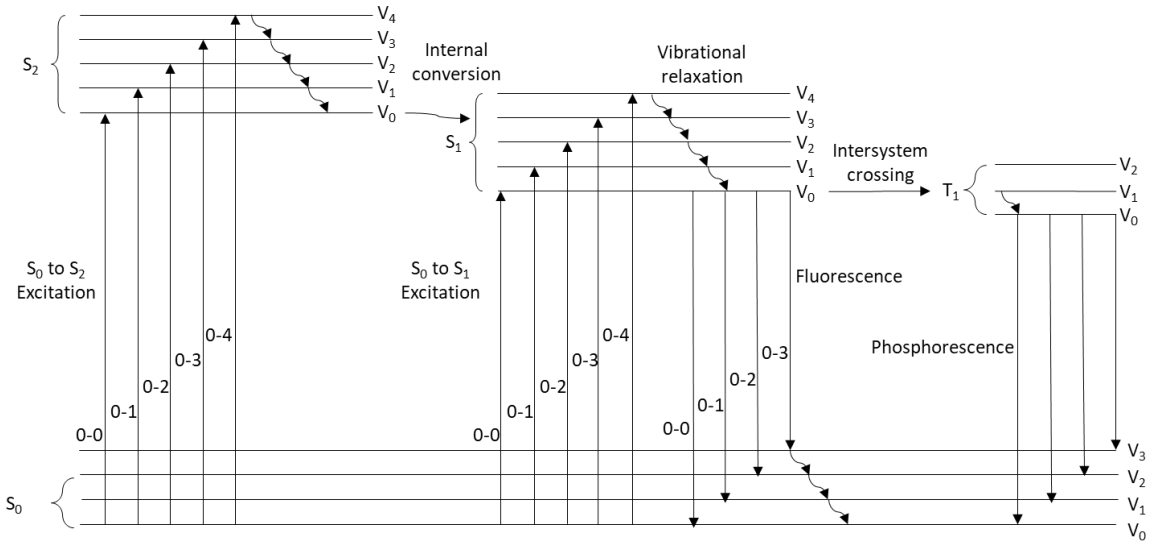


Figure 1 Perrin-Jablonski diagram.

Emission of a photon as fluorescence only occurs from the first excited singlet state (S_1). This is known as Kasha's rule.[10] Azulene is the only known exception to this rule as it emits fluorescence from the second excited singlet state (S_2). This is due to the large energy gap between the S_2 and S_1 levels.[10] As excitation does not significantly alter the compound's vibrational energy levels, this causes the emission and absorption spectra to become mirror images.[11] This is known as the mirror image rule. A further rule regarding fluorescence is the Kasha-Vavilov rule.[12] This states that the quantum yield of photoluminescence is independent of the excitation wavelength, however, there are expectations to this rule.[10]

Vibrational energy levels as well as electronic energy levels are also present in a compound. This results in fine-structure of the absorption and emission spectra from vibronic transitions. The absorption and emission spectra of anthracene is an example of this (**Figure 2 b**).[11] As anthracene is excited to the first excited state, numerous vibrational levels can be occupied. With excitation to V_0 energy level of S_1, this is defined as a 0-0 vibronic transition (**Figure 2 a**). The 0-0 vibronic excitation involves absorption of a photon at 376 nm. Excitation to the V_1 level involves a 0-1 vibronic transition which corresponds to absorption at 356 nm. The 0-2 transition to the V_2 level is observed with absorption at 339 nm. From the mirror image rule, these vibronic transitions are present in the emission spectrum (**Figure 2 b**). Emission to the V_0 level from a 0-0 transition is observed at 378 nm. With relaxation to the V_1 state from a 0-1 transition, an emission at 400 nm is observed. Emission at 423 nm is observed for a 0-2 transition to the V_2 level of the ground singlet state.[11] If the chromophore can freely rotate, then rotational energy levels will also be present.

With rotational energy levels being very small, this leads to broadening of both the absorption and emission spectra. Therefore, vibronic fine structure is not observed in non-rigid compounds.

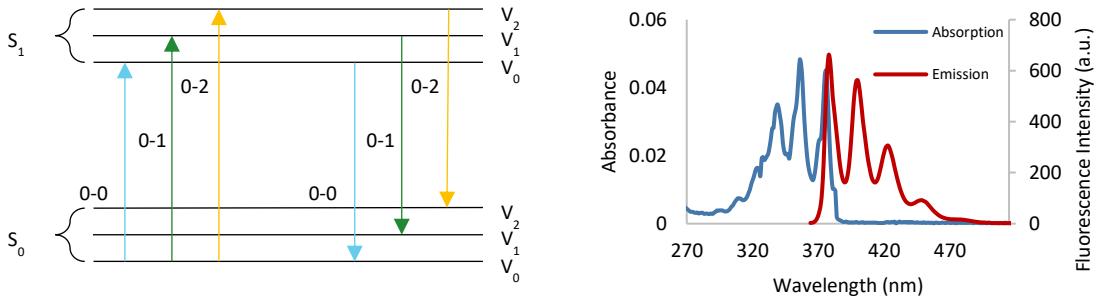


Figure 2 Vibrational energy levels (left). Absorption and emission spectrum of anthracene in ethanol (5 µM) (right).

The photoluminescent properties of a fluorophore can be quantified.[13,14] The molar attenuation coefficient is used to quantify how efficiently a compound can absorb photons. Quantum yield is used to determine how efficiently the compound converts absorbed photons into fluorescence. The molar attenuation coefficient and quantum yield can be combined to give a value known as brightness. This is used to quantify the total fluorescence intensity of the fluorophore.

The photoluminescence properties of a fluorophore can alter in different environments and this is known as solvatochromism.[15] As a fluorophore is excited, it gains a larger dipole moment. The surrounding solvent dipoles will reorient to stabilise the dipole moment. With increasing solvent polarity, the effect of this energy reduction becomes more pronounced. Therefore, in more polar solvents the emission becomes red-shifted and lower in energy. An example of this is with 4-dimethylamino-4'-nitrostilbene (**1**) (**Figure 3**). In pentane, it emits with a maximum of 465 nm. When **1** was dissolved in dioxane, the emission maximum was at 598 nm and when it was dissolved in DMF, the emission maximum occurred at 830 nm.

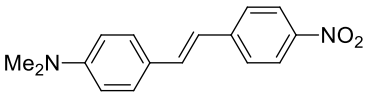


Figure 3 4-Dimethylamino-4'-nitrostilbene (**1**).

If a compound has an electron-donating group conjugated to an electron-accepting group, an intramolecular charge transfer (ICT) state can form. The formation of an ICT state is dependent upon solvent polarity. In polar solvents, the separated charges can be stabilised, resulting in the

ICT becoming more favourable. In non-polar solvents, these charges cannot be stabilised, resulting in a locally excited state (LE) being more favourable. Therefore, in polar solvents, emission will occur only from the ICT state and in non-polar solvents, emission will only occur from the LE state. For some intermediate solvents, such as methanol, emission may occur from both ICT and LE states giving dual emission. For formation of the ICT state, rotation of the functional groups present is sometimes required. This rotation gives a twisted internal charge transfer (TICT) state. In addition, the quantum yield of a compound can vary in different solvents. Typically, the use of high polar hydrogen-bonding solvents reduces the quantum yield of compounds.[16]

1.1.3 Two-photon excitation

Commonly a single photon is used in exciting a compound to its excited state. However, multiple photons can also be used to excite a compound (**Figure 4**). With multiple photons being used to excite the compound, the total energy required to reach the excited state is divided between the photons. For example, with a two-photon process, this corresponds to a doubling of the excitation wavelength. If a compound requires photons of 350 nm to reach the first excited state with a one-photon process, then 700 nm can be used for a two-photon process.

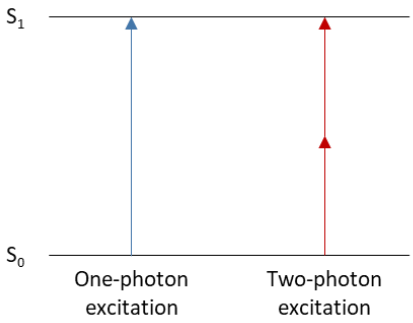


Figure 4 Two-photon excitation.

For biological imaging, multi-photon excitation offers many advantages over traditional one-photon excitation, such as reduced phototoxicity, reduced photobleaching and greater tissue penetration.[17] Reduced phototoxicity can be achieved with the use of higher excitation wavelengths. Some fluorophores are excited with UV light and this can be detrimental to the biological system being studied. For example, mammalian cells undergo apoptosis when irradiated with UV light.[18] Whereas, if a two-photon process is used, the resulting higher excitation wavelengths could avoid cell apoptosis. The advantages of two-photon microscopy have been demonstrated with the monitoring of mitochondrial distribution in hamster embryos.[19] After 8 h of one-photon microscopy, it was found that embryo development had ceased. Whereas, the use

of two-photon microscopy over a 24 h time span with frequent imaging, resulted in viable embryos, still capable of foetus development. Furthermore, photobleaching is also reduced with the use of two-photon excitation.[2] As fluorescence emission increases quadratically with excitation intensity, photobleaching only occurs within the vicinity of the focal plane, minimising damage.

From its high sensitivity and real-time analysis, fluorescence has been used to investigate biological processes.[20] There exists an "imaging window" between 650 nm and 1450 nm where tissue penetration is the largest, as the components of tissue have low absorbance at these wavelengths (**Figure 5**).[21] Therefore, having fluorophores that operate at these wavelengths allows for deep tissue penetration. An example of this has been demonstrated with quantum dots.[22] The use of two-photon excitation (900 nm) allowed for high-resolution fluorescence imaging up to depths of 2 mm. This was not achievable using a one-photon process with light of 450 nm.

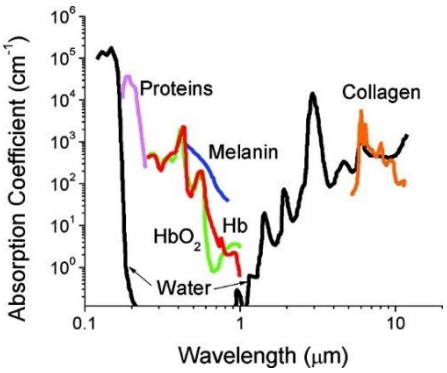


Figure 5 Imaging window. Reprinted with permission from (*Chem. Mater.*, 2012, **24**, 812–827). Copyright (2012) American Chemical Society.

1.1.4 Quenching of fluorescence

Fluorescence can be quenched through a number of different processes and this results in a decrease of intensity. The inclusion of heavy atoms can lead to a reduction of the fluorescence quantum yield (**Table 1**).[9] This is due to increased efficiency of intersystem crossing to the triplet state from spin-orbit coupling, although from this, increased phosphorescence is observed. For naphthalene, a quantum yield of 0.55 is measured but with the inclusion of an iodine atom, this is decreased to 0.0005.[23] It should be noted that the quantum yield of phosphorescence is increased to 0.38.[24] This "heavy atom effect" has also been shown to operate both externally and over long distances.[25]

Compound	ϕ_F	ϕ_P
Naphthalene	0.55	0.051
1-Fluoronaphthalene	0.84	0.056
1-Chloronaphthalene	0.058	0.30
1-Bromonapthalene	0.0026	0.27
1-Iodonaphthalene	<0.0005	0.38

Table 1 Fluorescence and phosphorescence quantum yield values for halogenated naphthalene.

The inclusion of nitro functional groups into polyaromatic compounds can also lead to fluorescence quenching, resulting in the fluorescence of aromatic nitro compounds not being detectable.[9,26] It has been determined that 1-nitronaphthalene has the largest intersystem crossing rate of any organic molecule.[27] This results in many aromatic nitro compounds being phosphorescent.[9] For example, carbazole (**2**) has excellent fluorescent properties.[9] However, with 3-nitrocarbazole (**3)**, no fluorescence is detected.[28] Instead, high levels of phosphorescence is observed with **3** when measured in a solid matrix at 77 K. However, for some nitroaromatics, the intersystem crossing is not fully efficient. So, some quenching may be due to high rates of S_1 to S_0 non-radiative internal conversion resulting from the strong electron-withdrawing nature of the nitro group.[9]

2
Φ_F = 0.42 in MeCN
Φ_P = 0.24 in solid matrix (77 K)

3
Φ_F = 0.00 in MeCN
Φ_P = 0.47 in solid matrix (77 K)

Figure 6 Quantum yields of **2** and **3**.

Quenching of fluorescence can also occur with excited-state proton transfer.[29] The acidity and basicity properties of a compound can differ between the ground state and excited state. For example, phenol has a pka of 10.6 in the ground state. This pKa value is decreased to 3.6 when phenol is excited.[9] Therefore, the fluorescence of compounds such as naphthol can be quenched in water from proton transfer.[30] Fluorescence can also be diminished with collisions with quenchers. Dissolved oxygen is a well-known quencher for aromatic hydrocarbons.[31] Similar quenching is observed between 1-chloronaphthalene and ethyl iodide and between anthracene

and diethylaniline.[32,33] The presence of nitro-aromatic compounds has also been shown to quench fluorescence, with a photo-induced electron transfer from the fluorophore to the aromatic nitro compound.[33] This quenching mechanism has been used in the detection of nitro-containing explosive compounds such as 2,4,6-trinitrotoluene.

1.1.5 Methods for improving the fluorescence of chromophores

To improve the photoluminescence properties of a compound, a number of strategies can be implemented. For example, the conjugation present in the fluorophore can be increased. The increased conjugation from biphenyl (**4**) to *para*-terphenyl (**5**) leads to higher quantum yield, molar attenuation coefficient and longer absorption and emission wavelengths (**Figure 7**).[34]

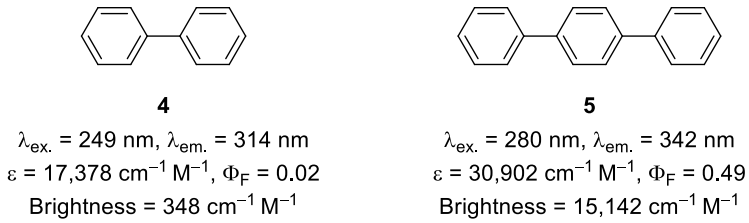


Figure 7 Photoluminescent properties of **4** and **5**.

The quantum yield and molar attenuation coefficient is also increased in more rigid and planar compounds.[35–37] The influence of rigidity on the quantum yield value is demonstrated with 4,4'-di-*tert*-butylbiphenyl (**6**) and fluorene (**7**). Due to the increase in rigidity, the quantum yield increased from 0.26 to 0.70 (**Figure 8**).[38] The same phenomenon is also observed in donor-π-acceptor systems. While alkene **8** has a quantum yield of 0.15, the more rigid alkyne **9** has a value of 0.83.[39]

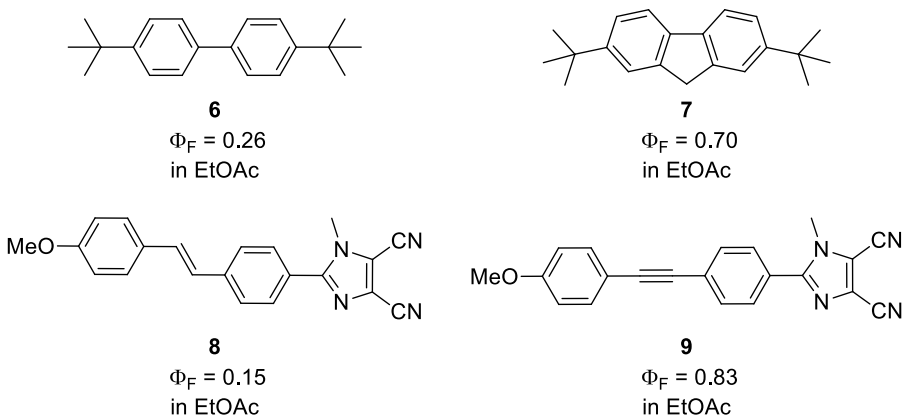
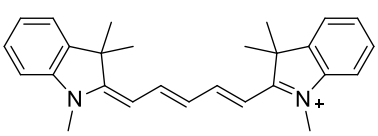


Figure 8 Quantum yield values of compounds **6**–**9**.

The strategy of rigidifying compounds has shown to be very successful at creating improved fluorophores for biological imaging. This was shown with far-red cyanine dye **10** (**Figure 9**).[37] Although, the compound is heavily used in fluorescence microscopy, it has a small quantum yield value in aqueous solutions This is due to excited state *trans-cis* polyene rotation which quenches the fluorescence. To overcome this, the fluorophore was rigidified and this gave octacyclic compound **11**. With this increased rigidity, a substantial increase of the quantum yield from 0.15 for **10** to 0.69 for **11** in PBS solution was observed.

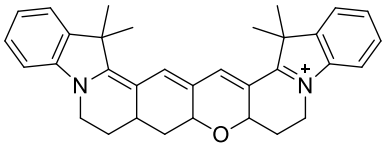


10

$\lambda_{ex.}$ = 638 nm, $\lambda_{em.}$ = 657 nm

ε = 214,000 $cm^{-1}M^{-1}$, Φ_F = 0.15

Brightness = 32,100 $cm^{-1}M^{-1}$



11

$\lambda_{ex.}$ = 662 nm, $\lambda_{em.}$ = 677 nm

ε = 206,000 $cm^{-1}M^{-1}$, Φ_F = 0.69

Brightness = 142,140 $cm^{-1}M^{-1}$

Figure 9 Photoluminescent properties of **10** and **11**.

As mentioned, it is preferable for fluorophores to have high absorption and emission wavelengths for imaging. A method to increase these wavelengths is with push and pull electronic systems. For example, alkyne **12** has an emission wavelength of 361 nm in ethyl acetate (**Figure 10**).[39] When a push-pull system is introduced by incorporating an electron-donating methoxy functional group **9**, the emission wavelength is increased to 396 nm. When the more electron-donating dimethylamino group is used **13,** the emission wavelength is increased to 515 nm. The excitation wavelengths of these compounds are also increased.

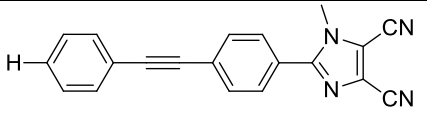
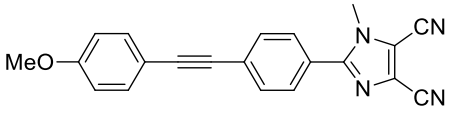
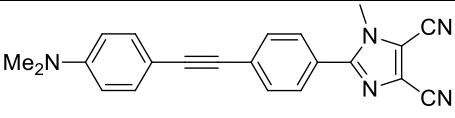
	**12** λ_{Ex} = 304 nm, $\lambda_{em.}$ = 361 nm in EtOAc
	**9** λ_{Ex} = 319 nm, $\lambda_{em.}$ = 396 nm in EtOAc
	**13** $\lambda_{Ex.}$ = 360 nm, $\lambda_{em.}$ = 515 nm in EtOAc

Figure 10 Excitation and emission wavelengths of compounds **9**, **12** and **13**.

1.1.6 Introduction to fluorescent amino acids

Fluorescence imaging is highly important for investigating peptide-protein and protein-protein interactions using environmentally sensitive and highly fluorescent dyes that are available.[40,41] When studying biological processes, the proteinogenic amino acids, L-phenylalanine, L-tyrosine and L-tryptophan **14**–**16** can be used as fluorophores (**Figure 11**).[13,14,42] From its small quantum yield and low molar attenuation coefficient, fluorescence from L-phenylalanine is only detected in proteins without L-tryptophan and L-tyrosine residues.[14] Therefore, L-phenylalanine has very limited applications. L-Tyrosine has much greater fluorescence intensity than L-phenylalanine, but it lacks environmental sensitivity.[14] Whereas, with its bigger brightness value and high environmental sensitivity, L-tryptophan has found more application in fluorescent microscopy.[14] The study of protein structure has been studied with time-resolved fluorescence using L-tryptophan residues.[43] This is carried out with proteins containing only a single L-tryptophan residue. Otherwise, the replacement of all but one L-tryptophan residue can be achieved with mutagenesis. It has also been shown that the fluorescence from L-tryptophan residues can be used in the diagnosis of cataracts.[34]

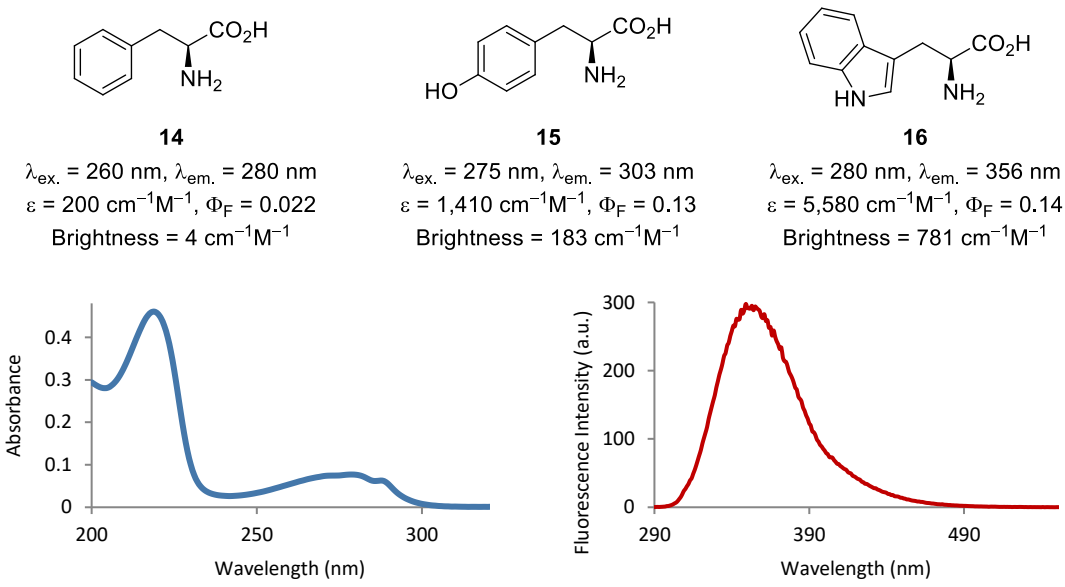


Figure 11 Structure and fluorescent properties of amino acids **14**–**16** and absorption, emission spectra of tryptophan in MeOH (10 µM).

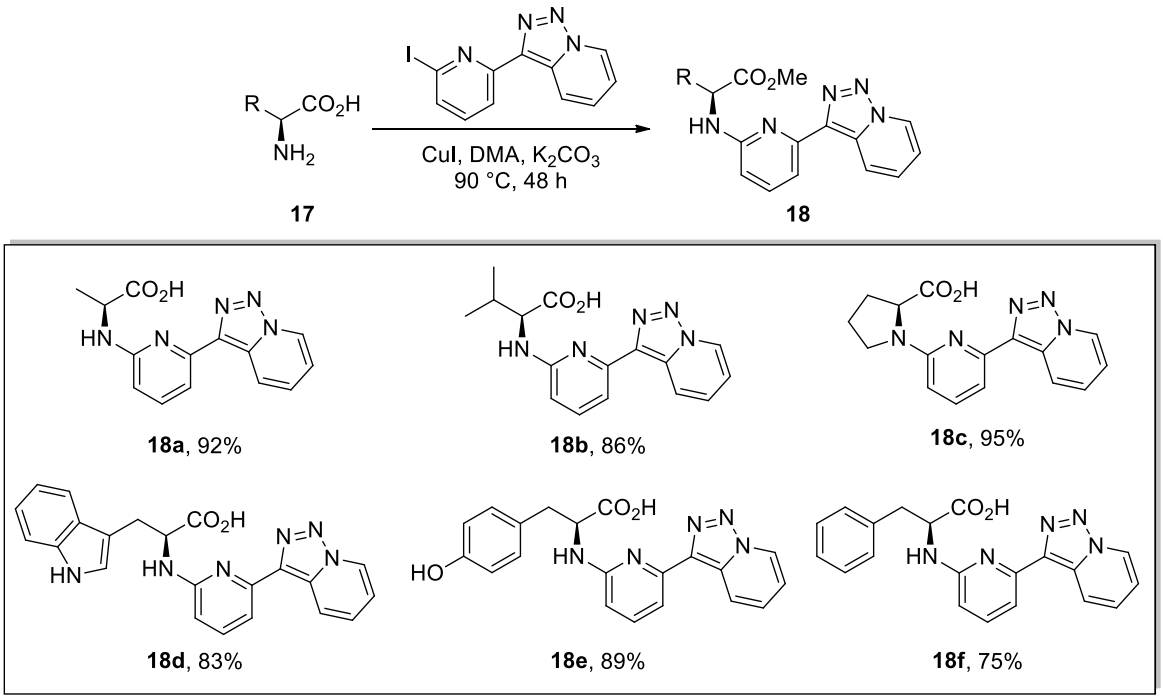
However, due to the issues associated with naturally occurring amino acids such as low brightness, nonspecific labelling and low excitation wavelengths, it is often it is best to install a superior fluorophore. Fluorescent protein such as green fluorescent protein can be used to label peptides and proteins. Although, the size of a fluorescent protein may affect the folding and function of the

protein being studied.[14,40] Small, highly fluorescent amino acids can also be used. Due to their small size, they may not affect the nature of protein being studied.[14,20,40,41,45] Furthermore, they can be specifically placed anywhere in the protein/peptide to give site-specific information.

1.1.7 Synthesis of fluorescent amino acids

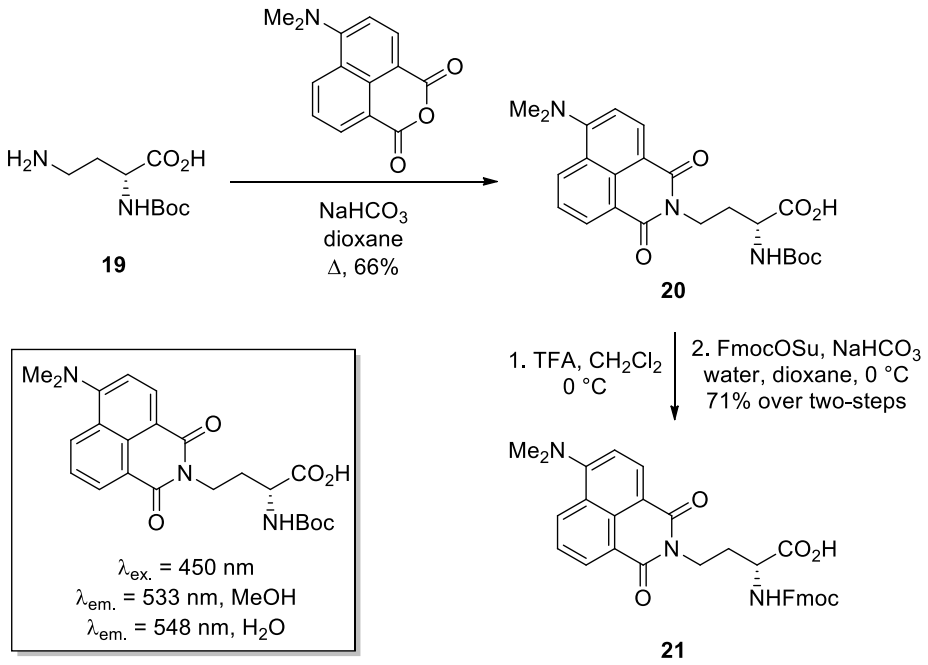
Fluorescent amino acids have been prepared using a number of different approaches.[45] Most syntheses of fluorescent amino acids have used a chiral pool strategy, that took advantage of the diverse range of natural α-amino acids available. This approach avoids the use of enantioselective catalysts and chiral resolution.

One approach that had been used for the synthesis of fluorescent amino acids was functionalisation of the α-amino group.[46] For example, it was shown that L-amino acids **18a**–**18f** could be prepared from the proteinogenic amino acids with 3-(6-iodo-2-pyridinyl)[1,2,3]triazolo[1,5-a]pyridine in high yields (**Scheme 1**).[46] High excitation (333–357 nm) and emission wavelengths (405–451 nm) were obtained from this library of compounds. It was then investigated if these amino acids could be used as metal sensors. It is known that Cu(II) and Zn(II) can diminish or enhance fluorescence respectively.[47] When copper(II) was added to the amino acids, the fluorescence was strongly quenched. There was no enhancement in fluorescence with the addition of zinc(II). It was also shown that fluorescence was enhanced and blue-shifted by 20 nm with the addition of oxalic acid. As the fluorophore was installed onto the α-amino group, this limited the use of amino acids **18a**–**18f** in peptide synthesis.



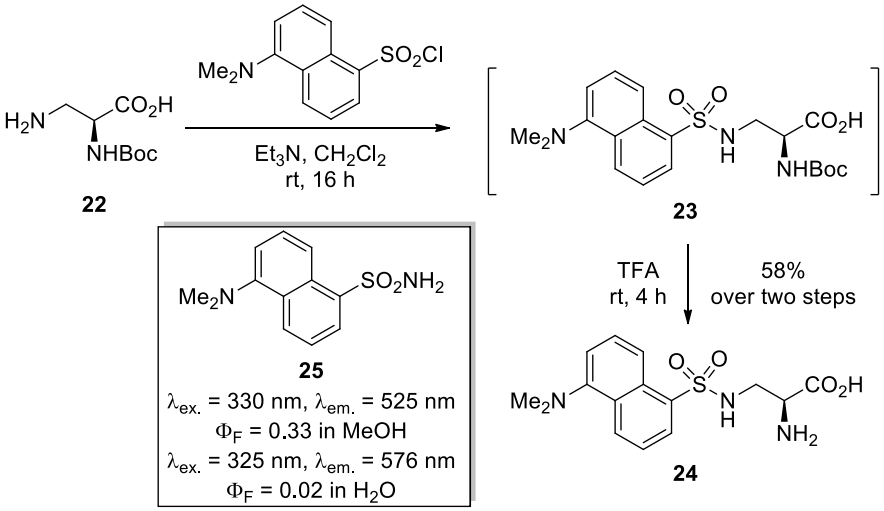
Scheme 1 Synthesis of fluorescent amino acids **18a**–**18f**.

Fluorescent amino acids are more commonly synthesised by modification of the side-arms of the natural amino acids. Lysine, 2,3-diaminopropionic acid, serine, tyrosine and aspartic acid have all been used to prepare fluorescent amino acids. For example, D-lysine has been used in the synthesis of dimethylamino substituted naphthalimide derived amino acid **21** (**Scheme 2**).[48] This was achieved by a condensation reaction of Boc protected D-lysine **19** with dimethylamino substituted naphthalic anhydride and gave **20** in 66% yield. The fluorescent properties of amino acid **20** was next investigated. It was found that the deprotected amino acid had an excitation wavelength of 450 nm and an emission wavelength of 533 nm in methanol. In water, a bathochromic shift was observed with amino acid emitting at 548 nm. A Fmoc protected analogue **21** was then prepared from **20** in 71% yield over two-steps. This allowed for the synthesis of a tripeptide by solid-phase peptide synthesis (SPPS) using **21.** It was thought that the use of D-amino acid **21** in peptide synthesis would be highly attractive due to its resistance to enzymatic degradation.



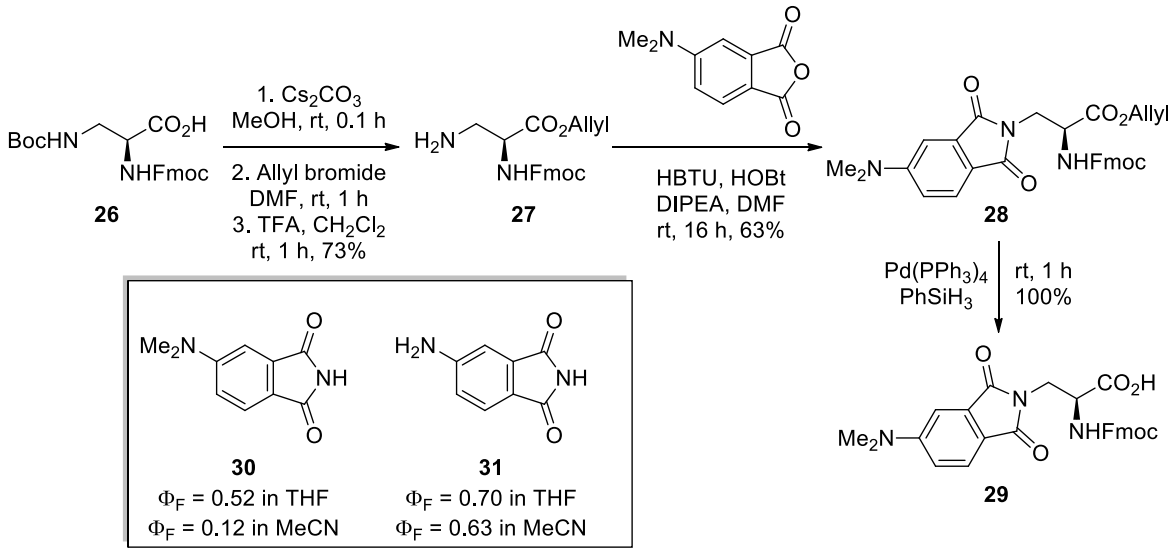
Scheme 2 Synthesis and photophysical properties of naphthalimide derived amino acid **20**.

The amino acid, L-2,3-diaminopropionic acid, has been used for the preparation of fluorescent amino acids.[49] Dansyl derived fluorescent amino acid **24** was prepared by the reaction of protected analogue **22** with dansyl chloride (**Scheme 3**).[50] After deprotection with TFA, target compound **24** was isolated in a 58% yield over the two steps. It had been shown that dansyl fluorophore **25** has favourable fluorescent properties in methanol.[51] Amino acid **24** was incorporated into human superoxide dismutase protein. Unfolding of this protein in the presence of guanidinium chloride was investigated by fluorescence microscopy.



Scheme 3 Synthesis of dansyl derived amino acid **24**.

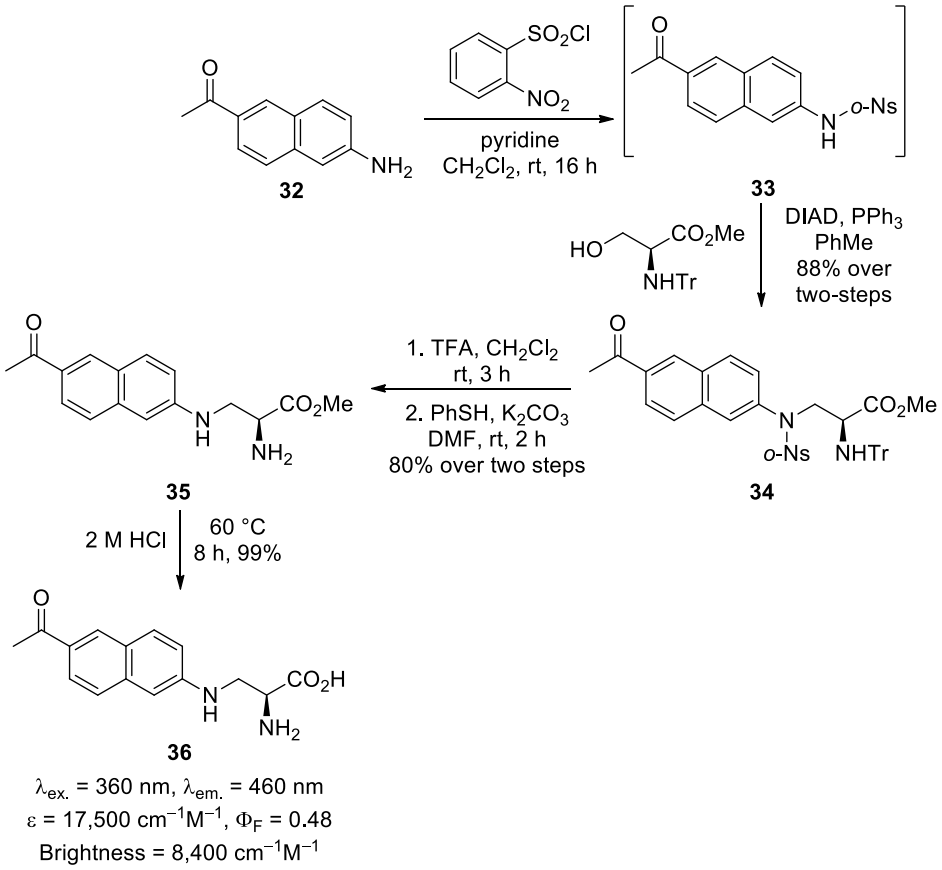
L-2,3-Diaminopropionic acid has also been used for the synthesis of phthalimide derived amino acid **29** (**Scheme 4**).[52] This was accomplished by protection of the commercially available amino acid **26** with allyl bromide and removal of the Boc protecting group with TFA gave **27** in 73% yield. This allowed for coupling with the dimethylamino substituted phthalic anhydride. It was found that the reaction of **27** with the anhydride did not result in ring closure when standard conditions were used. No cyclisation occurred even when the reaction was heated in piperidine or toluene. HBTU and HOBt were used to complete cyclisation and this gave **28** in a 63% yield. Removal of the allyl protecting group was achieved with tetrakis(triphenylphosphine)palladium and phenylsilane and this resulted in formation of Fmoc amino acid **29** in a 100% yield. The dimethylamino substituted phthalimide heterocycle (DAP) **30** had some interesting fluorescent properties.[53] In MeOH, **30** has an absorption wavelength of 396 nm and an emission maximum of 534 nm, which makes it a useful fluorophore for studying protein-protein interactions.[52] The quantum yield of **30** is sensitive to solvent polarity. In THF it had a value of 0.52, which decreased to 0.12 in MeCN. This observed decrease in quantum yield with solvent polarity was due to a nonradiative twisted intramolecular charge transfer (TICT) state.[53] This is not evident with 4-aminophthalimide (**31**) as a TICT state isn't possible and therefore, it's quantum yield value is independent of solvent polarity.



Scheme 4 Synthesis of phthalimide derived amino acid **29**.

L-Serine has also been used for the synthesis of fluorescent amino acids. Fluorescent amino acid **32** has been prepared from serine using a Fukuyama-Mitsunobu reaction (**Scheme 5**).[54] A sulfonamide protecting group was used to lower the pKa of 2-aminonaphthalene **32** and allowed for a successful Mitsunobu reaction.[55] Synthesis of **34** was achieved with the reaction of **32** with

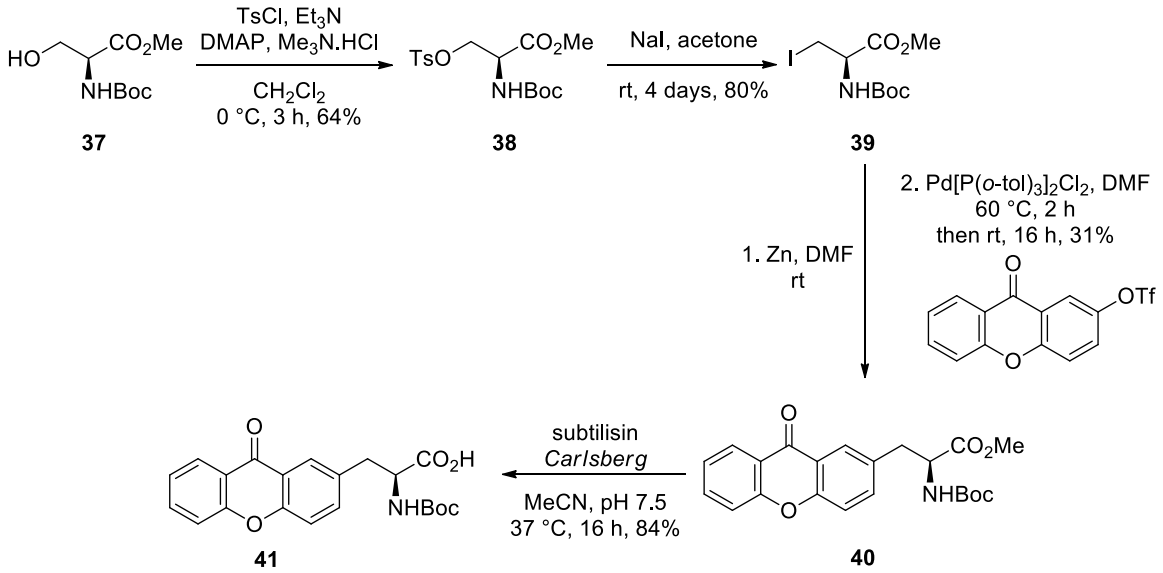
nosyl chloride under basic conditions and this gave intermediate **33**. This was used in the next step without any purification. A L-serine analogue was then coupled with **33** using DIAD and triphenylphosphine, which gave **34** in 88% yield over the two-steps. It was then shown that **34** could be deprotected stepwise. The trityl protecting group was removed with trifluoroacetic acid and the nosyl group was then removed with thiophenol and potassium carbonate in DMF. This gave **35** in 80% yield over the two-steps. The methyl ester was then hydrolysed with 2 M HCl at 60 °C, which gave fully deprotected analogue **36** in 99% yield. Amino acid **36** was found to possess a high quantum yield and solvatochromic properties.[56]



Scheme 5 Synthesis of 2-aminonaphthyl amino acid **36** and fluorescent properties in EtOH.

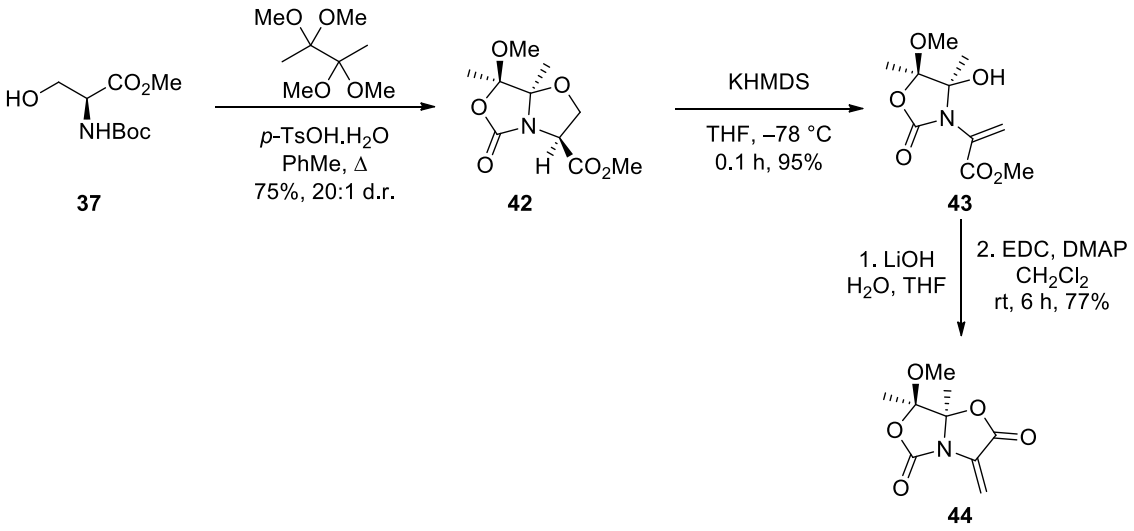
L-Serine analogue **37** was also used as a starting material for the synthesis of xanthone derived amino acid **41** through a palladium catalysed Negishi coupling (**Scheme 6**).[57] This was achieved by activation of serine analogue **37** with tosyl chloride under basic conditions. This gave **38** in 64% yield. Iodine functionalised amino acid **39** was prepared from **38** with sodium iodide using a Finkelstein-type reaction in 80% yield. The reaction of amino acid **39** with activated zinc gave the organozinc reagent required for the Negishi reaction. This was performed with dichlorobis(tri-*o*-tolylphosphine)palladium(II) and a xanthone triflate in DMF and gave **40** in a 31% yield. Enzymatic hydrolysis of the methyl ester gave amino acid **41** in a 84% yield. Amino acid **41** displayed typical

xanthone fluorescent characteristics. This included more enhanced fluorescence intensity in water than organic solvents.[58] This is due to ultrafast intersystem crossing resulting in very limited fluorescence in most solvents. In water, the quantum yield can be 100 times larger from a delayed fluorescence mechanism.



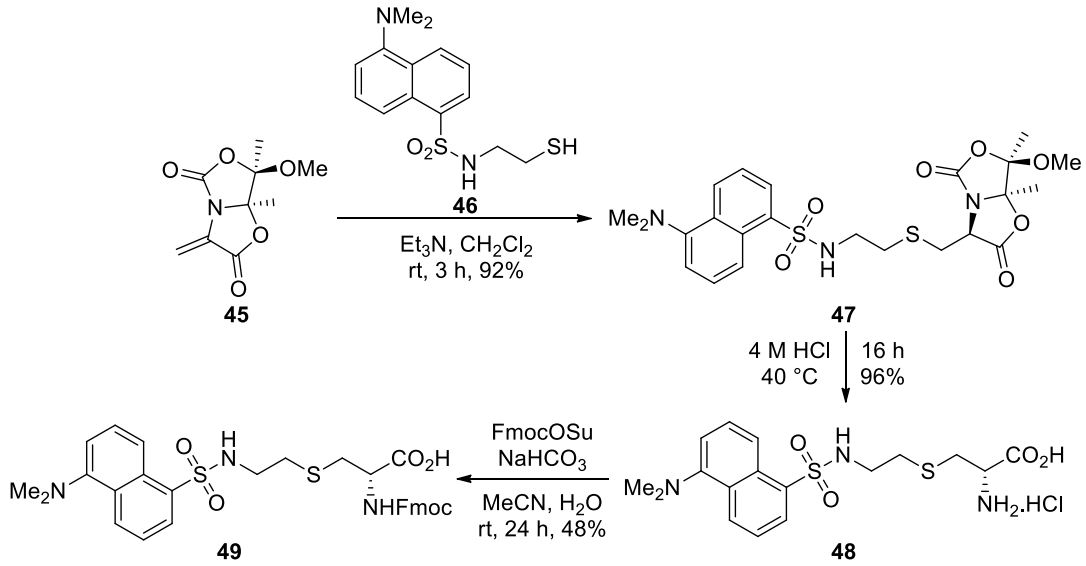
Scheme 6 Synthesis of xanthone-substituted amino acid **41**.

Bicyclic oxazolidone **44** has also been used to prepare fluorescent amino acids (**Scheme 7**).[59] **44** was prepared in four-steps from L-serine analogue **37**. Initially, the reaction of serine analogue **37** with 2,2,3,3-tetramethoxybutane under acidic conditions gave **42** in 75% yield. When **42** was reacted with KHMDS in THF this gave alkene **43** in 95% yield. Bicyclic intermediate **44** was prepared by ester hydrolysis of **43** with LiOH, followed by lactonisation with EDC and DMAP in a 77% yield over the two-steps.[60]



Scheme 7 Synthesis of intermediate **44**.

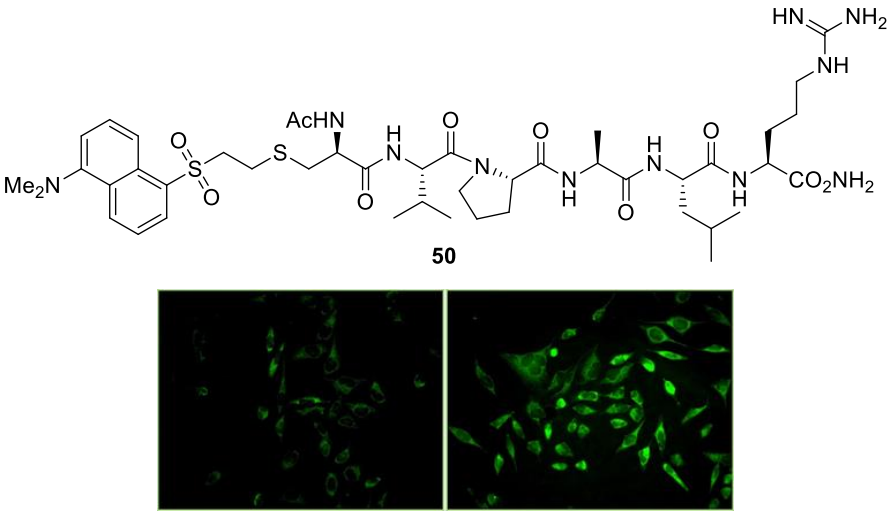
With **45** prepared, it was then functionalised with *N*-dansyl 2-aminoethanethiol **46**.[61] This was by a 1,4-conjugate addition under basic conditions that gave **47** in a 92% yield. Acid hydrolysis of **47** with 4 M HCl gave fluorescent D-amino acid **48** in 96% yield. The Fmoc protecting group was installed on amino acid **48** with FmocOSu and sodium hydrogen carbonate, which gave Fmoc analogue **49** in 48% yield.



Scheme 8 Synthesis of Fmoc protected dansyl derived amino acid **49**.

Dansyl derived amino acid **49** was then incorporated into a short cell-penetrating peptide **50** (**Figure 12**). As the synthesised peptide contained only natural amino acids, this resulted in **50**

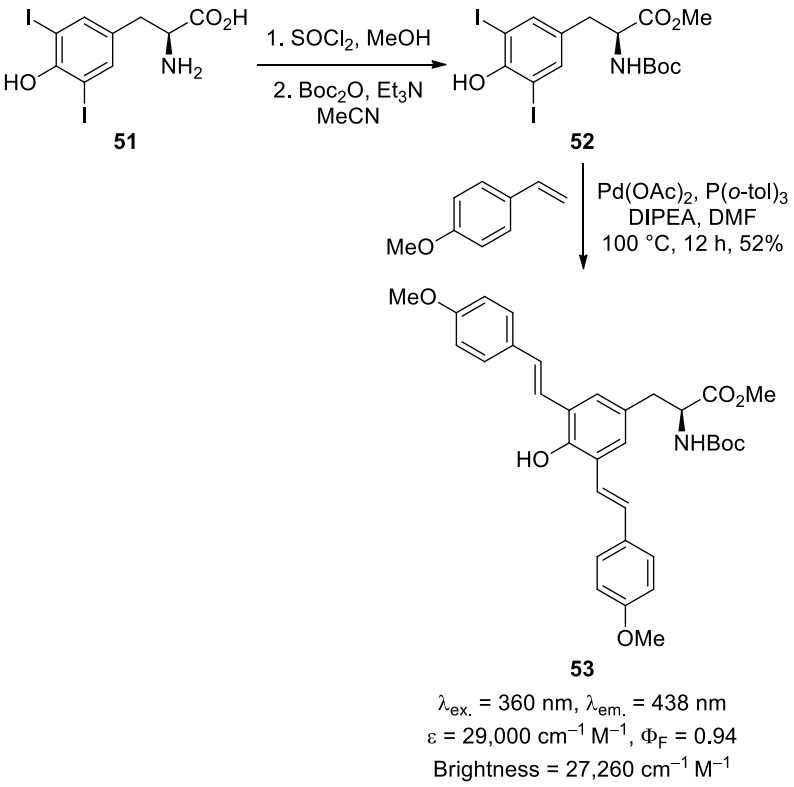
being stable for up to 4 h in human plasma and being non-cytotoxic up to 200 µM.[61] The potential of this fluorescent peptide was demonstrated with the imaging of HeLa cells.



50

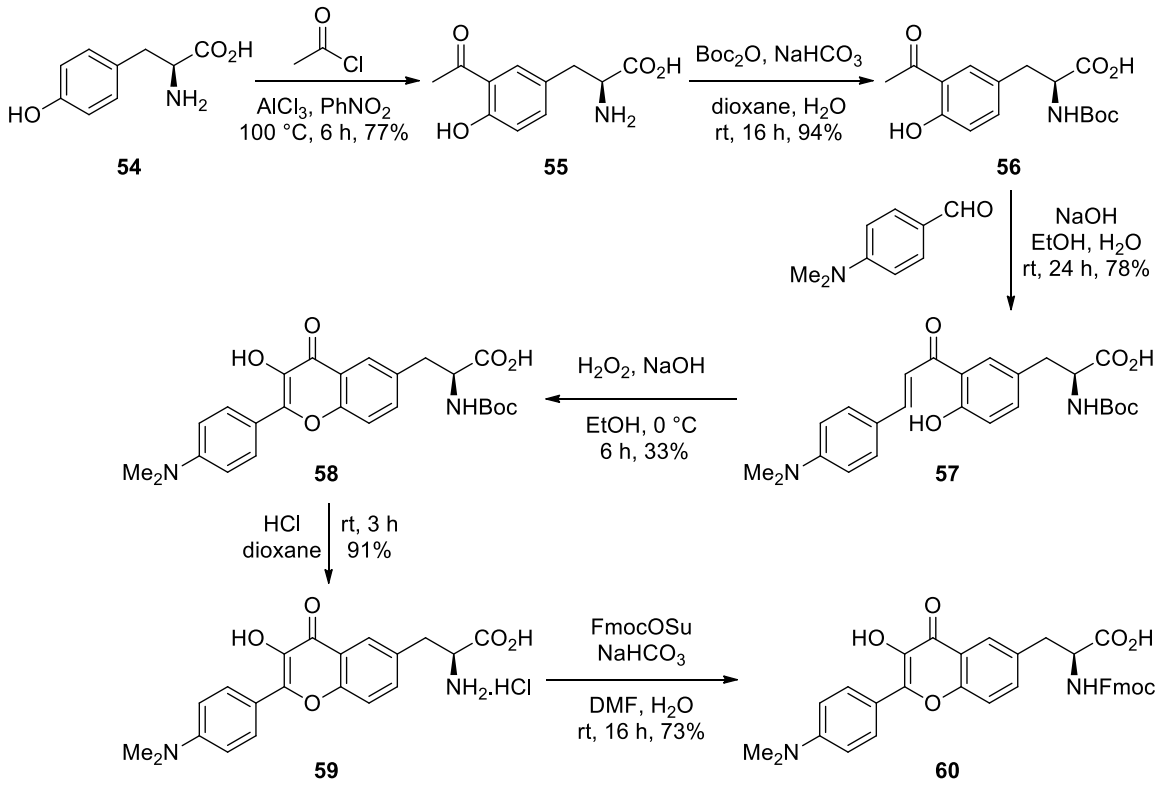
Figure 12 Peptide **50** and fluorescence imaging of HeLa cells, control (left) and with peptide (right).[61] Reprinted with permission from (*Chem. Eur. J.*, 2018, **24**, 7991). Copyright (2018) Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

As L-tyrosine is already fluorescent, only a few modifications are required to prepare an amino acid that has excellent fluorescent properties. For example, amino acid **53** was prepared using a palladium catalysed Heck reaction (**Scheme 9**).[62] Starting from commercially available 3',5'-diiodo-L-tyrosine (**51**) this was protected by esterification and standard Boc-protection, which gave **52**. A Heck reaction was performed using **52** and 4'-methoxystyrene, which gave **53** in 52% yield. With the increased conjugation, amino acid **53** was a better fluorophore than L-tyrosine. It had a higher quantum yield and molar attenuation coefficient values and longer excitation and emission wavelengths.



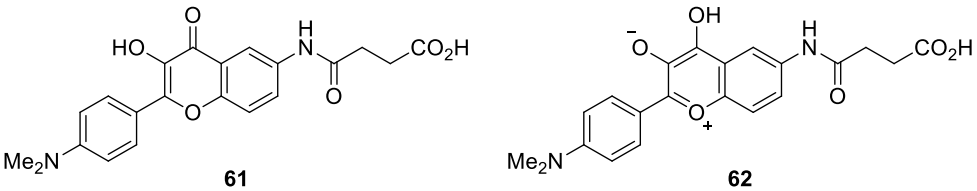
Scheme 9 Synthesis of amino acid **53** via a Heck cross-coupling reaction and fluorescent properties in DMSO.

It was also demonstrated that flavone derived amino acid **59** could be synthesised from L-tyrosine (**54**) (**Scheme 10**).[63] This was achieved by Friedel-Crafts acylation of **54** using acetyl chloride and gave **55** in 77% yield. Tyrosine derivative **55** was protected with di-*tert*-butyl dicarbonate and this gave **56** in 94% yield. An aldol condensation was then carried out with **57**, 4-dimethylaminobenzaldehyde and sodium hydroxide in ethanol and this gave chalcone **58** in 78% yield. Flavone **59** was synthesised from **58** using the Algar-Flynn-Oyamada reaction with sodium hydroxide and hydrogen peroxide in 33% yield. Target amino acid **59** was prepared by Boc group deprotection with HCl in dioxane. The Fmoc protecting group was installed on **59** under basic conditions and this gave **60** in 73% yield. With SPPS, Fmoc amino acid **60** was incorporated into two peptide chains. The peptide chains were based upon Melittin, a 26-mer peptide chain that is found in the venom of the honeybee (*Apis mellifera*).[64] For one peptide the hydrophobic leucine residue was replaced at the 9-position, for the other peptide a tryptophan residue at the 19-position was replaced. These fluorescent peptides were then used in image HeLa cells using two-photon excitation with a wavelength of 830 nm.



Scheme 10 Synthesis of dimethylamino substituted flavone derived amino acid **59**.

One interesting aspect regarding the fluorescence of **59** is the dual emission that is observed. This is due to an excited state intramolecular proton transfer (ESIPT) (**Table 2**).[65] This is demonstrated with compound **61**. In its excited state, a proton transfer takes place and this resulted in tautomer **62**. The fluorescence from this state is emitted at a higher wavelength. Depending upon the solvent used, fluorescence will either take place from LE **61** or ESIPT state **62**. Less polar solvents favour the ESIPT state. For **61** in toluene, the emission is almost exclusively from the ESIPT state (95% of total emission) with an emission maximum wavelength of 566 nm. As the solvent polarity is increased, the proportional emission from the ESIPT is decreased. When **61** is dissolved in MeCN, most of the emission comes from the LE state with an emission maximum of 516 nm. In aqueous buffer solution and methanol, emission is exclusively from LE state **61** with emission maxima at 533 and 550 nm, respectively.



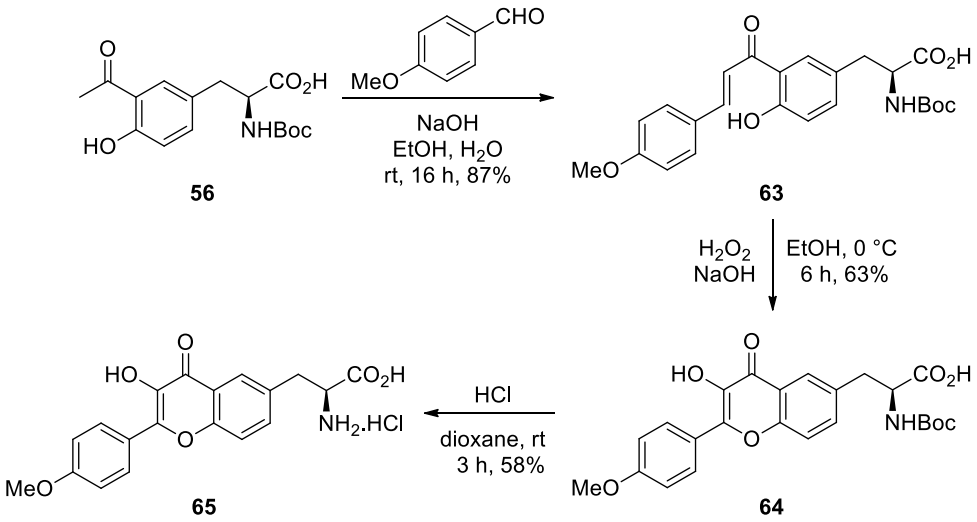
61
LE state
$\lambda_{em.}$ = 470 nm

62
ESIPT state
$\lambda_{em.}$ = 570 nm

Solvent	LE emission (nm)	ESIPT emission (nm)	% ESIPT emission	ϕ_F
Toluene	466	566	95	0.226
EtOAc	487	567	75	0.095
Acetone	503	573	61	0.101
MeCN	516	575	30	0.154
MeOH	533	N/A	0	0.262
PBS solution	550	N/A	0	0.004

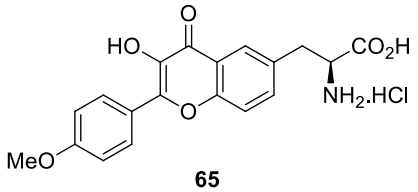
Table 2 Excited state **61**, ESIPT state **62** and photoluminescent properties.

To improve the fluorescent properties in aqueous environments of **59**, the dimethylamino functional group was replaced with a methoxy functional group. This was accomplished using the same synthetic approach. Ketone **56** was reacted with 4-methoxybenzaldehyde and this gave **63** in 87% yield (**Scheme 11**).[66] Subsequent Algar-Flynn-Oyamada reaction with sodium hydroxide and hydrogen peroxide gave **64** in 63% yield. Removal of the Boc protecting group with HCl in dioxane gave methoxy substituted flavone derived amino acid **65** in 58% yield.



Scheme 11 Synthesis of methoxy substituted flavone derived amino acid **65**.

As the fluorescent properties of **65** were studied, differences were found in comparison with **61**.[63,66,67] The fluorescence quantum yield of methoxy analogue **65** had far less sensitivity to solvent polarity (**Table 3**). With **65** dissolved in methanol, a quantum yield of 0.067 was measured. This value increased to 0.078 when **65** was dissolved in aqueous PBS solution. Dual emission was still observed with approximately a 1:1 ratio of emission from LE and ESIPT states when **65** was dissolved in methanol.



65

Solvent	Excitation Wavelength (nm)	LE emission (nm)	ESIPT emission (nm)	% Emission from ESIPT	ϕ_F
1,4-Dioxane	355	416	540	96	0.159
DMF	355	421	539	90	0.068
EtOH	360	429	535	66	0.072
MeOH	357	434	535	51	0.067
PBS solution	355	442	N/A	0	0.078

Table 3 Solvatochromic properties of methoxy substituted flavone derived amino acid **65**.

Based on the favourable properties of **65**, it was incorporated into a peptide corresponding to the zinc finger domain of the nucleocapsid protein of the human immunodeficiency virus.[66] Two fluorescent-tagged peptide chains were prepared with amino acid **65**. Either the alanine residue at position 30 or the tryptophan residue at position 37 was replaced with **65**. These fluorescent peptides were then used for the imaging of HeLa cells with two-photon FLIM (**Figure 13**). With each pixel in the image, the fluorescence lifetime was determined with a biexponential fit. From this, the image was coloured to represent the fluorescence lifetime of that pixel. No lifetimes under 2.5 and 1.7 ns were found, from which it was concluded that no unbound peptide was present within the cells. The lifetimes found suggested that the fluorescent peptides were bound to nucleic acids.

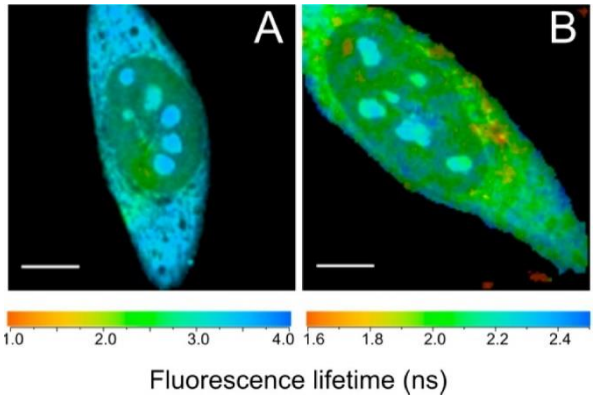
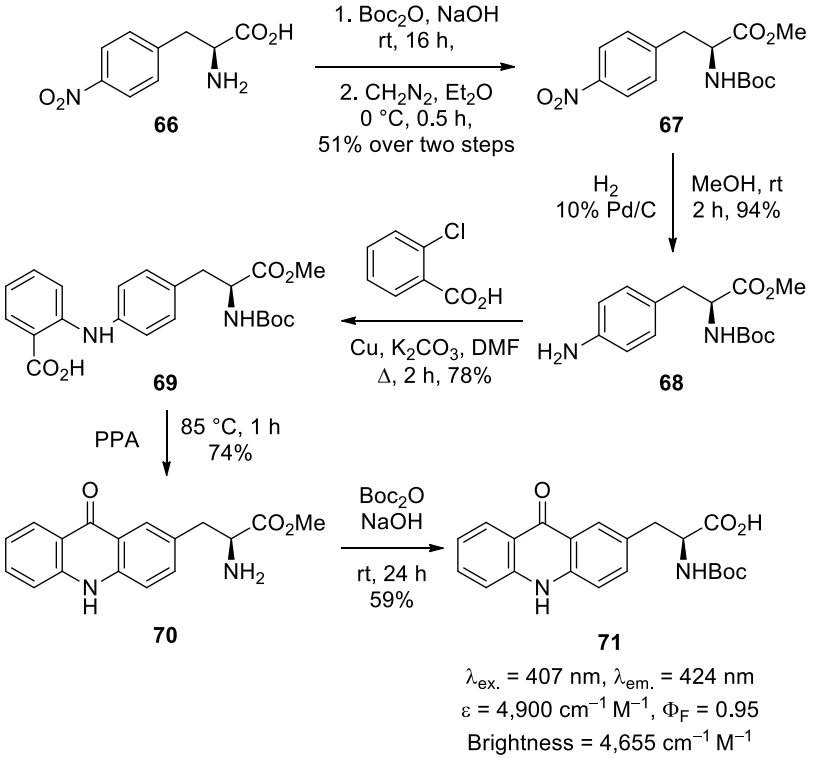


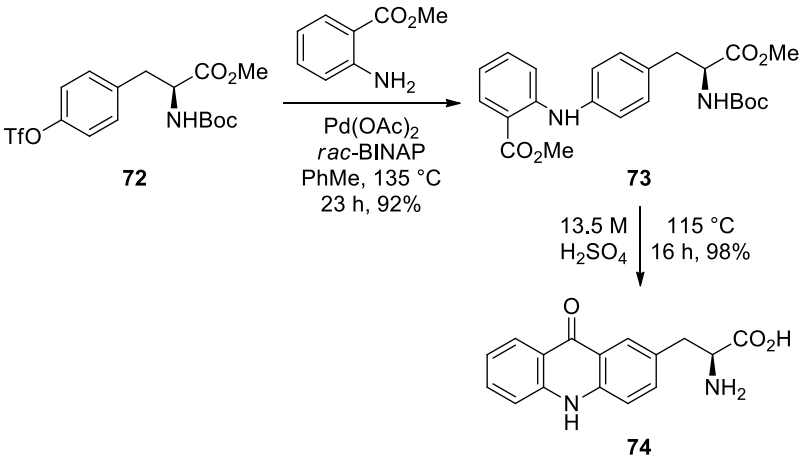
Figure 13 2-Photon FLIM imaging with fluorescent peptide tagged with **65**. Reprinted with permission from (*J. Phys. Chem.*, 2015, **119**, 2585–2595). Copyright (2014) American Chemical Society.

The commercially available amino acid, 4'-nitro-L-phenylalanine (**66**) has also been used to synthesise fluorescent amino acids (**Scheme 12**).[68] For example, the synthesis of fluorescent amino acid **71** was achieved from **66** in six steps. Initially, **66** was protected with di-*tert*-butyl dicarbonate and then diazomethane and gave **67** in 51% yield over the two steps. Reduction of **67** was carried out with 10% palladium on carbon and hydrogen in methanol and gave amino acid **68** in 94% yield. An Ullmann-Jourdan condensation was then carried out with **68** and 2-chloroenzoic acid and gave **69** in 78% yield. Formation of an acridone ring system was carried out with **69** and hot polyphosphoric acid (PPA). This resulted in Boc deprotection and gave **70** in a 74% yield. The Boc protecting group was re-installed with di-*tert*-butyl dicarbonate and sodium hydroxide, which resulted in ester hydrolysis and gave **71** in a 59% yield. Unlike, xanthone derived amino acid **41**, amino acid **71** had excellent fluorescent properties. It had excellent quantum yields in water (ϕ_F = 0.95), MeOH (ϕ_F = 0.74), DMSO (ϕ_F = 0.76) but a low quantum yield in THF (ϕ_F = 0.21). In water, **71** could be excited with light at 388 and 407 nm. This is very useful as it allowed for **71** to be excited selectively over tryptophan and tyrosine when used for investigating peptides and proteins. From vibronic transitions, two emission maxima were observed in water at 424 and 445 nm.



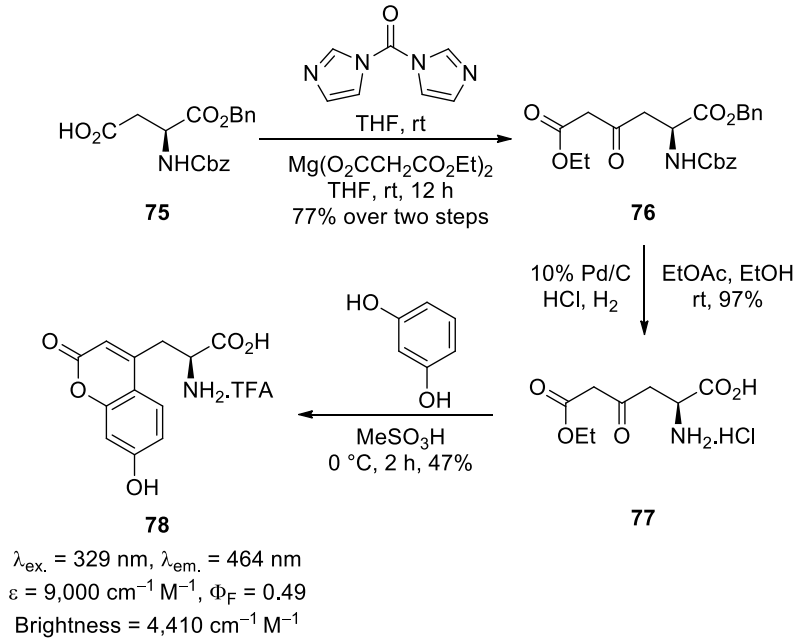
Scheme 12 Synthesis and of acridone derived amino acid **71** and fluorescent properties in water.

As acridone derived amino acid **71** had useful fluorescent properties an improved synthesis was published.[69] It was found that **71** could be synthesised in five steps with an 87% overall yield from L-tyrosine (**15**) (**Scheme 13**). Protected analogue **72** was prepared in three steps and with a 96% overall from **15**. Coupling of **72** with 2-methyl anthranilate was achieved using a Buchwald-Hartwig reaction and gave **73** in a 92% yield. It was also found that the efficiency of the Friedel-Crafts cyclisation could be improved using 13.5 M sulfuric acid and gave **74** in a 98% yield. From this more efficient synthesis of **74**, large quantities of the amino acid could be synthesised, allowing for milligram quantities of a fluorescent protein to be prepared. The protein calmodulin was selectively labelled with **74** at the 13, 41, 100 and 113 positions.[69] Then, it's binding to a peptide was investigated by fluorescence spectroscopy. It was found that the fluorescence emission of the protein became more redshifted as higher equivalents of peptide were introduced to the protein, signifying protein-peptide interactions.



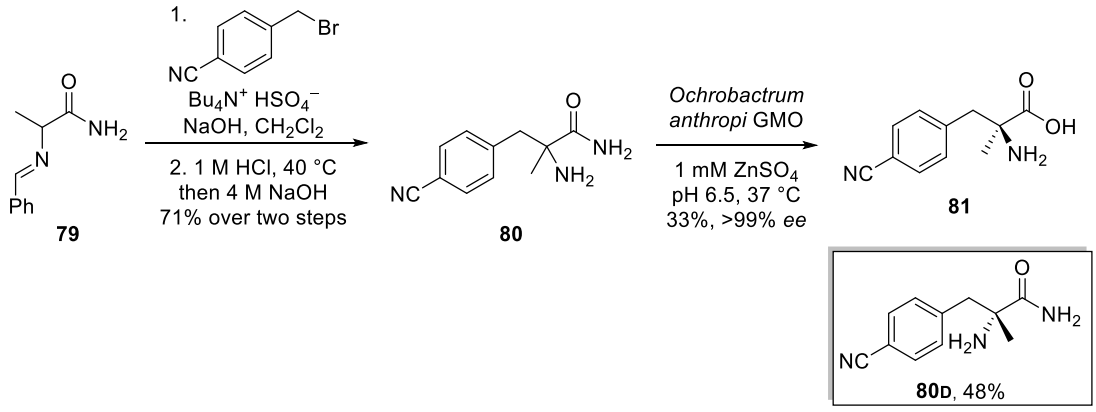
Scheme 13 Improved synthesis of acridone derived amino acid **74**.

L-Aspartic acid has also been used for the synthesis of fluorescent amino acids.[70] Protected analogue **75** was used in the synthesis of coumarin derived amino acid **78** (**Scheme 14**). This was achieved by synthesis of β-ketoester **76** from **75** in 77% yield. Removal of the protecting groups by hydrogenolysis, using 10% palladium on carbon gave **77** in 97% yield. The allowed for the key step, a Pechmann condensation with benzene-1,3-diol. The reaction was facilitated with methanesulfonic acid and gave coumarin derived amino acid **78** in a 47% yield. The imaging of HeLa cells was then carried out with a 17-mer peptide chain containing **78**.



Scheme 14 Synthesis of coumarin derived amino acid **78** and fluorescent properties in EtOH.

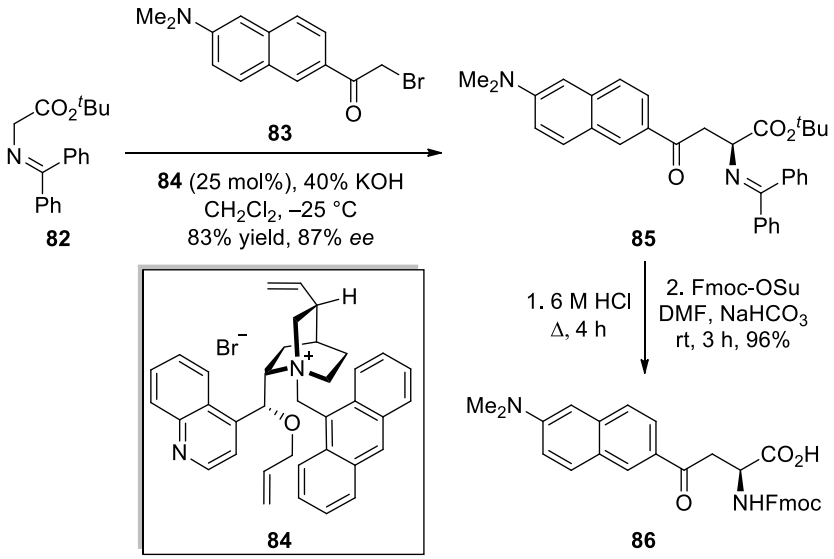
Although, the majority of fluorescent amino acids are synthesised from enantiopure starting materials, in some cases chirality is introduced by either resolution or enantioselective catalysis. It has been shown that the GMO enzyme *Ochrobactrum anthropi* can be used to resolve a racemic mixture of amino acids (**Scheme 15**).[71] Racemic benzylidene protected alanine amide **79** was alkylated with use of a phase transfer catalyst and this phenylalanine derivative **80** in 71% yield over two steps. Treatment of racemic amino acid **80** with GMO *Ochrobactrum anthropi* resulted in amide hydrolysis of the *S*-enantiomer and gave 4'-cyano-L-phenylalanine **81** in 33% yield with >99% *ee* and the *R*-enantiomer **80D** was returned in 48% yield. Compared to phenylalanine (**14**), amino acid **81** had a quantum yield that was five times larger and had an excitation wavelength of 235 nm with emission at 295–305 nm. In addition, it was found that the emission of **81** was sensitive to its environment. Amino acid **81** was then utilised in SPPS for the preparation of a decapeptide. This was used for investigating peptide-membrane interactions.



Scheme 15 Enzymatic resolution of **80**.

Enantioselective catalysis was used in the synthesis of charge transfer based fluorescent amino acid **86** (**Scheme 16**).[72] A cinchonidine organic catalyst was used in the coupling reaction of prochiral amino acid **82** with α-bromoketone **83**. It was reported that reaction temperature was crucial in allowing for an efficient transformation. At temperatures below −30 °C, the alkylation reaction did not occur and at elevated temperatures the stereoselectivity of the reaction was diminished. When the reaction was performed at −25 °C, a yield of 83% was achieved for **85** with 87% *ee*. Target amino acid **86** was prepared after acid hydrolysis and Fmoc protection in 96% yield over the two steps. It was found that the fluorescence emitted from amino acid **86** was highly sensitive to solvent polarity.[73] In heptane, the emission maximum was recorded at 409 nm. A bathochromic shift was observed when **86** was dissolved in water with an emission maximum at 542 nm. Amino acid **86** was then incorporated into the 15-mer peptide, S-tag, which was used to

bind to the S-protein. From fluorescent measurements, the binding affinity constants of the modified S-tags to the S-protein were determined.



Scheme 16 Synthesis of charge-transfer amino acid **86**.

1.1.8 Summary

There is much interest in the application of fluorescent amino acids for biological imaging and in particular investigating protein-protein and protein-peptide interactions.[4,20,39,40,44] This is due to the wide diversity of environmentally sensitive fluorescent amino acids available and their high brightness. In addition, when a fluorescent amino acid is incorporated into a protein or peptide unlike GFP its small size will not affect the folding or the function of the protein.[40]

2.1 Synthesis of Novel Fluorescent Pyrazoloquinazoline Functionalised α-Amino Acids

2.1.1 Previous work in the Sutherland group

Previous work within the group has focused on the synthesis of a diverse range of fluorescent α-amino acids (**Figure 14**). Originally, a fluorescent enone amino acid **87** was prepared with a HWE reaction between a β-keto phosphonate ester and 4-dimethylamino-1-naphthaldehyde. The charge transfer between the electron-rich amino group and the electron-deficient enone system resulted in a high excitation wavelength (393 nm) and a high emission maximum (540 nm).[74] With the enone being highly reactive due to conjugation with the dimethylamino group, it was proposed that this compound may not be suitable for long-term storage. Therefore, it was thought more robust fluorescent amino acids could be attained if the enone functional group was converted into a more stable heterocycle.[75] It was first shown that these enone derived amino acids could undergo reaction with phenylhydrazine for the preparation of the dihydropyrazole ring system. Subsequent oxidation of the dihydropyrazole ring with DDQ gave the aromatic pyrazole heterocycle **88**. The reaction of different enones with phenylhydrazine and DDQ gave a small library of pyrazole substituted amino acids. From this library, it was found that naphthyl-substituted pyrazole amino acid **88** was the most fluorescent amino acid.

Next, it was shown that pyridine heterocycles could be constructed from enone derived amino acids.[76] This was achieved by reaction of the enones with ethyl vinyl ether in an ytterbium catalysed, inverse electron-demand hetero-Diels-Alder reaction, giving dihydropyran derived amino acids **89**. A modified Knoevenagel-Stobbe reaction with hydroxylamine was then used to convert the dihydropyran into the pyridine heterocycle. Using this methodology, a small library of pyridine derived amino acids were synthesised. From this library, the 4-methoxyphenyl substituted pyridine derived amino acid **90** exhibited the best photoluminescence properties. This was due to the charge-transfer between the electron-rich methoxy group and the electron-deficient pyridine ring system. The 4-methoxyphenyl substituted pyridine derived amino acid had high values for both its molar attenuation coefficient (25,920 $cm^{-1} M^{-1}$) and quantum yield (0.46) resulting in a brightness value of 11,923 $cm^{-1} M^{-1}$.

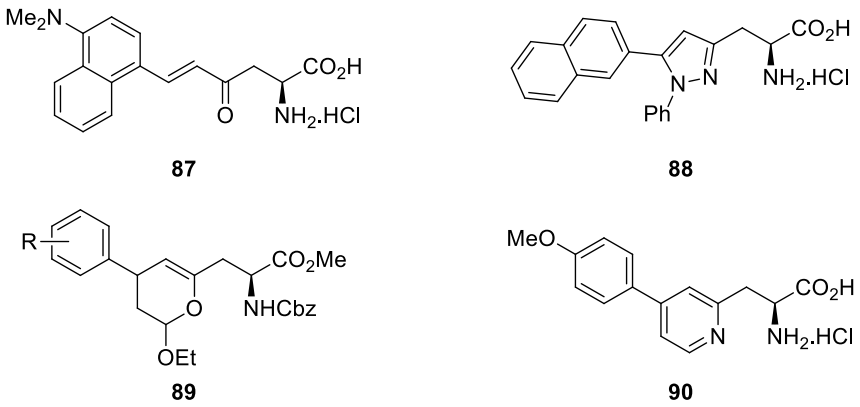


Figure 14 Previously synthesised fluorescent amino acids.

The emission of 4-methoxyphenyl substituted pyridine derived amino acid **90** was found to be highly sensitive to solvent polarity. In non-polar THF, the amino acid had an emission maximum of 308 nm (**Figure 15**). However, when dissolved in highly polar PBS solution, the emission maximum increased to 423 nm.

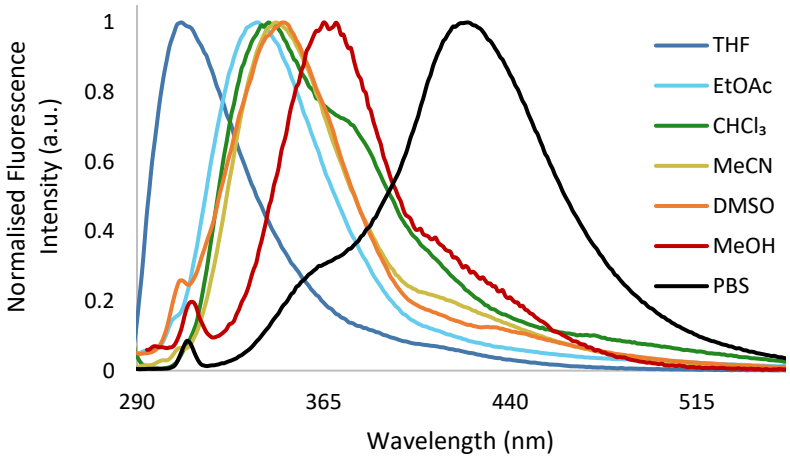


Figure 15 Emission spectra of amino acid **90** in a range of solvents.

The environmental sensitivity observed for 4-methoxyphenylpyridine **90** is due to an ICT state.[11] After excitation, electron flow from the methoxy group to the pyridine ring system gives ICT state **91** (**Figure 16**). This can only occur in polar solvents, as the highly polar ICT state is stabilised with solvent interactions. For non-polar states, emission occurs from a locally excited (LE) state that resembles the ground state. The presence of shoulder peaks in the emission spectra in chloroform and PBS solution is an indication of **90** possessing both LE and ICT states.

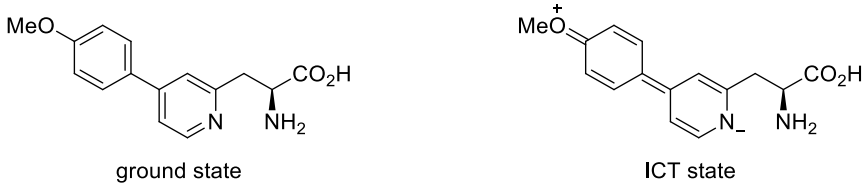


Figure 16 Ground state **90** and internal charge-transfer (ICT) state **91** for **90**.

2.1.2 Aim of the project

The amino acids previously developed did exhibit some interesting fluorescent properties. However, it was proposed that if more rigid and planar amino acids were synthesised, the photoluminescence properties could be improved. Therefore, it was thought that incorporating rigidity into one of the previously synthesised fluorescent amino acids, a superior fluorophore could be prepared. From the previously prepared amino acids, the pyrazole functionalised amino acid **92** was the obvious substrate for introducing rigidity. This was due to the presence of a heteroatom in close proximately to the adjoined phenyl ring that would be useful for tethering the two rings together **93** (**Figure 17**). There is a class of heterocycle known as pyrazoloquinazolines **94** that exhibit useful bioactivities, such as being antagonists for adenosine receptor **95**, AMPA receptor **96** and Gly/NMDA antagonist **97**.[77–80] It was proposed that this would make an excellent template for a new generation of rigid, planar fluorescent α-amino acids.

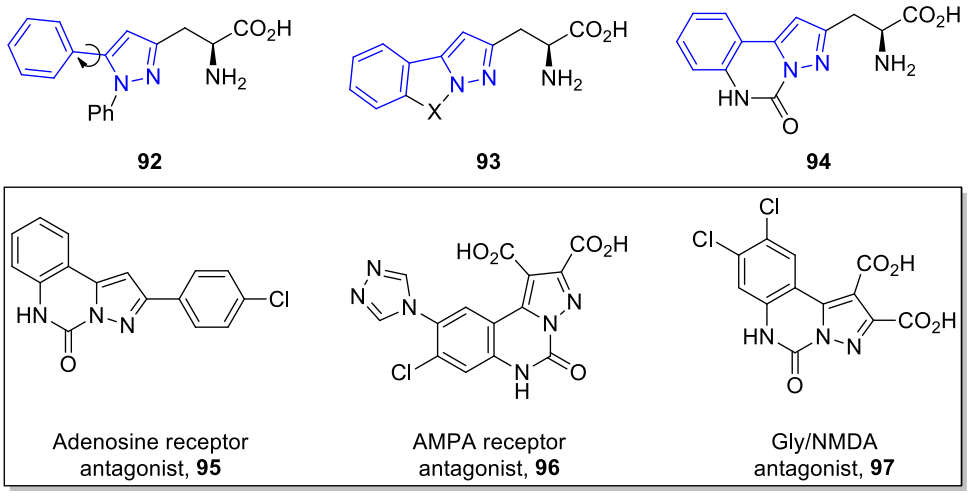


Figure 17 Conceptual design of the rigid pyrazoloquinazoline amino acid **94** and bioactive pyrazoloquinazoline compounds **95**–**97**.[77,78,80,81]

The aims of this project were to develop a synthetic route to pyrazoloquinazoline derived amino acid **94** and then prepare a small library of analogues. After a library was synthesised, the photoluminescence properties of the pyrazoloquinazoline derived amino acids would be measured with UV-Vis absorption and fluorescence spectroscopy. Amino acids **94, 98**–**101** were

chosen as target compounds for this library (**Figure 18**). The target compounds include the highly conjugated benzo-fused pyrazoloquinazoline **98**, the electron-deficient fluorine substituted pyrazoloquinazoline **99** and the electron-rich dimethoxy- and dimethylamino substituted pyrazoloquinazoline amino acids **100** and **101**.

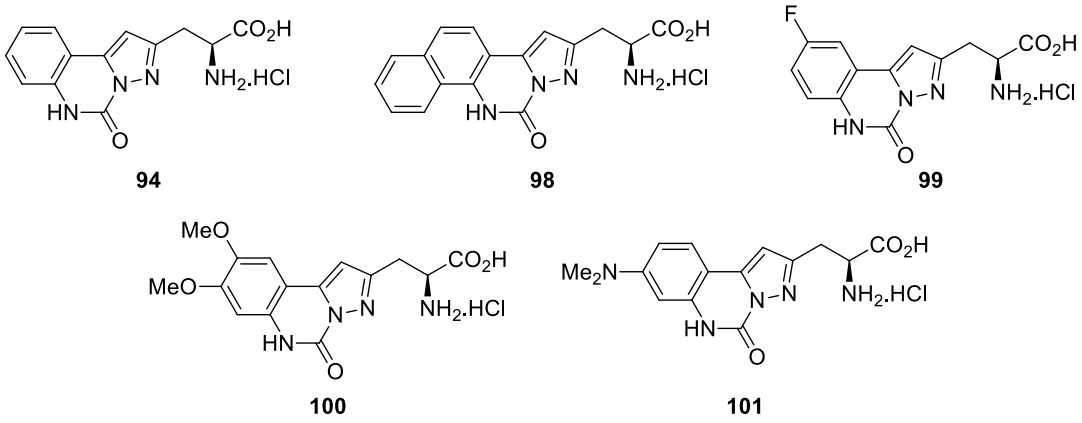
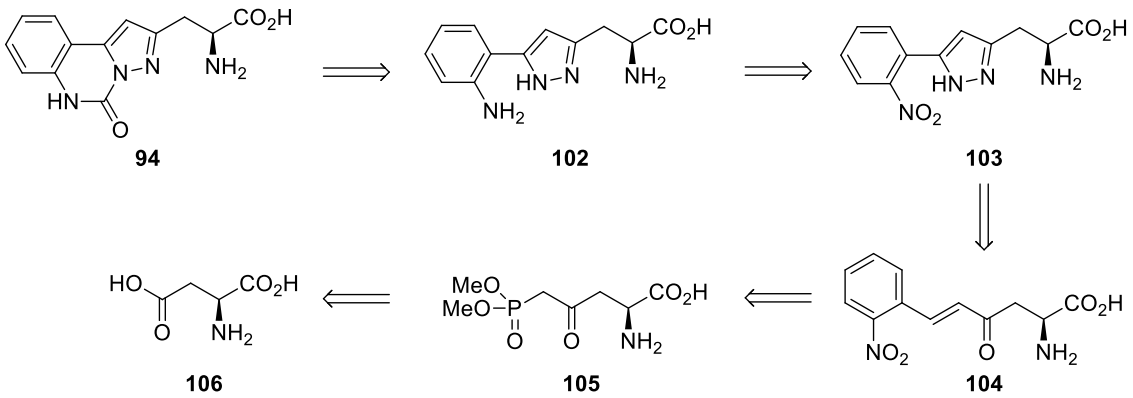


Figure 18 Target pyrazoloquinazoline amino acid compounds.

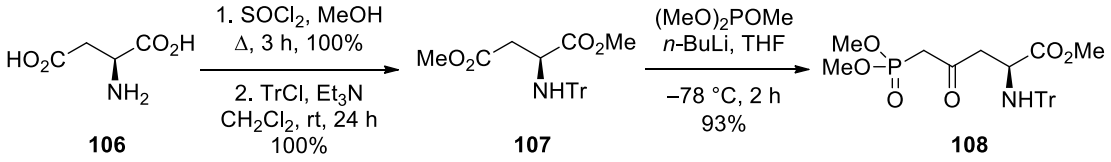
2.1.3 Synthesis of pyrazoloquinazoline derived amino acid

A synthetic route to pyrazoloquinazoline derived amino acid **94** was designed with the following retrosynthetic analysis (**Scheme 17**). It was proposed that a double disconnection could be achieved between the carbonyl carbon and the two adjoining nitrogen atoms. This disconnection gives 2-aminophenyl substituted pyrazole **102**. Preparation of the urea group could be achieved with triphosgene, carbonyl diimidazole or disuccinimidyl carbonate.[82,83] Amine **102** could be prepared from nitro derivative **103** with a functional group interconversion. Previous work within the group has shown that pyrazole derived amino acids can be synthesised from enones.[75] Thus, amino acid **103** could be prepared from enone derived amino acid **104**, hydrazine hydrate and then subsequent oxidation. Enone **104** could be prepared from the HWE reaction with β-keto-phosphonate ester **105** and 2-nitrobenzaldehyde. Analogues of enone **104** could be obtained by using different 2-nitrobenzaldehydes, allowing for the synthesis of target compounds **94, 98**–**101**. It has previously been shown that β-keto-phosphonate ester **105** can be synthesised from L-aspartic acid (**106**).[74] Hence, all pyrazoloquinazoline derived amino acids **94, 98**–**101** could be synthesised from L-aspartic acid (**106**).



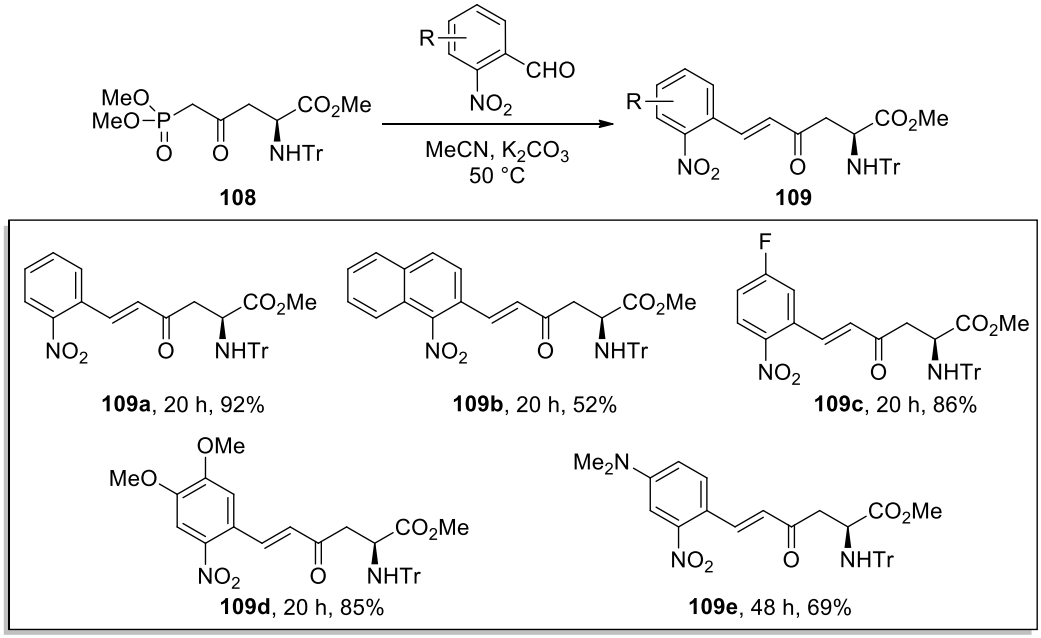
Scheme 17 Retrosynthetic analysis of pyrazoloquinazoline amino acid **94**.

Literature conditions were followed for the synthesis of β-keto-phosphonate ester **108** from L-aspartic acid (**106**) (**Scheme 18**).[74–76,84] This involved treatment of L-aspartic acid (**106**) with thionyl chloride in MeOH, which gave dimethyl-L-aspartate in quantitative yield. The trityl protecting group was installed with triphenylmethyl chloride under basic conditions, which produced dimethyl *N*-trityl-L-aspartate (**107**) in quantitative yield. The bulky trityl protecting group was required for the regioselective synthesis of β-keto-phosphonate ester amino acid **108**. Due to the large bulk of the trityl group, this shields the α-ester functional group from nucleophilic attack. When the anion of dimethyl methylphosphonate was reacted with protected amino acid **107**, this gave **108** in excellent yield as a single regioisomer.



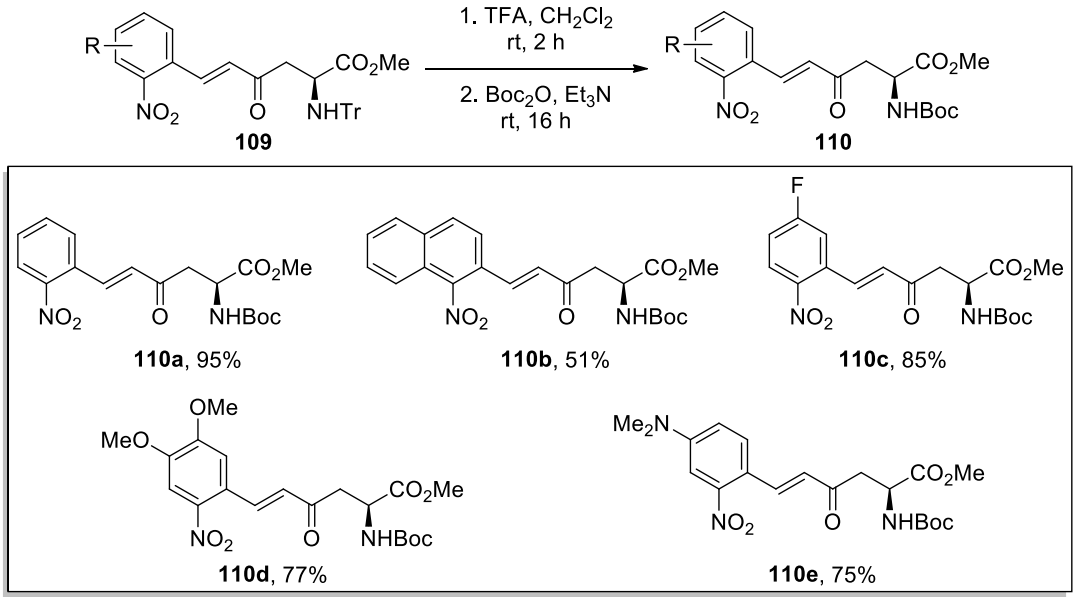
Scheme 18 Synthesis of β-keto-phosphonate ester **108** from L-aspartic acid (**106**).

HWE reactions were then carried out with β-keto-phosphonate ester **108** with various 2-nitrobenzaldehydes under mild conditions (**Scheme 19**). Due to the strong electron-withdrawing nitro group being in conjugation with the aldehyde, the reactions were highly efficient. Most of the reactions were completed within 24 h and gave enone amino acids **109a**–**109e** in good to excellent yields (52%–92%). Formation of the *E*-enone was confirmed by ^{1}H NMR spectroscopy with the appearance of doublets at 6.53 ppm and 7.89 ppm, with a large coupling constant of 16.2 Hz.



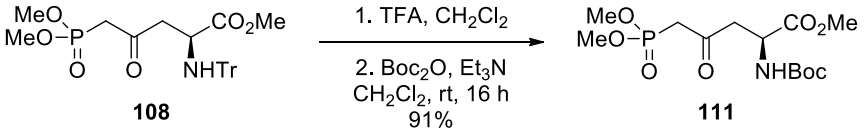
Scheme 19 Synthesis of enones **109a**–**109e** using a HWE reaction.

While it was required for the preparation of β-keto-phosphonate ester **108**, the trityl group inhibits subsequent reactions of the enone due to its steric bulk.[76] Hence, prior to the formation of the pyrazole ring, the trityl protecting group was replaced with a smaller protecting group. To achieve this, enones **109a**–**109e** were initially treated with trifluoroacetic acid (**Scheme 20**). The Boc protecting group was then installed with di-*tert*-butyl dicarbonate under basic conditions and this gave enones **110a**–**110e**, from good to excellent yields over the two-steps.



Scheme 20 Synthesis of Boc-protected enones **110a**–**110e**.

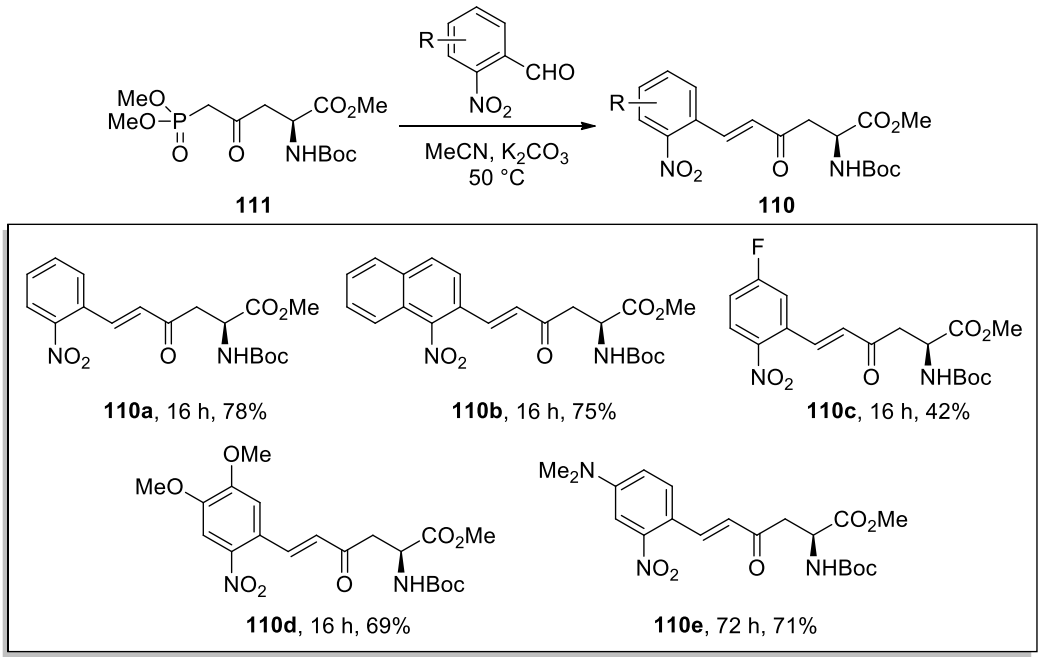
It was proposed a more efficient synthesis to Boc enones **110a**–**110e** could be achieved by reprotection of the β-keto phosphonate ester **108** followed by HWE reaction of this with various aldehydes. This would allow the introduction of the different aromatic groups at a later stage of the synthetic route. This was investigated by trityl deprotection of phosphonate ester **108** with TFA and then subsequent Boc protection with di-*tert*-butyl decarbonate, which gave **111** (**Scheme 21**). This transformation was achieved in high yield.



108 **111**

Scheme 21 Synthesis of Boc protected β-keto-phosphonate ester **111** from trityl protected β-keto-phosphonate ester **108**.

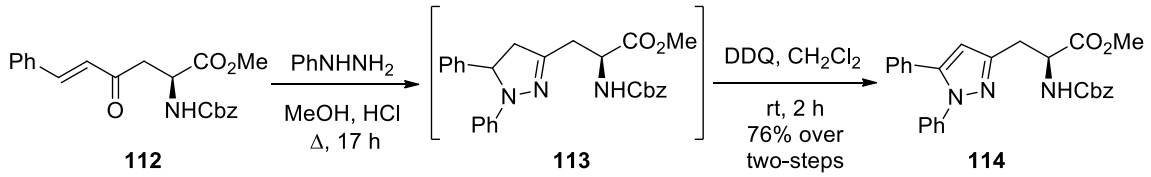
The HWE reaction was then trialled with Boc protected β-keto-phosphonate ester **111** and 2-nitrobenzaldehydes (**Scheme 22**). From this, it was found that good yields were achieved for enones **110a**-**110e,** in short reaction times. Only when the electron-rich, 4-dimethyl-2-nitrobenzaldehyde was used did the reaction require multiple days to reach completion. The lowest yield was obtained when using the electron-poor, 5-fluoro-2-nitrobenzaldehyde aldehyde.



Scheme 22 HWE reaction with Boc protected β-keto-phosphonate ester **111**.

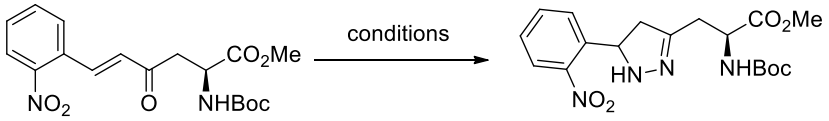
As previously mentioned, work within the group has demonstrated that pyrazole derived amino acid **114** can be prepared from enone **112** (**Scheme 23**).[75] This was achieved by reaction of **112**

with phenylhydrazine under acidic conditions, which gave dihydropyrazole **113**. Treatment of **113** with the oxidising agent DDQ gave pyrazole derived amino acid **114** in 76% yield over the two-steps.



Scheme 23 Previous synthesis of pyrazole amino acid **114**.[75]

Due to the presence of the acid-labile Boc protecting group, these acidic conditions were not used. As forcing conditions were required to react enone **112** with phenylhydrazine, therefore forcing conditions were initially tried with enone **110a** (**Table 4**). When the reaction was attempted with a large excess of hydrazine hydrate under reflux conditions, no desired target compound was obtained due to the reaction of the ester with hydrazine (entry 1). It has previously been reported that hydrazine will form the hydrazide by reaction with methyl esters in alcoholic solvents.[85] The number of equivalents of hydrazine hydrate was then reduced from 10 to 5 under the same conditions but, this still resulted in reaction with the ester functional group (entry 2). However, when the reaction temperature was reduced to room temperature and only one equivalent of hydrazine was used, it was found that the ester protecting group was still intact. Unfortunately, there was incomplete conversion from the enone to the dihydropyrazole (entry 3). Therefore, while keeping the reaction at room temperature, the quantity of hydrazine hydrate was gradually increased (entries 4 and 5). This resulted in an increase in yield for dihydropyrazole **115a**. From this, it was observed that 5 equivalents of hydrazine hydrate gave the best yield of **115a** (entry 6). It was then found that full conversion of enone **110a** occurred within 4 hours when 5 equivalents of hydrazine were used (entry 7). This gave dihydropyrazole **115a** in 86% yield.

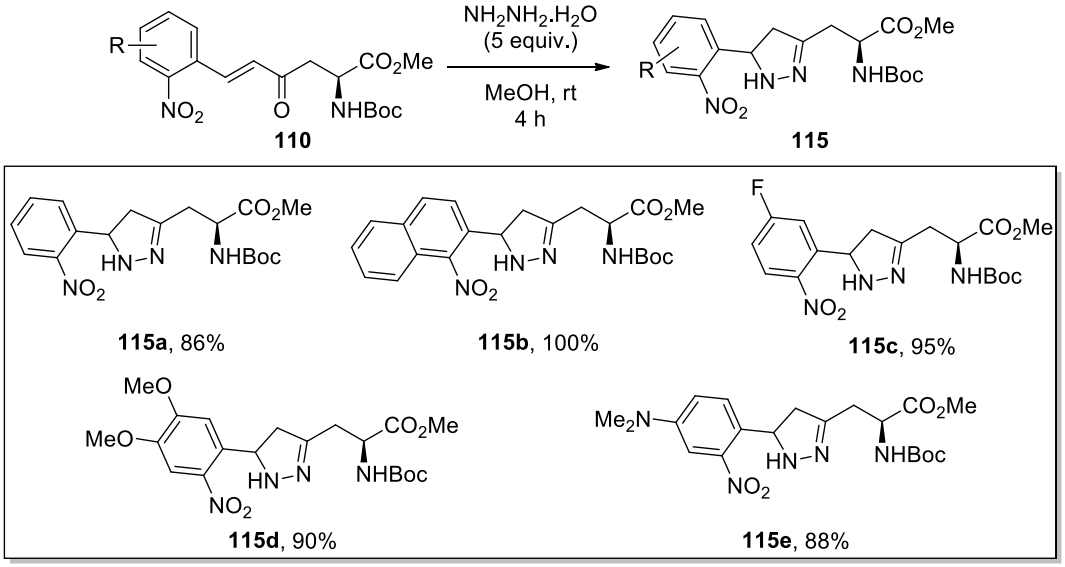


110a 115a

Entry	$N_2H_4.H_2O$ (equiv.)	Temperature	Time (h)	Yield (%)
1	10	reflux	17	0
2	5	reflux	3	0
3	1	rt	16	0
4	2	rt	16	31
5	4	rt	16	60
6	5	rt	16	83
7	5	rt	4	86

Table 4 Optimisation study for the formation of dihydropyrazole **115a**.

Dihydropyrazoles **115a**–**115e** were prepared from enone derived amino acids **110a**–**110e** using these optimised conditions. In all cases, full conversion to the cyclised products was achieved within 4 h. It was found by ^{1}H NMR spectroscopy that all the cyclised products were obtained in a 1:1 mixture of diastereomers.

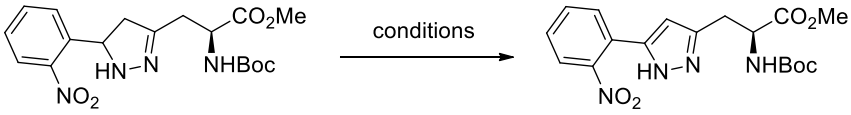


110 115

Scheme 24 Synthesis of dihydropyrazole derived amino acids **115a**–**115e.**

The oxidation to pyrazole derived amino acid **116a** from **115a** was next investigated. There are many oxidising reagents available for this transformation. It was reported by Huang *et al*. that 1,3,5-triaryl-4-alkylpyrazoles can be prepared from dihydropyrazole precursors with manganese dioxide at room temperature.[86] When these conditions were attempted with dihydropyrazole

115a, only a 4% yield of pyrazole **116a** was obtained (**Table 5**, entry 1). It was also reported that both 10% palladium on carbon and dihydropyran can be used as oxidising agents in pyrazole formation.[87,88] When these oxidants were used with dihydropyrazole **115a,** there was no conversion to pyrazole **116a** (entries 2 and 3). When dihydropyrazole **115a** was reacted with benzoquinone, pyrazole **116a** was isolated in a 28% yield (entry 4). When the oxidation reaction was performed with the stronger oxidising agent DDQ, the yield was reduced to 18% (entry 5). Performing the reaction with DDQ at lower temperature (−15 °C) gave an optimised yield of 65% (entry 6).

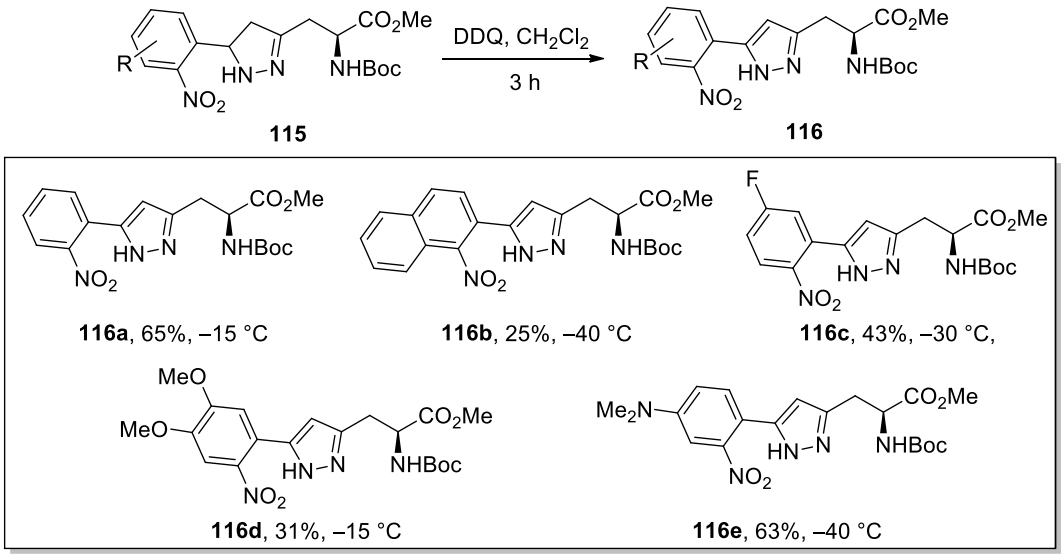


115a **116a**

Entry	Reagent	Temperature (°C)	Time (h)	Yield (%)
1	MnO_2	20	24	4
2	10% Pd/C	20	24	0
3	dihydropyran	60	24	0
4	benzoquinone	20	24	28
5	DDQ	20	2.5	18
6	DDQ	−15	3	65

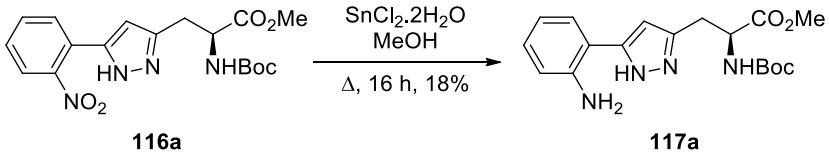
Table 5 Optimisation study for the oxidation of dihydropyrazole **115a** to the pyrazole **116a**.

Pyrazole derived amino acids **116a**–**116e** were then prepared from dihydropyrazoles **115a**–**115e** with the optimised conditions (**Scheme 25**). It was found that higher yields were obtained for pyrazoles **116b**, **116c** and **116e** when the DDQ oxidation was conducted at a lower temperature. The DDQ oxidation for **115a** and **115d** was also attempted at lower temperatures but there was no improvement in yield. With all these compounds **116a**–**116e**, a characteristic singlet was observed by ^{1}H NMR spectroscopy between 6.11–6.41 ppm for the H-4′ hydrogen atom, which confirmed pyrazole ring formation.



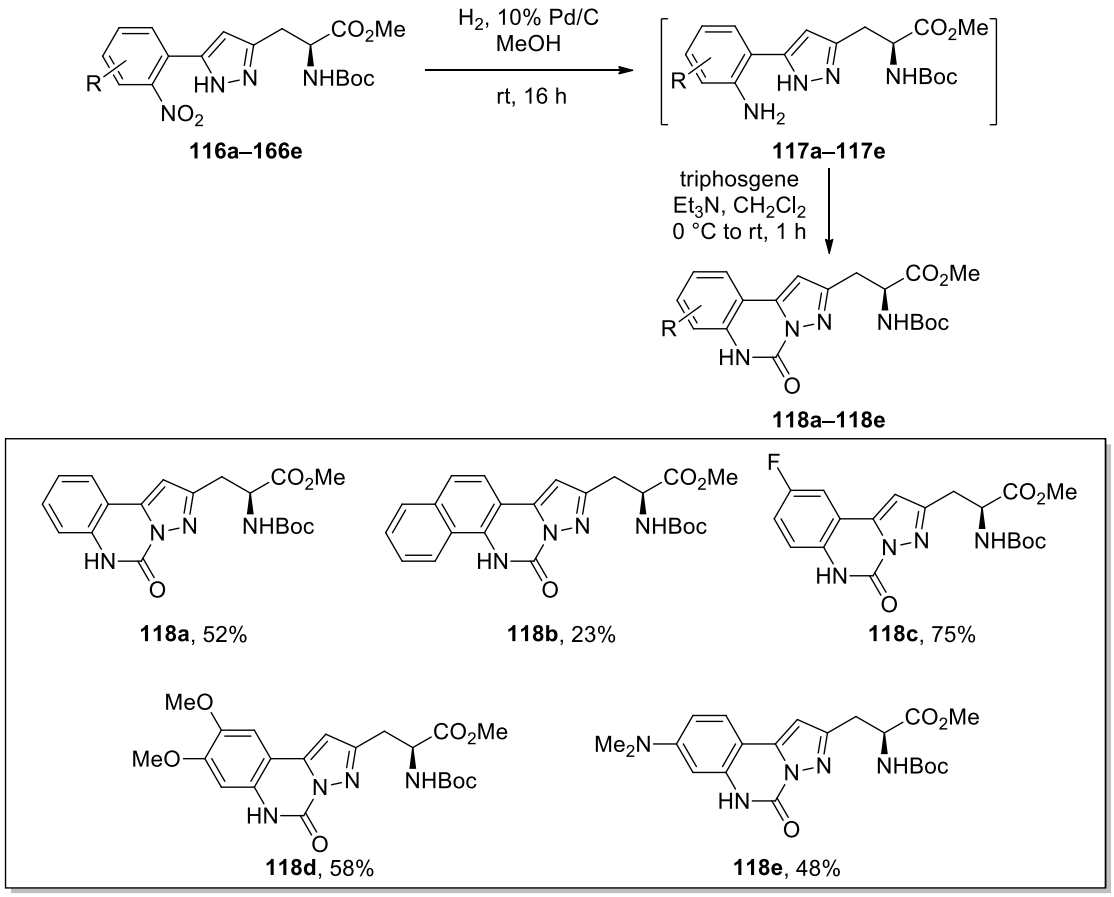
Scheme 25 Pyrazole products **116a**–**116e**.

With pyrazole derived amino acids **116a**–**116e** having been prepared, the reduction of the aromatic nitro group was next investigated. Initially, tin dichloride was used for the reduction of 2-nitrophenyl substituted pyrazole **116a** to amine **117a** (**Scheme 26**). From this, it was found due to the acidity of the reaction conditions, this did not result in a very efficient transformation.



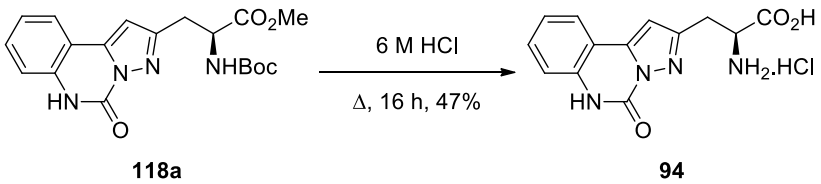
Scheme 26 Synthesis of 2-aminophenyl substituted pyrazole **117a** with tin dichloride.

Nitro group reduction was then attempted by hydrogenation with 10% palladium on carbon (**Scheme 27**). When 2-nitrophenyl substituted pyrazole amino acids **116a**–**116e** were subjected to hydrogenation conditions, full conversion to amines **117a**–**117e** were observed after 16 h. This was confirmed by ^{1}H NMR spectroscopy with the aromatic hydrogen atoms appearing upfield in the spectra of the products. As the transformations yielded no byproducts, simple removal of the palladium catalyst by filtration through Celite® allowed the material to be used in the next step without further purification. The reaction of amines **117a**–**117e** with triphosgene and triethylamine in dichloromethane gave **118a**–**118e** in good yields (48%–75%) over the two-steps, with only **118b** being prepared in a low yield (23%), even after multiple attempts. Formation of pyrazoloquinazoline derived amino acids **118a**–**118e** was confirmed by ^{1}H NMR spectroscopy with a characteristic NH signal at 10.40–11.30 ppm from the urea moiety. Due to the success of these conditions for the preparation of pyrazoloquinazolines **118a**–**118e**, the use of carbonyl diimidazole or disuccinimidyl carbonate were not investigated.



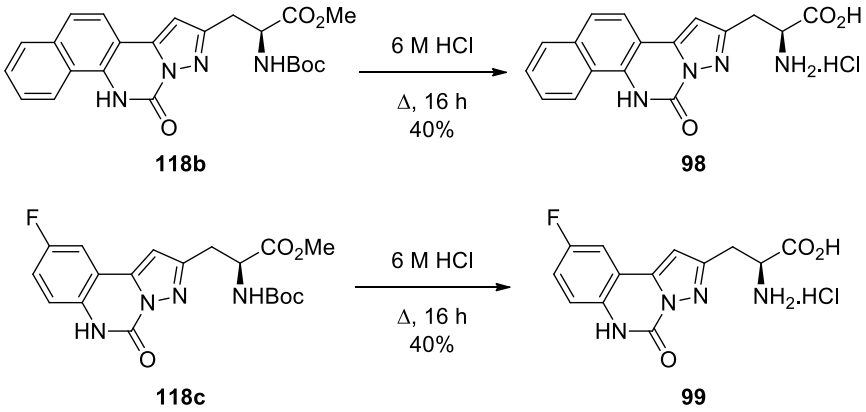
Scheme 27 Synthesis of pyrazoloquinazoline amino acids **118a**–**118e**.

Before measurement of the photoluminescence properties of these pyrazoloquinazoline derived amino acids **118a**–**118e**, the protecting groups were removed. To achieve this, pyrazoloquinazoline **118a** was subjected to 6 M hydrochloric acid under reflux conditions (**Scheme 28**). This removed both the Boc protecting group and methyl ester after 16 h and gave **94** in 47% yield after recrystallisation.



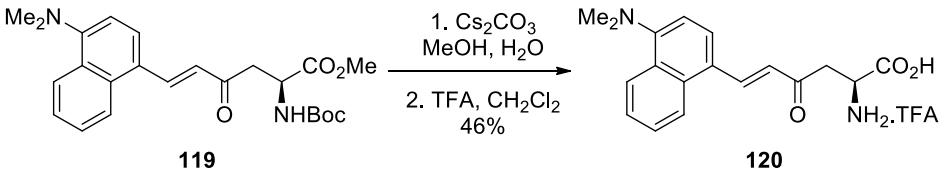
Scheme 28 Acid deprotection of amino acid **94**.

The same deprotection conditions were also used with benzo-fused pyrazoloquinazoline **118b** and 9'-fluoropyrazoloquinazoline **118c** compounds (**Scheme 29**). After 16 h, amino acids **98** and **99** were isolated after recrystallisation in 40% yield.



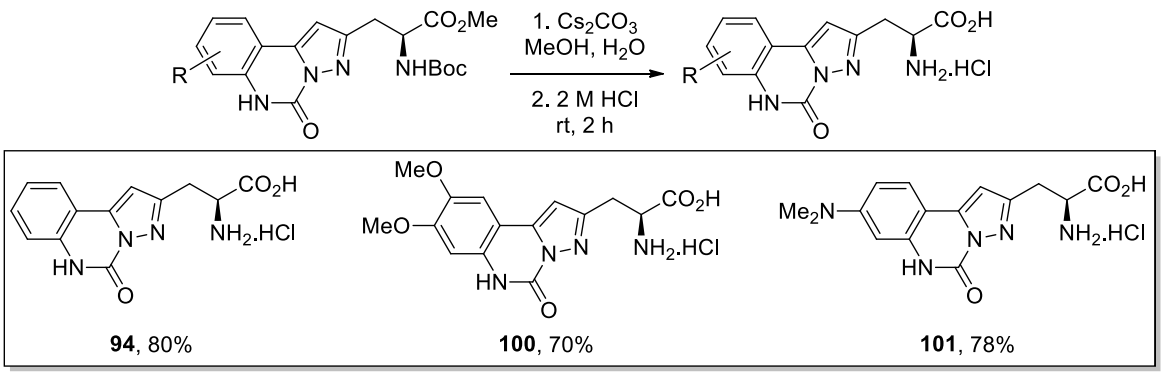
Scheme 29 Preparation of amino acids **98** and **99**.

The one-step acid deprotection was then attempted with dimethoxy and dimethylamino substituted pyrazoloquinazoline derived amino acids **118d** and **118e**. For these amino acids, this resulted in decomposition of the material. It was proposed that the electron-rich nature of these amino acids made them more susceptible to decomposition under harsh acidic reaction conditions. Hence, a milder deprotection strategy was implemented for the preparation of amino acids **100** and **101**. It had previously been shown within the group that ester hydrolysis can be accomplished with the mild base caesium carbonate.[74] In preparing enone derived amino acid **120**, caesium carbonate was used to hydrolysis the ester group of **119**. With the ester group hydrolysed, TFA was used for deprotection of the Boc group and this gave **120** in 46% yield over the two-steps (**Scheme 30**). Therefore, it was envisioned that similar conditions could be used for deprotection of amino acids **118d** and **118e**.



Scheme 30 Two-step deprotection strategy of enone derived amino acid **119**.[74]

Pyrazoloquinazoline derived amino acids **118d** and **118e** were both subjected to this two-step deprotection method. It was found that caesium carbonate gave the carboxylic acids from the methyl esters cleanly in both cases within 16 h (**Scheme 31**). Boc group deprotection was achieved for both analogues with 2 M hydrochloric acid at room temperature and this gave **100** and **101** in 70% and 78% yield, respectively. Owing to the high yields obtained with this approach, amino acid **118a** was also deprotected with these conditions and this resulted in an increase in yield from 47% to 80% for **94**.



Scheme 31 Two-step deprotection of pyrazoloquinazoline amino acids **94**, **100** and **101**.

2.1.4 Photoluminescence of pyrazoloquinazoline derived amino acids

With a library of pyrazoloquinazoline amino acids **94**, **98**–**101** synthesised, the photoluminescence properties of these compounds were studied. Initially, UV/Vis absorption and fluorescence spectroscopy were used to record the absorbance and emission spectra of all amino acids in methanol (5 µM). When pyrazoloquinazoline derived amino acid **94** was studied by absorption spectroscopy, a structured spectrum was obtained with multiple absorption maxima at 225, 257, 272, 307 and 321 nm (**Figure 19**). As mentioned previously, fluorophores that are planar in the ground state generally give rise to more structured absorption spectra, due to vibronic transitions.[35,36]

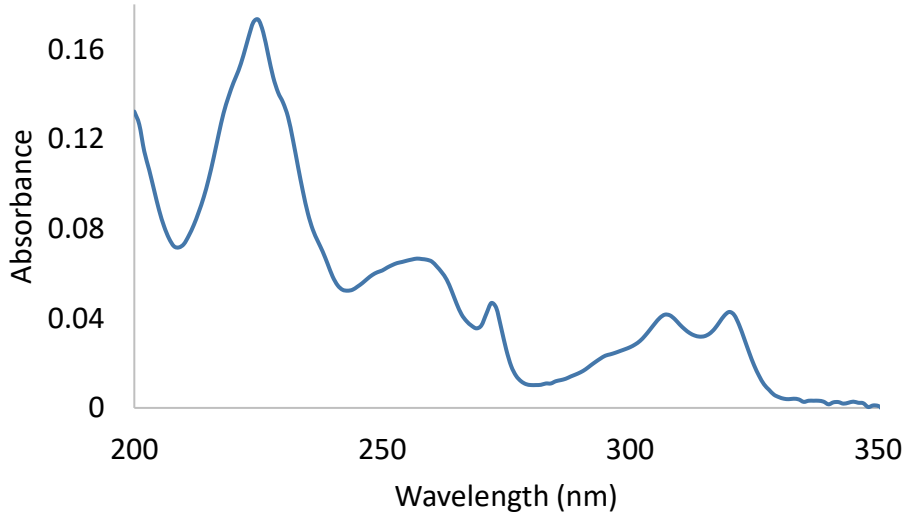


Figure 19 UV-Vis absorption spectra of pyrazoloquinazoline amino acid **94** in MeOH (5 µM).

The molar attenuation coefficient of these maxima were calculated. This was accomplished by measuring the absorption of **94** at different concentrations and using the Beer-Lambert Law (**Figure 20**). Five concentrations were used in order to determine an accurate value, with the

gradient of a concentration and absorption plot being the molar attenuation coefficient. For **94**, the absorbance maximum at 321 nm had a molar attenuation coefficient of 8,253 $cm^{-1}\,M^{-1}$.

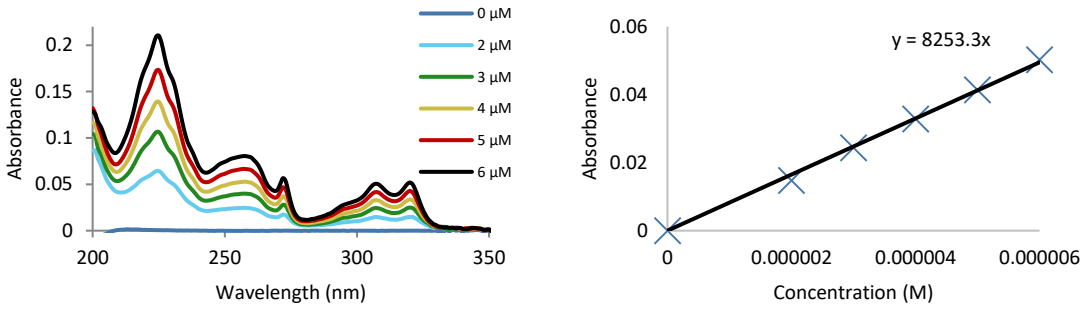


Figure 20 Calculation of the molar attenuation coefficient of amino acid **94** in MeOH.

This process was repeated for all the other maxima (**Table 6**). As all the molar attenuation values are very large, it can be concluded that all the absorption maxima account for $\pi \rightarrow \pi^*$ electronic transitions. The absorption spectra obtained for pyrazoloquinazoline amino acid **94** closely resembles the absorption spectra of 9,10-anthraquinone. The absorption spectra of 9,10-anthraquinone has absorption maxima at 253 nm (51,500 $cm^{-1}\,M^{-1}$), 263 nm (20,000 $cm^{-1}\,M^{-1}$), 272 nm (20,000 $cm^{-1}\,M^{-1}$) and 325 nm (5,600 $cm^{-1}\,M^{-1}$) and it has been reported that all these bands are due to $\pi \rightarrow \pi^*$ electronic transitions.[89–91] For 9,10-anthraquinone, a further weak absorption band is observed at 405 nm (90 $cm^{-1}\,M^{-1}$) and this due to an $n \rightarrow \pi^*$ transition. No such transition was observed for pyrazoloquinazoline derived amino acid **94**.

Wavelength (nm)	Molar Attenuation Coefficient ($cm^{-1}\,M^{-1}$)
225	34,810
257	13,279
272	9,337
307	8,280
321	8,253

Table 6 Molar attenuation coefficients for amino acid **94** in MeOH.

To understand how these absorption bands affect the emission of amino acid **94**, a 5 µM sample in MeOH was excited at these wavelengths and the emission spectra were recorded (**Figure 21**). With all these excitation wavelengths, the same emission spectrum was obtained. Therefore, as it can be concluded that emission is occurring from the lowest excited level, amino acid **94** obeys Kasha's rule.[10] However, it was found that emission spectra varied in intensity depending upon the excitation wavelength. It was found that exciting amino acid **94** at 321 nm gave the most

intense fluorescence. While this absorbance maximum has the longest wavelength, it is also the lowest in energy. Therefore, this electronic transition at 321 nm must be the S_0 to S_1 transition.[8,10] It can be concluded that the transition at 307 nm while also being excited to the S_1 state, is to the higher vibrational level. The absorption maximum at 272 nm is the electronic transition to the second excited state, S_0 to S_2. With all these different excitation wavelengths giving different fluorescence emission intensity, it can be concluded that amino acid **94** does not fulfil Vavilov's rule, as the quantum yield value does vary with excitation wavelength.[10] Furthermore, with the most intense fluorescence being emitted by excitation at 321 nm, it is the S_0 to S_1 electronic transition that is the most efficient at producing fluorescence.

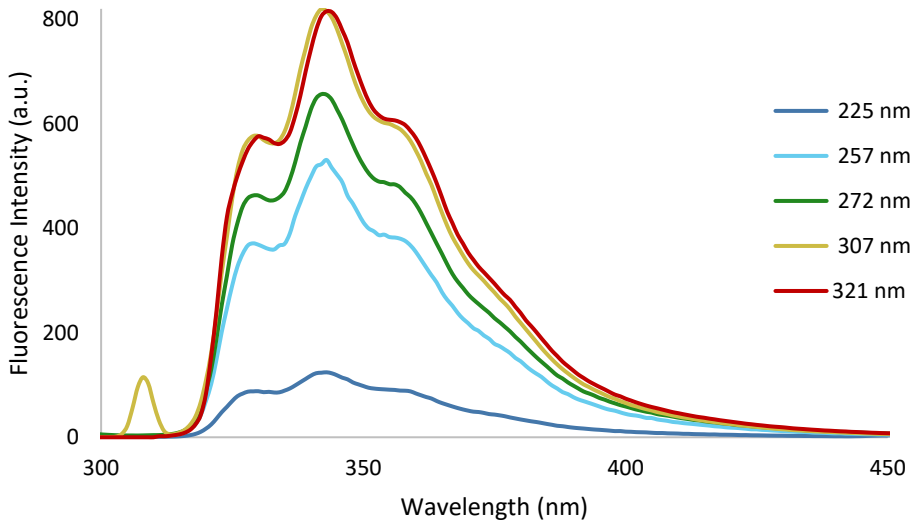


Figure 21 Emission spectra of amino acid **94** in MeOH (5 µM) at different excitation wavelengths.

The absorption and emission spectra of pyrazoloquinazoline derived amino acids **94**, **98**–**101** were recorded in MeOH (5 µM) (**Figure 22**, **Figure 23**, **Figure 24** and **Figure 25**). These amino acids were excited at the wavelength that gave the most intense fluorescence. All amino acids obeyed the "mirror image" rule with respect to their S_1 absorption band and their emission band. For amino acids **94**, **98**–**101**, vibronic fine structure was observed in both the absorption and emission spectra owing to the rigidity and planarity of these compounds. However, for the dimethylamino substituted pyrazoloquinazoline amino acid **101,** no vibronic fine structure was observed.

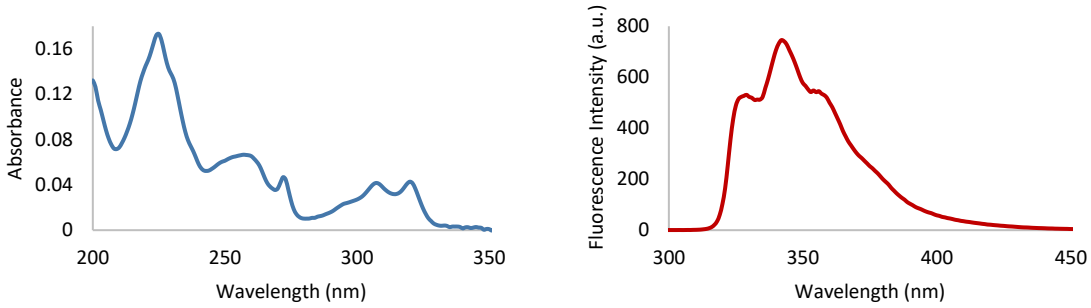


Figure 22 Absorption and emission spectra of amino acid **94** in MeOH (5 µM), excited at 321 nm.

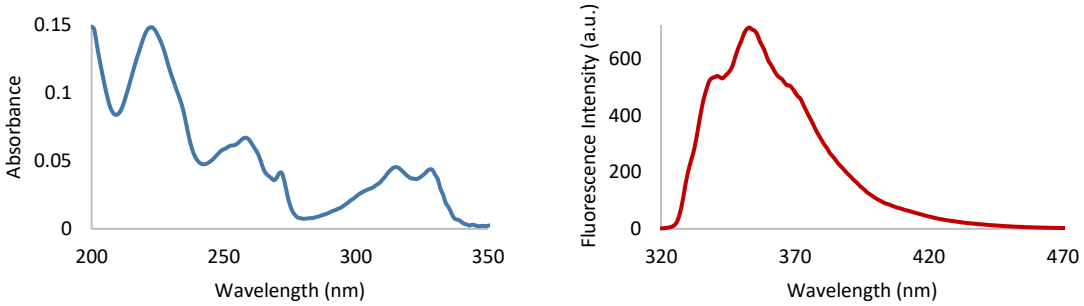


Figure 23 Absorption and emission spectra of amino acid **99** in MeOH (5 µM), excited at 328 nm.

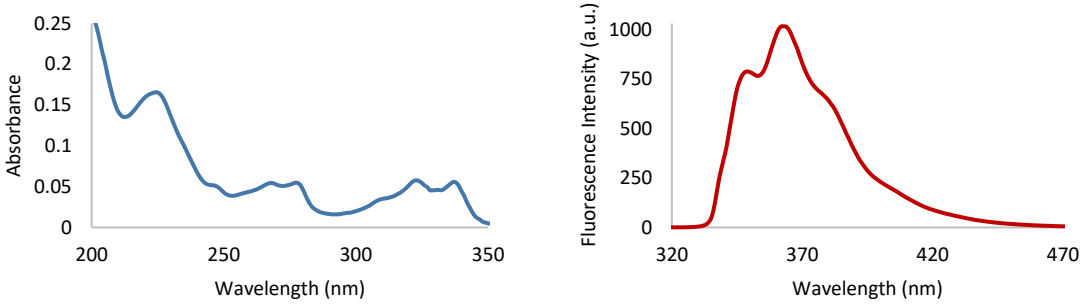


Figure 24 Absorption and emission spectra of amino acid **100** in MeOH (5 µM), excited at 337 nm.

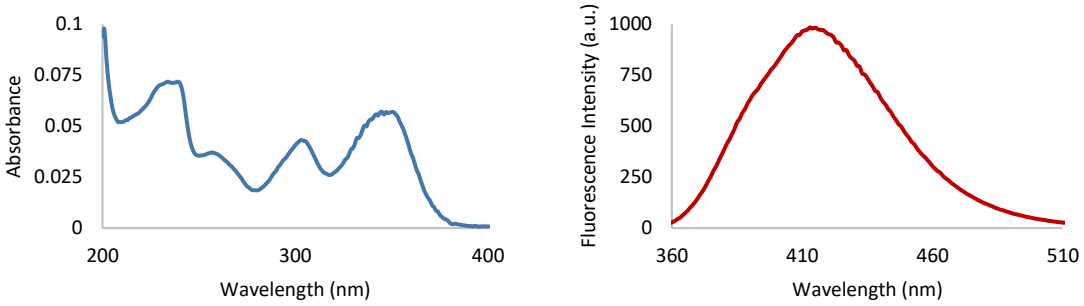


Figure 25 Absorption and emission spectra of amino acid **101** in MeOH (5 µM), excited at 349 nm.

When benzo-fused pyrazoloquinazoline amino acid **98** was studied with UV-Vis spectrometry, it also gave an absorption spectrum with multiple maxima (**Figure 26**). It had absorption maxima at 228 nm, 269 nm, 279 nm, 334 nm and 350 nm when dissolved in MeOH. When amino acid **98** was then excited with these wavelengths, the resulting emission spectra were recorded. As with **94**, **99**–**101**, the same emission spectrum was produced regardless of excitation wavelength. Unlike the other amino acids, excitation of **98** to the S_2 electronic level with light of 279 nm, gave the most intense fluorescence. When **98** was excited to the S_1 level with a wavelength 350 nm there was a reduction in the fluorescence intensity.

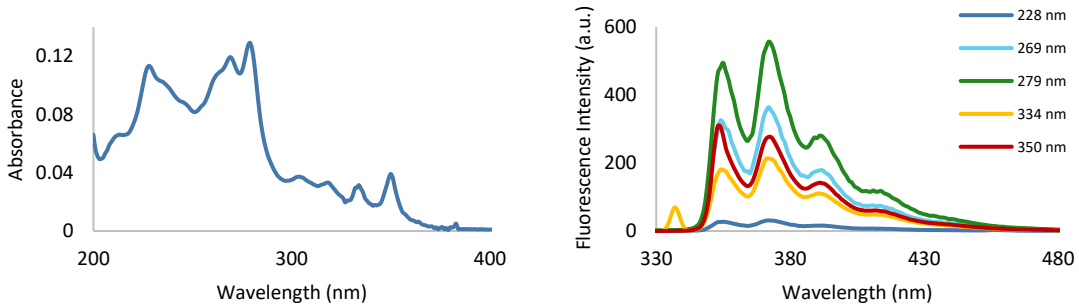


Figure 26 Absorption and emission spectra of amino acid **98** in MeOH (5 µM).

Several trends of the normalised emission spectra of the pyrazoloquinazoline derived amino acids **94**, **98**–**101** were noted (**Figure 27**). The inclusion of electron-donating groups or increased conjugation resulted in a bathochromic shift of the emission spectrum. For example, **94** has an emission maximum of 342 nm. This is increased to 372 nm as conjugation is increased, with the benzo-fused pyrazoloquinazoline amino acid **98**. When electron-donating groups are added, the emission maximum is also increased. Addition of two methoxy groups increased the emission maximum to 363 nm and the addition of a dimethylamino group increased the emission maximum to 414 nm. The emission maximum is also increased when the electron-withdrawing fluorine atom was substituted into the pyrazoloquinazoline ring system and this gave an emission maximum of 353 nm.

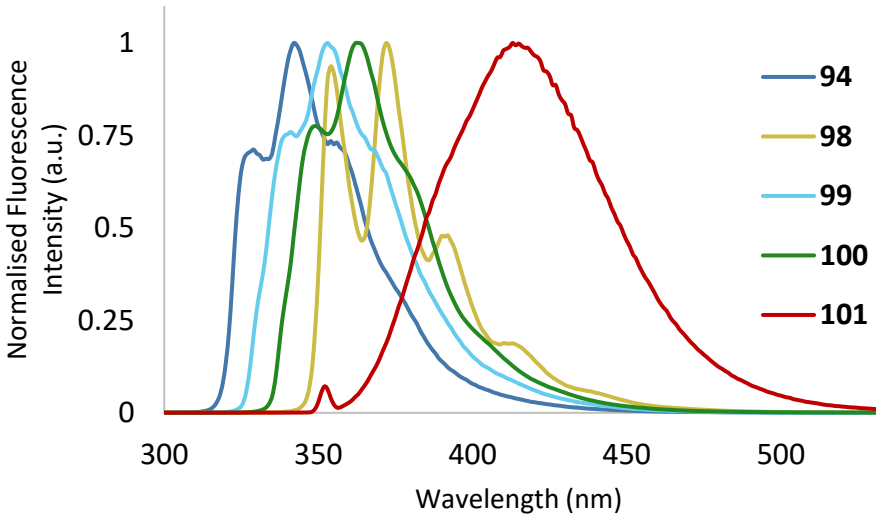


Figure 27 Emission spectra of pyrazoloquinazoline amino acids **94**, **98**–**101** in MeOH (5 μM).

The photoluminescence properties of these amino acids were furthered quantified by calculating the quantum yield values **94**, **98**–**101**. This was done using the comparative method with two known standards, L-tryptophan and anthracene.[92] The absorption and emission spectra of the amino acids and the two standards were measured at five different concentrations (**Figure 28**).

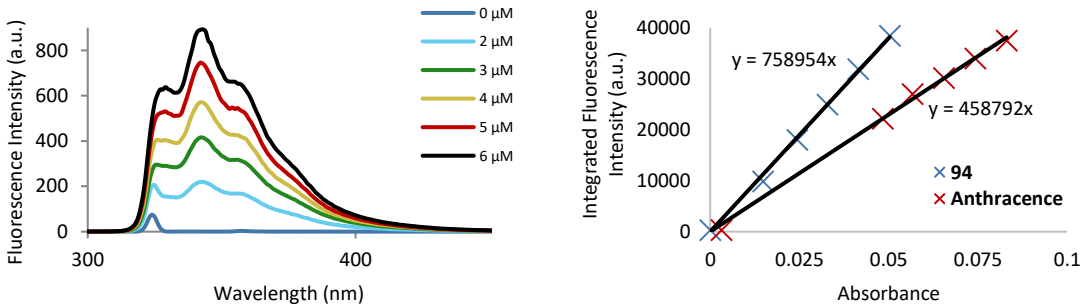


Figure 28 Calculation of the quantum yield value of pyrazoloquinazoline amino acid **94** in MeOH at 321 nm, relative to the known standard anthracene.

From a plot of integrated fluorescence intensity against the absorption, a gradient value was calculated, and used in **Equation 1**. With the use of a known standard, a quantum yield value for a test compound can be found.

$$\Phi_x = \Phi_{ST}\left(\frac{Grad_x}{Grad_{ST}}\right)\left(\frac{\eta_x^2}{\eta_{ST}^2}\right)$$

Equation 1 Calculation of quantum yield. Where Φ_X is the quantum yield of the test sample, Φ_{ST} is the quantum yield of the known standard, $Grad_X$ is the gradient from the test compound plot,

$Grad_{ST}$ is the gradient from the known standard plot and η is the refractive index of the solvent used in the fluorescence measurements.

Quantum yield values, molar attenuation coefficients and brightness values were calculated for all pyrazoloquinazoline amino acids **94**, **98**–**101** (**Table 7**). As expected, with these amino acids being highly planar and rigid, high quantum yields and brightness values were obtained.[35,93] The quantum yield value and brightness value was also calculated for pyrazole amino acid **88**, so that these two classes of amino acids could be compared. From this, it was found that pyrazoloquinazoline amino acids were much brighter. Pyrazole amino acid **88** had a quantum yield value of 0.04, whereas amino acid **98** had a quantum yield value of 0.47. Therefore, it can be concluded that rigidity did enhance the photoluminescent properties of amino acid **88** and hence, amino acids **94**, **98**–**101** have more potential for fluorescence imaging.

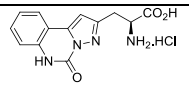
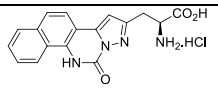
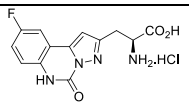
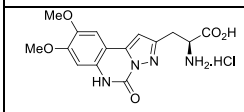
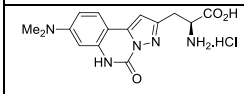
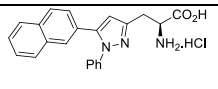
Compound	Excitation Wavelength (nm)	Molar Attenuation Coefficient ($cm^{-1}\,M^{-1}$)	Emission Maximum (nm)	Stokes Shift (nm)	Quantum Yield (ϕ_F)	Brightness ($cm^{-1}\,M^{-1}$)
 **94**	321	8,253	342	21	0.42	3,466
 **98**	279 (350)	24,830 (7,731)	373 (372)	94 (22)	0.27 (0.47)	6,704 (3,634)
 **99**	328	9,977	353	25	0.47	4,689
 **100**	337	11,209	363	26	0.56	6,277
 **101**	349	12,202	415	66	0.48	5,857
 **88**	251	23,650	356	105	0.04	946

Table 7 Photoluminescence properties of amino acids **94**, **98**–**101** and amino acid **88**.

After the photoluminescent properties were measured, it was concluded that the dimethylamino substituted amino acid **101** had the best optical properties. For example, it had a high excitation wavelength, emission maxima and brightness value. Therefore, the photoluminescence properties

of **101** were investigated further. The fluorescence lifetime of amino acid **101** was measured to determine if it would be a suitable fluorophore for FLIM (**Figure 29**).[17,94] It was found that **101** had an amplitude weighted lifetime of 1.81 ns, which is consistent with other similar fluorophores of this type.[95] It has been reported that flavanols, catechin and epicatechin, have been used in two-photon fluorescence lifetime imaging.[17] The flavanol catechin has a fluorescence lifetime of 1.1 ns and a quantum yield of 0.02 in MeOH at 37 °C. Based on these literature examples, it can be concluded that amino acid **101** may be a suitable fluorophore for FLIM microscopy.

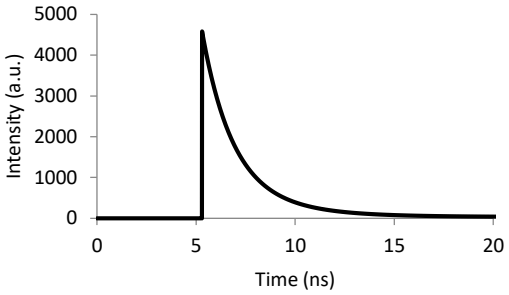


Figure 29 Lifetime measurement of amino acid **101** in MeOH (10 µM).

The photoluminescence characteristics of **101** in different solvents was next investigated. The absorption and emission spectra of **101** were measured in THF, MeCN, DMSO, MeOH and PBS (**Figure 30**). It was found there was only very slight variations in the absorption spectra, whereas for the emission spectra, **101** did display solvatochromic behaviour. A bathochromic shift was observed when amino acid **101** was dissolved in more polar solvents. When dissolved in THF, a emission maximum of 374 nm was obtained. This increased to 379 nm in MeCN, 382 nm in DMSO, 415 nm in MeOH and 424 nm in aqueous PBS solution. This bathochromic shift is due to solvent relaxation around the excited compound. With this variation in emission wavelength, this would allow amino acid **101** to be used as an environment-sensitive fluorescence probe.[72]

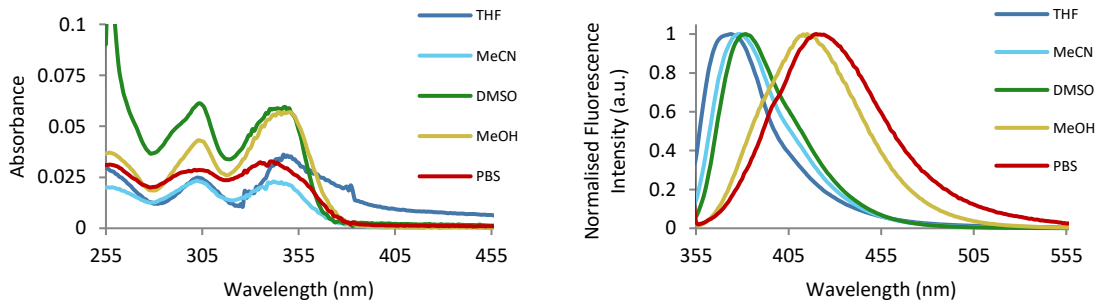


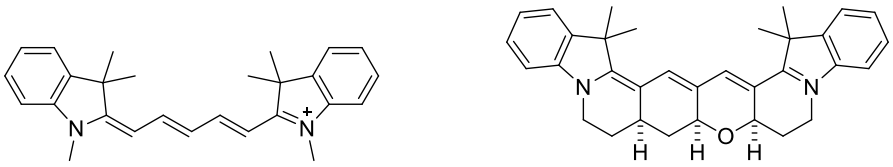
Figure 30 UV-Vis absorption and emission spectra of amino acid **101** in different solvents.

The quantum yield value of **101** was next calculated in the various solvents, except for THF and MeCN. It was proposed when **101** was dissolved in these non-polar solvents aggregation occurred. Therefore, quantum yield measurements in these solvents would not be reliable. This phenomenon has previously been reported with the fluorophores PRODAN and FR0 in hexane.[96] From measuring quantum yield values for **101** in DMSO, MeOH and PBS, it was found that the quantum yield value decreased as solvent polarity increased. In DMSO, a value of 0.92 was recorded. This decreased to 0.48 in MeOH and 0.01 in PBS. This decrease in quantum yield could be due to hydrogen bonding between **101** and the solvents, quenching the fluorescence.[16,30] With this significant difference in quantum yield, this would allow **101** to be used in binding titration experiments. It was shown that fluorescence titration could be performed with a chromophore of similar fluorescent properties.[67] This was incorporated into a peptide and exhibited weak fluorescence in aqueous solutions. Upon addition of peptide to vesicles, this resulted in fluorescence being turned on with interactions with the hydrophobic membrane. It was found that a ratio of one peptide per 100 lipids resulted in maximum fluorescence intensity, thus indicating full saturation of the membrane surface. Therefore, amino acid **101** has the potential to be used in similar applications.

Solvent	**Excitation Wavelength (nm)**	**Emission Maximum (nm)**	**Stokes shift (nm)**	**Molar Attenuation Coefficient ($cm^{-1} M^{-1}$)**	**Quantum Yield (ϕ_F)**	**Brightness ($cm^{-1} M^{-1}$)**
THF	347	374	26	6,820	N/A	N/A
MeCN	342	379	31	4,380	N/A	N/A
DMSO	348	382	40	14,582	0.92	13,415
MeOH	349	415	66	12,202	0.48	5,857
PBS	341	424	76	6,313	0.01	63

Table 8 Summary of the photoluminescence properties of amino acid **101** in various solvents.

With the rigid structure of amino acid **101**, it was next investigated how its emission varied with temperature. It is known that the photoluminescence properties of some fluorophores change with temperature. For example, the quantum yield of pentamethine cyanine **10** varies with temperature, due to photoisomerisation via *trans*-to-*cis* polyene rotation (**Figure 31**).[37] This rotation is more efficient at higher temperatures resulting in greater energy loss. However, the quantum yield of rigid analogue **11** shows no variance with temperature, as *trans*-to-*cis* polyene rotation is no longer possible.



10 11

Figure 31 Chemical structures of pentamethine cyanine **10** and restrained indocyanine **11**.

The emission spectra of amino acid **101** and pyrazole derived amino acid **88** were recorded in MeOH between 10–60 °C (**Figure 32**). From this, it was found that the emission intensity of **101** was consistent throughout the temperature range. However, for **88**, the fluorescence intensity did decrease when the temperature was increased. At higher temperatures, the π conjugation between pyrazole and naphthyl rings was disrupted through increased bond rotation. As the phenyl ring is tethered to the pyrazole ring in **101**, this rotation isn't possible and resulted in the fluorescence intensity being constant over the studied temperature range.

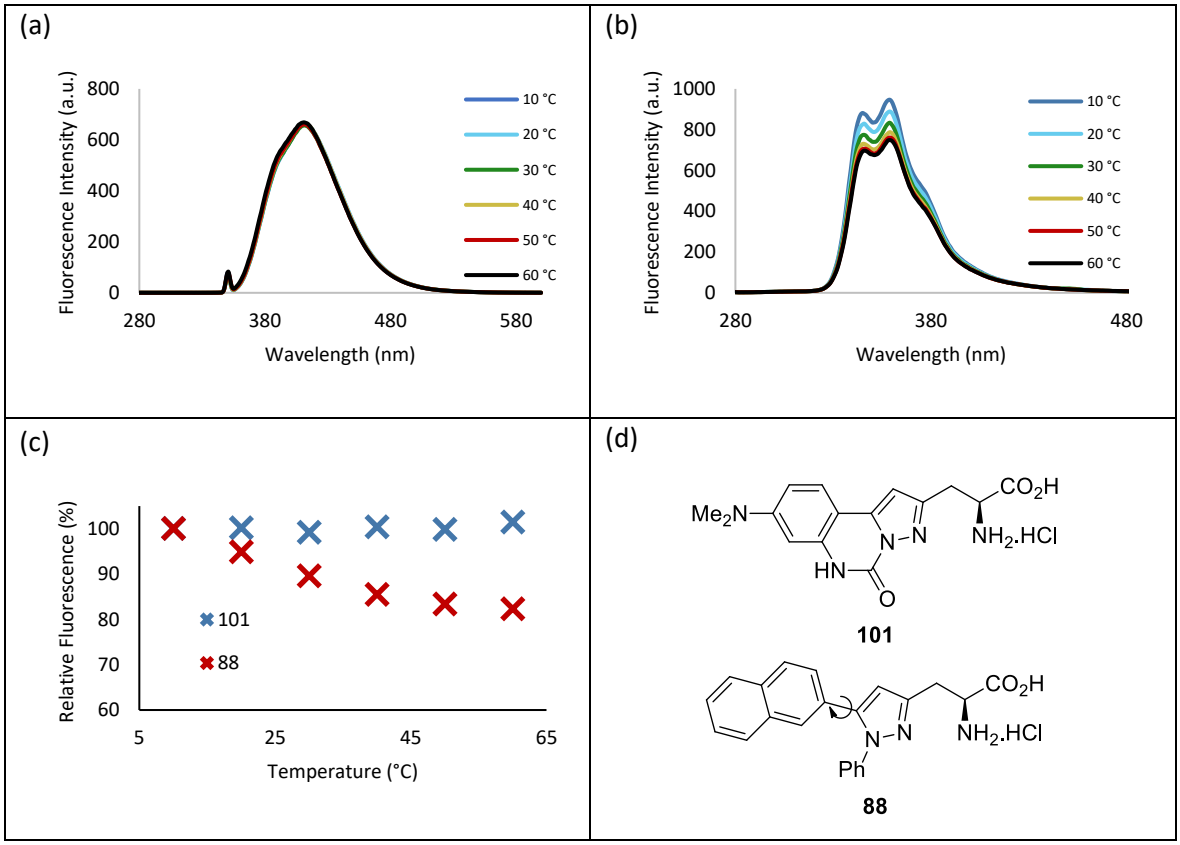


Figure 32 (a) Emission spectra of amino acid **101** in MeOH (5 µM). (b) Emission spectra of pyrazole **88** in MeOH (5 µM). (c) Comparison of emission intensity of **101** and **88**. (d) Structures of **101** and **88**.

It was next investigated whether amino acid **101** could be excited using a two-photon process. For biological imaging this would be highly advantageous as this would allow amino acid **101** to be

excited at 700 nm instead of 350 nm. With excitation at 700 nm, this would result in reduced photobleaching and phototoxicity.[17,18,21] This work was carried out by David Nobis and Eilidh Malcolm of the Steven Magennis research group at the University of Glasgow. Evidence of two-photon excitation was achieved with a log-log plot of laser power and fluorescence intensity. For a two-photon process, a gradient of two is required.[11] A sample of amino acid **101** in MeOH (203 µM) was excited with light at 800 nm (with a full width at half maximum (FWHM) of 135 nm) at four different laser powers: 79, 157, 117 and 228 mW and the emission intensity was recorded. This was plotted with a log-log plot and the resulting gradient was 1.96 ± 0.03. This is indicative of a two-photon process (**Figure 33**).

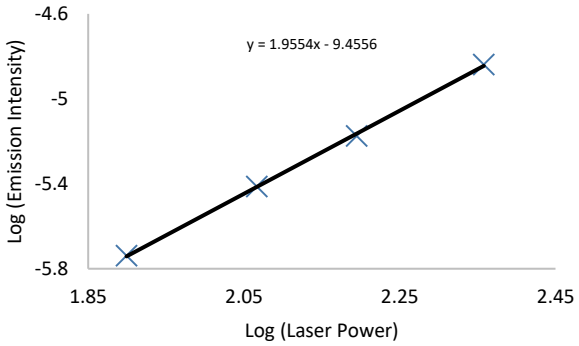


Figure 33 Log-log plot of **101** with light centred at 800 nm with FWHM of 135 nm.

As it was shown that **100** can be excited by a two-photon process, the efficiency of this process was assessed by calculating the cross-sectional area. This was achieved by using **Equation 2** and a known standard.[97]

$$\frac{\sigma_2^S \Phi^S}{\sigma_2^R \Phi^R} = \frac{\eta^R \left(n^S_{\lambda emission}\right)^2 n^R_{\lambda excitation} C^R F^S \langle P^R \rangle^2}{\eta^S \left(n^R_{\lambda emission}\right)^2 n^S_{\lambda excitation} C^S F^R \langle P^S \rangle^2}$$

Equation 2 Calculation for cross-sectional area. Where Φ is the quantum yield of the fluorescence, η accounts for the wavelength dependence of the detection efficiency, n is the refractive index of the solvent, C is the concentration, F is the integrated fluorescence signal and P is the excitation power. The superscripts R and S denote reference and sample respectively. The equation is simplified by performing the measurements with the same solvent and at the same wavelength (700 nm ± 10 nm). This allows for the following $\eta^R = \eta^S$ and $n_{\lambda emission} = n_{\lambda excitation} = n$ simplifications and gives **Equation 3**.

$$\frac{\sigma_2^S \phi^S}{\sigma_2^R \phi^R} = \frac{C^R F^S \langle P^R \rangle^2}{C^S F^R \langle P^S \rangle^2}$$

Equation 3 Simplified calculation for cross-sectional area.

Rhodamine B was used as the standard as it has a molar attenuation coefficient of 106,000 cm^{-1} M^{-1}, a two-photon cross-section area of 240 GM (at 700 nm) and a quantum yield value of 0.70.[98,99] The cross-section of **101** was calculated at three different concentrations and this gave a value of 0.38 ± 0.06 GM. From these measurements, the emission spectrum of **101** is obtained from the two photon-excitation (**Figure 34**). This emission spectrum obtained is very similar to the emission spectrum obtained with a one-photon excitation process.

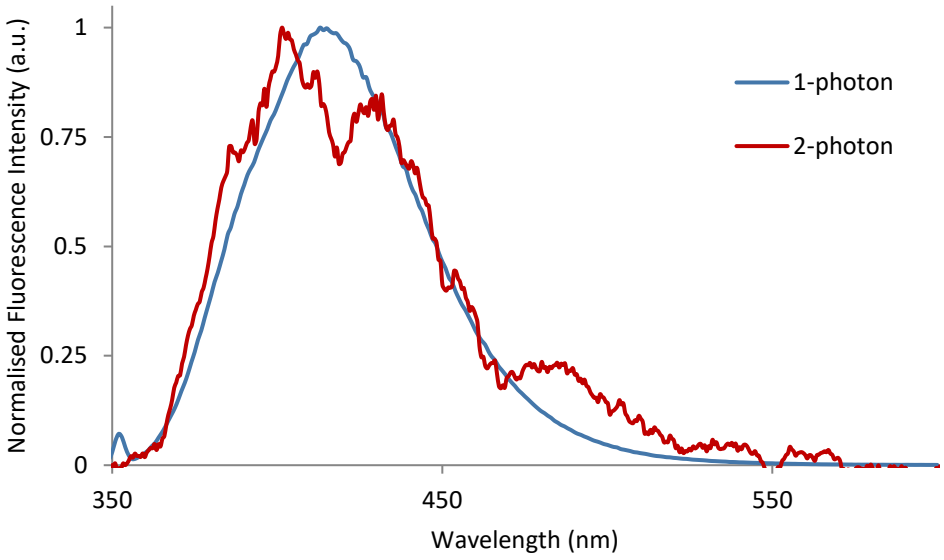


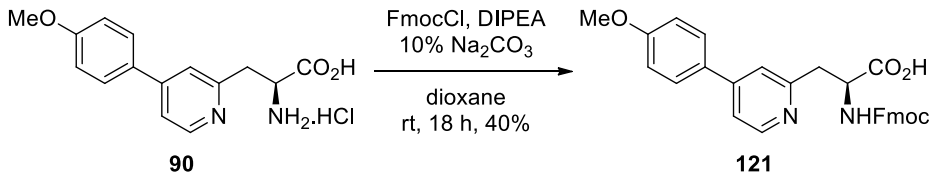
Figure 34 Emission of **101** with 1-photon and 2-photon excitation.

2.1.5 Synthesis of a fluorescent cell-penetrating peptide

The synthetic utility of amino acid **101** for use in SPPS was next investigated by its incorporation into a short peptide. From previous work within the group, it was shown that amino acid **90** could be incorporated into a short peptide chain.[76] The peptide sequence chosen was five residues long and consisted of Val-Pro-Thr-Leu-Lys. It was based upon the Bax-binding domain of the Ku70 protein. Although it consisted of only five amino acid residues, it was shown to have cell-penetrating properties and low cytotoxicity. Therefore, this peptide sequence was also used for amino acid **101** in SPPS.

When amino acid **90** was used in SPPS, it was protected with the Fmoc protecting group (**Scheme 32**).[76] This was done to allow for protecting group removal with piperidine during peptide

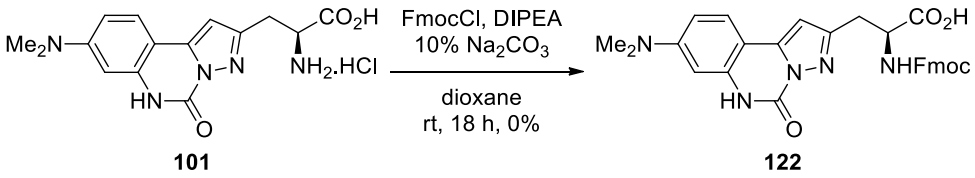
synthesis. The Fmoc protecting group was installed with fluorenylmethyloxycarbonyl chloride under basic conditions to give **121** in a 40% yield.



90 **121**

Scheme 32 Synthesis of Fmoc amino acid **121**.

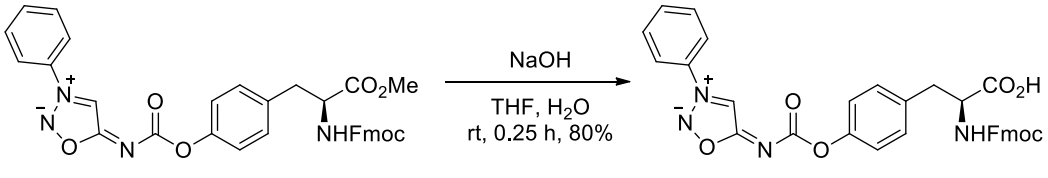
For the synthesis of a peptide containing **101**, the preparation of Fmoc protected amino acid **122** was attempted. It was found that the reaction of **101** with fluorenylmethyloxycarbonyl chloride under basic conditions gave **122** (**Scheme 33**). However, attempted purification with column chromatography with silica gel, eluting with dichloromethane and methanol was not successful. Therefore, this approach to the Fmoc amino acid was abandoned.



101 **122**

Scheme 33 Attempted synthesis of Fmoc amino acid **122**.

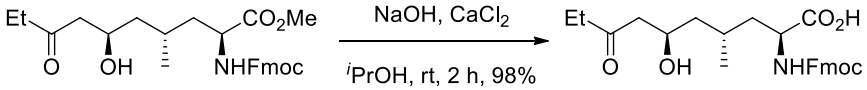
From the literature, there are examples of synthetic amino acids being prepared for peptide synthesis with selective ester hydrolysis of Fmoc esters. For example, in the preparation of novel amino acids capable of NO release, Fmoc sydnonimine amino acid **123** was selectively hydrolysed with sodium hydroxide to give Fmoc acid **124** in a yield of 80% (**Scheme 34**).[100]



123 **124**

Scheme 34 Selective hydrolysis of Fmoc protected sydnonimine amino ester **124**.[100]

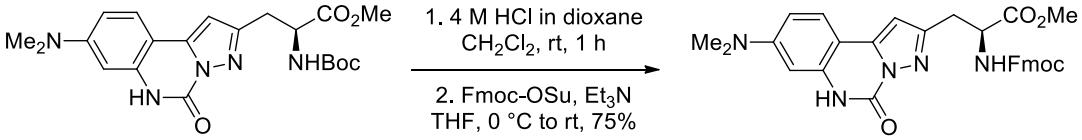
It was also shown that calcium chloride can be used in the selective hydrolysis of methyl esters.[101] For example, a combination of calcium chloride and sodium hydroxide was used to hydrolyse ester **125** to acid **124** without Fmoc deprotection in a 98% yield (**Scheme 35**).



125 **126**

Scheme 35 Selective ester hydrolysis of **124** with sodium hydroxide and calcium chloride.[101]

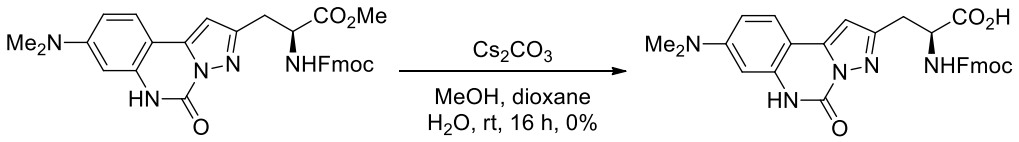
Therefore, it was proposed that selective ester hydrolysis of **127** would give an easier purification of **122**. It was found that **127** could be prepared from **118e** using literature conditions in 75% yield (**Scheme 36**).[100]



118e **127**

Scheme 36 Synthesis of Fmoc ester **127**.

With access to **127**, selective ester hydrolysis and preparation of **122** was next investigated. Based on literature precedent (**Scheme 34** and **35**) for base hydrolysis of Fmoc-protected amino esters and our experience of amino ester hydrolysis Cs_2CO_3 was chosen for this reaction. When this was attempted with caesium carbonate in MeOH and water, this resulted in a heterogeneous reaction mixture (**Scheme 37**). It was found that addition of dioxane gave a homogenous reaction mixture, but the hydrolysis was very slow and there was minimal conversion to **122**. When an extra equivalent of caesium carbonate was added and the reaction mixture was stirred at room temperature for 16 h, this resulted in both ester hydrolysis and Fmoc deprotection.

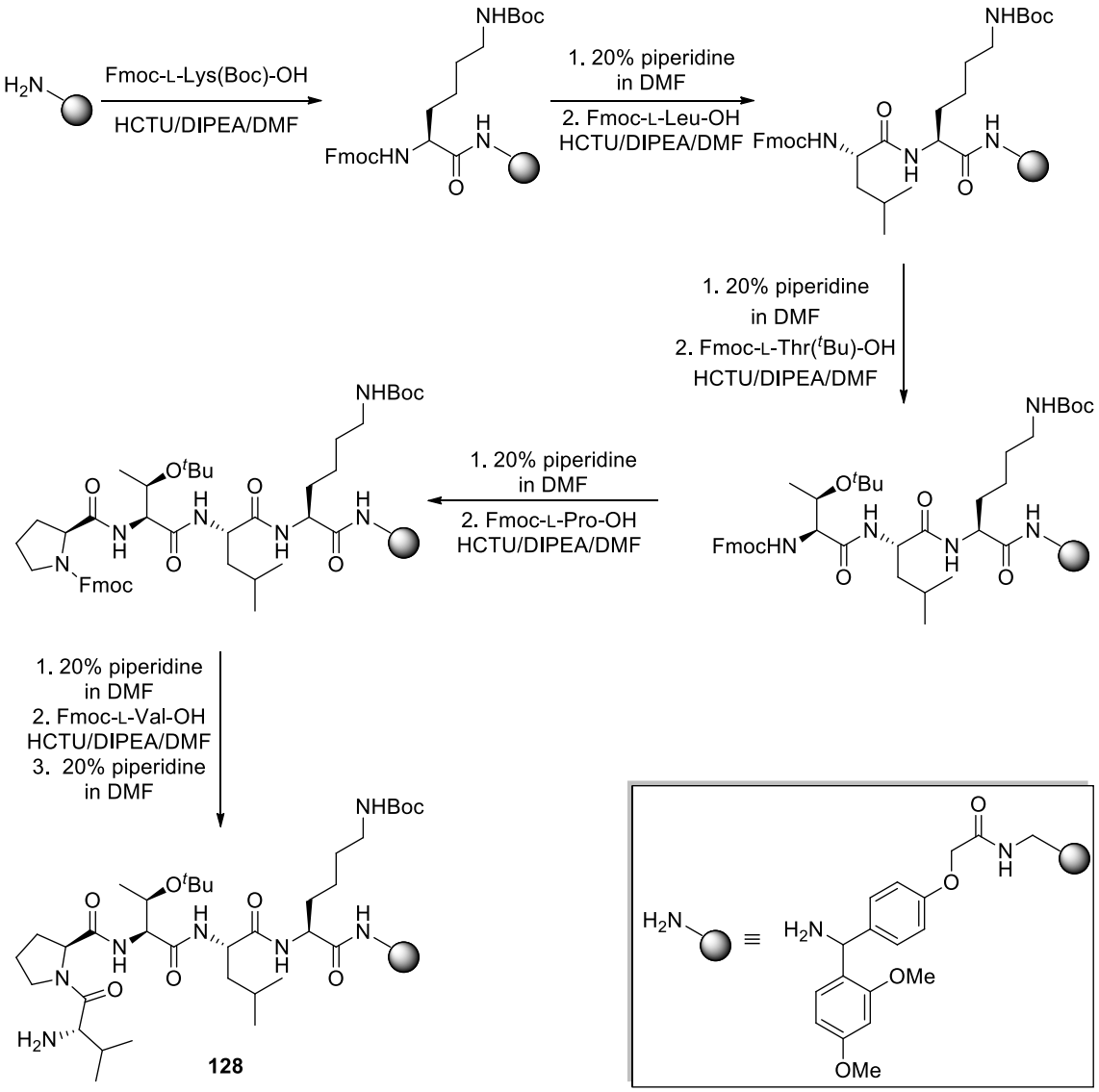


127 **122**

Scheme 37 Attempted synthesis of Fmoc acid **122**.

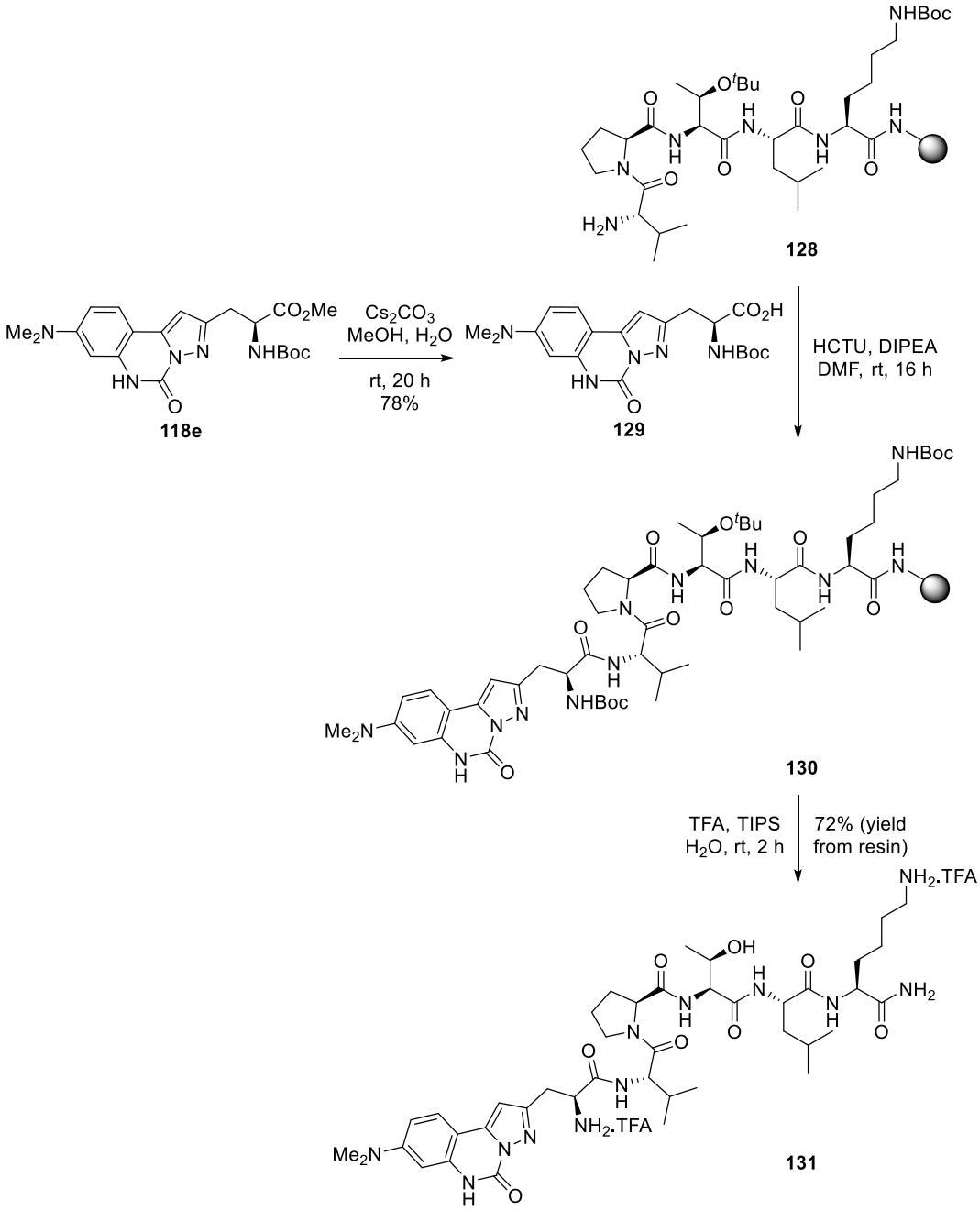
With the difficulties in synthesising **122** and with amino acid being incorporated last in the peptide, it was proposed that amino acid **101** could be incorporated as Boc-derivative **129**. As the cleavage of the peptide chain from the resin is accomplished with trifluoroacetic acid, it was thought that this would also remove the Boc group. This approach was investigated by synthesising pentapeptide **128**. This was achieved by SPPS synthesis with TentaGel™ S RAM resin on a 0.100 mmol scale (**Scheme 38**). Peptide couplings were carried out using a four-fold excess of the amino acid, amide coupling reagent (HCTU) and eight equivalents of *N*,*N*-diisopropylethylamine. Fmoc

deprotection was carried out using 20% piperidine in DMF. The structure and purity of pentapeptide **128** was confirmed with analytical HPLC and LCMS.



Scheme 38 Synthesis of **128** using automated SPPS.

With access to pentapeptide **128**, the use of **101** in peptide synthesis was then explored. Boc acid **129** was prepared by ester hydrolysis of **118e** with caesium carbonate in a yield of 71%. The peptide coupling was carried out manually, using two equivalents of **129** and at room temperature for 16 h (**Scheme 39**). From HPLC analysis, it was shown that the reaction resulted in the formation of only one product and LCMS confirmed the structure of hexapeptide **130**. After cleavage from the resin with trifluoroacetic acid and triisopropylsilane and purification by prep-HPLC, hexapeptide **131** was obtained in 72% yield (based on starting resin).



Scheme 39 Synthesis of fluorescent cell-penetrating hexapeptide **131**.

As **131** had been successfully prepared, its photoluminescence properties were next investigated, to ascertain if the fluorescent characteristics of **101** had altered by incorporation into a peptide. This was achieved by measurement of the absorption and emission spectra of **131** in MeOH (5 µM). It was found that there were small differences in the absorption spectra between amino acid **101** and peptide **131** (**Figure 35**). A hypsochromic shift in the emission spectrum of peptide **131** compared to amino acid **101** was observed. Amino acid **101** had an emission maximum of 414 nm, whereas peptide **131** has an emission maximum of 389 nm. It also had a shoulder peak at approximately 405 nm.

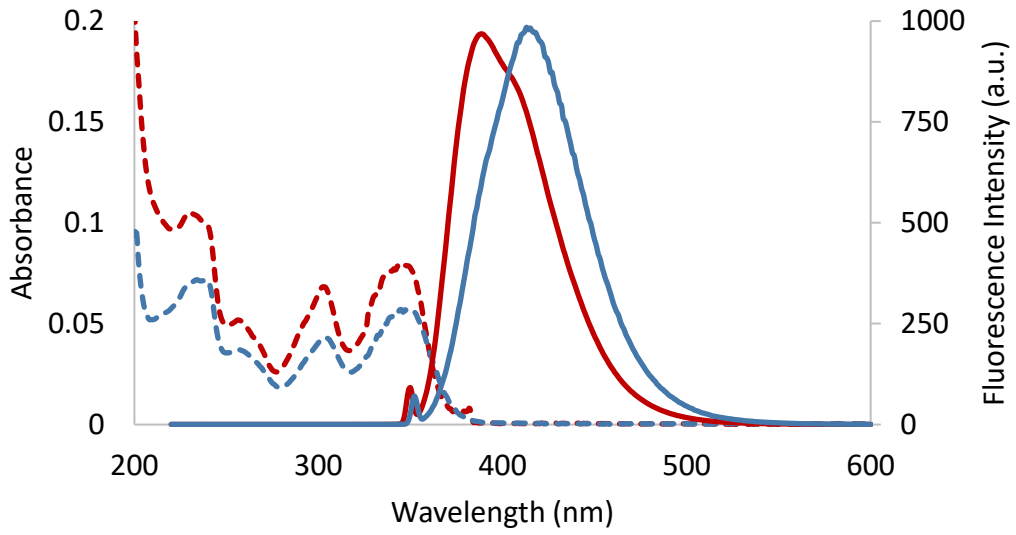


Figure 35 Absorption and emission spectra of amino acid **101** (blue) and peptide **131** (red).

The molar attenuation coefficient and quantum yield were then measured for **131** (**Table 9**). It was found that the brightness value for the fluorophore as part of a peptide increased from 5,857 to 6,900 cm^{-1} M^{-1}. The increase in brightness was solely due to an increase in the molar attenuation coefficient value from 12,202 to 15,333 cm^{-1} M^{-1}. Alongside this increase in brightness, there was also a decrease in the Stokes shift of the fluorophore from 65 nm to 40 nm.

	Excitation Wavelength (nm)	**Molar Attenuation Coefficient (cm^{-1} M^{-1})**	**Emission Maximum (nm)**	**Stokes Shift (nm)**	**Quantum Yield (ϕ_F)**	**Brightness (cm^{-1} M^{-1})**
Amino acid **101**	349	12,202	414	65	0.48	5,857
Peptide **131**	349	15,333	389	40	0.45	6,900

Table 9 Summary of photoluminescence for amino acid **101** and peptide **131**.

The emission spectrum of peptide **131** was also measured in an aqueous solution of PBS (**Figure 36**). This resulted in a bathochromic shift, with the emission maximum increasing from 389 nm in MeOH to 412 nm in PBS solution. So, when incorporated within a peptide, the fluorophore showed greater solvatochromic sensitivity than amino acid **101**.

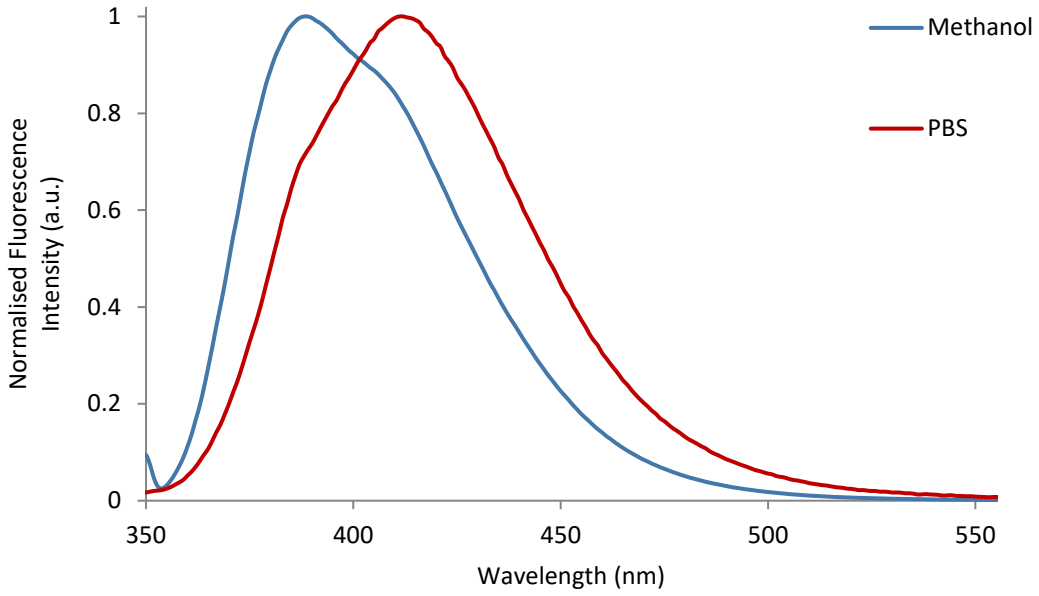


Figure 36 Solvatochromic behaviour of peptide **131**.

2.1.5 Summary

To conclude, a synthetic route to pyrazoloquinazoline functionalised amino acids was developed. This allowed access to five pyrazoloquinazoline derived amino acids **94**, **98**–**101** (**Figure 37**). The photoluminescence characteristics of these five amino acids were analysed by absorption and fluorescence spectroscopy. Their molar attenuation coefficient, quantum yield and brightness values were measured and calculated. It was found that the incorporation of rigidity into the fluorophore significantly improved the fluorescent characteristics of **94**, **98**–**101** compared to pyrazole **88**. For example, all pyrazoloquinazoline amino acids **94**, **98**–**101** had quantum yield values between 0.42 to 0.56, whereas pyrazole amino acid **88** had a quantum yield of 0.04. The improved photoluminescence properties are due to the restricted rotation of the fluorophore in the excited state as this reduces non-radiative decay processes, thus giving a more fluorescent compound.[102] This restricted rotation was further investigated with a temperature study where the fluorescence of **88** decreased with increasing temperature, due to faster bond rotation. Whereas for **101,** no decrease in fluorescence was observed due to restricted rotation. From this library of pyrazoloquinazoline derived amino acids, the dimethylamino substituted amino acid **101** showed the best optical properties. In particular, the highest emission wavelength and the largest Stokes shift.

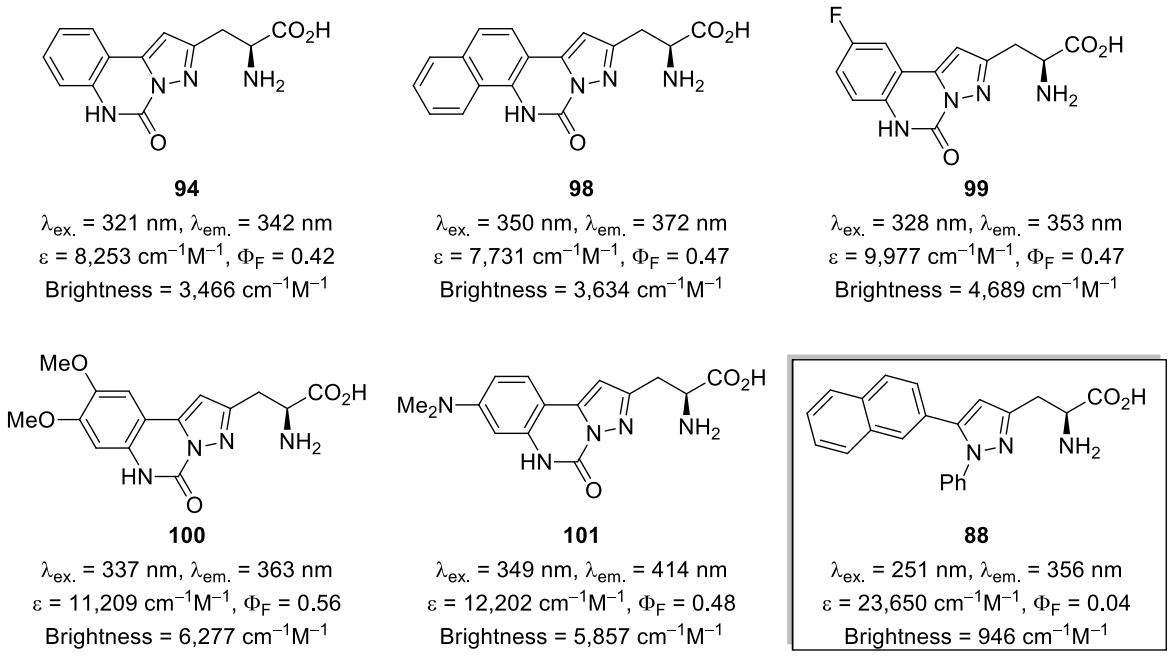


Figure 37 Structures of synthesised amino acids and their key fluorescent properties.

As amino acid **101** had the best photophysical properties, it was chosen for further study. It exhibited solvatochromic properties, with potential application as an environmentally sensitive chromophore. It was found that **101** could be excited by two-photon spectroscopy and it had a two-photon cross-section area of 0.38 ± 0.06 GM. It was then shown that **101** could be used in SPPS, with the preparation of a fluorescent cell-penetrating hexapeptide **131** in 72% yield (**Figure 38**). The fluorescence properties of peptide **131** showed a small hypsochromic shift compared to amino acid **101**.

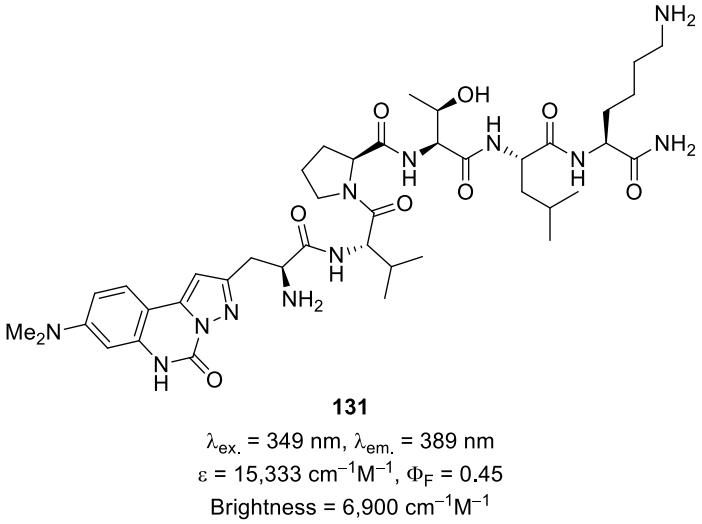
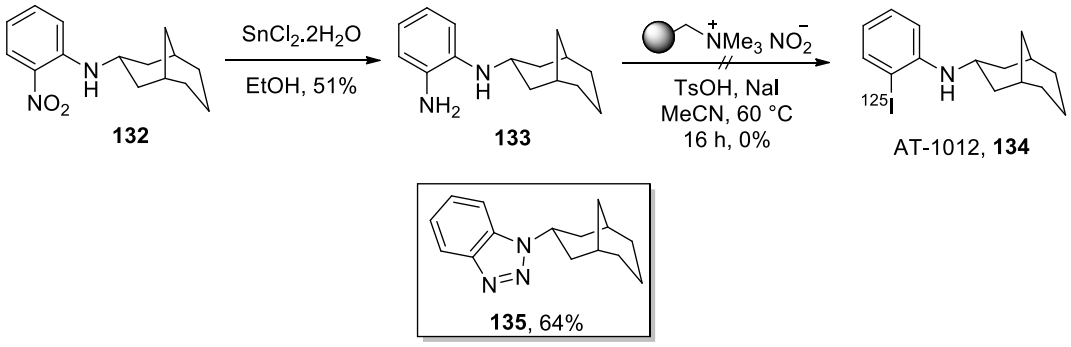


Figure 38 Fluorescent cell-penetrating hexapeptide **131** and its photoluminescence properties.

2.2 Synthesis of Novel Fluorescent Benzotriazole Functionalised α-Amino Acids

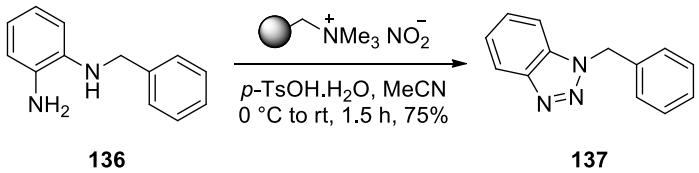
2.2.1 Introduction

The compound AT-1012 (**134**) is an antagonist for the α3β4 nicotinic acetylcholine receptor (nAChR). Preparation of a radio-labelled analogue of this compound was previously attempted from *ortho*-nitroaniline **132** within the group.[103] A tin dichloride reduction of the aromatic nitro group gave diaminophenyl **133** (**Scheme 40**). The radioactive iodine-125 atom was to be installed via the diazonium salt using a resin supported nitrite reagent.[104] After 16 h at 60 °C, full consumption of diaminophenyl starting material was achieved, but there was no radio-labelled product **134**, instead, a benzotriazole product was isolated in 64% yield (see box). The formation of benzotriazoles from *ortho*-diaminobenzenes upon treatment with sodium nitrite, under acidic conditions has been widely reported in the literature.[105–109]



Scheme 40 Attempted synthesis of AT-1012.

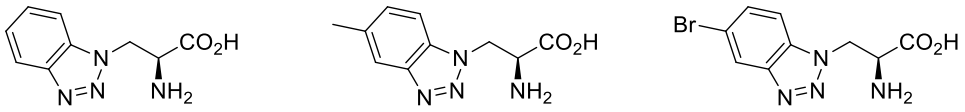
Further work within the group utilised the nitrite resin-bound reagent for the mild synthesis of benzotriazoles.[110] For example, *N*-benzyl benzotriazole **137** could be prepared from *ortho*-diaminobenzene **136** under mildly acidic conditions at ambient temperature with no release of toxic nitrogen oxides (**Scheme 41**).[105]



Scheme 41 Formation of *N*-benzyl benzotriazole **137** with the resin-supported nitrite reagent.

2.2.2 Aim of the project

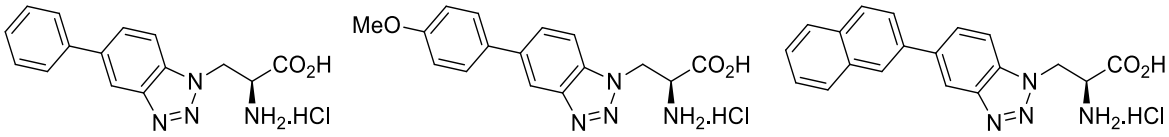
Considering this elegant heterocycle synthesis, it was proposed that this methodology could be used to prepare benzotriazole substituted amino acids. There are three aims to this project. Firstly, this methodology would be used for the preparation of simple benzotriazole-derived amino acids **138**–**140** (**Figure 39**).



138 **139** **140**

Figure 39 Simple benzotriazole target compounds.

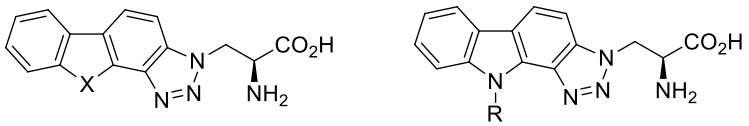
After the development of this approach, the use of cross-coupling chemistry would be used to prepare conjugated products **141**–**143** (**Figure 40**). The fluorescent properties of these conjugated amino acids **141**–**143** should be much superior to original amino acids **138**–**140**, due to the extended π system.[111]



141 **142** **143**

Figure 40 Conjugated benzotriazole compounds.

Finally, the benzotriazole core would be rigidified to improve the photophysical properties of the amino acids (**Figure 41**). This could be achieved by tethering the phenyl ring with the benzotriazole heterocycle to give **144**. A nitrogen atom could be used to tether the two rings together to give a carbazole triazole fused ring system **145**. It is expected that this increase in rigidity would improve properties such as quantum yield.

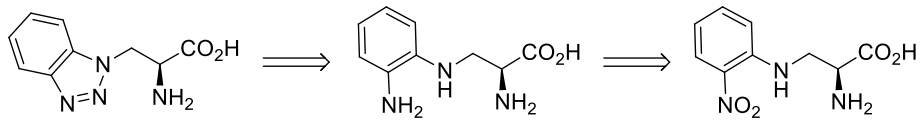


144 **145**

Figure 41 Rigid benzotriazoles.

2.2.3 Synthesis of benzotriazole-derived amino acids

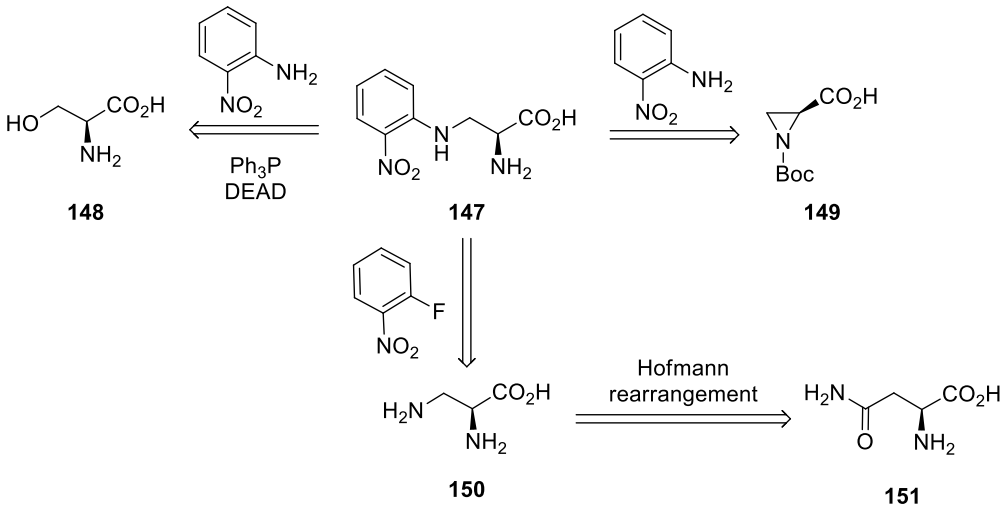
From retrosynthetic analysis, it was proposed that benzotriazole-derived amino acid **138** could be prepared from 2-nitroaniline amino acid **147** (**Scheme 42**). After nitro group reduction to amine **146**, the previously developed conditions for benzotriazole ring formation could be applied to give **138**.[110]



138 **146** **147**

Scheme 42 Retrosynthetic analysis of benzotriazole amino acid **138**.

In preparing amino acid **147**, three approaches were considered (**Scheme 43**). The first of these involved a Mitsunobu reaction with L-serine **148** and 2-nitroaniline. It has previously been shown that L-serine can be used in Mitsunobu reactions without racemisation and that anilines can be used as nucleophiles for Mitsunobu reactions.[112,113] Typically nucleophiles in the Mitsunobu reaction must be acidic, with a pKa of 11 or below.[55] Alternatively, a nucleophilic aromatic substitution with 3-amino-L-alanine **150** and 2-fluoronitrobenzene could be used to give **147**. 3-Amino-L-alanine could be prepared from L-asparagine (**151**) using a Hofmann rearrangement. Finally, a ring-opening reaction of chiral aziridine **149** with 2-nitroaniline to give amino acid **147** could also be used.[114]

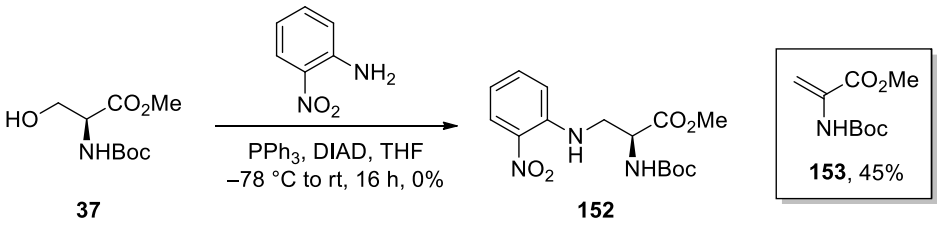


148 **147** **149** **150** **151**

Scheme 43 Retrosynthetic analysis of amino acid **147**.

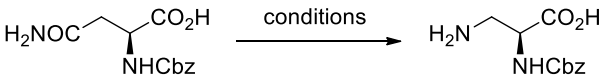
Synthesis of intermediate 2-nitroaniline **152** was first attempted with a Mitsunobu reaction with L-serine analogue **37** (**Scheme 44**). From this reaction, it was found that after 16 h there was no product formation. Instead, starting material **37** and alkene **153** were isolated in 42% yield and

45% yield, respectively. This showed that an elimination reaction was taking place, instead of the desired substitution reaction.



Scheme 44 Attempted Mitsunobu synthesis of amino acid **152**.

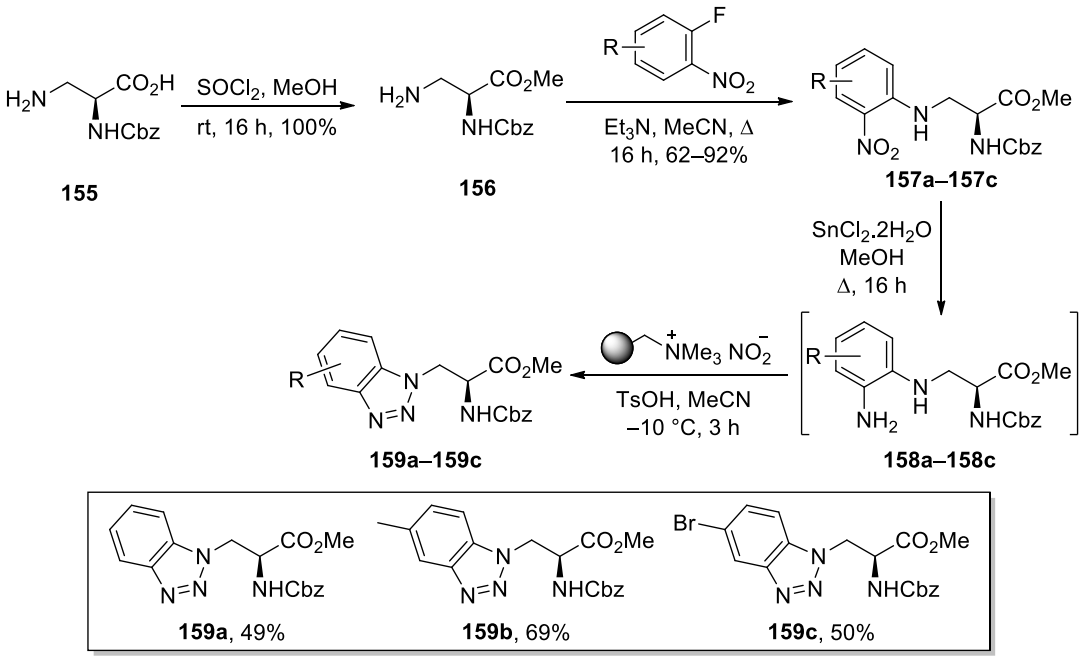
A new approach was required. Therefore, it was proposed that the preparation of amino acid **147** by nucleophilic substitution would be investigated next. The Hofmann rearrangement of amino acids is well documented.[115–118] The reaction was first attempted with the hypervalent iodine species (diacetoxyiodo)benzene (PIDA) in THF, but this only resulted in a 22% yield after 20 h (**Table 10**, entry 1).[116] It has been reported in the literature that bis-[(trifluoroacetoxy)iodo)benzene (PIFA) can also be an effective reagent for the Hofmann rearrangement.[119] When PIFA was used to prepare **155** in THF an increased yield of 34% was obtained (entry 2). With low yields being obtained for this transformation other conditions were investigated. It has been reported that the inclusion of pyridine is beneficial for the transformation.[115] When pyridine was used in combination with PIFA in aqueous acetonitrile a yield of 49% for **155** was obtained (entry 3). It has been reported that very high yields can be obtained when the Hofmann rearrangement of **154** is carried out in a solvent mixture of ethyl acetate, acetonitrile and water.[117,118] When this transformation was first carried out with PIFA, no product was given (entry 4). However, when PIDA was used, a quantitative yield of **155** was achieved (entry 5). It was found that **155** had very low solubility, resulting in precipitation during the reaction and easy isolation.



Entry	Reagent	Pyridine	Solvent	Temperature	Time (h)	Yield (%)
1	PIDA	No	THF	0 °C to rt	20	22
2	PIFA	No	THF	0 °C to rt	20	34
3	PIFA	Yes	MeCN, H_2O	rt	20	49
4	PIFA	No	EtOAc, MeCN, H_2O	15 °C to rt	3	0
5	PIDA	No	EtOAc, MeCN, H_2O	15 °C to rt	3	100

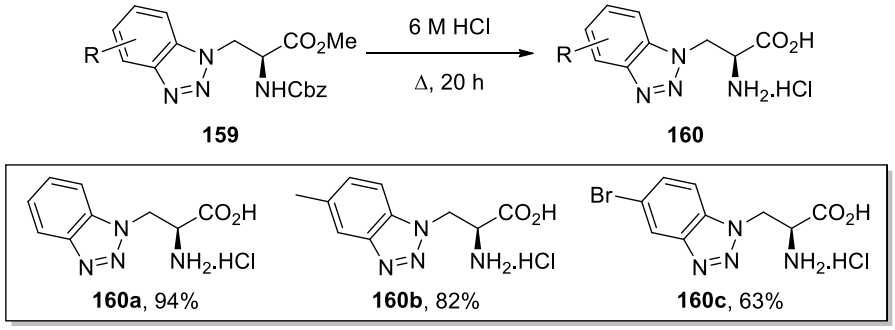
Table 10 Optimisation of Hofmann rearrangement.

With **155** being prepared, the carboxylic acid was then protected as the methyl ester with thionyl chloride in methanol, which gave methyl 3-amino-L-alaninate **156** in quantitative yield (**Scheme 45**).[120] Compound **156** was then used in a nucleophilic aromatic substitution with 2-fluoronitrobenzenes with triethylamine.[121] The reaction was monitored by TLC and from this, it was observed that complete consumption of starting material was achieved after 16 h with the formation of a yellow compound. It is known that *ortho* and *para*-nitroanilines have a strong yellow colour, due to their strong absorbance in the visible region. For example, 2-nitroaniline has a strong absorbance at 405 nm with a molar attenuation coefficient of 6000 $cm^{-1}M^{-1}$.[122] Hence, it can be concluded that the reaction was progressing to *o*-nitroaniline **157** due to the development of this yellow compound. From the reaction, yields of 62–92% of the coupled products were obtained with a 16 h reaction time. The structure of the products was confirmed by ^{1}H, ^{13}C NMR spectroscopy and mass spectrometry. Coupled products **157a**–**157c** were reduced to 1,2-diaminophenyl derived amino acids **158a**–**158c** with tin dichloride. As this reaction resulted in the loss of the push-pull system in the phenyl ring, the yellow nitroaniline colour disappeared during the course of the reaction. It was also observed with ^{1}H NMR spectroscopy that the hydrogen atoms on the disubstituted phenyl ring migrated upfield as the phenyl ring became more electron-rich. Anilines **158a**–**158c** were immediately reacted with the resin-bound nitrite and *p*-tosic acid, which gave *N*-Cbz protected benzotriazoles **159a**–**159c**. Originally these reactions were run at room temperature, but it was found better yields could be obtained by lowering the reaction temperature. When the reactions were run at −10 °C for 3 h, good yields (49–69%) over the two-steps were achieved for the synthesis of **159a**–**159c**. Formation of these benzotriazoles gave a characteristic downfield signal (7.77–8.03 ppm) with ^{1}H NMR spectroscopy of the aromatic hydrogen atom *ortho* to the triazole ring. This helped identify the formation of the benzotriazole products. It was proposed that brominated analogue **159c** would be a highly valuable synthetic building block, due to its aryl halide functionality, which could be used for palladium cross-coupling chemistry.



Scheme 45 Synthesis of benzotriazole-derived amino acids.

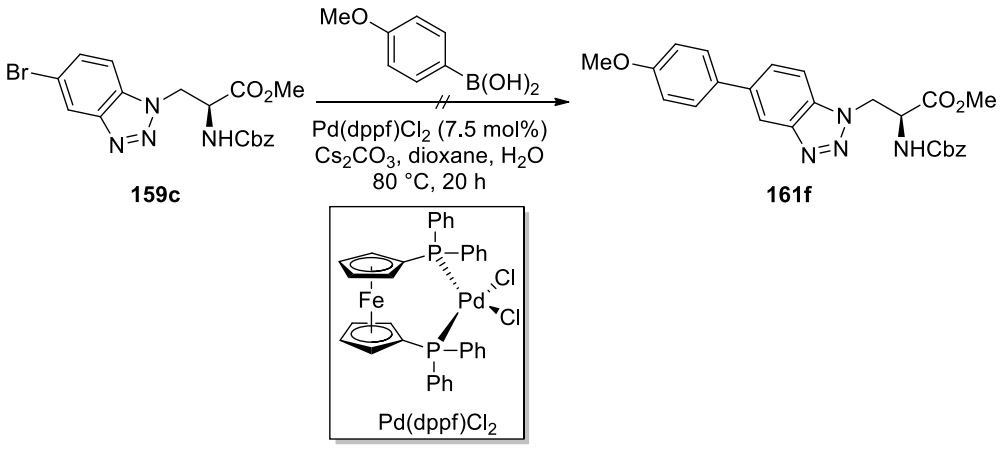
To access the parent amino acids, compounds **159a**–**159c** were deprotected. This was achieved in one-step by treatment with 6 M hydrochloric acid (**Scheme 46**). It was found that this gave analogues **160a**–**160c** in 63%–94% yields after purification by recrystallisation from methanol and diethyl ether.



Scheme 46 One-step acid deprotection to amino acids **160a**–**160c**.

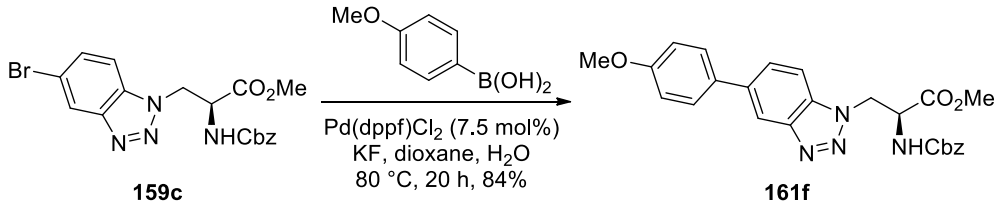
It was found that these simple benzotriazoles, **160a**–**160c** were not fluorescent. Therefore, investigations began on further functionalisation of the benzotriazole derived amino acids. It was proposed that the conjugation of these benzotriazole amino acids could be increased using the Suzuki-Miyaura reaction. Due to the wide range of boronic acid coupling partners available, a wide range of conjugated products could be synthesised. With this increase in conjugation, it was proposed that some of these Suzuki-Miyaura products would be fluorescent. Initially, the Suzuki cross-coupling reaction was attempted using, [1,1′-bis(diphenylphosphino)ferrocene]palladium(II) dichloride, 4-methoxyphenylboronic acid and caesium carbonate in a solvent mixture of water and dioxane (**Scheme 47**). It was found after reaction at 80 °C for 20 h, that none of the desired

Suzuki product **161f** could be isolated, as the methyl ester had been hydrolysed by the caesium carbonate base.



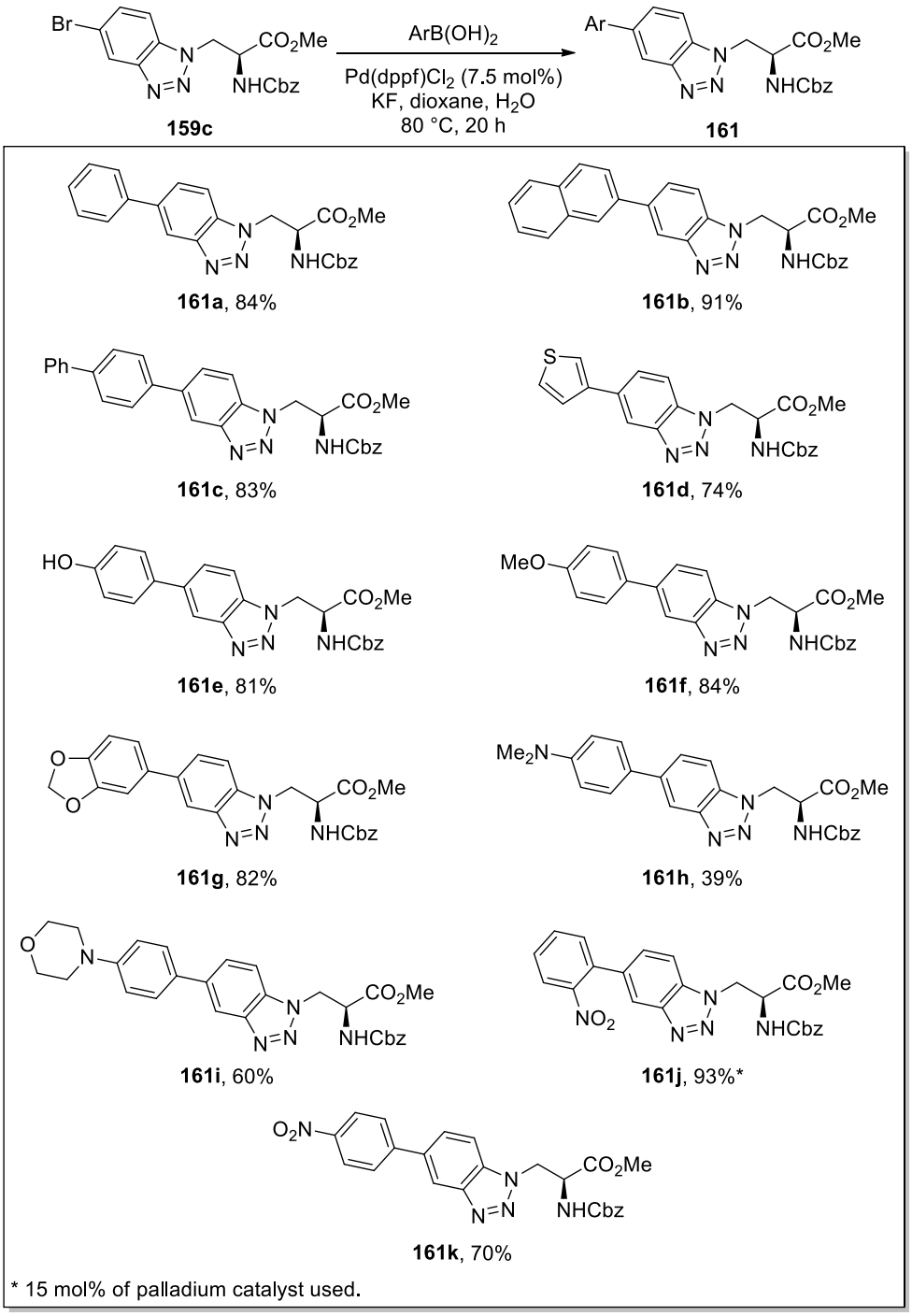
Scheme 47 Attempted Suzuki-Miyaura reaction with caesium carbonate.

To remedy this, the hydrolytic base caesium carbonate was replaced with the non-hydrolytic base potassium fluoride (**Scheme 48**). After reaction at 80 °C for 20 h, coupled product **161f** was isolated in an excellent 84% yield.



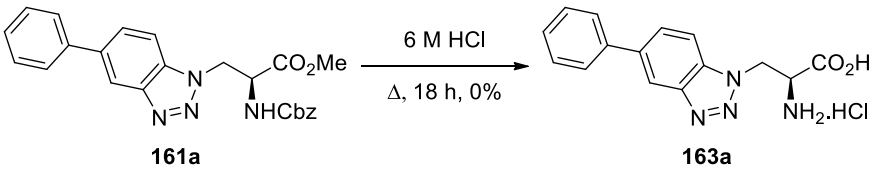
Scheme 48 Suzuki-Miyaura reaction with potassium fluoride.

Having developed the optimised conditions for the Suzuki-Miyaura reaction, the substrate scope was then investigated (**Scheme 49**). The reaction of bromide analogue **159c** with a range of boronic acids gave coupled products **161a**–**161k**. Simple aryl boronic acids gave coupled products **161a**–**161c** in excellent 83–91% yields. The sulfur-containing thiophene heterocycle could also be coupled to the benzotriazole using 3-thiopheneboronic acid in good yield. The functional groups, hydroxy, methoxy, dioxole, nitro and amine were all tolerated in this reaction, giving the coupled products **161e**–**161k**, from low to excellent yield (39–84%). It should be noted that while the majority of these reactions required a catalyst loading of 7.5 mol%, in synthesising compound **161j**, 15 mol% was required to reach full conversion. It is proposed that this reaction was less efficient due to the presence of an *ortho*-substituent.



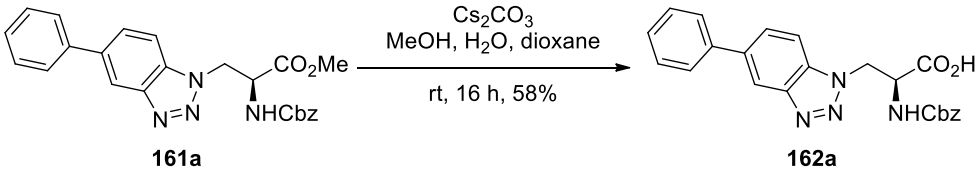
Scheme 49 Synthesis of Suzuki coupled products.

Before the photoluminescence properties of these coupled products **161a**–**161k** was assessed, the protecting groups were removed. Owing to the successful deprotection of the simple benzotriazole amino acids **160a**–**160c** with a one-pot acidic deprotection, this was attempted for amino acid **161a** (**Scheme 50**). It was observed under these conditions that the protected amino acid degraded, yielding no product.



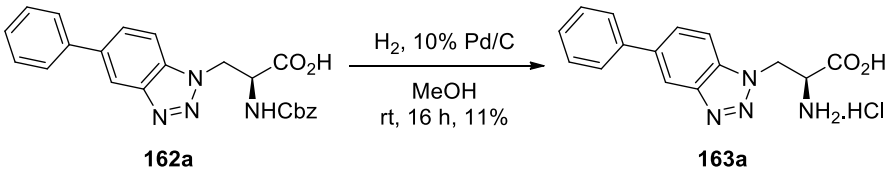
Scheme 50 Attempted one-pot deprotection of amino acid **161a**.

Therefore, a mild two-step deprotection strategy was next investigated to gain access to the target amino acids. Following a literature procedure, ester hydrolysis of **161a** with caesium carbonate gave acid **162a** in 58% yield (**Scheme 51**). It was reported that the hydrolysis was carried out in a solution of methanol and water.[74,75] When this was attempted, this resulted in a heterogeneous reaction mixture and no ester hydrolysis occurred. It was found that the addition of a minimal amount of 1,4-dioxane resulted in a homogenous reaction mixture and gave hydrolysed product **163a** cleanly. The reaction was also trialled in a solvent mixture of 1,4-dioxane and water, but this resulted in no ester hydrolysis.



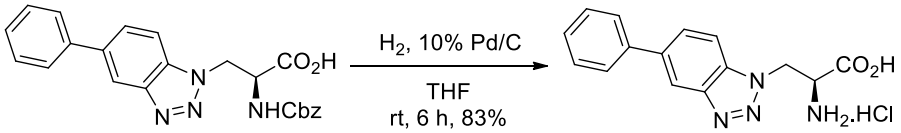
Scheme 51 Ester hydrolysis of **161a** with caesium carbonate.

To avoid the use of acidic conditions, hydrogenolysis of the Cbz group was attempted, following a previously reported procedure (**Scheme 52**).[123] When the reaction was trialled with **162a** in methanol, using 10% palladium on carbon, a very low yield of deprotected amino acid **163a** was obtained.



Scheme 52 Hydrogenolysis of Cbz amino acid **162a** in methanol.

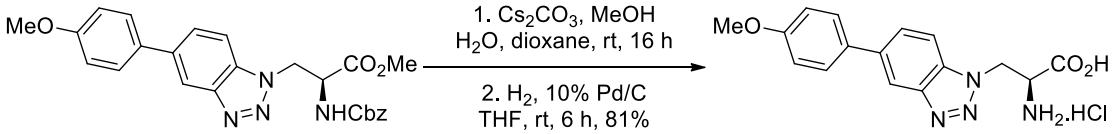
From the literature, it had been reported that hydrogenolysis mediated deprotection of the Cbz group can be achieved when THF is used as the solvent.[124] Therefore, this was attempted with Cbz protected benzotriazole amino acid **162a** (**Scheme 53**). After 6 h under hydrogenation conditions, complete deprotection was achieved and the target amino acid **163a** was isolated in 83% yield. It was found that forming the HCl amino acid salt was crucial in the amino acid having good solubility and allowing for a more straightforward purification by recrystallisation.



162a **163a**

Scheme 53 Hydrogenolysis of Cbz protected amino acid **162a** in THF.

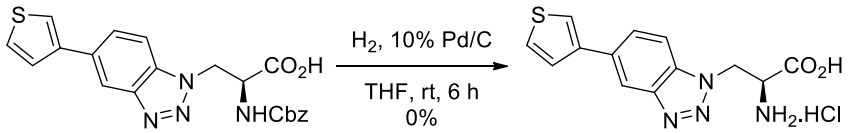
With the success of preparing amino acid **163a** with hydrogenolysis, this approach was then investigated for the other substrates. It was found that the 4-methoxyphenyl substituted benzotriazole amino acid **161f** could also be deprotected using this protocol (**Scheme 54**), giving deprotected amino acid **163f** in excellent yield over the two steps.



161f **163f**

Scheme 54 Deprotection of benzotriazole amino acid **163f** with hydrogenolysis.

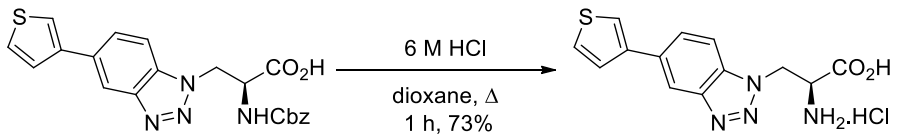
When other benzotriazole amino acids were subjected to these hydrogenolysis conditions, no Cbz deprotection was achieved. For example, when thiophene derived benzotriazole amino acid **162d** was subjected to hydrogenolysis conditions for 6 h, only starting material was returned (**Scheme 55**). The hydrogenolysis of amino acid **162d** was also attempted with palladium black. Again, this only returned starting material.



162d **163d**

Scheme 55 Attempted hydrogenolysis of amino acid **163d**.

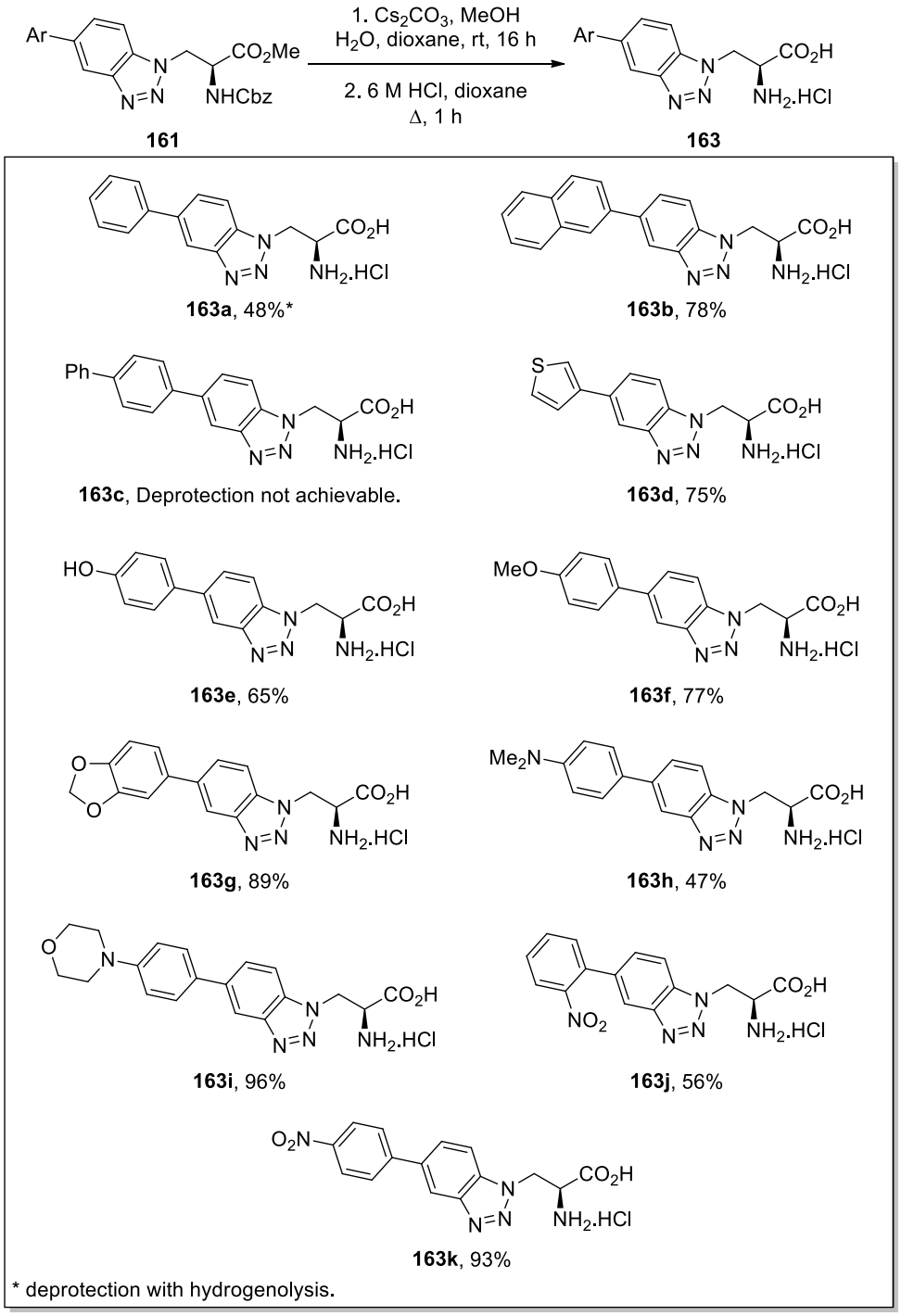
It has been reported that the acidic removal of Cbz protecting groups can be achieved in very short reaction times.[125] This was demonstrated for 2-[(2*S*)-1-benzyloxycarbonyl-2-pyrrolidinyl]pyridine, where the Cbz protecting group was removed in an excellent yield of 92%, after a reaction time of 1 h.[125] Hence, these literature conditions were trialled for amino acid **162d** (**Scheme 56**). It was found that after a reaction time of 1 h, full deprotection of the Cbz group was achieved with target compound **163d** being isolated in 73% yield. In performing the reaction, it was found that the addition of a minimal amount of 1,4-dioxane was crucial in ensuring a homogenous reaction mixture.



162d **163d**

Scheme 56 Acid deprotection of Cbz-protected amino acid **163d**.

Having developed an effective deprotection protocol, the other amino acids **163b**–**163k** were trialled under these conditions (**Scheme 57**). Nine of the ten benzotriazole amino acids tested gave excellent yields for the target compounds **163b**, **163d**–**163k**, with only highly lipophilic biaryl substituted benzotriazole amino acid **163c** not successful. For amino acid **161c**, ester hydrolysis to the *N*-Cbz protected carboxylic acid proceeded well. However, the resulting carboxylic acid had very poor solubility in most solvents. When the acidic removal of the Cbz group was attempted, using the optimised conditions, this resulted in a heterogeneous reaction mixture. Even with extended heating, the reaction would not become homogenous and therefore no deprotection occurred. When large amounts of 1,4-dioxane were added to the reaction mixture, a homogenous reaction mixture could be achieved but this then led to slow Cbz removal. With extended reaction times, the material then degraded under these forcing conditions before deprotection was complete.



Scheme 57 Two-step deprotection of benzotriazole amino acids.

2.2.3 Optical properties of benzotriazole-derived amino acids

With target benzotriazole derived amino acids **163a**, **163b 163d–163k** being synthesised, the photoluminescence properties were then measured (**Table 11**). Fluorescence spectroscopy was carried out with the benzotriazoles being excited by the wavelengths corresponding to their absorption maxima from their absorption spectra. As with the pyrazoloquinazoline amino acids **94**, **98–101**, the Stokes shift, molar attenuation coefficient, quantum yields and brightness of these benzotriazole amino acids **163a**, **163b 163d–163k** were calculated. No fluorescence was

observed for amino acids **160a**–**160c** and **163j** and **163k**, with only scattered excitation and Raman scattering being recorded. This was confirmed using methanol as a blank sample, which gave identical emission spectra.[11] Therefore brightness and quantum yield values could not be calculated for these compounds.

For amino acids **163a**, **163b 163d**–**163l**, mega-Stokes shifts were observed between the excitation and emission wavelength maxima.[126] Due to the lack of rigidity and planarity, the quantum yields of these benzotriazole derived amino acids were lower and more varied than pyrazoloquinazoline derived amino acids **94**, **98**–**101**. In addition, no vibronic fine structure was observed due to rotation between the aryl rings being allowed. The presence of rotational energy levels led to broad absorption and emission spectra. Like the previously reported pyridine-derived amino acids, the highest quantum yield values were obtained for naphthyl and 4-methoxyphenyl analogues **163b** and **163f**. 4-Methoxyphenyl substituted benzotriazole **163f** had one of the highest molar attenuation coefficients, which made it the brightest compound from this series. Alongside, the high brightness value, 4-methoxyphenyl substituted benzotriazole amino acid **163f** also had a Stokes shift of 162 nm making it the lead compound of this series.

It should be noted that the 4-methoxyphenyl **163f** and 4-hydroxyphenyl substituted benzotriazole amino acid **163e** displayed very different fluorescent properties. While the 4-methoxyphenyl substituted benzotriazole was a very bright fluorophore, the 4-hydroxyphenyl substituted benzotriazole showed almost no fluorescence. This is due to excited-state proton transfer, the same phenomenon has been observed for hydroxyl- and methoxyflavones.[127] Another observation is the significant decrease in brightness between 4-methoxyphenyl substituted benzotriazole **163f** and benzodioxole substituted benzotriazole amino acid **163g**. This trend was also observed with the previously synthesised pyridine amino acids.[128]

Both 4-dimethyaminophenyl- and 4-morpholinephenyl substituted benzotriazole amino acids **163h** and **163l** had limited fluorescence. It has been reported that fluorescent properties can be improved by the replacement of dialkylamino functional groups with azetidine rings.[129] Therefore, the fluorescence properties of a 4-azetidinephenyl substituted benzotriazole amino acid would be of interest.

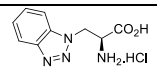
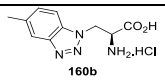
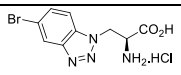
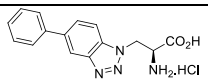
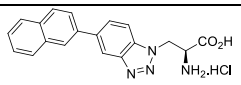
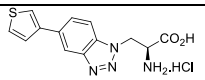
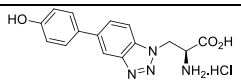
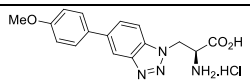
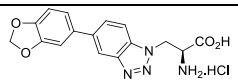
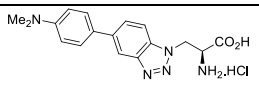
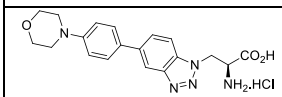
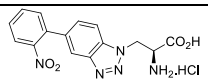
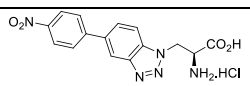
Compound	Excitation Wavelength (nm)	Molar Attenuation Coefficient ($cm^{-1}M^{-1}$)	Emission Maximum (nm)	Stokes shift (nm)	Quantum yield (ϕ_F)	Brightness ($cm^{-1}M^{-1}$)
 **160a**	282	4,824	N/A	N/A	N/A	N/A
 **160b**	290	6,117	N/A	N/A	N/A	N/A
 **160c**	294	4,601	N/A	N/A	N/A	N/A
 **163a**	250	22,633	363	120	0.0024	55
 **163b**	280	10,309	417	131	0.18	1866
 **163d**	251	19,903	384	127	0.03	533
 **163e**	260	26,729	436	176	0.0081	216
 **163f**	256	23,034	418	162	0.17	3857
 **163g**	258	18,504	454	196	0.02	350
 **163h**	290	17,212	382	92	0.0016	27
 **163i**	285	19,212	384	99	0.0043	82
 **163j**	290	24,360	N/A	N/A	N/A	N/A
 **163k**	224 312	26,308 17,224	N/A	N/A	N/A	N/A

Table 11 Photoluminescence properties of the synthesised amino acids **160a**–**160c**, **163a**, **163b** and **163d**–**163k**.

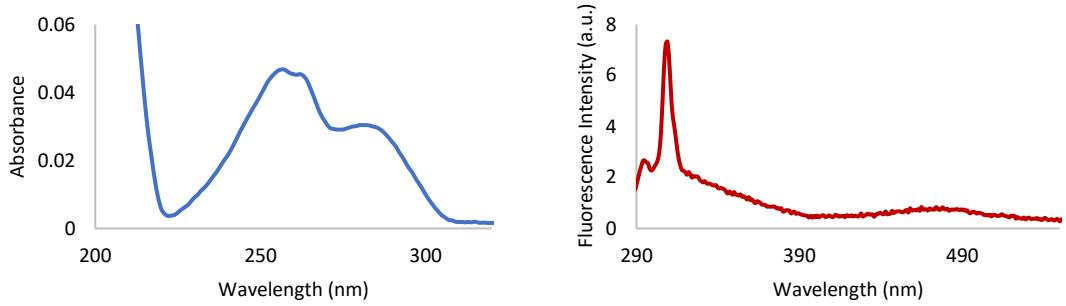


Figure 42 Absorption and emission spectra of amino acid **160a**, in MeOH (excitation at 282 nm).

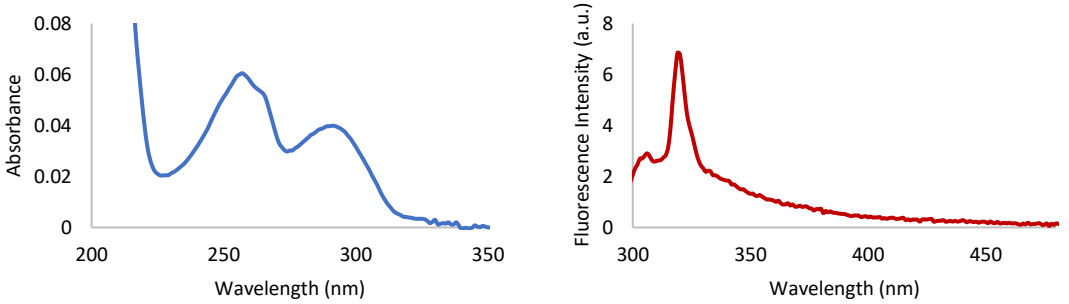


Figure 43 Absorption and emission spectra of amino acid **160b**, in MeOH (excitation at 290 nm).

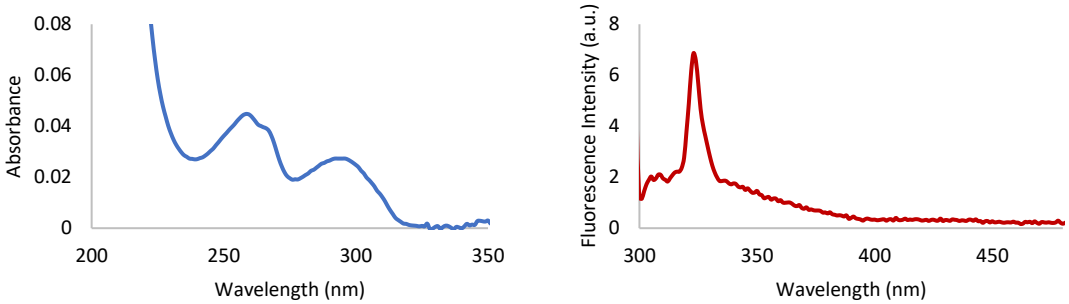


Figure 44 Absorption and emission spectra of amino acid **160c**, in MeOH (excitation at 294 nm).

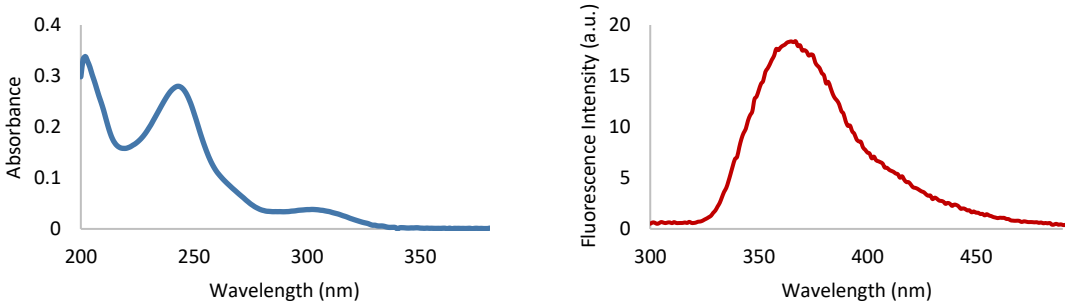


Figure 45 Absorption and emission spectra of amino acid **163a**, in MeOH (excitation at 250 nm).

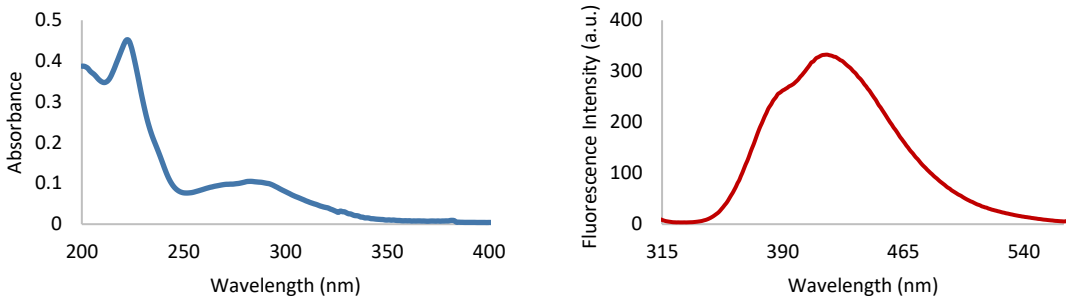


Figure 46 Absorption and emission spectra of amino acid **163b**, in MeOH (excitation at 280 nm).

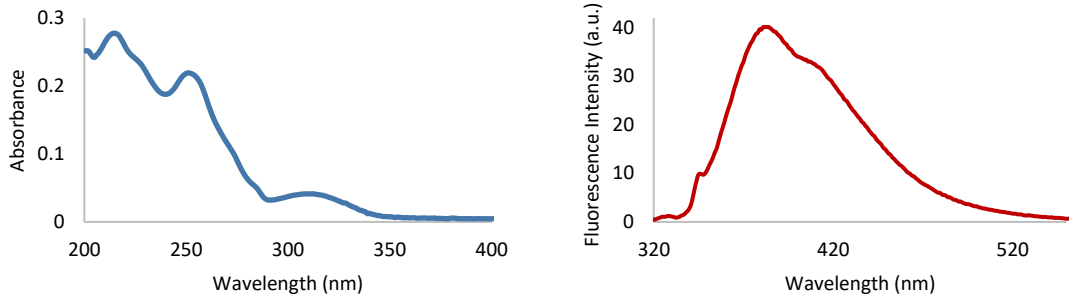


Figure 47 Absorption and emission spectra of amino acid **163d**, in MeOH (excitation at 251 nm).

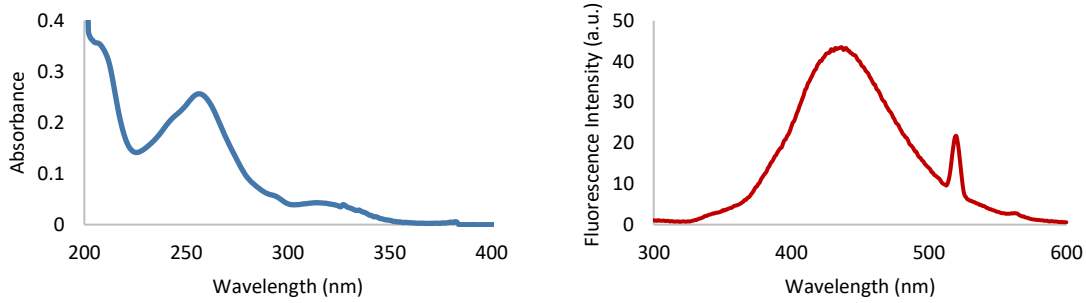


Figure 48 Absorption and emission spectra of amino acid **163e**, in MeOH (excitation at 260 nm).

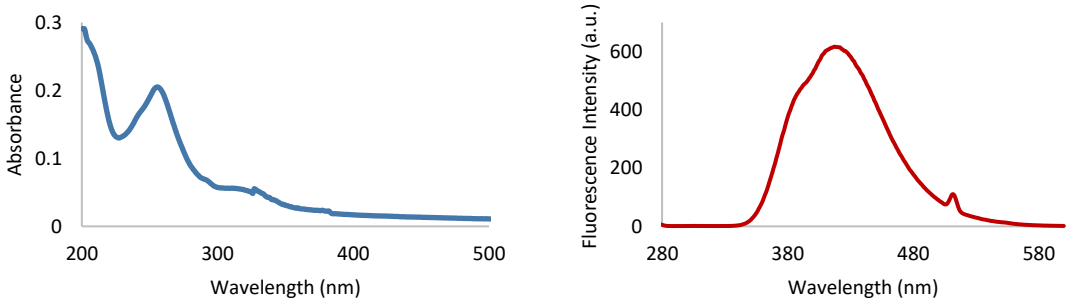


Figure 49 Absorption and emission spectra of amino acid **163f**, in MeOH (excitation at 256 nm).

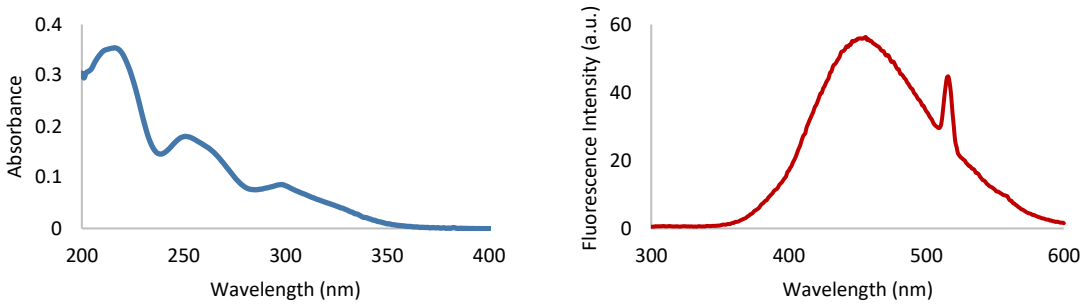


Figure 50 Absorption and emission spectra of amino acid **163g**, in MeOH (excitation at 258 nm).

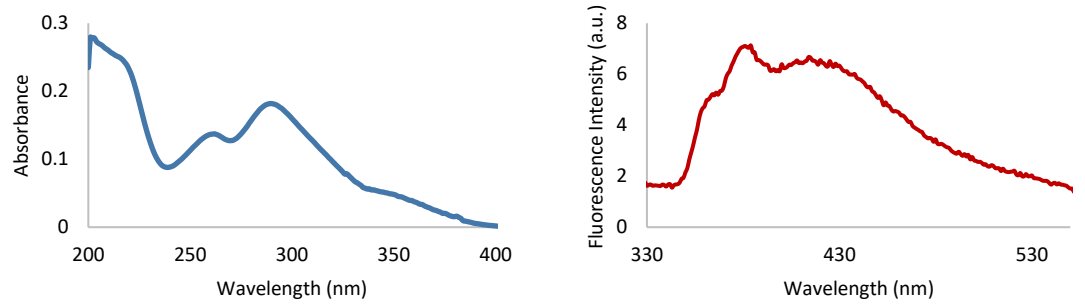


Figure 51 Absorption and emission spectra of amino acid **163h**, in MeOH (excitation at 287 nm).

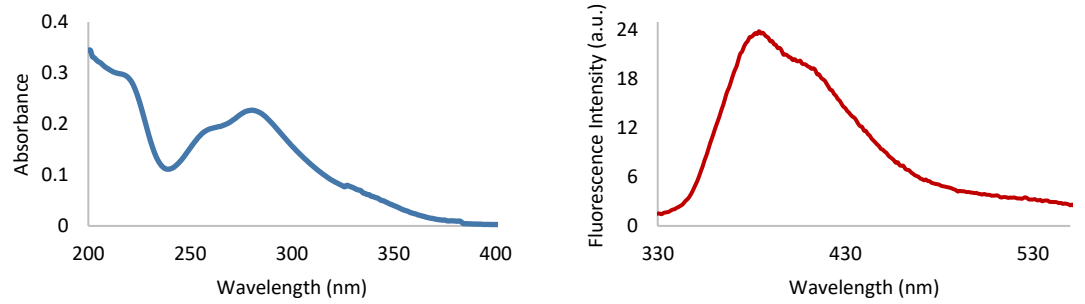


Figure 52 Absorption and emission spectra of amino acid **163i**, in MeOH (excitation at 285 nm).

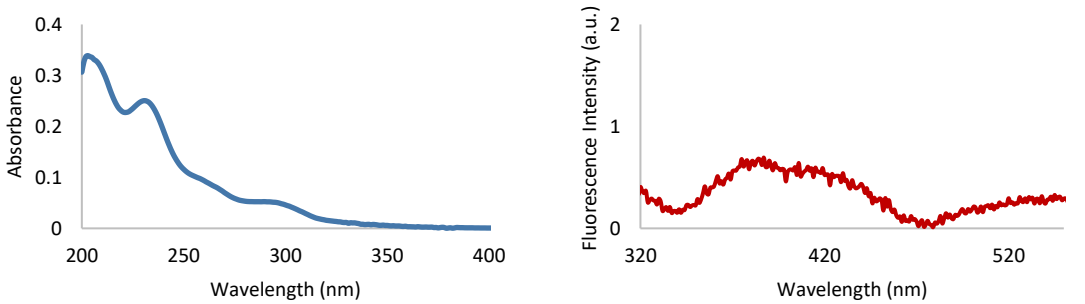


Figure 53 Absorption and emission spectra of amino acid **163j**, in MeOH (excitation at 290 nm).

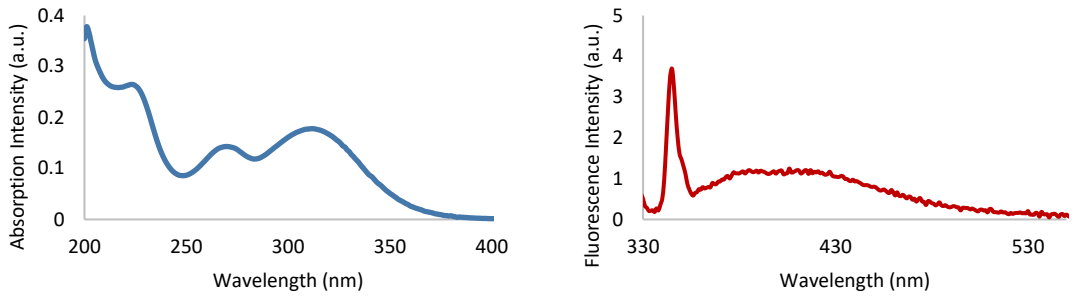


Figure 54 Absorption and emission spectra of amino acid **163k**, in MeOH (excitation at 260 nm).

Compound **163f** the lead compound in this series, was studied further with a brief solvatochromic study (**Figure 55**). The absorption and emission spectra of the amino acid were measured in THF, methanol and PBS solution. It was found that the absorption spectra were very similar in all the solvents. However, it was observed that the emission spectra of the amino acid displayed strong solvent effects. As the solvent polarity increased, the emission maximum also increased. In THF, an emission maximum of 372 nm was recorded, while in methanol, the emission maximum was observed at 417 nm. In aqueous PBS solution, the emission maximum was found at 449 nm. Furthermore, there is evidence of a change in the excited state between polar and non-polar solvents with a shoulder peak present in the emission spectrum recorded in methanol. From this, it can be concluded that in non-polar solvents the compound exists in a locally excited state before the emission of a photon, whereas in polar solvents an ICT state present.

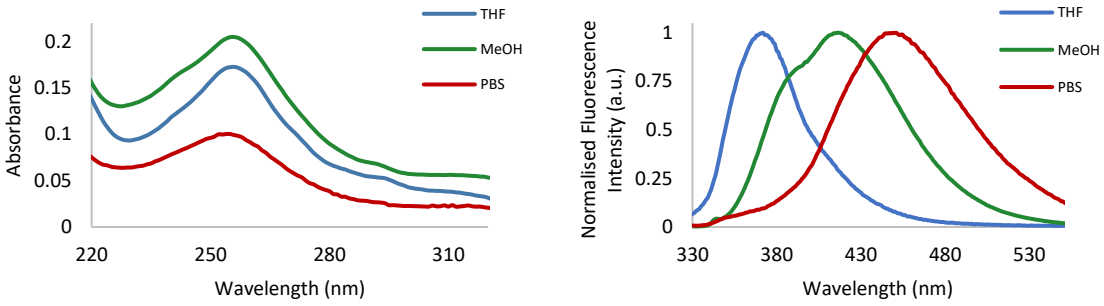
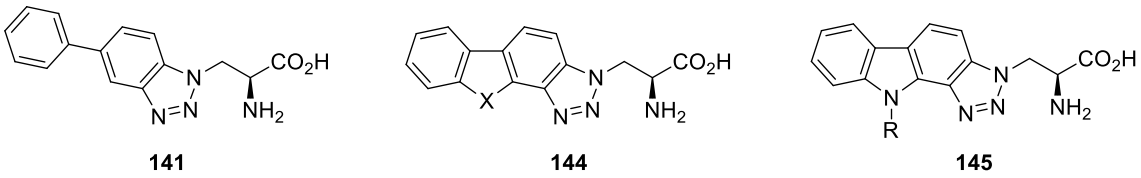


Figure 55 Solvatochromic study of **163f**.

2.2.4 Carbazole derived amino acids

With the photoluminescence properties of pyrazole amino acids being significantly improved by introducing rigidity to the structure, it was proposed a similar approach could be undertaken for the benzotriazole derived amino acids. With amino acid **141**, the phenyl ring could distort out of the plane of the benzotriazole ring system, leading to reduced conjugation and fluorescence (**Figure 56**). Therefore, a tether **144** could be incorporated, constraining the phenyl and benzotriazole rings into the same plane. As carbazoles are well-established tricyclic aromatic

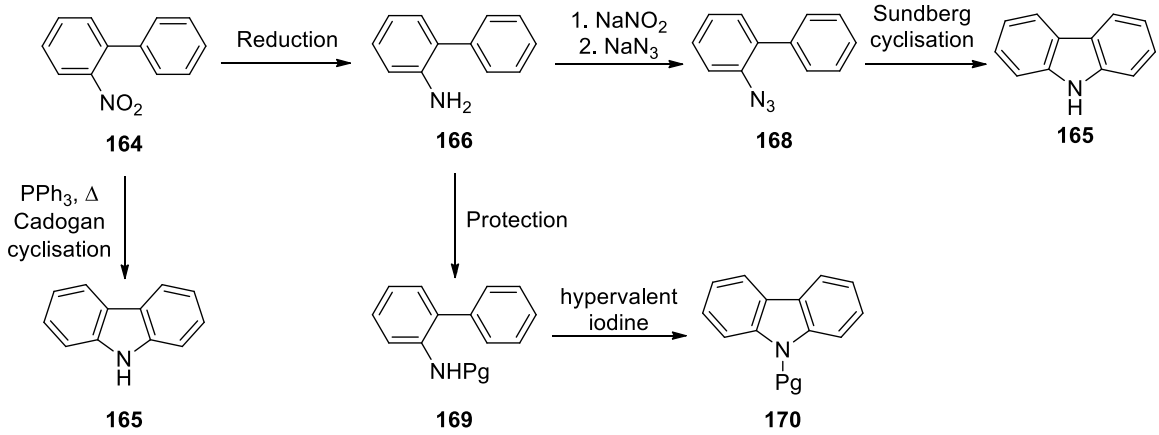
heterocycles, it was thought this ring system could be used to introduce rigidity into these benzotriazole fluorophores **145**.



141 **144** **145**

Figure 56 Introducing rigidity into benzotriazole amino acids.

There are several ways to prepare carbazoles from 2-nitrobiphenyl (**164**) (**Figure 57**) and it was proposed that one of these methods would be applicable for benzotriazole derived amino acids. Carbazole (**165**) can be prepared in one-step from **164** via a Cadogan cyclisation at high temperatures with triphenylphosphine.[130] The reaction proceeds through a nitrene intermediate as the triphenylphosphine is oxidised to the triphenylphosphine oxide. Once the nitrene is formed, an intramolecular cyclisation can take place giving the carbazole. This has the advantage of reducing the nitro group and performing the cyclisation reaction in the same step. However, extremely harsh conditions are generally needed for this reaction to occur. If **164** is reduced to 2-aminobiphenyl (**166**) much milder chemistry can be implemented to form the desired carbazole. From **166**, azide compound **168** can be prepared from the diazonium salt.[131] Then, **168** can undergo a Sundberg cyclisation reaction to give carbazole (**165**).[131] Otherwise, after the amino group of **166** has been protected with a suitable nitrogen protecting group, it has been shown that carbazole **170** can be accessed with treatment of **169** with a hypervalent iodine species.[30–32]

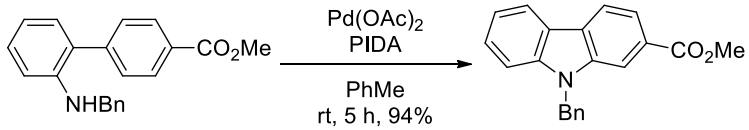


164 **166** **168** **165**

165 **169** **170**

Figure 57 Synthetic approaches to carbazoles

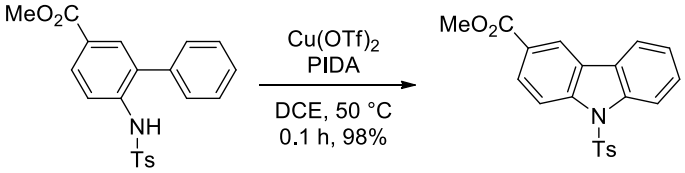
Lately, there has been a plethora of research in the use of the hypervalent iodine species in carbazole cyclisation chemistry. For example, it has been shown that this transformation can be carried out with the use of a palladium catalyst and PIDA (**Scheme 58**). Under mild conditions, *N*-benzyl protected carbazole **172** was formed in 94% yield from aniline **171**.



171 172

Scheme 58 Palladium catalysed carbazole formation with benzyl-protected aniline **171**.

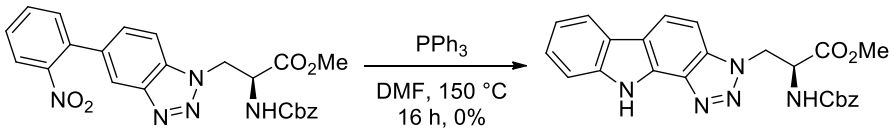
It was then later reported, that the same cyclisation can also be performed with a copper catalyst and a tosyl-protected aniline (**Scheme 59**).[134] At 50 °C, the tosyl-aniline **173** was converted to carbazole **174** in excellent yield with copper(II) triflate and PIDA



173 174

Scheme 59 Copper catalysed carbazole formation with tosyl-protected aniline **173**.

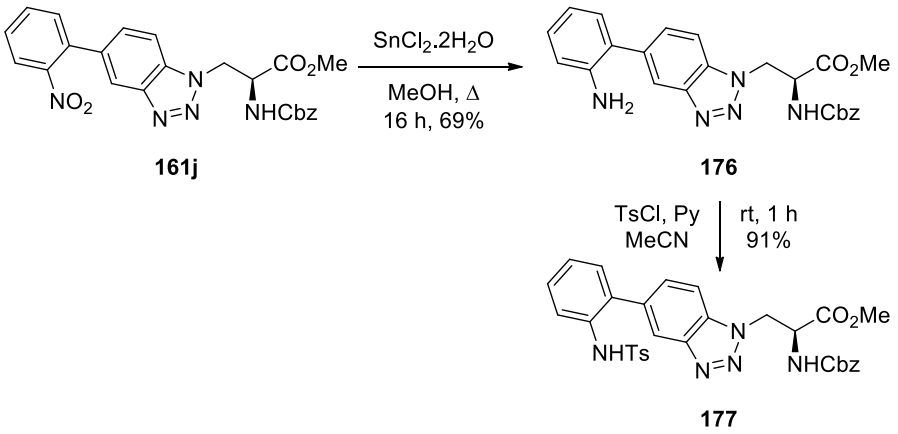
In an attempt to form the carbazole ring, a Cadogan cyclisation reaction was first attempted with 2-nitrophenyl substituted benzotriazole amino acid **161j** (**Scheme 60**). No product formation was observed. A Graebe–Ullmann reaction may be occurring resulting in decomposition of the material.[135,136]



161j 175

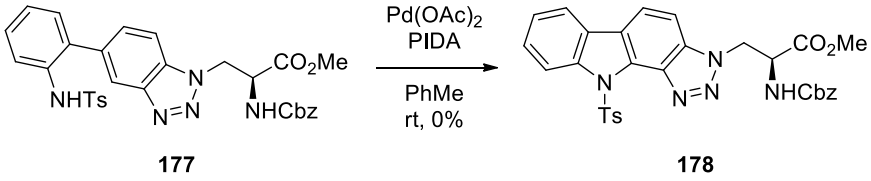
Scheme 60 Attempted Cadogan cyclisation of benzotriazole **161j**.

With substrate **161j** unable to perform the Cadogan cyclisation reaction, an alternative approach was required. It was proposed that metal-catalysed carbazole ring formation with a hypervalent iodine oxidant had the greatest prospect of success, owing to the very mild reaction conditions needed for ring closure. For this approach to be investigated, the 2-nitrophenyl substituted benzotriazole compound was converted to 2-aminophenyl substituted benzotriazole **176** (**Scheme 61**). This was achieved by tin dichloride reduction of the aromatic nitro group to give **176** in 69% yield. Protection of **176** was then carried out with 4-methylbenzenesulfonyl chloride (TsCl) under basic conditions, this gave the Ts protected analogue **177** in a 91% yield from **176**.



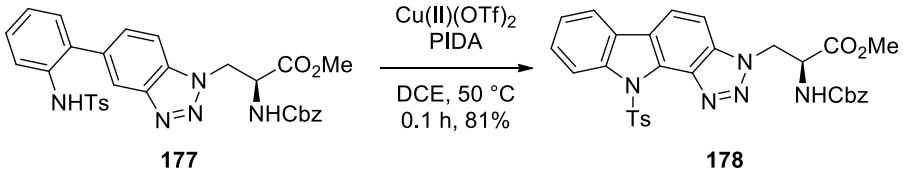
Scheme 61 Synthesis of protected aniline **177**.

With access to **177,** the formation of the carbazole ring system with a transition metal catalyst and PIDA was next investigated.[132,134] The transformation was first attempted using palladium-catalysed conditions (**Scheme 62**). However, no formation of **178** was observed, even with extended reaction times and heating.[134]



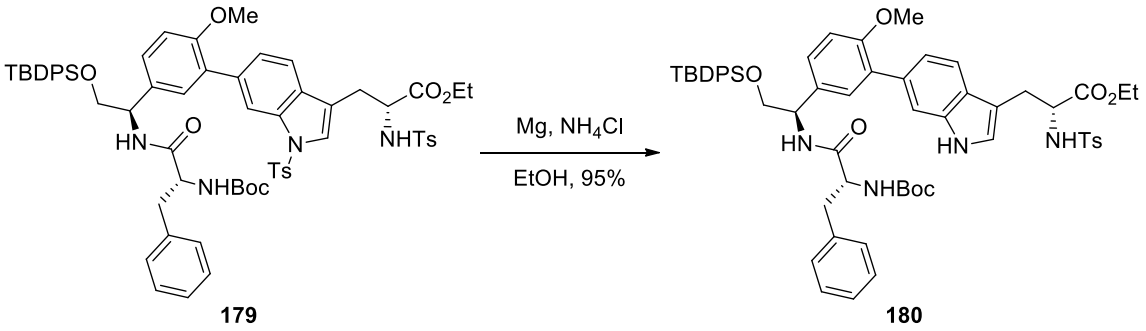
Scheme 62 Attempted carbazole formation with palladium catalyst.[134]

The transformation was then attempted using copper-catalysed conditions (**Scheme 63**). Full conversion of **177** to the carbazole product was observed after a reaction time of 0.1 h at 50 °C and this gave **178** in 81% yield.



Scheme 63 Copper catalysed carbazole ring formation.

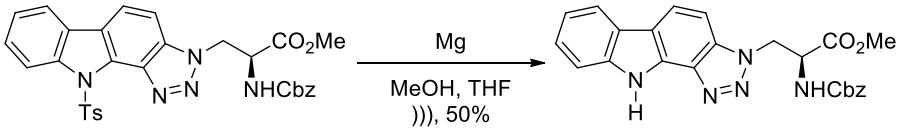
Prior to the measurement of any photoluminescent properties, the protecting groups of **178** had to be removed. From the literature, it was found that magnesium metal can be used to remove aromatic tosyl groups when dissolved in alcoholic solvents.[137–139] For example, the reaction of compound **179** with magnesium metal gave **180** in a 95% yield (**Scheme 64**).[137]



179 180

Scheme 64 Selective tosyl deprotection of compound **179**.

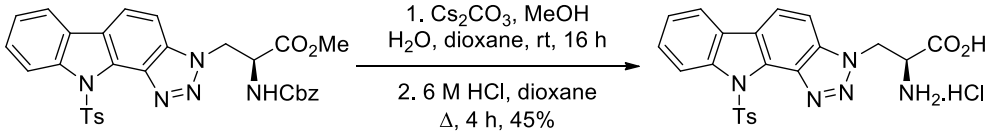
When similar conditions were used with **178**, tosyl deprotection did occur, which gave **181**, in 50% yield (**Scheme 65**). However, it was found that this result was not reproducible, and a sufficient amount of product could not be prepared for subsequent steps with this approach.



178 181

Scheme 65 Tosyl deprotection of carbazole **178**.

Therefore, the other protecting groups of **178** were removed instead using a combination of caesium carbonate and 6 M HCl, which gave **182** in a good 45% overall yield (**Scheme 66**).



178 182

Scheme 66 Ester hydrolysis and Cbz deprotection of **182**.

The photoluminescence properties of **182** were then measured in methanol (10 μM) (**Figure 58**). The Stokes shift, molar attenuation coefficient, quantum yield and brightness values were calculated for **182**. These values were then compared with the phenyl substituted benzotriazole amino acid **163a** (**Table 12**). With the introduction of the nitrogen tether, the photoluminescence properties were improved. Carbazole **182** has longer excitation and emission wavelengths, a larger Stokes shift and is significantly brighter.

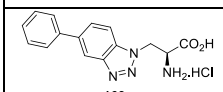
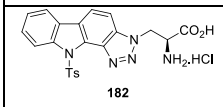
Compound	Excitation Wavelength (nm)	Molar Attenuation Coefficient ($cm^{-1}M^{-1}$)	Emission Maximum (nm)	Stokes shift (nm)	Quantum yield (ϕ_F)	Brightness ($cm^{-1}M^{-1}$)
 163a	243	22,633	363	120	0.0024	55
 182	270	18,399	411	141	0.034	622

Table 12 Comparison of benzotriazole **163a** and carbazole amino acid **182**.

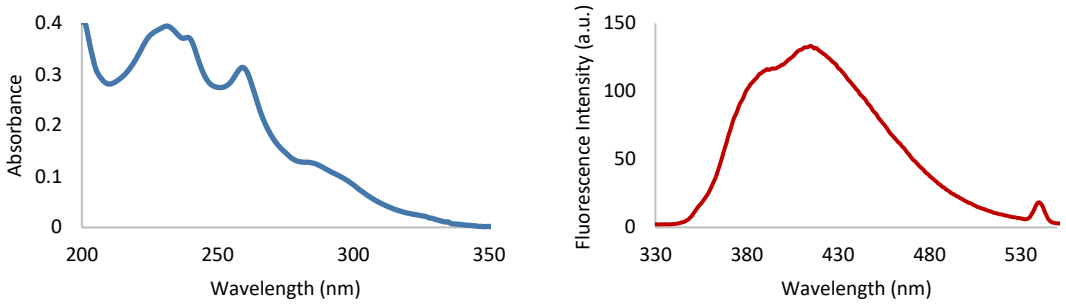


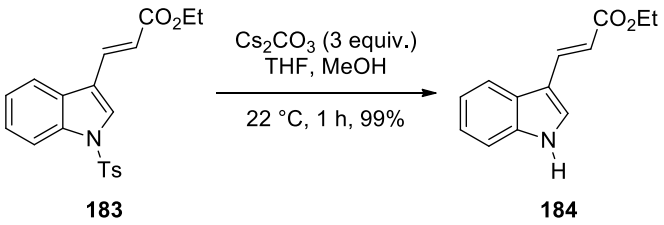
Figure 58 Absorption and emission spectra of carbazole **182** (MeOH, 10 µM), excited at 270 nm.

2.2.5 Summary

In conclusion, a route to benzotriazole derived amino acids has been established. Originally this allowed for the synthesis of three simple benzotriazole amino acids **160a**–**160c**. As expected, none of these amino acids were fluorescent. As a bromide analogue had been synthesised, this allowed for further functionalisation via the Suzuki-Miyaura reaction. This gave a small library of polyaromatic benzotriazole substituted amino acids **163a**, **163b** and **163d**–**163k**, and from this library, many of these displayed interesting photoluminescence properties. To improve these properties, more rigid analogues were studied. It was found that 2-nitrophenyl substituted benzotriazole **161j** could be reduced to 2-aminophenyl substituted benzotriazole **176**. After tosyl protection of **176**, could undergo a copper-catalysed C-H functionalisation reaction, which gave carbazole **178** in high yield. Initial work suggests that the fluorescence properties of this carbazole derived amino acids is superior to the original benzotriazole functionalised amino acids. One drawback of this synthesis was the use of the tosyl protecting group. While this allowed for the efficient formation of the carbazole ring system its removal afterwards was challenging. It was found that magnesium metal was capable of removing the protecting group, but the transformation was not reproducible. Therefore, other protecting groups should be considered for this route.

2.2.6 Future work for benzotriazole derived amino acids

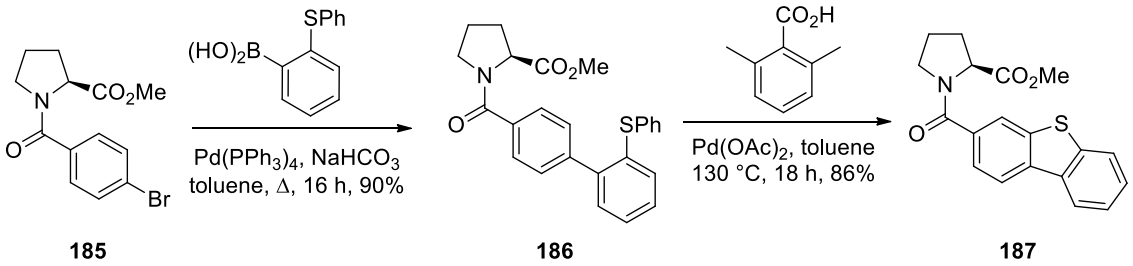
It has been shown that aromatic tosyl groups can be removed with excess caesium carbonate (**Scheme 67**).[140] Therefore, tosyl deprotection of carbazole **178** could be trialled using these conditions to give the fully deprotected amino acid.



Scheme 67 Deprotection of indole **183** with caesium carbonate.

Otherwise, for the carbazole ring formation alternative protecting groups could also be investigated. For the copper catalysed cyclisation, the acetamide was reported to be a suitable protecting group.[134] With the palladium catalysed carbazole ring formation, methyl, benzyl, allyl and *t*-butyl were all shown to be suitable protecting groups for the ring-closing reaction.[132] The use of one of these alternative protecting groups may allow for easier preparation of **181**.

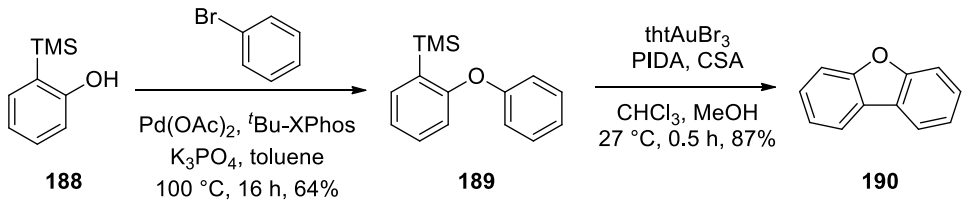
In addition to amino acid **181**, other heterocycles could be pursued to increase rigidity within the benzotriazole side-chain. It has been reported that dibenzothiophene derived amino acid **187** can be synthesised from proline derivative **185**, in 77% yield over the two-steps (**Scheme 68**).[141] This was achieved using a Suzuki-Miyaura reaction of amino acid **185** with (2-phenylthio)phenylboronic acid, which gave coupled product **186**. Palladium catalysis with a carboxylic acid ligand under high temperatures gave **187** from **186**. A similar approach could be investigated with 5'-bromo substituted benzotriazole **159c**.



Scheme 68 Synthesis of dibenzothiophene amino acid **187**.

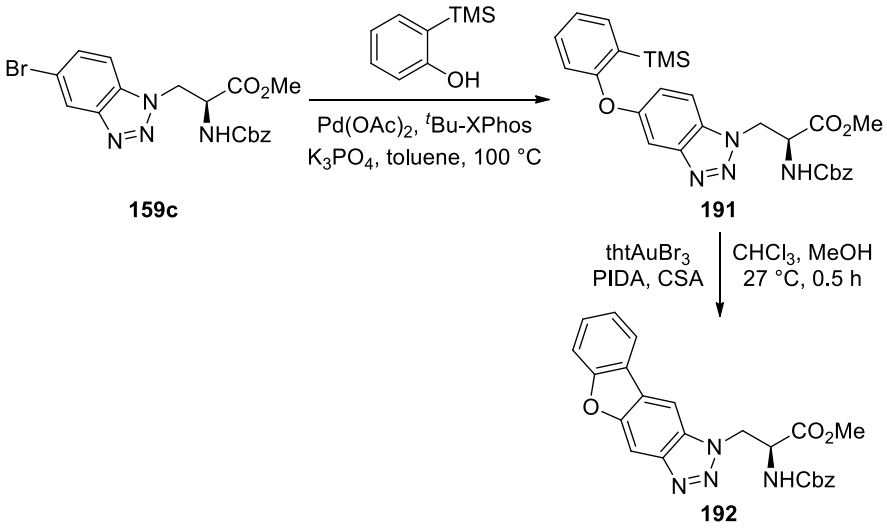
A dibenzofuran ring system could be another ring system for installing rigidity into benzotriazole amino acid. It has been recently reported that dibenzofuran (**190**) could be prepared from silyl phenol **188** (**Scheme 69**).[142] With palladium catalysis, phenol **188** was converted to *ortho*-silyl

ether **189** in good yield. A gold-catalysed intramolecular arylation then gave **190** from silyl ether **189**.



Scheme 69 Preparation of dibenzofuran (**190**).

From this, a synthetic route to give a dibenzofuran analogue can be proposed (**Scheme 70**). Starting with 5′-bromo substituted analogue **159c**, etherification with 2-(trimethylsilyl)phenol would give silyl **191**. Then a gold catalysed cyclisation would result in benzofuran product **192**. An alternative ring-closing strategy would be the Pschorr reaction from 2-nitrophenol using literature conditions.[110,143]



Scheme 70 Synthetic route to dibenzofuran derived amino acid **192**.

2.2.7 General future work for fluorescent amino acids

As discussed above, the replacement of dimethylamino functional groups with azetidine rings can improve photoluminescence properties.[129] For example, when the dimethylamino functional group was replaced with an azetidine ring in fluorophores **193** and **195** the quantum yield was greatly increased (**Figure 59**).

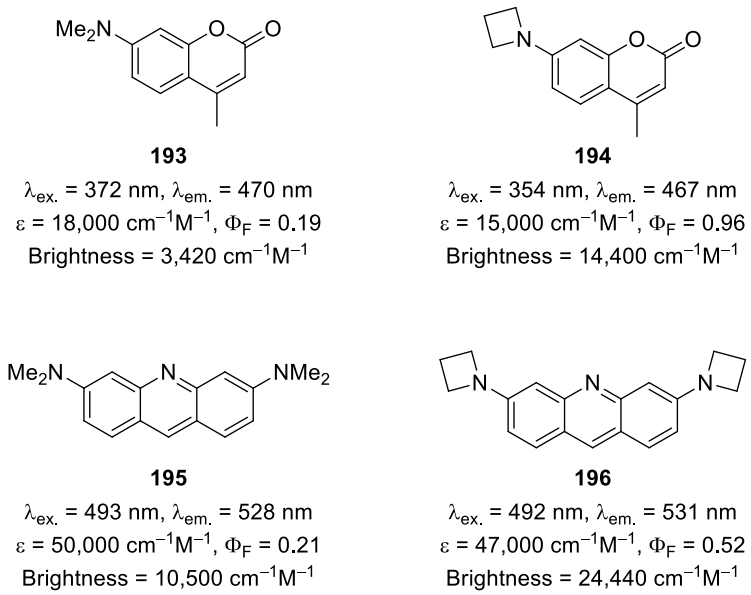


Figure 59 Comparison of photoluminescence properties of dimethylamino- and azetidine functionalised fluorophores.

For this reason, it would be interesting to prepare azetidine analogues of amino acids previously synthesised in the group (**Figure 60**). Once the target **197**–**201** azetidine functionalised amino acids had been synthesised, their photoluminescence properties would be measured and compared to the current library.

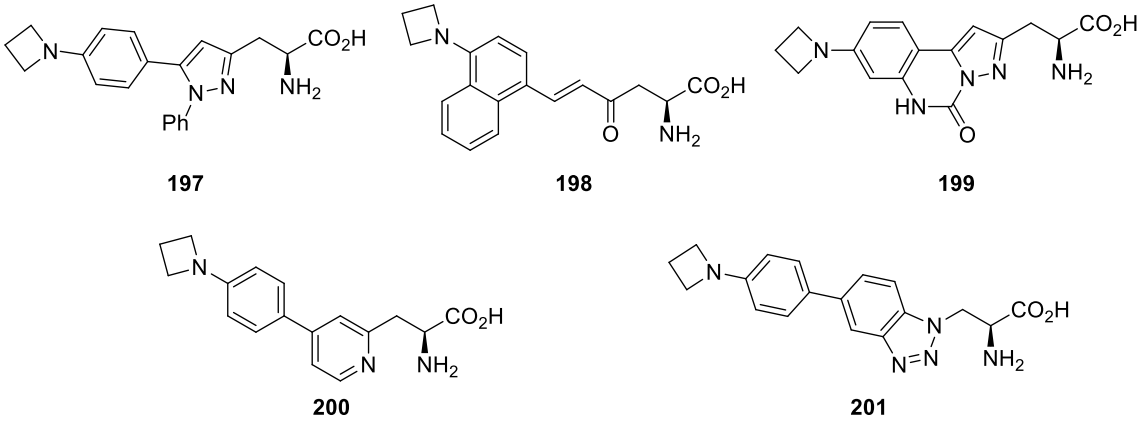
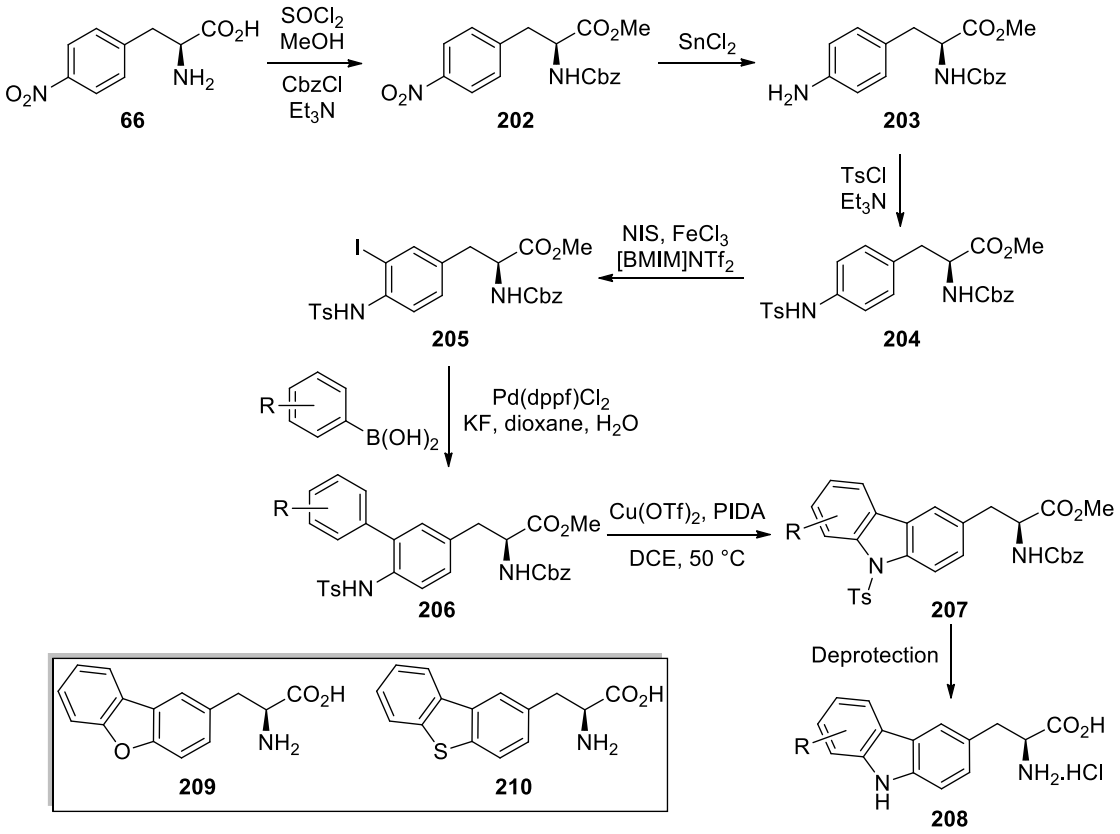


Figure 60 Potential azetidine functionalised target compounds.

Finally, the chemistry developed for the synthesis of **178** (**Scheme 63**), could be used for the synthesis of carbazole functionalised amino acid **208** from 4′-nitro-L-phenylalanine (**66**) (**Scheme 71**). This could be achieved by reduction of the protected analogue **202** with tin dichloride to give aniline **203**, followed by protection with TsCl to produce **204**. Then, a selective *ortho*-iodination reaction using NIS, iron(III) chloride and [BMIM]NTf_2 would give halogenated product **205**.[144] The Suzuki-Miyaura reaction could be used with iodide functionalised amino acid **205** and a range of boronic acids to give a library of polyaromatic derived amino acids **206**.[43,44] A ring-closing reaction

could then be carried out with the previously used conditions to give a collection of carbazole products **207**. Assessment of the photoluminescence properties of these carbazole derived amino acids **207** would be carried out after the removal of protecting groups. From the literature, it has already been shown that carbazole compounds possess favourable fluorescent properties.[147,148] It is also envisioned that dibenzofuran and dibenzothiophene derived amino acids **209** and **210** could also be prepared from L-tyrosine and 4′-mercapto-L-phenylalanine respectively.[118,141,149]



Scheme 71 Proposed synthesis of carbazole functionalised amino acid **208**.

2.3 Bioconjugation

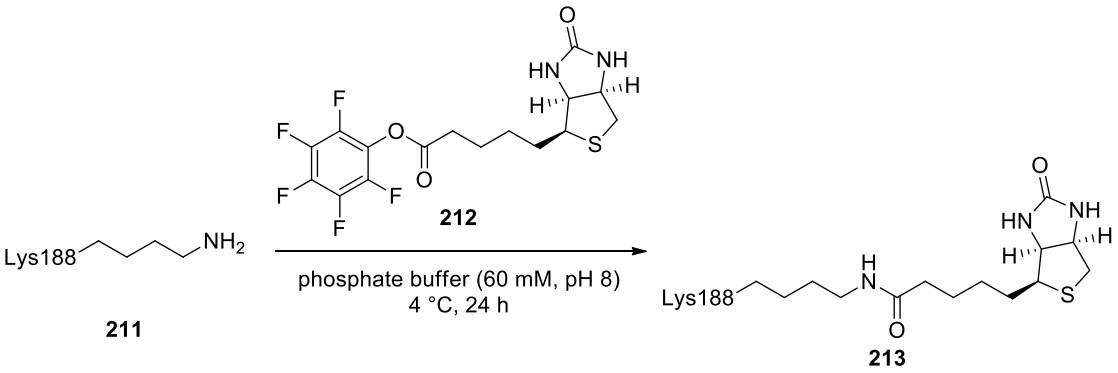
2.3.1 Introduction

Bioconjugation is the coupling of two compounds with a covalent bond, with one of these being a biomolecule.[150,151] This allows for the properties of the biomolecule to be improved. Polyethyleneglycol can be conjugated to therapeutic proteins to extend their *in vivo* half-lives.[152] Biomolecules can also be functionalised through bioconjugation for the introduction of organic fluorophores, quantum dots and biotin.[153–155] This facilitates the study of biological processes.

2.3.2 Bioconjugation with native amino acids

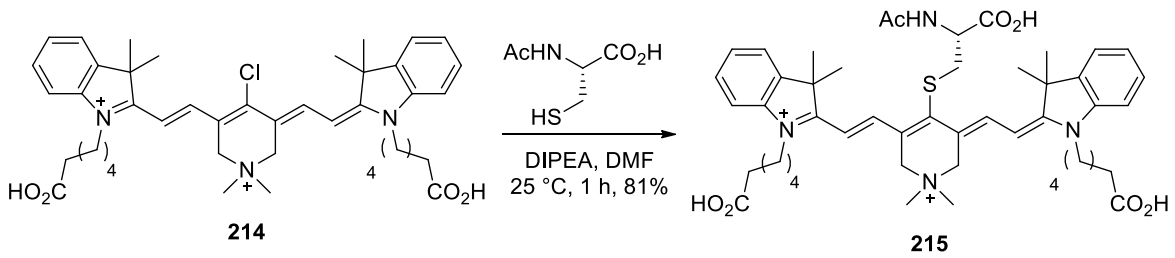
Bioconjugation of proteins can be achieved by coupling with side-chains of amino acids.[151] The amino acids: lysine, cysteine, tryptophan, histidine, tyrosine, arginine, aspartic acid, glutamic acid and methionine have all been targets for bioconjugation reactions.[151]

Lysine residue 188 of human kappa antibodies **211** was selectively modified with biotin using a fluorophenyl ester **212** (**Scheme 72**).[156] By performing the coupling at 4 °C in an aqueous phosphate buffer solution, biotinylation was selective even in the presence of approximately 40 other lysine residues. The selectivity achieved was due to the neighbouring His189 and Asp151 residues accelerating the rate of labelling at the Lys188 site. Further control of site selectivity was accomplished with lower reaction temperatures and performing the reaction with flow chemistry.



Scheme 72 Bioconjugation of the human kappa antibody.

The amino acid cysteine is an attractive target for bioconjugation owing to the very high nucleophilicity of the thiol side-arm and its low abundance in living systems.[151] Fluorophore QuatCy (**214**) has been found to react with cysteine (**Scheme 73**) and with cysteine residues in proteins at 37 °C.[157] It was shown that QuatCy is highly selective and will not react with lysine, proline and tyrosine residues. When QuatCy was reacted with the protein vimentin, an abundant intracellular protein with only one cysteine residue, Cys328, the adduct was fully formed within 1 h.[157]

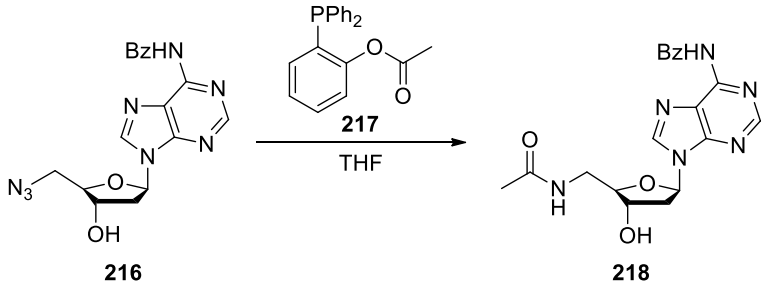


Scheme 73 Reaction of QuatCy (**214**) with *N*-acetamide cysteine.

2.3.3 Bioorthogonal reactions

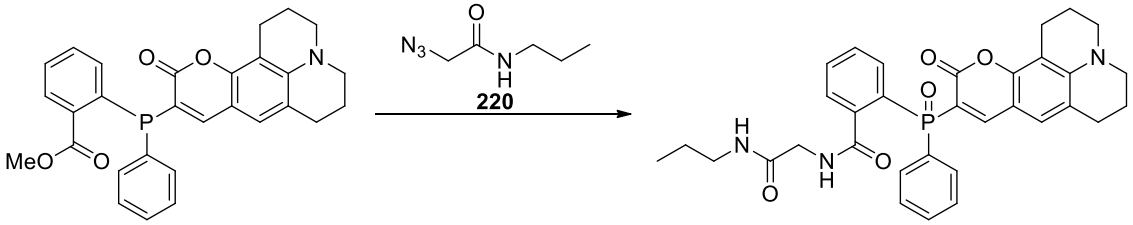
A major disadvantage of using native amino acids for bioconjugation is that if the targeted functionality is present multiple times within the protein, then control of site selectivity is very challenging.[156,158] Hence, bioorthogonal chemistry has been developed to allow the specific labelling of protein residues.

Staudinger ligation is one such method where a biomolecule can be selectively functionalised.[157,159,160] An azide-functionalised biomolecule can react with 2-(diphenylphosphino)phenyl acetate resulting in an amide bond. For example, the reaction of azidonucleoside **216** with phosphine **217**, gave **218** in 95% HPLC yield (**Scheme 74**).



Scheme 74 Staudinger ligation of azidonucleoside **216**.

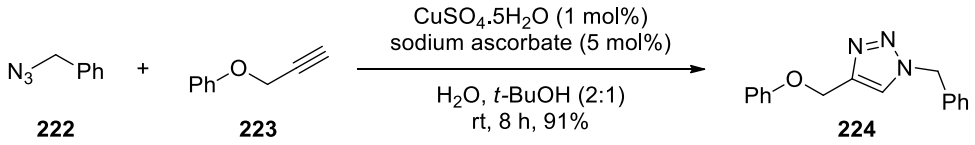
The use of this conjugation strategy has been highlighted with the ligation of phosphine dye **219** with protein murine dihydrofolate reductase.[161] For this protein, a native methionine residue had been replaced with an azidohomoalanine residue to allow for the Staudinger ligation to take place. The mechanism of this process is demonstrated with the reaction of **219** with **220** (**Scheme 75**).[161] Before the Staudinger reaction, the lone pair of electrons present on the phosphorus quenches the fluorescence. This **219** a quantum yield of 0.011, after the ligation amide **221** has a quantum yield of 0.65 in PBS solution.



219 **221**

Scheme 75 Staudinger ligation of phosphine **219** with "turn on" fluorescence.

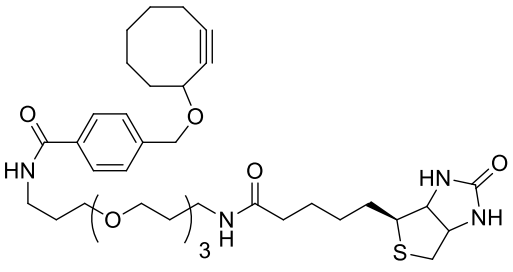
Another bioorthogonal reaction used extensively in bioconjugation coupling is the Huisgen cycloaddition with alkynes and azides, giving the corresponding triazole heterocycle.[153,162–166] The reaction can occur under very mild conditions and can be catalysed with copper. The reaction of azide **222** with alkyne **223** using a copper sulfate catalyst gave **224** in 91% yield (**Scheme 76**). Due to the rapid reaction, high yields, wide scope and generation of minimal byproducts, this process can be considered a click reaction.[167]



222 **223** **224**

Scheme 76 Huisgen cycloaddition of azide **222** and alkyne **223.**

As copper is toxic to bacterial and mammalian cells, the Huisgen reaction must occur under copper-free conditions if this reaction is to be used in biological systems. The use of cyclooctynes allows for triazole ring formation under metal-free conditions. This is due to the large ring strain present (18 kcal mol^{-1}).[165] Using a cyclooctyne functionalised with biotin **225** allowed for live-cell labelling of Jurkat cells incubated with an azide functionalised protein (**Figure 61**). No negative effects were observed of the cells during treatment with cyclooctyne **225**.

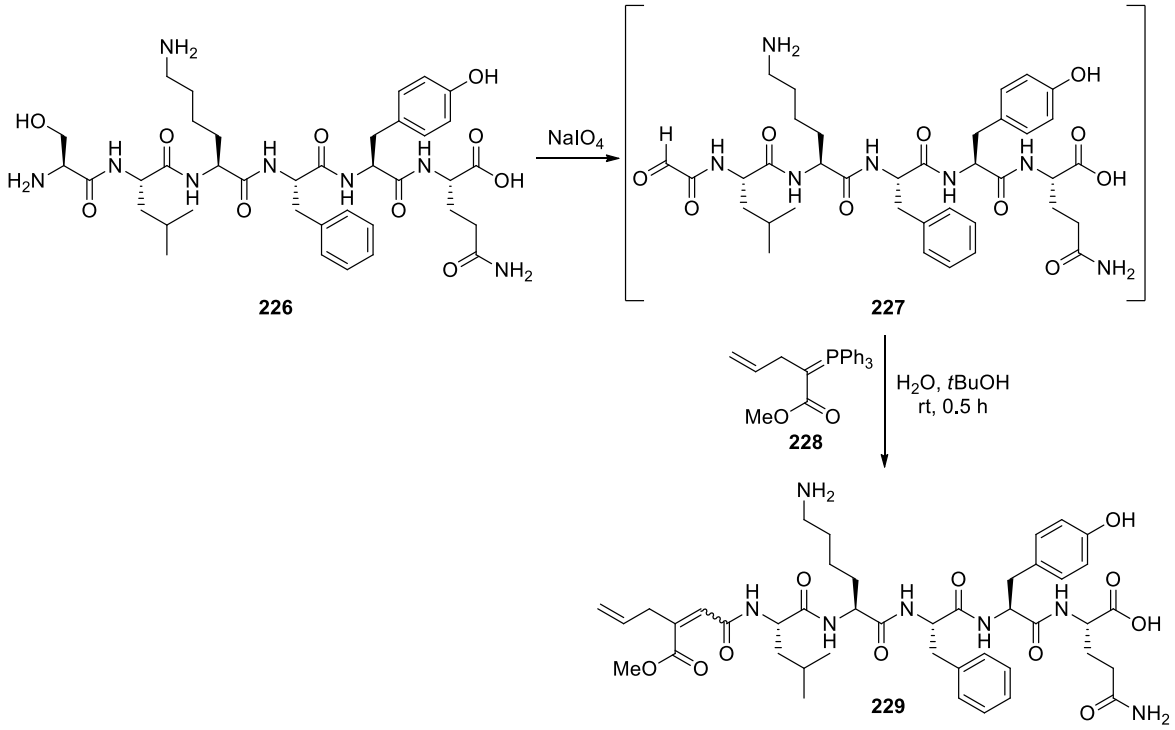


225

Figure 61 Cyclooctyne functionalised biotin **225**.

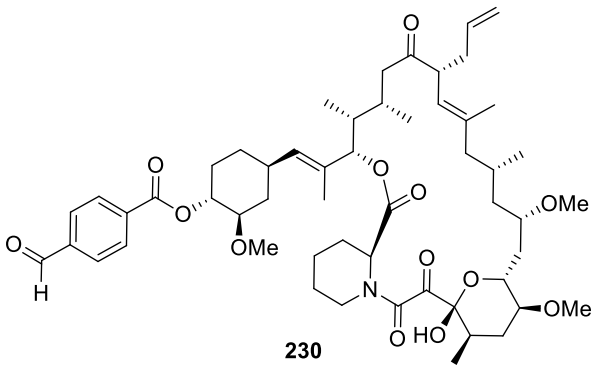
In addition to the Staudinger ligation and Huisgen reactions, the Wittig reaction has also been studied for bioconjugation. It has been reported that hexapeptide **226** could be functionalised with

Wittig reagent **228** (**Scheme 77**).[168] This is achieved with a Malaprade oxidation of the terminal serine residue of **226** with sodium periodate, which gave aldehyde **227**. The intermediate aldehyde could be used in the next reaction with **228** without any purification.[169,170] It was then shown that this methodology could also be applied to the protein interleukin 8, which also has an *N*-terminal serine residue.[168]

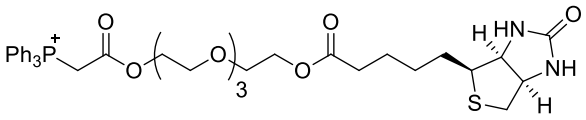


Scheme 77 Wittig modification of hexapeptide **226**.

It was also reported that aldehyde **230**, an analogue of the FK506 drug molecule, was coupled with biotin Wittig reagent **231** in a cellular environment (**Figure 62**).[171] HeLa cells were incubated with **230** for 8 h and then **231** was added. After 15 h, it was found that a successful Wittig coupling had taken place.



230



231

Figure 62 Aldehyde analogue of drug molecule FK506 **230** and biotin Wittig reagent **231.**

2.3.4 Aims of the project

From these excellent results, it was proposed that the HWE reaction could also be used in orthogonal bioconjugation reactions. Therefore, the aim of this project was to assess if a β-keto phosphonate ester analogue of **111** used earlier could also be used in bioconjugation. This will be investigated by synthesising a dipeptide phosphonate ester **232** and evaluating its performance in model HWE reactions with a range of aldehydes (**Figure 63**). It was then investigated if the use of electron-rich aldehydes for the HWE reaction would result in the synthesis of fluorescent charge-transfer enones. This would allow for the synthesis of fluorescent enone derived peptides for use in biological imaging.[74,128]

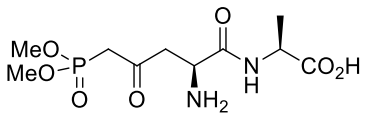
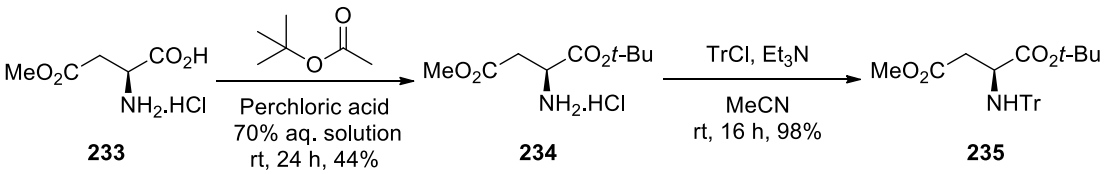


Figure 63 Dipeptide phosphonate ester **232**.

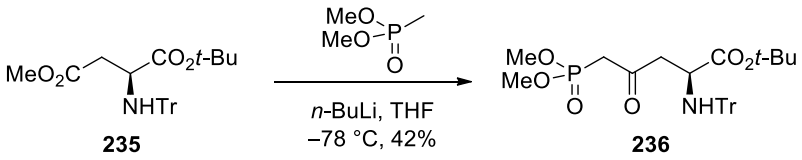
2.3.5 Synthesis

L-Aspartic acid β-methyl ester hydrochloride (**233**) was fully protected using acid-labile protecting groups (**Scheme 78**). This was achieved by the reaction of **233** with *tert*-butyl acetate and perchloric acid to give *tert*-butyl ester **234** in 44% yield.[172] The trityl protected amino acid **235** was prepared in 95% yield by reaction of **234** with trityl chloride in the presence of triethylamine in acetonitrile.



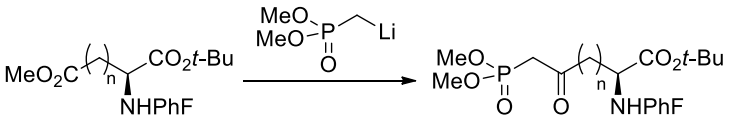
Scheme 78 Preparation of protected amino acid **235**.

Formation of β-keto phosphonate ester **236** from protected amino acid **235** was next investigated (**Scheme 79**). Using the previously mentioned conditions, 2.2 equivalents of dimethyl methylphosphonate, 2.3 equivalents of butyllithium, at -78 °C in THF resulted in a low yield of 42% of **236**. To improve the yield of **236**, different reaction conditions were trialled. A synthesis of **236** was reported when **235** was reacted with 4.5 equivalents of phosphorus anion in *tert*-butyl methyl ether, which gave a yield of 66%.[173] When the reaction was attempted using 4.5 equivalents of phosphorus anion in THF, a reduced yield of 31% for phosphonate ester **236** was obtained.



Scheme 79 Synthesis of phosphonate ester **236** in THF.

From the literature, the synthesis of β-keto phosphonate esters **238** from protected amino acids **237** was reported (**Table 13**).[174] When the reaction was first trialled in THF with the glutamate analogue and a reaction concentration of 0.5 M, a yield of 28% was achieved (entry 1). The reaction molarity was decreased from 0.5 M to 0.1 M and this resulted in an increased yield of 52% (entry 2). When the reaction was trialled in diethyl ether with a reaction concentration of 0.05 M, an improved yield of 84% was obtained (entry 3). The same transformation was then trialled on the aspartate analogue. The reaction was conducted in diethyl ether with a reaction concentration of 0.05 and a yield of 71% was obtained for the desired β-keto phosphonate ester (entry 4). It was then found that the same yield could be achieved when toluene was used as the reaction solvent with a higher reaction concentration of 0.1 M (entry 5).

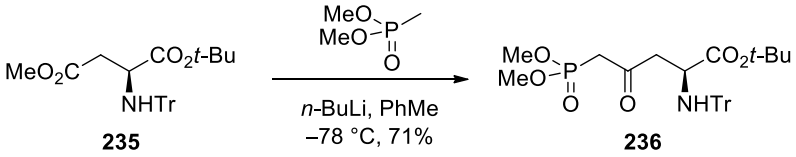


237 **238**

Entry	n	Solvent	M	Yield of 238
1	2	THF	0.5	28%
2	2	THF	0.1	52%
3	2	Et_2O	0.05	84%
4	1	Et_2O	0.05	71%
5	1	PhMe	0.1	71%

Table 13 Literature optimization for the synthesis of phosphonate ester **238**.

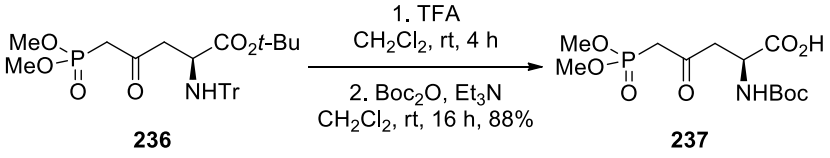
Therefore, from these literature results, the synthesis of β-keto phosphonate ester **236** was trialled in toluene. When amino acid **235** was subjected to these reaction conditions this gave **236** in 71% yield **(Scheme 80)**.



235 **236**

Scheme 80 Synthesis of phosphonate ester **236** in toluene.

For the β-keto phosphonate ester to be used in amide coupling reactions, the acid-labile *tert*-butyl ester group had to be removed and the trityl protecting group replaced. It was found that both protecting groups of **236** were removed with 50% TFA in dichloromethane after 4 h (**Scheme 81**). The deprotected intermediate was then reacted with di-*tert*-butyl dicarbonate in the presence of triethylamine, which gave **237** in 88% yield over the two steps.

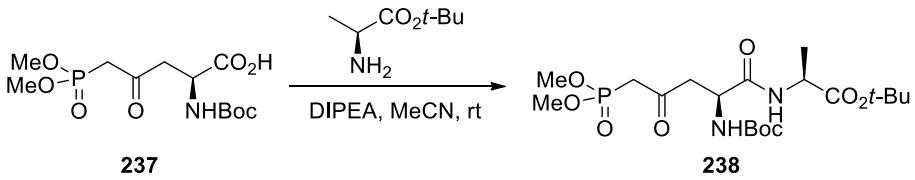


236 **237**

Scheme 81 Synthesis of Boc phosphonate ester **237**.

To investigate peptide coupling of phosphonate ester **237,** L-alanine *tert*-butyl ester hydrochloride was used as the coupling partner (**Table 14**). Various coupling reagents were then trialled to optimise this reaction. HBTU and HATU gave moderate yields for dipeptide **238** (entries 1 and 2). The use of the coupling reagent PyBOP® gave **238** in 68% yield after a reaction time of 16 h (entry 3). It was then found that the reaction time could be decreased from 16 h to 1 h (entry 4). The low

yields observed with HBTU and HATU were due to removal of the tetramethylurea (TMU) byproduct. TMU was removed with aqueous washes, but **238** also partitioned into the aqueous layer.

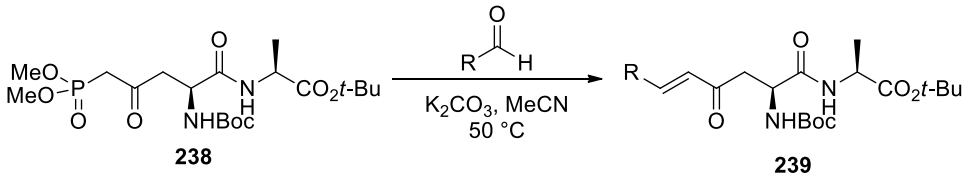


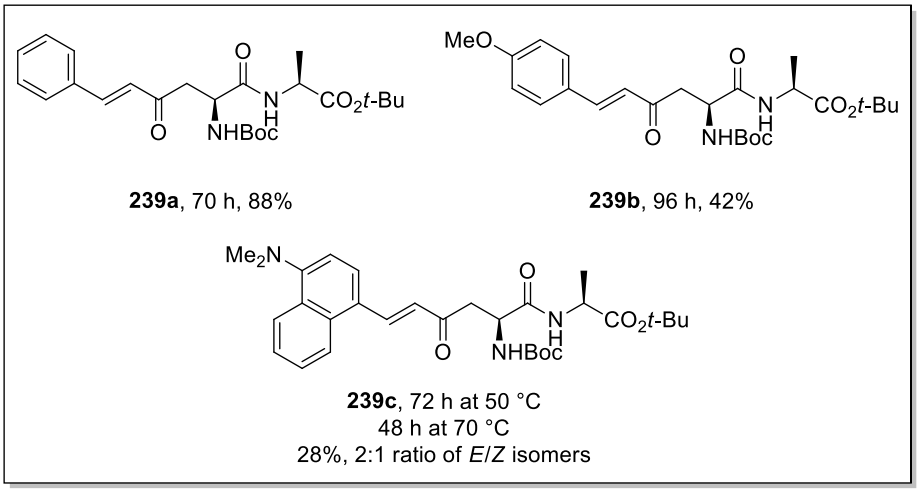
237 **238**

Entry	Coupling reagent	Reaction time (h)	Yield
1	HBTU	16	52%
2	HATU	16	53%
3	PyBOP®	16	68%
4	PyBOP®	1	71%

Table 14 Peptide coupling optimisation of phosphonate ester **238**.

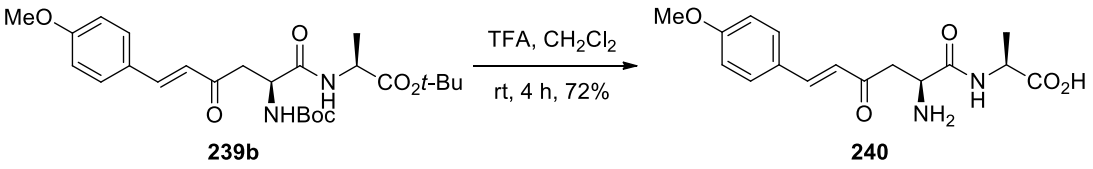
Dipeptide **238** was then trialled in HWE reactions with a range of benzaldehydes (**Scheme 82**). The reaction of **238** with benzaldehyde with standard conditions gave enone **239a** in 88% yield. When more electron-rich aldehydes were used the efficiency of this reaction decreased. For example, when 4-anisaldehyde was used this gave enone **239b** in 42% yield. Using the highly electron-rich aldehyde, 4-dimethylaminonaphthaldehyde, the reaction was very slow. To achieve full conversion, an extra equivalent of base, higher temperatures and extended reaction time were all required. This resulted in formation of **239c** in 28% yield with a 2:1 mixture of *E*/*Z* isomers.





Scheme 82 HWE reactions with dipeptide **238**.

To access deprotected dipeptide **240**, protected enone **239b** was reacted with 50% trifluoroacetic acid in dichloromethane, this gave **240** in 72% yield (**Scheme 83**).



239b **240**

Scheme 83 Deprotection of enone **239b**.

As this approach had generated a chromophore with a potential charge-transfer motif, the photoluminescent properties were investigated. The absorbance and emission of **240** were recorded in methanol (10 µM) (**Figure 64**). From UV/Vis spectroscopy it was found that peptide **240** absorbed strongly at 324 nm. When **240** was excited at 326 nm no fluorescence was detected. The emission spectrum of **240** was compared to a control spectrum of methanol and from this, it was concluded that only Raman scattering was being detected.

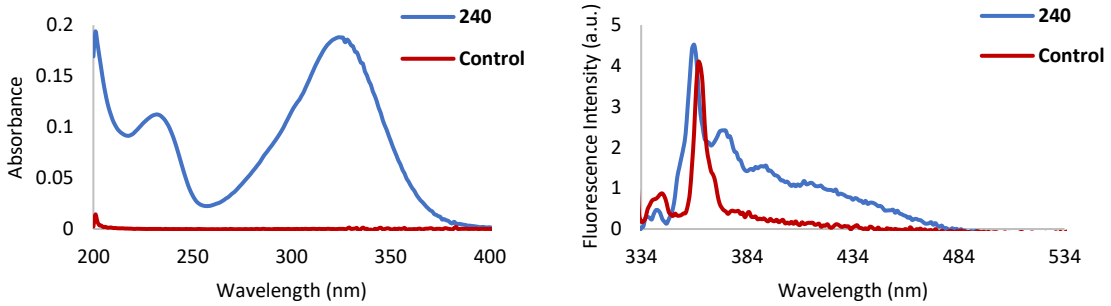


Figure 64 Absorption and emission spectra of **240** in methanol (10 µM), excited at 326 nm.

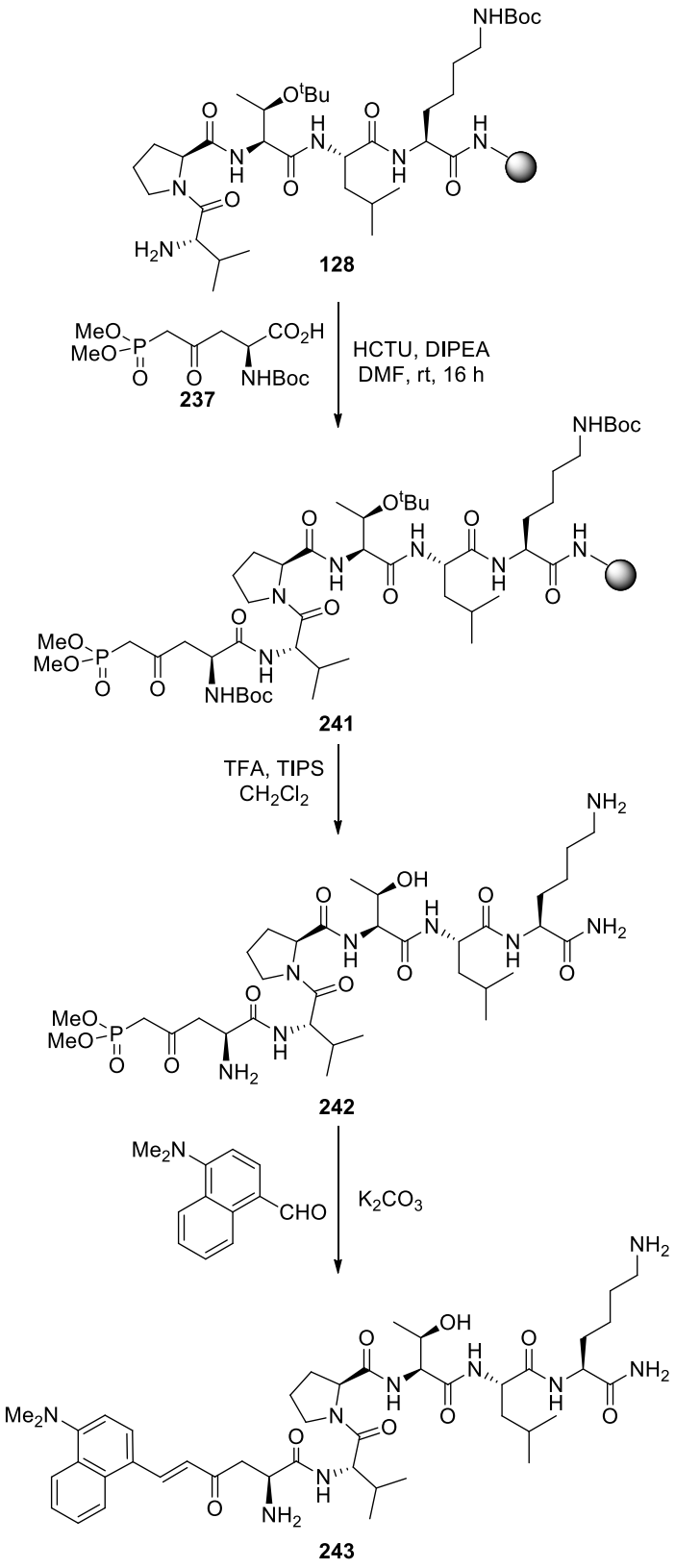
2.3.6 Summary

In conclusion, β-keto phosphonate ester dipeptide **238** was synthesised and shown to be an effective substrate for model HWE reactions, giving enones **239a**–**239c**. This demonstrates that the HWE reaction could be used in bioconjugation reactions, analogous to the previously reported Wittig bioconjugation reactions.[168,171] However, some optimisation, particularly for electron-rich aldehydes is required.

2.3.7 Future work

In order for the HWE reaction to be used in bioconjugation, the reactions conditions need to be optimised. More forcing conditions using barium hydroxide as base, in aqueous THF have been reported in the literature.[175,176] However, as the aim of this work is to perform HWE reaction for bioconjugation, it would be preferable to use the mildest and non-toxic conditions possible, as then the reaction could take place within living cells. There have been multiple reports of HWE reactions being carried out in water, using potassium carbonate as base, giving the enones in good yield.[177–179] In the event of solubility issues, the HWE reaction has also been carried out in water/IPA and water/THF mixtures[180,181]

Once optimised this procedure could be used to couple proteins or peptides with aldehyde functionalised compounds through a HWE reaction. From previous group work, it has both been shown that enone **87** has very favourable photoluminescent properties and that peptides are effective at imaging human fibroblast cells.[74,128] In the literature it has been shown that Wittig bioconjugation reactions can take place within the cellular environment.[170] Therefore, it is envisioned that Boc-protected phosphonate ester **237** could be installed upon cell-penetrating peptide **128**, to give **241** (**Scheme 84**). After deprotection and resin cleavage, peptide **242** could be incubated into a cell. The subsequent addition of aldehydes, such as 4-dimethylaminonaphthaldehyde would form enone **243** in the cellular environment with turn-on fluorescence for cell imaging.



Scheme 84 Proposed synthesis of fluorescent enone hexapeptide.

2.4 Acid-mediated stereoselective synthesis of 2,6-*trans*-4-oxopiperidines from amine-functionalised enones

2.4.1 Introduction

Both *trans*-2,6-dialkyl-4-oxopiperidines and *trans*-2,6-dialkylpiperidines are found in natural products and in bioactive compounds (**Figure 65**). For example (+)-myrtine (**244**) is a naturally occurring bicyclic alkaloid isolated from the shrub *Vaccinium myrtillus* (Ericaceae) containing the *trans*-2,6-alkyl-4-oxopiperidine ring system.[182,183] (−)-Cermizine C (**245**), isolated from the moss *Lycopodium cernuum* is another bicyclic alkaloid that has a *trans*-2,6-dialkylpiperidine ring system.[184] The alkaloid (−)-solenopsin A (**246**), which consists of a *trans*-2,6-dialkylpiperidine ring is believed to be the active compound in the venom of fire ants.[185,186] Finally the *trans*-2,6-dialkylpiperidine ring systems have also been found in highly bioactive compounds. For example, himbacine (**247**) has shown activity in the treatment of Alzheimer's disease.[187]

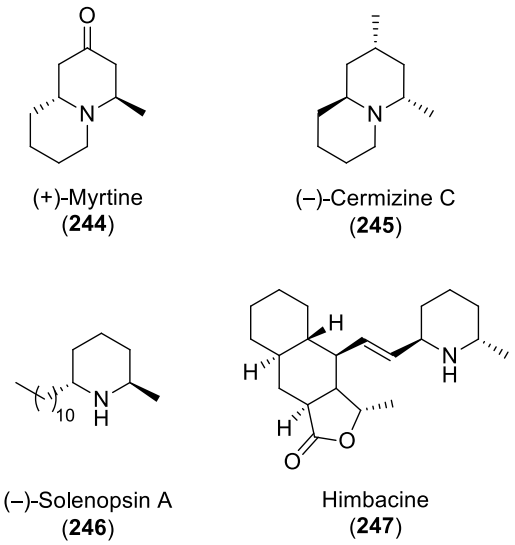
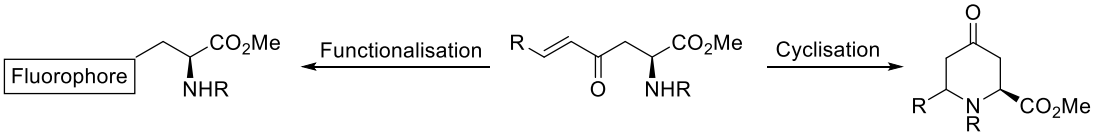


Figure 65 Structures of *trans*-2,6-dialkyl-4-oxopiperidines and *trans*-2,6-dialkylpiperidines natural products and bioactive compounds.

As well as undergoing functionalisation to afford fluorescent amino acids **249**, the previously mentioned enone derived amino acids **248** can also undergo a 6-*endo*-*trig* cyclisation process (**Scheme 85**). This is via an *aza*-1,4-conjugate addition of the enone system with the α-nitrogen atom to give the cyclised product. The products of these transformations are 2,6-disubstituted-4-oxopiperidines **250**. After cyclisation, the ketone can be functionalised to give the 2,4,6-trisubstituted piperidines products.

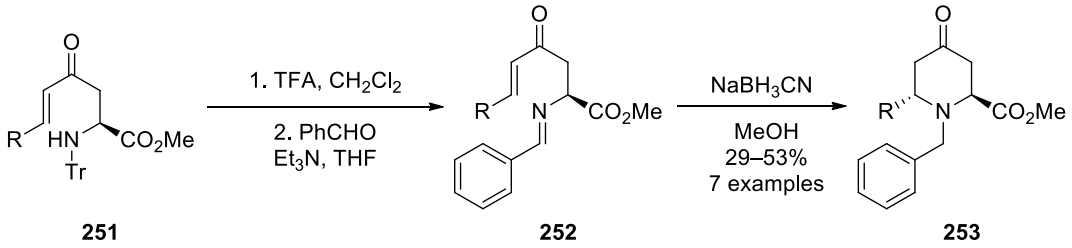


249 **248** **250**

Scheme 85 6-*endo-trig* cyclisation via an *aza*-1,4-conjugate addition.

2.4.2 Previous work in the Sutherland group

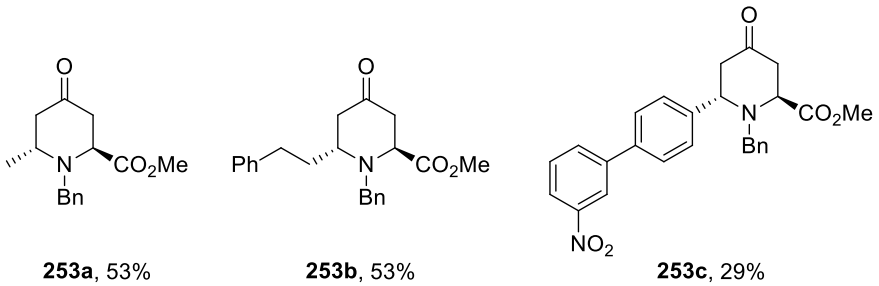
Work within the Sutherland group began by exploring a reductive amination process to induce a 6-*endo-trig* cyclisation to give the corresponding 4-oxopipecolic acids (**Scheme 86**).[84] Starting from the trityl protected amino acid **251,** cyclisation was achieved first by deprotection of the trityl-protecting group under mildly acidic conditions that gave the amine. This amine was then protected with benzaldehyde, which gave **252**. A chemoselective reduction of the imine in the presence of a ketone functional group induced cyclisation. Various reducing conditions were tested, and it was found that the sodium cyanoborohydride in methanol was the most efficient, yielding the *trans*-2,6-disubstituted-4-oxopipecolic acid product **253** after 1 h at room temperature in good yields.



251 **252** **253**

Scheme 86 Synthesis of *trans*-2,6-disubstituted-4-oxopipecolic acid **253**.

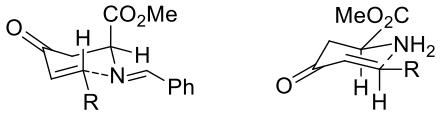
The scope of the reaction was broad with both alkyl and aryl-substituted enones being cyclised to pipecolic acids **253a** and **253b.** Generally, good yields were obtained over the three steps with only the very large and highly conjugated substituents giving low yields of the cyclised products **253c** (**Figure 66**).



253a, 53% **253b**, 53% **253c**, 29%

Figure 66 4-Oxopipecolic acid compounds **253a**–**253c**, produced via reductive amination.

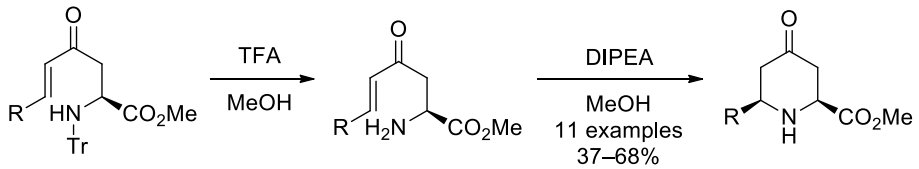
It was proposed that the diastereoselectivity observed was due to a Zimmerman-Traxler chair-like transition state with both the benzyl and R-group adopting in equatorial positions and the methyl ester in an axial position **254** (**Figure 67**). It was envisioned that the absence of the nitrogen protecting group would allow both the ester and R-group to occupy the favoured equatorial positions **255**. This allows for the *cis*-2,6-disubstituted-4-oxo-pipecolic acid to become the favoured product of the *aza*-1,4-conjugate cyclisation.



254 **255**

Figure 67 Transition states with *N*-protecting group **254** and without *N*-protecting group **255**.

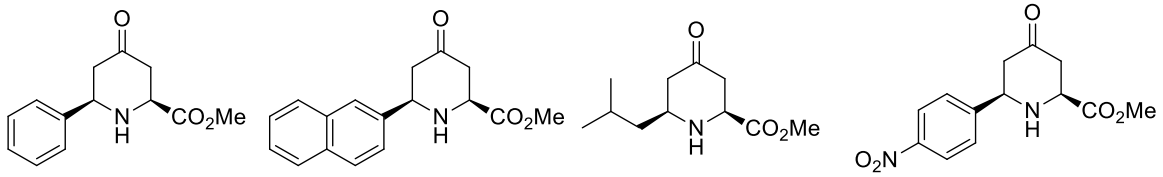
To investigate this hypothesis, trityl protected enone **255** was deprotected with trifluoroacetic acid and this gave free amine **256** (**Scheme 87**).[188] Then a range of different bases were tested to induce the 6-*endo*-*trig* cyclisation. Butyl lithium and lithium bis(trimethylsilyl)amide both yielded no product. The inorganic base sodium carbonate yielded no product when the reaction was carried out in dichloromethane but gave a modest yield of 41% of **257** (of both diastereoisomers) when performed in methanol. When the organic base, diisopropylethylamine was used the yield increased to 85% and a diastereotopic ratio of 75:25 in favour of the *cis* isomer was obtained with a phenyl substituent. Hence, without the *N*-protecting group present, the *cis* isomer becomes the favoured over the *trans* isomer.



255 **256** **257**

Scheme 87 Base-mediated cyclisation of enone **255**.

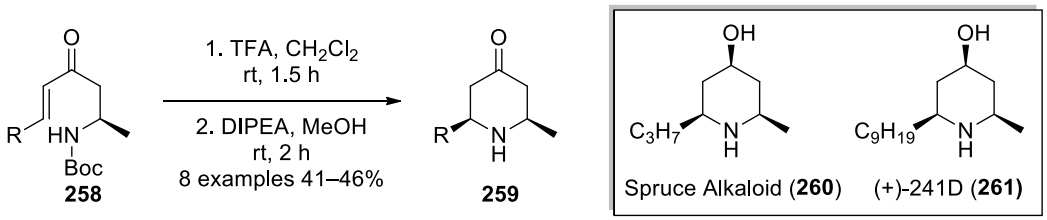
After the reaction was developed, it was shown that a wide range of *cis*-2,6-disubstituted-4-oxopipecolic acids **257a**–**257d** could be produced (**Figure 68**). Enones with aryl and alkyl side-chains were suitable substrates for this 6-*endo*-*trig* cyclisation process giving good isolated yields of the cyclised product. Electron deficient aryl substrates gave the lowest yields **257d** for this process.



257a, 56% **257b**, 66% **257c**, 68% **257d**, 37%

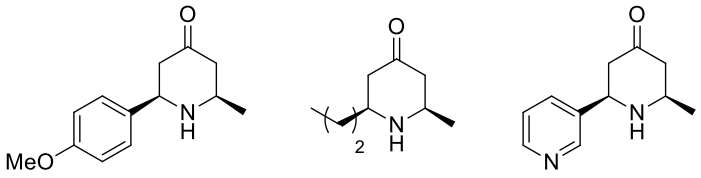
Figure 68 Base-mediated cyclisation products **257a**–**257d**.

It was proposed that *cis*-2,6-disubstituted-4-oxopiperidines **259** could be prepared using the same reaction conditions (**Scheme 88**). These compounds would be useful synthetic intermediates for the natural products, spruce alkaloid **260** and (+)-241 D **261**.[189] While the *cis*-2,6-disubstituted-4-oxopiperidines were produced under basic conditions it was observed that this reaction was less diastereoselective, giving approximately a 1:1 mixture of both diastereomers.



Scheme 88 Base-mediated cyclisation to 2,6-*cis*-4-oxopiperidines **259** from **258** and the natural products spruce alkaloid **260** and (+)-241D **261**.

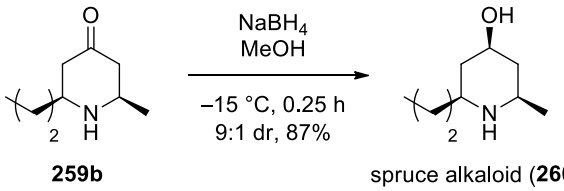
Nevertheless, from this transformation, a library of *cis*-2,6-disubstituted-4-oxopiperidines were synthesised in yields from 41% to 46% (**Figure 69**). Alkyl **259a**, aryl **259b** and heterocyclic **259c** side-arms were all tolerated in this transformation.



259a, 46% **259b**, 43% **259c**, 41%

Figure 69 *cis*-2,6-Disubstituted-4-oxopiperidines products from base mediated cyclisation.

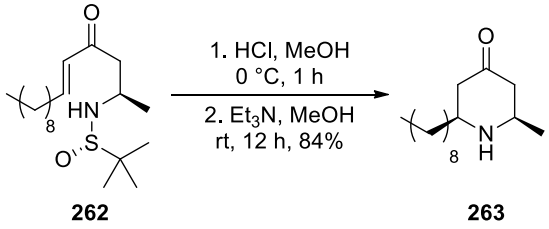
Finally, the utility of this methodology was demonstrated with the synthesis of (+)-241D and spruce alkaloid. **259b** underwent a diastereoselective reduction with sodium borohydride to give spruce alkaloid **260** in an excellent yield of 87% (**Scheme 89**). A yield of 84% was for the preparation of **261**.



Scheme 89 Synthesis of **260** by diastereoselective reduction with sodium borohydride.

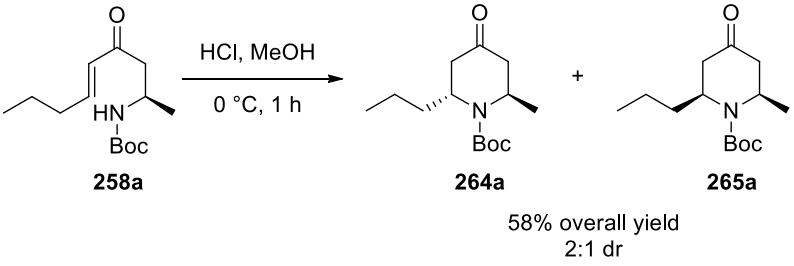
2.4.3 Aims of the project

In optimising conditions for the synthesis of *cis*-2,6-disubstituted-4-oxopiperidines **259**, various cyclisation conditions were trialled. A similar cyclisation process was reported with sulfoxide **262** (**Scheme 90**).[190] It was reported that the sulfoxide protecting group was removed using one equivalent of hydrochloric acid followed by 6-*endo*-*trig* cyclisation with triethylamine and this gave cyclised product **263** in 84% yield.



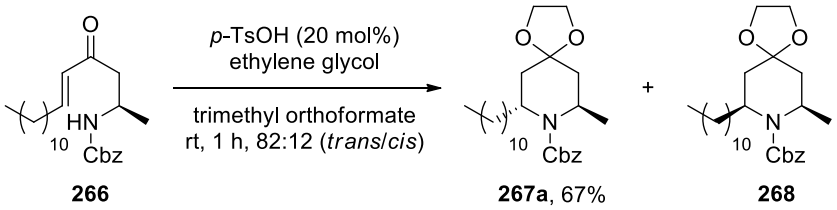
Scheme 90 Cyclisation of sulfoxide protected enone **262.**

As the above conditions resulted in the efficient transformation of **262** to **263** with an *aza*-1,4-conjugate addition, it was therefore thought that the same reaction conditions might be suitable for the cyclisation of Boc protected enone **258** to **259**. However, when **258a** was treated with hydrochloric acid at room temperature for one hour in methanol there was no Boc deprotection. Instead, it was found that a cyclisation reaction had occurred, which gave a mixture of *trans*-2,6- and *cis*-2,6-dialkyl-4-oxopiperidines **264a** and **265a** (**Scheme 91**). These compounds were isolated, and it was found that the combined yield of both isomers was 58% in a 2:1 ratio in favour of **264a**.[128]



Scheme 91 Attempted Boc deprotection of **258a**.

While an unexpected result, acid-mediated 6-*endo*-*trig* cyclisation reactions are known in the literature. It was reported that *N*-Cbz protected enone **266** underwent an acid-mediated intramolecular *aza*-Michael reaction which gave *trans*-2,6-disubstituted-4-ketalpiperidine **267a** in 67% yield (**Scheme 92**).[191] It was hypothesised that the first step of the reaction is the formation of the corresponding ketal, the size of the ketal is critical in determining the selectivity of the reaction. When ethylene glycol is used, this forms the cyclic 1,3-dioxolane ketal and this imposes steric control in the transition state to give a highly selective reaction.



266 **267a**, 67% **268**

Scheme 92 Acid-mediated cyclisation of Cbz protected enone **266**.

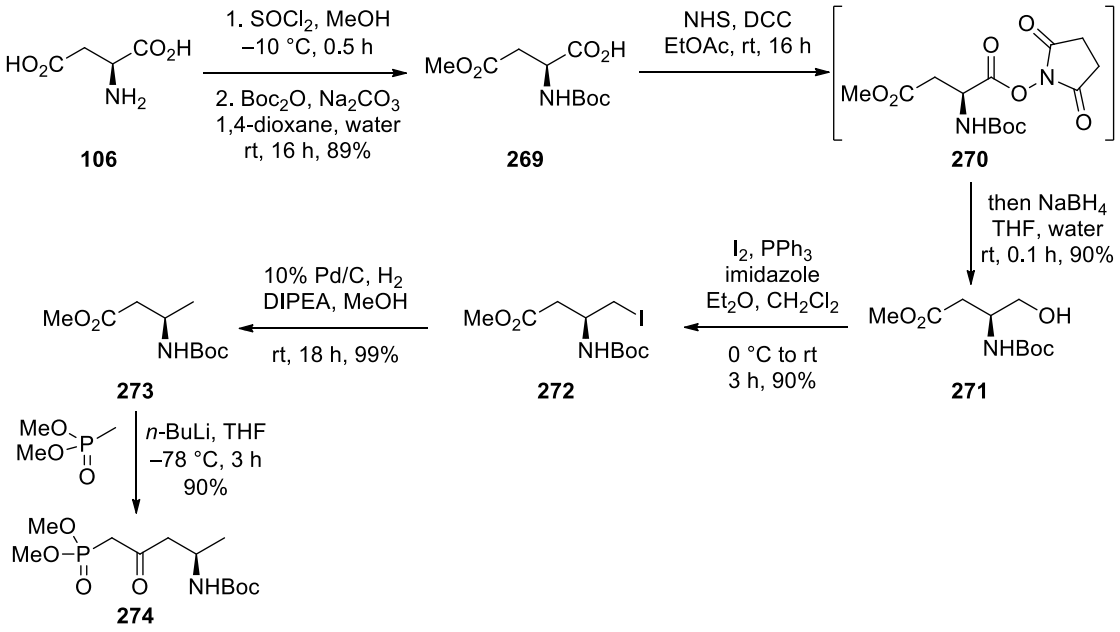
The aims of this project were to develop methodology for the acid-mediated cyclisation on Boc-protected enones **258**. Once optimised cyclisation conditions had been developed the synthesis of (+)-myrtine (**244**) and (−)-solenopsin (**246**) was then investigated.

2.4.4 Synthesis of Boc-protected enones

The enones required to explore this reaction were synthesised from the corresponding β-keto phosphonate ester **274**, which was prepared in a seven-step synthesis from L-aspartic acid (**106**) (**Scheme 93**). At −10 °C, the β-carboxylic acid of **106** was selectively converted to the methyl ester with thionyl chloride with no esterification occurred at the α-carboxylic acid. Boc protection under basic conditions with di-*tert*-butyl dicarbonate gave **269** in 89% yield over the two-steps.[192] Reduction of the remaining carboxylic acid group was next investigated.

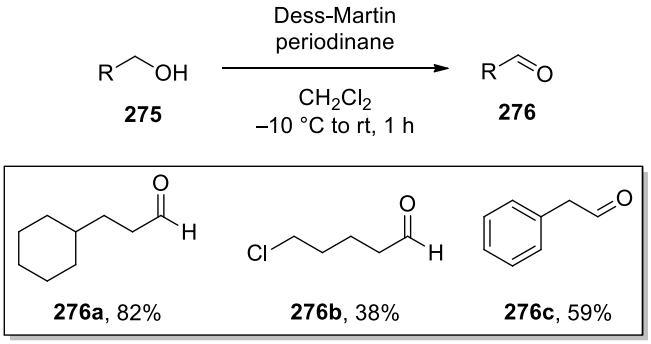
This was achieved by reaction of **269** with *N*-hydroxysuccinimide and *N*,*N*′-dicyclohexylcarbodiimide and this gave ester **270**. It was reported that **270** could be reduced with sodium borohydride.[193] It was found the reaction of **270** with sodium borohydride gave alcohol **271** in a 90% yield over the two steps. Using a literature procedure **271** was converted to alkyl-iodide **272** in a Appel reaction with iodine and triphenylphosphine with 90% yield.[192] Using a previously reported procedure hydrodehalogenation of **272** with palladium on carbon gave ester **273** in 99% yield.[194] It should be noted that diisopropylethylamine was added to quench the hydrogen iodide formed during the reaction and prevent poisoning of the Pd/C catalyst. β-Phosphonate ester **274** was prepared from **273** using standard conditions. It was found that **274** could be prepared in THF with 90% yield.[173,174] While the synthesis of **274** required, it was highly

efficient (64% overall yield) and therefore multi-gram preparation of this intermediate was possible.



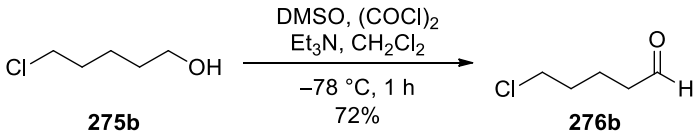
Scheme 93 Synthetic route to phosphonate ester **274** from L-aspartic acid (**106**).

With developed HWE conditions, **274** used in reactions with various carbonyl compounds and this gave a small library of enones. The majority of the carbonyl compounds tested were commercially available, with only **276a** and **276b** had to be prepared beforehand. Compounds **276a** and **276b** were prepared from commercially available alcohols **275a** and **275b** (**Scheme 94**). Phenylacetaldehyde **276c** was also prepared beforehand from benzyl alcohol **276c** for the HWE as this aldehyde has a limited shelf-life. Originally all these aldehydes were synthesised with Dess-Martin periodinane. This resulted in the formation of **276a** and **276c** in high yield but even with multiple attempts **276b** was only isolated in 38% yield



Scheme 94 Dess-Martin oxidation to give aldehydes **276a**–**276c**.

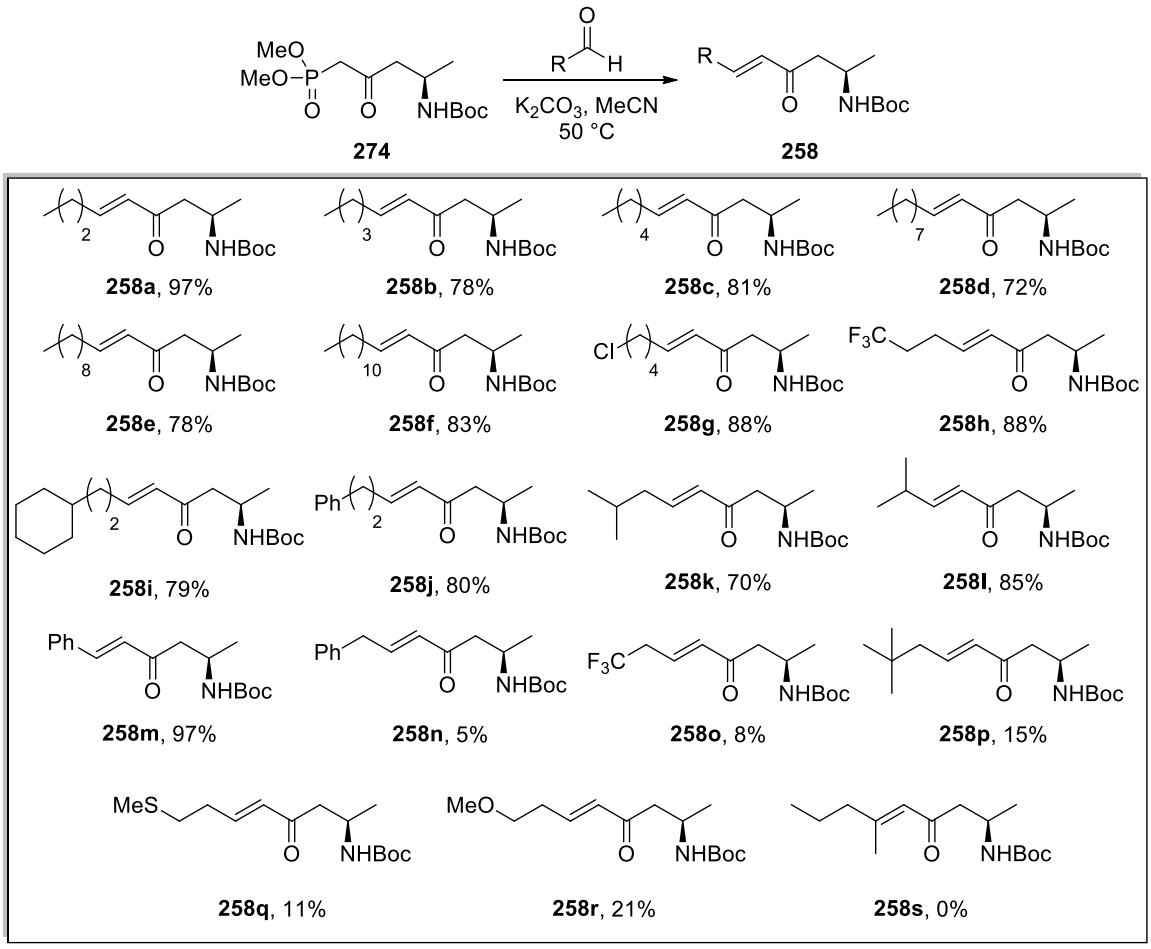
As the use of Dess-Martin periodinane in preparing **276b** resulted in low yield, different oxidations conditions were investigated. It was found that the Swern oxidation gave **276b** in high yield from **275b** (**Scheme 95**).



275b **276b**

Scheme 95 Swern oxidation of alcohol **275b** to give aldehyde **276b**.

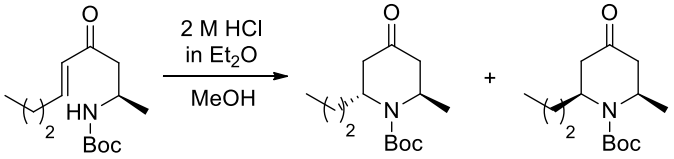
It was found that **274** was an excellent substrate for the HWE reaction, allowing for a small library of enones to be prepared (**Scheme 96**). In all the reactions only formation of the *E*-isomer was observed. The reaction of **274** with *n*-alkyl aldehydes gave the corresponding enones **258a**–**258f** in 72–97% yield. It was also found that functional aldehydes, such as **276b** could be used in this reaction and this gave chlorinated enone **258g** in 88% yield. The biologically relevant trifluoromethyl functional group was also tolerated in this reaction with 4,4,4-trifluorobutanal and this gave enone **258h** in 88% yield. Enones containing ring systems **258i**, **258j** and **258m** could all be prepared from **274** with excellent yields. Branched aldehydes were also tolerated in this reaction with isovaleraldehyde and isobutyraldehyde with **274** which gave enones **258k** and **258l.** On the other hand, when **274** was reacted with phenylacetaldehyde and 3,3,3-trifluoropropanal very low yields for enones **258n** and **258o** were given. This was due to base catalysed polymerisation of the aldehyde reagents.[195] Attempted synthesis of enone **258p**, from 3,3-dimethylbutanal and **274** gave yield of 15%, it was proposed that steric factors resulted in the low yield of this reaction. Low yields were also obtained for functionalised enones **258q** and **258r**. The HWE reaction was attempted with **274** and an alkyl ketone. When pentan-2-one was used in this reaction no conversion to enone **258s** was observed and only starting materials were returned from the reaction mixture. Only enones that were prepared in high yield were used as substrates in the subsequent acid-mediated 6-*endo*-*trig* reaction.



Scheme 96 Synthesised enones **258a**–**258s** from HWE reaction with **274**.

2.5.5 Optimisation and substrate scope of the acid-mediated 6-*endo*-*trig* reaction

To optimise the conditions for this reaction, alkyl enone **258a** was chosen as the model substrate (**Table 15**). As mentioned previously, treatment with one equivalent of hydrochloric acid at room temperature for 1 hour gave both diastereoisomers with a combined yield of 58% (entry 1). Increased amounts of hydrochloric acid led to decomposition and removal of the Boc group (entry 2). Whereas, when a catalytic amount (50 mol%) of hydrochloric acid was used this gave an incomplete reaction and a reduced yield of 40% (entry 3). Addition of the acid at 0 °C and slowly warming the reaction to 30 °C, gave a cleaner reaction and with a greater overall yield of both diastereomers (entry 4). A marginal gain in the combined yield was observed when the acid was added at −78 °C and then slowly warmed to room temperature (entry 5). On performing the reaction at reduced temperature, whilst there was an increase in yield there was no improvement in the selectivity of the reaction. However, under these improved reaction conditions, this allowed isolation of **264a** in a 49% yield.



258a **264a** **265a**

Entry	HCl (eq.)	Temperature (°C)	Time (h)	Combined Yield (%)[a]
1	1	20	1	58
2	5	20	1	0
3	0.5	20	3	40
4	1	0 to 20	2	75
5	1	−78 to 20	2	82

Table 15 Optimisation of acid-mediated 6-*endo-trig* cyclisation with **258a**. Reactions performed on a 0.04 M concentration. [a]Combined yield of both diastereoisomers after column chromatography.

To gain further understanding of this reaction, the cyclisation reaction of **258a** was monitored by ^{1}H NMR spectroscopy (**Figure 70**). From this, it was found **258a** was fully consumed within 2 h. Furthermore, it was observed **264a** forms first but over time the amount of **264a** decreases and the amount of **265a** increases.

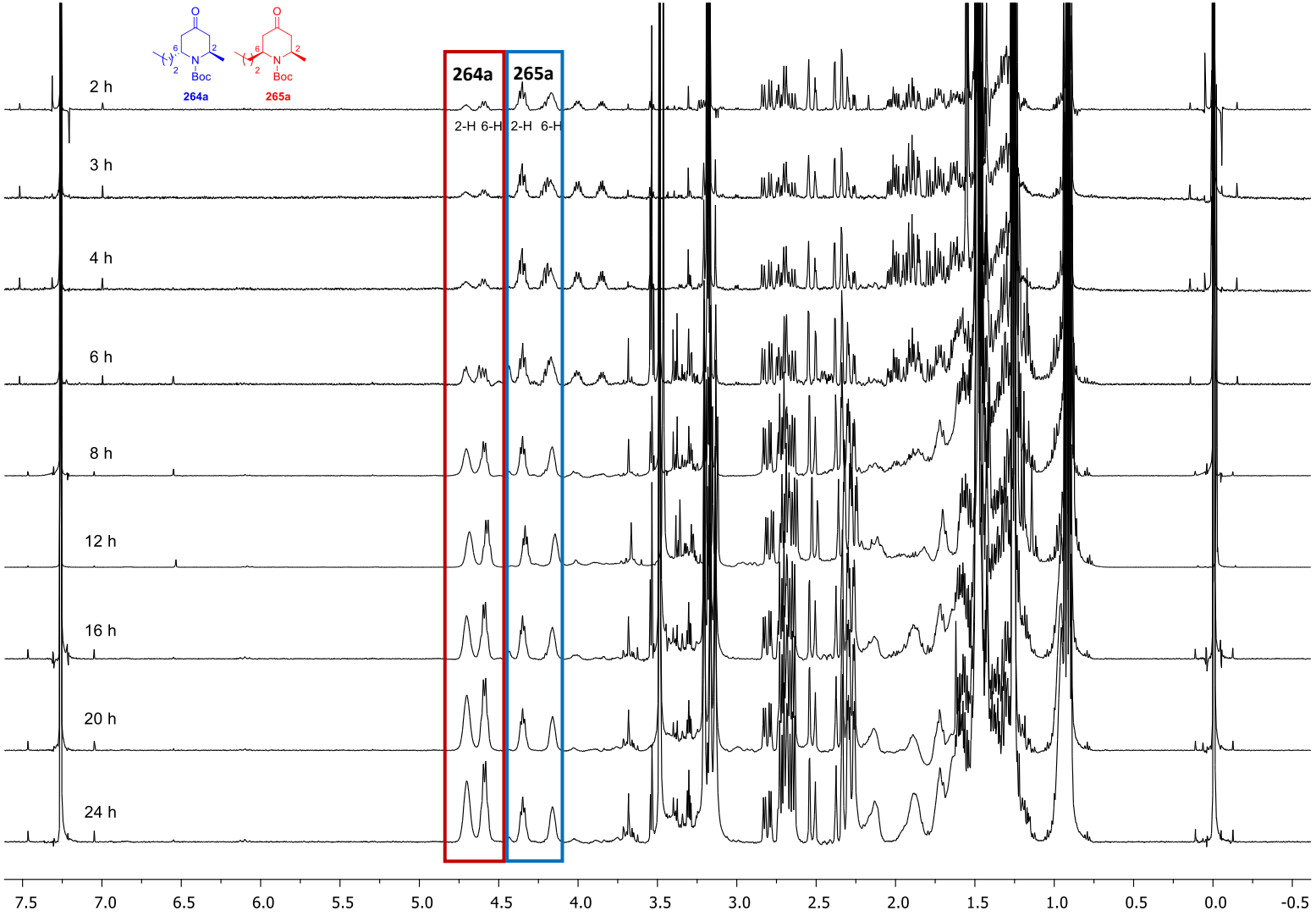


Figure 70 ^{1}H NMR spectra of **258a** subjected to reaction conditions for 24 hours.

Initially, a ratio of 3:1 in favour of *trans*-isomer **264a** was measured (**Figure 71**). As the reaction progressed the quantity of *cis* increased and after 8 h there was approximately a 1:1 ratio of **264a** to **265a**. After 24 h the reaction predominantly gave **265a** in a 2:1 ratio. The ratio marginally increased to 2.3:1 after 48 h of reaction.

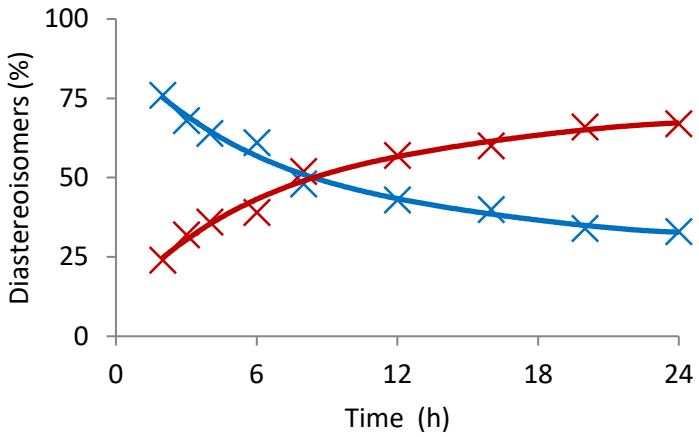
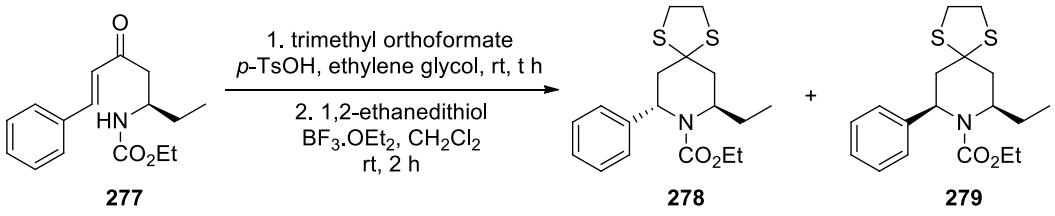


Figure 71 Plot of diastereomeric ratio of **264a** (blue), **265a** (red) and reaction time.

The same phenomenon has been previously reported with the cyclisation of enone **277** (**Table 16**).[191] It was postulated that the formation of *trans* isomer **278** occurred first, followed by epimerisation to the more stable *cis* isomer **279** with either a retro-Mannich or a retro-Michael reaction.



277 **278** **279**

t (h)	278 (%)	279 (%)
0.3	85	15
24	40	60

Table 16 Formation of **278** and **279** with reaction time.[191]

Further control experiments were performed by subjecting the separated **264a** and **265a** compounds to the reaction conditions for six hours (**Figure 72**). After this time, both isomers showed conversion to the other diastereomer, but this was more pronounced for the less stable *trans*-isomer. **264a** was converted to a mixture of the two isomers in a 2:1 ratio in favour of **264a**. While **265a** gave a final ratio of 5:1 in favour of **265a**.

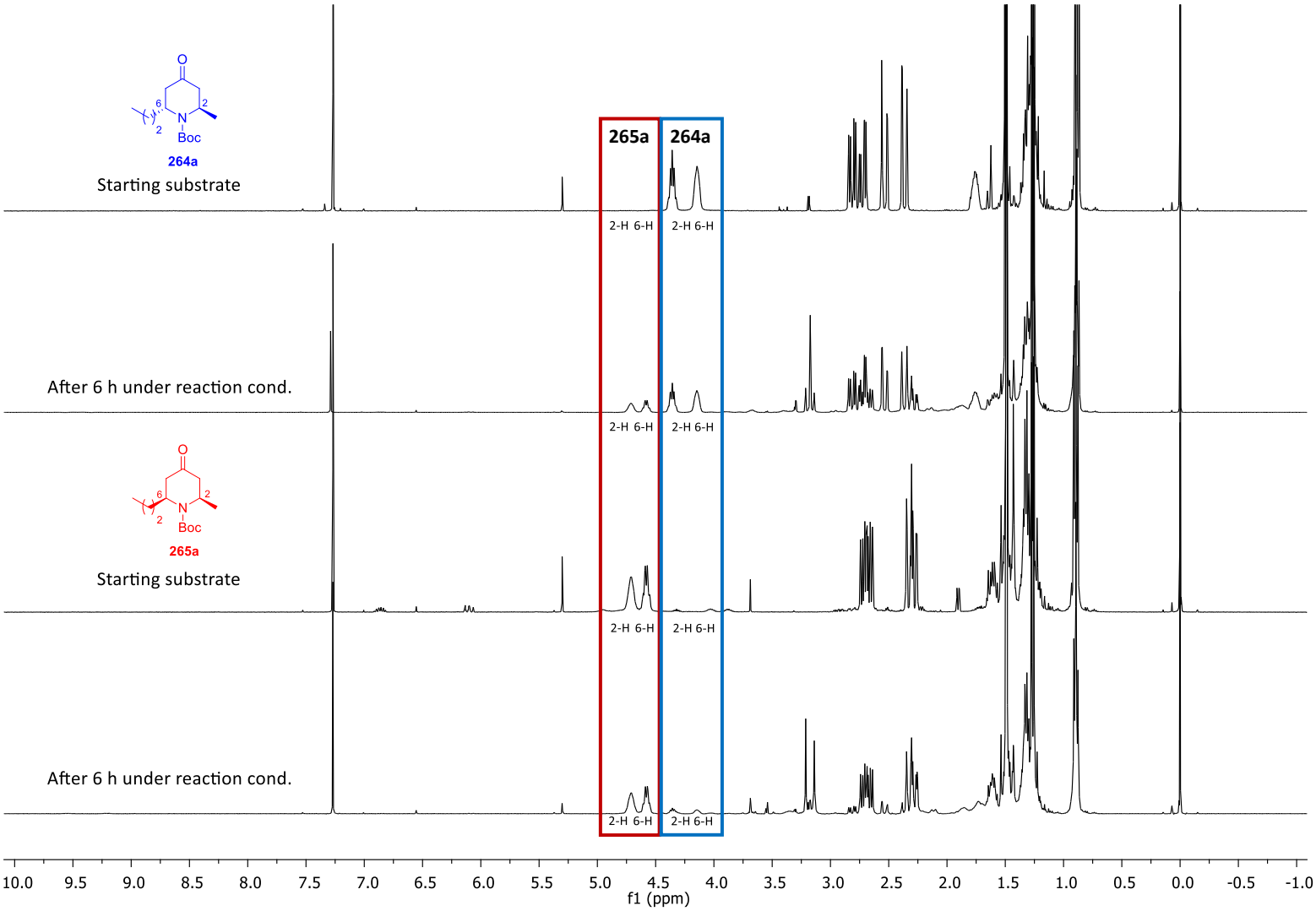
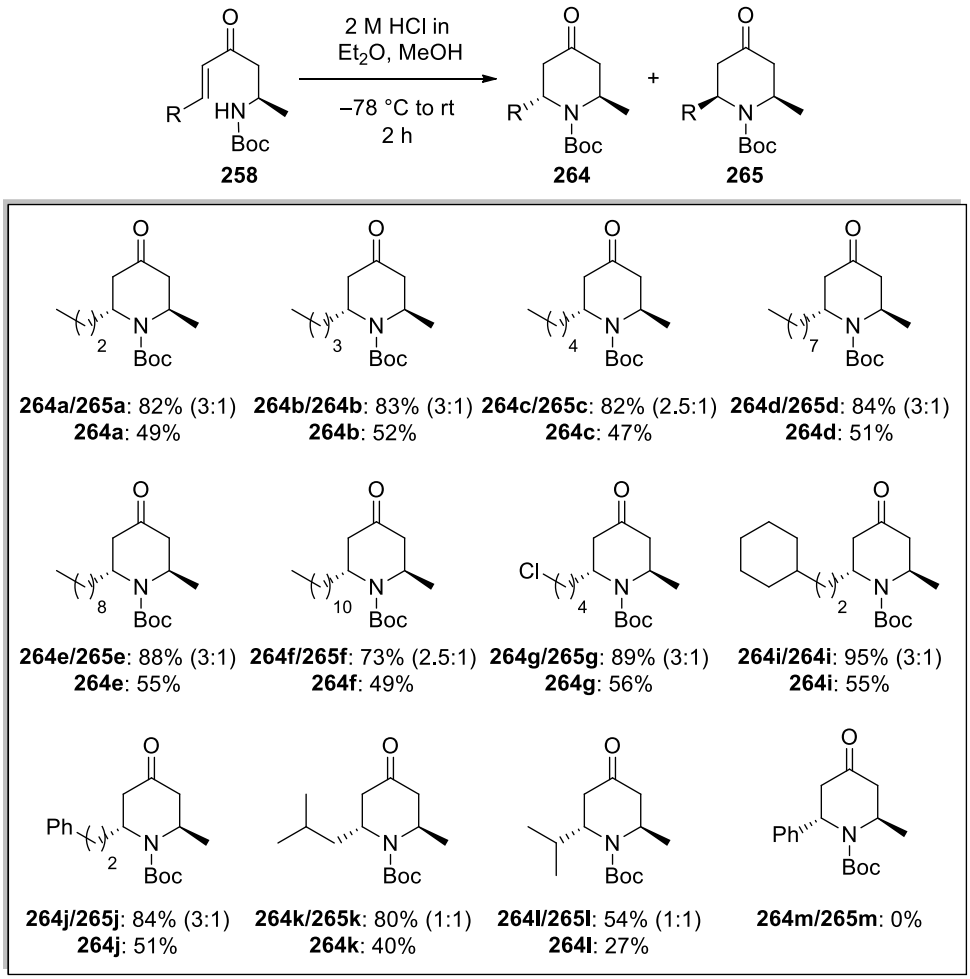


Figure 72 ^{1}H NMR spectra of isolated *trans*/*cis* products **264a** and **265a** re-subjected to reaction conditions for 6 hours.

From these experiments, it was concluded that the *cis* product **265a** is the thermodynamically favoured isomer and the *trans* product **264a** is the kinetically favoured isomer. In the reaction **264a** forms first and then this is epimerised to **265a**. From this it was decided, a reaction time of 2 hours was optimal to ensure a maximum yield of **264** is obtained. As this would be enough time for the starting material to be fully consumed and to minimise formation of the **265** isomer.

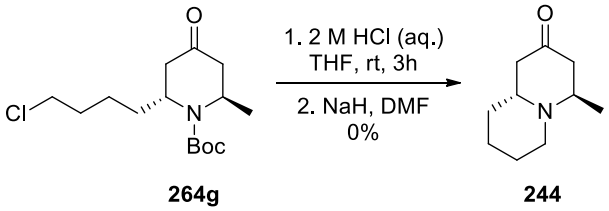
With the optimised reaction conditions established, the scope of this reaction was next investigated (**Scheme 97**). Enones with long alkyl side-arms were found to be excellent substrates for this reaction and after 2 h gave *trans*-2,6-dialkyl-4-oxopiperidines **264a**–**264f** in 47–56% yield. The cyclisation was also shown to accommodate functional groups, and this allowed for the preparation of **264g** in 56% yield. The synthesis of functional piperidines such as **264g** allowed for further functionalisation. These conditions were found not to be applicable for enone **258h**, with extended reaction times being required for full consumption of the starting material. From these extended reaction times, this led to a complicated product mixture from which no pure 4-oxopiperidine product could be isolated. It was found that enones containing ring systems **258i** and **258j** were also good substrates for this reaction, with the compounds **264i** and **264j** being formed in 51–54% yield. It was noted that increased steric bulk next to the newly forming ring as with *sec*-butyl **264k** and *iso*-propyl **264l** analogues resulted in the loss of stereoselectivity. This can be explained with A-values. With methyl and ethyl substituents the A-values are 1.70 and 1.75 kcal/mol respectively this indicates only minimal additional steric interactions is introduced as the alkyl substituent becomes longer. Therefore, *n*-propyl, *n*-butyl, *n*-pentyl, *n*-hexyl, *n*-nonyl and *n*-undecyl substituents all impart similar steric influence upon the ring system. However, for branched alkyl substituents there is a significant increase in the steric bulk. This is represented with the A-value increasing from 1.75 for an ethyl substituent to 2.15 kcal/mol for an *iso*-propyl substituent **258l**, as there is more steric bulk being in close proximity to the 4-oxopiperidine ring system. This explains the loss of stereoselectivity when compounds **264k** and **264l** are prepared, as the competing *cis* isomers **265k** and **265l** can place both substituents into equatorial positions. The conjugated enone **264m** was also tried with these reaction conditions. It was found that even with extended reaction times no cyclised products formed. It was hypothesised that the highly conjugated substrate is too stable to undergo cyclisation under these conditions.



Scheme 97 Acid-mediated cyclisation of enones **258a**–**258m**.

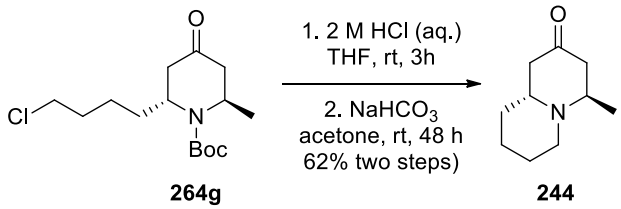
2.5.6 Synthesis of natural products, (+)-myrtine and (−)-solenopsin A

As it was found that *N*-Boc protected enones **258a**–**258l** can be efficiently converted to 2,6-*trans*-alkyl-4-oxopiperidines **264a–264l** under acidic conditions. The synthetic utility of these cyclic compounds was next explored with the synthesis of alkaloid natural products. The natural products (+)-myrtine (**244**) and (−)-solenopsin A (**246**) were chosen as target compounds. It was proposed that **244** could be synthesised from chlorinated enone **264g** by removal of the Boc group and then nitrogen alkylation. After Boc deprotection under acidic conditions, attempts to perform the *N*-alkylation using sodium hydride following a literature precedent, resulted in decomposition of the substrate and no product formation (**Scheme 98**).[196]



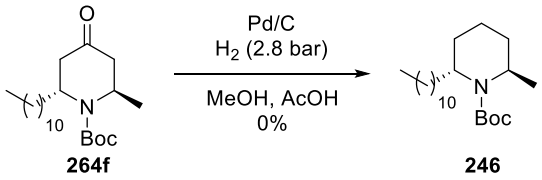
Scheme 98 Attempted synthesis of (+)-myrtine **244** with sodium hydride.

It was also reported that milder base, sodium hydrogen carbonate could also be used in intramolecular *N*-alkylations.[197,198] When sodium hydrogen carbonate was used in this reaction, this gave **244** cleanly with no decomposition in 62% yield over the two steps. (**Scheme 99**).



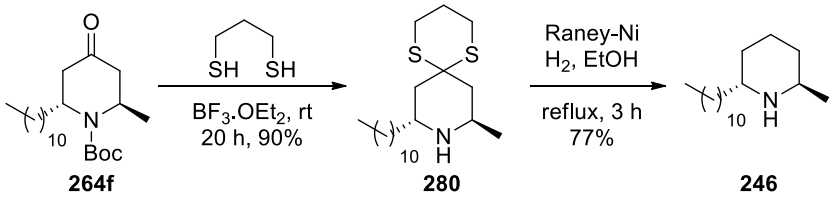
Scheme 99 Synthesis of (+)-myrtine (**244**) from 4-oxopiperidine **264g**.

(−)-Solenopsin A (**246**) was prepared from 4-oxopiperidine **264f**. Initially, removal of the ketone was attempted with literature high-pressure hydrogenation conditions (**Scheme 100**).[196] However, when this was attempted this only returned starting material and there was no formation of **246**.



Scheme 100 Attempted synthesis of (−)-solenopsin A (**246**) with high-pressure hydrogenation.

Therefore, a two-step approach to remove the ketone was performed. This involved synthesis of dithiane intermediate **280**, as the thioketal could be removed with a nickel catalyst.[191] Treatment of piperidine **264f** with 1,3-propanedithiol under Lewis acid conditions gave **280** in a 90% yield. The use of boron trifluoride resulted both in the installation of the cyclic dithioketal and removal of the Boc-protecting group (**Scheme 101**). Reduction of **280** with Raney® nickel gave **246** in 77% yield. Therefore, natural product **246** can be prepared from **264f** in 69% yield over two-steps.



Scheme 101 Synthesis of (−)-solenopsin A (**246**) with Raney® nickel.

2.5.7 Future work

Tri-substituted piperidines are found both in bio-active molecules, for example, eucatropine (**281**) is a mydriatic. Therefore, it would be of interest to synthesise **281** and other related compounds (**Figure 73**).[199,200]

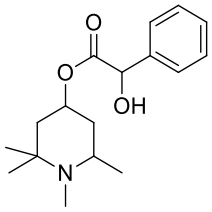
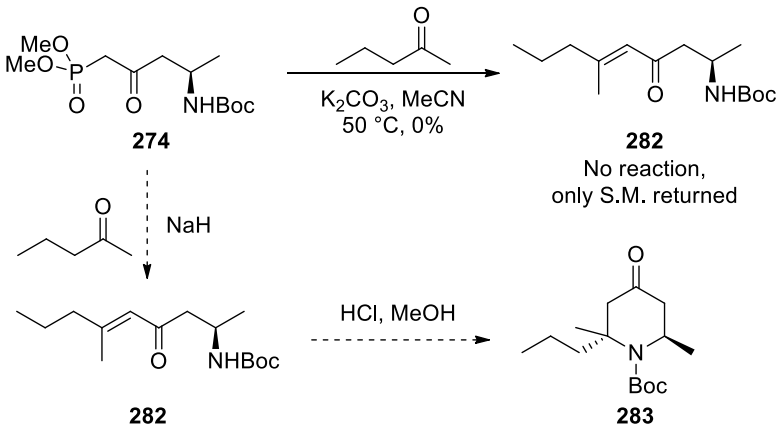


Figure 73 Chemical structure of eucatropine (**281**).

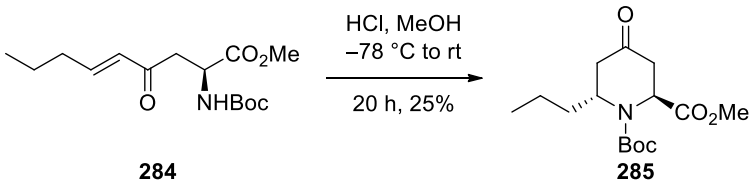
It is proposed that tri-substituted-2,6,6-piperidones **283** could be prepared from **274** and various ketones with the HWE reaction (**Scheme 102**). Initial investigations have found that the enone **282** could not be prepared using potassium carbonate as base. However, there are examples in literature where this transformation can be achieved with the use of sodium hydride.[201–203] Therefore, it is envisioned the use of sodium hydride could facilitate the preparation of **282**. Using the developed acid-mediated 6-*endo*-*trig* procedure would allow access to tri-substituted-2,6,6-piperidones **283**.



Scheme 102 Potential synthetic route to 2,6,6-trialkyl-4-oxopiperidines **283**.

As mentioned, previous work in the group has established a one-pot, three-step method for the synthesis of pipecolic acids **253**. Therefore, it would be of interest if these compounds could be prepared more efficiently using this acid-mediated procedure. The enones needed for this transformation can be easily prepared from phosphonate ester **111**. Then, the optimisation of the cyclisation step could then be carried out. Currently, it has been shown that pipecolic acid **285** can

be synthesised from enone **284** in low yield (**Scheme 103**). This result would serve as a starting point for optimisation of this process.

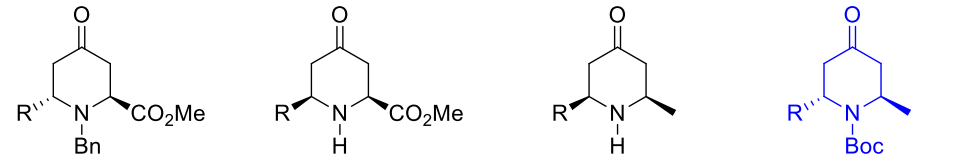


284 **285**

Scheme 103 Synthesis of 4-oxopipecolic acid **285**.

2.5.8 Summary

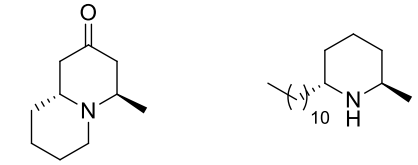
In summary, an acid-mediated stereoselective reaction has been developed and a library of eleven *trans*-2,6-dialkyl-4-oxopiperidines were synthesised. This complements the work already developed by the group (**Figure 74**). It was also shown that these products can isomerise under reaction conditions. Mechanistic studies showed that the *trans* compound is the kinetic product, whilst the *cis* compound is the thermodynamic product. Furthermore, despite the reaction being performed under acidic conditions, no significant Boc deprotected or ketal products were isolated, with the Boc-protected 4-oxopiperidines being isolated as the major product (54–95% yield).



Chem. Commun., 2011, **47**, 6569–6571 | *J. Org. Chem*., 2012, **77**, 10001–10009 | *J. Org. Chem*., 2018, **83**, 535–542 | This work *Org. Biomol. Chem*., 2018, **16**, 6410–6422

Figure 74 Group methodology to cyclic 4-oxopipecolic acid and 4-oxopiperidines.

With this understanding, it was shown that this methodology could be used for the efficient synthesis of two natural products (−)-solenopsin A (**244**) and (+)-myrtine (**246**) (**Figure 75**). Both compounds were synthesised from L-aspartic acid in eleven steps with overall yields of 18% and 20%, respectively.



(+)-Myrtine (**244**)
11 steps
20% overall yield

(−)-Solenopsin A (**246**)
11 steps
18% overall yield

Figure 75 Natural products accessed via the acid-mediated 6-*endo*-*trig* methodology.

3.0 Experimental

3.1 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 (UV254) 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 ppm and δ_C = 0.0 ppm), or solvent signals as the internal standard, for ^1H NMR spectra: $CHCl_3$ δ_H = 7.26 ppm, DMSO-d_5, δ_H = 2.50 ppm or CD_2HOH δ_H = 3.31 ppm. For ^{13}C NMR spectra $CDCl_3$ δ_C = 77.2 ppm DMSO-d_6 δ_C = 39.5 ppm and MeOD-d_4 δ_C = 49.0 ppm. Proton and carbon assignments are based on two-dimensional COSY, HSQC, HMBC and DEPT experiments. 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^{-1} g^{-1}. UV-Vis spectra were recorded on a Pekin Elmer Lambda 25 instrument. Fluorescence spectra were recorded on a Shimadzu RF- 5301PC spectrofluorophotometer. Emission data were measured using excitation and emission bandpass filters of 3 nm. Quantum yields were determined using a comparative method against two standards.[92] Anthracene (Φ = 0.27, in ethanol) and L-tryptophan (Φ = 0.14 in water) were used as standard references.[42,204] The integrated fluorescence intensity of each compound was determined from the emission spectra given. Measurements were performed at five different concentrations. Concentrations were chosen to ensure the absorption value was below 0.1 to avoid re-absorption effects. Integrated fluorescence intensity was plotted as a function of the measured absorbance and a linear fit is calculated with an intercept of zero. The resultant gradient was then used to calculate the quantum yield.

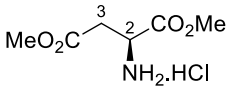
$$\Phi_x = \Phi_{ST}\left(\frac{Grad_x}{Grad_{ST}}\right)\left(\frac{\eta_x^2}{\eta_{ST}^2}\right)$$

Calculation of quantum yield. Subscript *ST* signifies the quantities associated with the quantum yield standard. Subscript *X* signifies the quantities associated with the novel compound. $Grad_X$ is

the determined gradient associated with the novel compound. $Grad_{ST}$ is the determined gradient associated with quantum yield standard. η is the refractive index of the solvent used in the fluorescence measurements. η = 1.333 for water, 1.361 for ethanol and 1.331 for methanol.[205,206]

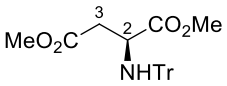
3.2 Experimental for pyrazoloquinazoline derived amino acids

Dimethyl (2*S*)-2-aminobutandioate hydrochloride (286)[207]



To a suspension of L-aspartic acid (**106**) (5.00 g, 37.6 mmol) in methanol (30 mL) at 0 °C under argon was added dropwise thionyl chloride (4.00 mL, 52.6 mmol). The reaction mixture was warmed to room temperature and then heated under reflux for 3 h. The solution was concentrated in vacuo to give a colourless oil and triturated with diethyl ether which gave dimethyl (2*S*)-2-aminobutandioate hydrochloride as a colourless solid (7.55 g, 100%). Mp 115–116 °C (lit.[207] 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, OMe), 3.74 (3H, s, OMe), 4.35 (1H, t, *J* 5.5 Hz, 2-H), 8.72 (3H, s, $CHNH_3^+$); δ_C (101 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).

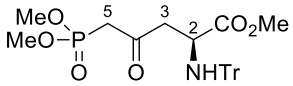
Dimethyl (2*S*)-2-(tritylamino)butandioate (107)[173]



To a solution of dimethyl (2*S*)-2-aminobutandioate hydrochloride (**286**) (7.46 g, 37.7 mmol) in dichloromethane (150 mL) at 0 °C was added dropwise triethylamine (11.0 mL, 75.4 mmol) and triphenylmethylchloride (10.5 g, 37.7 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 24 h. The reaction mixture was washed with 2 M citric acid (100 mL), water (100 mL), brine (100 mL), then dried ($MgSO_4$) and concentrated in vacuo to give a colourless oil. The crude product was purified by column chromatography (elution with 50% diethyl ether in petroleum ether) which gave **107** as a colourless solid (15.2 g, 100%). Mp 71–72 °C (lit. 70–71 °C); $[\alpha]_D^{24}$ +36.6 (c1.0, $CHCl_3$); 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, OMe), 3.67 (3H, s, OMe), 3.68–3.73 (1H, m, 2-H), 7.15–7.20 (3H, m, ArH), 7.23–7.28 (6H, m, ArH), 7.46–7.51 (6H, m, ArH); δ_C (101 MHz,

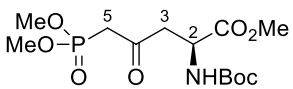
$CDCl_3$) 39.0 (CH_2), 50.5 (CH), 50.7 (CH_3), 52.4 (CH_3), 69.9 (C), 125.2 (3 × CH), 126.6 (6 × CH), 127.5 (6 × CH), 144.4 (3 × C), 169.7 (C), 172.6 (C); *m/z* (EI) 403 (M^+, 1%), 326 (35), 243 (100), 165 (30), 83 (70).

Methyl (2*S*)-5-(dimethyloxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (108)[173]



A solution of dimethyl methylphosphonate (3.00 mL, 27.3 mmol) in THF (50 mL) was cooled to –78 °C under an argon atmosphere. *n*-Butyl lithium (2.5 M in hexane, 11.0 mL, 28.6 mmol) was added dropwise and the reaction mixture stirred for 1 h. In a separate reaction vessel, a solution of dimethyl (2*S*)-2-(tritylamino)butandioate (**107**) (5.00 g, 12.4 mmol) in THF (100 mL) was cooled to –78 °C and then the dimethyl methylphosphonate/*n*-butyl lithium solution was cannulated into the flask and the reaction mixture stirred at –78 °C for 2 h to give a yellow solution. The reaction was quenched with a saturated solution of ammonium chloride (3 mL) and allowed to warm to room temperature. The mixture was concentrated *in vacuo*. The resulting residue was diluted with ethyl acetate (100 mL), washed with water (2 × 100 mL), brine (100 mL) then dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by column chromatography (elution with 40% ethyl acetate in dichloromethane) which gave **108** as a colourless solid (5.71 g, 93%). Mp 117–118 °C (lit.[173] 117–118.5 °C); $[\alpha]_D^{24}$ +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, ArH), 7.26 (6H, t, *J* 7.7 Hz, ArH), 7.47 (6H, d, *J* 7.7 Hz, ArH); δ_C (101 MHz, $CDCl_3$) 41.8 (d, $J_{C–P}$ 128 Hz, CH_2), 48.8 (CH_2), 52.0 (CH_3), 52.9 (CH_3), 53.0 (CH_3), 53.1 (CH), 71.3 (C), 126.6 (3 × CH), 127.9 (6 × CH), 128.8 (6 × CH), 145.7 (3 × C), 174.0 (C), 199.3 (C); *m/z* (CI) 496 (MH^+, 1%), 301 (5), 254 (90), 243 (100), 237 (55), 167 (45).

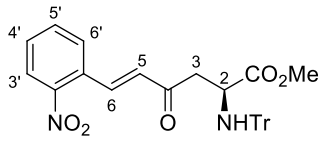
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-5-(dimethoxyphosphoryl)-4-oxopentanoate (111)



To a solution of methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**108**) (1.95 g, 3.94 mmol) in dichloromethane (40 mL) was added trifluoroacetic acid (3.02 mL, 39.4 mmol). The reaction mixture was stirred at room temperature for 2 h and then concentrated *in vacuo*. The

resulting residue was dissolved in water (50 mL) and washed with diethyl ether (2 × 50 mL). The aqueous layer was concentrated *in vacuo* to give the TFA salt. This was dissolved in dichloromethane (40 mL), cooled to 0 °C and, di-*tert*-butyl dicarbonate (1.81 mL, 7.87 mmol) and triethylamine (1.12 mL, 7.87 mmol) was added. The reaction mixture was warmed to room temperature and stirred for 16 h. The reaction mixture was diluted with dichloromethane (10 mL), washed with 1 M aqueous hydrochloric acid (50 mL) and brine (50 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash chromatography using silica gel, eluting with 2% methanol in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-5-(dimethoxyphosphoryl)-4-oxopentanoate (**111**) as a clear oil (1.27 g, 91%). ν_{max}/cm^{-1} (neat) 3291 (NH), 2957 (CH), 1713 (C=O), 1506, 1368, 1252, 1165, 1026; $[\alpha]_D^{22}$ +25.2 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.42 (9H, s, 3 × CH_3), 3.09 (2H, d, *J* 22.7 Hz, 5-H_2), 3.13 (1H, dd, *J* 18.4, 4.3 Hz, 3-*H*H), 3.29 (1H, dd, *J* 18.4, 4.7 Hz, 3-H*H*), 3.71 (3H, s, OCH_3), 3.75 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 4.50 (1H, ddd, *J* 8.4, 4.7, 4.3 Hz, 2-H), 5.45 (1H, d, *J* 8.4 Hz, NH); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 41.6 (d, $J_{C\text{-}P}$ 128 Hz, CH_2) 45.9 (CH_2), 49.5 (CH), 52.7 (CH_3), 53.2 (d, $J_{C\text{-}O\text{-}P}$ 5.4 Hz, CH_3), 53.3 (d, $J_{C\text{-}O\text{-}P}$ 5.1 Hz, CH_3), 80.2 (C), 155.5 (C), 171.7 (C), 199.9 (C); *m/z* (ESI) 376.1121 (MNa^+. $C_{13}H_{24}NNaO_8P$ requires 376.1132).

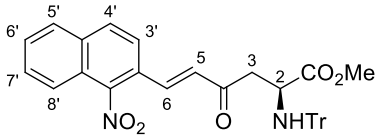
Methyl (2*S*,5*E*)-6-(2'-nitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (109a)



Methyl (2*S*)-5-(dimethyoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**108**) (2.50 g, 5.05 mmol), 2-nitrobenzaldehyde (1.53 g, 10.1 mmol) and potassium carbonate (0.733 g, 5.30 mmol) were stirred in anhydrous acetonitrile (50 mL) at 50 °C under argon for 20 h. The reaction mixture was concentrated *in vacuo*. The resulting residue was dissolved in ethyl acetate (50 mL) and washed with water (50 mL) and brine (50 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product. Purification by flash chromatography using silica gel, eluting with 50% diethyl ether in petroleum ether (40–60) gave methyl (2*S*,5*E*)-6-(2'-nitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**109a**) as a yellow solid (2.21 g, 92%). Mp 137–139 °C; ν_{max}/cm^{-1} (neat) 2922 (CH) 1736 (C=O), 1692 (C=O), 1524, 1343, 1201, 1172, 745, 706; $[\alpha]_D^{24}$ +39.9 (*c* 1.1, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 2.81 (1H, dd, *J* 15.7 6.9 Hz, 3-*H*H), 2.90–2.94 (2H, m, 3-H*H* and NH), 3.33 (3H, s, OCH_3), 3.81 (1H, ddd, *J* 9.8 6.9 5.1 Hz, 2-H), 6.53 (1H, d, *J* 16.1 Hz, 5-H), 7.17 (3H, tt, *J* 7.3 1.3 Hz, ArH), 7.24–7.27 (6H, m, ArH) 7.49–7.51 (6H, m, ArH), 7.55–7.58 (1H, m, 4'-H), 7.61 (1H, dd, *J* 7.8 1.5 Hz, 6'-H), 7.65–7.68 (1H, m, 5'-H), 7.89 (1H, d, *J* 16.1 Hz, 6-H), 8.07 (1H, dd, *J* 8.2 1.2 Hz, 3'-H); δ_C (121 MHz, $CDCl_3$) 45.2 (CH_2), 52.2 (CH_3), 53.7 (CH), 71.4 (C) 125.2

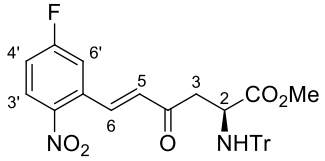
(CH), 126.7 (3 × CH), 128.1 (6 × CH), 128.9 (6 × CH), 129.2 (CH),130.6 (CH), 130.9 (C), 131.3 (CH), 133.8 (CH), 138.7(CH), 145.9 (3 × C), 148.5 (C), 174.5 (C), 197.4 (C); *m/z* (ESI) 543.1869 (MNa^+. $C_{32}H_{28}N_2NaO_5$ requires 543.1890).

Methyl (2*S*,5*E*)-6-(1′-nitronaphthalen-2′-yl)-4-oxo-2-(tritylamino)hex-5-enoate (109b)



Methyl (2*S*,5*E*)-6-(1′-nitronaphthalen-2′-yl)-4-oxo-2-(tritylamino)hex-5-enoate (**109b**) was synthesised as described for methyl (2*S*,5*E*)-6-(2′-nitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**109a**) using methyl (2*S*)-5-(dimethyoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**108**) (0.100 g, 0.202 mmol), 1-nitro-2-naphthaldehyde (0.0812 g, 0.404 mmol) and potassium carbonate (0.0307 g, 0.222 mmol) in anhydrous acetonitrile (4 mL). Purification by flash chromatography using silica gel, eluting with 50% diethyl ether in petroleum ether (40–60) gave methyl (2*S*,5*E*)-6-(1′-nitronaphthalen-2′-yl)-4-oxo-2-(tritylamino)hex-5-enoate (**109b**) as a yellow solid (0.0604 g, 52%). Mp 115–118 °C; ν_{max}/cm^{-1} (neat) 1735 (C=O), 1732 (C=O), 1612, 1595, 1526; $[\alpha]_D^{24}$ +45.2 (*c* 1.4, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.74 (1H, dd, *J* 15.7, 6.8 Hz, 3-*H*H), 2.84 (1H, dd, *J* 15.7, 4.8 Hz, 3-H*H*), 2.93 (1H, d, *J* 9.2 Hz, NH), 3.36 (3H, s, OCH_3), 3.75–3.83 (1H, m, 2-H), 6.78 (1H, d, *J* 16.0 Hz, 5-H), 7.19 (3H, tt, *J* 7.2, 1.2 Hz, 3 × ArH), 7.25–7.30 (6H, m, 6 × ArH), 7.48–7.54 (7H, m, 6-H and 6 × ArH), 7.62–7.71 (3H, m, 3′-H, 6′-H and 7′-H), 7.76–7.80 (1H, m, 5′-H), 7.90–7.96 (1H, m, 8′-H), 7.99 (1H, d, *J* 8.8 Hz, 4′-H); δ_C (126 MHz, $CDCl_3$) 45.5 (CH_2), 52.2 (CH_3), 53.6 (CH), 71.4 (C), 122.4 (CH), 122.5 (CH), 123.9 (C), 124.6 (C), 126.7 (3 × CH), 128.1 (6 × CH), 128.3 (CH), 128.8 (CH), 128.9 (6 × CH), 129.5 (CH), 131.2 (CH), 131.3 (CH), 134.6 (C), 135.0 (CH), 145.9 (3 × C), 148.8 (C), 174.4 (C), 196.9 (C); *m/z* (ESI) 593.2023 (MNa^+. $C_{36}H_{30}N_2NaO_5$ requires 593.2047).

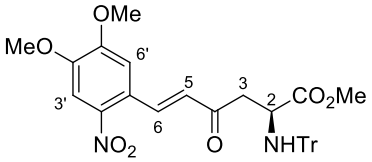
Methyl (2*S*,5*E*)-6-(5′-fluoro-2′-nitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (109c)



Methyl (2*S*,5*E*)-6-(5′-fluoro-2′-nitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**109c**) was synthesised as described for methyl (2*S*,5*E*)-6-(2′-nitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**109a**) using methyl (2*S*)-5-(dimethyoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**108**) (2.00

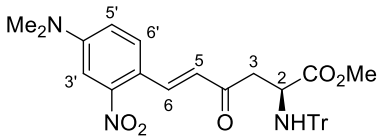
g, 4.04 mmol), 5-fluoro-2-nitrobenzaldehyde (1.37 g, 8.08 mmol) and potassium carbonate (0.614 g, 4.44) in anhydrous acetonitrile (40 mL). Purification by flash chromatography using silica gel, eluting with 50% diethyl ether in petroleum ether (40–60) gave methyl (2*S*,5*E*)-6-(5′-fluoro-2′-nitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**109c**) as a yellow solid (1.88 g, 86%). Mp 108–110 °C; ν_{max}/cm^{-1} (neat) 1734 (C=O), 1729 (C=O), 1523, 1341, 1276, 1213; $[\alpha]_D^{28}$ +36.1 (*c* 0.8, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 2.80 (1H, dd, *J* 15.7, 7.0 Hz, 3-*H*H), 2.90 (1H, dd, *J* 15.7, 5.0 Hz, 3-H*H*), 2.95 (1H, d, *J* 9.5 Hz, NH), 3.33 (3H, s, OCH_3), 3.76–3.87 (1H, m, 2-H), 6.50 (1H, d, *J* 16.0 Hz, 5-H), 6.14–7.20 (4H, m, ArH), 7.21–7.29 (7H, m, ArH), 7.50 (6H, d, *J* 7.5 Hz, ArH), 7.88 (1H, d, *J* 16.0 Hz, 6-H), 8.10 (1H, dd, *J* 9.1 5.0 Hz, ArH); δ_C (126 MHz, $CDCl_3$) 45.4 (CH_2), 52.1 (CH_3), 53.6 (CH), 71.3 (C), 116.0 (d, $^2J_{CF}$ 24.6 Hz, CH), 117.4 (d, $^2J_{CF}$ 23.4 Hz, CH), 126.6 (3 × CH), 128.0 (6 × CH), 128.1 (d, $^3J_{CF}$ 10.2 Hz, CH), 128.8 (6 × CH), 131.7 (CH), 134.1 (d, $^3J_{CF}$ 9.2 Hz, C), 137.6 (CH), 144.4 (C), 145.7 (3 × C), 164.9 (d, $^1J_{CF}$ 258.4 Hz, C), 174.3 (C), 196.9 (C); *m/z* (ESI) 561.1774 (MNa^+. $C_{32}H_{27}FN_2NaO_5$ requires 561.1796).

Methyl (2*S*,5*E*)-6-(4′,5′-dimethoxy-2′-nitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (109d)



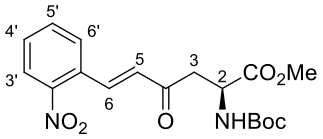
Methyl (2*S*,5*E*)-6-(4′,5′-dimethoxy-2′-nitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**109d**) was synthesised as described for methyl (2*S*,5*E*)-6-(2′-nitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**109a**) using methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**108**) (0.825 g, 1.67 mmol), 6-nitroveratraldehyde (0.705 g, 3.34 mmol) and potassium carbonate (0.254 g, 1.84 mmol) in anhydrous acetonitrile (20 mL). Purification by flash chromatography using silica gel, eluting with 80% diethyl ether in petroleum ether (40–60) gave methyl (2*S*,5*E*)-6-(4′,5′-dimethoxy-2′-nitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**109d**) as a yellow solid (0.822 g, 85%). Mp 106–108 °C; ν_{max}/cm^{-1} (neat) 3072 (NH), 2943 (CH), 1733 (C=O), 1663 (C=O), 1519, 1280, 1067; $[\alpha]_D^{32}$ +36.4 (*c* 0.7, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.84 (1H, dd, *J* 15.7, 6.9 Hz, 3-*H*H), 2.93–2.97 (2H, m, 3-H*H* and NH), 3.32 (3H, s, OCH_3), 3.82 (1H, m, 2-H), 3.98 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 6.46 (1H, d, *J* 16.2 Hz, 5-H), 6.95 (1H, s, 6′-H), 7.17 (3H, tt, *J* 7.3, 1.1 Hz, ArH), 7.23–7.26 (6H, m, ArH), 7.49–7.51 (6H, m, ArH), 7.66 (1H, s, 3′-H), 8.01 (1H, d, *J* 16.2 Hz, 6-H); δ_C (126 MHz, $CDCl_3$) 44.8 (CH_2), 52.2 (CH_3), 53.8 (CH), 56.7 (CH_3), 56.7 (CH_3), 71.4 (C), 108.2 (CH), 109.9 (CH), 125.4 (C), 126.7 (3 × CH) 128.1 (6 × CH), 129.0 (6 × CH), 130.4 (CH), 139.8 (CH), 141.5 (C), 145.9 (3 × C), 150.3 (C), 153.4 (C), 174.5 (C), 197.7 (C); *m/z* (ESI) 603.2073 (MNa^+. $C_{34}H_{32}N_2NaO_7$ requires 603.2102).

Methyl (2*S*,5*E*)-6-(4′-dimethylamino-2′-nitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (109e)



Methyl (2*S*,5*E*)-6-(4′-dimethylamino-2′-nitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**109e**) was synthesised as described for methyl (2*S*,5*E*)-6-(2′-nitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**109a**) using methyl (2*S*)-5-(dimethyoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**108**) (0.512 g, 1.03 mmol), 4-(dimethylamino)-2-nitrobenzaldehyde (0.401 g, 0.207 mmol) and potassium carbonate (0.157 g, 1.14 mmol) in anhydrous acetonitrile (10 mL). Purification by flash chromatography using silica gel, eluting with 50% diethyl ether in petroleum ether (40–60) gave methyl (2*S*,5*E*)-6-(1′-nitronaphthalen-2-yl)-4-oxo-2-(tritylamino)hex-5-enoate (**109e**) as a yellow solid (0.0604 g, 52%). Mp 117–118 °C; ν_{max}/cm^{-1} (neat) 3059 (NH), 2947 (CH), 1737 (C=O), 1593 (C=C), 1537, 1179; $[\alpha]_D^{23}$+70.3 (*c* 0.2, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.77 (1H, dd, *J* 15.5, 6.8 Hz, 3-*H*H), 2.85–2.94 (2H, m, 3-H*H* and NH), 3.08 (6H, s, NMe_2), 3.30 (3H, s, OCH_3), 3.71–3.84 (1H, m, 2-H), 6.48 (1H, d, *J* 16.1 Hz, 5-H), 6.85 (1H, dd, *J* 9.0, 1.8 Hz, 5′-H), 7.12–7.20 (3H, m, ArH), 7.20–7.28 (7H, m, ArH), 7.50 (6H, d, *J* 7.9 Hz, ArH), 7.54 (1H, d, *J* 9.0 Hz, 6′-H) 7.78 (1H, d, *J* 16.1, 6-H); δ_C (101 MHz, $CDCl_3$) 40.3 (2 × CH_3), 44.9 (CH_2), 52.1 (CH_3), 53.8 (CH), 71.4 (C), 107.0 (CH), 115.7 (CH), 116.0 (C), 126.6 (3 × CH), 127.0 (CH), 128.0 (6 × CH), 129.0 (6 × CH), 129.4 (CH), 138.4 (CH), 146.0 (3 × C), 150.7 (C), 151.5 (C), 174.6 (C), 197.6 (C); *m/z* (ESI) 586.2297 (MNa^+. $C_{34}H_{33}N_3NaO_5$ requires 586.2312).

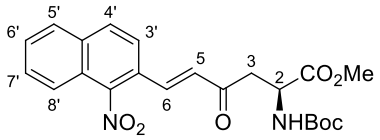
Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(2′-nitrophenyl)-4-oxohex-5-enoate (110a)



Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-5-(dimethoxyphosphoryl)-4-oxopentanoate (**111**) (0.100 g, 0.283 mmol), 2-nitrobenzaldehyde (0.0855 g, 0.566 mmol) and potassium carbonate (0.0430 g, 0.311 mmol) were stirred in anhydrous acetonitrile (3 mL) at 50 °C under argon for 16 h. The reaction mixture was concentrated *in vacuo*. The resulting residue was dissolved in ethyl acetate (50 mL) and washed with water (50 mL) and brine (50 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product. Purification by flash chromatography using silica gel, eluting with 30% ethyl acetate in petroleum ether gave methyl (2*S*, 5*E*)-2-(*tert*-

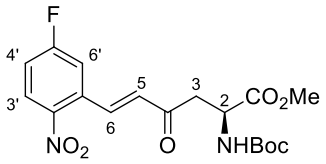
butoxycarbonylamino)-6-(2′-nitrophenyl)-4-oxohex-5-enoate (**110a**) as a yellow solid (0.0836 g, 78%). Mp 84–86 °C; ν_{max}/cm^{-1} (neat) 3560 (NH), 2981 (CH), 1720 (C=O), 1694 (C=O), 1621, 1605, 1525, 1339, 1159, 1091, 979, 747; $[\alpha]_D^{29}$ +37.7 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.39 (9H, s, 3 × CH_3), 3.22 (1H, dd, *J* 18.0, 4.4 Hz, 3-*H*H), 3.42 (1H, dd, *J* 18.0, 4.4 Hz, 3-H*H*), 3.70 (3H, s, OCH_3), 4.60 (1H, dt, *J* 8.8, 4.4 Hz, 2-H), 5.55 (1H, d, *J* 8.8 Hz, NH), 6.55 (1H, d, *J* 16.2 Hz, 5-H), 7.54 (1H, ddd, *J* 8.5, 7.0, 2.0 Hz, 4′-H), 7.60–7.66 (2H, m, 5′-H and 6′-H), 7.98 (1H, d, *J* 16.2 Hz, 6-H), 8.02 (1H, dd, *J* 8.5, 0.9 Hz, 3′-H); δ_C (126 MHz, $CDCl_3$) 28.3 (3 × CH_3), 42.5 (CH_2), 49.5 (CH), 52.7 (CH_3), 80.0 (C), 125.1 (CH), 129.1 (CH), 130.1 (CH), 130.5 (C), 130.8 (CH), 133.8 (CH), 139.3 (CH), 148.4 (C), 155.5 (C) 171.9 (C), 197.3 (C); *m/z* (ESI) 401.1304 (MNa^+. $C_{18}H_{22}N_2NaO_7$ requires 401.1319).

Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(1′-nitronaphthalen-2′-yl)-4-oxohex-5 -enoate (110b)



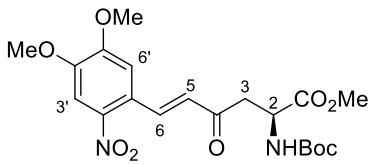
Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(1′-nitronaphthalen-2′-yl)-4-oxohex-5-enoate (**110b**) was synthesised as described for methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(2′-nitrophenyl)-4-oxohex-5-enoate (**110a**) using methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-5-(dimethoxyphosphoryl)-4-oxopentanoate (**111**) (0.0830 g, 0.235 mmol), 3-nitro-2-naphthaldehyde (0.0944 g, 0.469 mmol) and potassium carbonate (0.0436 g, 0.260 mmol) in anhydrous acetonitrile (3 mL). Purification by flash chromatography using silica gel, eluting with 50% to 80% diethyl ether in petroleum ether gave methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(1′-nitronaphthalen-2′-yl)-4-oxohex-5-enoate (**110b**) as a yellow solid (0.0757 g, 75%). Mp 88–90 °C; ν_{max}/cm^{-1} (neat) 3373 (NH), 2982 (CH), 1735 (C=O), 1692 (C=O), 1669 (C=O), 1523, 1367,1298, 1167, 747; $[\alpha]_D^{26}$ +30.2 (*c* 0.7, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.45 (9H, s, 3 × CH_3), 3.24 (1H, dd, *J* 18.0, 4.2 Hz, 3-*H*H), 3.45 (1H, dd, *J* 18.0, 4.2 Hz, 3-H*H*), 3.76 (3H, s, OCH_3), 4.65 (1H, dt, *J* 8.6, 4.2 Hz, 2-H), 5.55 (1H, d, *J* 8.6 Hz, NH), 6.85 (1H, d, *J* 16.1 Hz, 5-H), 7.62 (1H, d, *J* 16.1 Hz, 6-H), 7.63–7.70 (2H, m, 6′-H and 7′-H), 7.72 (1H, d, *J* 8.7 Hz, 3′-H), 7.75–7.81 (1H, m, 5′-H), 7.90–7.95 (1H, m, 8′-H), 8.00 (1H, d, *J* 8.7 Hz, 4′-H); δ_C (126 MHz, $CDCl_3$) 28.5 (3 × CH_3), 43.2 (CH_2), 49.6 (CH), 52.9 (CH_3), 80.3 (C), 122.4 (CH), 122.5 (CH), 123.7 (C), 124.6 (C), 128.3 (CH), 128.9 (CH), 129.5 (CH), 130.3 (CH), 131.3 (CH), 134.7 (C), 135.8 (CH), 148.9 (C), 155.7 (C), 171.9 (C), 196.9 (C); *m/z* (ESI) 451.1457 (MNa^+. $C_{22}H_{24}N_2NaO_7$ requires 451.1476).

Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(5′-fluoro-2′-nitrophenyl)-4-oxohex-5-enoate (110c)



Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(5′-fluoro-2′-nitrophenyl)-4-oxohex-5-enoate (**110c**) was synthesised as described for methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(2′-nitrophenyl)-4-oxohex-5-enoate (**110a**) using methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-5-(dimethoxyphosphoryl)-4-oxopentanoate (**111**) (0.100 g, 0.313 mmol), 5-fluoro-2-nitrobenzaldehyde (0.0960 g, 0.566 mmol) and potassium carbonate (0.0430 g, 0.311 mmol) in anhydrous acetonitrile (3 mL). Purification by flash chromatography using silica gel, eluting with 50% diethyl ether in petroleum ether gave methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(5′-fluoro-2′-nitrophenyl)-4-oxohex-5-enoate (**110c**) as a yellow solid (0.0470 g, 42%). Mp 83–86 °C; ν_{max}/cm^{-1} (neat) 2921 (CH), 1748 (C=O), 1701 (C=O), 1614, 1583, 1526, 1343, 1161, 729; $[\alpha]_D^{30}$ +24.2 (*c* 1.3, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.45 (9H, s, 3 × CH_3), 3.30 (1H, dd, *J* 18.0, 4.2 Hz, 3-*H*H), 3.47 (1H, dd, *J* 18.0, 4.2 Hz, 3-H*H*), 3.76 (3H, s, OCH_3), 4.67 (1H, dt, *J* 8.6, 4.2 Hz, 2-H), 5.64 (1H, d, *J* 8.6 Hz, NH), 6.62 (1H, d, *J* 16.1 Hz, 5-H), 7.29 (1H, ddd, *J* 9.2, 6.9, 2.8 Hz, 4′-H), 7.36 (1H, dd, *J* 8.8, 2.8 Hz, 6′-H), 8.04 (1H, d, *J* 16.1 Hz, 6-H), 8.19 (1H, dd, *J* 9.2, 5.0 Hz, 3′-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 42.7 (CH_2), 49.5 (CH), 52.7 (CH_3), 80.1 (C), 116.1 (d, $^2J_{CF}$ 24.6 Hz, CH), 117.5 (d, $^2J_{CF}$ 23.4 Hz, CH), 128.1 (d, $^3J_{CF}$ 10.0 Hz, CH), 130.9 (CH), 133.9 (d, $^3J_{CF}$ 9.2 Hz, C), 138.4 (CH), 144.3 (d, $^4J_{CF}$ 2.9 Hz, C), 155.5 (C), 164.9 (d, $^1J_{CF}$ 258.0 Hz, C), 171.8 (C), 197.0 (C); *m/z* (ESI) 419.1215 (MNa^+. $C_{18}H_{21}FN_2NaO_7$ requires 419.1225).

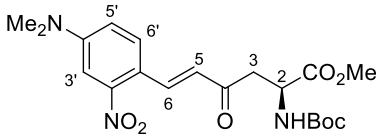
Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(4′,5′-dimethoxy-2′-nitrophenyl)-4 -oxohex-5-enoate (110d)



Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(4′,5′-dimethoxy-2′-nitrophenyl)-4-oxohex-5-enoate (**110d**) was synthesised as described for methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(2′-nitrophenyl)-4-oxohex-5-enoate (**110a**) using methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-5-

(dimethoxyphosphoryl)-4-oxopentanoate (**111**) (0.321 g, 0.909 mmol), 6-nitroveratraldehyde (0.384 g, 1.82 mmol) and potassium carbonate (0.138 g, 0.999 mmol) in anhydrous acetonitrile (9 mL). Purification by flash chromatography using silica gel, eluting with 50% to 80% diethyl ether in petroleum ether gave methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(4′,5′-dimethoxy-2′-nitrophenyl)-4-oxohex-5-enoate (**110d**) as a yellow solid (0.275 g, 69%). Mp 75–78 °C; ν_{max}/cm^{-1} (neat) 3384 (NH), 2981 (CH), 1747 (C=O), 1707 (C=O), 1670 (C=O), 1521, 1331, 1280, 1218, 1165, 1066; $[\alpha]_D^{29}$ +28.8 (*c* 1.1, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.37 (9H, s, 3 × CH_3), 3.21 (1H, dd, *J* 17.8, 4.3 Hz, 3-*H*H), 3.39 (1H, dd, *J* 17.8, 4.0 Hz, 3-H*H*), 3.68 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 4.58–4.59 (1H, m, 2-H), 5.53 (1H, d, *J* 8.7 Hz, NH), 6.46 (1H, d, *J* 16.1 Hz, 5-H), 6.93 (1H, s, 6′-H), 7.58 (1H, s, 3′-H), 8.05 (1H, d, *J* 16.1 Hz, 6-H); δ_C (126 MHz, $CDCl_3$) 28.3 (3 × CH_3), 42.1 (CH_2), 49.5 (CH), 52.6 (CH_3), 56.5 (CH_3) 56.6 (CH_3), 79.9 (C), 108.0 (CH), 109.8 (CH), 124.9 (C), 129.1 (CH), 140.1 (CH), 141.3 (C), 150.2 (C), 153.2 (C), 155.5 (C), 171.9 (C), 197.4 (C); *m/z* (ESI) 461.1517 (MNa^+. $C_{20}H_{26}N_2NaO_9$ requires 461.1531).

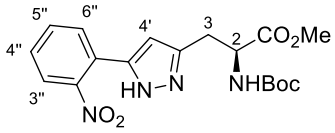
Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(4'-dimethylamino-2′-nitrophenyl)-4-oxohex-5-enoate (110e)



Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(4′-dimethylamino-2′-nitrophenyl)-4-oxohex-5-enoate (**110e**) was synthesised as described for methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(2′-nitrophenyl)-4-oxohex-5-enoate (**110a**) using methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-5-(dimethoxyphosphoryl)-4-oxopentanoate (**111**) (0.697 g, 1.97 mmol), 4-(dimethylamino)-2-nitrobenzaldehyde (0.767 g, 3.95 mmol) and potassium carbonate (0.300 g, 2.17 mmol) in anhydrous acetonitrile (10 mL). The reaction was stirred at 50 °C under argon for 72 h. Purification by flash chromatography using silica gel, eluting with 60% to 100% diethyl ether in petroleum ether gave methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(4′-dimethylamino-2′-nitrophenyl)-4-oxohex-5-enoate (**110e**) as a red solid (0.603 g, 82%). Mp 102–104 °C; ν_{max}/cm^{-1} (neat) 3378 (NH), 2953 (CH), 1750 (C=O), 1717 (C=O), 1590, 1541, 1368, 1168; $[\alpha]_D^{25}$ +49.1 (*c* 0.4, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.39 (9H, s, 3 × CH_3), 3.04 (6H, s, 2 × CH_3), 3.15 (1H, dd, *J* 17.8, 4.3 Hz, 3-*H*H), 3.38 (1H, dd, *J* 17.8, 4.3 Hz, 3-H*H*), 3.69 (3H, s, OCH_3), 4.57 (1H, dt, *J* 8.8, 4.3 Hz, 2-H), 5.55 (1H, d, *J* 8.8 Hz, NH), 6.47 (1H, d, *J* 16.0 Hz, 5-H), 6.80 (1H, dd, *J* 8.9, 2.7 Hz, 5'-H), 7.08 (1H, d, *J* 2.7 Hz, 3'-H), 7.51 (1H, d, *J* 8.9 Hz, 6'-H), 7.83 (1H, d, *J* 16.0 Hz, 6-H); δ_C (126 MHz, $CDCl_3$) 28.3 (3 × CH_3), 40.1 (2 × CH_3), 42.1 (CH_2), 49.6 (CH), 52.6 (CH_3), 79.9 (C), 106.7 (CH), 115.3 (C), 115.5 (CH), 125.4 (CH), 129.2 (CH), 138.8

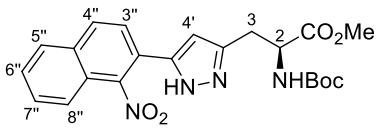
(CH), 150.6 (C), 151.4 (C), 155.5 (C), 172.1 (C), 197.3 (C); *m/z* (ESI) 444.1725 (MNa^+. $C_{20}H_{27}N_3NaO_7$ requires 444.1741).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5′-(2″-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (116a)



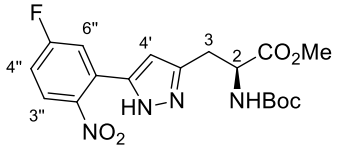
Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(2′-nitrophenyl)-4-oxohex-5-enoate (**110a**) (0.277 g, 0.733 mmol) was dissolved in methanol (15 mL) and hydrazine monohydrate (65%, 0.176 mL, 3.67 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature for 4 h, poured into 1 M aqueous hydrochloric acid (50 mL) and extracted with dichloromethane (3 × 30 mL). The combined organic layers were washed with water (50 mL), brine (50 mL) and concentrated *in vacuo* to give dihydropyrazole **115a** as a yellow solid (0.247 g, 86%). Dihydropyrazole **115a** (0.194 g, 0.494 mmol) was dissolved in anhydrous dichloromethane (20 mL), cooled to −15 °C and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.112 g, 0.494 mmol) was added. After stirring at −15 °C for 3 h, the reaction mixture was concentrated. Purification by flash chromatography using silica gel, eluting with 0% to 20% acetonitrile in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino-3-[5′-(2′′-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (**116a**) as a yellow solid (0.126 g, 65%). Mp 88–90 °C; ν_{max}/cm^{-1} (neat) 3298 (NH), 2923 (CH), 1743 (C=O), 1694 (C=O), 1528, 1366, 1160, 752; $[\alpha]_D^{29}$ +12.4 (*c* 1.1, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.42 (9H, s, 3 × CH_3), 3.19 (1H, dd, *J* 14.8, 5.5 Hz, 3-*H*H), 3.25 (1H, dd, *J* 14.8, 5.5 Hz, 3-H*H*), 3.76 (3H, s, OCH_3), 4.54–4.70 (1H, m, 2-H), 5.42 (1H, d, *J* 8.1 Hz, NH), 6.23 (1H, s, 4′-H), 7.46 (1H, td, *J* 8.0, 1.4 Hz, 4′′-H), 7.59 (1H, td, *J* 8.0, 1.2 Hz, 5′′-H), 7.68 (1H, dd, *J* 8.0, 1.4 Hz, 6′′-H), 7.72 (1H, dd, *J* 8.0, 1.2 Hz, 3′′-H); δ_C (126 MHz, $CDCl_3$) 28.3 (3 × CH_3), 29.4 (CH_2), 52.7 (CH_3), 53.1 (CH), 80.4 (C), 104.7 (CH), 123.6 (CH), 126.5 (C), 128.7 (CH), 130.8 (CH), 132.0 (CH), 141.6 (C), 145.4 (C), 149.0 (C), 155.5 (C), 172.1 (C); *m/z* (ESI) 413.1423 (MNa^+. $C_{18}H_{22}N_4NaO_6$ requires 413.1432).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5′-(1″-nitronaphthalen-2″-yl)-1′*H*-pyrazol-3′-yl]propanoate (116b)



Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5′-(1′′-nitronaphthalen-2′′-yl)-1′*H*-pyrazol-3′-yl]propanoate (**116b**) was synthesised as described for methyl (2*S*)-2-(*tert*-butoxycarbonylamino-3-[5′-(2′′-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (**116a**) using methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(1′-nitronaphthalen-2′-yl)-4-oxohex-5-enoate (**110b**) (0.469 g, 1.10 mmol) and hydrazine monohydrate (0.264 mL, 5.48 mmol) in methanol (15 mL). This gave dihydropyrazole **115b** as a yellow solid (0.490 g, 100%). 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (0.122 g, 0.538 mmol) was added to a solution of dihydropyrazole **115b** (0.238 g, 0.538 mmol) in dichloromethane (20 mL) at −40 °C and stirred for 3 h. Purification by flash chromatography using silica gel, eluting with 0% to 20% acetonitrile in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5′-(1′′-nitronaphthalen-2′′-yl)-1′*H*-pyrazol-3′-yl]propanoate (**116b**) as a yellow solid (0.0581 g, 25%). Mp 86–90 °C; ν_{max}/cm^{-1} (neat) 3312 (NH), 2922 (CH), 1741 (C=O), 1697 (C=O), 1530, 1368, 1162, 751, 732; $[\alpha]_D^{20}$ +26.0 (*c* 0.6, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.42 (9H, s, 3 × CH_3), 3.24 (1H, dd, *J* 15.2, 6.0 Hz, 3-*H*H), 3.32 (1H, dd, *J* 15.2, 5.3 Hz, 3-H*H*), 3.77 (3H, s, OCH_3), 4.67 (1H, m, 2-H), 5.46 (1H, d, *J* 6.9 Hz, NH), 6.41 (1H, s, 4′-H), 7.53–7.66 (2H, m, 6′′-H and 7′′-H), 7.70 (1H, d, *J* 8.4 Hz, 5′′-H), 7.83 (1H, d, *J* 8.6 Hz, 3′′-H), 7.88 (1H, d, *J* 8.0 Hz, 8′′-H), 7.95 (1H, d, *J* 8.6 Hz, 4′′-H); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 29.4 (CH_2), 52.9 (CH_3), 53.2 (CH), 80.7 (C), 104.6 (CH), 121.7 (CH), 124.8 (C), 125.6 (CH), 127.6 (CH), 128.1 (CH), 128.9 (CH), 130.4 (CH), 133.2 (2 × C), 141.5 (C), 145.5 (C), 145.7 (C), 155.6 (C), 172.1 (C); *m/z* (ESI) 463.1573 (MNa^+. $C_{22}H_{24}N_4NaO_6$ requires 463.1588).

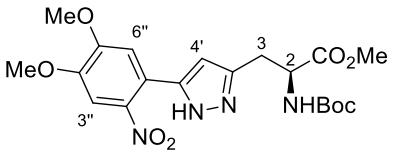
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5′-(5″-fluoro-2″-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (116c)



Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5′-(5′′-fluoro-2′′-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (**116c**) was synthesised as described for methyl (2*S*)-2-(*tert*-butoxycarbonylamino-3-[5′-(2′′-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (**116a**) using (2*S*,5*E*)-2-(*tert*-

butoxycarbonylamino)-6-(5′-fluoro-2′-nitrophenyl)-4-oxohex-5-enoate (**110c**) (0.479 g, 1.21 mmol) and hydrazine monohydrate (0.291 mL, 6.05 mmol) in methanol (15 mL). This gave dihydropyrazole **115c** as a yellow solid (0.474 g, 95%). 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (0.0320 g, 0.141 mmol) was added to a solution of dihydropyrazole **115c** (0.0579 g, 0.141 mmol) in dichloromethane (10 mL) at −30 °C and stirred for 3 h. Purification by flash chromatography using silica gel, eluting with 0% to 20% acetonitrile in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5′-(5′′-fluoro-2′′-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (**116c**) as a yellow solid (0.0260 g, 45%). Mp 82–84 °C; ν_{max}/cm^{-1} (neat) 3287 (NH), 2981 (CH), 1741 (C=O), 1696 (C=O), 1529, 1366, 1161, 887, 734; $[\alpha]_D^{26}$ +20.2 (*c* 0.8, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.42 (9H, s, 3 × CH_3), 3.18 (1H, dd, *J* 15.0, 5.1 Hz, 3-*H*H), 3.26 (1H, dd, *J* 15.0, 5.1 Hz, 3-H*H*), 3.76 (3H, s, OMe), 4.64 (1H, br s, 2-H), 5.44 (1H, d, *J* 5.0 Hz, NH), 6.22 (1H, s, 4′-H), 7.12 (1H, ddd, *J* 8.9, 7.3, 2.6 Hz, 4′′-H), 7.41 (1H, dd, *J* 9.0, 2.6 Hz, 6′′-H), 7.76 (1H, dd, *J* 8.9, 5.0 Hz, 3′′-H); δ_C (126 MHz, $CDCl_3$) 28.4 (3 × CH_3), 29.5 (CH_2), 52.9 (CH_3), 53.1 (CH), 80.8 (C), 104.9 (CH), 115.6 (d, $^2J_{CF}$ 23.5 Hz, CH), 117.8 (d, $^2J_{CF}$ 24.5 Hz, CH), 126.5 (d, $^3J_{CF}$ 9.7 Hz, CH), 130.1 (d, $^3J_{CF}$ 9.2 Hz, C), 141.1 (C), 145.2 (d, $^4J_{CF}$ 2.7 Hz, C), 145.4 (C), 155.6 (C), 163.9 (d, $^1J_{CF}$ 254.6 Hz, C), 172.0 (C); *m/z* (ESI) 431.1321 (MNa^+. $C_{18}H_{21}FN_4NaO_6$ requires 431.1337).

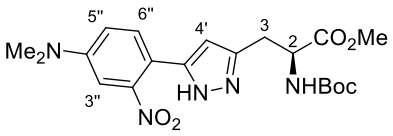
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5′-(4′′,5′′-dimethoxy-2′′-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (116d)



Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5′-(4′′,5′′-dimethoxy-2′′-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (**116d**) was synthesised as described for methyl (2*S*)-2-(*tert*-butoxycarbonylamino-3-[5′-(2′′-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (**116a**) using methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(4′,5′-dimethoxy-2′-nitrophenyl)-4-oxohex-5-enoate (**110d**) (0.573 g, 1.31 mmol) and hydrazine monohydrate (0.315 mL, 6.55 mmol) in methanol (15 mL). This gave dihydropyrazole **115d** as a yellow solid (0.532 g, 90%). 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (0.145 g, 0.637 mmol) was added to a solution of dihydropyrazole **115d** (0.288 g, 0.637 mmol) at −15 °C and stirred for 3 h. Purification by flash chromatography using silica gel, eluting with 0% to 20% acetonitrile in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5′-(4′′,5′′-dimethoxy-2′′-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (**116d**) as a yellow solid (0.0955 g, 34%). Mp 79–82 °C; ν_{max}/cm^{-1} (neat) 3395 (NH), 2925 (CH), 1711 (C=O), 1522, 1337, 1268, 1221, 1163, 1045, 790; $[\alpha]_D^{29}$ +9.1 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.42 (9H, s, 3 × CH_3), 3.10–3.32 (2H, m, 3-H_2), 3.76 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 3.96 (3H, s, OCH_3), 4.65 (1H, br s, 2-H), 5.51 (1H, d, *J* 8.1

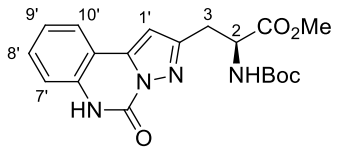
Hz, NH), 6.18 (1H, s, 4′-H), 7.04 (1H, s, 6′′-H), 7.44 (1H, s, 3′′-H); δ_C (126 MHz, $CDCl_3$) 28.3 (3 × CH_3), 29.7 (CH_2), 52.7 (CH_3), 53.1 (CH), 56.4 (CH_3), 56.5 (CH_3), 80.4 (C), 105.4 (CH), 107.7 (CH), 113.0 (CH), 121.3 (C), 141.3 (C), 142.1 (C), 145.5 (C), 148.6 (C), 152.2 (C), 155.5 (C), 172.1 (C); *m/z* (ESI) 473.1632 (MNa^+. $C_{20}H_{26}N_4NaO_8$ requires 473.1643).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5'-(4''-dimethylamino-2''-nitrophenyl)-1'*H*-pyrazol-3'-yl]propanoate (116e)



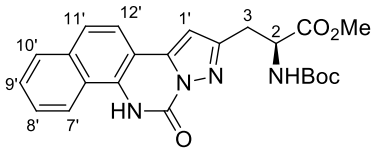
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5'-(4''-dimethylamino-2''-nitrophenyl)-1'*H*-pyrazol-3'-yl]propanoate (**116e**) was synthesised as described for methyl (2*S*)-2-(*tert*-butoxycarbonylamino-3-[5′-(2′′-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (**116a**) using methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-5-(dimethoxyphosphoryl)-4-oxopentanoate (**110e**) (0.291 g, 0690 mmol) and hydrazine monohydrate (0.166 mL, 3.45 mmol) in methanol (10 mL). This gave dihydropyrazole **115e** as a yellow solid (0.263 g, 88%). 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (0.695 g, 3.06 mmol) was added to a solution of dihydropyrazole **115e** (1.33 g, 3.06 mmol) in dichloromethane (60 mL) at −40 °C and stirred for 3 h. Purification by flash chromatography using silica gel, eluting with 3% methanol in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5'-(4''-dimethylamino-2''-nitrophenyl)-1'*H*-pyrazol-3'-yl]propanoate (**116e**) as a yellow solid (0.960 g, 72%). Mp 107–110 °C; ν_{max}/cm^{-1} (neat) 3301 (NH), 2983 (CH), 1713 (C=O), 1625 (C=O), 1531, 1367, 1163; $[\alpha]_D^{26}$ +24.6 (*c* 0.7, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.42 (9H, s, 3 × CH_3), 3.02 (6H, s, 2 × CH_3), 3.18 (2H, d, *J* 5.2 Hz, 3-H_2), 3.73 (3H, s, OCH_3), 4.61 (1H, br s, 2-H), 5.50 (1H, d, *J* 6.5, NH), 6.11 (1H, s, 4′-H), 6.82 (1H, dd, *J* 8.8, 2.7 Hz, 5′′-H), 6.95 (1H, d, *J* 2.7 Hz, 3''-H), 7.43 (1H, d, *J* 8.8 Hz, 6''-H); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 29.9 (CH_2), 40.3 (2 × CH_3), 52.7 (CH_3), 53.2 (CH), 80.3 (C), 104.6 (CH), 106.5 (CH), 112.5 (C), 115.1 (CH), 131.7 (CH), 143.6 (C), 149.9 (C), 150.3 (2 × C), 155.6 (C), 172.3 (C); *m/z* (ESI) 456.1840 (MNa^+. $C_{20}H_{27}N_5NaO_6$ requires 456.1854).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c] quinazolin-5′-yl)propanoate (118a)



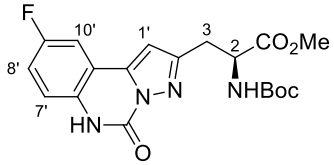
To a solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino-3-[5′-(2′′-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (**116a**) (0.0871 g, 0.223 mmol) in methanol (5 mL) was added 10% palladium on carbon (0.0120 g, 0.0112 mmol). The reaction mixture was purged with hydrogen for 0.5 h and then stirred under an atmosphere of hydrogen at room temperature for 16 h. The reaction mixture was filtered through Celite®, which was washed with methanol. The filtrate was concentrated *in vacuo* and the resulting aniline was dissolved in dichloromethane (10 mL). Triphosgene (0.0265 g, 0.089 mmol) and triethylamine (0.063 mL, 0.0446 mmol) was added at −10 °C. After stirring at room temperature for 1 h, the reaction mixture was diluted with dichloromethane (40 mL), washed with 1 M aqueous hydrochloric acid (50 mL), water (50 mL) and brine (50 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product. Purification by flash chromatography using silica gel, eluting with 90% ethyl acetate in petroleum ether gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate (**118a**) as a white solid (0.0448 g, 52%). Mp 123–126 °C; ν_{max}/cm^{-1} (neat) 3248 (NH), 2978 (CH), 1715 (C=O), 1598, 1491, 1343, 1162, 734; $[\alpha]_D^{30}$ +13.4 (*c* 1.3, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.38 (9H, s, 3 × CH_3), 3.32 (1H, dd, *J* 14.8, 7.0 Hz 3-*H*H), 3.40 (1H, dd, *J* 14.8, 5.0 Hz, 3-H*H*), 3.76 (3H, s, OCH_3), 4.67–4.80 (1H, m, 2-H), 5.44 (1H, d, *J* 8.3 Hz, NH), 6.75 (1H, s, 1′-H), 7.24 (1H, t, *J* 7.4 Hz, 9′-H), 7.36–7.48 (2H, m, 7′-H and 8′-H), 7.72 (1H, d, *J* 7.4 Hz, 10′-H), 11.24 (1H, s, NH); δ_C (126 MHz, $CDCl_3$) 28.4 (3 × CH_3), 31.5 (CH_2), 52.7 (CH_3), 53.1 (CH), 80.2 (C), 101.0 (CH), 112.7 (C), 116.6 (CH), 123.8 (CH), 124.1 (CH), 130.5 (CH), 133.8 (C), 142.0 (C), 146.6 (C), 154.3 (C), 155.5 (C), 172.3 (C); *m/z* (ESI) 409.1467 (MNa^+. $C_{19}H_{22}N_4NaO_5$ requires 409.1482).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(5′-oxo-5′,6′-dihydrobenzo[h]pyrazole [1′,5′-c]quinazolin-2′-yl)propanoate (118b)



Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(5′-oxo-5′,6′-dihydrobenzo[h]pyrazole[1′,5′-c]quinazolin-2′-yl)propanoate **(118b**) was synthesised as described for methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate (**118a**) using methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5′-(1′′-nitronaphthalen-2′′-yl)-1′*H*-pyrazol-3′-yl]propanoate (**116b**) (0.133 g, 0.301 mmol) with 10% palladium on carbon (0.0160 g, 0.0151 mmol) in methanol (10 mL). The resulting aniline was reacted with triphosgene (0.0357 g, 0.120 mmol) and triethylamine (0.0854 mL, 0.602 mmol) in dichloromethane (10 mL). Purification by flash chromatography using silica gel, eluting with 30% to 60% ethyl acetate in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(5′-oxo-5′,6′-dihydrobenzo[h]pyrazole[1′,5′-c]quinazolin-2′-yl)propanoate (**116b**) as a white solid (0.0300 g, 23%). Mp 140–142 °C; ν_{max}/cm^{-1} (neat) 3278 (NH), 2975 (CH), 1709 (C=O), 1344, 1165; $[\alpha]_D^{23}$ +17.6 (*c* 0.3, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.41 (9H, s, 3 × CH_3), 3.31–3.47 (2H, m, 3-H), 3.82 (3H, s, OCH_3), 4.72–4.86 (1H, m, 2-H), 5.60 (1H, d, *J* 8.3 Hz, NH), 6.72 (1H, s, 1′-H), 7.51–7.61 (3H, m, 9′-H, 11′-H and 12′-H), 7.70 (1H, t, *J* 8.0 Hz, 8′-H), 7.82 (1H, d, *J* 8.0 Hz, 10′-H), 8.39 (1H, d, *J* 8.0 Hz, 7′-H), 10.50 (1H, s, NH); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 31.4 (CH_2), 52.8 (CH_3), 53.1 (CH), 80.2 (C), 100.9 (CH), 108.5 (C), 120.4 (CH), 120.9 (CH), 121.7 (C), 124.6 (CH), 127.8 (CH), 127.9 (CH), 128.9 (CH), 129.7 (C), 133.9 (C), 142.4 (C), 146.0 (C), 154.7 (C), 155.6 (C), 172.5 (C); *m/z* (ESI) 459.1634 (MNa^+. $C_{23}H_{24}N_4NaO_5$ requires 459.1639).

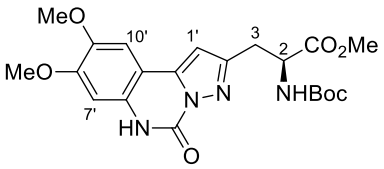
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(9′-fluoro-5′-oxo-5′,6′-dihydropyrazole [1′,5′-c]quinazolin-2′-yl)propanoate (118c)



Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(9′-fluoro-5′-oxo-5′,6′-dihydropyrazole[1′,5′-c]quinazolin-2′-yl)propanoate (**118c**) was synthesised as described for methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate (**118a**)

using methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5′-(5′′-fluoro-2′′-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (**116c**) (0.142 g, 0.347 mmol) with 10% palladium on carbon (0.0185 g, 0.0174 mmol) in methanol (10 mL). The resulting aniline was reacted with triphosgene (0.0412 g, 0.139 mmol) and triethylamine (0.0982 mL, 0.694 mmol) in dichloromethane (10 mL). Purification by flash chromatography using silica gel, eluting with 50% ethyl acetate in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(9′-fluoro-5′-oxo-5′,6′-dihydropyrazole[1′,5′-c]quinazolin-2′-yl)propanoate (**118c**) as a white solid (0.105 g, 75%). Mp 135–137 °C; ν_{max}/cm^{-1} (neat) 3250 (NH), 2979 (CH), 1720 (C=O), 1497, 1367, 1268, 1169; $[\alpha]_D^{26}$ +16.2 (*c* 0.9, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.39 (9H, s, 3 × CH_3), 3.32 (1H, dd, *J* 14.7, 7.3 Hz, 3-*H*H), 3.41 (1H, dd, *J* 14.7, 5.0 Hz, 3-H*H*), 3.77 (3H, s, OCH_3), 4.69–4.78 (1H, m, 2-H), 5.41 (1H, d, *J* 8.3 Hz, NH), 6.75 (1H, s, 1′-H), 7.20 (1H, td, *J* 8.7, 2.5 Hz, 8′-H), 7.37–7.42 (1H, m, 10′-H), 7.46 (1H, dd, *J* 8.7, 4.2 Hz, 7′-H), 11.30 (1H, s, NH); δ_C (126 MHz, $CDCl_3$) 28.3 (3 × CH_3), 31.4 (CH_2), 52.6 (CH_3), 53.0 (CH), 80.2 (C), 101.6 (CH), 109.6 (d, $^2J_{CF}$ 24.6 Hz, CH), 113.6 (d, $^3J_{CF}$ 9.2 Hz, C), 118.3 (d, $^2J_{CF}$ 23.4 Hz, CH), 118.4 (d, $^3J_{CF}$ 8.5 Hz, CH), 130.1 (d, $^4J_{CF}$ 1.7 Hz, C), 141.0 (C), 146.3 (C), 154.4 (C), 155.4 (C), 158.9 (d, $^1J_{CF}$ 244.5 Hz, C), 172.1 (C); *m/z* (ESI) 427.1378 (MNa^+. $C_{19}H_{21}FN_4NaO_5$ requires 427.1388).

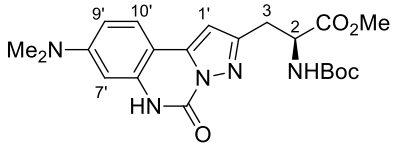
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(8′,9′-dimethoxy-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate (118d)



Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(8′,9′-dimethoxy-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate (**118d**) was synthesised as described for methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate (**118a**) using methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5′-(4′′,5′′-dimethoxy-2′′-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (**116d**) (0.0280 g, 0.0622 mmol) with 10% palladium on carbon (0.00330 g, 0.00311 mmol) in methanol (5 mL). The resulting aniline was reacted with triphosgene (0.00740 mg, 0.0249 mmol) and triethylamine (0.0176 mL, 0.124 mmol) in dichloromethane (5 mL). Purification by flash chromatography using silica gel, eluting first with 60% ethyl acetate in dichloromethane and then with 3% methanol in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(8′,9′-dimethoxy-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate (**118d**) as a white solid (0.0156 g, 58%). Mp 128–130 °C; ν_{max}/cm^{-1} (neat) 3250 (NH), 2929 (CH), 1719 (C=O), 1509, 1278, 1226, 1165, 1021; $[\alpha]_D^{28}$ +1.6 (*c* 0.9, $CHCl_3$); δ_H(500 MHz, $CDCl_3$)

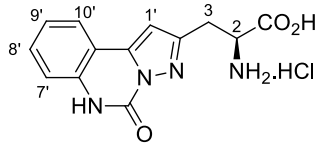
1.39 (9H, s, 3 × CH_3), 3.29 (1H, dd, *J* 14.8, 7.4 Hz, 3-*H*H), 3.37 (1H, dd, *J* 14.8, 4.9 Hz, 3-H*H*), 3.77 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 4.68–4.80 (1H, m, 2-H), 5.42 (1H, d, *J* 8.3 Hz, NH), 6.59 (1H, s, 1′-H), 6.83 (1H, s, 7′-H), 7.04 (1H, s, 10′-H), 11.05 (1H, s, NH); δ_C (126 MHz, $CDCl_3$) 28.4 (3 × CH_3), 31.6 (CH_2), 52.7 (CH_3), 53.1 (CH), 56.5 (CH_3), 56.7 (CH_3), 80.2 (C), 99.2 (CH), 99.3 (CH), 105.0 (CH), 105.3 (C), 128.7 (C), 142.2 (C), 146.6 (C), 146.7 (C), 152.1 (C), 154.4 (C), 155.6 (C), 172.4 (C); *m/z* (ESI) 469.1676 (MNa^+. $C_{21}H_{26}N_4NaO_7$ requires 469.1694).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(8'-dimethylamino-5'-oxo-5'-6'-dihydropyrazolo[1',5'-c]quinazolin-2'-yl)propanoate (118e)



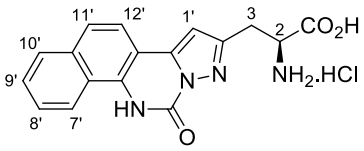
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(8'-dimethylamino-5'-oxo-5'-6'-dihydropyrazolo[1',5'-c]quinazolin-2'-yl)propanoate (**118e**) was synthesised as described for methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate (**118a**) using methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5'-(4''-dimethylamino-2''-nitrophenyl)-1'*H*-pyrazol-3'-yl]propanoate (**116e**) (0.0814 g, 0.188 mmol) with 10% palladium on carbon (0.0100 g, 0.00940 mmol) in methanol (10 mL). The resulting aniline was reacted with triphosgene (0.0223 g, 0.0752 mmol) and triethylamine (0.0532 mL, 0.376 mmol) in dichloromethane (10 mL). Purification by flash chromatography using silica gel, eluting with 80% to 100% ethyl acetate in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(8'-dimethylamino-5'-oxo-5'-6'-dihydropyrazolo[1',5'-c]quinazolin-2'-yl)propanoate (**118e**) as a yellow solid (0.0384 g, 48%). Mp 174–176 °C; ν_{max}/cm^{-1} (neat) 3271 (NH), 2978 (CH), 1705 (C=O), 1628 (C=O), 1528, 1373, 1157, 910, 725; $[\alpha]_D^{27}$ +6.0 (*c* 0.7, $CHCl_3$); δ_H(400 MHz, $CDCl_3$) 1.39 (9H, s, 3 × CH_3), 3.01 (6H, s, 2 × CH_3), 3.26 (1H, dd, *J* 14.4, 7.2 Hz, 3-*H*H), 3.33 (1H, dd, *J* 14.4, 4.8 Hz, 3-H*H*), 3.76 (3H, s, OCH_3), 4.64–4.77 (1H, m, 2-H), 5.44 (1H, d, *J* 8.2 Hz, NH), 6.36–6.46 (2H, m, 1'-H and 7'-H), 6.56 (1H, dd, *J* 8.8, 1.5 Hz, 9'-H), 7.47 (1H, d, *J* 8.8 Hz, 10'-H), 10.44 (1H, s, NH); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 31.4 (CH_2), 40.4 (2 × CH_3), 52.6 (CH_3), 53.1 (CH), 80.0 (C), 96.9 (CH), 98.0 (CH), 102.0 (C), 109.3 (CH), 124.8 (CH), 135.6 (C), 142.9 (C), 146.8 (C), 151.9 (C), 154.1 (C), 155.6 (C), 172.5 (C); *m/z* (ESI) 452.1900 (MNa^+. $C_{21}H_{27}N_5NaO_5$ requires 452.1904).

(2*S*)-2-Amino-3-(5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (94)



Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate (**118a**) (0.0417 g, 0.108 mmol) was dissolved in methanol (3.5 mL) and a solution of caesium carbonate (0.0457 g, 0.140 mmol) in water (1.5 mL) was added. The reaction mixture was stirred at room temperature for 16 h before being concentrating *in vacuo*. The resulting residue was dissolved in water (20 mL), acidified to pH 1 with 1 M aqueous hydrochloric acid and extracted with dichloromethane (3 × 30 mL). The organic layers were combined and washed with brine (50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting residue was dissolved in 2 M aqueous hydrochloric acid (5 mL) and stirred at room temperature for 2 h. The reaction mixture was concentrated *in vacuo* and the resulting residue was purified by recrystallisation from methanol and diethyl ether to give (2*S*)-2-amino-3-(5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (**94**) as a white solid (0.0268 g, 80%). Mp 280–283 °C (decomposition); ν_{max}/cm^{-1} (neat) 3373 (NH), 2479 (CH), 1701 (C=O), 1599, 1490, 1355, 1119, 973; $[\alpha]_D^{27}$ –6.6 (*c* 0.5, MeOH); δ_H (500 MHz, CD_3OD) 3.43 (1H, dd, *J* 16.3, 8.3 Hz, 3-*H*H), 3.56 (1H, dd, *J* 16.3, 3.9 Hz, 3-H*H*), 4.44 (1H, dd, *J* 8.3, 3.9 Hz, 2-H), 7.02 (1H, s, 1′-H), 7.25–7.36 (2H, m, 7′-H and 9′-H), 7.48–7.54 (1H, m, 8′-H), 7.93 (1H, d, *J* 7.8 Hz, 10′-H); δ_C (126 MHz, CD_3OD) 30.0 (CH_2), 53.4 (CH), 102.2 (CH), 113.7 (C), 117.0 (CH), 125.1 (CH), 125.3 (CH), 132.0 (CH), 135.6 (C), 143.5 (C), 147.0 (C), 154.3 (C), 171.3 (C); *m/z* (ESI) 295.0795 (MNa^+. $C_{13}H_{12}N_4NaO_3$ requires 295.0802).

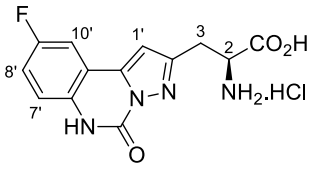
(2*S*)-2-Amino-3-(5′-oxo-5′,6′-dihydrobenzo[h]pyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (98)



Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(5′-oxo-5′,6′-dihydrobenzo[h]pyrazole[1′,5′-c]quinazolin-2′-yl)propanoate (**118b**) (0.0293 g, 0.0671 mmol) was suspended in 6 M aqueous hydrochloric acid (5 mL). The reaction mixture was stirred under reflux for 16 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo* and recrystallisation from

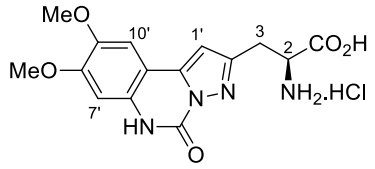
methanol and diethyl ether gave (2*S*)-2-amino-3-(5′-oxo-5′,6′-dihydrobenzo[h]pyrazolo[1′,5′-c]quinazolin-2-yl)propanoic acid hydrochloride (**98**) as an off-white solid (0.0089 g, 37%). Mp 278–279 °C (decomposition); ν_{max}/cm^{-1} (neat) 3178 (NH), 2975 (CH), 1725 (C=O), 1509, 1350, 1177, 801; $[\alpha]_D^{23}$ –7.0 (*c* 0.2, MeOH); δ_H (500 MHz, CD_3OD) 3.48 (1H, dd, *J* 16.4, 8.3 Hz, 3-*H*H), 3.61 (1H, dd, *J* 16.4, 3.9 Hz, 3-H*H*), 4.55 (1H, dd, *J* 8.3, 3.9 Hz, 2-H), 7.10 (1H, s, 1′-H), 7.64–7.72 (2H, m, 8′-H and 9′-H), 7.77 (1H, d, *J* 8.7 Hz, 11′-H), 7.92 (1H, d, *J* 8.7 Hz, 12′-H), 7.95 (1H, dd, *J* 8.1, 1.0 Hz, 10′-H), 8.50 (1H, d, *J* 8.1 Hz, 7′-H); δ_C (126 MHz, CD_3OD) 29.9 (CH_2), 53.1 (CH), 102.1 (CH), 109.6 (C), 121.7 (CH), 122.5 (CH), 123.3 (C), 125.9 (CH), 128.6 (CH), 129.1 (CH), 130.1 (CH), 131.7 (C), 135.7 (C), 144.0 (C), 147.4 (C), 154.6 (C), 170.9 (C); *m/z* (ESI) 345.0948 (MNa^+. $C_{17}H_{14}N_4NaO_3$ requires 345.0958).

(2*S*)-2-Amino-3-(9′-fluoro-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (99)



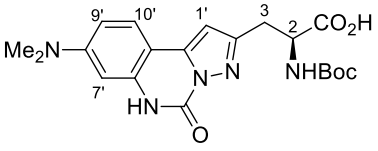
(2*S*)-2-Amino-3-(9′-fluoro-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (**99**) was synthesised as described for (2*S*)-2-amino-3-(5′-oxo-5′,6′-dihydrobenzo[h]pyrazolo[1′,5′-c]quinazolin-2-yl)propanoic acid hydrochloride (**98**) using methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(9′-fluoro-5′-oxo-5′,6′-dihydropyrazole[1′,5′-c]quinazolin-2′-yl)propanoate (**118c**) (0.0469 g, 0.116 mmol). Recrystallisation from methanol and diethyl ether gave (2*S*)-2-amino-3-(9′-fluoro-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (**99**) as a white solid (0.0150 g, 40%). Mp 275–276 °C (decomposition); ν_{max}/cm^{-1} (neat) 3175 (NH), 2950 (CH), 1710 (C=O), 1506, 1358, 1268, 1191, 828; $[\alpha]_D^{27}$ −14.5 (*c* 0.1, MeOH); δ_H (400 MHz, DMSO-d_6) 3.19–3.45 (2H, m, 3-H_2), 4.34 (1H, t, *J* 6.5 Hz, 2-H), 7.18 (1H, s, 1′-H), 7.35–7.47 (2H, m, 8′-H and 10′-H), 7.94 (1H, dd, *J* 8.8, 1.6 Hz, 7′-H), 8.35 (2H, br s, NH_2), 11.93 (1H, s, NH); δ_C (101 MHz, DMSO-d_6) 29.0 (CH_2), 51.6 (CH), 102.4 (CH), 109.7 (d, $^2J_{CF}$ 24.9 Hz, CH), 113.0 (d, $^3J_{CF}$ 9.6 Hz, C), 117.8 (d, $^3J_{CF}$ 8.9 Hz, CH), 118.0 (d, $^2J_{CF}$ 24.9 Hz, CH), 131.0 (d, $^4J_{CF}$ 1.4 Hz, C), 140.6 (d, $^4J_{CF}$ 3.3 Hz, C), 143.9 (C), 151.6 (C), 157.8 (d, $^1J_{CF}$ 239.5 Hz, C), 169.9 (C); *m/z* (ESI) 313.0706 (MNa^+. $C_{13}H_{11}FN_4NaO_3$ requires 313.0707).

(2*S*)-2-Amino-3-(8′,9′-dimethoxy-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (100)



(2*S*)-2-Amino-3-(8′,9′-dimethoxy-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (**100**) was synthesised as described for (2*S*)-2-amino-3-(5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (**94**) using methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(8′,9′-dimethoxy-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate (**118d**) (0.0226 g, 0.0507 mmol) and caesium carbonate (0.0215 g, 0.0658 mmol). Recrystallisation from methanol and diethyl ether gave (2*S*)-2-amino-3-(8′,9′-dimethoxy-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (**100**) as a white solid (0.0152 g, 82%). Mp 268–270 °C (decomposition); ν_{max}/cm^{-1} (neat) 3375 (NH), 2919 (CH), 1715 (C=O), 1507, 1284, 1229, 1011, 791; $[\alpha]_D^{25}$ −7.6 (*c* 0.4, MeOH); δ_H (500 MHz, CD_3OD) 3.45 (1H, dd, *J* 16.0, 9.3 Hz, 3-*H*H), 3.59 (1H, dd, *J* 16.0, 3.5 Hz, 3-H*H*), 3.86 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 4.57 (1H, dd, *J* 9.3, 3.5 Hz, 2-H), 6.56 (1H, s, 1′-H), 6.89 (1H, s, 10′-H), 7.25 (1H, s, 7′-H); δ_C (126 MHz, CD_3OD) 30.1 (CH_2), 53.6 (CH), 56.5 (CH_3), 56.9 (CH_3), 99.4 (CH), 100.8 (CH), 105.8 (C), 106.4 (CH), 130.1 (C), 143.4 (C), 146.7 (C), 147.8 (C), 153.4 (C), 154.0 (C), 171.0 (C); *m/z* 355.0998 (MNa^+. $C_{15}H_{16}N_4NaO_5$ requires 355.1013).

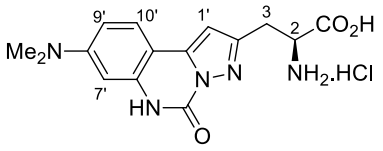
(2*S*)-2-(*tert*-butoxycarbonylamino)-3-(8'-dimethylamino-5'-oxo-5'-6'-dihydropyrazolo[1',5'-c]quinazolin-2'-yl)propanoic acid (129)



Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(8′-dimethylamino-5'-oxo-5'-6'-dihydropyrazolo[1',5'-c]quinazolin-2'-yl)propanoate (**118e**) (0.188 g, 0.438 mmol) was dissolved in methanol (8.5 mL) and a solution of caesium carbonate (0.187 g, 0.575 mmol) in water (3.5 mL) was added. The reaction mixture was stirred at room temperature for 20 h and then concentrated *in vacuo*. The resulting residue was dissolved in water (50 mL) and acidified to pH 1 with 1 M aqueous hydrochloric acid. Solid sodium chloride was added and extracted with dichloromethane (3 × 50 mL). The organic

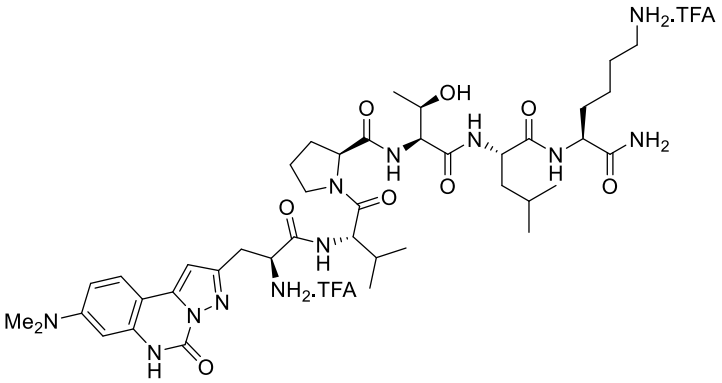
layers were combined and washed with brine (50 mL). The brine was re-extracted with dichloromethane (30 mL). The organic layers were dried ($MgSO_4$) and concentrated *in vacuo* to give (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(8'-dimethylamino-5'-oxo-5'-6'-dihydropyrazolo[1',5'-c]quinazolin-2'-yl)propanoic acid (**129**) as a yellow solid (0.143 g, 78%). Mp 295–297 °C; ν_{max}/cm^{-1} (neat) 3414 (NH), 2976 (CH), 1708 (C=O), 1629, 1533, 1387, 1163; $[\alpha]_D^{23}$ +15.3 (*c* 0.9, $CHCl_3$); δ_H (400 MHz, CD_3OD) 1.39 (9H, s, 3 × CH_3), 3.03 (6H, s, 2 × CH_3), 3.13 (1H, dd, *J* 14.6, 9.2 Hz, 3-*H*H), 3.28–3.33 (1H, m, 3-H*H*), 4.52 (1H, dd, *J* 9.2, 4.8 Hz, 2-H), 6.41 (1H, d, *J* 2.3 Hz, 7'-H), 6.65 (1H, s, 1'-H), 6.69 (1H, dd, *J* 8.9, 2.3 Hz, 9'-H), 7.61 (1H, d, *J* 8.9 Hz, 10'-H); δ_C (101 MHz, CD_3OD) 28.7 (3 × CH_3), 31.9 (CH_2), 40.3 (2 × CH_3), 54.7 (CH), 80.6 (C), 97.4 (CH), 99.1 (CH), 103.0 (C), 110.3 (CH), 125.9 (CH), 137.3 (C), 143.9 (C), 147.2 (C), 153.5 (C), 156.0 (C), 157.9 (C), 175.2 (C); *m/z* 438.1738 (MNa^+. $C_{20}H_{25}N_5NaO_5$ requires 438.1748).

(2*S*)-2-Amino-3-(8′-dimethylamino-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (101)



(2*S*)-2-(*tert*-Butoxycarbonylamino)-3-(8'-dimethylamino-5'-oxo-5'-6'-dihydropyrazolo[1',5'-c]quinazolin-2'-yl)propanoic acid (**129**) (0.0375 g, 0.0903 mmol) was dissolved in 2 M aqueous hydrochloric acid (5 mL) and stirred at room temperature for 2 h. The reaction mixture was concentrated *in vacuo* and the resulting residue was purified by recrystallisation from methanol and diethyl ether. This gave (2*S*)-2-amino-3-(8′-dimethylamino-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (**101**) as a yellow solid (0.0322 g, 100%). Mp 208–210 °C (decomposition); ν_{max}/cm^{-1} (neat) 3423 (NH), 2854 (CH), 1713 (C=O), 1612, 1497, 1335, 1211, 810; $[\alpha]_D^{32}$ –10.7 (*c* 0.3, MeOH); δ_H (400 MHz, CD_3OD) 3.11 (6H, s, 2 × CH_3), 3.40 (1H, dd, *J* 16.6, 8.3 Hz, 3-*H*H), 3.52 (1H, dd, *J* 16.6, 3.9 Hz, 3-H*H*), 4.49 (1H, dd, *J* 8.3, 3.9 Hz, 2-H), 6.66 (1H, d, *J* 2.3 Hz, 7'-H), 6.80 (1H, s, 1'-H), 6.91 (1H, dd, *J* 8.9, 2.2 Hz, 9'-H), 7.80 (1H, d, *J* 8.9 Hz, 10'-H); δ_C (126 MHz, CD_3OD) 29.9 (CH_2), 43.9 (2 × CH_3), 53.1 (CH), 103.8 (C), 109.1 (C), 113.9 (CH), 127.1 (CH), 136.9 (CH), 137.0 (CH), 143.2 (C), 146.9 (C), 149.3 (C), 154.4 (C), 170.8 (C); *m/z* 338.1224 (MNa^+. $C_{15}H_{17}N_5NaO_3$ requires 338.1224).

Hexapeptide 131



The pentapeptide was synthesised on a Biotage Initiator+ Alstra peptide synthesiser using a Fmoc/^{t}Bu protecting group strategy on a 0.1 mmol synthetic scale using Rink Amide ChemMatrix® resin. The resin-bound peptide 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 2-(6-chloro-1-*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU) in DMF (4 equivalents) and 2 M diisopropylethylamine in NMP (8 equivalents). Fmoc groups were removed using 20% piperidine in DMF. The resin-bound pentapeptide (0.296 g, 0.100 mmol) was treated with 20% piperidine in DMF (2 mL) and shaken for 0.25 h. The solution was filtered, and the resin-bound peptide was washed with dichloromethane (3 × 2 mL), isopropanol (3 × 2 mL) and DMF (3 × 2 mL). The resin-bound peptide was suspended in DMF (2 mL) followed by addition of (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(8'-dimethylamino-5'-oxo-5'-6'-dihydropyrazolo[1',5'-c]quinazolin-2'-yl)propanoic acid (**129**) (0.0834 g, 0.200 mmol), diisopropylethylamine (0.0700 mL, 0.400 mmol) and 0.5 M HCTU in DMF (0.400 mL, 0.200 mmol). The mixture was shaken for 18 h. The solution was filtered, and the resin-bound peptide was washed with dichloromethane (3 × 2 mL), isopropanol (3 × 2 mL) and DMF (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 **131** was precipitated from a solution of ice-cold diethyl ether (2 mL), centrifuged at 4500 rpm for 5 minutes and the precipitate was washed with ice-cold diethyl ether (3 × 2 mL). Peptide **131** 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 µm, 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 to give a yellow solid (0.0614 g, 0.0720 mmol, 72%). Peptide **131** 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 H2O + 0.1% TFA) and B (5% H2O in MeCN + 0.1% TFA). Two gradients were used to characterise **131**, a gradient from 0–100% solution B over 20 minutes (Figure 1) and a gradient from 0–100% solution B over 50 minutes (Figure 2). Analytical RP-HPLC data is reported as column retention time (tR) in minutes (Table 1). 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 (%)	853.5043 $[M+H]^+$	853.5045 $[M+H]^+$
11.56	99	20.25	98		

Table 1: RP-HPLC gradients and HRMS data.

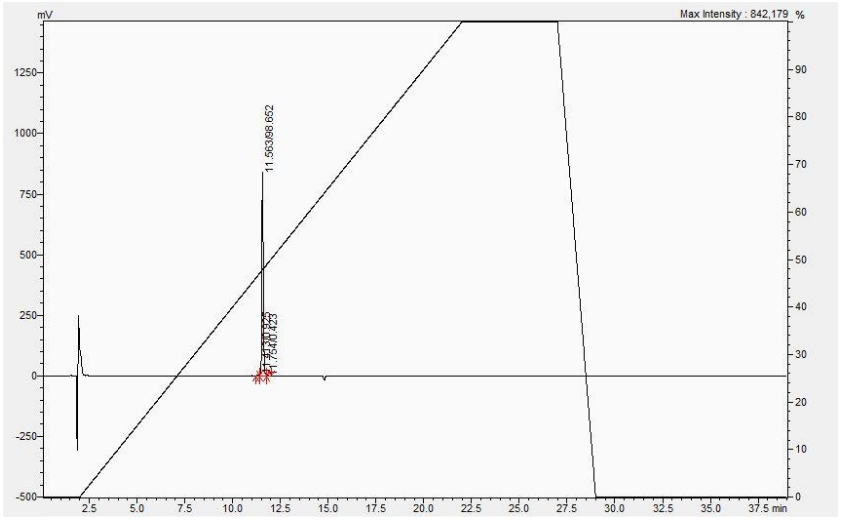


Figure 1: Analytical HPLC 20-minute gradient.

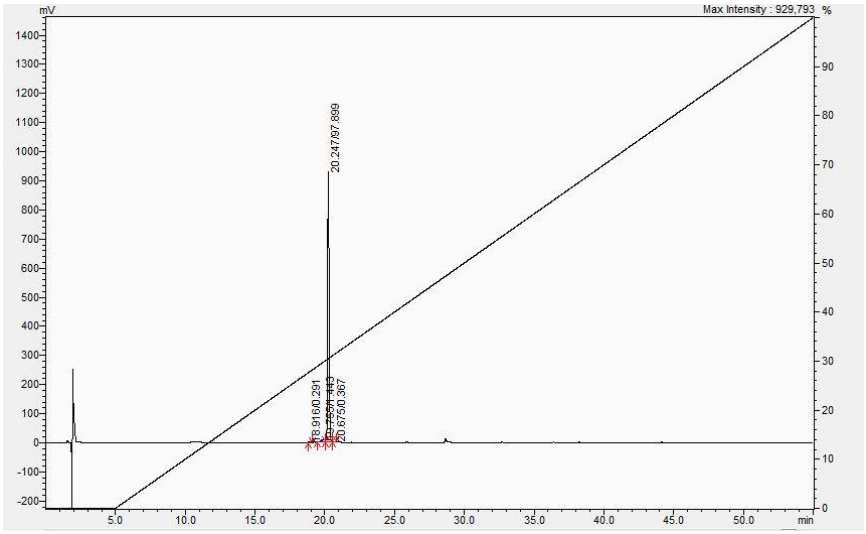
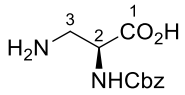


Figure 2: Analytical HPLC 50-minute gradient.

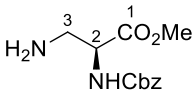
3.3 Experimental for benzotriazole derived amino acids

(2*S*)-2-[(Benzyloxycarbonyl)amino]-3-aminopropionic acid (155)[208]



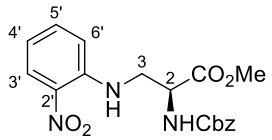
To a suspension of N_{α}-(benzyloxycarbonyl)amino-L-asparagine (**154**) (5.00 g, 18.8 mmol) in acetonitrile (24 mL), ethyl acetate (24 mL) and water (12 mL) was added (diacetoxyiodo)benzene (7.26 g, 22.5 mmol) and this reaction mixture was stirred at 16 °C for 0.5 h. The reaction mixture was warmed to room temperature for and stirred for a further 3 h. The resulting solid filtered and washed with cold ethyl acetate (50 mL) and diethyl ether (50 mL) to give (2*S*)-2-[(benzyloxycarbonyl)amino]-3-aminopropionic acid (**155**) (4.48 g, 100%) as a white solid. Mp 208–211 °C; $[\alpha]_D^{23}$ −7.9 (*c* 1.2, 1 M NaOH), lit.[208] $[\alpha]_D^{23}$ −7.7 (*c* 0.4, 1 M NaOH); δ_H (400 MHz, DMSO-d_6/TFA) 2.99–3.06 (1H, m, 3-*H*H), 3.21–3.28 (1H, m, 3-H*H*), 4.30 (1H, dt, *J* 8.9, 4.6 Hz, 2-H), 5.07 (2H, s, OCH_2Ph), 7.28–7.37 (5H, m, Ph), 7.70 (1H, d, *J* 8.9 Hz, NH), 7.91 (2H, s, NH_2); δ_C (101 MHz, DMSO-d_6/TFA) 40.4 (CH_2), 51.9 (CH), 66.0 (CH_2), 128.0 (2 × CH), 128.0 (CH), 128.5 (2 × CH), 136.8 (C), 156.4 (C), 170.9 (C); *m/z* (ESI) 261 (MNa^+. 100%).

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-aminopropanoate hydrochloride (156)[209]



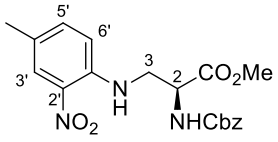
To a suspension of (2*S*)-2-[(benzyloxycarbonyl)amino]-3-aminopropionic acid (**155**) (4.48 g, 18.8 mmol) in methanol (30 mL) at 0 °C under argon was added thionyl chloride (1.78 mL, 24.4 mmol) dropwise. After warming to room temperature, the reaction mixture was stirred for 16 h, concentrated *in vacuo* to give the crude product. This was triturated with diethyl ether to give methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-aminopropanoate hydrochloride (**156**) (4.74 g, 100%) as a white solid. Mp 165–168 °C, lit.[209] 169–171 °C; $[\alpha]_D^{22}$ −41.4 (*c* 1.1, MeOH), lit.[2] $[\alpha]_D^{21}$ −43.1 (*c* 2.5, MeOH); δ_H (400 MHz, CD_3OD) 3.23 (1H, dd, *J* 13.2, 8.8 Hz, 3-*H*H), 3.45 (1H, dd, *J* 13.2, 5.1 Hz, 3-H*H*), 3.78 (3H, s, OCH_3), 4.51 (1H, dd, *J* 8.8, 5.1 Hz, 2-H), 5.14 (2H, s, OCH_2Ph), 7.30–7.40 (5H, m, Ph); δ_C (101 MHz, CD_3OD) 41.3 (CH_2), 53.1 (CH), 53.4 (CH_3), 68.2 (CH_2), 129.0 (2 × CH), 129.2 (CH), 129.5 (2 × CH), 137.8 (C), 158.7 (C), 170.9 (C); *m/z* (ESI) 275 (MNa^+. 100%).

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[(2′-nitrophenyl)amino]propanoate (157a)



To a solution of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-aminopropanoate hydrochloride (**156**) (0.250 g, 0.991 mmol) in acetonitrile (7.5 mL) was added 2-fluoronitrobenzene (0.310 mL, 2.97 mmol) and triethylamine (0.410 mL, 2.97 mmol). The reaction mixture was degassed with argon for 0.15 h and heated under reflux for 16 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. The resulting residue was dissolved in ethyl acetate (50 mL), washed with 1 M aqueous hydrochloric acid (50 mL), water (50 mL) and brine (50 mL). The organic layer was dried and concentrated *in vacuo* to give the crude product. Purification by flash chromatography using silica gel, eluting with 0% to 20% ethyl acetate in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[(2′-nitrophenyl)amino]propanoate (**157a**) as a yellow solid (0.330 g, 89%). Mp 82–84 °C; ν_{max}/cm^{-1} (neat) 3348 (NH), 1721 (C=O), 1512, 1265, 1234, 741; $[\alpha]_D^{25}$ +50.8 (*c* 0.3, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.64–3.74 (5H, m, 3-H_2 and OCH_3), 4.57 (1H, dt, *J* 6.7, 5.7 Hz, 2-H), 5.04 (2H, s, OCH_2Ph), 5.63 (1H, d, *J* 6.7 Hz, NH), 6.69 (1H, br t, *J* 8.6 Hz, 4′-H), 6.98 (1H, d, *J* 8.6 Hz, 6′-H), 7.27–7.51 (6H, m, 5′-H and Ph), 8.16 (1H, dd, *J* 8.6, 1.2 Hz, 3′-H), 8.22 (1H, t, *J* 5.6 Hz, NH); δ_C (101 MHz, $CDCl_3$) 44.6 (CH_2), 53.1 (CH_3), 53.6 (CH), 67.4 (CH_2), 113.7 (CH), 116.3 (CH), 127.0 (CH), 128.3 (2 × CH), 128.4 (CH), 128.7 (2 × CH), 132.8 (C), 136.0 (C), 136.4 (CH), 144.9 (C), 155.9 (C), 170.8 (C); *m/z* (ESI) 396.1158 (MNa^+. $C_{18}H_{19}N_3NaO_6$ requires 396.1166).

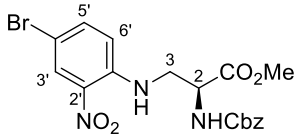
Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[(2′-nitro-4′-methylphenyl)amino]propanoate (157b)



Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[(2′-nitro-4′-methylphenyl)amino]propanoate (**157b**) was synthesised as described for methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[(2′-nitrophenyl)amino]propanoate (**157a**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-aminopropanoate hydrochloride (**156**) (2.85 g, 11.3 mmol), 4-fluoro-3-nitrotoluene (5.26 g, 33.9 mmol) and triethylamine (4.72 mL, 33.9 mmol) in acetonitrile (50 mL). Purification by flash chromatography using silica gel, eluting with 0% to 20% ethyl acetate in dichloromethane gave

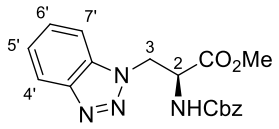
methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[(2′-nitro-4′-methylphenyl)amino]propanoate (**157b**) as a viscous orange oil (2.70 g, 62%). ν_{max}/cm^{-1} (neat) 3364 (NH), 2955 (CH), 1721 (C=O), 1520 (C=C), 1273, 1227, 1057; $[\alpha]_D^{24}$ +50.4 (*c* 1.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.18 (3H, s, CH_3), 3.61–3.74 (5H, m, 3-H_2 and OCH_3), 4.59 (1H, q, *J* 6.3 Hz, 2-H), 5.04 (2H, s, OC*H*$_2$Ph), 5.90 (1H, d, *J* 6.3 Hz, NH), 6.82 (1H, d, *J* 8.5 Hz, 6′-H), 7.16 (1H, d, *J* 8.5 Hz, 5′-H), 7.20–7.32 (5H, m, Ph), 7.87 (1H, s, 3′-H), 8.05 (1H, t, *J* 6.3 Hz, NH); δ_C (101 MHz, $CDCl_3$) 19.8 (CH_3), 44.4 (CH_2), 52.8 (CH_3), 53.4 (CH), 67.1 (CH_2), 113.6 (CH), 125.6 (C), 126.1 (CH), 128.0 (2 × CH), 128.1 (CH), 128.4 (2 × CH), 132.2 (C), 136.0 (C), 137.6 (CH), 142.9 (C), 155.9 (C), 170.8 (C); *m/z* (ESI) 410.1313 (MNa^+. $C_{19}H_{21}N_3NaO_6$ requires 410.1323).

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[(4′-bromo-2′-nitrophenyl)amino]propanoate (157c)



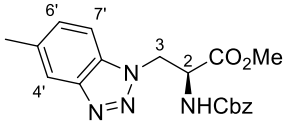
Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[(4′-bromo-2′-nitrophenyl)amino]propanoate (**157c**) was synthesised as described for methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[(2′-nitrophenyl)amino]propanoate (**157a**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-aminopropanoate hydrochloride (**156**) (3.97 g, 13.8 mmol), 5-bromo-2-fluoronitrobenzene (5.09 mL, 41.3 mmol) and triethylamine (5.76 mL, 41.3 mmol) in acetonitrile (50 mL). Purification by flash chromatography using silica gel, eluting with 0% to 20% ethyl acetate in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[(4′-bromo-2′-nitrophenyl)amino]propanoate (**157c**) as a yellow solid (5.75 g, 92%). Mp 86–89 °C; ν_{max}/cm^{-1} (neat) 3364 (NH), 2955 (CH), 1721 (C=O), 1612 (C=O), 1504, 1227, 1065; $[\alpha]_D^{25}$ +14.3 (*c* 1.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.60–3.76 (5H, m, 3-H_2 and OCH_3), 4.60 (1H, dt, *J* 6.9, 5.8 Hz, 2-H), 5.06 (1H, d, *J* 12.2 Hz, OC*H*HPh), 5.11 (1H, d, *J* 12.2 Hz, OCH*H*Ph), 6.06 (1H, d, *J* 6.9 Hz, NH), 6.81 (1H, d, *J* 9.1 Hz, 6′-H), 7.20–7.30 (5H, m, Ph), 7.36 (1H, dd, *J* 9.1, 1.5 Hz, 5′-H), 8.16 (1H, d, *J* 1.5 Hz, 3′-H), 8.19 (1H, t, *J* 6.0 Hz, NH); δ_C (101 MHz, $CDCl_3$) 44.2 (CH_2), 52.8 (CH_3), 53.2 (CH), 67.0 (CH_2), 107.0 (C) 115.3 (CH), 127.9 (2 × CH), 128.1 (CH), 128.3 (2 × CH), 128.6 (CH), 132.5 (C), 135.8 (C), 138.7 (CH), 143.6 (C), 155.9 (C), 170.4 (C); *m/z* (ESI) 474.0276 (MNa^+. $C_{18}H_{18}{}^{79}BrN_3NaO_6$ requires 474.0271).

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (159a)



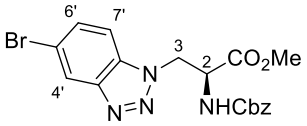
To a solution of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[(2′-nitrophenyl)amino] propanoate (**157a**) (0.320 g, 0.857 mmol) in methanol (12 mL) was added tin(II) dichloride dihydrate (0.970 g, 4.29 mmol). The reaction mixture was heated under reflux and stirred under argon for 24 h. After cooling to room temperature, the reaction was concentrated *in vacuo* and the resulting residue was dissolved in ethyl acetate (50 mL) and mixed with a saturated solution of aqueous sodium hydrogen carbonate (30 mL). The biphasic mixture was filtered through Celite® and the organic layer was separated from the aqueous layer. The product was further extracted from the aqueous layer with ethyl acetate (2 × 20 mL). The combined organic layers were washed with brine (50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by flash chromatography using silica gel, eluting with 10% diethyl ether in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[(2′-aminophenyl)amino]propanoate (**158a**) as a pink solid (0.244 g, 83%). This material was then used immediately for the next step. To a stirring solution of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[(2′-aminophenyl)amino]propanoate (**158a**) (0.244 g, 0.711 mmol) in acetonitrile (6 mL) at −10 °C was added *p*-toluenesulfonic acid (0.216 g, 1.14 mmol) and polymer-supported nitrate (0.609 g, containing 2.13 mmol of NO_2^-). The reaction mixture was stirred at −10 °C for 3 h. Afterwards, the reaction mixture was diluted with dichloromethane (50 mL) and the resin was removed by filtration. The filtrate was washed with sat. aqueous sodium carbonate (50 mL) and brine (50 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product. Purification by flash chromatography using silica gel, eluting with 0% to 10% diethyl ether in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**159a**) as a white solid (0.148 g, 59%). Mp 82–85 °C; ν_{max}/cm^{-1} (neat) 3325 (NH), 2955 (CH), 1713 (C=O), 1504, 1211, 1057, 741; $[\alpha]_D^{25}$ +37.0 (*c* 0.4, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.75 (3H, s, OCH_3), 4.88 (1H, dt, *J* 7.0, 4.4 Hz, 2-H), 5.05–5.18 (4H, m, 3-H_2 and O*C*H_2Ph), 5.66 (1H, d, *J* 7.0 Hz, NH), 7.29–7.45 (8H, m, 5′-H, 6′-H, 7′-H and Ph), 8.03 (1H, br d, *J* 8.0, 4′-H); δ_C (101 MHz, $CDCl_3$) 48.7 (CH_2), 53.3 (CH_3), 54.4 (CH), 67.4 (CH_2), 109.2 (CH), 120.2 (CH), 124.2 (CH), 127.9 (CH), 128.3 (2 × CH), 128.5 (CH), 128.7 (2 × CH), 133.9 (C), 136.0 (C), 145.8 (C), 155.8 (C), 169.5 (C); *m/z* (ESI) 377.1223 (MNa^+. $C_{18}H_{18}N_4NaO_4$ requires 377.1220).

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-methyl-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (159b)



Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-methyl-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**159b**) was synthesised as described for methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**159a**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[(2′-nitro-4′-methylphenyl)amino]propanoate (**157b**) (2.70 g, 6.96 mmol). This gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-methyl-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**159b**) as a white solid (0.997 g, 69%). Mp 79–83 °C; ν_{max}/cm^{-1} (neat) 3314 (NH), 2951 (CH), 1717 (C=O), 1500, 1215, 1057, 1022, 741; $[\alpha]_D^{25}$ +36.5 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.48 (3H, s, CH_3), 3.73 (3H, s, OCH_3), 4.86 (1H, dt, *J* 7.0, 4.6 Hz, 2-H), 5.01–5.17 (4H, m, 3-H_2 and OCH_2Ph), 5.68 (1H, d, *J* 7.0 Hz, NH), 7.21 (1H, dd, *J* 8.5, 1.4 Hz, 6′-H), 7.22–7.31 (6H, m, 7′-H and Ph), 7.76 (1H, br s, 4′-H); δ_C (101 MHz, $CDCl_3$) 21.6 (CH_3), 48.6 (CH_2), 53.3 (CH_3), 54.3 (CH), 67.3 (CH_2), 108.7 (CH), 119.1 (CH), 128.3 (2 × CH), 128.4 (CH), 128.7 (2 × CH), 130.1 (CH), 132.4 (C), 134.2 (C), 136.1 (C), 146.4 (C), 155.8 (C), 169.5 (C); *m/z* (ESI) 391.1374 (MNa^+. $C_{19}H_{20}N_4NaO_4$ requires 391.1377).

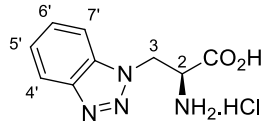
Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-bromo-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (159c)



Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-bromo-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**159c**) was synthesised as described for methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**159a**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[(4′-bromo-2′-nitrophenyl)amino] propanoate (**157c**) (9.00 g, 19.9 mmol). This gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-bromo-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**159c**) as a pink solid (4.25 g, 66%). Mp 110–114 °C; ν_{max}/cm^{-1} (neat) 3321 (NH), 2954 (CH), 1717 (C=O), 1512, 1211, 1057, 752; $[\alpha]_D^{22}$ −22.2 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 3.77 (3H, s, OCH_3), 4.84 (1H, dt, *J* 6.6, 4.5 Hz, 2-H), 5.00–5.20 (4H, m,

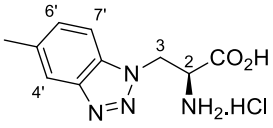
3-H_2 and OCH_2Ph), 5.69 (1H, d, *J* 6.6 Hz, NH), 7.22–7.47 (7H, m, 6′-H, 7′-H and Ph), 8.15 (1H, d, *J* 1.0 Hz, 4′-H); δ_C (126 MHz, $CDCl_3$) 48.6 (CH_2), 53.3 (CH_3), 54.2 (CH), 67.3 (CH_2), 110.4 (CH), 117.4 (C), 122.6 (CH), 128.3 (2 × CH), 128.4 (CH), 128.6 (2 × CH), 131.2 (CH), 132.8 (C), 135.8 (C), 146.7 (C), 155.6 (C), 169.1 (C); *m/z* (ESI) 455.0325 (MNa^+. $C_{18}H_{17}{}^{79}BrN_4NaO_4$ requires 455.0325).

(2*S*)-2-Amino-3-(1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoic acid hydrochloride (160a)



A solution of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**159a**) (0.0834 g, 0.236 mmol) in 6 M hydrochloric acid solution (10 mL) was heated under reflux for 20 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*. Purification by recrystallisation from methanol and diethyl ether gave (2*S*)-2-amino-3-(1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoic acid hydrochloride (**160a**) as a pale brown solid (0.0537 g, 94%). Mp 190–192 °C; ν_{max}/cm^{-1} (neat) 2893 (CH), 1728 (C=O), 1234, 1165, 756; $[\alpha]_D^{24}$ +11.9 (*c* 0.7, MeOH); δ_H (400 MHz, CD_3OD) 4.79 (1H, dd, *J* 5.8, 4.1 Hz, 2-H), 5.26 (1H, dd, *J* 15.5, 4.1 Hz, 3-*H*H), 5.37 (1H, dd, *J* 15.5, 5.8 Hz, 3-H*H*), 7.49 (1H, ddd, *J* 8.4, 7.0, 0.9 Hz, 5′-H), 7.63 (1H, ddd, *J* 8.5, 7.0, 0.9 Hz, 6′-H), 7.84 (1H, dt, *J* 8.5, 0.9 Hz, 7′-H), 8.05 (1H, dt, *J* 8.4, 0.9 Hz, 4′-H); δ_C (101 MHz, CD_3OD) 48.0 (CH_2), 53.5 (CH), 111.1 (CH), 120.3 (CH), 126.0 (CH), 129.5 (CH), 134.9 (C), 146.8 (C), 168.9 (C); *m/z* (ESI) 229.0706 (MNa^+. $C_9H_{10}N_4NaO_2$ requires 229.0696).

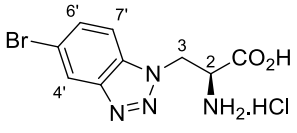
(2*S*)-2-Amino-3-(5′-methyl-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoic acid hydrochloride (160b)



(2*S*)-2-Amino-3-(5′-methyl-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (**160b**) was synthesised as described for (2*S*)-2-amino-3-(1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (**160a**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-methyl-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**159b**) (0.159 g, 0.432 mmol) and 6 M aqueous hydrochloric (10 mL) to give (2*S*)-2-amino-3-(5′-methyl-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoic acid hydrochloride (**160b**) as a white solid (0.0911 g, 82%) Mp 180–184 °C; ν_{max}/cm^{-1} (neat) 2835 (CH), 1744 (C=O), 1505, 1435, 1227, 810; $[\alpha]_D^{19}$ +17.9 (*c* 1.0, MeOH); δ_H (400 MHz, CD_3OD) 2.52 (3H,

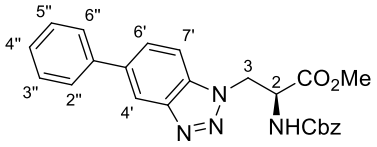
s, CH_3), 4.80 (1H, dd, *J* 5.6, 4.2 Hz, 2-H), 5.25 (1H, dd, *J* 15.4, 4.2 Hz, 3-*H*H), 5.34 (1H, dd, *J* 15.4, 5.6 Hz, 3-H*H*), 7.47 (1H, dd, *J* 8.6, 1.5 Hz, 6′-H), 7.73 (1H, dd, *J* 8.6, 0.8 Hz, 7′-H), 7.77 (1H, dd, *J* 1.5, 0.8 Hz, 4′-H); δ_C (101 MHz, CD_3OD) 21.4 (CH_3), 48.0 (CH_2), 53.5 (CH), 110.8 (CH), 119.0 (CH), 131.6 (CH), 133.4 (C), 136.6 (C), 147.2 (C), 168.9 (C); *m/z* (ESI) 243.0847 (MNa^+. $C_{10}H_{12}N_4NaO_2$ requires 243.0852).

(2*S*)-2-Amino-3-(5′-bromo-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoic acid hydrochloride (160c)



(2*S*)-2-Amino-3-(5′-bromo-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoic acid hydrochloride (**160c**) was synthesised as described for (2*S*)-2-amino-3-(1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoic acid hydrochloride (**160a**) using (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-bromo-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**159c**) (0.137 g, 0.317 mmol) and 6 M aqueous hydrochloric acid (6 mL) to give (2*S*)-2-amino-3-(5′-bromo-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoic acid hydrochloride (**160c**) as a white solid (0.0641, 63%). Mp 168–172 °C; ν_{max}/cm^{-1} (neat) 3402 (NH), 2847 (OH), 1744 (C=O), 1474, 1211, 802; $[\alpha]_D^{22}$ +9.1 (*c* 0.7, MeOH); δ_H (500 MHz, CD_3OD) 4.74 (1H, dd, *J* 5.9, 4.0 Hz, 2-H), 5.24 (1H, dd, *J* 15.5, 4.0 Hz, 3-*H*H), 5.34 (1H, dd, *J* 15.5, 5.9 Hz, 3-H*H*), 7.73 (1H, dd, *J* 8.8, 1.7 Hz, 6′-H), 7.78 (1H, dd, *J* 8.8, 0.5 Hz, 7′-H), 8.26 (1H, dd, *J* 1.7, 0.5 Hz, 4′-H); δ_C (126 MHz, CD_3OD) 48.3 (CH_2), 53.6 (CH), 112.9 (CH), 119.0 (C), 123.1 (CH), 132.7 (CH), 134.0 (C), 148.1 (C), 169.0 (C); *m/z* (ESI) 284.9978 (MH^+. $C_9H_{10}{}^{79}BrN_4O_2$ requires 284.9982).

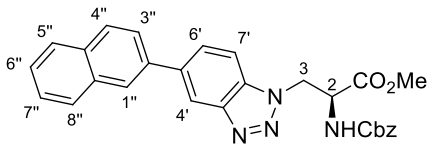
Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-phenyl-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (161a)



To a microwave vial containing phenylboronic acid (0.0463 g, 0.379 mmol), potassium fluoride (0.0441 g, 0.759 mmol) and [1,1′-bis(diphenylphosphino)ferrocene]palladium(II) dichloride-dichloromethane complex (0.0155 g, 0.0189 mmol) was added a solution of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-bromo-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**159c**) (0.109 g, 0.253 mmol) in 1,4-dioxane (3.6 mL) and water (0.4 mL). The reaction mixture was

degassed with argon for 0.15 h, the vial was sealed under argon and stirred at 80 °C for 20 h. The reaction mixture was filtered through Celite® and washed with ethyl acetate (50 mL). The filtrate was washed with 1 M aqueous hydrochloric acid (50 mL), water (50 mL) and brine (50 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product. Purification by flash chromatography using silica gel, eluting with 5% diethyl ether in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-phenyl-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**161a**) as a white solid (0.0912 g, 84%). Mp 136–138 °C; ν_{max}/cm^{-1} (neat) 3323 (NH), 2952 (CH), 1718 (C=O), 1507, 1214, 1060, 757, 698; $[\alpha]_D^{23}$ +8.3 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.78 (3H, s, OCH_3), 4.90 (1H, dt, *J* 7.0, 4.3 Hz, 2-H), 5.05–5.25 (4H, m, 3-H_2 and OCH_2Ph), 5.63 (1H, d, *J* 7.0 Hz, NH), 7.30–7.43 (6H, m, ArH and Ph), 7.44–7.55 (3H, m, 3 × ArH), 7.59–7.70 (3H, m, 3 × ArH), 8.20 (1H, br s, 4′-H); δ_C (101 MHz, $CDCl_3$) 48.6 (CH_2), 53.3 (CH_3), 54.3 (CH), 67.3 (CH_2), 109.2 (CH), 117.9 (CH), 127.5 (2 × CH), 127.6 (CH), 128.1 (CH), 128.3 (2 × CH), 128.4 (CH), 128.6 (2 × CH), 129.0 (2 × CH), 133.2 (C), 135.9 (C), 138.0 (C), 140.4 (C), 146.5 (C), 155.7 (C), 169.3 (C); *m/z* (EI) 430.1654 (M^+. $C_{24}H_{22}N_4O_4$ requires 430.1641).

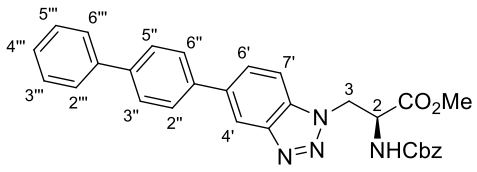
Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(naphthalen-2′′-yl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (161b)



Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(naphthalen-2′′-yl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161b**) was synthesised as described for methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-phenyl-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**161a**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-bromo-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**159c**) (0.105 g, 0.242 mmol), 2-naphthylboronic acid (0.0834 g, 0.485 mmol), [1,1′-bis(diphenylphosphino)ferrocene]palladium(II) dichloride-dichloromethane complex (0.0148 g, 0.0182 mmol) and potassium fluoride (0.0422 g, 0.726 mmol) in dioxane (3.6 mL) and water (0.4 mL). Purification with flash chromatography using silica gel, eluting with 5% diethyl ether in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(naphthalen-2′′-yl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161b**) as a white solid (0.105 g, 91%). Mp 72–74 °C; ν_{max}/cm^{-1} (neat) 3335 (NH), 2950 (CH), 1713 (C=O), 1508, 1211, 1018, 777, 729; $[\alpha]_D^{20}$ +11.7 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.81 (3H, s, OCH_3), 4.93 (1H, dt, *J* 7.1, 4.3 Hz, 2-H), 5.02–5.30 (4H, m, 3-H_2 and OCH_2Ph), 5.76 (1H, d, *J* 7.1 Hz, NH), 7.25–7.62 (11H, m, 6 × ArH and Ph), 7.81 (1H, d, *J* 8.4 Hz, ArH), 7.90 (1H, br d, *J* 8.3 Hz, 7′-H), 7.93 (1H, d, *J* 8.1 Hz, ArH), 8.13 (1H, br s, 4′-H); δ_C (101 MHz,

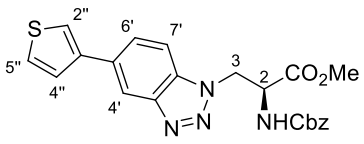
$CDCl_3$) 48.7 (CH_2), 53.3 (CH_3), 54.3 (CH), 67.3 (CH_2), 108.7 (CH), 120.8 (CH), 125.4 (CH), 125.7 (CH), 126.0 (CH), 126.3 (CH), 127.5 (CH), 128.1 (CH), 128.2 (2 × CH), 128.4 (2 × CH), 128.6 (2 × CH), 130.7 (CH), 131.7 (C), 133.2 (C), 133.8 (C), 135.9 (C), 137.1 (C), 139.1 (C), 146.0 (C), 155.7 (C), 169.4 (C); *m/z* (ESI) 503.1671 (MNa^+. $C_{28}H_{24}N_4NaO_4$ requires 503.1690).

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(biphenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (161c)



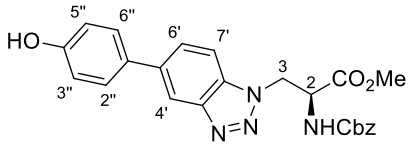
Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(biphenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161c**) was synthesised as described for methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-phenyl-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**161a**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-bromo-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**159c**) (0.105 g, 0.242 mmol), 4-biphenylboronic acid (0.0959 g, 0.484 mmol), [1,1′-bis(diphenylphosphino)ferrocene]palladium(II) dichloride-dichloromethane complex (0.0148 g, 0.0182 mmol) and potassium fluoride (0.0422 g, 0.726 mmol) in dioxane (3.6 mL) and water (0.4 mL). Purification with flash chromatography using silica gel, eluting with 5% diethyl ether in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(biphenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161c**) as a white solid (0.102 g, 83%). Mp 176–178 °C; ν_{max}/cm^{-1} (neat) 3307 (NH), 2954 (CH), 1715 (C=O), 1481, 1214, 764, 732; $[\alpha]_D^{24}$ +13.6 (*c* 1.0, $CHCl_3$), δ_H (400 MHz, $CDCl_3$) 3.79 (3H, s, OCH_3), 4.91 (1H, dt, *J* 7.1, 4.3 Hz, 2-H), 5.05–5.25 (4H, m, 3-H_2 and O*C*H_2Ph), 5.65 (1H, d, *J* 7.1 Hz, NH), 7.29–7.42 (6H, m, ArH and Ph), 7.44–7.53 (3H, m, 3 × ArH), 7.64–7.70 (7H, m, 7 × ArH), 8.25 (1H, dd, *J* 1.3, 0.7 Hz, 4′-H); δ_C (101 MHz, $CDCl_3$) 48.7 (CH_2), 53.3 (CH_3), 54.3 (CH), 67.3 (CH_2), 109.4 (CH), 117.8 (CH), 127.1 (2 × CH) 127.5 (CH), 127.7 (2 × CH), 127.9 (2 × CH), 128.0 (CH), 128.3 (2 × CH), 128.4 (CH), 128.6 (2 × CH), 128.9 (2 × CH), 133.3 (C), 135.9 (C), 137.5 (C), 139.3 (C), 140.5 (2 × C), 146.5 (C), 155.7 (C), 169.3 (C); *m/z* (ESI) 529.1831 (MNa^+. $C_{30}H_{26}N_4NaO_4$ requires 529.1846).

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(thiophen-3′′-yl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (161d)



Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(thiophen-3′′-yl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161d**) was synthesised as described for methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-phenyl-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**161a**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-bromo-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**159c**) (0.111 g, 0.256 mmol), 3-thiopheneboronic acid (0.0654 g, 0.511 mmol), [1,1′-bis(diphenylphosphino)ferrocene]palladium(II) dichloride-dichloromethane complex (0.0157 g, 0.0192 mmol) and potassium fluoride (0.0446 g, 0.768 mmol) in dioxane (3.6 mL) and water (0.4 mL). Purification with flash chromatography using silica gel, eluting with 5% diethyl ether in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(thiophen-3′′-yl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161d**) as a white solid (0.0826 g, 74%). Mp 144–145 °C; ν_{max}/cm^{-1} (neat) 3321 (NH), 2957 (CH), 1744 (C=O), 1713 (C=O), 1514, 1214, 788; $[\alpha]_D^{23}$ +18.0 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.77 (3H, s, OCH_3), 4.89 (1H, dt, *J* 7.1, 4.3 Hz, 2-H), 5.02–5.25 (4H, m, 3-H_2 and O*C*H_2Ph), 5.64 (1H, d, *J* 7.1 Hz, NH), 7.28–7.47 (8H, m, 7′-H, 4′′-H and 5′′-H and Ph), 7.49 (1H, dd, *J* 2.8, 1.5 Hz, 2′′-H), 7.62 (1H, dd, *J* 8.7, 1.3 Hz, 6′-H), 8.20 (1H, dd, *J* 1.3, 0.7 Hz, 4′-H); δ_C (101 MHz, $CDCl_3$) 48.6 (CH_2), 53.3 (CH_3), 54.3 (CH), 67.3 (CH_2), 109.4 (CH), 116.9 (CH), 120.9 (CH), 126.5 (CH), 126.7 (CH), 127.5 (CH), 128.3 (2 × CH_2), 128.4 (CH), 128.6 (2 × CH_2), 132.5 (C), 133.1 (C), 135.9 (C), 141.5 (C), 146.4 (C), 155.7 (C), 169.3 (C); *m/z* (ESI) 459.1081 (MNa^+. $C_{22}H_{20}N_4NaO_4S$ requires 459.1097).

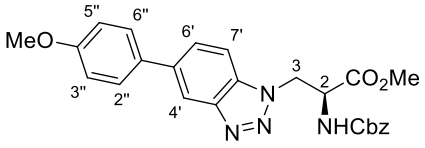
Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(4′′-hydroxyphenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (161e)



Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(4′′-hydroxyphenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161e**) was synthesised as described for methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-phenyl-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**161a**) using methyl (2*S*)-2-

[(benzyloxycarbonyl)amino]-3-(5′-bromo-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**159c**) (0.106 g, 0.244 mmol), 4-hydroxyphenylboronic acid (0.0672 g, 0.487 mmol), [1,1′-bis(diphenylphosphino)ferrocene]palladium(II) dichloride-dichloromethane complex (0.0150 g, 0.0183 mmol) and potassium fluoride (0.0425 g, 0.732 mmol) in dioxane (3.6 mL) and water (0.4 mL). Purification with flash chromatography using silica gel, eluting with 15% diethyl ether in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(4′′-hydroxyphenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161e**) as a white solid (0.0887 g, 81%). Mp 103–106 °C; ν_{max}/cm^{-1} (neat) 3318 (NH) 2955 (CH), 1740 (C=O), 1690 (C=O), 1522, 1211, 1063, 754, 698; $[\alpha]_D^{23}$ −11.4 (*c* 1.0, MeOH); δ_H (400 MHz, CD_3OD), 3.77 (3H, s, OCH_3), 4.80–4.85 (1H, m, 2-H), 4.98 (2H, s, OC*H*$_2$Ph), 5.09 (1H, dd, *J* 14.5, 8.1 Hz, 3-*H*H), 5.21 (1H, dd, *J* 14.5, 4.9 Hz, 3-H*H*), 6.90 (2H, d, *J* 8.7 Hz, 3′′-H and 5′′-H), 7.18–7.32 (5H, m, Ph), 7.51 (2H, d, *J* 8.7 Hz, 2′′-H and 6′′-H), 7.64–7.76 (2H, m, 6′-H and 7′-H), 8.05 (1H, br s, 4′-H); δ_C (101 MHz, CD_3OD), 47.4 (CH_2), 51.9 (CH_3), 54.2 (CH), 66.3 (CH_2), 109.9 (CH), 115.1 (CH), 115.4 (2 × CH), 127.4 (2 × CH), 127.5 (CH), 127.6 (CH), 128.0 (2 × CH), 128.2 (2 × CH), 131.4 (C), 132.8 (C), 136.5 (C), 138.3 (C), 146.0 (C), 156.6 (C), 157.2 (C), 169.7 (C); *m/z* (ESI) 469.1466 (MNa^+. $C_{24}H_{22}N_4NaO_5$ requires 469.1482).

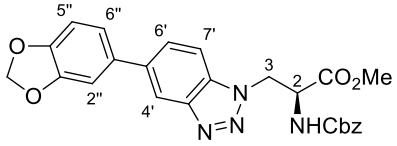
Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(4′′-methoxyphenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (161f)



Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(4′′-methoxyphenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161f**) was synthesised as described for methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-phenyl-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**161a**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-bromo-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**159c**) (0.200 g, 0.462 mmol), 4-methoxyphenylboronic acid (0.105 g, 0.693 mmol), [1,1′-bis(diphenylphosphino)ferrocene]palladium(II) dichloride-dichloromethane complex (0.0289 g, 0.0346 mmol) and potassium fluoride (0.0805 g, 1.39 mmol) in dioxane (3.6 mL) and water (0.4 mL). Purification with flash chromatography using silica gel, eluting with 15% diethyl ether in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(4′′-methoxyphenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161f**) as a white solid (0.179 g, 84%). Mp 139–142 °C; ν_{max}/cm^{-1} (neat) 3333 (NH), 2932 (CH), 1721 (C=O), 1604, 1512, 1342, 1250, 1026; $[\alpha]_D^{21}$ +7.6 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.77 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 4.89 (1H, dt, *J* 7.2, 4.3 Hz, 2-H), 5.04–5.22 (4H, m, 3-H_2 and OC*H*$_2$Ph), 5.68 (1H, d, *J* 7.2 Hz, NH), 7.02 (2H, d, *J* 8.8

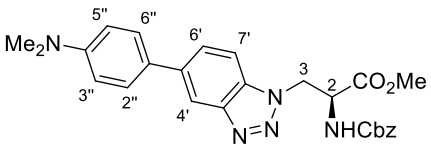
Hz, 3''-H and 5''-H), 7.28–7.40 (5H, m, Ph), 7.43 (1H, br d, *J* 8.7 Hz, 7'-H), 7.55 (2H, d, *J* 8.8 Hz, 2''-H and 6''-H), 7.59 (1H, dd, *J* 8.7, 1.5 Hz, 6'-H), 8.13 (1H, dd, *J* 1.5, 0.7 Hz, 4'-H); δ_C (101 MHz, $CDCl_3$) 48.6 (CH_2), 53.2 (CH_3), 54.3 (CH), 55.4 (CH_3), 67.3 (CH_2), 109.2 (CH), 114.4 (2 × CH), 117.2 (CH), 127.9 (CH), 128.3 (2 × CH), 128.4 (CH), 128.5 (2 × CH), 128.6 (2 × CH), 132.9 (2 × C), 135.9 (C), 137.6 (C), 146.5 (C), 155.7 (C), 159.4 (C), 169.3 (C); *m/z* (ESI) 483.1623 (MNa^+. $C_{25}H_{24}N_4NaO_5$ requires 483.1639).

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5'-(3'',4''-methylenedioxyphenyl)-1*H*-benzo[*d*][1.2.3]triazol-1'-yl]propanoate (161g)



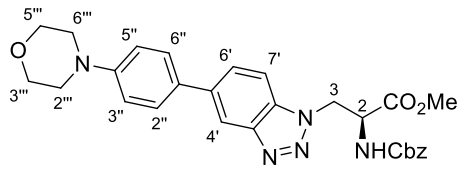
Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5'-(3'',4''-methylenedioxyphenyl)-1*H*-benzo[*d*][1.2.3]triazol-1'-yl]propanoate (**161g**) was synthesised as described for methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5'-phenyl-1*H*-benzo[*d*][1.2.3]triazol-1'-yl)propanoate (**161a**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5'-bromo-1*H*-benzo[*d*][1.2.3]triazol-1'-yl)propanoate (**159c**) (0.104 g, 0.239 mmol), 3,4-(methylenedioxy)phenylboronic acid (0.0793 g, 0.478 mmol), [1,1'-bis(diphenylphosphino)ferrocene]palladium(II) dichloride-dichloromethane complex (0.0146 g, 0.0179 mmol) and potassium fluoride (0.0417 g, 0.717 mmol) in dioxane (3.6 mL) and water (0.4 mL). Purification with flash chromatography using silica gel, eluting with 5% diethyl ether in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5'-(3'',4''-methylenedioxyphenyl)-1*H*-benzo[*d*][1.2.3]triazol-1'-yl]propanoate (**161g**) as a white solid (0.0926 g, 82%). Mp 160–162 °C; ν_{max}/cm^{-1} (neat) 3322, (NH), 2930 (CH), 1715 (C=O), 1510, 1480, 1225, 1038, 799, 735; $[\alpha]_D^{24}$ +13.4 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.78 (3H, s, OCH_3), 4.89 (1H, dt, *J* 7.0, 4.3 Hz, 2-H), 5.03–5.22 (4H, m, 3-H_2 and O*C*H_2Ph), 5.65 (1H, d, *J* 7.0 Hz, NH), 6.03 (2H, s, OCH_2O), 6.92 (1H, dd, *J* 7.5, 0.9 Hz, 6''-H), 7.04–7.11 (2H, m, 2''-H and 5''-H), 7.29–7.40 (5H, m, Ph), 7.42 (1H, br d, *J* 8.6 Hz, 7'-H), 7.54 (1H, dd, *J* 8.6, 1.3 Hz, 6'-H), 8.10 (1H, dd, *J* 1.3, 0.6 Hz, 4'-H); δ_C (101 MHz, $CDCl_3$) 48.6 (CH_2), 53.3 (CH_3), 54.3 (CH), 67.3 (CH_2), 101.3 (CH_2), 108.0 (CH), 108.7 (CH), 109.2 (CH), 117.4 (CH), 121.1 (CH), 128.0 (CH), 128.3 (2 × CH), 128.4 (CH), 128.6 (2 × CH), 133.1 (C), 134.7 (C), 135.9 (C), 137.7 (C), 146.4 (C), 147.4 (C), 148.3 (C), 155.7 (C), 169.3 (C); *m/z* (ESI) 497.1411 (MNa^+. $C_{25}H_{22}N_4NaO_6$ requires 497.1432).

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(4′′-dimethylaminophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (161h)



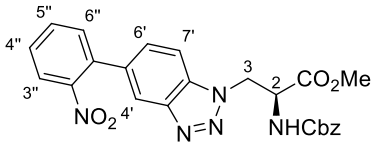
Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(4′′-dimethylaminophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161h**) was synthesised as described for methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-phenyl-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**161a**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-bromo-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**159c**) (0.106 g, 0.245 mmol), 4-dimethylaminobenzeneboronic acid (0.0810 g, 0.491 mmol), [1,1′-bis(diphenylphosphino)ferrocene]palladium(II) dichloride-dichloromethane complex (0.0150 g, 0.0184 mmol) and potassium fluoride (0.0430 g, 0.735 mmol) in dioxane (3.6 mL) and water (0.4 mL). Purification with flash chromatography using silica gel, eluting with 20% diethyl ether in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(4′′-dimethylaminophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161h**) as a white solid (0.0453 g, 39%). Mp 139–140 °C; ν_{max}/cm^{-1} (neat) 3347 (NH), 2947 (CH), 1719 (C=O), 1609, 1528, 1489, 1439, 1213, 1061, 802; $[\alpha]_D^{20}$ +11.5 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.02 (6H, s, NMe_2), 3.76 (3H, s, OCH_3), 4.89 (1H, dt, *J* 7.2, 4.2 Hz, 2-H), 5.04–5.22 (4H, m, 3-H_2 and O*C*H_2Ph), 5.66 (1H, d, *J* 7.2 Hz, NH), 6.84 (2H, d, *J* 8.9 Hz, 3′′-H and 5′′-H), 7.28–7.39 (5H, m, Ph), 7.41 (1H, br d, *J* 8.7 Hz, 7′-H), 7.53 (2H, d, *J* 8.9 Hz, 2′′-H and 6′′-H), 7.61 (1H, dd, *J* 8.7, 1.4 Hz, 6′-H), 8.13 (1H, dd, *J* 1.4, 0.6 Hz, 4′-H); δ_C (101 MHz, $CDCl_3$) 40.5 (2 × CH_3), 48.6 (CH_2), 53.2 (CH_3), 54.3 (CH), 67.3 (CH_2), 109.0 (CH), 112.9 (2 × CH), 116.3 (CH), 127.8 (CH), 128.1 (2 × CH), 128.2 (2 × CH), 128.3 (CH), 128.6 (2 × CH), 132.6 (C), 135.9 (C), 138.1 (2 × C), 146.7 (C), 150.2 (C), 155.7 (C), 169.4 (C); *m/z* (ESI) 496.1937 (MNa^+. $C_{26}H_{27}N_5NaO_4$ requires 496.1955).

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(4′′-morpholinophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (161i)



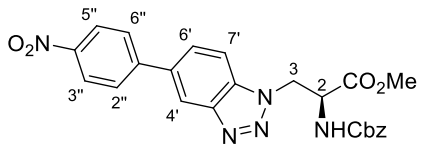
Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(4′′-morpholinophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161i**) was synthesised as described for methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-phenyl-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**161a**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-bromo-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**159c**) (0.0810 g, 0.187 mmol), 4-morpholinophenylboronic acid (0.116 g, 0.561 mmol), [1,1′-bis(diphenylphosphino)ferrocene]palladium(II) dichloride-dichloromethane complex (0.0115 g, 0.0140 mmol) and potassium fluoride (0.0326 g, 0.561 mmol) in dioxane (3.6 mL) and water (0.4 mL). Purification with flash chromatography using silica gel, eluting with 20% diethyl ether in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(4′′-morpholinophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161i**) as a white solid (0.0578 g, 60%). Mp 159–160 °C; ν_{max}/cm^{-1} (neat) 3302 (NH), 2953 (CH), 1717 (C=O), 1522, 1485, 1227, 1117, 926, 729; $[\alpha]_D^{20}$ +5.3 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.23 (4H, t, *J* 4.5 Hz, 2′′′-H_2 and 6′′′-H_2), 3.76 (3H, s, OCH_3), 3.90 (4H, t, *J* 4.5 Hz, 3′′′-H_2 and 5′′′-H_2), 4.88 (1H, dt, *J* 7.1, 4.3 Hz, 2-H), 5.04–5.24 (4H, m, 3-H_2 and O*C*H_2Ph), 5.76 (1H, d, *J* 7.1 Hz, NH), 7.04 (2H, d, *J* 8.5 Hz, 3′′-H and 5′′-H), 7.29–7.39 (5H, m, Ph), 7.43 (1H, d, *J* 8.7 Hz, 7′-H), 7.55 (2H, d, *J* 8.5 Hz, 2′′-H and 6′′-H), 7.59 (1H, dd, *J* 8.7, 1.4 Hz, 6′-H), 8.13 (1H, br s, 4′-H); δ_C (101 MHz, $CDCl_3$) 48.6 (CH_2), 49.3 (2 × CH_2), 53.2 (CH_3), 54.3 (CH), 66.7 (2 × CH_2), 67.3 (CH_2), 109.2 (CH), 116.1 (2 × CH), 116.9 (CH), 127.7 (CH), 128.2 (2 × CH), 128.3 (3 × CH), 128.6 (2 × CH), 132.0 (C), 132.9 (C), 135.9 (C), 137.5 (C), 146.6 (C), 150.5 (C), 155.7 (C), 169.4 (C); *m/z* (ESI) 538.2039 (MNa^+. $C_{28}H_{29}N_5NaO_5$ requires 538.2061).

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(2″-nitrophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (161j)



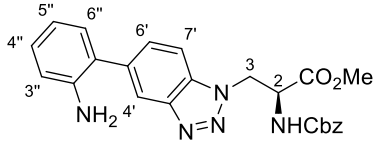
Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(2″-nitrophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161j**) was synthesised as described for methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-phenyl-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**161a**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-bromo-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**159c**) (0.601 g, 1.39 mmol), 2-nitrophenylboronic acid (0.463 g, 2.78 mmol), [1,1′-bis(diphenylphosphino)ferrocene]palladium(II) dichloride-dichloromethane complex (0.170 g, 0.209 mmol) and potassium fluoride (0.242 g, 4.17 mmol) in dioxane (9 mL) and water (1 mL). Purification with flash chromatography using silica gel, eluting with 5% diethyl ether in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(4″-nitrophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161j**) after recrystallisation from diethyl ether as a white solid (0.614 g, 93%). Mp 90–92 °C; ν_{max}/cm^{-1} (neat) 3348 (NH), 2950 (CH), 1720 (C=O), 1528, 1350, 1219, 1057, 741; $[\alpha]_D^{20}$ +9.9 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.77 (3H, s, OCH_3), 4.90 (1H, dt, *J* 6.9, 4.2 Hz, 2-H), 5.04–5.26 (4H, m, 3-H_2 and OCH_2Ph), 5.65 (1H, d, *J* 6.9 Hz, NH), 7.29 (1H, dd, *J* 8.6, 1.4 Hz, 6′-H), 7.30–7.40 (5H, m, Ph), 7.44 (1H, d, *J* 8.6 Hz, 7′-H), 7.48 (1H, dd, *J* 7.5, 1.3 Hz, 6″-H), 7.56 (1H, ddd, *J* 8.0, 7.5, 1.3 Hz, 4″-H), 7.68 (1H, ddd, *J* 8.0, 7.5, 1.1 Hz, 5″-H), 7.96 (1H, dd, *J* 8.0, 1.1 Hz, 3″-H), 8.01 (1H, br s, 4′-H); δ_C (101 MHz, $CDCl_3$) 48.7 (CH_2), 53.3 (CH_3), 54.3 (CH), 67.4 (CH_2), 109.3 (CH), 119.3 (CH), 124.5 (CH), 128.3 (2 × CH), 128.4 (2 × CH), 128.6 (2 × CH), 128.7 (CH), 132.4 (CH), 132.7 (CH), 133.5 (C), 134.0 (C), 135.7 (C), 135.9 (C), 145.9 (C), 149.2 (C), 155.7 (C), 169.3 (C); *m/z* (ESI) 498.1375 (MNa^+. $C_{24}H_{21}N_5NaO_6$ requires 498.1384).

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(4″-nitrophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (161k)



Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(4″-nitrophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161k**) was synthesised as described for methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-phenyl-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**161a**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-bromo-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**159c**) (0.105 g, 0.241 mmol), 4-nitrophenylboronic acid (0.0806 g, 0.483 mmol), [1,1′-bis(diphenylphosphino)ferrocene]palladium(II) dichloride-dichloromethane complex (0.0148 g, 0.0181 mmol), potassium fluoride (0.0420 g, 0.723 mmol) in dioxane (3.6 mL) and water (0.4 mL). Purification with flash chromatography using silica gel, eluting with 5% diethyl ether in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(4″-nitrophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161k**) as a white solid (0.0805 g, 70%). Mp 178–180 °C; ν_{max}/cm^{-1} (neat) 1983, 1721 (C=O), 1512, 1342, 1219, 748; $[\alpha]_D^{20}$ −46.3 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.81 (3H, s, OCH_3), 4.90 (1H, dt, *J* 6.8, 4.2 Hz, 2-H), 5.02–5.30 (4H, m, 3-H_2 and OCH_2Ph), 5.60 (1H, d, *J* 6.8 Hz, NH), 7.30–7.42 (5H, m, Ph), 7.52 (1H, br d, *J* 8.7 Hz, 7′-H), 7.60 (1H, dd, *J* 8.7, 1.3 Hz, 6′-H), 7.77 (2H, d, *J* 8.9 Hz, 2″-H and 6″-H), 8.27 (1H, dd, *J* 1.3, 0.8 Hz, 4′-H), 8.35 (2H, d, *J* 8.9 Hz, 3″-H and 5″-H); δ_C (101 MHz, $CDCl_3$) 48.7 (CH_2), 53.4 (CH_3), 54.3 (CH), 67.3 (CH_2), 110.0 (CH), 118.9 (CH), 124.3 (2 × CH), 127.7 (CH), 128.2 (2 × CH), 128.3 (2 × CH), 128.4 (CH), 128.6 (2 × CH), 134.0 (C), 135.4 (C), 135.9 (C), 146.3 (C), 146.8 (C), 147.3 (C), 155.6 (C), 169.2 (C); *m/z* (ESI) 498.1365 (MNa^+. $C_{24}H_{21}N_5NaO_6$ requires 498.1384).

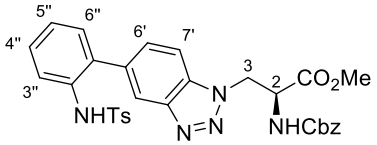
Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(2″-aminophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (176)



To a solution of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(4″-nitrophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161j**) (0.604 g, 1.27 mmol) in methanol (20 mL) was added tin(II) dichloride dihydrate (1.15 g, 5.08 mmol). The resulting reaction mixture was heated under reflux under an atmosphere of argon for 20 h. The solvent was then removed *in vacuo* and

the resulting residue was dissolved in ethyl acetate (50 mL). A saturated aqueous solution of sodium hydrogen carbonate (50 mL) was added and the biphasic mixture was filtered through Celite®. The organic phase was separated and the aqueous layer was washed with ethyl acetate (2 × 50 mL). The combined organic layers were combined, washed with water (50 mL), brine (50 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product. Purification by flash chromatography using silica gel, eluting with 15% diethyl ether in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-(2′′-aminophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoate (**176**) as a pale yellow solid (0.390 g, 69%). Mp 48–50 °C; ν_{max}/cm^{-1} (neat) 3364 (NH), 2955 (CH), 1713 (C=O), 1616, 1504, 1483, 1211, 1055, 1018, 745; $[\alpha]_D^{20}$ +17.3 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.73 (2H, br s, NH_2), 3.79 (3H, s, OCH_3), 4.90 (1H, dt, *J* 7.1, 4.2, 2-H), 5.04–5.27 (4H, m, 3-H_2 and OC*H*$_2$Ph), 5.68 (1H, d, *J* 7.1 Hz, NH), 6.80 (1H, dd, *J* 8.0, 1.0 Hz, 3′′-H), 6.86 (1H, td, *J* 7.5, 1.0 Hz, 5′′-H), 7.14 (1H, dd, *J* 7.5, 1.6 Hz, 6′′-H), 7.20 (1H, ddd, *J* 8.0, 7.5, 1.6 Hz, 4′′-H), 7.27–7.42 (5H, m, Ph), 7.48 (1H, d, *J* 8.6 Hz, 7′-H), 7.52 (1H, dd, *J* 8.6, 1.2 Hz, 6′-H), 8.10 (1H, br s, 4′-H); δ_C (101 MHz, $CDCl_3$) 48.6 (CH_2), 53.3 (CH_3), 54.3 (CH), 67.3 (CH_2), 109.4 (CH), 115.8 (CH), 118.8 (CH), 120.0 (CH), 126.5 (C), 128.2 (2 × CH), 128.4 (CH), 128.6 (2 × CH), 128.9 (CH), 129.8 (CH), 130.8 (CH), 133.1 (C), 135.9 (2 × C), 143.7 (C), 146.3 (C), 155.7 (C), 169.3 (C); *m/z* (ESI) 468.1636 (MNa^+. $C_{24}H_{23}N_5NaO_4$ requires 468.1642).

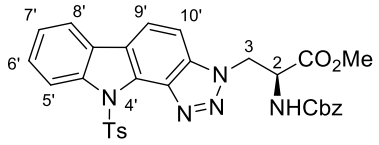
Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-{5′-[2′′-(4′′′-methylphenylsulfonylamino)phenyl]-1*H*-benzo[*d*][1.2.3]triazol-1′-yl}propanoate (177)



To a solution of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(2′′-aminophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**176**) (0.347 g, 0.769 mmol) in acetonitrile (10 mL) was added pyridine (0.186 mL, 2.31 mmol) and 4-toluenesulfonyl chloride (0.440 g, 2.31 mmol). The reaction mixture was stirred at room temperature for 1 h and then concentrated *in vacuo*. The resulting residue was dissolved in ethyl acetate (50 mL), washed with 1 M aqueous hydrochloric acid (50 mL), water (50 mL), brine (50 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product. Purification by flash chromatography using silica gel, eluting with 15% diethyl ether in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-{5′-[2′′-(4′′′-methylphenylsulfonylamino)phenyl]-1*H*-benzo[*d*][1.2.3]triazol-1′-yl}propanoate (**177**) as a white solid (0.417 g, 91%). Mp 72–74 °C; ν_{max}/cm^{-1} (neat) 3347 (NH), 2953 (CH), 1717 (C=O), 1506, 1335 1213, 1159, 1092, 754; $[\alpha]_D^{15}$ +5.8 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.40 (3H, s, CH_3), 3.82 (3H, s,

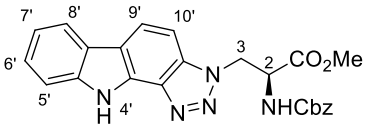
OCH_3), 4.90 (1H, dt, *J* 6.9, 4.3 Hz, 2-H), 5.07–5.25 (4H, m, 3-H_2 and O*C*H_2Ph), 5.68 (1H, d, *J* 6.9 Hz, NH), 6.49 (1H, s, NH), 7.00 (1H, dd, *J* 8.5, 0.9 Hz, 3''-H), 7.09–7.17 (3H, m, 3 × ArH), 7.20 (1H, td, *J* 7.5, 1.1 Hz, 4''-H), 7.28–7.47 (10H, m, 5 × ArH and Ph), 7.69 (1H, d, *J* 8.0 Hz, 7'-H); δ_C (101 MHz, $CDCl_3$) 21.6 (CH_3), 48.7 (CH_2), 53.3 (CH_3), 54.3 (CH), 67.4 (CH_2), 109.8 (CH), 120.4 (CH), 122.3 (CH), 125.3 (CH), 127.0 (2 × CH), 128.4 (3 × CH), 128.6 (2 × CH), 128.9 (CH), 129.1 (CH), 129.7 (2 × CH), 130.7 (CH), 133.3 (C), 133.4 (C), 133.5 (C), 133.9 (C), 135.9 (C), 136.1 (C), 144.2 (C), 145.9 (C), 155.7 (C), 169.3 (C); *m/z* (ESI) 622.1714 (MNa^+. $C_{31}H_{29}N_5NaO_6S$ requires 622.1731).

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-toluenesulfonyl)-[1,2,3]triazolo[2,3-c]carbazol-1(4'H)-yl]propanoate (178)



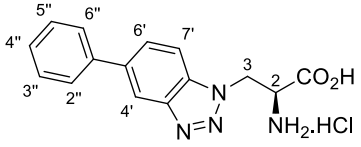
To a solution of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-{5'-[2''-(4'''-methylphenylsulfonylamino)phenyl]-1*H*-benzo[*d*][1.2.3]triazol-1'-yl}propanoate (**177**) (0.0450 g, 0.0750 mmol) in anhydrous dichloroethane (1 mL) at 50 °C was added copper(II) trifluoromethanesulfonate (0.00136 g, 0.00375 mmol). To the reaction mixture was added (diacetoxyiodo)benzene (0.0364 g, 0.113 mmol) in dichloroethane (1 mL) over 0.1 h. The reaction mixture was stirred at 50 °C for 0.15 h before being filtered through Celite®, washed with ethyl acetate (20 mL) and concentrated *in vacuo* to give the crude product. Purification by flash chromatography with silica gel, eluting with 5% diethyl ether in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-toluenesulfonyl)-[1,2,3]triazolo[2,3-c]carbazol-1(4'H)-yl]propanoate (**178**) as a white solid (0.0364 g, 81%). Mp 163–165 °C; ν_{max}/cm^{-1} (neat) 3368 (NH), 2954 (CH), 1721 (C=O), 1511, 1379, 1236, 1211, 1190, 1052, 971, 778, 738; $[\alpha]_D^{20}$ +4.3 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.26 (3H, s, CH_3), 3.77 (3H, s, OCH_3), 4.92 (1H, dt, *J* 7.1, 4.3 Hz, 2-H), 5.00–5.27 (4H, m, 3-H_2 and O*C*H_2Ph), 5.71 (1H, d, *J* 7.1 Hz, NH), 7.14 (2H, d, *J* 8.3 Hz, 3'''-H and 5'''-H), 7.27–7.35 (5H, m, Ph), 7.37 (1H, d, *J* 8.6 Hz, 10'-H), 7.41–7.48 (1H, m, 7'-H), 7.56 (1H, ddd, *J* 8.5, 7.4, 1.2 Hz, 6'-H), 7.88 (1H, d, *J* 8.6 Hz, 9'-H), 7.95 (1H, d, *J* 7.4 Hz, 5'-H), 8.16 (2H, d, *J* 8.3 Hz, 2''-H and 6''-H), 8.57 (1H, br d, *J* 8.5 Hz, 8'-H); δ_C (101 MHz, $CDCl_3$) 21.6 (CH_3), 48.6 (CH_2), 53.3 (CH_3), 54.1 (CH), 67.2 (CH_2), 105.1 (CH), 115.4 (CH), 119.2 (CH), 120.3 (CH), 121.6 (C), 123.8 (CH), 125.6 (C), 126.9 (CH), 127.4 (C), 127.8 (2 × CH), 128.2 (2 × CH), 128.3 (CH), 128.6 (2 × CH), 129.6 (2 × CH), 134.8 (C), 135.0 (C), 135.7 (C), 135.9 (C), 138.4 (C), 144.9 (C), 155.7 (C), 169.2 (C); *m/z* (ESI) 620.1560 (MNa^+. $C_{31}H_{27}N_5NaO_6S$ requires 620.1574).

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-([1,2,3]triazolo[2,3-c]carbazol-1(4'H)-yl)propanoate (181)



To a solution of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-toluenesulfonyl)-[1,2,3]triazolo[2,3-c]carbazol-1(4'H)-yl]propanoate (**178**) (0.0359 g, 0.0699 mmol), in anhydrous methanol (3 mL) and tetrahydrofuran (1 mL) was added magnesium (0.0168 g, 0.691 mmol). The reaction mixture was sonicated for 0.5 h, diluted with dichloromethane (50 mL), washed with 1 M aqueous hydrochloric acid (50 mL), aqueous saturated sodium hydrogen carbonate solution (50 mL) and brine (50 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product. Purification by flash chromatography using silica gel, eluting with 20% diethyl ether in dichloromethane, then recrystallisation from chloroform gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-([1,2,3]triazolo[2,3-c]carbazol-1(4'H)-yl)propanoate (**181**) as a white solid (0.0133 g, 50%). Mp 196–198 °C, ν_{max}/cm^{-1} (neat) 3350 (NH), 3173 (CH), 1744 (C=O), 1690 (C=O), 1533, 1250, 1219, 1117, 1067, 1005, 741; $[\alpha]_D^{20}$ +6.8 (*c* 0.1, DMSO); δ_H (500 MHz, DMSO-d_6) 3.69 (3H, s, OCH_3), 4.78 (1H, ddd, *J* 9.2, 8.7, 5.0 Hz, 2-H), 4.90 (1H, d, *J* 12.7 Hz, OC*H*HPh), 4.94 (1H, d, *J* 12.7 Hz, OCH*H*Ph), 5.08 (1H, dd, *J* 14.4, 9.2 Hz, 3-*H*H), 5.18 (1H, dd, *J* 14.4, 5.0 Hz, 3-H*H*), 7.10–7.30 (6H, m, 7'-H and Ph), 7.41 (1H, t, *J* 7.8 Hz, 6'-H), 7.52 (1H, d, *J* 8.7 Hz, 10'-H), 7.61 (1H, d, *J* 7.8 Hz, 5'-H), 7.98 (1H, d, *J* 8.7 Hz, NH), 8.19 (1H, d, *J* 7.8 Hz, 8'-H), 8.26 (1H, d, *J* 8.7 Hz, 9'-H), 12.5 (1H, s, NH); δ_C (126 MHz, DMSO-d_6) 48.3 (CH_2), 52.9 (CH_3), 54.3 (CH), 66.0 (CH_2), 101.4 (CH), 112.2 (CH), 117.5 (C), 120.0 (CH). 120.1 (CH), 121.5 (CH), 123.6 (C), 124.9 (CH), 127.9 (2 × CH), 128.2 (CH), 128.7 (2 × CH), 129.3 (C), 133.9 (C), 134.0 (C), 137.1 (C), 139.2 (C), 156.2 (C), 170.3 (C); *m/z* (ESI) 466.1484 (MNa^+. $C_{24}H_{21}N_5NaO_4$ requires 466.1486).

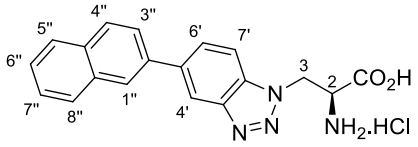
(2*S*)-2-Amino-3-(5'-phenyl-1*H*-benzo[*d*][1.2.3]triazol-1'-yl)propanoic acid hydrochloride (163a)



To a solution of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5'-phenyl-1*H*-benzo[*d*][1.2.3]triazol-1'-yl)propanoate (**159a**) (0.0438 g, 0.102 mmol) in a mixture of methanol (4 mL) and 1,4-dioxane (2 mL) was added a solution of caesium carbonate (0.0430 g, 0.132 mmol) in water (2 mL). The

reaction mixture was stirred at room temperature for 20 h and then concentrated *in vacuo.* The resulting residue was dissolved in water (100 mL) and acidified to pH 1 with 1 M aqueous hydrochloric acid. The aqueous layer was extracted with dichloromethane (3 × 30 mL) and the combined organic layers were washed with 1 M aqueous hydrochloric acid (50 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-phenyl-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoic acid (**162a**) as a white solid (0.0227 g, 58%). This was used for the next reaction without any further purification. To a solution of (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-phenyl-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoic acid (**162a**) (0.0355 g, 0.0852 mmol) in tetrahydrofuran (5 mL) was added 10% palladium on carbon (0.0181 g, 0.0170 mmol). The solution was purged with hydrogen for 1 h and stirred under an atmosphere of hydrogen for 5 h. After addition of 1 M aqueous hydrochloric acid (0.200 mL), the reaction mixture was filtered through Celite® with tetrahydrofuran and concentrated *in vacuo* to give the crude product. Purification by recrystallisation from methanol and diethyl ether gave (2*S*)-2-amino-3-(5′-phenyl-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoic acid hydrochloride (**163a**) as a yellow solid (0.0225 g, 83%). Mp 176–178 °C; ν_{max}/cm^{-1} (neat) 3695 (NH/OH), 2932 (CH), 2068, 1682 (C=O), 1296, 980; $[\alpha]_D^{20}$ +1.2 (*c* 0.5, DMSO); δ_H (400 MHz, CD_3OD) 4.79 (1H, dd, *J* 5.7, 4.1 Hz, 2-H), 5.28 (1H, dd, *J* 15.5, 4.1 Hz, 3-*H*H), 5.38 (1H, dd, *J* 15.5, 5.7 Hz, 3-H*H*), 7.40 (1H, t, *J* 7.5 Hz, 4′′-H), 7.49 (2H, t, *J* 7.5 Hz, 3′′-H and 5′′-H), 7.70 (2H, d, *J* 7.5 Hz, 2′′-H and 6′′-H), 7.89 (1H, d, *J* 8.7 Hz, 7′-H), 7.93 (1H, dd, *J* 8.7, 0.9 Hz, 6′-H), 8.23 (1H, br s, 4′-H); δ_C (101 MHz, CD_3OD) 46.8 (CH_2), 52.2 (CH), 110.0 (CH), 116.5 (CH), 127.1 (2 × CH), 127.5 (CH), 128.2 (CH), 128.8 (2 × CH), 132.9 (C), 138.7 (C), 140.1 (C), 146.3 (C), 167.6 (C); *m/z* (ESI) 283.1191 (MH^+. $C_{15}H_{15}N_4O_2$ requires 283.1190).

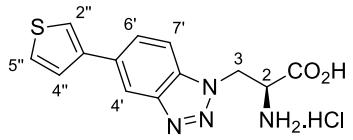
(2*S*)-2-Amino-3-[5′-(naphthalen-2′′-yl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (163b)



(2*S*)-2-[(Benzyloxycarbonyl)amino]-3-(5′-(naphthalen-2′′-yl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)proponic acid (**162b**) was synthesised as described for (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-phenyl-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)propanoic acid (**162a**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(naphthalen-2′′-yl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161b**) (0.103 g, 0.213 mmol) and caesium carbonate (0.0904 g, 0.277 mmol). This gave (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-(naphthalen-2′′-yl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)proponic acid (**162b**) as a white solid (0.0994 g, 100%). This was used for the next reaction without any

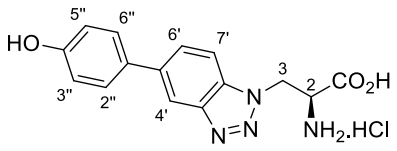
further purification. To a solution of (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(5′-(naphthalen-2′′-yl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl)proponic acid (**162b**) (0.0994 g, 0.213 mmol) in 1,4-dioxane (1 mL) was added 6 M aqueous hydrochloric acid (5 mL). The reaction mixture was heated under reflux for 1 h, then concentrated *in vacuo* to give the crude product. Purification by recrystallisation from methanol and diethyl ether gave (2*S*)-2-amino-3-[5′-(naphthalen-2′′-yl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (**163b**) as a white solid (0.0994 g, 78%). Mp 224–226 °C; ν_{max}/cm^{-1} (neat) 2833 (CH), 1734 (C=O), 1568, 1510, 1441, 1400, 1238, 1143, 774; $[\alpha]_D^{20}$ +8.1 (*c* 0.5, DMSO); δ_H (400 MHz, CD_3OD) 4.74 (1H, dd, *J* 5.8, 4.0 Hz, 2-H), 5.33 (1H, dd, *J* 15.4, 4.0 Hz, 3-*H*H), 5.42 (1H, dd, *J* 15.4, 5.8 Hz, 3-H*H*), 7.38–7.66 (4H, m, 4 × ArH), 7.73 (1H, dd, *J* 8.7, 1.2 Hz, 6′-H), 7.76 (1H, d, *J* 8.5 Hz, ArH), 7.89–8.05 (3H, m, 3 × ArH), 8.09 (1H, br s, 4′-H); δ_C (101 MHz, CD_3OD) 47.9 (CH_2), 52.7 (CH), 109.7 (CH), 119.5 (CH), 125.0 (CH), 125.1 (CH), 125.7 (CH), 126.0 (CH), 127.1 (CH), 127.9 (CH), 128.2 (CH), 130.7 (CH), 131.5 (C), 132.9 (C), 134.0 (C), 137.9 (C), 138.9 (C), 145.8 (C), 168.1 (C); *m/z* (ESI) 355.1155 (MNa^+. $C_{19}H_{16}N_4NaO_2$ requires 355.1165).

(2*S*)-2-Amino-3-[5′-(thiophen-3′′-yl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (163d)



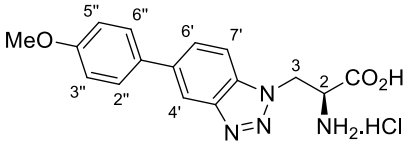
(2*S*)-2-Amino-3-[5′-(thiophen-3′′-yl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (**163d**) was synthesised as described for (2*S*)-2-amino-3-[5′-(naphthalen-2′′-yl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (**163b**) using (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(thiophen-3′′-yl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161d**) (0.0810 g, 0.186 mmol) and caesium carbonate (0.0785 g, 0.241 mmol). Purification by recrystallisation from methanol and diethyl ether gave (2*S*)-2-amino-3-[5′-(thiophen-3-yl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (**163d**) as a white solid (0.0453 g, 75%). Mp 174–176 °C; ν_{max}/cm^{-1} (neat) 2884 (OH), 1740 (C=O), 1587, 1233, 1207, 775, 590; $[\alpha]_D^{20}$ +2.5 (*c* 0.5, DMSO); δ_H (400 MHz, CD_3OD) 4.81 (1H, dd, *J* 5.7, 4.1 Hz, 2-H), 5.27 (1H, dd, *J* 15.5, 4.1 Hz, 3-*H*H), 5.37 (1H, dd, *J* 15.5, 5.7 Hz, 3-H*H*), 7.54 (1H, dd, *J* 5.0, 2.8 Hz, 5′′-H), 7.56 (1H, dd, *J* 5.0, 1.5 Hz, 4′′-H), 7.76 (1H, dd, *J* 2.8, 1.5 Hz, 2′′-H), 7.85 (1H, d, *J* 8.8 Hz, 7′-H), 7.98 (1H, dd, *J* 8.8, 1.4 Hz, 6′-H), 8.26 (1H, br s, 4′-H); δ_C (101 MHz, CD_3OD) 46.7 (CH_2), 52.2 (CH), 110.1 (CH), 115.4 (CH), 121.0 (CH), 125.9 (CH), 126.5 (CH), 127.6 (CH), 132.7 (C), 133.3 (C), 141.0 (C), 146.2 (C), 167.5 (C); *m/z* (ESI) 289.0750 (MH^+. $C_{13}H_{13}N_4O_2S$ requires 289.0754).

(2*S*)-2-Amino-3-[5′-(4′′-hydroxyphenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (163e)



(2*S*)-2-Amino-3-[5′-(4′′-hydroxyphenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (**163e**) was synthesised as described for (2*S*)-2-amino-3-[5′-(naphthalen-2′′-yl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (**163b**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(4′′-hydroxyphenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161e**) (0.0803 g, 0.180 mmol) and caesium carbonate (0.0762 g, 0.234 mmol). Purification by recrystallisation from methanol and diethyl ether gave (2*S*)-2-amino-3-[5′-(4′′-hydroxyphenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (**163e**) as a white solid (0.0392 g, 65%). Mp 188–190 °C; ν_{max}/cm^{-1} (neat) 3335 (NH/OH), 3007 (CH), 1728 (C=O), 1609, 1483, 1267, 1236, 816; $[\alpha]_D^{20}$ +5.8 (*c* 0.5, MeOH); δ_H (400 MHz, CD_3OD) 4.82 (1H, dd, *J* 5.6, 4.2 Hz, 2-H), 5.28 (1H, dd, *J* 15.4, 4.2 Hz, 3-*H*H), 5.38 (1H, dd, *J* 15.4, 5.6 Hz, 3-H*H*), 6.91 (2H, d, *J* 8.7 Hz, 3′′-H and 5′′-H), 7.54 (2H, d, *J* 8.7 Hz, 2′′-H and 6′′-H), 7.86 (2H, m,6′-H and 7′-H), 8.11 (1H, br s, 4′-H); δ_C (101 MHz, CD_3OD) 46.8 (CH_2), 52.2 (CH), 109.9 (CH), 115.3 (CH), 155.5 (2 × CH), 128.0 (CH), 128.2 (2 × CH), 131.2 (C), 132.5 (C), 138.8 (C), 146.1 (C), 157.3 (C), 167.5 (C); *m/z* (ESI) 299.1134 (MH^+. $C_{15}H_{15}N_4O_3$ requires 299.1139).

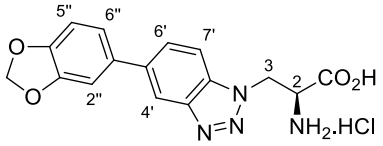
(2*S*)-2-Amino-3-[5′-(4′′-methoxyphenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (163f)



(2*S*)-2-Amino-3-[5′-(4′′-methoxyphenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (**163f**) was synthesised as described for (2*S*)-2-amino-3-[5′-(naphthalen-2′′-yl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (**163b**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(4′′-methoxyphenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161f**) (0.155 g, 0.336 mmol) and caesium carbonate (0.142 g, 0.436 mmol). Purification by recrystallisation from methanol and diethyl ether gave (2*S*)-2-amino-3-[5′-(4′′-methoxyphenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (**163f**) as a white

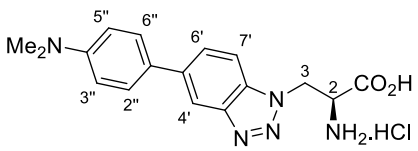
solid (0.0906 g, 77%). Mp 214–216 °C; ν_{max}/cm^{-1} (neat) 2832 (OH), 1744 (C=O), 1612, 1489, 1250, 810; $[\alpha]_D^{20}$ +6.5 (*c* 0.5, MeOH); δ_H (400 MHz, CD_3OD) 3.85 (3H, s, OCH_3), 4.79 (1H, dd, *J* 5.7, 4.1 Hz, 2-H), 5.27 (1H, dd, *J* 15.5, 4.1 Hz, 3-*H*H), 5.37 (1H, dd, *J* 15.5, 5.7 Hz, 3-H*H*), 7.04 (2H, d, *J* 8.8 Hz, 3''-H and 5''-H), 7.63 (2H, d, *J* 8.8 Hz, 2''-H and 6''-H), 7.81–7.92 (2H, m, 6'-H and 7'-H), 8.15 (1H, br s, 4'-H); δ_C (101 MHz, CD_3OD) 46.8 (CH_2), 52.2 (CH), 54.4 (CH_3), 110.0 (CH), 114.1 (2 × CH), 115.6 (CH), 128.0 (CH), 128.1 (2 × CH), 132.4 (C), 132.6 (C), 138.4 (C), 146.3 (C), 159.8 (C), 167.6 (C); *m/z* (ESI) 313.1290 (MH^+. $C_{16}H_{17}N_4O_3$ requires 313.1295).

(2*S*)-2-Amino-3-[5'-(3'',4''-methylenedioxyphenyl)-1*H*-benzo[*d*][1.2.3]triazol-1'-yl]propanoic acid hydrochloride (163g)



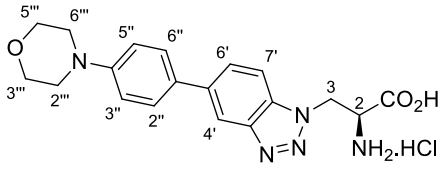
(2*S*)-2-Amino-3-[5'-(3'',4''-methylenedioxyphenyl)-1*H*-benzo[*d*][1.2.3]triazol-1'-yl]propanoic acid hydrochloride (**163g**) was synthesised as described for (2*S*)-2-amino-3-[5'-(naphthalen-2''-yl)-1*H*-benzo[*d*][1.2.3]triazol-1'-yl]propanoic acid hydrochloride (**163b**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5'-(3'',4''-methylenedioxyphenyl)-1*H*-benzo[*d*][1.2.3]triazol-1'-yl]propanoate (**161g**) (0.0898 g, 0.189 mmol) and caesium carbonate (0.0801 g, 0.246 mmol). Purification by recrystallisation from methanol and diethyl ether gave (2*S*)-2-amino-3-[5'-(3'',4''-methylenedioxyphenyl)-1*H*-benzo[*d*][1.2.3]triazol-1'-yl]propanoic acid hydrochloride (**163g**) as a white solid (0.0610 g, 89%). Mp 203–205 °C; ν_{max}/cm^{-1} (neat) 2886 (OH), 1724 (C=O), 1479, 1248, 1227, 1038, 800; $[\alpha]_D^{20}$ +1.6 (*c* 1.0, DMSO); δ_H (400 MHz, CD_3OD) 4.80 (1H, dd, *J* 5.7, 4.1 Hz, 2-H), 5.27 (1H, dd, *J* 15.5, 4.1 Hz, 3-*H*H), 5.37 (1H, dd, *J* 15.5, 5.7 Hz, 3-H*H*), 6.01 (2H, s, OCH_2O), 6.94 (1H, d, *J* 7.8, 0.5 Hz, 5''-H), 7.14–7.26 (2H, m, 2''-H and 6''-H), 7.81–7.95 (2H, m, 6'-H and 7'-H), 8.14 (1H, br s, 4'-H); δ_C (101 MHz, CD_3OD) 46.7 (CH_2), 52.2 (CH), 101.3 (CH_2), 107.3 (CH), 108.3 (CH), 109.9 (CH), 116.0 (CH), 120.8 (CH), 128.1 (CH), 132.7 (C), 134.3 (C), 138.5 (C), 146.2 (C), 147.7 (C), 148.5 (C), 167.5 (C); *m/z* (ESI) 327.1074 (MH^+. $C_{16}H_{15}N_4O_4$ requires 327.1088).

(2*S*)-2-Amino-3-[5'-(4''-dimethylaminophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1'-yl]propanoic acid hydrochloride (163h)



(2*S*)-2-Amino-3-[5′-(4′′-dimethylaminophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (**163h**) was synthesised as described for (2*S*)-2-amino-3-[5′-(naphthalen-2′′-yl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (**163b**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(4′′-dimethylaminophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161h**) (0.0368 g, 0.0777 mmol) and caesium carbonate (0.0329 g, 0.101 mmol). Purification by recrystallisation from methanol and diethyl ether gave (2*S*)-2-amino-3-[5′-(4′′-dimethylaminophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (**163h**) as a white solid (0.0132 g, 47%). Mp 166–168 °C; ν_{max}/cm^{-1} (neat) 3393 (NH), 2851 (OH), 1732 (C=O), 1485, 1234, 808; $[\alpha]_D^{20}$ +1.6 (*c* 0.5, MeOH); δ_H (400 MHz, CD_3OD) 3.37 (6H, s, 2 × CH_3), 4.84 (1H, dd, *J* 5.4, 4.3 Hz, 2-H), 5.32 (1H, dd, *J* 15.5, 4.3 Hz, 3-*H*H), 5.42 (1H, dd, *J* 15.5, 5.4 Hz, 3-H*H*), 7.86 (2H, d, *J* 8.7 Hz, 3′′-H and 5′′-H), 7.92–8.09 (4H, m, 6′-H, 7′-H, 2′′-H and 6′′-H), 8.31 (1H, br s, 4′-H); δ_C (101 MHz, CD_3OD) 46.0 (2 × CH_3), 46.8 (CH_2), 52.2 (CH), 110.7 (CH), 117.3 (CH), 121.1 (2 × CH), 127.9 (CH), 129.2 (2 × CH), 133.5 (C), 136.3 (C), 142.0 (C), 142.4 (C), 146.1 (C), 167.5 (C); *m/z* (ESI) 326.1612 (MH^+. $C_{17}H_{20}N_5O_2$ requires 362.1612).

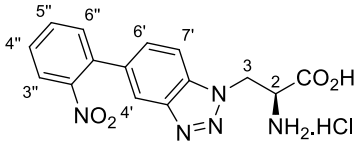
(2*S*)-2-Amino-3-[5′-(4′′-morpholinophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (163i)



(2*S*)-2-Amino-3-[5′-(4′′-morpholinophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (**163i**) was synthesised as described for (2*S*)-2-amino-3-[5′-(naphthalen-2′′-yl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (**163b**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(4′′-morpholinophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161i**) (0.116 g, 0.224 mmol) and caesium carbonate (0.0948 g, 0.291 mmol). Purification by recrystallisation from methanol and diethyl ether gave (2*S*)-2-amino-3-[5′-(4′′-morpholinophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (**163i**) as a white solid (0.0957 g, 97%). Mp 172–174 °C; ν_{max}/cm^{-1} (neat) 3366 (NH), 2866 (OH), 1738 (C=O), 1487, 1121, 1059, 889, 806; $[\alpha]_D^{20}$ +3.5 (*c* 1.0, MeOH); δ_H (400 MHz, CD_3OD) 3.79 (4H, t, *J* 4.6 Hz, 2′′′-H and 6′′′-H), 4.17 (4H, t, *J* 4.6 Hz, 3′′′-H and 5′′′-H), 4.83 (1H, dd, *J* 5.6, 4.2 Hz, 2-H), 5.31 (1H, dd, *J* 15.5, 4.2 Hz, 3-*H*H), 5.41 (1H, dd, *J* 15.5, 5.6, 3-H*H*), 7.89 (2H, d, *J* 8.8 Hz, 3′′-H and 5′′-H), 7.93–8.01 (4H, m, 6′-H, 7′-H, 2′′-H and 6′′-H), 8.31 (1H, br s, 4′-H); δ_C (101 MHz, CD_3OD) 46.8 (CH_2), 52.1 (CH),

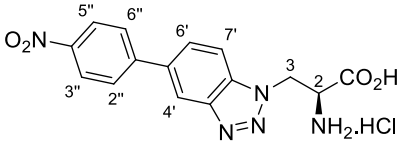
54.9 (2 × CH_2), 64.1 (2 × CH_2), 110.6 (CH), 117.2 (CH), 121.5 (2 × CH), 127.9 (CH), 129.1 (2 × CH), 133.4 (C), 136.3 (C), 141.3 (C), 141.9 (C), 146.2 (C), 167.5 (C); *m/z* (ESI) 368.1708 (MH^+. $C_{19}H_{22}N_5O_3$ requires 368.1717).

(2*S*)-2-Amino-3-[5′-(2′′-nitrophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (163j)



(2*S*)-2-Amino-3-[5′-(2′′-nitrophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (**163j**) was synthesised as described for (2*S*)-2-amino-3-[5′-(naphthalen-2′′-yl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (**163b**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(2′′-nitrophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161j**) (0.0577 g, 0.121 mmol) and caesium carbonate (0.0513 g, 0.157 mmol). Purification by recrystallisation from methanol and diethyl ether gave (2*S*)-2-amino-3-[5′-(2′′-nitrophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (**163j**) as a white solid (0.0244 g, 56%). Mp 186–187 °C; ν_{max}/cm^{-1} (neat) 2862 (OH), 1744 (C=O), 1524, 1350, 1236; $[\alpha]_D^{20}$ +0.4 (*c* 0.5, MeOH); δ_H (400 MHz, CD_3OD) 4.82 (1H, dd, *J* 5.9, 4.0 Hz, 2-H), 5.30 (1H, dd, *J* 15.5, 4.0 Hz, 3-*H*H), 5.39 (1H, dd, *J* 15.5, 5.9 Hz, 3-H*H*), 7.57 (1H, dd, *J* 8.7, 1.5 Hz, 6′-H), 7.59 (1H, dd, *J* 7.8, 1.2 Hz, 6′′-H), 7.65 (1H, ddd, *J* 8.1, 7.8 Hz, 1.2 Hz, 4′′-H), 7.77 (1H, td, *J* 7.8, 1.3 Hz, 5′′-H), 7.89 (1H, dd, *J* 8.7, 0.7 Hz, 7′-H), 7.99 (1H, dd, *J* 8.1, 1.3 Hz, 3′′-H), 8.02 (1H, dd, *J* 1.5, 0.7 Hz, 4′-H); δ_C (101 MHz, CD_3OD) 46.8 (CH_2), 52.1 (CH), 110.0 (CH), 118.2 (CH), 124.0 (CH), 128.7 (CH), 128.8 (CH), 132.0 (CH), 132.5 (CH), 133.2 (C), 134.9 (C), 135.0 (C), 145.7 (C), 149.4 (C), 167.5 (C); *m/z* 350.0848 (MNa^+. $C_{15}H_{13}N_5NaO_4$ requires 350.0860).

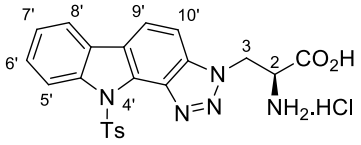
(2*S*)-2-Amino-3-[5′-(4′′-nitrophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (163k)



(2*S*)-2-Amino-3-[5′-(4′′-nitrophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (**163k**) was synthesised as described for (2*S*)-2-amino-3-[5′-(naphthalen-2′′-yl)-1*H*-

benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (**163b**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5′-(4′′-nitrophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoate (**161k**) (0.0529 g, 0.111 mmol) and caesium carbonate (0.0469 g, 0.144 mmol). Purification by recrystallisation from methanol and diethyl ether gave (2*S*)-2-amino-3-[5′-(4′′-nitrophenyl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (**163k**) as a white solid (0.0375 g, 93%). Mp 168–170 °C; ν_{max}/cm^{-1} (neat) 2899 (OH), 1735 (C=O), 1595, 1514, 1344, 750; $[\alpha]_D^{20}$ +4.8 (*c* 1.0, DMSO); δ_H (400 MHz, CD_3OD) 4.81 (1H, dd, *J* 5.7, 4.1 Hz, 2-H), 5.31 (1H, dd, *J* 15.5, 4.1 Hz, 3-*H*H), 5.41 (1H, dd, *J* 15.5, 5.7 Hz, 3-H*H*), 7.94–8.05 (4H, m, 6′-H, 7′-H, 2′′-H and 6′′-H), 8.36 (2H, d, *J* 8.9 Hz, 3′′-H and 5′′-H), 8.39 (1H, br s, 4′-H); δ_C (101 MHz, CD_3OD) 46.8 (CH_2), 52.2 (CH), 110.7 (CH), 117.8 (CH), 123.8 (2 × CH), 127.9 (CH), 128.1 (2 × CH), 133.7 (C), 136.0 (C), 146.2 (C), 146.4 (C), 147.4 (C), 167.6 (C); *m/z* (ESI) 328.1035 (MH^+. $C_{15}H_{14}N_5O_4$ requires 328.1040).

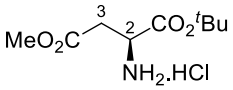
(2*S*)-2-Amino-3-[4′-(4′′-toluenesulfonyl)-[1,2,3]triazolo[2,3-c]carbazol-1(4′H)-yl]propanoic acid hydrochloride (182)



(2*S*)-2-Amino-3-[4′-(4′′-toluenesulfonyl)-[1,2,3]triazolo[2,3-c]carbazol-1(4′H)-yl]propanoic acid hydrochloride (**182**) was synthesised as described for (2*S*)-2-amino-3-[5′-(naphthalen-2′′-yl)-1*H*-benzo[*d*][1.2.3]triazol-1′-yl]propanoic acid hydrochloride (**163b**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4′-(4′′-toluenesulfonyl)-[1,2,3]triazolo[2,3-c]carbazol-1(4′H)-yl]propanoate (**178**) (0.0319 g, 0.0534 mmol) and caesium carbonate (0.0226 g, 0.0694 mmol). Purification by recrystallisation from methanol and diethyl ether gave (2*S*)-2-(amino)-3-[4′-(4′′-toluenesulfonyl)-[1,2,3]triazolo[2,3-c]carbazol-1(4′H)-yl]propanoic acid hydrochloride (**182**) as a white solid (0.0117 g, 45%). Mp 180–182 °C; ν_{max}/cm^{-1} (neat) 2843 (OH), 1734 (C=O), 1597, 1441, 1389, 1240, 1179, 677; $[\alpha]_D^{20}$ +4.9 (*c* 0.2, MeOH); δ_H (400 MHz, CD_3OD) 2.18 (3H, s, CH_3), 5.00 (1H, br s, 2-H), 5.40 (1H, d, *J* 4.3 Hz, 3-H_2), 7.07 (2H, d, *J* 8.0 Hz, 2′′-H and 6′′-H), 7.31 (1H, t, *J* 7.6 Hz, 6′-H), 7.46–7.55 (1H, ddd, *J* 8.5, 7.6, 0.9 Hz, 7′-H), 7.67 (2H, d, *J* 8.0 Hz, 3′′-H and 5′′-H), 7.73 (1H, d, *J* 7.6 Hz, 5′-H), 7.85 (1H, d, *J* 8.6 Hz, 10′-H), 8.10 (1H, d, *J* 8.6 Hz, 9′-H), 8.39 (1H, d, *J* 8.5 Hz, 8′-H); δ_C (101 MHz, CD_3OD) 21.4 (CH_3), 48.7 (CH_2), 49.8 (CH), 108.0 (CH), 116.5 (CH), 120.7 (CH), 122.2 (CH), 124.2 (C), 125.8 (CH), 127.2 (C), 127.6 (C), 128.0 (2 × CH), 128.2 (CH), 130.7 (2 × CH), 135.8 (C), 135.9 (C), 136.1 (C), 139.3 (C), 147.1 (C), 169.2 (C); *m/z* (ESI) 450.1224 (MH^+. $C_{22}H_{20}N_5O_4S$ requires 450.1231).

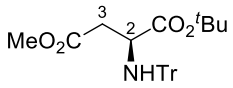
3.4 Experimental for bioconjugation project

L-Aspartic acid-α-*tert*-butyl-β-methyl diester hydrochloride (234)[210]



To a suspension of L-aspartic acid 4-methyl ester hydrochloride (**233**) (2.00 g, 10.9 mmol) in *tert*-butyl acetate (75 mL) at 0 °C was added 70% aqueous perchloric acid (2.17 mL, 25.0 mmol) dropwise. The reaction mixture was stirred at room temperature for 24 h, poured onto saturated aqueous potassium carbonate solution (145 mL) and the resulting mixture was extracted with diethyl ether (3 × 100 mL). The combined organic layers were combined and washed with saturated aqueous potassium carbonate solution (3 × 50 mL), brine (50 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give a clear oil. The crude product was dissolved in diethyl ether (50 mL), cooled to 0 °C and 2 M ethereal HCl (5.5 mL) was added, this afforded a white solid. The solid was filtered and washed with cold diethyl ether to give L-aspartic acid-α-*tert*-butyl-β-methyl diester hydrochloride (**234**) as a white solid (1.14 g, 44%). Spectroscopic data was consistent with the literature.[210] Mp 126–128 °C; $[\alpha]_D^{15}$ +8.8 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.49 (9H, s, 3 × CH_3), 3.17 (1H, dd, *J* 17.6, 5.1 Hz, 3-*H*H), 3.28 (1H, dd, *J* 17.6, 5.1 Hz, 3-H*H*), 4.38 (1H, t, *J* 5.1 Hz, 2-H), 8.84 (3H, s, NH_3); δ_C (101 MHz, $CDCl_3$) 27.8 (3 × CH_3), 34.1 (CH_2), 50.0 (CH_3), 52.5 (CH), 84.7 (C), 166.6 (C), 170.2 (C); *m/z* (ESI) 204 (MH^+ 100%).

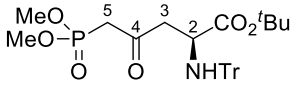
***N*-Trityl-L-aspartic acid-α-*tert*-butyl-β-methyl diester (235)**[210]



To a solution of L-aspartic acid-α-*tert*-butyl-β-methyl diester hydrochloride (**234**) (1.14 g, 4.75 mmol) in anhydrous acetonitrile (40 mL) at 0 °C was added triethylamine (1.32 mL, 9.50 mmol) and triphenylmethyl chloride (1.32 g, 4.75 mmol). The reaction mixture was stirred at room temperature for 16 h, then concentrated *in vacuo*. The resulting residue was dissolved in ethyl acetate (100 mL), washed with 1 M aqueous hydrochloric acid (50 mL), water (50 mL), brine (50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The compound was purified by flash chromatography using silica gel, eluting with 10% diethyl ether in petroleum ether. Addition of petroleum ether (40–60) precipitated the triphenylmethanol by-product as white crystals, which were isolated by filtration. The filtrate was concentrated *in vacuo* to give *N*-trityl-L-aspartic acid-α-*tert*-butyl-β-methyl diester (**235**) as a colourless oil (2.08 g, 98%). Spectroscopic data was

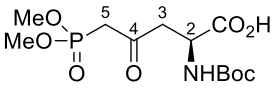
consistent with the literature.[210] $[\alpha]_D^{20}$ +22.7 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.23 (9H, s, 3 × CH_3), 2.39 (1H, dd, *J* 14.8, 7.4 Hz, 3-*H*H), 2.50 (1H, dd, *J* 14.8, 4.2 Hz, 3-H*H*), 3.01 (1H, d, *J* 8.5 Hz, NH), 3.57 (1H, ddd, *J* 8.5, 7.4, 4.2 Hz, 2-H), 7.13–7.20 (3H, m 3 × ArH), 7.22–7.29 (6H, m, 6 × ArH), 7.45–7.55 (6H, m, 6 × ArH); δ_C (126 MHz, $CDCl_3$) 27.8 (3 × CH_3), 40.2 (CH_2), 51.5 (CH_3), 54.0 (CH), 71.4 (C), 81.2 (C), 126.5 (3 × CH), 127.9 (6 × CH), 128.8 (6 × CH), 146.0 (3 × C), 171.3 (C), 172.4 (C); *m/z* (ESI) 468 (MNa^+ 100%).

tert-Butyl (2*S*)-5-(dimethyloxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (236)[173]



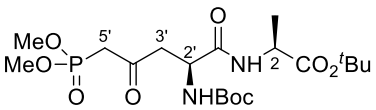
A solution of dimethyl methylphosphonate (1.24 mL, 11.5 mmol) in anhydrous toluene (115 mL) was cooled to −78 °C under an argon atmosphere. *n*-Butyl lithium (2.5 M in hexane, 4.58 mL, 11.5 mmol) was added dropwise and the reaction mixture was stirred for 0.5 h at −78 °C. In a separate reaction vessel, a solution of *N*-trityl-L-aspartic acid-α-*tert*-butyl-β-methyl diester (**235**) (2.04 g, 4.58 mmol) in toluene (50 mL) was cooled to −78 °C and then the dimethyl methylphosphonate ester/*n*-butyl lithium solution was cannulated into the flask and the reaction mixture stirred at −78 °C for 3 h. The solution was slowly warmed to room temperature and the reaction was quenched with saturated aqueous ammonium chloride (5 mL). The biphasic mixture was concentrated *in vacuo*, dissolved in ethyl acetate (50 mL) and washed with water (2 × 50 mL) and brine (50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash chromatography using silica gel, eluting with 10% to 30% ethyl acetate in dichloromethane gave *tert*-butyl (2*S*)-5-(dimethyloxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**236**) as a white solid (1.74 g, 71%). Mp 90–91 °C, lit.[173] 90–91 °C; $[\alpha]_D^{20}$ +7.5 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.26 (9H, s, 3 × CH_3), 2.62 (1H, dd, *J* 15.4, 5.0 Hz, 3-*H*H), 2.67 (1H, dd, *J* 15.4, 3.3 Hz, 3-H*H*), 3.50–3.59 (3H, m, 5-H_2 and NH), 3.75 (3H, d, $J_{H\text{-}C\text{-}O\text{-}P}$ 1.6 Hz, OCH_3), 3.78 (3H, d, $J_{H\text{-}C\text{-}O\text{-}P}$ 1.6 Hz, OCH_3), 7.15–7.21 (3H, m, 3 × ArH), 7.22–7.33 (6H, m, 6 × ArH), 7.42–7.58 (6H, m, 6 × ArH); δ_C (101 MHz, $CDCl_3$) 27.8 (3 × CH_3), 41.6 (d, $J_{C\text{-}P}$ 128.5 Hz, CH_2), 48.7 (CH_2), 53.0 (d, $J_{C\text{-}O\text{-}P}$ 6.5 Hz, CH_3), 53.0 (d, $J_{C\text{-}O\text{-}P}$ 6.5 Hz, CH_3), 53.5 (CH), 71.4 (C), 81.4 (C), 126.5 (3 × CH), 127.9 (6 × CH), 128.8 (6 × CH), 146.0 (3 × C), 172.4 (C), 199.5 (d, $J_{C\text{-}C\text{-}P}$ 6.5 Hz, C); *m/z* (ESI) 560 (MNa^+ 100%).

(2*S*)-5-(Dimethyloxyphosphoryl)-4-oxo-2-(*tert*-butoxycarbonylamino)pentanoate (237)



To a solution of *tert*-butyl (2*S*)-5-(dimethyloxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**236**) (0.172 g, 0.321 mmol) in dichloromethane (2.5 mL) was added trifluoroacetic acid (2.5 mL) and this was stirred at room temperature for 4 h. The reaction mixture was concentrated *in vacuo* and the resulting residue was dissolved in water (20 mL) and washed with diethyl ether (2 × 20 mL). The aqueous layer was concentrated *in vacuo*, azeotroping with diethyl ether. This was suspended in dichloromethane (5 mL) and di-*tert*-butyl dicarbonate (0.147 mL, 0.642 mmol) and triethylamine (0.0895 mL, 0.642 mmol) were added. The reaction mixture was stirred at room temperature for 16 h, with the reaction slowly becoming homogenous. The reaction was concentrated *in vacuo* and the resulting residue was dissolved in ethyl acetate (20 mL), washed with brine (2 × 20 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Trituration with hexane gave (2*S*)-5-(dimethyloxyphosphoryl)-4-oxo-2-(*tert*-butoxycarbonylamino)pentanoate (**237**) as a colourless oil (0.0960 g, 88%). ν_{max}/cm^{-1} (neat) 3366 (NH), 2974 (CH), 2164, 1717 (C=O), 1510, 1368, 1248, 1167, 1036; $[\alpha]_D^{20}$ +32.8 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.43 (9H, s, 3 × CH_3), 2.86–3.51 (4H, m, 3-H_2 and 5-H_2), 3.78 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 4.53 (1H, br s, 2-H), 5.64 (1H, d, *J* 7.7 Hz, NH), 9.24 (1H, br s, OH); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 41.0 (d, $J_{C\text{-}P}$ 130 Hz, CH_2), 45.9 (CH_2), 49.4 (CH), 53.4 (d, $J_{C\text{-}O\text{-}P}$ 6.5 Hz, CH_3), 53.6 (d, $J_{C\text{-}O\text{-}P}$ 6.5 Hz, CH_3), 80.2 (C), 155.7 (C), 173.4 (C), 199.9 (C); *m/z* (ESI) 362.0966 (MNa^+. $C_{12}H_{22}NNaO_8P$ requires 362.0975).

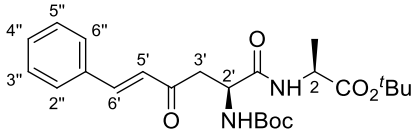
***tert*-Butyl (2*S*,2'*S*)-2-[2'-(*tert*-butoxycarbonylamino)-5'-(dimethoxyphosphoryl)-4'-oxopentanamido]propanoate (238)**



To a solution of (2*S*)-5-(dimethyloxyphosphoryl)-4-oxo-2-(*tert*-butoxycarbonylamino)pentanoate (**237**) (0.0564 g, 0.166 mmol), *tert*-butyl L-alaninate (0.0332 g, 0.183 mmol) and 1-hydroxybenzotriazole hydrate (0.0127 g, 0.0830 mmol) in acetonitrile (5 mL) at 0 °C was added PyBop® (0.130 g, 0.249 mmol) and *N*,*N*-diisopropylethylamine (0.867 mL, 0.498 mmol). The reaction mixture was warmed to room temperature, stirred for 1 h and concentrated *in vacuo*. The resulting residue was dissolved in ethyl acetate (50 mL), washed with 1 M aqueous hydrochloric acid (50 mL, for good separation the addition of a minimum amount of brine was

required), brine (50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash chromatography using silica gel, eluting first with 20% acetonitrile in dichloromethane and then 4% methanol in dichloromethane gave *tert*-butyl (2*S*,2′*S*)-2-[2′-(*tert*-butoxycarbonylamino)-5′-(dimethoxyphosphoryl)-4′-oxopentanamido]propanoate (**238**) as a colourless oil (0.0552 g, 71%). ν_{max}/cm^{-1} (neat) 3300 (NH), 2978 (CH), 1717 (C=O), 1520 (NH), 1456, 1368, 1250, 1153, 1028; $[\alpha]_D^{20}$ +14.8 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.36 (3H, d, *J* 7.1 Hz, 2-CH_3), 1.40–1.52 (18H, m, 6 × CH_3), 2.97 (1H, dd, *J* 17.7, 5.1 Hz, 3′-*H*H), 3.10–3.36 (3H, m, 3′-H*H* and 5′-H_2), 3.78 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 4.37 (1H, quin., *J* 7.1 Hz, 2-H), 4.55 (1H, dt, *J* 8.0, 5.1 Hz, 2′-H), 5.63 (1H, d, *J* 8.0 Hz, NH), 7.11 (1H, d, *J* 7.1 Hz, NH); δ_C (126 MHz, $CDCl_3$) 18.3 (CH_3), 27.9 (3 × CH_3), 28.3 (3 × CH_3), 41.8 (d, $J_{C\text{-}P}$ 129 Hz, CH_2), 45.5 (CH_2), 48.9 (CH), 50.7 (CH), 53.1 (d, $J_{C\text{-}O\text{-}P}$ 6.4 Hz, CH_3), 53.2 (d, $J_{C\text{-}O\text{-}P}$ 6.4 Hz, CH_3), 80.4 (C), 81.9 (C), 155.5 (C), 170.2 (C), 171.6 (C), 201.1 (C); *m/z* (ESI) 489.1966 (MNa^+. $C_{19}H_{35}N_2NaO_9P$ requires 489.1972).

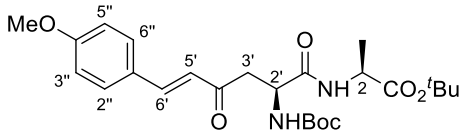
tert-Butyl (2*S*,2′*S*,5′*E*)-2-[2′-(_tert_-butoxycarbonylamino)-4′-oxo-6′-phenylhex-5′-enamido]propanoate (239a)



To a solution of *tert*-butyl (2*S*,2′*S*)-2-[2′-(*tert*-butoxycarbonylamino)-5′-(dimethoxyphosphoryl)-4′-oxopentanamido]propanoate (**238**) (0.0298 g, 0.0639 mmol) in anhydrous acetonitrile (2 mL) was added potassium carbonate (0.00971 g, 0.0703 mmol) and benzaldehyde (0.0130 mL, 0.128 mmol). The reaction mixture was heated to 50 °C for 70 h and then concentrated *in vacuo*. The resulting residue was dissolved in ethyl acetate (50 mL) and washed with water (50 mL), brine (50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash chromatography using silica gel, eluting with 50 to 60% diethyl ether in petroleum ether (40–60) gave *tert*-butyl (2*S*,2′*S*)-2-[2′-(*tert*-butoxycarbonylamino)-4′-oxo-6′-phenylhex-5′-enamido]propanoate (**239a**) as a clear oil (0.0250 g, 88%). ν_{max}/cm^{-1} (neat) 3306 (NH), 2978 (CH), 1728 (C=O), 1657 (C=O), 1526 (NH), 1368, 1152, 748, 691; $[\alpha]_D^{20}$ +78.5 (*c* 0.25, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.38 (3H, d, *J* 6.9 Hz, 2-CH_3), 1.41–1.50 (18H, m, 6 × CH_3), 2.99 (1H, dd, *J* 17.8, 5.0 Hz, 3′-*H*H), 3.54 (1H, br d, *J* 17.8 Hz, 3′-H*H*), 4.41 (1H, quin., *J* 6.9 Hz, 2-H), 4.61 (1H, br s, 2′-H), 5.77 (1H, d, *J* 7.6 Hz, NH), 6.73 (1H, d, *J* 16.2 Hz, 5′-H), 7.16 (1H, d, *J* 6.9 Hz, NH), 7.31–7.46 (3H, m, 3′′-H, 4′′-H and 5′′-H), 7.49–7.63 (3H, m, 6′-H, 2′′-H and 6′′-H); δ_C (101 MHz, $CDCl_3$) 18.4 (CH_3), 28.0 (3 × CH_3), 28.3 (3 × CH_3), 42.2 (CH_2), 49.0 (CH), 50.6 (CH), 80.3 (C), 81.9 (C), 125.9 (CH), 128.5 (2 × CH), 129.0 (2 × CH), 130.8 (CH), 134.2 (C), 144.0 (CH),

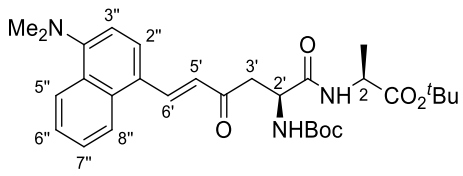
155.5 (C), 170.5 (C), 171.7 (C), 199.0 (C); *m/z* (ESI) 469.2302 (MNa^+. $C_{24}H_{34}N_2NaO_6$ requires 469.2309).

tert-Butyl (2*S*,2′*S*,5′*E*)-2-[2′-(_tert_-butoxycarbonylamino)-4′-oxo-6′-(4′′-methoxyphenyl)hex-5′-enamido]propanoate (239b)



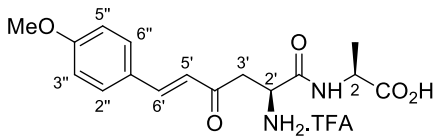
tert-Butyl (2*S*,2′*S*,5′*E*)-2-[2′-(*tert*-butoxycarbonylamino)-4′-oxo-6′-(4′′-methoxyphenyl)hex-5′-enamido]propanoate (**239b**) was synthesised as described for *tert*-butyl (2*S*,2′*S*)-2-[2′-(*tert*-butoxycarbonylamino)-4′-oxo-6′-phenylhex-5′-enamido]propanoate (**239a**) using *tert*-butyl (2*S*,2′*S*)-2-[2′-(*tert*-butoxycarbonylamino)-5′-(dimethoxyphosphoryl)-4′-oxopentanamido]propanoate (**238**) (0.0503 g, 0.108 mmol), *p*-anisaldehyde (0.0262 mL, 0.216 mmol) and potassium carbonate (0.0164 g, 0.119 mmol) in anhydrous acetonitrile (4 mL). Purification by flash chromatography using silica gel, eluting with 0 to 20% diethyl ether in dichloromethane gave *tert*-butyl (2*S*,2′*S*,5′*E*)-2-[2′-(*tert*-butoxycarbonylamino)-4′-oxo-6′-(4′′-methoxyphenyl)hex-5′-enamido]propanoate (**239b**) as a colourless oil (0.0217 g, 42%). ν_{max}/cm^{-1} (neat) 3321 (NH), 2978 (CH), 1655 (C=O), 1599, 1572, 1510 (NH), 1368, 1250, 1169, 1153, 1028, 733; $[\alpha]_D^{20}$ +95.6 (*c* 0.5, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.37 (3H, d, *J* 7.1 Hz, 2-CH_3), 1.41–1.49 (18H, m, 6 × CH_3), 2.96 (1H, dd, *J* 17.7, 5.9 Hz, 3′-*H*H), 3.50 (1H, br d, 17.7 Hz, 3′-H*H*), 3.84 (3H, s, OCH_3), 4.40 (1H, quin., *J* 7.2 Hz, 2-H), 4.59 (1H, br s, 2′-H), 5.78 (1H, d, *J* 7.8 Hz, NH), 6.61 (1H, d, *J* 16.2 Hz, 5′-H), 6.91 (2H, d, *J* 8.8 Hz, 3′′-H and 5′′-H), 7.16 (1H, d, *J* 7.2 Hz, NH), 7.49 (2H, d, *J* 8.8 Hz, 2′′-H and 6′′-H), 7.54 (1H, d, *J* 16.2 Hz, 6′-H); δ_C (101 MHz, $CDCl_3$) 18.4 (CH_3), 28.0 (3 × CH_3), 28.3 (3 × CH_3), 42.1 (CH_2), 49.0 (CH), 50.6 (CH), 55.4 (CH_3), 80.3 (C), 81.8 (C), 114.5 (2 × CH), 123.6 (CH), 126.9 (C), 130.3 (2 × CH), 143.8 (CH), 155.5 (C), 161.9 (C), 170.6 (C), 171.7 (C), 198.9 (C); *m/z* (ESI) 499.2405 (MNa^+. $C_{25}H_{36}N_2NaO_7$ 499.2415).

***tert*-Butyl (2*S*,2′*S*,5′*E*)-2-[2′-(*tert*-butoxycarbonylamino)-4′-oxo-6′-(4″-dimethylaminonaphthalen-1′-yl)hex-5′-enamido]propanoate (239c)**



tert-Butyl-(2*S*,2′*S*,5′*E*) 2-[2′-(*tert*-butoxycarbonylamino)-4′-oxo-6′-(4″-dimethylaminonaphthalen-1′-yl)hex-5′-enamido]propanoate (**239c**) was synthesised as described for *tert*-butyl (2*S*,2′*S*)-2-[2′-(*tert*-butoxycarbonylamino)-4′-oxo-6′-phenylhex-5′-enamido]propanoate (**239a**) using *tert*-butyl (2*S*,2′*S*)-2-[2′-(*tert*-butoxycarbonylamino)-5′-(dimethoxyphosphoryl)-4′-oxopentanamido]propanoate (**238**) (0.0580 g, 0.124 mmol), 4-dimethylamino-1-naphthaldehyde (0.0297 g, 0.249 mmol) and potassium carbonate (0.0189 g, 0.136 mmol) in anhydrous acetonitrile (4 mL). The reaction mixture was heated at 50 °C for 72 h. Potassium carbonate (0.00857 g, 0.0620 mmol) was added and the reaction continued to be stirred at 70 °C for 48 h. Additional potassium carbonate (0.00857 g, 0.0620 mmol) was added and the reaction mixture was heated at 70 °C for a further 24 h. The mixture was then concentrated *in vacuo*. Purification by flash chromatography using silica gel, eluting with 60% diethyl ether in petrolumn ether (40–60) gave *tert*-butyl (2*S*,2′*S*,5′*E*)-2-[2′-(*tert*-butoxycarbonylamino)-4′-oxo-6′-(4″-dimethylaminonaphthalen-1′-yl)hex-5′-enamido]propanoate (**239c**) as a yellow oil (0.0189 g, 28%, 71:29 ratio of *E*/*Z* isomers). ν_{max}/cm^{-1} (neat) 3329 (NH), 2978 (CH), 1713 (C=O), 1667 (C=O), 1512, 1391, 1368, 1155, 1047, 768, 733; $[\alpha]_D^{20}$ +51.4 (*c* 0.5, $CHCl_3$); NMR data is given for the *E*-isomer: δ_H (400 MHz, $CDCl_3$) 1.39 (3H, d, *J* 7.2 Hz, 2-CH_3), 1.45 (9H, s, 3 × CH_3), 1.47 (9H, s, 3 × CH_3), 2.96 (6H, s, NMe_2), 3.05 (1H, dd, *J* 16.5, 5.5 Hz, 3′-*H*H), 3.60 (1H, br d, *J* 16.5 Hz, 3′-H*H*), 4.43 (1H, quin., *J* 7.2 Hz, 2-H), 4.64 (1H, br s, 2′-H), 5.82 (1H, d, *J* 7.2 Hz, NH), 6.76 (1H, d, *J* 15.8 Hz, 5′-H), 7.04 (1H, d, *J* 8.0 Hz, 3″-H), 7.21 (1H, d, *J* 7.2 Hz, NH), 7.41–7.61 (2H, m, 6″-H and 7″-H), 7.75 (1H, d, *J* 8.0 Hz, 2″-H), 8.18 (1H, dd, *J* 8.0, 1.3 Hz, 8″-H), 8.23 (1H, dd, *J* 8.3, 1.3 Hz, 5″-H), 8.42 (1H, d, *J* 15.8 Hz, 6′-H); δ_C (101 MHz, $CDCl_3$) 18.5 (CH_3), 28.0 (3 × CH_3), 28.3 (3 × CH_3), 44.9 (2 × CH_3), 45.0 (CH_2), 49.0 (CH), 50.6 (CH), 81.8 (C), 81.8 (C), 113.3 (CH), 123.6 (CH), 125.2 (C), 125.2 (CH), 125.3 (CH), 125.7 (CH), 126.1 (CH), 126.8 (CH), 128.3 (C), 133.1 (C), 140.9 (CH), 152.5 (C), 154.0 (C), 170.6 (C), 171.7 (C), 198.8 (C); *m/z* (ESI) 562.2867 (MNa^+. $C_{30}H_{41}N_3NaO_6$ requires 562.2888).

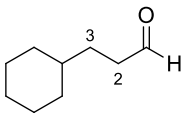
(2*S*,2′*S*,5′*E*)-2-[2′-Amino-4′-oxo-6′-(4′′-methoxyphenyl)hex-5′-enamido]propanoic acid trifluoroacetate (240)



To a solution of *tert*-butyl (2*S*,2′*S*,2*E*)-2-[2′-(*tert*-butoxycarbonylamino)-4′-oxo-6′-(4′′-methoxyphenyl)hex-5′-enamido]propanoate (**239b**) (0.0234 g, 0.0491 mmol) in dichloromethane (2 mL) was added trifluoroacetic acid (2 mL). The reaction mixture was stirred at room temperature for 4 h. The reaction mixture was concentrated *in vacuo* and the product was precipitated with diethyl ether. The solid was filtered and washed with diethyl ether to give (2*S*,2′*S*,5′*E*)-2-[2′-amino-4′-oxo-6′-(4′′-methoxyphenyl)hex-5′-enamido]propanoic acid trifluoroacetate (**240**) as a white solid (0.0153 g, 72%). Mp 94–96 °C; ν_{max}/cm^{-1} (neat) 3549 (NH), 3121 (OH), 1674 (C=O), 1597, 1512 (NH), 1396, 1258, 1173, 816; $[\alpha]_D^{20}$ −11.0 (*c* 0.1, MeOH); δ_H (500 MHz, CD_3OD) 1.44 (3H, d, *J* 7.3 Hz, 2-CH_3), 3.26 (1H, dd, *J* 18.7, 9.8 Hz, 3′-*H*H), 3.48 (1H, dd, *J* 18.7, 3.1 Hz, 3-H*H*), 4.34 (1H, dd, *J* 9.8, 3.1 Hz, 2′-H), 4.44 (1H, q, *J* 7.3 Hz, 2-H), 6.79 (1H, d, *J* 16.2 Hz, 5′-H), 6.99 (2H, d, *J* 8.8 Hz, 3′′-H and 5′′-H), 7.63 (2H, d, *J* 8.8 Hz, 2′′-H and 6′′-H), 7.72 (1H, d, *J* 16.2 Hz, 6′-H); δ_H (126 MHz, CD_3OD) 16.2 (CH_3), 40.5 (CH_2), 48.3 (CH), 48.9 (CH), 54.5 (CH_3), 114.2 (2 × CH), 122.2 (CH), 126.7 (C), 130.2 (2 × CH), 144.8 (CH), 162.4 (C), 168.3 (C), 174.4 (C), 195.9 (C); *m/z* (ESI) 343.1276 (MNa^+. $C_{16}H_{20}N_2NaO_5$ requires 343.1264).

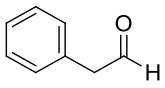
3.5 Experimental for acid-mediated 6-*endo-trig* project

3-Cyclohexylpropanal (276a)[211]



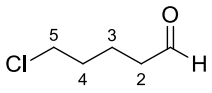
3-Cyclohexyl-1-propanol (**275a**) (0.400 mL, 2.56 mmol) was added dropwise to a solution of Dess-Martin periodinane (1.63 g, 3.84 mmol) in dichloromethane (15 mL) at 0 °C. The resulting reaction mixture was stirred at room temperature for 1 h, diluted with dichloromethane (50 mL) and washed with 1 M aqueous sodium thiosulfate solution (2 × 30 mL), brine (50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash chromatography using silica gel, eluting with 20% diethyl ether in petroleum ether (40–60) gave 3-cyclohexylpropanal (**276a**) as a clear oil (0.295 g, 82%). δ_H (400 MHz, $CDCl_3$) 0.81–0.99 (2H, m, CH_2), 1.06–1.34 (4H, m, 2 × CH_2), 1.52 (2H, br q, *J* 7.6 Hz, 3-H_2), 1.58–1.78 (5H, m, CH and 2 × CH_2), 2.42 (2H, td, *J* 7.6, 1.9 Hz, 2-H_2), 9.76 (1H, t, *J* 1.9 Hz, 1-H); δ_C (101 MHz, $CDCl_3$) 26.2 (2 × CH_2), 26.5 (CH_2), 29.4 (CH_2), 33.1 (2 × CH_2), 37.2 (CH), 41.5 (CH_2), 203.0 (CH); *m/z* (ESI) 281 ($2MH^+$, 100%)

Phenylacetaldehyde (276c)[212]



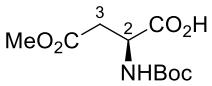
Phenylacetaldehyde (**276c**) was synthesised as described for 3-cyclohexylpropanal (**276a**) using 2-phenyl-1-ethanol (**275c**) (0.300 mL, 2.51 mmol), Dess-Martin periodinane (1.59 g, 3.76 mmol) in dichloromethane (15 mL). Purification by flash chromatography using silica gel, eluting with 25% TBME in cyclohexane gave phenylacetaldehyde (**276c**) as a clear oil (0.187 g, 59%). Spectroscopic data was consistent with literature.[212] δ_H (400 MHz, $CDCl_3$) 3.74 (2H, d, *J* 2.4 Hz, CH_2), 7.24–7.31 (2H, m, 2 × ArH), 7.34–7.50 (3H, m, 3 × ArH), 9.81 (1H, t, *J* 2.4 Hz, CH); δ_C (101 MHz, $CDCl_3$) 50.6 (CH_2), 127.4 (CH), 129.0 (2 × CH), 129.6 (2 × CH), 131.9 (C), 199.3 (CH).

5-Chloropentanal (276b)[213]



A solution of oxalyl chloride (0.438 mL, 5.18 mmol) in dichloromethane (15 mL) was added dropwise to a solution of DMSO (0.737 mL, 10.37 mmol) in dichloromethane (2.5 mL) at −78 °C. The resultant reaction mixture was stirred at −78 °C for 0.1 h. A solution of 5-chloro-1-pentanol (**275b**) (0.500 mL, 4.32 mmol) in dichloromethane (2.5 mL) was added to the reaction mixture. The resulting reaction mixture was stirred at −78 °C for 0.2 h. Triethylamine (3.10 mL, 21.6 mmol) was added to the reaction mixture and the reaction was warmed to room temperature. The reaction mixture was diluted with dichloromethane and washed with water (50 mL), brine (50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash chromatography using silica gel, eluting with dichloromethane gave 5-chloropentanal (**276b**) as a clear oil (0.376 g, 72%). Spectroscopic data was consistent with literature.[213] ν_{max}/cm^{-1} (neat) 2947, 2730, 1721 (C=O), 1242, 648; δ_H (400 MHz, $CDCl_3$) 1.72–1.86 (4H, m, 3-H_2 and 4-H_2), 2.40–2.56 (2H, m, 2-H_2), 3.44–3.61 (2H, m, 5-H_2), 9.77 (1H, t, *J* 1.5 Hz, 1-H); δ_C (101 MHz, $CDCl_3$) 19.5 (CH_2), 31.9 (CH_2), 43.1 (CH_2), 44.6 (CH_2), 201.9 (CH); *m/z* (ESI) 281 ($2MH^+$, 100%).

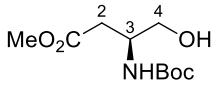
***N*-Boc-L-aspartic acid-α-methyl ester (269)**[214]



L-Aspartic acid (**106**) (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-α-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 which gave *N*-Boc-L-aspartic acid 4-methyl

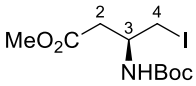
ester (**269**) as a white solid (8.27 g, 89%). Mp 54–56 °C (lit.,[214] 56 °C); δ_H (500 MHz, $CDCl_3$) 1.45 (9H, s, 3 × CH_3), 2.85 (1H, dd, *J* 17.2, 3.8 Hz, 3-*H*H), 3.04 (1H, dd, *J* 17.2, 3.8 Hz, 3-H*H*), 3.72 (3H, s, OCH_3), 4.55–4.67 (1H, m, 2-H), 5.55 (1H, d, *J* 8.0 Hz, NH); δ_C (126 MHz, $CDCl_3$) 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 (271)[215]



To a solution of *N*-Boc-L-aspartic acid 4-methyl ester (**269**) (8.27 g, 33.5 mmol) in ethyl acetate (200 mL) at 0 °C was added *N*-hydroxysuccinimide (4.24 g, 36.9 mmol). *N*,*N*'-Dicyclohexylcarbodiimide (7.05 g, 34.2 mmol) in ethyl acetate (20 mL) was then 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*. Purification by flash column chromatography using silica gel, eluting with 30% ethyl acetate in dichloromethane gave methyl (3*S*)-3-(*tert*-butoxycarbonylamino)-4-hydroxybutanoate (**271**) as a colourless oil (7.03 g, 90%). $[\alpha]_D^{26}$ +5.6 (*c* 1.0, $CHCl_3$), lit.[215] $[\alpha]_D^{23}$ +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%).

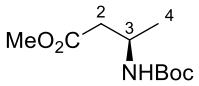
Methyl (3*S*)-3-(*tert*-butoxycarbonylamino)-4-iodobutanoate (272)[193]



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

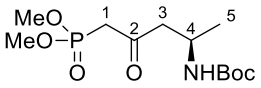
mmol) in three portions over 0.5 h. After stirring for a further 0.2 h, a solution of methyl (3*S*)-3-(*tert*-butoxycarbonylamino)-4-hydroxybutanoate (**271**) (7.03 g, 30.2 mmol) in a mixture of diethyl ether and dichloromethane (2:1, 50 mL) was added and the resulting mixture was stirred for 3 h at room temperature. The reaction mixture was filtered through Celite and the filtrate was concentrated *in vacuo.* Purification by flash column chromatography using silica gel, eluting with 30% diethyl ether in petroleum ether (40–60) gave methyl (3*S*)-3-(*tert*-butoxycarbonylamino)-4-iodobutanoate (**272**) (9.33 g, 90%) as a colourless oil. Spectroscopic data were consistent with the literature.[193] $[\alpha]_D^{33}$ +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), 28.5 (3 × CH_3), 38.5 (CH_2), 47.7 (CH), 52.0 (CH_3), 79.9 (C), 154.7 (C), 171.1 (C); *m/z* (ESI) 366 (MNa^+, 100%).

Methyl (3*R*)-3-(*tert*-butoxycarbonylamino)butanoate (273)[216]



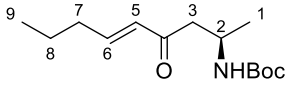
A solution of methyl (3*S*)-3-(*tert*-butoxycarbonylamino)-4-iodobutanoate (**272**) (9.33 g, 27.2 mmol), *N*,*N*-diisopropylethylamine (7.11 mL, 40.8 mmol) and 10% Pd/C (2.89 g, 2.72 mmol) in methanol (50 mL) were purged with hydrogen for 0.5 h. The reaction mixture was stirred under an atmosphere of hydrogen for 18 h at room temperature. The mixture 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 concentrated *in vacuo* which gave methyl (3*R*)-3-(*tert*-butoxycarbonylamino)butanoate (**273**) as a colourless oil (5.87 g, 99%). Spectroscopic data were consistent with the literature.[216] $[\alpha]_D^{26}$ +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), 40.7 (CH_2), 43.5 (CH), 51.6 (CH_3), 79.3 (C), 155.1 (C), 172.0 (C); *m/z* (ESI) 240 (MNa^+, 100%).

(4*R*)-4-(*tert*-Butoxycarbonylamino)-1-(dimethyloxyphosphoryl)pentan-2-one (274)



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 (**273**) (3.00 g, 13.8 mmol) in tetrahydrofuran (20 mL) was added dropwise. The resulting 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) and extracted with ethyl acetate (2 × 50 mL). The combined organic layers were combined, washed with brine (100 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by flash column chromatography using silica gel, eluting with 40% ethyl acetate in dichloromethane gave (4*R*)-4-(*tert*-butoxycarbonylamino)-1-(dimethyloxyphosphoryl)pentan-2-one (**274**) (3.84 g, 90%) as a colourless oil. 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, $CDCl_3$) 20.5 (CH_3), 28.3 (3 × CH_3), 41.8 (d, $J_{C\text{-}P}$ 127.5 Hz, CH_2), 43.2 (CH), 50.1 (CH_2), 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), 79.2 (C), 155.1 (C), 200.5 (C); *m/z* (ESI) 332.1225 (MNa^+. $C_{12}H_{24}NNaO_6P$ requires 332.1233).

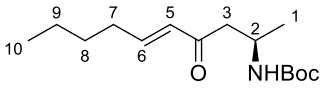
(2*R*,5*E*)-2-(*tert*-Butoxycarbonylamino)-4-oxonona-5-ene (258a)



(4*R*)-4-(*tert*-Butoxycarbonylamino)-1-(dimethyloxyphosphoryl)pentan-2-one (**274**) (0.421 g, 1.36 mmol) was dissolved in anhydrous acetonitrile (14 mL) and potassium carbonate (0.207 g, 1.50 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 stirred for 72 h. The solution was then concentrated *in vacuo*. the resulting residue was dissolved in ethyl acetate (20 mL), washed with water (2 × 15 mL) and brine (15 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography using silica gel, eluting with

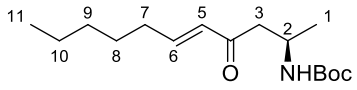
20% diethyl ether in petroleum ether (40–60) gave (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxonona-5-ene (**258a**) as a clear colourless oil (0.336 g, 87%). ν_{max}/cm^{-1} (neat) 3350 (NH), 2968 (CH), 1689 (C=O), 1516, 1365, 1166, 1053, 977; $[\alpha]_D^{26}$ +9.3 (*c* 1.0, $CHCl_3$); δ_H (500 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 (2H, 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), 21.3 (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-oxodec-5-ene (258b)



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 (**258a**) using (4*R*)-4-(*tert*-butoxycarbonylamino)-1-(dimethyloxyphosphoryl)pentan-2-one (**274**) (0.100 g, 0.326 mmol), valeraldehyde (0.070 mL, 0.652 mmol) and potassium carbonate (0.0496 g, 0.359 mmol) in acetonitrile (4 mL). Purification by flash column chromatography using silica gel, eluting with 20% diethyl ether in petroleum ether (40–60) gave (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxodec-5-ene (**258b**) (0.069 g, 78%) as a colourless oil. ν_{max}/cm^{-1} (neat) 3356 (NH), 2961 (CH), 1693 (C=O), 1516, 1366, 1248, 1171, 1053; $[\alpha]_D^{20}$ +6.1 (*c* 0.9, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.88 (3H, t, *J* 7.2 Hz; 10-H_3), 1.17 (3H, d, *J* 6.7 Hz, 1-H_3), 1.27–1.46 (13H, m, 3 × CH_3, 8-H_2, 9-H_2), 2.19 (2H, qd, *J* 7.1, 1.5 Hz, 7-H_2), 2.60 (1H, dd, *J* 15.9, 6.6 Hz, 3-*H*H), 2.82 (1H, dd, *J* 15.9, 4.9 Hz, 3-H*H*), 3.94–4.07 (1H, m, 2-H), 4.95 (1H, br s, NH), 6.06 (1H, dt, *J* 15.9, 1.5 Hz, 5-H), 6.82 (1H, dt, *J* 15.9, 7.1 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 13.7 (CH_3), 20.5 (CH_3), 22.2 (CH_2), 28.4 (3 × CH_3), 30.1 (CH_2), 32.1 (CH_2), 43.7 (CH), 45.7 (CH_2), 79.1 (C), 130.6 (CH), 148.4 (CH), 155.1 (C), 199.0 (C); *m/z* (ESI) 292.1890 (MNa^+. $C_{15}H_{27}NNaO_3$ requires 292.1889.

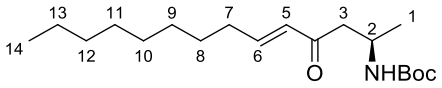
(2*R*,5*E*)-2-(*tert*-Butoxycarbonylamino)-4-oxoundec-5-ene (258c)



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 (**258a**) using (4*R*)-4-(*tert*-butoxycarbonylamino)-1-

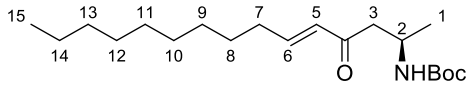
(dimethyloxyphosphoryl)pentan-2-one (**274**) (0.047 g, 0.151 mmol), hexanal (0.037 mL, 0.303 mmol) and potassium carbonate (0.0230 g, 0.166 mmol) in acetonitrile (4 mL). Purification by flash column chromatography using silica gel, eluting with 20% diethyl ether in petroleum ether (40–60) gave (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxoundec-5-ene (**258c**) as a colourless oil (0.035 g, 81%). ν_{max}/cm^{-1} (neat) 3341 (NH), 2928 (CH), 1694 (C=O), 1516, 1366, 1173, 1053; $[\alpha]_D^{24}$ +5.6 (*c* 0.7, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.89 (3H, t, *J* 6.9 Hz, 11-H_3), 1.20 (3H, d, *J* 6.7 Hz, 1-H_3), 1.24–1.36 (4H, m, 9-H_2 and 10-H_2), 1.40–1.52 (11H, m, 8-H_2 and 3 × CH_3), 2.14 (2H, qd, *J* 7.1, 1.4 Hz, 7-H_2), 2.63 (1H, dd, *J* 15.9, 6.5 Hz, 3-*H*H), 2.85 (1H, dd, *J* 15.9, 4.6 Hz, 3-H*H*), 3.95–4.07 (1H, m, 2-H), 4.94 (1H, br s, NH), 6.08 (1H, dt, *J* 15.8, 1.4 Hz, 5-H), 6.85 (1H, dt, *J* 15.8, 7.1 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 14.1 (CH_3), 20.6 (CH_3), 22.6 (CH_2), 27.9 (CH_2), 28.6 (3 × CH_3), 31.5 (CH_2), 32.6 (CH_2), 43.9 (CH), 45.8 (CH_2), 79.3 (C), 130.7 (CH), 148.7 (CH), 155.3 (C), 199.3 (C); *m/z* (ESI) 306.2039 (MNa^+. $C_{16}H_{29}NNaO_3$ requires 306.2040).

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



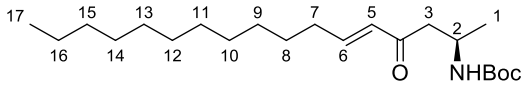
The reaction was carried out according to the above procedure for the synthesis of (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxonona-5-ene (**258a**) using (4*R*)-4-(*tert*-butoxycarbonylamino)-1-(dimethyloxyphosphoryl)pentan-2-one (**274**) (0.129 g, 0.416 mmol), nonanal (0.143 mL, 0.832 mmol) and potassium carbonate (0.0632 g, 0.458 mmol) in acetonitrile (4 mL). Purification by flash column chromatography using silica gel, eluting with 30% diethyl ether in petroleum ether (40–60) gave (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxotetradec-5-ene (**258d)** as a colourless oil (0.098 g, 72%). ν_{max}/cm^{-1} (neat) 3352 (NH), 2924 (CH), 1694 (C=O), 1501, 1366, 1246, 1169, 1053; $[\alpha]_D^{29}$ +6.5 (*c* 0.8, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.87 (3H, t, *J* 6.9 Hz, 14-H_3), 1.19 (3H, d, *J* 6.7 Hz, 1-H_3), 1.22–1.35 (10H, m, 9-H_2, 10-H_2, 11-H_2, 12-H_2 and 13-H_2), 1.38–1.50 (11H, m, 8-H_2 and 3 × CH_3), 2.20 (2H, qd, *J* 7.0, 1.5 Hz, 7-H_2), 2.62 (1H, dd, *J* 15.9, 6.5 Hz, 3-*H*H), 2.85 (1H, dd, *J* 15.9, 4.8 Hz, 3-H*H*), 3.95–4.09 (1H, m, 2-H), 4.95 (1H, br s, NH), 6.07 (1H, dt, *J* 15.9, 1.5 Hz, 5-H), 6.84 (1H, dt, *J* 15.9, 7.0 Hz, 6-H); δ_C (101 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 (2 × CH_2), 29.3 (CH_2), 31.8 (CH_2), 32.5 (CH_2), 43.8 (CH), 45.7 (CH_2), 79.2 (C), 130.6 (CH), 148.5 (CH), 155.2 (C), 199.1 (C); *m/z* (ESI) 348.2498 (MNa^+. $C_{19}H_{35}NNaO_3$ requires 348.2509).

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



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 (**258a**) using (4*R*)-4-(*tert*-butoxycarbonylamino)-1-(dimethyloxyphosphoryl)pentan-2-one (**274**) (0.405 g, 1.31 mmol), decanal (0.500 mL, 2.62 mmol) and potassium carbonate (0.199 g, 1.44 mmol) in acetonitrile (8 mL). Purification by flash column chromatography using silica gel, eluting with 30% diethyl ether in petroleum ether (40–60) gave (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxopentadec-5-ene (**258e**) (0.345 g, 78%) as a colourless oil. 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).

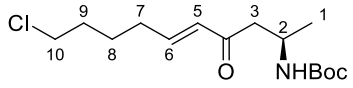
(2*R*,5*E*)-2-(*tert*-Butoxycarbonylamino)-4-oxoheptadec-5-ene (258f)



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 (**258a**) using (4*R*)-4-(*tert*-butoxycarbonylamino)-1-(dimethyloxyphosphoryl)pentan-2-one (**274**) (0.750 g, 2.42 mmol), dodecanal (1.08 mL, 4.85 mmol) and potassium carbonate (0.368 g, 2.66 mmol) in acetonitrile (10 mL). Purification by flash column chromatography using silica gel, eluting with 30% diethyl ether in petroleum ether (40–60) gave (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxoheptadec-5-ene (**258f**) as a colourless oil (0.734 g, 83%). ν_{max}/cm^{-1} (neat) 3354 (NH), 2925 (CH), 1694 (C=O), 1515, 1366, 1248, 1172, 1053; $[\alpha]_D^{22}$ +4.8 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.87 (3H, t, *J* 6.8 Hz, 17-H_3), 1.19 (3H, d, *J* 6.8 Hz, 1-H_3), 1.22–1.36 (16H, m, 9-H_2, 10-H_2, 11-H_2, 12-H_2, 13-H_2, 14-H_2, 15-H_2 and 16-H_2), 1.38–1.50 (11H, m, 8-H_2, 3 × CH_3), 2.20 (2H, qd, *J* 7.1, 1.4 Hz, 7-H_2), 2.63 (1H, dd, *J* 15.9, 6.6 Hz, 3-*H*H), 2.85 (1H, dd, *J* 15.9, 4.7 Hz, 3-H*H*), 3.93–4.09 (1H, m, 2-H), 4.96 (1H, br s, NH), 6.07 (1H, dt, *J* 15.8, 1.4 Hz, 5-H),

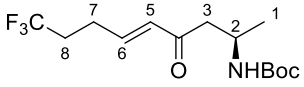
6.85 (1H, dt, *J* 15.8, 7.1 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 14.3 (CH_3), 20.6 (CH_3), 22.8 (CH_2), 28.2 (CH_2), 28.6 (3 × CH_3), 29.3 (CH_2), 29.5 (2 × CH_2), 29.7 (CH_2), 29.8 (2 × CH_2), 32.0 (CH_2), 32.7 (CH_2), 43.8 (CH), 45.8 (CH_2), 79.3 (C), 130.7 (CH), 148.7 (CH), 155.3 (C), 199.3 (C); *m/z* (ESI) 390.2985 (MNa^+. $C_{22}H_{41}NNaO_3$ requires 390.2979).

(2*R*,5*E*)-2-(*tert*-Butoxycarbonylamino)-10-chloro-4-oxodec-5-ene (258g)



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 (**258a**) using (4*R*)-4-(*tert*-butoxycarbonylamino)-1-(dimethyloxyphosphoryl)pentan-2-one (**274**) (0.114 g, 0.367 mmol), 5-chloropentanal (0.089 g, 0.735 mmol), potassium carbonate (0.0558 g, 0.404 mmol) in acetonitrile (4 mL). Purification by flash column chromatography using silica gel, eluting with 30% diethyl ether in petroleum ether (40–60) gave (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-10-chloro-4-oxodec-5-ene (**258g**) as a colourless oil (0.098 g, 88%). ν_{max}/cm^{-1} (neat) 3357 (NH), 2977 (CH), 1693 (C=O), 1663 (C=O), 1510, 1366, 1247, 1167, 1053; $[\alpha]_D^{22}$ +4.7 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.19 (3H, d, *J* 6.8 Hz, 1-H_3), 1.42 (9H, s, 3 × CH_3), 1.57–1.67 (2H, m, 8-H_2), 1.74–1.88 (2H, m, 9-H_2), 2.25 (2H, qd, *J* 6.9, 1.5 Hz, 7-H_2), 2.62 (1H, dd, *J* 15.9, 6.6 Hz, 3-*H*H), 2.85 (1H, dd, *J* 15.9, 4.7 Hz, 3-H*H*), 3.54 (2H, t, *J* 6.5 Hz, 10-H_2), 3.94–4.08 (1H, m, 2-H), 4.94 (1H, br s, NH), 6.09 (1H, dt, *J* 15.9, 1.5 Hz, 5-H), 6.83 (1H, dt, *J* 15.9, 6.9 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 20.6 (CH_3), 25.4 (CH_2), 28.5 (3 × CH_3), 31.7 (CH_2), 32.0 (CH_2), 43.8 (CH), 44.7 (CH_2), 46.0 (CH_2), 79.3 (C), 131.0 (CH), 147.2 (CH), 155.3 (C), 199.0 (C); *m/z* (ESI) 326.1484 (MNa^+. $C_{15}H_{26}{}^{35}ClNNaO_3$ requires 326.1493).

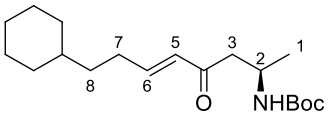
(2*R*,5*E*)-2-(*tert*-Butoxycarbonylamino)-9,9,9-trifluoro-4-oxonona-5-ene (258h)



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 (**258a**) using (4*R*)-4-(*tert*-butoxycarbonylamino)-1-(dimethyloxyphosphoryl)pentan-2-one (**274**) (0.0454 g, 0.147 mmol), 4,4,4-trifluorobutanal (0.031 mL, 0.294 mmol) and potassium carbonate (0.0223 g, 0.161) in acetonitrile (5 mL). Purification with flash chromatography using silica gel, eluting with 30% diethyl ether in petroleum ether (40–60) gave (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-9,9,9-trifluoro-4-oxonona-5-ene (**258h**)

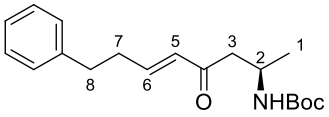
as a clear oil (0.040 g, 88%). ν_{max}/cm^{-1} (neat) 3361 (NH), 2979 (CH), 1696 (C=O), 1509, 1367, 1552, 1138; $[\alpha]_D^{20}$ +7.5 (*c* 0.3, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.19 (3H, d, *J* 6.8 Hz, 1-H_3), 1.42 (9H, s, 3 ×CH_3), 2.18–2.34 (2H, m, 8-H_2), 2.44–2.53 (2H, m, 7-H_2), 2.62 (1H, dd, *J* 15.9, 6.7 Hz, 3-*H*H), 2.85 (1H, dd, *J* 15.9, 4.9 Hz, 3-H*H*), 3.90–4.11 (1H, m, 2-H), 4.85 (1H, s, NH), 6.14 (1H, dt, *J* 15.9, 1.6 Hz, 5-H), 6.49 (1H, dt, *J* 15.9, 6.7 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 20.5 (CH_3), 24.9 (q, $^4J_{CF}$ 3.3 Hz, CH_2), 28.4 (3 × CH_3), 32.4 (q, $^3J_{CF}$ 29.2 Hz, CH_2), 43.7 (CH), 46.4 (CH_2), 79.3 (C), 126.5 (q, $^2J_{CF}$ 277 Hz, C), 131.5 (CH), 143.4 (CH), 155.1 (C), 198.3 (C); *m/z* (ESI) 332.1446 (MNa^+. $C_{14}H_{22}F_3NNaO_3$ requires 332.1449).

(2*R*,5*E*)-2-(*tert*-Butoxycarbonylamino)-8-cyclohexyl-4-oxooct-5-ene (258i)



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 (**258a**) using (4*R*)-4-(*tert*-butoxycarbonylamino)-1-(dimethyloxyphosphoryl)pentan-2-one (**274**) (0.044 g, 0.144 mmol), 3-cyclohexylpropanal (0.040 g, 0.288 mmol) and potassium carbonate (0.0219 g, 0.158 mmol) in acetonitrile (4 mL). Purification by flash column chromatography using silica gel, eluting with 30% diethyl ether in petroleum ether (40–60) gave (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-8-cyclohexyl-4-oxodec-5-ene (**258i**) as a colourless oil (0.037 g, 79%). ν_{max}/cm^{-1} (neat) 3341 (NH), 2924 (CH), 1694 (C=O), 1512, 1366, 1246, 1169, 1053; $[\alpha]_D^{24}$ +5.1 (*c* 0.9, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.80–0.96 (2H, m, CH_2), 1.09–1.28 (8H, m, 1-H_3, 8-CH, 2 × CH_2), 1.34 (2H, q, *J* 6.9 Hz, 8-H_2), 1.43 (9H, s, 3 × CH_3), 1.60–1.78 (4H, m, 2 × CH_2), 2.22 (2H, qd, *J* 6.9, 1.5 Hz, 7-H_2), 2.63 (1H, dd, *J* 15.8, 6.6 Hz, 3-*H*H), 2.85 (1H, dd, *J* 15.8, 4.8 Hz, 3-H*H*), 3.97–4.07 (1H, m, 2-H), 4.94 (1H, br s, NH), 6.08 (1H, dd, *J* 15.9, 1.5 Hz, 5-H), 6.85 (1H, dd, *J* 15.9, 6.9 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 20.7 (CH_3), 26.4 (2 × CH_2), 26.7 (CH_2), 28.6 (3 × CH_3), 30.1 (CH_2), 33.3 (2 × CH_2), 35.8 (CH_2), 37.3 (CH), 43.9 (CH), 45.8 (CH_2), 79.3 (C), 130.6 (CH), 149.0 (CH), 155.3 (C), 199.3 (C); *m/z* (ESI) 346.2343 (MNa^+. $C_{19}H_{33}NNaO_3$ requires 346.2353).

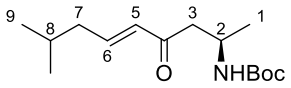
(2*R*,5*E*)-2-(*tert*-Butoxycarbonylamino)-4-oxo-8-phenyloct-5-ene (258j)



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 (**258a**) using (4*R*)-4-(*tert*-butoxycarbonylamino)-1-

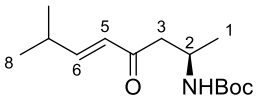
(dimethyloxyphosphoryl)pentan-2-one (**274**) (0.349 g, 1.13 mmol), hydrocinnamaldehyde (0.300 mL, 2.26 mmol) and potassium carbonate (0.172 g, 1.24 mmol). Purification by flash column chromatography using silica gel, eluting with 20% ethyl acetate in petroleum ether (40–60) gave (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxo-8-phenyloct-5-ene (**258j**) (0.288 g, 80%) as a pale yellow oil. 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), 28.5 (3 × CH_3), 34.2 (CH_2), 34.4 (CH_2), 43.7 (CH), 45.9 (CH_2), 79.2 (C), 126.3 (CH), 128.4 (2 × CH), 128.6 (2 × CH), 131.1 (CH), 140.7 (C), 147.0 (CH), 155.2 (C), 199.0 (C); *m/z* (ESI) 340.1868 (MNa^+. $C_{19}H_{27}NNaO_3$ requires 340.1883).

(2*R*,5*E*)-2-(*tert*-Butoxycarbonylamino)-8-methyl-4-oxonon-5-ene (258k)



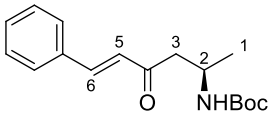
The reaction was carried out according to the above procedure for the synthesis of (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxonona-5-ene (**258a**) using (4*R*)-4-(*tert*-butoxycarbonylamino)-1-(dimethyloxyphosphoryl)pentan-2-one (**274**) (0.141 g, 0.455 mmol), isovaleraldehyde (0.097 mL, 0.910 mmol) and potassium carbonate (0.0692 g, 0.501 mmol) in acetonitrile (5 mL). Purification by flash column chromatography using silica gel, eluting with 40% diethyl ether in petroleum ether (40–60) gave (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-8-methyl-4-oxonon-5-ene (**258k**) (0.080 g, 70%) as a colourless oil. ν_{max}/cm^{-1} (neat) 3356 (NH), 2963 (CH), 1690 (C=O), 1504, 1366, 1165, 1250, 1049; $[\alpha]_D^{29}$ +7.5 (*c* 0.5, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.91 (6H, d, *J* 6.7 Hz, 8-CH_3 and 9-H_3), 1.18 (3H, d, *J* 6.8 Hz, 1-H_3), 1.41 (9H, s, 3 × CH_3), 1.67–1.83 (1H, m, 8-H), 2.09 (2H, td, *J* 7.5, 1.3 Hz, 7-H_2), 2.62 (1H, dd, *J* 15.9, 6.6 Hz, 3-*H*H), 2.84 (1H, dd, *J* 15.9, 4.8 Hz, 3-H*H*), 3.94–4.08 (1H, m, 2-H), 4.95 (1H, br s, NH), 6.06 (1H, dt, *J* 15.7, 1.3 Hz, 5-H), 6.81 (1H, dt, *J* 15.7, 7.5, 6-H); δ_C (101 MHz, $CDCl_3$) 20.6 (CH_3), 22.5 (2 × CH_3), 28.0 (CH), 28.5 (3 × CH_3), 41.9 (CH_2), 43.9 (CH), 45.9 (CH_2), 79.3 (C), 131.8 (CH), 147.3 (CH), 155.3 (C), 199.1 (C); *m/z* (ESI) 292.1873 (MNa^+. $C_{15}H_{27}NNaO_3$ requires 292.1883).

(2*R*,5*E*)-2-(*tert*-Butoxycarbonylamino)-7-methyl-4-oxooct-5-ene (258l)



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 (**258a**) using (4*R*)-4-(*tert*-butoxycarbonylamino)-1-(dimethyloxyphosphoryl)pentan-2-one (**274**) (0.160 g, 0.517 mmol), isobutyraldehyde (0.095 mL, 1.04 mmol) and potassium carbonate (Purification by flash column chromatography using silica gel, eluting with 30% diethyl ether in petroleum ether (40–60) gave (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-7-methyl-4-oxooct-5-ene (**258l**) (0.112 g, 85%) as a colourless oil. ν_{max}/cm^{-1} (neat) 3356 (NH), 2970 (CH), 1690 (C=O), 1512, 1366, 1250, 1165, 1049; $[\alpha]_D^{29}$ +9.4 (*c* 1.2, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.02 (6H, d, *J* 6.8 Hz, 7-CH_3 and 8-H_3), 1.15 (3H, d, *J* 6.8 Hz, 1-H_3), 1.38 (9H, s, 3 × CH_3), 2.35–2.47 (1H, m, 7-H), 2.60 (1H, dd, *J* 16.0, 6.6 Hz, 3-*H*H), 2.81 (1H, dd, *J* 16.0, 4.8 Hz, 3-H*H*), 3.90–4.05 (1H, m, 2-H), 4.97 (1H, br s, NH), 5.99 (1H, dd, *J* 16.0, 1.2 Hz, 5-H), 6.76 (1H, dd, *J* 16.0, 6.7 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 20.6 (CH_3), 21.3 (2 × CH_3), 28.5 (3 × CH_3), 31.2 (CH), 43.7 (CH), 45.8 (CH_2), 79.1 (C), 127.8 (CH), 154.3 (CH), 155.2 (C), 199.4 (C); *m/z* (ESI) 278.1722 (MNa^+. $C_{14}H_{25}NNaO_3$ requires 278.1727).

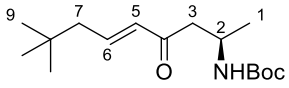
(2*R*,5*E*)-2-(*tert*-Butoxycarbonylamino)-4-oxo-6-phenylhex-5-ene (258m)



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 (**258a**) using (4*R*)-4-(*tert*-butoxycarbonylamino)-1-(dimethyloxyphosphoryl)pentan-2-one (**274**) (0.251 g, 0.810 mmol), benzaldehyde (0.160 mL, 1.62 mmol) and potassium carbonate (0.246 g, 1.78 mmol) in acetonitrile (8 mL). Purification by flash column chromatography using silica gel, eluting with 20% ethyl acetate in petroleum ether (40–60) gave (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxo-6-phenylhex-5-ene (**258m**) (0.227 g, 97%) as a white solid. Mp 59–62 °C; 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

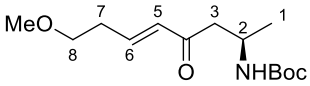
(3 × CH_3), 43.8 (CH), 46.6 (CH_2), 79.2 (C), 126.4 (CH), 128.4 (2 × CH), 129.0 (2 × CH), 130.6 (CH), 134.4 (C), 143.2 (CH), 155.2 (C), 198.9 (C); *m/z* (ESI) 312.1558 (MNa^+. $C_{17}H_{23}NNaO_3$ requires 312.1570).

(2*R*,5*E*)-2-(*tert*-Butoxycarbonylamino)-8,8-dimethyl-4-oxonona-5-ene (258p)



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 (**258a**) using (4*R*)-4-(*tert*-butoxycarbonylamino)-1-(dimethyloxyphosphoryl)pentan-2-one (**274**) (0.100 g, 0.323 mmol), 3,3-dimethylbutanal (0.081 mL, 0.647 mmol) and potassium carbonate (0.0469 g, 0.339 mmol) in acetonitrile (4 mL). Purification with flash chromatography using silica gel, eluting with 20% diethyl ether in petroleum ether (40–60) gave (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-8,8-dimethyl-4-oxonona-5-ene (**258p**) as a clear oil (0.015 g, 16%). ν_{max}/cm^{-1} (neat) 3353 (NH), 2960 (CH), 1695 (C=O), 1514, 1366, 1248, 1172, 1054; $[\alpha]_D^{20}$ +6.8 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.94 (9H, s, 2 × 8-CH_3 and 9-H_3), 1.20 (3H, d, *J* 6.7 Hz, 1-H_3), 1.43 (9H, s, 3 × CH_3), 2.09 (2H, dd, *J* 7.8, 1.3 Hz, 7-H), 2.63 (1H, dd, *J* 15.8, 6.6 Hz, 3-*H*H), 2.86 (1H, dd, *J* 15.8, 4.8 Hz, 3-H*H*), 3.89–4.14 (1H, m, 2-H), 4.93 (1H, s, NH), 6.07 (1H, dt, *J* 15.7, 1.3 Hz, 5-H), 6.88 (1H, dt, *J* 15.7, 7.8 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 20.6 (CH_3), 28.4 (3 × CH_3), 29.4 (3 × CH_3), 31.5 (CH_2), 43.8 (CH), 45.9 (CH_2), 46.7 (C), 79.3 (C), 132.5 (CH), 145.8 (CH), 155.2 (C), 198.8 (C); *m/z* (ESI) 306.2043 (MNa^+. $C_{16}H_{29}NNaO_3$ requires 306.2045).

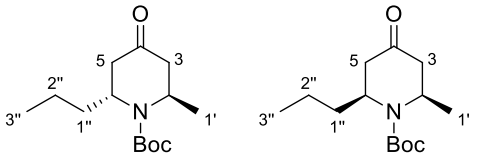
(2*R*,5*E*)-2-(*tert*-Butoxycarbonylamino)-8-methoxy-4-oxoocta-5-ene (258r)



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 (**258a**) using (4*R*)-4-(*tert*-butoxycarbonylamino)-1-(dimethyloxyphosphoryl)pentan-2-one (**274**) (0.0498 g, 0.161 mmol), 3-methoxypropanal (0.032 mL, 0.322 mmol) and potassium carbonate (0.0245 g, 0.177) in acetonitrile (4 mL). Purification with flash chromatography using silica gel, eluting with 30% diethyl ether in petroleum ether (40–60) gave (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-8-methoxy-4-oxoocta-5-ene (**258r**) as a clear oil (0.0093 g, 21%). ν_{max}/cm^{-1} (neat) 3348 (NH), 2976 (CH), 1696 (C=O), 1518, 1366, 1171, 1117; $[\alpha]_D^{20}$ +10.3 (*c* 0.3, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.20 (3H, d, *J* 6.8 Hz, 1-H_3), 1.43 (9H, s, 3 × CH_3), 2.48 (2H,

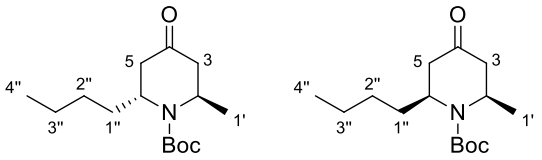
br qd, *J* 6.6, 1.5 Hz, 7-H_2), 2.65 (1H, dd, *J* 16.1, 6.5 Hz, 3-*H*H), 2.85 (1H, dd, *J* 16.1, 4.8 Hz, 3-H*H*), 3.34 (3H, s, OCH_3), 3.50 (2H, t, *J* 6.6 Hz, 8-H_2), 3.85–4.21 (1H, m, 2-H), 4.93 (1H, s, NH), 6.14 (1H, dt, *J* 16.0, 1.5 Hz, 5-H), 6.84 (1H, dt, *J* 16.0, 6.6 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 20.5 (CH_3), 28.4 (3 × CH_3), 32.8 (CH_2), 43.7 (CH), 45.8 (CH_2), 58.7 (CH_3), 70.7 (CH_3), 79.2 (C), 132.0 (CH), 144.5 (CH), 155.1 (C), 198.8 (C); *m/z* (ESI) 294.1674 (MNa^+. $C_{14}H_{25}NNaO_4$ requires 294.1681).

tert-Butyl (2*R*,6*R*)-2-methyl-4-oxo-6-propylpiperidine-1-carboxylate (264a) and _tert_-butyl (2*R*,6*S*)-2-methyl-4-oxo-6-propylpiperidine-1-carboxylate (265a)



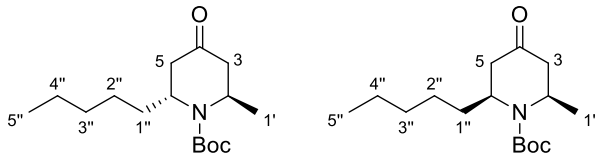
(2*R*,5*E*)-2-(*tert*-Butoxycarbonylamino)-4-oxonona-5-ene (**258a**) (0.0756 g, 0.296 mmol) was dissolved in methanol (7.4 mL) and cooled to −78 °C. 2 M Hydrochloric acid in diethyl ether (0.148 mL, 0.296 mmol) was added and the solution stirred at this temperature for 1 h and, then at room temperature for 1 h. Upon completion, the mixture was concentrated *in vacuo*. Purification by flash column chromatography using silica gel, eluting with 30% diethyl ether in petroleum ether (40–60) gave *tert*-butyl (2*R*,6*R*)-2-methyl-4-oxo-6-propylpiperidine-1-carboxylate (**264a**) (0.0369 g, 49%) and *tert*-butyl (2*R*,6*S*)-2-methyl-4-oxo-6-propylpiperidine-1-carboxylate (**265a**) (0.0253 g, 33%) as colourless oils. Data for (2*R*,6*R*)-2-methyl-4-oxo-6-propylpiperidine-1-carboxylate (**264a**): ν_{max}/cm^{-1} (neat) 2964 (CH), 1726 (C=O), 1688 (C=O), 1389, 1174, 1101; $[\alpha]_D^{22}$ +151.7 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.90 (3H, t, *J* 7.2 Hz, 3''-H_3), 1.20–1.39 (6H, m, 1'-H_3, 1''-*H*H and 2''-H_2), 1.49 (9H, s, 3 × CH_3), 1.65–1.78 (1H, m, 1''-H*H*), 2.35 (1H, dd, *J* 17.7, 2.0 Hz, 3-*H*H), 2.52 (1H, dd, *J* 17.9, 2.0 Hz, 5-*H*H), 2.70 (1H, ddd, *J* 17.9, 6.3, 1.4 Hz, 5-H*H*), 2.81 (1H, dd, *J* 17.7, 6.3 Hz, 3-H*H*), 4.10–4.22 (1H, m, 6-H), 4.29–4.40 (1H, m, 2-H); δ_C (101 MHz, $CDCl_3$) 14.0 (CH_3), 20.1 (CH_2), 22.9 (CH_3), 28.6 (3 × CH_3), 39.4 (CH_2), 41.4 (CH_2), 44.7 (CH_2), 46.6 (CH), 51.0 (CH), 80.0 (C), 154.7 (C), 208.3 (C); *m/z* (ESI) 278.1730 (MNa^+. $C_{14}H_{25}NNaO_3$ requires 278.1727). Data for *tert*-butyl (2*R*,6*S*)-2-methyl-4-oxo-6-propylpiperidine-1-carboxylate (**265a**): ν_{max}/cm^{-1} (neat) 2969 (CH), 1719 (C=O), 1690 (C=O), 1403, 1354, 1174, 1116; $[\alpha]_D^{22}$ −14.2 (*c* 0.5, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 0.91 (3H, t, *J* 7.3 Hz, 3''-H_3), 1.16–1.38 (5H, m, 1'-H_3 and 2''-H_2), 1.40–1.52 (10H, m, 1''-*H*H and 3 × CH_3), 1.54–1.62 (1H, m, 1''-H*H*), 2.23–2.35 (2H, m, 3-*H*H and 5-*H*H), 2.65 (1H, dd, *J* 15.0, 7.5 Hz, 5-H*H*), 2.70 (1H, dd, *J* 15.0, 8.0 Hz, 3-H*H*), 4.52–4.62 (1H, m, 6-H), 4.64–4.76 (1H, m, 2-H); δ_C (126 MHz, $CDCl_3$) 14.0 (CH_3), 20.2 (CH_2), 22.8 (CH_3), 28.6 (3 × CH_3), 39.3 (CH_2), 43.9 (CH_2), 45.6 (CH_2), 48.5 (CH), 52.6 (CH), 80.3 (C), 154.9 (C), 208.9 (C); *m/z* (ESI) 278.1715 (MNa^+. $C_{14}H_{25}NNaO_3$ requires 278.1727).

***tert*-Butyl (2*R*,6*R*)-6-butyl-2-methyl-4-oxopiperidine-1-carboxylate (264b) and *tert*-butyl (2*R*,6*S*)-6-butyl-2-methyl-4-oxopiperidine-1-carboxylate (265b)**



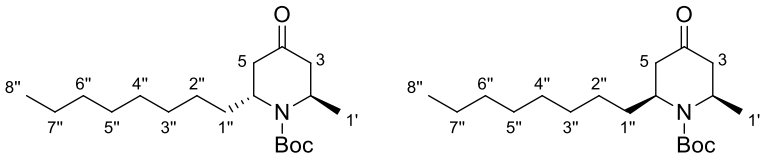
The reaction was carried out according to the above procedure for the synthesis of *tert*-butyl (2*R*,6*R*)-2-methyl-4-oxo-6-propylpiperidine-1-carboxylate (**264a**) using (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxodec-5-ene (**258b**) (0.0616 g, 0.229 mmol). Purification by flash column chromatography using silica gel, eluting with 20% diethyl ether in petroleum ether (40–60) gave *tert*-butyl (2*R*,6*R*)-6-butyl-2-methyl-4-oxopiperidine-1-carboxylate (**264b**) (0.0320 g, 52%) and *tert*-butyl (2*R*,6*S*)-6-butyl-2-methyl-4-oxopiperidine-1-carboxylate (**265b)** (0.0189 g, 31%) as a colourless oils. Data for *tert*-butyl (2*R*,6*R*)-6-butyl-2-methyl-4-oxopiperidine-1-carboxylate (**264b**): ν_{max}/cm^{-1} (neat) 2961 (CH), 1727 (C=O), 1691 (C=O), 1390, 1175; $[\alpha]_D^{20}$ +131.8 (*c* 0.2, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.88 (3H, t, *J* 7.0 Hz, 4''-H_3), 1.17–1.39 (8H, m, 1'-H_3, 1''-*H*H, 2''-H_2 and 3''-H_2), 1.49 (9H, s, 3 × CH_3), 1.68–1.86 (1H, m, 1''-H*H*), 2.35 (1H, dd, *J* 17.7, 1.9 Hz, 3-*H*H), 2.52 (1H, dd, *J* 17.9, 2.1 Hz, 5-*H*H), 2.72 (1H, ddd, *J* 17.9, 6.2, 1.4 Hz, 5-H*H*), 2.81 (1H, dd, *J* 17.7, 6.4 Hz, 3-H*H*), 4.04–4.24 (1H, m, 6-H), 4.30–4.44 (1H, m, 2-H); δ_C (101 MHz, $CDCl_3$) 14.0 (CH_3), 22.5 (CH_2), 22.7 (CH_3), 28.5 (3 × CH_3), 28.9 (CH_2), 36.9 (CH_2), 41.3 (CH_2), 44.6 (CH_2), 46.5 (CH), 51.2 (CH), 79.9 (C), 154.6 (C), 208.0 (C); *m/z* (ESI) 292.1886 (MNa^+. $C_{15}H_{27}NNaO_3$ requires 292.1889). Data for *tert*-butyl (2*R*,6*S*)-6-butyl-2-methyl-4-oxopiperidine-1-carboxylate (**265b**): ν_{max}/cm^{-1} 2929, 1692 (C=O), 1367, 1175; $[\alpha]_D^{20}$ −9.2 (*c* 0.1, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 0.89 (3H, t, *J* 7.1 Hz, 4''-H_3), 1.26 (3H, t, *J* 7.0 Hz, 1'-H_3), 1.29–1.38 (4H, m, 2''-H_2 and 3''-H_2), 1.44–1.53 (10H, m, 3 × CH_3 and 1''-*H*H), 1.55–1.66 (1H, m, 1''-H*H*), 2.24–2.37 (2H, m, 3-*H*H and 5-*H*H), 2.62–2.75 (2H, m, 3-H*H* and 5-H*H*), 4.48–4.64 (1H, m, 6-H), 4.64–4.80 (1H, m, 2-H); δ_C (126 MHz, $CDCl_3$) 14.1 (CH_3), 22.6 (CH_2), 22.8 (CH_3), 28.6 (3 × CH_3), 29.3 (CH_2), 36.8 (CH_2), 44.0 (CH_2), 45.7 (CH_2), 48.5 (CH), 52.9 (CH), 80.3 (C), 155.0 (C), 208.9 (C); *m/z* (ESI) 292.1887 (MNa^+. $C_{15}H_{27}NNaO_3$ requires 292.1889).

***tert*-Butyl (2*R*,6*R*)-2-methyl-4-oxo-6-pentylpiperidine-1-carboxylate (264c) and *tert*-butyl (2*R*,6*S*)-2-methyl-4-oxo-6-pentylpiperidine-1-carboxylate (265c)**



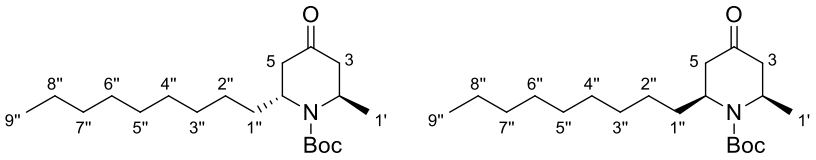
The reaction was carried out according to the above procedure for the synthesis of *tert*-butyl (2*R*,6*R*)-2-methyl-4-oxo-6-propylpiperidine-1-carboxylate (**264a**) using (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxoundec-5-ene (**258c**) (0.0852 g, 0.301 mmol). Purification by flash column chromatography using silica gel, eluting with 20% diethyl ether in petroleum ether (40–60) gave *tert*-butyl (2*R*,6*R*)-2-methyl-4-oxo-6-pentylpiperidine-1-carboxylate (**264c**) (0.0402 g, 47%) and (2*R*,6*S*)-2-methyl-4-oxo-6-pentylpiperidine-1-carboxylate (**265c**) (0.0302 g, 35%) as a colourless oils. Data for *tert*-butyl (2*R*,6*R*)-2-methyl-4-oxo-6-pentylpiperidine-1-carboxylate (**264c**): ν_{max}/cm^{-1} (neat) 2932 (CH), 1728 (C=O), 1690 (C=O), 1366, 1173; $[\alpha]_D^{25}$ +139.9 (*c* 0.4, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 0.86 (3H, t, *J* 6.8 Hz, 5''-H_3), 1.17–1.35 (10H, m, 1'-H_3, 1''-*H*H, 2''-H_2, 3''-H_2 and 4''-H_2), 1.49 (9H, s, 3 × CH_3), 1.67–1.80 (1H, m, 1''-H*H*), 2.36 (1H, dd, *J* 17.7, 1.9 Hz, 3-*H*H), 2.53 (1H, dd, *J* 17.9, 2.0 Hz, 5-*H*H), 2.71 (1H, ddd, *J* 17.9, 6.2, 1.3 Hz, 5-H*H*), 2.80 (1H, dd, *J* 17.7, 6.4 Hz, 3-H*H*), 4.05–4.22 (1H, m, 6-H), 4.25–4.40 (1H, m, 2-H); δ_C (126 MHz, $CDCl_3$) 14.1 (CH_3), 22.7 (CH_2), 22.9 (CH_3), 26.6 (CH_2), 28.6 (3 × CH_3), 31.7 (CH_2), 37.3 (CH_2), 41.4 (CH_2), 44.7 (CH_2), 46.6 (CH), 51.3 (CH), 80.0 (C), 154.7 (C), 208.3 (C); *m/z* (ESI) 306.2034 (MNa^+. $C_{16}H_{29}NNaO_3$ requires 306.2040). Data for (2*R*,6*S*)-2-methyl-4-oxo-6-pentylpiperidine-1-carboxylate (**265c**): ν_{max}/cm^{-1} 2931, 1690 (C=O), 1350, 1173; $[\alpha]_D^{20}$ −20.8 (*c* 0.2, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 0.81 (3H, t, *J* 7.1 Hz, 5''-H_3), 1.15–1.30 (9H, m, 1'-H_3, 2''-H_2, 3''-H_2 and 4''-H_2), 1.38–1.47 (10H, m, 3 × CH_3 and 1''-*H*H), 1.49–1.57 (1H, m, 1''-H*H*), 2.16–2.33 (2H, m, 3-*H*H and 5-*H*H), 2.53–2.72 (2H, m, 3-H*H* and 5-H*H*), 4.43–4.57 (1H, m, 6-H), 4.58–4.74 (1H, m, 2-H); δ_C (126 MHz, $CDCl_3$) 14.1 (CH_3), 22.7 (CH_2), 22.8 (CH_3), 26.8 (CH_2), 28.6 (3 × CH_3), 31.7 (CH_2), 37.1 (CH_2), 44.0 (CH_2), 45.7 (CH_2), 48.5 (CH), 52.9 (CH), 80.3 (C), 155.0 (C), 209.0 (C); *m/z* (ESI) 306.2039 (MNa^+. $C_{16}H_{29}NNaO_3$ requires 306.2040).

***tert*-Butyl (2*R*,6*R*)-2-methyl-6-octyl-4-oxopiperidine-1-carboxylate (264d) and *tert*-butyl (2*R*,6*S*)-2-methyl-6-octyl-4-oxopiperidine-1-carboxylate (265d)**



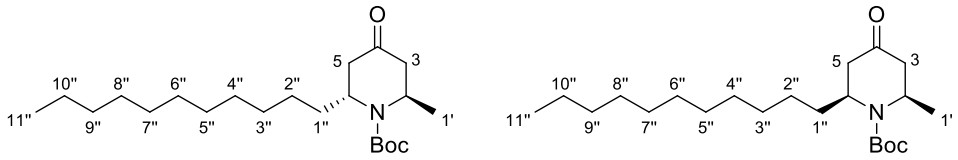
The reaction was carried out according to the above procedure for the synthesis of *tert*-butyl (2*R*,6*R*)-2-methyl-4-oxo-6-propylpiperidine-1-carboxylate (**264a**) using (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxotetraadec-5-ene (**258d**) (0.0977 g, 0.300 mmol). Purification by flash column chromatography using silica gel, eluting with 20% diethyl ether in petroleum ether (40–60) gave *tert*-butyl (2*R*,6*R*)-2-methyl-6-octyl-4-oxopiperidine-1-carboxylate (**264d**) (0.0495 g, 51%) and *tert*-butyl (2*R*,6*S*)-2-methyl-6-octyl-4-oxopiperidine-1-carboxylate (**265d**) (0.0318, 33%) as a colourless oils. Data for *tert*-butyl (2*R*,6*R*)-2-methyl-6-octyl-4-oxopiperidine-1-carboxylate (**264d**): ν_{max}/cm^{-1} (neat) 2924 (CH), 1728 (C=O), 1690 (C=O), 1366, 1172; $[\alpha]_D^{30}$ +158.2 (*c* 0.6, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.87 (3H, t, *J* 6.9 Hz, 8''-H_3), 1.15–1.35 (16H, m, 1'-H_3, 1''-*H*H, 2''-H_2, 3''-H_2, 4''-H_2, 5''-H_2, 6''-H_2, and 7''-H_2), 1.49 (9H, s, 3 × CH_3), 1.65–1.80 (1H, m, 1''-H*H*), 2.36 (1H, dd, *J* 17.8, 1.9 Hz, 3-*H*H), 2.53 (1H, dd, *J* 17.9, 2.0 Hz, 5-*H*H), 2.72 (1H, ddd, *J* 17.9, 6.2, 1.3 Hz, 5-H*H*), 2.81 (1H, dd, *J* 17.8, 6.4 Hz, 3-H*H*), 4.05–4.20 (1H, m, 6-H), 4.28–4.45 (1H, m, 2-H); δ_C (101 MHz, $CDCl_3$) 14.2 (CH_3), 22.8 (CH_2), 22.9 (CH_3), 26.9 (CH_2), 28.6 (3 × CH_3), 29.3 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 32.0 (CH_2), 37.3 (CH_2), 41.4 (CH_2), 44.7 (CH_2), 46.6 (CH), 51.3 (CH), 80.0 (C), 154.7 (C), 208.3 (C); *m/z* (ESI) 348.2515 (MNa^+. $C_{19}H_{35}NNaO_3$ requires 348.2509). Data for *tert*-butyl (2*R*,6*S*)-2-methyl-6-octyl-4-oxopiperidine-1-carboxylate (**265d**): ν_{max}/cm^{-1} 2924, 1690 (C=O), 1350, 1173; $[\alpha]_D^{20}$ −15.4 (*c* 0.2, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 0.87 (3H, t, *J* 7.0 Hz, 8''-H_3), 1.15–1.39 (15H, m, 1'-H_3, 2''-H_2, 3''-H_2, 4''-H_2, 5''-H_2, 6''-H_2 and 7''-H_2), 1.48–1.54 (10H, m, 3 × CH_3 and 1''-*H*H), 1.54–1.64 (1H, m, 1''-H*H*), 2.24–2.37 (2H, m, 3-*H*H and 5-*H*H), 2.62–2.76 (2H, m, 3-H*H* and 5-H*H*), 4.49–4.64 (1H, m, 6-H), 4.64–4.80 (1H, m, 2-H); δ_C (126 MHz, $CDCl_3$) 14.2 (CH_3), 22.8 (CH_2), 22.9 (CH_3), 27.1 (CH_2), 28.6 (3 × CH_3), 29.3 (CH_2), 29.5 (CH_2), 29.7 (CH_2), 32.0 (CH_2), 37.2 (CH_2), 44.0 (CH_2), 45.7 (CH_2), 48.5 (CH), 52.9 (CH), 80.3 (C), 154.9 (C), 209.0 (C); *m/z* (ESI) 348.2494 (MNa^+. $C_{19}H_{35}NNaO_3$ requires 348.2509).

***tert*-Butyl (2*R*,6*R*)-2-methyl-6-nonyl-4-oxopiperidine-1-carboxylate (264e) and *tert*-butyl (2*R*,6*S*)-2-methyl-6-nonyl-4-oxopiperidine-1-carboxylate (265e)**[217]



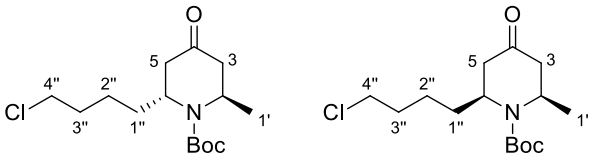
The reaction was carried out according to the above procedure for the synthesis of *tert*-butyl (2*R*,6*R*)-2-methyl-4-oxo-6-propylpiperidine-1-carboxylate (**264a**) using (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxopentadec-5-ene (**258e**) (0.0996 g, 0.148 mmol). Purification by flash column chromatography using silica gel, eluting with 20% diethyl ether in petroleum ether (40–60) gave *tert*-butyl (2*R*,6*R*)-2-methyl-6-nonyl-4-oxopiperidine-1-carboxylate (**264e**) (0.0444 g, 55%) and *tert*-butyl (2*R*,6*S*)-2-methyl-6-nonyl-4-oxopiperidine-1-carboxylate (**265e**) (0.329 g, 33%) as a colourless oils. Data for *tert*-butyl (2*R*,6*R*)-2-methyl-6-nonyl-4-oxopiperidine-1-carboxylate (**264e**): ν_{max}/cm^{-1} (neat) 2925 (CH), 1726 (C=O), 1691 (C=O), 1389, 1175, 1094; $[\alpha]_D^{23}$ +102.4 (*c* 1.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.87 (3H, t, *J* 6.8 Hz, 9''-H_3), 1.16–1.36 (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), 1.49 (9H, s, 3 × CH_3), 1.68–1.80 (1H, m, 1''-H*H*), 2.35 (1H, dd, *J* 17.7, 1.8 Hz, 3-*H*H), 2.52 (1H, dd, *J* 18.0, 1.8 Hz, 5-*H*H), 2.71 (1H, ddd, *J* 18.0, 5.5, 0.8 Hz, 5-H*H*), 2.80 (1H, dd, *J* 17.7, 6.4 Hz, 3-H*H*), 4.08–4.19 (1H, m, 6-H), 4.29–4.40 (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), 154.5 (C), 208.2 (C); *m/z* (ESI) 362.2662 (MNa^+. $C_{20}H_{37}NNaO_3$ requires 362.2666). Data for *tert*-butyl (2*R*,6*S*)-2-methyl-6-nonyl-4-oxopiperidine-1-carboxylate (**265e**): Spectroscopic data was consistent with the literature.[217] ν_{max}/cm^{-1} (neat) 2926, 1690 (C=O), 1353, 1173; $[\alpha]_D^{23}$ −13.4 (*c* 0.9, $CHCl_3$), lit.[217] $[\alpha]_D$ −21.5 (*c* 1.1, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 0.87 (3H, t, *J* 7.0 Hz, 9''-H_3), 1.17–1.40 (17H, m, 1'-H_3, 2''-H_2, 3''-H_2, 4''-H_2, 5''-H_2, 6''-H_2, 7''-H_2 and 8''-H_2), 1.44–1.55 (10H, 3 × CH_3 and 1''-*H*H), 1.55–1.65 (1H, m, 1''-H*H*), 2.24–2.36 (2H, m, 3-*H*H and 5-*H*H), 2.61–2.76 (2H, m, 3-H*H* and 5-H*H*), 4.53–4.62 (1H, m, 6-H), 4.66–4.76 (1H, m, 2-H); δ_C (126 MHz, $CHCl_3$) 14.2 (CH_3), 22.8 (CH_2), 22.8 (CH_3), 27.1 (CH_2), 28.6 (3 × CH_3), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 32.0 (CH_2), 37.2 (CH_2), 44.0 (CH_2), 45.7 (CH_2), 48.5 (CH), 52.9 (CH), 80.3 (C), 154.9 (C), 209.0 (C); *m/z* (ESI) 362 (MNa^+, 100%).

***tert*-Butyl (2*R*,6*R*)-2-methyl-4-oxo-6-undecylpiperidine-1-carboxylate (264f) and *tert*-butyl (2*R*,6*S*)-2-methyl-4-oxo-6-undecylpiperidine-1-carboxylate (265f)**[217]



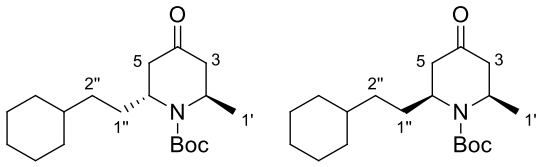
The reaction was carried out according to the above procedure for the synthesis of *tert*-butyl (2*R*,6*R*)-2-methyl-4-oxo-6-propylpiperidine-1-carboxylate (**264a**) using (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxoheptadec-5-ene (**258f**) (0.113 g, 0.307 mmol). Purification by flash column chromatography using silica gel, eluting with 20% diethyl ether in petroleum ether (40–60) gave *tert*-butyl (2*R*,6*R*)-2-methyl-4-oxo-6-undecylpiperidine-1-carboxylate (**264f**) (0.0551 g, 49%) and *tert*-butyl (2*R*,6*S*)-2-methyl-4-oxo-6-undecylpiperidine-1-carboxylate (**265f**) (0.0270 g, 24%) as a colourless oils. Data for *tert*-butyl (2*R*,6*R*)-2-methyl-4-oxo-6-undecylpiperidine-1-carboxylate (**264f**): ν_{max}/cm^{-1} (neat) 2924 (CH), 1726 (C=O), 1691 (C=O), 1389, 1174, 1095; $[\alpha]_D^{24}$ +92.7 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.88 (3H, t, *J* 6.9 Hz, 11''-H_3), 1.18–1.38 (22H, 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, 8''-H_2, 9''-H_2 and 10''-H_2), 1.49 (9H, s, 3 × CH_3), 1.66–1.80 (1H, m, 1''-H*H*), 2.36 (1H, dd, *J* 17.7, 1.9 Hz, 3-*H*H), 2.53 (1H, dd, *J* 17.9, 2.0 Hz, 5-*H*H), 2.71 (1H, ddd, *J* 17.9, 6.2, 1.3 Hz, 5-H*H*), 2.81 (1H, dd, *J* 17.7, 6.5 Hz, 3-H*H*), 4.07–4.23 (1H, m, 6-H), 4.27–4.43 (1H, m, 2-H); δ_C (101 MHz, $CDCl_3$) 14.3 (CH_3), 22.8 (CH_2), 22.9 (CH_3), 26.9 (CH_2), 28.7 (3 × CH_3), 29.5 (CH_2), 29.6 (CH_2), 29.7 (3 × CH_2), 29.8 (CH_2) 32.1 (CH_2), 37.3 (CH_2), 41.4 (CH_2), 44.7 (CH_2), 46.6 (CH), 51.3 (CH), 80.0 (C), 154.7 (C), 208.3 (C); *m/z* (ESI) 390.2963 (MNa^+. $C_{22}H_{41}NNaO_3$ requires 390.2979). Data for *tert*-butyl (2*R*,6*S*)-2-methyl-4-oxo-6-undecylpiperidine-1-carboxylate (**265f**): Spectroscopic data was consistent with the literature.[217] ν_{max}/cm^{-1} (neat) 2924, 2854, 1691 (C=O), 1353, 1172; $[\alpha]_D^{24}$ −11.0 (*c* 0.9, $CHCl_3$), lit.[217] $[\alpha]_D$ −19.7 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.88 (3H, t, *J* 6.9 Hz, 11''-H_3), 1.18–1.40 (21H, m, 1'-H_3, 2''-H_2, 3''-H_2, 4''-H_2, 5''-H_2, 6''-H_2, 7''-H_2, 8''-H_2, 9''-H_2 and 10''-H_2), 1.45–1.55 (10H, 3 × CH_3 and 1''-*H*H), 1.55–1.65 (1H, m, 1''-H*H*), 2.24–2.37 (2H, m, 3-*H*H and 5-*H*H), 2.62–2.77 (2H, m, 3-H*H* and 5-H*H*), 4.51–4.64 (1H, m, 6-H), 4.65–4.80 (1H, m, 2-H); δ_C (101 MHz, $CHCl_3$) 14.2 (CH_3), 22.8 (CH_3), 22.8 (CH_2), 27.1 (CH_2), 28.6 (3 × CH_3), 29.5 (2 × CH_2), 29.7 (4 × CH_2), 32.0 (CH_2), 37.2 (CH_2), 44.0 (CH_2), 45.7 (CH_2), 48.5 (CH), 52.9 (CH), 80.3 (C), 154.9 (C), 208.9 (C); *m/z* (ESI) 390 (MNa^+, 100%).

***tert*-Butyl (2*R*,6*R*)-6-(4''-chlorobutyl)-2-methyl-4-oxopiperidine-1-carboxylate (264g) and *tert*-butyl (2*R*,6*S*)-6-(4''-chlorobutyl)-2-methyl-4-oxopiperidine-1-carboxylate (265g)**



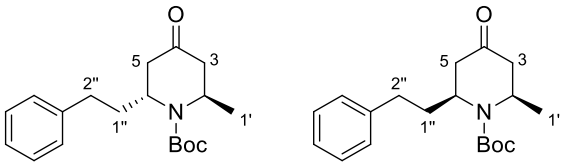
The reaction was carried out according to the above procedure for the synthesis of *tert*-butyl (2*R*,6*R*)-2-methyl-4-oxo-6-propylpiperidine-1-carboxylate (**264a**) using (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-10-chloro-4-oxodec-5-ene (**258g**) (0.0484 g, 0.0159 mmol). Purification by flash column chromatography using silica gel, eluting with 20% diethyl ether in petroleum ether (40–60) gave *tert*-butyl (2*R*,6*R*)-6-(4''-chlorobutyl)-2-methyl-4-oxopiperidine-1-carboxylate (**264g**) (0.0269 g, 56%) as a white solid and *tert*-butyl (2*R*,6*S*)-6-(4''-chlorobutyl)-2-methyl-4-oxopiperidine-1-carboxylate (**265g**) (0.0160 g, 33%) as a clear oil. Data for *tert*-butyl (2*R*,6*R*)-6-(4''-chlorobutyl)-2-methyl-4-oxopiperidine-1-carboxylate (**264g**): Mp 58–59 °C; ν_{max}/cm^{-1} (neat) 2975 (CH), 1725 (C=O), 1688 (C=O), 1390, 1366, 1174, 1097; $[\alpha]_D^{22}$ +120.1 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.25 (3H, d, *J* 6.6 Hz, 1'-H_3), 1.30–1.47 (3H, m, 1-*H*H and 2''-H_2), 1.49 (9H, s, 3 × CH_3), 1.69–1.84 (3H, m, 3''-H_2 and 1''-H*H*), 2.37 (1H, dd, *J* 17.8, 1.9 Hz, 3-*H*H), 2.52 (1H, dd, *J* 18.0, 2.0 Hz, 5-*H*H), 2.73 (1H, ddd, *J* 18.0, 6.0, 0.8 Hz, 5-H*H*), 2.78 (1H, dd, *J* 17.8, 6.4 Hz, 3-H*H*), 3.51 (2H, t, *J* 6.5 Hz, 4''-H_2), 4.09–4.24 (1H, m, 6-H), 4.26–4.43 (1H, m, 2-H); δ_C (101 MHz, $CDCl_3$) 22.8 (CH_3), 24.1 (CH_2), 28.6 (3 × CH_3), 32.3 (CH_2), 36.5 (CH_2), 41.5 (CH_2), 44.7 (CH_2), 44.8 (CH_2), 46.7 (CH), 51.0 (CH), 80.2 (C), 154.7 (C), 207.9 (C); *m/z* (ESI) 326.1480 (MNa^+. $C_{15}H_{26}{}^{35}ClNNaO_3$ requires 326.1493). Data for *tert*-butyl (2*R*,6*S*)-6-(4''-chlorobutyl)-2-methyl-4-oxopiperidine-1-carboxylate (**265g**): ν_{max}/cm^{-1} (neat) 2970 (CH), 1720 (C=O), 1682 (C=O), 1350, 1165; $[\alpha]_D^{24}$ −10.2 (*c* 1.2, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.26 (3H, d, *J* 7.0 Hz, 1'-H_3), 1.41–1.57 (12H, m, 3 × CH_3, 1''-*H*H and 2''-H_2), 1.60–1.69 (1H, m, 1''-H*H*), 1.73–1.86 (2H, m, 3''-H_2), 2.23–2.35 (2H, m, 3-*H*H and 5-*H*H), 2.64–2.76 (2H, m, 3-H*H* and 5-H*H*), 3.52 (2 H, t, *J* 6.5 Hz, 4''-H_2), 4.53–4.66 (1H, m, 6-H), 4.67–4.80 (1H, m, 2-H); δ_C (101 MHz, $CDCl_3$) 22.7 (CH_3), 24.2 (CH_2), 28.4 (3 × CH_3), 32.1 (CH_2), 36.2 (CH_2), 43.8 (CH_2), 44.8 (CH_2), 45.5 (CH_2), 48.6 (CH), 52.5 (CH), 80.4 (C), 154.8 (C), 208.5 (C); *m/z* (ESI) 326.1480 (MNa^+. $C_{15}H_{26}{}^{35}ClNNaO_3$ requires 326.1493).

***tert*-Butyl (2*R*,6*R*)-6-(2''-cyclohexylethyl)-2-methyl-4-oxopiperidine-1-carboxylate (264i) and *tert*-butyl (2*R*,6*S*)-6-(2''-cyclohexylethyl)-2-methyl-4-oxopiperidine-1-carboxylate (265i)**



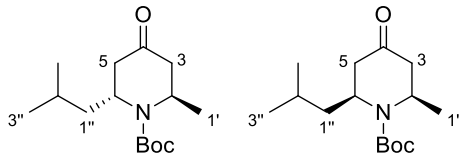
The reaction was carried out according to the above procedure for the synthesis of *tert*-butyl (2*R*,6*R*)-2-methyl-4-oxo-6-propylpiperidine-1-carboxylate (**264a**) using (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-8-cyclohexyl-4-oxodec-5-ene (**258i**) (0.0269 g, 0.0830 mmol). Purification by flash column chromatography using silica gel, eluting with 20% diethyl ether in petroleum ether (40–60) gave *tert*-butyl (2*R*,6*R*)-6-(2''-cyclohexylethyl)-2-methyl-4-oxopiperidine-1-carboxylate (**264i**) (0.0149 g, 55%) and *tert*-butyl (2*R*,6*S*)-6-(2''-cyclohexylethyl)-2-methyl-4-oxopiperidine-1-carboxylate (**265i**) (0.0108 g, 40%) as a colourless oils. Data for *tert*-butyl (2*R*,6*R*)-6-(2''-cyclohexylethyl)-2-methyl-4-oxopiperidine-1-carboxylate (**264i**): ν_{max}/cm^{-1} (neat) 2924 (CH), 1721 (C=O), 1690 (C=O), 1389, 1366, 1173, 1096; $[\alpha]_D^{25}$ +105.9 (*c* 0.4, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.75–0.95 (2H, m, CH_2), 1.05–1.38 (11H, m, 1'-H_3, 1''-*H*H, 2''-H_2, 3''-H, and 2 × CH_2), 1.49 (9H, s, 3 × CH_3), 1.60–1.84 (5H, m, 1''-H*H* and 2 × CH_2), 2.36 (1H, dd, *J* 17.8, 1.9 Hz, 3-*H*H), 2.52 (1H, dd, *J* 17.9, 2.0 Hz, 5-*H*H), 2.71 (1H, ddd, *J* 17.9, 6.2, 1.2 Hz, 5-H*H*), 2.80 (1H, dd, *J* 17.8, 6.4 Hz, 3-H*H*), 4.03–4.17 (1H, m, 6-H), 4.28–4.44 (1H, m, 2-H); δ_C (101 MHz, $CDCl_3$) 22.8 (CH_3), 26.4 (2 × CH_2), 26.7 (CH_2), 28.7 (3 × CH_3), 33.4 (CH_2), 33.6 (CH_2), 34.6 (CH_2), 34.7 (CH_2), 37.7 (CH), 41.4 (CH_2), 44.7 (CH_2), 46.6 (CH), 51.7 (CH), 80.0 (C), 154.7 (C), 208.3 (C); *m/z* (ESI) 346.2345 (MNa^+. $C_{19}H_{33}NNaO_3$ requires 346.2353). Data for *tert*-butyl (2*R*,6*S*)-6-(2''-cyclohexylethyl)-2-methyl-4-oxopiperidine-1-carboxylate (**265i**): ν_{max}/cm^{-1} (neat) 2924, 1689 (C=O), 1358, 1173; $[\alpha]_D^{25}$ −12.3 (*c* 0.3, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.78–0.95 (2H, m, CH_2), 1.04–1.35 (10H, m, 1'-CH_3, 2''-H_2, 3''-H and 2 × CH_2), 1.40–1.48 (1H, m, 1''-*H*H), 1.48 (9H, s, 3 × CH_3), 1.60–1.74 (5H, m, 1''-H*H* and 2 × CH_2), 2.20–2.36 (2H, m, 3-*H*H and 5-*H*H), 2.60–2.77 (2H, m, 3-H*H* and 5-H*H*), 3.52 (2 H, t, *J* 6.5 Hz, 4''-H_2), 4.46–4.62 (1H, m, 6-H), 4.63–4.78 (1H, m, 2-H); δ_C (101 MHz, $CDCl_3$) 22.7 (CH_3), 26.1 (CH_2), 26.6 (CH_2), 26.7 (CH_2), 28.4 (3 × CH_3), 33.3 (CH_2), 33.4 (CH_2), 34.3 (CH_2), 34.7 (CH_2), 37.5 (CH), 43.9 (CH_2), 45.6 (CH_2), 48.4 (CH), 53.1 (CH), 80.0 (C), 154.7 (C), 208.8 (C); *m/z* (ESI) 346.2341 (MNa^+. $C_{19}H_{33}NNaO_3$ requires 346.2353).

***tert*-Butyl (2*R*,6*R*)-2-methyl-4-oxo-6-(2''-phenylethyl)piperidine-1-carboxylate (264j) and *tert*-butyl (2*R*,6*S*)-2-methyl-4-oxo-6-(2''-phenylethyl)piperidine-1-carboxylate (265j)**



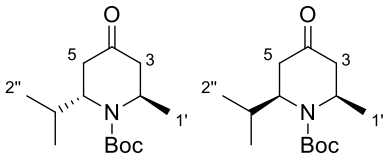
The reaction was carried out according to the above procedure for the synthesis of *tert*-butyl (2*R*,6*R*)-2-methyl-4-oxo-6-propylpiperidine-1-carboxylate (**264a**) using (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-4-oxo-8-phenyloct-5-ene (**258j**) (0.090 g, 0.284 mmol). Purification by flash column chromatography using silica gel, eluting with 20% ethyl acetate in petroleum ether (40–60) gave *tert*-butyl (2*R*,6*R*)-2-methyl-4-oxo-6-(2''-phenylethyl)piperidine-1-carboxylate (**264j**) (0.041 g, 46%) as a white solid and *tert*-butyl (2*R*,6*S*)-2-methyl-4-oxo-6-(2''-phenylethyl)piperidine-1-carboxylate (**265j**) (0.0276 g, 31%) as a clear oil. Data for *tert*-butyl (2*R*,6*R*)-2-methyl-4-oxo-6-(2''-phenylethyl)piperidine-1-carboxylate (**264j**): Mp 76–78 °C; ν_{max}/cm^{-1} (neat) 2973 (CH), 1724 (C=O), 1687 (C=O), 1389, 1366, 1173, 1096; $[\alpha]_D^{18}$ +97.7 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.27 (3H, d, *J* 6.7 Hz, 1'-H_3), 1.49 (9H, s, 3 × CH_3), 1.58–1.70 (1H, m, 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 and 2''-H_2), 2.77 (1H, dd, *J* 17.5, 5.6 Hz, 5-H*H*), 2.82 (1H, dd, *J* 17.8, 6.8 Hz, 3-H*H*), 4.20–4.30 (1H, m, 6-H), 4.31–4.43 (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) 340.1875 (MNa^+. $C_{19}H_{27}NNaO_3$ requires 340.1883). Data for *tert*-butyl (2*R*,6*S*)-2-methyl-4-oxo-6-(2''-phenylethyl)piperidine-1-carboxylate (**265j**): ν_{max}/cm^{-1} (neat) 2974 (CH), 1719 (C=O), 1687 (C=O), 1353, 1169; $[\alpha]_D^{19}$ +6.1 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.31 (3H, d, *J* 6.9 Hz, 1'-H_3), 1.48 (9H, s, 3 × CH_3), 1.74–2.02 (2H, m, 1''-H_2), 2.25–2.42 (2H, m, 3-*H*H and 5-*H*H), 2.54–2.78 (4H, m, 3'-H*H* 5-H*H* and 2''-H_2), 4.61–4.83 (2H, m, 2-H and 6-H), 7.13–7.33 (5H, m, Ph); δ_C (101 MHz, $CDCl_3$) 22.8 (CH_3), 28.4 (3 × CH_3), 33.4 (CH_2), 39.0 (CH_2), 43.7 (CH_2), 45.5 (CH_2), 48.4 (CH), 52.6 (CH), 80.4 (C), 126.1 (CH), 128.3 (2 × CH), 128.5 (2 × CH), 141.2 (C), 154.8 (C), 208.3(C); *m/z* (ESI) 340.1878 (MNa^+. $C_{19}H_{27}NNaO_3$ requires 340.1883).

***tert*-Butyl (2*R*,6*R*)-2-methyl-6-(2''-methylpropyl)-4-oxopiperidine-1-carboxylate (264k) and *tert*-butyl (2*R*,6*S*)-2-methyl-6-(2''-methylpropyl)-4-oxopiperidine-1-carboxylate (265k)**



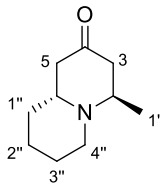
The reaction was carried out according to the above procedure for the synthesis of *tert*-butyl (2*R*,6*R*)-2-methyl-4-oxo-6-propylpiperidine-1-carboxylate (**264a**) using (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-8-methyl-4-oxonon-5-ene (**258k**) (0.0804 g, 0.298 mmol). Purification by flash column chromatography using silica gel, eluting with 20% diethyl ether in petroleum ether (40–60) gave *tert*-butyl (2*R*,6*R*)-2-methyl-6-(2''-methylpropyl)-4-oxopiperidine-1-carboxylate (**264k**) (0.032 g, 40%) and *tert*-butyl (2*R*,6*S*)-2-methyl-6-(2''-methylpropyl)-4-oxopiperidine-1-carboxylate (**265k**) (0.032 g, 40%) as colourless oils. Data for *tert*-butyl (2*R*,6*R*)-2-methyl-6-(2''-methylpropyl)-4-oxopiperidine-1-carboxylate (**264k**): ν_{max}/cm^{-1} (neat) 2960 (CH), 1726 (C=O), 1688 (C=O), 1389, 1369, 1175; $[\alpha]_D^{28}$ +34.4 (*c* 0.2, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 0.89 (3H, d, *J* 6.3 Hz, 3''-H_3), 0.92 (3H, d, *J* 6.3 Hz, 2''-CH_3), 1.26 (3H, d, *J* 6.7 Hz, 1'-H_3), 1.27–1.33 (1H, m, 2''-H), 1.50 (9H, s, 3 × CH_3), 1.52–1.58 (2H, m, 1''-H_2), 2.36 (1H, dd, *J* 17.8, 2.0 Hz, 3-*H*H), 2.52 (1H, dd, *J* 17.9, 2.0 Hz, 5-*H*H), 2.72 (1H, ddd, *J* 17.9, 6.1, 1.0 Hz, 5-H*H*), 2.82 (1H, dd, *J* 17.8, 6.5 Hz, 3-H*H*), 4.22–4.30 (1H, m, 6-H), 4.31–4.39 (1H, m, 2-H); δ_C (126 MHz, $CDCl_3$) 21.4 (CH_3), 22.9 (CH), 23.9 (CH_3), 25.5 (CH_3), 28.7 (3 × CH_3), 41.5 (CH_2), 44.7 (CH_2), 45.8 (CH_2), 46.7 (CH), 49.4 (CH), 80.1 (C), 154.6 (C), 208.2 (C); *m/z* (ESI) 292.1877 (MNa^+. $C_{15}H_{27}NNaO_3$ requires 292.1883). Data for *tert*-butyl (2*R*,6*S*)-2-methyl-6-(2''-methylpropyl)-4-oxopiperidine-1-carboxylate (**265k**): ν_{max}/cm^{-1} (neat) 2961 (CH), 1721 (C=O), 1686 (C=O), 1352, 1171, 1125; $[\alpha]_D^{28}$ −4.0 (*c* 0.3, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 0.91 (3H, d, *J* 6.5 Hz, 3''-H_3), 0.94 (3H, d, *J* 6.5 Hz, 2''-CH_3), 1.26 (3H, d, *J* 7.0 Hz, 1'-H_3), 1.33–1.44 (2H, m, 1''-H_2), 1.48 (9H, s, 3 × CH_3), 1.51–1.59 (1H, m, 2''-H), 2.25–2.33 (2H, m, 3-*H*H and 5-*H*H), 2.59–2.73 (2H, m, 3-H*H* and 5-H*H*), 4.58–4.82 (2H, m, 2-H and 6-H); δ_C (126 MHz, $CDCl_3$) 22.2 (CH_3), 23.0 (CH), 23.2 (CH_3), 25.4 (CH_3), 28.6 (3 × CH_3), 44.1 (CH_2), 45.6 (CH_2), 46.0 (CH_2), 48.4 (CH), 50.8 (CH), 80.4 (C), 154.8 (C), 208.8 (C); *m/z* (ESI) 292.1877 (MNa^+. $C_{15}H_{27}NNaO_3$ requires 292.1883).

***tert*-Butyl (2*R*,6*R*)-2-methyl-6-(1''-methylethyl)-4-oxopiperidine-1-carboxylate (264l) and *tert*-butyl (2*R*,6*S*)-2-methyl-6-(1''-methylethyl)-4-oxopiperidine-1-carboxylate (265l)**



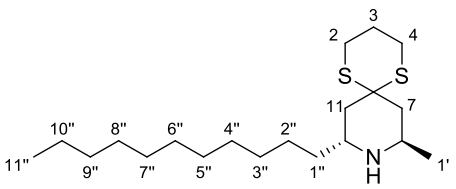
The reaction was carried out according to the above procedure for the synthesis of *tert*-butyl (2*R*,6*R*)-2-methyl-4-oxo-6-propylpiperidine-1-carboxylate (**264a**) using (2*R*,5*E*)-2-(*tert*-butoxycarbonylamino)-7-methyl-4-oxooct-5-ene (**258l**) (0.103 g, 0.403 mmol). Purification by flash column chromatography using silica gel, eluting with 20% diethyl ether in petroleum ether (40–60) gave *tert*-butyl (2*R*,6*R*)-2-methyl-6-(1''-methylethyl)-4-oxopiperidine-1-carboxylate (**264l**) (0.027 g, 27%) and *tert*-butyl (2*R*,6*S*)-2-methyl-6-(1''-methylethyl)-4-oxopiperidine-1-carboxylate (**265l**) (0.027 g, 27%) as colourless oils. Data for *tert*-butyl (2*R*,6*R*)-2-methyl-6-(1''-methylethyl)-4-oxopiperidine-1-carboxylate (**264l**): ν_{max}/cm^{-1} (neat) 2968 (CH), 1724 (C=O), 1688 (C=O), 1381, 1366, 1354, 1175; $[\alpha]_D^{27}$ +171.1 (*c* 0.2, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 0.92 (3H, d, *J* 6.5 Hz, 2''-H_3), 0.97 (3H, d, *J* 6.5 Hz, 1''-CH_3), 1.26 (3H, d, *J* 6.5 Hz, 1'-H_3), 1.49 (9H, s, 3 × CH_3), 1.60–1.72 (1H, m, 1''-H), 2.33 (2H, dd, *J* 17.5, 2.2 Hz, 3-*H*H), 2.59 (1H, dd, *J* 18.0, 2.6 Hz, 5-*H*H), 2.66 (1H, dd, *J* 18.0, 5.8 Hz, 5-H*H*), 2.79 (1H, dd, *J* 17.5, 6.5 Hz, 3-H*H*), 3.95–4.10 (1H, m, 6-H), 4.31 (1H, quin.d, *J* 6.5, 2.2 Hz, 2-H); δ_C (126 MHz, $CDCl_3$) 19.3 (CH_3), 20.4 (CH_3), 22.8 (CH_3), 28.6 (3 × CH_3), 35.1 (CH), 41.5 (CH_2), 44.8 (CH_2), 47.0 (CH), 56.3 (CH), 80.1 (C), 155.5 (C), 208.6 (C); *m/z* (ESI) 278.1722 (MNa^+. $C_{14}H_{25}NNaO_3$ requires 278.1727). Data for *tert*-butyl (2*R*,6*S*)-2-methyl-6-(1''-methylethyl)-4-oxopiperidine-1-carboxylate (**265l**): ν_{max}/cm^{-1} (neat) 2972 (CH), 1718 (C=O), 1687 (C=O), 1365, 1172, 1120, 1066; $[\alpha]_D^{23}$ −47.6 (*c* 0.5, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 0.93 (3H, d, *J* 6.5 Hz, 2''-H_3), 0.97 (3H, d, *J* 6.5 Hz, 1''-CH_3), 1.30 (3H, d, *J* 6.8 Hz, 1'-H_3), 1.48 (9H, s, 3 × CH_3), 1.76–1.84 (1H, m, 1''-H), 2.29 (1H, ddd, *J* 15.5, 5.4, 0.9 Hz, 3-*H*H), 2.53–2.57 (2H, m, 5-H_2), 2.67 (1H, dd, *J* 15.5, 7.7 Hz, 3-H*H*), 4.15–4.35 (1H, m, 6-H), 4.52–4.75 (1H, m, 2-H); δ_C (126 MHz, $CDCl_3$) 20.2 (CH_3), 20.4 (CH_3), 22.6 (CH_3), 28.4 (3 × CH_3), 33.0 (CH), 42.0 (CH_2), 45.5 (CH_2), 48.1 (CH), 58.5 (CH), 80.2 (C), 155.3 (C), 208.8 (C); *m/z* (ESI) 278.1720 (MNa^+. $C_{14}H_{25}NNaO_3$ requires 278.1727).

(+)-Myrtine (244)[198]



To a solution of *tert*-butyl (2*R*,6*R*)-6-(4''-chlorobutyl)-2-methyl-4-oxopiperidine-1-carboxylate (**264g**) (0.049 g, 0.161 mmol) in tetrahydrofuran (2 mL) was added 2 M hydrochloric acid solution. The reaction mixture was stirred at room temperature for 4 h. The reaction mixture was concentrated *in vacuo* to afford a yellow residue. This was dissolved in acetone (5 mL) and saturated sodium hydrogen carbonate solution (3 mL) and water (2 mL) were added. The reaction mixture was stirred at room temperature for 48 h. The reaction mixture was poured onto saturated sodium hydrogen carbonate solution (50 mL), which was extracted with dichloromethane (3 × 50 mL). The combined organic layers were combined and washed with brine (50 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product as a yellow oil. Purification by flash column chromatography using silica gel, eluting with 4% methanol in dichloromethane gave (+)-myrtine (**244**) as a pale yellow oil (0.017 g, 62%). $[\alpha]_D^{24}$ +8.9 (*c* 0.3, $CHCl_3$), lit.[198] $[\alpha]_D^{22}$ +10.5 (*c* 0.9, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 0.95 (3H, d, *J* 6.8 Hz, 1'-H_3), 1.15–1.35 (2H, m, 2''-*H*H and 3''-*H*H), 1.52–1.76 (4H, m, 1''-H_2, 2''-H*H* and 3''-H*H*), 2.14–2.28 (3H, m, 3-*H*H and 4''-H_2), 2.46 (1H, td, *J* 11.6, 2.8 Hz, 5-*H*H), 2.60–2.69 (1H, m, 6-H), 2.74–2.80 (1H, m, 5-H*H*), 2.83 (1H, dd, *J* 13.4, 6.8 Hz, 3-H*H*), 3.37 (1H, quin.d, *J* 6.8, 2.3 Hz, 2-H); δ_C (126 MHz, $CDCl_3$) 11.2 (CH_3), 23.5 (CH_2), 26.0 (CH_2), 34.4 (CH_2), 48.2 (CH_2), 48.8 (CH_2), 51.5 (CH_2), 53.6 (CH), 57.2 (CH), 209.7 (C); *m/z* (ESI) 168 (MH^+, 100%).

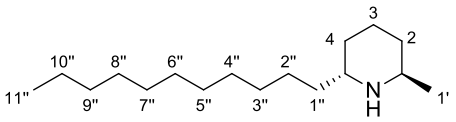
(8*R*,10*R*)-8-Methyl-10-undecyl-1,5-dithia-9-azaspiro[5.5]undecane (**280**)



A solution of *tert*-butyl (2*R*,6*R*)-2-methyl-4-oxo-6-undecylpiperidine-1-carboxylate (**264f**) (0.0570 g, 0.136 mmol) in anhydrous dichloromethane (5 mL) was cooled to 0 °C and 1,3-propanedithiol (0.136 mL, 1.36 mmol) and boron trifluoride diethyl etherate (0.042 mL, 0.340 mmol) was added dropwise. The reaction was warmed to room temperature and stirred for 20 h. The reaction

mixture was then diluted with dichloromethane (50 mL), washed with 1 M sodium hydroxide (50 mL) and brine (50 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product as a yellow oil. Purification by flash column chromatography using silica gel, eluting with 5% methanol in dichloromethane gave (8*R*,10*R*)-8-methyl-10-undecyl-1,5-dithia-9-azaspiro[5.5]undecane (**280**) as a clear oil (0.0500 g, 90%). ν_{max}/cm^{-1} (neat) 2922 (CH), 1465, 721; $[\alpha]_D^{24}$ +6.4 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.88 (3H, t, *J* 6.9 Hz, 11''-H_3), 1.16–1.41 (21H, m, 1'-H_3, 2''-H_2, 3''-H_2, 4''-H_2, 5''-H_2, 6''-H_2, 7''-H_2, 8''-H_2, 9''-H_2 and 10''-H_2), 1.58–1.76 (2H, m, 1''-H_2), 1.86 (1H, dd, *J* 14.0, 7.4 Hz, 7-*H*H), 1.90–2.04 (3H, m, 3-H_2 and 11-*H*H), 2.24 (1H, dd, *J* 14.2, 4.4 Hz, 11-H*H*), 2.29 (1H, dd, *J* 14.0, 3.9 Hz, 7-H*H*), 2.77–2.94 (4H, m, 2-H_2 and 4-H_2), 3.08–3.21 (1H, m, 10-H), 3.27–3.41 (1H, m, 8-H); δ_C (101 MHz, $CDCl_3$) 14.3 (CH_3), 21.7 (CH_3), 22.8 (CH_2), 25.6 (CH_2), 26.6 (2 × CH_2), 26.8 (CH_2), 29.7 (3 × CH_2), 29.8 (2 × CH_2), 32.1 (CH_2), 35.1 (CH_2), 41.3 (CH_2), 44.0 (CH_2), 45.0 (CH), 47.9 (CH_2), 50.2 (CH); *m/z* (ESI) 358.2583 (MH^+. $C_{20}H_{40}NS_2$ requires 358.2597).

(−)-Solenopsin A (246)[191]



To a solution of (8*R*,10*R*)-8-methyl-10-undecyl-1,5-dithia-9-azaspiro[5.5]undecane (**280**) (0.0710 g, 0.155 mmol) in ethanol (10 mL) was added a suspension of Raney nickel (1.58 g) in ethanol (10 mL). The reaction mixture was heated under reflux whilst purging with hydrogen for 0.5 h. The reaction was then stirred under reflux, under an atmosphere of hydrogen for a further 3 h. After cooling to room temperature, the reaction mixture was filtered through a Celite pad and then concentrated *in vacuo*. The resulting residue was dissolved in dichloromethane (50 mL) and washed with 2 M sodium hydroxide (50 mL) and brine (50 mL). The organic layer was dried ($MgSO_4$) and concentrated to give the crude product as a yellow oil. Purification by flash column chromatography using silica gel, eluting with 20% methanol in chloroform gave (−)-solenopsin A (**246**) as a clear oil (0.0301 g, 77%). $[\alpha]_D^{25}$ −1.0 (*c* 0.5, MeOH), lit.[191] $[\alpha]_D$ −1.21 (*c* 0.94, MeOH); δ_H (400 MHz, $CDCl_3$) 0.88 (3H, t, *J* 6.9 Hz, 11''-H_3), 1.07 (3H, d, *J* 6.5 Hz, 1'-H_3), 1.19–1.70 (26H, m, 3-H_2, 4-H_2, 5-H_2, 1''-H_2, 2''-H_2, 3''-H_2, 4''-H_2, 5''-H_2, 6''-H_2, 7''-H_2, 8''-H_2, 9''-H_2 and 10''-H_2), 2.87 (1H, qd, *J* 6.8, 4.4 Hz, 6-H), 3.06 (1H, quin.d, *J* 6.5, 3.6 Hz, 2-H); δ_C (101 MHz, $CDCl_3$) 14.3 (CH_3), 19.7 (CH_2), 21.4 (CH_3), 22.8 (CH_2), 26.6 (CH_2), 29.5 (CH_2), 29.7 (3 × CH_2), 29.8 (3 × CH_2), 29.9 (CH_2), 32.1 (CH_2), 33.1 (CH_2), 46.0 (CH), 51.0 (CH); *m/z* (EI) 253 (M^+, 11%), 238 (36), 210 (5), 126 (6), 111 (8), 98 (100).

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